# DEVELOPMENT OF ANTIBACTERIAL COMPOUNDS TO TARGET DRUG RESISTANT BACTERIA

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# Abstract

Nosocomial infections caused by resistant Gram-positive organisms are on the rise, presumably due to a combination of factors including prolonged hospital exposure, increased use of invasive procedures and pervasive antibiotic therapy. Compounding the problem is the emergence of multidrug-resistant (MDR) Gram-positive bacteria [e.g. methicillin resistant *Staphylococcus aureus* (MRSA), *Streptococcus pneumoniae* and *Enterococcus* spp.], which render treatment extremely difficult. As a result, last resort antibiotics (e.g. vancomycin, linezolid and daptomycin) are frequently deployed as treatment for these infections, which have the unintended consequence of selecting resistance to these agents. Although antibiotic stewardship and infection control measures are helpful, newer agents against MDR Gram-positive bacteria are urgently needed. Here we describe our efforts that lead to the identification of 5-aminoquinolone **111** with exceptionally potent Gram-positive activity with MICs $\leq$ 0.06 μg/mL against numerous clinical MRSA isolates. Preliminary mechanism of action and resistance studies demonstrate the 5-aminoquinolones are bacteriostatic but become cidal with 4-8 times MIC, do not select for resistance, and selectively disrupt bacterial membranes over eukaryotic membranes. While the precise molecular mechanism has not been elucidated, the lead compound is non-toxic displaying a therapeutic index of greater than 1000, is devoid of hemolytic activity and has attractive physicochemical properties (clogP = 3.8, MW = 441) that warrant further investigation of this promising antibacterial scaffold for treatment of Gram-positive infections.

Another infectious disease that has a massive burden upon the global society and shares the same concern of drug resistance is Tuberculosis (TB). *Mycobacterium tuberculosis* (*Mtb*), the causative infectious agent of TB, contains many essential biosynthetic pathways necessary for the

survival and virulence of *Mtb*, but are absent in humans making these pathways prime candidates for antimicrobial compounds. Chorismate biosynthesis is one such essential pathway that has been exploited as a route to TB chemotherapy. Chorismate is also a metabolic hub towards the biosynthesis of a wide array of aromatic small molecules such as folates, mycobactins, aromatic amino acids, and menaquinone in *Mtb.* Herein, we describe the synthesis of the epimers of 6-fluoroshikimate and methyl-6-fluoroshikimate, known inhibitors of chorismate-utilizing pathways, and biological evaluation of methyl (6*S*)-6-fluoroshikimate (**125**) in *Mycobacterium*. Initial supplementation studies indicate that methyl (6*S*)-6-fluoroshikimate may act upon unexpected chorismate-utilizing pathways.

# Table of Contents

List of Tables

# List of Figures

# List of Schemes

# List of Abbreviations

Multidrug-resistant (MDR), methicillin resistant *Staphylococcus aureus* (MRSA), antimicrobial resistance (AMR), Centers for Disease Control (CDC), World Health Organization (WHO), staphylococcal cassette chromosome *mec* (SCC*mec*), healthcare-associated MRSA (HA-MRSA), community-associated MRSA (CA-MRSA), cationic antimicrobial peptides (CAMPs), high-throughput screening (HTS), structure-activity relationships (SAR), polyphosphoric acid (PPA), nucleophilic aromatic substitution ($S_NAr$), potassium bis(trimethylsilyl)amide (KHMDS), trifluoroacetic anhydride (TFAA), Muellar-Hinton (MH), minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), transmission electron microscopy (TEM), fluorescence microscopy (FM), frequency of resistance (FOR), tuberculosis (TB), *Mycobacterium tuberculosis* (*Mtb*), multidrug-resistant (MDR-TB), extensively drug resistant tuberculosis (XDR-TB), MST (Menaquinone, Siderophore, and Tryptophan), salicylate synthase (SS), anthranilate synthase (AS), isochorismate synthase (IS), chorismate mutase (CM), 4-aminodeoxychorismate synthase (ADCS), 4-aminodeoxychorismate (ADC), *p*-aminobenzoic acid (PABA), *p*-aminosalicylic acid (PAS), 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), N,N'-dimethylaminopyridine (DMAP), *meta*-chloroperoxybenzoic acid (*m*CPBA)

# Chapter 1. Development and characterization of potent membrane disrupting agents to combat antibiotic resistant Gram-positive bacteria

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John Schultz designed, synthesized, and characterized all compounds along with confirmation of MIC values for select compounds.

## *1.1. Introduction*

Rising antimicrobial resistance (AMR) threatens global public health, and if unabated, may force us to return to a 'pre-antibiotic era' when infectious diseases caused nearly one-third of all reported deaths.[1] The Gram-positive bacterium methicillin-resistant *Staphylococcus aureus* (MRSA) is a prototypical multidrug-resistant organism listed by the Centers for Disease Control (CDC) as a top priority pathogen.[2,3] *S. aureus* is both a commensal microbe found in the nasal mucosa of ~30% of healthy adults[4] and a human opportunistic pathogen. Infections with *S. aureus* typically occur in immunocompromised individuals with underlying medical conditions—such as diabetes,[5] acquired immunodeficiency syndrome or defective neutrophil function[6]—following disruption of the host's cutaneous or mucosal barriers. Disruption of these barriers can be caused by injury, surgical procedures,[7] medical devices,[8] and drug use[9] which can lead to a litany of diseases, including sepsis, severe skin infections, catheter-associated infections and pneumonia.[8] In 2017 alone, severe cases of MRSA led to an estimated 119,000 systemic infections with a mortality rate of 17%.[10] While MRSA has historically been recognized for its role in healthcare-associated (HA) infections, community-associated (CA) infections have become more prevalent in the past 25 years, often leading to worse health outcomes.[11,12]

MRSA was first reported in 1961,[13] only one year after the introduction of the β-lactamase resistant penicillin known as methicillin into clinical practice. β-Lactam resistance in MRSA is due to expression of the altered penicillin-binding protein PBP2a,[14,15] which is only weakly inhibited by virtually all β-lactam antibiotics.[16] PBP2a is encoded by *mecA* or similar homologues that are part of a mobile genetic element called the staphylococcal cassette chromosome *mec* (SCC*mec*), which can be further classified into fourteen types (I-XIV).[17,18] SCC*mec* types I, II, and III are commonly found in healthcare-associated MRSA (HA-MRSA) while SCC*mec* IV and V are found

in both HA-MRSA and community-associated MRSA (CA-MRSA).[19] The different SCC*mec* types contain other genetic elements that confer resistance to other classes of antibacterial agents such as tetracyclines,[20] glycopeptides,[21] lipopeptides,[22] macrolides,[23, 24] and aminoglycosides.[23,25]

Despite the growing rise of antimicrobial resistance, there have only been six new first-in-class antibacterial drugs approved in the past 20 years.[26–30] Clinicians continue to rely almost exclusively on intravenously administered vancomycin for treatment of hospitalized patients with serious MRSA infections while intravenous daptomycin is used for MRSA bacteremia and endocarditis. Linezolid is an attractive oral switch therapy for MRSA infections and is widely used for treatment of pneumonia and skin and soft tissue infections.[31] Resistance to all three agents has been reported.[21,22,32] The limited treatment options, inadequate number of antibacterial agents in the drug pipeline, and emerging resistance to standard-of-care treatment options all point to the need for novel therapeutics with unconventional mechanisms of action.

The bacterial membrane has traditionally been overlooked in antibacterial drug research because membrane-targeting agents are generally considered poorly selective.[33] However, selectivity can be achieved by binding prokaryotic structural lipids,[34] membrane proteins,[35] and cell wall components[35,36] enabling discrimination from host cell membranes.[37] Bacterial membranes represent particularly promising antibacterial targets since they are essential under replicating and non-replicating conditions, as well as in planktonic and biofilm cultures. Moreover, the development of resistance to compounds targeting the bacterial membrane is more difficult than to classical antibiotics directed against proteins which are mutable.[37] The naturally occurring cationic antimicrobial peptides (CAMPs) that disrupt bacterial membranes are part of prokaryotes', eukaryotes' and plantaes' innate immune system[38,39] while several classes of FDA-approved antibiotics exert their activity through bacterial membrane disruption including the polymyxins,[40]

bacitracins,[41] lipopeptides,[42] and select lipoglycopeptides[43] (**Figure 1.1.1**). The potent antibacterial activity of the synthetic retinoids CD437 and CD1530 (**Figure 1.1.1**) was recently shown[44] to be caused by membrane disruption as the primary mechanism of action. These aforementioned membrane-targeting antibacterial agents are noted for their poor pharmacokinetic behavior and/or toxicity, which emanates from their amphipathic nature and undesirable physicochemical properties. Herein, we report our investigation of a membrane-disrupting aminoquinoline antibacterial scaffold that led to the identification of a highly potent and bacterial-selective Gram-positive antibacterial agent with attractive physicochemical properties.

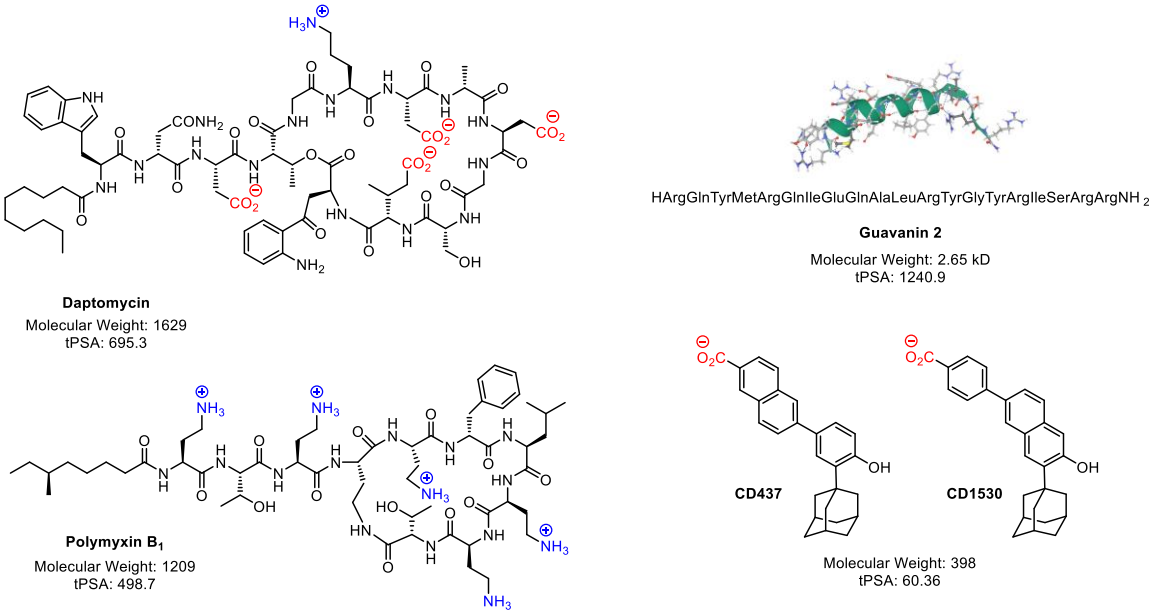



**Figure 1.1.1.** Therapeutic and experimental membrane-disrupting agents. Daptomycin is an FDA-approved antibiotic for treatment of gram-positive that inserts into the cytoplasmic membrane of the bacteria and permeabilizes it via membrane-associated oligomers. Guavanin 2 is a cationic antimicrobial peptide (CAMP) that disrupts membranes of bacteria via membrane hyperpolarization. Guavanin 2 structure[39] taken from PDB 5V1E. Polymyxin $B_1$ is part of the polymyxin class of antibiotics and is an FDA-approved antibiotic that disrupts membranes of Gram-negative bacteria.

## *1.2. Design and Synthesis of Anti-MRSA Compounds*

We previously reported the identification of the 4-quinolinol derivative DNAC-2 from a high-throughput screening (HTS) campaign with moderate activity (MIC = 8 µg/mL) against MRSA (**Figure 1.2.1**).[45] Intriguingly, DNAC-2 was found to target the membrane of Gram-positive bacteria resulting in partial membrane depolarization while displaying no overt toxicity towards eukaryotic membranes. In addition to DNAC-2, a few other substituted quinolines were identified with the same mechanism of action typified by quinoline **1** (**Figure 1.2.1**) indicating flexibility at the 4-position. We were attracted to the 4-substituted quinoline scaffold based on its promising activity, chemical tractability for analog synthesis, and prevalence in several approved drugs.[46] We initially sought to examine the structure-activity relationships (SAR) of **1** through substitution and replacement of the 4-aryl ring with more polar and non-planar substituents (**Figure 1.2.1**). In parallel, we wanted to explore modification and substitution to the quinoline heterocycle through introduction of nitrogen atoms and introduction of more polar substituents at the 2-, 7- and 8-positions to decrease the lipophilicity.

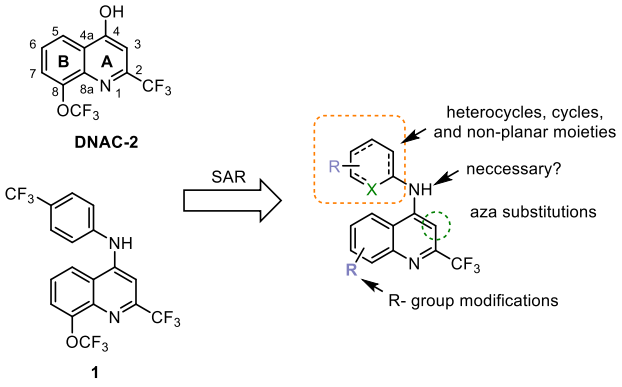
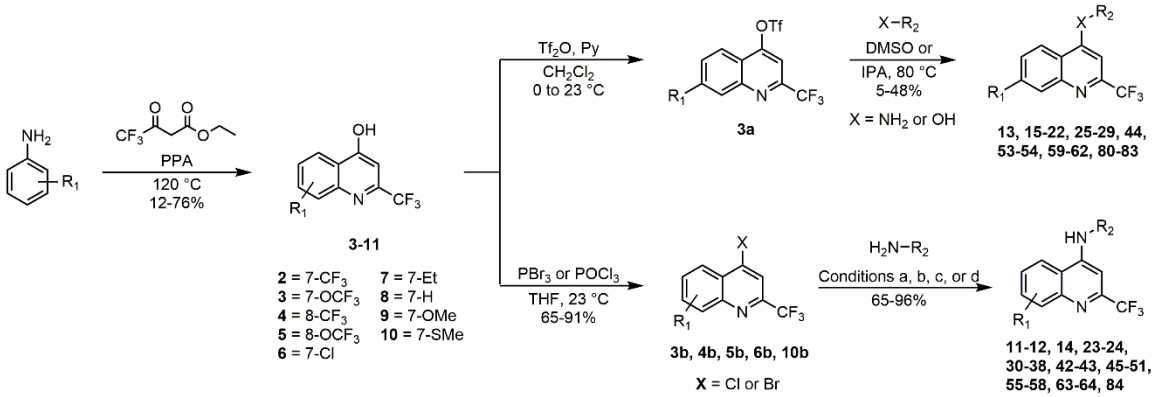



**Figure 1.2.1.** High throughput screen hits and SAR analysis of the 4-aminoquinoline scaffold.

## *1.3. Chemistry*

The first series of quinoline analogues were synthesized from a common set of quinolone building blocks **2-10** that were prepared via a modified Conrad-Limpach reaction[47,48] between substituted aniline derivatives and 4,4,4-trifluoroacetoacetate in neat polyphosphoric acid (PPA) at 120 °C.[49] *Meta*-substituted anilines typically formed a mixture of both the 5- and 7-regioisomers that were challenging to separate and led to reduced isolated yields of the desired 7-regioisomers, whereas *ortho*-substituted anilines exclusively afforded the 8-regioisomers. The quinolines were evaluated for antibacterial activity and only compounds **3–6** and **10** (DNAC-2 is the same as **6**) were found to be active (**Table 1.4.1**). Consequently, only these compounds were derivatized by introduction of a substituent at the 4-position. Quinolone **3** was converted to the corresponding triflate **3a** employing triflic anhydride and reacted with various amines and phenols by nucleophilic aromatic substitution ($S_NAr$)[50] to afford **13**, **15–22**, **25–29**, **44**, **53–54**, **59–62**, and **80-83** (**Scheme 1.3.1**). This strategy proved less effective for electron-deficient amines as well as quinolones with electron donating substituents. In these cases, we utilized a complimentary route by conversion of quinolones to the corresponding aryl chloride[51,52] or aryl bromides[53] **3b–6b** and **10b** followed by Buchwald-Hartwig amination[54–56] to provide **2**, **12**, **14**, **23–24**, **30–38**, **42–43**, **45–51**, **55–58**, **63-64** and **84** (**Scheme 1.3.1**).

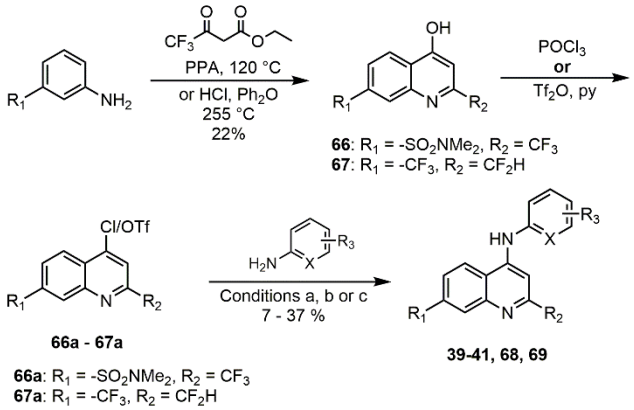
**Scheme 1.3.1.** 4-Aminoquinoline and 4-oxyquinoline synthesis




Conditions: [a] $K_3PO_4$, SPhos, $Pd_2(dba)_3 \cdot CH_2Cl_2$, THF, 55 °C, [b] *t*-BuONa, DPPF, $Pd(dppf)Cl_2 \cdot CH_2Cl_2$, THF, 55 °C [c] *t*-BuONa, XantPhos, $Pd_2(dba)_3$, dioxane 80 °C, [d] HCl, EtOH, reflux.

Compounds containing a difluoromethyl C-2 substituent were prepared analogously by Conrad-Limpach reaction between 3-trifluoromethylaniline and 4,4-difluoroacetoacetate to afford quinolone **67**, which was activated by triflic anhydride to **67a** and elaborated to **68** and **69** by $S_NAr$ substitution with 3,4-dichloroaniline and 3-trifluormethoxyaniline, respectively (**Scheme 1.3.2**). Analogs containing a dimethylaminosulfonyl C-7 substituent could not be synthesized using the usual PPA-mediated procedure and required substantially more thermal energy. Compound **66** was instead prepared by refluxing 3-(dimethylaminosulfonyl)aniline and 4,4,4-trifluoroacetoacetate at 255 °C in diphenyl ether (**Scheme 1.3.2**). Chlorination of **66** with $POCl_3$ yielded **66a** that was diversified to **39**–**41** by Buchwald-Hartwig amination.

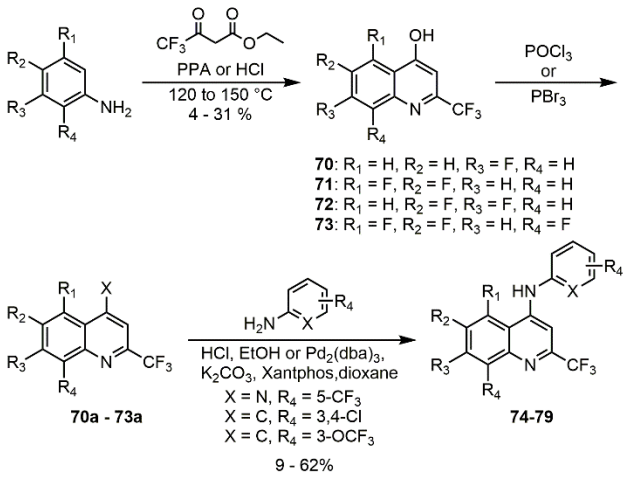
**Scheme 1.3.2.** 4-Aminoquinoline synthesis.




Conditions: [a]DMSO, 80 °C; [b]HCl, EtOH, reflux; [c]Pd2(dba)3, XantPhos, t-BuONa, dioxane 80 °C

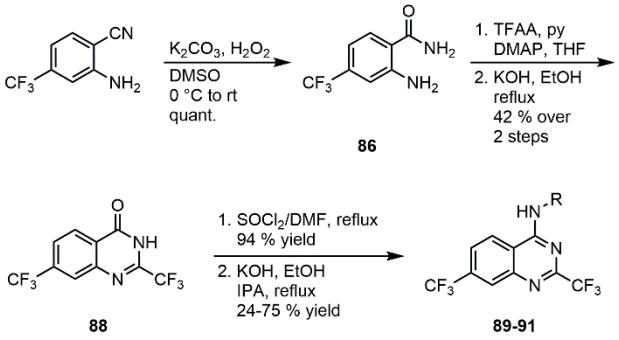
We synthesized a series of mono-, di- and tri-fluorinated analogs of the B-ring in an attempt to replace the lipophilic C-7 trifluoromethyl group. While *m*-fluoroaniline reacted with 4,4,4-trifluoroacetoacetate in neat polyphosphoric acid (PPA) at 100 °C to furnish **70**, the di- and tri-fluoroanilines required heating at 150 °C to effect cyclization to quinolones **71–73** (**Scheme 1.3.3**). Halogenation of **70–73** to quinolines **70a–73a** followed by Buchwald-Hartwig amination as described previously yielded **74–79**.

**Scheme 1.3.3.** Synthesis of fluorinated B-ring analogues.



In an attempt to reduce the lipophilicity of the aminoquinolines, we targeted the synthesis of quinazolines containing an additional nitrogen atom in the A-ring. Synthesis commenced from commercially available 2-amino-4-(trifluoromethyl)benzonitrile that was oxidized from the nitrile to the amide intermediate **86** by treatment with an alkaline solution of hydrogen peroxide (**Scheme 1.3.4**). Treatment of the resulting substituted aniline **86** with 2,2,2-trifluoroacetyl chloride furnished the bis-amide intermediate **87**, which was cyclized to the quinazolone **88** employing potassium hydroxide in ethanol at reflux. Chlorination of quinazolone **88** with thionyl chloride in DMF followed by $S_NAr$ substitution with 3,4-dichloroaniline, 3-trifluoromethoxyaniline, and 2-amino-5-trifluoromethylpyridine afforded the final 4-aminoquinazolines **89**–**91**.

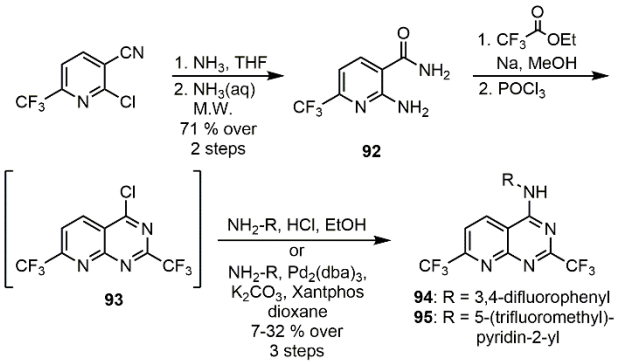
**Scheme 1.3.4.** Synthesis of 4-sminoquinazolines.




The pyrido[2,3-*d*]pyrimidine scaffold containing two additional nitrogen atoms was investigated as a quinoline isostere in an attempt to further decrease lipophilicity. The synthesis began by $S_NAr$ substitution of 2-chloro-3-cyano-6-trifluoromethylpyridine with ammonia in THF followed by base-promoted hydration of the cyano group to furnish amide **92** (**Scheme 1.3.5**). Subsequent condensation of **92** with ethyl trifluoroacetate and base-catalyzed annulation afforded an intermediate pyrido[2,3-*d*]pyrimidin-4-one derivative that was converted to **93** by $POCl_3$ mediated chlorination. $S_NAr$ substitution of **93** with 3,4-dichloroaniline yielded **94** while reaction

of **93** with 2-amino-5-trifluoromethylpyridine to provide **95** required the complimentary Buchwald-Hartwig amination.

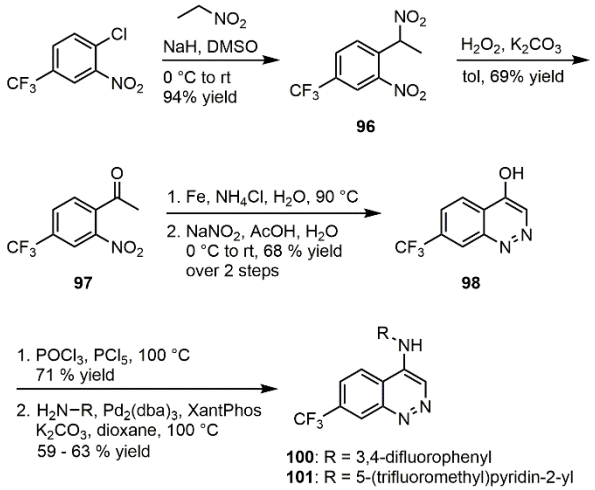
**Scheme 1.3.5.** Synthesis of pyrido[2,3-*d*]pyrimidines.




The cinnoline analogs were the final set of aza-analogs of the quinoline scaffold prepared (**Scheme 1.3.6**). Initial efforts to arrive at the o-acetylaniline intermediate (**97**) from Grignard alkylation of 2-cyano-5-(trifluoromethyl)aniline repeatedly gave very undesirable yields in our hands (5-19 % yields). Searching the literature we found a secondary route to **97** via a base-mediated alkylation of 2-chloro-5-(trifluoromethyl)nitrobenzene with nitroethanone followed by an oxidative Nef reaction from a modified procedure reported by Reid and Runge.[57] The reported conditions, of two equivalents of DBU, at 0 °C in ethyl acetate, for the initial alkylation of 2-chloro-5-(trifluoromethyl)nitrobenzene gave complete conversion to the *ortho*-DBU adduct of the nitrobenzene starting material which was noted as a trace byproduct in the initial manuscript.[57] Altering temperature, solvent, equivalents of base, and order of addition all afforded the *ortho*-DBU adduct while giving only trace amounts of the desired product. When DBN was used as the base the analogous *ortho*-DBN adduct of the nitrobenzene starting material was the sole product. Organic bases such as TEA and DIPEA gave either no conversion or trace amount of the degradation of the chloro starting material to the phenol. Lithium diisopropylamide and LiHMDS

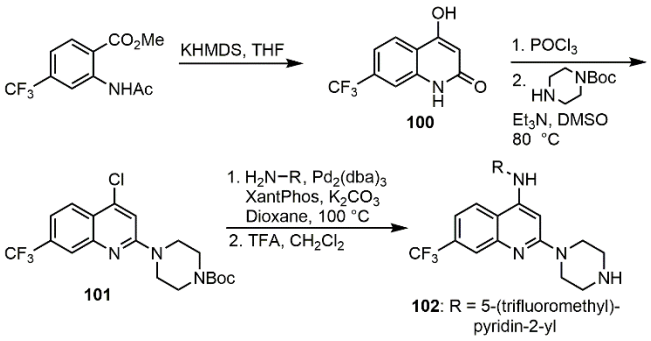
bases both saw significant degradation of the starting material to a mixture of isomers and a significant amount of the phenol byproduct. Inorganic bases such as cesium carbonate and sodium hydride both gave significant amounts of the desired product using both DMSO and THF as solvents with minimal or no degradation to the phenol. Optimal base and solvent were determined to be sodium hydride and DMSO which gave yields of **96** similar to what was reported in the initial Reid paper.[57] The subsequent oxidative Nef reaction of **96** gave the desired acetyl intermediate (**97**) without modification. In two steps, **97** was reduced to the aniline and subsequently treated with aqueous sodium nitrite in acetic acid resulting in the diazonium salt which allowed for acid cyclized annulation of the ketone to give cinnoline (**98)**. The cinnoline (**98**) was then converted to the chloride upon heating with a solution of phosphorus oxychloride and phosphorus pentachloride. The resulting halogenated cinnoline (**99**) was coupled to 3,4-difluoroaniline and 2-amino-5-trifluoromethylpyridine under Buchwald-Hartwig conditions providing **100** and **101**, respectively.

**Scheme 1.3.6.** Cinnoline synthesis.



We developed an alternate quinoline synthesis to explore modification of the C-2 position featuring a 2,4-dichloroquinoline intermediate. This was accomplished by intramolecular Claisen-like condensation of ethyl *N*-acetyl-2-amino-4-trifluoromethylbenzoate mediated by potassium bis(trimethylsilyl)amide (KHMDS) to afford a 4-hydroxyquinoline-(2*H*)-one (**100**) intermediate (**Scheme 1.3.7**). Compound **100** was converted to 2,4-dichloroquinoline by refluxing in phosphorus oxychloride followed by $S_NAr$ substitution by Boc-protected piperazine to give **101** along with the C-4 regioisomer (not shown). Buchwald-Hartwig coupling of **101** and 2-amino-5-trifluoromethylpyridine followed by TFA deprotection of the Boc group gave the desired analogue **102**. The regioisomeric analog **103**, described in the supporting information, was prepared from the corresponding C-4 piperazine intermediate isolated as a side-product in the preparation of **101**. The C-2 and C-4 morpholino substituted analogues **104** and **105** were synthesized in an analogous fashion.

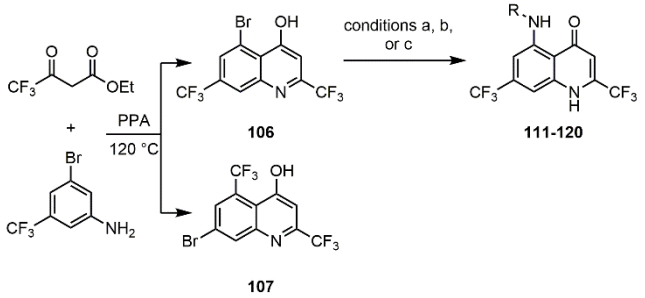
**Scheme 1.3.7.** Synthesis of a C-2 piperazine analogue.




We next conceived of a hybrid scaffold of the initial active quinoline-4-ones and the aryl-substituted 4-aminoquinolines, giving an aryl-substituted 5-aminoquinoline-4-ones scaffold (**Scheme 1.3.8**). The two-step synthesis started from condensation of 3-bromo-5-trifluoromethylaniline and ethyl 2,2,2-trifluoromethylacetoacetate in neat PPA to give two

separable regioisomers **106** and **107**. The structure of the regioisomers were assigned by $^{1}$H NMR analysis of the debrominated products generated by palladium-catalyzed dehalogenation (not shown). Following optimization of the Buchwald-Hartwig amination conditions, we were able to access the desired C-5 substituted aminoquinoline-4-ones **111–120** from **106**. The C-7 fluoro derivative **122** was synthesized analogously from 3-bromo-5-fluoroaniline as described in the experimentals.

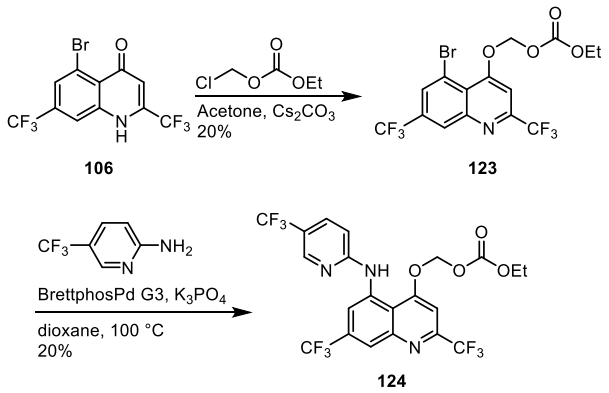
**Scheme 1.3.8.** C-5 and C-7 substituted quinoline-4-ones.




Conditions: [a]2-MeBuOH, $K_3PO_4$, tBuBrettPhos Pd G3, 90 °C; [b]dioxane, $K_3PO_4$, tBuBrettPhos, $Pd_2(dba)_3$, 100 °C [c]dioxane, $K_2CO_3$, $Pd_2(dba)_3$, JohnPhos, dioxane 100 °C

Based on the promising activity of quinolin-4-one **111**, but low solubility (**Table 1.4.8**), we sought to prepare an alkoxycarbonate prodrug for in vivo studies to increase solubility and bioavailability based on the precedent of Frueh and co-workers, who successfully applied this strategy in their preclinical development of their novel antimalarial quinolone ELQ-330.[58] Direct installation of the promoiety onto **111** was unsuccessful; however, the alkoxycarbonate promoiety could be introduced into 5-bromoquinoline **106** by cesium carbonate mediated alkylation of chloromethyl ethyl carbonate in acetone. The resulting methyloxycarbonate ester **123** was then converted to the desired final product **124** via a Buchwald-Hartwig amination with 2-amino-5-trifluoromethylpyridine (**Scheme 1.3.9**).

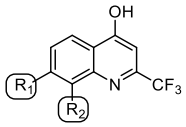
**Scheme 1.3.9.** Prodrug synthesis.




## *1.4. Microbiology.*

The antibacterial activity of compounds was initially determined against a clinical strain of methicillin-resistant *S. aureus* (FPR3757) in Muellar-Hinton (MH) broth according to the CLSI protocol to determine the minimum inhibitory concentration (MIC) that resulted in complete inhibition of observable growth. The first set of compounds evaluated were analogs at the C-7 and C-8 positions of the initial quinolone hit **DNAC-2** since the other HTS hits identified (data not shown) were substituted at these positions (**Table 1.4.1**). We first explored modification at the C-7 position with a small series of electron-donating and withdrawing substituents. The trifluoromethyl **2** and trifluoromethoxy **3** are the most potent with MICs of 4–8 μg/mL while chloro **6** is slightly weaker with an MIC of 20 μg/mL. However, analogs containing electron donating groups at C-7 including ethyl **7**, benzyloxy **8**, methoxy **9** and methylthio **10** are weakly active displaying MICs of 320–640 μg/mL indicating electron-donating substituents at C-7 are poorly tolerated. The impact of electronics is best illustrated with methoxy **9**, which is 80–160 less potent that the isosteric trifluoromethoxy **3**. The C-8 position was evaluated with trifluoromethyl **4** and trifluoromethoxy **5** containing the optimal C-7 substituents. Both **4** and **5** are equipotent to the corresponding C-7 analogs **2** and **3** indicating some flexibility of the quinolone scaffold.

**Table 1.4.1.** Substituted quinoline-4-one analogues.

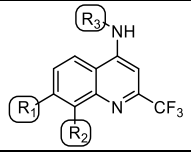



| Compd | $R_1$ | $R_2$ | MIC μg/mL | cLogP |
|---|---|---|---|---|
| **2** | $CF_3$ | H | 5 | 2.2 |
| **3** | $OCF_3$ | H | 4-8 | 2.4 |
| **4** | H | $CF_3$ | 2-4 | 2.2 |
| **5** | H | $OCF_3$ | 5 | 2.4 |
| **6** | chloro | H | 20 | 2.0 |
| **7** | ethyl | H | 320 | 2.3 |
| **8** | benzylether | H | 640 | 3.1 |
| **9** | methoxy | H | 640 | 1.3 |
| **10** | thiomethyl | H | 640 | 1.9 |

We next explored the SAR of the C-4 aryl substituent of 4-aminoquinoline **1** whose MIC is 8 μg/mL (**Table 1.4.2**). Our first series of compounds contain a 7-trifluoromethyl rather than the 8-trifluoromethoxy substituent found in **1**. The 2′-, 3′-, and 4′-chlorophenyl analogs **15–17** helped to define the steric requirements for activity: 2′-chlorophenyl **15** is inactive while both 3′-chlorophenyl **16** and 4′-chlorophenyl **17** possess MICs of 0.125–0.25 μg/mL, which represents a dramatic 32–64-fold increase in potency over **1**. Given the enhanced potency of the chloro-substituted analogs, we conducted a halogen scan and evaluated 3′-fluorophenyl **18**, 3′-bromophenyl **19** and 3′-iodophenyl **20**. The more lipophilic halogens **19** and **20** maintain potent activity with MICs of 0.25 μg/mL while the fluoro analog has a substantial 16–64-fold loss of potency. We also explored a couple of 3′,4′-disubstituted analogs with 3′,4′-fluorophenyl **21** and 3′,4′-dichlorophenyl **22**. Both analogs display further improvements in potency relative to the corresponding mono-halogenated analogs and the MIC of 3′,4′-dichlorophenyl **22** decreased to

0.0625 μg/mL, the lowest value among the series of analogs described in Table 2. Additionally, a broader array of substituents were explored at the 3′- and 4′-positions of the aryl ring including phenyl **11**, 3′-acetyl **13**, 3′-cyanophenyl **14**, 3′-hydroxymethylphenyl **23**, 3′-methylthiophenyl **24**, 3′-methoxyphenyl **25**, 3′-trifluoromethylphenyl **26**, 3′-trifluoromethoxyphenyl **27**, 3′-(morpholino)phenyl **29**, 4′-(morpholino)phenyl **30** and 3′,4′-(methylenedioxy)phenyl **28**. Analogs containing polar acetyl, cyano, methoxy, hydroxymethyl, methylenedioxy, and morpholino substituents are inactive or weakly active with MICs generally >16 μg/mL. By contrast, analogs containing lipophilic groups including methylthio and trifluoromethyl are potent with MICs of 0.25–0.50 μg/mL, the exception being trifluoromethoxy **27**, whose MIC is 8 μg/mL. In an attempt to decrease the lipophilicity, we replaced the phenyl ring of 3′-trifluoromethylphenyl **26** by a pyridine to furnish 5′-(trifluoromethyl)pyridin-2-yl **31**, which fortuitously maintains activity providing an identical MIC to **26** while decreasing the calculated logP by 1.2 units to 6.0. While introduction of an appropriately substituted arylamino group led to a substantial enhancement in activity relative to the simple quinolones shown in Table 1, this boost in potency came at the expense of substantially increased lipophilicity. This is exemplified by 3′-trifluoromethylphenyl **26** whose 16-fold improvement in potency relative to the parent quinolone **3** is offset by a 5.1 unit increase in the calculated logP. Attempts to decrease lipophilicity by introduction of polar substituents onto the aryl ring led to sharp reductions in potency. However, heterocyclic replacement of the phenyl ring by a pyridine is tolerated providing a means to partially address the increased lipophilicity of the *N*-(arylamino)quinolines.

**Table 1.4.2.** Aryl 4-aminoquinoline analogues.

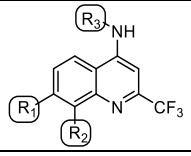



| Compound | $R_1$ | $R_2$ | $R_3$ | MIC (μg/mL) | cLogP |
|---|---|---|---|---|---|
| **1** | H | $OCF_3$ | 3-(trifluoromethoxy)phenyl | 8 | 7.9 |
| **11** | $CF_3$ | H | phenyl | 128 | 6.4 |
| **12** | $CF_3$ | H | 2-isopropylphenyl | 320 | 7.8 |
| **13** | $CF_3$ | H | 3-acetylphenyl | >128 | 5.9 |
| **14** | $CF_3$ | H | 3-cyanophenyl | >32 | 5.9 |
| **15** | $CF_3$ | H | 2-chlorophenyl | >16 | 7.1 |
| **16** | $CF_3$ | H | 3-chlorophenyl | 0.25 | 7.1 |
| **17** | $CF_3$ | H | 4-chlorophenyl | 0.12 | 7.1 |
| **18** | $CF_3$ | H | 3-fluorophenyl | 4-16 | 6.5 |
| **19** | $CF_3$ | H | 3-bromophenyl | 0.25 | 7.3 |
| **20** | $CF_3$ | H | 3-iodophenyl | 0.25 | 7.5 |
| **21** | $CF_3$ | H | 3,4-difluorophenyl | 0.125-0.25 | 6.6 |
| **22** | $CF_3$ | H | 3,4-dichloropheyl | 0.0625 | 7.7 |
| **23** | $CF_3$ | H | 3-(hydroxymethyl)phenyl | >16 | 5.3 |
| **24** | $CF_3$ | H | 3-thiomethylphenyl | 0.25-0.5 | 7.0 |
| **25** | $CF_3$ | H | 3-methoxyphenyl | >64 | 6.3 |
| **26** | $CF_3$ | H | 3-(trifluoromethyl)phenyl | 0.25 | 7.3 |
| **27** | $CF_3$ | H | 3-(trifluoromethoxy)phenyl | 8 | 7.4 |
| **28** | $CF_3$ | H | 3,4-(methylenedioxy)phenyl | >4 | 6.2 |
| **29** | $CF_3$ | H | 3-(morpholino)phenyl | >16 | 5.8 |
| **30** | $CF_3$ | H | 4-(morpholino)phenyl | >16 | 5.8 |

The SAR of the *N*-(arylamino)quinoline scaffold was further probed at the C-7 and C-8 positions with 7-methoxy, 7-trifluoromethoxy, 7-(dimethylamino)sulfonyl, 8-trifluoromethyl, and 8-trifluoromethoxy substituents with the C-4 aryl moiety selected from representatives of **12–31** including 3′,4′-difluorophenyl **21** (MIC of 0.125-0.25 μg/mL), 3′,4′-dichlorophenyl **22** (MIC of 0.0625 μg/mL), 3′-(trifluoromethoxy)phenyl **27** (MIC of 8 μg/mL), and 5′-(trifluoromethyl)pyridin-2-yl **31** (MIC of 0.25 μg/mL). Replacement of the C-7 trifluoromethyl

group by a trifluoromethoxy group in **32–34** yielded flat SAR with MICs ranging from 0.5–1 μg/mL. The SAR trend from this limited set of compounds did not parallel the SAR observed with the 7-trifluoromethyl series of compounds. The isosteric 7-methoxy analogs **35–38** were largely inactive (MICs of ≥ 32 μg/mL), a result consistent with the quinolone SAR described in Table 1. The observation that electron-withdrawing substituents at C-7 are favorable prompted exploration of the 7-dimethylaminosulfonyl group with **39–41** since sulfonamides are electron-withdrawing and considerably more polar than a trifluoromethyl group. Unfortunately, this set of compounds was only weakly active with MICs ranging from 8 to >32 μg/mL suggesting optimal quinoline substituents at C-7 should not only be electron-withdrawing, but also nonpolar. Analogs bearing trifluoromethyl and trifluoromethoxy substituents at C-8 exhibited remarkably flat SAR with MICs of 0.5–2.0 μg/mL. The SAR trend was inconsistent with the 7-trifluoromethyl substituted analogs, whose MICs varied over 128-fold for the same set of C-4 aryl substituents. Taken together, the SAR from **32–47** demonstrates substitution at C-7 is preferred and optimal substituents at this position should be non-polar and strongly electron-withdrawing.

**Table 1.4.3.** Additional aryl 4-aminoquinoline analogues.

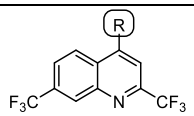


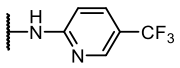
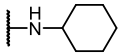
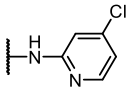
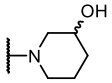
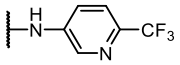
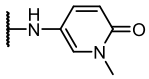
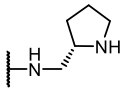
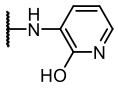
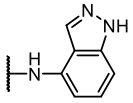
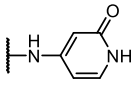
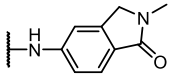
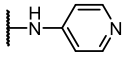
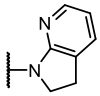
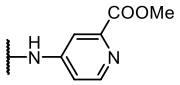
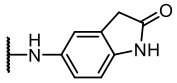
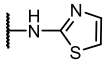
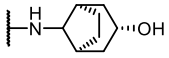
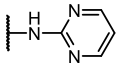

| Compound | $R_1$ | $R_2$ | $R_3$ | MIC (μg/mL) | cLogP |
|---|---|---|---|---|---|
| **1** | H | $OCF_3$ | 3-(trifluoromethoxy)phenyl | 8 | 7.9 |
| **11** | $CF_3$ | H | phenyl | 128 | 6.4 |
| **21** | $CF_3$ | H | 3,4-difluorophenyl | 0.125-0.25 | 6.6 |
| **22** | $CF_3$ | H | 3,4-dichloropheyl | 0.0625 | 7.7 |
| **31** | $CF_3$ | H | 5-(trifluoromethyl)pyridin-2-yl | 0.25 | 6.1 |
| **32** | $OCF_3$ | H | 3,4-dichlorophenyl | 0.5-1 | 8.2 |
| **33** | $OCF_3$ | H | 3-(trifluoromethoxy)phenyl | 1 | 7.9 |
| **34** | $OCF_3$ | H | 5-(trifluoromethyl)pyridin-2-yl | 1 | 6.5 |
| **35** | OMe | H | 3,4-dichlorophenyl | 2 | 6.0 |
| **36** | OMe | H | 3,4-difluorophenyl | >32 | 4.9 |
| **37** | OMe | H | 3-(trifluoromethoxy)phenyl | >32 | 5.7 |
| **38** | OMe | H | 5-(trifluoromethyl)pyridin-2-yl | 32 | 4.3 |
| **39** | $S(O)_2N(Me$ | H | 3,4-difluorophenyl | >32 | 5.1 |
| **40** | $S(O)_2N(Me$ | H | 3-(trifluoromethoxy)phenyl | 8 | 5.9 |
| **41** | $S(O)_2N(Me$ | H | 5-(trifluoromethyl)pyridin-2-yl | 8 | 4.5 |
| **42** | H | $OCF_3$ | 3,4-dichlorophenyl | 0.5 | 8.2 |
| **43** | H | $OCF_3$ | 3-(trifluoromethoxy)phenyl | 1 | 7.8 |
| **44** | H | $OCF_3$ | 5-(trifluoromethyl)pyridin-2-yl | 1 | 6.5 |
| **45** | H | $CF_3$ | 3,4-dichlorophenyl | 0.5-2 | 8.2 |
| **46** | H | $CF_3$ | 3-(trifluoromethoxy)phenyl | 1 | 7.9 |
| **47** | H | $CF_3$ | 5-(trifluoromethyl)pyridin-2-yl | 0.5 | 6.5 |

The promising activity of compound **31** containing a 5′-(trifluoromethyl)pyridin-2-yl-amino moiety appended to C-4 of the quinoline prompted us to explore more diverse heterocyclic substituents at C-4 (**Table 1.4.4**). A primary objective in these analogues was to decrease the overall lipophilicity through introduction of polar atoms and to reduce the planarity by increasing the $sp^3$ character since lipophilic and planar molecules tend to have poor solubility that adversely impacts drug disposition properties. Replacement of the 5′-(trifluoromethyl)pyridin-3-yl-amino

group at C-4 with a closely related 4′-(chloro)pyridin-2-yl-amino group in **48** led to an 8-fold loss of activity while transposition of the pyridine nitrogen by one atom in 2′-(trifluoromethyl)pyridin-5-yl-amino **49** completely abolished activity. These findings foreshadowed our unsuccessful attempts to modify the C-4 substituent. Thus pyridones **50**–**52**, pyridine **53**, picolinate **54**, pyrimidine **56**, cyclohexane **57**, hydroxypiperidine **58**, morpholine **59**, aminomethylpyrrolidine **60**, indazole **61**, isoindole **62**, pyrrolopyridine **63**, indolone **64** and azabicyclooctanol **65** were inactive at the highest concentration evaluated (MIC > 32 μg/mL). Only, aminothiazole **55** demonstrated moderate activity with an MIC of 2 μg/mL.

**Table 1.4.4.** Heterocyclic 4-substitutions.

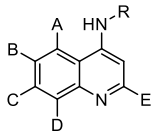


| Cmpd | R | MIC µg/mL | cLogP | Cmpd | R | MIC µg/mL | cLogP |
|---|---|---|---|---|---|---|---|
| **31** |  | 0.25 | 6.0 | **57** |  | >16 | 6.6 |
| **48** |  | 2 | 5.8 | **58** |  | >32 | 4.0 |
| **49** |  | >32 | 6.0 | **59** |  | >32 | 4.0 |
| **50** |  | >32 | 4.2 | **60** |  | >32 | 4.9 |
| **51** |  | >128 | 5.4 | **61** |  | >32 | 6.0 |
| **52** |  | >32 | 3.7 | **62** |  | >32 | 5.2 |
| **53** |  | >32 | 5.1 | **63** |  | >32 | 5.3 |
| **54** |  | >32 | 4.7 | **64** |  | >32 | 5.0 |
| **55** |  | 1-2 | 4.9 | **65** |  | >32 | 5.3 |
| **56** |  | >32 | 4.2 | | | | |

Further structural modifications were focused on reducing the calculated LogP by modifications of the quinoline core employing the optimal C-4 substituents: 3′,4′-dichlorophenyl,

3′-(trifluoromethoxy)phenyl and 5′-(trifluoromethyl)pyridin-2-yl from compounds **22, 27,** and **31**, respectively. The trifluoromethyl groups at the C-2 and C-7 positions contribute significantly to the overall lipophilicity, thus the next series of analogs explored replacement of the trifluoromethyl group by difluoromethyl and aryl fluorides, which were predicted to lower the LogP by approximately 0.7 units per trifluoromethyl group (**Table 1.4.5**). Replacement of the C-2 trifluoromethyl group of **22** with a difluoromethyl group afforded compound **68**, which is 16-fold less potent than **22**. Conversely, compound **69** exhibits a 16-fold increase in potency relative to the corresponding trifluoromethyl analog **27**. We cannot reconcile the disparate impact on potency of the difluoromethyl group based on this limited set of analogs, but the difluoromethyl group appears to level the SAR as both **68** and **69** have similar MICs. Replacement of the C-7 trifluoromethyl group by a fluorine was explored with analogs **74–79**. Substitution of the C-7 trifluoromethyl group by a 7-fluoro moiety in **74–76** led to uniform 4–8-fold reductions in potency relative to the corresponding trifluoromethyl analogs **22**, **27** and **31** providing MICs ranging from 0.5–2.0 μg/mL. Given the more predictable SAR of the aryl fluoride analogs, we sought to introduce additional fluorine atoms in the B-ring of **76** at the 5, 6, and 8-positions with difluorinated analogs **77–78** and trifluorinated analog **79**. While fluorine was poorly tolerated at the 5 and 6-positions, the 6,7-difluoro analog **78** fully regained the activity of the parent trifluoromethyl analog **31**. Collectively, these results indicate modest attenuation of the cLogP can be achieved by replacement of the lipophilic trifluoromethyl groups with fluorine atoms while maintaining potent activity.

**Table 1.4.5.** Fluorine substitutions

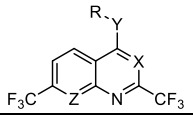



| Compound | A | B | C | D | E | R | MIC µg/mL | cLogP |
|---|---|---|---|---|---|---|---|---|
| **68** | H | H | $CF_3$ | H | $CHF_2$ | 3,4-dichlorophenyl | 1 | 6.3 |
| **69** | H | H | $CF_3$ | H | $CHF_2$ | 3-(trifluoromethoxy)phenyl | 0.5 | 6.6 |
| **74** | H | H | F | H | $CF_3$ | 3,4-dichlorophenyl | 0.5 | 7.0 |
| **75** | H | H | F | H | $CF_3$ | 3-(trifluoromethoxy)phenyl | 2 | 6.7 |
| **76** | H | H | F | H | $CF_3$ | 5-(trifluoromethyl)pyridin-2-yl | 1 | 5.3 |
| **77** | F | F | H | H | $CF_3$ | 5-(trifluoromethyl)pyridin-2-yl | 16-32 | 5.3 |
| **78** | H | F | F | H | $CF_3$ | 5-(trifluoromethyl)pyridin-2-yl | 0.25 | 5.3 |
| **79** | F | F | H | F | $CF_3$ | 5-(trifluoromethyl)pyridin-2-yl | >32 | 5.5 |

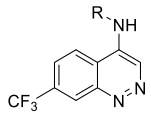
With extensive coverage of the C2, C-4, C-7 and C-8 positions of the 4-aminoquinoline, the SAR campaign moved towards heteroatom modifications of the 4-aminoquinoline core. We first studied the importance of the 4-amino group and specifically the importance of an H-bond donor at this position with ether analogues **80–83**, *N*-methyl derivative **84** and amide **85** (**Table 1.4.6**). Compounds **80–85** are inactive with MICs greater than 32 µg/mL indicating an NH moiety is essential for activity and a one atom linker is preferred. We then explored quinazoline analogs **89–91** containing a single aza substitution at the C-3 position, which retains a similar pharmacophore while lowering the calculated LogP by 1.5 units. The 3′,4′-dichlorophenyl **89**, and 5′-(trifluoromethyl)pyridin-2-yl **91** quinazoline analogues are 8-fold less active than the parent quinolines **22, 31** while the trifluoromethoxy **90** derivative has an opposite 8-fold increase in potency relative to the parent quinoline **27**. The aza substitution thus appears to flatten the SAR as the potency of **89–91** varies only 4-fold from 0.5–2.0 µg/mL Introduction of another nitrogen atom

into the quinazoline at the C-8 position led to pyridopyrimidine derivatives **94–95** and an attendant decrease in logP by almost 3 units. Unfortunately, both pyridopyrimidines **94** and **95** have drastically reduced activity with MICs ≥ 32 μg/mL.

**Table 1.4.6.** Heteroatom Exchanges




| Compound | X | Y | Z | R | MIC μg/mL | cLogP |
|---|---|---|---|---|---|---|
| **80** | CH | O | CH | 3-chlorophenyl | >32 | 6.8 |
| **81** | CH | O | CH | 3-fluorophenyl | >32 | 6.2 |
| **82** | CH | O | CH | 3,4-dichlorophenyl | >32 | 7.4 |
| **83** | CH | O | CH | 3-(trifluoromethoxy)phenyl | >32 | 7.1 |
| **84** | CH | NMe | CH | 4-trifluoromethylphenyl | >32 | 7.4 |
| **85** | CH | N(C=O) | CH | 5-(trifluoromethyl)pyridin-2-yl | >32 | 5.3 |
| **89** | N | NH | CH | 3,4-dichlorophenyl | 0.5 | 6.9 |
| **90** | N | NH | CH | 3-(trifluoromethoxy)phenyl | 1 | 6.6 |
| **91** | N | NH | CH | 5-(trifluoromethyl)pyridin-2-yl | 2 | 5.1 |
| **94** | N | NH | N | 3,4-difluorophenyl | 32 | 4.0 |
| **95** | N | NH | N | 5-(trifluoromethyl)pyridin-2-yl | >32 | 5.8 |

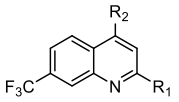



| Compound | R | MIC μg/mL | cLogP |
|---|---|---|---|
| **100** | 3,4-difluorophenyl | >32 | 4.8 |
| **101** | 5-(trifluoromethyl)pyridin-2-yl | >32 | 4.1 |

A few remaining miscellaneous modifications to reduce the cLogP of the 4-aminoquinoline scaffold are described in Tables 1.4.6 and 1.4.7. The cinnoline analogues **100** and **101** lacking a C-2 trifluoromethyl group and containing a nitrogen atom at C-2 are unsurprisingly inactive (**Table 1.4.6**). Remarkably, introduction of a piperazine at C-2 with **102** was reasonably well tolerated yielding an MIC of 0.5–1.0 μg/mL while the morpholine analogue **104** is inactive (**Table 1.4.7**).

The piperazine and morpholine constitutional isomers **103-104** have modest activity with MICs of 4–8 μg/mL.

**Table 1.4.7.** C-2 and C-4 substituted quinolines.

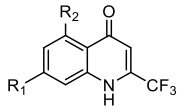



| Compd | $R_1$ | $R_2$ | MIC μg/mL | cLogP |
|---|---|---|---|---|
| **102** | piperazinyl | 5-(trifluoromethyl)pyridin-2-yl-amino | 0.5-1 | 5.1 |
| **103** | 5-(trifluoromethyl)pyridin-2-yl-amino | piperazinyl | 4-8 | 5.1 |
| **104** | morpholino | 5-(trifluoromethyl)pyridin-2-yl-amino | >32 | 5.1 |
| **105** | 5-(trifluoromethyl)pyridin-2-yl-amino | morpholino | 8 | 5.1 |

The final series of compounds investigated was a hybrid scaffold of the initial active quinol-4-one (**Table 1.4.1**) and the aryl-substituted 4-aminoquinoline (**Table 1.4.2**) to afford an aryl-substituted 5-aminoquinolin-4-one scaffold (**Table 1.4.8**) in an attempt to lower the calculated logP. All of the 5-aminoquinolin-4-ones **111–122** with the exception of **119** showed good to outstanding antibacterial activity with MICs ranging from <0.0625 to 2 μg/mL while simultaneously decreasing the calculated LogP by two and up to five units. The optimal C-4 substituents in the 4-aminoquinoline series yielded extremely potent 5-aminoquinolone analogues **111–113** with MICs less than 0.0625 μg/mL and attendant dramatic reductions in lipophilicity. Compound **111** was the first potent derivative synthesized with a calculated LogP of less than four. Given the impressive activity of **111**, **112**, and **113** we sought to further examine closely related substituents containing polar substituents and/or greater $sp^3$ character including 5-fluoropyridin-2-yl-amino **114**, 6-(trifluoromethyl)pyridazin-3-yl-amino **115**, 5-dimethylaminopyridin-2-yl-amino **116**, 3-(*N*,*N*-dimethylsulfonamide)pyridine-6-yl-amino **117**, 4-amino-1*H*-indazolyl **118**,

morpholino **119**, and 4-(trifluoromethyl)cyclohex-1-yl-amino **120**, whose calculated LogPs ranged from 2.0 to 3.9. The SAR exhibited substantially greater flexibility than observed in the 4-aminoquinoline series (**Table 1.4.5**) and many of these analogs including **114–118** had respectable MICs ranging from 0.125–2 μg/mL (**Table 1.4.8**). Lastly, we prepared **122** incorporating a 7-fluoro substituent in place of the trifluoromethyl group of **111** in an attempt to further modulate the lipophilicity. Unfortunately, **122** loses some potency with an MIC of 1 μg/mL which indicates that the 5-position is much more mailable to functional group modification than the 7-position with **114** having eight fold greater activity than **122** with the same fluoro modification. Collectively, the results from the last series of 5-aminoquinoline-4-one demonstrate high antibacterial activity can be achieved by introduction of appropriate substituents at C-5 of this scaffold and that the C-5 position is somewhat permissive to modification tolerating more polar as well as nonplanar groups.

**Table 1.4.8.** 5 and 7 Substituted quinolinones.




| Compound | $R_1$ | $R_2$ | MIC μg/mL | cLogP |
|---|---|---|---|---|
| **111** | $CF_3$ | 5-(trifluoromethyl)pyridin-2-yl-amino | <0.0625 | 3.8 |
| **112** | $CF_3$ | 3,4-dichloroanilino | <0.0625 | 5.2 |
| **113** | $CF_3$ | 3,4-difluoroanilino | <0.0625 | 4.1 |
| **114** | $CF_3$ | 5-(fluoro)pyridin-2-yl-amino | 0.125 | 3.1 |
| **115** | $CF_3$ | 6-(trifluoromethyl)pyridazine-3-yl-amino | 1 | 2.9 |
| **116** | $CF_3$ | 5-dimethylaminopyridin-2-yl-amino | 2 | 3.6 |
| **117** | $CF_3$ | (N,N-dimethyl-6-sulfamoyl)pyridin-2-yl-amino | 2 | 2.1 |
| **118** | $CF_3$ | 1*H*-indazol-4-yl-amino | 2 | 3.6 |
| **119** | $CF_3$ | morpholino | >32 | 2.1 |
| **120** | $CF_3$ | 4-(trifluoromethyl)cyclohexyl-amino | 0.125 | 3.9 |
| **122** | F | 5-(trifluoromethyl)pyridin-2-yl-amino | 1 | 3.1 |

We selected a few of the most potent compounds from the 4-aminoquinoline (**22**, **31**) and 5-aminoquinolin-4-one (**111, 120**) series for evaluation against a panel of other MRSA strains and representative gram-positive and gram-negative pathogens (**Table 1.4.9**). Compounds **111** and **120** show excellent activity (MIC 0.125-<0.06 μg/mL) towards all six *S. aureus* strains while **31** also displays very good activity with MICs ranging from 0.125–0.25 μg/mL. The compounds maintain activity against *Staphylococcus epidermidis*; however, **31**, **111** and **120** still maintain some potency against *Enterococcus faecalis* and *Enterococcus faecium* (MIC of 2-8 μg/mL for **111** and **120**), which contribute heavily, along with MRSA, to healthcare-associated infections.[7] The compounds are inactive against the Gram-negative bacilli *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterococcus cloacae* as well as the fungus *Candida albicans* at the highest concentration (128 μg/mL) tested.

While the MIC data confirmed that these compounds were at least bacteriostatic, we wanted to test for bactericidal properties of both the aminoquinoline and aminoquinolone scaffolds. The minimum bactericidal concentration (MBC) of **22**, **31**, **111** and **120** was evaluated against the MRSA clinical strain *S. aureus* FPR3757 (**Table 1.4.10**). Compounds **22**, **31**, and **120** possessed MBCs values equal to 8 to 32 times of MIC. The kinetics of bacterial killing was assessed in vitro using time-kill assays by incubating compounds at 1× their MIC with an initial inoculum of $10^5$ colony forming units (CFU) of *S. aureus* and removing aliquots at various time points to determine the residual CFU by plating. Compounds **22, 31**, **111,** and **120** were bacteriostatic at 1×MIC as the number of CFU remains flat at three to six hours (**Figure 1.4.3**). In contrast, the positive control daptomycin, was rapidly bactericidal at one hour after exposure.

**Table 1.4.9.** Antimicrobial susceptibility.

| | | MIC (μg/mL) | | | |
|---|---|---|---|---|---|
| **Species** | **Strain** | **22** | **31** | **111** | **120** |
| *S. aureus* | FPR3757 | 0.0625 | 0.25 | <0.06 | 0.125 |
| | MW2 | 0.125 | 0.125 | <0.06 | 0.125 |
| | COL | 0.25 | 0.25 | <0.06 | <0.06 |
| | N315 | 0.25 | 0.25 | <0.06 | 0.125 |
| | NRS71 | 0.25 | 0.25 | <0.06 | 0.0625 |
| *S. epidermidis* | NIH04008 | 0.125 | 0.125 | <0.06 | 0.125 |
| | NIH04003 | 0.25 | 0.5 | <0.06 | 0.125 |
| *E. faecalis* | ATCC700802 | 0.5 | 16 | 2 | 2 |
| | DHMC #1 | 0.25 | 1 | 2 | 0.125 |
| *E. faecium* | ATCC19579 | 0.125 | 1 | <0.06 | 0.25 |
| | DHMC #1 | 0.25 | 4 | 2 | 0.25 |
| *E. coli* | DHMC-1 | >128 | >128 | >128 | >128 |
| *K. pneumoniae* | 7117 | >128 | >128 | >128 | >128 |
| *E. cloacae* | ND-21 | >128 | >128 | >128 | >128 |
| *C. albicans* | SC5314 | >256 | >256 | 256 | >256 |

**Table 1.4.10.** Bactericidal activity of lead compounds in MRSA.[a]

| **Compound** | **MIC (μg/mL)** | **MBC (μg/mL)** | **Fold Difference** |
|---|---|---|---|
| **22** | 0.0625 | 2 | 32x |
| **31** | 0.25 | 8 | 32x |
| **111** | 0.0625 | 2 | 32x |
| **120** | 0.5 | 4 | 8x |

[a]$10^5$ *Staphylococcus aureus* FPR3757 cells (USA300 CA-MRSA) used per well. All MICs and MBCs conducted in triplicate.

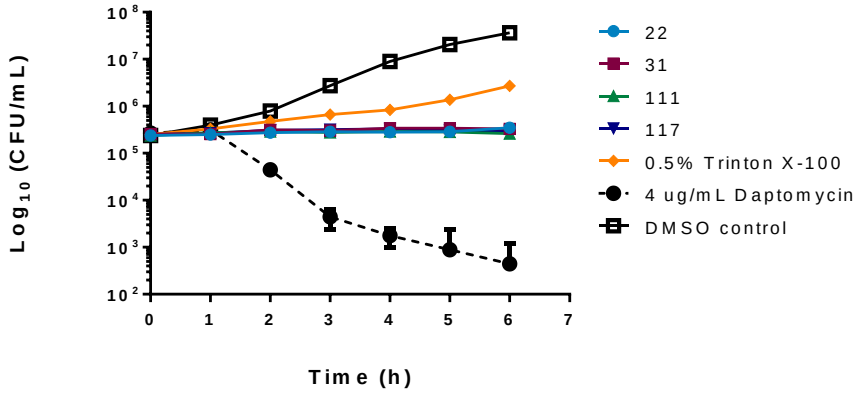



**Figure 1.4.3.** *In vitro* time-kill analysis of MRSA. Bacterial killing was monitored by measuring the CFU for six hours when treated with compounds **22**, **31**, **111**, and **117** at 1× the MIC of each compound. The CFU at each timepoint was determined by plating and then compared to a DMSO control. Three independent experiments were performed and one representative experiment is shown.

## *1.5. Mechanism of action studies.*

Classical macromolecular synthesis assays were performed to provide insight on the putative mechanism of action of the most promising quinoline **31** and a favorable quinolone **122** candidate. Addition of compounds **31** and **122** interferes with all major metabolic activities in the cell as distinguished by macromolecular synthesis assays. More specifically, radiolabeled precursors [$^3$H]-L-isoleucine, [$^3$H]-thymidine, [$^3$H]-uridine, and [$^3$H]-glucosamine were added to a culture of *Staphylococcus simulans* ($OD_{600}$ = 0.4) as a surrogate for *S. aureus* in Mueller-Hinton cation (MHC) adjusted medium at 37 °C along with compounds at 0.5×, 1× and 5× MIC. The control antibiotics ciprofloxacin, rifampicin, vancomycin and tetracycline were included as inhibitors of DNA, RNA, cell wall, and protein synthesis, respectively. The cells were quenched at various time points with 10% trichloroacetic acid (TCA), filtered, washed and the amount of precursor incorporation was quantified by scintillation counting. Cells treated with **31** or **122** show a clear

concentration-dependent inhibition of DNA, RNA, protein and cell-wall synthesis (**Figure 1.5.1** and **Figure S18**). At 5× MIC, both **31** and **122** completely inhibited all macromolecular processes, a profile that is consistent with disruption of the cellular membrane.[45,59–62]

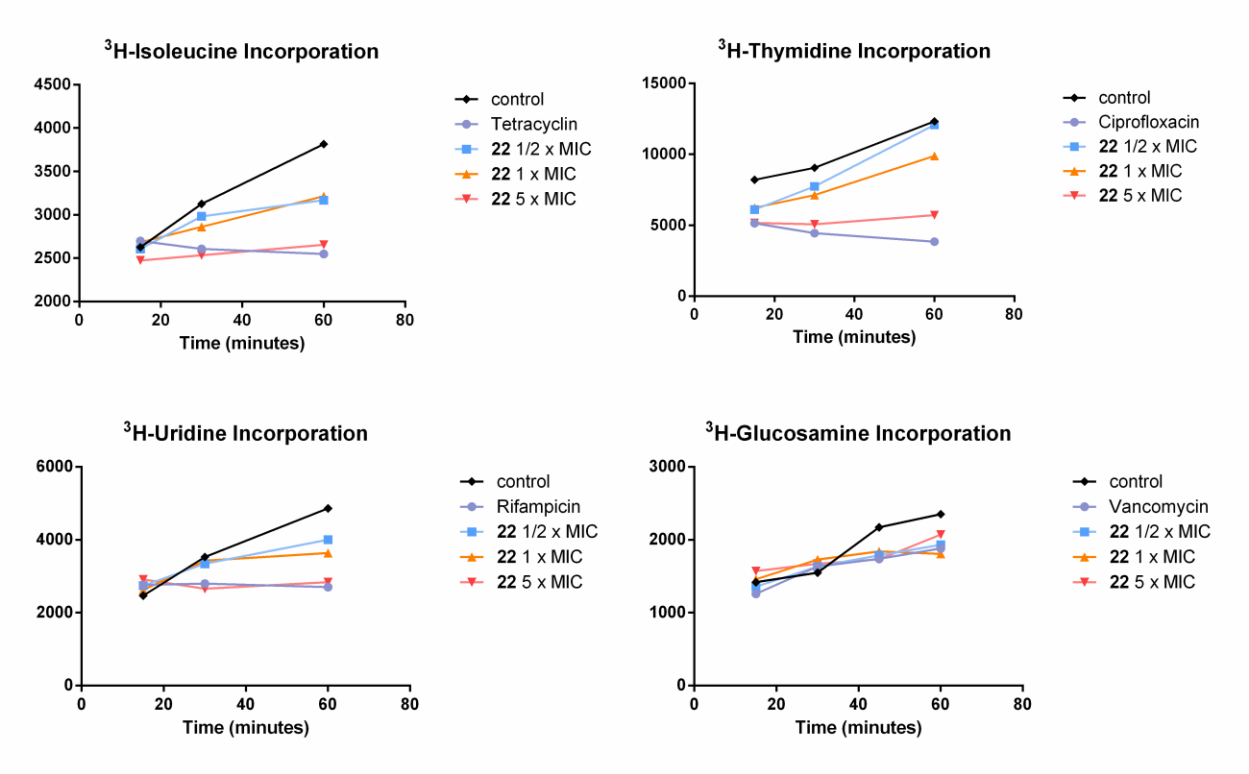



**Figure 1.5.1.** Macromolecular Synthesis Assays. Representative data with **122**. Time-course for inhibition of incorporation of radiolabeled precursors [$^3$H]-L-isoleucine (protein), [$^3$H]-thymidine (DNA), [$^3$H]-uridine (RNA), and [$^3$H]-glucosamine (cell wall) in *S. simulans* by **122** (at 0.5×, 1× and 5× MIC). Data are expressed as the percentage of inhibition relative to the DMSO only negative control. The positive control antibiotics denoted by closed circles were used at 10× MIC. Data represent the mean ± SD of triplicate experiments.

While macromolecular synthesis assays suggested membrane disruption as a likely mechanism, we wanted to provide additional evidence for bacterial membrane damage. Transmission electron microscopy (TEM) and fluorescence microscopy (FM) allowed us to directly observe the effects of the novel 4-aminoquinolines on 5-aminoquinolones on the cell

morphology of staphylococci. *S. aureus* (strain USA300) treated with **31**, **117** and **122.** After 10-minute exposure, the cells displayed cross-wall-septum formation with reduced splitting compared to the untreated cells in TEM images (**Figure 1.5.2**). Cells treated with **31, 117** and **122** cells also displayed mesosome-like membrane inclusions, membrane "wrinkling," and bulging of the septum (blue arrows). These cellular defects were not seen in any of the control cells treated with DMSO. Fluorescent microscopy was performed on a *S. aureus* (strain COL) strain after treatment with either DMSO, **117** or **122** for 30 minutes followed by staining with FM 4-64 (red membrane stain), bodipy-vancomycin (Van-FL, green stain for cell wall), and Hoechst (blue DNA stain). FM 4-64 staining revealed membrane defects (**Figure 1.5.3 B** and **D)** in the cells treated with **117** and **122** that included large bulges and bulging septum formation. DNA and cell wall staining showed little or no change compared to the DMSO control (**Figure 1.5.3 A** and **C**). Taken together, these results indicated that **117** and **122** affected the cell membrane with gross morphological changes in the membrane but did not have a major impact on the cell wall, consistent with the macromolecular synthesis assays.

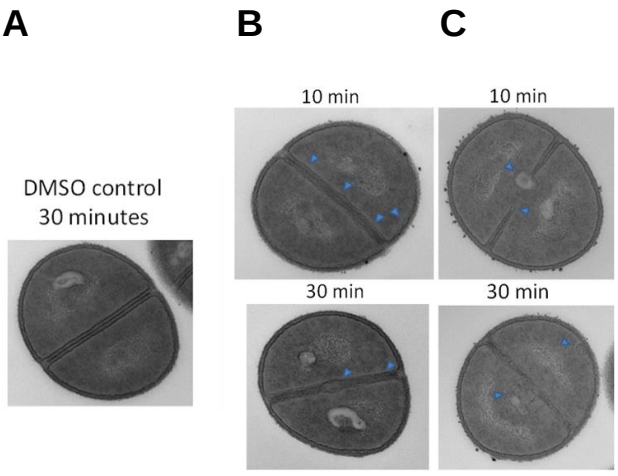



**Figure 1.5.2.** Transmission electron microscopy (TEM) imaging of MRSA USA300 treated with **22** and **31.** Membrane disruption highlighted by blue arrows by compounds **22** (**B**) and **31** (**C**)

after 10 minutes (top row) and 30 minutes (bottom row) of exposure at 1× MIC compared to a DMSO control (**A**).

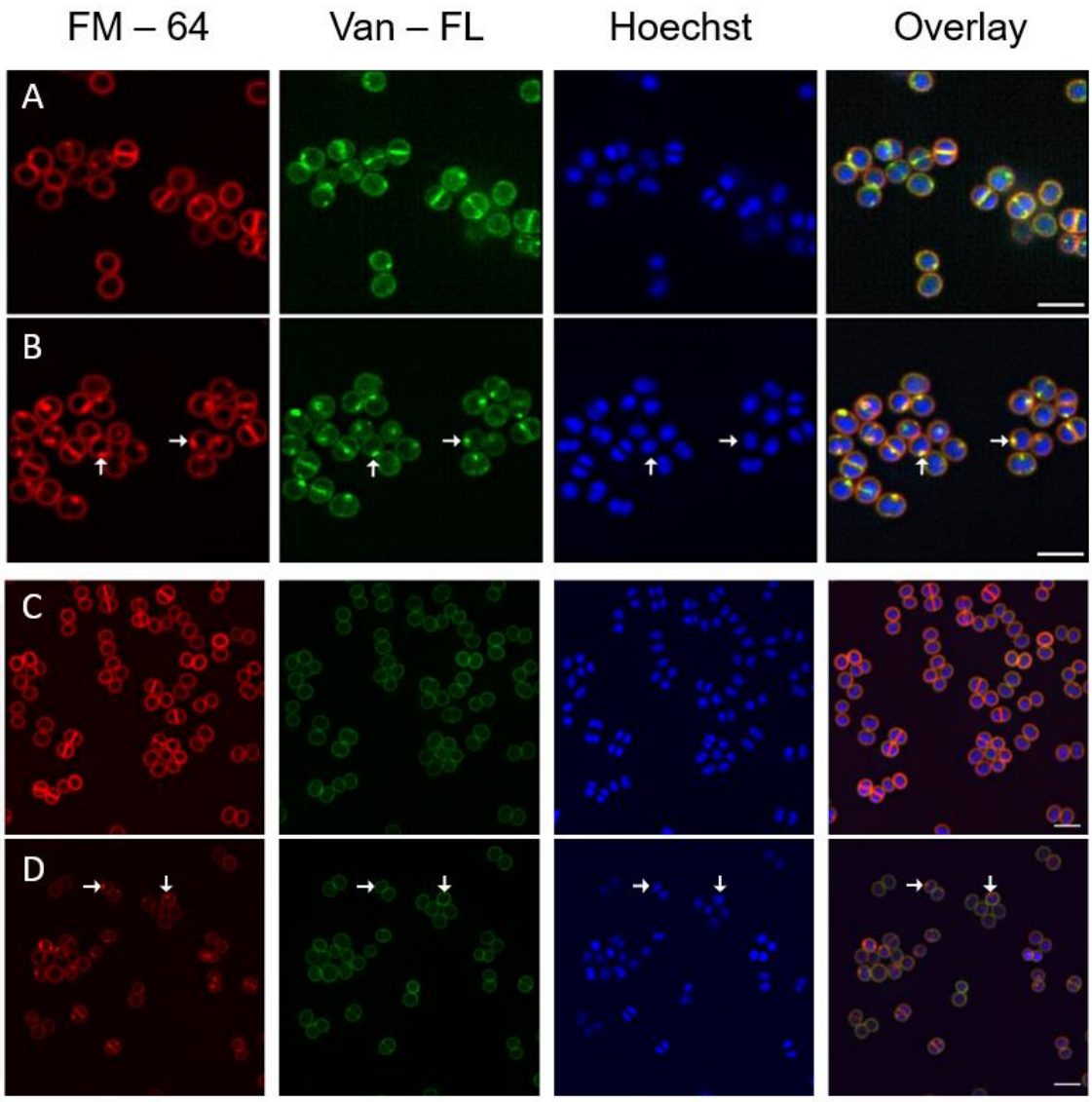



**Figure 1.5.3.** Fluorescent microscopy (FM) analysis of membrane, cell wall, and DNA in MRSA. FM of COL MRSA strain treated with compound **117 (C** row) or **122** (**D** row) at 1× MIC and DMSO (**A** and **C** rows) for 30 minutes followed by staining with FM-64 (far left column, 0.5 μg/mL), Va7n-FL (second column, 1 μg/mL), and Hoechst (third column, 1 μg/mL) for 5 minutes and washed with 1× PBS before imaging (fourth column, overlay). Arrows indicate the location of "mesosome-like" structures displayed in each image.

We next sought to determine membrane selectivity of representative 4-aminoquinolines (**31**, **76** and **78**) and the most potent 5-aminoquinolin-4-ones (**108–111**, **117** and **122**) by a hemolytic assay employing washed sheep erythrocytes.[63] None of the compounds except **78**, displayed any hemolysis at 32 µg/mL compared to the positive control Triton X-100 (100% lysis) (**Figure 1.5.4**). The promising 5-aminoquinolin-4-ones tested (**108–111, 117, 122**) displayed greater than 500-fold selectivity for *S. aureus* membranes over erythrocyte membranes. The high membrane selectivity of the 5-aminoquinolin-4-ones in this assay is impressive and is in accordance with the observed therapeutic index ($CC_{50}$/MIC).

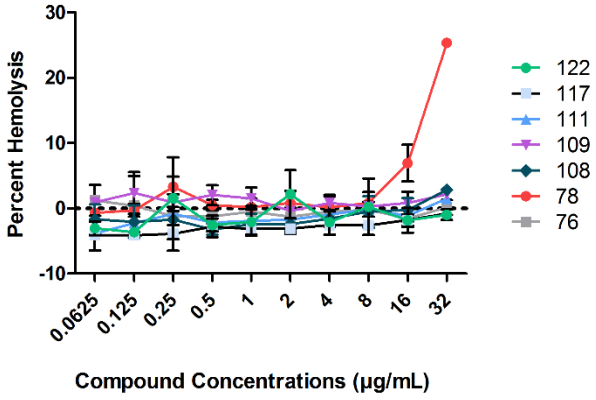



**Figure 1.5.4.** Percent hemolysis analysis of sheep erythrocytes. Concentration-dependent hemolysis was measured by monitoring the $OD_{540}$ of PBS-washed sheep erythrocytes. Complete hemolysis (100%) was confirmed by treatment of erythrocytes with 2% Triton X-100. Data points represent the mean ± SD of triplicate experiments.

In order to identify potential genetic determinants of intrinsic resistance, we screened the Nebraska Transposon Mutant Library (NTML) of *S. aureus* JE2 against compound **22** at sub-inhibitory concentrations. Identified among the 51 mutants that displayed reduced survival were 6 membrane associated ATPases, 5 permeases, 3 lipo-proteins, 2 transcriptional factors and a

conserved hypothetical xenobiotic resistance effector (XRE) Interestingly, one of the identified ATPases, VraF, is the cognate ATPase of the membrane permease VraG which has been implicated in proper sensing of membrane perturbation from antimicrobial peptides.[64] Another membrane permease identified was the efflux pump NorB responsible for resistance/efflux of quinolone antibiotics (e.g. ciprofloxacin, norfloxacin and moxifloxacin).[65] These results suggest that our compounds of interest may affect the bacterial membrane and, thus, corroborate our prior assessments.

We next assessed frequency of resistance to these compounds through a multi-step resistance selection process by serially passaging *S. aureus* FPR3757 in duplicate in subinhibitory and inhibitory concentrations of compounds **22** and **31**. After 65 days of serial passage, no development of resistance was noted against compound **22**, but one of the two isolates tested developed two-fold resistance (0.25 ug/mL to 0.5 ug/mL) against compound **31**. The genome sequences of these two strains, obtained from two colonies of each isolate, were first evaluated and then compared to those obtained from screening of the transposon library. Sequencing data revealed 4 single nucleotide variations (SNVs) in intergenic regions and non-silent SNVs in quorum sensing signal receptor *agrC*, the clumping factor *clfA* and lysyl transferase gene *mprF*. Notably, the hypothetical XRE protein, identified with the NTML screen, was also identified by the resistant mutant screen. Further work is necessary to understand the independent contribution and biological effects of these mutations as well as those found in uncharacterized intergenic regions to compound.

## *1.6. Pharmacokinetic analysis of analogue 111.*

With the drastic improvement of physical properties of our 5-aminoquinolin-4-one scaffolds as compared to the initial 4-aminoquinoline hits afforded by our synthetic campaign we selected

compound **111** to assay the pharmacokinetic (PK) properties. Single dose *in vivo* PK studies were performed in female CD-1 mice and plasma concentrations of **111** were determined by LC-MS/MS at various timepoints over a 24-hour period following both intravenous (i.v.) and oral administration (p.o.) (**Figure 1.6.1**). PK parameters were determined by noncompartmental PK analysis (**Table 1.6.1**). Following i.v. dosing at 0.5 mg/kg, **111** demonstrated a volume of distribution ($V_d$) of 0.32 L/kg, clearance (CL) of 119 mL/(kg*h), and a half-life ($t_{1/2}$) of 1.88 h. Single p.o. dosing at 10 mg/kg showed an area under the curve ($AUC_{0\text{-}24hr}$) of 43,349 h*ng/mL and a maximum concentration (Cmax) of 3,100 ng/mL and a bioavailability (F) of 52%. Plasma protein-binding of **111** was also assayed by Rapid Equilibrium Device (RED) dialysis, and compound **111** displayed extensive protein-binding of 99.7%. Despite the extensive protein-binding displayed by **111,** the favorable oral drug exposure, along with the potent MIC, suggest that **111** warrants further efficacy testing in animal models of MRSA infections. Following i.v. dosing at 0.5 mg/kg total plasma concentrations reached well above the MIC (<0.0625 ug/mL) for about the first 9 h of the study (Figure 11). Plasma protein-binding of **111** was also assayed by Rapid Equilibrium Device (RED) dialysis, and compound **111** displayed extensive protein-binding of 99.7%. Despite the extensive protein-binding displayed by **111,** the favorable oral drug exposure, along with the potent MIC, suggest that **111** warrants further efficacy testing in animal models of MRSA infections.

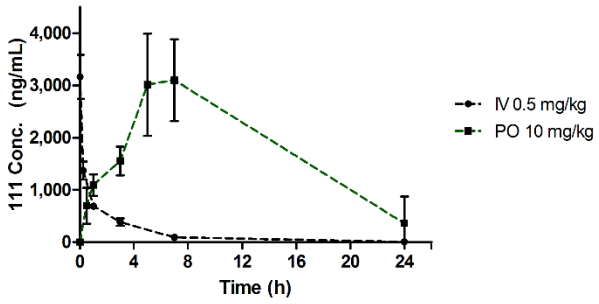



**Figure 1.6.1.** Mean plasma concentration versus time curves after single p.o. (10 mg/kg) and i.v. (0.5 mg/kg) administration of compound **111** to mice. Error bars represent standard deviation of the mean ($n$ = 3).

**Table 1.6.1.** *In vivo* pharmacokinetic parameters of **111** in female CD-1 mice ($n$ = 3, mean ± SD)

| **Pharmacokinetic Indices** | **Analogue 111** |
|---|---|
| dose i.v., p.o. (mg/kg) | 0.5, 10 |
| $AUC_{0\text{-}24h}$ (i.v., h*ng/mL) | 4200 ± 308 |
| $V_d$ (i.v., mL/kg) | 322. ± 23.6 |
| CL (i.v., mL/(kg*h)) | 119. ± 9.2 |
| $t_{1/2}$ (i.v., h) | 1.88 ± 0.08 |
| $AUC_{0\text{-}24h}$ (p.o., h*ng/mL) | 43300 ± 543. |
| Cmax (p.o., ng/mL) | 3100 ± 780. |
| F (%) | 51.6 ± 5.2 |

## *1.7. Conclusions*

The SAR of the quinolone DNAC-2 (MIC = 8 μg/mL) was systematically explored through the synthesis of more than 100 analogues that examined modification to every position of the scaffold. Strongly electron-withdrawing substituents ($CF_3$ and $OCF_3$) were optimal at the C-2 and C-7 positions while electron-donating substituents abolished activity. Introduction of a 4-arylamino group at C-4 led to a dramatic increase in potency culminating in 3,4-dichlorophenylamino **18** with

an MIC of 0.06 μg/mL that was offset by a large increase in the calculated logP to 7.7. A great deal of effort was subsequently expended to maintain this outstanding antibacterial activity against Gram+ organisms while reducing the lipophilicity and planarity of the molecule. Isosteric replacement of the C-4 group with a more polar 5′-(trifluoromethyl)pyridin-2-yl-amino moiety in **31** helped to decrease the calculated logP to 6.1 with an attendant 4-fold loss of potency, but non-conservative changes to introduce more polar or non-planar heterocycles were not allowed. Examination of replacements for the lipophilic 2- and 7-trifluoromethyl groups revealed 6,7-difluoro substitution of the quinoline in **78** was tolerated while reducing the calculated logP to 5.3; however, further attempts to modulate the lipophilicity through introduction of nitrogen atoms into the quinoline scaffold indicated potency and lipophilicity could not be separated. A breakthrough in the SAR was observed by the synthesis of a hybrid 5-aminoquinolin-4-one scaffold by combining the quinol-4-one core of DNAC-2 with an *N*-aryl substituent at C-5, typified by **111** containing an 5′-(trifluoromethyl)pyridin-2-yl-amino group at C-5, whose MIC was less than 0.06 μg/mL with a calculated logP of 3.8.

The 4-aminoquinoline and 5-aminoquinolone scaffolds represented by **31** and **111** are narrow-spectrum agents with antibacterial activity against strictly Gram-positive organisms, with no activity against Gram-negative bacilli or fungi. Staphylococci species including several multidrug-resistant MRSA strains and *S. epidermidis* are most sensitive with MICs of ≤0.06–0.12 μg/mL, but *E. faecalis*, and *E. faecium* are susceptible with MICs ranging from 2–8 μg/mL. The compounds are bacteriostatic, but become bactericidal with 8-32 times MIC (2-8 μg/mL). The 4-aminoquinoline and 5-aminoquinolone are non-toxic displaying no cytotoxicity at the highest concentration evaluated, translating to a therapeutic indexes of greater than 1000. Preliminary resistance and mechanism of action studies demonstrate these compounds selectively disrupt

bacterial membrane. Overall, compound 5-aminoquinolone **111** is the most promising derivative identified from these studies based on its exceptionally potent activity, excellent therapeutic index, selective membrane-disruption and attractive physicochemical properties including improved solubility which lends to oral formulation. In this regard, these compounds are distinguished from other membrane-active agents that tend to be large amphipathic molecules.

## *1.8 Experimental methods*

**MIC assays.** MICs were performed with compounds according to CLSI standards.[66] Briefly, bacterial strains were grown in either Trypticase soy or Luria-Bertani broth (TSB, LB; Difco) overnight and then diluted to an $OD_{600}$ of 1 (~$10^9$ CFU/mL) in Mueller-Hinton Broth (MBH; Difco). Strains were then diluted to $10^6$ CFU/mL in MHB and 100 uL aliquots were applied to a 96-well plate. Amended to each aliquot were 100 uL two-fold serial dilutions of compounds beginning at 32 ug/mL. Plates were then incubated stationary at 37°C overnight for 24 hours and MICs were assessed. *Candida albicans* was grown overnight at 30°C in YPD (yeast extract, peptone; Difco, dextrose; Fischer). Overnight culture is then diluted to an $OD_{600}$ of 0.1 in 200 uL of RPMI 1640 (Gibco) and 60 uL of this dilution is amended to 5 mL of RPMI 1640 to make the working inoculi. To a 96-well plate, 100 uL aliquots of inoculum were dispensed followed by 100 uL two-fold serial dilutions of compounds beginning at 32 ug/mL. Plates were then incubated stationary at 35°C overnight for 24 hours and MICs were assessed.

**Determination of time-kill curves.** Time-kill assays were conducted according to CLSI standards.[67] Briefly, *S. aureus* FPR3757 was grown overnight in TSB and then diluted to ~$10^6$ CFU/mL in MHB. 100 mL MHB cultures of $10^5$ CFU/mL were then amended with compounds 22, 31, 111 and 117 at 1X MIC or 4 ug/mL Daptomycin or 0.5% Triton X-100. Cultures

were then allowed to incubate for 6 hours over which aliquots were taken every hour beginning with time point 0 and subsequently plated on Trypticase soy agar (TSA; Difco). CFU counts were then assessed versus growth controls and bactericidal effect was determined by a lethality percentage of 90% at 6 h, which is equivalent to a lethality of 99.9% at 24 h.

**Determination of MBC values.** Compound bactericidal activity was assessed according to CLSI standards (Same reference as above). Briefly, *S. aureus* FPR3757 was grown in TSB overnight and then diluted to an $OD_{600}$ of 1 (~$10^9$ CFU/mL) in MHB. Culture was then diluted to $10^6$ CFU/mL in MHB and 100 µL aliquots were applied to a 96-well plate. Amended to each aliquot were 100 µL two-fold serial dilutions of compounds beginning at 32 µg/mL. Following stationary incubation at 37°C for 24 hours, dilutions of wells corresponding to 0.5X MIC to 32X MIC were plated and incubated overnight at 37°C. The minimal bactericidal concentration was determined as the lowest compound concentration at which cell viability fell below 99.9% of the initial inoculum.

**Nebraska Transposon Mutant Library (NTML) susceptibility screening.** Non-essential genetic contributions to compound resistance were assessed through the USA300 JE2 *bursa aurelias* transposon mutagenized library. MHB was amended with half MIC concentration of compound **22** and 200 µL aliquots were then added to wells in enough 96-well plates to cover all 1,952 unique mutants. Mutants were inoculated into each well by a 96 solid pin microplate replicator from frozen stock plates. Plates were then incubated overnight

stationary at 37°C. Library hits were deemed to be mutants that failed to grow at half MIC concentrations of compound **22**.

**Frequency of resistance (FOR).** Resistance development to compounds **22** and **31** were assessed by a multi-step resistance study. Briefly, *S. aureus* FPR3757 was serially passaged in duplicate in sub-inhibitory concentrations of compound **22** and **31** for a total of 65 days. Upon completion of passaging, all four strains were genomically sequenced (Dartmouth Genomics and Molecular Biology Shared Resources) and genetic variations were identified and processed by CLC Genomic Workbench (Dartmouth CLC Genomic Workbench Server).

**Macromolecular synthesis assays.** The effect of compounds on macromolecular synthesis was studied by monitoring the incorporation of $^{3}H$- or $^{14}C$-labeled precursors (5-[$^{3}H$] thymidine, [$^{3}H$] glucosamine hydrochloride, [$^{3}H$] uridine and l-[$^{14}C$] isoleucine) as described previously (22). An overnight culture of *Staphylococcus simulans* 22 grown in MHC was diluted 50-fold into fresh medium and cultured at 37 °C to an $OD_{600}$ of about 0.5. Cultures were aliquoted, diluted to an $OD_{600}$ of 0.1 and allowed to regrow to an $OD_{600}$ of 0.4. The respective labelled precursor was then added to each culture (final concentration 1 µCi/ml); compound was added at 0.5X , 1X or 2X MIC, while another aliquot was run with 10X MIC of a control antibiotic and one without any antibiotic. Control antibiotics were vancomycin (3.1 µg/ml) to inhibit cell wall synthesis, tetracycline (0.4 µg/ml) to inhibit protein synthesis, ciprofloxacin (0.3 µg/ml) to inhibit DNA synthesis and rifampicin (0.01 µg/ml) to inhibit RNA synthesis. Incorporation of labeled precursors was monitored for up to 60 min and representative aliquots taken at 0, 5, 15, 30, 45 and 60 minutes. Macromolecules were precipitated with ice-cold TCA (10%) and incubated for at least 30 min on ice before being filtered through glass microfiber

filters (Whatman). Filters were washed with 5 ml of TCA (2.5%) containing 10 mm unlabeled metabolite, dried, counted and the data expressed as mean of the counts incorporated from triplicate samples.

**Florescence microscopy.** Bacterial strains were incubated overnight in TSB at 37 °C, back diluted in fresh TSB and allowed to grow until mid-exponential phase ($OD_{600}$ ~ 0.5). Each culture was then divided into flasks with antibiotic (compounds **117** or **122** at 1x MIC), DMSO or TSB alone. Cultures were incubated for 5 or 30 min, after which the cells were pelleted, washed in 1x PBS buffer and mounted on microscope slides with pads of 1.5% agarose in 1x PBS. For staining of membrane, cell wall or DNA, cells were incubated with FM 4–64 (0.5 μg/mL), BODIPY FL vancomycin (1 μg/mL) or Hoechst 33342 (1 μg/mL) (all from Molecular Probes) for 5 min at 23 °C with shaking and washed with 1x PBS before being imaged. Cells were imaged using a Zeiss Axio Observer microscope equipped with a Photometrics CoolSNAP HQ2 camera (Roper Scientific) and Zen Blue software (Zeiss) or by Super-Resolution Structured Illumination Microscopy (SR-SIM) in an ELYRA PS.1 Microscope (Zeiss) using a sCMOS camera (Andor) and a Plan-Apochromat 63x/1.4 oil DIC M27 objective (Zeiss). SR-SIM images were acquired using 5 phase shifts and 5 grid rotations for each channel, reconstructed and analyzed with Zen Black Software (Zeiss). For channel alignment, multicolored-beads were imaged to determine the experimental point spread function (PSF), which was used for the alignment of the different channels.

**Transmission electron microscopy.** Transmission electron microscopy (TEM) was used to analyze membrane disruption in *S. aureus* FPR3757 after exposure to daptomycin and compounds **22** and **31**. Overnight cultures of FPR3757 were diluted 1:100 and allowed to

grow at 37°C with shaking until an $OD_{600}$ of 1.1. These cultures were then diluted to an $OD_{600}$ of 0.45 and allowed to grow at 37°C with shaking for an additional 30 minutes. To each culture, 1X MIC of compounds, 2 μg/mL daptomycin or 0.5 uL of DMSO was amended and the cultures were incubated for 1 hour at 37°C with shaking. Cell pellets were then collected at room temperature by centrifugation at 2000 rpm for 10 minutes, washed once in PBS and then collected again by centrifugation. The PBS was completely removed and pellets were resuspended in 10X volume of fixative (2% glutaraldehyde/ 1% paraformaldehyde in 0.1 M sodium cacodylate, pH 7.4) then left at room temperature for 15 minutes. Cells were collected by centrifugation and then resuspended in fixative at room temperature for 1 hour. The cells are then collected, resuspended in fixative and then allowed to fix at room temperature for 24 hours on a rotator. Pellets are collected then washed 3 times with 0.1 M sodium cacodylate for 15 minutes each and then post-fixed at room temperature with 1% osmium tetroxide in 0.1 M sodium cacodylate for 2 hours on a rotator. Fixed cells were then rinsed in 0.1 M sodium cacodylate and then distilled water followed by en bloc staining in 1-2% aqueous uranyl acetate in the dark for 30 minutes. Stained cells are then rinsed with distilled water and collected at 3000 rpm. The samples are then dehydrated with an ethanol series (30%, 50%, 70%, 95%) for twenty minutes followed by 3 times at 100% for 1 hour each on a rotator and finally with 100% ethanol overnight on a rotator. The samples are then changed in propylene oxide twice for 30 minutes each and the pellet is then immersed in LX112: propylene oxide (1.5:1) several times over 6-8 hours and then under vacuum for 24 hours. Pellets are then placed in specimen embedding capsules and desiccated for 24 hours followed by

polymerization by heating to 45°C for 8 hours and then for 40 hours at 60°C. Samples were then sectioned, thin sectioned, stained and imaged by the Dartmouth College Electron Microscopy lab.

**Red blood cell lysis assay.** Hemolytic activity of compounds were evaluated as described previously.[68] Briefly, 100 µL of 4% sheep erythrocytes (Rockland Immunochemicals, Limerick, PA, USA) were added to either 100 µL of two-fold serially diluted compounds in PBS, 0.2% DMSO or 2% Triton X-100 in a 96-well plate and then incubated stationary at 37°C for 1 hour. The plate was then centrifuged at 500xg for 5 minutes and 50 µL of the supernatant was transferred from each well to a new 96-well plate and measured for absorbance at 540 nm. Percent hemolysis was calculated according to the following equation:

$$\text{Percent hemolysis} = \left(\frac{\text{Compound Sample } A_{540} - 0.1\%\text{ DMSO } A_{540}}{1\%\text{ Triton X-100 } A_{540} - 0.1\%\text{ DMSO } A_{540}}\right) \text{ x } 100$$

**Pharmacokinetics studies.** All animal studies were ethically reviewed and carried out in accordance with the Institutional Animal Care and Use Committee of Hackensack Meridian Health. Six-week old CD-1 female mice (20-25 g) were used in pharmacokinetic studies. **111** was administered as a single dose by intravenous injection at 0.5 mg/kg using 5% Dimethylacetamide (DMA):95% (4% Cremophor EL) vehicle and by oral gavage at 10 mg/kg in 5% DMA:60% Polyethylene Glycol 300 (PEG 300):35% (5% Dextrose in Water) vehicle. Aliquots of 20 µL of blood were taken by puncture of the lateral tail vein from each mouse (n = 3 per route and dose) at 1 minute, 15 minute, 1, 3, 7, and 24 hours post-dose following intravenous injection and at 30 minutes, 1, 3, 5, 7, and 24 hours post-dose following oral gavage and captured in CB300 blood collection tubes containing $K_2$EDTA and stored on ice. Plasma was recovered

after centrifugation and stored at -80 °C until analyzed by high pressure liquid chromatography coupled to tandem mass spectrometry.

**Pharmacokinetic LC-MS/MS analytical methods.** Neat 1 mg/mL DMSO stocks of **111** were serial diluted in 50/50 Acetonitrile water to create standard curves and quality control spiking solutions. Standards and QCs were created by adding 10 µLs of spiking solutions to 90 µLs of drug free plasma (CD-1 $K_2$EDTA Mouse, Bioreclamation IVT). 10 µLs of control, standard, QC, or study sample were added to 100 µLs of Acetonitrile/Methanol 50/50 protein precipitation solvent containing 10ng/mL of the internal standard Verapamil (Sigma Aldrich). Extracts were vortexed for 5 minutes and centrifuged at 4000 RPM for 5 minutes. 75 µLs of supernatant was transferred for HPLC-MS/MS analysis and diluted with 75 µLs of Milli-Q deionized water.

LC-MS/MS analysis was performed on a Sciex Applied Biosystems Qtrap 6500+ triple-quadrupole mass spectrometer coupled to a Shimadzu Nexera X2 UHPLC system to quantify each drug in plasma. Chromatography was performed on a Agilent SB-C8 (2.1x30 mm; particle size, 3.5 µm) using a reverse phase gradient. Milli-Q deionized water with 0.1% formic acid was used for the aqueous mobile phase and 0.1% formic acid in acetonitrile for the organic mobile phase. Multiple-reaction monitoring of parent/daughter transitions in electrospray positive-ionization mode was used to quantify the analytes. The following MRM transitions were used for **111** (441.98/146.10) and Verapamil (455.40/165.00). Sample analysis was accepted if the concentrations of the quality control samples were within 20% of the nominal concentration. Data processing was performed using Analyst software (version 1.6.2; Applied Biosystems Sciex).

**Plasma protein binding.** In triplicate, 200 µLs of $K_2$EDTA mouse plasma containing 1000 ng/mL of **111** was placed in the donor chamber of the rapid equilibration dialysis (RED) insert. 350 µL of PBS was placed in the receiver chamber. The base plate containing RED inserts was sealed and incubated at 37 °C while stirring at 300 RPM for 4 hours. After 4 hours of equilibration, 10 µL aliquots of sample were taken from the both the donor chamber and receiver chambers. Aliquots were extracted and analyzed by LC-MS/MS as specified above previously detailed. Unbound fraction was calculated by dividing the concentration in the receiver compartment chamber by the concentration in the donor compartment chamber.

**General materials and methods.** Chemicals and solvents were purchased from Acros Organics, Alfa Aesar, Sigma-Aldrich, and TCI America and were used as received. An anhydrous solvent dispensing system using two packed columns of neutral alumina was used for drying THF, MeOH, Toluene, and $CH_2Cl_2$, while two packed columns of molecular sieves were used to dry DMF, and the solvents were dispensed under argon (Ar). EtOAc and hexanes were purchased from Fisher Scientific. TLC analyses were performed on TLC silica gel plates 60F254 from EMD Chemical Inc. and were visualized with UV light. Purification by flash chromatography was performed using a medium-pressure flash chromatography system equipped with flash column silica cartridges with the indicated solvent system. Preparative reversed-phase HPLC purification was performed on a Phenomenex Gemini 10 µm C18 250 × 20 mm column operating at 21.0 mL/min with detection at 254 nm with the indicated solvent system (Method A). Analytical reversed-phase HPLC was performed on a Waters Symmetry 5 µm C18 4.6 × 20 mm column operating at 0.5 mL/min with detection at 250 nm employing a linear gradient from 5% to 95% MeOH in water for 9 min (Method B). $^{1}$H and $^{13}$C spectra were recorded on either 400, 500, or 600 MHz NMR spectrometers. Proton chemical shifts are reported in ppm from an internal

standard of residual chloroform (7.27), methanol (3.31), or dimethyl sulfoxide (2.50); carbon chemical shifts are reported in ppm from an internal standard of residual chloroform (77.0), methanol (49.1), or dimethyl sulfoxide (39.5). Proton chemical data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, dt = doublet of triplets, t = triplet, q = quartet, pentet = pent, m = multiplet, ap = apparent, br = broad, ovlp = overlapping), coupling constant(s), integration. All compounds were determined to be >90 % by analytical reverse-phase HPLC (purities for each final compound are given in the experimental section below).

## General chemistry procedures

**General Procedure A.** To a stirring mixture of polyphosphoric acid (1 g/1 mmol aniline) and the ethyl beta-ketoester (37 mmol, 1.0 equiv) added the aniline (37 mmol, 1.0 equiv) dropwise at 100°C. Raised the temperature to 120°C and stirred vigorously for 4 h at which point the reaction was taken off heat and quenched with water once the reaction cooled to 50°C. The resulting precipitate was filtered and washed with water followed by multiple washes with saturated $NaHCO_3$. The solid was dissolved in MeOH:$CH_2Cl_2$ (1:1) and dried over $MgSO_4$. Product was purified via flash column chromatography or trituration with hexanes.

**General Procedure B.** To a flame dried round bottom flask under a nitrogen atmosphere containing toluene (0.1 M) added the quinoline-4-one (2.8 mmol, 1.0 equiv), phosphorus pentoxide (5.6 mmol, 2 equiv), and tetrabutylammonium bromide (4.3 mmol, 1.5 equiv) in one portion. The flask was backfilled with argon and heated to 95°C. After 3.5 hours decanted the top organic layer and the lower layer was extracted with refluxing toluene (2x 30 mL). The toluene layers were combined, diluted with EtOAc, washed with saturated $NaHCO_3$ (2 × 30 mL), water (30 mL), dried over $MgSO_4$ and concentrated under vacuum.

**General Procedure C.** To a flame dried Schlenk tube under a nitrogen atmosphere added the bromoquinoline (1.0 equiv), potassium phosphate tribasic (1.4 equiv), the arylamine (1.2 equiv), SPhos (0.15 equiv), and $Pd_2(dba)_3{\cdot}CH_2Cl_2$ (0.05 equiv) were all added in one portion and the flask was backfilled with argon. Dry THF (0.15 M) was added and the reaction mixture was heated to 55°C and stirred for 24 h. Reaction mixture was directly concentrated on to silica gel for flash column chromatography.

**General Procedure D.** To a stirring slurry of the quinoline-4-one (0.80 mmol, 1.0 equiv) and pyridine (0.8 mmol, 1.0 equiv) in $CH_2Cl_2$ (0.66 M) at 0°C was added trifluoromethanesulfonic anhydride (0.96 mmol, 1.2 equiv) dropwise. The ice bath was removed and the reaction was allowed to warm to room temperature and stirred for 3 h. The reaction was quenched with saturated aqueous $NH_4Cl$ and extracted with EtOAc (3×25 mL). The organic extracts were combined and dried over $MgSO_4$ to afford the crude triflate.

To a solution of the crude triflate in DMSO (0.2 M) was added the corresponding aniline derivative (1.2 mmol, 1.5 equiv) and heated at 80 °C for 12-18 h. The reaction was then diluted in MeCN and purified by *Prep*.-HPLC (method A).

**General Procedure E.** A mixture of haloquinoline (1 equiv), aryl amine (1 equiv), and aqueous conc. HCl (0.1 equiv, 12 M) in EtOH (0.15 M) was degassed and purged with $N_2$ for 3 times, then the mixture was stirred at 80 °C for 2 h under $N_2$ atmosphere. After removal of solvent, the obtained residue was purified by *Prep*.-HPLC.

**General Procedure F.** To a flame dried flask under a nitrogen atmosphere added the haloquinoline (1.0 equiv), the arylamine (1.3 equiv), $K_2CO_3$ (3 equiv), $Pd_2(dba)_3$ (0.2 equiv), Xantphos (0.4 equiv) and degassed dioxane (0.2 M), the flask was purged with $N_2$ three times, and then the mixture was stirred at 100 °C for 2 h under $N_2$ atmosphere. The reaction mixture was

concentrated under reduced pressure and the resulting residue was purified by *Prep*.-HPLC (method A).

**General Procedure G.** To a flame dried Schlenk tube under a nitrogen atmosphere added the bromoquinoline (1.0 equiv), sodium *tert*-butoxide (0.51 mmol, 1.2 equiv), aromatic amine (1.2 equiv), DPPF (0.1 equiv), and Pd(dppf)$Cl_2$·$CH_2Cl_2$ (0.05 equiv) were all added in one portion and the flask was backfilled with argon. Dry THF (0.15 M) was added and the reaction mixture was heated to 55°C and stirred for 20 h. Reaction mixture was directly concentrated on to silica gel for flash column chromatography.

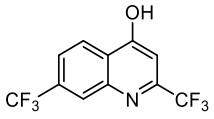
**General Procedure H.** To a stirring slurry of the quinoline-4-ol (0.80 mmol, 1.0 equiv) and pyridine (0.8 mmol, 1.0 equiv) in $CH_2Cl_2$ (0.66 M) at 0°C was added trifluoromethanesulfonic anhydride (0.96 mmol, 1.2 equiv) dropwise. The ice bath was removed and the reaction was allowed to warm to room temperature and stirred for 3 h. The reaction was quenched with saturated aqueous $NH_4Cl$ and extracted with EtOAc (3×25 mL). The organic extracts were combined and dried over $MgSO_4$ to afford the crude triflate.

To a solution of the crude triflate in DMSO (0.2 M) was added the corresponding phenol derivative (1.68 mmol, 2.1 equiv), $CsCO_3$ (1.76 mmol, 2.2 equiv) and heated at 80 °C for 12-18 h. The reaction was then diluted in MeCN and purified by HPLC (method A).

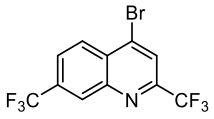
**General Procedure I.** To a mixture of 5-bromo-2,7-bis(trifluoromethyl)quinolin-4(1*H*)-one (**106**, 1 equiv 167 µmol), 6-amino-*N*,*N*-dimethyl-pyridine-3-sulfonamide (1.5 equiv), [2-(2-aminophenyl)phenyl]-methylsulfonyloxy-palladium;dicyclohexyl-[3,6-dimethoxy-2-(2,4,6-triisopropylphenyl)phenyl]phosphane (0.2 equiv), $K_3PO_4$ (3 equiv) was added degassed 2-methylbutan-2-ol (0.16 M) that was then purged with $N_2$ three times, then the mixture was stirred

at 100 °C for 12 h under $N_2$ atmosphere. The reaction mixture was concentrated under reduced pressure and purified by *Prep*.-HPLC.

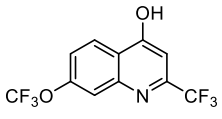
## Synthetic procedures for compounds 2-124



**2,7-bis(Trifluoromethyl)quinolin-4-ol (2).** The title compound was prepared from ethyl 4,4,4-trifluoroacetoacetate (5.44 mL, 37.2 mmol) added 3-trifluoromethylaniline (4.65 mL, 37.2 mmol) using General Procedure A. Flash column chromatography afforded the product (7.66 g, 73% yield) as a pale-yellow solid: HPLC 93.1%, $t_R$ = 6.68 min, $k'$ = 14.53 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 8.42 (d, $J$ = 8.4. Hz, 1H), 8.21 (s, 1H), 7.72 (d, $J$ = 8.7Hz ,1H), 6.97 (s, 1H); HRMS (ESI+) $m/z$ calcd for $C_{11}H_6F_6NO^+$ $[M + H]^+$ 282.0348, found 282.0350 (error 0.61 ppm).

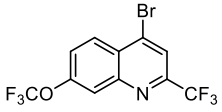


**4-Bromo-2,7-bis(trifluoromethyl)quinoline (2b).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinolin-4-one (**2,** 1.0 g, 2.8 mmol) using General Procedure B. Flash column chromatography ($SiO_2$) afforded the product (850 mg, 86% yield) as a white solid: $R_f$ = 0.3 (Hex); HPLC purity 98.3%, $t_R$ = 7.58 min, $k'$ = 16.63 (method B); $^1$H NMR (500 MHz, $CDCl_3$) δ 8.58 (s, 1H), 8.43 (d, $J$ = 8.8 Hz, 1H), 8.15 (s, 1H), 7.96 (d, $J$ = 8.7 Hz, 1H); Could not obtain HRMS via ESI TOF HRMS.

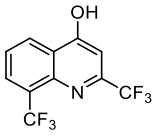


**7-(Trifluoromethoxy)-2-(trifluoromethyl)quinolin-4-ol (3).** The title compound was prepared from ethyl 4,4,4-trifluoroacetoacetate (4.95 mL, 33.9 mmol) added 3-(trifluoromethoxy)aniline (4.65 mL, 33.9 mmol) using General Procedure A. Flash column chromatography afforded the product (8.34 g, 76% yield) as an off-white powder: HPLC purity 97.8%, $t_R$ = 6.71 min, $k'$ = 14.60 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 12.70 (s, 1H), 8.35

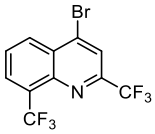
(d, $J$ = 8.7 Hz , 1H), 7.94 (s, 1H), 7.64 (br s, 1H), 7.15 (br s, 1H); HRMS (ESI$^+$) $m/z$ calcd for $C_{11}H_6F_6NO_2^+$ $[M + H]^+$ 298.0297, found 298.0311 (error 4.56 ppm).



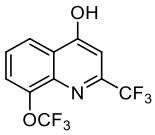

**4-Bromo-7-(trifluoromethoxy)-2-(trifluoromethyl)quinoline (3b).** The title compound was prepared from 7-(trifluoromethoxy)-2-(trifluoromethyl)quinolin-4-one (**3,** 1.0 g, 2.8 mmol) using General Procedure B. Flash column chromatography afforded the product (850 mg, 86% yield) as a white solid: $R_f$ = 0.8 (2:5 EtOAc/Hex); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 8.52 (s, 1H), 8.43 (d, $J$ = 9.2 Hz, 1H), 8.22 (s, 1H), 7.97 (dd, $J$ = 9.2, 2.0 Hz, 1H) Could not obtain HRMS via ESI TOF HRMS.



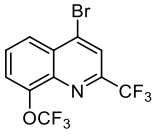

**2,8-bis(Trifluoromethyl)quinolin-4-ol (4).** The title compound was prepared from ethyl 4,4,4-trifluoroacetoacetate (5.45 mL, 37.3 mmol) added 2-trifluoromethylaniline (4.65 mL, 37.2 mmol) using General Procedure A. Flash column chromatography afforded the product (7.4 g, 71% yield) as a pale-yellow solid: HPLC purity 95.1%, $t_R$ = 6.80 min, $k'$ = 14.81 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 12.81 (br. s, 1H), 8.52 (d, $J$ = 8.3 Hz, 1H), 8.28 (d, $J$ = 6.8 Hz, 1H), 7.80 (t, $J$ = 8.1 Hz, 1H), 7.29 (s, 1H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 164.2, 148.7 (q, $^2J_{C-F}$ = 33.6 Hz), 144.8, 130.4 (d, $J$ = 4.5 Hz), 127.7, 126.6 (q, $^2J_{C-F}$ = 29.1 Hz), 126.5, 124.3 (q, $^1J_{C-F}$ = 273 Hz), 122.4, 121.7 (q, $^1J_{C-F}$ 275 Hz), 101.5 (d, $J$ = 1.82 Hz); HRMS (ESI) $m/z$ calcd for $C_{11}H_6F_6NO^+$ $[M + H]^+$ 282.0348, found 282.0337 (error 4.04 ppm).




**4-Bromo-2,8-bis(trifluoromethyl)quinoline (4b).** The title compound was prepared from 2,8-bis(trifluoromethyl)quinoline-4-one (**4**, 1.0 g, 3.0 mmol) using General Procedure B. Flash column chromatography afforded the product in 66% yield as a white solid (680 mg, 66% yield): $R_f$ = 0.8 (3:10 EtOAc/Hex); HPLC purity 95.8%, $t_R$ = 7.32 min, $k'$ = 16.03 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 8.63 (s, 1H), 8.60 (d, $J$ = 8.5 Hz, 1H), 8.48 (d, $J$ = 7.3 Hz, 1H), 8.08 (t, $J$ = 7.9 Hz, 1H); Could not obtain HRMS via ESI TOF HRMS.

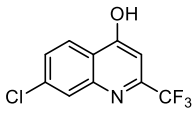



**8-(Trifluoromethoxy)-2-(trifluoromethyl)quinolin-4-ol (5).** The title compound was prepared from ethyl 4,4,4-trifluoroacetoacetate (2.78 mL, 19.1 mmol) added 2-(trifluoromethoxy)aniline (2.31 mL, 19.5 mmol) using General Procedure A. Flash column chromatography afforded the product (3.83 g, 71% yield) as a white powder: HPLC purity 99.9%, $t_R$ = 6.65 min, $k'$ = 14.46 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 12.73 (br s, 1H), 8.24 (d, $J$ = 8.5 Hz, 1H), 7.90 (d, $J$ = 7.6 Hz, 1H), 7.72 (t, $J$ = 8.0 Hz, 1H), 7.25 (s, 1H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 163.5, 148.36 (q, $^2J_{C\text{-}F}$ = 33.7 Hz), 144.1, 141.4, 126.9, 123.3, 122.9, 121.9, 121.3 (q, $^1J_{C\text{-}F}$ = 276 Hz), 120.4 (q, $^1J_{C\text{-}F}$ = 257 Hz), 101.1; HRMS (ESI) $m/z$ calcd for $C_{11}H_6F_6NO_2^+$ $[M + H]^+$ 298.0297, found 298.0288 (error 3.17 ppm).

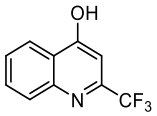



**4-Bromo-8-(trifluoromethoxy)-2-(trifluoromethyl)quinoline (5b).** The title compound was prepared from 8-(trifluoromethoxy)-2-(trifluoromethyl)quinolin-4-one (**5,** 1.0 g, 2.8 mmol) using General Procedure B. Flash column chromatography afforded the product in 84% yield as a white solid (810 mg, 84% yield): $R_f$ = 0.4 (2:5 EtOAc/Hex); $^1$H NMR (500 MHz,

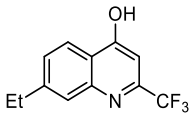
$(CD_3)_2SO$)) δ 8.59 (d, *J* = 3.0 Hz, 1H), 8.33 (d, *J* = 8.7 Hz, 1H), 8.11 (d, *J* = 7.4 Hz, 2H), 8.02 (td, *J* = 8.2, 2.0 Hz, 1H); Could not obtain HRMS via ESI TOF HRMS.




**7-Chloro-2-(trifluoromethyl)quinolin-4-ol (6).** The title compound was prepared from ethyl 4,4,4-trifluoroacetoacetate (0.79 mL, 5.4 mmol) added 3-chlorolaniline (0.42 mL, 4.0 mmol) using General Procedure A. Flash column chromatography afforded the product (118 mg, 12% yield) as a pale-yellow solid: HPLC purity 96.6%, $t_R$ = 6.28 min, $k'$ = 13.6 (method B); HPLC purity 99.0%, $t_R$ = 5.65 min, $k'$ = 12.13 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 12.61 (s, 1H), 8.22 (d, *J* = 9.4 Hz, 1H), 8.07 (s, 1H), 7.67 (d, *J* = 6.4 Hz, 1H), 7.09 (s. 1H); $^{13}$C NMR (151 MHz, $(CD_3)_2SO$) δ 165.6, 147.9, 136.3, 127.4, 126.5, 124.9, 121.3 (q, *J* = 276 Hz), 120.9, 120.6, 101.4; HRMS (ESI) *m/z* calcd for $C_{10}H_6ClF_3NO^+$ $[M + H]^+$ 248.0085, found 248.0076 (error 3.45 ppm).

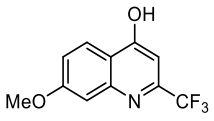



**2-(trifluoromethyl)quinolin-4-ol (7).** The title compound was prepared from ethyl 4,4,4-trifluoroacetoacetate (1.11 mL, 7.37 mmol) added aniline (0.58 g, 6.2 mmol) using General Procedure A. Flash column chromatography ($SiO_2$) using a stepwise gradient of MeOH:$CH_2Cl_2$:TEA (1:99:1 to 3:97:1) afforded the product (106 mg, 8% yield) as a white powder: $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 12.36 (s, 1H), 8.20 (d, *J* = 8.3 Hz, 1H), 7.97 (d, *J* = 8.5 Hz, 1H), 7.82 (t, *J* = 7.6 Hz, 1H), 7.61 (t, *J* = 7.4 Hz, 1H), 7.04 (br s, 1H).

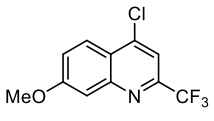



**7-Ethyl-2-(trifluoromethyl)quinolin-4-ol (8).** The title compound was prepared from ethyl 4,4,4-trifluoroacetoacetate (0.79 mL, 5.4 mmol) added 3-ethylaniline (0.62 mL, 4.9

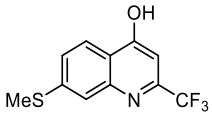
mmol) using General Procedure A. Flash column chromatography afforded the product (61 mg, 5% yield) as a white powder: HPLC purity 98.7%, $t_R$ = 6.33 min, $k'$ = 13.7 (method A); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 7.26 (d, $J$ = 5.1 Hz, 1H), 7.24 (s, 1H), 6.62 (br s, 1H), 3.34 (q, $J$ = 7.6 Hz, 1H), 1.25 (t, $J$ = 7.3 Hz, 1H); HRMS (ESI) $m/z$ calcd for $C_{12}H_{11}F_3NO^+$ $[M + H]^+$ 242.0787, found , 242.0778 (error 3.76 ppm).



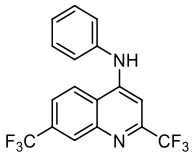

**5-Methoxy-2-(trifluoromethyl)quinolin-4-ol (9).** The title compound was prepared from ethyl 4,4,4-trifluoroacetoacetate (0.79 mL, 5.4 mmol) added 3-methoxylaniline (0.45 mL, 4.0 mmol) using General Procedure A. Flash column chromatography afforded the product (118 mg, 12% yield) as a pale-yellow solid: HPLC purity 98.3%, $t_R$ = 5.50 min, $k'$ = 11.78 (method B); $^1$H NMR (400MHz, $(CD_3OD)$ δ 8.11 (d, $J$ = 8.8 Hz, 1H), 7.54 (s, 1H), 7.37 (dd, $J$ = 8.7, 1.8 Hz, 1H), 6.73 (s, 1H), 2.60 (s, 3H); HRMS (ESI+) $m/z$ calcd for $C_{11}H_9F_3NO_2^+$ $[M + H]^+$ 244.0580, found 244.0590 (error 4.25 ppm).



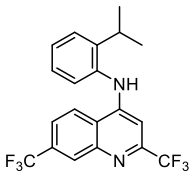

**4-Chloro-7-methoxy-2-(trifluoromethyl)quinoline (9b).** A mixture of 7-methoxy-2-(trifluoromethyl)-1H-quinolin-4-one (**9**, 100. mg, 411 μmol) and $POCl_3$ (1.6 g, 10.8 mmol) was degassed and purged with $N_2$ for 3 times, and then the mixture was stirred at 120 °C for 2 h under $N_2$ atmosphere. LCMS showed reactant was consumed completely and one main peak with desired mass was detected. The reaction mixture was quenched by slow addition $H_2O$ (10 mL) at 0°C, then extracted with EtOAc (5 mL × 3). The combined organic layers were washed with brine (5 mL), dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to afford the crude title compound (110.0 mg, crude) as a yellow oil.



**7-Thiomethyl-2-(trifluoromethyl)quinolin-4-ol (10).** The title compound was prepared from ethyl 4,4,4-trifluoroacetoacetate (0.79 mL, 5.4 mmol) added 3-methoxylaniline (0.62 mL, 4.9 mmol) using General Procedure A. Flash column chromatography afforded the product (61 mg, 5% yield) as a white solid: $^{1}$H NMR (400 MHz, ($CD_3OD$) δ 8.11 (d, $J$ = 8.77 Hz, 1H), 7.54 (s, 1H), 7.37 (dd, $J$ = 8.73, 1.85 Hz, 1H), 6.73 (s, 1H), 2.60 (s, 3H).

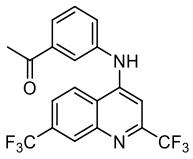


**4-Phenylamino-2,7-bis(trifluoromethyl)quinoline (11).** The title compound was prepared from 4-bromo-2,7-bis(trifluoromethyl)quinoline (70 mg, 0.20 mmol) and aniline (22 µL, 0.24 mmol) using General Procedure C. Reaction was stopped after 24 h. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 1:5) afforded the title compound (69 mg, 98% yield) as a light-yellow powder: $R_f$ = 0.42 (1:5 EtOAc/Hexanes); HPLC purity 99.5%, $t_R$ = 7.38 min, $k'$ = 16.17 (method B); $^{1}$H NMR (500 MHz, $(CD_3)_2SO$) δ 9.81 (s, 1H), 8.76 (d, $J$ = 8.8 Hz, 1H), 8.34 (s, 1H), 7.98 (d, $J$ = 8.8 Hz, 1H), 7.53 (t, $J$ = 7.8 Hz, 2H), 7.45 (d, $J$ = 7.7 Hz, 2H), 7.31 (t, $J$ = 7.3 Hz, 1H), 7.09 (s, 1H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 150.9, 148.6 (q, $^{2}J_{C\text{-}F}$ = 33 Hz), 147.0, 138.8, 131.0 (q, $^{2}J_{C\text{-}F}$ = 33 Hz), 129.8, 127.0 (d, $^{3}J_{C\text{-}F}$ = 4.5 Hz), 125.8, 124.6, 124.0, 123.8 (q, $^{1}J_{C\text{-}F}$ = 273 Hz), 121.7, 121.5 (q, $^{1}J_{C\text{-}F}$ = 276 Hz), 121.4, 96.9; HRMS (ESI+) $m/z$ calcd for $C_{17}H_{11}F_6N_2^+$ $[M + H]^+$ 357.0821, found 357.0824 (error 0.73 ppm).

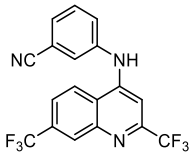


**4-[(2-Isopropyl)phenylamino]-2,7-bis(trifluoromethyl)quinoline (12).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinoline-4-one (140 mg, 0.49 mmol)

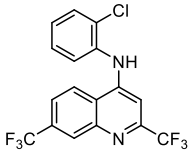
and 2-isopropylaniline (150 uL, 1.0 mmol) using General Procedure D. Purification by HPLC (method A) afforded the product (43 mg, 22% yield) as a yellow powder: HPLC purity 85.2%, $t_R$ = 7.63 min, $k'$ = 16.8 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 9.69 (br. s, 1H), 8.82 (d, $J$ = 8.8 Hz, 1H), 8.31 (s, 1H), 7.96 (d, $J$ = 8.6 Hz, 1H), 7.53 (d, $J$ = 7.7 Hz, 1H), 7.44 (t, $J$ = 7.4 Hz, 1H), 7.37 (m, 1H), 7.31 (m, 1H), 6.31 (s, 1H), 3.06 (dt, $J$ = 13.6, 6.8 Hz, 1H), 1.10 (d, $J$ = 6.8 Hz, 6H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 153.3, 149.0 (q, $^2J_{C\text{-}F}$ = 33.6 Hz), 147.3, 146.6, 135.7, 131.5 (q, $^2J_{C\text{-}F}$ = 32.7 Hz), 129.0 , 128.8, 128.0, 127.5, 127.5, 124.9, 124.3 (q, $^1J_{C\text{-}F}$ = 273 Hz), 122.1, 122.0 (q, $^1J_{C\text{-}F}$ = 276 Hz), 121.2, 96.7, 28.0, 23.7; HRMS (ESI+) $m/z$ calcd for $C_{20}H_{17}F_6N_2^+$ [M + H]$^+$ 399.1290, found 399.1294 (error 0.93 ppm).




**4-[(3-Acetyl)phenylamino]-2,7-bis(trifluoromethyl)quinoline (13).** The title compound was prepared from 4-bromo-2,7-bis(trifluoromethyl)quinoline (70 mg, 0.20 mmol) and 3'-aminoacetophenone (32 mg, 0.24 mmol) using General Procedure C. Reaction was stopped after 40 h. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 1:5) afforded the title compound (48 mg, 61% yield) as an orange powder: $R_f$ = 0.17 (1:5 EtOAc/Hexanes); HPLC purity 99.3%, $t_R$ = 7.21 min, $k'$ = 15.78 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 9.93 (s, 1H), 8.75 (d, $J$ = 8.8 Hz, 1H), 8.36 (s, 1H), 8.01 (d, $J$ = 8.9 Hz, 1H), 7.97 (s, 1H), 7.87 (d, $J$ = 7.6 Hz, 1H), 7.75 (d, $J$ = 8.2 Hz, 1H), 7.66 (t, $J$ = 7.8 Hz, 1H), 7.18 (s, 1H), 2.61 (s, 3H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 197.5, 150.5, 148.7 (q, $^2J_{C\text{-}F}$ = 33 Hz), 147.0, 139.5, 138.3, 131.1 (q, $^2J_{C\text{-}F}$ = 33 Hz), 130.2, 127.9, 127.1, 125.3, 124.6, 123.8 (q, $^1J_{C\text{-}F}$ = 273 Hz), 122.8, 122.0, 121.6, 121.4 (q, $^1J_{C\text{-}F}$ = 276 Hz), 97.4, 26.8; HRMS (ESI+) $m/z$ calcd for C19H13F6N2O+ [M + H]+ 399.0927, found 399.0918 (error 2.04 ppm).

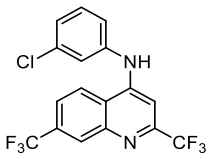



**4-[(3-Cyano)phenylamino]-2,7-bis(trifluoromethyl)quinoline (14).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinoline-4-one (140 mg, 0.48 mmol) and 3-aminobenzonitrile (88 mg, 0.73 mmol) using General Procedure D. Purification by HPLC (method A) afforded the product (50 mg, 27% yield) as a yellow powder: HPLC purity 99.8%, $t_R$ = 7.20 min, $k'$ = 15.74 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 8.90 (s, 1H) 8.69 (d, $J$ = 8.9 Hz, 1H), 8.36 (s, 1H), 8.00 (dd, $J$ = 8.9, 1.1 Hz, 1H), 7.89 (s, 1H), 8.00 (dt, $J$ = 6.8, 2.2 Hz, 1H), 7.69 (m, 2H), 7.23 (s, 1H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 149.9, 148.7 (q, $^2J_{C\text{-}F}$ = 32.7 Hz), 147.0 140.3, 131.12 (q, $^2J_{C\text{-}F}$ = 32.7 Hz), 131.08, 128.7, 127.9, 127.1 (d, $^3J_{C\text{-}F}$ = 4.5 Hz), 126.3, 124.6, 123.7 (q, $^1J_{C\text{-}F}$ = 273 Hz), 122.1 (d, $^3J_{C\text{-}F}$ = 2.7 Hz), 121.8, 121.4 (q, $^1J_{C\text{-}F}$ = 276 Hz), 118.3, 112.6, 98.2; HRMS (ESI+) *m*/*z* calcd for $C_{18}H_{10}F_6N_3^+$ $[M + H]^+$ 382.0773, found 382.0764 (error 2.44 ppm).

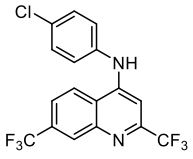



**4-[(2-Chloro)phenylamino]-2,7-bis(trifluoromethyl)quinoline (15).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinoline-4-one (140 mg, 0.48 mmol) and 2-chloroaniline (95 mg, 0.73 mmol) using General Procedure D. Purification by HPLC (method A) afforded the product (46 mg, 24% yield) a white powder: HPLC purity 97.6%, $t_R$ = 7.34 min, $k'$ = 16.15 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) 9.88 (s, 1H), 8.79 (d, $J$ = 8.9 Hz, 1H), 8.36 (s, 1H), 8.01 (dd, $J$ = 8.9, 1.4 Hz, 1H), 7.73 (dd, $J$ = 8, 1.1 Hz, 1H), 7.59 (s, 1H), 7.54 (td, $J$ = 7.6, 1.3 Hz, 1H), 7.48 (td, $J$ = 7.7, 1.4 Hz, 1H), 6.41 (s, 1H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 151.2, 148.5 (q, $^2J_{C\text{-}F}$ = 32.7 Hz), 146.8, 135.4, 131.1 (q, $^2J_{C\text{-}F}$ = 32.7 Hz), 131.0, 130.8, 129.5, 129.2, 128.9, 127.1 (d, $^3J_{C\text{-}F}$ = 4.5 Hz), 124.5, 123.7 (q, $^1J_{C\text{-}F}$ = 272 Hz), 122.0, 121.4 (q, $^1J_{C\text{-}F}$ = 276 Hz),

120.9, 97.2; HRMS (ESI$^+$) *m*/*z* calcd for $C_{17}H_{10}C_{l1}F_6N_2^+$ [M + H]$^+$ 391.0431, found 391.0447 (error 4.09 ppm).

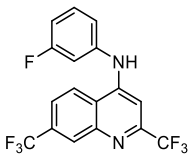



**4-[(3-Chloro)phenylamino]-2,7-bis(trifluoromethyl)quinoline (16).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinoline-4-one (140 mg, 0.48 mmol) and 3-chloroaniline (88 mg, 0.73 mmol) using General Procedure D. The reaction was then diluted in MeCN and purified by HPLC affording the product as a yellow powder (44 mg, 24% yield): HPLC purity 99.2%, $t_R$ = 7.65 min, $k'$ = 16.8 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 9.82 (s, 1H) 8.70 (d, *J* = 8.9 Hz, 1H), 8.34 (s, 1H), 7.98 (d, *J* = 8.8 Hz, 1H), 7.53-7.50 (m, 2H), 7.44 (, *J* = 8 Hz, 1H) 7.31 (dd, *J* = 7.7, 1.1 Hz, 1H), 7.20 (s, 1H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 150.1, 148.7 (q, $^2J_{C\text{-}F}$ = 33 Hz), 147.0, 140.7, 133.9, 131.3, 131.1 (q, $^2J_{C\text{-}F}$ = 33 Hz), 127.1 (d, $^3J_{C\text{-}F}$ = 3.6 Hz), 125.1, 124.5, 123.7 (q, $^1J_{C\text{-}F}$ = 273 Hz), 123.1, 122.0 121.7 (q, $^1J_{C\text{-}F}$ = 276 Hz), 97.9; HRMS (ESI) *m*/*z* calcd for $C_{17}H_{10}ClF_6N_2^+$ [M + H]$^+$ 391.0431, found 391.0442 (error 2.87 ppm).

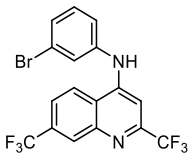



**4-[(4-Chloro)phenylamino]-2,7-bis(trifluoromethyl)quinoline (17).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinoline-4-one (225 mg, 0.80 mmol) and 4-chloroaniline (0.14 mL, 1.2 mmol) using General Procedure D. Purification by HPLC (method A) afforded the product (61 mg, 20% yield) as a yellow powder: HPLC purity 99.3%, $t_R$ = 7.64 min, $k'$ = 16.76 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 9.82 (s, 1H), 8.72 (d, *J* = 8.9 Hz, 1H), 8.35 (s, 1H), 7.99 (d, *J* = 8 Hz, 1H), 7.55 (d, *J* = 8.6 Hz, 1H), 7.47 (d, *J* = 8.7 Hz, 1H), 7.13 (s, 1H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 150.5, 148.7 (q, $^2J_{C\text{-}F}$ = 32.7 Hz), 147.0, 137.9, 131.1 (q, $^2J_{C\text{-}F}$ = 32.7 Hz), 129.7, 129.4, 127.1 (d, $^3J_{C\text{-}F}$ = 3.6 Hz), 125.4, 124.6, 123.8 (q, $^1J_{C\text{-}F}$ = 272 Hz),

121.9. 121.6. 121.4 (q, $^{1}J_{C\text{-}F}$ = 276 Hz), 97.4; HRMS (ESI+) *m*/*z* calcd for $C_{17}H_{10}Cl_1F_6N_2^+$ $[M + H]^+$ 391.0431, found 391.0434 (error 0.98 ppm).

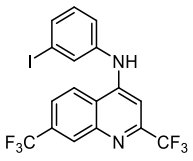



**4-[(3-Fluoro)phenylamino]-2,7-bis(trifluoromethyl)quinoline (18).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinoline-4-one (140 mg, 0.48 mmol) and 3-fluoroaniline (82 mg, 0.73 mmol) using General Procedure D. Purification by HPLC (method A) afforded the product (62 mg, 34% yield) as a yellow powder: HPLC purity 99.4%, $t_R$ = 7.46 min, $k'$ = 16.36 (method B); $^{1}$H NMR (500 MHz, $(CD_3)_2SO$) δ 9.84 (s, 1H) 8.71(d, *J* = 8.9 Hz, 1H), 8.34 (s, 1H), 7.99 (d, *J* = 8.8 Hz, 1H), 7.53 (q, *J* = 8 Hz, 1H), 7.3 (m, 2H), 7,23 (s, 1H), 7.09 (td, *J* = 8.4, 2 Hz, 1H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 162.8 (d, $^{1}J_{C\text{-}F}$ = 244 Hz), 150.2, 148.7 (q, $^{2}J_{C\text{-}F}$ = 32.7 Hz), 147.0, 141.0 (d, $^{3}J_{C\text{-}F}$ = 10 Hz), 131.4 (d, $^{3}J_{C\text{-}F}$ = 10 Hz), 131.1 (q, $^{2}J_{C\text{-}F}$ = 32.7 Hz), 127.1 (d, $^{3}J_{C\text{-}F}$ = 3.6 Hz), 124.6, 123.7 (q, $^{1}J_{C\text{-}F}$ = 273 Hz), 122.0, 121.7, 121.4 (q, $^{1}J_{C\text{-}F}$ = 276 Hz), 119.1, 112.0 (d, $^{2}J_{C\text{-}F}$ = 21 Hz), 110.2 (d, $^{2}J_{C\text{-}F}$ = 24 Hz), 98.0; HRMS ($ESI^+$) *m*/*z* calcd for $C_{17}H_{10}F_7N_2^+$ $[M + H]^+$ 375.0727, found 375.0721 (error 1.47 ppm).

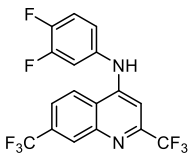



**4-[(3-Bromo)phenylamino]-2,7-bis(trifluoromethyl)quinoline (19).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinoline-4-one (140 mg, 0.48 mmol) and 3-bromoaniline (130 mg, 0.73 mmol) using General Procedure D. Purification by HPLC (method A) afforded the product (116 mg, 55% yield) as a yellow powder: HPLC purity 99.2%, $t_R$ = 7.70 min, $k'$ = 16.91 (method B); $^{1}$H NMR (500 MHz, $(CD_3)_2SO$) δ 9.82 (s, 1H), 8.69 (d, *J* = 8.8 Hz, 1H), 8.34 (s, 1H), 7.99 (dd, *J* = 8.8, 1 Hz, 1H), 7.63 (s, 1H), 7.50-7.44 (m, 3H), 7.20 (s, 1H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 150.1, 148.7 (q, $^{2}J_{C\text{-}F}$ = 32.7 Hz), 147.0, 140.8, 131.6, 131.1 (q, $^{2}J_{C\text{-}$

$_F$ = 32.7 Hz), 128.0. 127.1 (d, $^3J_{C\text{-}F}$ = 3.6 Hz), 126.0, 124.5, 123.7 (q, $^1J_{C\text{-}F}$ = 273 Hz), 122.3, 122.1, 122.0, 121.7, 121.4 (q, $^1J_{C\text{-}F}$ = 276 Hz), 97.8; HRMS (ESI$^+$) *m*/*z* calcd for $C_{17}H_{10}Br_1F_6N_2^+$ [M + H]$^+$ 434.9926, found 434.9919 (error 1.53 ppm).

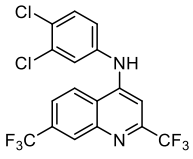



**4-[(3-Iodo)phenylamino]-2,7-bis(trifluoromethyl)quinoline (20).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinoline-4-one (230 mg, 0.80 mmol) and 3-iodoaniline (270 mg, 1.2 mmol) using General Procedure D. Purification by HPLC (method A) afforded the product (108 mg, 28% yield) as a yellow powder: HPLC purity 98.6%, $t_R$ = 7.75 min, $k'$ = 17.02 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 9.78 (s, 1H), 8.69 (d, *J* = 8.9 Hz, 1H), 8.34 (s, 1H), 7.99 (d, *J* = 8.9 Hz, 1H), 7.79 (s, 1H), 7.63 (d, *J* = 7.8 Hz, 1H), 7.51 (dd, *J* = 8, 1 Hz, 1H), 7.29 (t, *J* = 7.9 Hz, 1H), 7.17 (s, 1H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 150.2, 148.6 (q, $^2J_{C\text{-}F}$ = 32.7 Hz), 147.0, 140.5, 133.9, 131.9, 131.5, 131.0 (q, $^2J_{C\text{-}F}$ = 32.7 Hz), 127.1 (d, $^3J_{C\text{-}F}$ = 4.5 Hz), 124.5, 123.7 (q, $^1J_{C\text{-}F}$ = 273 Hz), 122.5, 122.0, 121.6, 121.4 (q, $^1J_{C\text{-}F}$ = 276 Hz), 97.6, 95.3; HRMS (ESI$^+$) *m*/*z* calcd for $C_{17}H_{10}F_6I_1N_2^+$ [M + H]$^+$ 482.9787, found 482.9783 (error 0.85 ppm).

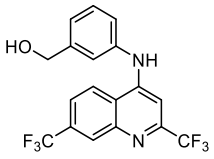



**4-[(3,4-Difluoro)phenylamino]-2,7-bis(trifluoromethyl)quinoline (21).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinoline-4-one (230 mg, 0.80 mmol) and 3,4-difluoroaniline (160 mg, 1.2 mmol) using General Procedure D. Purification by HPLC (method A) afforded the product (82 mg, 26% yield) as a white powder: HPLC purity 99.6%, $t_R$ = 7.49 min, $k'$ = 16.43 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 9.80 (br. s, 1H) 8.69 (d, *J* = 8.9 Hz, 1H), 8.33 (s, 1H), 7.97 (dd, *J* = 8.8, 1 Hz, 1H), 7.58-7.52 (m, 2H), 7.31-7.29 (m, 1H) 7.11 (s, 1H); $^{13}$C NMR (126 MHz, $(CD_3)SO$) δ 150.6, 149.8 (dd, $^1J_{C\text{-}F}$ = 247, $^2J_{C\text{-}F}$ = 13 Hz), 148.7 (q, $^2J_{C\text{-}$

$_F$ = 33 Hz), 147.1 (dd, $^1J_{C\text{-}F}$ = 244, $^2J_{C\text{-}F}$ = 12 Hz), 146.9, 136.0 (dd, $J_{C\text{-}F}$ = 8.2, 2.7 Hz), 131.1 (q, $^2J_{C\text{-}F}$ = 33 Hz), 124.5, 123.7 (q, $^1J_{C\text{-}F}$ = 273 Hz), 122.0, 121.5, 121.4 (q, $^1J_{C\text{-}F}$ = 274 Hz), 120.8 (dd, $J_{C\text{-}F}$ = 6.4, 2.7 Hz), 118.4 (d, $^2J_{C\text{-}F}$ = 18 Hz), 113.4 (d, $^2J_{C\text{-}F}$ = 18 Hz), 97.5; HRMS (ESI$^+$) *m*/*z* calcd for $C_{17}H_{17}F_6N_2^+$ [M + H]$^+$ 393.0633, found 393.0622 (error 2.85 ppm).

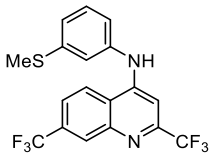



**4-[(3,4-Dichloro)phenylamino]-2,7-bis(trifluoromethyl)quinoline (22).** The title compound was prepared from 4-bromo-2,7-bis(trifluoromethyl)quinoline (350 mg, 1.0 mmol) and 3,4-dichloroaniline (200 mg, 1.2 mmol) using General Procedure C. Reaction was stopped after 20 h. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 1:1) afforded the title compound as a yellow powder (410 mg, 94% yield): $R_f$ = 0.63 (3:10 EtOAc/Hexanes); HPLC purity 99.5%, $t_R$ = 7.88 min, $k'$ = 17.33 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 9.84 (s, 1H), 8.68 (d, *J* = 8.9 Hz, 1H), 8.37 (s, 1H), 8.01 (d, *J* = 8.8 Hz, 1H), 7.73-7.70 (m, 2H), 7.48 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.26 (s, 1H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 149.9, 148.7 (q, $^2J_{C\text{-}F}$ = 33 Hz), 147.0, 139.5, 131.9, 131.5, 131.1 (q, $^2J_{C\text{-}F}$ = 33 Hz), 127.2, 126.8, 124.8, 124.5, 123.7 (q, $^1J_{C\text{-}F}$ = 273 Hz), 123.1, 122.1, 121.8, 121.4 (q, $^1J_{C\text{-}F}$ = 275 Hz), 98.4; HRMS (ESI$^+$) *m*/*z* calcd for $C_{17}H_9F_6Cl_2N_2^+$ [M + H]$^+$ 425.0041, found 425.0032 (error 2.22 ppm).

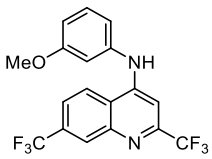



**4-[(3-hydroxymethyl)phenylamino]-2,7-bis(trifluoromethyl)quinoline (23).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinoline-4-one (140 mg, 0.48 mmol) and 3-aminobenzonitrile (82 µL, 0.73 mmol) using General Procedure D. Purification by HPLC (method A) afforded the product (22 mg, 12% yield) as a yellow powder: HPLC purity 98.5%, $t_R$ = 6.99 min, $k'$ = 15.26 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 9.81 (s, 1H) 8.76

(d, $J$ = 8.9 Hz, 1H), 8.32 (s, 1H), 7.97 (d, $J$ = 8.9 Hz, 1H), 7.46 (t, $J$ = 7.8 Hz, 1H), 7.39 (s, 1H), 7.32 (d, $J$ = 7.8 Hz, 1H), 7.24 (d, $J$ = 7.6 Hz, 1H), 7.10 (s, 1H), 5.31 (t, $J$ = 5.3 Hz, 1H), 4.56 (d, $J$ = 4.7 Hz, 2H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 150.9, 148.6 (q, $^2J_{C\text{-}F}$ = 32.7 Hz), 147.0, 144.6, 138.6, 131.0 (q, $^2J_{C\text{-}F}$ = 32.7 Hz), 129.4, 127.0 (d, $^3J_{C\text{-}F}$ = 3.6 Hz), 124.6, 123.8 (q, $^1J_{C\text{-}F}$ = 273 Hz), 123.7, 122.1, 121.9, 121.7, 121.5 (q, $^1J_{C\text{-}F}$ = 276 Hz), 121.4, 96.9, 62.5; HRMS (ESI$^+$) $m/z$ calcd for $C_{18}H_{13}F_6N_2O^+$ $[M + H]^+$ 387.0927, found 387.0929 (error 0.56 ppm).

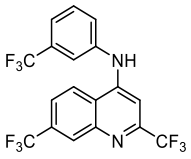



**4-[(3-(Thiomethyl)phenylamino]-2,7-bis(trifluoromethyl)quinoline (24).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinoline-4-one (225 mg, 0.80 mmol) and 3-thiomethylaniline (0.152 mL, 1.2 mmol) using General Procedure D. Purification by HPLC (method A) afforded the product (46 mg, 15% yield) as a yellow powder: HPLC purity 97.8%, $t_R$ = 7.61 min, $k'$ = 16.69 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 9.80 (s, 1H) 8.74 (d, $J$ = 8.9 Hz, 1H), 8.34 (s, 1H), 7.99 (d, $J$ = 8.9 Hz, 1H), 7.44 (t, $J$ = 7.9 Hz, 1H), 7.31 (s, 1H), 7.24 (d, $J$ = 7.9 Hz, 1H), 7.18-7.17 (m, 2H), 2.50 (s, 3H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 150.6, 148.6 (q, $^2J_{C\text{-}F}$ = 33 Hz), 147.0, 140.0, 139.6, 131.0 (q, $^2JF4\text{-}8_{C\text{-}F}$ = 33 Hz), 130.1, 127.1 (d, $^3J_{C\text{-}F}$ = 4.5 Hz), 124.5, 123.7 (q, $^1J_{C\text{-}F}$ = 273 Hz), 122.9, 121.81 (d, $^3J_{C\text{-}F}$ = 2.7 Hz), 121.6, 121.5 (q, $^1J_{C\text{-}F}$ = 276 Hz), 120.6, 119.8, 97.5, 14.5; HRMS (ESI$^+$) $m/z$ calcd for $C_{18}H_{10}F_7N_2O^+$ $[M + H]^+$ 403.0676, found 403.0691 (error 3.72 ppm).

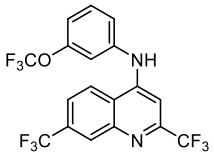



**4-[(3-Methoxy)phenylamino]-2,7-bis(trifluoromethyl)quinoline (25).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinoline-4-one (225 mg, 0.80 mmol) and 3-methoxyaniline (0.134 mL, 1.2 mmol) using General Procedure D. Purification by HPLC

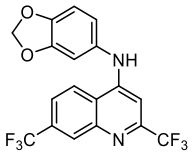
(method A) afforded the product (115 mg, 37% yield) as a light-brown powder: HPLC purity 96.4%, $t_R$ = 7.43 min, $k'$ = 16.27 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 9.77 (s, 1H), 8.73 (d, $J$ = 8.9 Hz, 1H), 8.33 (s, 1H) 7.97 (d, $J$ = 8.8 Hz, 1H), 7.41 (t, $J$ = 8.0 Hz, 1H), 7.17 (s, 1H), 7.03 (d, $J$ = 8.2 Hz, 1H), 7.00 (s, 1H), 6.87 (dd, $J$ = 8.2, 1.7 Hz, 1H), 3.79 (s, 3H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 160.4, 150.7, 148.6, (q, $^2J_{C\text{-}F}$ = 32.7 Hz), 140.1, 131.1 (q, $^2J_{C\text{-}F}$ = 32.7 Hz), 130.6, 127.0 (d, $^3J_{C\text{-}F}$ = 4.5 Hz), 124.6, 123.7 (q, $^1J_{C\text{-}F}$ = 273 Hz), 121.8, 121.50, 121.50 (q, $^1J_{C\text{-}F}$ = 275 Hz), 115.7, 111.3, 109.5, 97.4, 55.2; HRMS (ESI$^+$) $m/z$ calcd for $C_{18}H_{13}F_6N_2O^+$ $[M + H]^+$ 387.0927, found 387.0935 (error 2.09 ppm).




**2,7-bis(trifluoromethyl)-4-[3-(trifluoromethyl)phenylamino]quinoline (26).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinoline-4-one (140 mg, 0.48 mmol) and 3-trifluoromethylaniline (91 uL, 1.0 mmol) using General Procedure D. Purification by HPLC (method A) afforded the product (42 mg, 20% yield) as a tan powder: HPLC purity 99.6%, $t_R$ = 7.63 min, $k'$ = 16.75 (method B); NMR (500 MHz, $(CD_3)_2SO$) δ 9.94 (s, 1H), 8.72 (d, $J$ = 8.72 Hz, 1H), 8.37 (s, 1H), 8.72 (dd, $J$ = 8.8, 1.3 Hz, 1H), 7.81-7.76 (m, 2H), 7.72 (t, $J$ = 7.9 Hz, 1H), 7.60 (d, $J$ = 7.8 Hz, 1H), 7.25 (s, 1H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 150.0, 148.7 (q, $^2J_{C\text{-}F}$ = 34 Hz), 147.0, 140.1, 131.1 (q, $^2J_{C\text{-}F}$ = 33 Hz), 130.9, 130.5 (q, $^2J_{C\text{-}F}$ = 32 Hz), 127.1 (d, $^3J_{C\text{-}F}$ = 4.5 Hz), 126.6, 124.5, 123.9 (q, $^1J_{C\text{-}F}$ = 273 Hz), 123.7 (q, $^1J_{C\text{-}F}$ = 273 Hz), 122.1 (d, $^3J_{C\text{-}F}$ = 2.7 Hz), 121.8, 121.5 (d, $^3J_{C\text{-}F}$ = 3.6 Hz), 121.4 (q, $^1J_{C\text{-}F}$ = 275 Hz), 119.7 (d, $^3J_{C\text{-}F}$ = 3.6 Hz), 97.9; HRMS (ESI) $m/z$ calcd for $C_{18}H_{10}F_9N_2^+$ $[M + H]^+$ 425.0695, found 425.0715 (error 4.71 ppm).

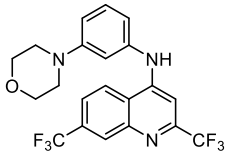


**4-[(3-Trifluoromethoxy)phenylamino]-2,7-bis(trifluoromethyl)quinoline (27).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinoline-4-one (500 mg, 1.5 mmol) and 3-trifluoromethoxyaniline (309 mg, 1.7 mmol) using General Procedure C. Reaction was stopped after 39 h. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 3:5) afforded the product as white powder (531 mg, 83% yield): using General Procedure F. Reaction was stopped after 20 h. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 3:5) afforded the product as a yellow powder (48 mg, 27% yield): HPLC purity 99.8%, $t_R$ = 7.69 min, $k'$ = 16.89 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 9.91 (s, 1H), 8.71 (d, $J$ = 8.8 Hz, 1H), 8.35 (s, 1H), 8.0 (d, $J$ = 8.7 Hz, 1H), 7.61 (t, $J$ = 8.1 Hz, 1H), 7.50-7.45 (m, 2H), 7.26-7.22 (m, 2H); $^{13}$C NMR (126 FMHz, $(CD_3)_2SO$) δ 150.0, 149.0, 148.7 (q, $^2J_{C\text{-}F}$ = 33 Hz), 147.0, 141.0, 131.1 (q, $^2J_{C\text{-}F}$ = 33 Hz), 127.1, 124.6, 123.7 (q, $^1J_{C\text{-}F}$ = 274 Hz), 122.1, 121.8, 121.4 (q, $^1J_{C\text{-}F}$ = 276 Hz), 120.1 (q, $^1J_{C\text{-}F}$ = 257 Hz), 117.4, 115.6, 98.0; HRMS (ESI+) $m/z$ calcd for $C_{18}H_{10}F_9N_2O^+$ $[M + H]^+$ 441.0644, found 441.0644 (error 3.57 ppm).

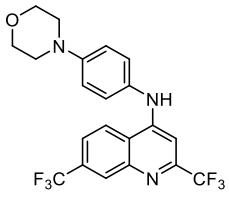


**4-[3,4-(Methylenedioxy)phenylamino]-2,7-bis(trifluoromethyl)quinoline (28).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinoline-4-one (230 mg, 0.80 mmol) and 3,4-(methylenedioxy)aniline (170 mg, 1.2 mmol) using General Procedure D. Purification by HPLC (method A) afforded the product (84 mg, 26% yield) as a black powder: HPLC purity 97.9%, $t_R$ = 7.81 min, $k'$ = 16.01 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 9.67 (s, 1H), 8.71 (d, $J$ = 8.8 Hz, 1H), 8.30 (s, 1H), 7.94 (d, $J$ = 8.9, 1.4 Hz, 1H), 7.04 (m, 2H), 6.88 (m, 2H), 6.11 (s, 2H); $^{13}$C NMR (126 MHz, $(CD_3)SO$) δ 151.8, 148.6 (q, $^2J_{C\text{-}F}$ = 33 Hz), 148.2, 146.9,

145.6, 132.4, 130.9 (q, $^{2}J_{C\text{-}F}$ = 33 Hz), 127.0 (d, $^{3}J_{C\text{-}F}$ = 4.5 Hz), 124.4, 123.8 (q, $^{1}J_{C\text{-}F}$ = 273 Hz), 121.6, 121.5 (q, $^{1}J_{C\text{-}F}$ = 276 Hz), 121.1, 118.4, 108.9, 106.5, 101.7, 96.6; HRMS (ESI+) *m*/*z* calcd for $C_{18}H_{11}F_6N_2O_2^+$ $[M + H]^+$ 401.0719, found 401.0728 (error 2.22 ppm).

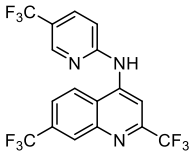


**4-[3-(Morpholino)phenylamino]-2,7-bis(trifluoromethyl)quinoline (29).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinoline-4-one (163 mg, 0.58 mmol) and 3-(morpholino)aniline (159 mg, 0.87 mmol) using General Procedure D. Purification by HPLC (method A) afforded the product (102 mg, 40% yield) as a yellow powder: HPLC purity 70.4%, $t_R$ = 7.46 min, $k'$ = 16.36 (method B); NMR (500 MHz, $(CD_3)_2SO$) δ 9.73 (s, 1H), 8.75 (d, *J* = 8.9 Hz, 1H), 8.33 (s, 1H), 7.97 (d, *J* = 8.9 Hz, 1H), 7.36 (d, *J* = 8 Hz, 1H), 7.13 (s, 1H), 6.97 (s, 1H), 6.89 (m, 2H), 3.75 (t, *J* = 4.7 Hz, 4H), 3.16 (t, *J* = 4.8 Hz, 4H); $^{13}$C NMR (126 MHz, $(CD_3)SO$) δ 152.3, 151.0, 148.8 (q, $^{2}J_{C\text{-}F}$ = 32.7 Hz), 147.0, 139.5, 130.9 (q, $^{2}J_{C\text{-}F}$ = 32.7 Hz), 130.2, 127.0 (d, $^{3}J_{C\text{-}F}$ = 4.5 Hz), 124.5, 123.8 (q, $^{1}J_{C\text{-}F}$ = 273 Hz), 121.6, 121.5 (q, $^{1}J_{C\text{-}F}$ = 276 Hz), 114.35, 112.6, 110.6, 97.2, 66.0, 48.1; HRMS ($ESI^+$) *m*/*z* calcd for $C_{21}H_{18}F_6N_3O^+$ $[M + H]^+$ 442.1349, found 442.1367 (error 4.07 ppm).

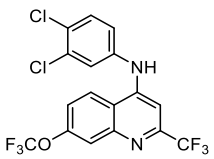


**4-[4-(Morpholino)phenylamino]-2,7-bis(trifluoromethyl)quinoline (30).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinoline-4-one (163 mg, 0.58 mmol) and 4-(morpholino)aniline (159 mg, 0.87 mmol) using General Procedure D. Purification by HPLC (method A) afforded the product (70 mg, 33 % yield) as a yellow powder: HPLC purity 99.9%, $t_R$ = 7.25 min, $k'$ = 15.86 (method B); $^{1}$H NMR (500 MHz, $(CD_3)_2SO$) δ 9.65 (s, 1H), 8.73

(d, $J$ = 8.9 Hz, 1H), 8.29 (s, 1H), 7.94 (d, $J$ = 8.9 Hz, 1H), 7.28 (d, $J$ = 8.8 Hz, 2H), 7.08 (d, $J$ = 8.9 Hz, 2H), 6.87 (s, 1H), 3.76 (t, $J$ = 4.7 Hz, 4H), 3.16 (t, $J$ = 4.7 Hz, 4H); $^{13}$C NMR (126 MHz, $(CD_3)SO$) δ 151.8, 149.3, 148.6 (q, $^2J_{C\text{-}F}$ = 32.7 Hz), 147.0, 130.9 (q, $^2J_{C\text{-}F}$ = 32.7 Hz), 129.6, 126.9 (d, $^3J_{C\text{-}F}$ = 3.6 Hz), 125.9, 124.2, 123.9 (q, $^1J_{C\text{-}F}$ = 273 Hz), 121.55 (q, $^1J_{C\text{-}F}$ = 275 Hz), 121.45, 121.1, 115.9, 96.2, 66.1, 48.2; HRMS (ESI$^+$) $m/z$ calcd for $C_{21}H_{18}F_6N_3O^+$ $[M + H]^+$ 442.1349, found 442.1362 (error 2.94 ppm).

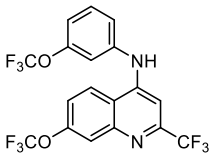



**2,7-bis(Trifluoromethyl)-4-[5-(trifluoromethyl)pyridin-2-ylamino]quinoline (31).** The title compound was prepared from 4-bromo-2,7-bis(trifluoromethyl)quinoline (150 mg, 0.43 mmol) and 2-amino-5-trifluoromethylpyridine (83 mg, 0.51 mmol) using General Procedure C. Reaction was stopped after 20 h. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 1:5) afforded the title compound (97 mg, 54% yield) as a yellow powder: $R_f$ = 0.27 (1:5 EtOAc/Hexanes); HPLC purity 99.0%, $t_R$ = 7.71 min, $k'$ = 16.94 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 10.28 (s, 1H), 9.04 (s, 1H), 8.81 (d, $J$ = 8.9 Hz, 1H), 8.71 (br. s, 1H), 8.37 (s, 1H), 8.12 (d, $J$ = 8.2 Hz, 1H), 8.02 (d, $J$ = 8.8 Hz, 1H), 7.56 (d, $J$ = 8.8 Hz, 1H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 157.0, 148.6 (q, $^2J_{C\text{-}F}$ = 34 Hz), 146.6, 145.9, 144.8 (d, $^3J_{C\text{-}F}$ = 3.6 Hz), 135.2, 130.1 (q, $^2J_{C\text{-}F}$ = 33.7 Hz), 127.4 (d, $^3J_{C\text{-}F}$ = 4.5 Hz), 124.6, 124.1 (q, $^1J_{C\text{-}F}$ = 272 Hz), 123.7 (q, $^1J_{C\text{-}F}$ = 273 Hz), 122.5, 122.2, 121.5 (q, $^1J_{C\text{-}F}$ = 274 Hz), 118.8 (q, $^2J_{C\text{-}F}$ = 32.7 Hz), 113.9, 104.3; HRMS (ESI+) $m/z$ calcd for $C_{17}H_9F_9N_3^+$ $[M + H]^+$ 426.0647, found 426.0631 (error 3.76 ppm).

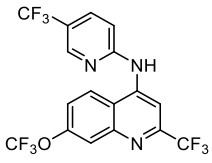



**4-[(3,4-Dichloro)phenylamino]-7-(trifluoromethoxy)-2-(trifluoromethyl)-quinoline (32).** The title compound was prepared from 4-bromo-7-(trifluoromethoxy)-2-

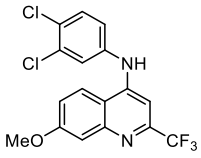
(trifluoromethyl)quinoline (200 mg, 0.56 mmol) and 3,4-dichloroaniline (108 mg, 0.67 mmol) using General Procedure C. Reaction was stopped after 20.5 h. Flash column chromatography afforded the product (68 mg, 27% yield) as a yellow powder from an EtOAc/Hex (0:1 to 1:1) stepwise gradient. $R_f$ = 0.28 (3:20 EtOAc/Hexanes); $^1$H NMR (500 MHz, ($CDCl_3$) δ 8.17 (d, *J* = 9.1 Hz, 1H), 8.09 (s, 1H), 7.54 (d, *J* = 7.2 Hz, 1H), 7.49 (dd, *J* = 9.1, 2.2 Hz, 1H), 7.54 (d, *J* = 2.5, Hz, 1H), 7.22 (dd, *J* = 8.6, 2.5 Hz, 1H), 7.17 (s, 1H); $^{13}$C NMR (151 MHz $(CD_3)_2SO$) δ 149.89, 149.87, 148.6 (q, *J* = 33.2 Hz), 148.5, 139.6, 131.9, 131.5, 126.7, 125.3, 124.8, 123.1f, 121.4 (q, *J* = 276 Hz), 120.5, 120.1 (q, *J* = 258 Hz), 119.5, 118.5, 97.5 (q, *J* = 2.6 Hz); HRMS (ESI+) *m*/*z* calcd for $C_{17}H_9Cl_2F_6N_2O^+$ $[M + H]^+$ 440.9991, found 440.9991 (error 0.12 ppm).




**7-(Trifluoromethoxy)-4-[3-(trifluoromethoxy)phenylamino]-2-(trifluoromethyl)quinoline (33).** The title compound was prepared from 4-bromo-7-(trifluoromethoxy)-2-(trifluoromethyl)quinoline (200 mg, 0.56 mmol) and 3,4-dichloroaniline (108 mg, 0.67 mmol) using General Procedure C. Reaction was stopped after 22.5 h. Flash column chromatography afforded the product (139 mg, 55% yield) as a white powder from an EtOAc/Hex (0:1 to 1:1) stepwise gradient: $R_f$ = 0.31 (3:20 EtOAc/Hexanes); HPLC purity 99.7%, $t_R$ = 7.71 min, $k'$ = 16.95 (method B); $^1$H NMR (500 MHz, $CDCl_3$) δ 8.04 (d, *J* = 0.8 Hz, 1H), 8.02 (d, *J* = 9.3 Hz, 1H), 7.53-7.49 (m, 2H), 7.31-7.27 (m, 2H), 7.20 (s, 1H), 7.15 (dd, *J* = 8.3, 0.9 Hz, 1H), 6.88 (br s, 1H); $^{13}$C NMR (151 MHz, $CD_3OD$) δ 152.2, 152.1 (q, *J* = 2.1 Hz), 151.4 (q, *J* = 1.9 Hz), 151.0 (q, *J* = 33.8 Hz), 150.3, 142.5, 132.4, 130.1, 129.7, 125.6, 123.0, 122.9 (q, *J* = 275 Hz), 121.9 (q, *J* = 256 Hz), 121.5, 120.0, 118.8, 117.2, 98.5 (q, *J* = 2.7 Hz); HRMS (ESI+) *m*/*z* calcd for $C_{18}H_{10}F_9N_2O_2^+$ $[M + H]^+$ 457.0593, found 457.0594 (error 0.21 ppm).

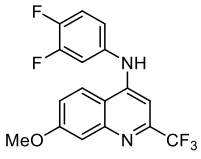


**7-(Trifluoromethoxy)-2-(trifluoromethyl)-4-[5-(trifluoromethyl)pyridin-2-ylamino]quinoline (34).** The title compound was prepared from 4-bromo-2,7-bis(trifluoromethyl)quinoline (350 mg, 1.0 mmol) and 2-amino-5-(trifluoromethyl)pyridine (190 mg, 1.2 mmol) using General Procedure C. Reaction was stopped after 20 h. Flash column chromatography afforded the product as a yellow powder (350 mg, 82% yield) from an EtOAc/Hex (0:1 to 2:5) stepwise gradient. $R_f$ = 0.64 (1:5 EtOAc/Hexanes); $^1$H NMR (500 MHz, ($CDCl_3$) δ 8.72 (s, 1H), 8.64 (s, 1H), 8.56 (s, 1H), 8.15 (d, *J* = 8.7 Hz, 1H), 7.96 (d, *J* = 8.5 Hz, 1H), 7.86 (d, *J* = 8.8 Hz, 1H), 7.64 (br. s, 1H), 7.19 (d, *J* = 8.6 Hz, 1H); $^{13}$C NMR (151 MHz, MeOD) δ 158.5, 151.8 (q, *J* = 1.6 Hz), 151.0 (q, *J* = 33.8 Hz), 150.0, 147.8, 146.2 (q, *J* = 4.5 Hz), 136.3 (q, *J* = 3.3 Hz), 125.5, 125.48 (q, *J* = 270 Hz), 123.03 (q, *J* = 275 Hz), 122.1, 121.9 (q, *J* = 258 Hz), 121.39 (q, *J* = 33 Hz), 120.49, 120.4, 114.5, 105.1 (q, *J* = 2.1 Hz).

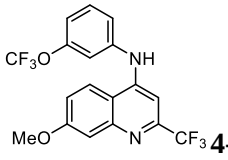


**4-(3,4-dichlorophenylamino)-7-methoxy -2- (trifluoromethyl)quinoline (35).** A mixture of 7-methoxy-2-(trifluoromethyl)-1H-quinolin-4-one (**10**, 100. mg, 411 μmol) and $POCl_3$ (1.6 g, 10.8 mmol) was degassed and purged with $N_2$ for 3 times, and then the mixture was stirred at 120 °C for 2 h under $N_2$ atmosphere. LCMS showed reactant was consumed completely and one main peak with desired mass was detected. The reaction mixture was quenched by addition $H_2O$ (10 mL) at 0°C, then extracted with EtOAc (5 mL × 3). The combined organic layers were washed with brine (5 mL), dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to afford crude 4-chloro-7-methoxy-2-(trifluoromethyl)quinoline (**10a**, 110.0 mg, crude) as a yellow oil, which was used in the next step without further purification.

The title compound was prepared from a mixture of 4-chloro-7-methoxy-2-(trifluoromethyl)quinoline (**10a**, 40 mg, 152.9 µmol), 3,4-dichloroaniline (24.8 mg, 152.9 µmol), and HCl (12 M, 1.3 µL) in EtOH (1 mL) according to according to General Procedure E. Reaction was stopped after 2 h where the solvent was removed and the obtained residue was purified by *Prep*.-HPLC: (column: Kromasil 150*25mm*10um; mobile phase: [water (0.04% $NH_3H_2O$+10 mM $NH_4HCO_3$)-ACN]; B%:45%-75%, 10min) to afford the title compound (8.82 mg, 14.7% yield, 98.6% purity) as a white solid. $^1$H NMR (400MHz, $CDCl_3$) δ 7.82 (d, *J* = 9.3 Hz, 1H), 7.57 - 7.47 (m, 2H), 7.41 (d, *J* = 2.4 Hz, 1H), 7.30 (br d, *J* = 2.4 Hz, 1H), 7.19 (dd, *J* = 2.4, 8.6 Hz, 1H), 7.14 (s, 1H), 6.67 (s, 1H), 3.98 (s, 3H); MS (ESI) $[M+H]^+$ = 387.0; HRMS (ESI) *m*/*z* calcd for $C_{17}H_{12}Cl_2F_3N_2O^+$ $[M + H]^+$ 387.0273, found 387.0273 (error 0.04 ppm).

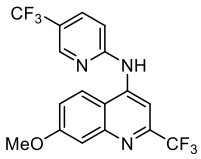



**4-(3,4-difluorophenylamino)-7-methoxy -2- (trifluoromethyl)quinoline (36).** The title compound was synthesized according to the synthetic procedure reported for **35**: $^1$H NMR (400 MHz, $(CD_3)_2SO$) δ 9.45 (s, 1H), 8.34 (d, *J* = 9.3 Hz, 1H), 7.59 - 7.46 (m, 2H), 7.41 (d, *J* = 2.6 Hz, 1H), 7.34 (dd, *J*=2.4, 9.3 Hz, 1H), 7.29 - 7.20 (m, 1H), 6.94 (s, 1H), 3.94 (s, 3H); MS (ESI) $[M+H]^+$ = 355.0; HRMS (ESI) *m*/*z* calcd for $C_{17}H_{12}F_5N_2O^+$ $[M + H]^+$ 355.0864, found 355.0874 (error 2.84 ppm).




**4-[(3-trifluoromethoxy)phenylamino]-7-methoxy -2- (trifluoromethyl) quinoline (37).** The title compound was synthesized according to the synthetic procedure reported for **35**: $^1$H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, *J* = 9.3 Hz, 1H), 7.54 - 7.43 (m, 2H), 7.30 (d, *J* = 2.6 Hz, 1H), 7.25 - 7.23 (m, 1H), 7.18 (s, 1H), 7.10 (br d, *J* = 8.4 Hz, 1H), 6.77 (s, 1H), 3.98 (s, 3H);

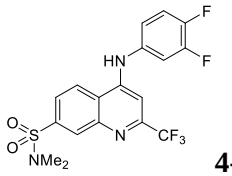
MS (ESI) $[M+H]^+$ = 403.0; HRMS (ESI) *m/z* calcd for $C_{18}H_{13}F_6N_2O_2^+$ $[M + H]^+$ 403.0876, found 403.0895 (error 4.88 ppm).



**7-methoxy-2-(trifluoromethyl)-4-[5-(trifluoromethyl)pyridin-2-ylamino]quinoline (38).** A mixture of 7-methoxy-2-(trifluoromethyl)-1H-quinolin-4-one (**10**, 100. mg, 411 μmol) and $POCl_3$ (1.6 g, 10.8 mmol) was degassed and purged with $N_2$ for 3 times, and then the mixture was stirred at 120 °C for 2 h under $N_2$ atmosphere. LCMS showed reactant was consumed completely and one main peak with desired mass was detected. The reaction mixture was quenched by addition $H_2O$ (10 mL) at 0°C, then extracted with EtOAc (5 mL × 3). The combined organic layers were washed with brine (5 mL), dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to afford crude 4-chloro-7-methoxy-2-(trifluoromethyl)quinoline (**10a**, 110.0 mg, crude) as a yellow oil, which was used in the next step without further purification.

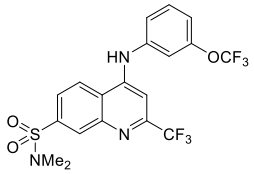
A mixture of 4-chloro-7-methoxy-2-(trifluoromethyl)quinoline (**10a**, 100 mg, 382 μmol), 5-(trifluoromethyl) pyridin-2-amine (74.4 mg, 459 μmol), $K_2CO_3$ (159 mg, 1.2 mmol), $Pd_2(dba)_3$ (70.0 mg, 76.4 μmol), Xantphos (88.5 mg, 153 μmol) in dioxane (2 mL) was degassed and purged with $N_2$ for 3 times, and then the mixture was stirred at 100 °C for 2 h under $N_2$ atmosphere. The reaction mixture was concentrated under reduced pressure and the resulting residue was purified by *Prep*.-HPLC: (column: Kromasil 150*25mm*10um; mobile phase: [water (0.04% $NH_3H_2O$+10 mM $NH_4HCO_3$)-ACN]; B%:50%-70%, 10min) to afford the title compound (30 mg, 19% yield, 98.14% purity) as a white solid. $^1$H NMR (400MHz, $(CD_3)_2SO$) δ 10.12 (br s, 1H), 8.79 - 8.70 (m, 2H), 8.50 (br d, *J* = 9.3 Hz, 1H), 8.17 - 8.08 (m, 1H), 7.54 (br d, *J* = 8.8 Hz, 1H), 7.49 (br d, *J* = 1.8

Hz, 1H), 7.45 - 7.39 (m, 1H), 3.96 (s, 3H); MS (ESI) $[M+H]^+$ = 388.0; HRMS (ESI) *m*/*z* calcd for $C_{17}H_{12}F_6N_3O^+$ $[M + H]^+$ 388.0879, found 388.0889 (error 2.54 ppm).

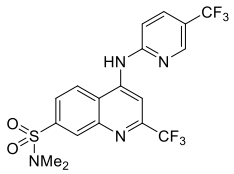



**4-(3,4-difluoroanilino)-*N*,*N*-dimethyl-2-(trifluoromethyl)quinoline-7-sulfonamide (39).** To *N*,*N*-dimethyl-4-oxo-2-(trifluoromethyl)-1*H*-quinoline-7-sulfonamide (**66**, 100 mg, 312 µmol) was added $POCl_3$ (1.45 mL, 15.6 mmol). The mixture was stirred at 120 °C for 1 h. The reaction mixture was quenched by addition $H_2O$ (10 mL) at 0 °C and extracted with EtOAc (10 mL × 3). The combined organic layers were washed with brine (10 mL), dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to 4-chloro-*N*,*N*-dimethyl-2-(trifluoromethyl)quinoline-7-sulfonamide (**66a**, 100 mg, crude) as a brown oil.

A mixture of 4-chloro-*N*,*N*-dimethyl-2-(trifluoromethyl)quinoline-7-sulfonamide (**66a**, 90 mg, 266 µmol), 3,4-difluoroaniline (68.6 mg, 531 µmol), HCl (12 M, 2.21 µL) in EtOH (2 mL) was degassed and purged with $N_2$ three times, then the mixture was stirred at 80 °C for 12 h under $N_2$ atmosphere. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by *Prep*.-HPLC: (column: Kromasil 150 × 25mm × 10 um; mobile phase: [water (0.04% $NH_3H_2O$ + 10 mM $NH_4HCO_3$)-ACN]; B%: 30%-60%, 20 min) gave the title compound (42.2 mg, 36.7% yield, 99.6% purity) as a white solid: $^1$H NMR (400 MHz, $(CD_3)_2SO$) δ 9.84 (s, 1H), 8.71 (d, *J* = 9.0 Hz, 1H), 8.28 (d, *J* = 1.5 Hz, 1H), 7.98 (dd, *J* = 1.8, 8.8 Hz, 1H), 7.65 - 7.51 (m, 2H), 7.36 - 7.26 (m, 1H), 7.12 (s, 1H), 2.71 (s, 6H). MS (ESI) $[M+H]^+$ = 432.0; HRMS (ESI) *m*/*z* calcd for $C_{18}H_{13}F_5N_3O_2S^-$ $[M - H]^-$ 430.0654, found 430.0674 (error 4.71 ppm).

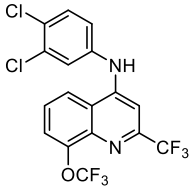


**4-(3-trifluoromethoxyanilino)-*N,N*-dimethyl-2-(trifluoromethyl) quinoline-7-sulfonamide (40).** The title compound was synthesized according to the synthetic procedure reported for **39**: $^1$H NMR (400 MHz, $(CD_3)_2SO$) δ 9.95 (s, 1H), 8.73 (d, *J* = 8.8 Hz, 1H), 8.30 (d, *J* =1.8 Hz, 1H), 8.00 (dd, *J* =1.8, 8.8 Hz, 1H), 7.66 - 7.60 (m, 1H), 7.54 - 7.45 (m, 2H), 7.32 - 7.19 (m, 2H), 2.72 (s, 6H); MS (ESI) $[M+H]^+$ = 480.0; HRMS (ESI) *m*/*z* calcd for $C_{19}H_{14}F_6N_3O_3S^-$ $[M - H]^-$ 478.0666, found 430.0678 (error 2.57 ppm).

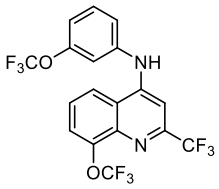


***N,N*-dimethyl-2-(trifluoromethyl)-4-{[5-(trifluoromethyl)pyridin-2-yl]amino}quinoline-7- sulfonamide (41).** A mixture of 4-chloro-*N,N*-dimethyl-2-(trifluoromethyl)quinoline-7-sulfonamide (100 mg, 295 µmol), 2-amino-5-(trifluoromethyl)pyridine (71.8 mg, 443 µmol), $Pd_2(dba)_3$ (54.1 mg, 59.0 µmol), Xantphos (68.3 mg, 118 µmol) and $K_2CO_3$ (122 mg, 886 µmol) in dioxane (2 mL) was degassed and purged with $N_2$ three times, and then the mixture was stirred at 100 °C for 12 h under $N_2$ atmosphere. 3-Mercaptopropyltrimethoxysilane (100 mg) was added and stirred at 25 °C for 2 h. The solvent was removed under vacuum to form a residue. The residue was purified by *Prep*.-HPLC: (column: Kromasil 150 × 25 mm × 10 µm; mobile phase: [water (0.04% $NH_3H_2O$ + 10 mM $NH_4HCO_3$)-ACN]; B%: 55%-75%, 20 min) to afford the title compound (9.11 mg, 6.57% yield, 98.9% purity) as a white solid: $^1$H NMR (400 MHz, $(CD_3)_2SO$) δ 10.44 (br s, 1H), 9.09 (s, 1H), 8.90 (d, *J* = 8.8 Hz, 1H), 8.81 (s, 1H), 8.38 (d, *J* = 1.8 Hz, 1H), 8.24 - 8.15 (m, 1H), 8.11 - 8.03 (m, 1H), 7.62 (d, *J*

= 8.8 Hz, 1H), 2.73 (s, 6H); MS (ESI) $[M+H]^+$ = 465.0; HRMS (ESI) *m*/*z* calcd for $C_{18}H_{13}F_6N_4O_2S^-$ $[M - H]^-$ 463.0669, found 430.0684 (error 3.34 ppm).

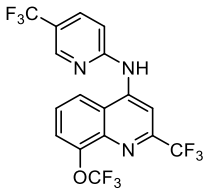



**4-[(3,4-Dichloro)phenylamino]-7-(trifluoromethoxy)-2-(trifluoromethyl) quinoline (42).** The title compound was prepared from 2-(trifluoromethyl)-8-(trifluoromethoxy)-4-bromoquinoline (200 mg, 0.55 mmol) and 3,4-dichloroaniline (108 mg, 0.67 mmol) using General Procedure C. Reaction was stopped after 17 h. Flash column chromatography ($SiO_2$) using a stepwise gradient of TBME/Hex (0:1 to 1:1) afforded the product (204 mg, 83% yield) as a tan powder. $R_f$ = 0.3 (3:10 EtOAc/Hexanes); HPLC purity 90.4%, $t_R$ = 7.74 min, $k'$ = 16.98 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 10.32 (s, 1H), 9.03 (s, 1H), 8.78 (s, 1H), 8.67 (d, *J* = 8.5 Hz, 1H), 8.17 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.96 (d, *J* = 7.6, 1H), 7.85 (t, *J* = 8.2, 1H), 7.61 (d, *J* = 8.8, 1H); HRMS (ESI) *m*/*z* calcd for $C_{17}H_9Cl_2F_6N_2O^+$ $[M + H]^+$ 440.9991, found 440.9974 (error 3.71 ppm).

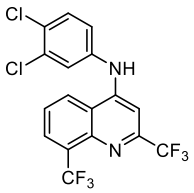



**7-(Trifluoromethoxy)-4-[3-(trifluoromethoxy)phenylamino]-2-(trifluoromethyl)quinoline (43).** The title compound was prepared from 2-(trifluoromethyl)-8-(trifluoromethoxy)-4-bromoquinoline (200 mg, 0.55 mmol) and 3-(trifluoromethoxy)aniline (118 mg, 0.67 mmol) using General Procedure C. Reaction was stopped after 17 h. Flash column chromatography ($SiO_2$) using a stepwise gradient of TBME/Hex (0:1 to 1:1) afforded the product (153 mg, 62% yield) as a tan powder: $R_f$ = 0.28 (3:10 EtOAc/Hexanes); HPLC purity 99.2%, $t_R$ = 7.57 min, $k'$ = 16.60 (method B); $^1$H NMR (500 MHz, $CDCl_3$) δ 7.92 (dd, *J* = 8.5, 0.8 Hz, 1H), 7.73-7.71 (m, 1H), 7.64 (t, *J* = 8.1 Hz, 1H), 7.52 (t, *J* = 8.2 Hz, 1H), 7.35 (s, 1H), 7.28 (dd, *J* = 8.4,

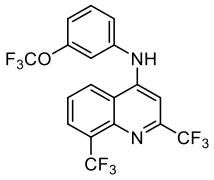
1.8 Hz, 1H), 7.20 (s, 1H), 7.15 (dd, *J* = 8.3, 0.9 Hz, 1H), 6.88 (s, 1H); $^{13}$C NMR (151 MHz, $CD_3OD$) δ 142.3, 141.9 (q, *J* = 1.5 Hz), 140.8 (q, *J* = 34.0 Hz), 137.4, 133.8, 133.2, 122.9, 117.9, 114.4, 113.6, 113.5 (q, *J* = 275 Hz), 113.3, 112.8 (q, *J* = 256 Hz), 112.6, 112.2 (q, *J* = 256 Hz), 109.1, 107.5, 89.5 (q, *J* = 2.4 Hz); HRMS (ESI) *m*/*z* calcd for $C_{18}H_{10}F_9N_2O_2^+$ $[M + H]^+$ 457.0593, found 457.0603 (error 2.22 ppm).




**7-(Trifluoromethoxy)-2-(trifluoromethyl)-4-[5-(trifluoromethyl)pyridin-2-ylamino]quinoline (44).** The title compound was prepared from 2-(trifluoromethyl)-8-(trifluoromethoxy)-4-bromoquinoline (200 mg, 0.55 mmol) and 2-amino-5-trifluoromethylpyridine (108 mg, 0.67 mmol) using General Procedure C. Reaction was stopped after 20 h. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 1:1) afforded the product (42 mg, 17% yield) as a white powder: $R_f$ = 0.12 (3:10 EtOAc/Hexanes); $^1$H NMR (600 MHz, $(CD_3)_2SO$) δ 10.32 (s, 1H), 9.03 (s, 1H), 8.77 (s, 0H), 8.67 (d, *J* = 8.6 Hz, 1H), 8.17 (dd, *J* = 8.9, 2.6 Hz, 1H), 7.95 (d, *J* = 7.6 Hz, 1H), 7.84 (t, *J* = 8.2 Hz, 1H), 7.61 (d, *J* = 8.8 Hz, 1H); $^{13}$C NMR (151 MHz, $(CD_3)_2SO$) δ 166.7, 157.3 (q, *J* = 33.6 Hz), 155.6, 154.5 (q, *J* = 4.4 Hz), 154.0, 150.5, 144.9, 136.5, 133.6 (q, *J* = 271.1 Hz), 132.3, 131.6, 131.5, 131.0 (q, *J* = 275.5 Hz), 129.9 (q, *J* = 257.0 Hz), 128.2 (q, *J* = 32.5 Hz), 123.5, 113.5 (d, *J* = 2.9 Hz); HRMS (ESI) *m*/*z* calcd for $C_{17}H_9F_9N_3O^+$ $[M + H]^+$ 442.0596, found 442.0598 (error 0.46 ppm).

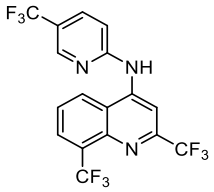


**4-[(3,4-Dichloro)phenylamino]-2,8-bis(trifluoromethyl)quinoline (45).** The title compound was prepared from 4-bromo-2,8-bis(trifluoromethyl)quinoline (200 mg, 0.58 mmol) and 3,4-dichloroaniline (110 mg, 0.70 mmol) using General Procedure C. Reaction was stopped after 20 h. Flash column chromatography afforded the product as a white powder (130 mg, 53% yield) from an EtOAc/Hex (0:1 to 1:1) stepwise gradient: $R_f$ = 0.63 (3:10 EtOAc/Hexanes); HPLC purity 98.2%, $t_R$ = 7.75 min, $k'$ = 17.02 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 9.88 (s, 1H), 8.79 (d, *J* = 8.5 Hz, 1H), 8.35 (d, *J* = 7.2 Hz, 1H), 7.90 (t, *J* = 7.09Hz, 1H), 7.79 (d, *J* = 8.6, Hz, 1H), 7.76 (d, *J* = 2.4, Hz, 1H), 7.54 (dd, *J* = 8.7,2.5, Hz, 1H), 7.32 (s, 1H); $^{13}$C NMR (151 MHz, MeOD) δ 151.6, 150.0 (q, *J* = 34.4 Hz), 146.3, 141.1, 134.4, 132.6, 130.3 (q, *J* = 5.6 Hz), 129.7 (q, *J* = 29.8 Hz), 129.3, 127.3, 126.7, 126.0, 125.3 (q, *J* = 273 Hz), 123.8, 122.9 (q, *J* = 275 Hz), 122.1, 99.2 (q, *J* = 2.6 Hz); HRMS (ESI) *m*/*z* calcd for $C_{17}H_7Cl_2F_6N_2^-$ [M - H]$^-$ 422.9896, found 422.9876 (error 4.73 ppm).

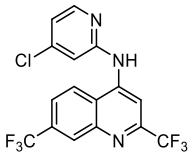


**4-[3-(Trifluoromethoxy)phenylamino]-2,8-bis(trifluoromethyl)quinoline (46).** The title compound was prepared from 4-bromo-2,8-bis(trifluoromethyl)quinoline (200 mg, 0.58 mmol) and 3-(trifluoromethoxy)aniline (124 mg, 0.70 mmol) using General Procedure C. Reaction was stopped after 20 h. Flash column chromatography afforded the product as a yellow powder (140 mg, 55% yield) from an EtOAc/Hex (0:1 to 1:1) stepwise gradient: $R_f$ = 0.23 (1:5 EtOAc/Hexanes); HPLC purity 98.0%, $t_R$ = 7.58 min, $k'$ = 16.63 (method B); $^1$H NMR (500 MHz,

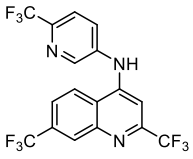
$(CD_3)_2SO$) δ 9.89 (s, 1H), 8.78 (d, *J* = 8.6 Hz, 1H), 8.30 (d, *J* = 6.7 Hz, 1H), 7.85 (d, *J* = 7.0 Hz, 1H), 7.63 (td, *J* = 8.2, 2.1 Hz, 1H), 7.5 (m, 2H), 7.27 (m, 2H); $^{13}$C NMR (151 MHz, $CD_3SD$) δ 151.7, 151.4 (q, *J* = 1.9 Hz), 150.0 (q, *J* = 34.2 Hz), 146.4, 142.8, 132.3, 130.3 (q, *J* = 5.5 Hz), 129.7 (q, *J* = 29.6 Hz), 127.4, 126.7, 125.3 (q, *J* = 272.6 Hz), 122.9 (q, *J* = 274.6 Hz), 122.61, 122.1, 121.9 (q, *J* = 255.9 Hz), 118.5, 116.8, 99.0 (q, *J* = 2.5 Hz); HRMS (ESI) *m*/*z* calcd for $C_{18}H_8F_9N_2O^-$ [M - H]$^-$ 439.0498, found 439.0499 (error 0.11 ppm).




**2,8-bis(Trifluoromethyl)-4-[5-(trifluoromethyl)pyridin-2-ylamino]quinoline (47).** The title compound was prepared from 4-bromo-2,8-bis(trifluoromethyl)quinoline (200 mg, 0.58 mmol) and 2-amino-5-(trifluoromethyl)pyridine (110 mg, 0.70 mmol) us General Procedure C. Reaction was stopped after 21 h. Flash column chromatography afforded the product as a white powder (170 mg, 68% yield) from an EtOAc/Hex (0:1 to 1:1) stepwise gradient: $R_f$ = 0.25 (3:10 EtOAc/Hexanes); HPLC purity 98.3%, $t_R$ = 7.55 min, *k*′ = 16.58 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 10.40 (s, 1H), 9.06 (s, 1H), 8.93 (d, *J* = 8.5 Hz, 1H), 8.81 (s, 1H), 8.34 (d, *J* = 7.2 Hz, 1H), 8.2 (dd, *J* = 8.9, 2.4 Hz, 1H), 7.93 (t, *J* = 7.9 Hz, 1H), 7.62 (d, *J* = 8.8 Hz, 1H); $^{13}$C NMR (151 MHz, $CD_3OD$) δ 158.7, 150.0 (q, *J* = 34.4 Hz), 147.4, 146.2 (q, *J* = 4.5 Hz), 146.0, 136.3 (q, *J* = 3.3 Hz), 130.1 (q, *J* = 5.4 Hz), 129.7 (q, *J* = 29.6 Hz), 125.5 (q, *J* = 270.3 Hz), 127.3, 127.2, 125.2 (q, *J* = 272.7 Hz), 123.0 (q, *J* = 274.8 Hz), 122.7, 121.2 (q, *J* = 33.1 Hz), 114.3, 105.7 (q, *J* = 2.5 Hz); HRMS (ESI) *m*/*z* calcd for $C_{17}H_7F_9N_3^-$ [M - H]$^-$ 424.0502, found 424.0519 (error 4.06 ppm).

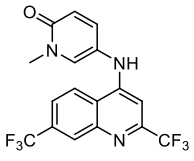


**4-[(4-Chloropyrid-2-yl)amino]-2,7-bis(trifluoromethyl)quinoline (48).** The title compound was prepared from 4-bromo-2,7-bis(trifluoromethyl)quinoline (100 mg, 0.28 mmol) and 3-chloroaniline (44 mg, 0.34 mmol) using General Procedure G. Reaction was stopped after 22 h. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 7:20) afforded the title compound (55 mg, 50% yield) as a light-yellow powder: $R_f$ = 0.65 (3:10 EtOAc/Hexanes); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 10.02 (br. s, 1H), 9.05 (s, 1H), 8.81 (d, $J$ = 8.8 Hz, 1H), 8.39-8.37 (m, 2H), 8.04 (d, $J$ = 8.7 Hz, 1H), 7.55, s, 1H), 7.20-7.19 (m, 1H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 155.2, 148.8, 148.6 (q, $^2J_{C\text{-}F}$ = 33 Hz), 146.6, 146.2, 143.8, 130.8 (q, $^2J_{C\text{-}F}$ = 32 Hz), 127.4 (q, $^3J_{C\text{-}F}$ = 4.5 Hz), 124.4, 123.7 (q, $^1J_{C\text{-}F}$ = 273 Hz), 122.4, 121.9, 121.6 (q, $^1J_{C\text{-}F}$ = 276 Hz), 118.1, 103.4; HRMS ($ESI^+$) $m/z$ calcd for $C_{16}H_9ClF_6N_3^+$ $[M + H]^+$ 392.0384, found 392.0397 (error 3.33 ppm).

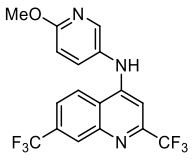


**4-{[6-(trifluoromethyl)pyridin-3-yl]amino}-2,7-bis(trifluoromethyl)quinoline (49).** The title compound was prepared from 4-bromo-2,7-bis(trifluoromethyl)quinoline (100 mg, 0.28 mmol) and 5-amino-2-trifluoromethylpyridine (55 mg, 0.34 mmol) using General Procedure G. Reaction was stopped after 22 h. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 1:5) afforded the title compound (75 mg, 62% yield) as a light-yellow powder: $R_f$ = 0.42 (1:5 EtOAc/Hexanes); HPLC purity 97.5%, $t_R$ = 7.35 min, $k'$ = 16.10 (method B); $^1$H NMR (600 MHz, $CD_3OD$) δ 8.77 (d, $J$ = 2.6 Hz, 1H), 8.57 (d, $J$ = 8.9 Hz, 1H), 8.39 (s, 1H), 8.07 (dd, $J$ = 8.5, 2.6 Hz, 1H), 7.92 (dd, $J$ = 8.9, 1.9 Hz, 1H), 7.90 (d, $J$ = 8.5 Hz, 1H), 7.51 (s, 1H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 151.1 (q, $^2J_{C\text{-}}$

$_F$ = 34 Hz), 151.0, 148.9, 144.8, 144.1 (q, $^2J_{C\text{-}F}$ = 35 Hz), 141.2, 133.9 (q, $^2J_{C\text{-}F}$ = 33 Hz), 130.6, 128.4 (q, $^3J_{C\text{-}F}$ = 4.4 Hz), 125.2 (q, $^1J_{C\text{-}F}$ = 272 Hz), 125.0, 123.89 (q, $^3J_{C\text{-}F}$ = 2.9 Hz), 123.86, 123.1 (q, $^1J_{C\text{-}F}$ = 272Hz), 122.8 (q, $^3J_{C\text{-}F}$ = 2.8 Hz), 122.8 (q, $^1J_{C\text{-}F}$ = 275 Hz), 101.1 (q, $^3J_{C\text{-}F}$ = 2.5 Hz); HRMS (ESI$^+$) *m*/*z* calcd for $C_{17}H_9F_9N_3^+$ [M + H]$^+$ 426.0647, found 426.0647 (error 1.78 ppm).

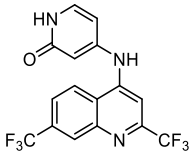



**4-[(1-Methyl-pyridin-5-yl-2-one)amino]-2,7-bis(trifluoromethyl)quinoline (50).** The title compound was prepared from 4-bromo-2,7-bis(trifluoromethyl)quinoline (150 mg, 0.43 mmol) and 5-amino-1-methyl-pyridin-2-one oxalate (65 mg, 0.51 mmol) using General Procedure C. Reaction was stopped after 20 h. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 2:5) afforded the product as a yellow powder (43 mg, 26% yield): $R_f$ = 0.11 (1:5 EtOAc/Hexanes); HPLC purity 99.8%, $t_R$ = 6.57 min, $k'$ = 14.27 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 9.51 (s, 1H), 8.70 (d, *J* = 8.9 Hz, 1H), 8.23 (s, 1H), 7.96 (d, *J* = 8.5 Hz, 1H), 7.93 (d, *J* = 2.5 Hz, 1H), 7.53 (dd, *J* = 9.5, 2.7 Hz, 1H), 6.76 (s, 1H), 6.54 (d, *J* = 9.5 Hz, 1H), 3.48 (s, 3H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 161.0, 152.6, 148.7 (q, $^2J_{C\text{-}F}$ = 33 Hz), 146.7, 140.3, 137.9, 131.0 (q, $^2J_{C\text{-}F}$ = 33 Hz), 127.0, 124.3, 123.7 (q, $^1J_{C\text{-}F}$ = 273 Hz), 121.8, 121.5 (q, $^1J_{C\text{-}F}$ = 276 Hz), 120.9, 120.1, 117.3, 96.8, 37.0; HRMS (ESI+) *m*/*z* calcd for $C_{17}H_{12}F_6N_3O^+$ [M + H]$^+$ 388.0879, found 388.0885 (error 1.55 ppm).

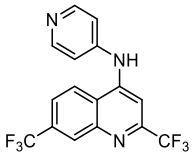



**4-[2-(Methoxy)pyrid-5-ylamino]-2,7-bis(trifluoromethyl)quinoline (51).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinoline-4-one (135 mg, 0.48 mmol) and 5-amino-2-methoxypyridine (132 mg, 1.0 mmol) using General Procedure D. Purification by HPLC (method A) afforded the product (68 mg, 36 % yield) as a yellow powder: HPLC purity

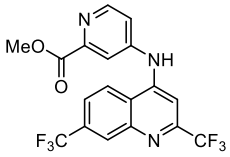
99.1%, $t_R$ = 7.26 min, $k'$ = 15.9 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 9.73 (br. s, 1H), 8.73 (d, $J$ = 8.8 Hz, 1H), 8.33 (s, 1H), 8.26 (d, $J$ = 1.8 Hz, 1H), 7.98 (d, $J$ = 8.7 Hz, 1H), 7.83 (dd, $J$ = 8.6, 2.1 Hz, 1H), 6.99 (d, $J$ = 8.7 Hz, 1H), 6.80 (s, 1H), 3.91 (s, 3H); $^{13}$C NMR (126 MHz, $(CD_3)SO$) δ 161.7, 151.9, 148.7 (q, $^2J_{C\text{-}F}$ = 33 Hz), 146.9, 143.8, 137.0, 131.0 (q, $^2J_{C\text{-}F}$ = 33 Hz), 129.2, 127.0 (d, $^3J_{C\text{-}F}$ = 4.5 Hz), 124.4, 123.8 (q, $^1J_{C\text{-}F}$ = 273 Hz), 121.8 (d, $^3J_{C\text{-}F}$ = 2.7 Hz), 121.5 (q, $^1J_{C\text{-}F}$ = 276 Hz), 121.2, 111.5, 96.5, 53.5; HRMS (ESI$^+$) $m/z$ calcd for $C_{17}H_{12}F_6N_3O^+$ $[M + H]^+$ 388.0879, found 388.0883 (error 1.04 ppm).



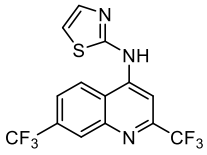

**4-[(1*H*-pyridin-4-yl-2-one)amino]-2,7-bis(trifluoromethyl)quinoline (52).** The title compound was prepared from 4-bromo-2,7-bis(trifluoromethyl)quinoline (150 mg, 0.42 mmol) and 4-amino-1*H*-pyridin-2-one (57 mg, 0.51 mmol) using General Procedure G. Reaction was stopped after 14 h. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 1:5) afforded the title compound (86 mg, 55% yield) as a light-yellow powder: $R_f$ = 0.22 (1:5 EtOAc/Hexanes); HPLC purity 90.4%, $t_R$ = 6.25 min, $k'$ = 13.53 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 8.68 (s, 1H), 8.25 (s, 1H), 8.08 (d, $J$ = 8.9 Hz, 1H), 7.90 (d, $J$ = 8.8 Hz, 1H), 7.46 (d, $J$ = 7.4 Hz, 1H), 6.56 (br. s, 2H), 5.97 (dd, $J$ = 7.4, 1.4 Hz, 1H), 5.39 (s, 1H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 161.7, 158.35, 149.6, 148.8 (q, $^2J_{C\text{-}F}$ = 35 Hz), 146.5, 137.8, 131.5 (q, $^2J_{C\text{-}F}$ = 33 Hz), 128.2, 127.5, 126.3, 124.9, 123.5 (q, $^1J_{C\text{-}F}$ = 273 Hz), 121.0 (q, $^1J_{C\text{-}F}$ = 275 Hz), 118.8, 100.4, 91.6; HRMS (ESI$^+$) $m/z$ calcd for $C_{16}H_{10}F_6N_3O^+$ $[M + H]^+$ 374.0723, found 374.0726 (error 1.01 ppm).



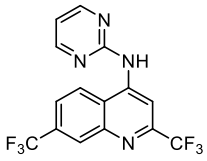

**4-[(pyridin-4-yl)amino]-2,7-bis(trifluoromethyl)quinoline (53).** The title compound was prepared from 4-bromo-2,7-bis(trifluoromethyl)quinoline (100 mg, 0.28 mmol) and 4-aminopyridine (33 mg, 0.34 mmol) using General Procedure G. Reaction was stopped after 24 h. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 1:5) afforded the title compound (34 mg, 34% yield) as a light-yellow powder:




**Methyl 4-{[2,7-bis(trifluoromethyl)quinolin-4-yl]amino}picolinate (54).** The title compound was prepared from 4-bromo-2,7-bis(trifluoromethyl)quinoline (70 mg, 0.20 mmol) and 4-amino-pyridine-2-carboxylic acid methyl ester (37 mg, 0.24 mmol) using General Procedure C. Reaction was stopped after 16 h. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (3:10 to 1:0) afforded the title compound (25 mg, 30% yield) as yellow powder: $R_f$ = 0.10 (1:1 EtOAc/Hexanes); HPLC purity 98.7%, $t_R$ = 7.10 min, $k'$ = 15.51 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 10.15 (s, 1H), 8.65 (d, $J$ = 8.9 Hz, 1H), 8.60 (d, $J$ = 5.4 Hz, 1H), 8.43 (s, 1H), 8.05 (d, $J$ = 8.9 Hz, 1H), 7.75 (s, 1H), 7.63 (dd, $J$ = 5.3, 1.8 Hz, 1H), 3.89 (s, 3H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 165.2, 151.0, 148.8, 148.7 (obs. q, $^2J_{C\text{-}F}$ = 34 Hz), 148.6, 147.7, 147.1, 131.3 (q, $^2J_{C\text{-}F}$ = 33 Hz), 127.3, 124.9, 123.7 (q, $^1J_{C\text{-}F}$ = 273 Hz), 123.1, 122.8, 121.3 (q, $^1J_{C\text{-}F}$ = 275 Hz), 116.0, 115.4, 102.4, 52.5; HRMS (ESI+) $m/z$ calcd for $C_{18}H_{12}F_6N_3O_2^+$ [M + H]+ 416.0828, found 416.0824 (error 1.08 ppm).

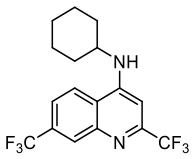


**4-(thiazol-2-ylamino)-2,7-bis(trifluoromethyl)quinoline (55).** The title compound was prepared from 4-bromo-2,7-bis(trifluoromethyl)quinoline (150 mg, 0.43 mmol) and 2-aminothiazole (52 mg, 0.51 mmol) using General Procedure C. Reaction was stopped after 16 h. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 1:5) afforded the title compound (93 mg, 60% yield) as a yellow powder: $R_f$ = 0.21 (1:5 EtOAc/Hexanes); $^1$H NMR (400 MHz, $CD_3OD$) δ 9.07 (s, 1H), 8.61 (d, $J$ = 8.9 Hz, 1H), 8.37 (s, 1H), 7.89 (dd, $J$ = 9.0, 1.9 Hz, 1H), 7.52 (d, $J$ = 3.7 Hz, 1H), 7.15 (d, $J$ = 3.7 Hz, 1H); $^{13}$C NMR (151 MHz, MeOD) δ 163.7, 151.4 (q, $^2J_{C\text{-}F}$ = 34.0 Hz), 148.4, 140.2, 133.3 (q, $^2J_{C\text{-}F}$ = 32.9 Hz), 128.4 (q, $^3J_{C\text{-}F}$ = 4.5 Hz), 125.2 (q, $^1J_{C\text{-}F}$ = 272 Hz), 124.4, 124.3, 123.9 (q, $^3J_{C\text{-}F}$ = 3.4 Hz), 123.0 (q, $^1J_{C\text{-}F}$ = 275 Hz), 122.7, 113.7, 104.2 (q, $^3J_{C\text{-}F}$ = 2.6 Hz);

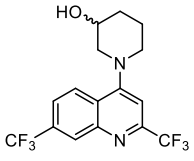


**4-[(Pyrimidn-2-yl)amino]-2,7-bis(trifluoromethyl)quinoline (56).** The title compound was prepared from 4-bromo-2,7-bis(trifluoromethyl)quinoline (150 mg, 0.43 mmol) and 2-aminopyrimidine (49 mg, 0.51 mmol) using General Procedure C. Reaction was stopped after 20 h. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 2:5) afforded the product as a yellow powder (117 mg, 76% yield): $R_f$ = 0.11 (1:5 EtOAc/Hexanes); HPLC purity 99.8%, $t_R$ = 7.18 min, $k'$ = 15.70 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 10.71 (s, 1H), 9.00 (s, 1H), 8.96 (d, $J$ = 9.1 Hz, 1H), 8.77 (d, $J$ = 4.7 Hz, 2H), 8.45 (s, 1H), 7.99 (d, $J$ = 8.9 Hz, 1H), 7.20 (t, $J$ = 4.8 Hz, 1H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 159.3, 158.5, 148.5 (q, $^2J_{C\text{-}F}$ = 33 Hz), 146.7, 146.2, 130.8 (q, $^2J_{C\text{-}F}$ = 33 Hz), 127.2, 125.3, 123.7 (q, $^1J_{C\text{-}F}$ = 273 Hz), 122.5,

121.6 (q, $^{1}J_{C\text{-}F}$ = 276 Hz), 115.7, 105.1; HRMS (ESI+) *m*/*z* calcd for $C_{15}H_9F_6N_4^+$ [M + H]$^+$ 359.0726, found 359.0722 (error 0.97 ppm).

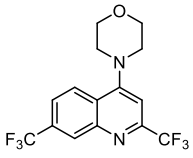



**4-(Cyclohexylamino)-2,7-bis(trifluoromethyl)quinoline (57).** The title compound was prepared from 4-bromo-2,7-bis(trifluoromethyl)quinoline (200 mg, 0.56 mmol) and cyclohexylamine (68 mg, 0.68 mmol) using General Procedure C. Reaction was stopped after 20 h. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 1:5) afforded the title compound (100 mg, 47% yield) as a yellow powder: $R_f$ = 0.56 (1:5 EtOAc/Hexanes); HPLC purity 99.3%, $t_R$ = 7.59 min, $k'$ = 16.66 (method B); $^{1}$H NMR (500 MHz, $(CD_3)_2SO$) δ 8.68 (d, *J* = 8.9 Hz, 1H), 8.19 (s, 1H), 7.82 (dd, *J* = 8.8, 1.1 Hz, 1H), 7.65 (d, *J* = 7.8 Hz, 1H), 6.90 (s, 1H), 3.68 (m, 1H), 1.98 (d, *J* = 7.3 Hz, 2H), 1.77 (m, 2H), 1.66 (d, *J* = 12.3 Hz, 1H), 1.43 (m, 4H), 1.19 (m, 1H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 151.1, 148.9 (q, $^{2}J_{C\text{-}F}$ = 32.7 Hz), 146.7, 130.5 (q, $^{2}J_{C\text{-}F}$ = 32.7 Hz), 126.7 (d, $^{3}J_{C\text{-}F}$ = 4.5 Hz), 124.5, 123.9 (q, $^{1}J_{C\text{-}F}$ = 273 Hz), 121.8 (q, $^{1}J_{C\text{-}F}$ = 276 Hz), 120.9, 120.6, 94.7, 51.2, 31.6, 25.3, 24.6; HRMS (ESI+) *m*/*z* calcd for C17H17F6N2$^+$ [M + H]$^+$ 363.1290, found 363.1283 (error 1.99 ppm).

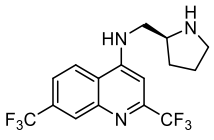



**4-(3-hydroxypiperidin-1-yl)-2,7-bis(trifluoromethyl)quinoline (58).** The title compound was prepared from 4-bromo-2,7-bis(trifluoromethyl)quinoline (150 mg, 0.43 mmol) and 3-hydroxypiperidine (53 mg, 0.52 mmol) using General Procedure C. Reaction was stopped after 20 h. Flash column chromatography ($SiO_2$) using a stepwise gradient of MeOH/$CH_2Cl_2$ (0:1 to 1:10) afforded the title compound (16 mg, 10% yield) as a yellow powder: (1:5 EtOAc/Hexanes); HPLC purity 99.1%, $t_R$ = 7.00 min, $k'$ = 15.26 (method B); $^{1}$H NMR (500 MHz, $(CD_3)_2SO$) δ 8.39

(s, 1H), 8.34 (d, *J* = 8.8 Hz, 1H), 7.91 (d, *J* = 9.0 Hz, 1H), 7.35 (s, 1H), 5.01 (s, 1H), 3.87 (s, 1H), 3.47 (d, *J* = 12.1 Hz, 1H), 3.17 (t, *J* = 10.8 Hz, 1H), 3.06 (dd, *J* = 12.1, 7.9 Hz, 1H), 1.95 (d, *J* = 10.4 Hz, 3H), 1.77 – 1.68 (m, 1H), 1.50 (q, *J* = 9.2 Hz, 1H); HRMS ($ESI^+$) *m*/*z* calcd for $C_{16}H_{15}F_6N_2O^+$ $[M + H]^+$ 365.1083, found 365.1099 (error 4.46 ppm).



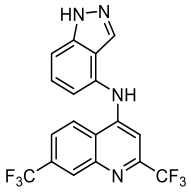
**4-(2,7-bis(Trifluoromethyl)quinolin-4-yl)morpholine (59).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinoline-4-one (140 mg, 0.48 mmol) and morpholine (90 uL, 1.0 mmol) using General Procedure D. The reaction was then diluted in MeCN and purified by HPLC affording the product as a white powder (46 mg, 27% yield): HPLC purity 98.8%, $t_R$ = 7.03 min, $k'$ = 15.33 (method B); $^1H$ NMR (500 MHz, $(CD_3)_2SO$) δ 8.43 (s, 1H), 8.34 (d, *J* = 8.7, 1H), 7.91 (d, *J* = 8.7, 1H), 7.41 (s, 1H), 3.89 (s, 4H), 3.37 (s, 4H); $^{13}C$ NMR (126 MHz, $(CD_3)SO$) δ 159.0, 149.2 (q, $^2J_{C\text{-}F}$ = 33.6 Hz), 147.1, 130.7 (q, $^2J_{C\text{-}F}$ = 32.7 Hz), 127.6 (d, $^3J_{C\text{-}F}$ = 3.6 Hz), 126.6, 124.8, 123.7 (q, $^1J_{C\text{-}F}$ = 273 Hz), 122.3 (d, $^3J_{C\text{-}F}$ = 2.7 Hz), 121.4 (q, $^1J_{C\text{-}F}$ = 276 Hz), 105.7, 65.9, 52.0; HRMS (ESI) *m*/*z* calcd for $C_{15}H_{13}F_6N_2O^+$ $[M + H]^+$ 351.0927, found 351.0923 (error 1.13 ppm).



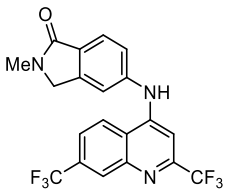
**(S)-2-{[(2,7-bis(trifluoromethyl)quinolin-4-yl]amino}methyl pyrrolidine (60).** The title compound was prepared from 2,7-bis(trifluoromethyl)-4-bromoquinoline (150 mg, 0.42 mmol) and *tert*-butyl (S)-2-aminomethylpyrrolidine-1-carboxylate (104 mg, 0.51 mmol) using General Procedure G. Crude Boc protected product was isolated in 77% yield as a white foam (150 mg, 0.32 mmol); $R_f$ = 0.74-8

5 (3:7 EtOAc/Hexanes).

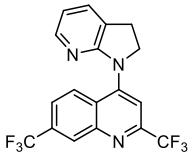
The boc protected product was then treated with 4M HCl in methanol for 4 h while stirring. The reaction mixture was then quenched with the addition of $NaHCO_3$ sat. (5 mL) and the organics were concentrated under vacuum. The aqueous layer was then extracted with EtAOc (3x 10 mL), dried over $MgSO_4$, and concentrated under vacuum to afford the title compound (85 mg, 82 % yield) as a white powder: HPLC purity 91.1%, $t_R$ = 6.26 min, $k'$ = 13.57 (method B); $^1$H NMR (400 MHz, $CDCl_3$) δ 10.60 (s, 1H), 10.26 (s, 1H), 8.82 (d, *J* = 8.81 Hz, 1H), 8.34 (s, 1H), 8.10 (s, 1H), 7.70 (d, *J* = 8.43 Hz, 1H), 6.71 (s, 1H), 5.44 – 5.12 (m, 2H), 4.08 (s, 1H), 3.96 (s, 1H), 3.74 (d, *J* = 14.36 Hz, 1H), 2.99 (t, *J* = 6.28 Hz, 1H), 2.37 – 1.75 (m, 3H), HRMS (ESI$^+$) *m*/*z* calcd for $C_{16}H_{16}F_6N_3^+$ [M + H]$^+$ 364.1243, found 364.1251 (error 2.33 ppm).




**4-(1*H*-indazol-4-yl)amino-2,7-bis(trifluoromethyl)quinoline (61).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinoline-4-one (204 mg, 0.72 mmol) and 4-amino-1*H*-indazole (149 mg, 1.09 mmol) using General Procedure D. Purification by HPLC (method A) afforded the product (33 mg, 12% yield) as a tan powder: HPLC purity 98.6%, $t_R$ = 7.01 min, $k'$ = 15.30 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 13.32 (br s, 1H), 10.06 (s, 1H), 8.88 (d, *J* = 8.8 Hz, 1H), 8.36 (s, 1H), 8.00 (d, *J* = 8.8 Hz, 1H), 7.94 (s, 1H), 7.52 (d, *J* = 8.3 Hz, 1H), 7.46 (t, *J* = 7.8 Hz, 1H), 7.18 (d, *J* = 7.1 Hz, 1H), 6.90 (s, 1H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 150.8, 148.5 (q, $^2J_{C\text{-}F}$ = 34 Hz), 147.0, 141.5, 132.0, 131.2, 131.0 (q, $^2J_{C\text{-}F}$ = 32 Hz), 127.1, 126.9, 124.9, 123.8 (q, $^1J_{C\text{-}F}$ = 273 Hz), 121.8, 121.6, 121.5 (q, $^1J_{C\text{-}F}$ = 275 Hz), 118.3, 115.3, 108.3, 98.2; HRMS (ESI+) *m*/*z* calcd for $C_{18}H_{10}F_6N_4^+$ [M + H]$^+$ 397.0882, found 397.0891 (error 2.27 ppm).

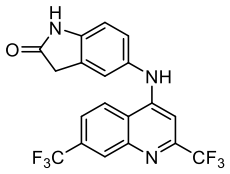


**5-{[2,7-bis(Trifluoromethyl)quinolin-4-yl]methylamino}1-methylisoindolin -2-one (62).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinoline-4-one (204 mg, 0.72 mmol) and 5-amino-2,3-dihydro-2-methyl-1*H*-isoindolo-1-one (182 mg, 1.09 mmol) using General Procedure D. Purification by HPLC (method A) afforded the product (58 mg, 19% yield) as a tan powder: HPLC purity 91.9%, $t_R$ = 6.95 min, $k'$ = 15.16 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 9.96 (s, 1H), 8.75 (d, $J$ = 8.9 Hz, 1H), 8.38 (s, 1H), 8.02 (dd, $J$ = 8.8, 1.9 Hz, 1H), 7.76 (d, $J$ = 8.1 Hz, 1H), 7.65 (s, 1H), 7.52 (dd, $J$ = 8.1, 1.9 Hz, 1H), 7.29 (s, 1H), 4.50 (s, 2H), 3.08 (s, 3H); $^{13}$C NMR (151 MHz, DMSO) δ 166.8, 150.2, 148.8 (q, $^2J_{C\text{-}F}$ = 33 Hz), 147.1, 143.7, 142.0, 131.20 (q, $^2J_{C\text{-}F}$ = 33 Hz), 129.3, 127.2 (d, $J$ = 3.9 Hz), 124.7, 124.1, 123.8 (q, $^1J_{C\text{-}F}$ = 273 Hz), 122.4, 122.1 (d, $J$ = 2.7 Hz), 121.9, 121.5 (q, $^1J_{C\text{-}F}$ = 275 Hz), 117.4, 98.3, 51.4, 29.0; HRMS (ESI+) $m/z$ calcd for $C_{20}H_{14}F_6N_3O^+$ $[M + H]^+$ 426.1036, found 426.1038 (error 0.58 ppm).

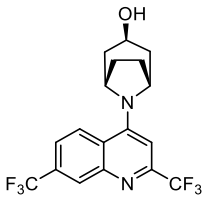


**4-(2,3-Dihydro-1*H*-pyrrolo[2,3-b]pyridin-1-yl)-2,7-bis(trifluoromethyl) quinoline (63).** The title compound was prepared from 4-bromo-2,7-bis(trifluoromethyl)quinoline (150 mg, 0.43 mmol) and 2,3-dihydro-1*H*-pyrrolo[2,3b]pyridine (62 mg, 0.51 mmol) using General Procedure C. Reaction was stopped after 19.5 h. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 3:5) afforded the product as a yellow powder (69 mg, 42% yield): $R_f$ = 0.20 (3:10 EtOAc/Hexanes); HPLC purity 98.6%, $t_R$ = 7.10 min, $k'$ = 15.52 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 8.49 (s, 1H), 8.24 (d, $J$ = 8.9 Hz, 1H), 7.97 (s, 1H), 7.87-

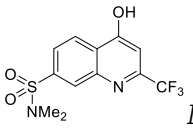
7.84 (m, 2H), 1.01 (d, $J$ = 7.2 Hz, 1H), 6.88 (dd, $J$ = 7.1, 5.2 Hz, 1H), 4.34 (t, $J$ = 8 Hz, 1H), 3.26 (t, $J$ = 7.9 Hz, 1H); $^{13}$C NMR (126 MHz, $(CD_3)SO$) δ 150.2, 148.5 (q, $^2J_{C\text{-}F}$ = 34 Hz), 147.1, 145.0, 133.1, 130.9 (q, $^2J_{C\text{-}F}$ = 33 Hz), 128.6, 127.1 (d, $^3J_{C\text{-}F}$ = 4.5 Hz), 125.7, 125.4, 123.7 (q, $^1J_{C\text{-}F}$ = 273 Hz), 121.5, 121.4 (q, $^1J_{C\text{-}F}$ = 276 Hz), 117.0, 108.8, 52.0, 26.0; HRMS (ESI$^+$) $m/z$ calcd for $C_{18}H_{12}F_6N_3^+$ $[M + H]^+$ 384.0930, found 384.0927 (error 0.78 ppm).




**5-{[2,7-bis(Trifluoromethyl)quinolin-4-yl]amino}indolin-2-one (64).** The title compound was prepared from 4-bromo-2,8-bis(trifluoromethyl)quinoline (150 mg, 0.43 mmol) and 5-amino-1,3-dihydro-indol-2-one (77 mg, 0.51 mmol) using General Procedure C. Reaction was stopped after 20 h. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 3:5) afforded the product as a yellow powder (48 mg, 27% yield) using General Procedure F. Reaction was stopped after 20 h. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 3:5) afforded the product as a yellow powder (48 mg, 27% yield): HPLC purity 90.4%, $t_R$ = 6.83 min, $k'$ = 14.87 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 10.52 (s, 1H), 9.71 (s, 1H), 8.73 (d, $J$ = 8.8 Hz, 1H), 8.29 (s, 1H), 7.93 (d, $J$ = 8.7 Hz, 1H), 7.26 (s, 1H), 7.23 (d, $J$ = 8.2 Hz, 1H), 6.95 (d, $J$ = 8.1 Hz, 1H), 6.86 (s, 1H), 3.56 (s, 2H); $^{13}$C NMR (126 MHz, $(CD_3)SO$) δ 176.4, 151.9, 148.6 (q, $^2J_{C\text{-}F}$ = 33 Hz), 147.0, 142.1, 131.9, 130.9 (q, $^2J_{C\text{-}F}$ = 33 Hz), 127.5, 127.0 (d, $^3J_{C\text{-}F}$ = 4.5 Hz), 124.7, 124.6, 123.8 (q, $^1J_{C\text{-}F}$ = 273 Hz), 122.0, 121.54 (q, $^1J_{C\text{-}F}$ = 276 Hz), 121.52 (d, $^3J_{C\text{-}F}$ = 2.7 Hz), 121.1, 109.9, 96.3; HRMS (ESI$^+$) $m/z$ calcd for $C_{19}H_{12}F_6N_3O^+$ $[M + H]^+$ 412.0879, found 412.0896 (error 4.15 ppm).

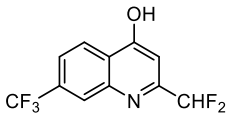


**(1*R*,3*R*,5*S*)-8-(2,7-bis(Trifluoromethyl)quinolin-4-yl)-8-azabicyclo[3.2.1]octan-3-ol (65).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinoline-4-one (65 mg, 0.23 mmol) and (1*R*,3*R*,5*S*)-8-azabicyclo[3.2.1]octan-3-ol (36 mg, 0.27 mmol) using General Procedure C. Purification by HPLC (method A) afforded the product (23 mg, 26 % yield) as a yellow powder: HPLC purity 99.4%, $t_R$ = 7.19 min, $k'$ = 15.72 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 8.31-8.28 (m, 2H), 7.84 (d, $J$ = 8.7 Hz, 1H), 7.25 (s, 1H), 4.44 (s, 2H), 4.04 (s, 1H), 2.29-2.22 (m, 4H), 1.89-1.86 (m, 4H); $^{13}$C NMR (126 MHz, $(CD_3)SO$) δ 155.6, 148.5 (q, $J$ = 34.6 Hz), 147.6, 130.4 (q, $J$ = 32.2 Hz), 127.1 (q, $J$ = 3.8 Hz), 124.2, 123.8 (q, $J$ = 269 Hz), 121.6 (q, $J$ = 271 Hz), 121.3, 102.3, 62.6, 58.9, 38.8, 26.6; HRMS (ESI+) $m/z$ calcd for $C_{18}H_{17}F_6N_2O^+$ [M + H]$^+$ 391.1240, found 391.1257 (error 4.22 ppm).

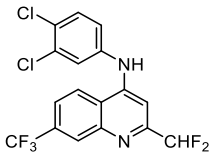


***N*,*N*-dimethyl-2-(trifluoromethyl)quinoline-4(1*H*)-one-7-sulfonamide (66).** To a solution of 3-amino-*N*,*N*-dimethyl-benzenesulfonamide (1 g, 4.99 mmol) in $Ph_2O$ (3 mL) were added ethyl 4,4,4-trifluoro-3-oxo-butanoate (1.09 mL, 7.49 mmol) and HCl (12 M, 208 µL). The mixture was stirred at 265 °C for 2 h. The reaction mixture was quenched by addition NaOH (10%, 50 mL) at 0 °C, then diluted with TBME 20 mL and extracted with TMBE (20 mL × 2). The obtained *aq*. layer was acidified by *conc*. HCl (10 mL), then extracted with EtOAc (20 mL × 3), the combined organic layers were washed with brine (20 mL), dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to give a residue. The residue was purified by *Prep*.-HPLC: (column: Phenomenex Luna C18 200 × 40 mm × 10 um; mobile phase: [water (0.1% TFA)-ACN];

B%: 30%-50%, 10 min) to afford the title compound (400 mg, 25.0% yield) as a white solid: $^1$H NMR (400 MHz, $(CD_3)_2SO$) δ 8.54 (d, *J* = 8.8 Hz, 1H), 8.42 (s, 1H), 7.97 (d, *J* = 8.8 Hz, 1H), 7.22 (br s, 1H), 2.81 (s, 6H).

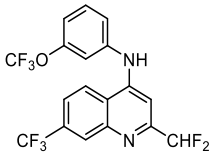



**2-difluoromethyl-7-trifluoromethylquinolin-4-ol (67).** The title compound was prepared from ethyl 4,4-difluoroacetoacetate (1.18 mL, 8.58 mmol) added 3-trifluoromethylaniline (0.97 mL, 7.80 mmol) using General Procedure A. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 1:5) afforded the title compound (0.501 g, 22% yield) as a pale-yellow solid: $R_f$ = 0.20 (1:5 EtOAc/Hexanes); $t_R$ = 7.46 min, $k'$ = 16.36 (method B); $^1$H NMR (400 MHz, $CD_3OD$) δ 8.43 (d, *J* = 8.6 Hz ,1H), 8.09 (s, 1H), 7.69 (dd, *J* = 8.6, 1.7 Hz, 1H), 6.91 (t, *J* = 54.0 Hz, 1H), 6.66 (s, 1H).; HRMS (ESI) *m*/*z* calcd for $C_{11}H_7F_5NO^+$ $[M + H]^+$ 264.0442, found 264.0441 (error 0.35 ppm).

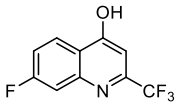



**4-[3,4-(dichlorophenyl)amino]-2-difluoromethyl-7-(trifluoromethyl) quinoline (68).** The title compound was prepared from 2-difluormethyl-7-trifluoromethylquinolin-4-ol (**67,** 184 mg, 0.70 mmol) and 3,4-dichloroaniline (0.127 mL, 1.05 mmol) using General Procedure B. Purification by HPLC (method A) afforded the product (38 mg, 13% yield) as a light-brown powder: HPLC purity 98.6%, $t_R$ = 7.69 min, $k'$ = 16.88 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 9.69 (s, 1H), 8.65 (d, *J* = 8.9 Hz, 1H), 8.32 (s, 1H), 7.97 (d, *J* = 8.6 Hz, 1H), 7.73 (d, *J* = 8.6 Hz, 1H), 7.67 (d, *J* = 1.8 Hz, 1H), 7.45 (dd, *J* = 8.6, 1.7 Hz, 1H), 7.24 (s, 1H), 6.97 (t, *J* = 55 Hz, 1H); $^{13}$C NMR (151 MHz, $CD_3OD$) δ 154.8 (t, $^2J_{C\text{-}F}$ = 25.0 Hz), 152.8, 147.1, 140.4,

134.5, 134.0 (q, $^{2}J_{C\text{-}F}$ = 33.0 Hz), 132.7, 130.1, 126.5, 126.1 (q, $^{3}J_{C\text{-}F}$ = 4.3 Hz), 125.09 (q, $^{1}J_{C\text{-}F}$ = 272 Hz), 125.07, 124.4, 123.2 (q, $^{3}J_{C\text{-}F}$ = 3.1 Hz), 122.8, 114.6 (t, $^{1}J_{C\text{-}F}$ = 241 Hz) , 100.1 (t, $^{3}J_{C\text{-}F}$ = 3.7 Hz); HRMS (ESI) *m*/*z* calcd for $C_{17}H_{10}Cl_2F_5N_2^+$ $[M + H]^+$ 407.0136, found 407.0136 (error 0.01 ppm).

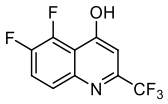



**4-[3-(trifluoromethoxy)phenylamino]-2-difluoromethyl-7-(trifluoromethyl) quinoline (69).** The title compound was prepared from 2-difluormethyl-7-trifluoromethylquinolin-4-ol (**67**, 184 mg, 0.70 mmol) and 3-trifluormethoxyaniline (0.140 mL, 1.05 mmol) using General Procedure B. Purification by HPLC (method A) afforded the product (53 mg, 18% yield) as a light-brown powder: HPLC purity 98.1%, $t_R$ = 7.53 min, $k'$ = 16.50 (method B); $^{1}$H NMR (500 MHz, $(CD_3)_2SO$) δ 9.80 (s, 1H), 8.68 (d, *J* = 8.9 Hz, 1H), 8.30 (s, 1H), 7.96 (dd, *J* = 8.8, 1.3 Hz, 1H), 7.61 (t, *J* = 8.2 Hz, 1H), 7.47 (d, *J* = 8.1 Hz, 1H), 7.41 (s, 1H), 7.26 (s, 1H), 7.21 (d, *J* = 8.2 Hz, 1H), 6.96 (t, *J* = 55 Hz, 1H); $^{13}$C NMR (151 MHz, $CD_3OD$) δ 154.1 (t, $^{2}J_{C\text{-}F}$ = 25.4 Hz), 153.6, 151.5 (q, $^{3}J_{C\text{-}F}$ = 2.1 Hz), 146.2, 141.8, 134.4 (q, $^{2}J_{C\text{-}F}$ = 33.7 Hz), 132.6, 125.3, 125.2 (q, $^{3}J_{C\text{-}F}$ = 3.2 Hz), 125.1 (q, $^{1}J_{C\text{-}F}$ = 271 Hz), 123.5, 123.4 (q, $^{3}J_{C\text{-}F}$ = 3.2 Hz), 122.6, 121.9 (q, $^{1}J_{C\text{-}F}$ = 256 Hz), 119.6, 117.7, 114.3 (t, $^{1}J_{C\text{-}F}$ = 241 Hz), 100.0; HRMS (ESI) *m*/*z* calcd for $C_{18}H_{11}F_8N_2O^+$ $[M + H]^+$ 423.0738, found 423.07525 (error 3.40 ppm).

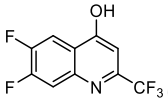



**7-fluoro-2-(trifluoromethyl)quinolin-4-ol (70).** To a mixture of ethyl 4,4,4-trifluoro-3-oxo-butanoate (2.63 mL, 18.0 mmol) and polyphosphoric acid (8.52 g, 25.20 mmol) was added 3-fluoroaniline (1.72 mL, 18.0 mmol) dropwise at 100 °C. The temperature was then increased to 150 °C for 2 h. The reaction was cooled to ambient temperature, diluted with aqueous

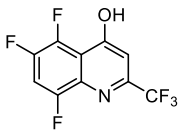
sodium hydroxide (5%, 50 mL). The precipitate formed was dissolved in aqueous sodium hydroxide (10%, 30 mL). After some insoluble material had been removed by filtration, the clear solution was acidified with *conc*. HCl. The solid obtained was collected. The mixture was purified by *Prep*.-HPLC: (column: Phenomenex Luna C18 200 × 40 mm × 10 um; mobile phase: [water (0.1% TFA)-ACN]; B%: 20%-40%, 10 min) to afford the title compound (300 mg, 7.21% yield) as a yellow solid: $^1$H NMR (400 MHz, $(CD_3)_2SO$) δ 8.32 - 8.22 (m, 1H), 7.74 (br d, *J* = 9.3 Hz, 1H), 7.54 (br s, 1H), 7.04 (br s, 1H).



**5,6-difluoro-2-(trifluoromethyl)quinolin-4-ol/6,7-difluoro-2-(trifluoromethyl) quinoline-4-ol (71/72).** To a mixture of 3,4-difluoroaniline (3 g, 23 mmol) and ethyl 4,4,4-trifluoro-3-oxo-butanoate (5.09 mL, 34.8 mmol) was added PPA (13.7 g, 34.9 mmol). The mixture was stirred at 150 °C for 4 h. The reaction was diluted with *aq*. NaOH (5%, 50 mL) after letting the reaction cool. The precipitate formed was dissolved in *aq*. NaOH (10%, 80 mL). After some insoluble material had been removed by filtration, the clear solution was acidified with *conc*. HCl (10 mL). The residue was purified by *Prep*.-HPLC (Phenomenex luna C18 250 × 50 mm × 10 µm, water (0.1% TFA)-ACN) to afford the **71** (350 mg, 4.15% yield) as a white solid and **72** (800 mg, 2.20 mmol, 9.48% yield) as a white solid: Compound **71** $^1$H NMR (400 MHz, $(CD_3)_2SO$) 8.14 - 7.94 (m, 2H), 7.11 (br s, 1H);

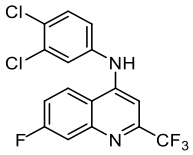


Compound **72** $^1$H NMR (400 MHz, $CD_3OD$) δ 7.85 - 7.69 (m, 2H), 6.95 (br s, 1H).



**5,6,8-trifluoro-2-(trifluoromethyl)quinolin-4-ol (73).** To a mixture of 2,4,5-trifluoroaniline (2 g, 13.6 mmol) and ethyl 4,4,4-trifluoro-3-oxo-butanoate (2.98 mL, 20.4 mmol)

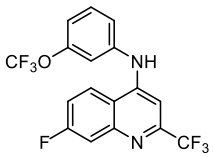
was added PPA (20.0 g, 50.8 mmol). The mixture was stirred at 150 °C for 1 h. When cold, the mixture was diluted with *aq*. NaOH (5%, 100 mL). The precipitate formed was dissolved in *aq*. NaOH (10%, 30 mL). After some insoluble material had been removed by filtration, the clear solution was acidified with *conc*. HCl (12 M). The solid formed was filtered and dried affording the crude title compound (1.1 g, crude) as a white solid: $^1$H NMR (400 MHz, $(CD_3)_2SO$) δ 8.12 - 7.97 (m, 1H), 7.43 (s, 1H).



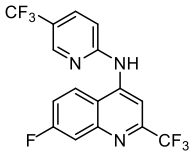

**4-[(3,4-dichlorophenyl)amino]-7-fluoro-2-(trifluoromethyl)quinoline (74).** To a flask under $N_2$ atmosphere added $POCl_3$ (1.21 mL, 13.0 mmol) and 7-fluoro-2-(trifluoromethyl)quinolin-4-ol (**70**, 100 mg, 433 µmol) The solution was degassed and purged with $N_2$ for 3 times, then the mixture was stirred at 110 °C for 1 h under $N_2$ atmosphere. The reaction mixture was quenched by addition $H_2O$ (10 mL) at 0 °C and extracted with EtOAc (10 mL × 3). The combined organic layers were washed with brine (10 mL), dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to give the crude title compound (150 mg, crude) as a yellow solid:

To a solution of 4-chloro-7-fluoro-2-(trifluoromethyl)quinoline (42 mg, 168 µmol) and 3,4- dichloroaniline (32.7 mg, 202 µmol) in EtOH (2 mL) was added HCl (12 M, 7.01 µL). The mixture was stirred at 80 °C for 8 h. The solvent was removed under reduced pressure to give a residue. The residue was purified by *Prep*.-HPLC: column: Kromasil 150 × 25mm × 10 um; mobile phase: [(water (0.04% $NH_3{\cdot}H_2O$ + 10mM $NH_4HCO_3$) -ACN]; B%: 45%-75%, 10 min) to give the title compound (40.2 mg, 62% yield, 98% purity) as a white solid: $^1$H NMR (400 MHz, $CDCl_3$) δ 8.00 - 7.91 (m, 1H), 7.85 - 7.78 (m, 1H), 7.55 (d, *J* = 8.6 Hz, 1H), 7.43 (br d, *J* = 2.6 Hz, 2H), 7.23

- 7.18 (m, 1H), 7.17 (s, 1H), 6.73 (s, 1H); MS (ESI) $[M+H]^+$ = 374.9; HRMS (ESI) *m*/*z* calcd for $C_{16}H_9Cl_2F_4N_2^+$ $[M + H]^+$ 375.0073, found 375.0063 (error 2.88 ppm).



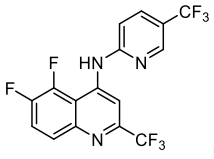
**7-fluoro-4-[3-(trifluoromethoxy)phenylamino]-2-(trifluoromethyl)quinoline (75).** The title compound was synthesized according to the synthetic procedure reported for **74**: $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.02 - 7.94 (m, 1H), 7.86 - 7.79 (m, 1H), 7.51 (t, *J* = 8.1 Hz, 1H), 7.47 - 7.38 (m, 1H), 7.30 - 7.27 (m, 3H), 7.20 (br s, 1H), 7.14 (br d, *J* = 8.2 Hz, 1H), 6.82 (br s, 1H); MS (ESI) $[M+H]^+$ = 391.0; HRMS (ESI) *m*/*z* calcd for $C_{17}H_{10}F_7N_2O^+$ $[M + H]^+$ 391.0676, found 391.0680 (error 1.17 ppm).



**7-fluoro-2-(trifluoromethyl)-4-{[5-(trifluoromethyl)pyridin-2-yl]amino}quinoline (76).** To a flask under $N_2$ atmosphere added $POCl_3$ (1.21 mL, 13.0 mmol) and 7-fluoro-2-(trifluoromethyl)quinolin-4-ol (**70**, 100 mg, 433 µmol) The solution was degassed and purged with $N_2$ for 3 times, then the mixture was stirred at 110 °C for 1 h under $N_2$ atmosphere. The reaction mixture was quenched by addition $H_2O$ (10 mL) at 0 °C and extracted with EtOAc (10 mL × 3). The combined organic layers were washed with brine (10 mL), dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to give the crude title compound (150 mg, crude) as a yellow solid:

To a flask under $N_2$ atmosphere added 4-chloro-7-fluoro-2-(trifluoromethyl)-quinoline (60 mg, 240 µmol), 5-(trifluoromethyl) pyridin-2-amine (58.5 mg, 361 µmol), $Pd_2(dba)_3$ (22.0 mg, 24 µmol), Xantphos (27.8 mg, 48.1 µmol) and $K_2CO_3$ (100 mg, 721 µmol) in dioxane (1 mL). The solvent was degassed and the flask was purged with $N_2$ three times. The mixture was stirred at 100

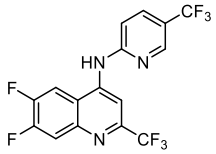
°C for 2 h under $N_2$ atmosphere. The solvent was removed under reduced pressure to give a residue. The residue was purified by *Prep*.-HPLC: (HUAPU C8 Extreme BDS 150 × 30 5μ, water (10 mM $NH_4HCO_3$)-ACN) to afford the title compound (39.5 mg, 42% yield, 95.5% purity) as a yellow solid: $^1$H NMR (400 MHz, $(CD_3)_2SO$) δ 10.28 (br s, 1H), 8.89 (s, 1H), 8.81 - 8.61 (m, 2H), 8.23 - 8.07 (m, 1H), 7.92 - 7.81 (m, 1H), 7.79 - 7.66 (m, 1H), 7.55 (br d, $J$ = 8.8 Hz, 1H); $^{13}$C NMR (151 MHz, DMSO) δ 163.1 (d, $^1J_{C\text{-}F}$ = 250 Hz), 157.4, 149.0 (d, $^3J_{C\text{-}F}$ = 13 Hz), 148.3 (d, $^2J_{C\text{-}F}$ = 33 Hz), 146.3, 145.0 (q, $^3J_{C\text{-}F}$ = 4.1 Hz), 135.3, 125.6 (d, $^3J_{C\text{-}F}$ = 10 Hz), 124.2 (d, $^1J_{C\text{-}F}$ = 271 Hz), 120.8 (q, $^1J_{C\text{-}F}$ = 276 Hz), 118.4 (d, $^2J_{C\text{-}F}$ = 31.7 Hz), 117.8, 117.5 (d, $^2J_{C\text{-}F}$ = 25 Hz), 113.9, 113.2 (d, $^2J_{C\text{-}F}$ = 20 Hz), 102.8; MS (ESI) $[M+H]^+$ = 376.0; HRMS (ESI) *m*/*z* calcd for $C_{16}H_9F_7N_3^+$ $[M + H]^+$ 376.0679, found 376.0688 (error 2.31 ppm).



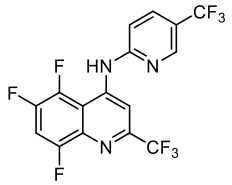

**5,6-difluoro-2-(trifluoromethyl)-*N*-[5-(trifluoromethyl)pyridin-2-yl]quinolin-4-amine (77).** To 5,6-difluoro-2-(trifluoromethyl) quinolin-4-ol (**71**, 100 mg, 275 μmol) was added $POCl_3$ (1.82 mL, 19.6 mmol) and stirred at 120 °C for 1 h. The reaction mixture was quenched by addition $H_2O$ (10 mL) at 0 °C, then extracted with EtOAc (10 mL × 2). The combined organic layers were washed with brine (10 mL), dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to give crude 4-chloro-5,6-difluoro-2-(trifluoromethyl)quinoline (**71a**, 100 mg, crude) as a yellow oil.

To a flask under $N_2$ atmosphere added crude 4-chloro-5,6-difluoro-2-(trifluoromethyl)quinoline (**71a**, 40 mg, 149 μmol), 5-(trifluoromethyl)pyridin-2-amine (36.4 mg, 224 μmol), $Pd_2(dba)_3$ (13.7 mg, 15.0 μmol), Xantphos (17.3 mg, 29.9 μmol) and *t*-BuONa (43.1 mg, 448 μmol) in dioxane (1 mL). The solvent was degassed and the flask was purged with $N_2$

three times. The mixture was stirred at 100 °C for 2 h under $N_2$ atmosphere. The mixture was concentrated under reduced pressure to give a residue which was purified by *Prep*.-HPLC (column: Kromasil 150 × 25 mm × 10 um; mobile phase: [water (0.04% $NH_3 \cdot H_2O$ + 10 mM $NH_4HCO_3$)-ACN]; B%: 50%-70%, 20 min) to afford the title compound (15.3 mg, 26.1% yield) as a yellow solid: $^1$H NMR (601 MHz, $(CD_3)_2SO$) δ 10.07 (s, 1H), 8.97 (s, 1H), 8.77 (d, *J* = 2.6 Hz, 1H), 8.71 (dd, *J* = 12.5, 8.4 Hz, 1H), 8.17 – 8.14 (m, 2H), 7.54 (d, *J* = 8.8 Hz, 1H); $^{13}$C NMR (151 MHz, $(CD_3)_2SO$) δ 157.1, 151.9 (dd, $^1J_{C\text{-}F}$ = 254, 16.2 Hz), 149.6 (dd, $^1J_{C\text{-}F}$ = 250, 15.4 Hz), 147.8 (q, $^2J_{C\text{-}F}$ = 34 Hz), 145.6 (d, $^3J_{C\text{-}F}$ = 4.9 Hz), 145.3 (d, $^2J_{C\text{-}F}$ = 11.2 Hz), 144.9 (q, $^3J_{C\text{-}F}$ = 4.4 Hz), 135.3 (q, $^3J_{C\text{-}F}$ = 3.4 Hz), 124.1 (q, $^1J_{C\text{-}F}$ = 271 Hz), 121.6 (q, $^1J_{C\text{-}F}$ = 275 Hz), 118.7 (q, $^2J_{C\text{-}F}$ = 32.7 Hz), 117.6 (d, $^3J_{C\text{-}F}$ = 7.5 Hz), 116.4 (d, $^2J_{C\text{-}F}$ = 16.3 Hz), 113.8, 109.5 (d, $^2J_{C\text{-}F}$ = 20.2 Hz), 103.1; HRMS (ESI) *m*/*z* calcd for $C_{16}H_8F_8N_3^+$ $[M + H]^+$ 394.0585, found 394.0570 (error 3.80 ppm).



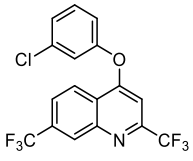

**6,7-difluoro-2-(trifluoromethyl)-*N*-[5-(trifluoromethyl)pyridin-2-yl] quinolin-4-amine (78).** The title compound was synthesized according to the synthetic procedure reported for **77** using **72** as the starting material: $^1$H NMR (400 MHz, $(CD_3)_2SO$) δ 10.09 (s, 1H), 8.98 (s, 1H), 8.78 (s, 1H), 8.72 (dd, J=8.5, 12.5 Hz, 1H), 8.21 - 8.13 (m, 2H), 7.55 (d, J=8.8 Hz, 1H). MS (ESI) $[M+H]^+$ = 394.0; HRMS (ESI) *m*/*z* calcd for $C_{16}H_8F_8N_3^+$ $[M + H]^+$ 394.0585, found 394.0593 (error 1.97 ppm).




**5,6,8-trifluoro-2-(trifluoromethyl)-*N*-[5-(trifluoromethyl)pyridin-2-yl]quinolin-4-amine (79).** To 5,6,8-trifluoro-2-(trifluoromethyl)quinolin-4-ol (**73**, 1.1 g, crude)

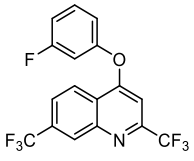
was added $POCl_3$ (1.21 mL, 13.0 mmol) and stirred at 120 °C for 1 h. The reaction mixture was quenched by addition $H_2O$ (10 mL) at 0°C, then extracted with EtOAc (10 mL × 2). The combined organic layers were washed with brine (10mL), dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to afford crude 4-chloro-5,6,8-trifluoro-2-(trifluoromethyl)quinoline (100 mg, crude) as a yellow oil.

To a flask under $N_2$ atmosphere added 4-chloro-5,6,8-trifluoro-2-(trifluoromethyl)quinoline (**73a**, 90 mg, 315 µmol), 5-(trifluoromethyl)pyridin-2-amine (61.3 mg, 378 µmol), $Pd_2(dba)_3$ (28.9 mg, 31.5 µmol), Xantphos (36.5 mg, 63.0 µmol) and $K_2CO_3$ (130.7 mg, 945 µmol) in dioxane (1 mL). The solvent was degassed and the flask was purged with $N_2$ three times. The mixture was then stirred at 100 °C for 2 h under $N_2$ atmosphere. The mixture was concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (column: Kromasil 150 × 25mm × 10 um; mobile phase: [water (0.04% $NH_3H_2O$+ 10mM $NH_4HCO_3$)-ACN]; B%: 55%-75%, 20 min) to afford the title compound (35.9 mg, 27.1% yield, 97.9% purity) as a pink solid: $^1$H NMR (400 MHz, $(CD_3)_2SO$) δ 10.04 (br s, 1H), 8.76 (s, 1H), 8.69 (s, 1H), 8.30 - 8.08 (m, 2H), 7.42 (d, *J* = 8.8 Hz, 1H); MS (ESI) $[M+H]^+$ = 412.1; HRMS (ESI) *m*/*z* calcd for $C_{16}H_5F_9N_3^-$ $[M - H]^-$ 410.0345, found 410.0363 (error 4.25 ppm).

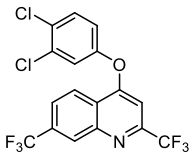



**4-[(3-chlorophenyloxy]-2,7-bis(trifluoromethyl)quinoline (80).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinoline-4-one (**3**, 281 mg, 1.0 mmol) and 3-chlorophenol (286 mg, 2.1 mmol) using General Procedure H. Purification by HPLC (method A) afforded the product (24 mg, 6% yield) as a white powder: HPLC purity 99.0%, $t_R$ = 7.89 min, $k'$ = 17.35 (method B); $^1$H NMR (500 MHz, $CD_3OD$) δ 8.65 (d, *J* = 8.8 Hz, 1H), 8.48 (s, 1H), 8.01

(d, $J$ = 8.7 Hz, 1H), 8.58 (t, $J$ = 8.2 Hz, 1H), 7.47-7.45 (m, 2H), 7.30 (d, $J$ = 8.9 Hz, 1H), 6.97 (s, 1H); $^{13}$C NMR (126 MHz, ($CD_3OD$) δ 165.0, 155.6, 151.7 (q, $^2J_{C\text{-}F}$ = 35 Hz), 149.2, 137.3, 134.7 (q, $^2J_{C\text{-}F}$ = 33 Hz), 133.2, 132.2, 128.4, 128.2 (d, $^3J_{C\text{-}F}$ = 4.5 Hz), 125.3 (q, $^1J_{C\text{-}F}$ = 271 Hz), 125.2, 124.9 122.9, 122.6 (q, $^1J_{C\text{-}F}$ = 275 Hz), 120.9, 102.8; HRMS (ESI$^+$) $m/z$ calcd for $C_{17}H_9ClF_6NO^+$ $[M + H]^+$ 392.0271, found 392.0289 (error 4.47 ppm).

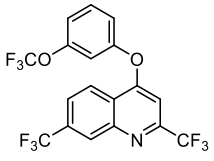



**4-[(3-fluorophenyloxy]-2,7-bis(trifluoromethyl)quinoline (81).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinoline-4-one (**3**, 281 mg, 1.0 mmol) and 3-fluorophenol (286 mg, 2.1 mmol) using General Procedure H. Purification by HPLC (method A) afforded the product (50 mg, 13% yield) as a white powder: HPLC purity 99.8%, $t_R$ = 7.60 min, $k'$ = 16.68 (method B); $^1$H NMR (500 MHz, $CD_3OD$) δ 8.65 (d, $J$ = 8.8 Hz, 1H), 8.48 (s, 1H), 8.01 (d, $J$ = 8.7 Hz, 1H), 8.58 (t, $J$ = 8.2 Hz, 1H), 7.47-7.45 (m, 2H), 7.30 (d, $J$ = 8.9 Hz, 1H), 6.97 (s, 1H); $^{13}$C NMR (126 MHz, ($CD_3OD$) δ 165.0, 155.6, 151.7 (q, $^2J_{C\text{-}F}$ = 35 Hz), 149.2, 137.3, 134.7 (q, $^2J_{C\text{-}F}$ = 33 Hz), 133.2, 132.2, 128.4, 128.2 (d, $^3J_{C\text{-}F}$ = 4.5 Hz), 125.3 (q, $^1J_{C\text{-}F}$ = 271 Hz), 125.2, 124.9 122.9, 122.6 (q, $^1J_{C\text{-}F}$ = 275 Hz), 120.9, 102.8; HRMS (ESI$^+$) $m/z$ calcd for $C_{17}H_9ClF_6NO^+$ $[M + H]^+$ 392.0271, found 392.0289 (error 4.47 ppm).

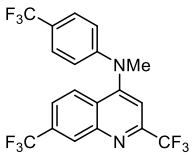



**4-[(3,4-Dichlorophenyloxy]-2,7-bis(trifluoromethyl)quinoline (82).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinoline-4-one (**3**, 281 mg, 1.0 mmol) and 3,4-dichlorophenol (286 mg, 2.1 mmol) using General Procedure H. Purification by HPLC (method A) afforded the product (33 mg, 7% yield) as a white powder: $^1$H NMR (500 MHz, $CD_3OD$) δ 8.65 (d, $J$ = 8.8 Hz, 1H), 8.48 (s, 1H), 8.01 (d, $J$ = 8.7 Hz, 1H), 8.58 (t, $J$ = 8.2 Hz, 1H),

7.47-7.45 (m, 2H), 7.30 (d, $J$ = 8.9 Hz, 1H), 6.97 (s, 1H); $^{13}$C NMR (126 MHz, ($CD_3OD$) δ 165.0, 155.6, 151.7 (q, $^2J_{C\text{-}F}$ = 35 Hz), 149.2, 137.3, 134.7 (q, $^2J_{C\text{-}F}$ = 33 Hz), 133.2, 132.2, 128.4, 128.2 (d, $^3J_{C\text{-}F}$ = 4.5 Hz), 125.3 (q, $^1J_{C\text{-}F}$ = 271 Hz), 125.2, 124.9 122.9, 122.6 (q, $^1J_{C\text{-}F}$ = 275 Hz), 120.9, 102.8; HRMS ($ESI^+$) $m/z$ calcd for $C_{17}H_9ClF_6NO^+$ $[M + H]^+$ 392.0271, found 392.0289 (error 4.47 ppm).



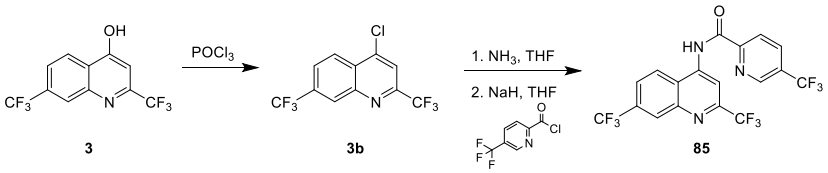

**4-[(3-trifluoromethoxy)phenyloxy]-2,7-bis(trifluoromethyl)quinoline (83).** The title compound was prepared from 2,7-bis(trifluoromethyl)quinoline-4-one (281 mg, 1.0 mmol) and 3-(trifluoromethoxy)phenol (288 uL, 2.1 mmol) using General Procedure H. Purification by HPLC (method A) afforded the product (28 mg, 6% yield) as a white powder: HPLC purity 99.0%, $t_R$ = 7.85 min, $k'$ = 17.27 (method B); $^1$H NMR (500 MHz, $CD_3OD$) δ 8.65 (d, $J$ = 8.8 Hz, 1H), 8.48 (s, 1H), 8.00 (d, $J$ = 8.8 Hz, 1H), 7.69 (t, $J$ = 8.6 Hz, 1H), 7.37 (d, $J$ = 6.4 Hz, 3H), 6.99 (s, 1H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 164.8, 155.8, 152.0, 151.7 (q, $^2J_{C\text{-}F}$ = 35 Hz), 149.3, 134.7 (q, $^2J_{C\text{-}F}$ = 33 Hz), 133.4, 128.3 (d, $^3J_{C\text{-}F}$ = 4.5 Hz), 125.3 (d, $^3J_{C\text{-}F}$ = 2.7 Hz), 125.24 (q, $^1J_{C\text{-}F}$ = 272 Hz), 125.2, 124.9, 122.5 (q, $^1J_{C\text{-}F}$ = 275 Hz), 122.0 (q, $^1J_{C\text{-}F}$ = 256 Hz), 121.2, 120.5, 120.0, 115.9, 102.8; HRMS ($ESI^+$) $m/z$ calcd for $C_{18}H_9F_9NO_2^+$ $[M + H]^+$ 442.0484, found 442.0483 (error 0.19 ppm).

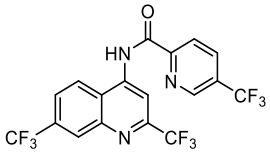



***N*-Methyl-2,7-bis(trifluoromethyl)-*N*-[5-(trifluoromethyl)phenyl] quinoline-4-amine (84).** The title compound was prepared from 4-bromo-2,7-bis(trifluoromethyl)quinoline (70 mg, 0.20 mmol) and *N*-methyl-3-trifluoromethylaniline (35 μL, 0.24 mmol) using General Procedure C. Reaction was stopped after 40 h. Flash column chromatography ($SiO_2$) using a

stepwise gradient of EtOAc/Hex (0:1 to 1:5) afforded the title compound (35 mg, 40% yield) as a yellow powder: (1:5 EtOAc/Hexanes); HPLC purity 97.6%, $t_R$ = 7.68 min, $k'$ = 16.83 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 8.55 (s, 1H), 7.99 (s, 1H), 7.87 (s, 2H), 7.57 (d, $J$ = 8.5 Hz, 2H), 7.07 (d, $J$ = 8.5 Hz, 2H), 3.62 (s, 3H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 154.6, 151.5, 149.4 (q, $^2J_{C\text{-}F}$ = 35 Hz), 147.5, 131.0 (q, $^2J_{C\text{-}F}$ = 33 Hz), 129.0, 128.7, 128.2, 127.7, 128.0, 126.6, 123.53 (q, $^1J_{C\text{-}F}$ = 273 Hz), 123.48, 122.4 (q, $^1J_{C\text{-}F}$ = 268 Hz), 121.4 (q, $^1J_{C\text{-}F}$ = 275 Hz), 118.5, 113.5, 41.5; HRMS (ESI+) $m/z$ calcd for $C_{19}H_{12}F_9N_2^+$ $[M + H]^+$ 439.0851, found 439.0855 (error 0.81 ppm).

**Scheme S9: 4-Amidoquinoline Analogue Synthesis**

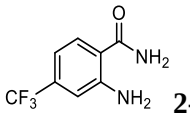


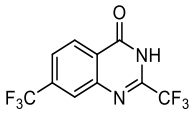
***N*-(2,7-bis(trifluoromethyl)quinolin-4-yl)-5-(trifluoromethyl) picolinamide (85).** To a flask under $N_2$ atmosphere added $POCl_3$ (3.50 mL, 37.7 mmol) and 2,7-bis(trifluoromethyl)quinolin-4(1*H*)-one (**3,** 288 mg, 1.02 mmol). The solution was degassed and purged with $N_2$ for 3 times, then the mixture was stirred at 110 °C for 1 h under $N_2$ atmosphere. LC-MS showed reactant was consumed completely and one main peak with desired mass was detected. The reaction mixture was quenched by addition $H_2O$ (10 mL) at 0 °C and extracted with EtOAc (10 mL × 3). The combined organic layers were washed with brine (10 mL), dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to give the crude 4-chloro-2,7-bis(trifluoromethyl)quinoline (292 mg, crude) as a yellow solid and carried on to the next step without further purification.

To a pressure vessel under $N_2$ atmosphere at -78 °C added 4-chloro-2,7-bis(trifluoromethyl)quinoline (**3b**, 238 mg, 794 μmol) and $NH_3$ (7 M in THF, 21.64 mL). The mixture was degassed and purged with $N_2$ for 3 times and sealed, then the mixture was stirred at 80 °C for 12 h under $N_2$ atmosphere. LC-MS showed reactant was consumed completely and desired mass was detected. The reaction mixture was concentrated under reduced pressure to give the crude 2,7-bis(trifluoromethyl)quinolin-4-amine (180 mg, crude) as a yellow solid:

To a solution of 2,7-bis(trifluoromethyl)quinolin-4-amine (64 mg, 228 µmol) in THF (1 mL) was added NaH (18.3 mg, 457 µmol, 60% purity) at 0 °C and stirred at that temperature for 0.5 h. To the mixture was added a solution of 5-(trifluoromethyl)pyridine-2-carbonyl chloride (144 mg, 685 µmol) in THF (0.5 mL) which was allowed to warm to 25 °C and stirred for 12 h. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by *Prep*.-HPLC: column: (Kromasil 150 × 25mm × 10 µm; mobile phase: [(water (0.04% $NH_3H_2O$ + 10 mM $NH_4HCO_3$)-ACN]; B%: 45%-65%, 20 min) to afford the title compound (6.45 mg, 6.16% yield, 98.83% purity) as a white solid: $^1$H NMR (400 MHz, $(CD_3)_2SO$) δ 11.62 (br s, 1H), 9.26 (s, 1H), 8.73 (s, 1H), 8.62 - 8.57 (m, 2H), 8.52 (d, *J* = 8.8 Hz, 1H), 8.44 (d, *J* = 8.2 Hz, 1H), 8.15 (dd, *J* = 1.5, 9.0 Hz, 1H); MS (ESI) $[M+H]^+$ = 454.0; HRMS (ESI) *m*/*z* calcd for $C_{18}H_7F_9N_3O^-$ $[M - H]^-$ 452.0451, found 452.0451 (error 2.68 ppm).

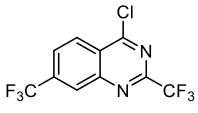



**2-Amino-4-(trifluoromethyl)benzamide (86).** To a stirred solution of 2-amino-4-(trifluoromethyl)benzonitrile (2.00 g, 10.5 mmol) in DMSO (6.6 mL) at 0 ºC was added potassium carbonate (306 mg, 2.22 mmol) and hydrogen peroxide (30 % aqueous solution, 2.19 mL, 21.4 mmol). The mixture was allowed to warm up to room temperature and stirred for 2 h. The reaction was then separated between water (100 mL) and EtOAc (100 mL). The organics were separated and washed with brine (50 mL), dried over $MgSO_4$, and concentrated under vacuum. The reaction was taken off heat and concentrated under vacuum onto silica gel. Flash column chromatography afforded the product (3.94 g, 88% yield) as a pale-yellow oil. $R_f$ = 0.4 (2:5 EtOAc/Hex); $^1$H NMR (500 MHz, $CDCl_3$) δ 8.21 (s, 1H), 7.95 (m, 1H), 7.88 (m, 1H), 4.44 (q, *J* = 7.1 Hz, 2H), 1.38 (t, *J* = 7.1 Hz, 3H).



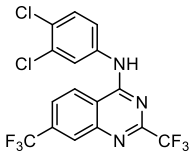
**2,7-bis(Trifluoromethyl)quinazolin-4-one (87).** To a stirred solution of 2-amino-4-(trifluoromethyl)benzamide (525 mg, 2.56 mmol) in THF (9 mL) was added pyridine (5.14 mmol, 0.42 mL), trifluoroacetic anhydride (0.39 mL, 2.8 mol), and DMAP (6 mg, 0.05 mmol) at ambient temperature and then stirred at that temperature for 3 h. After completion of the reaction, as indicated by TLC, the reaction mixture was diluted with water (10 mL) and extracted with $CH_2Cl_2$ (3 × 10 mL). The organic extracts were combined, dried with $Na_2SO_4$ and evaporated to dryness under reduced pressure to afford the crude 2-(2,2,2-trifluoroacetamido)-4-(trifluoromethyl)benzamide intermediate (0.5 g) as a white powder. The crude trifluoroacetamide was carried on to the next step without further purification: $R_f$ = 0.08 in 1:5 EtOAc/Hex); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 13.53 (s, 1H), 8.71 (s, 1H), 8.66 (d, *J* = 1.82 Hz, 1H), 8.23 (s, 1H), 8.15 (d, *J* = 8.27 Hz, 1H), 7.73 (dd, *J* = 8.33, 2.11 Hz, 1H).

To a solution of the crude trifluoroacetamide intermediate in EtOH: $H_2O$ (8mL, 1:1) was added 1 M KOH (5 mL) at room temperature. The reaction mixture was heated at reflux for 1 h. The reaction mixture was then cooled to ambient temperature, diluted with saturated $NH_4Cl$ (10 mL) and EtOAc (10 mL) and extracted with EtOAc (2 x 10 mL). The organics extracts were combined, concentrated under vacuum, and dried over $MgSO_4$ to give the title compound (302 mg, 42% yield over two steps) as a white powder: $R_f$ = 0.16 (3:10 EtOAc/Hex); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 8.38 (d, *J* = 8.29 Hz, 1H), 8.19 (s, 1H), 7.98 (d, *J* = 8.39 Hz, 1H).

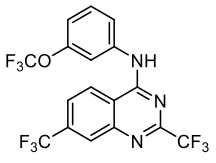


**4-Chloro-2,7-bis(trifluoromethyl)quinazoline (88).** To a round bottom flask dissolved 2,7-bis(trifluoromethyl)quinazolin-4-one (70 mg, 0.25 mmol) in thionyl chloride:DMF (1.5 mL, 1.2:0.3) and heated to reflux for 2.5 h. The reaction was cooled to room temperature and

most of the solvents were removed under vacuum. The crude material was then separated between $CH_2Cl_2$ (10 mL) and saturated $NaHCO_3$ (10 mL), washed with $NaHCO_3$ (1x10 mL), brine (1x 10 mL), dried over $MgSO_4$, and concentrated under vacuum. Flash column chromatography afforded the product (70 mg, 94% yield) as a yellow powder. $R_f$ = 0.5 (3:10 EtOAc/Hex); $^1$H NMR (500 MHz, ($CDCl_3$) δ 8.54 (m, 2H), 8.08 (m, 1H).

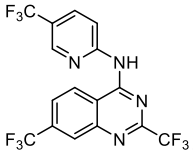


**4-(3,4-Dichlorophenylamino)-2,7-bis(trifluoromethyl)quinazoline (89).** To a round bottom flask dissolved 4-chloro-2,7-bis(trifluoromethyl)quinazoline (**88**, 35 mg, 0.12 mmol) in IPA (0.5 mL, 0.24M) and then added 3,4-dichloroaniline (18 mg, 0.12 mmol) in one portion. Heated the reaction to reflux for 17 h, let cool to ambient temperature, and then concentrated under vacuum. Flash column chromatography afforded the product (37 mg, 75% yield) as a white powder: $R_f$ = 0.5 (3:10 EtOAc/Hex); HPLC purity 91.2%, $t_R$ = 7.63 min, $k'$ = 16.72 (method B); $^1$H NMR (400 MHz, ($CDCl_3$) δ 8.38 (s, 1H), 8.09 (d, $J$ = 8.7 Hz, 1H), 8.05 (d, $J$ = 2.6 Hz, 1H), 7.89 (dd, $J$ = 8.6, 1.8 Hz, 1H), 7.76 (s, 1H), 7.72 (dd, $J$ = 8.8, 2.6 Hz, 1H), 7.49 (d, $J$ = 8.7 Hz, 1H); $^{13}$C NMR (151 MHz, MeOD) δ 160.3, 154.7 (q, $J$ = 35.6 Hz), 150.6, 139.5, 136.5 (q, $J$ = 33 Hz), 133.3, 131.4, 128.9, 125.7, 127.1 (q, $J$ = 4.0 Hz), 125.2 (q, $J$ = 3.0 Hz), 125.1, 124.81 (q, $J$ = 273 Hz), 123.0, 120.51 (q, $J$ = 276 Hz), 118.0.



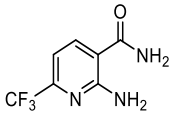
**3-[(trifluoromethoxy)phenylamino]-2,7-bis(trifluoromethyl)quinazoline (90).** To a round bottom flask dissolved 4-chloro-2,7- bis(trifluoromethyl)quinazoline (**88**, 35 mg, 0.12 mmol) in IPA (0.5 mL, 0.24 M) was added 3-(trifluoromethoxy)aniline (21 mg, 0.12 mmol) in one portion. Heated the reaction to reflux for 15.5 h, let cool to ambient temperature, and then

concentrated under vacuum. Flash column chromatography afforded the product (36 mg, 70% yield) as a white powder. $R_f$ = 0.45 (3:10 EtOAc/Hex); HPLC purity 99.0%, $t_R$ = 7.68 min, $k'$ = 16.88 (method B); $^1$H NMR (500 MHz, ($CDCl_3$) δ 8.39 (s, 1H), 8.11 (d, *J* = 8.7 Hz, 1H), 8.02 (dq, *J* = 2.4, 1.2 Hz, 1H), 7.90 (dd, *J* = 8.7, 1.8 Hz, 1H), 7.80 (s, 1H), 7.66 (ddd, *J* = 8.2, 2.2, 0.9 Hz, 1H), 7.46 (t, *J* = 8.2 Hz, 1H), 7.09 (ddt, *J* = 8.2, 2.2, 1.0 Hz, 1H); $^{13}$C NMR (151 MHz, $CDCl_3$) δ 157.9, 153.7 (q, *J* = 37.2 Hz), 149.8 (q, *J* = 1.9 Hz), 149.6, 138.7, 135.9 (q, *J* = 33.6 Hz), 130.4, 127.9 (q, *J* = 4.3 Hz), 124.6 (q, *J* = 3.2 Hz), 123.2 (q, *J* = 273 Hz), 121.8, 120.8 (q, *J* = 258 Hz), 119.3, 119.1 (q, *J* = 276 Hz), 117.6, 116.7, 114.3; HRMS (ESI$^+$) *m*/*z* calcd for $C_{17}H_9F_9N_3O^+$ [M + H]$^+$ 442.0596, found 442.0595 (error 0.31 ppm).



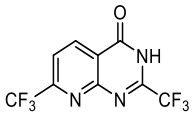
**4-[5-(Trifluoromethyl)pyrid-2-yl]-2,7-bis(trifluoromethyl)quinazoline (91).** To a round bottom flask dissolved 4-chloro-2,7-bis(trifluoromethyl)quinazoline (**88,** 35 mg, 0.12 mmol) in IPA (0.5 mL, 0.24M) and then added 2-amino-5-(trifluoromethyl)pyridine (19 mg, 0.12 mmol) in one portion. Heated the reaction to reflux for 17 h, let cool to ambient temperature, and then concentrated under vacuum. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 1:5) afforded the title compound (12 mg, 24% yield) as a pale-yellow solid: $R_f$ = 0.20 (1:5 EtOAc/Hexanes); HPLC purity 91.4%, $t_R$ = 7.73 min, $k'$ = 16.98 (method B); $^1$H NMR (500 MHz, ($CDCl_3$) δ 8.93 (d, *J* =8.7 Hz, 1H), 8.66 (br. s, 1H), 8.47 (m, 2H), 8.27 (d, *J* =8.3 Hz, 1H), 8.13 (d, *J* =8.9 Hz, 1H), 7.97 (d, *J* =8.6 Hz, 1H); $^{13}$C NMR (151 MHz, $CD_3OD$) δ 160.0, 156.1, 154.2 (q, *J* = 36.2 Hz), 150.7, 146.6, 136.8 (q, *J* = 33.3 Hz), 136.7 (q, *J* = 3.2 Hz), 127.3 (q, *J* = 4.3 Hz), 125.6 (q, *J* = 3.0 Hz), 126.1, 125.2 (q, *J* = 271 Hz), 124.7 (q, *J* = 272 Hz), 123.5 (q, *J*

= 33.3 Hz), 121.2 (q, *J* = 275 Hz), 118.9, 116.6; HRMS (ESI$^+$) *m*/*z* calcd for $C_{16}H_6F_9N_4^-$ [M - H]$^-$ 425.0454, found 425.0443 (error 2.57 ppm).



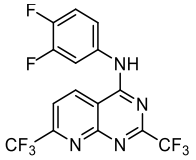
**2-amino-6-(trifluoromethyl)nicotinamide (92).** A mixture of 2-chloro-6-(trifluoromethyl)-nicotinonitrile (1.5 g, 7.26 mmol), $NH_3$ (7 M in THF, 70 mL) was degassed and purged with $N_2$ three times,and heated at 80 °C for 12 h under $N_2$ atmosphere. The reaction mixture was concentrated under reduced pressure to give the crude 2-amino-6-(trifluoromethyl)nicotinonitrile (1.3 g, crude) as a white solid.

To a microwave tube added the crude 2-amino-6-(trifluoromethyl)nicotinonitrile (1 g, 5.34 mmol) and aqueous $NH_3$ (21.4 mL, 139 mmol, 25% purity). The sealed tube was stirred at 80 °C for 18 h under microwave. The reaction mixture was concentrated under reduced pressure. Flash column chromatography ($SiO_2$) afforded the title compound (1.05 g, 81% yield, 85% purity) as a pale-yellow solid from a EtOAc/Petroleum ether (0 to 7:10) stepwise gradient: $^1$H NMR (400 MHz, $(CD_3)_2SO$) δ 8.15 (br s, 1H), 8.14 - 8.12 (d, *J* = 7.9 Hz, 1H), 7.61 (br s, 3H), 7.00 (d, *J* = 7.9 Hz, 1H).



**2,7-bis(trifluoromethyl)pyrido[2,3-*d*]pyrimidin-4(3*H*)-one (93).** To a flask charged with MeOH (10 mL) at 0 °C under an $N_2$ atmosphere added Na (842 mg, 36.7 mmol) portionwise. The resulting mixture was stirred until Na was consumed completely. 2-amino-6-(trifluoromethyl)nicotinamide (**92**, 940 mg, 4.58 mmol) and ethyl 2,2,2-trifluoroacetate (5.21 g, 36.66 mmol, 5.06 mL) were added into the generated NaOMe, then the mixture was stirred at 70 °C for 12 h. LC-MS showed reactant was consumed completely and desired mass was detected. The reaction mixture was concentrated under reduced pressure to give crude 2,7-

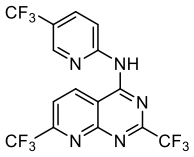
bis(trifluoromethyl)pyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**93**, 1.3 g, crude) as a yellow gum: $^1$H NMR (400 MHz, $(CD_3)_2SO$) δ 8.62 (d, *J* = 7.9 Hz, 1H), 7.74 (d, *J* = 8.2 Hz, 1H).



***N*-(3,4-difluorophenyl)-2,7-bis(trifluoromethyl)pyrido[2,3-*d*]pyrimidin-4-amine (94).** To a flask under $N_2$ atmosphere added 2,7-bis(trifluoromethyl)pyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**93**, 200 mg, 706 μmol) to $POCl_3$ (2.00 mL, 21.52 mmol). The flask was purged with $N_2$ 3 times, then the mixture was stirred at 110 °C for 1 h under $N_2$ atmosphere. LC-MS showed reactant was consumed completely and one main peak with desired mass was detected. The reaction mixture was diluted with $H_2O$ (30 mL) and extracted with EtOAc (10 mL × 3). The combined organic layers were dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to give the title compound (189 mg, crude) as a red solid.

To a flask under $N_2$ atmosphere added 4-chloro-2,7-bis(trifluoromethyl)pyrido[2,3-*d*]pyrimidine (80 mg, 265 μmol), 3,4- difluoroaniline (31.9 μL, 318 μmol,), HCl (2.21 μL, 12 M) in EtOH (1 mL) was degassed and purged with $N_2$ for 3 times, then the mixture was stirred at 80 °C for 12 h under $N_2$ atmosphere. LC-MS showed reactant was consumed completely and desired mass was detected. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by *Prep*.-HPLC (column: Kromasil 150 × 25mm × 10 μm; mobile phase: [water (0.04% $NH_3H_2O$ + 10 mM $NH_4HCO_3$)-ACN]; B%: 50%-70%, 10 min) to afford the title compound (47.7 mg, 45 % yield, 97.58% purity) as a yellow solid: $^1$H NMR (400 MHz, $(CD_3)_2SO$) δ 10.94 (s, 1H), 9.40 (d, *J* = 8.4 Hz, 1H), 8.37 (d, *J* = 8.6 Hz, 1H), 8.08 (ddd, *J* = 2.6, 7.5, 13.0 Hz, 1H), 7.71 - 7.64 (m, 1H), 7.61 - 7.51 (m, 1H); $^{13}$C NMR (151 MHz, DMSO) δ 160.0, 157.1, 155.4 (q, *J* = 35.6 Hz), 152.3 (q, *J* = 34.7 Hz), 148.8 (dd, *J* = 244, 13.2 Hz), 146.5 (dd, *J* = 244, 12.6 Hz),

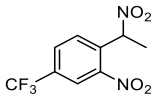
137.3, 134.8 (dd, *J* = 8.8, 3.1 Hz), 120.83 (q, *J* = 276 Hz), 119.51, 119.48 (q, *J* = 276.4 Hz), 119.1 (dd, *J* = 6.4, 3.3 Hz), 117.5 (d, *J* = 18.0 Hz), 113.1, 111.8 (d, *J* = 21.6 Hz); HRMS (ESI) *m*/*z* calcd for $C_{15}H_5F_8N_4$- $[M - H]^-$ 393.0392, found 393.0388 (error 1.11 ppm).



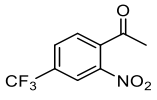
**2,7-bis(trifluoromethyl)-*N*-(5-(trifluoromethyl)pyridin-2-yl)pyrido[2,3-*d*]pyrimidin -4-amine (95).** To a flask under $N_2$ atmosphere added 2,7-bis(trifluoromethyl)pyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**93**, 200 mg, 706 μmol) to $POCl_3$ (2.00 mL, 21.52 mmol). The flask was purged with $N_2$ 3 times, then the mixture was stirred at 110 °C for 1 h under $N_2$ atmosphere. LC-MS showed reactant was consumed completely and one main peak with desired mass was detected. The reaction mixture was diluted with $H_2O$ (30 mL) and extracted with EtOAc (10 mL × 3). The combined organic layers were dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to give the title compound (189 mg, crude) as a red solid.

To a flask under $N_2$ atmosphere added 4-chloro-2,7-bis(trifluoromethyl)pyrido[2,3-*d*]pyrimidine (100 mg, 331 μmol), 5- (trifluoromethyl)pyridin-2-amine (64.5 mg, 398 μmol), $K_2CO_3$ (137 mg, 995 μmol), $Pd_2(dba)_3$ (60.7 mg, 66.3 μmol), Xantphos (76.8 mg, 133 μmol). Added dioxane (2 mL) which was degassed and then the flask was purged with $N_2$ 3 times. The mixture was stirred at 100 °C for 2 h under $N_2$ atmosphere. LC-MS showed reactant was consumed completely and desired mass was detected. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by *Prep*.-HPLC (column: Kromasil 150 × 25mm × 10 um; mobile phase: [water (0.04% $NH_3H_2O$ + 10 mM $NH_4HCO_3$)-ACN]; B%: 50%-70%, 10 min) to give the title compound (15.9 mg, 11.0% yield, 97.6% purity) as a yellow solid: $^1$H NMR (400 MHz, $(CD_3)_2SO$) δ 8.93 (d, *J* = 8.1 Hz, 1H), 7.88 - 7.78 (m, 2H), 7.78 - 7.69 (m,

1H), 7.32 (d, $J$ = 7.3 Hz, 1H); $^{13}$C NMR (151 MHz, $(CD_3)_2SO$) δ 159.9, 158.9, 156.9 (d, $^2J_{C\text{-}F}$ = 36 Hz), 150.2 (d, $^2J_{C\text{-}F}$ = 38 Hz), 145.2 (q, $^2J_{C\text{-}F}$ = 33 Hz), 138.2, 137.6, 124.2, 121.8 (d, $^1J_{C\text{-}F}$ = 274 Hz), 121.3 (d, $^1J_{C\text{-}F}$ = 275 Hz), 121.1, 120.0 (d, $^1J_{C\text{-}F}$ = 276 Hz), 117.6, 117.1, 113.6; MS (ESI) $[M+H]^+$ = 428.0; HRMS (ESI) *m*/*z* calcd for $C_{15}H_5F_9N_5^-$ $[M - H]^-$ 426.0407, found 426.0404 (error 0.56 ppm).

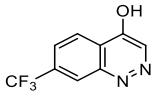



**2-Nitro-1-(1-nitroethyl)-4-(trifluoromethyl)benzene (96).** To a stirred solution of 4-chloro-3-nitrobenzotrifluoride (1.00 g, 4.43 mmol) and nitroethane (0.66 µL, 8.87 mmol) in DMSO at 10 °C was added sodium hydride (60% on mineral oil, 697 mg, 18.178 mmol). Reaction was left to sir for 18 h. Reaction was quenched with addition of cold aqueous HCl (1 M, 10 mL) and then extracted with EtOAc (3x10 mL). Organics were combined, dried over $Na_2SO_4$, and concentrated onto silica gel. Column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hexanes (0:1 to 1:10 EtOAc/Hexanes) afforded the product (1.1 g, 94 % yield) as a yellow oil: $R_f$ = 0.34 (1:5 EtOAc/Hexanes); $^1$H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.98 (dd, $J$ = 8.3, 1.9 Hz, 1H), 7.78 (d, $J$ = 8.3 Hz, 1H), 6.29 (q, $J$ = 6.9 Hz, 1H), 2.03 (d, $J$ = 6.9 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 148.6, 134.1, 133.2 (q, $^2J_{C\text{-}F}$ = 35 Hz), 130.7 (q, $^3J_{C\text{-}F}$ = 3.4 Hz), 129.7, 122.8 (q, $^3J_{C\text{-}F}$ = 3.9 Hz), 122.5 (q, $^1J_{C\text{-}F}$ = 273 Hz), 80.4, 19.7.




**1-[2-Nitro-4-(trifluoromethyl)phenyl]ethan-1-one (97).** To a suspension of the alkyl nitro intermediate (**97**, 1.69 g, 6.40 mmol) and potassium carbonate (1.36 g, 9.75 mmol) in toluene (2 mL) was added hydrogen peroxide solution (30% solution in water, 4 mL, 35 mmol). Reaction was quenched after 18 h upon addition of HCl (10 mL) and then extracted with $CH_2Cl_2$ (2x20 mL). Organics were combined, dried over $Na_2SO_4$, and concentrated onto silica gel for

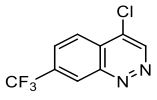
column chromatography. Column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hexanes (0:1 to 1:10 EtOAc/Hexanes) afforded the product (1.43 g, 69 % yield) as a light yellow foam: $R_f$ = 0.11 (1:10 EtOAc/Hexanes); $^1$H NMR (400 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.99 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.58 (d, *J* = 7.9 Hz, 1H), 2.59 (s, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 198.6, 141.2, 133.2 (q, $^2J_{C\text{-}F}$ = 34 Hz), 131.2 (q, $^3J_{C\text{-}F}$ = 3.5 Hz), 128.4, 122.5 (q, $^1J_{C\text{-}F}$ = 273 Hz), 122.0 (q, *J* = 3.8 Hz), 30.3.



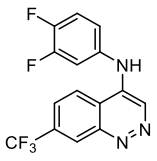

**7-(trifluoromethyl)cinnolin-4-ol (98).** To a suspension of 1-[2-nitro-4-(trifluoromethyl)phenyl]ethan-1-one (**97**, 2.1 g, 8.11 mmol) in deionized water (12 mL) added iron filings (1.36 g, 24.3 mmol) and ammonium chloride (1.30 g, 24.3 mmol). The reaction was heated at 90 ℃ for 21 h in a sealed container after which it was quenched with aqueous NaOH (1M, 15 mL). Aqueous layer was extracted with EtOAc (3x20 mL), organics were combined, dried over $Na_2SO_4$, and concentrated onto silica gel. Crude reaction mixture adsorbed to silica gel was filtered over a plug of celite with EtOAc to afford the crude 1-[2-amino-4-(trifluoromethyl)phenyl]ethan-1-one intermediate (1.33 g) as a yellow foam: $R_f$ = 0.20 (1:10 EtOAc/Hexanes); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.4 Hz, 1H), 6.89 (s, 1H), 6.84 (dd, *J* = 8.4, 1.8 Hz, 1H), 6.44 (s, 2H), 2.60 (s, 3H); HRMS (ESI) *m*/*z* calcd for $C_9H_9F_3NO^+$ $[M + H]^+$ 204.0630, found 204.0630 (error 0.31 ppm).

To a solution of the crude 1-[2-amino-4-(trifluoromethyl)phenyl]ethan-1-one intermediate (0.90 g, 3.3 mmol) in concentrated acetic acid (14 mL, 250 mmol) at 0 ℃ was added a solution of $NaNO_2$ (459 mg, 6.65 mmol) in $H_2O$ (9 mL) dropwise over 15 minutes. The mixture was left to warm to room temp for 3 h where the reaction was quenched with addition of saturated aqueous $NaHCO_3$ (10 mL) and extracted with EtOAc (3x10 mL). Organics were combined, dried

over $Na_2SO_4$, and concentrated. Flash column chromatography ($SiO_2$) with a stepwise gradient of EtOAc/Hexanes (0:1 to 2:5) afforded the title compound (480 mg, 68 % yield over 2 steps) as an orange powder: $R_f$ = 0.16 (1:5 EtOAc/Hexanes); $^1$H NMR (400 MHz, $(CD_3)_2SO$) δ 13.71 (s, 1H), 8.21 (d, *J* = 8.6 Hz, 1H), 7.90 (s, 1H), 7.85 (s, 1H), 7.66 (dd, *J* = 8.7, 1.7 Hz, 1H); $^{13}$C NMR (101 MHz, $(CD_3)_2SO$) δ 169.8, 141.3, 140.4, 133.2 (q, $^2J_{C\text{-}F}$ = 32 Hz), 126.1, 124.4, 123.3 (q, $^1J_{C\text{-}F}$ = 273 Hz), 120.1 (q, $^3J_{C\text{-}F}$ = 3.3 Hz), 114.4 (q, *J* = 4.5 Hz); HRMS (ESI) *m*/*z* calcd for $C_9H_6F_3N_2O^+$ $[M + H]^+$ 215.0427, found 215.0424 (error 1.43 ppm).

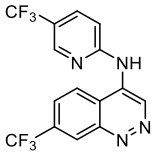



**4-chloro-7-(trifluoromethyl)cinnoline (99).** Heated a mixture of 7-(trifluoromethyl)cinnolin-4-ol (**98**, 97 mg, 0.45 mmol), $POCl_3$ (0.43 mL, 4.5 mmol), and $PCl_5$ (94 mg, 0.45 mmol) at 90 °C for 1 h. Reaction mixture was poured over cold water (5 mL) and then washed with $NaHCO_3$ (10 mL). Extracted aqueous layer with $CH_2Cl_2$ (3x10 mL), combined organics, dried over $Na_2SO_4$, and concentrated onto silica gel. Flash column chromatography ($SiO_2$) with a stepwise gradient of EtOAc/Hexanes (0:1 to 2:5) afforded the title compound (75 mg, 71 % yield) as a tan solid: $R_f$ = 0.49 (3:10 EtOAc/Hexanes); $^1$H NMR (400 MHz, $CDCl_3$) δ 9.46 (s, 1H), 8.87 (s, 1H), 8.34 (d, *J* = 8.8 Hz, 1H), 8.02 (d, *J* f= 8.9 Hz, 1H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 150.0, 145.7, 134.9, 133.6 (q, $^2J_{C\text{-}F}$ = 33.6 Hz), 128.4 (q, $^3J_{C\text{-}F}$ = 4.5 Hz), 127.8 (q, $^3J_{C\text{-}F}$ = 3.0 Hz), 126.3, 125.0, 123.2 (q, $^1J_{C\text{-}F}$ = 273 Hz).



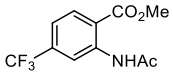

***N*-(3,4-difluorophenyl)-7-(trifluoromethyl)cinnolin-4-amine (100).** To a flask under an argon atmosphere added 3,4-difluoroaniline (16.8 mg, 129 µmol), 4-chloro-7-(trifluoromethyl)cinnoline (**99**, 25 mg, 101 µmol), potassium carbonate (45 mg, 320 µmol),

$Pd_2(dba)_3$ (9.8 mg, 11 µmol), Xantphos (12 mg, 22 µmol) and degassed dioxane (2 mL). The flask was purged with argon three times, sealed, and then then stirred at 100 °C for 3 h. The reaction mixture was then concentrated on to silica gel under reduced pressure, filtered over a pad of celite, and then reduced under vacuum to give a residue. Flash column chromatography ($SiO_2$) with a stepwise gradient of EtOAc/Hexanes (0:1 to 3:5) afforded the title compound (24 mg, 63 % yield, 98.7% purity) as a yellow solid: $R_f$ = 0.33 (1:5 EtOAc/Hexanes); $^1$H NMR (400 MHz, $CD_3OD$) δ 8.75 (s, 1H), 8.54 (d, *J* = 8.9 Hz, 1H), 8.40 (s, 1H), 7.95 (dd, *J* = 8.9, 1.8 Hz, 1H), 7.49 – 7.32 (m, 2H), 7.40 – 7.17 (m, 1H); $^{13}$C NMR (151 MHz, $CD_3OD$) δ 152.1 (dd, *J* = 249, 13.7 Hz), 150.0 (dd, *J* = 247, 12.5 Hz), 147.1, 143.8, 137.0, 134.7 (q, *J* = 32.9 Hz), 132.4, 125.0, 124.8 (q, *J* = 272 Hz), 124.5, 124.0, 121.9 (dd, *J* = 6.2, 3.4 Hz), 119.9, 119.6 (d, *J* = 18.6 Hz), 114.8 (d, *J* = 19.1 Hz); HRMS (ESI) *m*/*z* calcd for $C_{15}H_7F_5N_3^-$ $[M - H]^-$ 324.0566, found 324.0560 (error 1.63 ppm).

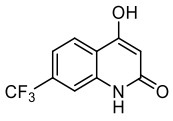



***N*-(5-trifluoromethylpyridin-2-yl)-7-(trifluoromethyl)cinnolin-4-amine (101).** To a flask under an argon atmosphere added 2-amino-5-(trifluoromethyl)pyridine (21.5 mg, 129 µmol), 4-chloro-7-(trifluoromethyl)cinnoline (**99**, 25 mg, 101 µmol), potassium carbonate (45 mg, 320 µmol), $Pd_2(dba)_3$ (9.8 mg, 11 µmol), Xantphos (12 mg, 22 µmol) and degassed dioxane (2 mL). The flask was purged with argon three times, sealed, and then then stirred at 100 °C for 3 h. The reaction mixture was then concentrated on to silica gel under reduced pressure, filtered over a pad of celite, and then reduced under vacuum to give a residue. Flash column chromatography ($SiO_2$) with a stepwise gradient of EtOAc/Hexanes (0:1 to 2:5) afforded the title compound (23 mg, 59 % yield, 98.7% purity) as a white solid: $^1$H NMR (601 MHz, $(CD_3)_2SO$) δ

10.47 (s, 1H), 8.90 (d, *J* = 8.9 Hz, 1H), 8.77 (s, 1H), 8.67 (s, 1H), 8.31 (br s, 1H), 8.16 (zdd, *J* = 8.7, 2.6 Hz, 1H), 8.11 (dd, *J* = 8.9, 1.9 Hz, 1H), 7.65 (d, *J* = 8.8 Hz, 1H); $^{13}$C NMR (151 MHz, $(CD_3)_2SO$) δ 164.7, 157.1, 145.5 (q, $^3J_{C\text{-}F}$ = 4.2 Hz), 141.2, 136.2, 135.4 (q, $^3J_{C\text{-}F}$ = 3.2 Hz), 130.6 (q, $^2J_{C\text{-}F}$ = 32.3 Hz), 126.1, 125.3, 124.6, 124.2 (q, $^1J_{C\text{-}F}$ = 271 Hz), 123.6 (q, $^1J_{C\text{-}F}$ = 273 Hz), 124.1, 119.2, 118.7 (q, $^2J_{C\text{-}F}$ = 32.3 Hz), 113.8; MS (ESI) $[M+H]^+$ = 359.0; HRMS (ESI) *m*/*z* calcd for $C_{15}H_7F_6N_4^-$ $[M - H]^-$ 357.0580, found 357.0595 (error 4.19 ppm).

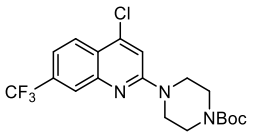



**methyl 2-acetamido-4-(trifluoromethyl)benzoate (102).** To a mixture of methyl 2-amino-4-(trifluoromethyl)benzoate (4.4 g, 20 mmol), TEA (3.35 mL, 24.1 mmol) in THF (40 mL) was added acetyl chloride (1.58 mL, 22.1 mmol) dropwise at -20 °C, then the mixture was stirred at 18 °C for 12 h under $N_2$ atmosphere. The reaction mixture was diluted with $H_2O$ (100 mL) and extracted with EtOAc (30 mL × 3). The combined organic layers were dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to give the title compound (3.94 g, 75.1% yield) as a pink solid: $^1$H NMR (400 MHz, $CDCl_3$) δ 11.12 (br s, 1H), 9.08 (s, 1H), 8.14 (d, *J* = 8.4 Hz, 1H), 7.32 (dd, *J* = 1.3, 8.4 Hz, 1H), 3.98 (s, 3H), 2.27 (s, 3H).



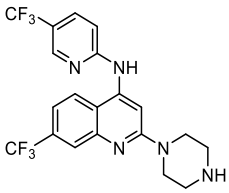

**4-hydroxy-7-(trifluoromethyl)quinolin-2(1*H*)-one (103).** To a stirred solution of methyl 2-acetamido-4-(trifluoromethyl)benzoate (3.94 g, 15.1 mmol) in THF (40 mL) was dropwise added KHMDS (1 M in THF, 45.3 mL) at -78 °C over 0.5 h, then the mixture was slowly warmed to 18 °C and stirred for 3.5 h under $N_2$ atmosphere. The reaction mixture was diluted with $H_2O$ (100 mL) and extracted with EtOAc (50 mL × 2). The *aq*. layer was acidified with HCl (4M, 10 mL). The precipitate formed was collected by filtration to give the title compound solid (2.6 g,

11.4 mmol, 75.2% yield) as a pink: $^1$H NMR (400 MHz, $(CD_3)_2SO$) δ 11.70 (br s, 1H), 11.49 (br s, 1H), 7.97 (d, *J* = 8.3 Hz, 1H), 7.58 (s, 1H), 7.43 (dd, *J* = 1.3, 8.4 Hz, 1H), 5.85 (s, 1H).




***tert*-butyl 4-[4-chloro-7-(trifluoromethyl)quinolin-2-yl]piperazine-1-carboxylate (104).** To 2-hydroxy-7-(trifluoromethyl)-1*H*-quinolin-4-one (**100**, 2.1 g, 9.16 mmol) was added $POCl_3$ (41.3 g, 269 mmol, 25 mL) in one portion, then the mixture was stirred at 110 °C for 12 h under $N_2$ atmosphere. The reaction mixture was diluted with $H_2O$ (400 mL) and extracted with EtOAc (100 mL × 3). The combined organic layers were dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to give a residue to give crude 2,4-dichloro-7-(trifluoromethyl)quinoline (2.8 g, crude) as a red solid.

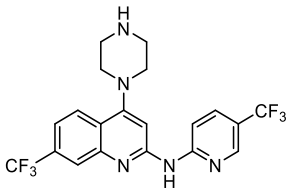
A mixture of 2,4-dichloro-7-(trifluoromethyl)quinoline (50 mg, 188 μmol), *tert*-butyl piperazine-1-carboxylate (35.0 mg, 188 μmol), TEA (26.2 μL, 188 μmol) in DMSO (1 mL) was degassed and purged with $N_2$ for 3 times, then the mixture was stirred at 60 ℃ for 8 h under $N_2$ atmosphere. The reaction mixture was diluted with $H_2O$ (10 mL) and extracted with EtOAc (5 mL × 3). The combined organic layers were dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to give a residue. The residue was purified by *Prep*.-TLC ($SiO_2$) from a petroleum ether/EtOAc gradient (15:1) to afford the title compound (5 mg, 6.4 % yield) as a yellow solid: $^1$H NMR (400 MHz, $CDCl_3$) δ 8.30 (s, 1H), 8.19 (d, *J* = 8.6 Hz, 1H), 7.68 (d, *J* = 8.6 Hz, 1H), 7.30 (s, 1H), 3.92 (br s, 4H), 3.73 - 3.67 (m, 4H), 1.50 (s, 9H).



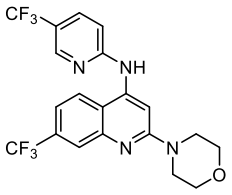
**2-(piperazin-1-yl)-7-(trifluoromethyl)-4-{[(5-(trifluoro-methyl)pyridin-2-yl]amino}quinoline (105).** To a flask under $N_2$ atmosphere added *tert*-butyl-4-[4-chloro-7-(trifluoromethyl)-2-quinolyl]piperazine-1-carboxylate (**101**, 100 mg, 240 µmol), 2-amino-5-(trifluoromethyl)pyridine (46.8 mg, 289 µmol), $K_2CO_3$ (99.7 mg, 721 µmol), Xantphos (55.7 mg, 96.2 µmol) and $Pd_2(dba)_3$ (44.0 mg, 48.1 µmol) in dioxane (2 mL). The solvent was degassed and the flask was purged with $N_2$ three times. The mixture was then stirred at 100 °C for 6 h under $N_2$ atmosphere. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was purified by *Prep*.-TLC ($SiO_2$) from a petroleum ether/EtOAc gradient (3:1) to afford crude *tert*-butyl-4-(7-(trifluoromethyl)-4-[(5-(trifluoromethyl)pyridin-2-yl)amino]quinolin-2-yl) piperazine-1-carboxylate (70 mg, 51.1% yield, 95% purity) as a yellow oil: MS (ESI) $[M+H]^+$ = 542.2.

To a stirred solution of *tert*-butyl-4-(7-(trifluoromethyl)-4-{[5-(trifluoromethyl)-2-pyridyl]amino}-2-quinolyl)piperazine-1-carboxylate (60 mg, 111 µmol) in DCM (2 mL) was added TFA (32.82 µL, 443 µmol) at 18 °C, then the mixture was stirred at 18 °C for 12 h under $N_2$ atmosphere. The reaction pH was adjusted to 7 by addition *aq*. $NH_3{\cdot}H_2O$ (25%). The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by *Prep*.-HPLC: (column: Kromasil 150 × 25mm × 10 um; mobile phase: [(water (0.04% $NH_3H_2O$ + 10 mM $NH_4HCO_3$)-ACN]; B%: 30%-60%, 20 min) to afford the title compound (12.6 mg, 25.8% yield, 99.9% purity) as a white solid: $^1$H NMR (400 MHz, $(CD_3)_2SO$) δ 9.72 (br s, 1H), 8.65 (s, 1H), 8.34 (d, *J* = 8.7 Hz, 1H), 8.22 (s, 1H), 8.04 (dd, *J* = 2.4, 8.9 Hz, 1H), 7.80 (s, 1H), 7.47 (dd, *J* = 1.6, 8.7 Hz, 1H), 7.41 (d, *J* = 8.9 Hz, 1H), 3.65 - 3.58 (m, 4H), 2.84 - 2.77 (m, 4H); $^{13}$C NMR

(151 MHz, $(CD_3)_2SO$) δ 158.8, 158.2, 147.7, 145.1 (q, $^3J_{C\text{-}F}$ = 4.3 Hz), 143.7, 134.7 (q, $^3J_{C\text{-}F}$ = 3.0 Hz), 129.6 (q, $^2J_{C\text{-}F}$ = 31.6 Hz), 124.4 (q, $^1J_{C\text{-}F}$ = 271 Hz), 124.3 (q, $^1J_{C\text{-}F}$ = 272 Hz), 123.8, 123.43 (q, $^3J_{C\text{-}F}$ = 4.2 Hz), 118.9, 117.00 (q, $^2J_{C\text{-}F}$ = 32.4 Hz), 115.93 (q, $^3J_{C\text{-}F}$ = 3.4 Hz), 112.4, 98.8, 45.8, 45.7; MS (ESI) $[M+H]^+$ = 442.1; HRMS (ESI) *m*/*z* calcd for $C_{20}H_{16}F_6N_5^-$ $[M - H]^-$ 440.1315, found 440.1326 (error 2.32 ppm).

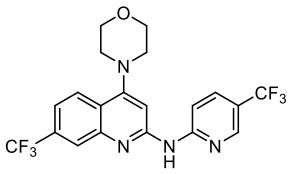


**4-(piperazin-1-yl)-7-(trifluoromethyl)-2-{[5-(trifluoromethyl)pyridin-2-yl]amino}quinoline (106).** The title compound was prepared according to the procedure for **104** and **105**: $^1$H NMR (400 MHz, $(CD_3)_2SO$) δ 10.51 (s, 1H), 8.79 (br d, *J* = 9.0 Hz, 1H), 8.64 (br s, 1H), 8.13 - 8.02 (m, 3H), 7.58 (br d, *J* = 8.4 Hz, 1H), 7.19 (s, 1H), 3.09 - 2.95 (m, 8H); $^{13}$C NMR (151 MHz, $(CD_3)_2SO$) δ $^{13}$C NMR (151 MHz, DMSO) δ 157.8, 156.5, 154.8, 147.2, 145.1 (q, $^3J_{C\text{-}F}$ = 4.3 Hz), 135.3 (q, $^3J_{C\text{-}F}$ = 3.2 Hz), 129.4 (q, $^2J_{C\text{-}F}$ = 31.9 Hz), 125.5, 124.5 (q, $^3J_{C\text{-}F}$ = 4.5 Hz), 124.3 (q, $^1J_{C\text{-}F}$ = 271 Hz), 124.2 (q, $^1J_{C\text{-}F}$ = 272 Hz), 122.2, 118.0 (q, $^3J_{C\text{-}F}$ = 4.3 Hz), 117.8 (q, $^2J_{C\text{-}F}$ = 32.3 Hz), 112.1, 103.0, 53.2, 45.5; MS (ESI) $[M - H]^-$ = 440.0; HRMS (ESI) *m*/*z* calcd for $C_{20}H_{16}F_6N_5^-$ $[M - H]^-$ 440.1315, found 440.1330 (error 3.40 ppm).

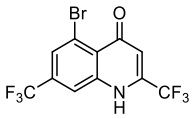


**2-(morpholino)-7-(trifluoromethyl)-4-{[5-(trifluoromethyl)pyridin-2-yl]amino}quinoline (107).** The title compound was prepared according to the procedure for **104** and **105** using morpholine: $^1$H NMR (400 MHz, $CD_3OD$) δ 9.77 (s, 1H), 8.65 (s, 1H), 8.38 (d, *J* = 8.6 Hz, 1H), 8.26 (s, 1H), 8.04 (dd, *J* = 8.9, 2.6 Hz, 1H), 7.83 (s, 1H), 7.51 (dd, *J* = 8.8, 2.0 Hz,

1H), 7.43 (d, $J$ = 8.8 Hz, 1H), 3.74 (t, $J$ = 4.8 Hz, 4H), 3.66 (t, $J$ = 4.8 Hz, 4H); $^{13}$C NMR (151 MHz, $(CD_3)_2SO$) δ 158.9, 158.2, 147.5, 145.0 (q, $^3J_{C\text{-}F}$ = 4.4 Hz), 144.0, 134.8 (q, $^3J_{C\text{-}F}$ = 3.6 Hz), 129.6 (q, $^2J_{C\text{-}F}$ = 31.8 Hz), 124.4 (q, $^1J_{C\text{-}F}$ = 271 Hz), 124.3 (q, $^1J_{C\text{-}F}$ = 273 Hz), 123.8, 123.6 (q, $^3J_{C\text{-}F}$ = 4.3 Hz), 119.1, 117.1 (q, $^2J_{C\text{-}F}$ = 32.5 Hz), 116.3 (q, $^3J_{C\text{-}F}$ = 3.2 Hz), 112.6, 98.5, 66.1, 45.0; MS (ESI) $[M - H]^-$ = 441.1.

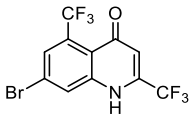


**4-(morpholino)-7-(trifluoromethyl)-2-{[5-(trifluoromethyl)pyridin-2-yl]amino}quinoline (108).** The title compound was prepared according to the procedure for **104** and **105** using morpholine: $^1$H NMR (400 MHz, $CD_3OD$) δ 8.70 (d, $J$ = 8.9 Hz, 1H), 8.55 (s, 1H), 8.14 (d, $J$ = 8.7 Hz, 1H), 8.09 (s, 1H), 8.05 - 7.99 (m, 1H), 7.59 - 7.52 (m, 1H), 7.11 (s, 1H), 4.03 - 3.96 (m, 4H), 3.27 - 3.21 (m, 4H); MS (ESI) $[M+H]^+$ = 443.1.

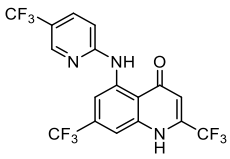


**5-Bromo-2,7-bis(trifluoromethyl) quinolin-4-ol (109).** To a stirring mixture of polyphosphoric acid (19 g) and ethyl 4,4,4-trifluoroacetoacetate (2.38 mL, 15.8 mmol) added 3-bromo-5-trifluoromethylaniline (1.98 mL, 15.8 mmol) dropwise at 100°C. Raised the temperature to 120°C and stirred vigorously for 4 h at that temperature. At 4 h the reaction was taken off heat and quenched with water once the reaction cooled to 50°C. The resulting precipitate was filtered and washed with water followed by multiple washes with saturated $NaHCO_3$. The solid was dissolved in MeOH:$CH_2Cl_2$ (1:1), dried over $MgSO_4$, and concentrated under vacuum. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 1:5) afforded the title compound (2.98 g, 52% yield) as a light-yellow powder: HPLC purity 98.5%, $t_R$ = 7.17 min, $k'$ = 15.65 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 9.07 (br. s, 1H), 7.88 (s, 1H), 7.48 (s,

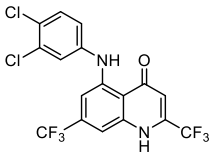
1H), 6.43 (s, 1H); HRMS (ESI) *m*/*z* calcd for $C_{11}H_3BrF_6NO^-$ [M - H]$^-$ 357.9308, found 357.9309 (error 0.41 ppm).



**7-Bromo-2,5-bis(trifluoromethyl)quinolin-4-ol (110).** To a stirring mixture of polyphosphoric acid (1 g/1 mmol aniline) and ethyl 4,4,4-trifluoroacetoacetate (2.38 mL, 15.8 mmol) added 3-bromo-5-trifluoromethylaniline (1.98 mL, 15.8 mmol) dropwise at 100°C. Raised the temperature to 120°C and stirred vigorously for 4 h at that temperature. At 4 h the reaction was taken off heat and quenched with water once the reaction cooled to 50ºC. The resulting precipitate was filtered and washed with water followed by multiple washes with saturated $NaHCO_3$. The solid was dissolved in MeOH:$CH_2Cl_2$ (1:1), dried over $MgSO_4$, and concentrated under vacuum. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 1:5) afforded the title compound (1.87 g, 33% yield) as a light-yellow powder: $R_f$ = 0.24 (1:5 EtOAc/Hex); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 8.95 (br s, 1H), 8.36 (d, *J* = 1.6 Hz, 1H), 7.96 (s, 1H), 6.99 (s, 1H).

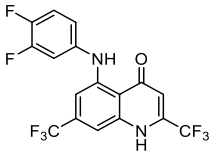


**5-[(5-trifluoromethylpyridin-2-yl)amino]-2,7-bis(trifluoromethyl) quinolin-4(1*H*)-one (111).** The title compound was synthesized according to General Procedure I: $^1$H NMR (400 MHz, $(CD_3OD)$ δ 9.19 (s, 1H), 8.62 (s, 1H), 7.98 - 7.83 (m, 1H), 7.45 (s, 1H), 7.04 (d, *J* = 8.6 Hz, 1H), 6.61 (s, 1H); MS (ESI) $[M+H]^+$ = 442.0.



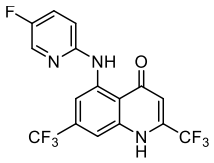
**5-[(3,4-dichlorophenyl)amino]-2,7-bis(trifluoromethyl)quinolin-4(1*H*)-one (112).** The title compound was synthesized according to General Procedure I: $^1$H NMR (400 MHz,

($CD_3OD$) δ 7.53 (d, *J* = 8.6 Hz, 1H), 7.47 (d, *J* = 2.6 Hz, 1H), 7.31 - 7.24 (m, 1H), 7.19 (s, 1H), 7.05 (d, *J* = 1.1 Hz, 1H), 6.54 (s, 1H); HRMS (ESI) *m*/*z* calcd for $C_{17}H_7Cl_2F_6N_2O^-$ [M - H]$^-$ 438.9845, found 438.9836 (error 2.08 ppm).

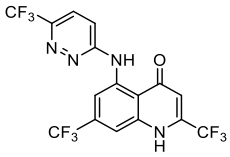



**5-[(3,4-difluorophenyl)amino]-2,7-bis(trifluoromethyl)quinolin-4(1*H*)-one (113).** To a flask under $N_2$ atmosphere added 5-bromo-2,7-bis(trifluoromethyl)quinolin-4-ol (**109**, 50.0 mg, 139 µmol), 3,4- difluoroaniline (26.9 mg, 208 µmol), [2-(2-aminophenyl)phenyl]-methylsulfonyloxy-palladium; dicyclohexyl-[3,6-dimethoxy-2-(2,4,6-triisopropylphenyl)phenyl] phosphane (25.2 mg, 27.8 µmol), $K_3PO_4$ (88.4 mg, 417 µmol) in 2-methyl-2-butanol (2 mL). The solvent was degassed and the flask was purged with $N_2$ three times. The mixture was stirred at 100 °C for 8 h under $N_2$ atmosphere. 3-Mercaptopropyltrimethoxysilane (100 mg) was added and stirred for 2 h. The reaction mixture was filtered and concentrated under reduced pressure. The residue was purified by *Prep*.-HPLC: (column: Kromasil 150 × 25 mm × 10 µm; mobile phase: [water (0.04% $NH_3H_2O$ + 10 mM $NH_4HCO_3$)-ACN]; B%: 35% -55%, 20 min) to afford the title compound (11.9 mg, 20.7% yield, 98.75% purity) as a yellow solid: $^1$H NMR (400 MHz, $CDCl_3$) δ 11.61 (s, 1H), 9.05 (s, 1H), 7.18 (dd, *J* = 18.2, 9.2 Hz, 1H), 7.19 – 7.10 (m, 1H), 7.04 (ddt, *J* = 8.4, 6.4, 2.7 Hz, 1H), 6.95 (s, 1H), 6.90 (s, 1H), 6.54 (d, *J* = 2.0 Hz, 1H); $^{13}$C NMR (151 MHz, MeOD) δ 182.8, 152.0 (dd, *J* = 248, 13.5 Hz), 149.8, 148.8 (dd, *J* = 245, 12.6 Hz), 144.6, 137.9 (dd, *J* = 8.7, 3.4 Hz), 136.4 (q, *J* = 31.9 Hz), 125.2, 124.8 (q, *J* = 272.3 Hz), 121.6 (q, *J* = 274.1 Hz), 120.9 (dd, *J* = 6.1, 3.3 Hz),Z 119.2 (d, *J* = 18.5 Hz), 115.1, 113.6 (d, *J* = 19.0 Hz), 109.1 (q, *J* = 2.9 Hz) 104.1

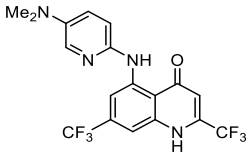
(dd, *J* = 12.8, 3.5 Hz), 100.8 (q, *J* = 3.5 Hz); HRMS (ESI) *m*/*z* calcd for $C_{17}H_7F_8N_2O^-$ [M - H]$^-$ 407.0436, found 407.0447 (error 2.61 ppm).



**5-[(5-fluoropyridin-2-yl)]amino]-2,7-bis(trifluoromethyl)quinolin-4(1*H*)-one (114).** The title compound was prepared according to General Procedure I: $^1$H NMR (400 MHz, $CD_3OD$) δ 9.01 (s, 1H), 8.24 (d, *J*=2.9 Hz, 1H), 7.54 (dt, *J*=3.1 Hz, 8.6 Hz, 1H), 7.32 (s, 1H), 6.98 (dd, *J*=3.4 Hz, 8.9 Hz, 1H), 6.58 (s, 1H); MS (ESI) $[M+H]^+$ =392.0; HRMS (ESI) *m*/*z* calcd for $C_{16}H_7F_7N_3O^-$ [M - H]$^-$ 390.0483, found 390.0494 (error 2.79 ppm).

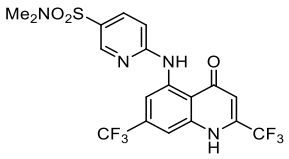


**2,7-bis(trifluoromethyl)-5-{[6-(trifluoromethyl)pyridazin-3-yl]amino} quinolin-4(1*H*)-one (115).** The title compound was prepared from **109** (80 mg, 222 μmol) and 6-(trifluoromethyl)pyridazin-3-amine (54.4 mg, 333 μmol) using General Procedure I. The residue was purified by *Prep*.-HPLC: [water (0.04% $NH_3{\cdot}H_2O$ + 10 mM $NH_4HCO_3$)-ACN]; B%: 30%-50%, 10 min) to afford the title compound (13.0 mg, 12.5% yield, 94.4% purity) as a white solid: $^1$H NMR (400 MHz, $(CD_3)_2SO$) δ13.94 (s, 1H), 13.45 - 12.98 (m, 1H), 9.08 (br s, 1H), 8.05 (br d, *J* = 9.3 Hz, 1H), 7.67 (s, 1H), 7.59 (br d, *J* = 9.5 Hz, 1H), 6.72 (s, 1H); MS (ESI) $[M+H]^+$ = 442.9; HRMS (ESI) *m*/*z* calcd for $C_{16}H_6F_9N_4O^-$ [M - H]$^-$ 441.0403, found 441.0424 (error 4.61 ppm).

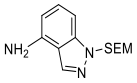


**5-{[5-(dimethylamino)pyridin-2-yl]amino}-2,7-bis(trifluoromethyl) quinolin-4(1*H*)-one (116).** The title compound was prepared from **109** (60 mg, 167 μmol) and *N*$^5$,*N*$^5$-dimethylpyridine-2,5-diamine (34.3 mg, 250 μmol) using General Procedure I: The residue

was purified by *Prep*.-HPLC: [water (0.04% $NH_3 \cdot H_2O$ + 10 mM $NH_4HCO_3$)-ACN]; B%: 30%-50%, 10 min) to afford the title compound (7.33 mg, 10.4% yield, 98.8% purity) as a yellow solid: $^1$H NMR (400 MHz, $CD_3OD$) δ 8.42 (s, 1H), 7.92 (d, *J* = 2.9 Hz, 1H), 7.31 (dd, *J* = 3.1 Hz, 8.8 Hz, 1H), 7.19 (s, 1H), 6.97 (d, *J* = 9.0 Hz, 1H), 6.55 (s, 1H), 2.96 (s, 6H); MS (ESI) $[M+H]^+$ = 417.0; HRMS (ESI) *m*/*z* calcd for $C_{18}H_{13}F_6N_4O^-$ $[M - H]^-$ 415.0987, found 415.0999 (error 3.00 ppm).

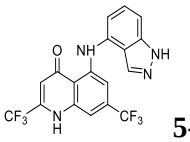



***N*,*N*-dimethyl-6-{[4-oxo-2,7-bis(trifluoromethyl)quinolin-5-yl]amino} pyridine-3-sulfonamide (117).** The title compound was prepared from 5-bromo-2,7-bis(trifluoromethyl)quinolin-4(1*H*)-one (**109**, 60 mg, 167 µmol) and 6-amino-*N*,*N*-dimethyl-pyridine-3-sulfonamide (50.3 mg, 250 µmol) using General Procedure I. The residue was purified by *Prep*.-HPLC: [water (0.04% $NH_3 \cdot H_2O$ + 10 mM $NH_4HCO_3$)-ACN]; B%: 25%-45%, 10 min) to give the title compound (10.2 mg, 12.7% yield, 99.8% purity) as a white solid: $^1$H NMR (400 MHz, $CD_3OD$) δ 9.22 (s, 1H), 8.71 (d, *J* = 2.2 Hz, 1H), 7.98 (dd, *J* = 2.5 Hz, 8.7 Hz, 1H), 7.48 (s, 1H), 7.06 (d, *J* = 8.6 Hz, 1H), 6.64 (s, 1H), 2.74 (s, 6H); $^{13}$C NMR (151 MHz, $(CH_3)_2SO$) δ 180.0, 156.7, 147.6, 143.1, 142.9, 136.9, 132.8, 123.5 (q, $^1J_{C\text{-}F}$ = 274 Hz), 120.3 (q, $^1J_{C\text{-}F}$ = 275 Hz), 115.0, 113.4, 108.6, 108.0, 105.7, 40.1, 37.5; MS (ESI) $[M+H]^+$ = 481.0; HRMS (ESI) *m*/*z* calcd for $C_{18}H_{13}F_6N_4O_3S^-$ $[M - H]^-$ 479.0618, found 479.0623 (error 1.04 ppm).




**1-[(2-(trimethylsilyl)ethoxy)methyl]-1*H*-indazol-4-amine.** Was prepared according to literature procedure Merck Sharp & Dohme LimitedWO2004/46133, 2004, A1 Location in patent: Page 29: $^1$H NMR (400 MHz, $CDCl_3$) δ 8.00 - 7.95 (m, 1H), 7.24 - 7.17 (m, 1H), 6.97 (d, *J*

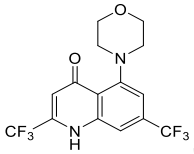
= 8.4 Hz, 1H), 6.40 (d, *J* = 7.5 Hz, 1H), 5.73 - 5.68 (m, 2H), 3.60 - 3.50 (m, 2H), 1.25 (t, *J* = 7.1 Hz, 2H), 0.94 - 0.85 (m, 2H), 0.04 - 0.10 (m, 9H).



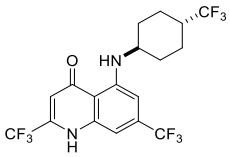
**5-[(1*H*-indazol-4-yl)amino]-2,7-bis(trifluoromethyl)quinolin-4(1*H*)-one (118).** To a microwave tube added **109** (200 mg, 555 µmol), 1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-indazol-4-amine (219 mg, 833 µmol), $K_3PO_4$ (354 mg, 1.67 mmol) and [2-(2-aminophenyl)phenyl]-methylsulfonyloxy-palladium;dicyclohexyl-[3,6-dimethoxy-2-(2,4,6-triisopropylphenyl)phenyl]phosphane (50.4 mg, 55.5 µmol) and were taken up in 2-methylbutan-2-ol (1 mL). The sealed tube was heated at 140 °C for 6 hr in microwave reactor. The reaction mixture was concentrated under reduced pressure. The residue was purified by *Prep*.-HPLC: [water (0.04% $NH_3 \cdot H_2O$)-ACN]; B%: 25%-55%, 10 min) to afford 2,7-bis(trifluoromethyl)-5-[(1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-indazol-4-yl)amino]quinolin-4(1*H*)-one (40 mg, 13.3% yield) as a yellow gum.

To a solution of 2,7-bis(trifluoromethyl)-5-[(1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-indazol-4-yl)amino] quinolin-4(1*H*)-one (**S1,** 40 mg, 74 µmol) in DCM (2 mL) was added TFA (1 mL, 14 mmol). The mixture was stirred at 20 °C for 1 h. The reaction mixture was concentrated under reduced pressure. Then the crude mixture was dissolved in MeOH (2 mL) and was added $K_2CO_3$ (102 mg, 737 µmol). The mixture was stirred at 20 °C for 12 h. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was purified by *Prep*.-HPLC: [water (0.04% $NH_3 \cdot H_2O$ + 10 mM $NH_4HCO_3$)-ACN]; B%: 45%-70%, 10 min) to afford the title compound (5.64 mg, 17.1% yield, 92.3% purity) as a yellow solid: $^1$H NMR (400 MHz, $(CD_3)_2SO$) δ 13.26 (s, 1H), 12.92 (br s, 1H), 12.51 (br s, 1H), 7.96 (s, 1H), 7.39 (t, *J* = 7.8 Hz, 1H), 7.33 (d, *J* = 8.4 Hz, 1H), 7.30 (s, 1H), 7.17 (d, *J* = 7.3 Hz, 1H), 7.07 (s, 1H), 6.63 (s, 1H); MS (ESI) $[M+H]^+$

= 413.0; HRMS (ESI) *m*/*z* calcd for $C_{18}H_9F_6N_4O^-$ [M - H]$^-$ 411.0686, found 411.0703 (error 4.12 ppm).

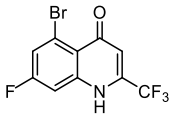


**5-morpholino-2,7-bis(trifluoromethyl)quinolin-4(1*H*)-one (119).** To a microwave tube under $N_2$ atmosphere added **109** (200 mg, 555 µmol), morpholine (147 µL, 1.67 mmol), $K_3PO_4$ (354 mg, 1.67 mmol), [2-(2-aminophenyl)phenyl]-methylsulfonyloxy-palladium;dicyclohexyl-[3,6-dimethoxy-2-(2,4,6-triisopropylphenyl)phenyl]phosphane (100.71 mg, 111.10 µmol) and 2-methylbutan-2-ol (2 mL). The sealed tube was heated at 120 °C for 5 h in microwave reactor. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by *Prep*.-TLC ($SiO_2$) from an EtOAc/petroleum ether gradient (1:3) to give the title compound (11.2 mg, 5.47% yield, 99.8% purity) as a yellow solid: $^1$H NMR (400 MHz, $CD_3OD$) δ 8.27 (br s, 1H), 7.95 (br s, 1H), 7.21 (br s, 1H), 4.10 - 4.04 (m, 2H), 3.91 - 3.82 (m, 2H), 3.38 - 3.32 (m, 2H), 3.19 - 3.12 (m, 2H); $^1$H NMR (400 MHz, $CDCl_3$) δ 15.09 (s, 1H), 8.39 (s, 1H), 7.63 (s, 1H), 7.18 (s, 1H), 4.13 (br d, *J* = 12.1 Hz, 2H), 3.96 - 3.84 (m, 2H), 3.34 - 3.23 (m, 2H), 3.13 (br d, *J* = 11.9 Hz, 2H); MS (ESI) $[M+H]^+$ = 367.0; HRMS (ESI) *m*/*z* calcd for $C_{15}H_{11}F_6N_2O_2^-$ [M - H]$^-$ 365.0730, found 365.0728 (error 0.51 ppm).

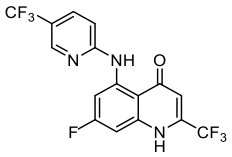


**2,7-bis(trifluoromethyl)-5-[(1*r*,4*r*)-4-(trifluoromethyl)cyclohexylamino] quinolin-4(1*H*)-one (120).** To a microwave tube under $N_2$ atmosphere added **106** (60 mg, 167 µmol), 4-(trifluoromethyl) cyclohexylamine (83.6 mg, 500 µmol), $K_3PO_4$ (106 mg, 500 µmol) and [2-(2-aminophenyl) phenyl]-methylsulfonyloxy-palladium;dicyclohexyl-[3,6-dimethoxy-2-(2,4,6-triisopropylphenyl) phenyl]phosphane (30.2 mg, 33.3 µmol) were taken up in 2-methylbutan-2-ol

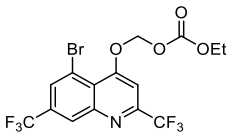
(2 mL). The sealed tube was heated at 135 °C for 6 h in a microwave reactor. The reaction mixture was concentrated under reduced pressure to form a residue. The residue was purified by *Prep*.-HPLC: [water (0.04% $NH_3 \cdot H_2O$ + 10 mM $NH_4HCO_3$)-ACN]; B%: 40%-60%, 10 min) to afford the title compound (11.5 mg, 14.9% yield, 96.6% purity) as a yellow solid: $^1$H NMR (400 MHz, $CD_3OD$) δ 6.91 (s, 1H), 6.57 (s, 1H), 6.43 (s, 1H), 3.47 (br t, *J* = 11.0 Hz, 1H), 2.26 (br d, *J* = 10.8 Hz, 3H), 2.05 (br d, *J* = 11.7 Hz, 2H), 1.65 - 1.48 (m, 2H), 1.44 - 1.30 (m, 2H); MS (ESI) $[M+H]^+$ = 447.1; $^{13}$C NMR (151 MHz, MeOD) δ 180.1, 151.8, 144.6, 137.0 (q, $^2J_{C\text{-}F}$ = 32.0 Hz), 129.7, 129.2 (q, $^1J_{C\text{-}F}$ = 278 Hz), 125.1 (q, $^1J_{C\text{-}F}$ = 273 Hz), 121.6 (q, $^1J_{C\text{-}F}$ = 274 Hz), 114.1, 109.0, 100.0, 99.1 (q, $^3J_{C\text{-}F}$ = 3.3 Hz), 61.3, 51.5, 42.3 (q, $^2J_{C\text{-}F}$ = 26.6 Hz), 25.0 (q, $^3J_{C\text{-}F}$ = 2.6 Hz), 31.9, 14.5; HRMS (ESI) *m*/*z* calcd for $C_{18}H_{14}F_9N_2O^-$ $[M - H]^-$ 445.0968, found 445.0953 (error 3.43 ppm).



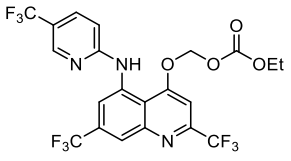

**5-bromo-7-fluoro-2-(trifluoromethyl)quinolin-4(1*H*)-one (121).** To a mixture of 3-bromo-5-fluoroaniline (100 mg, 526 µmol) and ethyl 4,4,4-trifluoro-3-oxo-butanoate (115 µL, 789 µmol) was added PPA (4.92 g, 14.6 mmol), the mixture was purged with $N_2$ three times, then the mixture was stirred at 150 °C for 1 h under $N_2$ atmosphere. When cold, the mixture was diluted with *aq*. NaOH (5%, 80 mL). The precipitate formed was dissolved in *aq*. NaOH (10%, 40 mL). After some insoluble material had been removed by filtration, the clear solution was acidified with *conc*. HCl (10 mL). The formed solid was collected. The residue was purified by *Prep*.-HPLC: [water (0.1% TFA)-ACN]; B%: 40%-50%, 9 min) to afford the title compound (120 mg, 73.5% yield) as a white solid: $^1$H NMR (400 MHz, $CD_3OD$) δ 7.57 (dd, *J* = 2.4, 8.4 Hz, 1H), 7.44 (dd, *J* = 2.3, 9.4 Hz, 1H), 6.70 (s, 1H).



**7-fluoro-2-(trifluoromethyl)-5-{[5-(trifluoromethyl)pyridin-2-yl]amino}quinolin-4(1*H*)-one (122).** To a mixture of 5-bromo-7-fluoro-2-(trifluoromethyl)quinolin-4(1*H*)-one (**118**, 65 mg, 180 µmol), 5-(trifluoromethyl)pyridin-2-amine (33 mg, 200 µmol), *tert*-butylBrettPhos (12 mg, 25 µmol), $K_3PO_4$ (77 mg, 360 µmol) was added degassed dioxane (2 mL) and purged with argon gas, then the mixture was stirred at 100 °C for 12 h under $N_2$ atmosphere. The initial dark purple solution changed to dark green within minutes upon heating. TLC showed significant product formation after 39 h where the reaction was then cooled to room temperature, filtered over a pad of celite and concentrated onto silica gel for column chromatography. Column chromatography ($SiO_2$) using a gradient of triethylamine:EtOAc:Hexanes (2:30:70) followed by a step-wise gradient of triethylamine: MeOH:$CH_2Cl_2$ (2:3:97) to triethylamine:MeOH:$CH_2Cl_2$ (2:20:80) afforded the product (40 mg, 24 % yield) as a yellow powder: $^1$H NMR (600 MHz, $CD_3OD$) δ 8.7 (dd, *J* = 13.0, 2.4 Hz, 1H), 8.6 (s, 1H), 7.9 (dd, *J* = 8.8, 2.5 Hz, 1H), 7.0 (d, *J* = 8.7 Hz, 1H), 6.8 (dd, *J* = 9.4, 2.4 Hz, 1H), 6.5 (d, *J* = 1.8 Hz, 1H); MS (ESI) $[M+H]^+$ = 392.1; HRMS (ESI) *m*/*z* calcd for $C_{16}H_{10}F_4N_3O^-$ $[M - H]^-$ 390.0483, found 390.0466 (error 4.24 ppm).




**{[5-bromo-2,7-bis(trifluoromethyl)quinolin-4-yl]oxy}methyl ethyl carbonate (123).** To a solution of 5-bromo-2,7-bis(trifluoromethyl)quinolin-4-one (**109**, 50 mg, 0.14 mmol) in acetone (1 mL) added cesium carbonate (91 mg, 0.28 mmol) and stirred for 30 min at 23oC. Added chloromethyl ethyl carbonate (33 uL, 0.28 mmol) dropwise and heated to 70oC and stirred at that temperature for 3 h. The reaction was diluted with 25 mL water, acidified with 1 M HCl, and extracted with EtOAc (2 x 25 mL). Organics were combined and concentrated onto silica gel under vacuum. Flash column chromatography from an EtOAc/hexanes stepwise gradient (0:1 to 1:10) afforded the product (24 mg, 37% yield) as a white powder: HPLC purity 96.9%, $t_R$ = 7.61 min, $k'$ = 16.70 (method B); $^1$H NMR (500 MHz, $(CD_3)_2SO$) δ 8.72 (d, $J$ = 1.8 Hz, 1H), 8.34 (s, 1H), 7.83 (s, 1H), 6.22 (s, 2H), 4.20 (q, $J$ = 7.1 Hz, 2H), 1.22 (t, $J$ = 7.1 Hz, 3H).




**{[2,7-bis(trifluoromethyl)-5-{[5-(trifluoromethyl)pyridin-2-yl]amino} quinolin-4-yl]oxy}methyl ethyl carbonate (124).** To a flame dried Schlenk tube under a nitrogen atmosphere added {[5-bromo-2,7-bis(trifluoromethyl)quinolin-4-yl]oxy}methyl ethyl carbonate (**120**, 70 mg, 0.15 mmol), potassium phosphate tribasic (64 mg, 0.30 mmol), 2-amino-5-(trifluoromethyl)pyridine (30 mg, 0.18 mmol), and BrettPhos Pd G3 (29 mg, 0.029 mmol) were all added in one portion and the flask was backfilled with argon. Dioxane (1.5 mL, 0.1 M) was added and sparged with argon for 15 minutes. The reaction vessel was sealed and heated to 100°C and stirred for 2.5 h. Reaction mixture was diluted with EtOAc (20 mL), filtered over a pad of silica gel, and concentrated on to silica gel. Flash column chromatography afforded the product (17 mg, 20% yield) as a yellow powder from an EtOAc/Hex (0:1 to 2:5) stepwise gradient. $R_f$ = 0.10 (1:5

EtOAc/Hexanes); $^{1}$H NMR (500 MHz, $CD_3OD$) δ 9.09 (d, *J* = 1.1 Hz, 1H), 8.52 (s, 1H), 8.02 (s, 1H), 7.89 (dd, *J* = 8.8, 2.2 Hz, 1H), 7.63 (s, 1H), 7.13 (d, *J* = 8.8 Hz, 1H), 6.22 (s, 2H), 4.22 (q, *J* = 7.1 Hz, 2H), 1.25 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (126 MHz, $(CD_3)_2SO$) δ 152.8 (q, $^{2}J_{C\text{-}F}$ = 35 Hz), 150.2, 150.0, 146.8, 144.5, 136.2 (q, $^{2}J_{C\text{-}F}$ = 32 Hz), 132.1, 130.4, 128.0, 125.1 (q, $^{1}J_{C\text{-}F}$ = 273 Hz), 124.3 (q, $^{1}J_{C\text{-}F}$ = 270 Hz), 122.5 (q, $^{1}J_{C\text{-}F}$ = 275 Hz), 118.9, 118.2, 117.3 (q, $^{2}J_{C\text{-}F}$ = 35 Hz), 112.7, 98.4, 91.1, 58.3, 49.6, 18.4; HRMS (ESI-) *m*/*z* calcd for $C_{21}H_{13}F_9N_3O_4^-$ $[M - H]^-$ 542.0768, found 542.0745 (error 4.28 ppm).

# Chapter 2. Investigation of (6*S*)-6- fluoroshikimate and methyl (6*S*)-6-fluoroshikimate in potentiation of p-aminosalicylic acid (PAS) and inhibition of chorismate-utilizing pathways in *M. tuberculosis*

John R. Schultz, Dr. Surendra Dawadi, Dr. Yuske Minato, Dr. Anthony Baughn, Dr.

Courtney C. Aldrich

This work was performed in collaboration with Dr. Surendra Dawadi, Dr. Yusuke Minato, Dr. Anthony Baugh, and Dr. Courtney C. Aldrich.

John R. Schultz synthesized compounds towards (6*S*)-6- fluoroshikimate (**126**-**140**). Dr. Surendra Dawadi completed the synthesis starting from **141** to (6*S*)-6- fluoroshikimate (**125**) and methyl (6*S*)-6-fluoroshikimate (**142, 143, 144**). Dr. Minato carried out initial MIC experiments in *M. tuberculosis* (H37Rv) and supplementation studies.

## *2.1. Introduction*

Tuberculosis (TB), caused by the bacterium *Mycobacterium tuberculosis* (*Mtb*), is a communicable disease that targets the lungs and is transmitted via inhaled aerosolized droplets expelled from a person with active TB disease. TB infection has massive burden upon global society with an estimated 10 million new cases of active infections and 1.4 million deaths in 2018.[69] Even more concerning, is that it is estimated that a quarter of the world's population is a carrier of *M. tuberculosis*, causing them to be at a higher risk for developing active TB disease. It has been estimated that around 10% of those individuals infected with *Mtb* are at high risk of developing active TB.[70] While there are effective treatment regiments for TB infections, recovery is a lengthy process with a 2-month intensive treatment phase using a combination therapy of all four first-line agents (isoniazid, rifampicin, ethambutol, and pyrazinamide), followed by a 4-7 month continuation phase of isoniazid and rifampicin.[71] The long duration of treatment, coupled with common adverse reactions to first-line drugs, lends itself to treatment disruptions which has been suggested to compound issues of drug resistance and reactivation of latent *Mycobacterium tuberculosis* infections.[72]

Resistance to first line agents in *Mtb* has been a growing concern as well. Drug resistance in TB is defined as being either multidrug-resistant (MDR-TB), which is resistant to both first line agents isoniazid and rifampin, or extensively drug resistant tuberculosis (XDR-TB), which is also resistant to a fluoroquinolone and a second-line agent.[73,74] In 2019, approximately 206,000 people with MDR-TB or rifampicin-resistant TB were detected globally which was a 10% increase from 2018. Treating MDR-TB becomes much more difficult with current regiments lasting 9-11 months and are still only successful in 57% of patients.[75] Even with the rise of resistance, only two new drugs, bedaquiline[76] and delamanid,[77] have been approved for use against drug-resistant TB since

the approval of rifampicin in 1963. The rise of MDR-TB and XDR-TB, the long treatment timelines of current drug regiments, and the high prevalence of TB globally outlines the requirement for new antitubercular agents with novel mechanisms that bolster our weakening defense against TB.

Imperative to successful antibacterial drug discovery is the selective disruption of bacterial cells over host cells. This selectivity is normally realized through inhibition of essential biosynthetic pathways that are present only in bacterium. The chorismate pathway (**Figure 2.1.1**) is not found in mammals, yet is present in bacteria, fungi, plants, algae and apicomplexan parasites[78] making the chorismate pathway a prime candidate for chemotherapy. Chorismate is also uniquely important in *Mycobacterium tuberculosis* as it is a key building block of many aromatic metabolites (Figure 2.1.1) such as folates, aromatic amino acids, menquinone, and mycobactins that all serve key roles in the survival of the bacterium. Genes corresponding to enzymes in the chorismate pathway (*aroB, aroD/aroQ, aroE, aroK, aroF*) and each of the resulting chorismate-utilizing pathways (folate biosynthesis: *pabB, folP, folC; folE*; mycobactin biosynthesis: *trpE2, mtbG, mtbC,* tryptophan biosynthesis: *trpE, trpD, trpC, trpB, trpA* and meanquinone biosynthesis: *menD, menC, menE*) have been predicted to be essential for growth of *Mtb* according to high-throughput insertional transposon mutagenesis studies highlighting the importance of these pathways.[79,80]

The shikimate or chorismate pathway (**Figure 2.1.1**) is seven steps in total that synthesizes chorismate from D-erythrose 4-phosphate and phosphoenolpyruvate. Many of these enzymes in this pathway have been exploited as potential drug targets in *Mtb*.[81] Yet relatively few compounds have been shown to be successful inhibitors of chorismate-utilizing enzymes in *Mtb*.[82] Chorismate-

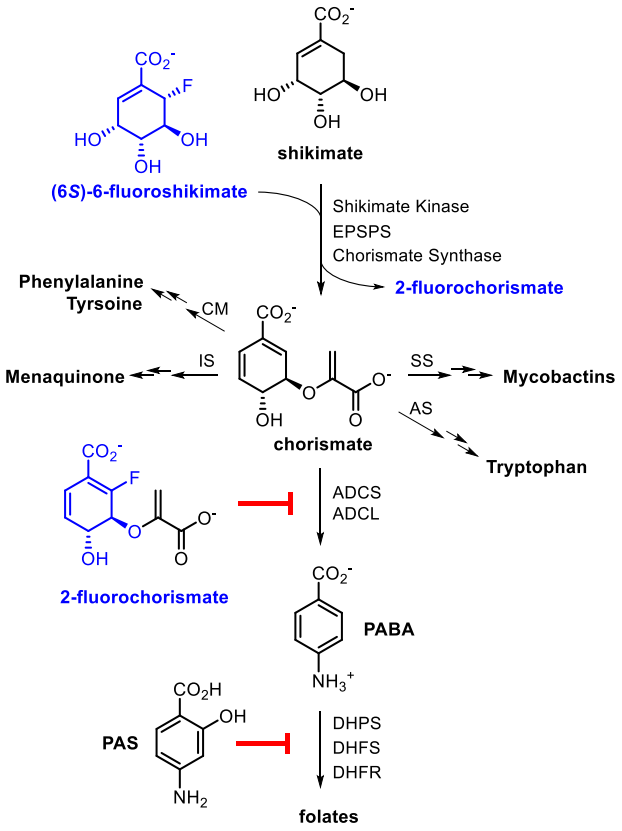



**Figure 2.1.1.** The *Mycobacterium tuberculosis* chorismate pathway and chorismate-utilizing pathways.The biosynthetic pathway of shikimate to chorismate, folates, mycobactins, aromatic amino acids, and menaquinone. Also shown are known inhibitors of the pathway (6S)-6-fluoroshikimate, 2-fluorochorismate, and *p*-aminosalicylic acid (PAS). (6S)-6-Fluoroshikimate is bioconverted via shikimate kinase, EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), and chorismate synthase to 2-florochorismate, a known 4-aminodeoxychorismate synthase (ADCS, PabAB) inhibitor in multiple organisms. Select chorismate-utilizing enzymes are also shown: salicylate synthase (SS, MtbI/TrpE2), anthranilate synthase (AS, TrpE), isochorismate synthase (IS, putative *menF*), chorismate mutase (CM, AroQ/*Rv0948c*), 4-aminodeoxychorismate lyase (ADCL). *Mtb* folate biosynthesis enzymes: DHPS (dihydropteroate synthase, FolP1), DHFS (dihydrofolate synthase, FolC), DHFR (dihydrofolate reductase, DfrA)

utilizing enzymes (**Figure 2.1.1**) salicylate synthase (SS, MbtI), anthranilate synthase (AS, TrpE), and isochorismate synthases (IS, punitive MenF) are part of a larger enzyme class labeled the MST (Menaquinone, Siderophore, and Tryptophan) family due to their similar mechanism and active-site structure.[83] All enzymes in this class are $Mg^{2+}$ dependent either for binding of substrates or

activation of nucleophiles based upon the transition states proposed.[84–88] Salicylate synthase (SS, MbtI) has been one of the few chorismate-utilizing enzymes that has been successfully targeted multiple times by small molecule inhibitors in *Mtb*.[31,89–91] MbtI has been investigated as an appealing drug target as MbtI catalyzes the first step in mycobactin synthesis. Mycobactins (*Mtb* specific siderophores) are small-molecule iron chelators produced by *Mtb* for iron mobilization. *Mtb* mutants unable to produce siderophores have shown to be essential to cause active infections *in vivo* or replicate under iron-restricted conditions. MbtI is a bifunctional enzyme that performs the two step transformation of chorismate to salicylate which is normally carried out by two distinct enzymes (isochorismate synthase/isochorismate lyase) in other organisms.[92] Isochorismate synthase (IS) catalyzes the first step towards menaquinone/ubiquinone in bacteria and has yet to be characterized in *Mtb*. In *Mtb,* a putative dedicated enzyme (e.g. MenF in *E.coli*)[92] or multifunctional enzyme (e.g. EntC in *E.coli*)[88,93] has been suggested to carry out this step. MbtI also has chorismate mutase activity[87] *in vitro* further highlighting the similarities of the MST enzyme class that MbtI, isochorismate synthase, and anthranilate synthase (AS) are a part of.

Chorismate is also a biosynthetic precursor to the aromatic amino acids phenylalanine, tryptophan, and tyrosine (**Figure 2.1.2**) in many infectious agents including *Mtb*. The first committed step to tryptophan is performed by anthranilate synthase (AS), another chorismate-utilizing MST enzyme. The *Mtb* AS is a bifunctional enzyme comprised of two distinct subunits TrpG (glutamine amidotransferase) and TrpE (anthranilate synthase). The generic mechanism of AS can be seen in Figure 2.1.2. AS is part of the MST enzyme class and therefore has a very similar mechanism to isochorismate synthase, salicylate synthase (MbtI), and anthranilate synthase (TrpE). The initial portion of the reaction is $S_N2''$ nucleophilic addition of ammonia, sourced from TrpG, at the C-2 position of chorismate followed by elimination of the enolpyruvate side chain and

subsequent aromatization. TrpG is also allosterically regulated by tryptophan and contains a tryptophan binding site.[85,94] Tryptophan biosynthesis is also essential for virulence *in vivo* as tryptophan auxotrophic strains are not lethal in mice infection models.[95] While TrpG inhibition would be expected to be a good anti-*Mtb* drug target, very few reported *Mtb* TrpG inhibitors exist.[86,96]Simple fluorinated analogues of anthranilate, initially thought to inhibit *Mtb* TrpG, have been proposed to be substrates of TrpG and the subsequent tryptophan biosynthetic pathway leading to cytotoxicity from build-up and incorporation of fluorotryptophan in proteins.[97]

Interestingly, while the biosynthetic route to tryptophan contains multiple genes (*trpE, trpD, trpC, trpB, trpA*) deemed essential for optimal growth,[79,80] in *Mtb*, the biosynthetic pathway from chorismate to phenylalanine and tyrosine contain only conditionally essential genes.[98] It should be noted that no transaminase for the final step of Phe and Tyr biosynthesis has been annotated in *Mtb,* yet there are multiple other essential transaminases. The first step in Phe and Tyr biosynthesis is the transformation of chorismate to prephenate performed by chorismate mutase (CM). The C-3 to C-1 Claisen rearrangement of the enolpyruvate side chain performed by this enzyme (**Figure 2.1.2**) is one of the very few known enzymes that perform pericyclic reactions.[99,100] *In silico* and *in vitro* screening of *Mtb* CM has suggested multiple inhibitors, yet CM has not been validated as a drug target in *Mtb*.[101] *Mtb* also expresses two unique chorismate mutase enzymes[102] which may lead to issues with targeted inhibitors of *Mtb* CM. There are no characterized *Mtb* auxotrophs of glycine, phenylalanine and tyrosine further indicating that this is either an unstudied area or there is little susceptibility to this pathway in *Mtb*.[103] It has also been shown that the salicylate synthase (SS) MbtI has chorismate mutase activity[87] *in vitro* which could lead to further protective redundancies of chorismate mutase when under chemotherapeutic pressure.

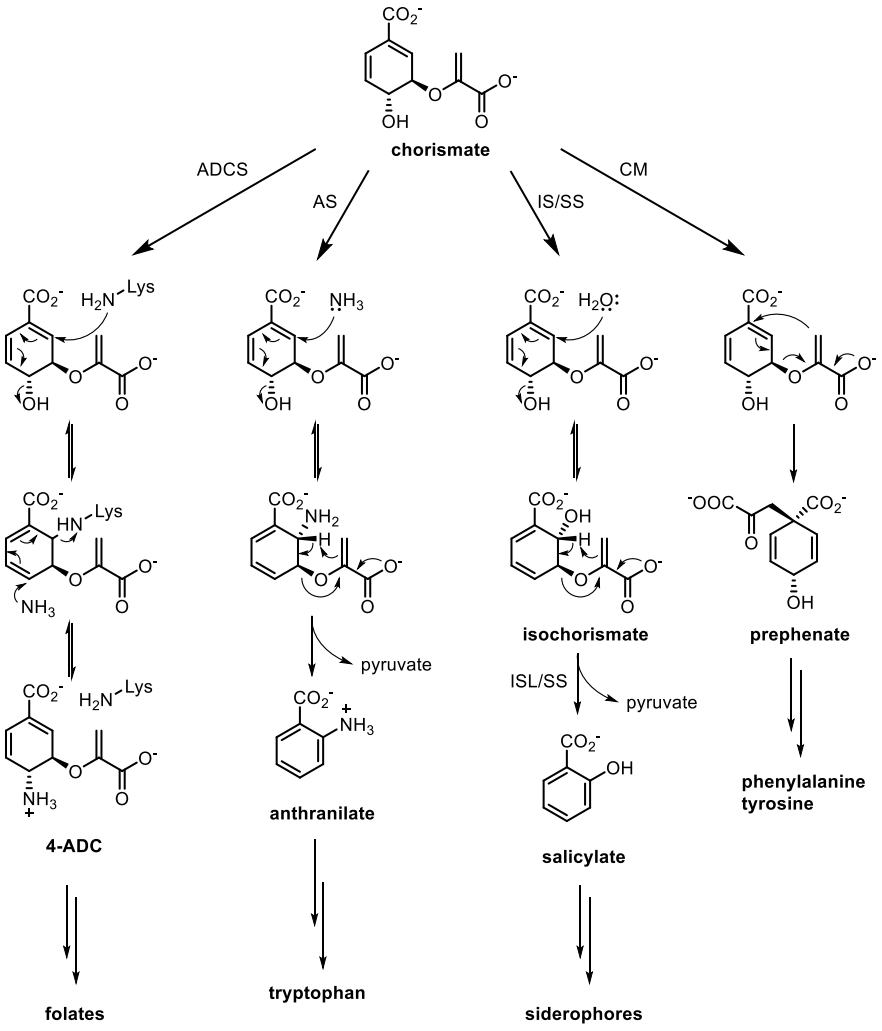



**Figure 2.1.2**. The mechanisms of chorismate utilizing enzymes. 4-ADC (4-aminodoexychorismate), ADCS (4-aminodeoxychorismate synthase), AS (anthranilate synthase), IS (isochorismate synthase), SS (salicylate synthase), ISL (isochorismate lyase), CM (chorismate mutase).

The final chorismate-utilizing enzyme in *Mtb* is PabB, a 4-aminodeoxychorismate synthase (ADCS). PabB is a heteromer comprised of PabB (4-aminodeoxychorismate synthase) that facilitates the successive $S_N2''$ aza substitution of the C-4 hydroxyl of chorismate while the ammonia source required for the transformation is released by PabA, a glutamine

amidotransferase.[104,105] The proposed mechanism of ADC synthase is shown in Figure 2.1.2. The resulting 4-aminodeoxychorismate is then converted to *para*-aminobenzioc acid via PabC (ADC lyase, **Figure 2.1.1**) which leads into folate biosynthesis. Small-molecule inactivation studies of *E.coli* ADCS PabB and *Serratia marcescens* anthranilate synthase (AS) gave insights to the structure-activity relationships of AS and ADCS inhibitors. Inactivation by competitive inhibitors (**Figure 2.1.3A**) was quite uniform between all of the inhibitors used with only 2-10 fold differences in $K_i$ between AS and ADCS over all compounds tested.[106]

PabB was has also recently been validated as a bactericidal target in *Mtb*.[107] *Mtb* mutants deficient in *pabB* were auxotrophic to *p*-aminobenzoic acid (PABA), the product of the second step in folate biosynthesis (**Figure 2.1.1**), and showed loss of viability during PABA starvation studies. These *Mtb pabB* mutants also showed substantially increased the activity of anti-folate drugs such as *p*-aminosalicylic acid (PAS, **Figure 2.1.1**). PAS is one of the first anti-tuberculosis drugs, which is currently a second-line anti-tubercular drug used for drug resistant TB.[108] PAS is an antimetabolite that inhibits folate biosynthesis after bio-conversion to its active metabolite, hydroxydihydrofolate.[109–111] A knockout of *pabB* in a PAS resistant strain[110] regained activity to PAS even in the presence of 1 ng/mL PABA.[107] Small molecule inhibition of this pathway in *Mtb* was also shown to be possible.[107] Compound MAC173979 (**Figure 2.1.3A**), a known inhibitor of *E.coli* PABA biosynthesis, displayed exceptionally low MIC values of 75 ng/mL in the previously mentioned *pabB* mutant MAC173979 also displayed a mild synergistic effect with anti-folate drug PAS.[107] Though MAC173979 was able to drive increased levels of PAS bioactivation, thereby dramatically enhancing drug action, this compound is highly undesirable from a drug-candidate standpoint. MAC173979 compound is a dichloro-nitrophenyl propanone that contains multiple

structural alerts from a metabolic standpoint, contains a reactive Michael-like acceptor, and is most likely somewhat non-specific due to low recovery of activity when supplemented with PABA.

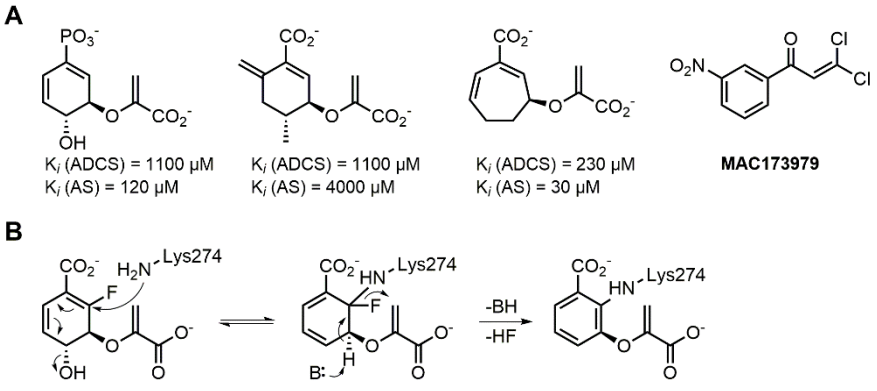



**Figure 2.1.3.** Known chorismate-utilizing enzyme inhibitors (A) and mechanism of inactivation of *E.coli* ADCS by 2-fluorochorismate (B).

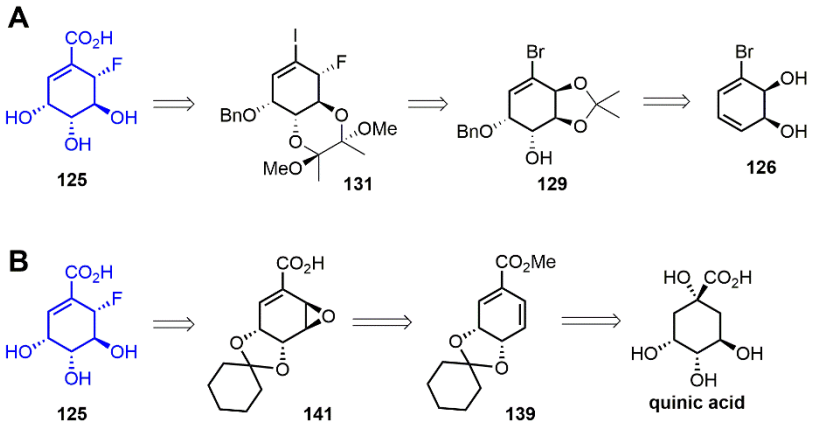
One particularly interesting chorismate pathway inhibitor (6S)-6-fluoroshikimate (Figure 2.1.1) has been studied in infectious agents such as *E coli*[104,112,113] and *P. falciparum*[114,115] but has yet to be characterized in any Mycobacterium. Out of the two isomers, (6S)-6-fluoroshikimate is known to be at least 100-fold more active than (6R)-6-fluoroshikimate against *E. coli* with an MIC of 0.25 µg/mL. In *E. coli* (6S)-6-fluoroshikimate acts as a mechanism-based-irreversible inhibitor of ADC (4-aminodeoxychorismate) synthase upon activation to 2-fluorochorismate. It was shown that (6S)-6-fluoroshikimate acts as a substrate of three shikimate-utilizing enzymes: shikimate kinase, EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), and chorismate synthase, affording the ADCS inhibitor 2-fluorochorismate.[104,113] The PabB-catalyzed aza substitution is readily reversible, but 2-fluorochorismate is able to trap the enzyme bound intermediate upon release of a fluoride atom (**Figure 2.1.3B**). ADCS also shares very a similar mechanism (**Figure 2.1.2**) with isochorismate synthase[116] and anthranilate synthase[117] which may cause pleiotropic effects upon inhibition of the mechanistically similar chorismate-utilizing enzymes. To confirm

this hypothesis of PAS potentiation by PABA biosynthesis inhibitors and to also determine the antibacterial activities in *M. tuberculosis*, we have recently accomplished the synthesis of both epimers of 6-fluoroshikimate. Herein, we report the synthesis and biological evaluation of epimers of 6-fluoroshikimate and methyl-6-fluoroshikimate.

## *2.2. Synthesis of (6S)-6-F-shikimate and (6R)-6-F-shikimate*

We envisioned using two unique routes to (6*S*)-6-fluoroshikimate (**125**), one based on the Humphreys et al. total synthesis[118] of fluoroshikimate (see **Figure 2.2.1A**) and another based off of the first synthesis of fluoroshikimate by the Sutherland[119] and Davies[120] labs (see **Figure 2.2.1B**).

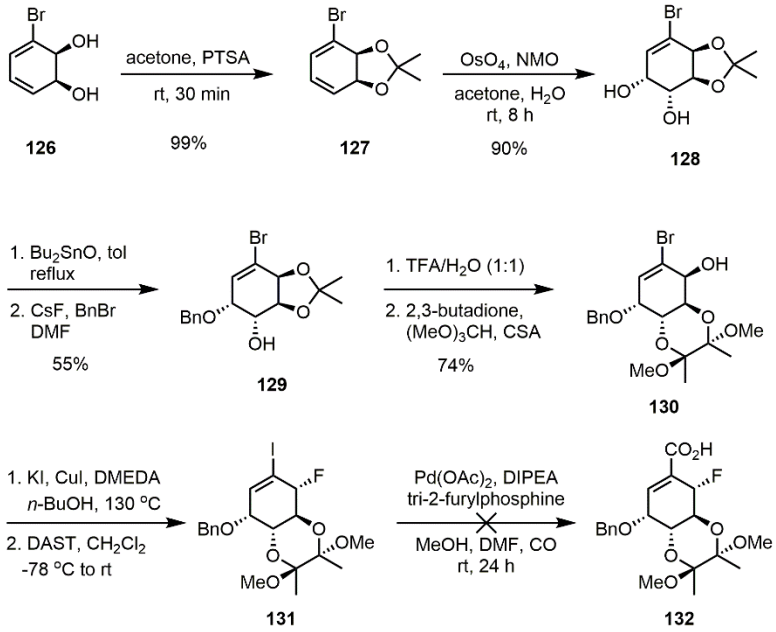
**Figure 2.2.1**. Routes used to (6*S*)-fluoroshikimate.




The first attempted synthesis (**Scheme 2.2.1**) of (6*S*)-6-F-shikimate was through a route inspired by Humphreys et al. total synthesis[118] of fluoroshikimate which started from a halogenated arene cis-dihydrodiol (**126**), obtained from the chemoenzymatic toluene-dioxygenase catalyzed cis-dihydroxylation of bromobenzene.[121] The syn-diol of **126** was readily protected to the acetonide **127** and then dihydroxylated (**128**). The osmium tetroxide dihydroxylation proceeded anti to the acetonide along with good chemoselectivity to the un-substituted (C3-C4) alkene as described previously.[122] The *O*-benzylmonosubstituted diol **129** was afforded over two steps by first forming

a stannylene acetal intermediate followed by alkylation of benzyl bromide under basic conditions to give the desired 3-benzyl protected intermediate (**129**).[118,123] To free the C2 hydroxyl of **129** a protecting group exchange was employed where first the acetonide was removed by treatment with aqueous TFA to give the triol intermediate followed by selective ketalisation of the anti-dol with 2,3-butadione to give **130**. The subsequent "aromatic Finkelstein" reaction gave poor yields and a mixture of the bromo starting material as was somewhat consistent with the experience of Humphreys and co-workers.[118] The subsequent palladium catalyzed carbonylation did not work in our hands and a secondary route (**Scheme 2.2.2**) from intermediate **129** was attempted.

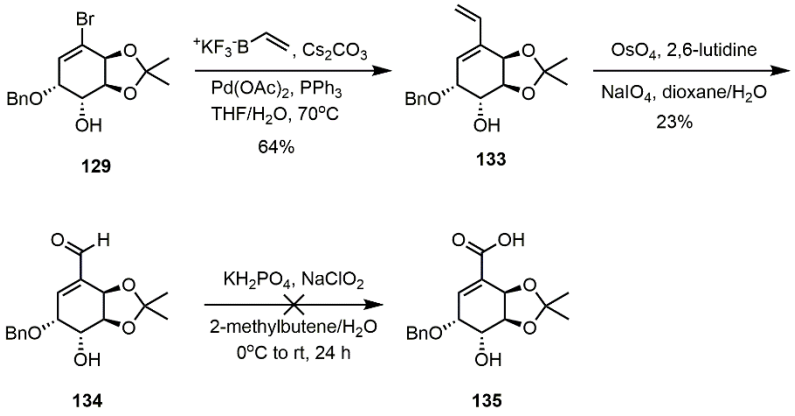
**Scheme 2.2.1.** Attempted synthesis of (6*S*)-6-F-shikimate.




With methods to easily access **129** already outlined in Scheme **2.2.1** we decided to attempt to form the C1 carboxyl group from a key vinyl intermediate **133**. Suzuki coupling of the bromo intermediate (**129**) and a vinyl borane salt[124] gave good yields of the diene (**133**), but subsequent oxidative cleavage of the diene proved difficult. The osmium tetroxide and sodium periodate

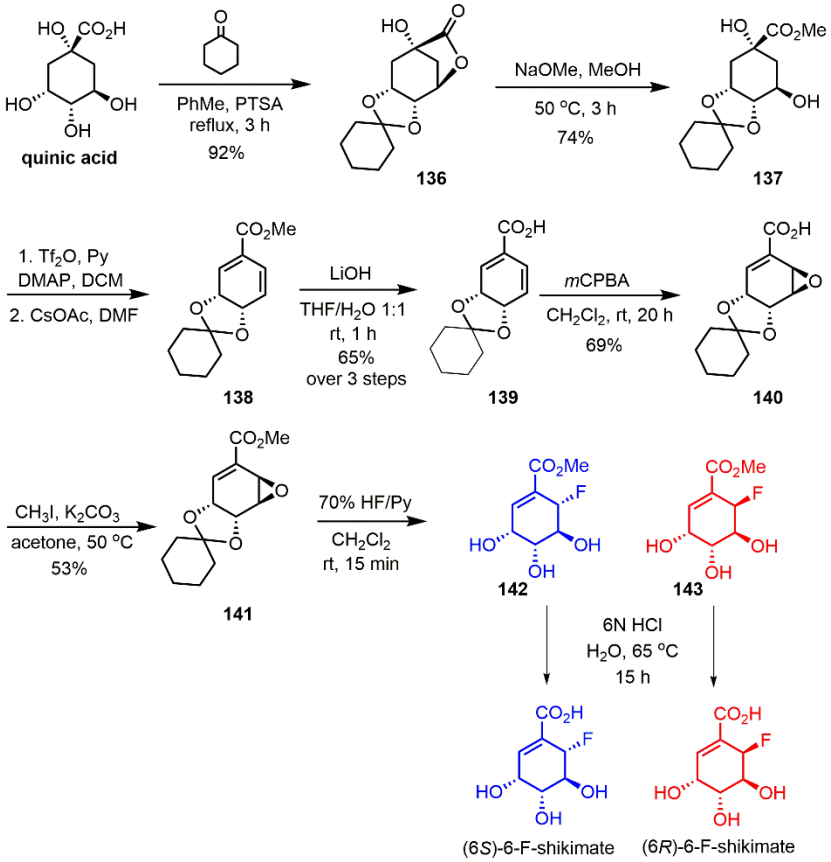
mediated oxidative cleavage of the diene gave ring-opened intermediates along with the desired aldehyde product **134**. Moving forward with the small amount of the resulting aldehyde the oxidation to the acid proved to be difficult and we decided to change to the initial routes to the epimers of fluoroshikimate laid out in **Scheme 2.2.3.**

**Scheme 2.2.2.** Adjusted route to vinyl intermediate.




The successful route to both epimers of 6-fluoroshikimate was through our route that was inspired by Sutherland[119] and Davies[120] labs (Scheme 2.2.3) that started from (-)-quinic acid. The route started from formation of the known lactone (**136**) along with syn-diol protection of (-)-quinic acid. The protected lactone intermediate (**136**) was then opened with sodium methoxide to give the protected methyl ester (**137**). The two free alcohols from **137** were then activated with to the triflates by treatment with triflic anhydride, pyridine, and DMAP which was partially eliminated to a mixture of the diene, singly eliminated triflate, and triflate. After a short workup the diene-triflate mixture was then fully eliminated to the diene-ester (**138**), which was unstable on silica gel and therefore purified after saponification of the ester to the acid-diene (**139**) giving a reasonable yield (62%) over 3 steps.

**Scheme 2.2.3.** Synthesis of (6*S*)-6-F-shikimate and (6*R*)-6-F-shikimate.




Epoxidation of the diene-ester **138** was attempted initially to form **141** directly, but, as reported previously, treatment with *m*CPBA eliminated nearly all regioselectivity towards the desired C5-C6 alkene giving an inseparable mixture of epoxides. Oxidation of the diene (**139**) with *m*CPBA was mostly chemoselective to the electron rich alkene and facially selective due to the bulky spirocyclic diol protection group to give the epoxide (**140**). The acid-epoxide intermediate (**141**) was then protected again with methyl iodide to prevent degradation that was seen in the subsequent fluorination step. Fluorination of the protected ester-epoxide (**141**) with treatment of Olah′s reagent afford both epimers of methyl (6)-fluoroshikimate (**142** and **143**) in a ratio of 3:2 determined by $^{19}$F NMR. Degradation of the products was noted though the reaction had not

reached completion. The fluoro-esters were then separated via preparative HPLC and carried on to the final ester hydrolysis to give both (6*S*)-fluoroshikimate (**125**) and (6*R*)-fluoroshikimate (**144**). Structure of each epimer was confirmed by comparison of previously reported $^{1}$H NMR.[118–120]

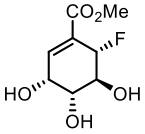
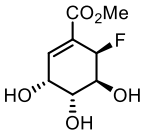
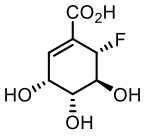
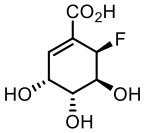
## *2.3. Evaluation of activity and discussion*

With epimers of both 6-fluoroshikimate (**125**, **144**) and methyl 6-fluoroshikimic acid (**142**, **143**) in hand we wanted to test the antibacterial activity in *Mycobacterium tuberculosis* (H37Rv). Inhibition of growth in *Mtb* by MIC was compared to *Escherichia coli* (K12). Both epimers of 6-Fluoroshikimate (**125, 144**) are known inhibitors of *E.coli* with a reported MIC of 0.25 μg/mL for (6*S*)-6-Fluoroshikimate (**125**) and 64 μg/mL for the R-epimer (**144**).[112] The esters **142** and **143** had never been tested in *E. coli* . In our hands the R-epimer (**144**) of fluoroshikimate had a much lower MIC of 16 μg/mL in *E. coli* (**Table 2.3.1**). (6*S*)-6-Fluoroshikimate (**125**) maintained the same activity as previously reported. In *M. tuberculosis* though the acids (**125** and **144**) showed no activity below 64 μg/mL while both the *R*-(**142**) and *S*-(**143**) 6-fluoroshikimate esters displayed moderate anti-tubercular activity with MIC values of 8 and 16 μg/mL, respectively (**Table 2.3.1**).

The increased activity of the esters compared to the acids of fluoroshikimate could be explained in a few ways. *Mtb* has a fortified and waxy cell envelope comprised of a variety of mycolic acids, phenolic glycolipids, and sulfolipids that could prevent permeabilization of the ionized acids while the increased lipophilicity of the methyl esters (**142**, **143**) could allow these compounds to permeabilize the *Mtb* membrane.[125–127] It should be noted that resistance in *E.coli* to **125** mainly arises due to mutation of *shiA*, a shikimate specific transporter.[128] No transporter is similarly annotated in *Mtb*. Secondly, the long time-course (14 days) of the MIC assays in *Mtb* could have caused significant degradation of the acids. It was already shown in the chemistry section that the vinyl methyl esters (**141**, **142**, **143**) were much more stable than their vinyl acids

counterparts, albeit in organic solvents. Further investigations into the stability of (6*S*)-6-F-shikimate will need to be performed. Finally, bioactivation of (6*S*)-6-fluoroshikimate (**125**) to the mechanism-based inhibitor 2-fluorochorismate that occurs in *E. coli* may be disallowed in *Mtb* due to differences in the binding sites of the *Mtb* homologues of shikimate kinase, 5-enolpyruvylshikimate-3-phosphate synthase, and chorismate synthase.

**Table 2.3.1.** MIC activity of 6-F-shikimate and methyl 6-F-shikimate epimers.

| Cmpd # | Structure | MIC μg/mL[a] | |
|---|---|---|---|
| | | ***E. coli*** **(K12)** | ***M. tuberculosis*** **(H37Rv)** |
| **142** |  | 16 | 8 |
| **143** |  | 16 | 16 |
| **125** |  | 0.25 | >64 |
| **144** |  | 16 | >64 |

With fluoroshikimate analogues being plausible general chorismate-utilizing enzyme inhibitors, we wanted to test the possibility of pleiotropic effects upon inhibition multiple chorismate-utilizing pathways (see **Figure 2.2.1**) We first started with supplementation studies of aromatic amino acids to question if the anti-tubercular methyl (6S)-6-fluroshikimate (**142**) is

preventing synthesis of intracellular tryptophan, tyrosine/phenylalanine, or both aromatic amino acid synthetic pathways. Supplementation of individual aromatic amino acids at 10 µg/mL concentrations in *M. bovis* (**Table 2.3.2**) did not affect the activity of **142** while the addition of all three amino acids (10 µg/mL each) inhibited activity of **142** in *M. bovis*. The ability of amino acid supplementation to recover activity in *M. bovis* indicates that **142** may act as either a general chorismate-utilizing enzyme inhibitor or targets aromatic amino acid biosynthesis specifically. In *E.coli*, susceptibility to (6S)-6-fluroshikimate (**125**) went unchanged upon supplementation of amino acids as reported by Davies et al.[112] (**Table 2.3.2**) However, supplementation of PABA overcame the inhibition of **125** in *E.* coli, representative of on-target inhibition of PabB and the resulting folate pathway (see **Figure 2.1.1** and **Figure 2.1.3**) .

**Table 2.3.2.** Supplementation studies of methyl (6S)-6-fluroshikimate.

| | | MIC (µg/mL) | |
|---|---|---|---|
| **Species** | **Condition** | **142** | **125** |
| *Mycobacterium bovis* | control | 32 | - |
| | +Phe[a] | 32 | - |
| | +Trp[a] | 32 | - |
| | +Try[a] | 32 | - |
| | +Phe, Trp, and Try[a] | >64 | - |
| *Escherichia coli* | control | - | 0.25 |
| | +Phe[b] | - | 0.25 |
| | +Trp[b] | - | 0.25 |
| | +Try[b] | - | 0.25 |
| | +Phe, Trp, and Try[b] | - | 0.25 |
| | +PABA[c] | - | >64 |

[a]Amino acids supplemented at 10 µg/mL. [b]Amino acids supplemented at 100 µg/mL. [c]*p*-aminobenzoic acid (PABA) supplemented at 0.001 ug/mL, *E.coli* data is included from Davies et al.[112]

## *2.4. Conclusion*

We were able to successfully synthesize both epimers of 6-fluoroshikimate (**125**, **144**) and methyl 6-fluoroshikimate (**142, 143**) as potential anti-tubercular agents. The acid epimers **125** and **144** both had no anti-tubercular activity while methyl 6-fluoroshikimate (**142, 143**) displayed moderate activity against *M. tuberculosis* (H37Rv) in MIC assays. Supplementation studies in *M. bovis* with the most active methyl (6*S*)-6-fluoroshikimate (**142**) revealed that these analogues may act on the aromatic amino acid biosynthetic pathway. Further investigations need to be performed on the methyl ester analogues to assess if they can inhibit other chorismate-utilizing pathways in *Mtb*, such as the menaquinone pathway, folate pathway, and siderophore pathway. Investigations also need to be done upon the possible bioactivation of the methyl 6-fluoroshikimate epimers (**142, 143**) to 2-fluorochorismate in *Mtb*. The most active analogue methyl (6*S*)-6-fluoroshikimate (**142**) seems to be an interesting inhibitor of the shikimate pathway of *Mtb*.

## *Experimental procedures*

### General chemistry procedures

Chemicals and solvents were purchased from Acros Organics, Alfa Aesar, Sigma-Aldrich, and TCI America and were used as received. An anhydrous solvent dispensing system using two packed columns of neutral alumina was used for drying THF, MeOH, Toluene, and $CH_2Cl_2$, while two packed columns of molecular sieves were used to dry DMF, and the solvents were dispensed under argon (Ar). EtOAc and hexanes were purchased from Fisher Scientific. TLC analyses were performed on TLC silica gel plates 60F254 from EMD Chemical Inc. and were visualized with UV light or $KMnO_4$ stains. Optical rotations values were obtained on a polarimeter using a 1 dm cell. Purification by flash chromatography was performed using a medium-pressure flash chromatography system equipped with flash column silica cartridges with the indicated solvent

system or manual silica gel columns. Preparative reversed-phase HPLC purification was performed on a Phenomenex Gemini 10 μm C18 250 × 20 mm column operating at 21.0 mL/min with detection at 254 nm with the indicated solvent system (Method A). $^{1}H$ and $^{13}C$ spectra were recorded on either 400, 500, or 600 MHz NMR spectrometers. Proton chemical shifts are reported in ppm from an internal standard of residual chloroform (7.26), methanol (3.31), or dimethyl sulfoxide (2.50); carbon chemical shifts are reported in ppm from an internal standard of residual chloroform (77.0), methanol (49.1), or dimethyl sulfoxide (39.5). Proton chemical data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, dt = doublet of triplets, t = triplet, q = quartet, pentet = pent, m = multiplet, ap = apparent, br = broad, ovlp = overlapping), coupling constant(s), integration.

## MIC experiments

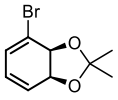
The minimum inhibitory concentration ($MIC_{90}$) was defined as the concentration of drug required to inhibit 90% of growth relative to a no drug control. Drugs were added using a $log_2$ dilution scheme, cultures were incubated at 37 °C and the $OD_{600}$ was measured at day 14 to determine the $MIC_{90}$. *M. tuberculosis* H37Rv was grown to mid log phase in Middlebrook 7H9 broth supplemented with 0.2% glycerol, 10% OADC and 0.05% tyloxapol prior to dilution to an OD600 of 0.01 in media containing compounds.

## Supplementation studies

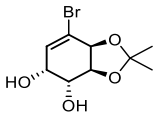
The minimum inhibitory concentrations (MICs) of Methyl-(6S)-6-fluroshikimate were determined by using the microdilution method. Twofold dilution series of Methyl-(6S)-6-fluroshikimate in Middlebrook 7H9 medium supplemented with oleate-albumin-dextrose-catalase (OADC; 10%, vol/vol), glycerol (0.2%, vol/vol), and tyloxapol (0.05%, vol/vol) (7H9-glycerol) was prepared in 96-well flat-bottom plates (TPP). *Mycobacterium bovis* BCG strain Montréal was

grown in 7H9-glycerol to mid-log phase. The cultures were inoculated into each well containing 7H9-glycerol to $OD_{600}$ 0.01. MICs were read spectrophotometrically ($OD_{600}$) to determine the minimum amount of Methyl-(6S)-6-fluroshikimate required to inhibit at least 50% of growth relative to a no drug control after 14 days incubation at 37 °C.

## Synthesis of compounds 125-144

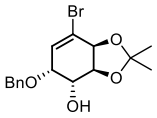



**(3a*S*,7a*S*)-4-bromo-2,2-dimethyl-3a,7a-dihydrobenzo[d][1,3]dioxole (127).** To a solution of cis-(1*S*, 2*S*)-1,2-dihydroxy-3-bromocyclohexa-3,5-diene (**126**, 203 mg, 1.06 mmol) in acetone (1 mL) was added 2,2-dimethoxypropane (3.95 mmol, 0.5 mL) and *p*-toluenesulfonic acid monohydrate (17 mg, 0.09 mmol). Reaction was quenched with addition of aqueous sat. $NaHCO_3$ (5 mL) and extracted with EtOAc (3x10 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated under reduced pressure to afford the title compound (245 mg, 99% yield) as a colorless oil $^1$H NMR (500 MHz, $CDCl_3$) δ 6.35 (d, *J* = 6.09 Hz, 1H), 5.97 (ddd, *J* = 9.54, 2.72, 1.23 Hz, 1H), 5.88 (dd, *J* = 9.62, 6.08 Hz, 1H), 4.73 (d, *J* = 1.76 Hz, 2H), 1.44 (d, *J* = 7.50 Hz, 7H).

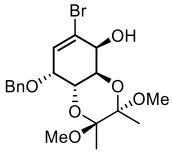



**((3a*S*,4*R*,5*R*,7a*S*)-7-bromo-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3] dioxole-4,5-diol (128).** To a solution of (3a*S*,7a*S*)-4-bromo-2,2-dimethyl-3a,7a-dihydrobenzo[d][1,3]dioxole (**128**, 477 mg, 2.06 mmol) in acetone:water (3:1, 30 mL) was added 4-methylmorpholine 4-oxide (374 mg, 3.09 mmol) and osmium tetroxide (2.5% in butanol, 400 uL) and stirred for 25 h. Osmium tetroxide was quenched by addition of saturated aqueous $NaHSO_3$ (10 mL) and the extracted with EtOAc (3x20 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated onto silica gel under reduced pressure. Flash column chromatography

($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 9:1) afford the title compound (493 mg, 90% yield) as a yellow oil: $R_f$ = 0.25 (1:1 EtOAc/Hexanes); $^1$H NMR (500 MHz, $CDCl_3$) δ 6.16 (dd, *J* = 3.21, 0.96 Hz, 1H), 4.66 (dd, *J* = 5.53, 1.44 Hz, 1H), 4.45 (t, *J* = 5.39 Hz, 1H), 4.38 (dtd, *J* = 6.94, 3.58, 1.34 Hz.

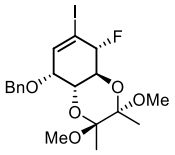



**(3a*S*,4*R*,5*R*,7a*S*)-5-(benzyloxy)-7-bromo-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-4-ol (129).** To a solution of (3a*S*,7a*S*)-4-bromo-2,2-dimethyl-3a,7a-dihydrobenzo[d][1,3]dioxole (**127**, 249 mg, 0.939 mmol) in methanol:toluene (1:1, 3 mL) was added dibutyltinoxide (280 mg, 1.12 mmol) and then refluxed at 110℃ for 4 h. Reaction was cooled to ambient temperature and concentrated. The crude stannylene acetal intermediate was transferred to a dry flask and dissolved in a solution of benzyl bromide (321 mg, 1.88 mmol), tetrabutylammonium bromide (61 mg, 0.188 mmol), and toluene (3 mL). The reaction mixture was then heated to reflux at 110 ℃ for 6 h. The reaction was cooled to ambient temperature and concentrated onto silica gel for column chromatography. Flash column chromatography ($SiO_2$), along with a KF pad, using a stepwise gradient of EtOAc/Hex (0:1 to 9:1) afford the title compound (179 mg, 54% yield) as a colorless oil: $R_f$ = 0.7 (1:1 EtOAc/Hexanes); $^1$H NMR (500 MHz, $CDCl_3$) δ 7.48 – 7.29 (m, 5H), 6.15 (dd, *J* = 2.76, 1.30 Hz, 1H), 4.66 (d, *J* = 1.55 Hz, 1H), 4.65 (q, *J* = 11.4 Hz, 2H), 4.47 (t, *J* = 5.10 Hz, 1H), 4.33 (ddt, *J* = 4.77, 3.53, 1.86 Hz, 1H), 4.17 – 4.11 (m, 1H), 1.40 (d, *J* = 7.79 Hz, 6H).




**(2*S*,3*S*,4aR,5*S*,8*R*,8a*R*)-8-(benzyloxy)-6-bromo-2,3-dimethoxy-2,3-dimethyl-2,3,4a,5,8,8a-hexahydrobenzo[b][1,4]dioxin-5-ol (130).** To a solution of (3a*S*,4*R*,5*R*,7a*S*)-5-

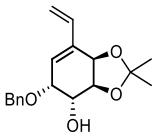
(benzyloxy)-7-bromo-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxol-4-ol (**129**, 320 mg, 0.90 mmol) in methanol (3 mL) was added (1*S*)-(+)-10-camphorsulfonic acid (22 mg, 0.095 mmol), trimethyl orthoformate (1.0 g, 9.5 mmol), 2,3-butanedione (340 mg, 4.0 mmol), and boron trifluorodiethyl etherate (13 mg, 0.09 mmol) which was added dropwise. The reaction mixture was heated to reflux at 80 °C for 23 h. The reaction was cooled to ambient temperature and concentrated onto silica gel for column chromatography. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 1:1) afford the title compound (287 mg, 74% yield) as a yellow oil: $R_f$ = 0.5 (1:1 EtOAc/Hexanes); $^1$H NMR matched previously reported values.[129]



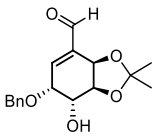

**(2S,3S,4aS,5R,8R,8aR)-8-(benzyloxy)-6-bromo-5-fluoro-2,3-dimethoxy-2,3-dimethyl-2,3,4a,5,8,8a-hexahydrobenzo[b][1,4]dioxine (131).** To a solution of (2*S*,3*S*,4aR,5*S*,8*R*,8a*R*)-8-(benzyloxy)-6-bromo-2,3-dimethoxy-2,3-dimethyl-2,3,4a,5,8,8a-hexahydrobenzo[b][1,4]dioxin-5-ol (**130**, 34 mg, .079 mmol) in n-butanol (1 mL) was added copper(I) iodide (0.008 mmol, 2 mg), patossium iodide (20 mg, 0.12 mmol), and N,N'-dimethylethylenediamine (5 µL, 0.04 mmol). Reaction was refluxed at 120 °C for 24 h. Reaction mixture was washed with aqueous sat. $NH_4Cl$ (5 mL) and extracted with EtOAc (3x10 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated under reduced pressure onto silica gel. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 1:1) afford the iodo intermediate (crude 25 mg,) as a yellow oil: $R_f$ = 0.45 (1:1 EtOAc/Hexanes); $^1$H NMR matched previously reported values.[118]

To a dry flask. with teflon stir bar added the allylic alcohol (108 mg, 0.23 mmol) and dissolved in dry $CH_2Cl_2$ (1.5 mL) and flushed with nitrogen. The reaction mixture was cooled at -

78 °C and added DAST (76 mg, 0.47 mmol) dropwise. Let warm to ambient temperature and stirred for 2 h. The reaction mixtuAdded sodium carbonate and 4 mL of methanol after cooling to -20°C on an ethylene glycol and dry ice bath and filtered off the solids. Concentrated organic layers onto silica gel for column chromatography. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 3:1) afford the crude title compound (58 mg, 26% yield) F19 NMR shows one dominant fluorinated isomer plus crude material in the HNMR. $^1$H NMR matched previously reported values.[118]

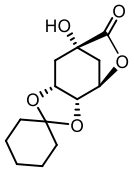



**(3aS,4R,5R,7aR)-5-(benzyloxy)-2,2-dimethyl-7-vinyl-3a,4,5,7a-tetra-hydrobenzo[d][1,3]dioxol-4-ol (134).** To a solution of the protected vinyl bromide intermediate (**129**, 101 mg, 0.33 mmol) in a solution of cis-(1S, 2S)-1,2-dihydroxy-3-bromocyclohexa-3,5-diene (**2**, 203 mg, 1.06 mmol) in acetone (1 mL) was added 2,2-dimethoxypropane (3.95 mmol, 0.5 mL) and *p*-toluenesulfonic acid monohydrate (17 mg, 0.09 mmol). Reaction was quenched with addition of aqueous sat. $NaHCO_3$ (5 mL) and extracted with EtOAc (3x10 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated under reduced pressure to afford the title compound (245 mg, 99% yield) as a colorless oil $^1$H NMR (500 MHz, $CDCl_3$) δ 7.44 – 7.29 (m, 5H), 6.34 (dd, *J* = 17.6, 10.9 Hz, 1H), 5.83 (d, *J* = 3.59 Hz, 1H), 5.55 (d, *J* = 17.6 Hz, 1H), 5.30 (s, 1H), 5.24 (d, *J* = 11 Hz, 1H), 4.88 (d, *J* = 5.94 Hz, 1H), 4.68 (d, *J* = 3.96 Hz, 2H), 4.46 (t, *J* = 6.02 Hz, 1H), 4.21 (t, *J* = 3.75 Hz, 1H), 4.14 (q, *J* = 4.88 Hz, 1H), 1.42 (s, 4H), 1.41 (s, 3H).

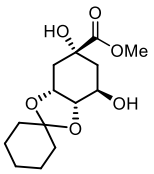



**(3aR,6R,7R,7aS)-6-(benzyloxy)-7-hydroxy-2,2-dimethyl-3a,6,7,7a-tetrahydrobenzo[d][1,3]dioxole-4-carbaldehyde (135).** To a solution of the protected diene

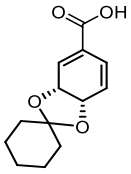
intermediate (**134**, 101 mg, 0.33 mmol) in dioxane:$H_2O$ (0.5 mL, 3:1) added 2-bromopyrimidine (105 mg, 0.66 mmol) followed by osmium tetroxide (135 mg, 0.013 mmol) dropwise. Sodium periodate (284 mg, 1.32 mmol) was then added in one portion. Reaction was quenched after 6 h upon addition of 5% aq. $NaHSO_4$ (5 mL). Aqueous layer was extracted with EtOAc (3x10 mL), organics were combined, dried over $MgSO_4$ and concentrated onto silica gel for column chromatography. Column chromatography ($SiO_2$) with an EtOAc/hexanes gradient (1:5) afforded the title compound (26 mg, 26 % yield) as a yellow oil: $^1$H NMR (500 MHz, $CDCl_3$) δ 9.55 (s, 1H), 7.57 – 7.32 (m, 5H), 6.70 (s, 1H), 5.03 (d, *J* = 5.95 Hz, 1H), 4.76 (s, 2H), 4.54 (t, *J* = 4.94 Hz, 1H), 4.46 (d, *J* = 4.45 Hz, 1H), 4.42 (s, 1H), 1.39 (s, 3H), 1.31 (s, 3H).




**(3a'R,4'R,7'S,8a'R)-7'-hydroxytetrahydrospiro[cyclohexane-1,2'-[4,7]methano [1,3] ioxolo[4,5-c]oxepin]-6'(4'H)-one (136).** A suspension of (-)-quinic acid (5.00 g, 26.0 mmol), cyclohexanone (13.5 mL, 130 mmol), and p-toluenesulfonic acid (200 mg, 1.04 mmol) in toluene (40 mL) was refluxed with a Dean-Stark trap until all the solids had dissolved. The reaction was cooled to room temperature and then poured into cold aqueous sat. $NaHCO_3$ (40 mL). The aqueous layer was extracted with $CH_2Cl_2$ (3 x 40 mL), the organic extracts were combined, washed successively with water and brine, dried over $Na_2SO_4$, filtered, and concentrated. The solid residue was triturated with hexanes, filtered, washed with hexanes, and dried to give the title compound (5.90 g, 89 % yield) as pale yellow needles: $^1$H NMR (400 MHz, $CDCl_3$) δ 4.73 (dd, *J* = 6.2, 2.6 Hz, 1H), 4.47 (ddd, *J* = 7.9, 6.6, 3.0 Hz, 1H), 4.30 (ddd, *J* = 6.5, 2.7, 1.4 Hz, 1H), 2.66 (d, *J* = 11.8 Hz, 1H), 2.39-2.16 (m, 2H), 2.18 (dd, *J* = 14.6, 3.0 Hz, 1H), 1.72-1.38 (m, 10H).



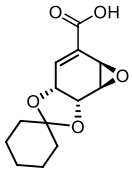
**methyl (3aS,4R,6R,7aR)-4,6-dihydroxyhexahydrospiro[benzo[d][1,3]dioxole-2,1'-cyclohexane]-6-carboxylate (137).** To a solution the lactone (**136**, 5.9 g, 23.23 mmol) and methanol:$CH_2Cl_2$ (1:1, 20 mL) at 0 ºC was added cold sodium methoxide (1 M in MeOH, 27 mL) dropwise. The reaction was to warm up to room temperature over 5 h and then quenched by the slow addition of glacial acetic acid (27 mmol, 1.6 mL). The aqueous layer was extracted with $CH_2Cl_2$ (3 x 30 mL) and the organic extracts were combined, washed successively with water and brine, dried over $Na_2SO_4$, filtered, and concentrated on to silica gel. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 1:1) afforded the title compound (4.1 g, 62 % yield) as a white foam: $R_f$ = 0.22 (1:1 EtOAc/Hexanes); $^1$H NMR (400 MHz, $CDCl_3$) δ 4.46-4.43 (m, 1H), 4.10 (dddd, *J* = 10.9, 5.9, 4.9, 1.3 Hz, 1H), 3.96 (ddd, *J* = 6.7, 5.6, 1.2 Hz, 1H), 3.79 (d, *J* = 1.2f Hz, 3H), 2.26-2.23 (m, 2H), 2.10-2.02 (m, 1H), 1.84 (ddd, *J* = 13.7, 10.8, 1.2 Hz, 1H), 1.73-1.35 (m, 10H).



**(3aR,7aS)-3a,7a-dihydrospiro[benzo[d][1,3]dioxole-2,1'-cyclohexane]-5-carboxylic acid (139).** To a solution of diol (**138**, 7.0 g, 24.4 mmol) in $CH_2Cl_2$ (200 mL) were added pyridine (9.85 mL, 122 mmol) and catalytic DMAP (388 mg, 3.18 mmol) and cooled to 0 ˚C. A solution of triflic anhydride (9.05 mL, 53.8 mmol) in $CH_2Cl_2$ (200 mL) was added slowly to the diol mixture at 0 ˚C with stirring. The reaction mixture was allowed to warm to rt while stirring for 4 h and then the reaction mixture was treated with aqueous $NaHCO_3$ and then extracted with $CH_2Cl_2$. The organic layer was dried over MgSO4, filtered, and evaporated to give a crude residue

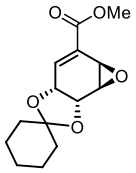
containing triflates and the product diene. The crude mixture was dissolved in DMF (40 mL) and CsOAc (4.83 g, 25.2 mmol) was added to the solution with stirring at rt. After 3 h, the reaction mixture was separated between cold t-butylmethylether (30 mL) and $H_2O$, and then extracted with t-butylmethylether (30 mL). The organic layers were combined washed with brine twice, dried over $MgSO_4$, filtered, and evaporated to afford crude diene ester (x, 4.2 g) which was unstable on silica gel and was moved on to the next step without further purification: $R_f$ = 0.62 (1:3 EtOAc/Hexanes); $^1$H NMR (400 MHz, $CDCl_3$) δ 6.85 (m, 1H), 6.51 (dd, *J* = 10.0, 1.2 Hz , 1H), 6.03 (dd, *J* = 10.0, 4.0 Hz , 1H), 4.79 (dd, *J* = 8.8, 3.7 Hz, 1H), 4.61 (ddt, *J* = 8.9, 4.1, 0.9 Hz, 1H) 3.78 (d, *J* = 0.8 Hz, 3H), 1.62-1.38 (m, 10 H).

To a solution of the crude diene-ester mixture (0.92 g, 3.68 mmol) in THF:water (1:1, 20 mL) at 0 °C was added LiOH (185 mg, 7.72 mmol) and stirred at that temperature until all the starting material was consumed (45 min). The pH was adjusted to ~2 by addition of 2H HCl and then extracted with EtOAc (20 mL). The organic layer was dried ($MgSO_4$) and evaporated to afford the crude acid. Flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 7:3) afforded the title compound (0.82 g, 60 % yield over 3 steps) as a white foam: $R_f$ = 0.2 (3:1 EtOAc/Hexanes); $^1$H NMR (400 MHz, $CDCl_3$) δ 7.01 (d, *J* = 3.6 Hz, 1H), 6.53 (d, *J* = 9.9 Hz, 1H), 6.09 (dd, *J* = 10.0, 4.1 Hz, 1H), 4.85 (dd, *J* = 8.8, 3.6 Hz, 1H), 4.65 (dd, *J* = 8.8, 4.1 Hz, 1H), 1.74 – 1.53 (m, 8H), 1.46 – 1.36 (m, 2H).

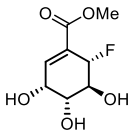



**(3a'R,5a'R,6a'R,6b'R)-3a',5a',6a',6b'-tetrahydrospiro[cyclohexane-1,2'-oxireno[2',3':3,4]benzo[1,2-d][1,3]dioxole]-5'-carboxylic acid (140).** To a solution of the diene-carboxylic acid (**139**, 0.82 g, 3.47 mmol) in $CH_2Cl_2$ (70 mL) was added mCPBA (0.86 g, 3.47

mmol) and stirred at rt for 20 h. The reaction mixture was concentrated and then triturated with $CH_2Cl_2$ (30 mL), filtered, and the filtrate was evaporated and purified by flash column chromatography ($SiO_2$) using a stepwise gradient of EtOAc/Hex (0:1 to 1:0) afforded the title compound (0.60 g, 69 % yield) as a white foam: $R_f$ = 0.35 (3:1 EtOAc/Hexanes); $^1$H NMR (400 MHz, $CDCl_3$) δ 10.02 (br s, 1H), 6.96 (s, 1H), 4.81 (d, *J* = 6.8 Hz , 1H), 4.60 (d, *J* = 6.9 Hz , 1H), 3.98 (d, *J* = 3.8 Hz, 1H), 3.71 (d, *J* = 3.5 Hz, 1H), 1.64-1.25 (m, 10H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 170.2, 142.7, 126.9, 112.0, 71.1, 70.1, 49.4, 46.0, 37.6, 35.4, 25.0, 24.1, 23.9.

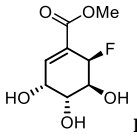



**methyl (3a'R,5a'R,6a'R,6b'R)-3a',5a',6a',6b'-tetrahydrospiro[cyclohexane-1,2'-oxireno[2',3':3,4]benzo[1,2-d][1,3]dioxole]-5'-carboxylate (141).** To a solution of the epoxide-carboxylic acid (**140**, 0.30 g, 1.19 mmol) in acetone (5 mL) was added $K_2CO_3$ (0.164 g, 1.19 mmol) and methyl iodide (0.338 g, 2.38 mmol) at room temperature. The reaction was heated to reflux for 2 h at which point the reaction mixture was filtered, the cake was washed with acetone, and concentrated. The crude methyl ester was then purified by flash column chromatography ($SiO_2$) using a EtOAc/Hex (1:5) gradient afforded the title compound (0.17 g, 54 % yield) as a colorless oil: $R_f$ = 0.75 (1:1 EtOAc/Hexanes); $^1$H NMR (400 MHz, $CDCl_3$) δ 6.80 (s, 1H), 4.77 (dd, *J* = 6.9, 2.1 Hz, 1H), 4.55 (dd, *J* = 6.8, 2.4 Hz, 1H), 3.99 – 3.93 (m, 1H), 3.81 (s, 3H), 3.66 (dd, *J* = 3.8, 2.1 Hz, 1H), 1.63 – 1.34 (m, 10H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 165.7, 140.4, 127.3, 111.8, 71.0, 70.2, 52.4, 49.5, 46.3, 37.6, 35.4, 25.0, 24.1, 23.9.

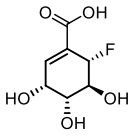



**methyl (3R,4R,5S,6S)-6-fluoro-3,4,5-trihydroxycyclohex-1-ene-1-carboxylate (142).** To a solution of the epoxide-ester (**141**, 0.17 g, 0.64 mmol) in dry $CH_2Cl_2$ (5 mL) was added

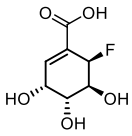
70% HF.pyridine (1.7 mL) and stirred at room temperature for 20 min. The reaction was quenched by dropwise addition of 2N NaOH until the pH of the aqueous layer reached ~5. The layers were separated and the aqueous layer was died under reduced pressure and reconstituted in MeOH, filtered and washed with MeOH. The combined MeOH layers were evaporated and was then purified by flash column chromatography ($SiO_2$) using a stepwise gradient of $CH_2Cl_2$/MeOH (1:0 to 3:20) afforded the title compound (84 mg, 64 % yield determined by NMR) as a mixture of two isomers in the ratio of 61:39 as determined by 1H and 19F NMR (see data). The separation of epimers was performed by Prep-HPLC using a C-18 column. Solvents were (A) 0.1% formic acid in $H_2O$ (B) 0.1% formic acid in acetonitrile. Detection was performed in 235 and 213 nm wavelengths. Retention time were 6.0 (first epimer) and 6.7 min (second epimer). The lyophilization of pooled fraction provided pure epimers as white solids (first epimer = 26 mg and second epimer = 13 mg): $^{1}$H NMR (400 MHz, MeOD) δ 6.95 (d, *J* = 4.4 Hz, 1H), 5.12 (dd, *J* = 47.9, 5.2 Hz, 1H), 4.39 (q, *J* = 4.2 Hz, 1H), 4.12 (ddd, *J* = 17.2, 8.4, 5.1 Hz, 1H), 3.82 (s, 3H), 3.67 (dd, *J* = 8.4, 4.1 Hz, 1H); $^{13}$C NMR (101 MHz, MeOD) δ 167.1, 142.01 (d, *J* = 6.1 Hz), 130.88 (d, *J* = 19.3 Hz), 90.21 (d, *J* = 172.8 Hz), 71.57 (d, *J* = 21.1 Hz), 70.50 (d, *J* = 6.8 Hz), 66.85 (d, *J* = 2.3 Hz), 52.6.




**methyl (3R,4R,5S,6R)-6-fluoro-3,4,5-trihydroxycyclohex-1-ene-1-carboxylate (143).** Using the method described for compound **142:** $^{1}$H NMR (400 MHz, MeOD) δ 7.04 (dd, *J* = 3.8, 2.5 Hz, 1H), 5.24 (dd, *J* = 46.4, 3.5 Hz, 1H), 4.38 (ddd, *J* = 10.2, 7.9, 2.5 Hz, 1H), 4.15 (ddd, *J* = 8.1, 3.6, 2.5 Hz, 1H), 3.82 (s, 3H), 3.74 (dt, *J* = 8.0, 2.4 Hz, 1H); $^{13}$C NMR (101 MHz, $MeOD_3$) δ 167.1, 147.8 (d, *J* = 7.1 Hz), 127.6 (d, *J* = 16.9 Hz), 87.7 (d, *J* = 169.5 Hz), 72.13, 72.13 (d, *J* = 26.9 Hz), 69.6 (d, *J* = 3.1 Hz), 52.6.




**(3R,4R,5S,6S)-6-fluoro-3,4,5-trihydroxycyclohex-1-ene-1-carboxylic acid (125).** To a solution of the fluoroshikimate ester (**142**, 16 mg) in deionized water (1 mL) was added HCl (0.1 mL. 6M in water) and heated to 65 $^{o}$C for 15 h. Reaction mixture was diluted in MeOH, filtered, and directly purified by Prep HPLC: $^{1}$H NMR (400 MHz, MeOD) δ 6.95 (dt, *J* = 4.5, 1.2 Hz, 1H), 5.12 (ddt, *J* = 47.7, 5.2, 0.9 Hz, 1H), 4.39 (td, *J* = 4.3, 2.6 Hz, 1H), 4.12 (ddd, *J* = 17.2, 8.4, 5.1 Hz, 1H), 3.82 (s, 3H), 3.67 (dd, *J* = 8.4, 4.1 Hz, 1H).




**(3R,4R,5S,6R)-6-fluoro-3,4,5-trihydroxycyclohex-1-ene-1-carboxylic acid (144).** Using the method described for compound **125**; $^{1}$H NMR matched previously reported NMR.[118–120]

# Bibliography

(1) NVSS - Mortality - Historical Data https://www.cdc.gov/nchs/nvss/mortality_historical_data.htm (accessed Jun 17, 2020).

(2) CDC. The biggest antibiotic-resistant threats in the U.S. https://www.cdc.gov/drugresistance/biggest-threats.html (accessed Jan 28, 2020).

(3) Antibiotic Resistance Threats in the United States, 2019. **2019**, 148.

(4) Wertheim, H. F.; Melles, D. C.; Vos, M. C.; van Leeuwen, W.; van Belkum, A.; Verbrugh, H. A.; Nouwen, J. L. The Role of Nasal Carriage in Staphylococcus Aureus Infections. *Lancet Infect. Dis.* **2005**, *5* (12), 751–762. https://doi.org/10.1016/S1473-3099(05)70295-4.

(5) Dryden, M.; Baguneid, M.; Eckmann, C.; Corman, S.; Stephens, J.; Solem, C.; Li, J.; Charbonneau, C.; Baillon-Plot, N.; Haider, S. Pathophysiology and Burden of Infection in Patients with Diabetes Mellitus and Peripheral Vascular Disease: Focus on Skin and Soft-Tissue Infections. *Clin. Microbiol. Infect.* **2015**, *21*, S27–S32. https://doi.org/10.1016/j.cmi.2015.03.024.

(6) Weinke, T.; Schiller, R.; Fehrenbach, F. J.; Pohle, H. D. Association BetweenStaphylococcus Aureus Nasopharyngeal Colonization and Septicemia in Patients Infected with the Human Immunodeficiency Virus. *Eur. J. Clin. Microbiol. Infect. Dis.* **1992**, *11* (11), 985–989. https://doi.org/10.1007/BF01967787.

(7) Percival, S. L.; Suleman, L.; Vuotto, C.; Donelli, G. Healthcare-Associated Infections, Medical Devices and Biofilms: Risk, Tolerance and Control. *J. Med. Microbiol.* **2015**, *64* (4), 323–334. https://doi.org/10.1099/jmm.0.000032.

(8) Hidron, A. I.; Edwards, J. R.; Patel, J.; Horan, T. C.; Sievert, D. M.; Pollock, D. A.; Fridkin, S. K.; National Healthcare Safety Network Team and Participating National Healthcare Safety Network Facilities. Antimicrobial-Resistant Pathogens Associated With Healthcare-Associated Infections: Annual Summary of Data Reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. *Infect. Control Hosp. Epidemiol.* **2008**, *29* (11), 996–1011. https://doi.org/10.1086/591861.

(9) Ronan, M. V.; Herzig, S. J. Hospitalizations Related to Opioid Abuse/Dependence and Associated Serious Infections from 2002 to 2012. *Health Aff. Proj. Hope* **2016**, *35* (5), 832–837. https://doi.org/10.1377/hlthaff.2015.1424.

(10) Kourtis, A. P. Vital Signs: Epidemiology and Recent Trends in Methicillin-Resistant and in Methicillin-Susceptible Staphylococcus Aureus Bloodstream Infections — United States. *MMWR Morb. Mortal. Wkly. Rep.* **2019**, *68*. https://doi.org/10.15585/mmwr.mm6809e1.

(11) Chambers, H. F.; DeLeo, F. R. Waves of Resistance: Staphylococcus Aureus in the Antibiotic Era. *Nat. Rev. Microbiol.* **2009**, *7* (9), 629–641. https://doi.org/10.1038/nrmicro2200.

(12) Lakhundi, S.; Zhang, K. Methicillin-Resistant Staphylococcus Aureus: Molecular Characterization, Evolution, and Epidemiology. *Clin. Microbiol. Rev.* **2018**, *31* (4). https://doi.org/10.1128/CMR.00020-18.

(13) Jevons, M. P. "Celbenin" - Resistant Staphylococci. *Br. Med. J.* **1961**, *1* (5219), 124–125.

(14) Lim, D.; Strynadka, N. C. J. Structural Basis for the β Lactam Resistance of PBP2a from Methicillin-Resistant Staphylococcus Aureus. *Nat. Struct. Biol.* **2002**, *9* (11), 870.

(15) Hartman, B. J.; Tomasz, A. Low-Affinity Penicillin-Binding Protein Associated with Beta-Lactam Resistance in Staphylococcus Aureus. *J. Bacteriol.* **1984**, *158* (2), 513–516.

(16) Kosowska-Shick, K.; McGhee, P. L.; Appelbaum, P. C. Affinity of Ceftaroline and Other β-Lactams for Penicillin-Binding Proteins from Staphylococcus Aureus and Streptococcus Pneumoniae. *Antimicrob. Agents Chemother.* **2010**, *54* (5), 1670–1677. https://doi.org/10.1128/AAC.00019-10.

(17) Shore, A. C.; Coleman, D. C. Staphylococcal Cassette Chromosome Mec: Recent Advances and New Insights. *Int. J. Med. Microbiol.* **2013**, *303* (6), 350–359. https://doi.org/10.1016/j.ijmm.2013.02.002.

(18) Urushibara, N.; Aung, M. S.; Kawaguchiya, M.; Kobayashi, N. Novel Staphylococcal Cassette Chromosome Mec (SCCmec) Type XIV (5A) and a Truncated SCCmec Element in SCC Composite Islands Carrying SpeG in ST5 MRSA in Japan. *J. Antimicrob. Chemother.* **2020**, *75* (1), 46–50. https://doi.org/10.1093/jac/dkz406.

(19) Bal, A. M.; Coombs, G. W.; Holden, M. T. G.; Lindsay, J. A.; Nimmo, G. R.; Tattevin, P.; Skov, R. L. Genomic Insights into the Emergence and Spread of International Clones of Healthcare-, Community- and Livestock-Associated Meticillin-Resistant Staphylococcus Aureus: Blurring of the Traditional Definitions. *J. Glob. Antimicrob. Resist.* **2016**, *6*, 95–101. https://doi.org/10.1016/j.jgar.2016.04.004.

(20) Côrtes, M. F.; Botelho, A. M.; Almeida, L. G.; Souza, R. C.; de Lima Cunha, O.; Nicolás, M. F.; Vasconcelos, A. T.; Figueiredo, A. M. Community-Acquired Methicillin-Resistant Staphylococcus Aureus from ST1 Lineage Harboring a New SCCmec IV Subtype (SCCmec IVm) Containing the TetK Gene. *Infect. Drug Resist.* **2018**, *11*, 2583–2592. https://doi.org/10.2147/IDR.S175079.

(21) Sievert, D. M.; Rudrik, J. T.; Patel, J. B.; McDonald, L. C.; Wilkins, M. J.; Hageman, J. C. Vancomycin-Resistant Staphylococcus Aureus in the United States, 2002–2006. *Clin. Infect. Dis.* **2008**, *46* (5), 668–674. https://doi.org/10.1086/527392.

(22) Mangili, A.; Bica, I.; Snydman, D. R.; Hamer, D. H. Daptomycin-Resistant, Methicillin-Resistant Staphylococcus Aureus Bacteremia. *Clin. Infect. Dis.* **2005**, *40* (7), 1058–1060. https://doi.org/10.1086/428616.

(23) Faridi, A.; Kareshk, A. T.; Fatahi-Bafghi, M.; Ziasistani, M.; Ghahraman, M. R. K.; Seyyed-Yousefi, S. Z.; Shakeri, N.; Kalantar-Neyestanaki, D. Detection of Methicillin-Resistant Staphylococcus Aureus (MRSA) in Clinical Samples of Patients with External Ocular Infection. *Iran. J. Microbiol.* **2018**, *10* (4), 215–219.

(24) Mohammadi, S.; Sekawi, Z.; Monjezi, A.; Maleki, M.-H.; Soroush, S.; Sadeghifard, N.; Pakzad, I.; Azizi-Jalilian, F.; Emaneini, M.; Asadollahi, K.; Pourahmad, F.; Zarrilli, R.; Taherikalani, M. Emergence of SCCmec Type III with Variable Antimicrobial Resistance Profiles and Spa Types among Methicillin-Resistant Staphylococcus Aureus Isolated from Healthcare- and Community-Acquired Infections in the West of Iran. *Int. J. Infect. Dis. IJID Off. Publ. Int. Soc. Infect. Dis.* **2014**, *25*, 152–158. https://doi.org/10.1016/j.ijid.2014.02.018.

(25) Mahairas, G. G.; Lyon, B. R.; Skurray, R. A.; Pattee, P. A. Genetic Analysis of Staphylococcus Aureus with Tn4001. *J. Bacteriol.* **1989**, *171* (7), 3968–3972.

(26) Butler, M. S.; Blaskovich, M. A.; Cooper, M. A. Antibiotics in the Clinical Pipeline at the End of 2015. *J. Antibiot. (Tokyo)* **2017**, *70* (1), 3–24. https://doi.org/10.1038/ja.2016.72.

(27) Research, C. for D. E. and. Novel Drug Approvals for 2016. *FDA* **2020**.

(28) Research, C. for D. E. and. Novel Drug Approvals for 2017. *FDA* **2020**.

(29) Research, C. for D. E. and. Novel Drug Approvals for 2018. *FDA* **2020**.

(30) Research, C. for D. E. and. New Drug Therapy Approvals 2019. *FDA* **2020**.

(31) Liu, C.; Bayer, A.; Cosgrove, S. E.; Daum, R. S.; Fridkin, S. K.; Gorwitz, R. J.; Kaplan, S. L.; Karchmer, A. W.; Levine, D. P.; Murray, B. E.; Rybak, M. J.; Talan, D. A.; Chambers, H. F. Clinical Practice Guidelines by the Infectious Diseases Society of America for the Treatment of Methicillin-Resistant Staphylococcus Aureus Infections in Adults and Children. *Clin. Infect. Dis.* **2011**, *52* (3), e18–e55. https://doi.org/10.1093/cid/ciq146.

(32) Gu, B.; Kelesidis, T.; Tsiodras, S.; Hindler, J.; Humphries, R. M. The Emerging Problem of Linezolid-Resistant Staphylococcus. *J. Antimicrob. Chemother.* **2013**, *68* (1), 4–11. https://doi.org/10.1093/jac/dks354.

(33) Silver, L. L. Challenges of Antibacterial Discovery. *Clin. Microbiol. Rev.* **2011**, *24* (1), 71–109. https://doi.org/10.1128/CMR.00030-10.

(34) Sohlenkamp, C.; Geiger, O. Bacterial Membrane Lipids: Diversity in Structures and Pathways. *FEMS Microbiol. Rev.* **2016**, *40* (1), 133–159. https://doi.org/10.1093/femsre/fuv008.

(35) Epand, R. M.; Walker, C.; Epand, R. F.; Magarvey, N. A. Molecular Mechanisms of Membrane Targeting Antibiotics. *Biochim. Biophys. Acta BBA - Biomembr.* **2016**, *1858* (5), 980–987. https://doi.org/10.1016/j.bbamem.2015.10.018.

(36) García-Lara, J.; Weihs, F.; Ma, X.; Walker, L.; Chaudhuri, R. R.; Kasturiarachchi, J.; Crossley, H.; Golestanian, R.; Foster, S. J. Supramolecular Structure in the Membrane of Staphylococcus Aureus. *Proc. Natl. Acad. Sci.* **2015**, *112* (51), 15725–15730. https://doi.org/10.1073/pnas.1509557112.

(37) Hurdle, J. G.; O'Neill, A. J.; Chopra, I.; Lee, R. E. Targeting Bacterial Membrane Function: An Underexploited Mechanism for Treating Persistent Infections. *Nat. Rev. Microbiol.* **2011**, *9* (1), 62–75. https://doi.org/10.1038/nrmicro2474.

(38) van 't Hof, W.; Veerman, E. C.; Helmerhorst, E. J.; Amerongen, A. V. Antimicrobial Peptides: Properties and Applicability. *Biol. Chem.* **2001**, *382* (4), 597–619. https://doi.org/10.1515/BC.2001.072.

(39) Porto, W. F.; Irazazabal, L.; Alves, E. S. F.; Ribeiro, S. M.; Matos, C. O.; Pires, Á. S.; Fensterseifer, I. C. M.; Miranda, V. J.; Haney, E. F.; Humblot, V.; Torres, M. D. T.; Hancock, R. E. W.; Liao, L. M.; Ladram, A.; Lu, T. K.; Fuente-Nunez, C. de la; Franco, O. L. In Silico Optimization of a Guava Antimicrobial Peptide Enables Combinatorial Exploration for Peptide Design. *Nat. Commun.* **2018**, *9* (1), 1–12. https://doi.org/10.1038/s41467-018-03746-3.

(40) Ortwine, J. K.; Kaye, K. S.; Li, J.; Pogue, J. M. Colistin: Understanding and Applying Recent Pharmacokinetic Advances. *Pharmacother. J. Hum. Pharmacol. Drug Ther.* **2015**, *35* (1), 11–16. https://doi.org/10.1002/phar.1484.

(41) Katz, B. E.; Fisher, A. A. Bacitracin: A Unique Topical Antibiotic Sensitizer. *J. Am. Acad. Dermatol.* **1987**, *17* (6), 1016–1024. https://doi.org/10.1016/S0190-9622(87)70292-8.

(42) Pogliano, J.; Pogliano, N.; Silverman, J. A. Daptomycin-Mediated Reorganization of Membrane Architecture Causes Mislocalization of Essential Cell Division Proteins. *J. Bacteriol.* **2012**, *194* (17), 4494–4504. https://doi.org/10.1128/JB.00011-12.

(43) Zhanel, G. G.; Schweizer, F.; Karlowsky, J. A. Oritavancin: Mechanism of Action. *Clin. Infect. Dis.* **2012**, *54* (suppl_3), S214–S219. https://doi.org/10.1093/cid/cir920.

(44) Kim, W.; Zhu, W.; Hendricks, G. L.; Tyne, D. V.; Steele, A. D.; Keohane, C. E.; Fricke, N.; Conery, A. L.; Shen, S.; Pan, W.; Lee, K.; Rajamuthiah, R.; Fuchs, B. B.; Vlahovska, P. M.; Wuest, W. M.; Gilmore, M. S.; Gao, H.; Ausubel, F. M.; Mylonakis, E. A New Class of Synthetic Retinoid Antibiotics Effective against Bacterial Persisters. *Nature* **2018**, *556* (7699), 103–107. https://doi.org/10.1038/nature26157.

(45) Nair, D. R.; Chen, J.; Monteiro, J. M.; Josten, M.; Pinho, M. G.; Sahl, H.-G.; Wu, J.; Cheung, A. A Quinolinol-Based Small Molecule with Anti-MRSA Activity That Targets Bacterial Membrane and Promotes Fermentative Metabolism. *J. Antibiot. (Tokyo)* **2017**, *70* (10), 1009–1019. https://doi.org/10.1038/ja.2017.79.

(46) Kumar, P. A Review on Quinoline Derivatives as Anti-Methicillin Resistant Staphylococcus Aureus (MRSA) Agents. *BMC Chem.* **2020**, *14* (1). https://doi.org/10.1186/s13065-020-00669-3.

(47) Conrad, M.; Limpach, L. Synthesen von Chinolinderivaten Mittelst Acetessigester. *Berichte Dtsch. Chem. Ges.* **1887**, *20* (1), 944–948. https://doi.org/10.1002/cber.188702001215.

(48) Garner, R.; Suschitzky, H. Syntheses of Heterocyclic Compounds. Part XI. Quinolines and Pyrones from the Reaction of N-Aminophenyl Heterocycles with β-Ketoesters in Polyphosphoric Acid. *J. Chem. Soc. C Org.* **1966**, No. 0, 186–189. https://doi.org/10.1039/J39660000186.

(49) Reitsema, R. H. The Chemistry of 4-Hydroxyquinolines. *Chem. Rev.* **1948**, *43* (1), 43–68. https://doi.org/10.1021/cr60134a002.

(50) Bunnett, J. F.; Zahler, R. E. Aromatic Nucleophilic Substitution Reactions. *Chem. Rev.* **1951**, *49* (2), 273–412. https://doi.org/10.1021/cr60153a002.

(51) Borrows, E. T.; Clayton, J. C.; Hems, B. A.; Long, A. G. S 43. The Synthesis of Thyroxine and Related Substances. Part II. The Preparation of Dinitrodiphenyl Ethers. *J. Chem. Soc. Resumed* **1949**, No. 0, S190–S199. https://doi.org/10.1039/JR949000S190.

(52) Ashley, J. N.; Harris, J. O.; Dalgliesh, C. E.; Stephenson, E. F. M.; Albert, A.; Magrath, D.; Baddiley, J.; Topham, A.; Reed, R. A. Notes. *J. Chem. Soc. Resumed* **1944**, No. 0, 677–679. https://doi.org/10.1039/JR9440000677.

(53) Kato, Y.; Okada, S.; Tomimoto, K.; Mase, T. A Facile Bromination of Hydroxyheteroarenes. *Tetrahedron Lett.* **2001**, *42* (29), 4849–4851. https://doi.org/10.1016/S0040-4039(01)00864-4.

(54) Guram, A. S.; Rennels, R. A.; Buchwald, S. L. A Simple Catalytic Method for the Conversion of Aryl Bromides to Arylamines. *Angew. Chem. Int. Ed. Engl.* **1995**, *34* (12), 1348–1350. https://doi.org/10.1002/anie.199513481.

(55) Louie, J.; Hartwig, J. F. Palladium-Catalyzed Synthesis of Arylamines from Aryl Halides. Mechanistic Studies Lead to Coupling in the Absence of Tin Reagents. *Tetrahedron Lett.* **1995**, *36* (21), 3609–3612. https://doi.org/10.1016/0040-4039(95)00605-C.

(56) Dorel, R.; Grugel, C. P.; Haydl, A. M. The Buchwald–Hartwig Amination After 25 Years. *Angew. Chem. Int. Ed.* **2019**, *58* (48), 17118–17129. https://doi.org/10.1002/anie.201904795.

(57) Reid, J. G.; Reny Runge, J. H. Addition of Nitroalkanes to Ortho-Halo-Nitrobenzenes: A New Synthesis of 4-Chloro-7-(Trifluoromethyl)Quinoline. *Tetrahedron Lett.* **1990**, *31* (8), 1093–1096. https://doi.org/10.1016/S0040-4039(00)88734-1.

(58) Frueh, L.; Li, Y.; Mather, M. W.; Li, Q.; Pou, S.; Nilsen, A.; Winter, R. W.; Forquer, I. P.; Pershing, A. M.; Xie, L. H.; Smilkstein, M. J.; Caridha, D.; Koop, D. R.; Campbell, R. F.; Sciotti, R. J.; Kreishman-Deitrick, M.; Kelly, J. X.; Vesely, B.; Vaidya, A. B.; Riscoe, M. K. Alkoxycarbonate Ester Prodrugs of Preclinical Drug Candidate ELQ-300 for Prophylaxis and Treatment of Malaria. *ACS Infect. Dis.* **2017**, *3* (10), 728–735. https://doi.org/10.1021/acsinfecdis.7b00062.

(59) Cotsonas King, A.; Wu, L. Macromolecular Synthesis and Membrane Perturbation Assays for Mechanisms of Action Studies of Antimicrobial Agents. *Curr. Protoc. Pharmacol.* **2009**, *Chapter 13*, Unit 13A.7. https://doi.org/10.1002/0471141755.ph13a07s47.

(60) Gerits, E.; Blommaert, E.; Lippell, A.; O'Neill, A. J.; Weytjens, B.; De Maeyer, D.; Fierro, A. C.; Marchal, K.; Marchand, A.; Chaltin, P.; Spincemaille, P.; De Brucker, K.; Thevissen, K.; Cammue, B. P. A.; Swings, T.; Liebens, V.; Fauvart, M.; Verstraeten, N.; Michiels, J. Elucidation of the Mode of Action of a New Antibacterial Compound Active against Staphylococcus Aureus and Pseudomonas Aeruginosa. *PloS One* **2016**, *11* (5), e0155139. https://doi.org/10.1371/journal.pone.0155139.

(61) Hobbs, J. K.; Miller, K.; O'Neill, A. J.; Chopra, I. Consequences of Daptomycin-Mediated Membrane Damage in Staphylococcus Aureus. *J. Antimicrob. Chemother.* **2008**, *62* (5), 1003–1008. https://doi.org/10.1093/jac/dkn321.

(62) Oliva, B.; Miller, K.; Caggiano, N.; O'Neill, A. J.; Cuny, G. D.; Hoemann, M. Z.; Hauske, J. R.; Chopra, I. Biological Properties of Novel Antistaphylococcal Quinoline-Indole Agents. *Antimicrob. Agents Chemother.* **2003**, *47* (2), 458–466. https://doi.org/10.1128/aac.47.2.458-466.2003.

(63) Lee, K.-H.; Oh, J.-E. Design and Synthesis of Novel Antimicrobial Pseudopeptides with Selective Membrane-Perturbation Activity. *Bioorg. Med. Chem.* **2000**, *8* (4), 833–839. https://doi.org/10.1016/S0968-0896(00)00019-5.

(64) Falord, M.; Karimova, G.; Hiron, A.; Msadek, T. GraXSR Proteins Interact with the VraFG ABC Transporter To Form a Five-Component System Required for Cationic Antimicrobial Peptide Sensing and Resistance in Staphylococcus Aureus. *Antimicrob. Agents Chemother.* **2012**, *56* (2), 1047–1058. https://doi.org/10.1128/AAC.05054-11.

(65) Truong-Bolduc, Q. C.; Dunman, P. M.; Strahilevitz, J.; Projan, S. J.; Hooper, D. C. MgrA Is a Multiple Regulator of Two New Efflux Pumps in Staphylococcus Aureus. *J. Bacteriol.* **2005**, *187* (7), 2395–2405. https://doi.org/10.1128/JB.187.7.2395-2405.2005.

(66) CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. Approved GUideline, CLSI Document M07-A10*, Tenth Edition.; Clinical and Laboratory Standards Institute: Clinical and Laboratory Standards Institute, Wayne, PA, 2015.

(67) CLSI. *Methods for Determining Bactericidal Activity of Antimicrobial Agents, Approved Guideline, CLSI Document M26-A*; Clinical and Laboratory Standards Institute: 950 West Valley Road Suite 2500, Wayne, PA 19087, USA, 1998.

(68) Nair, D. R.; Monteiro, J. M.; Memmi, G.; Thanassi, J.; Pucci, M.; Schwartzman, J.; Pinho, M. G.; Cheung, A. L. Characterization of a Novel Small Molecule That Potentiates β-Lactam Activity against Gram-Positive and Gram-Negative Pathogens. *Antimicrob. Agents Chemother.* **2015**, *59* (4), 1876–1885. https://doi.org/10.1128/AAC.04164-14.

(69) WHO | Global tuberculosis report 2019 http://www.who.int/tb/publications/global_report/en/ (accessed May 21, 2020).

(70) Lin, P. L.; Flynn, J. L. Understanding Latent Tuberculosis: A Moving Target. *J. Immunol. Baltim. Md 1950* **2010**, *185* (1), 15–22. https://doi.org/10.4049/jimmunol.0903856.

(71) Treatment of Tuberculosis | NEJM https://www-nejm-org.ezp2.lib.umn.edu/doi/10.1056/NEJMra1413919 (accessed May 8, 2021).

(72) Barry, C. E.; Boshoff, H. I.; Dartois, V.; Dick, T.; Ehrt, S.; Flynn, J.; Schnappinger, D.; Wilkinson, R. J.; Young, D. The Spectrum of Latent Tuberculosis: Rethinking the Biology and Intervention Strategies. *Nat. Rev. Microbiol.* **2009**, *7* (12), 845–855. https://doi.org/10.1038/nrmicro2236.

(73) Magiorakos, A.-P.; Srinivasan, A.; Carey, R. B.; Carmeli, Y.; Falagas, M. E.; Giske, C. G.; Harbarth, S.; Hindler, J. F.; Kahlmeter, G.; Olsson-Liljequist, B.; Paterson, D. L.; Rice, L. B.; Stelling, J.; Struelens, M. J.; Vatopoulos, A.; Weber, J. T.; Monnet, D. L. Multidrug-Resistant, Extensively Drug-Resistant and Pandrug-Resistant Bacteria: An International Expert Proposal for Interim Standard Definitions for Acquired Resistance. *Clin. Microbiol. Infect. Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis.* **2012**, *18* (3), 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x.

(74) Centers for Disease Control and Prevention (CDC). Emergence of Mycobacterium Tuberculosis with Extensive Resistance to Second-Line Drugs--Worldwide, 2000-2004. *MMWR Morb. Mortal. Wkly. Rep.* **2006**, *55* (11), 301–305.

(75) WHO: Global Tuberculosis Report. 2020 - Google Scholar https://scholar.google.com/scholar_lookup?title=Global%20Tuberculosis%20Report%202020&publication_year=2020&author=WHO (accessed May 8, 2021).

(76) Cox, E.; Laessig, K. FDA Approval of Bedaquiline — The Benefit–Risk Balance for Drug-Resistant Tuberculosis. *N. Engl. J. Med.* **2014**, *371* (8), 689–691. https://doi.org/10.1056/NEJMp1314385.

(77) Szumowski, J. D.; Lynch, J. B. Profile of Delamanid for the Treatment of Multidrug-Resistant Tuberculosis. *Drug Des. Devel. Ther.* **2015**, *9*, 677–682. https://doi.org/10.2147/DDDT.S60923.

(78) Roberts, C. W.; Roberts, F.; Lyons, R. E.; Kirisits, M. J.; Mui, E. J.; Finnerty, J.; Johnson, J. J.; Ferguson, D. J. P.; Coggins, J. R.; Krell, T.; Coombs, G. H.; Milhous, W. K.; Kyle, D. E.; Tzipori, S.; Barnwell, J.; Dame, J. B.; Carlton, J.; McLeod, R. The Shikimate Pathway and Its Branches in Apicomplexan Parasites. *J. Infect. Dis.* **2002**, *185 Suppl 1*, S25-36. https://doi.org/10.1086/338004.

(79) Sassetti, C. M.; Boyd, D. H.; Rubin, E. J. Genes Required for Mycobacterial Growth Defined by High Density Mutagenesis. *Mol. Microbiol.* **2003**, *48* (1), 77–84. https://doi.org/10.1046/j.1365-2958.2003.03425.x.

(80) Griffin, J. E.; Gawronski, J. D.; DeJesus, M. A.; Ioerger, T. R.; Akerley, B. J.; Sassetti, C. M. High-Resolution Phenotypic Profiling Defines Genes Essential for Mycobacterial Growth and Cholesterol Catabolism. *PLoS Pathog.* **2011**, *7* (9). https://doi.org/10.1371/journal.ppat.1002251.

(81) Nunes, J. E. S.; Duque, M. A.; de Freitas, T. F.; Galina, L.; Timmers, L. F. S. M.; Bizarro, C. V.; Machado, P.; Basso, L. A.; Ducati, R. G. Mycobacterium Tuberculosis Shikimate Pathway Enzymes as Targets for the Rational Design of Anti-Tuberculosis Drugs. *Molecules* **2020**, *25* (6). https://doi.org/10.3390/molecules25061259.

(82) Svarcova, M.; Kratky, M.; Vinsova, J. Investigation of Potential Inhibitors of Chorismate-Utilizing Enzymes. *Curr. Med. Chem.* **2015**, *22* (11), 1383–1399.

(83) Kolappan, S.; Zwahlen, J.; Zhou, R.; Truglio, J. J.; Tonge, P. J.; Kisker, C. Lysine 190 Is the Catalytic Base in MenF, the Menaquinone-Specific Isochorismate Synthase from Escherichia Coli: Implications for an Enzyme Family. *Biochemistry* **2007**, *46* (4), 946–953. https://doi.org/10.1021/bi0608515.

(84) Walsh, C. T.; Liu, J.; Rusnak, F.; Sakaitani, M. Molecular Studies on Enzymes in Chorismate Metabolism and the Enterobactin Biosynthetic Pathway. *Chem. Rev.* **1990**, *90* (7), 1105–1129. https://doi.org/10.1021/cr00105a003.

(85) Spraggon, G.; Kim, C.; Nguyen-Huu, X.; Yee, M.-C.; Yanofsky, C.; Mills, S. E. The Structures of Anthranilate Synthase of Serratia Marcescens Crystallized in the Presence of

(i) Its Substrates, Chorismate and Glutamine, and a Product, Glutamate, and (Ii) Its End-Product Inhibitor, l-Tryptophan. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98* (11), 6021–6026. https://doi.org/10.1073/pnas.111150298.

(86) Bashiri, G.; Johnston, J. M.; Evans, G. L.; Bulloch, E. M. M.; Goldstone, D. C.; Jirgis, E. N. M.; Kleinboelting, S.; Castell, A.; Ramsay, R. J.; Manos-Turvey, A.; Payne, R. J.; Lott, J. S.; Baker, E. N. Structure and Inhibition of Subunit I of the Anthranilate Synthase Complex of Mycobacterium Tuberculosis and Expression of the Active Complex. *Acta Crystallogr. D Biol. Crystallogr.* **2015**, *71* (11), 2297–2308. https://doi.org/10.1107/S1399004715017216.

(87) Zwahlen, J.; Kolappan, S.; Zhou, R.; Kisker, C.; Tonge, P. J. Structure and Mechanism of MbtI, the Salicylate Synthase from Mycobacterium Tuberculosis. *Biochemistry* **2007**, *46* (4), 954–964. https://doi.org/10.1021/bi060852x.

(88) Sridharan, S.; Howard, N.; Kerbarh, O.; Błaszczyk, M.; Abell, C.; Blundell, T. L. Crystal Structure of Escherichia Coli Enterobactin-Specific Isochorismate Synthase (EntC) Bound to Its Reaction Product Isochorismate: Implications for the Enzyme Mechanism and Differential Activity of Chorismate-Utilizing Enzymes. *J. Mol. Biol.* **2010**, *397* (1), 290–300. https://doi.org/10.1016/j.jmb.2010.01.019.

(89) Zhang, X.-K.; Liu, F.; Fiers, W. D.; Sun, W.-M.; Guo, J.; Liu, Z.; Aldrich, C. C. Synthesis of Transition-State Inhibitors of Chorismate Utilizing Enzymes from Bromobenzene Cis-1,2-Dihydrodiol. *J. Org. Chem.* **2017**, *82* (7), 3432–3440. https://doi.org/10.1021/acs.joc.6b02801.

(90) Pini, E.; Poli, G.; Tuccinardi, T.; Chiarelli, L. R.; Mori, M.; Gelain, A.; Costantino, L.; Villa, S.; Meneghetti, F.; Barlocco, D. New Chromane-Based Derivatives as Inhibitors of Mycobacterium Tuberculosis Salicylate Synthase (MbtI): Preliminary Biological Evaluation and Molecular Modeling Studies. *Mol. J. Synth. Chem. Nat. Prod. Chem.* **2018**, *23* (7). https://doi.org/10.3390/molecules23071506.

(91) Vasan, M.; Neres, J.; Williams, J.; Wilson, D. J.; Teitelbaum, A. M.; Remmel, R. P.; Aldrich, C. C. Inhibitors of the Salicylate Synthase (MbtI) from Mycobacterium Tuberculosis Discovered by High-Throughput Screening. *ChemMedChem* **2010**, *5* (12), 2079–2087. https://doi.org/10.1002/cmdc.201000275.

(92) Dahm, C.; Müller, R.; Schulte, G.; Schmidt, K.; Leistner, E. The Role of Isochorismate Hydroxymutase Genes EntC and MenF in Enterobactin and Menaquinone Biosynthesis in Escherichia Coli. *Biochim. Biophys. Acta* **1998**, *1425* (2), 377–386. https://doi.org/10.1016/s0304-4165(98)00089-0.

(93) Liu, J.; Quinn, N.; Berchtold, G. A.; Walsh, C. T. Overexpression, Purification and Characterization of Isochorismate Synthase (EntC), the First Enzyme Involved in the Biosynthesis of Enterobactin from Chorismate. *Biochemistry* **1990**, *29* (6), 1417–1425. https://doi.org/10.1021/bi00458a012.

(94) Morollo, A. A.; Eck, M. J. Structure of the Cooperative Allosteric Anthranilate Synthase from Salmonella Typhimurium. *Nat. Struct. Biol.* **2001**, *8* (3), 243–247. https://doi.org/10.1038/84988.

(95) Smith, D. A.; Parish, T.; Stoker, N. G.; Bancroft, G. J. Characterization of Auxotrophic Mutants of Mycobacterium Tuberculosis and Their Potential as Vaccine Candidates. *Infect. Immun.* **2001**, *69* (2), 1142–1150. https://doi.org/10.1128/IAI.69.2.1442-1150.2001.

(96) Negatu, D. A.; Yamada, Y.; Xi, Y.; Go, M. L.; Zimmerman, M.; Ganapathy, U.; Dartois, V.; Gengenbacher, M.; Dick, T. Gut Microbiota Metabolite Indole Propionic Acid

Targets Tryptophan Biosynthesis in Mycobacterium Tuberculosis. *mBio* **2019**, *10* (2). https://doi.org/10.1128/mBio.02781-18.

(97) Nurul Islam, M.; Hitchings, R.; Kumar, S.; Fontes, F. L.; Lott, J. S.; Kruh-Garcia, N. A.; Crick, D. C. Mechanism of Fluorinated Anthranilate-Induced Growth Inhibition in Mycobacterium Tuberculosis. *ACS Infect. Dis.* **2019**, *5* (1), 55–62. https://doi.org/10.1021/acsinfecdis.8b00092.

(98) Minato, Y.; Gohl, D. M.; Thiede, J. M.; Chacón, J. M.; Harcombe, W. R.; Maruyama, F.; Baughn, A. D. Genomewide Assessment of Mycobacterium Tuberculosis Conditionally Essential Metabolic Pathways. *mSystems* **2019**, *4* (4). https://doi.org/10.1128/mSystems.00070-19.

(99) Kast, P.; Grisostomi, C.; Chen, I. A.; Li, S.; Krengel, U.; Xue, Y.; Hilvert, D. A Strategically Positioned Cation Is Crucial for Efficient Catalysis by Chorismate Mutase *. *J. Biol. Chem.* **2000**, *275* (47), 36832–36838. https://doi.org/10.1074/jbc.M006351200.

(100) DeClue, M. S.; Baldridge, K. K.; Künzler, D. E.; Kast, P.; Hilvert, D. Isochorismate Pyruvate Lyase: A Pericyclic Reaction Mechanism? *J. Am. Chem. Soc.* **2005**, *127* (43), 15002–15003. https://doi.org/10.1021/ja055871t.

(101) Khanapur, M.; Alvala, M.; Prabhakar, M.; Shiva Kumar, K.; Edwin, R. K.; Sri Saranya, P. S. V. K.; Patel, R. K.; Bulusu, G.; Misra, P.; Pal, M. Mycobacterium Tuberculosis Chorismate Mutase: A Potential Target for TB. *Bioorg. Med. Chem.* **2017**, *25* (6), 1725–1736. https://doi.org/10.1016/j.bmc.2017.02.001.

(102) Schneider, C. Z.; Parish, T.; Basso, L. A.; Santos, D. S. The Two Chorismate Mutases from Both Mycobacterium Tuberculosis and Mycobacterium Smegmatis: Biochemical Analysis and Limited Regulation of Promoter Activity by Aromatic Amino Acids. *J. Bacteriol.* **2008**, *190* (1), 122–134. https://doi.org/10.1128/JB.01332-07.

(103) Yelamanchi, S. D.; Surolia, A. Targeting Amino Acid Metabolism of Mycobacterium Tuberculosis for Developing Inhibitors to Curtail Its Survival. *IUBMB Life* **2021**, *73* (4), 643–658. https://doi.org/10.1002/iub.2455.

(104) Bulloch, E. M. M.; Jones, M. A.; Parker, E. J.; Osborne, A. P.; Stephens, E.; Davies, G. M.; Coggins, J. R.; Abell, C. Identification of 4-Amino-4-Deoxychorismate Synthase as the Molecular Target for the Antimicrobial Action of (6S)-6-Fluoroshikimate. *J. Am. Chem. Soc.* **2004**, *126* (32), 9912–9913. https://doi.org/10.1021/ja048312f.

(105) Ye, Q. Z.; Liu, J.; Walsh, C. T. P-Aminobenzoate Synthesis in Escherichia Coli: Purification and Characterization of PabB as Aminodeoxychorismate Synthase and Enzyme X as Aminodeoxychorismate Lyase. *Proc. Natl. Acad. Sci.* **1990**, *87* (23), 9391–9395. https://doi.org/10.1073/pnas.87.23.9391.

(106) Walsh, C. T.; Erion, M. D.; Walts, A. E.; Delany, J. J.; Berchtold, G. A. Chorismate Aminations: Partial Purification of Escherichia Coli PABA Synthase and Mechanistic Comparison with Anthranilate Synthase. *Biochemistry* **1987**, *26* (15), 4734–4745. https://doi.org/10.1021/bi00389a021.

(107) Thiede, J. M.; Kordus, S. L.; Turman, B. J.; Buonomo, J. A.; Aldrich, C. C.; Minato, Y.; Baughn, A. D. Targeting Intracellular P-Aminobenzoic Acid Production Potentiates the Anti-Tubercular Action of Antifolates. *Sci. Rep.* **2016**, *6*. https://doi.org/10.1038/srep38083.

(108) PARA-AMINOSALICYLIC ACID IN THE TREATMENT OF TUBERCULOSIS. *The Lancet* **1946**, *247* (6384), 15–16. https://doi.org/10.1016/S0140-6736(46)91185-3.

(109) Zheng, J.; Rubin, E. J.; Bifani, P.; Mathys, V.; Lim, V.; Au, M.; Jang, J.; Nam, J.; Dick, T.; Walker, J. R.; Pethe, K.; Camacho, L. R. Para-Aminosalicylic Acid Is a Prodrug Targeting Dihydrofolate Reductase in Mycobacterium Tuberculosis ♦. *J. Biol. Chem.* **2013**, *288* (32), 23447–23456. https://doi.org/10.1074/jbc.M113.475798.

(110) Zhao, F.; Wang, X.-D.; Erber, L. N.; Luo, M.; Guo, A.; Yang, S.; Gu, J.; Turman, B. J.; Gao, Y.; Li, D.; Cui, Z.; Zhang, Z.; Bi, L.; Baughn, A. D.; Zhang, X.-E.; Deng, J.-Y. Binding Pocket Alterations in Dihydrofolate Synthase Confer Resistance to Para-Aminosalicylic Acid in Clinical Isolates of Mycobacterium Tuberculosis. *Antimicrob. Agents Chemother.* **2014**, *58* (3), 1479–1487. https://doi.org/10.1128/AAC.01775-13.

(111) Minato, Y.; Thiede, J. M.; Kordus, S. L.; McKlveen, E. J.; Turman, B. J.; Baughn, A. D. Mycobacterium Tuberculosis Folate Metabolism and the Mechanistic Basis for Para-Aminosalicylic Acid Susceptibility and Resistance. *Antimicrob. Agents Chemother.* **2015**, *59* (9), 5097–5106. https://doi.org/10.1128/AAC.00647-15.

(112) Davies, G. M.; Barrett-Bee, K. J.; Jude, D. A.; Lehan, M.; Nichols, W. W.; Pinder, P. E.; Thain, J. L.; Watkins, W. J.; Wilson, R. G. (6S)-6-Fluoroshikimic Acid, an Antibacterial Agent Acting on the Aromatic Biosynthetic Pathway. *Antimicrob. Agents Chemother.* **1994**, *38* (2), 403–406. https://doi.org/10.1128/AAC.38.2.403.

(113) Bornemann, S.; Ramjee, M. K.; Balasubramanian, S.; Abell, C.; Coggins, J. R.; Lowe, D. J.; Thorneley, R. N. F. Escherichia Coli Chorismate Synthase Catalyzes the Conversion of (6S)-6-Fluoro-5-Enolpyruvylshikimate-3-Phosphate to 6-Fluorochorismate: IMPLICATIONS FOR THE ENZYME MECHANISM AND THE ANTIMICROBIAL ACTION OF (6S)-6-FLUOROSHIKIMATE (∗). *J. Biol. Chem.* **1995**, *270* (39), 22811–22815. https://doi.org/10.1074/jbc.270.39.22811.

(114) Valenciano, A. L.; Fernández-Murga, M. L.; Merino, E. F.; Holderman, N. R.; Butschek, G. J.; Shaffer, K. J.; Tyler, P. C.; Cassera, M. B. Metabolic Dependency of Chorismate in Plasmodium Falciparum Suggests an Alternative Source for the Ubiquinone Biosynthesis Precursor. *Sci. Rep.* **2019**, *9* (1), 13936. https://doi.org/10.1038/s41598-019-50319-5.

(115) McConkey, G. A. Targeting the Shikimate Pathway in the Malaria Parasite Plasmodium Falciparum. *Antimicrob. Agents Chemother.* **1999**, *43* (1), 175–177. https://doi.org/10.1128/AAC.43.1.175.

(116) He, Z.; Stigers Lavoie, K. D.; Bartlett, P. A.; Toney, M. D. Conservation of Mechanism in Three Chorismate-Utilizing Enzymes. *J. Am. Chem. Soc.* **2004**, *126* (8), 2378–2385. https://doi.org/10.1021/ja0389927.

(117) Tamir, H.; Srinivasan, P. R. Studies of the Mechanism of Anthranilate Synthase Reaction*. *Proc. Natl. Acad. Sci. U. S. A.* **1970**, *66* (2), 547–551.

(118) Humphreys, J. L.; Lowes, D. J.; Wesson, K. A.; Whitehead, R. C. Arene Cis-Dihydrodiols—Useful Precursors for the Preparation of Antimetabolites of the Shikimic Acid Pathway: Application to the Synthesis of 6,6-Difluoroshikimic Acid and (6S)-6-Fluoroshikimic Acid. *Tetrahedron* **2006**, *62* (21), 5099–5108. https://doi.org/10.1016/j.tet.2006.03.029.

(119) Sutherland, J. K.; Watkins, W. J.; Bailey, J. P.; Chapman, A. K.; Davies, G. M. The Synthesis of 6α- and 6β-Fluoroshikimic Acids. *J. Chem. Soc. Chem. Commun.* **1989**, No. 18, 1386–1387. https://doi.org/10.1039/C39890001386.

(120) Bowles, S. A.; Campbell, M. M.; Sainsbury, M.; Davies, G. M. Reactivity Studies in the Shikimic Acid Series: The Synthesis of Racemic Methyl 6α-Fluoroshikimate. *Tetrahedron* **1990**, *46* (11), 3981–3992. https://doi.org/10.1016/S0040-4020(01)90533-2.

(121) Medium-Scale Preparation of Useful Metabolites of Aromatic Compounds via Whole-Cell Fermentation with Recombinant Organisms | Organic Process Research & Development https://pubs-acs-org.ezp3.lib.umn.edu/doi/10.1021/op020013s (accessed May 9, 2021).

(122) Hudlicky, T.; Price, J. D.; Rulin, F.; Tsunoda, T. Efficient and Enantiodivergent Synthesis of (+)- and (-)-Pinitol. *J. Am. Chem. Soc.* **1990**, *112* (25), 9439–9440. https://doi.org/10.1021/ja00181a081.

(123) Simas, A. B. C.; Pais, K. C.; da Silva, A. A. T. A More Convenient and General Procedure for O-Monobenzylation of Diols via Stannylenes: A Critical Reevaluation of the Bu2SnO Method. *J. Org. Chem.* **2003**, *68* (13), 5426–5428. https://doi.org/10.1021/jo026794c.

(124) Darses, S.; Genet, J.-P. Potassium Organotrifluoroborates: New Perspectives in Organic Synthesis. *Chem. Rev.* **2008**, *108* (1), 288–325. https://doi.org/10.1021/cr0509758.

(125) Brennan, P. J.; Nikaido, H. The Envelope of Mycobacteria. *Annu. Rev. Biochem.* **1995**, *64* (1), 29–63. https://doi.org/10.1146/annurev.bi.64.070195.000333.

(126) Bansal-Mutalik, R.; Nikaido, H. Mycobacterial Outer Membrane Is a Lipid Bilayer and the Inner Membrane Is Unusually Rich in Diacyl Phosphatidylinositol Dimannosides. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111* (13), 4958–4963. https://doi.org/10.1073/pnas.1403078111.

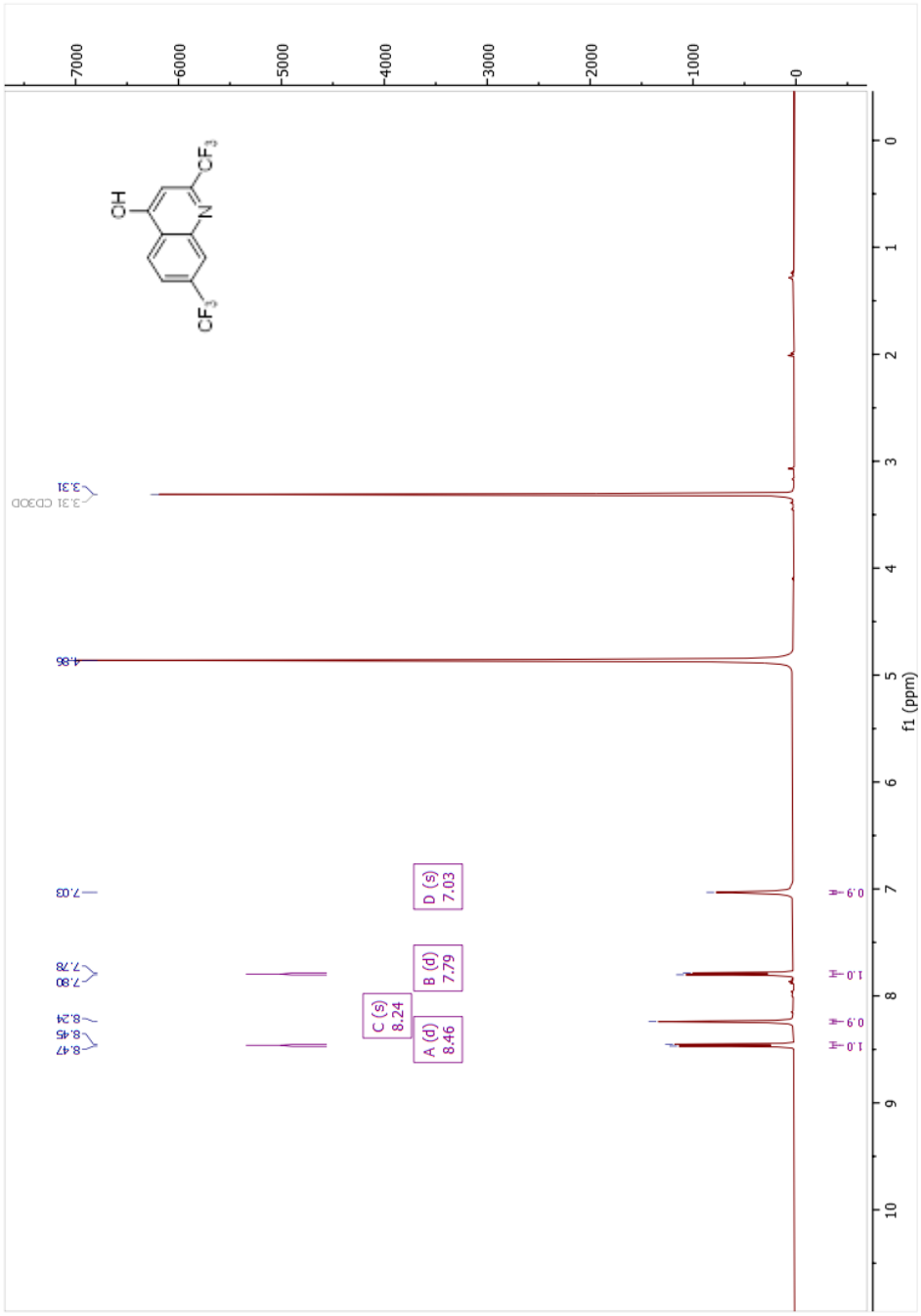
(127) Jackson, M.; Stadthagen, G.; Gicquel, B. Long-Chain Multiple Methyl-Branched Fatty Acid-Containing Lipids of Mycobacterium Tuberculosis: Biosynthesis, Transport, Regulation and Biological Activities. *Tuberc. Edinb. Scotl.* **2007**, *87* (2), 78–86. https://doi.org/10.1016/j.tube.2006.05.003.

(128) Ewart, C. D.; Jude, D. A.; Thain, J. L.; Nichols, W. W. Frequency and Mechanism of Resistance to Antibacterial Action of ZM 240401, (6S)-6-Fluoro-Shikimic Acid. *Antimicrob. Agents Chemother.* **1995**, *39* (1), 87–93. https://doi.org/10.1128/AAC.39.1.87.

(129) Begum, L.; Box, J. M.; Drew, M. G. B.; Harwood, L. M.; Humphreys, J. L.; Lowes, D. J.; Morris, G. A.; Redon, P. M.; Walker, F. M.; Whitehead, R. C. Difluorinated Analogues of Shikimic Acid. *Tetrahedron* **2003**, *59* (26), 4827–4841. https://doi.org/10.1016/S0040-4020(03)00697-5.

# Appendix. NMR Spectra of Synthesized Compounds

Compound 2 $^{1}$H NMR.



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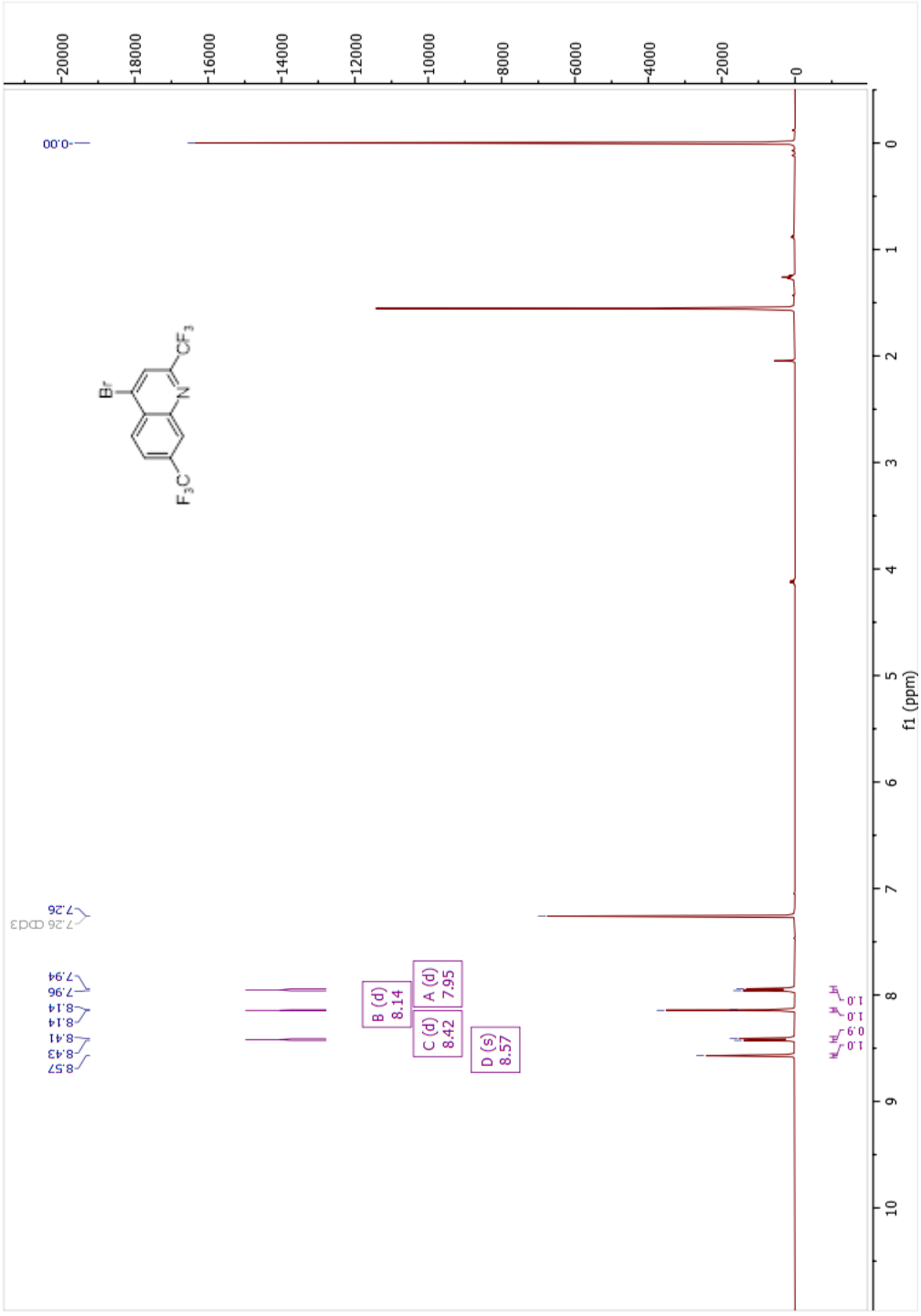

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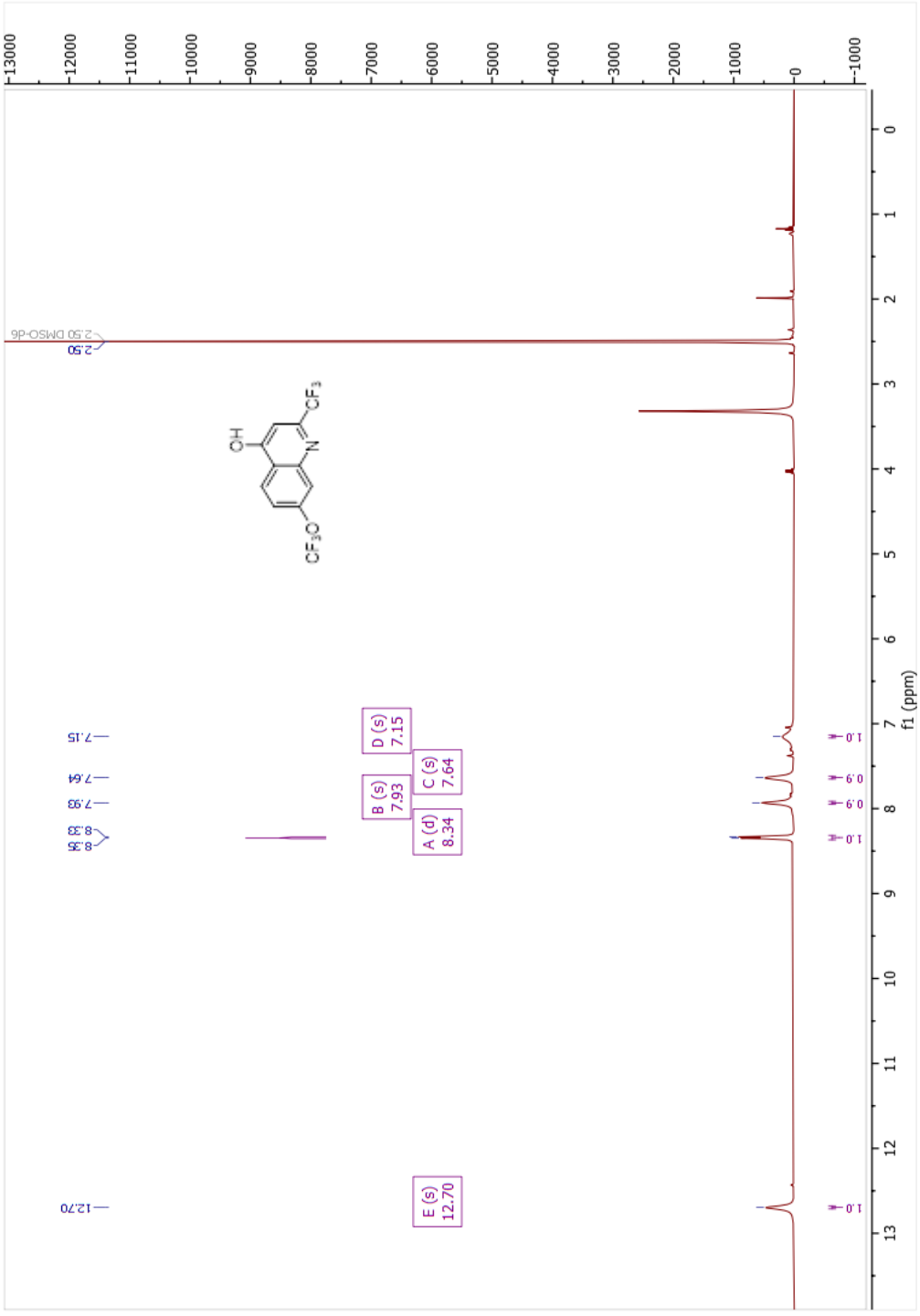
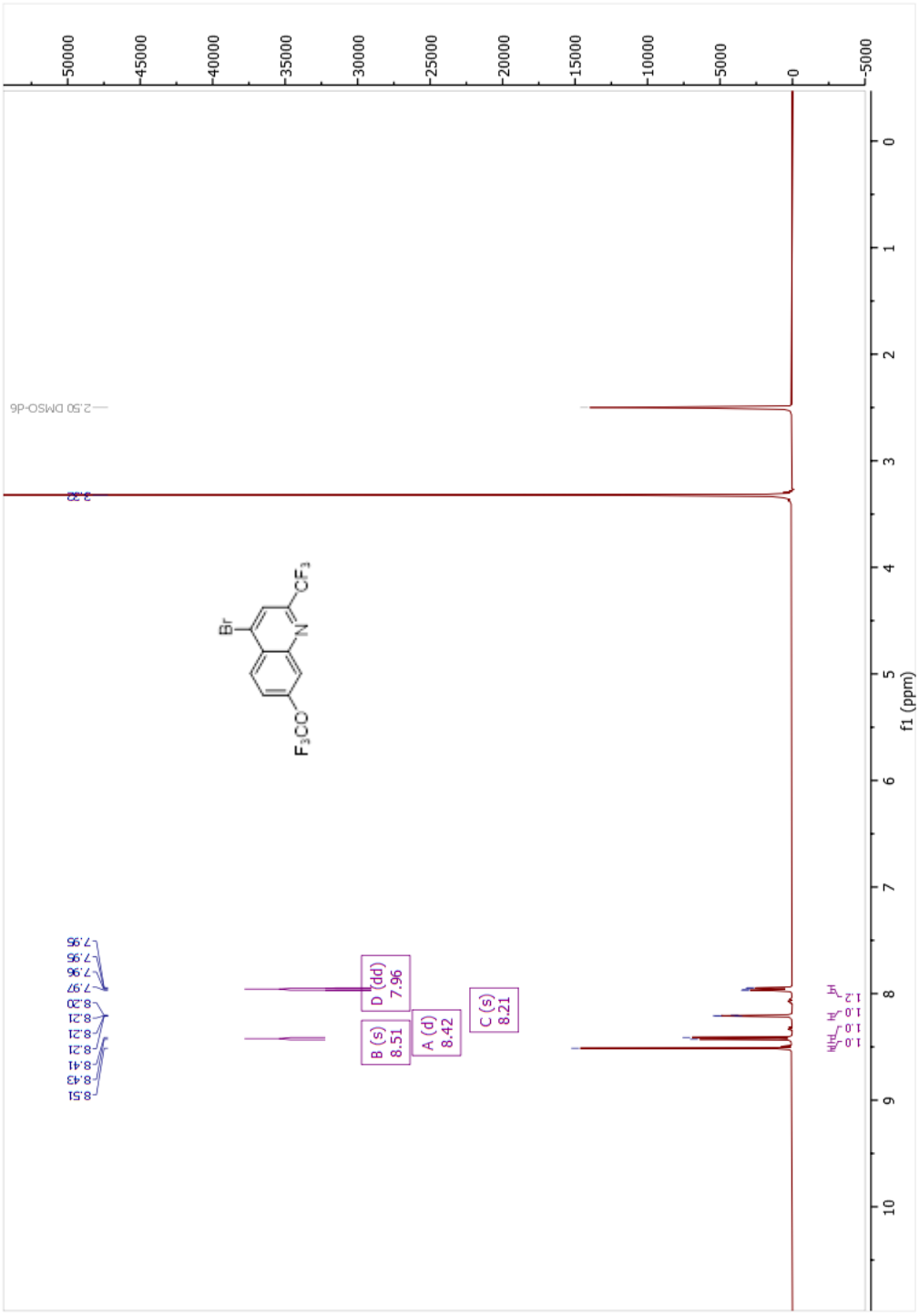


Compound 3b $^{1}H$ NMR.



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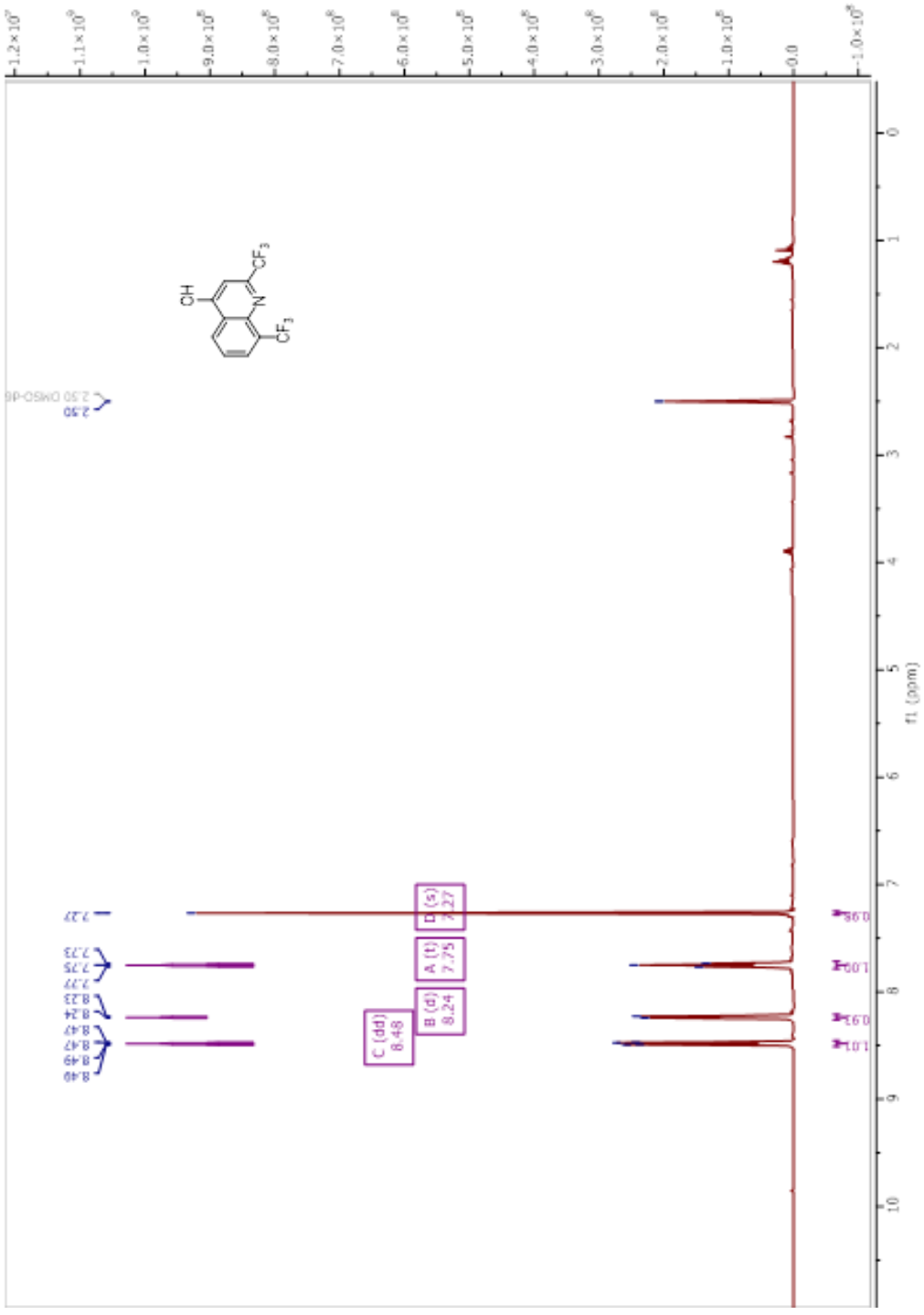

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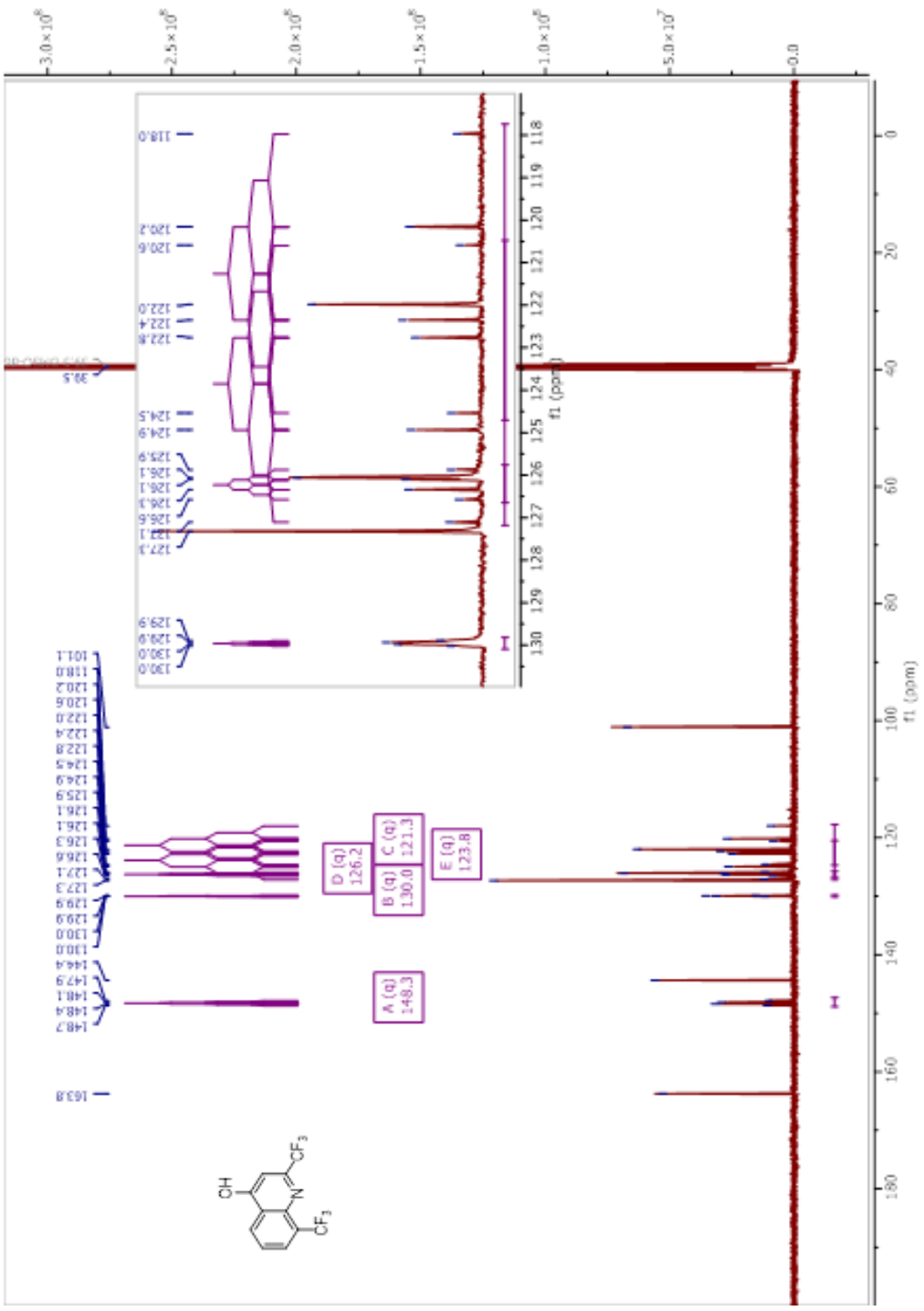
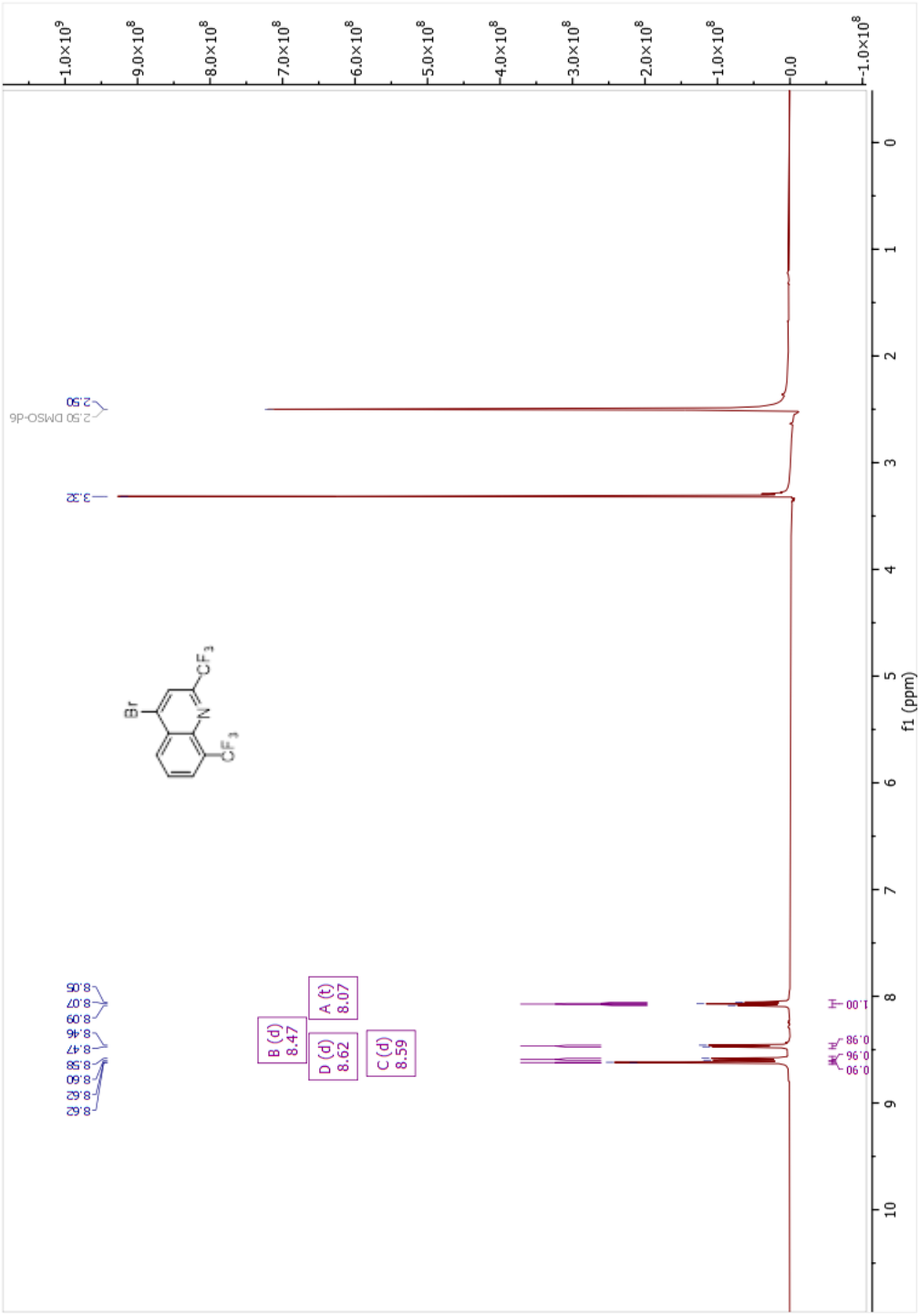


Compound 4b $^{1}H$ NMR.



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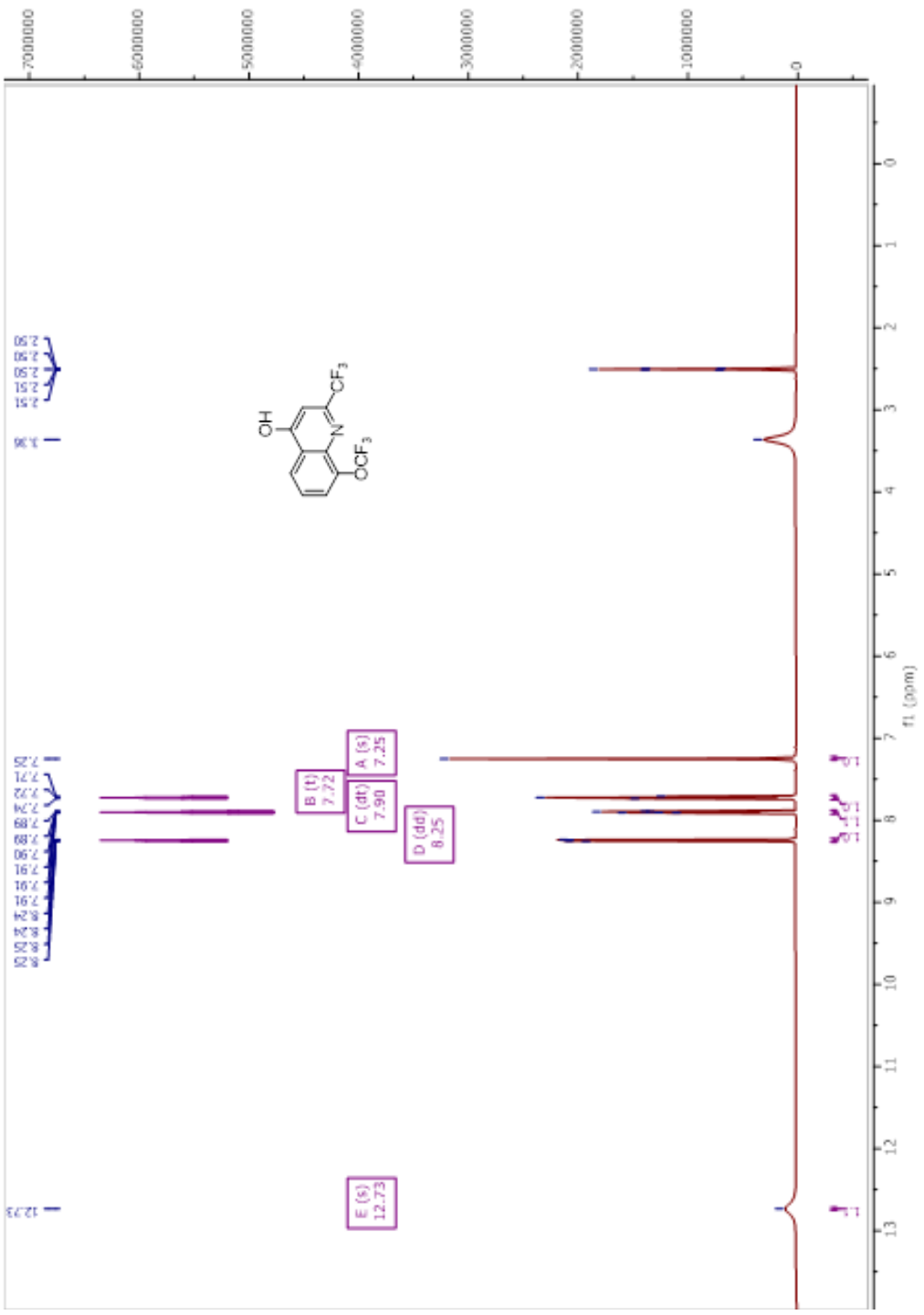

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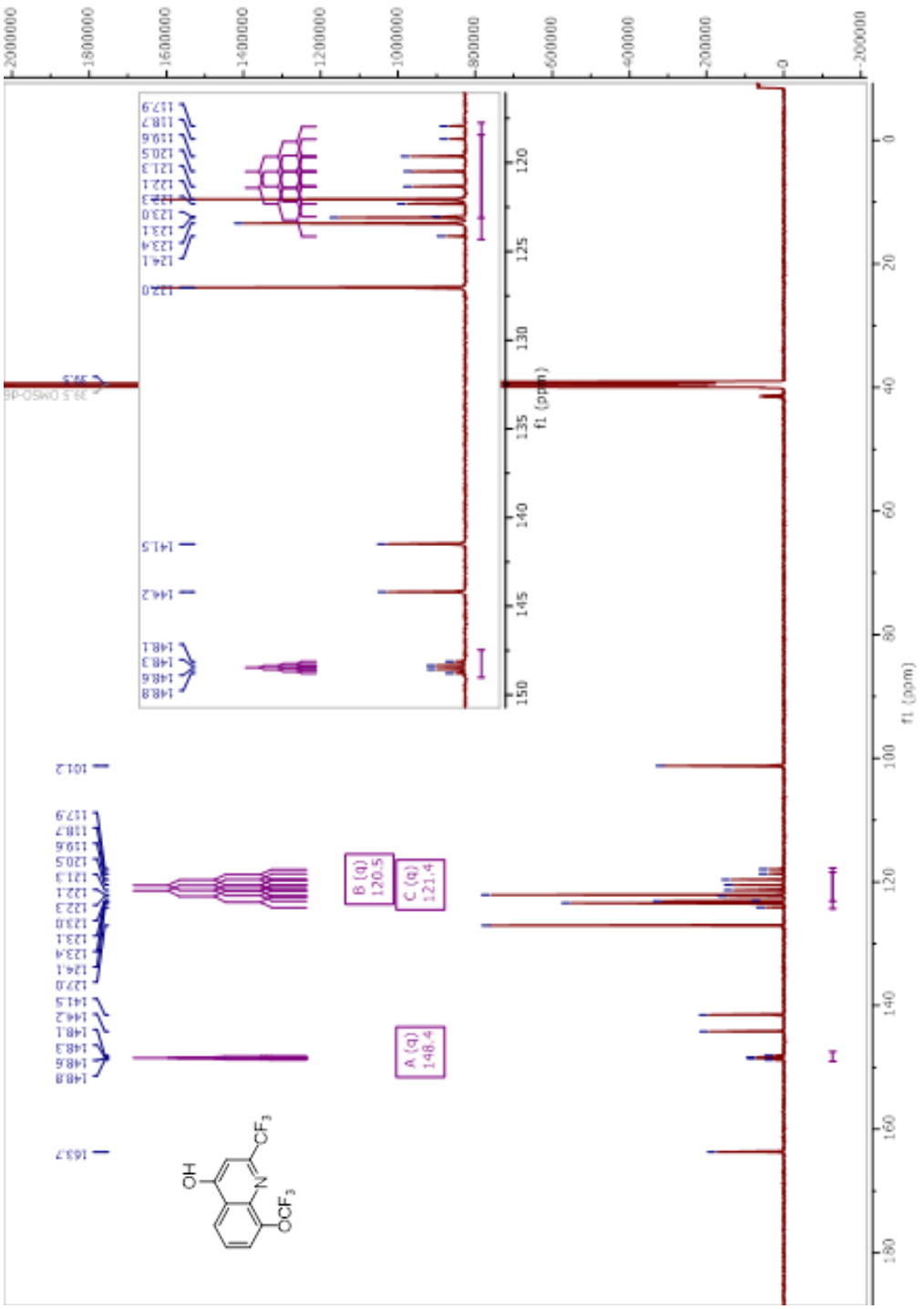

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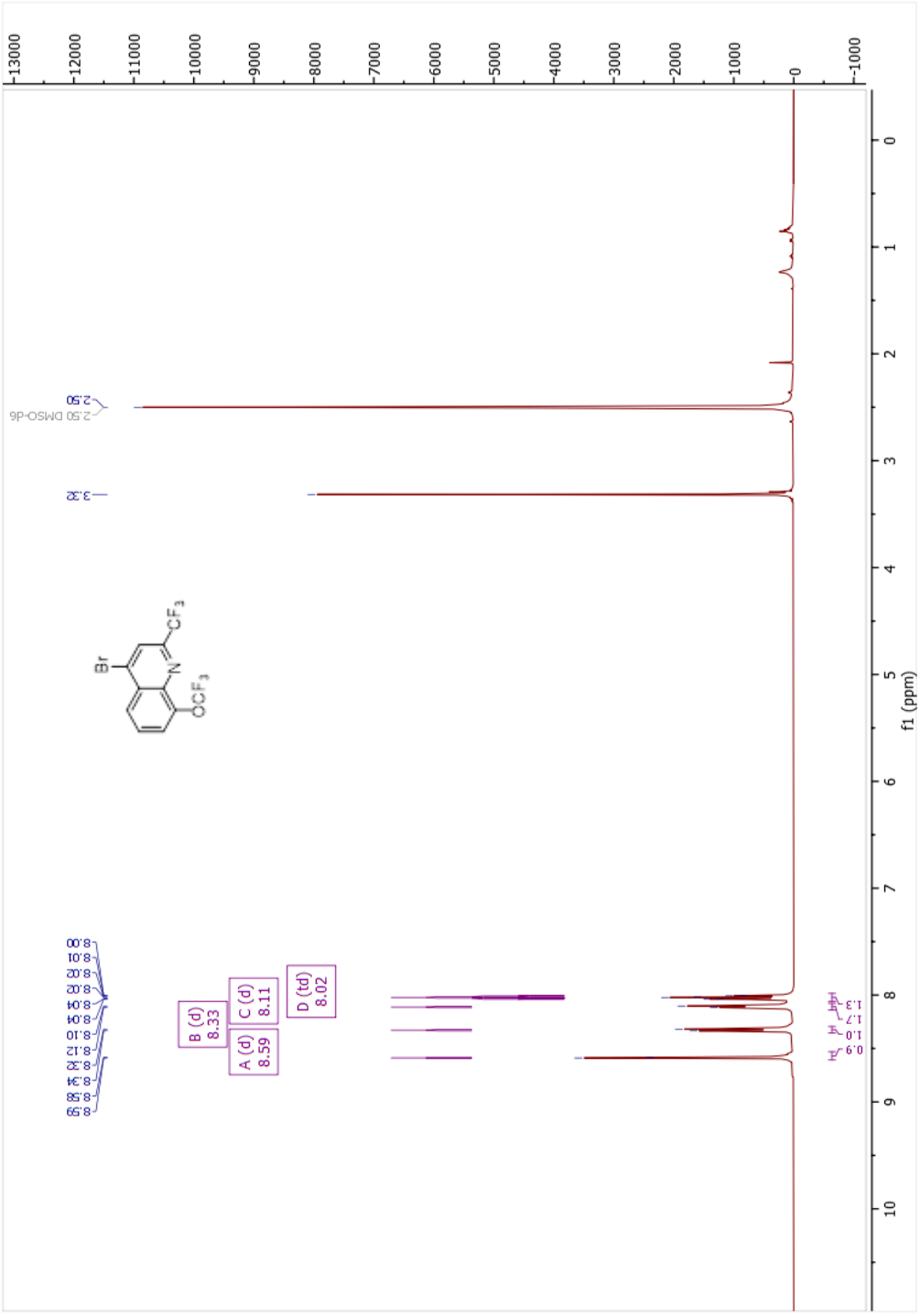

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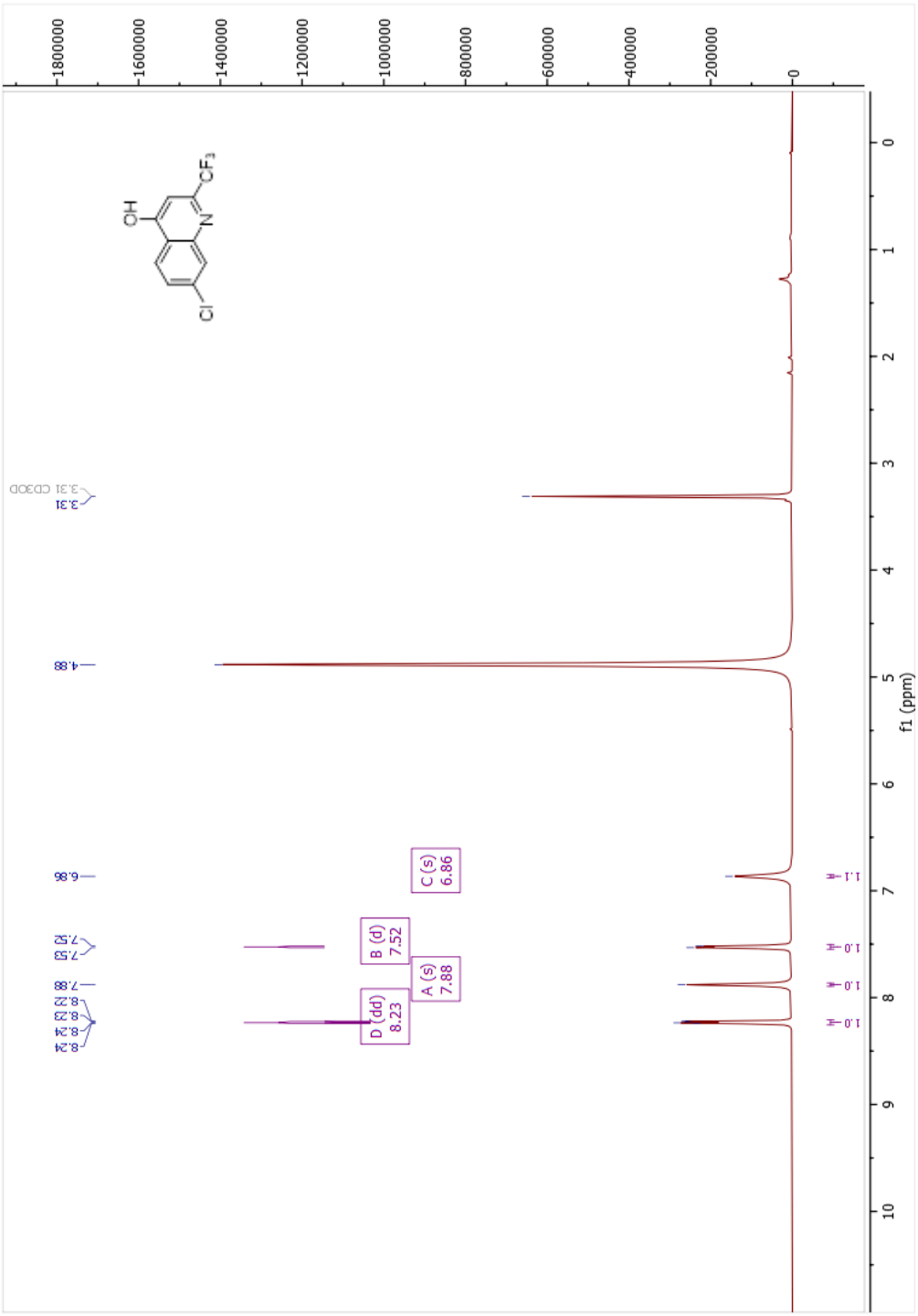

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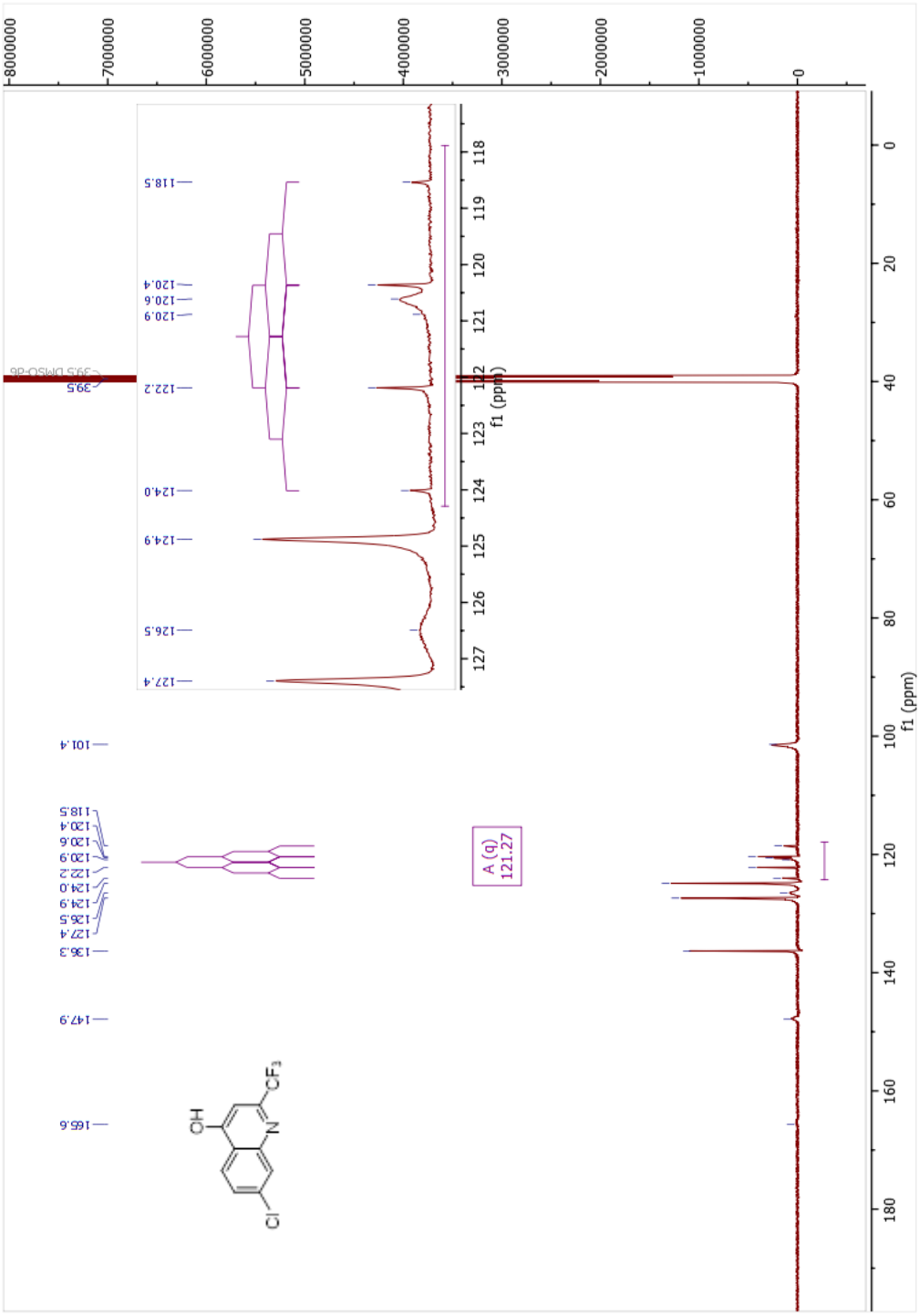

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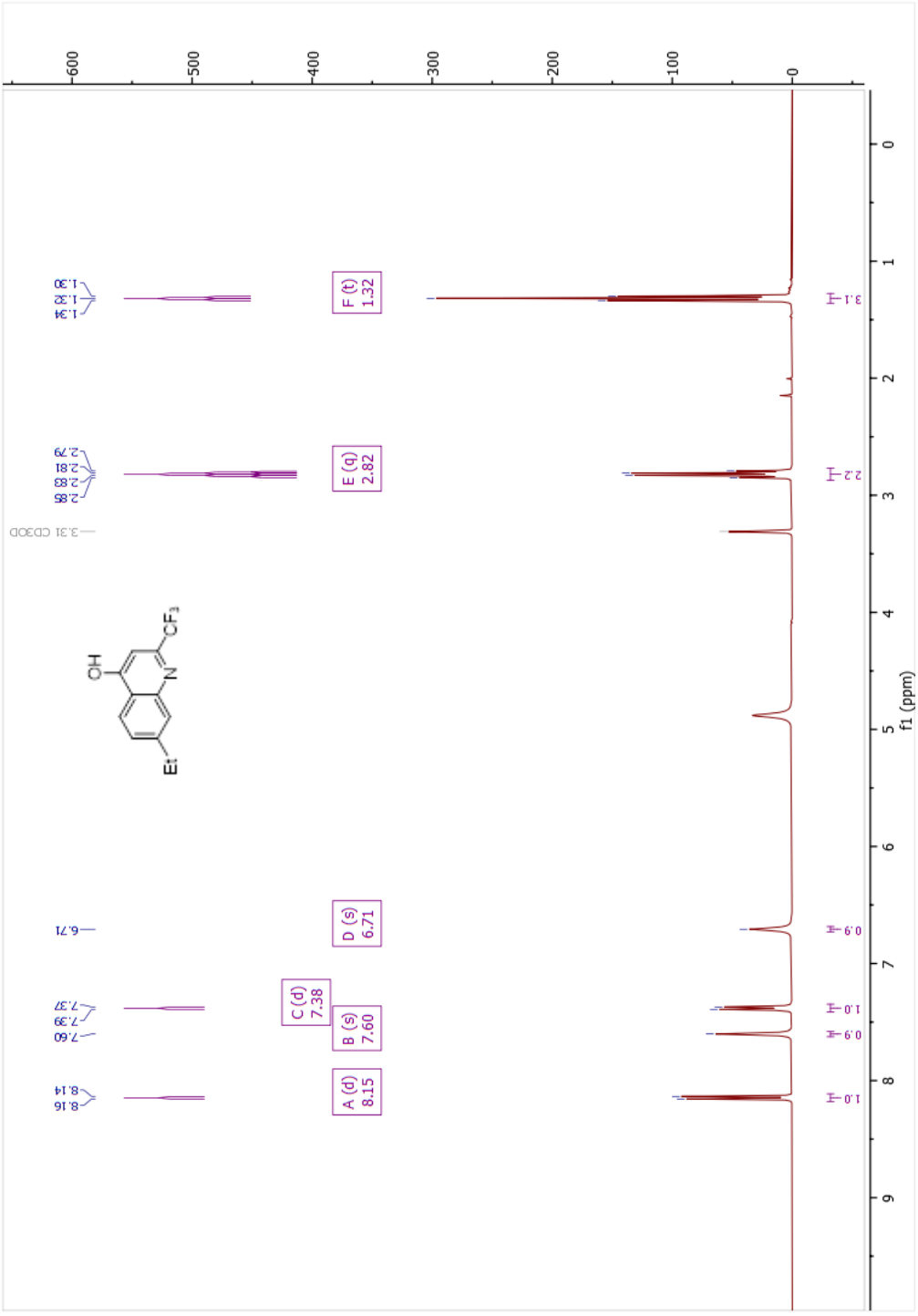

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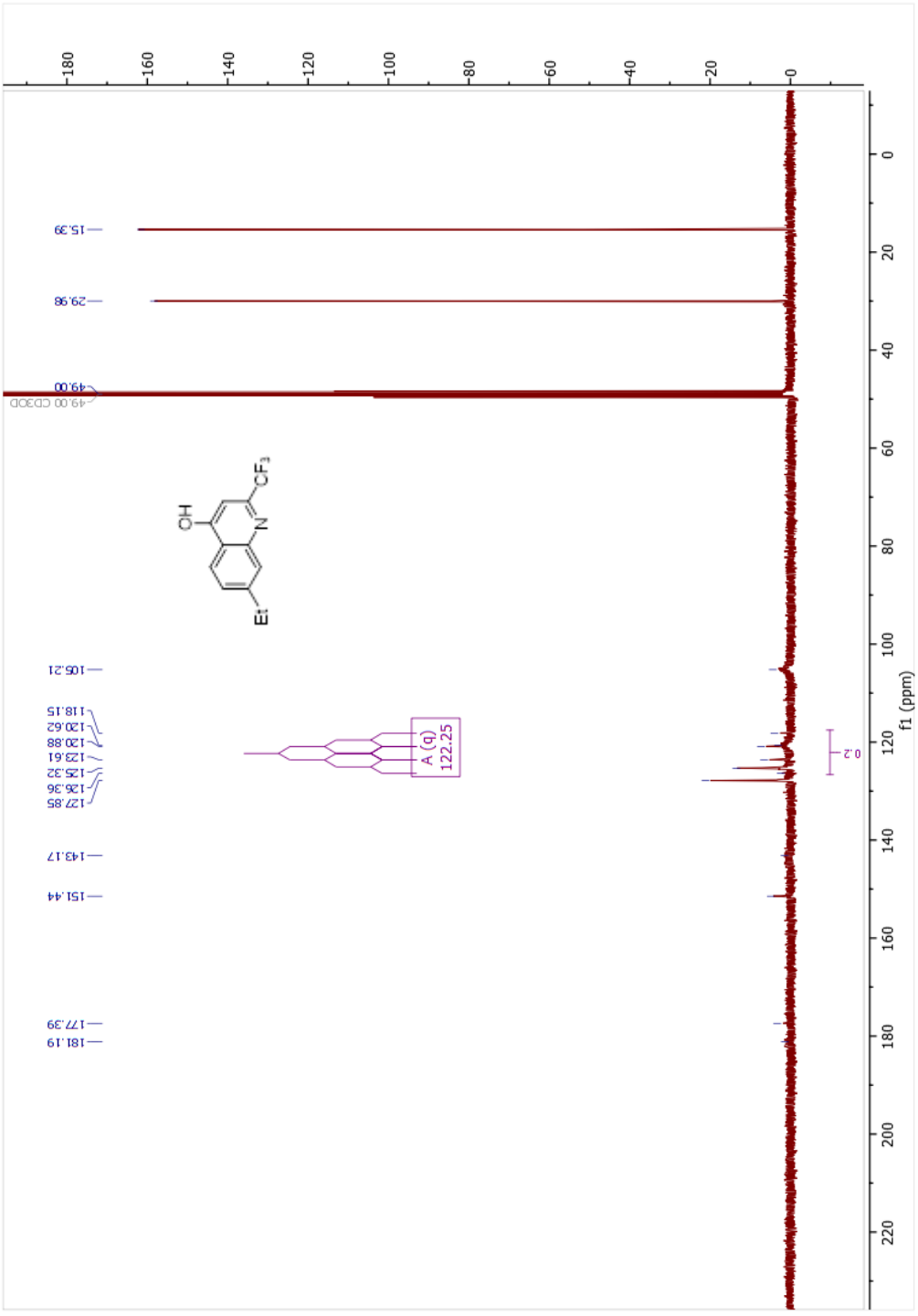
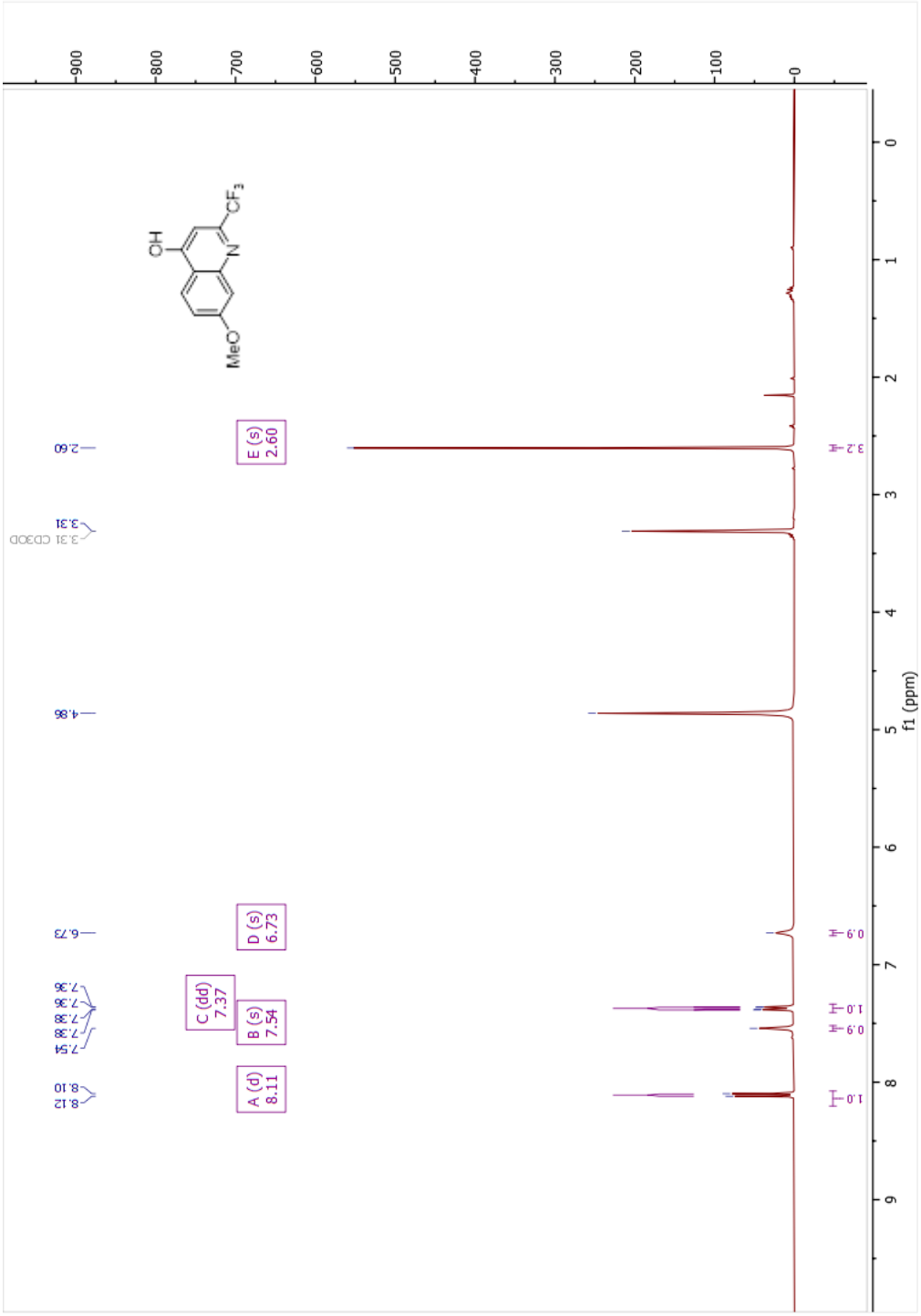
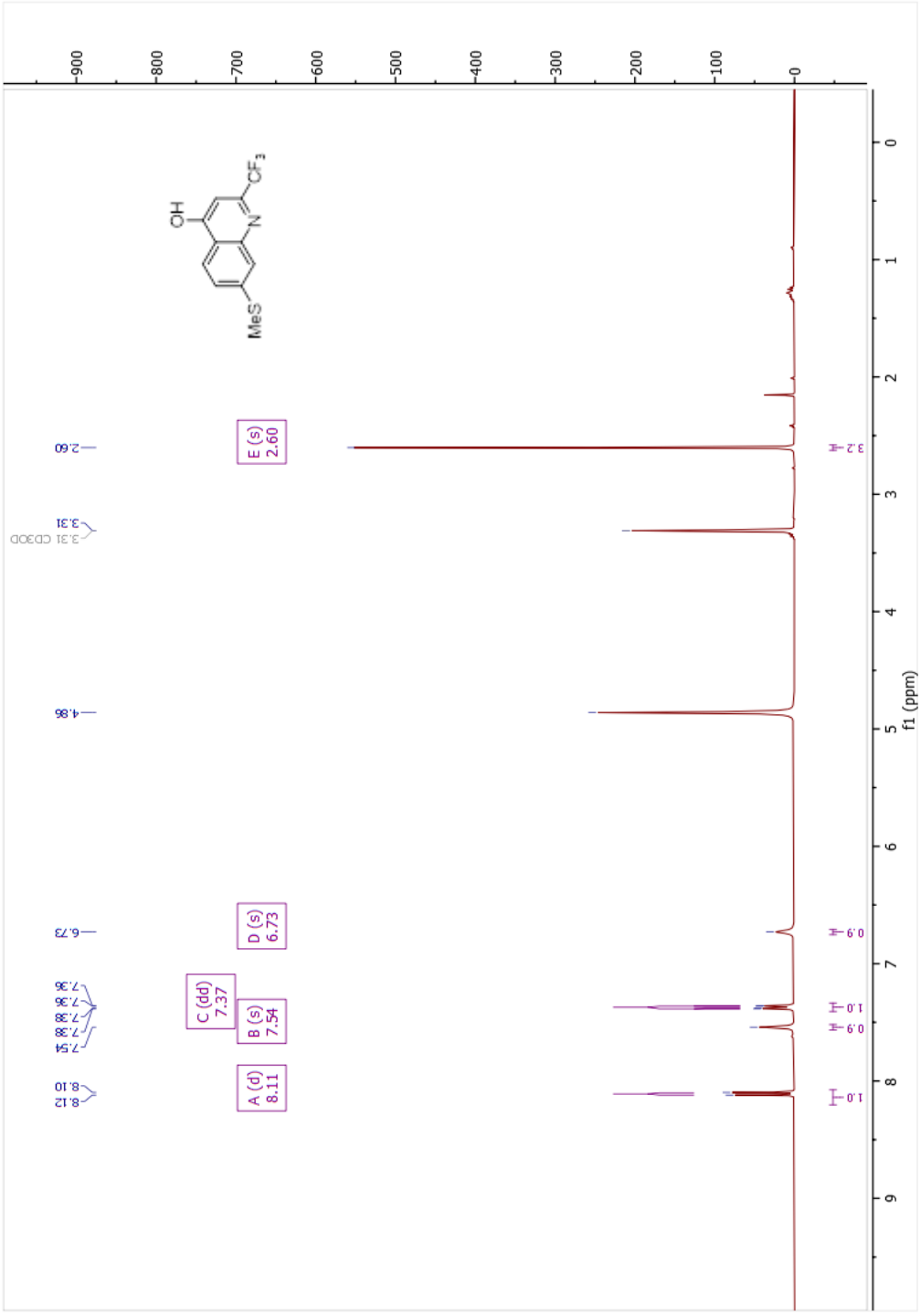


Compound 9 $^{1}$H NMR.



Compound 10 $^{1}H$ NMR.



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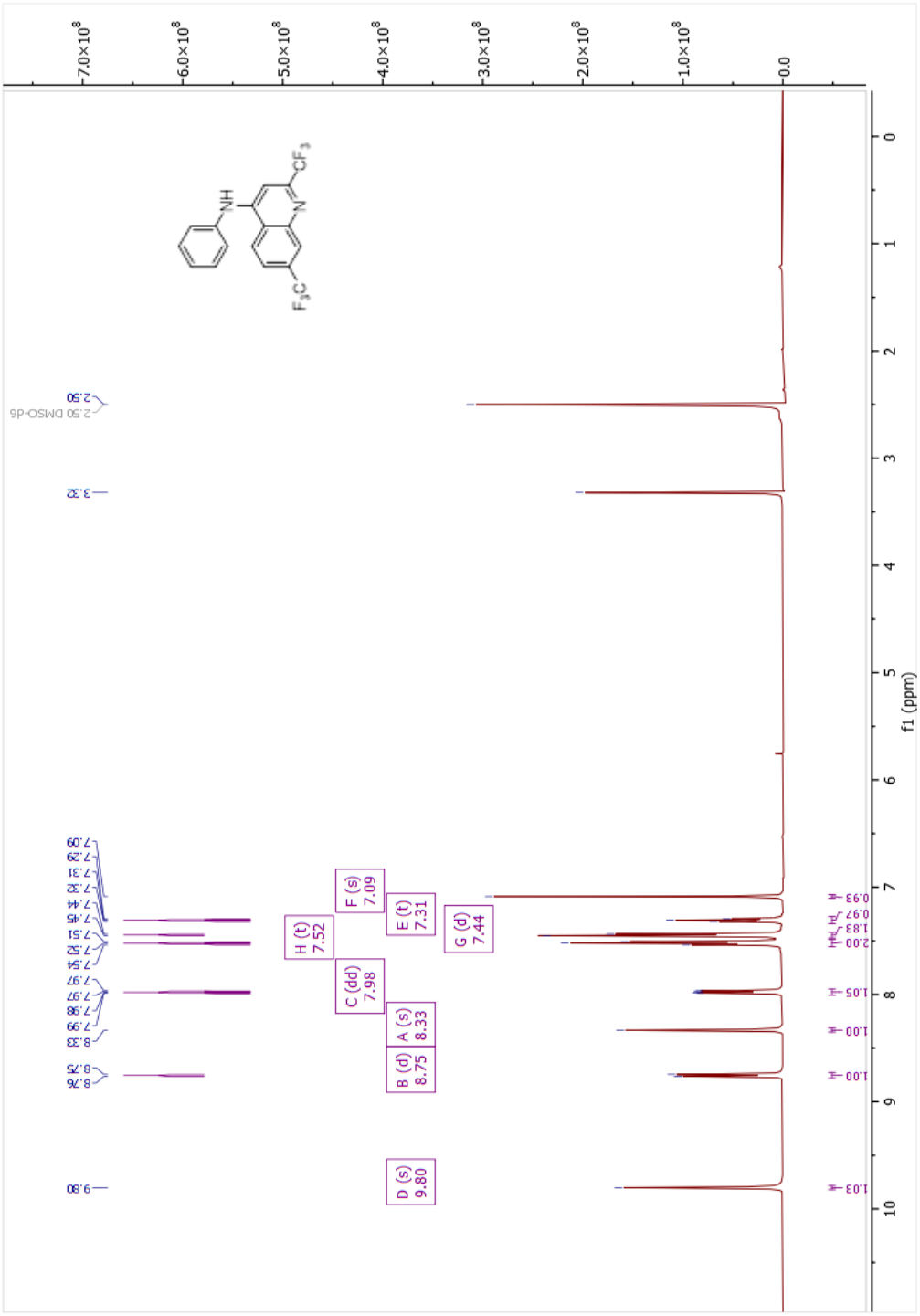

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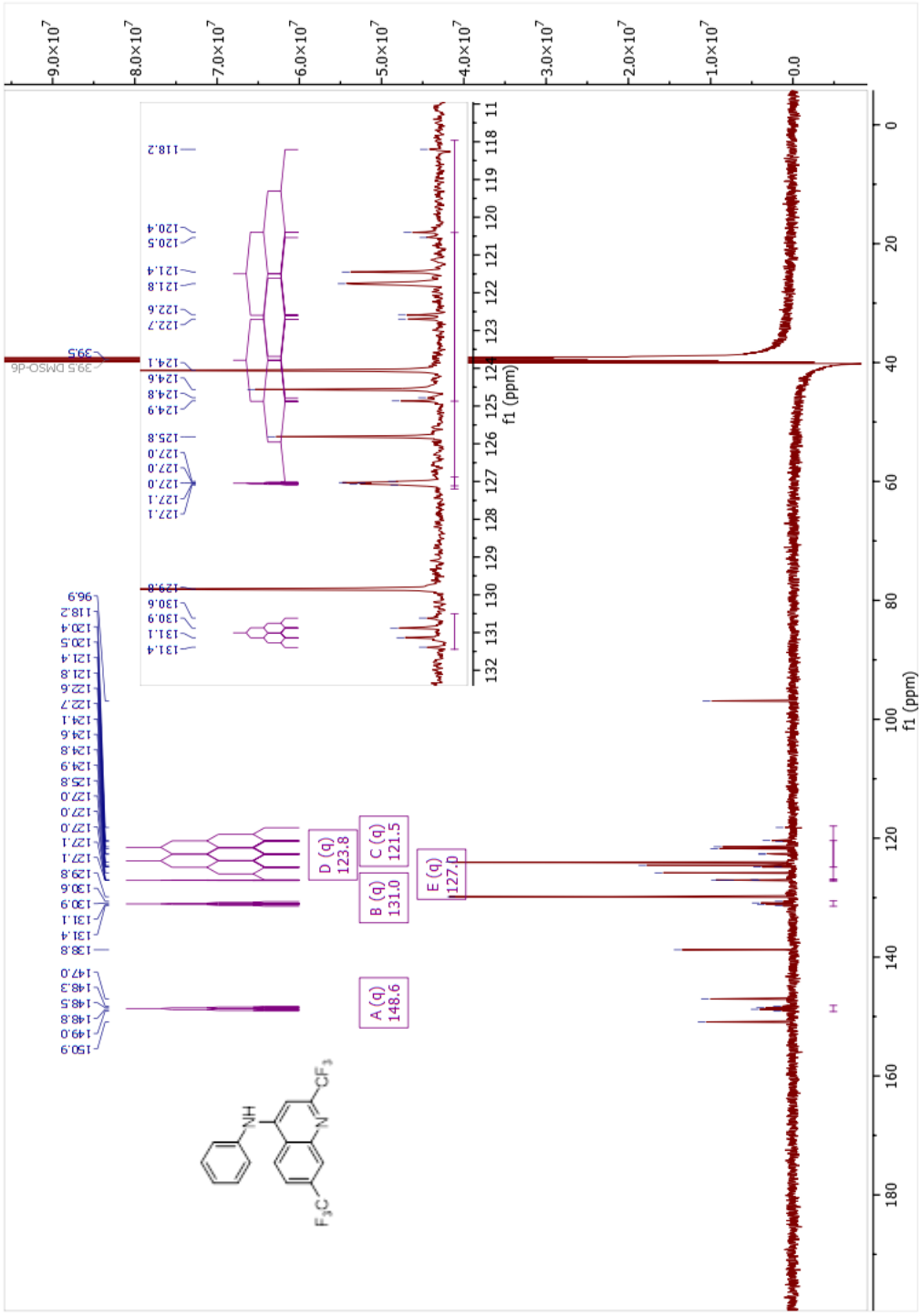

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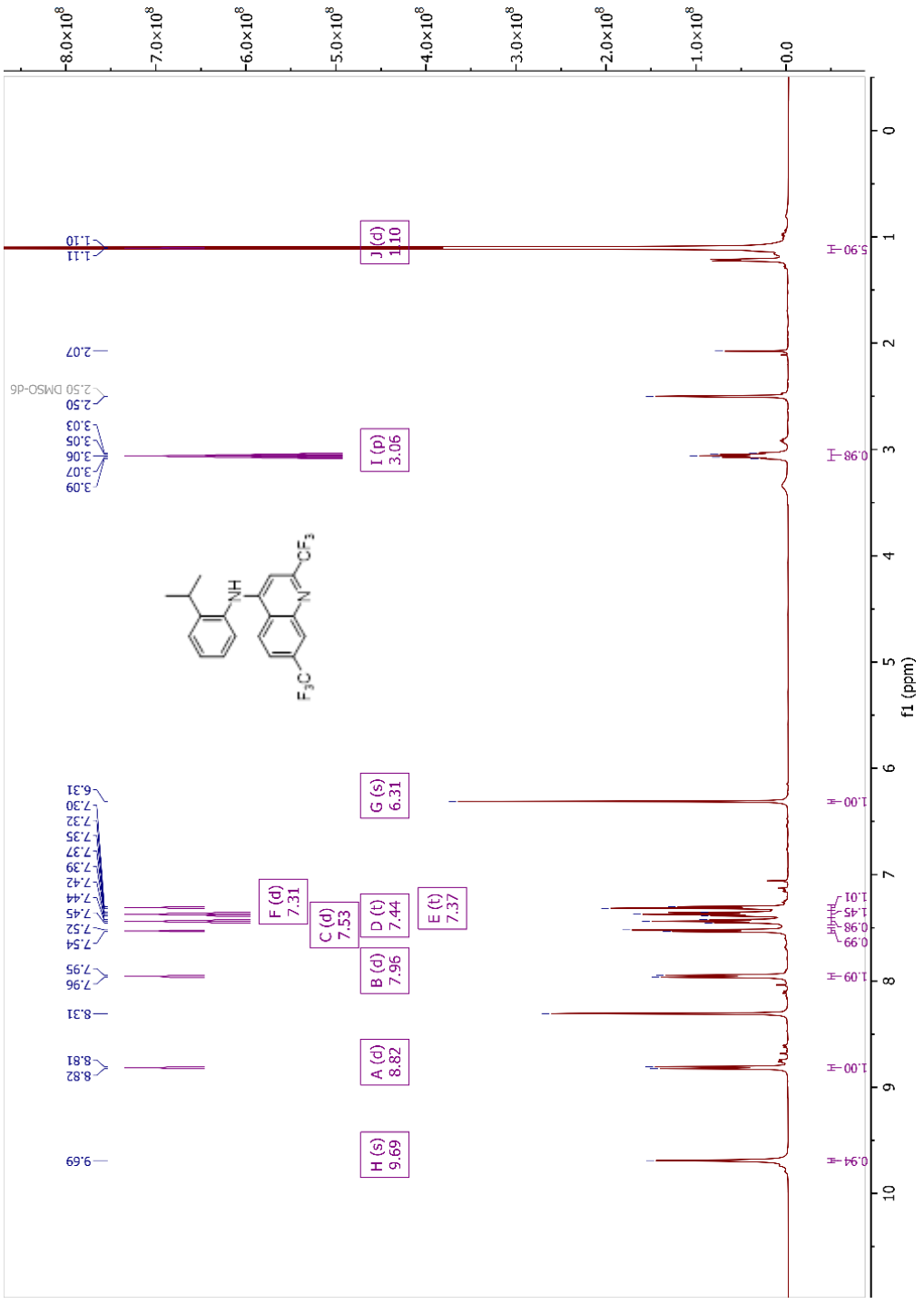

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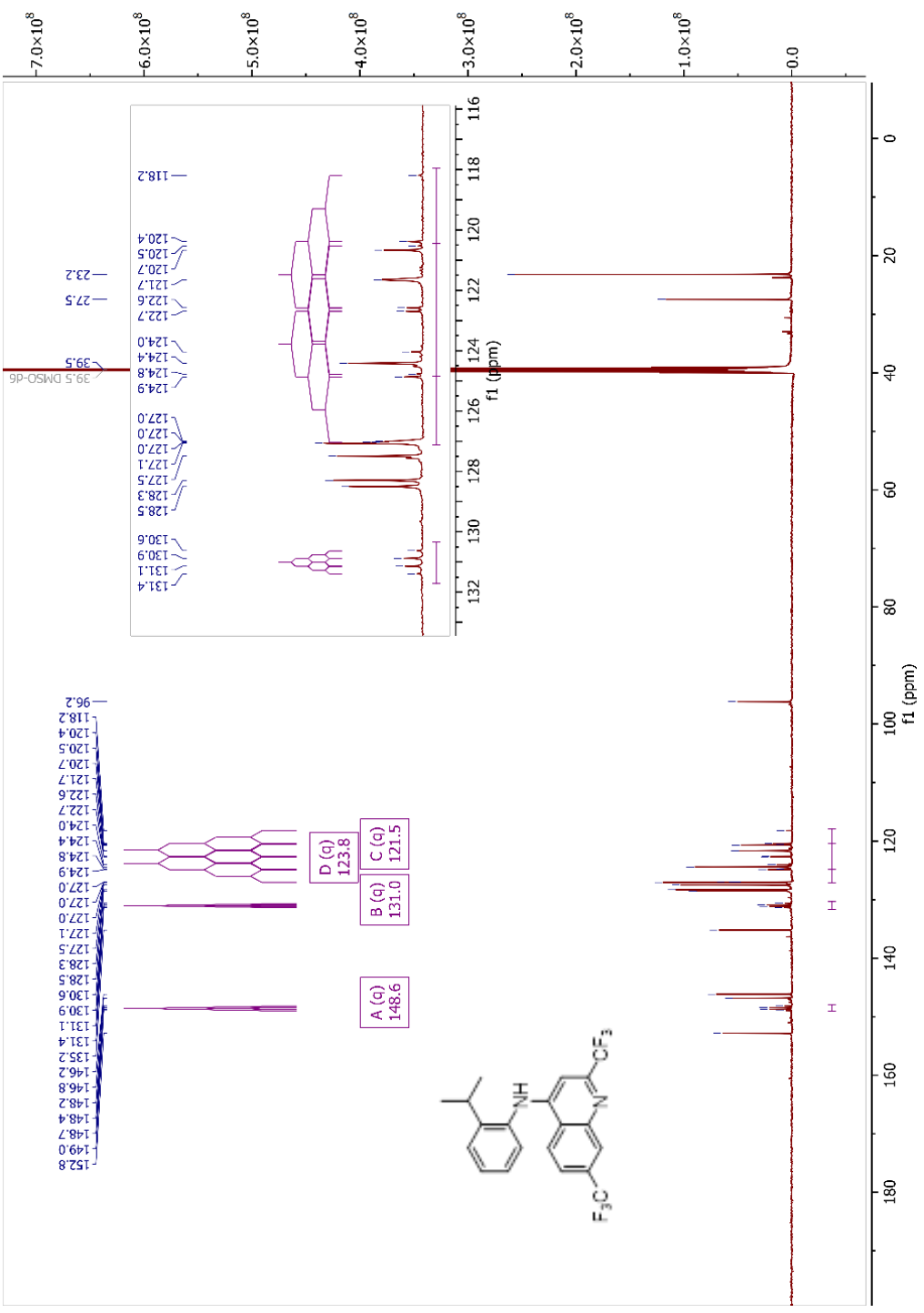
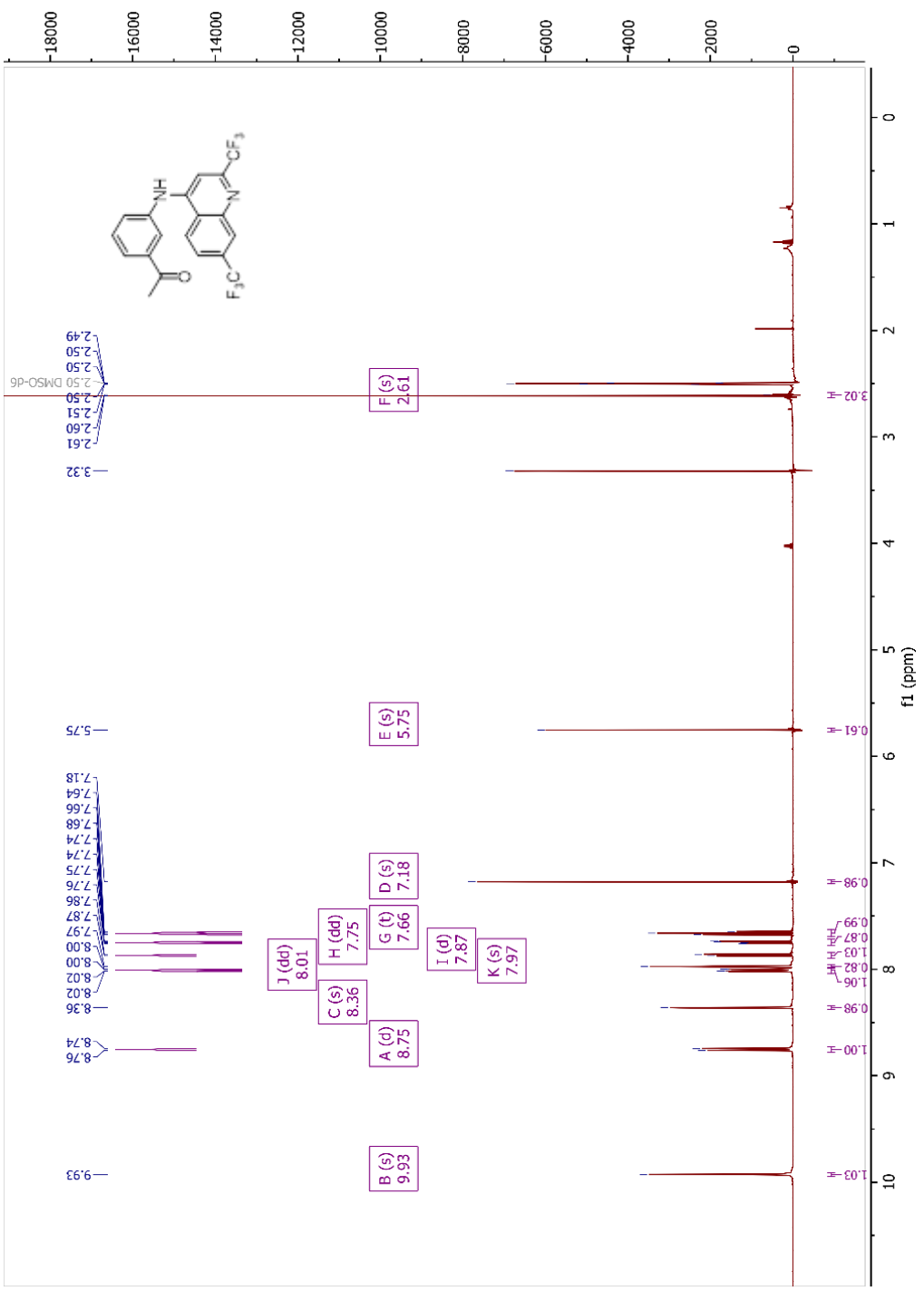
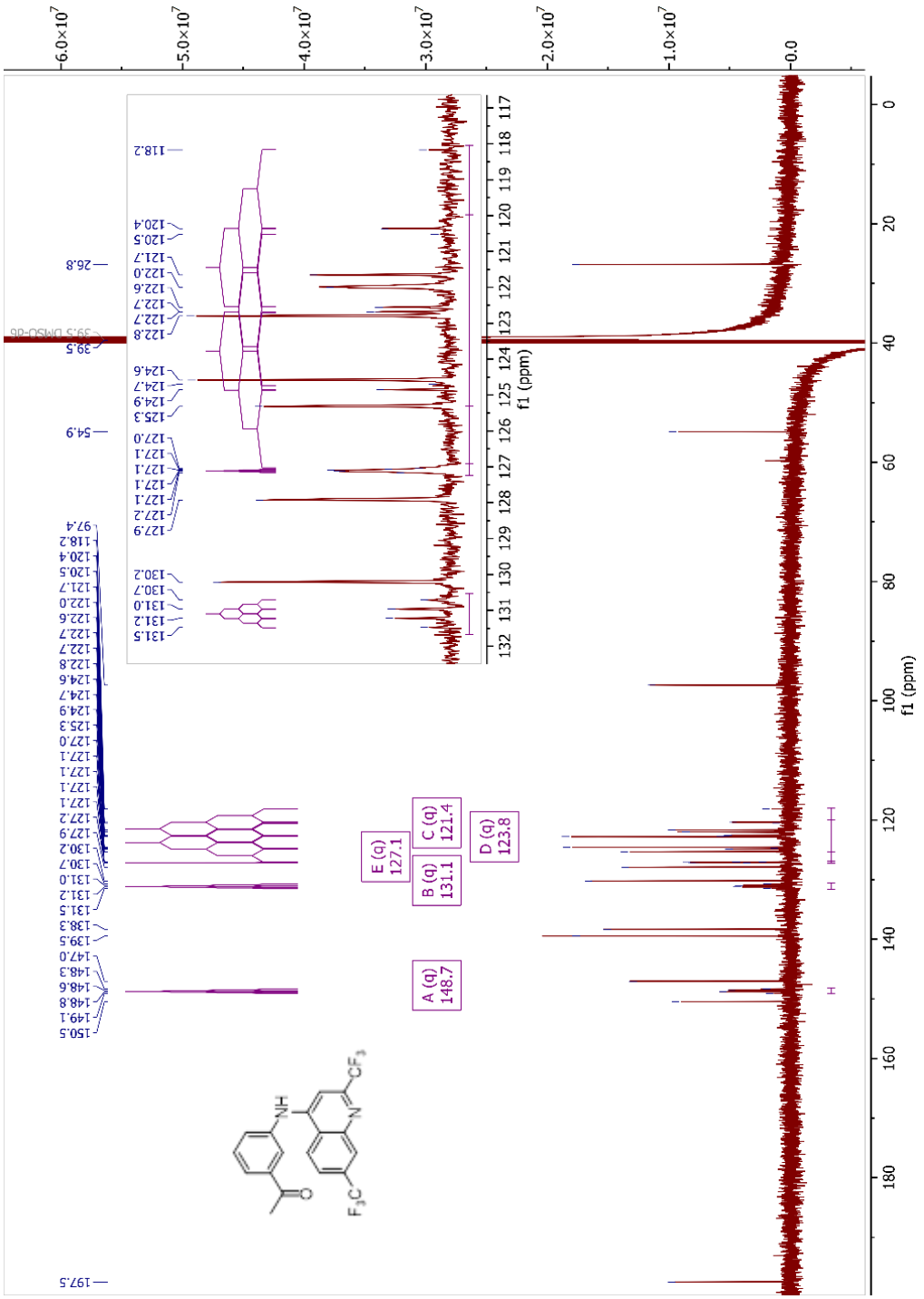


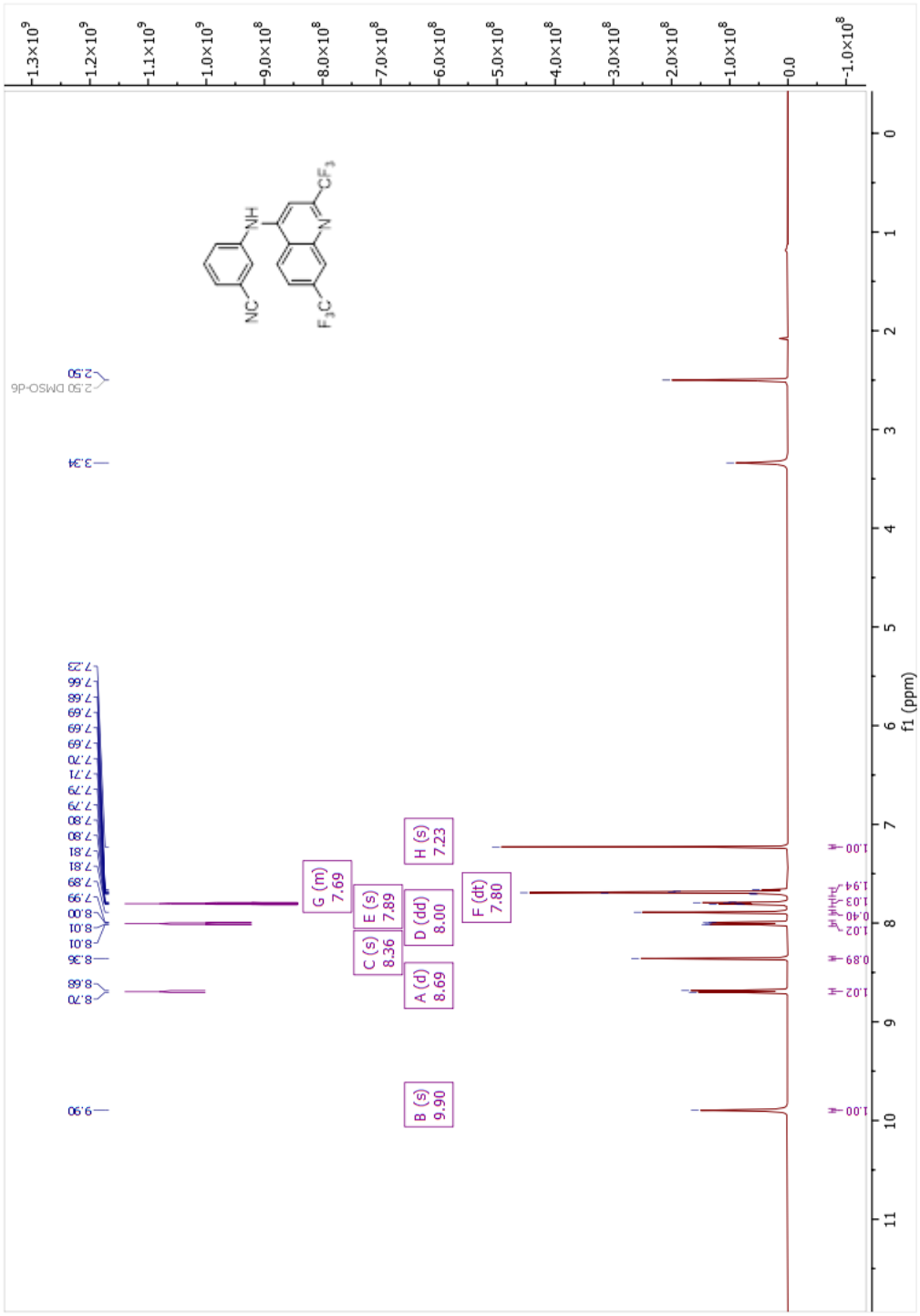
Compound 13 $^{1}$H NMR.



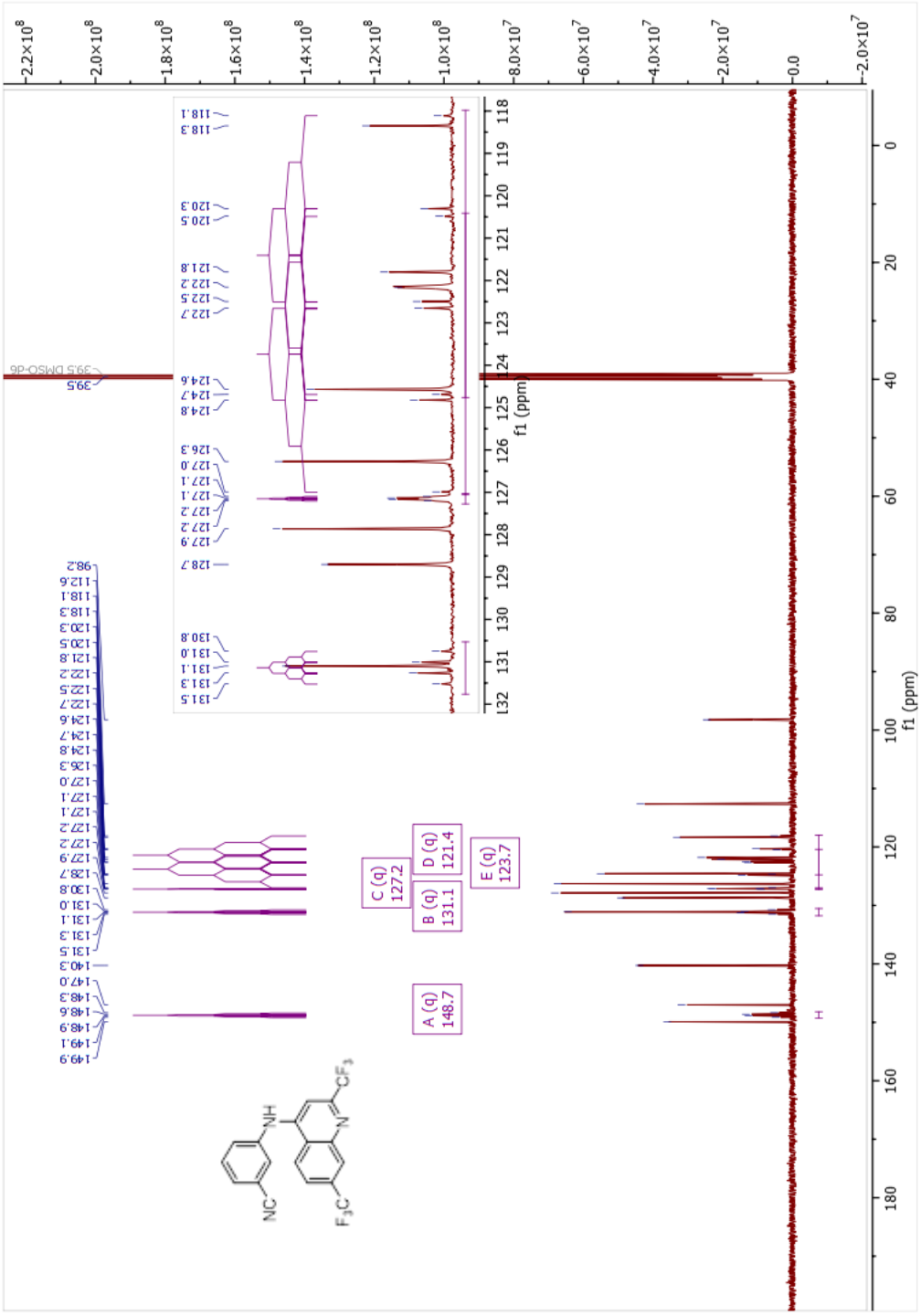
Compound 13 $^{13}C$ NMR.



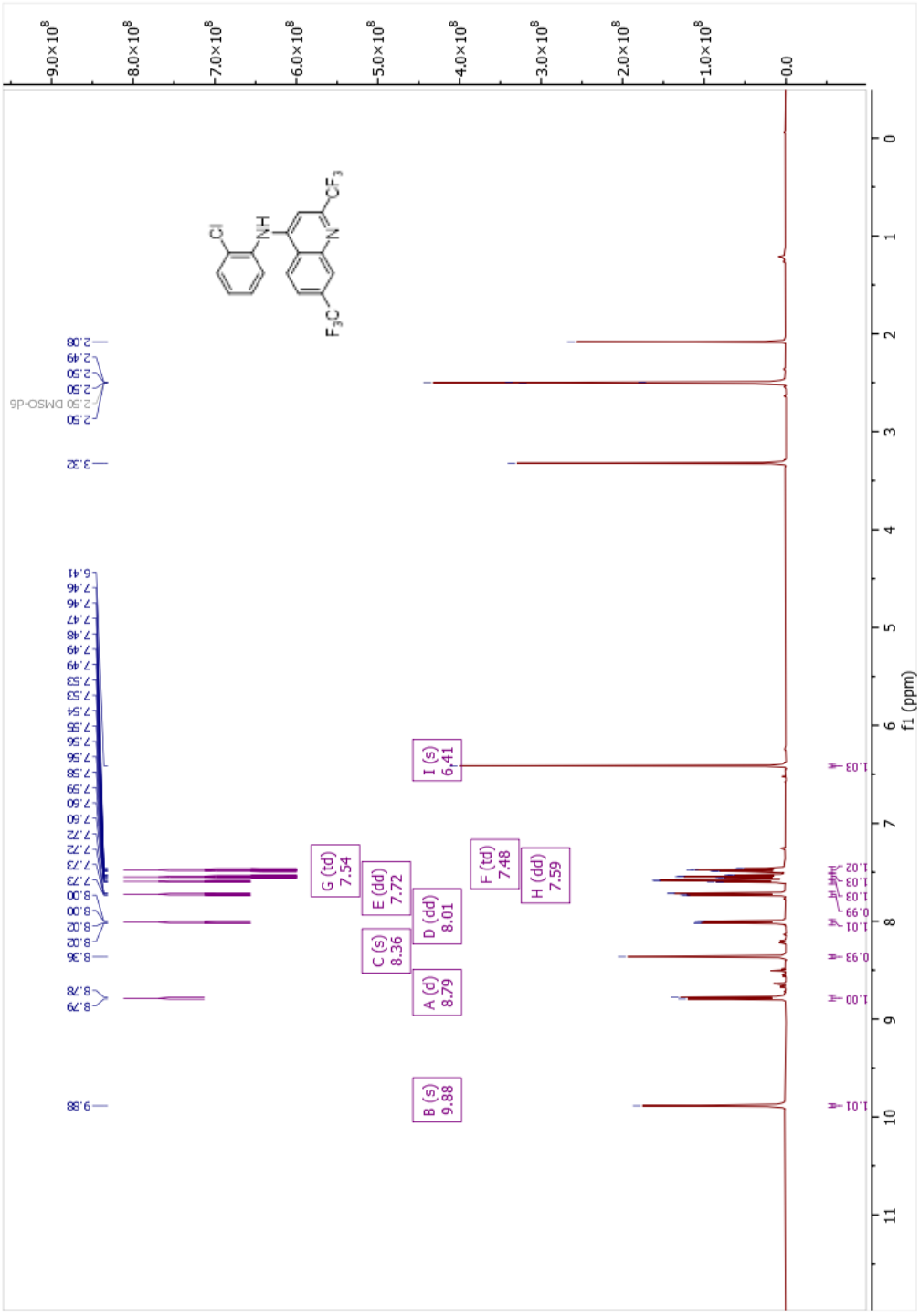
Compound 14 $^{1}$H NMR.



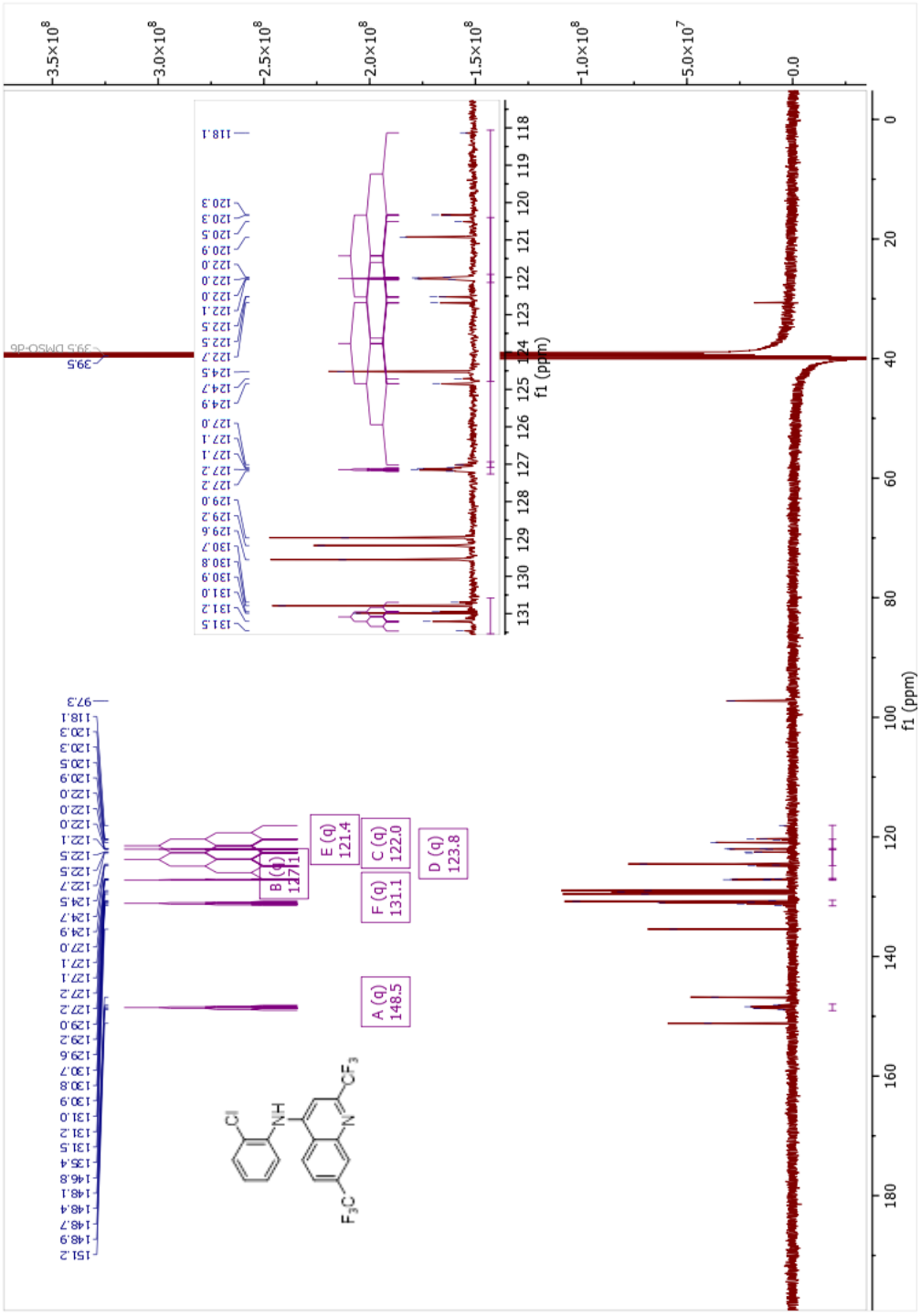
Compound 14 $^{13}C$ NMR.



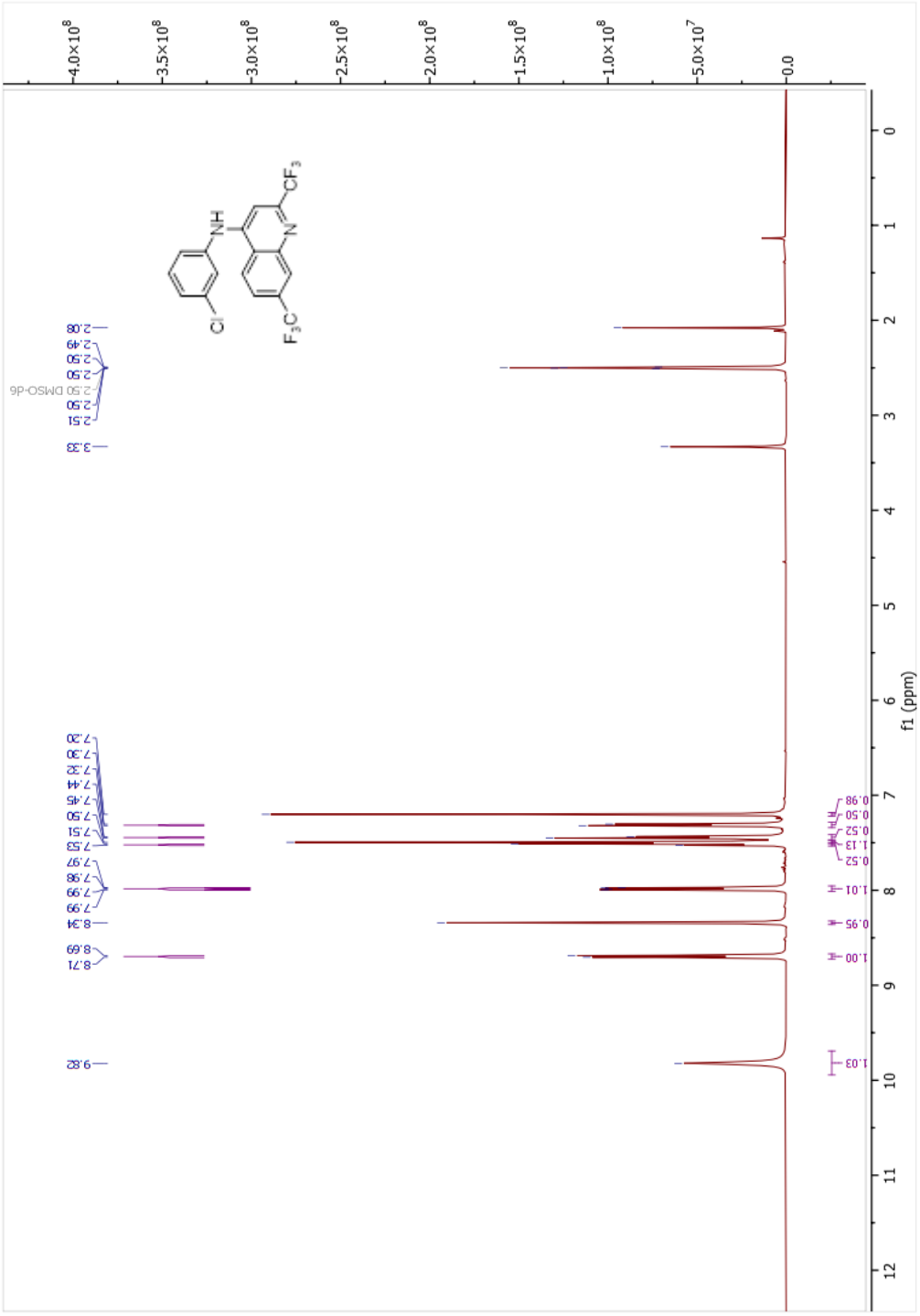
Compound 15 $^{1}H$ NMR.



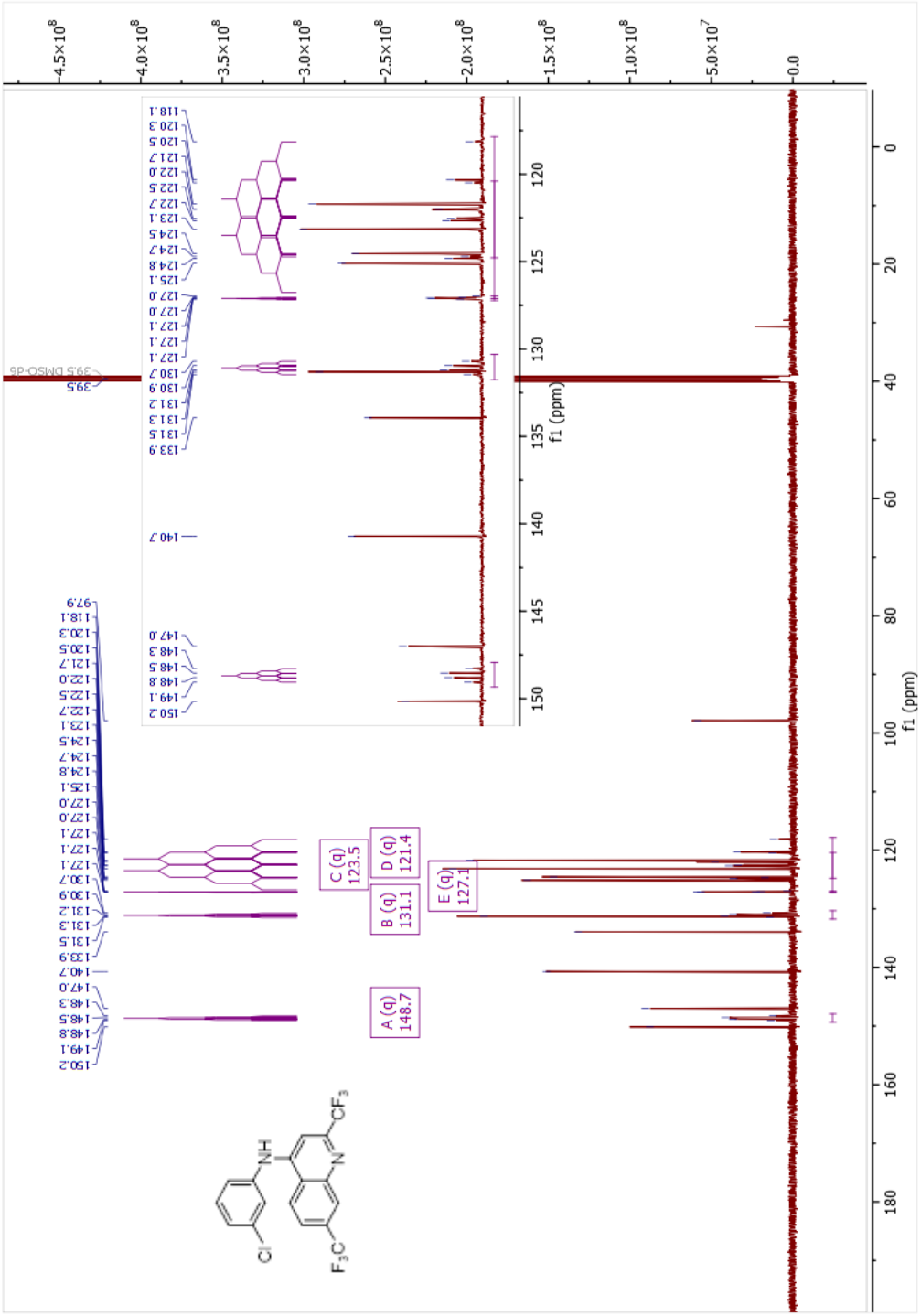
Compound 15 $^{13}C$ NMR.



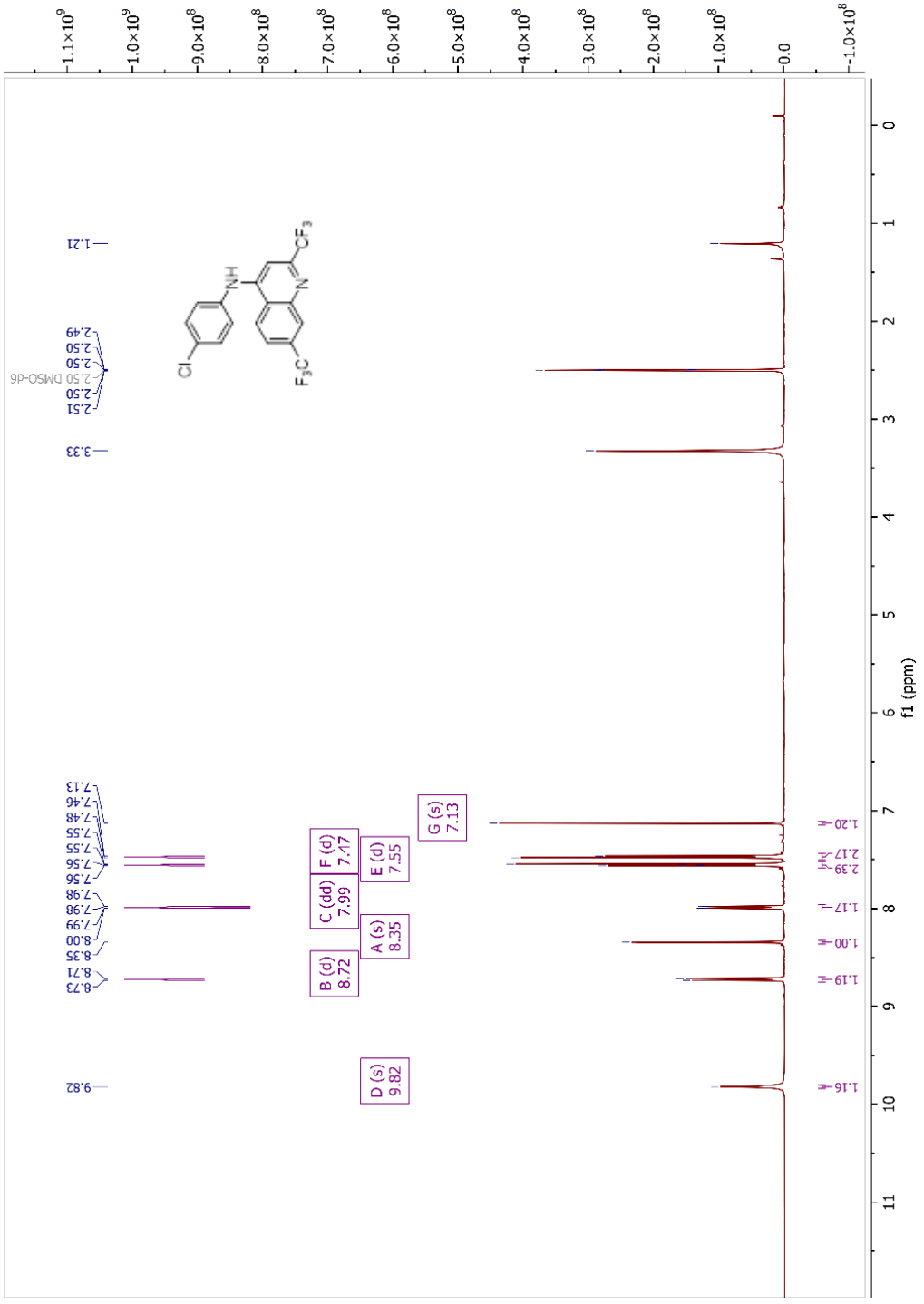
Compound 16 $^{1}$H NMR.



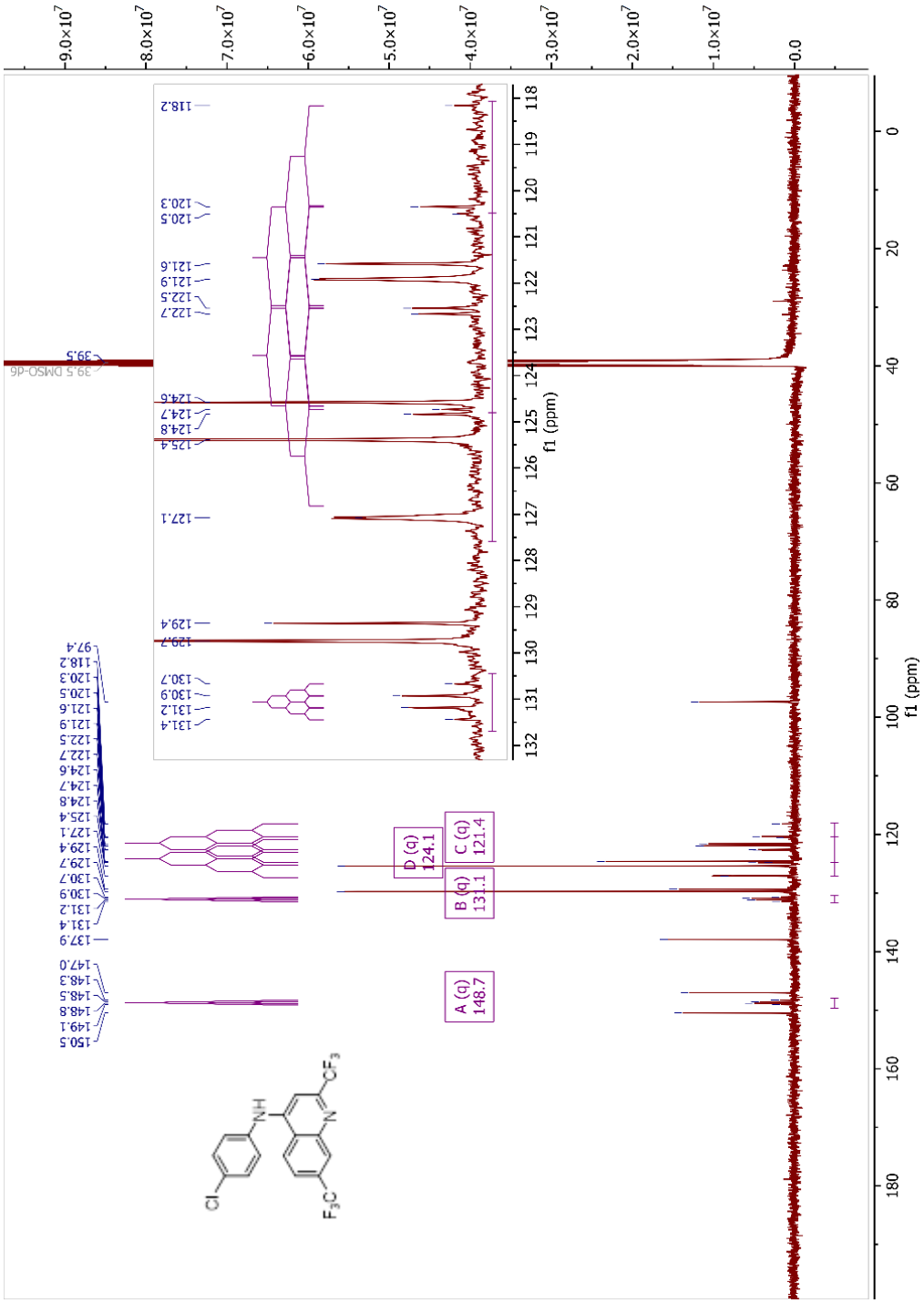
Compound 16 $^{13}C$ NMR.



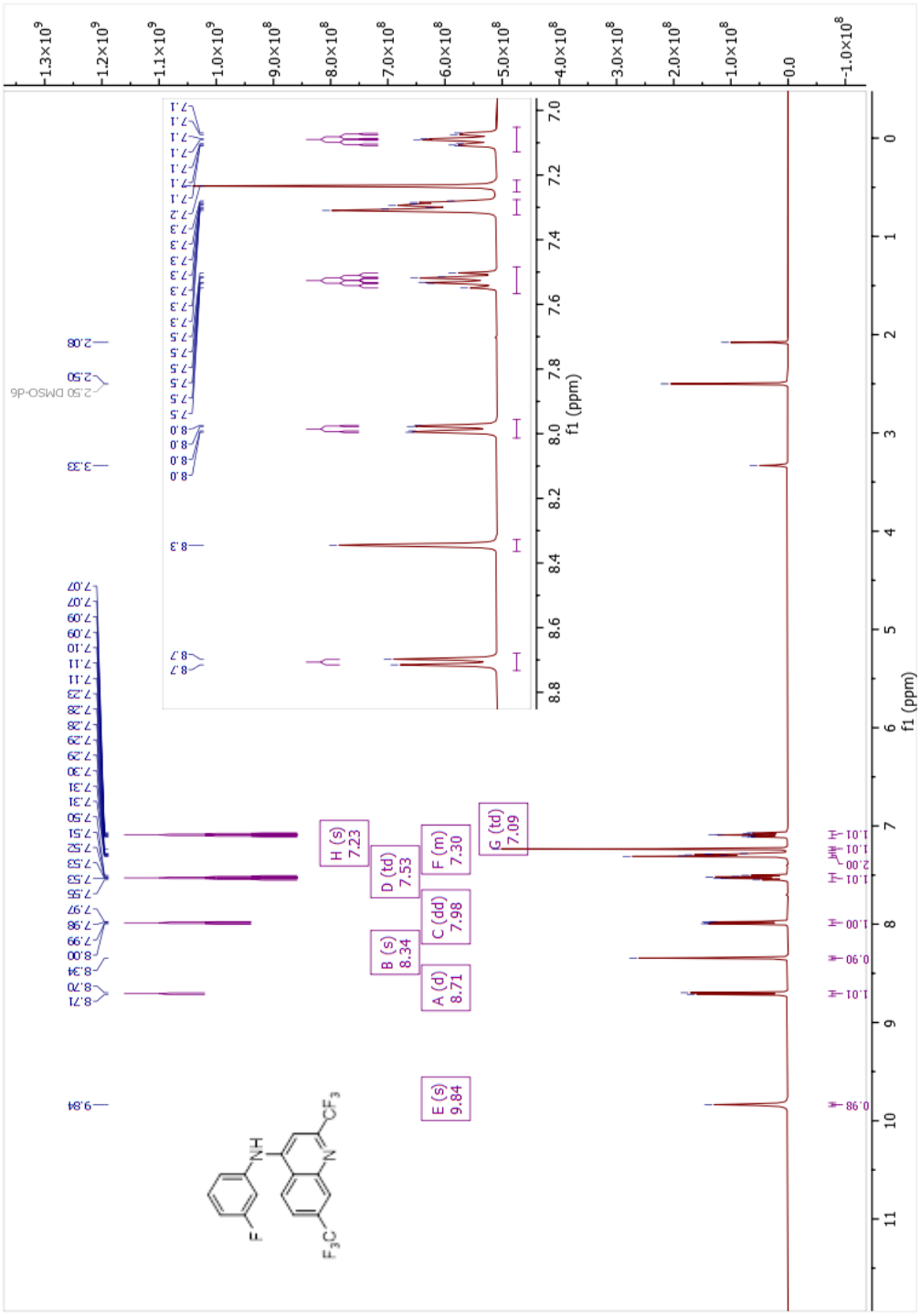
Compound 17 $^{1}$H NMR.



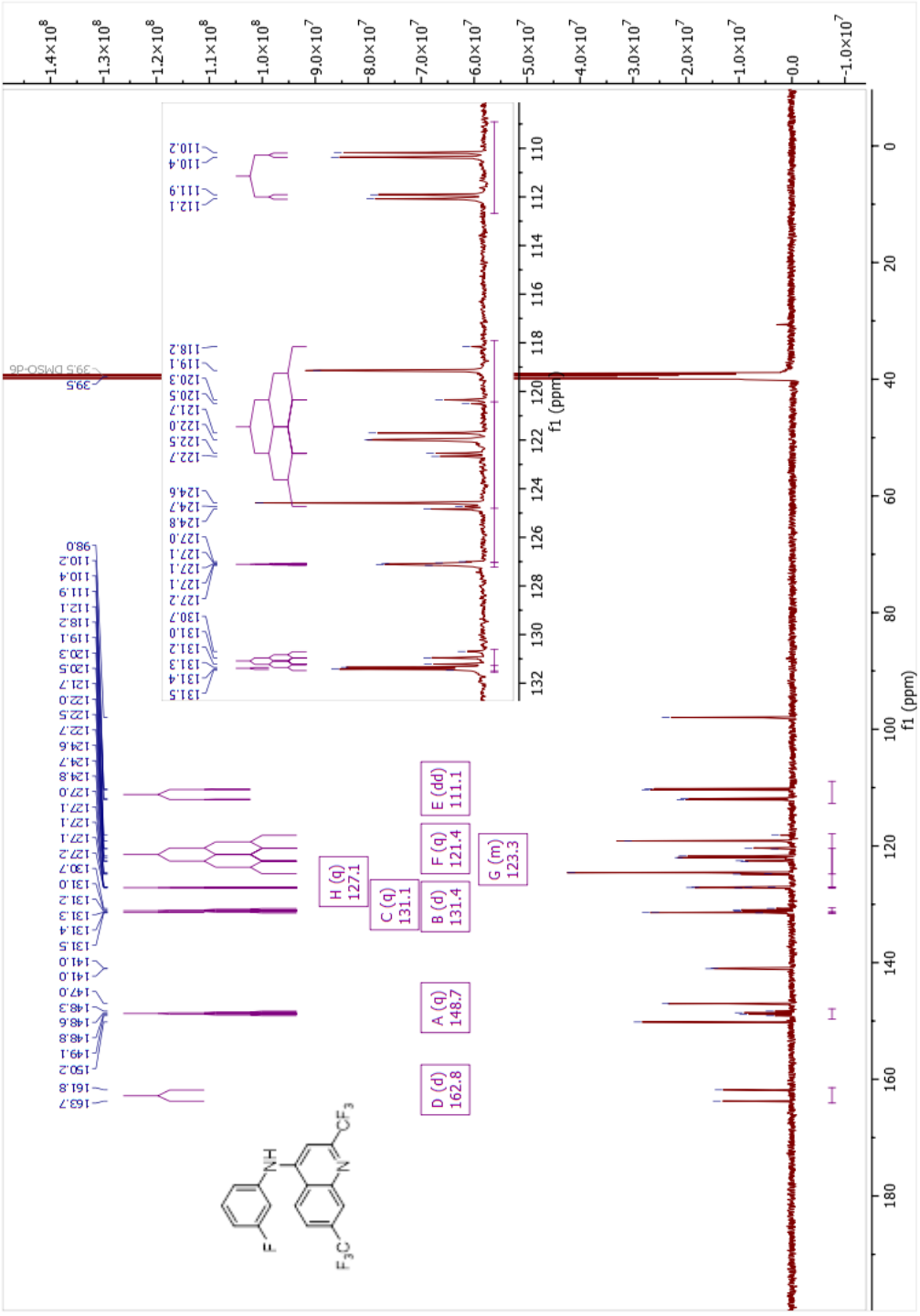
Compound 17 $^{13}C$ NMR.



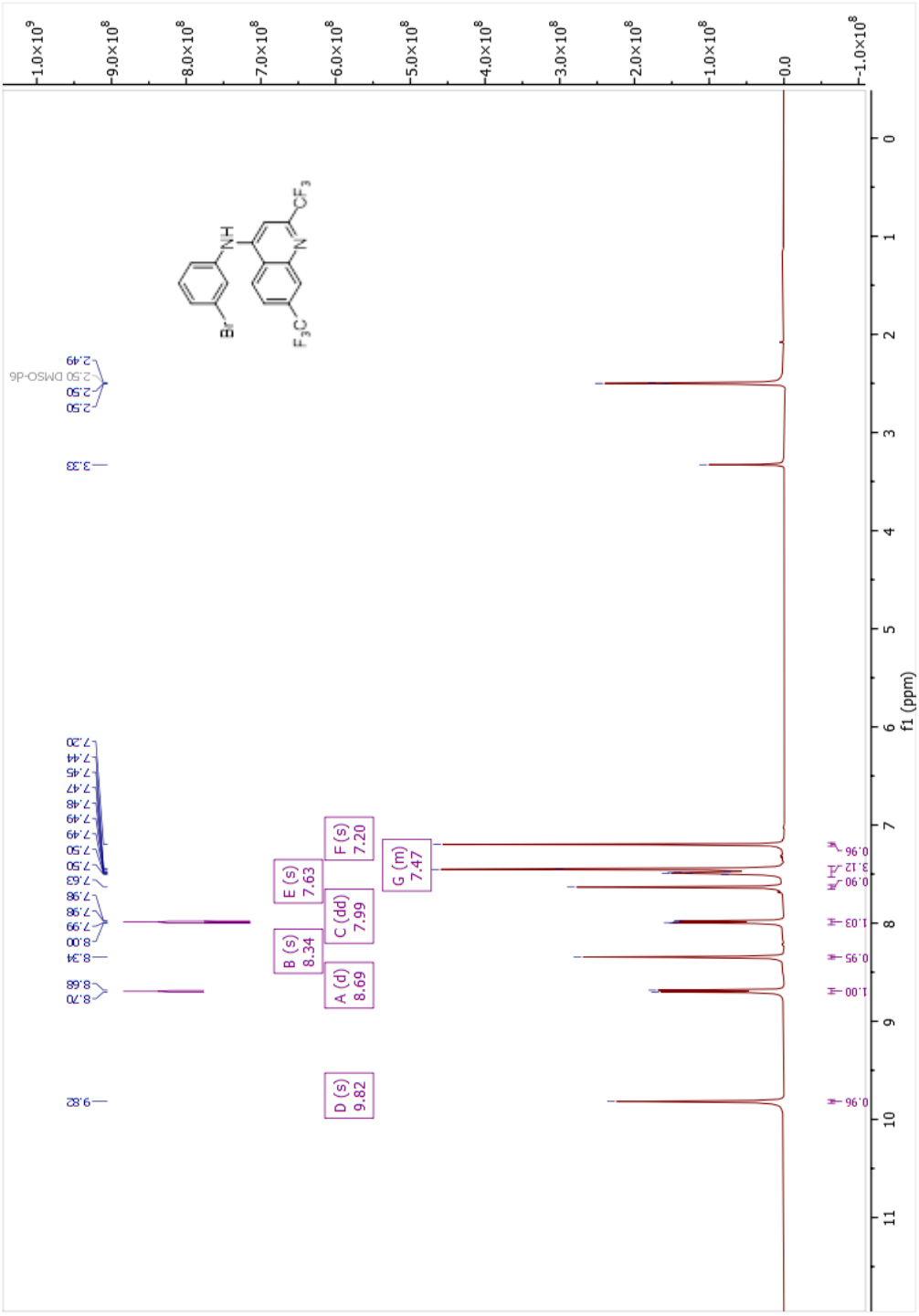
Compound 18 $^{1}$H NMR.



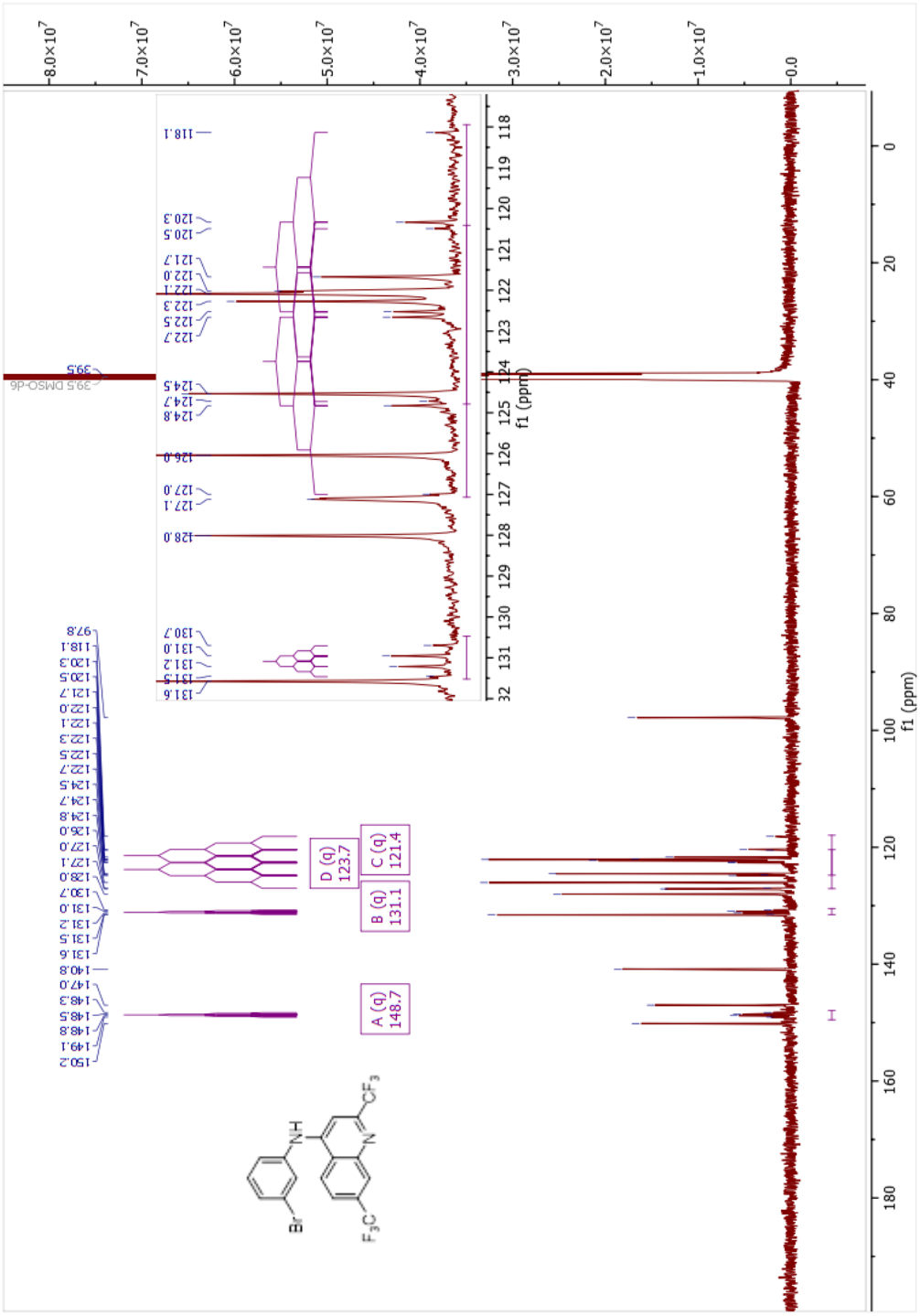
Compound 18 $^{13}C$ NMR.



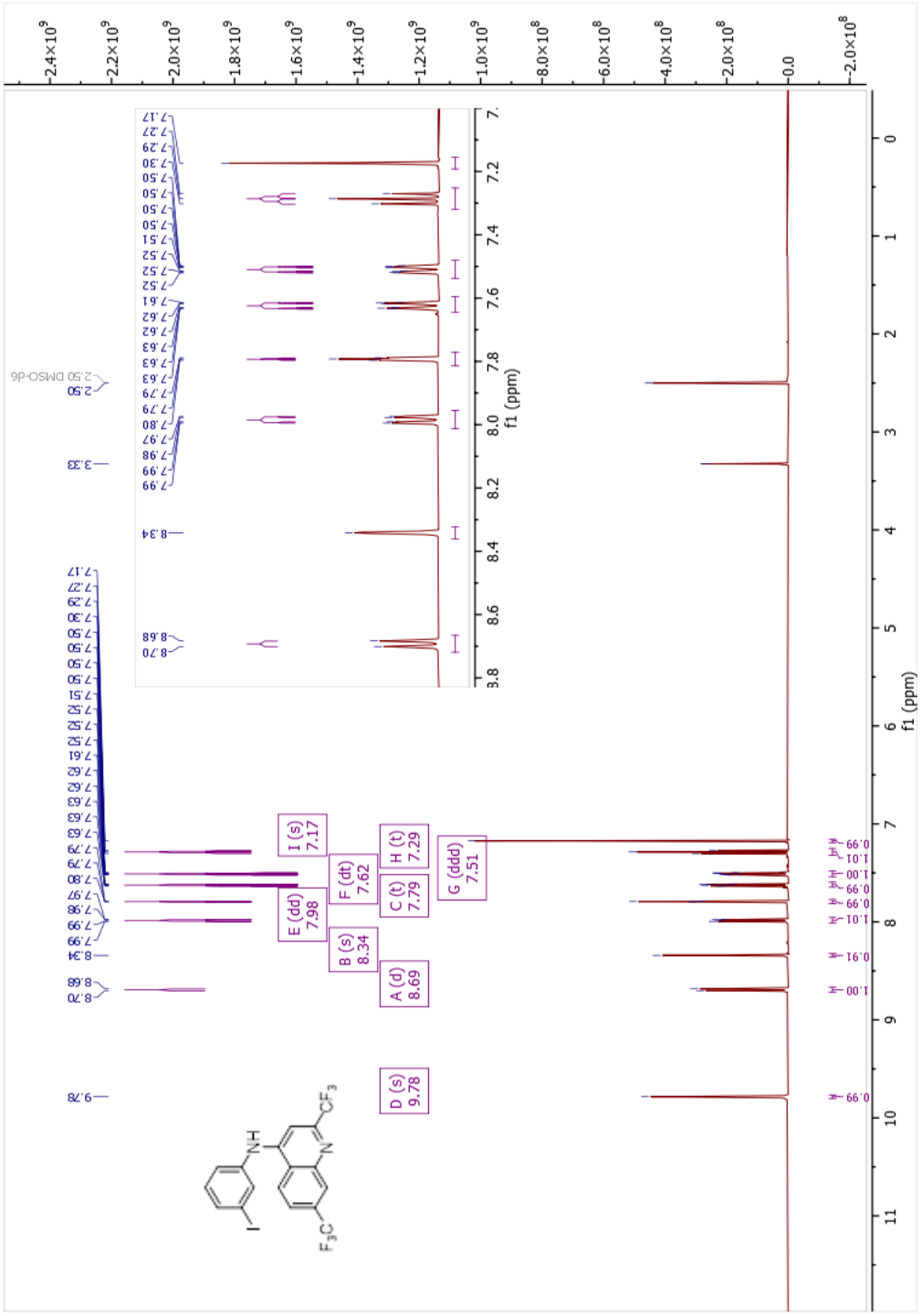
Compound 19 $^{1}$H NMR.



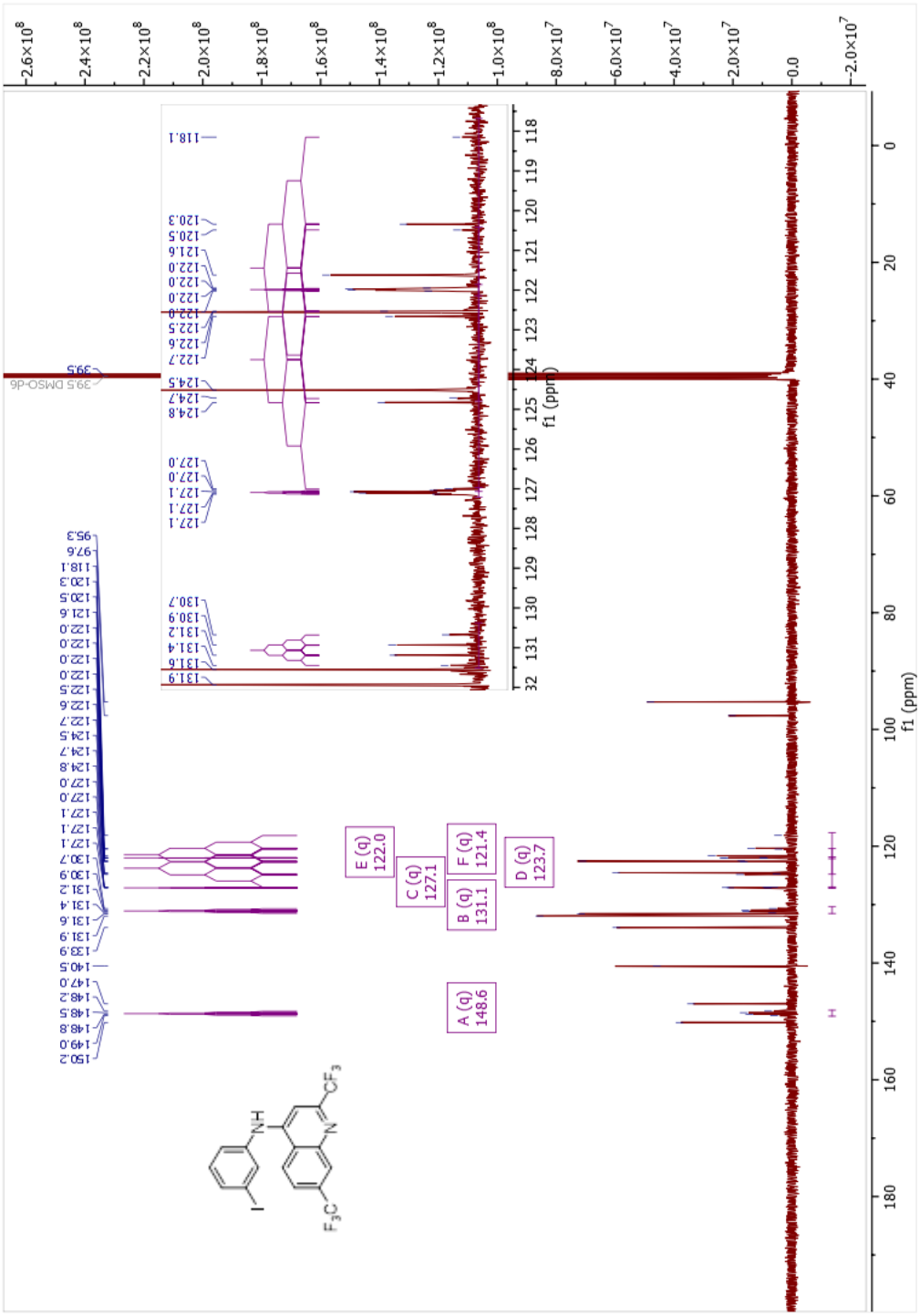
Compound 19 $^{13}C$ NMR.



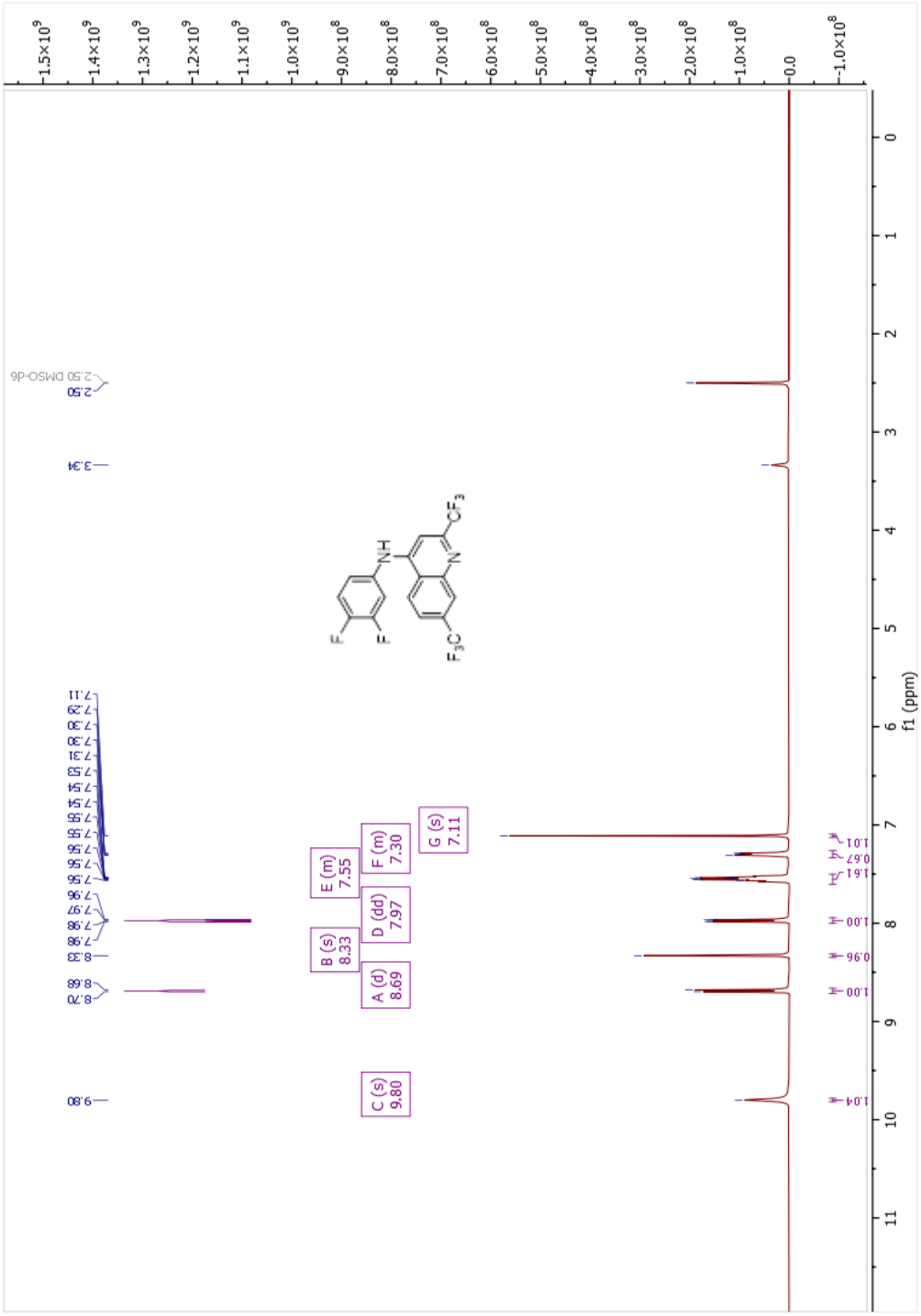
Compound 20 $^{1}H$ NMR.



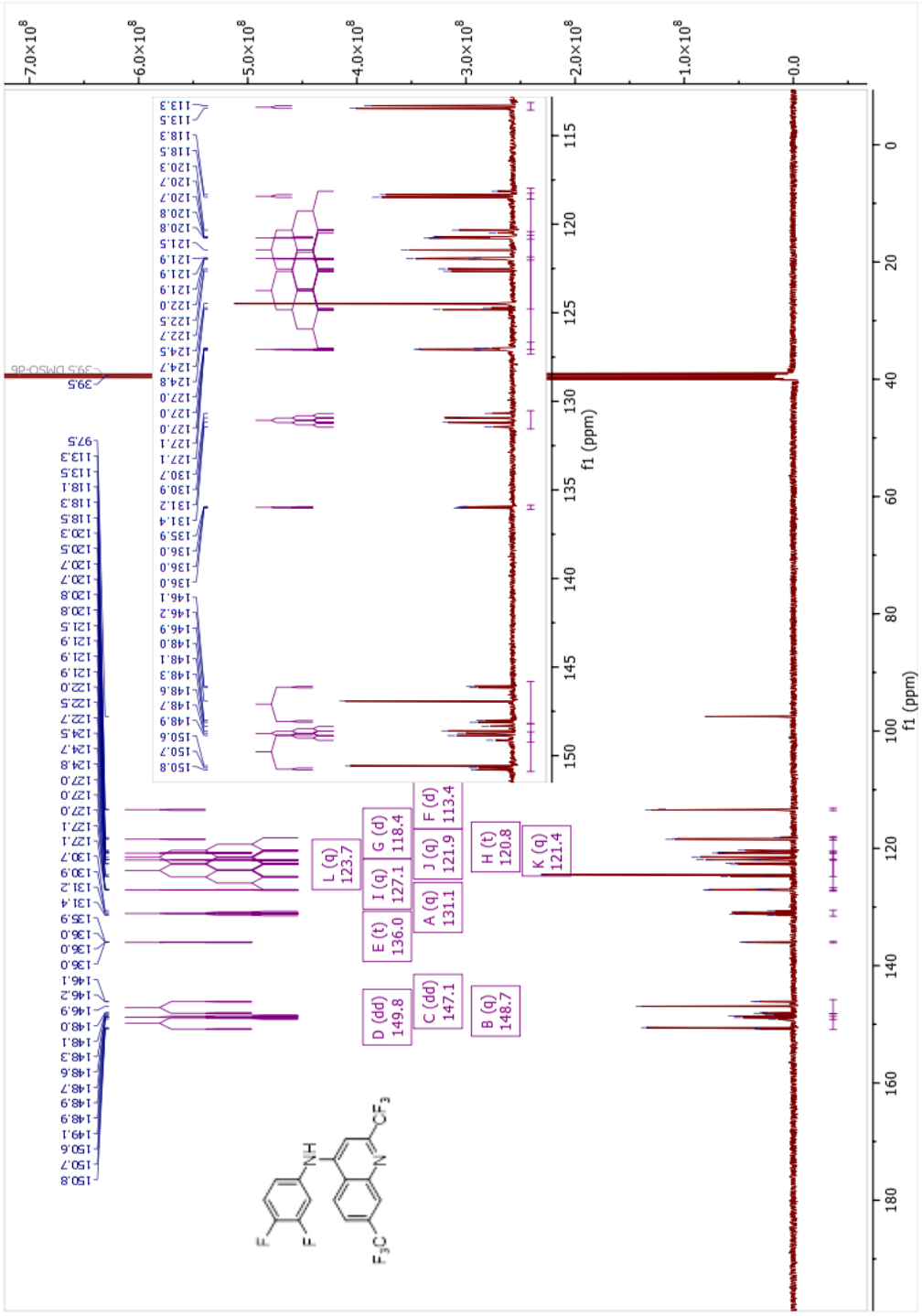
Compound 20 $^{13}C$ NMR.



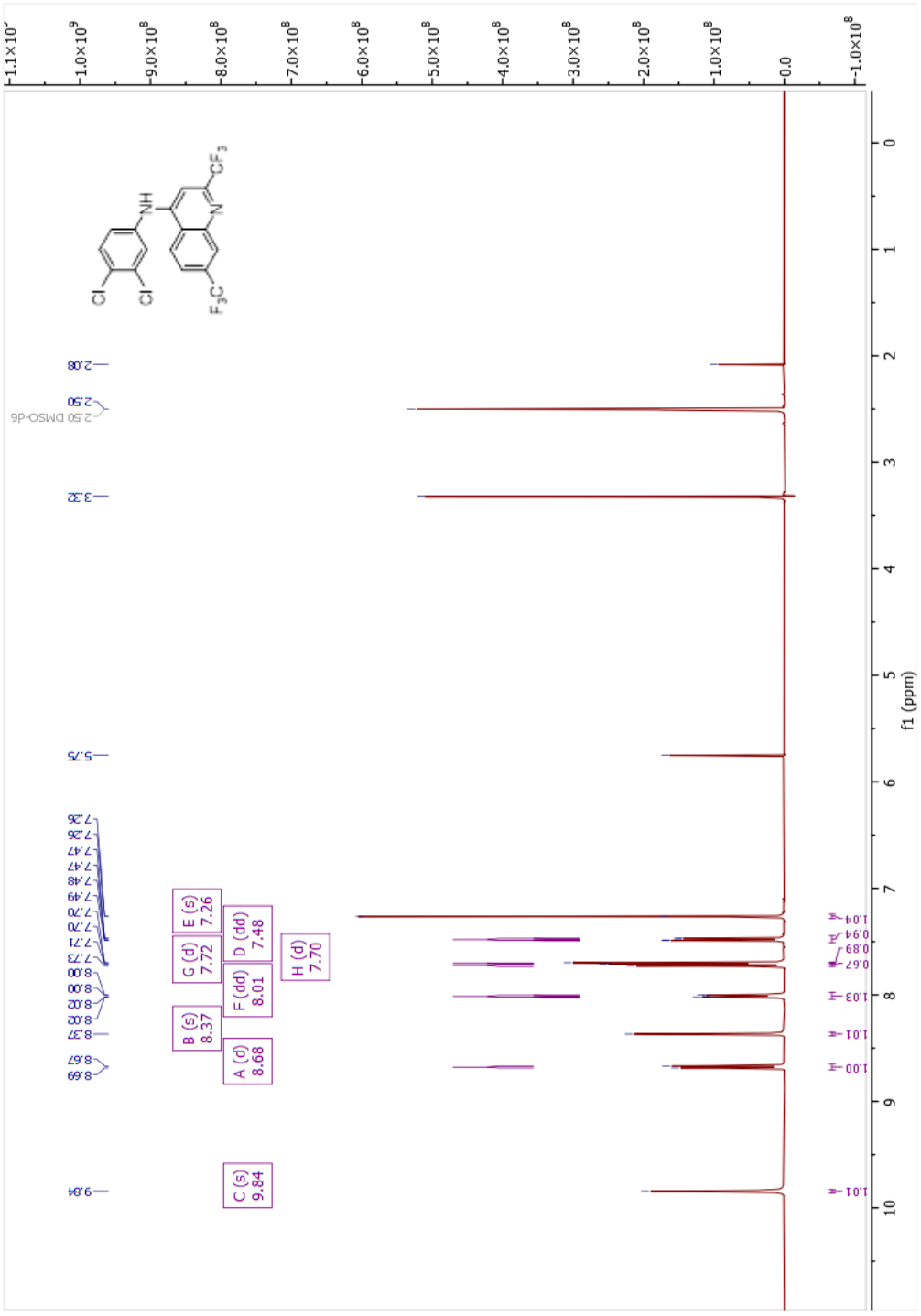
Compound 21 $^{1}$H NMR.



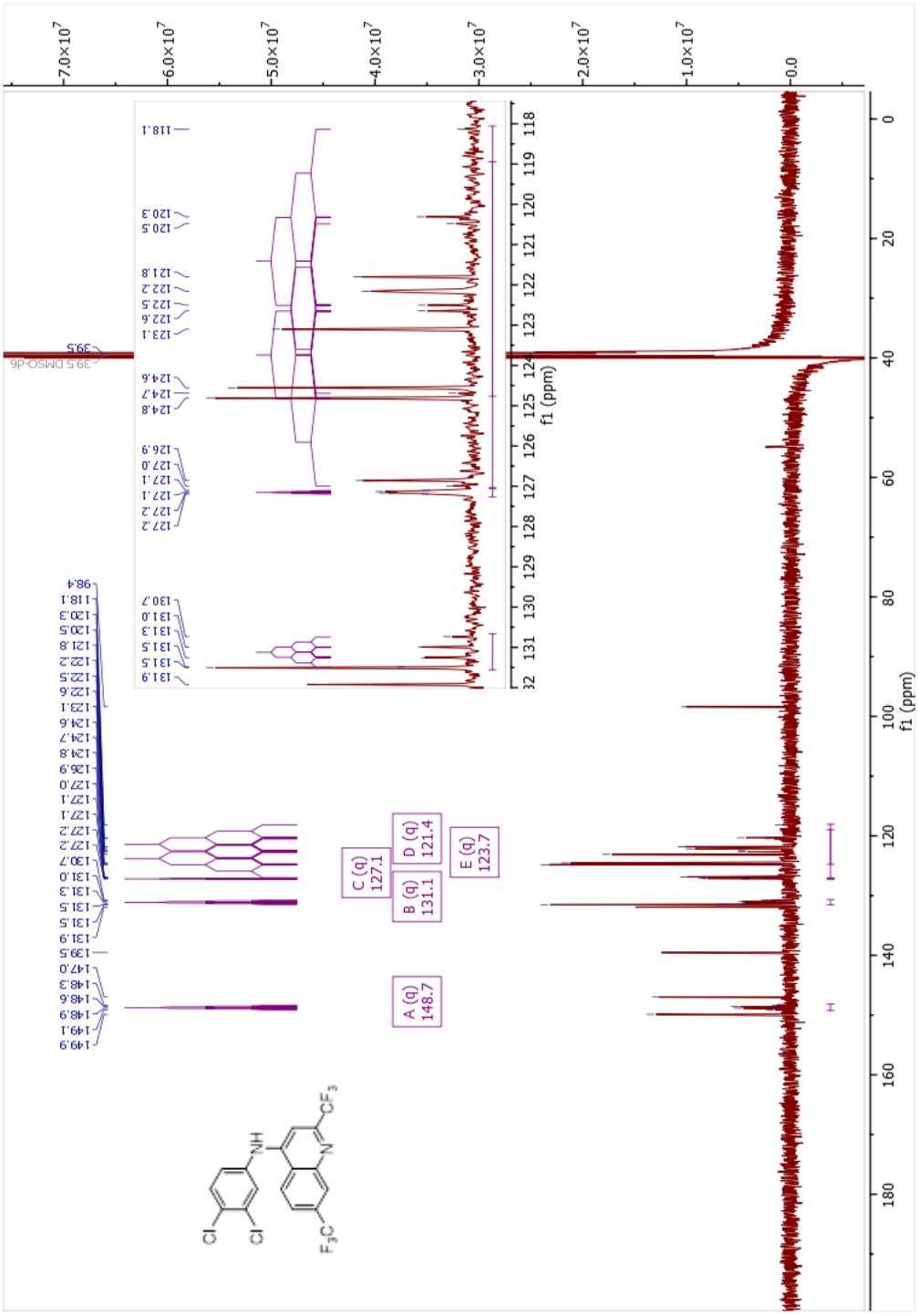
Compound 21 $^{13}C$ NMR.



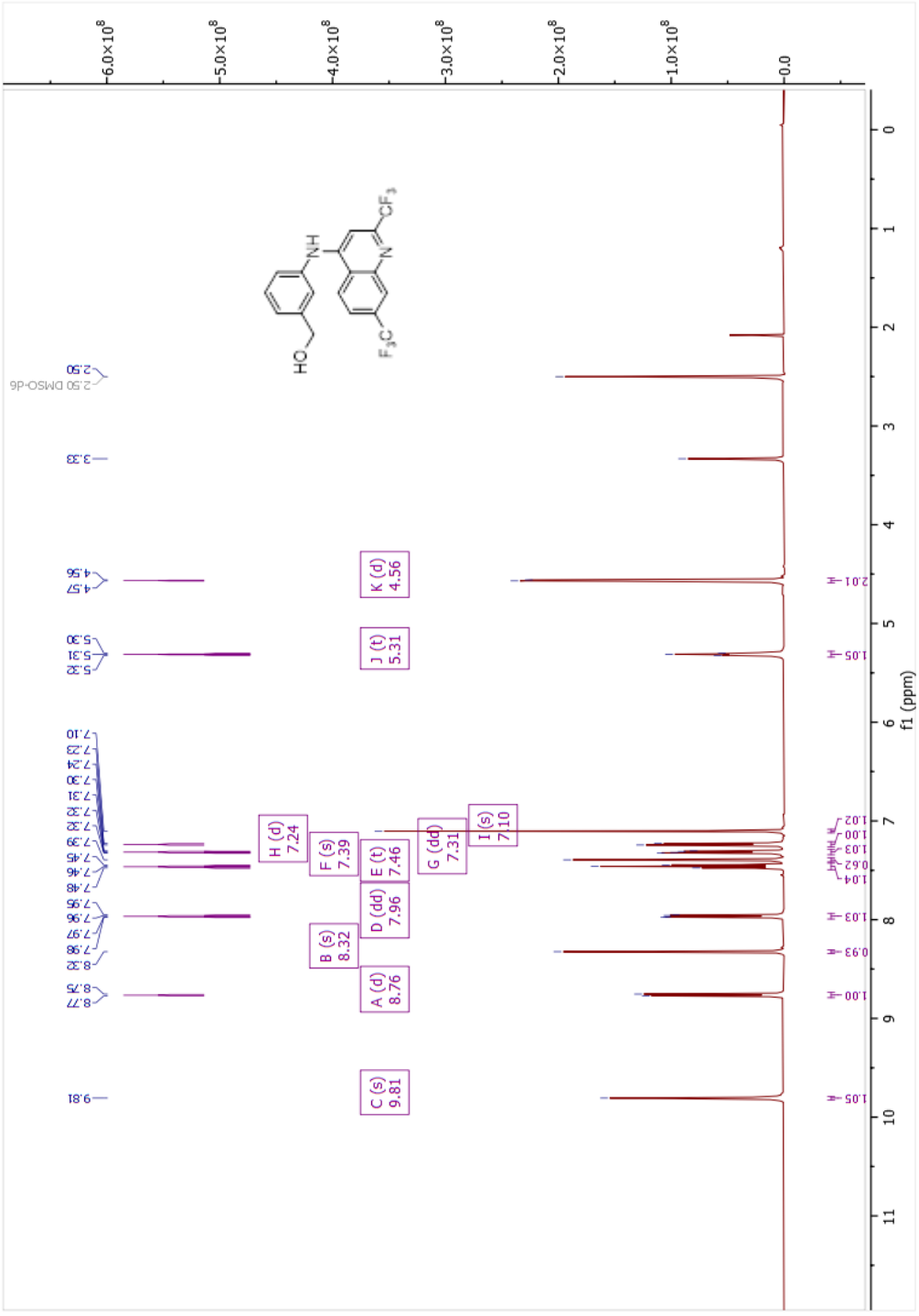
Compound 22 $^{1}$H NMR.



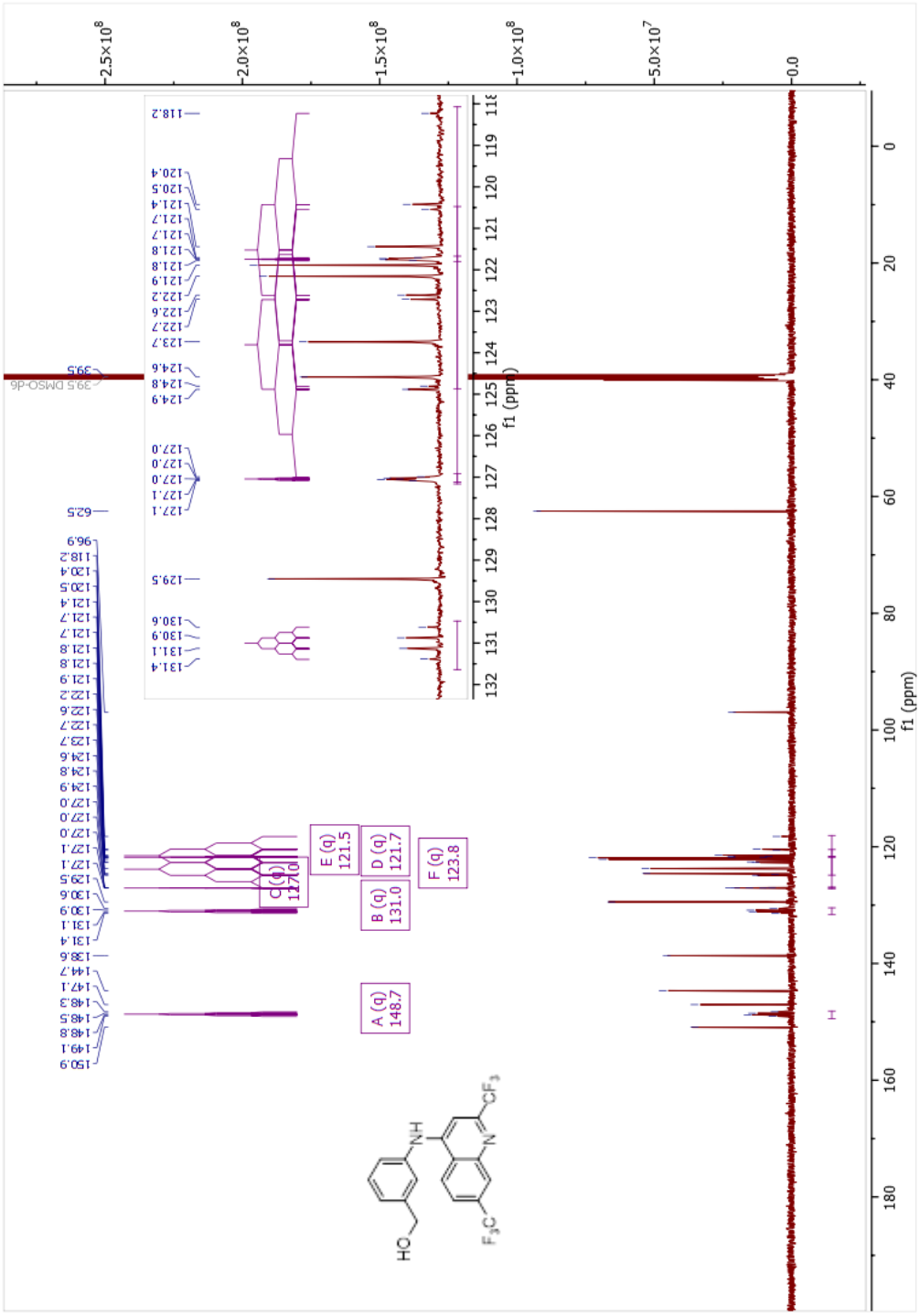
Compound 22 $^{13}C$ NMR.



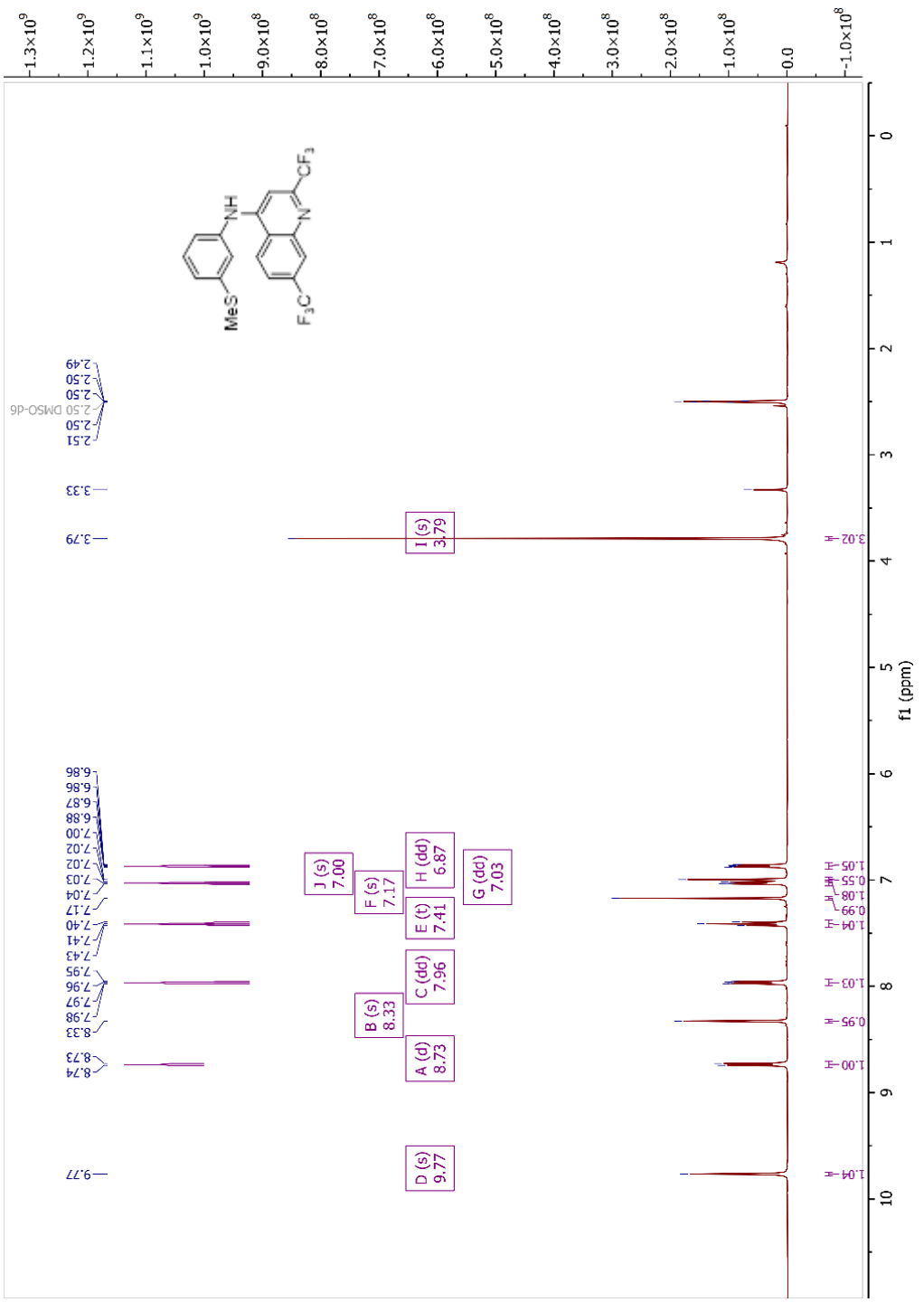
Compound 23 $^{1}$H NMR.



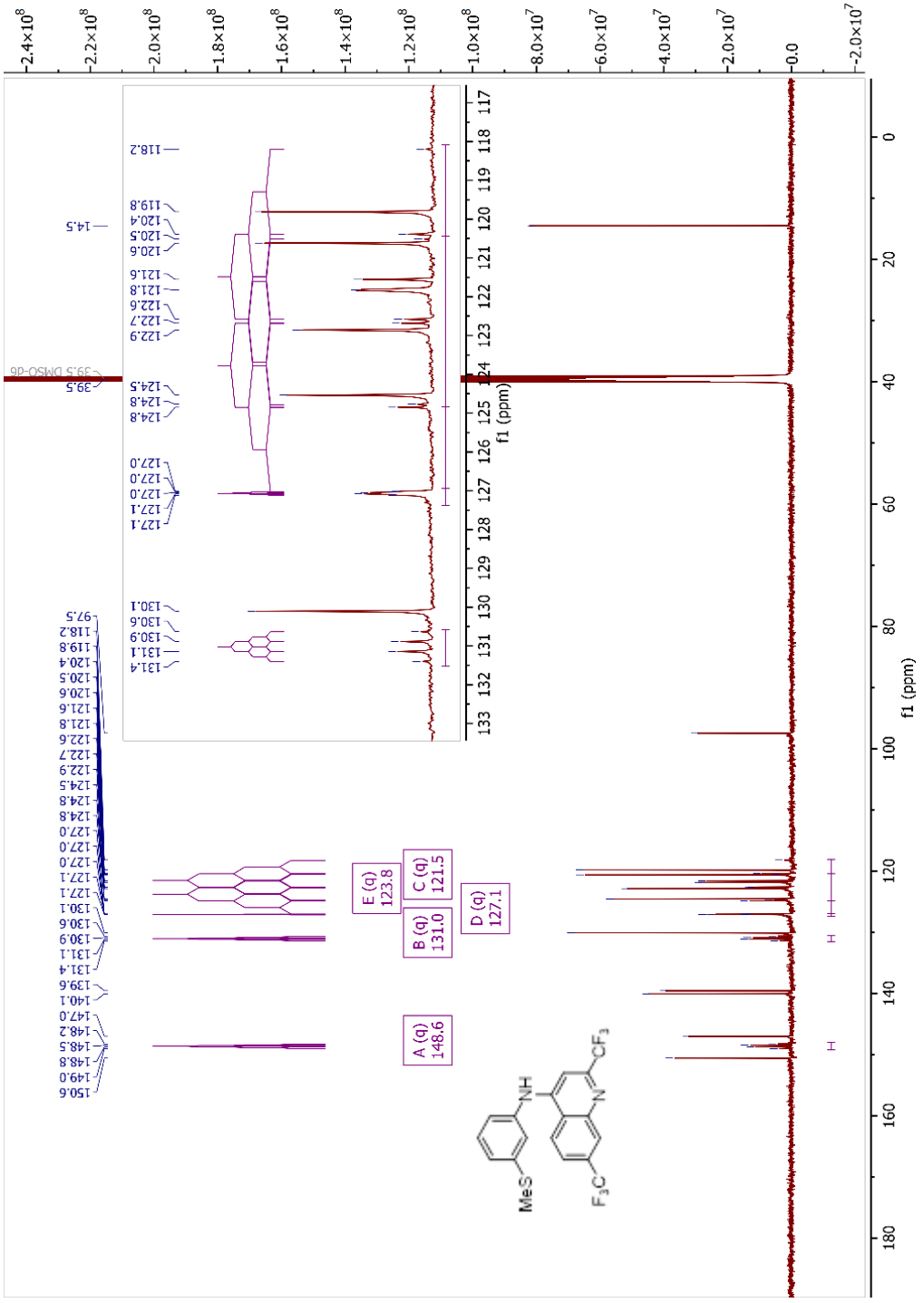
Compound 23 $^{13}C$ NMR.



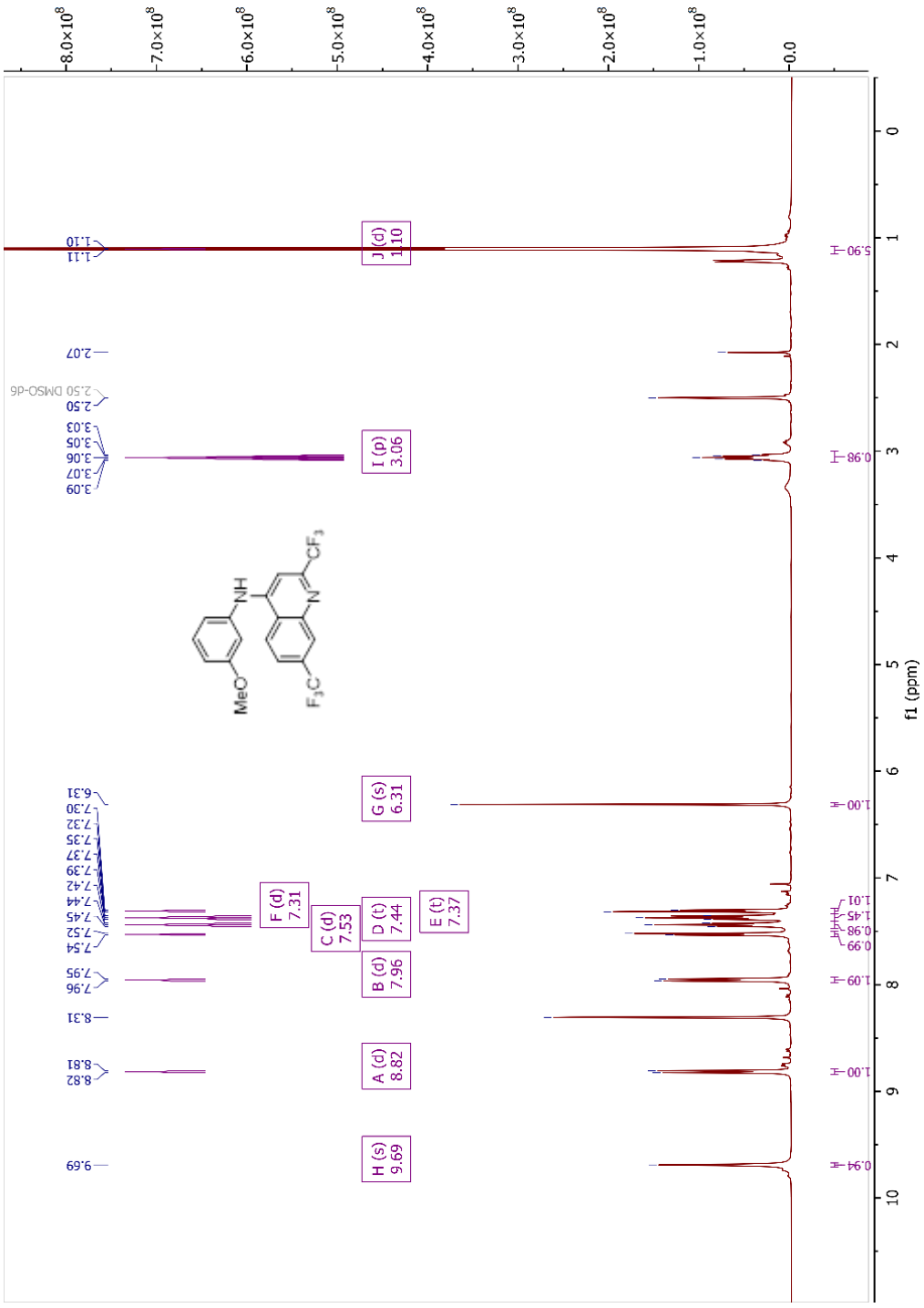
Compound 24 $^{1}$H NMR.



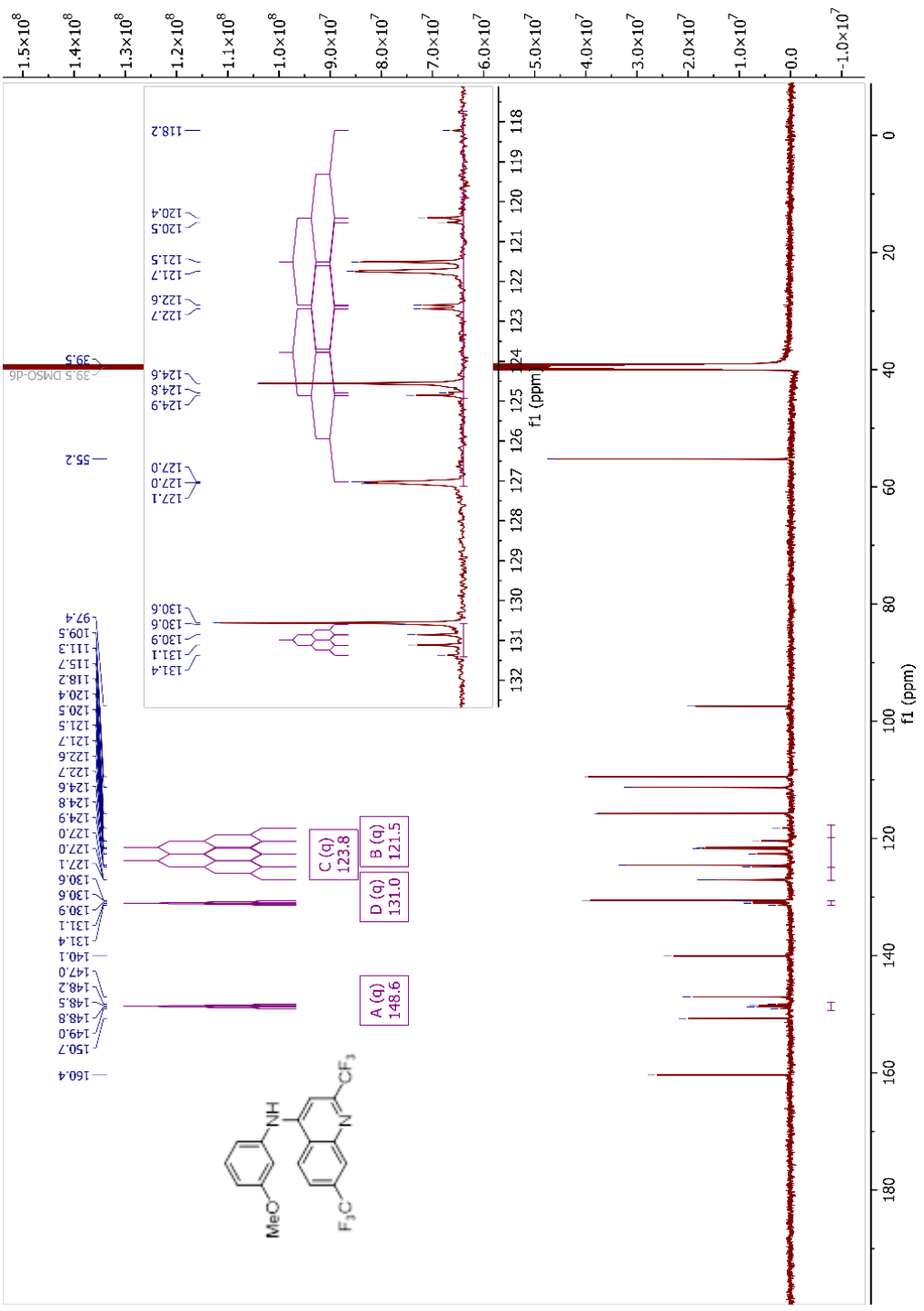
Compound 24 $^{13}C$ NMR.



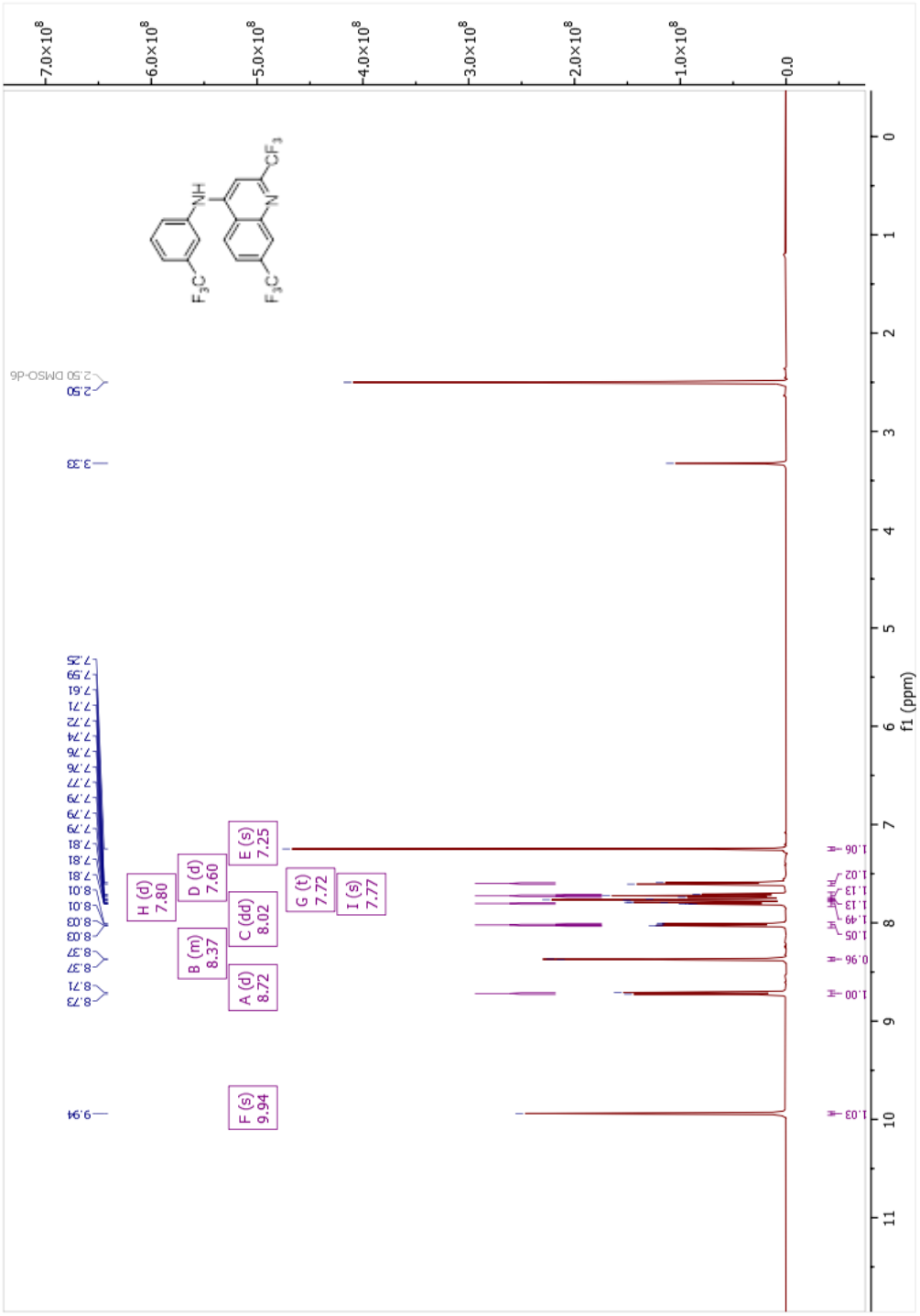
Compound 25 $^{1}$H NMR.



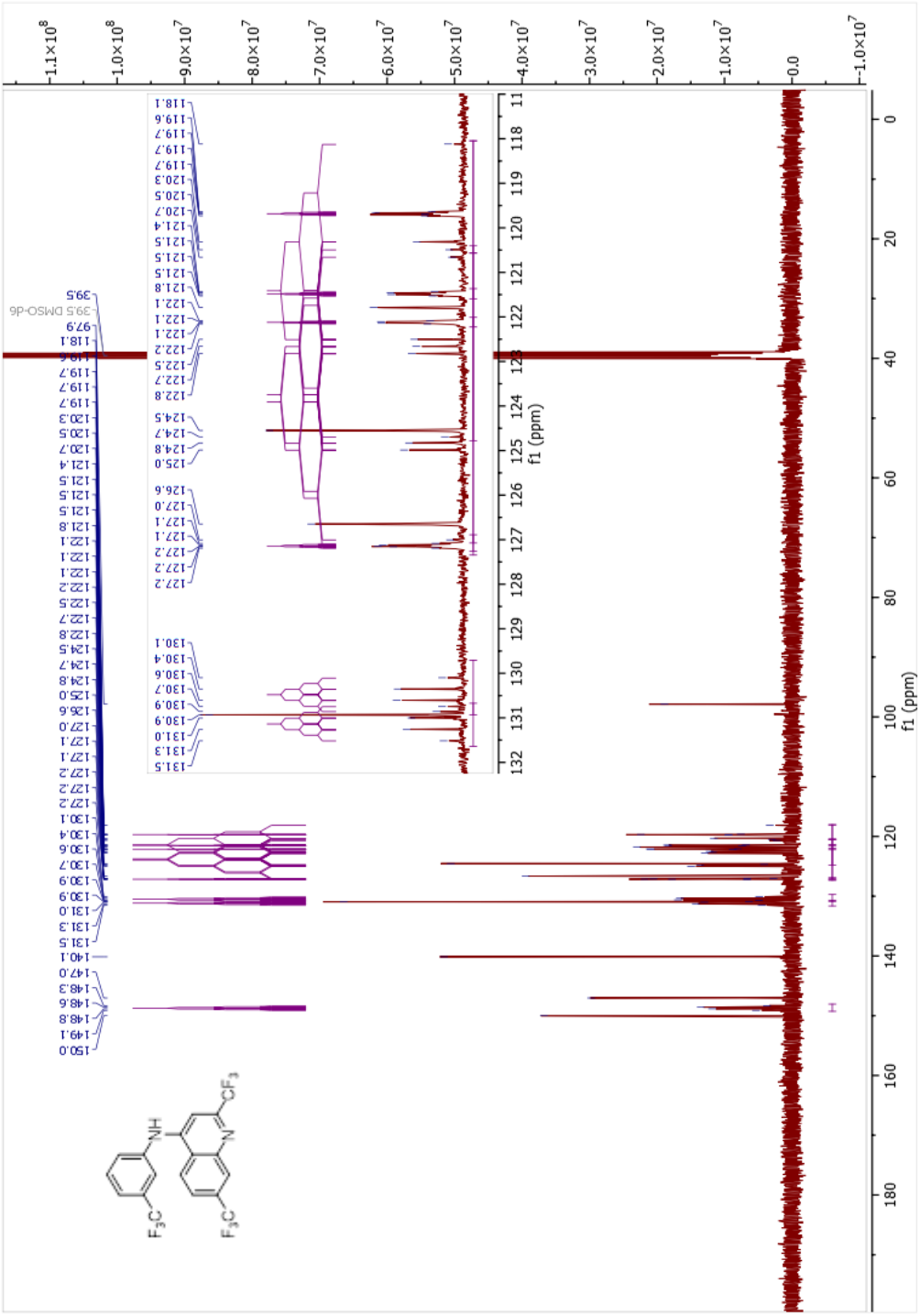
Compound 25 $^{13}C$ NMR.



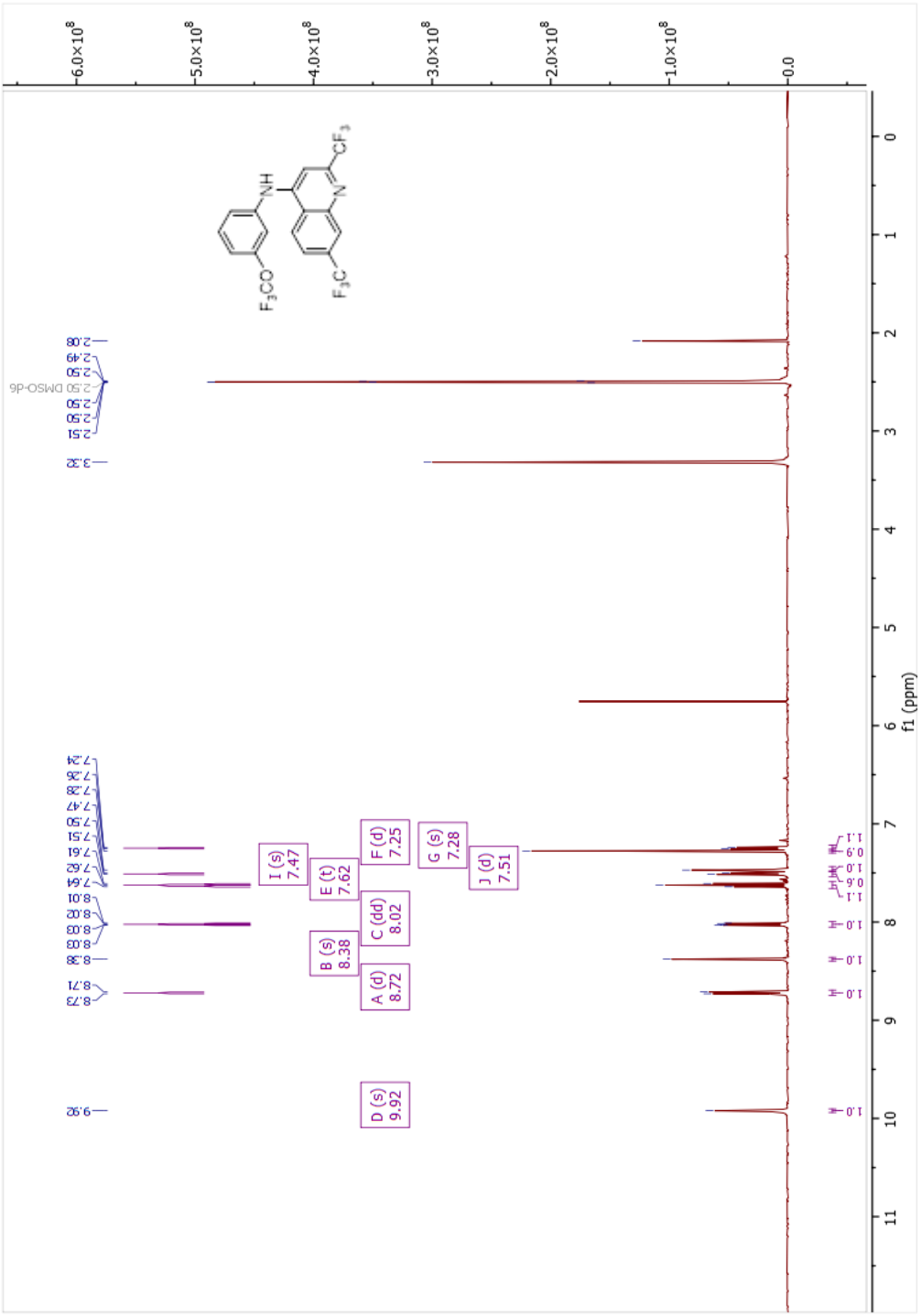
Compound 26 $^{1}$H NMR.



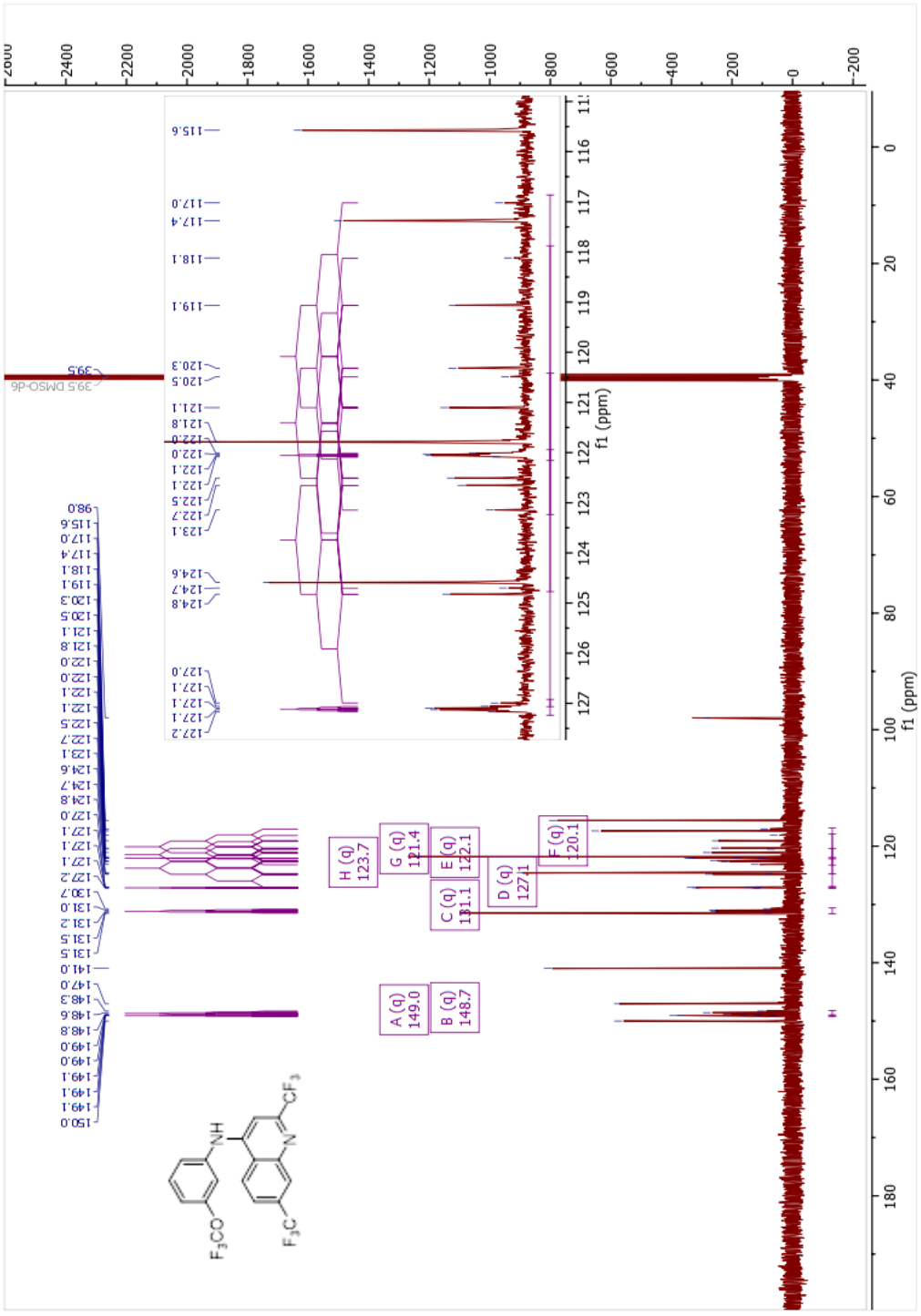
Compound 26 $^{13}C$ NMR.



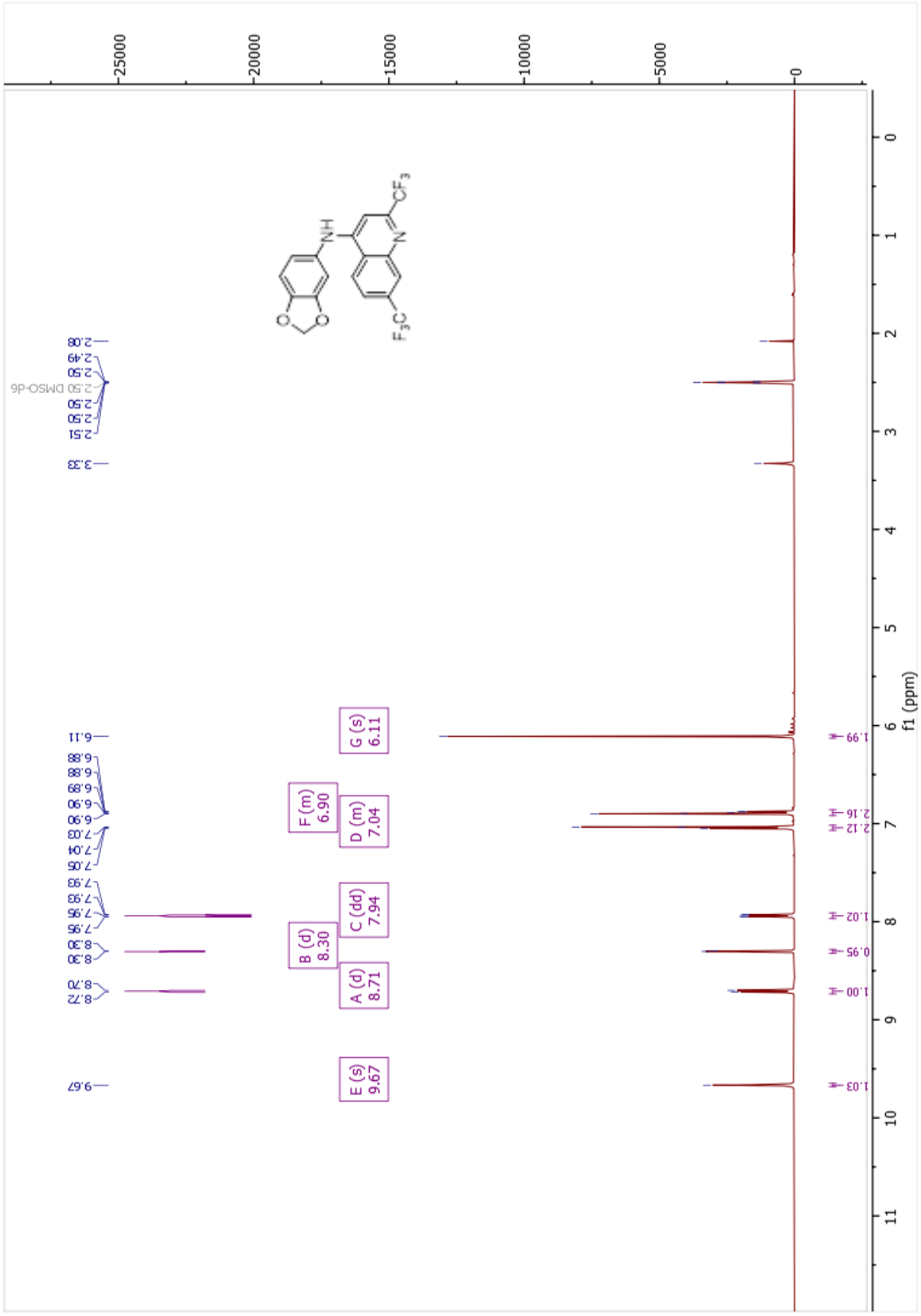
Compound 27 $^{1}$H NMR.



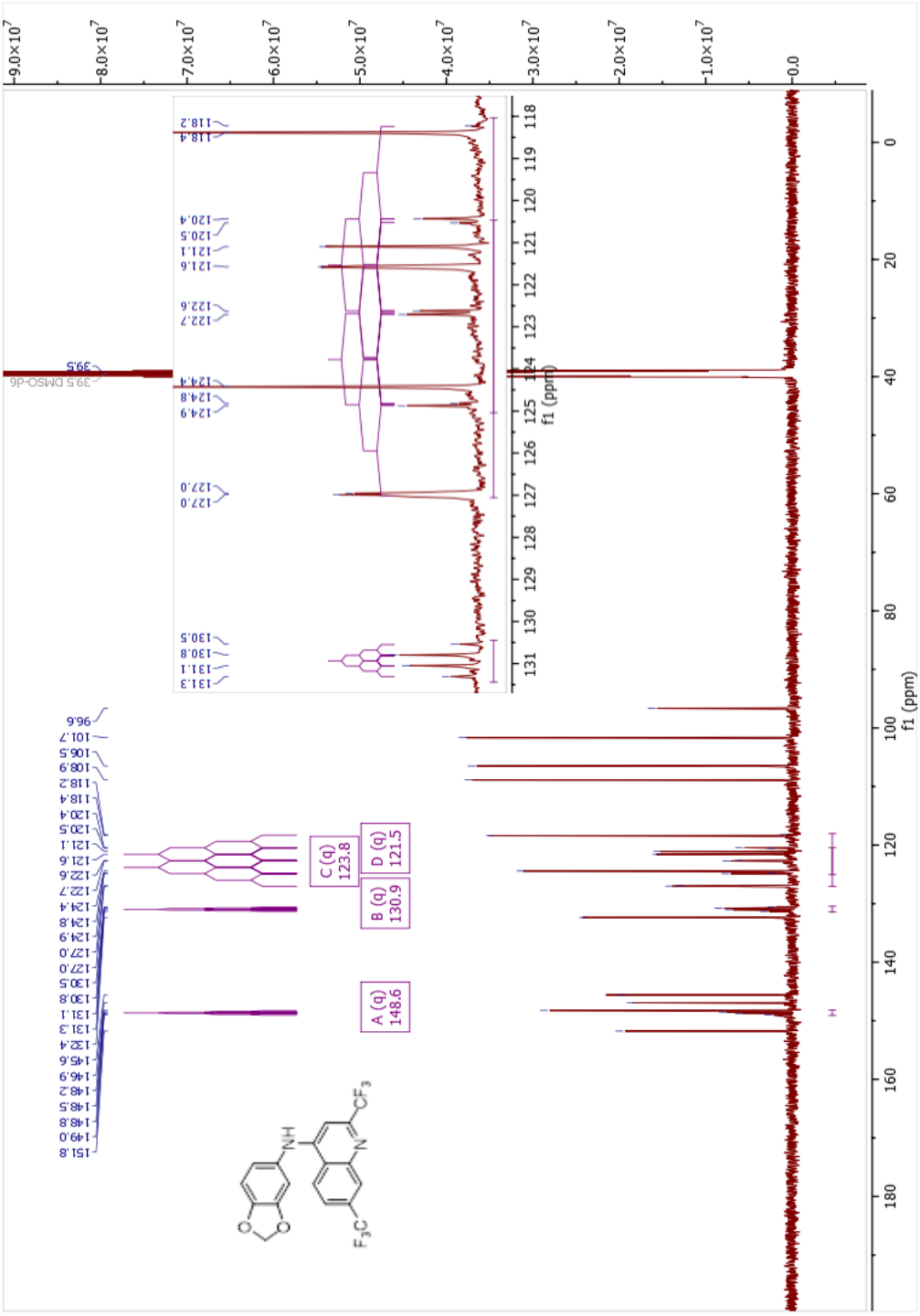
Compound 27 $^{13}C$ NMR.



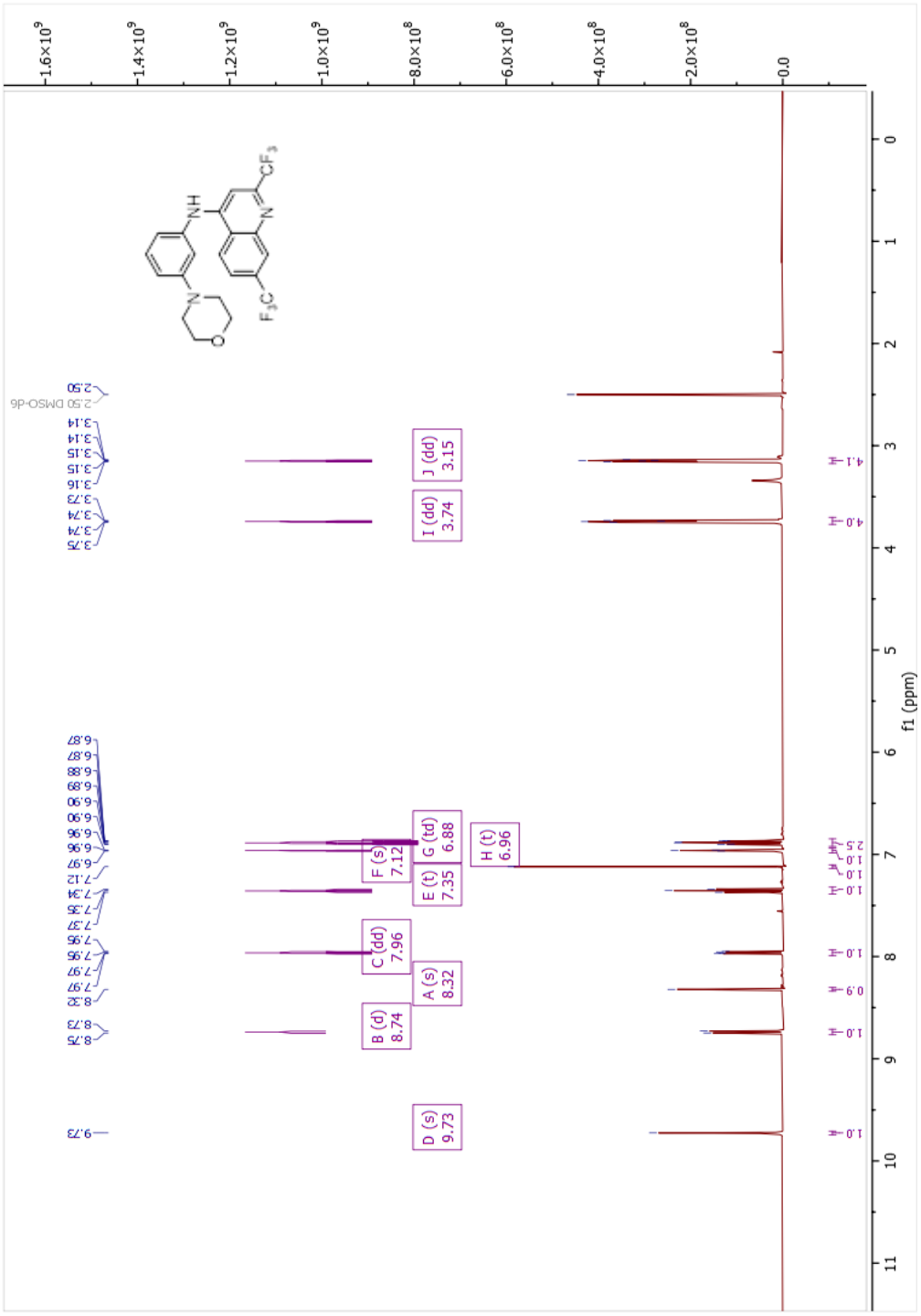
Compound 28 $^{1}H$ NMR.



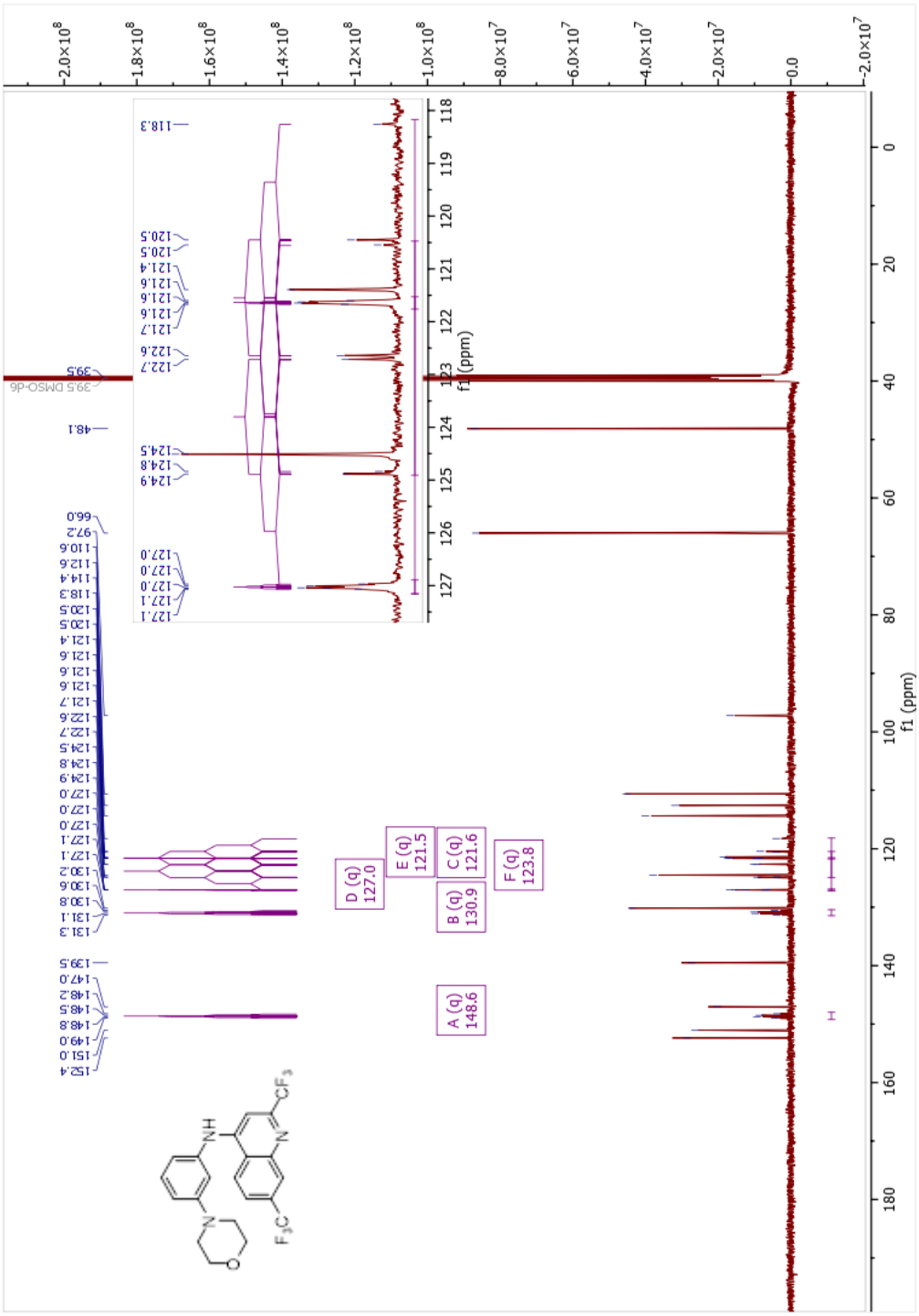
Compound 28 $^{13}C$ NMR.



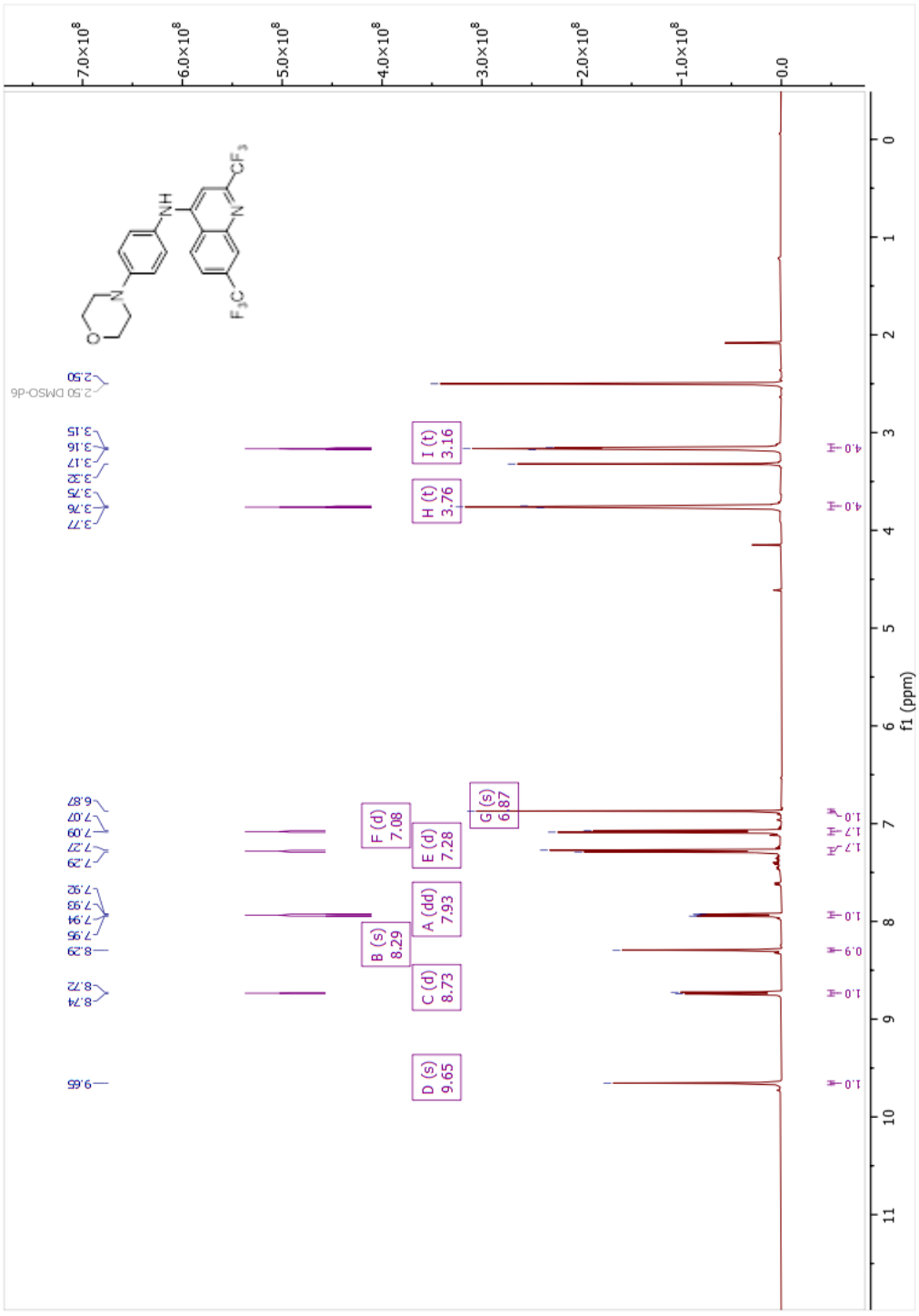
Compound 29 $^{1}$H NMR.



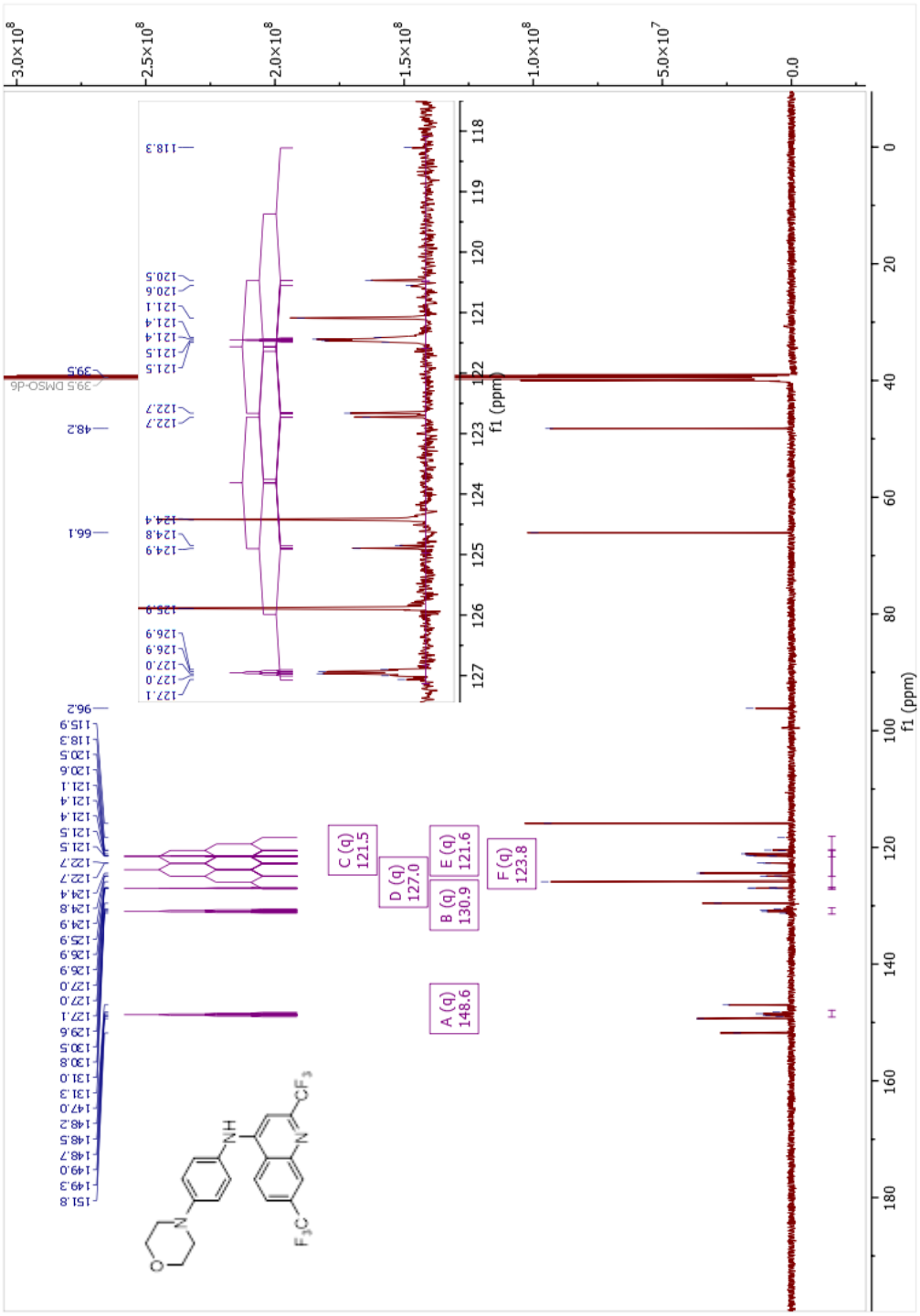
Compound 29 $^{13}C$ NMR.



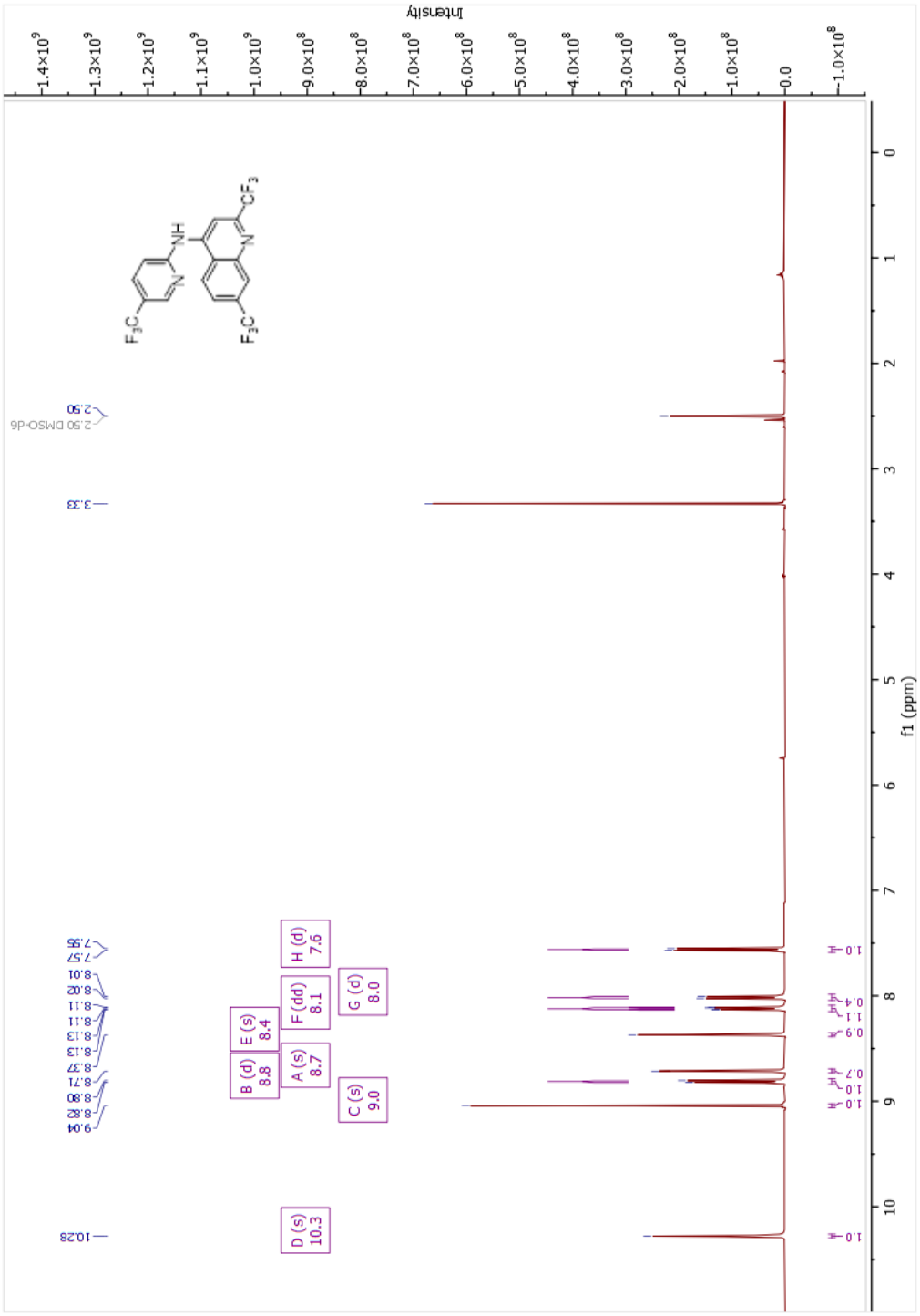
Compound 30 $^{1}$H NMR.



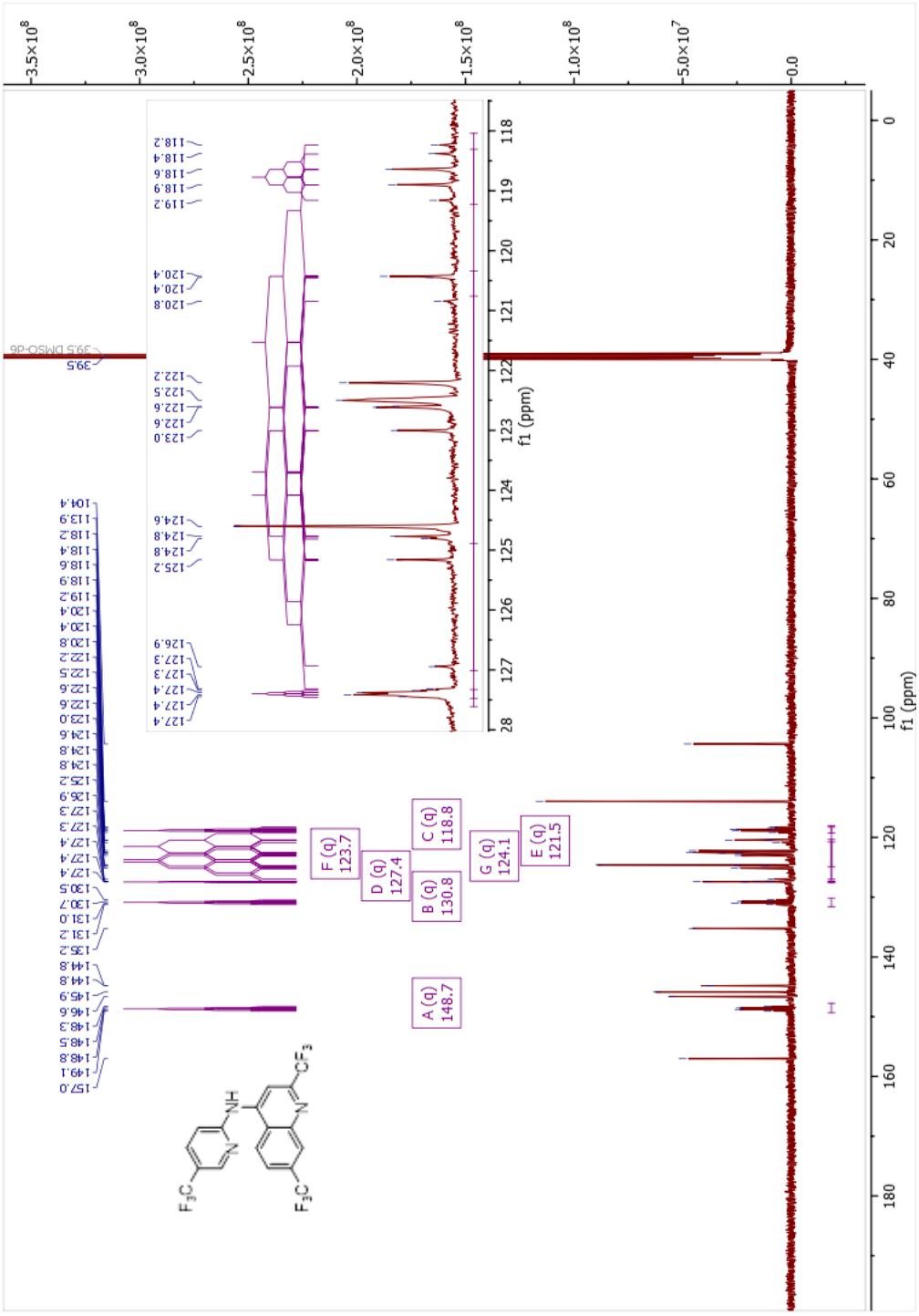
Compound 30 $^{13}$C NMR.



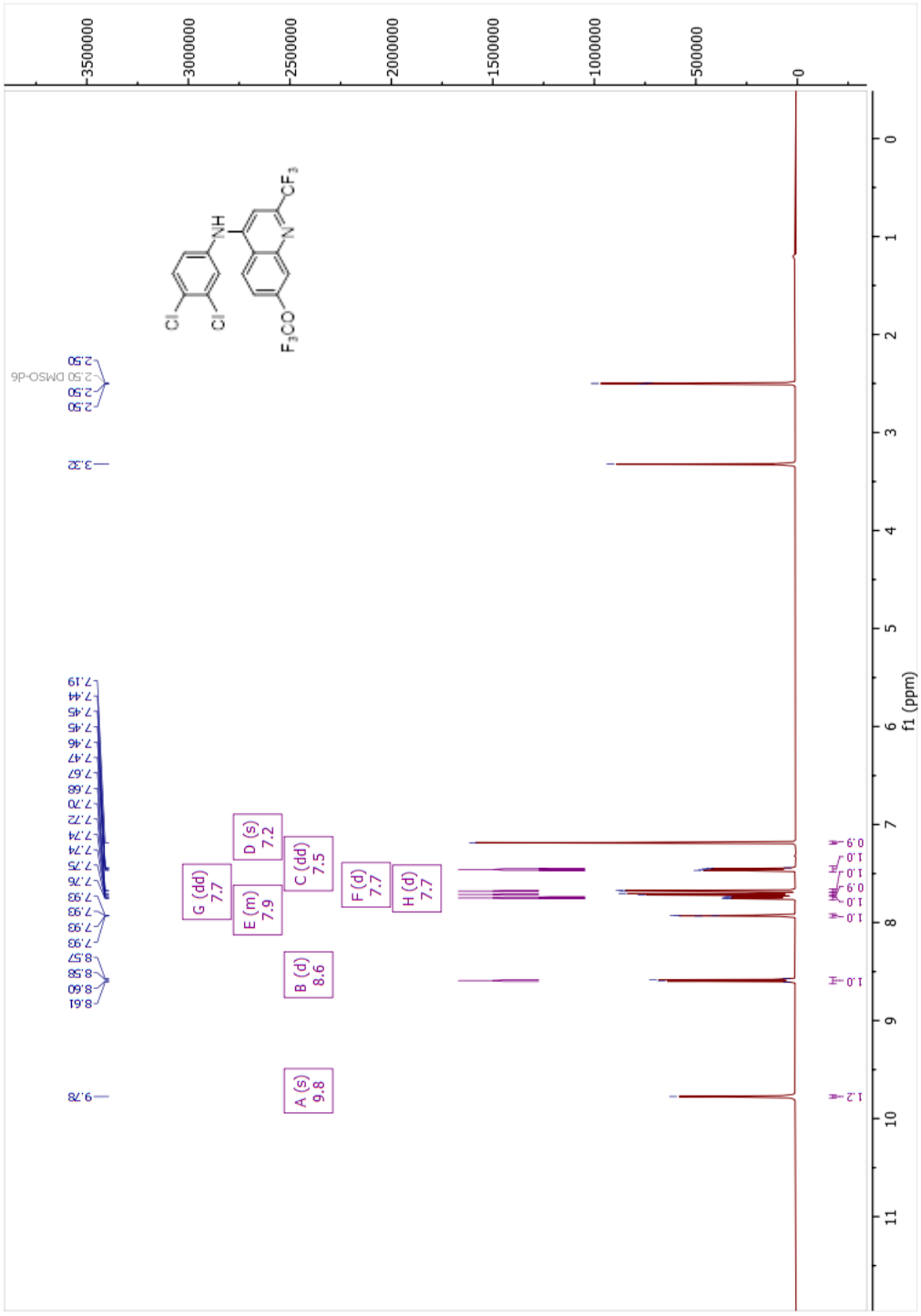
Compound 31 $^{1}$H NMR.



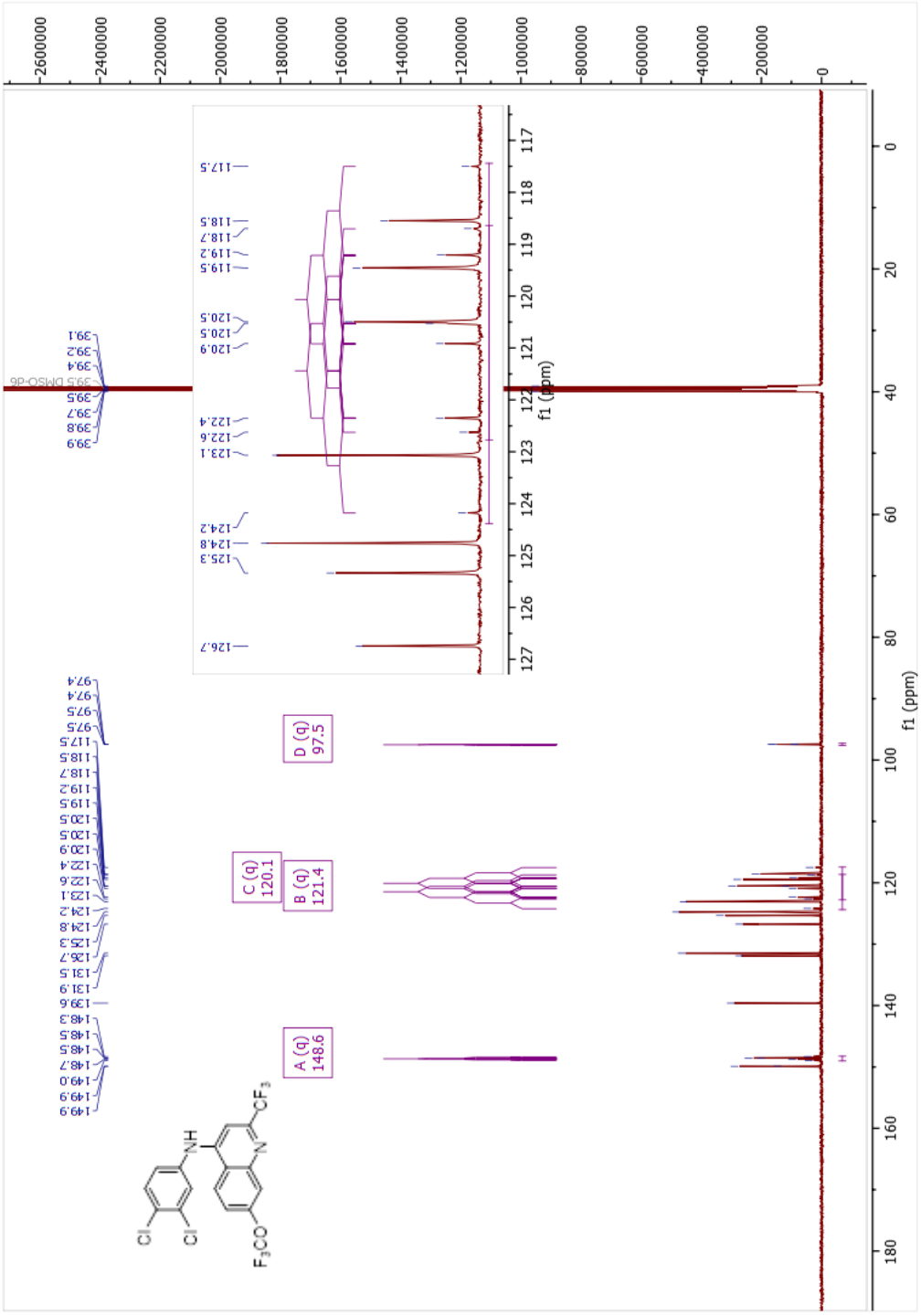
Compound 31 $^{13}C$ NMR.



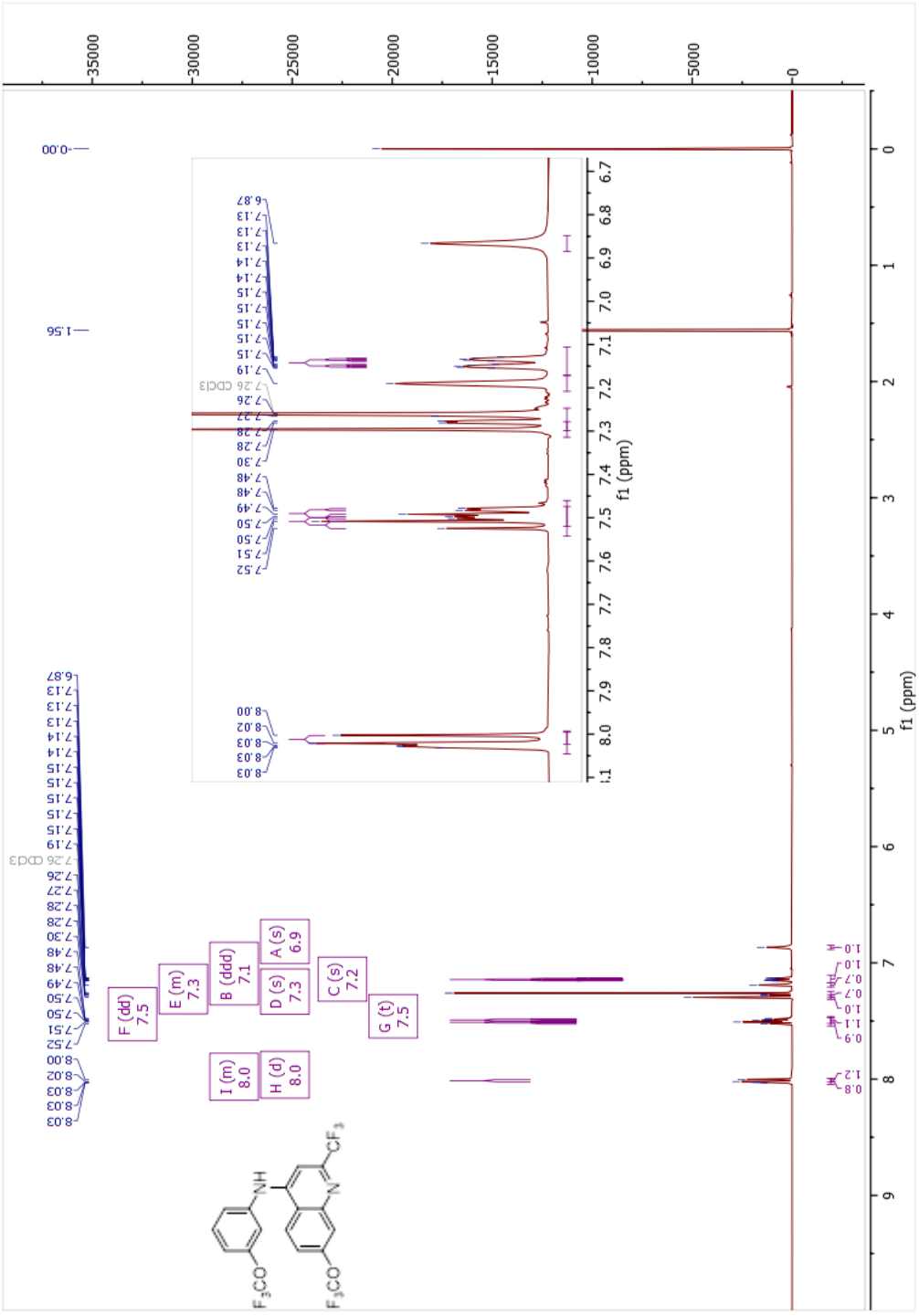
Compound 32 $^{1}$H NMR.



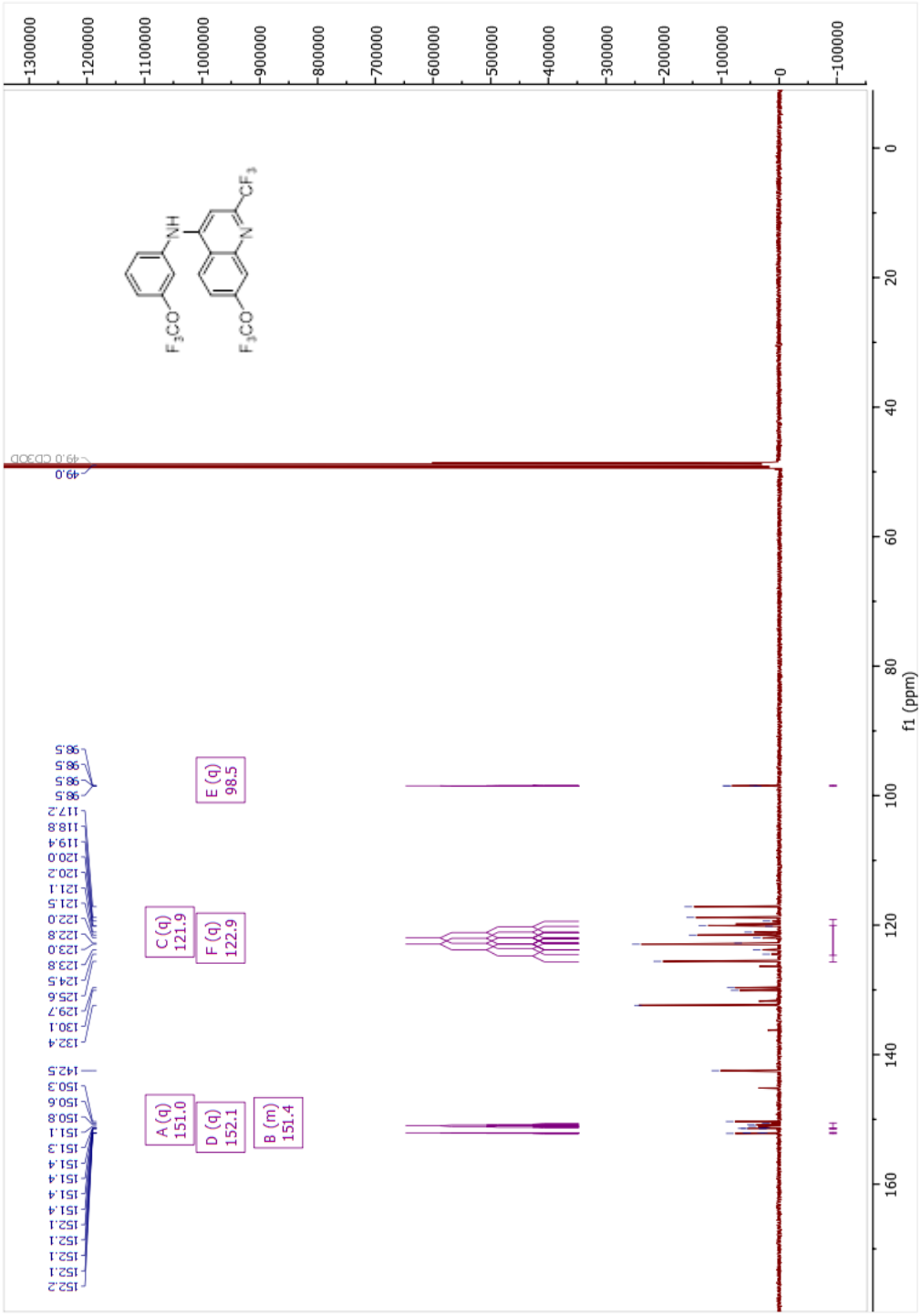
Compound 32 $^{13}C$ NMR.



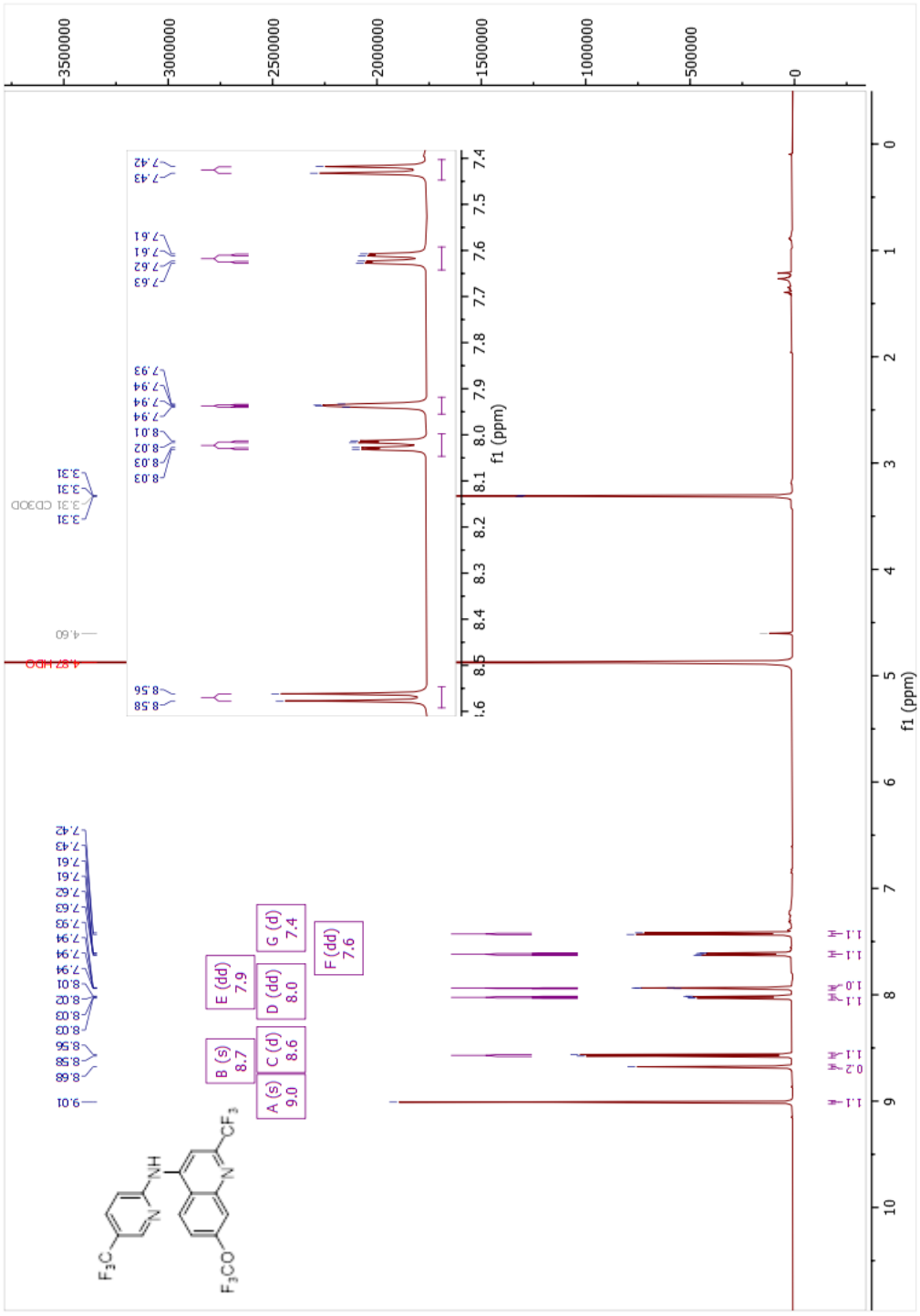
Compound 33 $^{1}$H NMR.



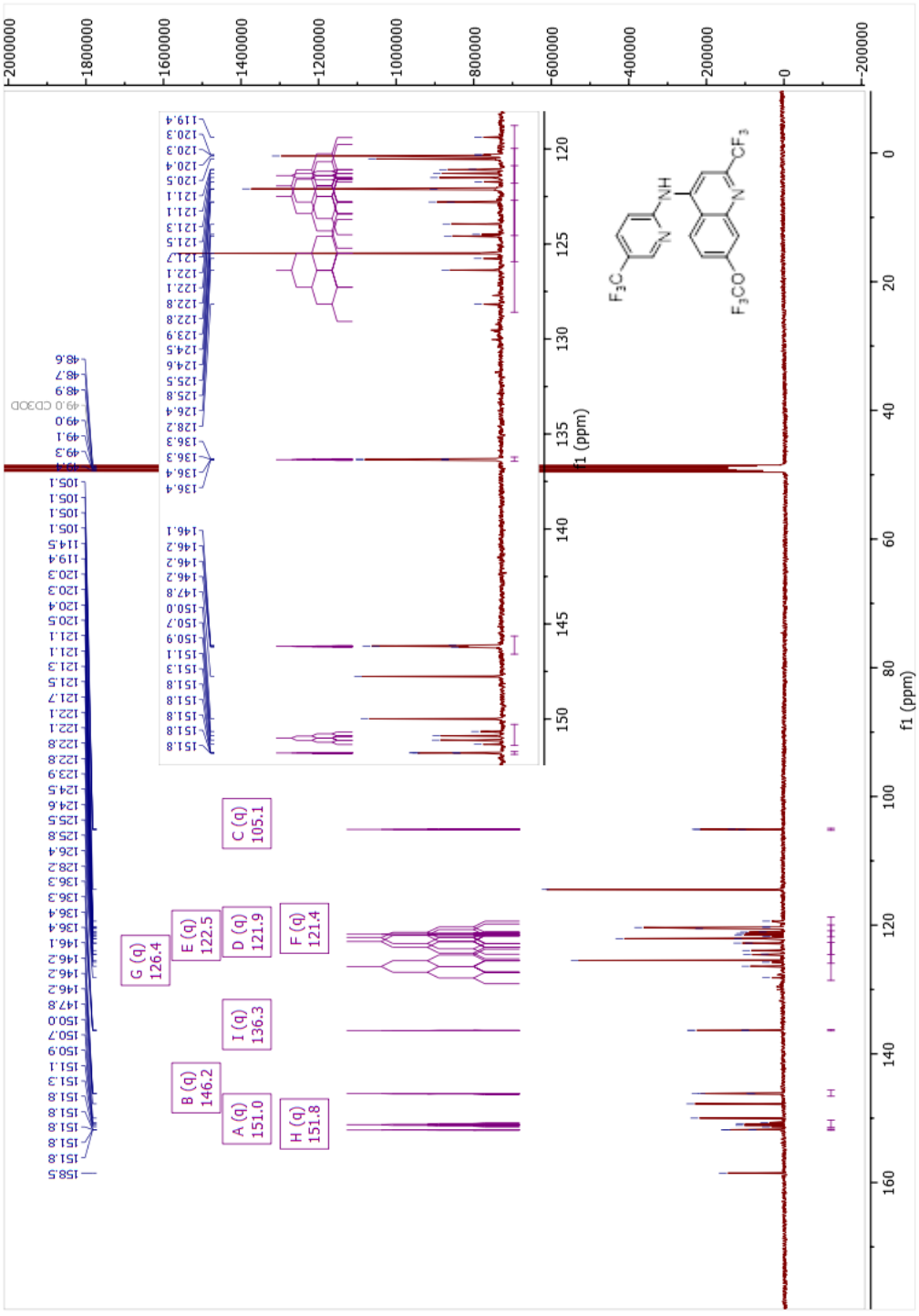
Compound 33 $^{13}C$ NMR.



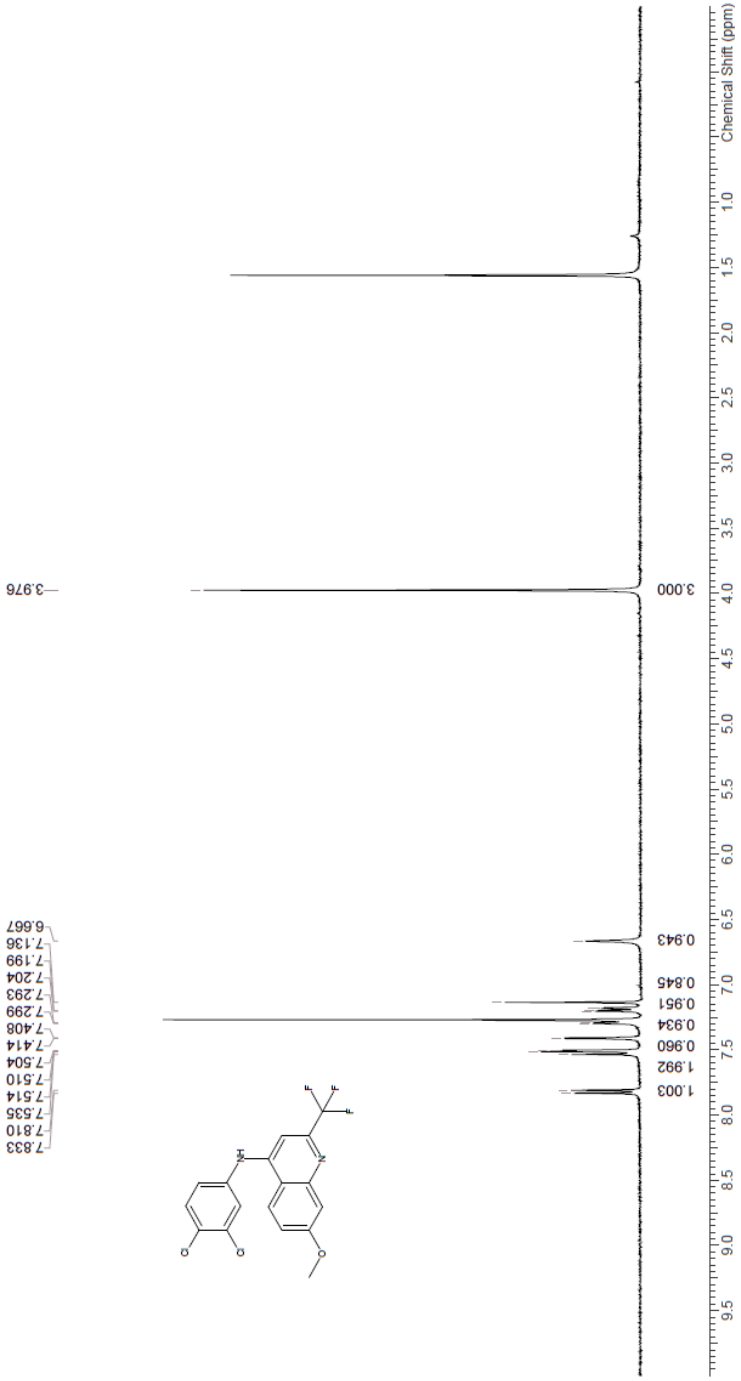
Compound 34 $^{1}$H NMR.



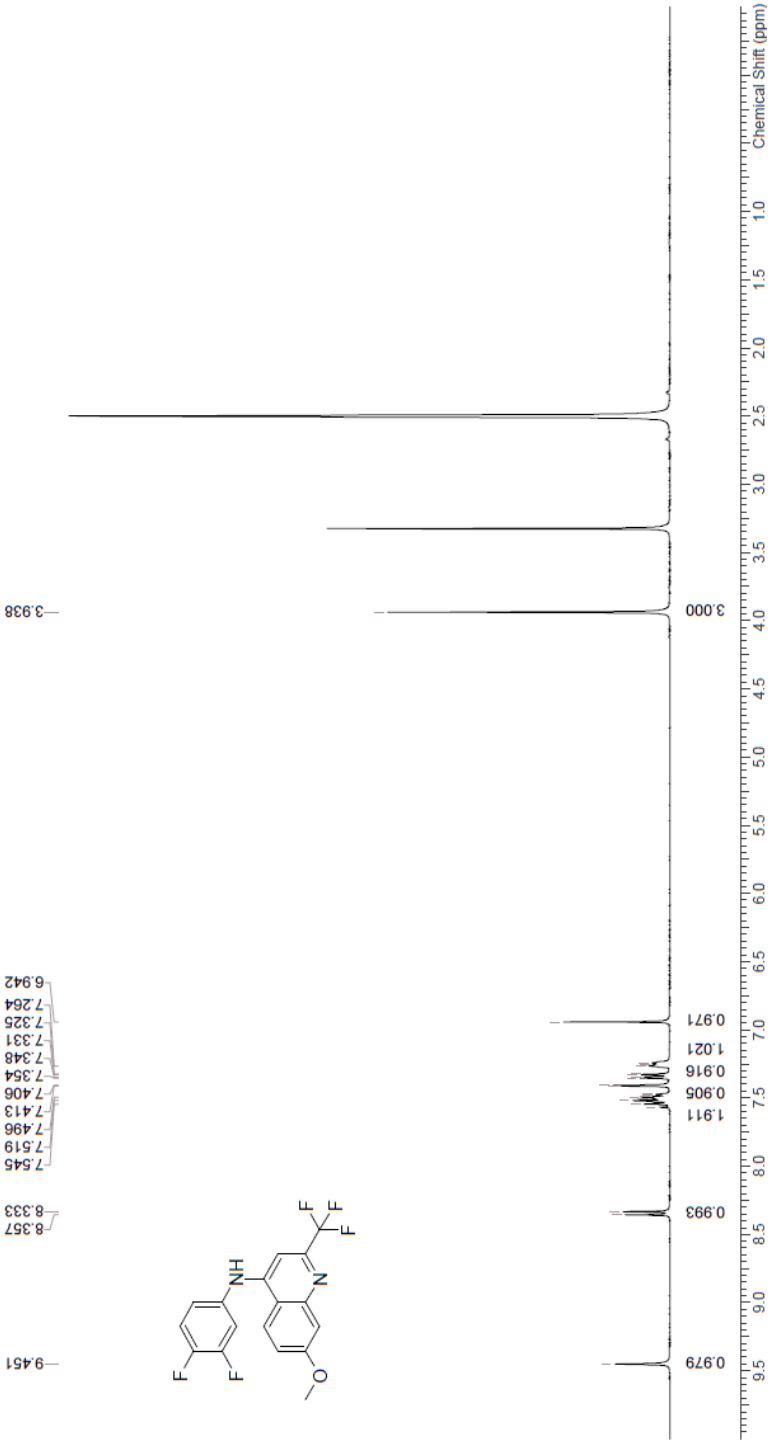
Compound 34 $^{13}C$ NMR.



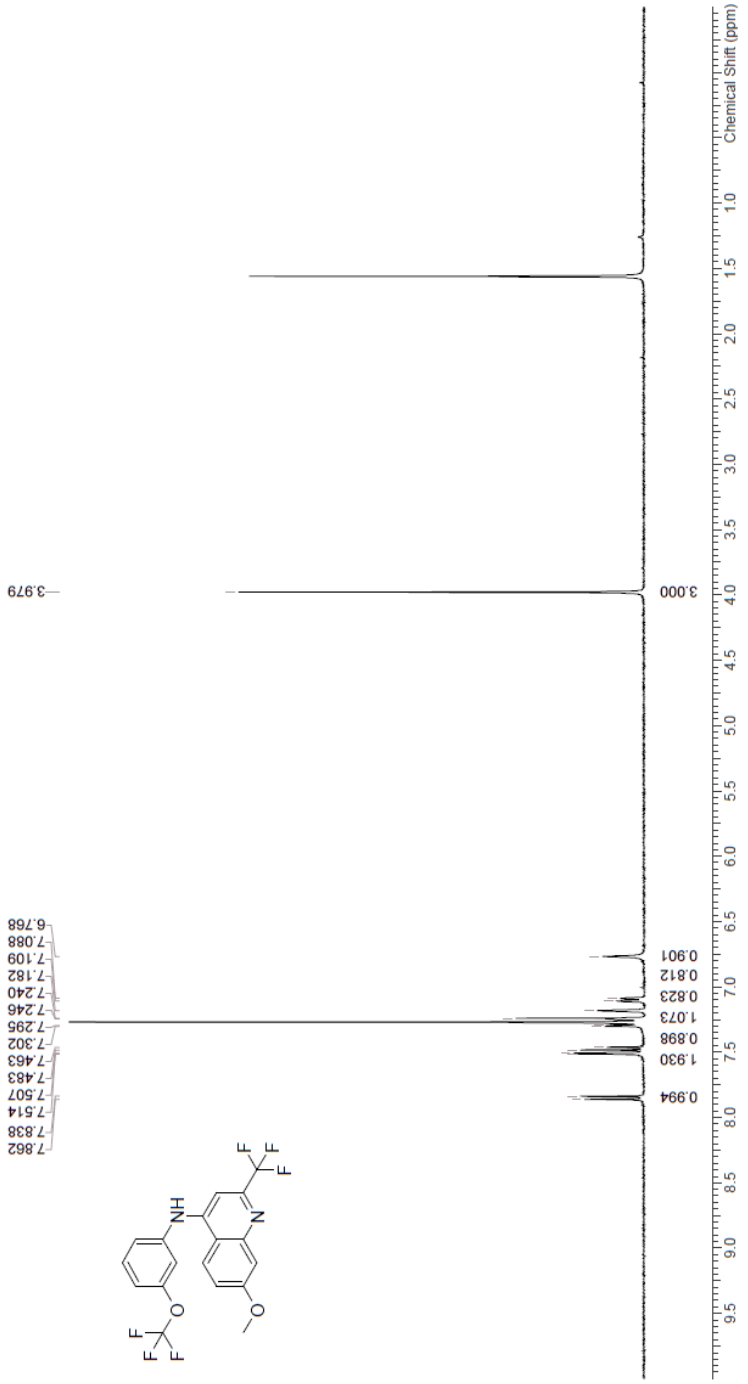
Compound 35 $^{1}$H NMR.



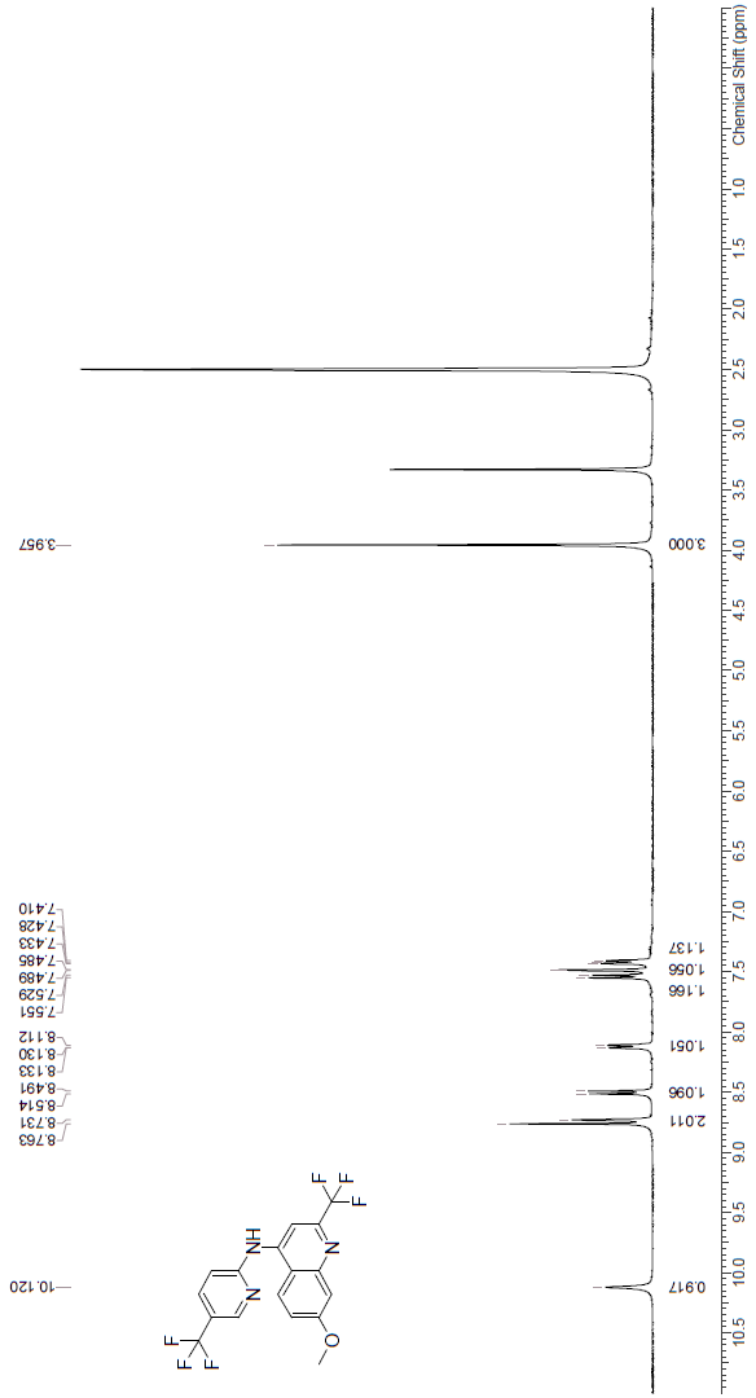
Compound 36 $^{1}H$ NMR.



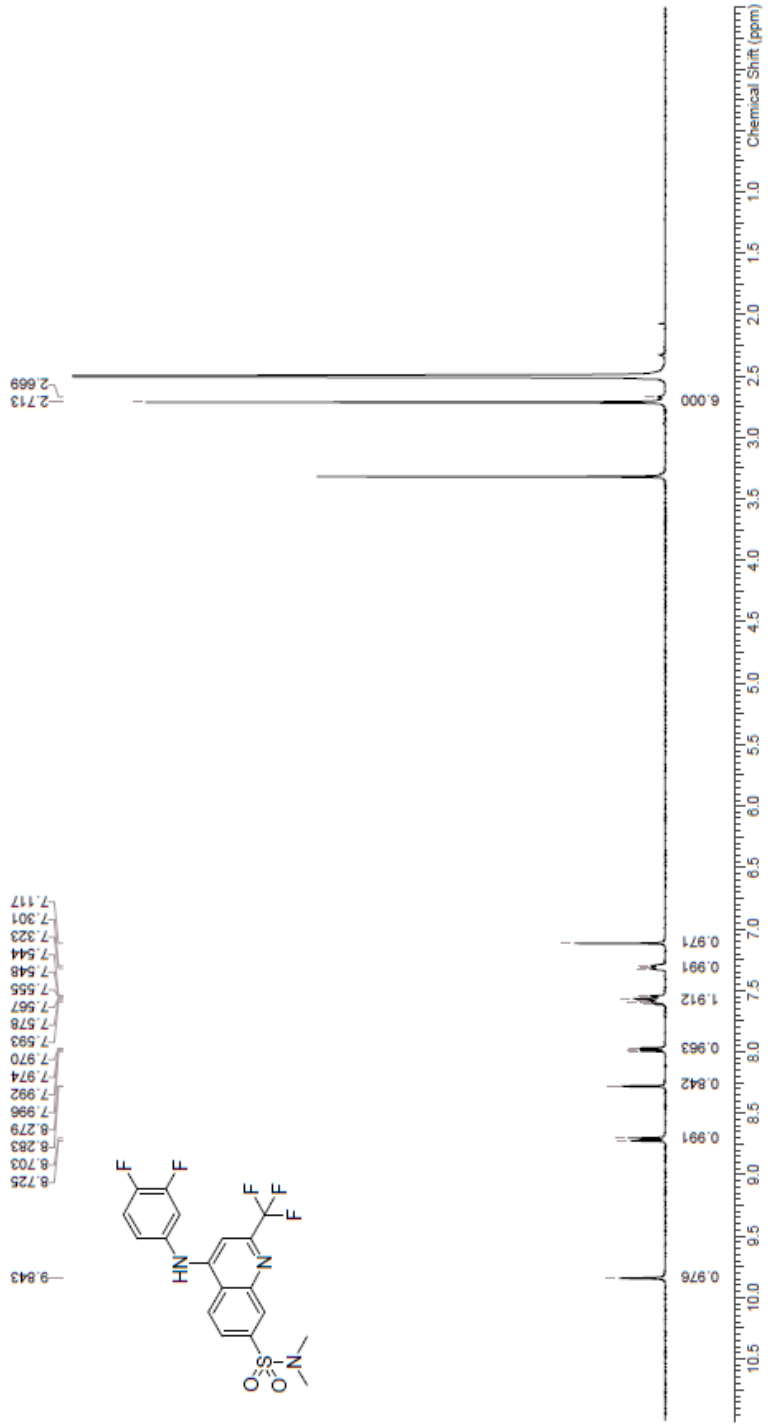
Compound 37 $^{1}$H NMR.



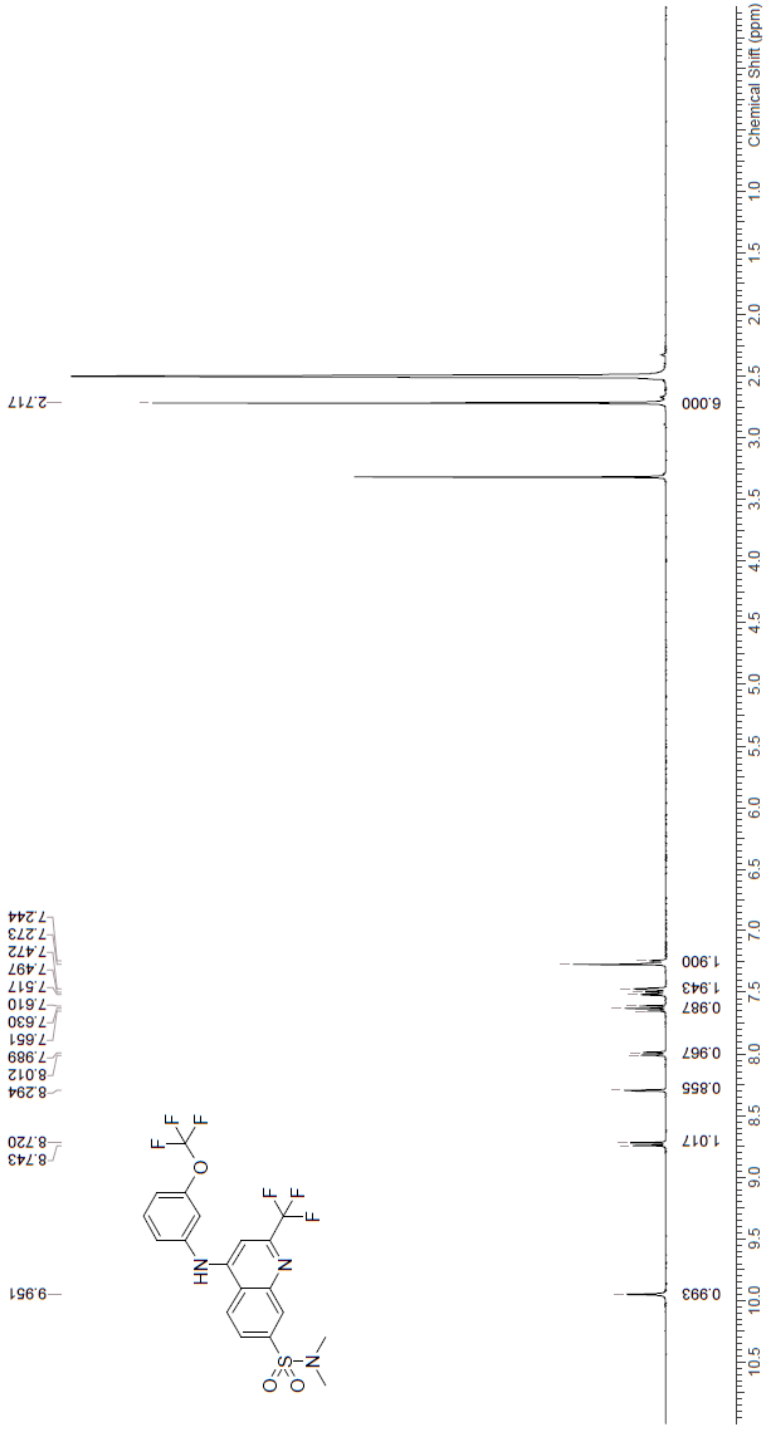
Compound 38 $^{1}$H NMR.



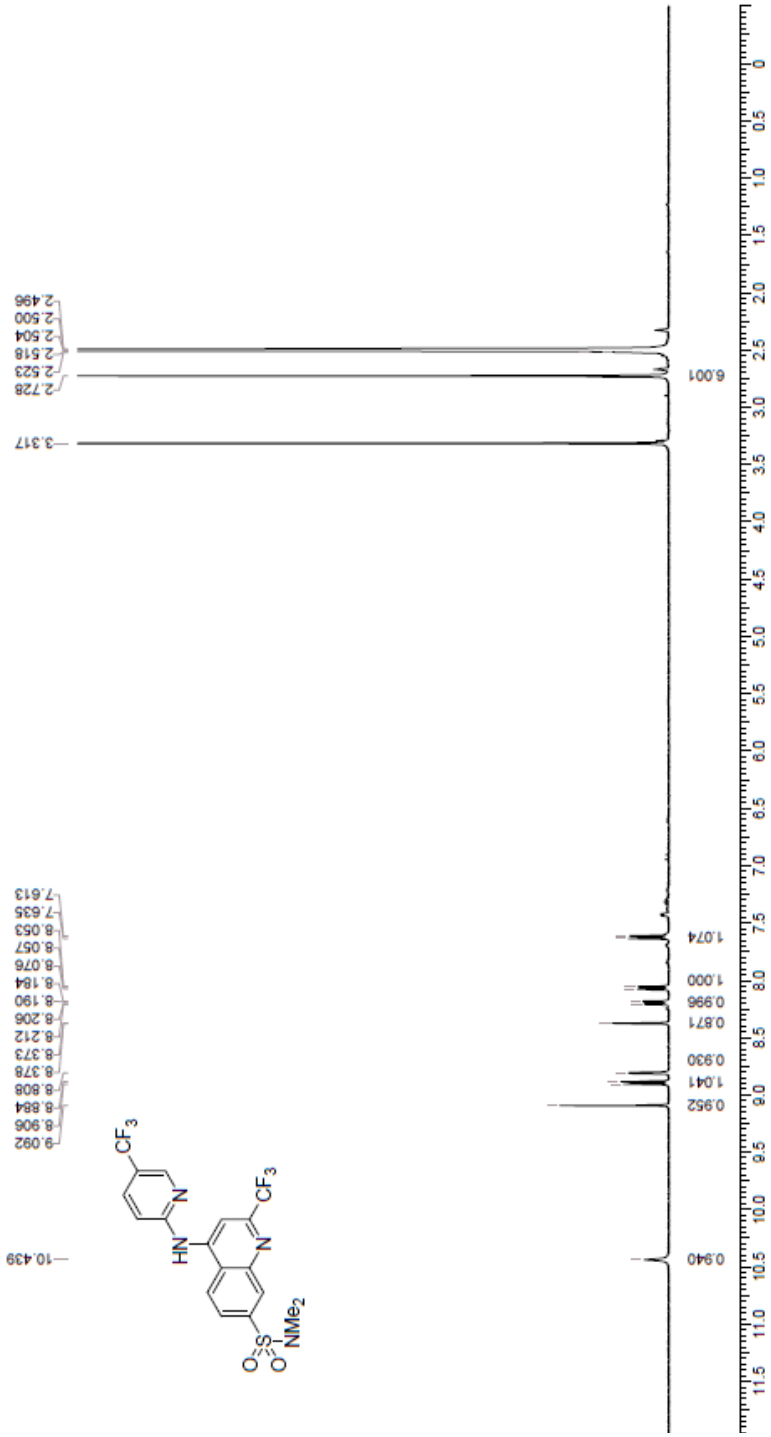
Compound 39 $^{1}$H NMR.



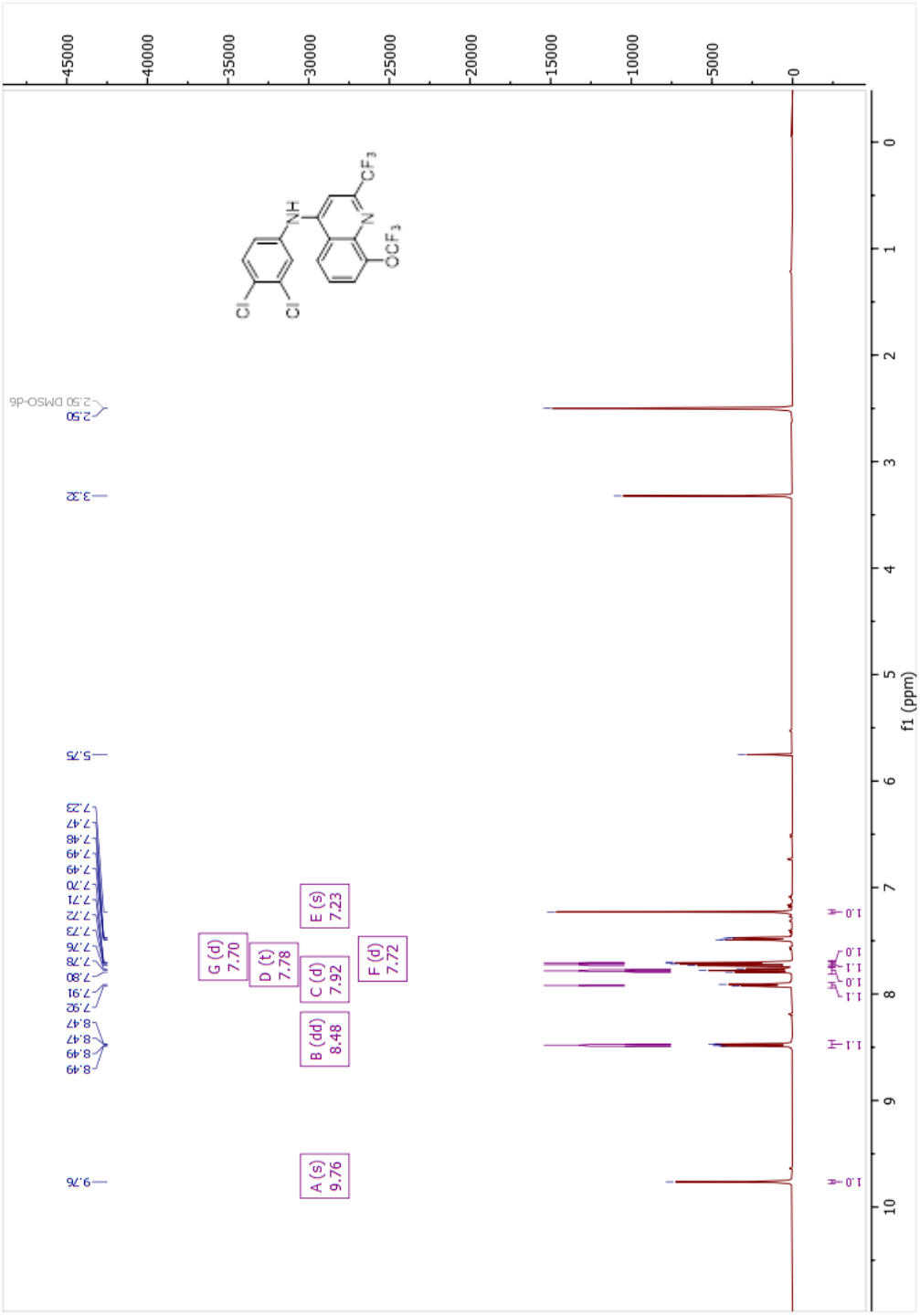
Compound 40 $^{1}$H NMR.



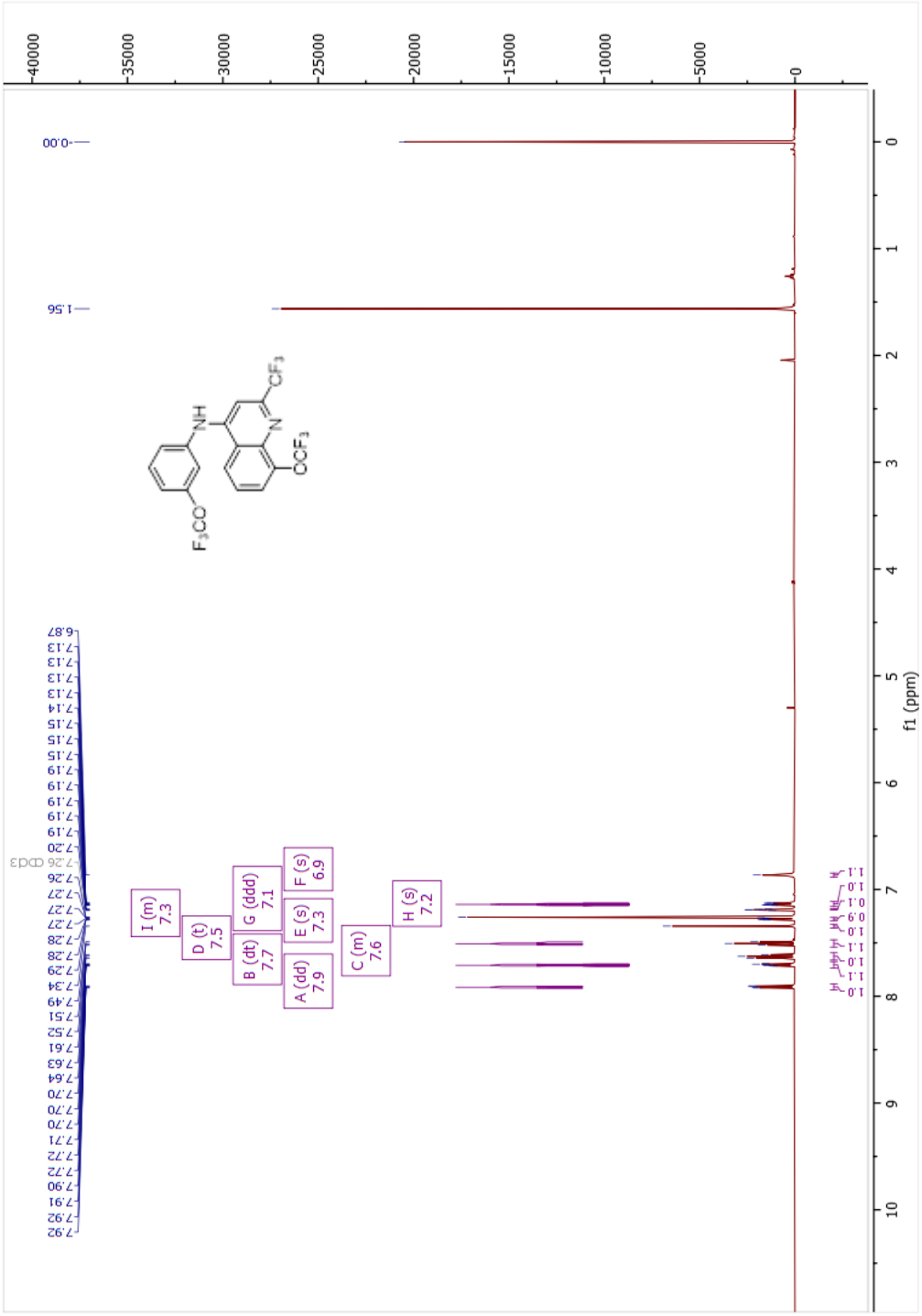
Compound 41 $^{1}$H NMR.



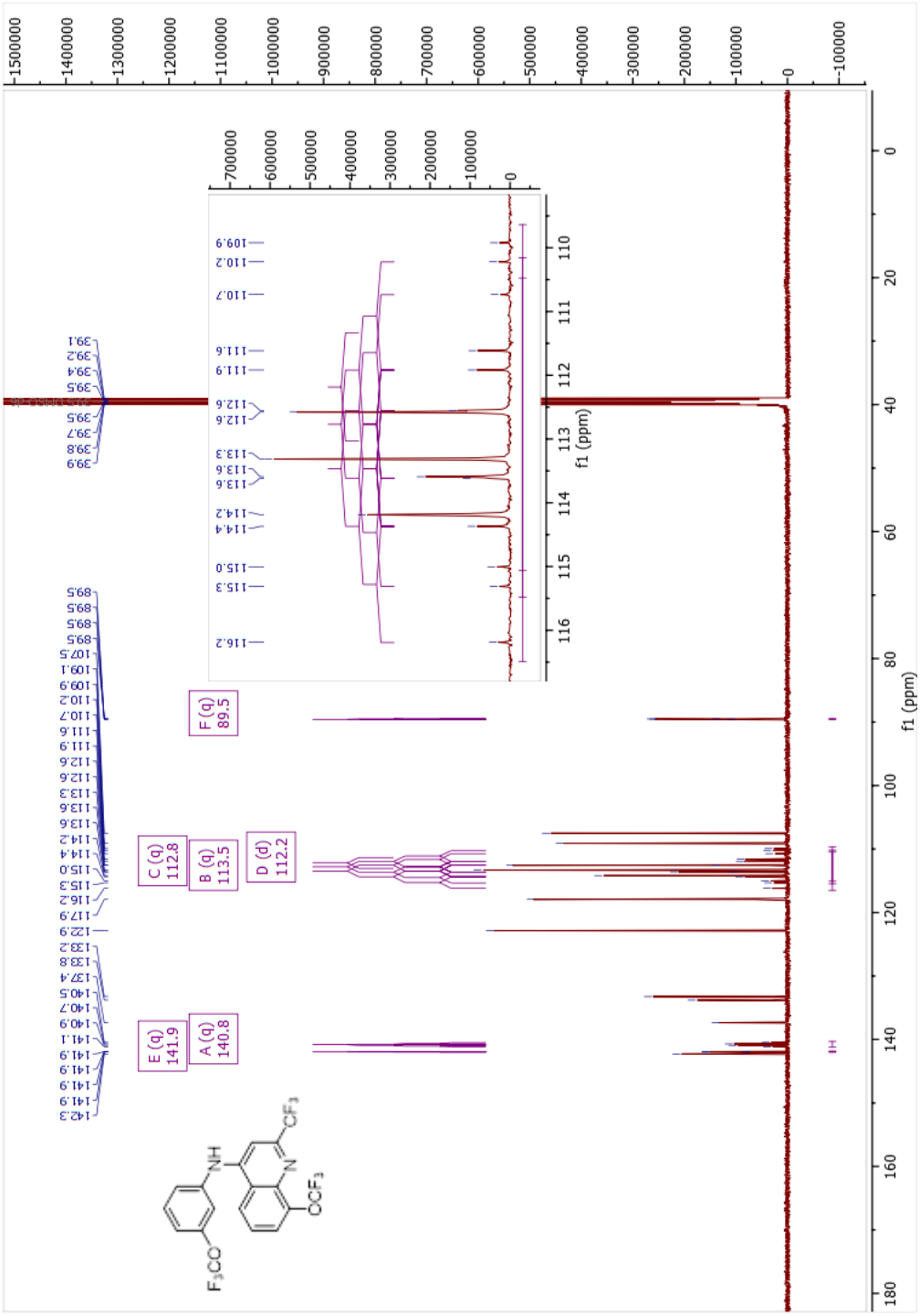
Compound 42 $^{1}$H NMR.



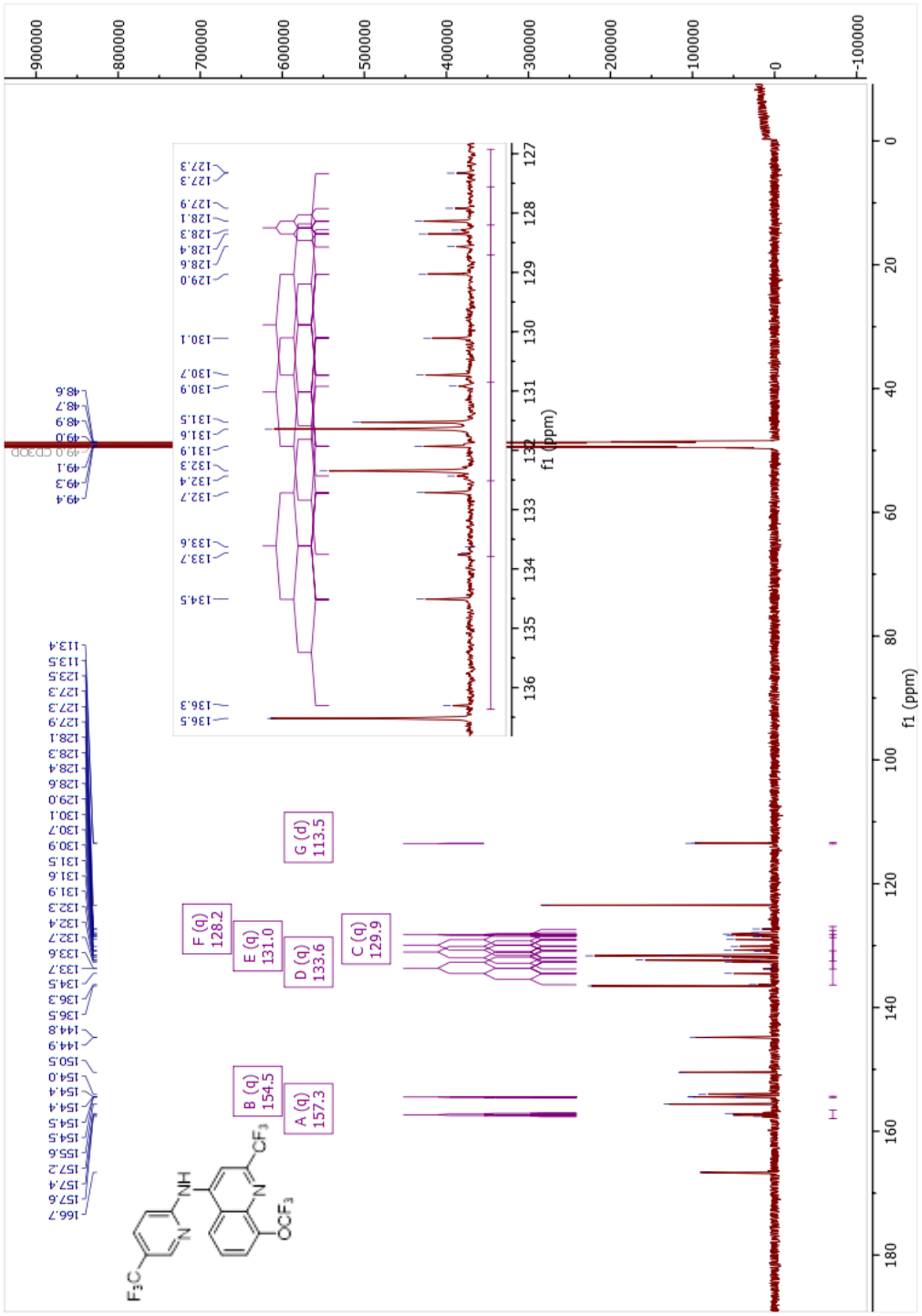
Compound 43 $^{1}H$ NMR.



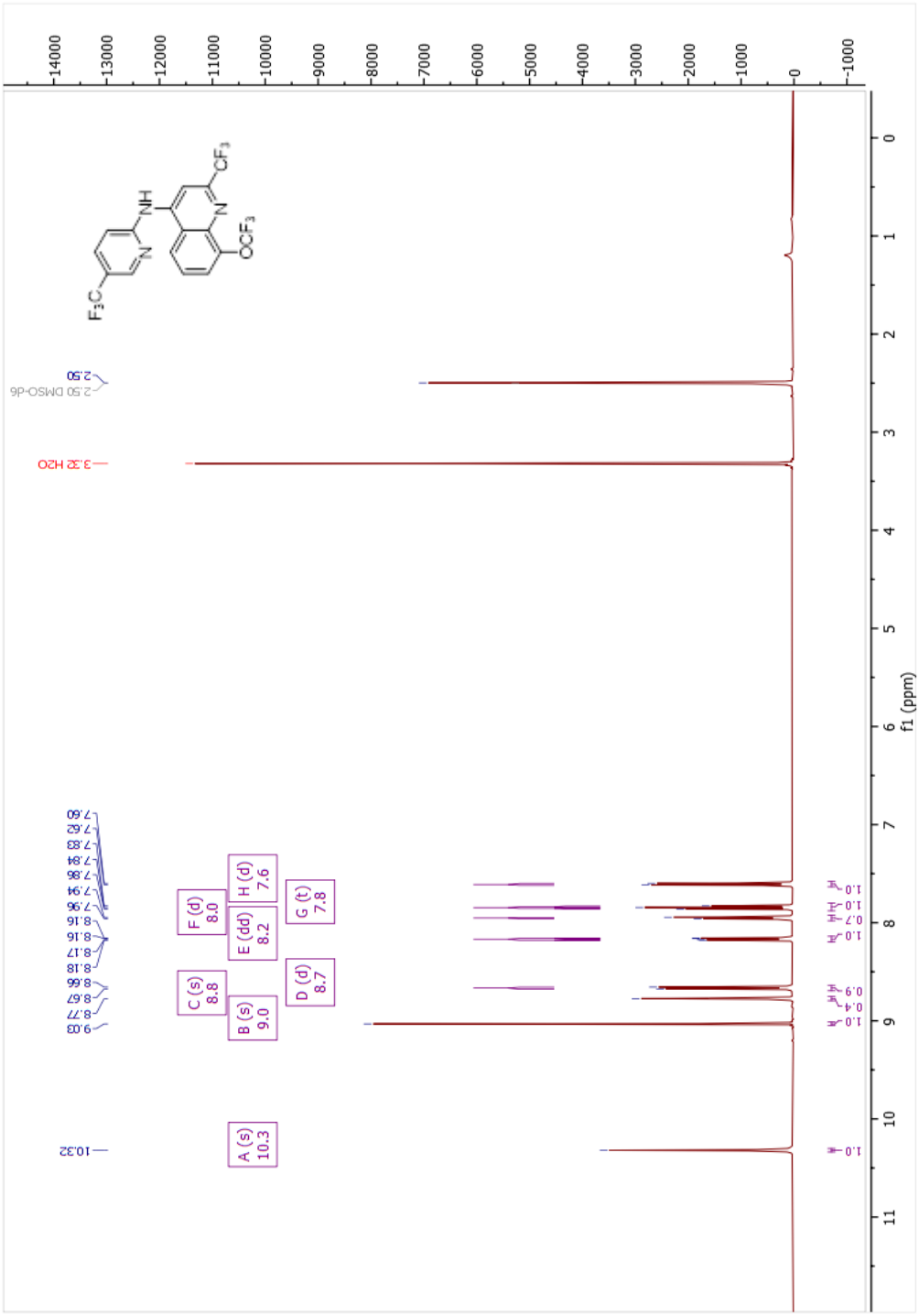
Compound 43 $^{13}C$ NMR.



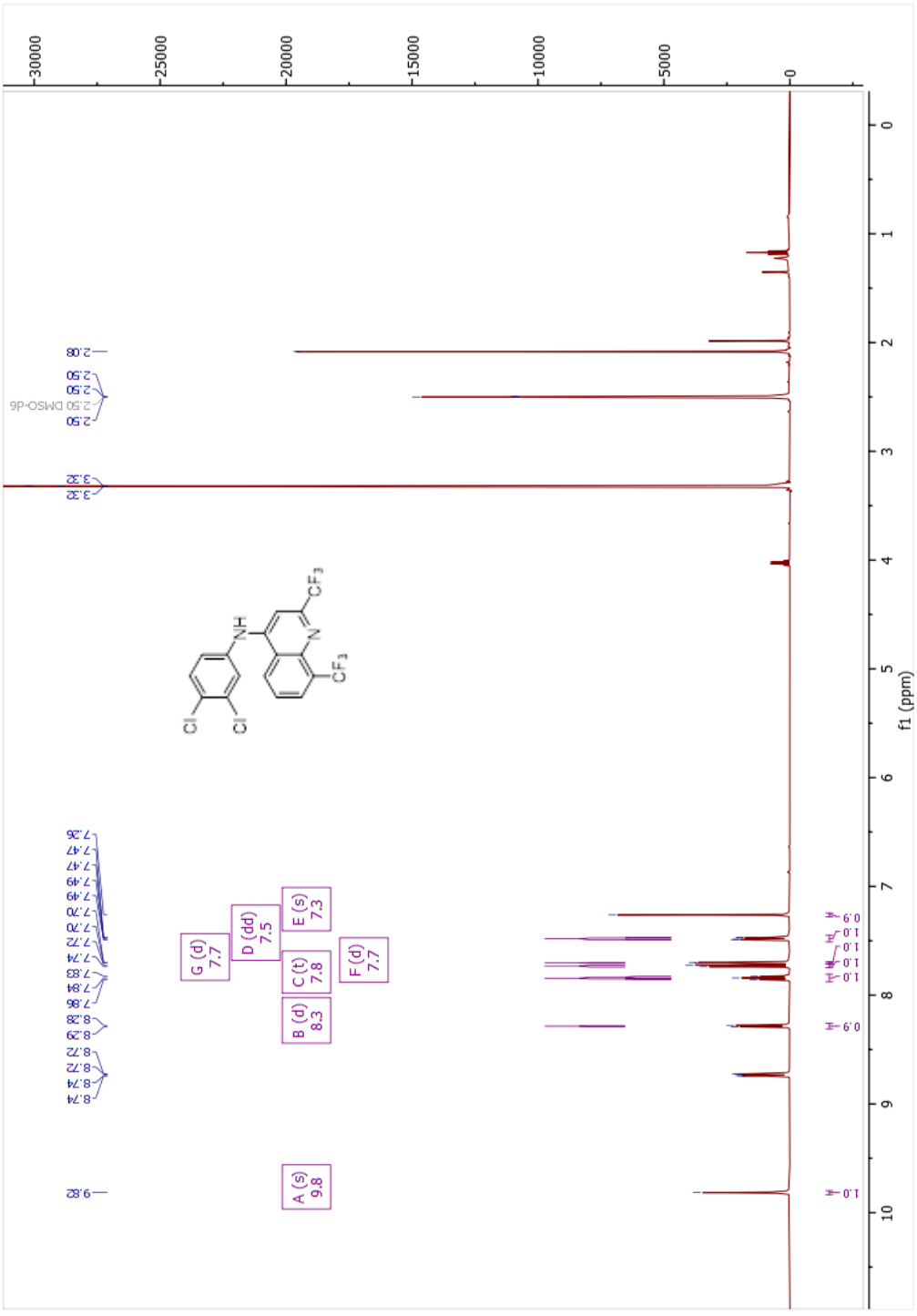
Compound 44 $^{1}$H NMR.



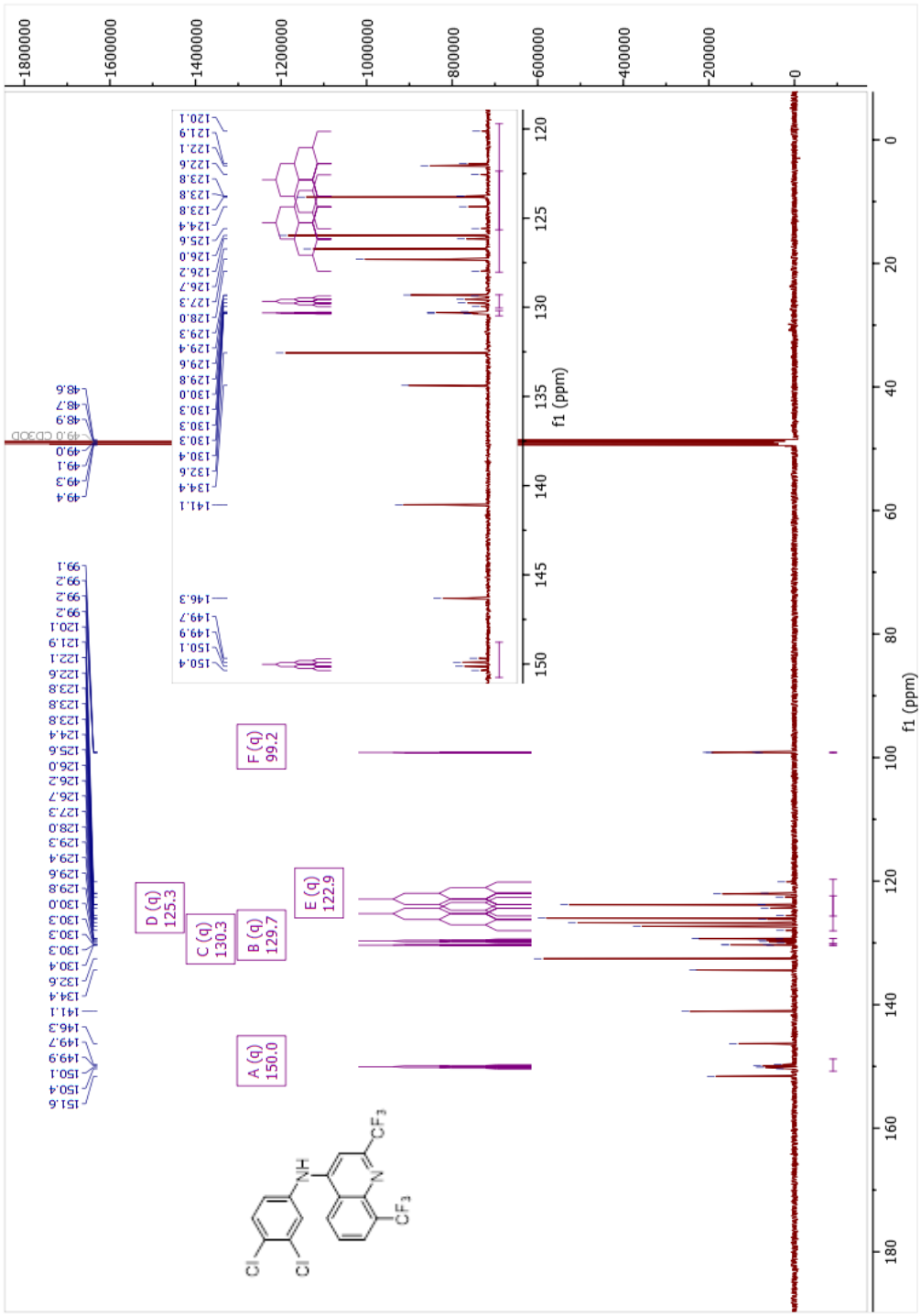
Compound 44 $^{13}C$ NMR.



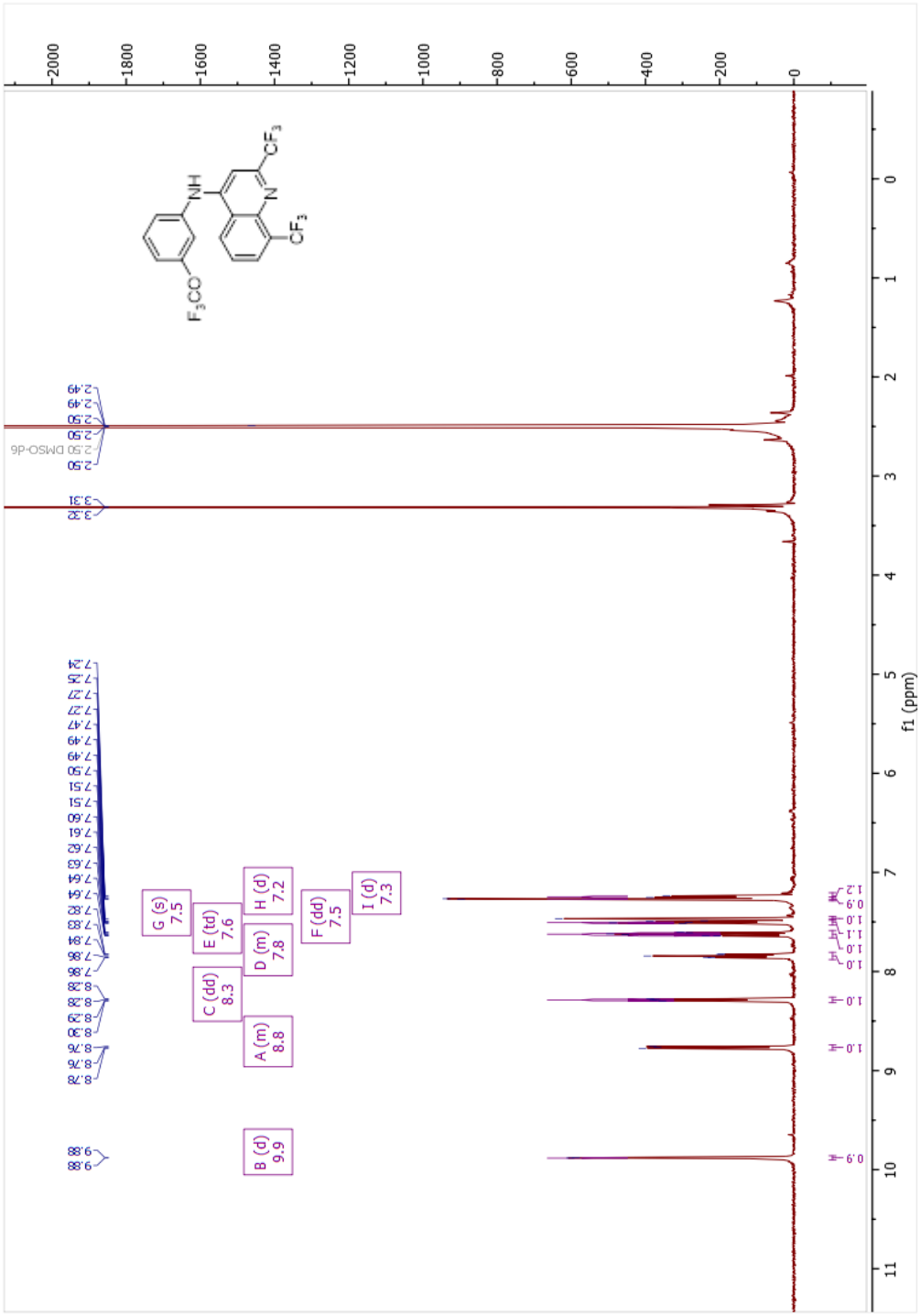
Compound 45 $^{1}$H NMR.



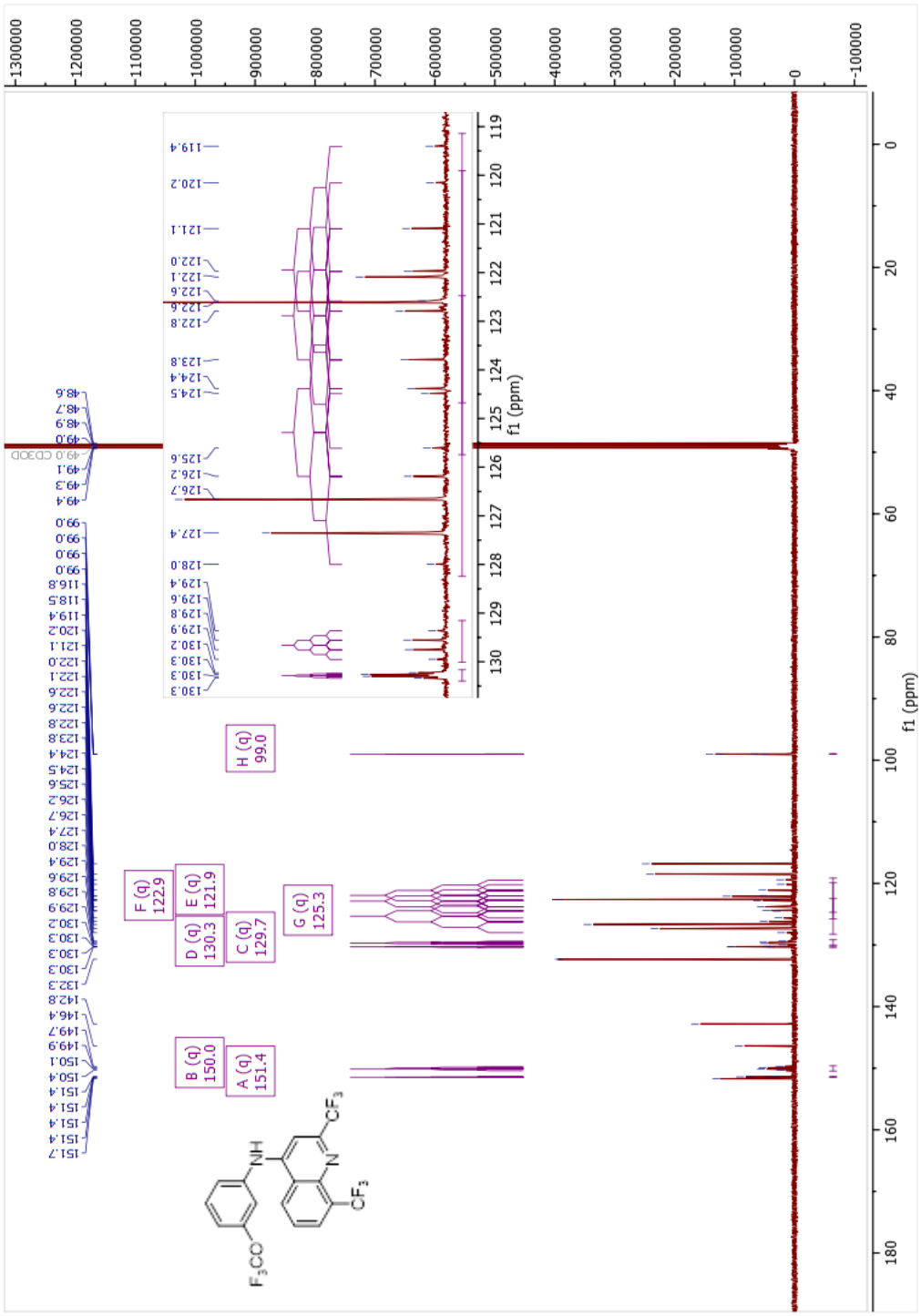
Compound 45 $^{13}C$ NMR.



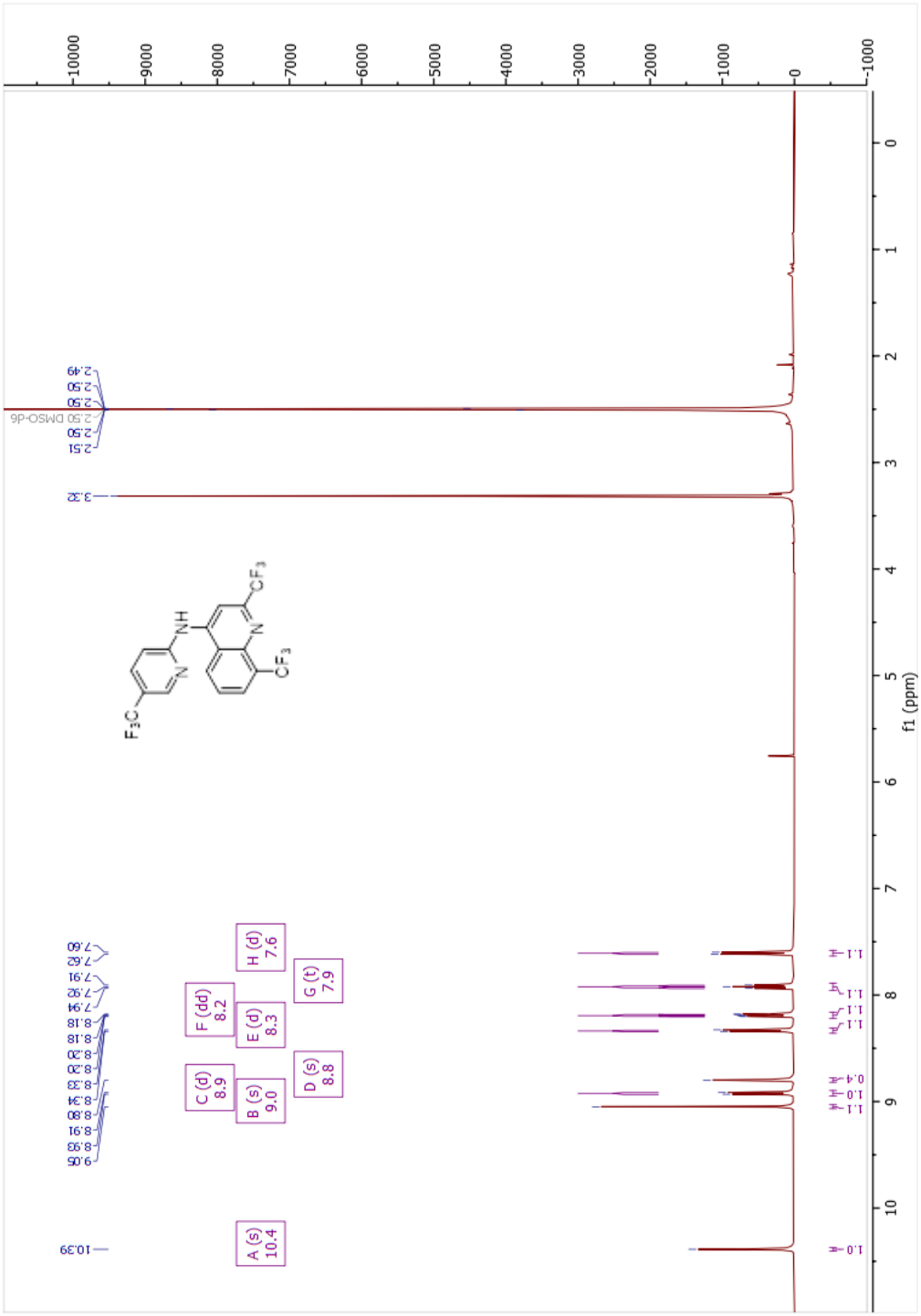
Compound 46 $^{1}H$ NMR.



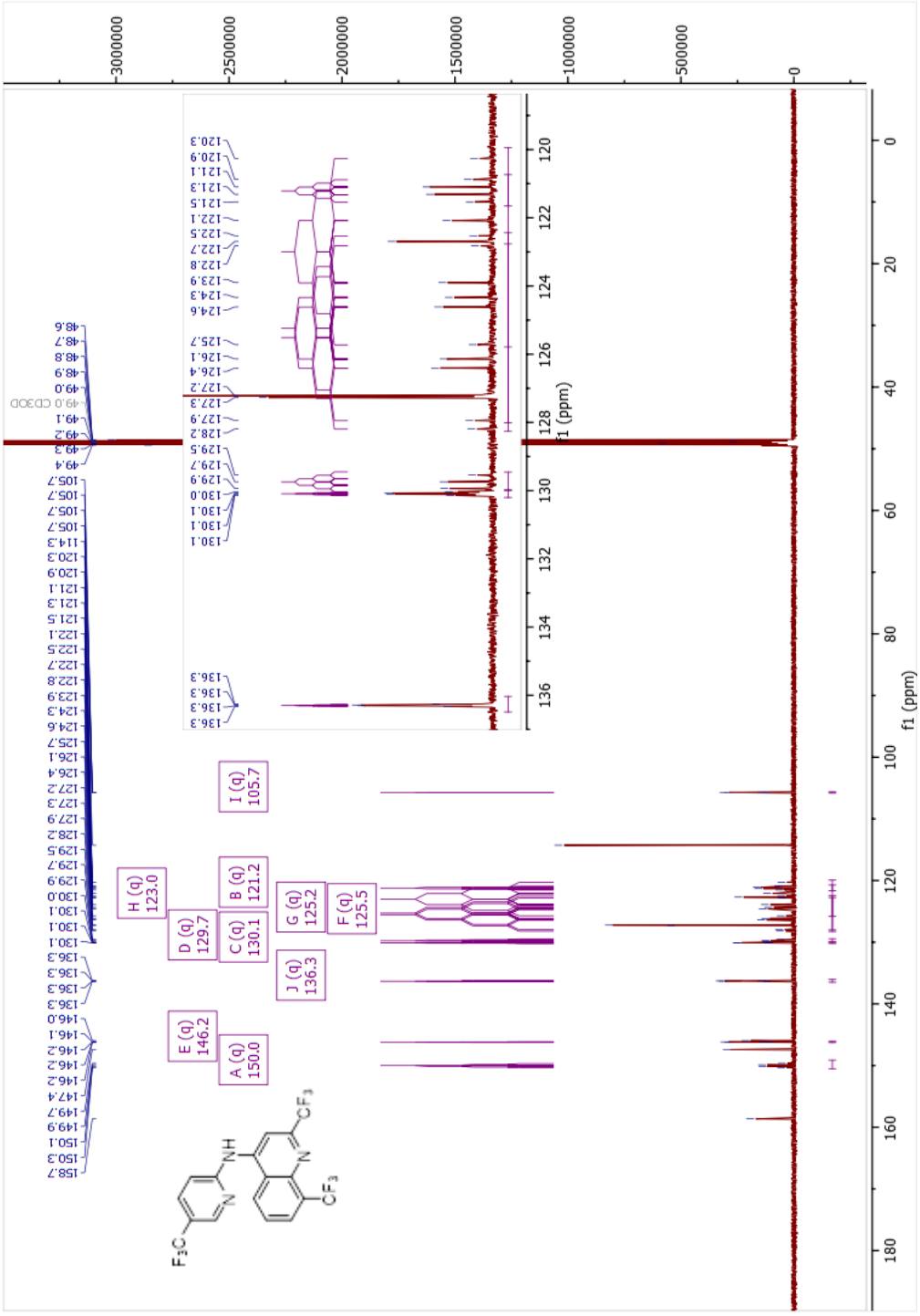
Compound 46 $^{13}C$ NMR.



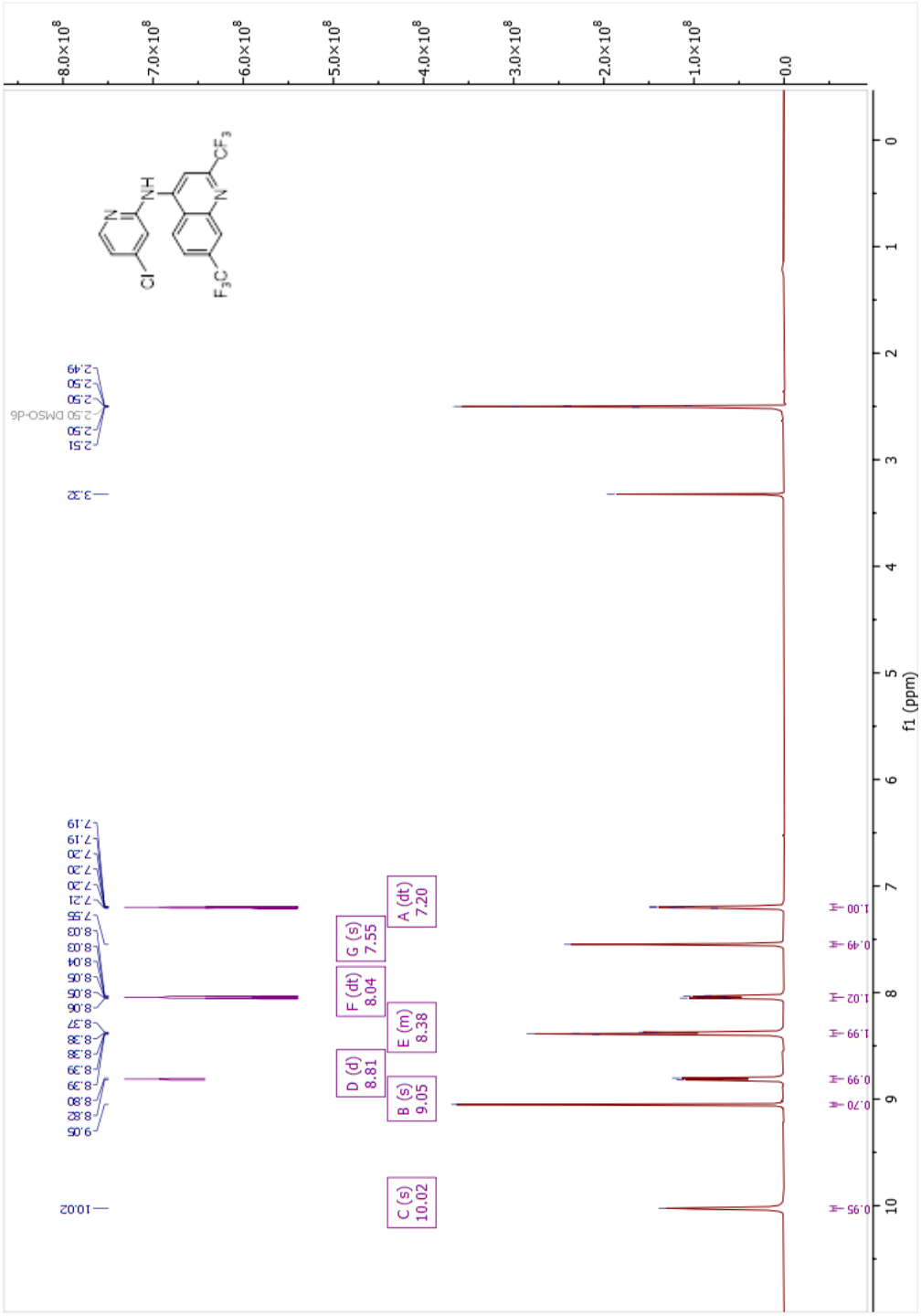
Compound 47 $^{1}$H NMR.



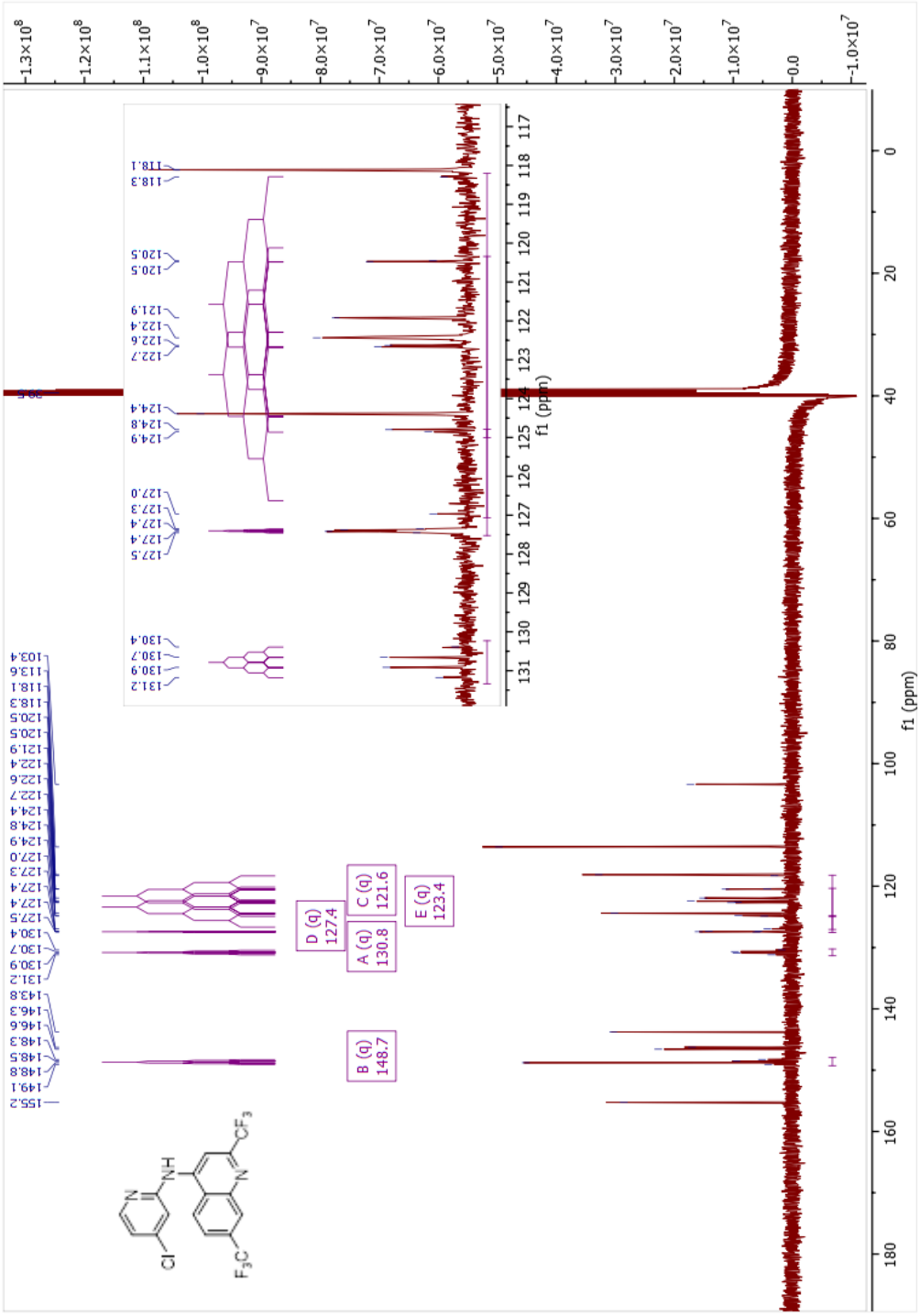
Compound 47 $^{13}C$ NMR.



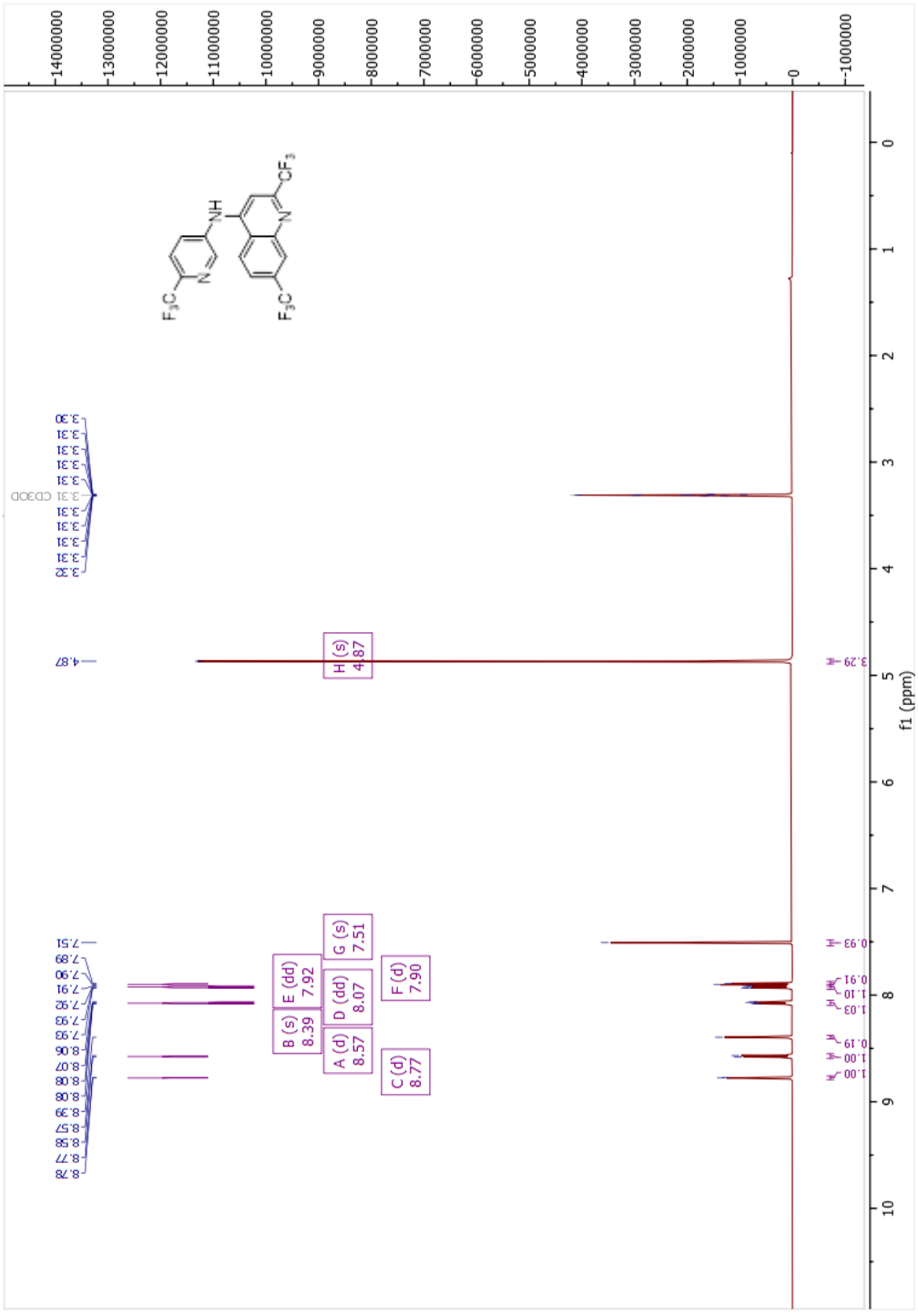
Compound 48 $^{1}H$ NMR.



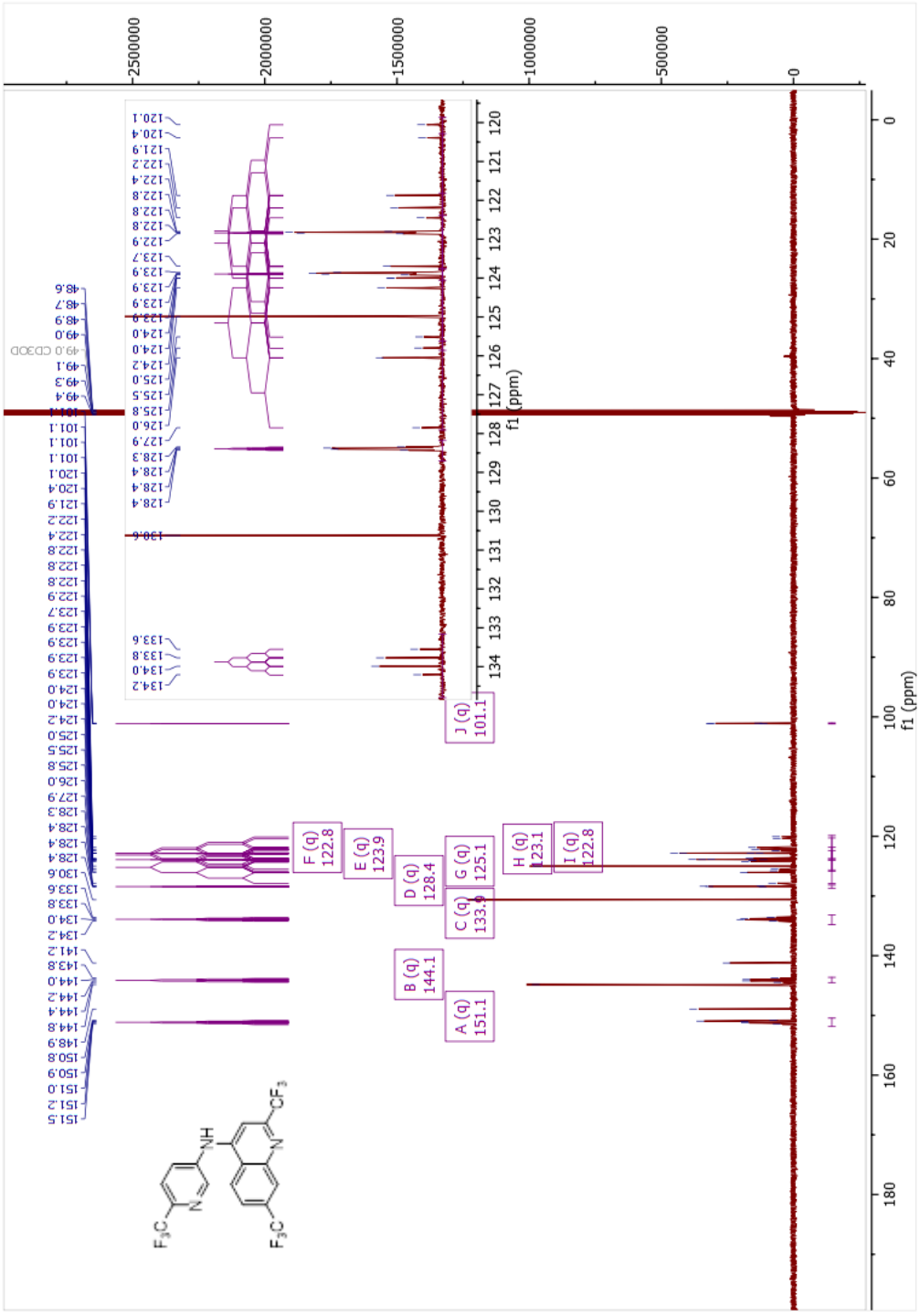
Compound 48 $^{13}$C NMR.



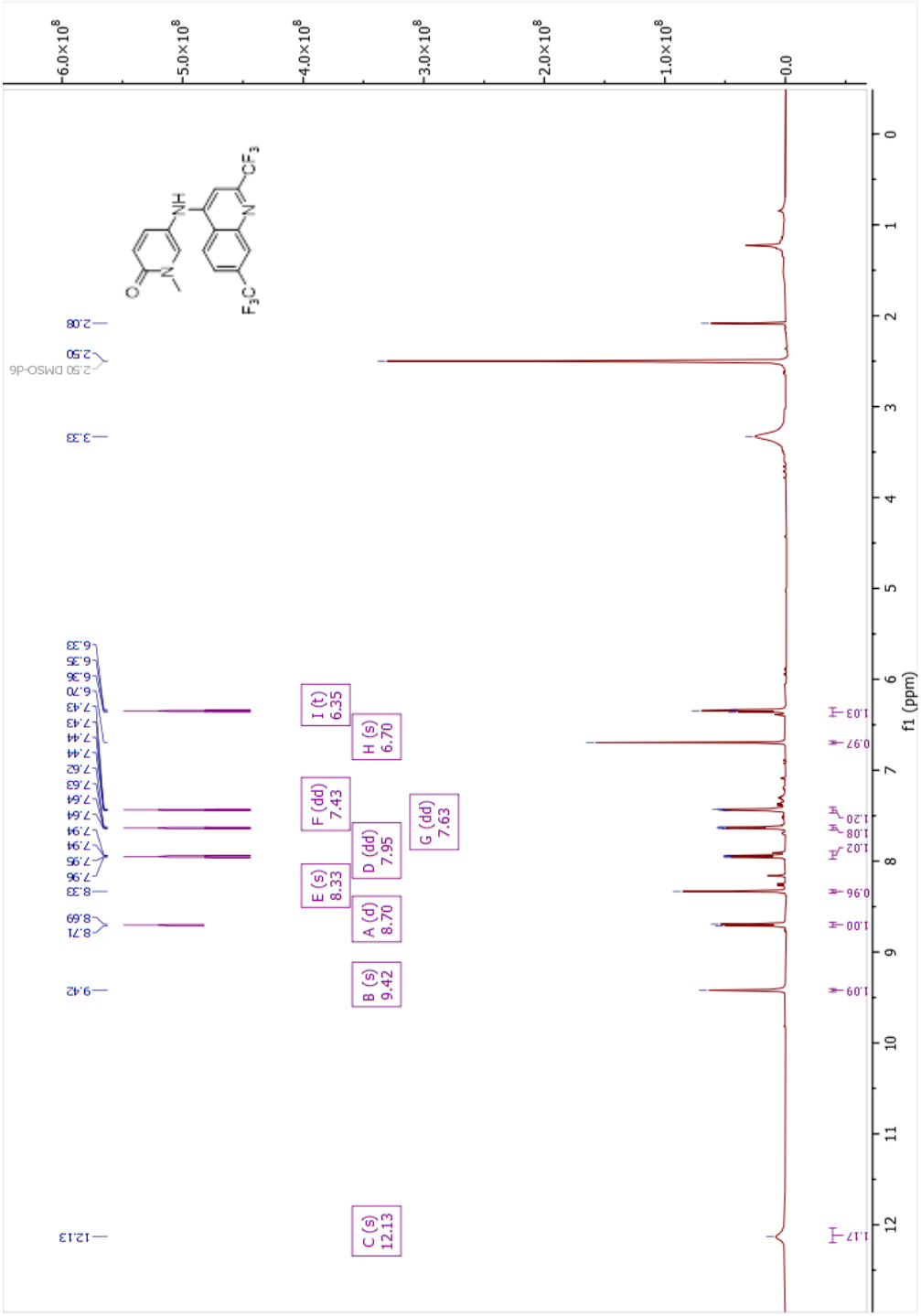
Compound 49 $^{1}$H NMR.



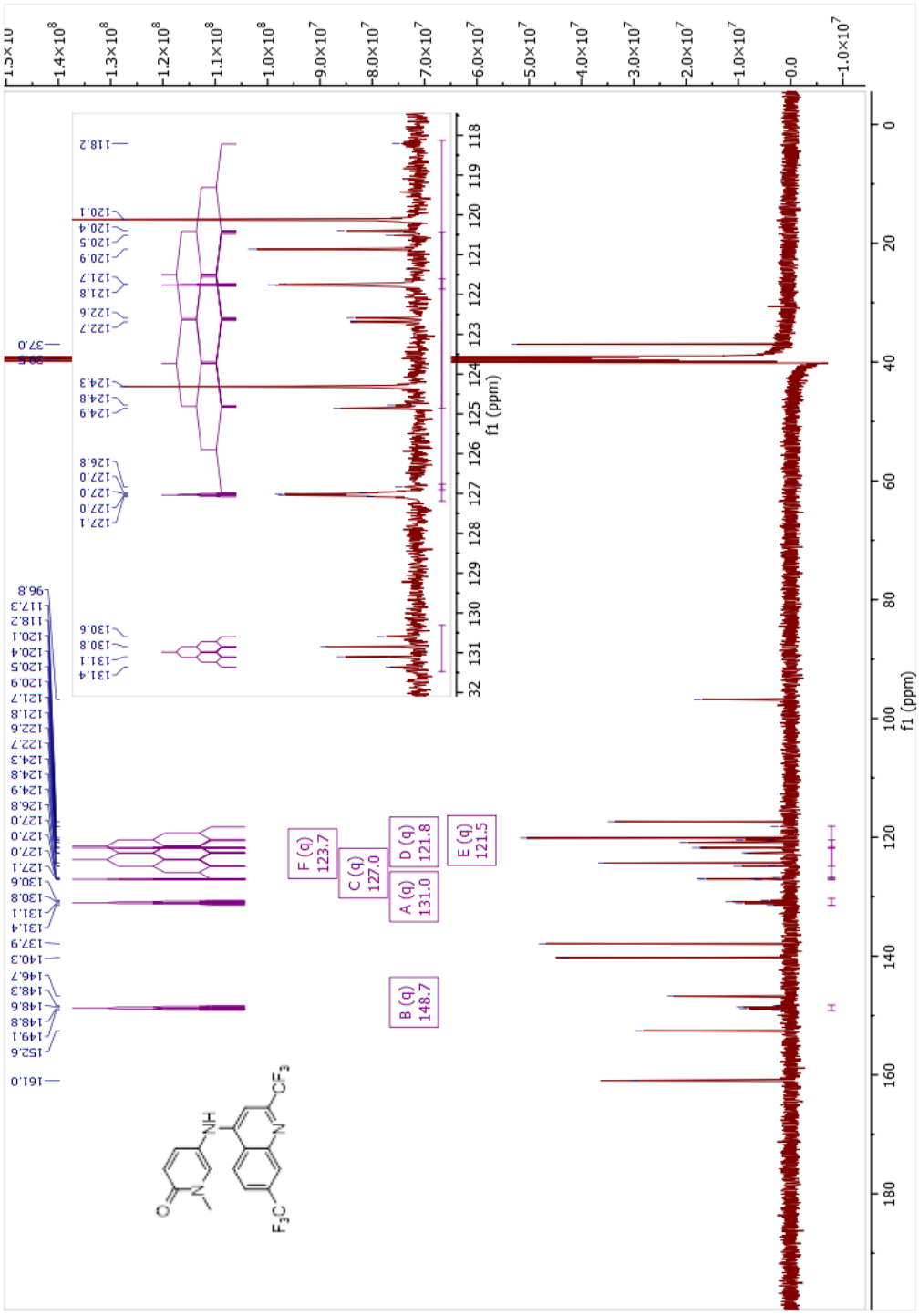
Compound 49 $^{13}C$ NMR.



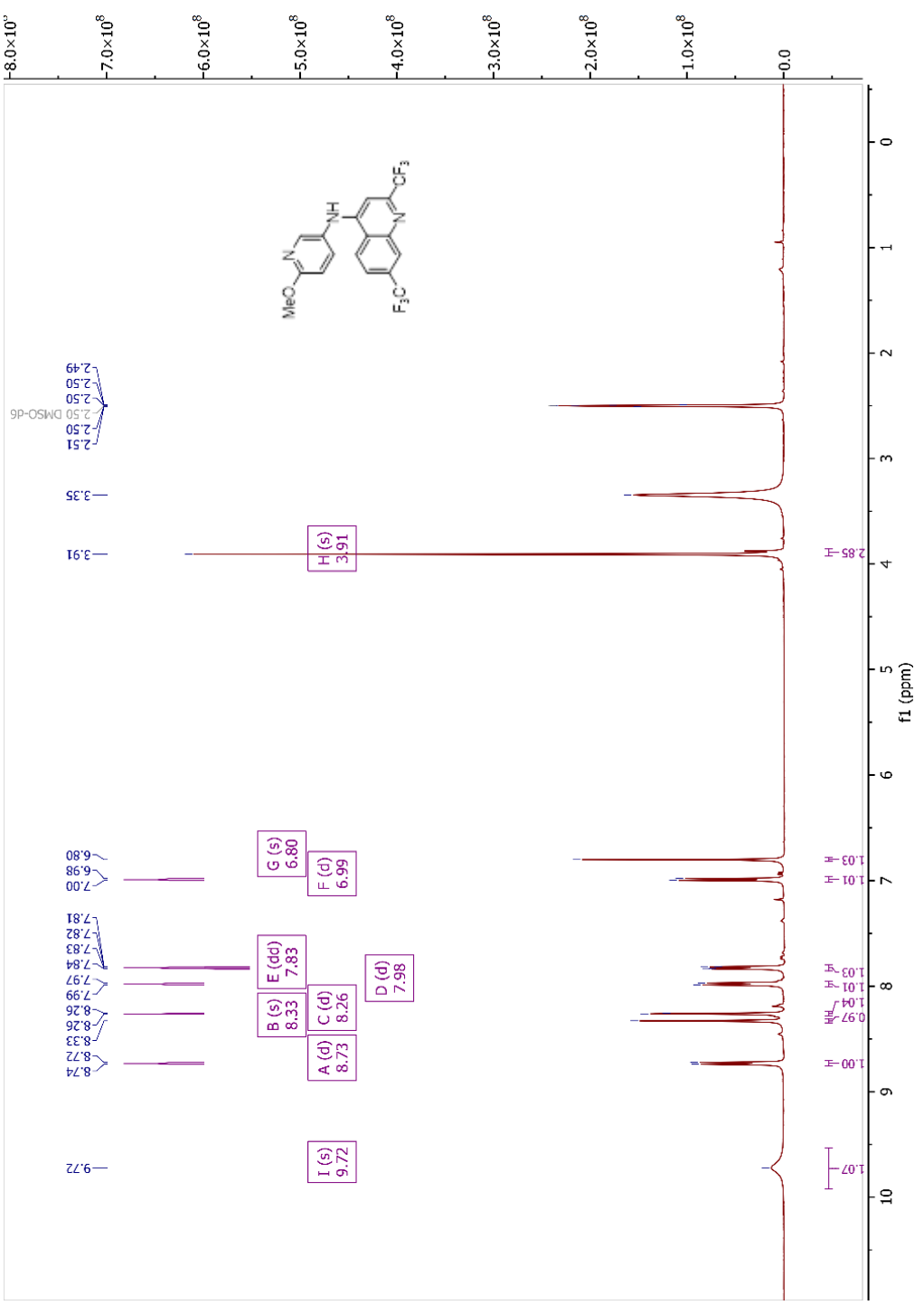
Compound 50 $^{1}$H NMR.



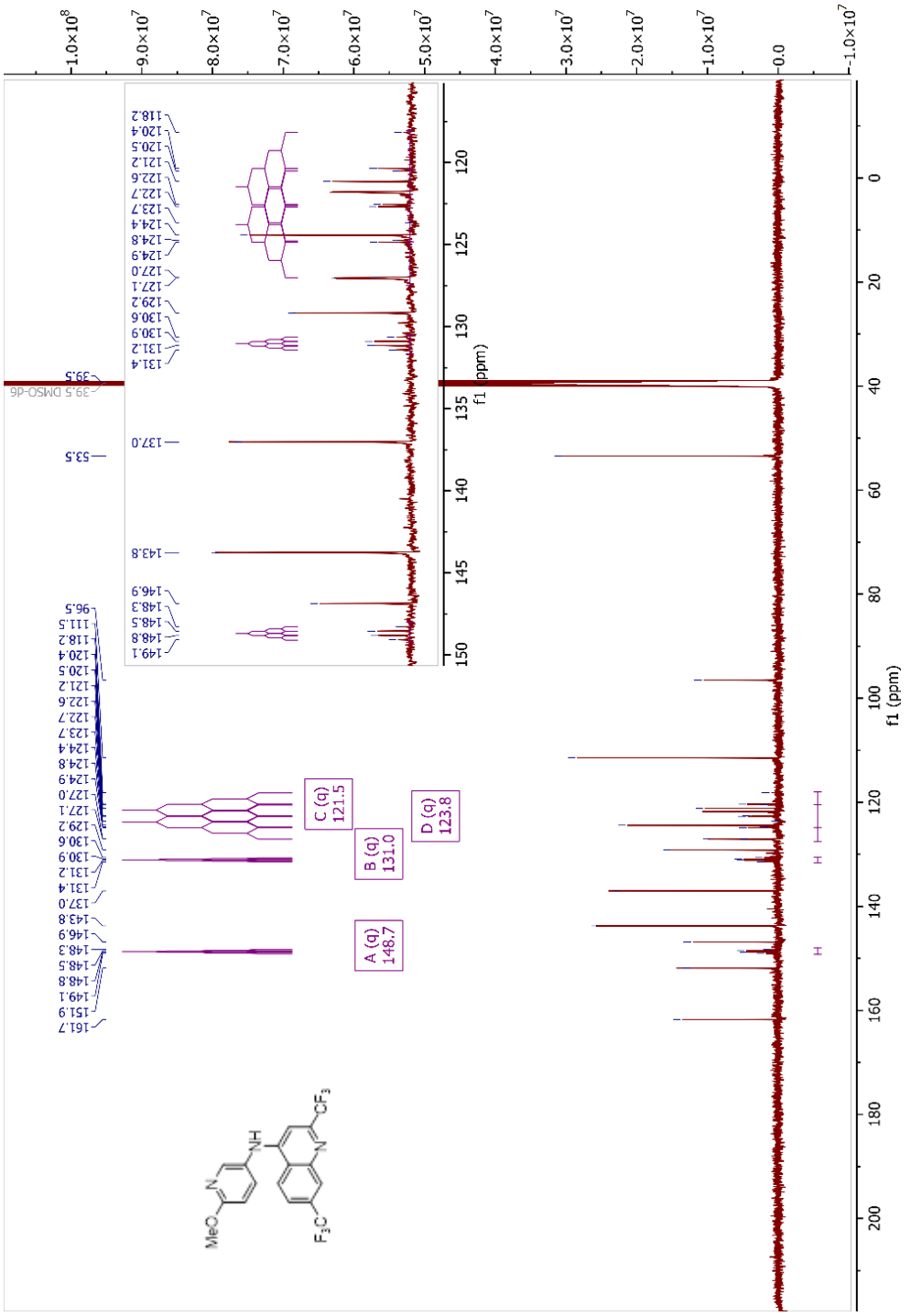
Compound 50 $^{13}C$ NMR.



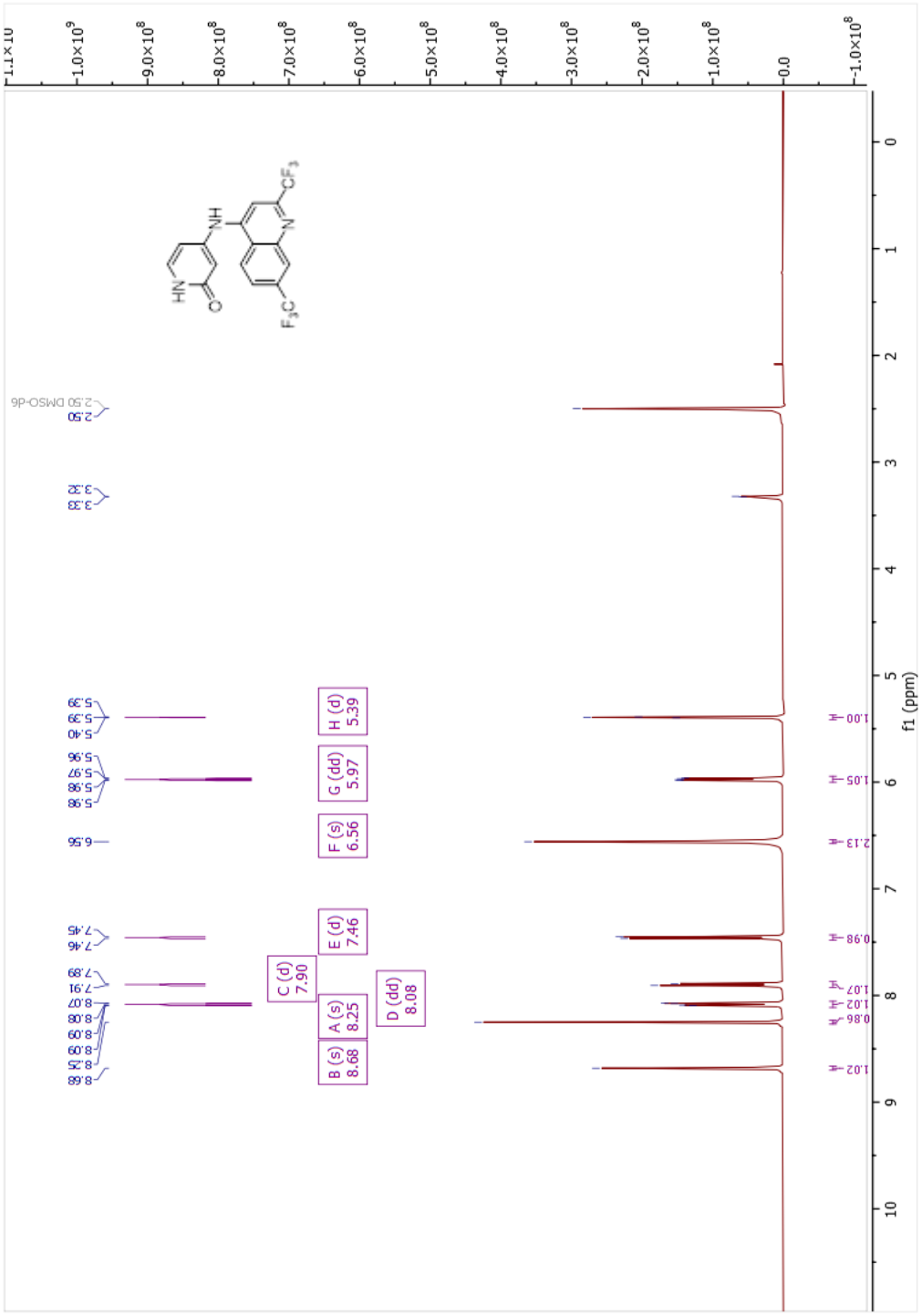
Compound 51 $^{1}$H NMR.



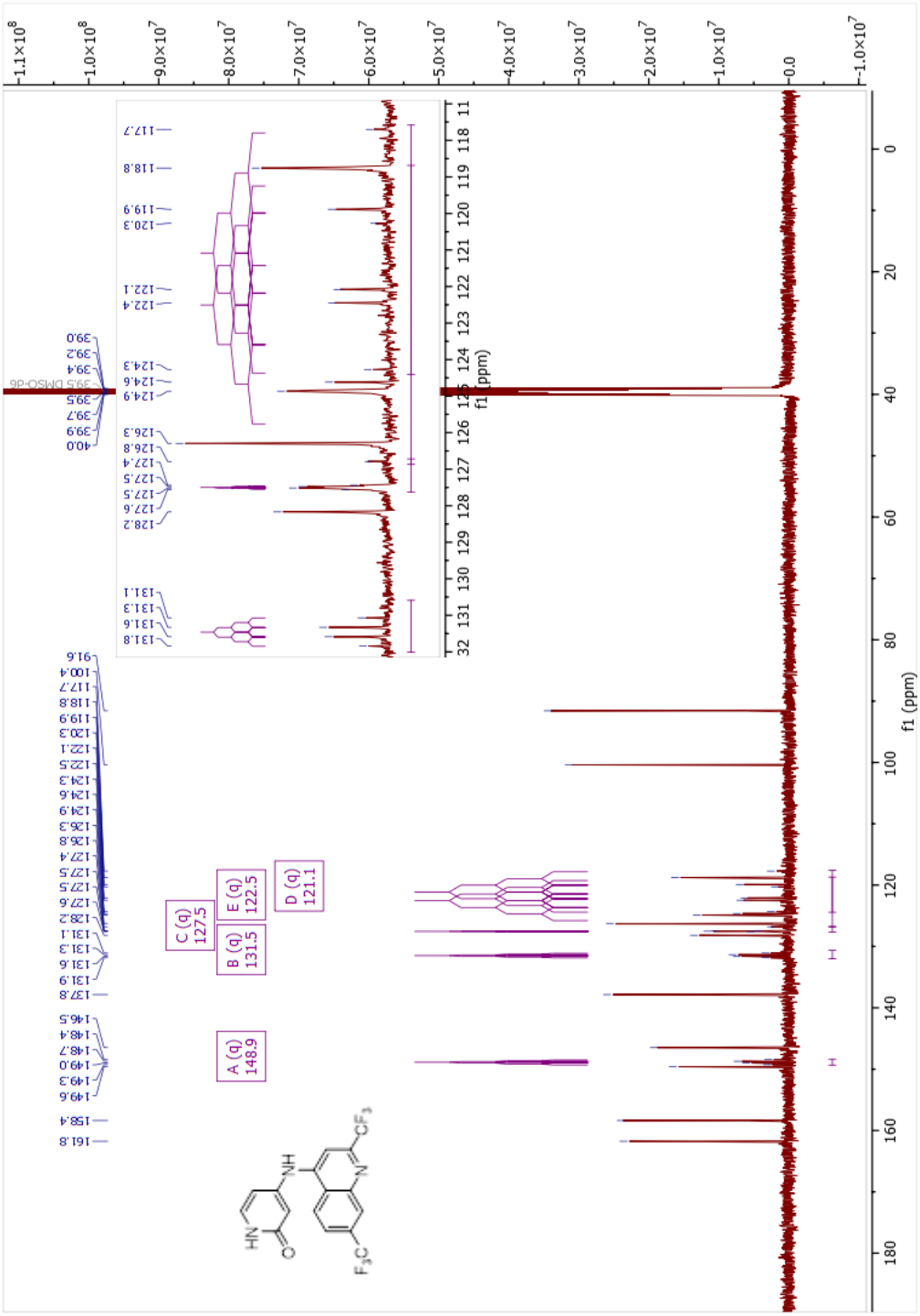
Compound 51 $^{13}C$ NMR.



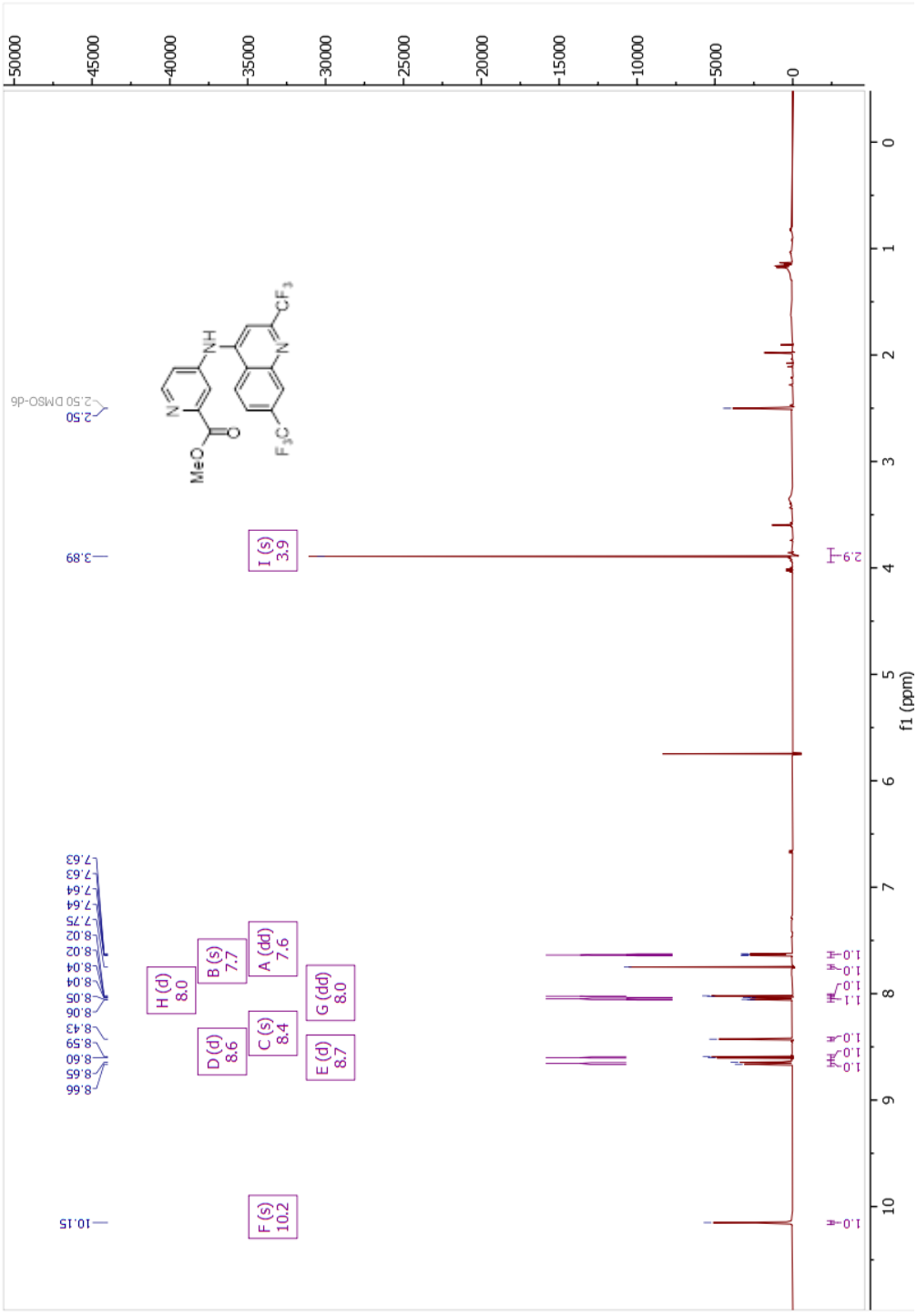
Compound 52 $^{1}$H NMR.



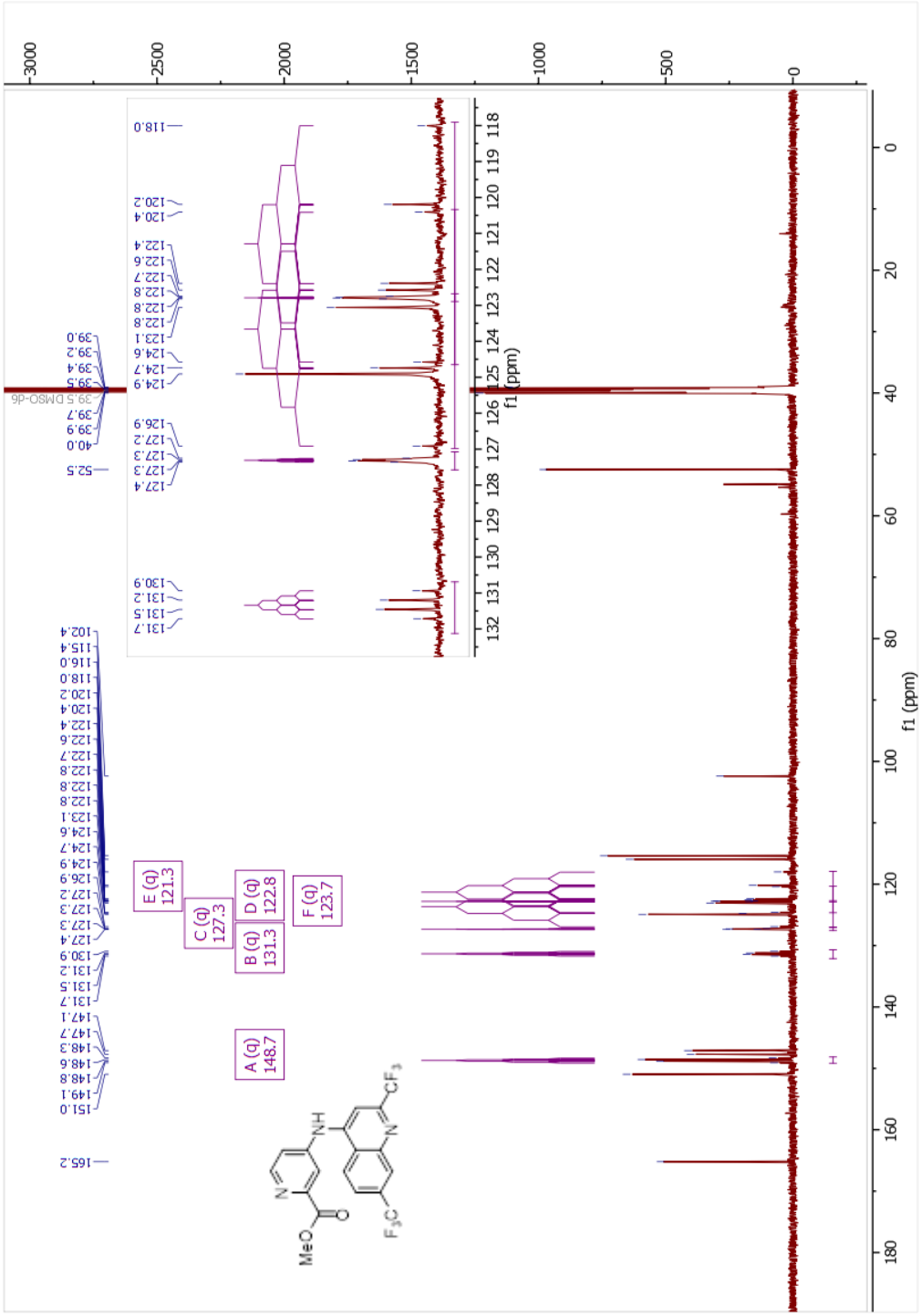
Compound 52 $^{13}C$ NMR.



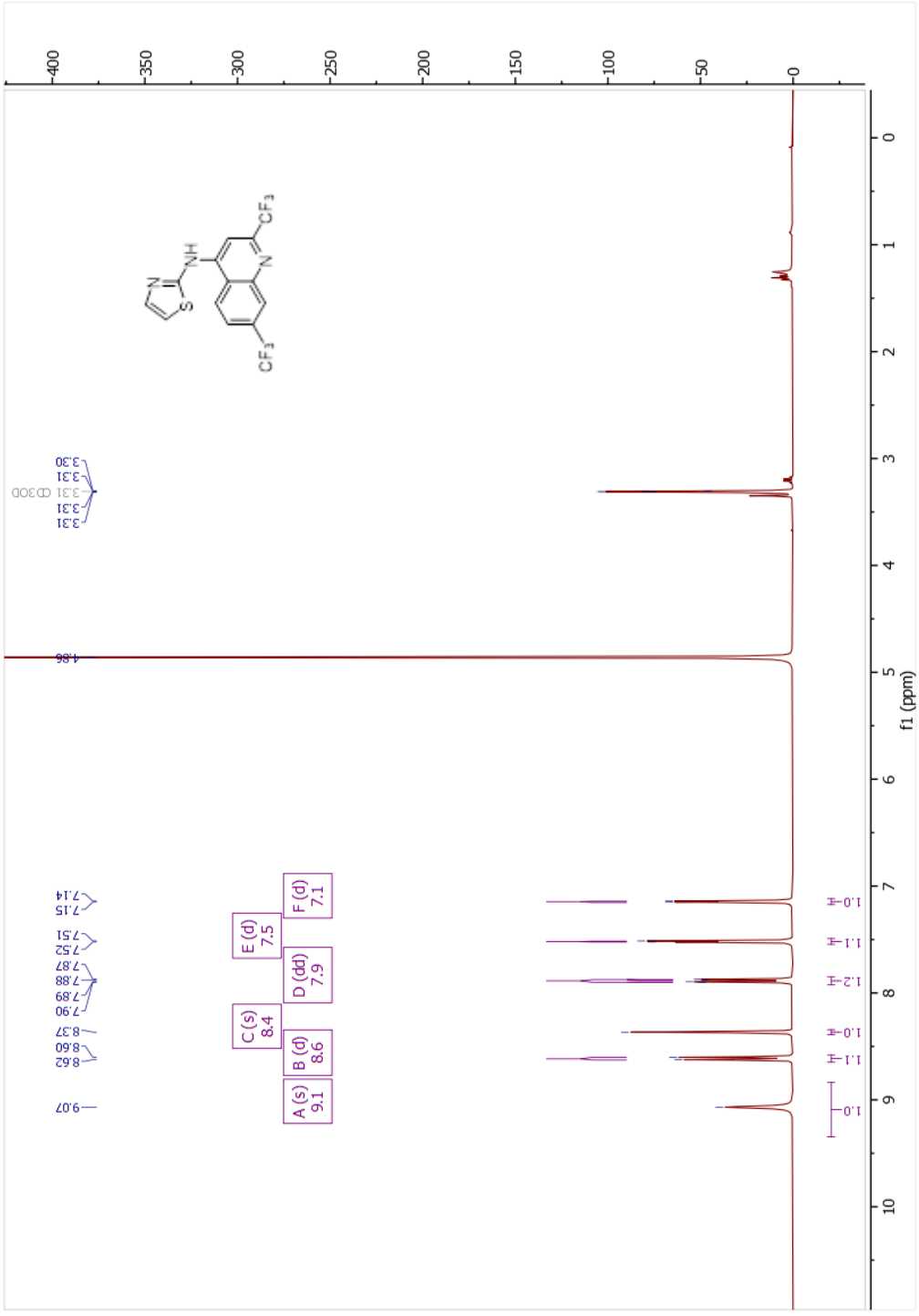
Compound 54 $^{1}$H NMR.



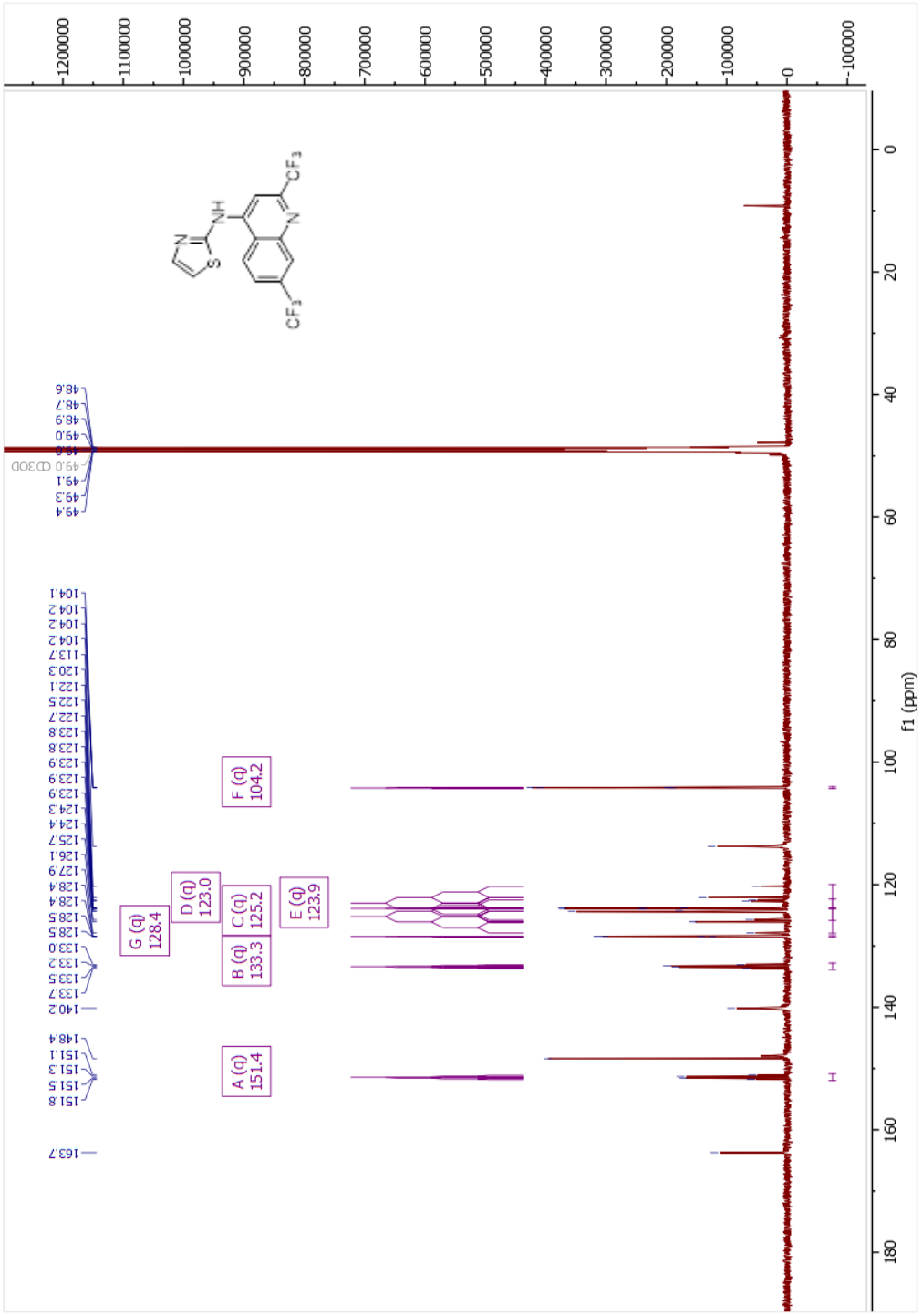
**C**ompound 54 $^{13}$C NMR.



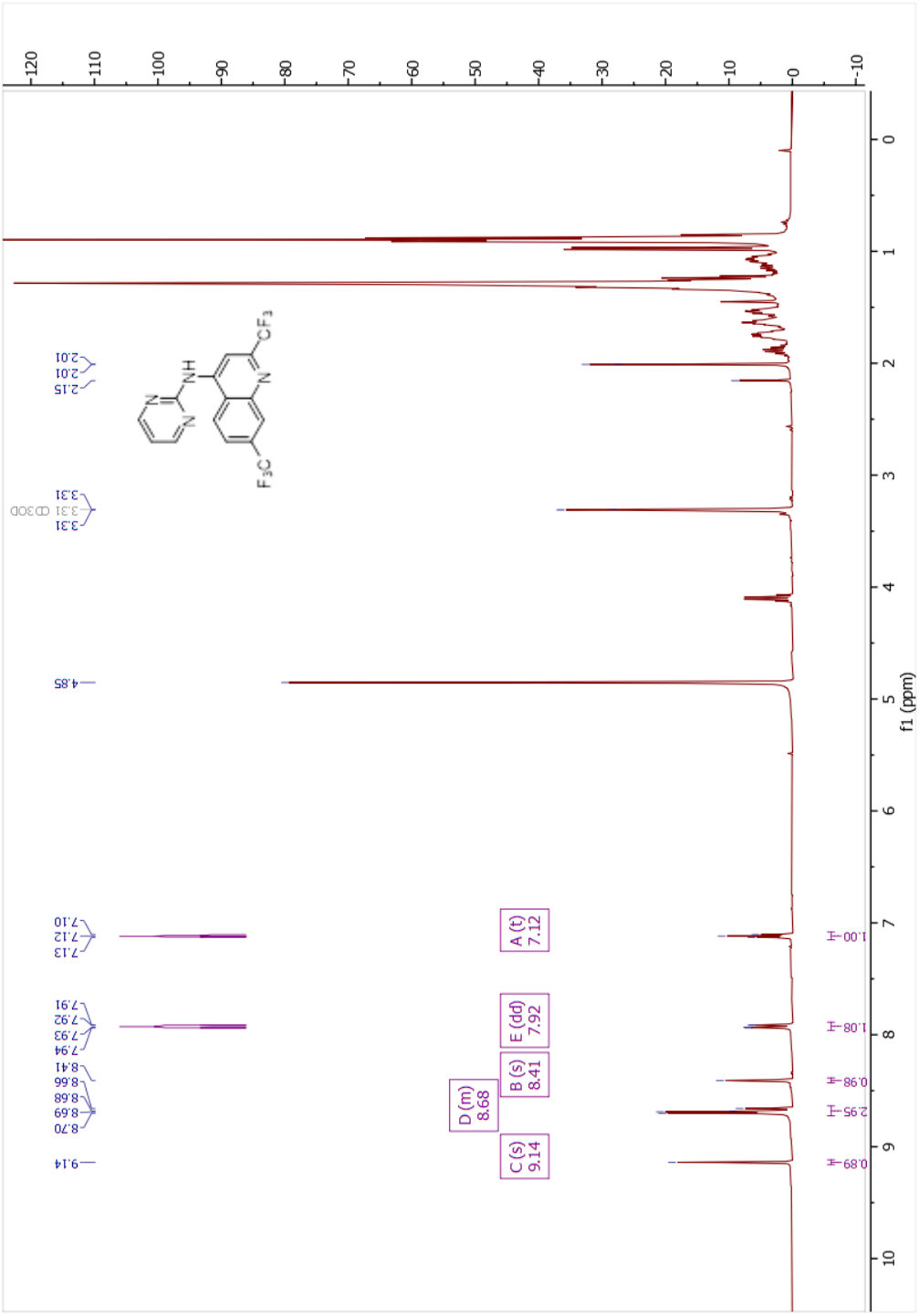
Compound 55 $^{1}$H NMR.



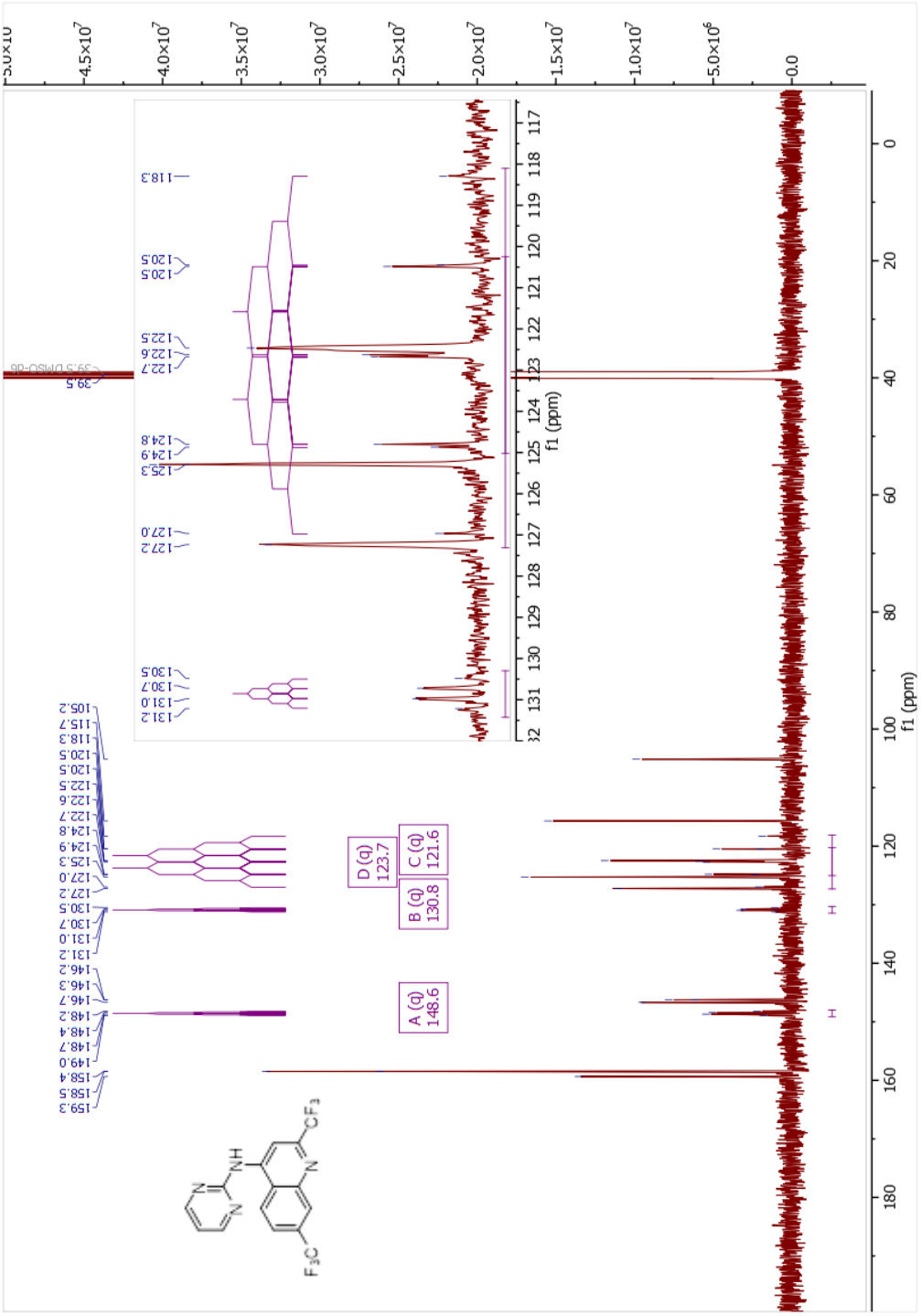
Compound 55 $^{13}C$ NMR.



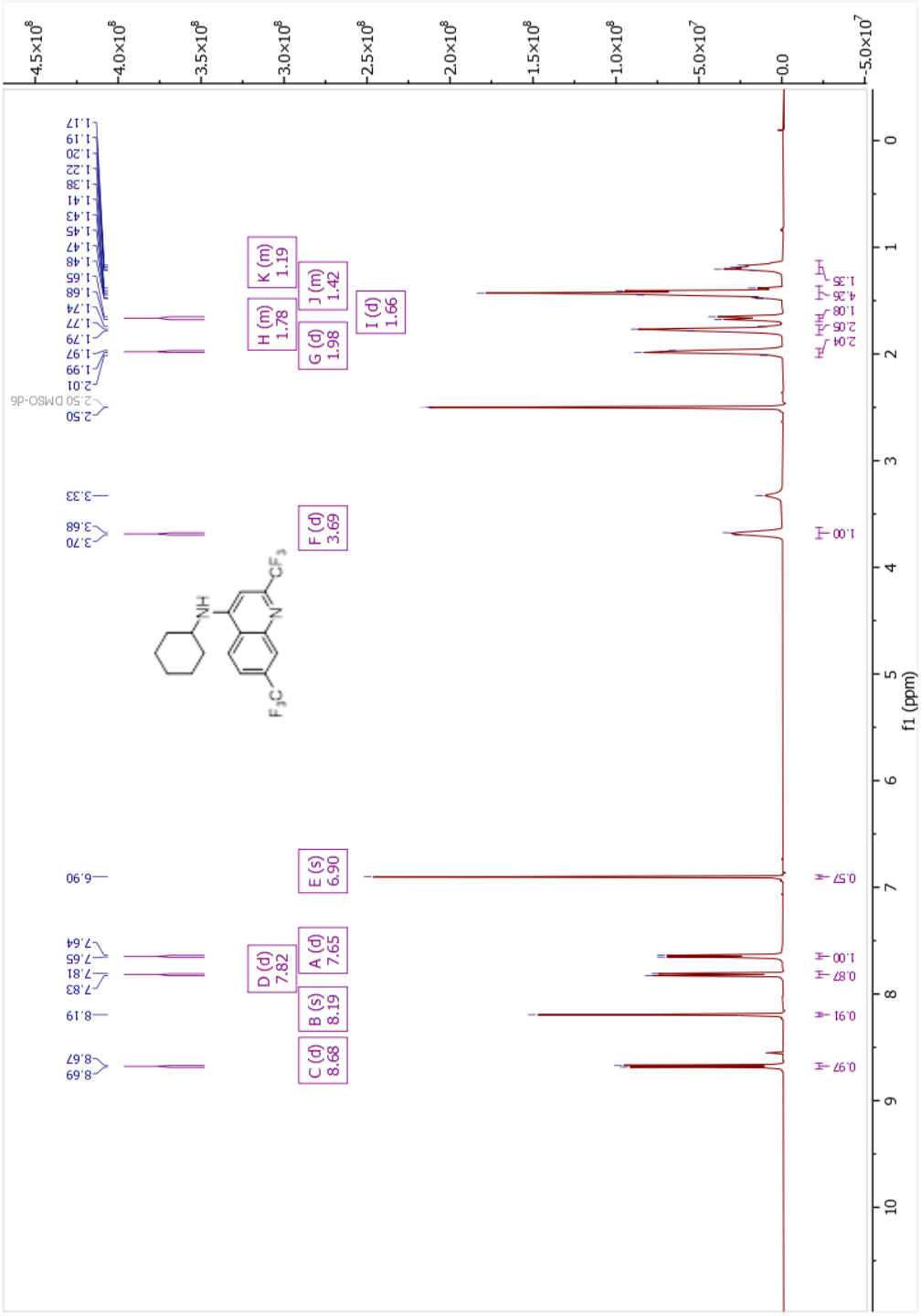
Compound 56 $^{1}$H NMR.



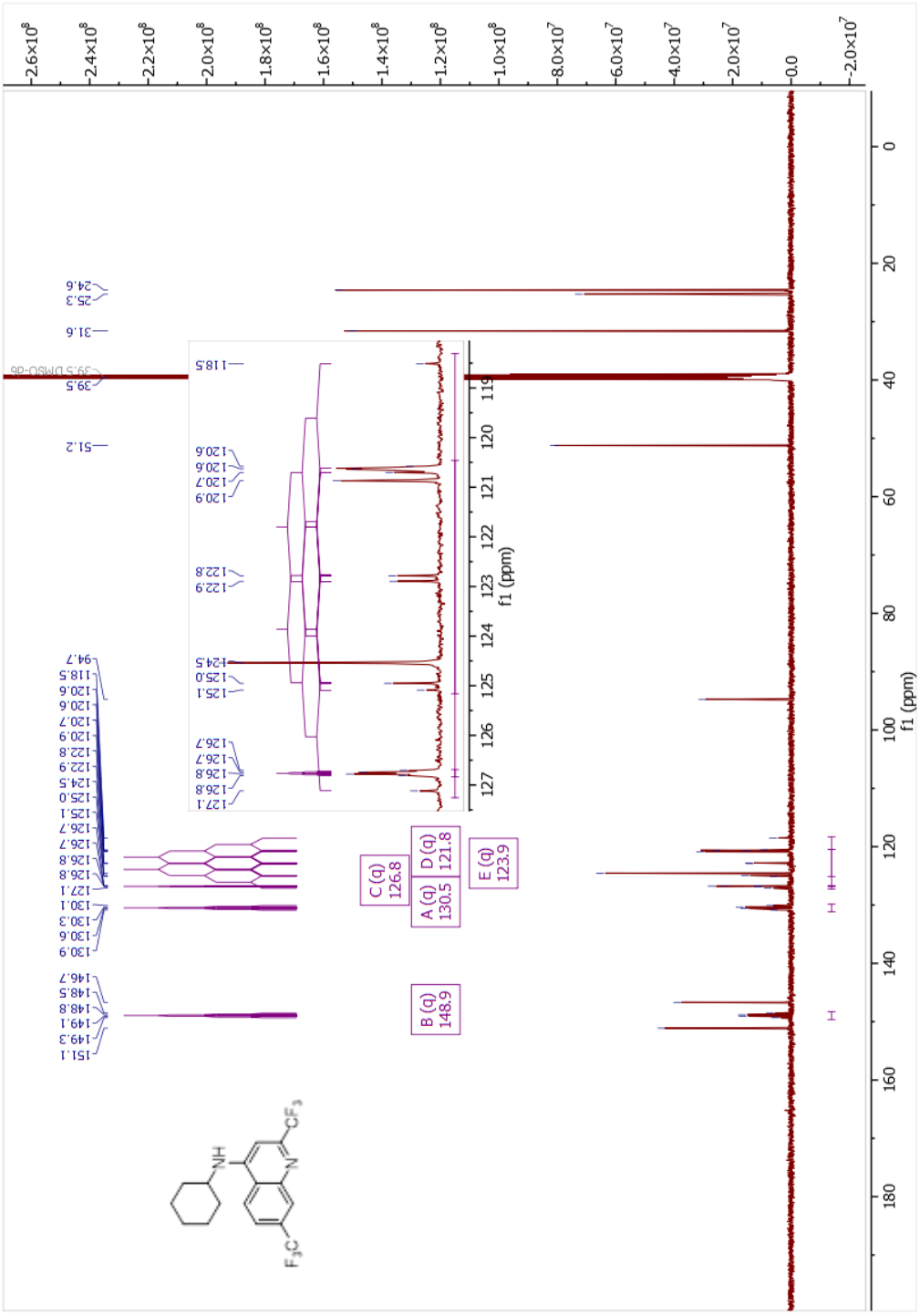
Compound 56 $^{13}C$ NMR.



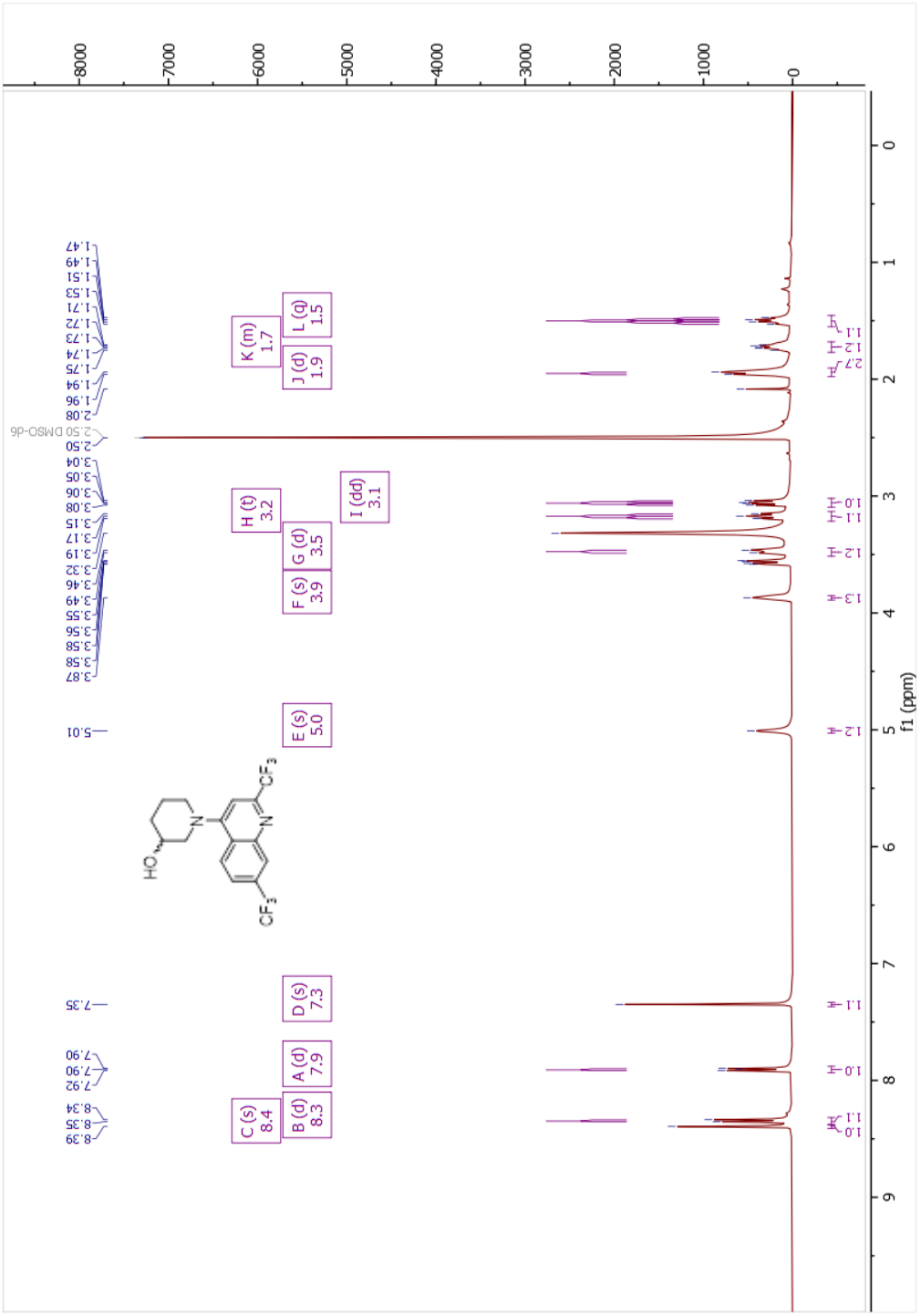
Compound 57 $^{1}H$ NMR.



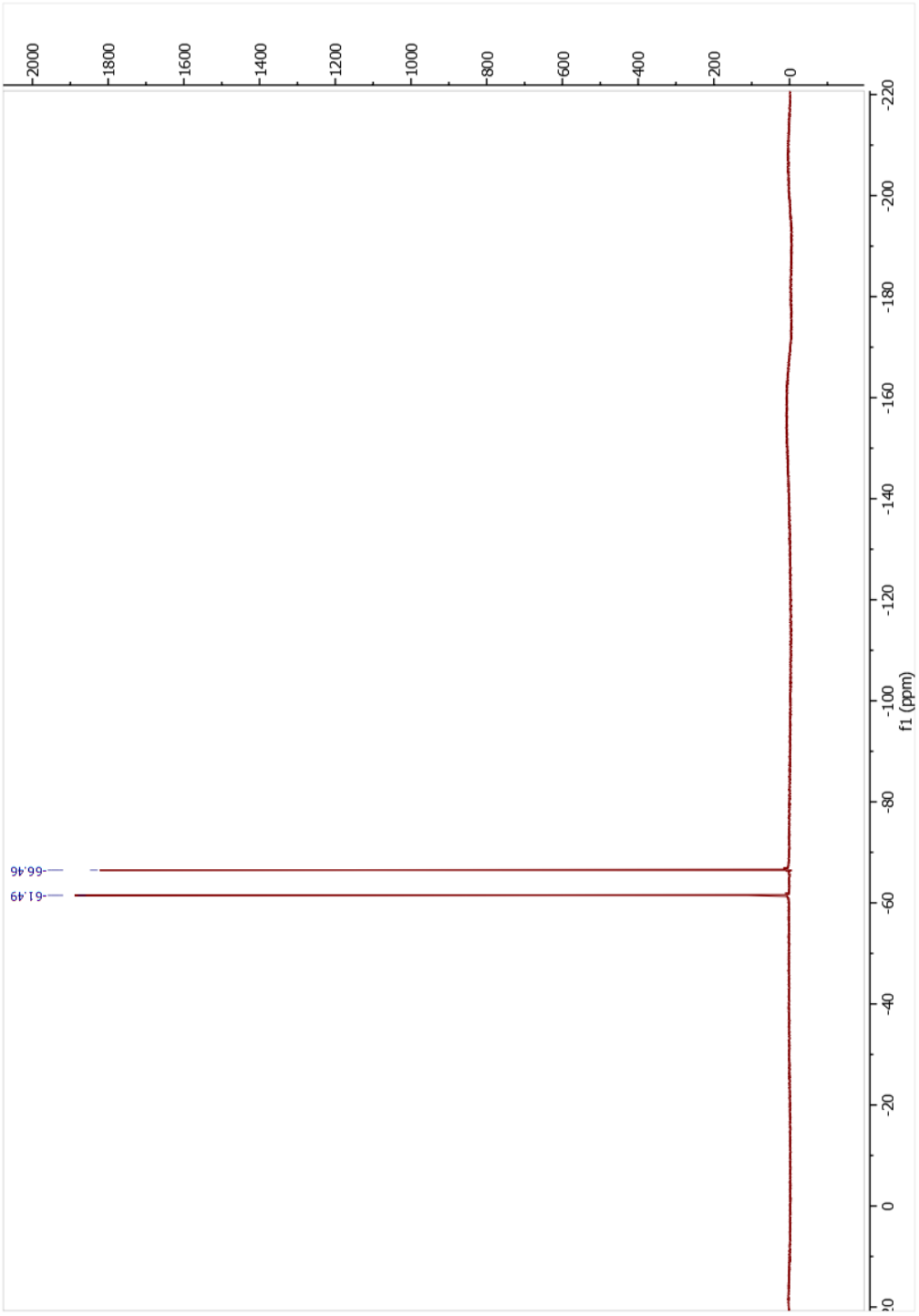
Compound 57 $^{13}C$ NMR.



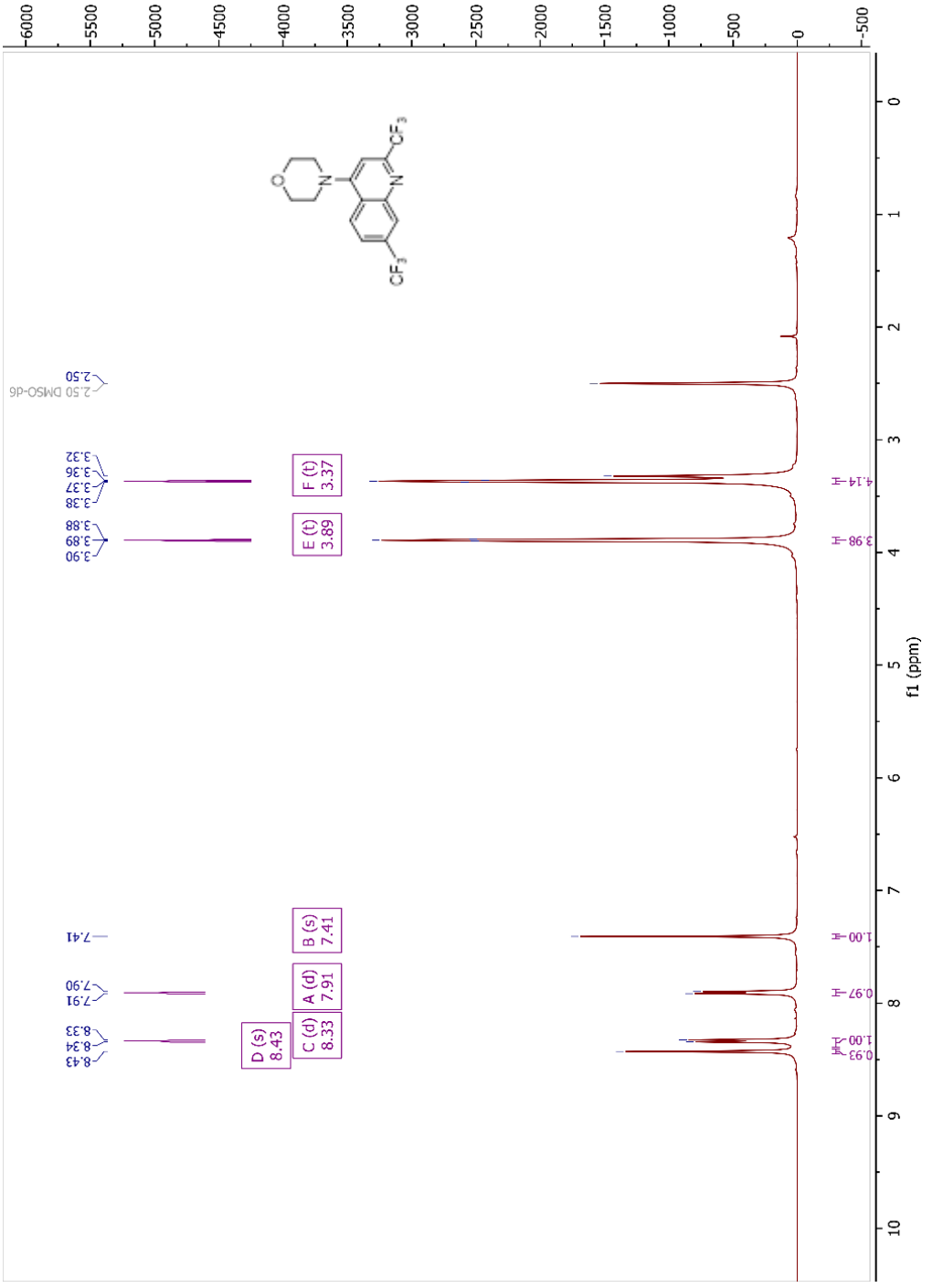
Compound 58 $^{1}H$ NMR.



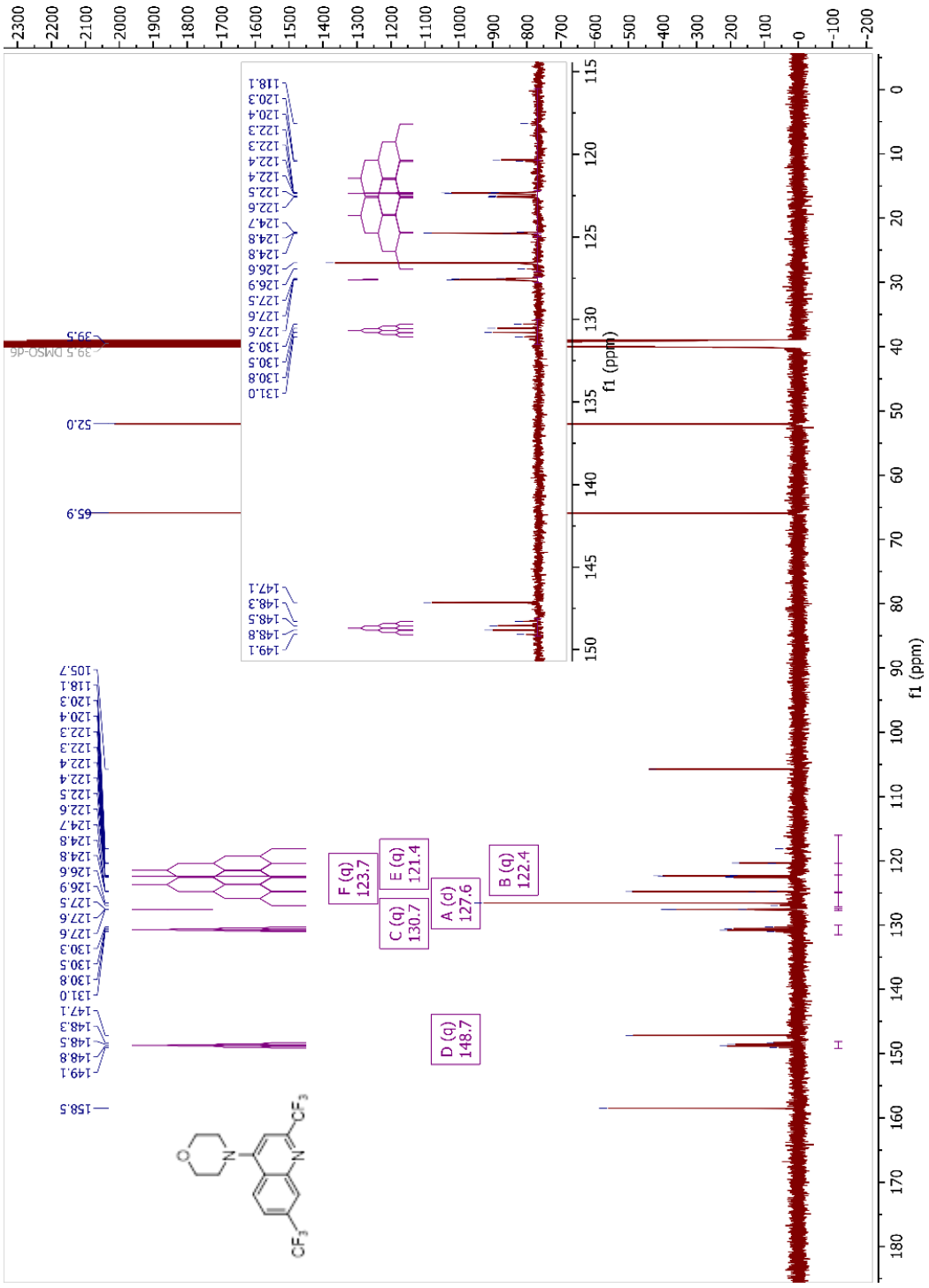
Compound 58 $^{19}F$ NMR.



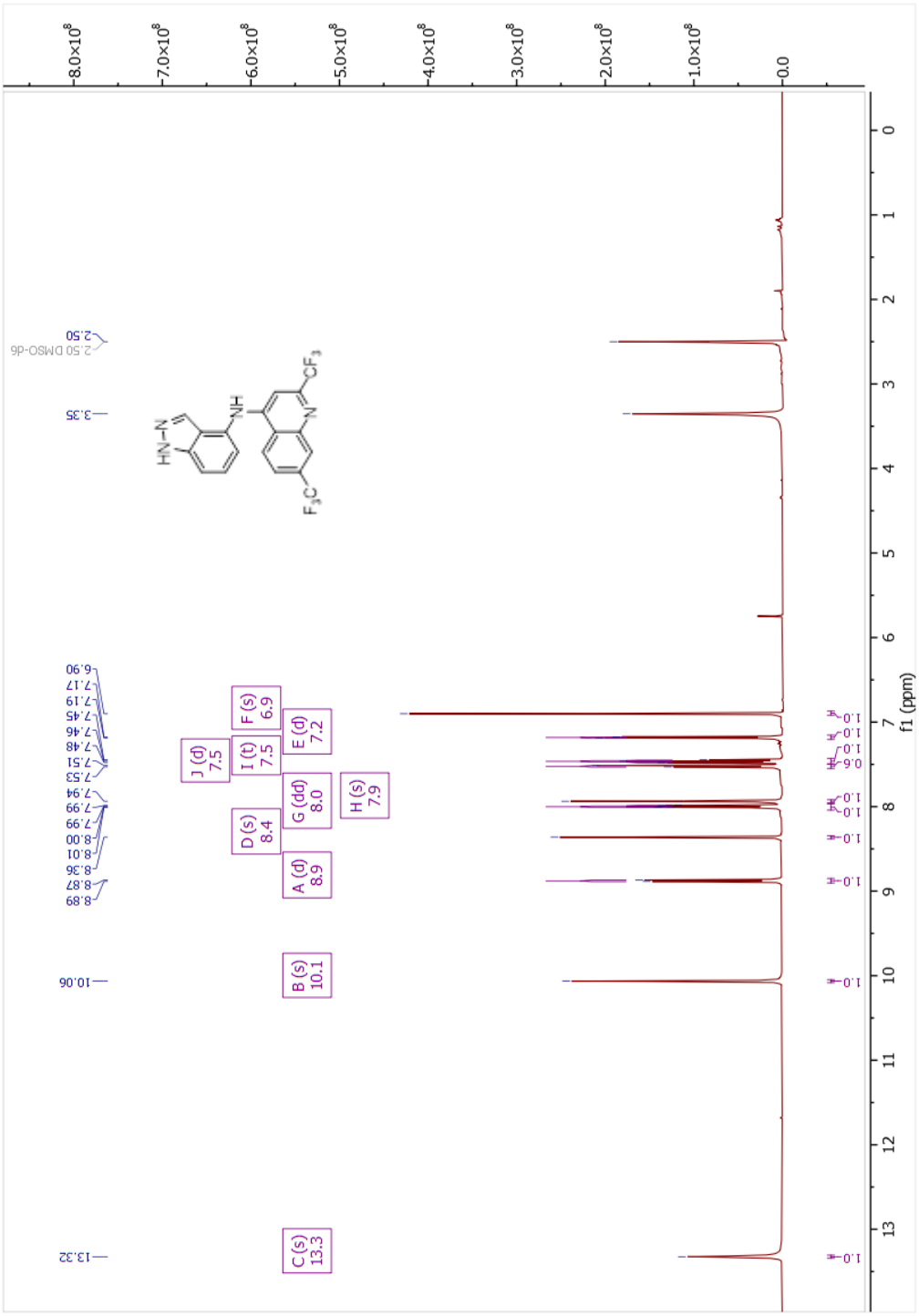
Compound 59 $^{1}$H NMR.



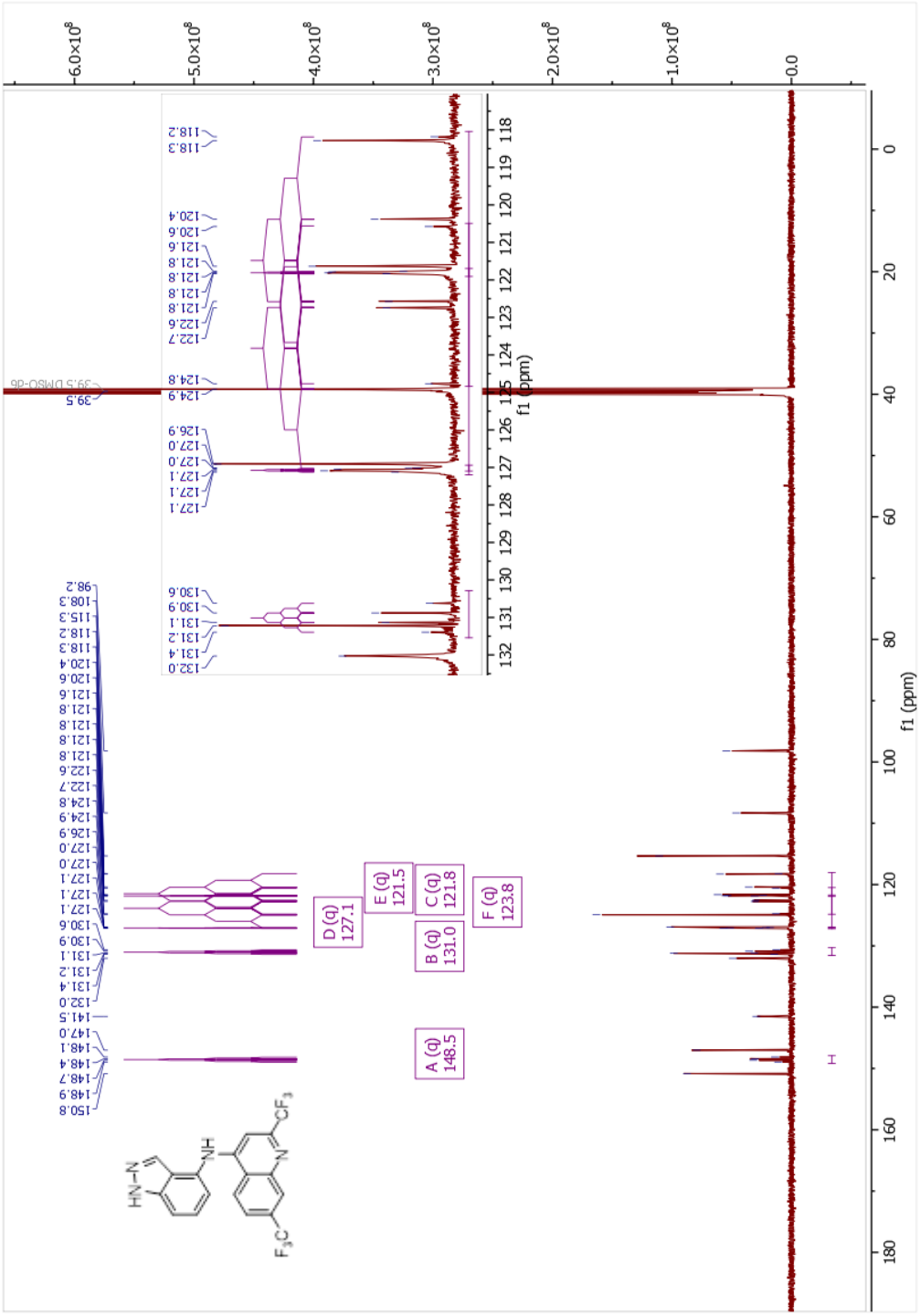
Compound 59 $^{13}C$ NMR.



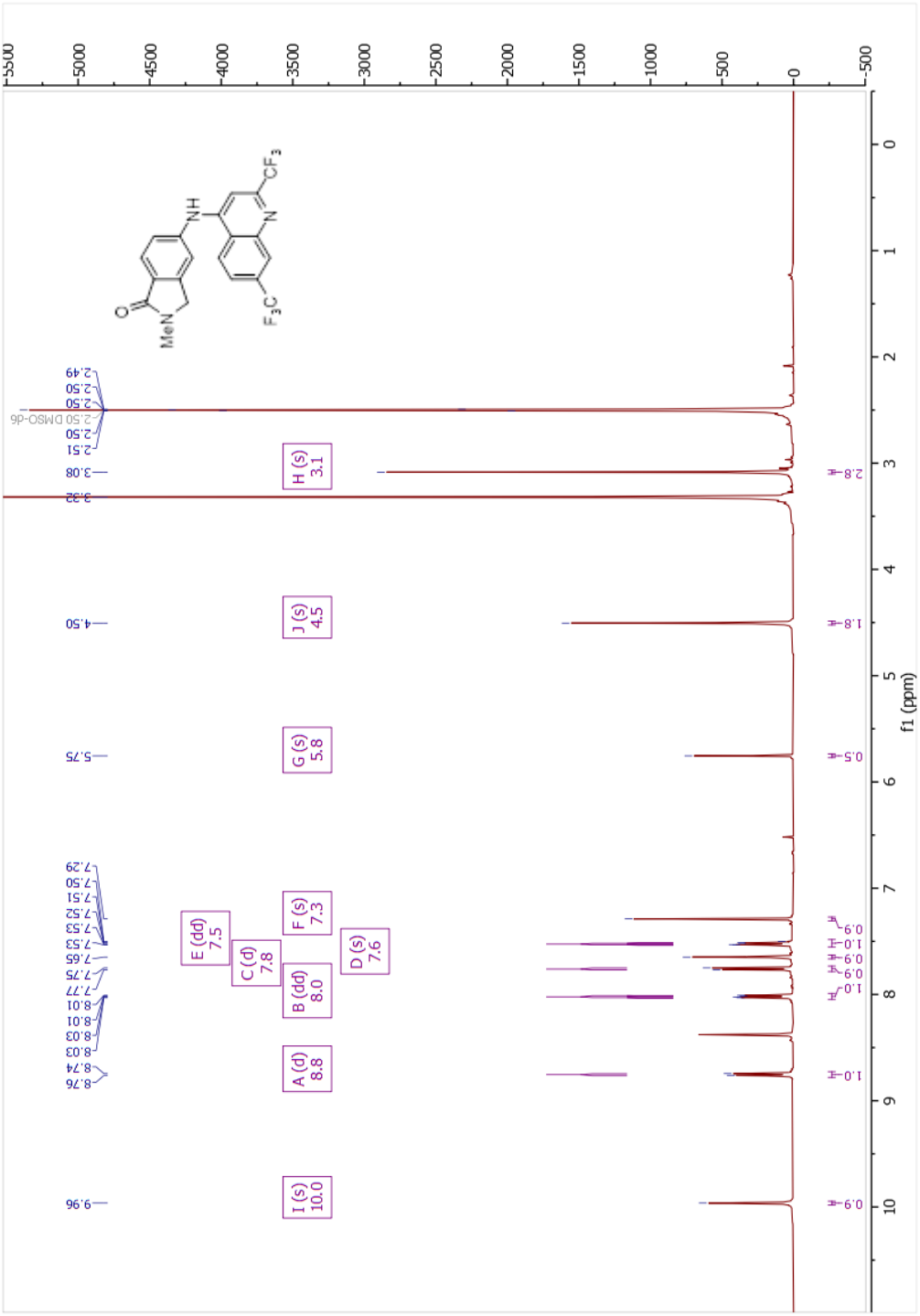
Compound 61 $^{1}$H NMR.



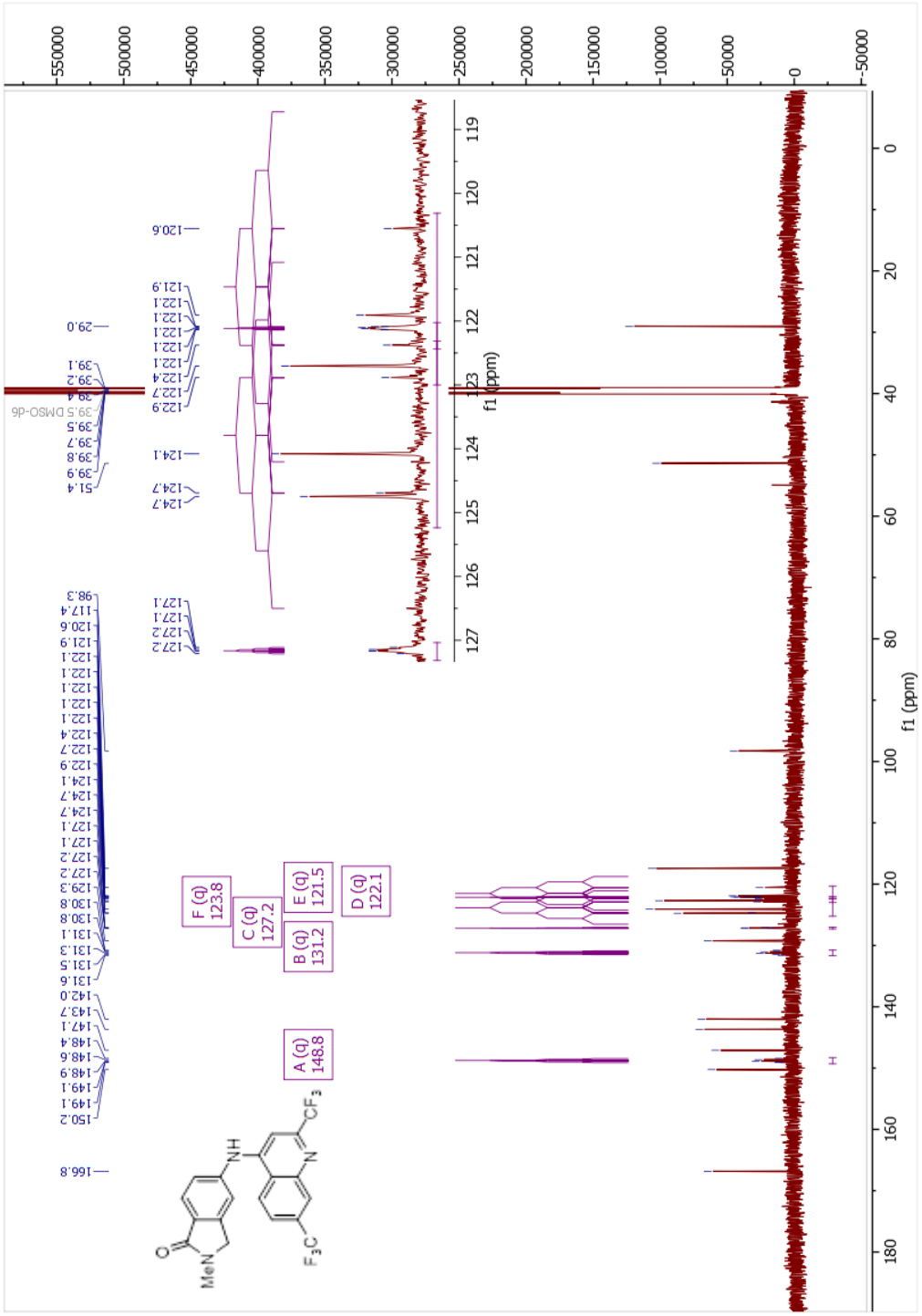
Compound 61 $^{13}C$ NMR.



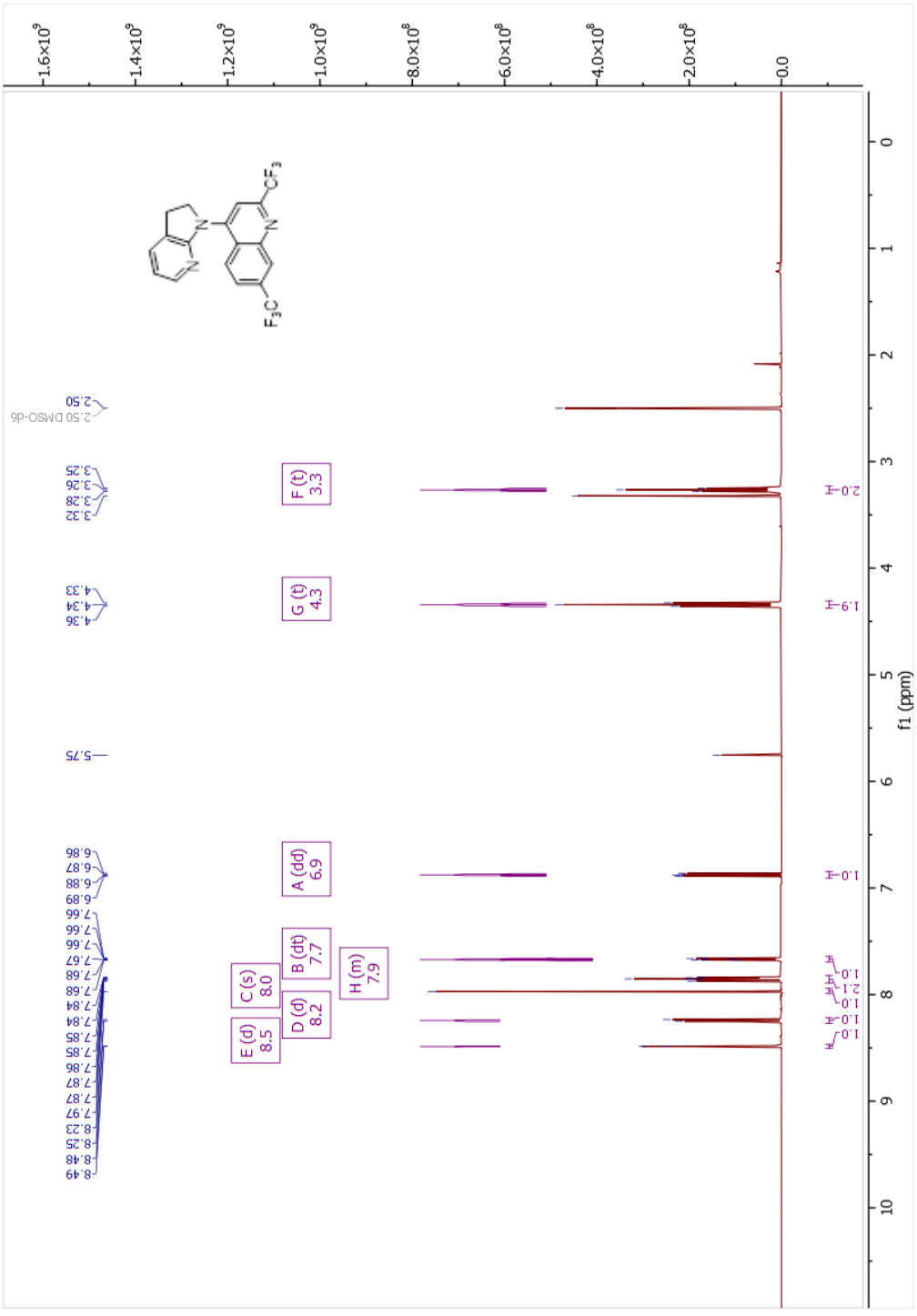
Compound 62 $^{1}$H NMR.



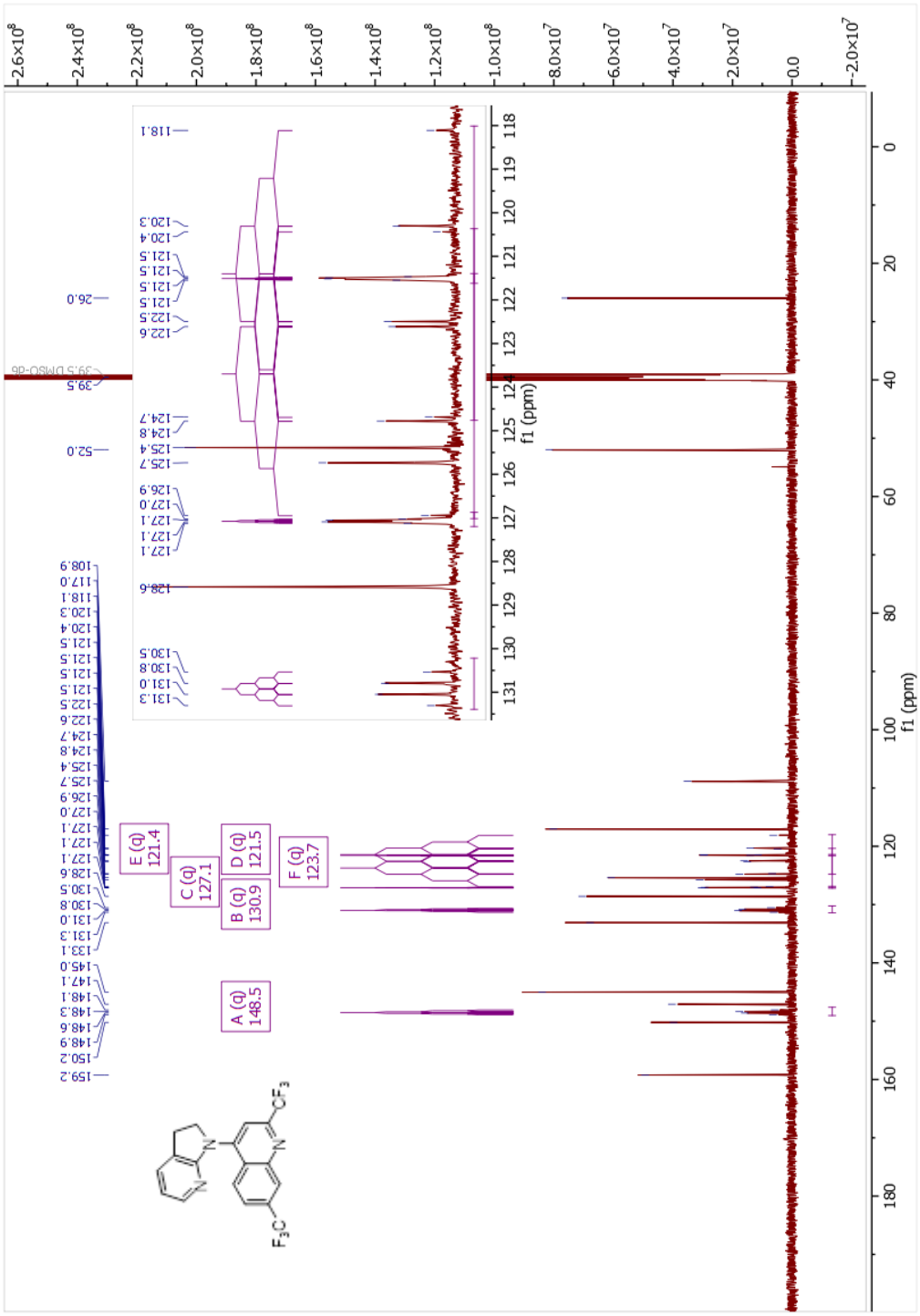
Compound 62 $^{13}$C NMR.



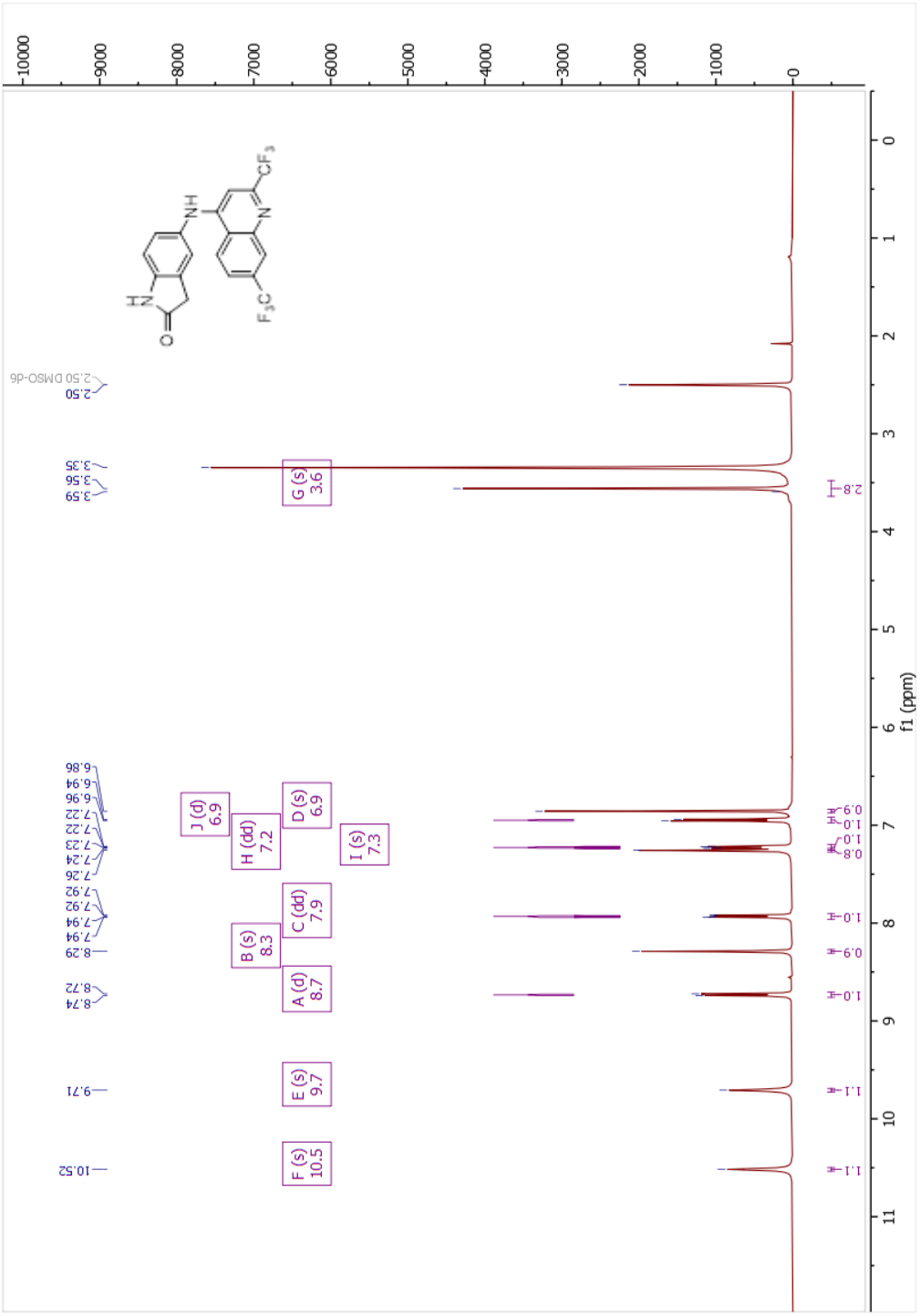
Compound 63 $^{1}$H NMR.



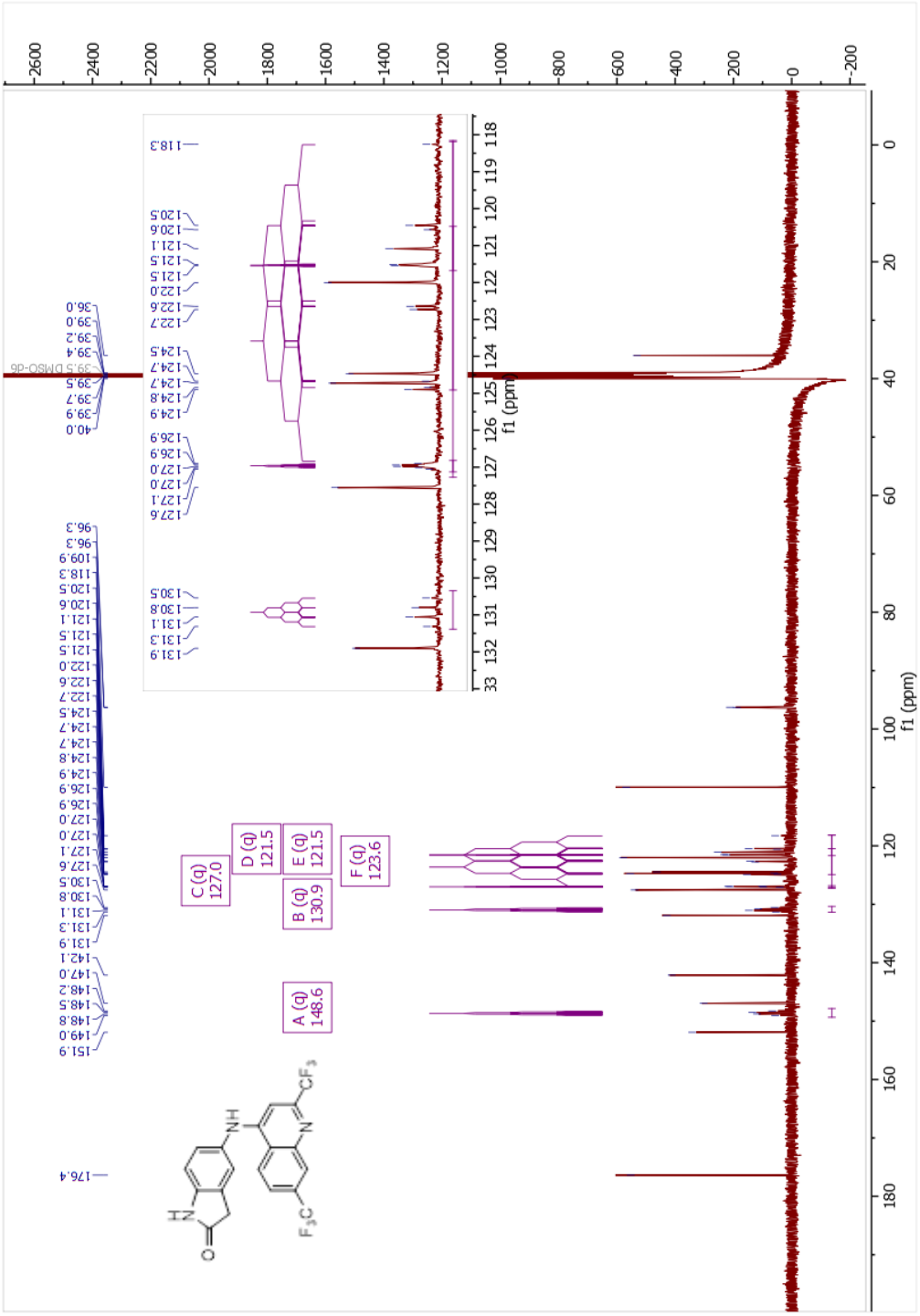
Compound 63 $^{13}C$ NMR.



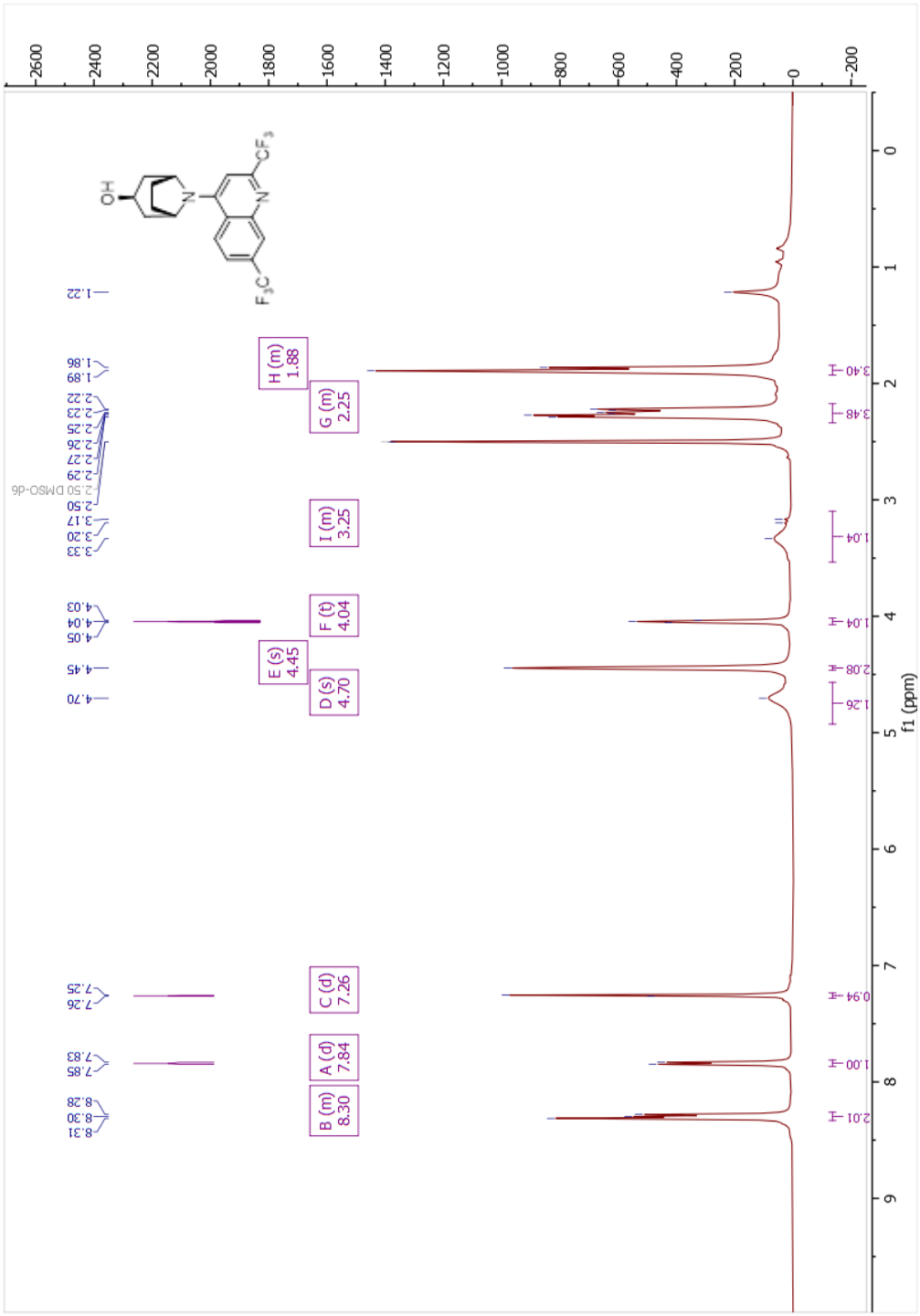
Compound 64 $^{1}$H NMR.



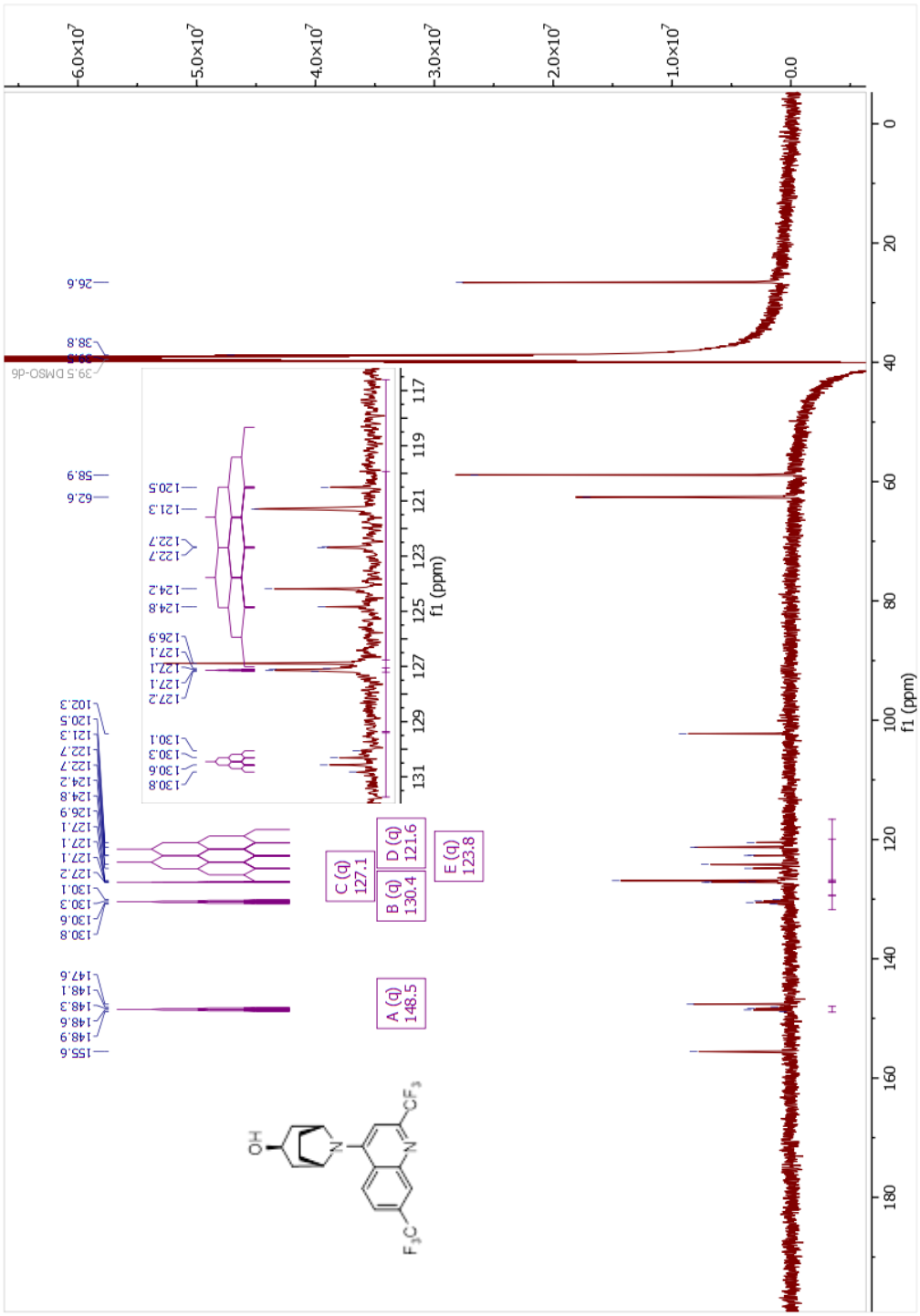
Compound 64 $^{13}$C NMR.



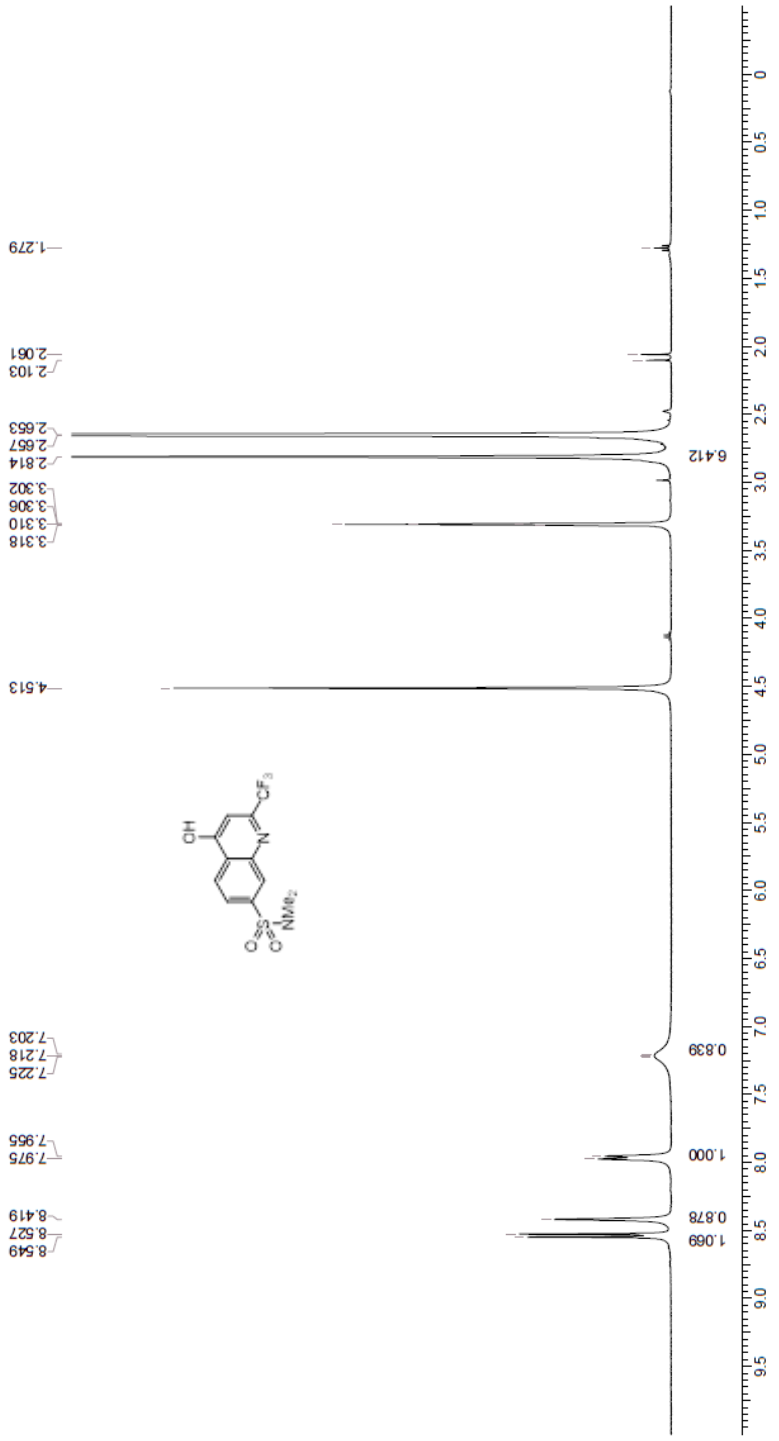
Compound 65 $^{1}$H NMR.



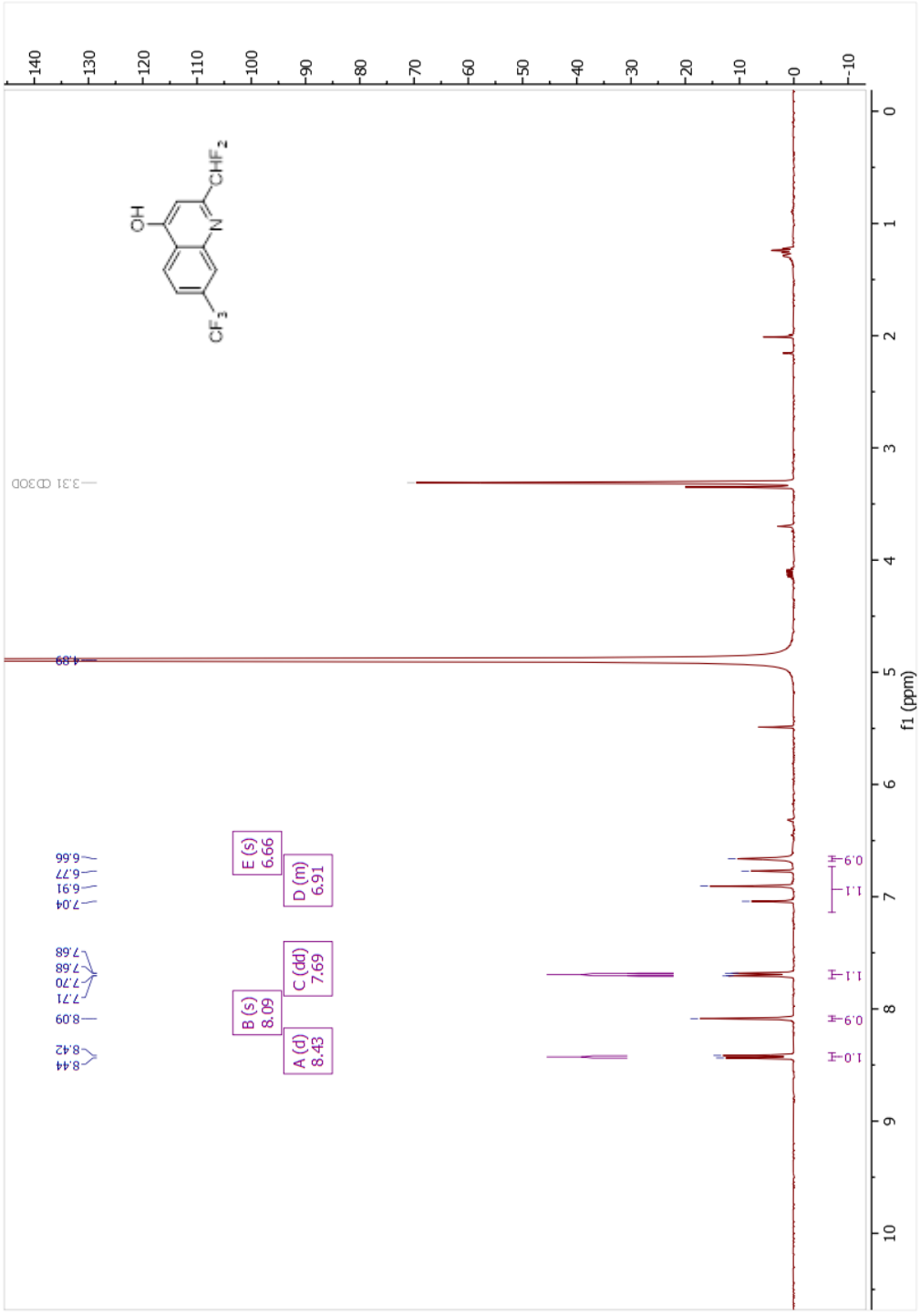
Compound 65 $^{13}$C NMR.



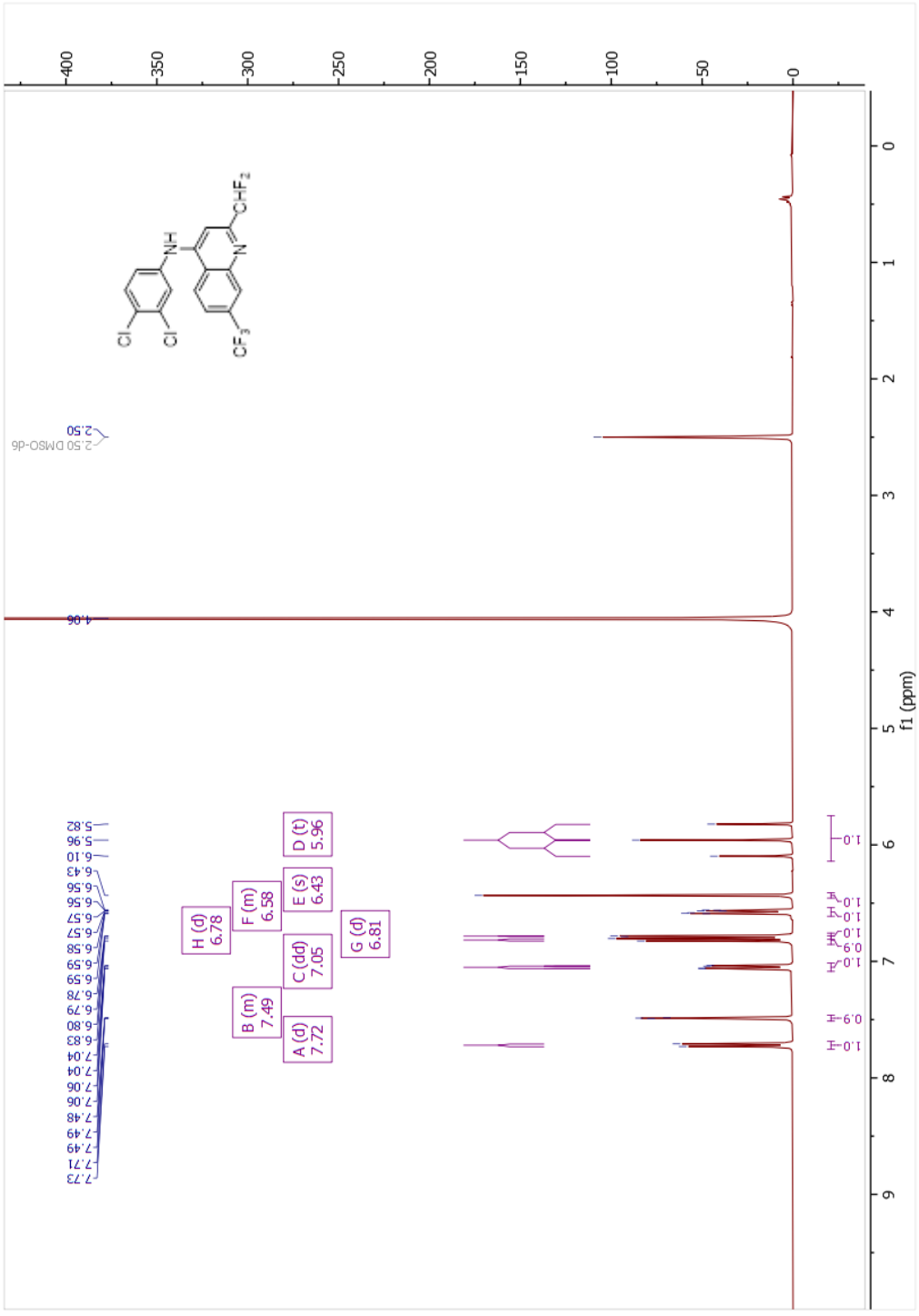
Compound 66 $^{1}$H NMR.



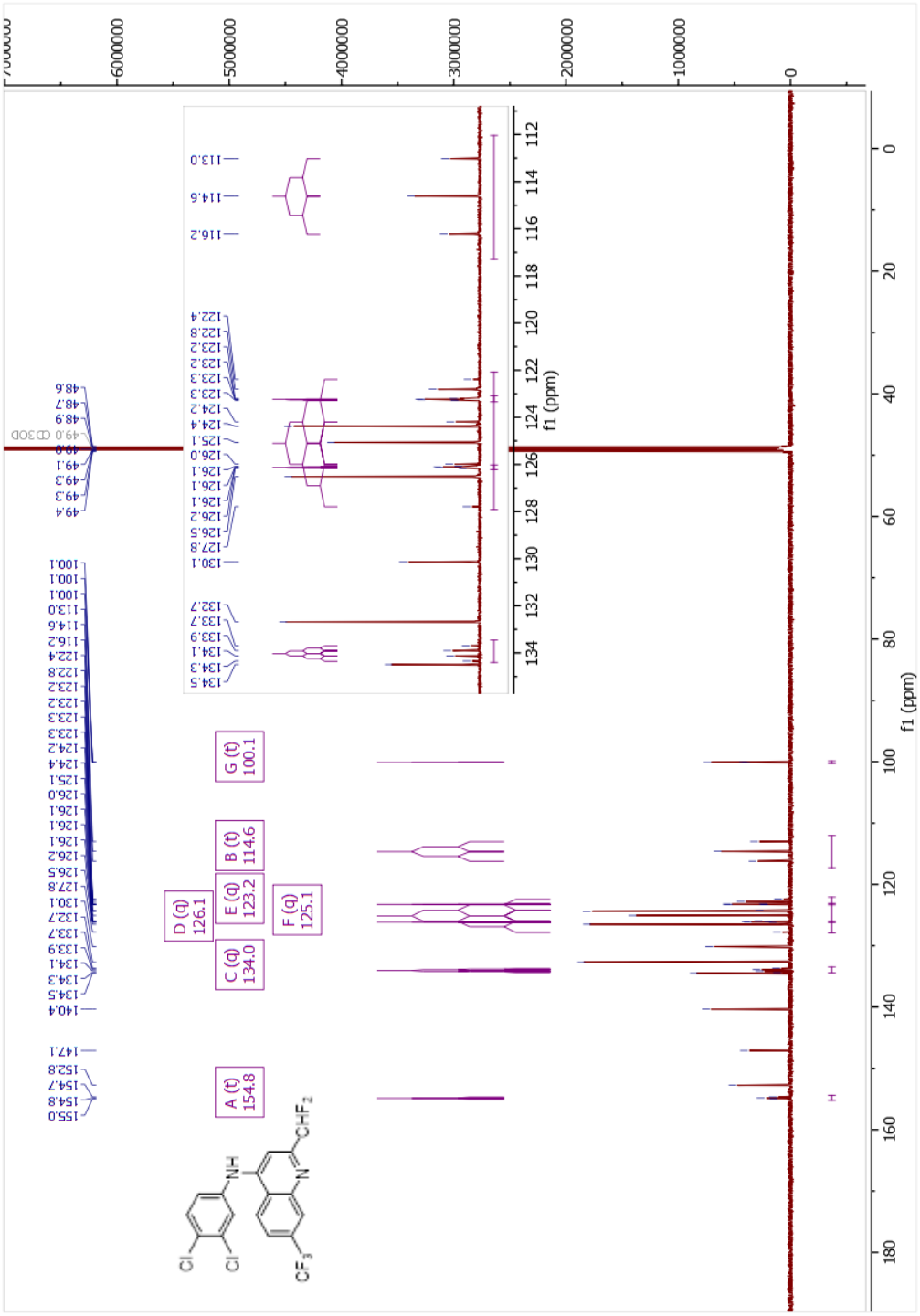
Compound 67 $^{1}$H NMR.



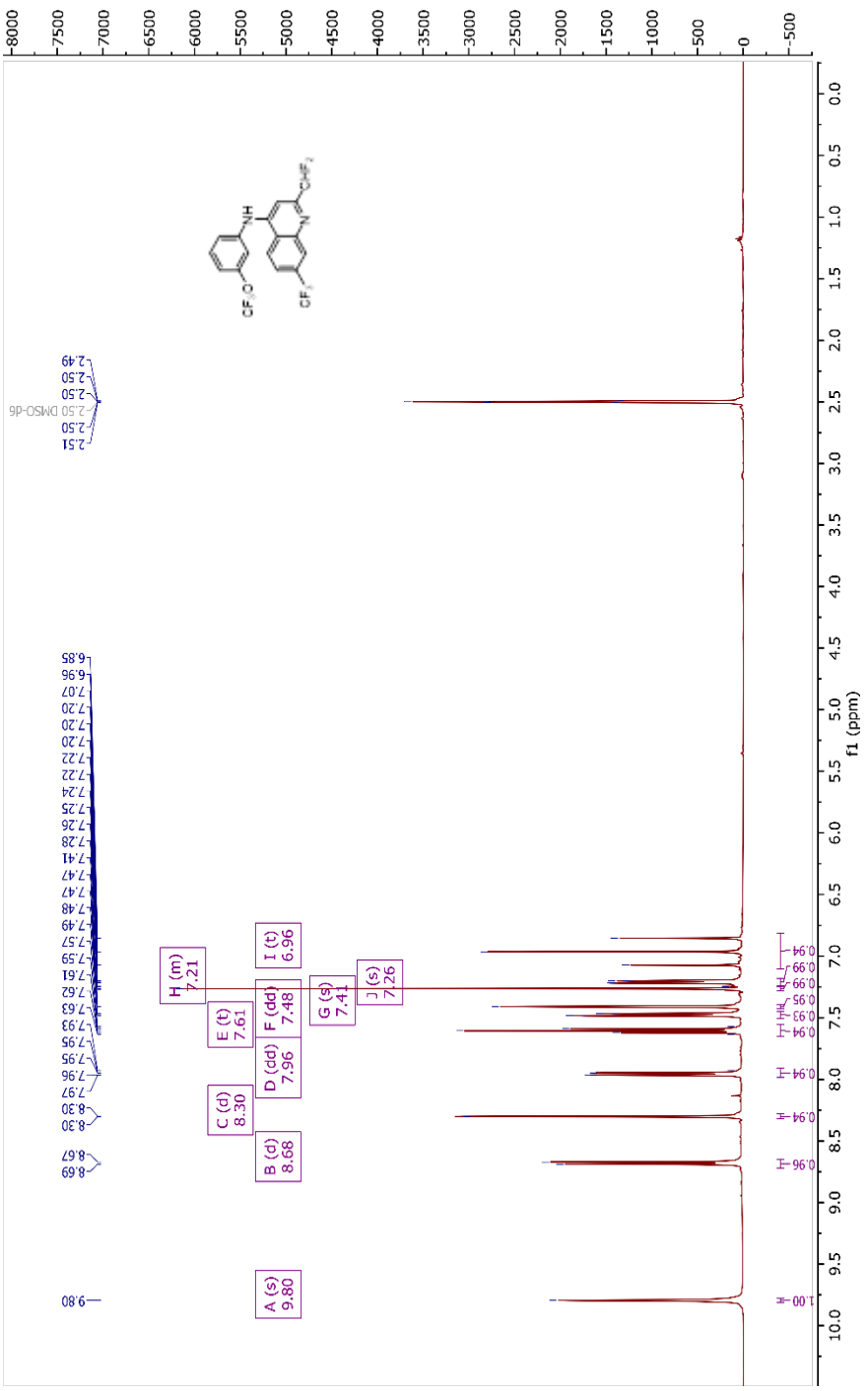
Compound 68 $^{1}$H NMR.



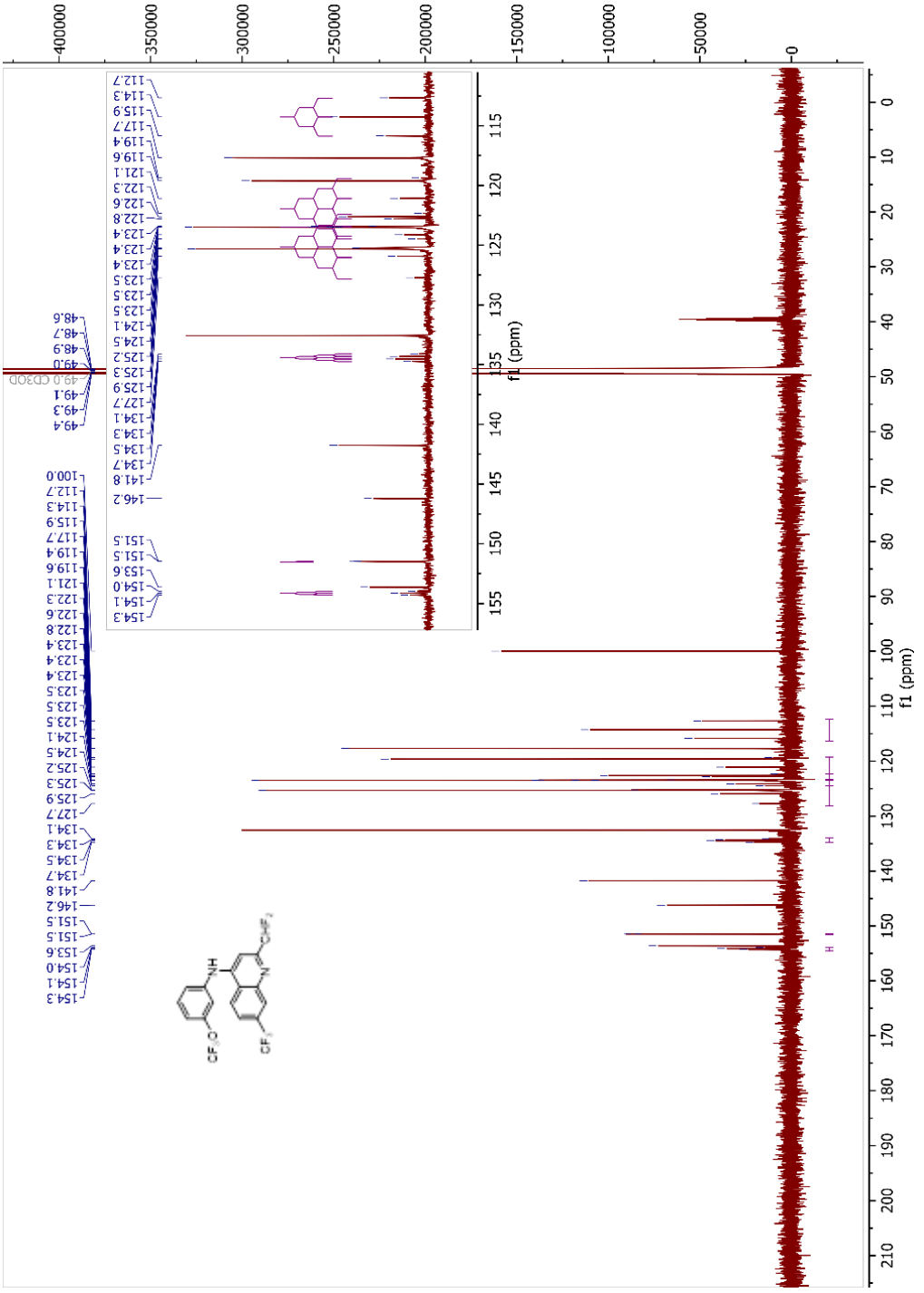
Compound 68 $^{13}C$ NMR.



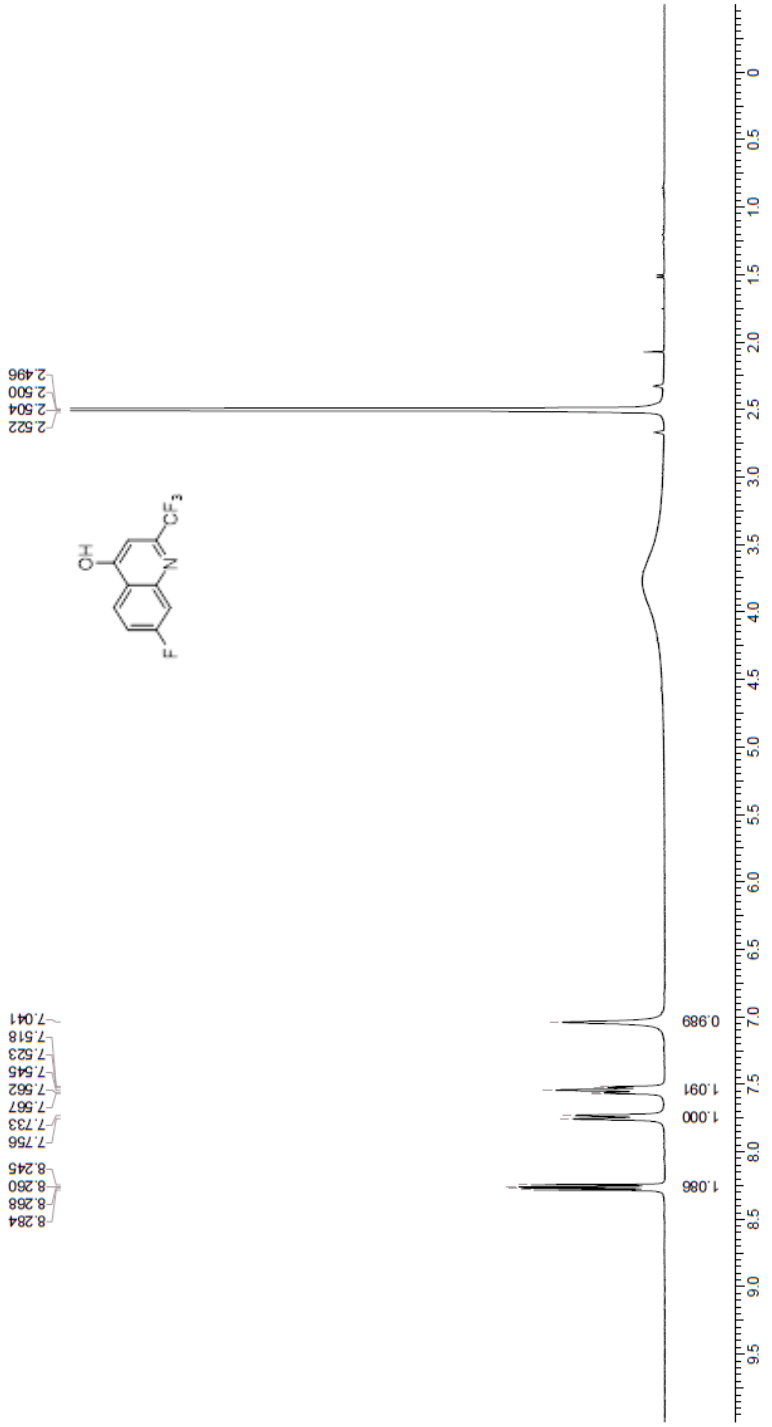
Compound 69 $^{1}$H NMR.



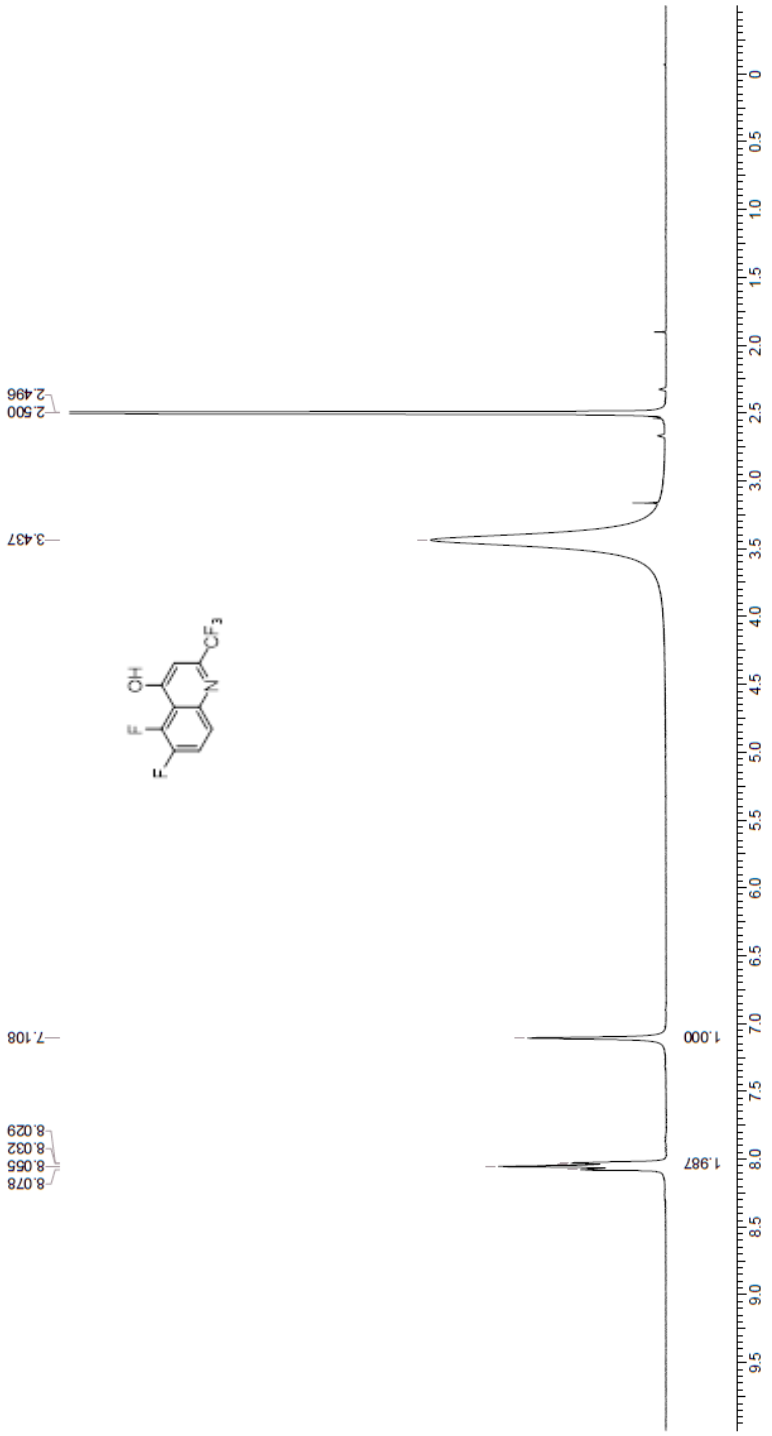
Compound 69 $^{13}C$ NMR.



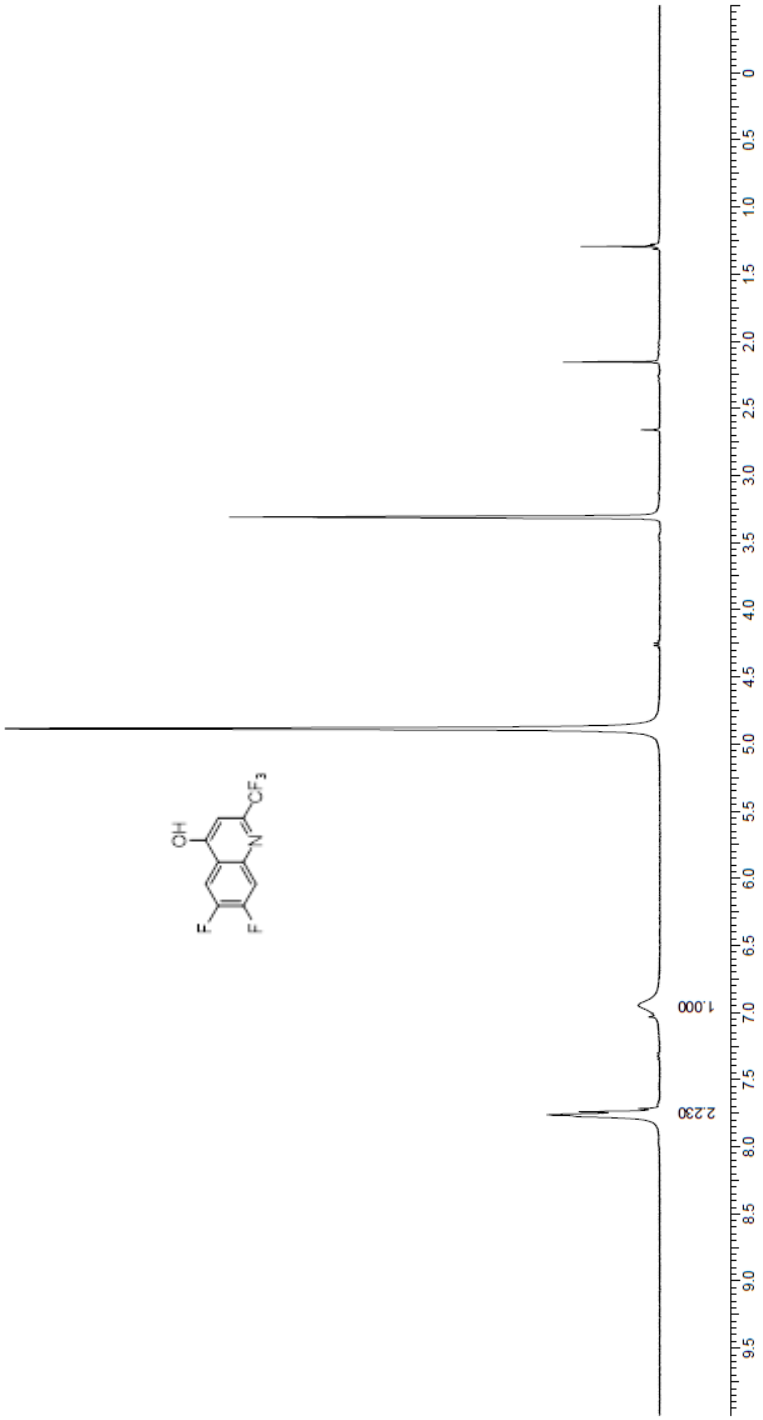
Compound 70 $^{1}$H NMR.



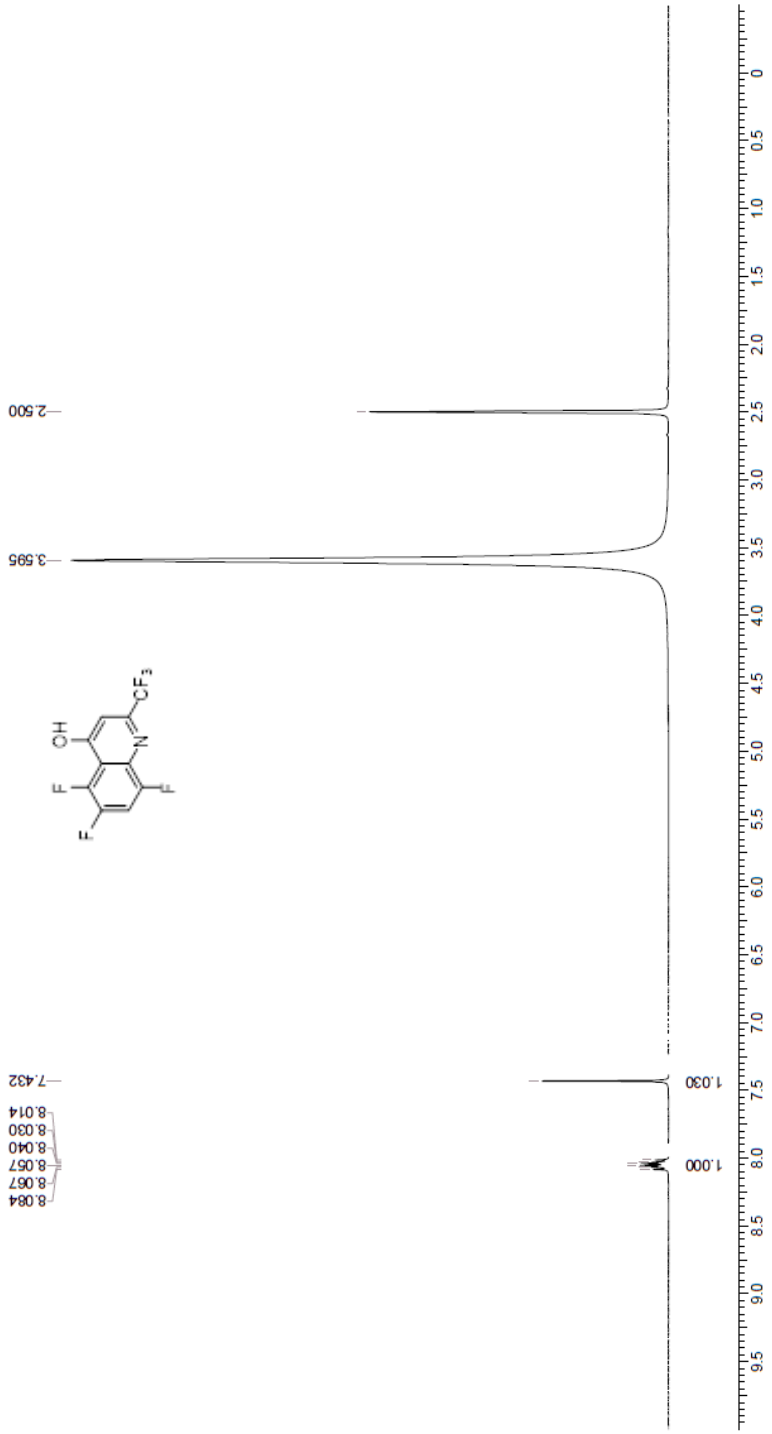
Compound 71 $^{1}$H NMR.



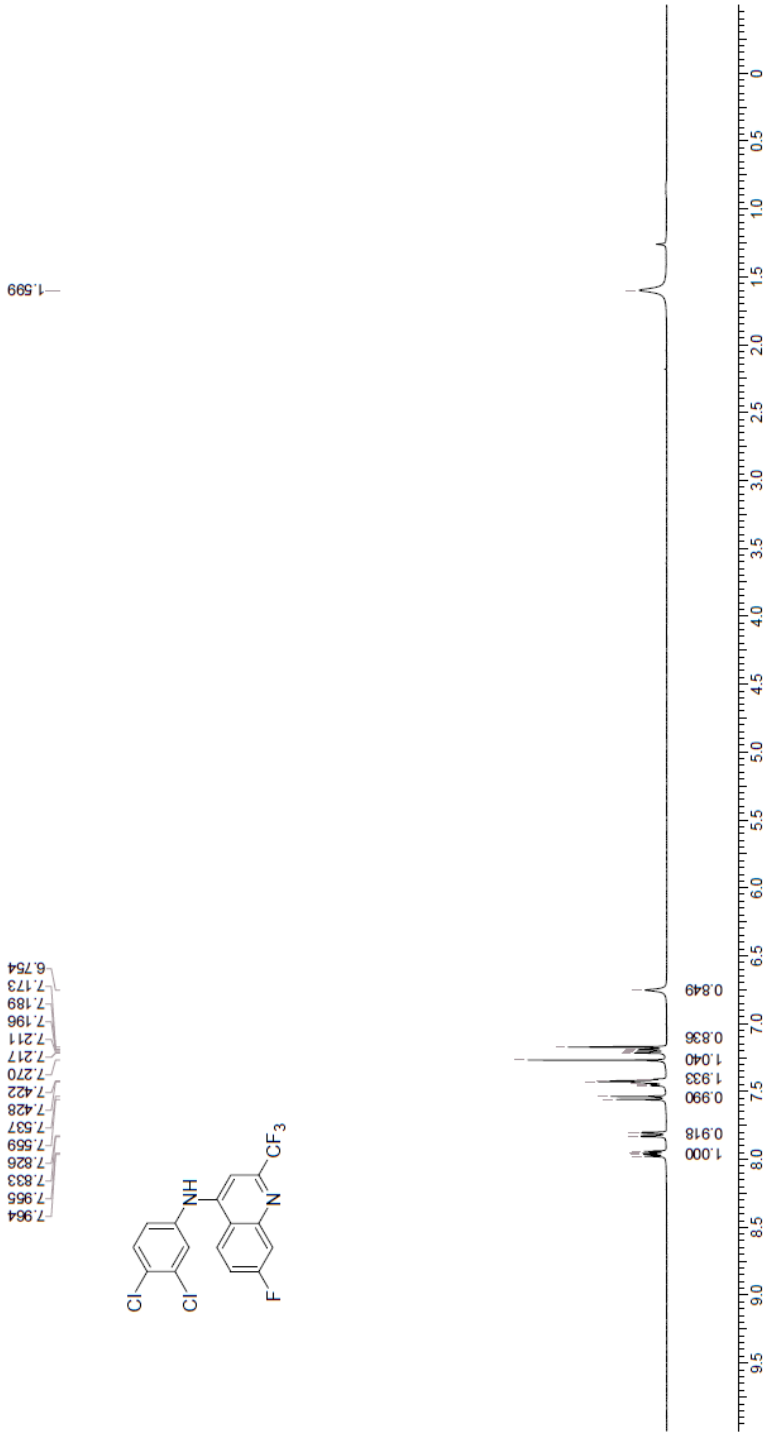
Compound 72 $^{1}H$ NMR.



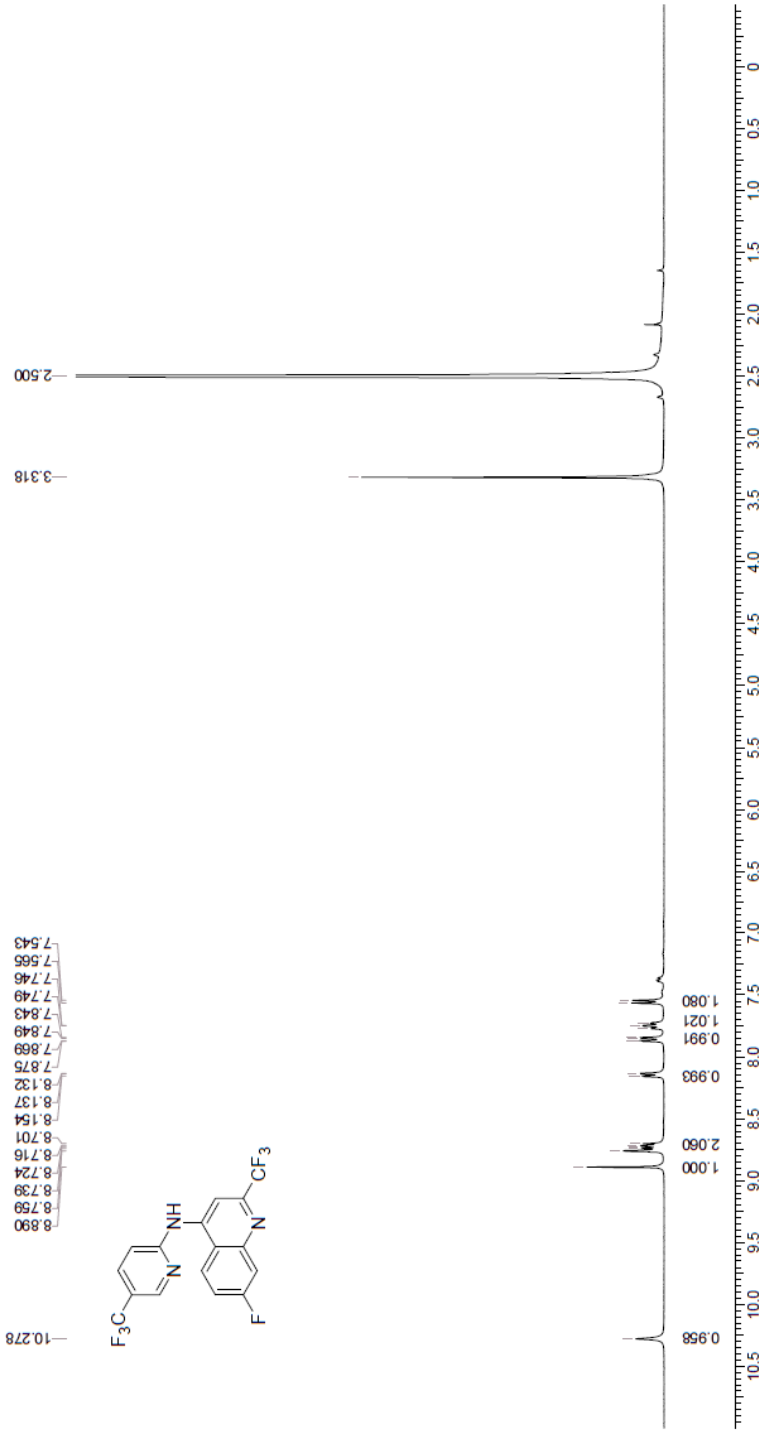
Compound 73 $^{1}$H NMR.



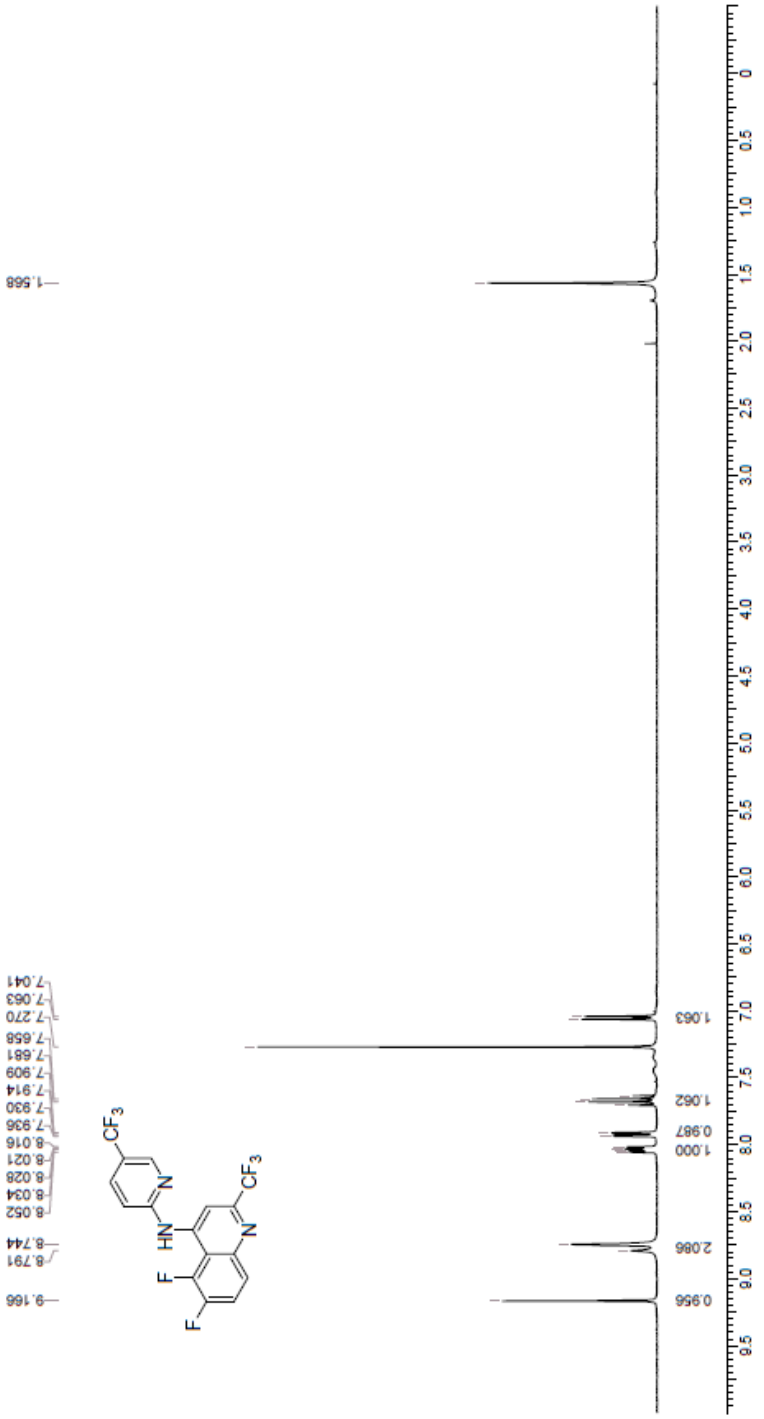
Compound 74 $^{1}$H NMR.



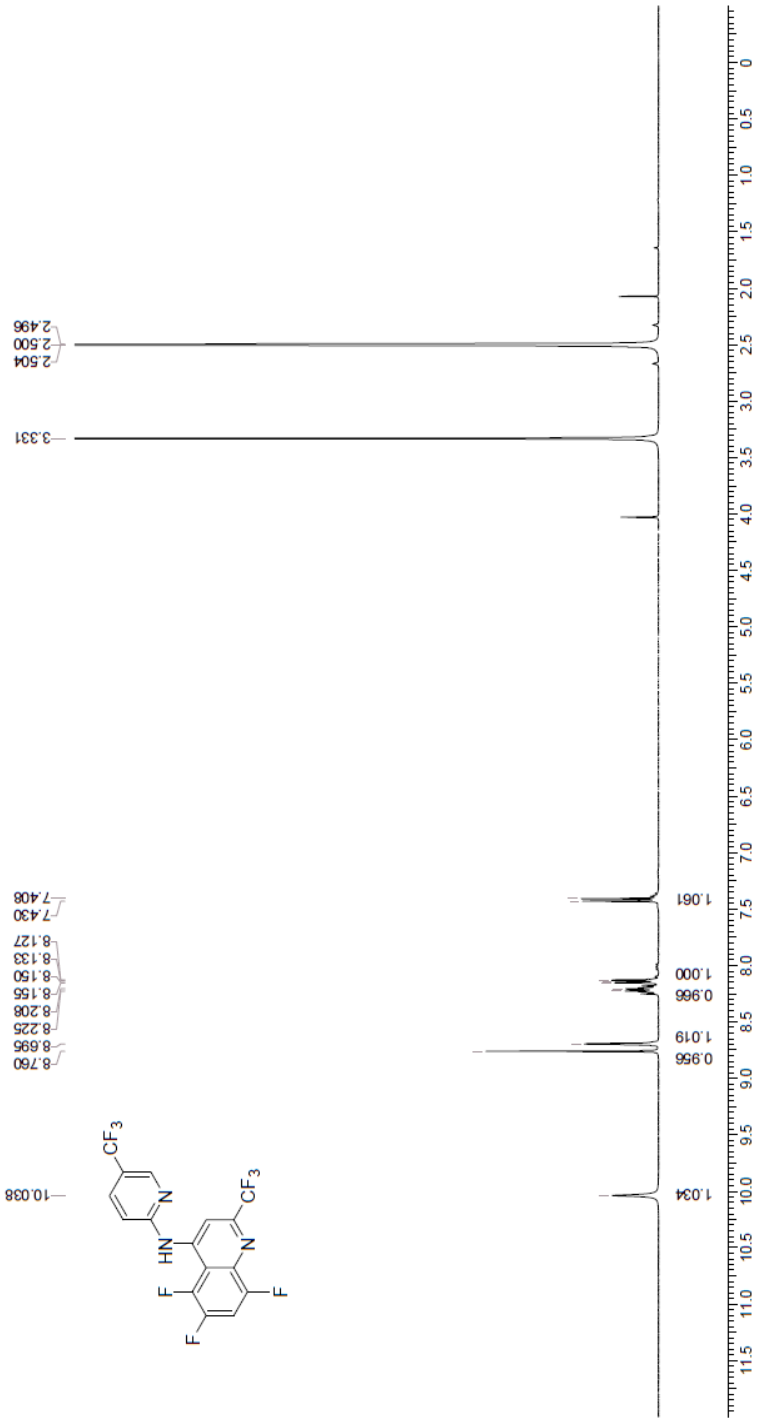
Compound 76 $^{1}$H NMR.



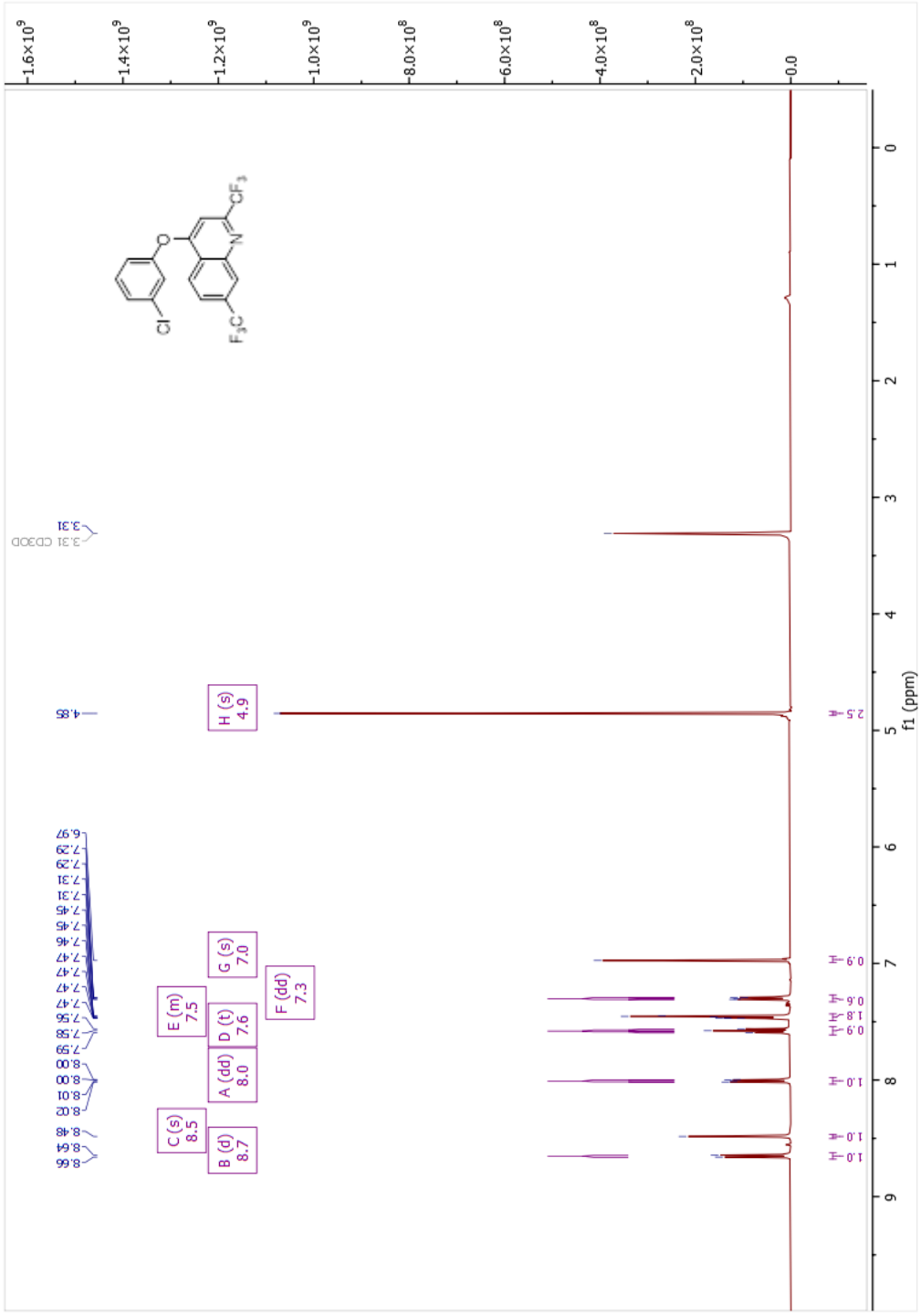
Compound 77 $^{1}$H NMR.



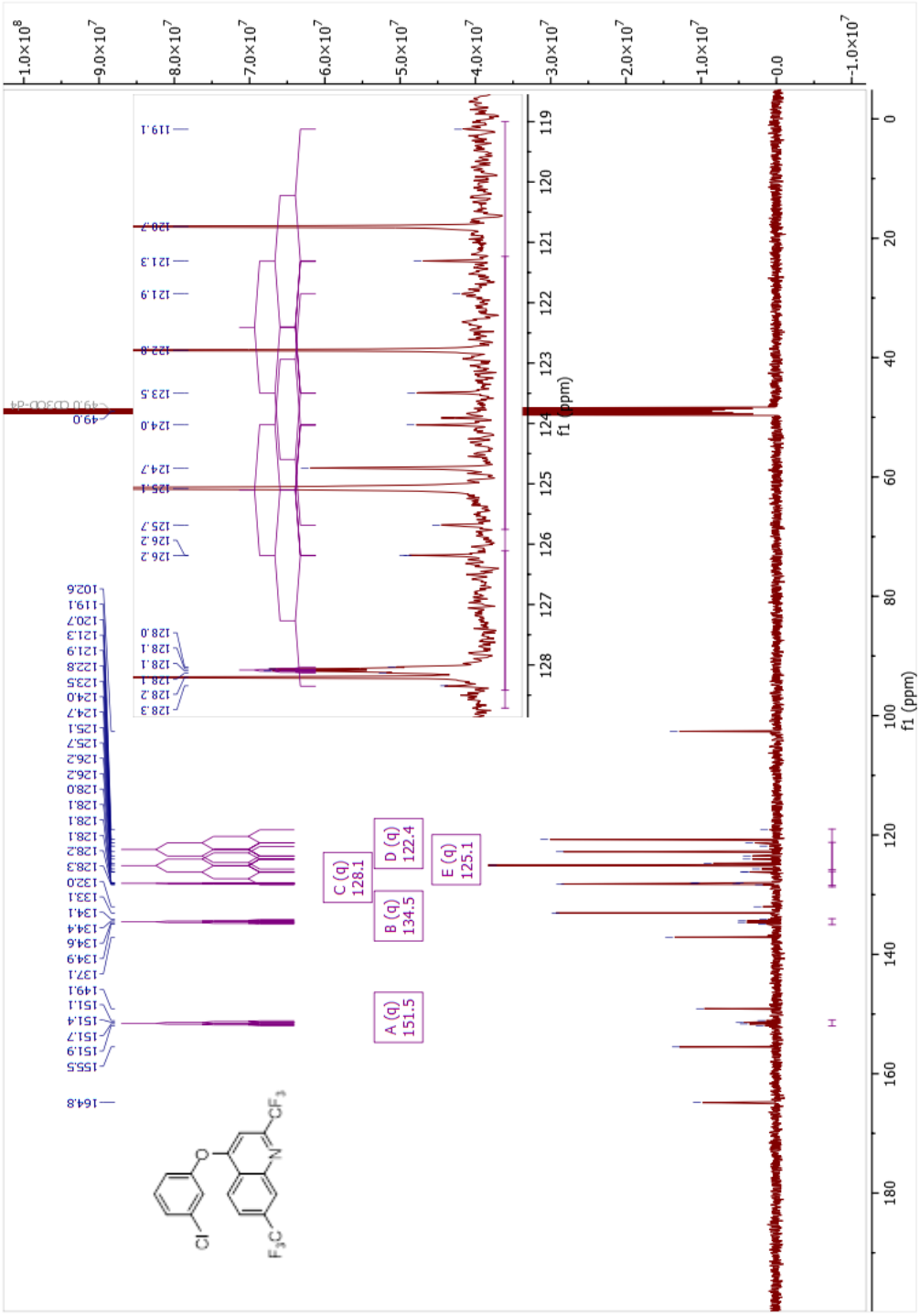
Compound 79 $^{1}$H NMR. **WX-X12Y03**



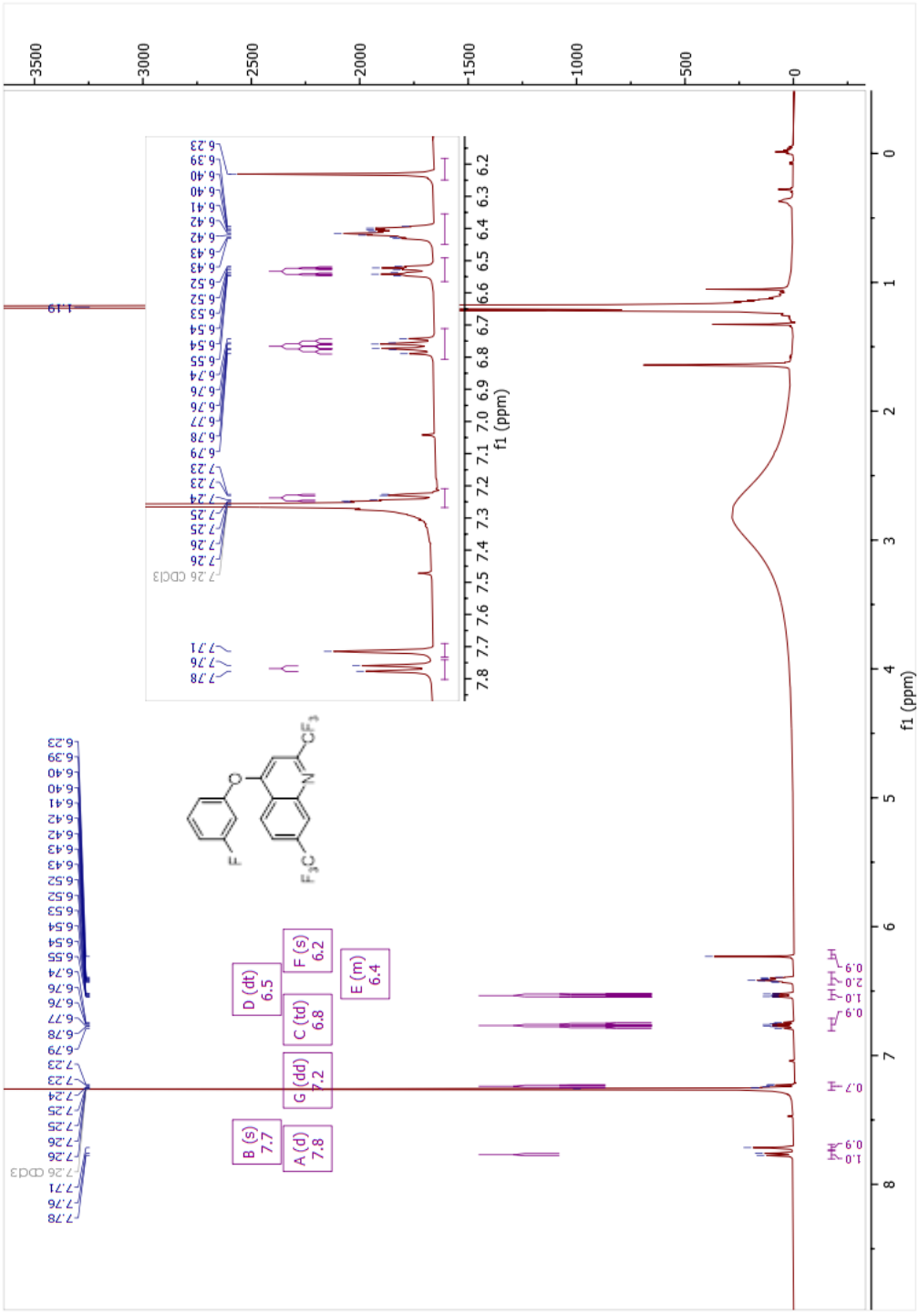
Compound 80 $^{1}$H NMR.



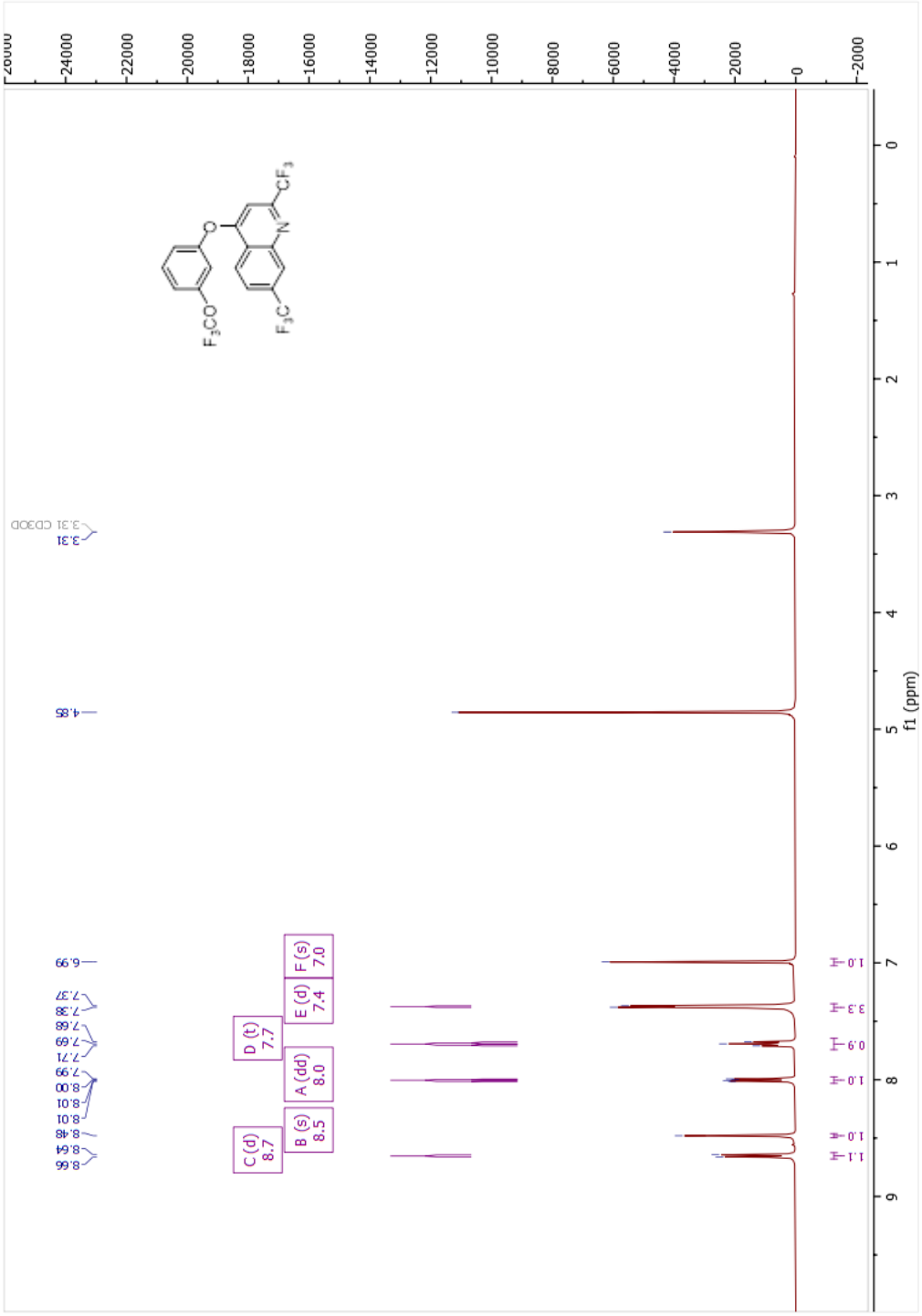
Compound 80 $^{13}C$ NMR.



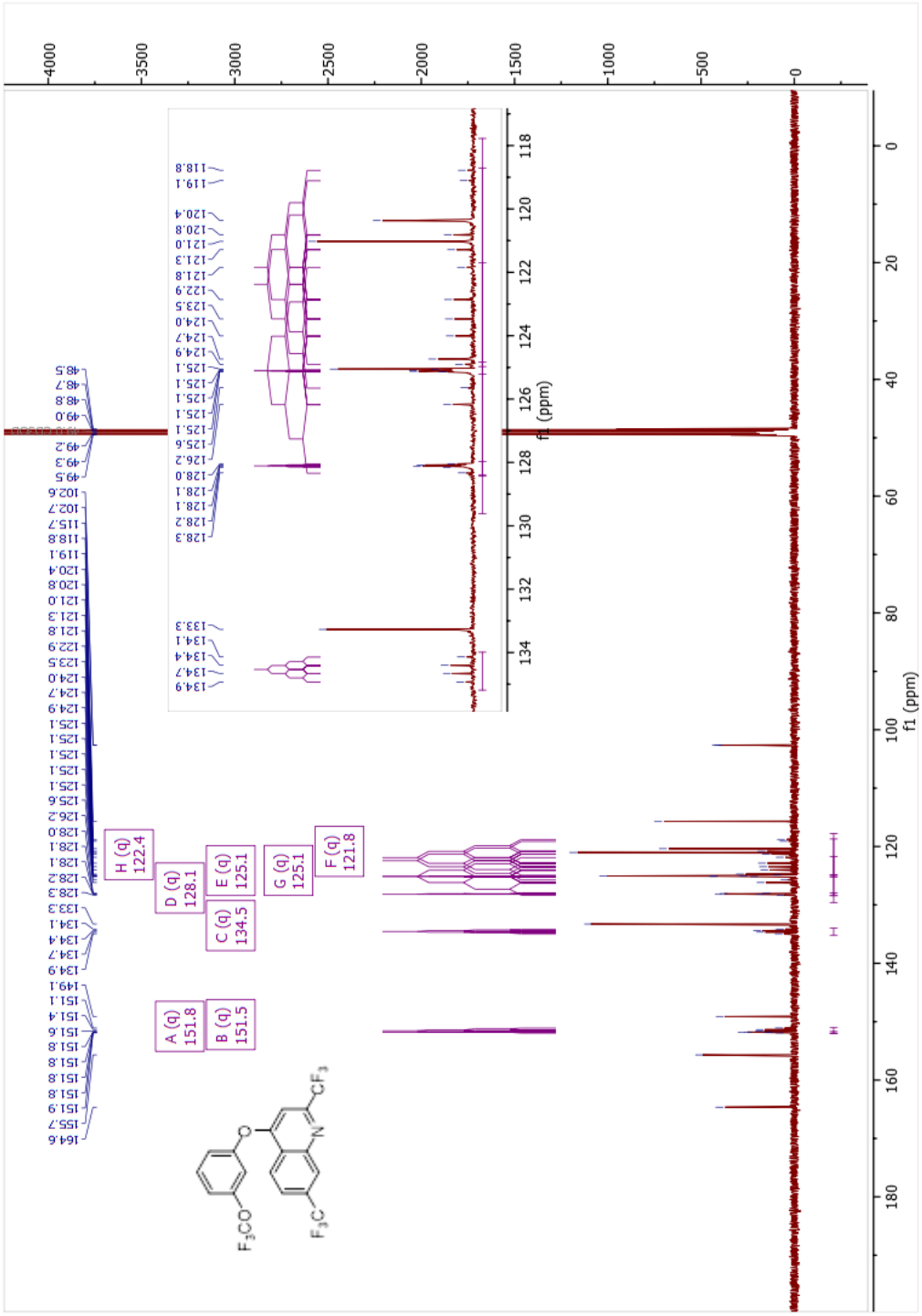
Compound 81 $^{1}$H NMR.



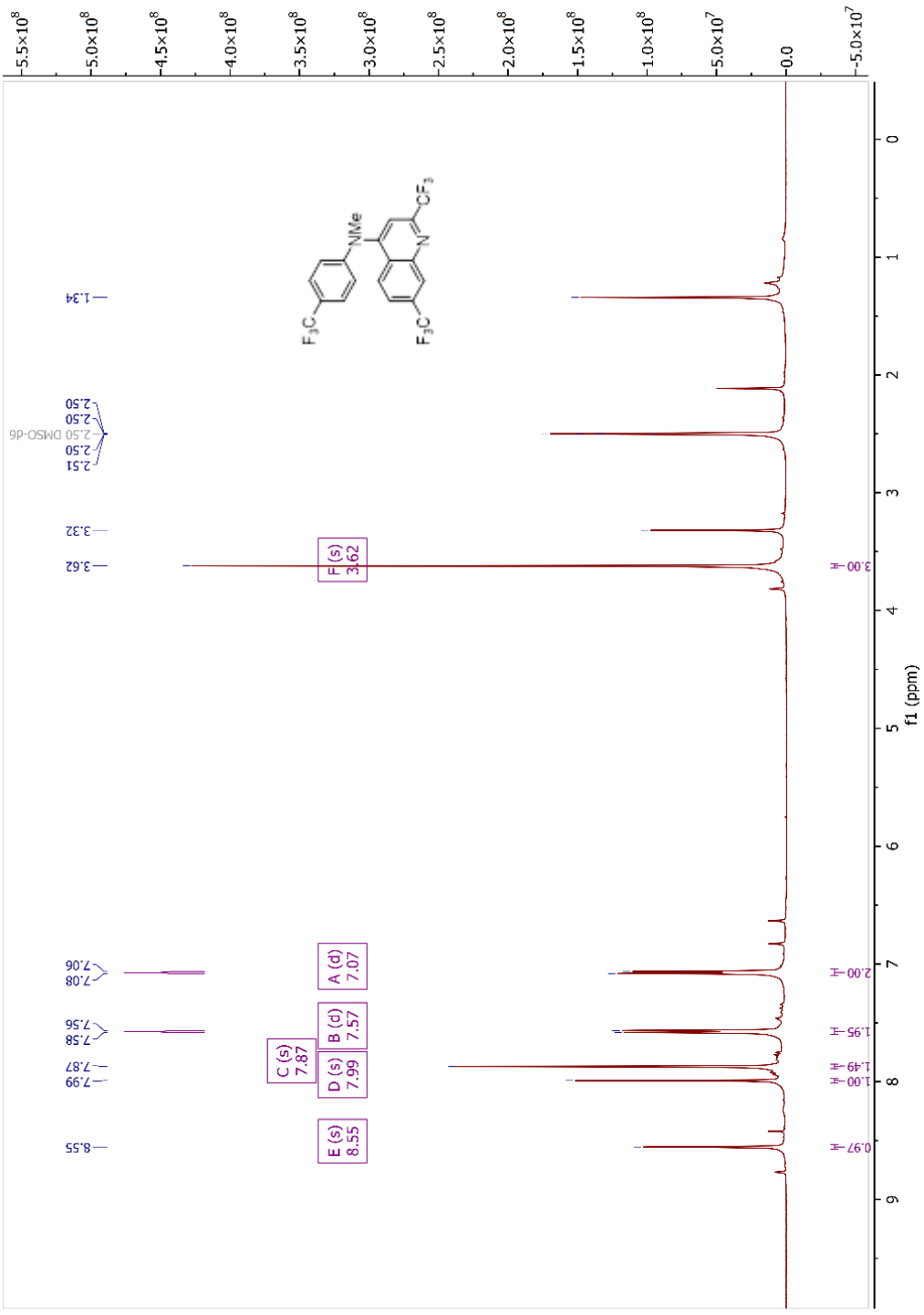
Compound 83 $^{1}$H NMR.



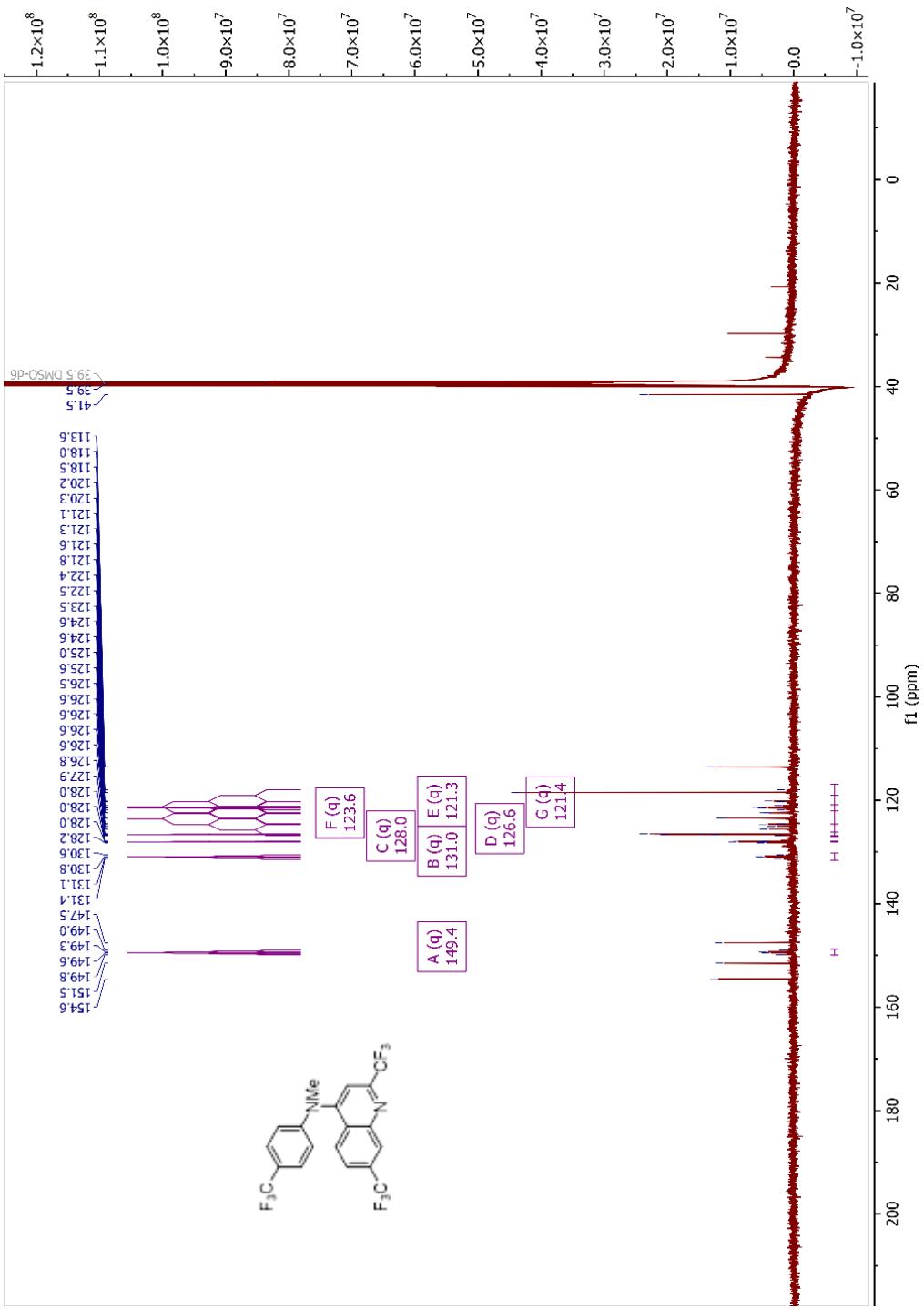
Compound 83 $^{13}C$ NMR.



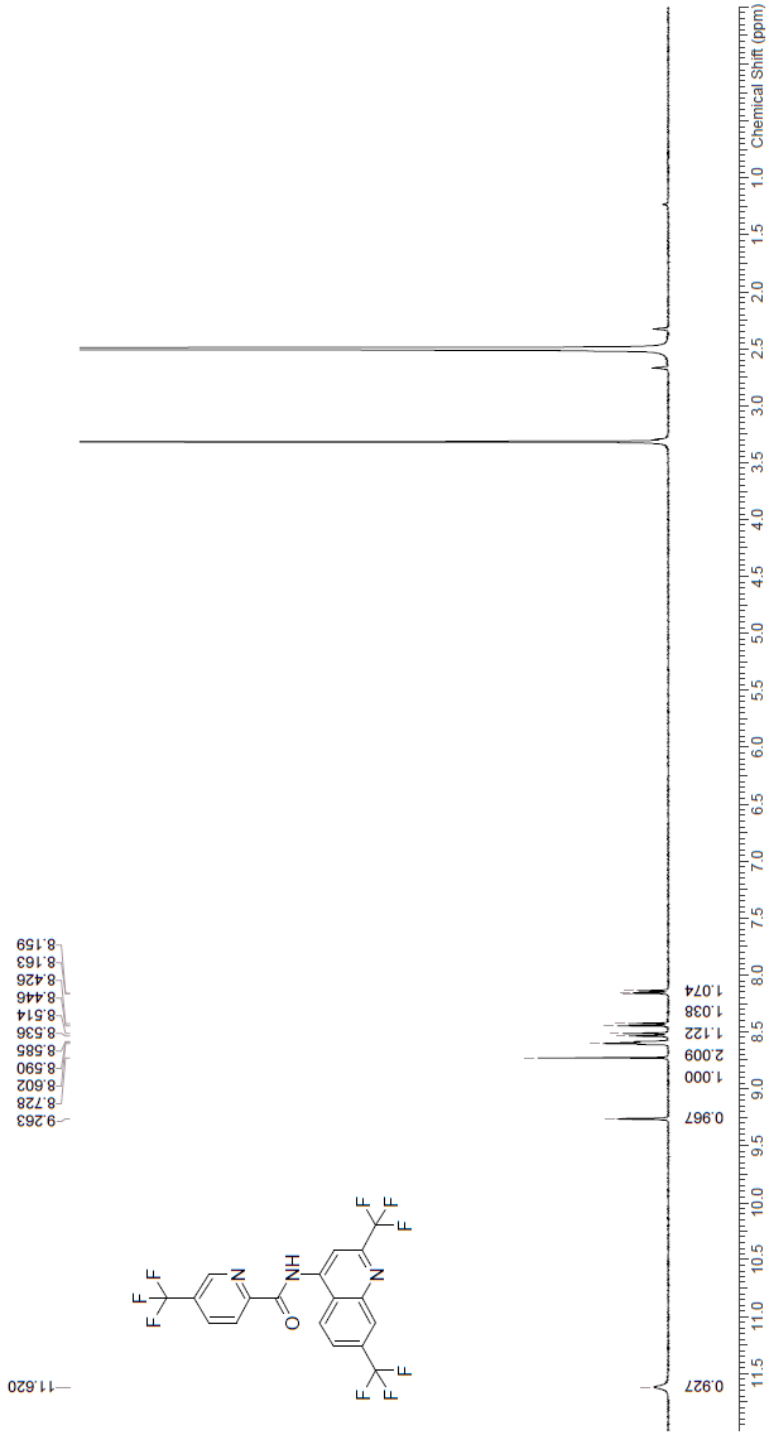
Compound 84 $^{1}$H NMR.



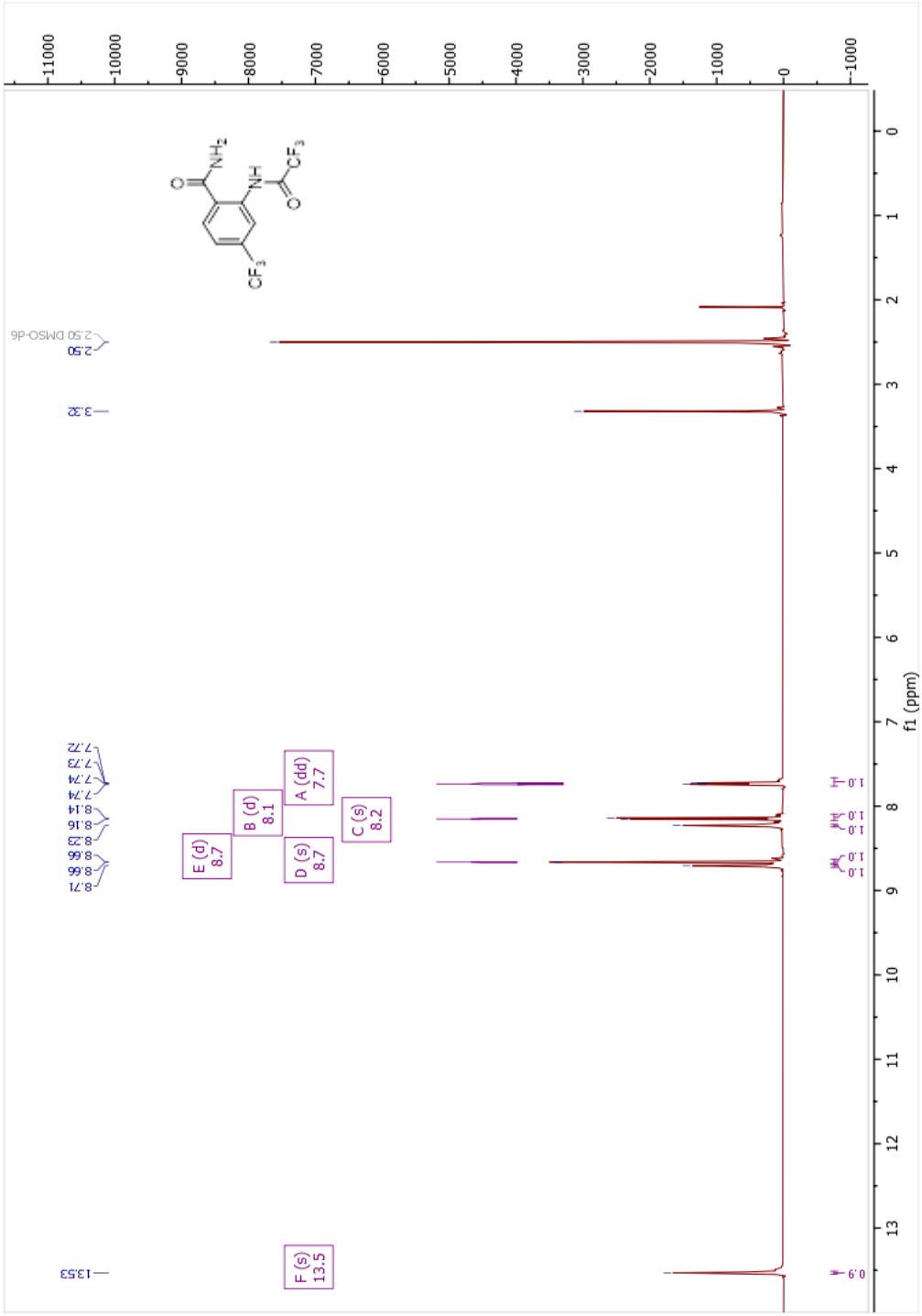
Compound 84 $^{13}C$ NMR.



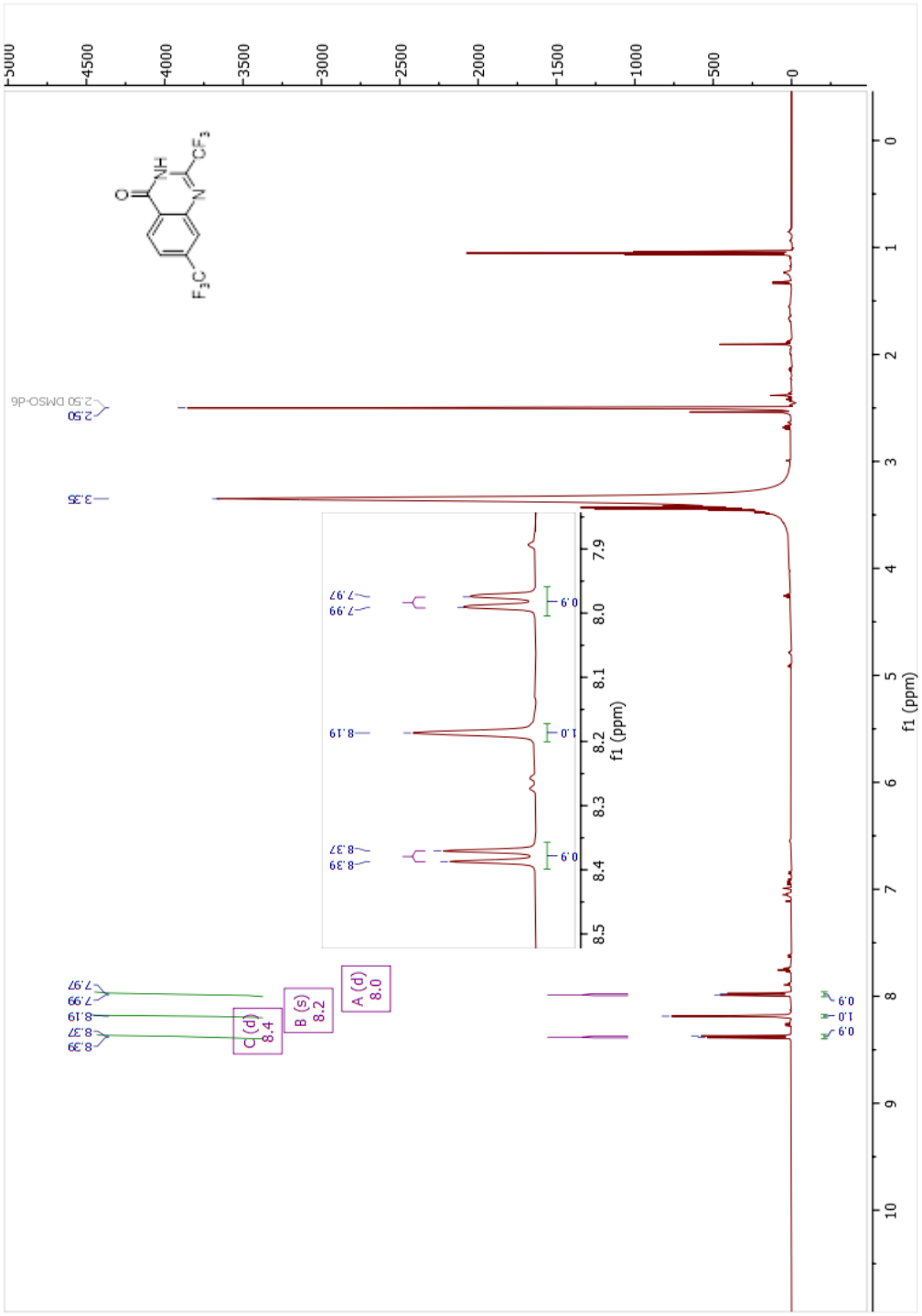
Compound 85 $^{1}$H NMR.



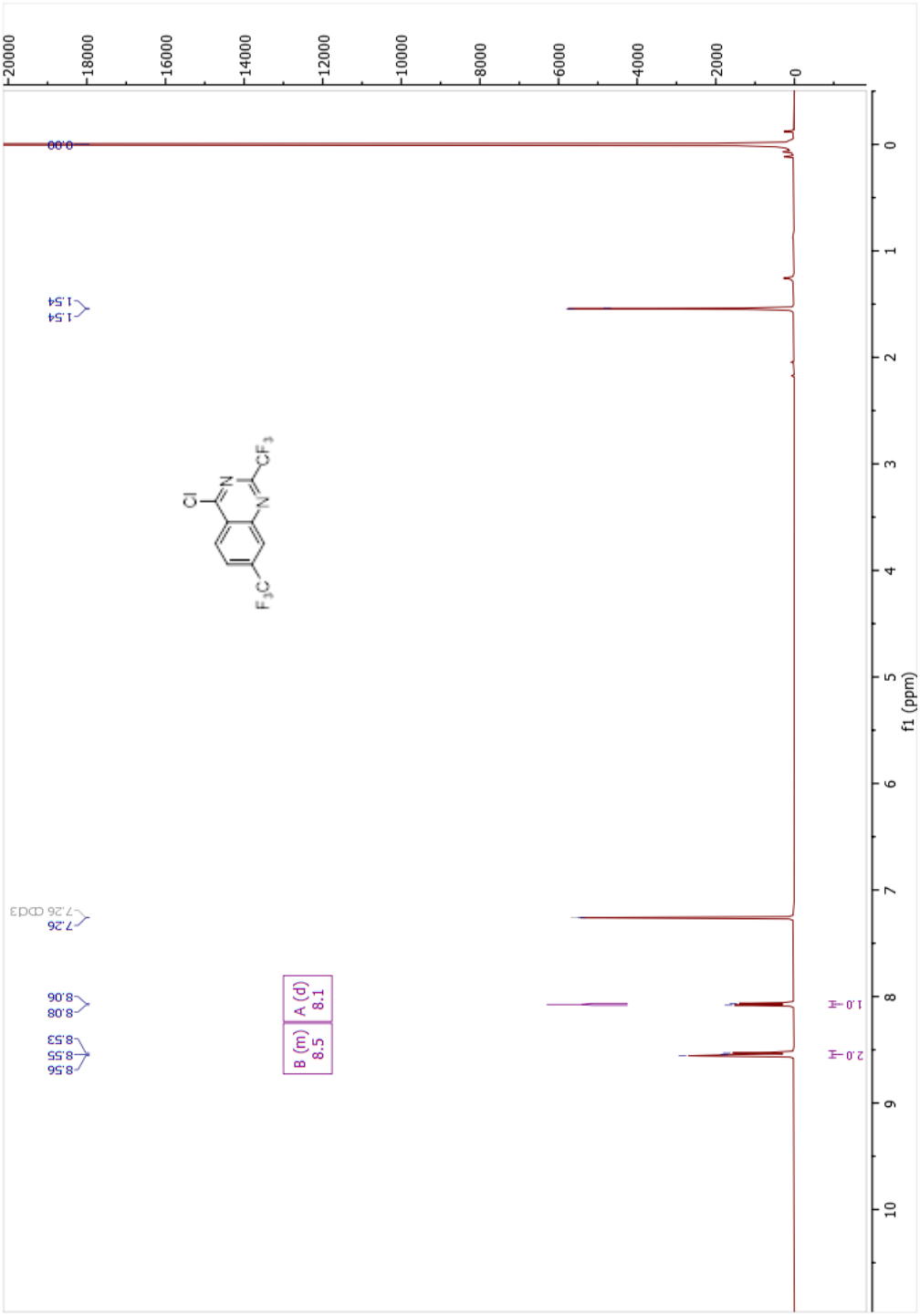
2-(2,2,2-trifluoroacetamido)-4-(trifluoromethyl)benzamide $^{1}$H NMR. **JRS-7-92**



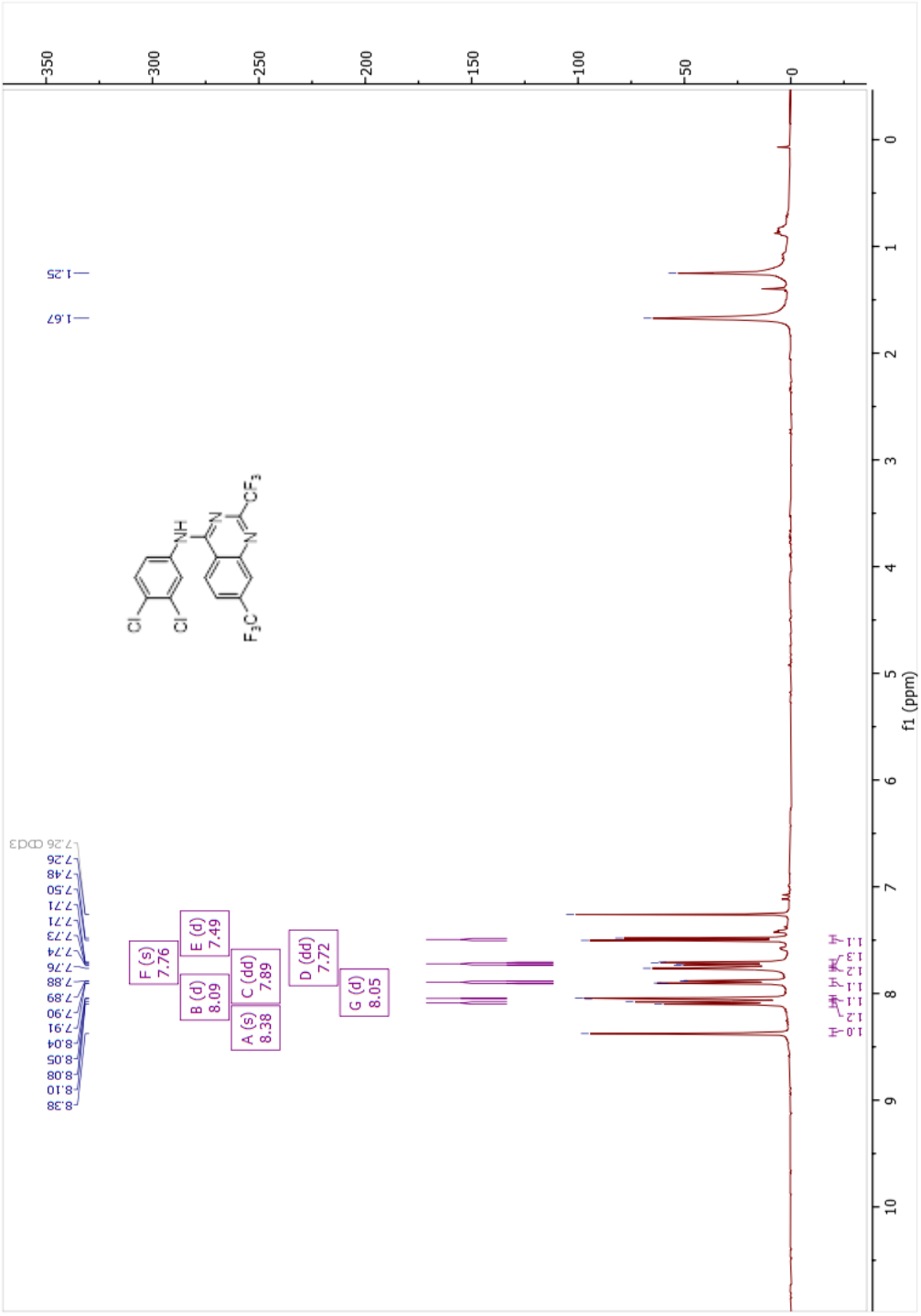
Compound 87 $^{1}H$ NMR.



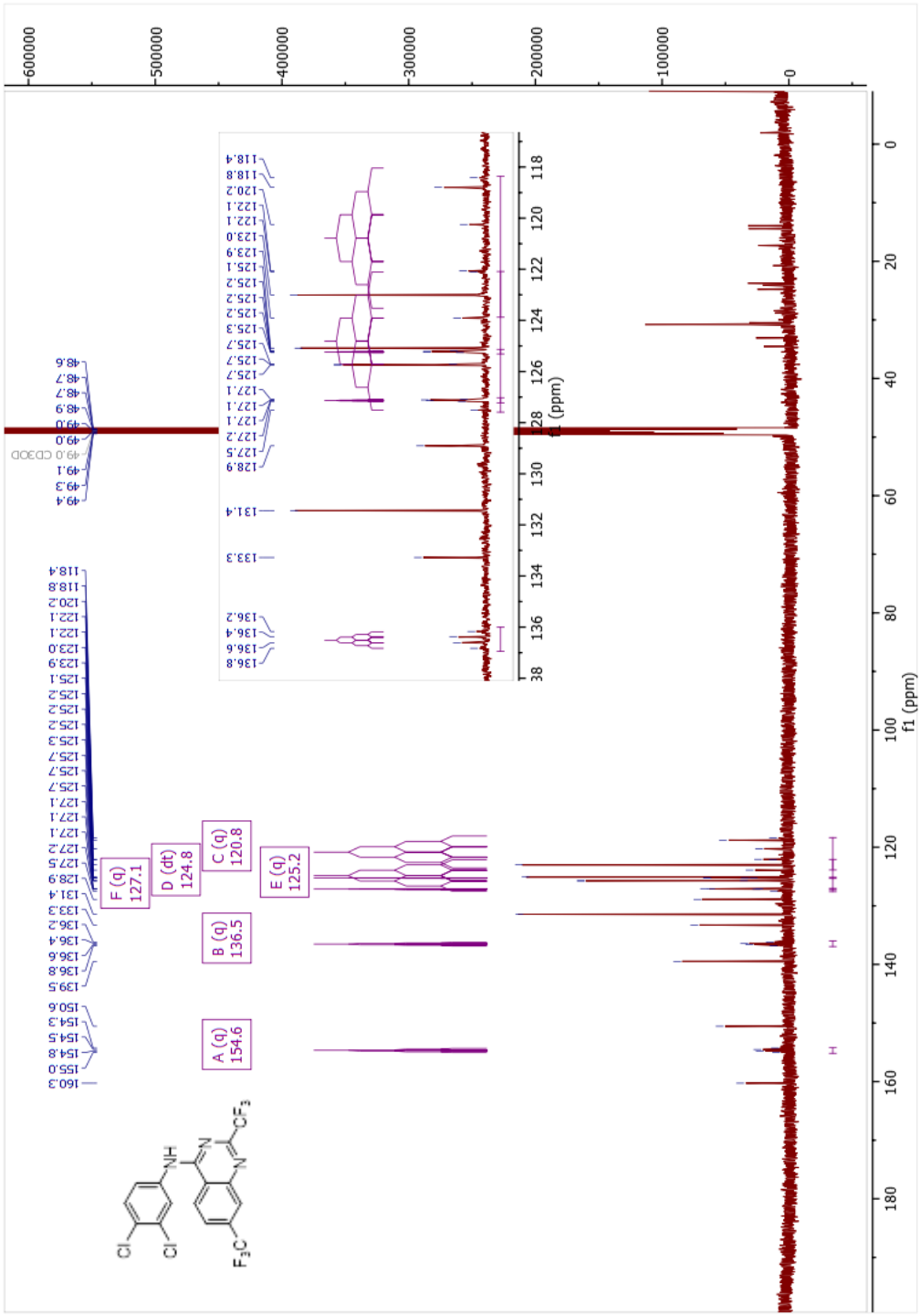
Compound 88 $^{1}$H NMR.



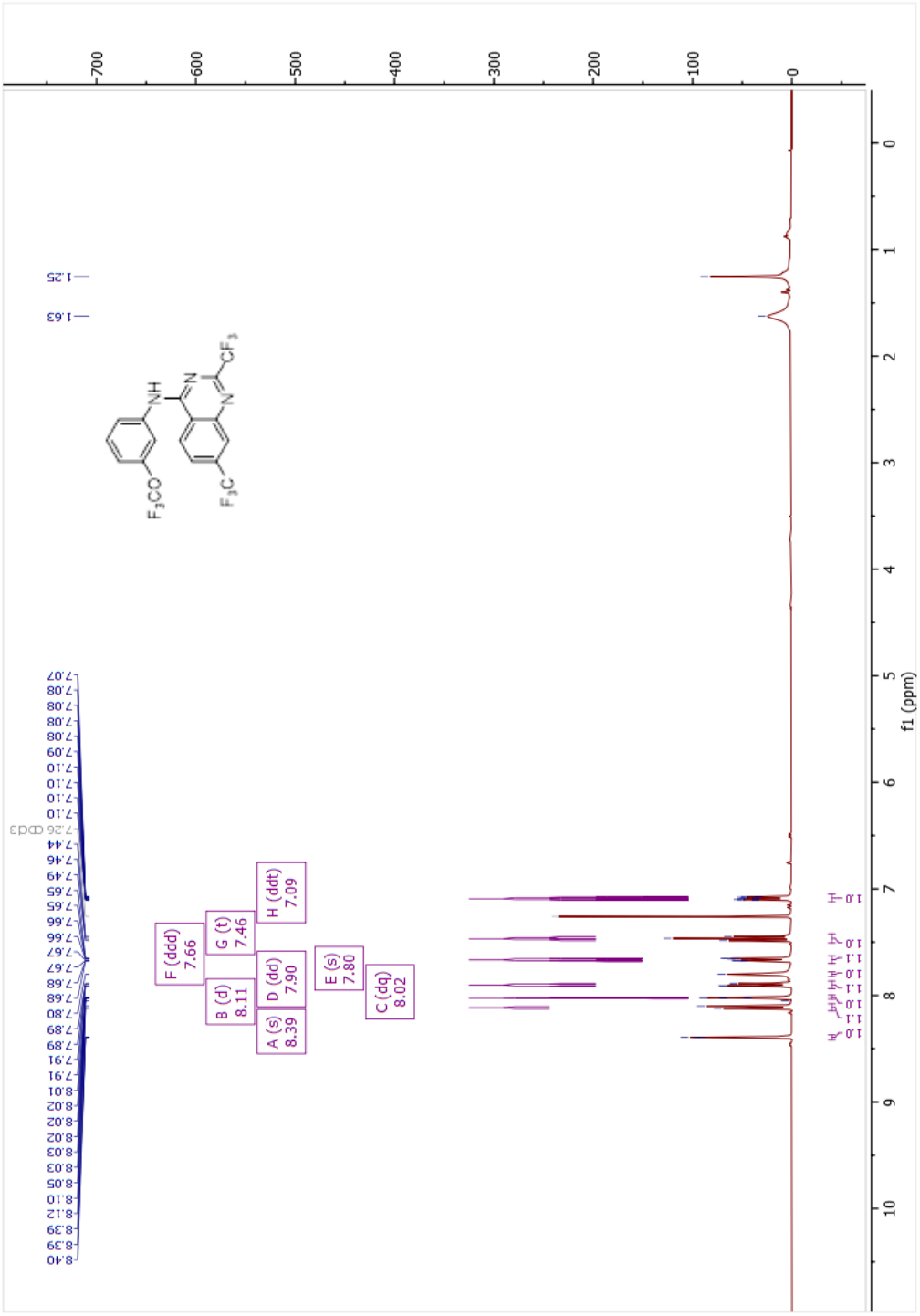
Compound 89 $^{1}$H NMR.



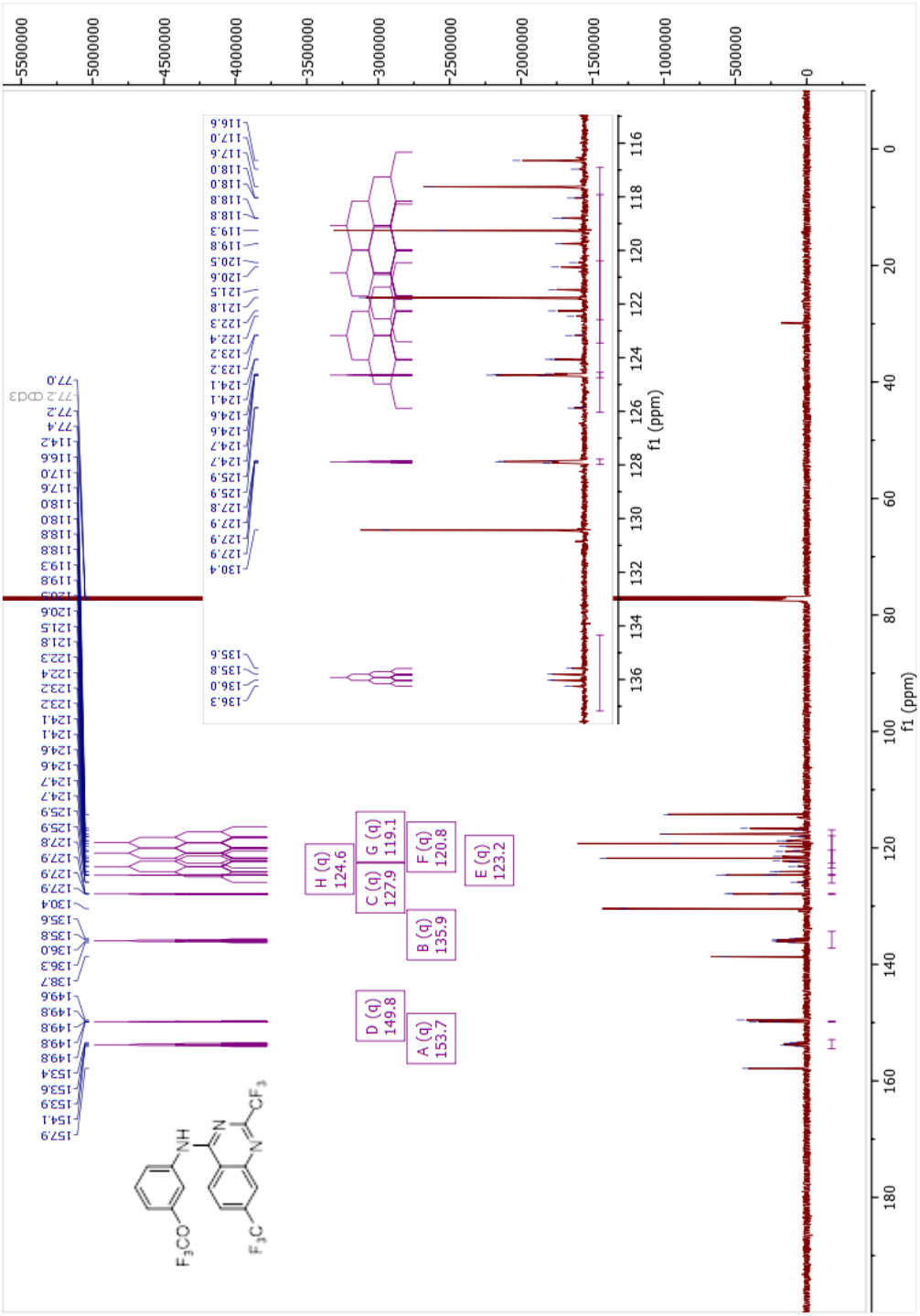
Compound 89 $^{13}C$ NMR.



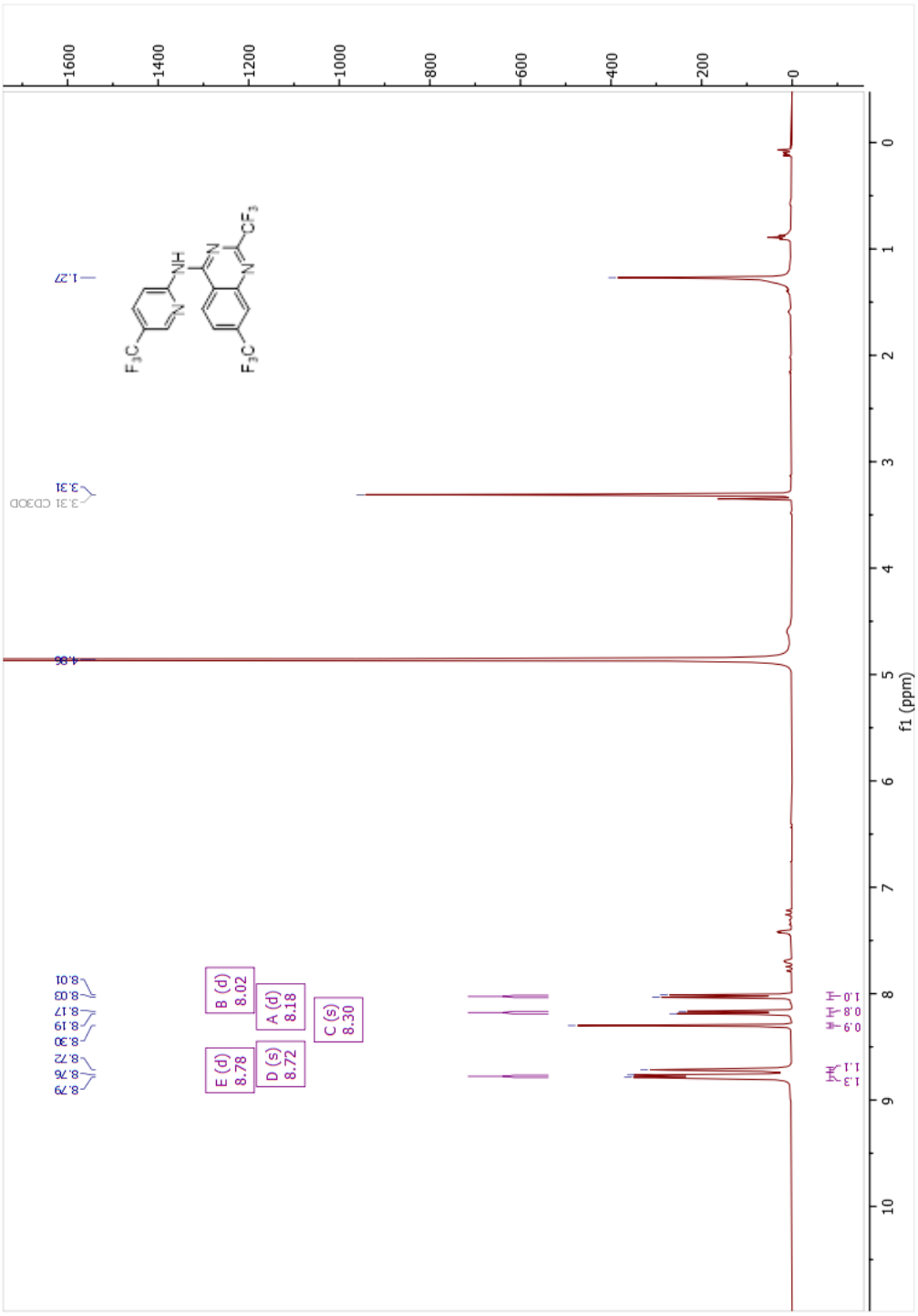
Compound 90 $^{1}$H NMR.



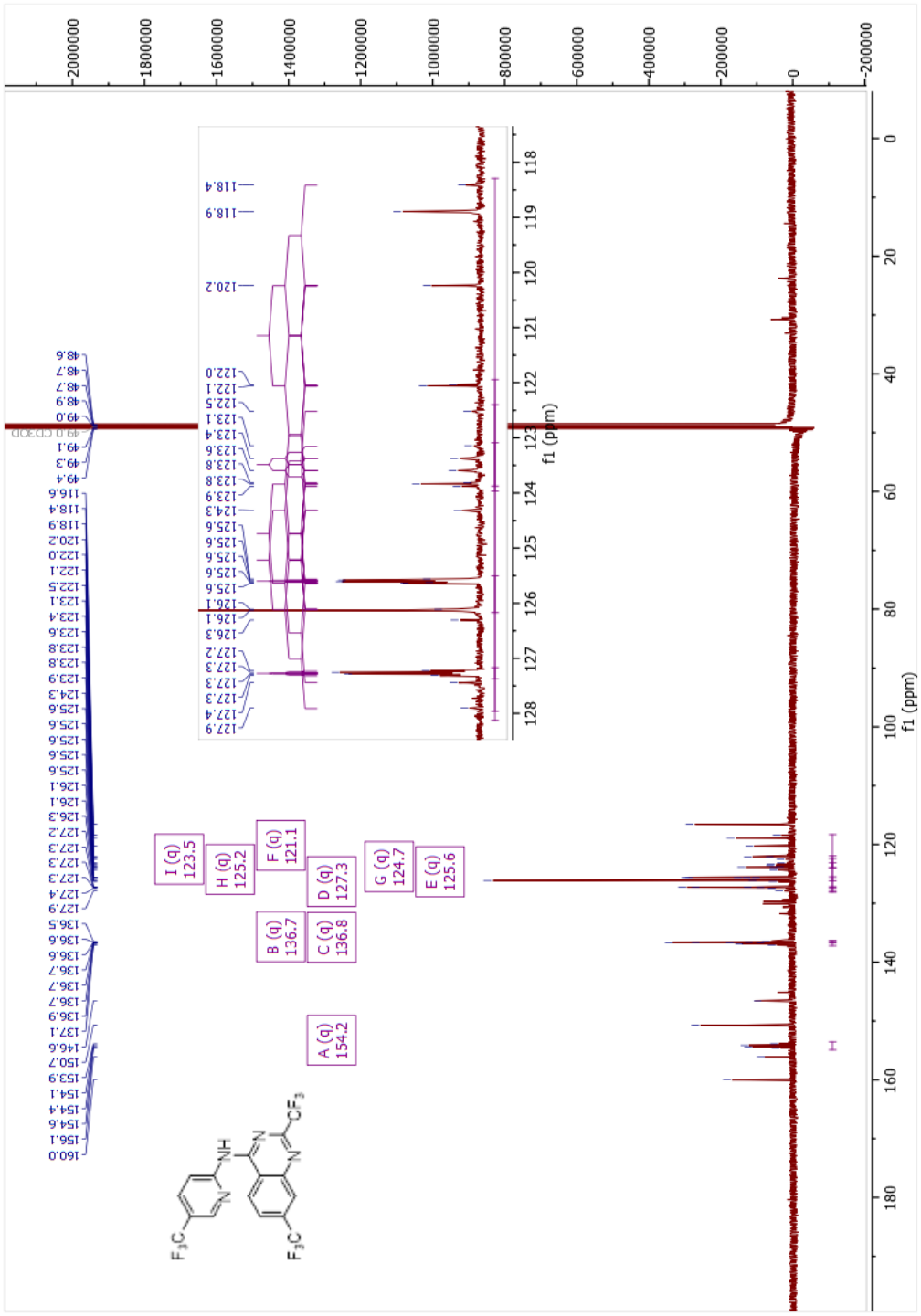
Compound 90 $^{13}C$ NMR.



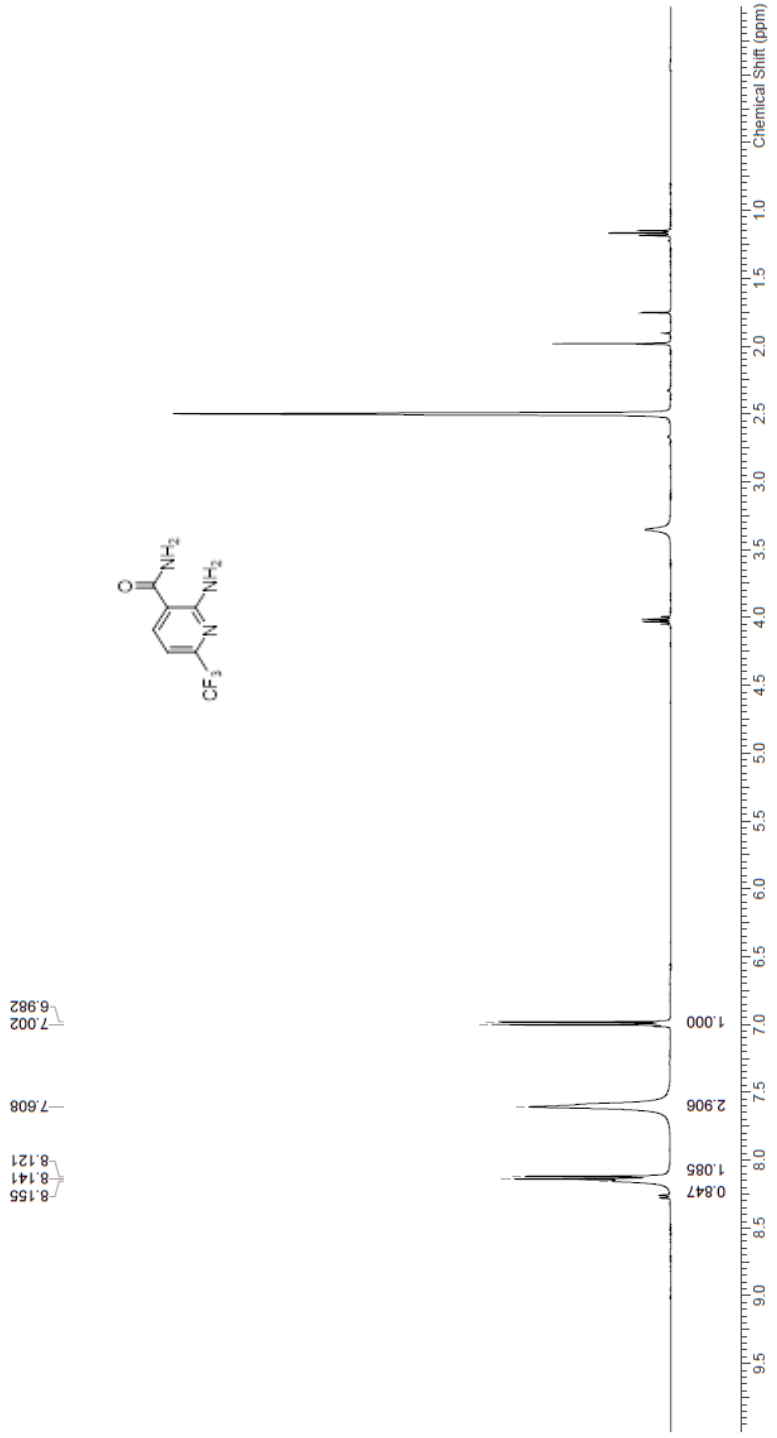
Compound 91 $^{1}$H NMR.



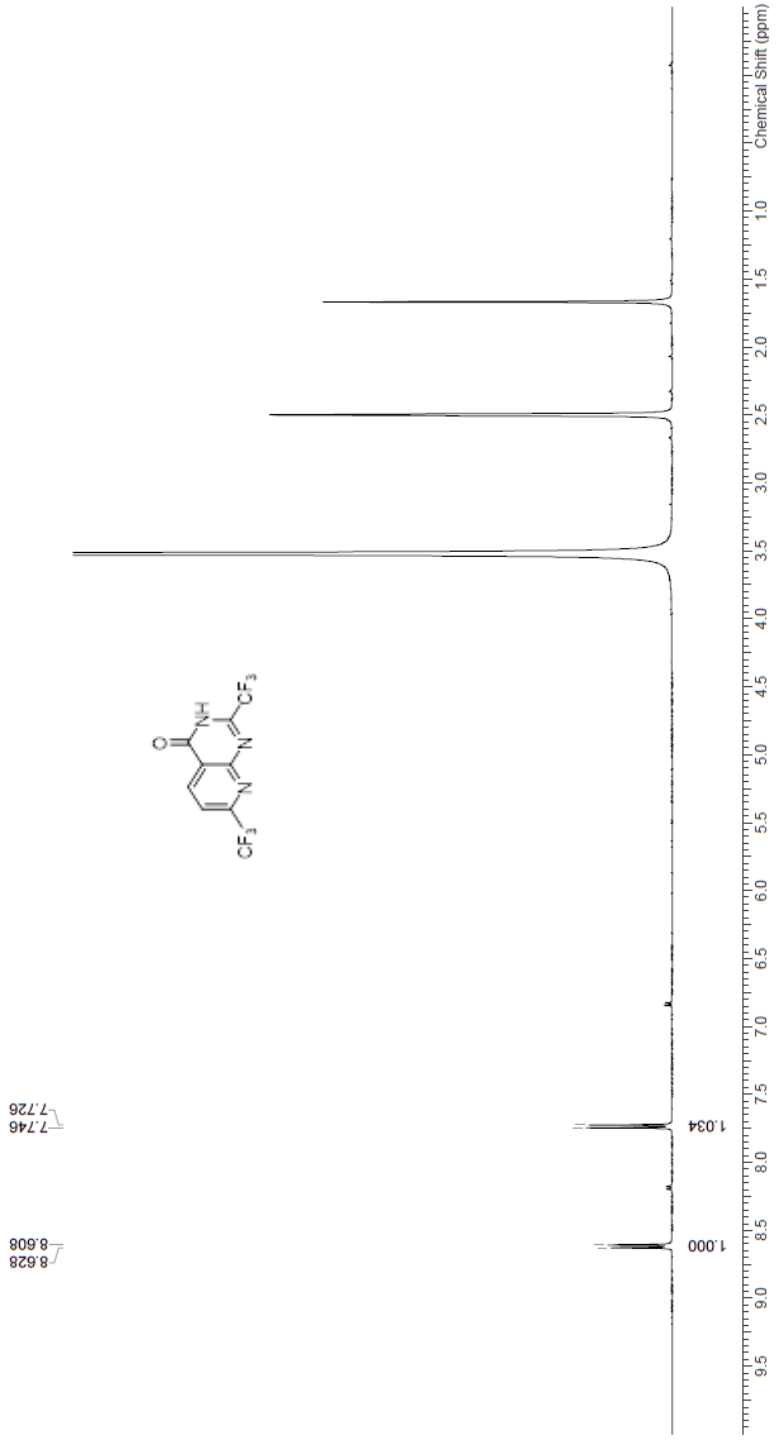
Compound 91 $^{13}C$ NMR.



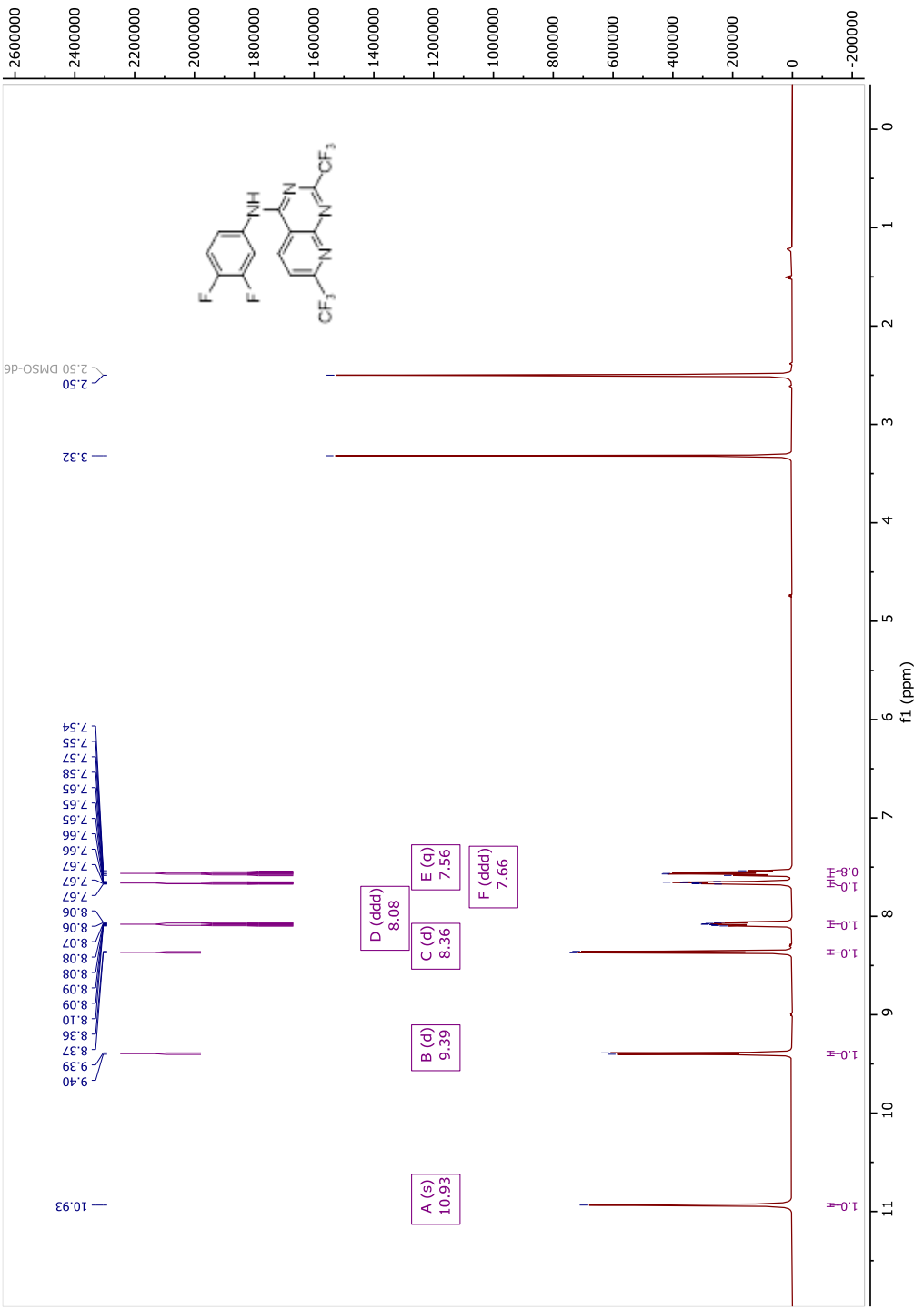
Compound 92 $^{1}H$ NMR.



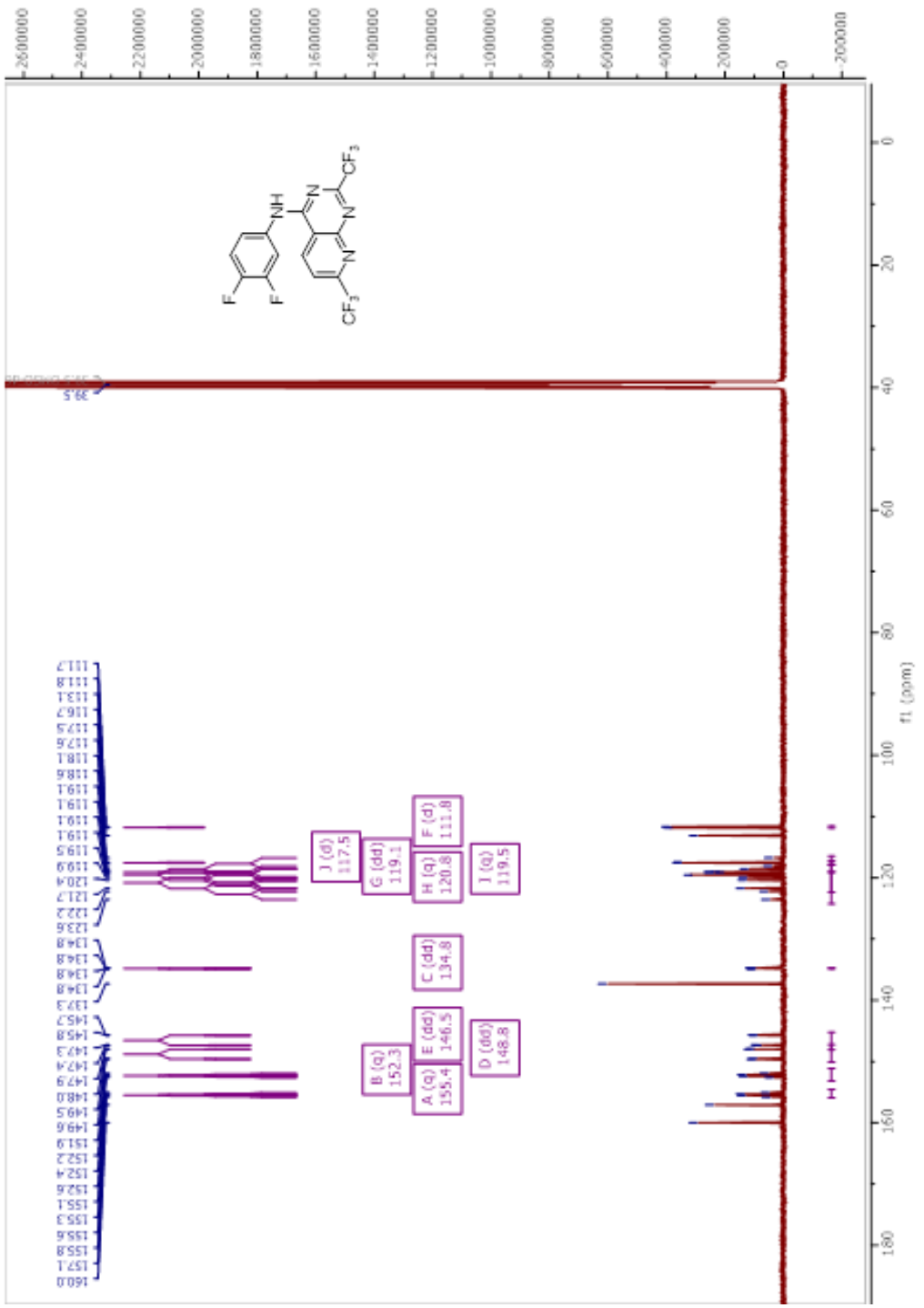
Compound 93 $^{1}$H NMR.



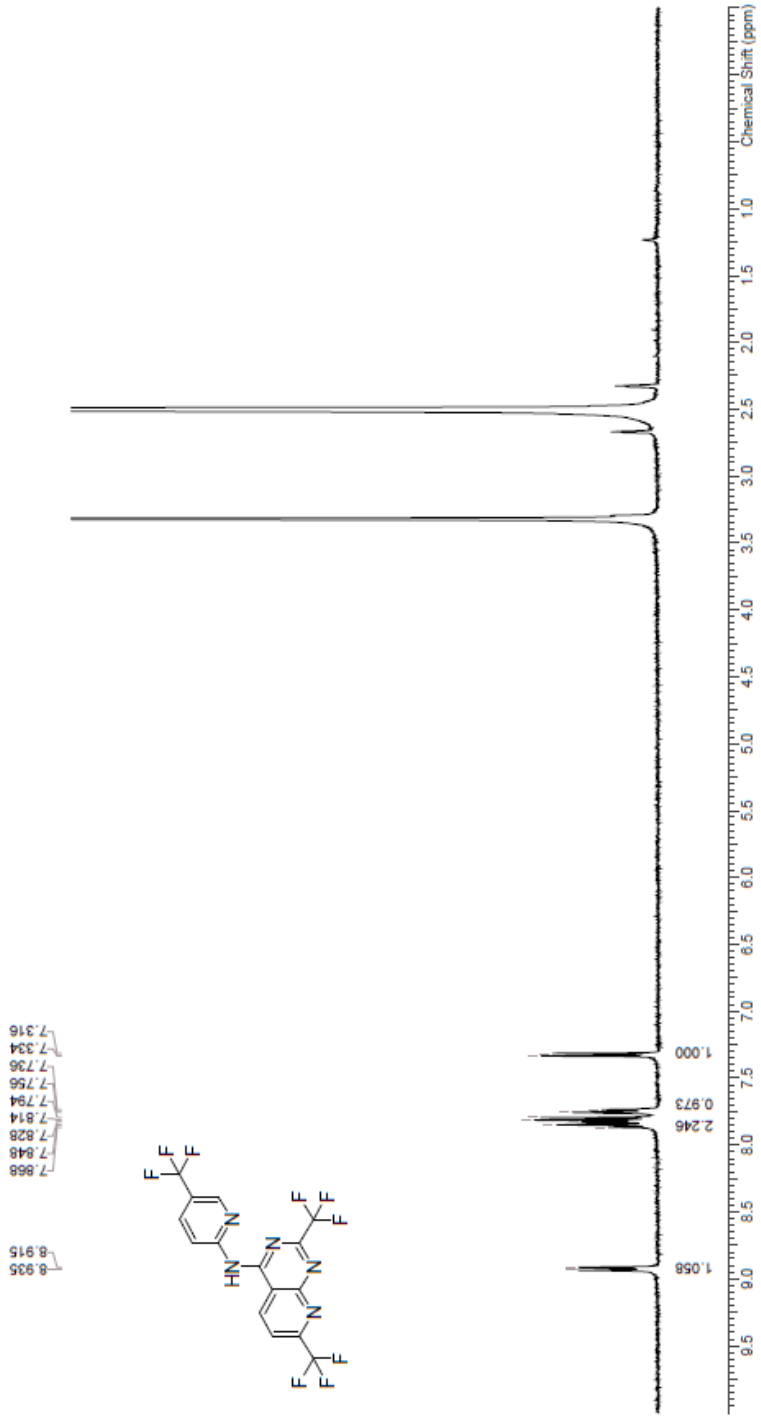
Compound 94 $^{1}$H NMR.



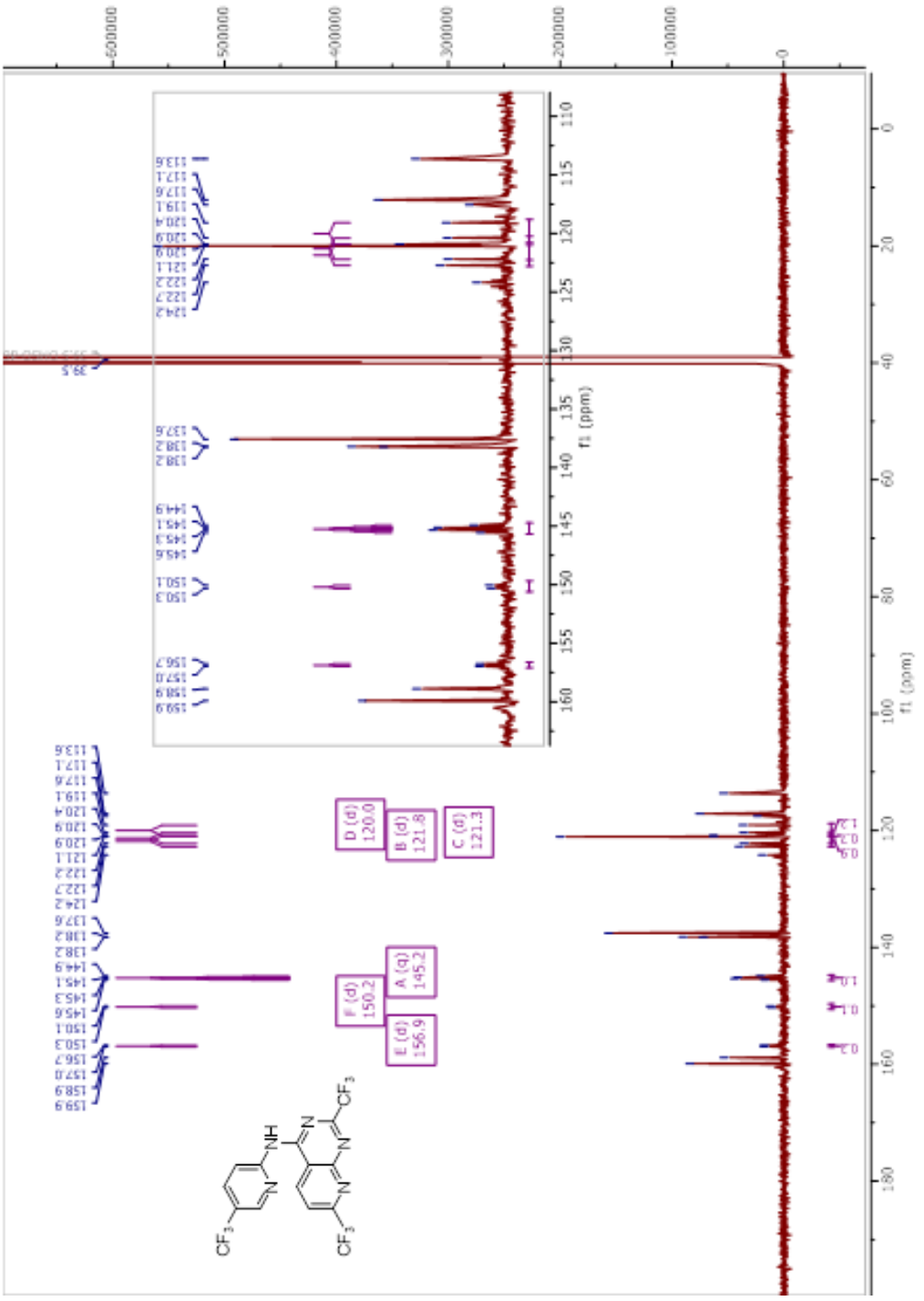
Compound 94 $^{13}C$ NMR.



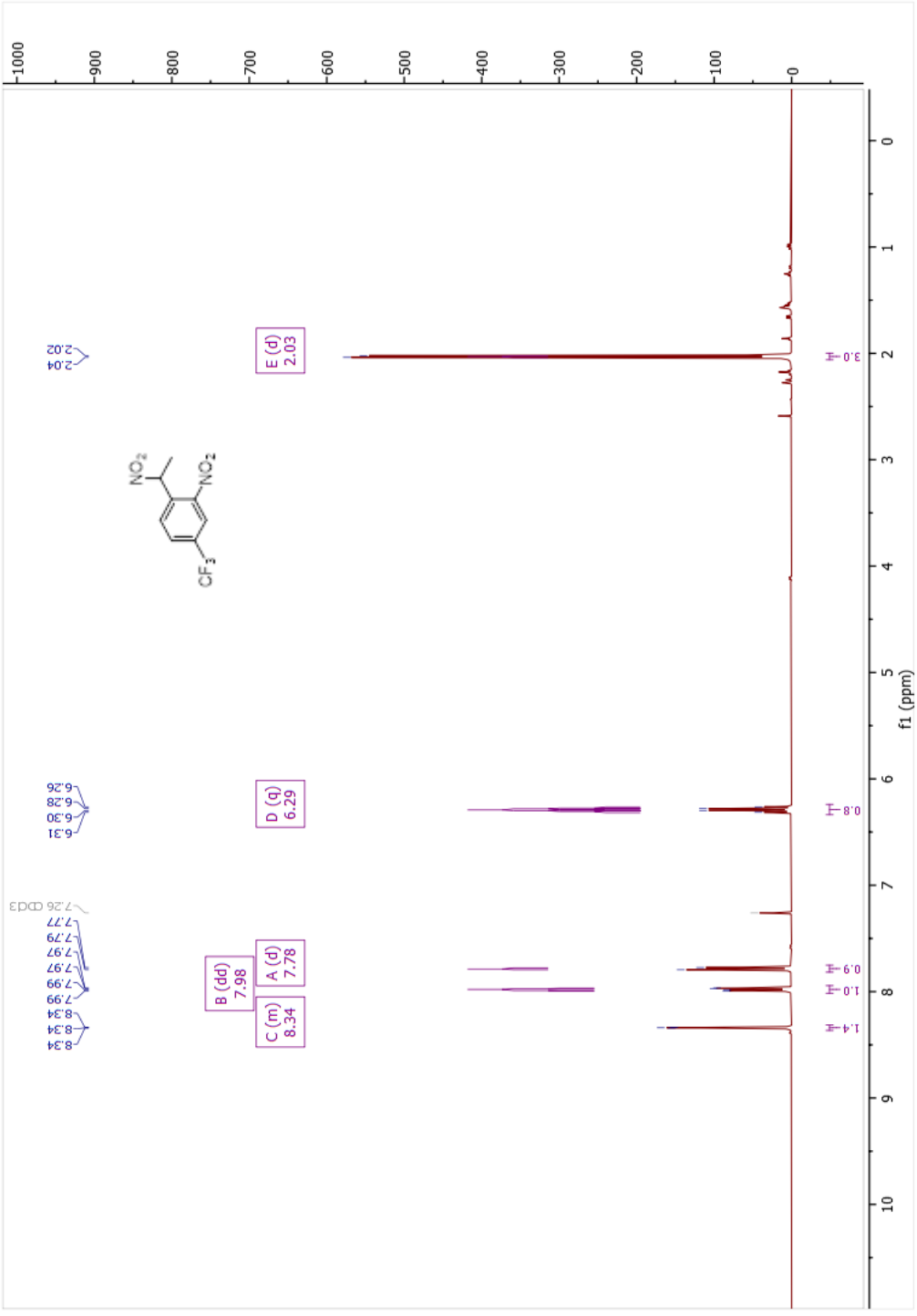
Compound 95 $^{1}$H NMR.



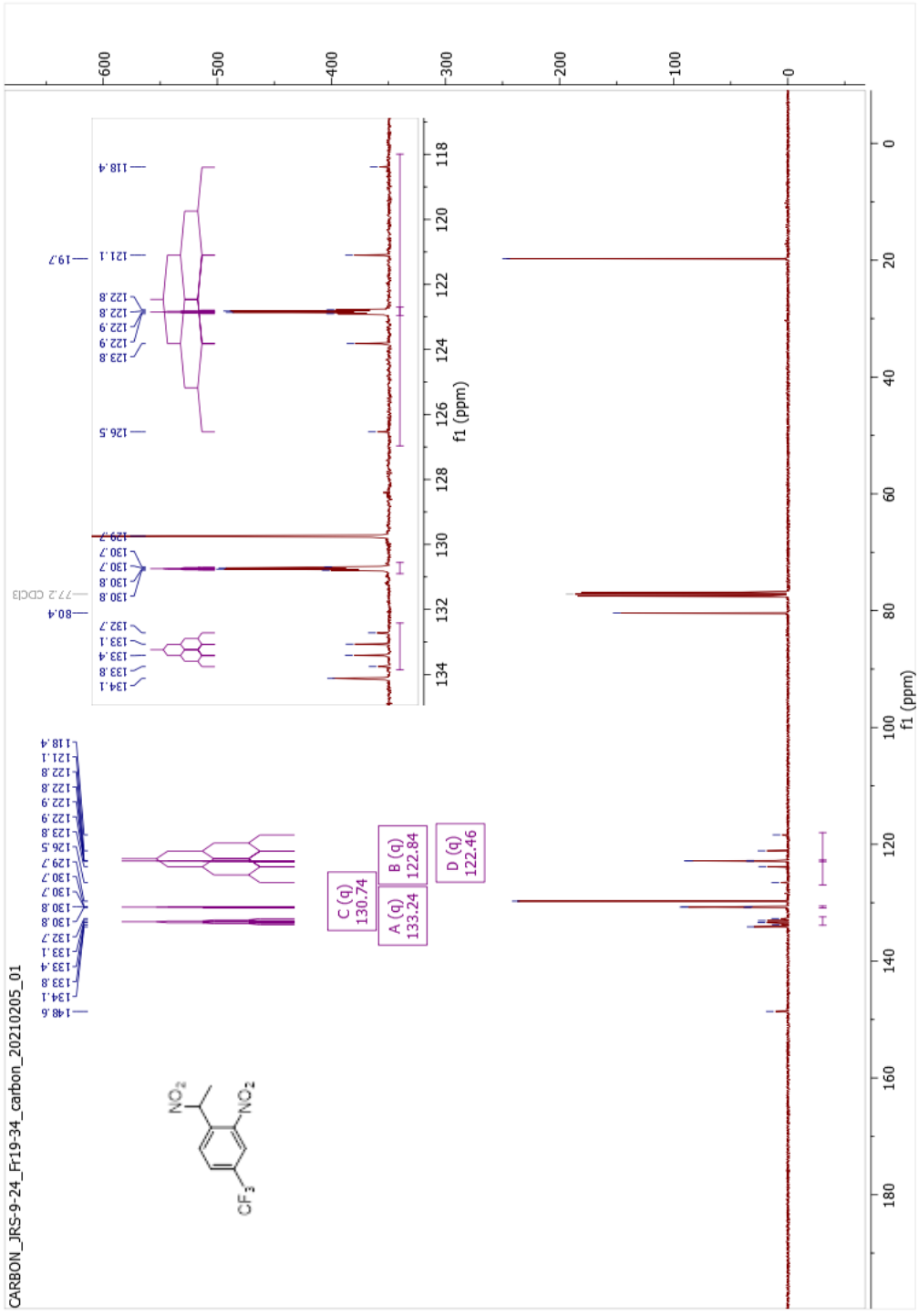
Compound 95 $^{13}C$ NMR.



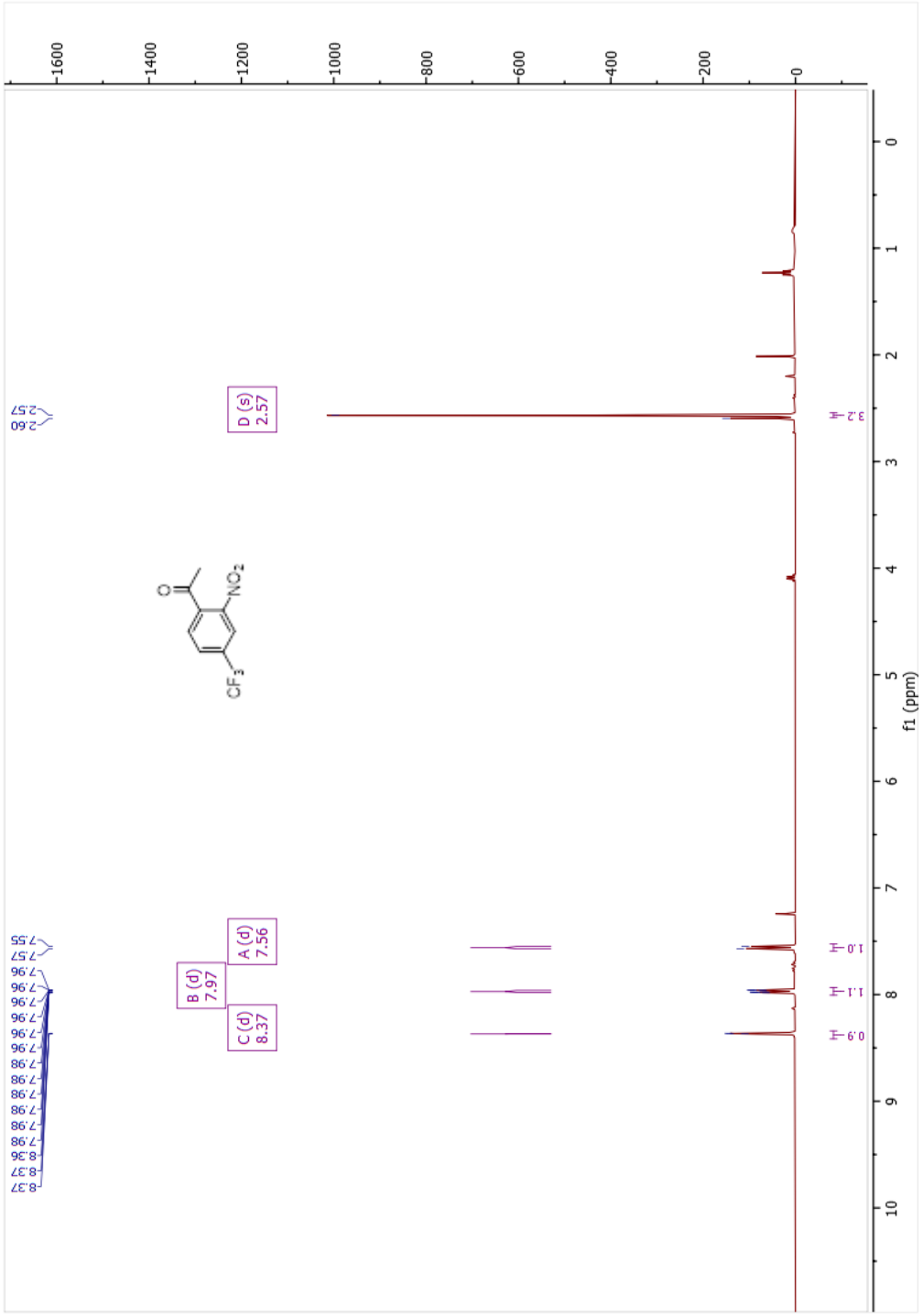
Compound 96 $^{1}H$ NMR.



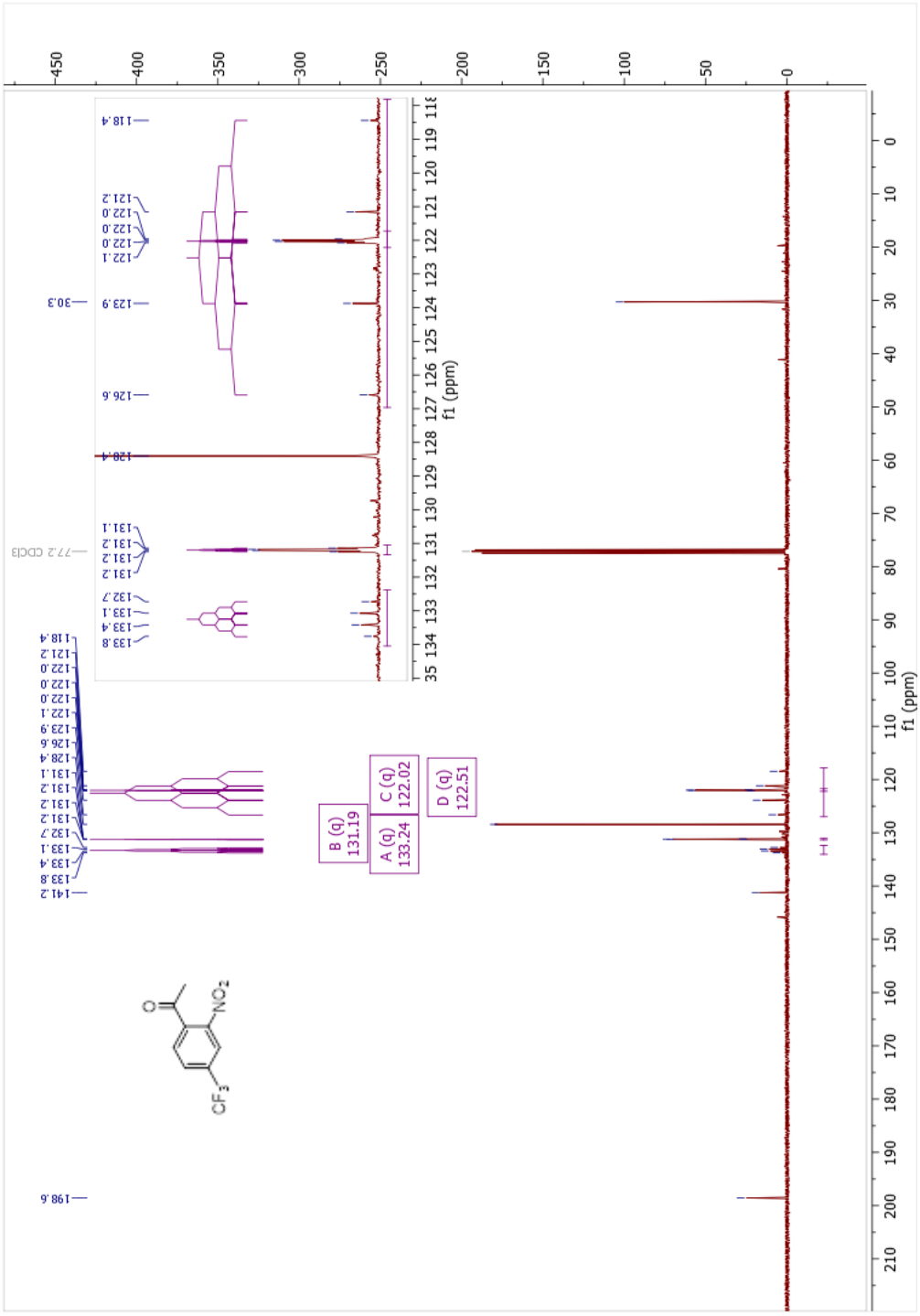
Compound 96 $^{13}$C NMR.



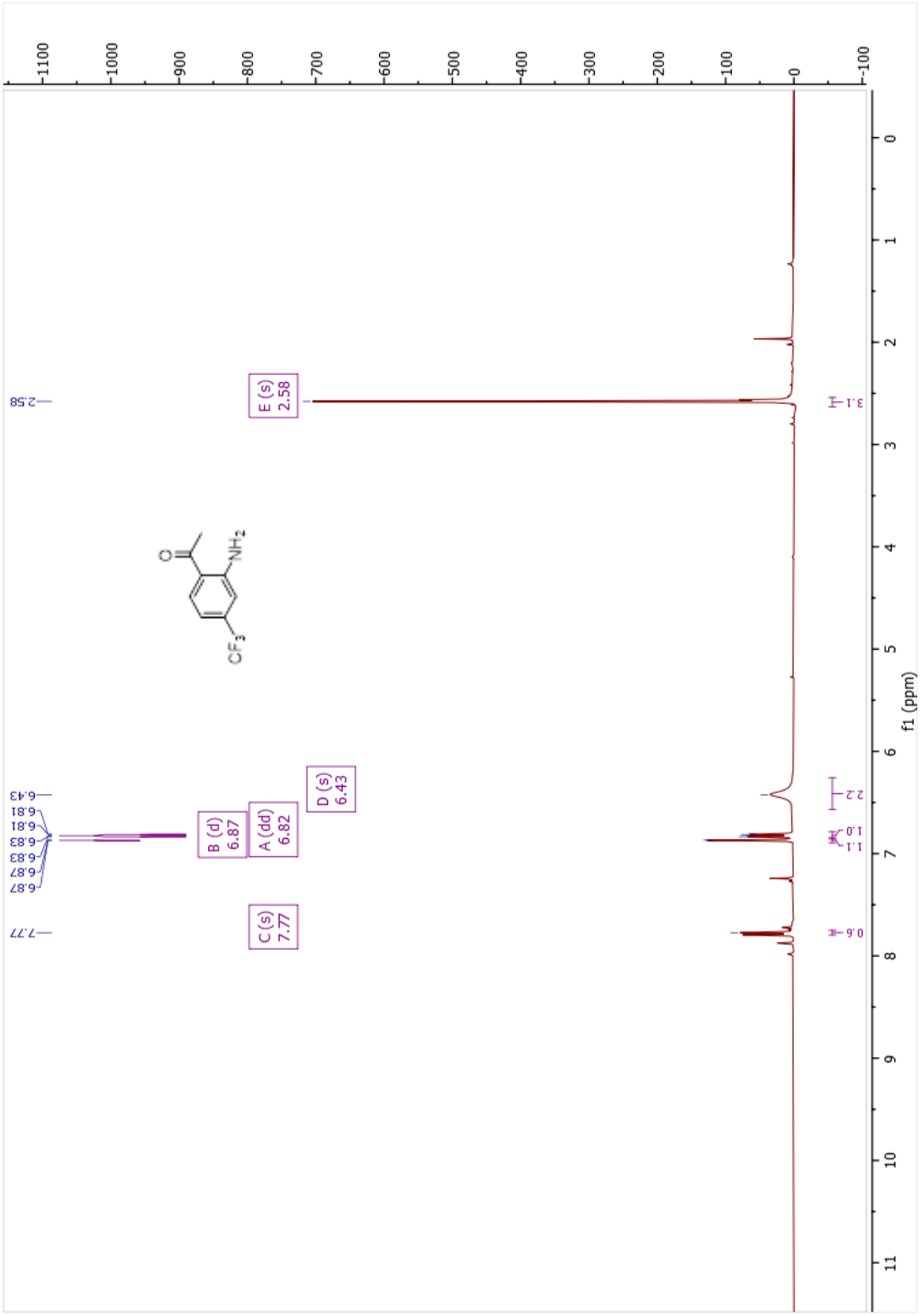
Compound 97 $^{1}$H NMR.



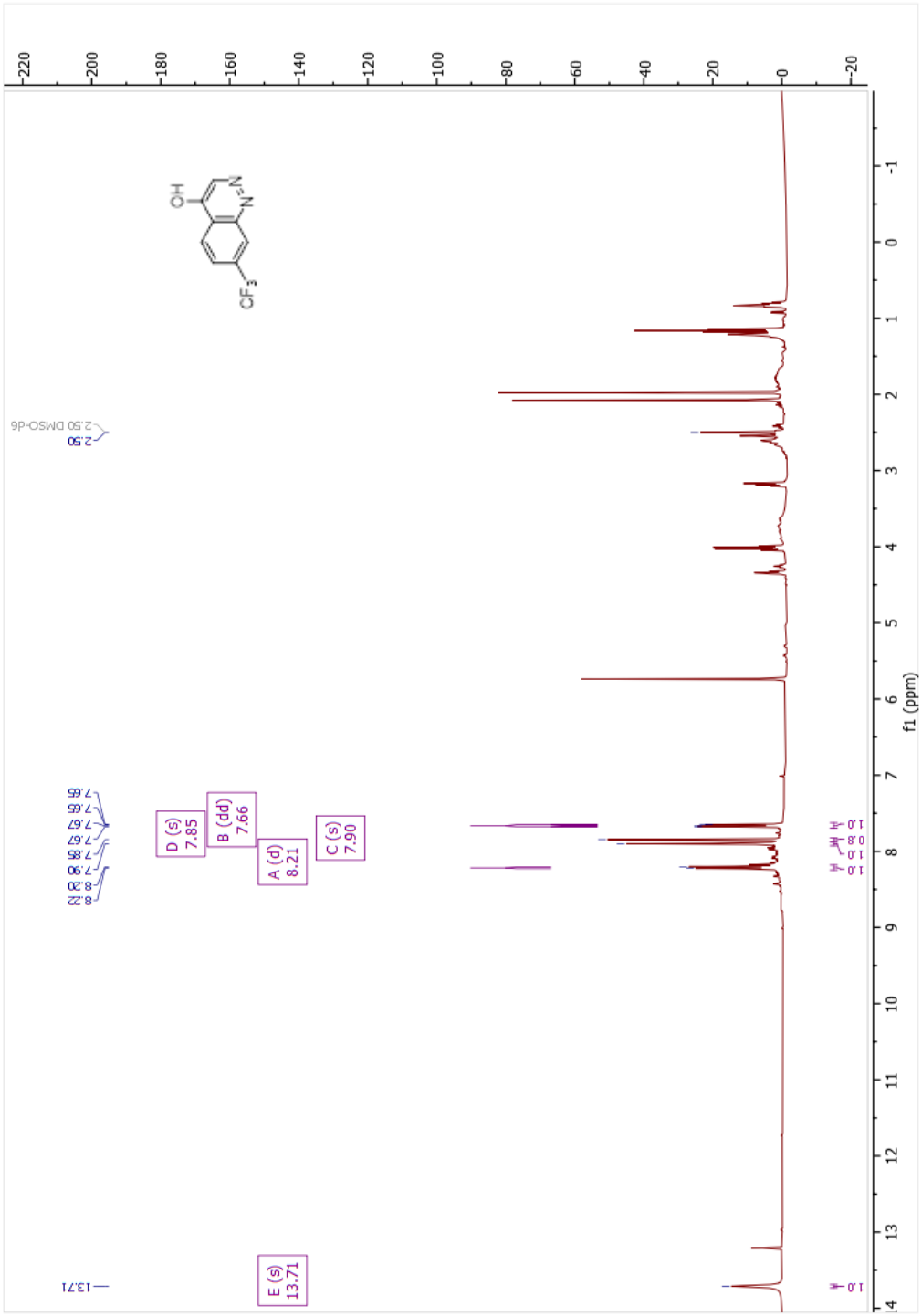
Compound 97 $^{13}C$ NMR.



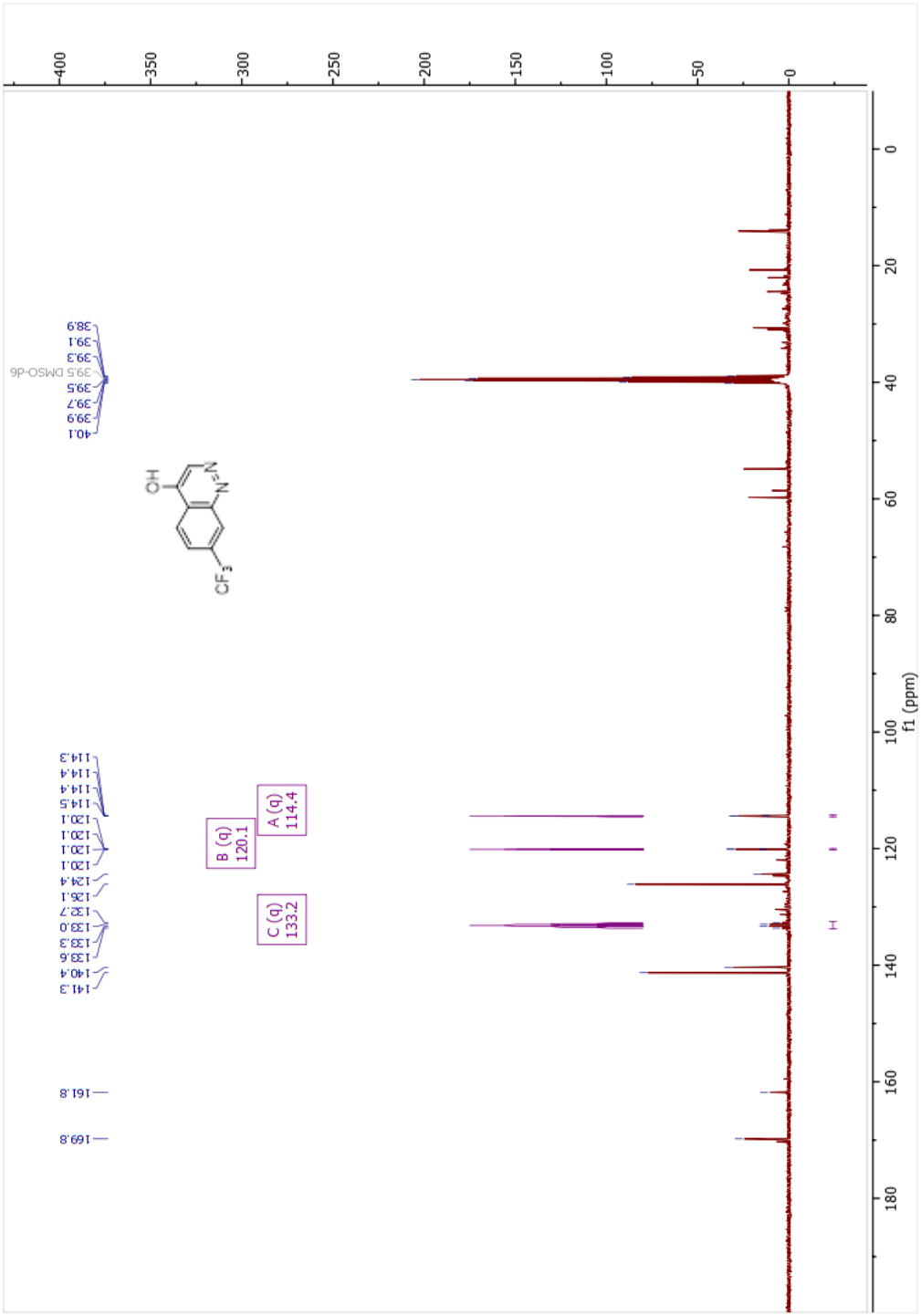
1-[2-amino-4-(trifluoromethyl)phenyl]ethan-1-one intermediate $^{1}$H NMR. **JRS-9-26/27**



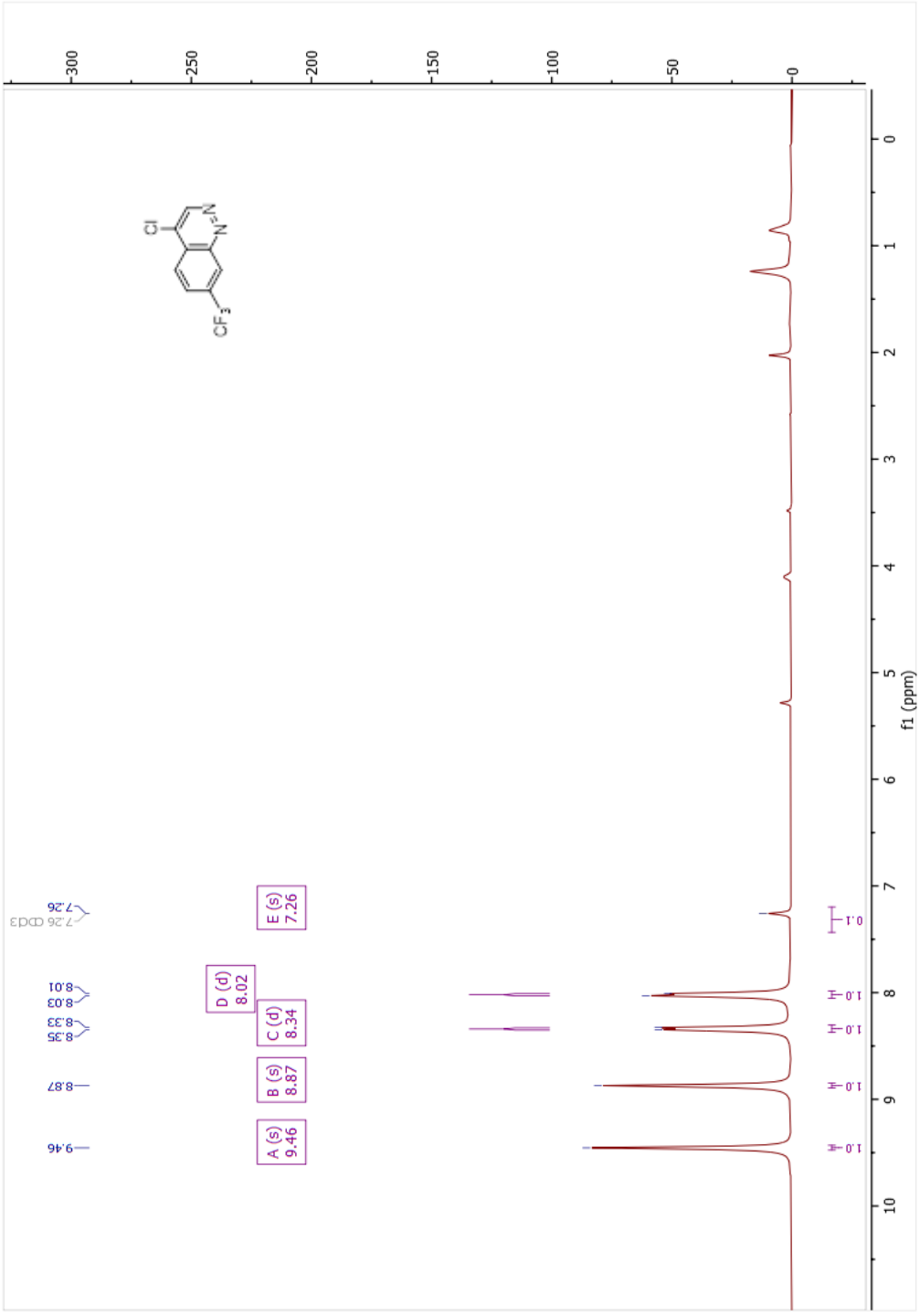
Compound 98 $^{1}$H NMR.



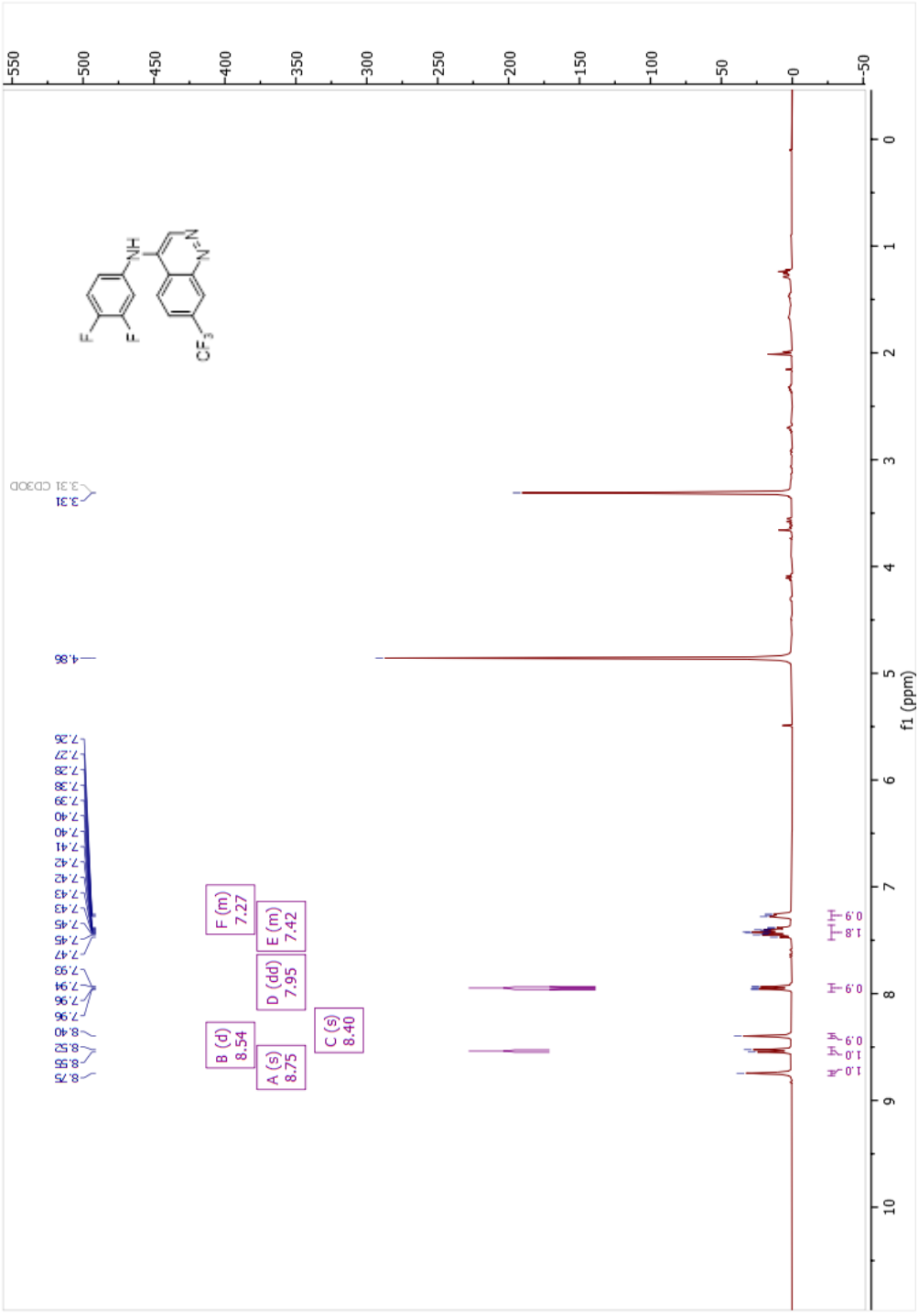
Compound 98 $^{13}C$ NMR.



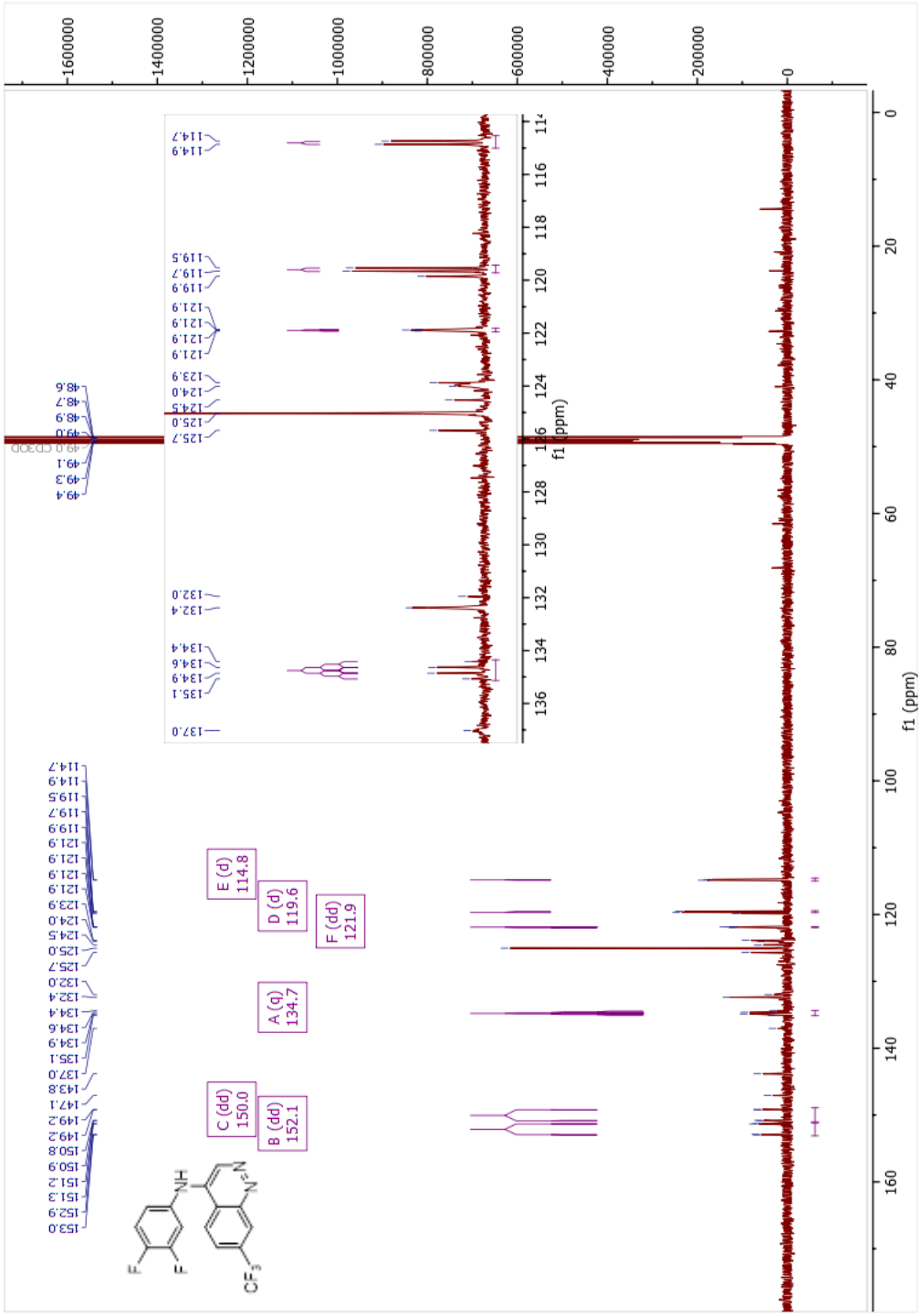
Compound 99 $^{1}H$ NMR.



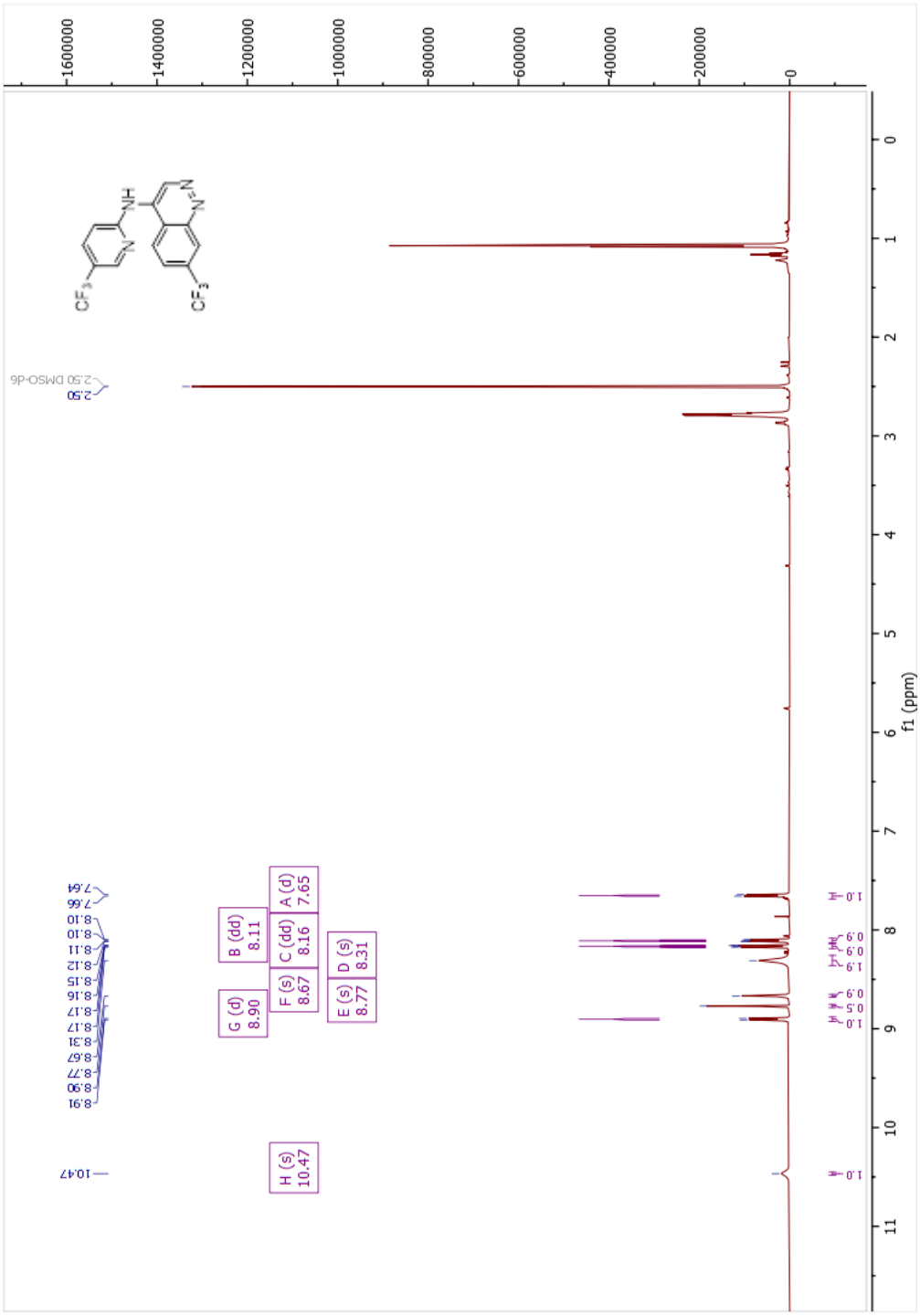
Compound 100 $^{1}$H NMR.



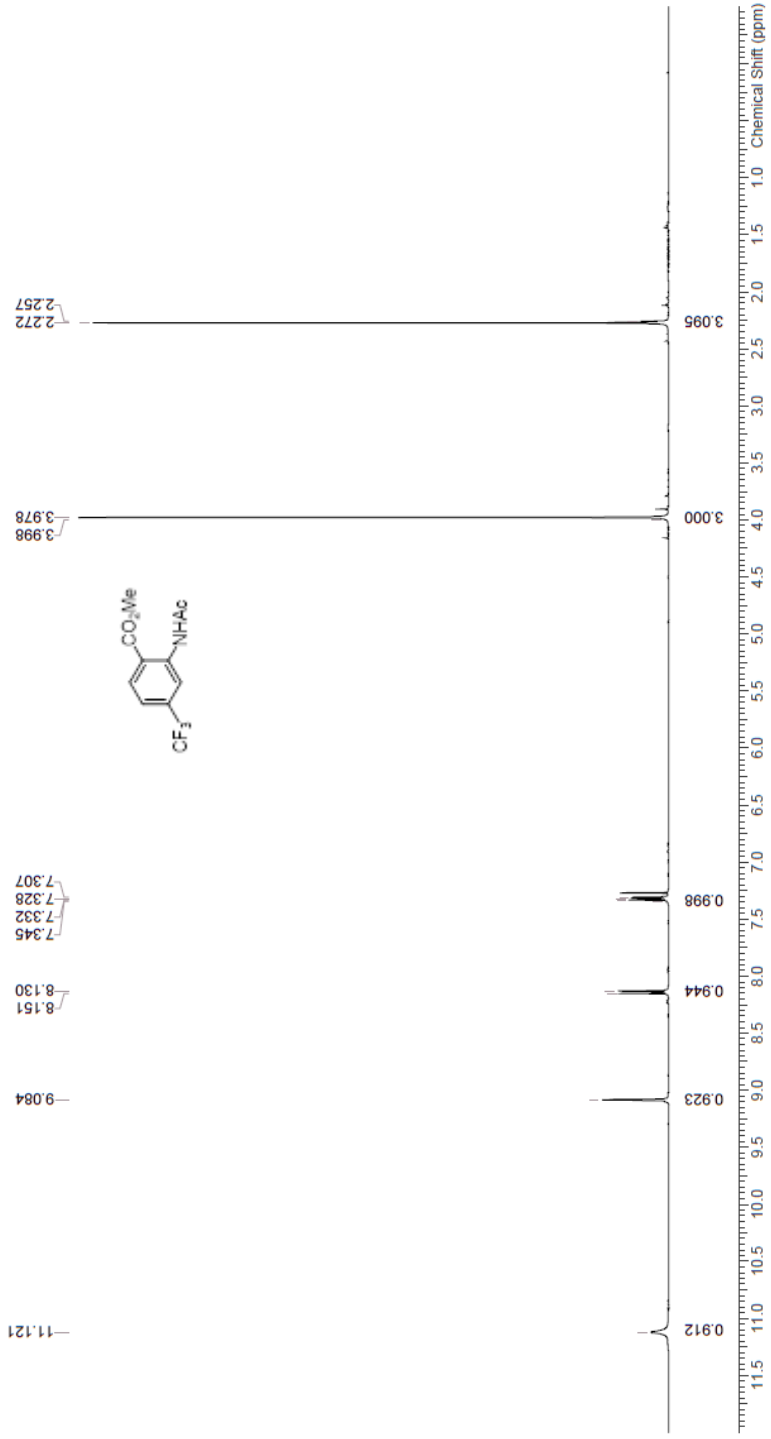
Compound 100 $^{13}C$ NMR.



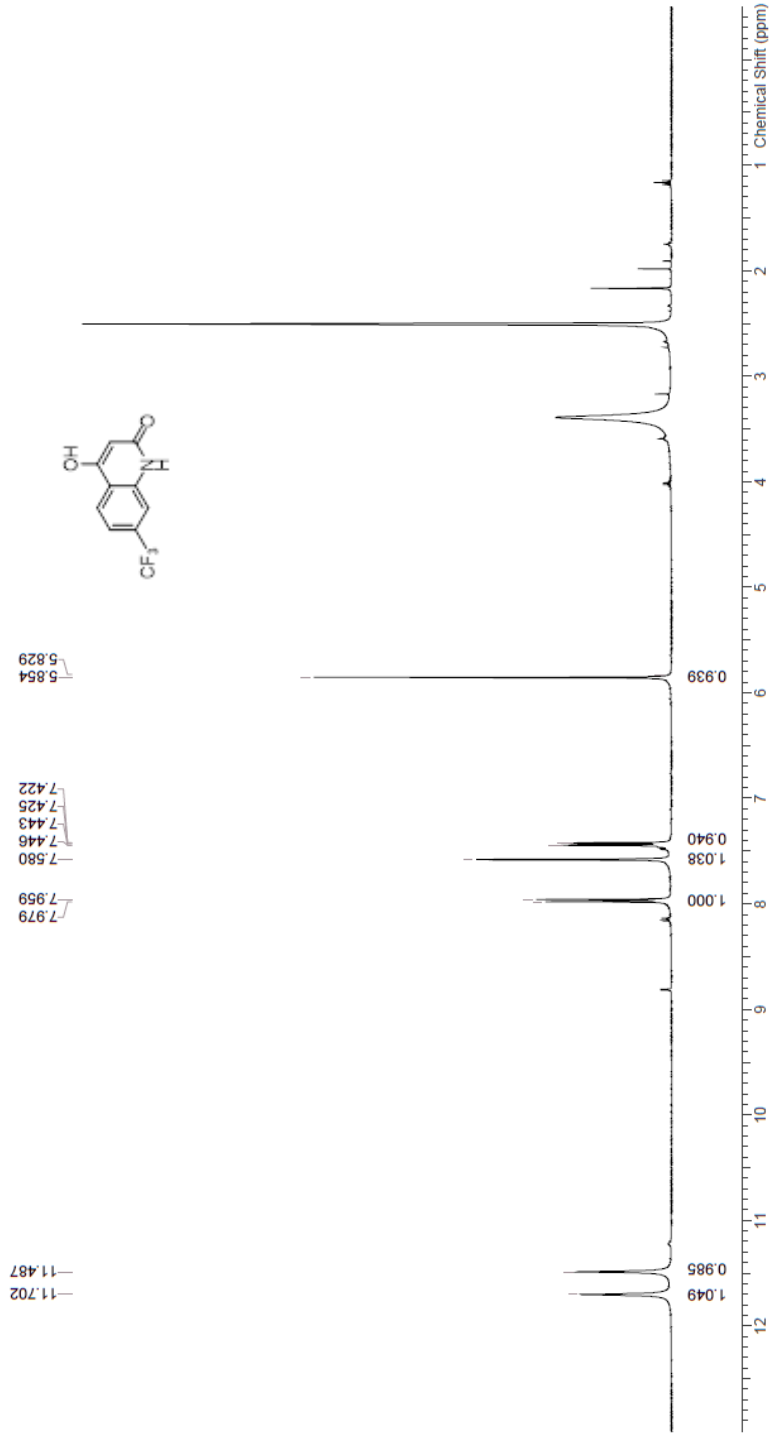
Compound 101 $^{1}$H NMR.



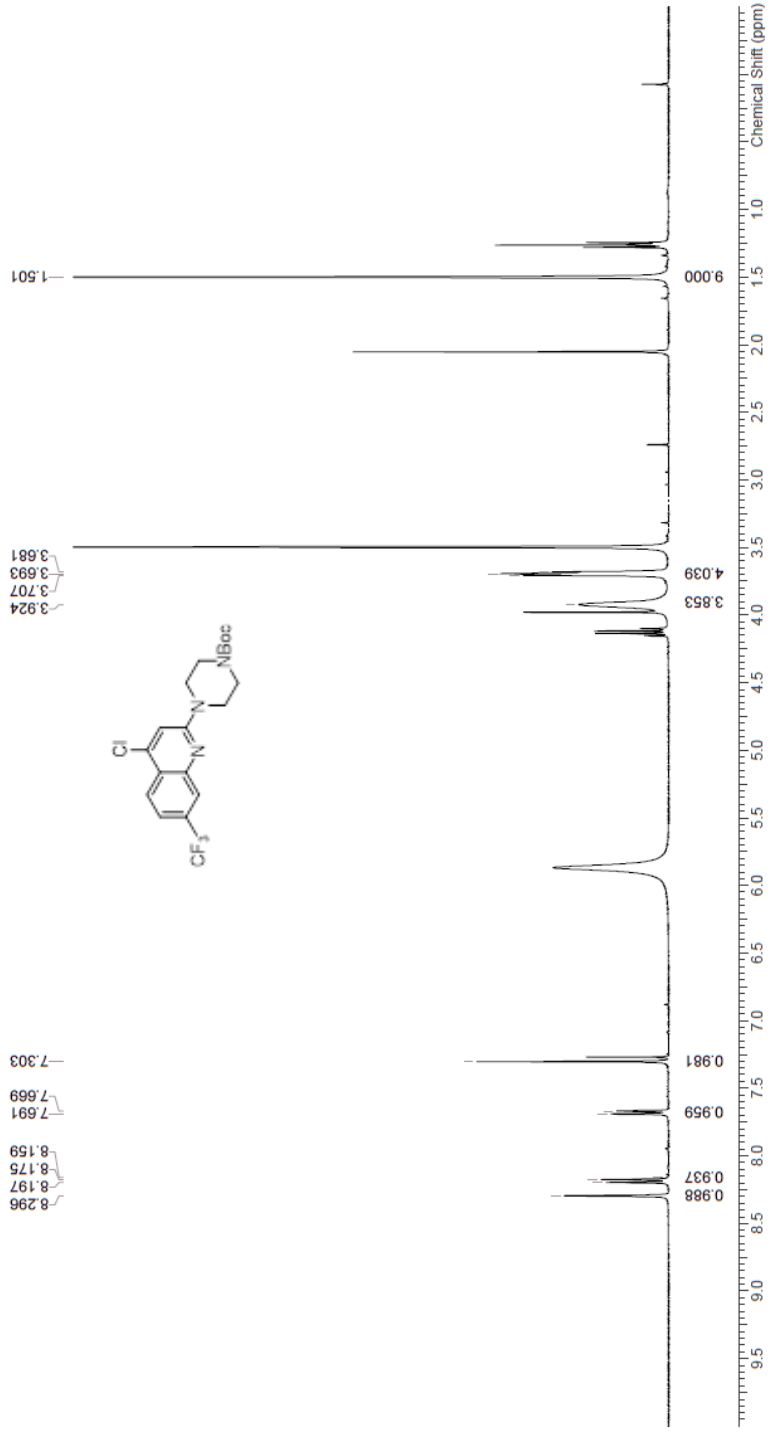
Compound 102 $^{1}$H NMR.



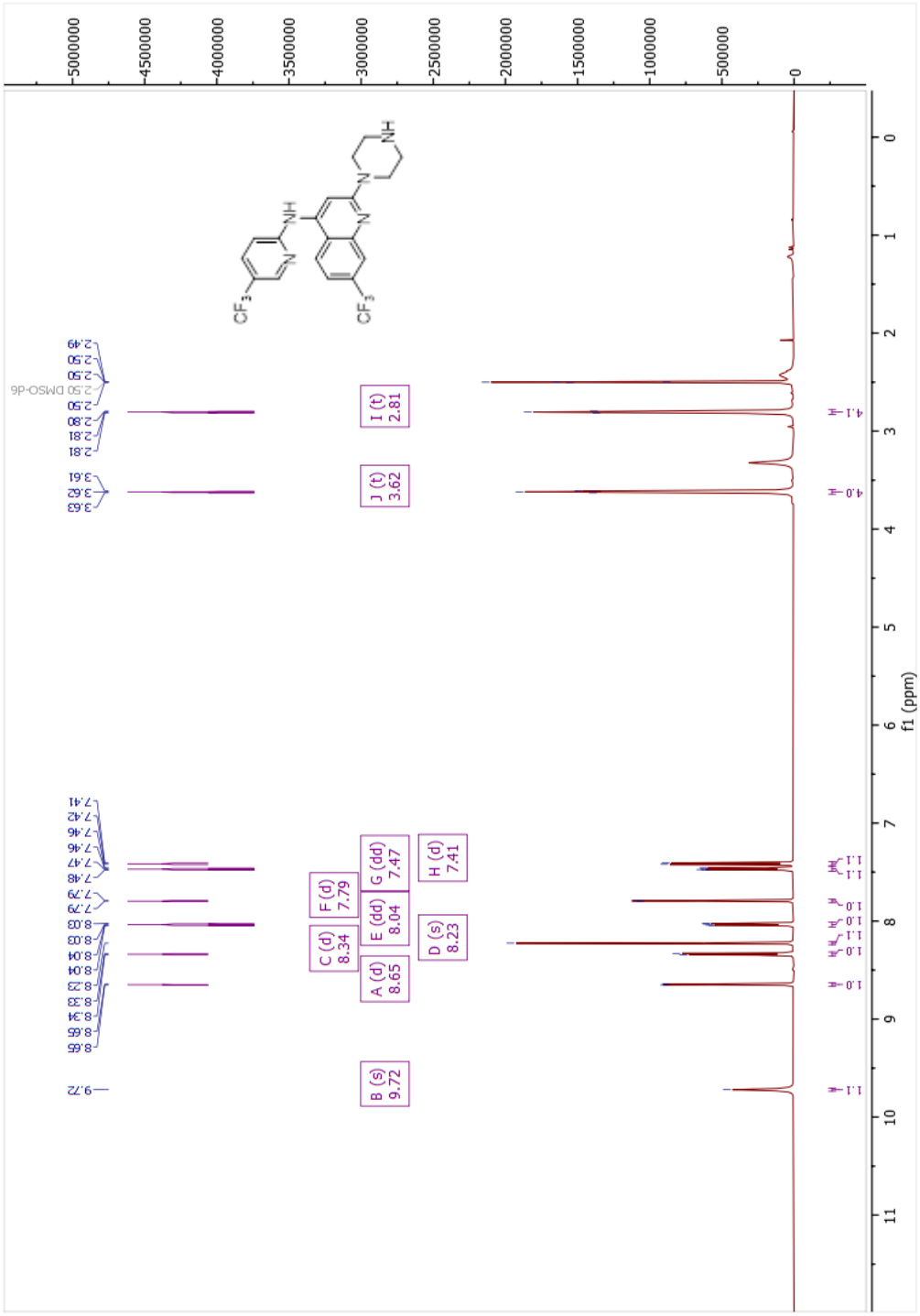
Compound 103 $^{1}H$ NMR.



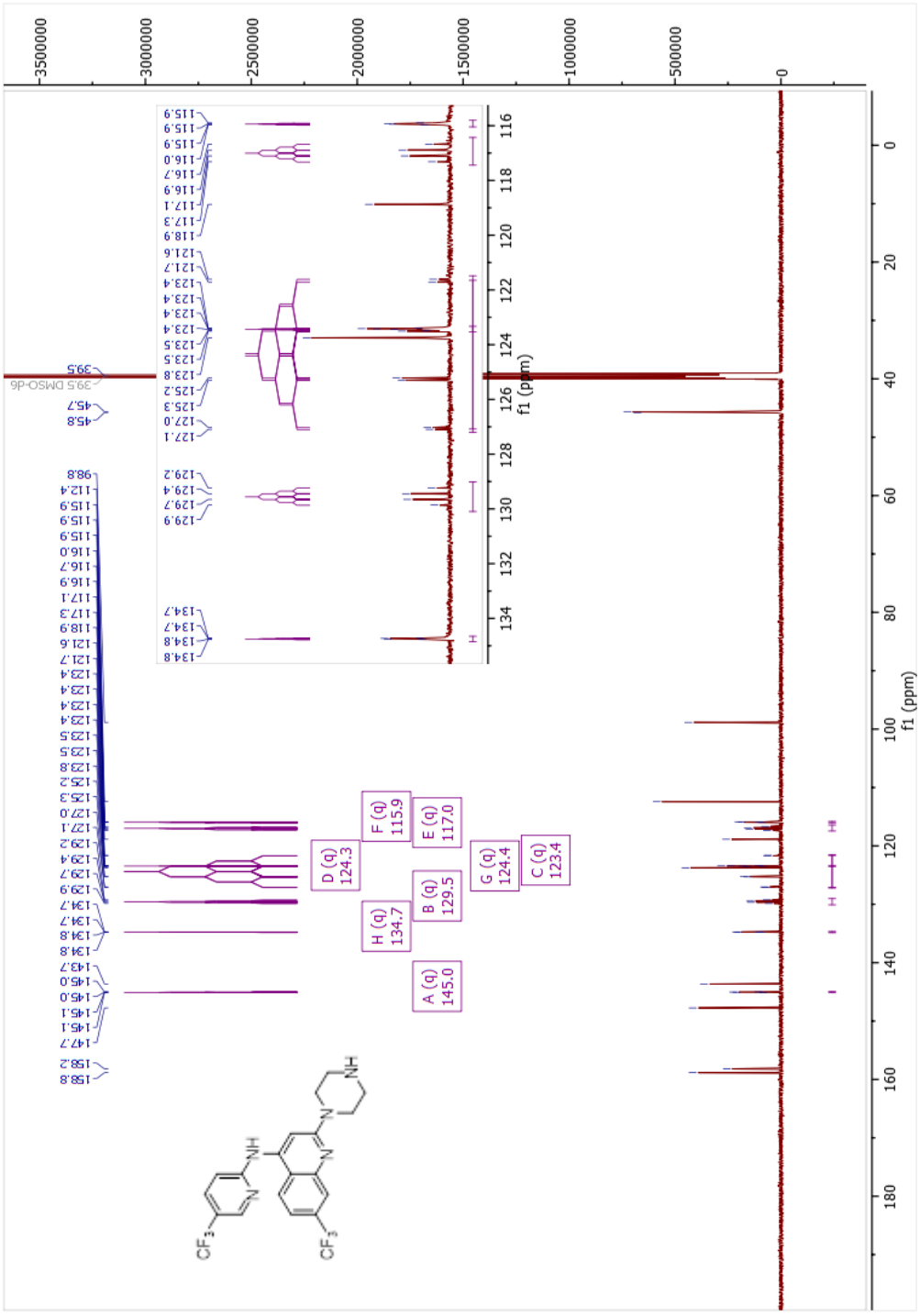
Compound 104 $^{1}$H NMR.



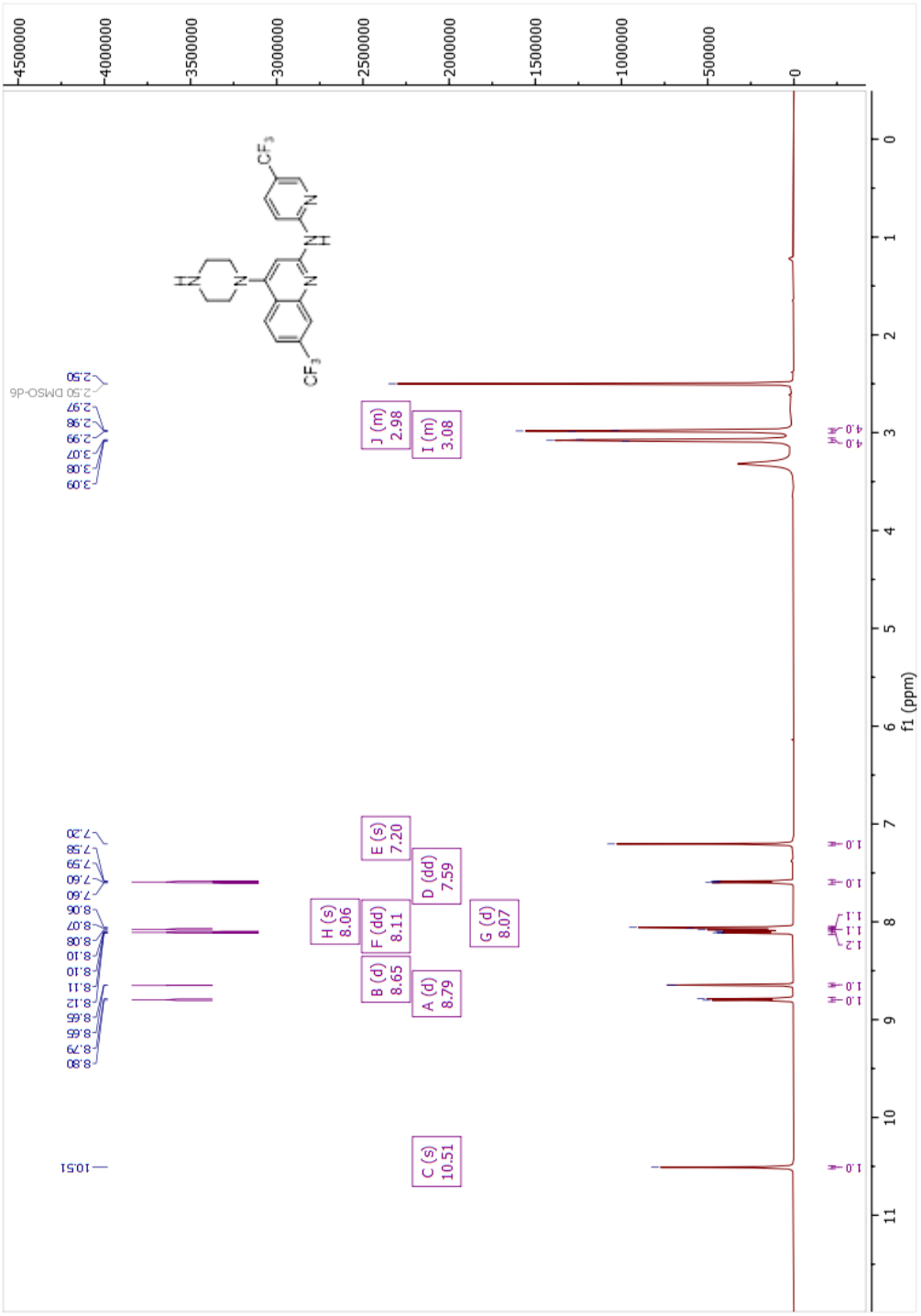
Compound 105 $^{1}H$ NMR.



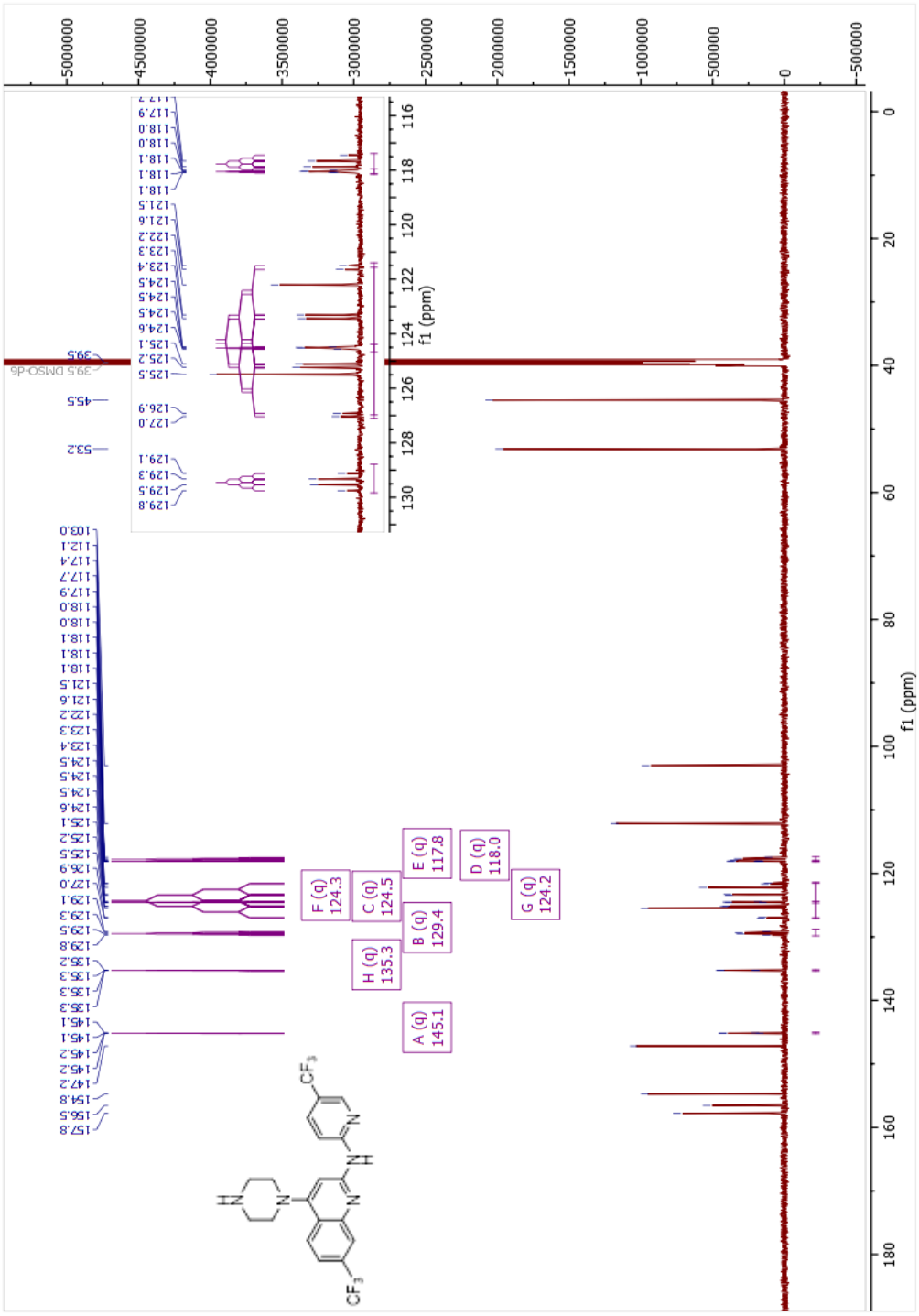
Compound 105 $^{13}C$ NMR.



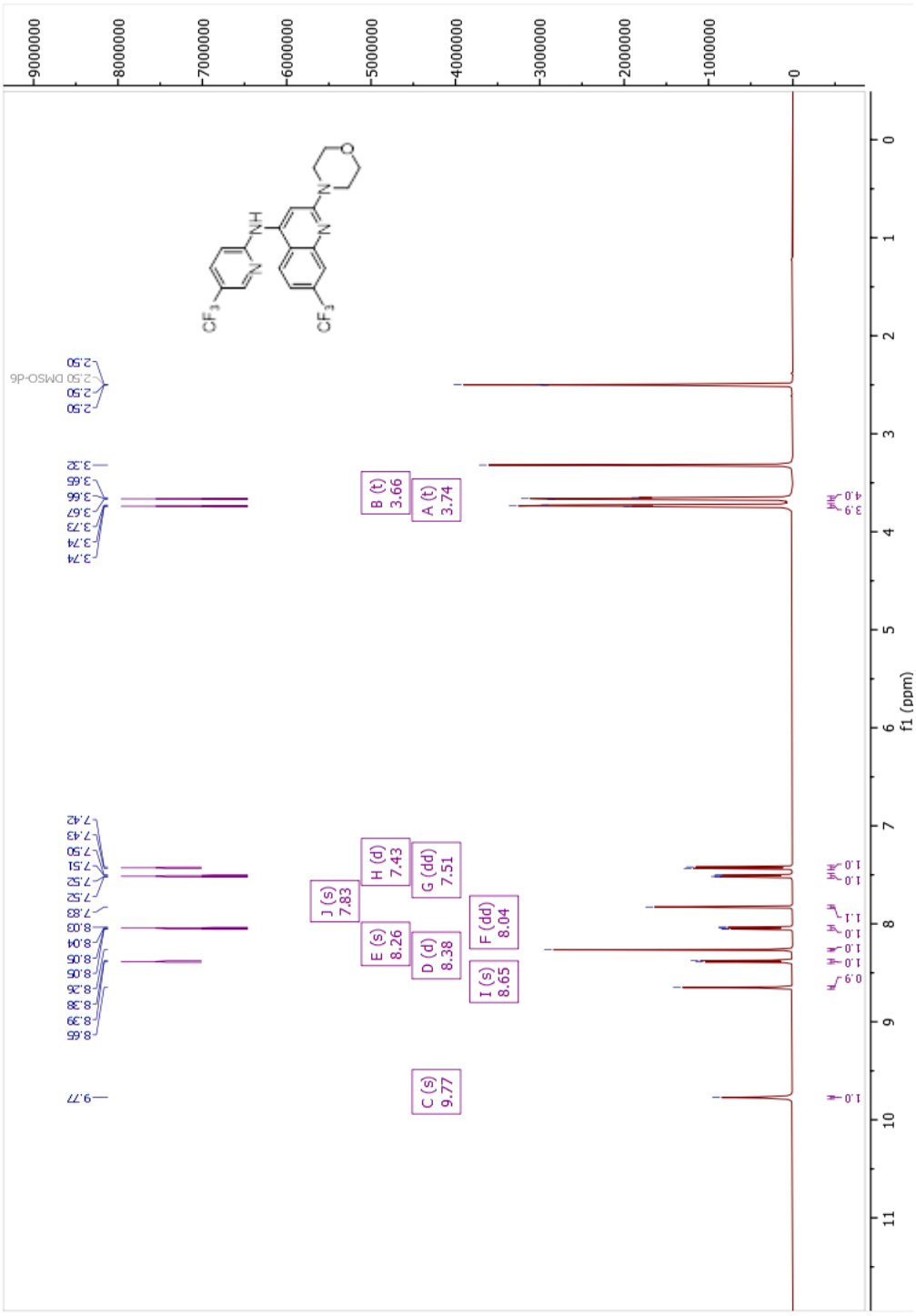
Compound 106 $^{1}H$ NMR.



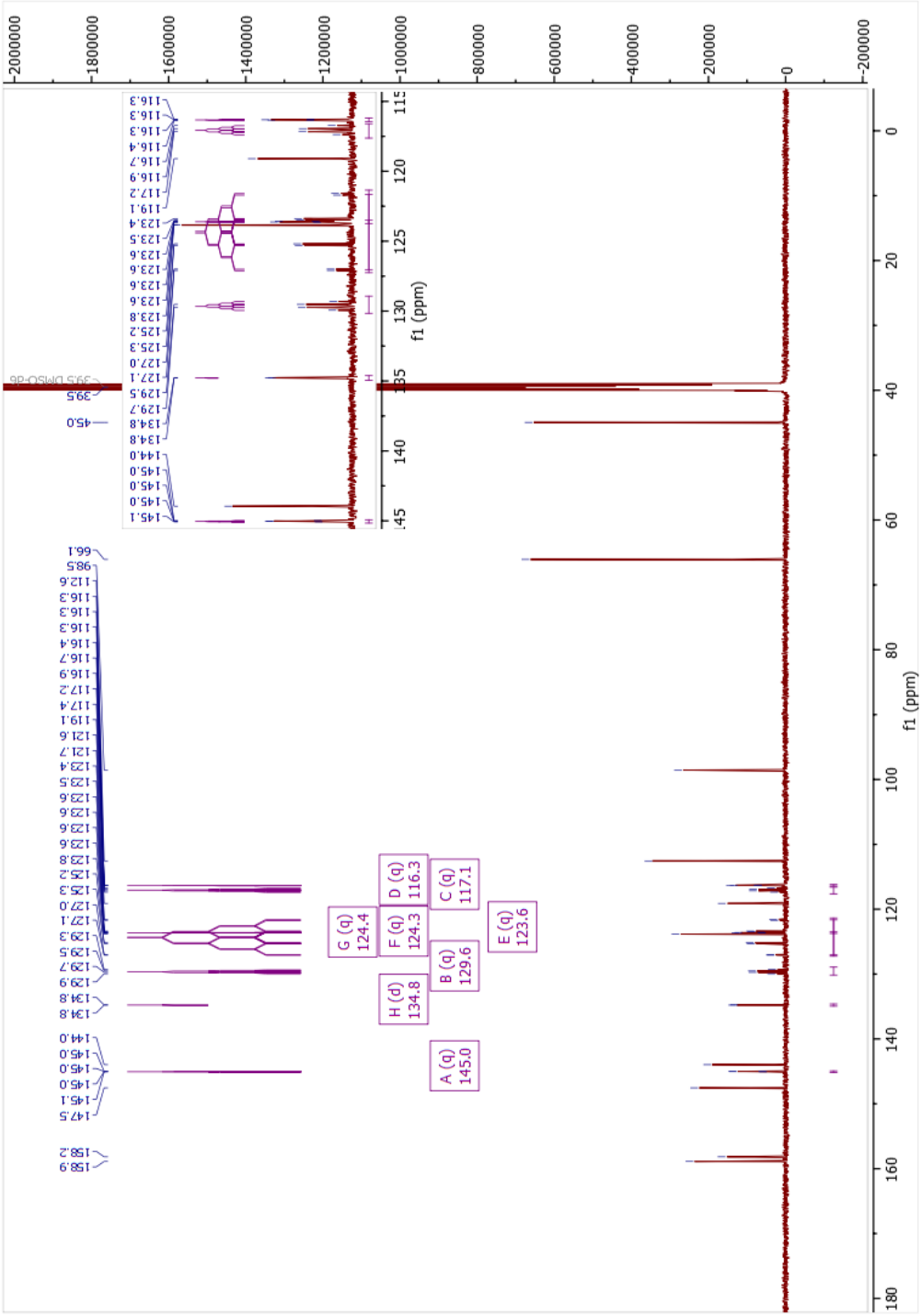
Compound 106 $^{13}$C NMR.



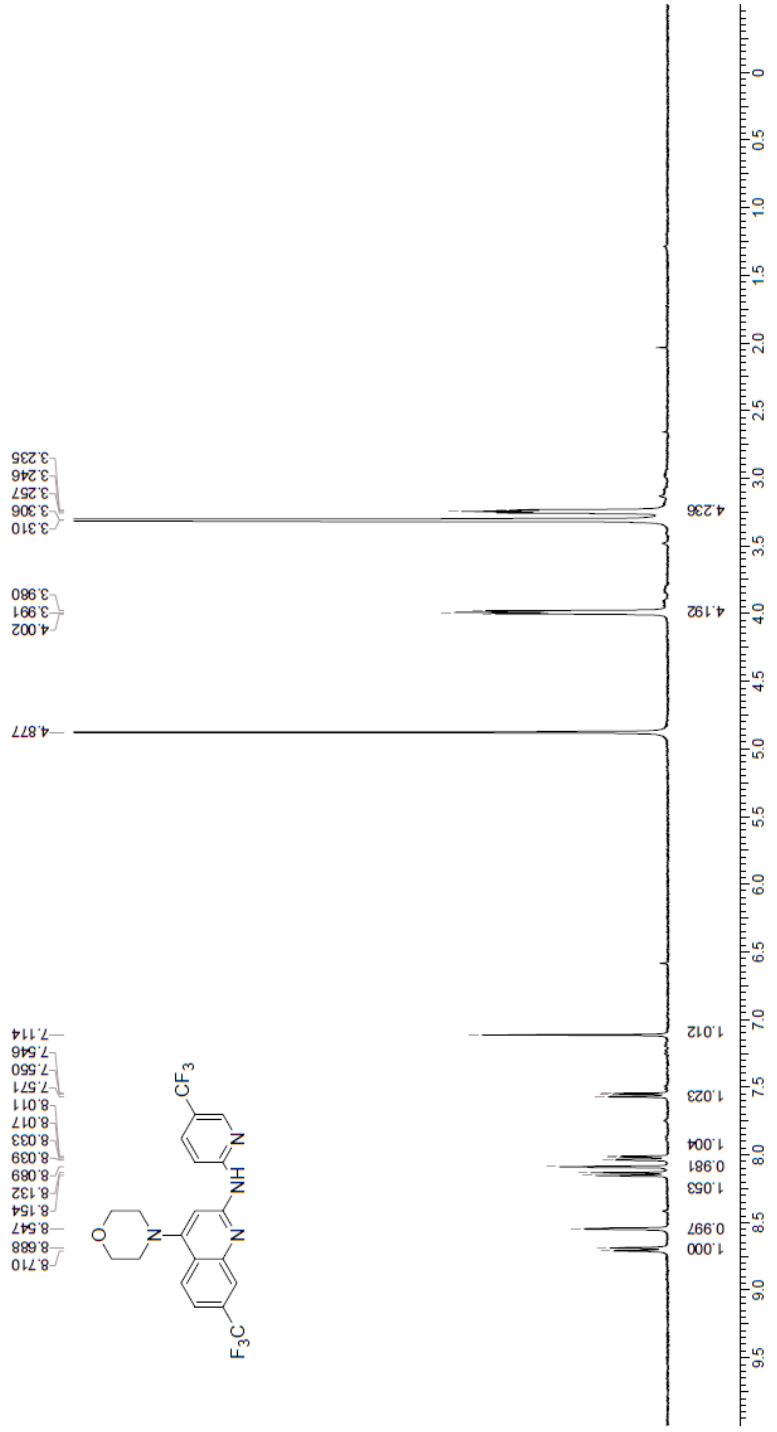
Compound 107 $^{1}$H NMR.



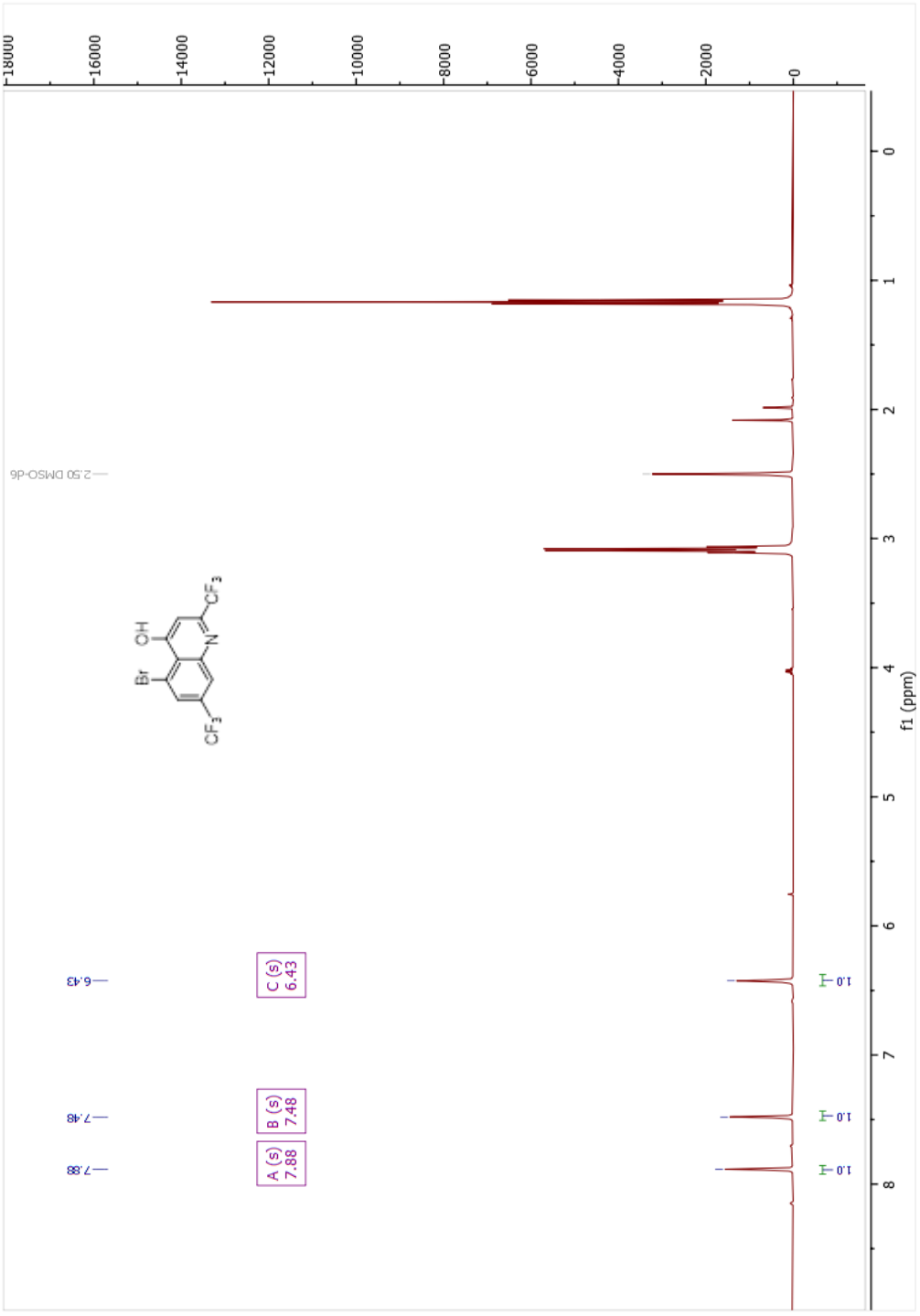
Compound 107 $^{13}$C NMR.



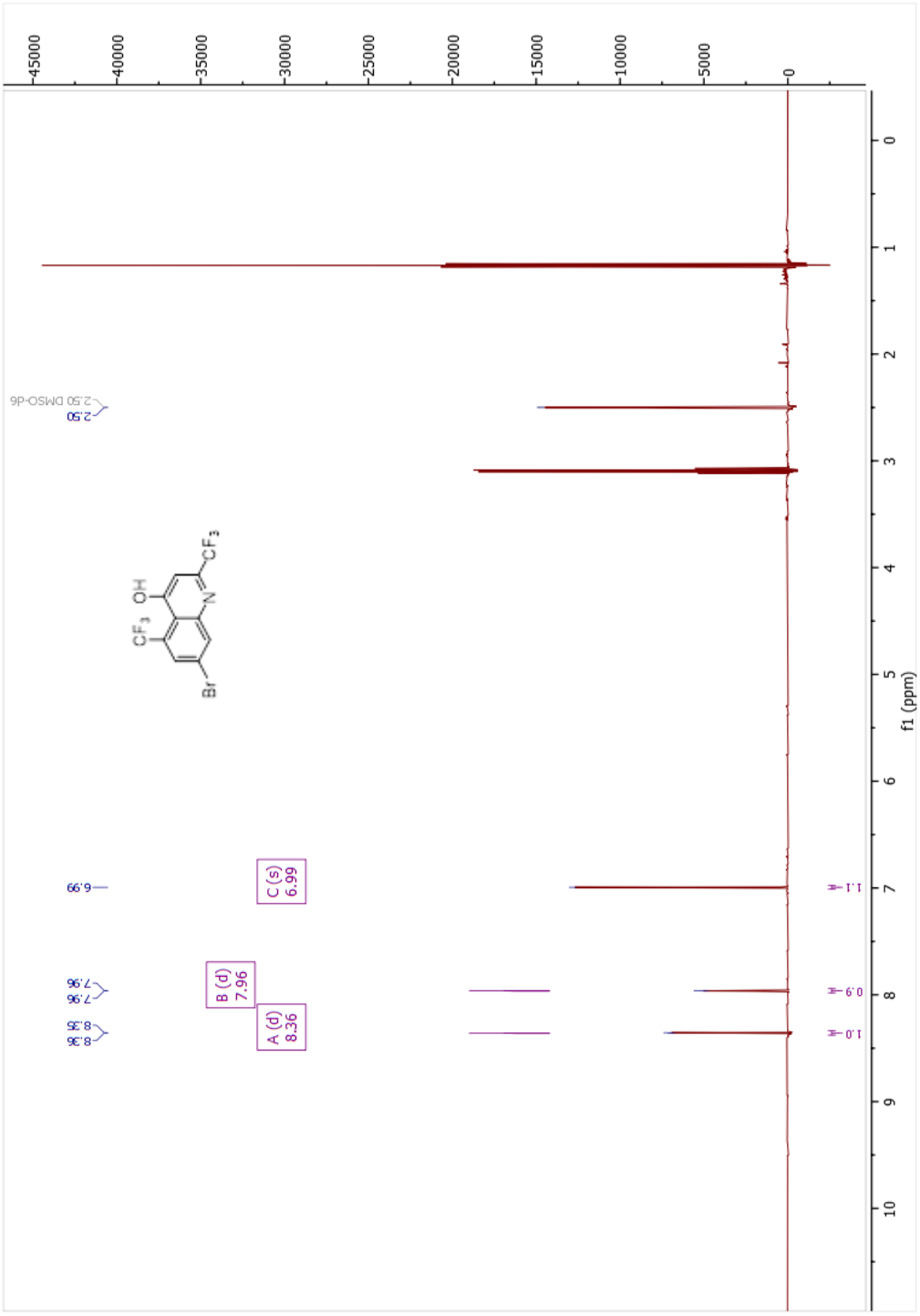
Compound 108 $^{1}H$ NMR.



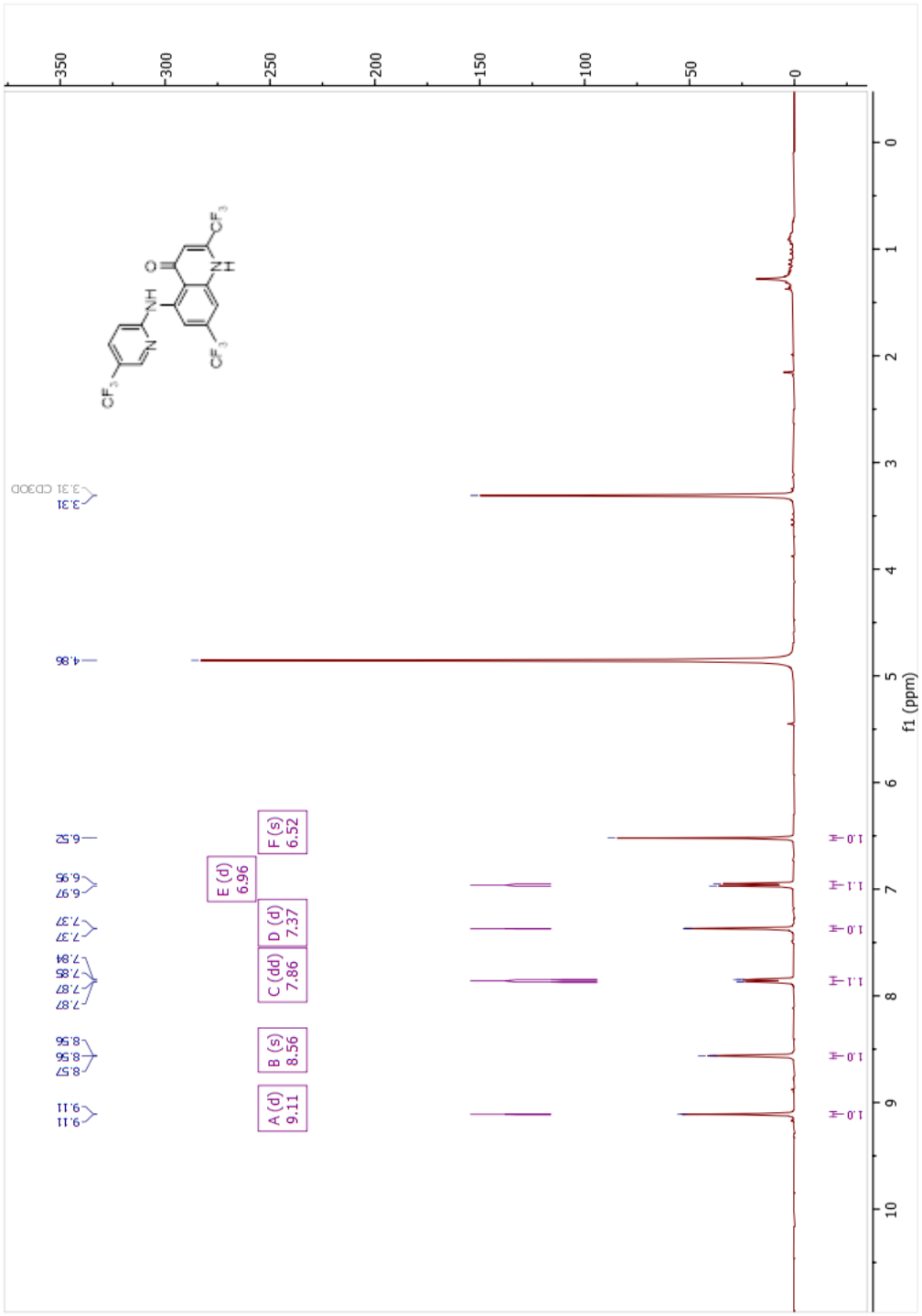
Compound 109 $^{1}H$ NMR



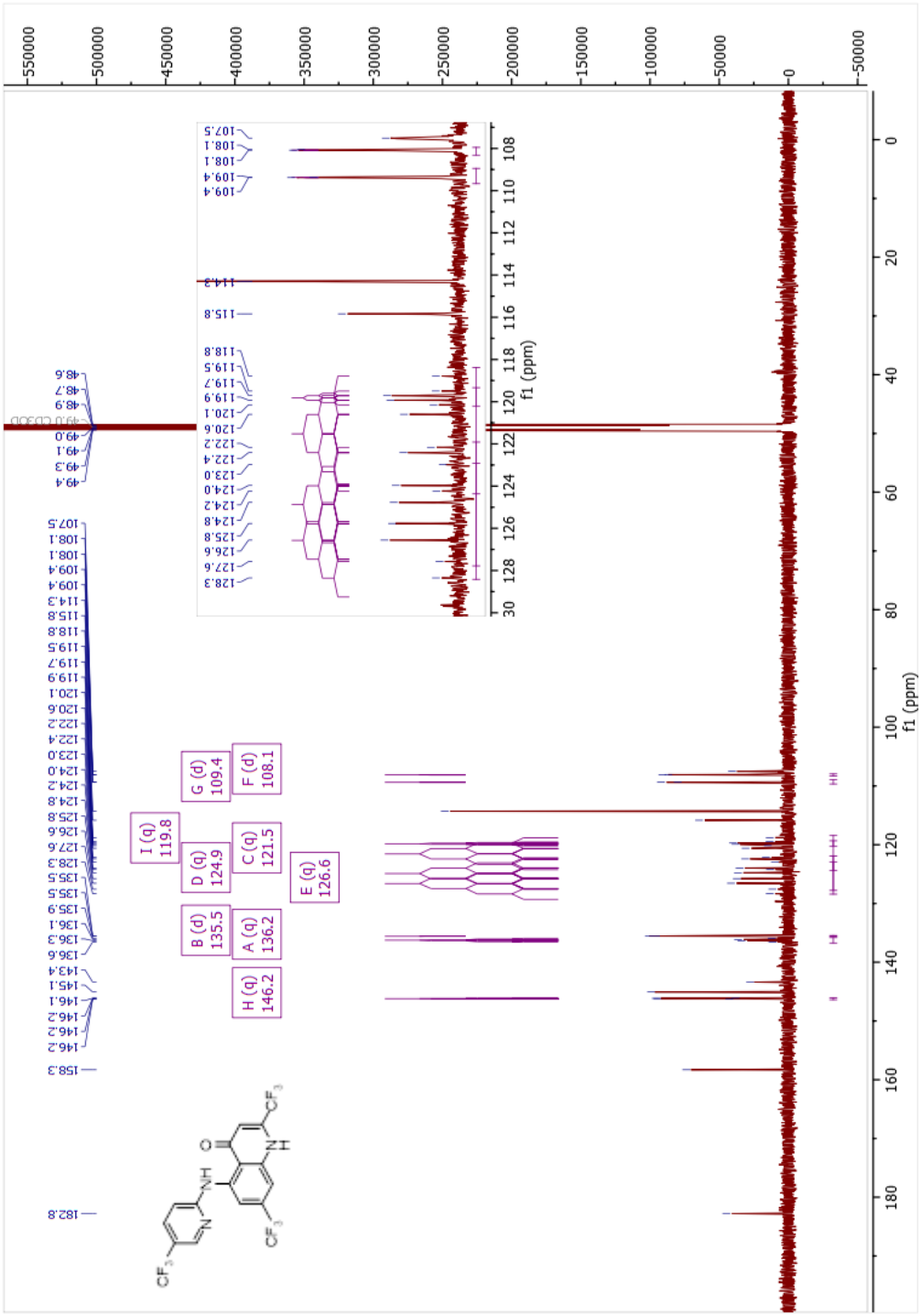
Compound 110 $^{1}H$ NMR.



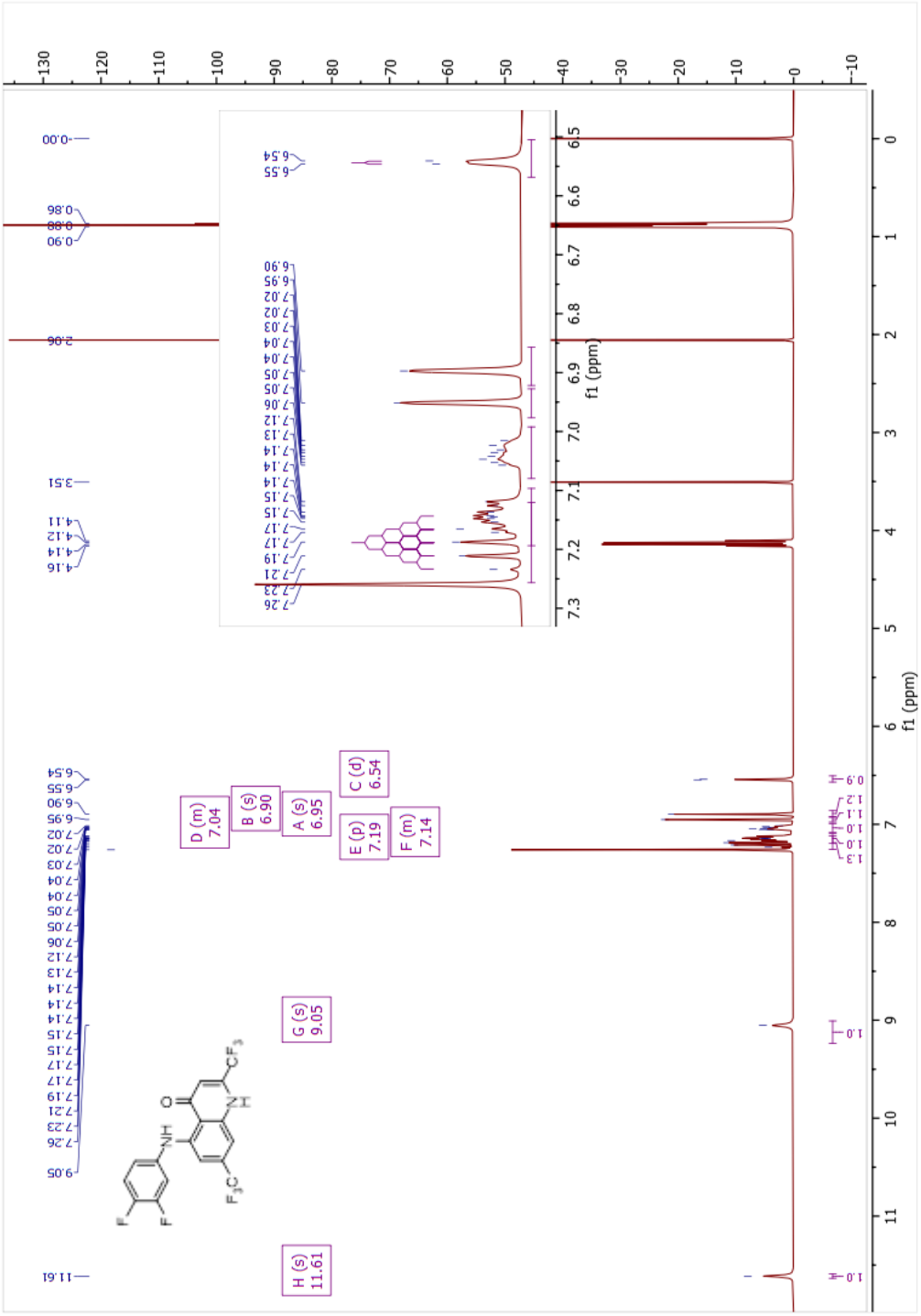
Compound 111 $^{1}H$ NMR.



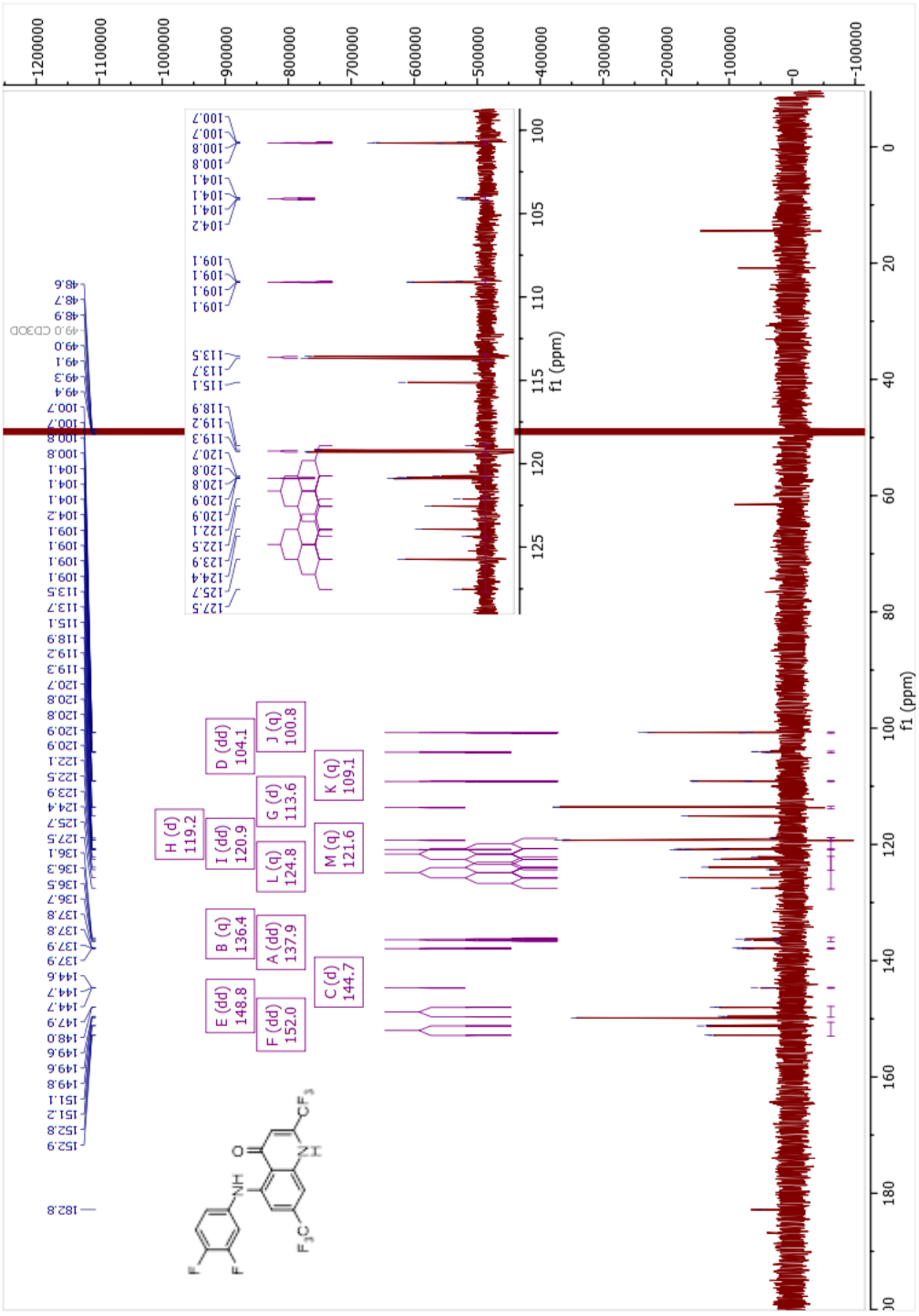
Compound 111 $^{13}C$ NMR.



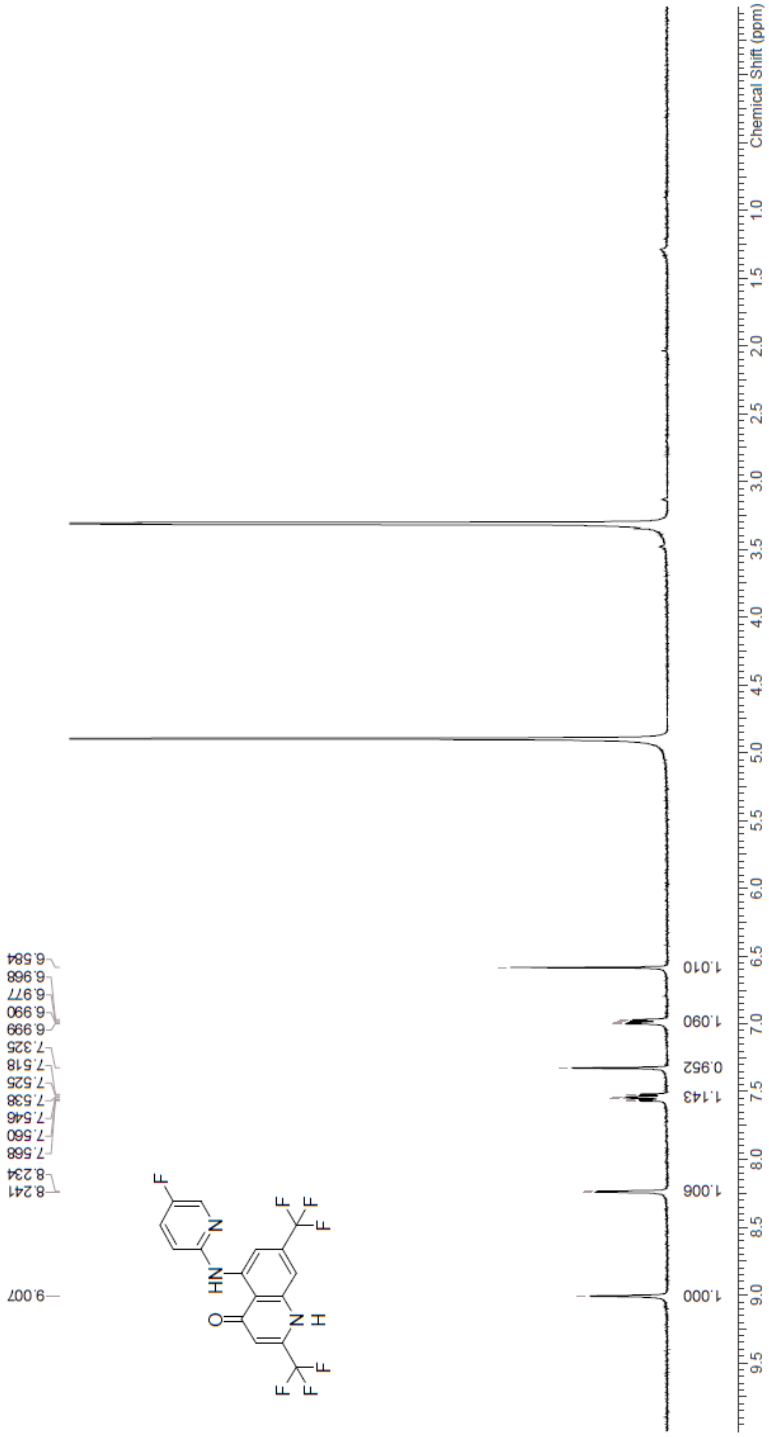
Compound 113 $^{1}H$ NMR.



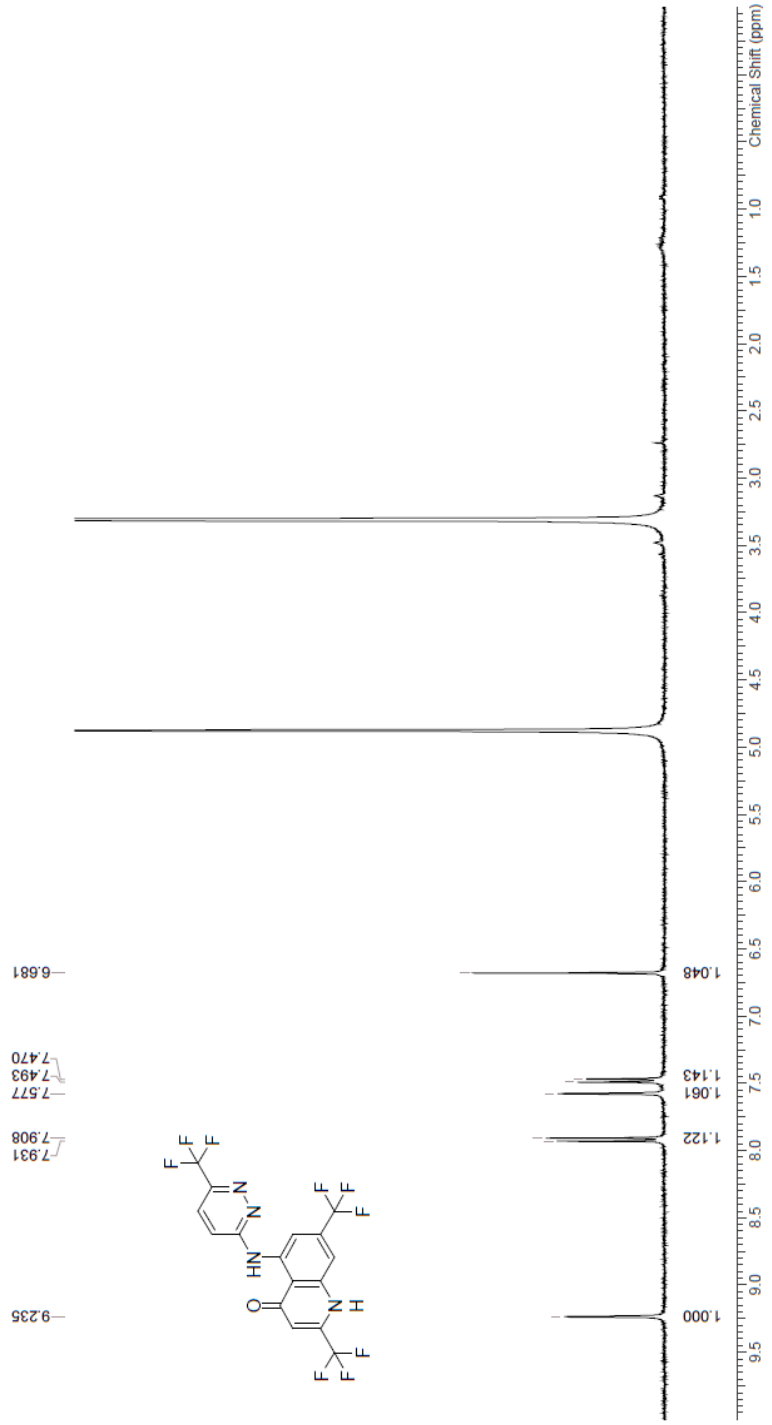
Compound 113 $^{13}$C NMR.



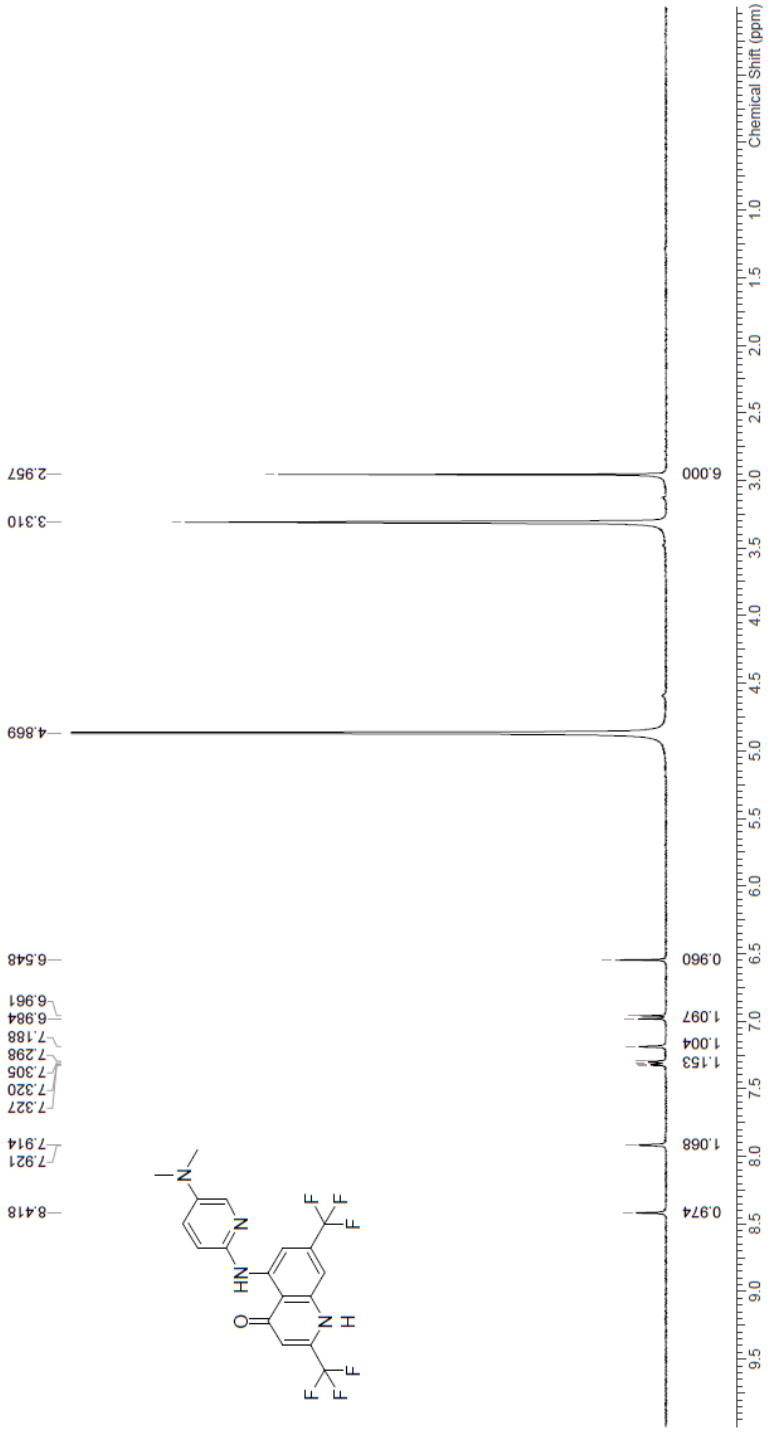
Compound 114 $^{1}$H NMR.



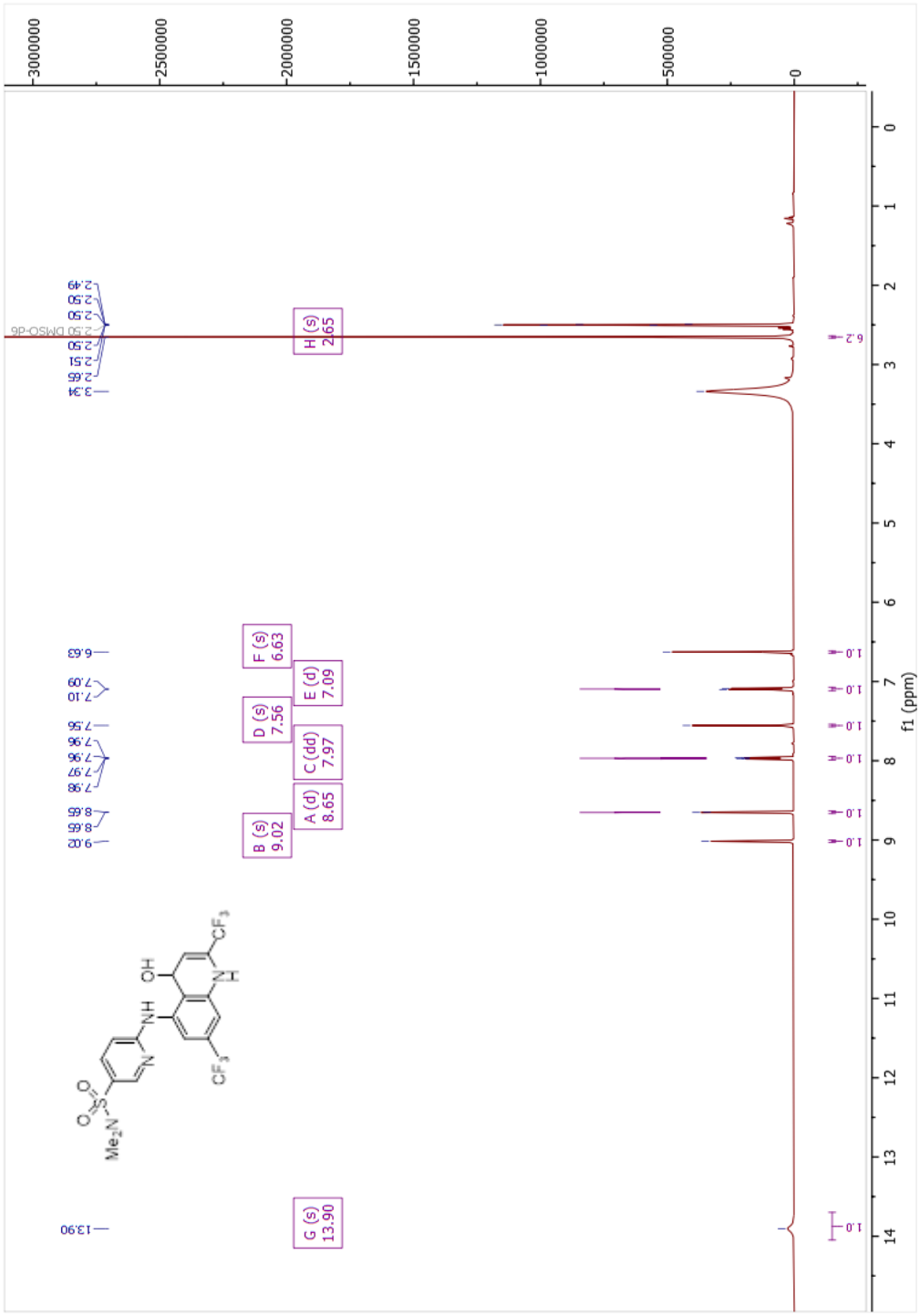
Compound 115 $^{1}$H NMR.



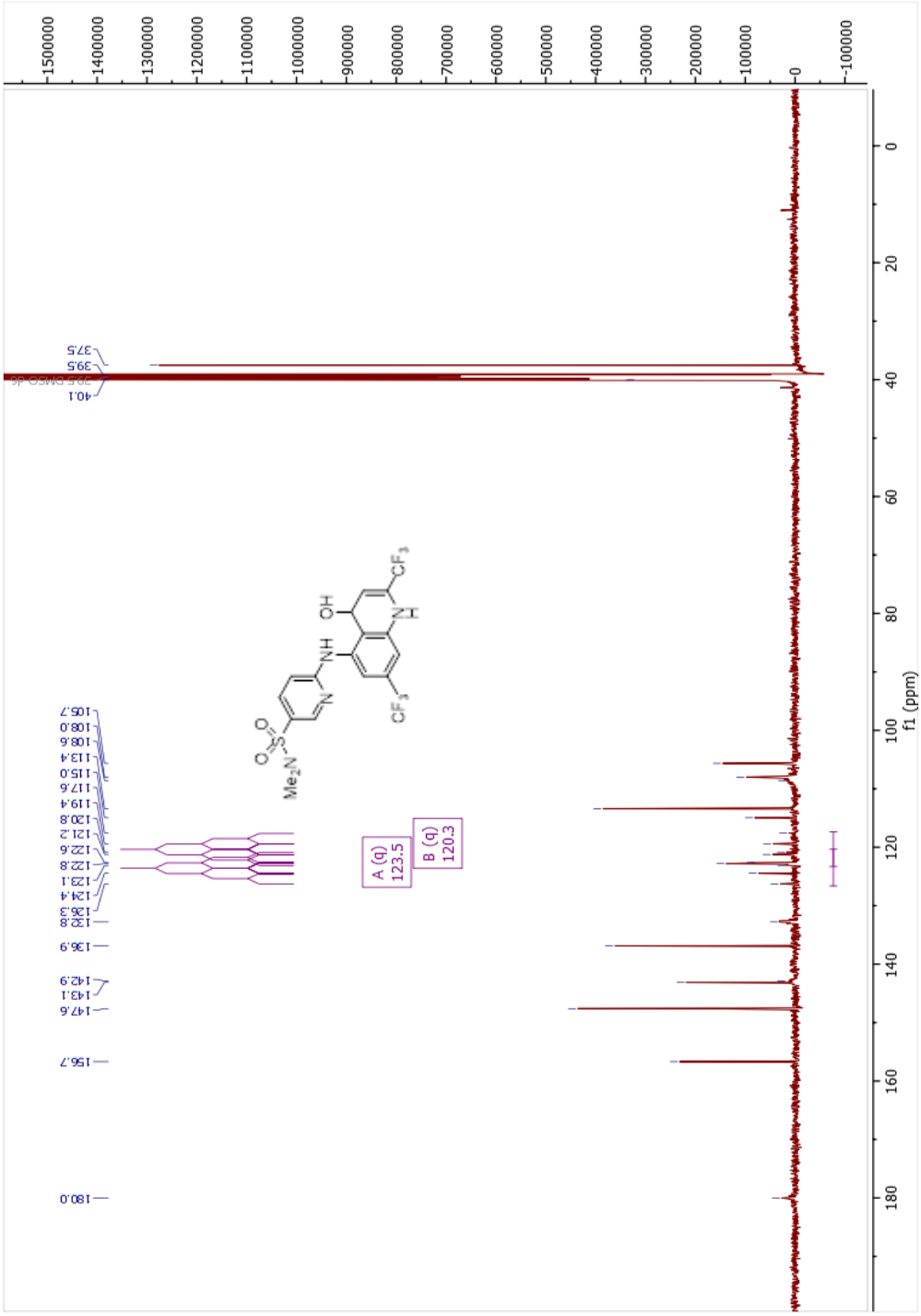
Compound 116 $^{1}$H NMR.



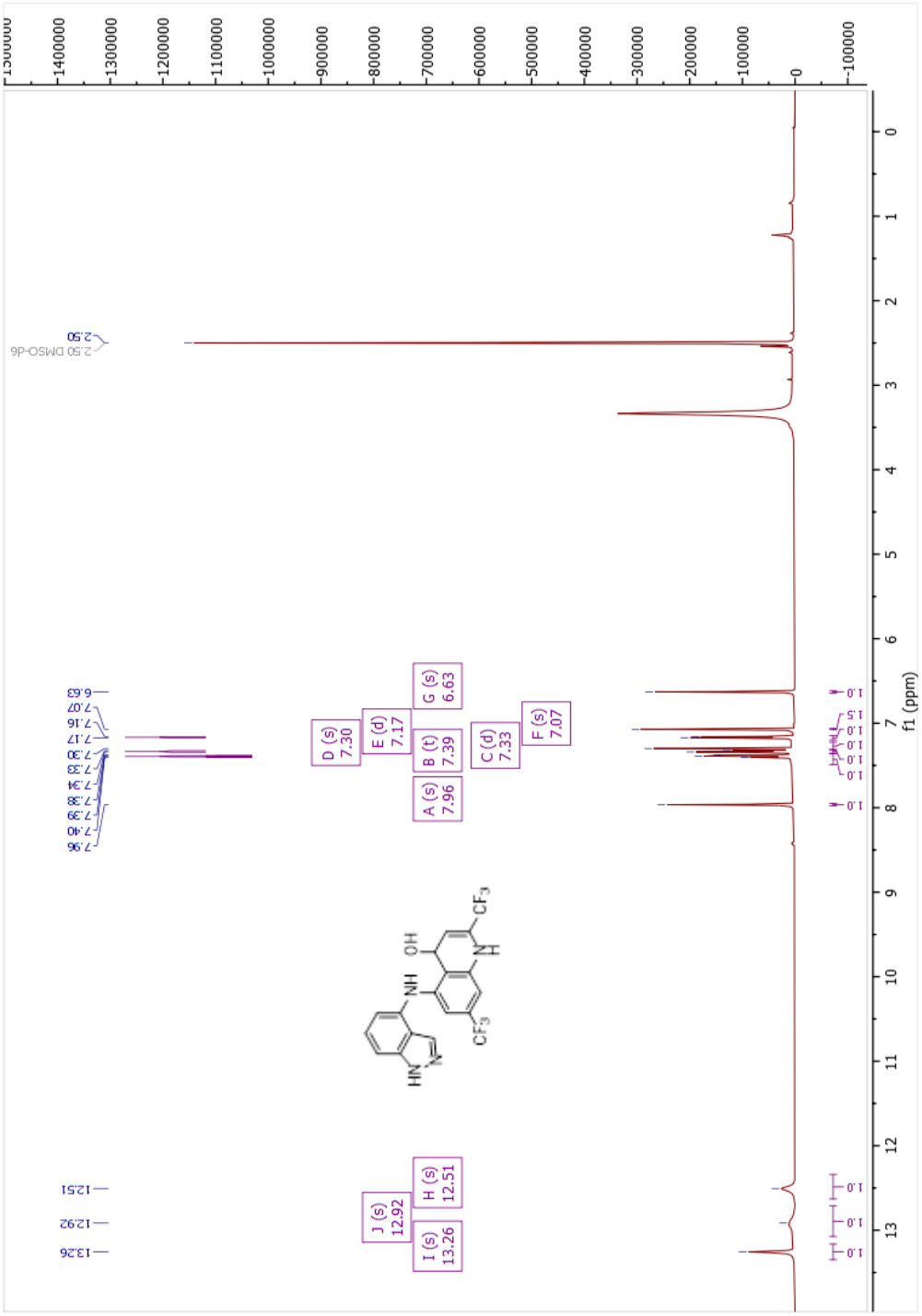
Compound 117 $^{1}H$ NMR.



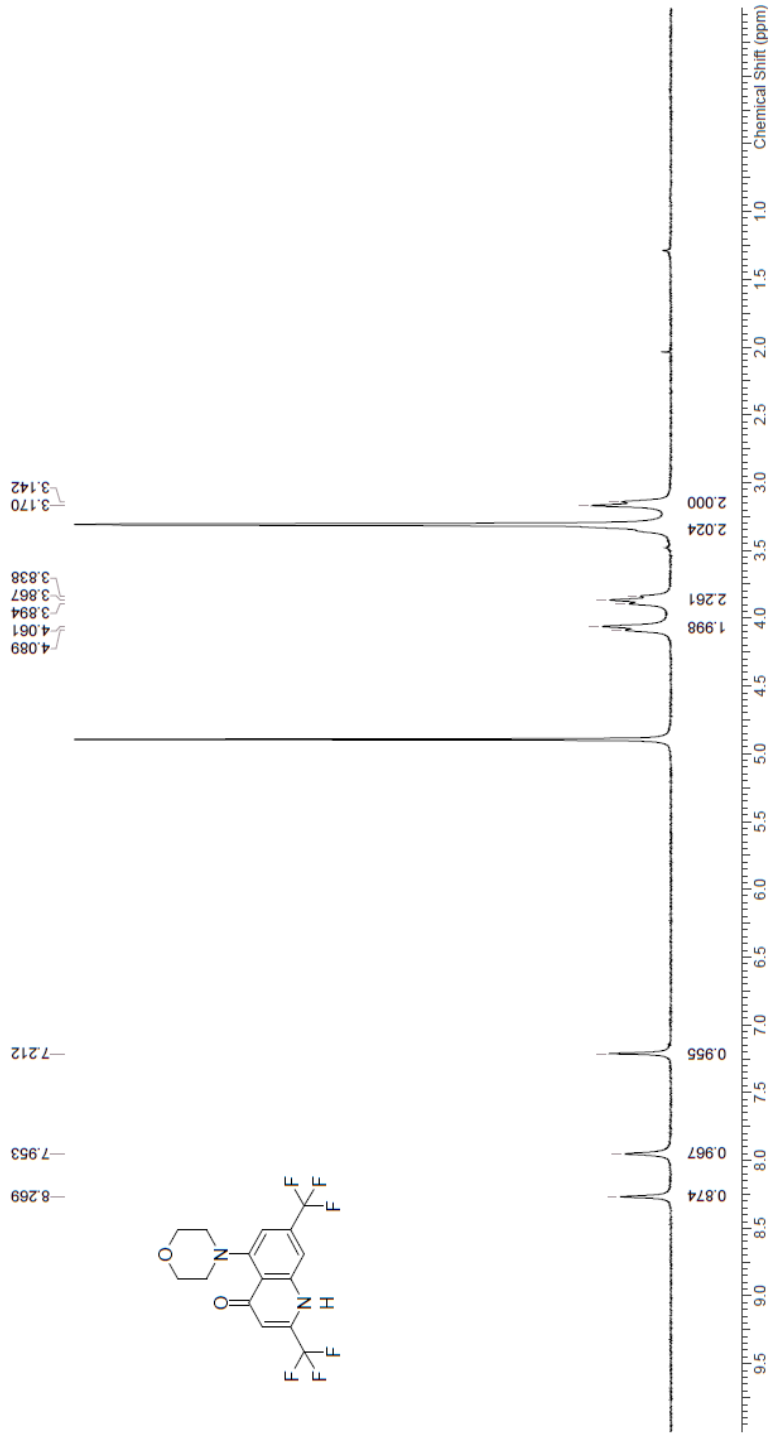
Compound 117 $^{13}C$ NMR.



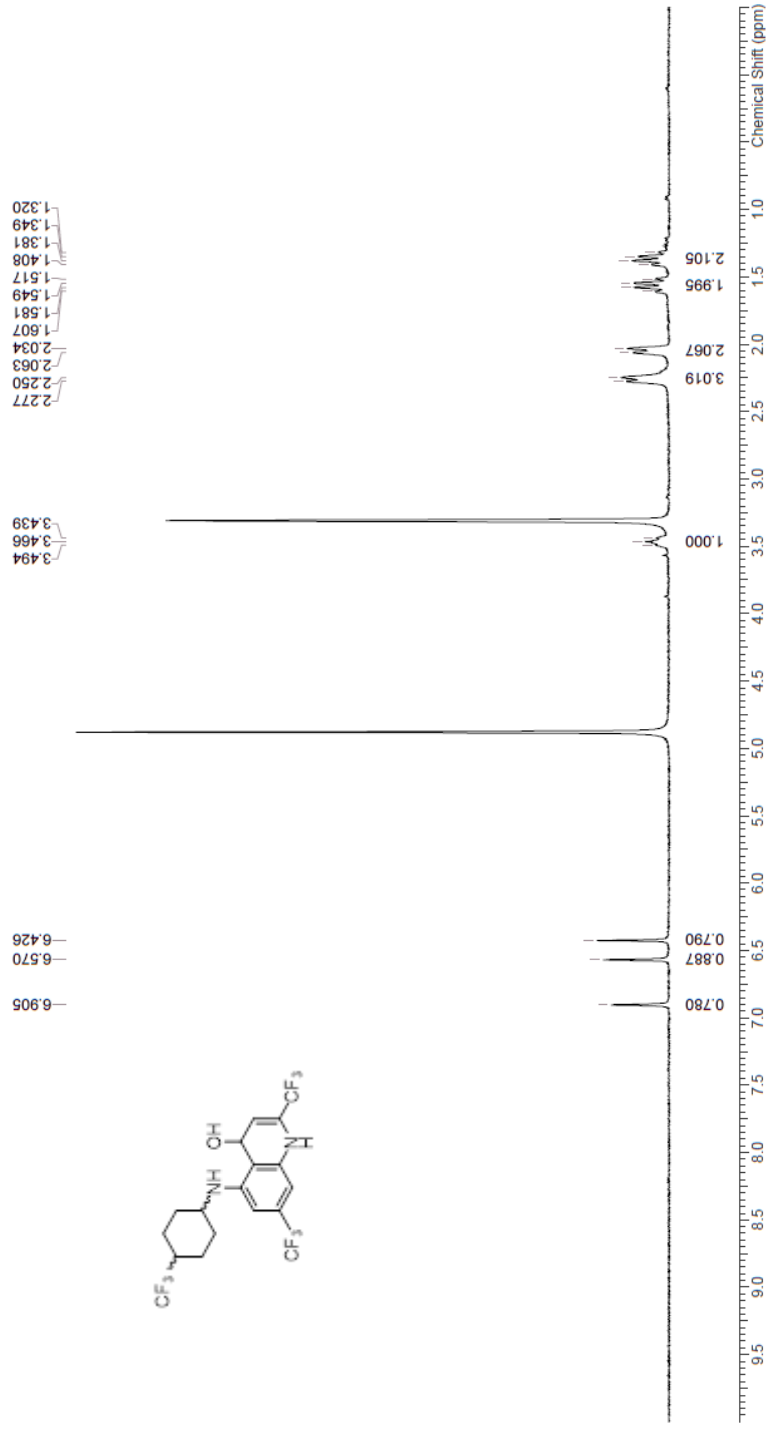
Compound 118 $^{1}H$ NMR.



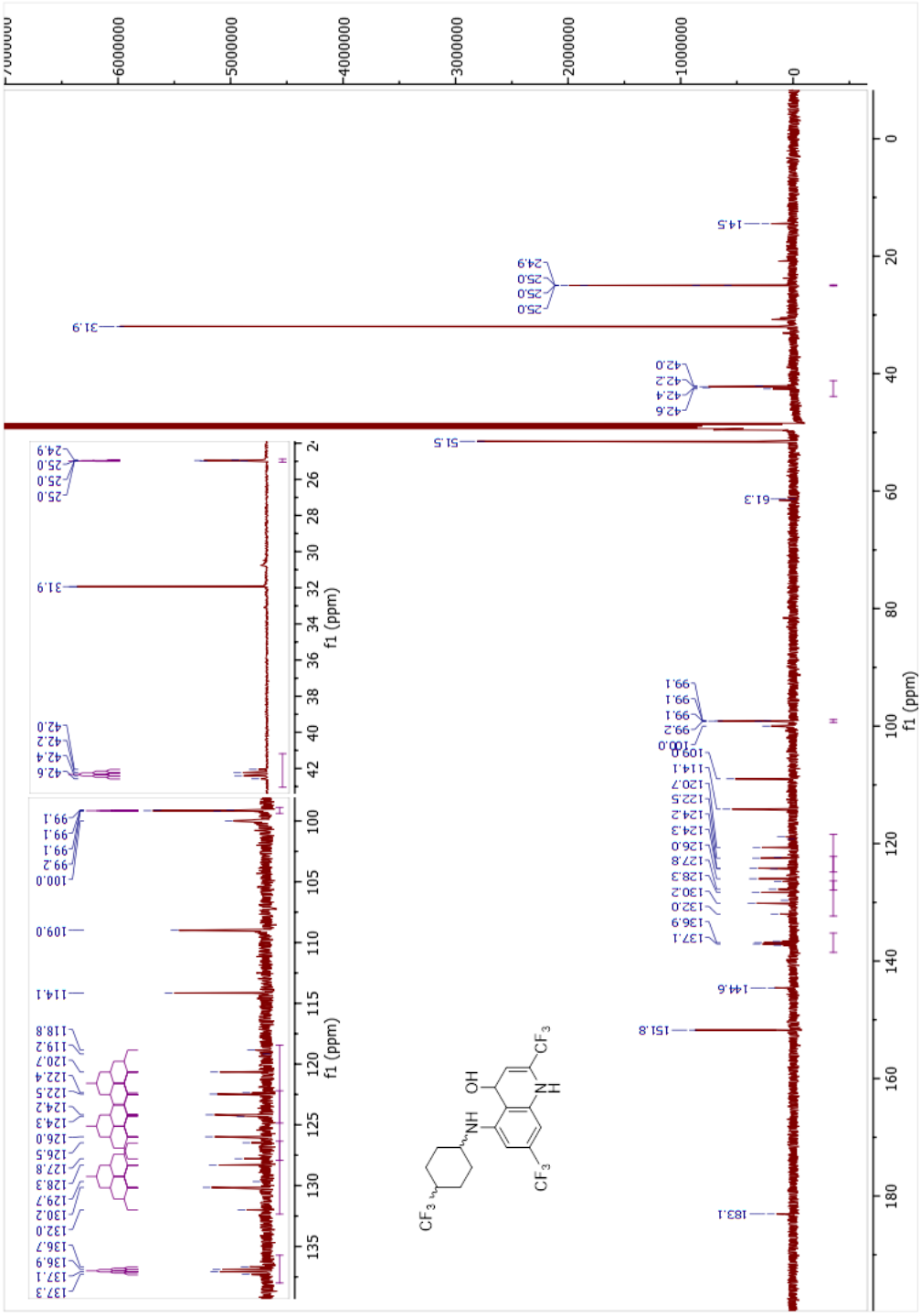
Compound 119 $^{1}H$ NMR.



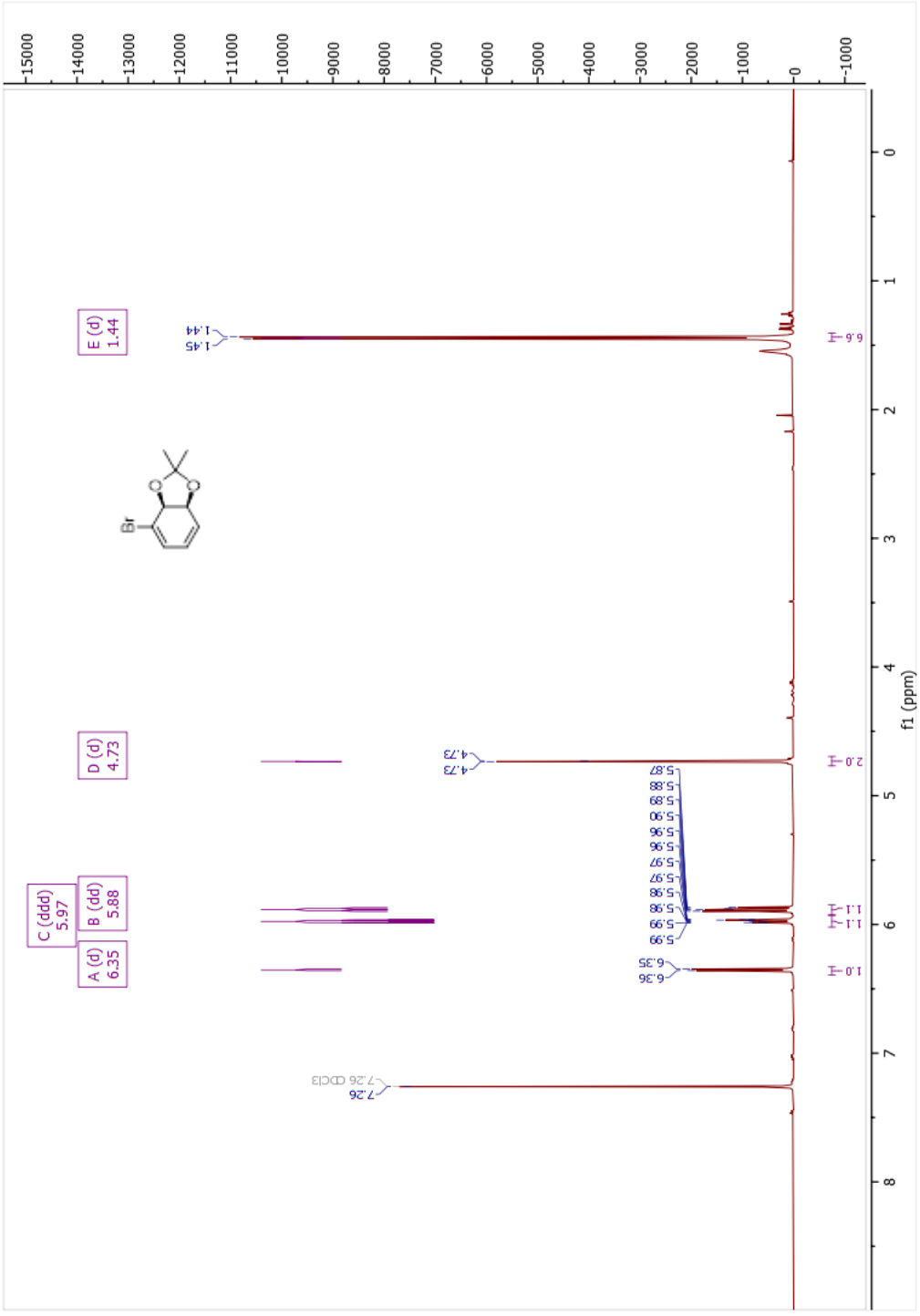
Compound 120 $^{1}$H NMR.



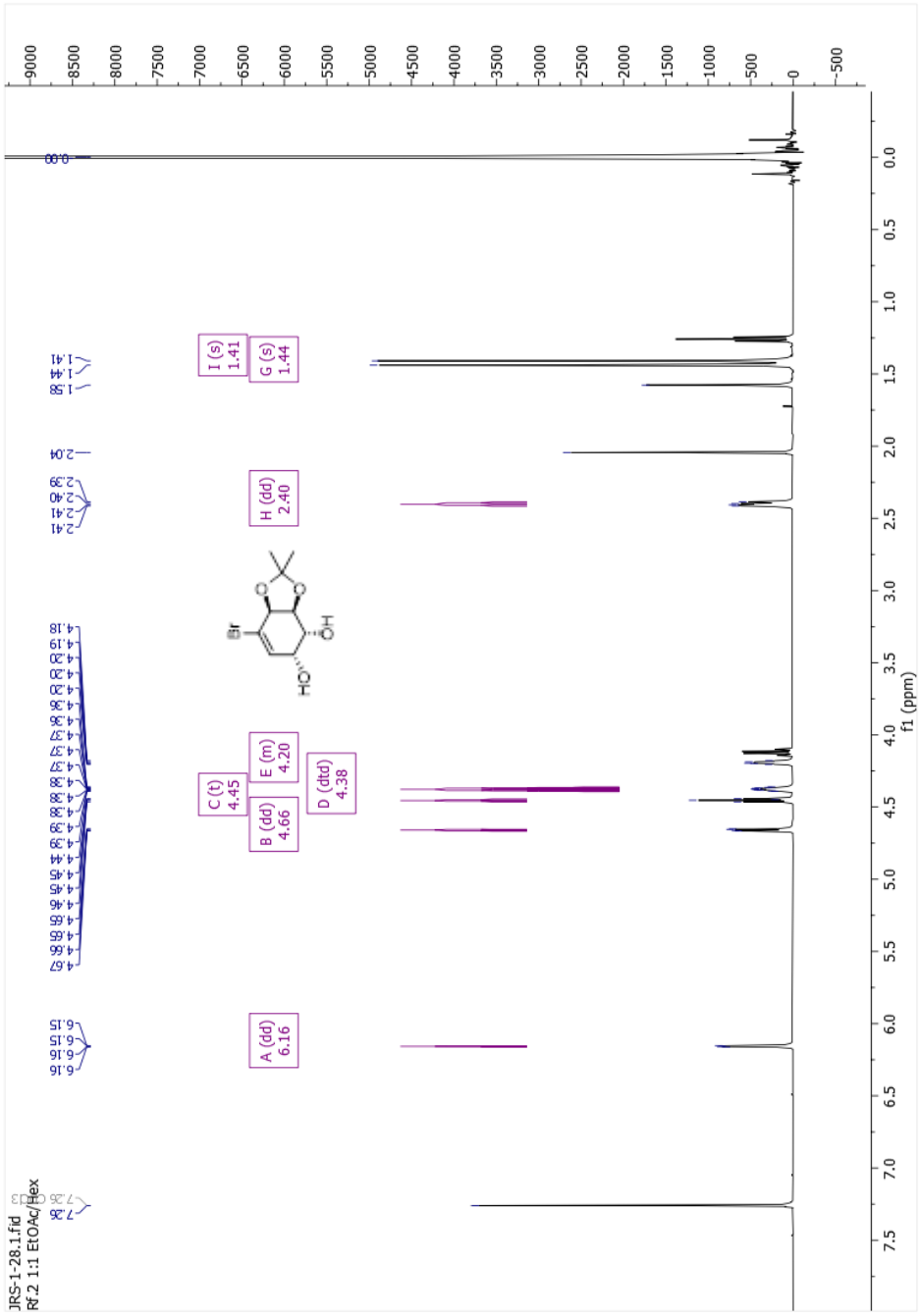
Compound 120 $^{13}C$ NMR.



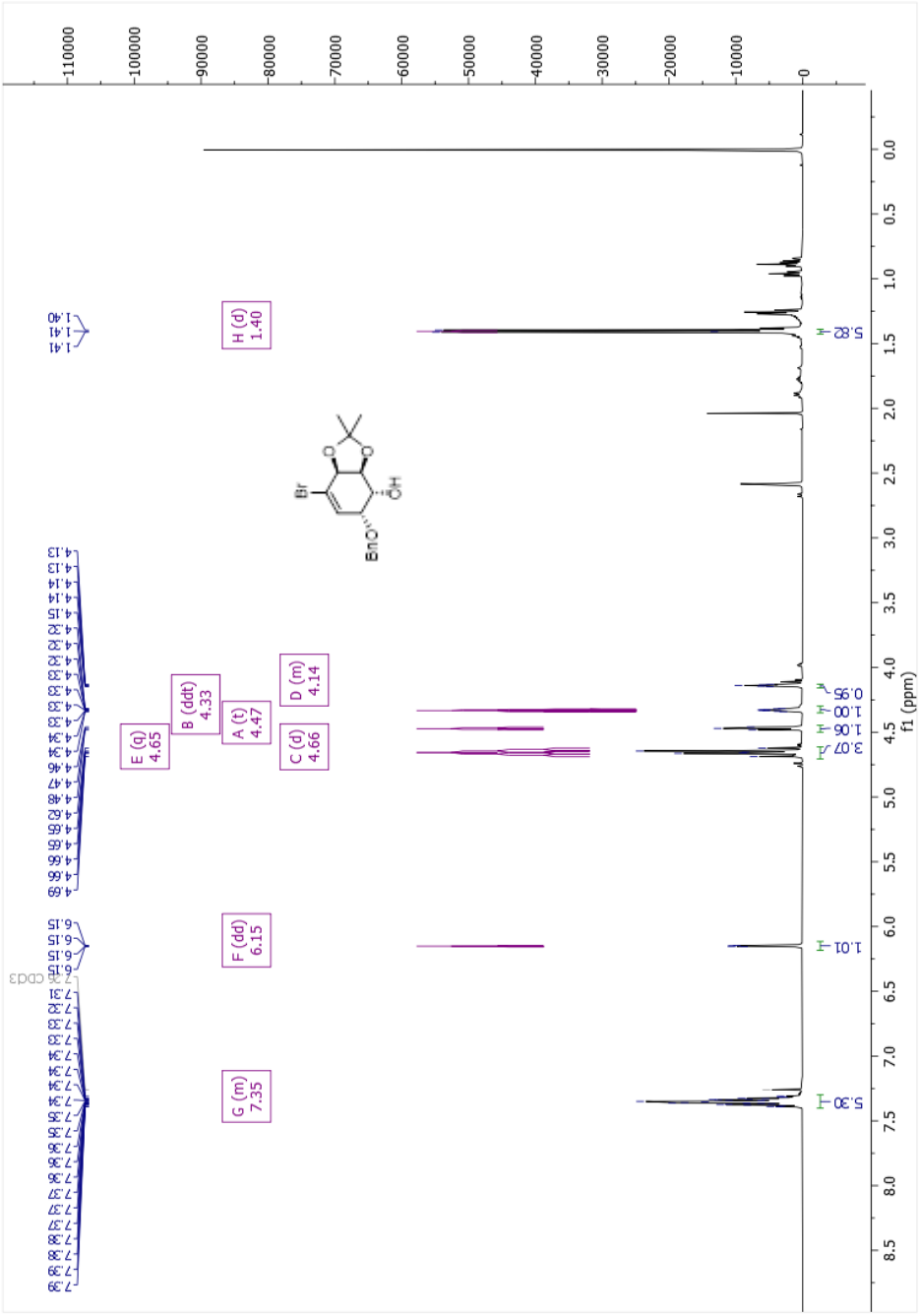
Compound 127 $^{1}$H NMR.



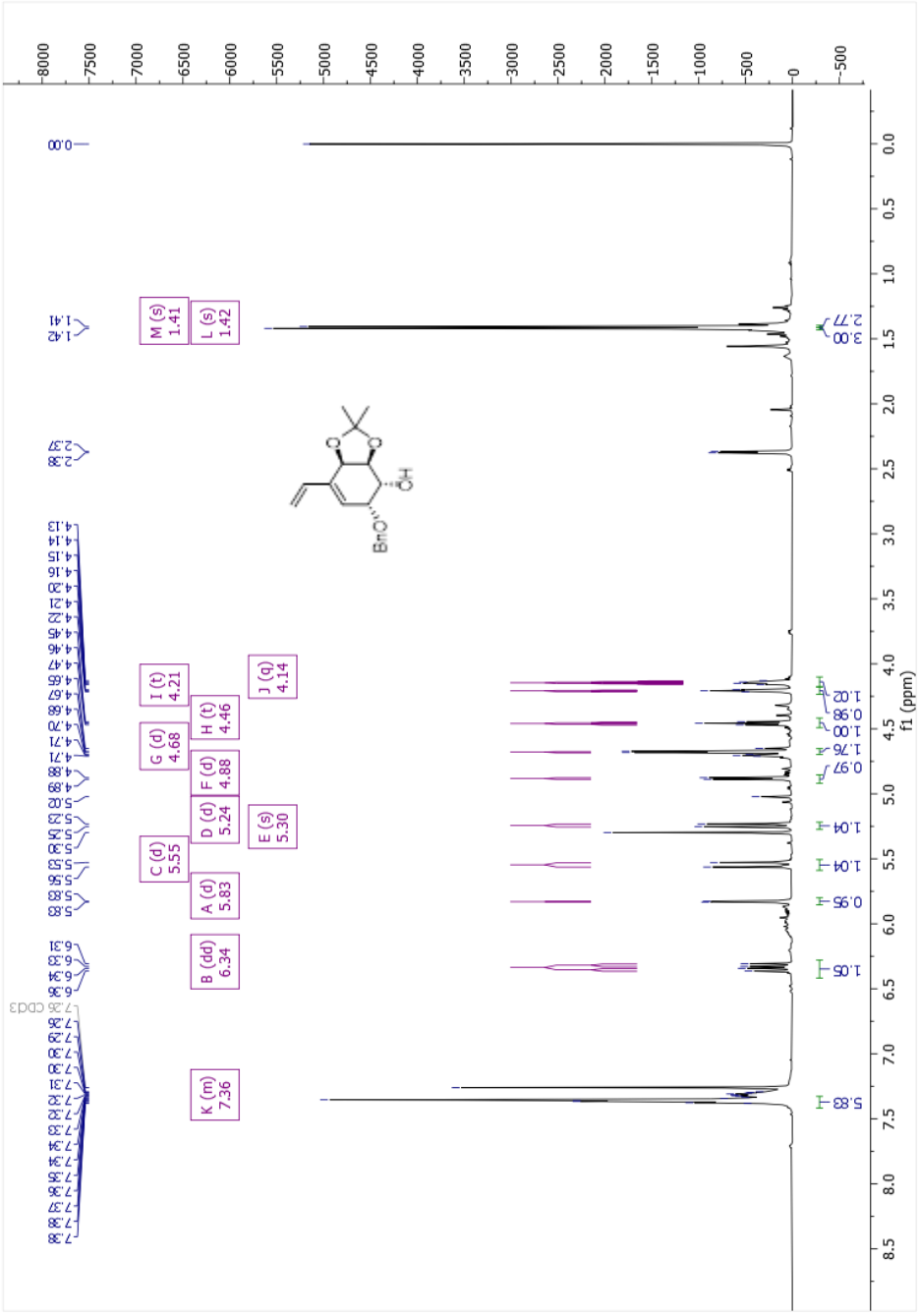
Compound 128 $^{1}H$ NMR.



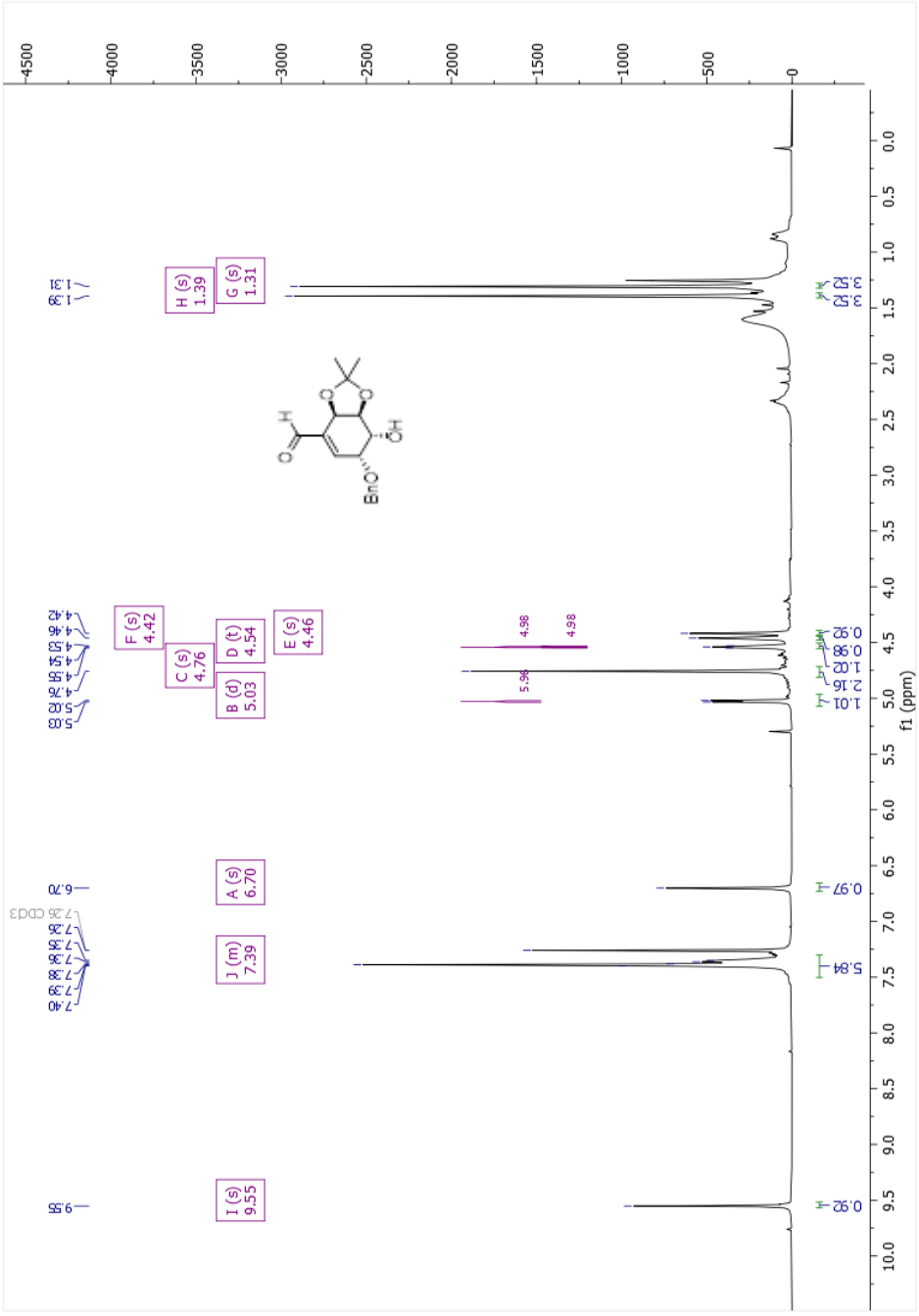
Compound 129 $^{1}H$ NMR.



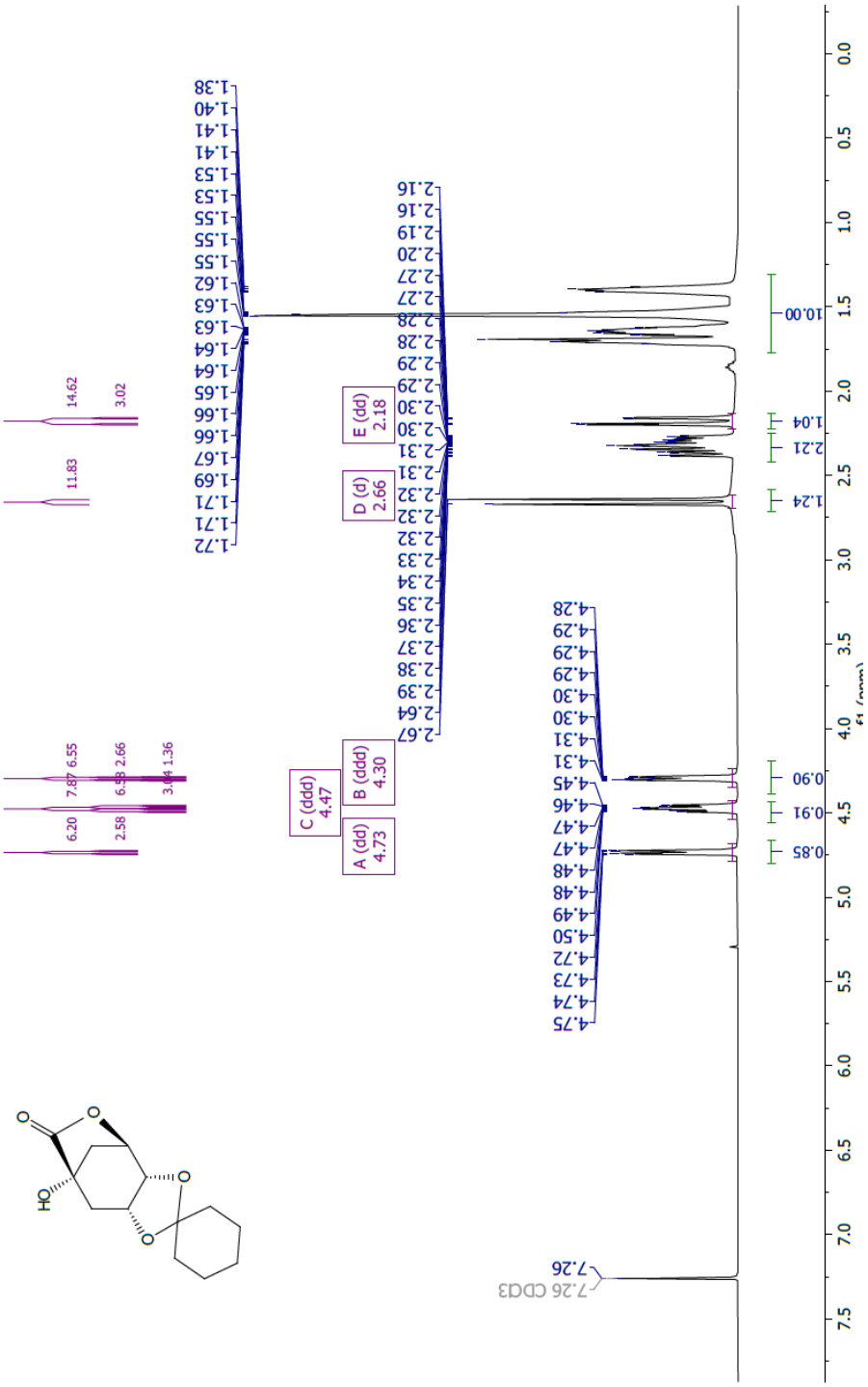
Compound 134 $^{1}H$ NMR.



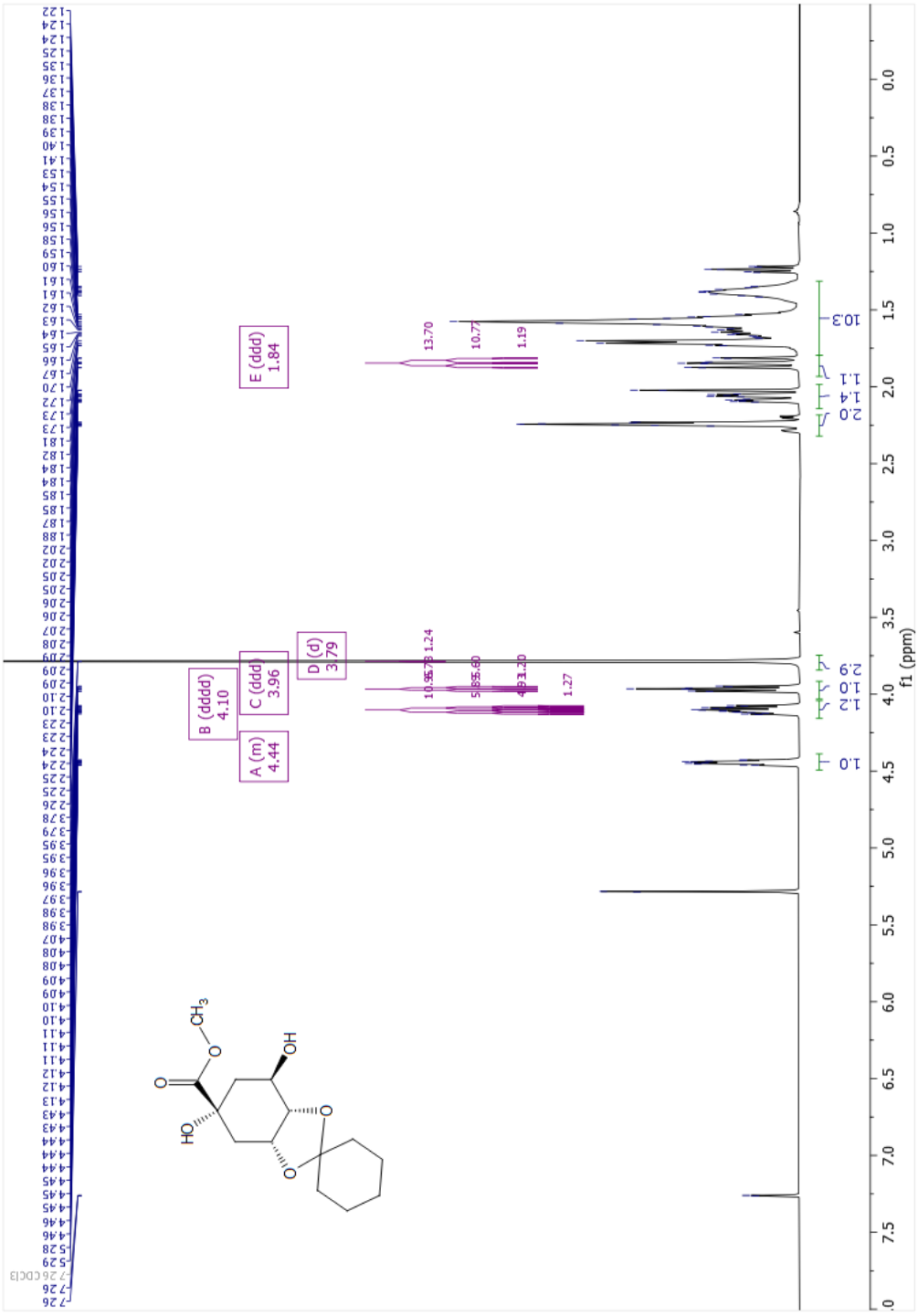
Compound 135 $^{1}$H NMR.



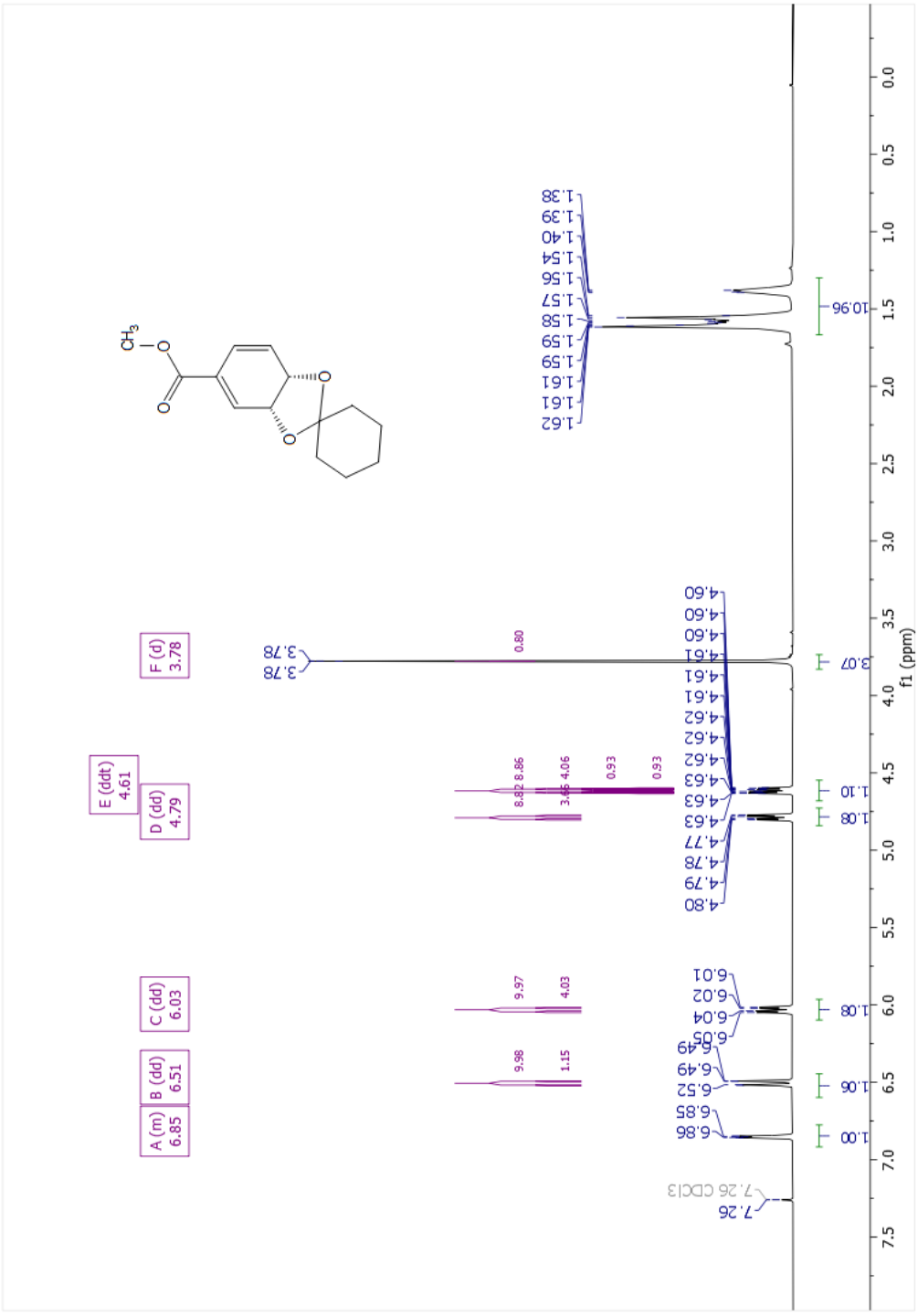
Compound 136 $^{1}H$ NMR.



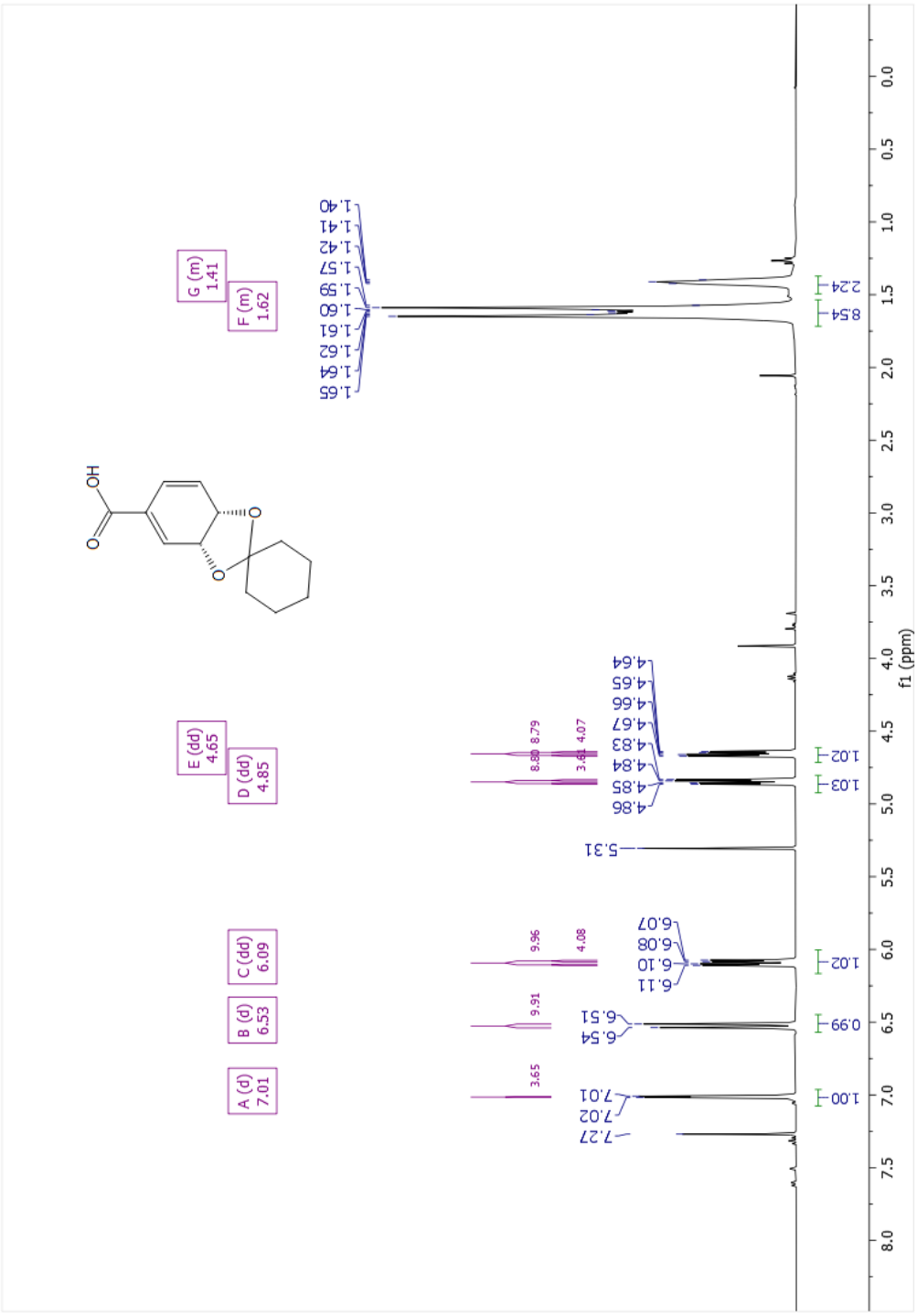
Compound 137 $^{1}H$ NMR.



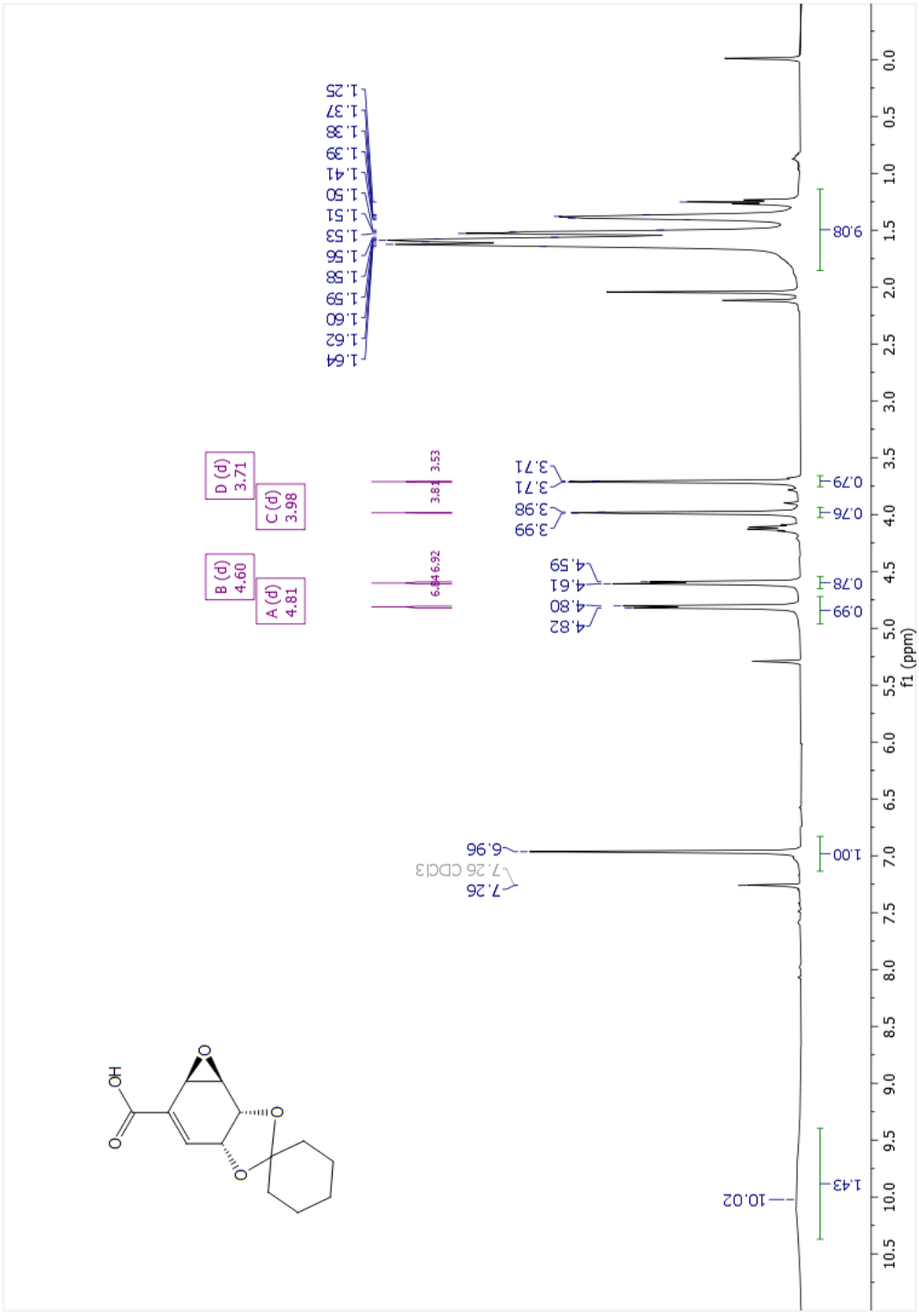
Compound 138 $^{1}H$ NMR.



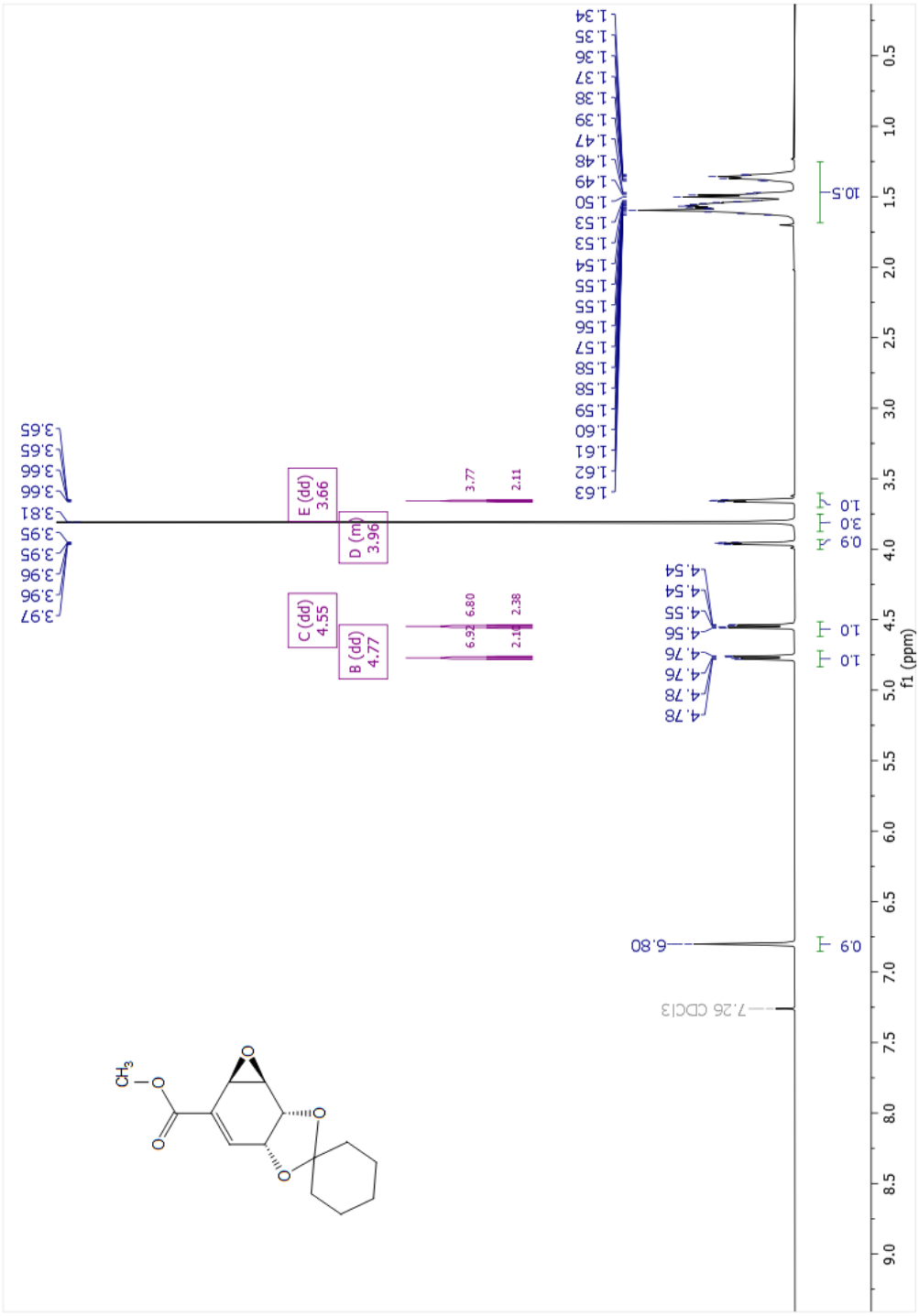
Compound 139 $^{1}H$ NMR.



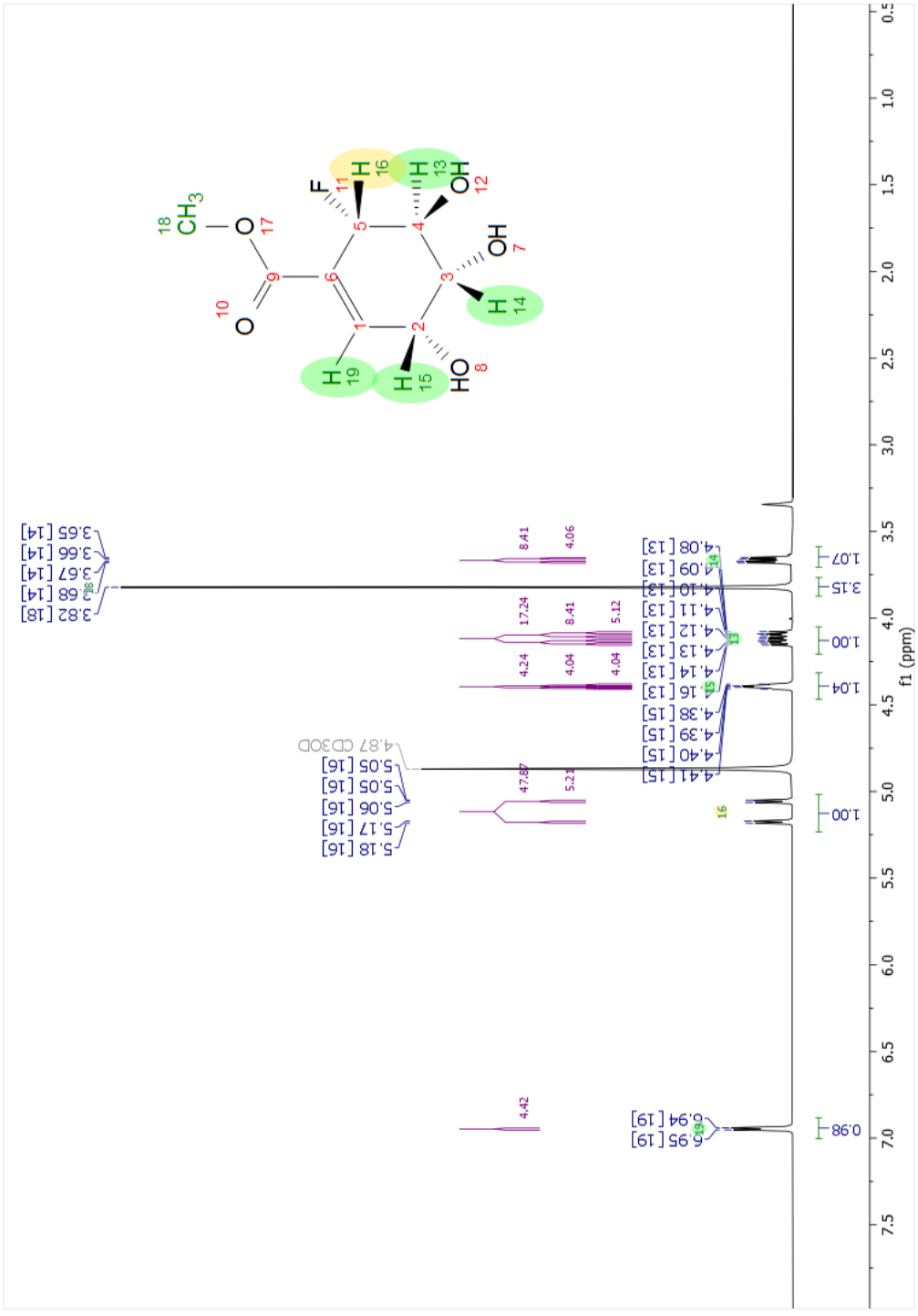
Compound 140 $^{1}$H NMR.



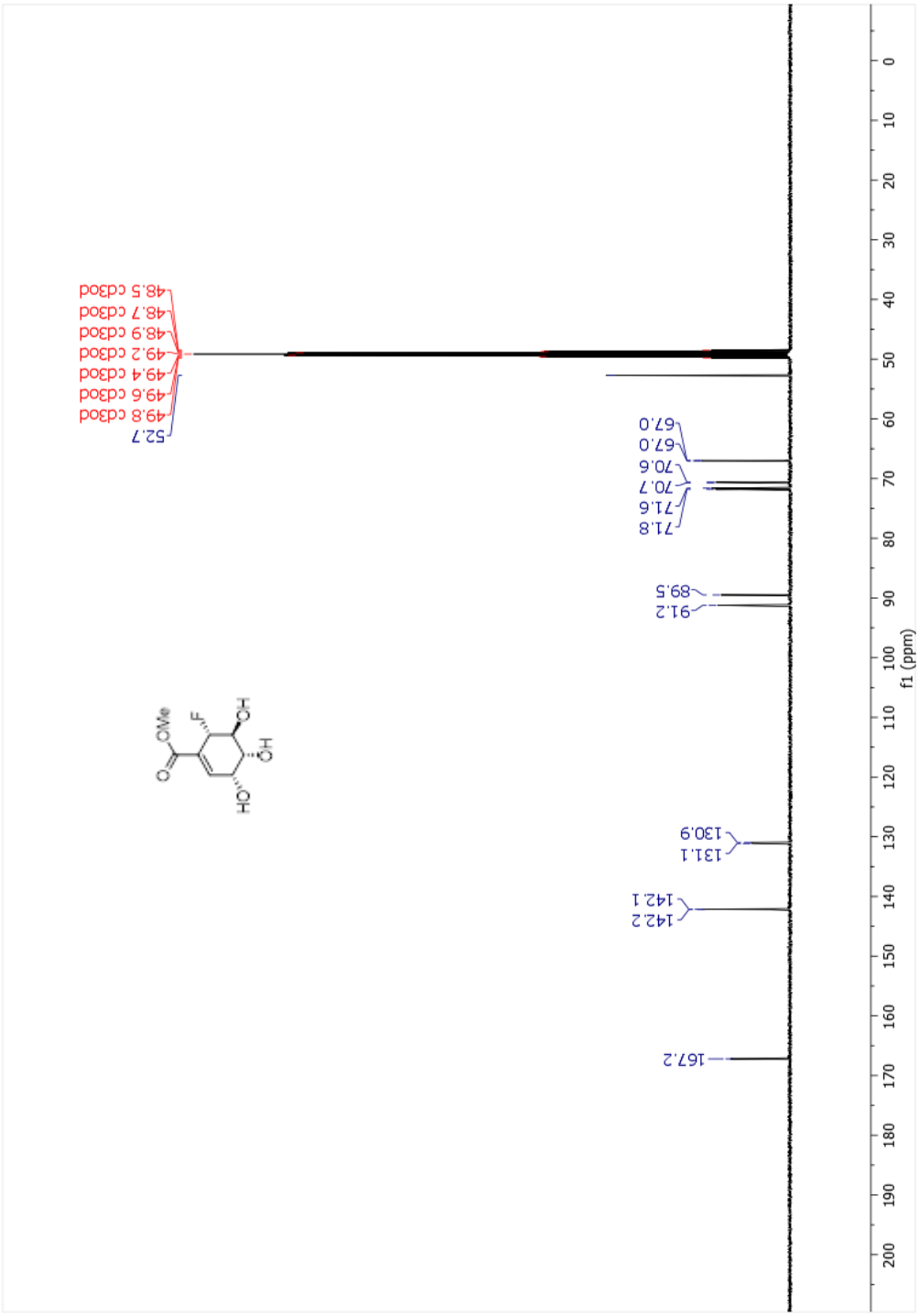
Compound 141 $^{1}$H NMR.



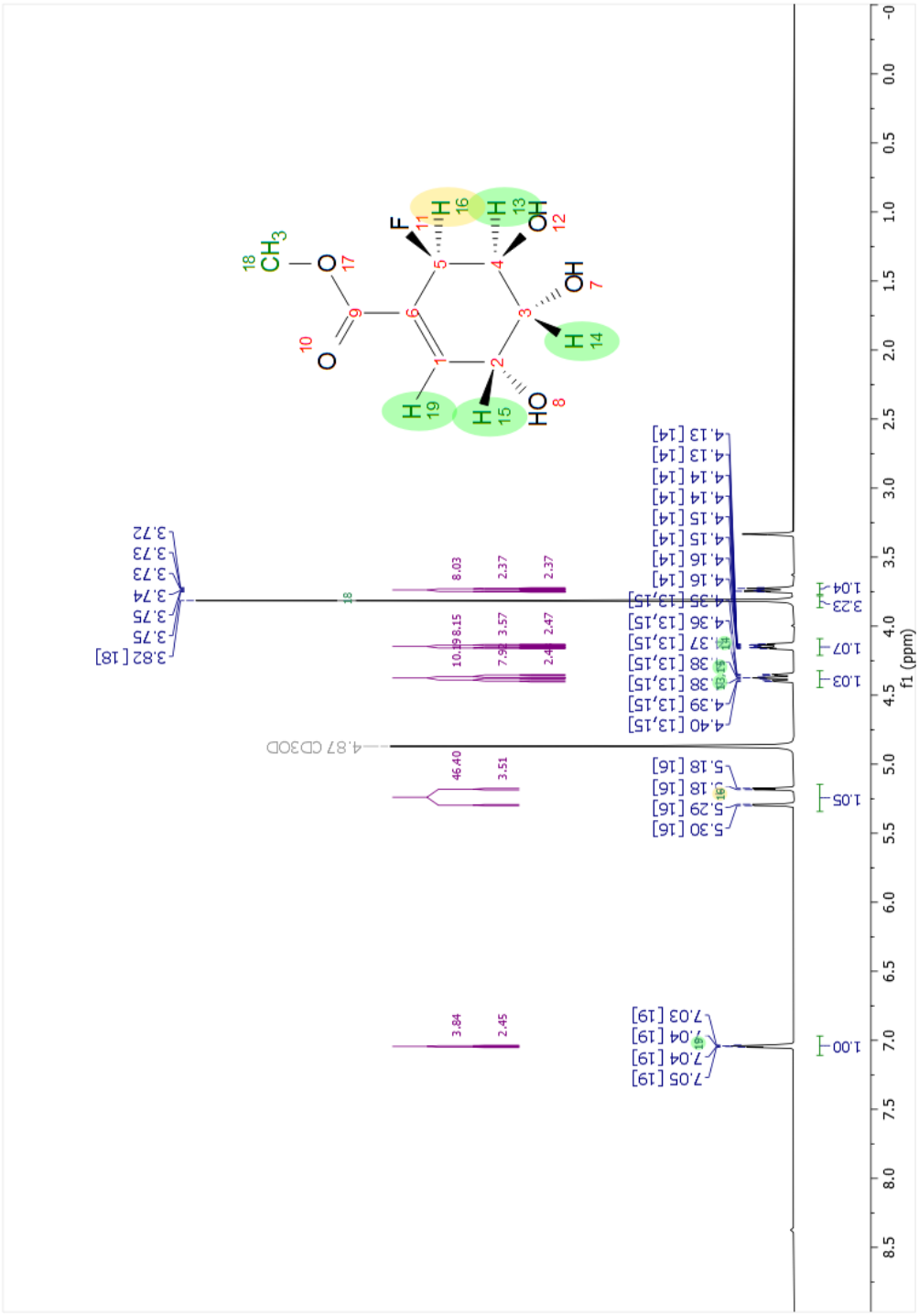
Compound 142 $^{1}$H NMR.



Compound 142 $^{13}C$ NMR.



Compound 143 $^{1}$H NMR.



Compound 143 $^{13}C$ NMR.