University of KwaZulu-Natal

Synthesis, Characterization and Antibacterial Activity of Curcumin and Curcumin-like derivatives

2018

Christina Kannigadu

Synthesis, Characterization and Antibacterial Activity of Curcumin and Curcumin-like derivatives

A Thesis

submitted in partial fulfillment for the requirements

for the award of the degree of

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in the

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College of Agriculture, Engineering & Science,

By

Christina Kannigadu

2018

Supervisor: Prof. N.A. Koorbanally

Co-Supervisor: Prof D. Ramjugernath

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A thesis submitted to the School of Chemistry, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, for the degree of Doctor of Philosophy.

This thesis has been prepared according to **Format 4** as outlined in the guidelines from the College of Agriculture, Engineering and Science which states:

This is a thesis in which chapters are written as a set of discrete research papers, with an overall introduction and final discussion, where one (or all) of the chapters have either been submitted for publication or already been published. Typically these chapters will have been published in internationally recognized, peer- reviewed journals.

Preface

I hereby declare that the thesis entitled "**Synthesis, characterization and antibacterial activity of curcumin and curcumin-like derivatives**" submitted to the University of KwaZulu-Natal for the award of degree of Doctor of Philosophy in Chemistry under the supervision of Professor Neil A. Koorbanally represents original work by the author and has not been submitted in full or part for any degree or diploma at this or any other University. Where use was made of the work of others it has been duly acknowledged in the text. This work was carried out at the School of Chemistry and Physics, University of KwaZulu-Natal, Westville campus, Durban, South Africa.

Signed: _____
Christina Kannigadu, MSc (UKZN)

As the candidate's supervisor, I have approved this dissertation for submission

Signed: _____
Prof. Neil A. Koorbanally, PhD (Natal)

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2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then,

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Acknowledgements

I would like to thank Professor Neil Koorbanally for his dedication, constant supervision, guidance, and time throughout my masters and doctoral studies. You have inspired me to be the best that I can possibly be, and have shown me that success is attainable as long as dedication and enthusiasm is present. I am honoured and grateful for the amount of faith and patience you have shown to me. Your time and dedication to me has never gone unnoticed. I have learnt alot from your academic expertise and I sincerely look forward to working with you again in the near future.

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To Thrineshen, my love, thank you for the dedication you have towards me, the advice that you have always offered willingly and the support you have always given to me. You have taught me the value of a lot of important things and I cannot express how much I appreciate having you in my life. You have been there for me through a lot of my rough times offering words of encouragement that meant more to me than you could have ever possibly realized.

Lastly, I would like to thank my loving family for all their support throughout my entire life. To my mum, thank you for the never failing encouragement, the constant upliftments and the love you have shown me. To my brothers, Shadrick and Shannon, thank you for everything you have done for me. You have guided me throughout my life and I am ever grateful for it. I cannot express my gratitude for all your sacrifices for me.

Abstract

This thesis consists of the synthesis, characterization and antibacterial activity of three series of curcumin and curcumin-like compounds. The curcumins were synthesised from acetylacetone and benzaldehydes whilst curcumin-like compounds were synthesised from acetone and either benzaldehydes or quinoline aldehydes. The curcumins were derivatised to pyrazolines and the curcumin-like compounds to ketopyrazoles with hydrazine hydrate and to spiro barbiturates with barbituric acid. The spiro barbiturates in turn were converted to oximes using hydroxylamine hydrochloride. A total of 52 new compounds were synthesised in this work. These compounds were characterized using ^{1}H, ^{13}C and 2D NMR spectroscopy and mass spectrometry. Both the ketopyrazoles and the spiro barbiturates contained sterogenic centers. Single crystal XRD was used to determine the configuration of these stereogenic centers, where the pyrazole was found to be in the 5*S* and the spiro barbiturates in the 7*R*,11*R* configuration.

The three sets of synthesised compounds was tested for their antibacterial activity against two Gram +ve strains (*Staphylococcus aureus* and methicillin resistant *S. aureus* (MRSA)) and four Gram -ve strains (*Salmonella typhimurium, Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli*). The best activity was seen by the 6-chloro, 6-bromo and 6-methyl derivatives of quinoline ketodienes, which were active against all six strains of Gram +ve and Gram -ve species at 0.98-31.3 μg mL^{-1}, with the exception of the 6-bromo derivative having lower activity against *S. aureus* at 250 μg mL^{-1}. This was followed by the curcumin pyrazolines where several compounds showed good antibacterial activity: the 3-Cl pyrazoline showed activity against both the Gram +ve MRSA (31.3 μg mL^{-1}) and Gram –ve *K. pneumonia* (7.8 μg mL^{-1}), the 2,4-difluoro pyrazoline showed activity against Gram –ve *K. pneumonia* (0.98 μg mL^{-1}), and the 3-methoxy-4-(4-chlorobenzyloxy) curcumin derivative

and 3-methoxy-4-hydroxy pyrazoline showed activity against both *S. aureus* and MRSA at 31.3-62.5 μg mL^{-1}. A further compound the 4-trifluoro curcumin pyrazoline was also active against the Gram –ve *E. coli* with a MBC of 31.3 μg mL^{-1}.

Amongst the ketopyrazoles, the chloro derivatives were active at low concentrations with MBC's between 15.6-62.5 μg mL^{-1} against either *S. aureus* or MRSA. In addition, the 4-bromo derivative was also active against MRSA and the 2-chloro derivative against *P. aeruginosa*, both with a MBC value of 31.3 μg mL^{-1}. In contrast, the spiro barbiturates showed activity against the Gram –ve *E. coli* and *P. aeruginosa* with MBC values ranging from 0.98-125 μg mL^{-1}. In particular, the 4-bromo derivative showed excellent activity, better than the standards levofloxacin and ciprofloxacin with MBC values of 0.98 μg mL^{-1}. Conversion to the oximes resulted in loss of activity against all the Gram –ve bacteria. However the 4-trifluoro and 4-bromo spiro barbiturate oximes showed weak activity against MRSA.

List of Abbreviations

^{1}H NMR - Proton nuclear magnetic resonance spectroscopy

^{13}C NMR - Carbon-13 nuclear magnetic resonance spectroscopy

°C - Degrees celsius

$CDCl_3$ - Deuterated chloroform

$CHCl_3$ - Chloroform

COSY - Correlated nuclear magnetic resonance spectroscopy

NOESY - Nuclear Overhauser effect spectroscopy

d - Doublet

DCC - *N,N*-dicyclohexylcarbodiimide

DCM - Dichloromethane

dd - Double doublet

DMSO - Dimethyl sulfoxide

dt - Doublet of triplets

EtOAc - Ethyl actetate

EtOH - Ethanol

FT- IR - Fourier transform - infrared spectroscopy

GC-MS - Gas chromatography - mass spectrometry

HCl - Hydrochloric acid

HMBC - Heteronuclear multiple bond coherence

HPLC - High pressure liquid chromatography

HRMS - High resolution mass spectrometry

HSQC - Heteronuclear multiple quantum coherence

Hz - Hertz

m - Multiplet

MeOH - Methanol

MBC- Minimum bactericidal concentration

MS - Mass spectrometry

s - Singlet

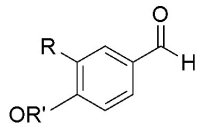
t - Triplet

td - Triplet of doublets

TLC - Thin Layer Chromatography

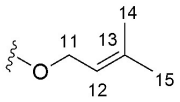
UV - Ultraviolet Spectroscopy

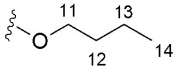
Compounds Synthesized in Chapter 2

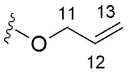


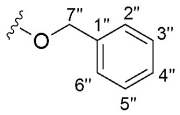
A-2p-x

R' = prenyl, allyl, n-butyl, benzyl,
4-fluorobenzyl, 4-chlorobenzyl

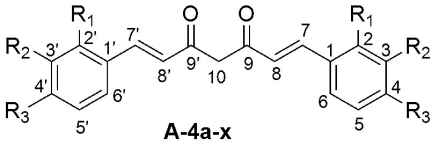
prenyl = 

butyl = 

allyl = 

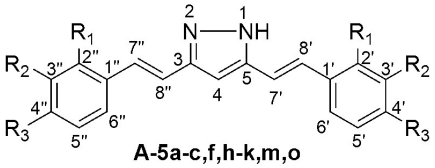
benzyl = 

	R-
A-2p	4-*O*prenyl
A-2q	4-*O*allyl
A-2r	3-OCH_3, 4-*O*allyl
A-2s	4-*O*butyl
A-2t	4-*O*benzyl
A-2u	3-OCH_3, 4-*O*benzyl
A-2v	4-*O*-(4-F)benzyl
A-2w	4-*O*-(4-Cl)benzyl
A-2x	3-OCH_3, 4-*O*-(4-Cl)benzyl



A-4a-x

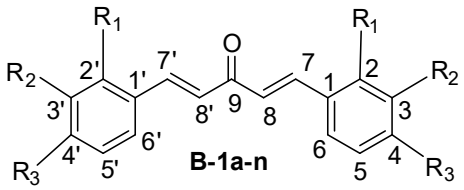
	R-
A-4a	2-Cl
A-4b	3-Cl
A-4c	4-Cl
A-4d	2-F
A-4e	3-F
A-4f	4-F
A-4g	2,4-F
A-4h	3,4-F
A-4i	2-CF_3
A-4j	3-CF_3
A-4k	4-CF_3
A-4l	2-OCH_3
A-4m	4-OCH_3
A-4n	2,4-diOCH_3
A-4o	3-OCH_3, 4-OH



A-5a-c,f,h-k,m,o

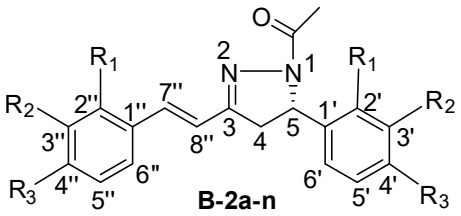
	R-
A-5a	2-Cl
A-5b	3-Cl
A-5c	4-Cl
A-5f	4-F
A-5g	2,4-F
A-5h	3,4-F
A-5i	2-CF_3
A-5j	3-CF_3
A-5k	4-CF_3
A-5m	4-OCH_3
A-5o	3-OCH_3, 4-OH

Compounds Synthesized in Chapter 3



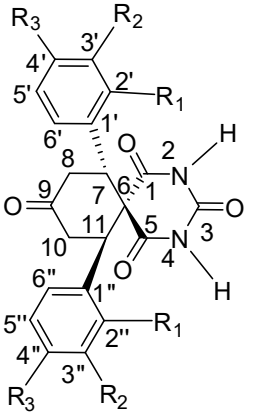
B-1a-n

	R-
B-1a	2-Cl
B-1b	3-Cl
B-1c	4-Cl
B-1d	2-CF_3
B-1e	3-CF_3
B-1f	4-CF_3
B-1g	3-F
B-1h	4-F
B-1i	2,4-diF
B-1j	3,4-diF
B-1k	2-OCH_3
B-1l	4-OCH_3
B-1m	4-Br
B-1n	4-NO_2



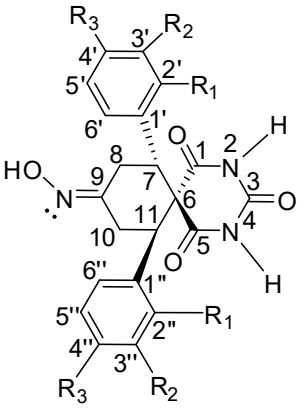
B-2a-n

	R-
B-2a	2-Cl
B-2b	3-Cl
B-2c	4-Cl
B-2d	2-CF_3
B-2e	3-CF_3
B-2f	4-CF_3
B-2g	3-F
B-2h	4-F
B-2i	2,4-diF
B-2j	3,4-diF
B-2k	2-OCH_3
B-2l	4-OCH_3
B-2m	4-Br
B-2n	4-NO_2



B-3a-n

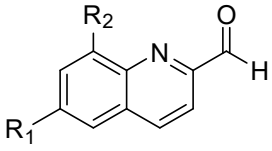
	R-
B-3a	2-Cl
B-3b	3-Cl
B-3c	4-Cl
B-3d	2-CF_3
B-3e	3-CF_3
B-3f	4-CF_3
B-3g	3-F
B-3h	4-F
B-3i	2,4-diF
B-3j	3,4-diF
B-3k	2-OCH_3
B-3l	4-OCH_3
B-3m	4-Br
B-3n	4-NO_2



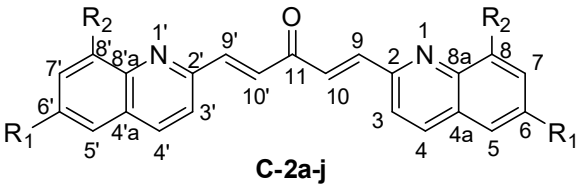
B-4a-n

	R-
B-4a	2-Cl
B-4b	3-Cl
B-4c	4-Cl
B-4d	2-CF_3
B-4e	3-CF_3
B-4f	4-CF_3
B-4g	3-F
B-4h	4-F
B-4i	2,4-diF
B-4j	3,4-diF
B-4l	4-OCH_3
B-4m	4-Br
B-4n	4-NO_2

Compounds Synthesized in Chapter 4



C-1a-j



C-2a-j

	R-
C-2a	6-Cl
C-2b	6-F
C-2c	6-Br
C-2d	6-Me
C-2e	6-OCH_3
C-2f	8-Cl
C-2g	8-F
C-2h	8-Br
C-2i	8-Me
C-2j	8-OCH_3

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Chapter 1 Introduction

1.1 Hybrid molecules

Hybrid molecules are normally derived from two or more different bioactive molecules that have complimentary pharmacophoric functions or different mechanisms of actions which can often display synergistic effects (Raghavan et al., 2015; Ai et al., 2016; Meunier, 2008; Liu et al., 2013; Rather et al., 2013). Synthesis of hybrid drugs with two or more pharmacophores along the same scaffold are much more effective together and have a wide range of pharmaceutical applications (Raghavan et al., 2015; Ai et al., 2016; Meunier, 2008; Rather et al., 2013; Liu et al., 2013). Hybrid molecules enhance the potency of drugs and avoids the administration of multiple drugs, thus reducing toxicity (Arnaud, 2007; Liu et al., 2013) and drug-drug interactions during combinatorial therapies (Wang et al., 2015a; Pokrovskaya and Baasov, 2010; Muregi et al., 2010).

Artemisinin-quinine (**Figure 1-1**) is an example of a hybrid drug that targets *Plasmodium falciparum,* a parasite responsible for the onset of malaria. Artemisinin by itself creates free radicals and binds to proteins of the parasite, thus killing it (Wang et al., 2015b). Replication of the parasite utilises the host's haemoglobin. Quinine targets the replication cycle of the parasite by preventing it from metabolising haemoglobin (Francis et al., 1997). Combined as one molecule, artemisinin-quinine is able to interact directly on the parasite while simultaneously hindering its replication, thus preventing any re-occurrence of the disease (Arnaud, 2007).

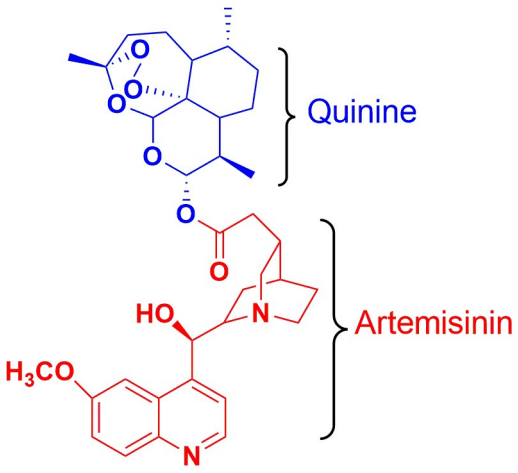


Figure 1-1: Artemisinin-quinine hybrid

A quinazolinone-thiazolidinone hybrid is formed by the combination of a quinazolinone to a thiazolidinone *via* an ethylamine linker (**Figure 1-2**). Quinazolinone inhibits penicillin-binding protein (PBP2a) by targeting both its allosteric and active sites thus impairing the formation of the cell wall in bacteria (Chang et al., 2016). Thiazolidinones can block some pathogenic mechanisms of bacteria and are inhibitors of the bacterial enzyme Mur B, an essential enzyme for the synthesis of bacterial cell walls (Gupta et al., 2016; Benson et al., 1995). Both these drugs have a wide range of biological activities, however when these scaffolds are combined they show good antibacterial activity against strains of *Bacillus cereus*, a bacteria responsible for food poisoning (Shah et al., 2014).

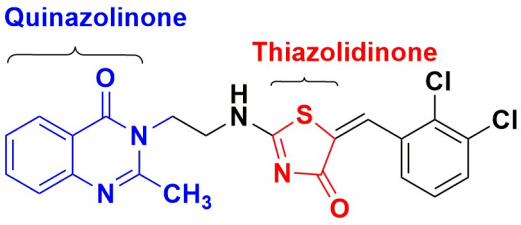


Figure 1-2: Quinazolinone-thiazolidinone hybrid (Shah et al., 2014)

Paclitaxel (PTX) is a mitotic inhibitor used as a chemotherapeutic drug in the treatment of cancer (Ayalew et al., 2017). Paclitaxel binds to tublin and inhibits the disassembly of microtubules preventing mitosis or cell division (Ayalew et al., 2017). It promotes apoptosis by binding and blocking the function of the apoptotic inhibitor protein B-cell Leukemia 2. Peptides are water soluble macromolecules that can be absorbed quickly through the bloodstream (Ayalew et al., 2017; Srivastava et al., 2015). To overcome Paclitaxel's low solubility and strong systemic side effects, a helical peptide was linked to creating a hybrid drug with the same potency but an increased solubility (**Figure 1-3**) (Ayalew et al., 2017; Srivastava et al., 2015).

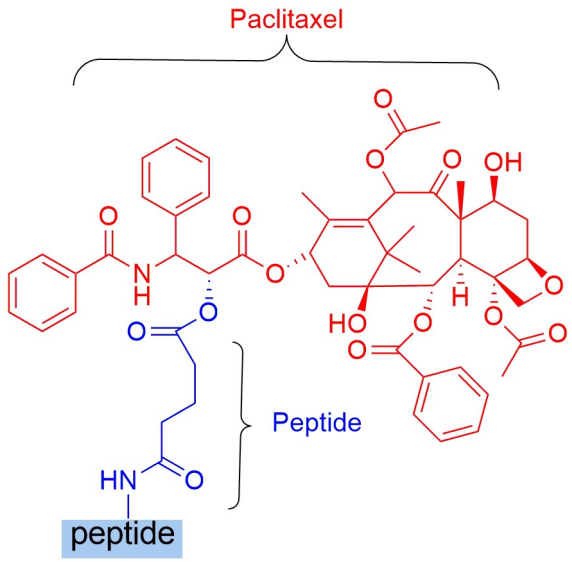


Figure 1-3: Paclitaxel-peptide hybrid drug (PTX-COL-CPP) (Ayalew et al., 2017)

Feruloyl-Donepezil (**Figure 1-4**) is a hybrid molecule which targets different degenerative symptoms of Alzheimer's disease. Donepezil by itself is an acetylcholinesterase inhibitor. Acetylcholinesterase is an enzyme responsible for the degradation of acetylcholine, a neurotransmitter responsible for memory retention (Dias et al., 2017). The donepezil moiety prevents over activity of acetylcholinesterase by inhibiting hydrolysis of acetylcholine

restoring the balance of neurotransmitters in the brain. Oxidative stresses triggers the progression of Alzheimer's disease by inducing the production of amyloid β which slows down brain function (Nagai et al., 2017). Ferulic acid is an oxidative stress inhibitor which binds to amyloid β and inhibits fibril formation protecting neuronal cells against amyloid β induced cytotoxicity (Nagai et al., 2017).

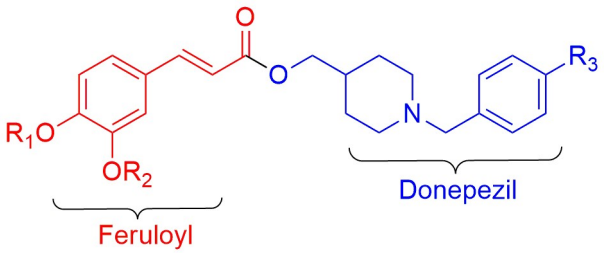


Figure 1-4: Feruloyl-donepezil hybrid derivatives (Dias et al., 2017)

A 1,2,3-triazole tethered β-lactam-chalcone (**Figure 1-5**) is an example of a hybrid molecule with three pharmacophores that showed potent anticancer activity (Kuhn et al., 2004; Singh et al., 2012). β-lactams are well known antibiotic drugs, which display anticancer activity by binding to DNA and inhibiting tumour growth (Kuhn et al., 2004; Singh et al., 2012). Chalcone and triazoles have the ability to induce apoptosis, and inhibit enzymes responsible for proliferation of cancer cells (Kuhn et al., 2004; Singh et al., 2012).

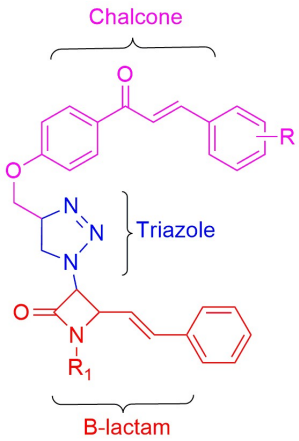


Figure 1-5: 1,2,3-Triazole tethered β-lactam-chalcone bifunctional hybrids (Singh et al., 2012)

1.2 Curcumin

Curcumin is a diarylheptanoid and the principle component in turmeric. Curcumin can exist in its keto and enol tautomeric forms, where the enol tautomer is more energetically stable in solution (**Figure 1-6**) (Akram et al., 2010, Ferrari et al., 2011; Lee et al., 2013).

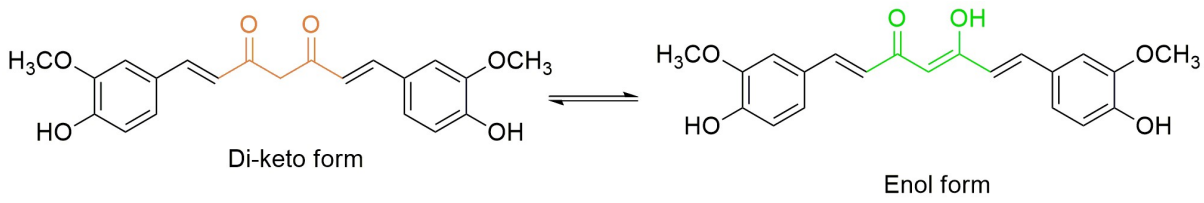
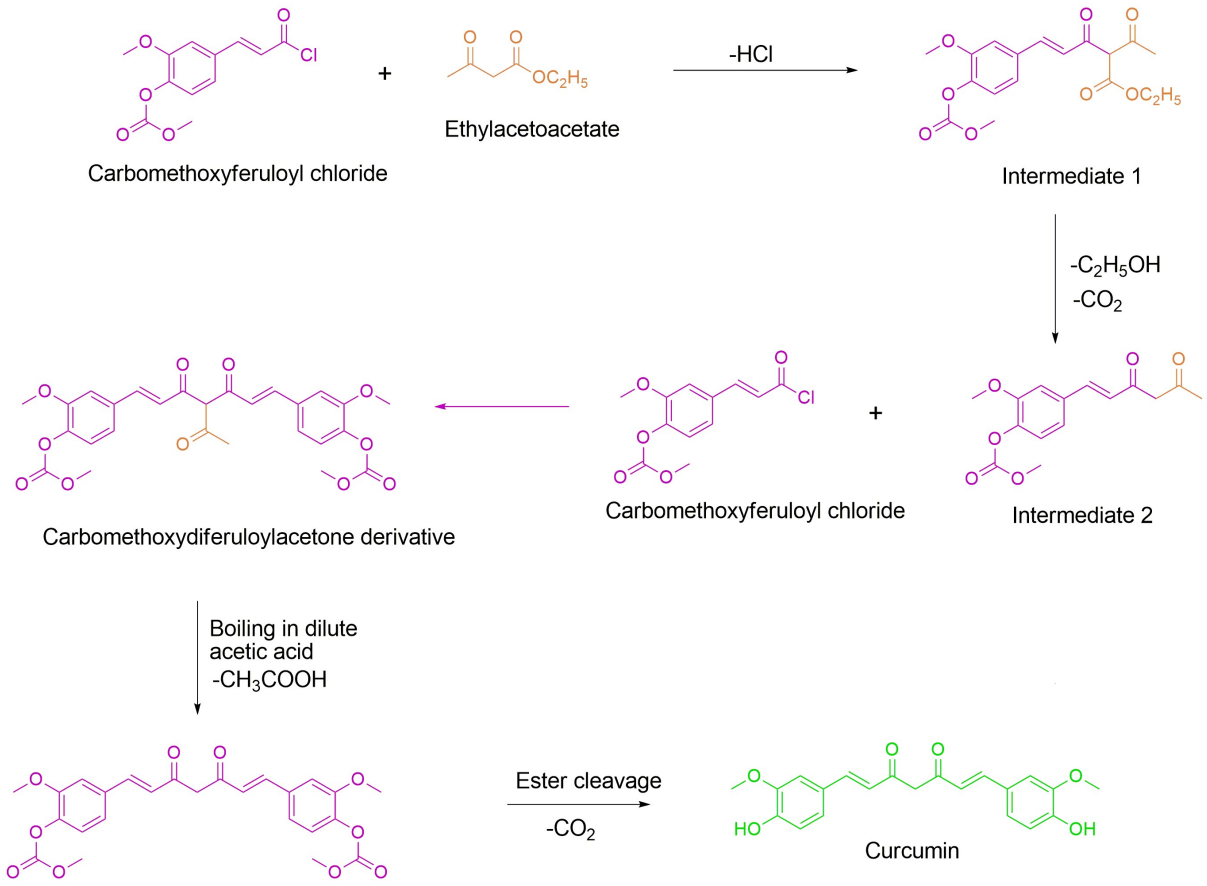


Figure 1-6: Structure of curcumin in its two tautomeric forms (Lee et al., 2013)

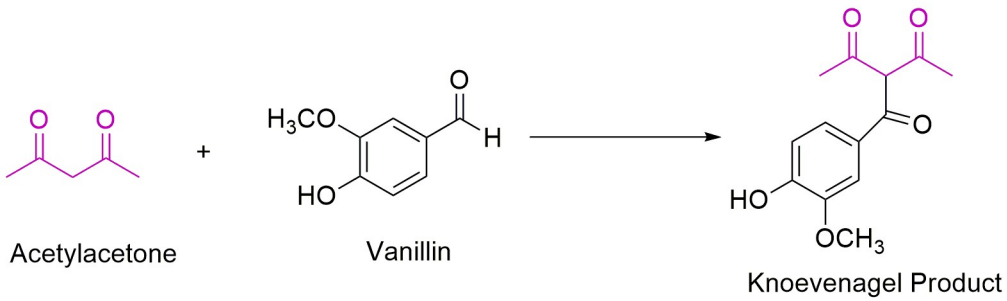
1.2.1 Curcumin synthesis

Curcumin was first synthesised in a five-step reaction from ethyl acetoacetate and carbomethoxy feruloyl chloride (**Scheme 1-1**). In this synthesis the starting materials first underwent a Knoevenagel addition, followed by saponification and decarboxylation to produce an α,β-unsaturated aldehyde, which was then reacted with carbomethoxyferuloyl chloride to produce a carbomethoxydiferuloylacetone derivative. This derivative was then cleaved with acetic acid, and subjected to saponification and decarboxylation to generate the curcumin (Lampe, 1918; Esatbeyoglu et al., 2012).

The reaction of acetylacetone with vanillin proved to be difficult since vanillin underwent Knoevenagel condensation with the central methylene group of acetylacetone rather than aldol condensation with its terminal methyl groups (**Scheme 1-2**). Therefore, this reaction produced low yields of curcumins (Esatbeyoglu et al., 2012).



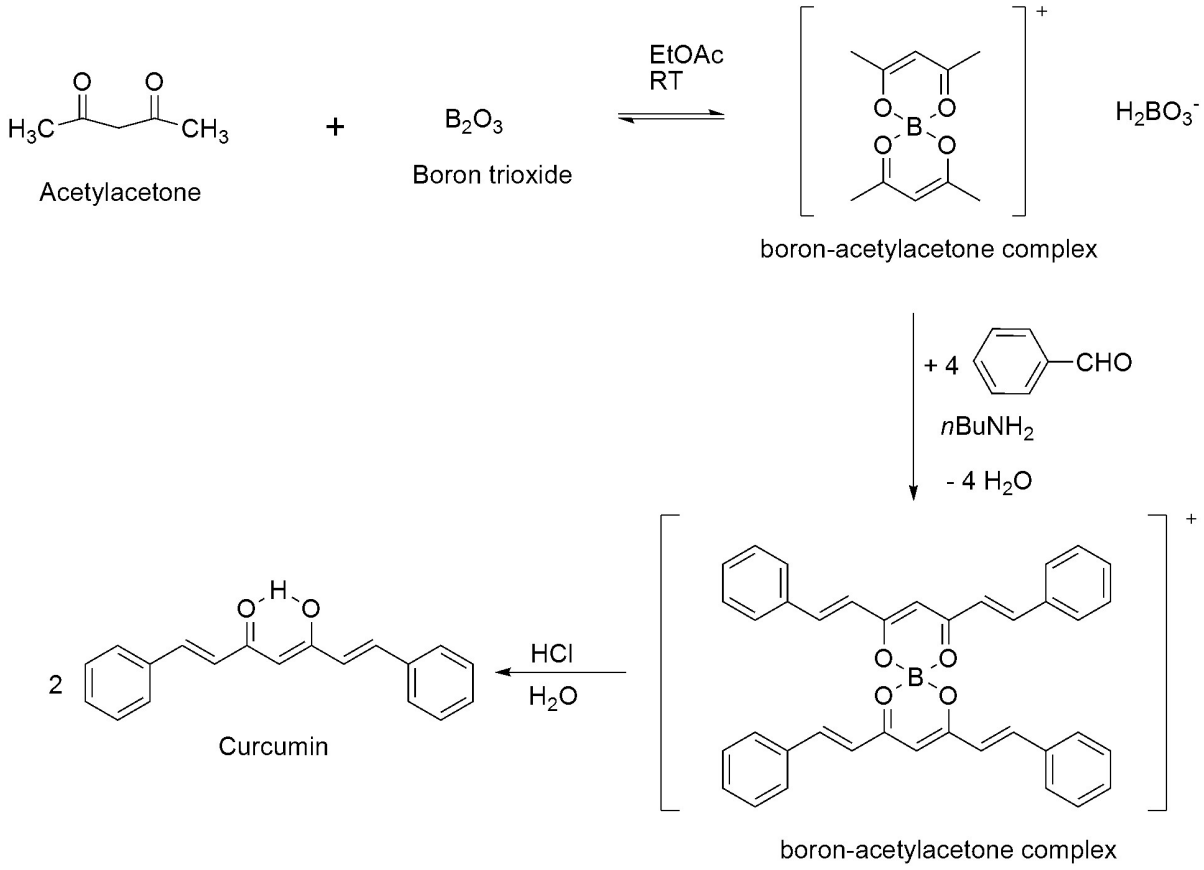
Scheme 1-1: Synthesis of curcumin from ethyl acetoacetate and carbomethoxyferuloyl chloride



Scheme 1-2: Synthesis of Knoevenagel product

To overcome this problem, the reaction was modified by reacting acetylacetone with boric anhydride to form a planar boron-acetylacetone complex. The presence of boron prevented the central methylene carbon of acetylacetone from reacting in a Knoevenagel condensation with vanillin. Thereafter, vanillin underwent an aldol type reaction with the boron-

acetylacetone complex where *n*-butylamine was used as a base to abstract the terminal methyl protons of acetylacetone. The water scavenger *n*-tributyl borate was used to drive the reaction to completion. These modifications produced curcumin in yields of 80% (Pabon, 1964; Esatbeyoglu et al., 2012). This has become a well known method for curcumin synthesis (**Scheme 1-3**) (Handler et al., 2007; Qui et al., 2008; Han et al., 2011; Leow et al., 2014; Okuda et al., 2016). Synthesis of curcumins involving no boric reagent resulted in lower yields (Rao and Sudheel, 2011).

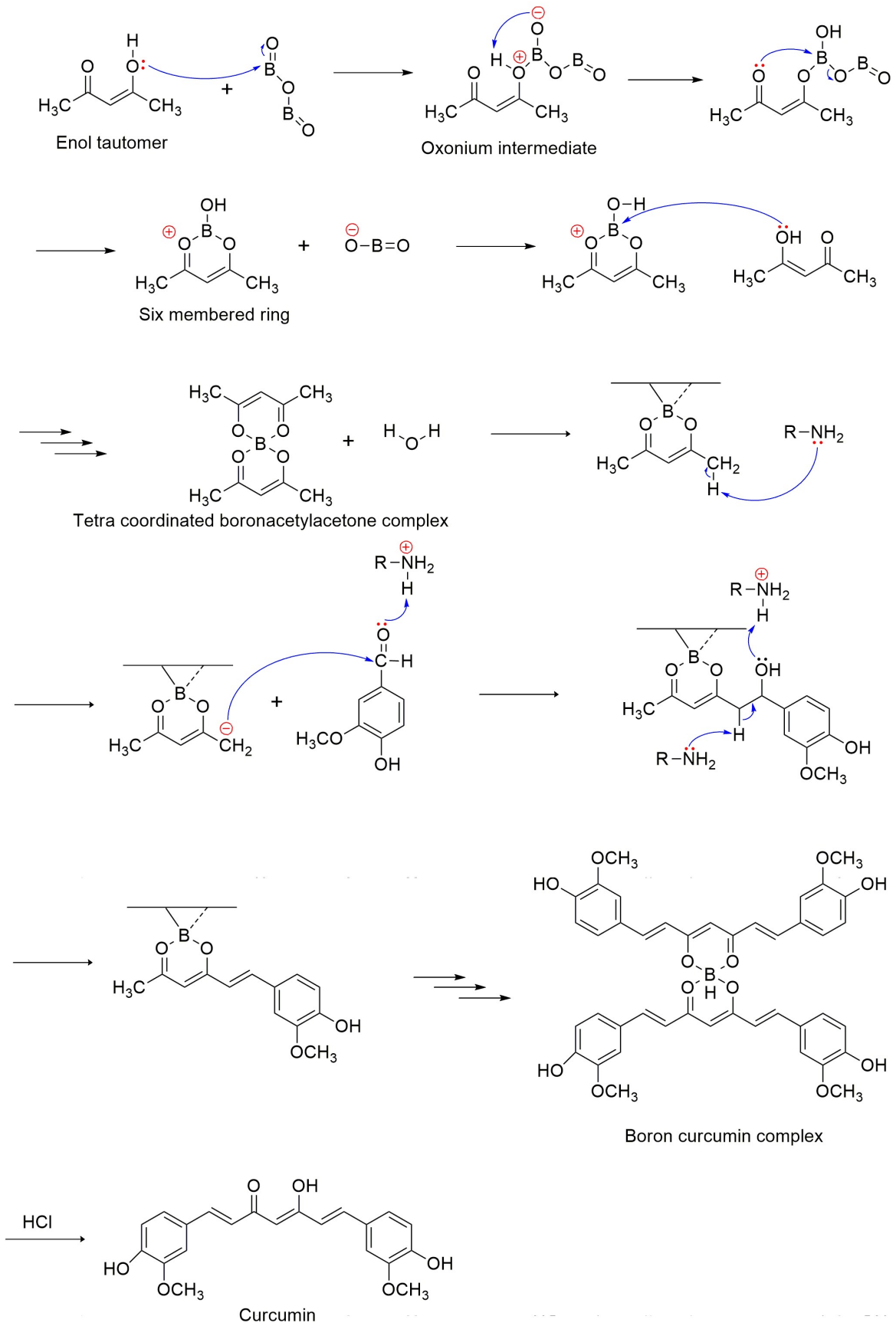


Scheme 1-3: Synthesis of curcumin

A proposed mechanism by which curcumin is synthesised from acetylacetone and vanillin is shown below (**Scheme 1-4**). In solution, acetylacetone converts to its enol tautomer which then reacts with boric anhydride forming an oxonium intermediate. On abstraction of a proton

from the oxonium intermediate, the carbonyl then intramolecularly attacks boron resulting in a six membered ring. The boron in the ring then undergoes an attack from another enol tautomer resulting in a tetra-coordinated boron acetylacetone complex. This complex allows the terminal hydrogens to be more accessible to a weak base such as butylamine, which abstracts them and leads to aldol condensation with benzaldehyde. A boron-curcumin complex is then formed and broken apart by addition of dilute acid resulting in two molecules of curcumin.

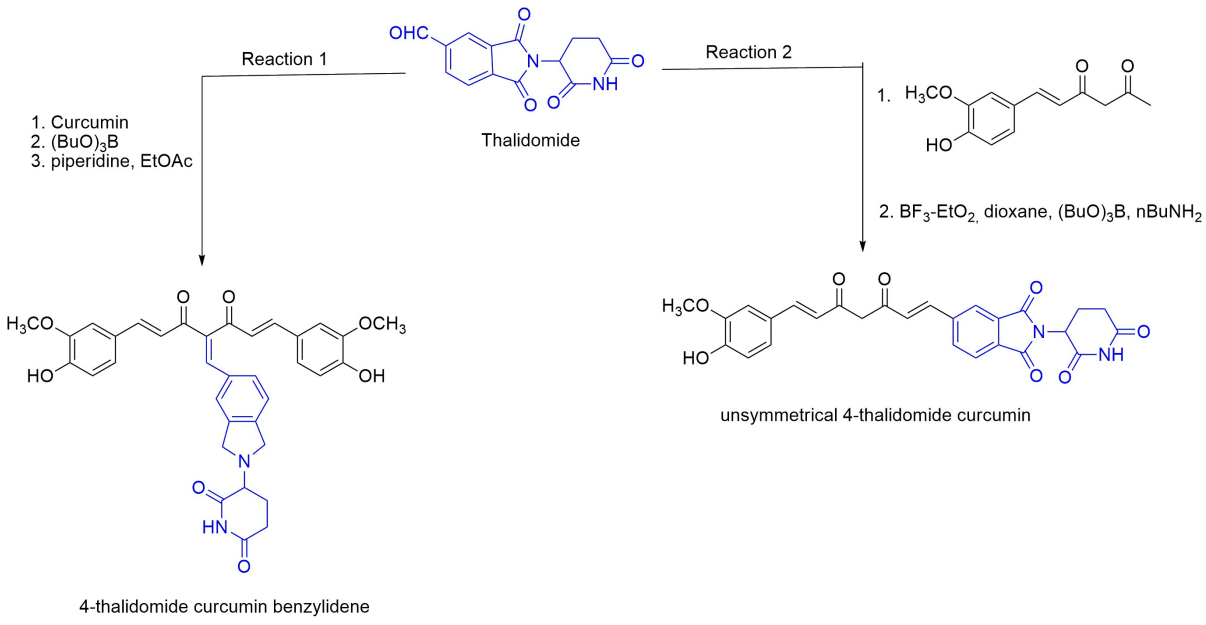
Modifying the carbonyl system of curcumin in an attempt to enhance stability and bioavailability of the diarylheptanoid scaffold (Sahu et al., 2016) has recently been investigated. The dicarbonyl system of curcumin makes it an interesting target for nucleophilic addition. This leads to the possibility of placing other pharmacophores onto the curcumin scaffold which could enhance the overall activity of the molecule.



Scheme 1-4: Proposed mechanism for the synthesis of curcumin

1.2.2 Curcumin hybrids

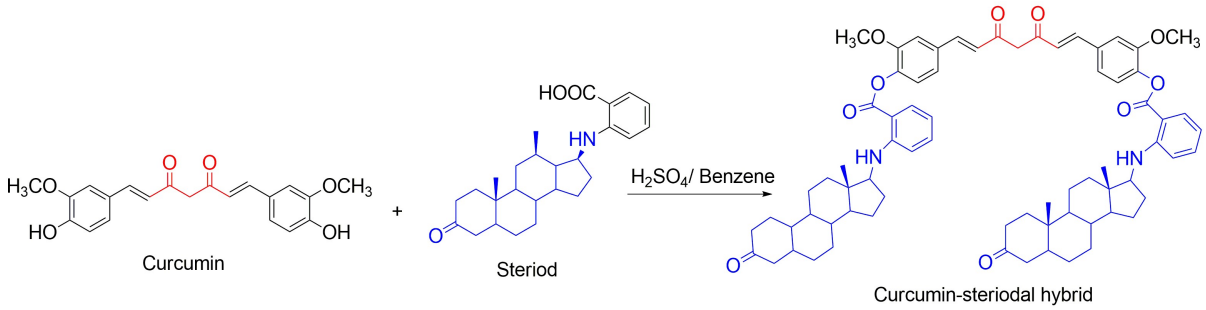
The curcumin structure allows for variability along its scaffold making it a perfect target for incorporation of other pharmacophores. Liu et al. (2013) have reported coupling of a thalidomide pharmacophore to different sites on the curcumin scaffold (**Scheme 1-5**). In the first case thalidomide is incorporated onto the methylene group of curcumin by a Knoevenagel condensation where butyl borate is used as a water scavenger to drive the reaction. In the second reaction, thalidomide is not reacted directly with curcumin but instead undergoes a reaction with a dicarbonyl intermediate forming curcumin in the process. In this reaction, $BF_3 \cdot Et_2O$ complexes to the carbonyl groups of the dicarbonyl intermediate preventing a Knoevenagel condensation and ensuring that the aldehyde group of thalidomide reacts at the terminal methyl group of the dicarbonyl intermediate, forming a curcumin-thalidomide hybrid (Liu et al., 2013).



Scheme 1-5: Synthesis of 4-thalidomide curcumin

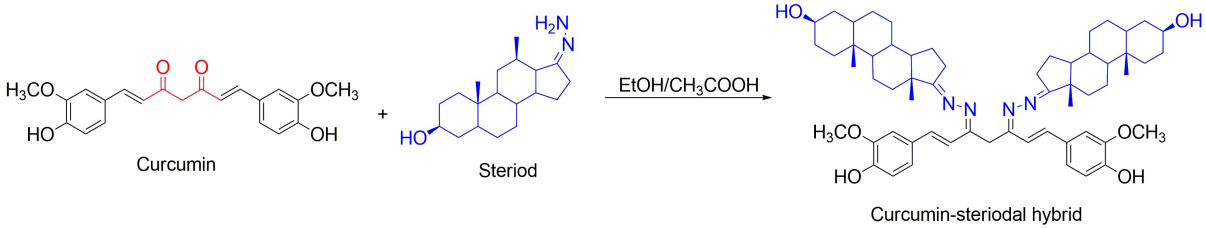
The free hydroxyl groups on the curcumin scaffold has made it a perfect target for esterification reactions. This was observed during the acid catalysed synthesis of curcumin-

steroidal hybrids where base sensitive steroidal carboxylic acids were linked to the aromatic rings of curcumin by ester formation at the *para* substituted hydroxyl groups (**Scheme 1-6**) (Elmegeed et al., 2015).



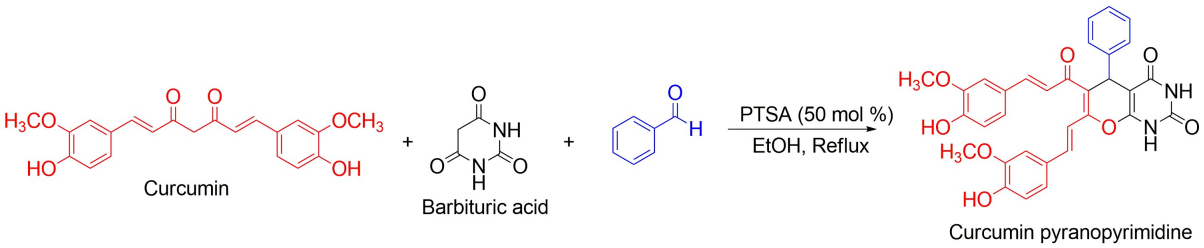
Scheme 1-6: Synthesis of curcumin steroidal hybrids with sulfuric acid and benzene

When reacted with hydrazine type steroids, curcumin formed imines (**Scheme 1-7**) at the carbonyl groups (Elmegeed et al., 2015).



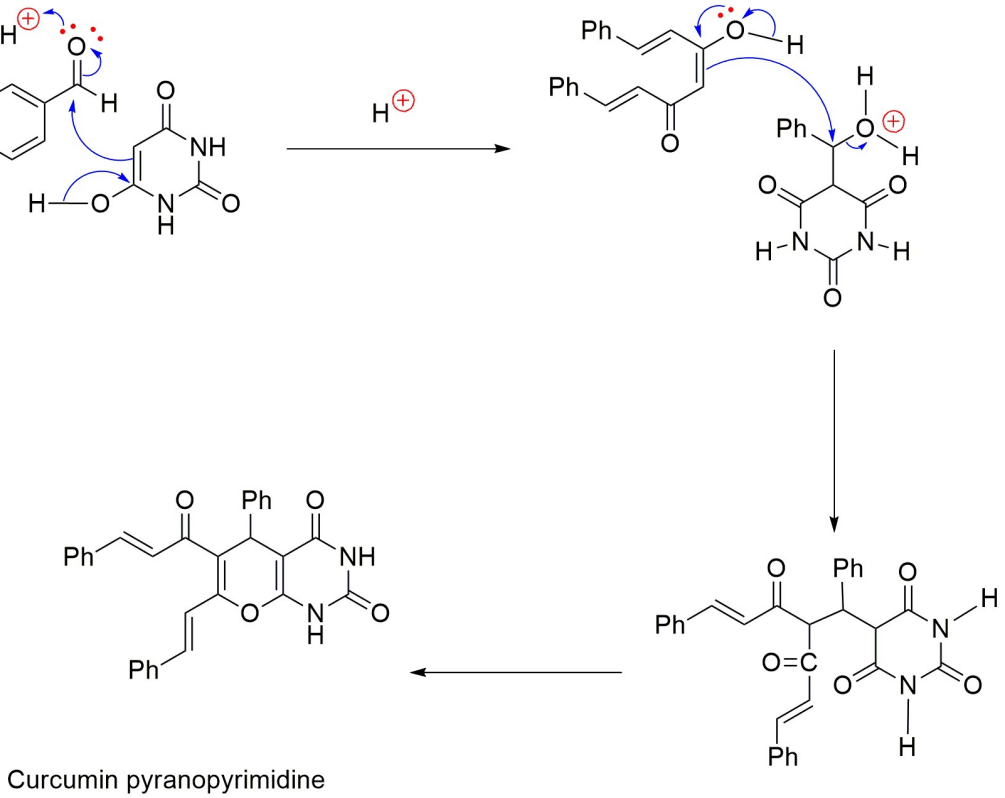
Scheme 1-7: Synthesis of curcumin steroidal hybrids with ethanol and acetic acid

The formation of a curcumin pyranopyrimidine illustrates curcumin's ability to participate in more than one reaction in a one pot synthesis with barbituric acid and benzaldehyde (Yousefi et al., 2015) (**Scheme 1-8**).



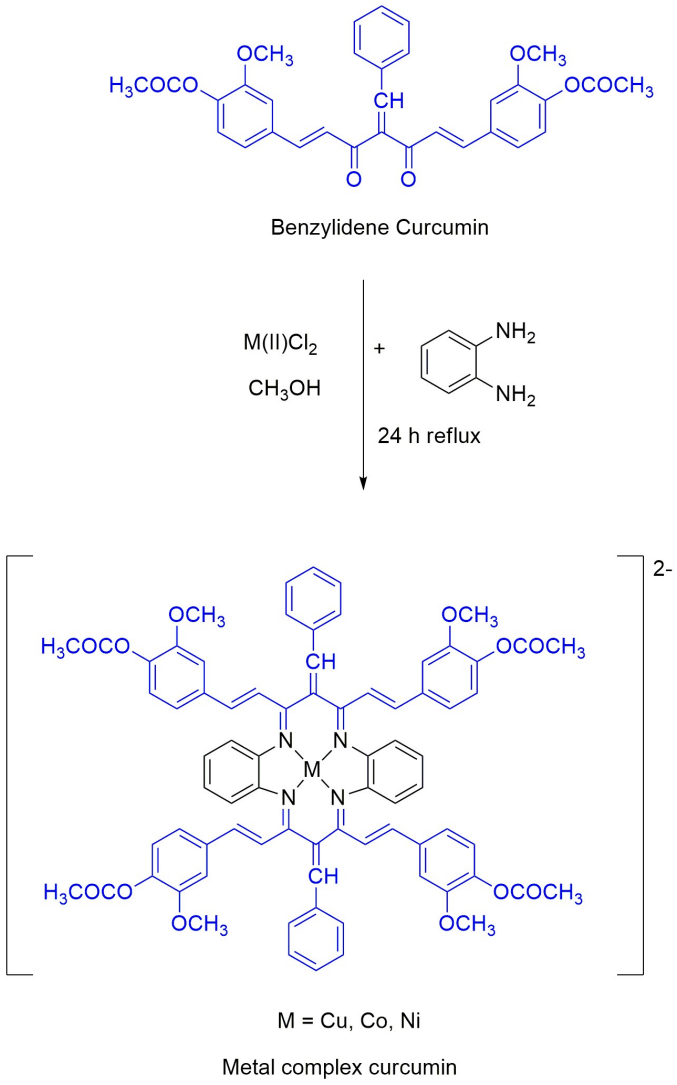
Scheme 1-8: Synthesis of curcumin pyranopyrimidine hybrids

In this reaction, acidic conditions favour enol formation for both barbituric acid and curcumin where barbituric acid attacks benzaldehyde to form an alcohol intermediate *in situ*. Enol formation of curcumin causes the central methylene group to act as a nucleophile and undergo Knoevenagel condensations with the alcohol intermediate to form a 1,5-dicarbonyl intermediate. The carbonyl group from the curcumin moiety cyclises with the carbonyl group of barbituric acid to form the desired pyranopyrimidine (**Scheme 1-9**).



Scheme 1-9: Proposed mechanism of the synthesis of curcumin pyranopyrimidine hybrids

Curcumin has served as a scaffold in the synthesis of metal complexes (Banerjee et al., 2015; Wanninger et al., 2015). In the synthesis of metal (II) azamacrocyclic diacetylcurcumin ligands, a divalent metal ion complexes to the *o*-phenyldiamine ligands which are already attached to the curcumin scaffold through an imine bond (**Scheme 1-10**) (Wanninger et al., 2015).

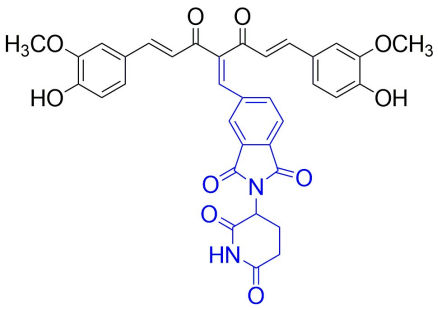


Scheme 1-10: Synthesis of metal (II) complexed curcumin containing azamacrocyclic diacetylcurcumin ligand (Wanninger et al., 2015)

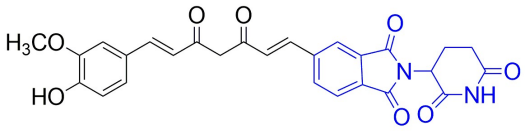
1.2.3 Bioactivity of curcumin and curcumin hybrids

Curcumin and its derivatives were found to have many interesting applications in traditional medicine (Lee et al., 2013) and diverse pharmacological properties such as antibacterial (Khan et al., 2012a; Cao et al., 2014), antidiabetic (Puneeth et al., 2015; Yousefi et al., 2015), anticancer (Adams et al., 2004; Simoni et al., 2008; Fuchs et al., 2009), antioxidant (Choudhury et al., 2015; Sahu et al., 2016), anti-inflammatory (Lang et al., 2009) and Alzheimer's diseases (Elmegeed et al., 2015; Okuda et al., 2016). Curcumins have the ability to cross the blood brain barrier, important for the treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases (Ahsan et al., 2015; Okuda et al., 2016). Curcumin and curcumin hybrids can successfully treat wounds, liver and biliary diseases, ulcers, psoriasis, arthritis, sinusitis, heart diseases, high cholesterol, diabetes, amyloidosis, cervical cancer, colon cancer and pancreatic cancer (Adams et al., 2004; Youssef et al., 2004; Simoni et al., 2008; Alok et al., 2015; Puneeth et al., 2015; Pulido-Moran et al., 2016). They have also been reports of curcumins being used in nanoformulations for the treatment of cancer (Yallapu et al., 2012).

Thalidomide coupled with curcumin hybrids is active against multiple myeloma. Multiple myeloma is a type of bone marrow cancer formed by malignant plasma cells. Thalidomide works against multiple myeloma by slowing down the blood vessel growth around the abnormal plasma. Curcumin is able to slow down proliferation of cancer cells and block carcinogens from forming. Together, this hybrid compound combats cancer by modulating the immune system and disturbing the microenvironment of tumour cell growth in the marrow. Liu et al. (2013) reported on the anticancer activity of a thalidomide coupled curcumin where 4-thalidomide curcumin benzylidene was found to show better activity than unsymmetrical 4-thalidomide curcumin on the lung cancer cell line U266 (**Figure 1-7**).



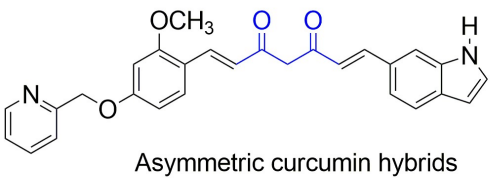
4-thalidomide curcumin benzylidene



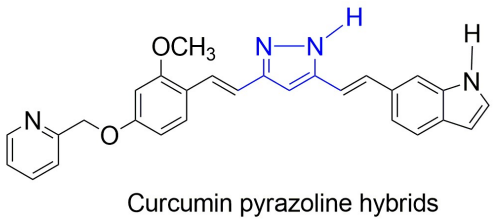
unsymmetrical 4-thalidomide curcumin

Figure 1-7: Thalidomide coupled with Curcumin and Curcumin-like derivatives

Okuda et al. (2016) synthesised hybrid curcumin molecules and tested them as potential tau and amyloid aggregation inhibitors for the treatment of Alzheimer's disease. Curcumin has anti-inflammatory and antioxidant abilities and is able to modulate various neurotransmitters in the brain slowing down the progression of Alzheimer's disease. Pyridines and indoles both possess significant anti-inflammatory and antioxidant activities. Like curcumins, they are able to cross the blood brain barrier and inhibit *β*-amyloid aggregation. The combination of these three moieties enhances the activity of the drug towards the treatment of neurological diseases. Okuda et al. (2016) also converted the curcumin hybrid into a pyrazoline curcumin hybrid and found it to be even more effective against *β*-amyloid aggregation than in its curcumin hybrid form (**Figure 1-8**).



Asymmetric curcumin hybrids



Curcumin pyrazoline hybrids

Figure 1-8: Curcumin hybrids and curcumin pyrazoline hybrids

Curcumin steroidal hybrid drugs are novel drugs active against neurological disorders such as Alzheimer's disease. Curcumin protects the brain from oxidative damage decreasing inflammation and amyloid accumulation. Steroids are able to regulate many of the body's physiological responses such as its stress response. The combination of this moiety on the curcumin scaffold makes it more effective against Alzheimer's disease (**Figure 1-9**) (Elmegeed et al., 2015).

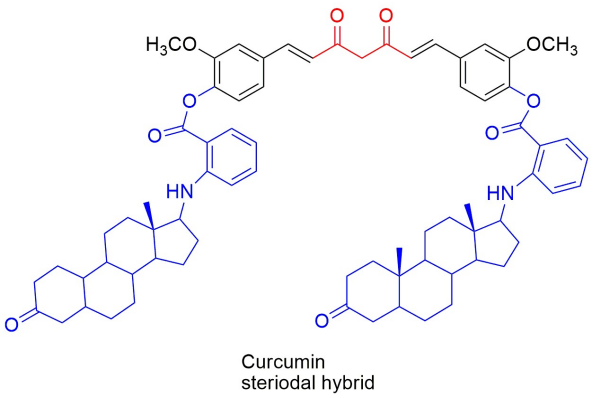
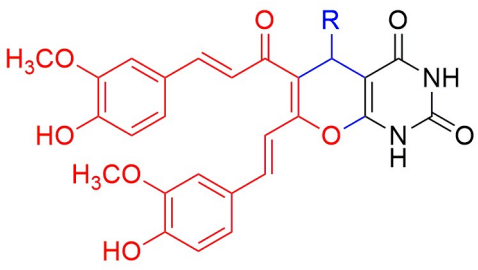


Figure 1-9: Curcumin steroidal hybrids

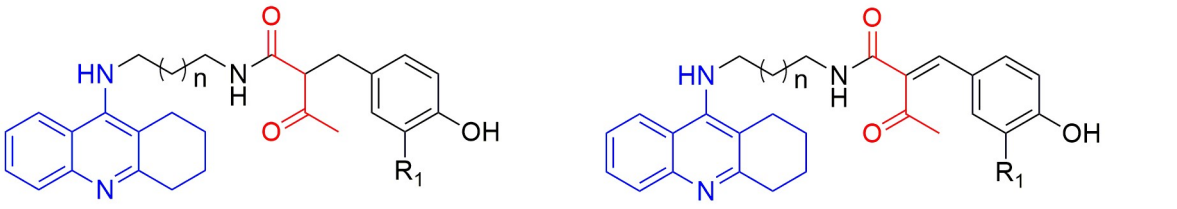
Yousefi et al. (2015) synthesised novel anti-diabetic curcumin based pyranopyrimidines as antioxidant inhibitors for amylase and glucosidase enzymes (**Figure 1-10**). They used curcumin as a core since it stimulates the pancreatic cells for production of insulin. The effectiveness of these drugs were tested against amylase and glucosidase as they are molecular targets for inhibiting postprandial hyperglycemia. Postprandial hyperglycemia is the exaggerated increase of blood sugar levels after a meal. They concluded that curcumin based pyranopyrimidine drugs were beneficial toward the treatment of diabetes since they are good antioxidants and were able to delay diabetic retinopathy.



Pyranopyrimidine

Figure 1-10: Curcumin pyranopyrimidine

Lui et al. (2017) synthesised a series of tacrine curcumin hybrids as multi-functional Alzheimer's agents. Tacrine is a cholinesterase inhibitor of acetylcholinesterase, responsible for preventing its over activity, thus restoring the balance of neurotransmitters in the brain. Acetylcholinesterase is an enzyme that catalyses the breakdown of the neurotransmitter acetylcholine. Curcumin is able to cross the blood brain barrier and slow down proliferation of Alzheimer's disease. These two moieties combined were reported to have good potency against Alzheimer's disease (**Figure 1-11**).



n = 1, 2, 4

R_1 = H or OCH_3

Figure 1-11: Tacrine Curcumin hybrids

1.3 Ketodienes

Ketodienes are commonly used as a component in sunscreen, ligands in organometallic chemistry or an intermediate in synthetic chemistry. This compound closely resembles the structure of curcumin and can be used to synthesise curcumin-like derivatives (**Figure 1-12**).

The difference between the curcumin and the ketodiene is that a curcumin has a dicarbonyl group whereas the ketodiene has a single carbonyl group. These ketodienes are important intermediates for synthesizing cyclic bioactive molecules such as pyrazolines, pyrimidines, isoxazoles and thiazolines (Levai et al., 2006; Abaee et al., 2009; Zhu et a., 2009; Handayani et al., 2012; Rahman et al., 2012; Nasr-Esfahani et al., 2016).

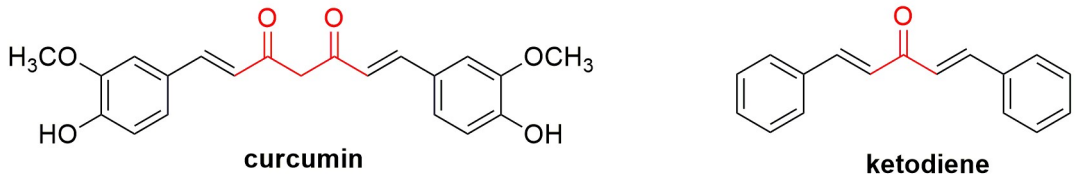
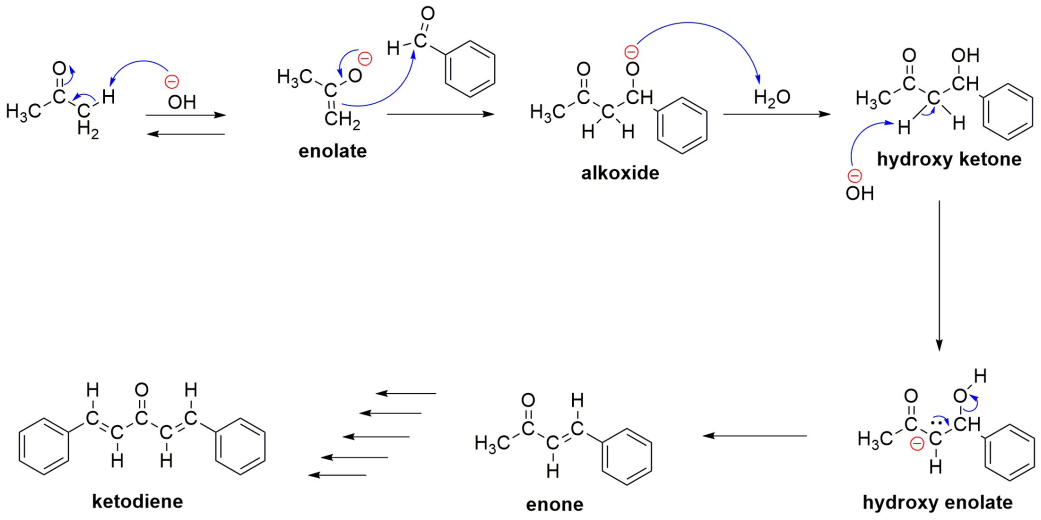


Figure 1-12: Structures of curcumin and ketodiene

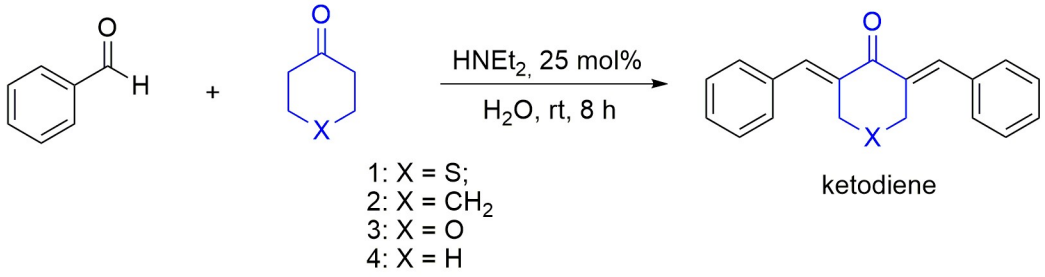
1.3.1 Synthesis of ketodienes

Ketodienes can be formed *via* the Claisen-Schmidt condensation, a reaction that is commonly used to synthesise chalcones. The Claisen-Schmidt condensation is a based catalysed reaction that involves the cross condensation of a ketone with an aldehyde in the presence of a base. The formation of a ketodiene involves the conversion of acetone to an enolate ion, which then attacks the carbonyl group of benzaldehyde, resulting in an alkoxide intermediate that undergoes protonation to form a hydroxyketone. A base abstracts a proton from the α carbon resulting in a conjugated enol intermediate that drives removal of the hydroxy group *via* an E1cB mechanism forming an α,β-unsaturated carbonyl system. This is repeated to form the ketodiene (**Scheme 1-11**).



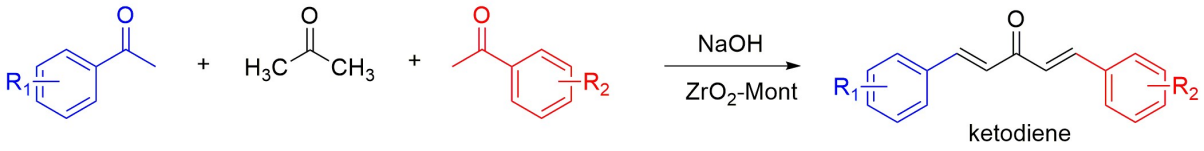
Scheme 1-11: Proposed mechanism of the synthesis of ketodienes

Ketodienes can be formed *via* a double crossed aldol condensation reaction of cyclohexanone with aromatic aldehydes under a based catalysed reaction with diethylamine and water at room temperature (**Scheme 1-12**). This method produces yields of 90% and above, is environmentally friendly, cost effective and relatively simple (Abaee et al., 2009).



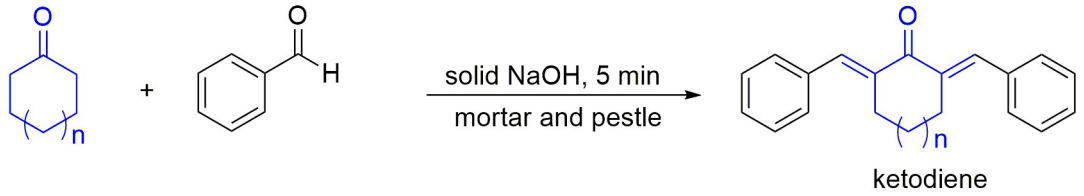
Scheme 1-12: Synthesis of ketodienes under aqueous conditions

Ketodienes can alternatively be formed in high yields through a double crossed condensation reaction of acetone, two different substituted aldehydes, sodium hydroxide and a ZrO_2-montmorillonitrile catalyst (**Scheme 1-13**). This method with no catalyst produces very low yields (Handayani et al., 2012).



Scheme 1-13: Synthesis of ketodienes with ZrO_2-montmorillonitrile

A solvent free method with cyclohexanone, aldehydes and solid sodium hydroxide was used to form ketodienes by grinding everything together with a mortar and pestle at room temperature over 5 minutes (**Scheme 1-14**) (Rahman et al., 2012).

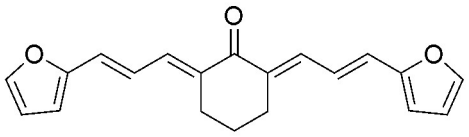


Scheme 1-14: Synthesis of ketodienes under solvent free conditions

1.3.2 Biological activity of ketodienes

Ketodiene products have a wide range of biological activities such as antibacterial, antimalarial, anticancer, anti-inflammatory, antioxidant, anthelmintic activity (Liang et al., 2008; Lee et al., 2009; Franco et al., 2012; Hosoya et al., 2012; Girija et al., 2013; Pan et al., 2015; Aguilera et al. 2016).

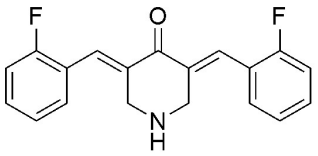
Aguilera et al. (2016) synthesised a series of symmetrical ketodienes derived from drugs that showed bactericidal activity against trypanosomatida, a bacterial parasite (**Figure 1-13**). Their studies found that the compound below was able to inhibit triosephosphate isomerase and cruzipain, enzymes important to the survival of the parasitic bacteria.



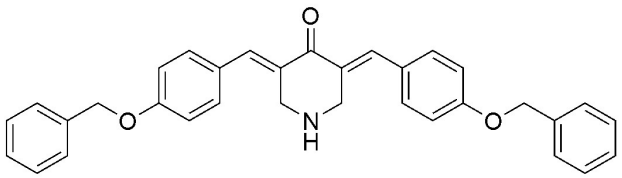
symmetrical diarylideneketone

Figure 1-13: Aldol derivative that is active against Triosephosphate isomerase and cruzipain

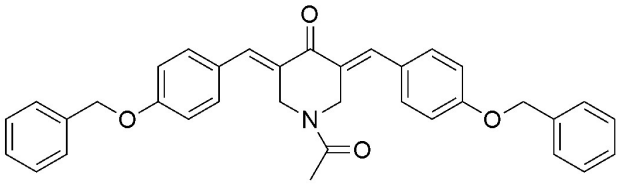
Qudjani et al. (2016) synthesised and evaluated a series of ketodienes for anticancer activity and found that these derivatives had good *in vitro* activity against human cervix carcinoma cell lines (HeLa) and pancreatic cell lines (Panc-1) (**Figure 1-14**). Docking studies also indicated that 3,5-bis(4-(benzyloxy)benzylidene)piperidin-4-one readily binds to the active site of human glyoxalase I protein *via* two strong hydrogen bonds engaging residues of Glu-99 and Lys-156.



3,5-bis(2-fluorobenzylidene)piperidin-4-one



3,5-bis(4-(benzyloxy)benzylidene)piperidin-4-one



acetyl-3,5-bis(4-(benzyloxy)benzylidene)piperidin-4-one

Figure 1-14: Diarylideneketone derivatives with good anticancer activity

Several diarylpentanoid analogues were found to strongly inhibit the activity of PLA2, COX, LOX, and mPGES-1 enzymes responsible for the onset of inflammation (**Figure 1-15**) (Ahmad et al., 2014).

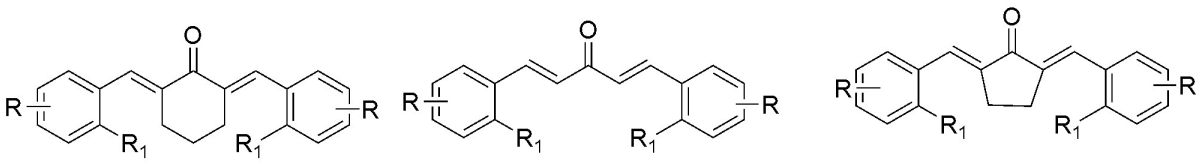


Figure 1-15: Synthetic scheme of diarylpentanoid analogues of curcumin

1.4 Pyrazolines

A pyrazoline is a heterocyclic molecule containing two nitrogen atoms adjacent to each other (**Figure 1-16**) and are usually incorporated into hybrid molecules since they have good biological activities (Rahman et al., 2010). This study focuses on the synthesis of pyrazolines as a scaffold for hybrid curcumin and curcumin-like molecules.

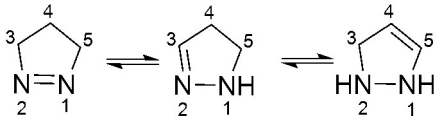
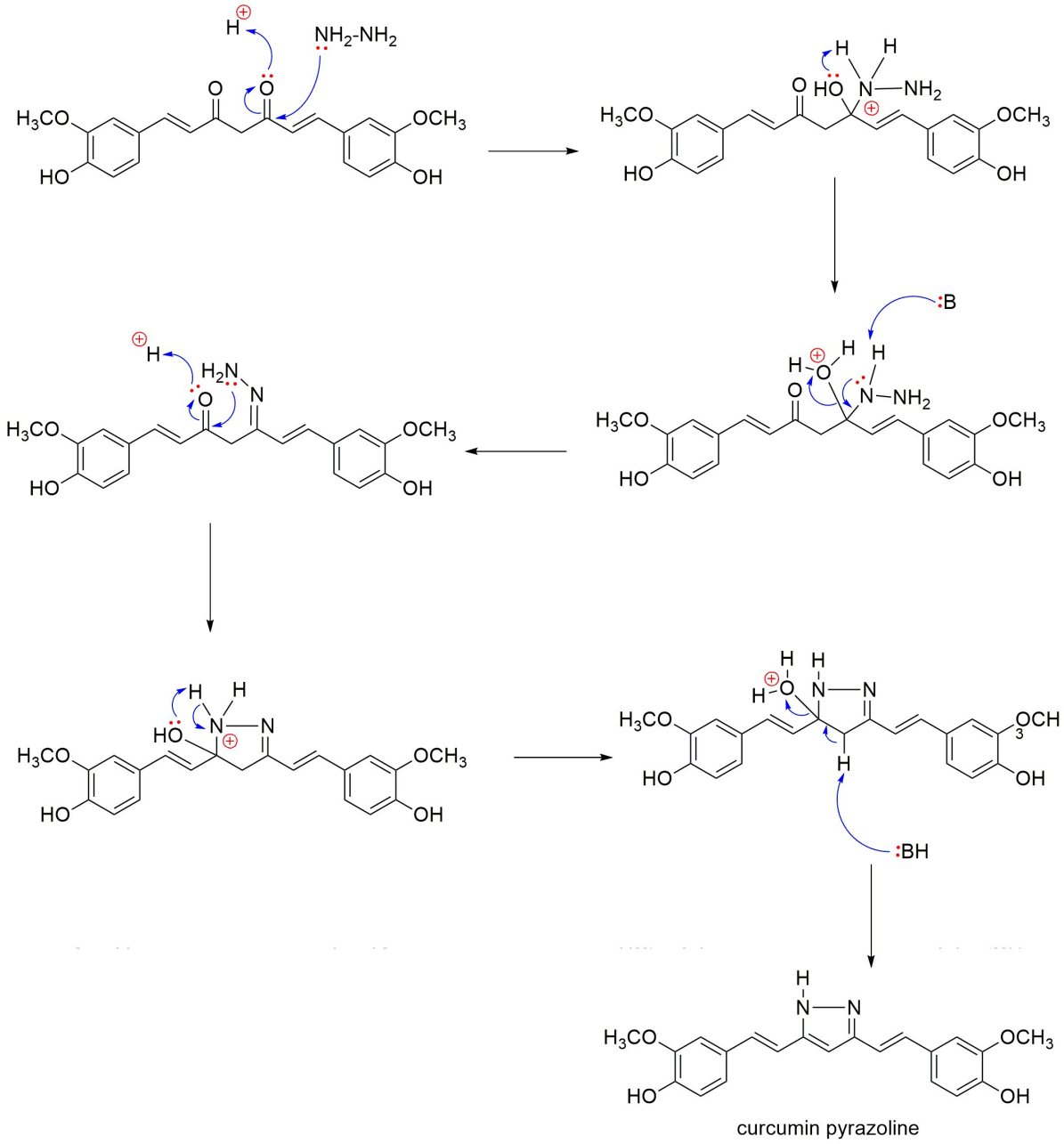


Figure 1-16: The three different reduced forms of pyrazoline

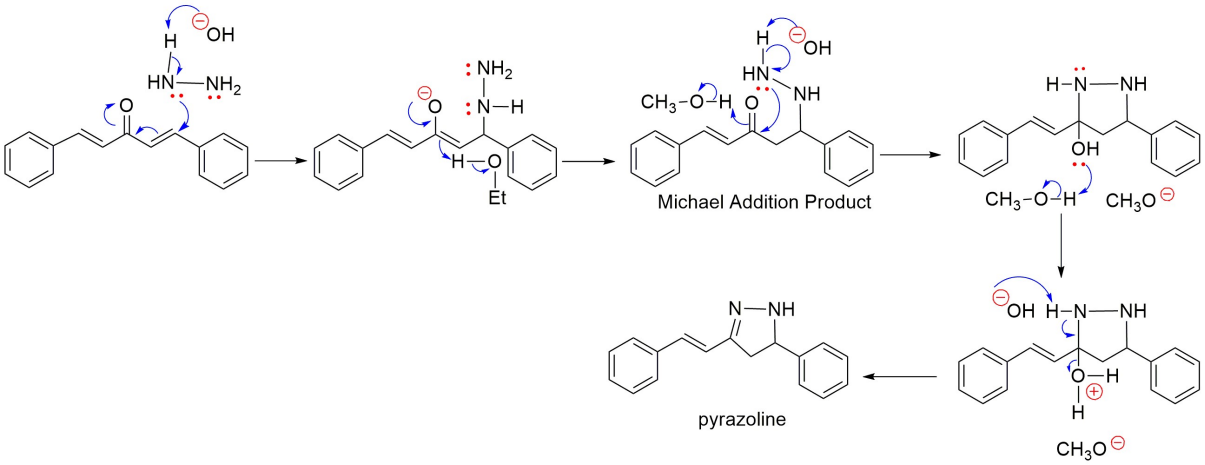
1.4.1 Pyrazoline synthesis

Curcumin and ketodienes both possess α,β-unsaturated carbonyl systems. When these systems react with hydrazine, they form pyrazolines (Levai, 2002; Chimenti et al., 2005; Chovatia et al., 2010; Punyapreddlwar et al., 2016). Pyrazolines derived from curcumins results in five membered ring systems with two double bonds, unlike pyrazolines derived from ketodienes, where a reduced pyrazole is formed with only one double bond. The two double bonds form as a result of the dicarbonyl system. The mechanism involves the nucleophilic hydrazine attacking one of the carbonyl groups to form an imine, which then cyclises further with the other carbonyl group. Subsequent removal of water is facilitated by formation of a C-C double bond forming the pyrazoline (**Scheme 1-15**).



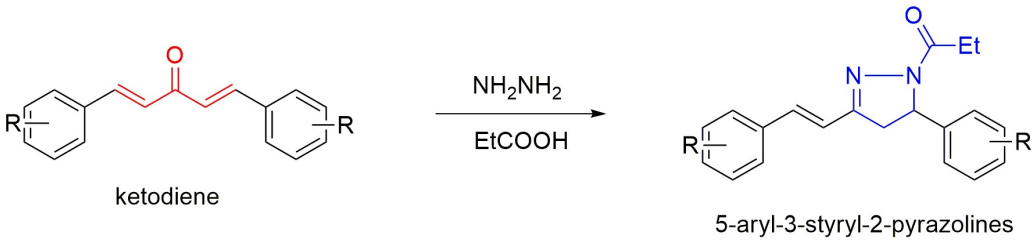
Scheme 1-15: Proposed mechanism for the synthesis of curcumin pyrazolines

The pyrazoline formed from a ketodiene occurs *via* a Michael Addition. This involves a nucleophilic attack on the *β*-carbon to form an enolate, which subsequently accepts a proton from the solvent. Nucleophilic addition can then occur between the second amino group and the carbonyl carbon. The oxygen atom from this carbonyl group then accepts two protons followed by elimination of water, forming the pyrazoline (**Scheme 1-16**).



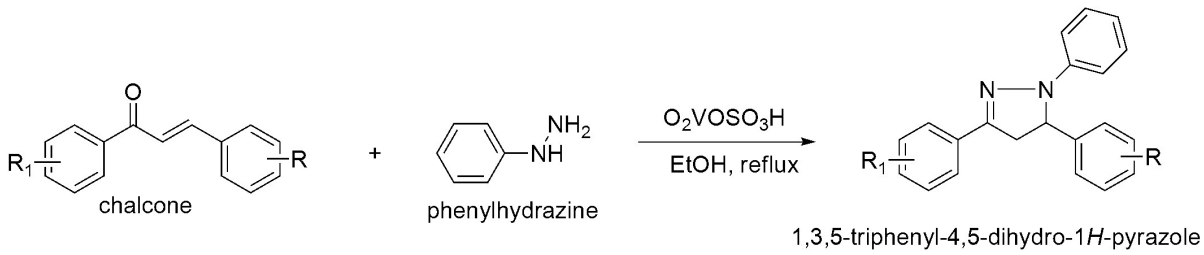
Scheme 1-16: Possible mechanism *via* Michael Addition for the formation of pyrazoline

The 5-aryl-3-styryl-2-pyrazolines are formed by a cyclocondensation reaction of ketodienes with hydrazine hydrate and hot propionic acid (**Scheme 1-17**). This reaction produces yields of 70% and above (Levai et al., 2006).



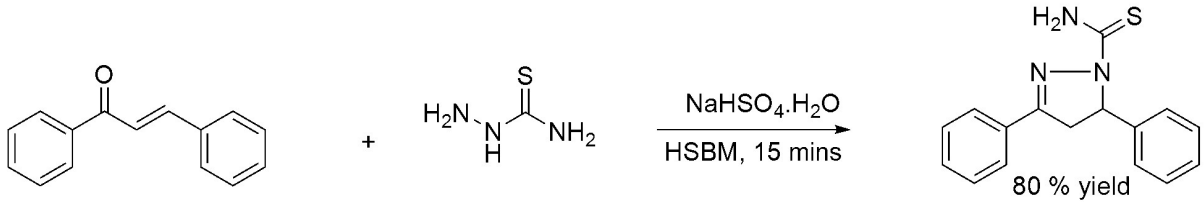
Scheme 1-17: Synthesis of 5-aryl-3-styryl-2-pyrazolines

Nasr-Esfahani et al. (2016) synthesised 1,3,5-triaryl-2-pyrazolines under mild conditions with high yields. They were formed by the cyclocondensation of chalcones with phenylhydrazine, ethanol and a nanorod vanadate sulfuric acid catalyst (**Scheme 1-18**).



Scheme 1-18: Synthesis of 1,3,5-triaryl-2-pyrazolines using a nanorod vanadatesulfuric acid catalyst

Zhu et al. (2009) reported an efficient mechanically activated solvent free synthesis of 1,3,5-triaryl-2-pyrazolines (**Scheme 1-19**) in the presence of $NaHSO_4.H_2O$ by high speed ball milling (HSBM). High speed ball milling is a process in which a compound is ground with one or more inert balls (eg. ceramic) that are rotating at high speeds around a horizontal axis. Their method produced good yields with short reaction times, is efficient, cheap, requires no solvent and is environmentally friendly.



Scheme 1-19: Synthesis of 1,3,5-triaryl-2-pyrazolines using solvent free HSBM conditions

As shown above, there are a variety of methods that can be used to synthesize pyrazolines, however, the simplest of these is refluxing a mono/diketo α,β-unsaturated system with hydrazine hydrate in acetic acid for 12 hours. Upon completion, the pyrazoline usually precipitates out in crushed ice (Chimenti, et al., 2005; Pujari et al., 2014).

1.4.2 Biological activity of pyrazolines

Pyrazolines have a wide range of medicinal properties such as antibacterial, anti-inflammatory, anticancer, anticonvulsant, antidepressant, and antioxidant amongst others (Rahman et al., 2010; Dipankar et al., 2011; Siddiqui et al., 2011; Bardalai and Panneerselvam, 2012; Lakshmi et al., 2016; Punyapreddlwar et al., 2016). Some examples of these are shown below.

The 1-[(aryl)thioacetyl]-3-(2-thienyl)-5-(4-chlorophenyl)-2-pyrazoline derivatives were synthesized and investigated for their antiproliferative effects on AsPC-1 human pancreatic adenocarcinoma, U87 and U251 human glioblastoma cell lines, where the compound in **Figure 1-17** showed good anticancer activity against AsPC-1 and U251 cancer cell lines and exhibited significant tumour selectivity (Karabacak et al., 2015).

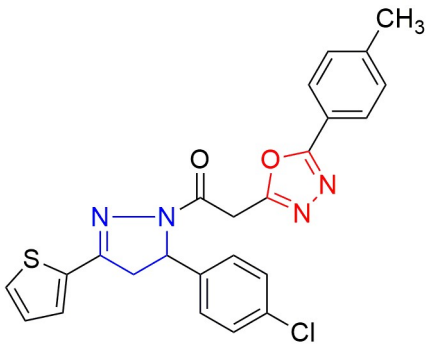


Figure 1-17: Pyrazoline derivative exhibiting antiproliferative activity against AsPC-1 and U251 cancer cell lines

The synthesis and *in vitro* antimalarial activity of a series of 1,3,5-trisubstituted pyrazolines (**Figure 1-18**) was evaluated and showed very good antimalarial activity against the chloroquine resistant strain (RKL9) with an IC_{50} value of 0.177 mM (Acharya et al., 2010).

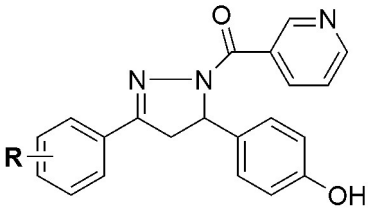


Figure 1-18: 1,3,5-trisubstituted pyrazolines with antimalarial activity

Jainey and Bhat evaluated a series of compounds for antitumour, analgesic and anti-inflammatory activity of their synthesized compounds against Ehrlich Ascites Carcinoma (EAC) tumour lines. Several compounds exhibited good anticancer and analgesic activity (**Figure 1-19**) (Jainey and Bhat, 2012).

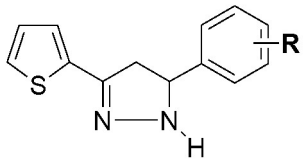


Figure 1-19: 2-pyrazolines derivatives exhibiting antitumour, analgesic and anti-inflammatory activity

A series of *N*-substituted 2-pyrazolines were synthesized from chalcone intermediates under ultrasonic irradiation and evaluated for their *in vivo* hyperglycaemic activity by an alloxan-induced diabetic model in rats. Alloxan is an analogue of glucose which selectively targets and destroys insulin producing cells located in the pancreas. The compound in **Figure 1-20** was found to be a good hyperglycaemic agent when compared with the standard drug insulin in reducing the blood glucose level (Santhi et al., 2013).

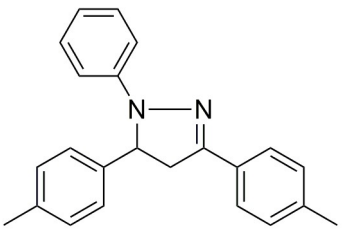
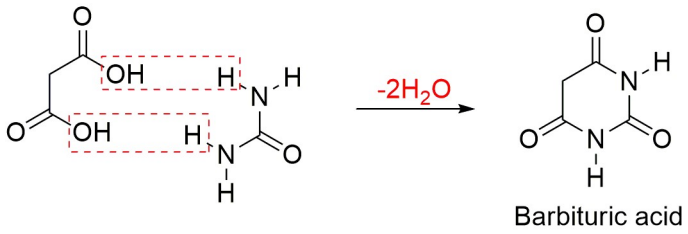


Figure 1-20: 2-pyrazoline derivative exhibiting antidiabetic activity

In this project, the carbonyl system of the curcumin and aldol product will be replaced by a pyrazoline ring in an attempt to determine if the activity of the diarylheptanoid scaffold is enhanced by the presence of a ring.

1.5 Barbiturates

Barbiturates are derivatives of barbituric acid, an organic pyrimidine first discovered by Adolf von Baeyer in 1864 when he combined urea with malonic acid (**Scheme 1-20**) (Huisgen, 1986).



Scheme 1-20: Synthesis of barbituric acid

Although it is not a pharmacologically active drug, its barbiturate derivatives possess many biological activities such as hypnotics, sedatives, anticonvulsants and anaesthetics. Over the years, these drugs have become famously known as "sleeping pills". A few examples of early barbiturate drugs are veronal, phenobarbital and pentobarbitone (**Figure 1-21**). Veronal was found to be an effective sleeping drug (Lopez-Munoz et al., 2005). Phenobarbital was marketed as a good hypnotic and anticonvulsant drug and later on discovered to be good at controlling epileptic seizures (Lopez-Munoz et al., 2005; Yasiry and Shorvon, 2012). Pentobarbitone is used as an anaesthetic during surgery and in higher doses results in death by respiratory arrest. Due to the latter, it was used for executions of convicted criminals (Yasiry and Shorvon, 2012).

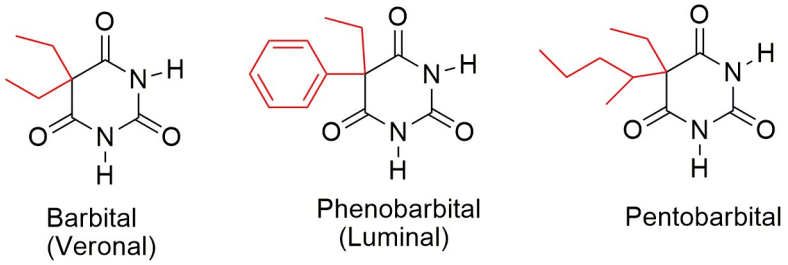
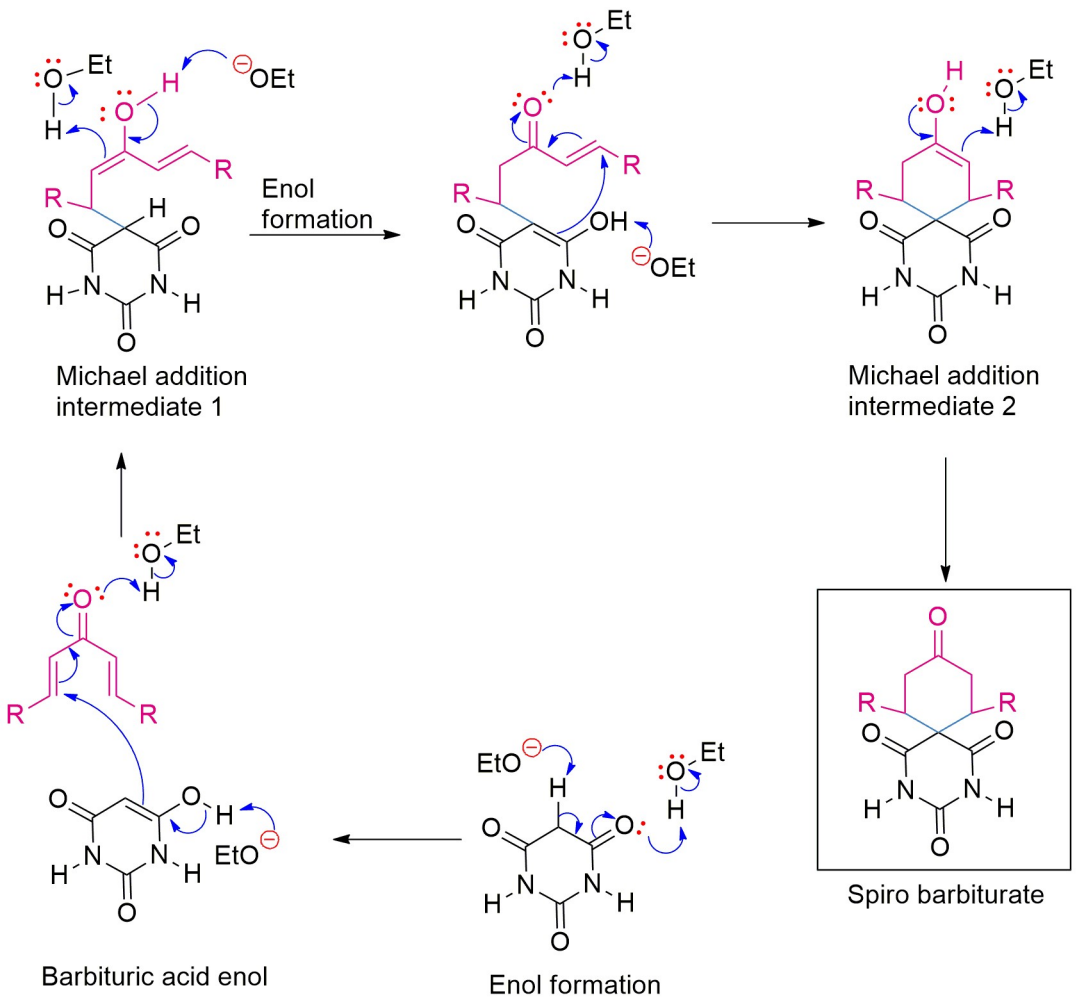


Figure 1-21: Derivatives of barbituric acid

1.5.1 Spirobarbiturate synthesis

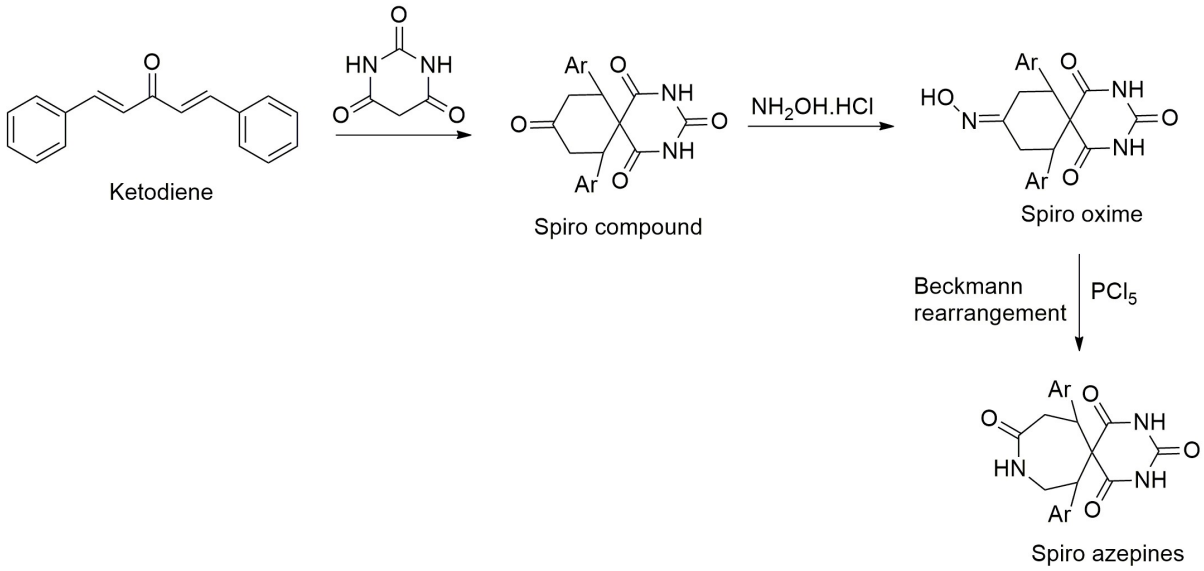
Barbiturates are cyclic ureas, synthesised by reacting urea with 1,3-diesters (Moussier et al., 2003). The proton on the α carbon of barbituric acid is very acidic and easily abstracted by a base. It can thus act as a nucleophile and add to a variety of electrophiles, including the Michael addition to α,β-unsaturated carbonyl compounds (Moussier et al., 2003; Kalita et al., 2014; Dhorajiya et al., 2016). When reacted with ketodienes (a molecule consisting of two α,β-unsaturated centers), a spiro barbiturate is formed (Behera et al., 2009; Kesharwani et al., 2009). The proposed mechanism of this reaction is shown below (**Scheme 1-21**).

In the mechanism (**Scheme 1-21**), the ethoxide anion abstracts a proton from the methylene group of barbituric acid to form an enol. This enol first attacks one half of the α,β-unsaturated group of the ketodiene by an intermolecular Michael addition forming an intermediate, which then undergoes the same process with the second α,β-unsaturated group, ultimately forming the spiro barbiturate.



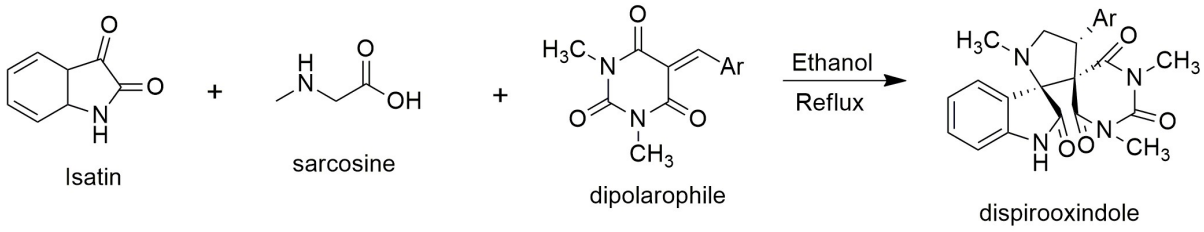
Scheme 1-21: Proposed mechanism for the synthesis of spiro barbiturates

An example of the synthesis of these spiro compounds can be seen in the synthesis of the spiro azepines in **Scheme 1-22** (Kesharwani et al., 2009). Ketodienes were first prepared from acetone and substituted aldehydes, reacted with barbituric acid, forming spiro compounds, further derivatised to oximes with hydroxylamine hydrochloride and transformed to spiro azepines with PCl_5 in a Beckmann rearrangement.



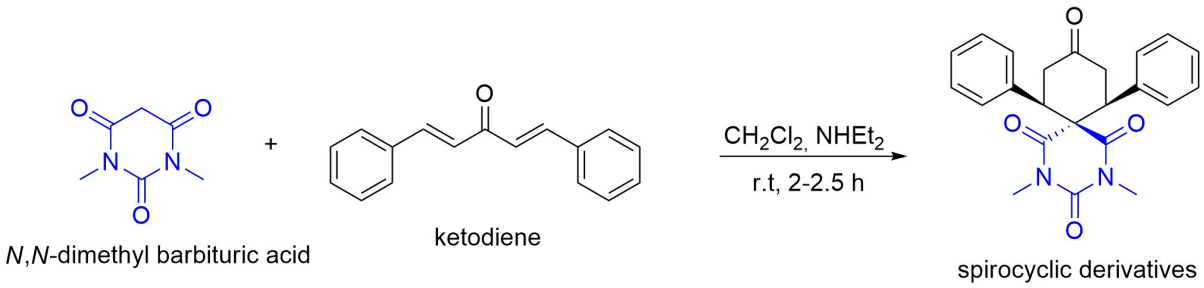
Scheme 1-22: Synthesis of spiro azepines

A one pot synthesis for dispirooxindoles was reported by Huang et al. (2012) (**Scheme 1-23**). In this reaction, an arylidene barbiturate was reacted with sarcosine and isatin to form the target molecules in a one pot reaction with yields of 75-90%.



Scheme 1-23: Synthesis of dispirooxindoles

These spirobarbiturates were also formed in a cascade [5+1] double Michael Addition reaction with yields of over 90% (**Scheme 1-24**) (Islam et al., 2015; Islam et al., 2017).



Scheme 1-24: Synthesis of spirocyclic derivatives by a cascade [5+1] double Michael addition

1.5.2 Biological activity of barbiturates

The barbiturate moiety is present in many synthetic molecules with various pharmaceutical and industrial applications. They have been used in cancer treatment (Dhorajiya et al., 2016) and found to possess anticonvulsant (Shiradkar et al., 2007), antitumour (Maquoi et al., 2004), antioxidant (Barakat et al., 2017) and antibacterial activity (Sokmen et al., 2013).

The spiro compounds and their oxime derivatives in **Scheme 1-22** were found to be good anticonvulsant agents, moreso than the spiroazepine derivatives (Kesharwani et al., 2007). A series of spiroquinolines formed from benzisoxazole-5-carbaldehyde and barbituric acid or thiobarbituric acid showed good anticancer activity against MCF-7 and KB cell lines with the spiroquinoline in **Figure 1-22** showing the highest cyctotoxicity (IC_{50} value of 90.2 µM for MCF-7 and 49.8 µM for KB cell line (Bhaskarachar et al., 2015).

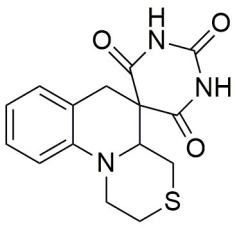
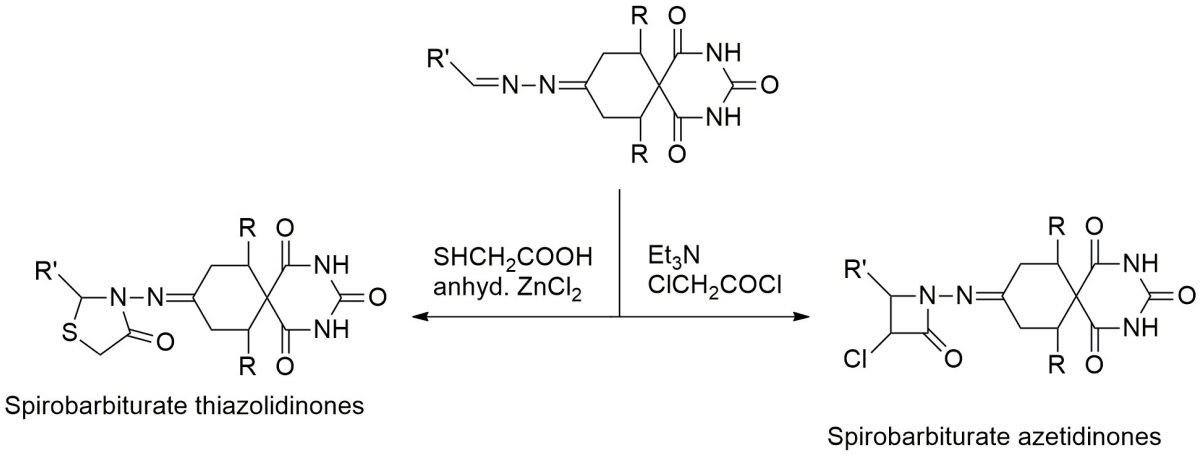


Figure 1-22: Potent anticancer spiroquinoline (Bhaskarachar et al., 2015)

A series of hybrid thiazolidinone or azetidinone spirobarbiturates were synthesized from Schiff base precursors, which showed good anticonvulsant activities (**Scheme 1-25**). The thiazolidinone hybrids were found to be more active than the azetidinone hybrids (Goel et al., 2005).



Scheme 1-25: Formation of spiro barbiturate thiazolidinone and azetidinone hybrids from their Schiff base precursors

Two 2-phenyl-1*H*-indole-3-carbaldehyde-based thiobarbiturates (**Figure 1-23**) showed good anticancer activity against a Human prostate cancer cell line DU145, a Human Dwivedi (DWD) cancer cell line, and a Human breast cancer cell line MCF-7 (Laxmi et al., 2015).

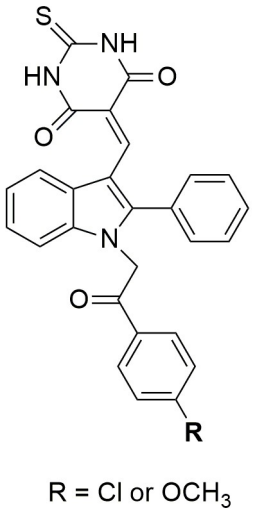


Figure 1-23: Structure of phenyl-1*H*-indole-3-carbaldehyde-based barbituric acid derivative

In this research, barbituric acid will be condensed with a series of substituted ketodienes in a Michael addition reaction. These compounds will be investigated for their antibacterial activity.

1.6 Oximes

Oximes are a class of organic compounds with an imine hydroxyl group having a general formula of $R^1R^2C{=}NOH$ (**Figure 1-24**). Oximes are used in industry to produce caprolactam, a precursor in the synthesis of nylon 6 (Thomas et al., 2005). They can also be used as intermediates in the formation of amides or nitriles (Kesharwani et al., 2009; Ghosh et al., 2016).

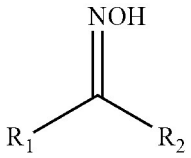
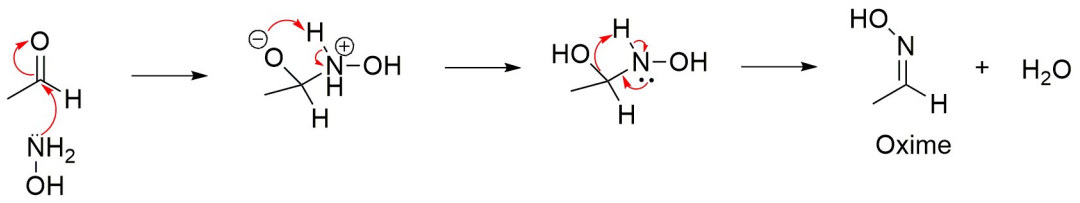


Figure 1-24: Structure of an oxime

1.6.1 Oxime synthesis

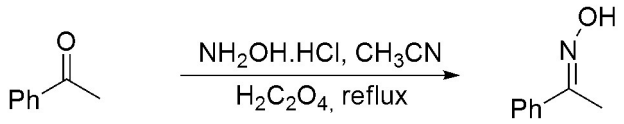
Oximes are synthesised by the condensation of hydroxylamine with either an aldehyde or ketone (**Scheme 1-26**). The condensation of aldehydes with hydroxylamine usually forms aldoximes while their condensation with ketones form ketoximes.



Scheme 1-26: Mechanism for the synthesis of oximes from hydroxylamine and acetaldehyde

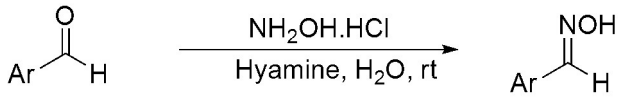
The presence of the oxygen increases the nucleophilicity of the nitrogen. When the hydroxyl oxygen abstracts a proton from NH, the lone pair on nitrogen forms an oxime, eliminating water in the process.

Oximes are formed fairly easy and even without a solvent by grinding the ketone or aldehyde, sodium hydroxide and hydroxylamine hydrochloride with a pestle and mortar (Damljanovic et al., 2005). They can also be formed using oxalic acid and acetonitrile together with the ketone and hydroxylamine (**Scheme 1-27**) (Ghozlojeh et al., 2015).



Scheme 1-27: Synthesis of oximes with oxalic acid

An eco-friendly synthesis of oximes from aldehydes using water as a solvent and the catalyst hyamine (a quaternary ammonium salt) together with hydroxylamine was reported by Lad et al. (2010) (**Scheme 1-28**). This method was not suitable for ketones as they do not react to form the desired product.



Scheme 1-28: Synthesis of oximes with hyamine

1.6.2 Biological activity of oximes

Oxime ether derivatives of cholesterol showed good antibacterial activity against Gram +ve *S. aureus* and *S. pyogenes*, and Gram –ve *S. typhimurium* and *E.coli*. The 3*β*-chloro

derivative (**Figure 1-25**) was found to have the best activity with a MIC value of 32 μg mL^{-1} against all bacterial strains tested against (Khan et al., 2012b).

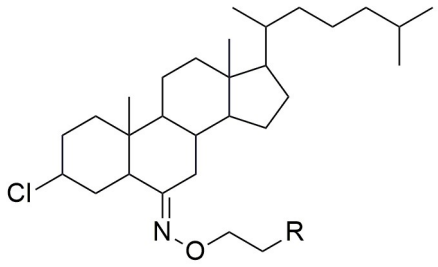


Figure 1-25: Structure of cholesterol oxime steroid

Metal complexes derived from 2-hydroxy-3-(hydroxyimino)-4-oxopent-2-ylidene) benzohydrazide ligands (**Figure 1-26**) were shown to be cytotoxic against human liver cancer cell lines (HepG2). The complexes were better than the ligands alone and exhibited IC_{50} values of 2.2 μM (El-Tabl., et al., 2015).

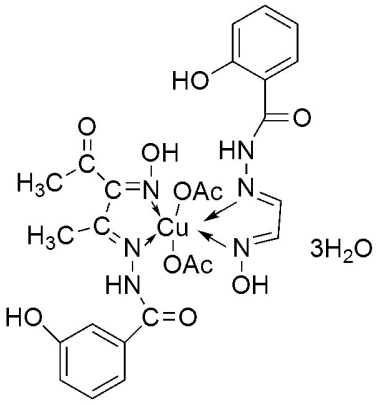
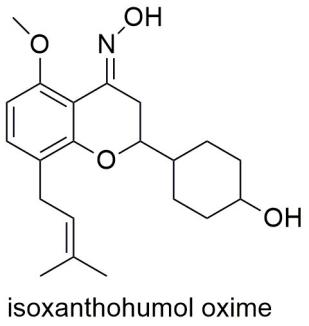


Figure 1-26: Structure of a copper complex of 2-hydroxy-3-(hydroxyimino)-4-oxopent-2-ylidene) benzohydrazide ligands

Isoxanthohumol oxime (**Figure 1-27**) was shown to have good antioxidant activity with an EC_{50} of 0.0411 mM in a DPPH assay (Potaniec et al., 2014). Isoxanthohumol, its parent compound is a prenylated flavonoid usually found in beer.



isoxanthohumol oxime

Figure 1-27: Structure of isoxanthohumol oxime

In this research, the spiro derivatives will be converted into spiro oximes and tested for their antibacterial activity.

1.7 Quinolines

Quinoline (1-*aza*-napthalene or benzo[*b*]pyridine) is a nitrogen containing heterocyclic compound with a molecular formula C_9H_7N (**Figure 1-28**). It is a weak tertiary base and can take part into both electrophilic and nucleophilic reactions (Marella et al., 2013). Quinoline was first extracted from coal tar bases in 1834 by Friedlieb Ferdinand Runge. The main sources of quinoline is found in petroleum, coal processing, wood preservation and shale oil (Prajapati et al., 2014). Some pharmaceutical compounds containing the quinoline moiety can be seen below (**Figure 1-29**).

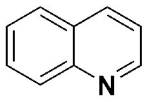


Figure 1-28: Structure of quinoline

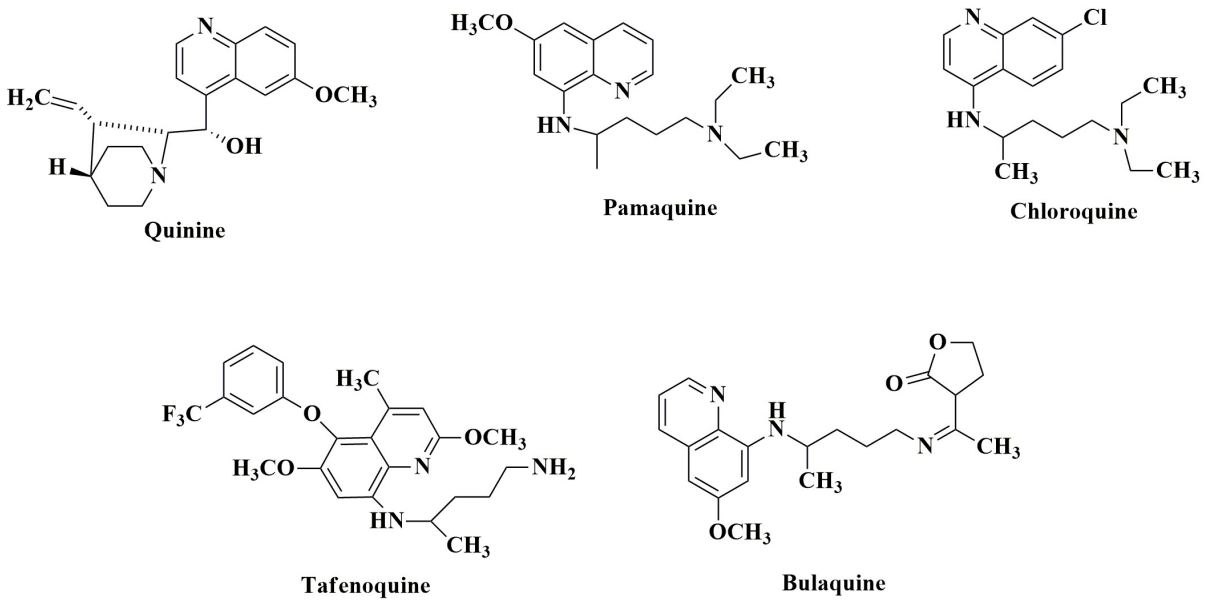


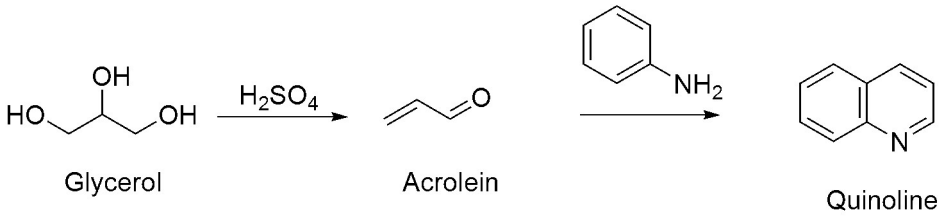
Figure 1-29: Structures of some pharmaceutical compounds containing the quinoline moiety (Marella et al., 2013)

The isolation of the quinoline ring system has led to many advances in heterocyclic chemistry since they have a diverse range of chemical and pharmacological properties (Marella et al., 2013; Prajapati et al., 2014). It was reported that compounds containing a quinoline scaffold have a broad range of biological activities such as antimalarial, anticancer, anti-inflammatory, antibacterial and antiepileptic amongst others (Azad et al., 2007; Kotra et al., 2009; Marella et al., 2013; David et al., 2015; Wei et al., 2015; Ramann et al., 2016). The most important use of the quinoline scaffold is its antimalarial activity. The pharmacueticals, chloroquine and mefloquine are quinoline based drugs currently used for the treatment of malaria (Ramann et al., 2016).

1.7.1 Quinoline synthesis

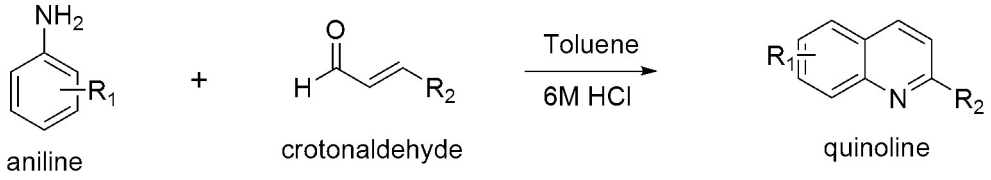
The quinoline moiety can be synthesized *via* the Skraup (Wang, 2010), Doebner-Miller (Gopaul and Koorbanally, 2016; Wang, 2010), Friedländer (Wang, 2010) and Combes synthesis (Wang, 2010).

The Skraup reaction involves the dehydration of glycerol with concentrated sulfuric acid to produce acrolein, which then reacts with aniline to form the quinoline after oxidation takes place (**Scheme 1-29**) (Wang, 2010).



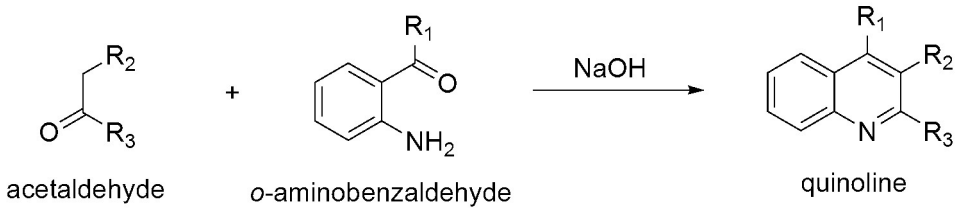
Scheme 1-29: Skraup reaction of Quinoline

The Doebner-Miller reaction is a milder modification of the original Skraup process and uses *α,β*-unsaturated aldhehydes such as crotonaldehyde. Condensation and oxidation reactions produce the quinoline in good yields. Modifications to this method led to a toluene/HCl solvent system preventing polymerization of crotonaldehyde and producing yields of up to 80% (**Scheme 1-30**) (Wang, 2010; Gopaul and Koorbanally, 2016).



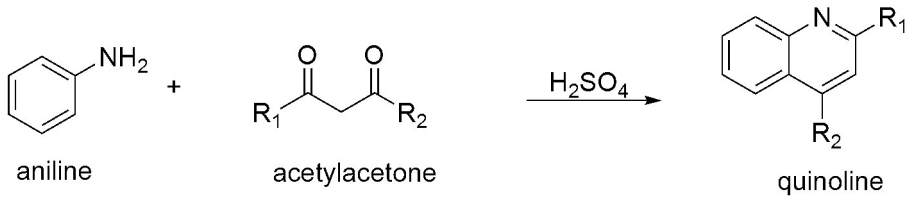
Scheme 1-30: Modified Doebner-Miller reaction of quinoline

The Friedländer condensation is a one pot synthesis in which *o*-aminobenzaldehyde is condensed with acetaldehyde in the presence of sodium hydroxide (**Scheme 1-31**) (Wang, 2010). This method is very successful and produces high yields of quinoline. A major disadvantage is that the aminobenzaldehyde precursor is unstable and readily undergoes self-condensation.



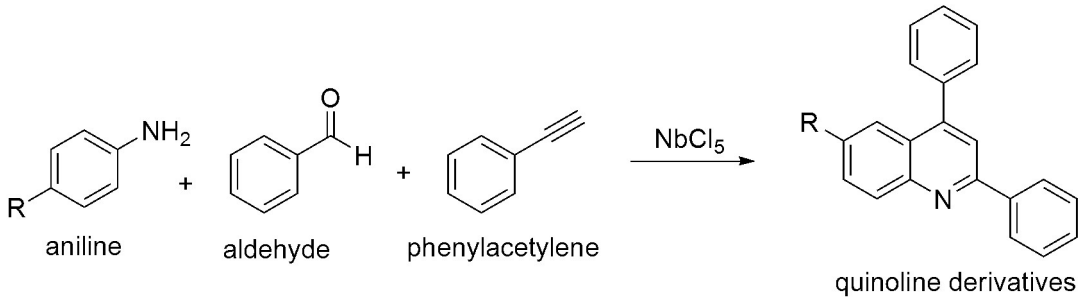
Scheme 1-31: Friedländer condensation reaction producing quinoline

The Coombes synthesis involves the condensation of primary aromatic amines with acetoacetate or other *β*-diketones followed by cyclization in the presence of sulfuric acid (**Scheme 1-32)**. This method provides rapid access to the 2,4-substituted quinoline skeleton but has low regioselectivity (Wang, 2010).



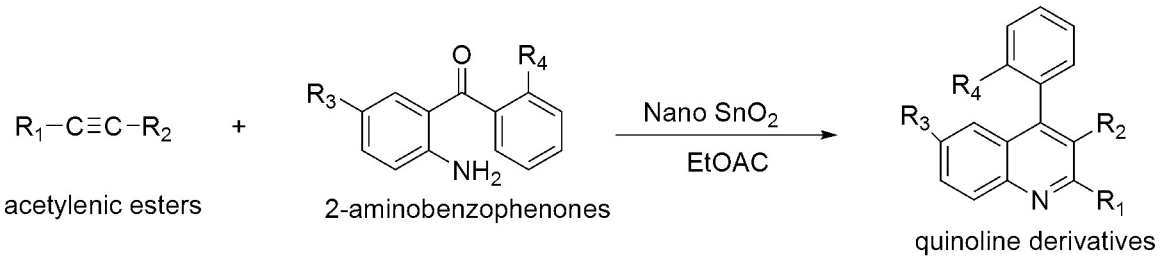
Scheme 1-32: Mechanism showing Coombes synthesis of quinoline

A multicomponent reaction can be used to synthesise quinoline derivatives by reacting together various substituted aldehydes, anilines and phenylacetylene with a niobium pentachloride catalyst (**Scheme 1-33**) (De Andrade et al., 2014). These reactions were carried out at room temperature with acetonitrile as the solvent. This reaction produced yields of greater than 90%.



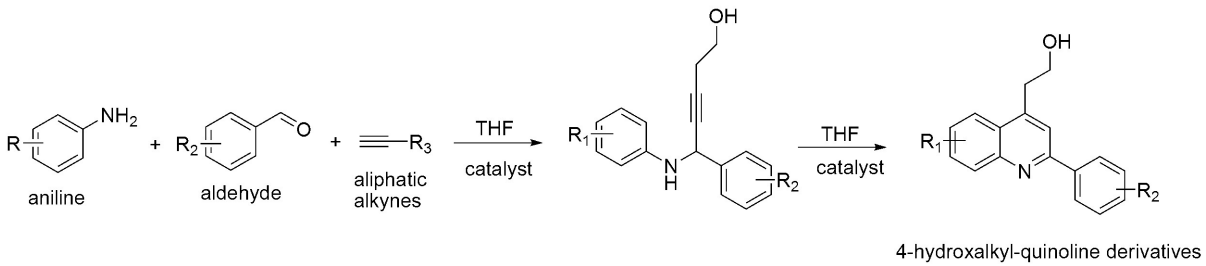
Scheme 1-33: Synthesis of quinoline derivatives

A three component reaction of acetylenic esters, 2-aminobenzophenones with ethyl acetate and SnO_2 nanoparticles were also used to synthesise quinolines with aromatic moeities at C-4 of the quinoline framework (**Scheme 1-34**). SnO_2 is used to catalyze the reaction by acting as a Lewis acid and increasing the electrophilicity of the carbonyl group on the 2-aminobenzophenone which allows the nucleophilic amine to attack the acetylenic ester and form quinoline derivatives in high yields (Qandalee et al., 2013).



Scheme 1-34: Synthesis of quinoline derivatives from acetylenic esters and 2-aminobenzophenones

A three component reaction was used to synthesise 4-hydroxyquinoline derivatives in good yields from anilines, aldehydes and aliphatic alkynes with either a copper chloride or gold chloride catalyst (**Scheme 1-35**) (Jiang et al., 2017).



Scheme 1-35: Synthesis of 4-hydroxalkyl quinoline derivatives

1.7.2 Biological activity of quinolines

Besides being well known for their antimalarial properties, quinolines also exhibit a diverse range of biological activities. Some of these are anticancer, anti-inflammatory, antibacterial and antiepileptic amongst others (Azad et al., 2007; Kotra et al., 2009; Marella et al., 2013; David et al., 2015; Wei, et al., 2015; Ramann et al., 2016). A few examples of reported pharmacological activities of quinoline hybrids are reported below.

A series of [(7-chloroquinolin-4-yl)amino]chalcone derivatives were found to be good antimalarial agents and showed activity against human prostate LNCaP tumour cells (Ferrer et al., 2009) (**Figure 1-30**).

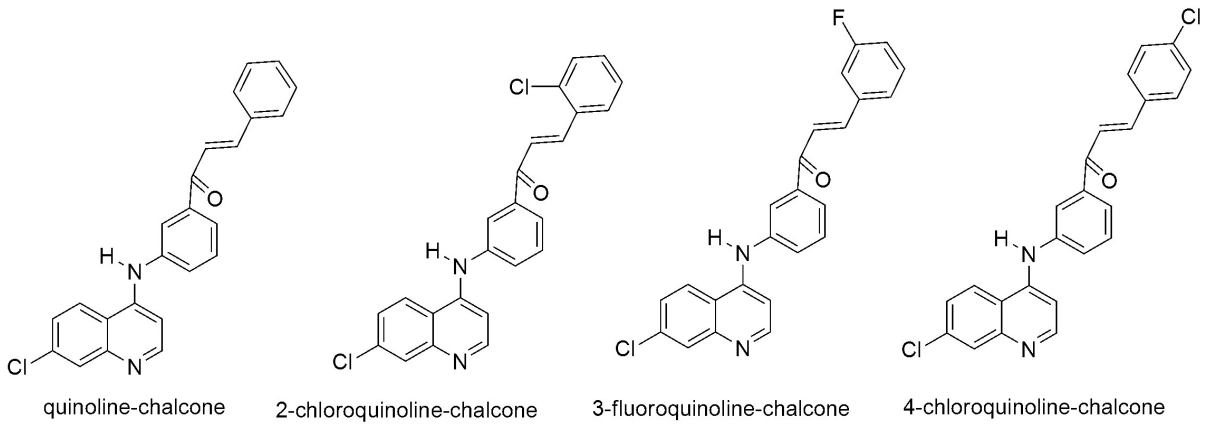


Figure 1-30: Examples of bioactive [(7-chloroquinolin-4-yl)amino]chalcone derivatives

A series of quinoline-lawsone hybrids (**Figure 1-31**) showed good activity against chloroquine-sensitive (RKL-2) and resistant (RKL-9) *Plasmodium falciparum* (Kashyap et al., 2016). The connecting bridge between these compounds were either aliphatic or aromatic/heteroaromatic diamines.

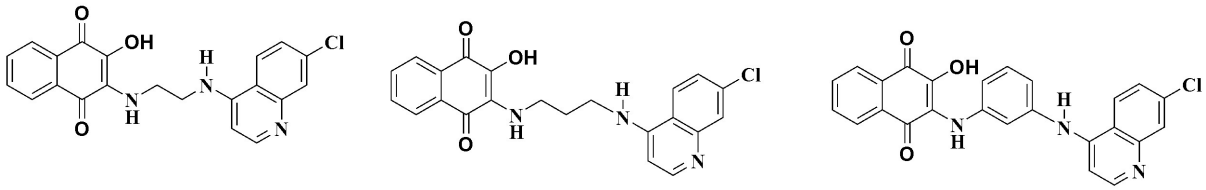


Figure 1-31: Quinoline-lawsone hybrids with antiplasmodial activity

Hybrid quinolines with pyridine and pyrazoline cores (**Figure 1-32**) were moderately active against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Pseudomonas aeruginosa*) and showed very good activity against the fungal strains *Candida albicans*, *Aspergillus niger* and *Aspergillus clavatus*. They also exhibited low cytotoxicity on HeLa cell lines (Desai et al., 2016).

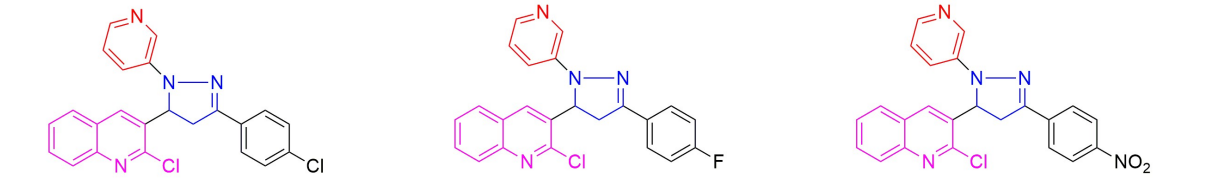


Figure 1-32: Hybrid quinolines with pyridine and pyrazoline frameworks

In this project, the quinoline moiety will be linked together with the aldol product of curcumin to form a hybrid drug that may be more effective than quinoline or curcumin alone.

1.8 Aims and Hypothesis

Since curcumins and curcumin like compounds have a broad range of bioactivity, it was hypothesised that combining this pharmacophore with other bioactive pharmacophores could lead to enhanced bioactivity. In particular, it was thought that the antibacterial activity of curcumins or curcumin like compounds could be enhanced by joining them to these other active pharmacophores leading to lead compounds for new and improved antibiotics.

Aims:

1. To synthesise small libraries of curcumin and curcumin-like hybrid molecules with ketodienes, quinolines, spiro barbiturates and pyrazolines.
2. To fully elucidate the structures of the synthesised compounds.
3. To test the synthesised compounds for their antibacterial activity against both Gram +ve and Gram –ve strains of bacteria.
4. To identify any potential lead compounds that could be developed into antibiotics.

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Chapter 2 The synthesis, structural elucidation and antibacterial activity of curcumin pyrazoline derivatives

***** The compounds referred to in the chapter are referred to in the Abstract, Conclusion and Appendices with **A** preceding the number of the compound. For example **5a** is referred to as **A-5a**.

Abstract

Eleven curcumin pyrazolines derivatives **5a-c**, **5f-k**, **5m** and **5o** were synthesised by the cyclocondensation reaction of various substituted curcumin derivatives with hydrazine hydrate and acetic acid. In addition, the curcumin intermediates **4s**, **4w**, and **4x** were also novel. The synthesis took place *via* a chelated boron intermediate forcing the aldehyde to react with terminal methyl groups instead of the alpha carbon flanked by the two carbonyl groups, resulting in a curcumin boron complex, which is hydrolysed to the curcumin intermediates. All the curcumin intermediates and pyrazolines were tested for their antibacterial activity against two Gram +ve strains (*Staphylococcus aureus* and methicillin resistant *S. aureus* (MRSA)) and four Gram -ve strains (*Salmonella typhimurium, Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli*). The results showed that **5b** (3-Cl derivative) showed activity against both the Gram +ve MRSA (31.3 μg mL^{-1}) and Gram –ve *K. pneumonia* (7.8 μg mL^{-1}). Compound **5g** (2,4-difluoro derivative) showed activity against Gram –ve *K. pneumonia* (0.98 μg mL^{-1}). Two other compounds **4w** (3-methoxy-4-(4-chlorobenzyloxy)) curcumin derivative and **5o** (3-methoxy-4-hydroxy) pyrazoline derivative showed activity against both *S. aureus* and MRSA at 31.3-62.5 μg mL^{-1}. A further compound **5k** (the 4-trifluoro derivative) was also active against the Gram –ve *E. coli* with a MBC of 31.3 μg mL^{-1}.

Keywords: Curcumin, curcumin-pyrazoline, antibacterial activity, boron complex

2.1 Introduction

Curcumin is a diarylheptanoid and the principal component in turmeric, a medicinal plant of the ginger family, Zingiberaceae (Akram et al., 2010; Lee et al., 2013). Curcumin and its derivatives have many interesting applications in traditional medicine such as antibacterial (Cao et al., 2014), antidiabetic (Puneet et al., 2015; Yousefi et al., 2015), anticancer (Adams et al., 2004; Simoni et al., 2008; Fuchs et al., 2009; Cao et al., 2014; Li et al., 2015), antioxidant (Cao et al., 2014; Choudhury et al., 2015; Li et al., 2015) and anti-inflammatory properties (Khan et al., 2012; Liang et al., 2009). Curcumins have the ability to cross the blood brain barrier, important in the treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases (Ahsan et al., 2015; Elmegeed et al., 2015; Okuda et al., 2016). Curcumins complexed to metals such as aluminium, vanadium, cobalt, nickel and copper was found to enhance its antitumour properties (Krishnankutty et al., 2003, John et al., 2012).

Curcumin can exist in two tautomeric forms, the enol and keto form, with the more energetically stable enol form being predominant in solution (Akram et al., 2010; Ferrari et al., 2011). They are synthesised from the aldol cross condensation reaction of a benzaldehyde with a chelated boron intermediate. The chelated boron intermediates are normally formed by reaction of boric oxide (B_2O_3) with acetylacetone (Handler et al., 2007; Qui et al., 2008; Han et al., 2011; Leow et al., 2014; Okuda et al., 2016). This intermediate is necessary to prevent the highly acidic methylene carbon from reacting with the aldehyde. An alternative method using boron trifluoride (BF_3), an inexpensive reagent compared to B_2O_3 was also carried out with success (Rao et al., 2011).

The dicarbonyl system of curcumin with the acidic methylene group makes it an interesting scaffold which can react with other electrophiles. This leads to the possibility of placing other pharmacophores onto the curcumin scaffold which could enhance the overall activity of the molecule. Recently, the dicarbonyl system of curcumins were reacted with various reagents such as hydrazines, aromatic aldehydes or aromatic aldehydes together with benzothiazoles, resulting in modified curcumin derivatives (Sahu et al., 2016). These modified curcumin systems showed enhanced anticancer activity to the curcumins themselves.

Pyrazoles on the other hand are five membered nitrogen containing heterocycles, formed by the cyclocondensation reaction of an α,β-unsaturated carbonyl system with hydrazine (Kapalanki et al., 2009; Marella et al., 2014; Raghav et al., 2014). These molecules possess a wide range of medicinal properties such as analgesic, anticancer and antimalarial (Kaplancikli et al., 2009; Pal et al., 2012; Marella et al., 2014; Raghav et al., 2014). Curcumin pyrazoles in particular showed the potential to be used in Parkinson's disease as these compounds were able to cross the blood brain barrier (Ahsan et al., 2015).

In this work, curcumins and their pyrazole derivatives were synthesised in order to assess the antibacterial activty of the synthesised compounds. Prenyloxy, butyloxy, allyloxy and benzyloxy groups were susbstituted onto the aromatic aldehydes prior to forming the curcumins. Curcumins containing these groups were reported to be very active having anti-inflammatory and anti-HIV activity and being active against Alzheimer's disease (Koeberle et al., 2014; Di Martino et al., 2015; Minassi et al., 2015).

2.2 Experimental

General Experimental Procedures

Reagent grade chemicals used in this study were purchased from Sigma Aldrich, South Africa. NMR spectra were recorded using a Bruker Avance III 400 MHz NMR spectrometer at room temperature with chemical shifts (δ) recorded against the internal standard, tetramethylsilane (TMS). IR spectra were recorded on a Perkin Elmer Spectrum 100 FTIR spectrometer with universal ATR sampling accessory. LC-MS was recorded on a Shimadzu LC-MS 2020 instrument and samples were run through a sample loop by direct injection. Samples were dissolved in methanol and analysed with a mobile phase of 95% acetonitrile/water containing 0.1% formic acid at a flow rate of 0.2 mL min^{-1}. IR spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrometer with universal ATR sampling accessory. Ultraviolet (UV) analyses were performed using a UV-VIS Shimadzu series 200 spectrophotometer in methanol. Melting point determinations were carried out using a Stuart Smart scientific melting point apparatus. HRMS was carried out on a Bruker microTOF-Q II ESI instrument operating at ambient temperatures with a sample concentration of 1 ppm. Column chromatography was carried out on 2 cm glass columns with silica gel as the stationary phase and varying solvent ratios of ethyl acetate and hexane as the mobile phase. In a typical separation, ~ 1.0 g of sample was loaded onto the column and fractions of 50 mL were collected.

Synthesis of prenyl, allyl, benzyloxy and butyloxy aldehydes (2p-x)

Vanillin (24.6 mmol; 2.0 g) or 4-hydroxybenzaldehyde (24.6 mmol; 2.0 g) was dissolved in acetone (50 mL) and dry potassium carbonate and potassium iodide added to the reaction mixture. Prenyl bromide (24.6 mmol; 1.5 mL or allyl bromide (24.6 mmol; 1.1 mL), was then added to the substituted aldehydes and the reaction refluxed for 1 h between 30-40 °C. To

vanillin or 4-hydroxybenzaldehyde, either *n*-butyl bromide or benzyl bromide was added (65.6 mmol), and the reaction refluxed at 100 °C. The butyloxy compounds took 12-16 h to complete, whereas the benzyloxy compounds reacted within 3h. The reaction was monitored by TLC. Upon completion of the reactions, the acetone was removed, the contents dissolved in water and extracted with CH_2Cl_2, dried over sodium sulfate and purified using column chromatography, ethyl acetate: n-hexane (20:80) to yield yellow oils for the prenyl, allyl and butyloxy aldehydes and white solids for the benzyloxy aldehydes. For the butyloxy aldehdyes, the solution after extraction was washed with 10% sodium hydroxide solution, then brine and water and dried over sodium sulfate.

Synthesis of Curcumin derivatives (4a-x)

Preparation of substituted curcumins ((1*E*,6*E*)-1,7-*bis*(phenyl)hepta-1,6-diene-3,5-dione derivatives) was synthesized according to the method published in Leow et al. (2015). Briefly, acetylacetone (10 mmol) was added to boric anhydride (5 mmol) in ethyl acetate and the reaction refluxed at 70 °C for 3 h. The boric complex that formed was filtered, dried and reacted with substituted aldehydes, tributyl borate and n-butylamine and left overnight refluxing at 70 °C. The chelated curcumin boron complexes (**3a-x**) that formed was then hydrolysed with dilute acid (HCl) at room temperature to pH 5, where the compounds **4a-x** precipitated out of solution. The structures of the synthesised compounds were verified by NMR spectroscopy. The intermediates **4s**, **4w** and **4x** were novel and the data are presented below. The known compounds were compared to data in the literature (John et al., 2006; Feng et al., 2009; Konatham et al., 2010; Di Martino et al., 2015; Leow et al., 2015; Groundwater et al., 2016; Laali et al., 2016; Lui et al., 2016).

(1E,6E)-1,7-bis(4-butoxyphenyl)hepta-1,6-diene-3,5-dione (**4s**)*,* yellow solid, mp 162-165 °C, IR υ_{max} (cm^{-1}): 3177 (C-H), 1600 (C=O), 1392 (C=C), 1301 (C-O). UV λ_{max} nm (log ε): 230 (4.40), 413 (4.29). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.96 (t, 2H, *J* = 7.3 Hz, H-14'/14), 1.52-1.43 (m, 2H, H-13'/13), 1.78-1.72 (m, 2H, H-12'/12), 3.98 (t, 2H, *J* = 6.5 Hz, H-11'/11), 5.75 (s, 1H, H-10), 6.45 (d, 2H, *J* = 15.9 Hz, H-8'/8), 6.88 (d, 4H, *J* = 8.8 Hz, 3'/3/5'/5), 7.47 (d, 4H, *J* = 8.8 Hz, H-2'/2/6'/6), 7.58 (d, 2H, *J* = 15.9 Hz, H-7'/7). ^{13}C NMR (100 MHz, $CDCl_3$): 13.8 (C-14'/14), 19.2 (C-13'/13), 31.2 (C-12'/12), 67.9 (C-11'/11), 101.3 (C-10), 114.9 (C-3'/3/5'/5), 121.7 (C-8'/8), 127.6 (1'/1), 129.8 (2'/2/6'/6), 140.2 (C-7'/7), 160.9 (C-4'/4), 183.3 (C-9'/9). HRMS (*pos*) *m/z* 443.2198 [M + Na] (Calculated for $C_{27}H_{32}O_4Na$, 443.2198)

(1E,6E)-1,7-bis(4-(4-chlorobenzyloxy)phenyl)hepta-1,6-diene-3,5-dione (**4w**), mp 188-191 °C, yellow solid, IR υ_{max} (cm^{-1}): 3037 (C-H), 1627 (C=O), 1491 (C=C), 1303 (C-O). UV λ_{max} nm (log ε): 221 (4.56), 356 (4.47). ^{1}H NMR (400 MHz, DMSO) δ 5.16 (s, 4H, H-7'''/7''), 5.87 (s, 1H, H-10), 6.66 (d, 2H, *J* = 16.0 Hz, H-8'/8), 7.05 (d, 4H, *J* = 8.6 Hz, H-3'/3/5'/5), 7.42-7.47 (m, 8H, H-2'''/2''/3'''/3''/5'''/5''/6'''/6''), 7.52 (d, 2H, *J* = 16.0 Hz, H-7'/7), 7.64 (d, 4H, *J* = 8.6 Hz, H-6'/6/2'/2). ^{13}C NMR (100 MHz, DMSO): 68.5 (7'''/7''), 100.8 (C-10), 115.2 (C-3'/3/5'/5), 120.7 (C-8'/8), 127.7 (C-1'/1), 128.4 (C-3'''/3''/5'''/5''), 129.5 (C-2'/2/6'/6), 129.9 (C-2'''/2''/6''/6'''), 135.8 (C-1'''/1''), 139.2 (C-7'/7), 159.7 (C-4'/4), 177.6 (C-9'/9). HRMS (*neg*) *m/z* 555.1133 [M - H] (Calculated for $C_{33}H_{25}Cl_2O_4$, 555.1130)

(1E,6E)-1,7-bis(4-(4-chlorobenzyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (**4x**), orange solid, mp 164-167 °C, IR υ_{max} (cm^{-1}): 2924 (C-H), 1637 (C=O), 1412 (C=C), 1302 (C-O). UV λ_{max} nm (log ε): 206 (5.02), 235 (4.51). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.09 (s, 6H, OC<u>H</u>$_3$), 5.11 (s, 4H, H-7'''/7''), 5.61 (s, 1H, H-10), 6.30 (d, 2H, *J* = 15.9 Hz, H-8'/8), 6.81 (d, 2H, *J* = 8.1 Hz, H-5'/5), 7.07-7.00 (m, 4H, H-2'/2/6'/6), 7.33 (m, 8H, H-

2'''/2''/3'''/3''/5'''/5''/6'''/6''), 7.50 (d, 2H, *J* = 15.9 Hz, H-7'/7). ^{13}C NMR (100 MHz, $CDCl_3$): 56.0 ($O\underline{C}H_3$), 70.2 (C-7'''/7''), 100.8 (C-10), 110.3 (C-2'/2), 113.6 (C-5'/5), 120.9 (C-8'/8), 122.0 (C-6'/6), 139.7 (C-7'/7), 149.7 (C-3'/3), 149.8 (C-4'/4), 177.6 (C-9'/9). HRMS (*neg*) *m/z* 615.1342 [M - H] (Calculated for $C_{35}H_{29}Cl_2O_6$, 615.1341)

Synthesis of pyrazoline derivatives (5a-c, f-k, m, o)

Hydrazine hydrate (19 mmol) was added to substituted curcumins (1.6 mmols) in glacial acetic acid (25 mL) and the reaction refluxed at 110 °C for 12-16 h, whilst being monitored by TLC. Upon completion, it was cooled and poured into ice water. The products **5a-c, f-k, m** and **o** precipitated out of solution. It was then filtered and recrystallized in ethanol. Products **5h** and **5j** did not precipitate. They were dissolved in water and extracted with CH_2Cl_2, concentrated and purified by column chromatography, ethyl acetate: n-hexane (20:80).

3,5-bis(2-chlorostyryl)-1H-pyrazole hydrate (**5a**), white solid; mp 177-180 °C, IR υ_{max} (cm^{-1}): 3154 (N-H), 1590 (C=C). UV λ_{max} nm (log ε): 289 (4.55). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.74 (s, 1H, H-4), 7.02 (d, *J* = 16.4 Hz, 2H, H-8''/8'), 7.20-7.16 (m, 4H, H-4''/4'/5''/5'), 7.35 (dd, 2H, *J* = 7.5, 1.6 Hz, H-3''/3'), 7.48 (d, 2H, 16.4 Hz, H-7''/7'), 7.65 (dd, 2H, *J* = 7.8, 1.8 Hz, H-6''/6'). ^{13}C NMR (100 MHz, $CDCl_3$): 101.1 (C-4), 120.1 (C-8''/8'), 126.4 (C-6''/6'), 126.8 (C-7''/7'), 127.0 (C-5''/5'), 128.9 (C-4''/4'), 129.9 (C-3''/3'), 133.5 (C-2''/2'), 134.6 (C-1''/1'). HRMS (*neg*) *m/z* 339.0454 [M - H] (Calculated for $C_{19}H_{13}Cl_2N_2$, 339.0456)

C-3/5 could not be detected

3,5-bis(3-chlorostyryl)-1H-pyrazole (**5b**), white solid; mp 166-169 °C, IR υ_{max} (cm^{-1}): 3175 (N-H), 1593 (C=C). UV λ_{max} nm (log ε): 242 (3.65), 328 (4.23). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.61 (s, 1H, H-4), 6.99 (d, 2H, *J* = 16.7 Hz, 7''/7'), 7.04 (d, 2H, *J* = 16.7 Hz, 8''/8'), 7.19 (m,

4H, H-4"/4'/5"/5'), 7.28-7.25 (m, 2H, H-6'/6), 7.38 (bs, 2H, H-2"/2'). ^{13}C NMR (100 MHz, $CDCl_3$): 101.0 (C-4), 118.5 (C-8"/8'), 124.7 (C-6"/6'), 126.4 (C-2"/2'), 128.1 (C-4"/4'), 129.9 (C-5"/5'), 130.0 (C-7"/7'), 134.7 (C-3"/3'), 138.2 (C-1"/1'), 146.7 (C-3/5). HRMS (*neg*) *m/z* 339.0460 [M - H] (Calculated for $C_{19}H_{13}Cl_2N_2$, 339.0456)

3,5-bis(4-chlorostyryl)-1H-pyrazole hydrate (**5c**), light yellow solid; mp 179-182 °C, IR υ_{max} (cm^{-1}): 3196 (N-H), 1550 (C=C). UV λ_{max} nm (log ε): 240 (4.87), 327 (4.47). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.59 (s, 1H, H-4), 6.94 (d, 2H, *J* = 16.5 Hz, H-8"/8'), 7.02 (d, 2H, *J* = 16.5 Hz, H-7"/7'), 7.27 (d, 4H, *J* = 8.6 Hz, H-3"/3'/5"/5'), 7.35 (d, 4H, *J* = 8.6 Hz, H-2"/2'/6"/6'). ^{13}C NMR (100 MHz, $CDCl_3$): 100.7 (C-4), 117.8 (C-8"/8'), 127.7 (C-5"/5'/3"/3'), 128.9 (C-2"/2'/6"/6'), 130.0 (C-7"/7'), 133.8 (C-4"/4'), 134.9 (C-1"/1'), 146.5 (C-3/5). HRMS (*neg*) *m/z* 339.0458 [M - H] (Calculated for $C_{19}H_{13}Cl_2N_2$, 339.0456)

3,5-bis(4-fluorostyryl)-1H-pyrazole (**5f**), white solid; mp 185-188 °C, IR υ_{max} (cm^{-1}): 3216 (N-H), 1597 (C=C). UV λ_{max} nm (log ε): 240 (4.25), 325 (4.19), 351 (4.29). ^{1}H NMR (400 MHz, DMSO) δ 6.74 (s, 1H, H-4), 7.05 (d, 2H, *J* = 16.6 Hz, H-8"/8'), 7.16 (d, 2H, *J* = 16.6 Hz, H-7"/7'), 7.22 (t, 4H, *J* = 8.8 Hz, H-3"/3'/5"/5'), 7.61 (dd, 4H, *J* = 8.8, 5.6 Hz, H-6"/6'/2"/2'). ^{13}C NMR (100 MHz, DMSO): 100.4 (C-4), 115.6 (d, *J* = 20.9 Hz, C-5"/5'/3"/3'), 116.5 (C-8"/8'), 128.2 (d, *J* = 8.1 Hz, C-2"/2'/6"/6'), 128.7 (C-7"/7'), 133.3 (C-1"/1'), 148.8 (C-3/5), 161.6 (d, *J* = 243.4 Hz, C-4"/4'). HRMS (*neg*) *m/z* 307.1053 [M - H] (Calculated for $C_{19}H_{13}F_2N_2$, 307.1047)

3,5-bis(2,4-difluorostyryl)-1H-pyrazole (**5g**), yellow solid, mp 190-193 °C, IR υ_{max} (cm^{-1}): 3194 (N-H), 1565 (C=C). UV λ_{max} nm (log ε): 285 (4.54). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.67 (s, 1H, H-4), 6.81 (ddd, 2H, *J* = 11.0, 8.8, 2.4 Hz, H-3"/3'), 6.86 (ddd, 2H, *J* = 10.5, 8.5, 2.3 Hz, H-5"/5'), 7.03 (d, 2H, *J* = 16.6 Hz, H-8"/8'), 7.14 (d, 2H, *J* = 16.6 Hz, H-7"/7'), 7.50 (ddd, 2H, *J* = 8.6, 6.4, 6.4 Hz, H-6"/6'). ^{13}C NMR (100 MHz, $CDCl_3$): 100.6 (C-4), 104.2 (t, *J* =

28.6, C-3"/3'), 111.6 (dd, *J* = 22.0, 3.7 Hz, C-5"/5'), 119.6 (C-8"/8'), 122.3 (C-7"/7'), 128.1 (dd, *J* = 9.8, 5.5 Hz, C-6"/6'), 147.3 (C-3/5), 162.5 (dd, *J* = 246.5, 11.6 Hz, C-2"/2'), 160.2 (dd, *J* = 214.8, 13.1 Hz, C-4"/4'). HRMS (*neg*) *m/z* 343.0859 [M - H] (Calculated for $C_{19}H_{11}F_4N_2$, 343.0858)

C-1"/1' could not be detected.

3,5-bis(3,4-difluorostyryl)-1H-pyrazole (**5h**), yellow solid; mp 166-169 °C, IR υ_{max} (cm^{-1}): 2920 (N-H), 1512 (C=C). UV λ_{max} nm (log ε): 241 (4.67), 324 (4.70), 352 (4.88). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.57 (s, 1H, H-4), 6.88 (d, 2H, *J* = 16.4 Hz, H-8"/8'), 6.95 (d, 2H, *J* = 16.4 Hz, H-7"/7'), 7.08-7.03 (m, 4H, H-5"/5'/6"/6'), 7.17-7.14 (m, 2H, H-2"/2'). ^{13}C NMR (100 MHz, $CDCl_3$): 100.9 (C-4), 114.6 (d, *J* = 18.8, C-2"/2'), 117.4 (d, *J* = 18.9 Hz, C-5"/5'), 118.5 (C-8"/8'), 122.8 (dd, *J* = 6.1, 3.4 Hz, C-6"/6'), 128.9 (C-7"/7'), 133.6 (dd, *J* = 5.4, 3.8 Hz, C-1"/1'), 146.7 (C-3/5), 150.2 (dd, *J* = 245.9, 43.2 Hz, C-3"/3'), 150.3 (dd, *J* = 240.6, 40.1 Hz, C-4"/4'). HRMS (*neg*) *m/z* 343.0859 [M - H] (Calculated for $C_{19}H_{11}F_4N_2$, 343.0858)

3,5-bis(2-(trifluoromethyl)styryl)-1H-pyrazole (**5i**), white solid; mp 172-175 °C, IR υ_{max} (cm^{-1}): 3187 (N-H), 1558 (C=C). UV λ_{max} nm (log ε): 300 (4.36). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.69 (s, 1H, H-4), 7.02 (d, 2H, *J* = 16.2 Hz, H-8"/8'), 7.34 (t, 2H, *J* = 7.7 Hz, H-4"/4'), 7.47 (dd, 2H, *J* = 16.2, 2.8 Hz, H-7"/7'), 7.49 (t, 2H, *J* = 7.6 Hz, H-5"/5'), 7.64 (d, 2H, *J* = 7.8 Hz, H-3"/3'), 7.70 (d, 2H, *J* = 7.8 Hz, H-6"/6'). ^{13}C NMR (100 MHz, $CDCl_3$): 101.3 (C-4), 121.3 (C-8"/8'), 124.3 (q, *J* = 272.1 Hz, $\underline{C}F_3$), 126.0 (q, *J* = 5.7 Hz, C-3"/3'), 126.9 (C-7"/7'), 126.8 (q, *J* = 1.8 Hz, C-6"/6'), 127.6 (q, *J* = 30.1 Hz, C-2"/2'), 127.7 (C-4"/4'), 132.0 (C-5"/5'), 135.5 (q, *J* = 1.7 Hz, C-1"/1'), 146.6 (C-3/5). HRMS (*neg*) *m/z* 407.0984 [M - H] (Calculated for $C_{21}H_{13}F_6N_2$, 407.0983)

3,5-bis(3-(trifluoromethyl)styryl)-1H-pyrazole (**5j**), white solid; mp 221-224 °C, IR υ_{max} (cm^{-1}): 3156 (N-H), 1591 (C=C). UV λ_{max} nm (log ε): 240 (4.79), 372 (4.24), ^{1}H NMR (400 MHz,

DMSO) δ 6.83 (s, 1H, H-4), 7.32 (d, 2H, *J* = 16.6 Hz, H-7"/7'), 7.26 (d, 2H, *J* = 16.6 Hz, H-8"/8'), 7.62 (bs, 4H, H-5"/5'/6"/6'), 7.90 (bs, 4H, H-2"/2'/4"/4'). ^{13}C NMR (100 MHz, DMSO): 101.9 (C-4), 118.3 (C-8"/8'), 123.2 (q, *J* = 14.5 Hz, C-5"/5'), 123.8 (q, *J* = 10.2 Hz, C-6"/6'), 127.9 (C-7"/7'), 130.1-130.6 (m, C-2"/2', 4"/4' and 3"/3'), 138.7 (C-1"/1'), 141.8 (C-3/5). HRMS (*neg*) *m/z* 407.0985 [M - H] (Calculated for $C_{21}H_{13}F_6N_2$, 407.0983)

3,5-bis(4-(trifluoromethyl)styryl)-1H-pyrazole (**5k**), white solid; mp 225-228 °C, IR υ_{max} (cm^{-1}): 3081 (N-H), 1578 (C=C). UV λ_{max} nm (log ε): 308 (3.96). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.70 (s, 1H, H-4), 7.11 (s, 4H, H-7"/7' and 8"/8'), 7.53 (d, 4H, *J* = 8.6 Hz, H-5"/5' and 3"/3'), 7.57 (d, 4H, *J* = 8.6 Hz, H-2"/2' and 6"/6'). ^{13}C NMR (100 MHz, $CDCl_3$): 101.3 (C-4), 119.7 (C-8"/8'), 124.1 (q, *J* = 270.2 Hz, $\underline{C}F_3$), 125.7 (q, *J* = 3.9 Hz, C-3"/3' and 5"/5'), 126.6 (C-2"/2' and 6"/6'), 129.7 (C-7"/7'), 129.9 (C-4"/4'), 139.8 (C-1"/1'), 146.7 (C-3/5). HRMS (*neg*) *m/z* 407.0977 [M - H] (Calculated for $C_{21}H_{13}F_6N_2$, 407.0983)

3,5-bis(4-methoxystyryl)-1H-pyrazole (**5m**), yellow solid; mp 171-174 °C, IR υ_{max} (cm^{-1}): 3383 (N-H), 1508 (C=C). UV λ_{max} nm (log ε): 299 (4.69). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.80 (s, 6H, $OC\underline{H}_3$), 6.56 (s, 1H, H-4), 6.85 (d, 4H, *J* = 8.6 Hz, H-3"/3' and 5"/5'), 6.88 (d, 2H, *J* = 16.4 Hz, H-8"/8'), 7.02 (d, *J* =16.4 Hz, H-7"/7'), 7.38 (d, 4H, *J* = 8.6 Hz, 2"/2' and 6"/6'). ^{13}C NMR (100 MHz, $CDCl_3$): 55.4 ($O\underline{C}H_3$), 99.5 (C-4), 114.2 (C-3"/3' and 5"/5'), 115.6 (C-8"/8'), 127.8 (C-2"/2' and 6"/6'), 129.4 (C-1"/1'), 130.5 (C-7"/7'), 146.8 (C-3/5), 159.6 (C-4"/4'). LCMS: *m/z* (rel. int.): 332 (100) [M^+]. HRMS (*neg*) *m/z* 331.1449 [M - H] (Calculated for $C_{21}H_{19}N_2O_2$, 331.1447)

4,4'-(1E,1'E)-2,2'-(1H-pyrazole-3,5-diyl)bis(ethene-2,1-diyl)bis(2-methoxyphenol) (**5o**), brown solid; mp 202-205 °C, IR υ_{max} (cm^{-1}): 3321 (O-H), 3321 (N-H), 1592 (C=C). UV λ_{max} nm (log ε): 327 (4.29). ^{1}H NMR (400 MHz, DMSO) δ 3.83 (s, 6H, $OC\underline{H}_3$), 6.62 (s, H-4), 6.76 (d, 2H, *J* = 8.0 Hz, H-5"/5'), 6.91 (d, 2H, *J* = 16.6 Hz, H-8"/8'), 6.92 (dd, 2H, *J* = 8.0, 1.8

Hz, H-6"/6'), 7.03 (d, 2H, *J* = 16.6 Hz, H-7"/7'), 7.14 (d, 2H, *J* = 1.8 Hz, H-2"/2'), 9.16 (s, O<u>H</u>). ^{13}C NMR (100 MHz, DMSO): 55.5 (O<u>C</u>H$_3$), 99.3 (C-4), 109.5 (C-2"/2'), 115.6 (C-8"/8' and 5"/5'), 120.0 (C-6"/6'), 128.3 (C-1"/1'), 129.5 (C-7"/7'), 146.7 (C-3/5), 147.8 (C-3"/3'), 167.9 (C-4"/4'). HRMS (*neg*) *m/z* 363.1348 [M - H] (Calculated for $C_{21}H_{19}N_2O_4$, 363.1345)

Antimicrobial assay

The antimicrobial activities of the synthesized compounds **4a-x**, **5a-c, 5f-k, m** and **o**, were investigated against two Gram +ve bacteria (*Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* Rosenbach ATCC BAA-1683 (MRSA) and four Gram -ve bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 31488, *Escherichia coli* ATCC 25922 and *Salmonella typhimurium* ATCC 14026).

Bacterial test organisms were grown on Mueller-Hinton agar (21 g L^{-1}) at 35-37 °C in a CO_2 incubator for 24 hours. Organisms were suspended in saline and the turbidity adjusted to a 0.5 McFarland standard. Mueller-Hinton agar plates were prepared by dissolving 38 g of agar in 1 L of water and pouring these into sterile petri dishes, which were allowed to set at room temperature. The Mueller-Hinton agar plate was inoculated with the required strain of bacteria by streaking a swab over the entire sterile agar surface evenly after it was firmly dipped into the adjusted suspension. Antibiotic assay discs were impregnated with 10 µL of each sample (1 mg mL^{-1} in DMSO) and placed onto Mueller-Hinton plates, left to incubate for 16-18 hours at 37°C, and the zones of inhibition measured in mm using a transparent ruler.

Minimum Bactericidal Concentration (MBC) Assay

All compounds that exhibited zones of inhibition during the disc diffusion assay were dissolved in DMSO (1 mg mL^{-1}) and serially diluted over ten dilutions with DMSO. The Mueller-Hinton agar plate was inoculated with the required strain of bacteria by streaking a swab over the entire sterile agar surface evenly after it has been firmly dipped into the adjusted suspension. 5 μL of each sample was then placed into the plate and left to incubate at 37 ºC for 18 h. This was performed in triplicate. The MBC was the lowest concentration where a zone of inhibition was observed. The DMSO control showed no growth inhibition of the bacterial strains in all cases.

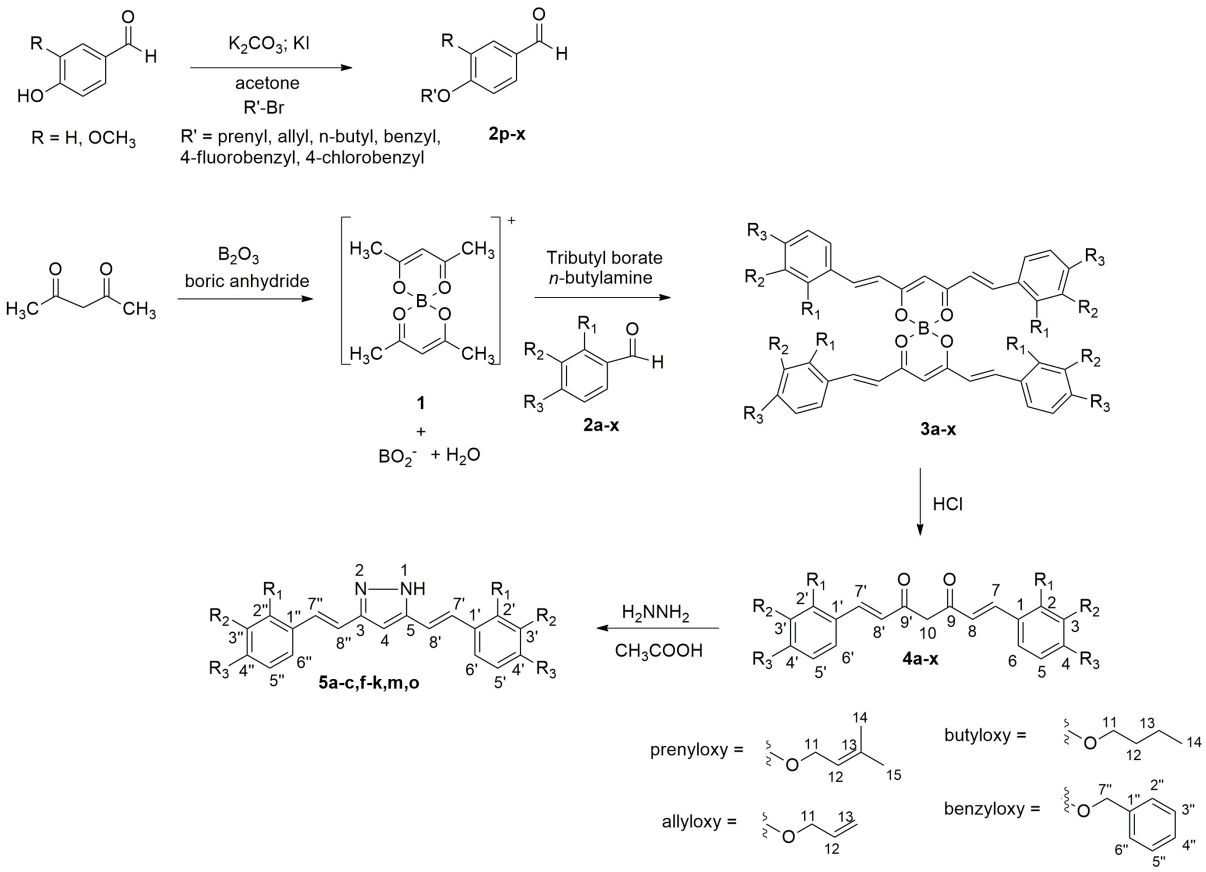
2.3 Results and Discussion

The synthesis of the curcumins **4a-x** starts with the formation of the chelated boron intermediate **1** from acetylacetone and boric anhydride. Regioselectivity is key in the formation of the curcumin since the benzaldehyde must react at the terminal methyl groups of acetylacetone. The chelated boron intermediate **1** is a sterically hindered boron complex enabling the substituted aldehyde to react with the terminal methyl group in an aldol condensation reaction in the presence of tribuyl borate and *n*-butylamine as a base to form the chelated curcumin boron complex **3a-x** (Handler et al., 2007; Qui et al., 2008; Han et al., 2011; Leow et al., 2014; Okuda et al., 2016). The bulky organic base catalyst n-butylamine is used to deprotonate the methyl groups at either end of acetylacetone. Tributylborate is used as a water scavenger to drive enone formation. Subsequent hydrolysis with HCl resulted in the formation of the curcumins **4a-x** in a 90% yield.

A range of curcumins were synthesised, with varying substituents at the *ortho*, *meta* and *para* positions on the phenyl ring (**Table 2-1**). All the synthesised curcumins were symmetrical since only one aldehyde was used in the synthesis of each of the compounds. Electron

withdrawing halogens, chlorine and fluorine and electron donating methoxy, hydroxy, *O*-prenyl (3-methylbut-2-en-1-yl), *O*-allyl (propen-3-yl), *O*-butyl and *O*-benzyl groups were substituted on the aromatic ring at various positions. The *O*-alkylated aldehydes were synthesised by reaction of either vanillin or 4-hydroxybenzaldehyde with the alkyl or benzyl bromides under basic conditions. Seventeen monosubstituted and seven disubstituted compounds were synthesised in order to carry out a structure activity relationship analysis in the antibiotic assays carried out. The 4-butyloxy curcumin (**4s)**, 4-chlorobenzyloxy curcumin (**4w)**, and 3'-methoxy-(4'-chlorobenzyloxy) curcumin (**4x)** were all new compounds.

Attempts were then made to convert the 24 curcumins to pyrazolines with glacial acetic acid and hydrazine hydrate. However, only eleven (**5a-c, f-k, m, o**) of the twenty-four curcumins converted to pyrazolines. The yields of the pyrazolines were between 65-85%. The 2' and 3'-fluorocurcumins (**4d-e**), the 2'-methoxycurcumin (**4l**), the 2',4'-dimethoxycurcumin (**4n**) and all the *O*-alkylated curcumins (**4p-x**) did not form pyrazolines under these reaction conditions. The cyclocondensation reaction is driven by the reaction of hydrazine hydrate with the dicarbonyl system of the curcumin and acetic acid acts as a solvent and catalyst. Only the 3-methoxy-4-hydroxy derivative **5o** amongst the pyrazolines had been previously synthesised. The other ten compounds **5a-m** were new.



Scheme 2-1: Synthetic scheme of curcumin pyrazolines

Table 2-1: Substitution patterns and yields of compounds **4** (curcumins) and **5** (pyrazoles)

No.	R_1	R_2	R_3	% yield (4)	% yield (5)
4a	Cl	H	H	90	70
4b	H	Cl	H	93	75
4c	H	H	Cl	95	85
4d	F	H	H	91	NR
4e	H	F	H	91	NR
4f	H	H	F	96	80
4g	F	H	F	90	75
4h	H	F	F	93	70
4i	CF_3	H	H	92	69
4j	H	CF_3	H	95	65
4k	H	H	CF_3	98	67
4l	OCH_3	H	H	92	NR
4m	H	H	OCH_3	94	85
4n	OCH_3	H	OCH_3	91	NR
4o	H	OCH_3	OH	98	85
4p	H	H	*O*-prenyl	90	NR
4q	H	H	*O*-allyl	97	NR
4r	H	OCH_3	*O*-allyl	92	NR
4s	H	H	*O*-butyl	96	NR
4t	H	H	*O*-benzyl	95	NR
4u	H	OCH_3	*O*-benzyl	90	NR
4v	H	H	*O*-(4-F benzyl)	90	NR
4w	H	H	*O*-(4-Cl benzyl)	95	NR
4x	H	OCH_3	*O*-(4-Cl benzyl)	93	NR

The structures of the curcumins were elucidated by their ^{1}H, ^{13}C and 2D NMR spectra. For example in **4c** (the 4-chloro derivative), a singlet resonance corresponding to H-10 is visible at δ 5.81 and integrates to one proton since keto-enol tautomerism occurs in solution, the enol tautomer being more stable than the keto form (Akram et al., 2010, Ferrari et al., 2011). The pair of doublets at δ 6.57 and δ 7.59 with a *J* coupling of 15.8 Hz indicated that the *α,β*-unsaturated moiety of H-8'/8 and H-7'/7 respectively formed and that the molecule was symmetrical since only one set of these doublets were seen, which integrated to four protons. The *ortho* coupled aromatic proton resonances of H-2'/2/6'/6 and H-3'/3/5'/5 occurred as doublets at δ 7.46 (*J* = 8.4 Hz) and 7.34 (*J* = 8.4 Hz) respectively. The carbonyl resonance occurred at δ 183.1 and C-10 occurred at δ 102.0.

A crystal structure of one of the curcumins **4m** (the 4-methoxy derivative) was solved in the C2/c space group with six molecules in the unit cell and shows the asymmetric unit containing the enol form of one half of the molecule (**Figure 2-1**), which confirms that the enol form is the most stable tautomer of the molecule.

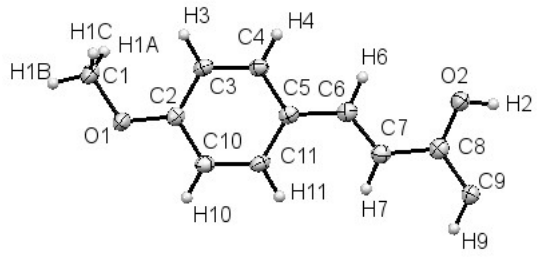


Figure 2-1: Ortep diagram of the asymmetric unit of 4-methoxycurcumin (**4m**)

Formation of the pyrazoles were indicated by the deshielded shifts of H-8'/8 and H-10, which for example in **5c** shifted from δ 6.57 to 6.94 (*J* = 16.4 Hz, H-8''/8') and from δ 5.81 to 6.59

respectively, and a shielded shift of H-7'/7 from δ 7.59 to 6.94 (J = 16.4 Hz, H-7''/7'). In the ^{13}C NMR spectrum the C-7'/7 and C-8'/8 resonances were also shifted from 139.3 and 124.5 respectively to 130.0 and 117.8 and C-2'/2/6'/6 and C-5'/5/3'/3 resonances from 129.3 and 129.2 to 128.9 and 127.7.

The overlaid spectra of **4c** and **5c** in **Figure 2-2** shows the differences in chemical shifts between the two compounds.

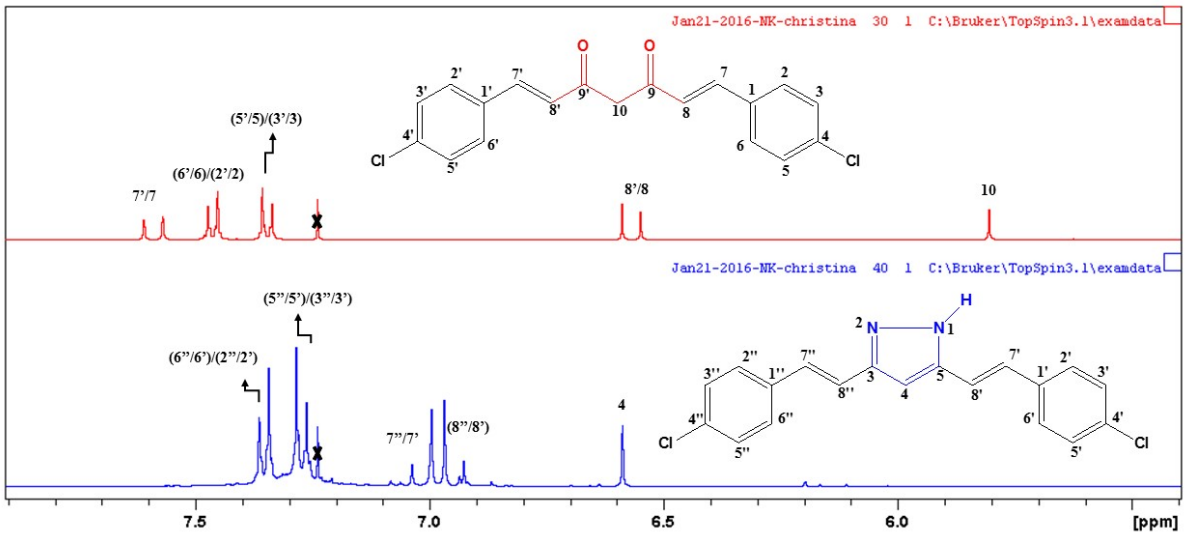


Figure 2-2: ^{1}H NMR spectrum of 4-chlorocurcumin (**4c**) and its 4-chloropyrazoline derivative (**5c**)

All curcumin intermediates and pyrazoline derivatives were tested against two Gram +ve bacterial strains (*S. aureus* and *S. aureus Rosenbach (*MRSA), and four Gram –ve strains, *S. typhimurium, P. aeruginosa*, *K. pneumonia* and *E. coli*. Preliminary screening using the disc diffusion method was used to select compounds to determine their minimum bactericidal concentration (MBC) values. All compounds that showed activity against one or more strains of bacteria were then selected for the MBC assay. The results of this assay is presented in **Table 2-2**.

Table 2-2: Minimum bactericidal concentration (MBC in μg mL^{-1}) of test compounds

Compound	Substitution	S. *aureus*	MRSA	*K. pneumonia*	*E. coli*
4b	3-Cl	-	-	-	-
4p	3-OMe, 4-Oprenyl	31.3	-	-	-
4w	3-Ome, 4-bnbr (4-Cl)	31.3	125	-	-
5a	2-Cl	31.3	31.3	-	500
5b	3-Cl	-	31.3	7.8	-
5c	4-Cl	-	-	-	-
5f	4-F	-	-	-	-
5g	2,4-F	-	-	0.9	-
5h	3,4-F	-	31.3	-	-
5i	2-CF_3	-	-	-	-
5j	3-CF_3	-	-	-	-
5k	4-CF_3	-	-	-	31.3
5m	4-OMe	500	-	-	500
5o	3-OMe, 4-OH	62.5	31.3	-	125
Ciprofloxacin		94.3	188.6	2.95	2.95
Levofloxacin		21.6	86.5	21.6	0.34

"-" denotes activity > 500 μg mL^{-1}

All compounds tested were not active against *S. typhimurium* and *P. aeruginosa* at concentrations of 500 μg mL^{-1} or less

Several of the compounds were active against both Gram +ve strains (*S. aureus* and MRSA) and against the Gram –ve *E. coli*. In one instance, compound **5b** and **5g** showed activity against *K. pneumonia* at a MBC of 7.8 μg mL^{-1} and 0.9 μg mL^{-1}. Compound **5b** also showed activity against MRSA at 31.3 μg mL^{-1}. Compounds **4p** and **4w** of the curcumin intermediates were active against *S. aureus* with MBCs of 31.3 μg mL^{-1}. Compound **4w** also showed activity against MRSA at 125 μg mL^{-1}. Compound **5a**, the 2-chloro pyrazoline derivative was active at 31.3 μg mL^{-1} for both *S. aureus* and MRSA. Compound **5h** was active at 31.3 μg mL^{-1} against MRSA and **5k** was active at 31.3 μg mL^{-1} against *E. coli*. Compound **5o** showed activity against three of the bacterial strains, 62.5 μg mL^{-1} against *S. aureus*, 31.3 μg mL^{-1} against MRSA and 125 μg mL^{-1} against *E. coli*. Those compounds showing activity at 31.3 μg mL^{-1} against *S. aureus* was comparable to that of levofloxacin, which showed a MBC value of 21.6 μg mL^{-1} against the same strain. Five of the compounds, **4w**, **5a**, **5b**, **5h** and **5o** showed better or comparable activity to both ciprofloxacin and

levofloxacin, which showed activity at 188.6 μg mL^{-1} and 86.5 μg mL^{-1} respectively against MRSA. Compound **5b** (7.8 μg mL^{-1}) showed better activity than levofloxacin (21.6 μg mL^{-1}) against *K. pneumonia*, but worse activity than ciprofloxacin (2.95 μg mL^{-1}). Compound **5g** (0.9 μg mL^{-1}) showed better activity than levofloxacin and ciprofloxacin against *K. pneumonia*.

In general, the 3,4-dioxygenated compounds **4w** and **5o** and the 2-chloropyrazoline **5a** showed good activity against both Gram +ve strains, *S. aureus* and MRSA. Amongst the pyrazolines, the 2-chloro substituent is preferred over the 2-trifluoromethyl group, however the 4-trifluoromethyl group is preferred for activity against *E. coli*.

2.4 Conclusion

Aldol cross condensation was used to synthesize 24 curcumins of which three were new. In addition, eleven of these were successfully converted to curcumin-pyrazolines of which ten were new, with varying substituents at the 2, 3 and 4 positions on the aromatic rings. Several of the compounds synthesised showed better antibacterial activity than ciprofloxacin and levofloxacin against *S. aureus* and MRSA. Compound **5b**, the 2-chloro derivative and **5g**, the 2,4-difluoro derivative was very active at 7.8 μg mL^{-1} and 0.9 μg mL^{-1} against *K. pneumonia*. A further two compounds, **5k** (4-trifluoromethyl derivative) and **5o** (3-methoxy-4-hydroxy derivative) were active against *E. coli*. No real trend could be observed between the different substituents and their antibacterial activity.

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Chapter 3 Synthesis and structural elucidation of a series of ketopyrazoles, spirobarbiturates and their oxime derivatives

***** The compounds referred to in this chapter are referred to in the Abstract, Conclusion and Appendices with **B** preceding the number of the compound. For example compound **2a** is referred to as **B-2a**.

Abstract

A series of fourteen substituted acetylated ketopyrazole derivatives (**2a-n)** and fourteen spiro barbiturates (**3a-n)** were synthesised where all the ketopyrazoles and six of the spiro barbiturates were new. The ketodienes (**1a-n**) was the key intermediate for both classes of compounds. The pyrazoles were synthesised by a cyclocondensation reaction of hydrazine hydrate and acetic acid and the spiro barbiturate derivatives (**3a-n)** by a Michael Addition with barbituric acid. The spiro barbiturates were further derivatised with hydroxylamine resulting in ten new spiro oxime derivatives. All these reactions were easily carried out and resulted in excellent yields of 90% or greater. Single crystal XRD of the pyrazoles and spiro barbiturates were carried out to determine the configuration of the stereogenic centers. The pyrazole was found to be in the 5*S* and the spiro barbiturates in the 7*R*, 11*R* configuration. The synthesised compounds were tested for their antibacterial activity against selected Gram +ve and Gram –ve bacteria where amongst the pyrazoles, the chloro derivatives were active at low concentrations with MBC's between 15.6-62.5 μg mL^{-1} against either *S. aureus* or MRSA. In addition, the 4-bromo derivative was also active against MRSA and the 2-chloro derivative against *P. aeruginosa*, both with a MBC value of 31.3 μg mL^{-1}. In contrast the spiro barbiturates showed activity against the Gram –ve *E. coli* and *P. aeruginosa* with MBC values ranging from 0.98-125 μg mL^{-1}. In particular, the 4-bromo derivative showed excellent activity, better than the standards levofloxacin and ciprofloxacin with MBC values of 0.98 μg mL^{-1}. Conversion to the oximes resulted in loss of activity against all the Gram –ve bacteria. However the 4-trifluoro and 4-bromo spiro barbiturate oximes showed weak activity against MRSA.

Keywords: ketodienes, ketopyrazoles, spiro barbiturates, spiro oximes, antibacterial activity

3.1 Introduction

Ketodienes are formed by the reaction of substituted aldehydes with ketones. When cyclic ketones such as cyclohexanone or cyclopentanone are used, cyclic ketodienes form (Lee et al., 2009; Hosoya et al., 2012; Ahmad et al., 2014 Peysan et al., 2014). A subset of these compounds, dibenzylidene ketones, are curcumin analogues that can undergo intramolecular Michael Additions with cyclic diones to form spirocyclic cyclohexanones (Kesharwani et al., 2009; Behera et al., 2009; Aggarwal et al., 2015). Spirocyclic compounds possess a bicyclic ring system with a unique characteristic in that both rings are joined by a single carbon, in many cases, a stereogenic carbon. These molecules have a wide variety of medicinal properties such as antibacterial, antimalarial, anticancer and antitubercular activity (Dandia et al., 2013; Denisov et al., 2013; Landani et al., 2016; Elmas et al., 2017) and have been synthesised by organocatalytic and organometallic methods (Companyo et al., 2010; Wang et al., 2013).

A hybrid molecule is one that has more than one pharmacophore which has the potential to synergistically improve overall activity of the drug (Arnaud et al., 2007; Muregi et al., 2010; Wang et al., 2015). Chalcones are distinguishable from ketodienes by its single vinyl system. The α,β-unsaturated carbonyl system of chalcones readily undergoes Aza-Michael Addition-cyclocondensations to form biologically active aza-cyclic pyrazolines (Rahman et al., 2010; Punyapreddlwar et al., 2016). The same reaction occurs when ketodienes are reacted with hydrazine hydrate, and various substituted phenyl hydrazines (Singh et al., 2012; Lakshmi et al., 2016).

Barbituric acid is a cyclic dione that belongs to a family of cyclic ureas called pyrimidines which have shown potent antioxidant, antidiabetic (Barakat et al., 2017), anti-microbial

(Sokmen et al., 2013; Dhorajiya et al., 2016), and anticancer activity (Maquoi et al., 2004) and potential use as sedatives, anaesthetics, and anti-epileptic drugs (López-Muñoz et al., 2005). The structure of these barbiturates is similar to the pyrimidine bases in DNA and has a good chance of being biologically active molecules. Furthermore, its similarity to 5-fluorouracil, a known anticancer agent makes it a good candidate for an anticancer drug. The dicarbonyl system of barbituric acid increases the acidity of the methylene proton making it easier to form a nucleophile, which can undergo addition reactions with α,β-unsaturated carbonyl systems.

Derivatisation of ketones into oximes may lead to enhanced bioactivity. These oximes are formed from the condensation of a ketone with hydroxylamine (Kesharwani et al., 2009; Rahman et al., 2013), and are usually used as precursors for the preparation of amides by the Beckmann rearrangement (Kesharwani et al., 2009). They have been known to have good antimicrobial (Elgendy et al., 2000), anticancer (Mernyk et al., 2013; Banday et al., 2014; Chen et al., 2015; Elsaied et al., 2017) and antioxidant activity (Potaniec et al., 2014).

This work focuses on using curcumin analogue ketodienes as an intermediate in the synthesis of novel vinyl acetylated pyrazoles, spiro barbiturate, and spiro oxime hybrids, which were tested for their antibacterial activities.

3.2 Experimental

General Experimental Procedures

Reagent grade chemicals were purchased from Merck and Sigma Aldrich, South Africa. TLC was performed using Merck Kieselgel 60 F_{254} plates. The samples were purified by column chromatography with silica gel (60–120 mesh) as the stationary phase and various ratios of

solvents. NMR spectra were acquired at 298 K using $CDCl_3$ and DMSO-d_6 using a Bruker Avance 400-MHz NMR spectrometer (9.4 T; Bruker, Germany) (400.22 MHz for ^1H, 100.63 MHz for ^{13}C). The FID resolution was 0.501 Hz/pt for ^1H and 0.734 Hz/pt for ^{13}C NMR spectra. Chemical shifts are reported in ppm and coupling constants (*J*) in Hz. The deuterated solvent resonances for $CDCl_3$ was 7.24 and 77.0 for ^1H and ^{13}C respectively and 2.50 and 39.2 for DMSO-d6, referenced to the internal standard TMS. All data was analysed using Bruker TopSpin 3.1 software. Infrared spectroscopy were recorded on a Perkin Elmer Spectrum 100 FTIR spectrometer with universal ATR sampling accessory. Melting points were carried out using a Stuart Smart Scientific melting point apparatus and HRMS was obtained on a Bruker microTOF-Q II ESI instrument operating at ambient temperatures.

Synthesis of substituted ketodiene intermediates (1a-n)

A 10 % (m/v) sodium hydroxide solution was added to a mixture of ethanol and water (3:2). Acetone (0.5 mL; 12.2 mmol) was then added to the mixture followed by the slow addition of the substituted aldehyde (24.2 mmol). The reaction was then refluxed for 30 min and monitored by TLC. On completion; the reaction was cooled on ice and the resulting precipitate filtered and recrystallized in ethanol to afford yellow crystals in yields of 70-85%.

Synthesis of ketopyrazoles derivatives (2a-n)

Substituted ketodiene intermediates (**1a-n**) (3.5 mmol) was dissolved in acetic acid (25 mL). Hydrazine hydrate (5.0 mL, 14.0 mmol) was then added and the reaction refluxed at 110 °C for 12 h. On completion, the reaction mixture was poured into ice water, extracted with dichloromethane and purified by column chromatography using ethyl acetate: n-hexane (20:80) resulting in the ketopyrazoles derivatives (**2a-n**) in yields of 89-97%.

(E)-1-(5-(2-chlorophenyl)-3-(2-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (***2a***) yellow-orange solid; mp 104-107 °C, IR (neat) υ_{max} 1666 (C=O), 1416 (C=C) cm^{-1}; HRMS *m/z* 381.0530 $[M + Na]^+$ (calcd. for $C_{19}H_{16}Cl_2N_2ONa$ 381.0537)

(E)-1-(5-(3-chlorophenyl)-3-(3-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (***2b***) yellow gummy solid; IR (neat) υ_{max} 1650 (C=O), 1414 (C=C) cm^{-1}; HRMS *m/z* 381.0527 $[M + Na]^+$ (calcd. for $C_{19}H_{16}Cl_2N_2ONa$ 381.0537)

(E)-1-(5-(4-chlorophenyl)-3-(4-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone ***(2c)*** yellow solid; mp 187-190 °C; IR (neat) υ_{max} 1646 (C=O), 1440 (C=C) cm^{-1}; HRMS *m/z* 381.0531 $[M + Na]^+$ (calcd. for $C_{19}H_{16}Cl_2N_2ONa$ 381.0537)

(E)-1-(5-(2-(trifluoromethyl)phenyl)-3-(2-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (***2d***) light yellow solid; mp 135-137 °C; IR (neat) υ_{max} 1667 (C=O), 1418 (C=C) cm^{-1}; HRMS *m/z* 449.1060 $[M + Na]^+$ (calculated for $C_{21}H_{16}F_6N_2ONa$ 449.1065)

(E)-1-(5-(3-(trifluoromethyl)phenyl)-3-(3-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (***2e***) yellow solid; mp 176-178 °C; IR (neat) υ_{max} 1656 (C=O), 1448 (C=C) cm^{-1}; HRMS *m/z* 449.1063 $[M + Na]^+$ (calcd. for $C_{21}H_{16}F_6N_2ONa$ 449.1065)

(E)-1-(5-(4-(trifluoromethyl)phenyl)-3-(4-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (***2f***) yellow solid; mp 135-136 °C; IR (neat) υ_{max} 1663 (C=O), 1410 (C=C) cm^{-1}; HRMS *m/z* 449.1052 $[M + Na]^+$ (calcd. for $C_{21}H_{16}F_6N_2ONa$ 449.1065)

(E)-1-(5-(3-fluorophenyl)-3-(3-fluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (***2g***) yellow gummy solid; IR (neat) υ_{max} 1655 (C=O), 1420 (C=C) cm^{-1}; HRMS *m/z* 349.1123 $[M + Na]^+$ (calcd. for $C_{19}H_{16}F_2N_2ONa$ 349.1128)

(E)-1-(5-(4-fluorophenyl)-3-(4-fluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (***2h***) yellow solid; mp 140-142 °C; IR (neat) υ_{max} 1653 (C=O), 1506 (C=C) cm^{-1}; HRMS *m/z* 349.1129 $[M + Na]^+$ (calcd. for $C_{19}H_{16}F_2N_2ONa$ 349.1128)

(E)-1-(5-(2,4-difluorophenyl)-3-(2,4-difluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (***2i***) white solid; mp 138-139 °C; IR (neat) υ_{max} 1600 (C=O), 1500 (C=C) cm^{-1}; HRMS *m/z* 385.0929 $[M + Na]^+$ (calcd. for $C_{19}H_{14}F_4N_2ONa$ 385.0940)

((E)-1-(5-(3,4-difluorophenyl)-3-(3,4-difluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (***2j***) green gummy solid; IR (neat) υ_{max} 1651 (C=O), 1412 (C=C) cm^{-1}; HRMS *m/z* 385.0943 $[M + Na]^+$ (calcd. for $C_{19}H_{14}F_4N_2ONa$ 385.0940)

(E)-1-(5-(4-methoxyphenyl)-3-(2-methoxystyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (***2k)*** orange solid; mp 136-142 °C; IR (neat) υ_{max} 1663 (C=O), 1597 (C=C) cm^{-1}; HRMS *m/z* 373.1534 $[M + Na]^+$ (calcd. for $C_{21}H_{22}N_2O_3Na$ 373.1528)

(E)-1-(5-(4-methoxyphenyl)-3-(4-methoxystyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (***2l***) white solid; mp 150-152 °C; IR (neat) υ_{max} 1649 (C=O), 1510 (C=C) cm^{-1}; HRMS *m/z* 373.1523 $[M + Na]^+$ (calcd. for $C_{21}H_{22}N_2O_3Na$ 373.1528)

(E)-1-(5-(4-bromophenyl)-3-(4-bromostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (***2m***) white solid; mp 206-207 °C; IR (neat) υ_{max} 1647 (C=O), 1439 (C=C) cm^{-1}; HRMS *m/z* 468.9538 $[M + Na]^+$ (calcd. for $C_{19}H_{16}Br_2N_2ONa$ 468.9527)

(E)-1-(5-(4-nitrophenyl)-3-(4-nitrostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (***2n***) yellow solid; mp 120-122 °C; IR (neat) υ_{max} 1648 (C=O), 1512 (C=C) (cm^{-1}); HRMS *m/z* 403.1012 $[M + Na]^+$ (calcd. for $C_{19}H_{16}N_4O_5Na$ 403.1018)

Synthesis of spiro barbiturate derivatives (3a-n)

Various substituted ketodienes (**1a-n**) (7.4 mmol) were dissolved in an ethanol-water mixture (1:1) followed by the addition of barbituric acid (1.0 g, 14.8 mmol) and the reaction left to reflux at 140 °C for 18 h. On completion, the reaction mixture was cooled on ice, filtered and washed with cold ethanol to afford white crystals.

7,11-bis(2-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone (***3a***) yellow-orange solid; mp 106-107 °C; IR (neat) υ_{max} 3036 (N-H), 1666 (C=O), 1416 (C=C) cm^{-1}; HRMS *m/z* 429.0407 [M - H] (calcd. for $C_{21}H_{15}Cl_2N_2O_4$ 429.0409)

7,11-bis(3-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone (***3b***) yellow gummy solid; IR (neat) υ_{max} 3211 (N-H), 1650 (C=O), 1414 (C=C) cm^{-1}; HRMS *m/z* 429.0407 [M - H] (calcd. for $C_{21}H_{15}Cl_2N_2O_4$ 429.0409)

7,11-bis(4-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone (***3c***) yellow solid; mp 188-190 °C; IR (neat) υ_{max} 3277 (N-H), 1646 (C=O), 1440 (C=C) cm^{-1}; HRMS *m/z* 429.0407 [M - H] (calcd. for $C_{21}H_{15}Cl_2N_2O_4$ 429.0409)

7,11-bis(2-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone (***3d***) white solid; mp 274-275 °C; IR (neat) υ_{max} 3068 (N-H), 1697 (C=O), 1445 (C=C) cm^{-1}; HRMS *m/z* 497.0927 [M - H] (calcd. for $C_{23}H_{15}F_6N_2O_4$ 497.0936)

7,11-bis(3-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone (***3e***) white solid; mp 242-243 °C; IR (neat) υ_{max} 3216 (N-H), 1687 (C=O), 1421 (C=C) cm^{-1}; HRMS *m/z* 497.0947 [M - H] (calcd. for $C_{23}H_{15}F_6N_2O_4$ 497.0936)

7,11-bis(4-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone (***3f***) light yellow solid; mp 260-261 °C; IR (neat) υ_{max} 3100 (N-H), 1687 (C=O), 1419 (C=C) cm^{-1}; HRMS *m/z* 497.0926 [M - H] (calcd. for $C_{23}H_{15}F_6N_2O_4$ 497.0936)

7,11-bis(3-fluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone (***3g***) white solid; mp 295-296 °C; IR (neat) υ_{max} 3074 (N-H), 1674 (C=O), 1586 (C=C) cm^{-1}; HRMS *m/z* 397.1004 [M - H] (calcd. for $C_{21}H_{15}F_2N_2O_4$ 397.1000)

7,11-bis(4-fluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone (***3h***) yellow solid; mp 141-142 °C; IR (neat) υ_{max} 3305 (N-H), 1653 (C=O), 1506 (C=C) cm^{-1}; HRMS *m/z* 397.1013 [M - H] (calcd. for $C_{21}H_{15}F_2N_2O_4$ 397.1000)

7,11-bis(2,4-difluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone (***3i***) white solid; mp 233-234 °C; IR (neat) υ_{max} 3260 (N-H), 1688 (C=O), 1502 (C=C) cm^{-1}; HRMS *m/z* 433.0811 [M - H] (calcd. for $C_{21}H_{13}F_4N_2O_4$ 433.0811)

7,11-bis(3,4-difluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone (***3j***) white solid; mp 297-298 °C; IR (neat) υ_{max} 3302 (N-H), 1692 (C=O), 1515 (C=C) cm^{-1}; HRMS *m/z* 433.0823 [M - H] (calcd. for $C_{21}H_{13}F_4N_2O_4$ 433.0811)

7,11-bis(2-methoxyphenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone (***3k***) white solid; mp 215-217 °C; IR (neat) υ_{max} 3198 (N-H), 1693 (C=O), 1599 (C=C) (cm^{-1}); HRMS *m/z* 421.1400 [M - H] (calcd. for $C_{23}H_{21}N_2O_6$ 421.1400)

7,11-bis(4-methoxyphenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone (***3l***) white solid; mp 149-151 °C; IR (neat) υ_{max} 3107 (N-H), 1649 (C=O), 1510 (C=C) cm^{-1}; HRMS *m/z* 421.1400 [M - H] (calcd. for $C_{23}H_{21}N_2O_6$ 421.1400)

7,11-bis(4-bromophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone (***3m***) white solid; mp 205-207 °C; IR (neat) υ_{max} 3283 (N-H), 1647 (C=O), 1439 (C=C) cm^{-1}; HRMS *m/z* 516.9402 [M - H] (calcd. for $C_{21}H_{15}Br_2N_2O_4$ 516.9399)

7,11-bis(4-nitrophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone (***3n***) yellow solid; mp 121-122 °C; IR (neat) υ_{max} 3061 (N-H), 1648 (C=O), 1512 (C=C) cm^{-1}; HRMS *m/z* 415.0891 [M - H] (calcd. for $C_{21}H_{15}N_4O_8$ 451.0890)

Synthesis of spiro oximes (4a-j, l-n)

Substituted spiro barbiturates (**3a-n**) (2.4 mmol) were dissolved in 25 mL methanol, after which sodium acetate (0.45 g, 4.8 mmol) and hydroxylamine hydrochloride (0.57 g, 7.2 mmol) were added and the reaction stirred at room temperature for 30 h. Thereafter, the methanol was removed and the contents of the flask dissolved in water and filtered to afford white crystals.

7,11-bis(2-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione (***4a***) white solid; mp 249-250 °C; IR (neat) υ_{max} 3238 (O-H), 3097 (N-H), 1689 (C=O), 1475 (C=C) cm^{-1}; HRMS *m/z* 444.0516 [M - H] (calcd. for $C_{21}H_{16}Cl_2N_3O_4$ 444.0518)

7,11-bis(3-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione (***4b***) white solid; mp 272-275 °C; IR (neat) υ_{max} 3388 (O-H), 3079 (N-H), 1686 (C=O), 1572 (C=C) cm^{-1}; HRMS *m/z* 444.0514 [M - H] (calcd. for $C_{21}H_{16}Cl_2N_3O_4$ 444.0518)

7,11-bis(4-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione (***4c***) white solid; mp 263-266 °C; IR (neat) υ_{max} 3201 (O-H), 3091 (N-H), 1693 (C=O), 1490 (C=C) cm^{-1}; HRMS *m/z* 444.0513 [M - H] (calcd. for $C_{21}H_{16}Cl_2N_3O_4$ 444.0518)

9-(hydroxyimino)-7,11-bis(2-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione (***4d***) white solid; mp 259-260 °C; IR (neat) υ_{max} 3373 (O-H), 3084 (N-H), 1694 (C=O), 1421 (C=C) cm^{-1}; HRMS *m/z* 512.1034 [M - H] (calcd. for $C_{23}H_{16}F_6N_3O_4$ 512.1045)

9-(hydroxyimino)-7,11-bis(3-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione (***4e***) white solid; mp 245-246 °C; IR (neat) υ_{max} 3399 (O-H), 3222 (N-H), 1690 (C=O), 1418 (C=C) cm^{-1}; HRMS *m/z* 512.1034 [M - H] (calcd. for $C_{23}H_{16}F_6N_3O_4$ 512.1045)

9-(hydroxyimino)-7,11-bis(4-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione (***4f***) white solid; mp 197-198 °C; IR (neat) υ_{max}: 3399 (O-H), 3222 (N-H), 1690 (C=O), 1418 (C=C) cm^{-1}; HRMS *m/z* 512.1044 [M - H] (calcd. for $C_{23}H_{16}F_6N_3O_4$ 512.1045)

7,11-bis(3-fluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione (***4g***) white solid; mp 263-264 °C; IR (neat) υ_{max} 3070 (O-H), 3070 (N-H), 1697 (C=O), 1486 (C=C) cm^{-1}; HRMS *m/z* 412.1115 [M - H] (calcd. for $C_{21}H_{16}F_2N_3O_4$ 412.1109)

7,11-bis(4-fluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione (***4h***) white solid; mp 274-275 °C; IR (neat) υ_{max} 3205 (O-H), 3078 (N-H), 1693 (C=O), 1509 (C=C) cm^{-1}; HRMS *m/z* 412.1113 [M - H] (calcd. for $C_{21}H_{16}F_2N_3O_4$ 412.1109)

7,11-bis(2,4-difluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione (***4i***) white solid; mp 245-246 °C; IR (neat) υ_{max} 3216 (O-H), 3085 (N-H), 1694 (C=O), 1503 (C=C) cm^{-1}; HRMS *m/z* 448.0915 [M - H] (calcd. for $C_{21}H_{14}F_4N_3O_4$ 448.0920)

7,11-bis(3,4-difluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione (***4j***) white solid; mp 281-283 °C; IR (neat) υ_{max} 3075 (O-H), 3075 (N-H), 1697 (C=O), 1516 (C=C) cm^{-1}; HRMS *m/z* 448.0912 [M - H] (calcd. for $C_{21}H_{14}F_4N_3O_4$ 448.0920)

9-(hydroxyimino)-7,11-bis(4-methoxyphenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione (***4l***) white solid; mp 246-247 °C; IR (neat) υ_{max}3396 (O-H), 1686 (C=O), 1512 (C=C) cm^{-1}; HRMS *m/z* 436.1511 [M - H] (calcd. for $C_{23}H_{22}N_3O_6$ 436.1509)

7,11-bis(4-bromophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione (***4m***) white solid; mp 279-280 °C; IR (neat) υ_{max} 3195 (O-H), 3098 (N-H), 1693 (C=O), 1487 (C=C) cm^{-1}; HRMS *m/z* 533.9484 [M - H] (calcd. for $C_{21}H_{16}Br_2N_3O_6$ 533.9487)

9-(hydroxyimino)-7,11-bis(4-nitrophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione (***4n***) brown-orange solid; mp 244-245 °C; IR (neat) υ_{max} 3210 (O-H), 3078 (N-H), 1690 (C=O), 1598 (C=C) cm^{-1}; HRMS *m/z* 466.0998 [M - H] (calcd. for $C_{21}H_{16}N_5O_8$ 466.0999)

Antimicrobial assay

The antibacterial activities of the synthesized compounds **2a-n**, **3a-n**, and **4a-j, l-n** was tested against four Gram -ve bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 31488, *Escherichia coli* ATCC 25922 and *Salmonella typhimurium* ATCC 14026) and two Gram +ve bacteria (*Staphylococcus aureus* ATCC 25923 and *Staphylococcus aureus* Rosenbach ATCC BAA-1683 (MRSA)).

Bacterial strains were grown on Mueller-Hinton agar (21 g L^{-1}) at 35-37 °C in a CO_2 incubator for 24 h. The microorganisms were suspended in saline and the turbidity adjusted to a 0.5 McFarland standard. Mueller-Hinton agar plates were prepared by dissolving 38 g of agar in 1 L of water in sterile petri dishes and allowed to set at room temperature. The Mueller-Hinton agar plates were inoculated with the required strain of bacteria by streaking a swab dipped into the adjusted suspension evenly over the entire sterile agar surface. A volume of 5 µL of a 1 mg mL^{-1} sample (in DMSO) was placed onto the Mueller-Hinton

plates and left to incubate for 16-18 h at 37 °C. Zones of inhibition were measured in mm using a transparent ruler.

Minimum Bactericidal Concentration (MBC) Assay

All compounds that showed zones of inhibition during the disc diffusion assay were serially diluted with DMSO. The Mueller-Hinton agar plate was inoculated with the required strain of bacteria above and 5 µL of each sample was placed onto the plate and left to incubate at 37 ^{o}C for 18 h. Experiments were performed in triplicate. The MBC was the lowest concentration where a zone of inhibition was observed. The DMSO control showed no growth inhibition of the bacterial strains in all cases.

Table 3-1: ^{1}H NMR of ketopyrazoles in $CDCl_3$ (**2a-n**)

	2a	**2b**	**2c**	**2d**	**2e**	**2f**	**2g**	**2h**	**2i**	**2j**	**2k**	**2l**	**2m**	**2n**
Pos.	**2-Cl**	**3-Cl**	**4-Cl**	**2-CF_3**	**3-CF_3**	**4-CF_3**	**3-F**	**4-F**	**2,4-F**	**3,4-F**	**2- OCH_3**	**4- OCH_3**	**4-Br**	**4-NO_2**
H-4a	3.73 (dd, *J* = 17.5, 11.9 Hz)	3.56 (dd, *J* = 17.4, 11.8 Hz)	3.56 (dd, *J* = 17.4, 11.7 Hz)	3.64 (dd, *J* = 17.6, 12.1 Hz)	3.63 (dd, *J* = 17.4, 11.8 Hz)	3.63 (dd, *J* = 17.3, 11.9 Hz)	3.57 (dd, *J* = 17.5, 11.9 Hz)	3.56 (dd, *J* = 17.3, 11.8 Hz)	3.59 (dd, *J* = 17.3, 12.0 Hz)	3.56 (dd, *J* = 17.3, 11.8 Hz)	3.56 (dd, *J* = 17.4, 11.8 Hz)	3.52 (dd, *J* = 17.2, 11.6 Hz)	3.56 (dd, *J* = 17.2, 11.8 Hz)	3.67 (dd, *J* = 17.4, 12.0 Hz)
H-4b	2.95 (dd, *J* = 17.5, 5.0 Hz)	2.95 (dd, *J* = 17.4, 4.6 Hz)	2.95 (dd, *J* = 17.4, 4.5 Hz)	2.87 (dd, *J* = 17.6, 5.3 Hz)	2.99 (dd, *J* = 17.4, 4.8 Hz)	2.99 (dd, *J* = 17.3, 4.8 Hz)	2.97 (dd, *J* = 17.5, 4.8 Hz)	3.00 (dd, *J* = 17.3, 4.9 Hz)	2.98 (dd, *J* = 17.3, 5.0 Hz)	2.94 (dd, *J* = 17.3, 4.6 Hz)	2.91 (dd, *J* = 17.4, 4.6 Hz)	2.98 (dd, *J* = 17.2, 4.2 Hz)	2.95 (dd, *J* = 17.2, 4.6 Hz)	3.00 (dd, *J* = 17.4, 5.0 Hz)
H-5	5.87 (dd, *J* = 11.9, 5.0 Hz)	5.47 (dd, *J* = 11.8, 4.6 Hz)	5.47 (dd, *J* = 11.7, 4.5 Hz)	5.89 (dd, *J* = 12.1, 5.3 Hz)	5.57 (dd, *J* = 11.8, 4.8 Hz)	5.57 (dd, *J* = 11.9, 4.8 Hz)	5.50 (dd, *J* = 11.9, 4.8 Hz)	5.49 (dd, *J* = 11.8, 4.9 Hz)	5.66 (dd, *J* = 12.0, 5.0 Hz)	5.46 (dd, *J* = 11.8, 4.6 Hz)	5.78 (dd, *J* = 11.8, 4.6 Hz)	5.46 (dd, *J* = 11.6, 4.2 Hz)	5.46 (dd, *J* = 11.8, 4.6 Hz)	5.61 (dd, *J* = 12.0, 5.0 Hz)
H-2'	-	7.43 (bs)	7.32 (d, *J* = 8.4 Hz)	-	7.44-7.42 (m)	7.57 (d, *J* = 8.2 Hz)	6.87 (dt, *J* = 9.5, 2.1 Hz)	7.16 (dd, *J* = 8.7, 5.2 Hz)	-	6.98-6.91 (m)	-	7.12 (d, *J* = 8.6 Hz)	7.06 (d, *J* = 8.3 Hz)	7.36 (d, *J* = 8.7 Hz)
H-3'	7.64 (dd, *J* = 7.4, 1.9 Hz)	-	7.12 (d, *J* = 8.4 Hz)	7.67 (d, *J* = 8.9 Hz)	-	7.31 (d, *J* = 8.2 Hz)	-	6.98 (t, *J* = 8.7 Hz)	6.84-6.77 (m)	-	6.95 (dd, *J* = 7.5, 1.6 Hz)	6.82 (d, *J* = 8.4 Hz)	7.43 (d, *J* = 8.3 Hz)	8.18 (d, *J* = 8.7 Hz)
H-4'	7.29-7.21 (m)	7.32-7.19 (m)	-	7.36 (t, *J* = 7.9 Hz)	7.44-7.42 (m)	-	7.02-6.97 (m)	-	-	-	6.89-6.85 (m)	-	-	-
H-5'	7.29-7.21 (m)	7.32-7.19 (m)	7.12 (d, *J* = 8.4 Hz)	7.49 (t, *J* = 7.5 Hz)	7.49 (t, *J* = 8.4 Hz)	7.31 (d, *J* = 8.2 Hz)	7.34-7.25 (m)	6.98 (t, *J* = 8.7 Hz)	6.88 (td, *J* = 8.1, 2.0 Hz)	7.16-7.05 (m)	7.20 (td, *J* = 9.1, 1.8 Hz)	6.82 (d, *J* = 8.4 Hz)	7.43 (d, *J* = 8.3 Hz)	8.18 (d, *J* = 8.7 Hz)
H-6'	7.06-7.02 (m)	7.32-7.19 (m)	7.32 (d, *J* = 8.4 Hz)	7.15 (d, *J* = 7.9 Hz)	7.36 (d, *J* = 7.6 Hz)	7.57 (d, *J* = 8.2 Hz)	7.02-6.97 (m)	7.16 (dd, *J* = 8.7, 5.2 Hz)	7.06 (dd, *J* = 14.8, 8.8 Hz)	6.98-6.91 (m)	6.89-6.85 (m)	7.12 (d, *J* = 8.6 Hz)	7.06 (d, *J* = 8.3 Hz)	7.36 (d, *J* = 8.7 Hz)
H-2''	-	7.15 (bs)	7.37 (d, *J* = 8.4 Hz)	-	7.69 (bs)	7.61 (d, *J* = 8.2 Hz)	7.15 (dt, *J* = 9.8, 2.2 Hz)	7.42 (dd, *J* = 8.7, 5.2 Hz)	-	7.26 (dd, *J* = 12.0, 7.9 Hz)	-	7.39 (d, *J* = 8.6 Hz)	7.30 (d, *J* = 8.3 Hz)	7.59 (d, *J* = 8.7 Hz)
H-3''	7.41-7.34 (m)	-	7.27 (d, *J* = 8.4 Hz)	7.64 (d, *J* = 9.0 Hz)	-	7.54 (d, *J* = 8.2 Hz)	-	7.04 (t, *J* = 8.7 Hz)	6.84-6.77 (m)	-	6.89-6.85 (m)	6.88 (d, *J* = 8.6 Hz)	7.48 (d, *J* = 8.3 Hz)	8.21 (d, *J* = 8.7 Hz)
H-4''	7.29-7.21 (m)	7.32-7.19 (m)	-	7.39 (t, *J* = 7.9 Hz)	7.55 (d, *J* = 7.6 Hz)	-	6.93 (td, *J* = 10.8, 2.4 Hz)	-	-	-	7.27 (td, *J* = 8.7, 1.6 Hz)	-	-	-
H-5''	7.29-7.21 (m)	7.32-7.19 (m)	7.27 (d, *J* = 8.4 Hz)	7.54 (t, *J* = 7.9 Hz)	7.49 (t, *J* = 8.4 Hz)	7.54 (d, *J* = 8.2 Hz)	7.34-7.25 (m)	7.04 (t, *J* = 8.7 Hz)	6.84-6.77 (m)	7.16-7.05 (m)	6.92 (t, *J* = 7.8 Hz)	6.88 (d, *J* = 8.6 Hz)	7.48 (d, *J* = 8.3 Hz)	8.21 (d, *J* = 8.7 Hz)
H-6''	7.41-7.34 (m)	7.09-7.06 (m)	7.37 (d, *J* = 8.4 Hz)	7.73 (d, *J* = 7.9 Hz)	7.62 (d, *J* = 7.6 Hz)	7.61 (d, *J* = 8.2 Hz)	7.20 (d, *J* =7.8 Hz)	7.42 (dd, *J* = 8.7, 5.2 Hz)	7.51 (ddd, *J* = 16.4, 8.5,	7.16-7.05 (m)	7.50 (dd, *J* = 7.7, 1.5 Hz)	7.39 (d, *J* = 8.6 Hz)	7.30 (d, *J* = 8.3 Hz)	7.59 (d, *J* = 8.7 Hz)

									6.2 Hz)					
	2a	**2b**	**2c**	**2d**	**2e**	**2f**	**2g**	**2h**	**2i**	**2j**	**2k**	**2l**	**2m**	**2n**
Pos.	**2-Cl**	**3-Cl**	**4-Cl**	**2-CF_3**	**3-CF_3**	**4-CF_3**	**3-F**	**4-F**	**2,4-F**	**3,4-F**	**2- OCH_3**	**4- OCH_3**	**4-Br**	**4-NO_2**
H-7''	7.16 (d, *J* = 16.5 Hz)	6.66 (d, *J* = 16.4 Hz)	6.68 (d, *J* = 16.3 Hz)	7.06 (bs)	6.77 (d, *J* = 16.4 Hz)	6.75 (d, *J* = 16.4 Hz)	6.69 (d, *J* = 16.4 Hz)	6.71 (d, *J* = 16.5 Hz)	6.81 (d, *J* = 16.4 Hz)	6.64 (d, *J* = 16.3 Hz)	7.08 (bs)	6.70 (d, *J* = 16.3 Hz)	6.66 (d, *J* = 16.3 Hz)	6.78 (d, *J* = 16.4 Hz)
H-8''	7.06 (d, *J* = 16.5 Hz)	7.08 (d, *J* = 16.4 Hz)	7.06 (d, *J* = 16.3 Hz)	7.06 (bs)	7.16 (d, *J* = 16.4 Hz)	7.17 (d, *J* = 16.4 Hz)	7.07 (d, *J* = 16.4 Hz)	6.99 (d, *J* = 16.5 Hz)	7.07 (d, *J* = 16.4 Hz)	6.97 (d, *J* = 16.3 Hz)	7.08 (bs)	6.95 (d, *J* = 16.3 Hz)	7.06 (d, *J* = 16.3 Hz)	7.23 (d, *J* = 16.4 Hz)
N-(CO) CH_3	2.43 (s)	2.35 (s)	2.33 (s)	2.38 (s)	2.36 (s)	2.36 (s)	2.35 (s)	2.33 (s)	2.35 (s)	2.33 (s)	2.39 (s)	2.32 (s)	2.33 (s)	2.37 (s)
OCH_3	-	-	-	-	-	-	-	-	-	-	3.85 (s)	3.81 (s)	-	-
OCH_3	-	-	-	-	-	-	-	-	-	-	3.83 (s)	3.75 (s)	-	-

OCH_3 is interchangeable

Table 3-2: ^{13}C NMR of ketopyrazoles in $CDCl_3$ (**2a-n**)

	2a	**2b**	**2c**	**2d**	**2e**	**2f**	**2g**	**2h**	**2i**	**2j**	**2k**	**2l**	**2m**	**2n**
Pos.	**2-Cl**	**3-Cl**	**4-Cl**	**2-CF_3**	**3-CF_3**	**4-CF_3**	**3-F**	**4-F**	**2,4-F**	**3,4-F**	**2- OCH_3**	**4- OCH_3**	**4-Br**	**4-NO_2**
C-3	155.1	154.3	154.5	154.1	154.0	154.1	154.3	154.7	154.9	154.0	156.7	155.4	154.4	153.6
C-4	40.2	40.9	40.9	41.4	41.0	40.8	41.0	41.0	39.9	40.9	40.3	41.0	40.8	40.7
C-5	57.8	59.5	59.3	56.7	59.6	59.7	59.5	59.2	54.5	59.0	55.5	59.2	59.4	59.6
C-1'	138.4	143.7	140.2	140.4	142.5	145.4	144.2 (d, *J* = 6.9 Hz)	137.6 (d, *J* = 3.01 Hz)	124.3 (dd, *J* = 14.0, 3.8 Hz)	138.6 (t, *J* = 4.5 Hz)	129.2	134.1	140.7	148.4
C-2'	133.7	126.9	128.2	127.9 (q, *J* = 29.9 Hz)	129.5 (q, *J* = 5.9 Hz)	127.1	112.5 (d, *J* = 22.3 Hz)	127.4 (d, *J* = 8.1 Hz)	160.6 (dd, *J* = 254.9, 12.1 Hz)	117.7 (d, *J* = 17.9 Hz)	156.0	126.9	127.4	126.6
C-3'	126.7	134.8	127.1	126.1 (q, *J* = 22.5 Hz)	131.3 (q, *J* = 32.0 Hz)	125.9 (q, *J* = 8.2 Hz)	163.0 (d, *J* = 246.9 Hz)	115.8 (d, *J* = 21.5 Hz)	104.4 (t, *J* = 26.1 Hz)	150.7 (dd, *J* = 248.9, 13.8 Hz)	125.5	114.2	132.1	124.3
C-4'	128.8	130.1	134.1	127.6	122.5	129.8	116.0 (d, *J* = 21.4 Hz)	163.2 (d, *J* = 250.6 Hz)	163.0 (dd, *J* = 254.9, 12.1 Hz)	150.5 (dd, *J* = 248.8, 13.4 Hz)	120.8	159.0	123.2	147.5
C-5'	130.0	130.3	127.1	132.1	124.7-124.6 (m)	125.9 (q, *J* = 8.2 Hz)	130.4 (d, *J* = 8.5 Hz)	115.8 (d, *J* = 21.5 Hz)	112.5 (dd, *J* = 20.0, 4.0 Hz)	117.8 (dd, *J* = 17.9, 9.4 Hz)	128.5	114.2	132.1	124.3
C-6'	125.8	125.2	128.2	125.1	129.0	127.1	121.2 (d, *J* = 2.8 Hz)	127.4 (d, *J* = 8.1 Hz)	128.5-128.1 (m)	121.7 (dd, *J* = 6.1, 3.4 Hz)	* 110.8	126.9	127.4	126.6

	2a	2b	2c	2d	2e	2f	2g	2h	2i	2j	2k	2l	2m	2n
Pos.	**2-Cl**	**3-Cl**	**4-Cl**	**2-CF_3**	**3-CF_3**	**4-CF_3**	**3-F**	**4-F**	**2,4-F**	**3,4-F**	**2- OCH_3**	**4- OCH_3**	**4-Br**	**4-NO_2**
C-1''	131.8	137.5	133.5	134.4	136.4	138.9	137.9 (d, *J* = 7.9 Hz)	131.9 (d, *J* = 3.4 Hz)	120.1 (dd, *J* = 12.0, 4.0 Hz)	132.8 (dd, *J* = 5.7, 4.2 Hz)	124.8	128.50	134.5	141.7
C-2''	133.8	125.7	129.2	126.5 (q, *J* = 31.2 Hz)	123.7 (q, *J* = 3.9 Hz)	127.1	113.3 (d, *J* = 21.9 Hz)	128.7 (d, *J* = 8.3 Hz)	160.1 (dd, *J* = 248.2, 12.9 Hz)	115.3 (d, *J* = 17.9 Hz)	157.1	128.47	128.4	127.5
C-3''	130.1	134.9	129.1	126.3 (q, *J* = 21.0 Hz)	131.5 (q, *J* = 29.8 Hz)	126.0 (q, *J* = 7.5 Hz)	163.0 (d, *J* = 246.9 Hz)	116.0 (d, *J* = 21.8 Hz)	104.5 (t, *J* = 25.4 Hz)	150.8 (dd, *J* = 252.6, 13.4 Hz)	*110.0	114.4	132.1	124.4
C-4''	127.3	128.0	134.9	128.6	125.6 (q, *J* = 3.0 Hz)	130.7	114.7 (d, *J* = 21.2 Hz)	162.2 (d, *J* = 246.1 Hz)	162.3 (dd, *J* = 245.1, 12.9 Hz)	150.6 (dd, *J* = 251.9, 13.8 Hz)	130.1	160.4	121.6	147.8
C-5''	127.1	129.0	129.1	132.9	124.7-124.6 (m)	126.0 (q, *J* = 7.5 Hz)	130.5 (d, *J* = 8.3 Hz)	116.0 (d, *J* = 21.8 Hz)	111.5 (dd, *J* = 22.0, 4.0 Hz)	117.8 (dd, *J* = 17.9, 9.4 Hz)	120.7	114.4	132.1	124.4
C-6''	130.1	123.8	129.2	127.1	130.0	127.1	123.0 (d, *J* = 2.7 Hz)	128.7 (d, *J* = 8.3 Hz)	128.5-128.1 (m)	123.5 (dd, *J* = 6.3, 3.5 Hz)	127.0	128.47	128.4	127.5
C-7''	133.0	135.8	136.0	132.6	135.7	135.6	136.0 (d, *J* = 2.8 Hz)	136.1	128.5-128.1 (m)	135.1 (t, *J* = 2.1 Hz)	132.0	137.0	136.0	134.7
C-8''	122.9	122.0	121.2	124.4	122.4	122.9	122.0	120.4 (d, *J* = 2.2 Hz)	122.3 (dd, *J* = 5.1, 2.4 Hz)	121.6 (d, *J* = 2.4 Hz)	121.2	118.5	121.3	124.6
C=O	168.8	168.8	168.7	168.6	168.9	168.9	168.8	168.7	168.8	168.8	168.5	168.5	168.7	169.0
CH_3	21.9	21.9	21.9	21.8	21.9	21.8	21.9	21.9	21.9	21.9	22.0	22.0	21.9	21.8
CF_3	-	-	-	124.0 (q, *J* = 274.5 Hz)	123.8 (q, *J* = 270.9 Hz)	124.0 (q, *J* = 271.2 Hz)	-	-	-	-	-	-	-	-
CF_3	-	-	-	124.1 (q, *J* = 273.8 Hz)	123.7 (q, *J* = 272.1 Hz)	124.1 (q, *J* = 273.3 Hz)	-	-	-	-	-	-	-	-
OCH_3	-	-	-	-	-	-	-	-	-	-	55.5	55.4	-	-
OCH_3	-	-	-	-	-	-	-	-	-	-	55.5	55.3	-	-

*OCH_3 and CF_3 is interchangeable; " * " denotes interchangeable carbon resonances*

Table 3-3: ^{1}H NMR of spiro barbiturates in DMSO-d_6 (**3a-n**)

	3a	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**	**3h**	**3i**	**3j**	**3k**	**3l**	**3m**	**3n**
Pos.	**2-Cl**	**3-Cl**	**4-Cl**	**2-CF_3**	**3-CF_3**	**4-CF_3**	**3-F**	**4-F**	**2,4-F**	**3,4-F**	**2-OCH_3**	**4-OCH_3**	**4-Br**	**4-NO_2**
H-2	12.0 (s)	11.7 (s)	11.6 (s)	12.4 (s)	11.7 (s)	11.6 (s)	11.5 (bs)	11.5 (s)	11.8 (s)	11.5 (s)	10.8 (s)	8.08 (s)	11.6 (s)	11.7 (s)
H-4	11.3 (s)	11.4 (s)	11.3 (s)	11.2 (s)	11.4 (s)	11.4 (s)	11.5 (bs)	11.3 (s)	11.3 (s)	11.5 (s)	10.8 (s)	7.94 (s)	11.3 (s)	11.4 (s)
H-7/H-11	4.64 (dd, *J* = 13.7, 5.2 Hz)	4.01 (dd, *J* = 14.0, 4.7 Hz)	4.00 (dd, *J* = 13.9, 4.8 Hz)	4.33 (dd, *J* = 13.6, 5.2 Hz)	4.16 (dd, *J* = 13.8, 4.8 Hz)	4.16 (dd, *J* = 13.8, 4.7 Hz)	4.02 (dd, *J* = 13.9, 4.7 Hz)	4.00 (dd, *J* = 14.0, 4.8 Hz)	4.40 (dd, *J* = 13.6, 5.2 Hz)	4.01 (dd, *J* = 13.9, 4.7 Hz)	4.30 (dd, *J* = 14.6, 2.8 Hz)	3.90 (dd, *J* = 14.6, 4.5 Hz)	3.98 (dd, *J* = 13.9, 4.6 Hz)	4.24 (dd, *J* = 13.8, 4.7 Hz)
H-8_{eq}/10_{eq}	2.53 (dd, *J* = 16.4, 5.2 Hz)	2.49 (dd, *J* = 15.6, 4.7 Hz)	2.46 (dd, *J* = 15.7, 4.8 Hz)	2.61 (dd, *J* = 16.9, 5.2 Hz)	2.55 (dd, *J* = 15.8, 4.8 Hz)	2.54 (dd, *J* = 15.8, 4.7 Hz)	2.48 (dd, *J* = 15.3, 4.7 Hz)	2.45 (dd, *J* = 15.5, 4.8 Hz)	2.50 (dd, *J* = 16.1, 5.2 Hz)	2.47 (dd, *J* = 16.3, 4.7 Hz)	2.41 (dd, *J* = 17.1, 3.0 Hz)	2.57 (dd, *J* = 16.1, 4.5 Hz) and)	2.46 (dd, *J* = 16.2, 4.6 Hz)	2.55 (dd, *J* = 16.0, 4.8 Hz)
H-8_{ax}/10_{ax}	3.25 (dd, *J* = 16.4, 13.7 Hz)	[a] 3.46 (t, *J* = 14.4 Hz)	[a] 3.45 (t, *J* = 15.0 Hz)	3.31 (dd, *J* = 16.9, 13.6 Hz)	[a] 3.52 (t, *J* = 15.4 Hz)	[a] 3.52 (t, *J* = 15.2 Hz)	[a] 3.48 (t, *J* = 15.3 Hz)	[a] 3.48 (t, *J* = 14.7 Hz)	[a] 3.32 (t, *J* = 14.6 Hz)	[a] 3.46 (t, *J* = 15.3 Hz)	[a] 3.62 (dd, *J* = 17.1, 14.6 Hz)	[a] 3.62 (t, *J* = 14.7 Hz	[a] 3.44 (t, *J* = 15.5 Hz)	[a] 3.51 (t, *J* = 15.7 Hz)
H-2'/ H-2''	-	7.13 (s)	7.12 (d, *J* = 8.5 Hz)	-	7.41 (bs)	7.35 (d. *J* = 8.2 Hz)	6.90 (dt, *J* = 10.2, 1.8 Hz)	[b] 7.16 (d, *J* = 7.8 Hz)	-	7.15 (td, *J* = 11.5, 1.9 Hz)	-	7.03 (d, *J* = 8.8 Hz)	7.06 (d, *J* = 8.5 Hz)	7.41 (d, *J* = 8.5 Hz)
H-3'/ H-3''	7.46-7.44 (m)	-	7.40 (d, *J* = 8.5 Hz)	7.53 (d, *J* = 7.6 Hz)	-	7.73 (d, *J* = 8.2 Hz)	-	[b] 7.16 (d, *J* = 7.8 Hz)	7.30-7.23 (m)	-	6.88 (d, *J* = 8.1 Hz)	6.75 (d, *J* = 8.8 Hz)	7.53 (d, *J* = 8.5 Hz)	8.21 (d, *J* = 8.5 Hz)
H-4'/ H-4''	7.35-7.28 (m)	7.37 (d, *J* = 7.2 Hz)	-	7.52 (t, *J* = 7.6 Hz)	7.68 (d, *J* = 7.9 Hz)	-	7.14 (td, *J* = 8.5, 2.5 Hz)	-	-	-	6.90 (t, *J* = 8.1 Hz)	-	-	-
H-5'/ H-5''	7.35-7.28 (m)	7.08 (td, *J* = 7.2, 1.9 Hz)	7.40 (d, *J* = 8.5 Hz)	7.69 (t, *J* = 7.6 Hz)	7.59 (t, *J* = 7.9 Hz)	7.73 (d, *J* = 8.2 Hz)	7.38 (td, *J* = 8.2, 6.8 Hz)	[b] 7.16 (d, *J* = 7.8 Hz)	7.11 (td, *J* = 8.2, 2.4 Hz)	7.43 (td, *J* = 10.7, 8.7 Hz)	7.21 (td, *J* = 8.1, 1.5 Hz)	6.75 (d, *J* = 8.8 Hz)	7.53 (d, *J* = 8.5 Hz)	8.21 (d, *J* = 8.5 Hz)
H-6'/ H-6''	7.35-7.28 (m)	7.37 (d, *J* = 7.2 Hz)	7.12 (d, *J* = 8.5 Hz)	7.74 (d, *J* = 7.6 Hz)	7.43 (d, *J* = 7.9 Hz)	7.35 (d. *J* = 8.2 Hz)	6.96 (d, *J* = 8.0 Hz)	[b] 7.16 (d, *J* = 7.8 Hz)	7.30-7.23 (m)	6.97-6.95 (m)	7.11 (d, *J* = 8.1 Hz)	7.03 (d, *J* = 8.8 Hz)	7.06 (d, *J* = 8.5 Hz)	7.41 (d, *J* = 8.5 Hz)
OCH_3	-	-	-	-	-	-	-	-	-	-	3.56 (s)	3.73 (s)	-	-

"a" resonance appears as a triplet since the double doublet coalesces into a triplet; "b" overlapping doublets; The 4-OCH_3 spectra was run in chloroform

Table 3-4: ^{13}C NMR of spiro barbiturates in DMSO-d_6 (**3a-n**)

	3a	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**	**3h**	**3i**	**3j**	**3k**	**3l**	**3m**	**3n**
Pos.	**2-Cl**	**3-Cl**	**4-Cl**	**2-CF_3**	**3-CF_3**	**4-CF_3**	**3-F**	**4-F**	**2,4-F**	**3,4-F**	**2- OCH_3**	**4-OCH_3**	**4-Br**	**4-NO_2**
C-1	171.9	171.5	171.5	173.0	171.5	171.2	171.6	171.7	171.0	171.5	171.5	171.6	171.5	171.0
C-3	148.8	148.6	148.7	148.8	148.4	148.6	148.7	148.7	148.8	148.5	149.7	147.4	148.7	151.6
C-5	169.5	170.5	170.5	168.8	170.5	170.2	170.5	170.6	170.2	170.3	171.5	170.0	170.5	170.0
C-6	55.4	58.4	58.6	55.3	58.5	58.3	58.5	58.9	56.4	58.6	55.9	61.1	58.5	58.0
C-7/ C-11	44.6	48.2	47.9	45.9	48.2	48.3	48.2	47.8	40.6	47.5	38.4	49.4	48.0	48.2
C-8/ C-10	43.1	42.3	42.3	44.6	42.2	42.1	42.3	42.7	42.3	42.3	41.1	43.2	42.3	41.9
C-9	205.6	206.3	206.6	204.6	206.1	206.1	206.4	206.7	205.6	206.1	209.8	208.1	206.5	205.7
C-1'/ C-1''	135.6	140.0	136.3	137.2	138.7	142.0	140.2 (d, *J* = 6.9 Hz)	133.7 (d, *J* = 3.0 Hz)	121.3 (dd, *J* = 14.6, 3.7 Hz)	135.1 (t, *J* = 4.3 Hz)	125.9	128.5	136.8	144.7
C-2'/ C-2''	133.3	127.8	128.8	127.6 (q, *J* = 29.2 Hz)	124.5 (q, *J* = 5.7 Hz)	128.8	114.6 (d, *J* = 21.7 Hz)	129.6 (d, *J* = 8.2 Hz)	161.6 (dd, *J* = 248.7, 12.8 Hz)	116.9 (d, *J* = 17.3 Hz)	156.4	128.9	130.0	129.5
C-3'/ C-3''	130.2	133.2	129.7	127.0 (q, *J* = 5.8 Hz)	129.4 (d, *J* = 30.5 Hz)	125.7 (q, *J* = 4.5 Hz)	161.8 (d, *J* = 245.1 Hz)	115.6 (d, *J* = 21.4 Hz)	104.3 (t, *J* = 26.0 Hz)	149.1 (dd, *J* = 247.5, 11.6 Hz)	110.1	114.5	131.7	123.9
C-4'/ C-4''	127.5	128.2	132.7	128.6	125.0 (q, *J* = 4.1 Hz)	128.7 (q, *J* = 31.9 Hz)	115.1 (d, *J* = 20.7 Hz)	161.4 (d, *J* = 246.2 Hz)	159.6 (dd, *J* = 251.6, 12.1 Hz)	149.0 (dd, *J* = 247.3, 12.3 Hz)	120.2	159.6	121.3	147.2
C-5' /C-5''	129.6	126.4	129.7	133.1	130.0	125.7 (q, *J* = 4.5 Hz)	130.9 (d, *J* = 8.6 Hz)	115.6 (d, *J* = 21.4 Hz)	112.1 (dd, *J* = 20.7, 3.6 Hz)	117.9 (d, *J* =17.3 Hz)	128.4	114.5	131.7	123.9
C-6' /C-6''	127.8	130.7	128.8	127.4	131.9	128.8	124.0 (d, *J* = 2.3 Hz)	129.6 (d, *J* = 8.2 Hz)	130.0 (dd, *J* = 9.5, 4.6 Hz)	124.9 (dd, *J* = 6.3, 3.2 Hz)	129.3	128.9	130.0	129.5
CF_3	-	-	-	123.8 (q, *J* = 275.1 Hz)	123.7 (q, *J* = 270.8 Hz)	124.1 (q, *J* = 272.1 Hz)	-	-	-	-	-	-	-	-
OCH_3								-	-	-	54.4	55.2	-	-

The 4-OCH_3 spectra was run in chloroform; C-1 and C-5 is interchangeable

Table 3-5: ^{1}H NMR of spiro oximes in DMSO-d_6 (**4a-j, l-n**)

	4a	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	**4h**	**4i**	**4j**	**4l**	**4m**	**4n**
Pos.	**2-Cl**	**3-Cl**	**4-Cl**	**2-CF_3**	**3-CF_3**	**4-CF_3**	**3-F**	**4-F**	**2,4-F**	**3,4-F**	**4-OCH_3**	**4-Br**	**4-NO_2**
H-2	11.8 (s)	11.5 (s)	11.4 (s)	12.1 (s)	11.5 (s)	11.2 (s)	11.4 (s)	11.4 (s)	11.7 (s)	11.5 (s)	11.2 (s)	11.4 (s)	11.6 (s)
H-4	11.2 (s)	11.4 (s)	11.3 (s)	11.2 (s)	11.4 (s)	11.2 (s)	11.3 (s)	11.2 (s)	11.3 (s)	11.4 (s)	11.1 (s)	11.3 (s)	11.4 (s)
H-7	4.15 (dd, *J* = 13.8, 4.1 Hz)	3.52 (dd, *J* = 13.9, 4.4 Hz)	3.51 (dd, *J* = 13.8, 4.3 Hz)	3.84 (dd, *J* = 13.6, 4.0 Hz)	3.67 (dd, *J* = 14.0, 4.2 Hz)	3.74 (dd, *J* = 13.9, 4.4 Hz)	3.54 (dd, *J* = 14.3, 4.5 Hz)	3.51 (dd, *J* = 13.9, 4.4 Hz)	3.85 (dd, *J* = 13.6, 4.1 Hz)	3.53 (dd, *J* = 13.8, 4.2 Hz)	3.42 (dd, *J* = 13.9, 4.2 Hz)	3.50 (dd, *J* = 13.6, 4.2 Hz)	3.73 (dd, *J* = 13.8, 4.5 Hz)
H-11	4.25 (dd, *J* = 14.1, 4.5 Hz)	3.62 (dd, *J* = 13.9, 4.4 Hz)	3.60 (dd, *J* = 13.8, 4.3 Hz)	3.92 (dd, *J* = 13.6, 4.0 Hz)	3.77 (dd, *J* = 14.0, 4.2 Hz)	3.65 (dd, *J* = 13.9, 4.4 Hz)	3.63 (dd, *J* = 14.3, 4.5 Hz)	3.61 (dd, *J* = 13.9, 4.4 Hz)	3.93 (dd, *J* = 13.6, 4.1 Hz)	3.62 (dd, *J* = 13.8, 4.2 Hz)	3.51 (dd, *J* = 13.9, 4.2 Hz)	3.59 (dd, *J* = 13.6, 4.2 Hz)	3.82 (dd, *J* = 13.8, 4.5 Hz)
H-8_{ax}	2.75 (t, *J* = 13.8 Hz)	2.89 (t, *J* = 13.9 Hz)	2.88 (t, *J* = 13.8 Hz)	2.79 (t, *J* = 13.6 Hz)	2.95 (t, *J* = 14.0 Hz)	2.95 (t, *J* = 13.9 Hz)	2.89 (t, *J* =14.3 Hz)	2.88 (t, *J* = 13.9 Hz)	2.85 (t, *J* = 13.6 Hz)	2.85 (t, *J* = 13.8 Hz)	2.86 (t, *J* = 13.9 Hz)	2.87 (t, *J* =13.6 Hz)	2.96 (t, *J* = 13.8 Hz)
H-8_{eq}	3.33 (dd, *J* = 13.8, 4.1 Hz)	3.31 (dd, *J* = 13.9, 4.4 Hz)	3.32 (dd, *J* = 13.8, 4.3 Hz)	3.45 (dd, *J* = 13.6, 4.0 Hz)	[d] 3.40	[d] 3.42	[d] 3.32	[d] 3.32	[d] 3.27	3.30 (dd, *J* = 13.8, 4.2 Hz)	[d] 3.28	[d] 3.29	[d] 3.43
H-10_{ax}	3.21 (t, *J* = 14.1 Hz)	3.31 (dd, *J* = 13.9, 4.4 Hz)	3.29 (dd, *J* = 13.8, 4.3 Hz)	3.27 (t, *J* = 13.6 Hz)	[d] 3.40	[d] 3.42	[d] 3.32	[d] 3.32	[d] 3.27	3.30 (dd, *J* = 13.8, 4.2 Hz)	[d] 3.28	[d] 3.29	[d] 3.43
H-10_{eq}	2.42 (dd, *J* = 14.1, 4.5 Hz)	2.43 (dd, *J* = 13.9, 4.4 Hz)	2.41 (dd, *J* = 13.8, 4.3 Hz)	2.48 (dd, *J* = 13.6, 4.0 Hz)	[d] 2.47	2.78 (dd, *J* = 13.9, 4.4 Hz)	2.43 (dd, *J* = 14.3, 4.5 Hz)	2.40 (dd, *J* = 13.9, 4.4 Hz)	2.41 (dd, *J* = 13.6, 4.1 Hz)	2.42 (dd, *J* = 13.8, 4.2 Hz)	2.33 (dd, *J* = 13.9, 4.2 Hz)	2.40 (dd, *J* = 13.6, 4.2 Hz)	[d] 2.47
H-2'	-	[c] 7.13 (bs)	7.13 (d, *J* = 8.5 Hz)	-	[c] 7.45-7.41 (m)	7.36 (d, *J* = 8.1 Hz)	[c] 6.91-6.88 (m)	[c] 7.16 (d, *J* = 7.2 Hz)	-	[c] 7.16-7.09 (m)	[c] 7.03 (d, *J* = 8.8 Hz)	[c] 7.07 (d, *J* = 8.5 Hz)	[c] 7.43 (d, *J* = 8.8 Hz)
H-2''	-	[c] 7.13 (bs)	7.13 (d, *J* = 8.5 Hz)	-	[c] 7.45-7.41 (m)	7.36 (d, *J* = 8.1 Hz)	[c] 6.91-6.88 (m)	[c] 7.16 (d, *J* = 7.2 Hz)	-	[c] 7.16-7.09 (m)	[c] 7.03 (d, *J* = 8.8 Hz	[c] 7.07 (d, *J* = 8.5 Hz)	[c] 7.43 (d, *J* = 8.8 Hz)
H-3'	[c] 7.46 (d, *J* = 7.6 Hz)	-	[c] 7.39 (d, *J* = 8.5 Hz)	7.51 (d, *J* = 8.3 Hz)	-	[c] 7.71 (d, *J* = 8.4 Hz)	-	[c] 7.15 (d, *J* = 7.2 Hz)	[c] 7.33-7.20 (m)	-	[c] 6.84 (d, *J* = 8.6 Hz)	[c] 7.52 (d, *J* = 8.5 Hz)	[c] 8.20 (d, *J* = 8.6 Hz)
H-3''	[c]7.46 (d, *J* = 7.6 Hz)	-	[c] 7.39 (d, *J* = 8.5 Hz)	7.52 (d, *J* = 8.3 Hz)	-	[c] 7.71 (d, *J* = 8.4 Hz)	-	[c] 7.15 (d, *J* = 7.2 Hz)	[c] 7.33-7.20 (m)	-	[c] 6.84 (d, *J* = 8.6 Hz)	[c] 7.52 (d, *J* = 8.5 Hz)	[c] 8.20 (d, *J* = 8.6 Hz)
H-4'	[c]7.35-7.28 (m)	[c] 7.36 (d, *J* = 7.2 Hz)	-	[c] 7.52 (t, *J* = 8.3 Hz)	[c] 7.67 (d, *J* = 7.5 Hz)	-	[c] 7.13 (td, *J* = 8.6, 1.8 Hz)	-	-	-	-	-	-
H-4''	[c]7.35-7.28 (m)	[c] 7.36 (d, *J* = 7.2 Hz)	-	[c]7.52 (t, *J* = 8.3 Hz)	[c] 7.67 (d, *J* = 7.5 Hz)	-	[c] 7.13 (td, *J* = 8.6, 1.8 Hz)	-	-	-	-	-	-
H-5'	[c]7.35-7.28 (m)	[c] 7.11-7.07 (m)	[c] 7.39 (d, *J* = 8.5 Hz)	[c] 7.68 (t, *J* = 8.3 Hz)	[c] 7.58 (t, *J* = 7.5 Hz)	[c] 7.71 (d, *J* = 8.4 Hz)	[c] 7.39-7.34 (m)	[c] 7.15 (d, *J* = 7.2 Hz)	[c] 7.11 (t, *J* = 8.3 Hz)	[c] 7.45-7.38 (m)	[c] 6.84 (d, *J* = 8.6 Hz)	[c] 7.52 (d, *J* = 8.5 Hz)	[c] 8.20 (d, *J* = 8.6 Hz)

	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j	4l	4m	4n
Pos.	**2-Cl**	**3-Cl**	**4-Cl**	**2-CF_3**	**3-CF_3**	**4-CF_3**	**3-F**	**4-F**	**2,4-F**	**3,4-F**	**4-OCH_3**	**4-Br**	**4-NO_2**
H-5''	[c]7.35-7.28 (m)	[c] 7.11-7.07 (m)	[c] 7.39 (d, *J* = 8.5 Hz)	[c] 7.68 (t, *J* = 8.3 Hz)	[c] 7.58 (t, *J* = 7.5 Hz)	[c] 7.71 (d, *J* = 8.4 Hz)	[c] 7.39-7.34 (m)	[c] 7.15 (d, *J* = 7.2 Hz)	[c] 7.11 (t, *J* = 8.3 Hz)	[c] 7.45-7.38 (m)	[c] 6.84 (d, *J* = 8.6 Hz)	[c] 7.52 (d, *J* = 8.5 Hz)	[c] 8.20 (d, *J* = 8.6 Hz)
H-6'	[c]7.35-7.28 (m)	[c]7.36 (d, *J* = 7.2 Hz)	7.12 (d, *J* = 8.5 Hz)	[c] 7.75 (d, *J* = 8.3 Hz)	[c] 7.45-7.41 (m)	7.34 (d, *J* = 8.1 Hz)	[c] 6.96 (d, *J* = 7.6 Hz)	[c] 7.16 (d, *J* = 7.2 Hz)	[c] 7.33-7.20 (m)	[c] 6.97-6.94 (m)	[c] 7.03 (d, *J* = 8.8 Hz)	[c] 7.06 (d, *J* = 8.5 Hz)	[c] 7.41 (d, *J* = 8.8 Hz)
H-6''	[c] 7.35-7.28 (m)	[c]7.36 (d, *J* = 7.2 Hz)	7.12 (d, *J* = 8.5 Hz)	[c] 7.75 (d, *J* = 8.3 Hz)	[c] 7.45-7.41 (m)	7.34 (d, *J* = 8.1 Hz)	[c] 6.96 (d, *J* = 7.6 Hz)	[c]7.16 (d, *J* = 7.2 Hz)	[c] 7.33-7.20 (m)	[c] 6.97-6.94 (m)	[c] 7.03 (d, *J* = 8.8 Hz)	[c] 7.06 (d, *J* = 8.5 Hz)	[c] 7.41 (d, *J* = 8.8 Hz)
N-OH	10.7 (s)	10.7 (s)	10.7 (s)	10.7 (s)	10.7 (s)	10.7 (s)	10.7 (s)	10.6 (s)	10.7 (s)	10.7 (s)	10.6 (s)	10.7 (s)	10.8 (s)
OCH_3	-	-	-	-	-	-	-	-	-	-	3.69 (s)	-	-

"c" resonances overlap; "d" resonance beneath solvent. Multiplicity cannot be determined; H-2 and H-4 is interchangeable

Table 3-6: ^{13}C NMR of spiro oximes in DMSO-d_6 (**4a-n**)

	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j	4l	4m	4n
Pos.	**2-Cl**	**3-Cl**	**4-Cl**	**2-CF_3**	**3-CF_3**	**4-CF_3**	**3-F**	**4-F**	**2,4-F**	**3,4-F**	**4-OCH_3**	**4-Br**	**4-NO_2**
C-1	171.7	171.7	171.7	172.8	171.6	171.4	171.8	171.9	170.8	171.7	172.3	171.7	171.2
C-3	148.8	148.7	148.7	148.8	148.5	148.6	148.7	148.8	148.8	148.7	149.0	148.7	148.5
C-5	169.6	170.3	170.3	168.9	170.3	170.0	170.4	170.5	170.3	170.2	170.8	170.3	169.8
C-6	56.2	59.3	59.5	55.9	59.4	59.2	59.3	59.8	57.2	59.4	60.1	59.3	58.9
C-7	44.9	48.1	47.9	46.5	48.2	48.3	48.2	47.9	40.7	47.5	48.0	48.0	48.2
C-8	26.6	25.4	25.4	28.2	25.4	25.2	25.4	25.7	26.0	25.4	25.9	25.3	25.2
C-9	153.2	153.5	153.7	152.6	153.4	153.4	153.6	154.0	153.2	153.4	154.6	153.7	153.0
C-10	33.6	32.4	32.4	35.2	32.3	32.2	32.5	32.7	32.7	32.4	33.0	32.4	32.0
C-11	46.1	49.4	49.2	47.6	49.5	49.6	49.5	49.1	42.0	48.8	49.3	49.2	49.5
C-1'	136.1	140.5	137.0	[c] 137.7 (q, *J* = 8.4 Hz)	139.3	142.6	140.8	134.3 (d, *J* = 6.7 Hz)	[c] 121.7-121.5 (m)	[c] 135.7 (dd, *J* = 9.5, 5.0 Hz)	130.2	137.4	145.4
C-1''	136.0	140.4	136.9	[c] 137.7 (q, *J* = 8.4 Hz)	139.2	142.5	140.7	134.2 (d, *J* = 6.7 Hz)	[c] 121.7-121.5 (m)	[c] 135.7 (dd, *J* = 9.5, 5.0 Hz)	130.1	137.3	145.3
C-2'	133.2	127.8	129.7	[c] 127.8 (q, *J* = 31.0 Hz)	[c] 124.4-124.3 (m)	[c] 128.8 (q, *J* = 3.3 Hz)	114.6 (d, *J* = 21.4, Hz)	[c] 129.8 (d, *J* = 7.9 Hz)	[c] 161.7 (dd, *J* = 248.9, 13.8 Hz)	[c] 116.9 (dd, *J* = 17.8, 6.6 Hz)	128.9	130.0	129.5
C-2''	133.1	127.7	129.6	[c] 127.8 (q, *J* = 31.0 Hz)	[c] 124.4-124.3 (m)	[c] 128.8 (q, *J* = 3.3 Hz)	114.4 (d, *J* = 21.4, Hz)	[c] 129.8 (d, *J* = 7.9 Hz)	[c] 161.7 (dd, *J* = 248.9, 13.8 Hz)	[c] 116.9 (dd, *J* = 17.8, 6.6 Hz)	128.8	129.9	129.4
C-3'	[c] 130.1	[c] 133.2	[c] 128.7	[c] 127.0 (q, *J* = 5.4 Hz)	[c] 129.3 (q, *J* = 33.0 Hz)	[c] 125.6 (q, *J* = 7.7 Hz)	[c] 169.8 (d, *J* = 243.3 Hz)	[c] 115.5 (d, *J* = 21.4 Hz)	[c] 104.2 (t, *J* = 27.9 Hz)	[c] 149.1 (dd, *J* = 248.3, 12.7 Hz)	[c] 113.9	[c] 131.6	[c] 123.8

	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j	4l	4m	4n
Pos.	**2-Cl**	**3-Cl**	**4-Cl**	**2-CF_3**	**3-CF_3**	**4-CF_3**	**3-F**	**4-F**	**2,4-F**	**3,4-F**	**4-OCH_3**	**4-Br**	**4-NO_2**
C-3''	[c] 130.1	[c] 133.2	[c] 128.7	[c] 127.0 (q, *J* = 5.4 Hz)	[c] 129.3 (q, *J* = 33.0 Hz)	[c] 125.6 (q, *J* = 7.7 Hz)	[c] 169.8 (d, *J* = 243.3 Hz)	[c] 115.5 (d, *J* = 21.4 Hz)	[c] 104.2 (t, *J* = 27.9 Hz)	[c] 149.1 (dd, *J* = 248.3, 12.7 Hz)	[c] 113.9	[c] 131.6	[c] 123.8
C-4'	127.6	128.1	[c] 132.6	128.5	[c] 124.9-124.8 (m)	[c] 128.2 (q, *J* = 37.8 Hz)	115.0 (d, *J* = 20.9, Hz)	[c] 161.6 (d, *J* = 245.1 Hz)	[c] 159.6 (dd, *J* = 250.3, 7.7 Hz)	[c] 149.0 (dd, *J* = 244.9, 13.2 Hz)	[c] 158.6	121.3	147.2
C-4''	127.5	128.0	[c] 132.6	127.2	[c] 124.9-124.8 (m)	[c] 128.2 (q, *J* = 37.8 Hz)	114.9 (d, *J* = 20.9 Hz)	[c] 161.6 (d, *J* = 245.1 Hz)	[c] 159.6 (dd, *J* = 250.3, 7.7 Hz)	[c] 149.0 (dd, *J* = 244.9, 13.2 Hz)	[c] 158.6	121.2	147.1
C-5'	129.5	126.5	[c] 128.7	[c] 133.0	[c] 130.0	[c] 125.6 (q, *J* = 4.4 Hz)	[c] 130.7 (d, *J* = 8.9 Hz)	[c] 115.5 (d, *J* = 21.4 Hz)	[c] 112.6 (dd, *J* = 21.6, 4.2 Hz)	[c] 117.8 (d, *J* = 16.9 Hz)	[c] 113.9	[c] 131.6	[c] 123.8
C-5''	129.4	126.4	[c] 128.7	[c] 133.0	[c] 130.0	[c] 125.6 (q, *J* = 4.4 Hz)	[c] 130.7 (d, *J* = 8.9 Hz)	[c] 115.5 (d, *J* = 21.4 Hz)	[c] 112.6 (dd, *J* = 21.6, 4.2 Hz)	[c] 117.8 (d, *J* = 16.9 Hz)	[c] 113.9	[c] 131.6	[c] 123.8
C-6'	127.8	[c] 130.6	129.7	[c] 127.2	131.9	[c] 128.8 (q, *J* = 3.3 Hz)	124.0 (d, *J* = 2.7 Hz)	[c] 129.7 (d, *J* = 7.9 Hz)	[c] 129.9-129.8 (m)	[c] 124.9 (dd, *J* = 9.7, 3.6 Hz)	128.9	130.0	129.5
C-6''	127.7	[c] 130.6	129.6	[c] 127.2	131.8	[c] 128.8 (q, *J* = 3.3 Hz)	123.9 (d, *J* = 2.7 Hz)	[c] 129.7 (d, *J* = 7.9 Hz)	[c] 129.9-129.8 (m)	[c] 124.9 (dd, *J* = 9.7, 3.6 Hz)	128.8	129.0	129.4
CF_3	-	-	-	124.0 (q, *J* = 274.9 Hz)	[c] 123.7 (q, *J* = 273.0 Hz)	[c] 124.1 (q, *J* = 273.0 Hz)	-	-	-	-	-	-	-
CF_3				123.9 (q, *J* = 274.9 Hz)	[c] 123.7 (q, *J* = 273.0 Hz)	[c] 124.1 (q, *J* = 273.0 Hz)	-		-	-	-		-
OCH_3	-	-	-	-	-	-	-	-	-	-	[c] 55.0	-	-
OCH_3	-	-	-	-	-	-	-	-	-	-	[c] 55.0	-	-

"c" resonances overlap; C-1 and C-5 is interchangeable

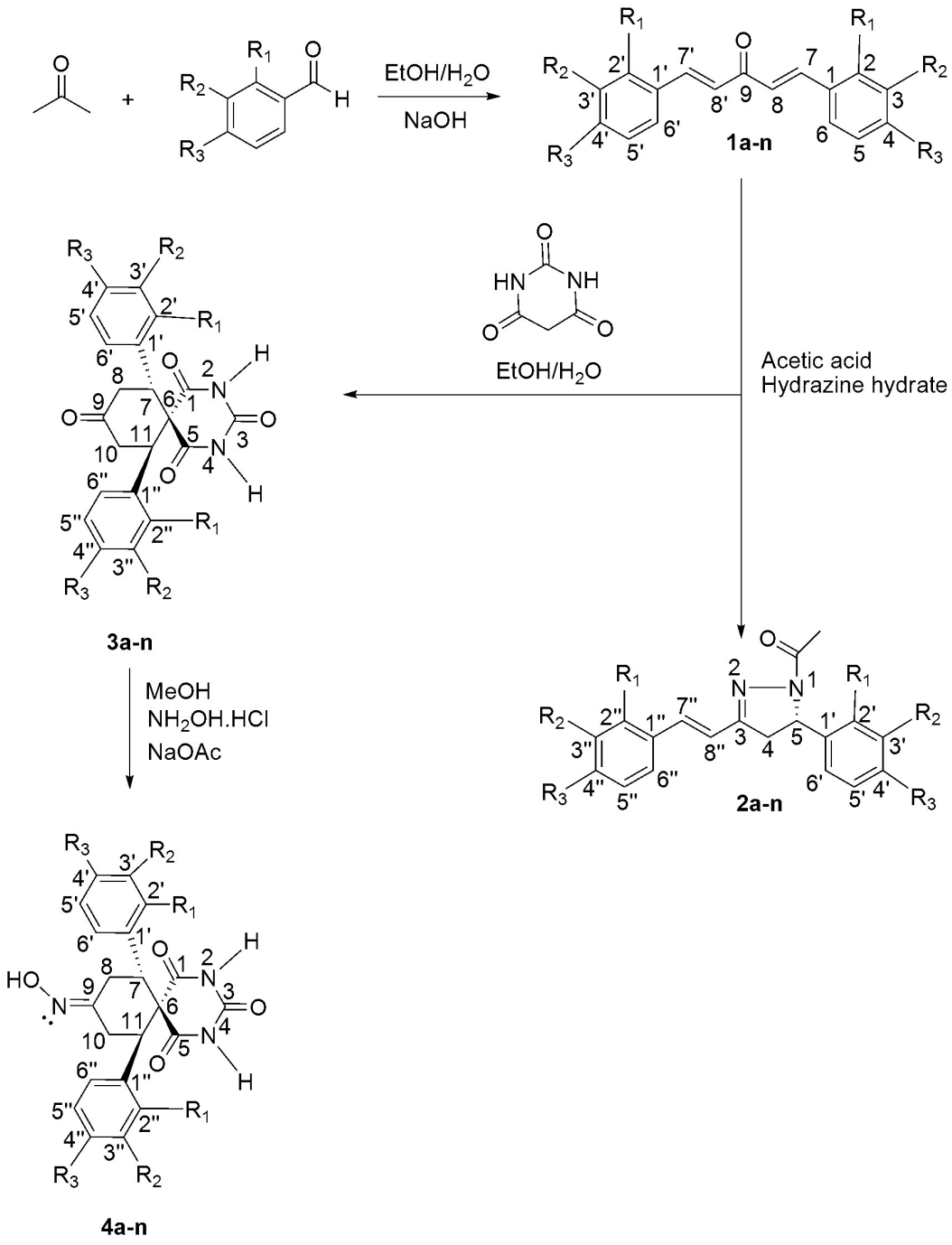
3.3 Results and Discussion

A series of ketopyrazoles and spiro barbiturates were formed from their corresponding ketodiene intermediates (**1a-n**). These ketodiene intermediates were formed in an aldol condensation reaction from acetone and substituted aromatic aldehydes under basic conditions in yields of 70-85%. The ketopyrazoles (**2a-n**) were formed in yields of 75-85% by a cyclocondensation reaction forming a pyrazole ring on one side of the molecule using acetic acid and hydrazine hydrate. The spiro barbiturates (**3a-n**) were formed *via* a Michael Addition reaction with barbituric acid. Ethanol behaves as a protic solvent in this reaction and further protonates the carbonyl group of the ketodiene intermediate, allowing for nucleophilic attack by barbituric acid at the β-carbon of the molecule forming a spiro compound. These spiro barbiturates (**3a-n**) were produced in very good yields of 84-96%. In a subsequent step they were converted to their oximes (**4a-j, l-n**) using hydroxylamine hydrochloride (**Scheme 3-1**; **Table 3-7**).

The structures of the synthesised molecules were determined from their ^{1}H and ^{13}C NMR spectra and their resonances assigned using 2D NMR spectra. Since the ketodiene is symmetrical, resonances for only one half of the molecule are observed. For example in **1c**, H-7 and H-8 both occurred as doublets at δ 7.64 and δ 7.00 with J = 15.9 Hz. The *ortho* coupled protons H-2/6 and H-3/5 occurred at δ 7.51 and δ 7.36 respectively with J = 8.3 Hz. The olefinic carbon resonances C-7 and C-8 occurred at δ 142.0 and δ 125.7 respectively and the carbonyl resonance C-9 occurred at δ 188.4.

The formation of the pyrazoline ring in **2c** was indicated by the appearance of an ABX system consisting of three double doublets, H-4a at δ 3.53 (J = 17.4, 11.9 Hz), H-4b at δ 2.94 (J = 17.4, 4.6 Hz) and H-5 at δ 5.47 (J = 11.8, 4.6 Hz). Based on the difference in dihedral

angles, H-4a undergoes a greater degree of coupling to H-5 since it experiences axial-axial coupling ($J_{H4a, H5}$ = 11.9 Hz) while H-4b undergoes axial-equatorial coupling to H-5 ($J_{H4b, H5}$ = 4.6 Hz). The geminal coupling between H-4a and H-4b is 17.4 Hz. The acetyl methyl group at N-1 occurred as a singlet at δ2.33 with the acetyl carbonyl resonance at δ168.7.



Scheme 3-1: Synthetic scheme for the synthesis of ketodienes (**1a-n**), ketopyrazoles (**2a-n**), spiro barbiturates (**3a-n**) and spiro oximes (**4a-j, l-n**)

Table 3-7: Substitution pattern and yields of compounds **2a-n**, **3a-n** and **4a-j, l-n**.

No.	R_1	R_2	R_3	% yield (1a-n)	% yield (2a-n)	% yield (3a-n)	% yield (4a-n)
a	Cl	H	H	73	90	83	90
b	H	Cl	H	78	89	86	96
c	H	H	Cl	85	96	95	98
d	CF_3	H	H	70	92	85	92
e	H	CF_3	H	75	96	88	94
f	H	H	CF_3	80	97	92	97
g	H	F	H	72	92	89	95
h	H	H	F	70	94	91	97
i	F	H	F	79	94	93	99
j	H	F	F	83	89	95	96
k	OCH_3	H	H	77	93	96	*NR*
l	H	H	OCH_3	85	98	87	98
m	H	H	Br	82	97	89	91
n	H	H	NO_2	80	96	93	93

The olefinic double bond H-7" and H-8" occurred at δ 6.68 and δ 7.05 with J = 16.4 Hz. Since the carbonyl group is replaced by the pyrazoline ring, electron delocalisation does not occur and H-8" becomes more shielded than H-7". This was confirmed by HMBC correlation from H-8" to C-4. The ^{13}C NMR spectrum of **2c** showed the presence of the acetyl carbonyl carbon attached to N-1 at δ 168.7 and the two C-N resonances, an imine carbon resonance C-3 at δ 154.6 and C-5 at δ 59.3. The C-4 resonance at δ 40.9 was assigned due to HMBC correlations to H-8" and H-5, and the C-3 resonance showed correlations to H-7", H-5 and H-4.

A crystal structure of one of the ketopyrazoles **2l** (the 4-methoxy derivative) was solved in the P21/n space group with four molecules in the unit cell. The unit consisting of the aromatic ring and the pyrazole ring at each ends of the double bond is almost planar (torsion angle of C(9)-C(10)-C(11)-C(20) being 178.09(12)° and C(7)-C(8)-C(9)-C(10) being 7.7(2)°) with the phenyl ring on the pyrazole moiety pointing away from this planar system with a dihedral angle of (89.8(39)°). The crystal structure also indicated that C-5 was in the *S* configuration.

The Ortep diagram of compound **2l** is shown in **Figure 3-1**.

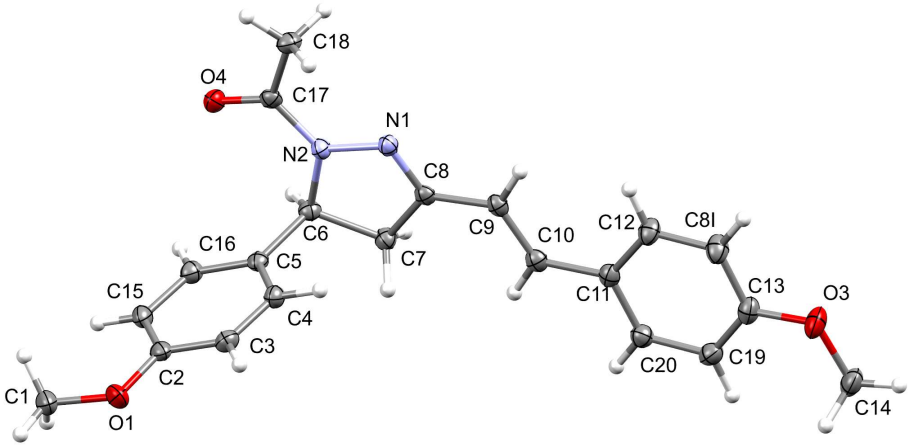


Figure 3-1: Ortep diagram of 4-methoxy ketopyrazole (**2l**)

The reaction of the ketodiene intermediate with barbituric acid resulted in symmetrical spiro barbiturates where the cyclohexanone ring consisted of three equivalent resonances for H-7, and H-11; H-8_{ax} and H-10_{ax}; and H-8_{eq} and H-10_{eq}. Each of these resonances occur as double doublets, H-7/11 being the most deshielded at δ 4.64 (J = 13.7, 5.2 Hz), followed by H-8_{ax}/10_{ax} at δ 3.25 (J = 16.4, 13.7 Hz) and H-8_{eq}/10_{eq} at δ 2.53 (J = 16.4, 5.2 Hz). The axial and equatorial protons resonances were determined by axial-axial coupling of 13.7 Hz and axial-equatorial coupling of 5.2 Hz. The aromatic proton resonances occurred between δ 7.28-7.46 and the two N-H resonances, H-2 and H-4 occured as singlets at δ 11.27 and δ 11.95.

The ^{13}C NMR spectrum of **3a** showed the carbonyl resonance of the cyclohexanone moiety, C-9 at δ 205.6. This was assigned due to it being the most deshielded resonance in the spectrum as well as HMBC correlations with H-8/10. The two carbonyl groups, C-1 and C-5 attached to the barbituric ring was in a similar environment and found at δ 171.9 and δ 169.5. These two carbon resonances showed HMBC correlations to H-2 and H-4, as well as H-7/11. The C-3 carbonyl resonance located between the two N-H groups distinctly appeared at δ

148.8. The aliphatic carbon C-6 appeared at δ 55.4. Similar to C-1 and C-5, they showed HMBC correlations to H-2, H-4 and H-7/11. The methylene carbons C-11/7 and C-8/10 were equivalent and appeared at δ 44.6 and δ 43.1. The aromatic carbons were found between δ 127.5 and δ 135.6.

The crystal structure of the spiro barbiturate **3k** (the 2-methoxy derivative) was crystallized in the monoclinic P21/n space group with one molecule in the asymmetric unit. The cyclohexanone ring and the barbiturate ring were at 60° to each other. Both the phenyl groups point away from the cyclohexanone ring with torsion angles of 44.6° for H(10)-C(10)-C(17)-C(18) and 44.8° for H(8)-C(8)-C(1)-C(2). The torsion angles between the methine and methylene group on the cyclohexanone ring were 64.4° for H(10)-C(10)-H(11A)-C(11), 178.2° for H(10)-C(10)-H(11B)-C(11), 47.6° for C(8)-H(8)-C(13)-H(13A) and 70.3° for C(8)-H(8)-C(13)-H(13B). The configuration at C-8 and C-10 was found to be *R* at both centers. The Ortep diagram of compound **3k** is given in **Figure 3-2**.

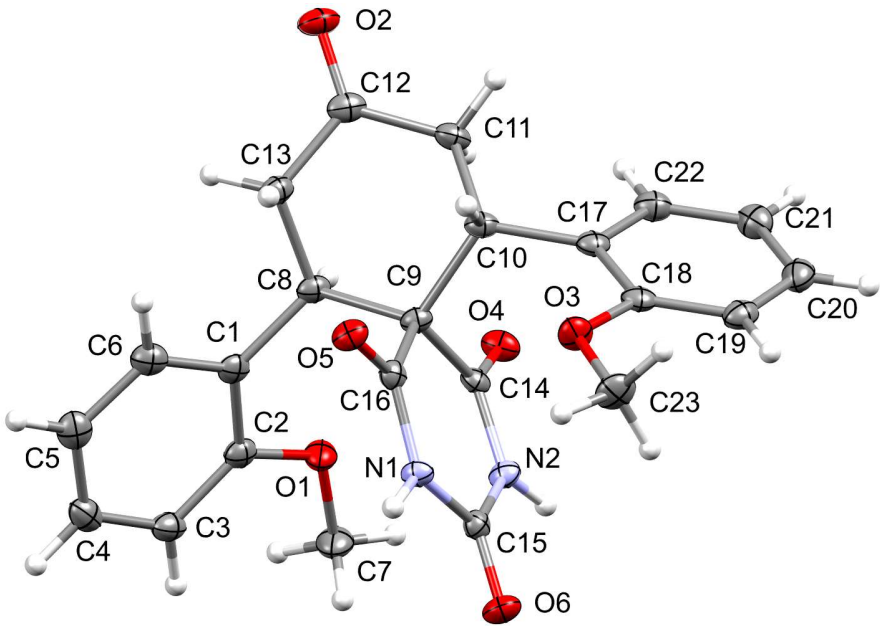


Figure 3-2: Ortep diagram of 2-methoxy ketopyrazole (**3k**)

When the spiro ketobarbiturates were converted to oximes, the hydroxyl proton of the oxime was observed at δ 10.74, with C-9 of the oxime occurring at δ 153.2. The equivalence of C-7 and C-11 and the methylene groups CH_2-8 and CH_2-10 was also observed to be lost. Unlike the ketones, where three sets of resonances were observed for H-7/11, H-8_{ax}/H-10_{ax} and H-8_{eq}/H10_{eq}, six separate resonances for H-7, H-11, H-8_{ax}, H-8_{eq}, H-10_{ax}, H-10_{eq} was now observed. The H-7 and H-11 resonances were methine protons and the other four were methylene protons. These other four consisted of two sets of methylene resonances, each set consisting of a double doublet and a triplet. One of the double doublets, H-8_{eq} was deshielded much more than the other and assigned based on a NOESY correlation to the N-OH resonance of the oxime and its *J* value of 4.1 Hz (axial-equatorial coupling). This was seen coupled to H-7 and H-8_{ax}, H-7 appearing as a double doublet at δ 4.15 with axial-axial coupling of 13.8 Hz and a smaller axial-equatorial coupling of 4.1 Hz. The H-8_{ax} proton appeared at δ 2.75 as a triplet with geminal coupling being equal to the axial-axial coupling (*J* = 13.8 Hz). A similar pattern is observed for H-11, H-10_{eq} and H-10_{ax}. The aromatic resonances appeared between δ 7.28-7.47.

All the synthesised compounds, ketopyrazoles (**2a-n**), spiro barbiturates (**3a-n**) and spiro oximes (**4a-j, l-n**) were evaluated for their antibacterial activity against two Gram +ve bacterial strains (*S. aureus* and *S. aureus Rosenbach* (MRSA)), and four Gram –ve strains, *S. typhimurium, P. aeruginosa*, *K. pneumonia* and *E. coli*. Preliminary screening by disc diffusion was first used to decide which compounds were best suited for further testing by the MBC assay. These results are presented in **Table 3-8** to **Table 3-10**.

The ketopyrazoles showed better activity against Gram +ve bacteria, *S. aureus* and MRSA than Gram -ve bacteria. Compounds **2a** (2-Cl), **2l** (4-OCH_3) and **2n** (4-NO_2) were active against *S. aureus* at 15.6, 125, and 125 μg mL^{-1} respectively. Compounds **2b** (3-Cl), **2c** (4-Cl), **2k** (2-OCH_3) and **2m** (4-Br) were active against MRSA at 31.3, 62.5, 125 and 31.3 μg mL^{-1} respectively. The chloro groups seemed to be the best substituent for antibacterial activity, since three of these compounds, **2a-c** had chloro groups substituted on the aromatic moiety. The other three compounds were 4-substituted with either electron donating methoxy or electron withdrawing bromo and nitro groups. The 4-bromo substituent (**2m**) showed good activity against MRSA at 31.3 μg mL^{-1}. The 2-chloro substituted ketopyrazole showed the best activity against *S. aureus* and *P. aeruginosa* at 15.6 and 31.3 μg mL^{-1} respectively. All the activities mentioned above were comparable or better than the standards, ciprofloxacin and levofloxacin (**Table 3-8Table 3-8**).

Table 3-8: Minimum bactericidal concentration (MBC in μg mL^{-1}) of test compounds **2a-n** (ketopyrazoles)

Compound	Substitution	S. *aureus*	MRSA	P. *aeruginosa*
2a	2-Cl	15.6	-	31.3
2b	3-Cl	-	31.3	-
2c	4-Cl	-	62.5	-
2k	2-OMe	250	125	-
2l	4-OMe	125	-	-
2m	4-Br	-	31.3	-
2n	4-NO_2	125	-	-
Ciprofloxacin		94.3	188.6	188.6
Levofloxacin		21.6	86.5	345

"-" denotes activity ≥ 500 μg mL^{-1}

All compounds tested were not active against *S. typhimurium* at concentrations of 500 μg mL^{-1} or less

The conversion to spiro barbiturates resulted in better activity against the Gram –ve *E. coli* and *P. aeruginosa* with seven of these compounds **3c** (4-Cl), **3f** (4-CF_3), **3k** (2-OCH_3), **3l** (4-OCH_3), **3m** (4-Br) and **3n** (4-NO_2) showing activity against either one or both *E. coli* and *P. aeruginosa*. Compounds **3c**, **3k** and **3l** showed activity against *E. coli* only with MBC values

of 31.3, 7.81 and 0.98 μg mL^{-1}. Compounds **3e** and **3f** showed activity against *P. aeruginosa* with a MBC value of 31.3 μg mL^{-1}. In addition, **3f** was also active against *S. aureus* at 31.3 μg mL^{-1}. The 4-nitro compound, **3n** was active against both *E. coli* and *P. aeruginosa* at 0.98 and 125 μg mL^{-1} respectively and broad spectrum antibacterial activity against all Gram –ve bacteria tested against was seen by the 4-bromo substituted derivative **3m** with a MBC value of 0.98 μg mL^{-1} against all bacterial strains. Thus, **3m** is an excellent lead compound for antibacterial activity against Gram –ve bacteria since it showed better activity than the standards ciprofloxacin and levofloxacin (**Table 3-9**).

Table 3-9: Minimum bactericidal concentration (MBC in μg mL^{-1}) of compounds **3a-n** (spiro barbiturates)

Compound	Substitution	S. *aureus*	S. *typhimurium*	*K. pneumonia*	*E. coli*	P. *aeruginosa*
3c	4-Cl	-	-	-	31.3	-
3e	3-CF_3	-	-	-	-	31.3
3f	4-CF_3	31.3	-	-	-	31.3
3k	2-OMe	-	-	-	7.81	-
3l	4-OMe	-	-	-	0.98	-
3m	4-Br	250	0.98	0.98	0.98	0.98
3n	4-NO_2	250	-	-	0.98	125
Ciprofloxacin		94.3	2.95	2.95	2.95	188.6
Levofloxacin		21.6	21.6	21.6	0.34	345

"-" denotes activity ≥ 500 μg mL^{-1}

All compounds tested were not active against MRSA at concentrations of 500 μg mL^{-1} or less

Derivatization of the spiro barbiturates to oximes resulted in loss of activity against all the Gram –ve bacteria tested. Although the spiro barbiturates were not active against MRSA, the oxime derivatives **4f** and **4m** did show some activity against MRSA at 125 and 250 μg mL^{-1} (**Table 3-10**). Thus, this indicated that the keto group in the cyclohexanone ring was responsible for the activity of the spirobarbiturates, since activity was lost when this group was derivatised to the oxime.

Table 3-10: Minimum bactericidal concentration (MBC in μg mL^{-1}) of compounds **4a-j, l-n** (spirobarbiturate oximes)

Compound	Substitution	S. *aureus*	MRSA
4f	4-CF_3	-	125
4m	4-Br	250	250
Ciprofloxacin		94.3	188.6
Levofloxacin		21.6	86.5

"-" denotes activity ≥ 500 μg mL^{-1}

All compounds tested were not active against *S. typhimurium*, *K. pneumonia*, *E. coli* and *P. aeruginosa* at concentrations of 500 μg mL^{-1} or less

3.4 Conclusion

A range of ketopyrazoles and spiro barbiturates were easily formed from their ketodiene precursor by reaction of hydrazine hydrate and barbituric acid in yields of 80% and above for the ketopyrazoles and 90% and above for the spiro barbiturates. The spiro barbiturates were converted to the oximes in yields of 90% and greater. The ketodiene precursors easily crystallized out of solution after the Claisen-Schmidt condensation of substituted benzaldehydes with acetone in yields of 70-85%. The spiro barbiturate reactions were also easily carried out as the products crystallized out of solution. Only in the case of the ketopyrazole the products were purified by column chromatography. Six of the ketopyrazoles, of which 3 were chlorinated showed good activity against either *S. aureus* or MRSA with the 2-chloro derivative being the most active in *S. aureus* and *P. aeruginosa* with MBC values of 15.6 and 31.3 μg mL^{-1}. Much better activity was seen by the spiro barbiturates where good activity was seen by seven of the compounds in at least one strain of bacteria. It was observed that these compounds were very active against the two Gram –ve bacteria *E. coli* and *P. aeruginosa*. The most active compound of the series was the 4-bromo derivative being active against all bacterial strains tested with the exception of MRSA. The MBC values for this compound was as low as 0.98 μg mL^{-1} for all the Gram –ve bacterial strains. The ketone group on the cyclohexanone moiety was responsible for this activtiy, since all activity was lost when this group was converted to the oxime.

3.5 References

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Chapter 4 The synthesis, structural elucidation and antimicrobial activity of symmetrical quinoline ketodiene derivatives

***** The compounds referred to in this chapter are referred to in the Abstract, Conclusion and Appendices with **C** preceding the number of the compound. For example **2a** is referred to as **C-2a**.

Abstract

Ten novel substituted quinoline ketodiene derivatives **2a-j** were synthesised by the aldol condensation reaction of acetone with substituted quinoline aldehydes and tested for their antibacterial activity against two Gram +ve (*Staphylococcus aureus* and methicillin resistant *S. aureus* (MRSA)) and four Gram -ve species (*Salmonella typhimurium, Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli*). This is the first report of curcumin-like molecules synthesised with a quinoline ring. All compounds were highly active against a minimum of three bacterial strains, in most cases better than levofloxacin and ciprofloxacin. The best activity was seen by **2a**, **2c** and **2d**, being active against all six strains of Gram +ve and Gram -ve species at 0.98-31.3 μg mL^{-1}, with the exception of **2c** having lower activity against *S. aureus* at 250 μg mL^{-1}.

Keywords: quinoline, ketodienes, antibacterial, curcumin

4.1 Introduction

Quinolines have a diverse range of chemical and pharmacological properties (Marella et al., 2013). Compounds containing a quinoline scaffold have a broad range of biological activities such as antimalarial, anticancer, anti-flammatory, antibacterial and antiepileptic amongst others (Marella et al., 2013; David et al., 2015; Kotra et al., 2009; Azad et al., 2007; Wei et al., 2015). Quinoline scaffolds are most popular for their antimalarial activity. Quinine, chloroquine and mefloquine are quinoline based drugs currently used for the treatment of malaria (Ramann et al., 2016). Quinolines can be synthesized *via* the Doebner-Miller (Gopaul and Koorbanally, 2016), Skraup (Wang, 2010), Friedländer (Wang, 2010) and Combes (Wang, 2010) methods.

Aldehydes and ketones are particularly useful precursors for aldol products and these functional groups can be used to design a large number of potentially bioactive molecules in medicinal chemistry. There are many ways of forming aldol products, however the most commonly used is the Claisen-Schmidt and Dieckmann reactions, and the Knoevenagel condensation. The Claisen-Schmidt condensation is a popular base-catalysed method which involves the cross condensation of an acetophenone with a benzaldehyde in the presence of a base. The Dieckmann reaction is similar to the Claisen-Schmidt condensation, however cyclic β-ketoesters are synthesised *via* an intramolecular aldol reaction. The Knoevenagel condensation is a modified version of an Aldol condensation and occurs when an aldehyde or ketone and an activated methylene group is converted to an α,β-unsaturated compound under an amine base catalyst (Clayden et al., 2007).

Symmetrical ketodienes are known to have antimalarial, anticancer, antioxidant and antibacterial activity amongst others (Lee et al., 2009, Ge et al., 2012; Ahmad et al., 2014;

Lee et al., 2014; Aguilera et al., 2016; Qudjani et al., 2016; Dohutia et al., 2017). Quinoline ketodienes can be formed *via* the aldol condensation of quinoline aldehydes and acetone (Cao et al., 2012).

We report the synthesis, structural elucidation and antibacterial activity of a series of quinoline-2-ketodienes synthesised from quinoline-2-carbaldehydes and acetone. This reaction forms a symmetrical *α,β*-unsaturated ketone chain between the two quinoline moieties. It is hypothesised that the *α,β*-unsaturated ketone chain between the two molecules will enhance the activity of the quinolines.

4.2 Experimental

General Experimental Procedures

Reagent grade chemicals and solvents were purchased from Sigma Aldrich and Merck, South Africa. NMR spectra were acquired on a Bruker Avance 400 MHz NMR instrument in deuterated DMSO or chloroform-D and recorded against the internal standard TMS and referenced to the solvent peak of DMSO at δ 2.50 or $CDCl_3$ at δ 7.24. Infrared spectra were acquired on a Perkin Elmer Spectrum 100 FTIR spectrometer with universal ATR sampling accessory. Melting points were determined on a Stuart Smart Scientific melting point instrument. High Resolution Mass Spectrometry was carried out on a Bruker microTOF-Q II ESI instrument. Samples were purified by column chromatography using silica gel as the stationary phase and varying ratios of hexane:ethyl acetate or dichloromethane:methanol as the mobile phase.

Synthesis of quinoline aldehydes (1a-j)

Substituted anilines (80.0 mmol) was dissolved in a mixture of HCl and water (3:2) and heated. Thereafter crotonaldehyde (120.0 mmol) in 40 mL toluene was added to the mixture and refluxed for 12 h. On completion, the reaction mixture was cooled and the toluene layer discarded. The remaining mixture was neutralised with sodium bicarbonate and extracted with ethyl acetate. Dioxane (50 mL) and selenium dioxide (46.2 mmols; 3.1 g) was then added to the extract and refluxed at 110 °C for 30 min. On completion, the reaction mixture was filtered, extracted with ethyl acetate and purified by column chromatography using n-hexane:ethyl acetate (98:2) to afford white crystals.

Synthesis of quinoline ketodienes (2a-j)

Substituted quinoline aldehydes (5.0 mmol) was dissolved in a mixture of toluene, ethanol and water (5:2:1). Thereafter, acetone (2.7 mmol; 0.16 mL) was added dropwise to the mixture using a graduated 1 mL syringe. The reaction was then refluxed for 12 h at 70 °C and monitored by TLC. On completion, the toluene was removed and the resultant mixture dissolved in water and extracted with dichloromethane. The extract was then purified by column chromatography using either hexane:ethyl acetate (1:1) or dichloromethane:methanol (98:2) depending on the derivative synthesised.

(1E,4E)-1,5-bis(6-chloroquinolin-2-yl)penta-1,4-dien-3-one (**2a**), brown solid, mp 122-124 °C, IR υ_{max} (cm^{-1}): 3003 (C-H), 1660 (C=O), 1487 (C=C). ^{1}H NMR (400 MHz, DMSO) δ 7.24 (d, 2H, *J* = 16.3 Hz, H-10'/10), 7.72 (d, 2H, *J* = 16.3 Hz, H-9'/9), 7.80 (dd, 2H, *J* = 9.0, 2.4 Hz, H-7'/7), 8.01 (d, 2H, *J* = 8.6 Hz, H-3'/3), 8.05 (d, 2H, *J* = 9.0 Hz, H-8'/8), 8.15 (d, 2H, *J* = 2.4 Hz, H-5'/5), 8.42 (d, 2H, *J* = 8.6 Hz, H-4'/4). ^{13}C NMR (100 MHz, DMSO): 121.9 (C-3'/3), 126.6 (C-5'/5), 128.4 (C-6'/6), 130.8 (C-7'/7), 131.2 (C-8'/8), 131.7 (C-4'a/4a), 132.0

(C-10'/10), 136.4 (C-4'/4), 141.9 (C-9'/9), 146.0 (C-8'a/8a), 154.0 (C-2'/2), 198.4 (C-11). HRMS (*pos*) *m/z* 427.0395 $[M + Na]^+$ (Calculated for $C_{23}H_{14}Cl_2N_2ONa$, 427.0381)

(1E,4E)-1,5-bis(6-fluoroquinolin-2-yl)penta-1,4-dien-3-one (**2b**), brown solid, mp 167-169 °C, IR υ_{max} (cm^{-1}): 2923 (C-H), 1503 (C=O), 1475 (C=C). ^1H NMR (400 MHz, DMSO): δ 7.80 (td, 2H, *J* = 8.8, 2.3 Hz, H-7'/7), 7.92 (d, 2H, *J* = 16.0 Hz, H-10'/10), 7.92 (dd, 2H, *J* = 9.8, 2.8 Hz, H-5'/5), 8.01 (d, 2H, *J* = 16.0 Hz, H-9'/9), 8.21 (d, 2H, *J* = 9.0 Hz, H-3'/3), 8.22 (d, 2H, *J* = 8.8 Hz, H-8'/8), 8.54 (d, 2H, *J* = 9.0 Hz, H-4'/4). ^{13}C NMR (100 MHz, DMSO): 111.1 (d, *J* = 21.9 Hz, C-5'/5), 120.4 (d, *J* = 25.5 Hz, C-7'/7), 121.7 (C-3'/3), 128.6 (d, *J* = 10.6 Hz, C-4'a/4a), 130.1 (C-10'/10), 132.1 (d, *J* = 9.1 Hz, C-8'/8), 136.6 (d, *J* = 4.8 Hz, C-4'/4), 142.6 (C-9'/9), 144.9 (C-8'a/8a), 153.2 (C-2'/2), 159.8 (d, *J* = 256.8 Hz, C-6'/6), 189.1 (C-11). HRMS (*pos*) *m/z* 395.0958 $[M + Na]^+$ (Calculated for $C_{23}H_{14}F_2N_2ONa$, 395.0972)

(1E,4E)-1,5-bis(6-bromoquinolin-2-yl)penta-1,4-dien-3-one (**2c**), brown solid, mp 141-142 °C, IR υ_{max} (cm^{-1}): 2924 (C-H), 1659 (C=O), 1483 (C=C). ^1H NMR (400 MHz, DMSO): δ7.24 (d, 2H, *J* = 16.4 Hz, H-10'/10), 7.72 (d, 2H, *J* = 16.4 Hz, H-9'/9), 7.91 (dd, 2H, *J* = 8.6, 2.1 Hz, H-7'/7), 7.97 (d, 2H, *J* = 8.6 Hz, H-8'/8), 8.01 (d, 2H, *J* = 8.6 Hz, H-3'/3), 8.31 (d, 2H, *J* = 2.1 Hz, H-5'/5), 8.41 (d, 2H, *J* = 8.6 Hz, H-4'/4). ^{13}C NMR (100 MHz, DMSO): 120.4 (C-6'/6), 121.8 (C-3'/3), 128.9 (C-4'a/4a), 129.9 (C-5'/5), 131.2 (C-8'/8), 132.1 (C-10'/10), 133.3 (C-7'/7), 136.3 (C-4'/4), 142.0 (C-9'/9), 146.1 (C-8'a/8a), 154.0 (C-2'/2), 198.5 (C-11). HRMS (*pos*) *m/z* 514.9379 $[M + Na]^+$ (Calculated for $C_{23}H_{14}Br_2N_2ONa$, 514.9371)

(1E,4E)-1,5-bis(6-methylquinolin-2-yl)penta-1,4-dien-3-one (**2d**), brown solid, mp 131-133 °C, IR υ_{max} (cm^{-1}): 2912 (C-H), 1650 (C=O), 1496 (C=C). ^1H NMR (400 MHz, $CDCl_3$): δ2.54 (s, 6H, C<u>H</u>$_3$), 7.58-7.56 (m, 4H, H-5'/5/7'/7), 7.67 (d, 2H, *J* = 8.5 Hz, H-3'/3), 7.71 (d, 2H, *J* = 15.7 Hz, H-10'/10), 7.97 (d, 2H, *J* = 15.7 Hz, H-9'/9), 8.04 (d, 2H, *J* = 9.0 Hz, H-8'/8), 8.12 (d, 2H, *J* = 8.5 Hz, H-4'/4). ^{13}C NMR (100 MHz, $CDCl_3$): 21.7 (<u>C</u>H$_3$), 120.9 (C-

3'/3), 126.4 (C-5'/5), 128.3 (C-6'/6), 129.47 (C-8'/8), 129.54 (C-11'/11), 132.5 (C-7'/7), 136.1 (C-4'/4), 137.7 (C-4'a/4a), 143.2 (C-9'/9), 147.0 (C-8'a/8a), 152.3 (C-2'/2), 189.6 (C-11). HRMS (*neg*) *m/z* 365.1667 [M - H] (Calculated for $C_{25}H_{19}N_2O$, 365.1654)

(1E,4E)-1,5-bis(6-methoxyquinolin-2-yl)penta-1,4-dien-3-one (**2e**), yellow-green solid, mp 219-220 °C, IR υ_{max} (cm^{-1}): 2938 (C-H), 1585 (C=O), 1477 (C=C). ^{1}H NMR (400 MHz, DMSO): δ 3.93 (s, 6H, OC<u>H</u>$_3$), 7.42 (d, 2H, *J* = 2.6 Hz, H-5'/5), 7.46 (dd, 2H, *J* = 9.1 Hz, 2.6, H-7'/7), 7.78 (d, 2H, *J* = 16.1 Hz, H-10'/10), 7.91 (d, 2H, *J* = 16.1 Hz, H-9'/9), 7.98 (d, 2H, *J* = 9.1 Hz, H-8'/8), 8.04 (d, 2H, *J* = 8.8 Hz, H-3'/3), 8.33 (d, 2H, *J* = 8.8 Hz, H-4'/4). ^{13}C NMR (100 MHz, DMSO): 55.6 (O<u>C</u>H$_3$), 105.7 (C-5'/5), 121.4 (C-3'/3), 122.9 (C-7'/7), 129.0 (C-10'/10), 129.2 (C-4'a/4a), 130.8 (C-8'/8), 135.6 (C-4'/4), 142.8 (C-9'/9), 143.8 (C-8'a/8a), 150.9 (C-2'/2), 158.1 (C-6'/6), 188.9 (C-11). HRMS (*pos*) *m/z* 397.1564 $[M + Na]^+$ (Calculated for $C_{25}H_{20}N_2O_3Na$, 397.1552)

(*1E,4E*)-1,5-bis(8-chloroquinolin-2-yl)penta-1,4-dien-3-one (**2f**), green solid, mp 119-121 °C, IR υ_{max} (cm^{-1}): 2921 (C-H), 1594 (C=O), 1494 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47 (t, 2H, *J* = 7.7 Hz, H-6'/6), 7.75 (d, 2H, *J* = 8.6 Hz, H-3'/3), 7.75 (dd, 2H, *J* = 7.6, 1.7 Hz, H-7'/7), 7.80 (d, 2H, *J* = 15.8 Hz, H-10'/10), 7.86 (dd, 2H, *J* = 7.6, 1.2 Hz, H-5'/5), 8.00 (d, 2H, *J* = 15.8 Hz, H-9'/9), 8.22 (d, 2H, *J* = 8.6 Hz, H-4'/4). ^{13}C NMR (100 MHz, $CDCl_3$): 121.8 (C-3'/3), 126.7 (C-7'/7), 127.3 (C-6'/6), 129.5 (C-10'/10), 130.3 (C-5'/5), 134.2 (C-8'/8), 137.4 (C-4'/4), 137.5 (C-4'a/4a), 142.7 (C-9'/9), 144.6 (C-8'a/8a), 154.1 (C-2'/2), 189.4 (C-11). HRMS (*pos*) *m/z* 427.0395 $[M + Na]^+$ (Calculated for $C_{23}H_{14}Cl_2N_2ONa$, 427.0381)

(1E,4E)-1,5-bis(8-fluoroquinolin-2-yl)penta-1,4-dien-3-one (**2g**), brown gummy solid, IR υ_{max} (cm^{-1}): 2921 (C-H), 1662 (C=O), 1501 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.13 (d, 2H, *J* = 16.9 Hz, H-10'/10), 7.42 (dd, 2H, *J* = 10.3, 7.4 Hz, H-7'/7), 7.50 (td, 2H, *J* = 7.8, 5.0 Hz, H-6'/6), 7.60 (d, 2H, *J* = 8.2 Hz, H-5'/5), 7.73 (d, 2H, *J* = 8.8 Hz, H-3'/3), 7.76 (d, 2H, *J* =

16.9 Hz, H-9′/9), 8.20 (d, 2H, *J* = 8.8 Hz, H-4′/4). ^{13}C NMR (100 MHz, $CDCl_3$): 114.5 (d, *J* = 19.1 Hz, C-7′/7), 120.6 (C-3′/3), 123.3 (d, *J* = 4.7 Hz, C-5′/5), 127.3 (d, *J* = 8.1 Hz, C-6′/6), 129.6 (C-4′a/4a), 132.5 (C-10′/10), 136.7 (d, *J* = 3.0 Hz, C-4′/4), 138.6 (d, *J* = 11.5 Hz, C-8′a/8a), 142.7 (C-9′/9), 153.9 (C-2′/2), 158.1 (d, *J* = 256.3 Hz, C-8′/8), 198.8 (C-11). HRMS (*pos*) *m/z* 395.0958 $[M + Na]^+$ (Calculated for $C_{23}H_{14}F_2N_2ONa$, 395.0972)

(1E,4E)-1,5-bis(8-bromoquinolin-2-yl)penta-1,4-dien-3-one (**2h**), brown solid, mp 162-165 °C, IR υ_{max} (cm^{-1}): 3386 (C-H), 1594 (C=O), 1494 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42 (t, 2H, *J* = 7.8 Hz, H-6′/6), 7.75 (d, 2H, *J* = 8.1 Hz, H-3′/3), 7.81 (d, 2H, *J* = 7.8 Hz, H-7′/7), 7.86 (d, 2H, *J* = 15.8 Hz, H-10′/10), 8.01 (d, 2H, *J* = 15.8 Hz, H-9′/9), 8.10 (d, 2H, *J* = 7.8 Hz, H-5′/5), 8.22 (d, 2H, *J* = 8.1 Hz, H-4′/4). ^{13}C NMR (100 MHz, $CDCl_3$): 122.0 (C-3′/3), 127.5 (C-7′/7), 127.8 (C-6′/6), 129.5 (C-4′a/4a/8′/8), 130.7 (C-10′/10), 133.9 (C-5′/5), 137.5 (C-4′/4), 142.5 (C-9′/9), 145.4 (C-8′a/8a), 154.3 (C-2′/2), 189.2 (C-11). HRMS (*pos*) *m/z* 514.9379 $[M + Na]^+$ (Calculated for $C_{23}H_{14}Br_2N_2ONa$, 514.9371)

(1E,4E)-1,5-bis(8-methylquinolin-2-yl)penta-1,4-dien-3-one (**2i**), green solid, mp 149-150 °C, IR υ_{max} (cm^{-1}): 3038 (C-H), 1592 (C=O), 1333(C=C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.87 (s, 6H, C$\underline{H}_3$), 7.44 (t, 2H, *J* = 7.4 Hz, H-6′/6), 7.58 (d, 2H, *J* = 7.4 Hz, H-7′/7), 7.65 (d, 2H, *J* = 7.4 Hz, H-5′/5), 7.67 (d, 2H, *J* = 8.6 Hz, H-3′/3), 7.76 (d, 2H, *J* = 15.8 Hz, H-10′/10), 7.96 (d, 2H, *J* = 15.8 Hz, H-9′/9), 8.16 (d, 2H, *J* = 8.6 Hz, H-4′/4). ^{13}C NMR (100 MHz, $CDCl_3$): 17.9 ($\underline{C}H_3$), 120.9 (C-3′/3), 125.5 (C-5′/5), 127.3 (C-6′/6), 128.2 (8′/8), 129.6 (C-10′/10), 130.1 (C-7′/7), 137.0 (C-4′/4), 138.1 (C-4′a/4a), 143.4 (C-9′/9), 147.4 (C-8′a/8a), 152.2 (C-2′/2), 189.9 (C-11). HRMS (*pos*) *m/z* 387.1472 $[M + Na]^+$ (Calculated for $C_{25}H_{20}N_2ONa$, 387.1473)

(1E,4E)-1,5-bis(8-methoxyquinolin-2-yl)penta-1,4-dien-3-one (**2j**), brown solid, mp 102-103 °C, IR υ_{max} (cm^{-1}): 3361 (C-H), 1633 (C=O), 1465 (C=C). ^{1}H NMR (400 MHz, DMSO): δ3.99 (s, 6H, OC<u>H</u>$_3$), 7.18 (d, 2H, *J* = 16.2 Hz, H-10'/10), 7.22 (d, 2H, *J* = 7.3 Hz, H-5'/5), 7.57-7.48 (m, 4H, H-7'/7/6'/6), 7.74 (d, 2H, *J* = 16.2 Hz, H-9'/9), 7.97 (d, 2H, *J* = 8.4 Hz, H-3'/3), 8.36 (d, 2H, *J* = 8.4 Hz, H-4'/4). ^{13}C NMR (100 MHz, DMSO): 55.7 (O<u>C</u>H$_3$), 108.9 (C-5'/5), 119.2 (C-7'/7), 121.0 (C-3'/3), 127.9 (C-6'/6), 128.8 (C-4'a/4a), 131.4 (C-10'/10), 136.8 (C-4'/4), 139.5 (C-8'a/8a), 142.8 (C-9'/9), 151.9 (C-2'/2), 155.3 (8'/8), 198.5 (C-11). HRMS (*pos*) *m/z* 397.1564 $[M + Na]^+$ (Calculated for $C_{25}H_{20}N_2O_3Na$, 397.1552)

Antimicrobial assay

The antimicrobial activities of the synthesized compounds **2a-j**, were determined against two Gram +ve bacteria (*Staphylococcus aureus* ATCC 25923 and *Staphylococcus aureus* Rosenbach ATCC BAA-1683 (MRSA)) and four Gram -ve bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 31488, *Escherichia coli* ATCC 25922 and *Salmonella typhimurium* ATCC 14026).

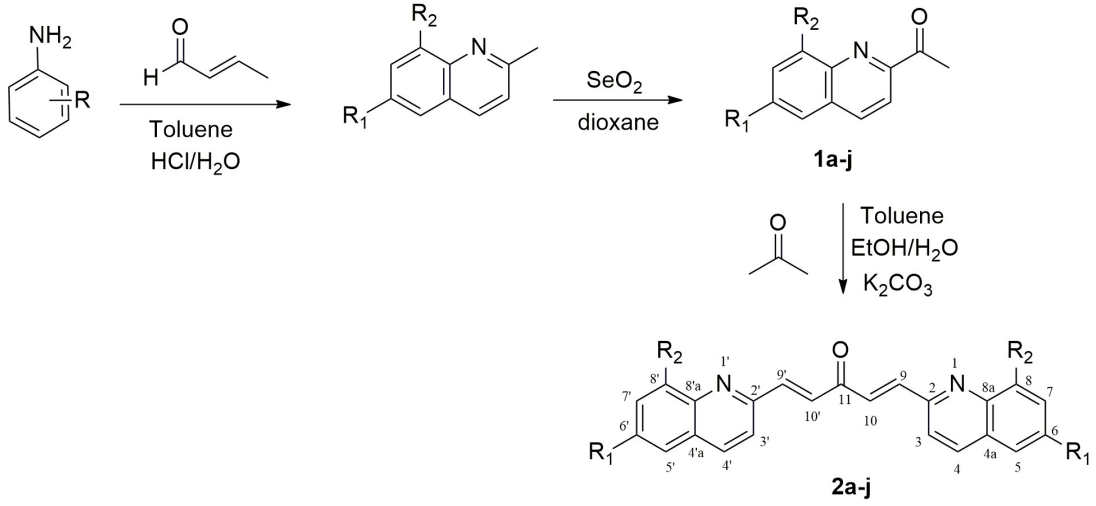
Bacterial strains were grown on Mueller-Hinton agar (21 g L^{-1}) at 35-37 °C in a CO_2 incubator for 24 hours. Organisms were suspended in saline and the turbidity adjusted to a 0.5 McFarland standard. Mueller-Hinton agar plates were prepared by dissolving 38 g of agar in 1 L of water in sterile petri dishes and allowed to set at room temperature. The Mueller-Hinton agar plate was inoculated with the required strain of bacteria by streaking a swab dipped into the adjusted suspension evenly over the entire sterile agar surface. The samples were dissolved in DMSO and 5 µL of a 1 mg mL^{-1} sample placed onto Mueller-Hinton plates, left to incubate for 16-18 hours at 37°C, and the zones of inhibition measured in mm using a transparent ruler.

Minimum Bactericidal Concentration (MBC) Assay

All compounds that exhibited zones of inhibition during the disc diffusion assay were dissolved in DMSO (1 mg mL^{-1}) and serially diluted. The Mueller-Hinton agar plate was inoculated with the required strain of bacteria and 5 µL of each concentration placed onto the plate and left to incubate at 37 ºC for 18 h. Experiments were performed in triplicate. The MBC was the lowest concentration where a zone of inhibition was observed. The DMSO control showed no growth inhibition of the bacterial strains in all cases.

4.3 Results and Discussion

A series of curcumin-like quinoline ketodienes were synthesised in three steps by forming 2-methylquinolines from substituted anilines and crotonaldehyde, oxidising them to quinoline-2-carbaldehydes (**1a-j**) and condensing them with acetone to yield the target compounds (**2a-j**) in yields of 50-65% (**Scheme 4-1**). A mixture of toluene, ethanol and water was used for the reaction, since products do not form when a mixture of ethanol and water (the normal solvents used in the Claisen condensation) is used without toluene. It is important to keep the temperature of the reaction at approximately 70 °C, as higher or lower temperatures result in lower yields of the ketodiene.



Scheme 4-1: Synthetic scheme for the synthesis of quinoline ketodienes (**2a-j**)

Table 4-1: Substitution patterns and yields of compounds **2a-j**

No.	R_1	R_2	Yield (%)
2a	Cl	H	55
2b	F	H	50
2c	Br	H	60
2d	CH_3	H	65
2e	OCH_3	H	65
2f	H	Cl	63
2g	H	F	56
2h	H	Br	52
2i	H	CH_3	64
2j	H	OCH_3	62

Formation of the quinoline ketodienes (**2a-j**) were determined from their ^{1}H and ^{13}C NMR spectra and assigned with the aid of 2D NMR. All the synthesised compounds were symmetrical about the carbonyl group. For example, the ^{1}H NMR spectrum of the 6'-chloro derivative (**2a**) (**Figure 4-1**) showed the presence of a pair of doublets with a *J* value of 16.3 Hz, typical of *trans*-olefinic protons and were attributed to H-9'/9 at δ 7.72 and H-10'/10 at δ 7.23. H-9'/9 was distinguished from H-10'/10 by an HMBC correlation to C-3'/3. The shielding of H-10'/10 is due to electron delocalisation of the α,β-unsaturated carbonyl group. The quinoline ring shows five aromatic resonances consisting of two coupled doublets at δ_H 8.01 and δ_H 8.42 (J = 8.6 Hz) assigned to H-3'/3 and H-4'/4. H-3'/3 is more dieshielded than H-4'/4 due to electron delocalisation of electrons from the quinoline ring onto the carbonyl group. The three other resonances on the quinoline ring, H-5'/5, H-7'/7 and H-8'/8 appeared as two double doublets and a doublet at δ_H 8.14 (J = 2.3 Hz), δ_H 7.80 (J = 9.0, 2.4 Hz) and δ_H 8.05 (J = 9.0 Hz) respectively.

The ^{13}C NMR spectrum showed the presence of the carbonyl carbon at δ 198.4 and the two C-N carbon resonances at δ 154.0 (C-2'/2) and 146.0 (C-8'a/8a). The C-2'/2 resonance showed HMBC correlations to H-3'/3, H-4'/4, H-9'/9 and H-10'/10, and the C-8'a/8a resonance showed correlations to H-4'/4, H-5'/5 and H-7'/7. An HMBC correlation between H-3'/3 and C-4'a/4a was used to distinguish between the C-4'a/4a and C-6'/6. This could be seen more clearly in the fluoro (**2b**) and bromo (**2c**) derivatives than the chloro derivative (**2a**).

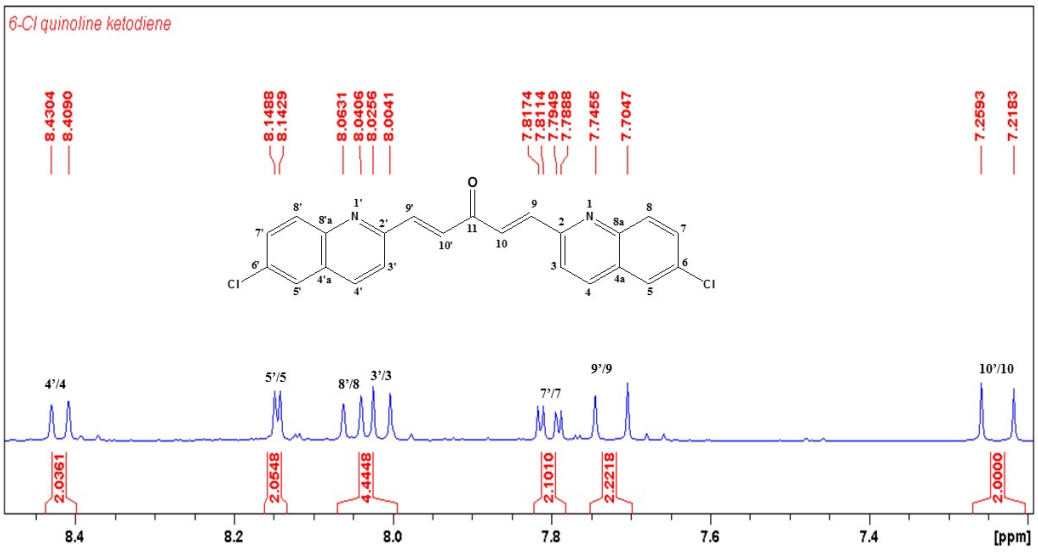


Figure 4-1: ^{1}H NMR of (*1E,4E*)-1,5-bis(6-chloroquinolin-2-yl)penta-1,4-dien-3-one (**2a**)

All quinoline ketodiene derivatives were tested against two Gram +ve bacterial strains (*S. aureus* and *S. aureus Rosenbach* (MRSA)), and four Gram –ve strains, *S. typhimurium, P. aeruginosa*, *K. pneumonia* and *E. coli*. Preliminary screening using the disc diffusion method was used to select compounds to determine their minimum bactericidal concentration (MBC) values. All compounds that showed activity against one or more strains of bacteria were then selected for the MBC assay. The results of this assay is presented in **Table 4-2Table 4-2**.

Table 4-2: Minimum Bactericidal Concentration (MBC in μg mL^{-1}) of compounds **2a-j**

Compound	Substitution	S. *aureus*	MRSA	S. *typhimurium*	*K. pneumonia*	*E. coli*	P. *aeruginosa*
2a	6-Cl	31.3	1.95	0.98	0.98	15.6	1.95
2b	6-F	0.24	1.95	-	-	0.98	0.24
2c	6-Br	250.0	15.6	0.98	0.98	0.98	15.6
2d	6-Me	15.6	1.95	0.98	0.98	0.98	0.98
2e	6-OMe	15.6	-	-	0.98	1.95	125.0
2f	8-Cl	-	1.95	-	0.98	0.98	3.9
2g	8-F	62.5	62.5	0.98	-	-	0.49
2h	8-Br	0.24	0.24	-	-	0.98	-
2i	8-Me	1.95	1.95	-	-	0.98	1.95
2j	8-OMe	500.0	-	0.98	-	0.98	0.24
Ciprofloxacin		94.3	188.6	2.95	2.95	2.95	188.6
Levofloxacin		21.6	86.5	21.6	21.6	0.34	345.0

"-" denotes activity > 500 μg mL^{-1}

All compounds showed excellent activity in at least three of the six bacterial strains tested. In general, substitution at 6' was better than at the 8' position. Compounds **2a** (6-chloro derivative), **2c** (6-bromo derivative), and **2d** (6-methyl derivative), showed broad spectrum antibacterial activity mostly at 0.98 or 1.95 μg mL^{-1} against all strains, which was either better than or comparable to the standards ciprofloxacin and levofloxacin. Slightly lower activity was seen by **2a** against *E. coli* and *S. aureus* at 15.6 and 31.3 μg mL^{-1} respectively and **2c** against *S. aureus*, MRSA and *P. aeruginosa* at 250.0, 15.6 and 15.6 μg mL^{-1} respectively. In a few cases 2-3 fold better activity than the standards were seen for **2b** against *S. aureus* and *P. aeruginosa*, **2h** against *S. aureus* and MRSA, and **2j** against *P. aeruginosa*, where the compounds had MBC values of 0.24 μg mL^{-1}.

4.4 Conclusion

Ten novel quinoline ketodiene derivatives were synthesised successfully by the aldol condensation of acetone and quinoline 2-carbaldehydes in yields of 50-65%. These derivatives possessed varied groups at the 6'/6 and 8'/8 positions consisting of electron withdrawing halogens chlorine, fluorine, bromine and electron donating methyl and methoxy

groups. This demonstrates that these curcumin-like quinolines can be synthesised with different types of quinoline molecules. The results indicate that all synthesised compounds were active against the bacterial strains tested with **2a**, **2c** and **2d** having the best activity against several of the strains with either better or comparable activity to levofloxacin or ciprofloxacin, the standards used in the assay.

4.5 References

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Chapter 5 Conclusion

Three series of curcumin and curcumin-like derivatives were synthesised, characterized and evaluated for its antibacterial activity against two Gram +ve bacteria (*Staphylococcus aureus* and methicillin resistant *S. aureus* (MRSA)) and four Gram –ve bacteria (*Salmonella typhimurium, Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli*).

Curcumin intermediates (**A4a-x**) were synthesised by an Aldol cross condensation reaction from substituted aldehydes and an acetoacetyl boron complex. These compounds were derivatised by a cyclocondensation reaction with hydrazine hydrate forming curcumin pyrazolines (**A5a-c, f-k, m** and **o)** in yields of 65-85%. In a similar aldol reaction, curcumin-like ketodienes (**B1a-n**) were synthesised with acetone and benzaldehydes by the Claisen-Schmidt condensation. These were then used as precursors to synthesise ketopyrazoles with acetic acid and hydrazine hydrate, and spiro barbiturates using barbituric acid. The yields of the ketopyrazoles were 89-98% and the spiro barbiturates were formed in yields of 83-96%. The spiro barbiturates were subsequently derivatised to oximes in yields of 91-99%. In the last set of compounds synthesised, 2-methylquinolines were synthesised from substituted anilines and crotonaldehyde followed by oxidation to form quinoline 2-carbaldehydes. They were then reacted with acetone to form quinoline ketodienes in yields of 50-65%.

A total of 52 new compounds were synthesised from the different sets of compounds. All these compounds were characterized using ^{1}H, ^{13}C and 2D NMR spectroscopy and high resolution mass spectrometry. The curcumin-like ketopyrazoles, spiro barbiturates and spiro-oximes were all chiral with a configuration of 5*S* for the ketopyrazole and 7*R*, 11*R* for the spirobarbiturates and oximes. These were determined by single crystal X-ray diffraction. The syntheses were all easy to carry out, with a simple, attractive design. These compounds could

easily be produced on a large scale from easily available starting materials, which makes their synthetic design highly desirable. Furthermore, two sets of compounds were produced in yields of approximately 60% and the other two in yields of approximately 90% indicating the success of these reactions.

Several curcumins and curcumin pyrazolines showed good activity against *S. aureus* and MRSA at 31.3 μg mL^{-1}. Two curcumin pyrazolines in particular, the 2-chloro, and 2,4-difluoro derivatives showed excellent activity against *K. pneumonia* at 7.8 μg mL^{-1} and 0.9 μg mL^{-1}, better than the standards, ciprofloxacin and levofloxacin. One of the derivatives, the 4-trifluoromethyl derivative was also active against *E. coli* at 31.3 μg mL^{-1}. The ketopyrazoles showed similar activity against *S. aureus* and MRSA with all chloro derivatives and a bromo derivative showing good activity against at least one of *S. aureus* or MRSA at 31.3 μg mL^{-1} or less with the exception of the 4-Cl derivative being active at 62.5 μg mL^{-1}. The most active of the ketopyrazoles was the 2-chloro derivative being active against *S. aureus* and *P. aeruginosa* with MBC values of 15.6 and 31.3 μg mL^{-1}.

The 4-OCH_3, 4-Br and 4-NO_2 spirobarbiturate derivatives were found to be very active against *E. coli* with MBC values of 0.98 μg mL^{-1}. The 4-Br derivative also showed excellent broad spectrum activity, being active against *S. typhimurium*, *K. pneumonia*, *E. coli* and *P. aeruginosa* at 0.98 μg mL^{-1}. The quinoline ketodienes showed the best activity amongst all series of compounds synthesised since three of the compounds (6-Cl, 6-Br and 6-CH_3 derivatives) showed broad spectrum activity across all four Gram –ve strains with MBC values as low as 0.98 μg mL^{-1}. A further three compounds (6-OCH_3, 8-Cl and 8-OCH_3 derivatives) showed activity in three of the four Gram –ve strains, also with excellent MBC

values of 0.98 μg mL^{-1} and almost all of the compounds were seen to be active against *E. coli* and *P. aeruginosa* with MBC values ranging from 0.98 – 15.6 μg mL^{-1}.

Thus, from this study, a lead scaffold for antibacterial activity was identified as the quinoline-ketodienes. Further work on derivatising this scaffold, possibly to pyrazolines to see whether this improves, retains or loses activity can be carried out and to see whether the ketodiene or quinoline moiety is the pharmacophore responsible for the activity.

University of KwaZulu-Natal

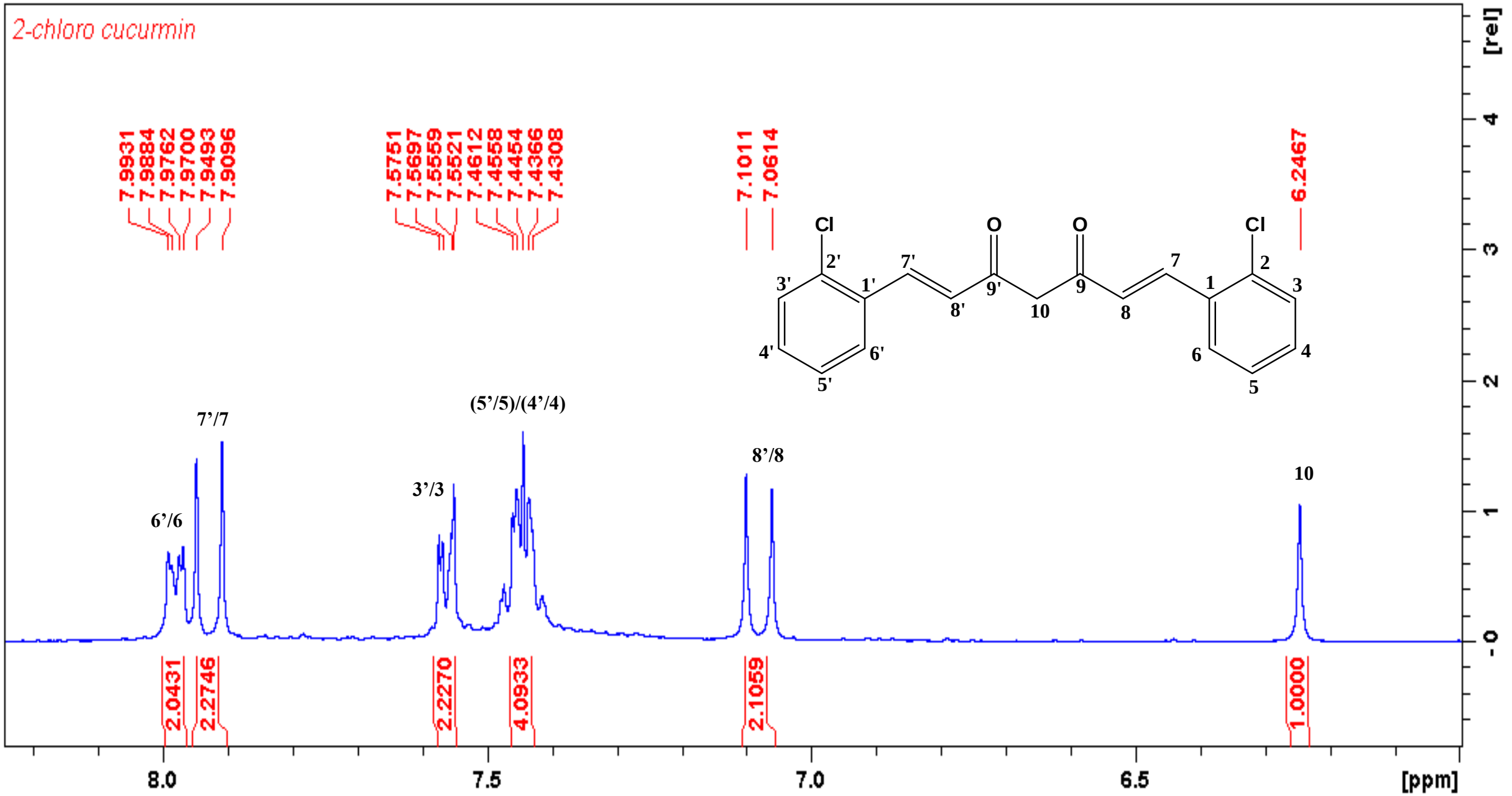
Synthesis, Characterization and Antibacterial Activity of Curcumin and Curcumin-like derivatives

Appendix A: Characterization data

2018

Christina Kannigadu

Table of Contents



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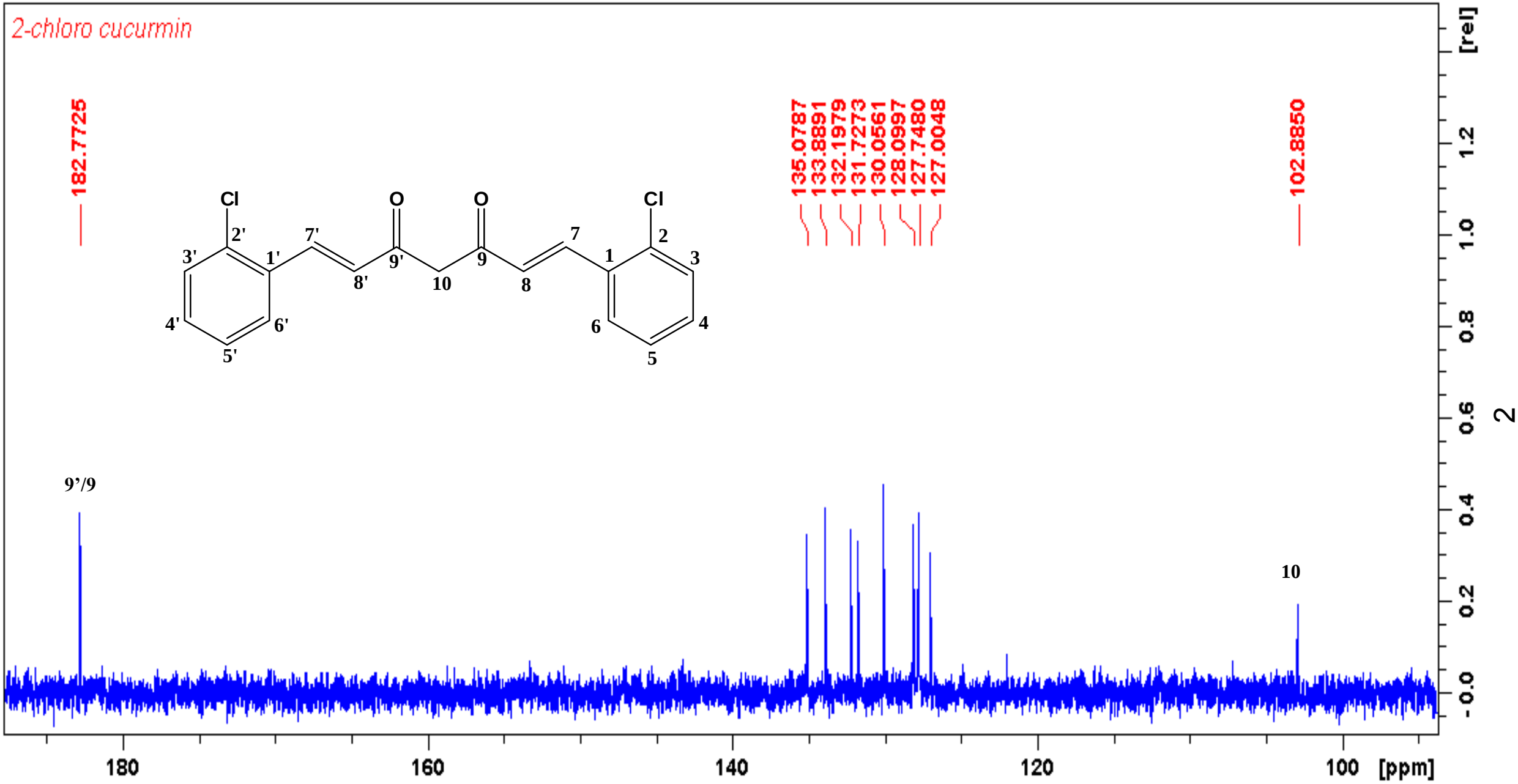

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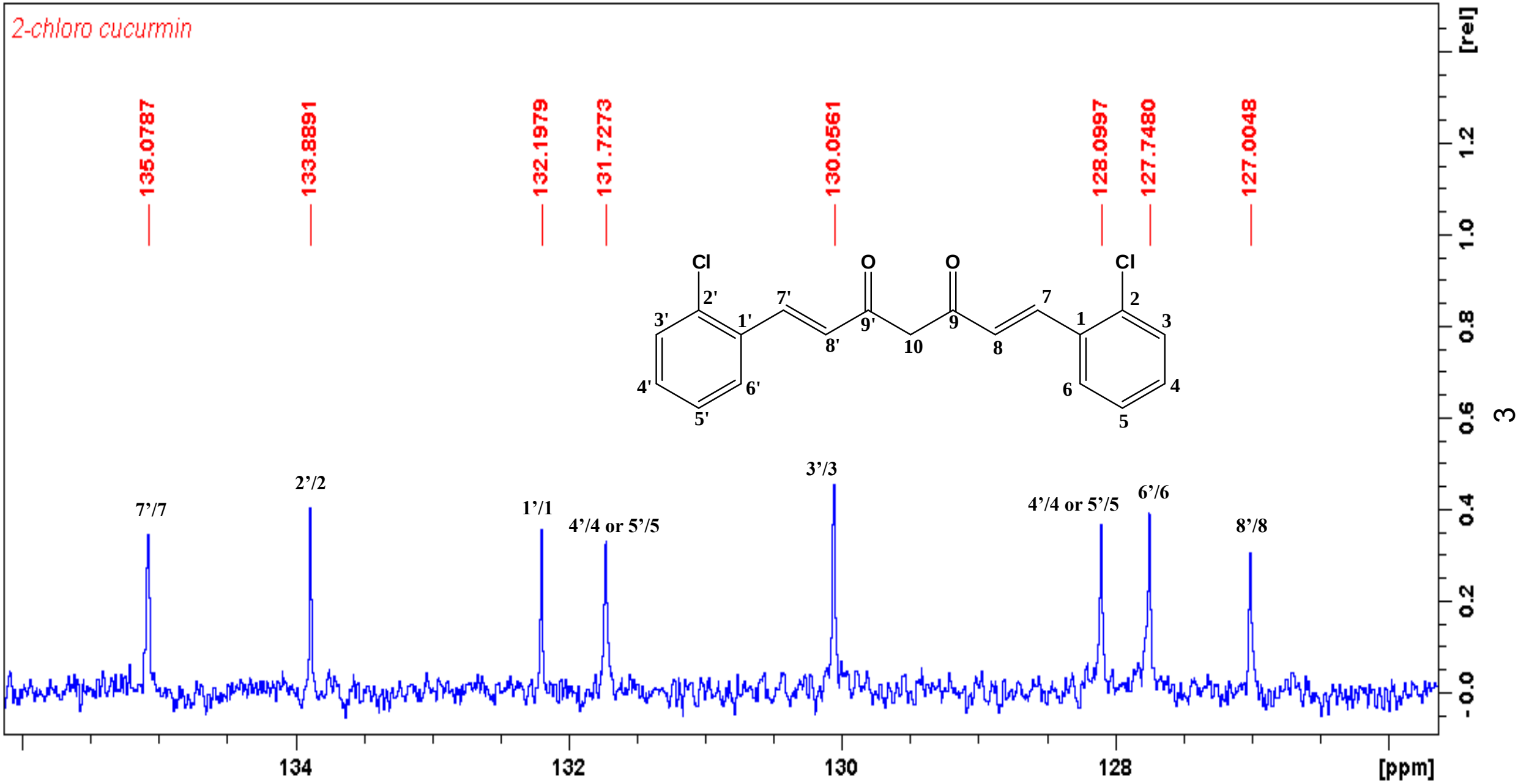

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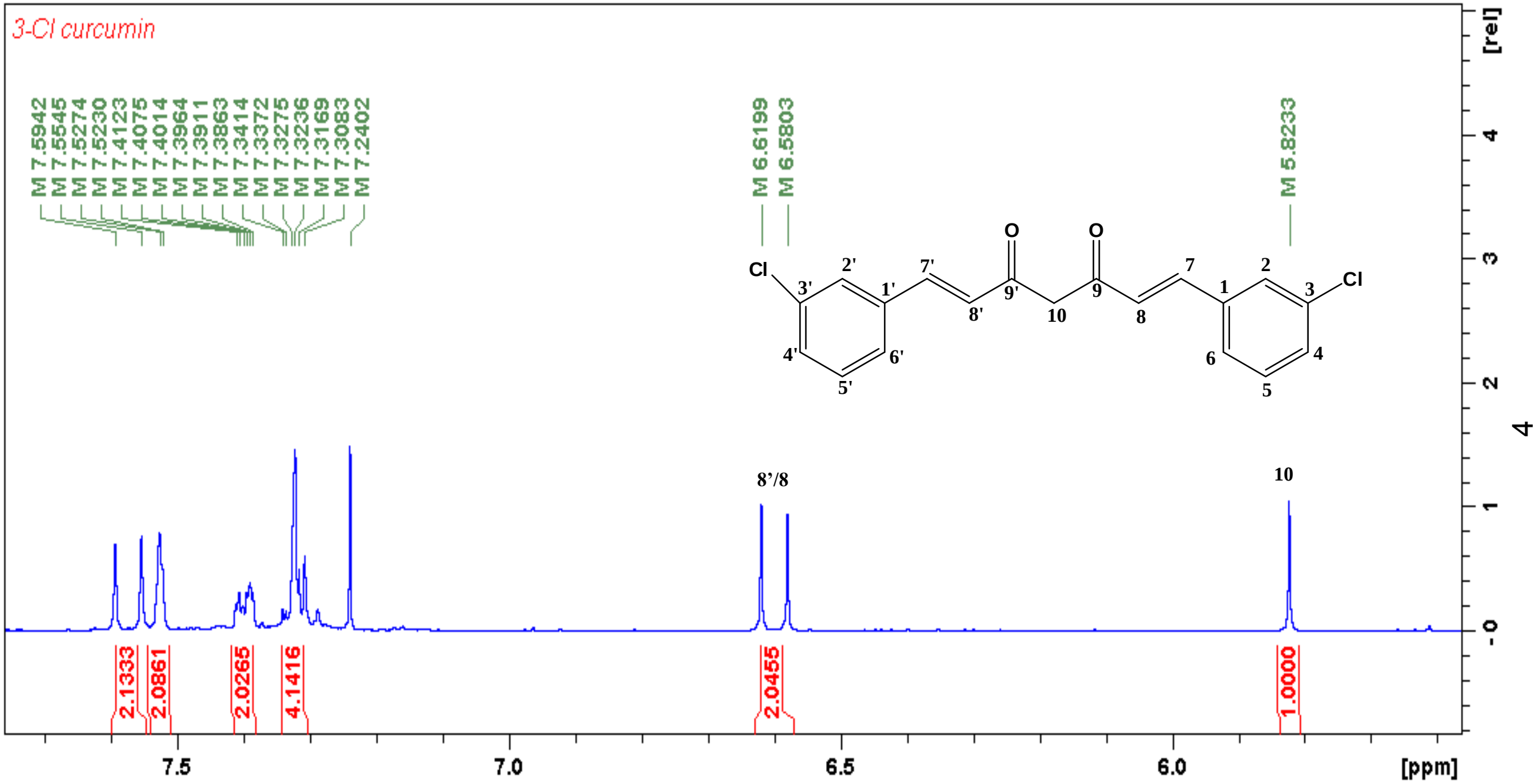

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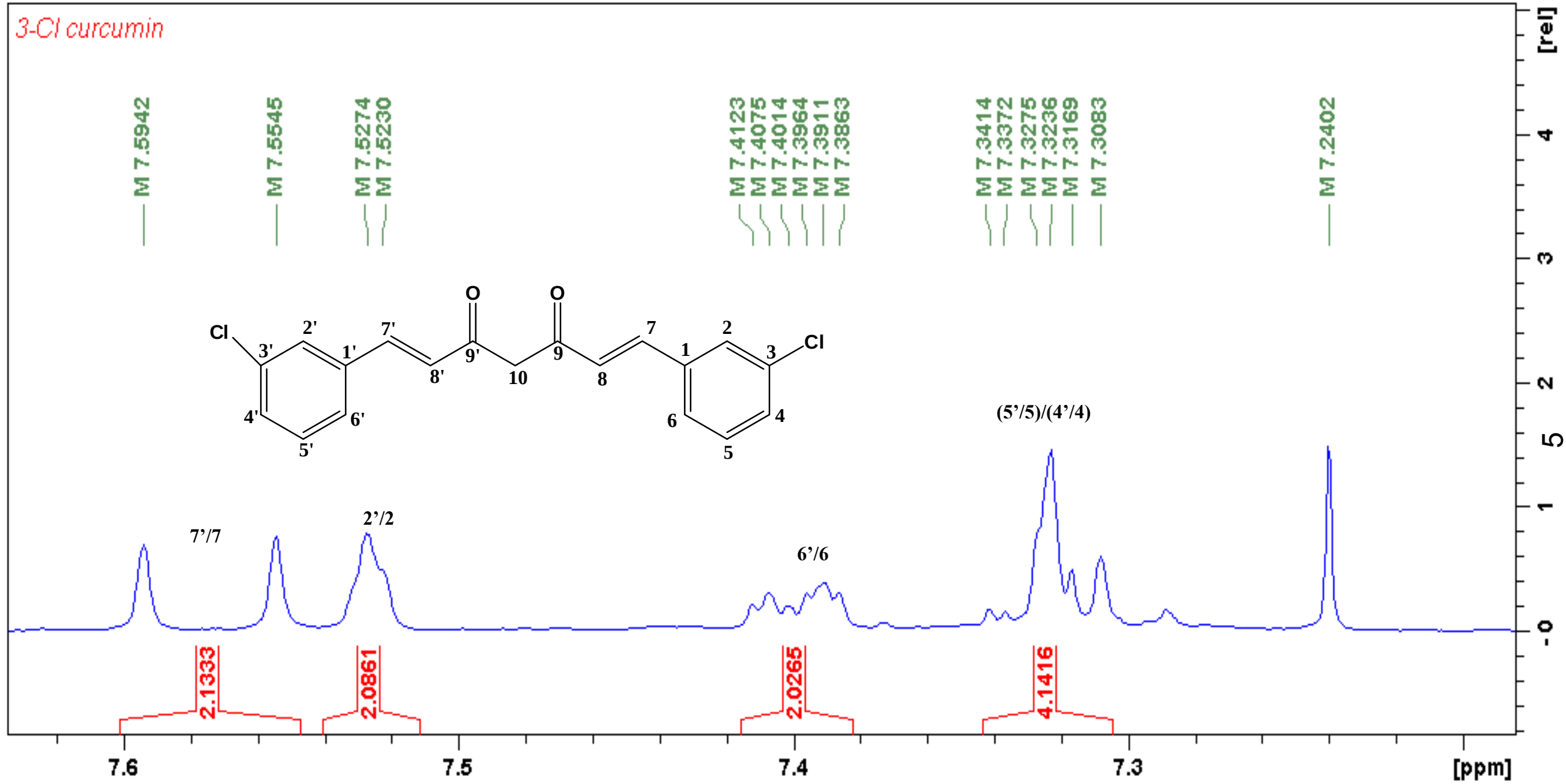

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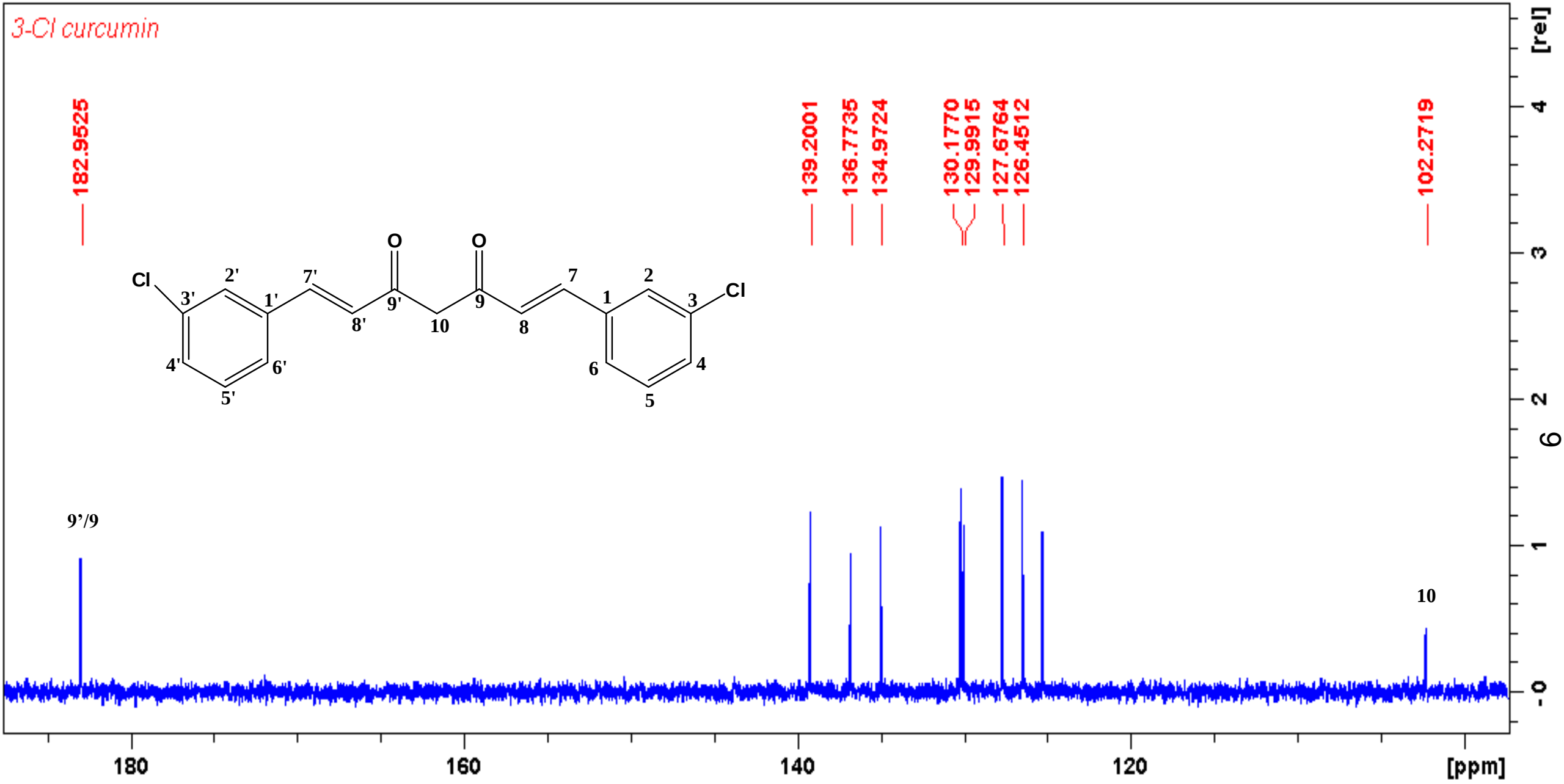

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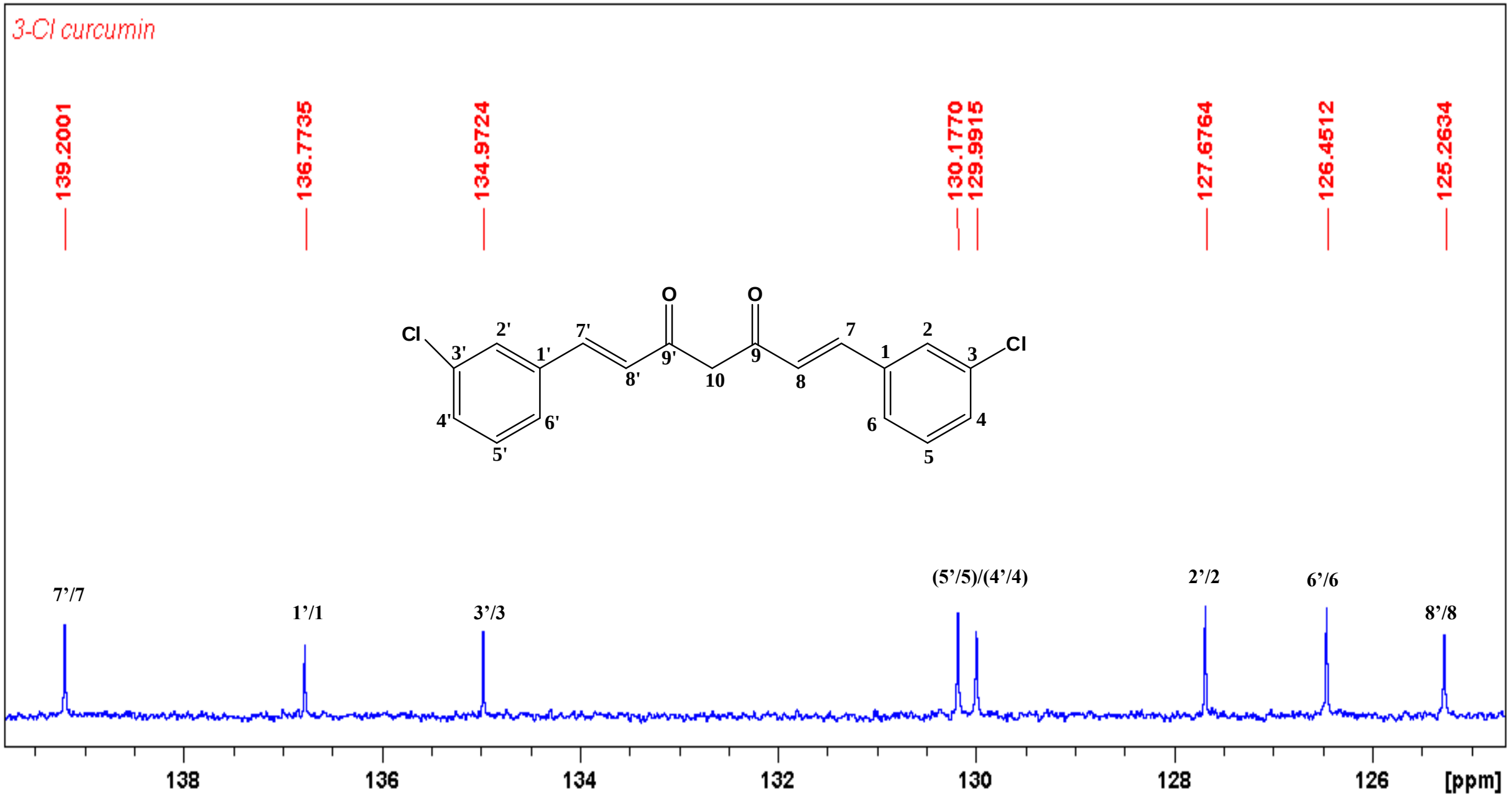

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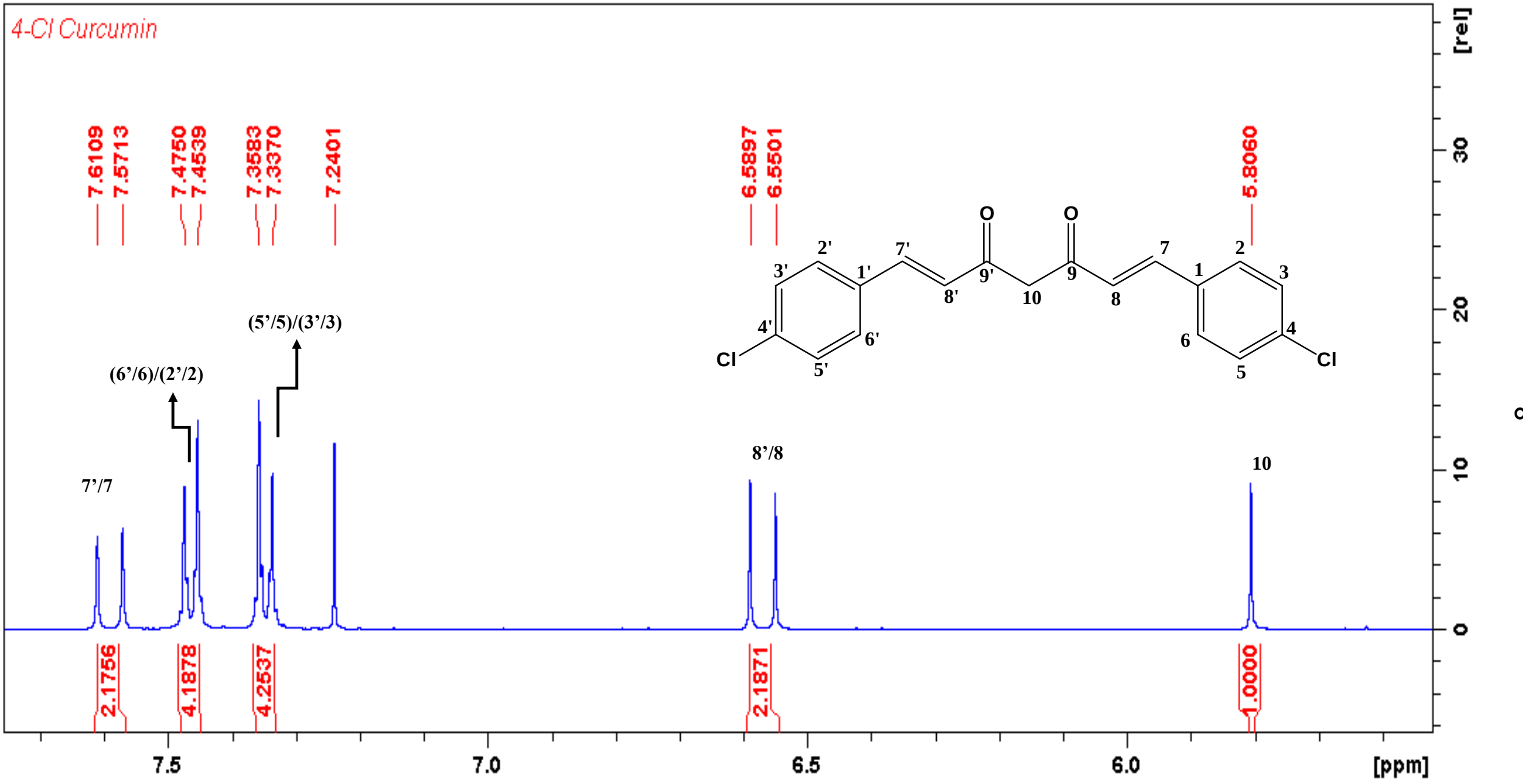

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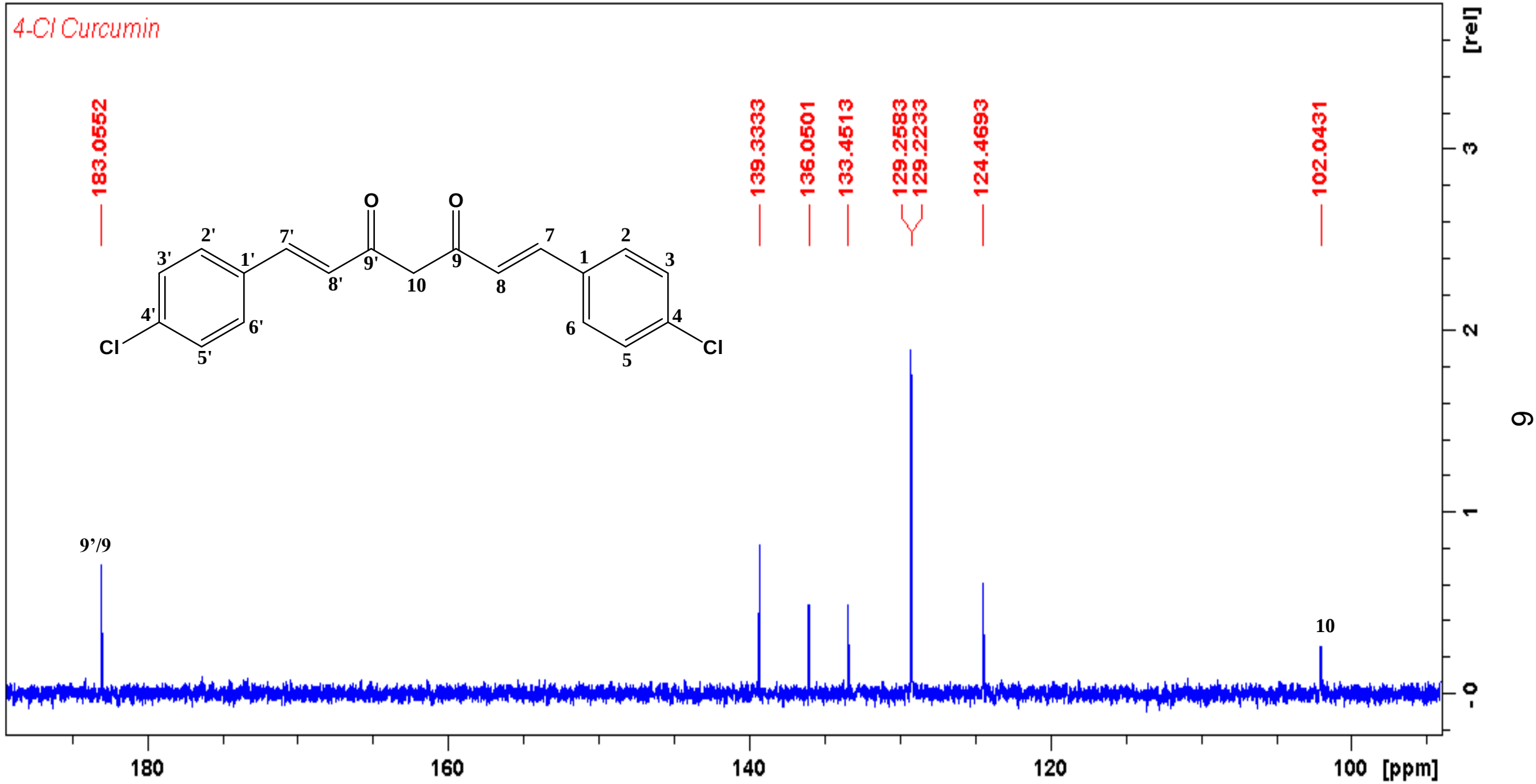

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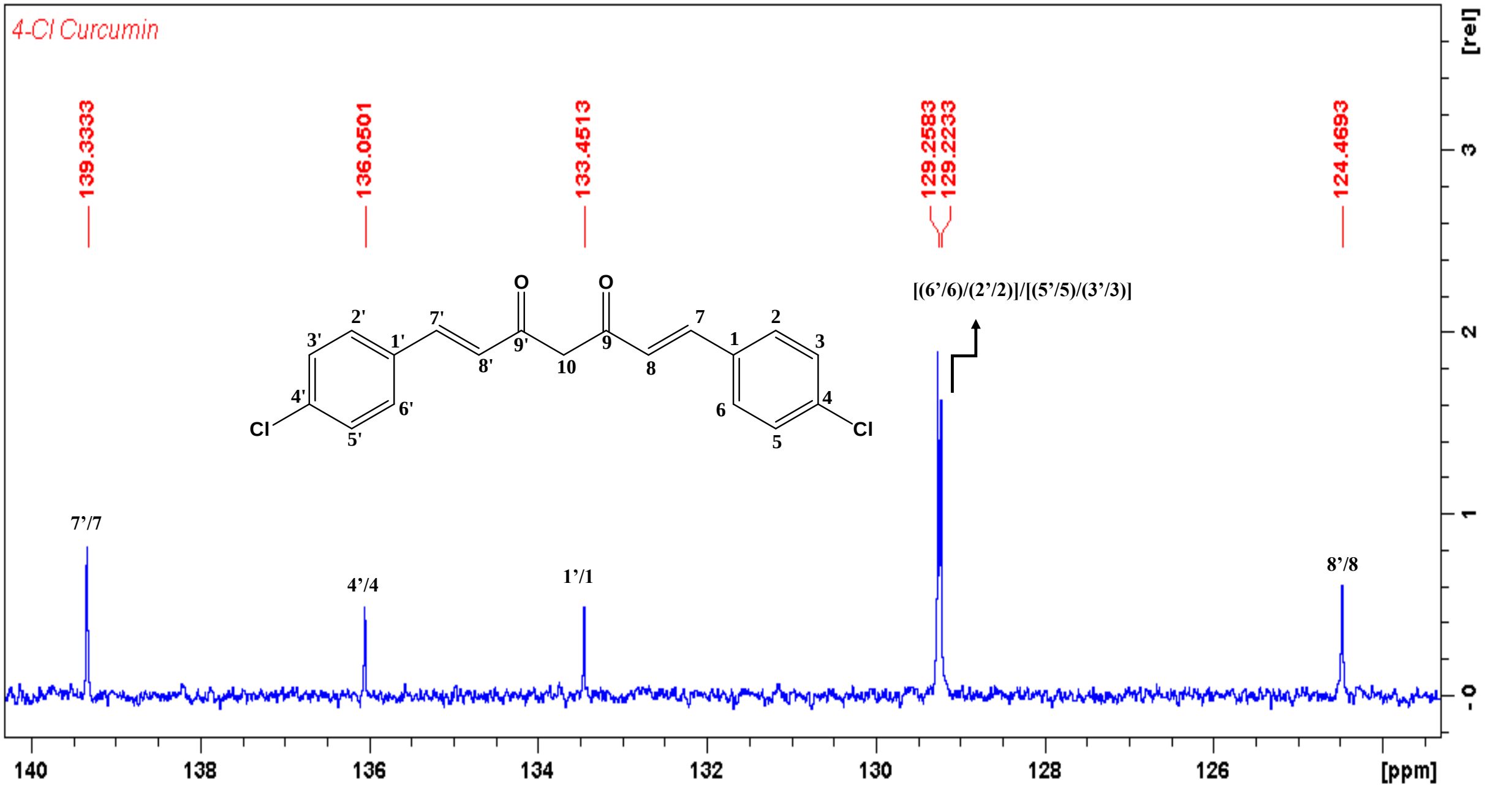

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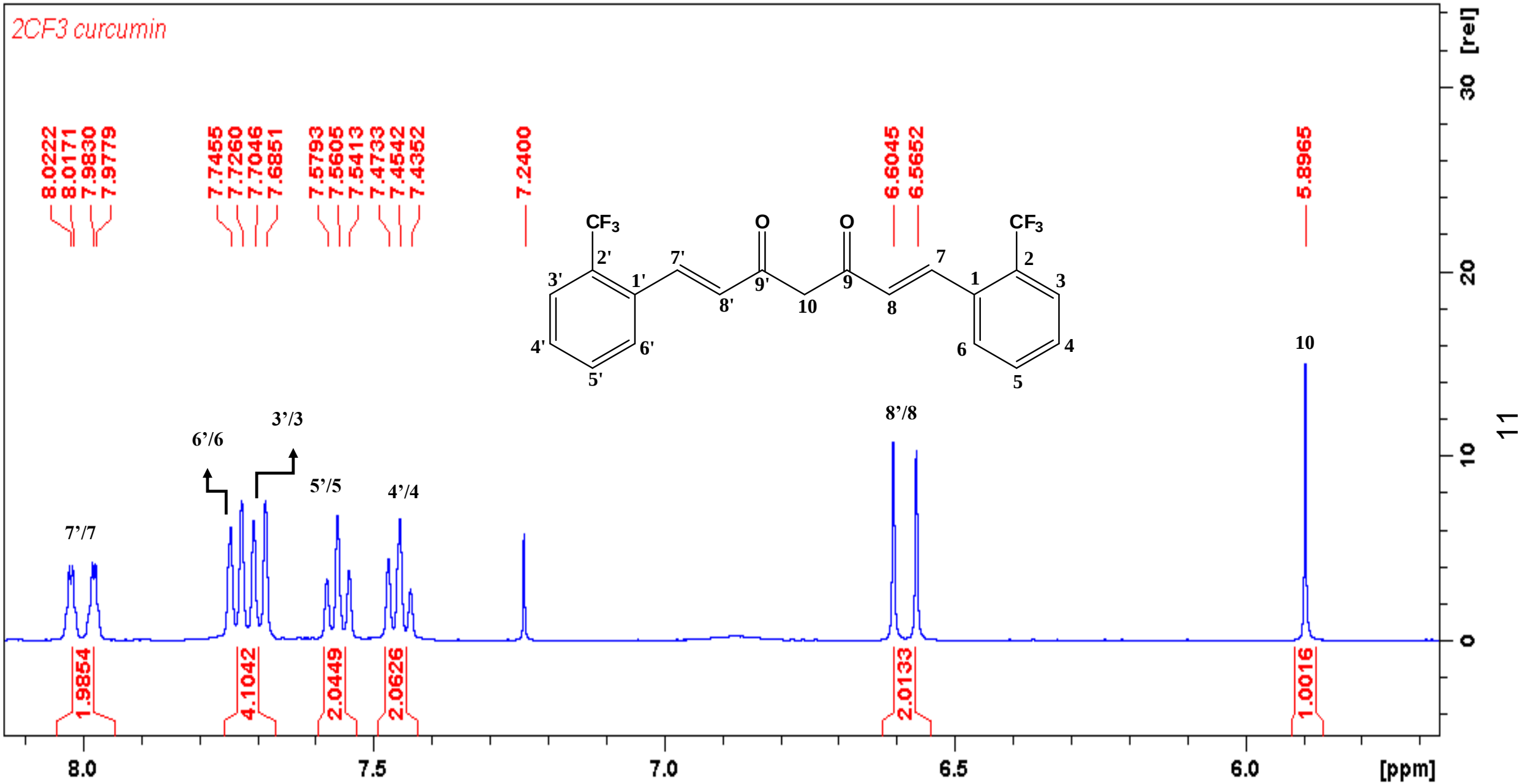

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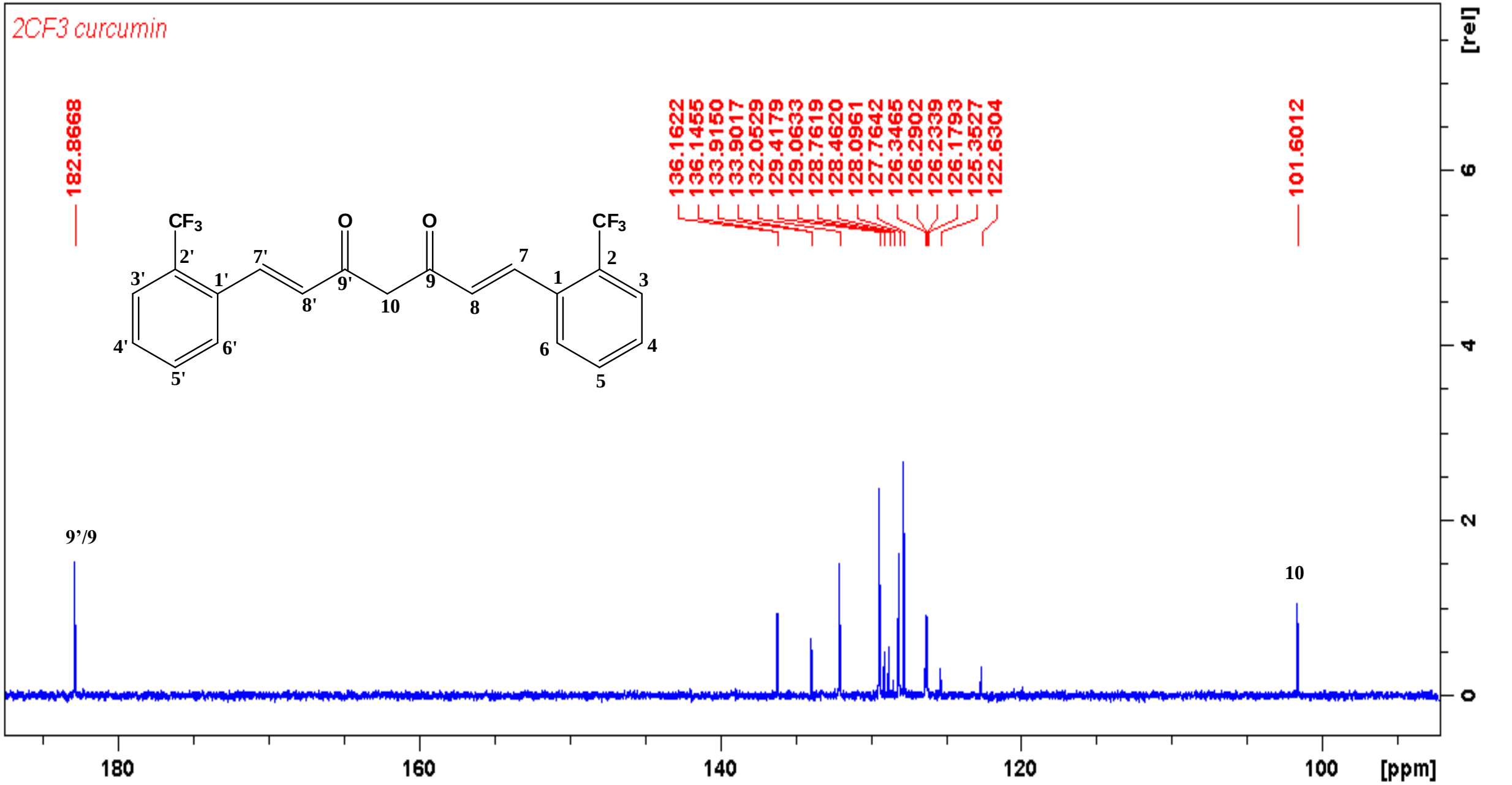

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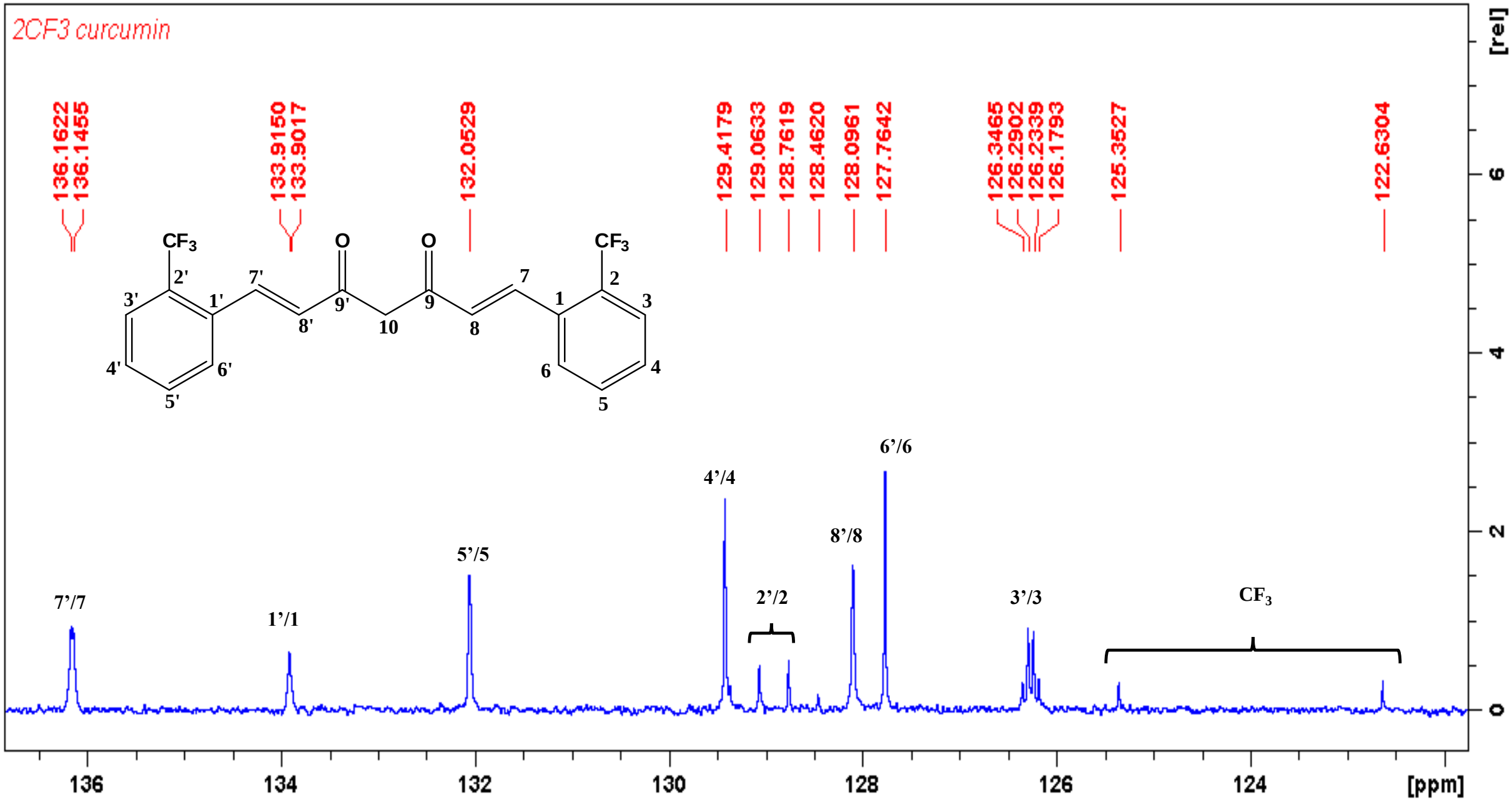

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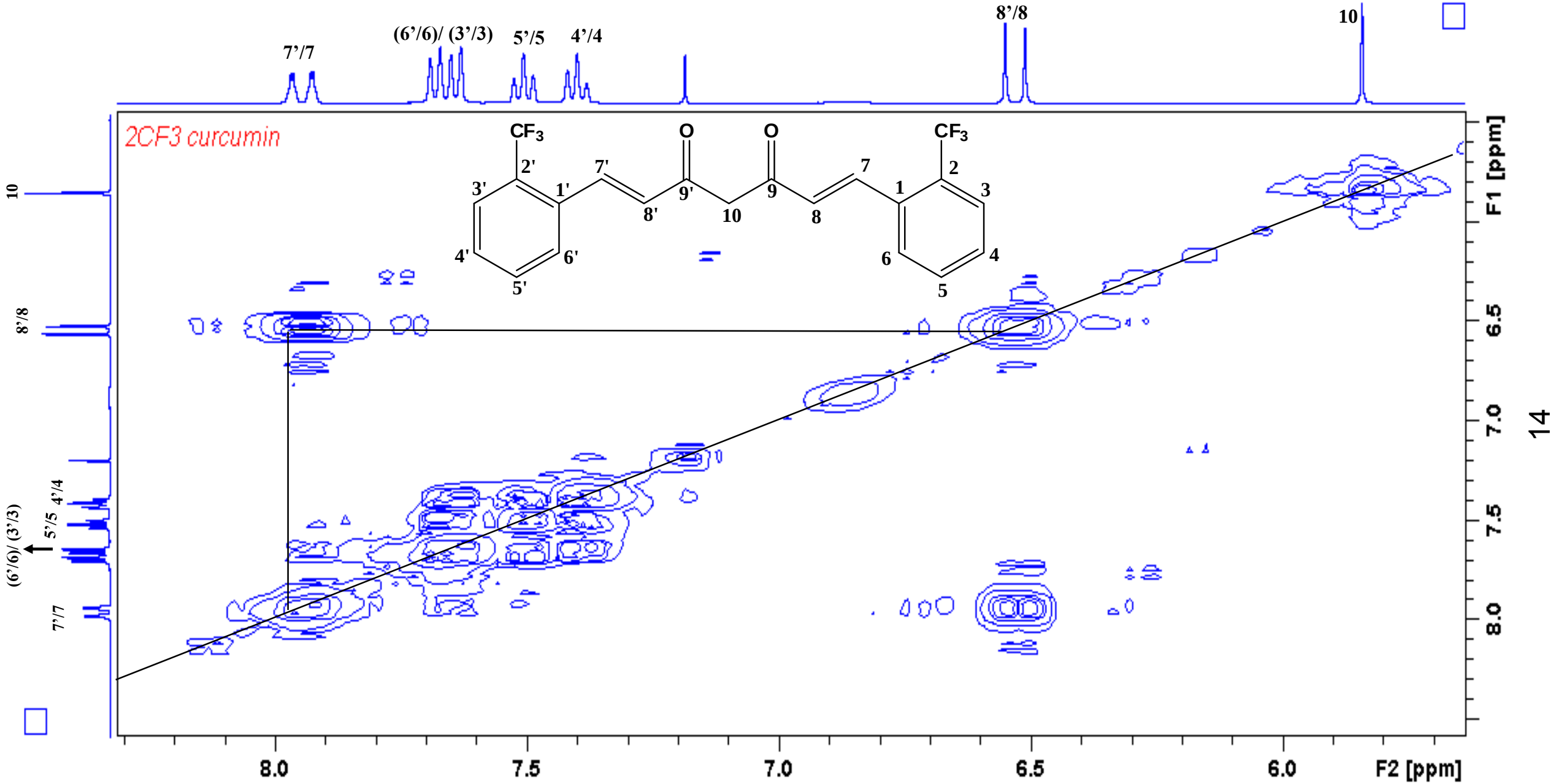

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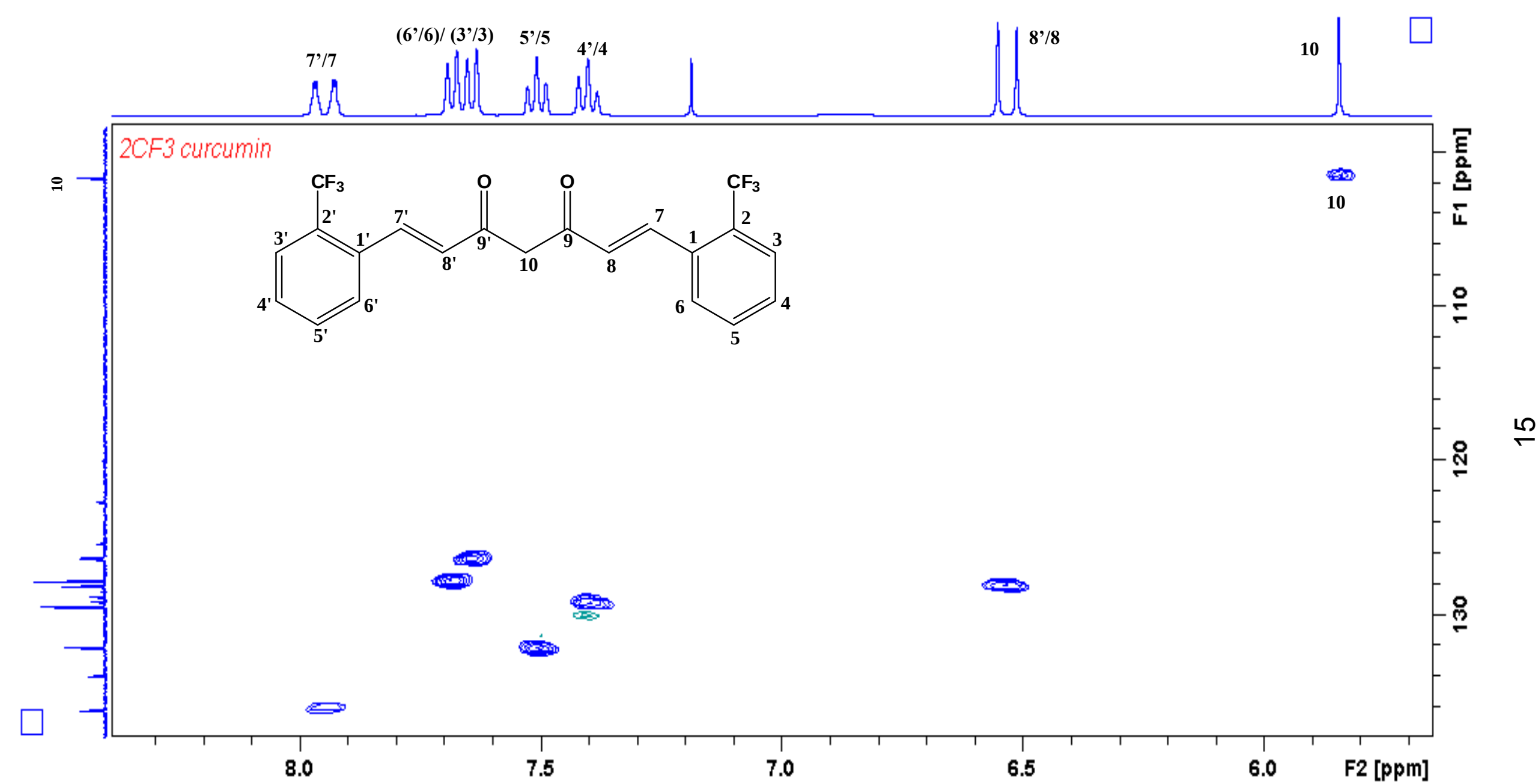

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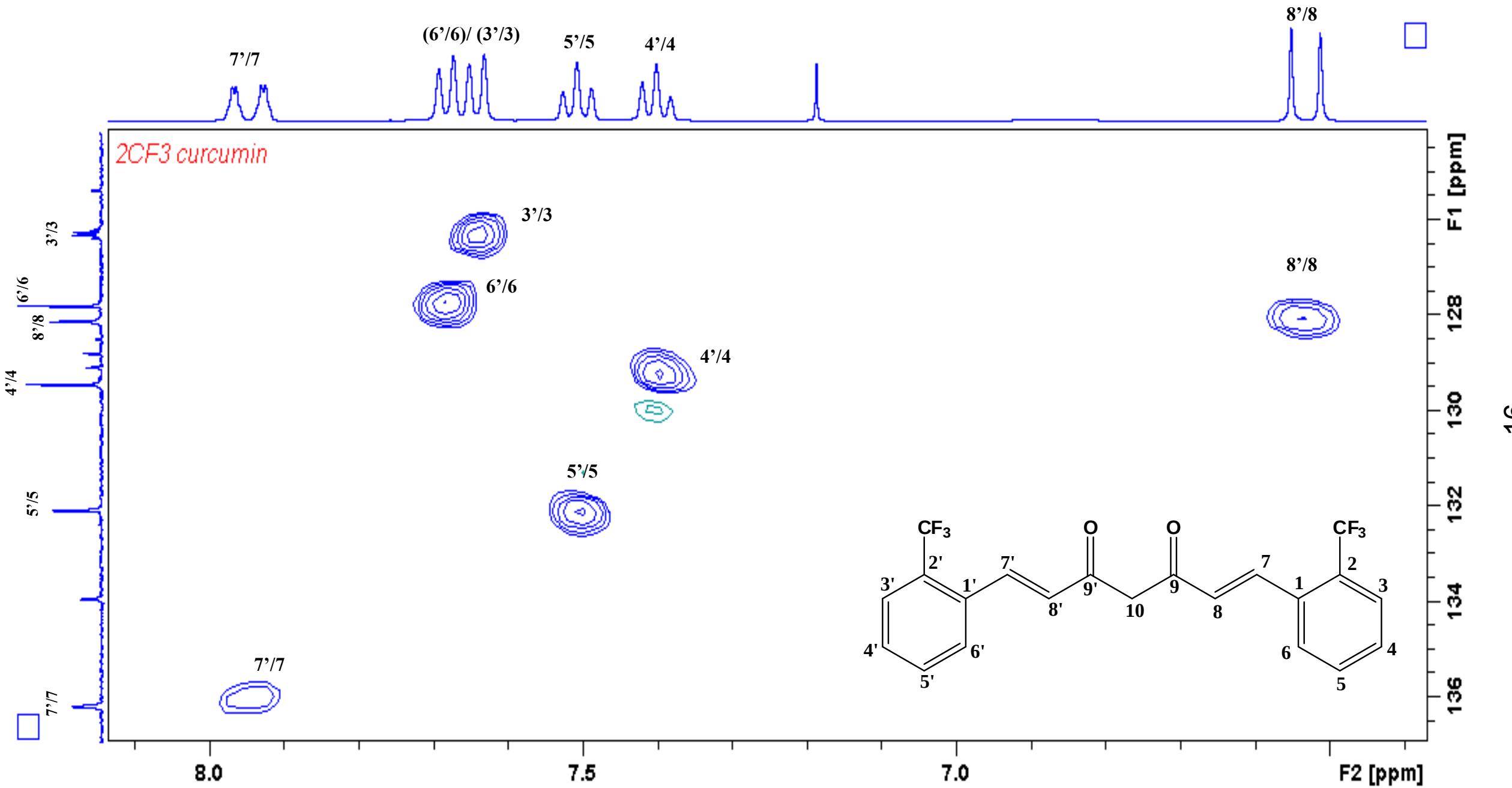

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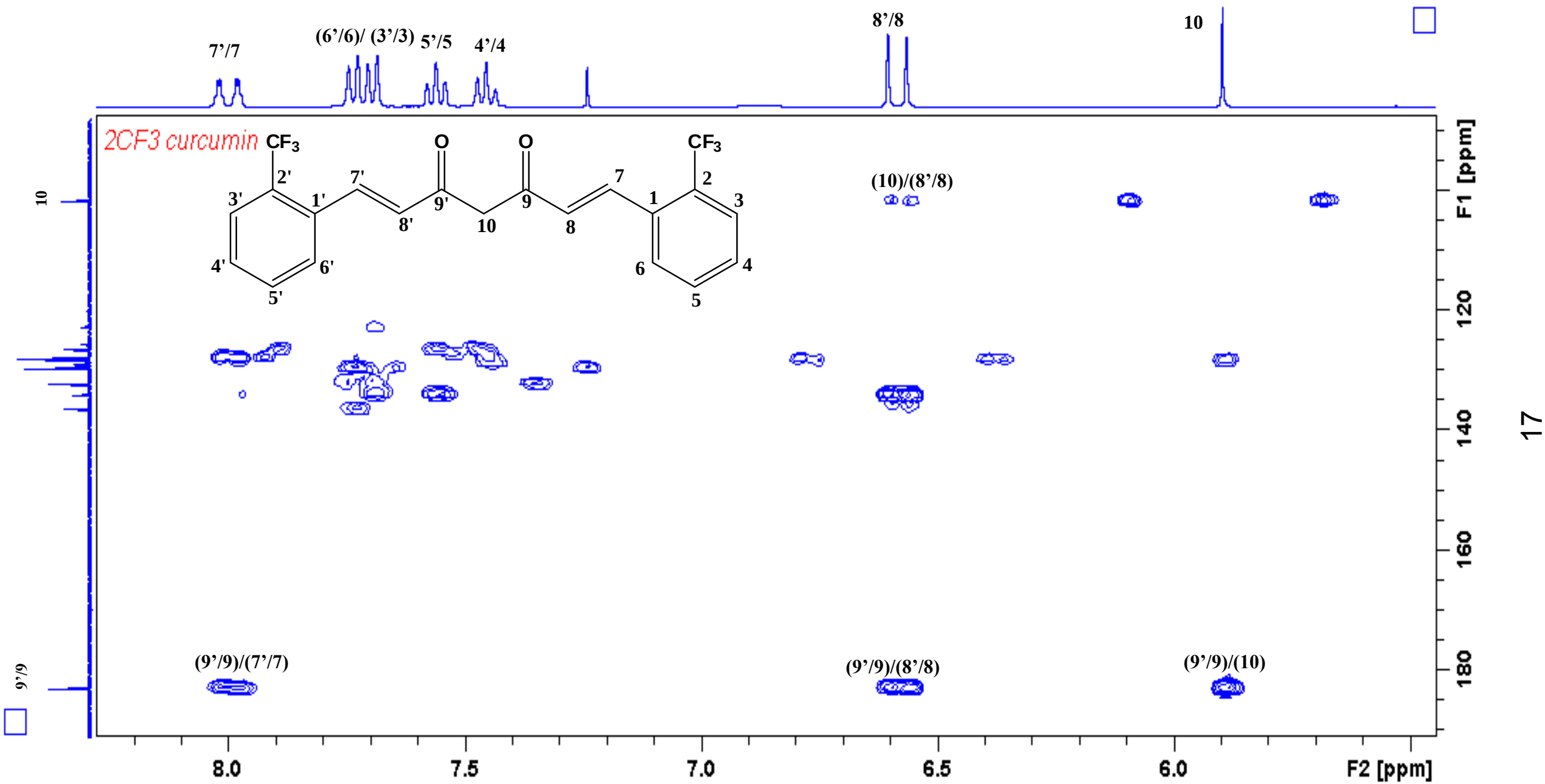

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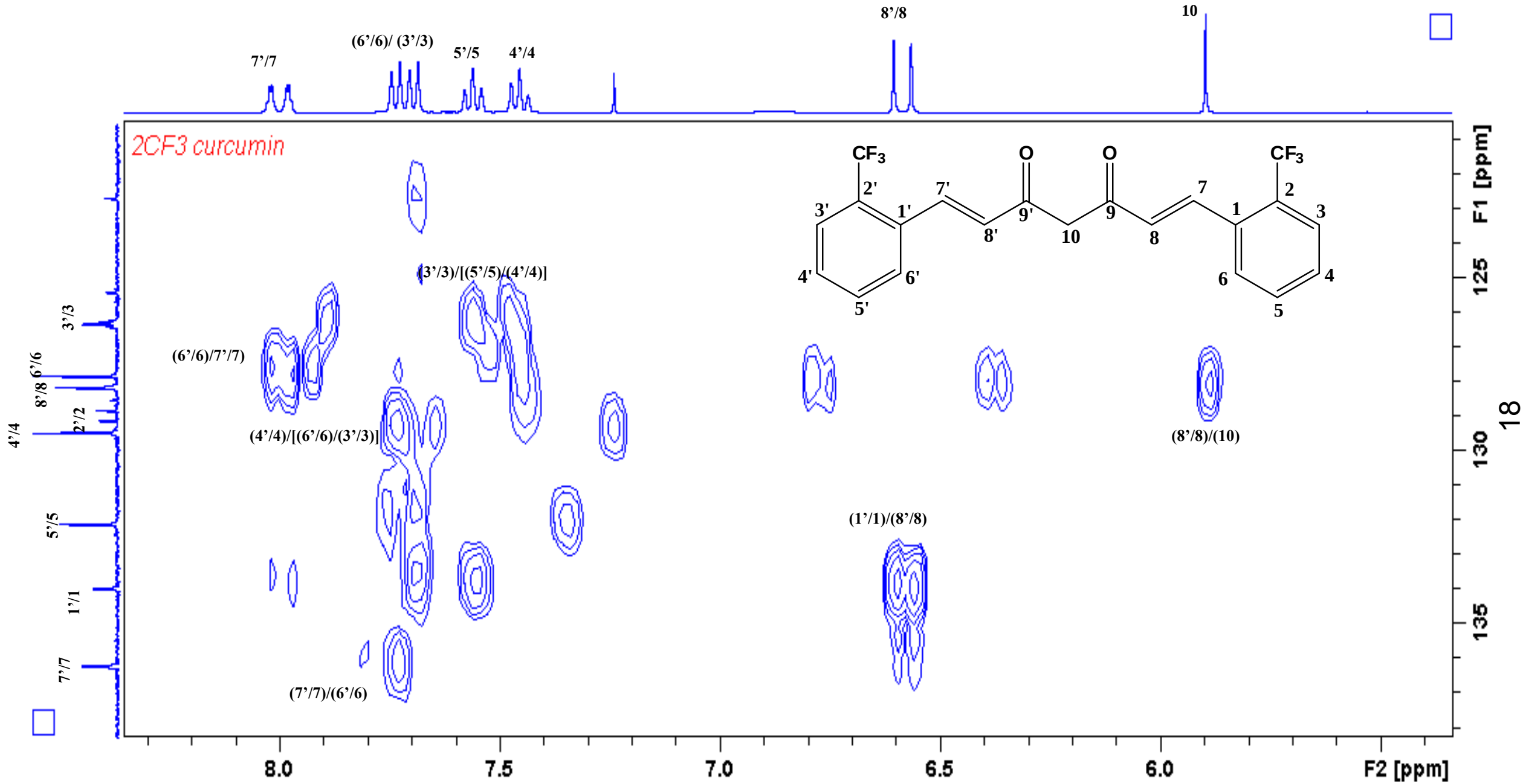

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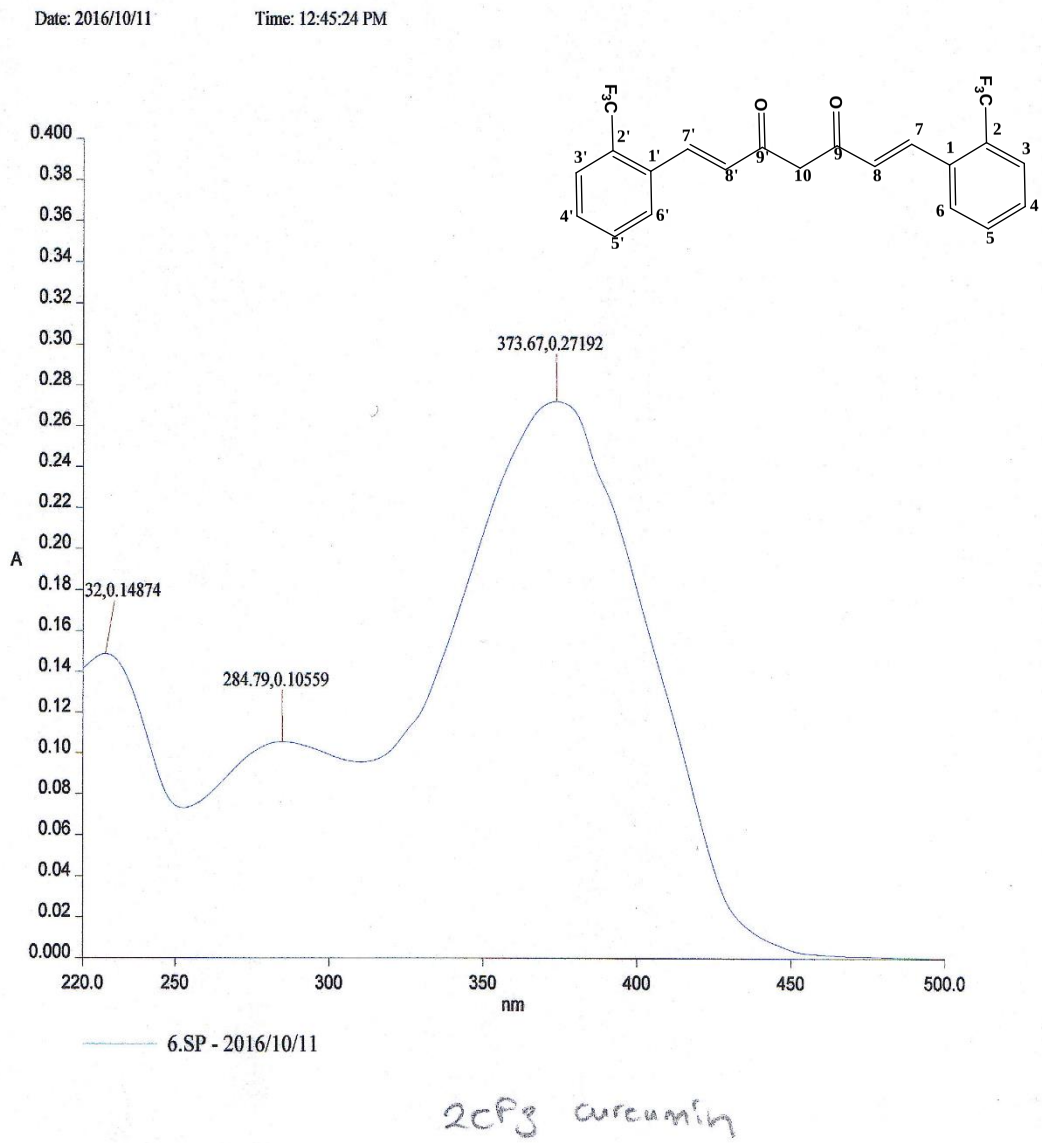
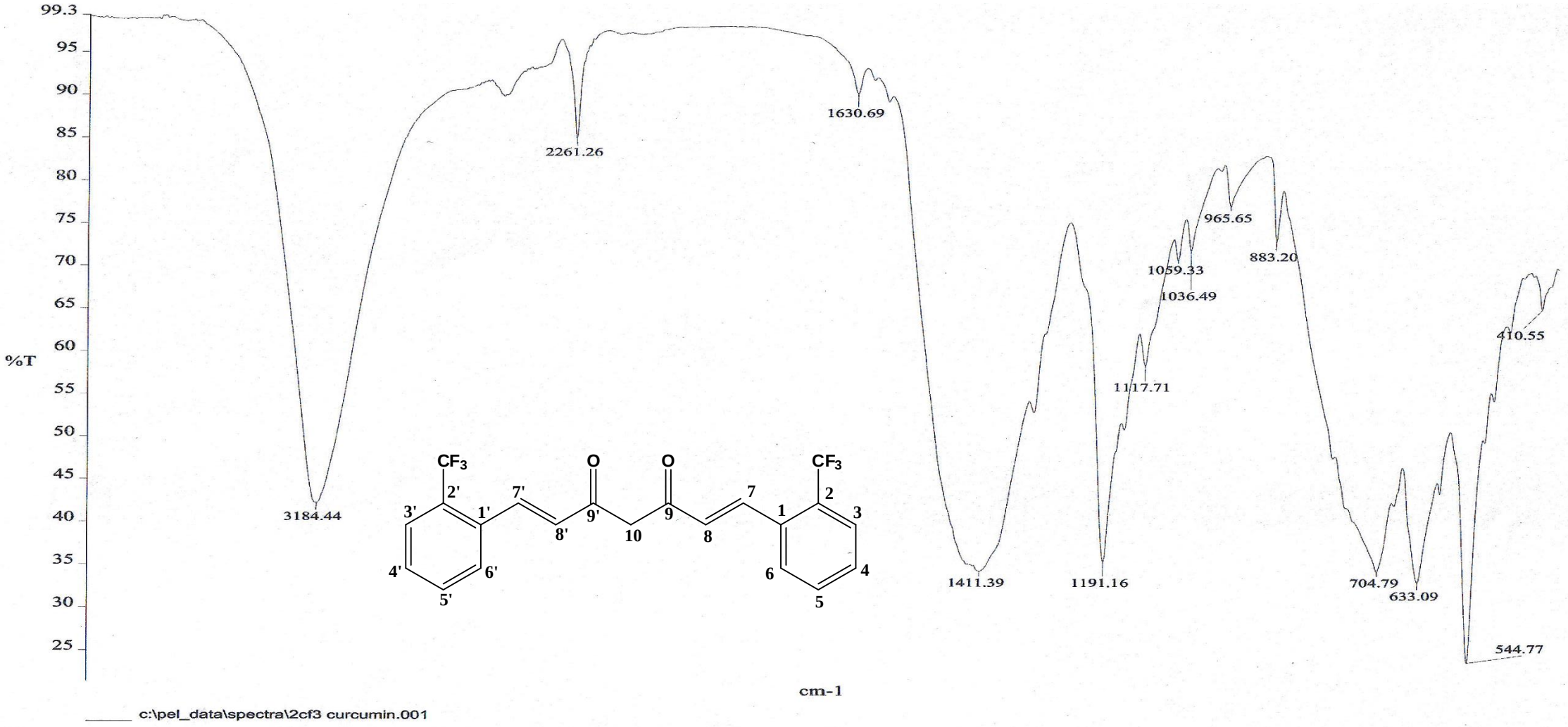


Ultraviolet Spectrum of Compound A-4d: (1E,6E)-1,7-bis(2-(trifluoromethyl)phenyl)hepta-1,6-diene-3,5-dione



Infrared Spectrum of Compound A-4d: (1E,6E)-1,7-bis(2-(trifluoromethyl)phenyl)hepta-1,6-diene-3,5-dione

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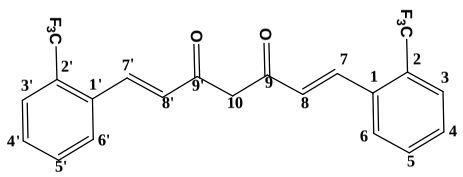

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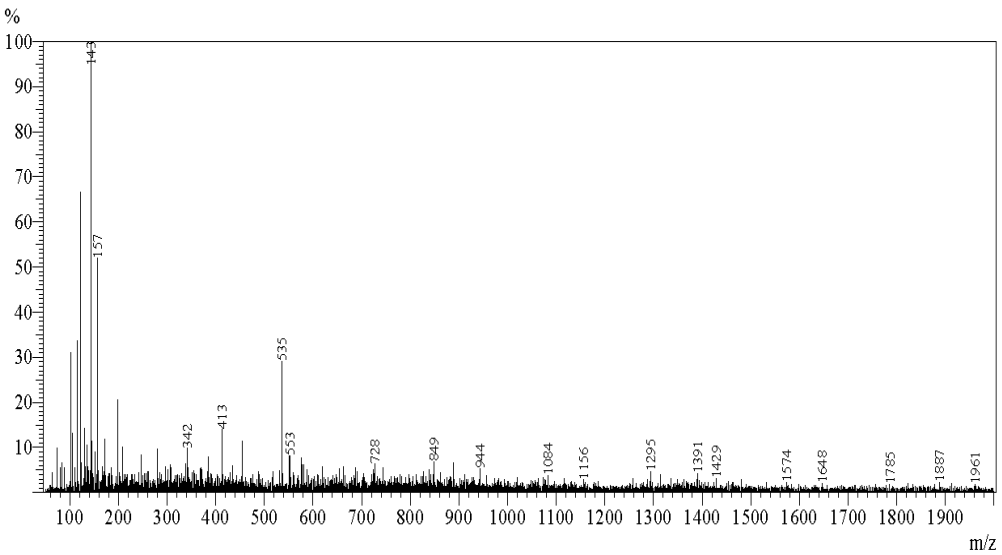
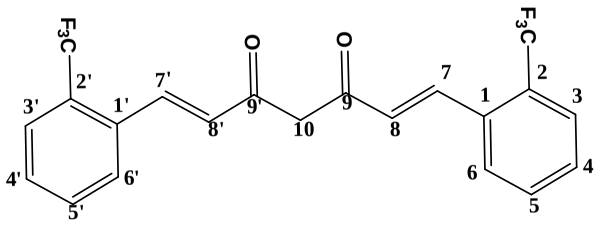


LCMS of Compound A-4d: (1E,6E)-1,7-bis(2-(trifluoromethyl)phenyl)hepta-1,6-diene-3,5-dione



2CF3 curcumin

Elemental Composition Report Page 1

Single Mass Analysis
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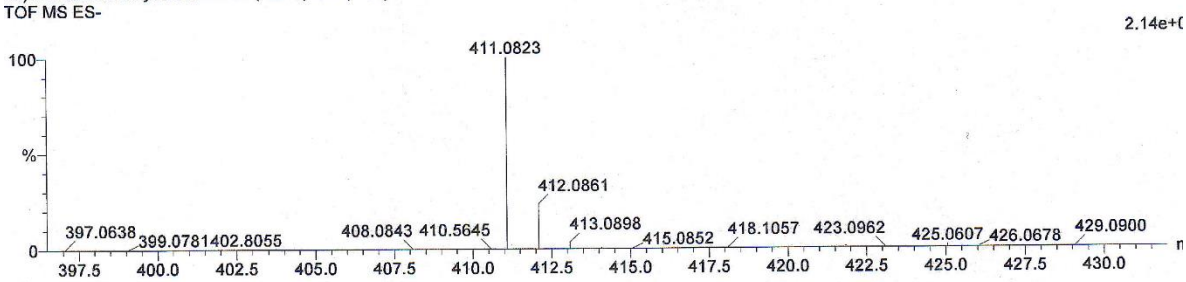
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
24 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-23 H: 10-15 O: 0-5 F: 5-10
13) 2-trifluoromethylcurcumin 33 (1.079) Cm (1:61)
TOF MS ES-

2.14e+(



Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
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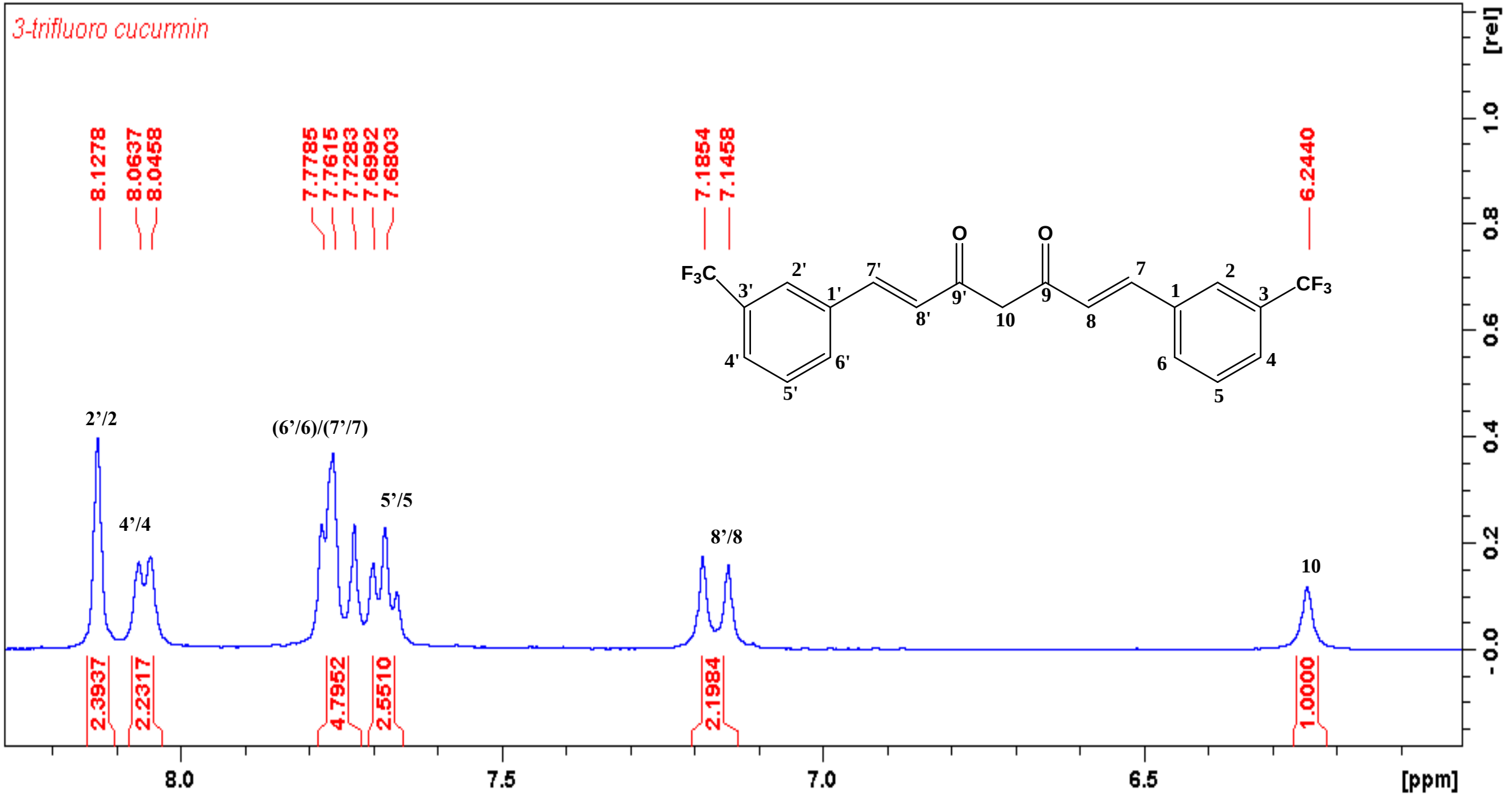

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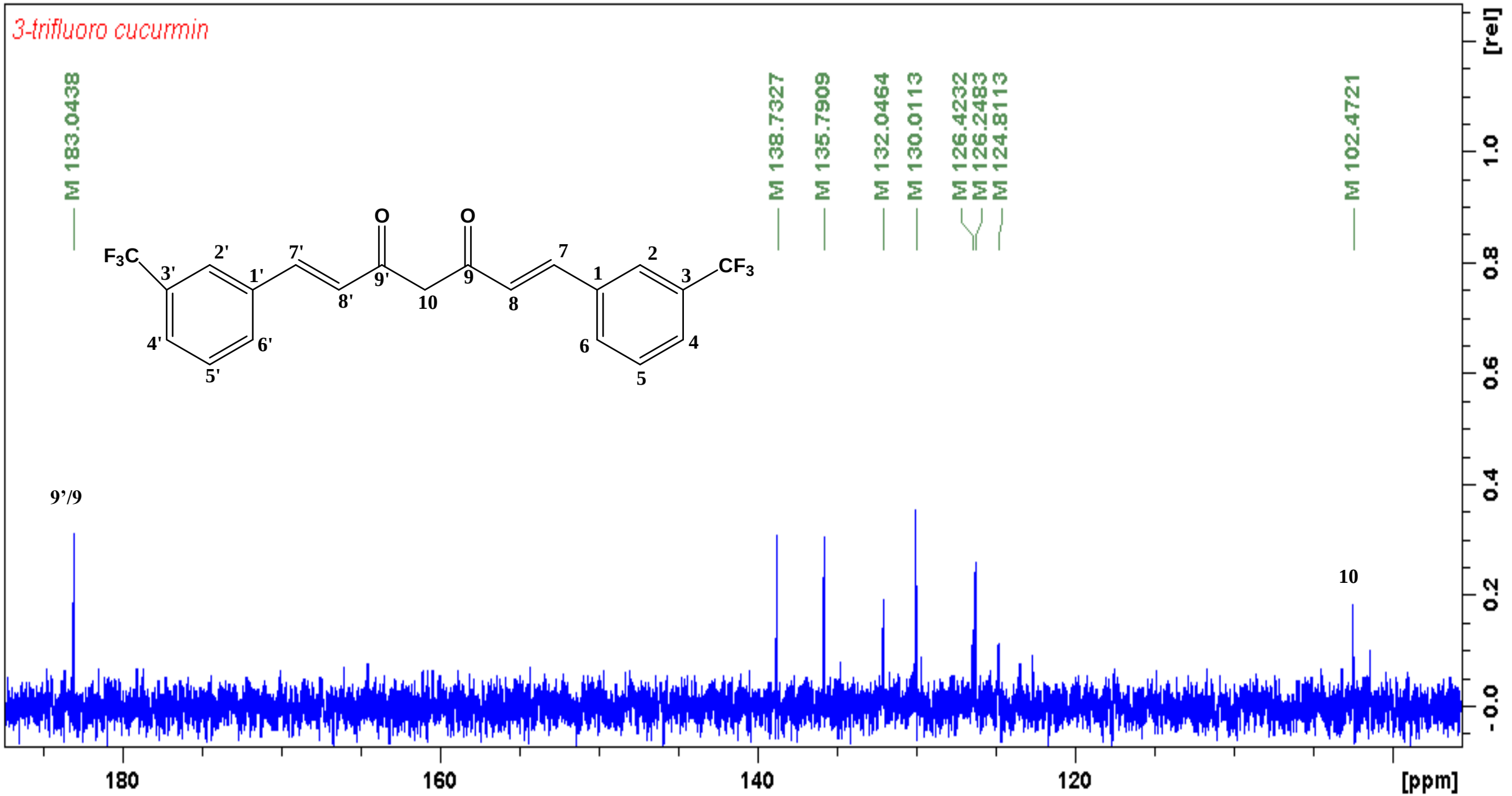

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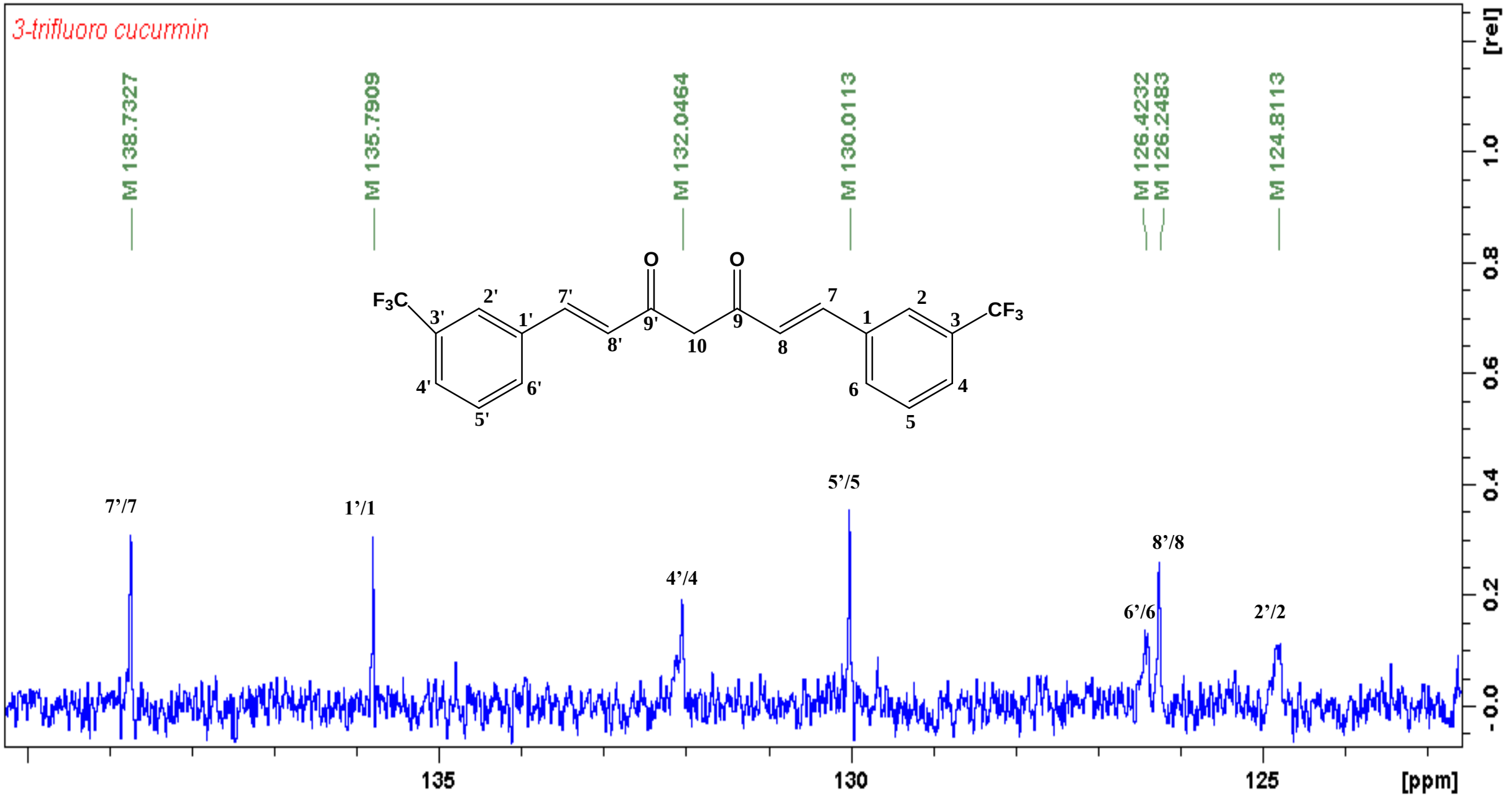

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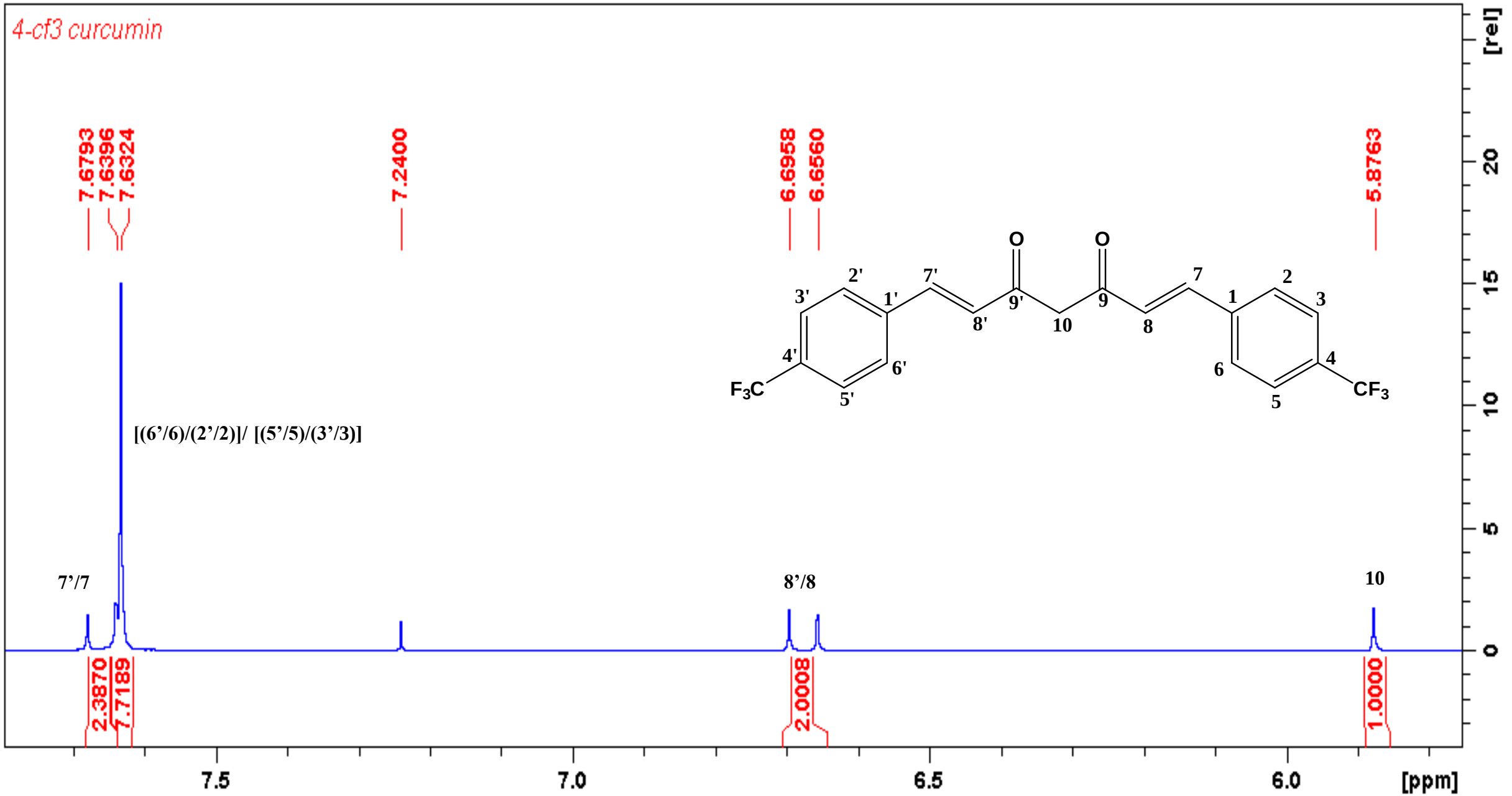

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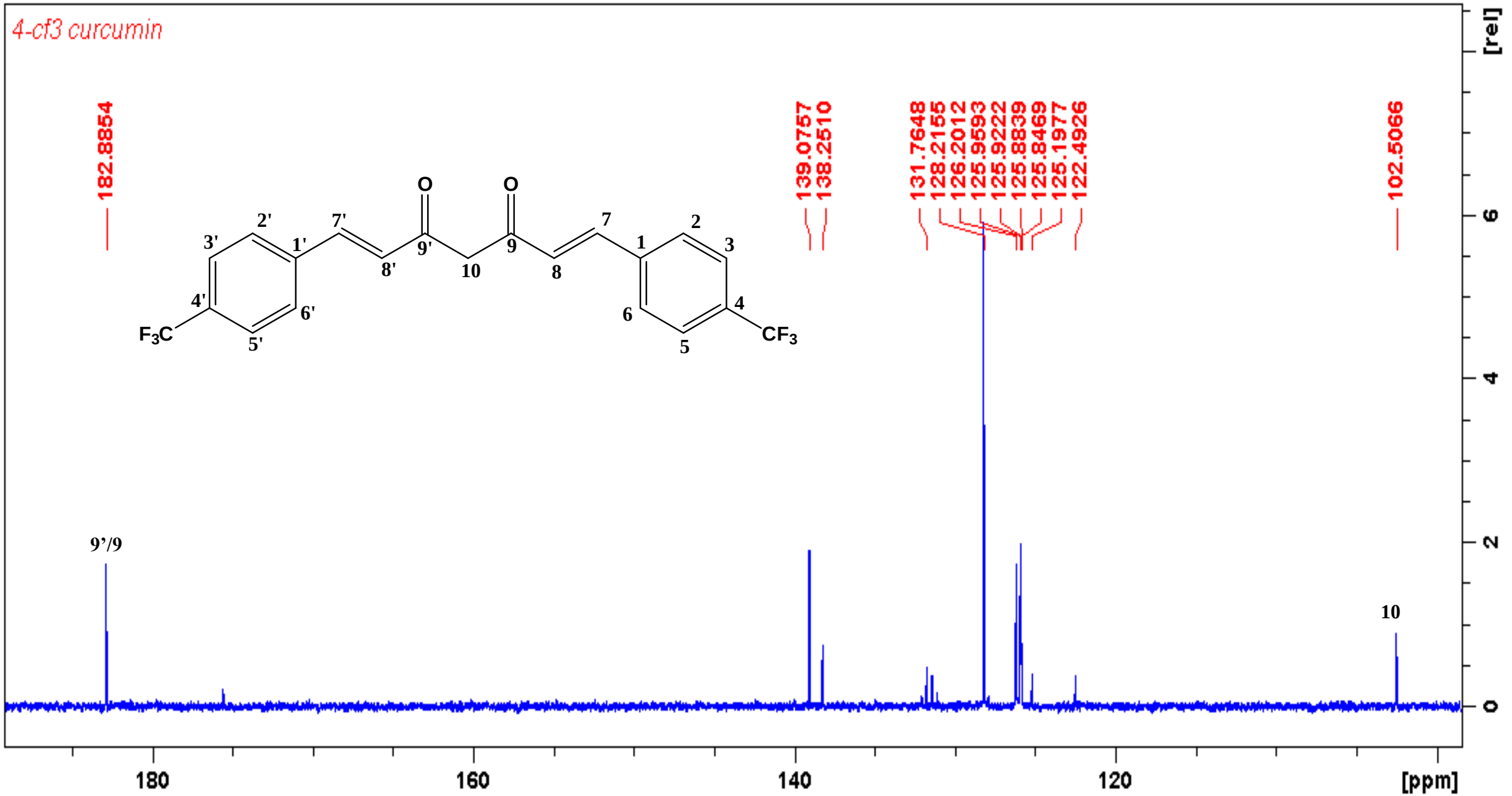

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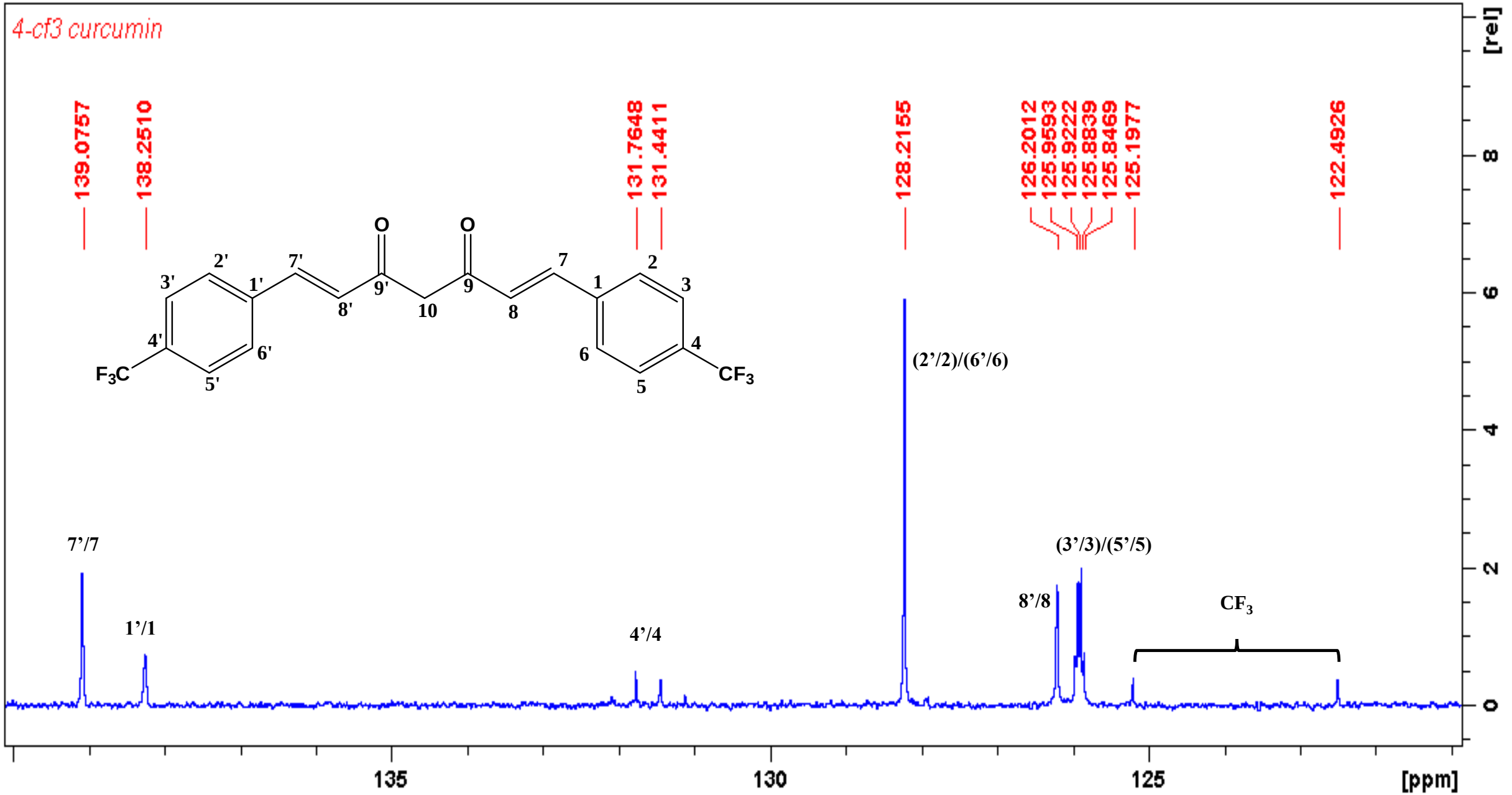

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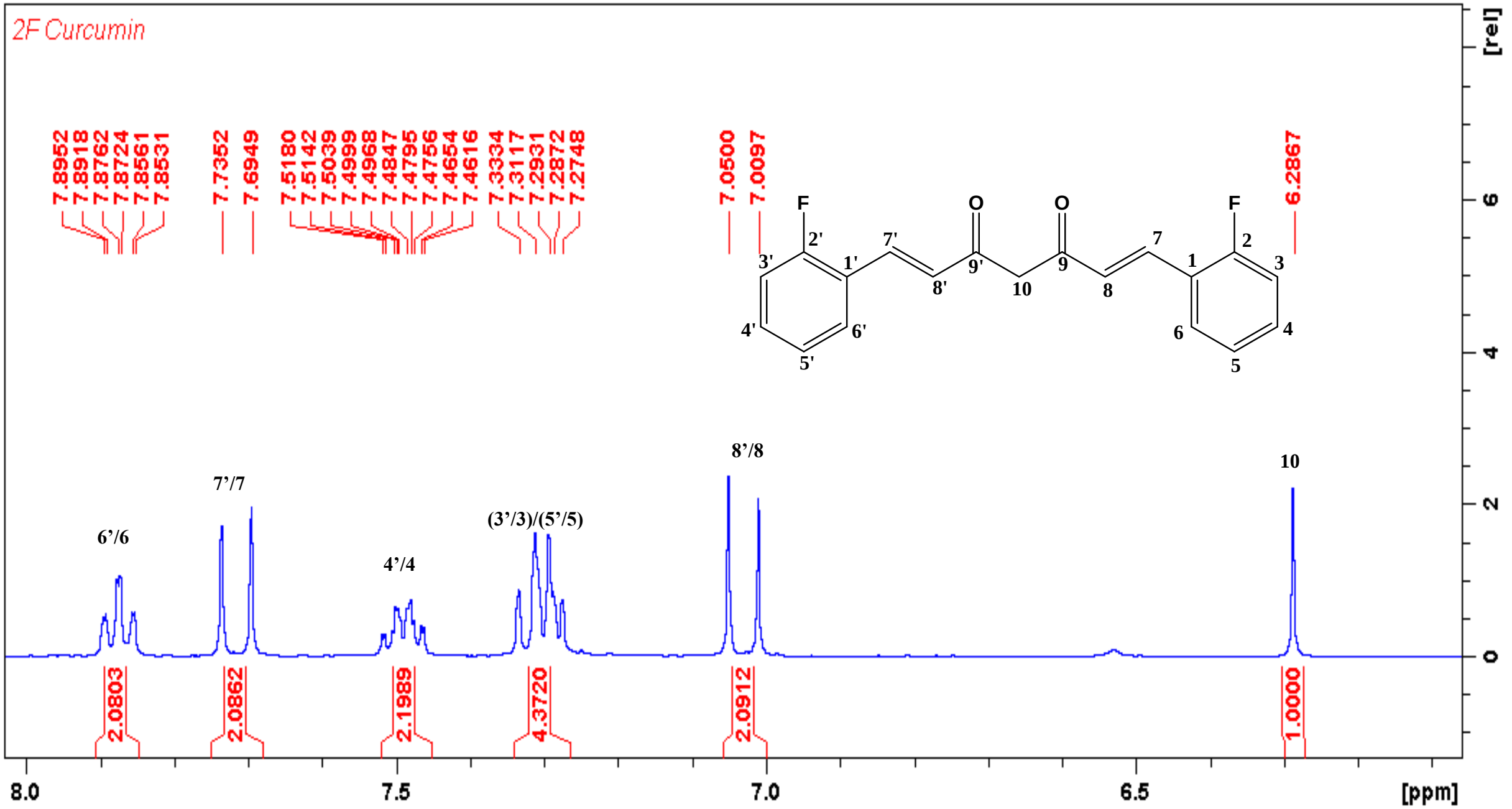

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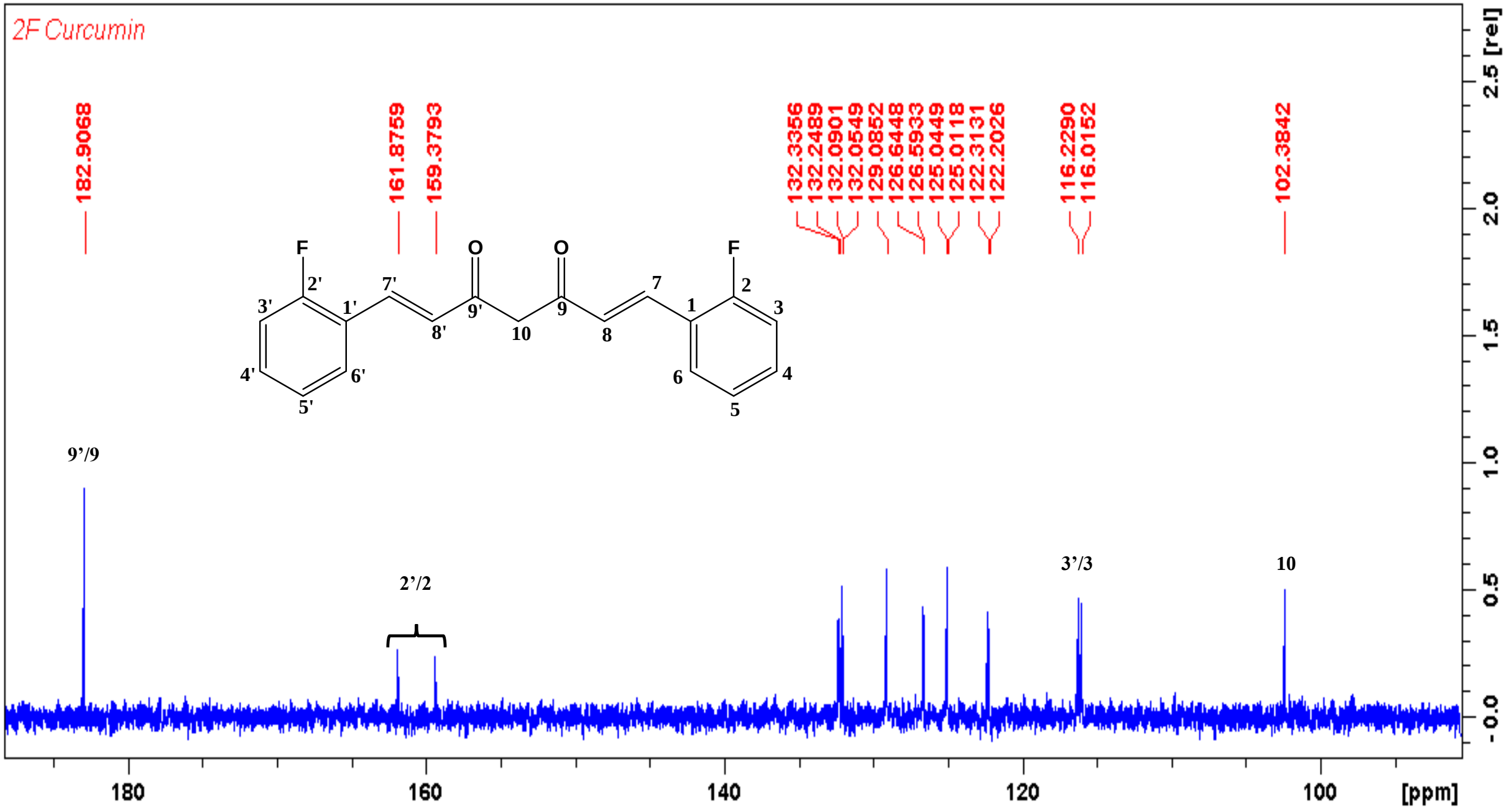
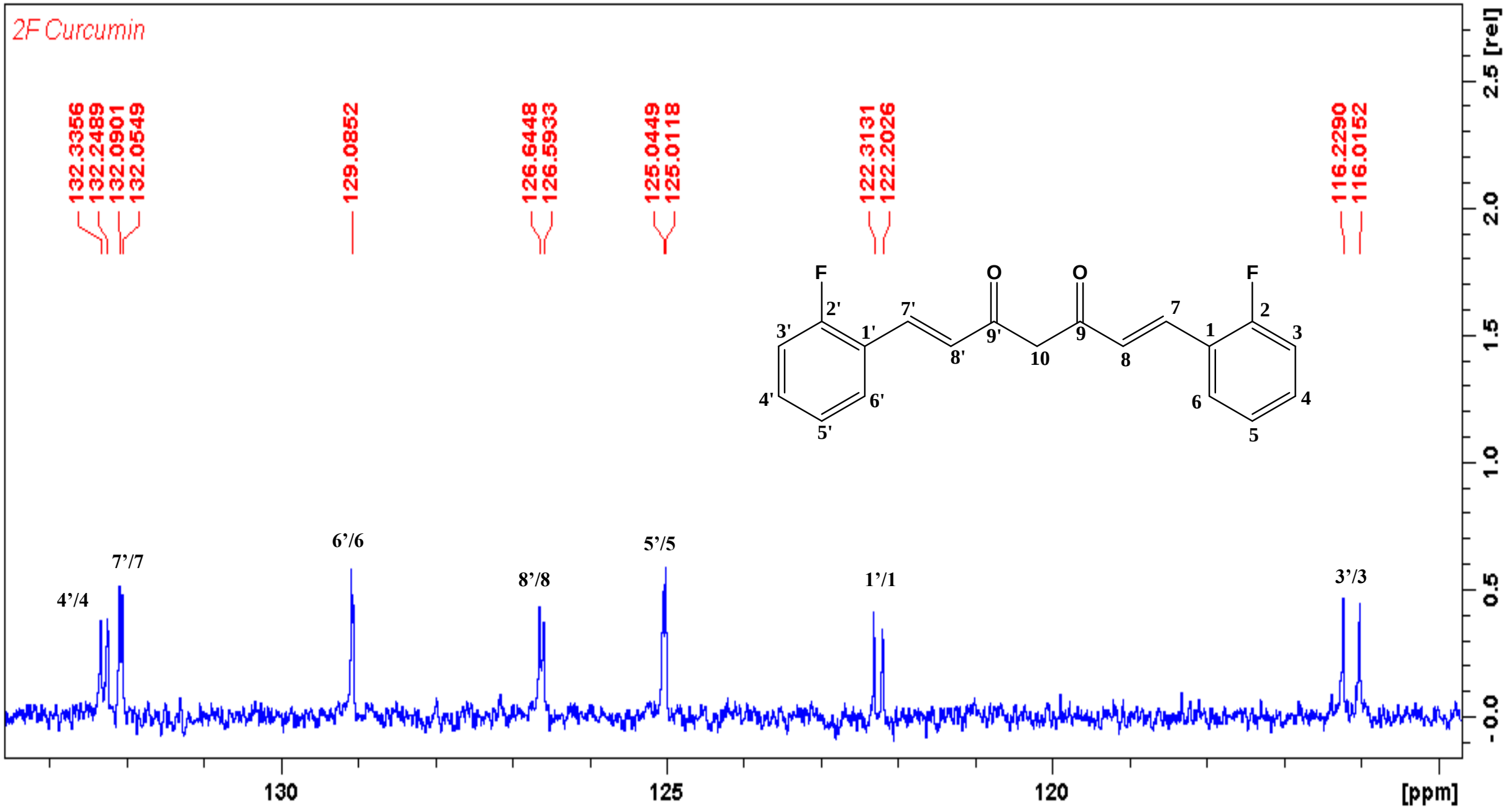


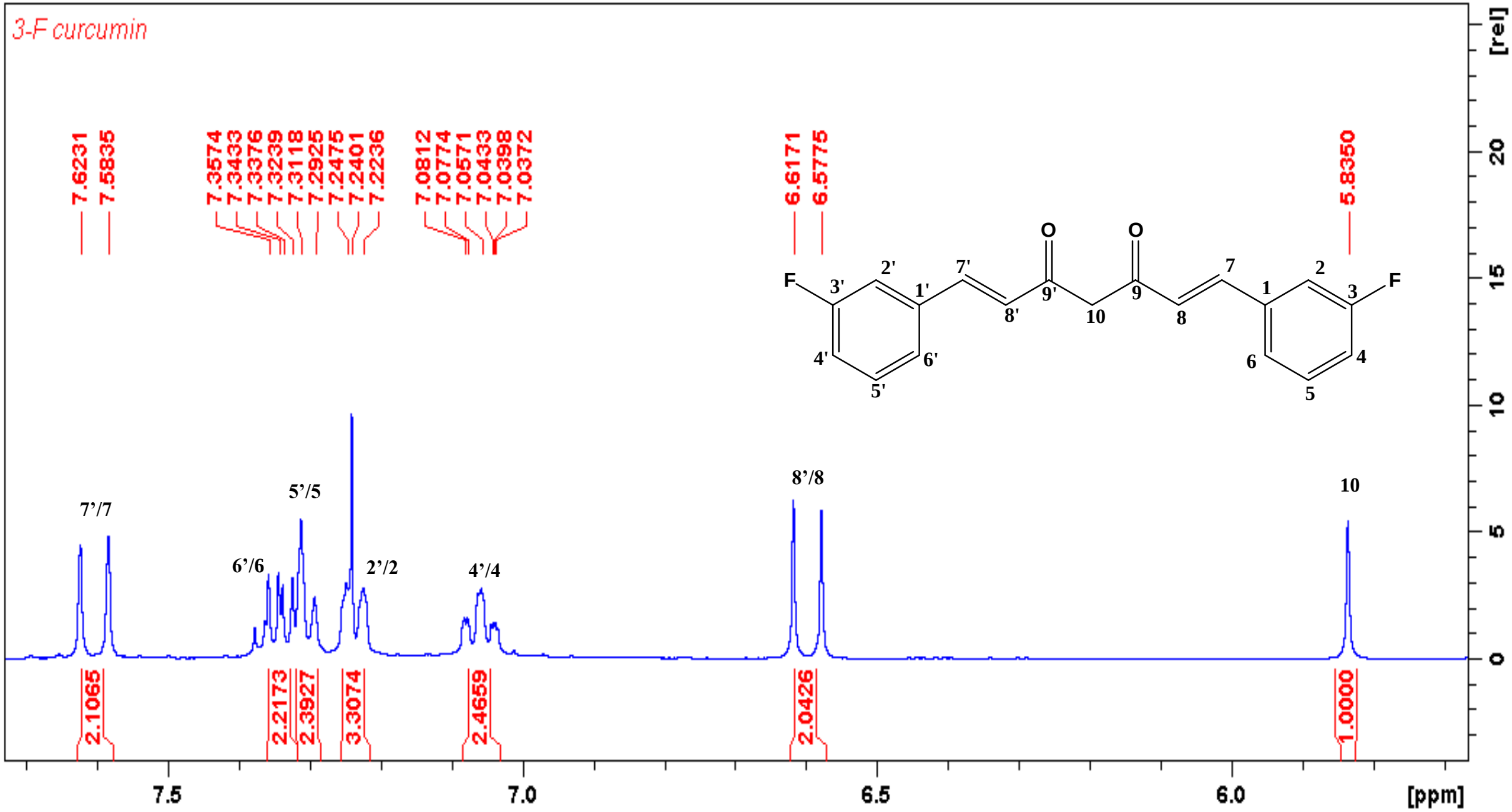
30

^{13}C Spectrum of Compound A-4g: (1E,6E)-1,7-bis(2-fluorophenyl)hepta-1,6-diene-3,5-dione

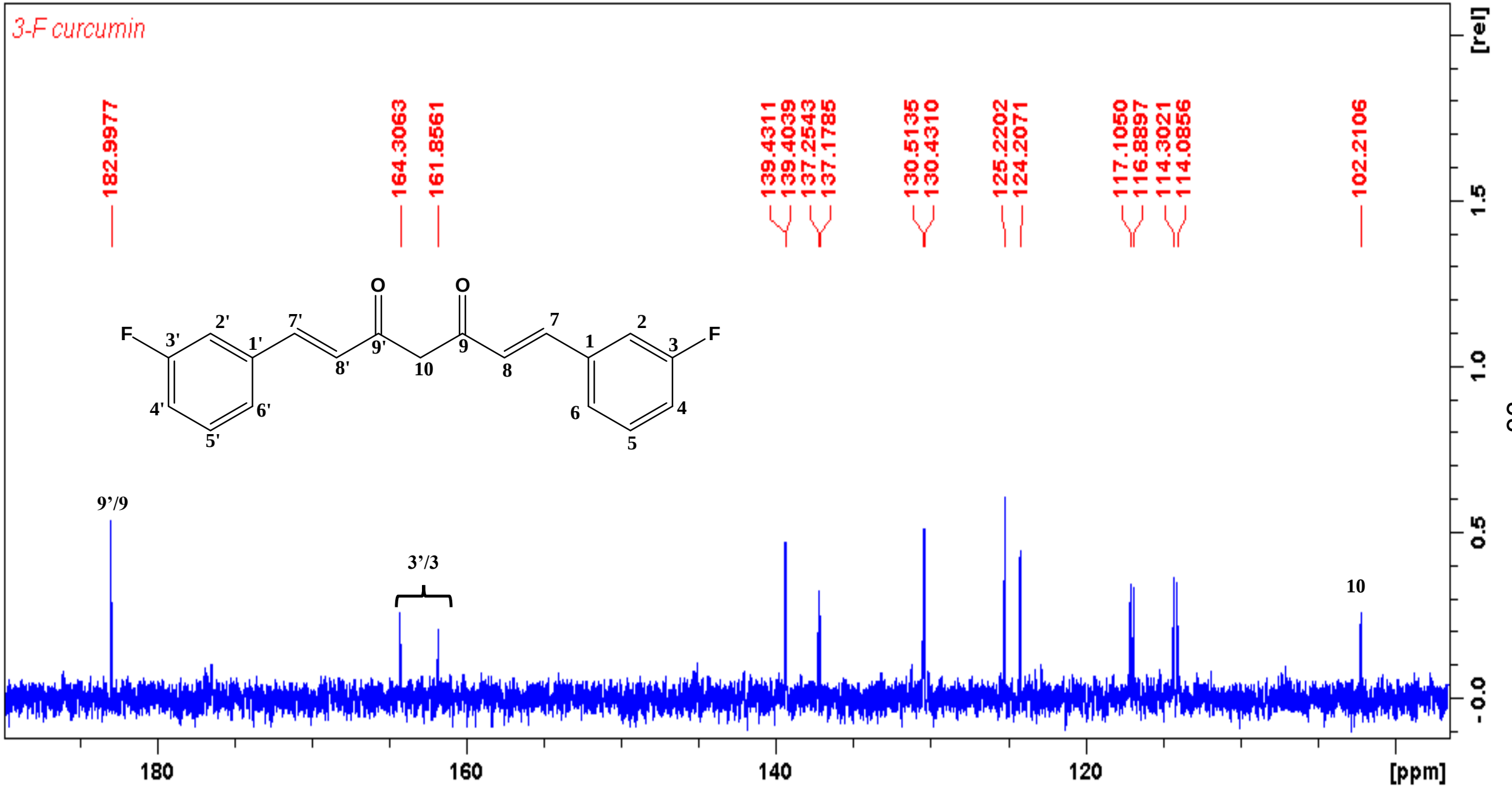


31

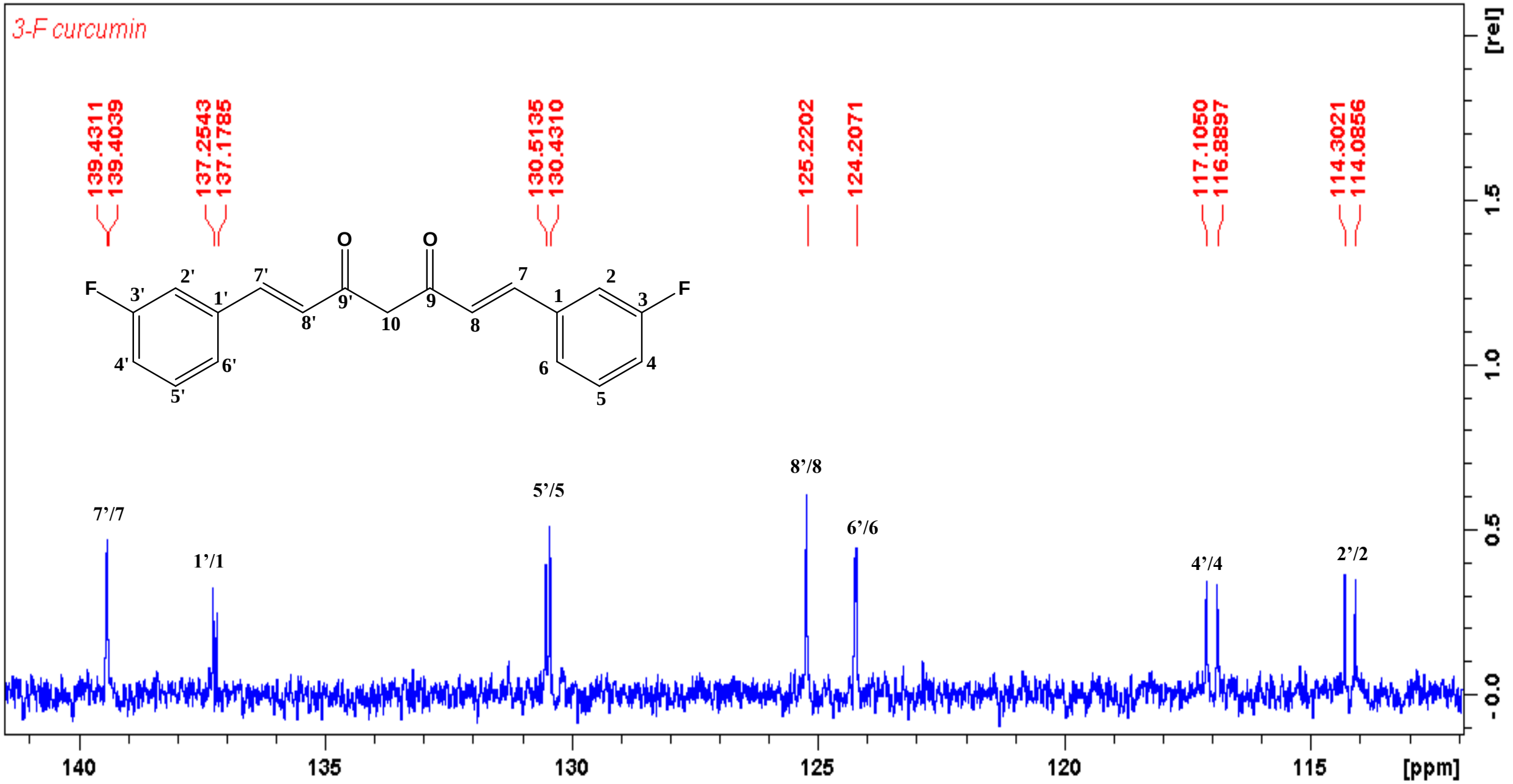
Expanded ^{13}C Spectrum of Compound A-4g: (1E,6E)-1,7-bis(2-fluorophenyl)hepta-1,6-diene-3,5-dione



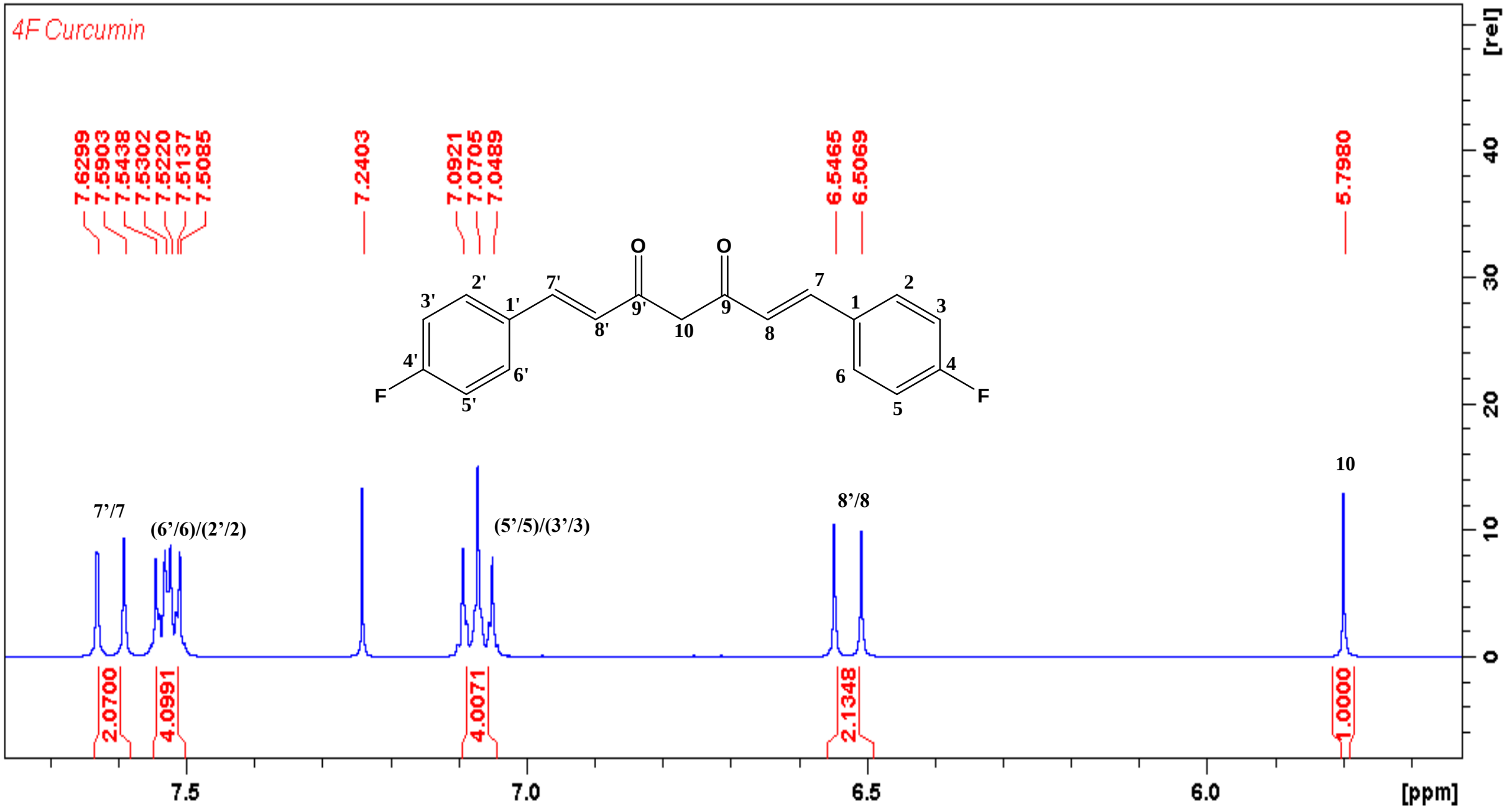
[1]H Spectrum of Compound A-4h: (1E,6E)-1,7-bis(3-fluorophenyl)hepta-1,6-diene-3,5-dione



^{13}C Spectrum of Compound A-4h: (1E,6E)-1,7-bis(3-fluorophenyl)hepta-1,6-diene-3,5-dione



Expanded ^{13}C Spectrum of Compound A-4h: (1E,6E)-1,7-bis(3-fluorophenyl)hepta-1,6-diene-3,5-dione



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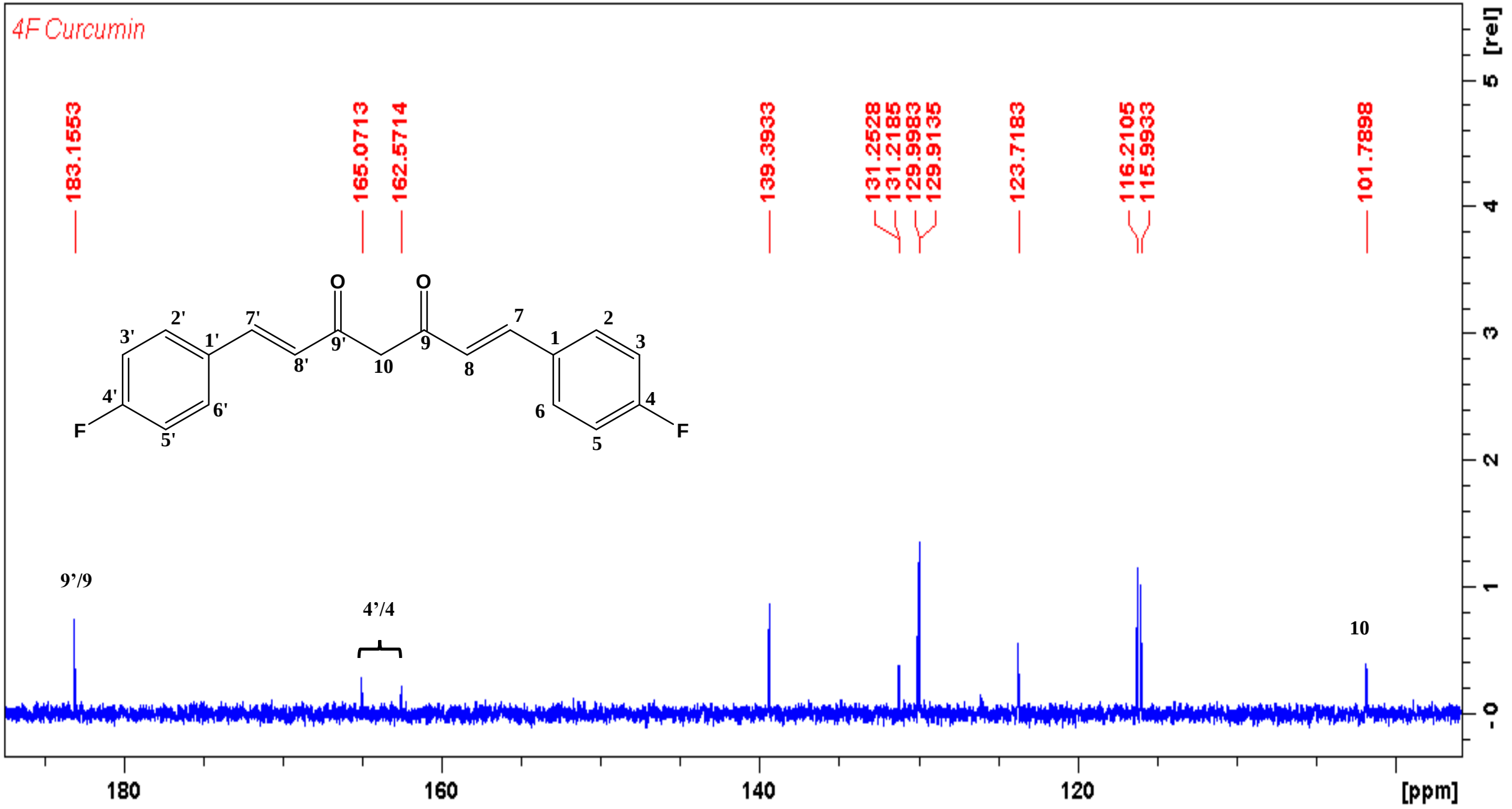
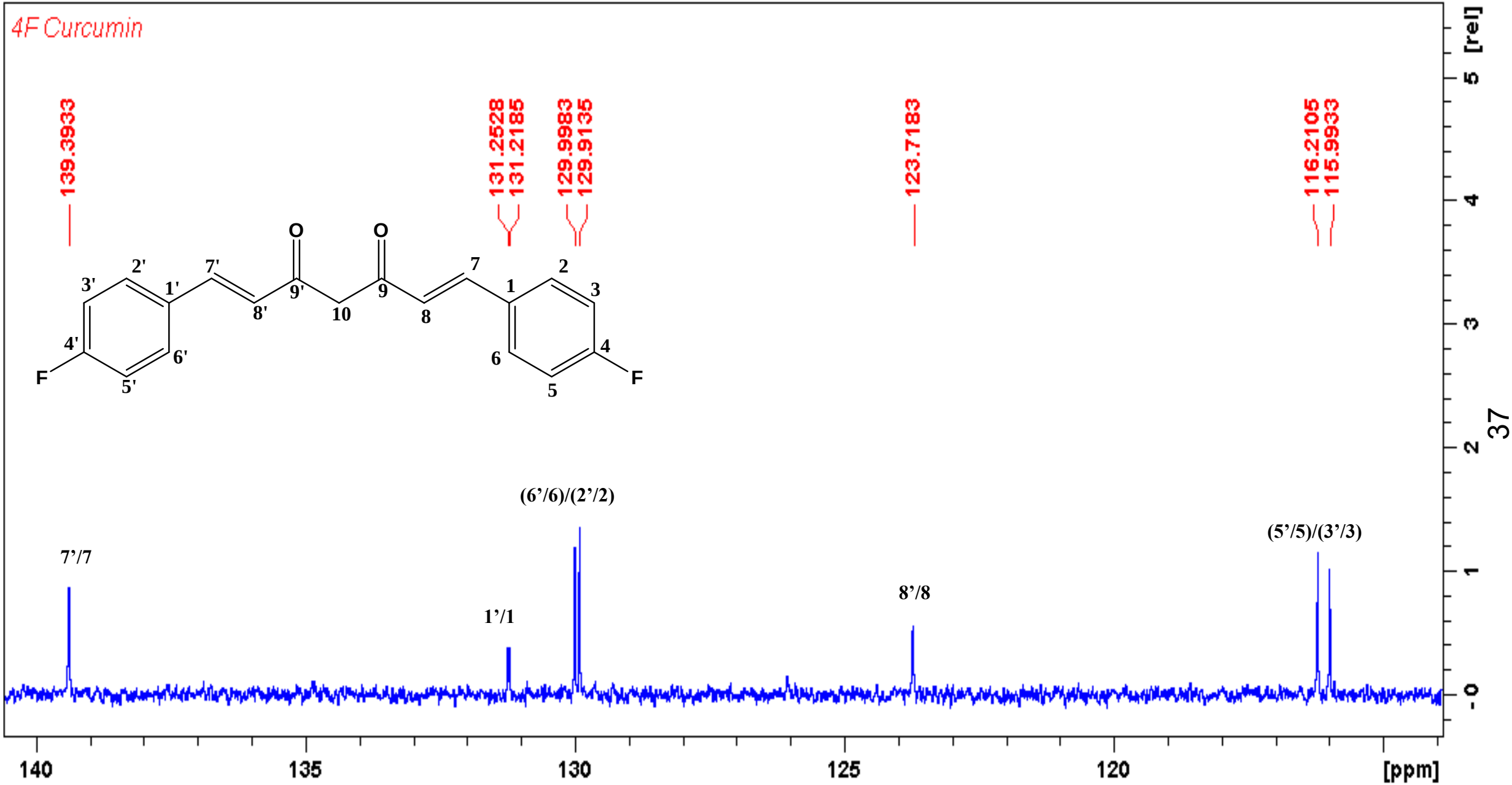
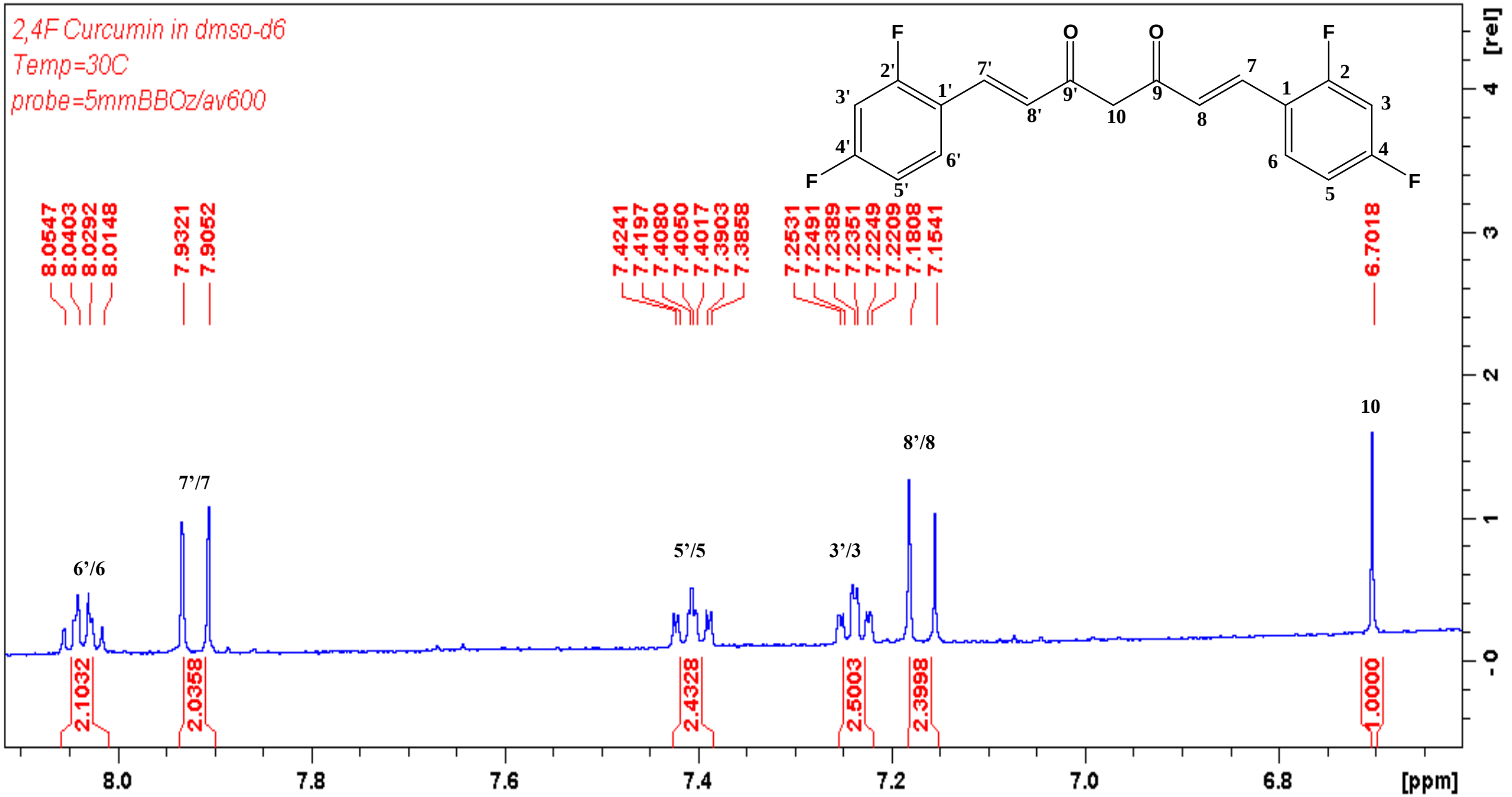
^{1}H Spectrum of Compound A-4i: (1E,6E)-1,7-bis(4-fluorophenyl)hepta-1,6-diene-3,5-dione



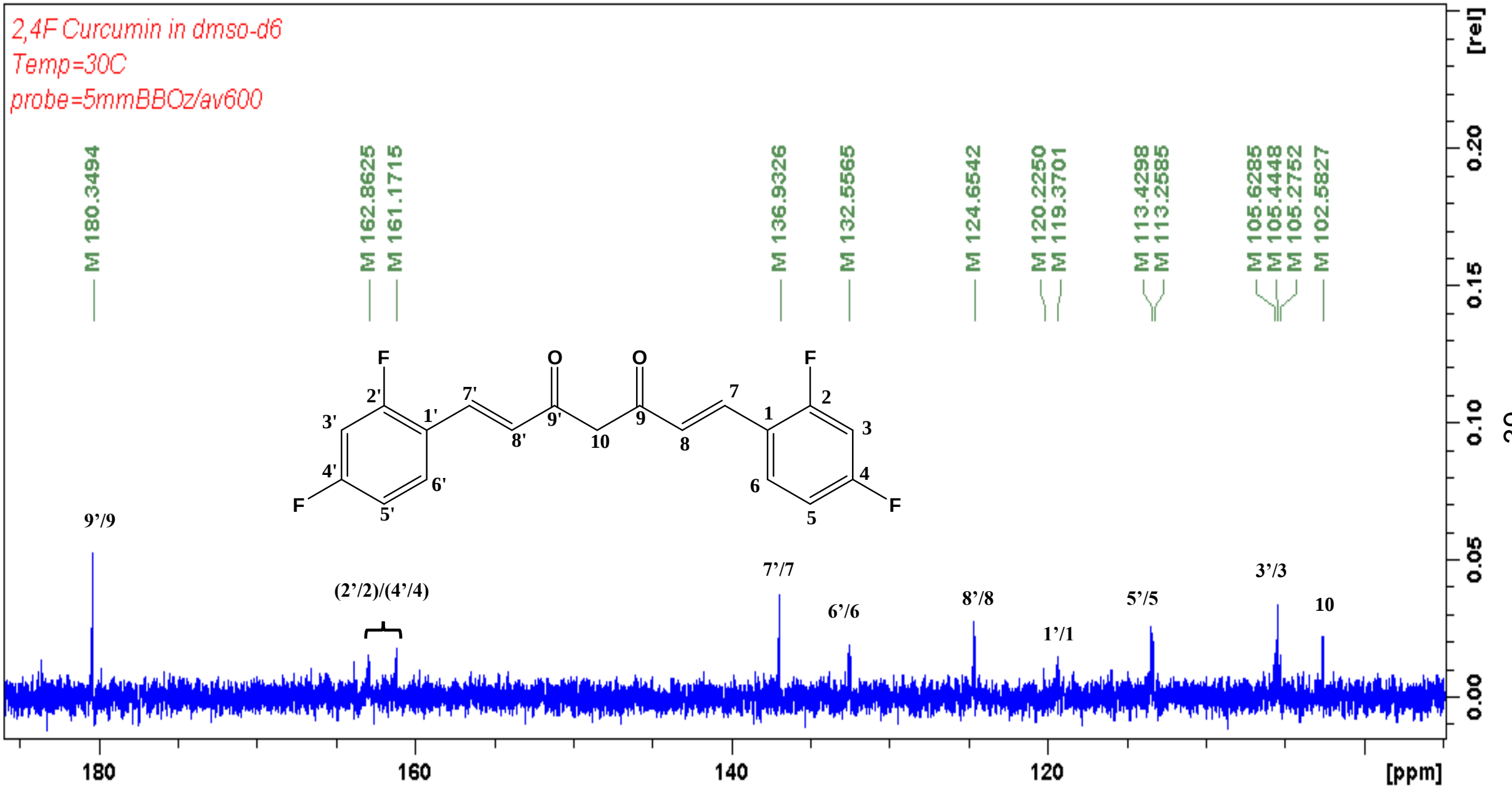
^{13}C Spectrum of Compound A-4i: (1E,6E)-1,7-bis(4-fluorophenyl)hepta-1,6-diene-3,5-dione



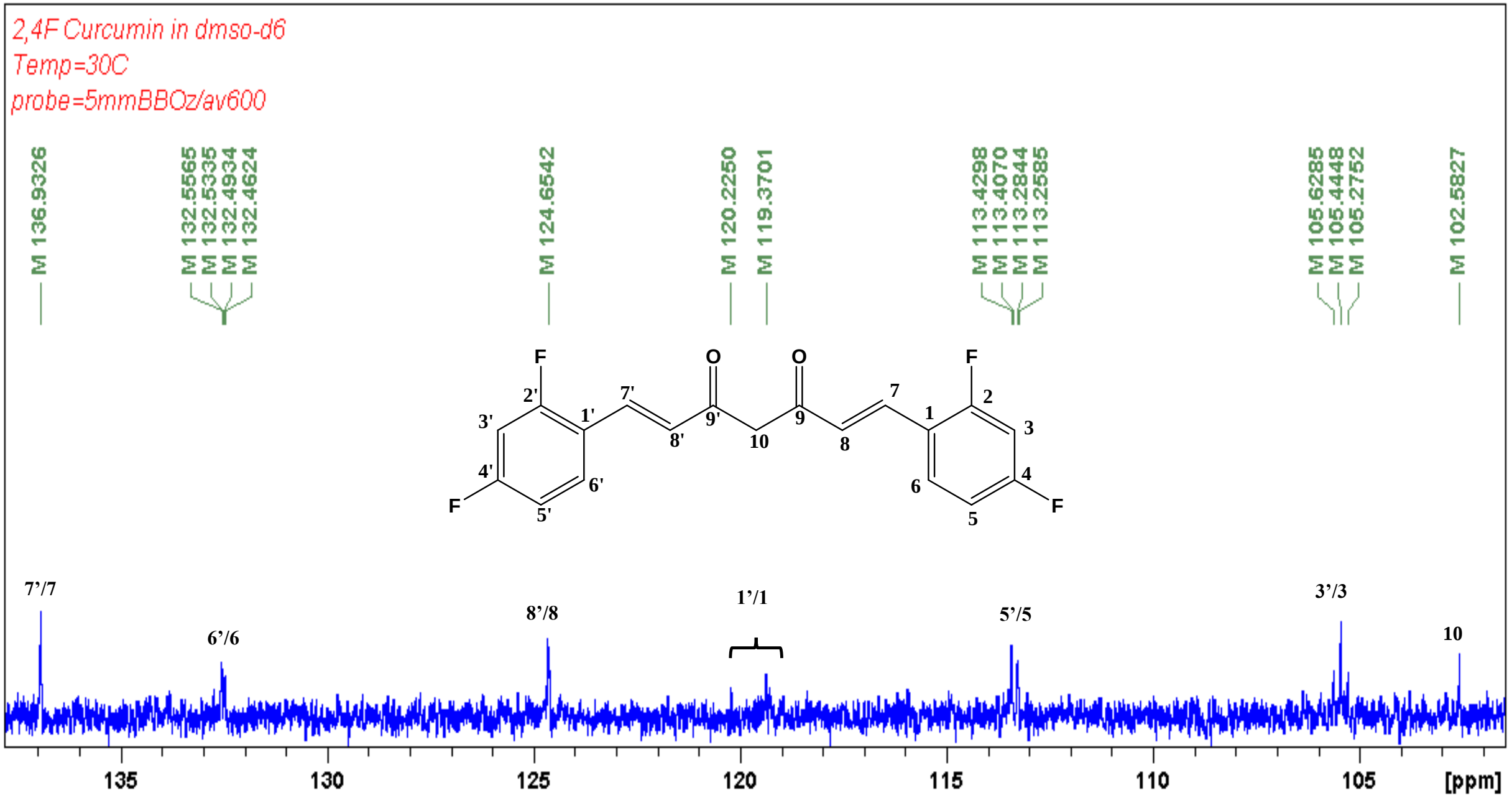
Expanded ^{13}C Spectrum of Compound A-4i: (1E,6E)-1,7-bis(4-fluorophenyl)hepta-1,6-diene-3,5-dione



^{1}H Spectrum of Compound A-4j: (1E,6E)-1,7-bis(2,4-difluorophenyl)hepta-1,6-diene-3,5-dione



^{13}C Spectrum of Compound A-4j: (1E,6E)-1,7-bis(2,4-difluorophenyl)hepta-1,6-diene-3,5-dione



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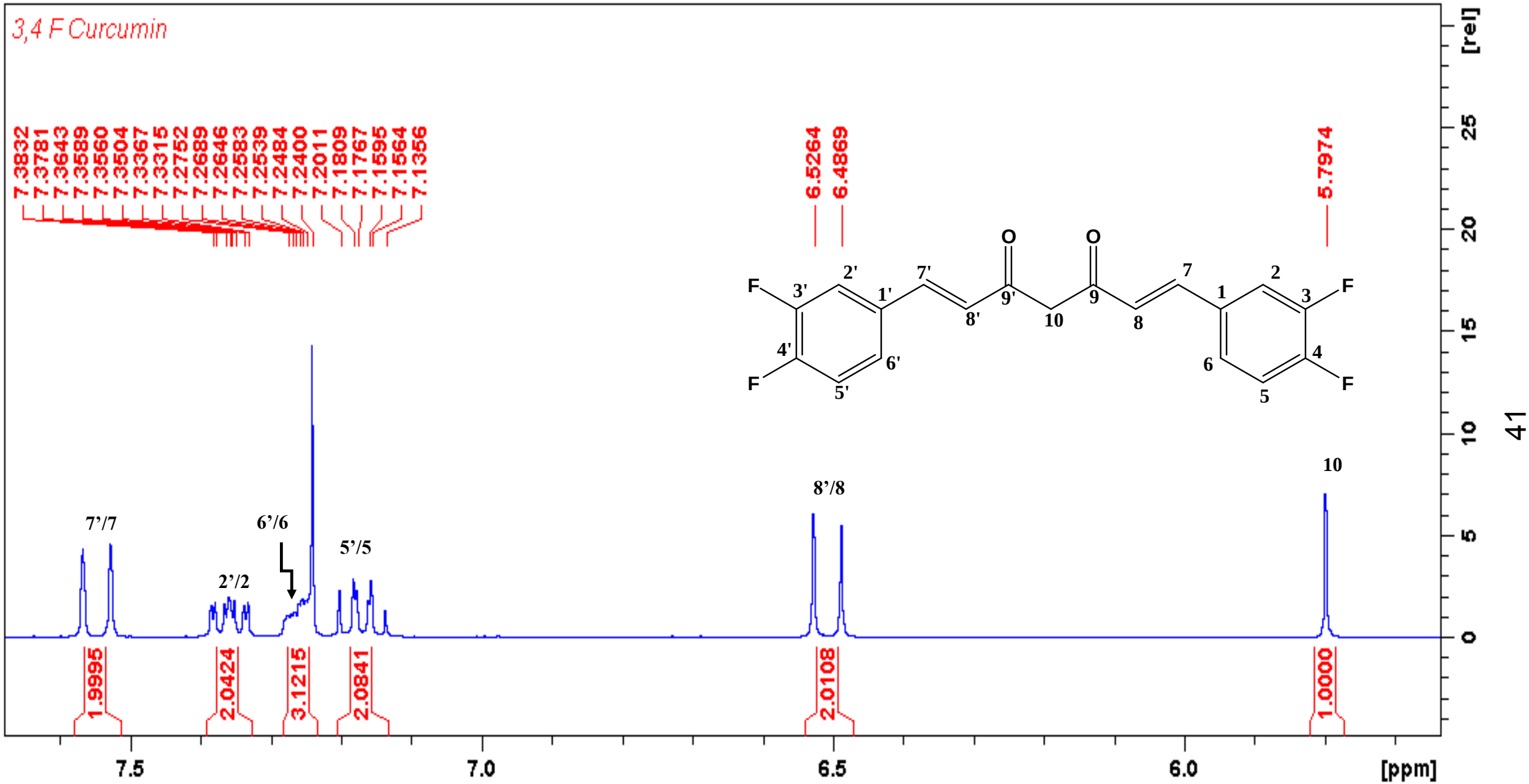
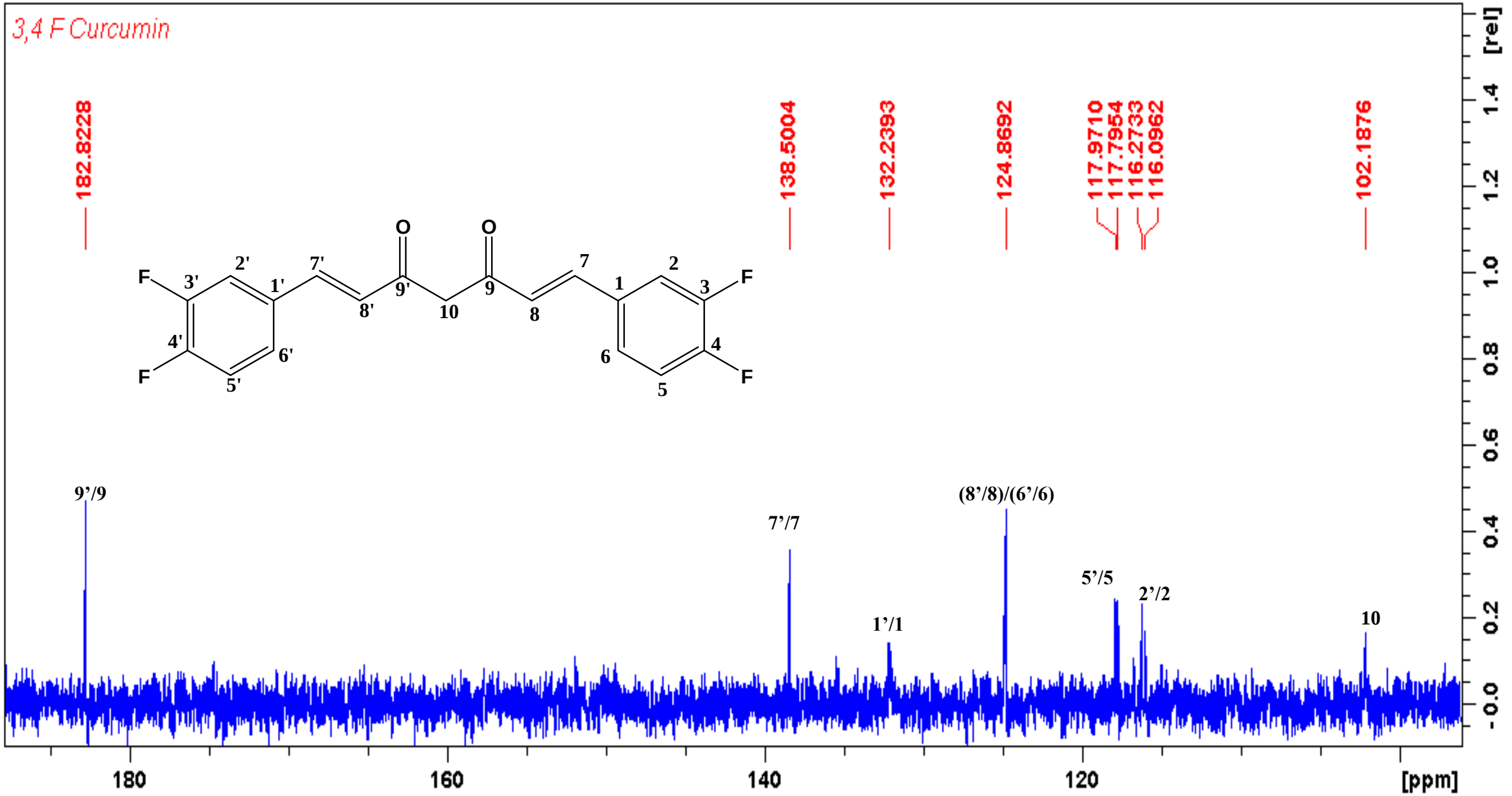
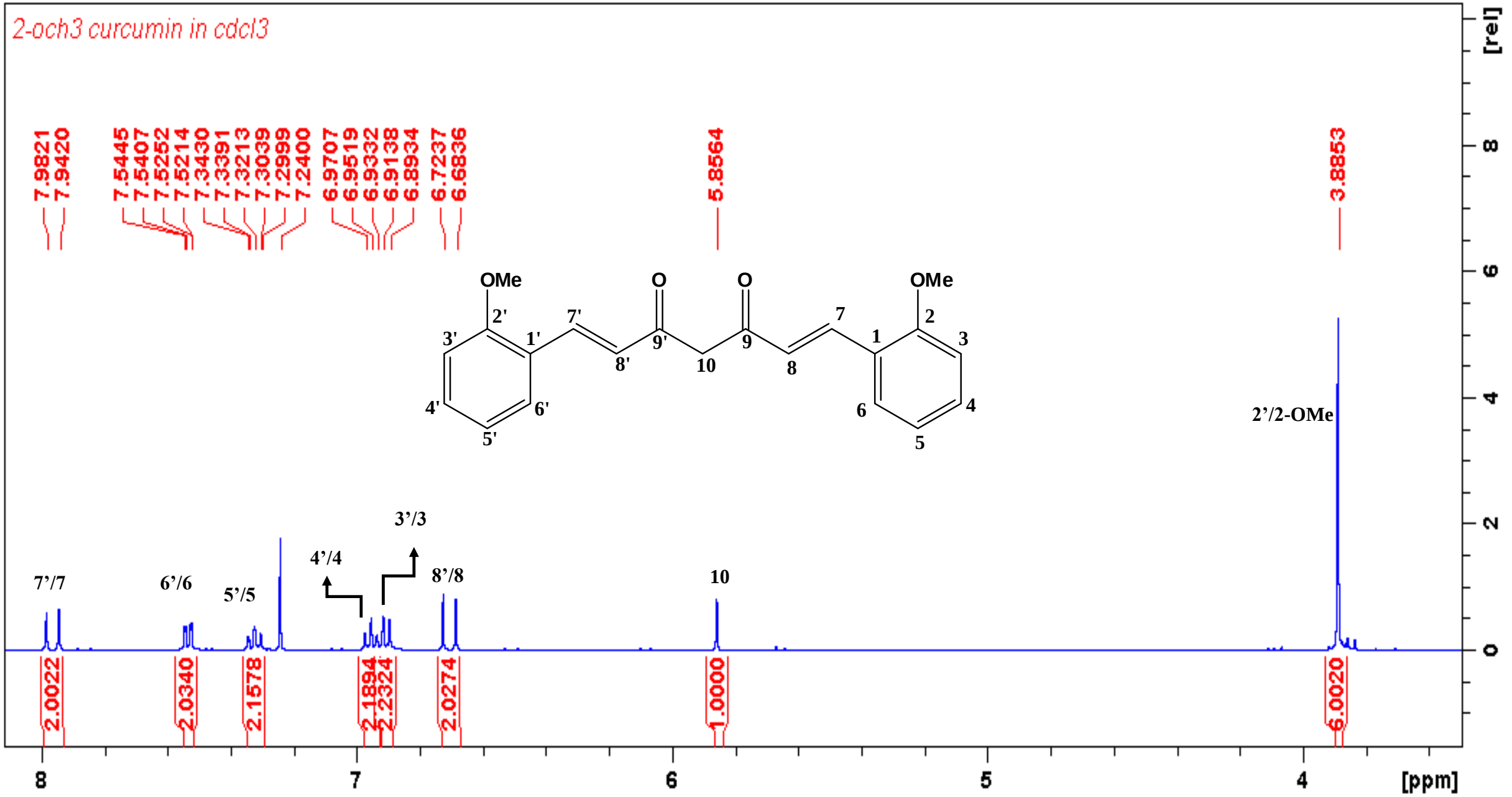
Expanded ^{13}C Spectrum of Compound A-4j: (1E,6E)-1,7-bis(2,4-difluorophenyl)hepta-1,6-diene-3,5-dione



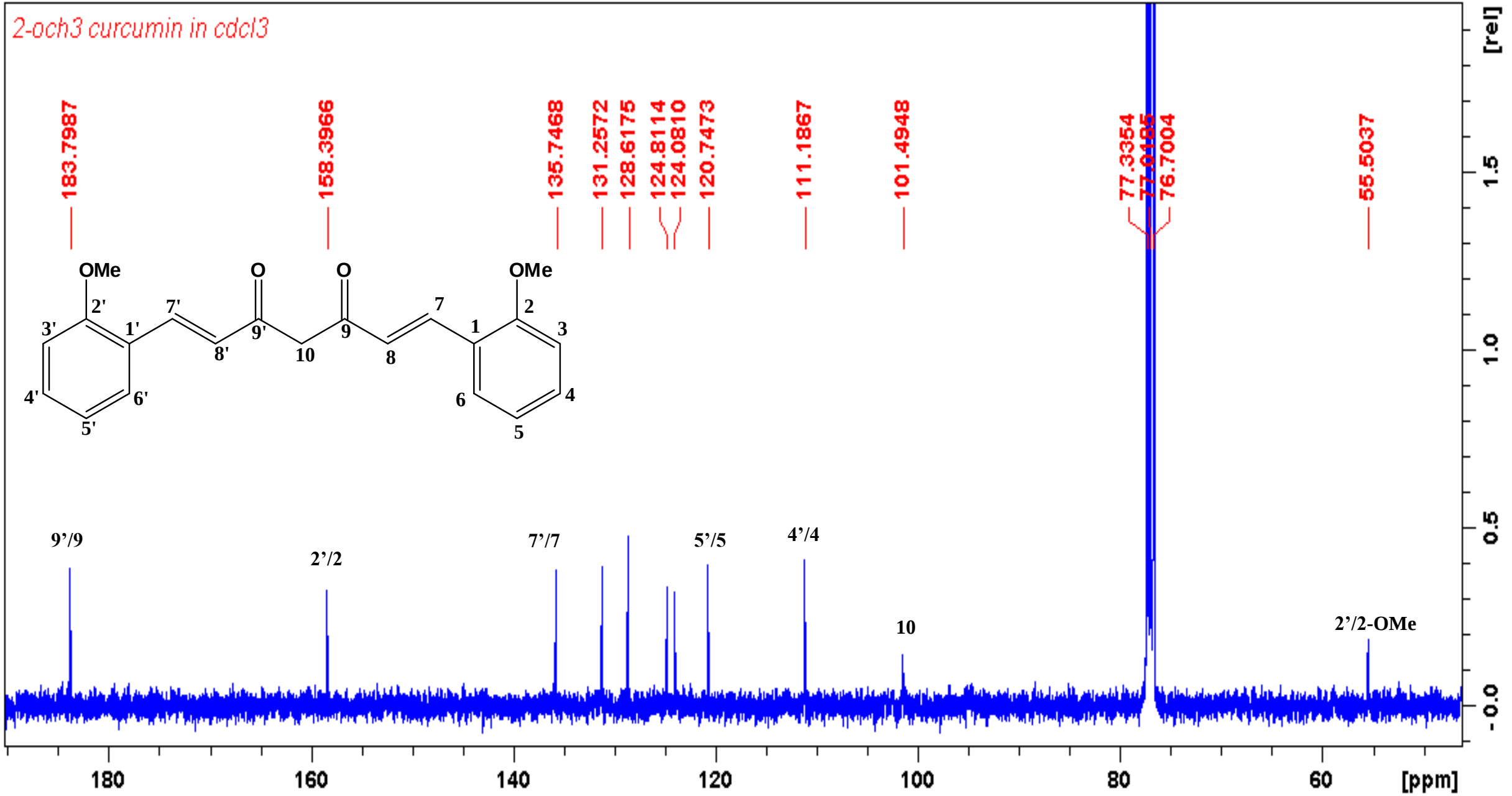
^{1}H Spectrum of Compound A-4k: (1E,6E)-1,7-bis(3,4-difluorophenyl)hepta-1,6-diene-3,5-dione



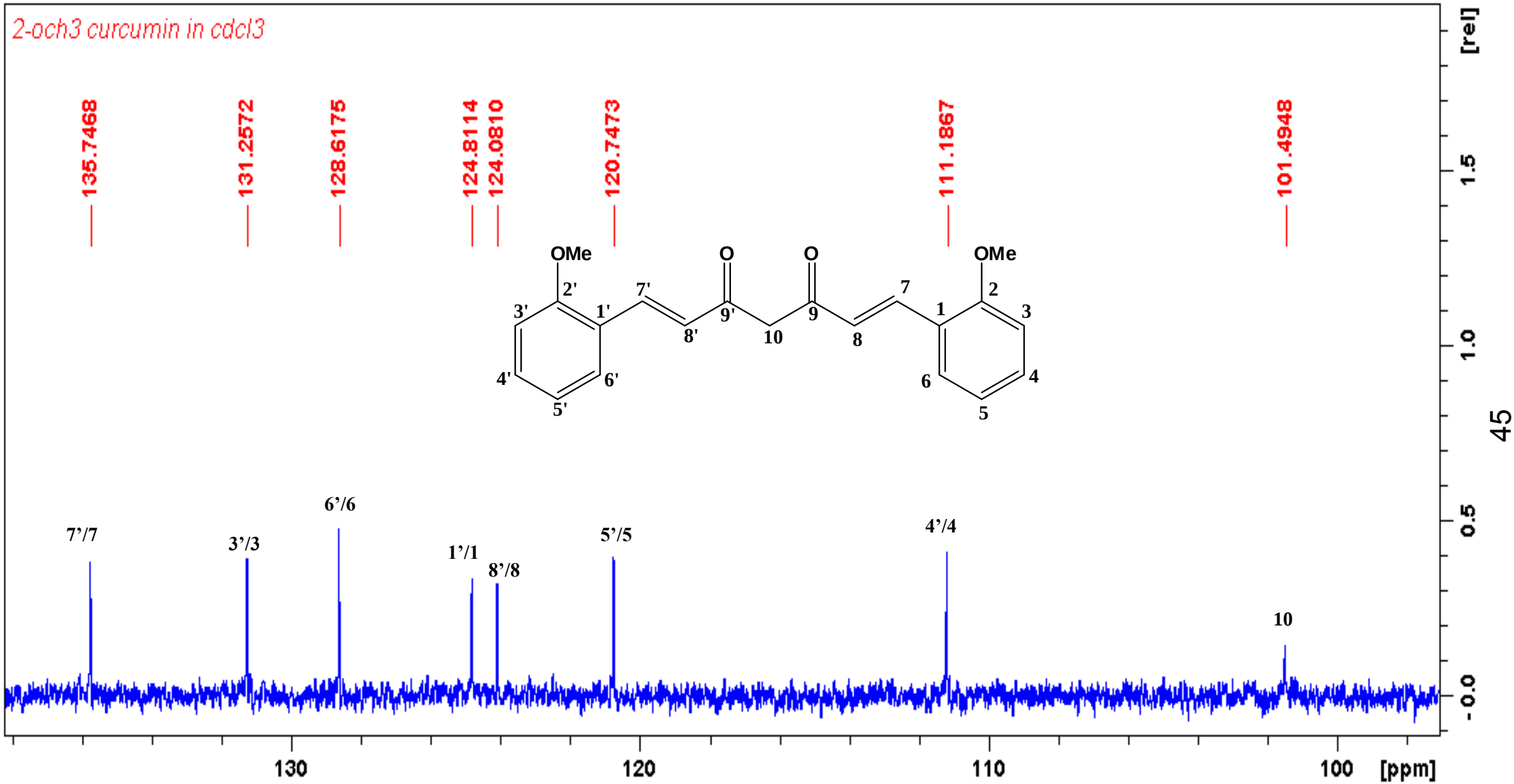
^{13}C Spectrum of Compound A-4k: (1E,6E)-1,7-bis(3,4-difluorophenyl)hepta-1,6-diene-3,5-dione



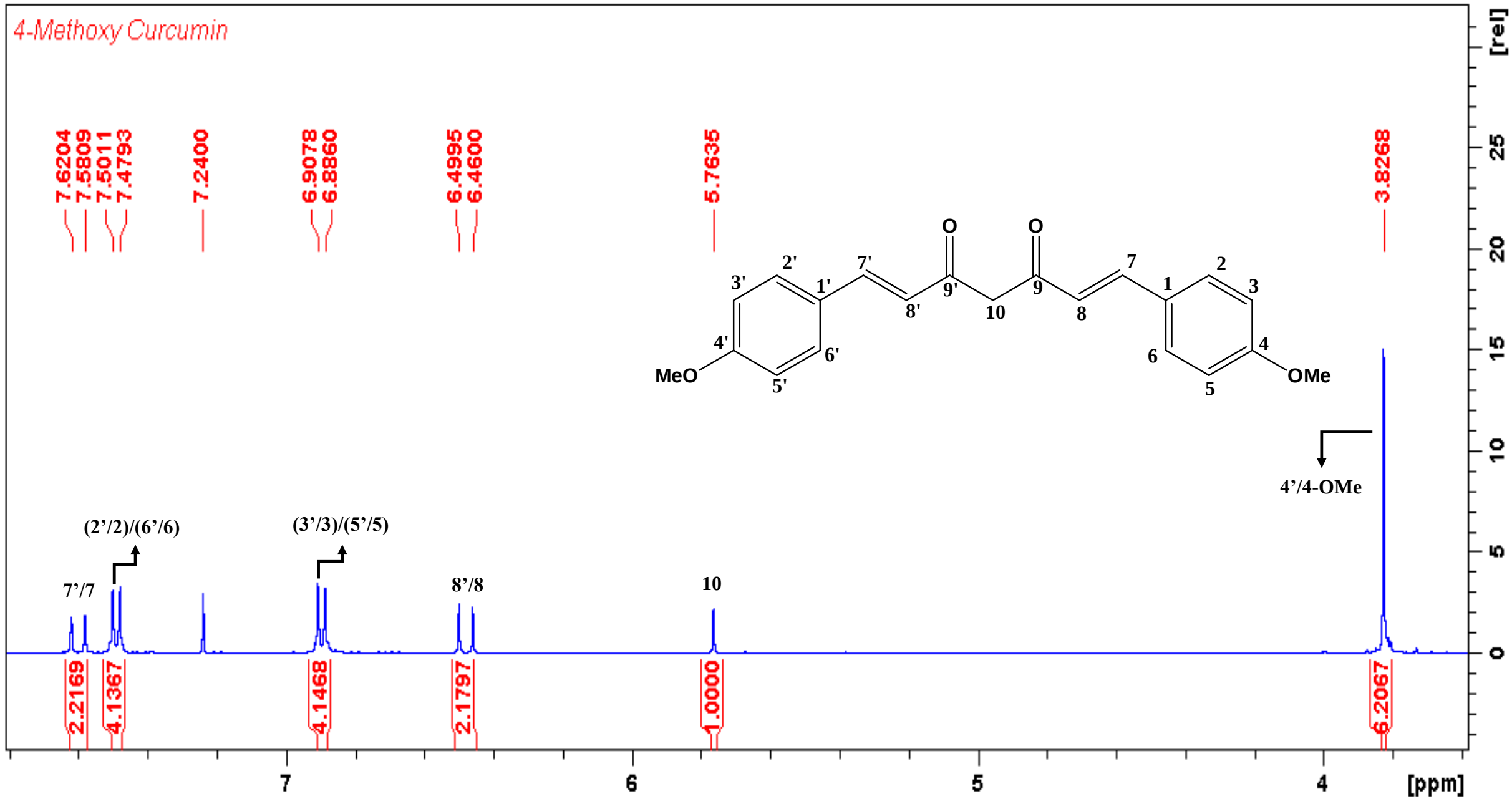
^{1}H Spectrum of Compound A-4l: (1E,6E)-1,7-bis(2-methoxyphenyl)hepta-1,6-diene-3,5-dione



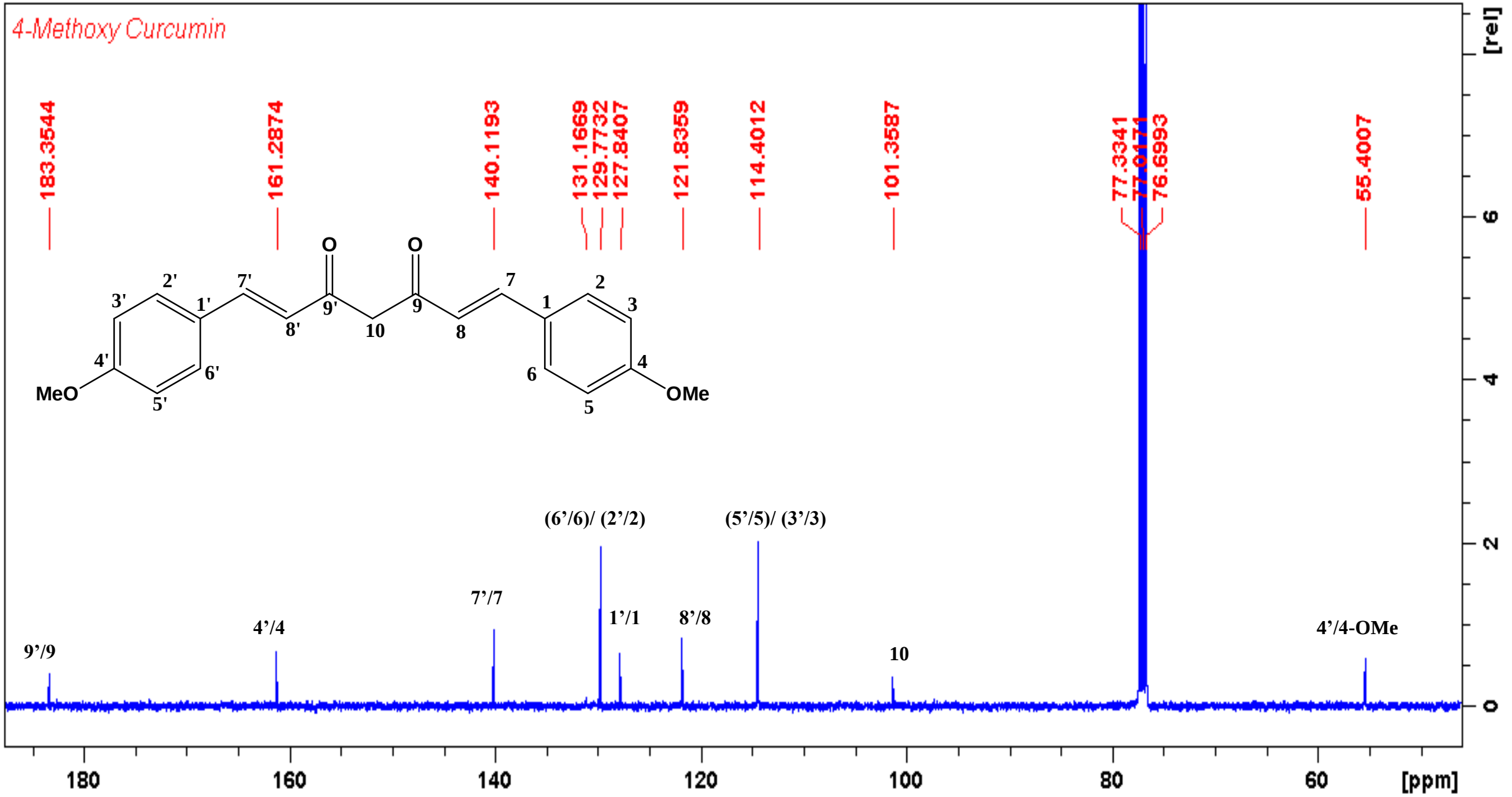
^{13}C Spectrum of Compound A-4l: (1E,6E)-1,7-bis(2-methoxyphenyl)hepta-1,6-diene-3,5-dione



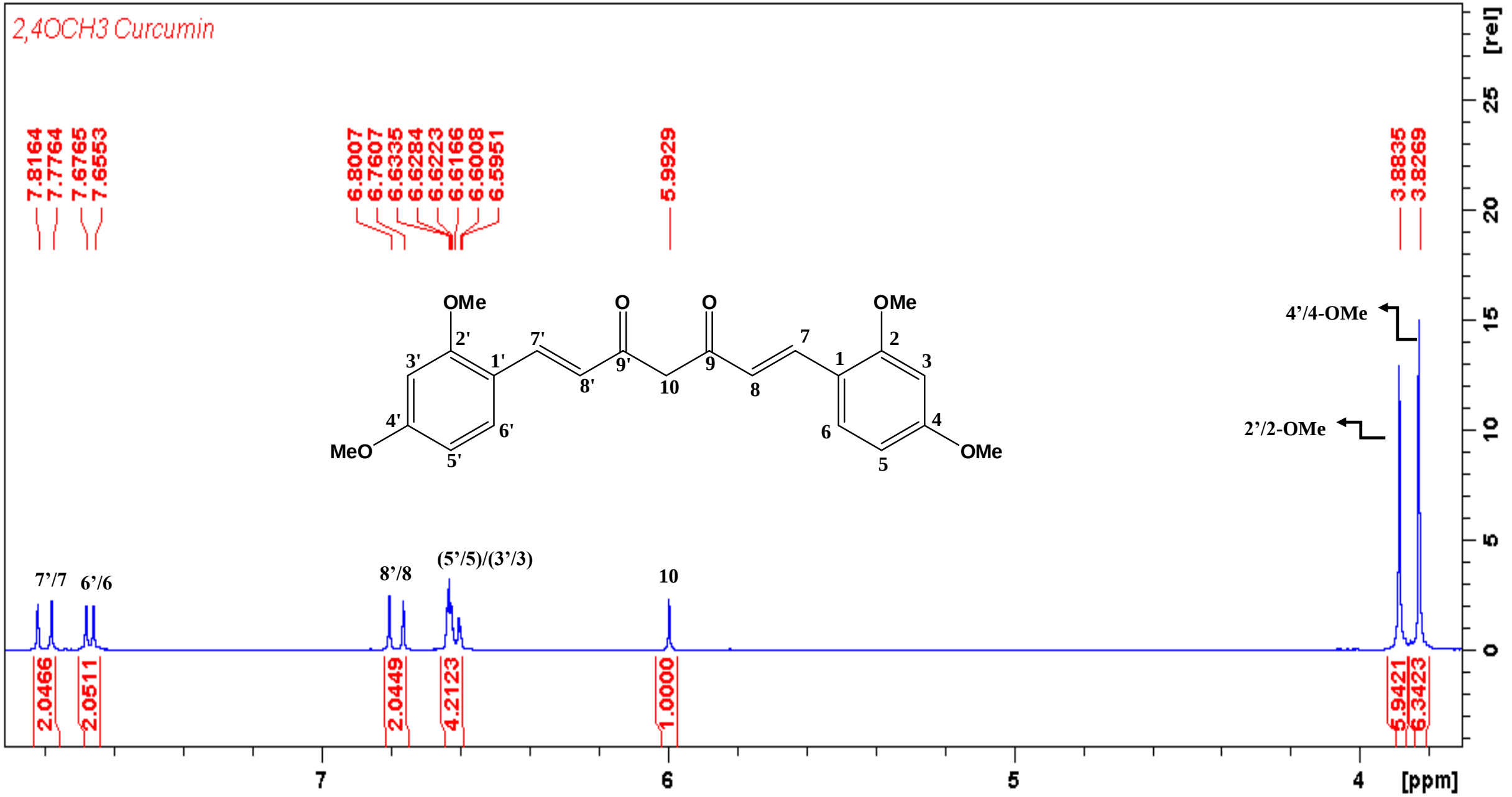
Expanded ^{13}C Spectrum of Compound A-4l: (1E,6E)-1,7-bis(2-methoxyphenyl)hepta-1,6-diene-3,5-dione



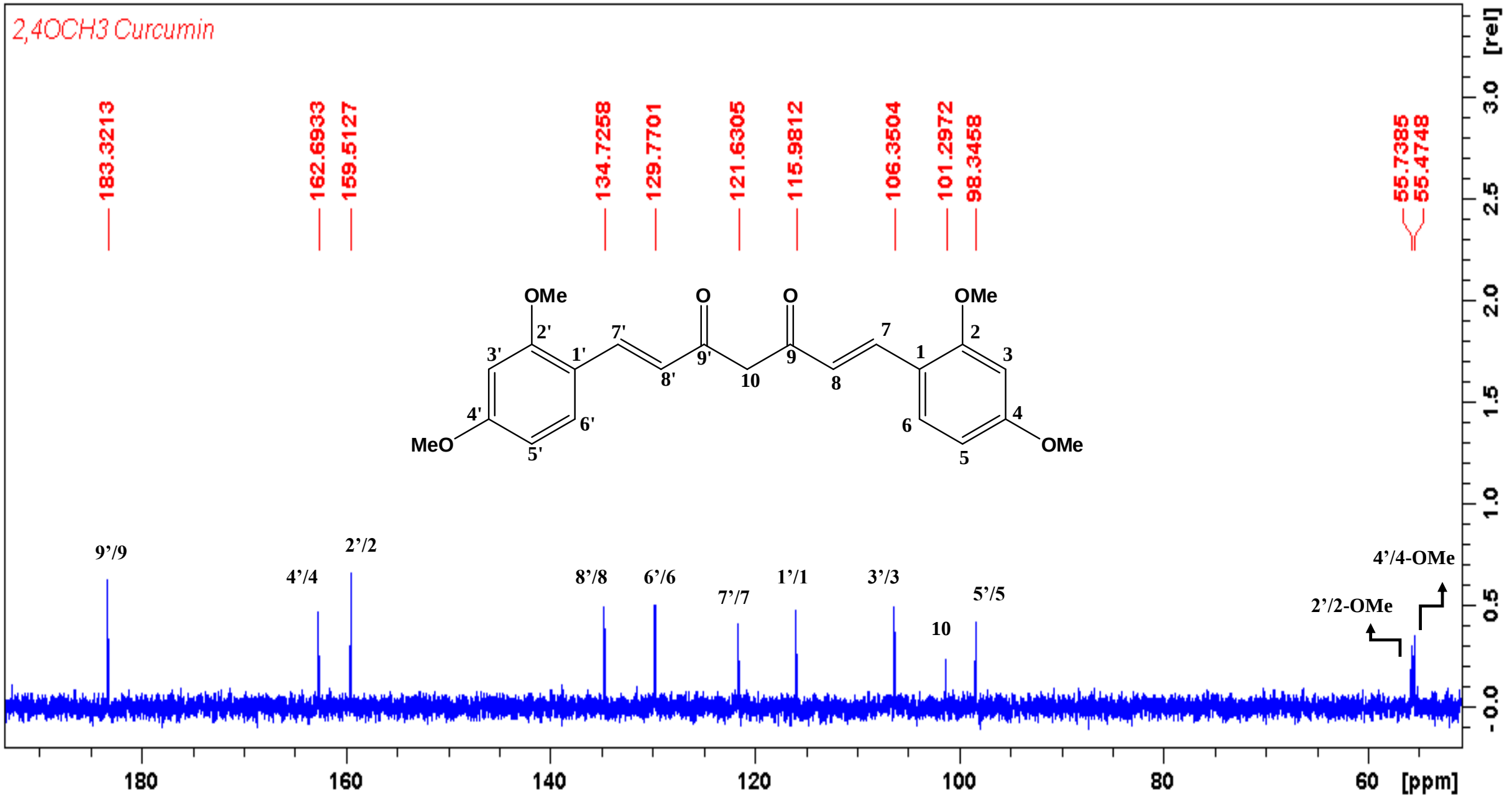
^{1}H Spectrum of Compound A-4m: (1E,6E)-1,7-bis(4-methoxyphenyl)hepta-1,6-diene-3,5-dione



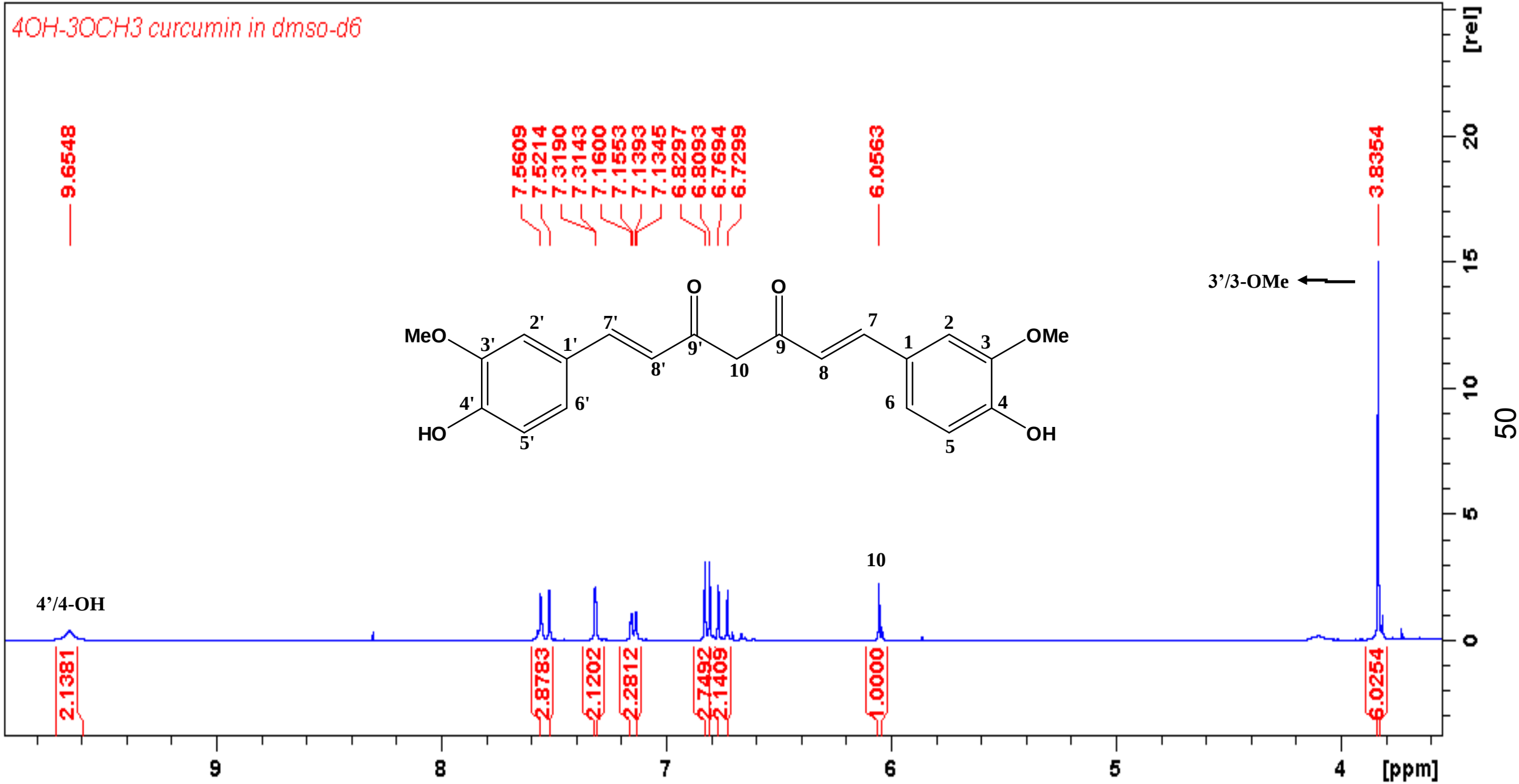
^{13}C Spectrum of Compound A-4m: (1E,6E)-1,7-bis(4-methoxyphenyl)hepta-1,6-diene-3,5-dione



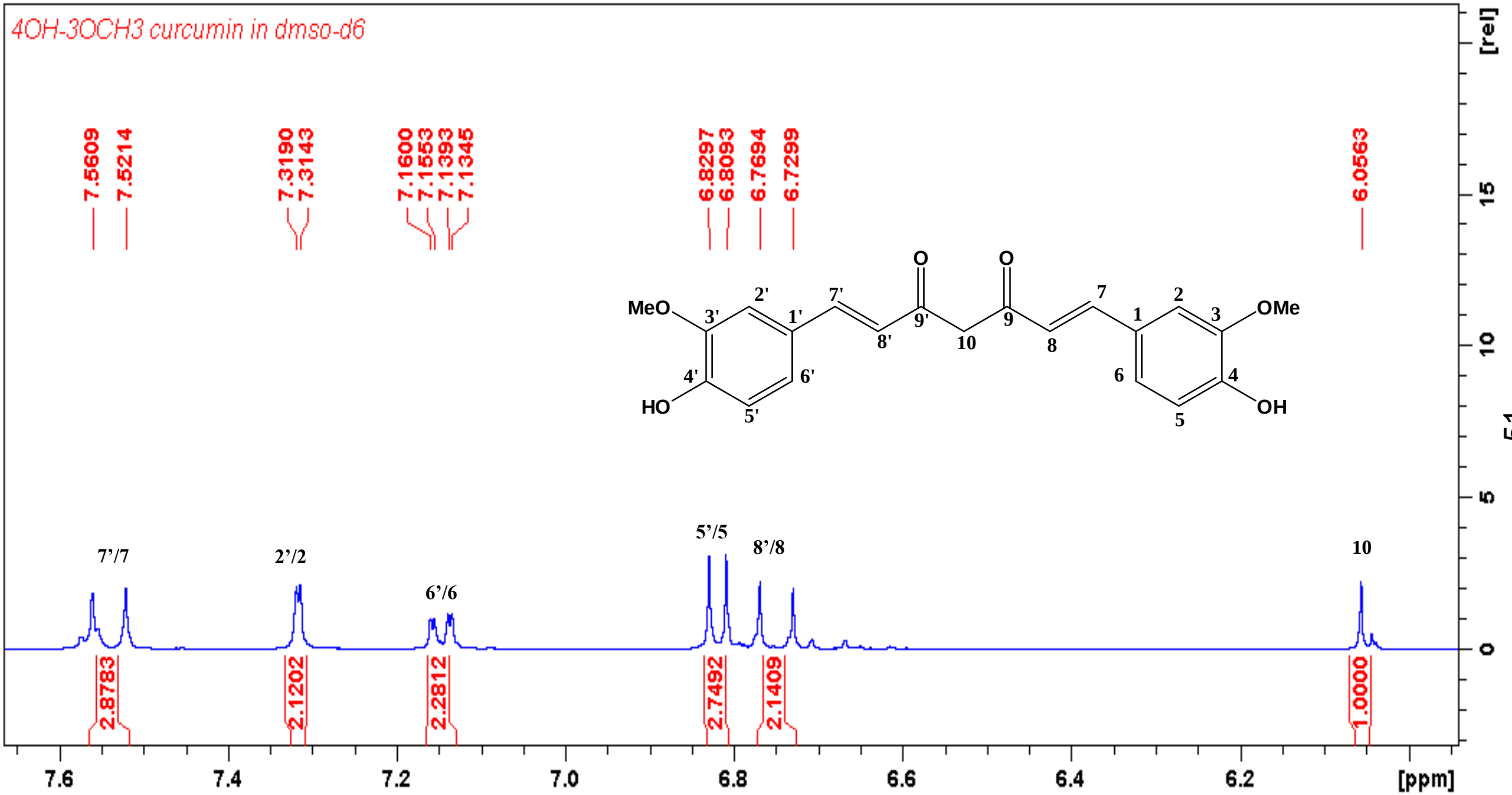
^{1}H Spectrum of Compound A-4n: (1E,6E)-1,7-bis(2,4-dimethoxyphenyl)hepta-1,6-diene-3,5-dione



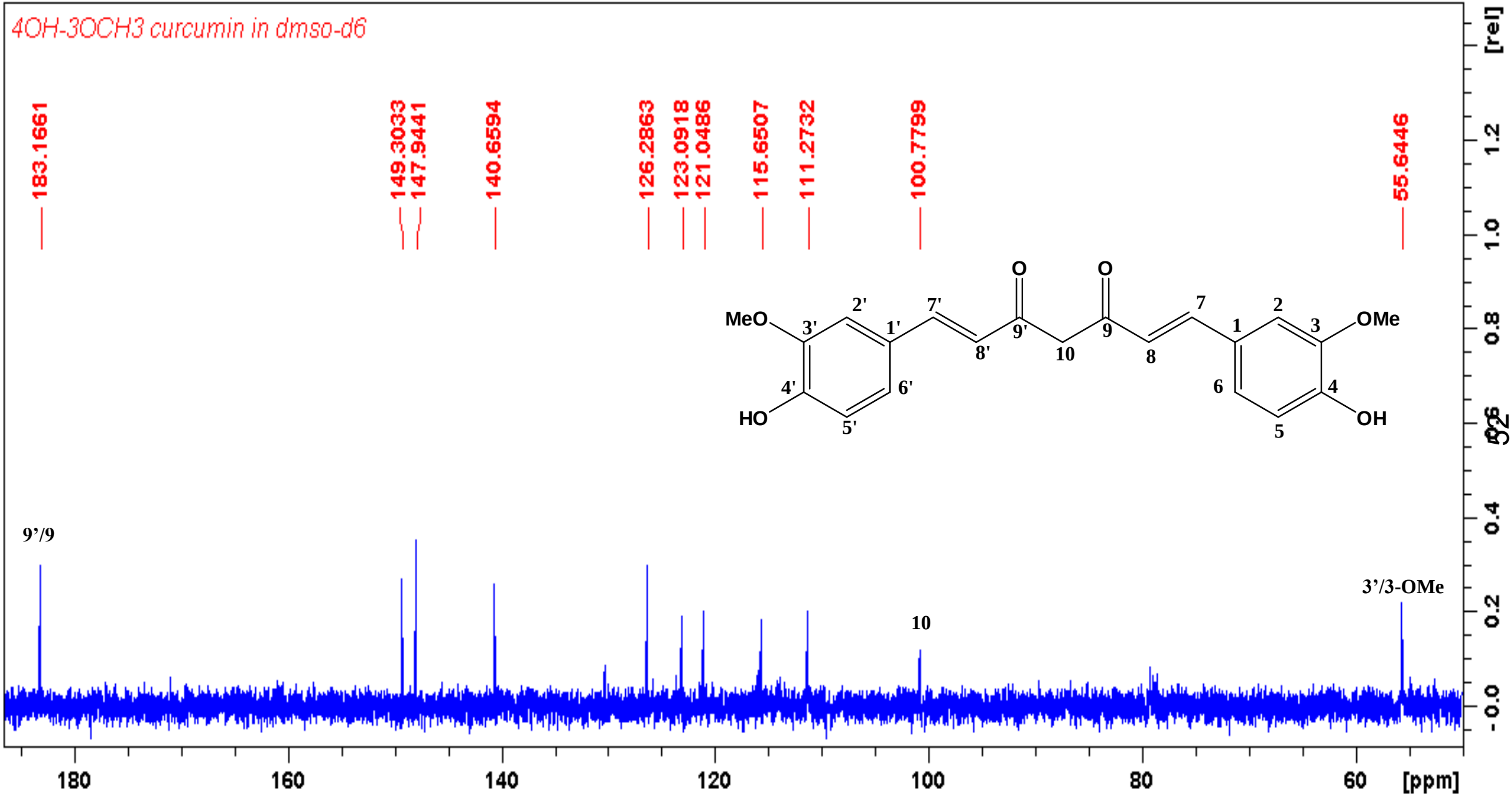
^{13}C Spectrum of Compound A-4n: (1E,6E)-1,7-bis(2,4-dimethoxyphenyl)hepta-1,6-diene-3,5-dione



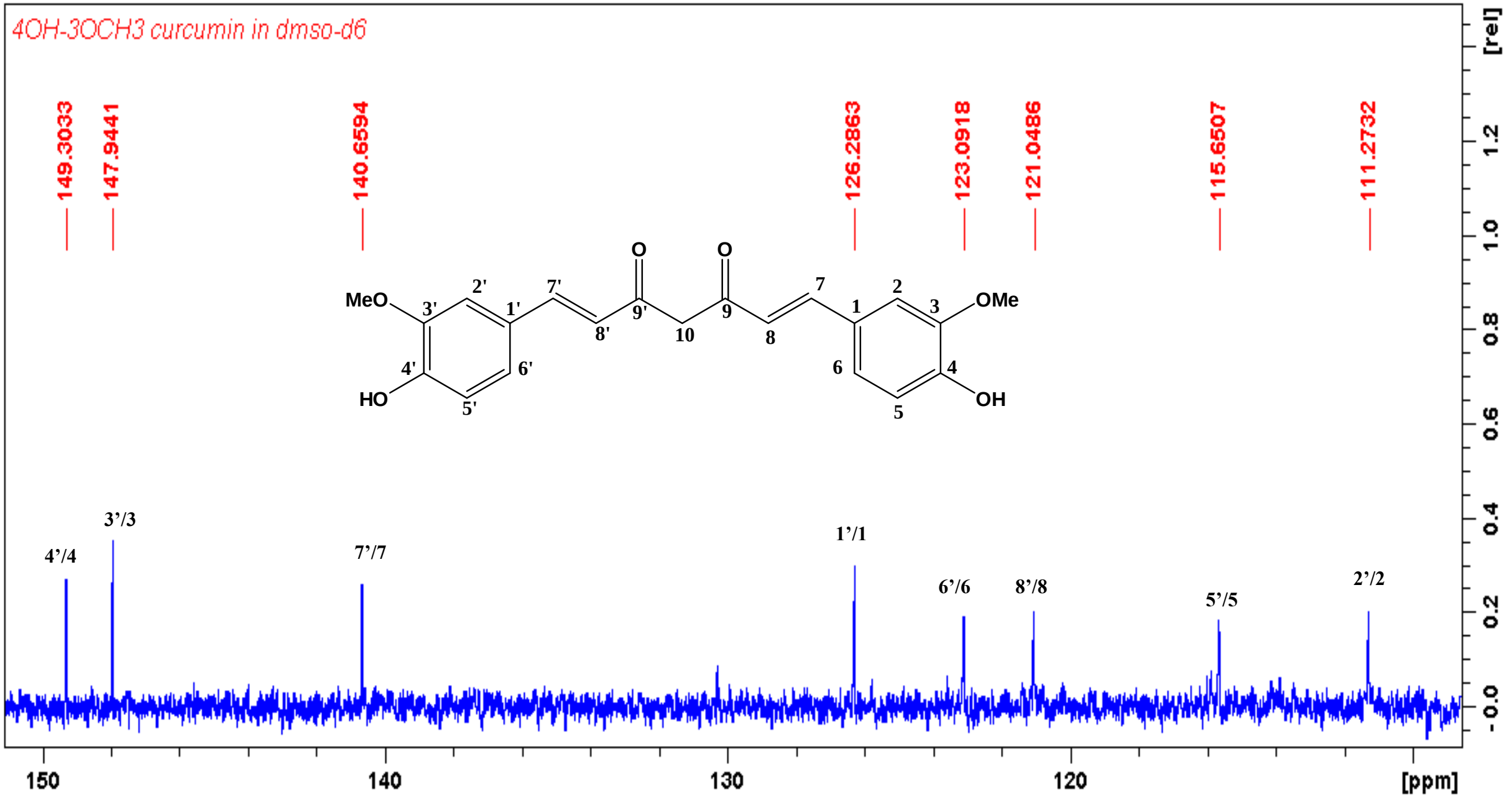
^{1}H Spectrum of Compound A-4o: (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



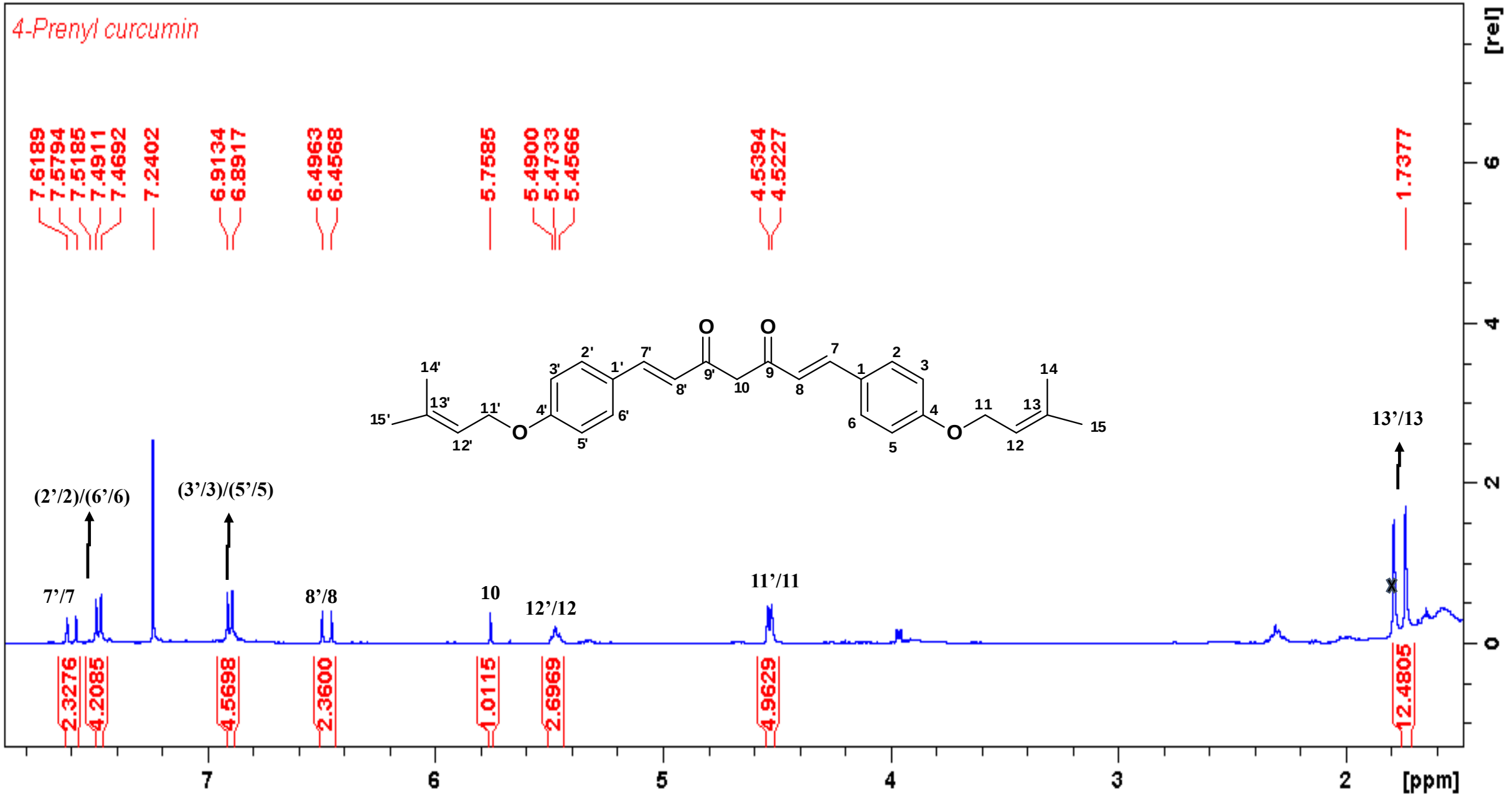
Expanded ^{1}H Spectrum of Compound A-4o: (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



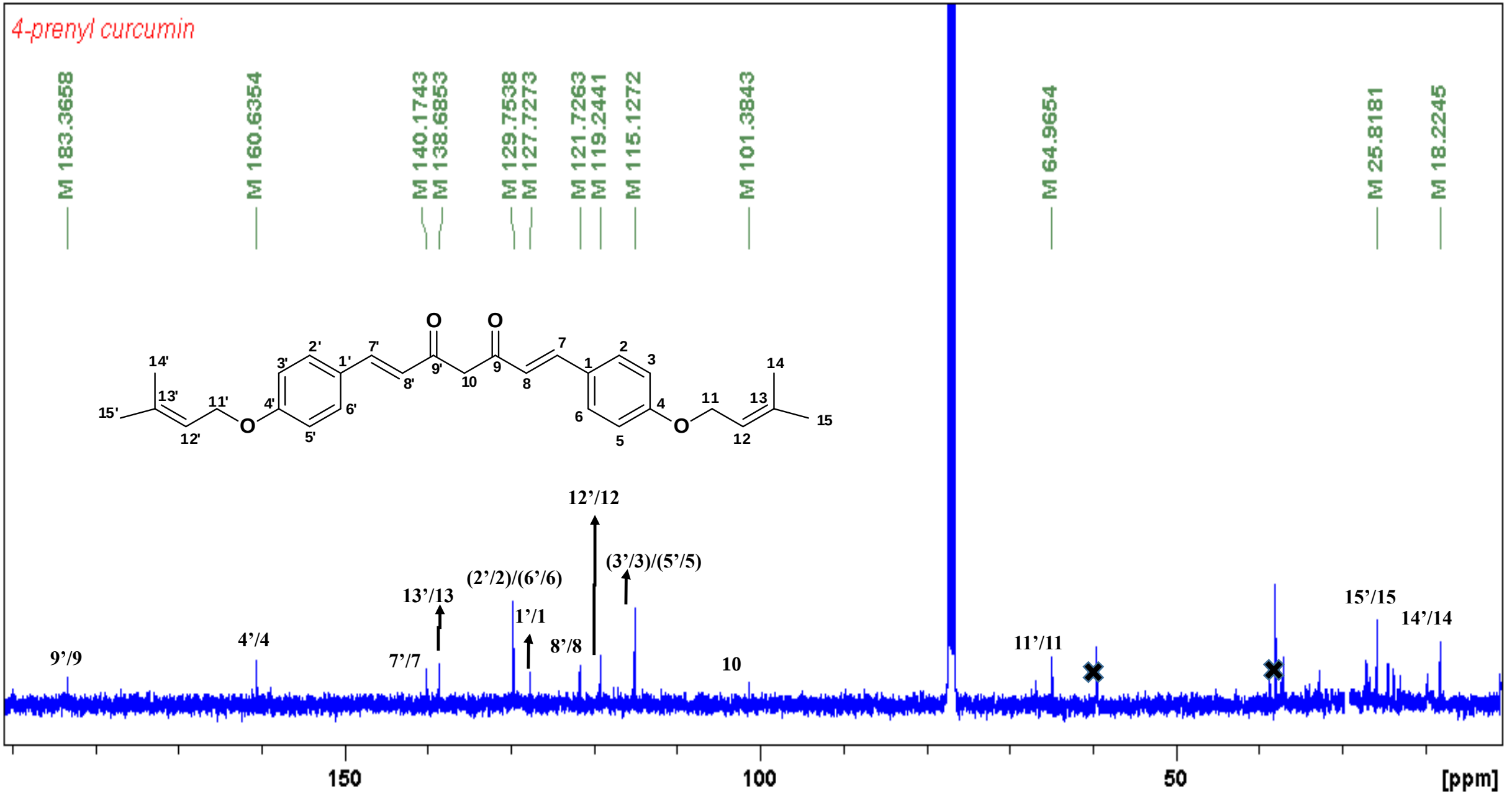
^{13}C Spectrum of Compound A-4o: (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



Expanded ^{13}C Spectrum of Compound A-4o: (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



^{1}H Spectrum of Compound A-4p: (1E,6E)-1,7-bis(4-(3-methylbut-2-enyloxy)phenyl)hepta-1,6-diene-3,5-dione



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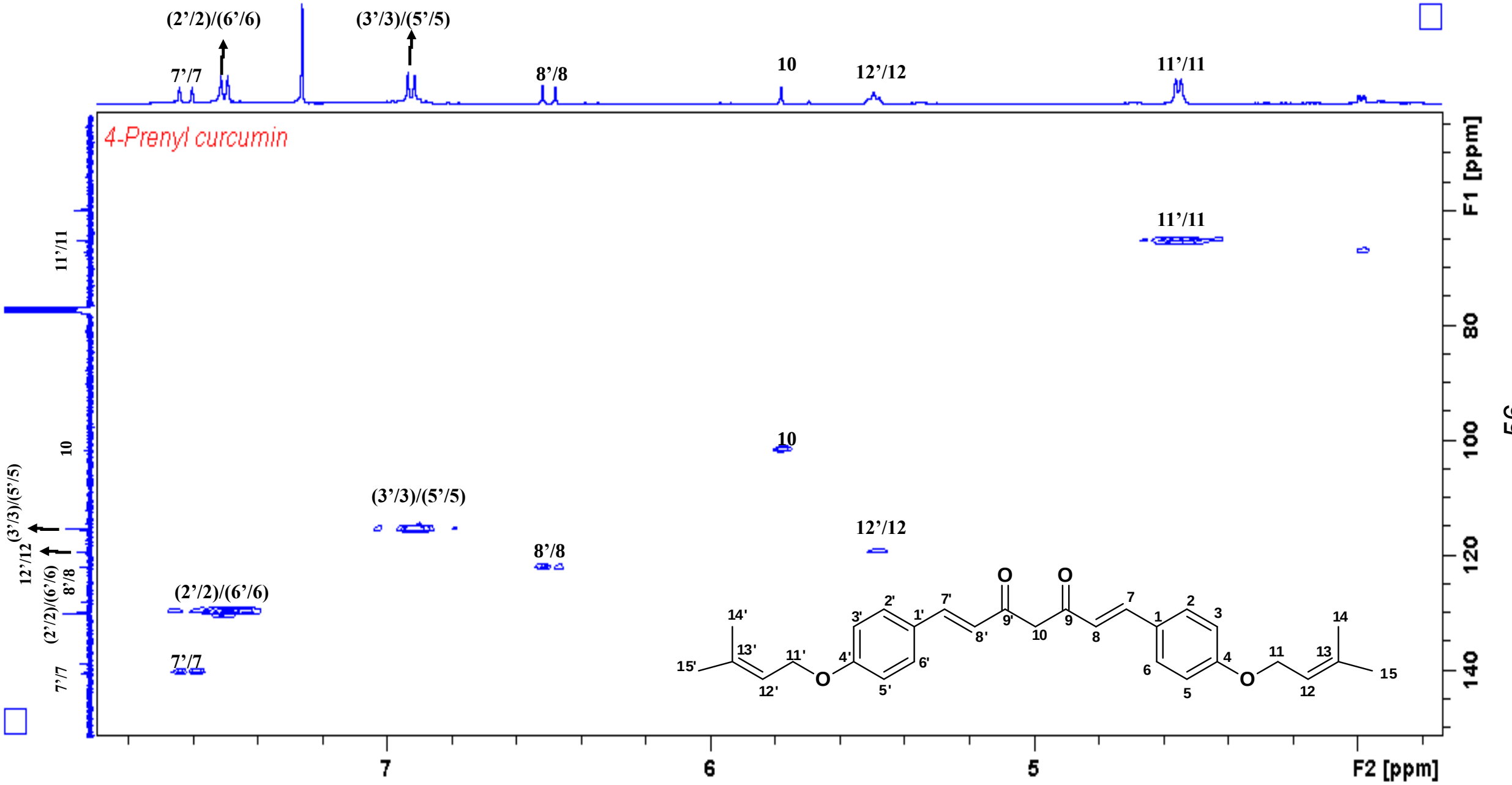
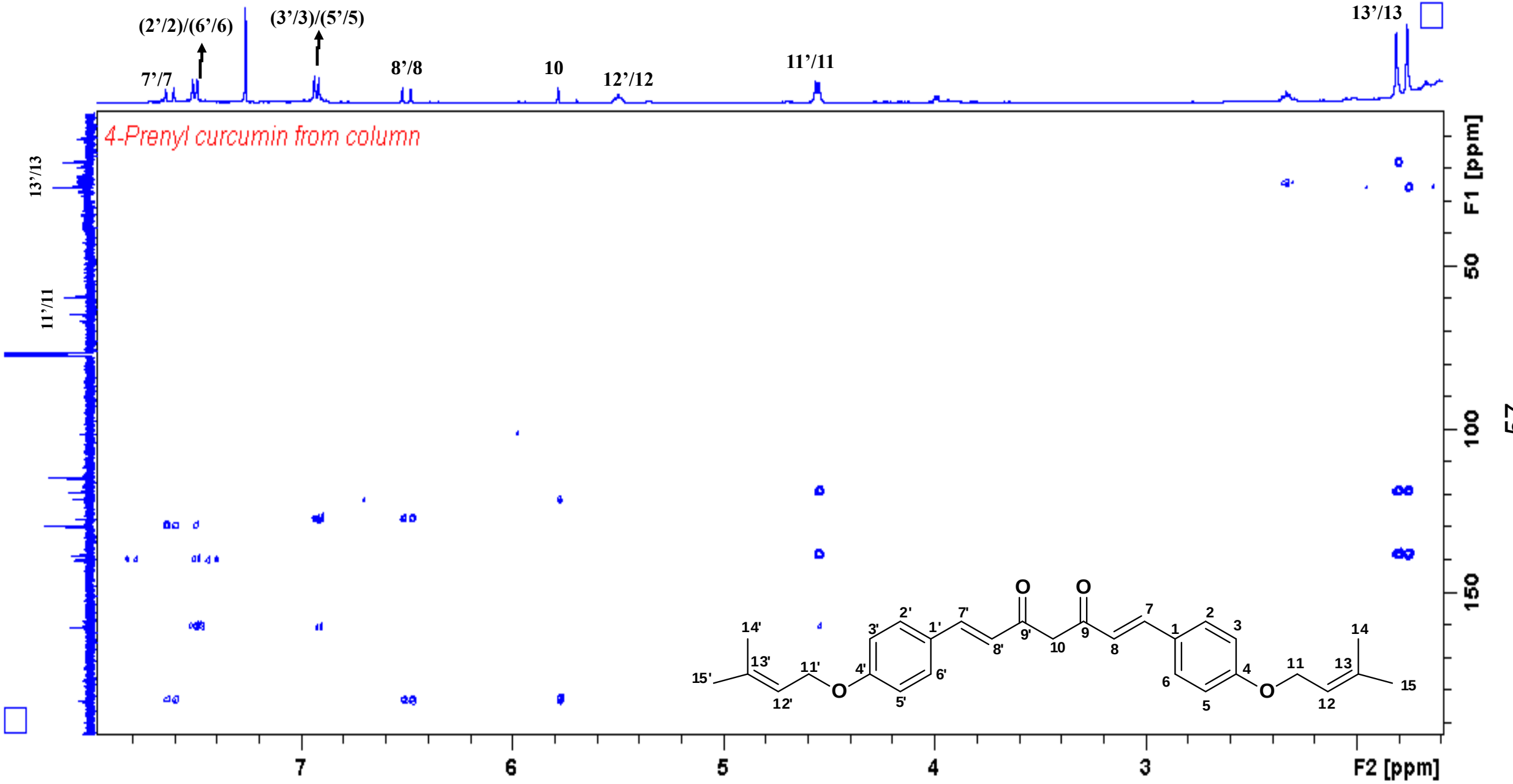
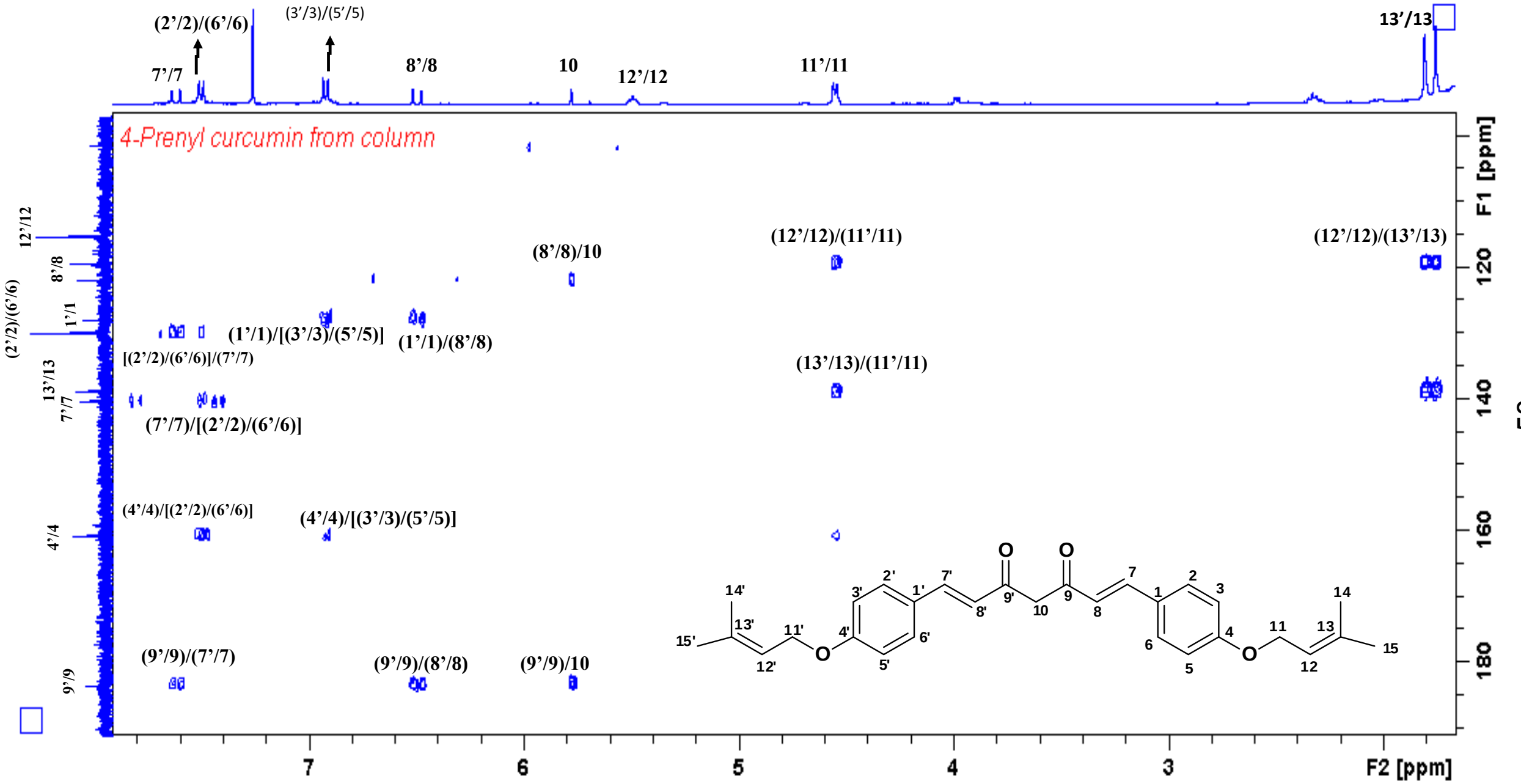
^{13}C Spectrum of Compound A-4p: (1E,6E)-1,7-bis(4-(3-methylbut-2-enyloxy)phenyl)hepta-1,6-diene-3,5-dione



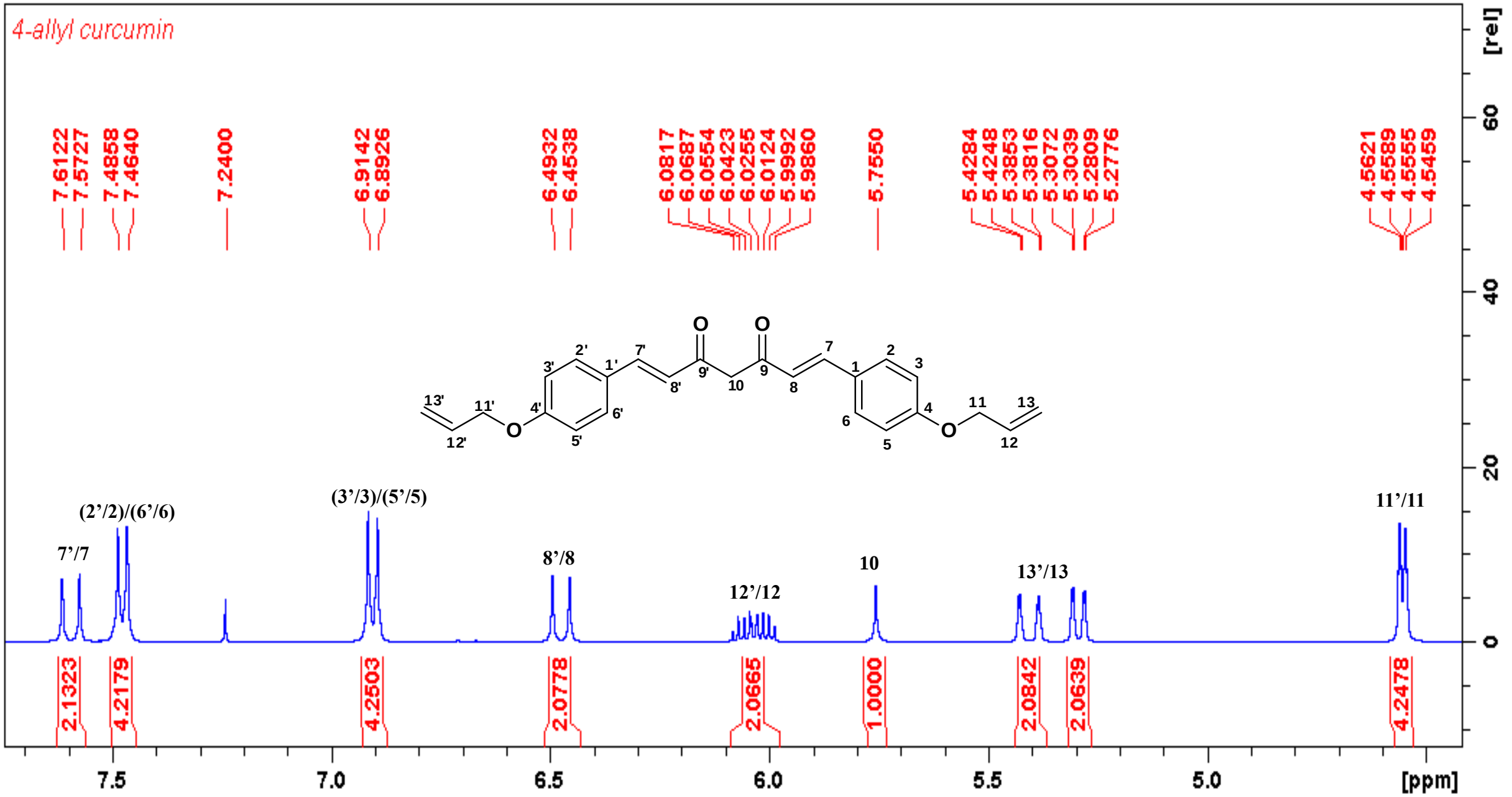
HSQC of Compound A-4p: (1E,6E)-1,7-bis(4-(3-methylbut-2-enyloxy)phenyl)hepta-1,6-diene-3,5-dione



HMBC of Compound A-4p: (1E,6E)-1,7-bis(4-(3-methylbut-2-enyloxy)phenyl)hepta-1,6-diene-3,5-dione



Expanded HMBC of Compound A-4p: (1E,6E)-1,7-bis(4-(3-methylbut-2-enyloxy)phenyl)hepta-1,6-diene-3,5-dione



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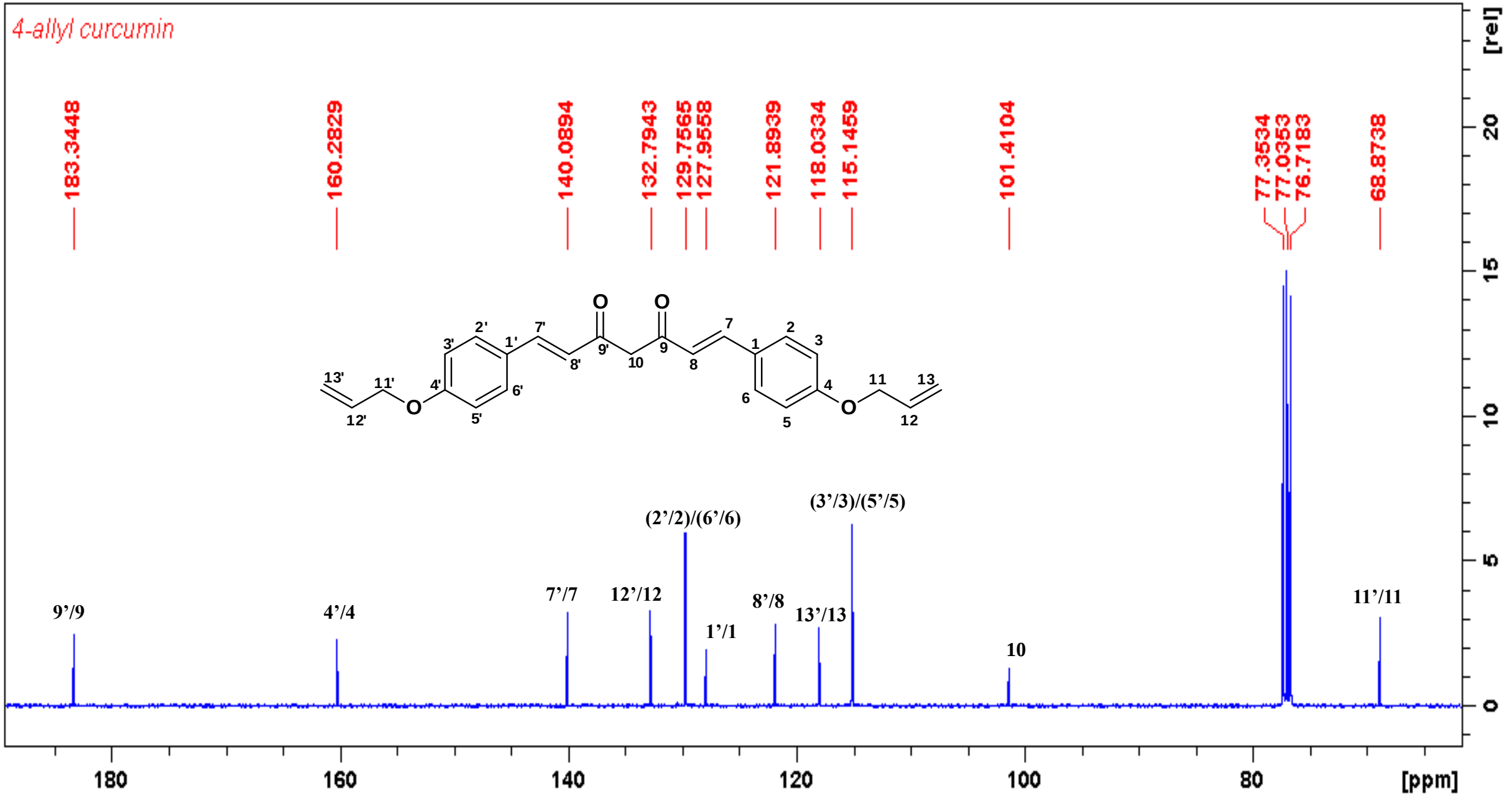
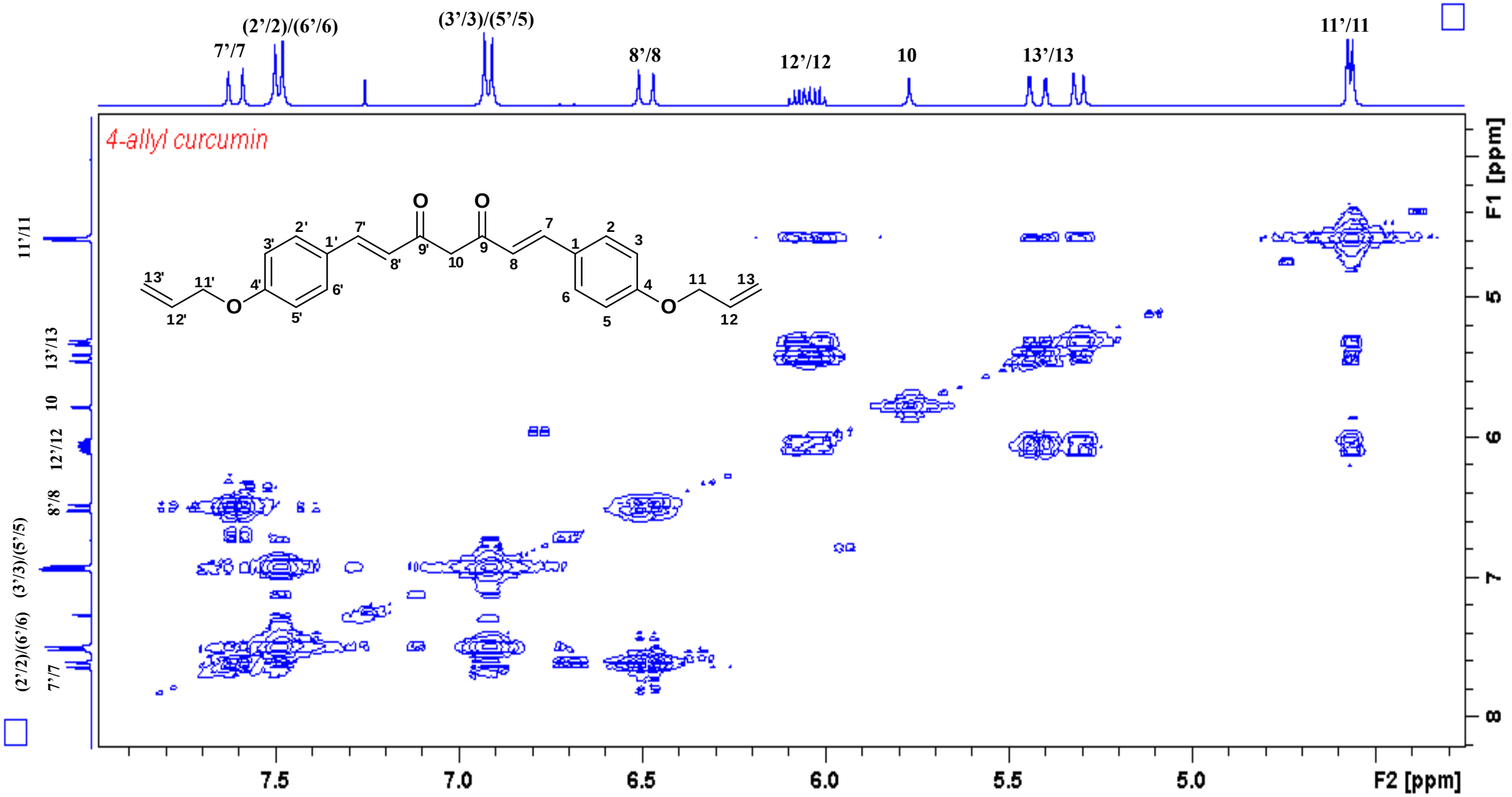
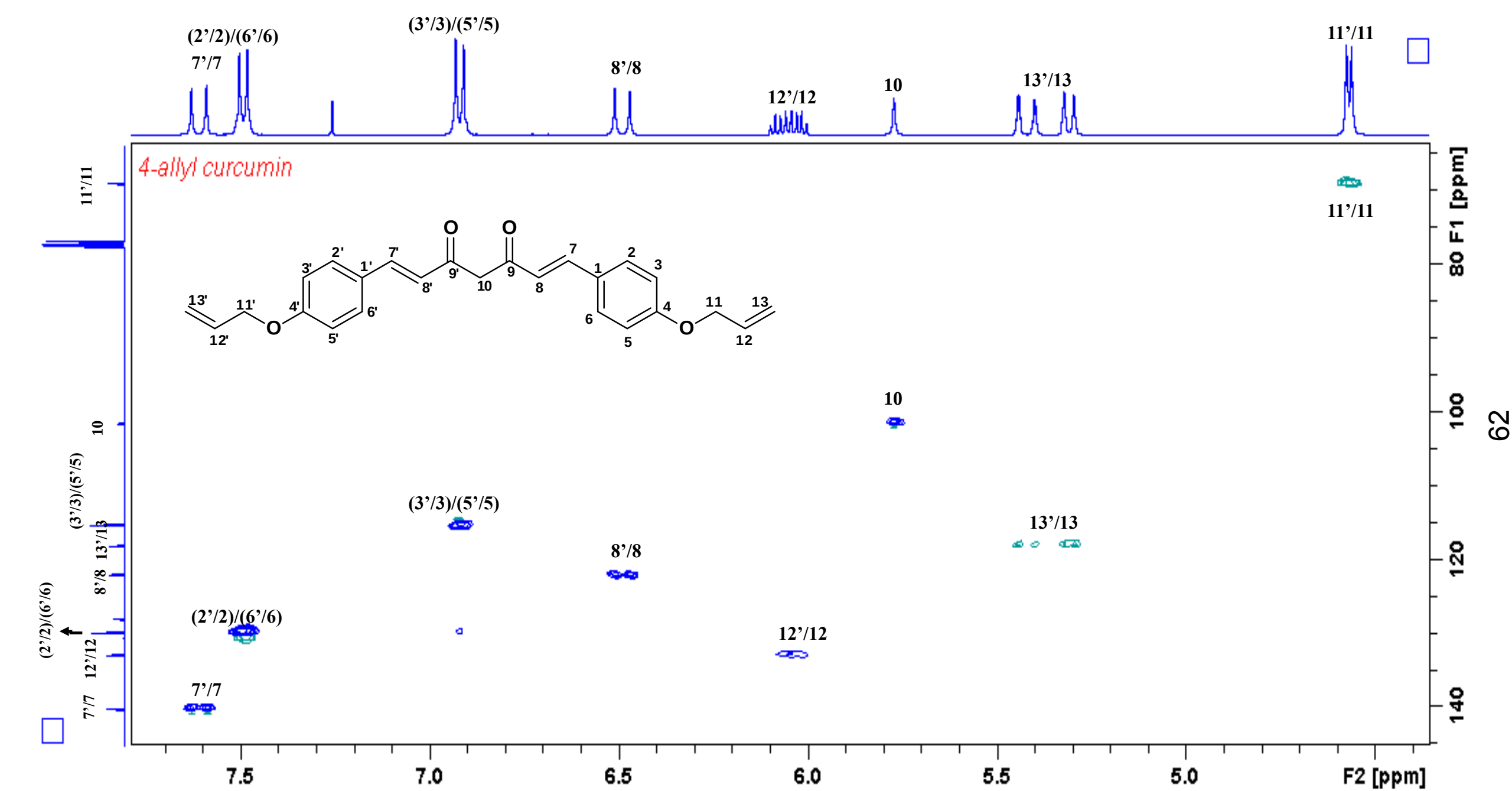
^{1}H spectrum of Compound A-4q: (1E,6E)-1,7-bis(4-(allyloxy)phenyl)hepta-1,6-diene-3,5-dione



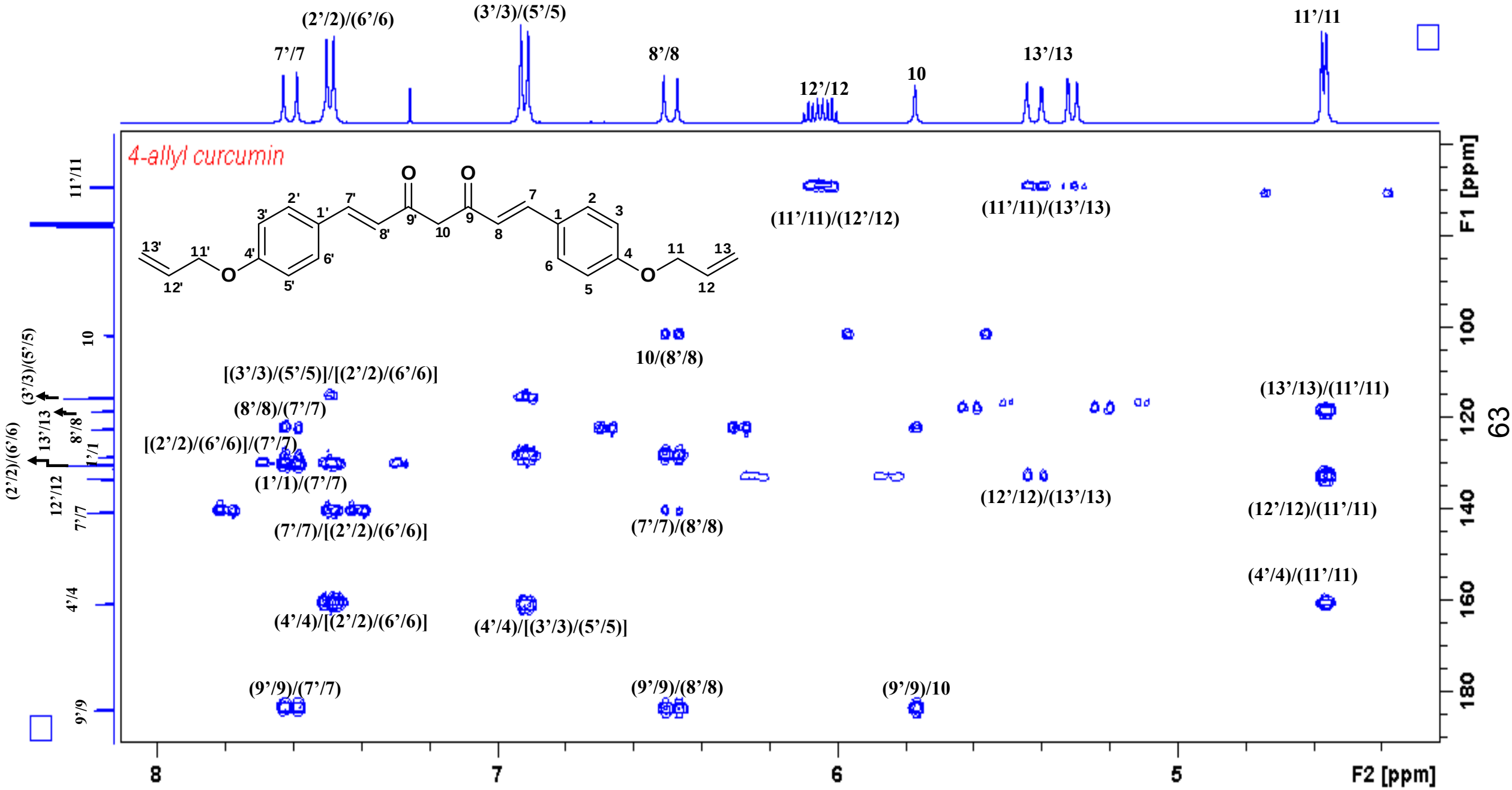
^{13}C spectrum of Compound A-4q: (1E,6E)-1,7-bis(4-(allyloxy)phenyl)hepta-1,6-diene-3,5-dione



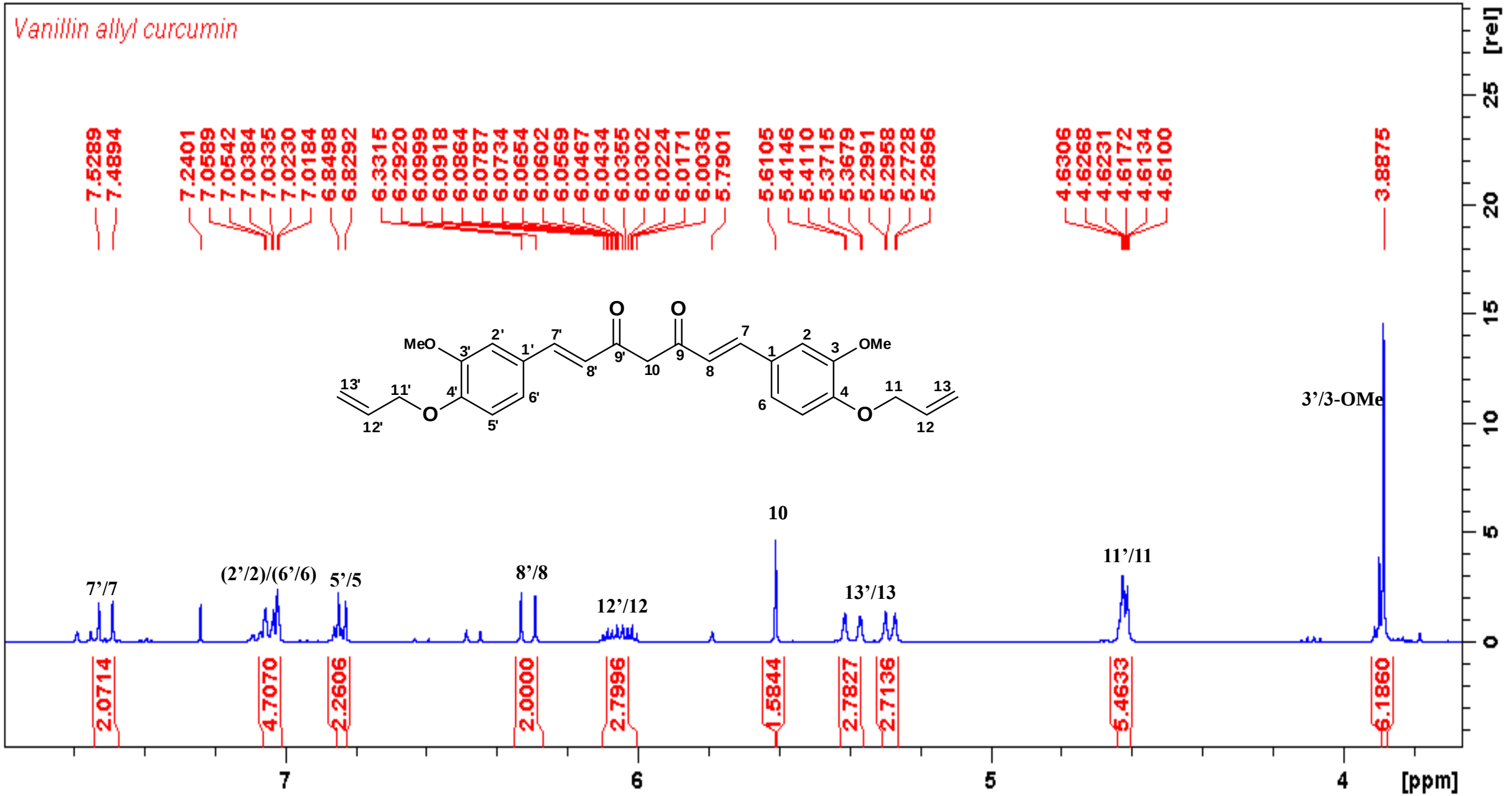
COSY of Compound A-4q: (1E,6E)-1,7-bis(4-(allyloxy)phenyl)hepta-1,6-diene-3,5-dione



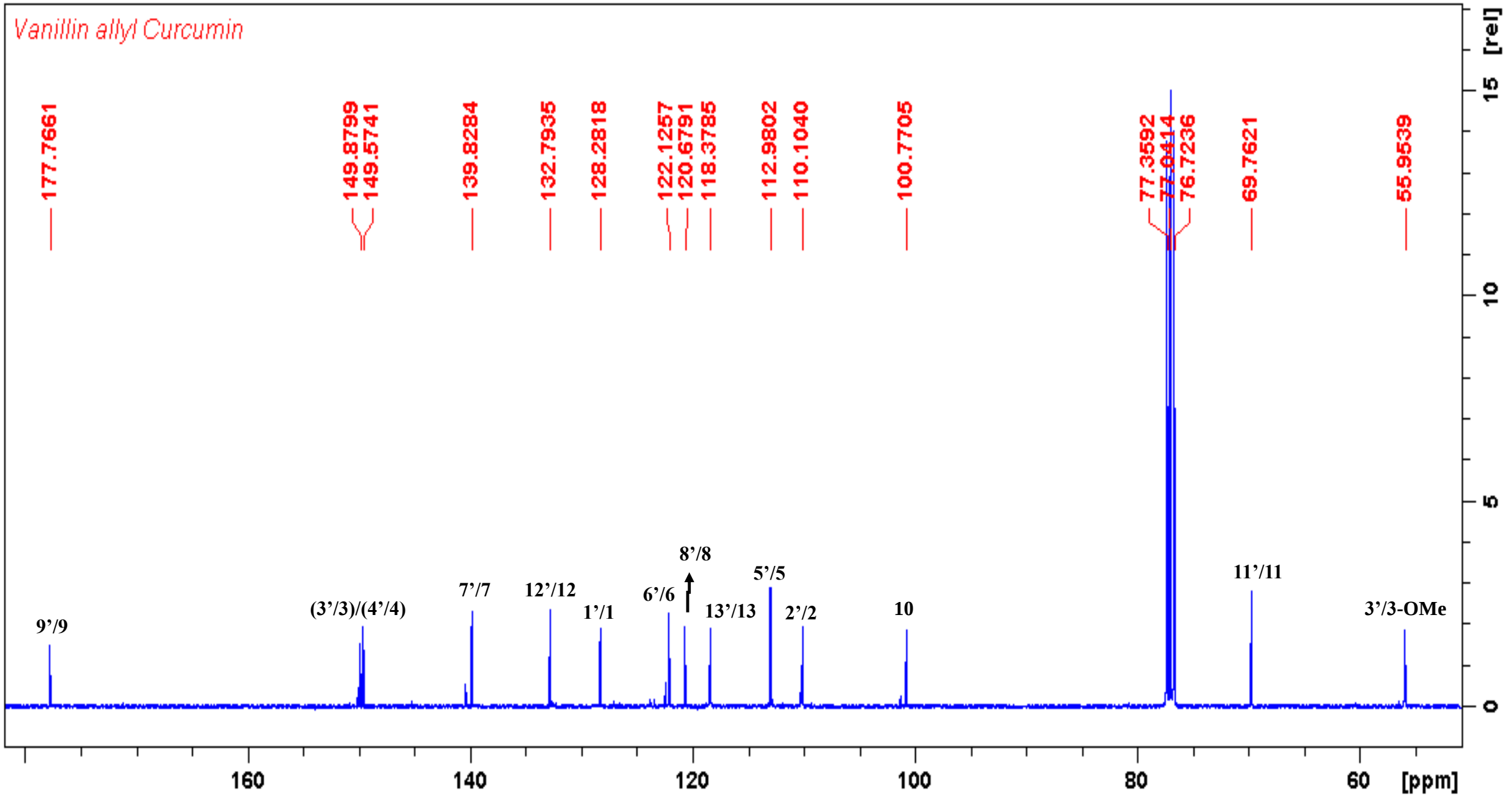
HSQC of Compound A-4q: (1E,6E)-1,7-bis(4-(allyloxy)phenyl)hepta-1,6-diene-3,5-dione



HMBC of Compound A-4q: (1E,6E)-1,7-bis(4-(allyloxy)phenyl)hepta-1,6-diene-3,5-dione



^{1}H spectrum of Compound A-4r: (1E,6E)-1,7-bis(4-(allyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



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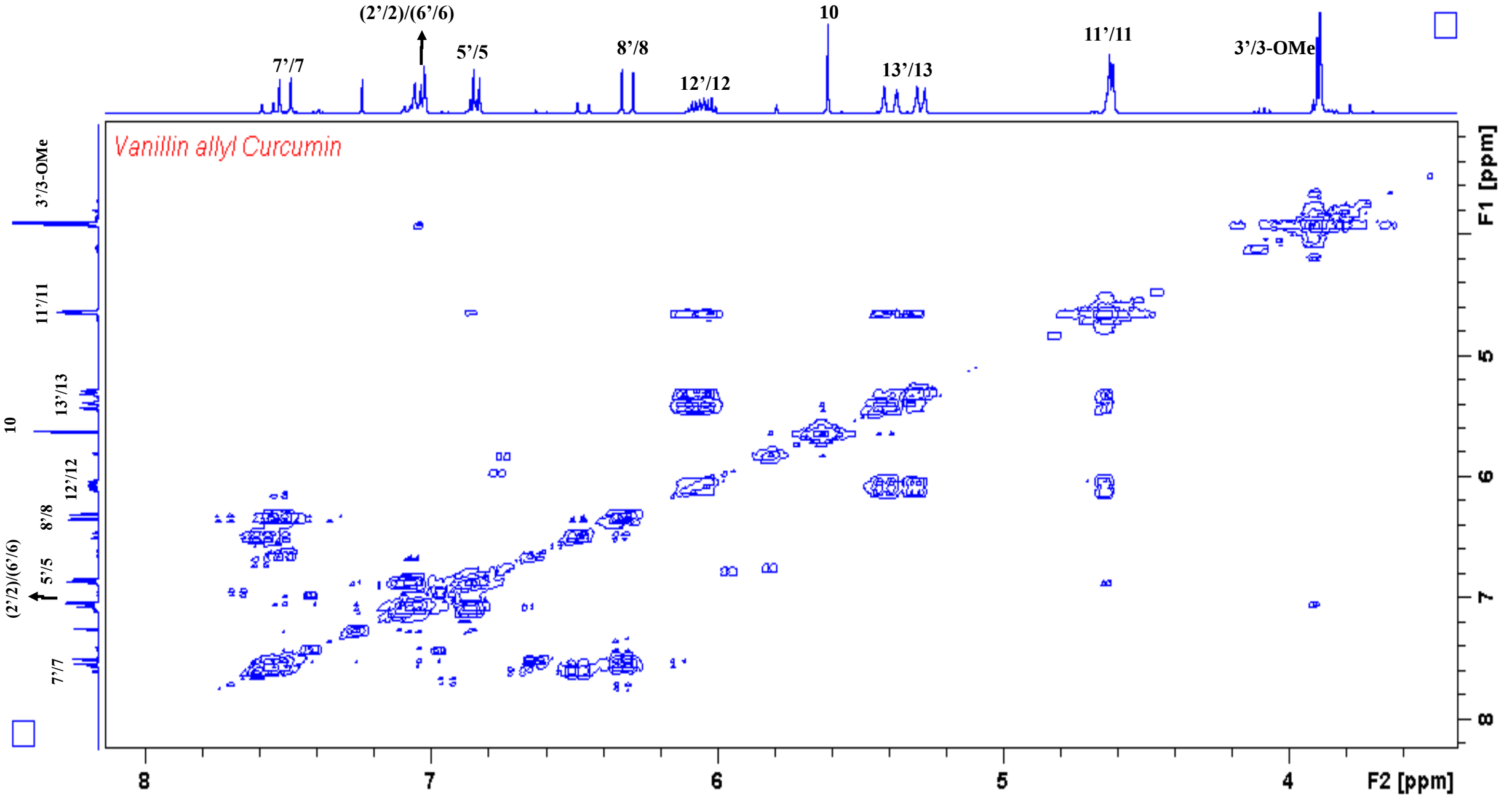
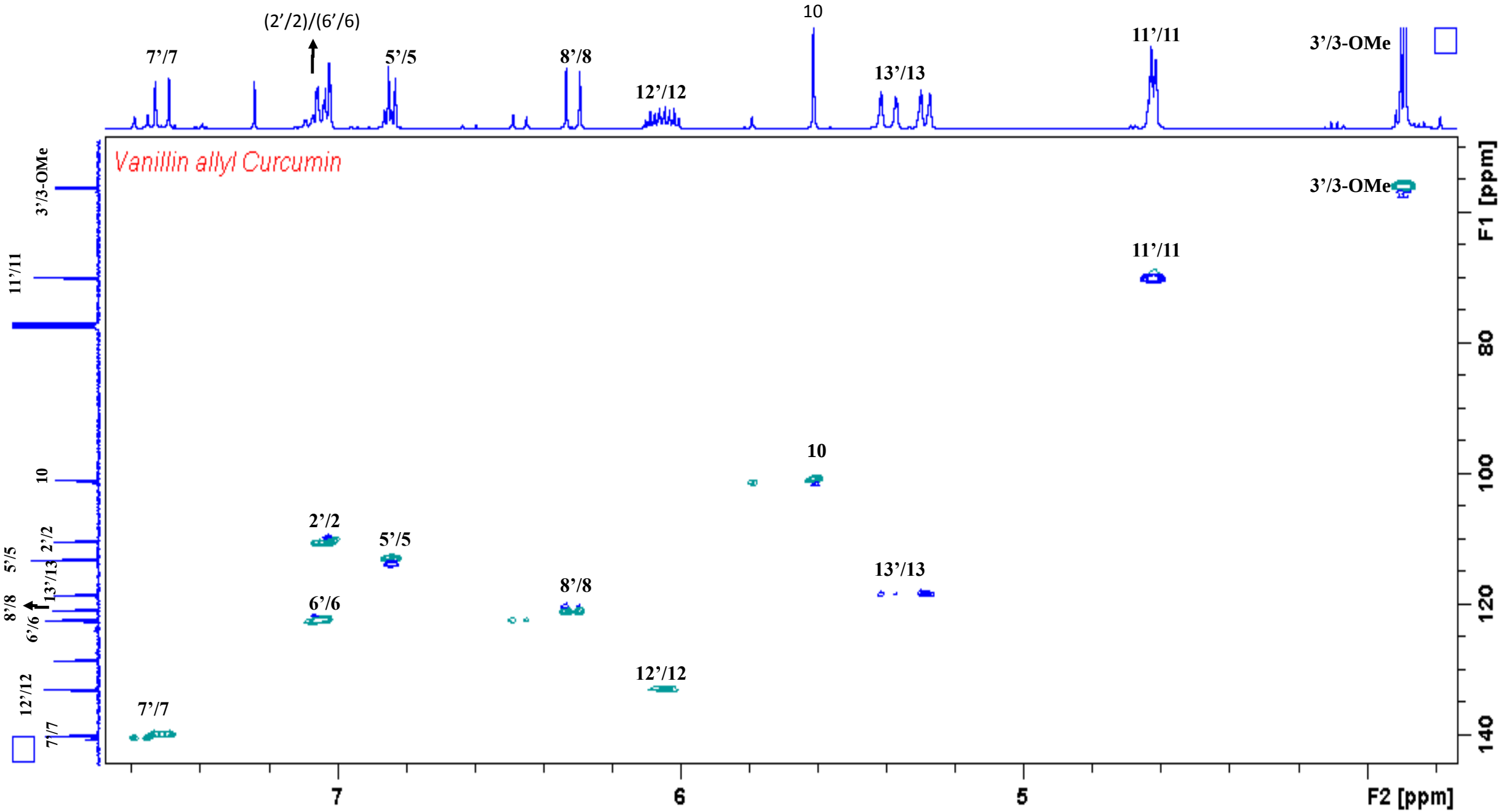
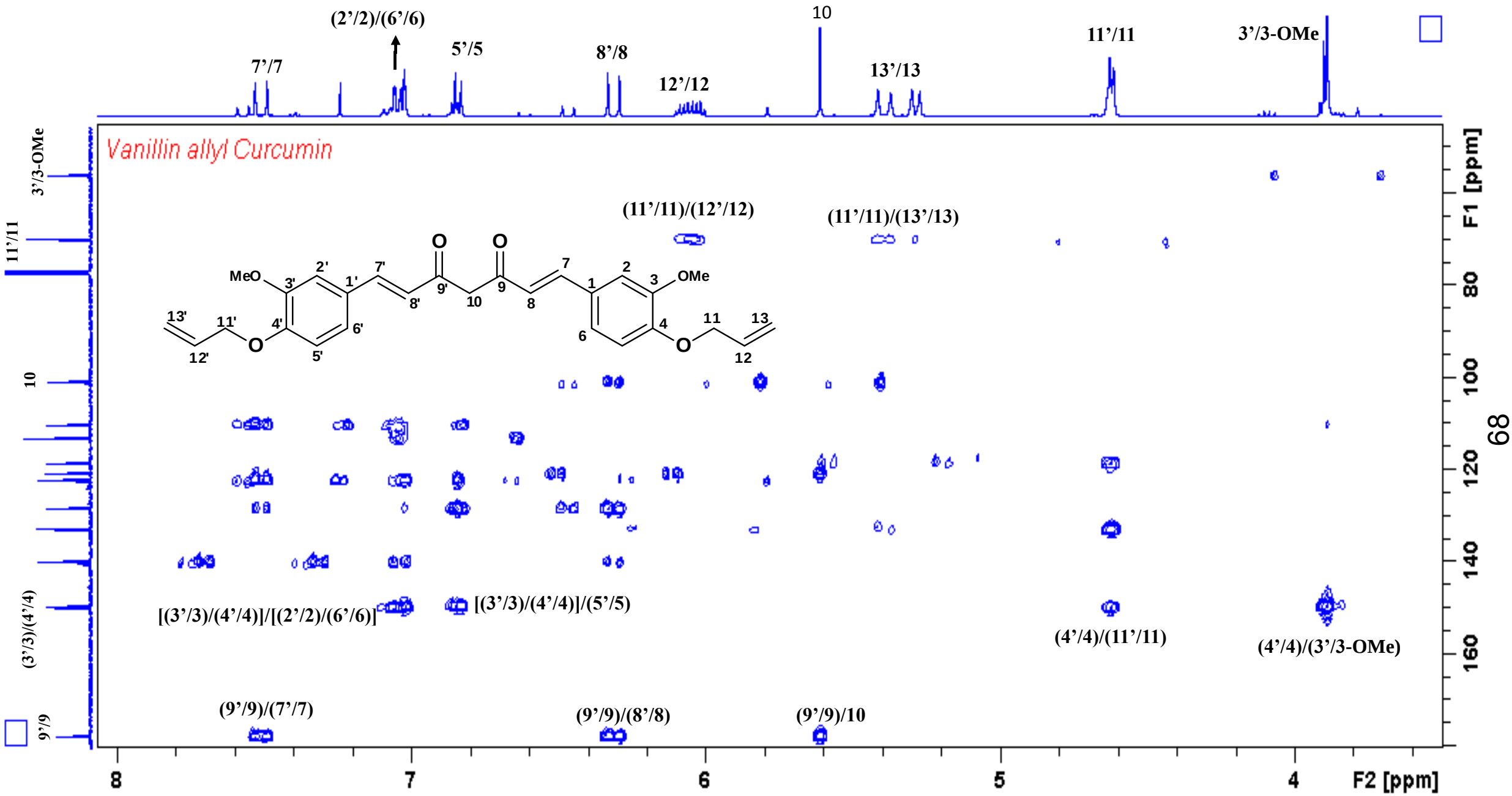
^{13}C spectrum of Compound A-4r: (1E,6E)-1,7-bis(4-(allyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



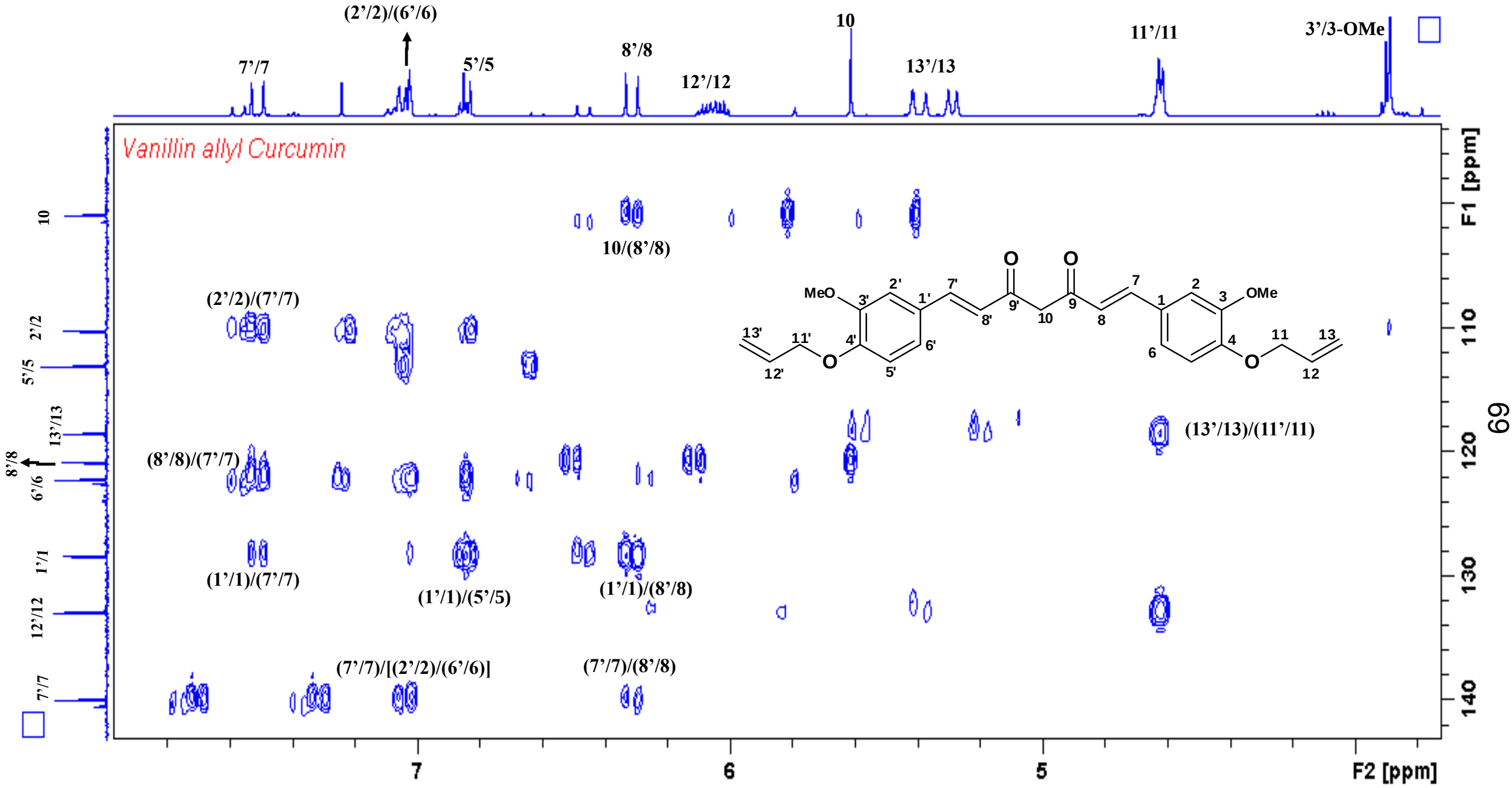
COSY of Compound A-4r: (1E,6E)-1,7-bis(4-(allyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



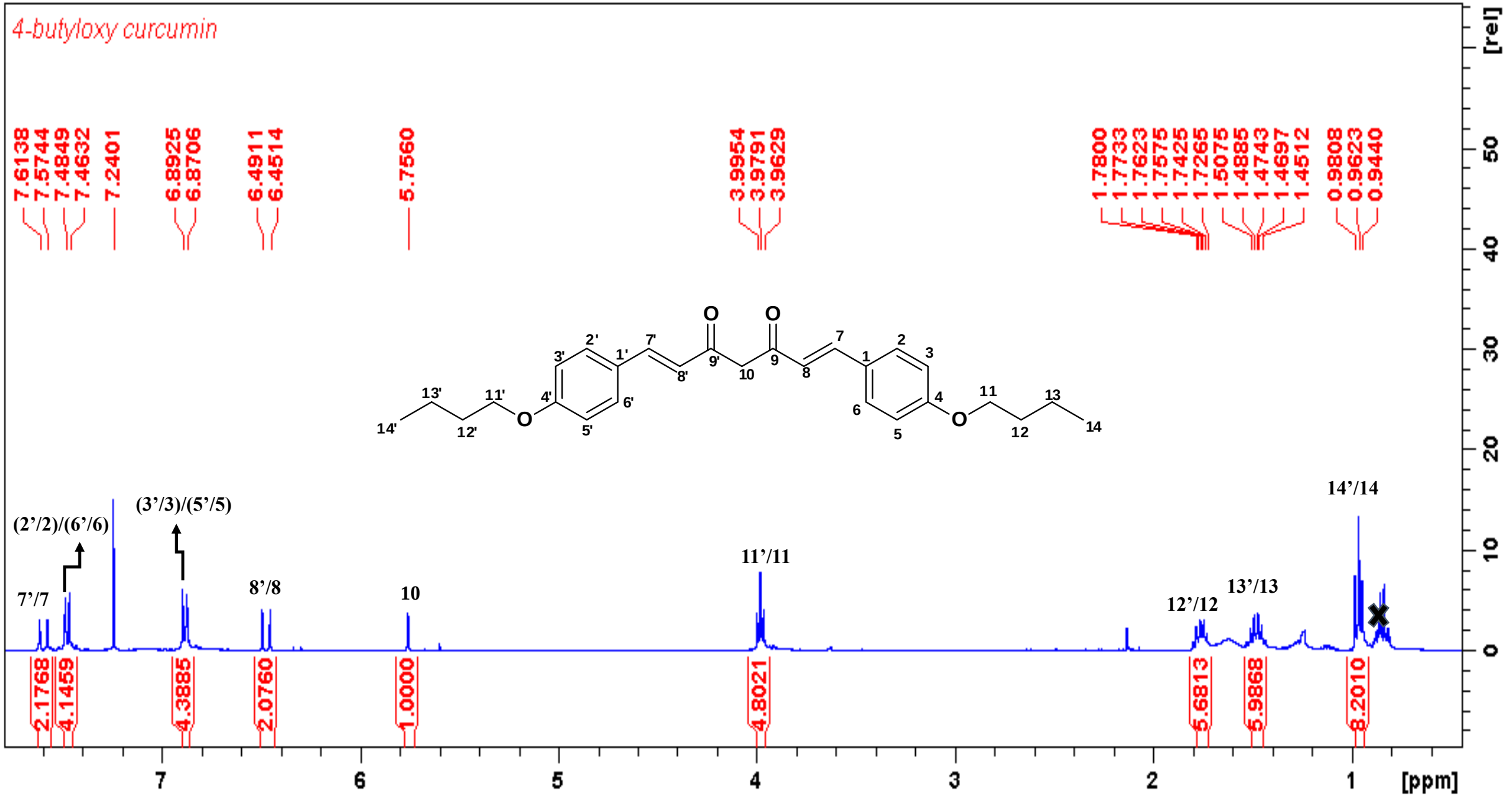
HSQC of Compound A-4r: (1E,6E)-1,7-bis(4-(allyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



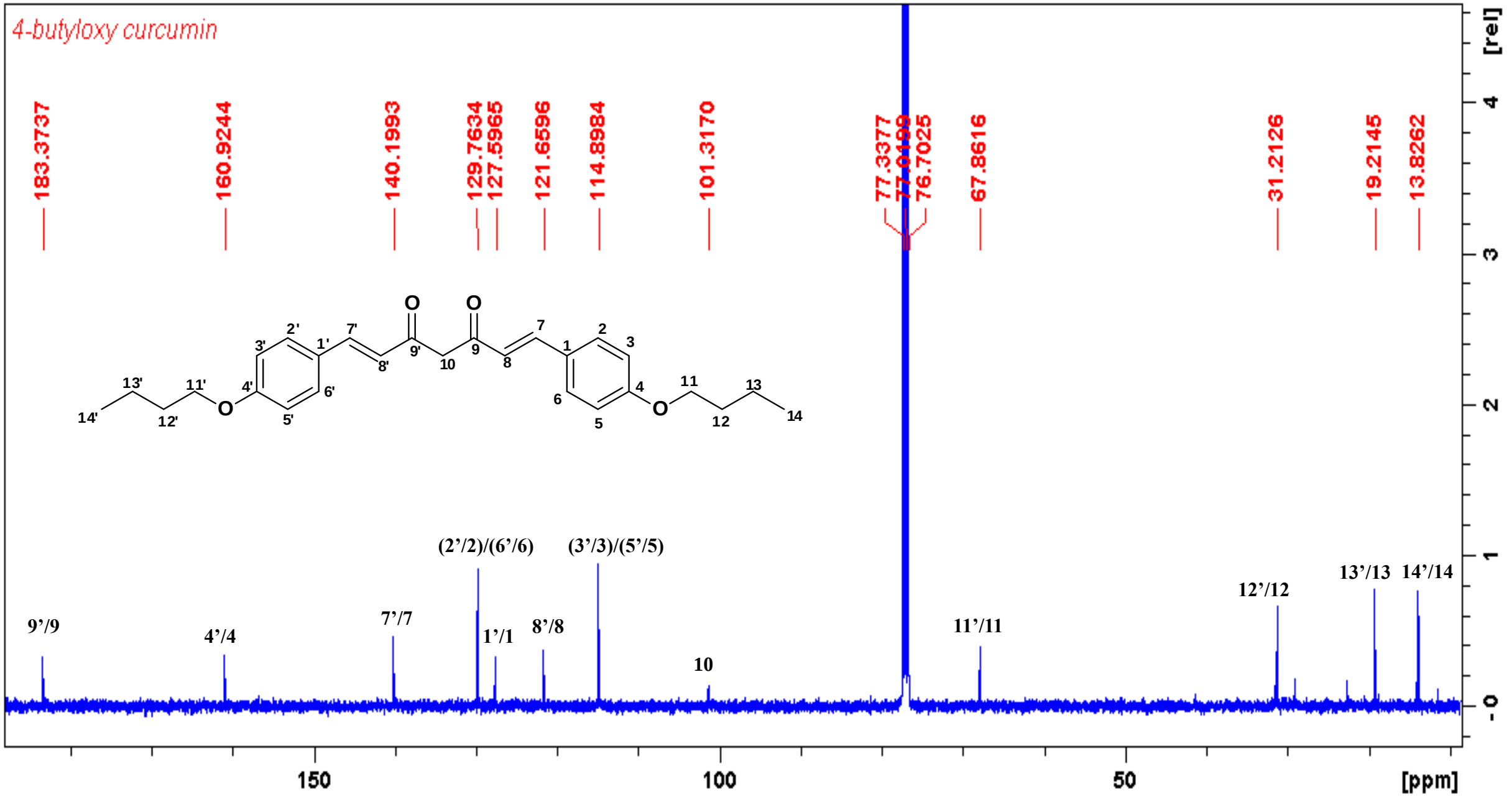
HMBC of Compound A-4r: (1E,6E)-1,7-bis(4-(allyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



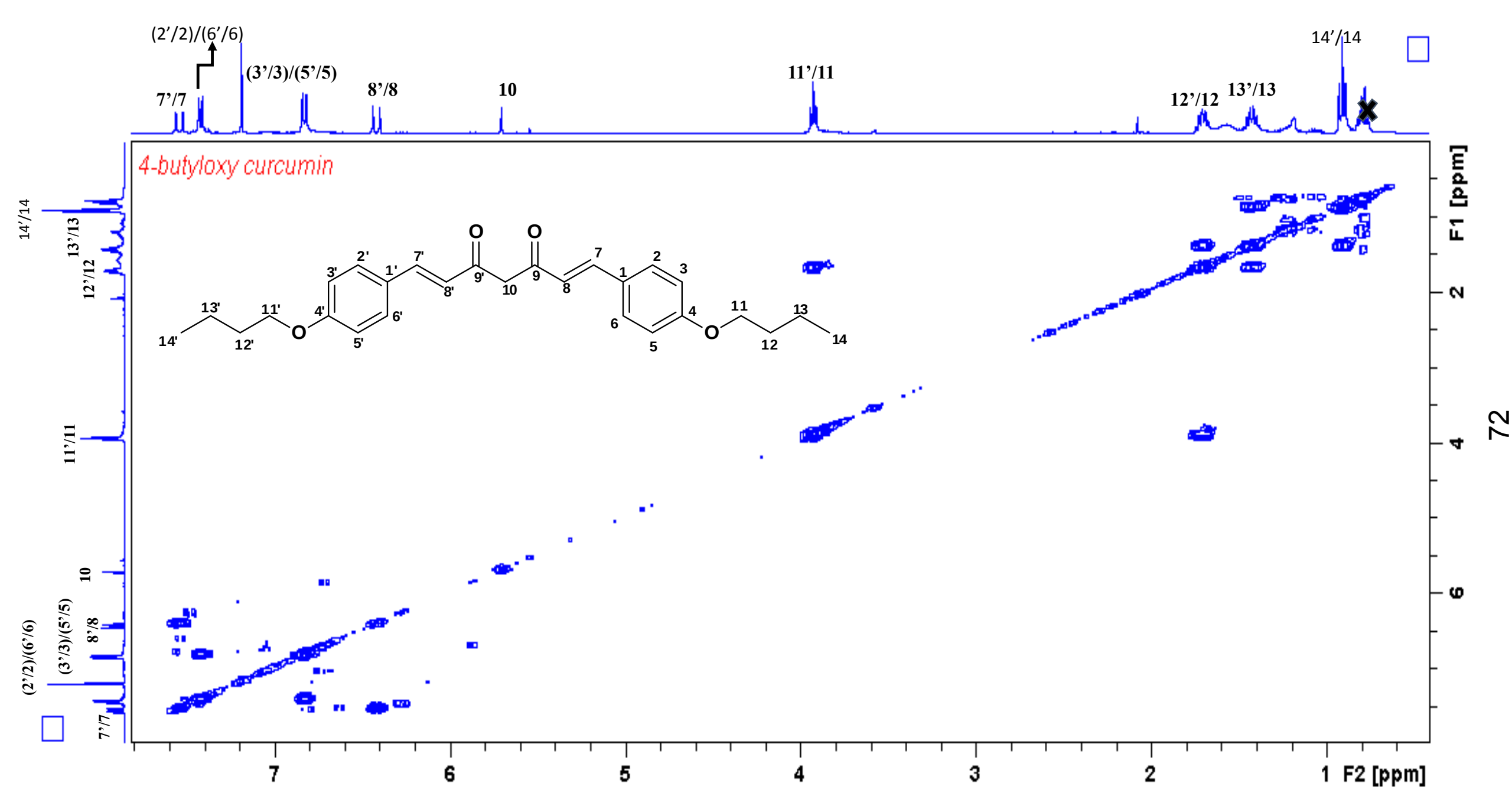
Expanded HMBC of Compound A-4r: (1E,6E)-1,7-bis(4-(allyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



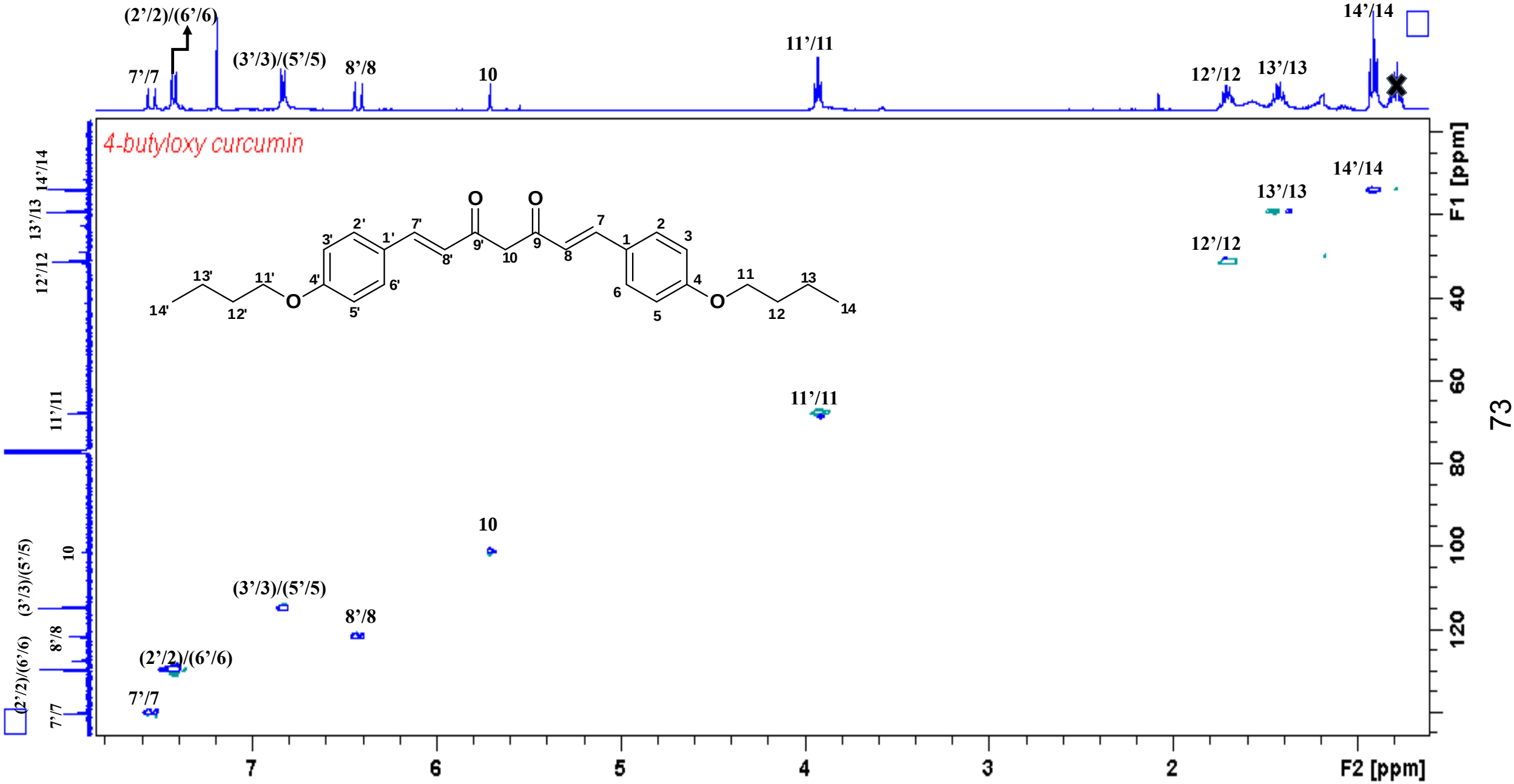
^{1}H spectrum of Compound A-4s: (1E,6E)-1,7-bis(4-butoxyphenyl)hepta-1,6-diene-3,5-dione



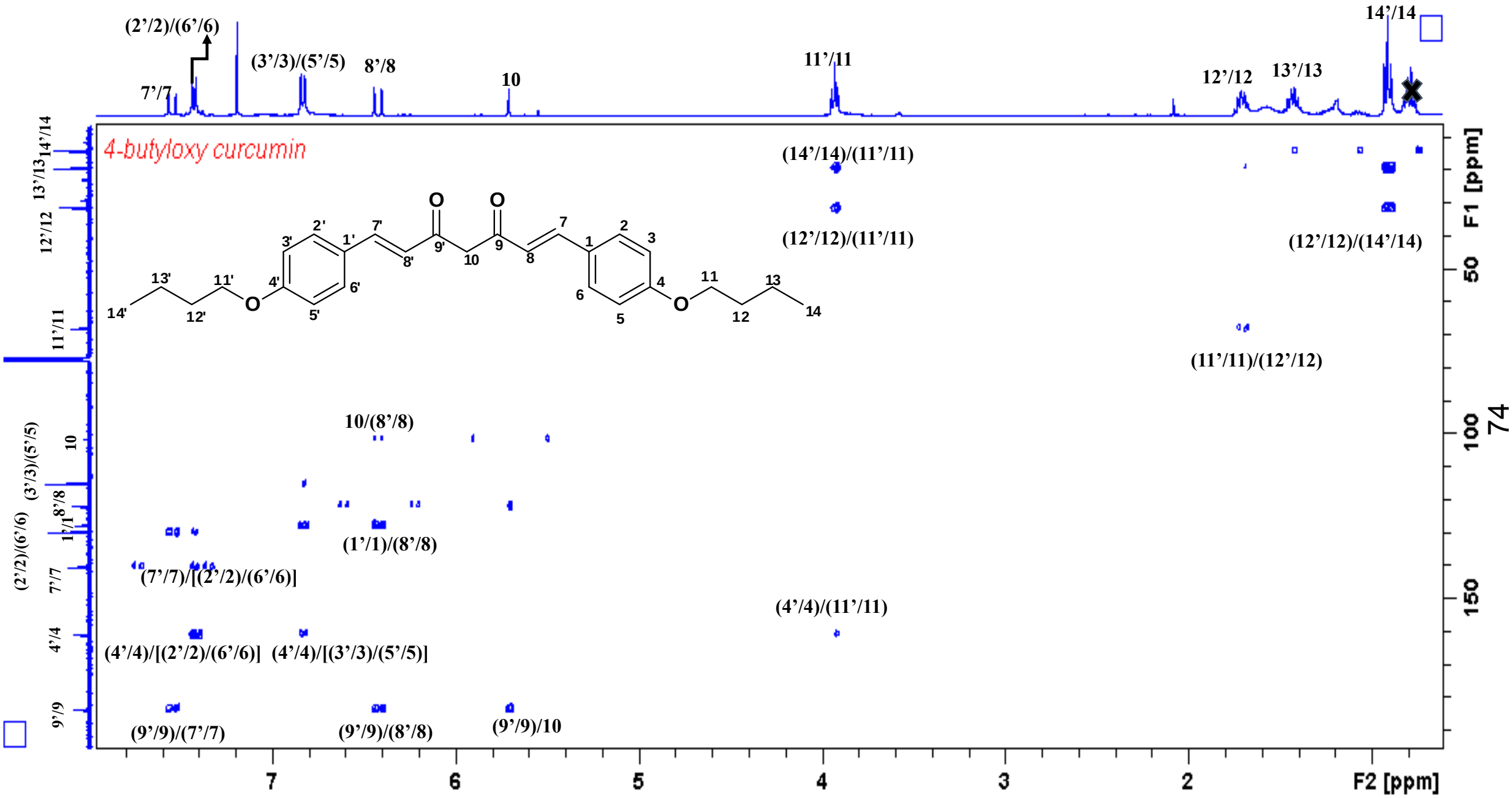
^{13}C spectrum of Compound A-4s: (1E,6E)-1,7-bis(4-butoxyphenyl)hepta-1,6-diene-3,5-dione



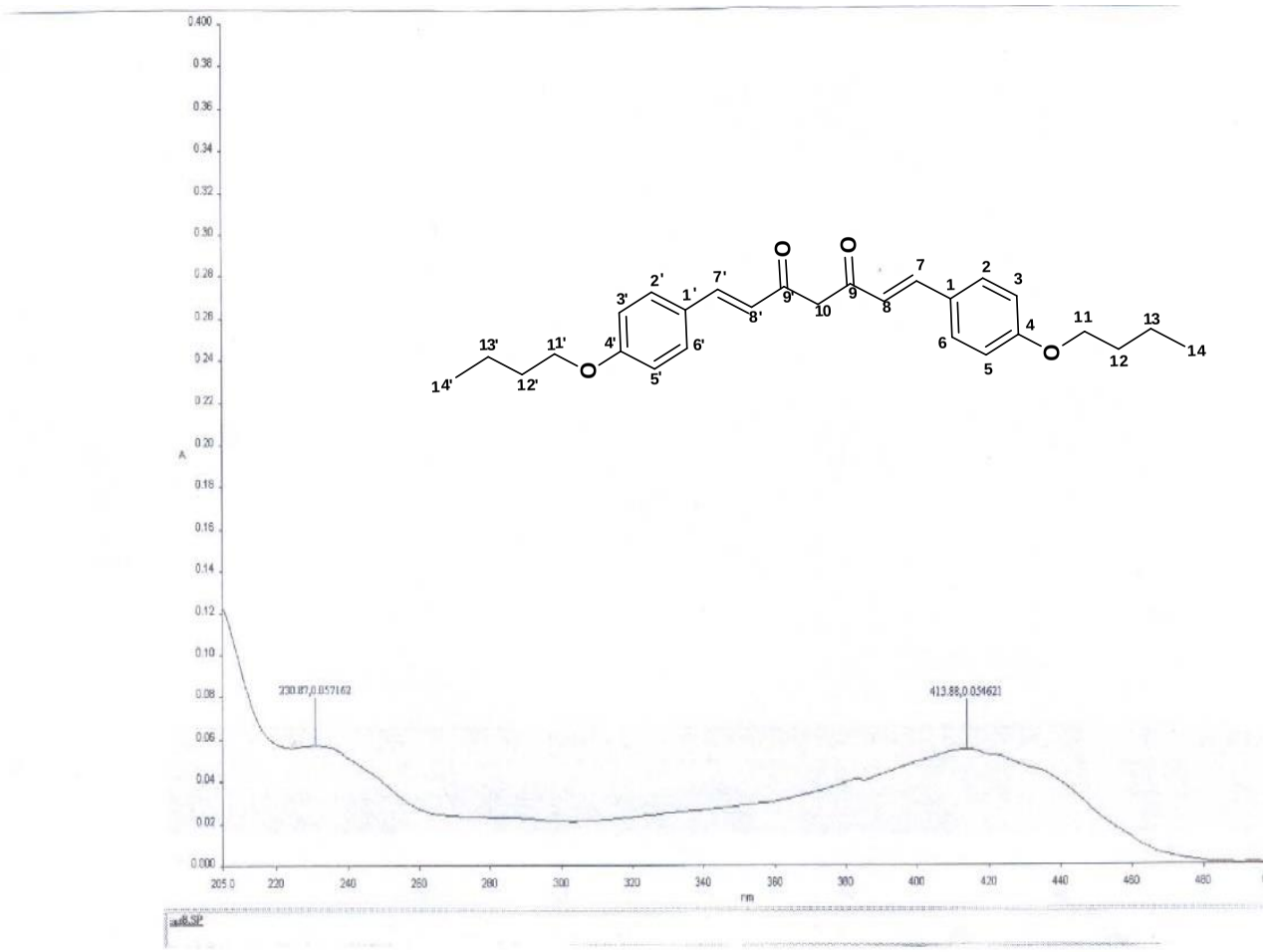
COSY of Compound A-4s: (1E,6E)-1,7-bis(4-butoxyphenyl)hepta-1,6-diene-3,5-dione



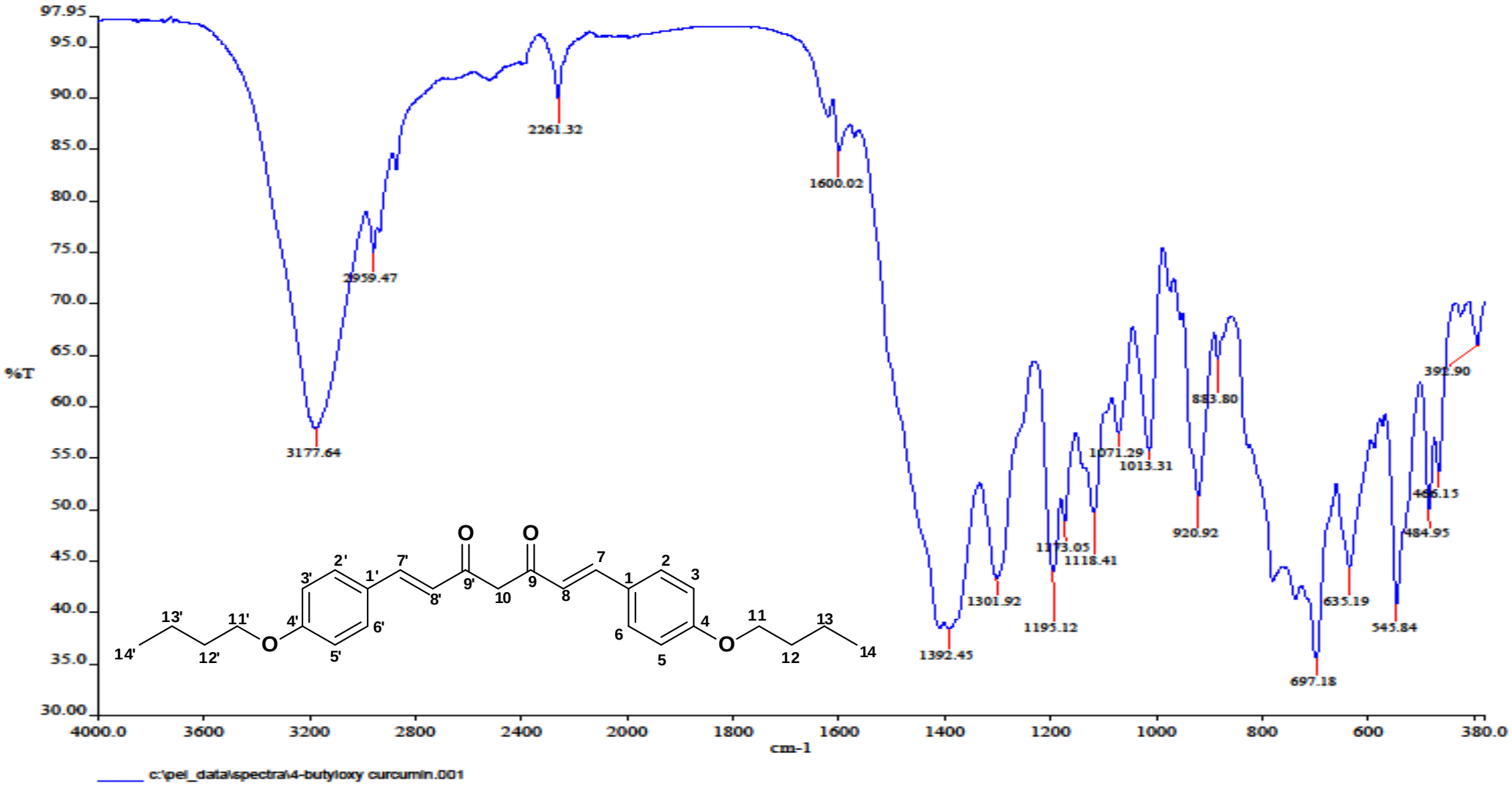
HSQC of Compound A-4s: (1E,6E)-1,7-bis(4-butoxyphenyl)hepta-1,6-diene-3,5-dione



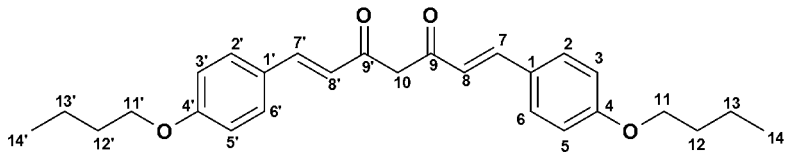
HMBC of Compound A-4s: (1E,6E)-1,7-bis(4-butoxyphenyl)hepta-1,6-diene-3,5-dione



Ultraviolet Spectrum of Compound A-4s: (1E,6E)-1,7-bis(4-butoxyphenyl)hepta-1,6-diene-3,5-dione



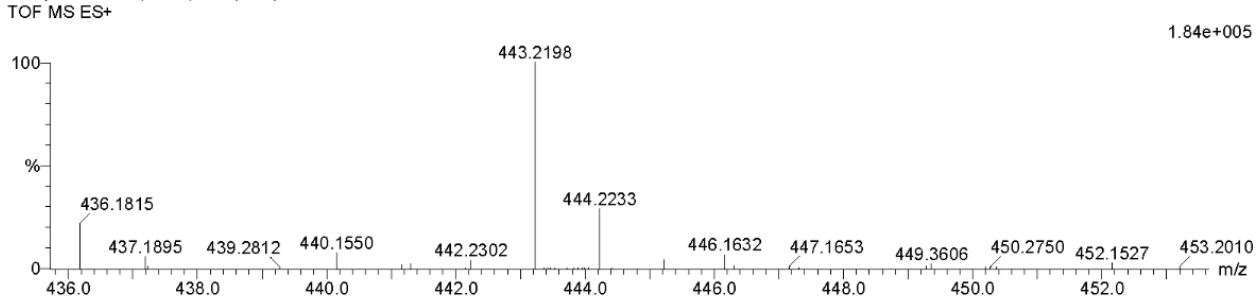
Infrared Spectrum of Compound A-4s: (1E,6E)-1,7-bis(4-butoxyphenyl)hepta-1,6-diene-3,5-dione



Elemental Composition Report **Page 1**

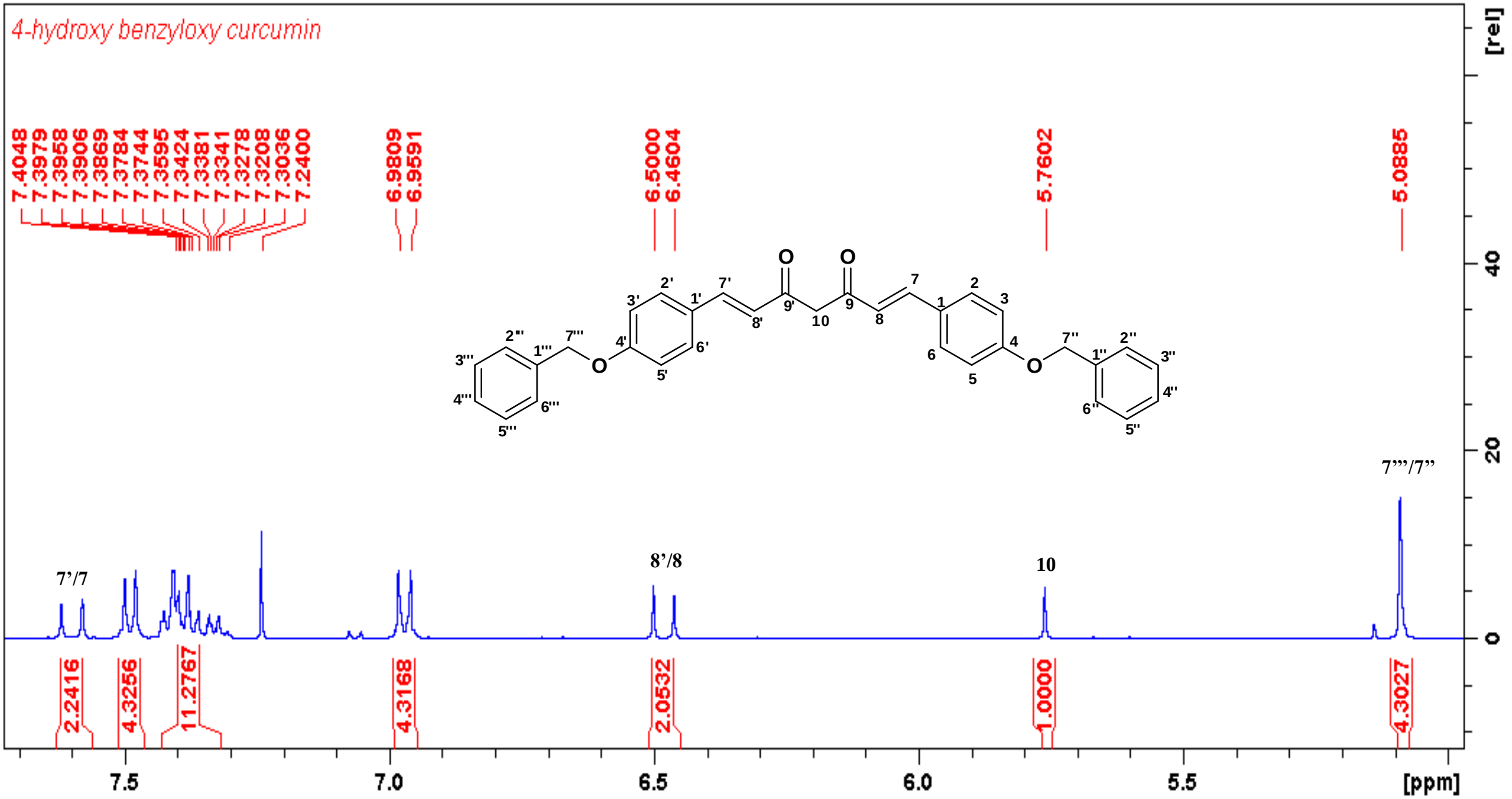
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
3 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 25-30 H: 30-35 O: 0-5 Na: 0-1
Compound 2 60 (1.991) Cm (1:61)
TOF MS ES+

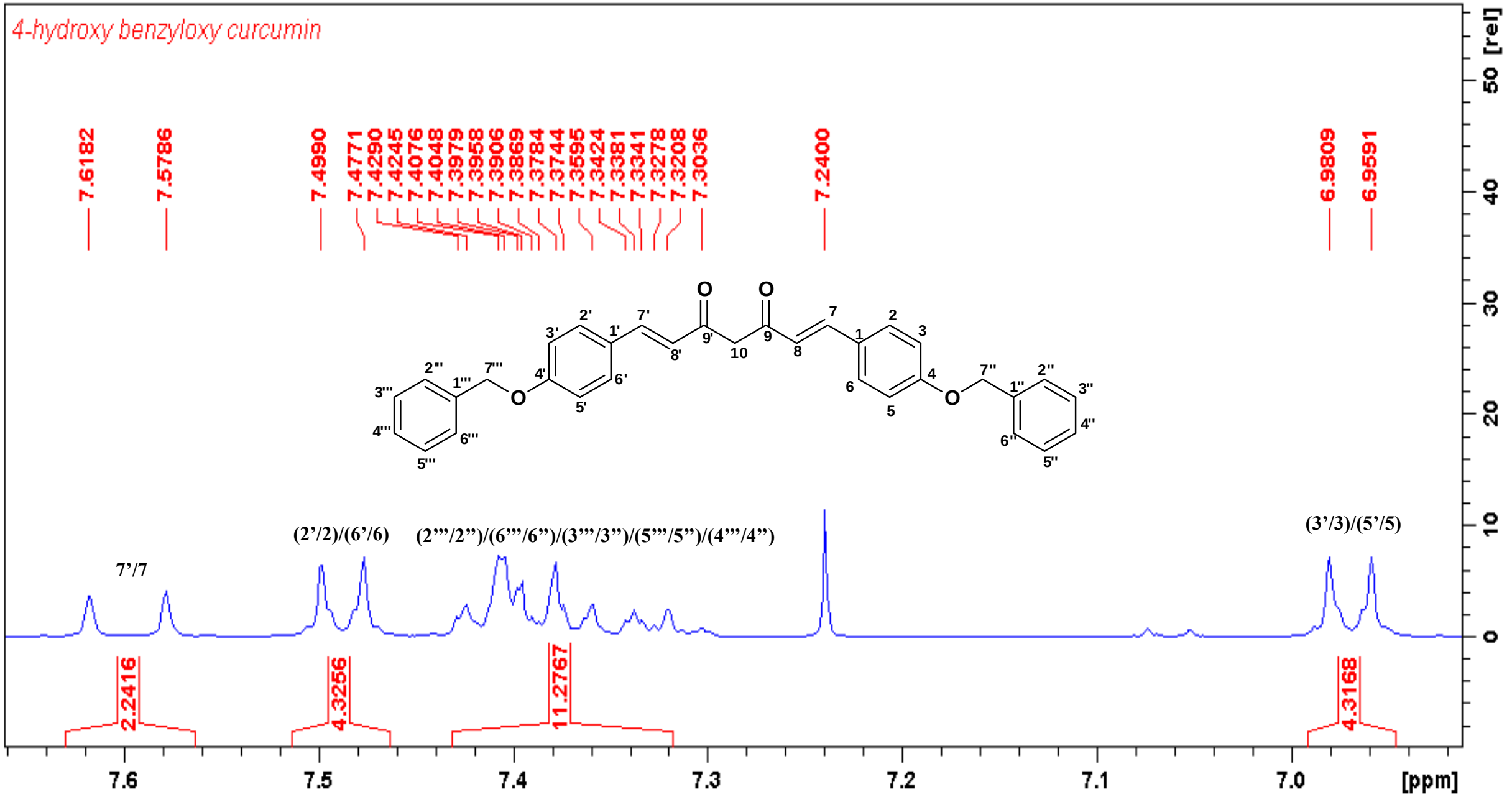


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
443.2198	443.2198	0.0	0.0	11.5	157.3	0.0	C27 H32 O4 Na

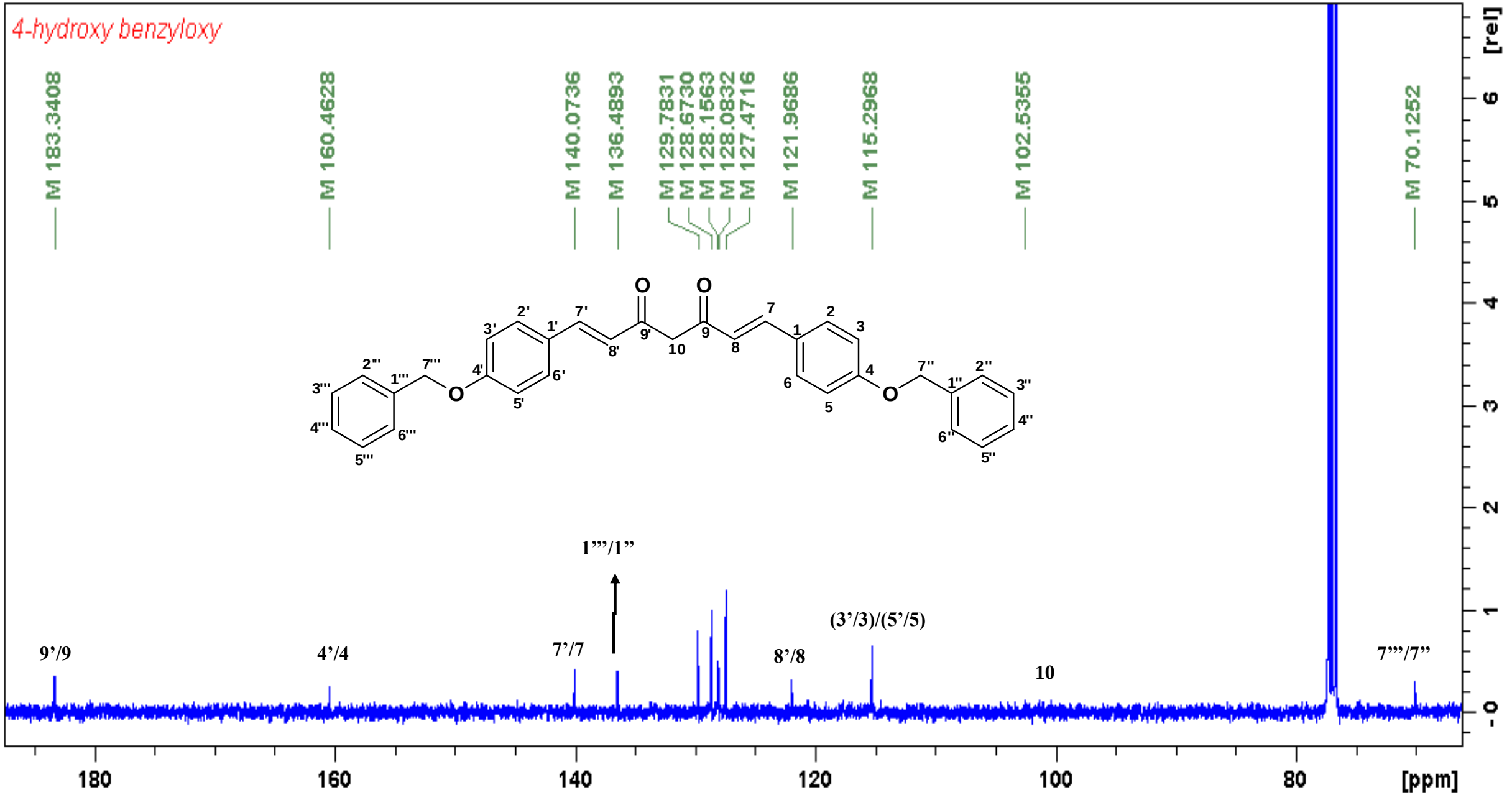
HRMS Spectrum of Compound A-4s: (1E,6E)-1,7-bis(4-butoxyphenyl)hepta-1,6-diene-3,5-dione



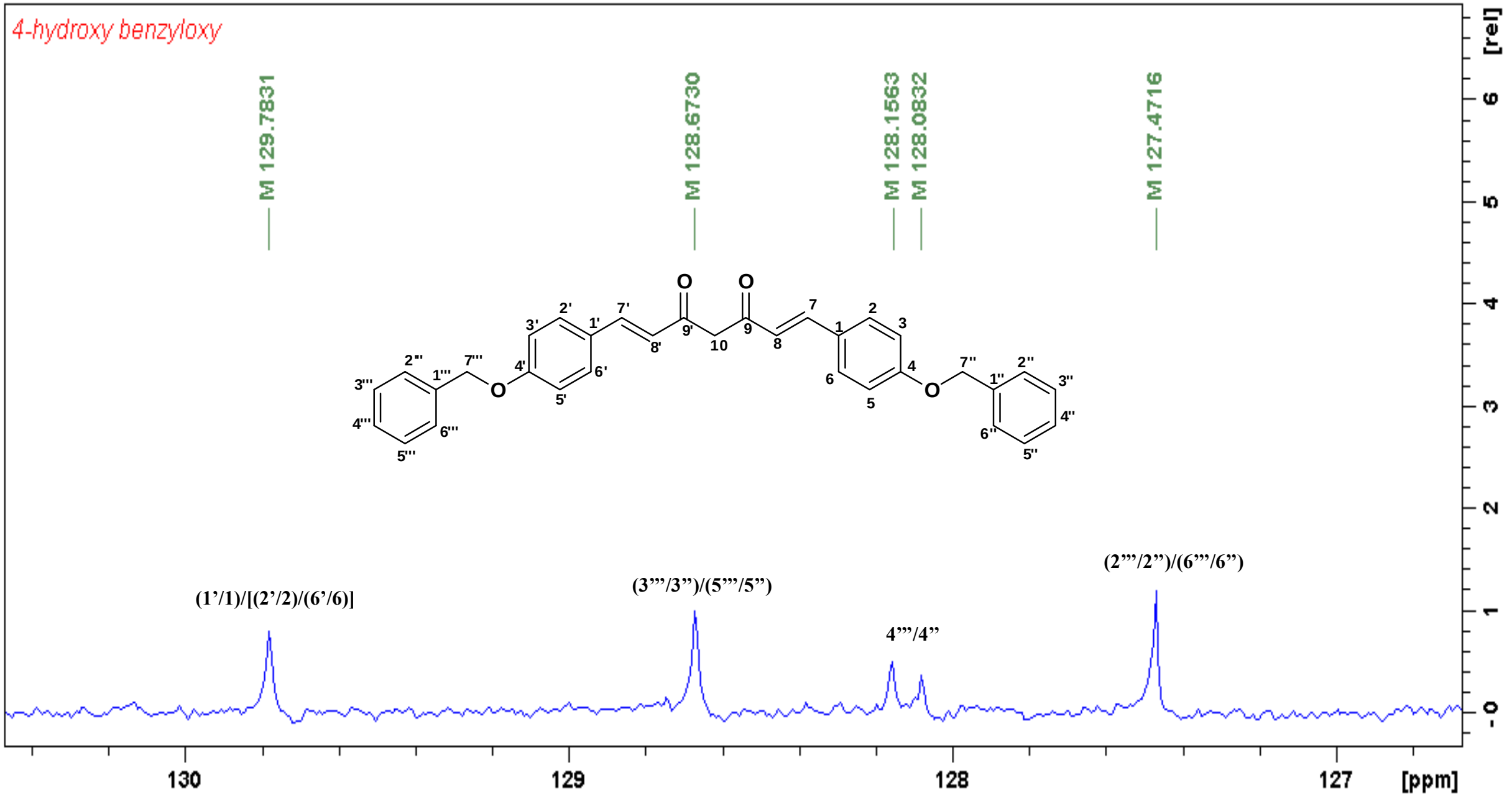
^{1}H Spectrum of Compound A-4t: (1E,6E)-1,7-bis(4-(benzyloxy)phenyl)hepta-1,6-diene-3,5-dione



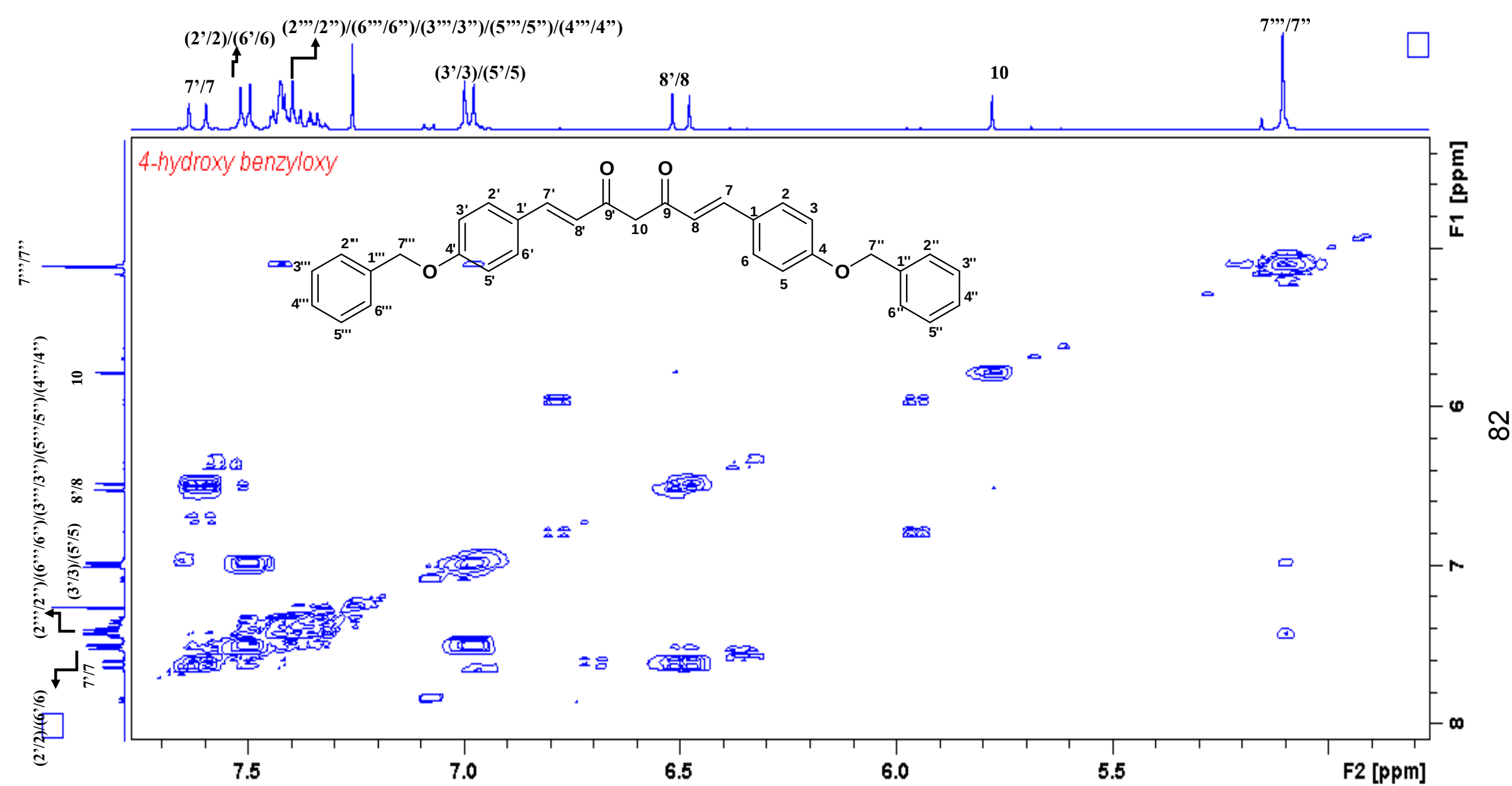
Expanded ^{1}H Spectrum of Compound A-4t: (1E,6E)-1,7-bis(4-(benzyloxy)phenyl)hepta-1,6-diene-3,5-dione



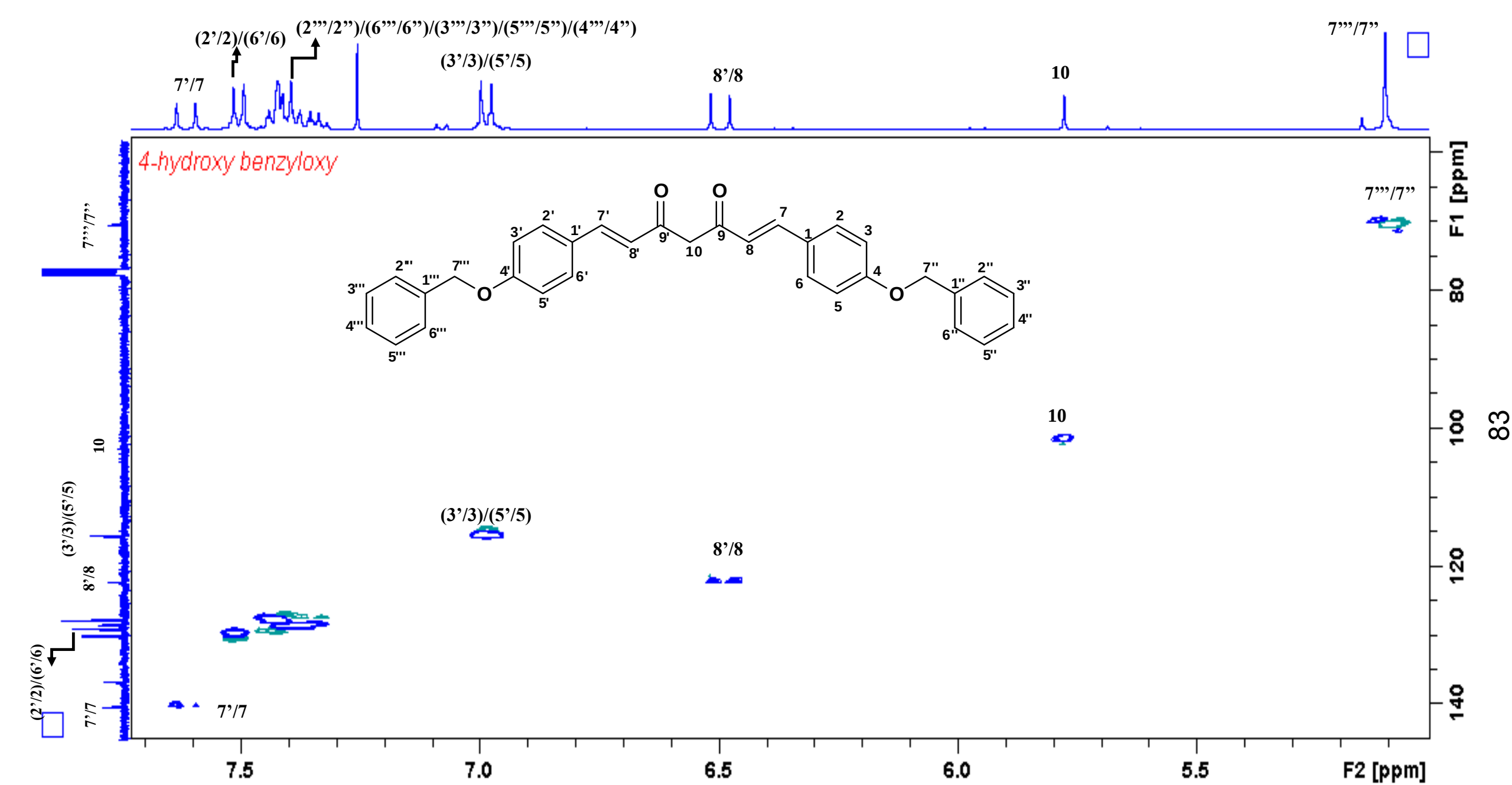
^{13}C Spectrum of Compound A-4t: (1E,6E)-1,7-bis(4-(benzyloxy)phenyl)hepta-1,6-diene-3,5-dione



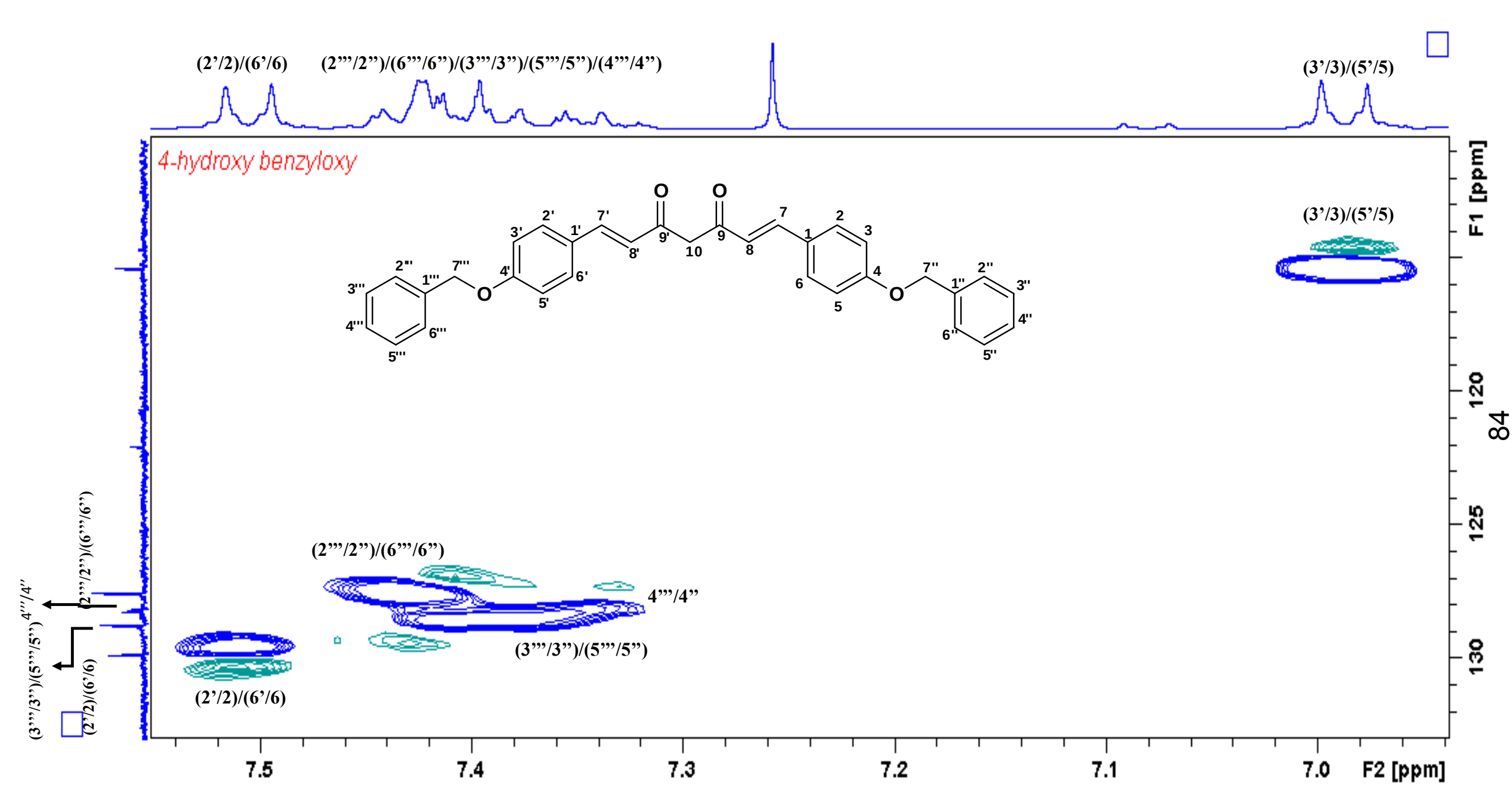
Expanded ^{13}C Spectrum of Compound A-4t: (1E,6E)-1,7-bis(4-(benzyloxy)phenyl)hepta-1,6-diene-3,5-dione



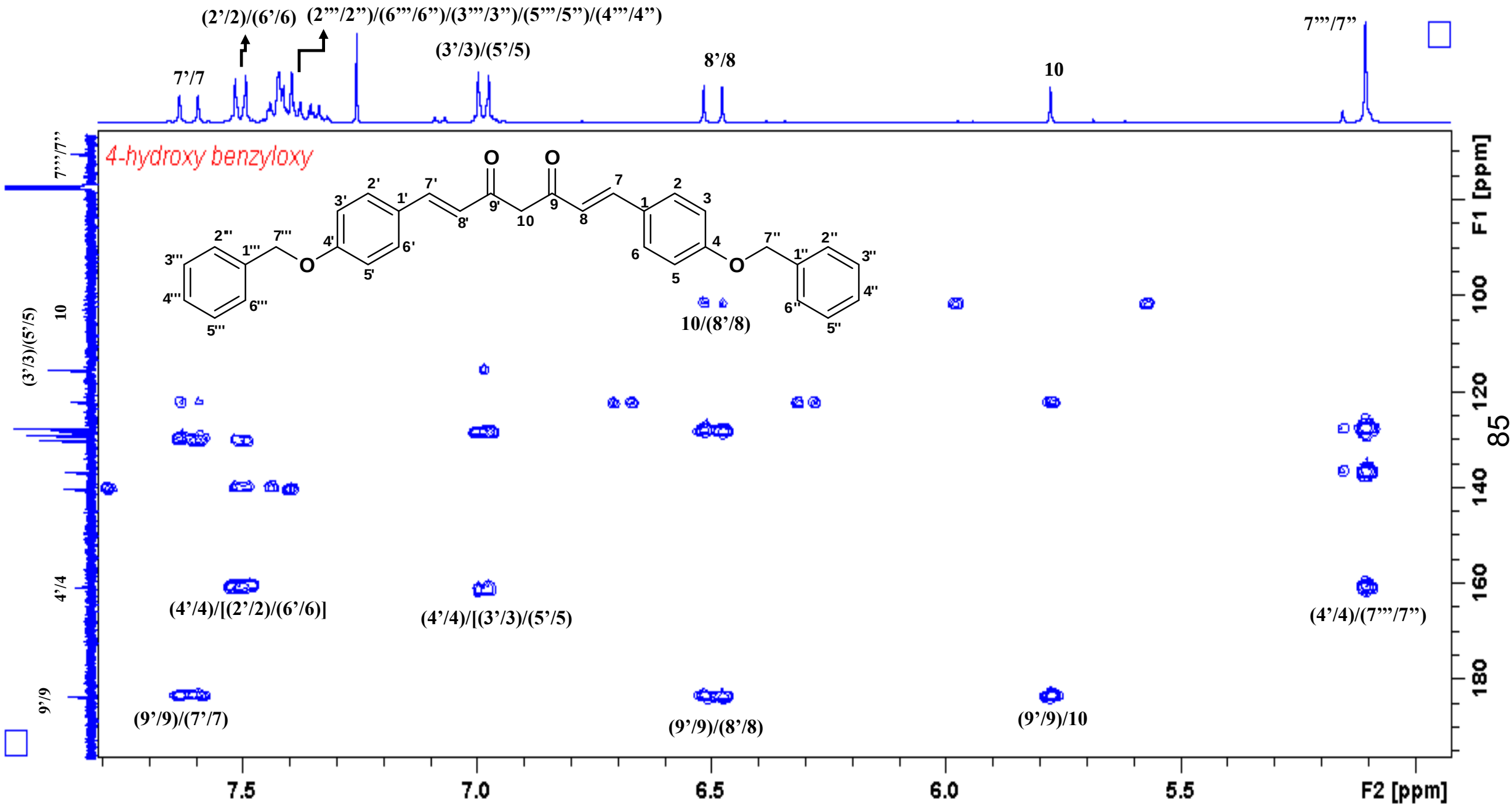
COSY of Compound A-4t: (1E,6E)-1,7-bis(4-(benzyloxy)phenyl)hepta-1,6-diene-3,5-dione



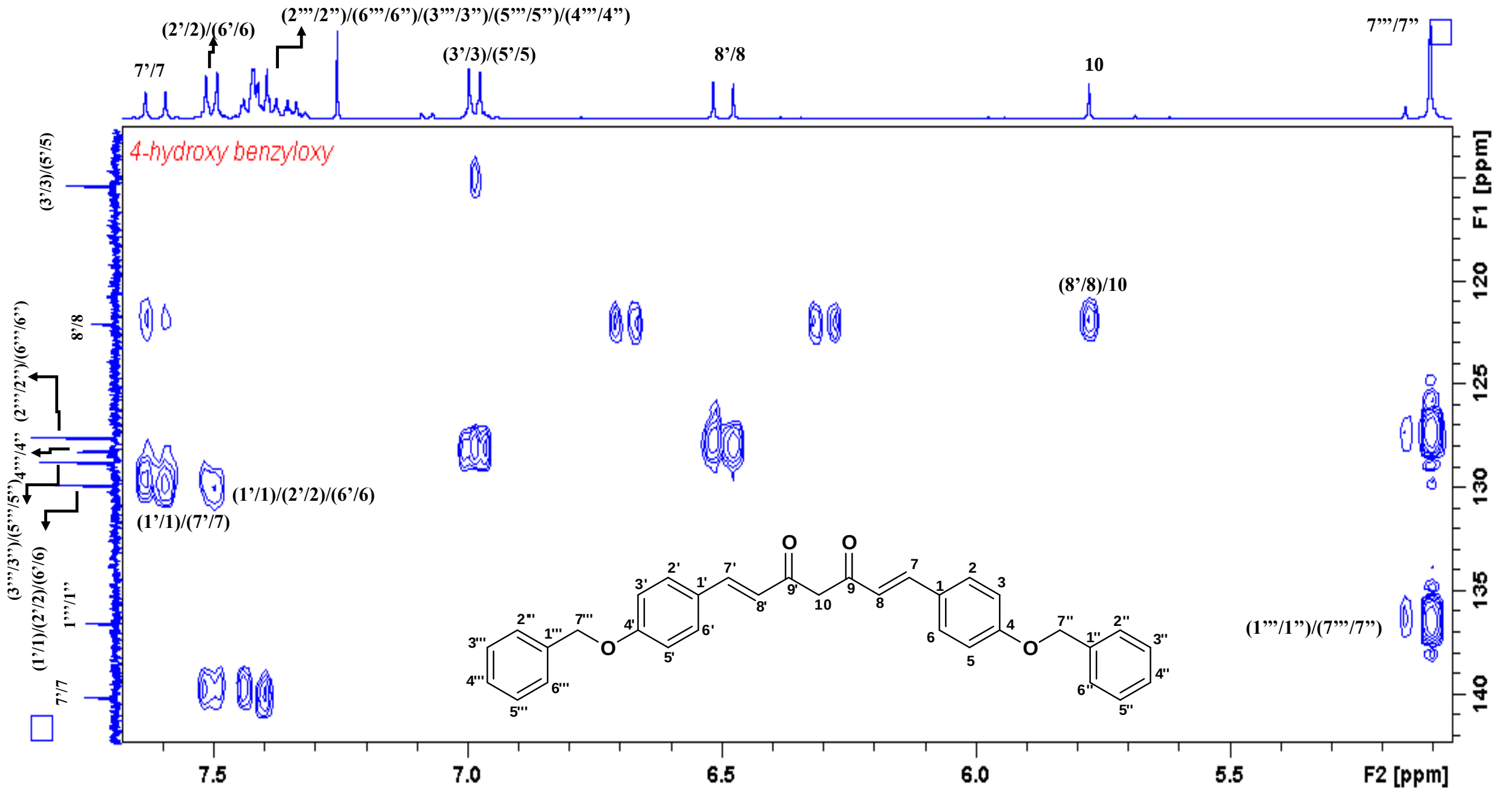
HSQC of Compound A-4t: (1E,6E)-1,7-bis(4-(benzyloxy)phenyl)hepta-1,6-diene-3,5-dione



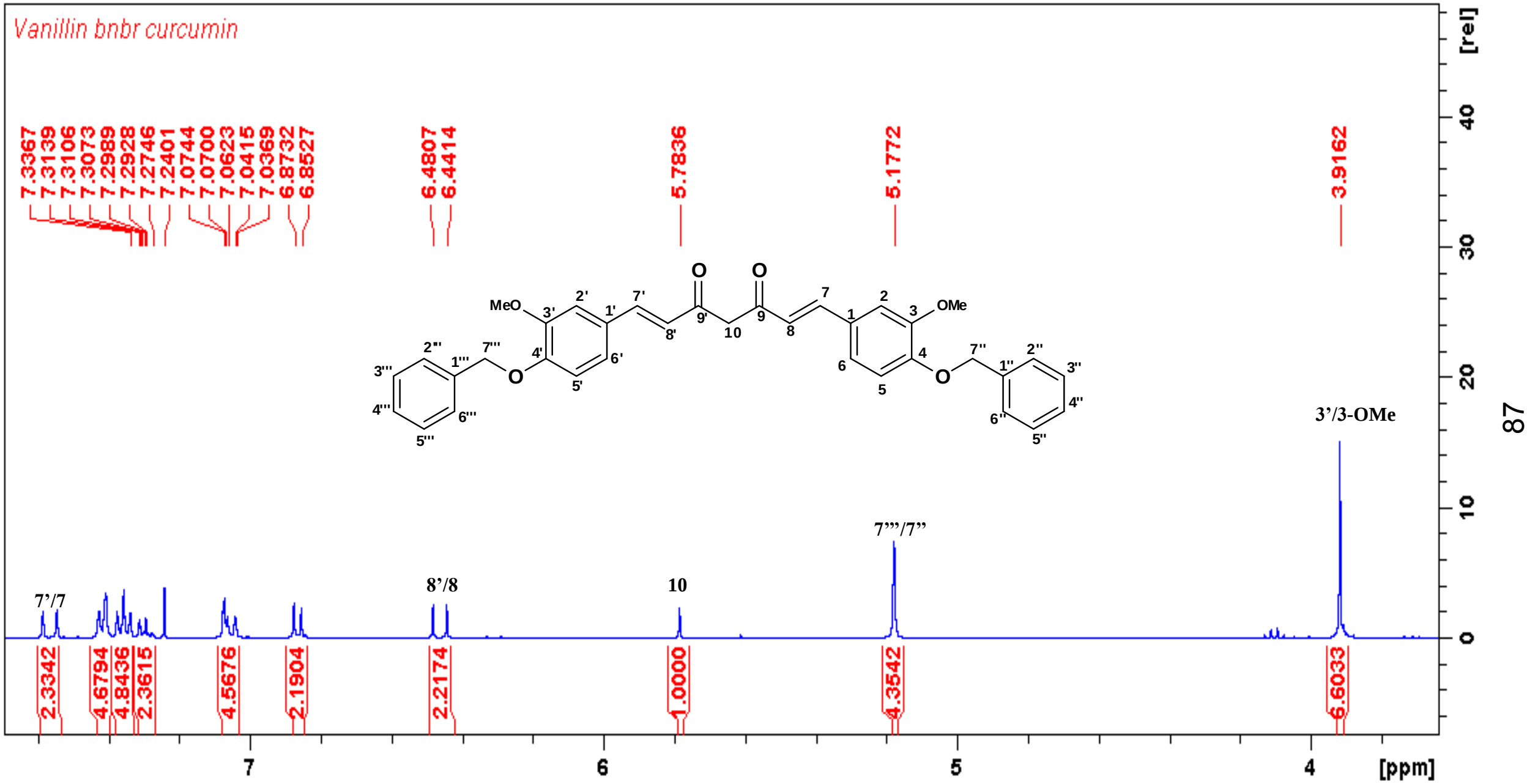
Expanded HSQC of Compound A-4t: (1E,6E)-1,7-bis(4-(benzyloxy)phenyl)hepta-1,6-diene-3,5-dione



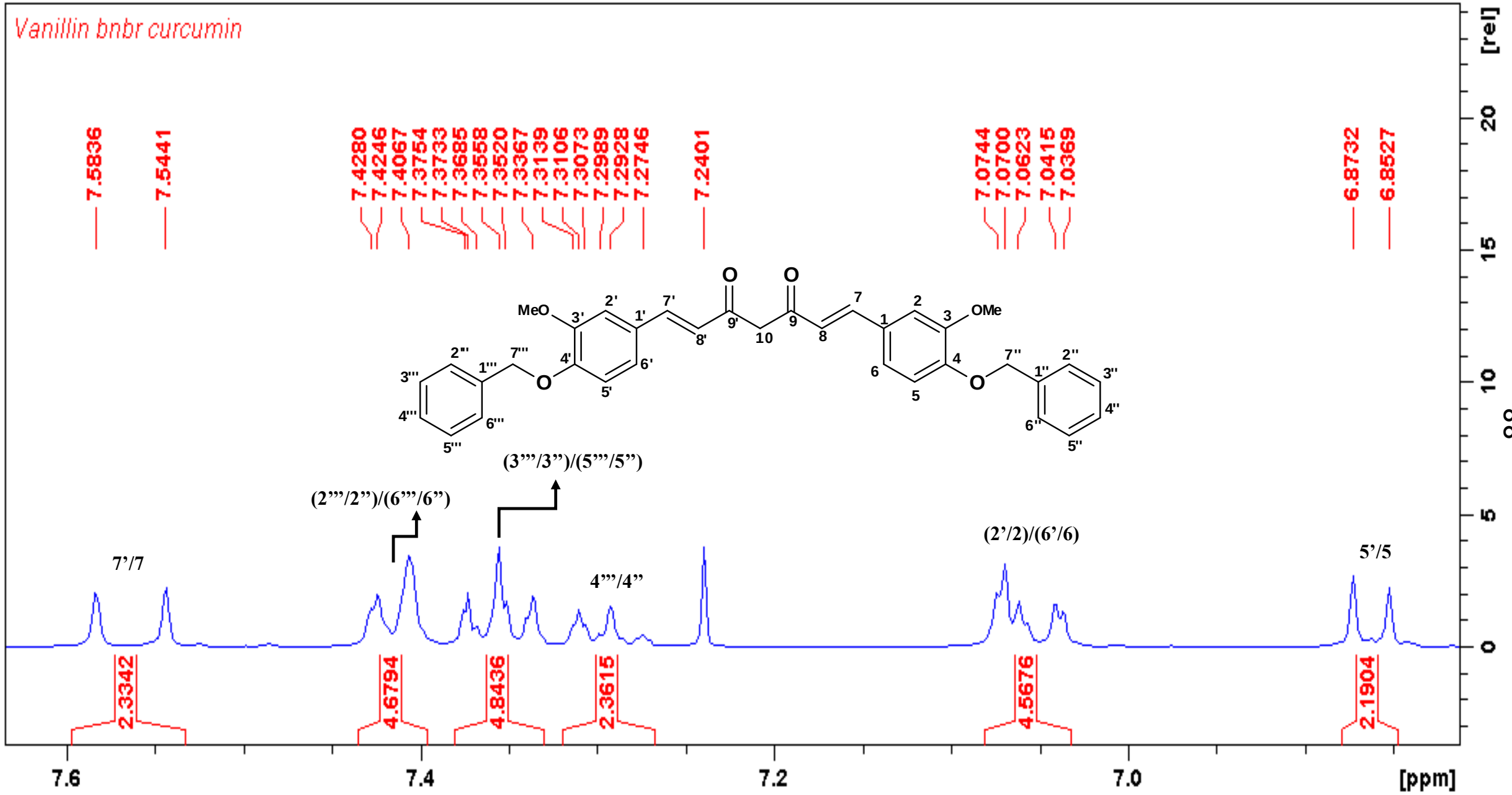
HMBC of Compound A-4t: (1E,6E)-1,7-bis(4-(benzyloxy)phenyl)hepta-1,6-diene-3,5-dione



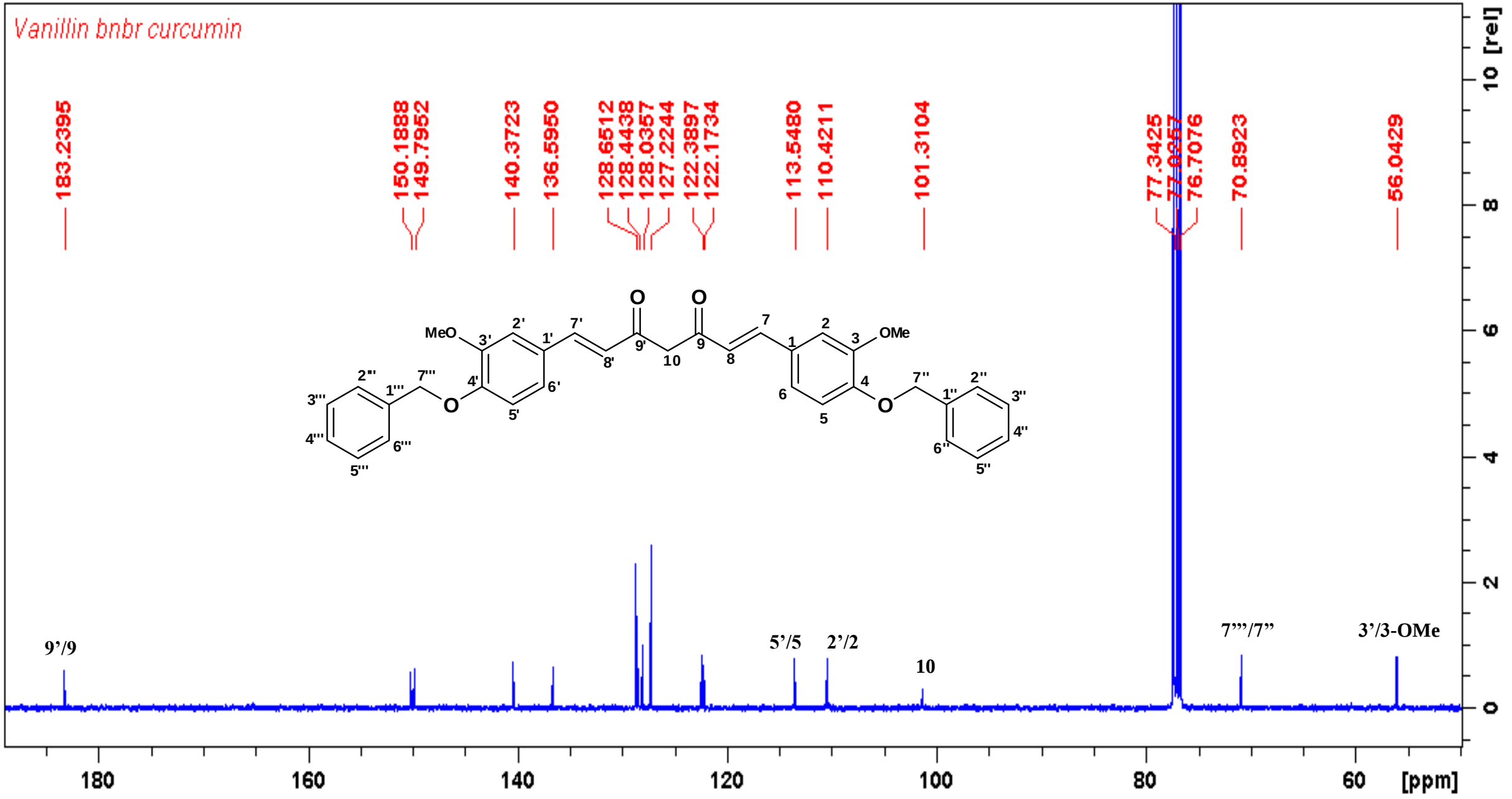
Expanded HMBC of Compound A-4t: (1E,6E)-1,7-bis(4-(benzyloxy)phenyl)hepta-1,6-diene-3,5-dione



^{1}H spectrum of Compound A-4u: (1E,6E)-1,7-bis(4-(benzyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



Expanded ^{1}H spectrum of Compound A-4u: (1E,6E)-1,7-bis(4-(benzyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



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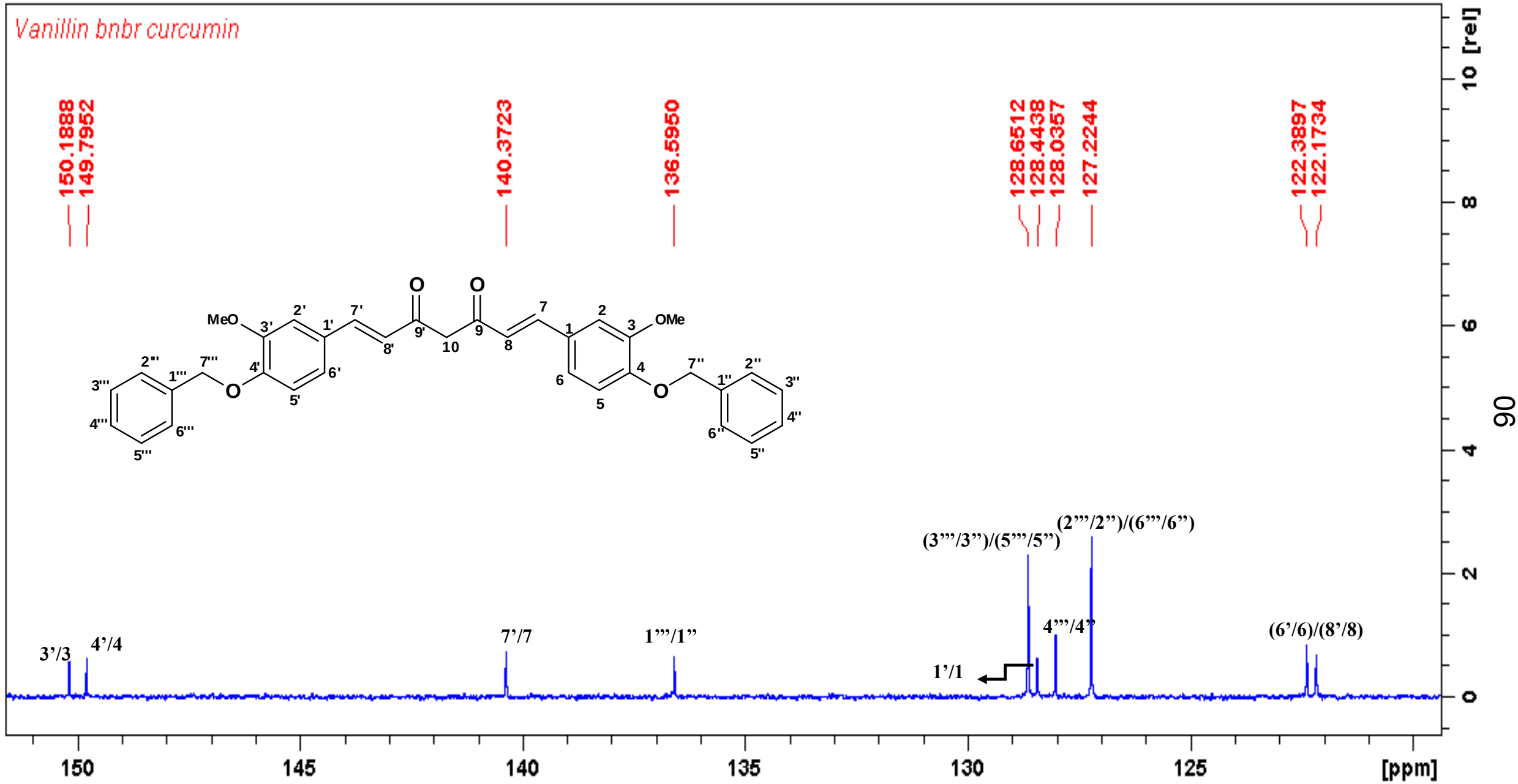
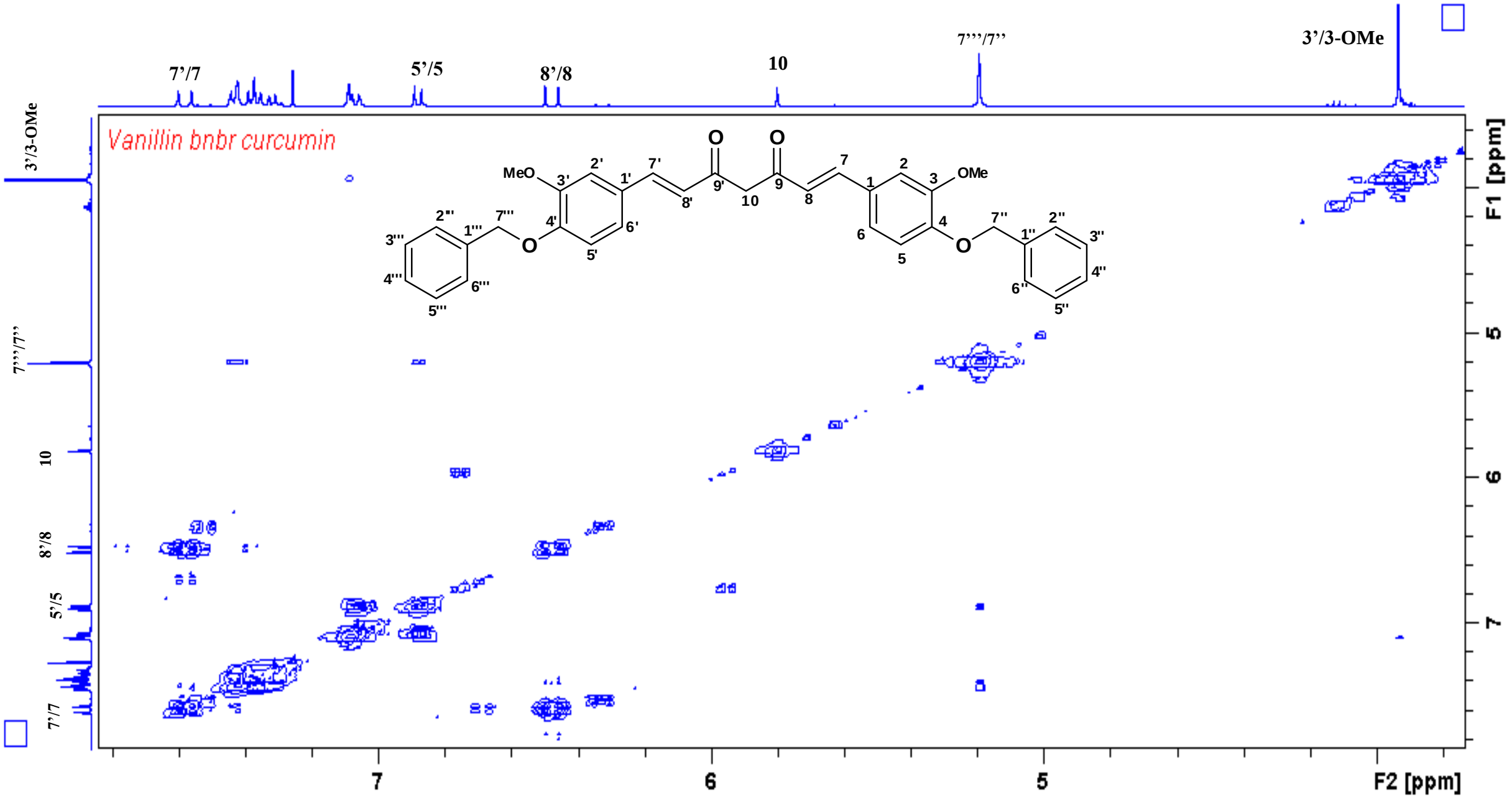
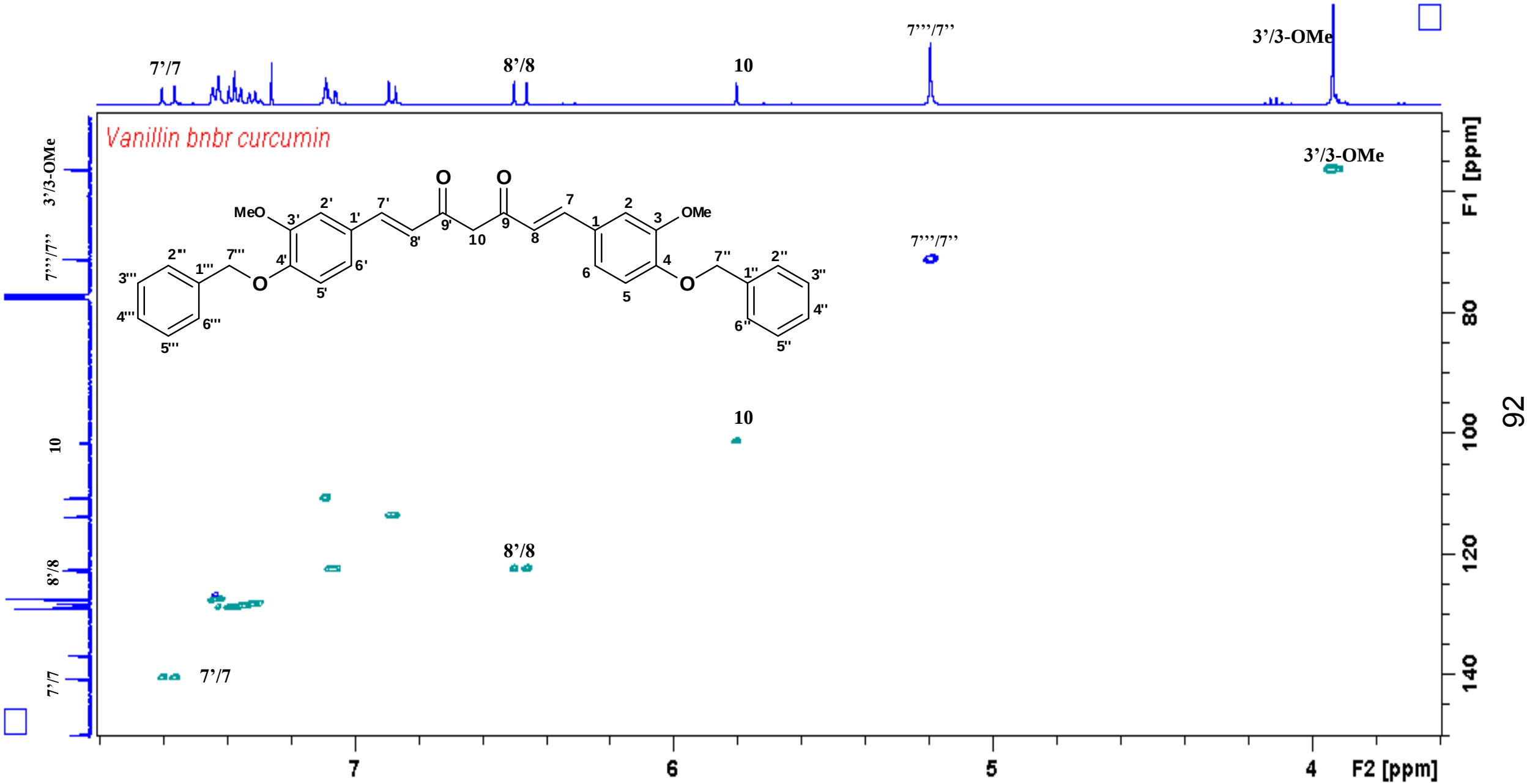
^{13}C spectrum of Compound A-4u: (1E,6E)-1,7-bis(4-(benzyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



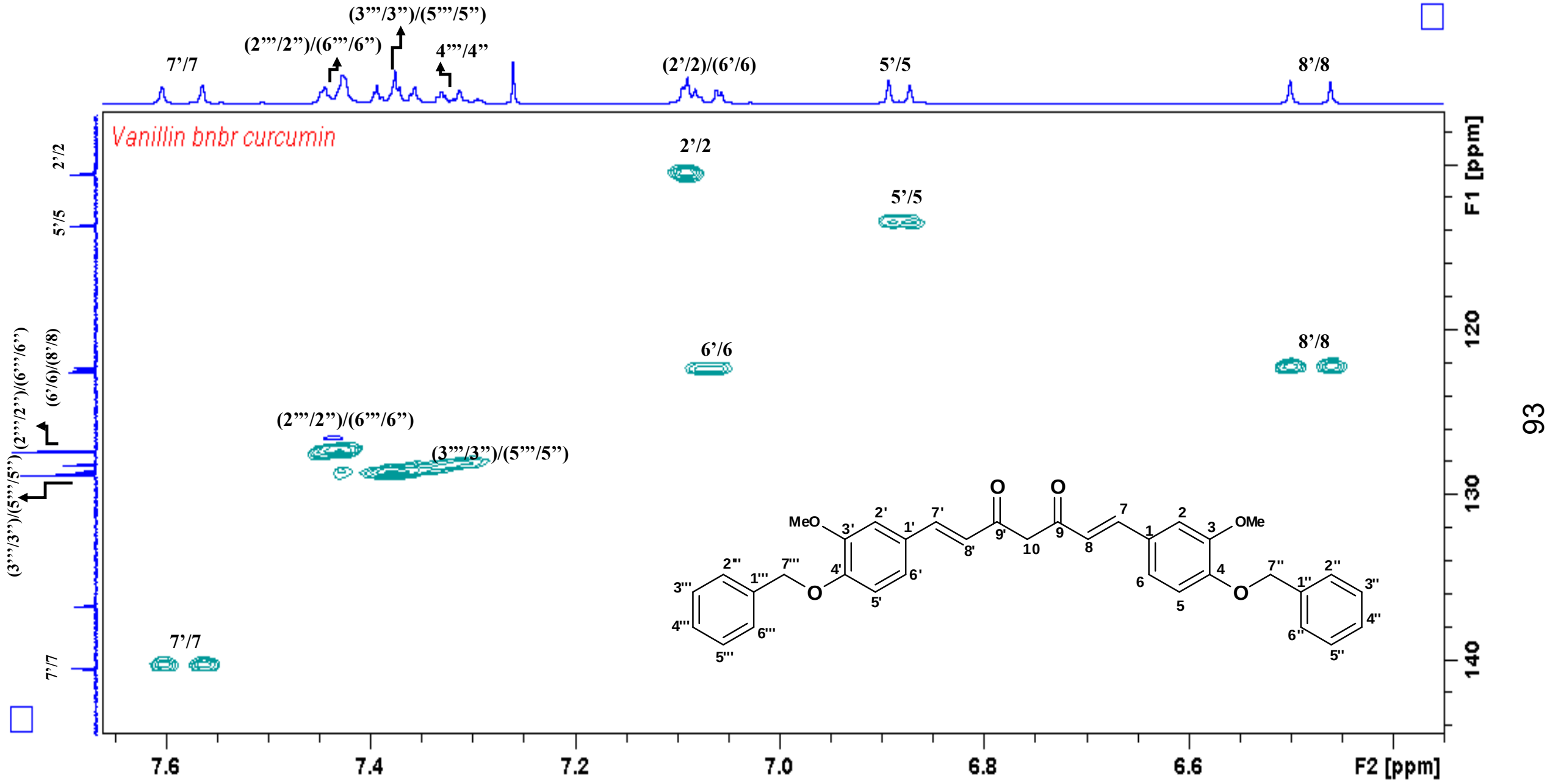
Expanded ^{13}C spectrum of Compound A-4u: (1E,6E)-1,7-bis(4-(benzyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



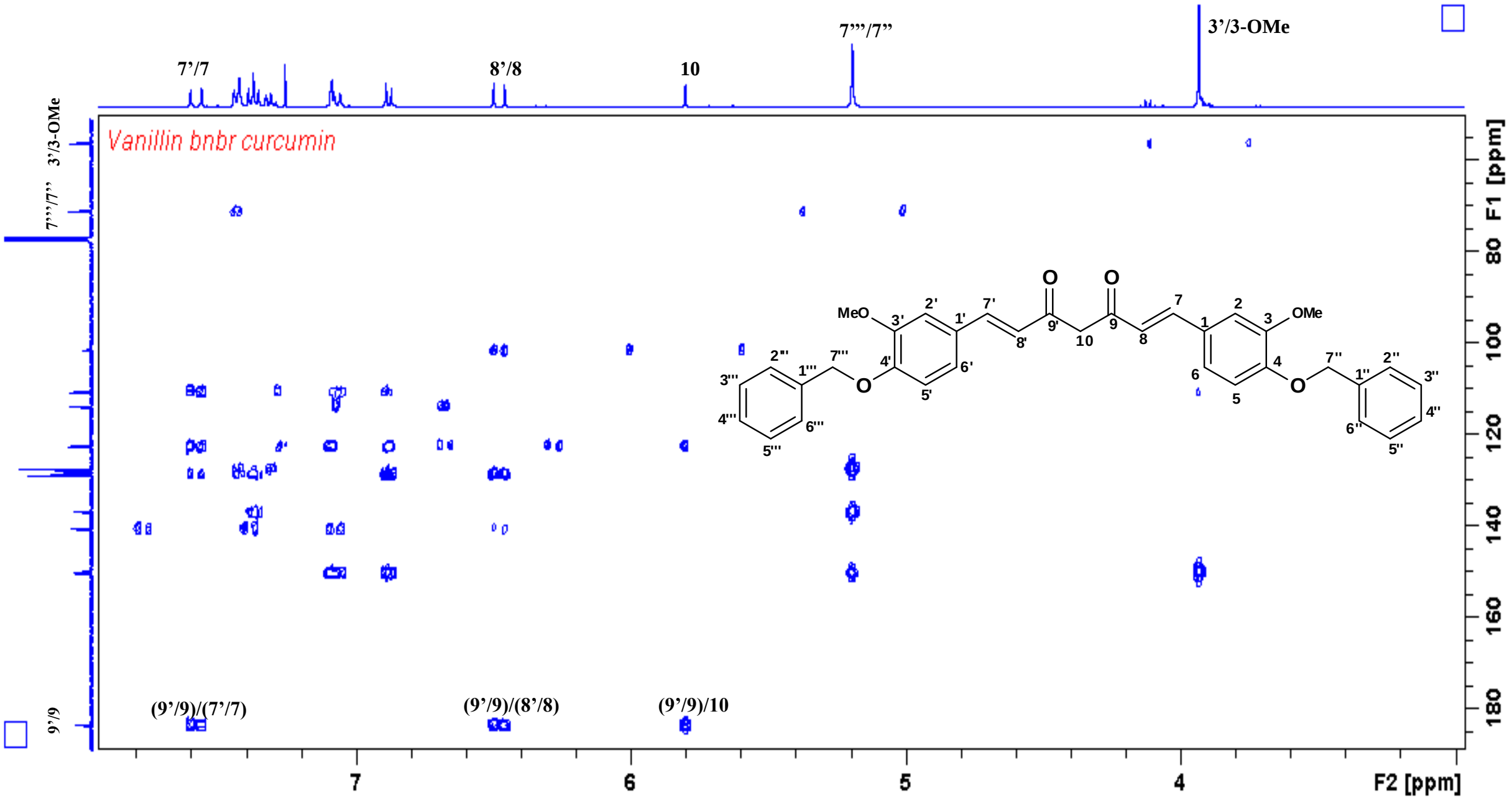
COSY of Compound A-4u: (1E,6E)-1,7-bis(4-(benzyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



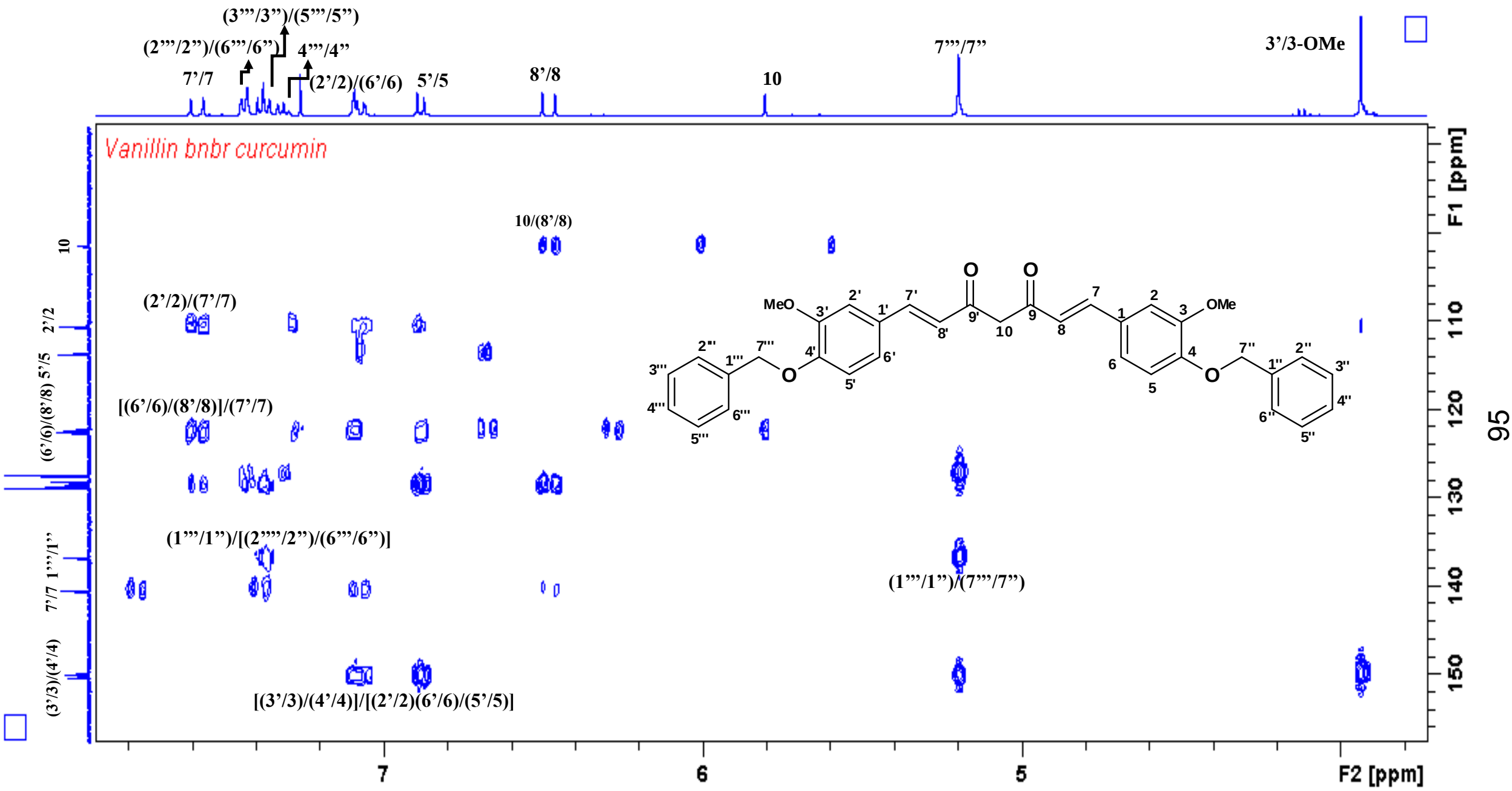
HSQC of Compound A-4u: (1E,6E)-1,7-bis(4-(benzyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



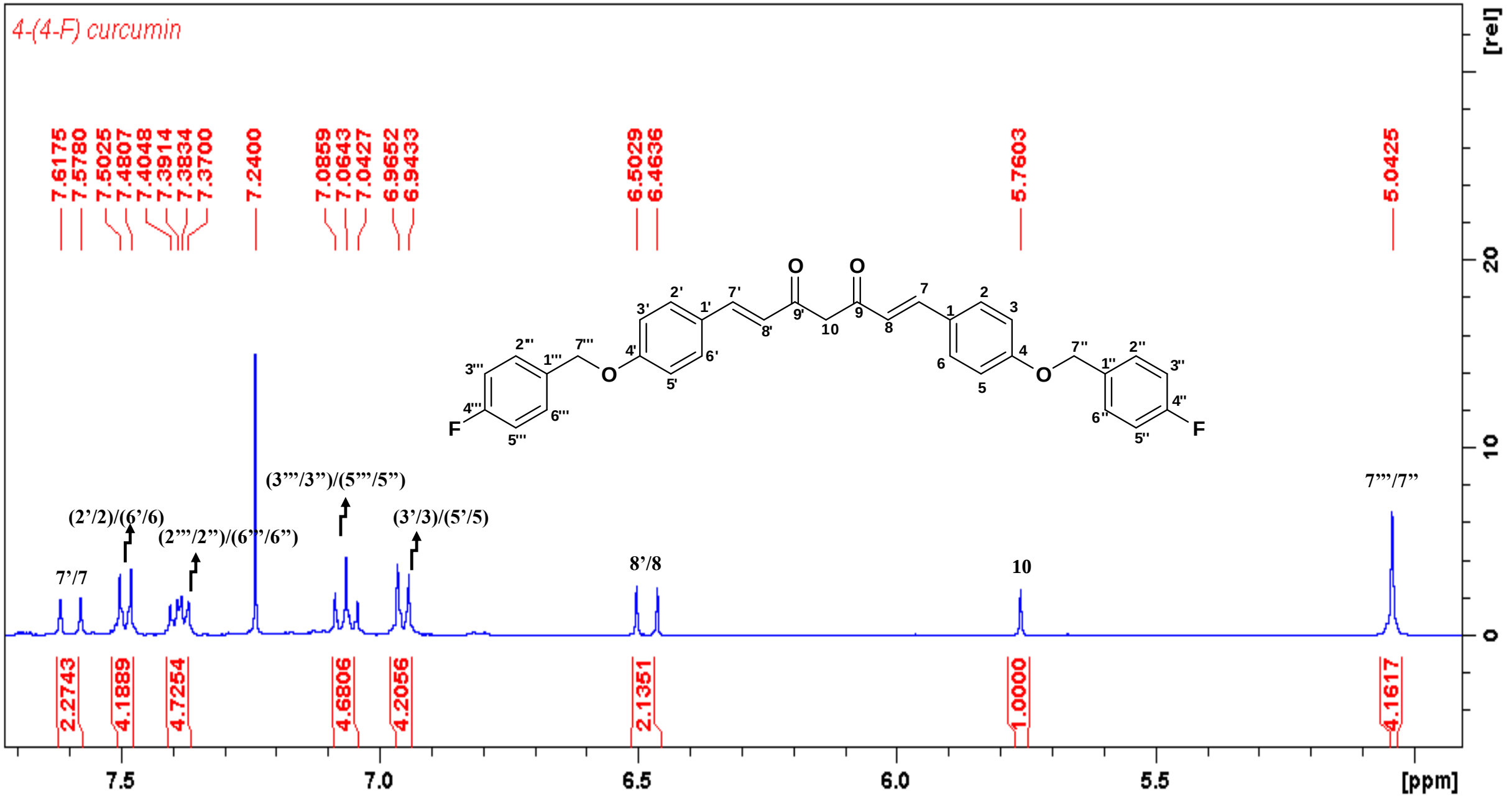
Expanded HSQC of Compound A-4u: (1E,6E)-1,7-bis(4-(benzyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



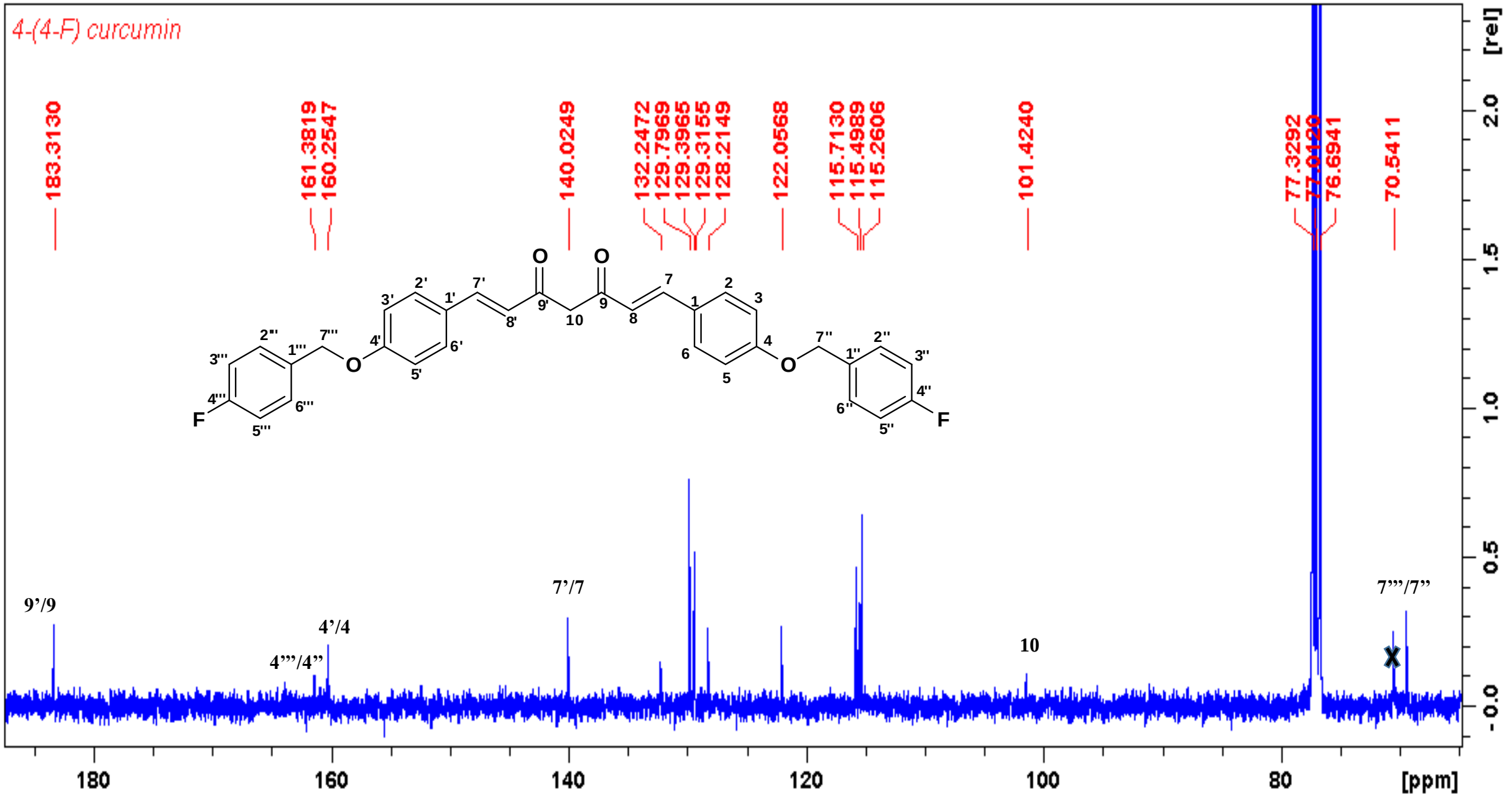
HMBC of Compound A-4u: (1E,6E)-1,7-bis(4-(benzyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



Expanded HMBC Compound A-4u: (1E,6E)-1,7-bis(4-(benzyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



^{1}H Spectrum of Compound A-4v: (1E,6E)-1,7-bis(4-(4-fluorobenzyloxy)phenyl)hepta-1,6-diene-3,5-dione



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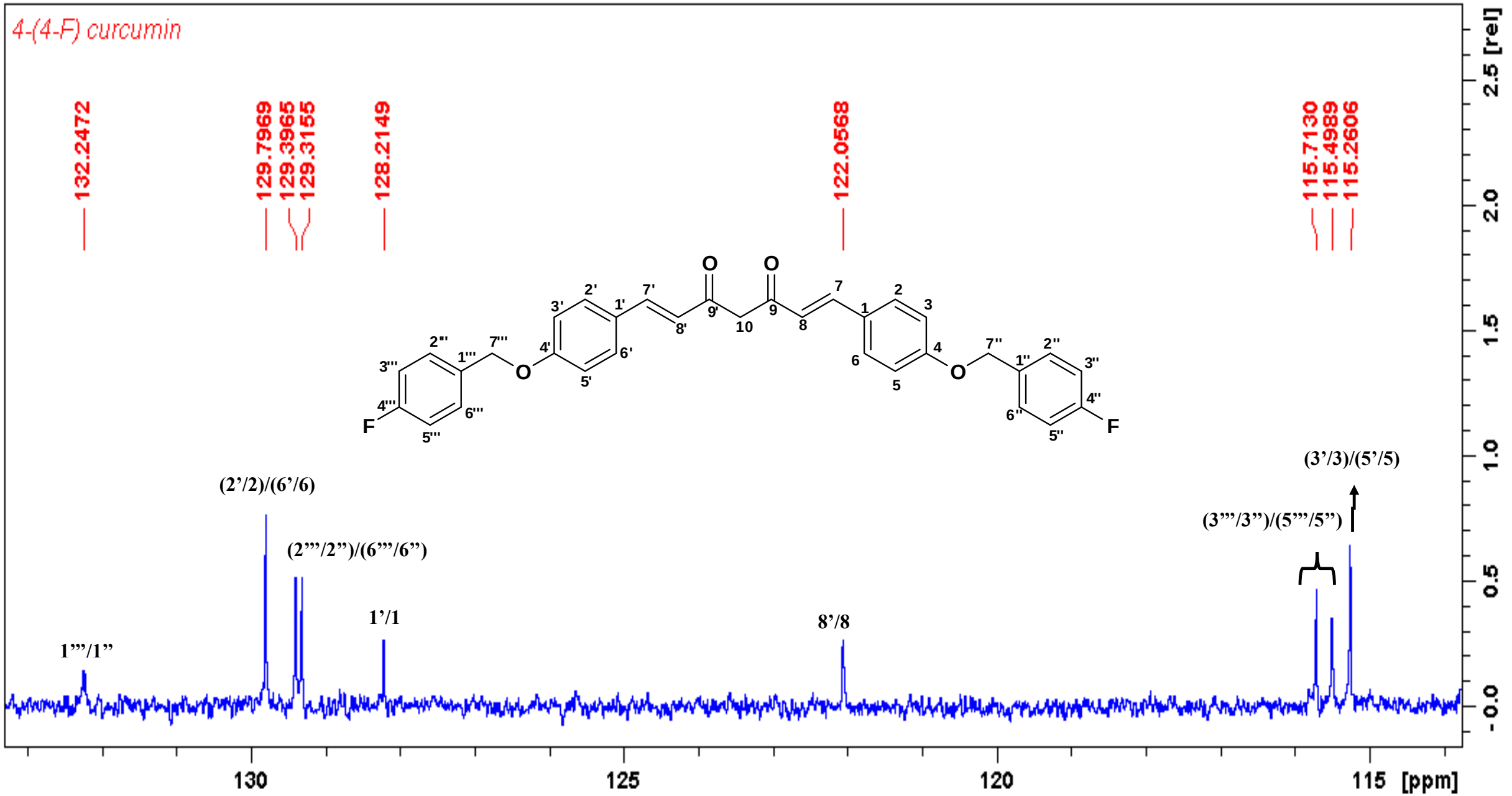
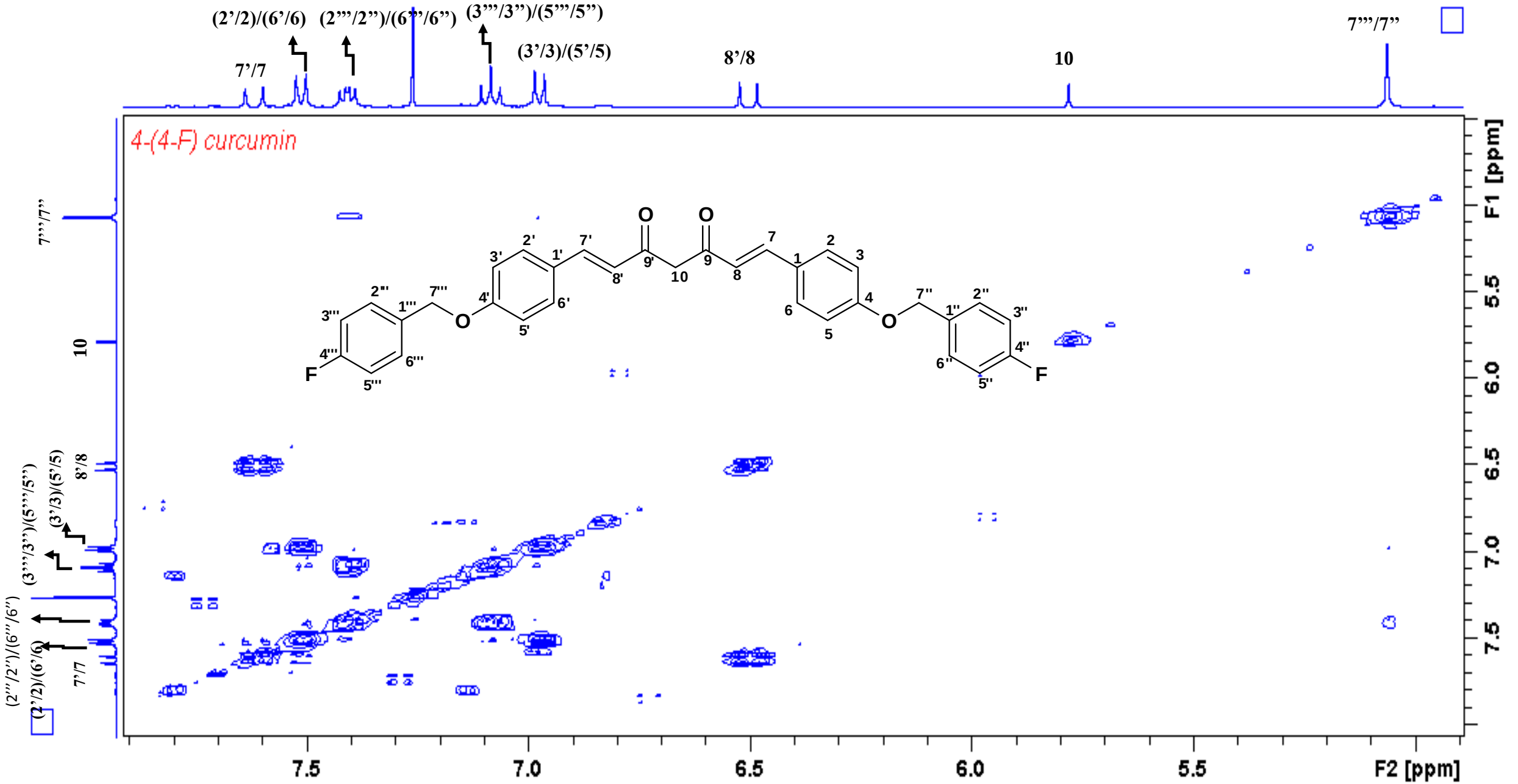
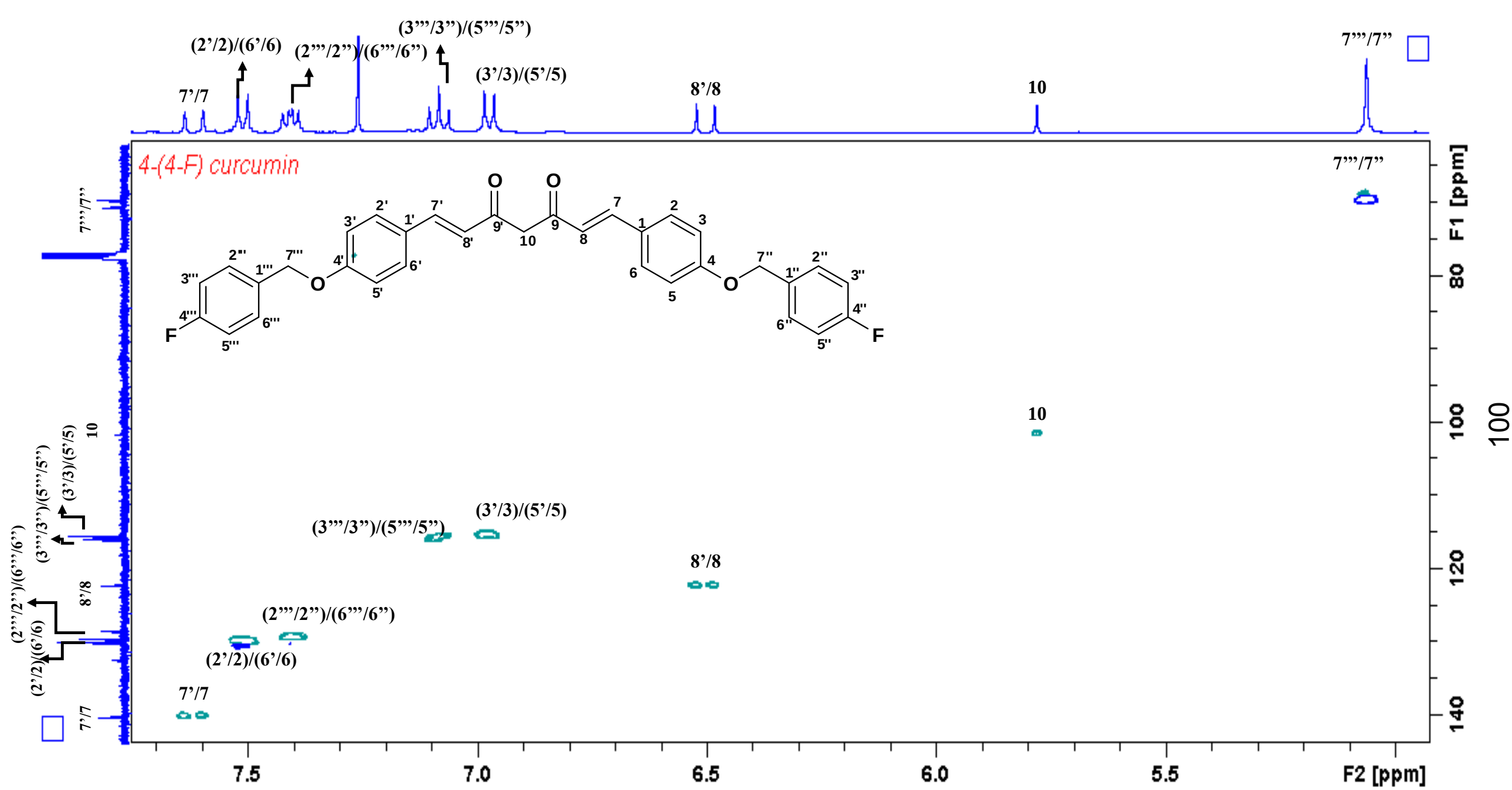
^{13}C Spectrum of Compound A-4v: (1E,6E)-1,7-bis(4-(4-fluorobenzyloxy)phenyl)hepta-1,6-diene-3,5-dione



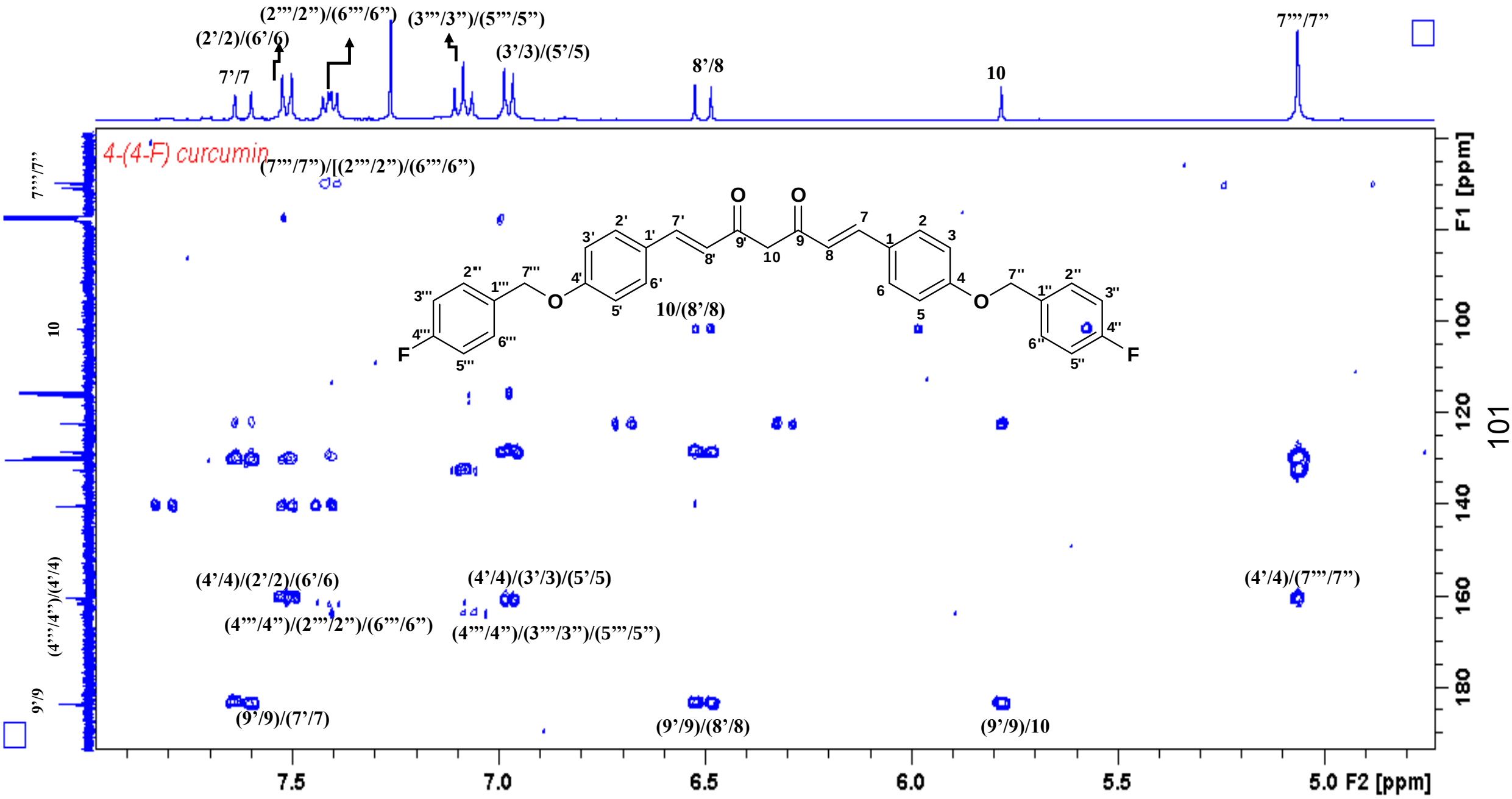
Expanded ^{13}C Spectrum of Compound A-4v: (1E,6E)-1,7-bis(4-(4-fluorobenzyloxy)phenyl)hepta-1,6-diene-3,5-dione



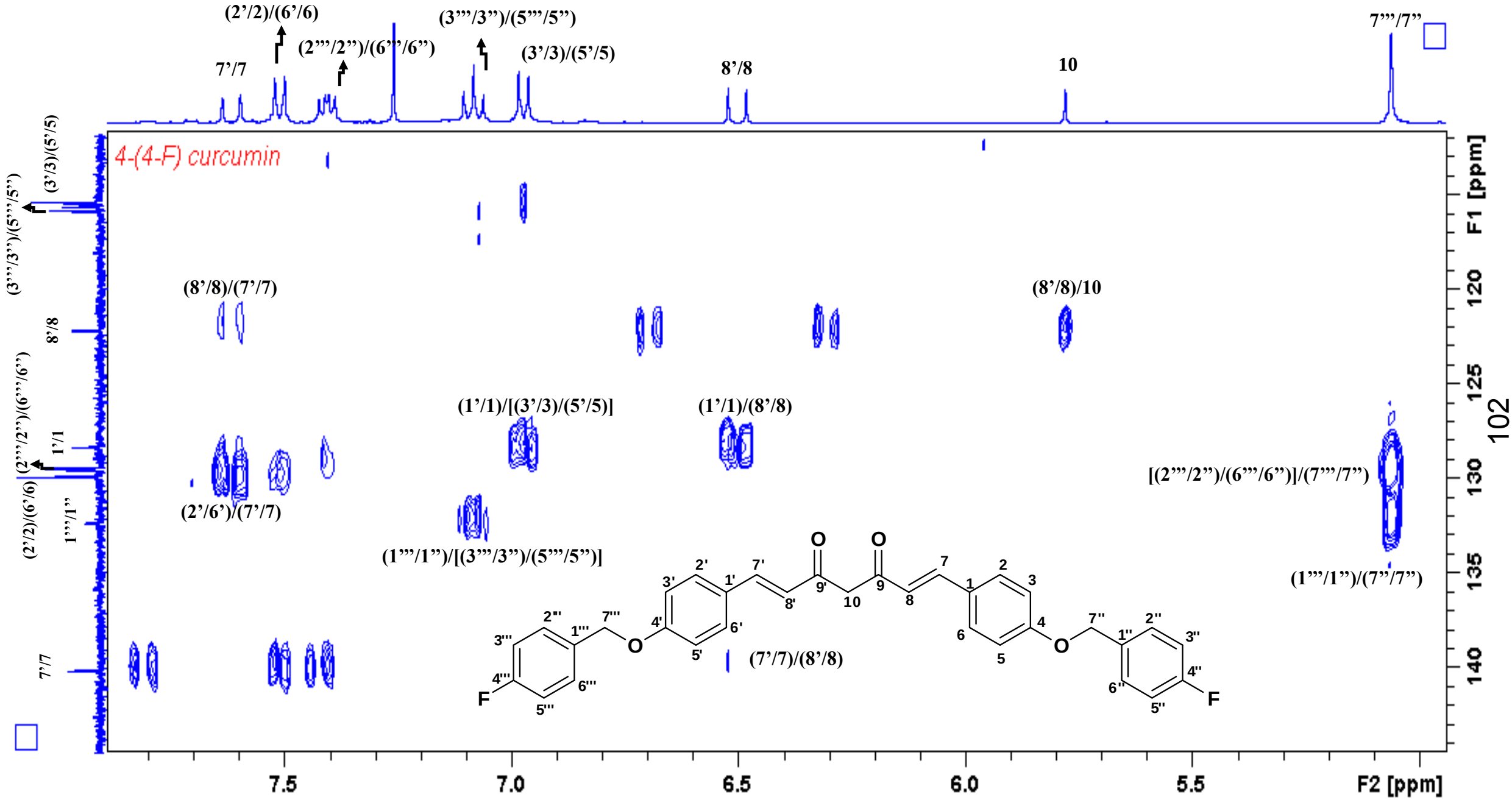
COSY of Compound A-4v: (1E,6E)-1,7-bis(4-(4-fluorobenzyloxy)phenyl)hepta-1,6-diene-3,5-dione



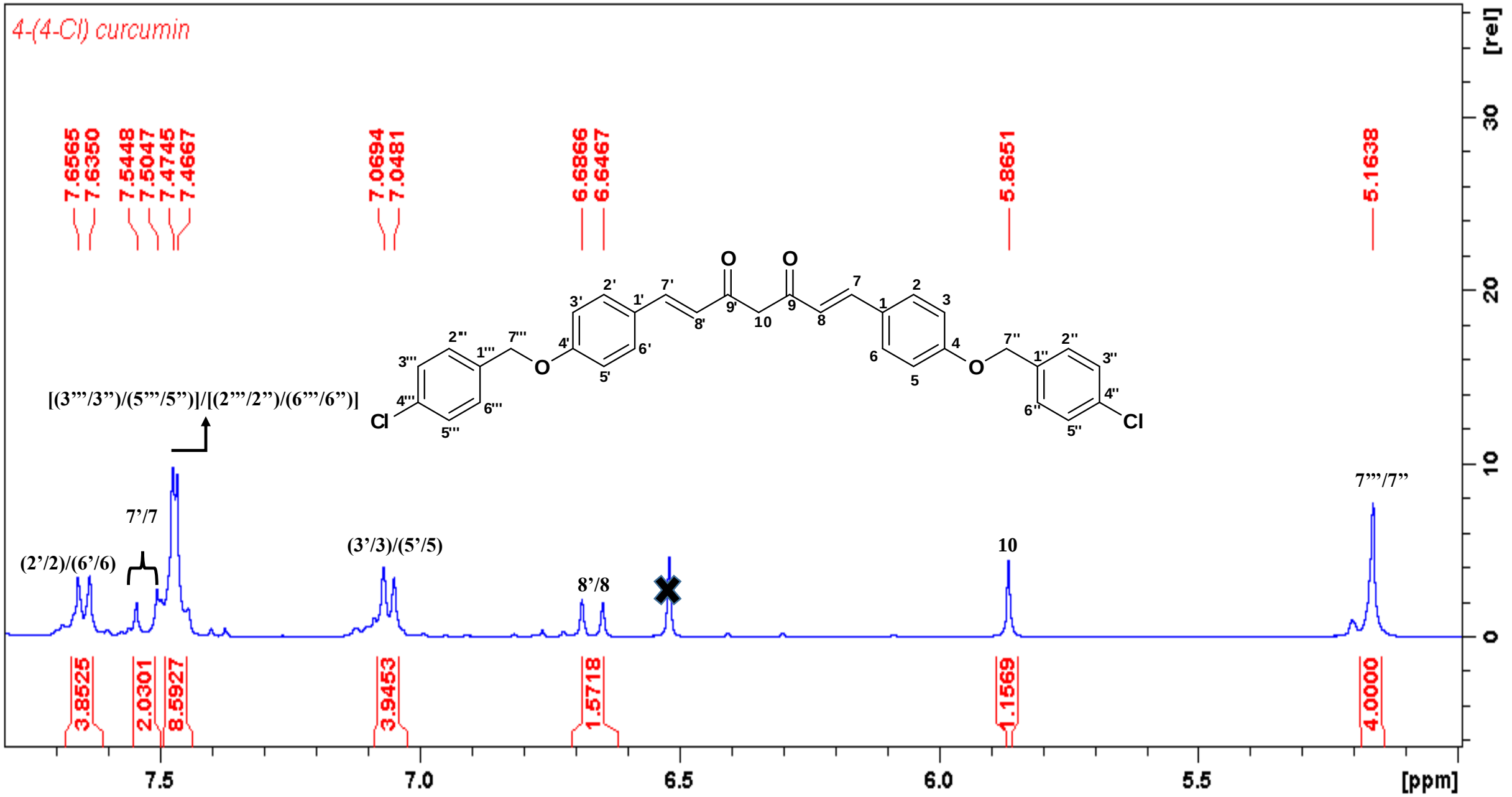
HSQC of Compound A-4v: (1E,6E)-1,7-bis(4-(4-fluorobenzyloxy)phenyl)hepta-1,6-diene-3,5-dione



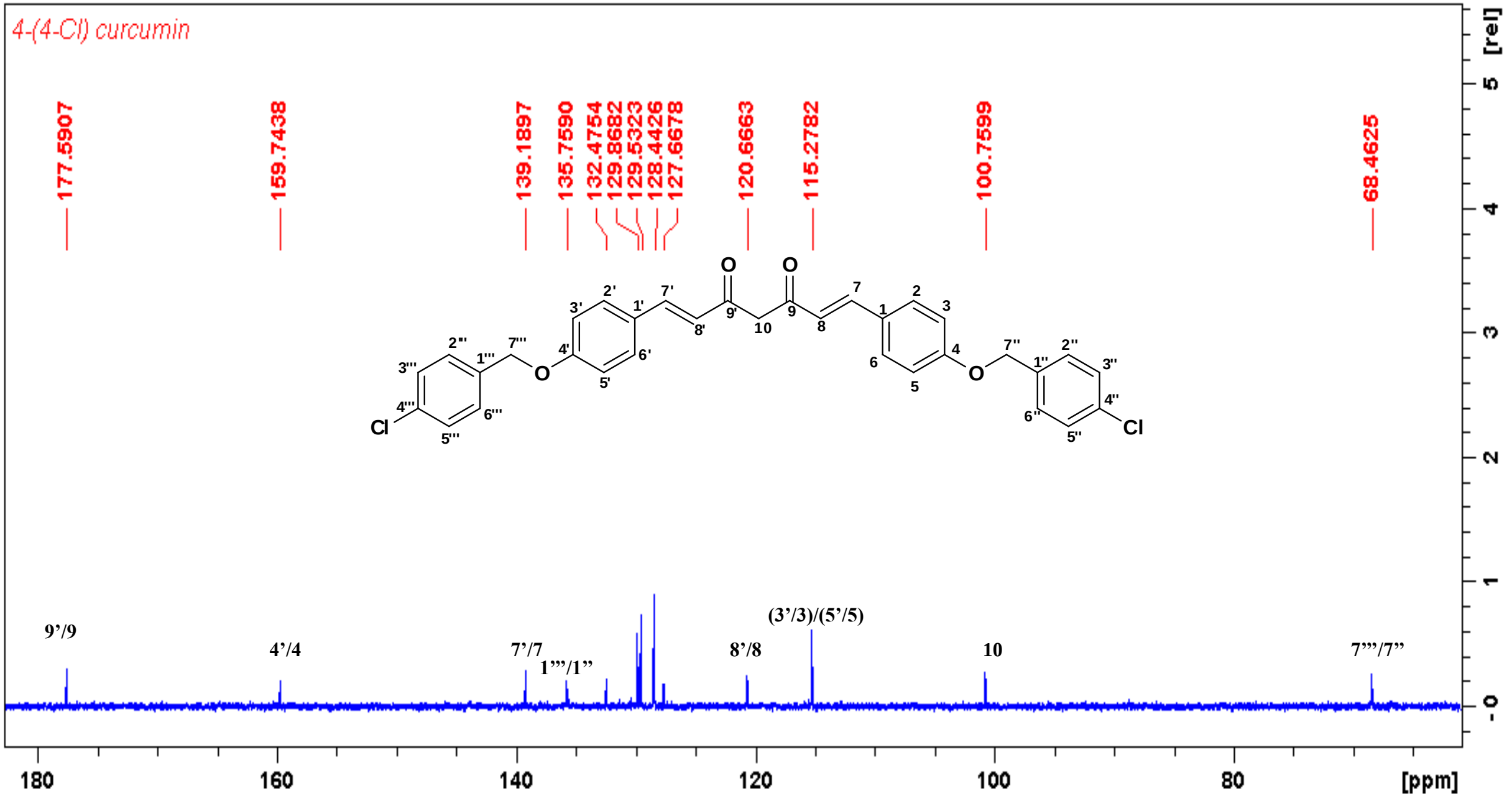
HMBC of Compound A-4v: (1E,6E)-1,7-bis(4-(4-fluorobenzyloxy)phenyl)hepta-1,6-diene-3,5-dione



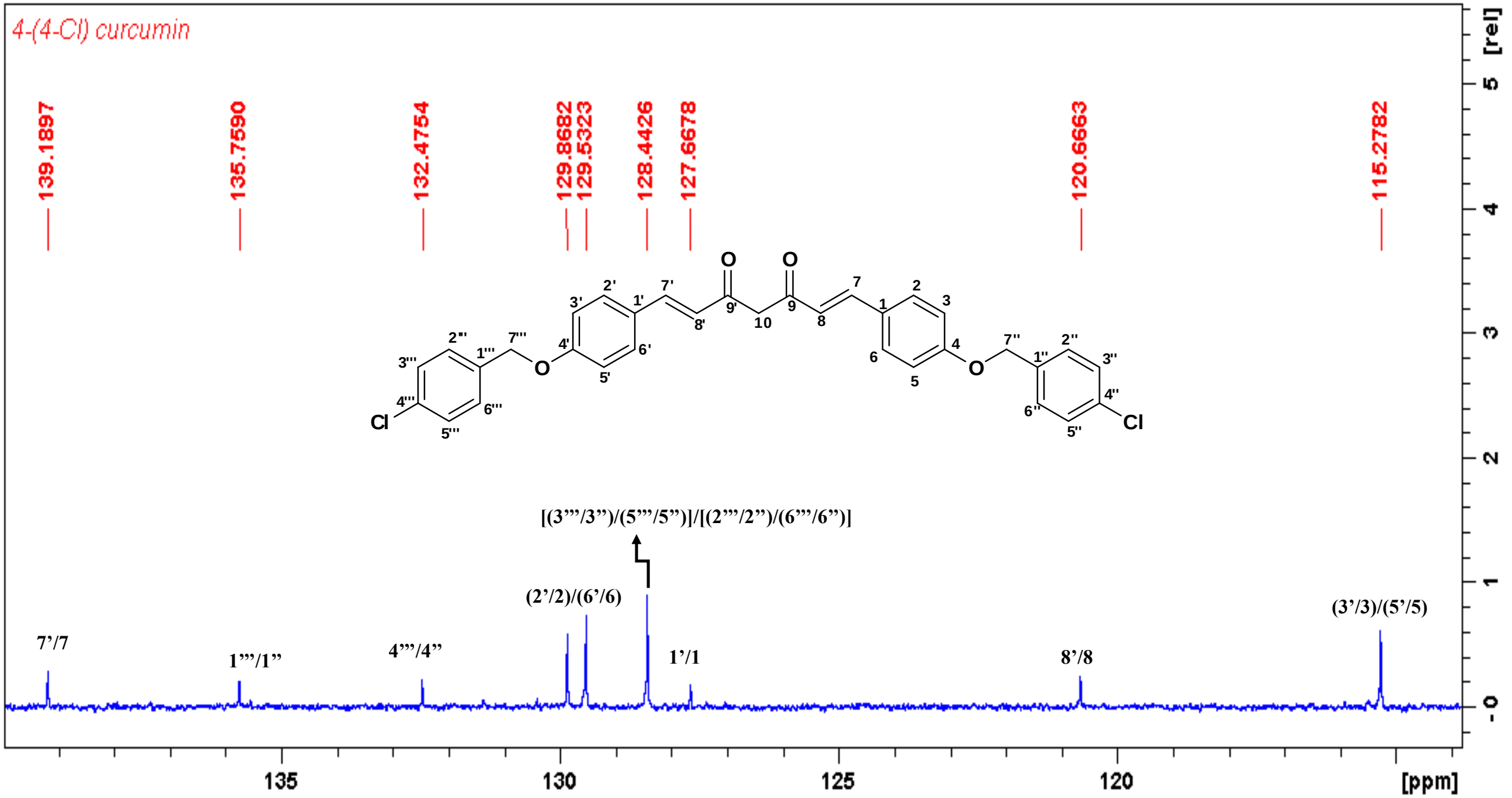
Expanded HMBC of Compound A-4v: (1E,6E)-1,7-bis(4-(4-fluorobenzyloxy)phenyl)hepta-1,6-diene-3,5-dione



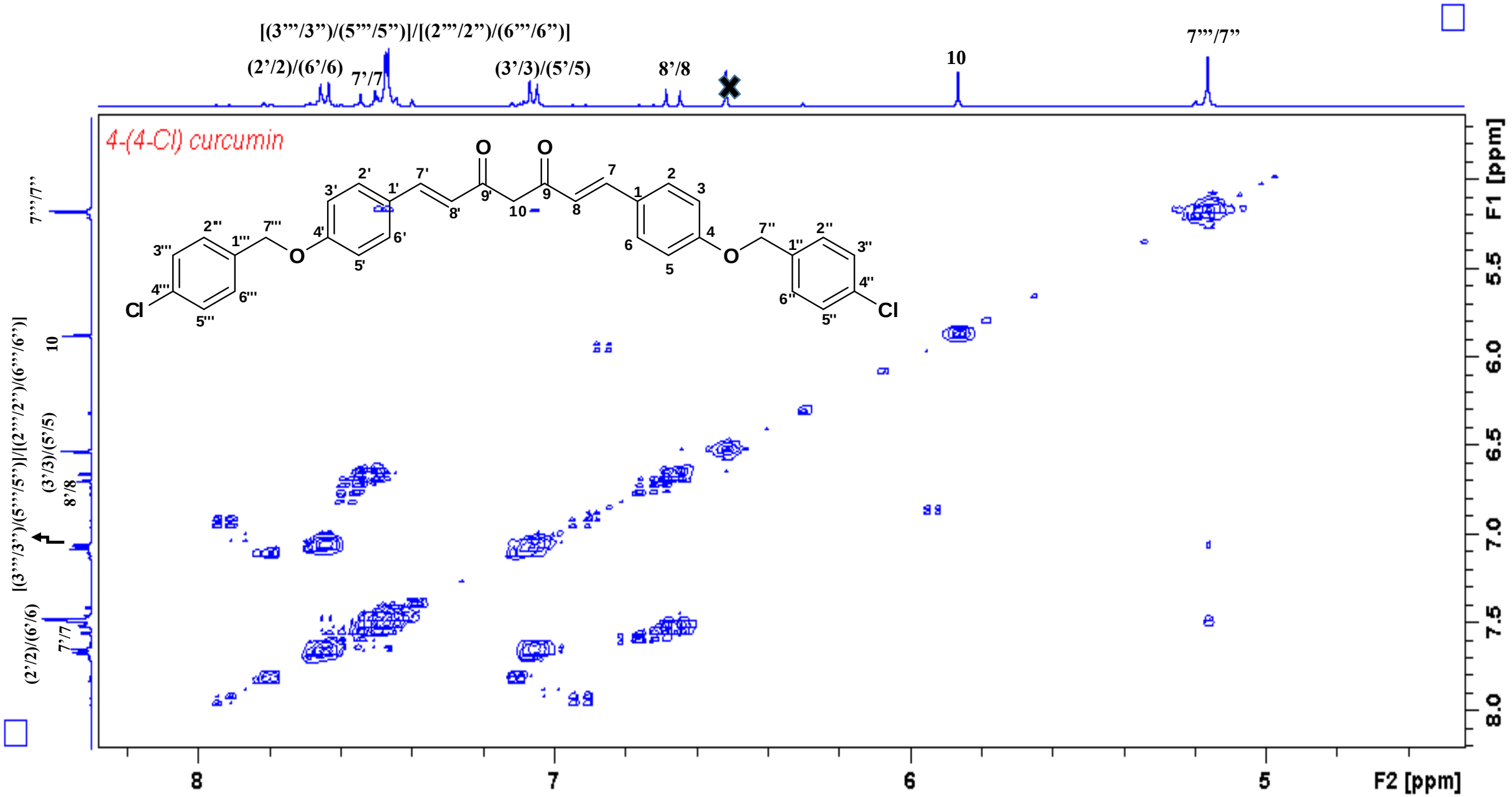
^{1}H spectrum of Compound A-4w: (1E,6E)-1,7-bis(4-(4-chlorobenzyloxy)phenyl)hepta-1,6-diene-3,5-dione



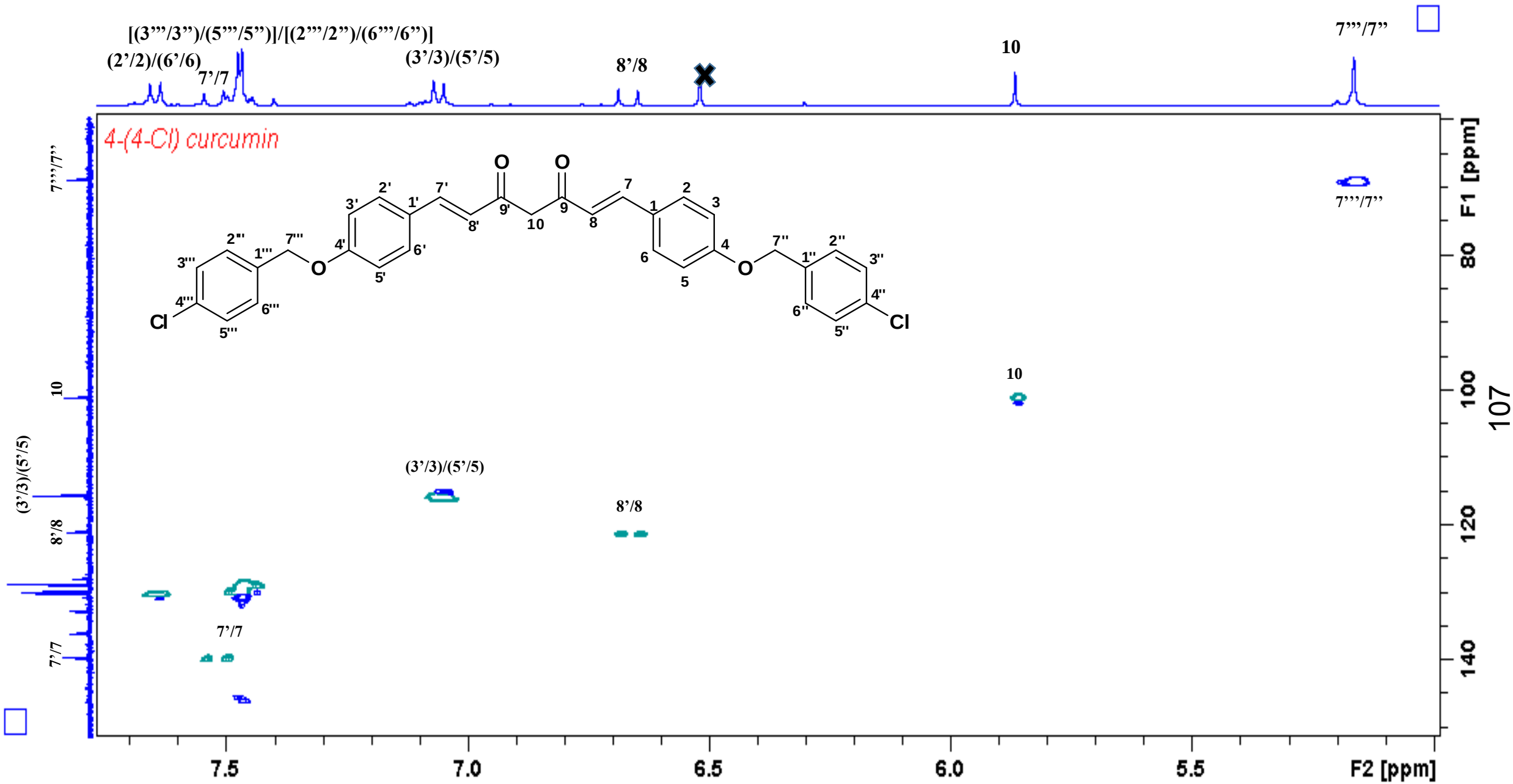
^{13}C spectrum of Compound A-4w: (1E,6E)-1,7-bis(4-(4-chlorobenzyloxy)phenyl)hepta-1,6-diene-3,5-dione



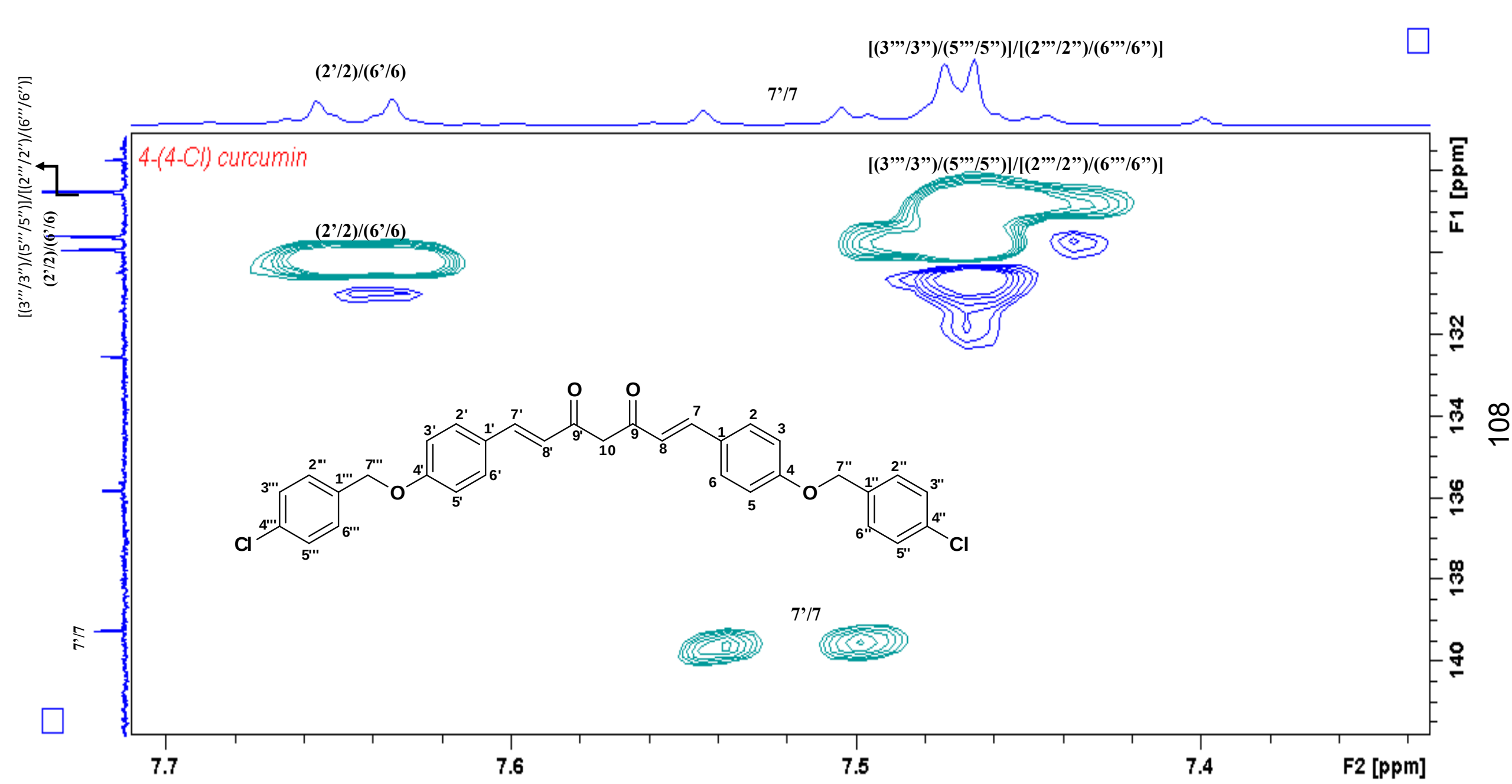
Expanded ^{13}C spectrum of Compound A-4w: (1E,6E)-1,7-bis(4-(4-chlorobenzyloxy)phenyl)hepta-1,6-diene-3,5-dione



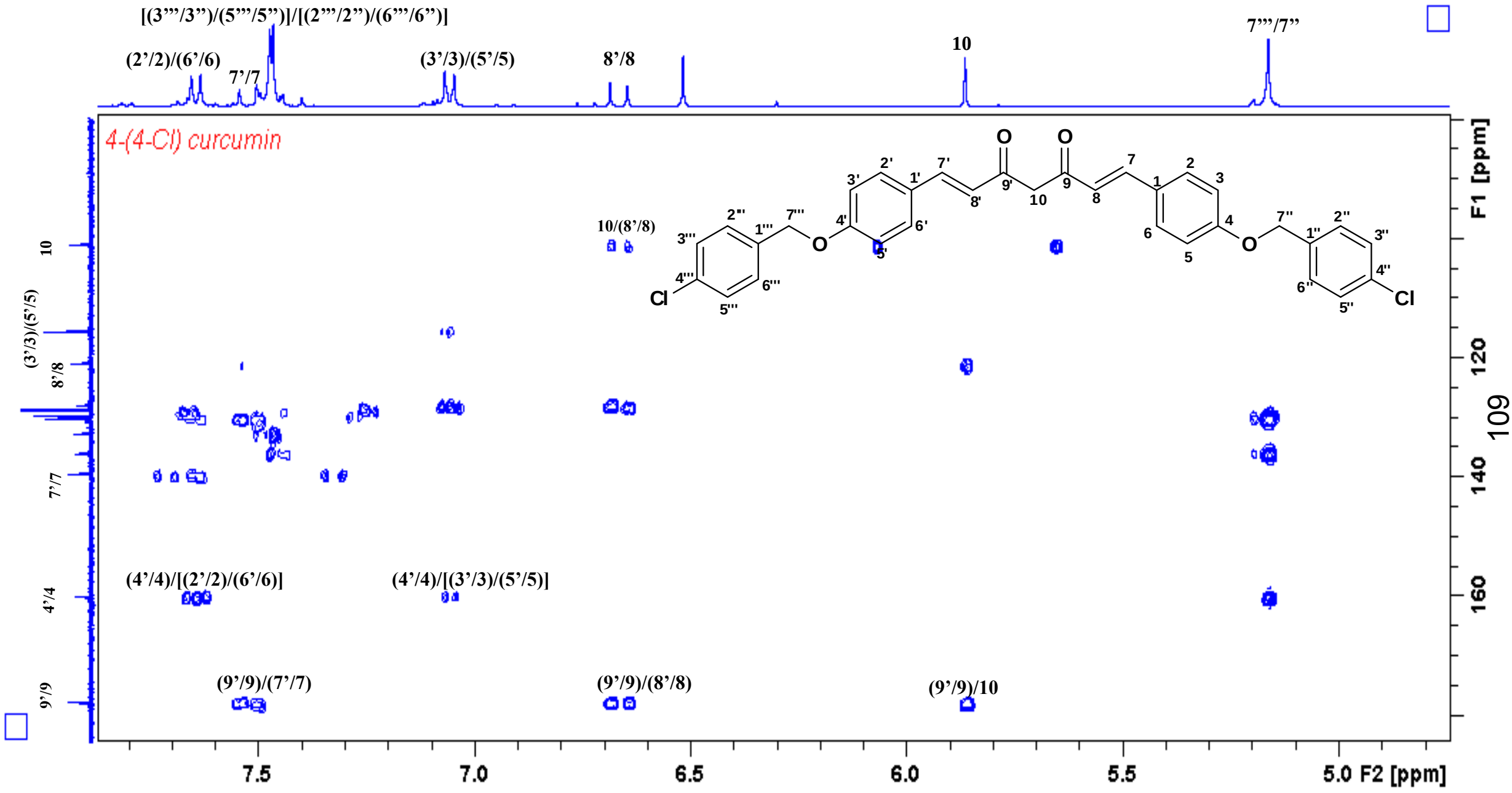
COSY of Compound A-4w: (1E,6E)-1,7-bis(4-(4-chlorobenzyloxy)phenyl)hepta-1,6-diene-3,5-dione



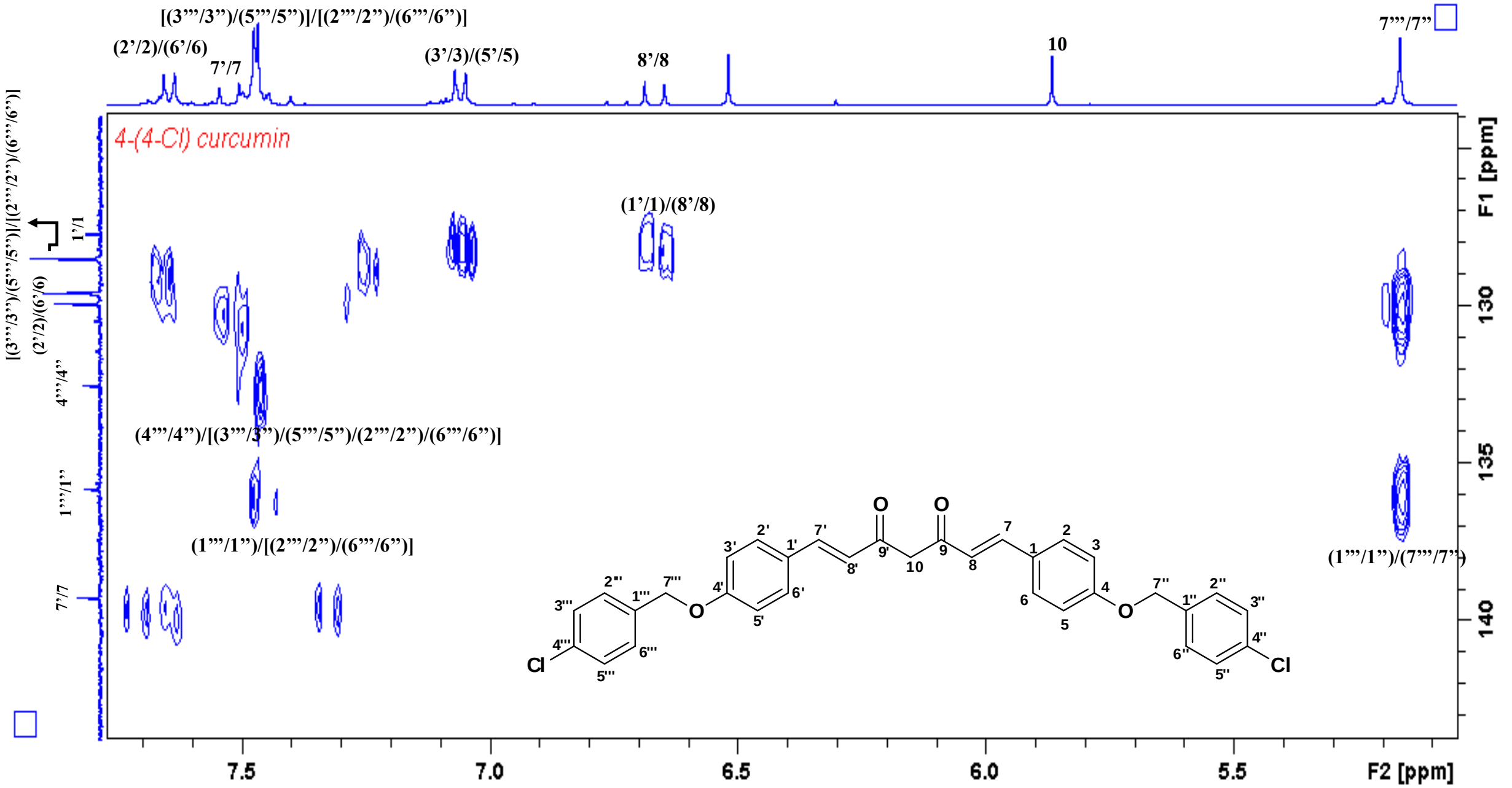
HSQC of Compound A-4w: (1E,6E)-1,7-bis(4-(4-chlorobenzyloxy)phenyl)hepta-1,6-diene-3,5-dione



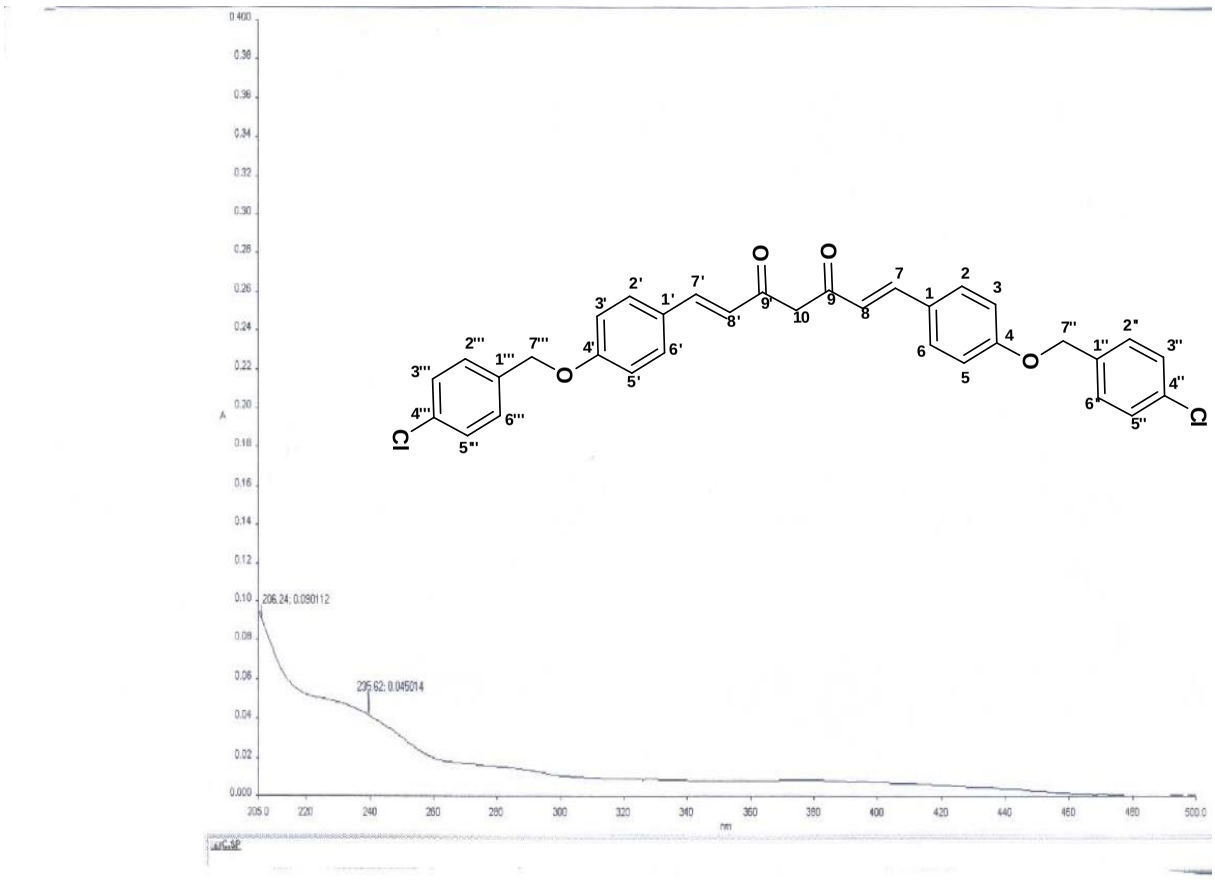
Expanded HSQC of Compound A-4w: (1E,6E)-1,7-bis(4-(4-chlorobenzyloxy)phenyl)hepta-1,6-diene-3,5-dione



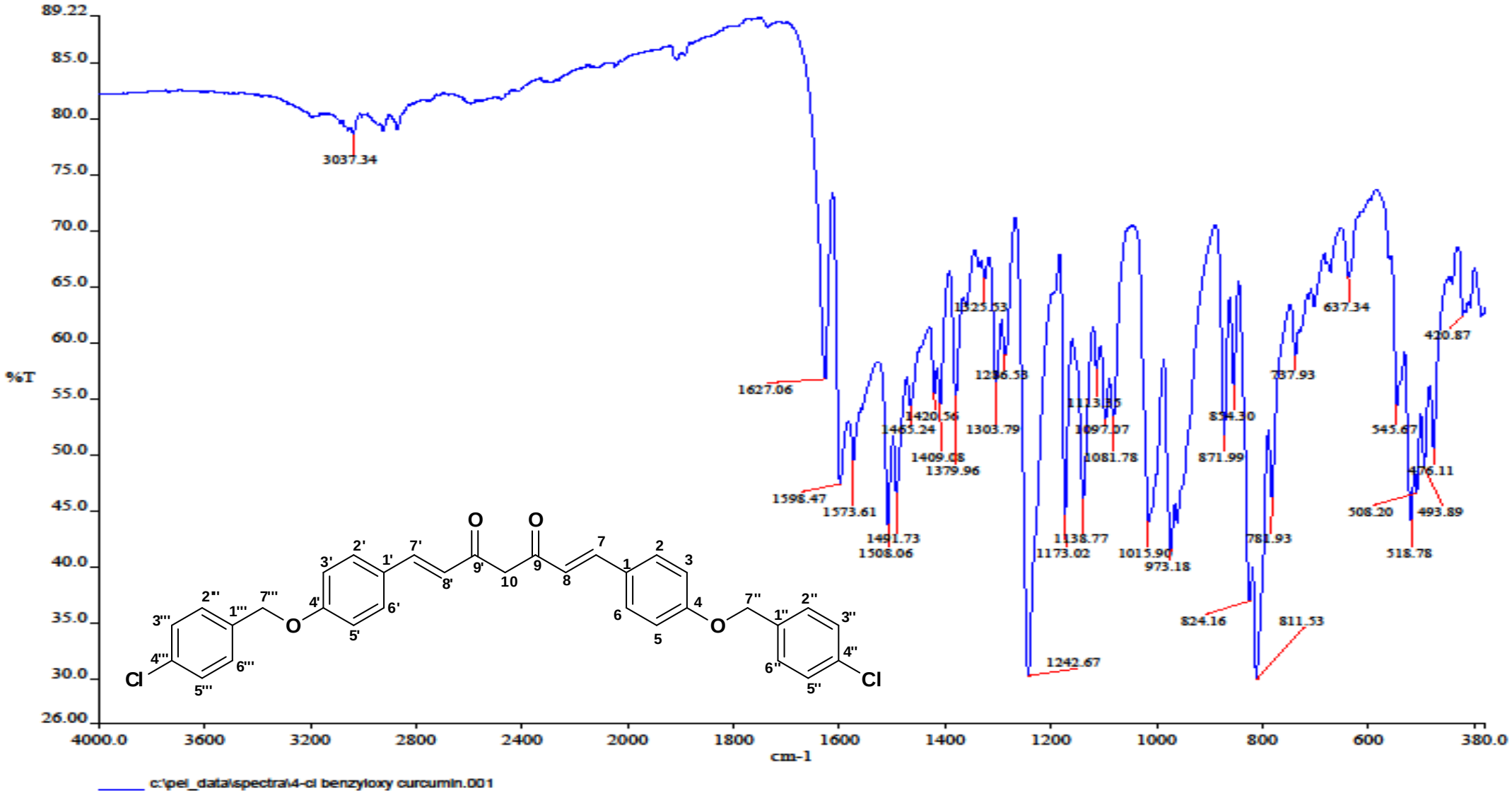
HMBC of Compound A-4w: (1E,6E)-1,7-bis(4-(4-chlorobenzyloxy)phenyl)hepta-1,6-diene-3,5-dione



Expanded HMBC of Compound A-4w: (1E,6E)-1,7-bis(4-(4-chlorobenzyloxy)phenyl)hepta-1,6-diene-3,5-dione



Ultraviolet Spectrum of Compound A-4w: (1E,6E)-1,7-bis(4-(4-chlorobenzyloxy)phenyl)hepta-1,6-diene-3,5-dione

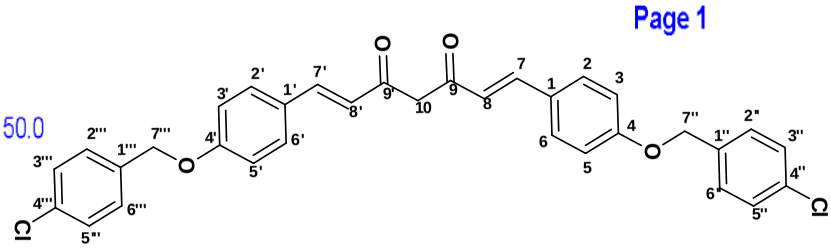


Infrared Spectrum of Compound A-4w: (1E,6E)-1,7-bis(4-(4-chlorobenzyloxy)phenyl)hepta-1,6-diene-3,5-dione

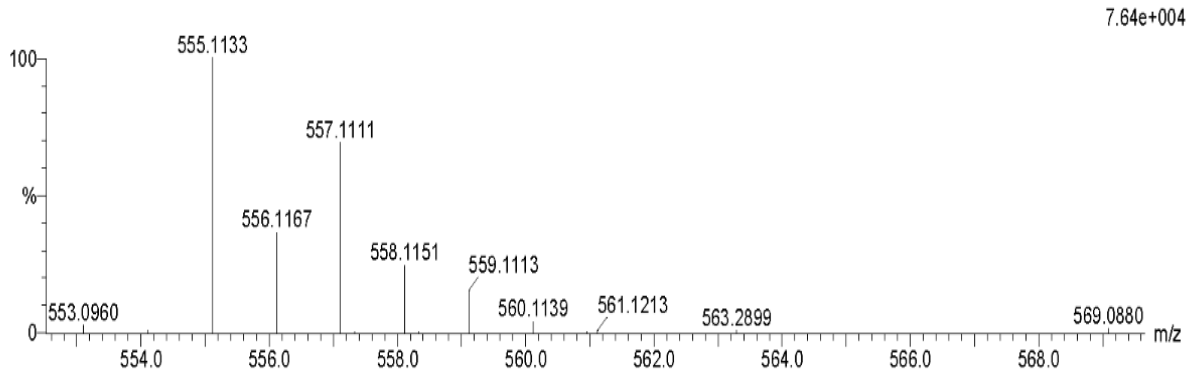
Elemental Composition Report **Page 1**

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

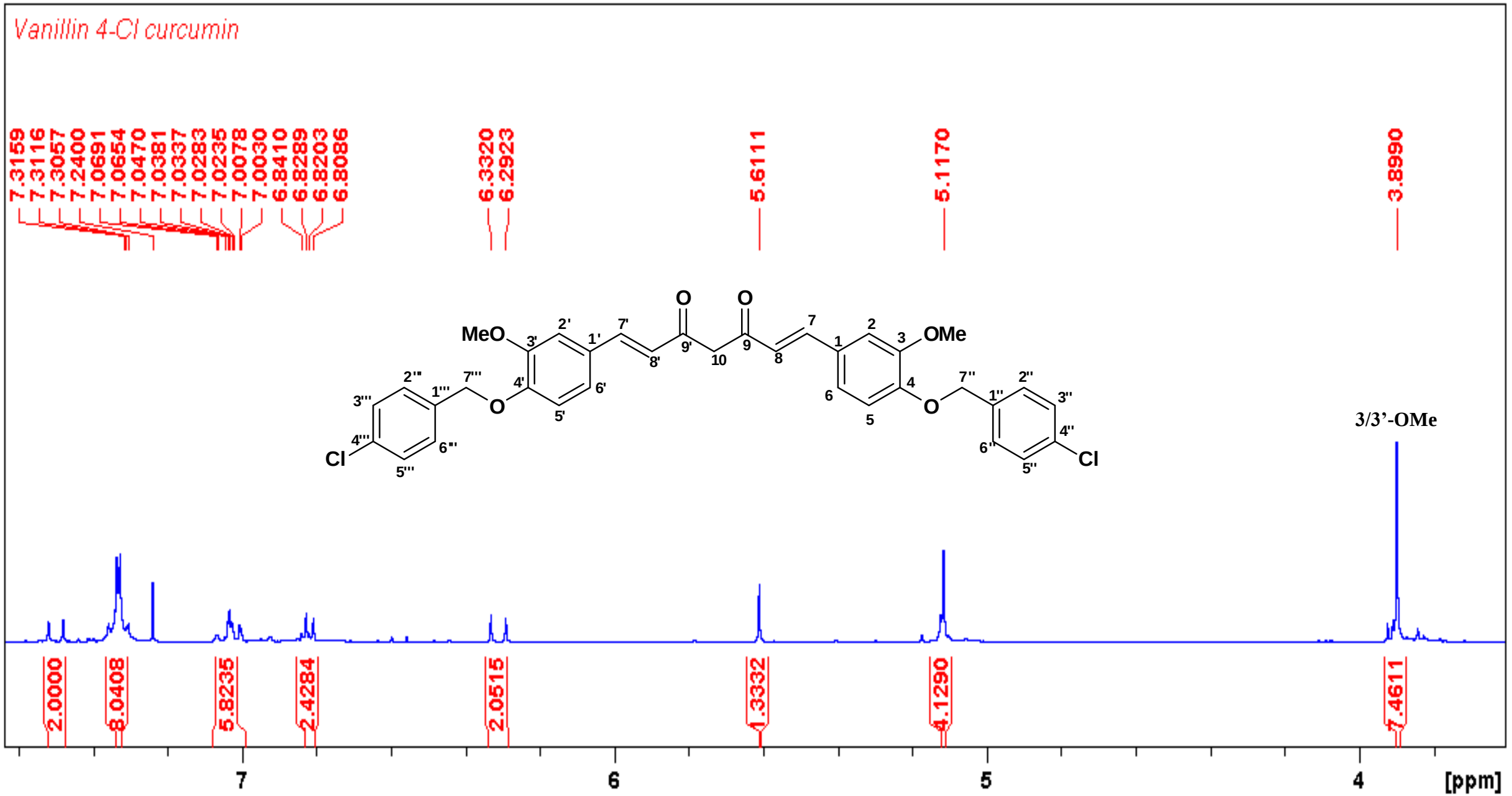


Monoisotopic Mass, Even Electron Ions
5 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 30-35 H: 25-30 O: 0-5 Cl: 0-2
12 4-Cl bnbr 60 (1.989) Cm (1:61)
TOF MS ES-

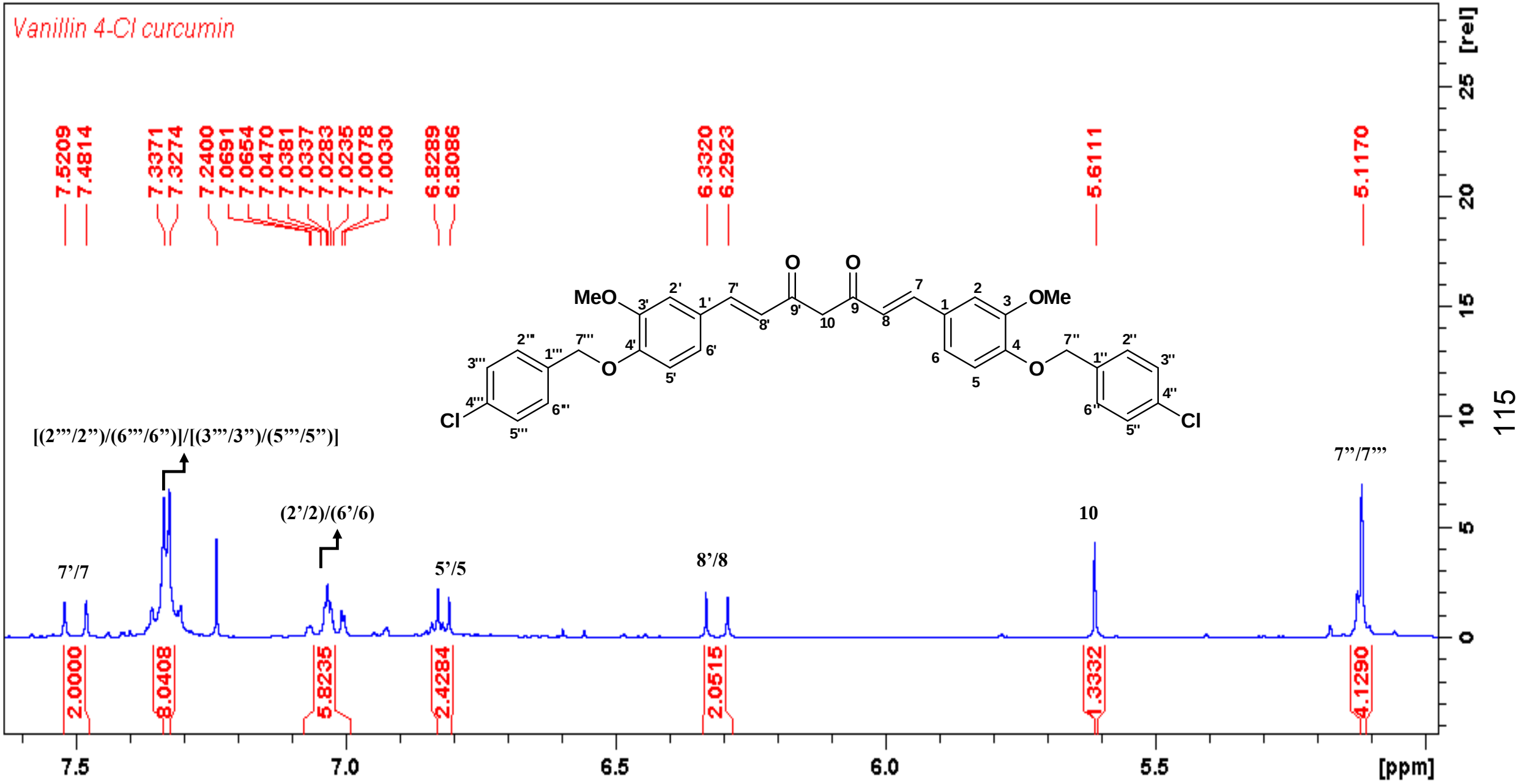


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
555.1133	555.1130	0.3	0.5	20.5	10.6	0.0	C33 H25 O4 Cl2

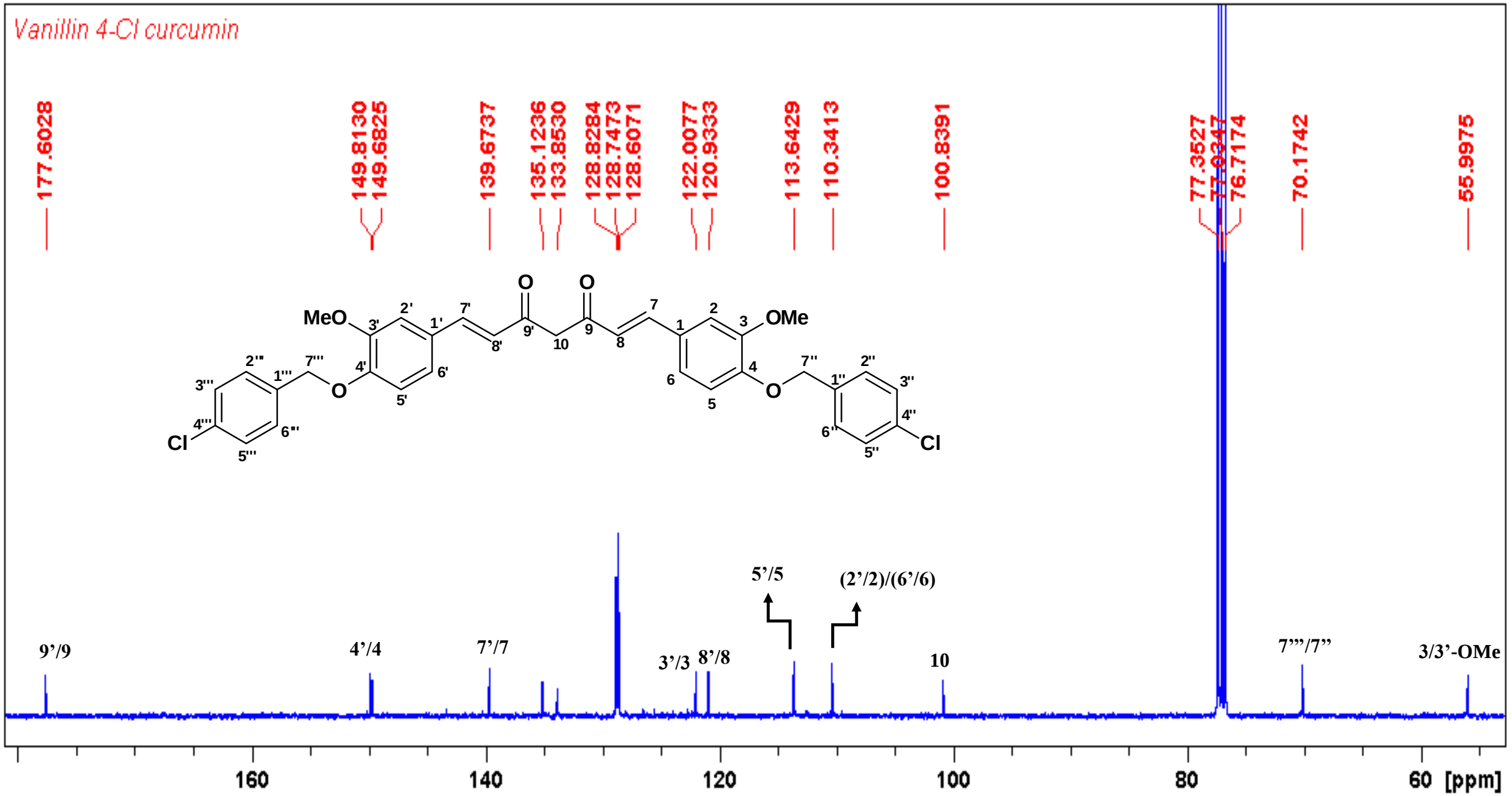
HRMS of Compound A-4w: (1E,6E)-1,7-bis(4-(4-chlorobenzyloxy)phenyl)hepta-1,6-diene-3,5-dione



^{1}H Spectrum of Compound A-4x: (1E,6E)-1,7-bis(4-(4-chlorobenzyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



Expanded ^{1}H Spectrum of Compound A-4x: (1E,6E)-1,7-bis(4-(4-chlorobenzyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



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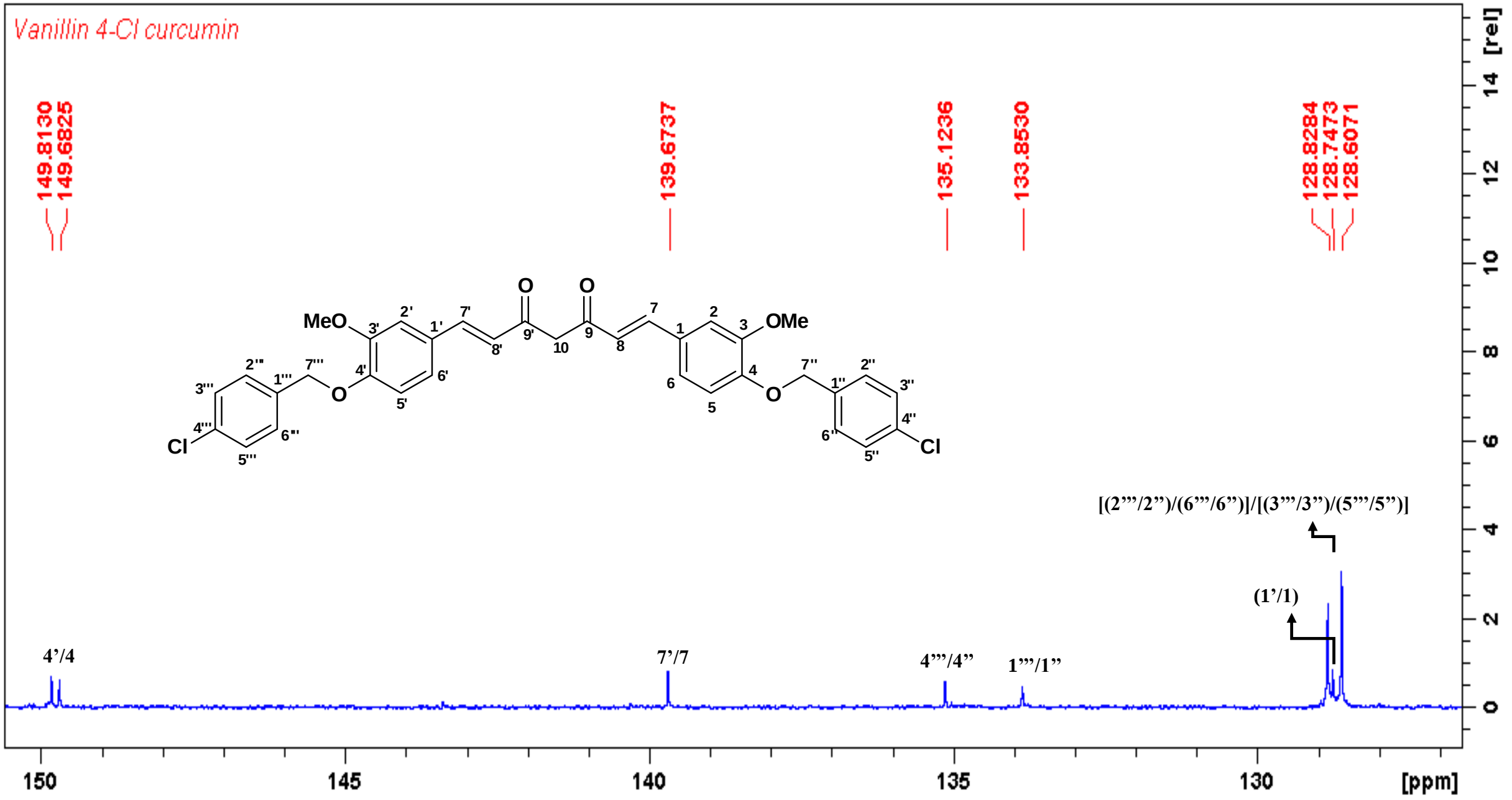
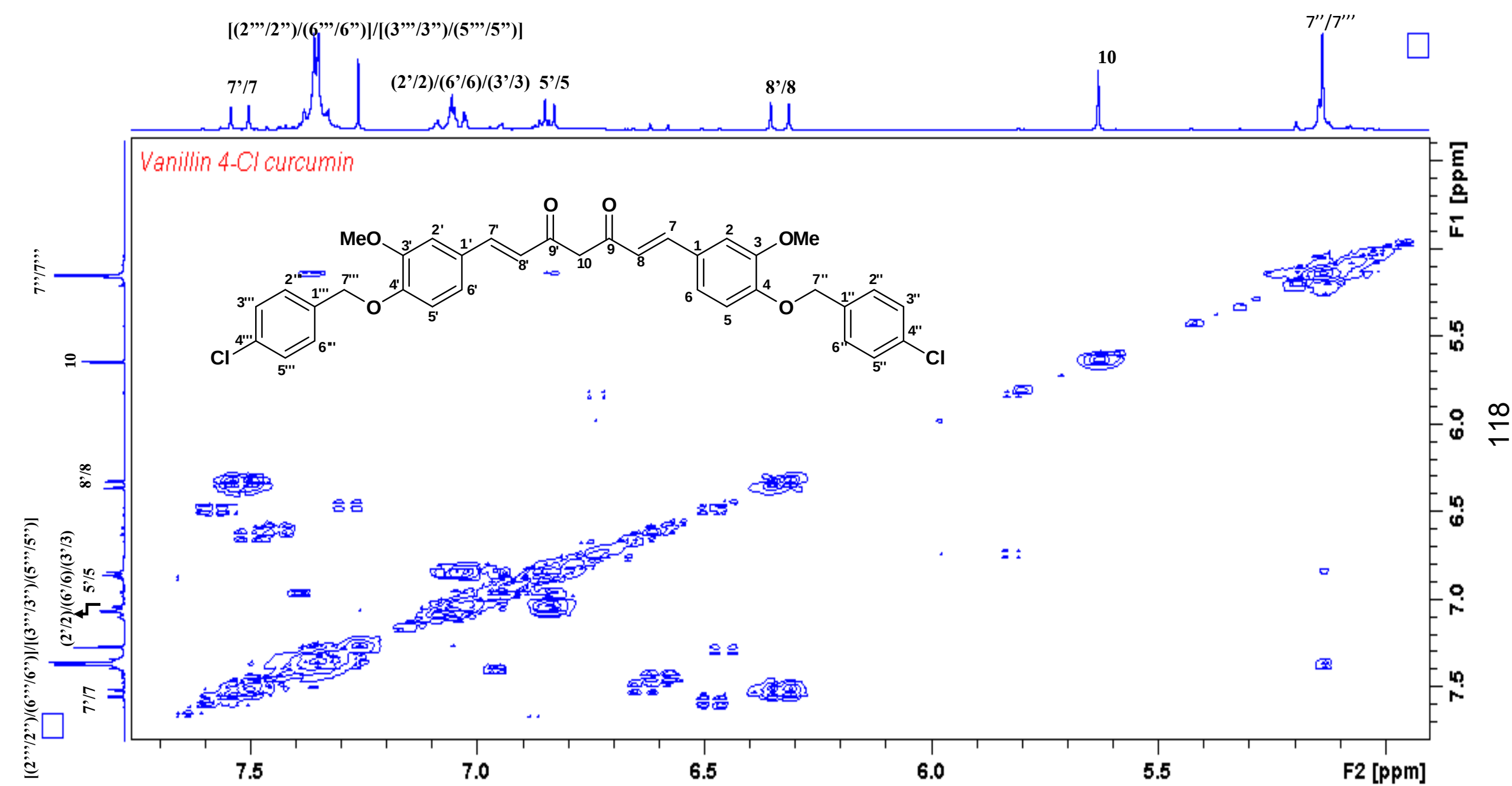
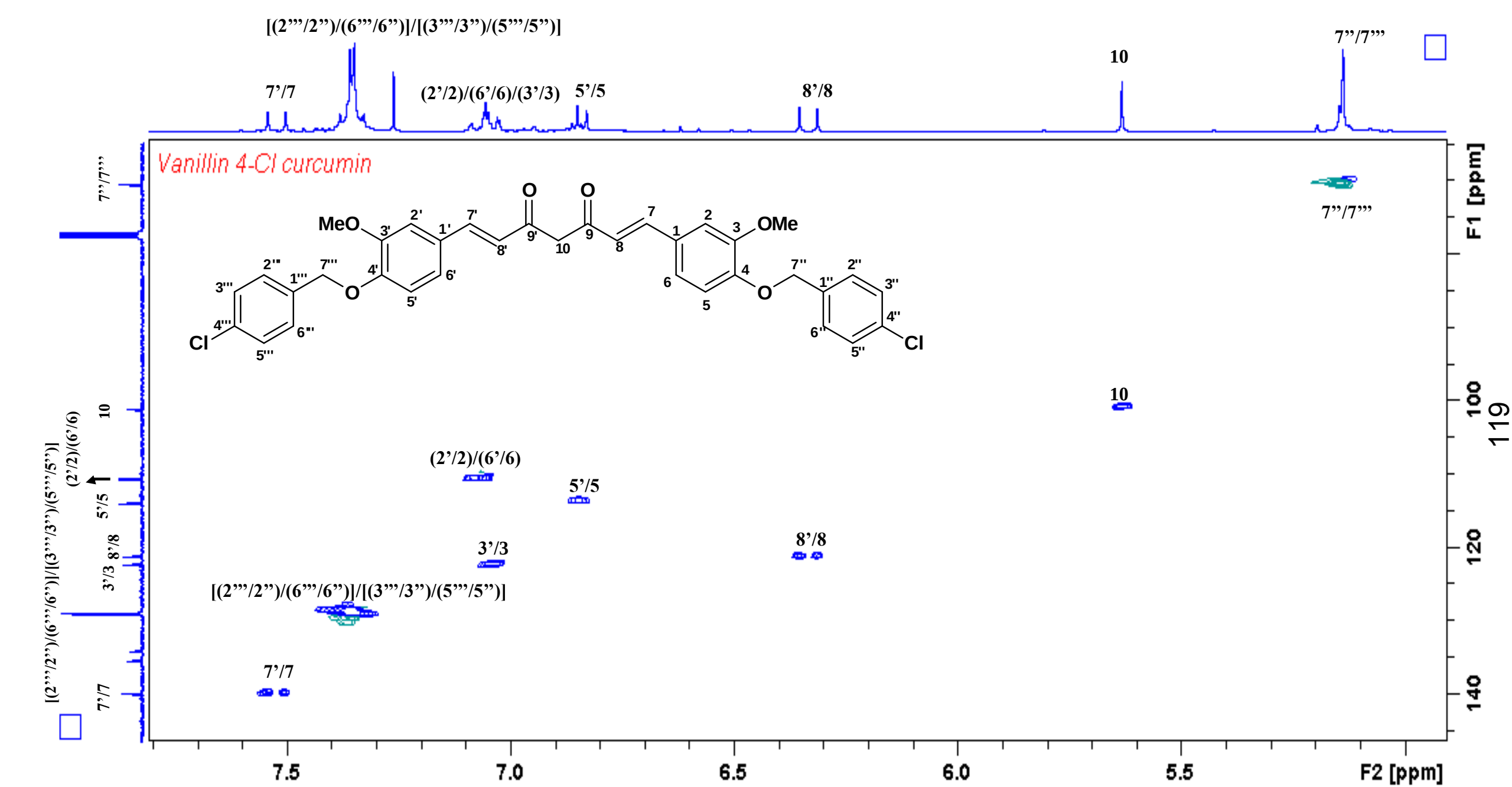
^{13}C Spectrum of Compound A-4x: (1E,6E)-1,7-bis(4-(4-chlorobenzyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



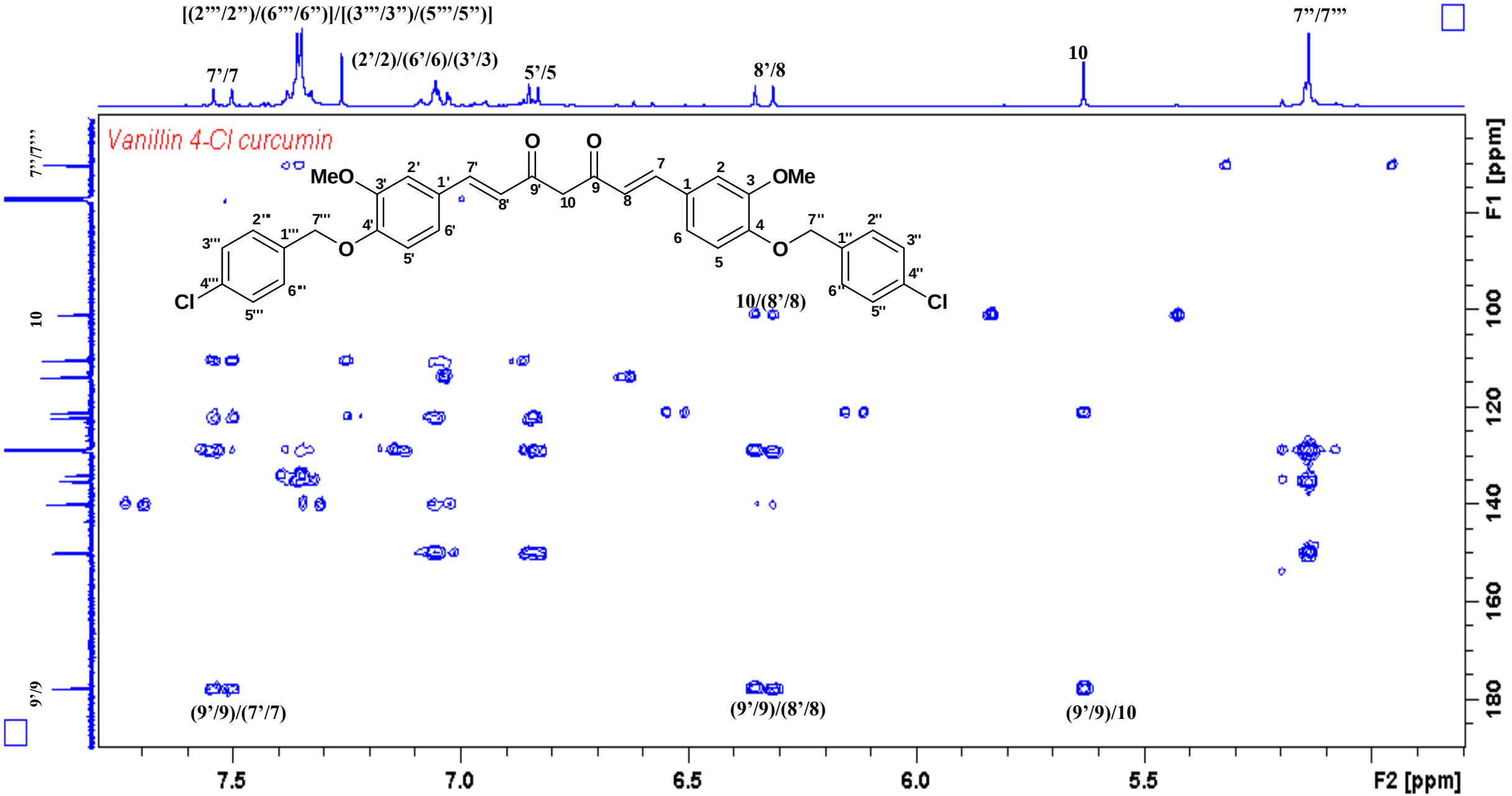
Expanded ^{13}C Spectrum of Compound A-4x: (1E,6E)-1,7-bis(4-(4-chlorobenzyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



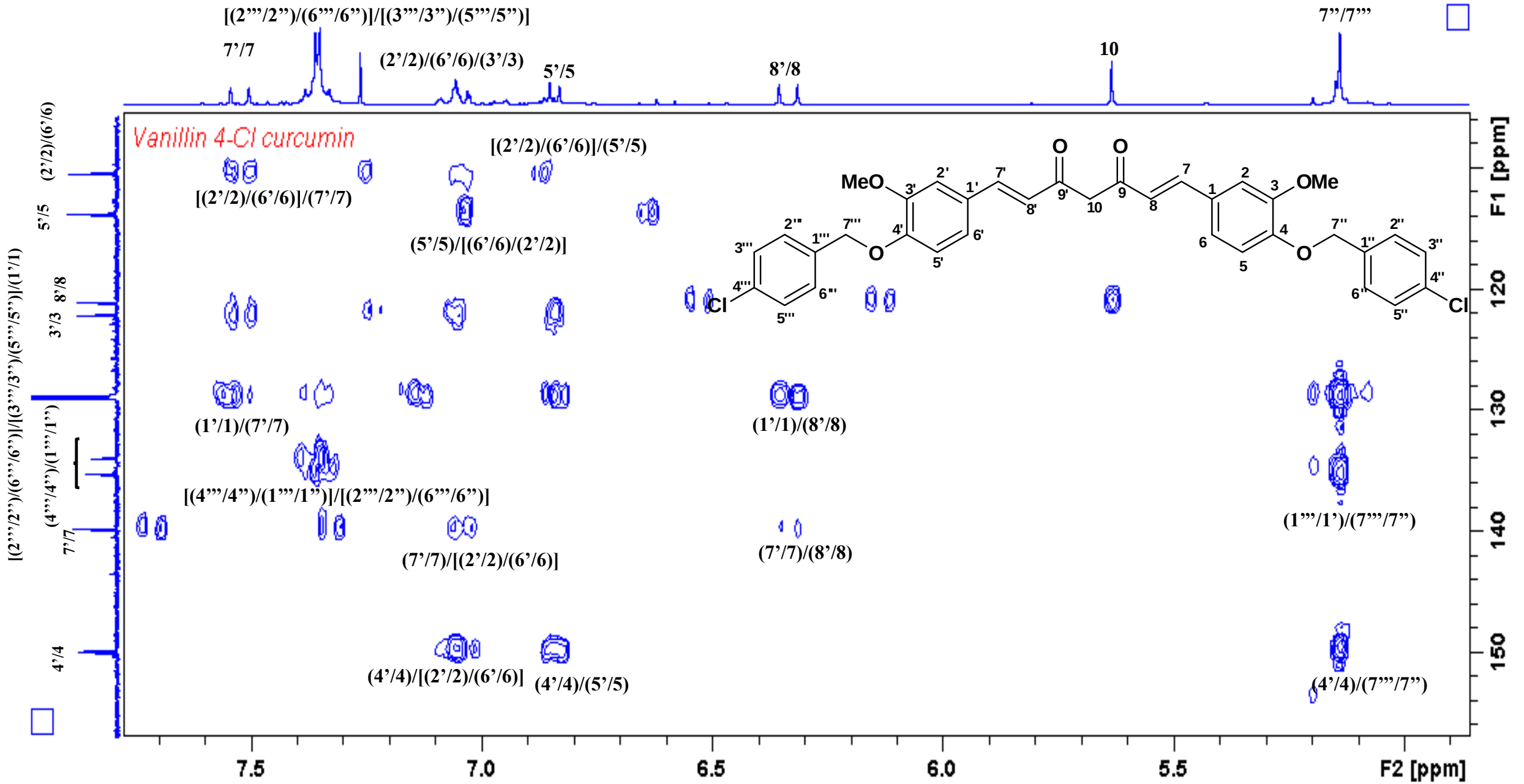
COSY of Compound A-4x: (1E,6E)-1,7-bis(4-(4-chlorobenzyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



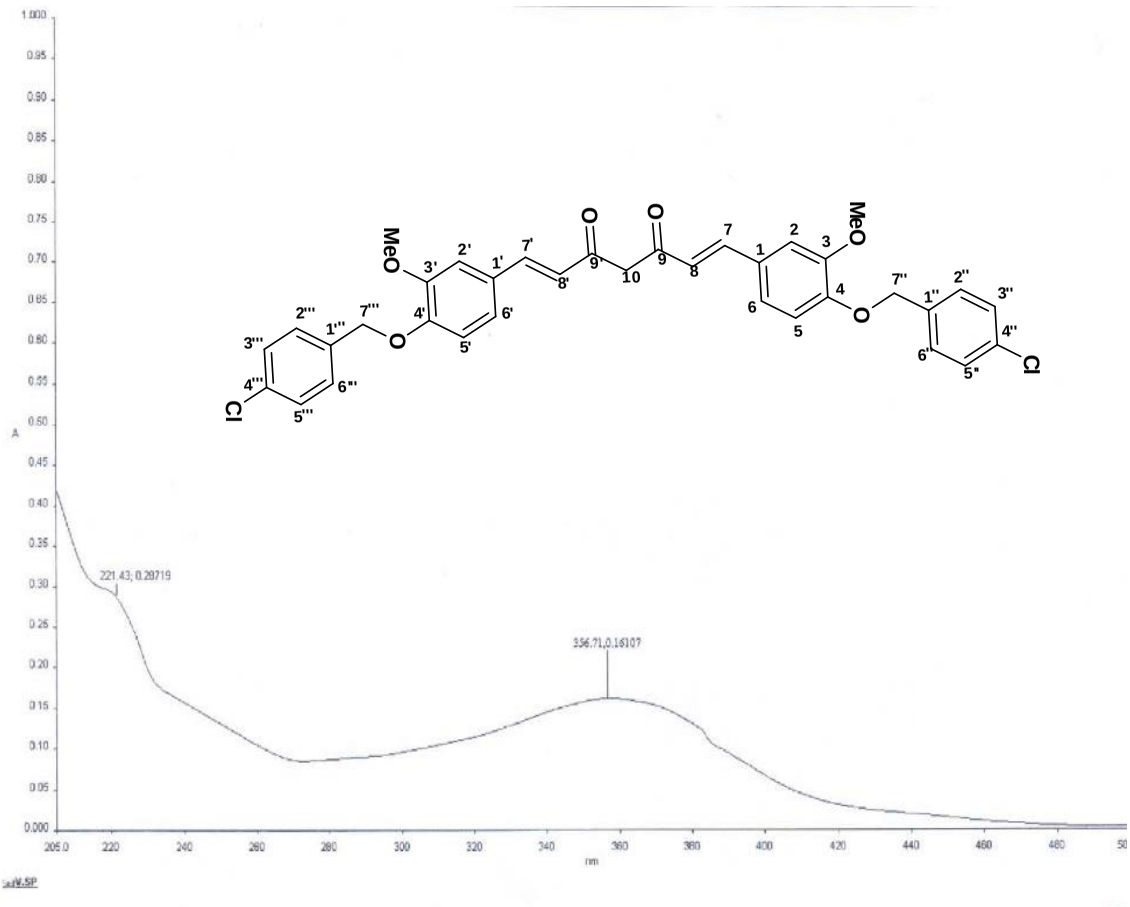
HSQC of Compound A-4x: (1E,6E)-1,7-bis(4-(4-chlorobenzyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



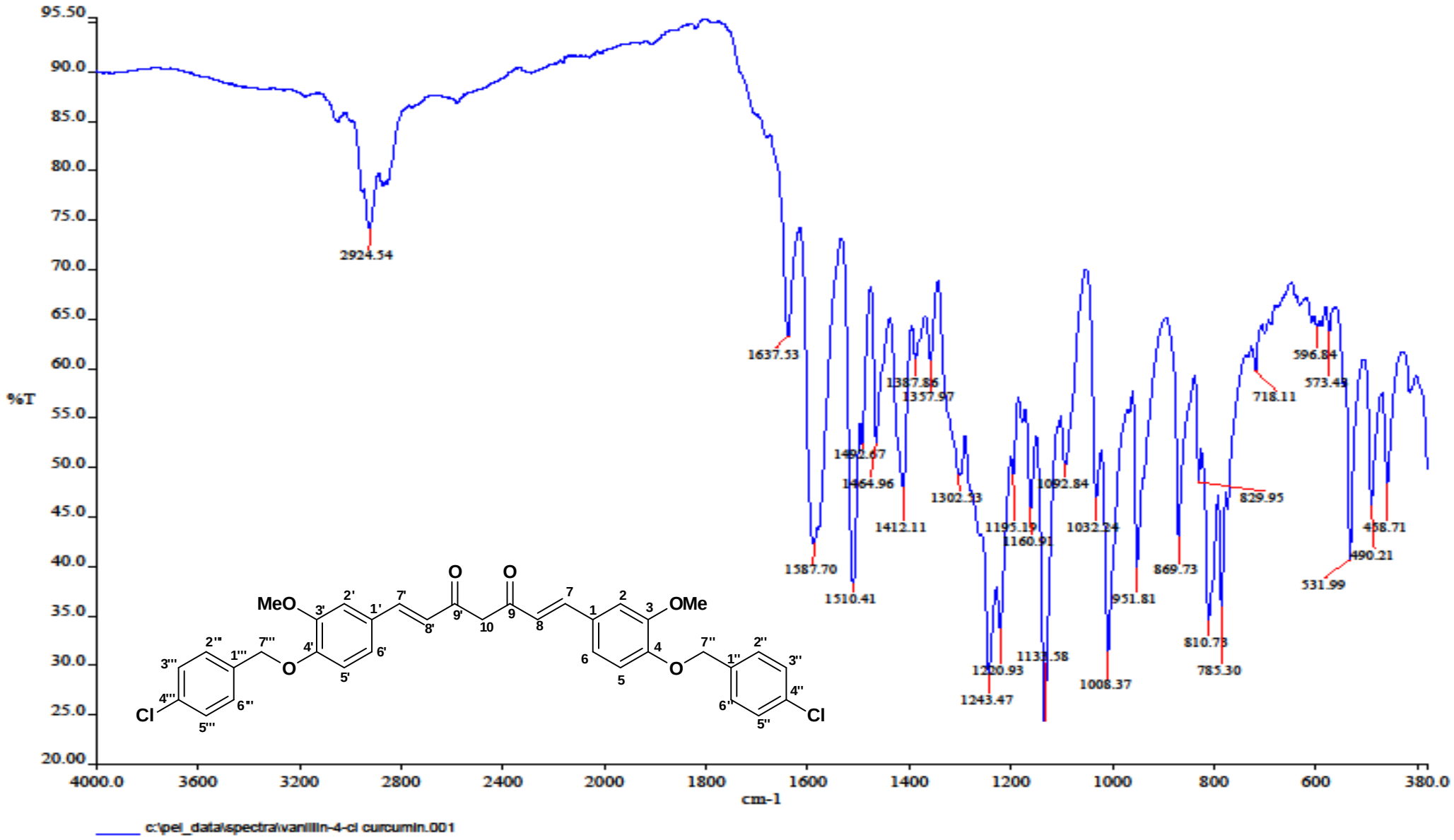
HMBC of Compound A-4x: (1E,6E)-1,7-bis(4-(4-chlorobenzyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



Expanded HMBC of Compound A-4x: (1E,6E)-1,7-bis(4-(4-chlorobenzyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione

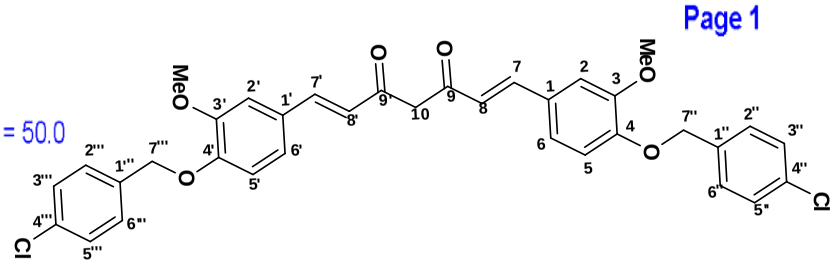


Ultraviolet Spectrum of Compound A-4x: (1E,6E)-1,7-bis(4-(4-chlorobenzyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



Infrared Spectrum of Compound A-4x: (1E,6E)-1,7-bis(4-(4-chlorobenzyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione

Elemental Composition Report

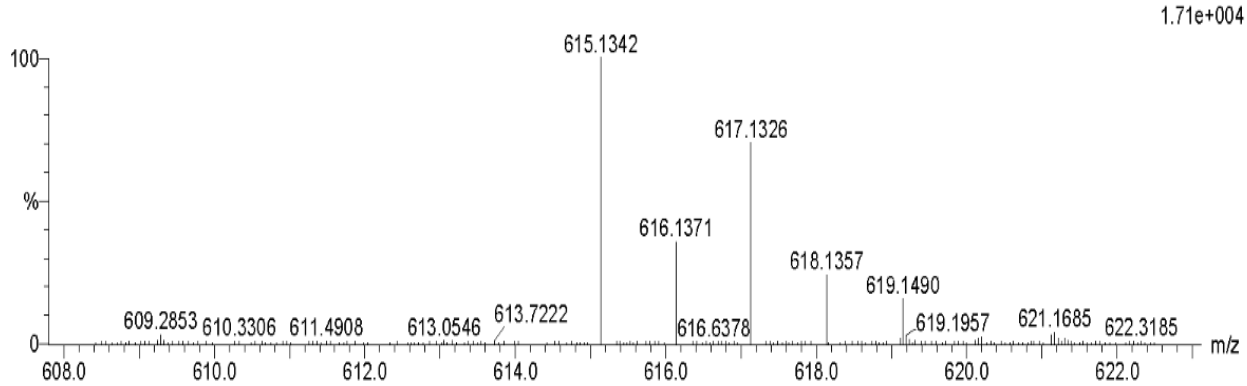


Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

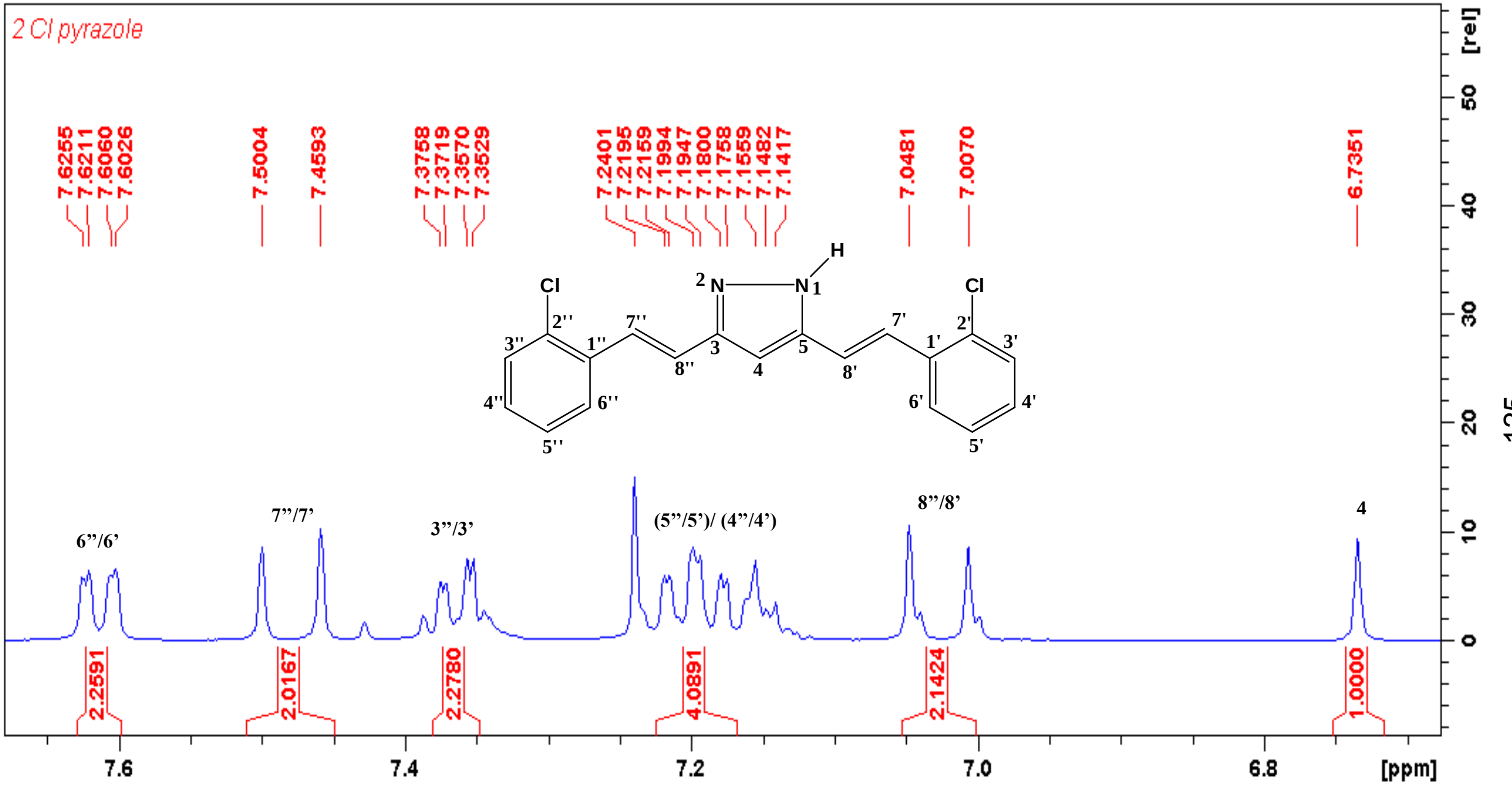
Monoisotopic Mass, Even Electron Ions
6 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 30-35 H: 25-30 O: 5-10 Cl: 0-2

13 Vanillin 4-Cl bnbr 2 (0.034) Cm (1:61)
TOF MS ES-

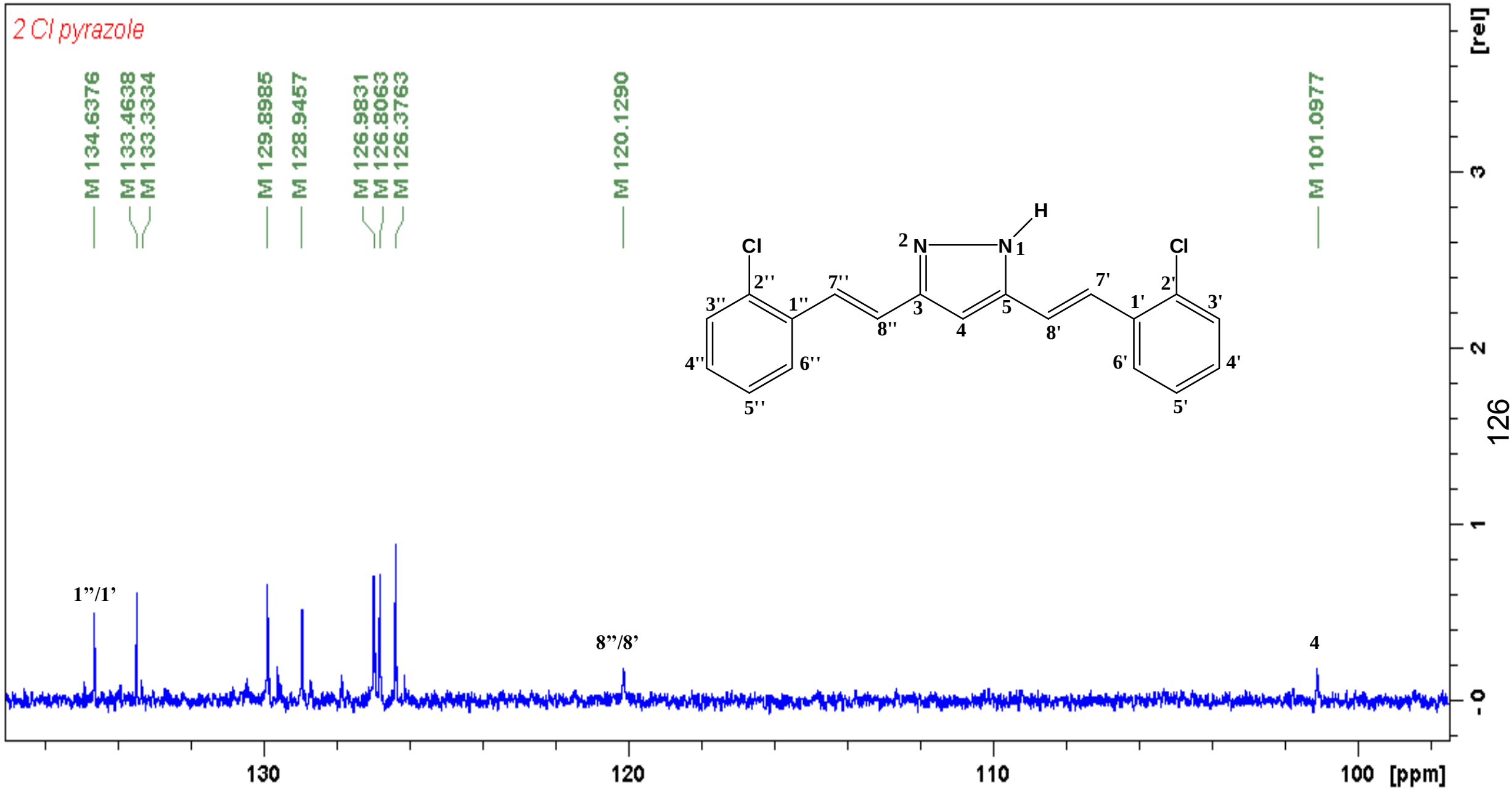


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
615.1342	615.1341	0.1	0.2	20.5	165.4	0.0	C35 H29 O6 Cl2

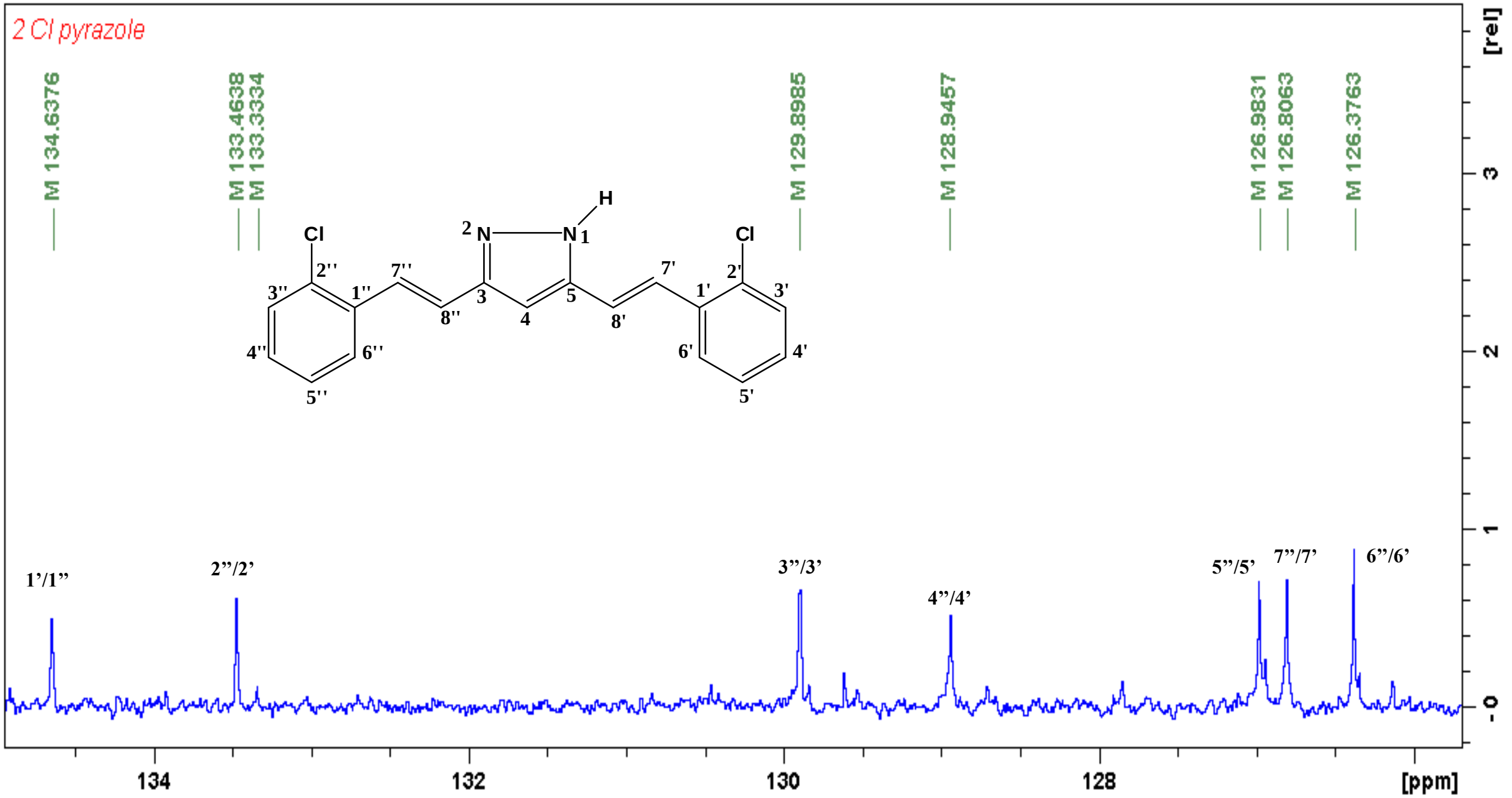
HRMS of Compound A-4x: (1E,6E)-1,7-bis(4-(4-chlorobenzyloxy)-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



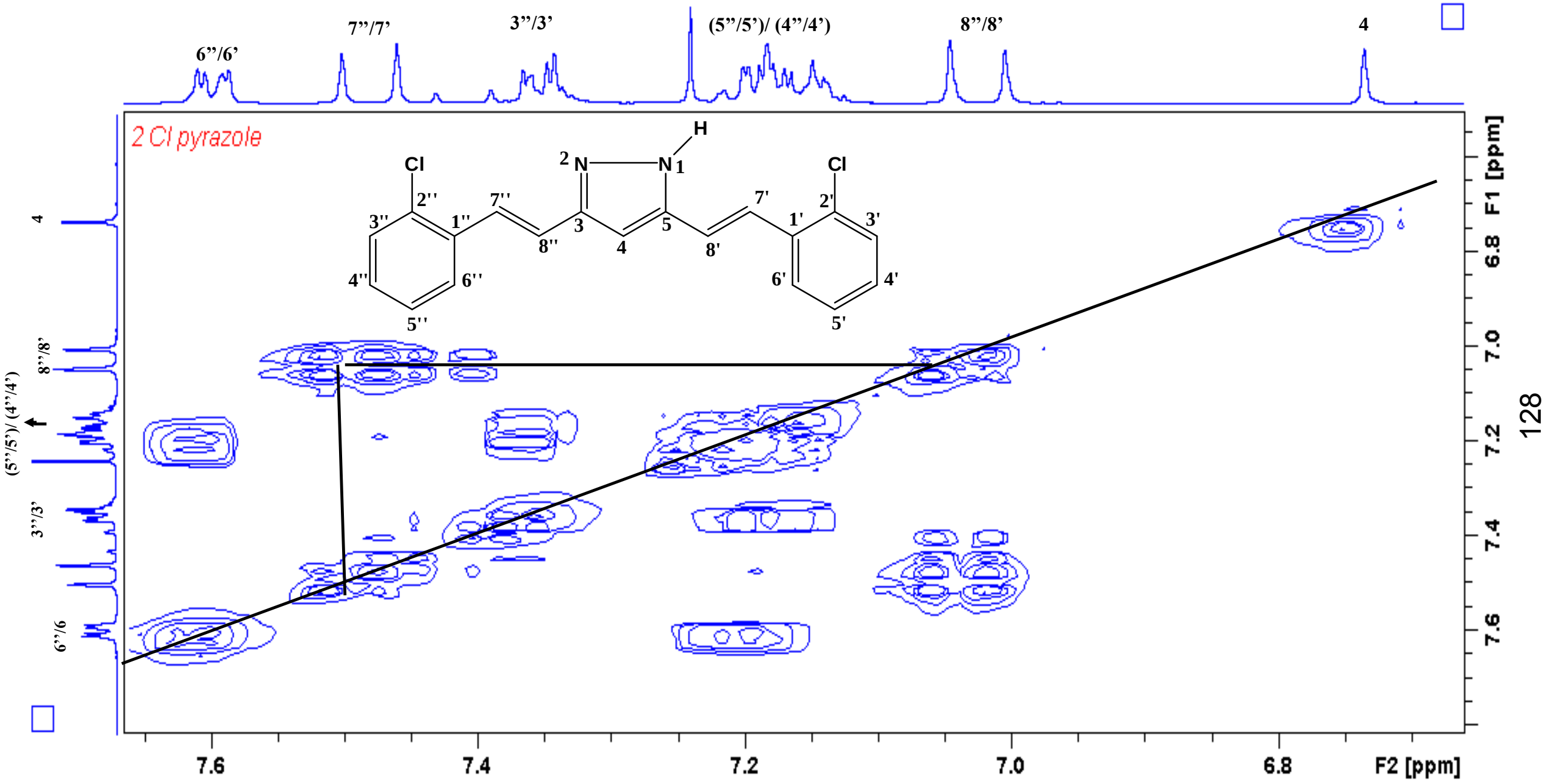
^{1}H Spectrum of Compound A-5a: 3,5-bis(2-chlorostyryl)-1H-pyrazole



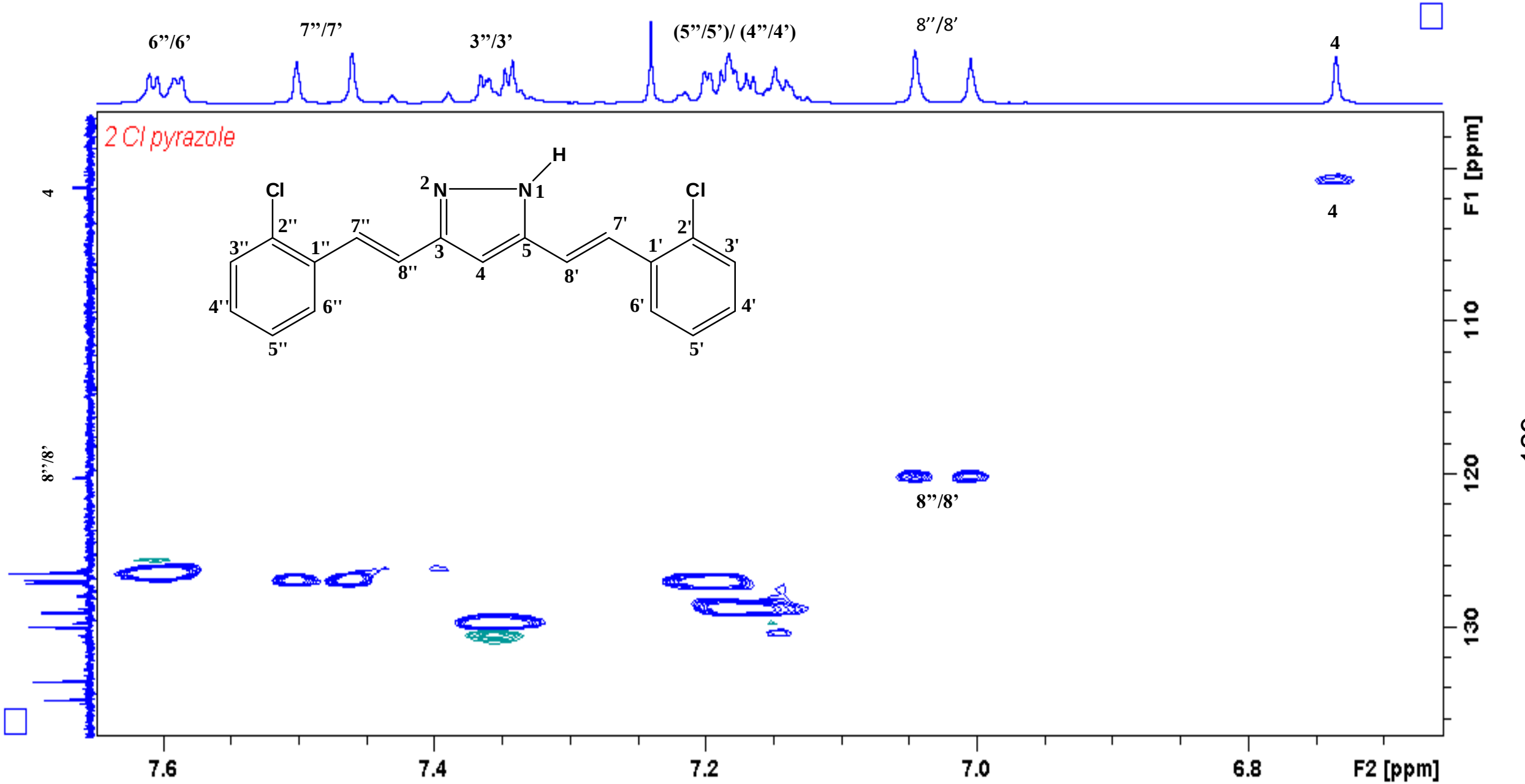
^{13}C Spectrum of Compound A-5a: 3,5-bis(2-chlorostyryl)-1H-pyrazole



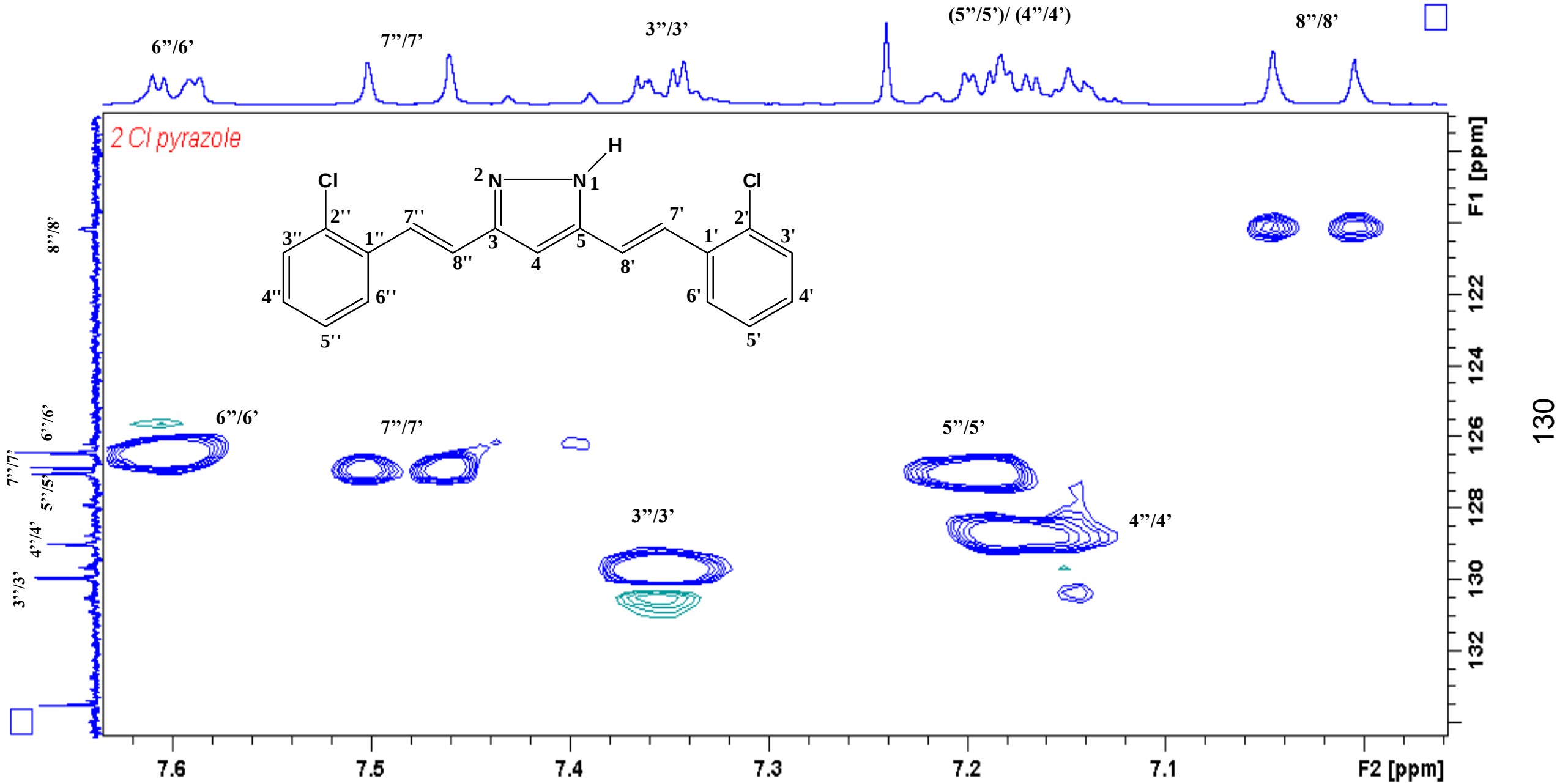
Expanded ^{13}C Spectrum of Compound A-5a: 3,5-bis(2-chlorostyryl)-1H-pyrazole



COSY of Compound A-5a: 3,5-bis(2-chlorostyryl)-1H-pyrazole

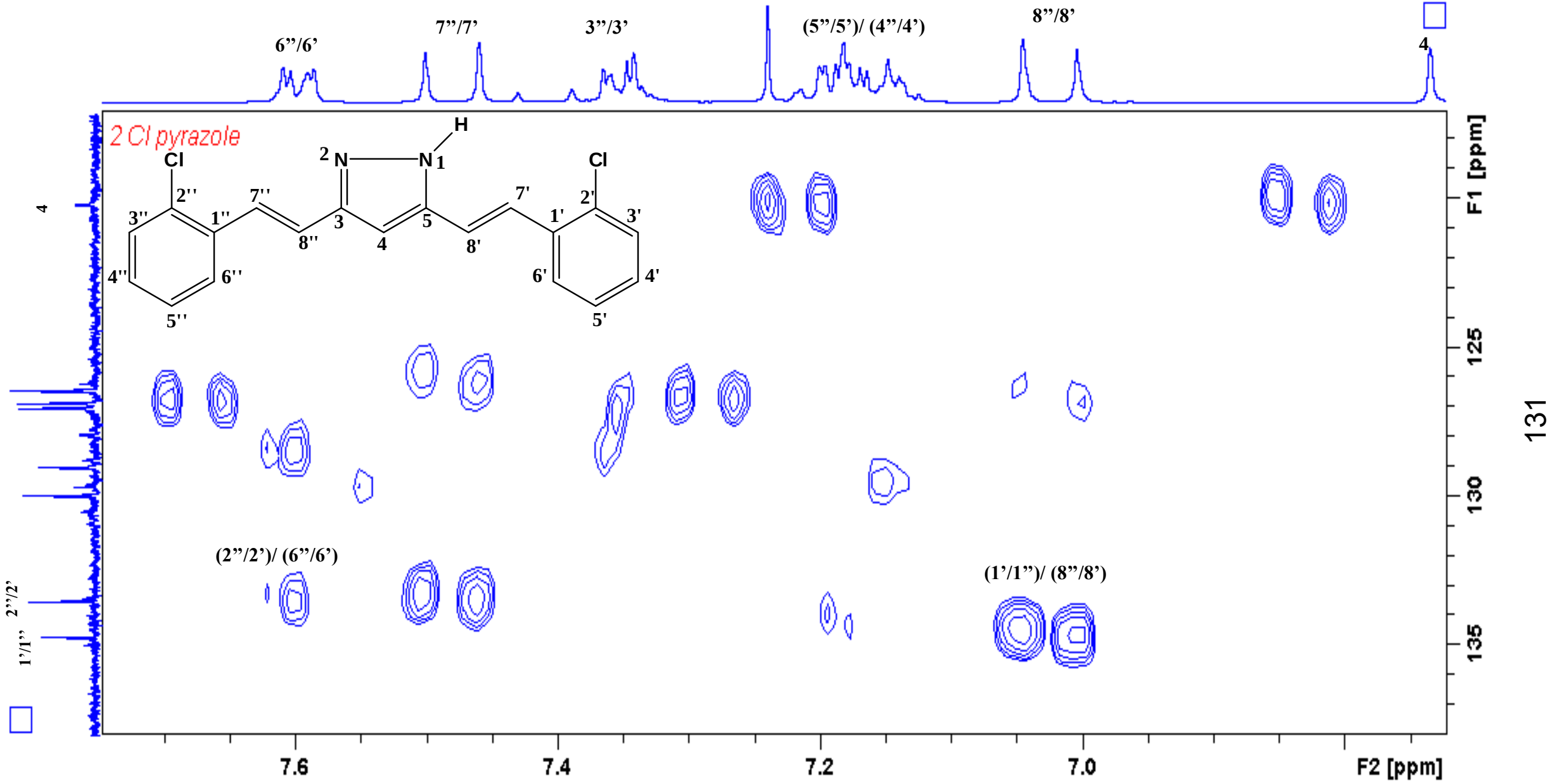


HSQC of Compound A-5a: 3,5-bis(2-chlorostyryl)-1H-pyrazole

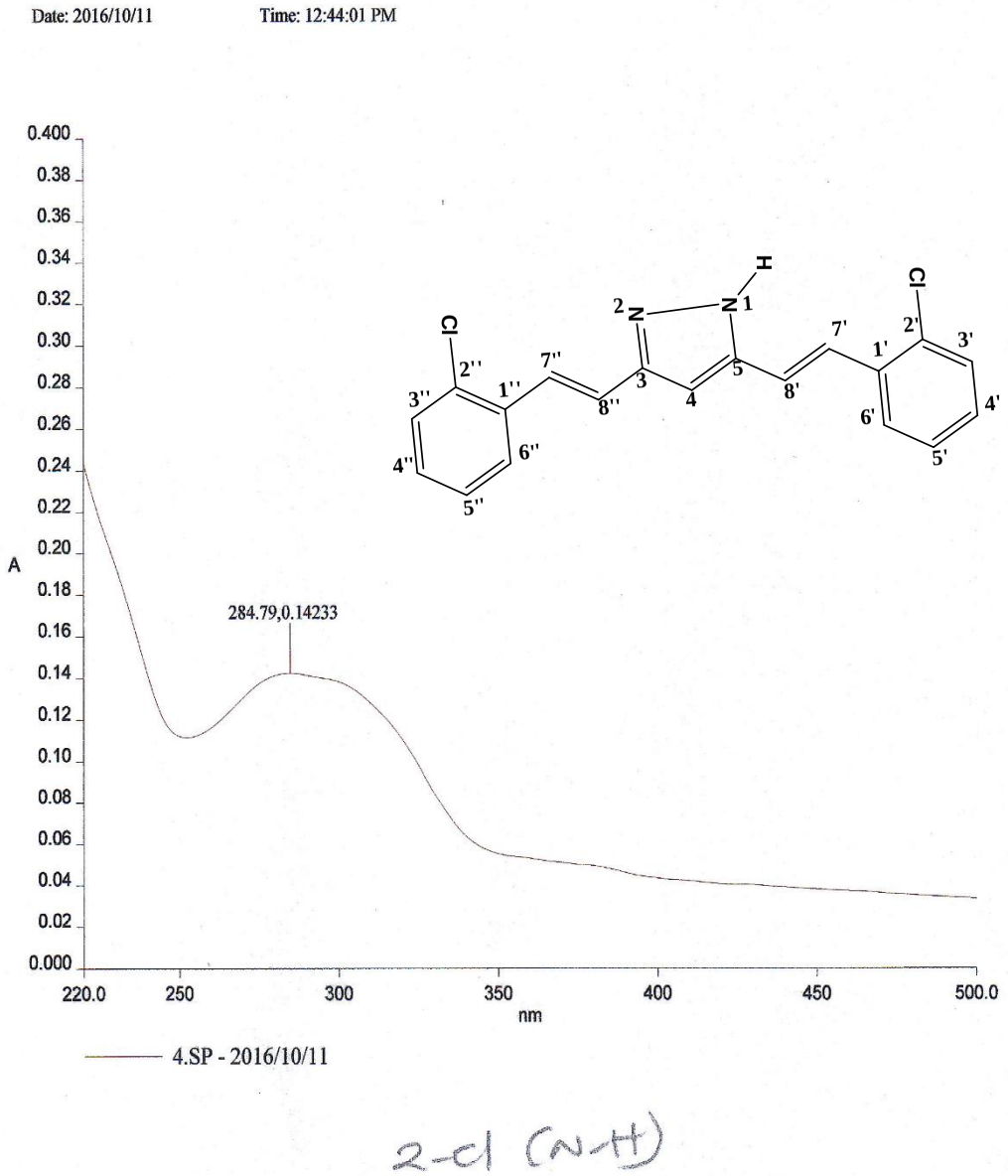


130

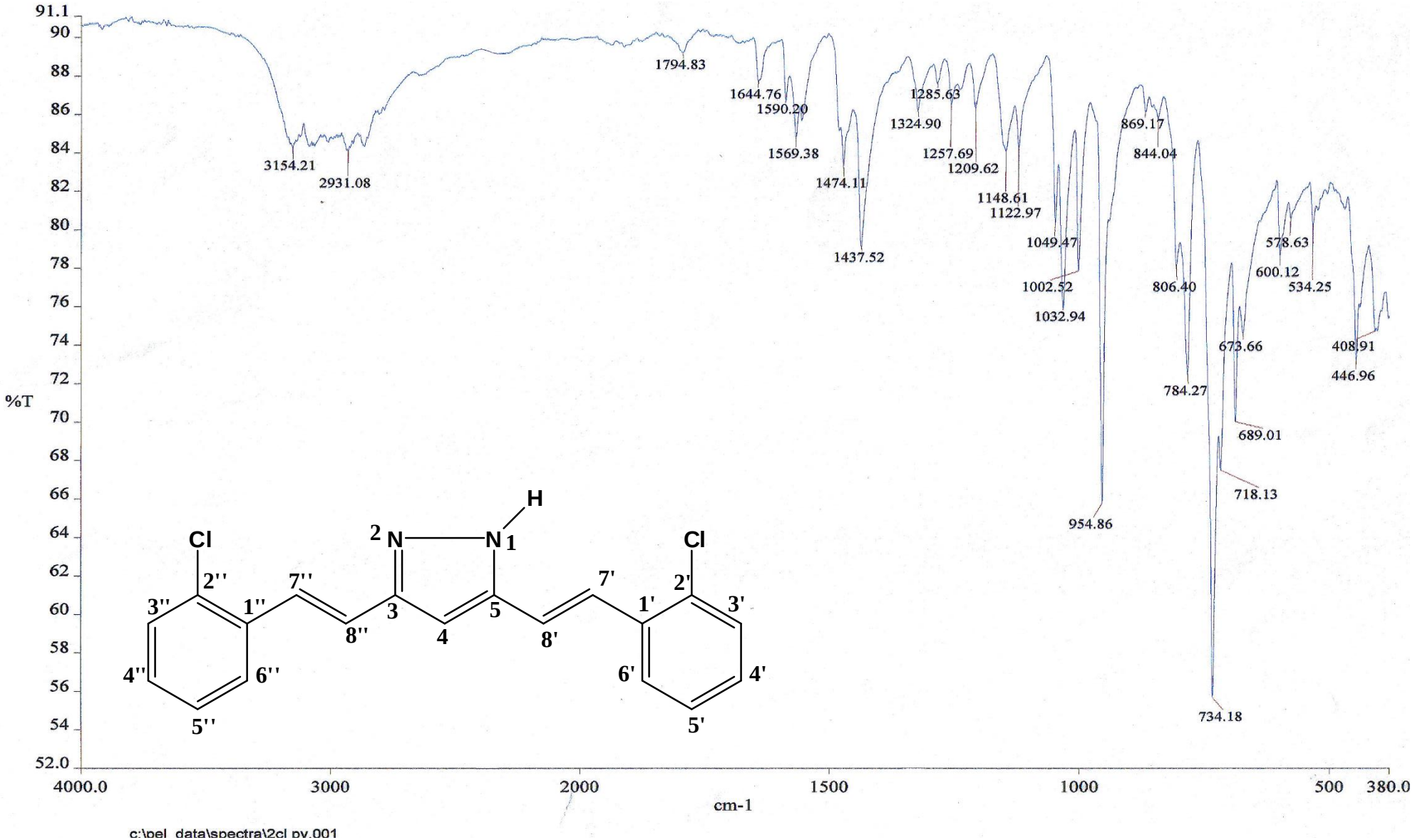
Expanded HSQC of Compound A-5a: 3,5-bis(2-chlorostyryl)-1H-pyrazole



HMBC of Compound A-5a: 3,5-bis(2-chlorostyryl)-1H-pyrazole



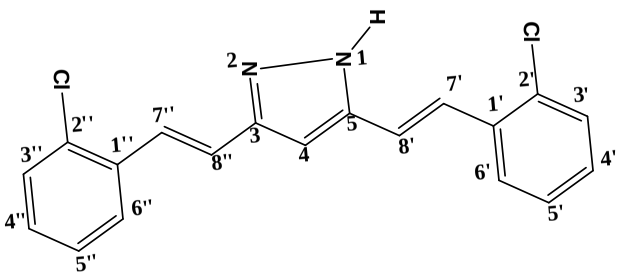
Ultraviolet Spectrum of Compound A-5a: 3,5-bis(2-chlorostyryl)-1H-pyrazole



Infrared Spectrum of Compound A-5a: 3,5-bis(2-chlorostyryl)-1H-pyrazole

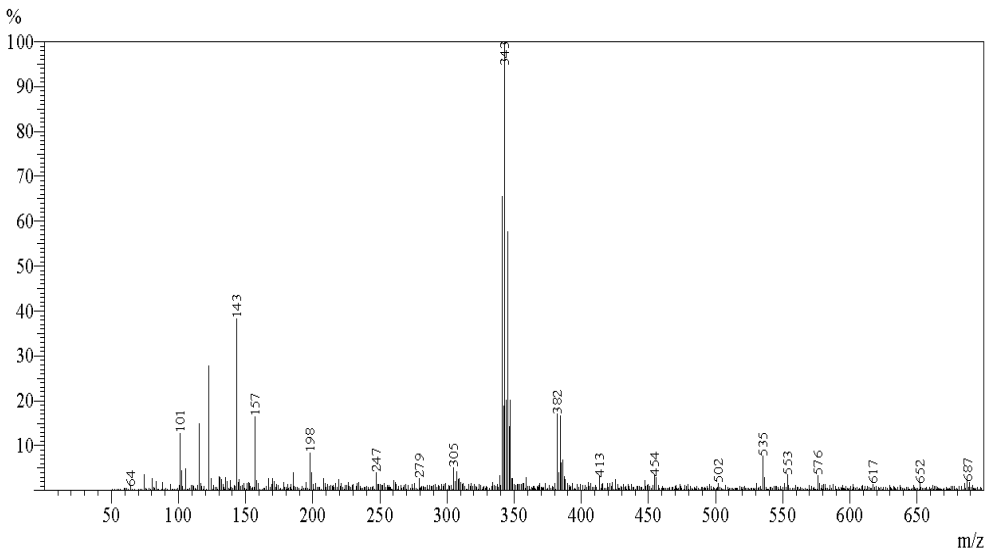
Sample Information

Date Acquired	: 2016/10/04 12:22:39 PM
Sample Type	: Unknown
Sample Name	: 14
Sample ID	: 14
Data File	: 14.lcd
Method File	: no column run.lcm
Report Format File	: DEFAULT.lsr
Tuning File	: 03022016.lct



MS Spectrum

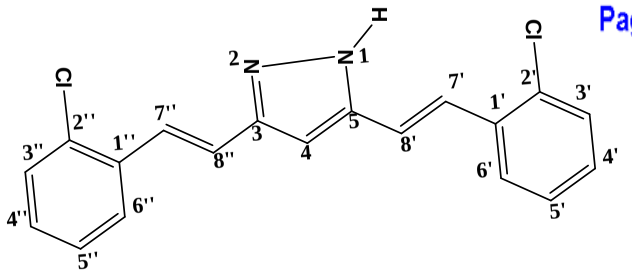
R.Time:1.067(Scan#:65)
MassPeaks:2109
Segment 1 - Event 1



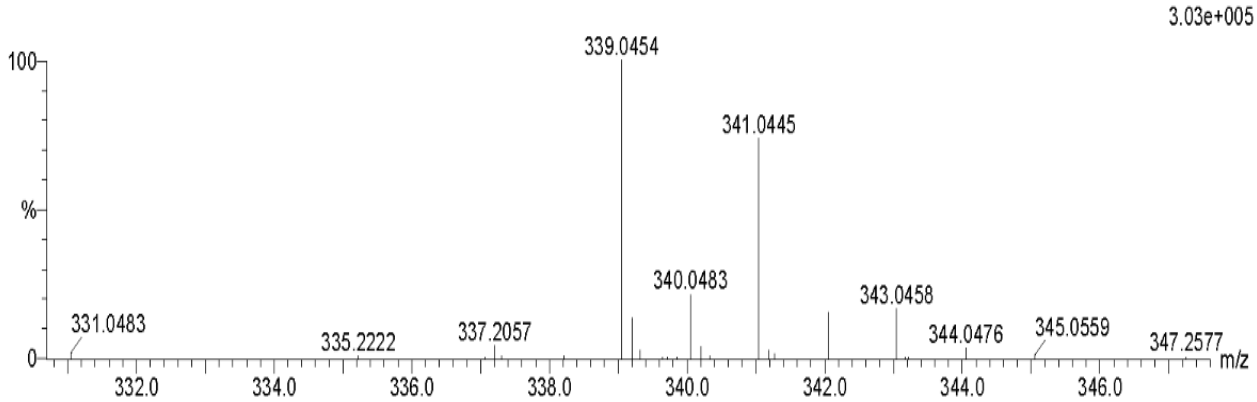
LC-MS of Compound A-5a: 3,5-bis(2-chlorostyryl)-1H-pyrazole

Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

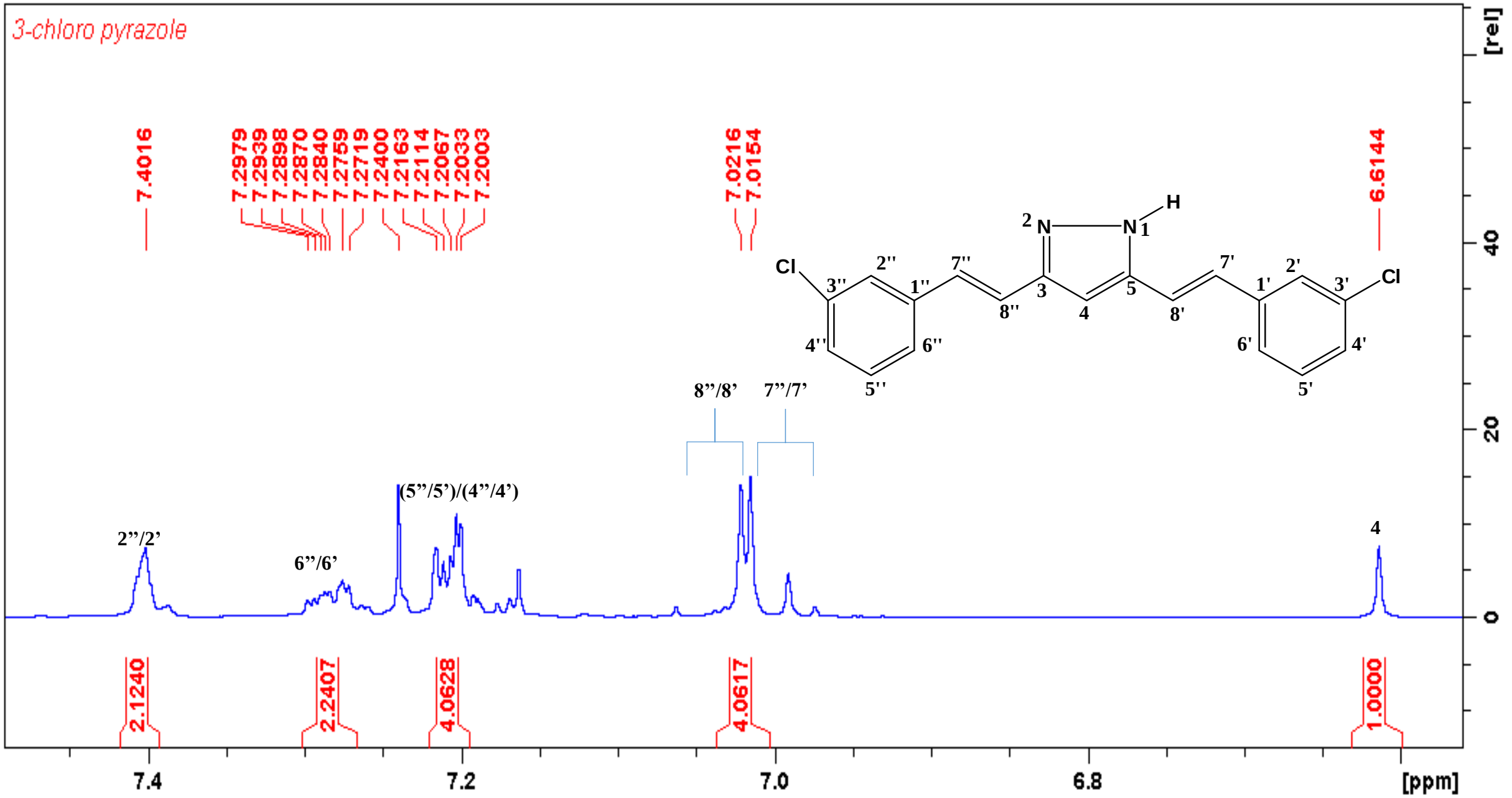


Monoisotopic Mass, Even Electron Ions
7 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 Cl: 0-2
1 2-Cl 2 (0.034) Cm (1:61)
TOF MS ES-

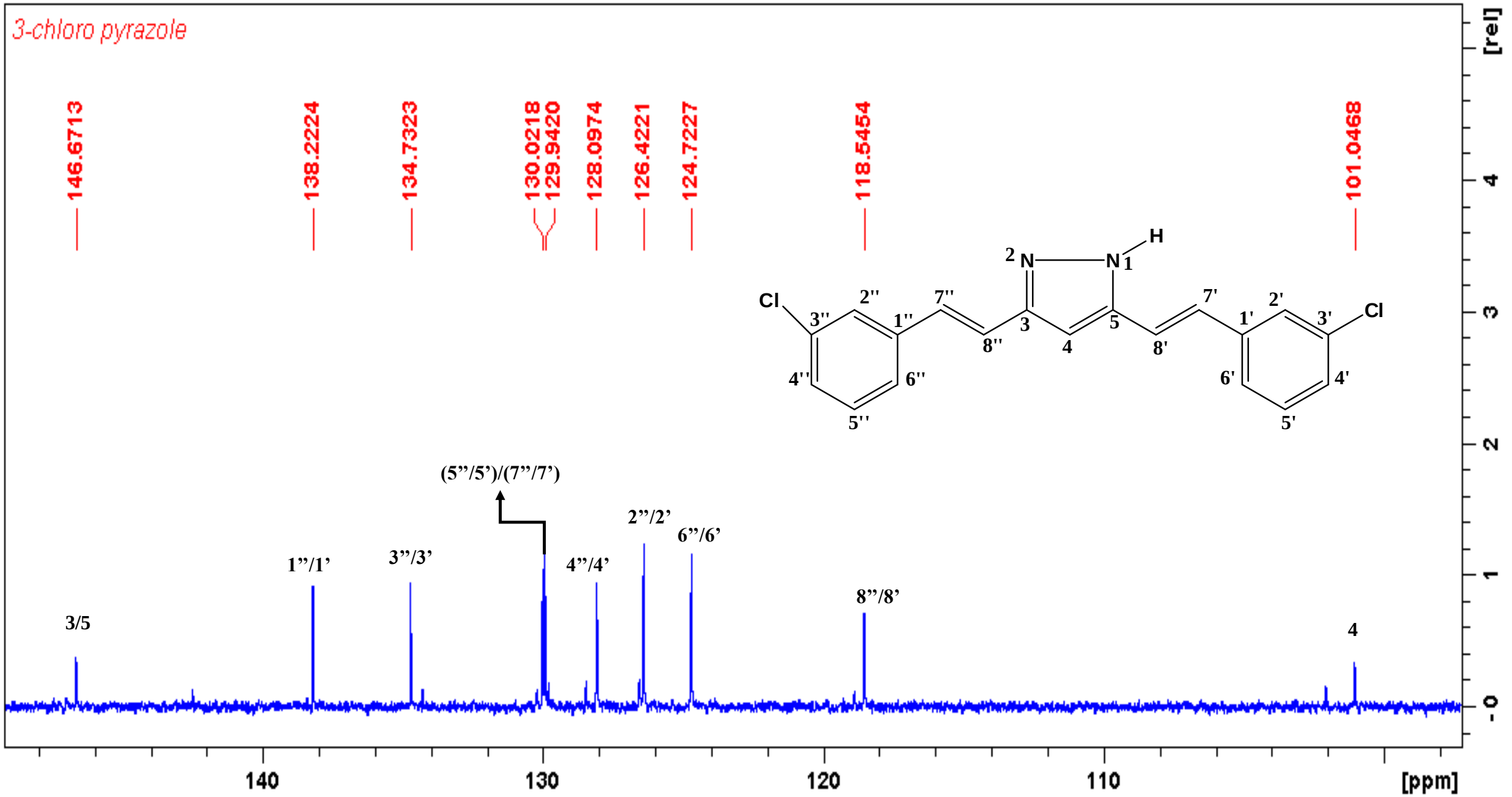


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
339.0454	339.0456	-0.2	-0.6	13.5	127.3	0.0	C19 H13 N2 Cl2

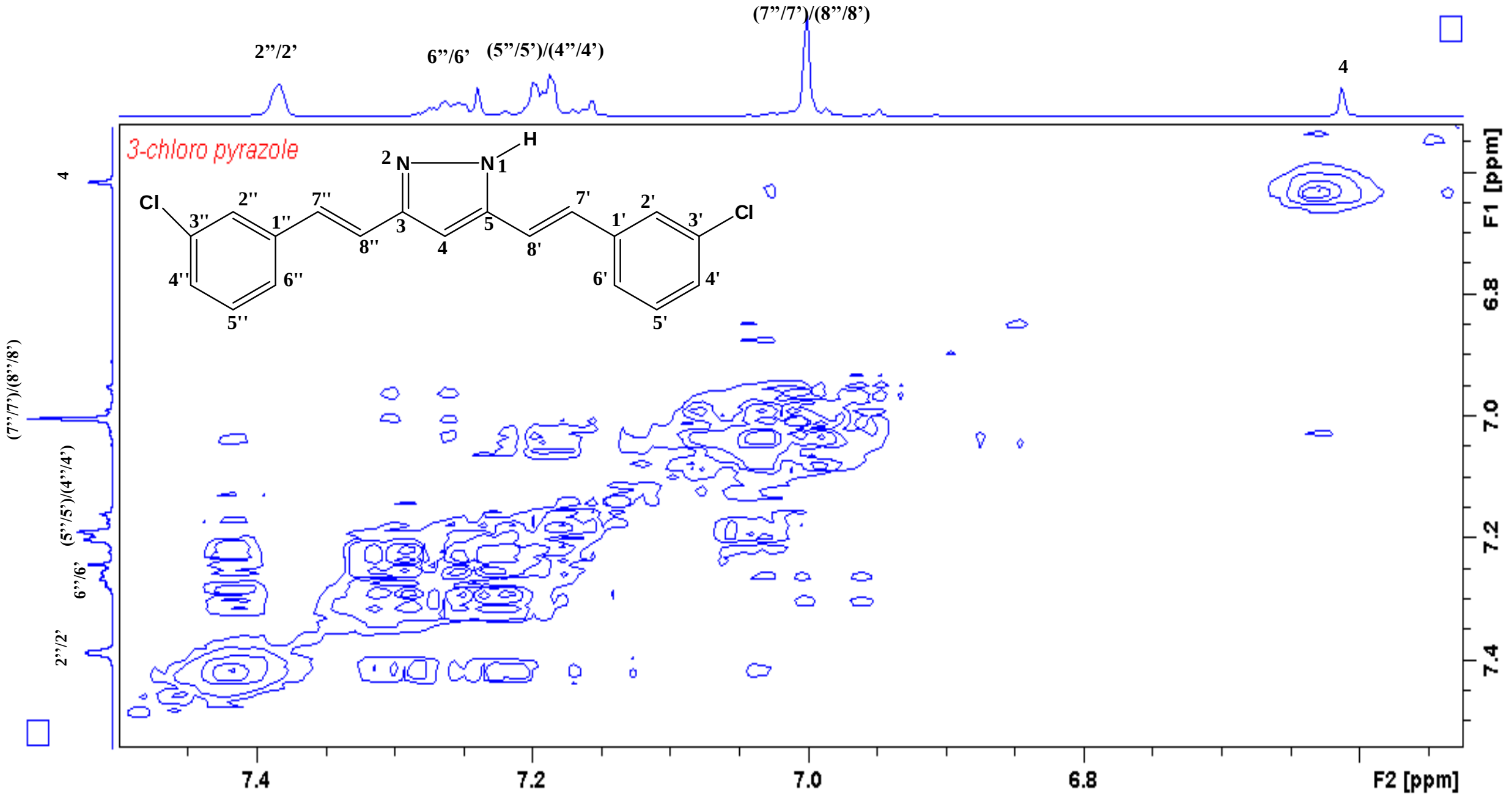
HRMS of Compound A-5a: 3,5-bis(2-chlorostyryl)-1H-pyrazole



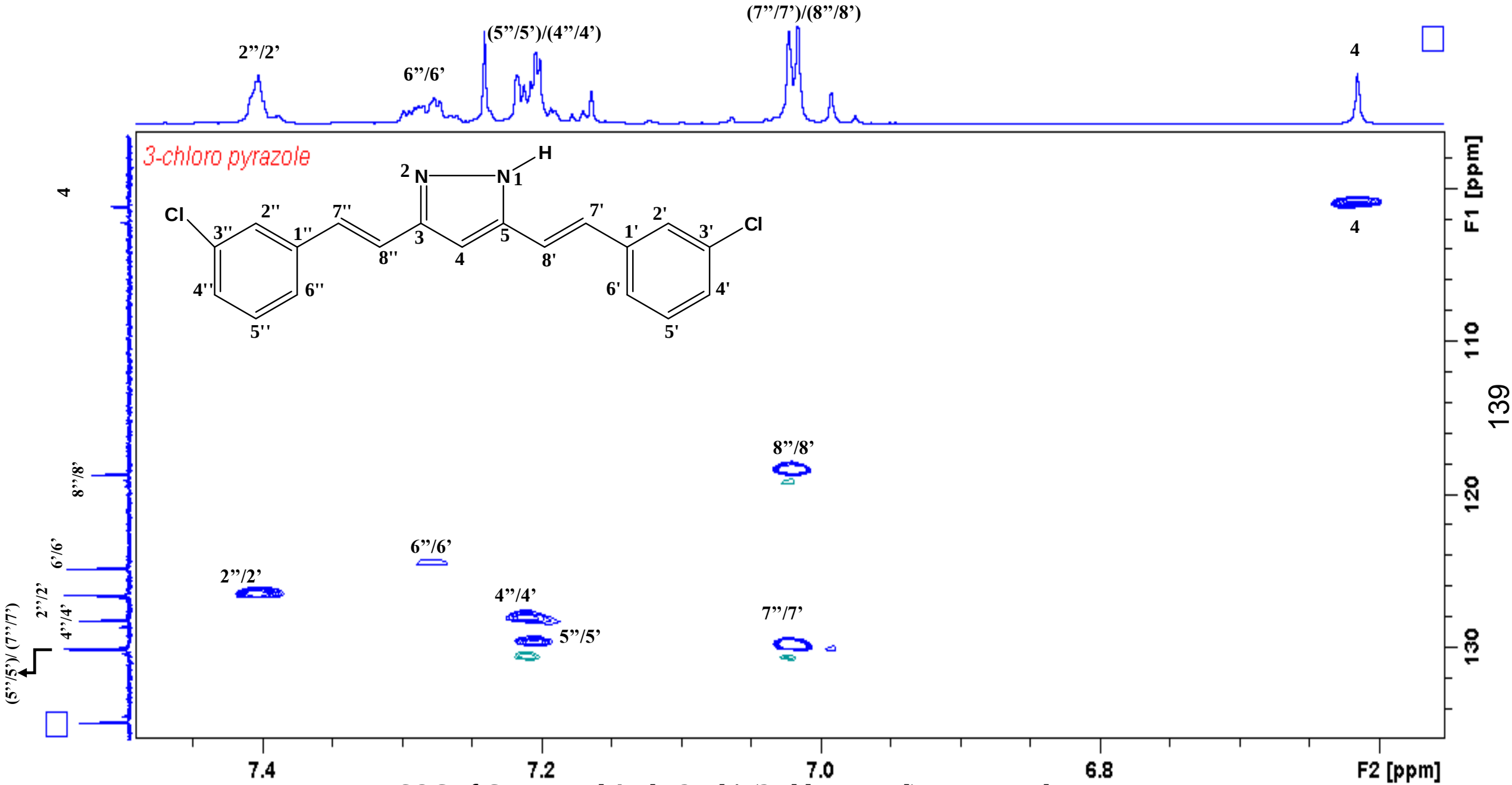
^{1}H Spectrum of Compound A-5b: 3,5-bis(3-chlorostyryl)-1H-pyrazole



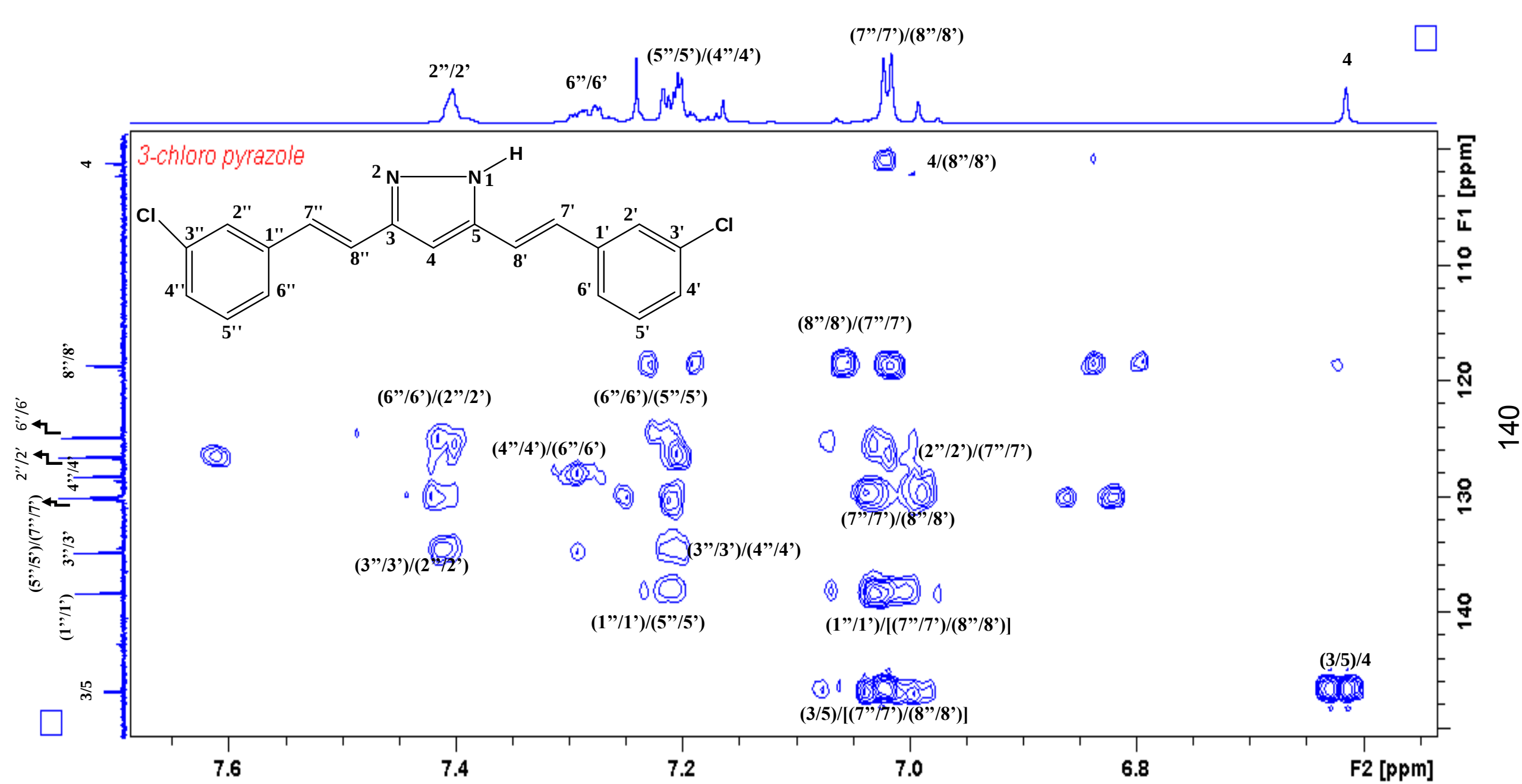
^{13}C Spectrum of Compound A-5b: 3,5-bis(3-chlorostyryl)-1H-pyrazole



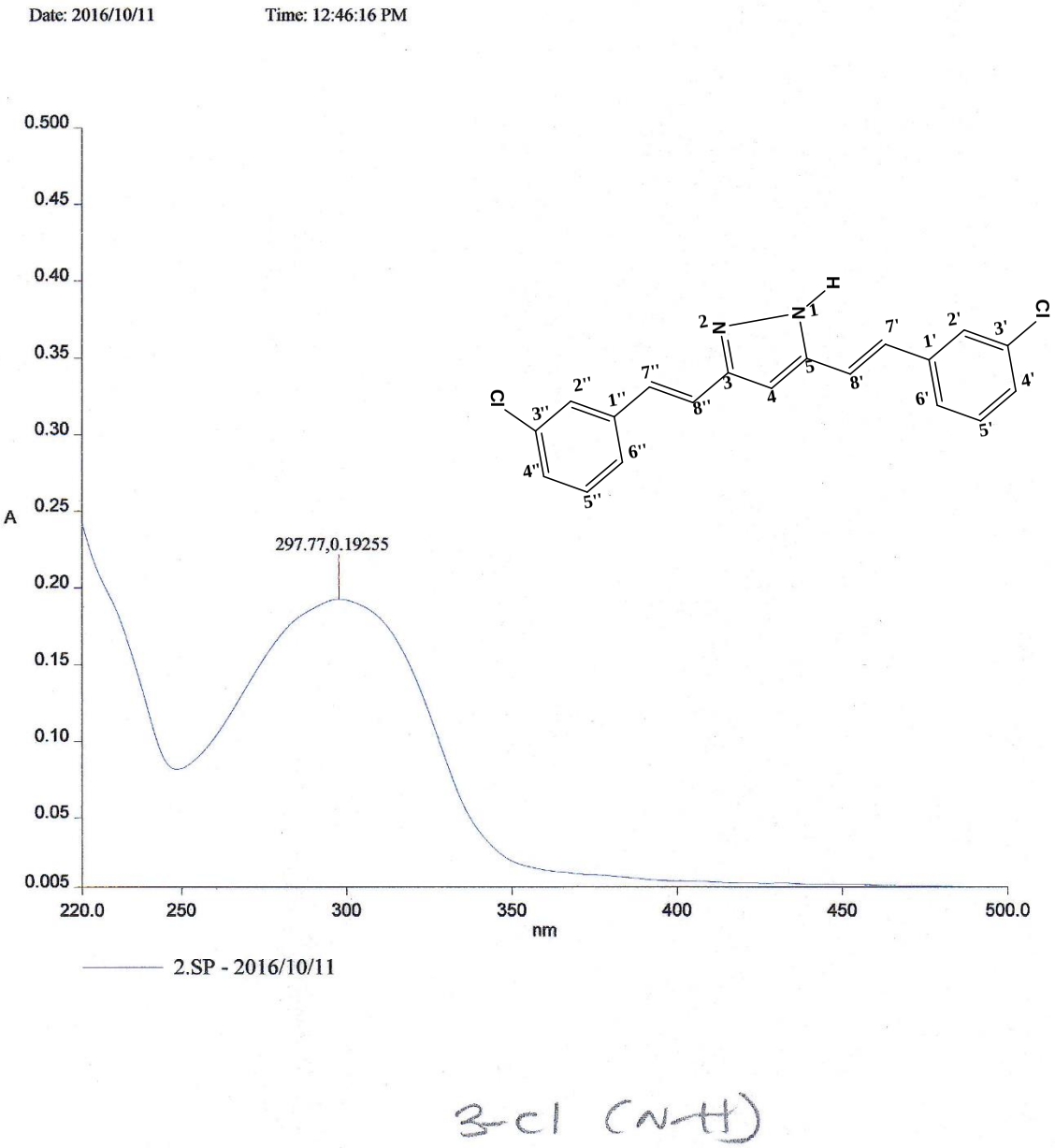
COSY of Compound A-5b: 3,5-bis(3-chlorostyryl)-1H-pyrazole



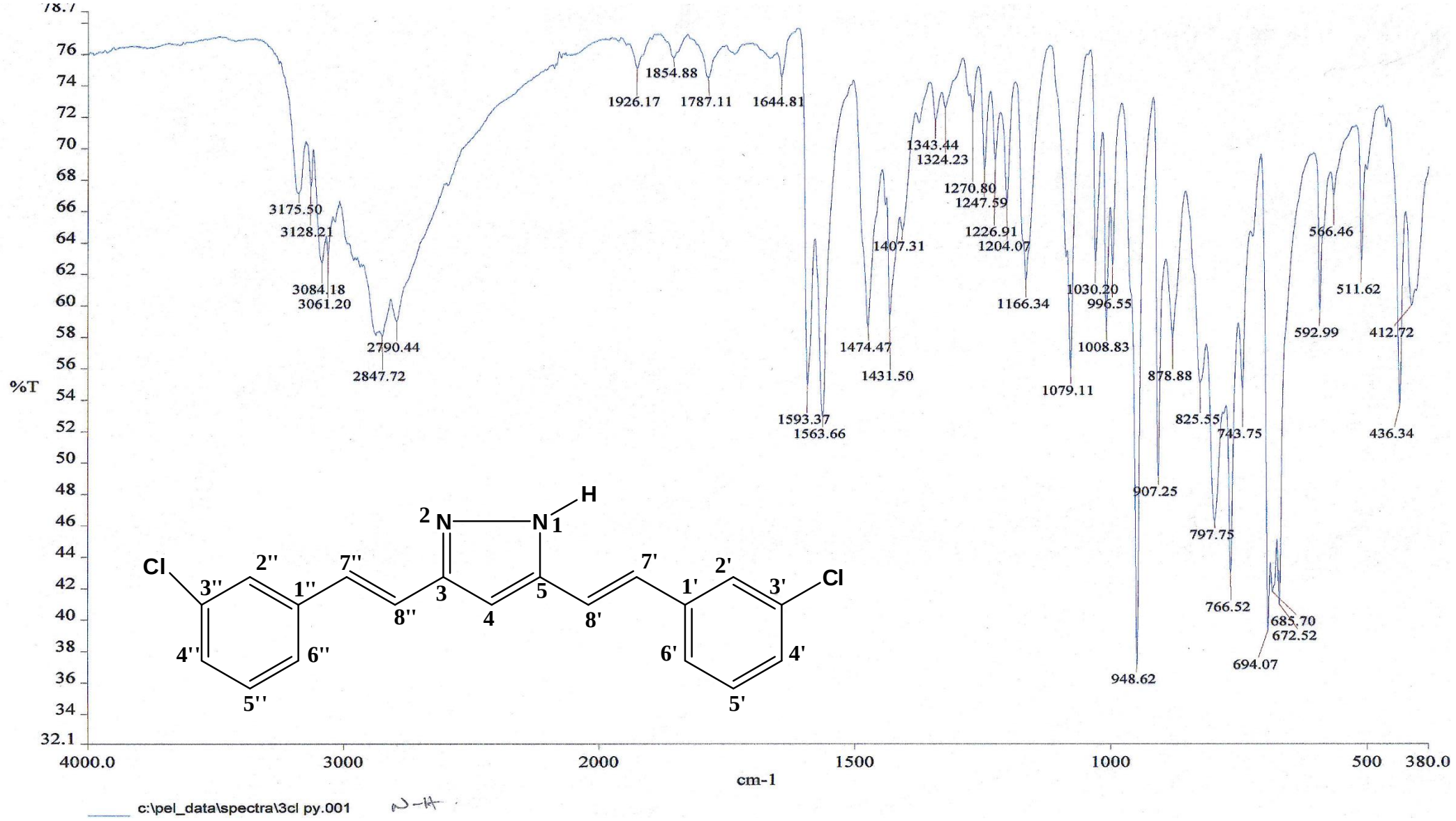
HSQC of Compound A-5b: 3,5-bis(3-chlorostyryl)-1H-pyrazole



HMBC of Compound A-5b: 3,5-bis(3-chlorostyryl)-1H-pyrazole



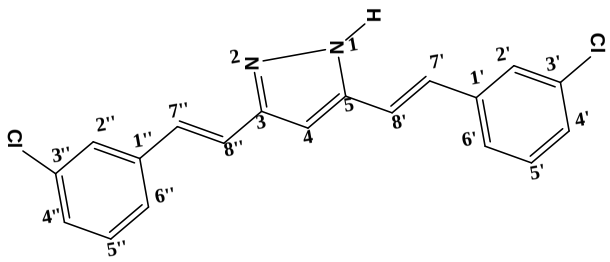
Ultraviolet Spectrum of Compound A-5b: 3,5-bis(3-chlorostyryl)-1H-pyrazole



Infrared Spectrum of Compound A-5b: 3,5-bis(3-chlorostyryl)-1H-pyrazole

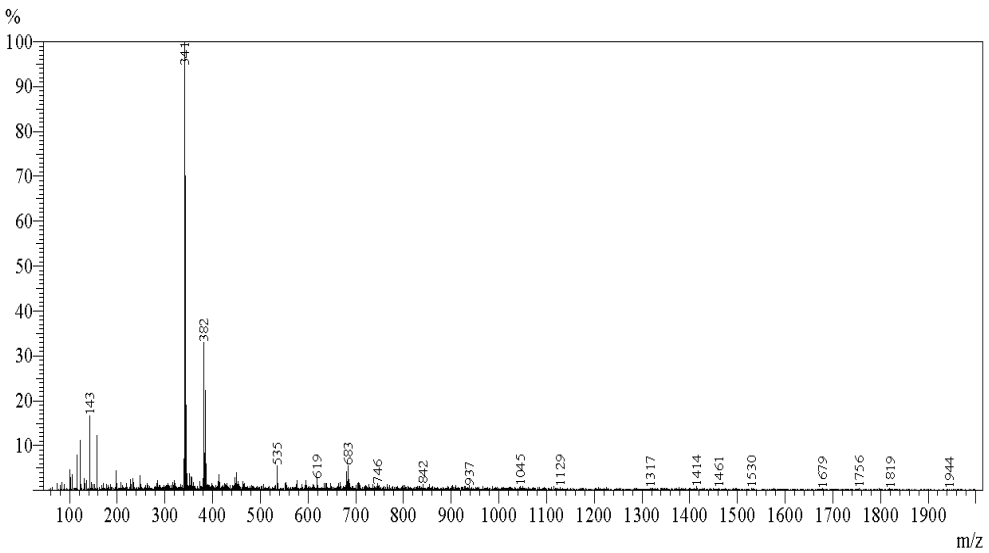
Sample Information

Date Acquired	: 2016/10/04 12:27:04 PM
Sample Type	: Unknown
Sample Name	: 15
Sample ID	: 15
Data File	: 15.lcd
Method File	: no column run.lcm
Report Format File	: DEFAULT.lsr
Tuning File	: 03022016.lct



MS Spectrum

R.Time:1.067(Scan#:65)
MassPeaks:2121
Segment 1 - Event 1

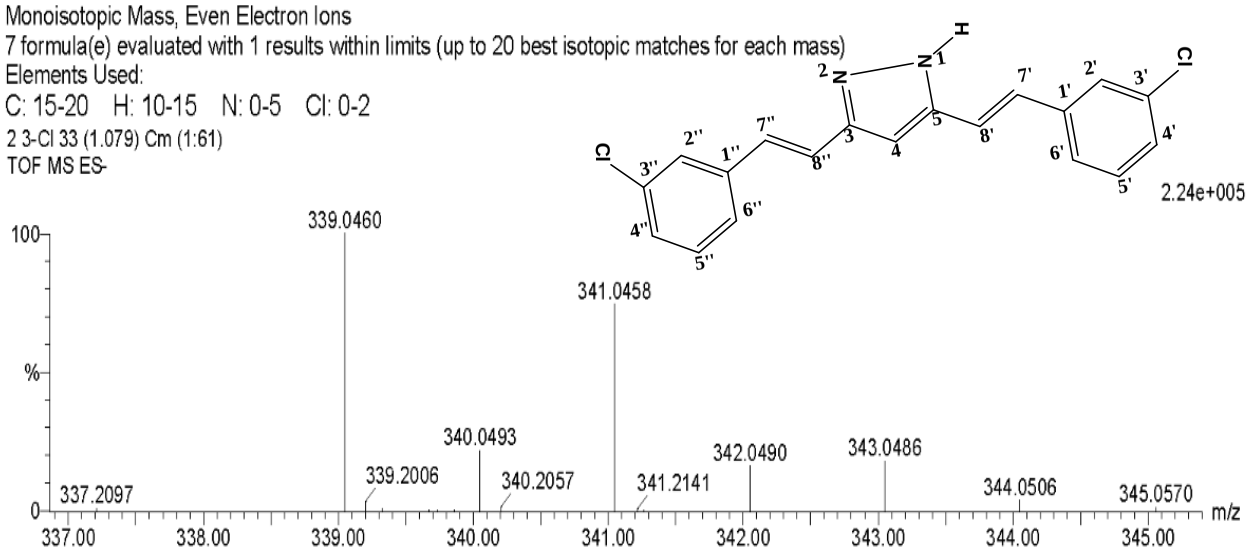


LC-MS of Compound A-5b: 3,5-bis(3-chlorostyryl)-1H-pyrazole

Single Mass Analysis

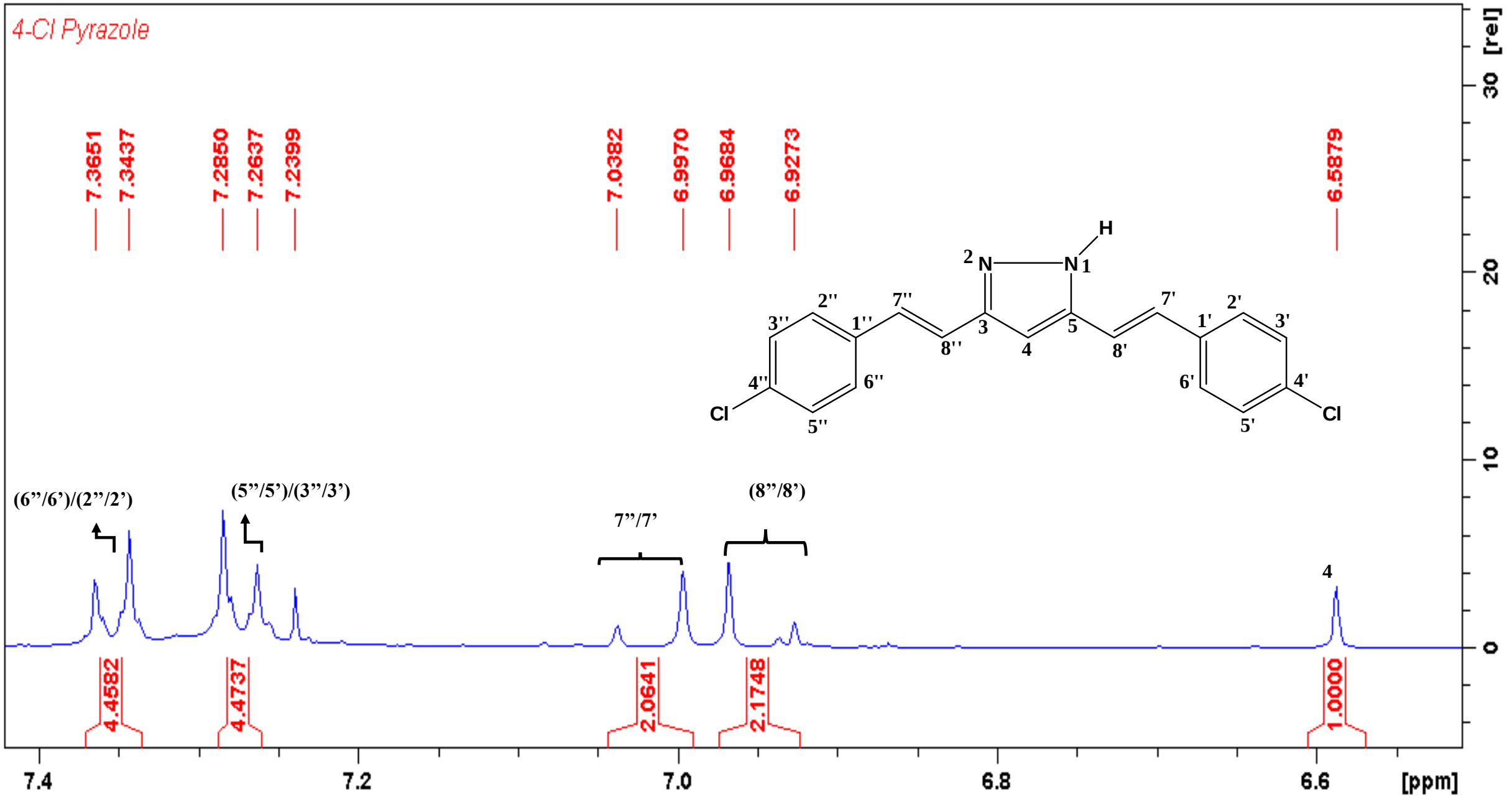
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
7 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 Cl: 0-2
2 3-Cl 33 (1.079) Cm (1:61)
TOF MS ES-

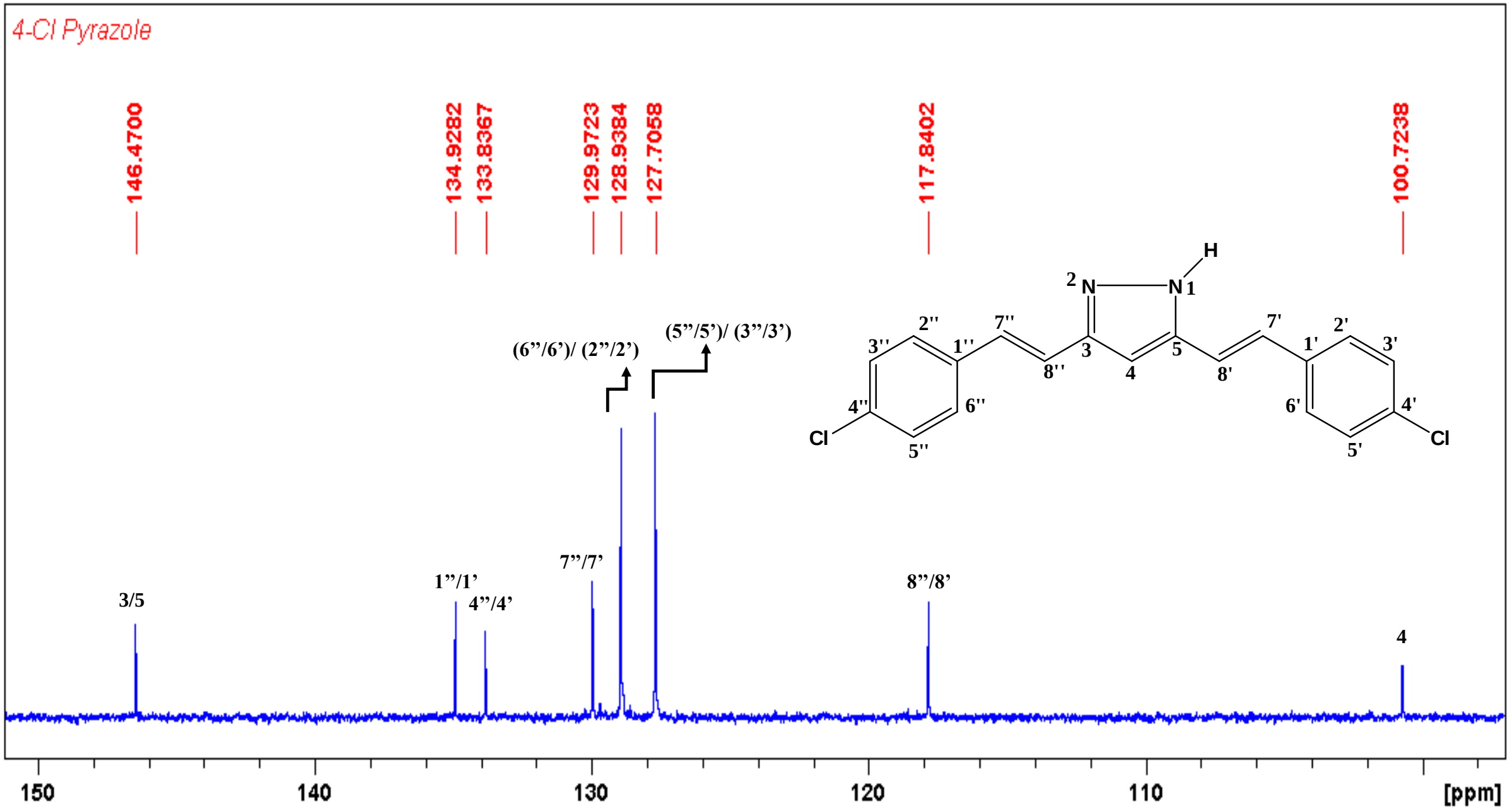


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
339.0460	339.0456	0.4	1.2	13.5	98.5	0.0	C19 H13 N2 Cl2

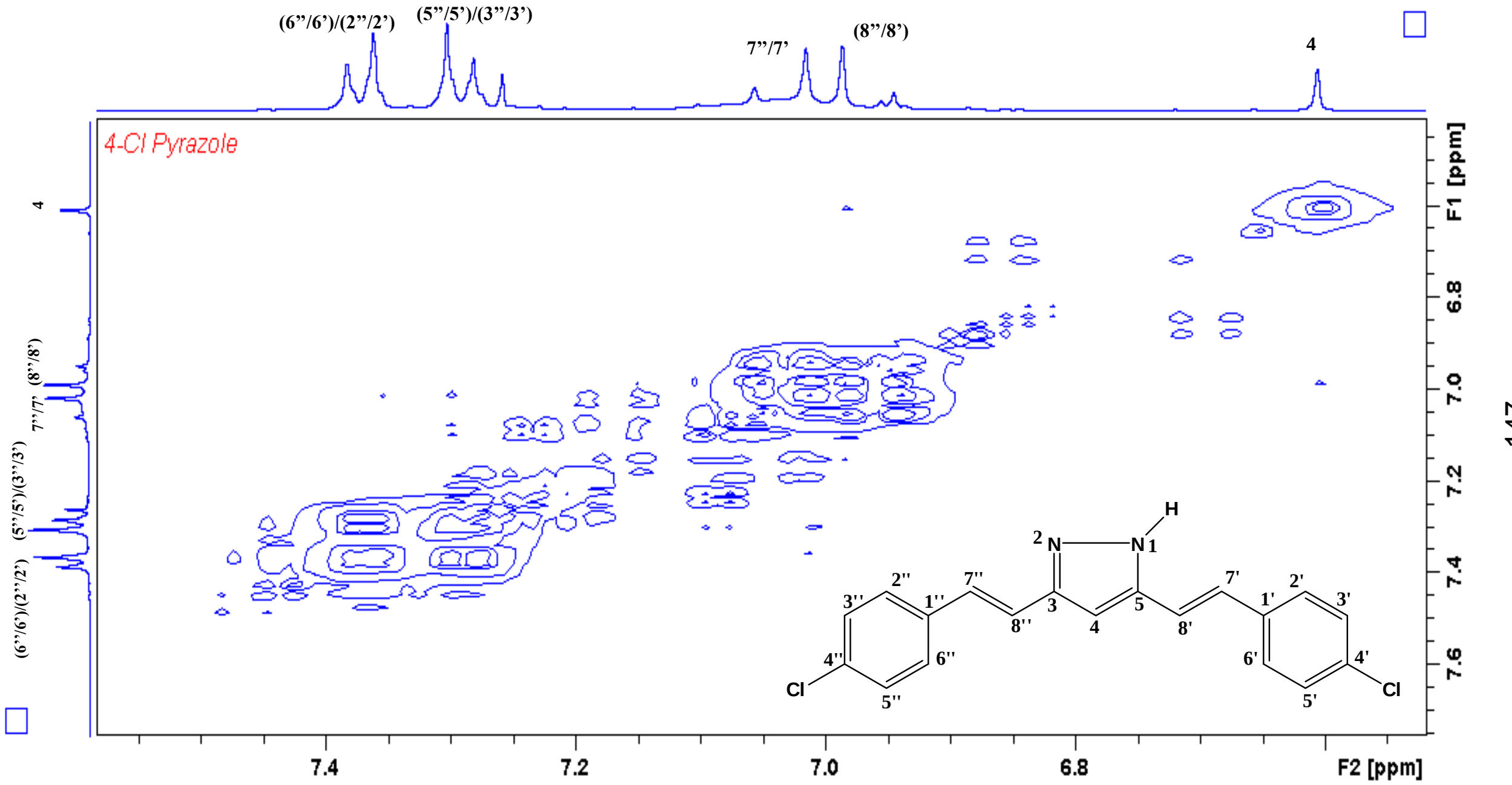
HRMS of Compound A-5b: 3,5-bis(3-chlorostyryl)-1H-pyrazole



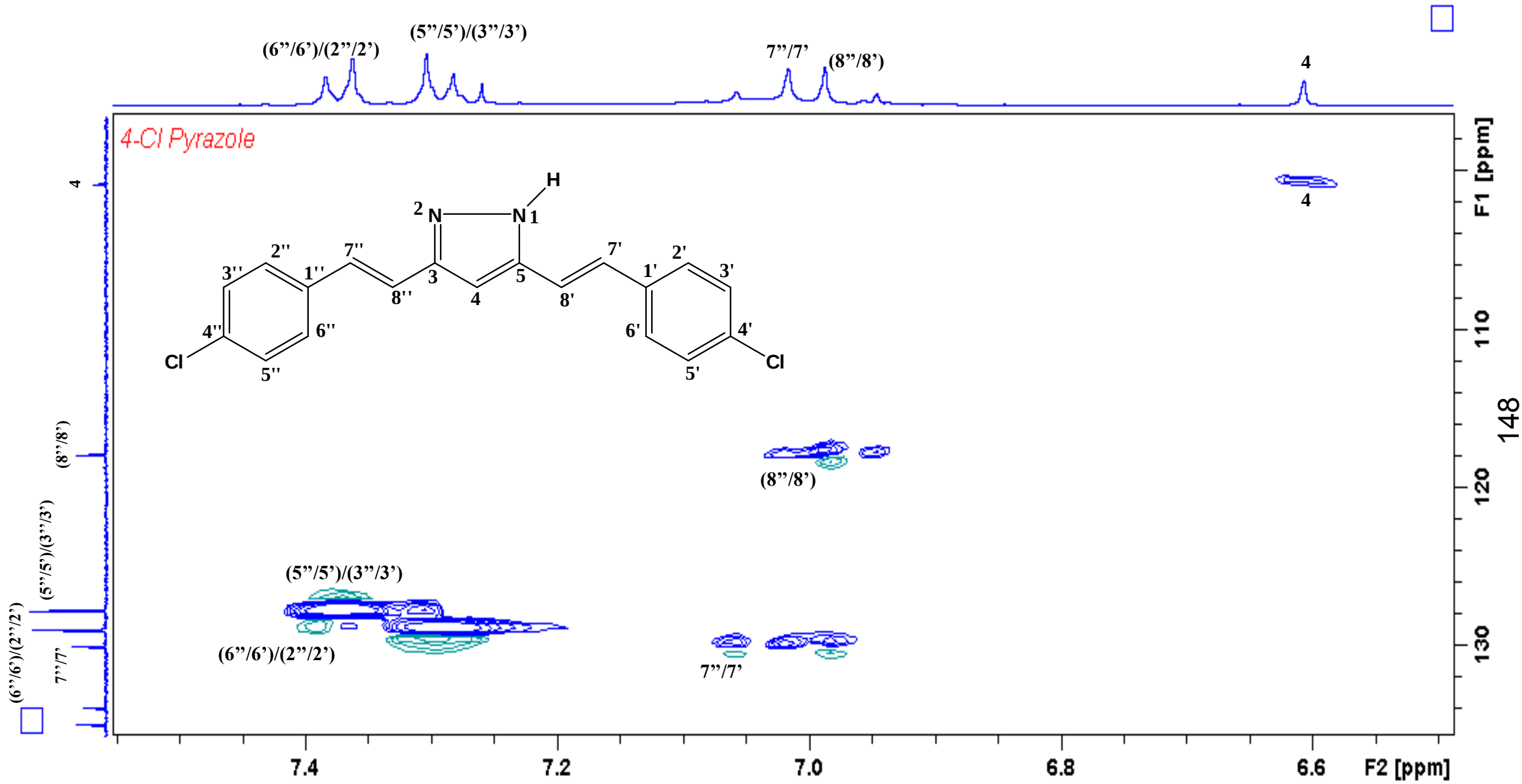
^{1}H Spectrum of Compound A-5c: 3,5-bis(4-chlorostyryl)-1H-pyrazole



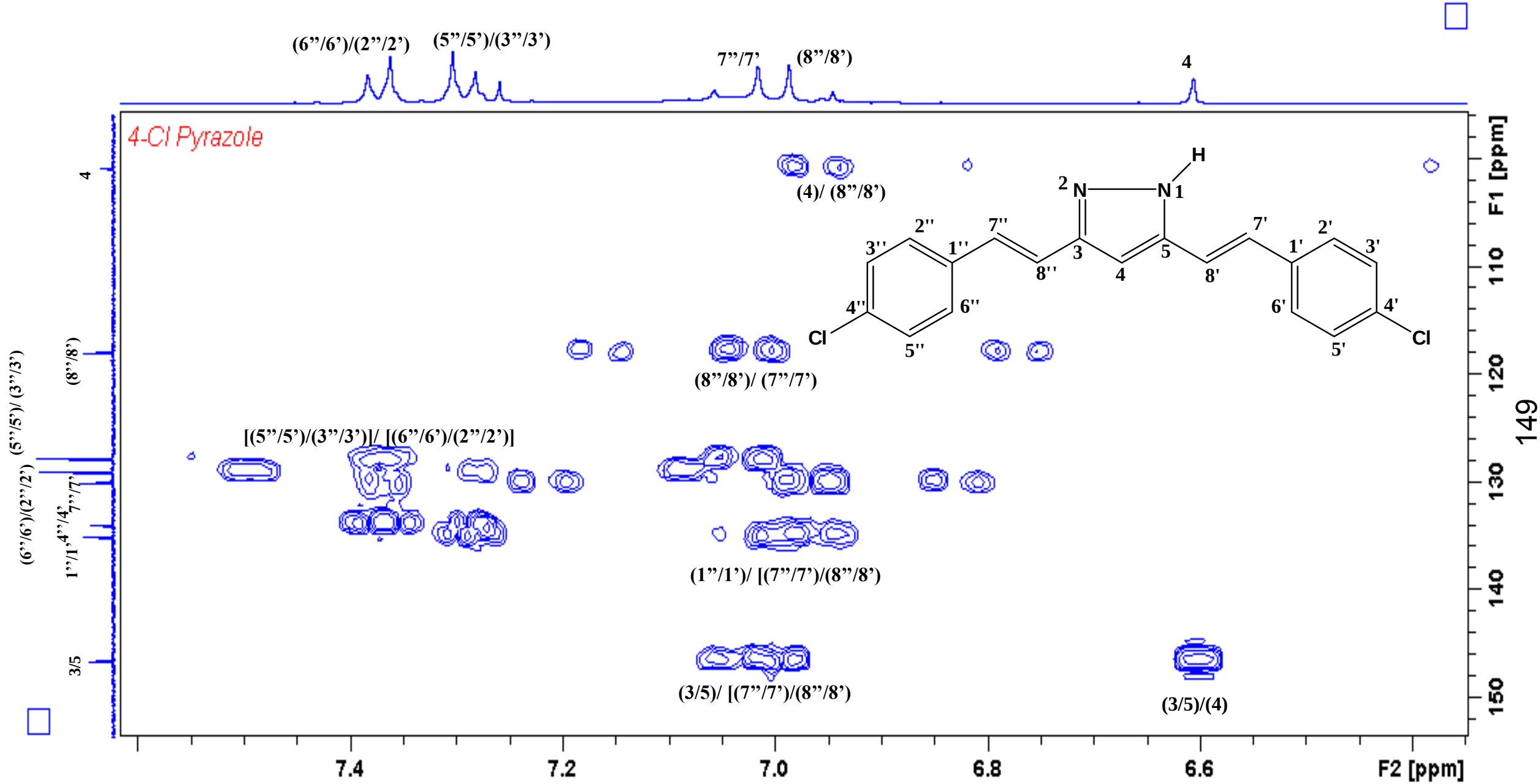
^{13}C Spectrum of Compound A-5c: 3,5-bis(4-chlorostyryl)-1H-pyrazole



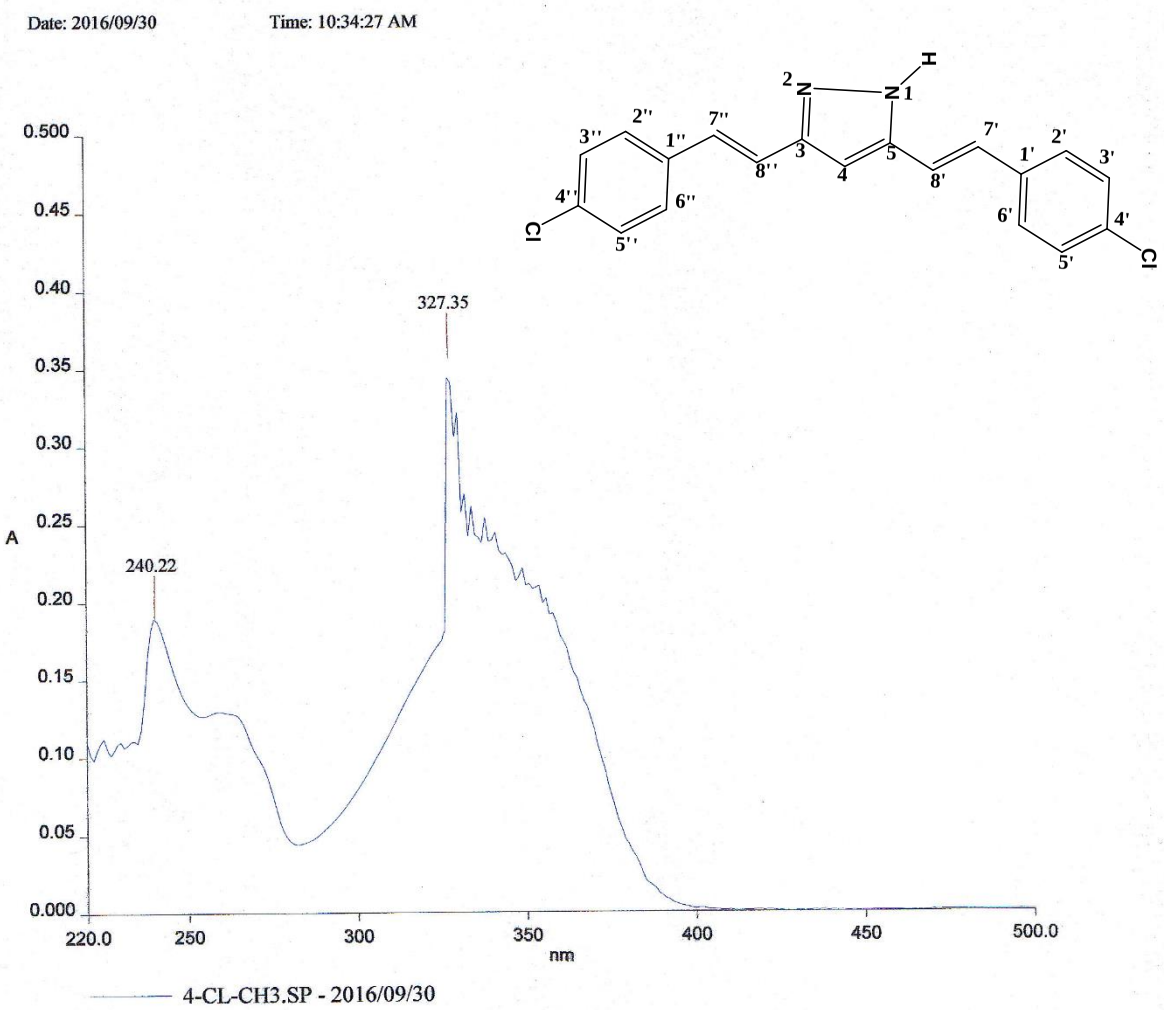
COSY of Compound A-5c: 3,5-bis(4-chlorostyryl)-1H-pyrazole



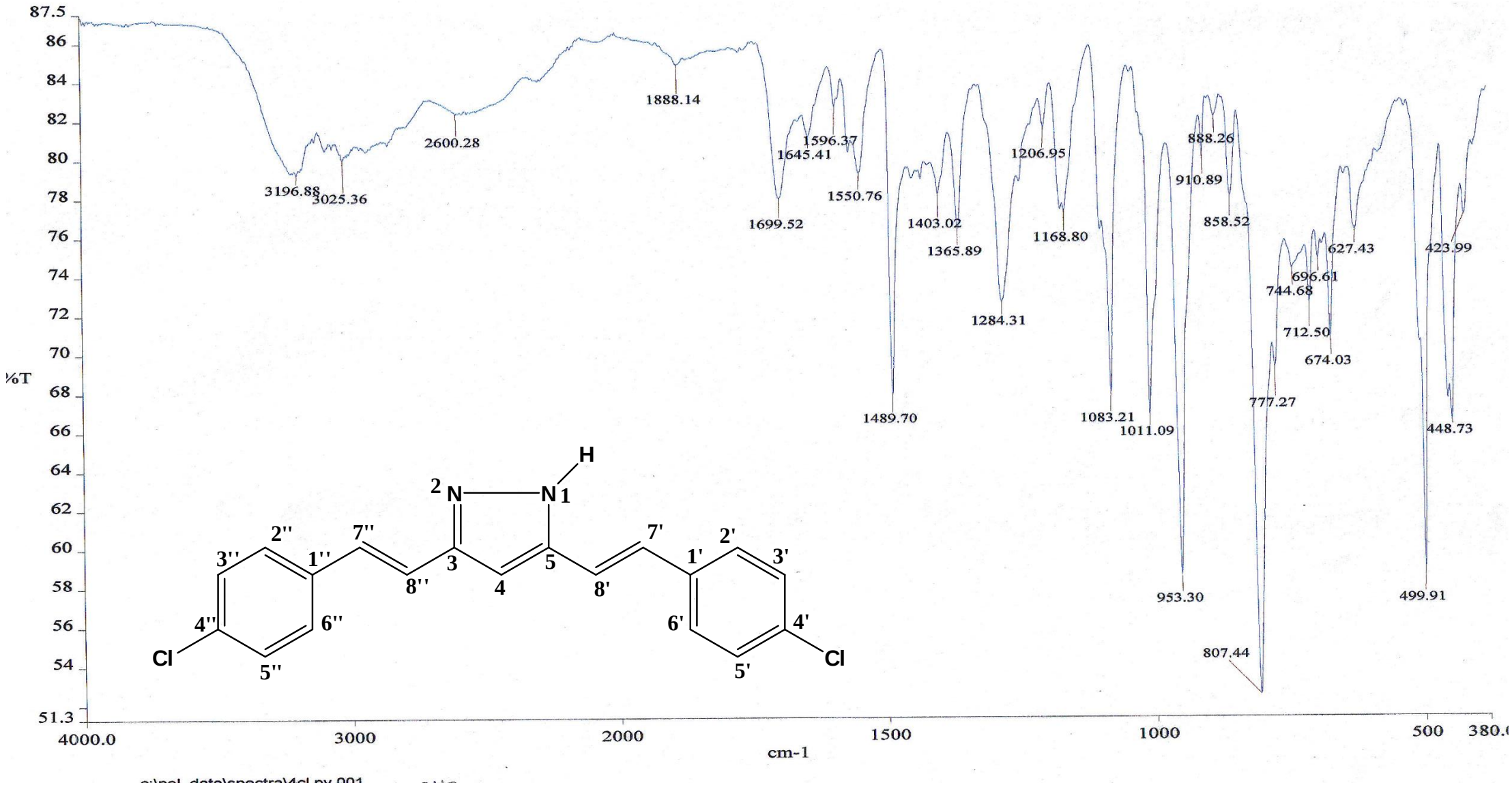
HSQC of Compound A-5c: 3,5-bis(4-chlorostyryl)-1H-pyrazole



HMBC of Compound A-5c: 3,5-bis(4-chlorostyryl)-1H-pyrazole



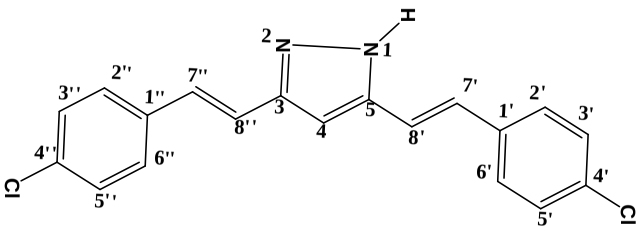
Ultraviolet Spectrum of Compound A-5c: 3,5-bis(4-chlorostyryl)-1H-pyrazole



Infrared Spectrum of Compound A-5c: 3,5-bis(4-chlorostyryl)-1H-pyrazole

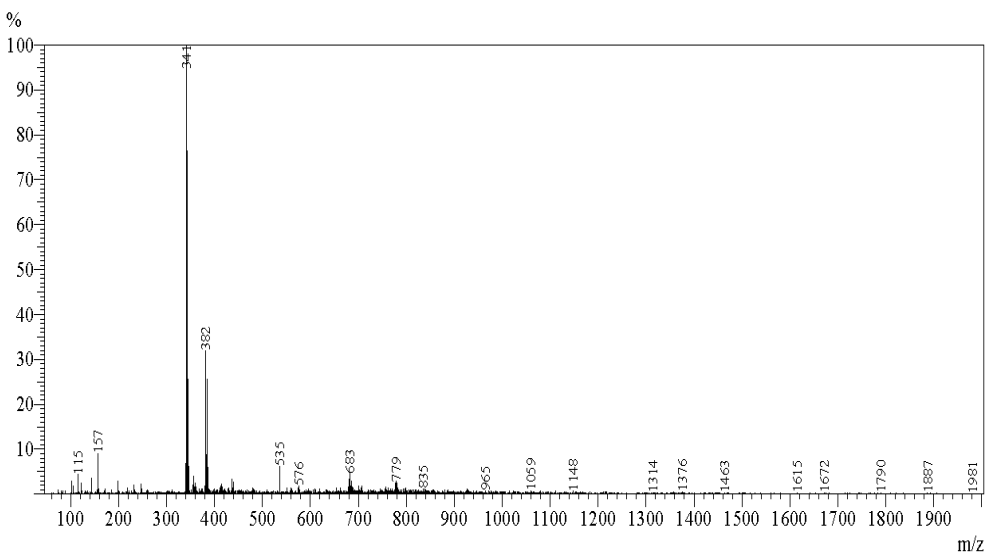
Sample Information

Date Acquired	: 2016/10/04 03:36:24 PM
Sample Type	: Unknown
Sample Name	: 3
Sample ID	: 3
Data File	: 3a.lcd
Method File	: no column run.lcm
Report Format File	: DEFAULT.lsr
Tuning File	: 03022016.lct



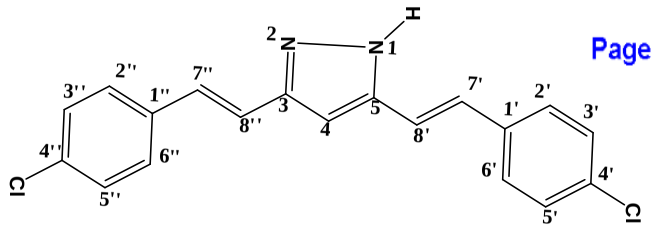
MS Spectrum

R.Time:1.067(Scan#:65)
MassPeaks:2114
Segment 1 - Event 1



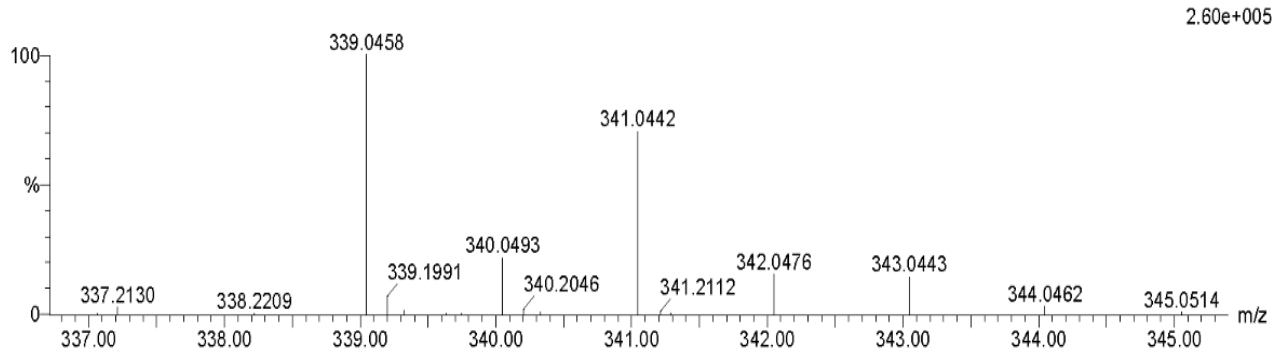
LC-MS of CompoundA-5c: 3,5-bis(4-chlorostyryl)-1H-pyrazole

Elemental Composition Report



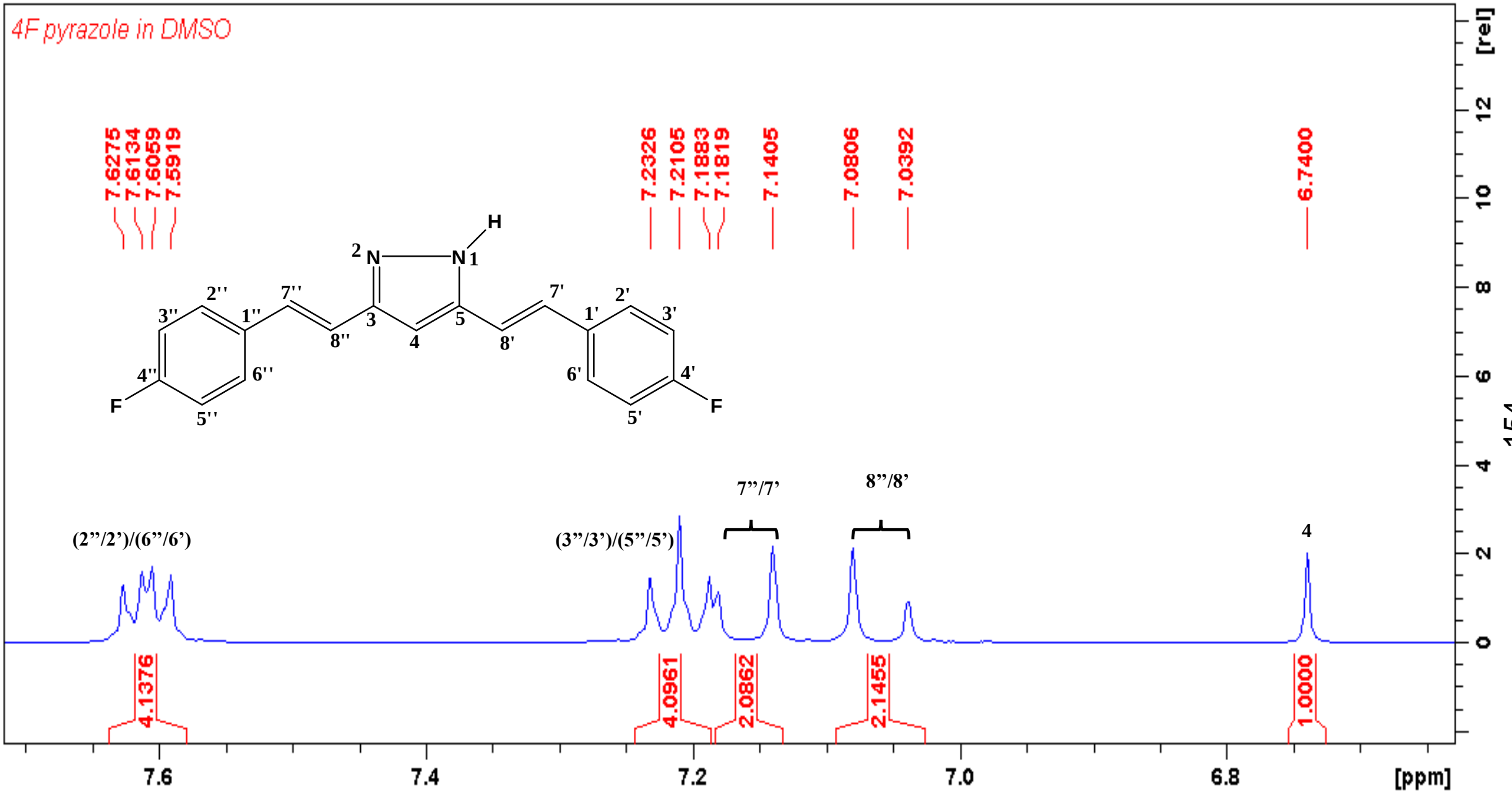
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
7 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 Cl: 0-2
3 4-Cl 40 (1.316) Cm (1:61)
TOF MS ES-

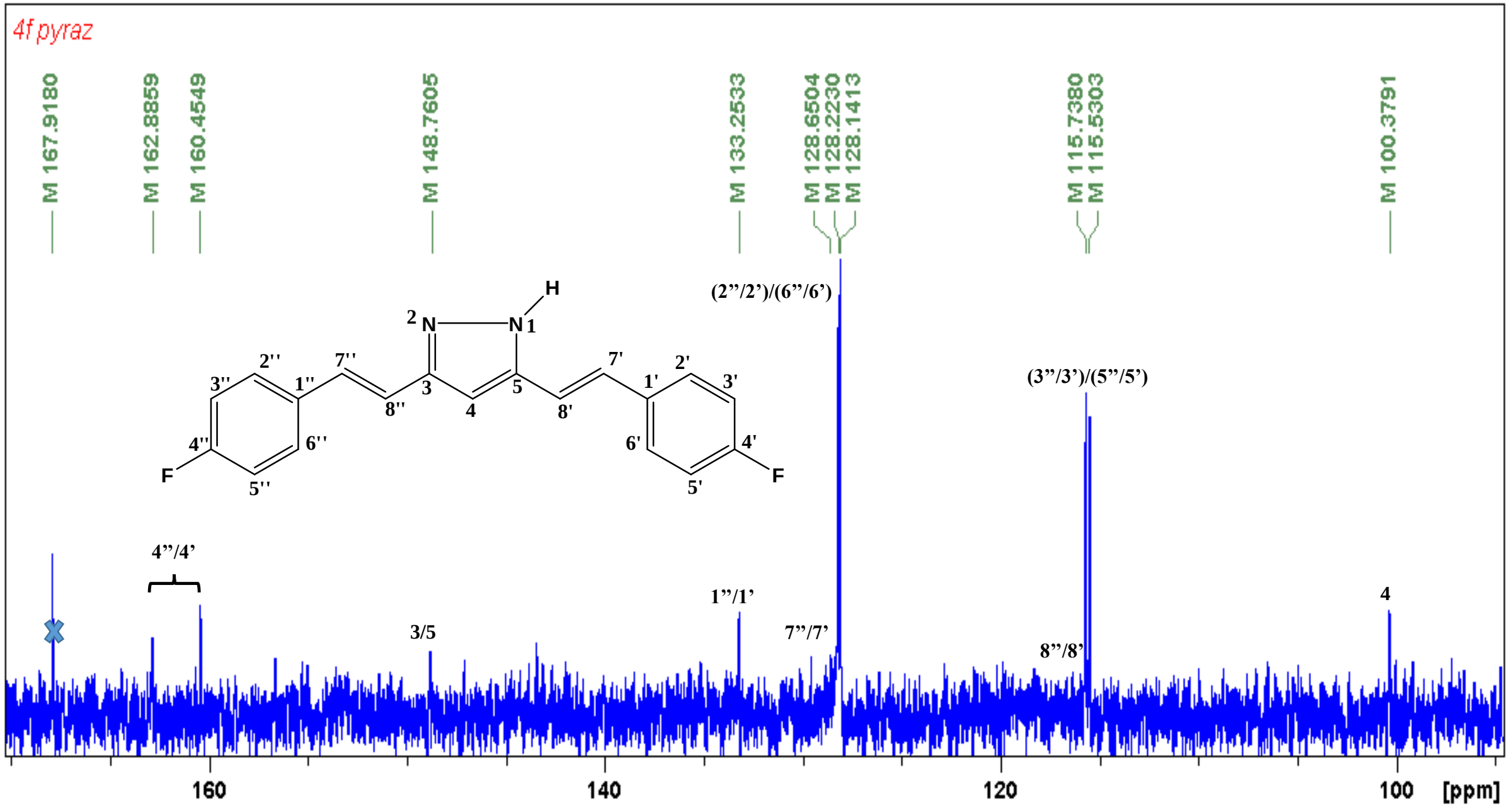


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
339.0458	339.0456	0.2	0.6	13.5	105.3	0.0	C19 H13 N2 Cl2

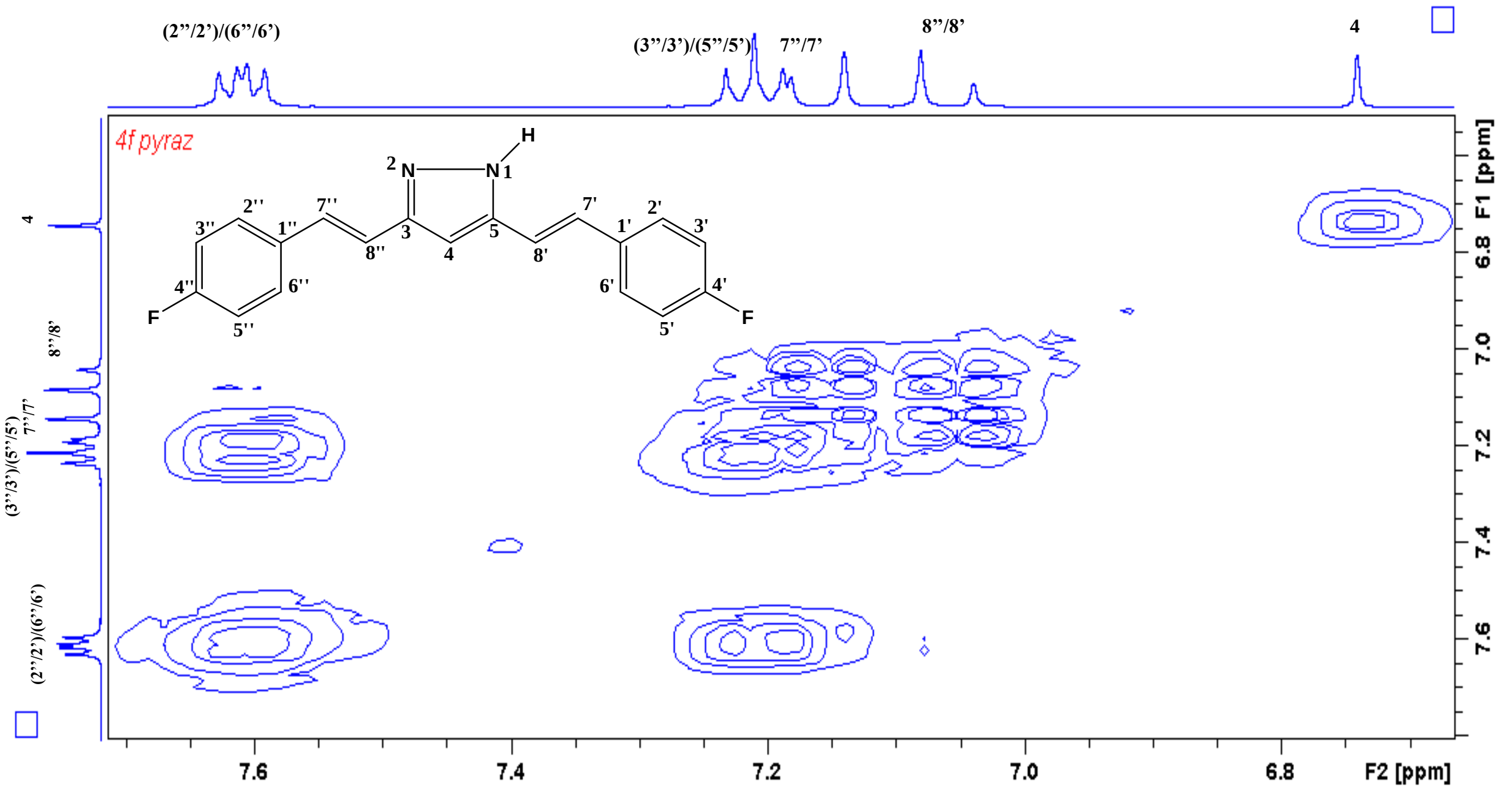
HRMS of Compound A-5c: 3,5-bis(4-chlorostyryl)-1H-pyrazole



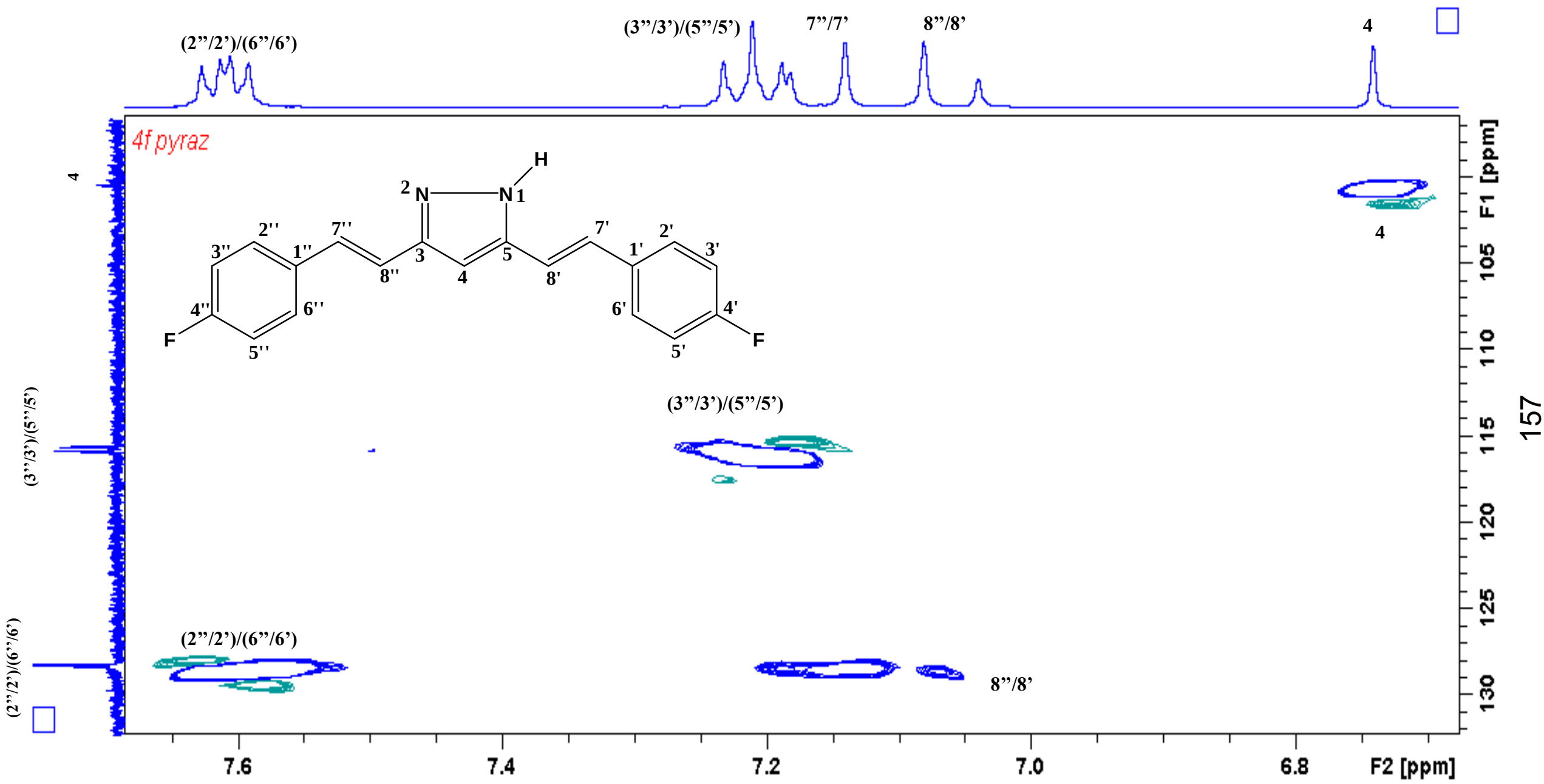
^{1}H Spectrum of Compound A-5f: 3,5-bis(4-fluorostyryl)-1H-pyrazole



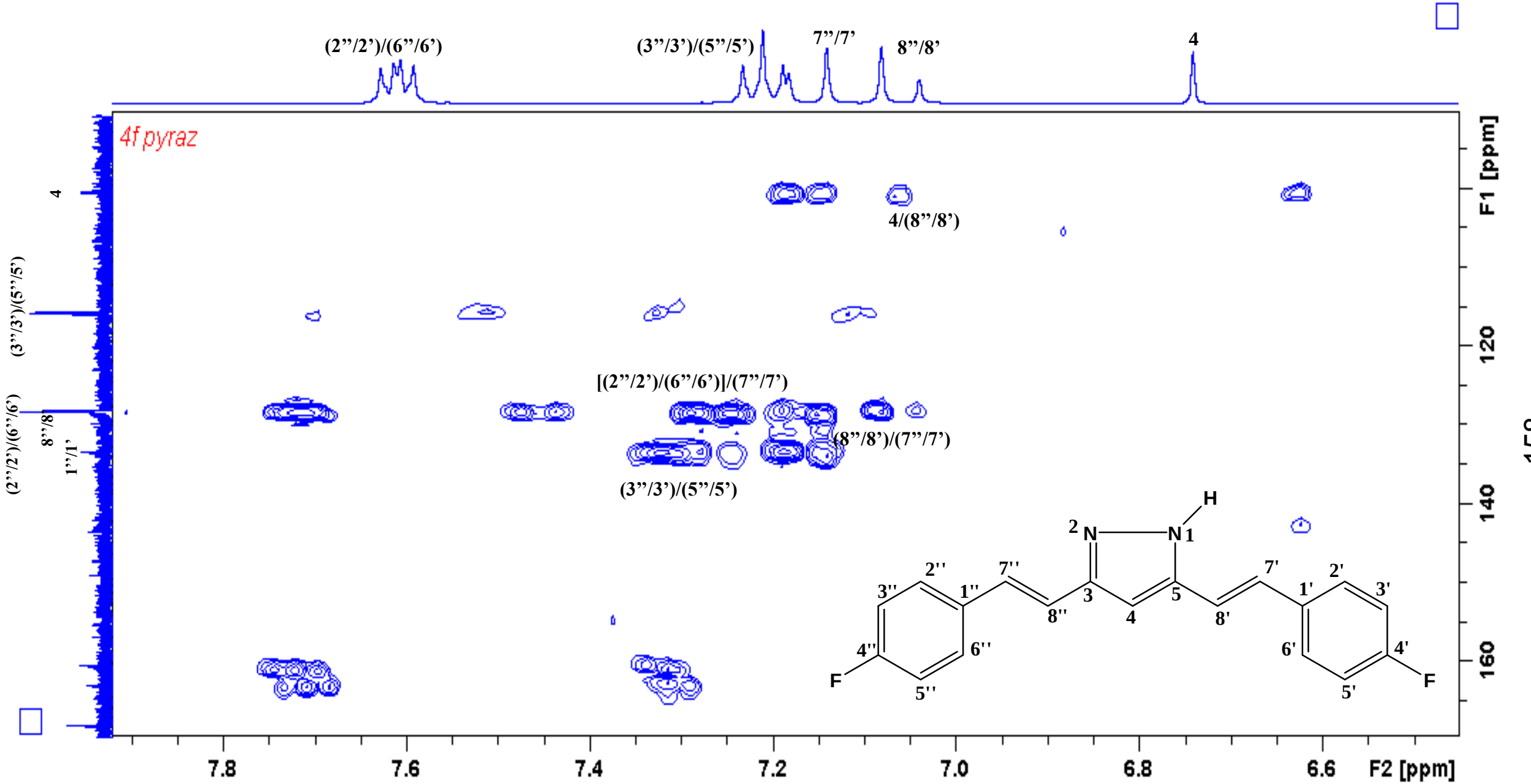
^{13}C Spectrum of Compound A-5f: 3,5-bis(4-fluorostyryl)-1H-pyrazole



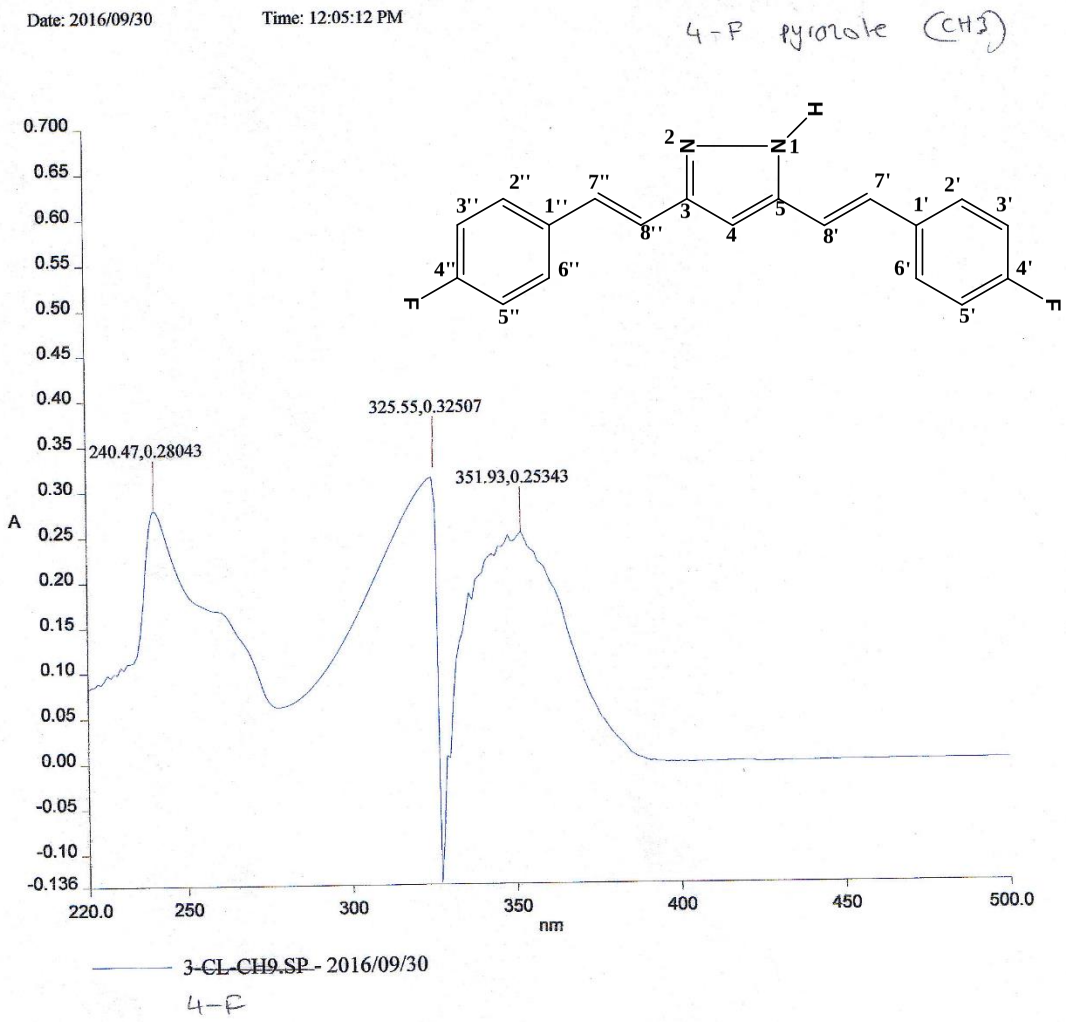
COSY of Compound A-5f: 3,5-bis(4-fluorostyryl)-1H-pyrazole



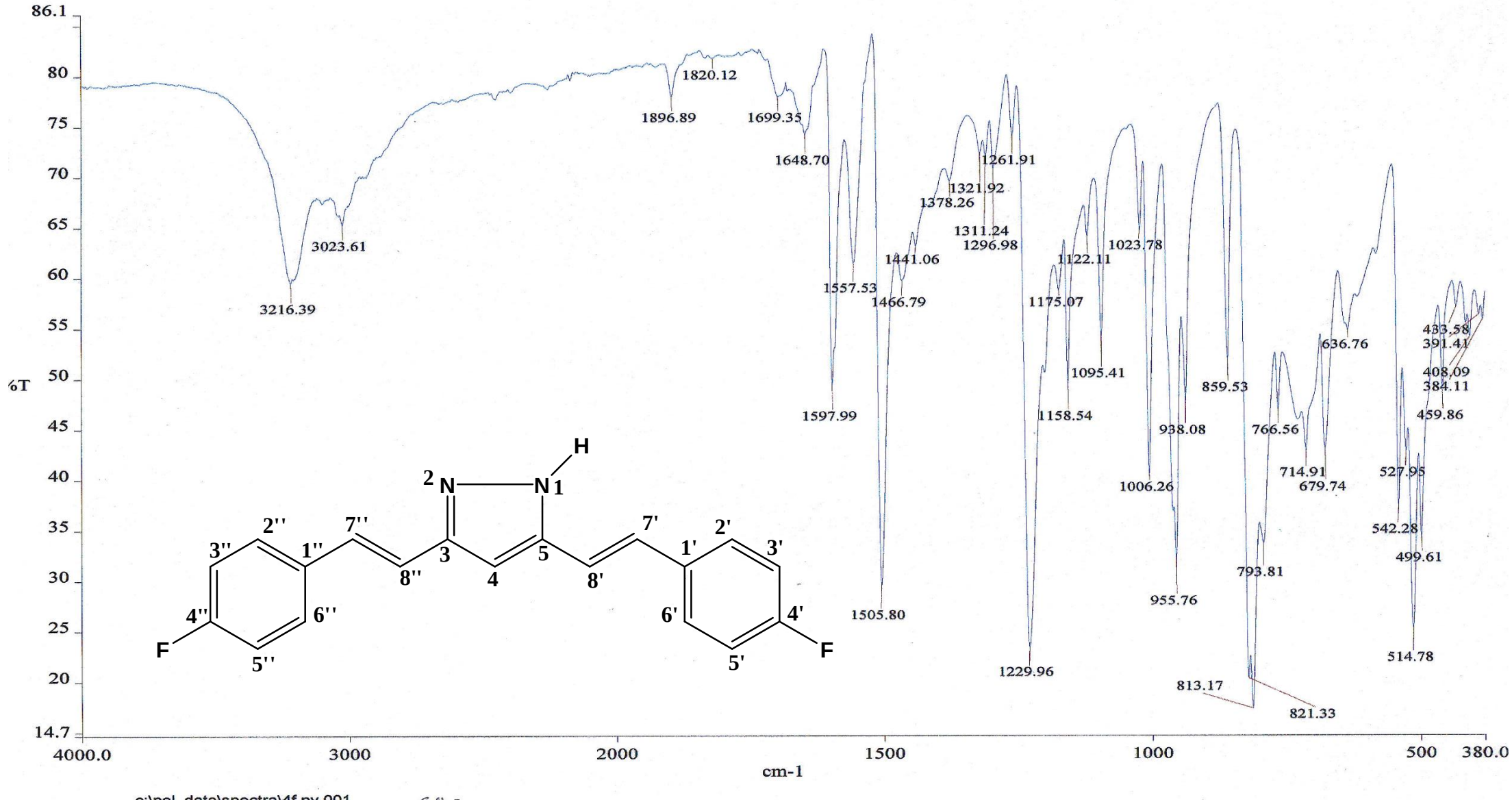
HSQC of Compound A-5f: 3,5-bis(4-fluorostyryl)-1H-pyrazole



HMBC of Compound A-5f: 3,5-bis(4-fluorostyryl)-1H-pyrazole



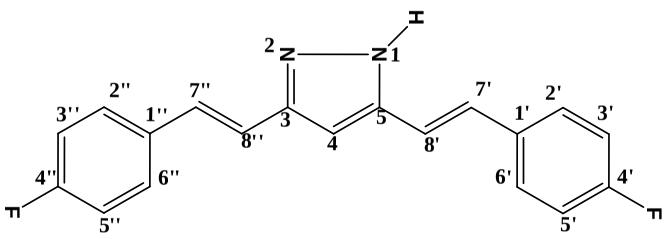
Ultraviolet Spectrum of Compound A-5f: 3,5-bis(4-fluorostyryl)-1H-pyrazole



Infrared Spectrum of Compound A-5f: 3,5-bis(4-fluorostyryl)-1H-pyrazole

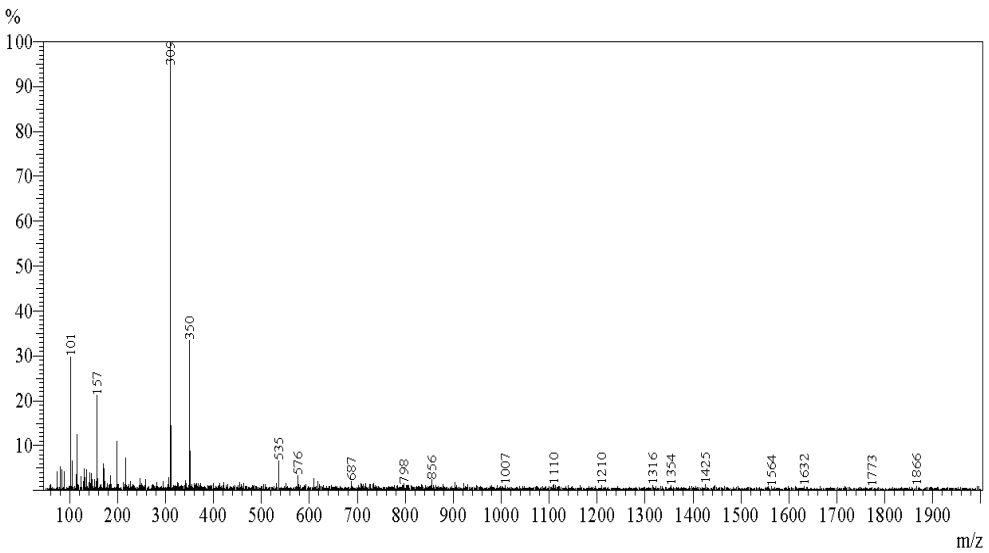
Sample Information

Date Acquired	: 2016/09/30 01:57:16 PM
Sample Type	: Unknown
Sample Name	: 8
Sample ID	: 8
Data File	: 8.lcd
Method File	: no column run.lcm
Report Format File	: DEFAULT.lsr
Tuning File	: 03022016.lct



MS Spectrum

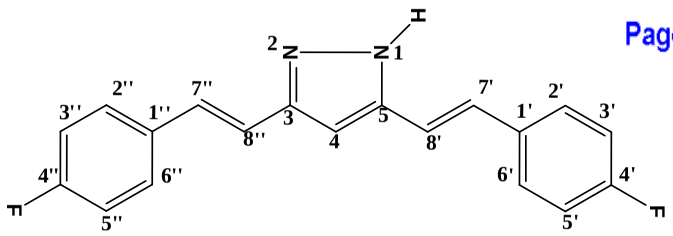
R.Time:1.067(Scan#:65)
MassPeaks:2064
Segment 1 - Event 1



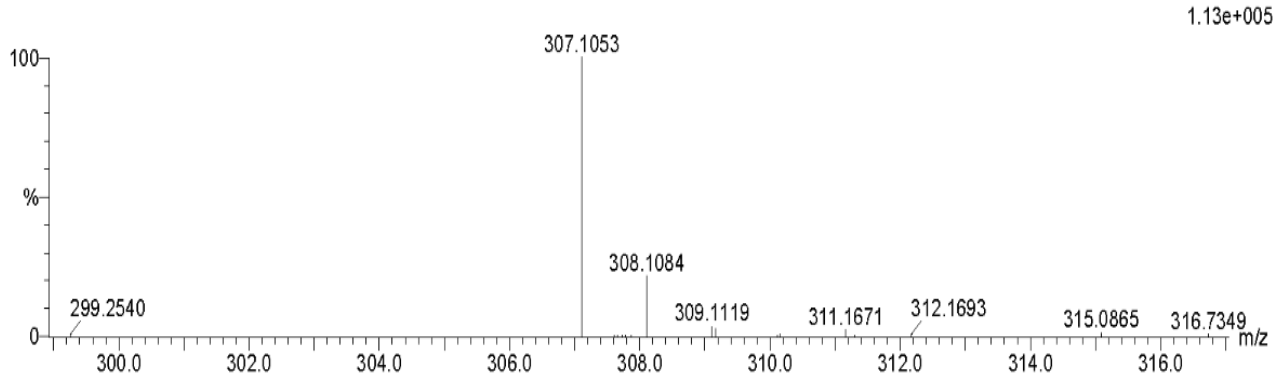
LC-MS of Compound A-5f: 3,5-bis(4-fluorostyryl)-1H-pyrazole

Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

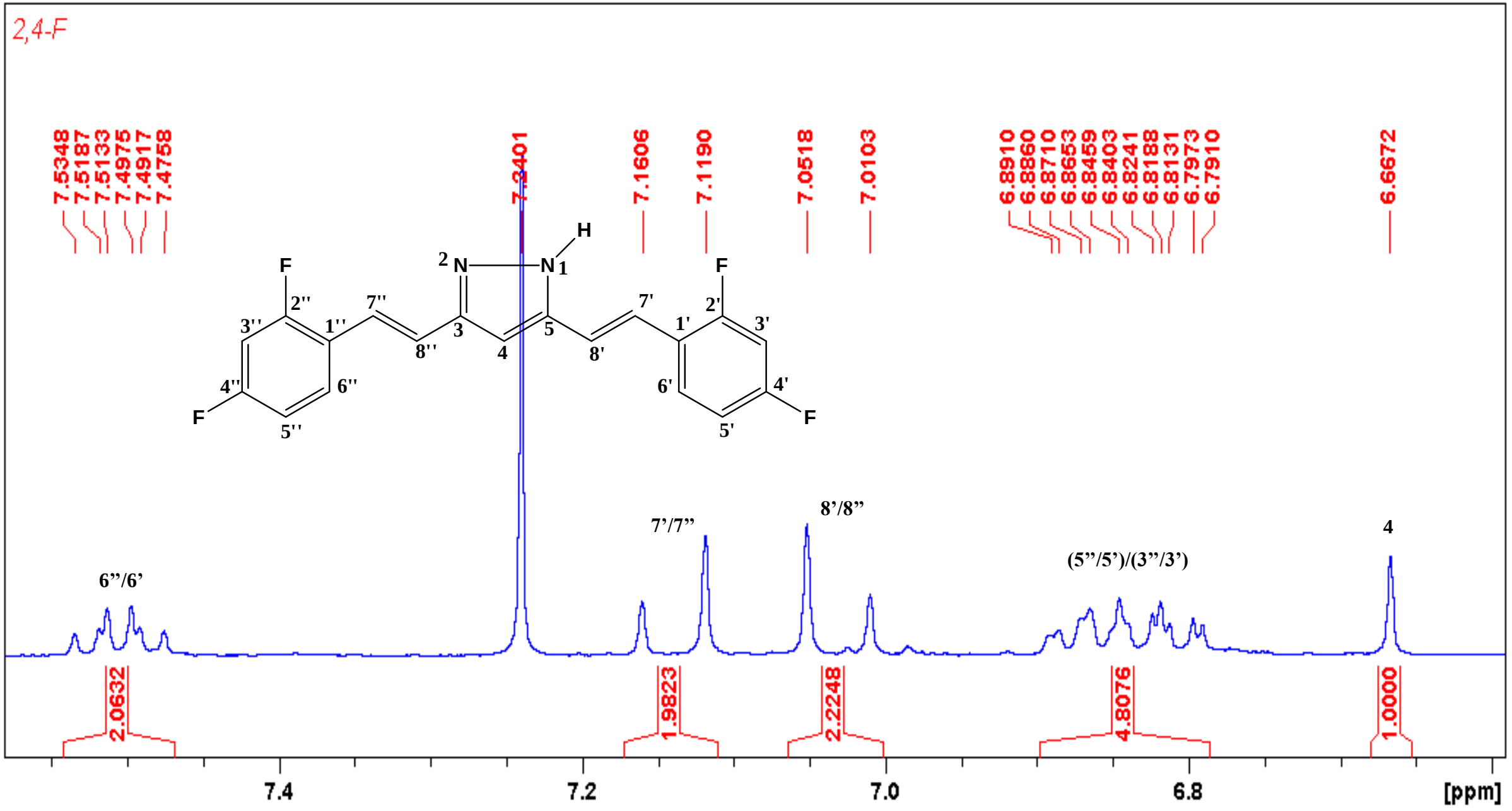


Monoisotopic Mass, Even Electron Ions
15 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 F: 0-5
8 4-F 2 (0.034) Cm (1:61)
TOF MS ES-

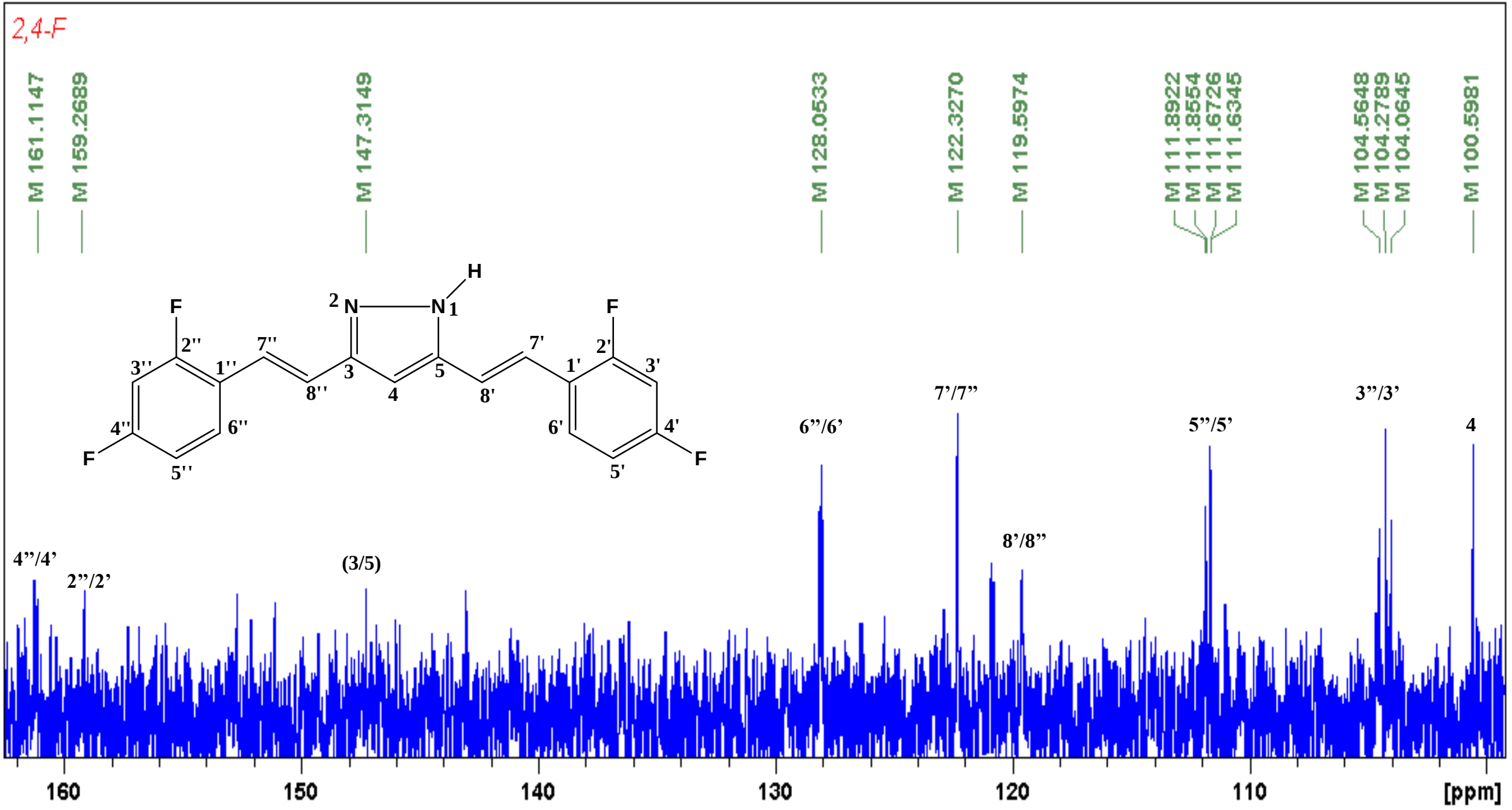


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
307.1053	307.1047	0.6	2.0	13.5	69.8	0.0	C19 H13 N2 F2

HRMS of Compound A-5f: 3,5-bis(4-fluorostyryl)-1H-pyrazole



^{1}H NMR of Compound A-5d: 3,5-bis(2,4-difluorostyryl)-1H-pyrazole



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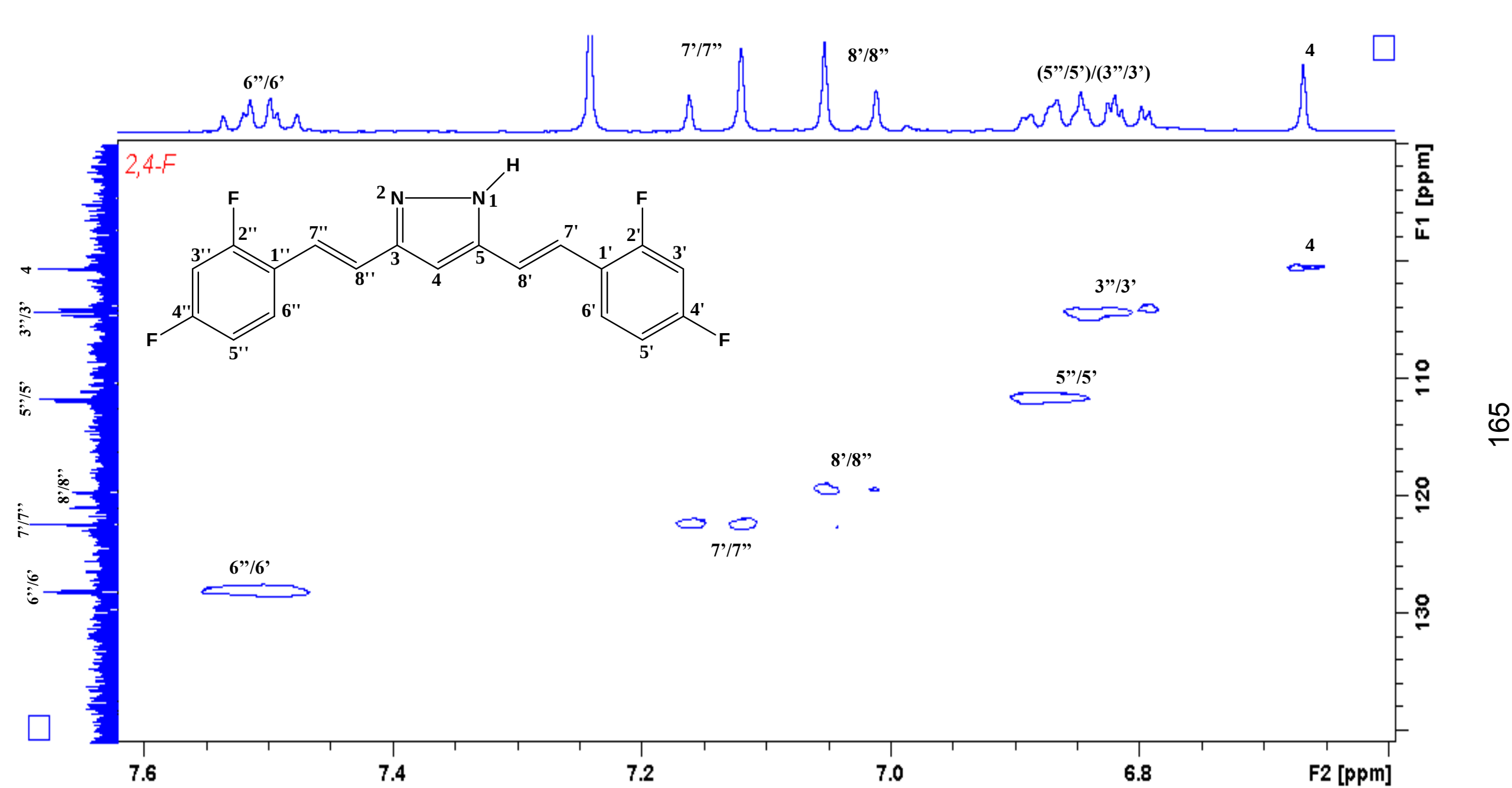
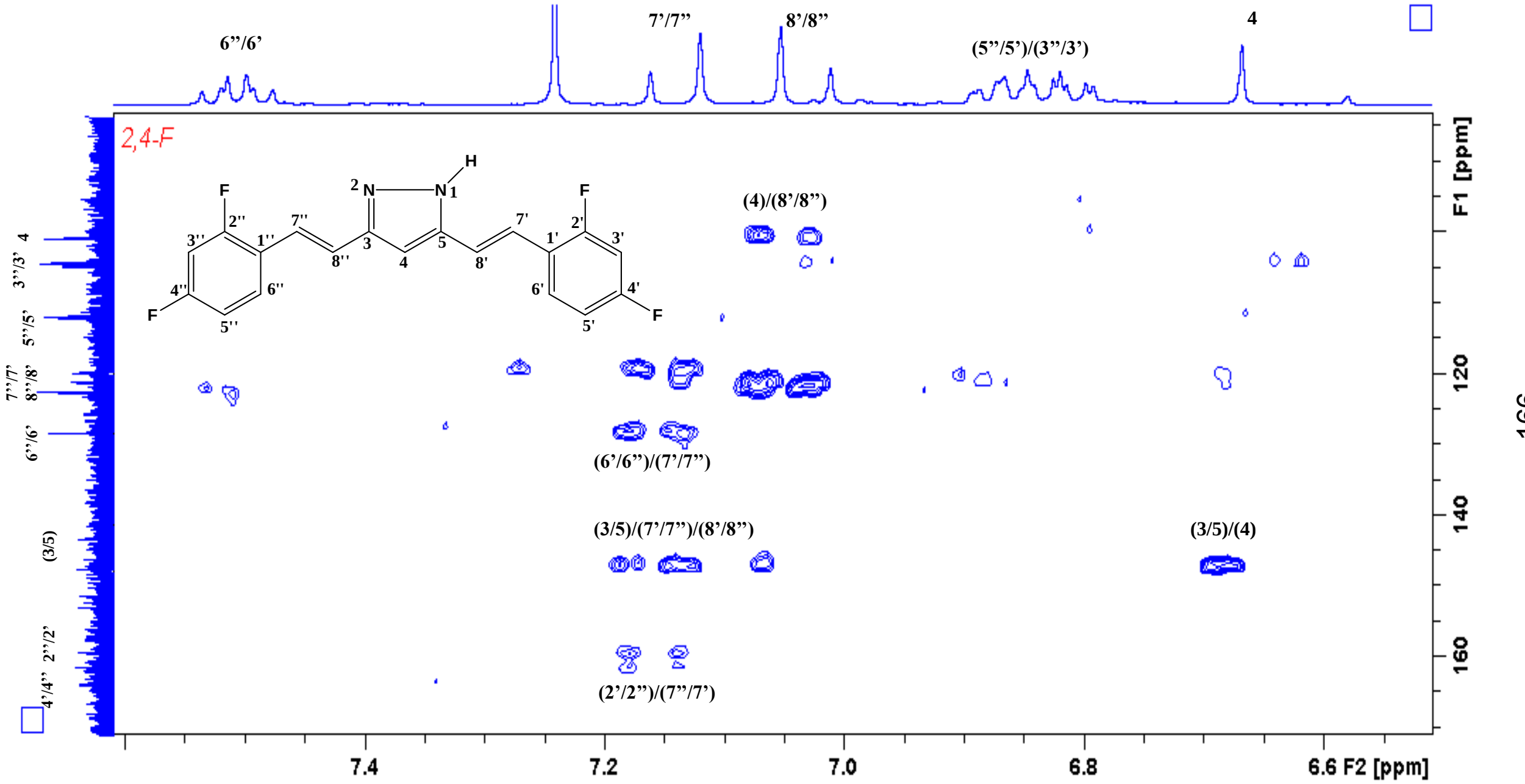
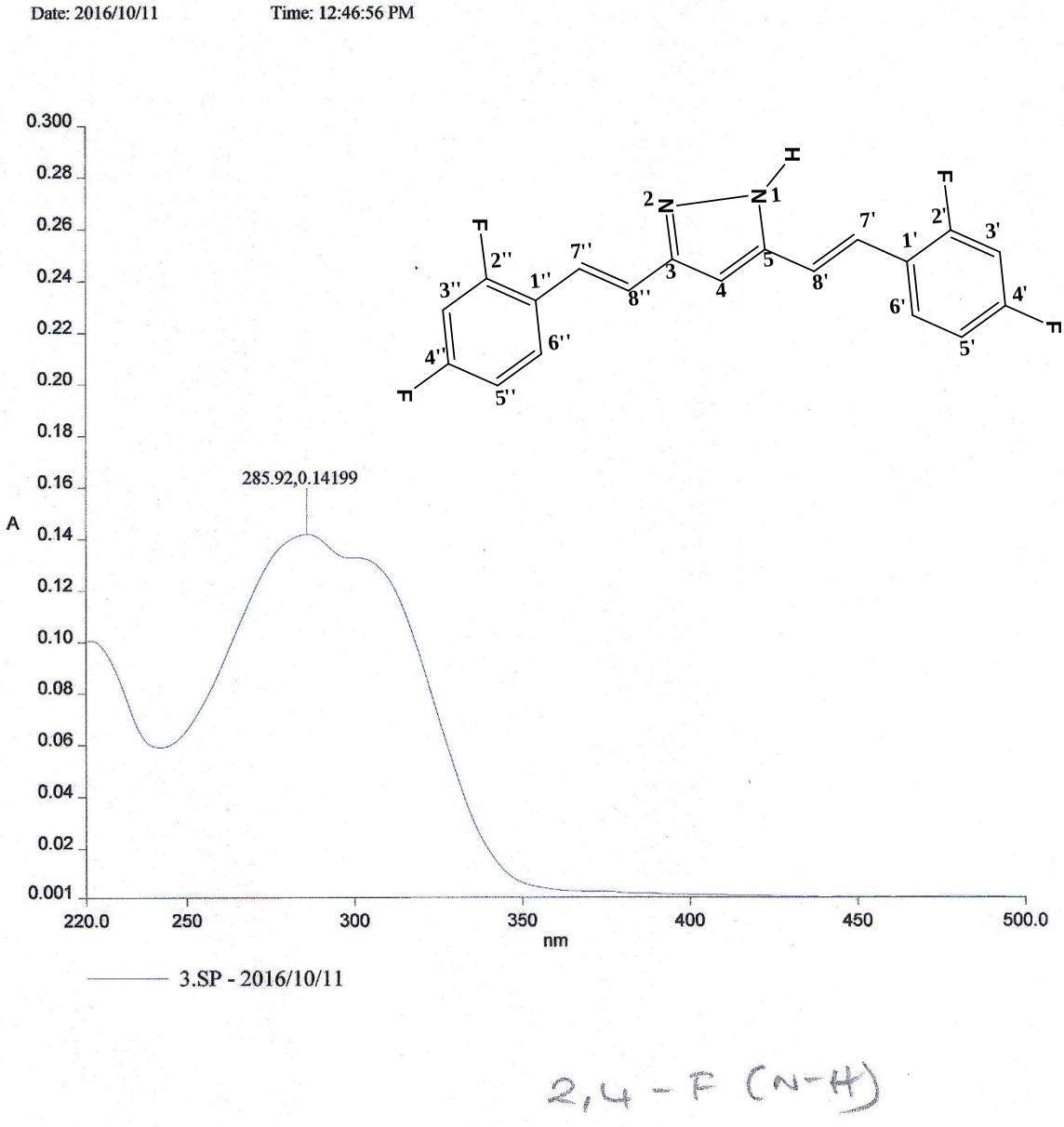
^{13}C NMR of Compound A-5d: 3,5-bis(2,4-difluorostyryl)-1H-pyrazole



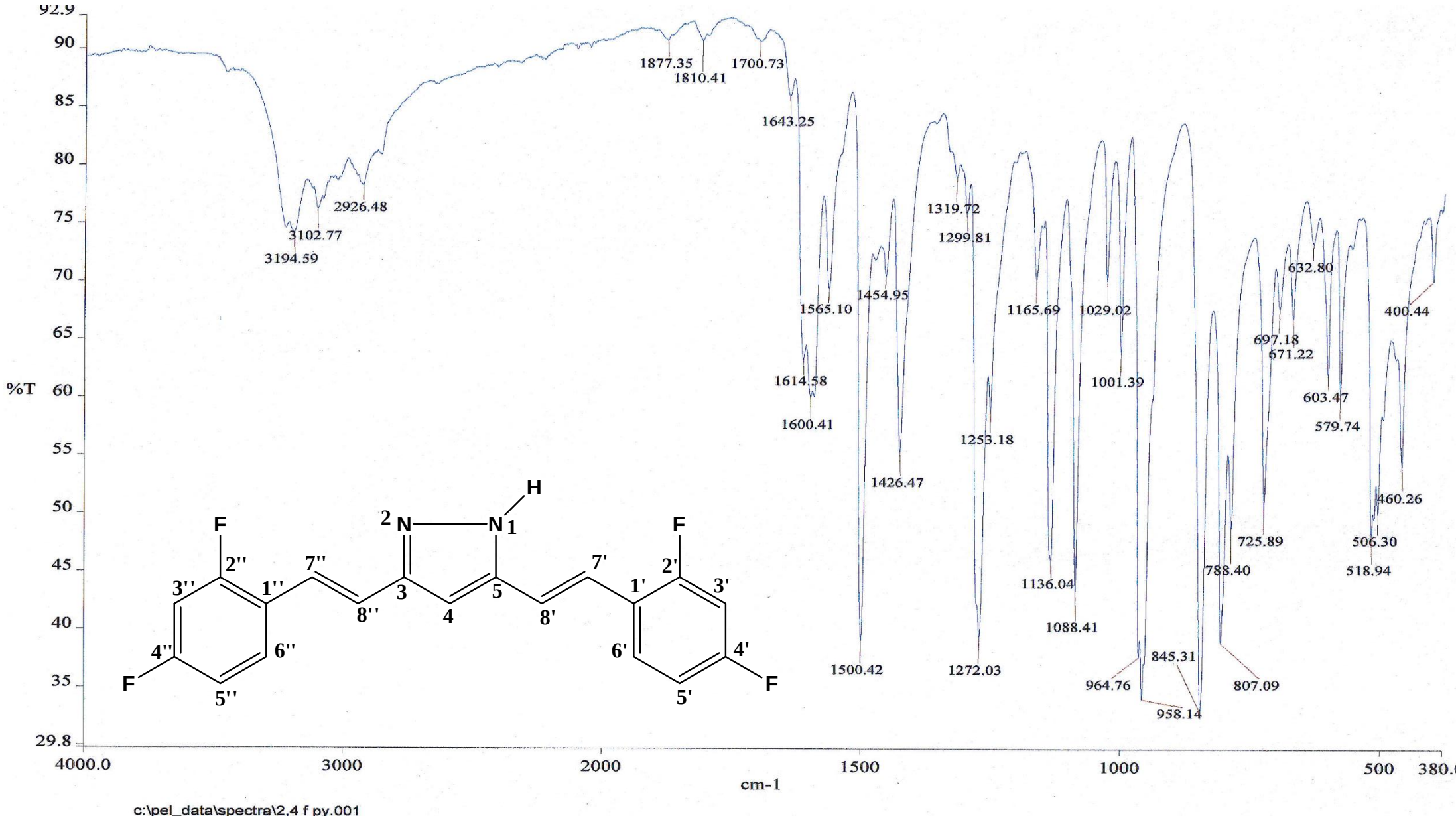
HSQC of Compound A-5d: 3,5-bis(2,4-difluorostyryl)-1H-pyrazole



HMBC of Compound A-5d: 3,5-bis(2,4-difluorostyryl)-1H-pyrazole



Ultraviolet Spectrum of Compound A-5d: 3,5-bis(2,4-difluorostyryl)-1H-pyrazole



Infrared Spectrum of Compound A-5d: 3,5-bis(2,4-difluorostyryl)-1H-pyrazole

Sample Information

Date Acquired	: 2016/10/04 04:22:44 PM
Sample Type	: Unknown
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Sample ID	: 17
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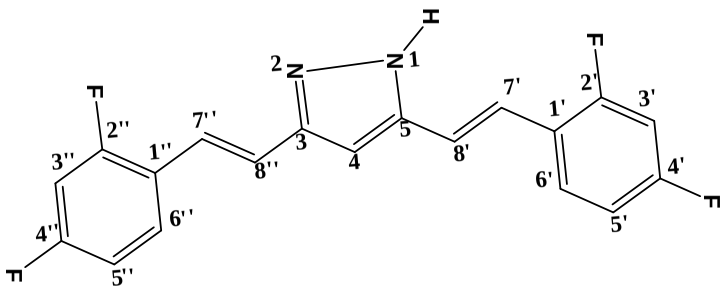
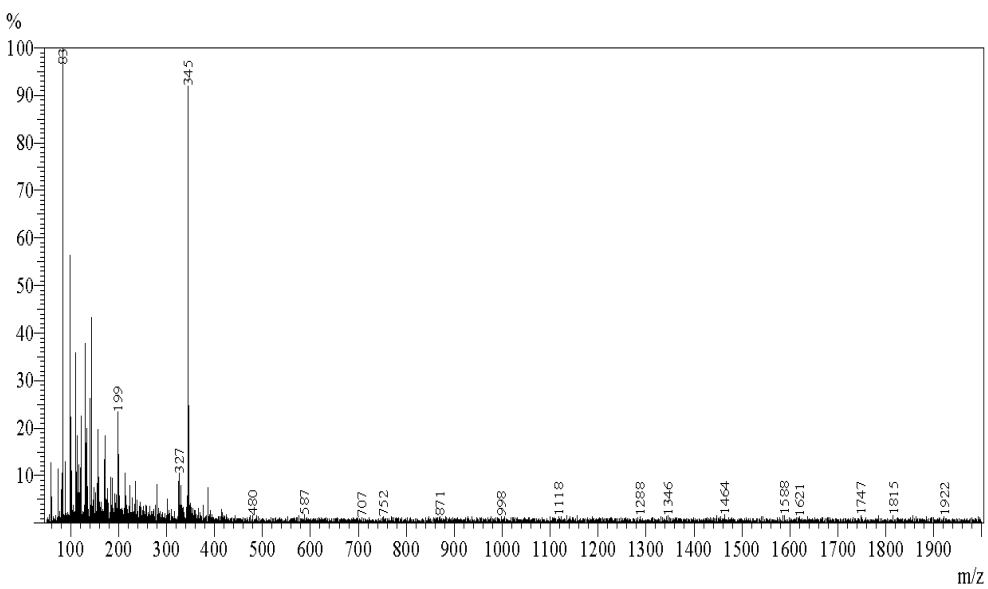
Method File	: no column run.lcm
Report Format File	: DEFAULT.lsr
Tuning File	: 03022016.lct



MS Spectrum

R.Time:1.067(Scan#:65)
MassPeaks:1919
Segment 1 - Event 1



LC-MS of Compound A-5d: 3,5-bis(2,4-difluorostyryl)-1H-pyrazole

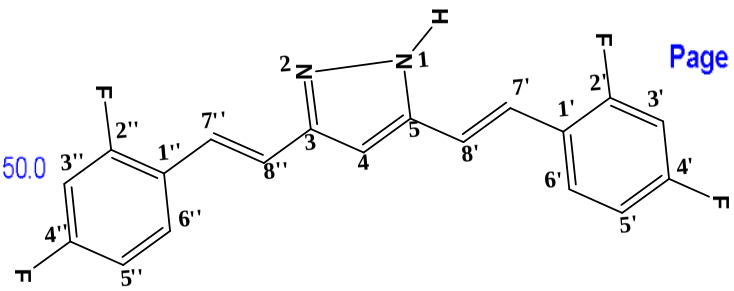
Elemental Composition Report Page 1

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 2



Monoisotopic Mass, Even Electron Ions

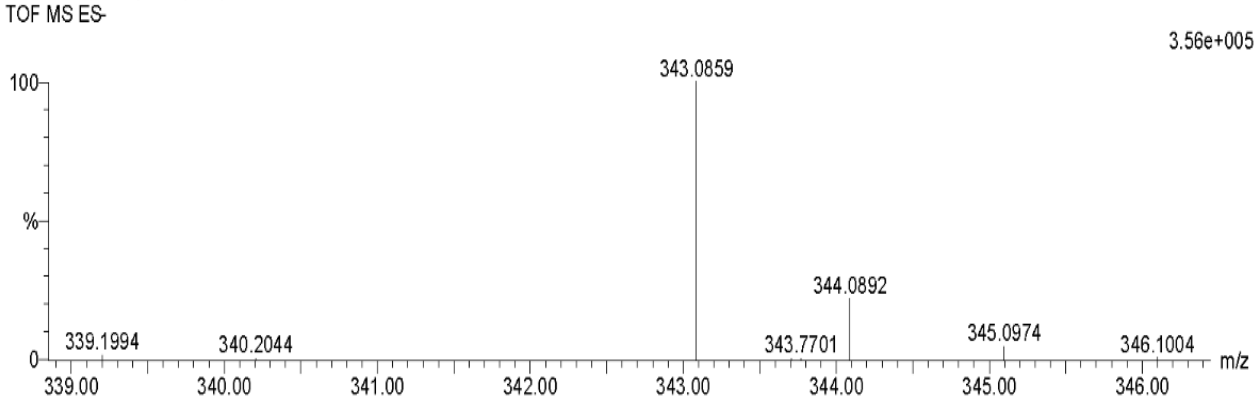
14 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)

Elements Used:

C: 15-20 H: 10-15 N: 0-5 F: 0-5

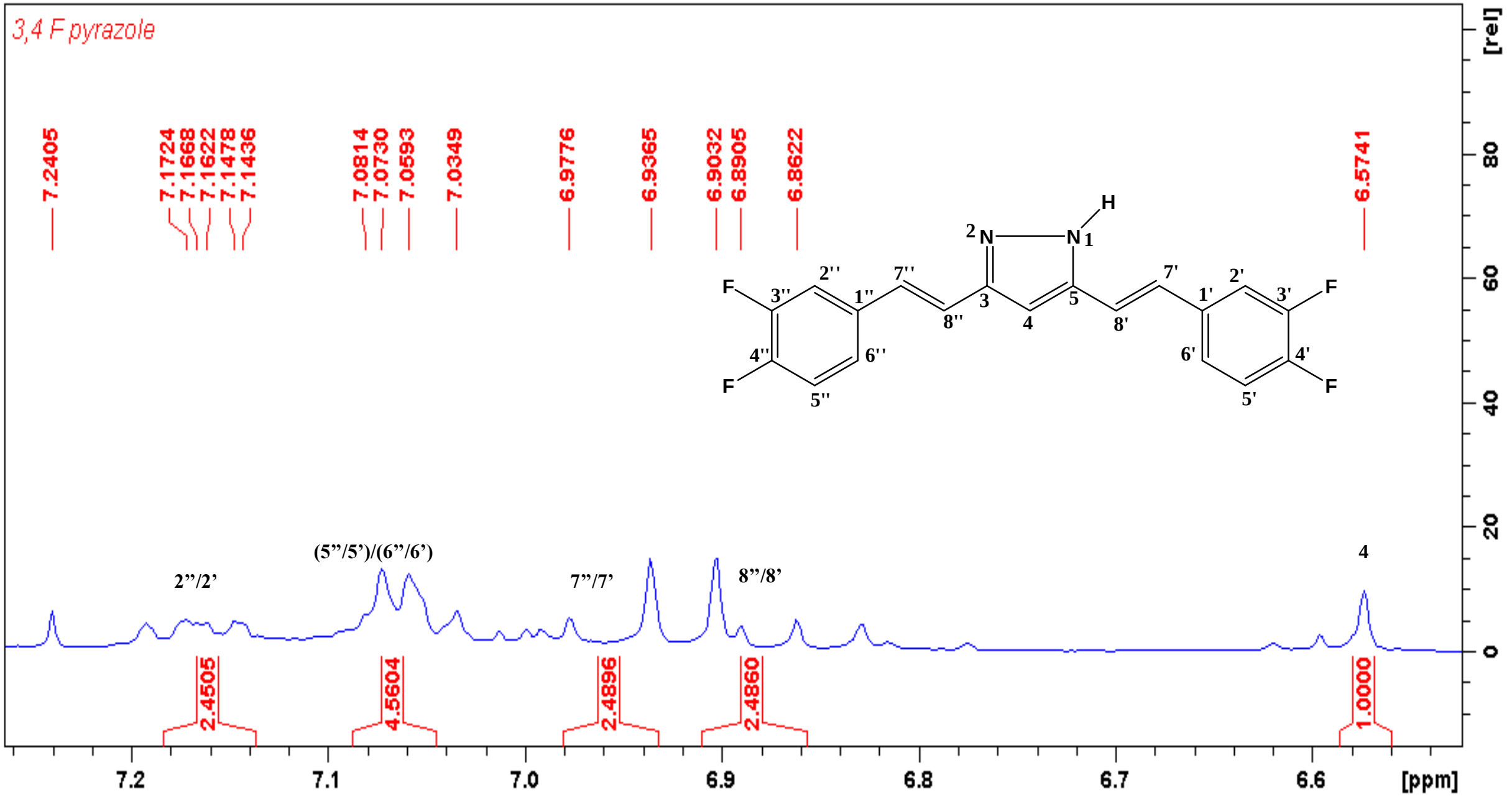
7 24-F 13 (0.405) Cm (1:61)

TOF MS ES-



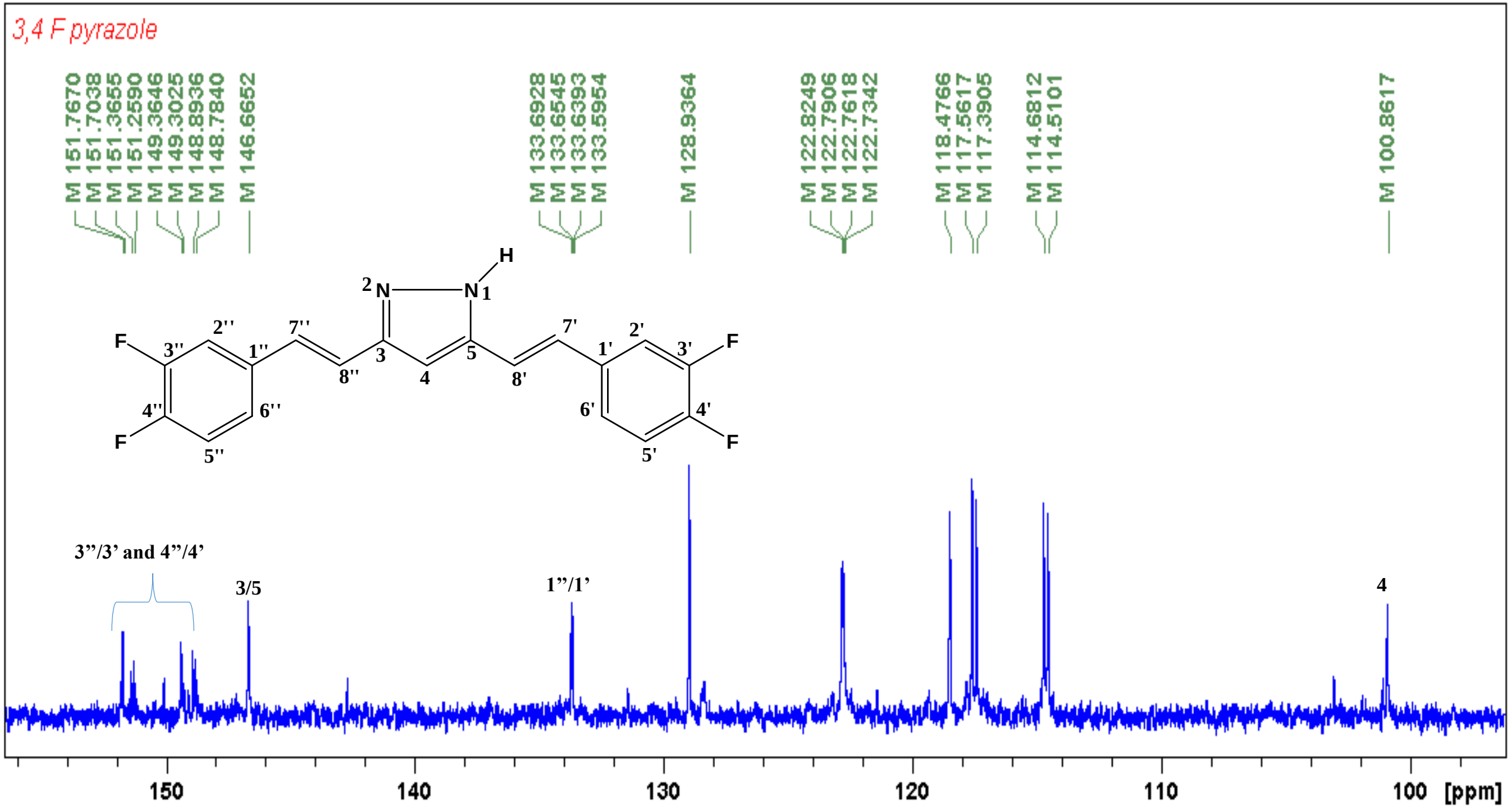
Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
343.0859	343.0858	0.1	0.3	13.5	36.9	0.0	C19 H11 N2 F4

HRMS of Compound A-5d: 3,5-bis(2,4-difluorostyryl)-1H-pyrazole



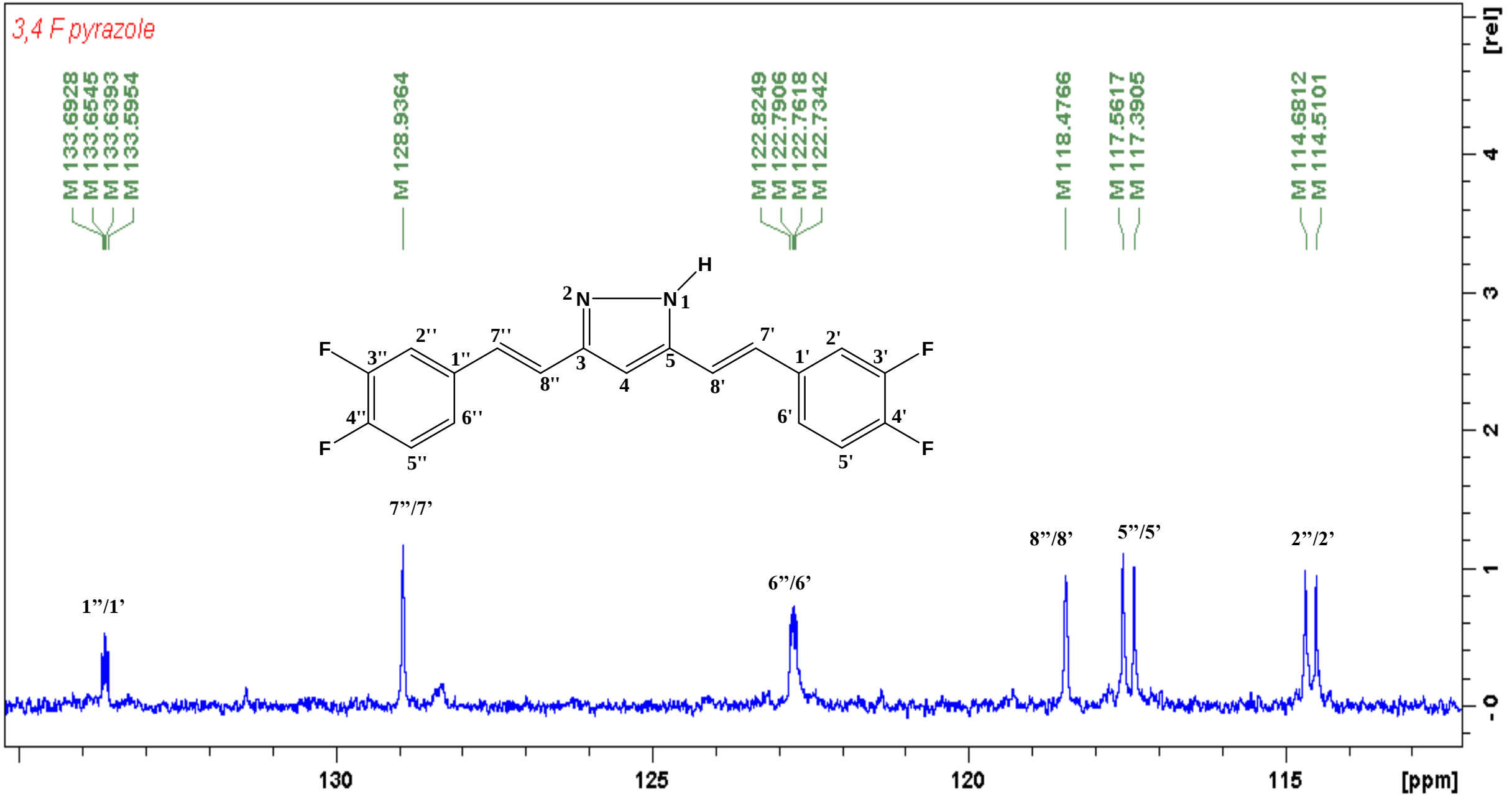
171

^{1}H Spectrum of Compound A-5h: 3,5-bis(3,4-difluorostyryl)-1H-pyrazole

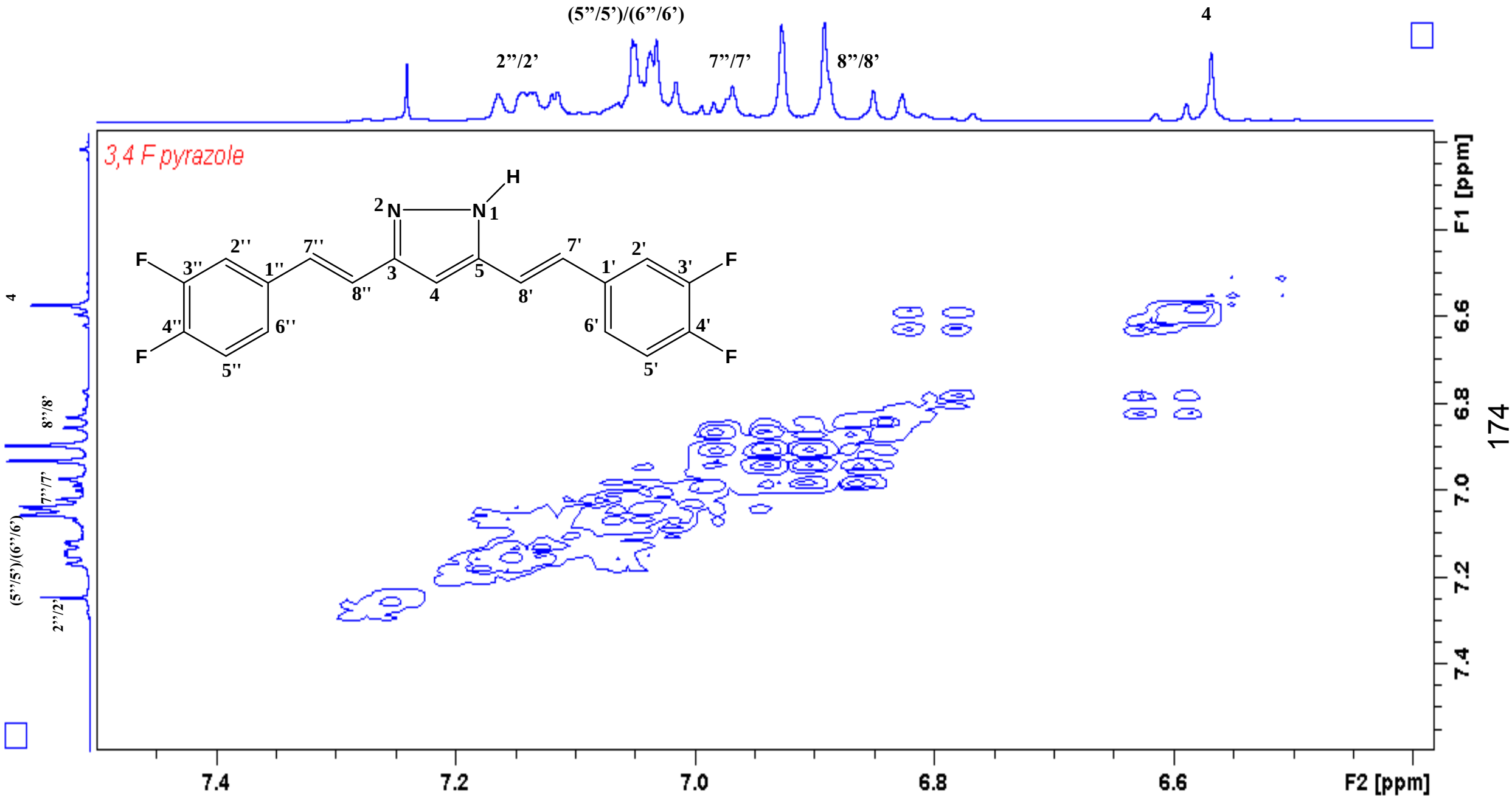


172

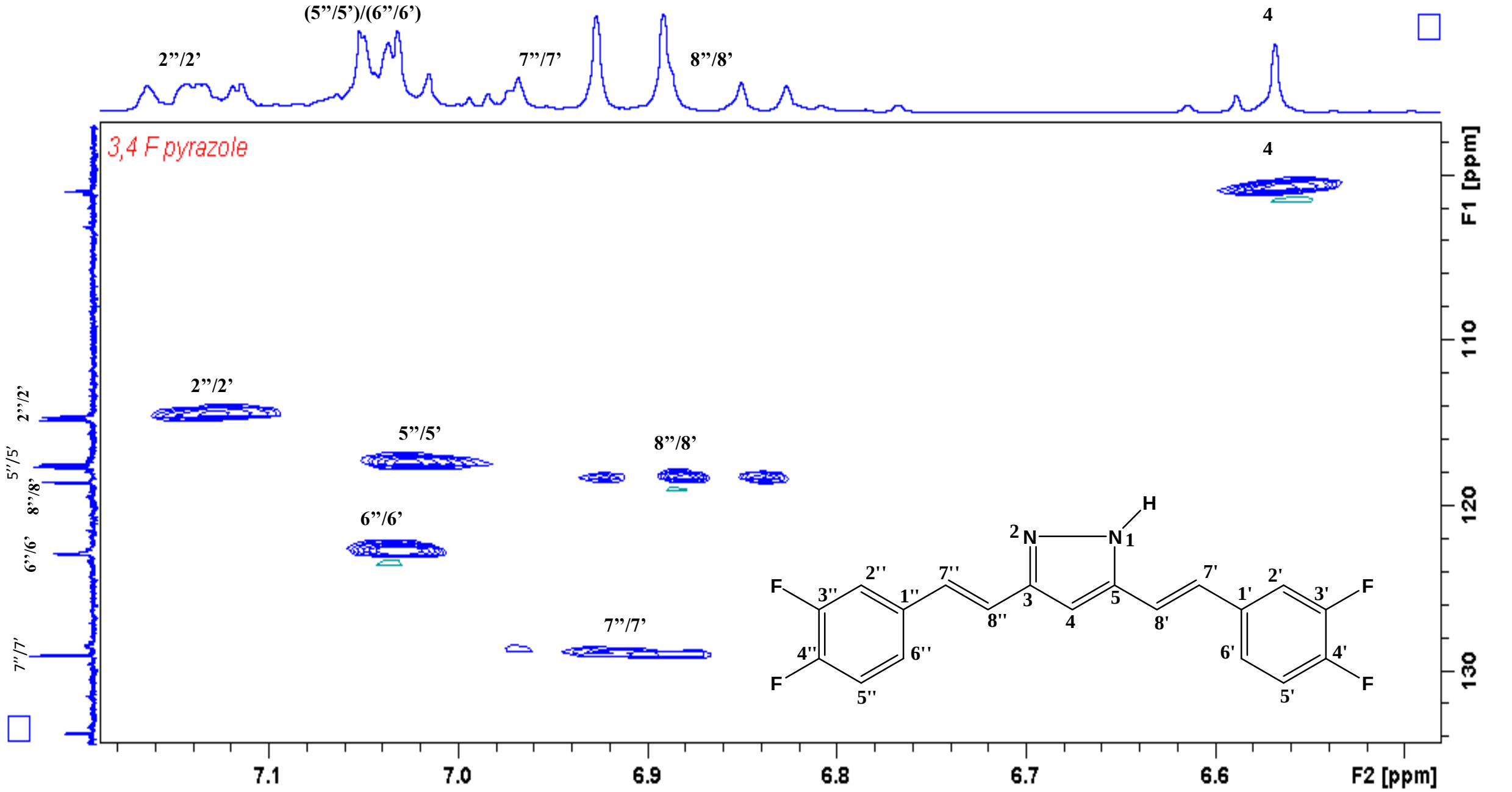
^{13}C Spectrum of Compound A-5h: 3,5-bis(3,4-difluorostyryl)-1H-pyrazole



Expanded ^{13}C Spectrum of Compound A-5h: 3,5-bis(3,4-difluorostyryl)-1H-pyrazole

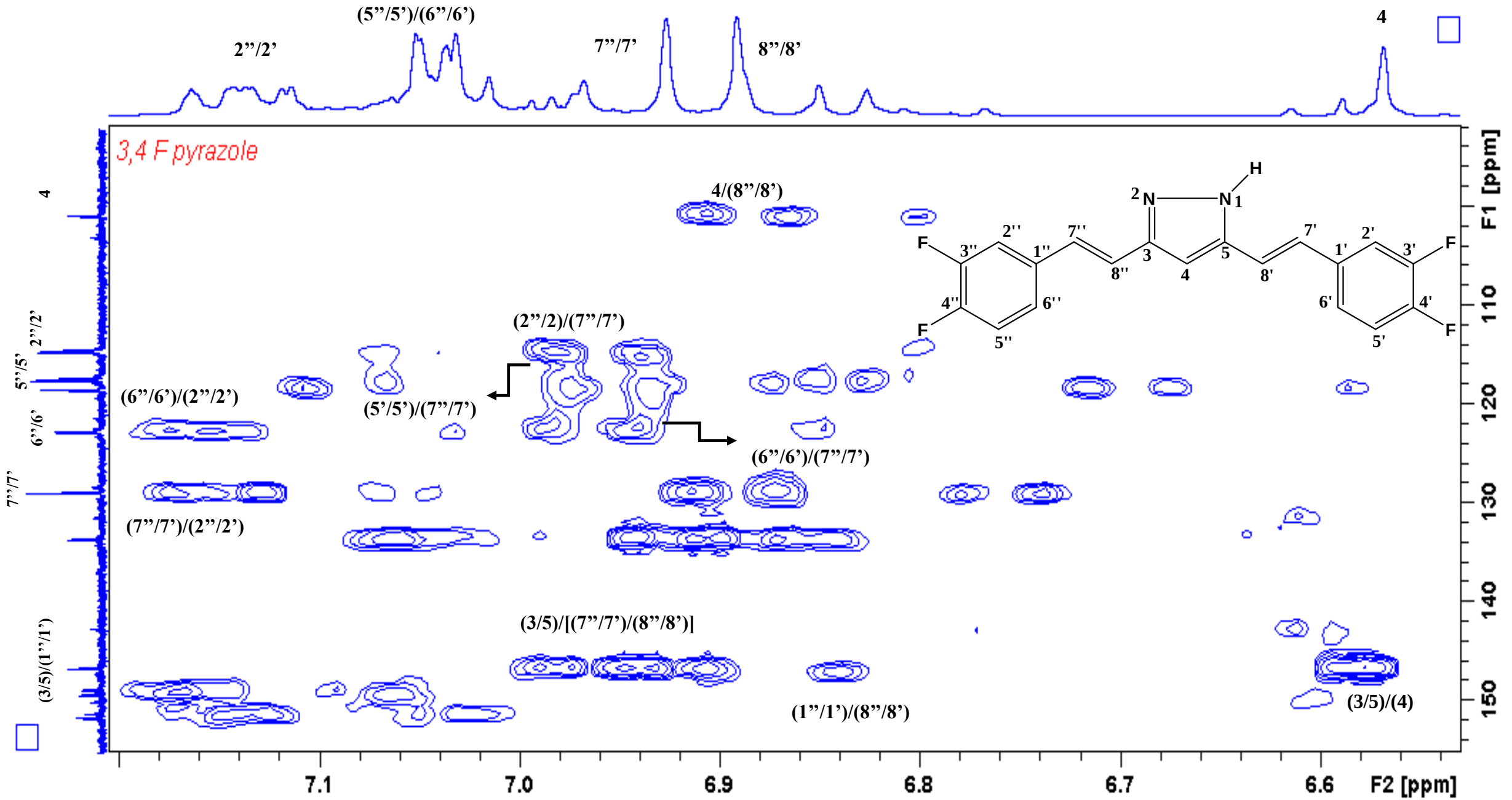


COSY of Compound A-5h: 3,5-bis(3,4-difluorostyryl)-1H-pyrazole



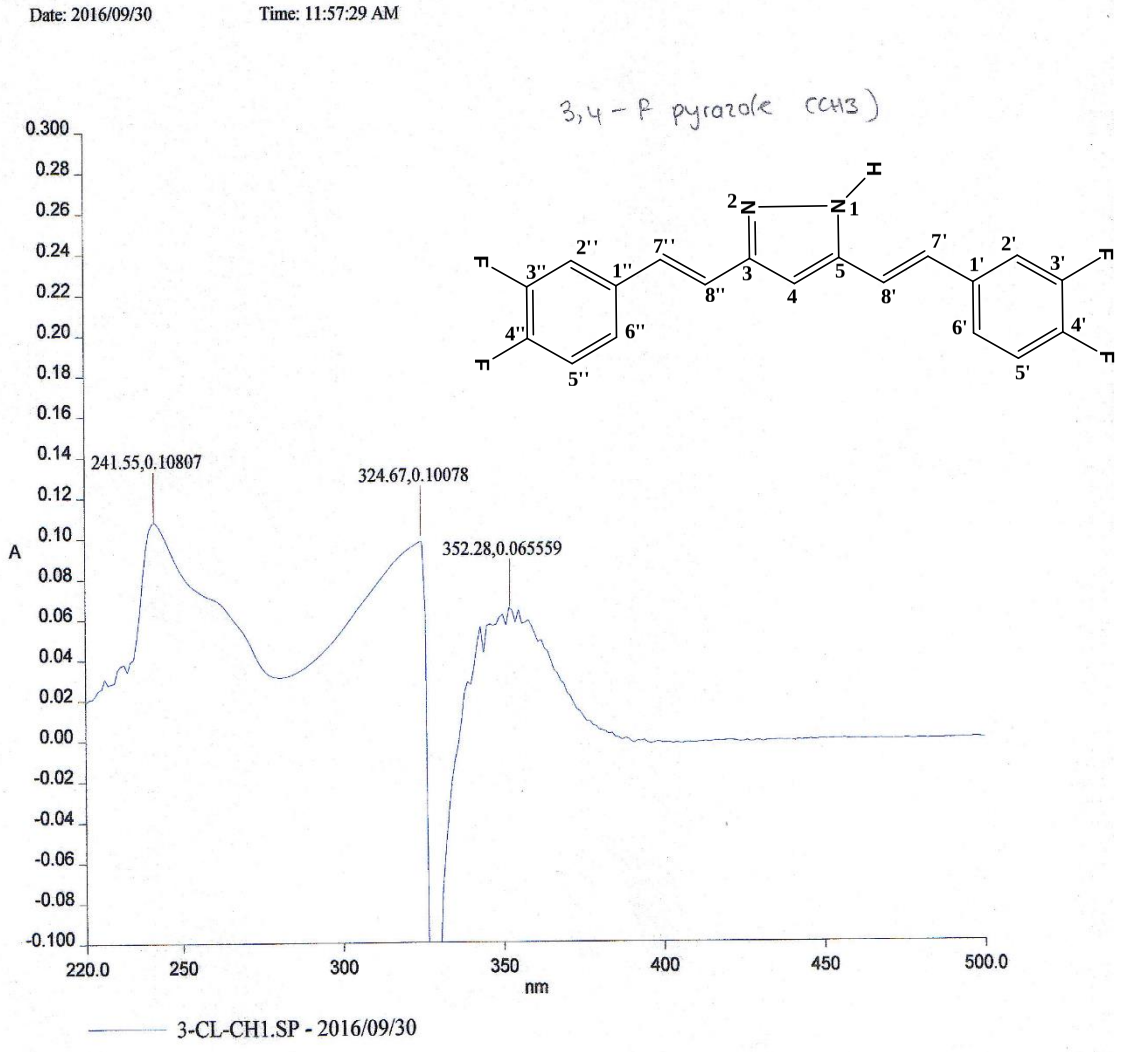
175

HSQC of Compound A-5h: 3,5-bis(3,4-difluorostyryl)-1H-pyrazole



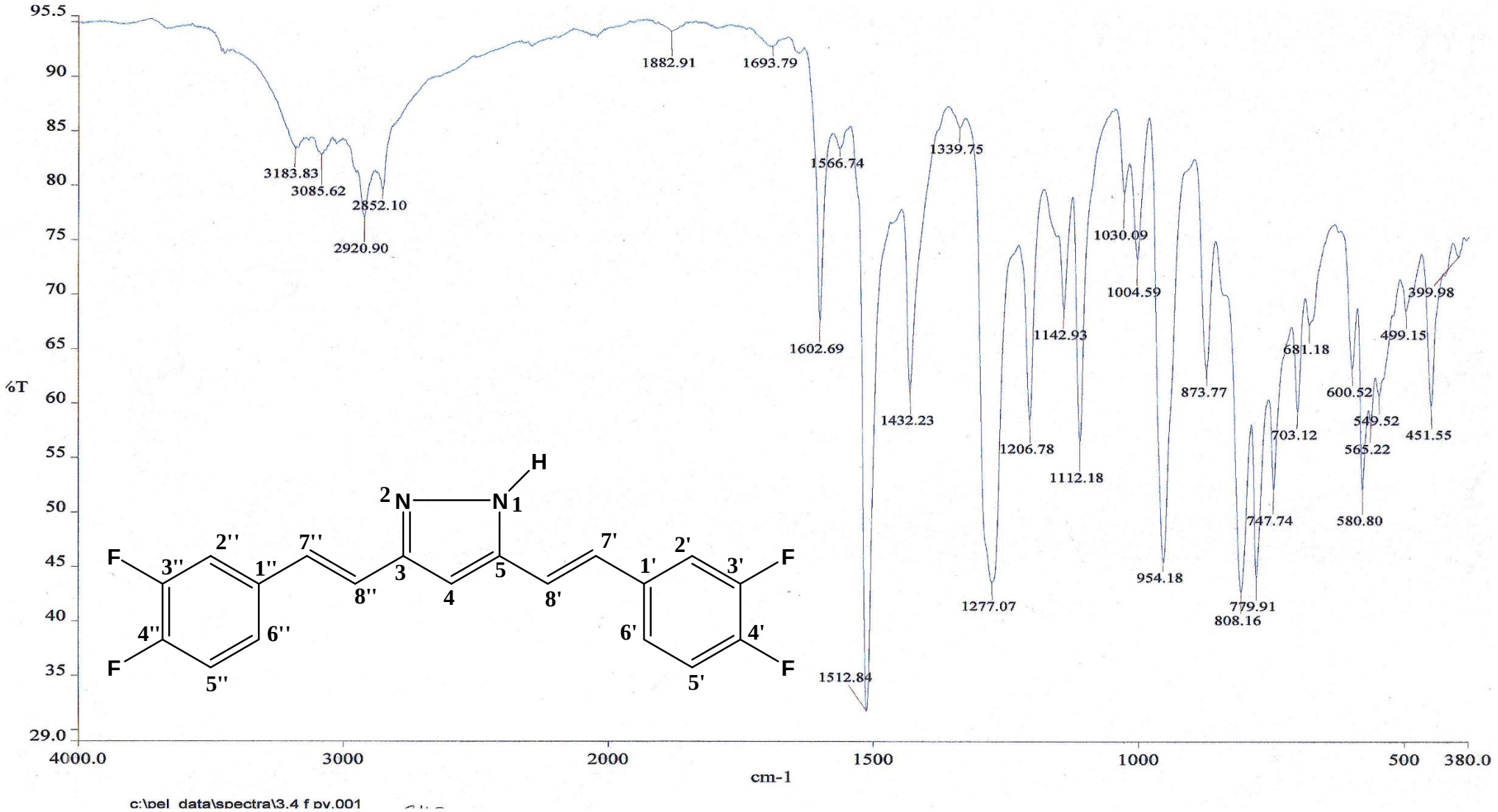
176

HMBC of Compound A-5h: 3,5-bis(3,4-difluorostyryl)-1H-pyrazole



Ultraviolet Spectrum of Compound A-5h: 3,5-bis(3,4-difluorostyryl)-1H-pyrazole

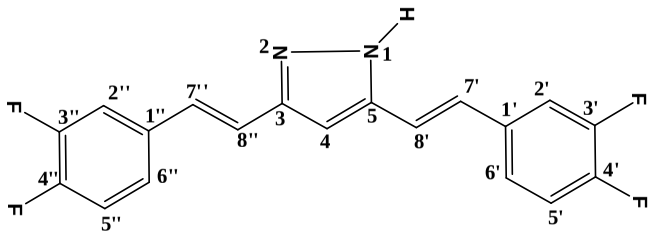
3,4 F



Infrared Spectrum of Compound A-5h: 3,5-bis(3,4-difluorostyryl)-1H-pyrazole

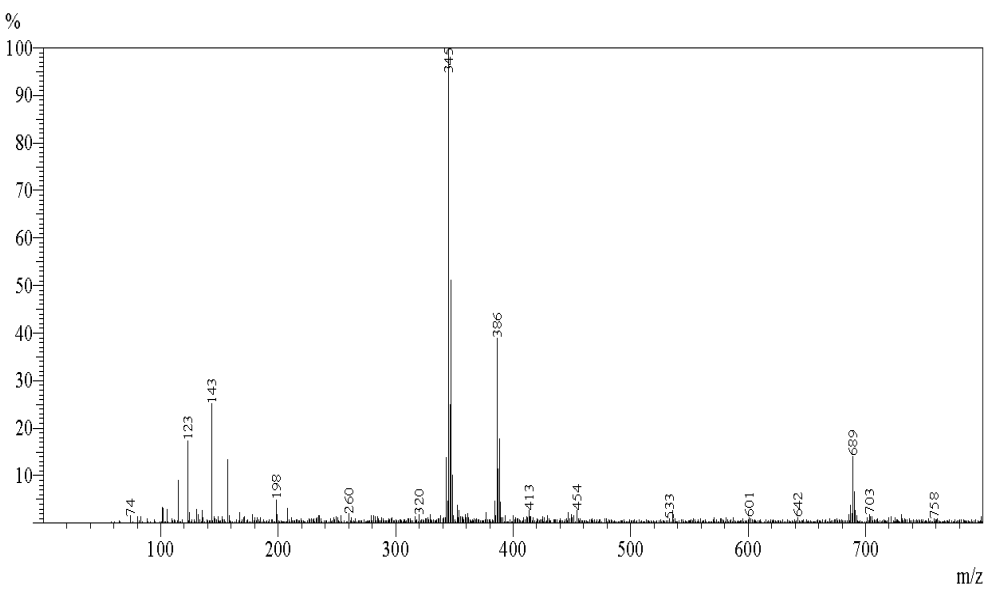
Sample Information

Date Acquired	: 2016/10/04 11:22:18 AM
Sample Type	: Unknown
Sample Name	: 10 right
Sample ID	: 10 right
Data File	: 10right1.lcd
Method File	: no column run.lcm
Report Format File	: DEFAULT.lsr
Tuning File	: 03022016.lct

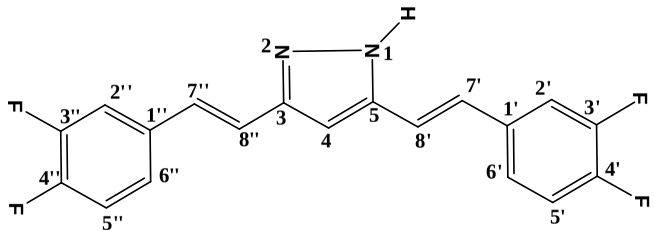


MS Spectrum

R.Time:1.067(Scan#:65)
MassPeaks:2093
Segment 1 - Event 1



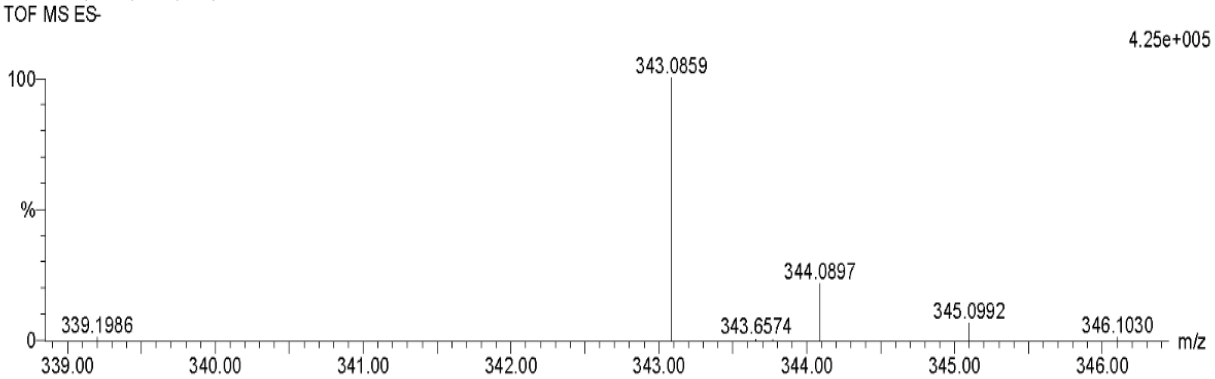
LC-MS of Compound A-5h: 3,5-bis(3,4-difluorostyryl)-1H-pyrazole



Single Mass Analysis

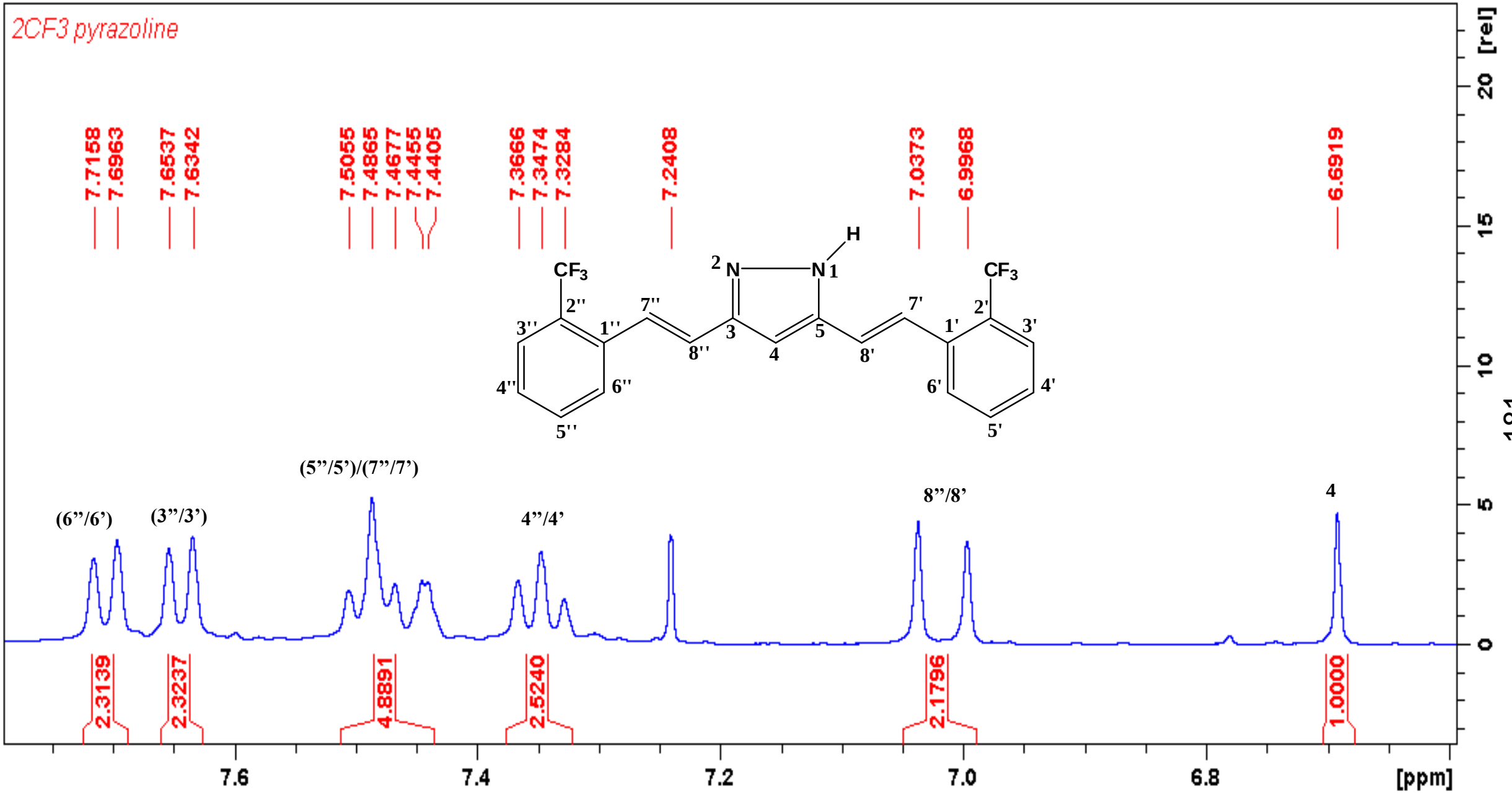
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
14 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 F: 0-5
9 34-F 61 (2.024) Cm (1:61)
TOF MS ES-

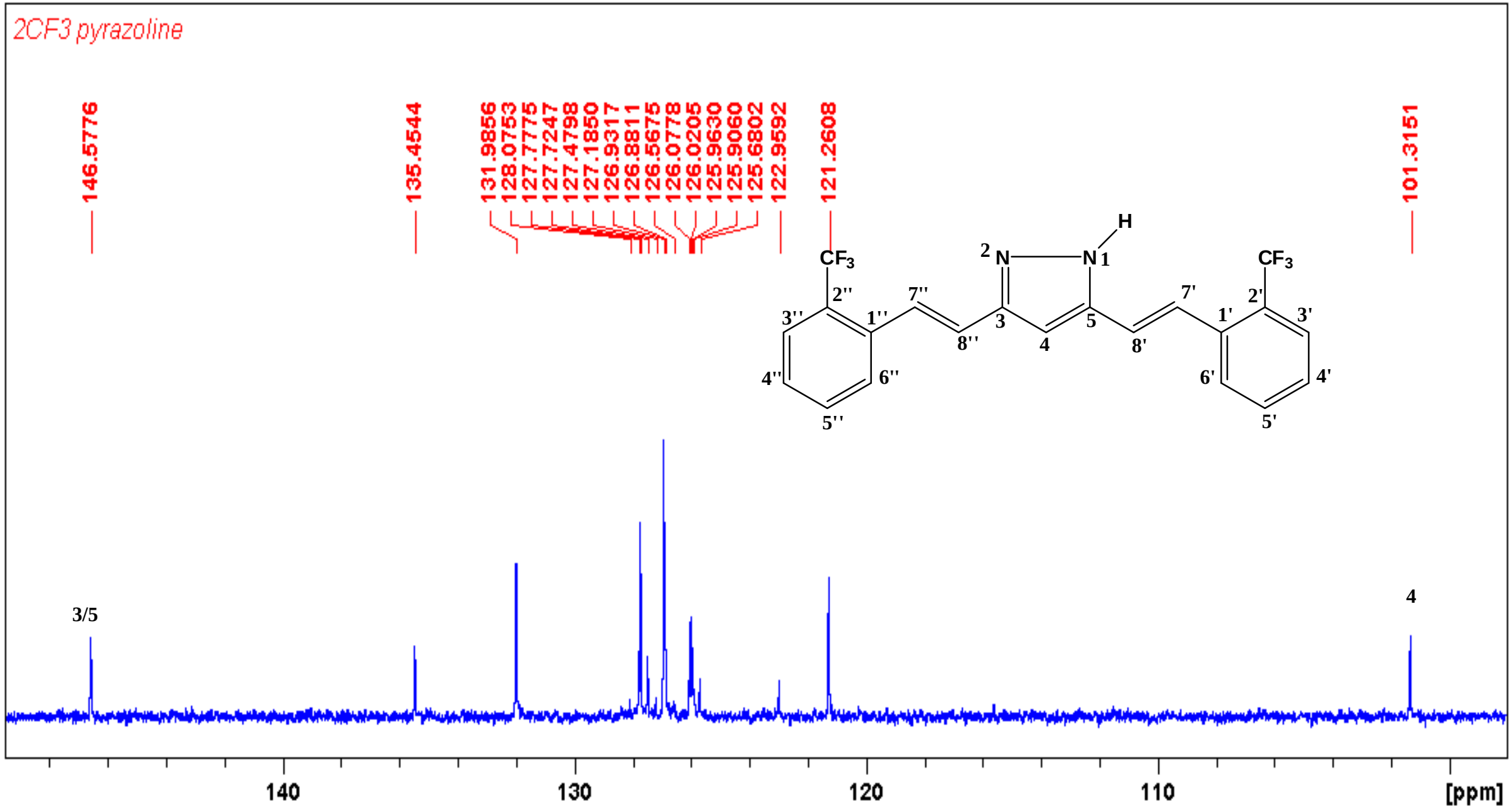


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
343.0859	343.0858	0.1	0.3	13.5	42.5	0.0	C19 H11 N2 F4

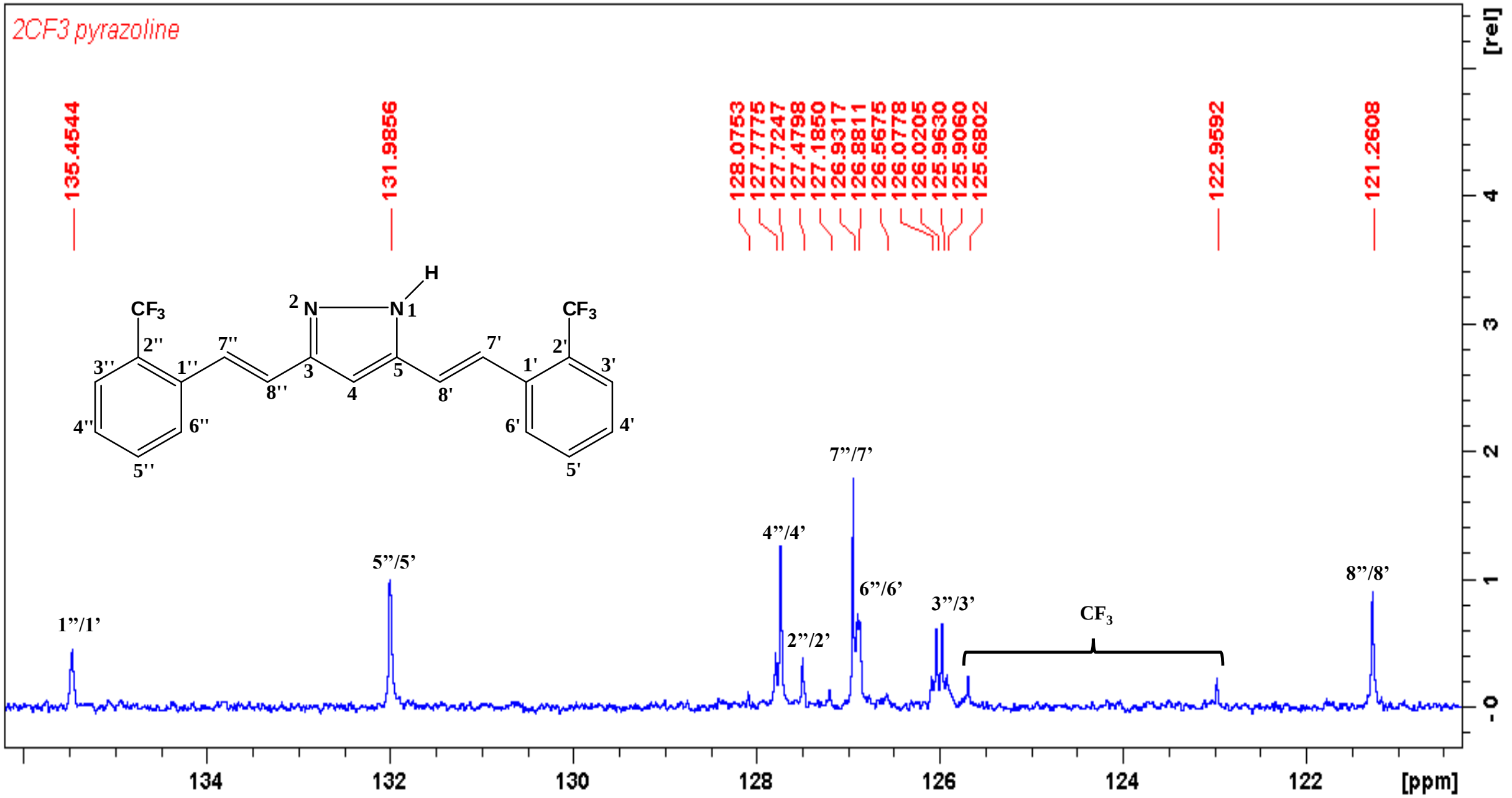
HRMS of Compound A-5h: 3,5-bis(3,4-difluorostyryl)-1H-pyrazole



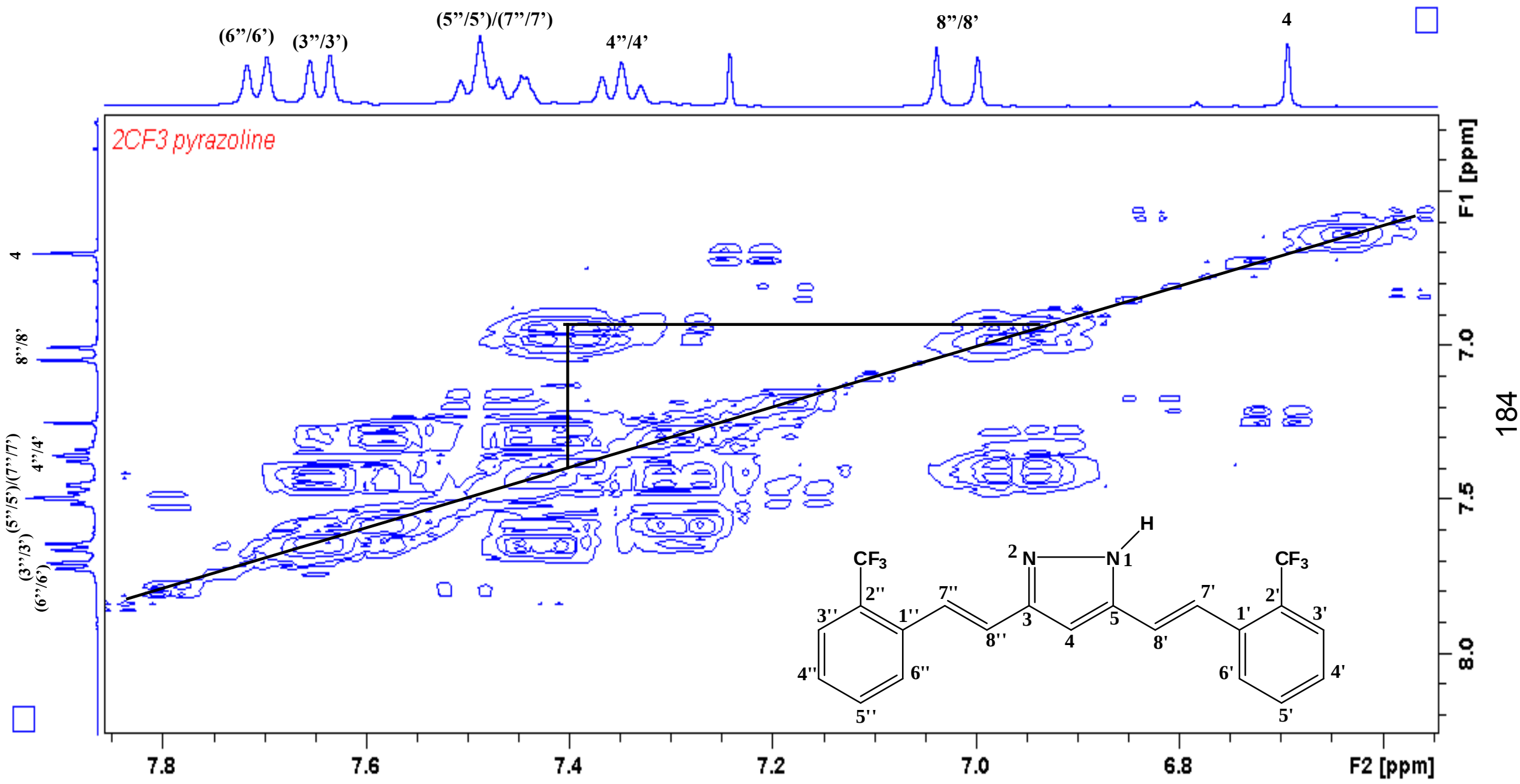
^{1}H Spectrum of Compound A-5i: 3,5-bis(2-(trifluoromethyl)styryl)-1H-pyrazole



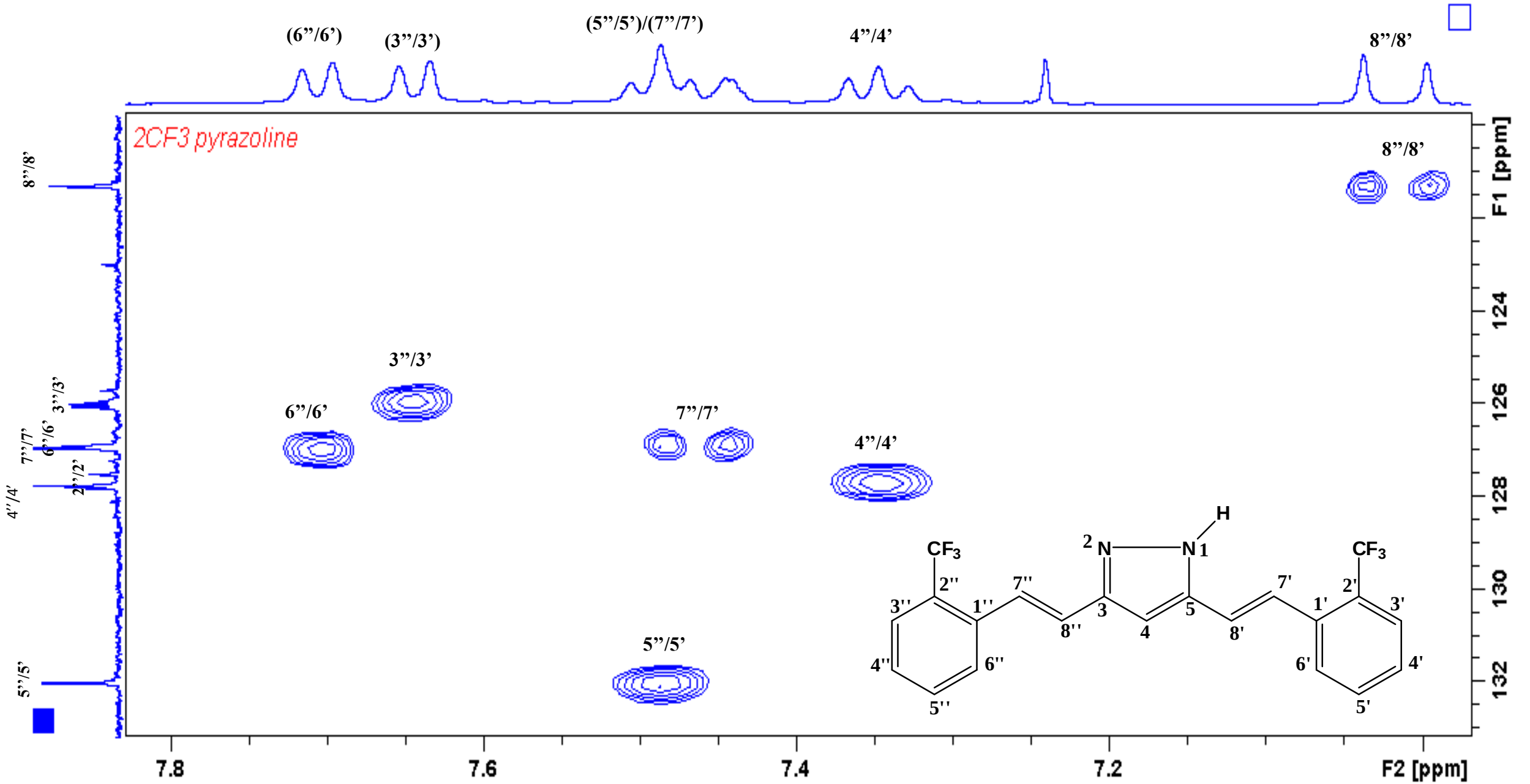
^{13}C Spectrum of Compound A-5i: 3,5-bis(2-(trifluoromethyl)styryl)-1H-pyrazole



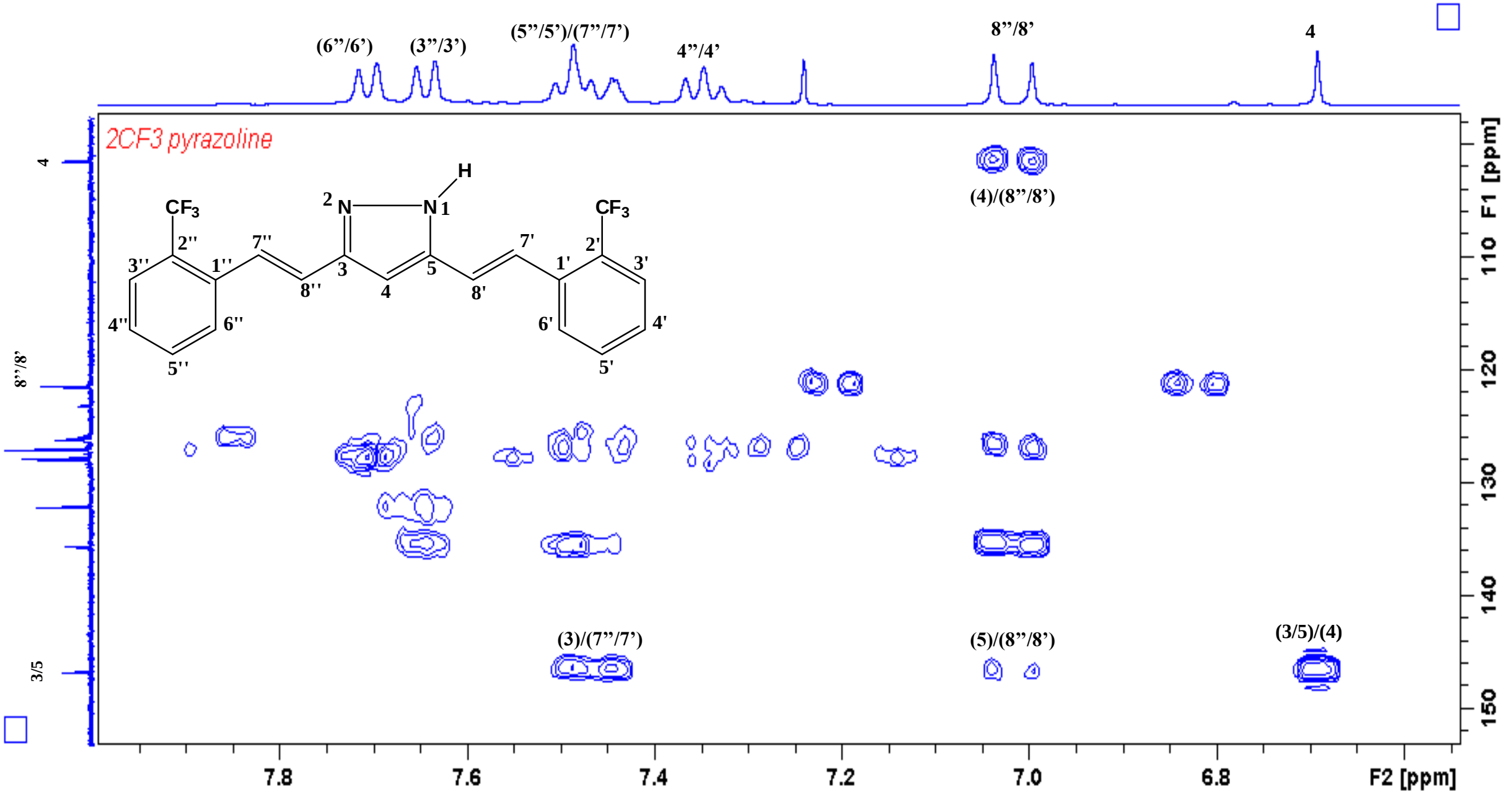
Expanded ^{13}C Spectrum of Compound A-5i: 3,5-bis(2-(trifluoromethyl)styryl)-1H-pyrazole



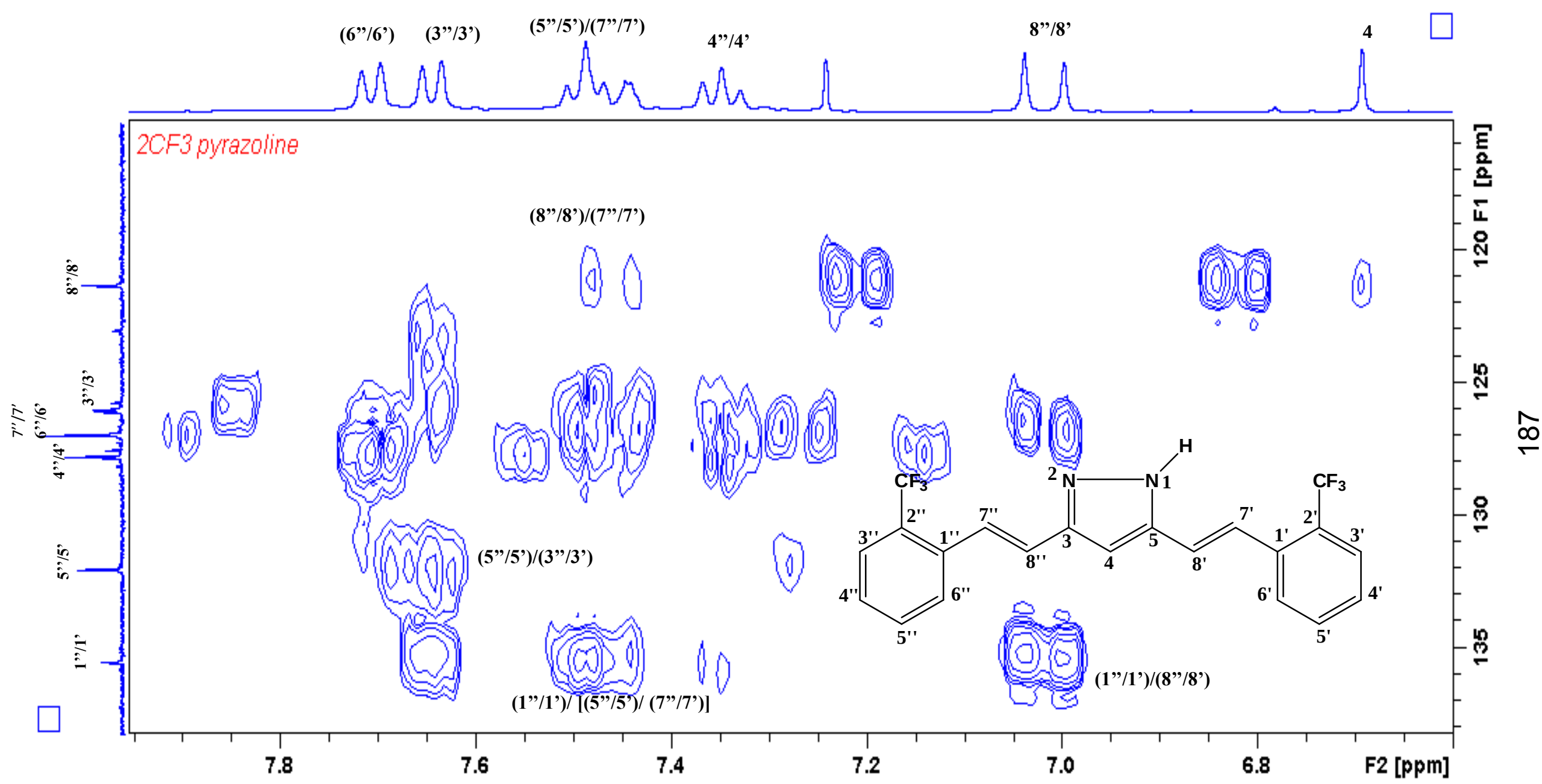
COSY of Compound A-5i: 3,5-bis(2-(trifluoromethyl)styryl)-1H-pyrazole



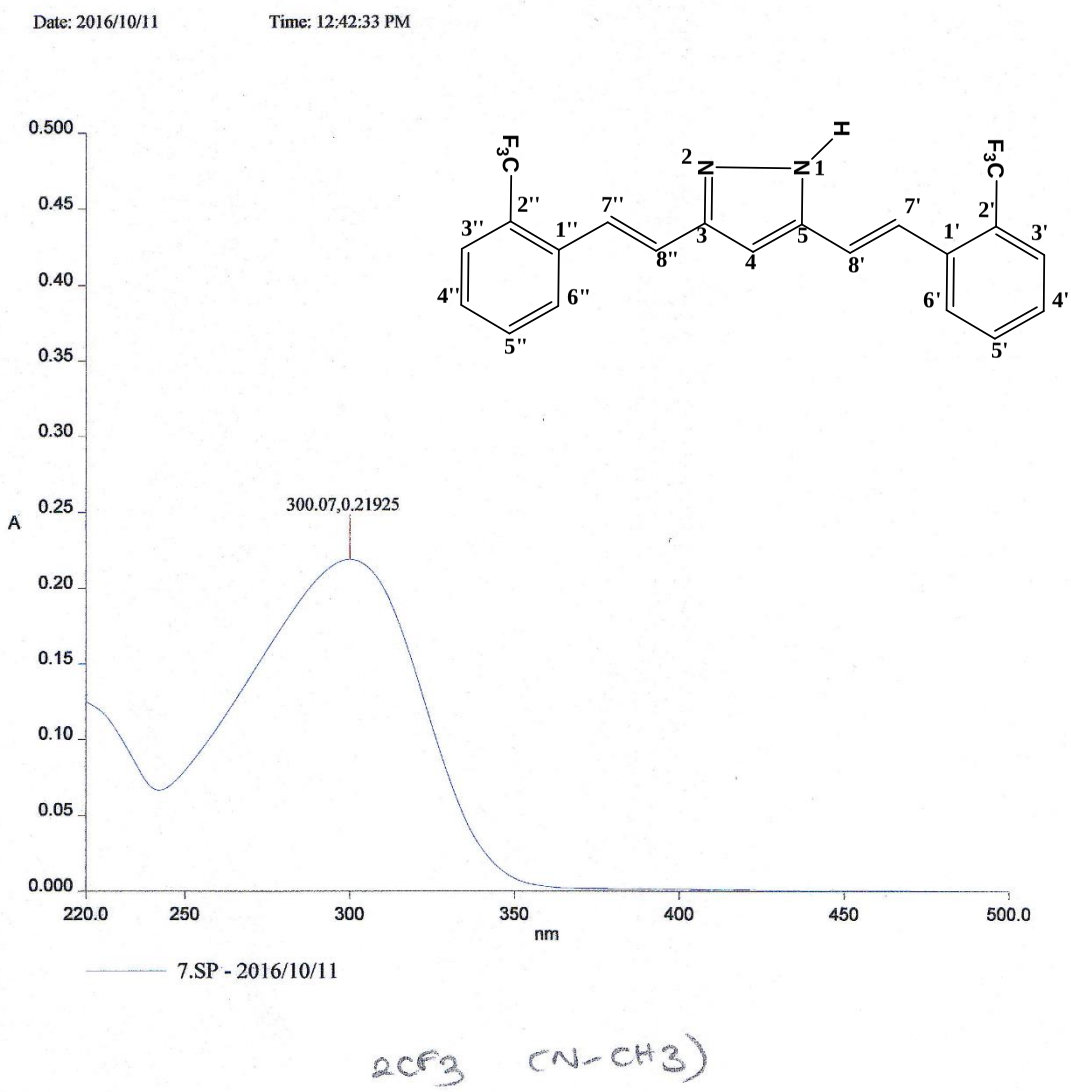
HSQC of Compound A-5i: 3,5-bis(2-(trifluoromethyl)styryl)-1H-pyrazole



HMBC of Compound A-5i: 3,5-bis(2-(trifluoromethyl)styryl)-1H-pyrazole

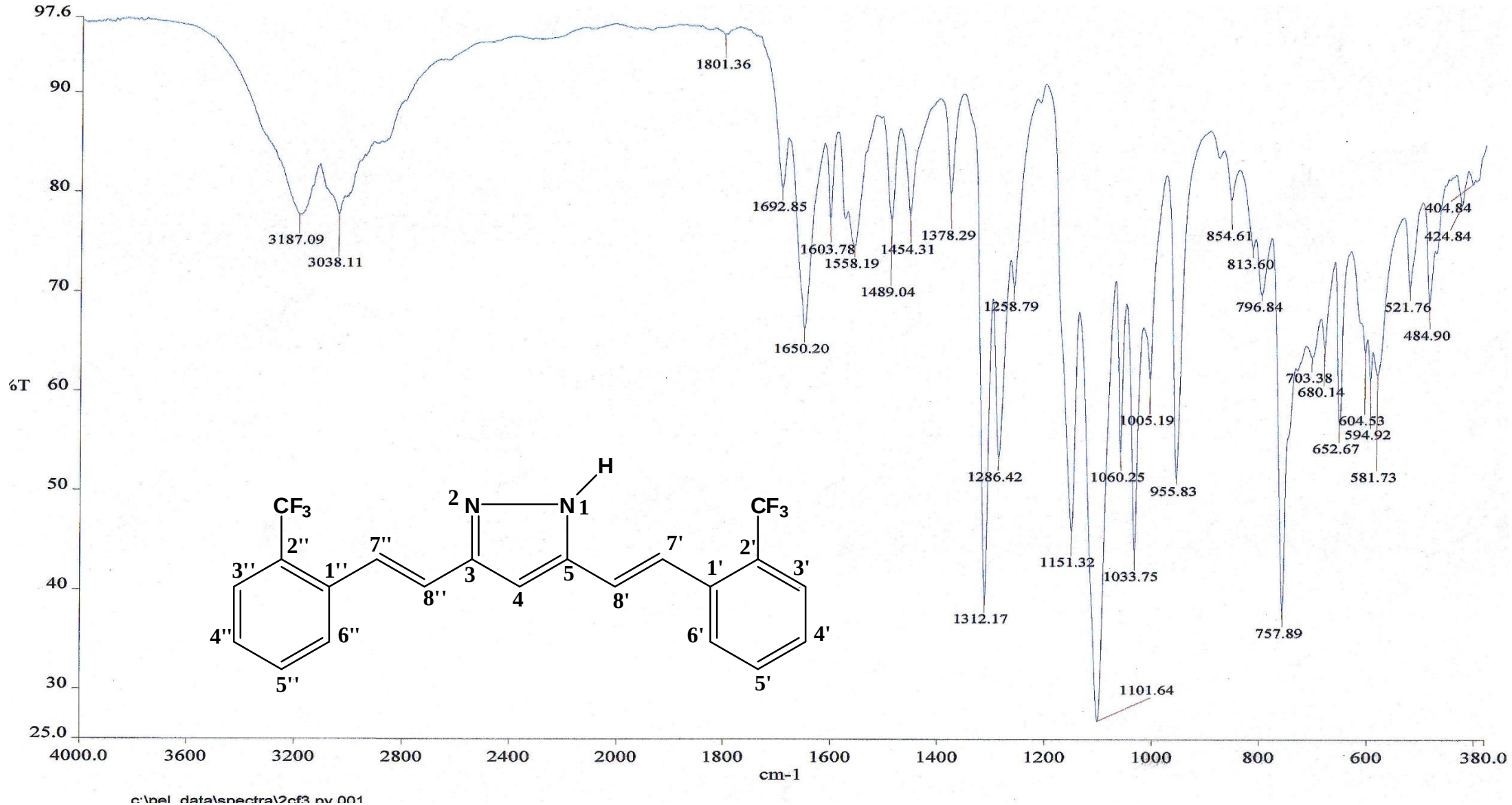


Expanded HMBC of Compound 3,5-bis(2-(trifluoromethyl)styryl)-1H-pyrazole



2CF3 (N-CH3)

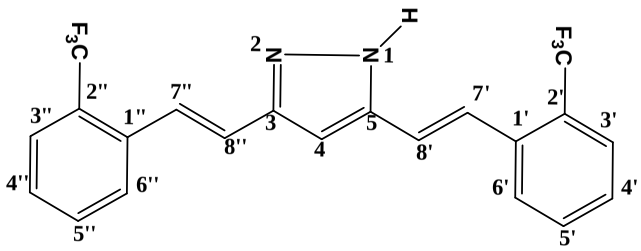
Ultraviolet Specrum of Compound A-5i: 3,5-bis(2-(trifluoromethyl)styryl)-1H-pyrazole



Infrared Spectrum of Compound A-5i: 3,5-bis(2-(trifluoromethyl)styryl)-1H-pyrazole

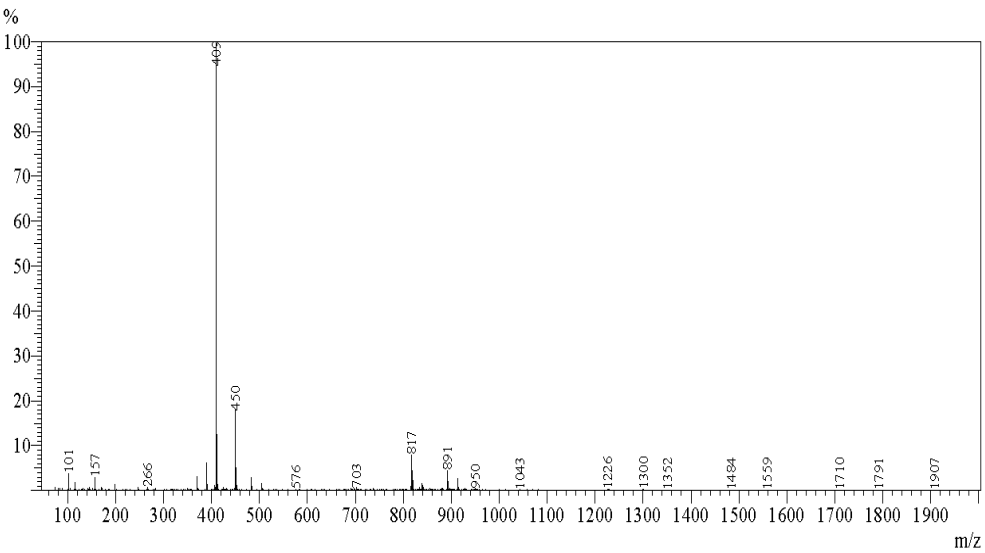
Sample Information

Date Acquired	: 2016/09/30 02:24:16 PM
Sample Type	: Unknown
Sample Name	: 4
Sample ID	: 4
Data File	: 4.lcd
Method File	: no column run.lcm
Report Format File	: DEFAULT.lsr
Tuning File	: 03022016.lct



MS Spectrum

R.Time:1.067(Scan#:65)
MassPeaks:2054
Segment 1 - Event 1

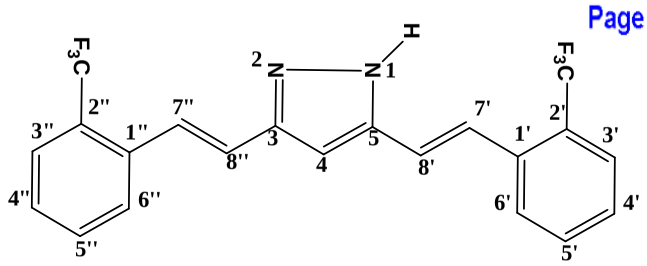


LC-MS of Compound A-5i: 3,5-bis(2-(trifluoromethyl)styryl)-1H-pyrazole

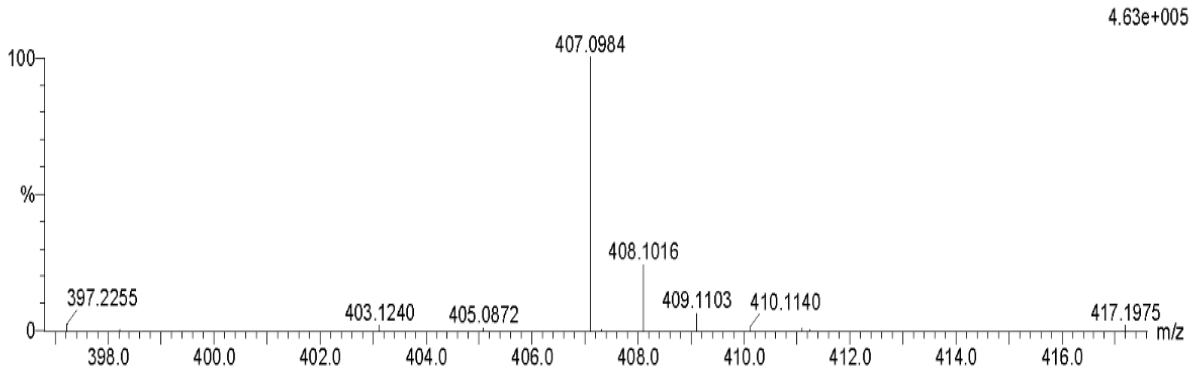
Elemental Composition Report Page 1

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

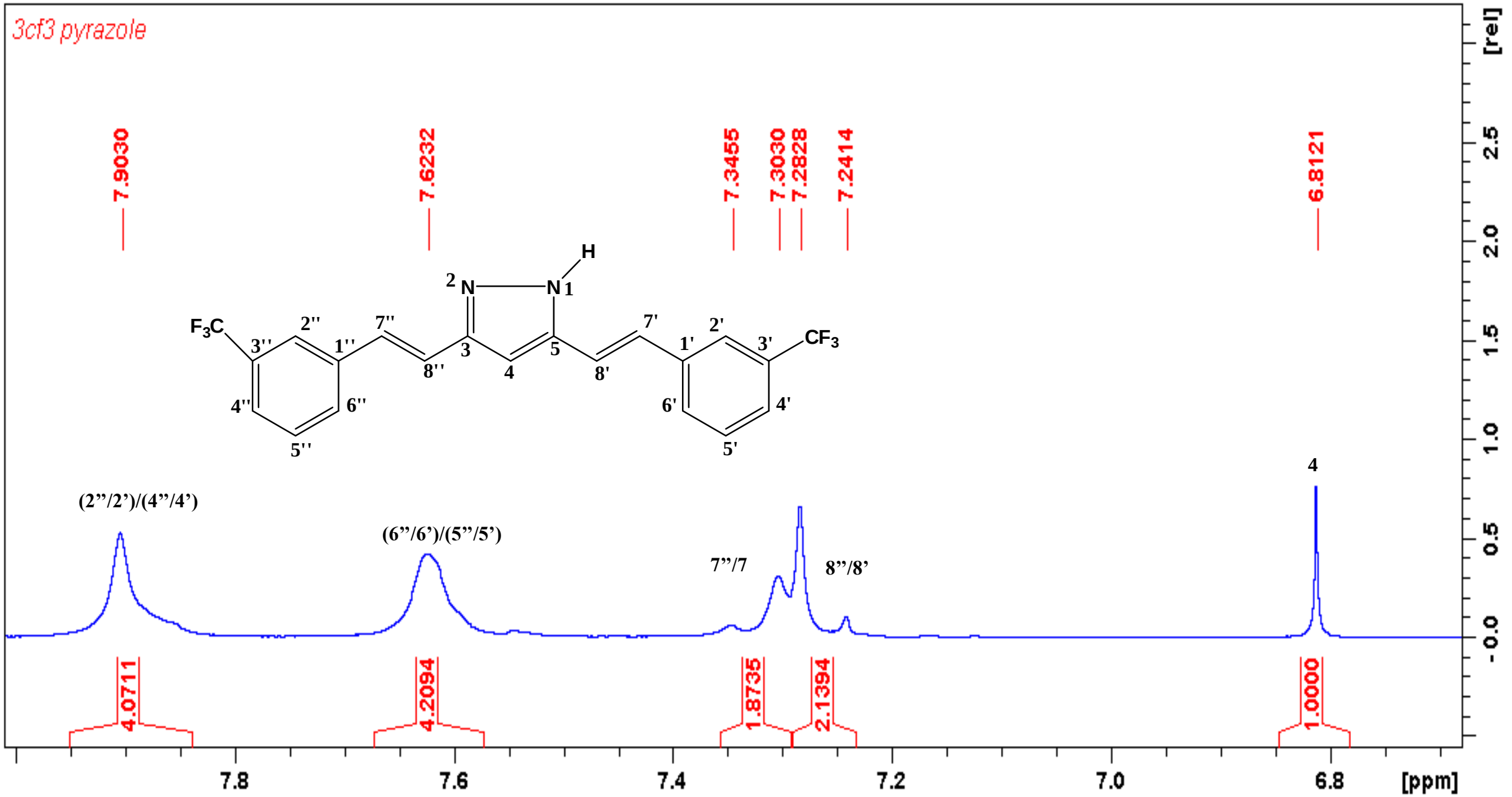


Monoisotopic Mass, Even Electron Ions
26 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 20-25 H: 10-15 N: 0-5 F: 5-10
4 2-CF3 57 (1.890) Cm (1:61)
TOF MS ES-

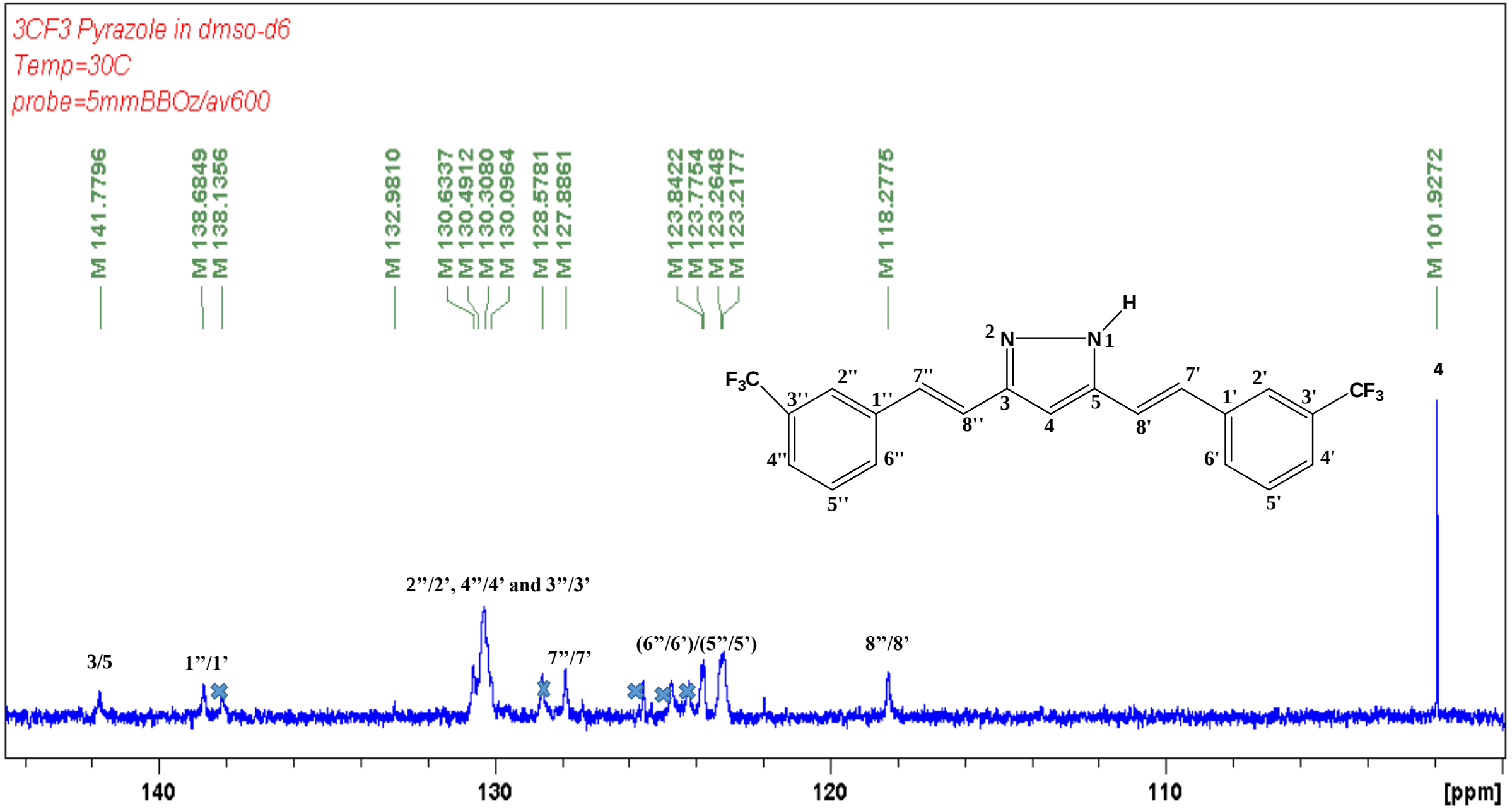


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
407.0984	407.0983	0.1	0.2	13.5	25.7	0.0	C21 H13 N2 F6

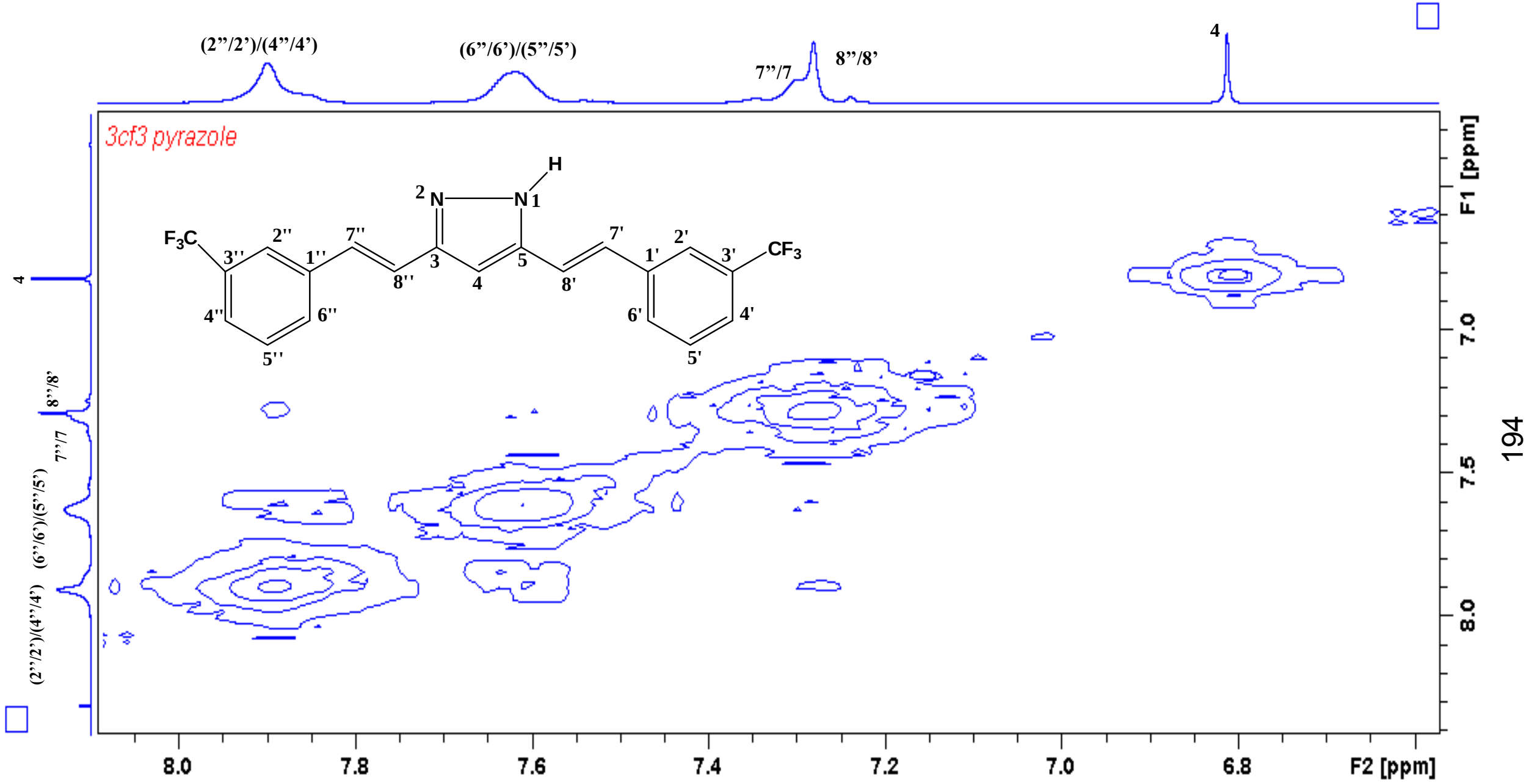
HRMS of Compound A-5i: 3,5-bis(2-(trifluoromethyl)styryl)-1H-pyrazole



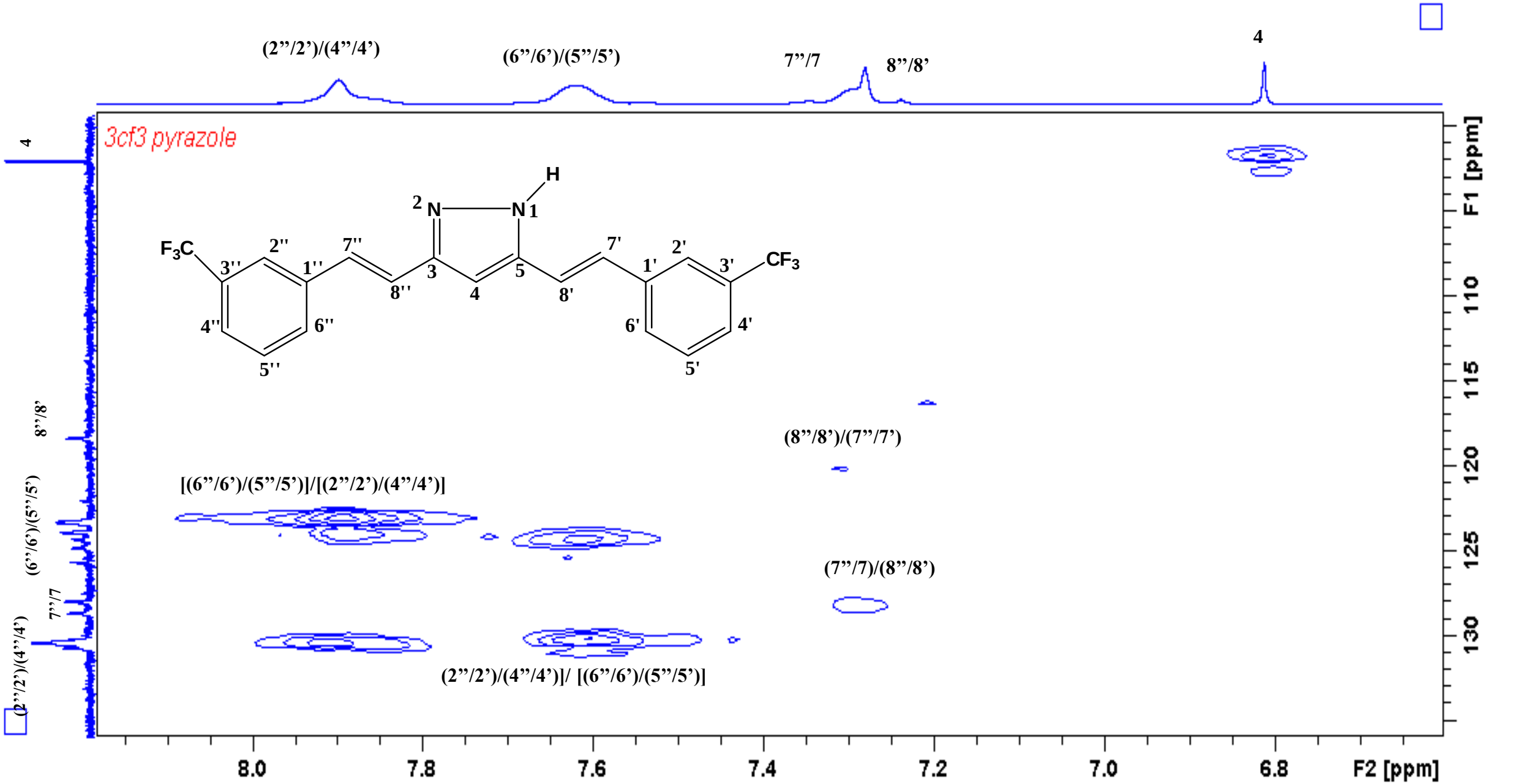
^{1}H Spectrum of Compound A-5j: 1-(3,5-bis(3-(trifluoromethyl)styryl)-1H-pyrazol-1-yl)ethanone



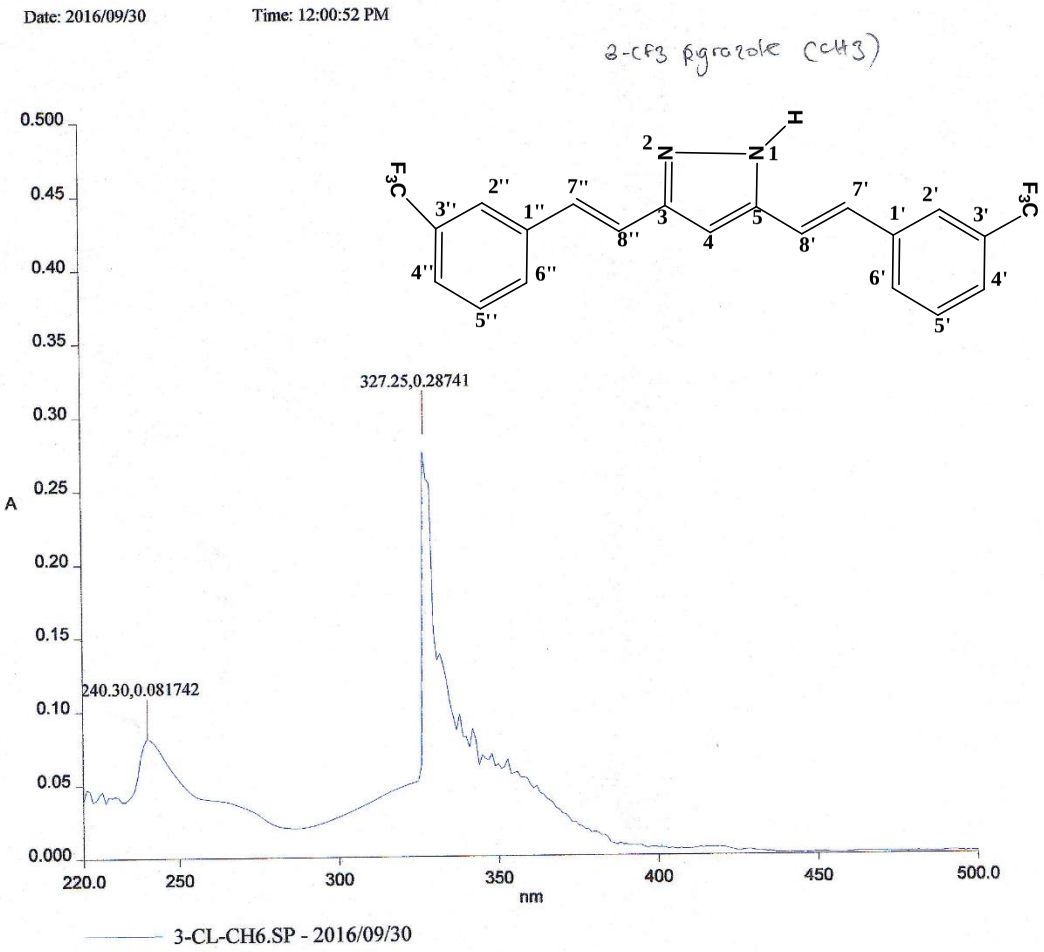
^{13}C Spectrum of Compound A-5j: 1-(3,5-bis(3-(trifluoromethyl)styryl)-1H-pyrazol-1-yl)ethanone



COSY of Compound A-5j: 1-(3,5-bis(3-(trifluoromethyl)styryl)-1H-pyrazol-1-yl)ethanone

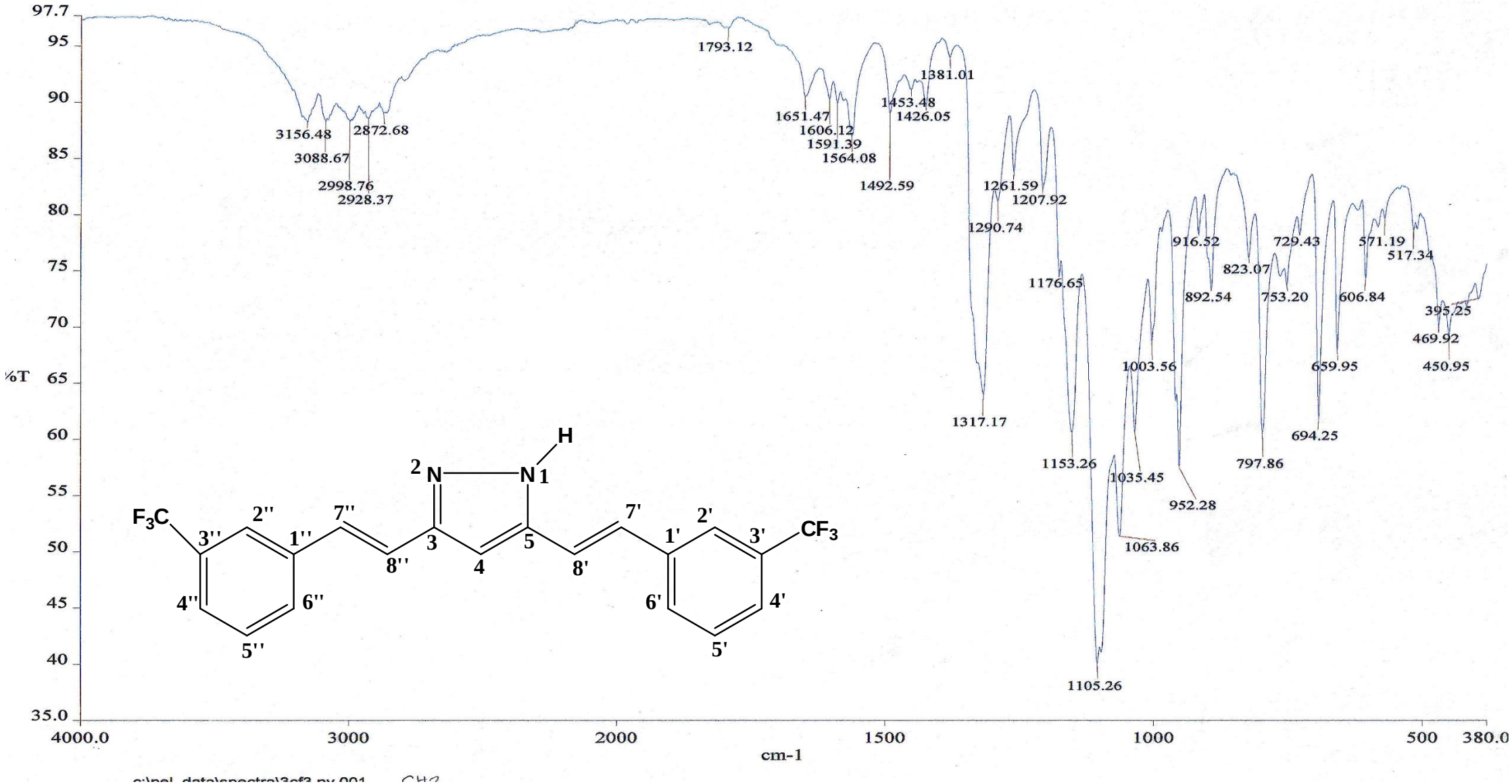


HSQC of Compound A-5j: 1-(3,5-bis(3-(trifluoromethyl)styryl)-1H-pyrazol-1-yl)ethanone



Ultraviolet Spectrum of Compound A-5j: 1-(3,5-bis(3-(trifluoromethyl)styryl)-1H-pyrazol-1-yl)ethanone

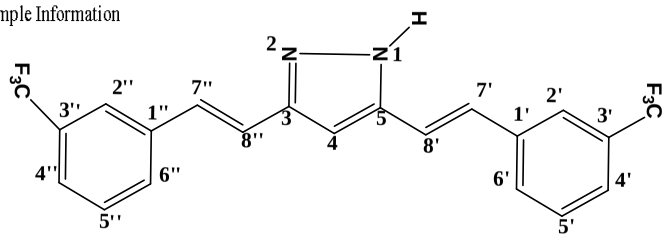
3 CF3



Infrared Spectrum of Compound A-5j: 1-(3,5-bis(3-(trifluoromethyl)styryl)-1H-pyrazol-1-yl)ethanone

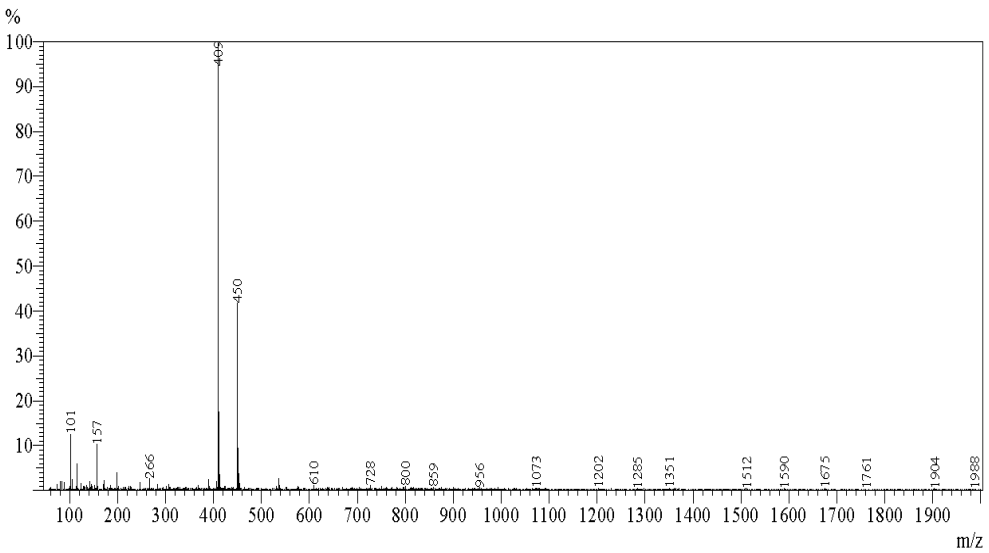
Sample Information

Date Acquired	: 2016/09/30 02:01:24 PM
Sample Type	: Unknown
Sample Name	: 5
Sample ID	: 5
Data File	: 5.lcd
Method File	: no column run.lcm
Report Format File	: DEFAULT.lsr
Tuning File	: 03022016.lct



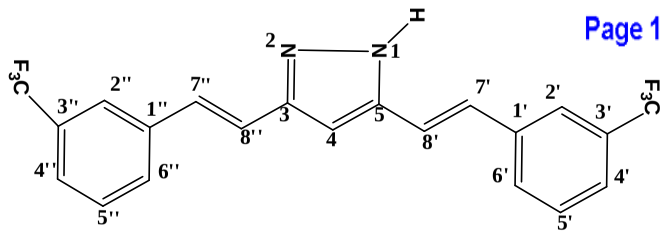
MS Spectrum

R.Time:1.067(Scan#:65)
MassPeaks:2038
Segment 1 - Event 1



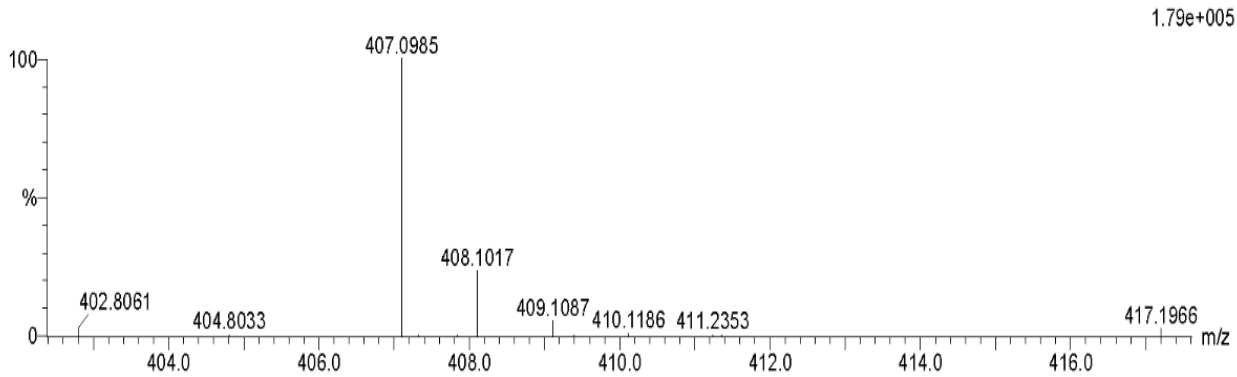
LC-MS of Compound A-5j: 1-(3,5-bis(3-(trifluoromethyl)styryl)-1H-pyrazol-1-yl)ethanone

Elemental Composition Report



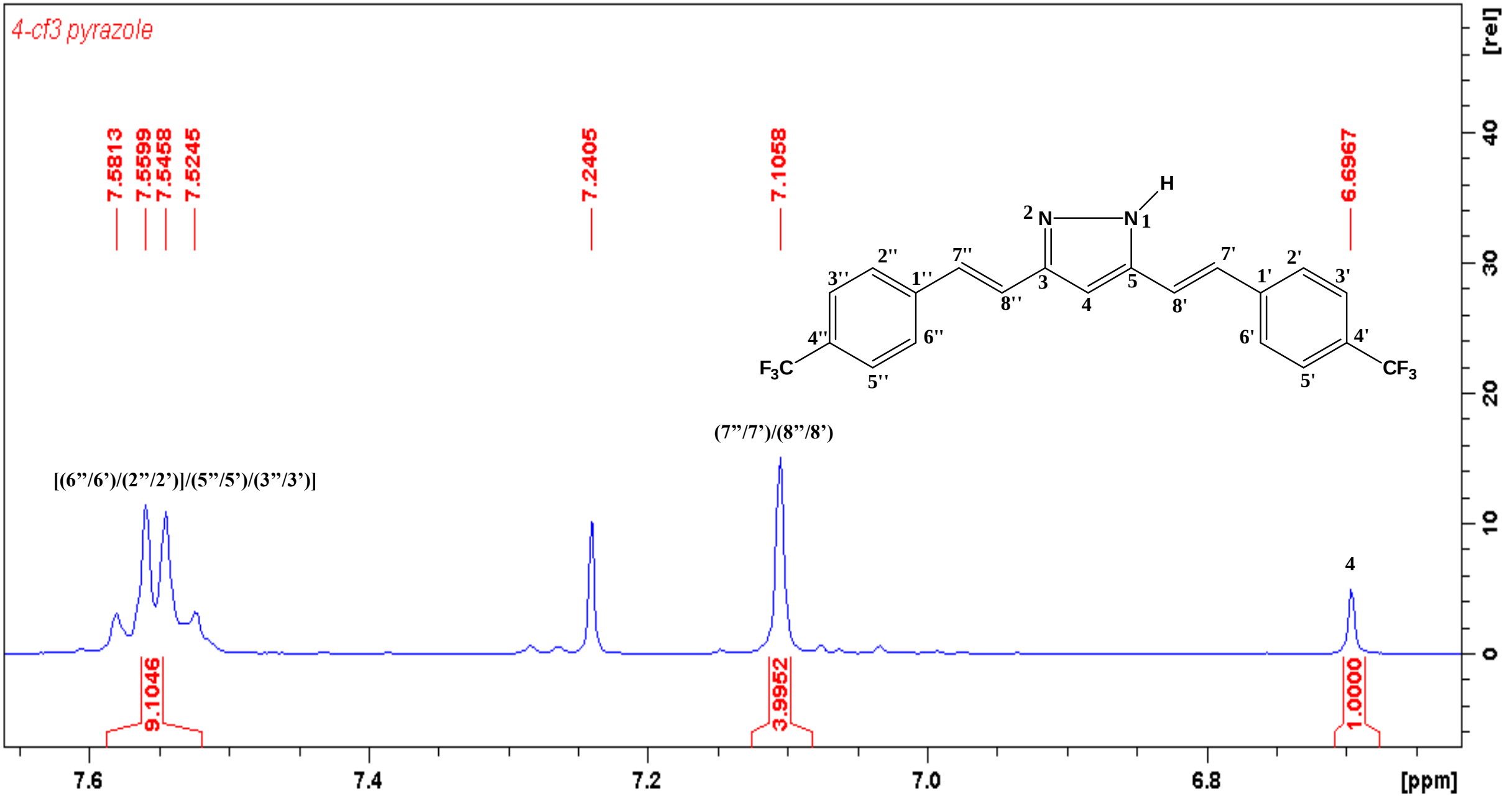
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
26 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 20-25 H: 10-15 N: 0-5 F: 5-10
5 3-CF3 54 (1.787) Cm (1:61)
TOF MS ES-

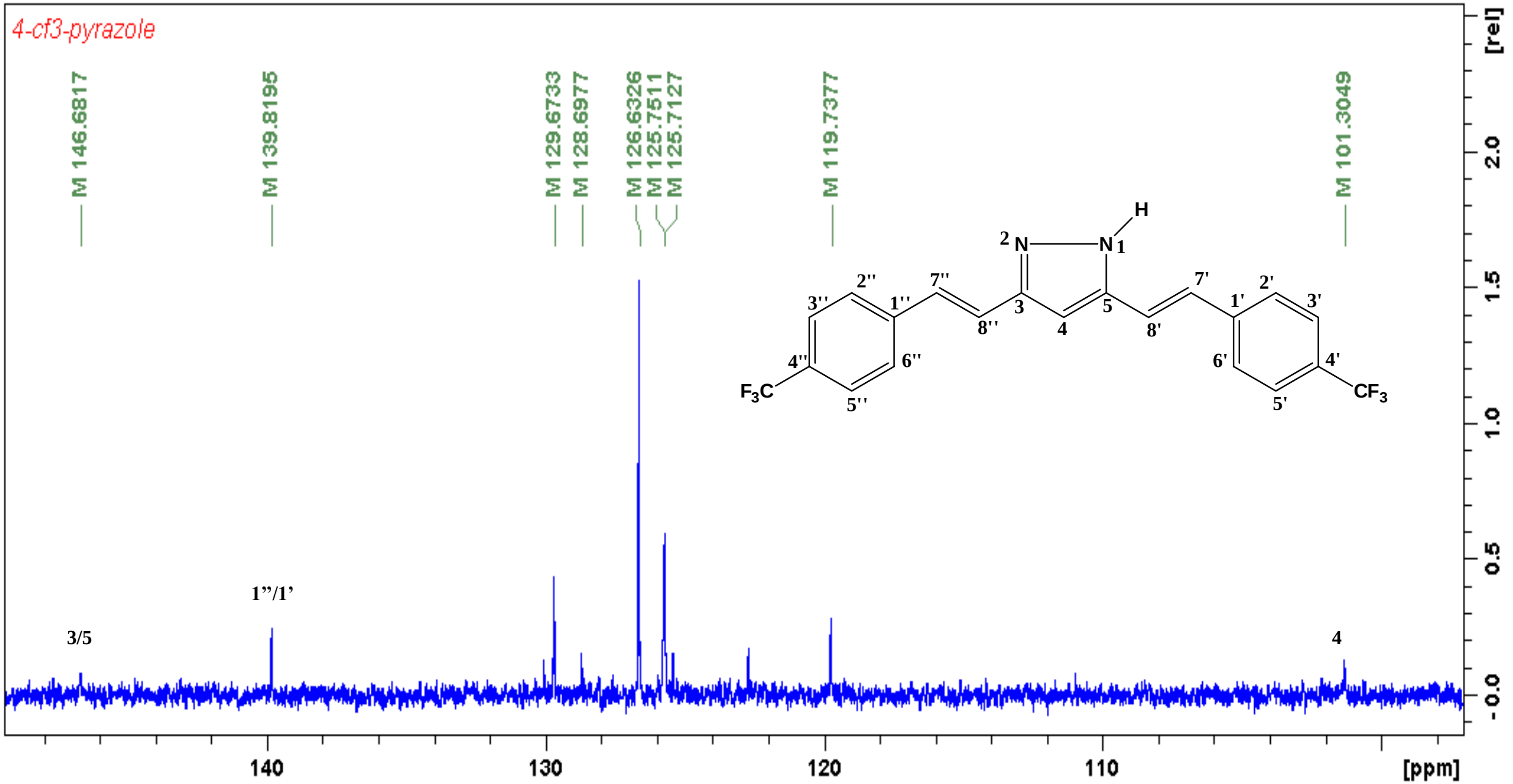


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
407.0985	407.0983	0.2	0.5	13.5	37.8	0.0	C21 H13 N2 F6

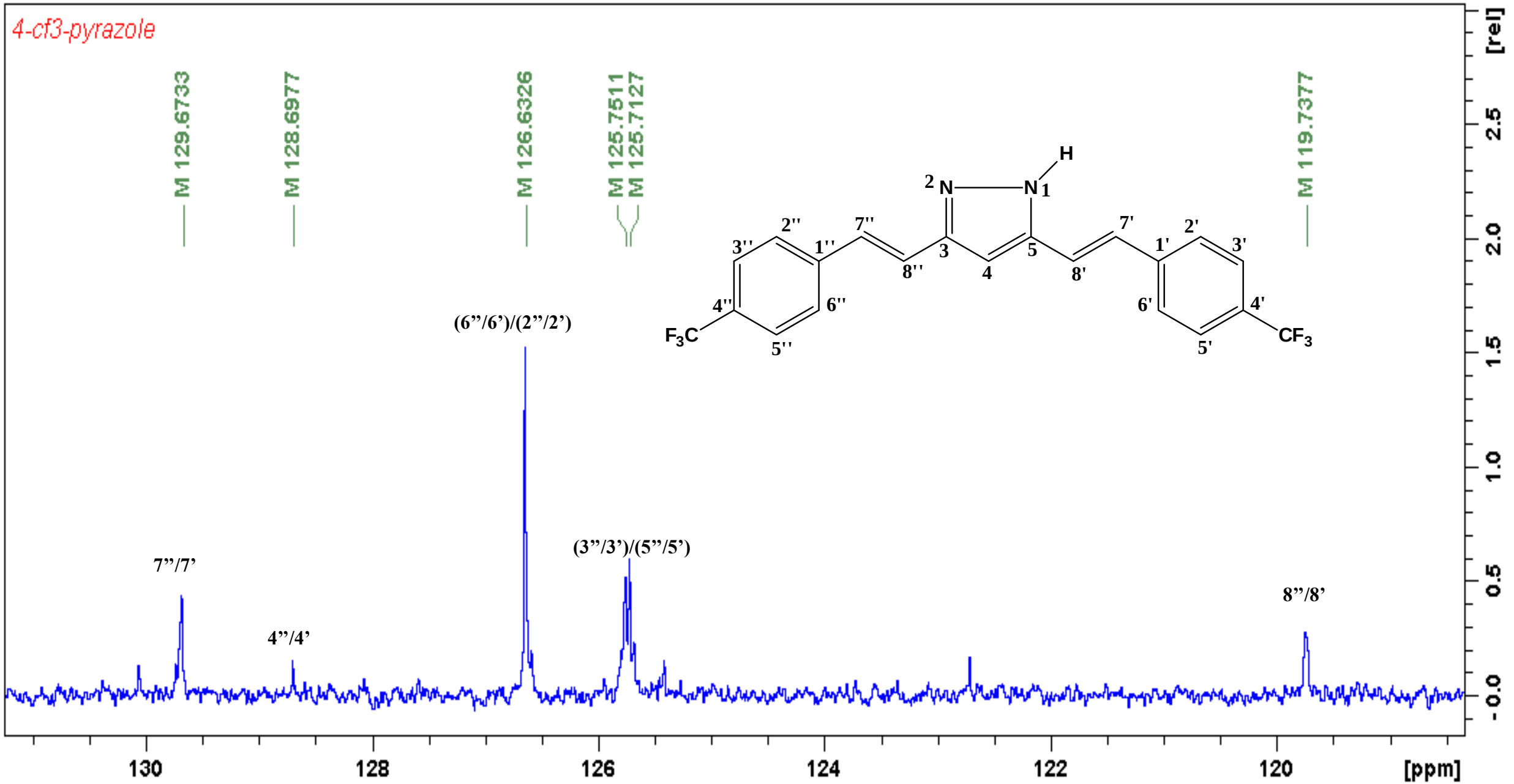
HRMS of Compound A-5j: 1-(3,5-bis(3-(trifluoromethyl)styryl)-1H-pyrazol-1-yl)ethanone



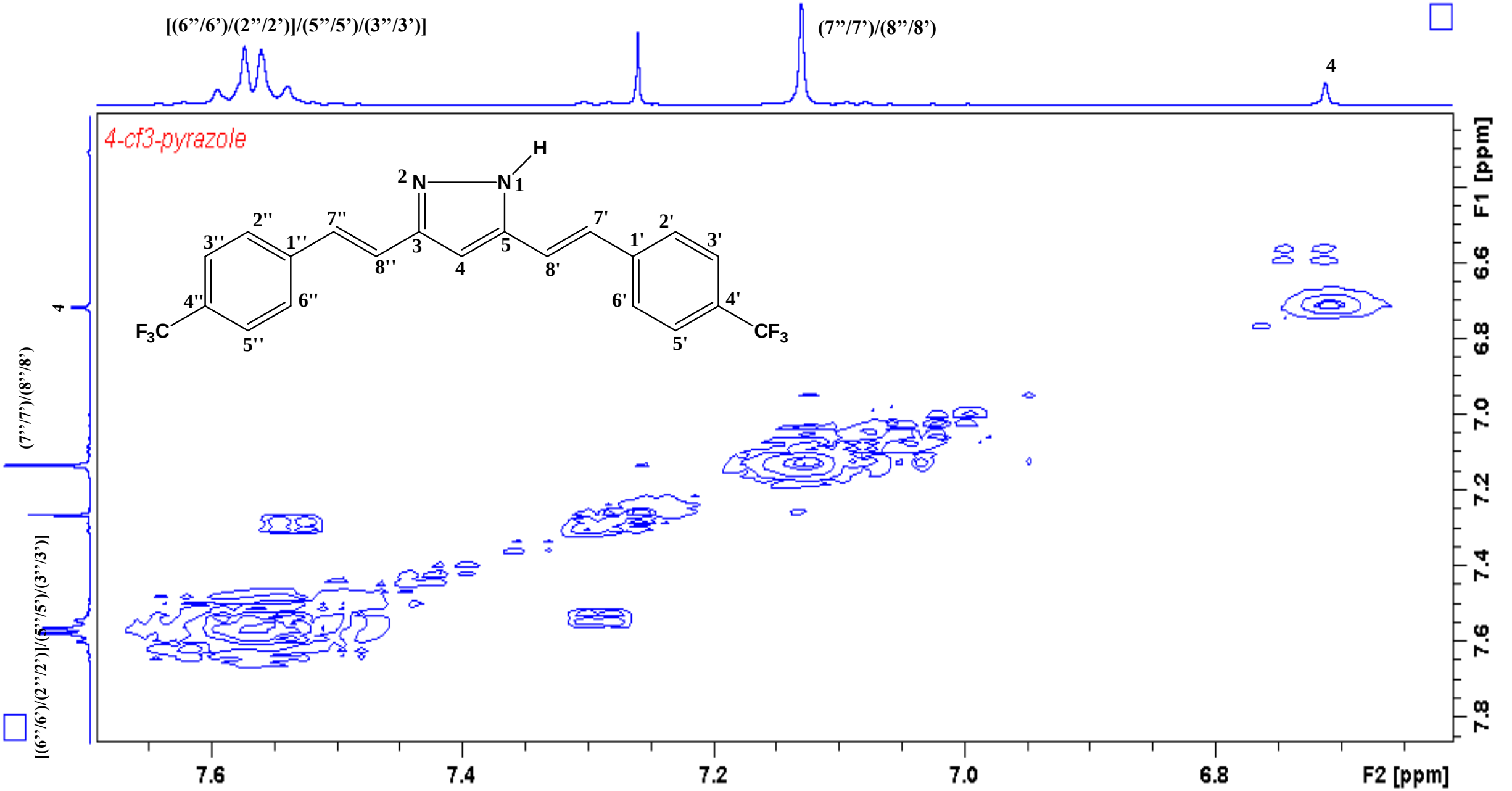
^{1}H Spectrum of Compound A-5k: 3,5-bis(4-(trifluoromethyl)styryl)-1H-pyrazole



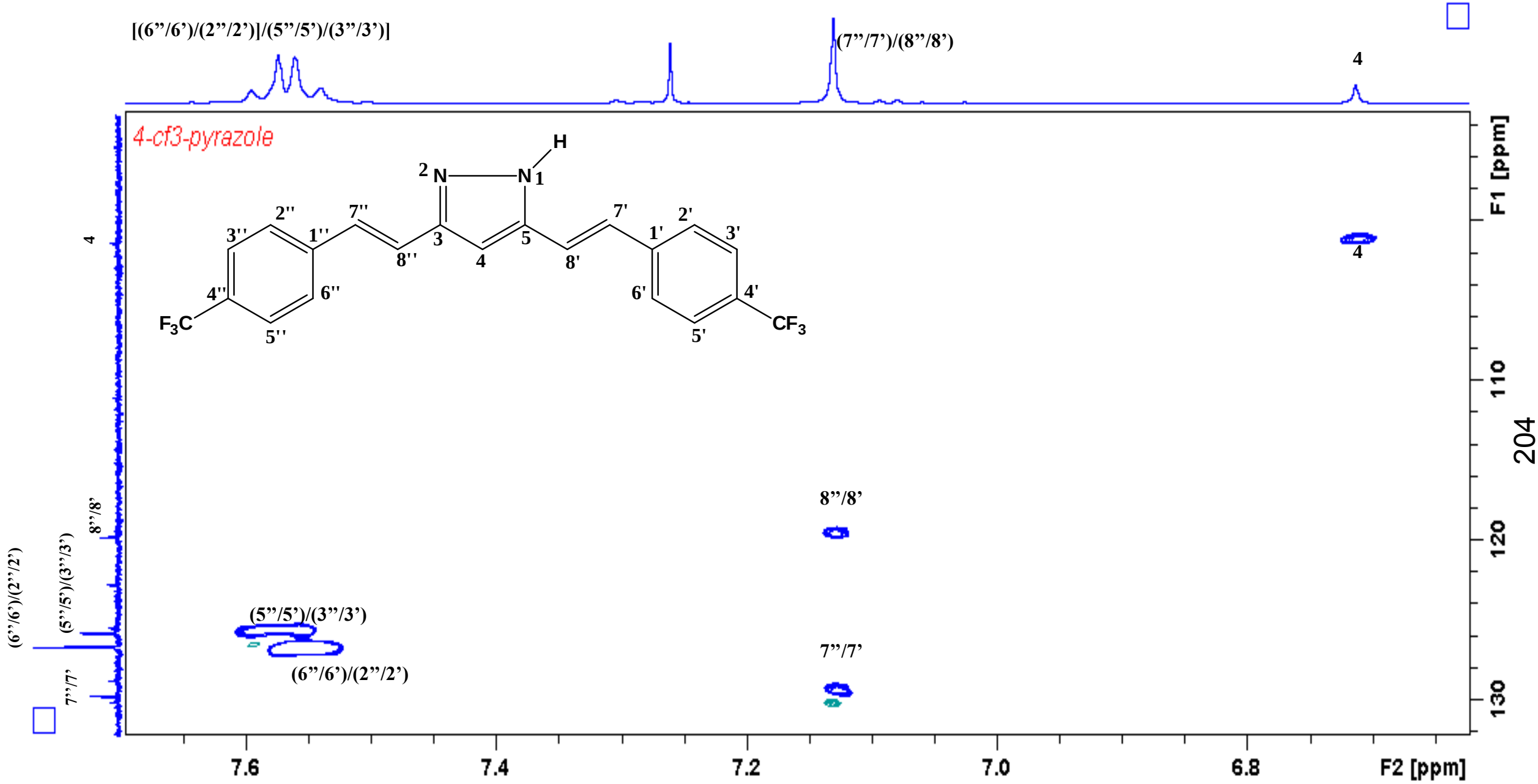
^{13}C Spectrum of Compound A-5k: 3,5-bis(4-(trifluoromethyl)styryl)-1H-pyrazole



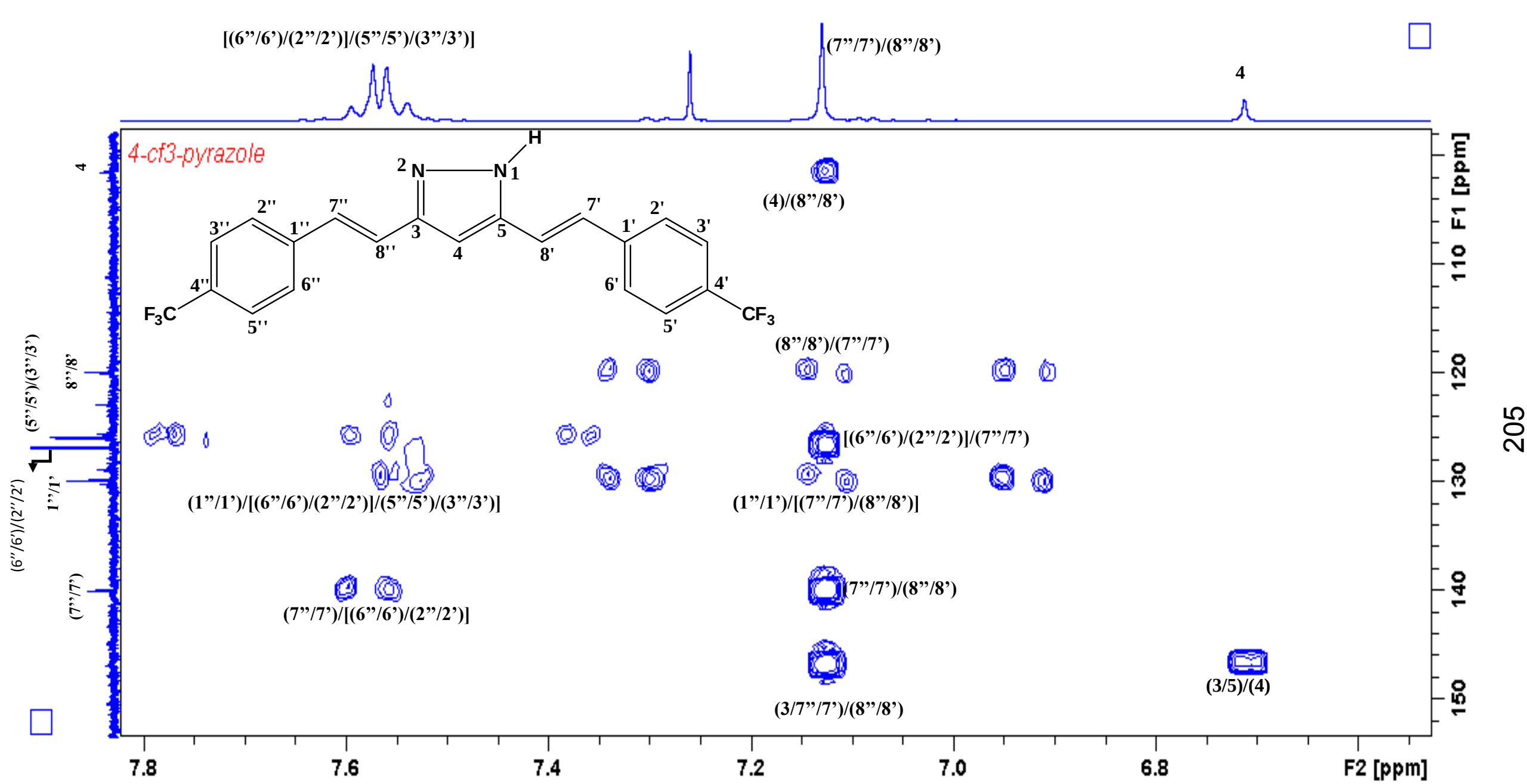
Expanded ^{13}C Spectrum of Compound A-5k: 3,5-bis(4-(trifluoromethyl)styryl)-1H-pyrazole



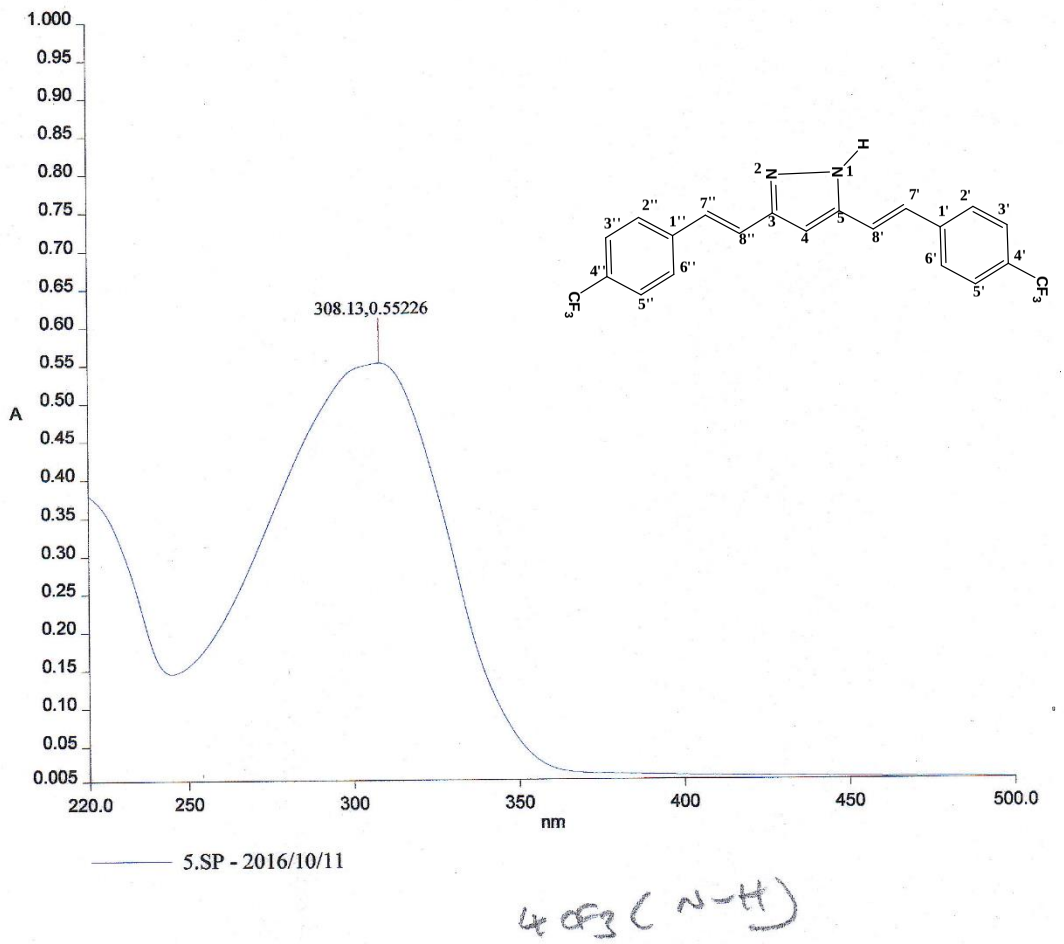
COSY Spectrum of Compound A-5k: 3,5-bis(4-(trifluoromethyl)styryl)-1H-pyrazole



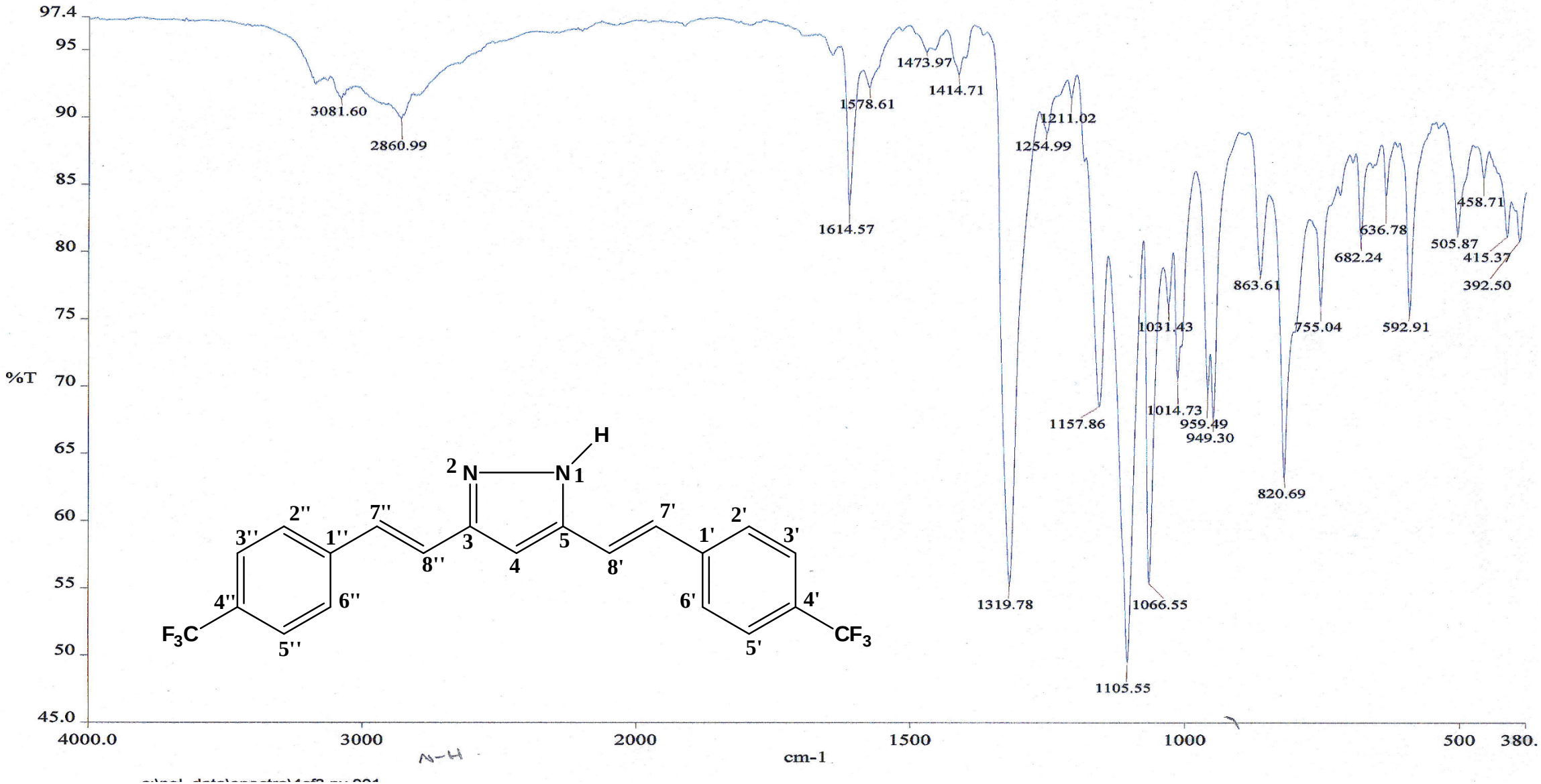
HSQC Spectrum of Compound A-5k: 3,5-bis(4-(trifluoromethyl)styryl)-1H-pyrazole



HMBC Spectrum of Compound A-5k: 3,5-bis(4-(trifluoromethyl)styryl)-1H-pyrazole



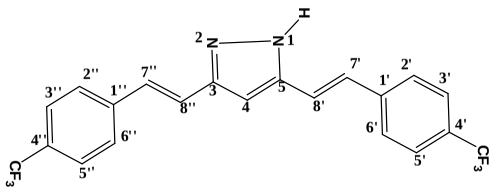
Ultraviolet Spectrum of Compound A-5k: 3,5-bis(4-(trifluoromethyl)styryl)-1H-pyrazole



Infrared Spectrum of Compound A-5k: 3,5-bis(4-(trifluoromethyl)styryl)-1H-pyrazole

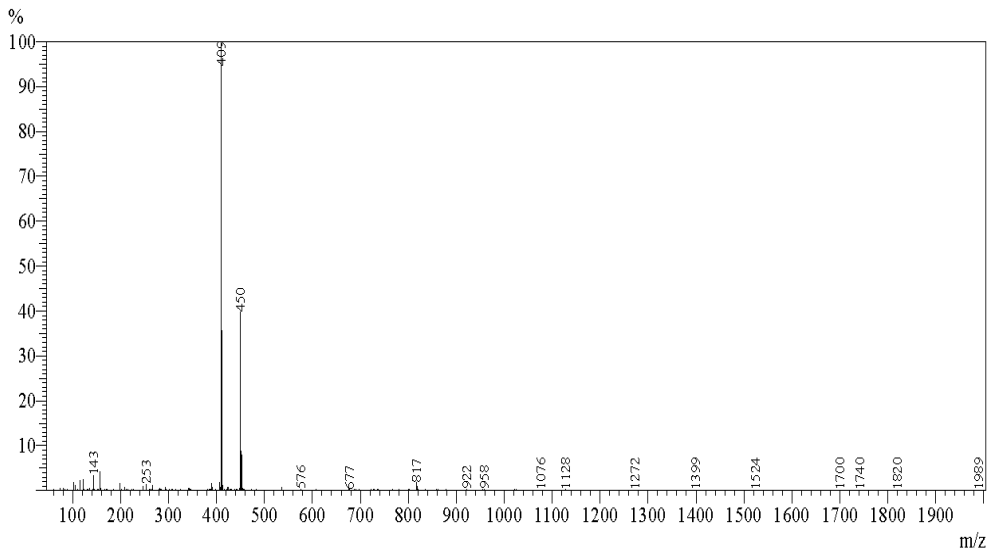
Sample Information

Date Acquired	: 2016/10/04 12:33:51 PM
Sample Type	: Unknown
Sample Name	: 16
Sample ID	: 16
Data File	: 16.lcd
Method File	: no column run.lcm
Report Format File	: mass spectrum %.lsr
Tuning File	: 03022016.lct



MS Spectrum

R.Time:1.067(Scan#:65)
MassPeaks:2090
Segment 1 - Event 1

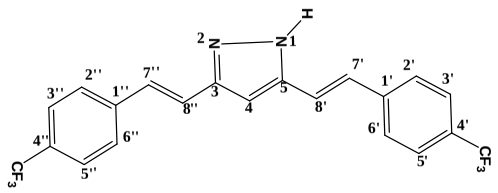


Ultraviolet of Compound A-5k: 3,5-bis(4-(trifluoromethyl)styryl)-1H-pyrazole

Elemental Composition Report Page 1

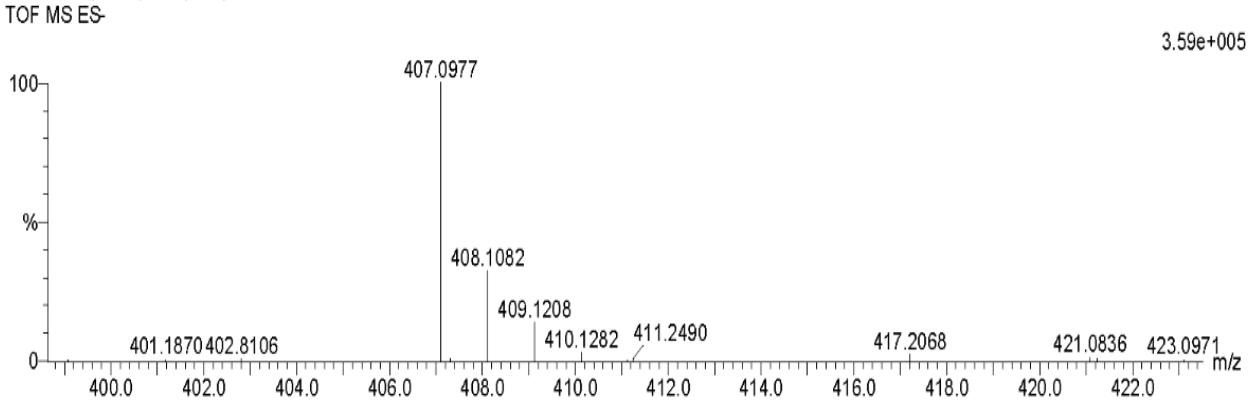
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2



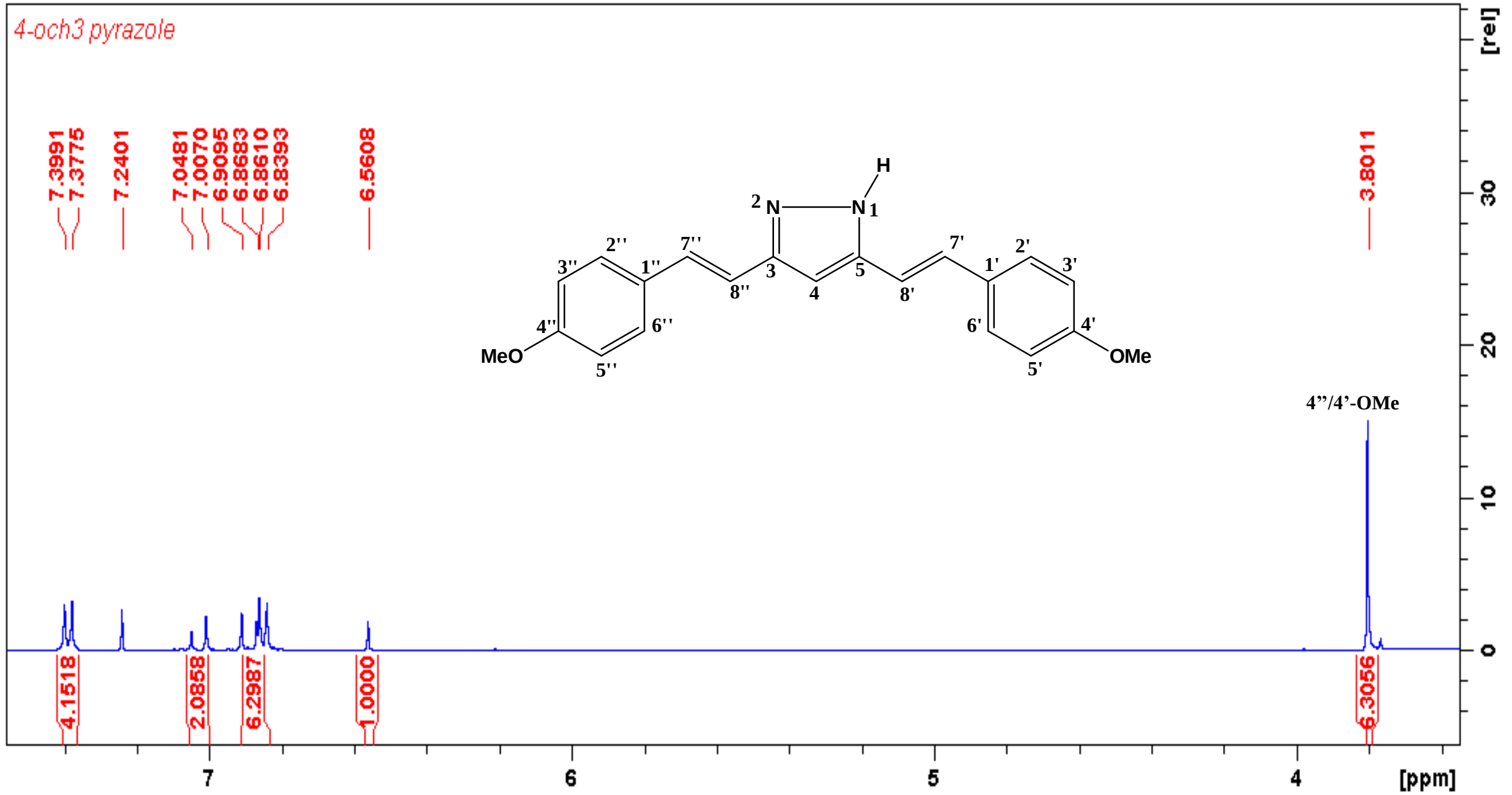
Monoisotopic Mass, Even Electron Ions
26 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 20-25 H: 10-15 N: 0-5 F: 5-10

6 4-CF3 7 (0.203) Cm (1:61)
TOF MS ES-

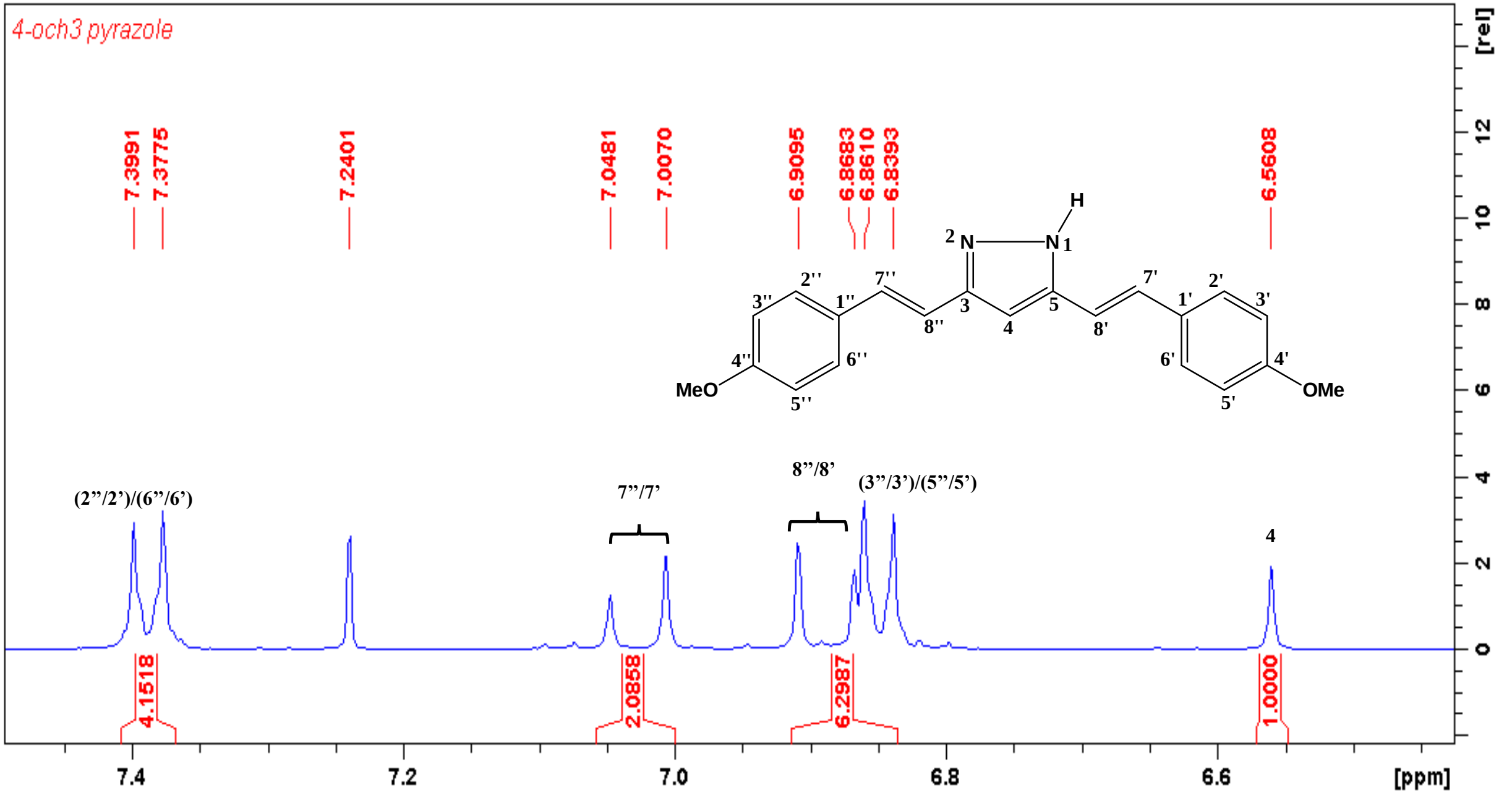


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
407.0977	407.0983	-0.6	-1.5	13.5	42.8	0.0	C21 H13 N2 F6

HRMS of Compound A-5k: 3,5-bis(4-(trifluoromethyl)styryl)-1H-pyrazole

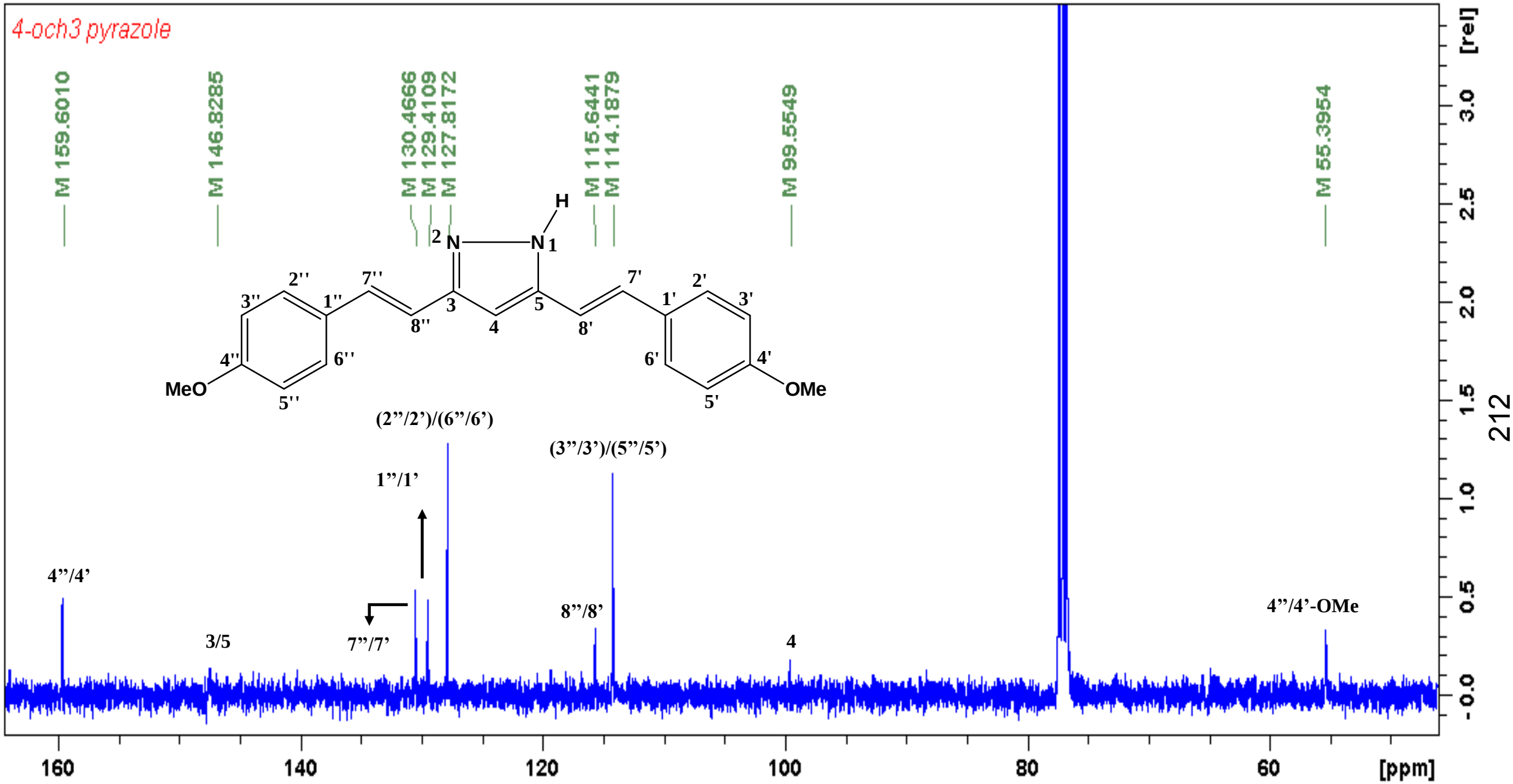


^{1}H Spectrum of Compound A-5m: 3,5-bis(4-methoxystyryl)-1H-pyrazole

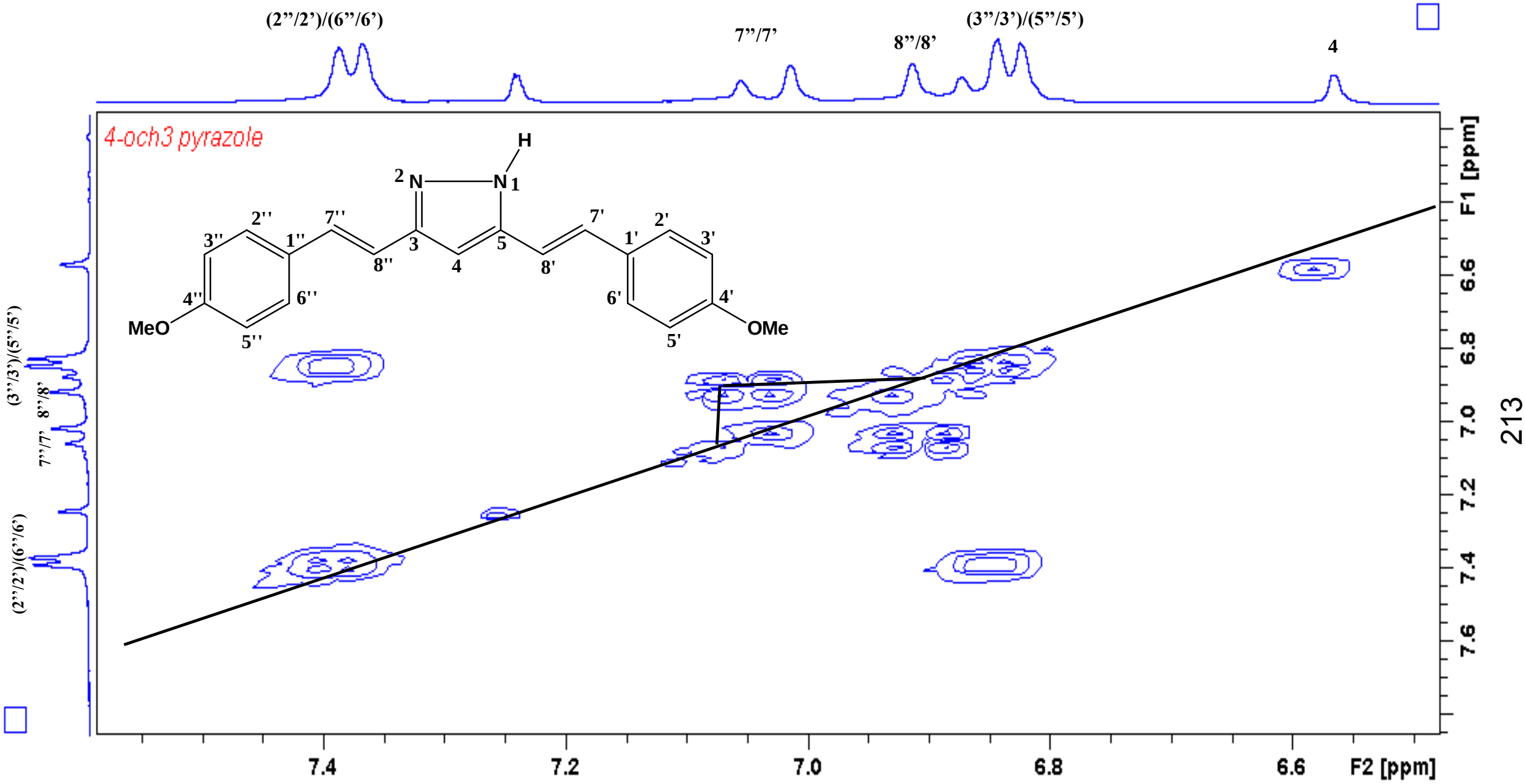


211

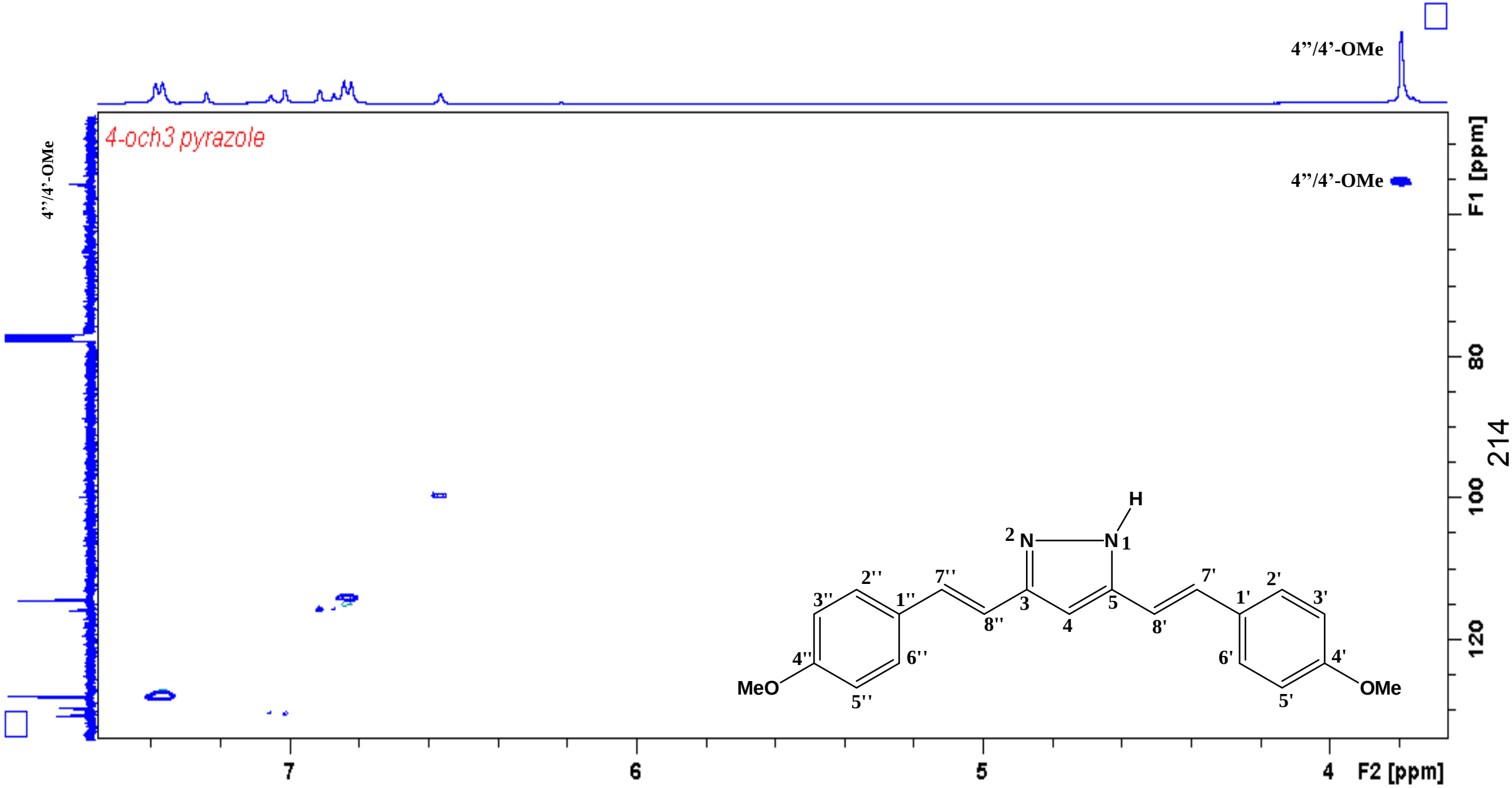
Expanded ^{1}H Spectrum of Compound A-5m: 3,5-bis(4-methoxystyryl)-1H-pyrazole



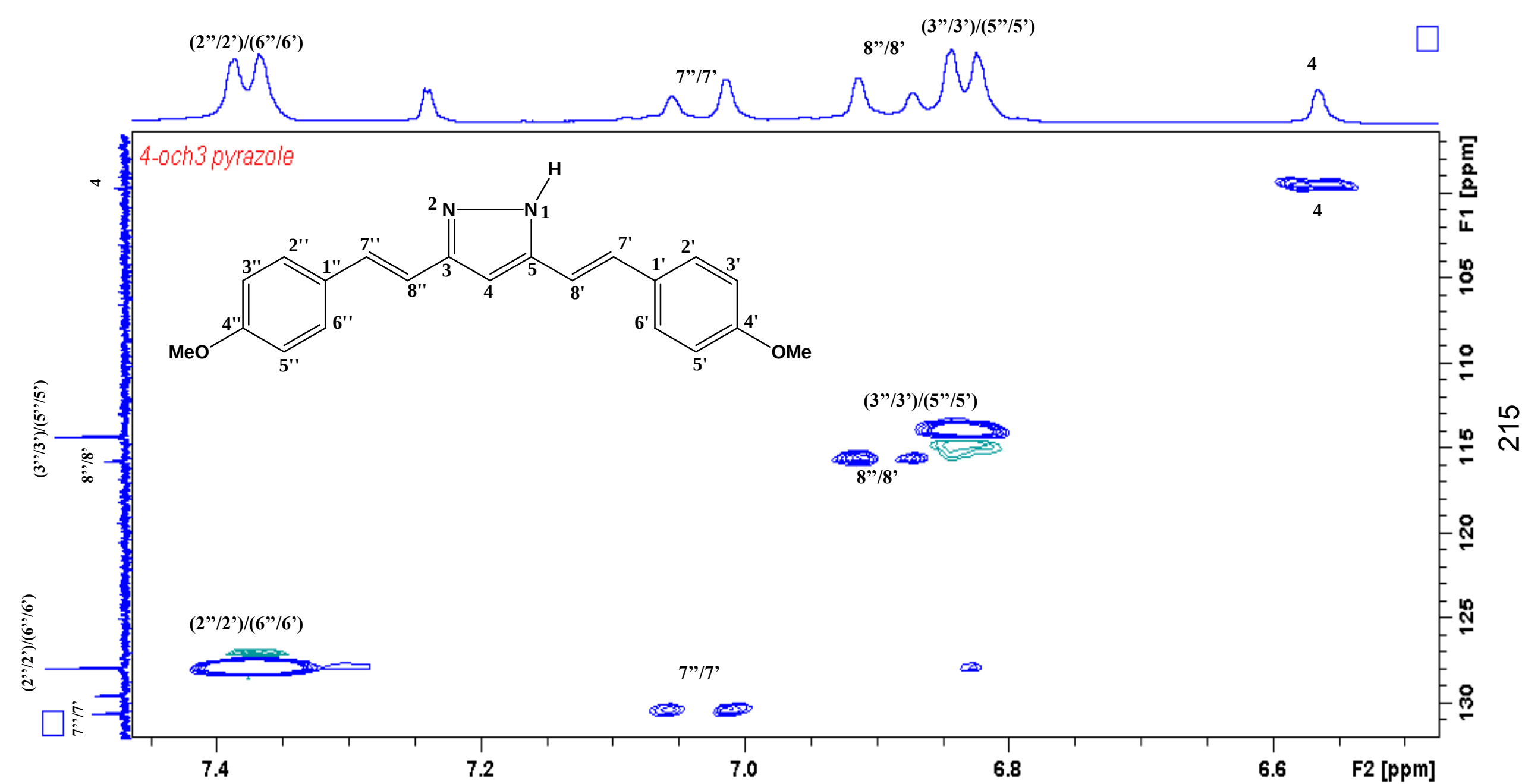
^{13}C Spectrum of Compound A-5m: 3,5-bis(4-methoxystyryl)-1H-pyrazole



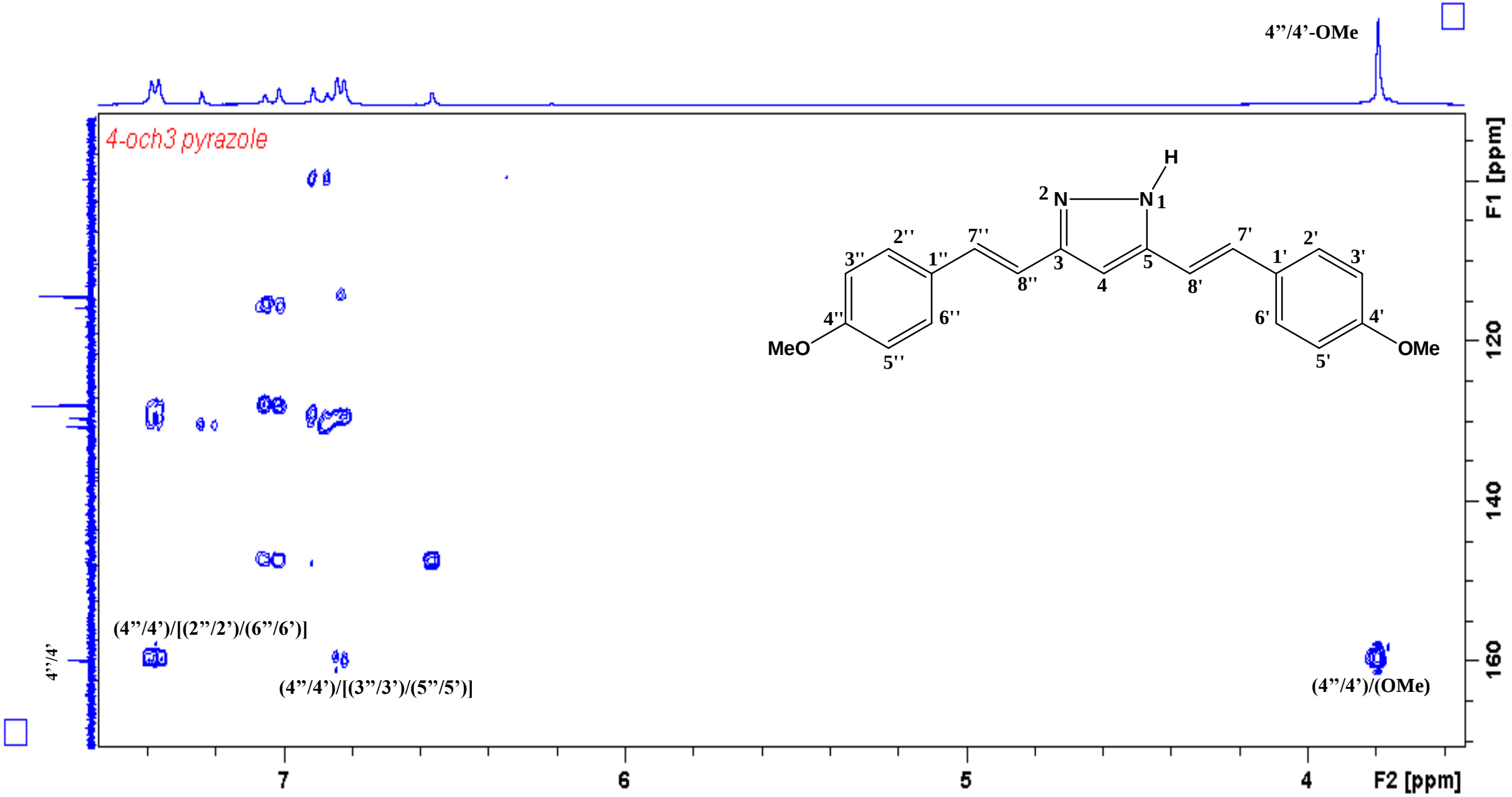
COSY of Compound A-5m: 3,5-bis(4-methoxystyryl)-1H-pyrazole



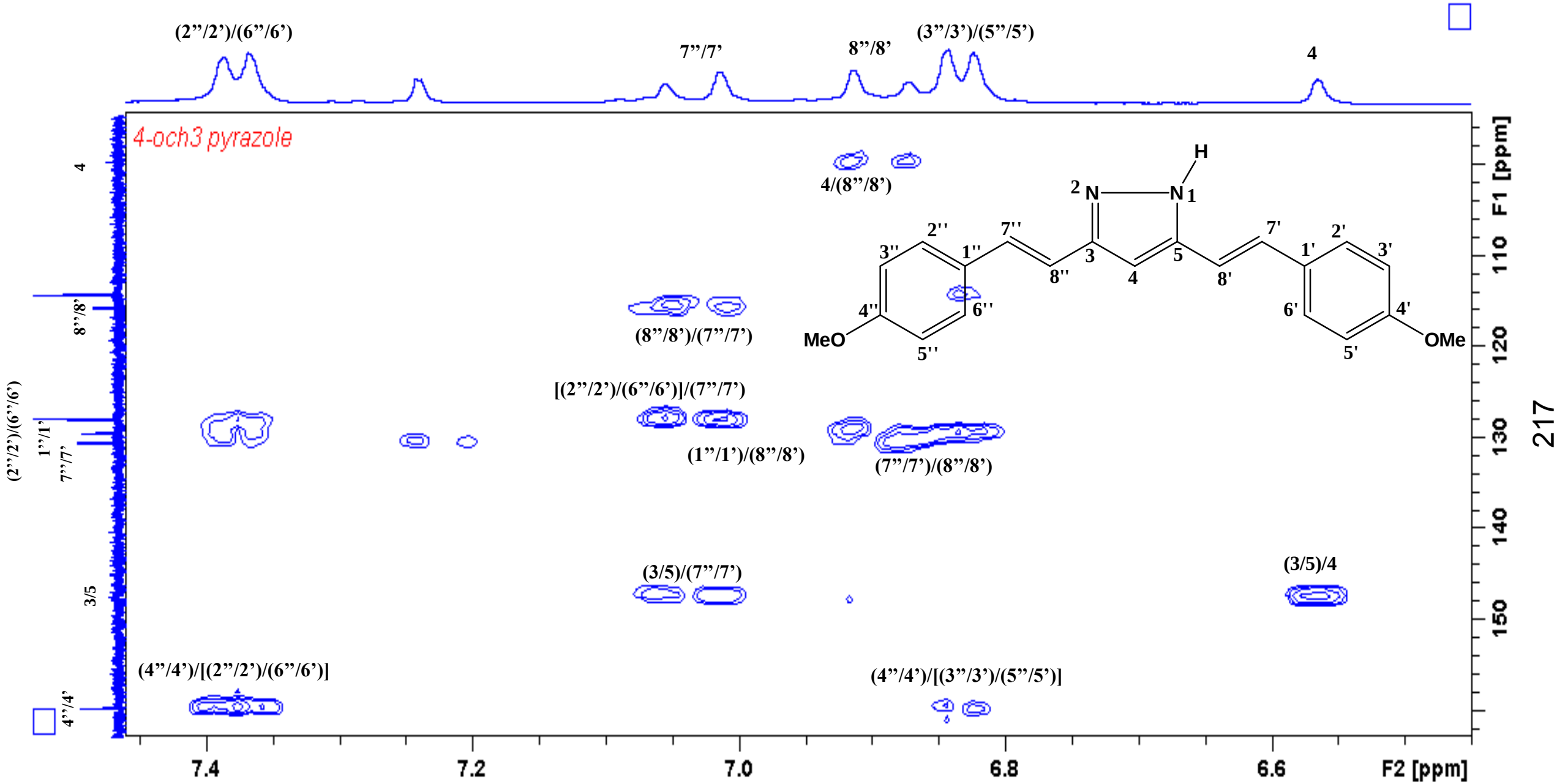
HSQC of Compound A-5m: 3,5-bis(4-methoxystyryl)-1H-pyrazole



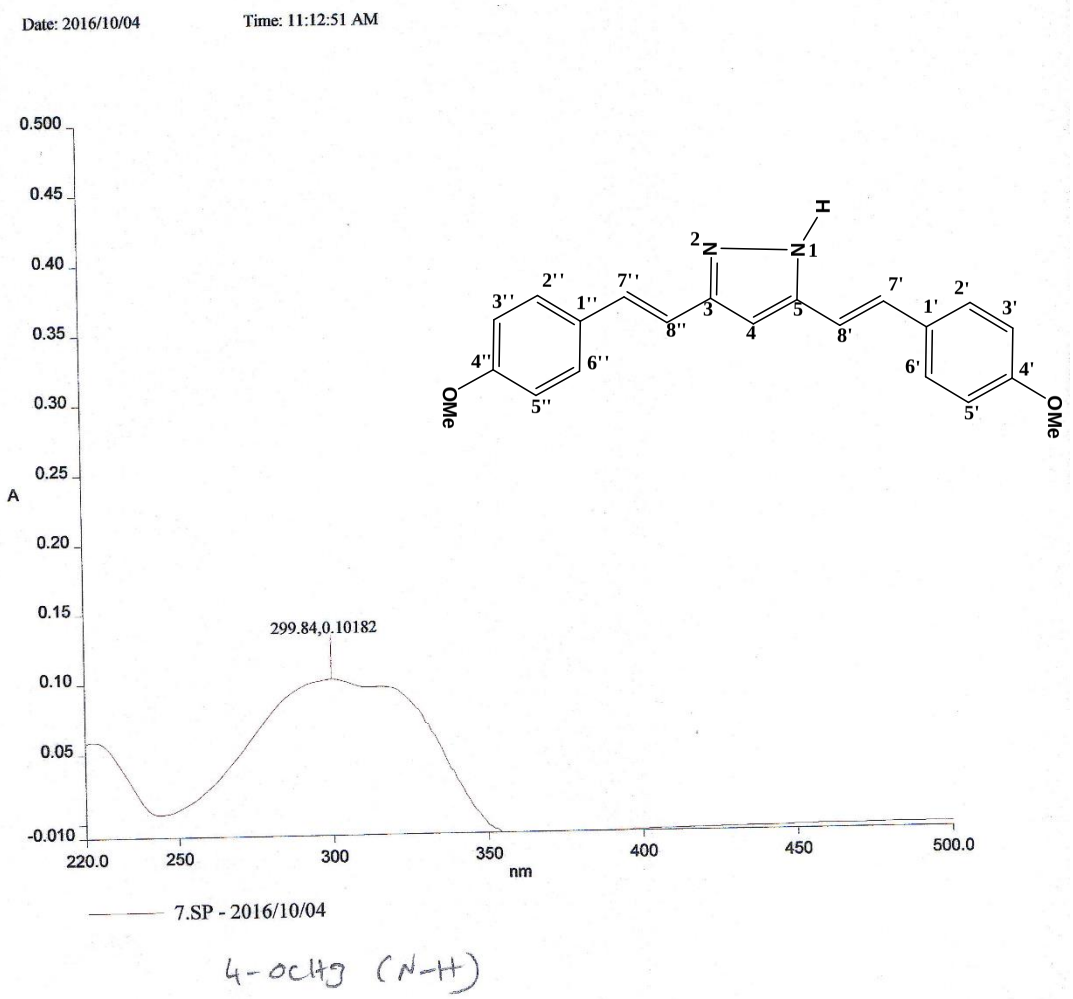
Expanded HSQC of Compound A-5m: 3,5-bis(4-methoxystyryl)-1H-pyrazole



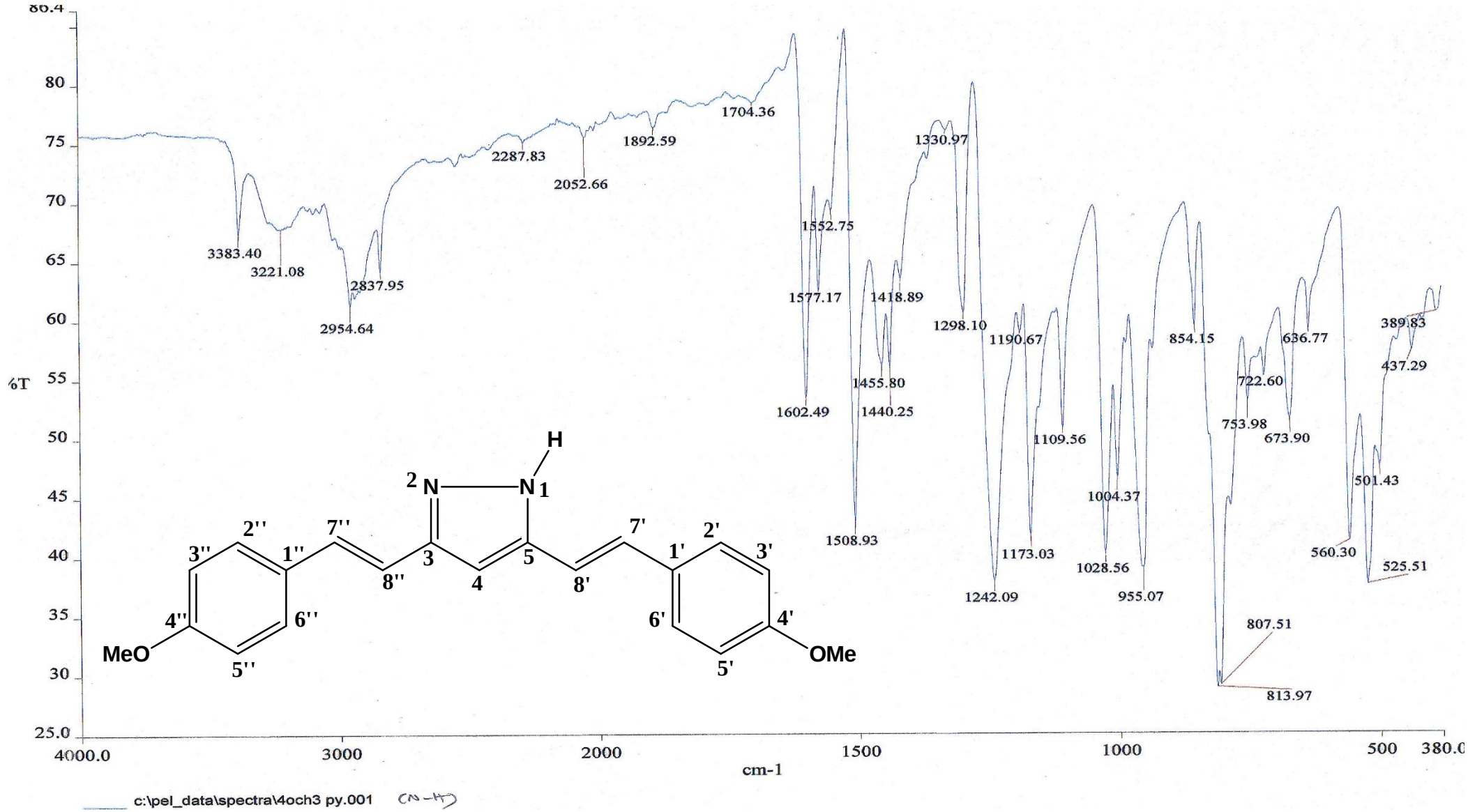
HMBC of Compound A-5m: 3,5-bis(4-methoxystyryl)-1H-pyrazole



Expanded HMBC of Compound A-5m: 3,5-bis(4-methoxystyryl)-1H-pyrazole



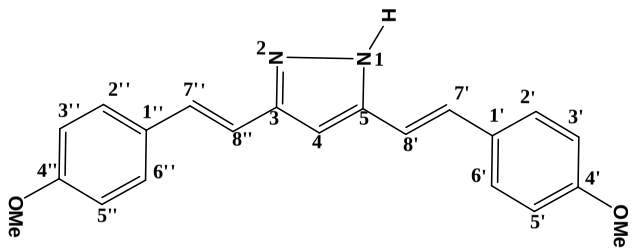
Ultraviolet Spectrum of Compound A-5m: 3,5-bis(4-methoxystyryl)-1H-pyrazole



Infrared Spectrum of Compound A-5m: 3,5-bis(4-methoxystyryl)-1H-pyrazole

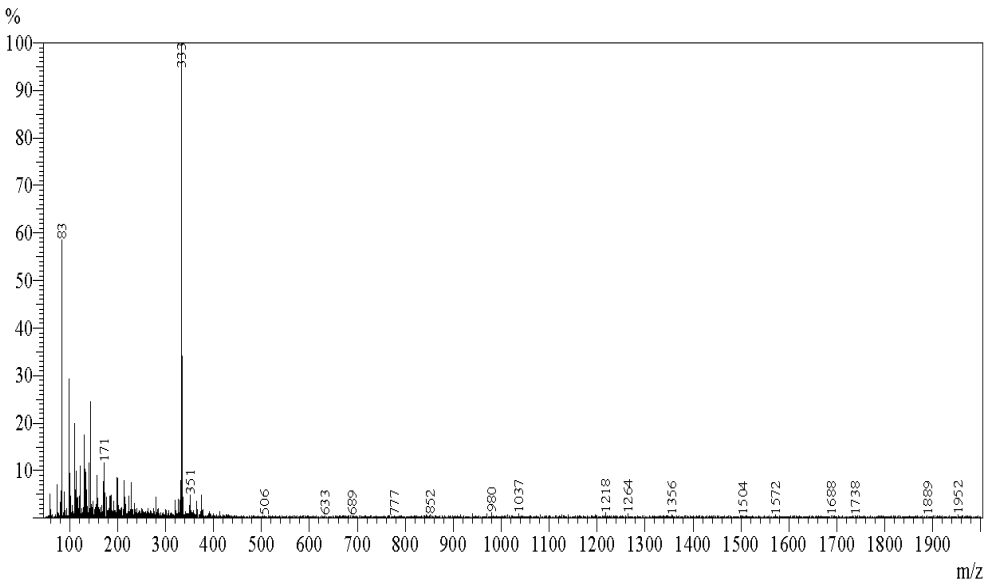
Sample Information

Date Acquired	: 2016/10/04 04:17:47 PM
Sample Type	: Unknown
Sample Name	: 18
Sample ID	: 18
Data File	: 18.lcd
Method File	: no column run.lcm
Report Format File	: DEFAULT.lsr
Tuning File	: 03022016.lct



MS Spectrum

R.Time:1.067(Scan#:65)
MassPeaks:1961
Segment 1 - Event 1

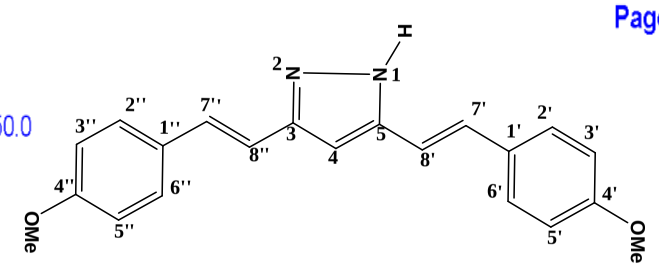


LC-MS of Compound A-5m: 3,5-bis(4-methoxystyryl)-1H-pyrazole

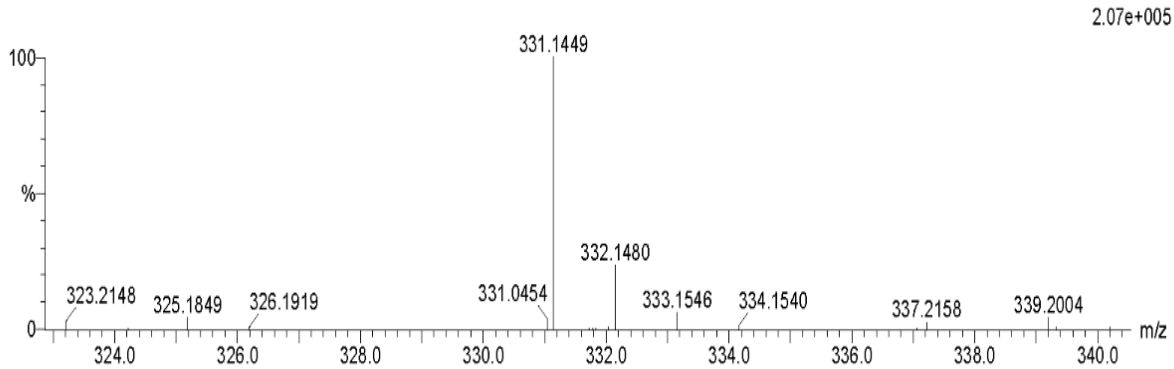
Elemental Composition Report Page 1

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

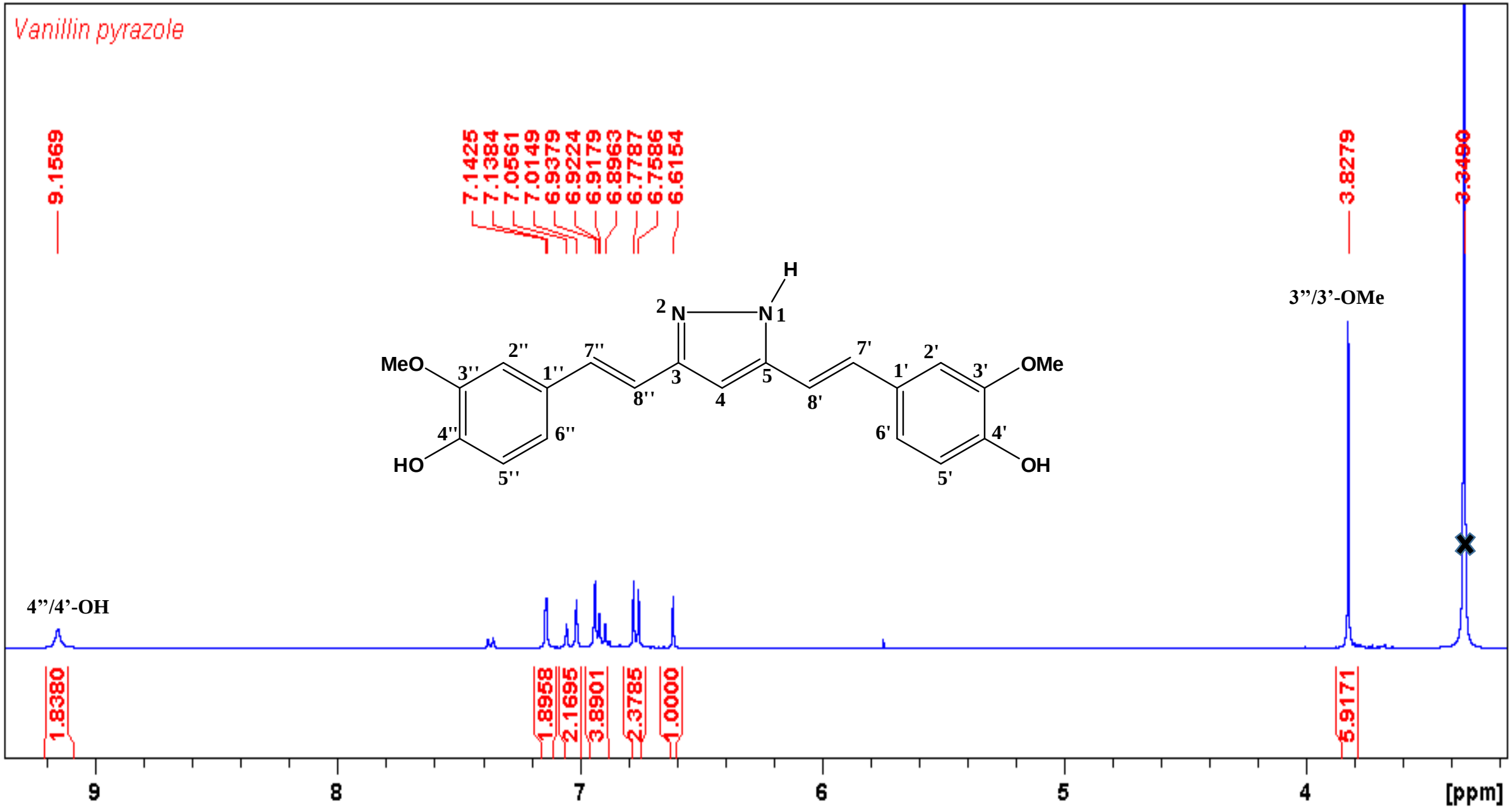


Monoisotopic Mass, Even Electron Ions
20 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 0-5
10 4-OCH3 42 (1.382) Cm (1:51)
TOF MS ES-

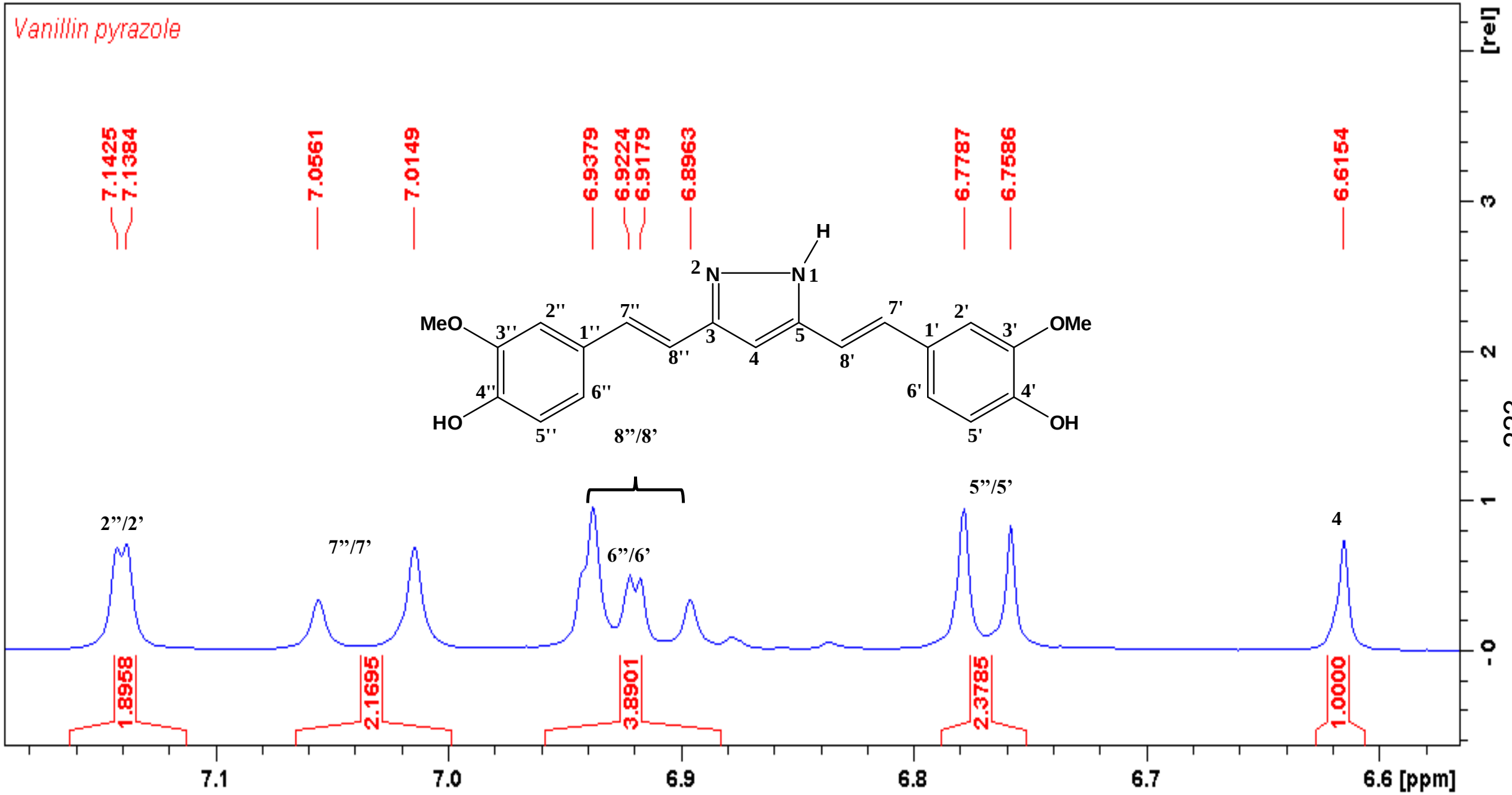


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
331.1449	331.1447	0.2	0.6	13.5	67.0	0.0	C21 H19 N2 O2

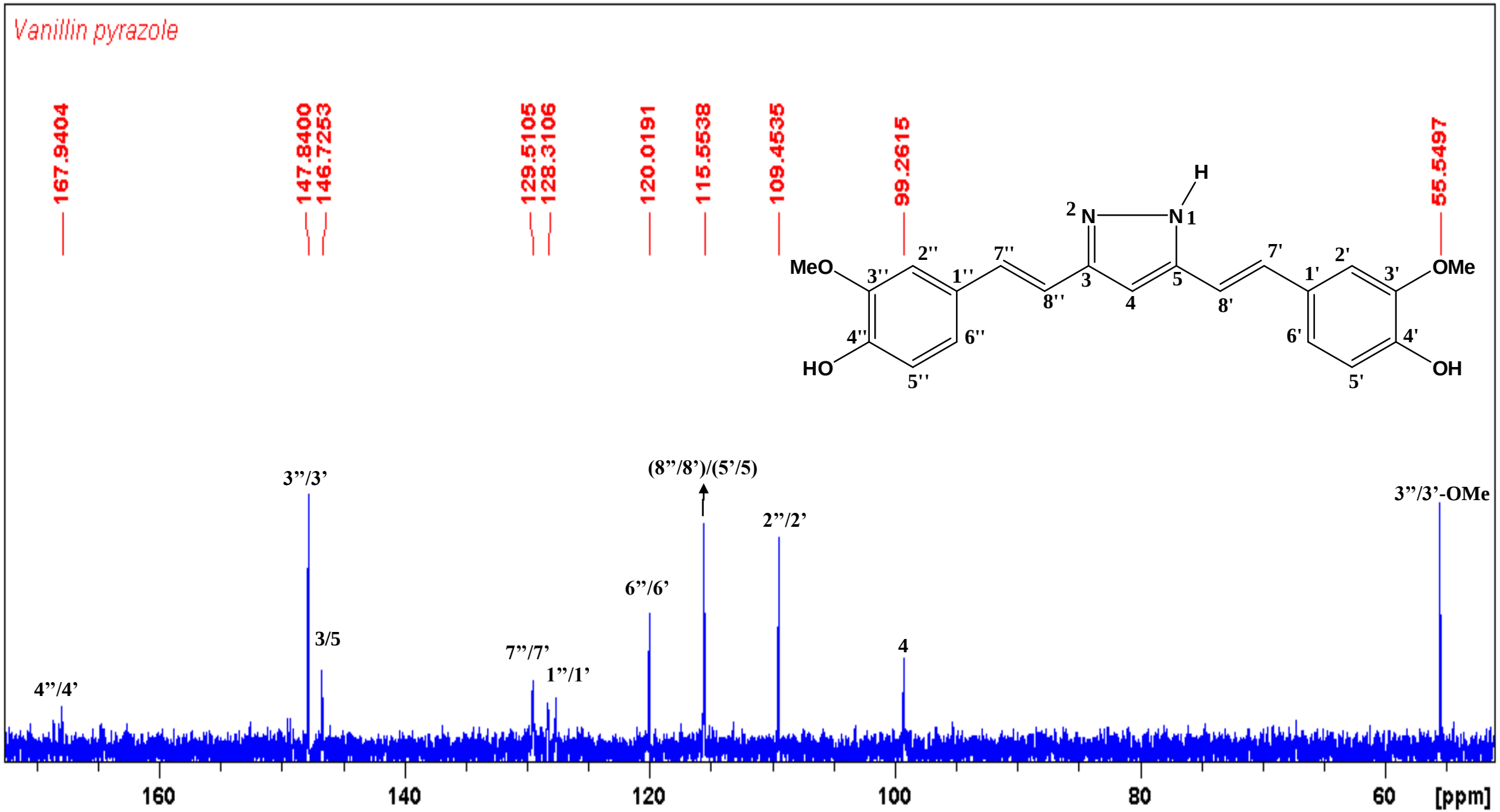
HRMS of Compound A-5m: 3,5-bis(4-methoxystyryl)-1H-pyrazole



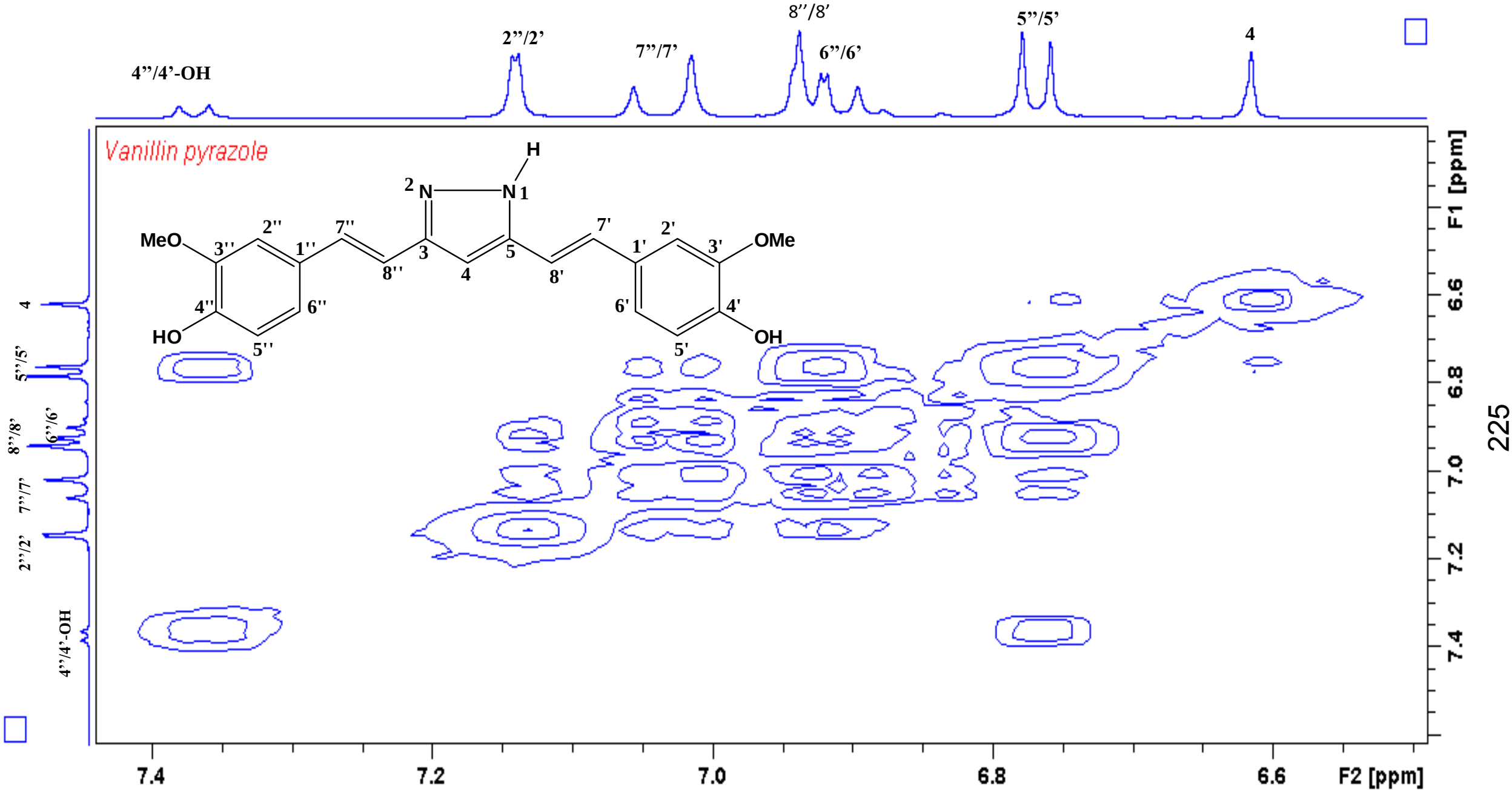
^{1}H Spectrum of Compound A-5o: 4,4'-(1E,1'E)-2,2'-(1H-pyrazole-3,5-diyl)bis(ethene-2,1-diyl)bis(2-methoxyphenol)



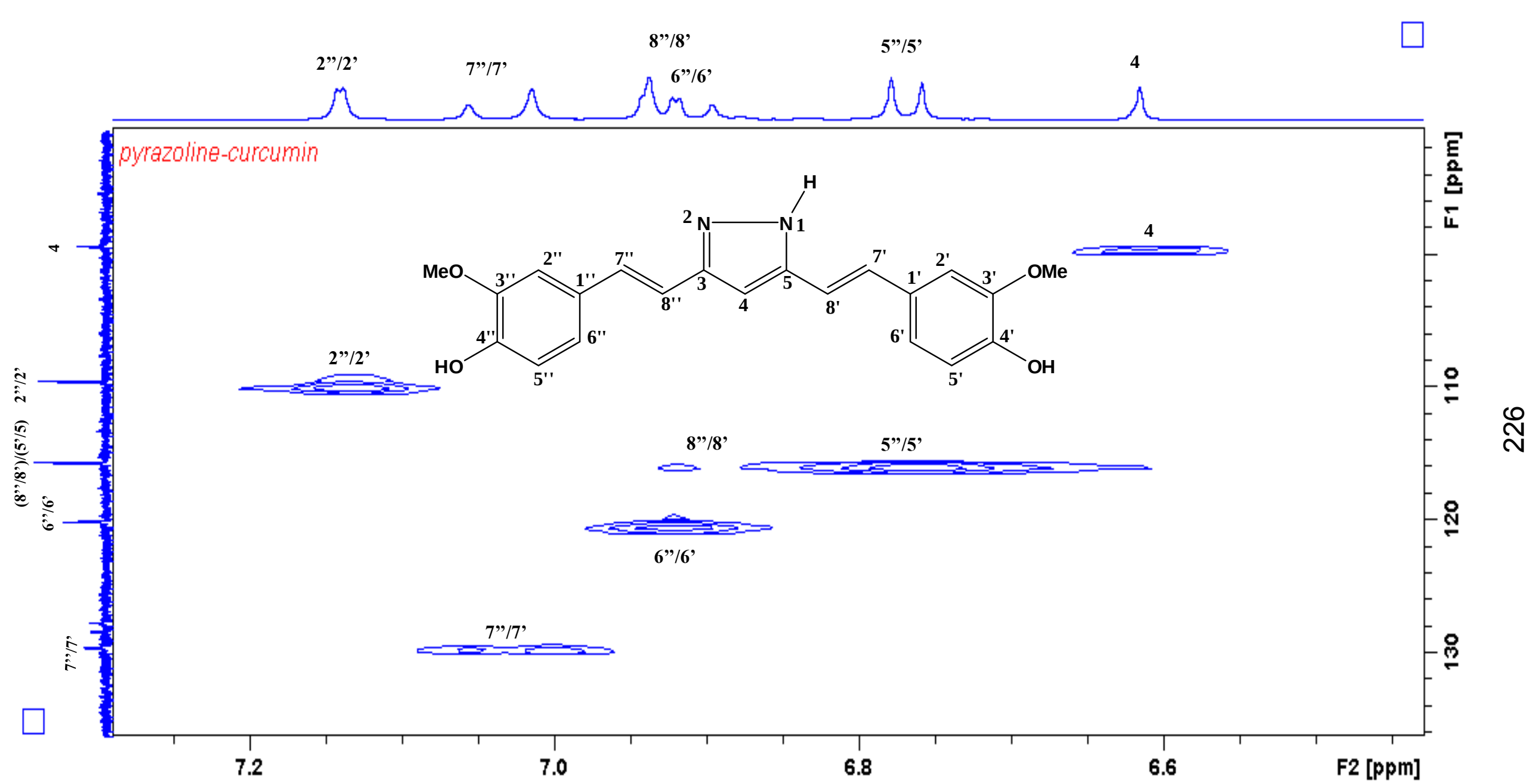
Expanded ^{1}H Spectrum of Compound A-5o: 4,4'-(1E,1'E)-2,2'-(1H-pyrazole-3,5-diyl)bis(ethene-2,1-diyl)bis(2-methoxyphenol)



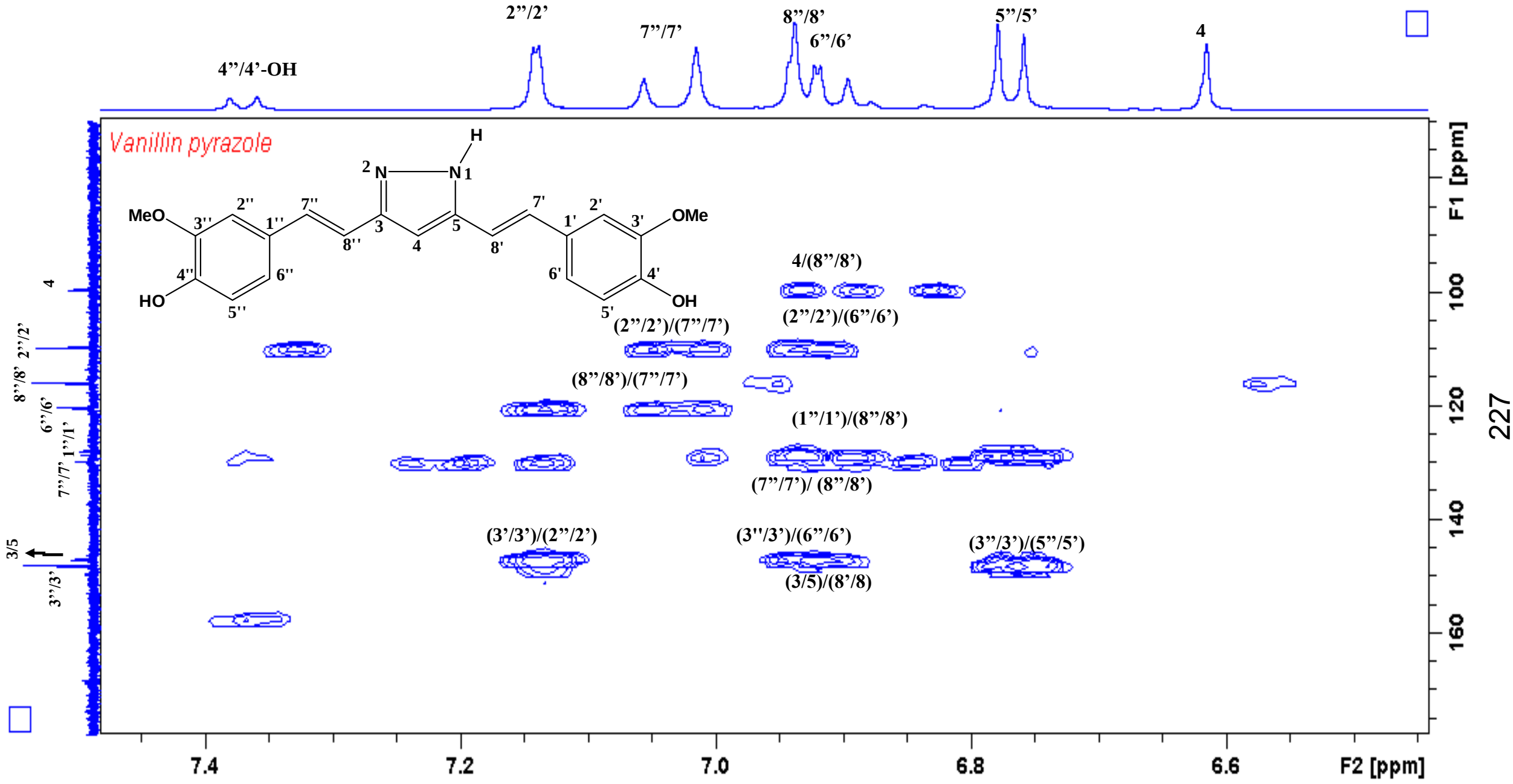
^{13}C Spectrum of Compound A-5o: 4,4'-(1E,1'E)-2,2'-(1H-pyrazole-3,5-diyl)bis(ethene-2,1-diyl)bis(2-methoxyphenol)



COSY of Compound A-5o: 4,4'-(1E,1'E)-2,2'-(1H-pyrazole-3,5-diyl)bis(ethene-2,1-diyl)bis(2-methoxyphenol)



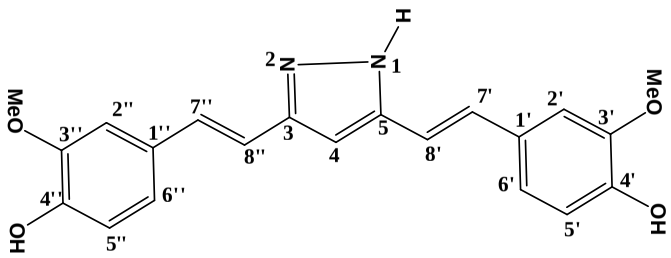
HSQC of Compound A-5o: 4,4'-(1E,1'E)-2,2'-(1H-pyrazole-3,5-diyl)bis(ethene-2,1-diyl)bis(2-methoxyphenol)



HMBC of Compound A-5o: 4,4'-(1E,1'E)-2,2'-(1H-pyrazole-3,5-diyl)bis(ethene-2,1-diyl)bis(2-methoxyphenol)

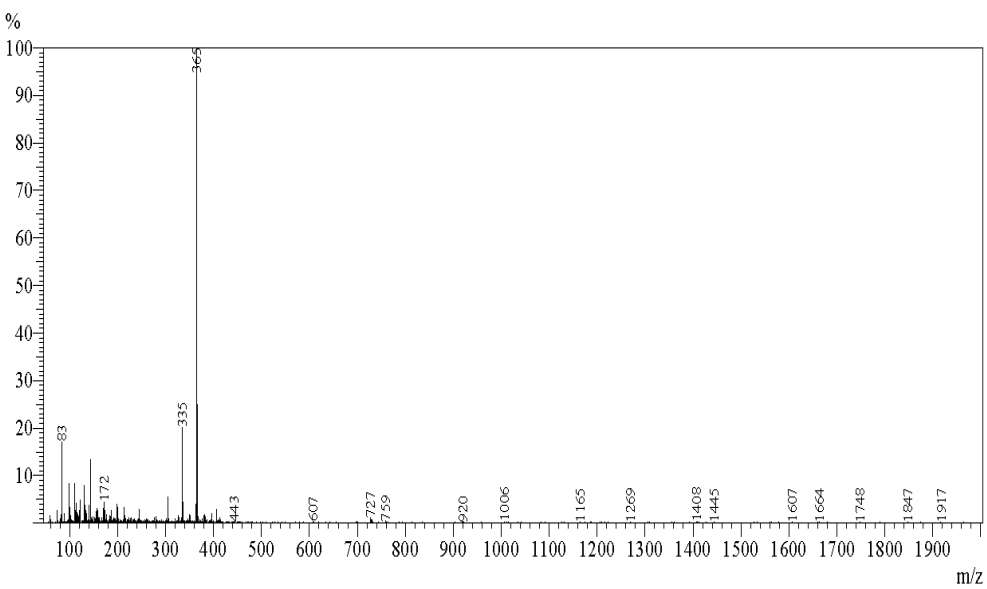
Sample Information

Date Acquired	: 2016/10/04 04:07:23 PM
Sample Type	: Unknown
Sample Name	: 12 run 2
Sample ID	: 12 run 2
Data File	: 12 run 2.lcd
Method File	: no column run.lcm
Report Format File	: DEFAULT.lsr
Tuning File	: 03022016.lct

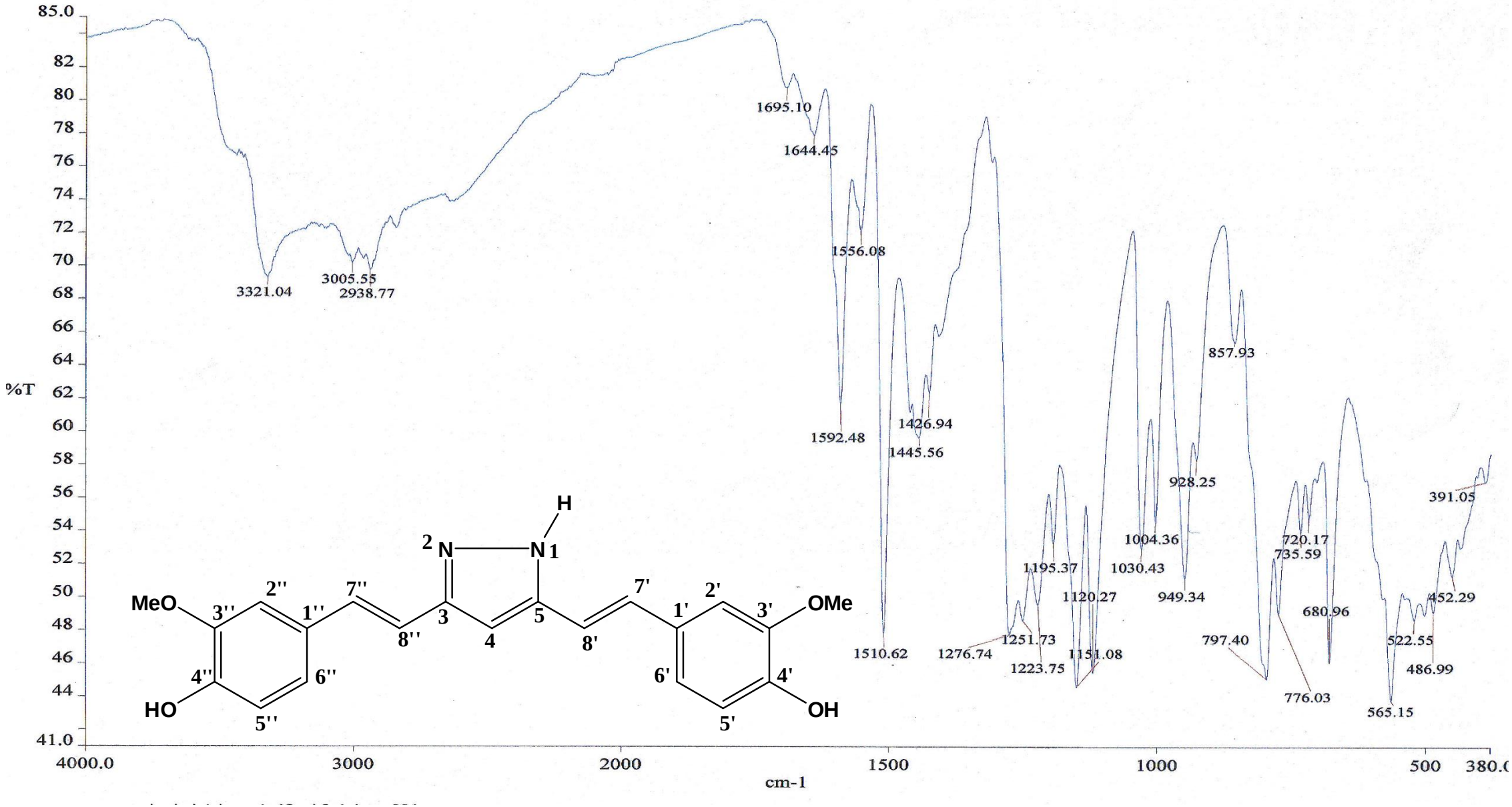


MS Spectrum

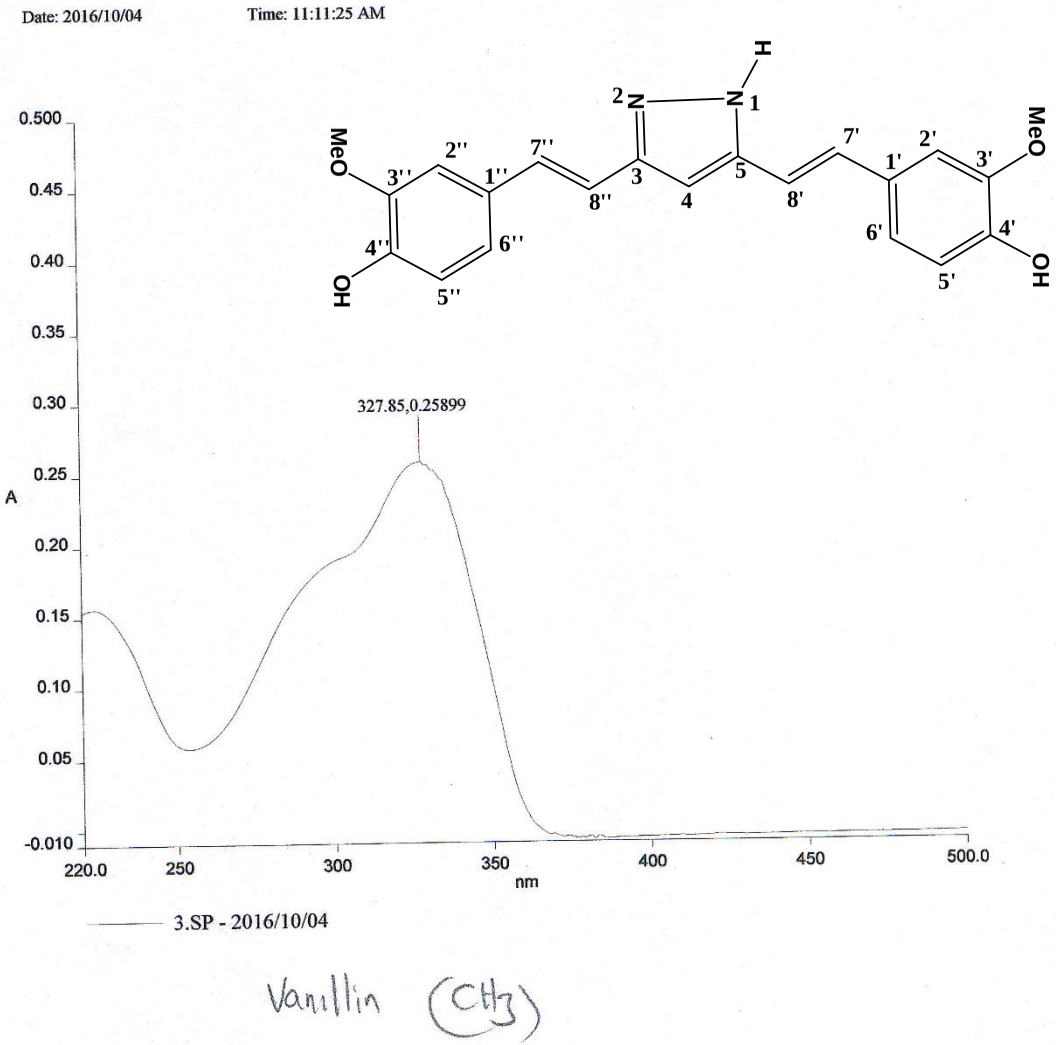
R.Time:1.067(Scan#:65)
MassPeaks:1941
Segment 1 - Event 1



LC-MS of Compound A-5o: 4,4'-(1E,1'E)-2,2'-(1H-pyrazole-3,5-diyl)bis(ethene-2,1-diyl)bis(2-methoxyphenol)



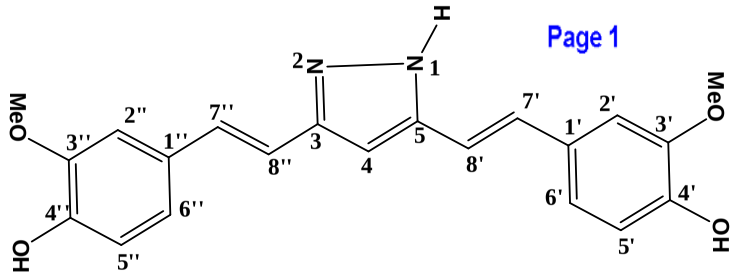
Infrared Spectrum of Compound A-5o: 4,4'-(1E,1'E)-2,2'-(1H-pyrazole-3,5-diyl)bis(ethene-2,1-diyl)bis(2-methoxyphenol)



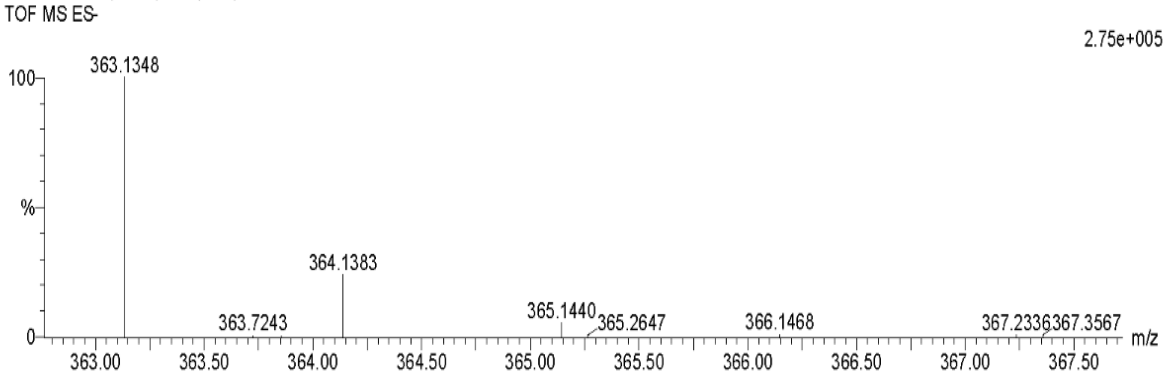
Ultraviolet Spectrum of Compound A-5o: 4,4'-(1E,1'E)-2,2'-(1H-pyrazole-3,5-diyl)bis(ethene-2,1-diyl)bis(2-methoxyphenol)

Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

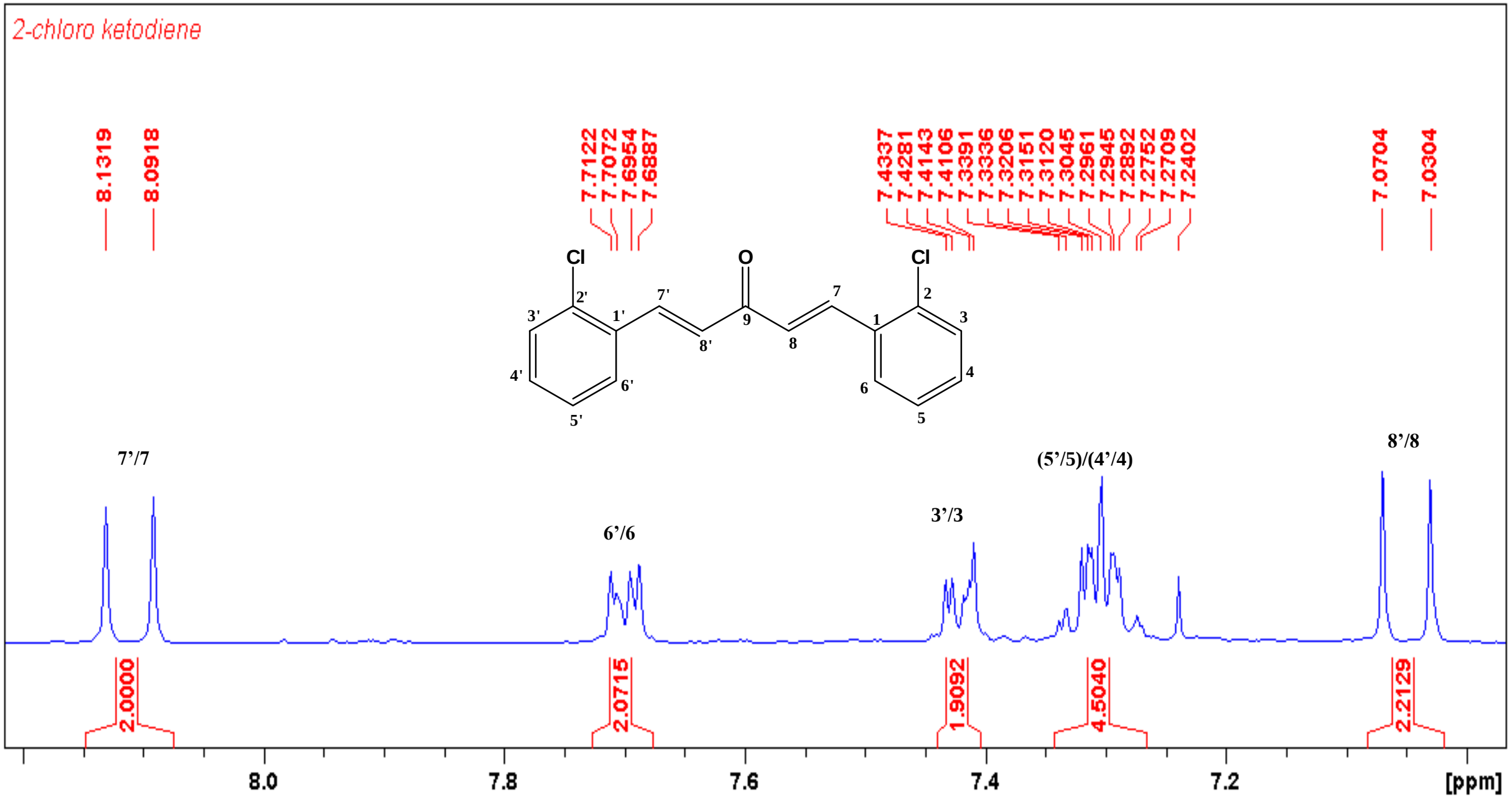


Monoisotopic Mass, Even Electron Ions
17 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 0-5
11 Vanillin 17 (0.540) Cm (1:61)
TOF MS ES-

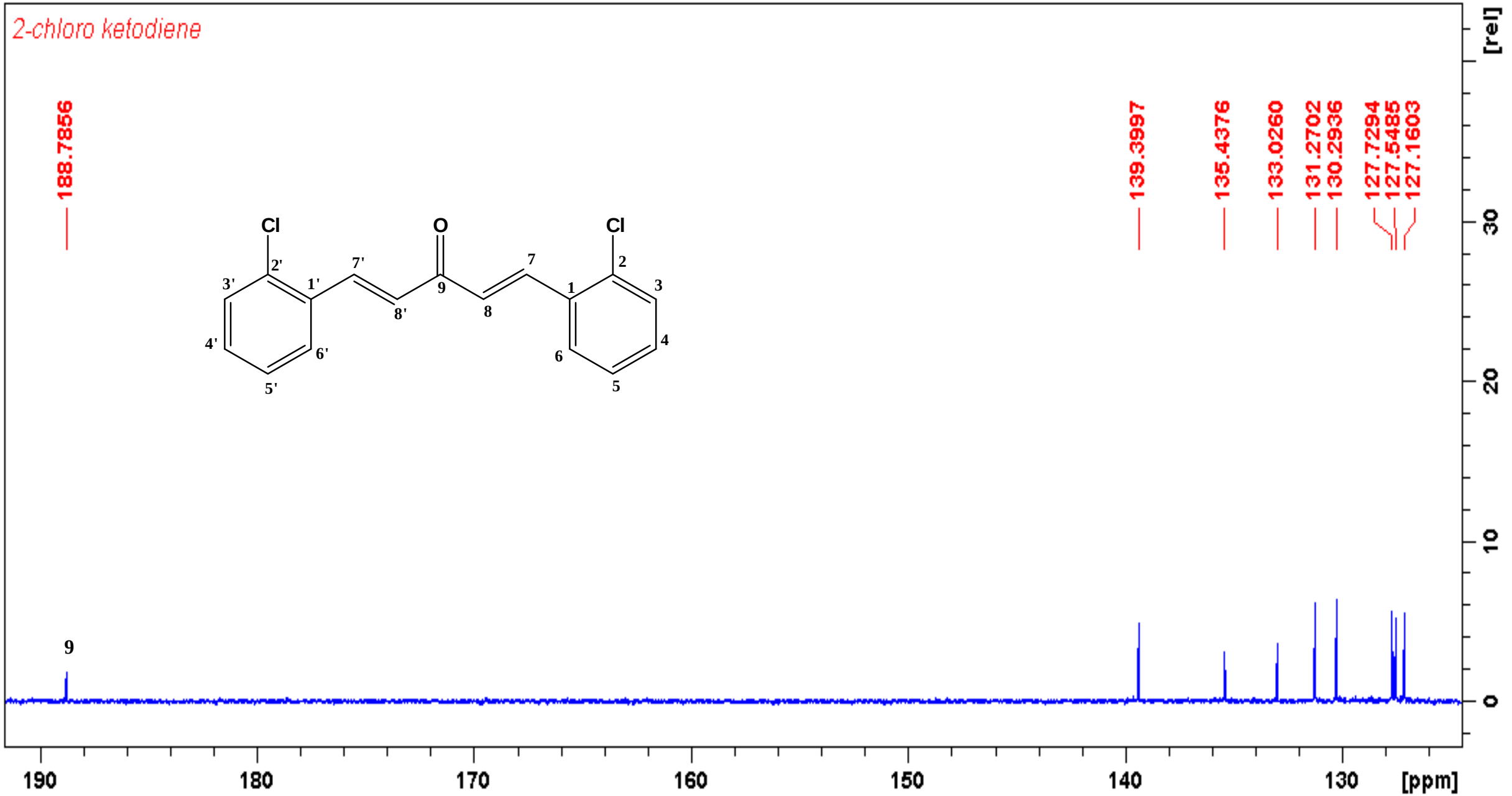


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
363.1348	363.1345	0.3	0.8	13.5	40.3	0.0	C21 H19 N2 O4

HRMS of Compound A-5o: 4,4'-(1E,1'E)-2,2'-(1H-pyrazole-3,5-diyl)bis(ethene-2,1-diyl)bis(2-methoxyphenol)



^{1}H Spectrum of Compound B-1a: (1E,4E)-1,5-bis(2-chlorophenyl)penta-1,4-dien-3-one



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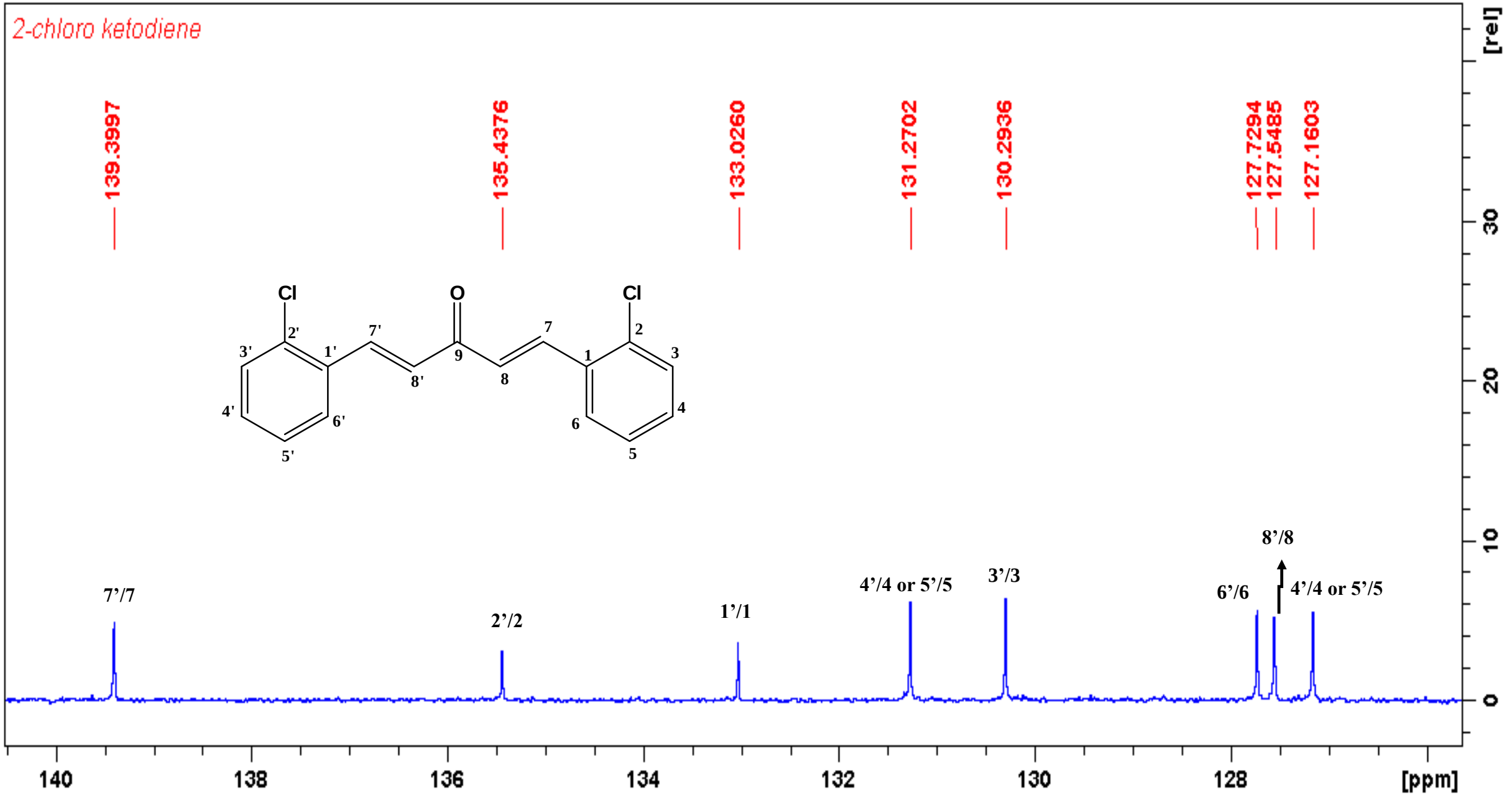
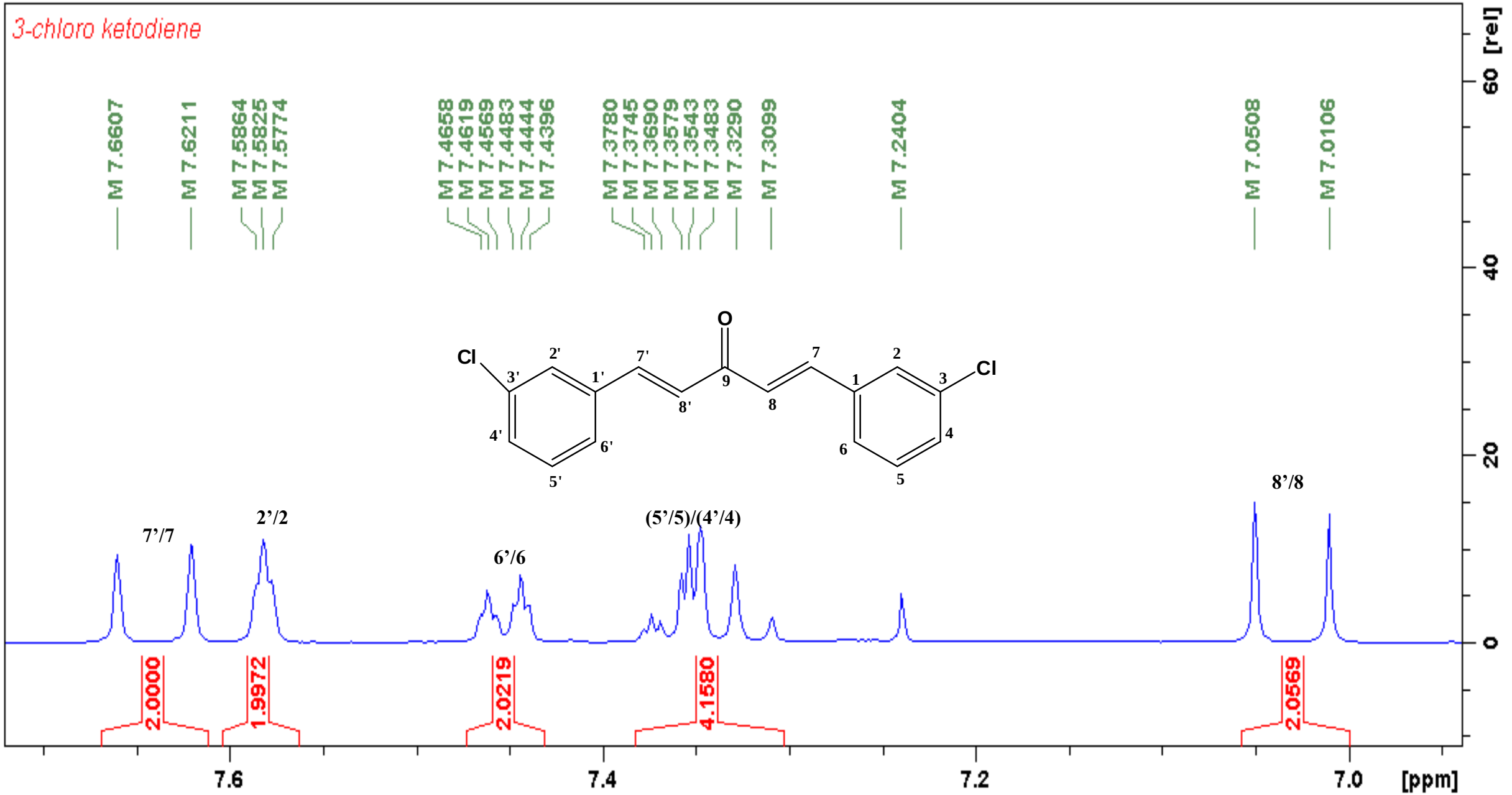
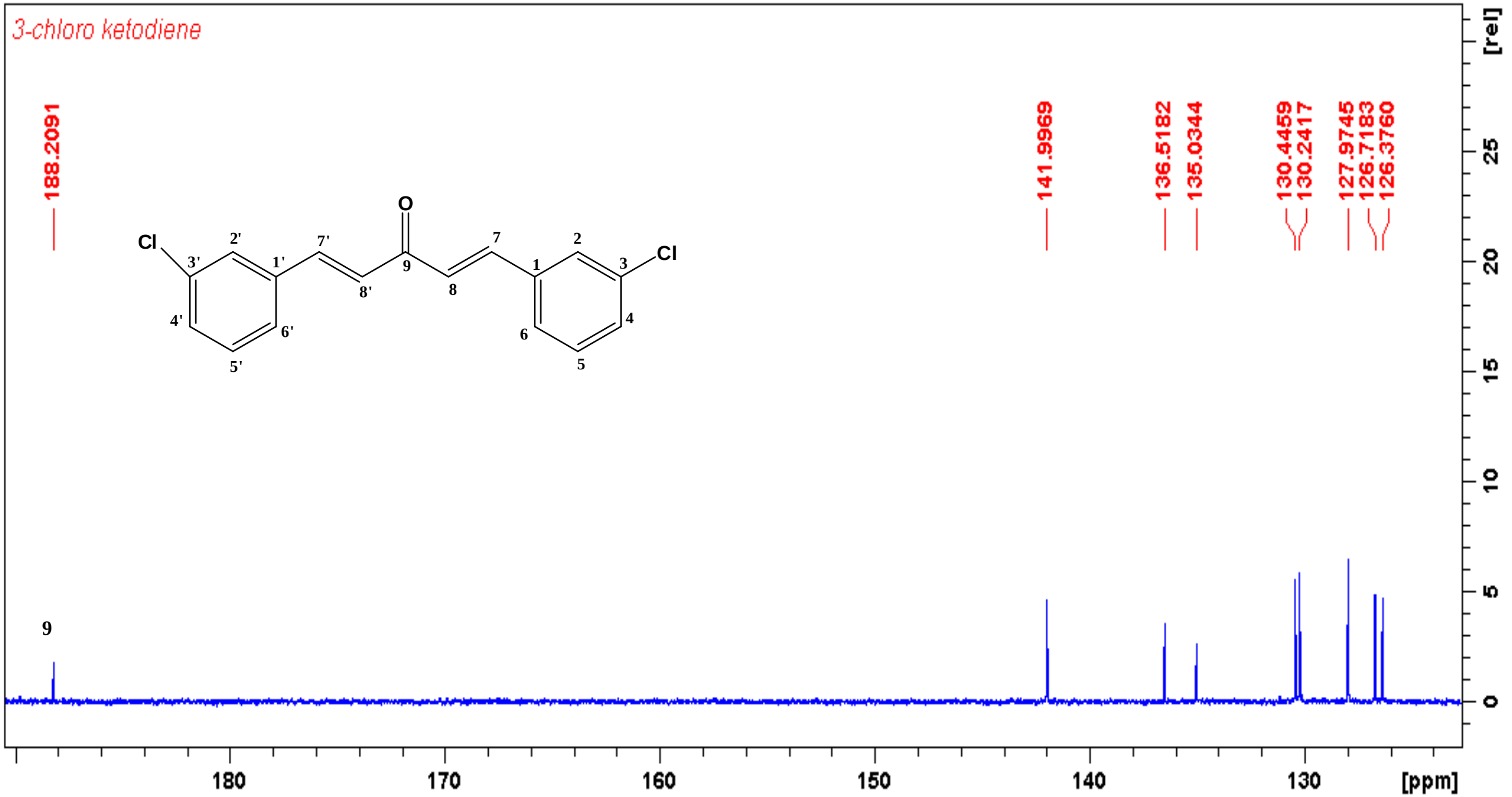
^{13}C Spectrum of Compound B-1a: (1E,4E)-1,5-bis(2-chlorophenyl)penta-1,4-dien-3-one



Expanded ^{13}C Spectrum of Compound B-1a: (1E,4E)-1,5-bis(2-chlorophenyl)penta-1,4-dien-3-one



^{1}H Spectrum of Compound B-1b: (1E,4E)-1,5-bis(3-chlorophenyl)penta-1,4-dien-3-one



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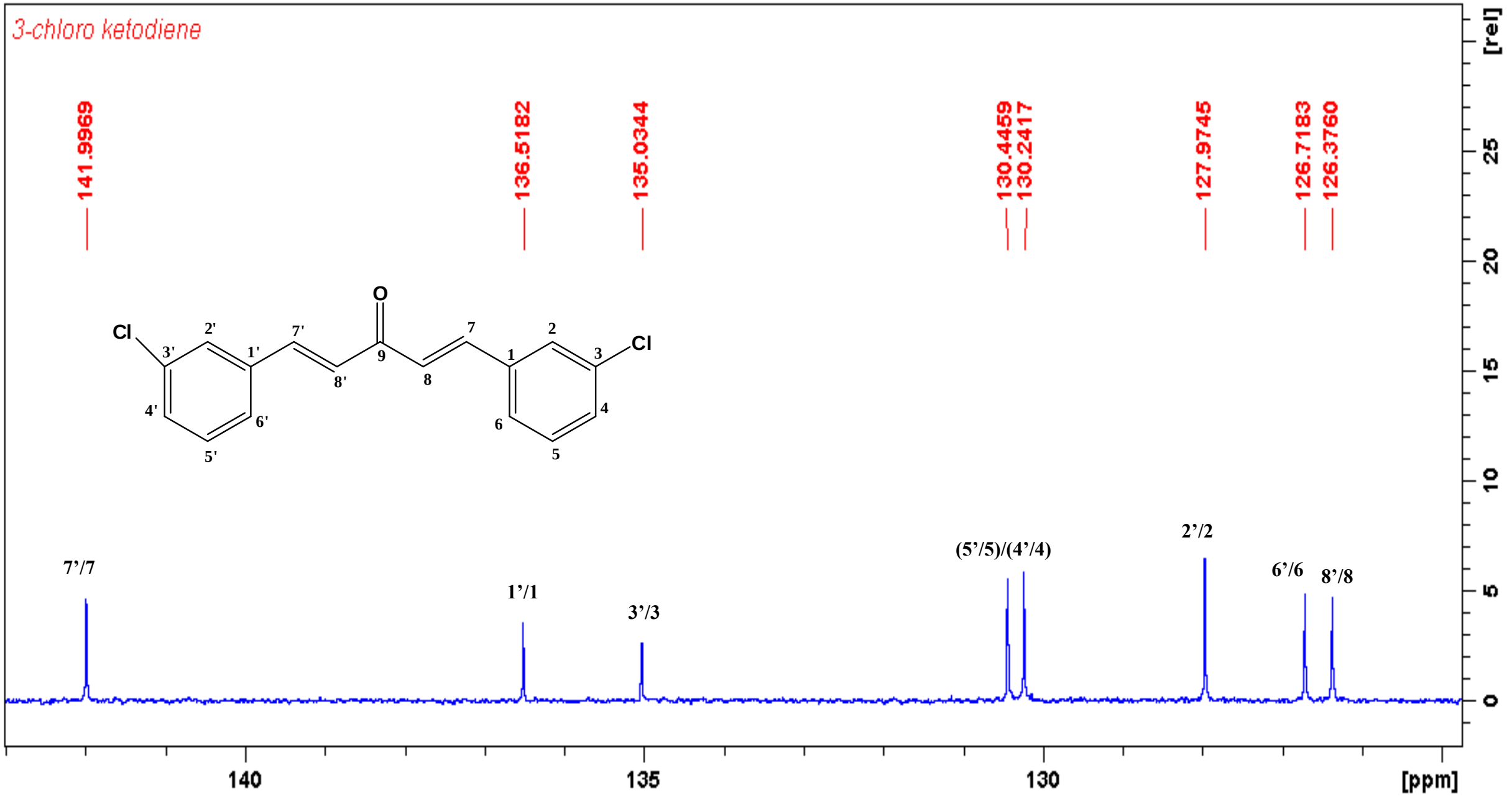
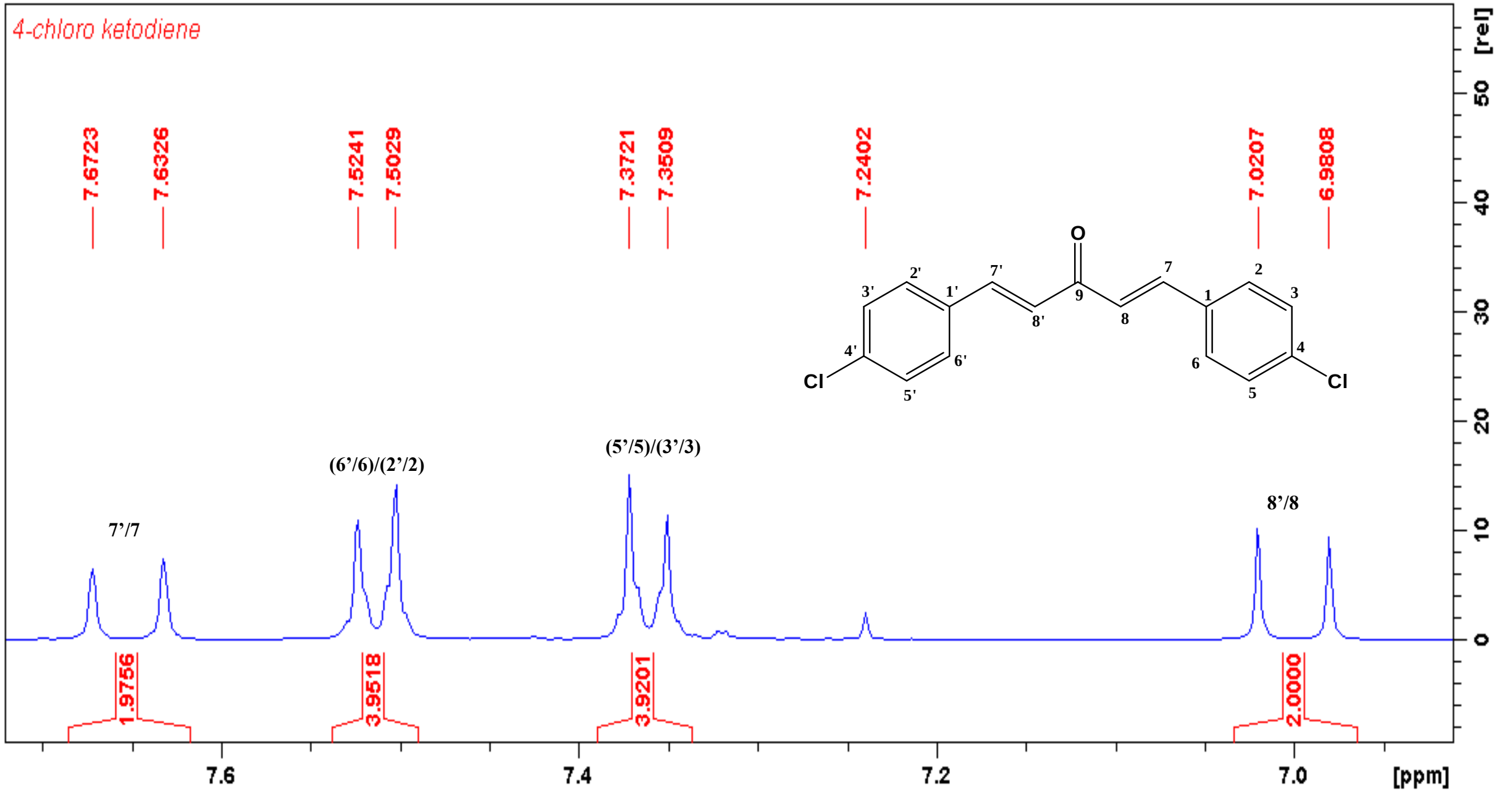
^{13}C Spectrum of Compound B-1b: (1E,4E)-1,5-bis(3-chlorophenyl)penta-1,4-dien-3-one



Expanded ^{13}C Spectrum of Compound B-1b: (1E,4E)-1,5-bis(3-chlorophenyl)penta-1,4-dien-3-one



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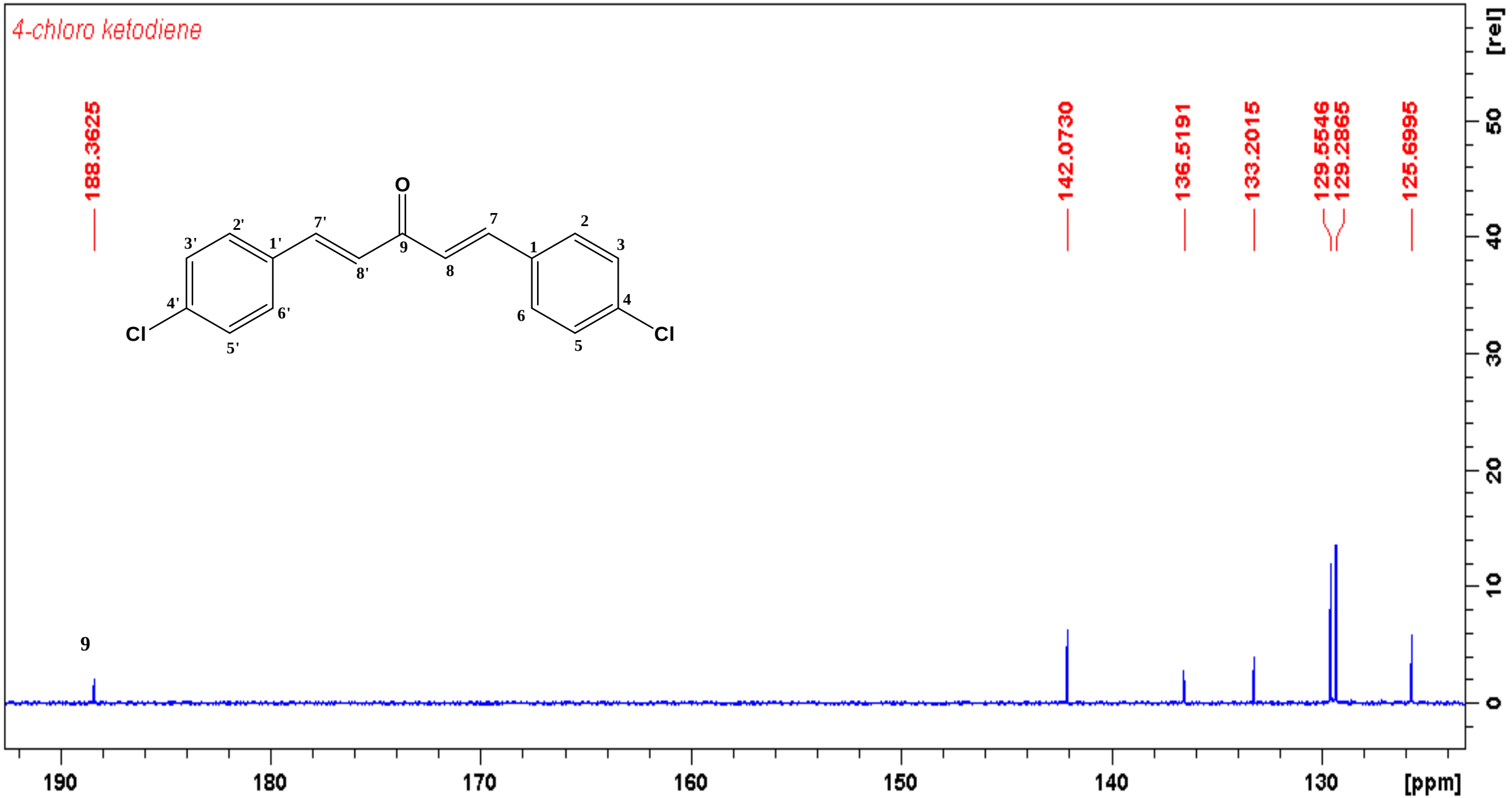
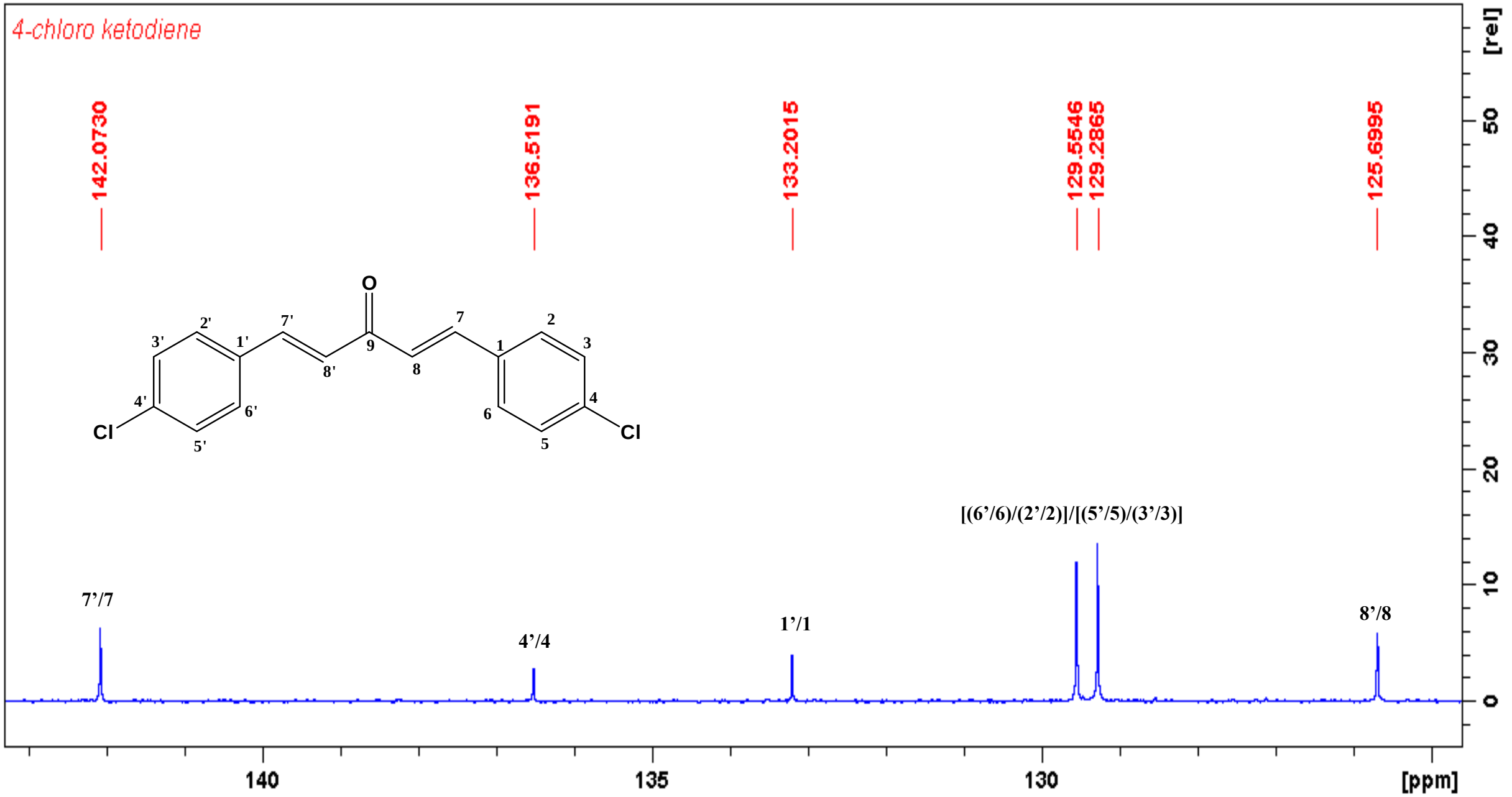
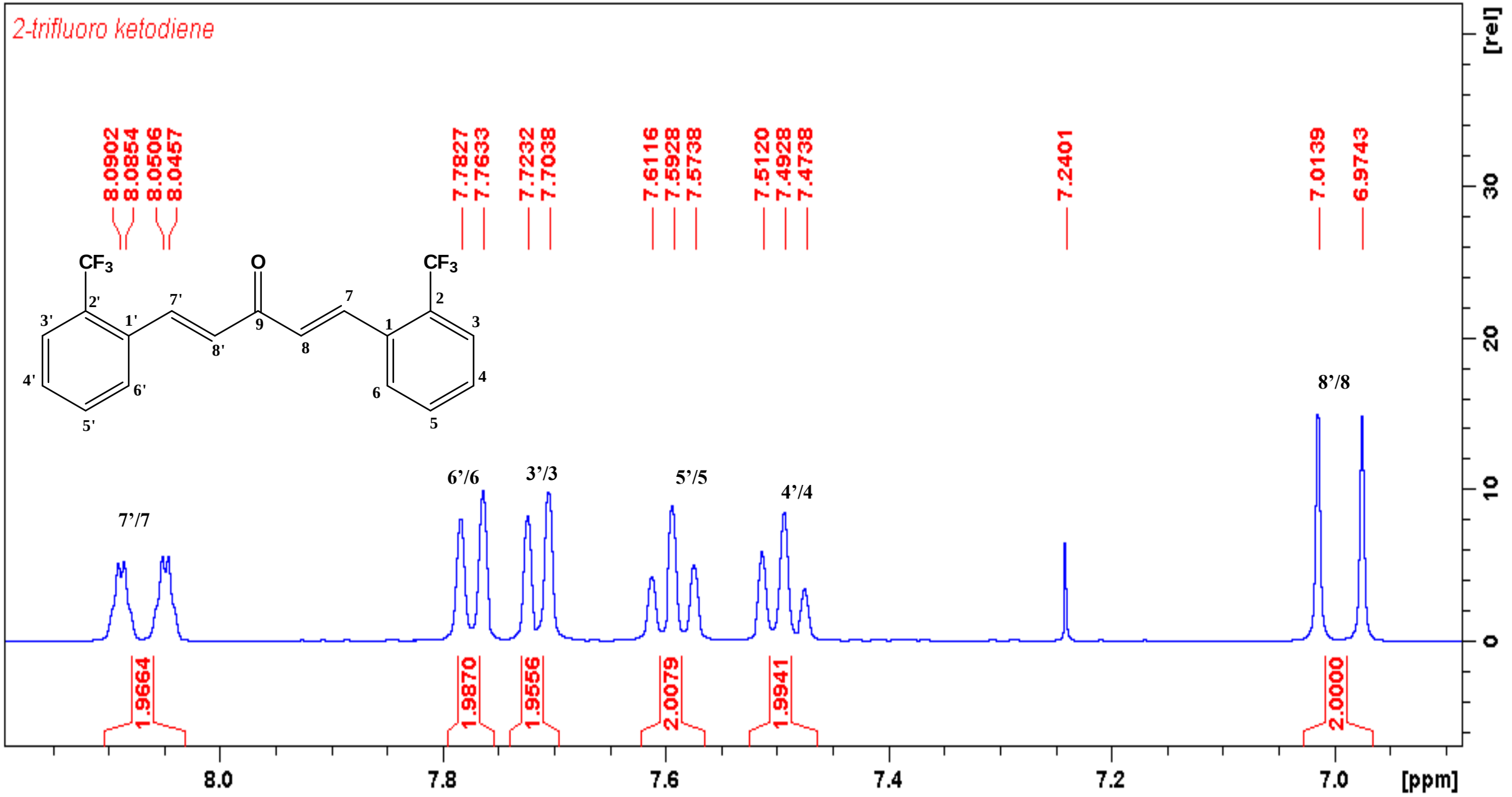
^{1}H Spectrum of Compound B-1c: (1E,4E)-1,5-bis(4-chlorophenyl)penta-1,4-dien-3-one



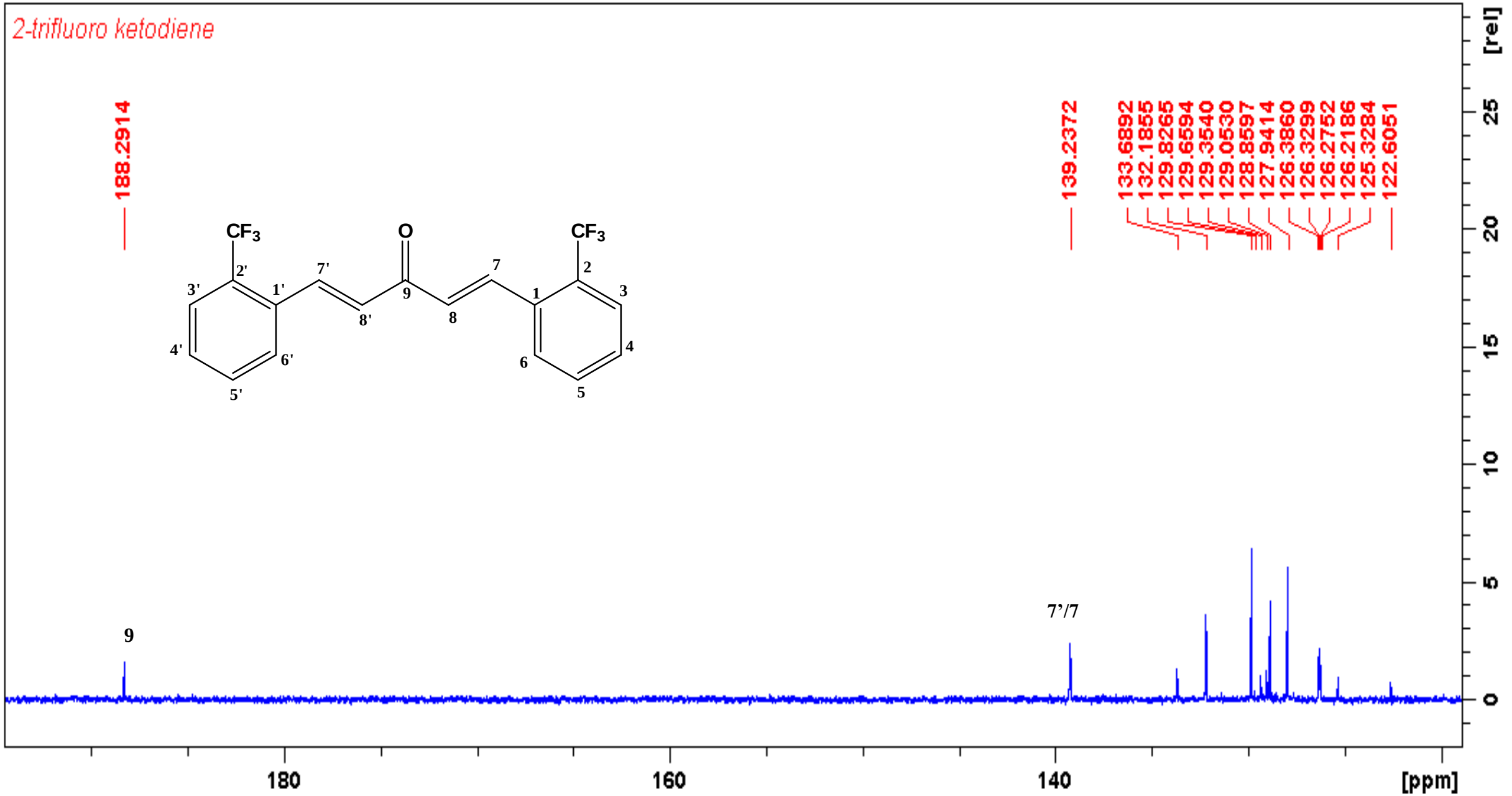
^{13}C Spectrum of Compound B-1c: (1E,4E)-1,5-bis(4-chlorophenyl)penta-1,4-dien-3-one



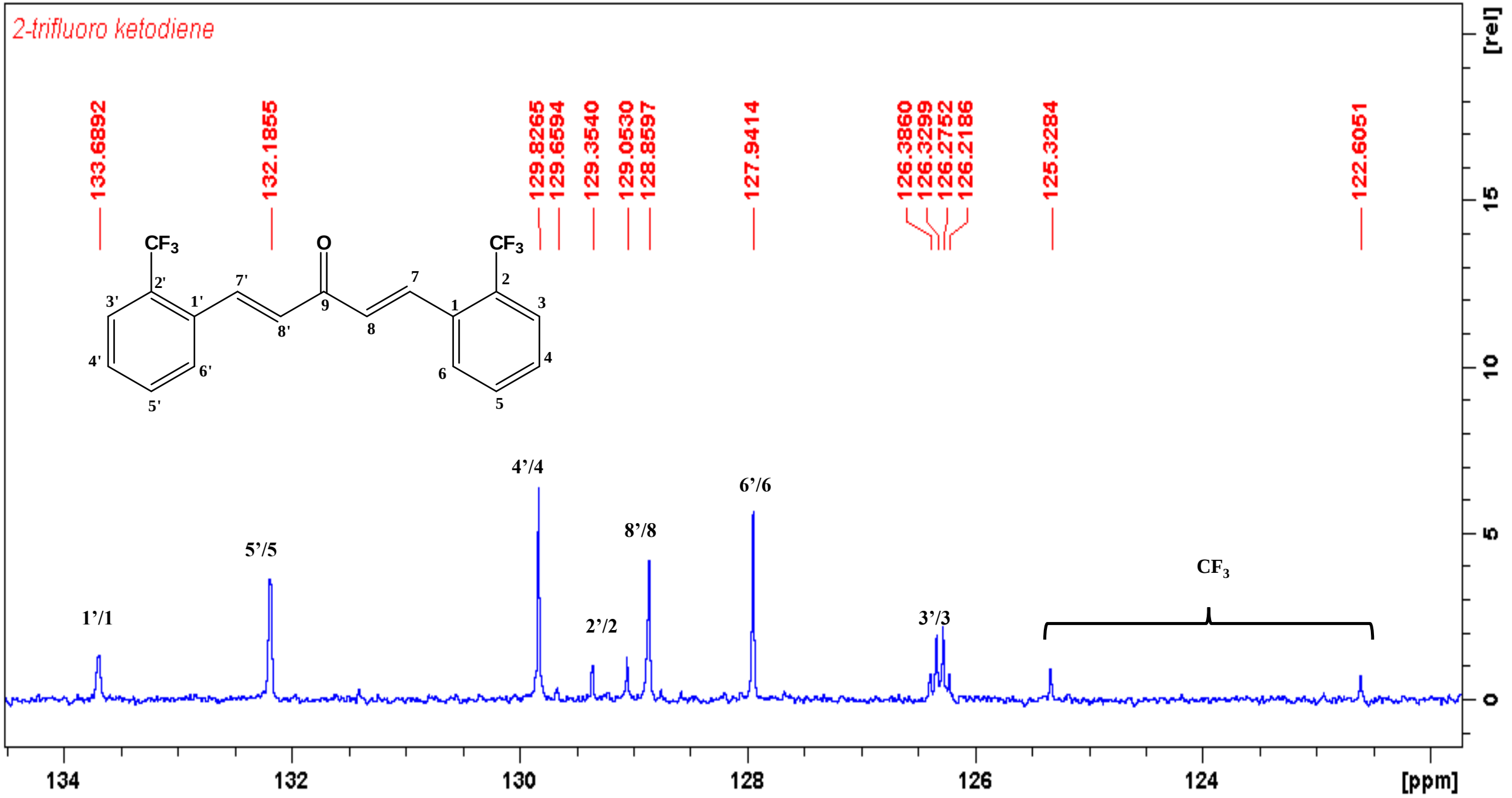
Expanded ^{13}C Spectrum of Compound B-1c: (1E,4E)-1,5-bis(4-chlorophenyl)penta-1,4-dien-3-one



^{1}H Spectrum of Compound B-1d: (1E,4E)-1,5-bis(2-(trifluoromethyl)phenyl)penta-1,4-dien-3-one



^{13}C Spectrum of Compound B-1d: (1E,4E)-1,5-bis(2-(trifluoromethyl)phenyl)penta-1,4-dien-3-one



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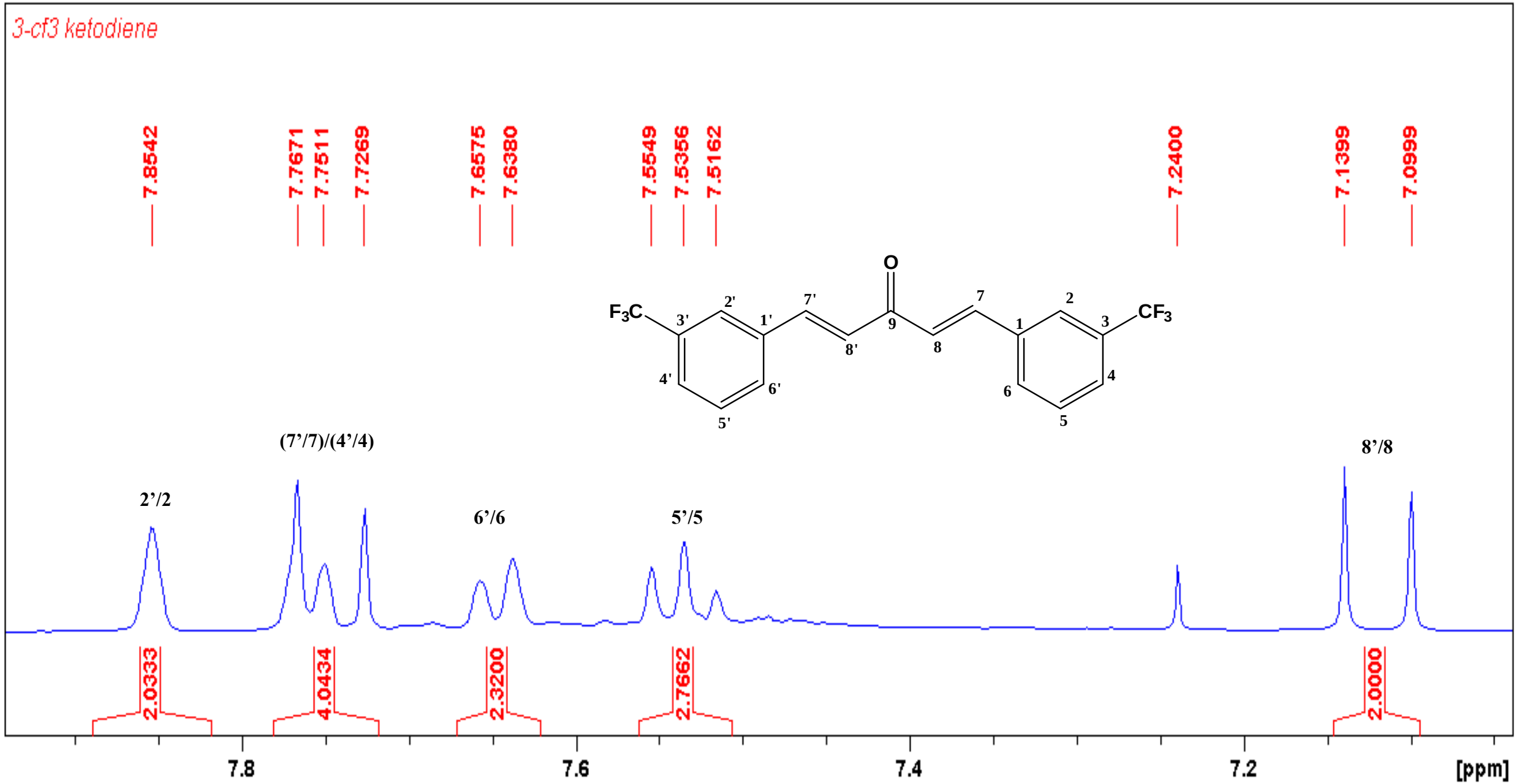
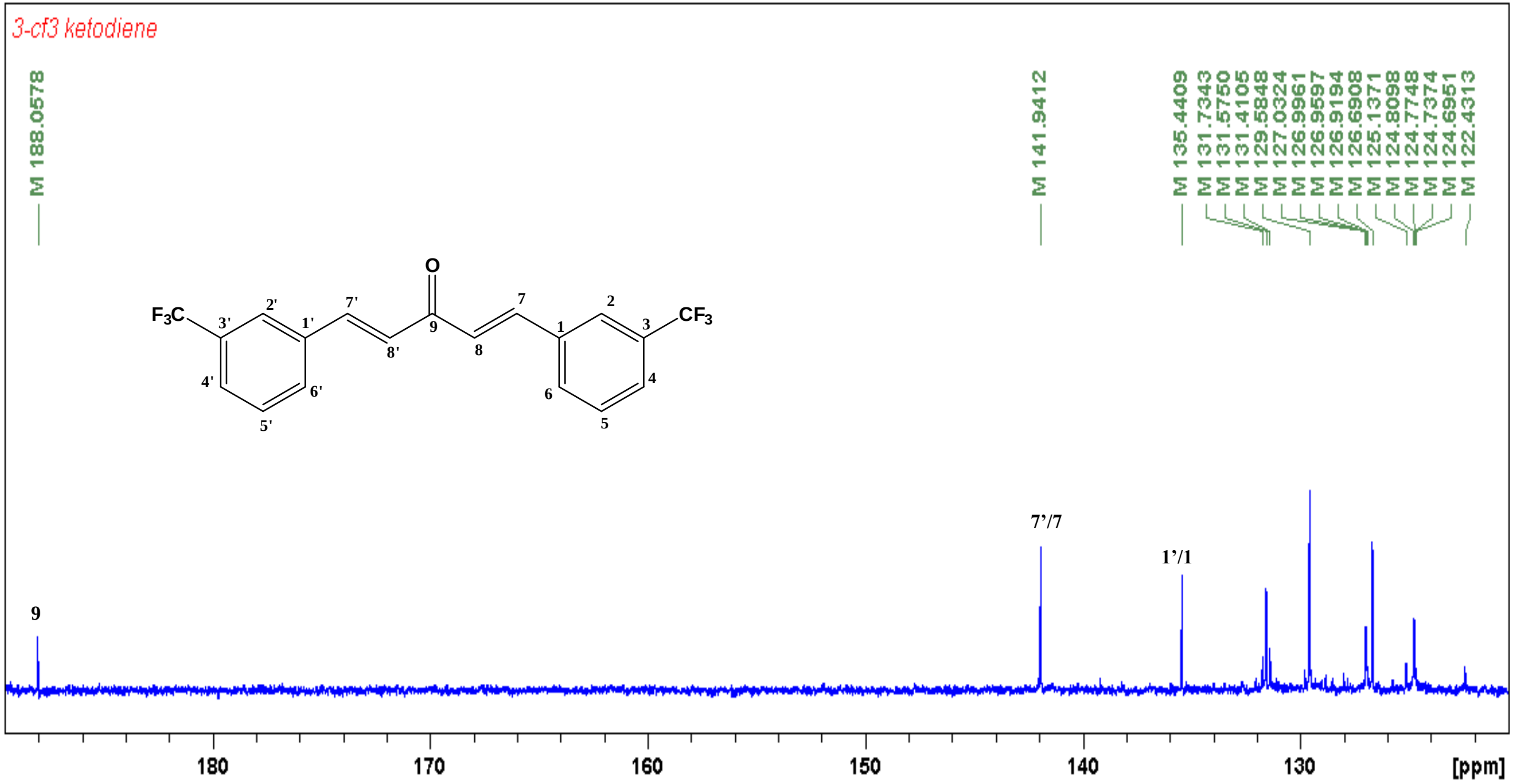
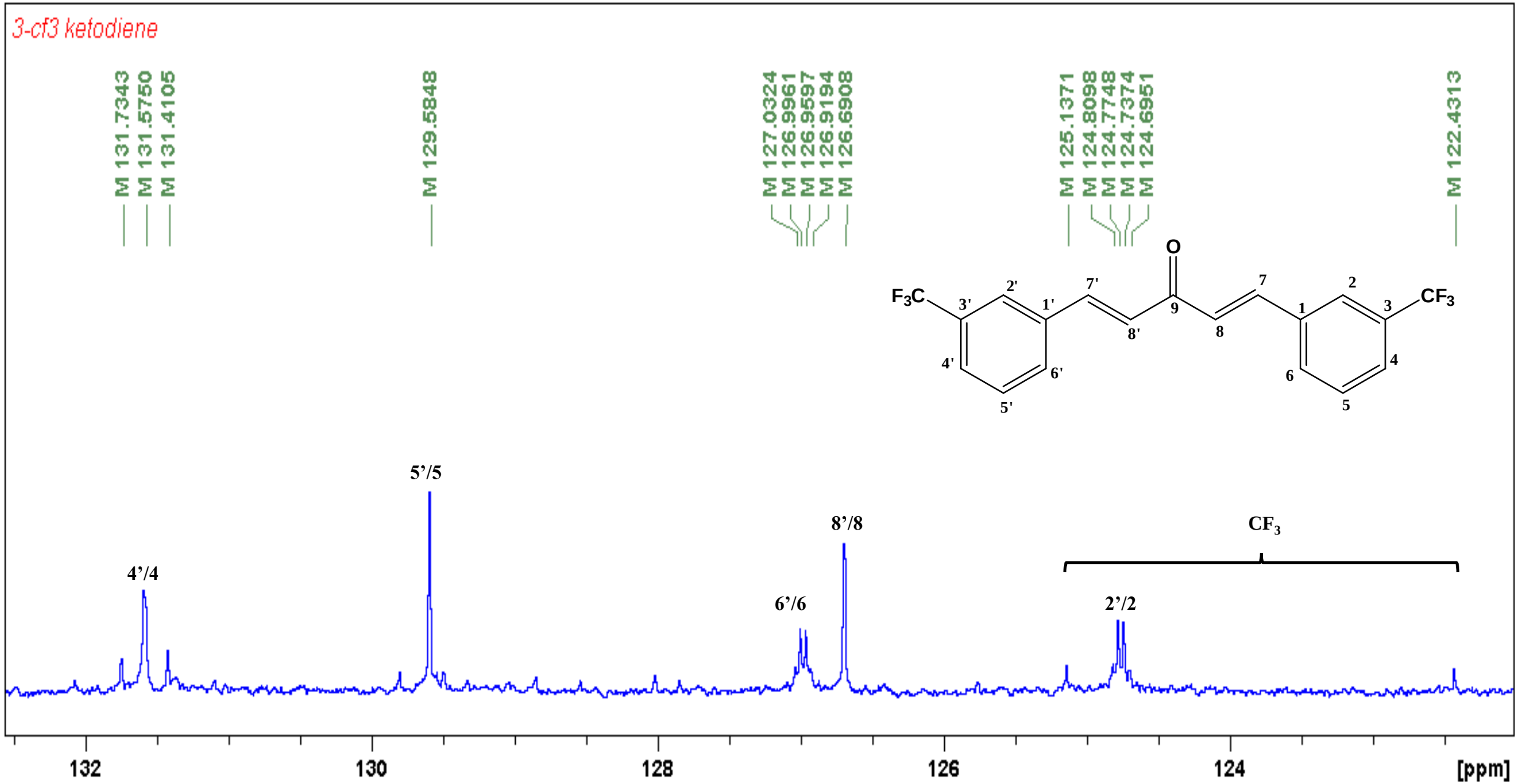
Expanded ^{13}C Spectrum of Compound B-1d: (1E,4E)-1,5-bis(2-(trifluoromethyl)phenyl)penta-1,4-dien-3-one



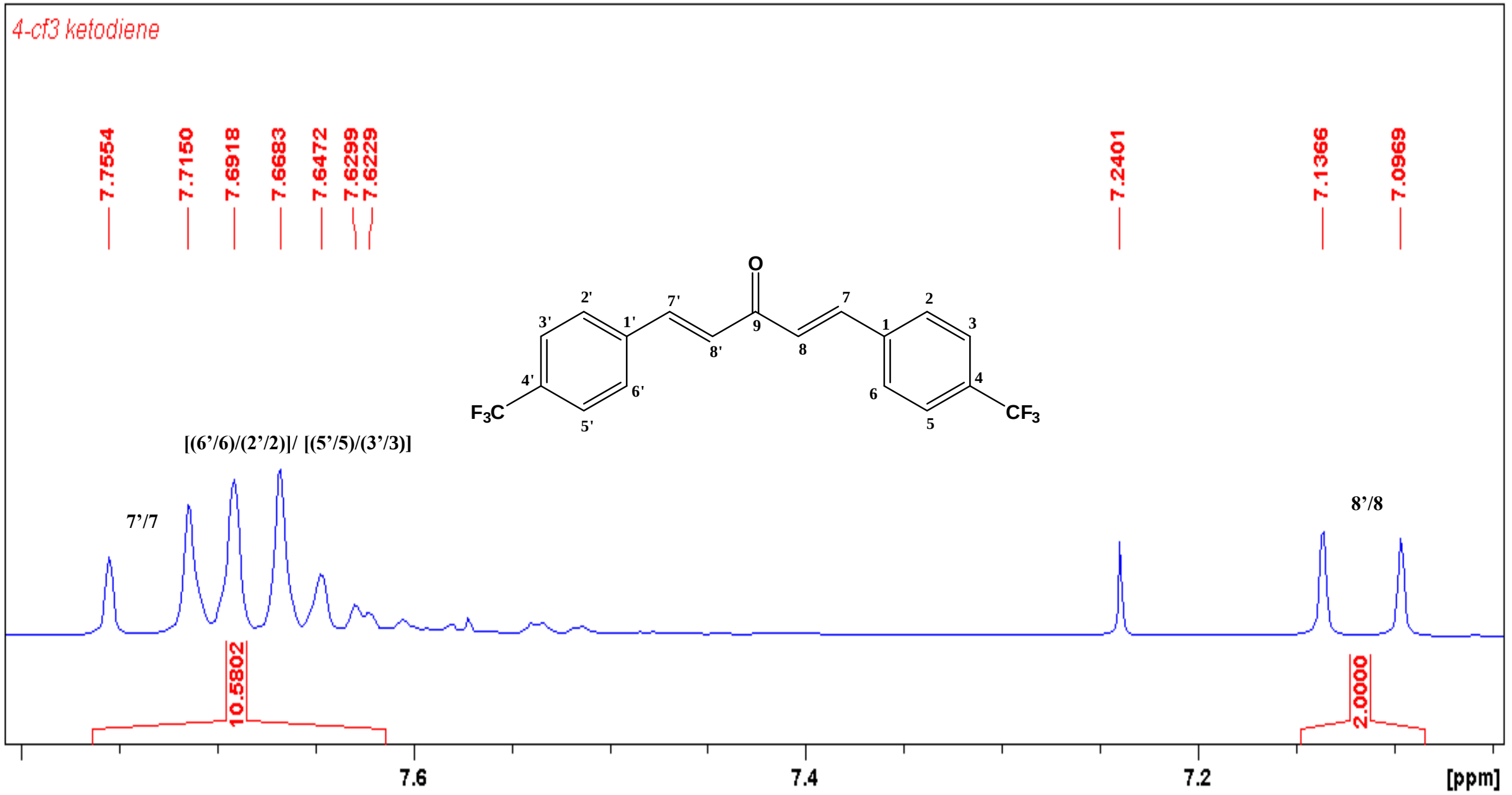
[1]H Spectrum of Compound B-1e: (1E,4E)-1,5-bis(3-(trifluoromethyl)phenyl)penta-1,4-dien-3-one



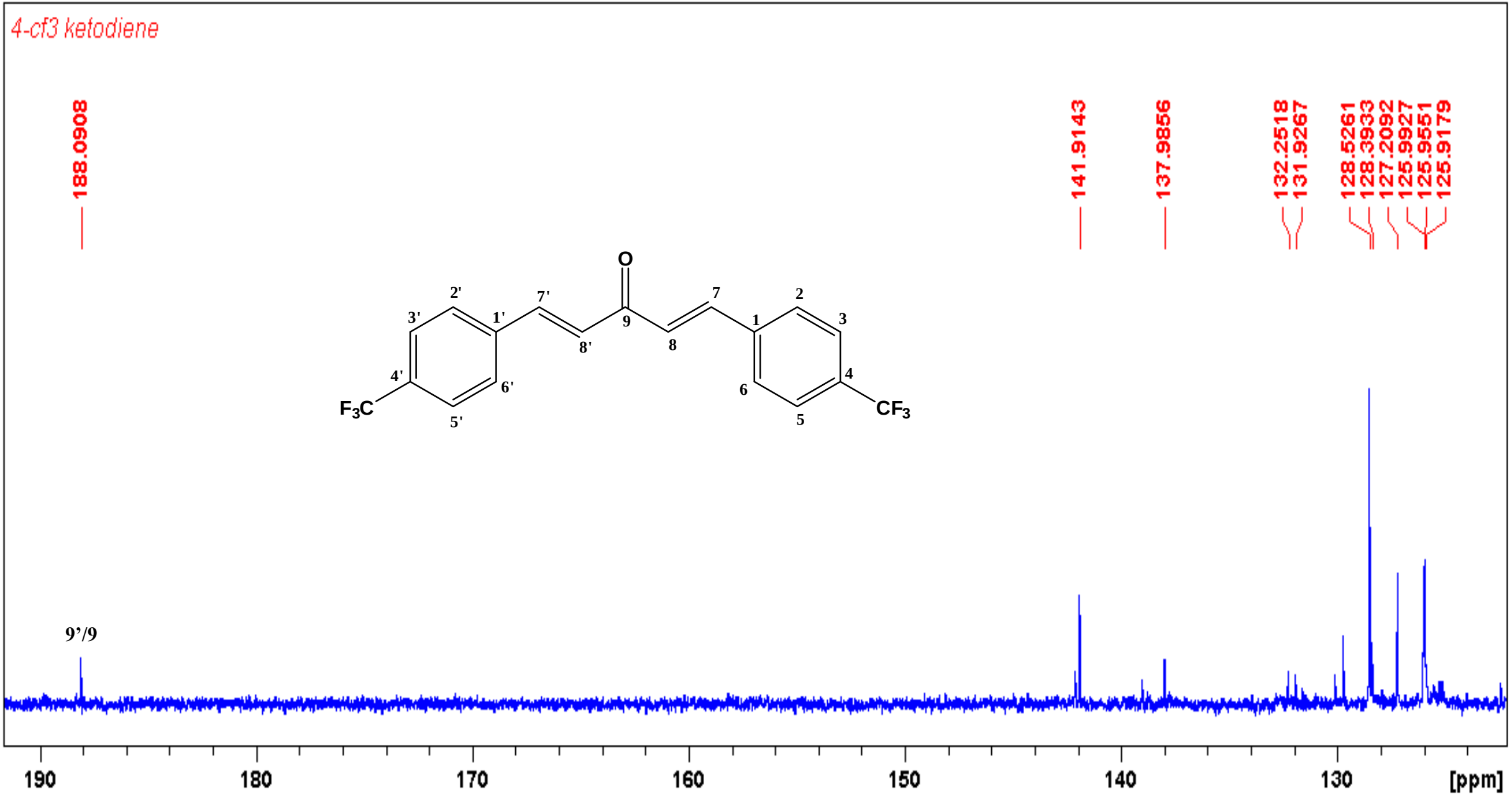
^{13}C Spectrum of Compound B-1e: (1E,4E)-1,5-bis(3-(trifluoromethyl)phenyl)penta-1,4-dien-3-one



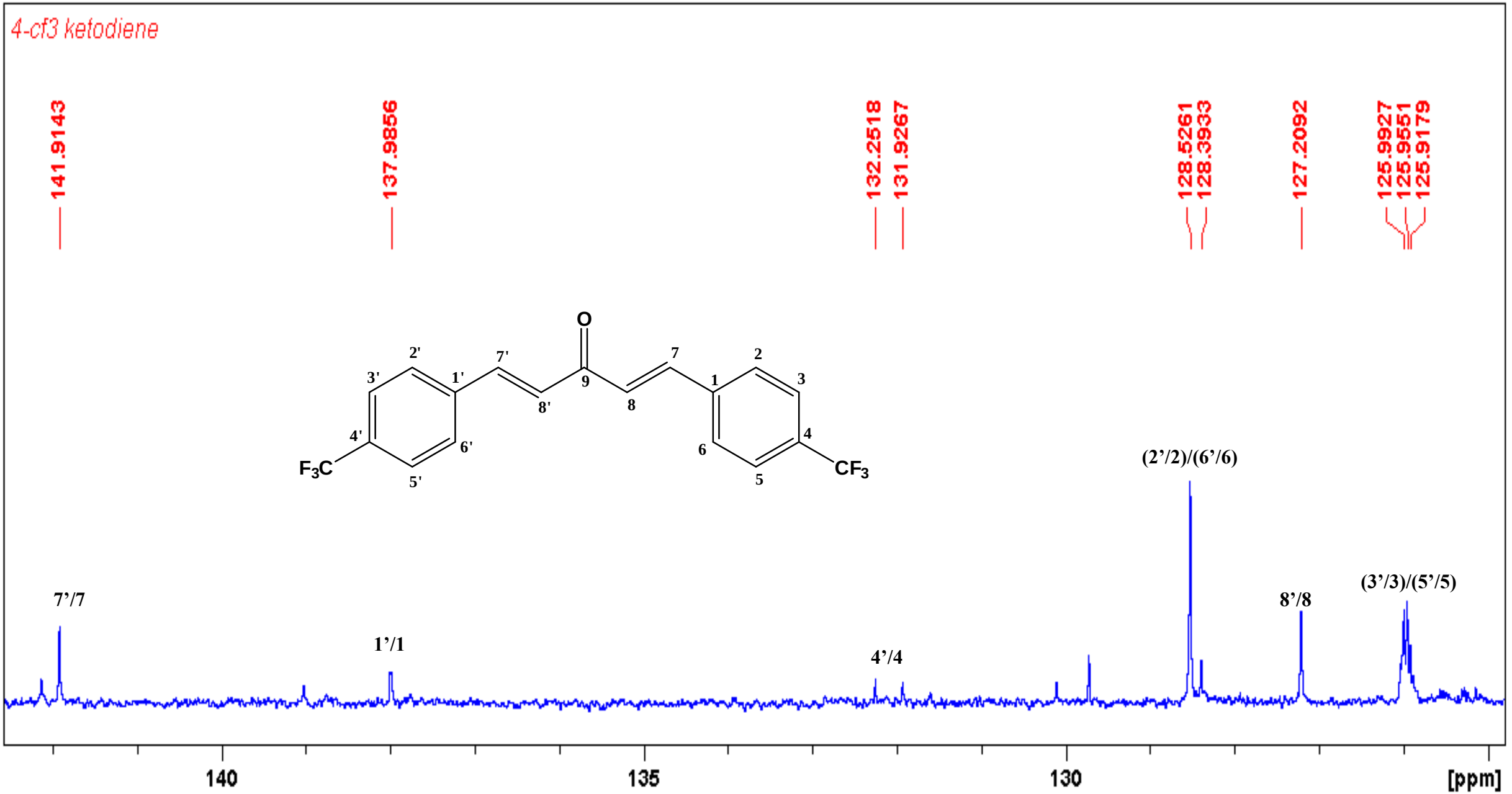
Expanded ^{13}C Spectrum of Compound B-1e: (1E,4E)-1,5-bis(3-(trifluoromethyl)phenyl)penta-1,4-dien-3-one



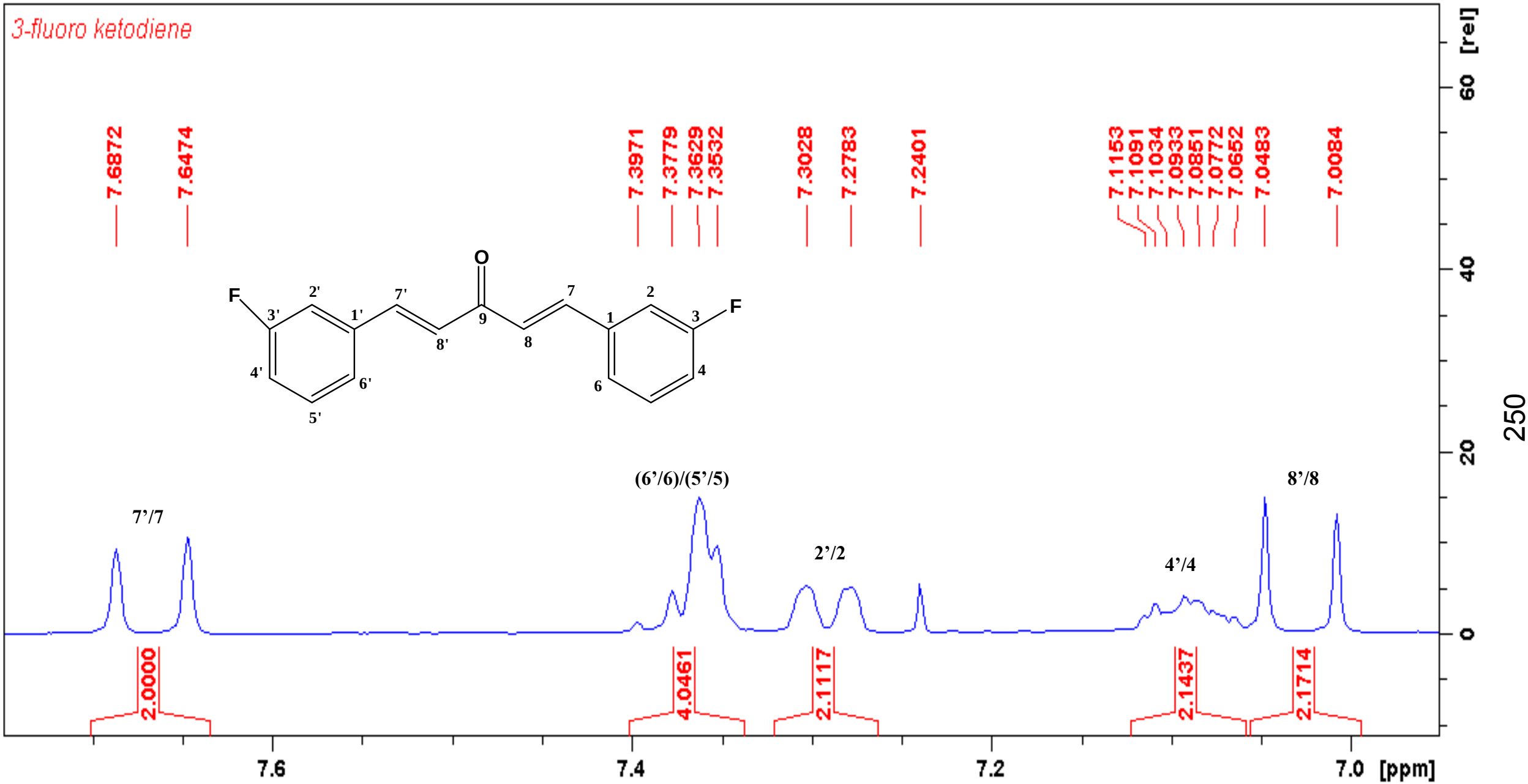
^{1}H Spectrum of Compound B-1f: (1E,4E)-1,5-bis(4-(trifluoromethyl)phenyl)penta-1,4-dien-3-one



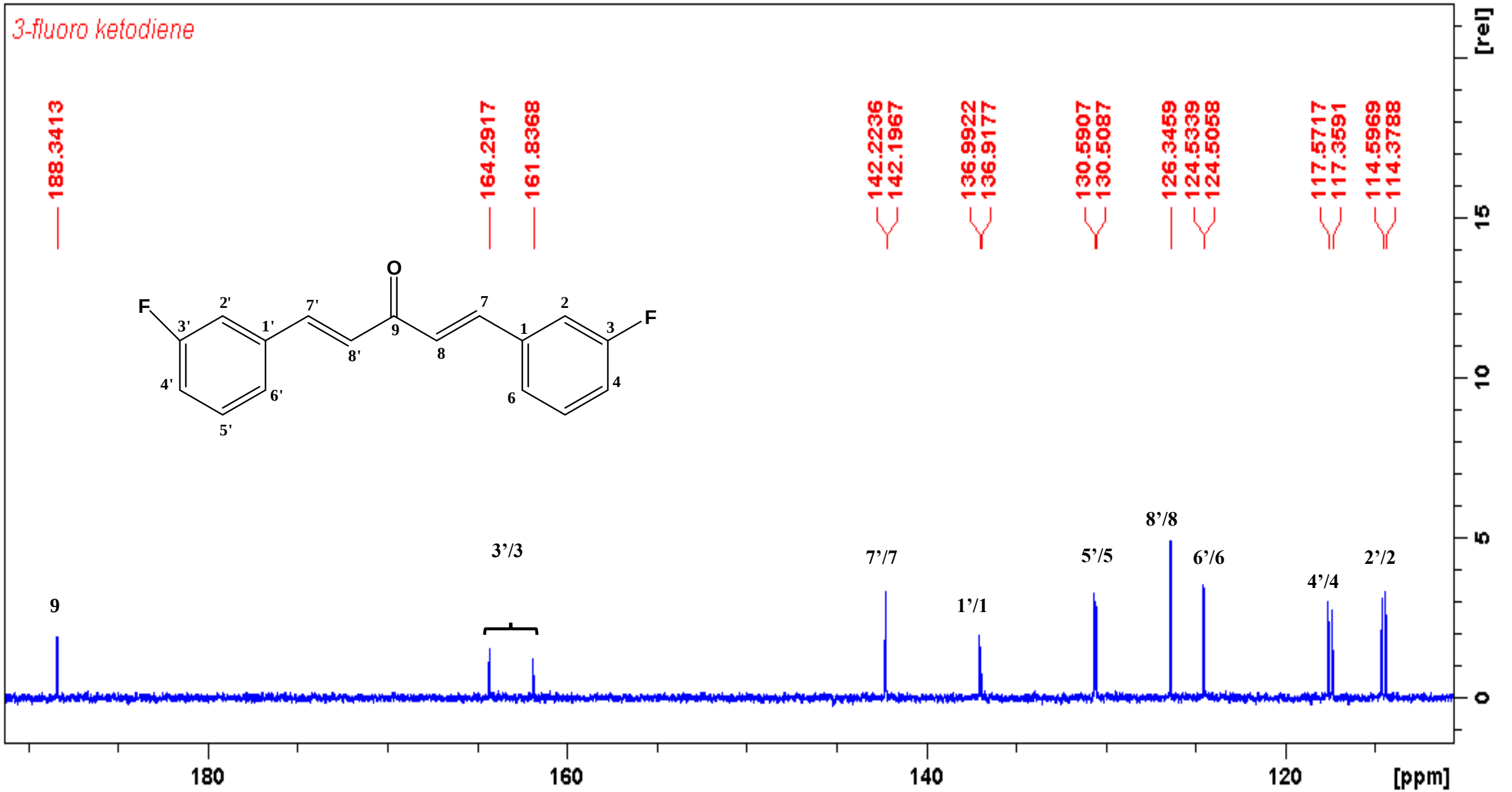
^{13}C Spectrum of Compound B-1f: (1E,4E)-1,5-bis(4-(trifluoromethyl)phenyl)penta-1,4-dien-3-one



Expanded ^{13}C Spectrum of Compound B-1f: (1E,4E)-1,5-bis(4-(trifluoromethyl)phenyl)penta-1,4-dien-3-one



^{1}H Spectrum of Compound B-1g: (1E,4E)-1,5-bis(3-fluorophenyl)penta-1,4-dien-3-one



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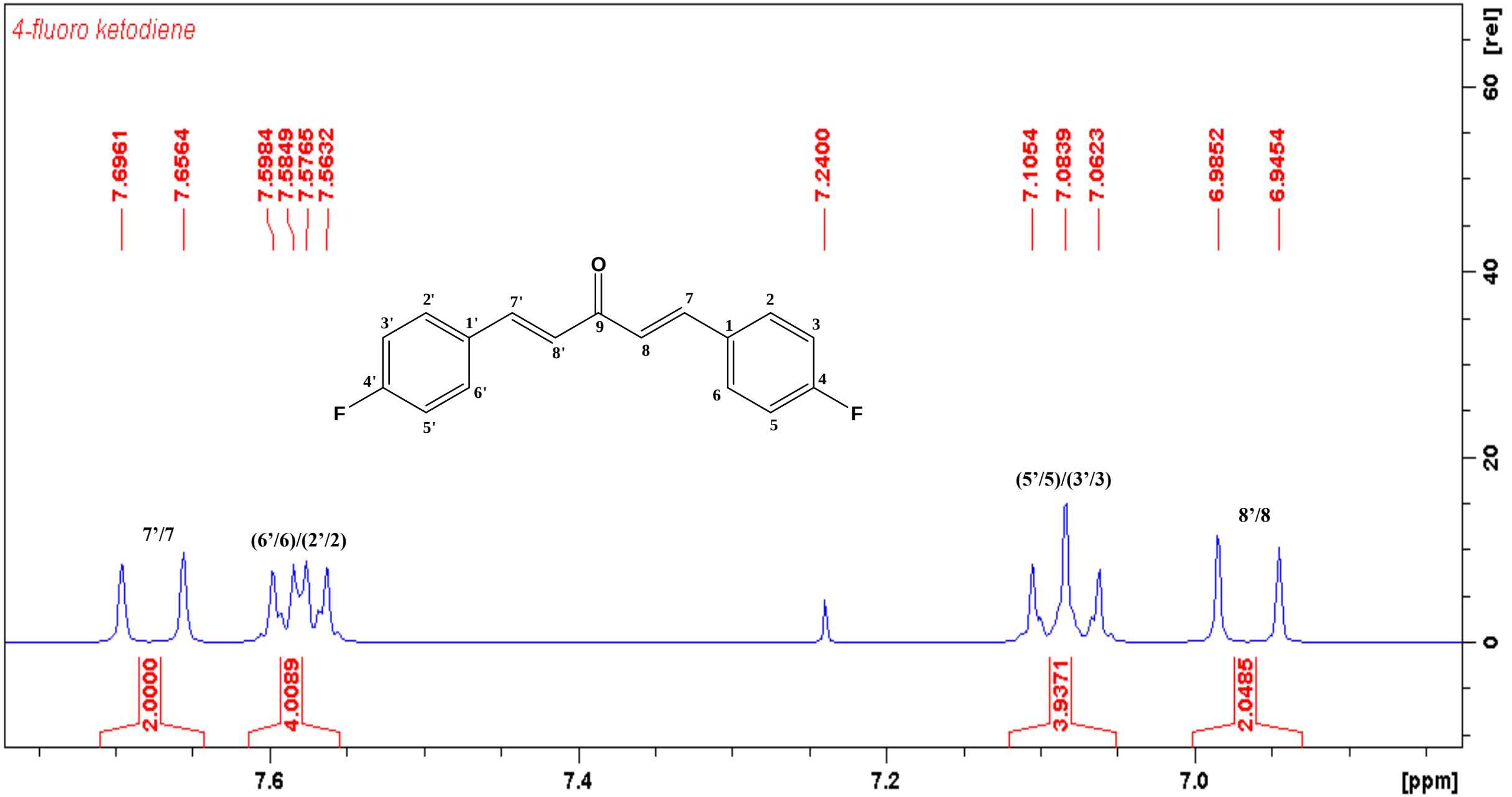
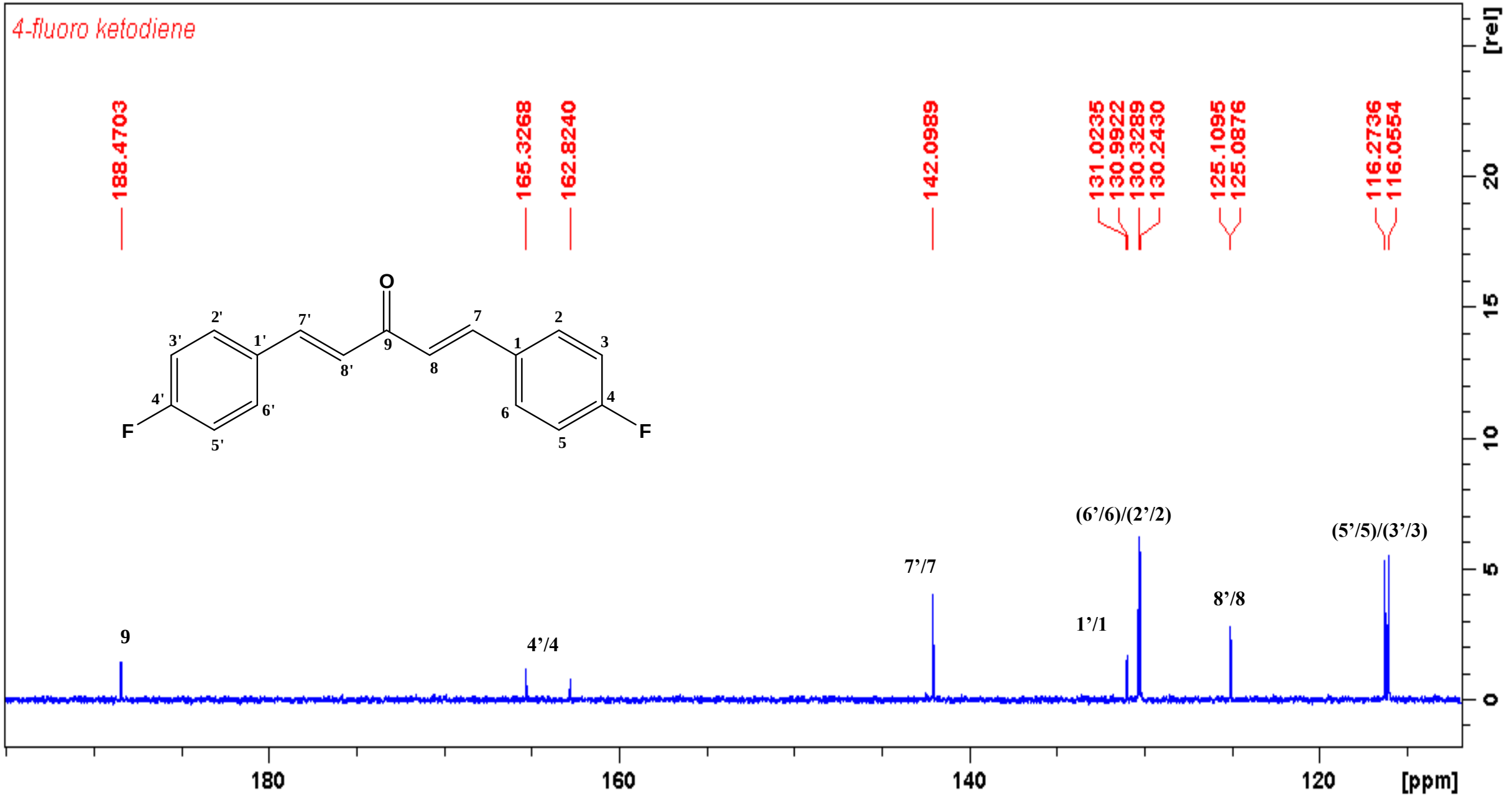
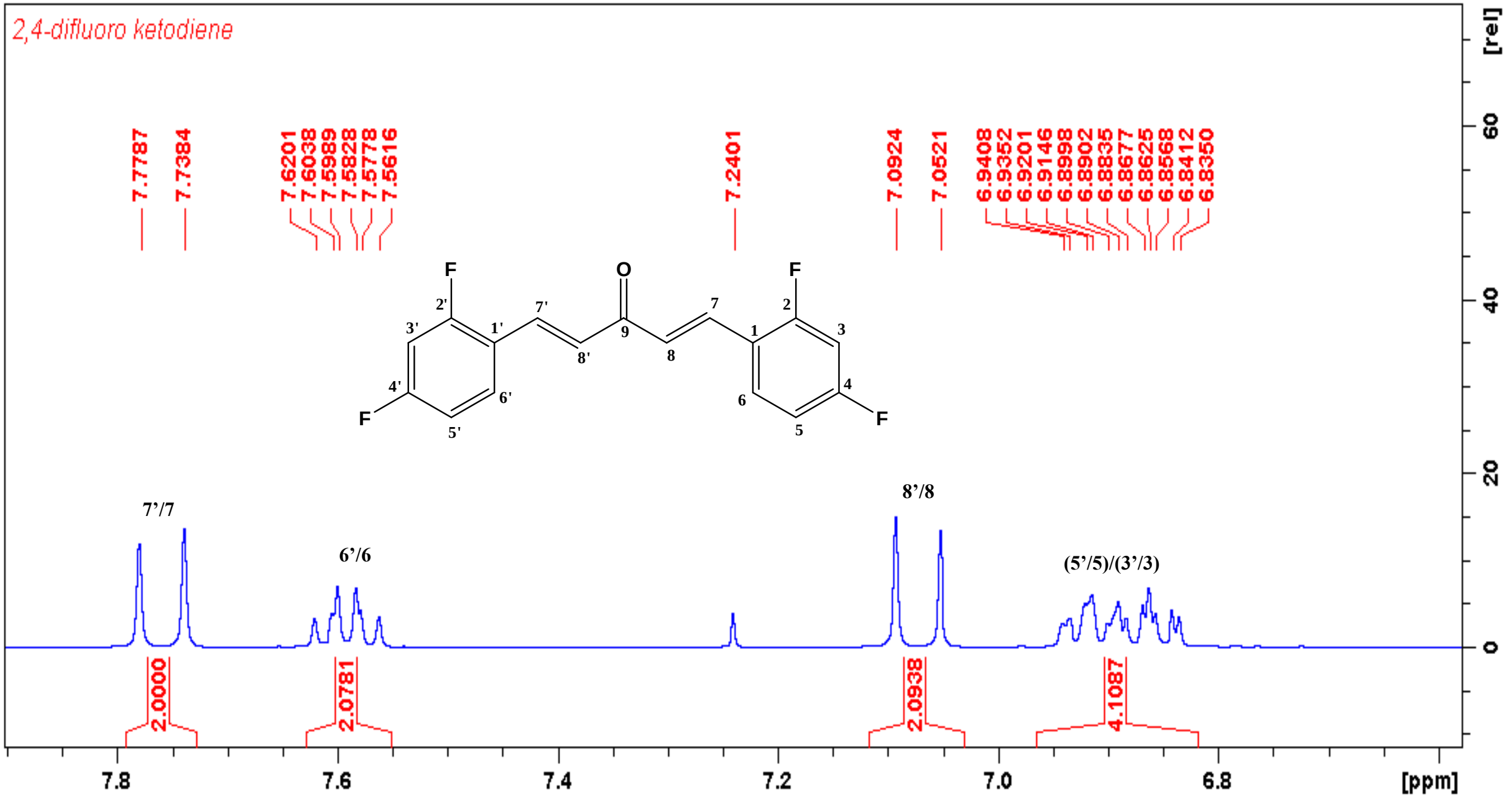
^{13}C Spectrum of Compound B-1g: (1E,4E)-1,5-bis(3-fluorophenyl)penta-1,4-dien-3-one



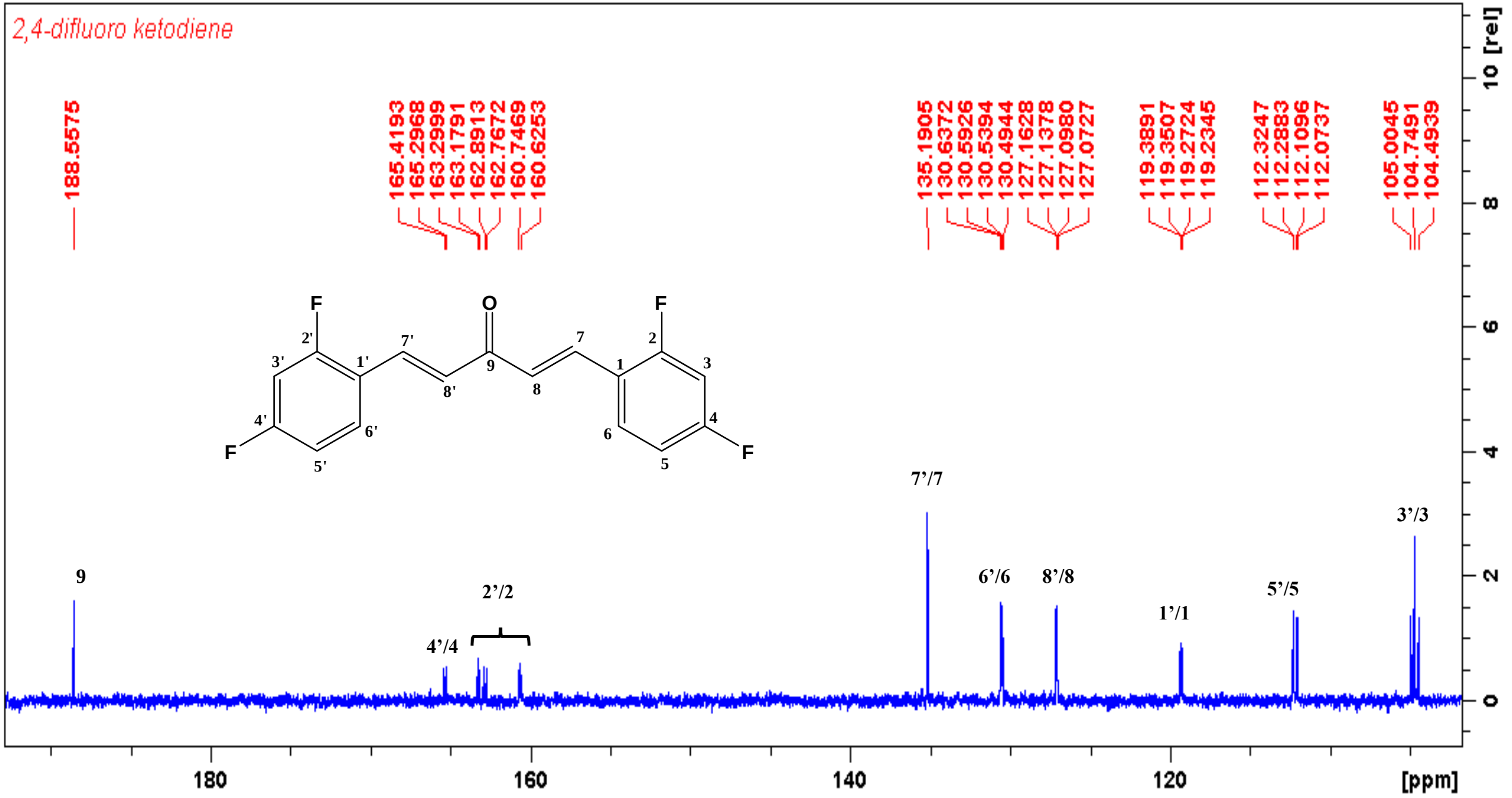
^{1}H Spectrum of Compound B-1h: (1E,4E)-1,5-bis(4-fluorophenyl)penta-1,4-dien-3-one



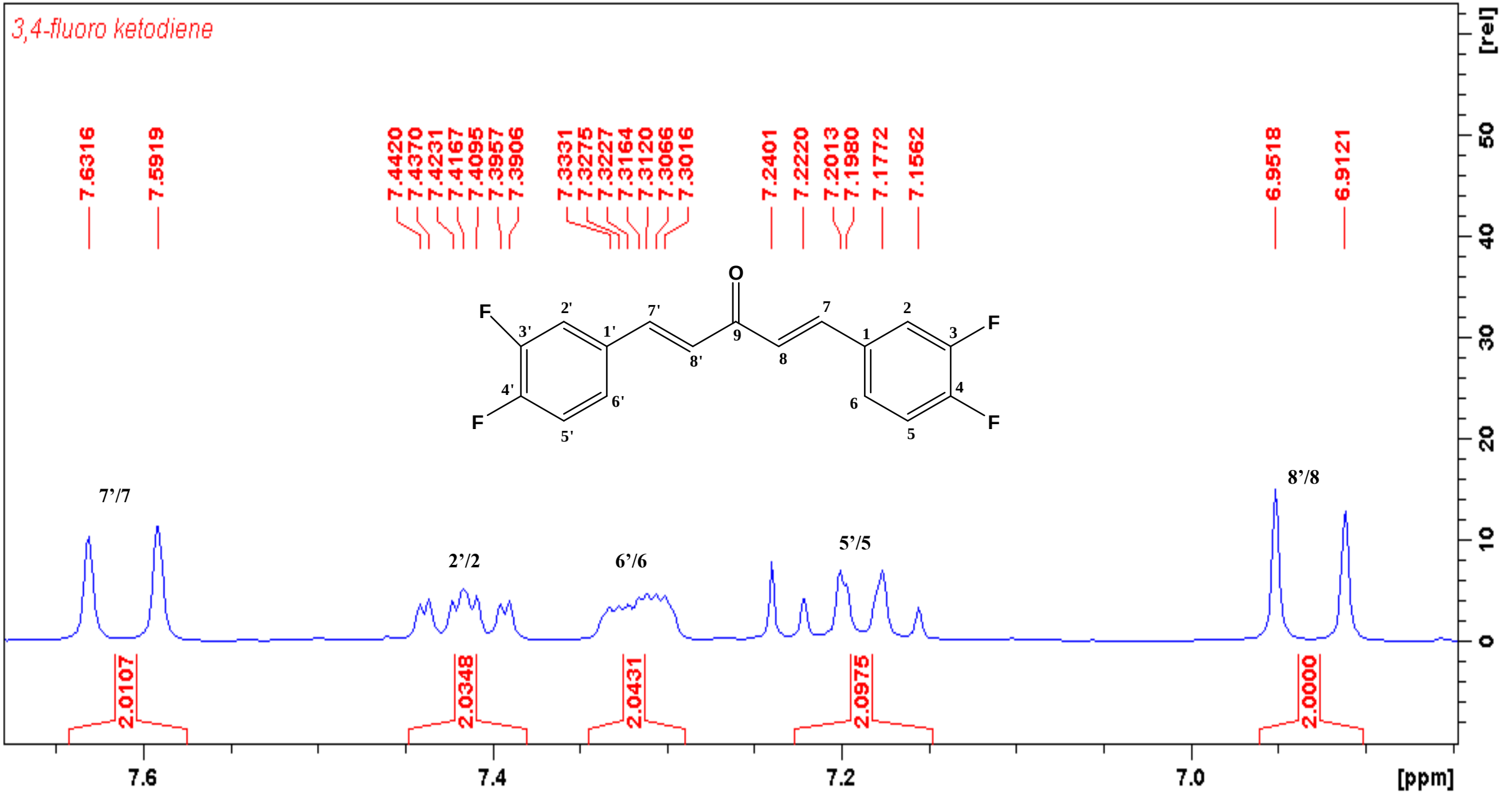
^{13}C Spectrum of Compound B-1h: (1E,4E)-1,5-bis(4-fluorophenyl)penta-1,4-dien-3-one



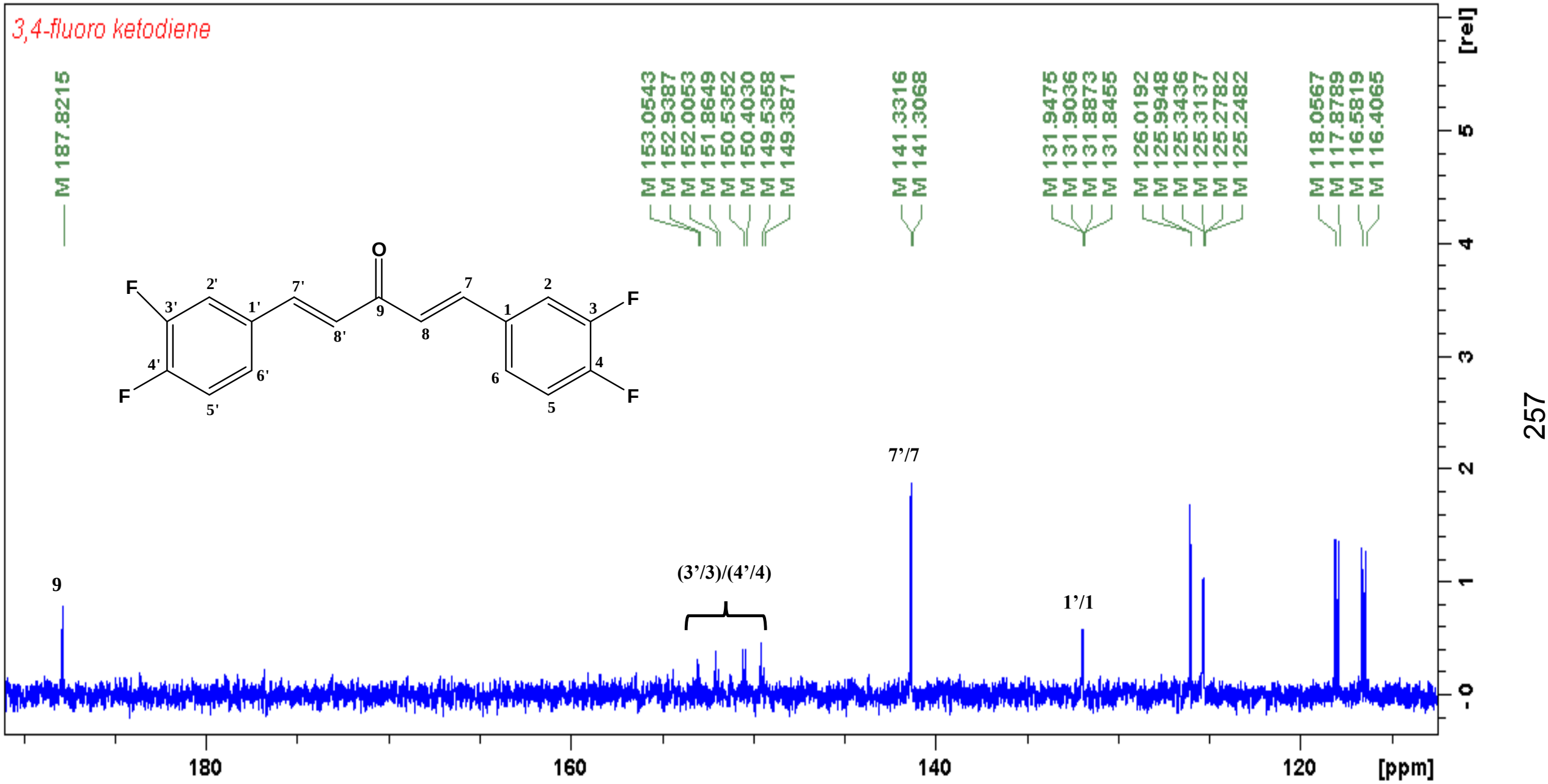
^{1}H Spectrum of Compound B-1i: (1E,4E)-1,5-bis(2,4-difluorophenyl)penta-1,4-dien-3-one



^{13}C Spectrum of Compound B-1i: (1E,4E)-1,5-bis(2,4-difluorophenyl)penta-1,4-dien-3-one



^{1}H Spectrum of Compound B-1j: (1E,4E)-1,5-bis(3,4-difluorophenyl)penta-1,4-dien-3-one



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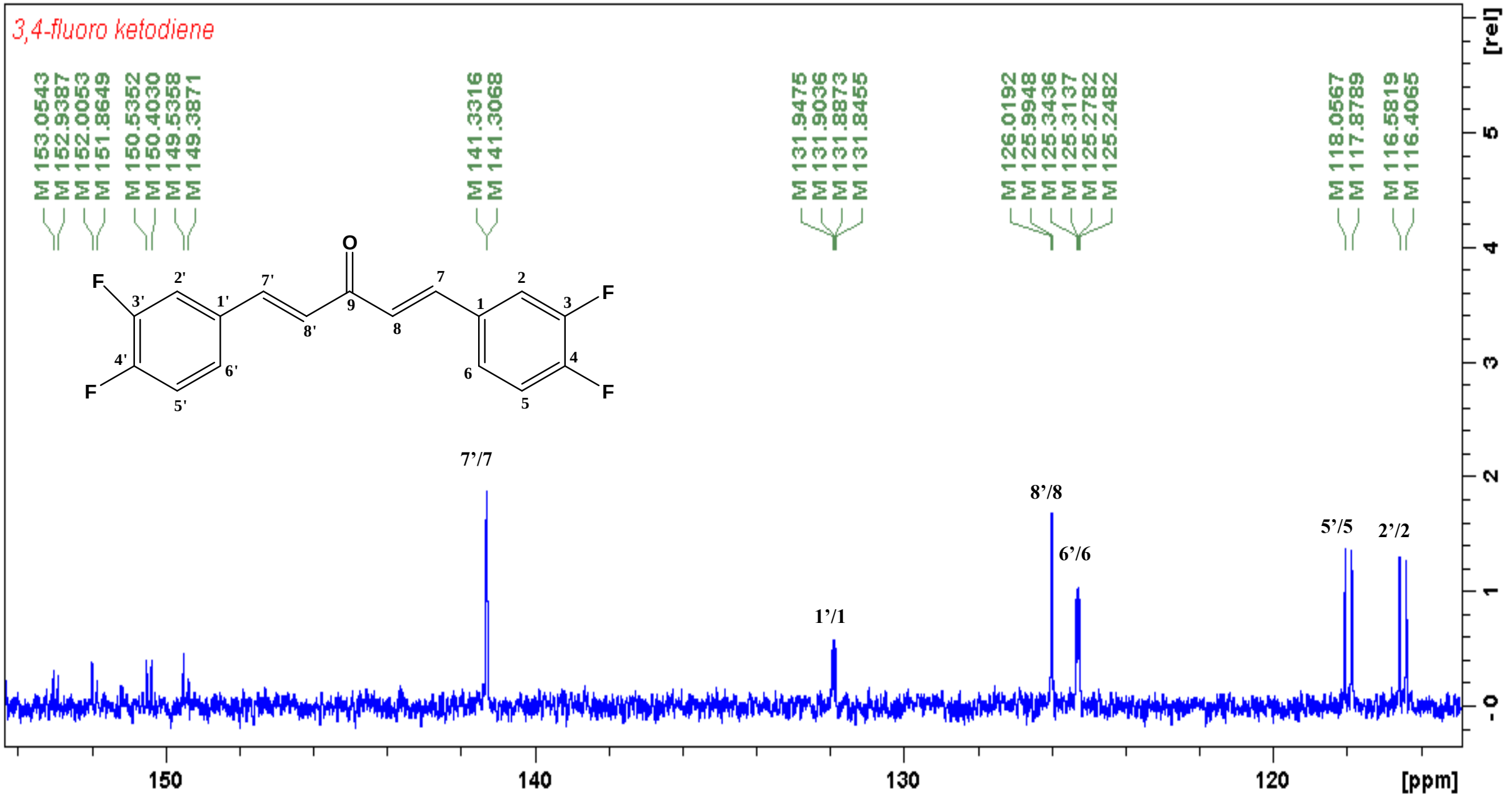
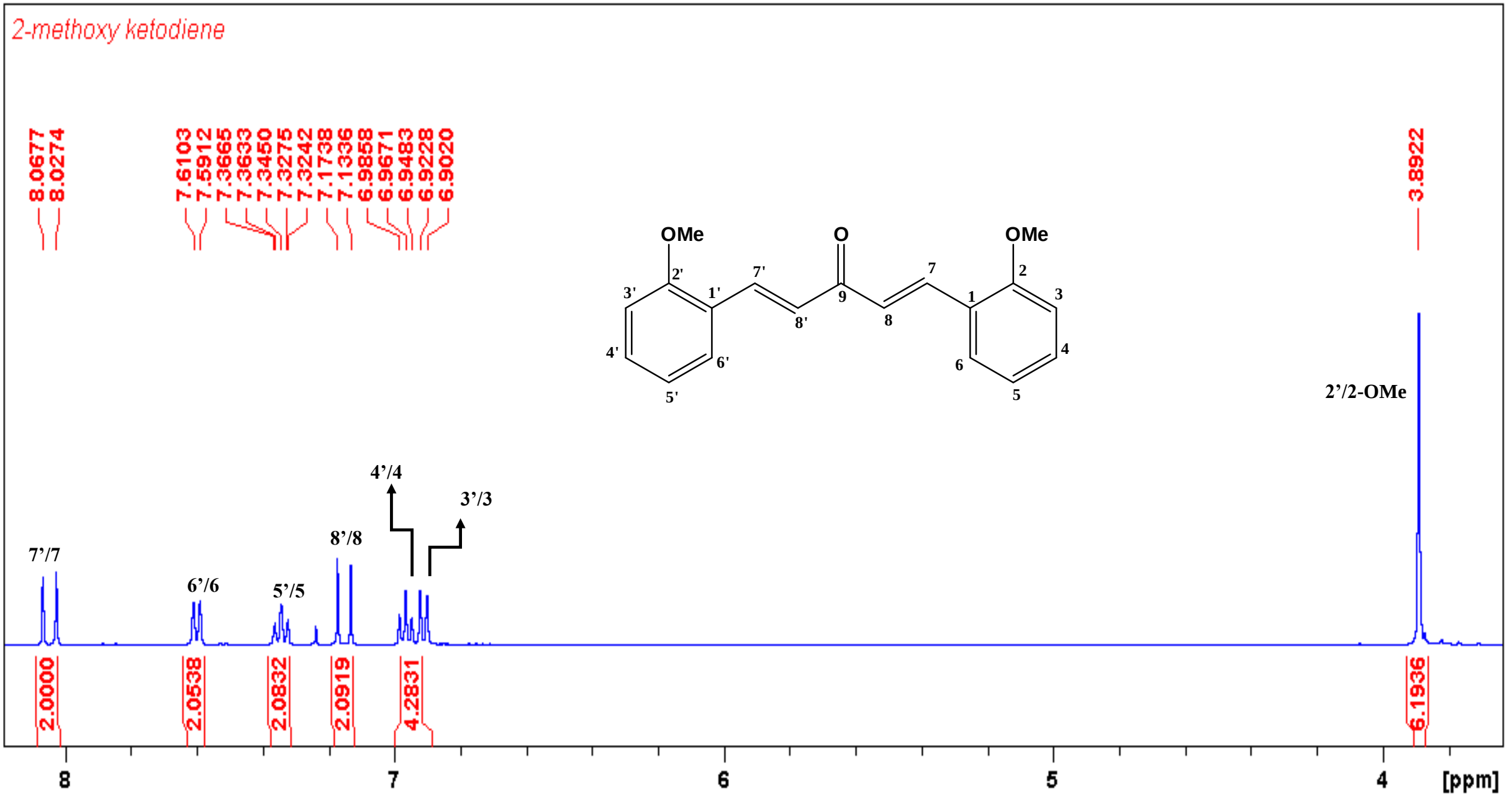
^{13}C Spectrum of Compound B-1j: (1E,4E)-1,5-bis(3,4-difluorophenyl)penta-1,4-dien-3-one



Expanded ^{13}C Spectrum of Compound B-1j: (1E,4E)-1,5-bis(3,4-difluorophenyl)penta-1,4-dien-3-one



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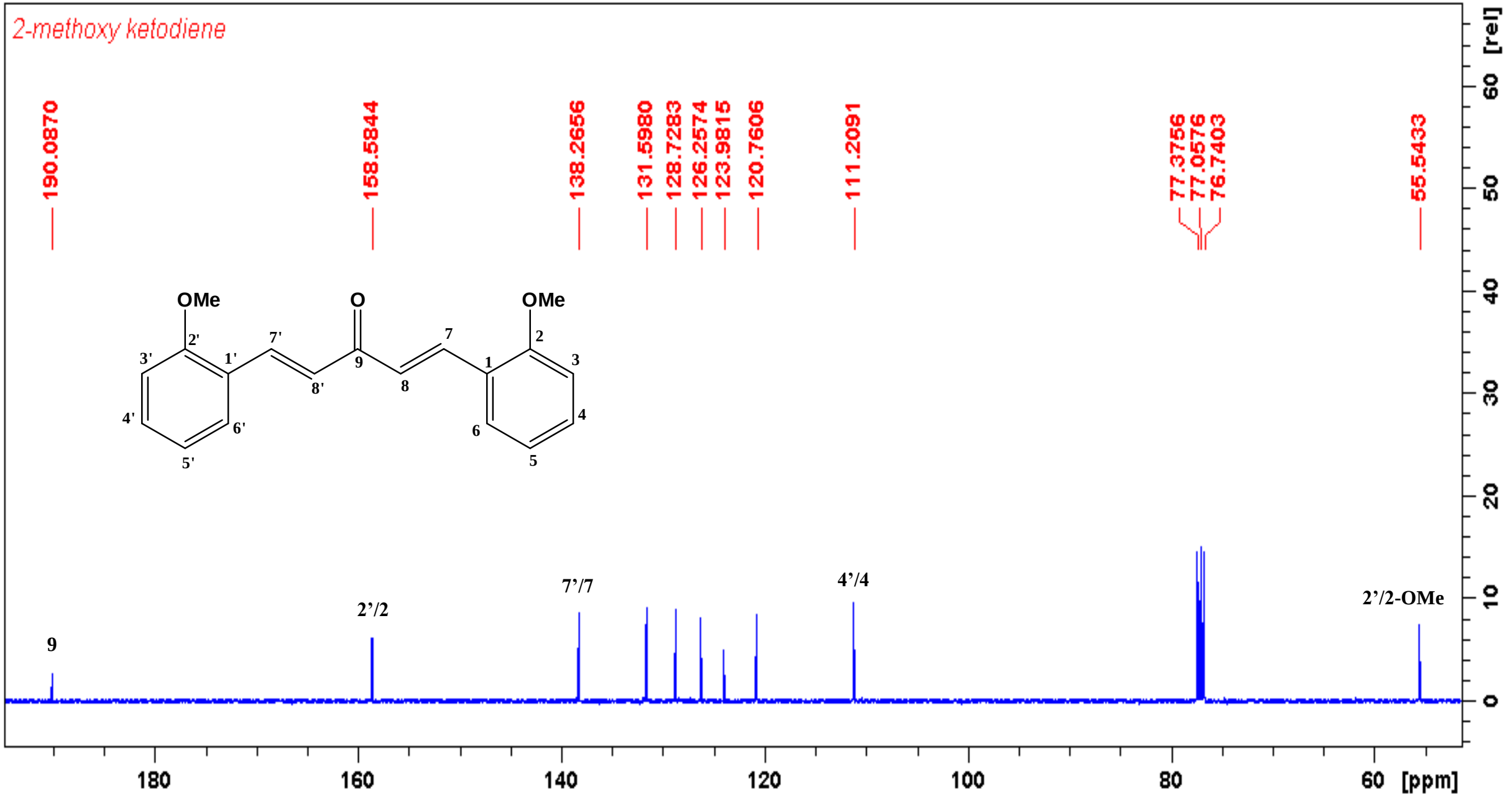
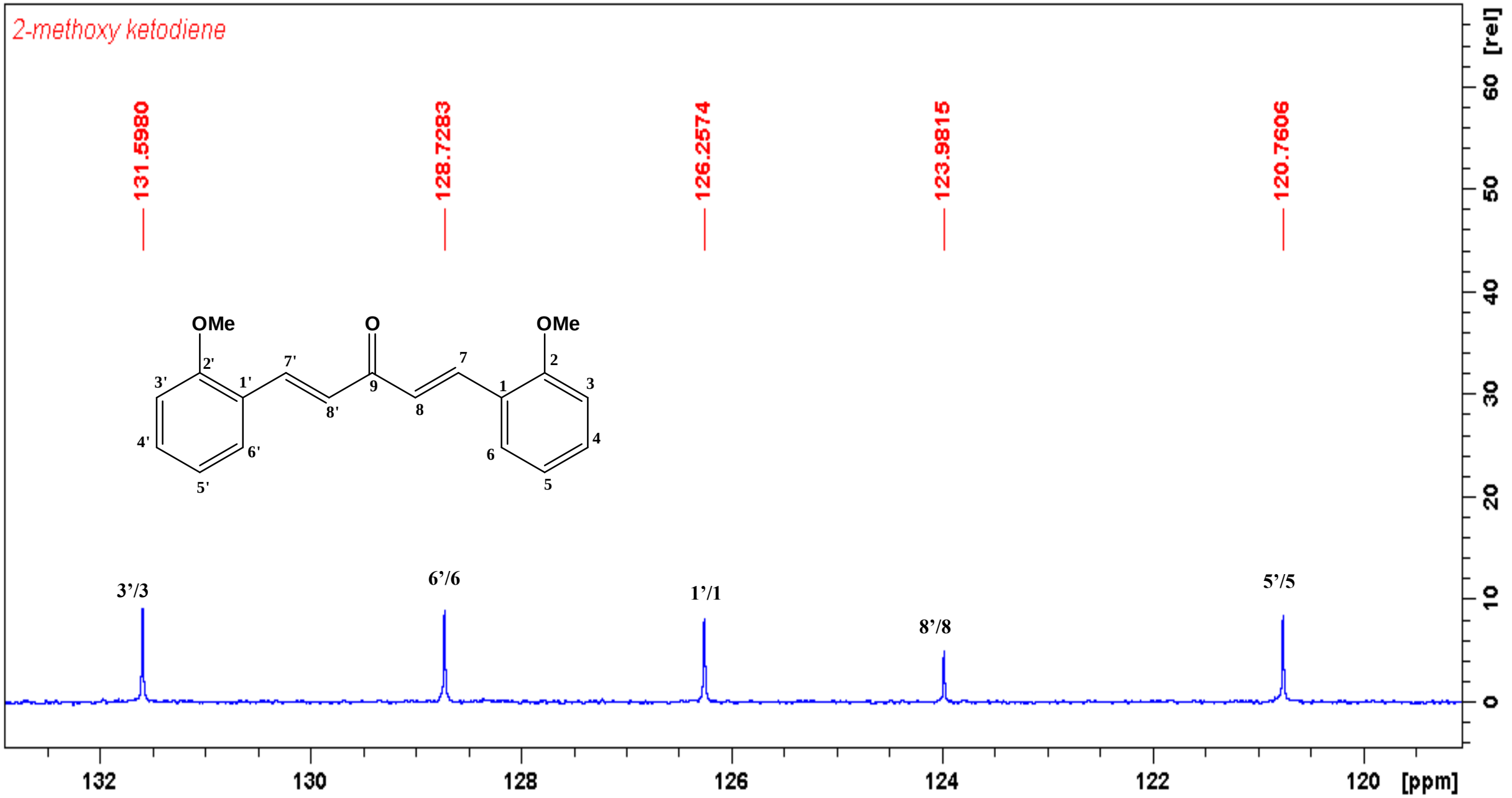
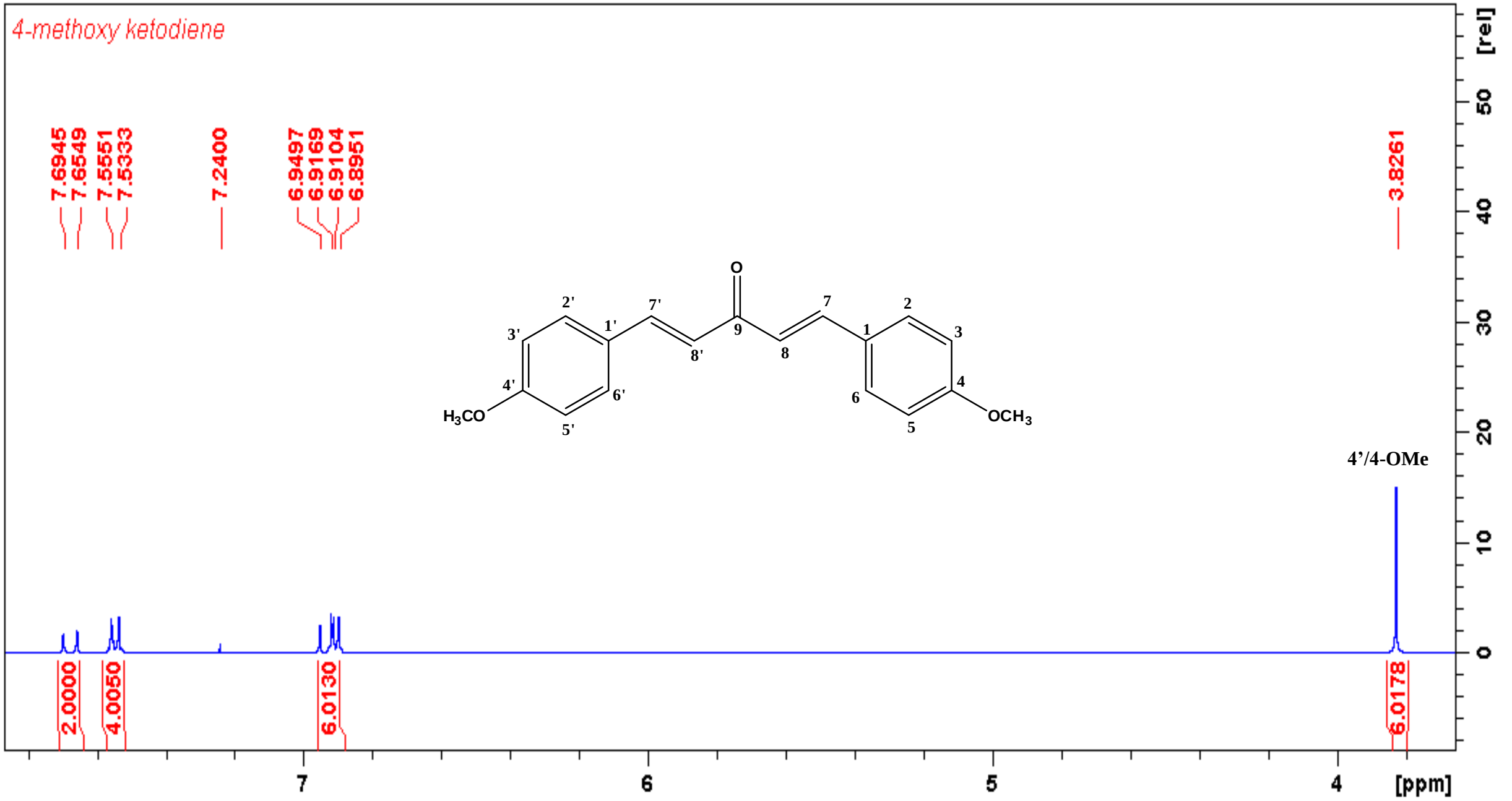
^{1}H Spectrum of Compound B-1k: (1E,4E)-1,5-bis(2-methoxyphenyl)penta-1,4-dien-3-one



^{13}C Spectrum of Compound B-1k: (1E,4E)-1,5-bis(2-methoxyphenyl)penta-1,4-dien-3-one



Expanded ^{13}C Spectrum of Compound B-1k: (1E,4E)-1,5-bis(2-methoxyphenyl)penta-1,4-dien-3-one



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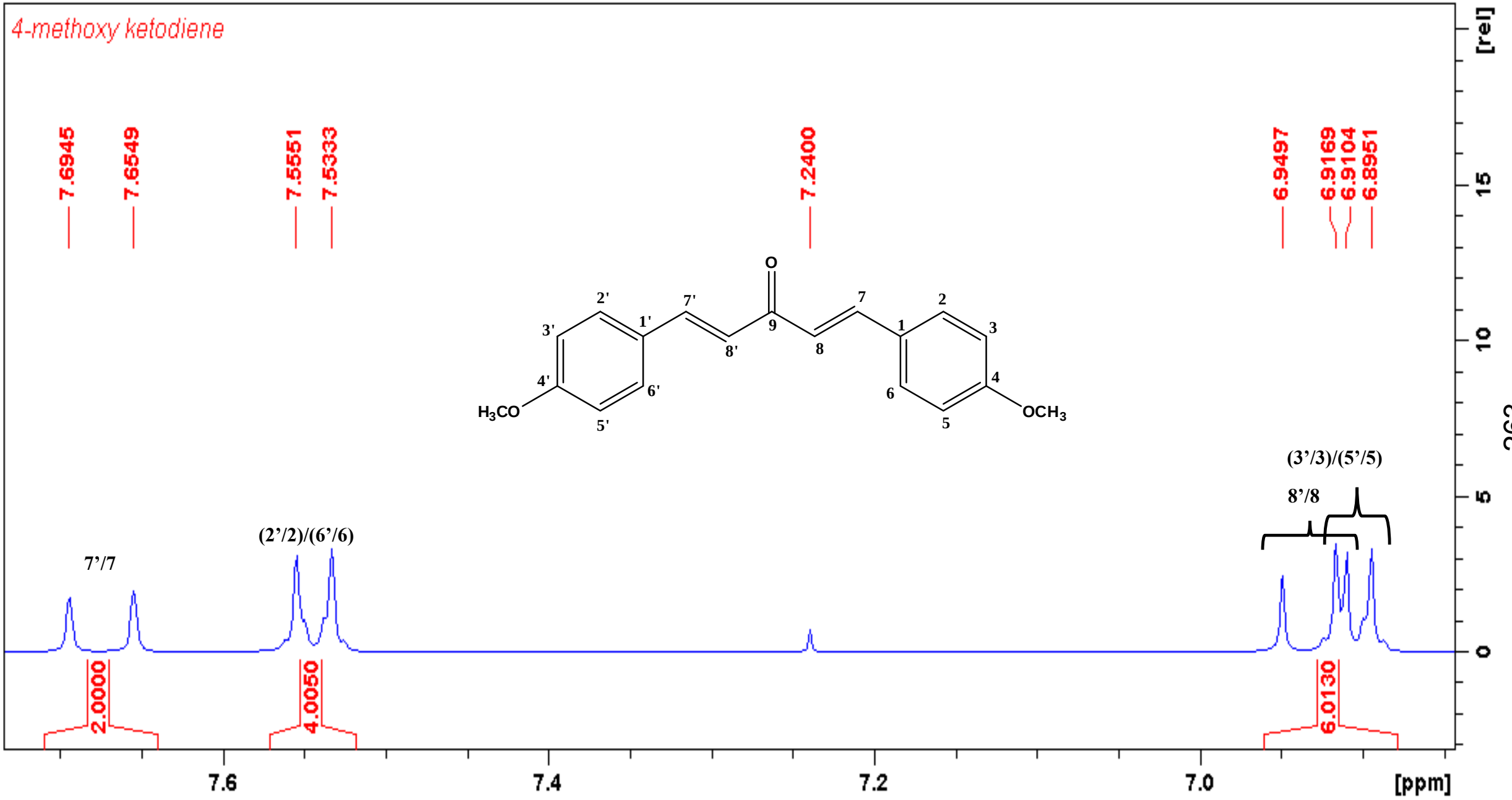
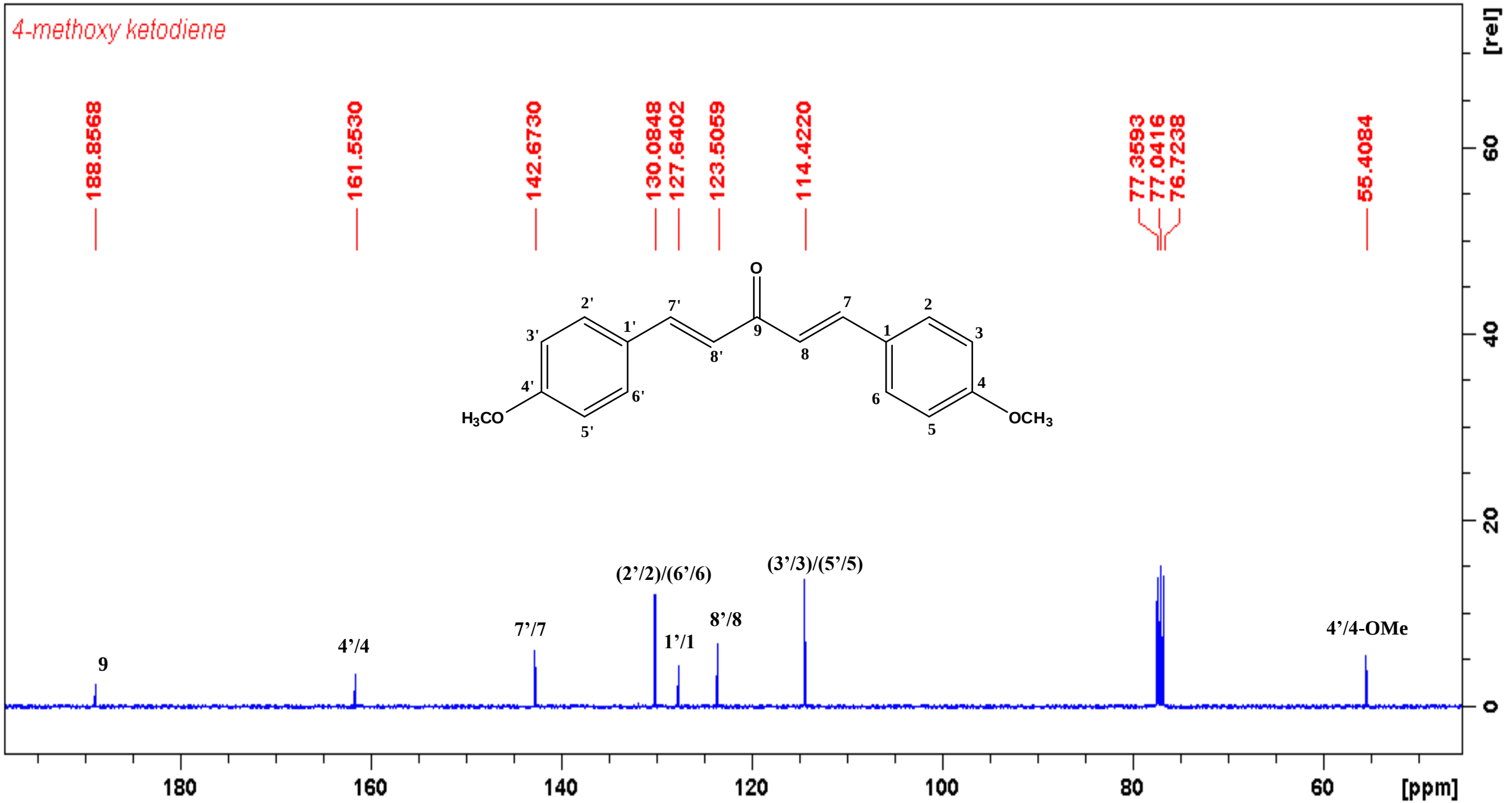
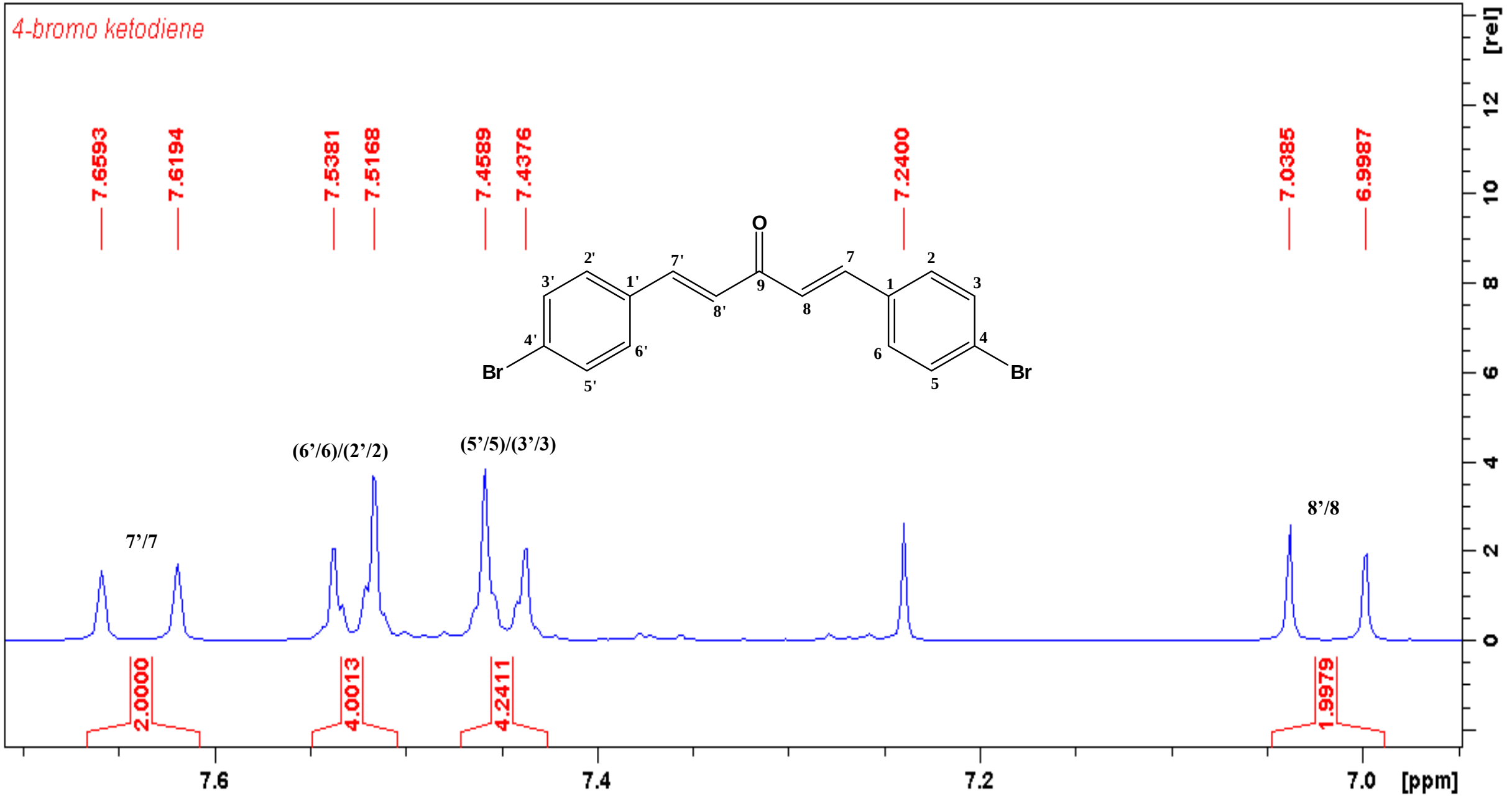
^{1}H Spectrum of Compound B-1l: (1E,4E)-1,5-bis(4-methoxyphenyl)penta-1,4-dien-3-one



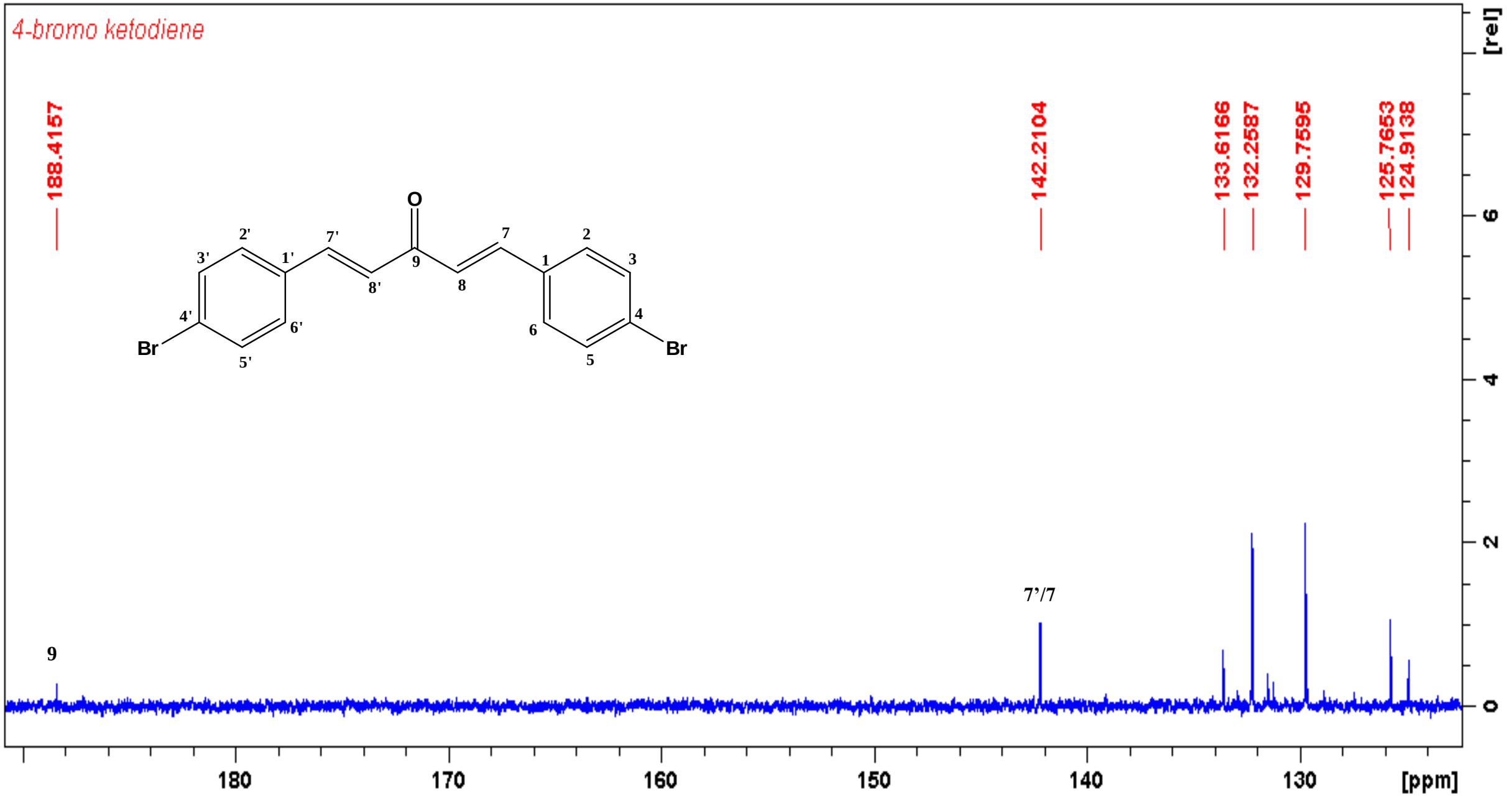
Expanded ^{1}H Spectrum of Compound B-1l: (1E,4E)-1,5-bis(4-methoxyphenyl)penta-1,4-dien-3-one



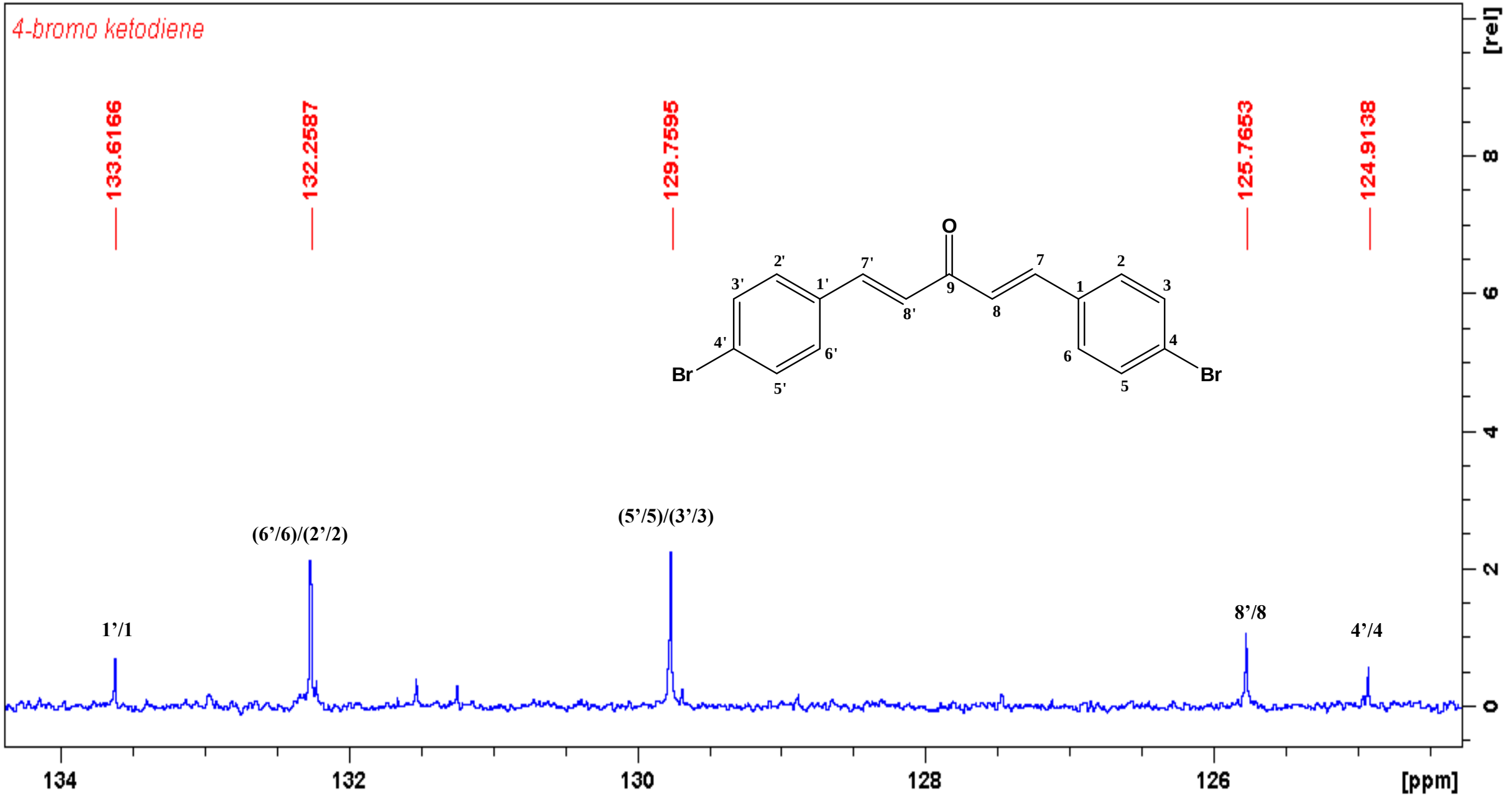
^{13}C Spectrum of Compound B-1l: (1E,4E)-1,5-bis(4-methoxyphenyl)penta-1,4-dien-3-one



^{1}H Spectrum of Compound B-1n: (1E,4E)-1,5-bis(4-bromophenyl)penta-1,4-dien-3-one



^{13}C Spectrum of Compound B-1n: (1E,4E)-1,5-bis(4-bromophenyl)penta-1,4-dien-3-one



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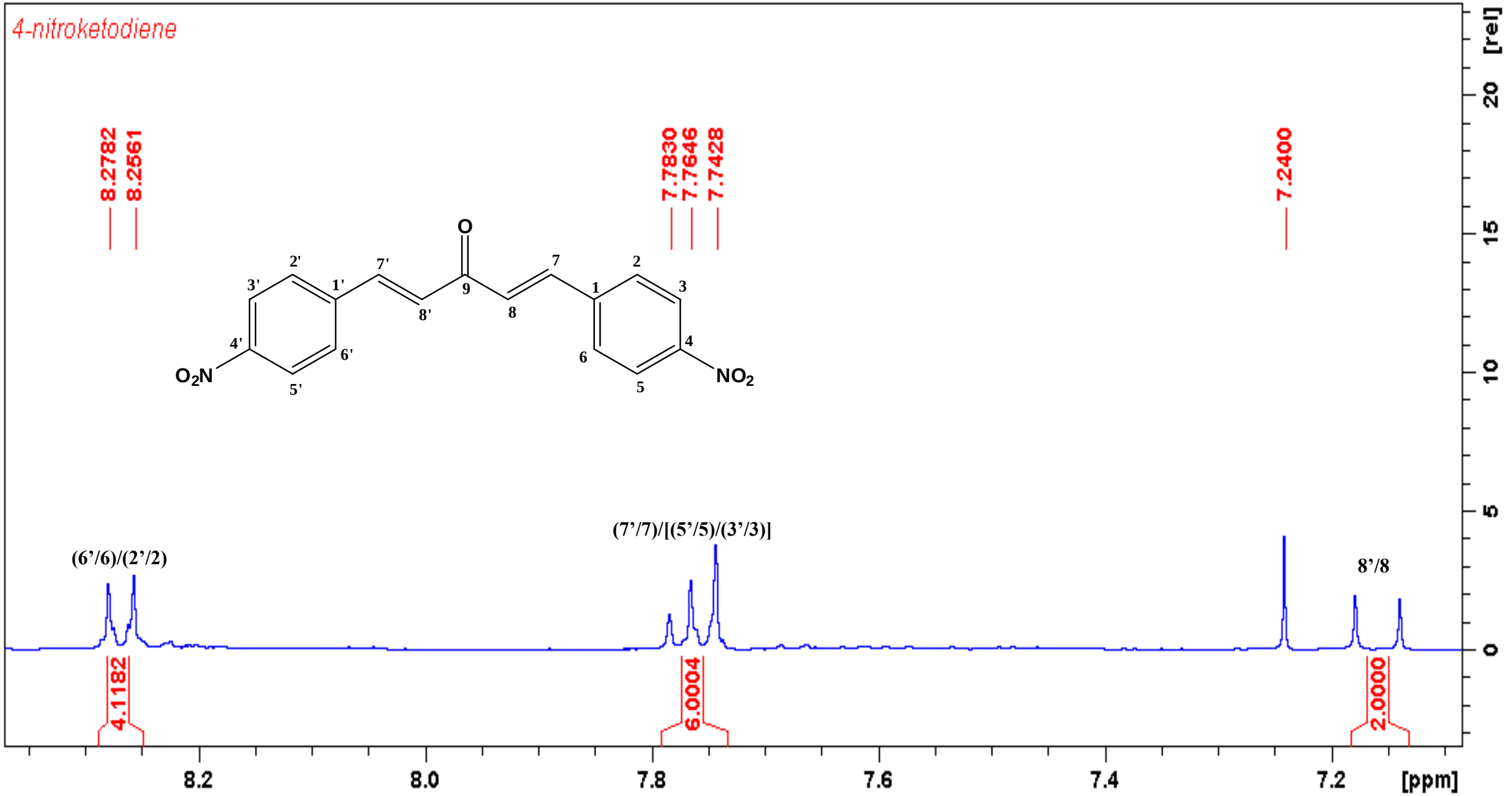
Expanded ^{13}C Spectrum of Compound B-1n: (1E,4E)-1,5-bis(4-bromophenyl)penta-1,4-dien-3-one



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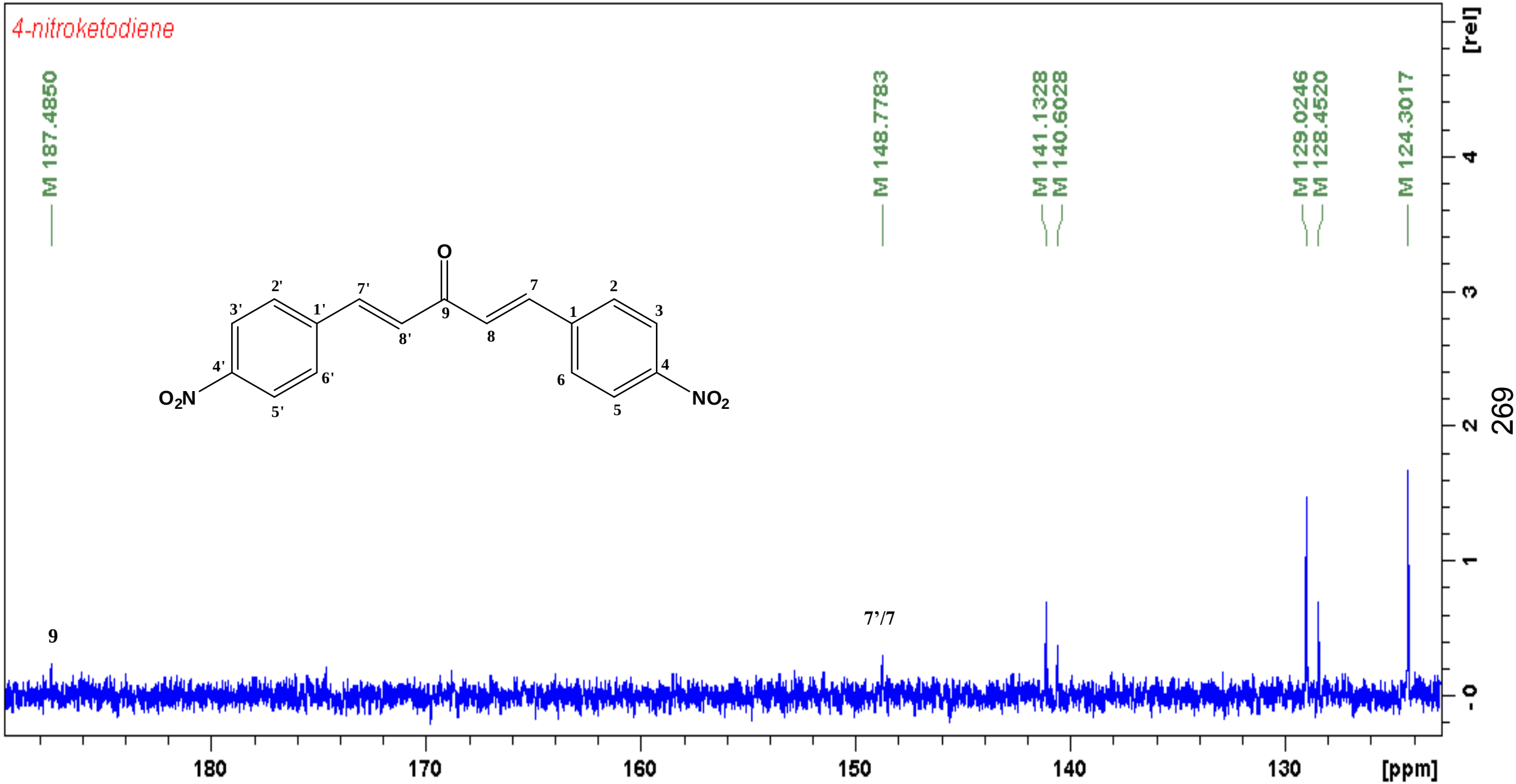
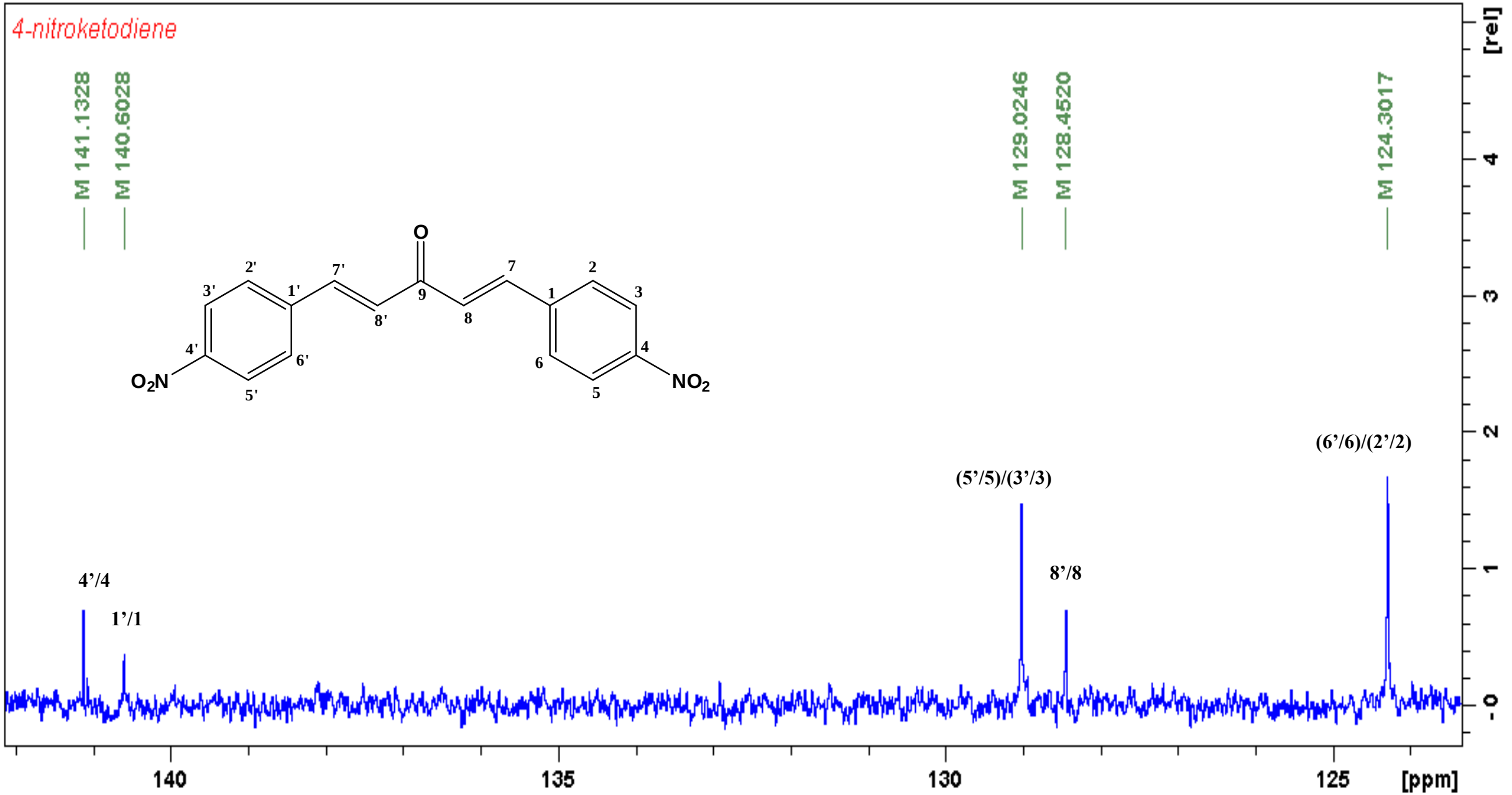
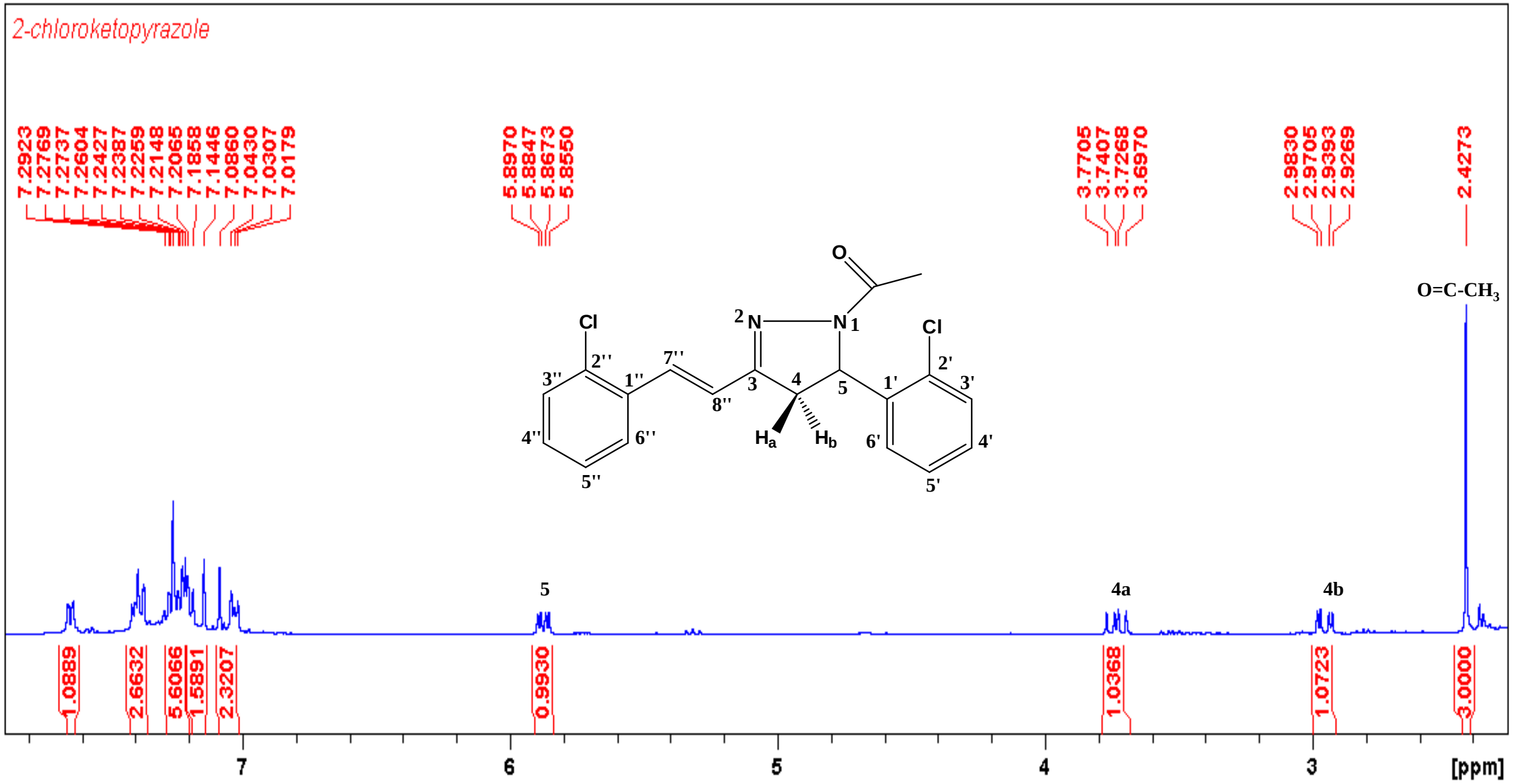
^{1}H Spectrum of Compound B-1o: (1E,4E)-1,5-bis(4-nitrophenyl)penta-1,4-dien-3-one



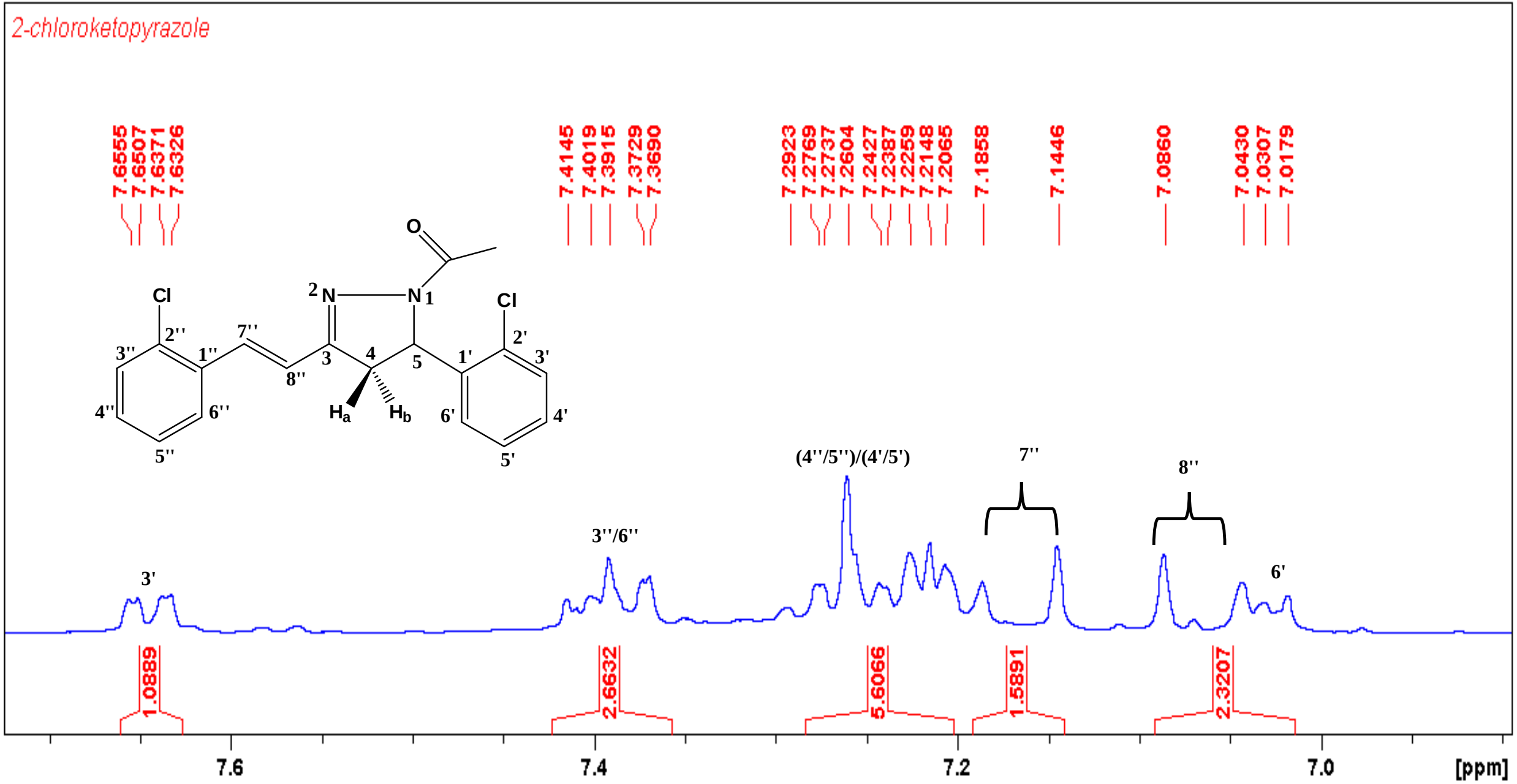
^{13}C Spectrum of Compound B-1o: (1E,4E)-1,5-bis(4-nitrophenyl)penta-1,4-dien-3-one



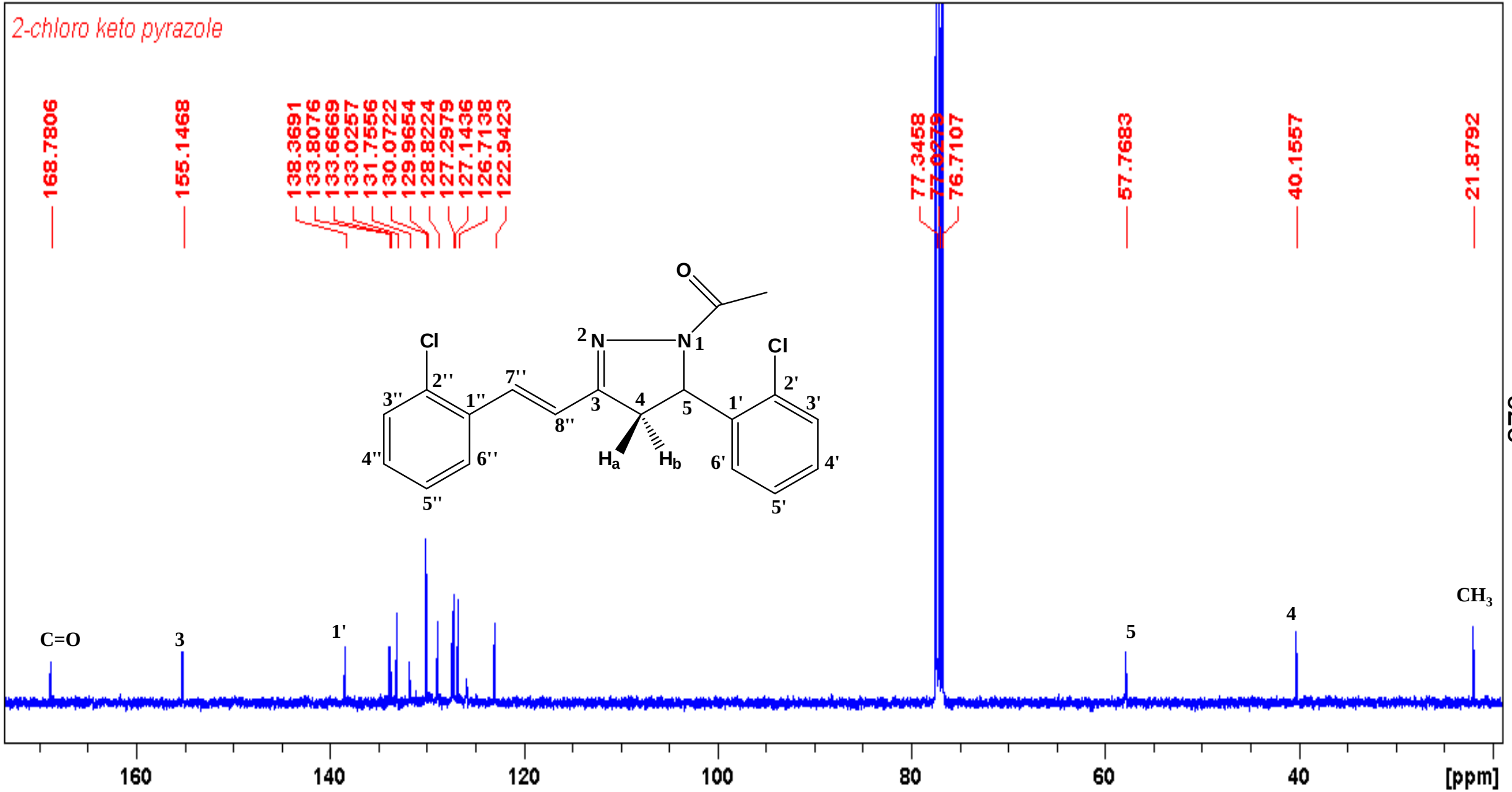
Expanded ^{13}C Spectrum of Compound B-1o: (1E,4E)-1,5-bis(4-nitrophenyl)penta-1,4-dien-3-one



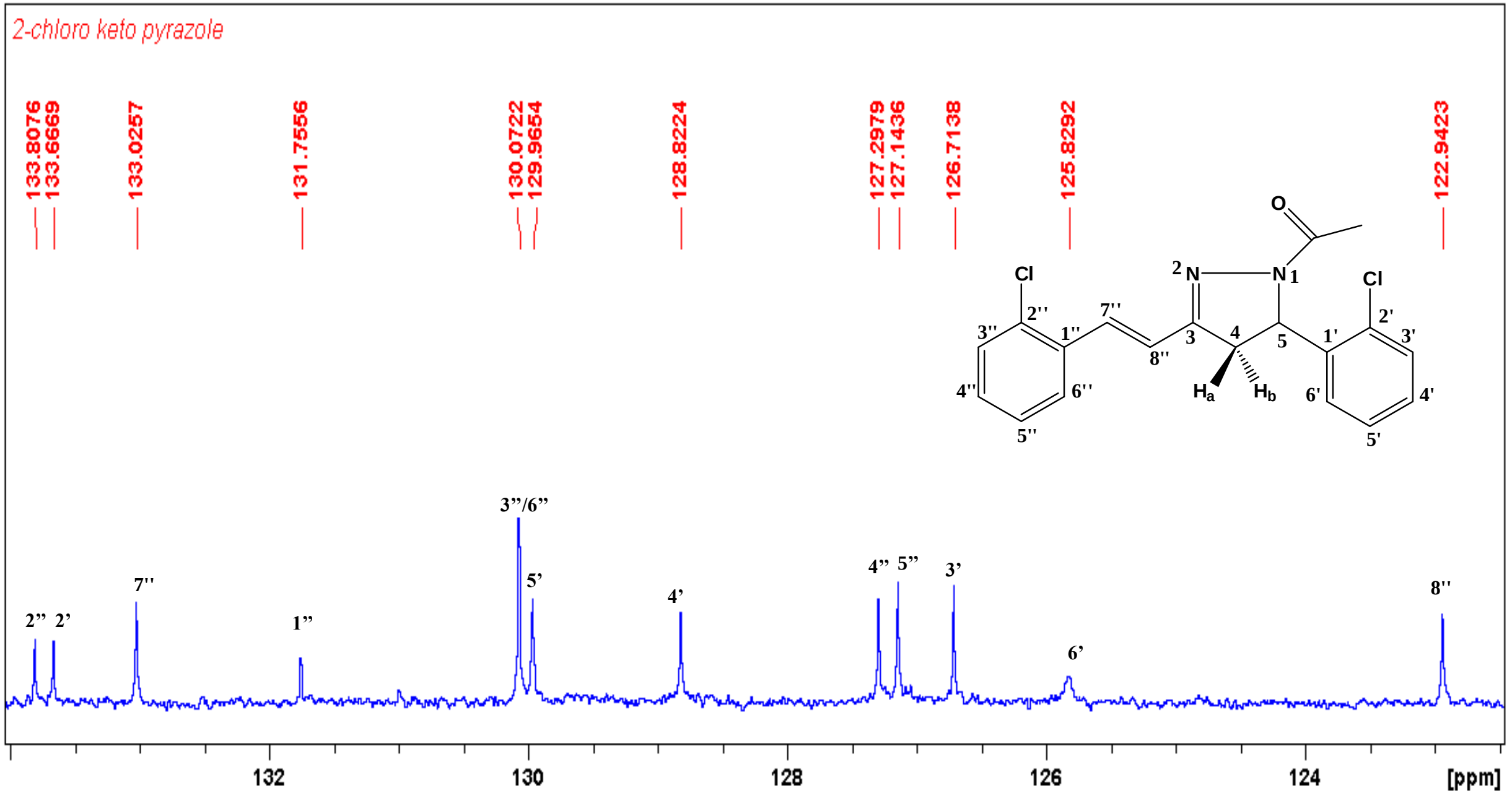
^{1}H Spectrum of Compound B-2a: (E)-1-(5-(2-chlorophenyl)-3-(2-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



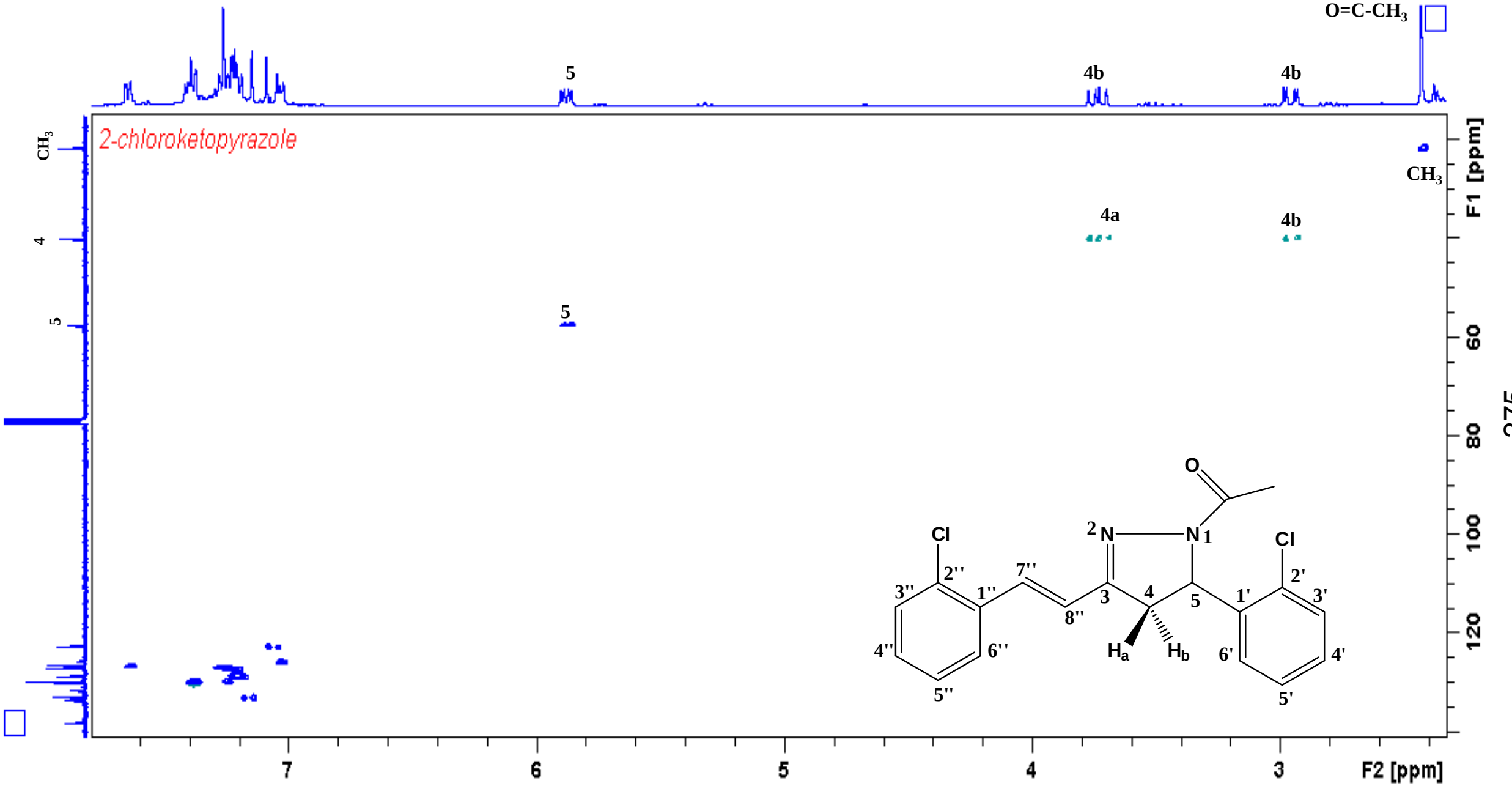
Expanded ^{1}H Spectrum of Compound B-2a: (E)-1-(5-(2-chlorophenyl)-3-(2-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



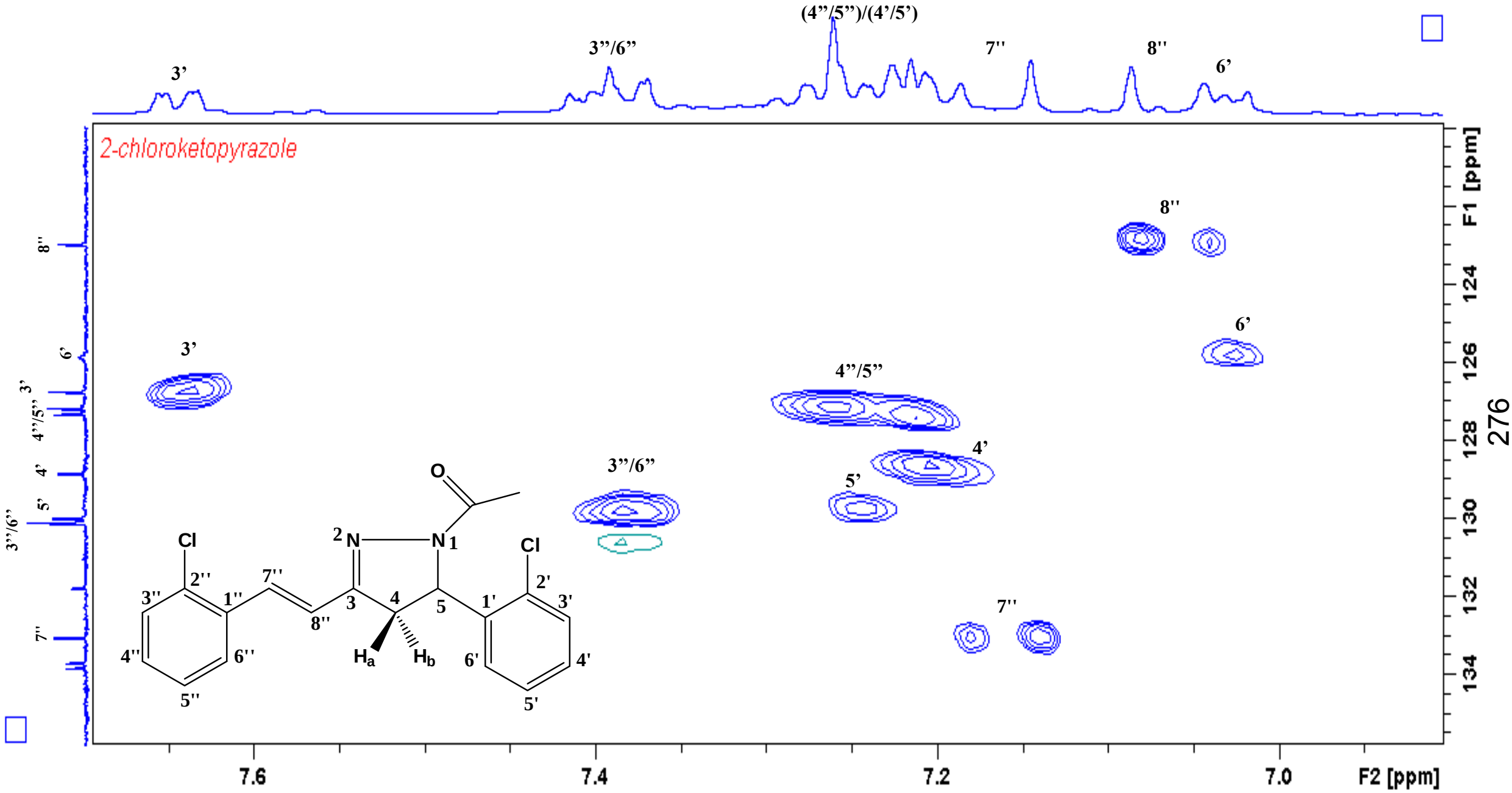
^{13}C Spectrum of Compound B-2a: (E)-1-(5-(2-chlorophenyl)-3-(2-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



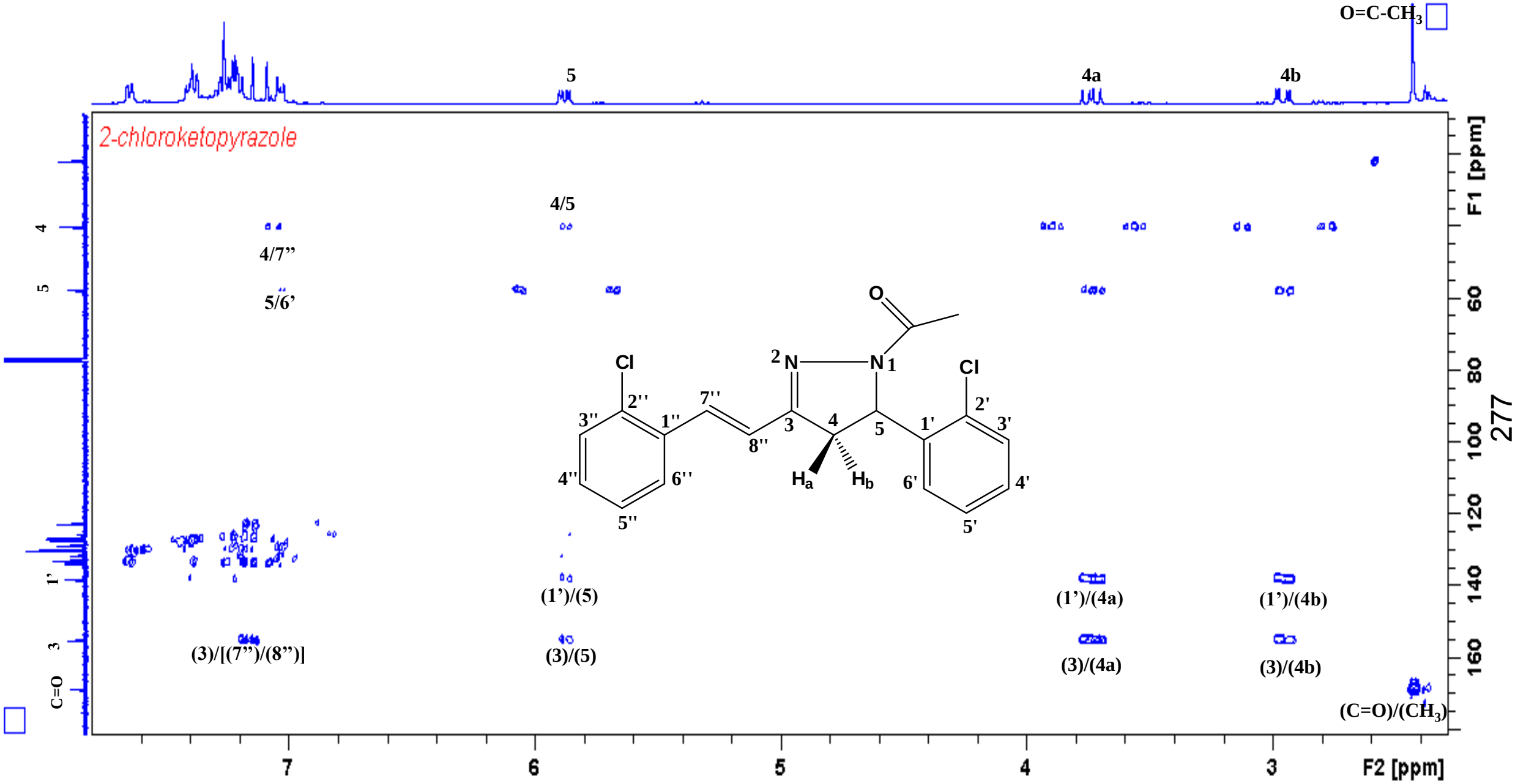
Expanded ^{13}C Spectrum of Compound B-2a: (E)-1-(5-(2-chlorophenyl)-3-(2-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



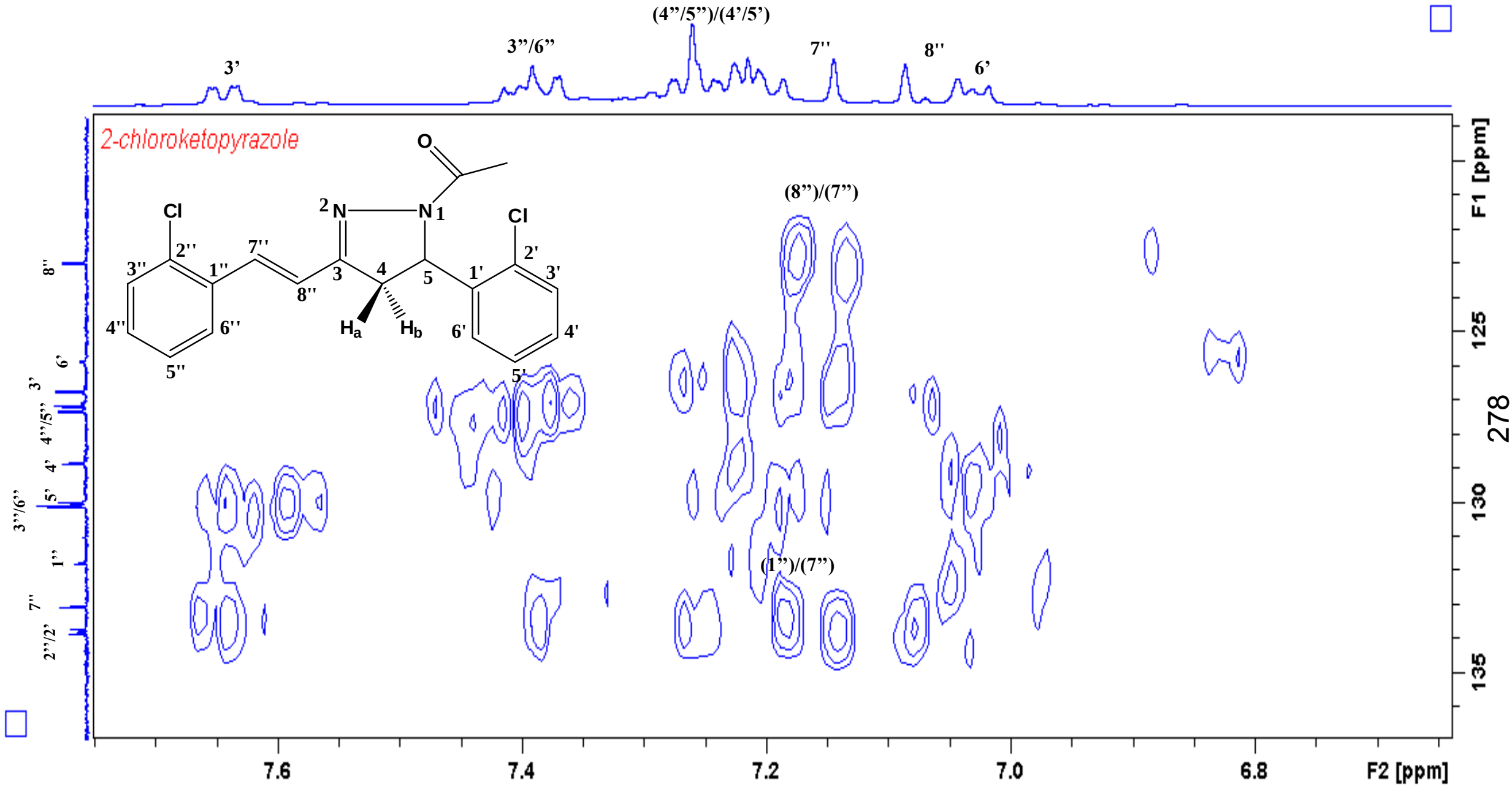
HSQC of Compound B-2a: (E)-1-(5-(2-chlorophenyl)-3-(2-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



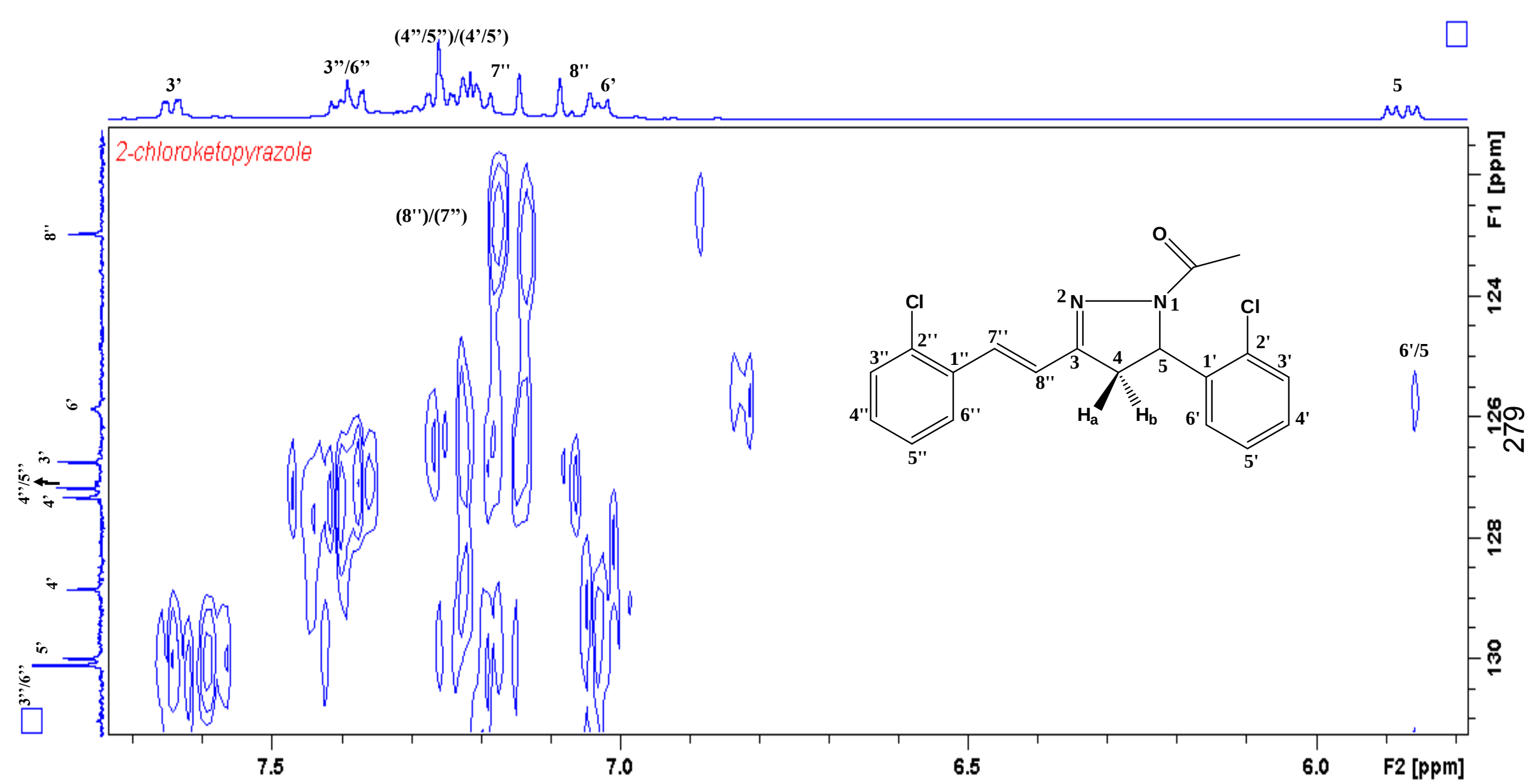
Expanded HSQC of Compound B-2a: (E)-1-(5-(2-chlorophenyl)-3-(2-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



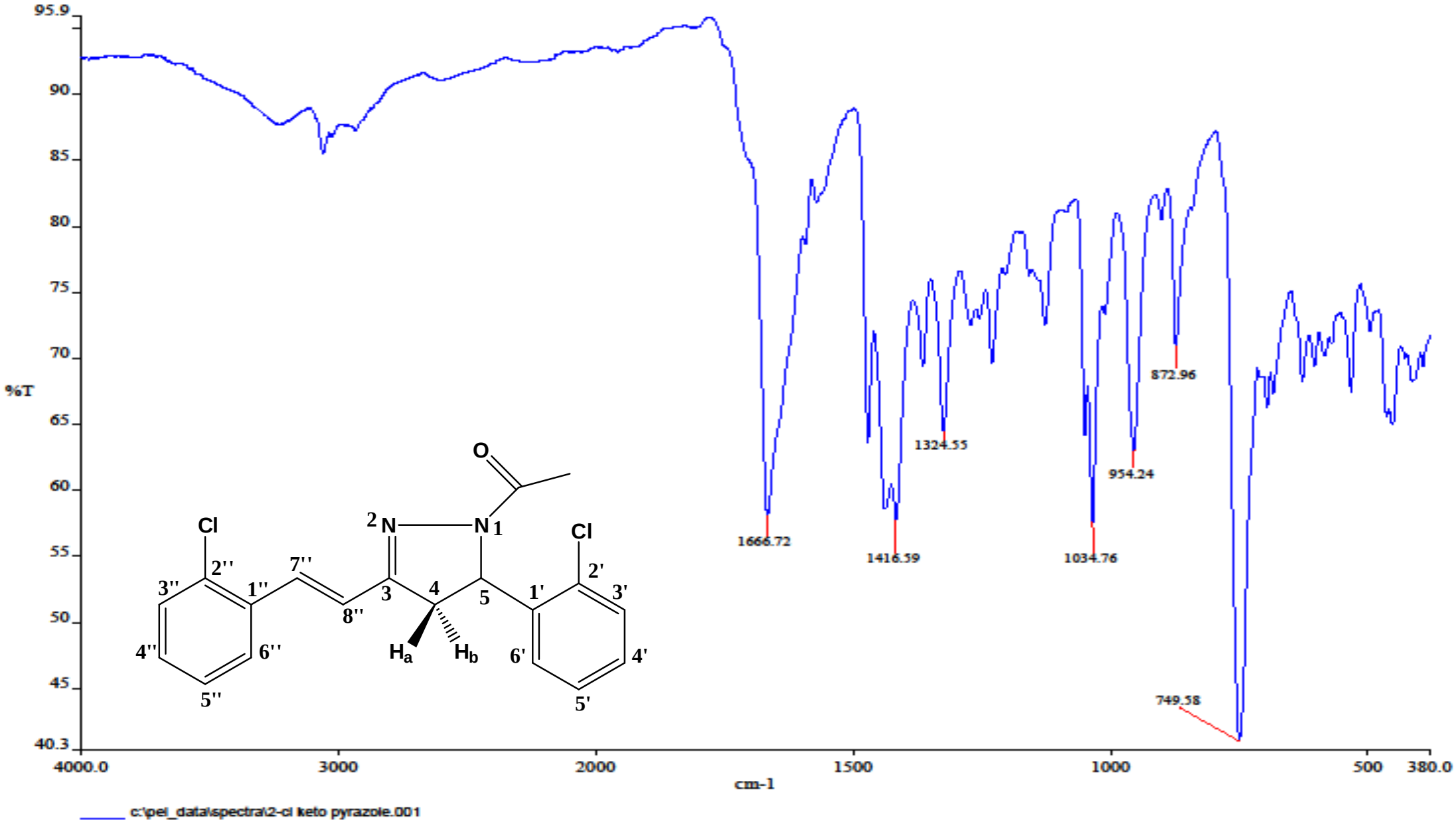
HMBC of Compound B-2a: (E)-1-(5-(2-chlorophenyl)-3-(2-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



Expanded HMBC of Compound B-2a: (E)-1-(5-(2-chlorophenyl)-3-(2-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

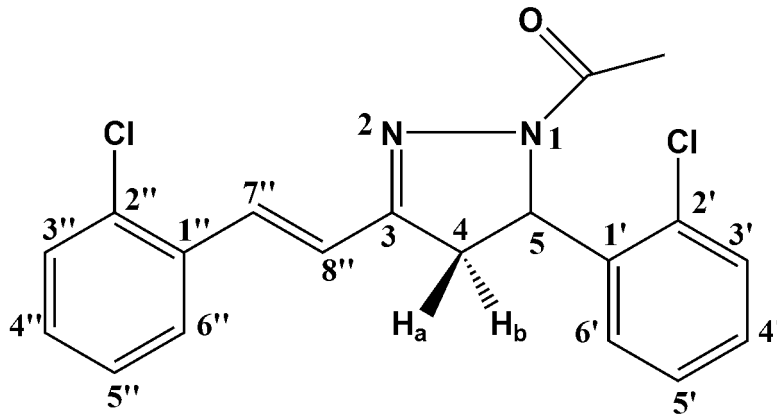


Expanded HMBC of Compound B-2a: (E)-1-(5-(2-chlorophenyl)-3-(2-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



280

Infrared Spectrum of Compound B-3a: (E)-1-(5-(2-chlorophenyl)-3-(2-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



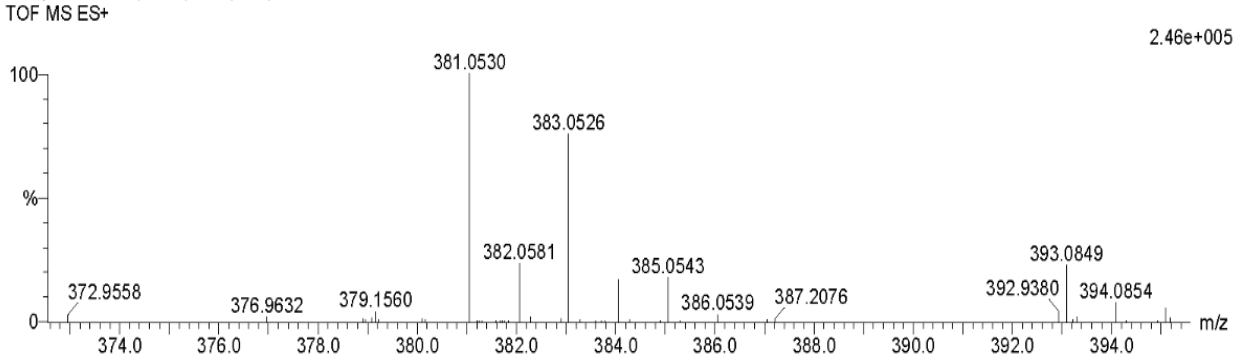
Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

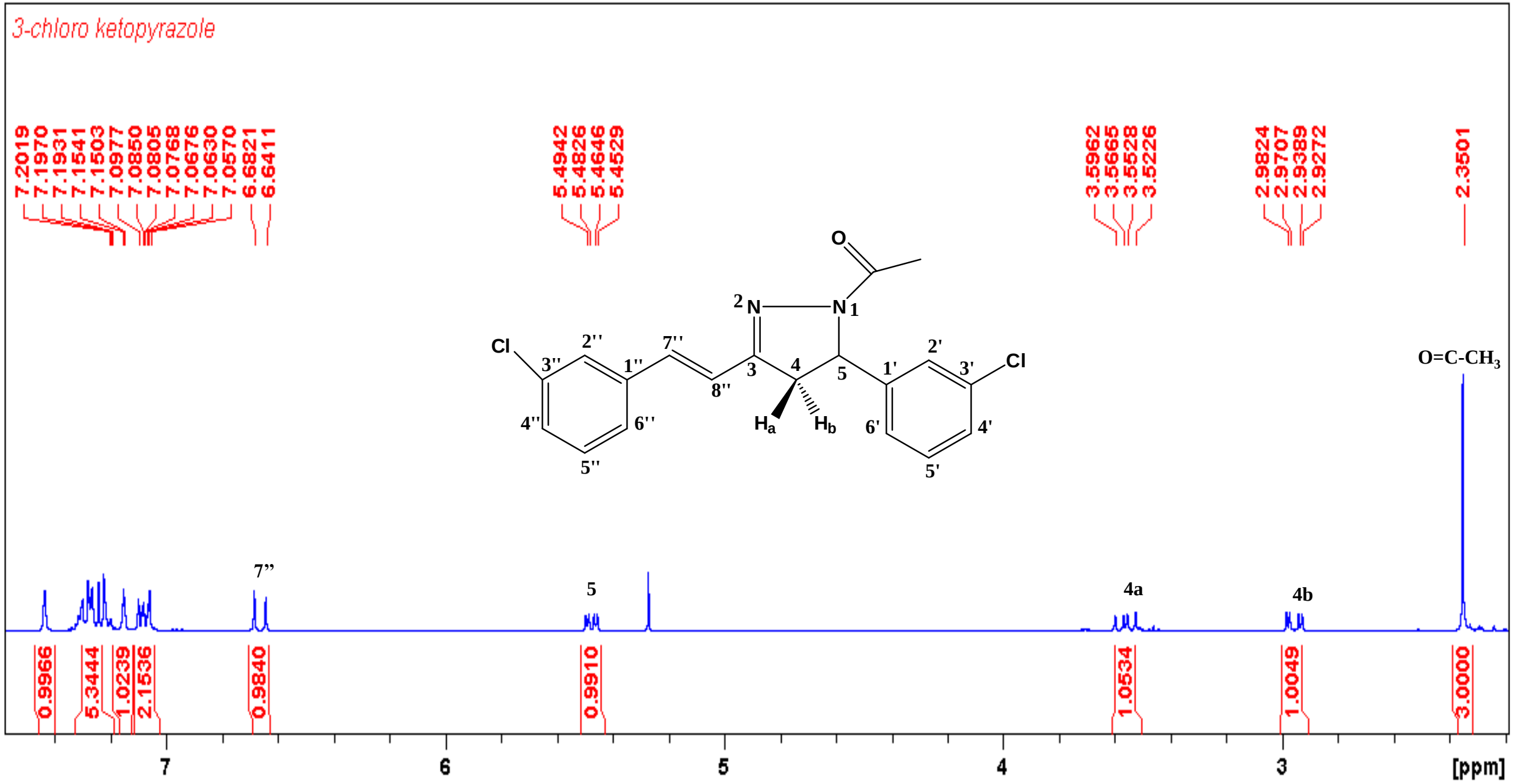
Monoisotopic Mass, Even Electron Ions
47 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 15-20 H: 15-20 N: 0-5 O: 0-5 Na: 1-1 Cl: 0-2

Compound 1 14 (0.439) Cm (1:61)
TOF MS ES+

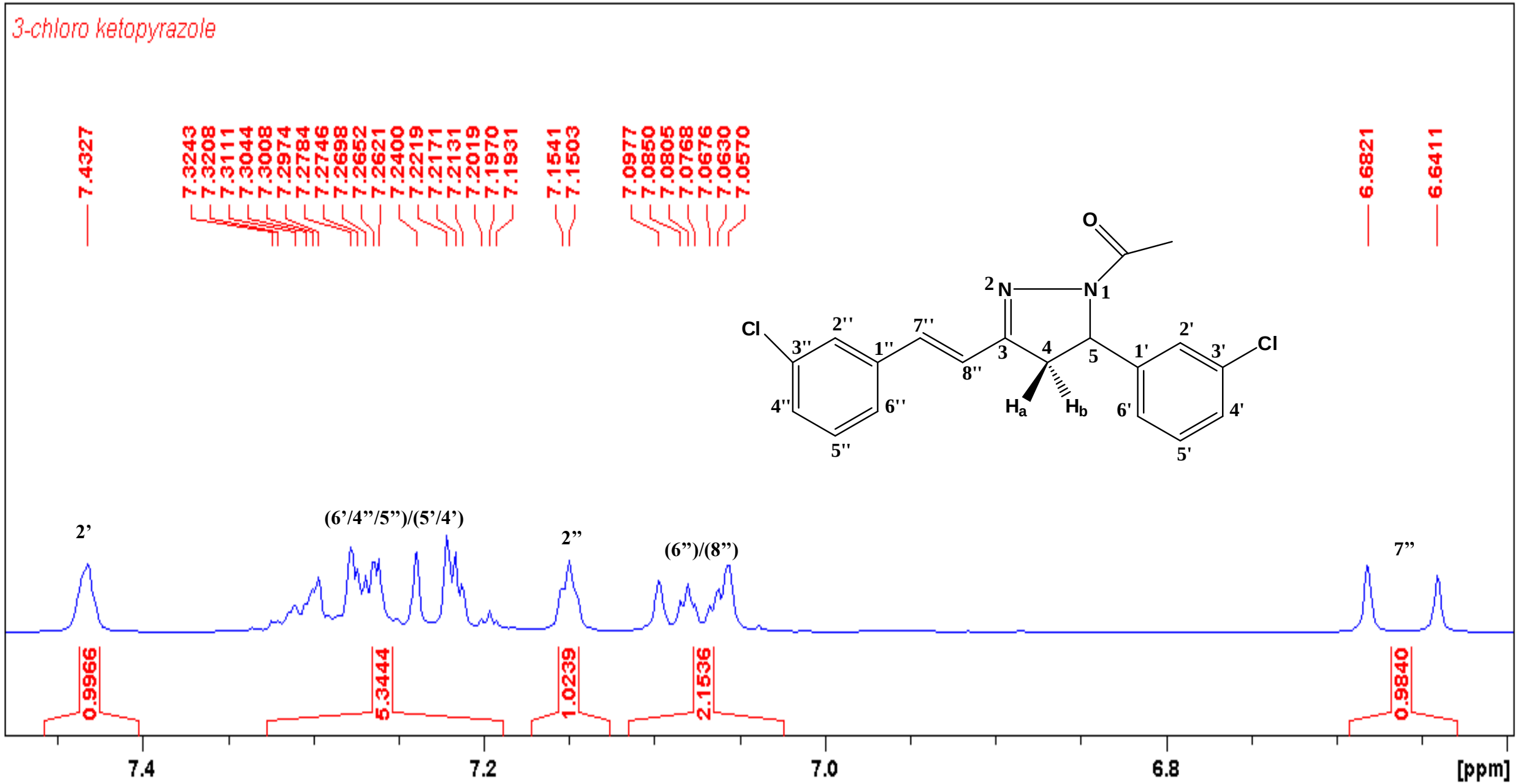


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
381.0530	381.0537	-0.7	-1.8	11.5	143.1	0.0	C19 H16 N2 O Na Cl2

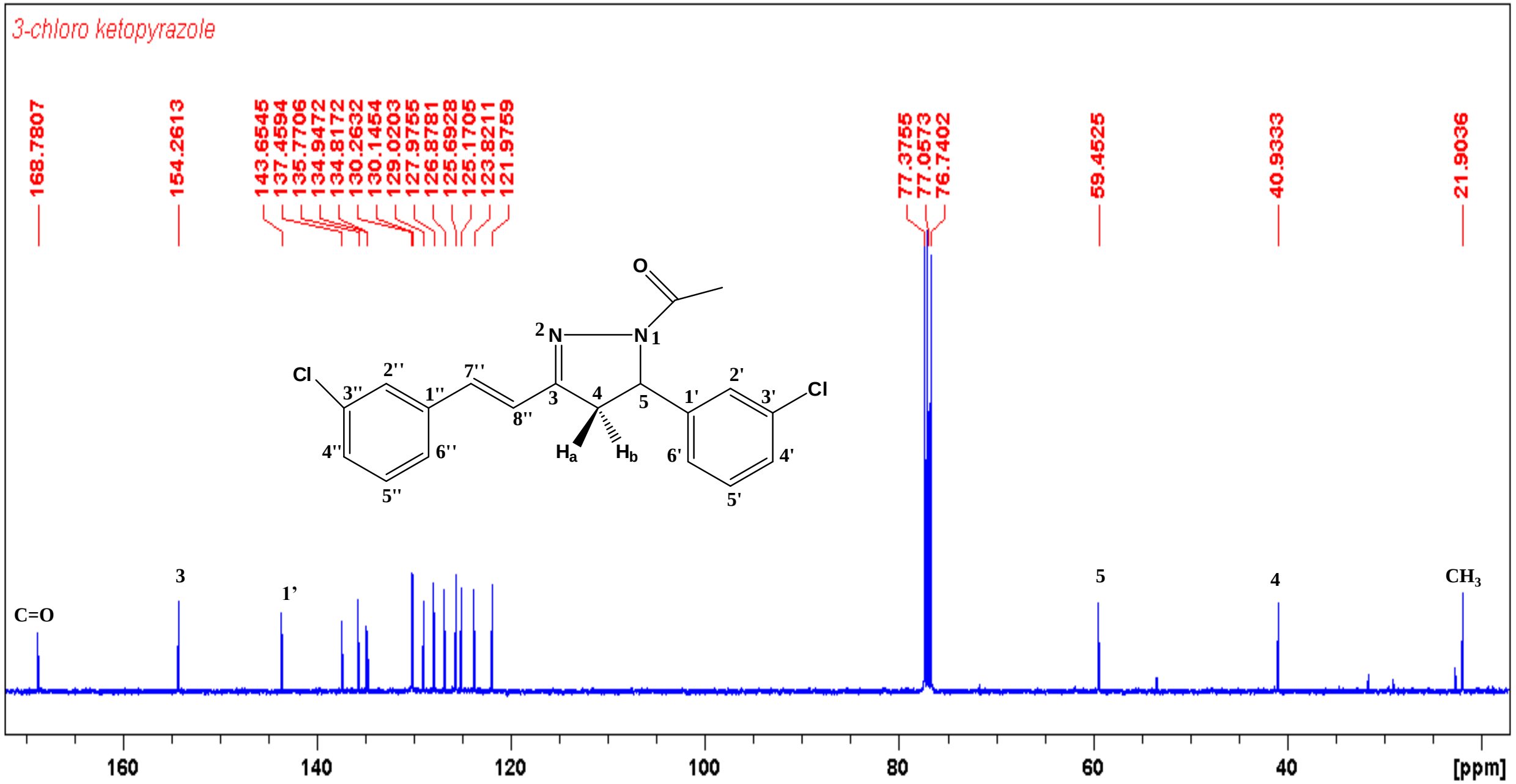
HRMS of Compound B-2a: (E)-1-(5-(2-chlorophenyl)-3-(2-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



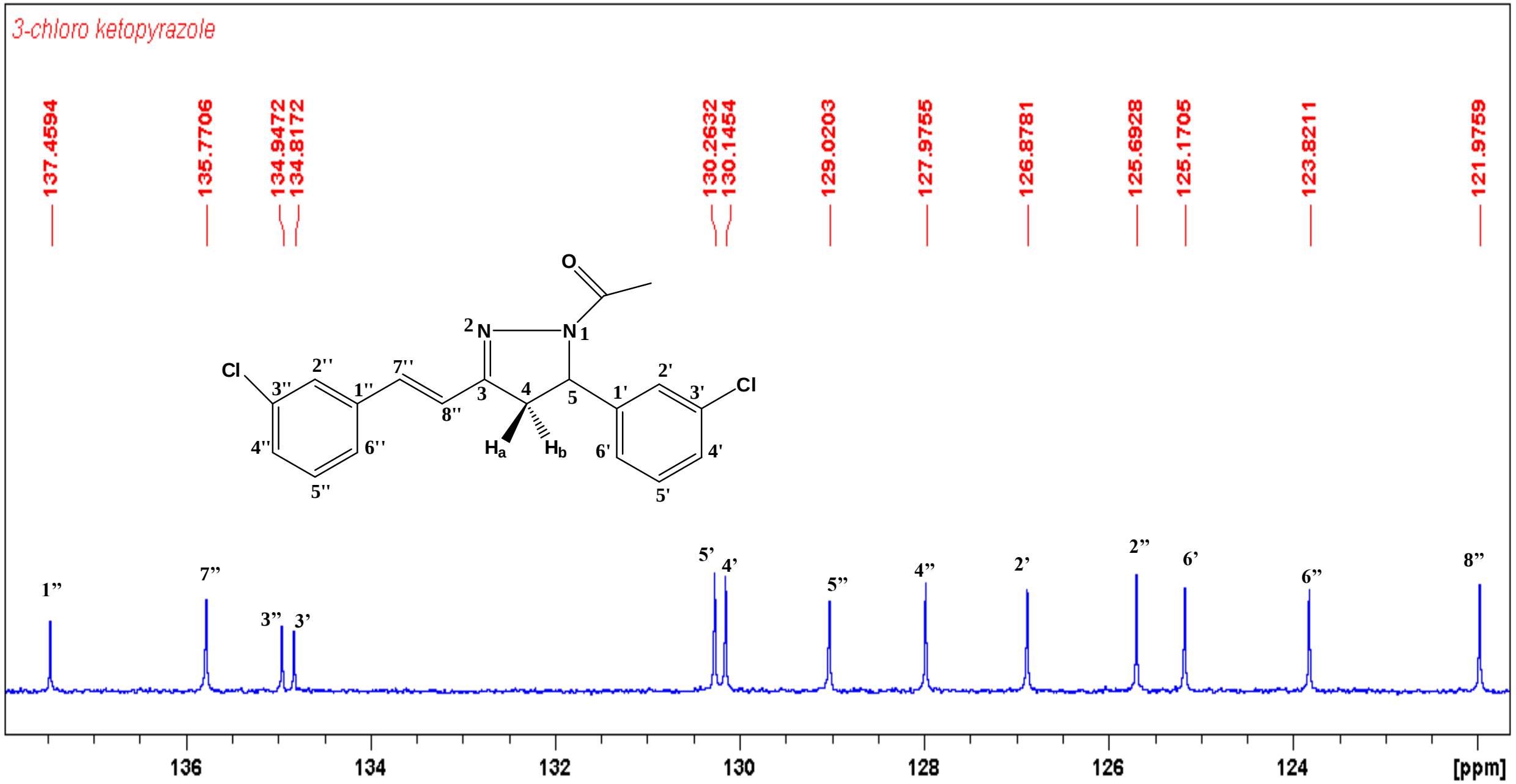
^{1}H Spectrum of Compound B-2b: (E)-1-(5-(3-chlorophenyl)-3-(3-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



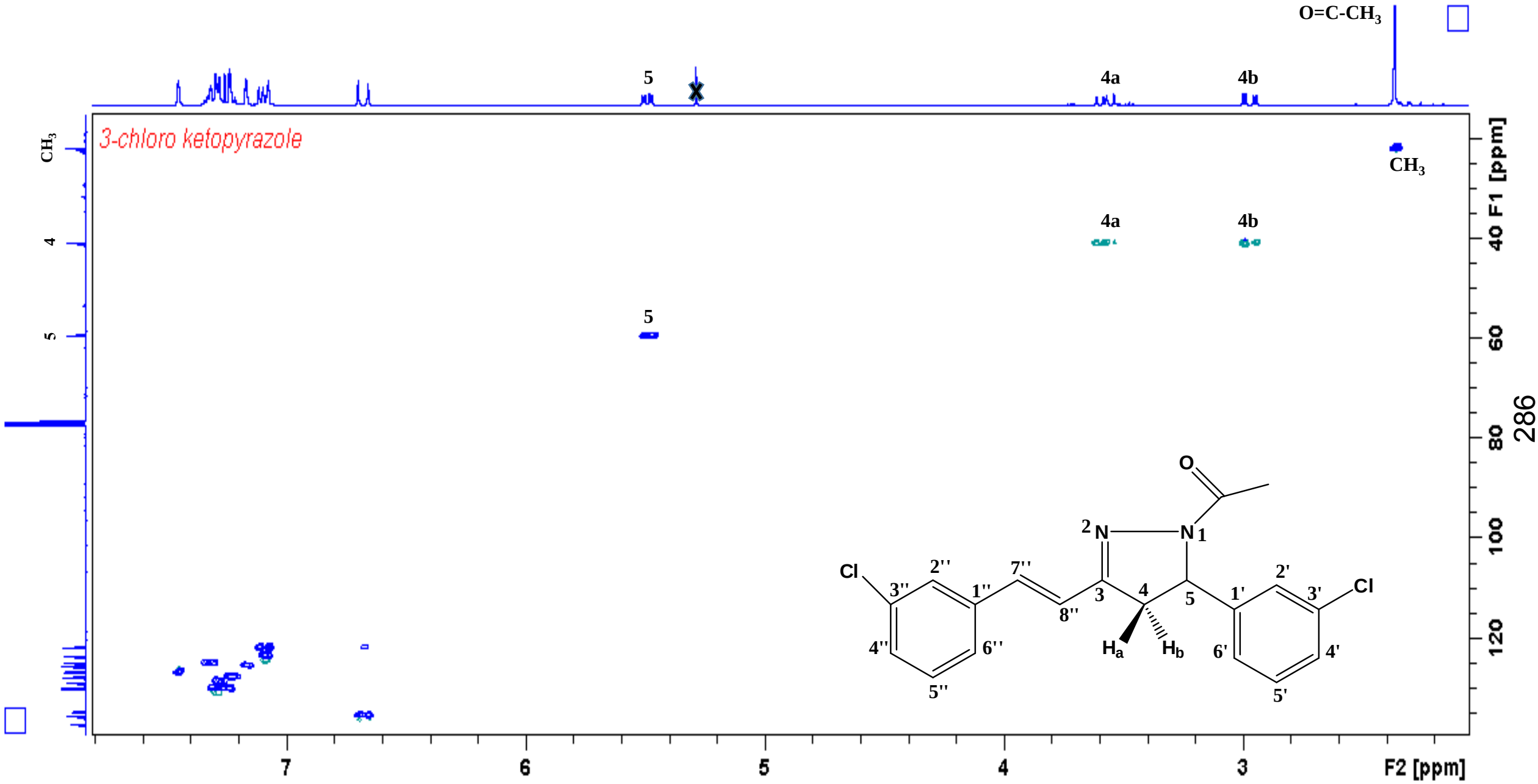
Expanded ^{1}H Spectrum of Compound B-2b: (E)-1-(5-(3-chlorophenyl)-3-(3-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



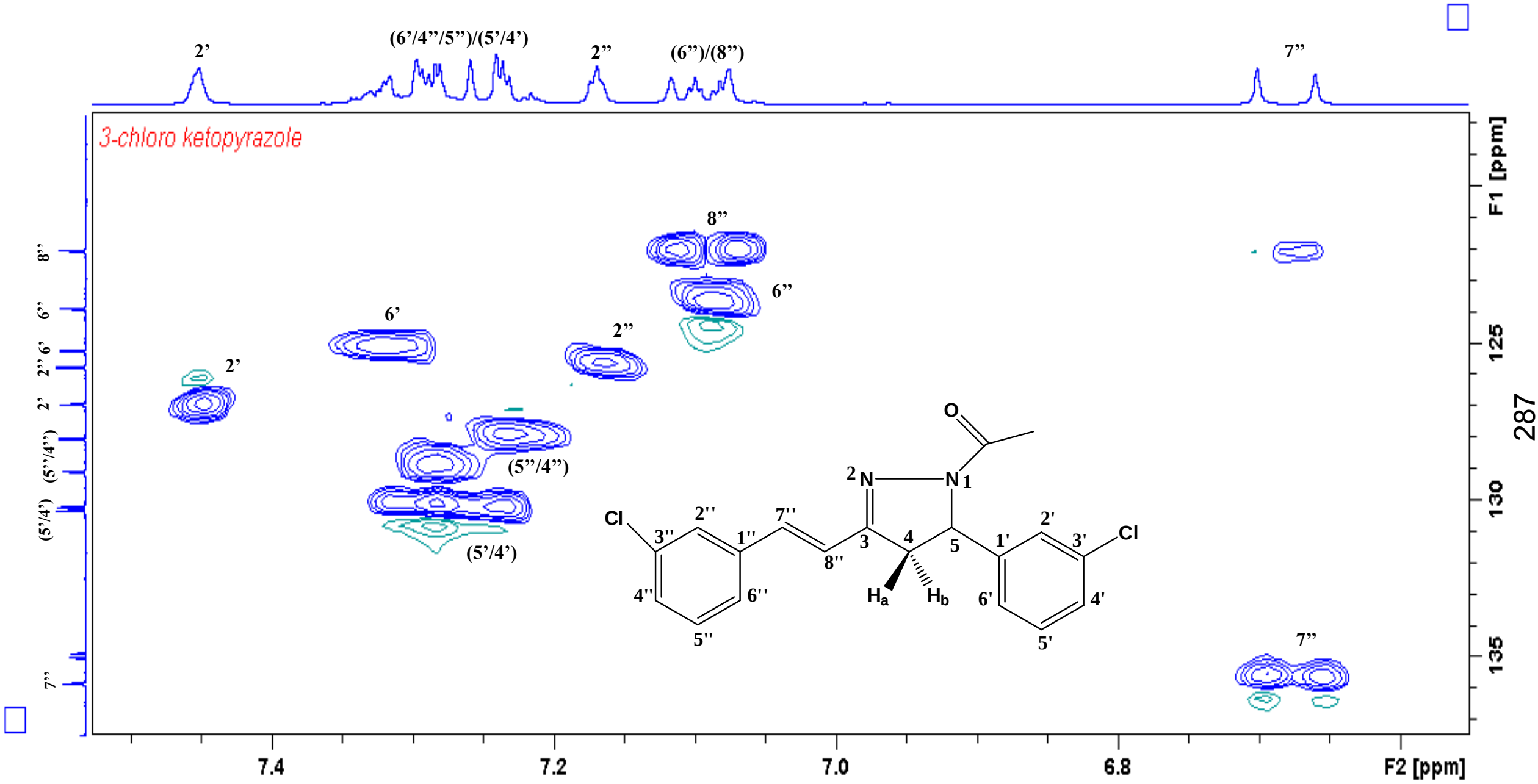
^{13}C Spectrum of Compound B-2b: (E)-1-(5-(3-chlorophenyl)-3-(3-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



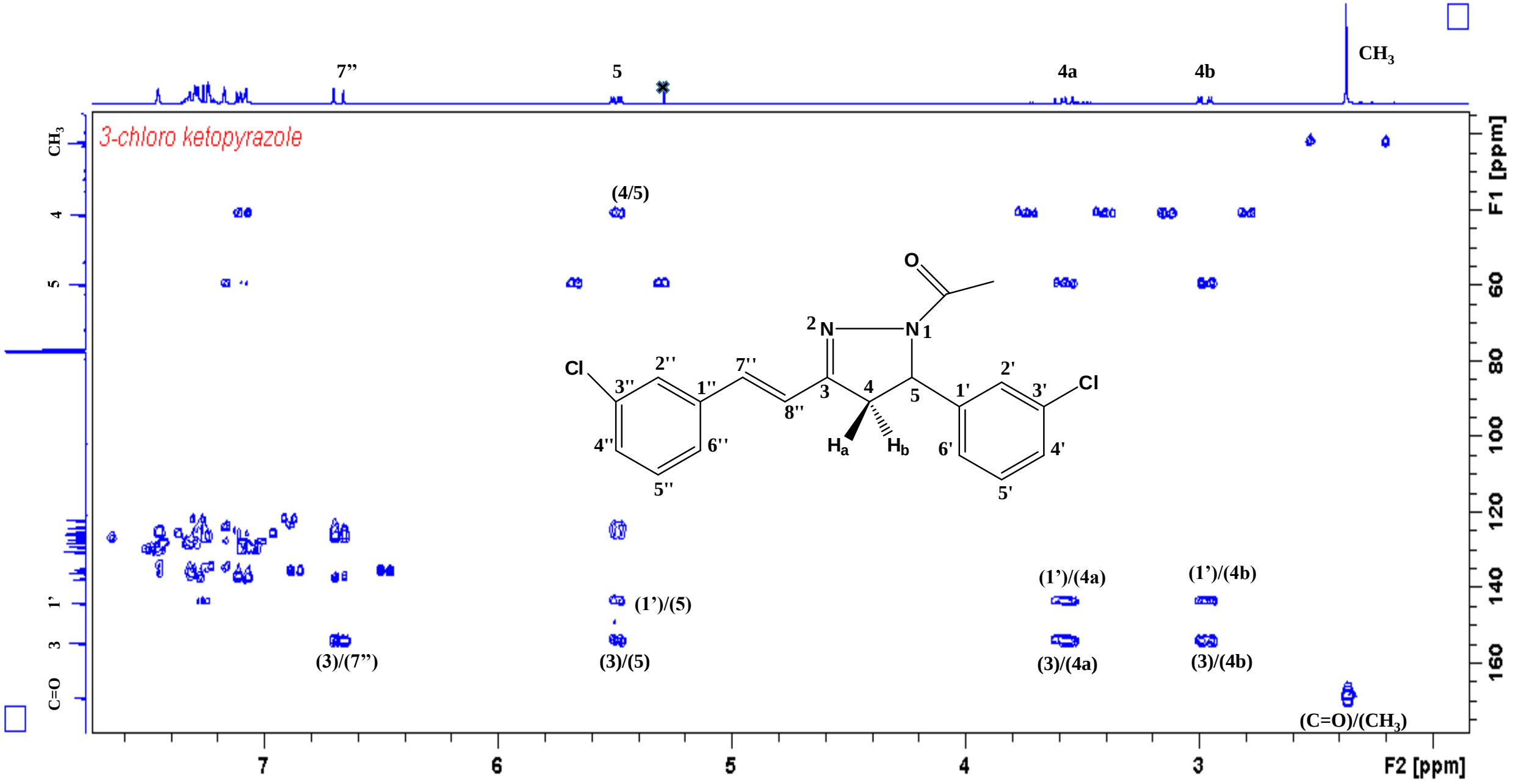
Expanded ^{13}C Spectrum of Compound B-2b: (E)-1-(5-(3-chlorophenyl)-3-(3-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



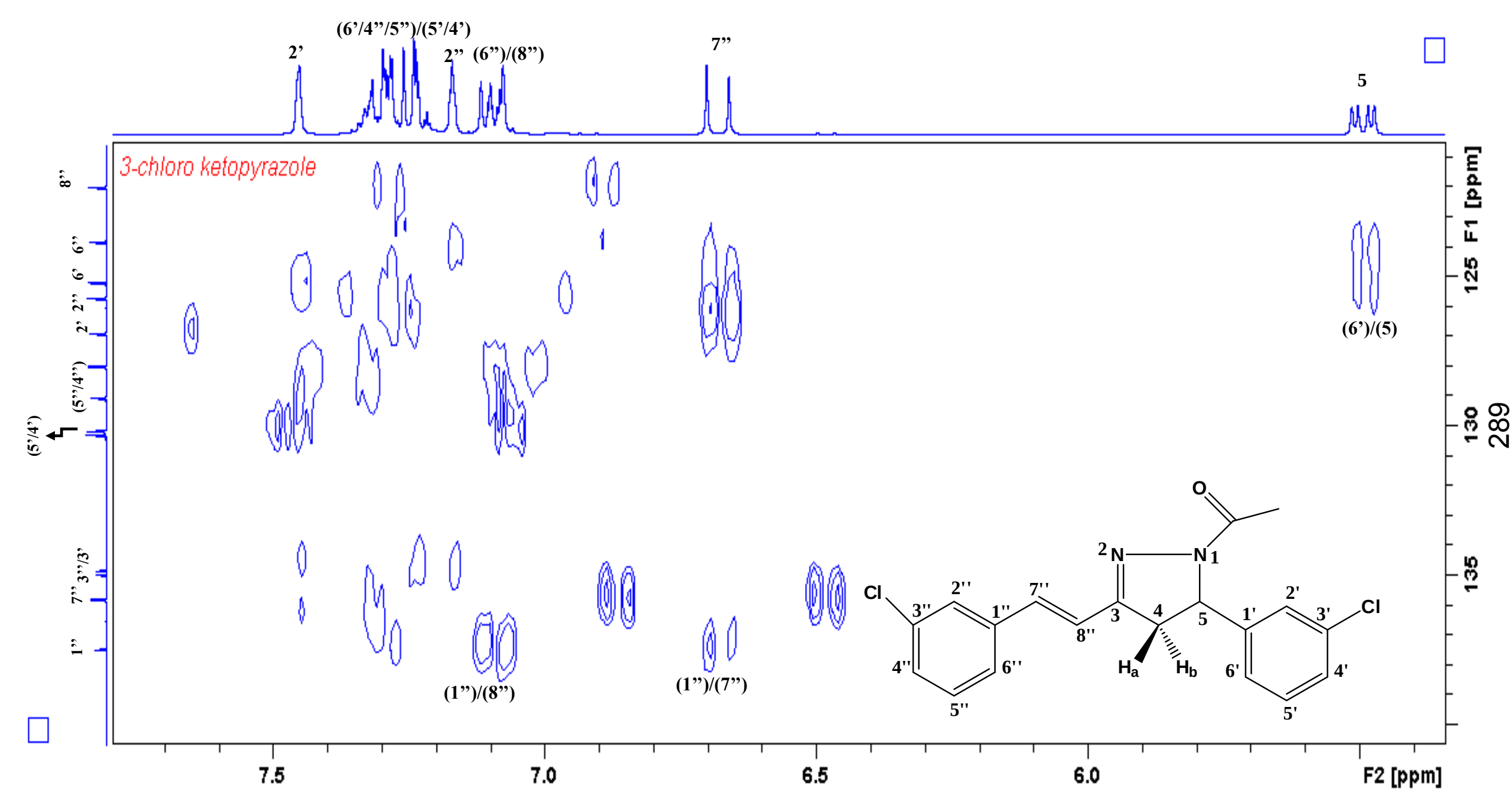
HSQC of Compound B-2b: (E)-1-(5-(3-chlorophenyl)-3-(3-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



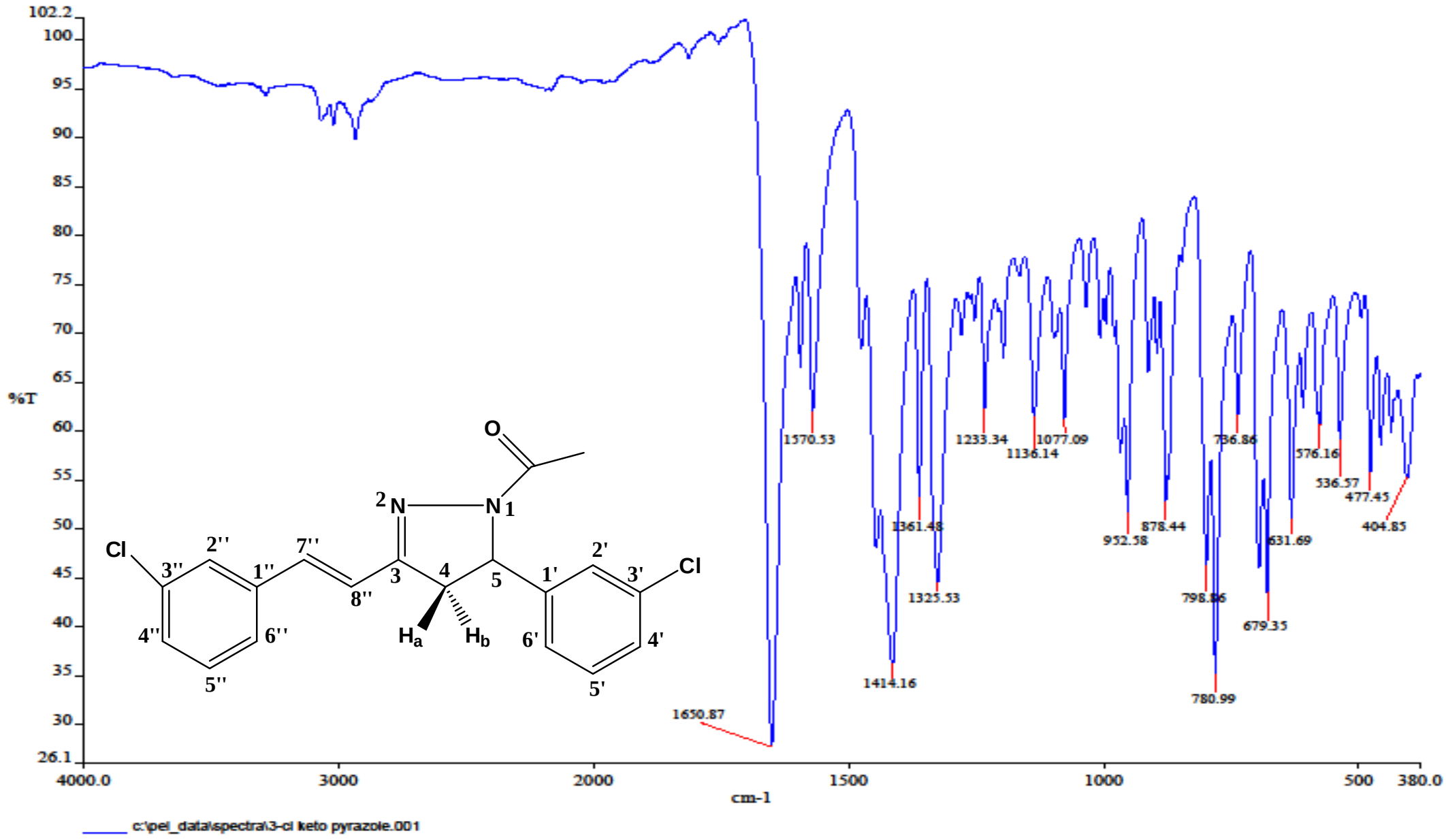
Expanded HSQC of Compound B-2b: (E)-1-(5-(3-chlorophenyl)-3-(3-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



HMBC of Compound B-2b: (E)-1-(5-(3-chlorophenyl)-3-(3-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



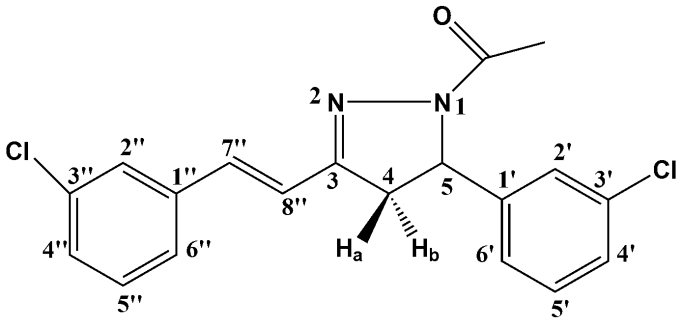
Expanded HMBC of Compound B-2b: (E)-1-(5-(3-chlorophenyl)-3-(3-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



290

Infrared Spectrum of Compound B-2b: (E)-1-(5-(3-chlorophenyl)-3-(3-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

Elemental Composition Report

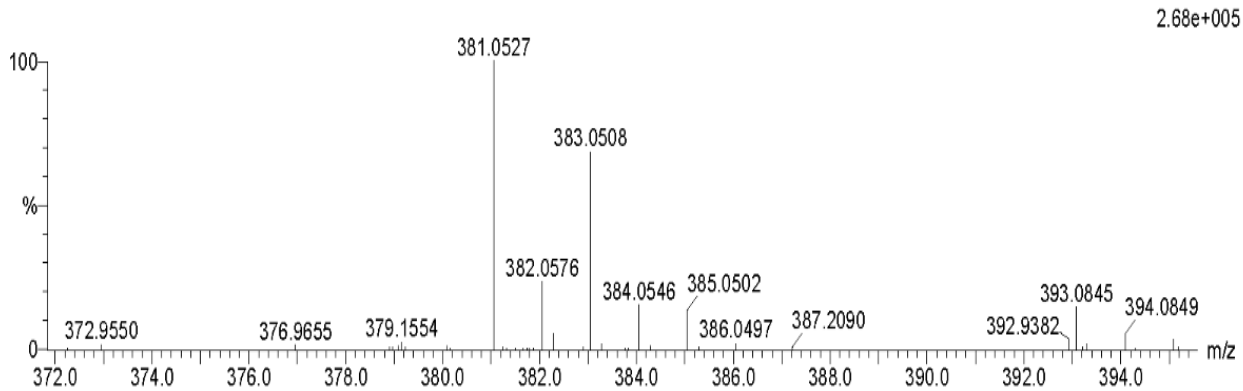


Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

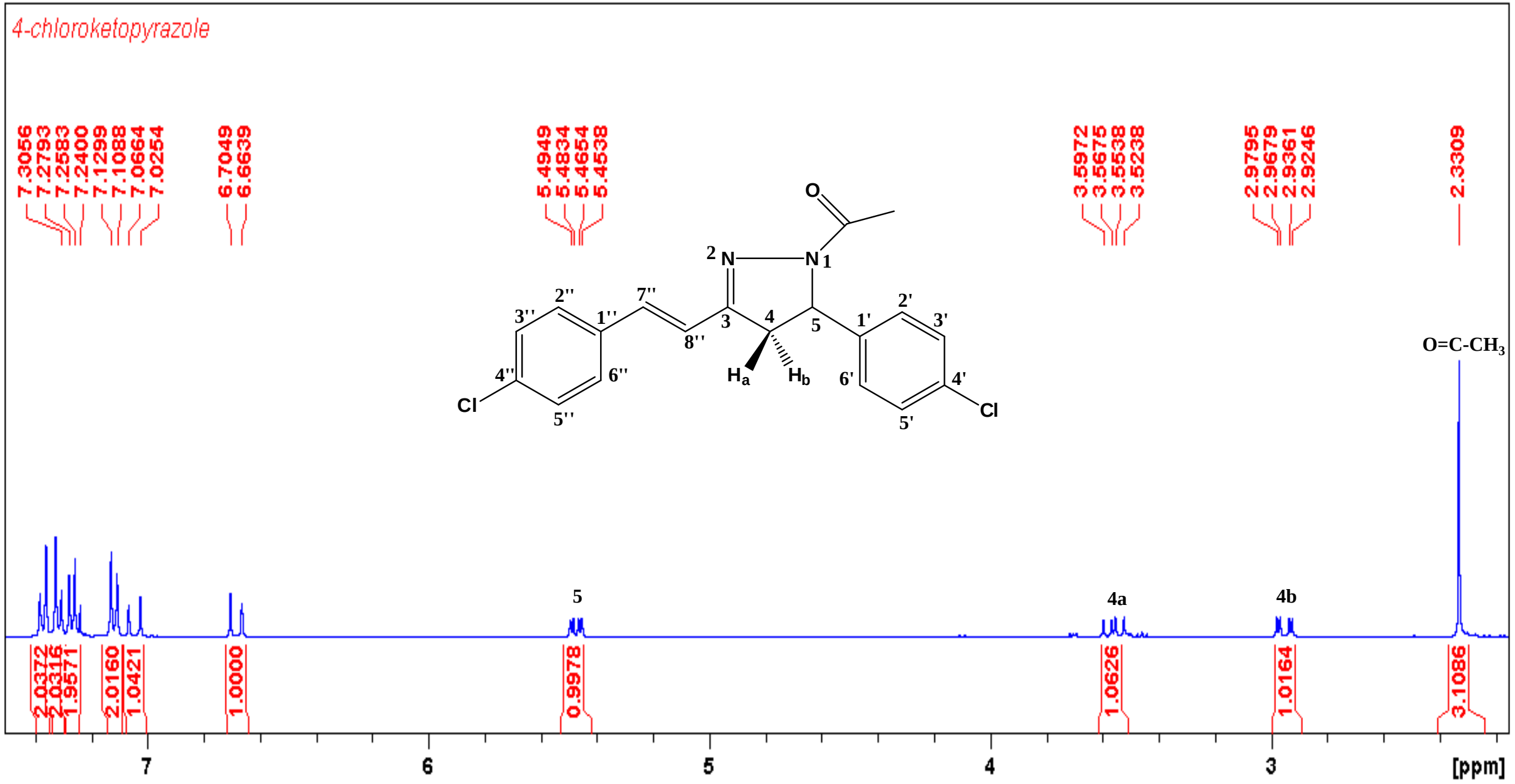
Monoisotopic Mass, Even Electron Ions
47 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 15-20 H: 15-20 N: 0-5 O: 0-5 Na: 1-1 Cl: 0-2

Compound 2 13 (0.405) Cm (1:61)
TOF MS ES+

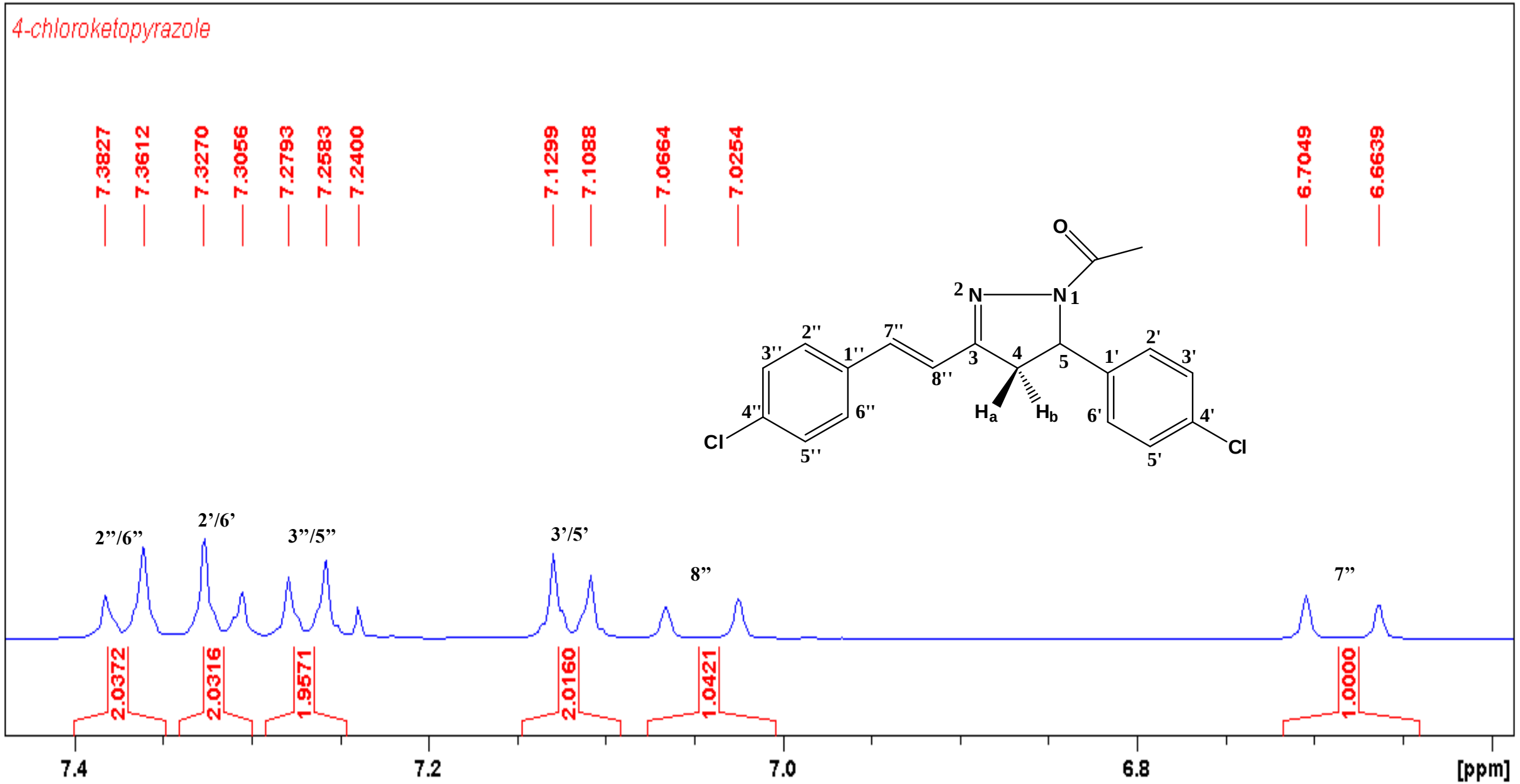


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
381.0527	381.0537	-1.0	-2.6	11.5	133.2	0.0	C19 H16 N2 O Na Cl2

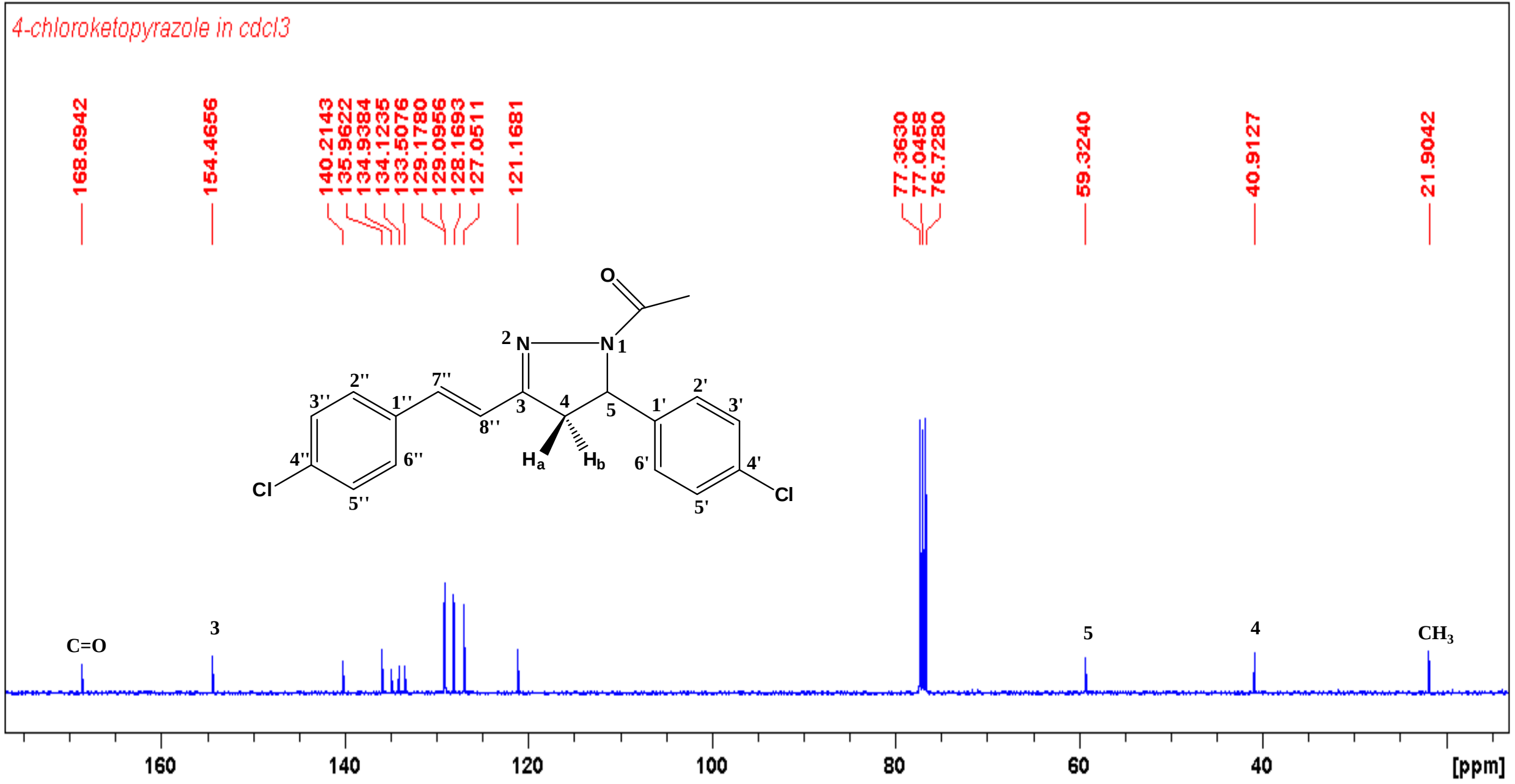
HRMS of Compound B-2b: (E)-1-(5-(3-chlorophenyl)-3-(3-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



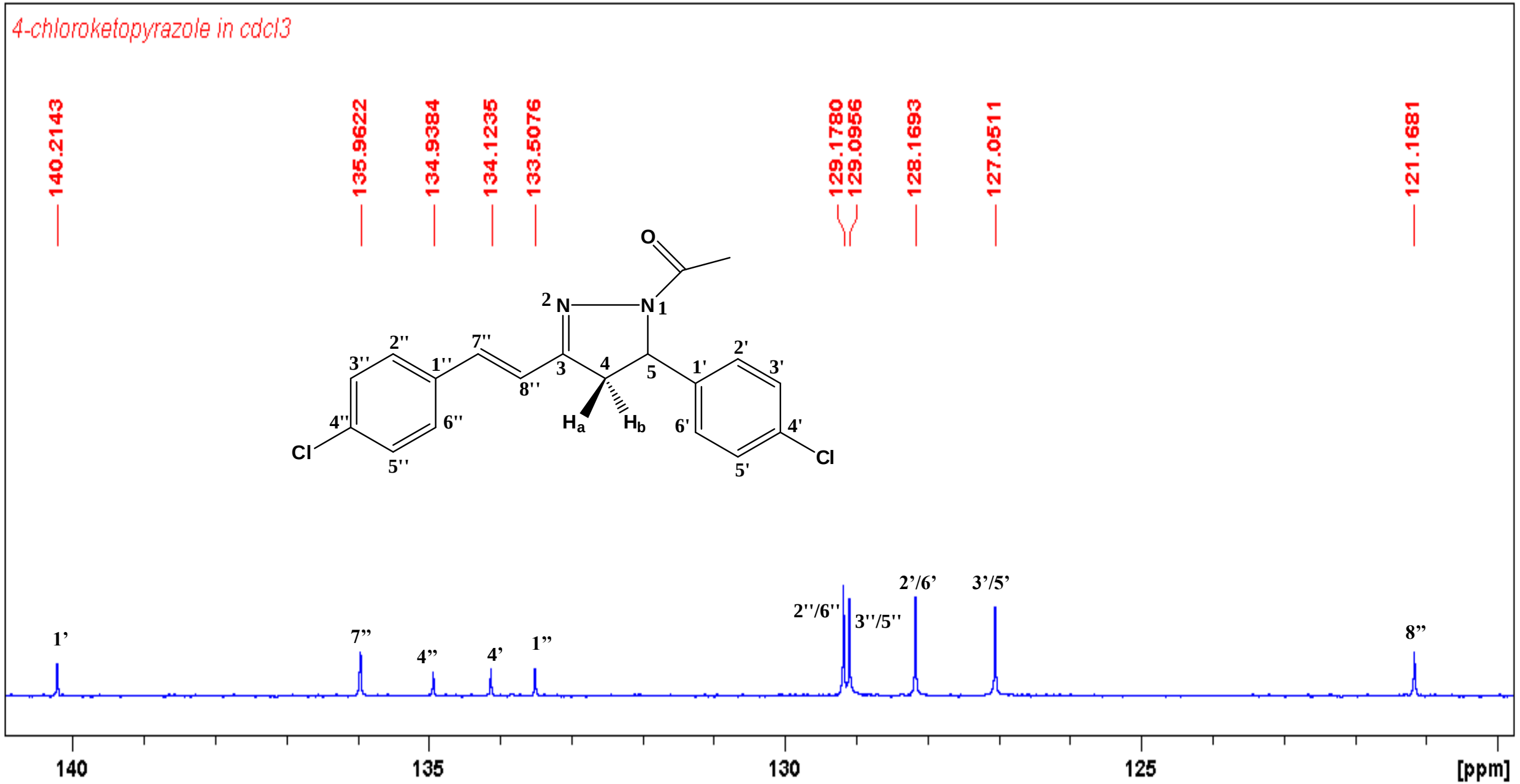
^{1}H Spectrum of Compound B-2c: (E)-1-(5-(4-chlorophenyl)-3-(4-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



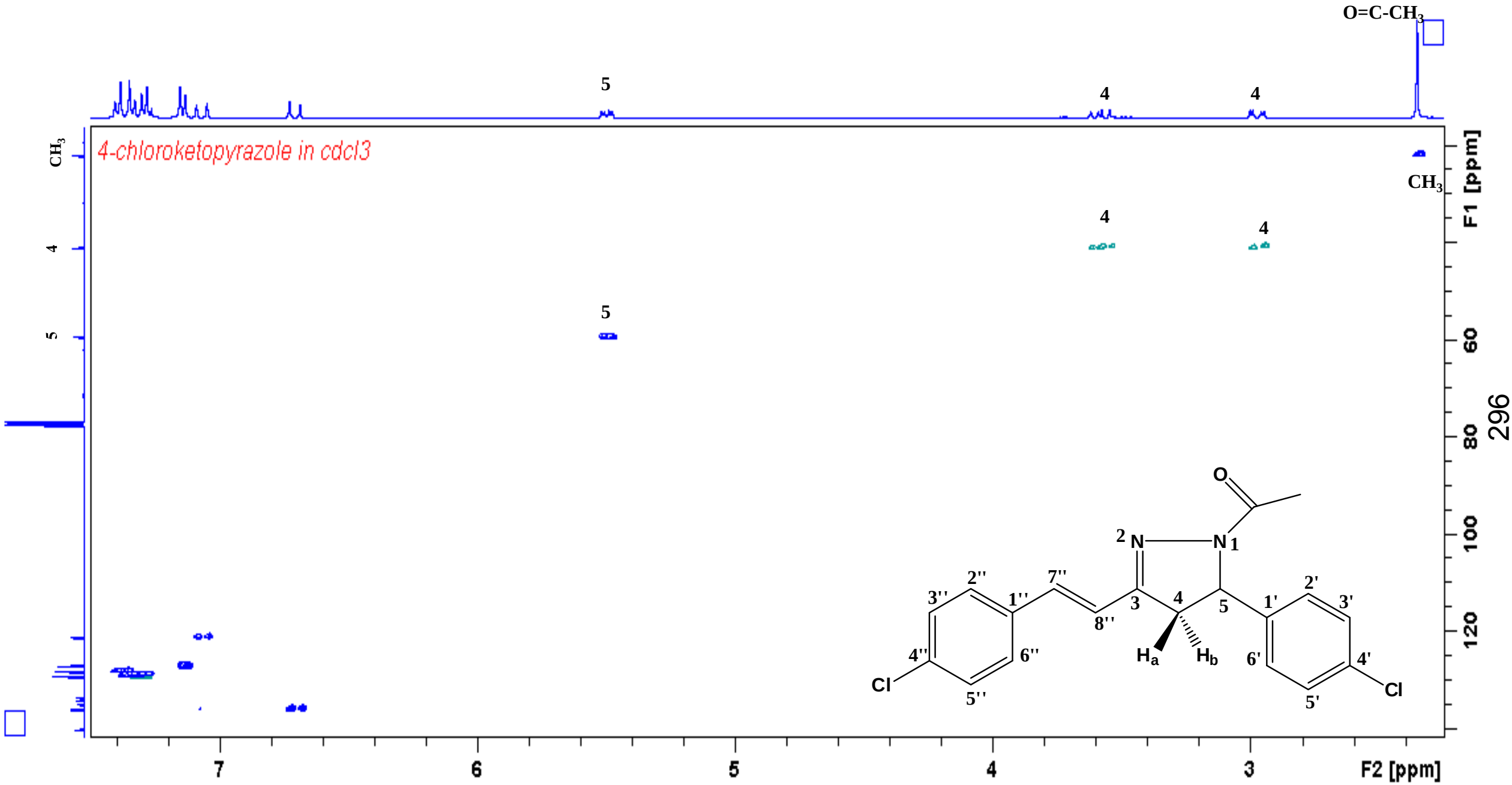
Expanded ^{1}H Spectrum of Compound B-2c: (E)-1-(5-(4-chlorophenyl)-3-(4-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



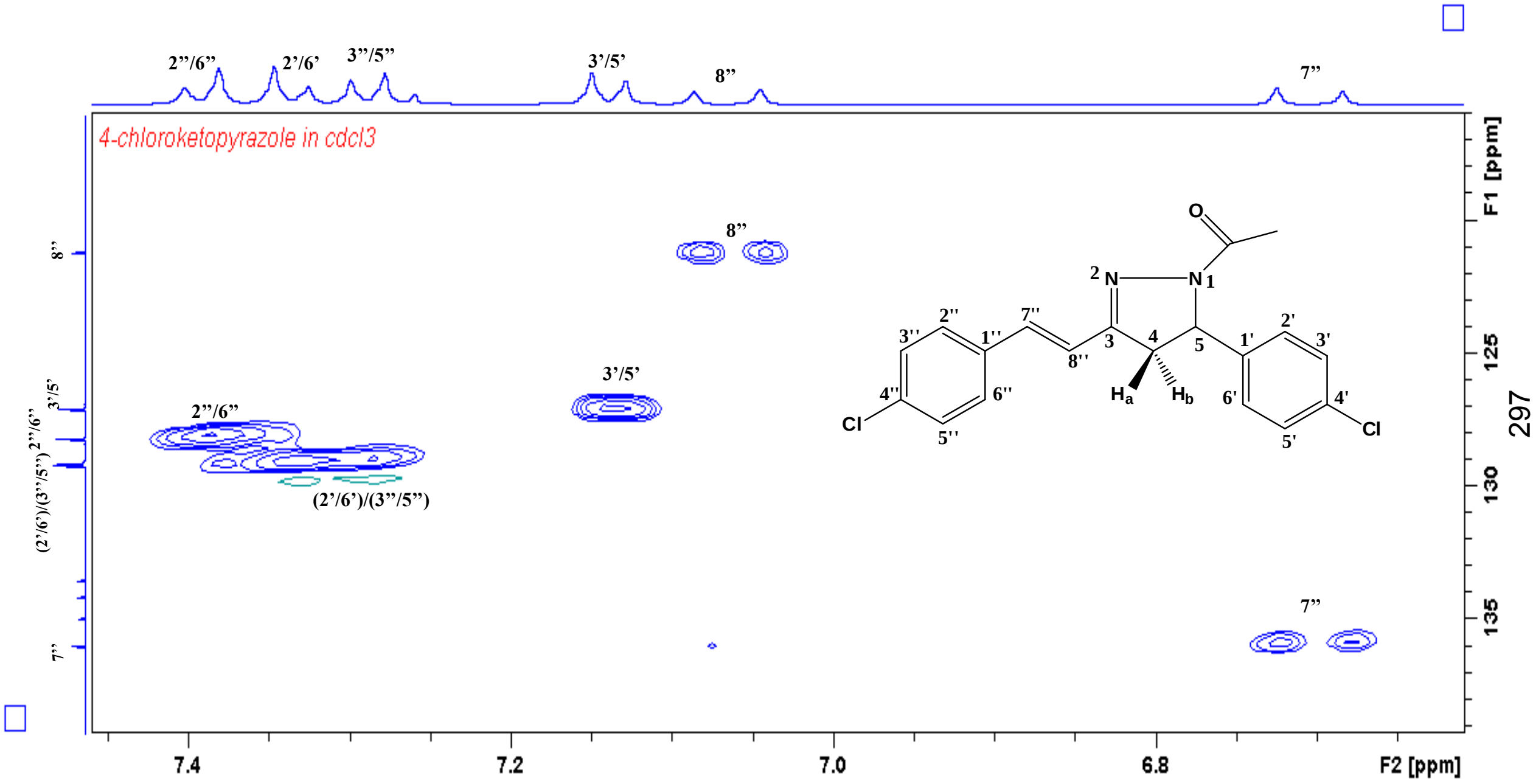
^{13}C Spectrum of Compound B-2c: (E)-1-(5-(4-chlorophenyl)-3-(4-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



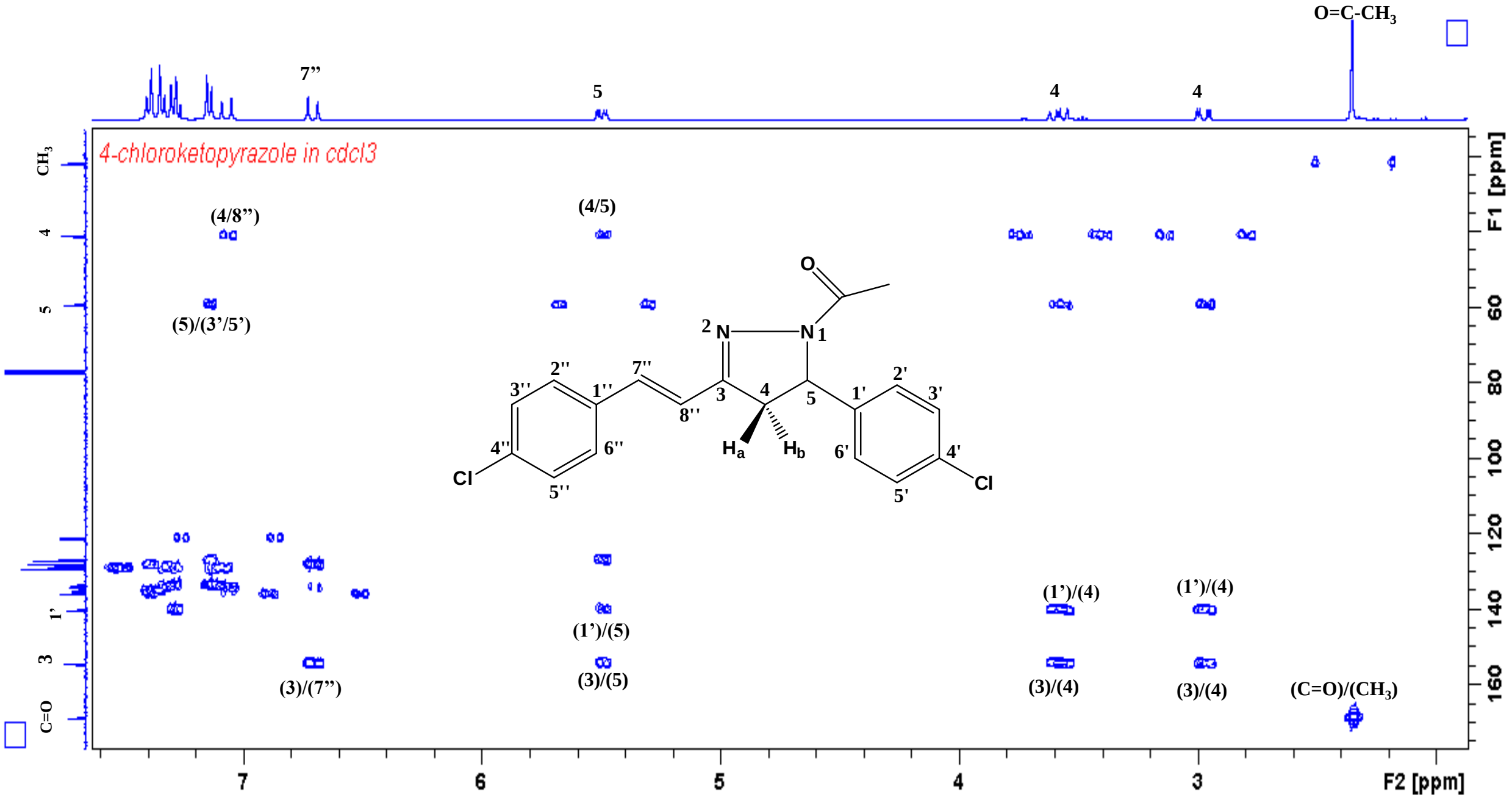
Expanded ^{13}C Spectrum of Compound B-2c: (E)-1-(5-(4-chlorophenyl)-3-(4-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



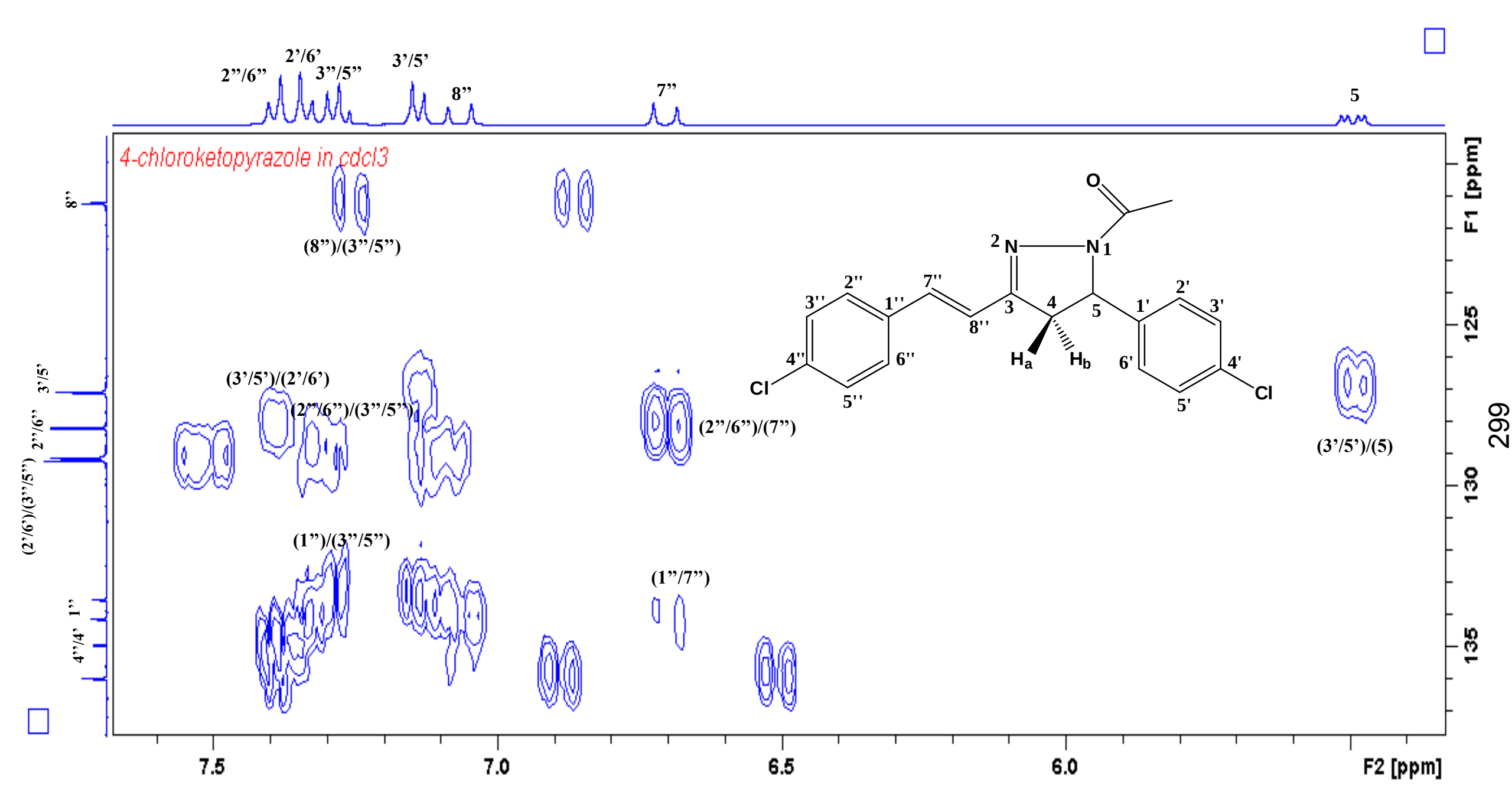
HSQC of Compound B-2c: (E)-1-(5-(4-chlorophenyl)-3-(4-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



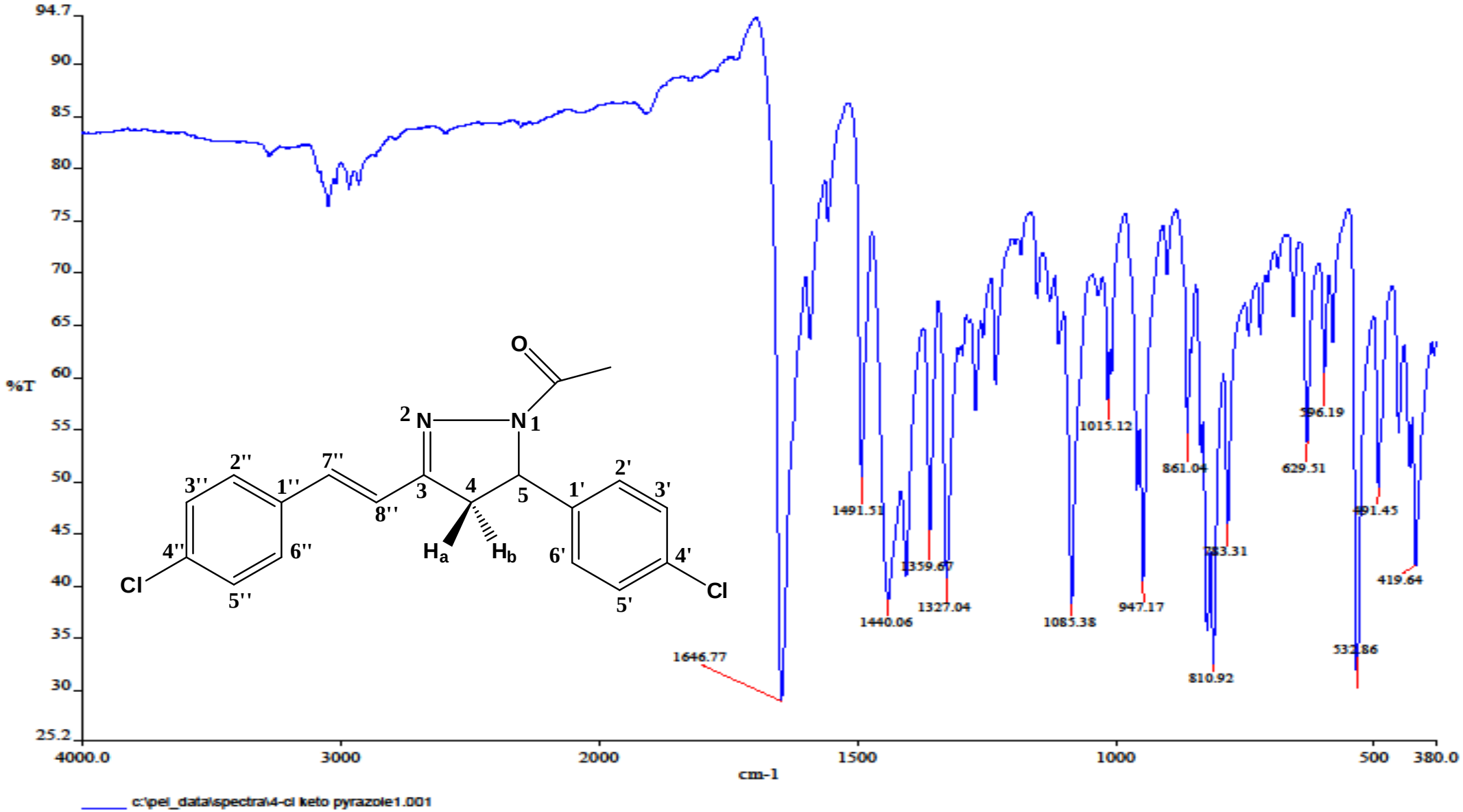
Expanded HSQC of Compound B-2c: (E)-1-(5-(4-chlorophenyl)-3-(4-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



HMBC of Compound B-2c: (E)-1-(5-(4-chlorophenyl)-3-(4-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

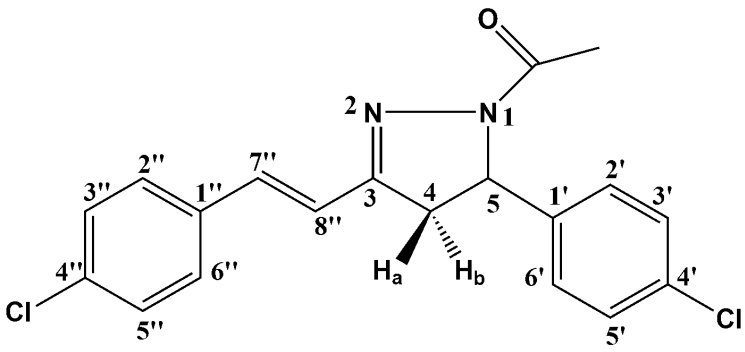


Expanded HMBC of Compound B-2c: (E)-1-(5-(4-chlorophenyl)-3-(4-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



300

Infrared Spectrum of Compound B-2c: (E)-1-(5-(4-chlorophenyl)-3-(4-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



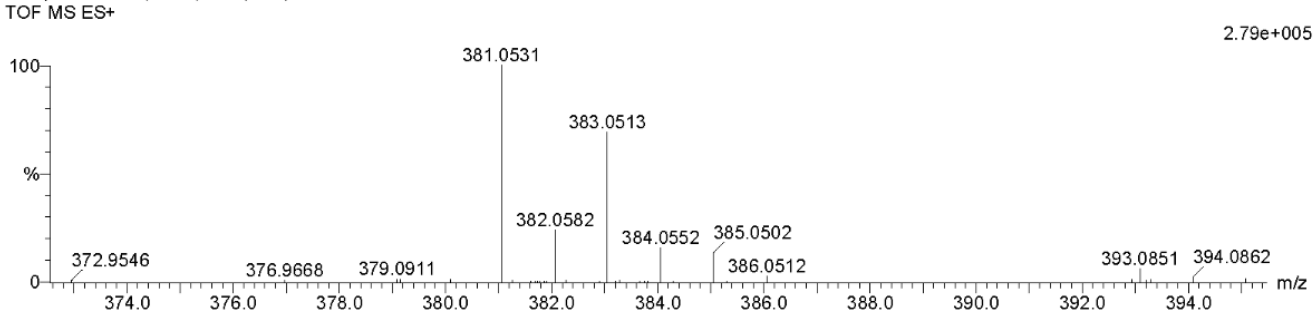
Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

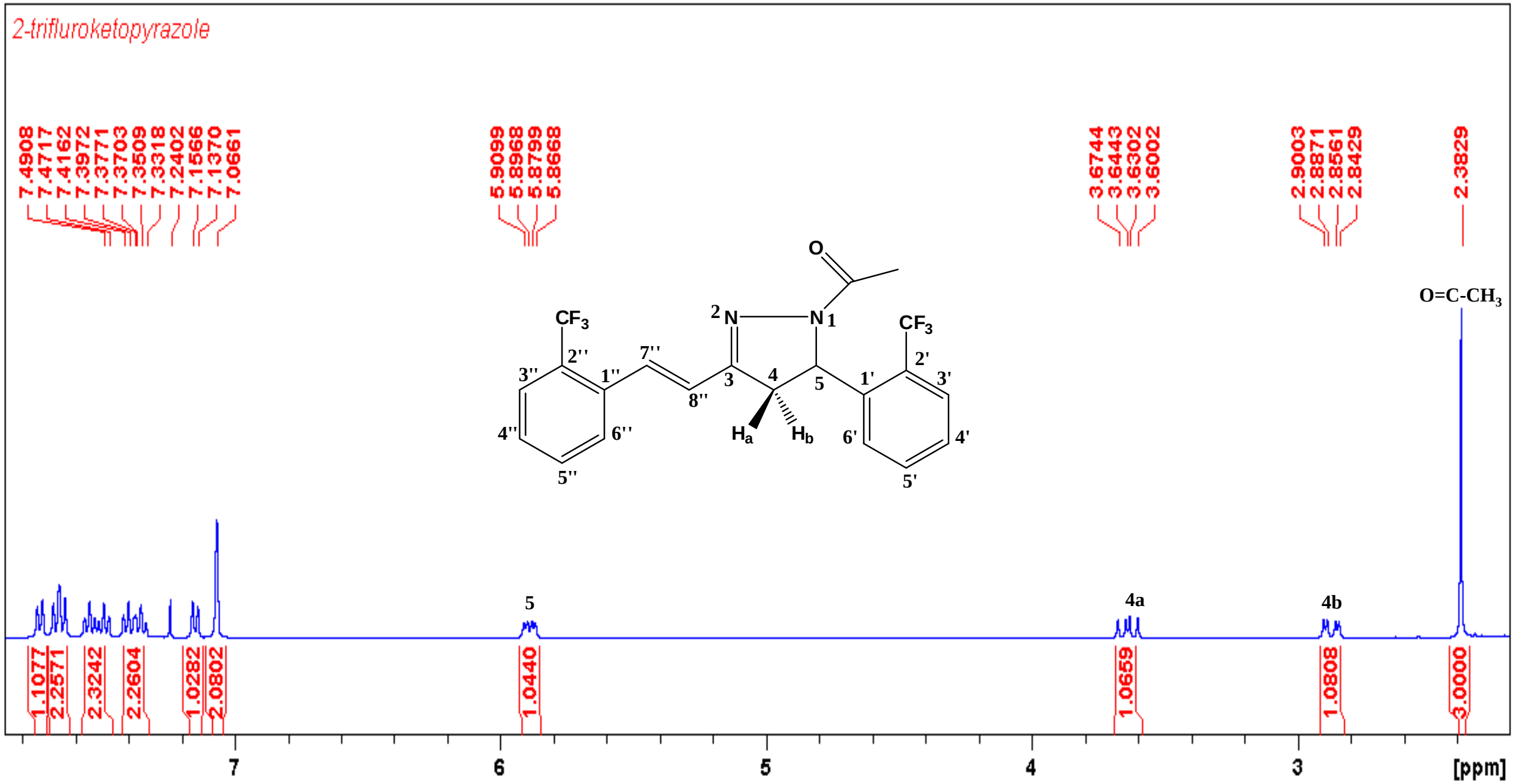
Monoisotopic Mass, Even Electron Ions
47 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 15-20 H: 15-20 N: 0-5 O: 0-5 Na: 1-1 Cl: 0-2

Compound 3 32 (1.080) Cm (1:60)
TOF MS ES+

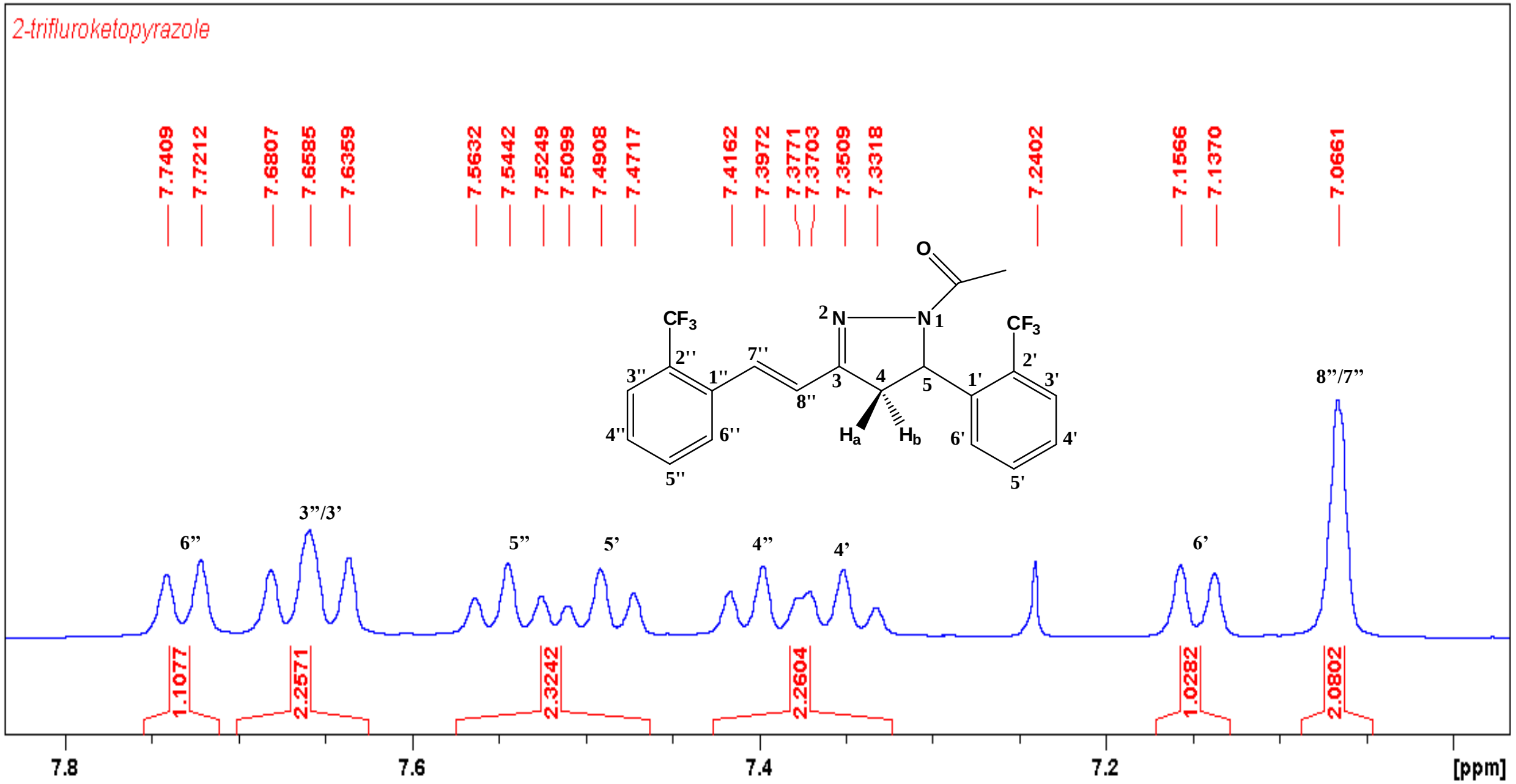


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
381.0531	381.0537	-0.6	-1.6	11.5	130.3	0.0	C19 H16 N2 O Na Cl2

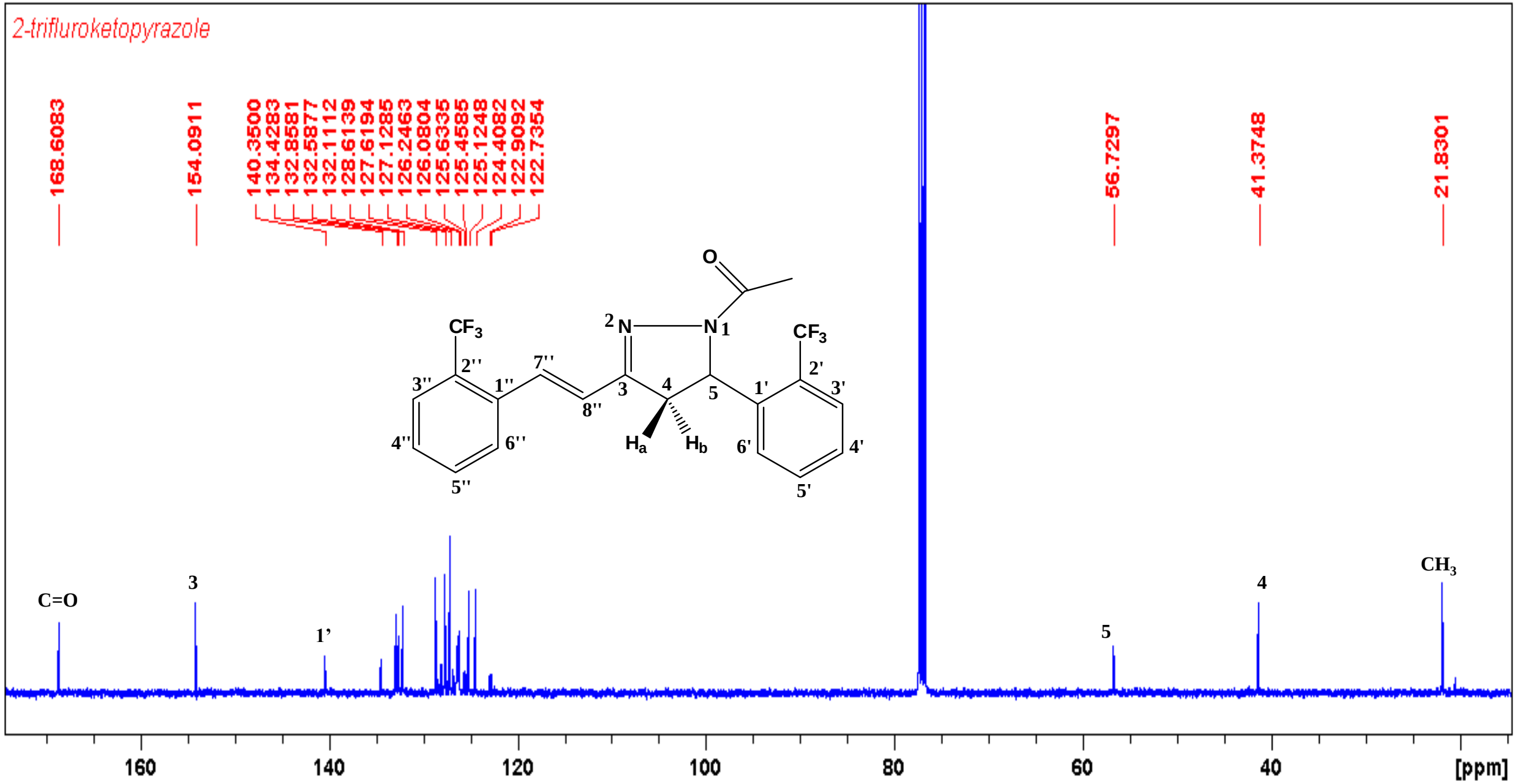
HRMS of Compound B-2c: (E)-1-(5-(4-chlorophenyl)-3-(4-chlorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



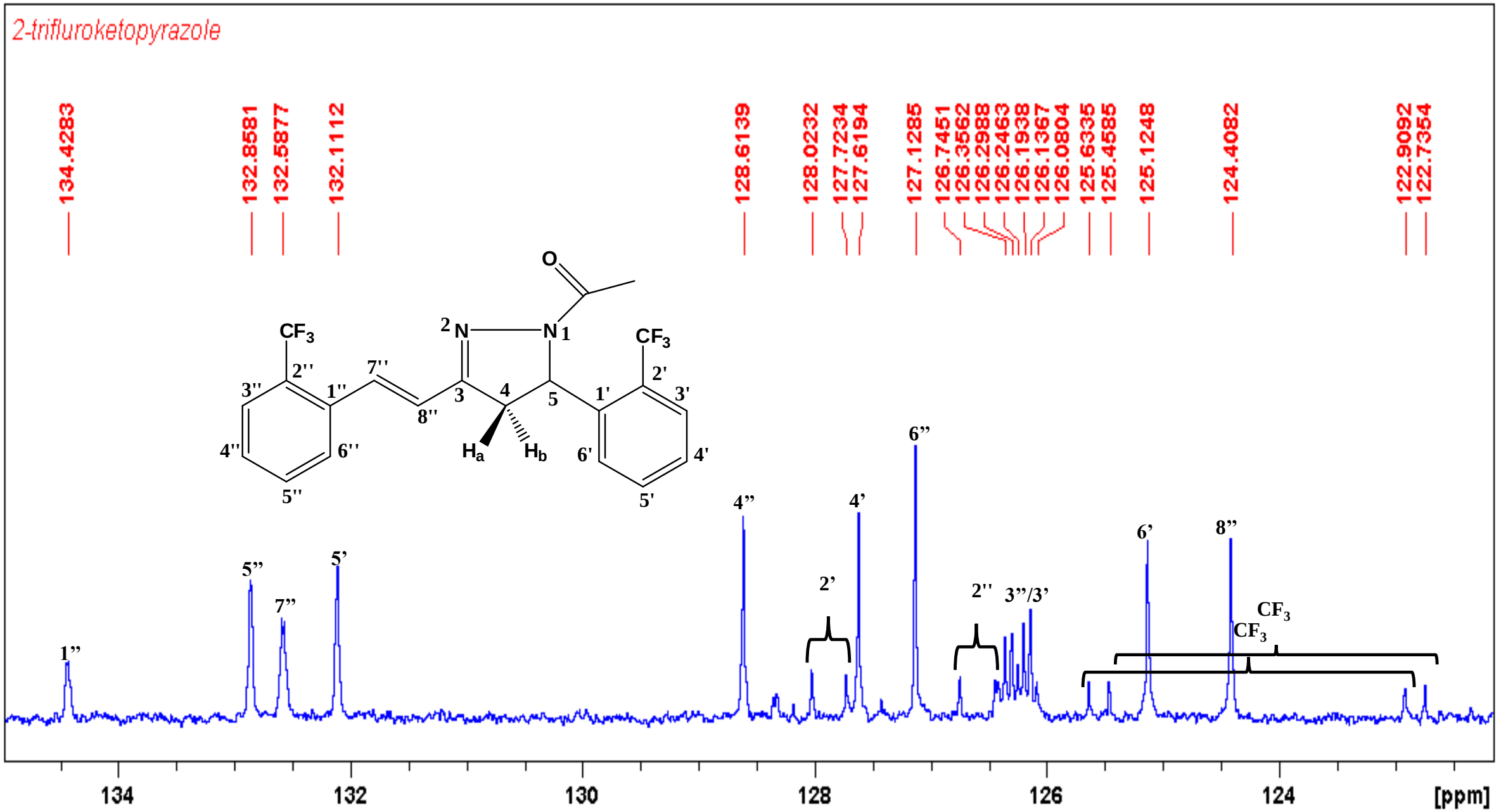
^{1}H Spectrum of Compound B-2d: (E)-1-(5-(2-(trifluoromethyl)phenyl)-3-(2-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



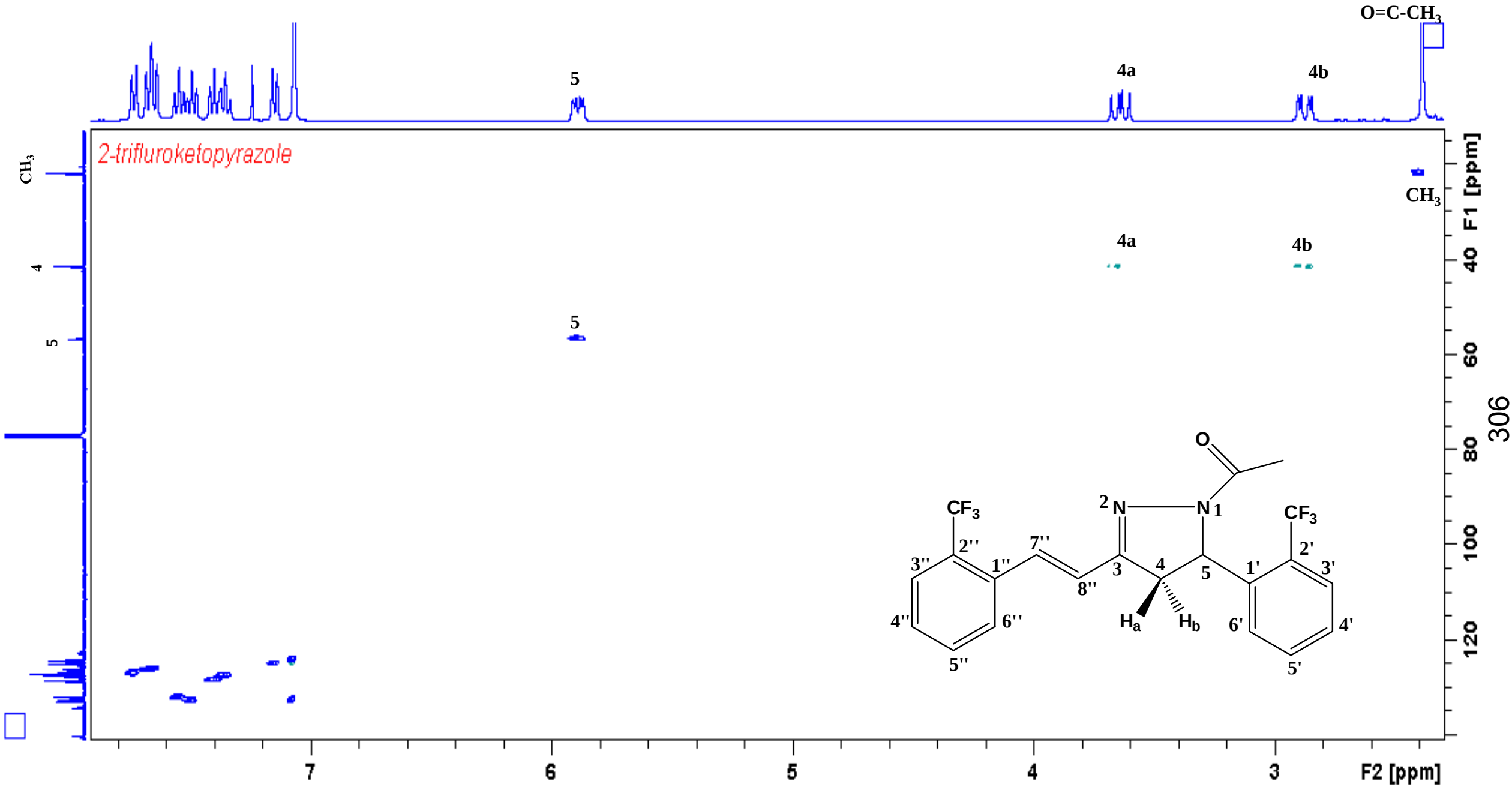
Expanded ^{1}H Spectrum of Compound B-2d: (E)-1-(5-(2-(trifluoromethyl)phenyl)-3-(2-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



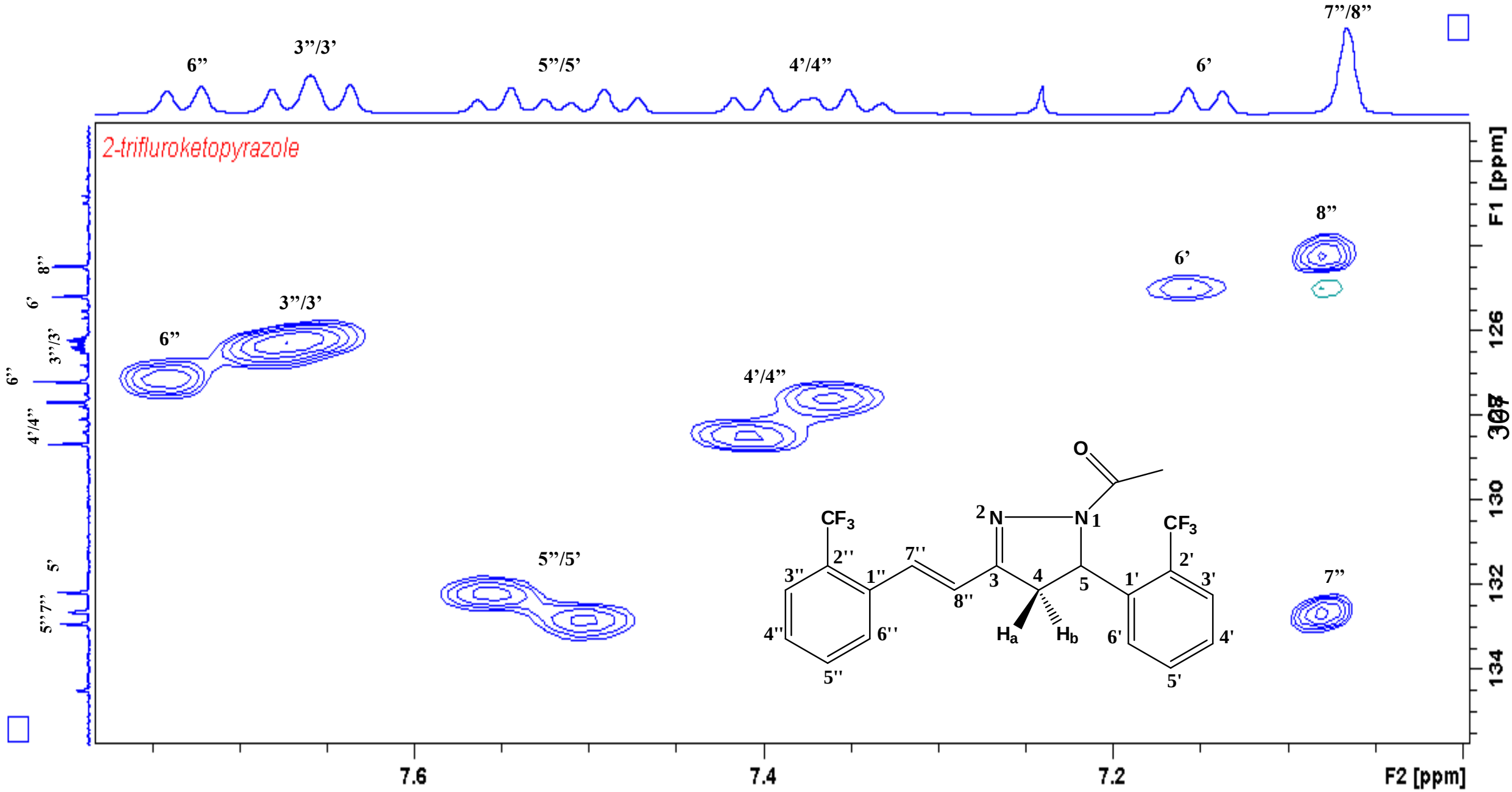
^{13}C Spectrum of Compound B-2d: (E)-1-(5-(2-(trifluoromethyl)phenyl)-3-(2-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



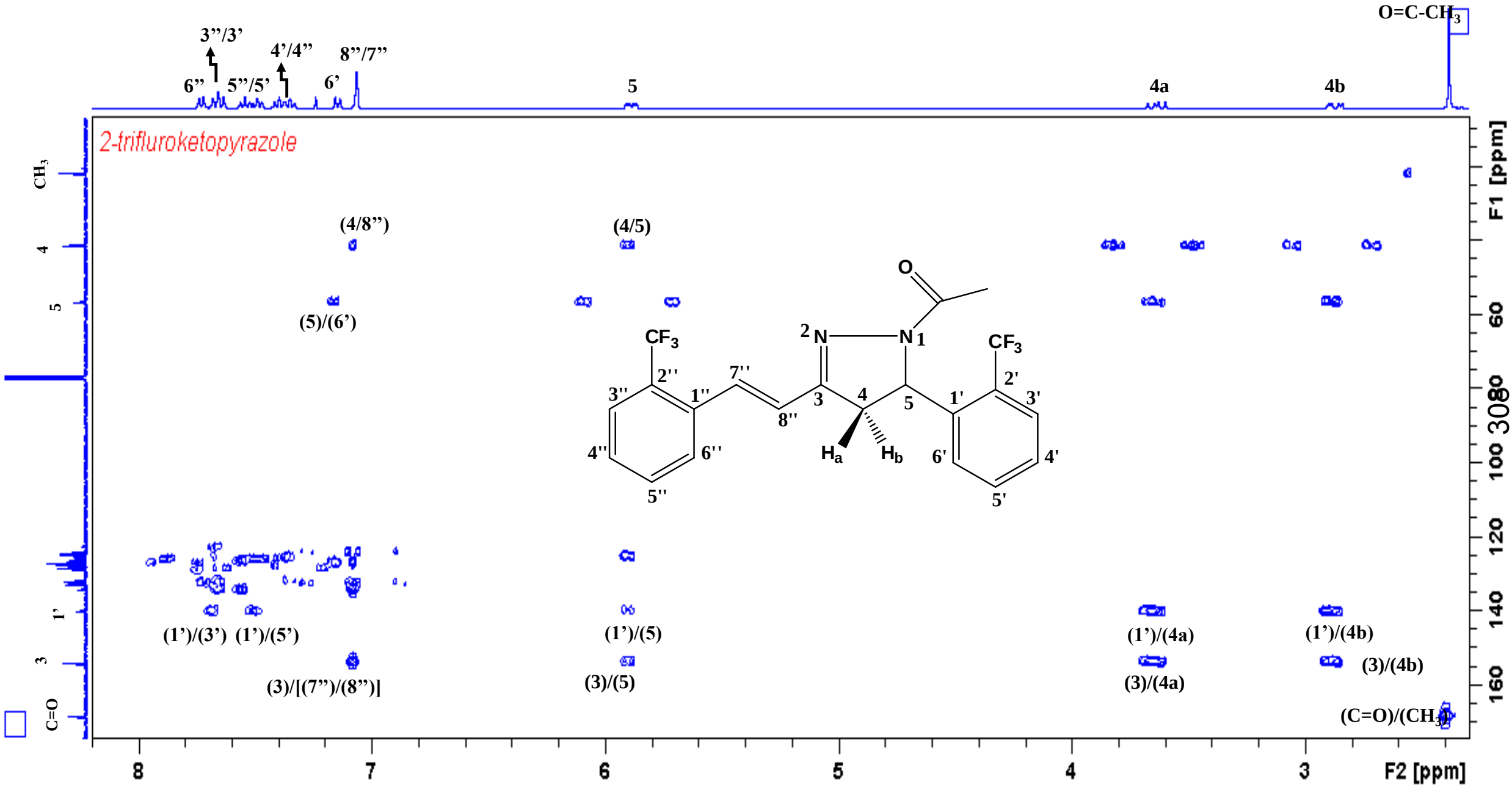
Expanded ^{13}C Spectrum of Compound B-2d: (E)-1-(5-(2-(trifluoromethyl)phenyl)-3-(2-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



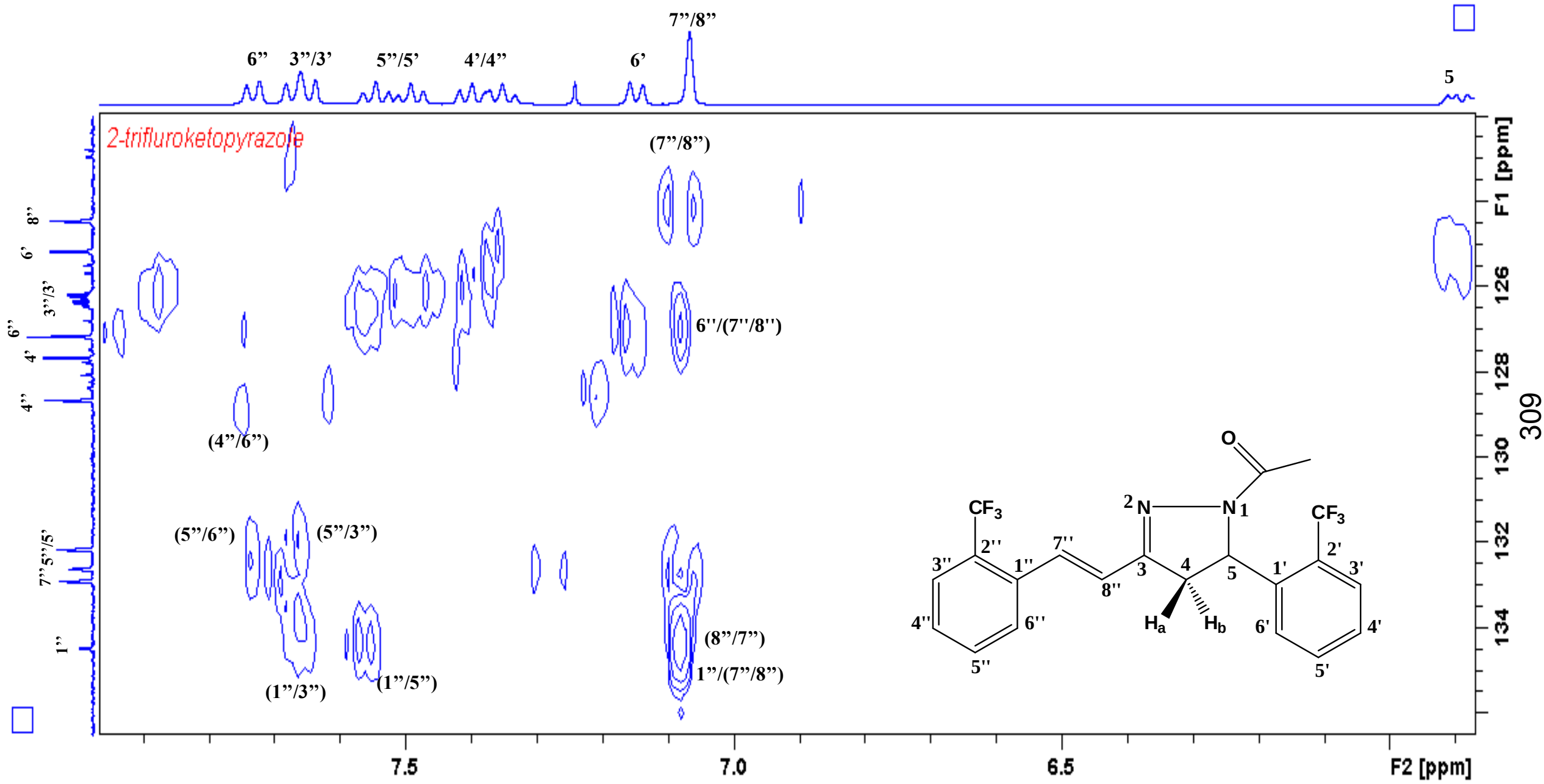
HSQC of Compound B-2d: (E)-1-(5-(2-(trifluoromethyl)phenyl)-3-(2-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



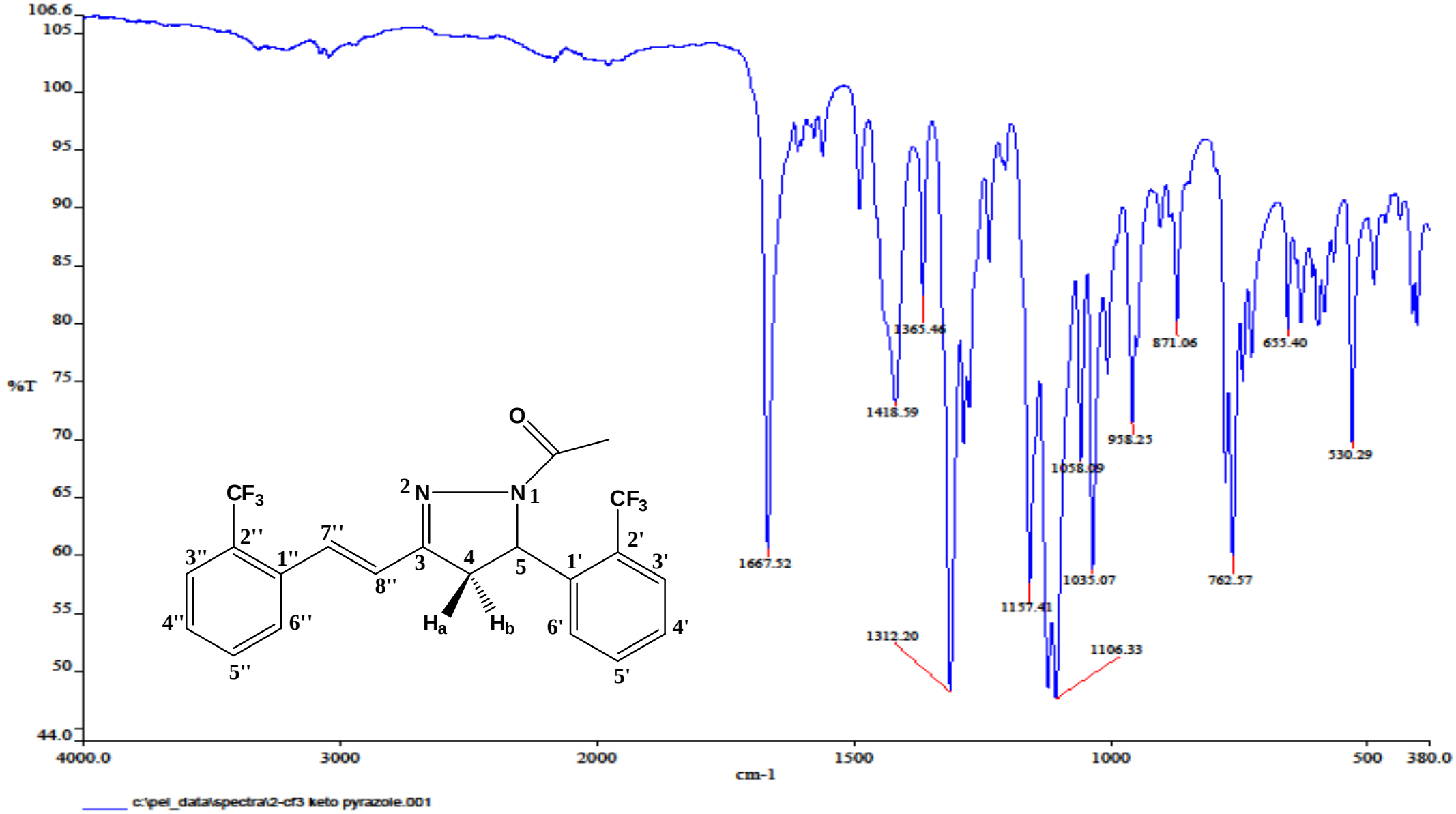
Expanded HSQC of Compound B-2d: (E)-1-(5-(2-(trifluoromethyl)phenyl)-3-(2-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



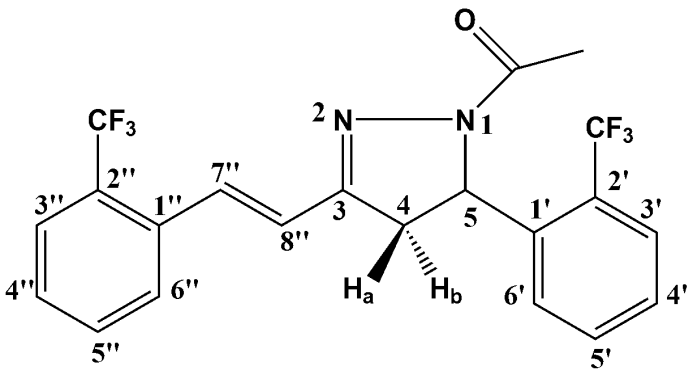
HMBC of Compound B-2d: (E)-1-(5-(2-(trifluoromethyl)phenyl)-3-(2-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



Expanded HMBC of Compound B-2d: (E)-1-(5-(2-(trifluoromethyl)phenyl)-3-(2-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



Infrared Spectrum of Compound B-2d: (E)-1-(5-(2-(trifluoromethyl)phenyl)-3-(2-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



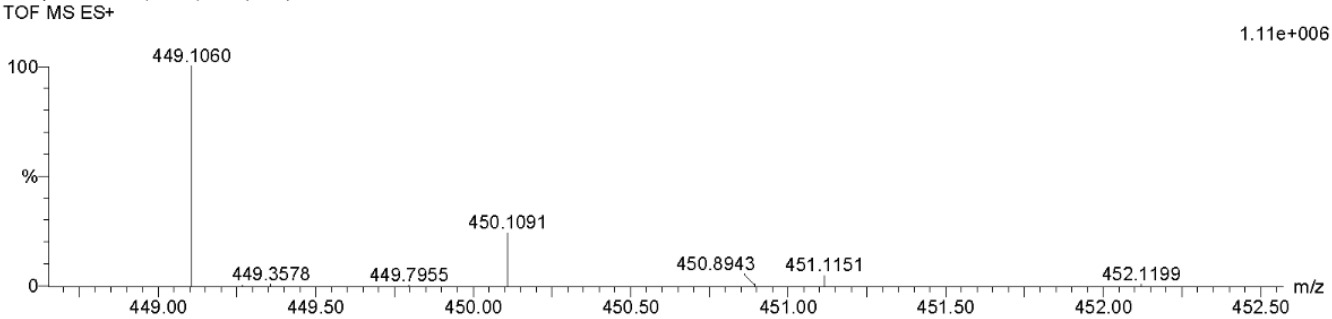
Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

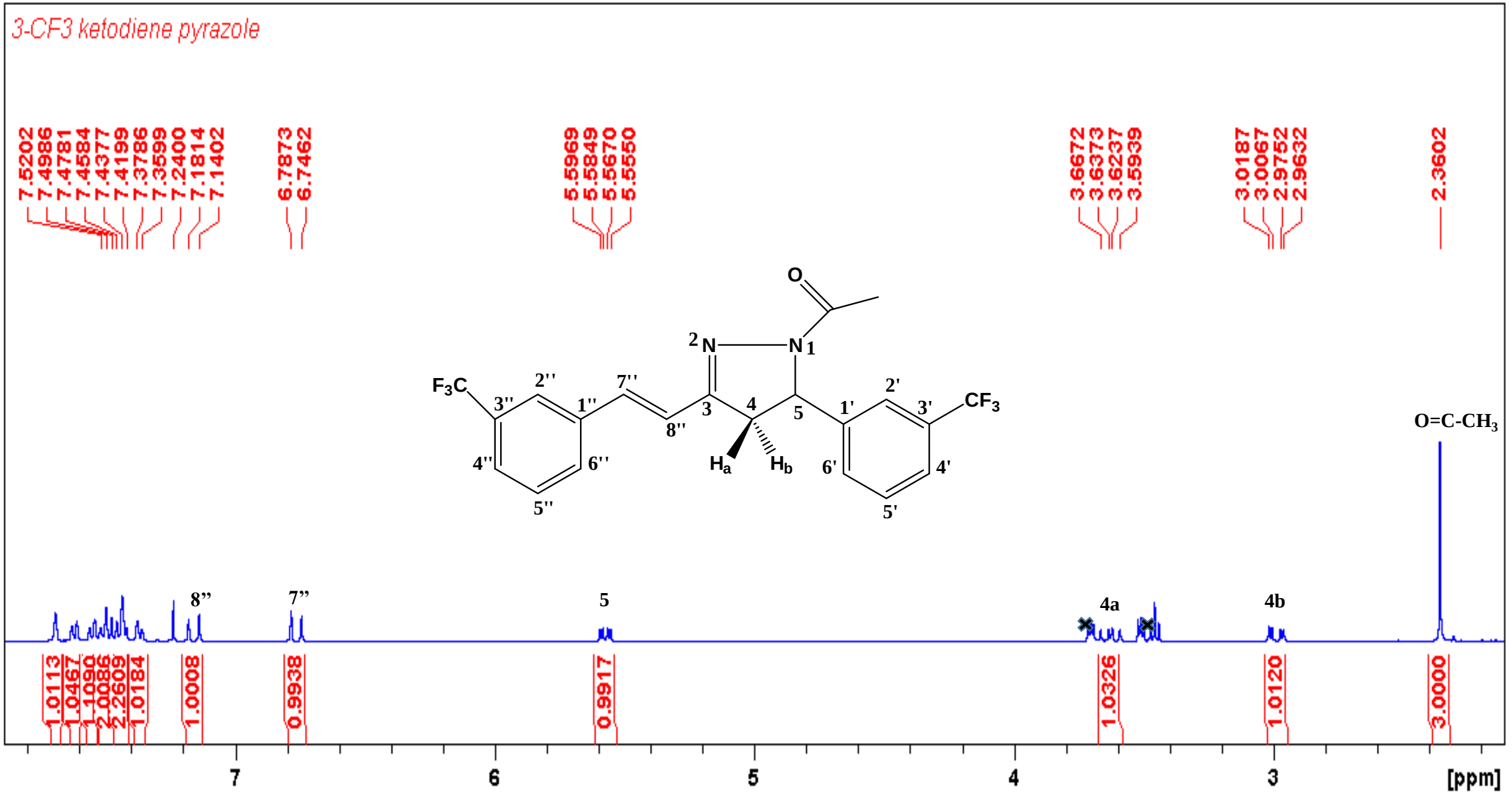
Monoisotopic Mass, Even Electron Ions
278 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 1-5 F: 5-10 Na: 0-1

Compound 4 56 (1.888) Cm (1:60)
TOF MS ES+



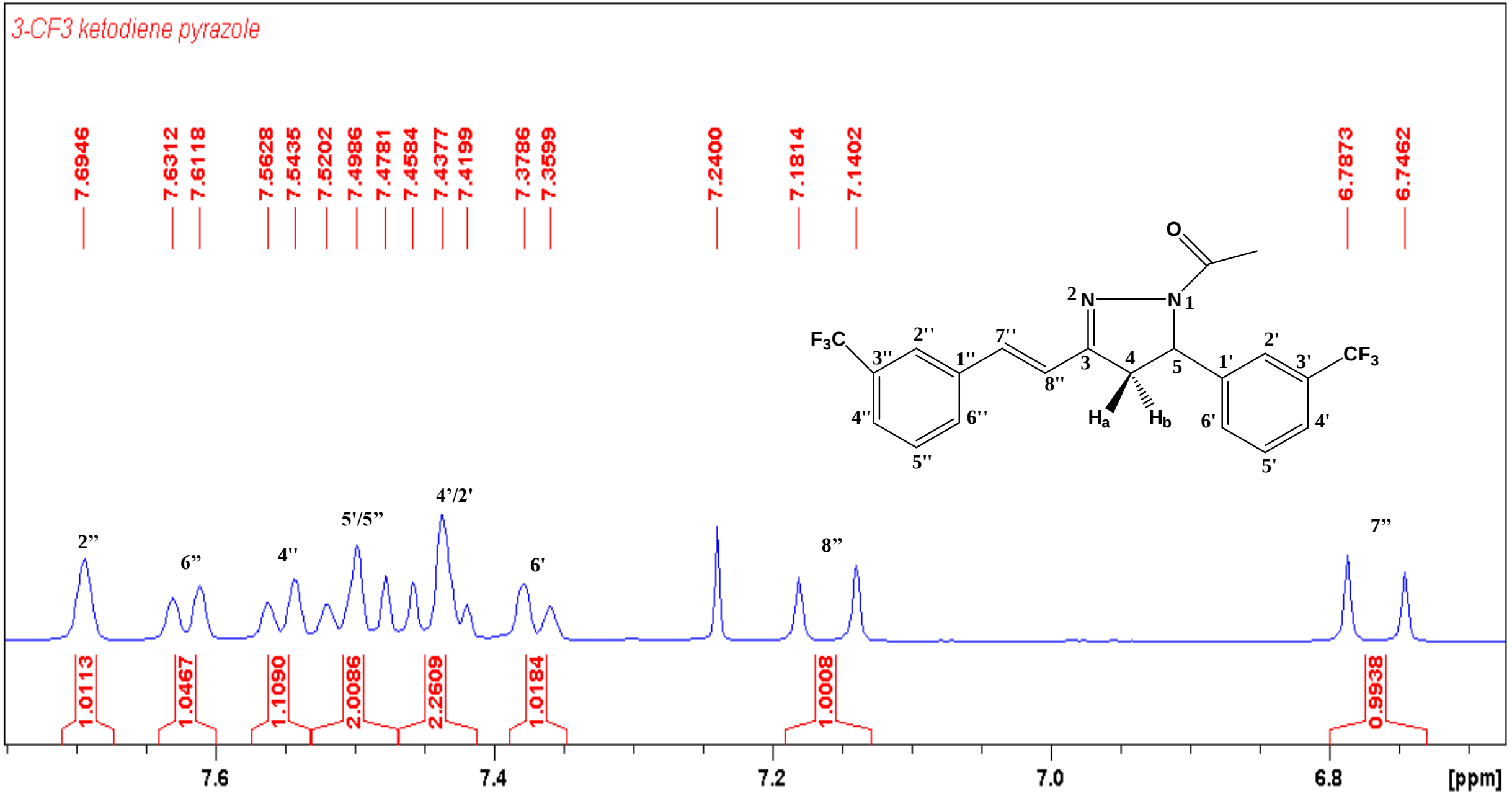
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
449.1060	449.1065	-0.5	-1.1	11.5	64.1	0.0	C21 H16 N2 O F6 Na

HRMS of Compound B-2d: (E)-1-(5-(2-(trifluoromethyl)phenyl)-3-(2-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

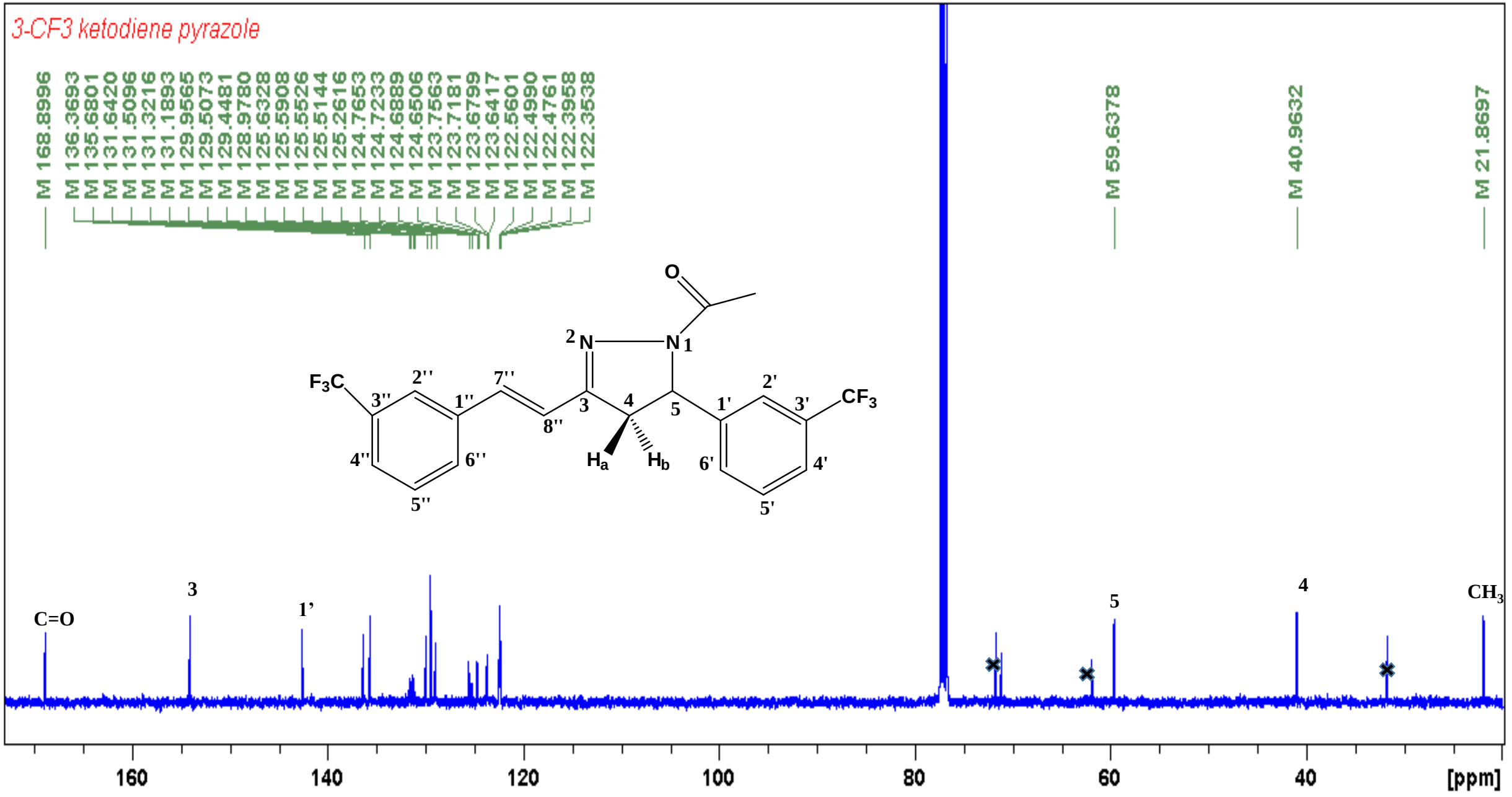


312

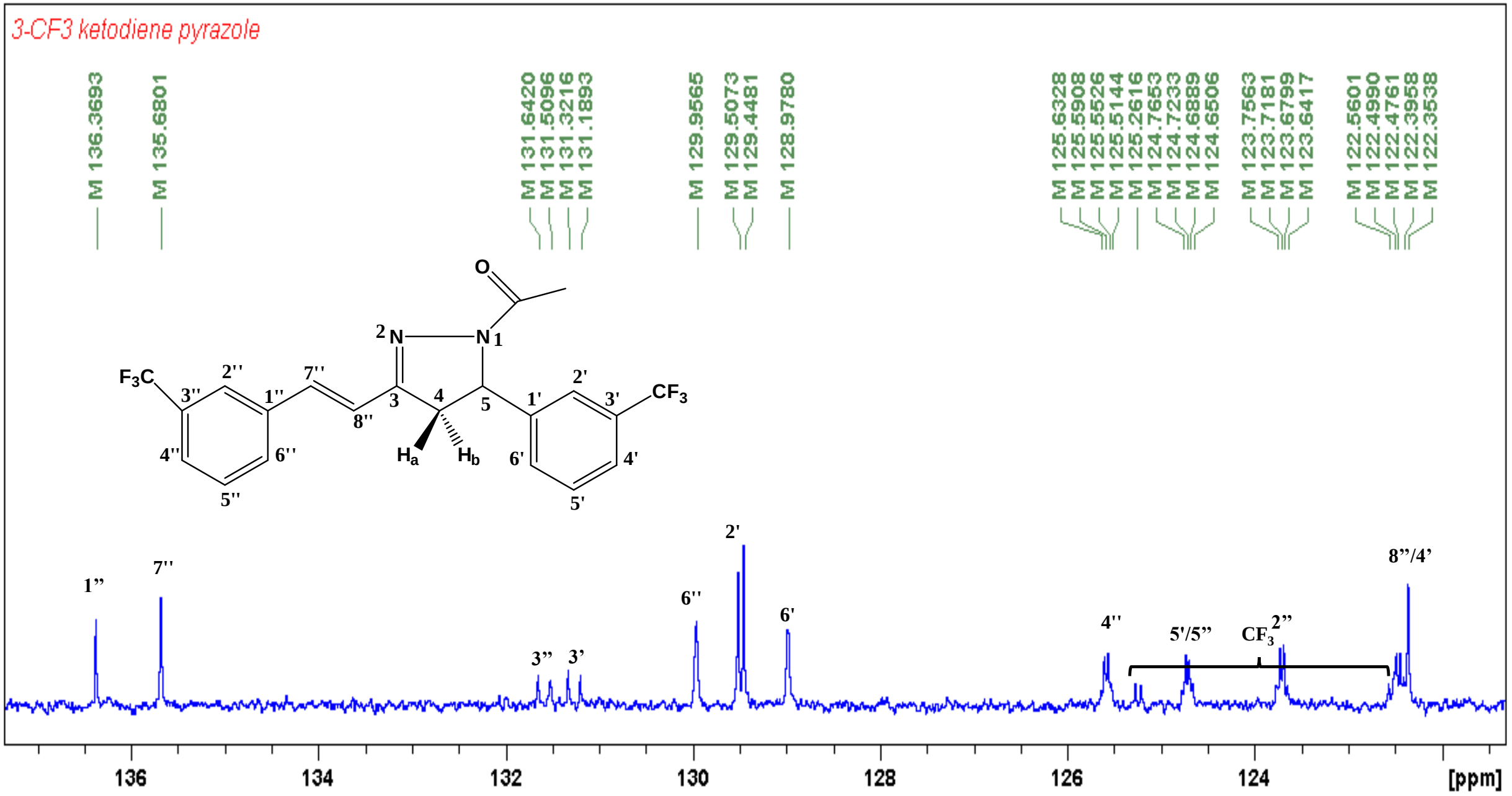
[1]H Spectrum of Compound B-2e: (E)-1-(5-(3-(trifluoromethyl)phenyl)-3-(3-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



Expanded ^{1}H Spectrum of Compound B-2e: (E)-1-(5-(3-(trifluoromethyl)phenyl)-3-(3-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

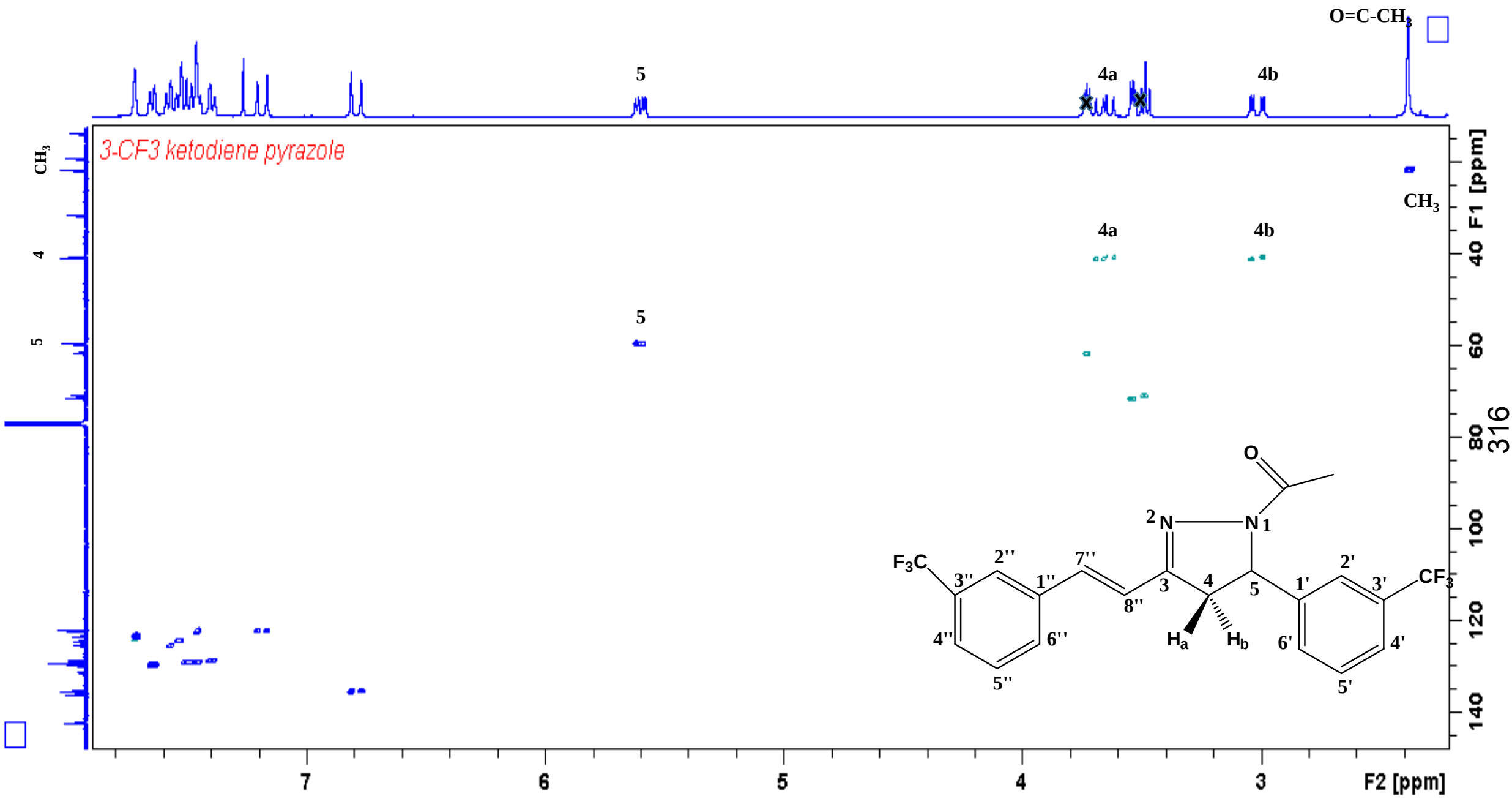


^{13}C Spectrum of Compound B-2e: (E)-1-(5-(3-(trifluoromethyl)phenyl)-3-(3-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

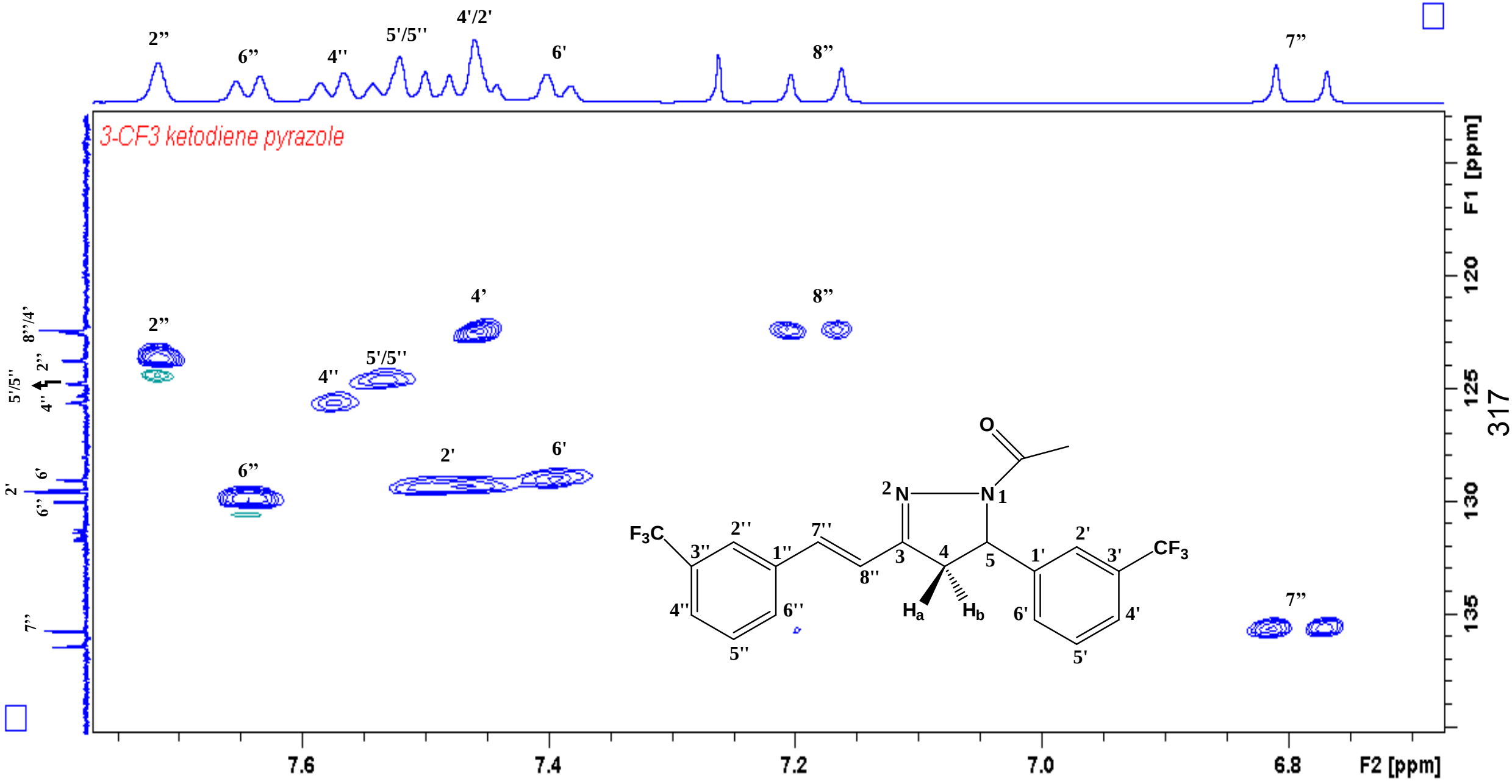


315

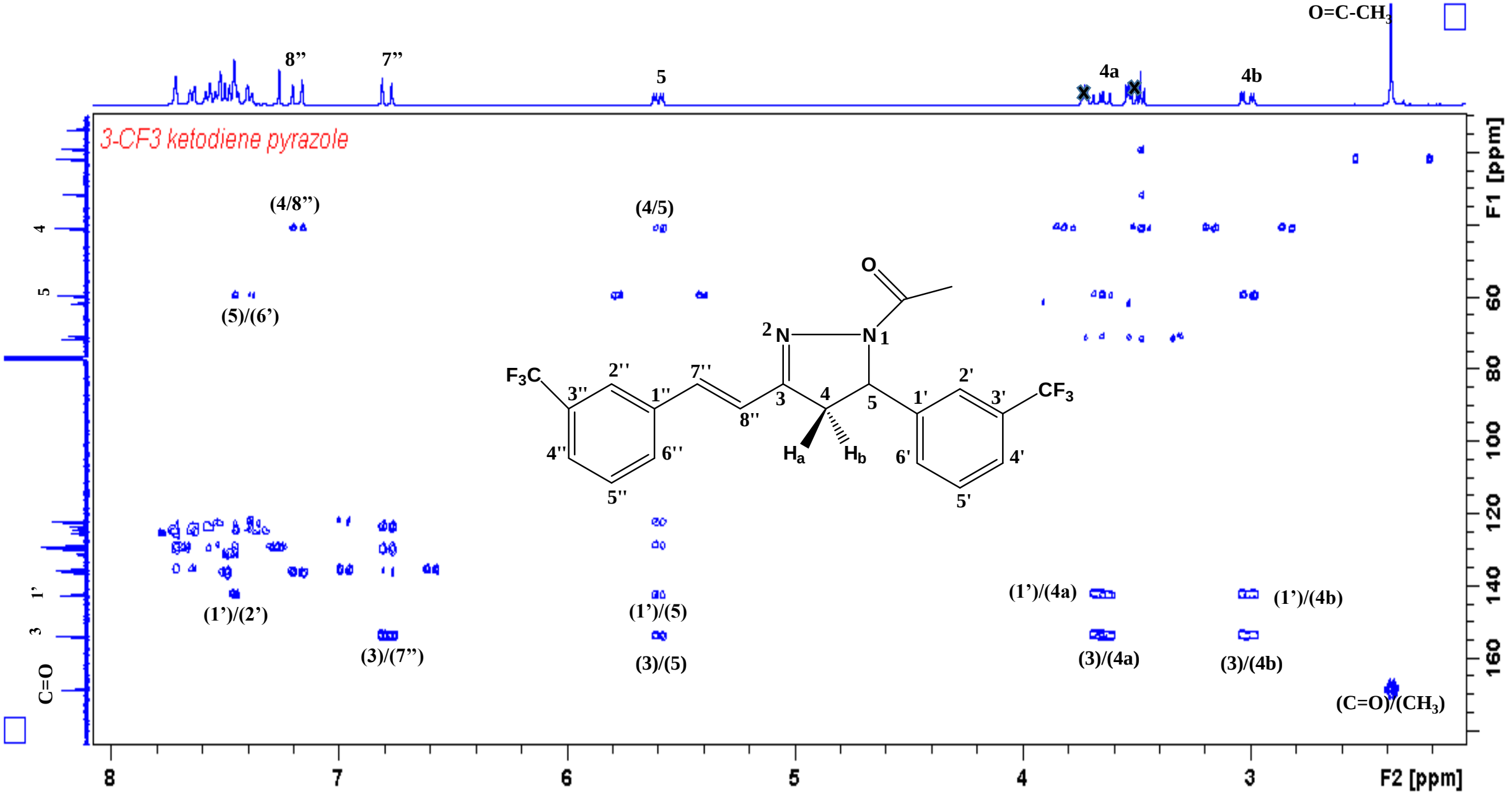
Expanded ^{13}C Spectrum of Compound B-2e: (E)-1-(5-(3-(trifluoromethyl)phenyl)-3-(3-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



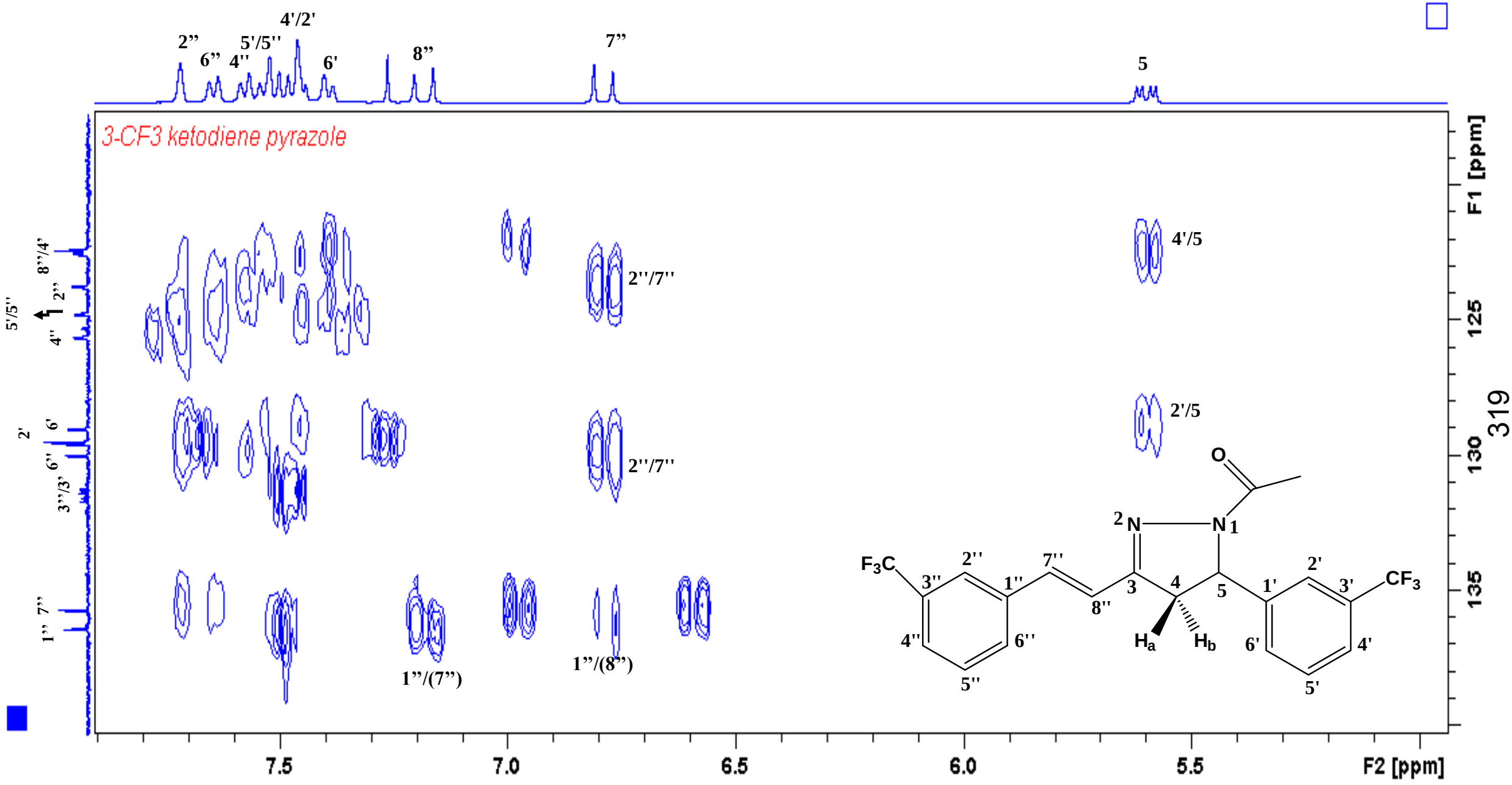
HSQC of Compound B-2e: (E)-1-(5-(3-(trifluoromethyl)phenyl)-3-(3-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



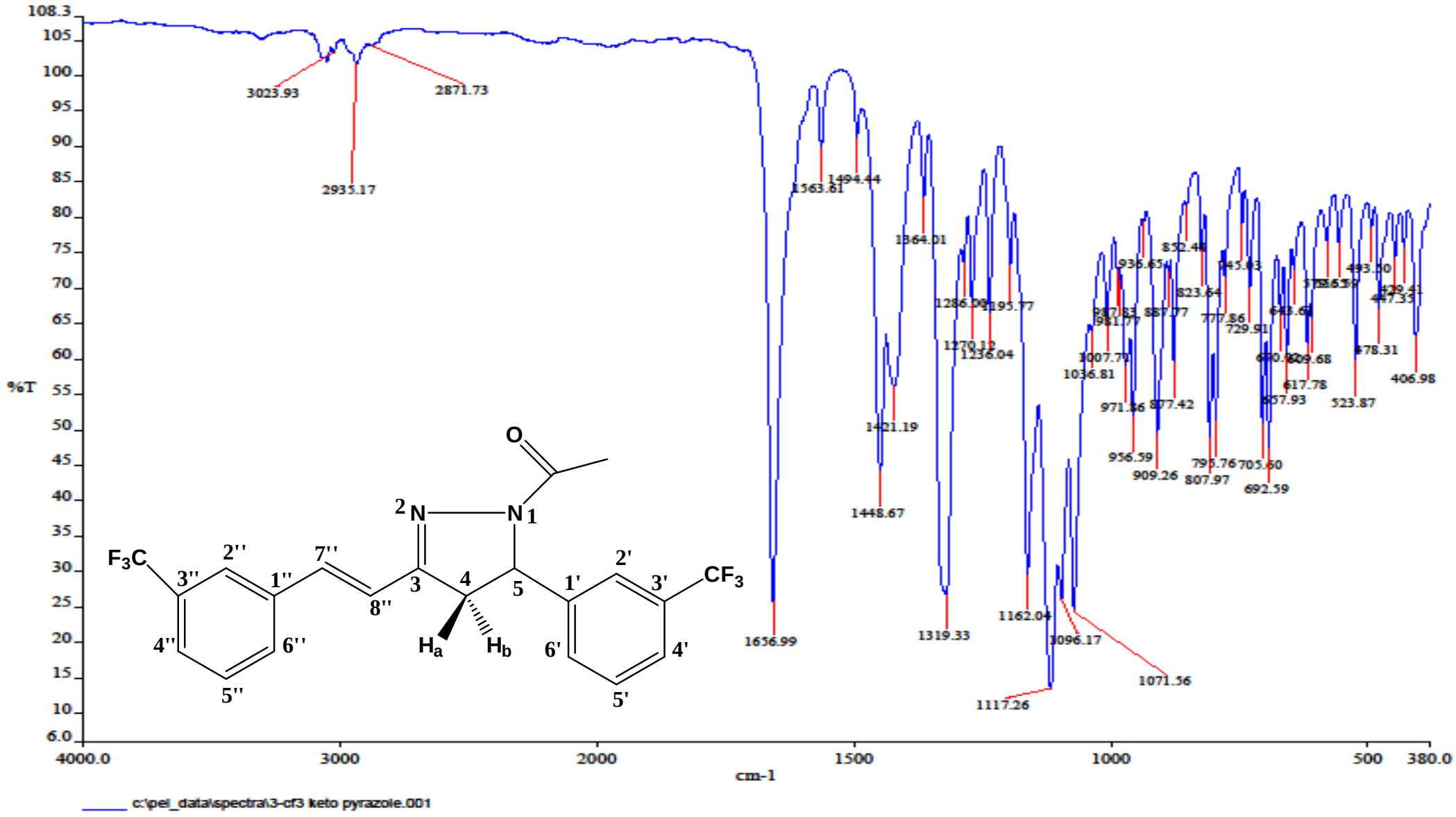
Expanded HSQC of Compound B-2e: (E)-1-(5-(3-(trifluoromethyl)phenyl)-3-(3-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



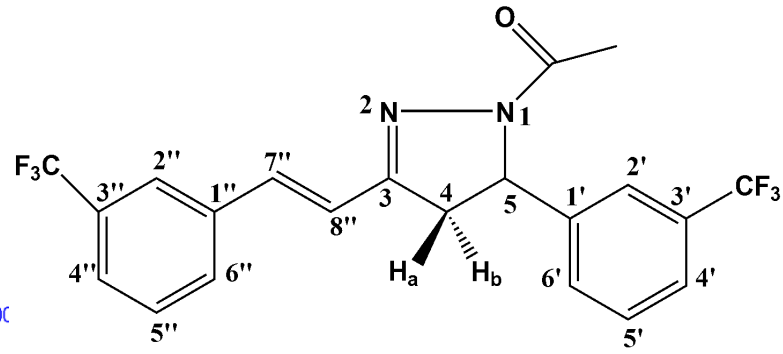
HMBC of Compound B-2e: (E)-1-(5-(3-(trifluoromethyl)phenyl)-3-(3-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



Expanded HMBC of Compound B-2e: (E)-1-(5-(3-(trifluoromethyl)phenyl)-3-(3-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



Infrared Spectrum of Compound B-2e: (E)-1-(5-(3-(trifluoromethyl)phenyl)-3-(3-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



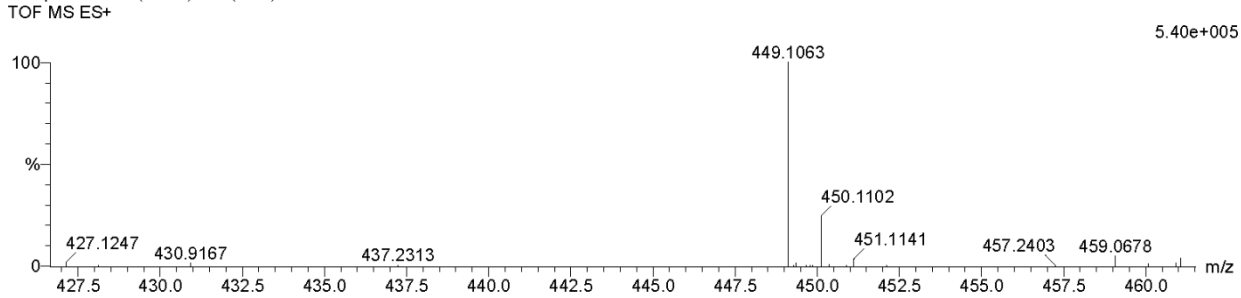
Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 10(
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

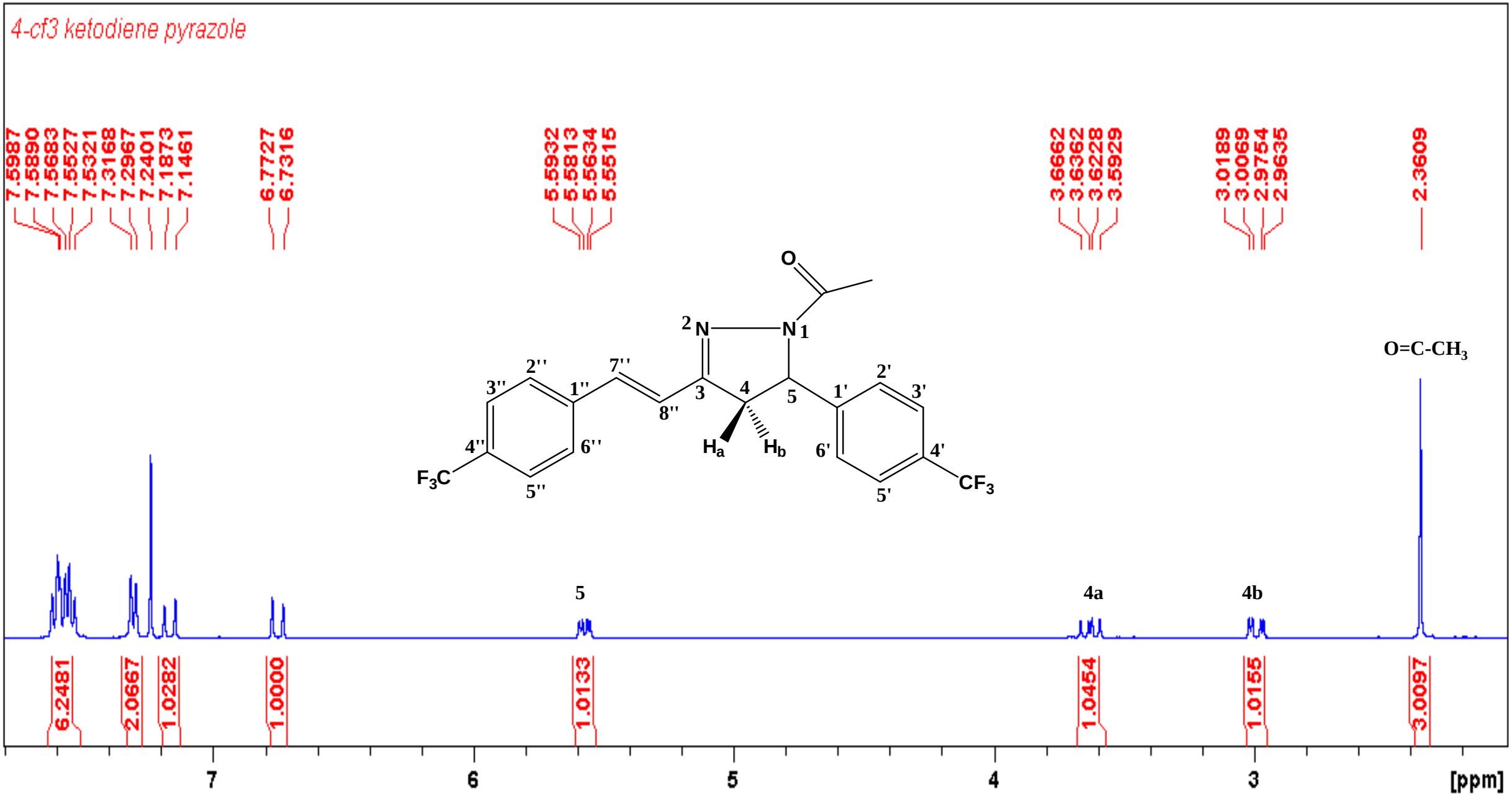
Monoisotopic Mass, Even Electron Ions
278 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 1-5 F: 5-10 Na: 0-1

Compound 5 50 (1.653) Cm (1:61)
TOF MS ES+



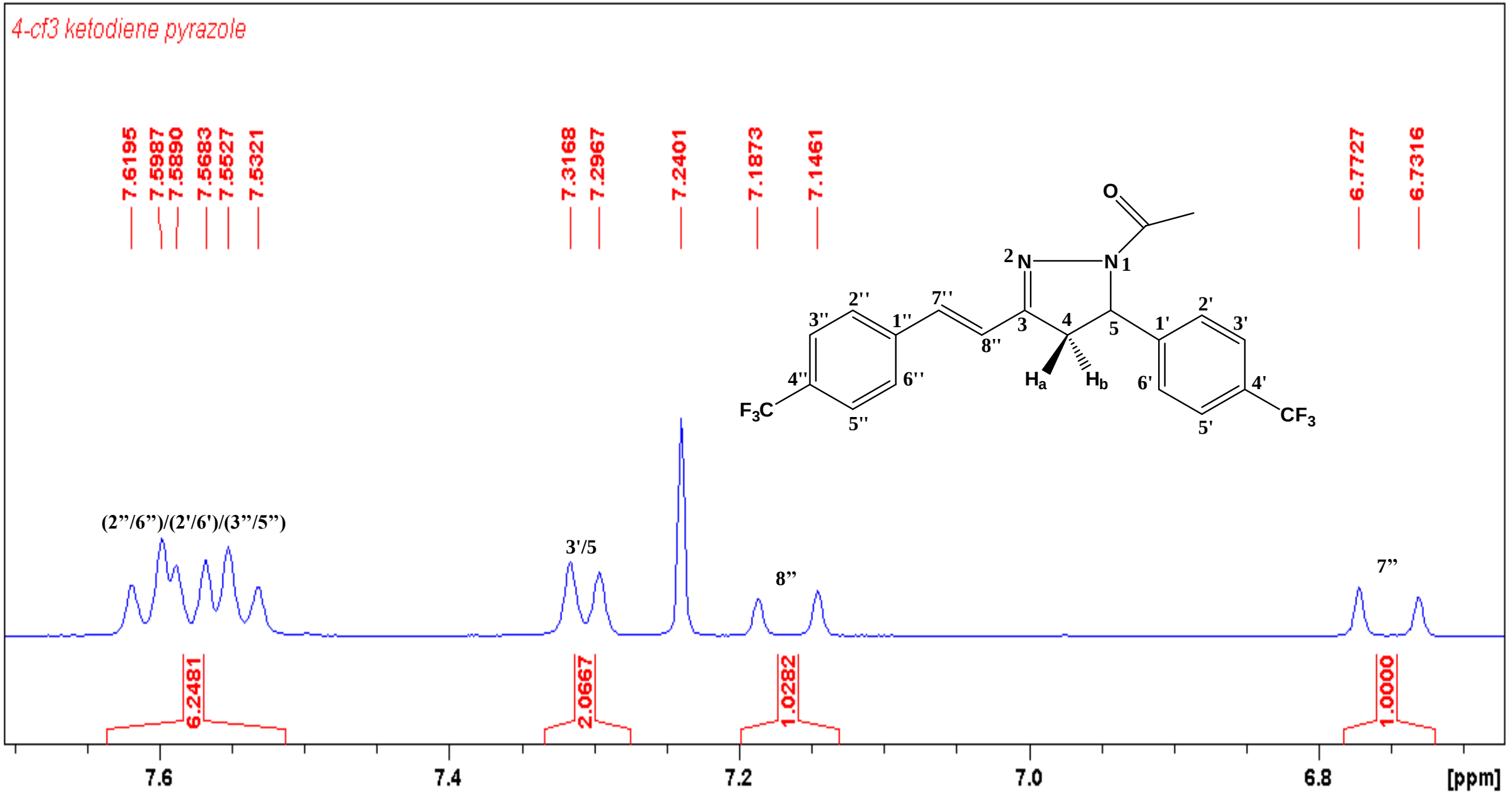
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
449.1063	449.1065	-0.2	-0.4	11.5	103.4	0.0	C21 H16 N2 O F6 Na

HRMS of Compound B-2e: (E)-1-(5-(3-(trifluoromethyl)phenyl)-3-(3-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



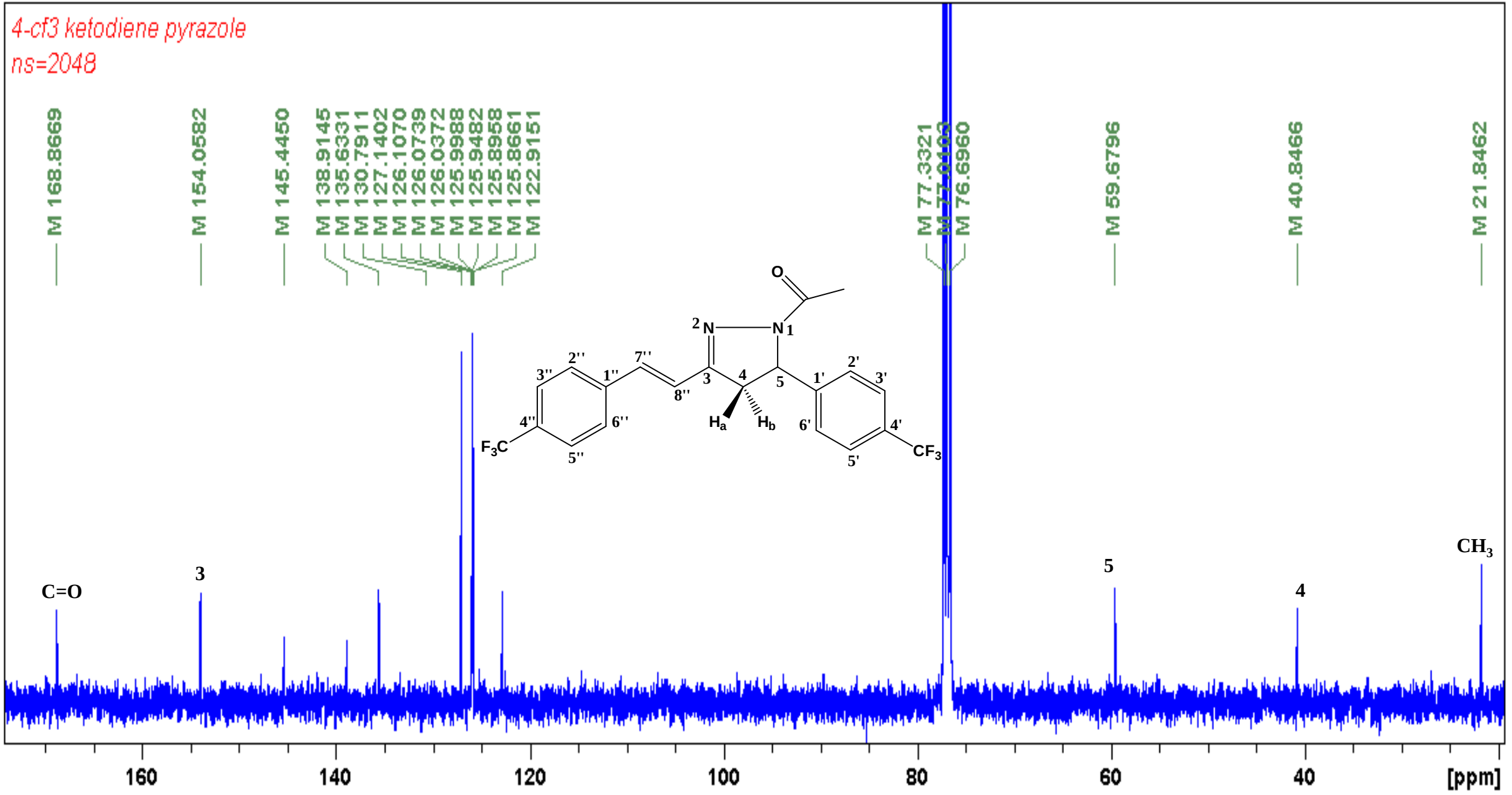
322

[1]H Spectrum of Compound B-2f: (E)-1-(5-(4-(trifluoromethyl)phenyl)-3-(4-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



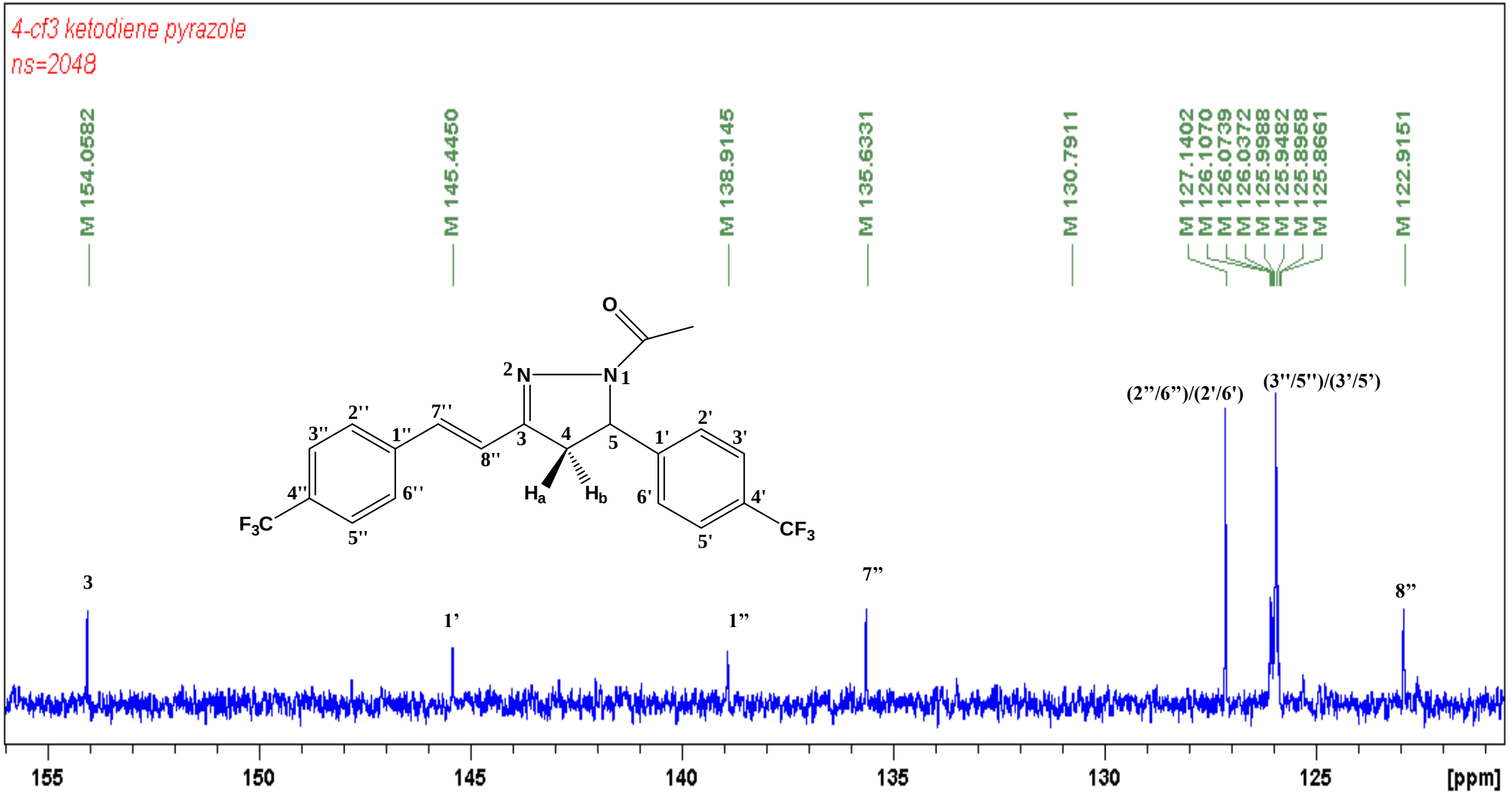
323

Expanded ^{1}H Spectrum of Compound B-2f: (E)-1-(5-(4-(trifluoromethyl)phenyl)-3-(4-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

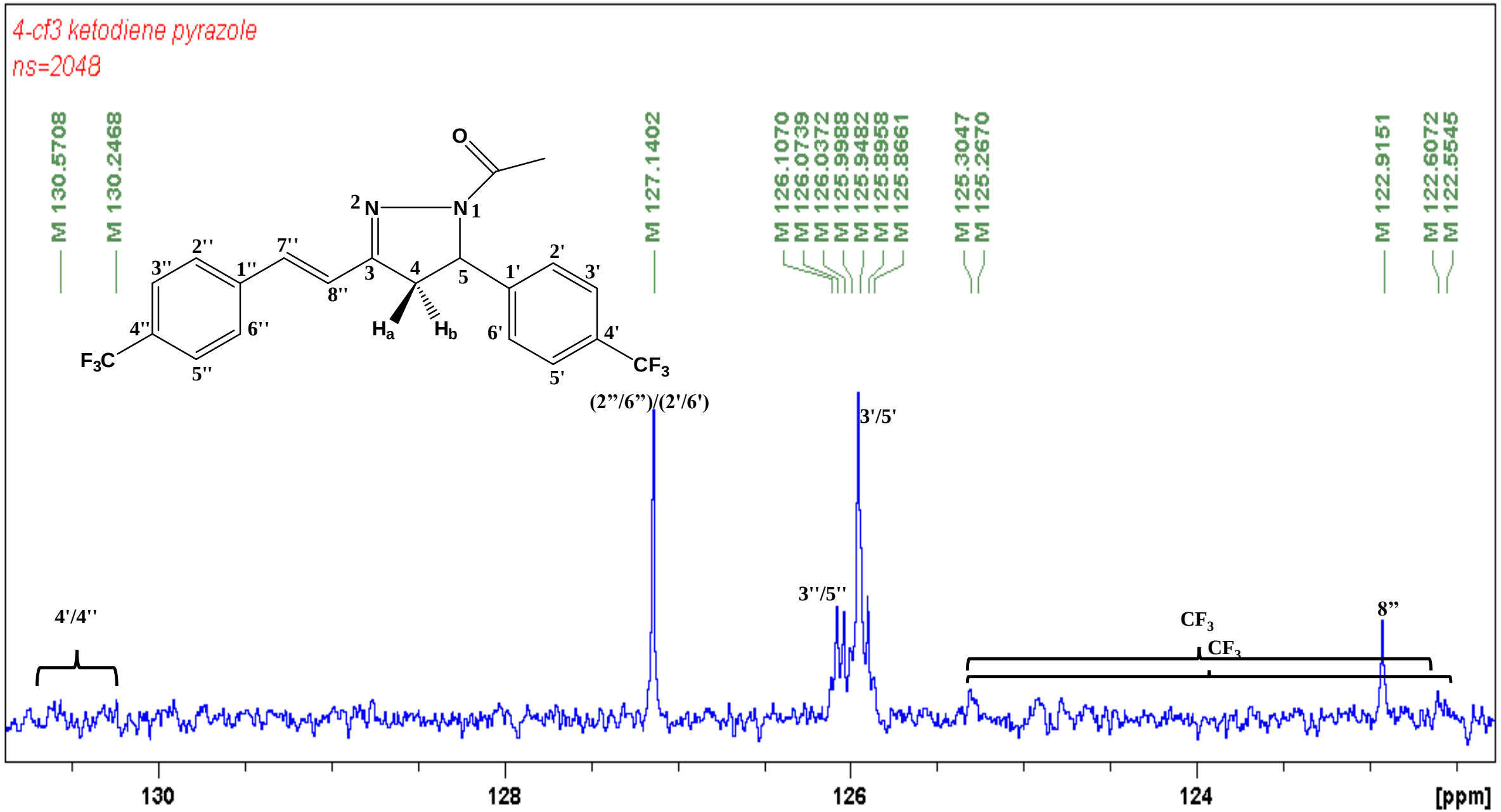


324

^{13}C Spectrum of Compound B-2f: (E)-1-(5-(4-(trifluoromethyl)phenyl)-3-(4-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

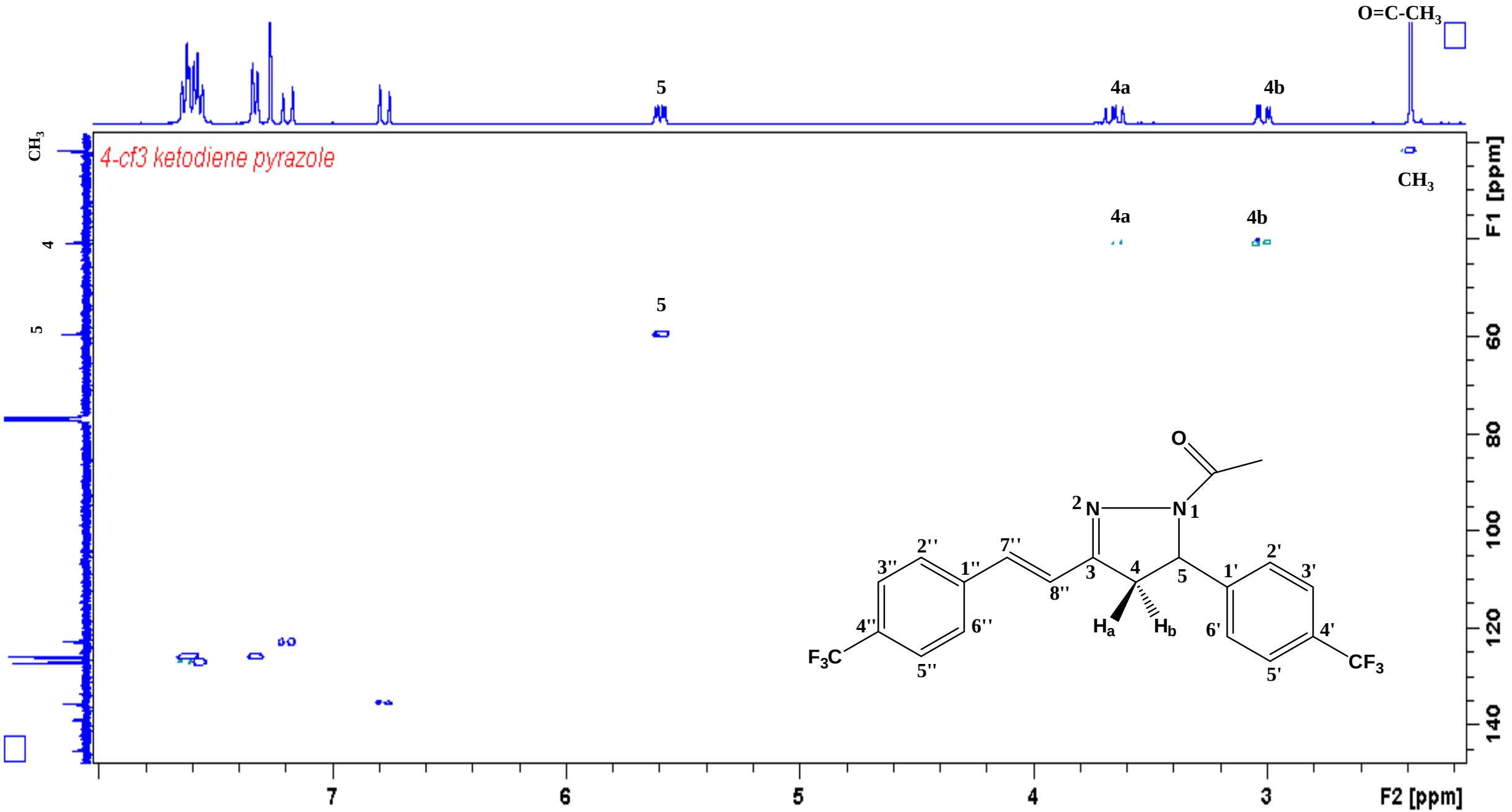


Expanded ^{13}C Spectrum of Compound B-2f: (E)-1-(5-(4-(trifluoromethyl)phenyl)-3-(4-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

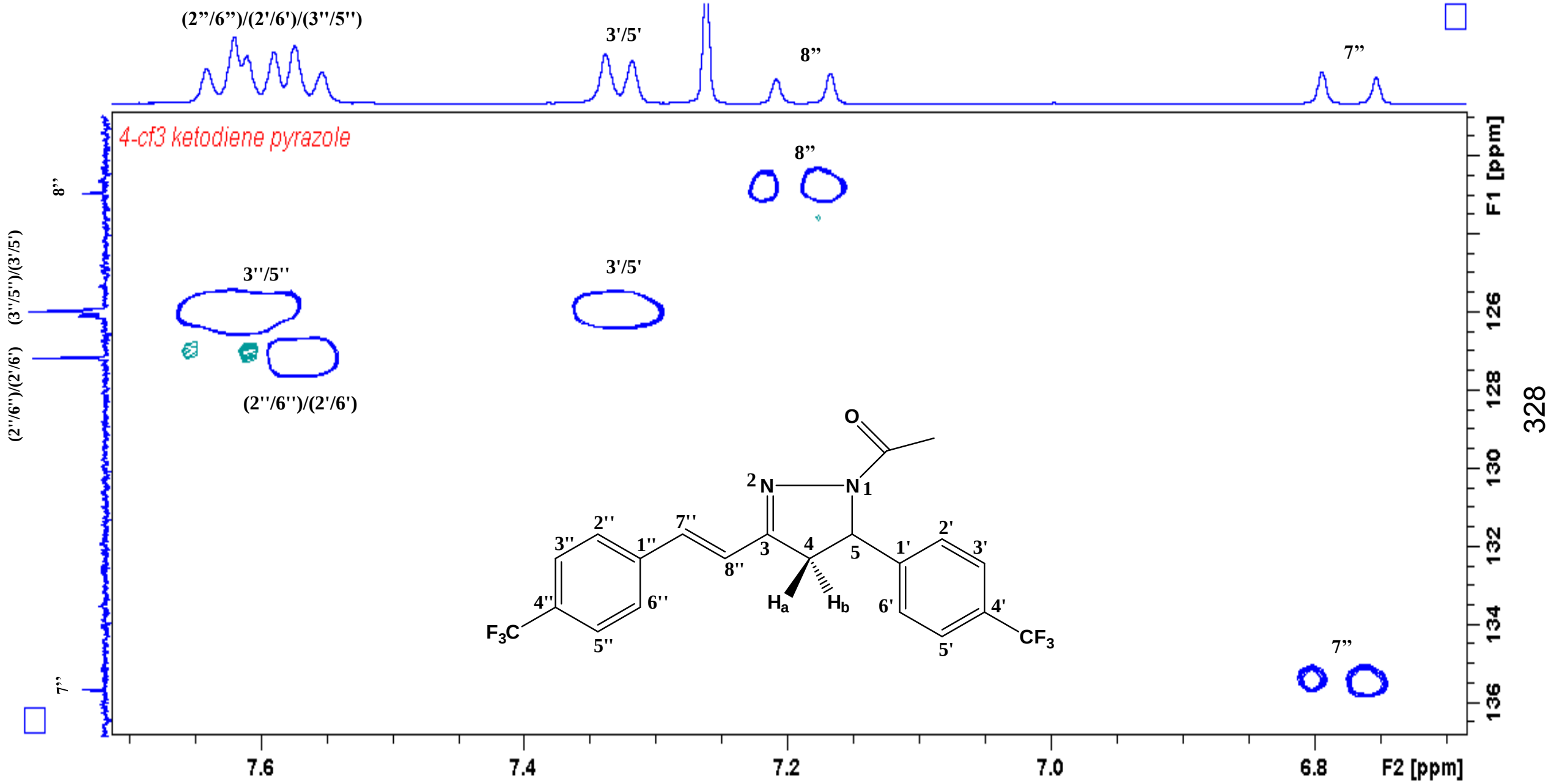


326

Expanded ^{13}C Spectrum of Compound B-2f: (E)-1-(5-(4-(trifluoromethyl)phenyl)-3-(4-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

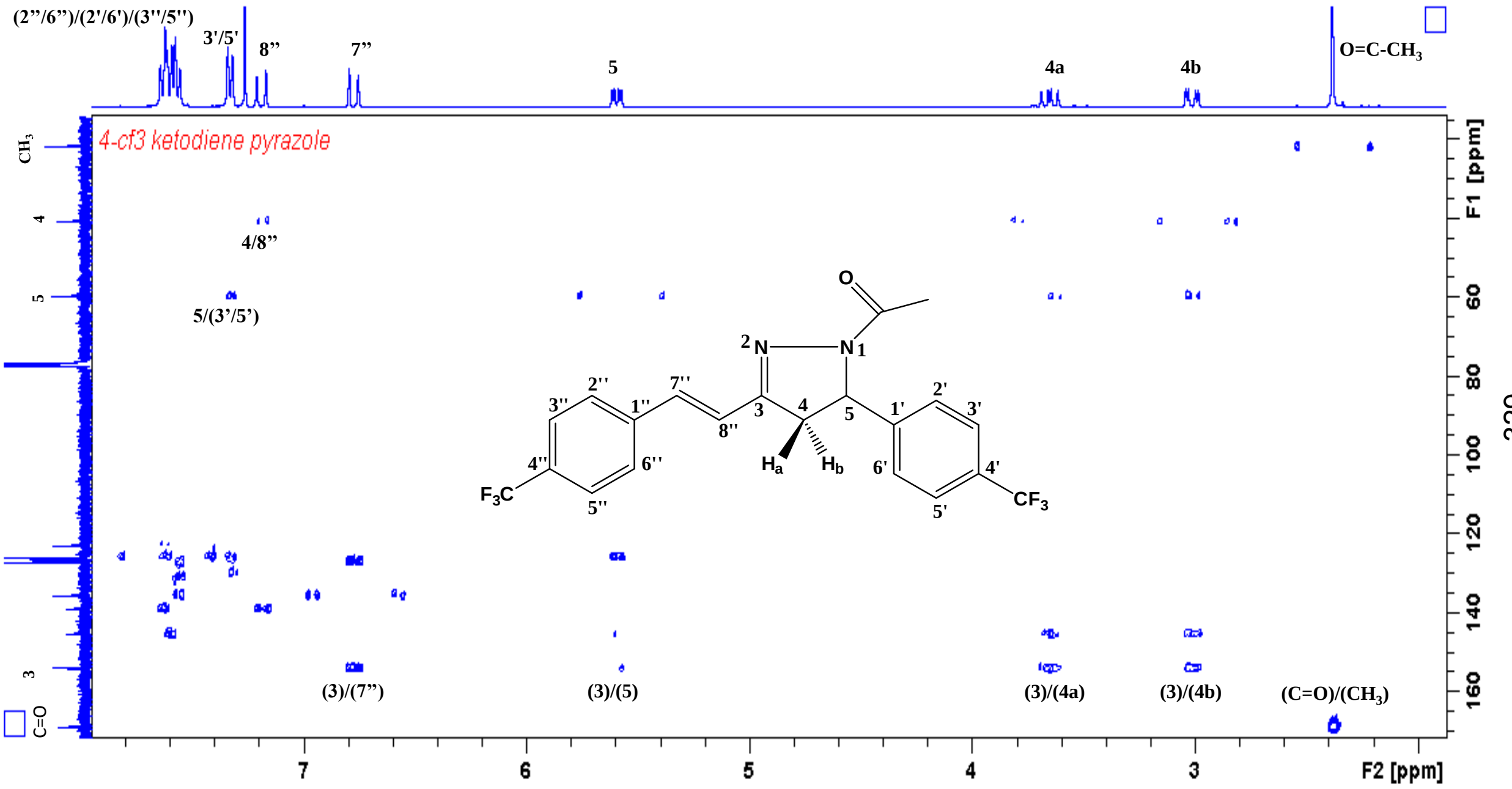


HSQC of Compound B-2f: (E)-1-(5-(4-(trifluoromethyl)phenyl)-3-(4-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

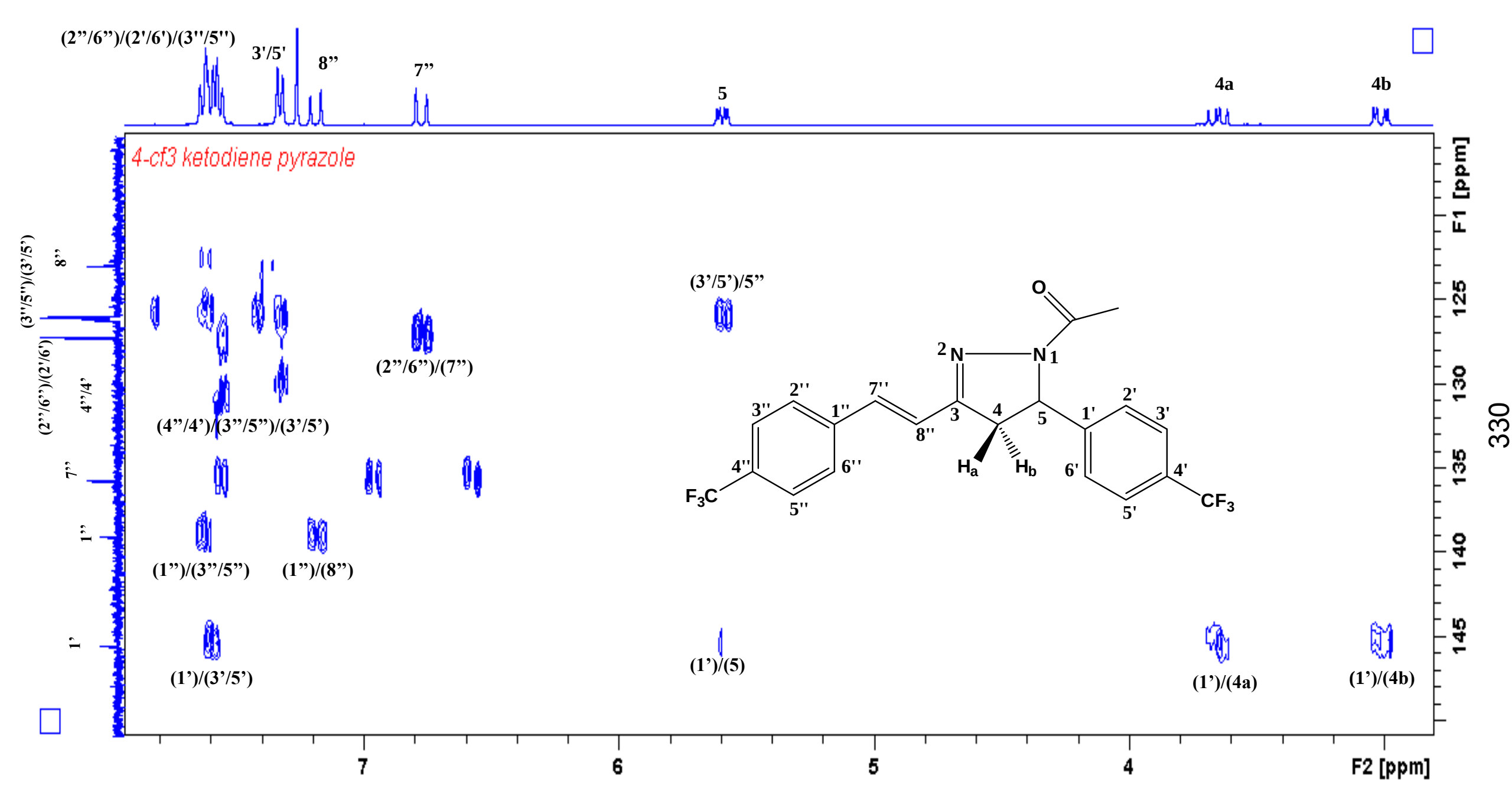


328

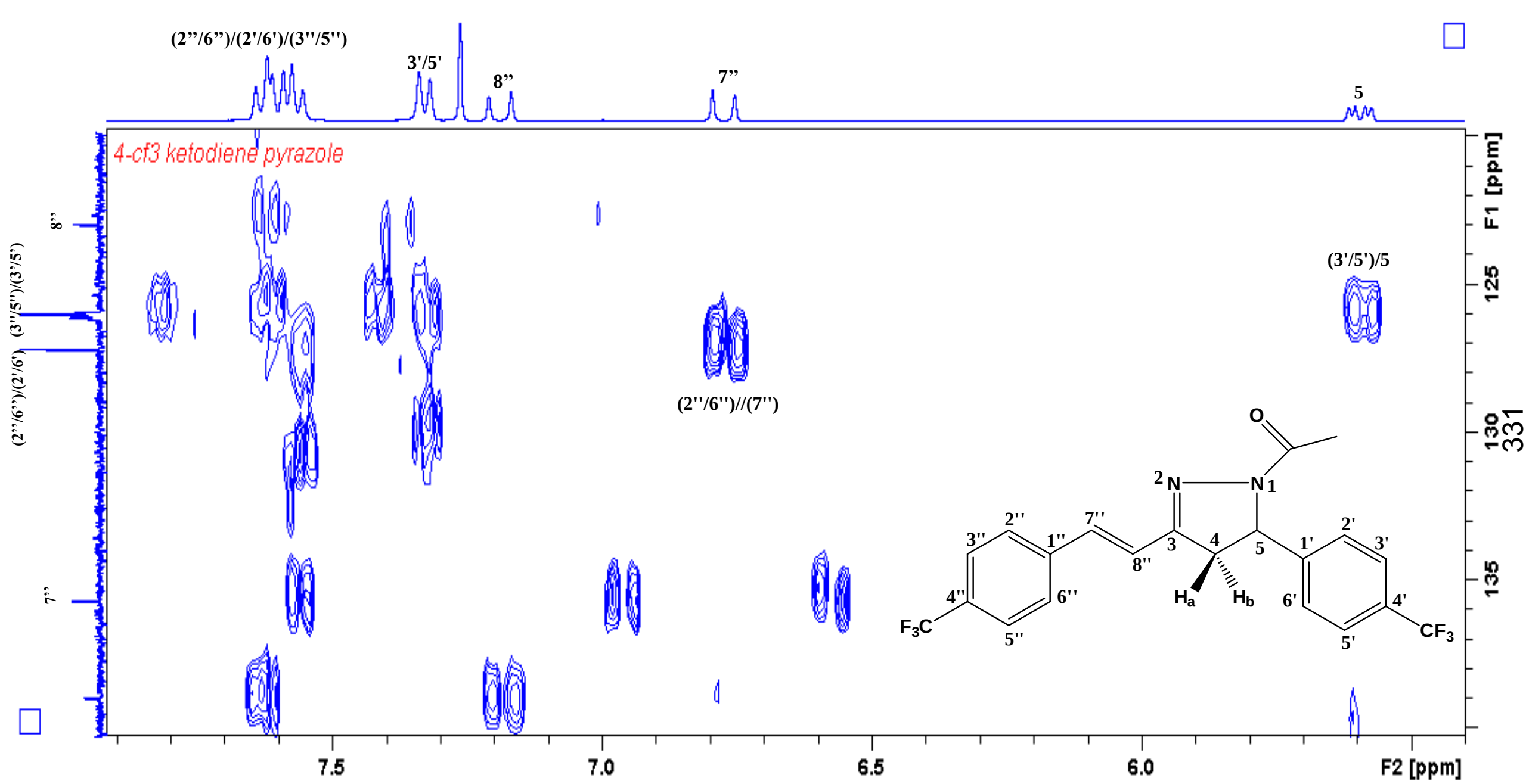
Expanded HSQC of Compound B-2f: (E)-1-(5-(4-(trifluoromethyl)phenyl)-3-(4-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



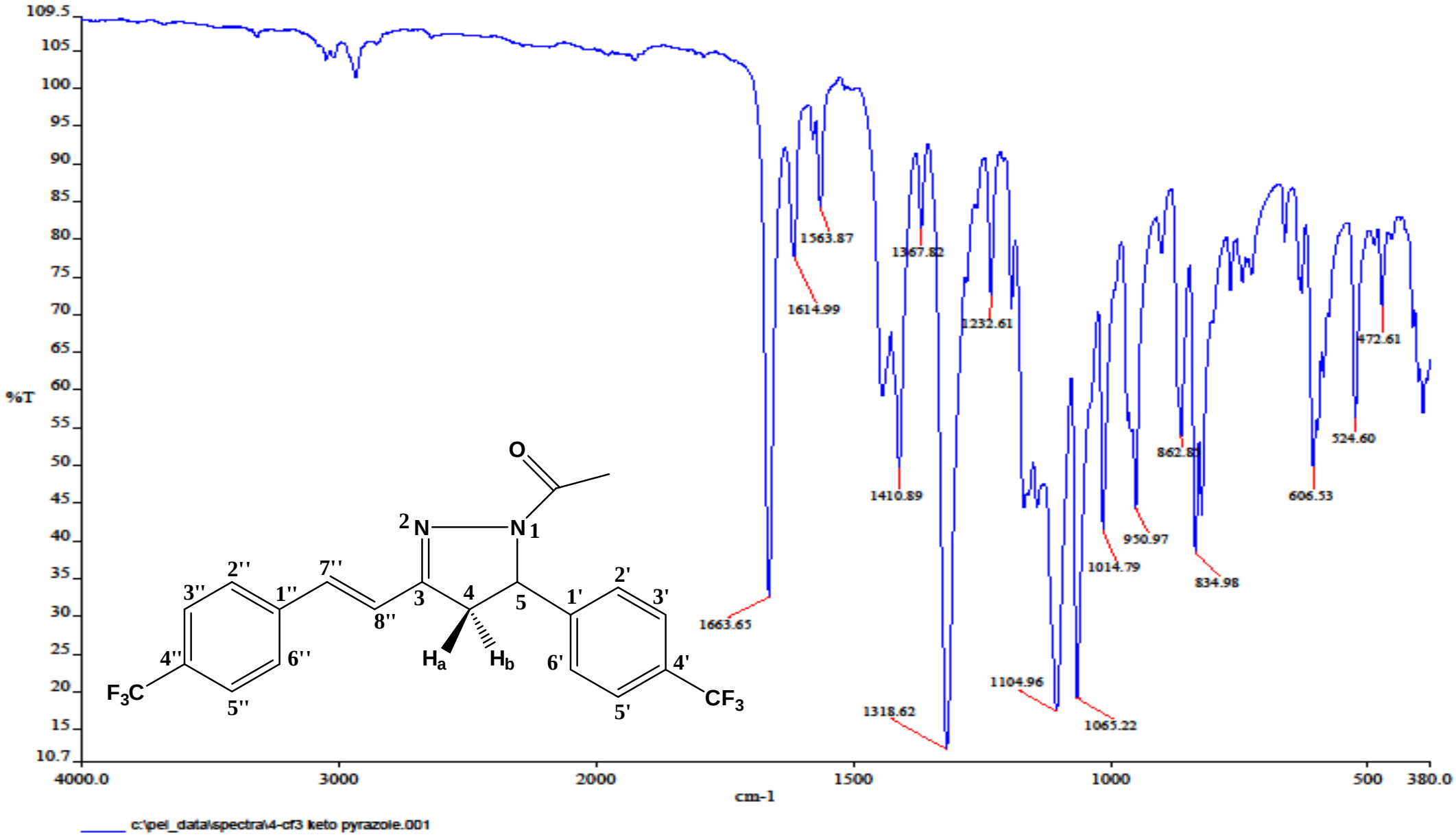
HMBC of Compound B-2f: (E)-1-(5-(4-(trifluoromethyl)phenyl)-3-(4-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



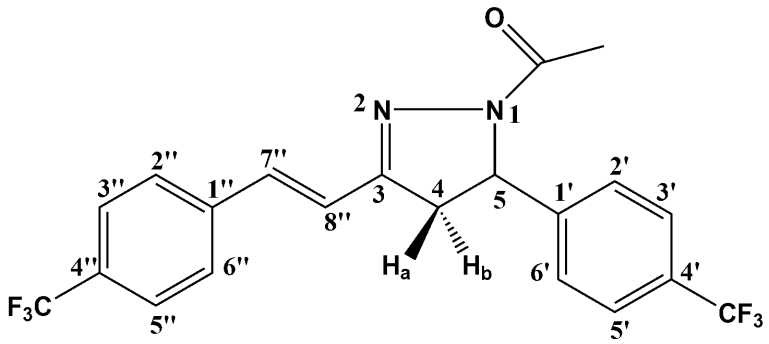
Expanded HMBC of Compound B-2f: (E)-1-(5-(4-(trifluoromethyl)phenyl)-3-(4-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



Expanded HMBC of Compound B-2f: (E)-1-(5-(4-(trifluoromethyl)phenyl)-3-(4-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



Infrared Spectrum of Compound B-2f: (E)-1-(5-(4-(trifluoromethyl)phenyl)-3-(4-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



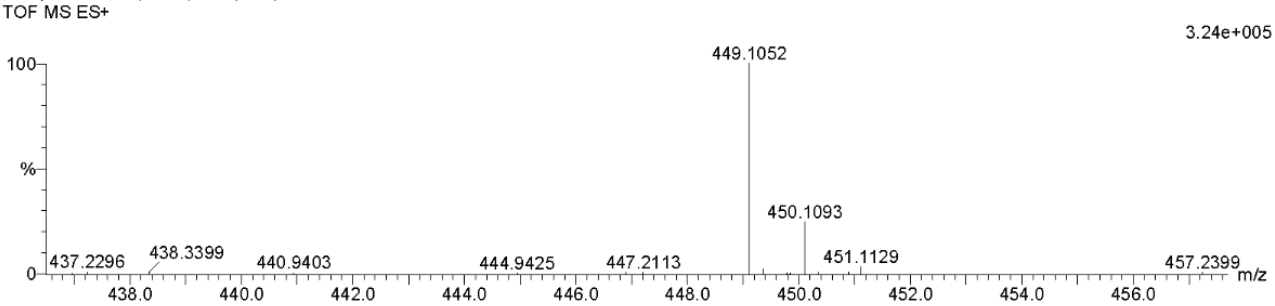
Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

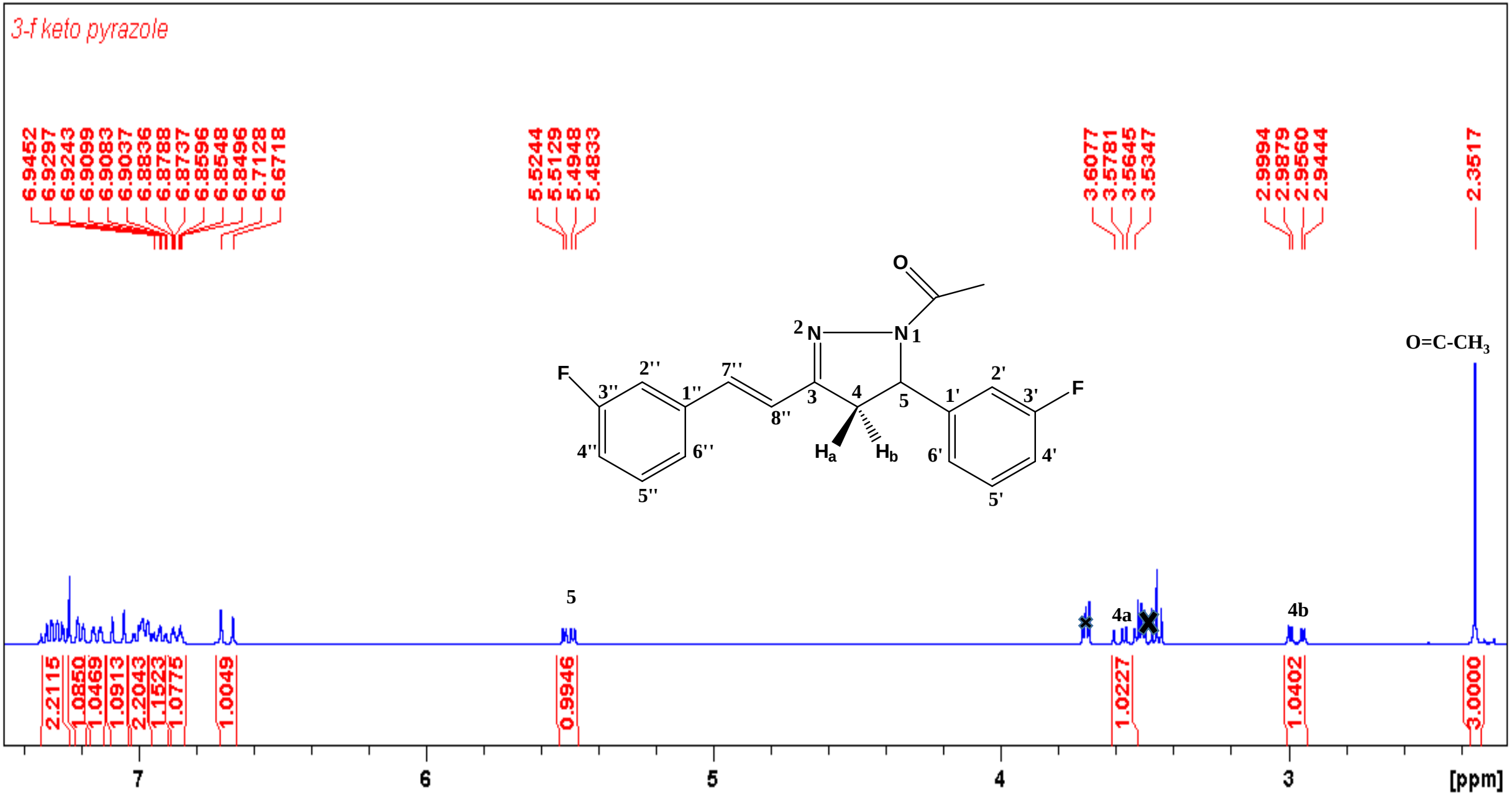
Monoisotopic Mass, Even Electron Ions
278 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 1-5 F: 5-10 Na: 0-1

Compound 6 10 (0.304) Cm (1:61)
TOF MS ES+

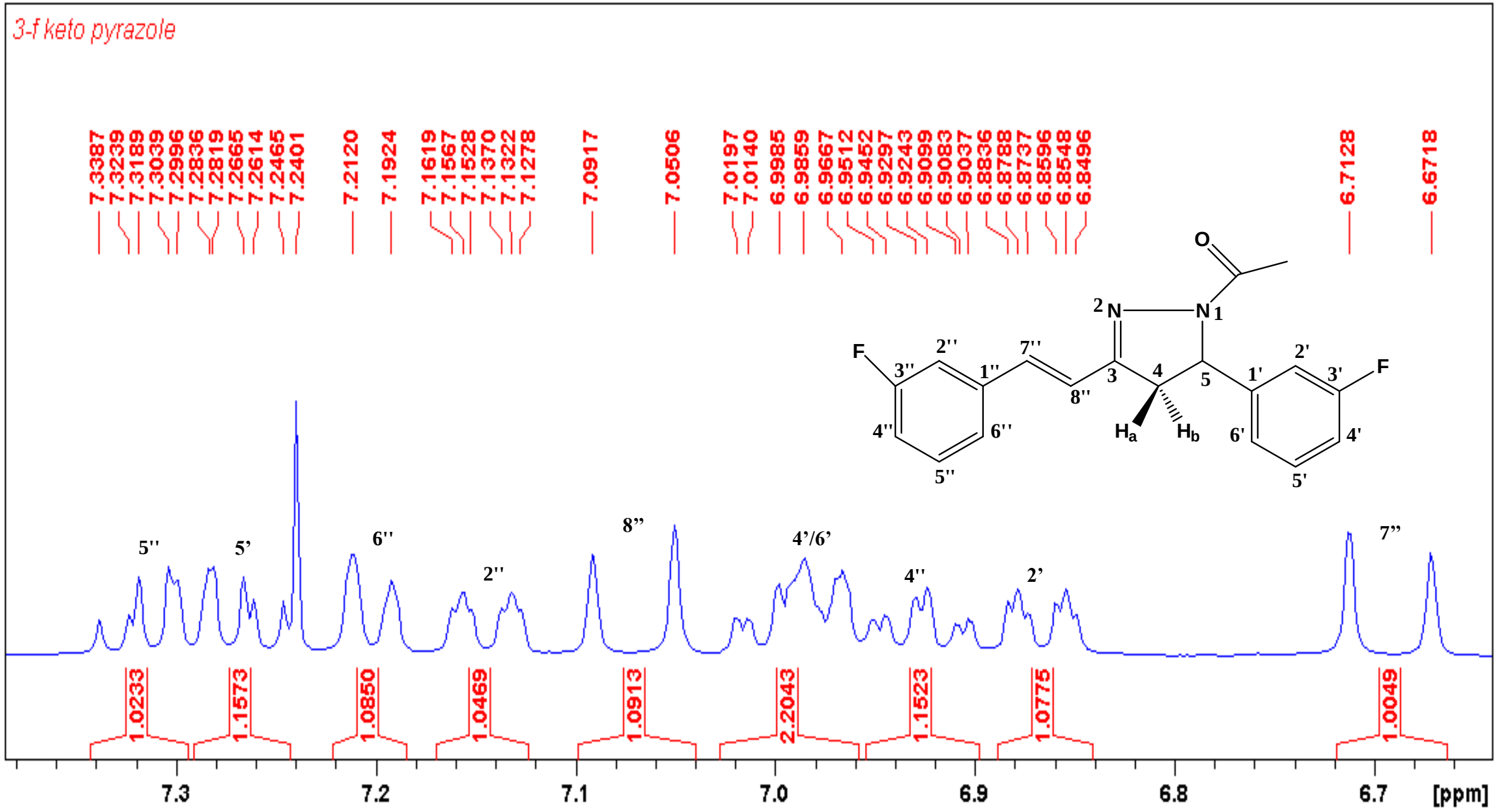


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
449.1052	449.1065	-1.3	-2.9	11.5	72.9	0.0	C21 H16 N2 O F6 Na

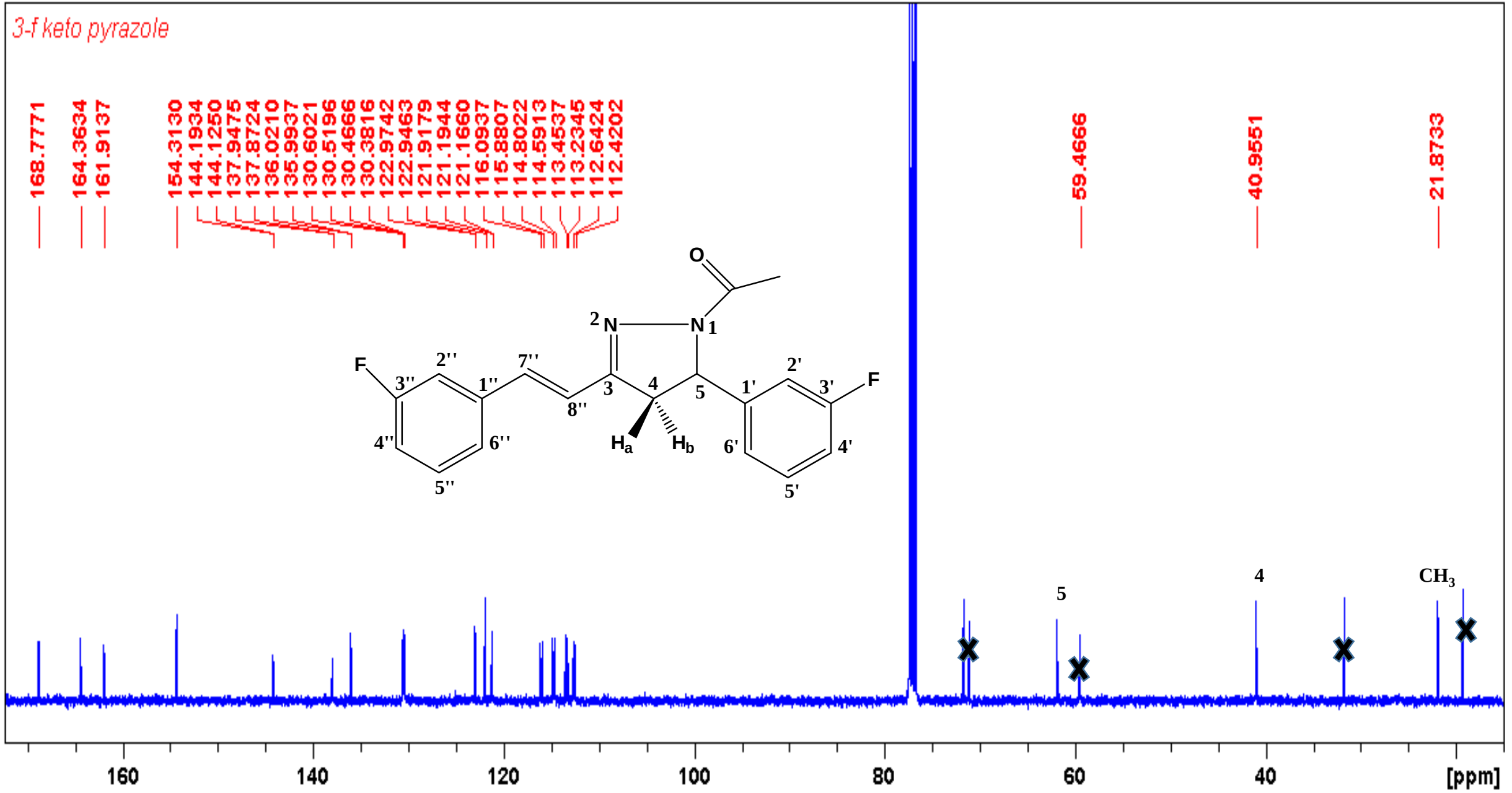
HRMS of Compound B-2f: (E)-1-(5-(4-(trifluoromethyl)phenyl)-3-(4-(trifluoromethyl)styryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



^{1}H Spectrum of Compound B-2g: (E)-1-(5-(3-fluorophenyl)-3-(3-fluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

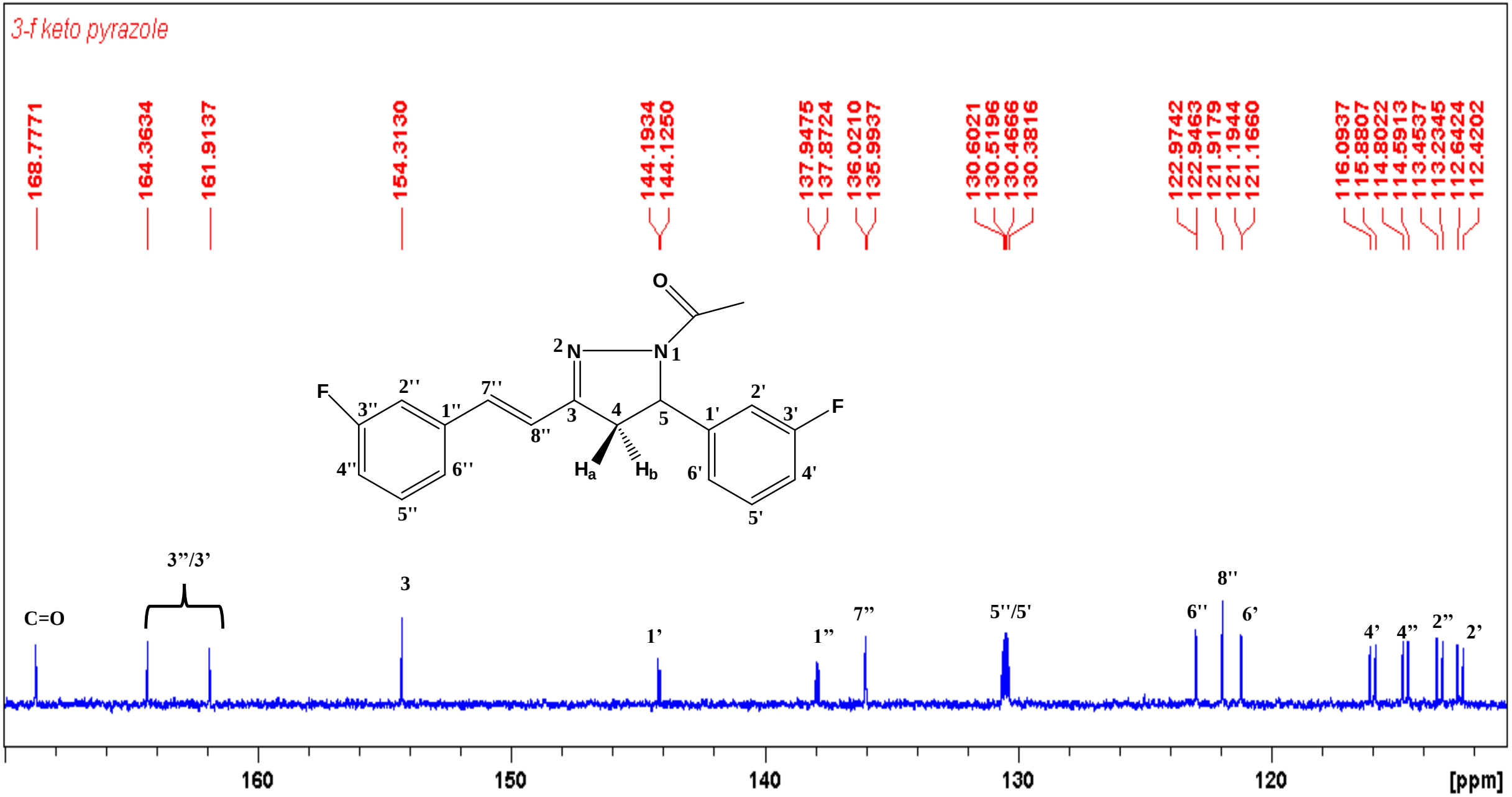


Expanded ^{1}H Spectrum of Compound B-2g: (E)-1-(5-(3-fluorophenyl)-3-(3-fluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



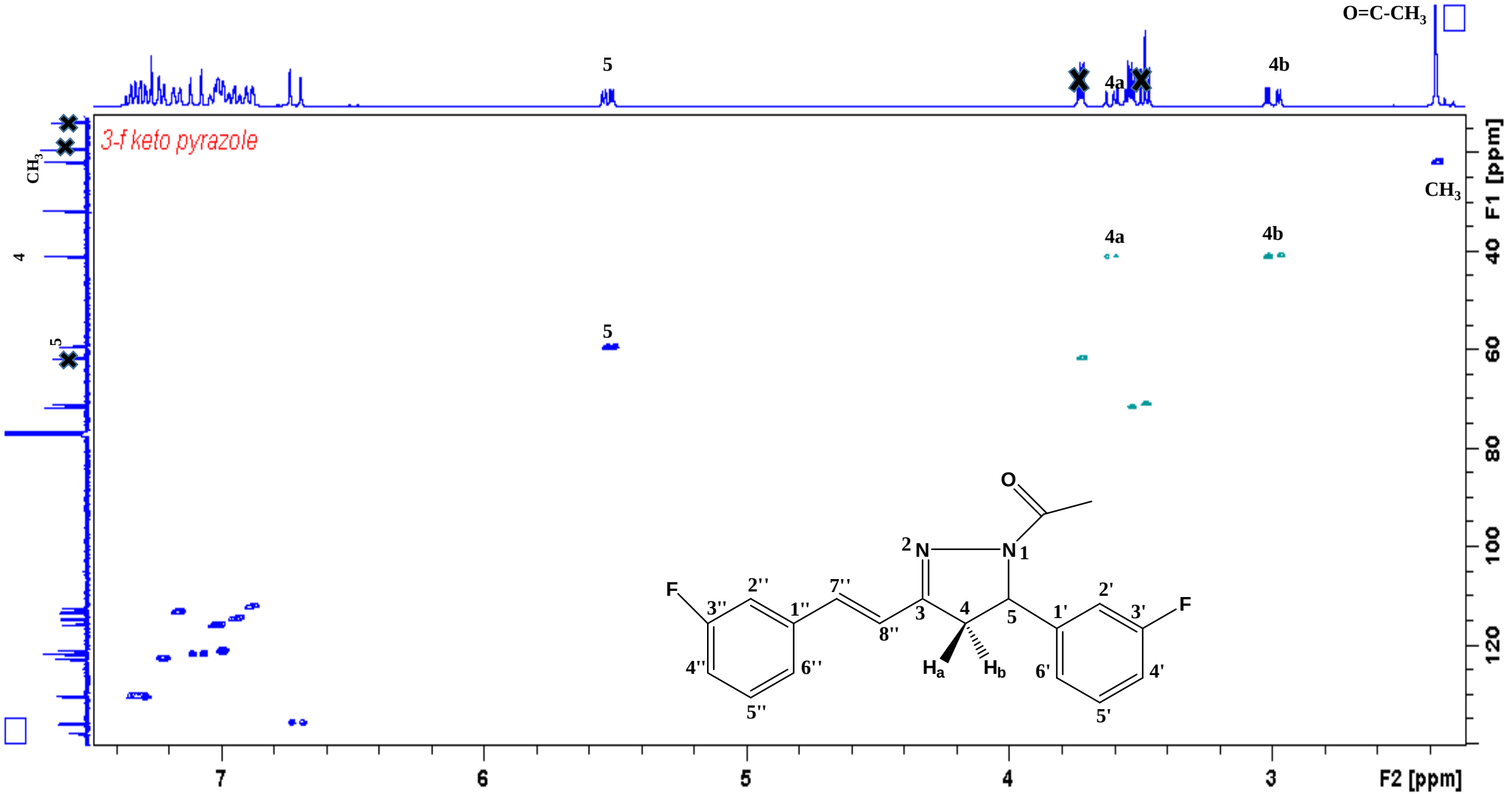
336

^{13}C Spectrum of Compound B-2g: (E)-1-(5-(3-fluorophenyl)-3-(3-fluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

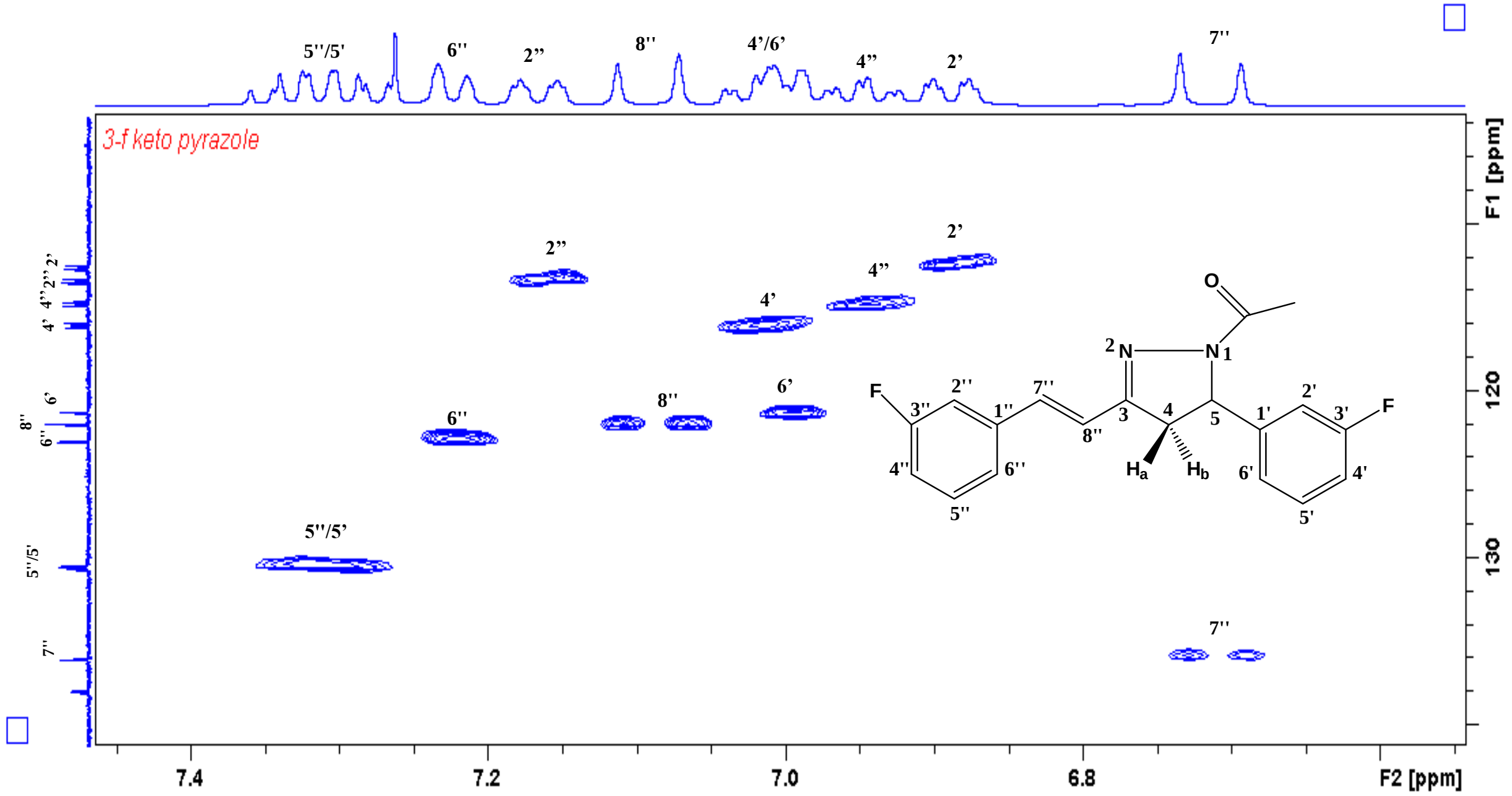


337

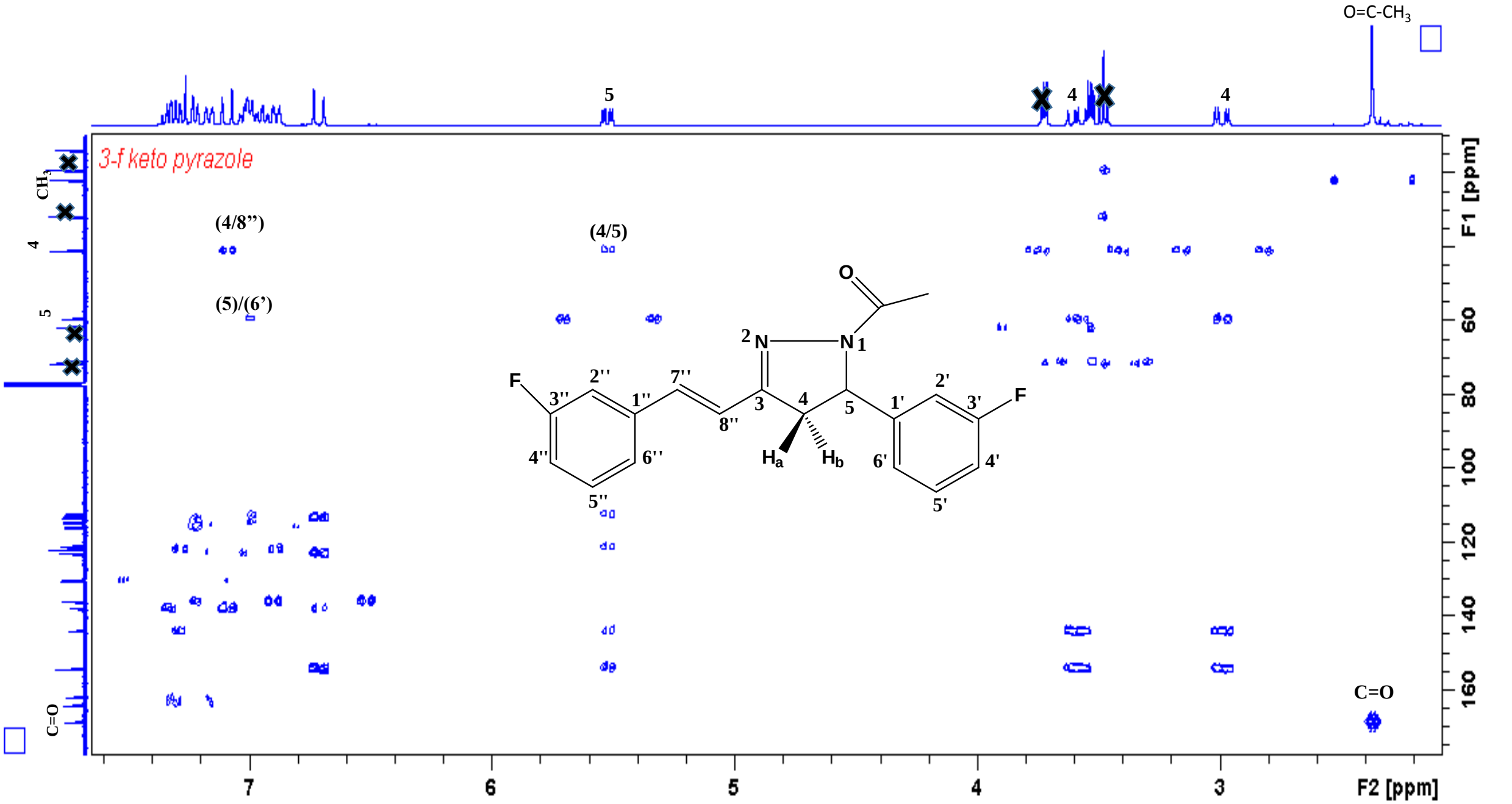
Expanded ^{13}C Spectrum of Compound B-2g: (E)-1-(5-(3-fluorophenyl)-3-(3-fluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



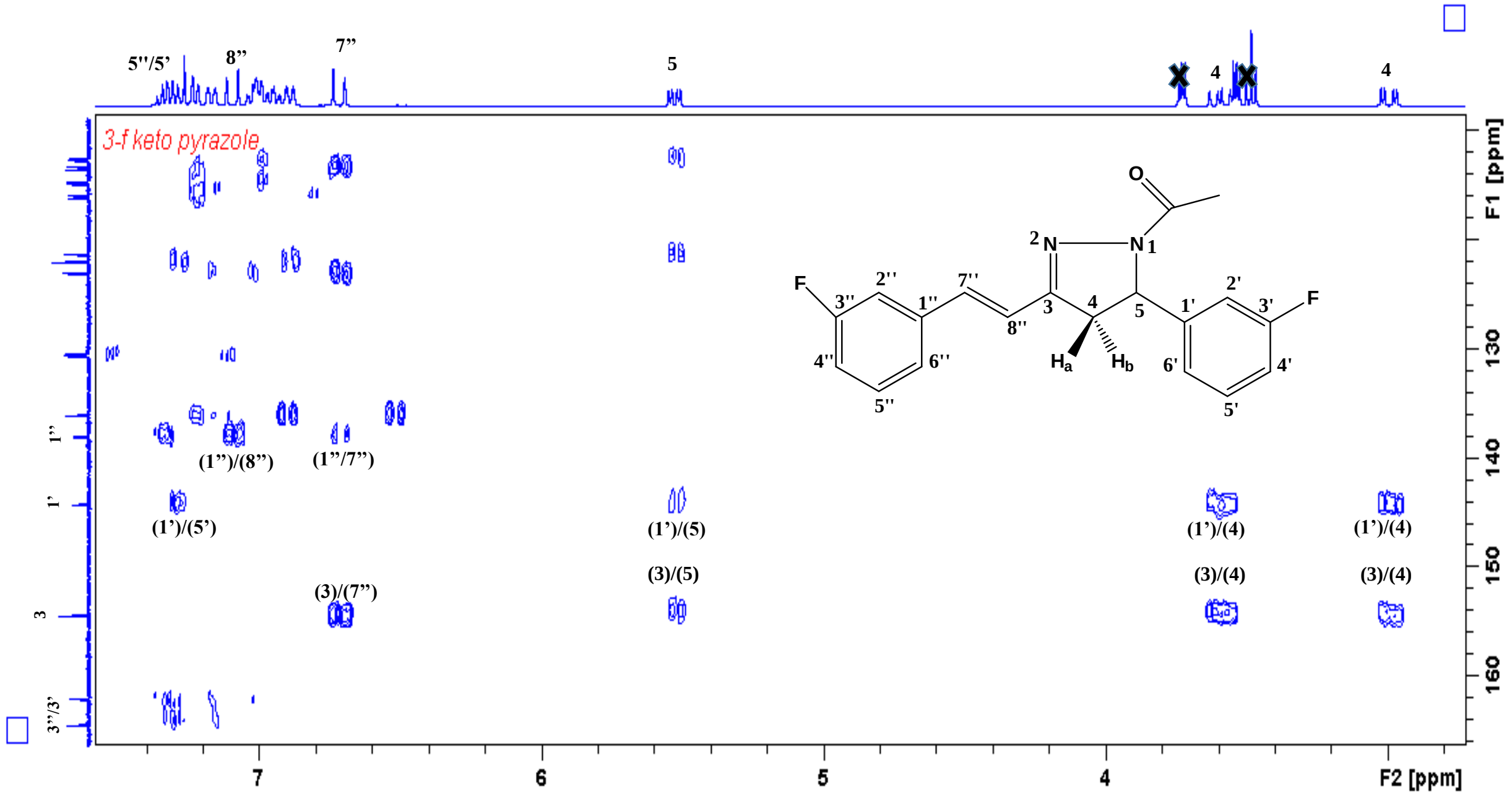
HSQC of Compound B-2g: (E)-1-(5-(3-fluorophenyl)-3-(3-fluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



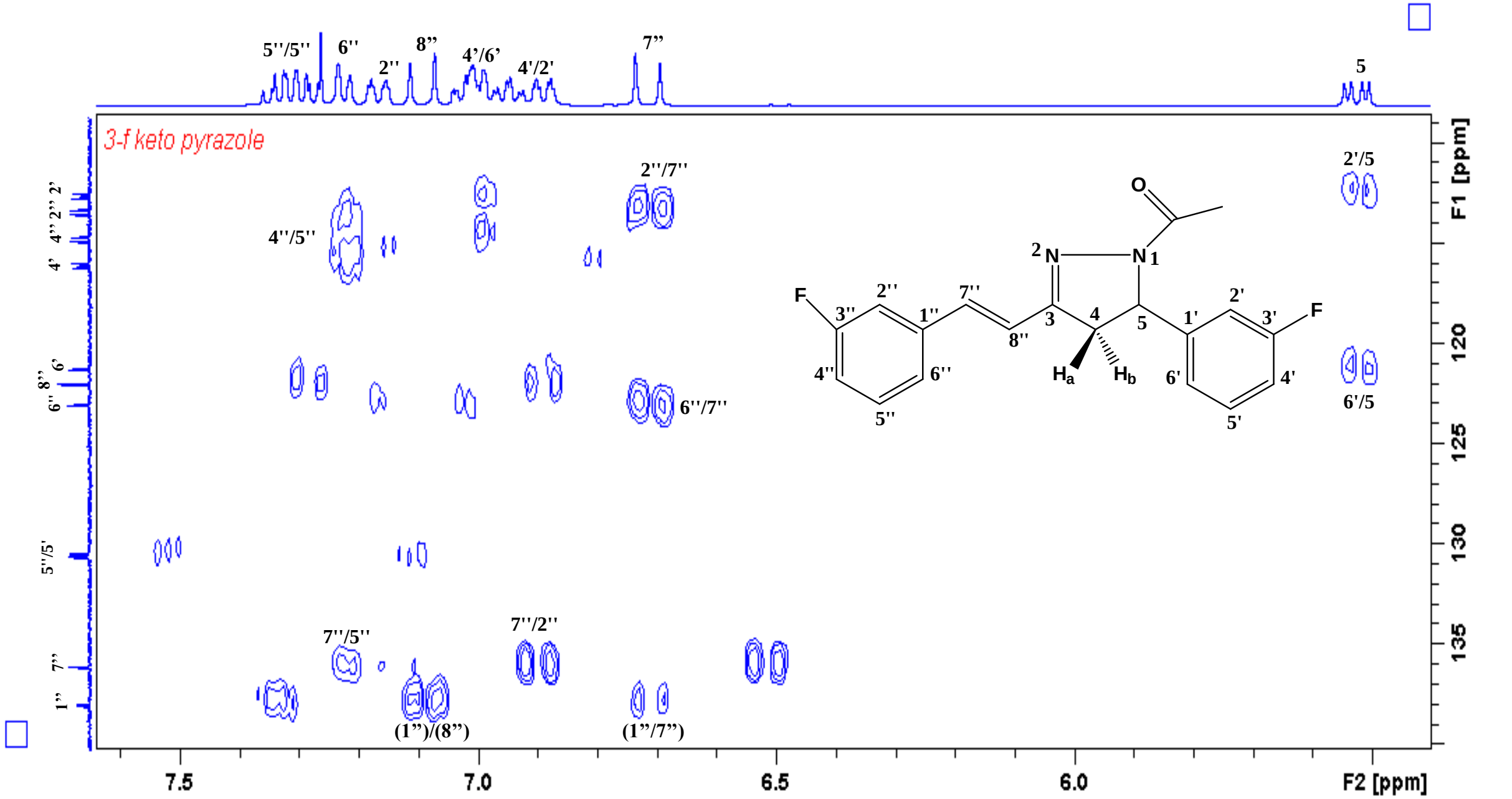
Expanded HSQC of Compound B-2g:(E)-1-(5-(3-fluorophenyl)-3-(3-fluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



HMBC of Compound B-2g: (E)-1-(5-(3-fluorophenyl)-3-(3-fluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

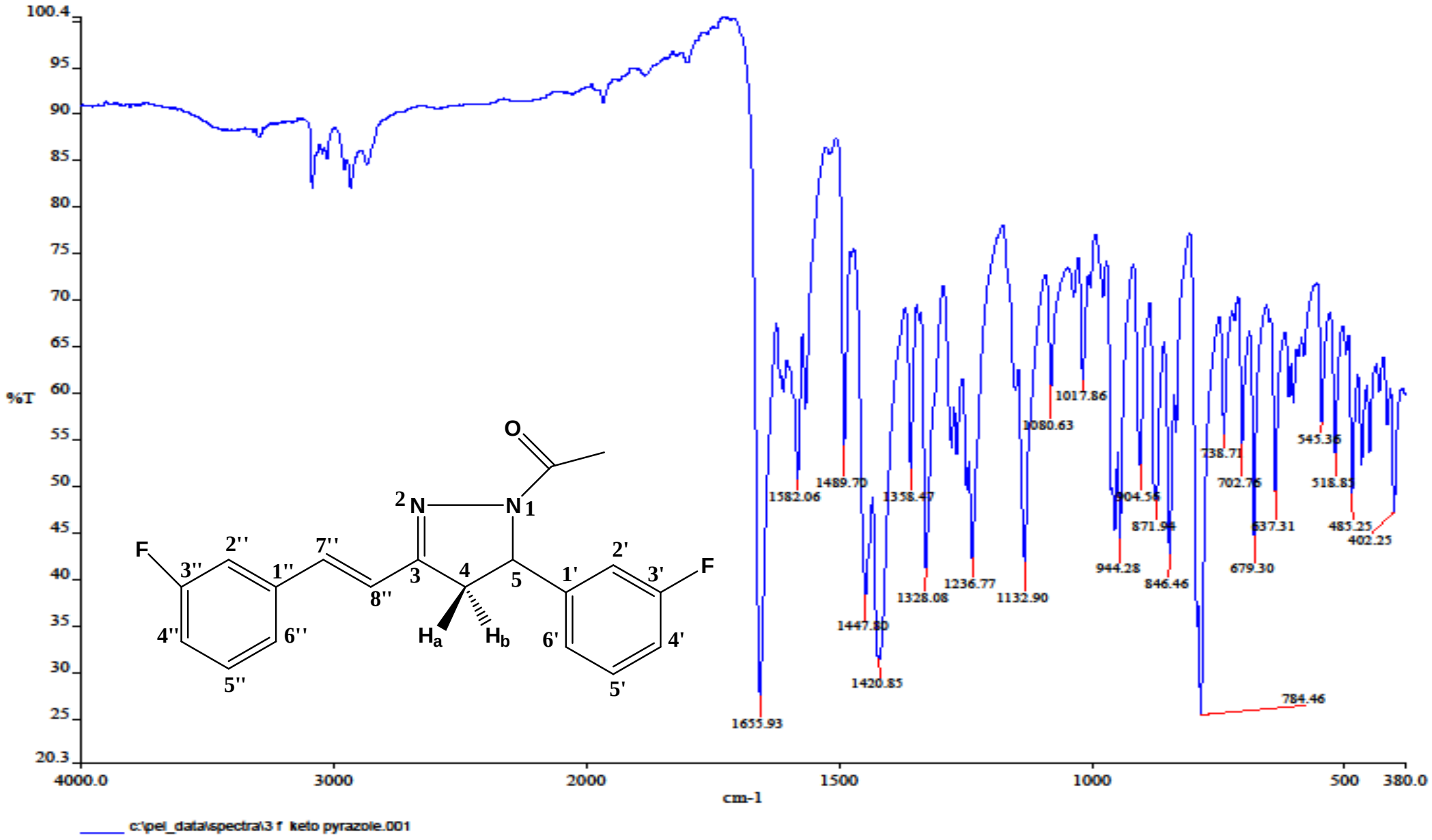


Expanded HMBC of Compound B-2g: (E)-1-(5-(3-fluorophenyl)-3-(3-fluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

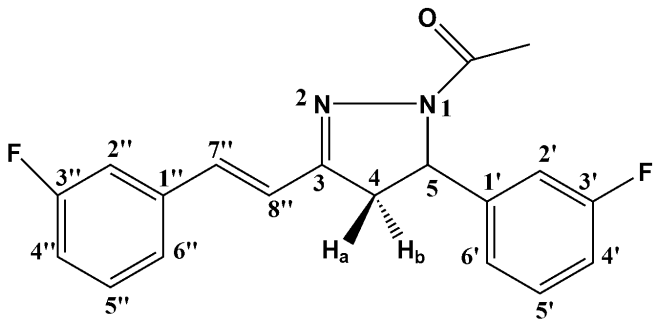


342

Expanded HMBC of Compound B-2g: (E)-1-(5-(3-fluorophenyl)-3-(3-fluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



Infrared Spectrum of Compound B-2g: (E)-1-(5-(3-fluorophenyl)-3-(3-fluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



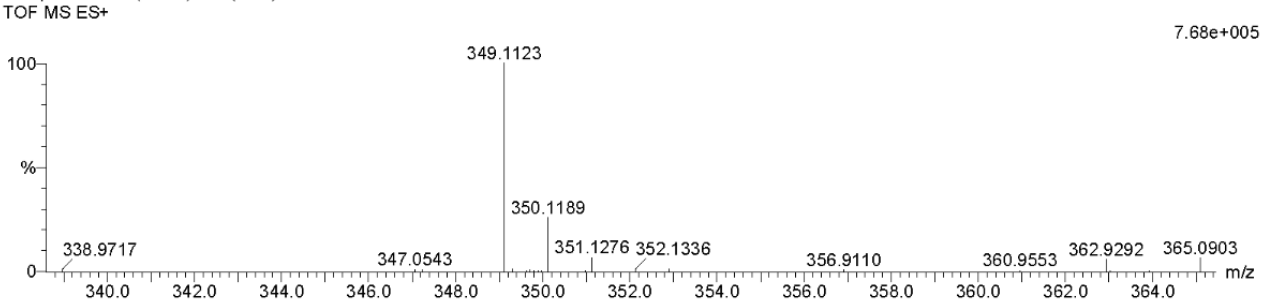
Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

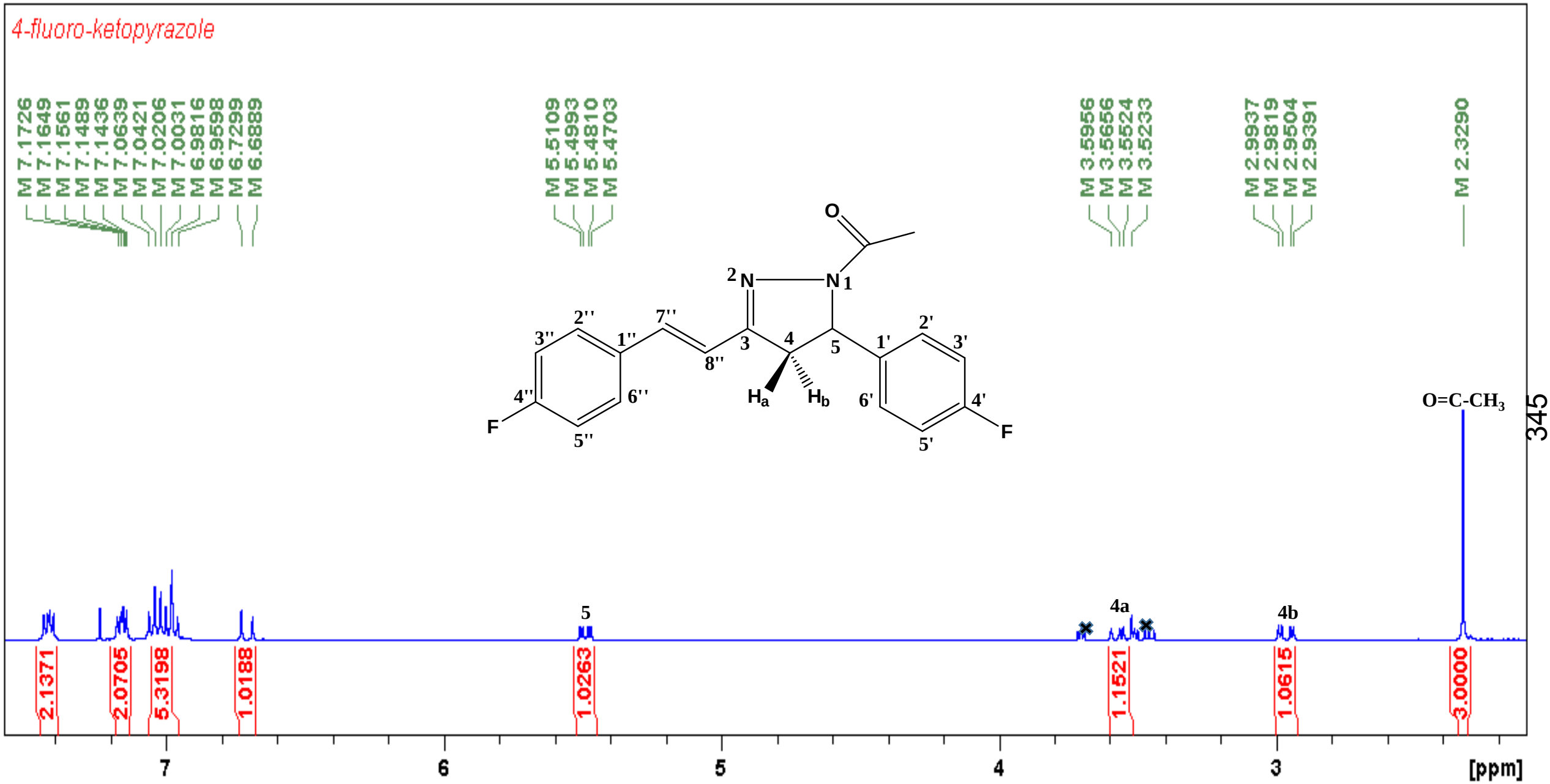
Monoisotopic Mass, Even Electron Ions
58 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 15-20 H: 15-20 N: 0-5 O: 0-1 F: 0-5 Na: 0-1

Compound 7 49 (1.618) Cm (1:61)
TOF MS ES+

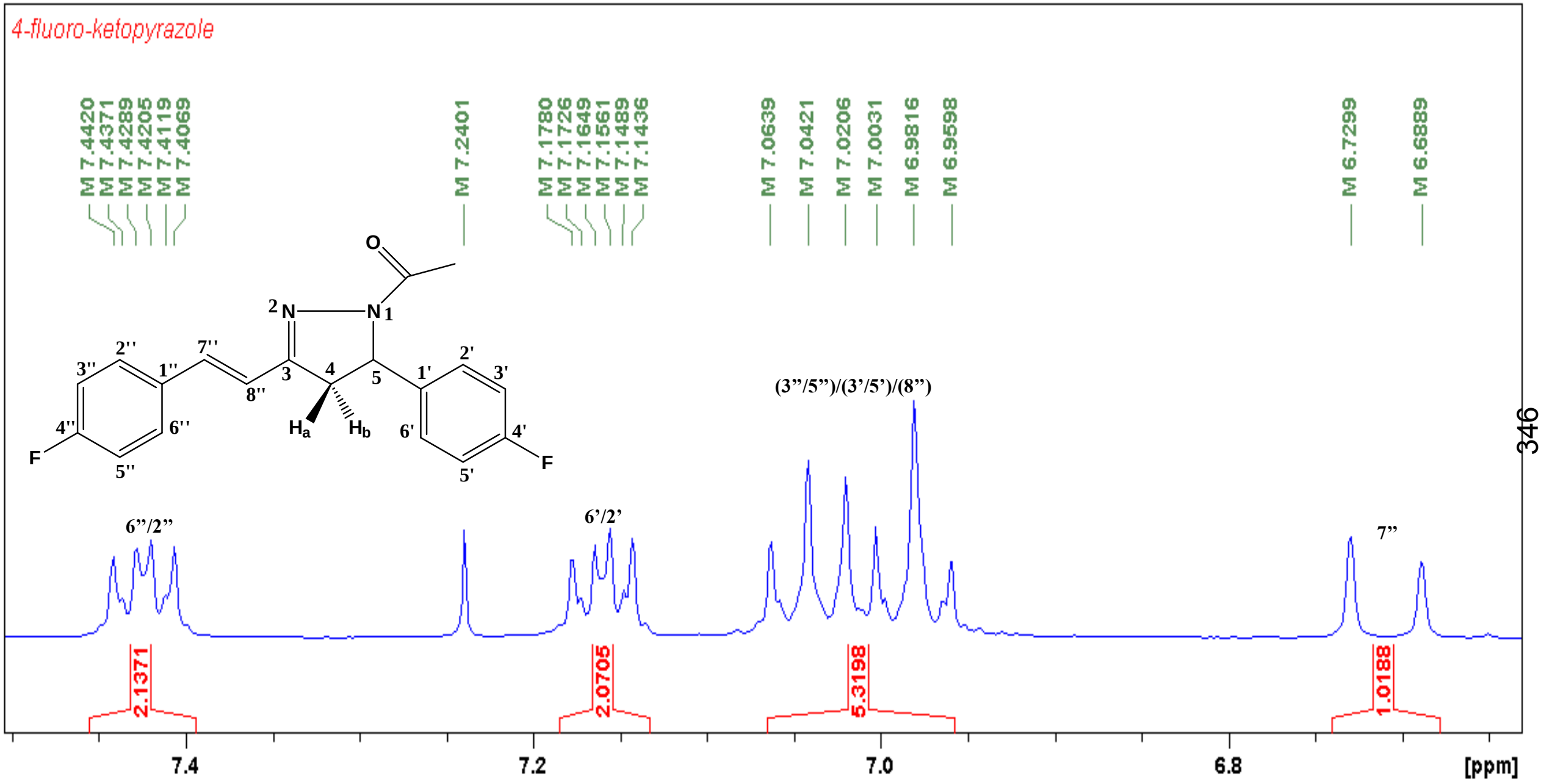


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
349.1123	349.1128	-0.5	-1.4	11.5	122.7	0.0	C19 H16 N2 O F2 Na

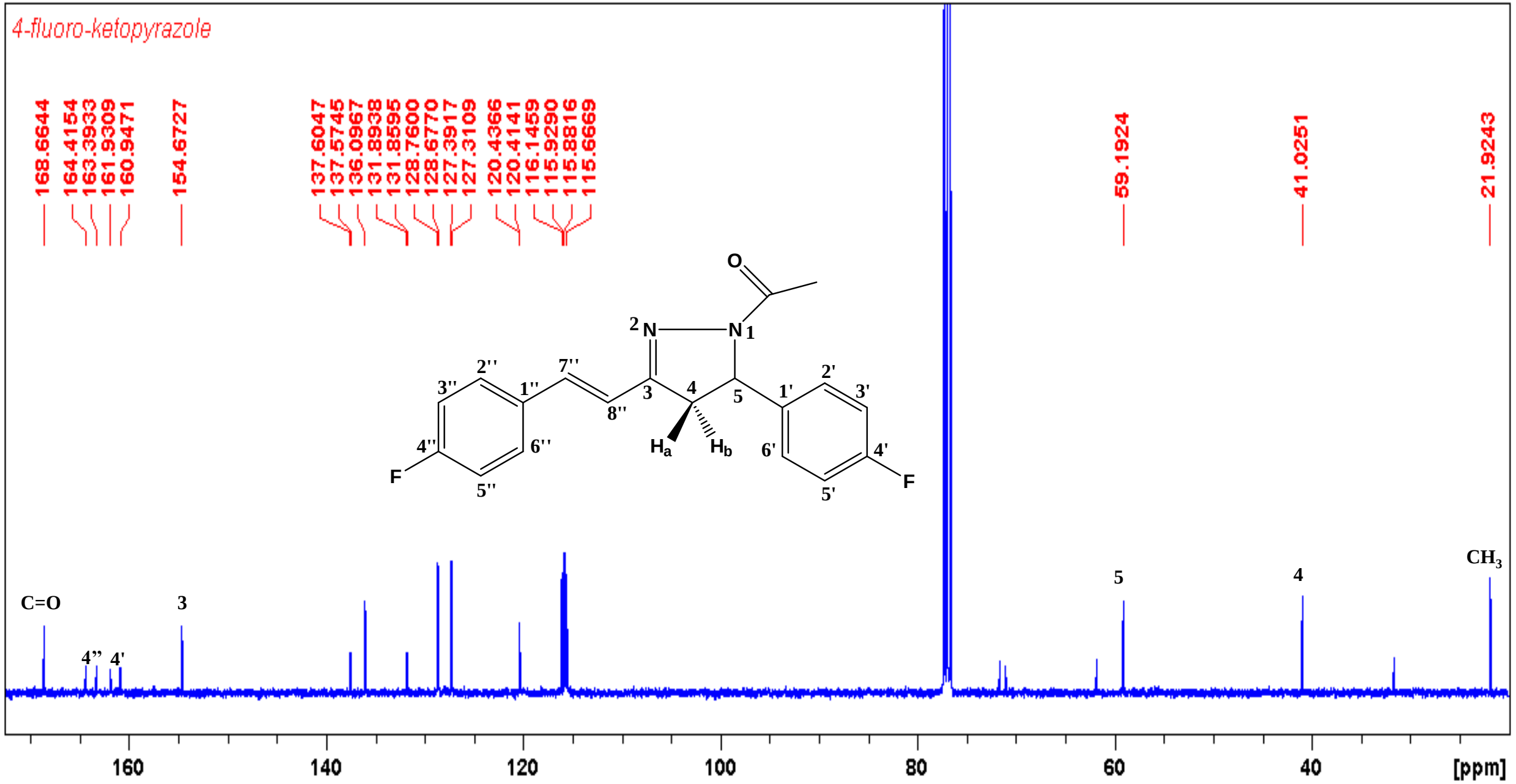
HRMS of Compound B-2g: (E)-1-(5-(3-fluorophenyl)-3-(3-fluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



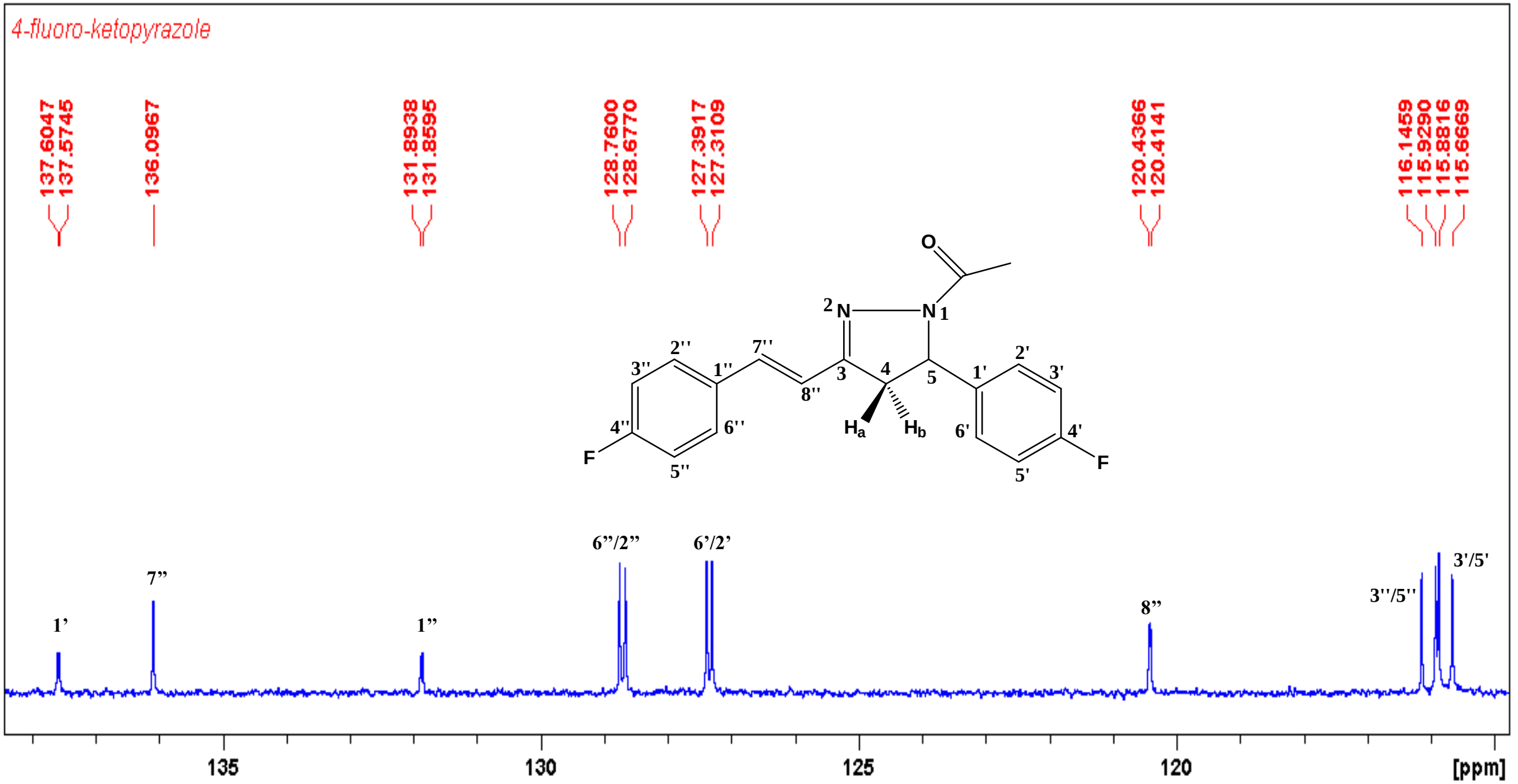
^{1}H Spectrum of Compound B-2h: (E)-1-(5-(4-fluorophenyl)-3-(4-fluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



Expanded ^{1}H Spectrum of Compound B-2h: (E)-1-(5-(4-fluorophenyl)-3-(4-fluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

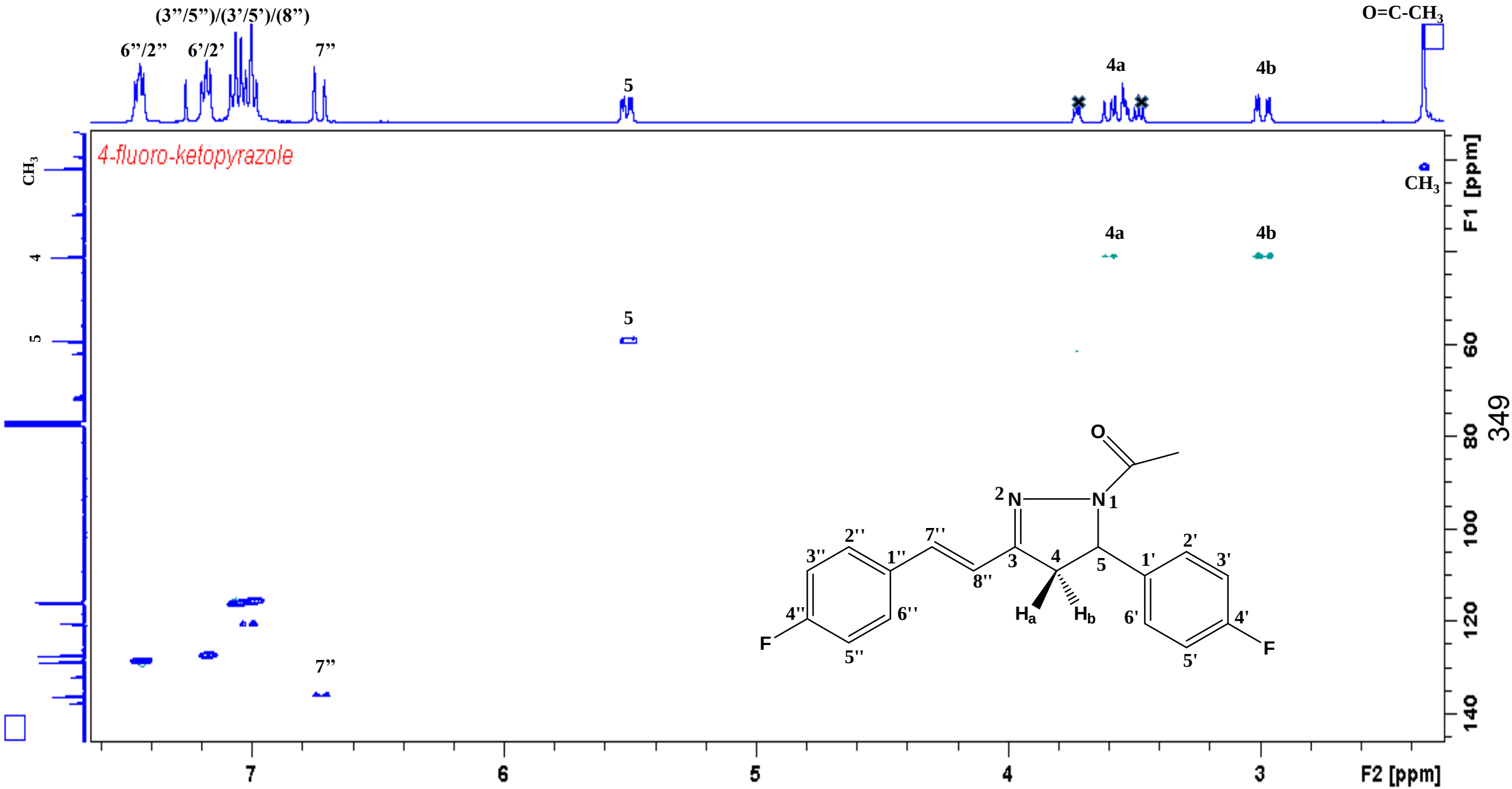


^{13}C Spectrum of Compound B-2h: (E)-1-(5-(4-fluorophenyl)-3-(4-fluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



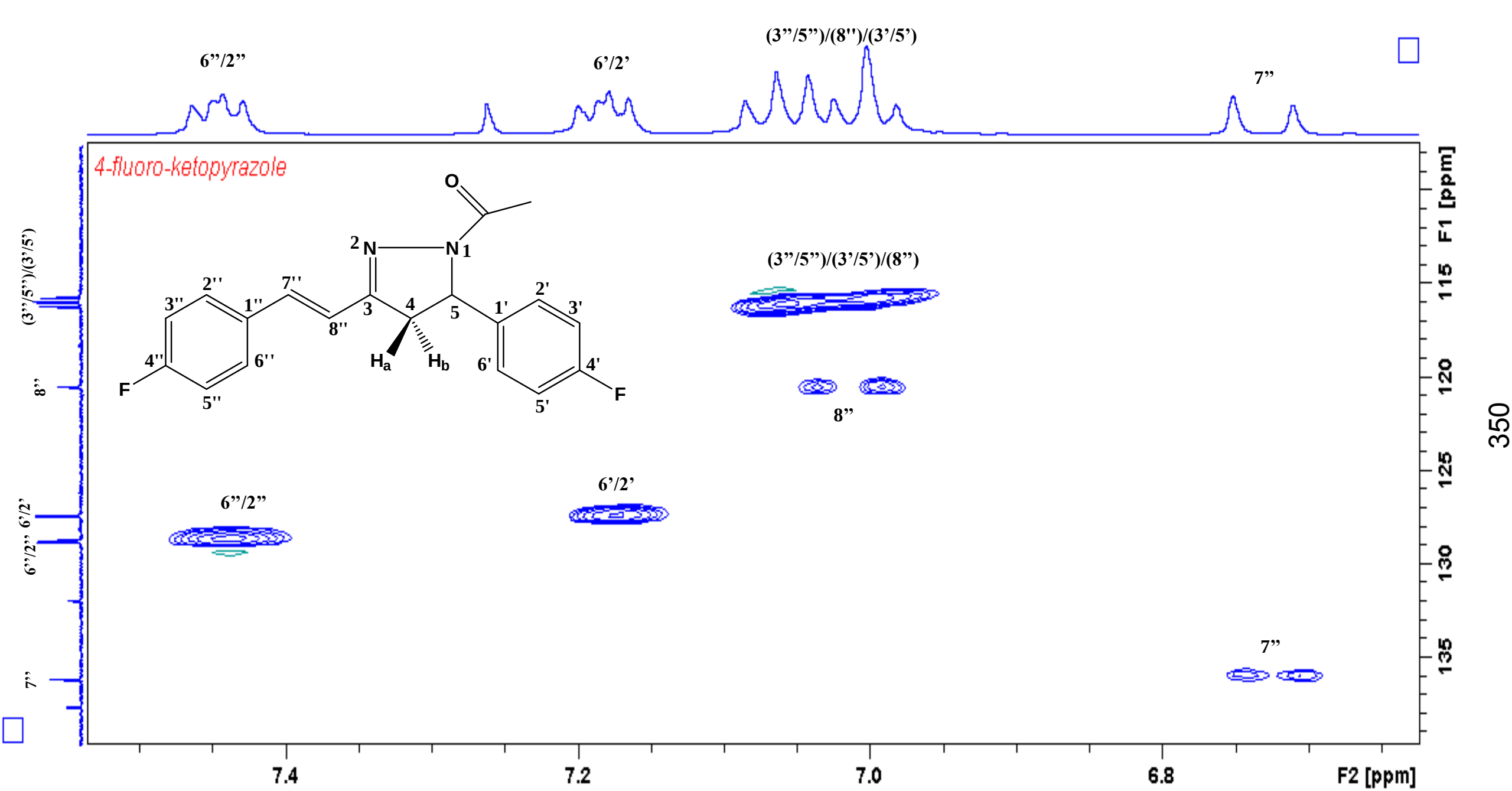
348

Expanded ^{13}C Spectrum of Compound B-2h: (E)-1-(5-(4-fluorophenyl)-3-(4-fluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

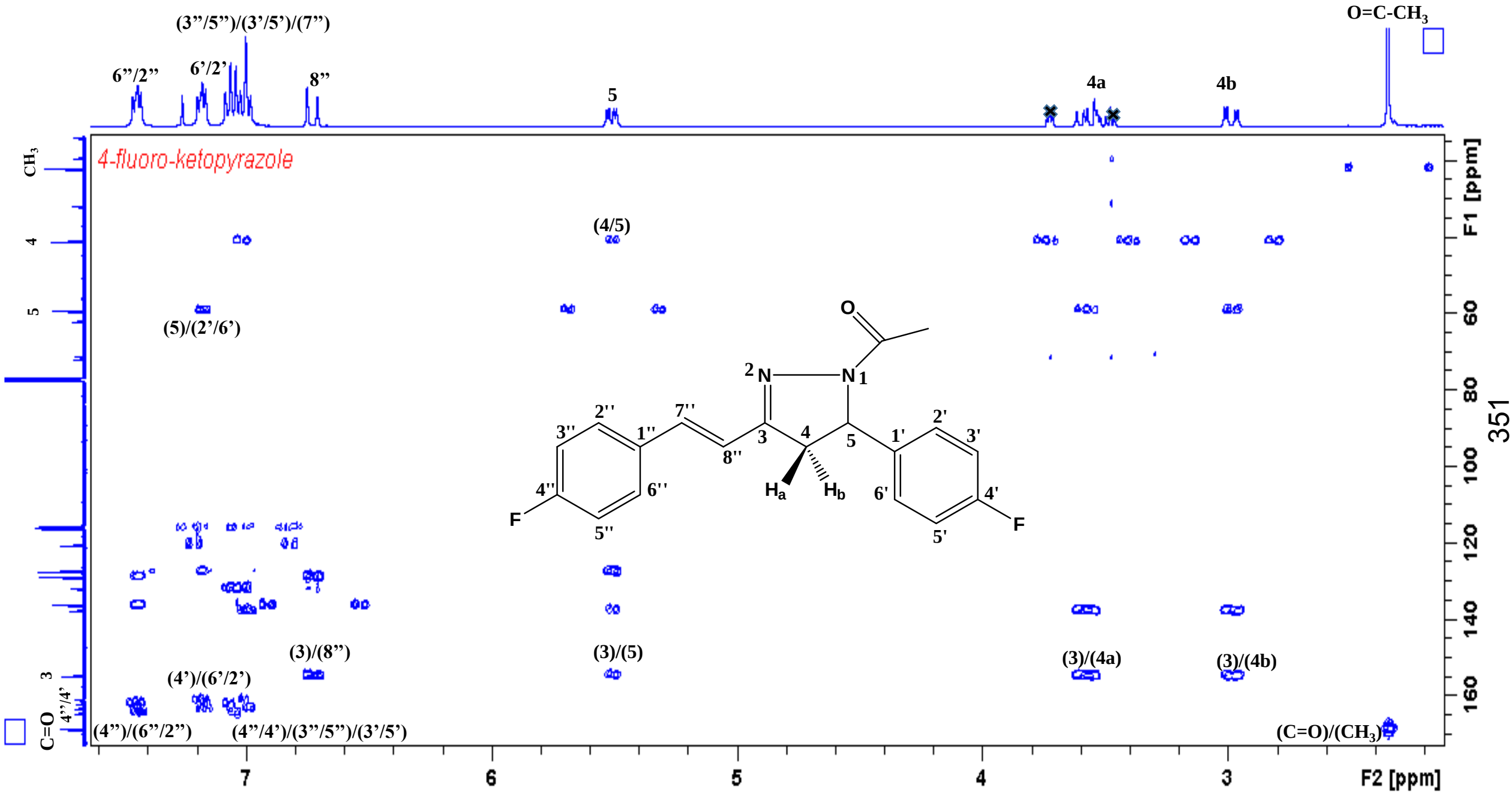


349

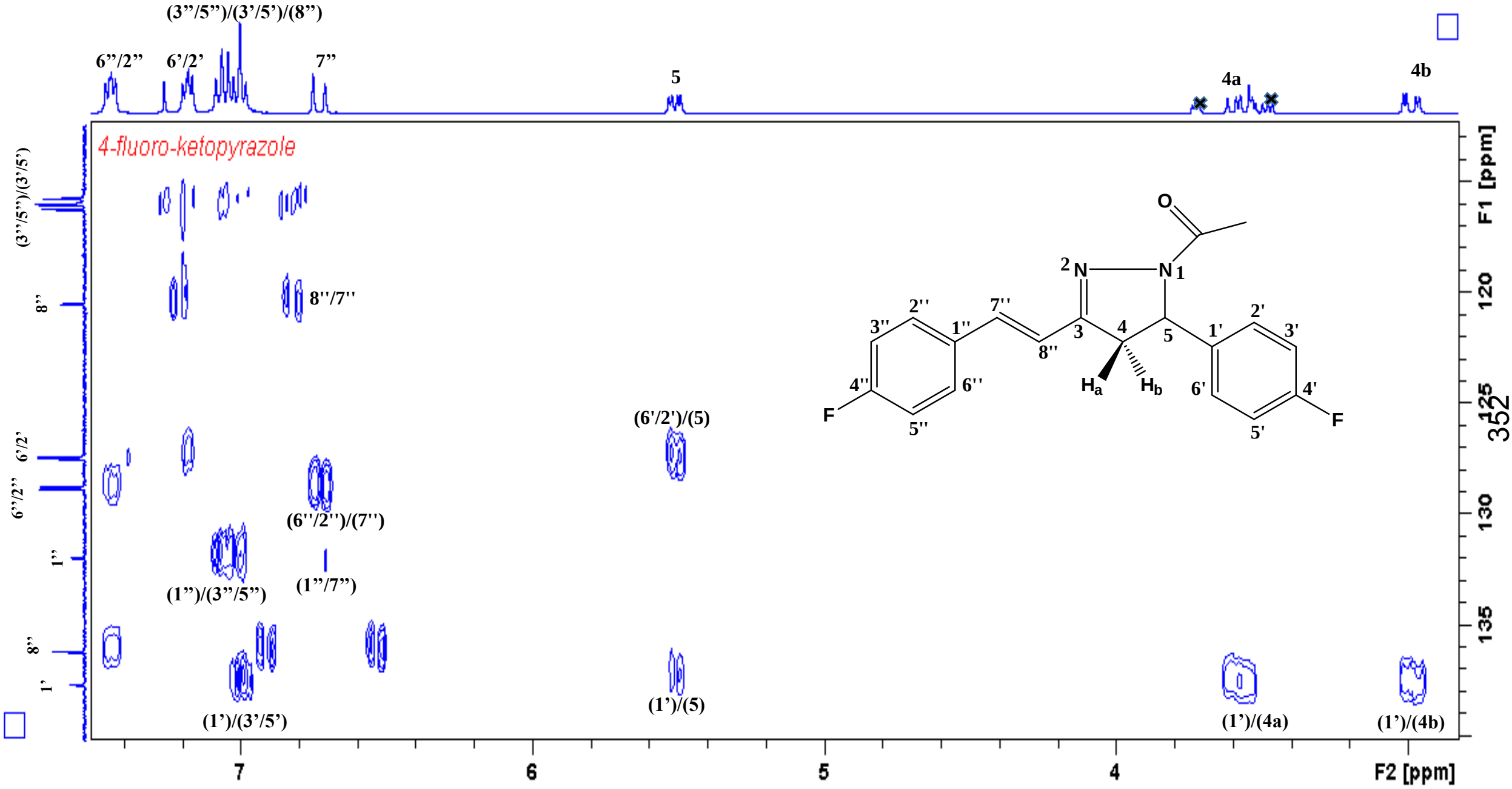
HSQC of Compound B-2h: (E)-1-(5-(4-fluorophenyl)-3-(4-fluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



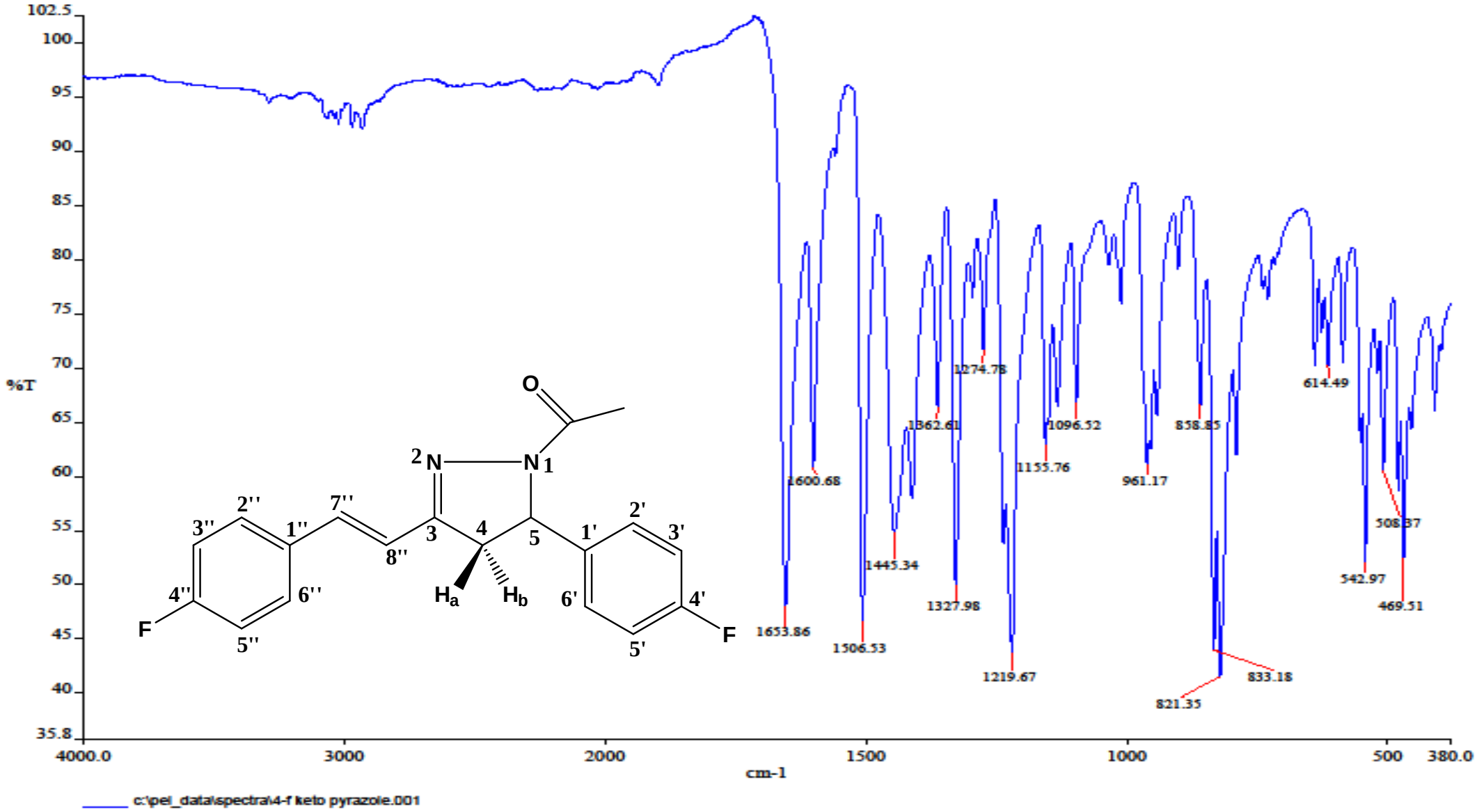
Expanded HSQC of Compound B-2h: (E)-1-(5-(4-fluorophenyl)-3-(4-fluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



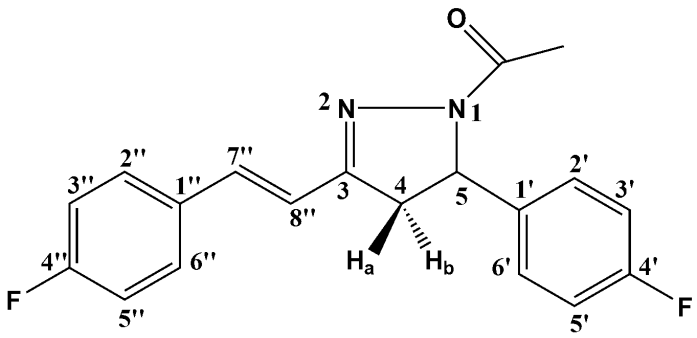
HMBC of Compound B-2h: (E)-1-(5-(4-fluorophenyl)-3-(4-fluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



Expanded HMBC of Compound B-2h: (E)-1-(5-(4-fluorophenyl)-3-(4-fluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



Infrared Spectrum of Compound B-2h: (E)-1-(5-(4-fluorophenyl)-3-(4-fluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



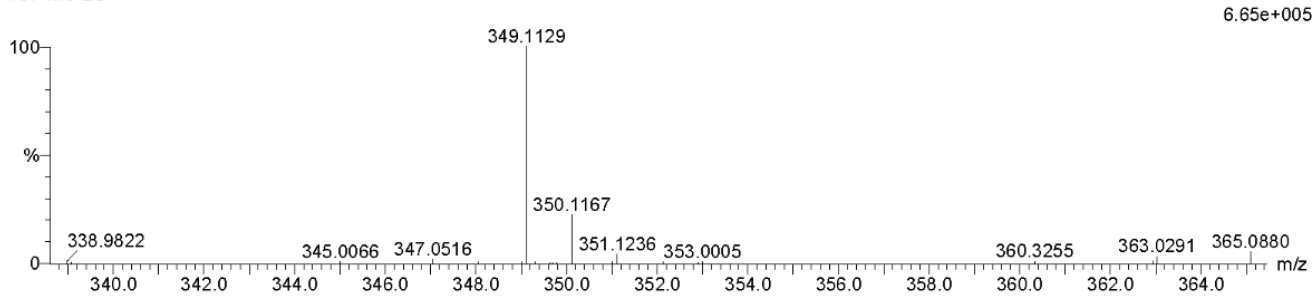
Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

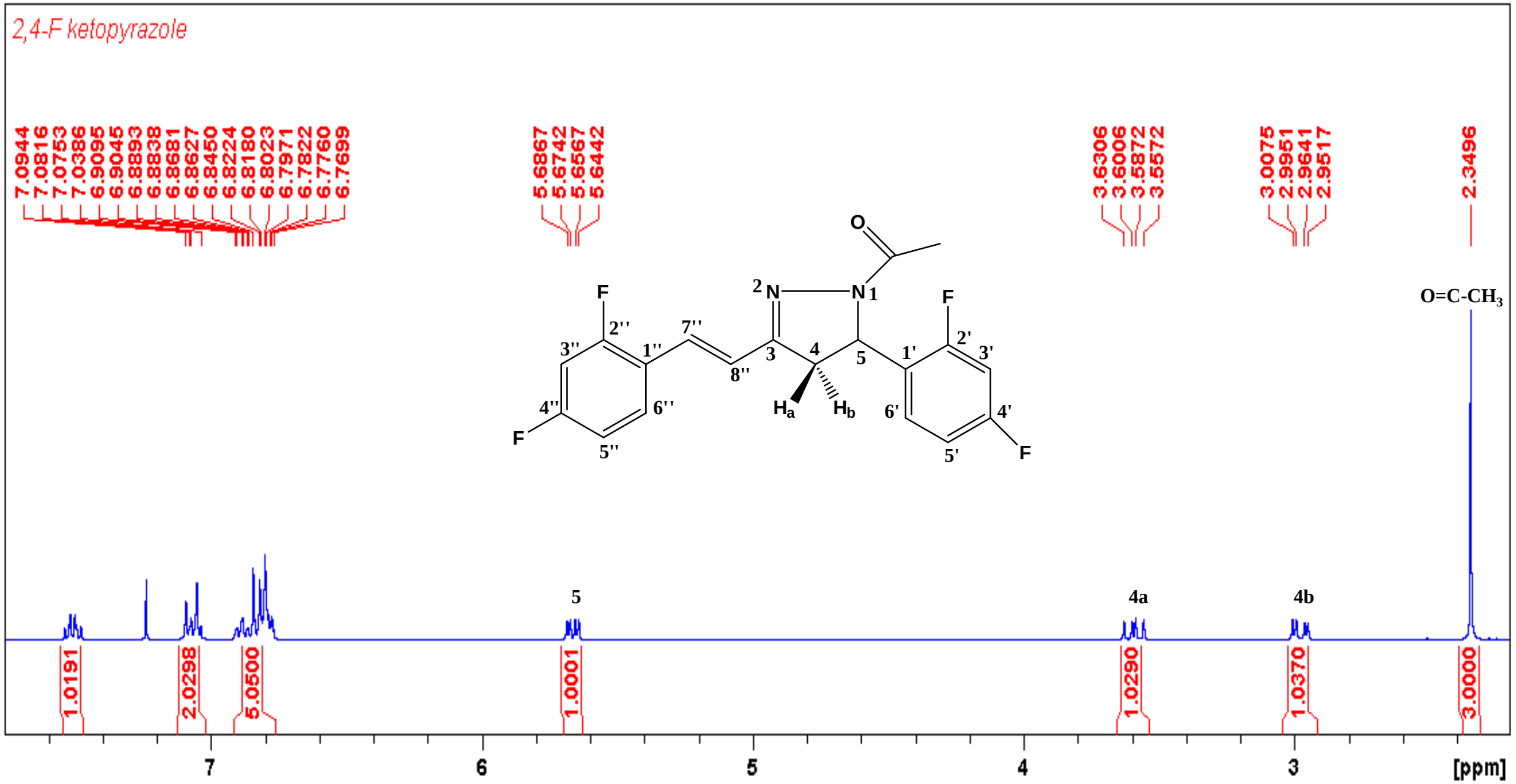
Monoisotopic Mass, Even Electron Ions
58 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 15-20 H: 15-20 N: 0-5 O: 0-1 F: 0-5 Na: 0-1

Compound 8 46 (1.518) Cm (1:61)
TOF MS ES+

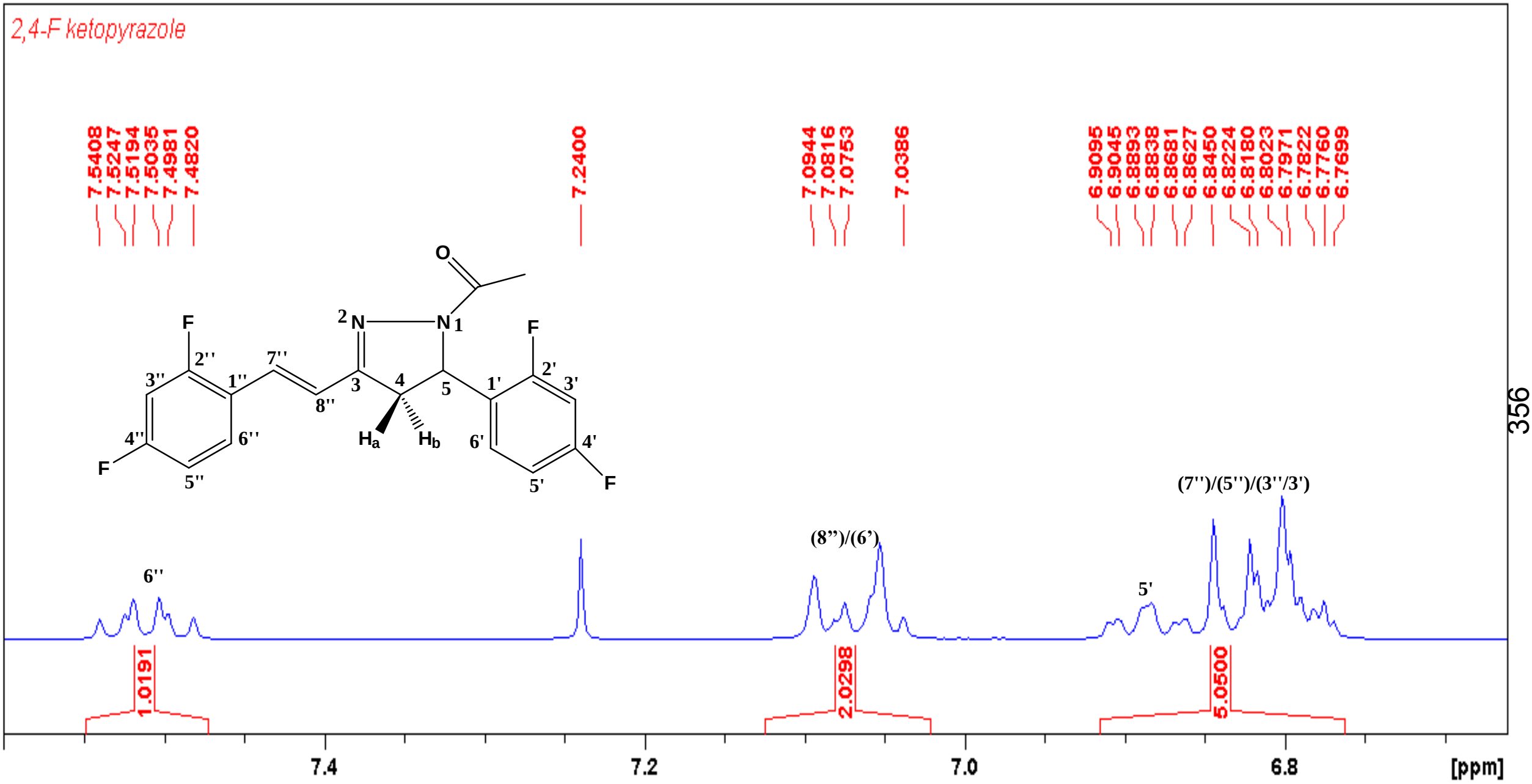


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
349.1129	349.1128	0.1	0.3	11.5	72.3	0.0	C19 H16 N2 O F2 Na

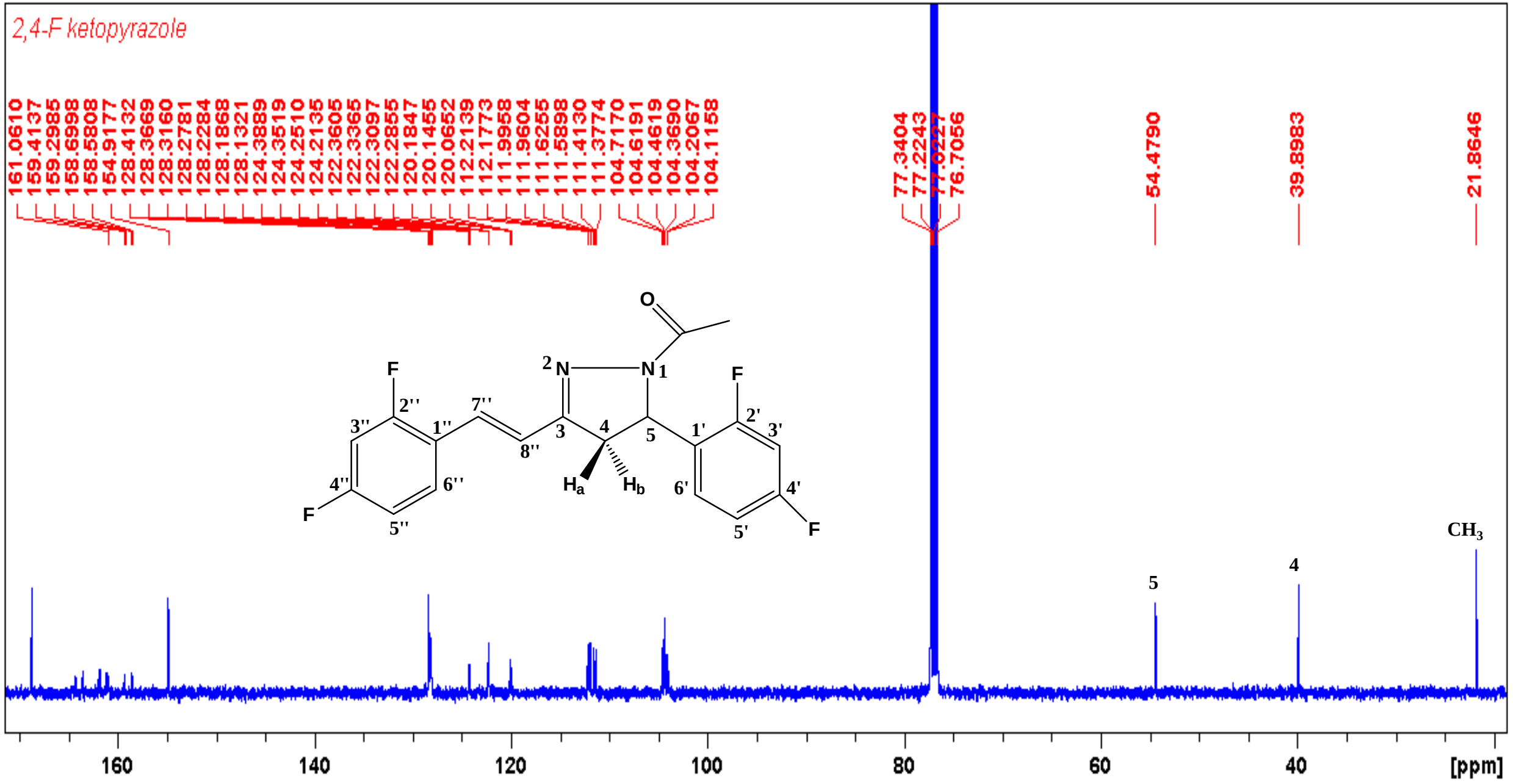
HRMS of Compound B-2h: (E)-1-(5-(4-fluorophenyl)-3-(4-fluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



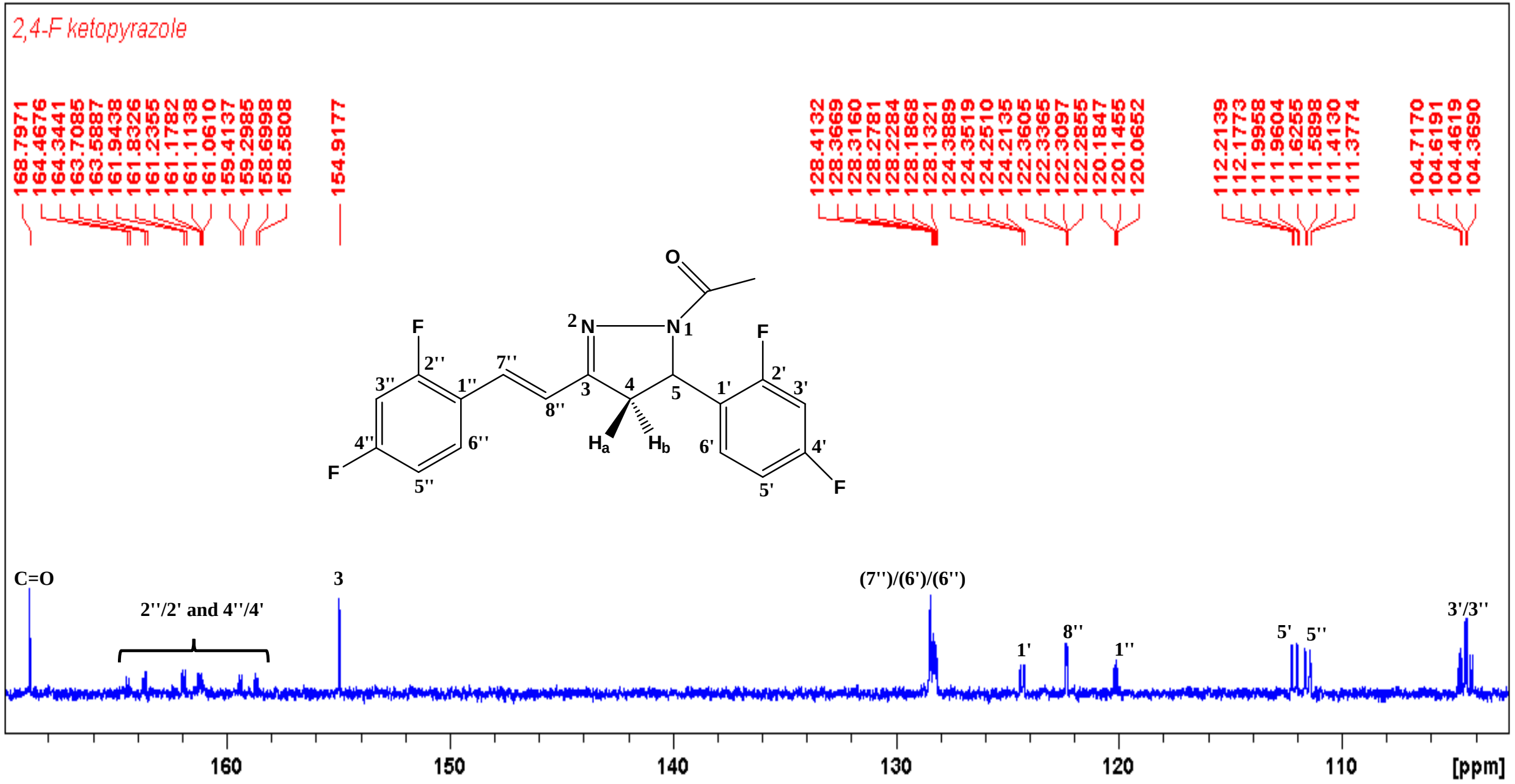
^{1}H Spectrum of Compound B-2i: (E)-1-(5-(2,4-difluorophenyl)-3-(2,4-difluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



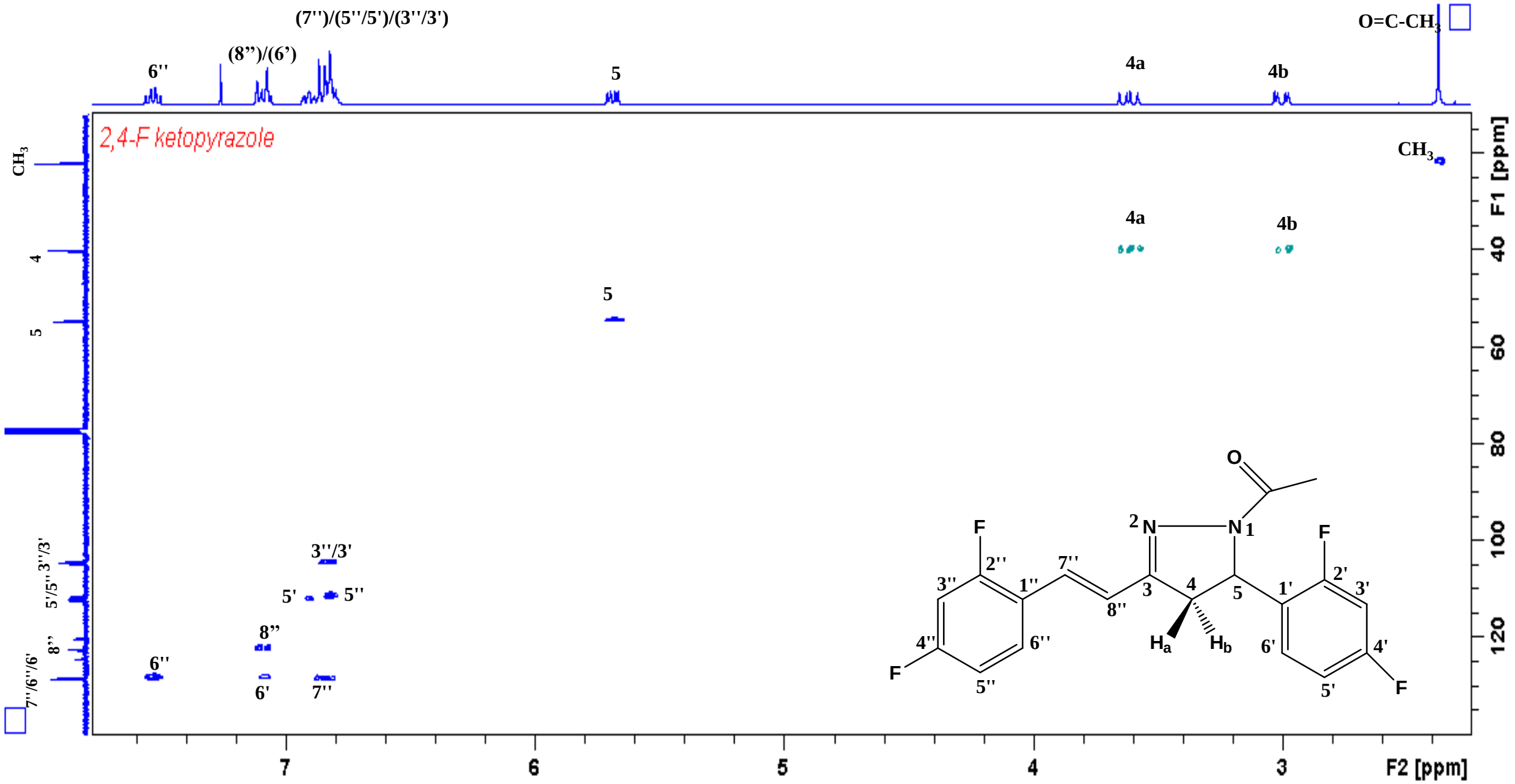
Expanded ^{1}H Spectrum of Compound B-2i: (E)-1-(5-(2,4-difluorophenyl)-3-(2,4-difluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



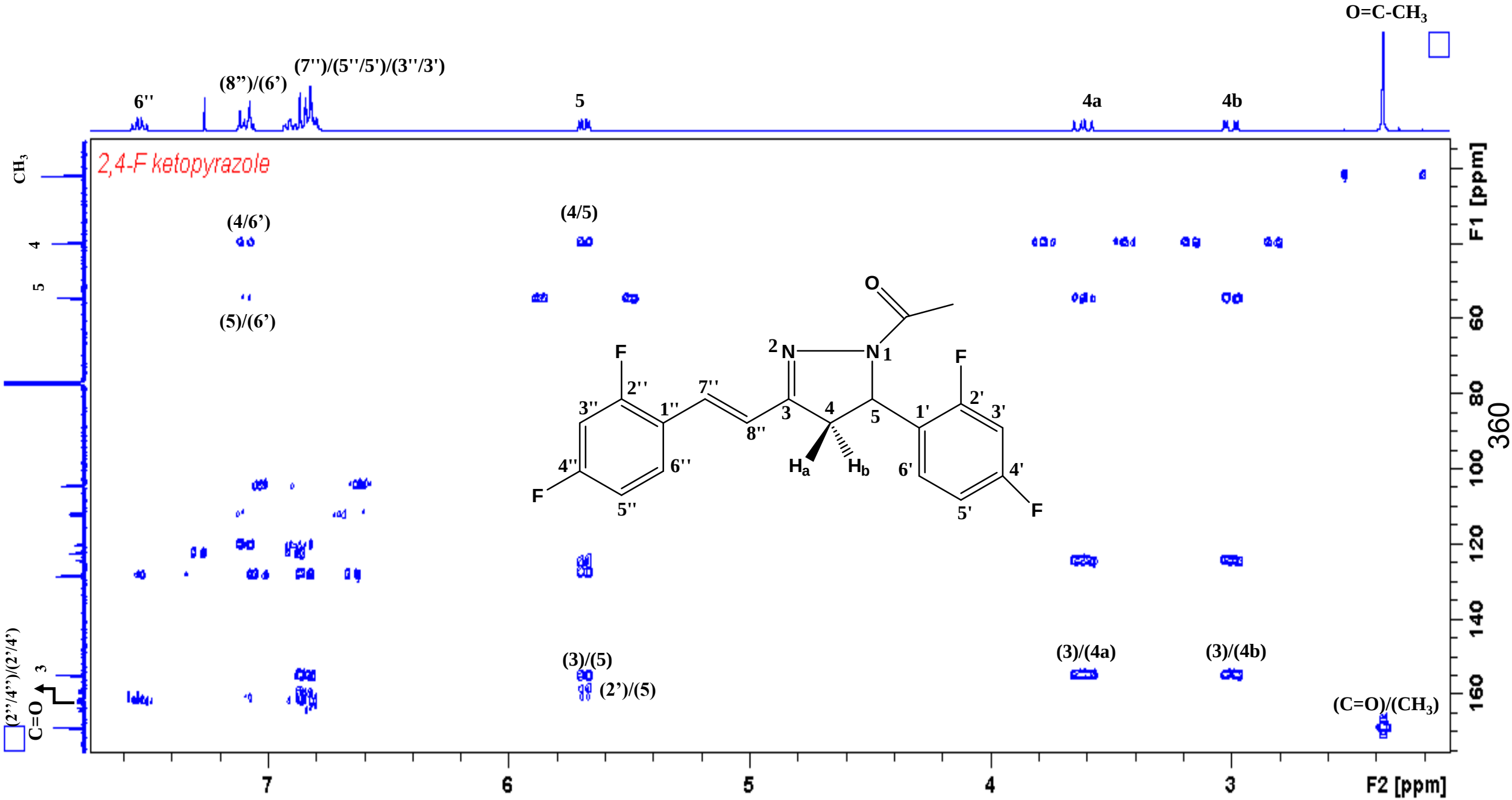
^{13}C Spectrum of Compound B-2i: (E)-1-(5-(2,4-difluorophenyl)-3-(2,4-difluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



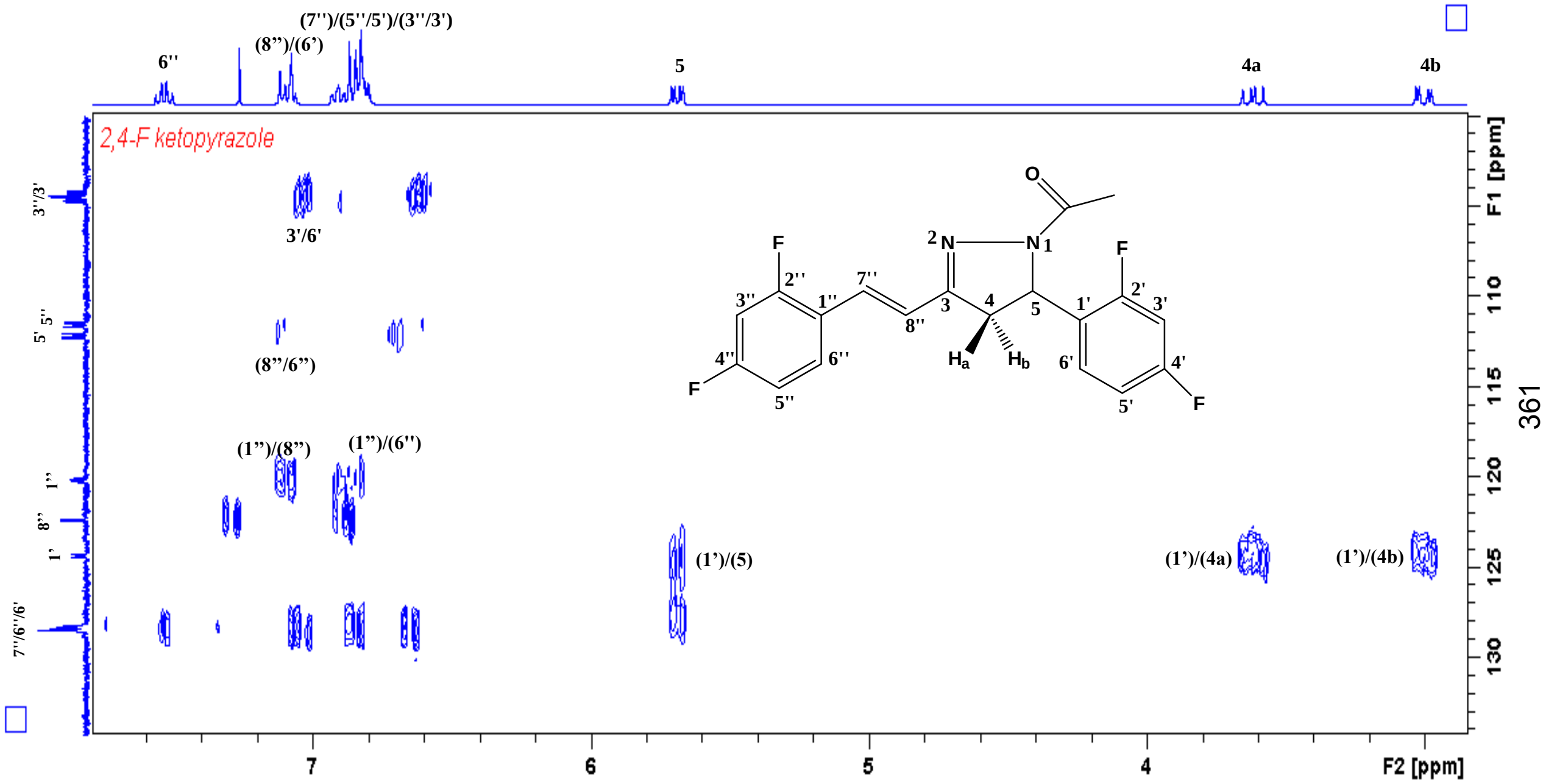
Expanded ^{13}C Spectrum of Compound B-2i:(E)-1-(5-(2,4-difluorophenyl)-3-(2,4-difluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



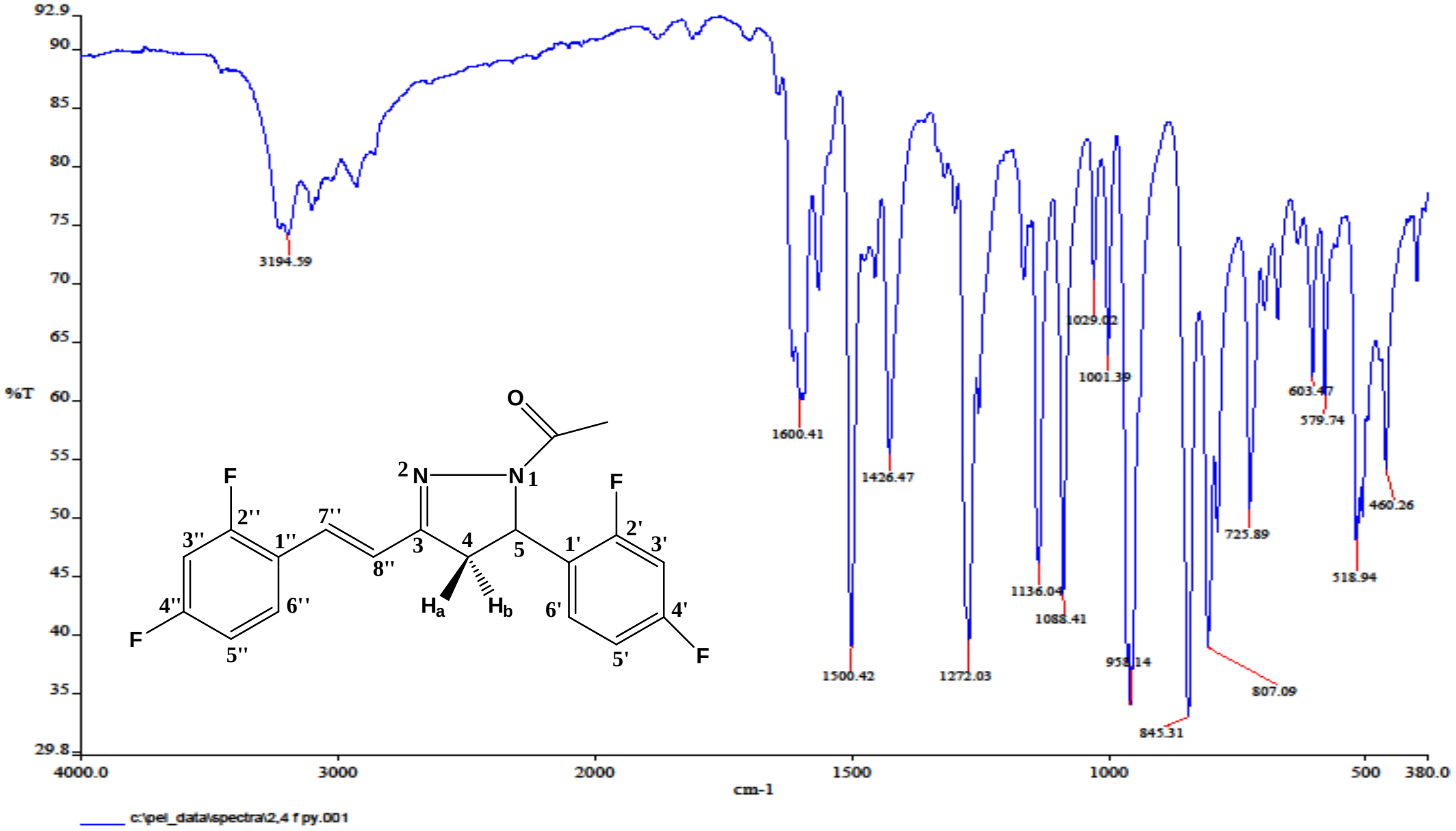
HSQC of Compound B-2i: (E)-1-(5-(2,4-difluorophenyl)-3-(2,4-difluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



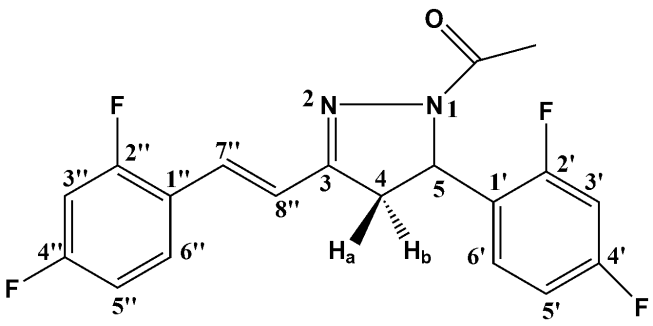
HMBC of Compound B-2i: (E)-1-(5-(2,4-difluorophenyl)-3-(2,4-difluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



Expanded HMBC of Compound B-2i: (E)-1-(5-(2,4-difluorophenyl)-3-(2,4-difluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



Infrared Spectrum of Compound B-2i: (E)-1-(5-(2,4-difluorophenyl)-3-(2,4-difluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



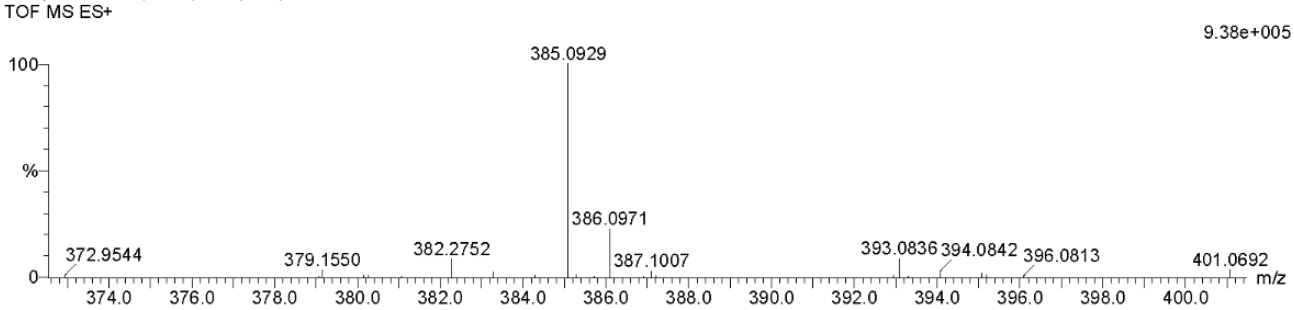
Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

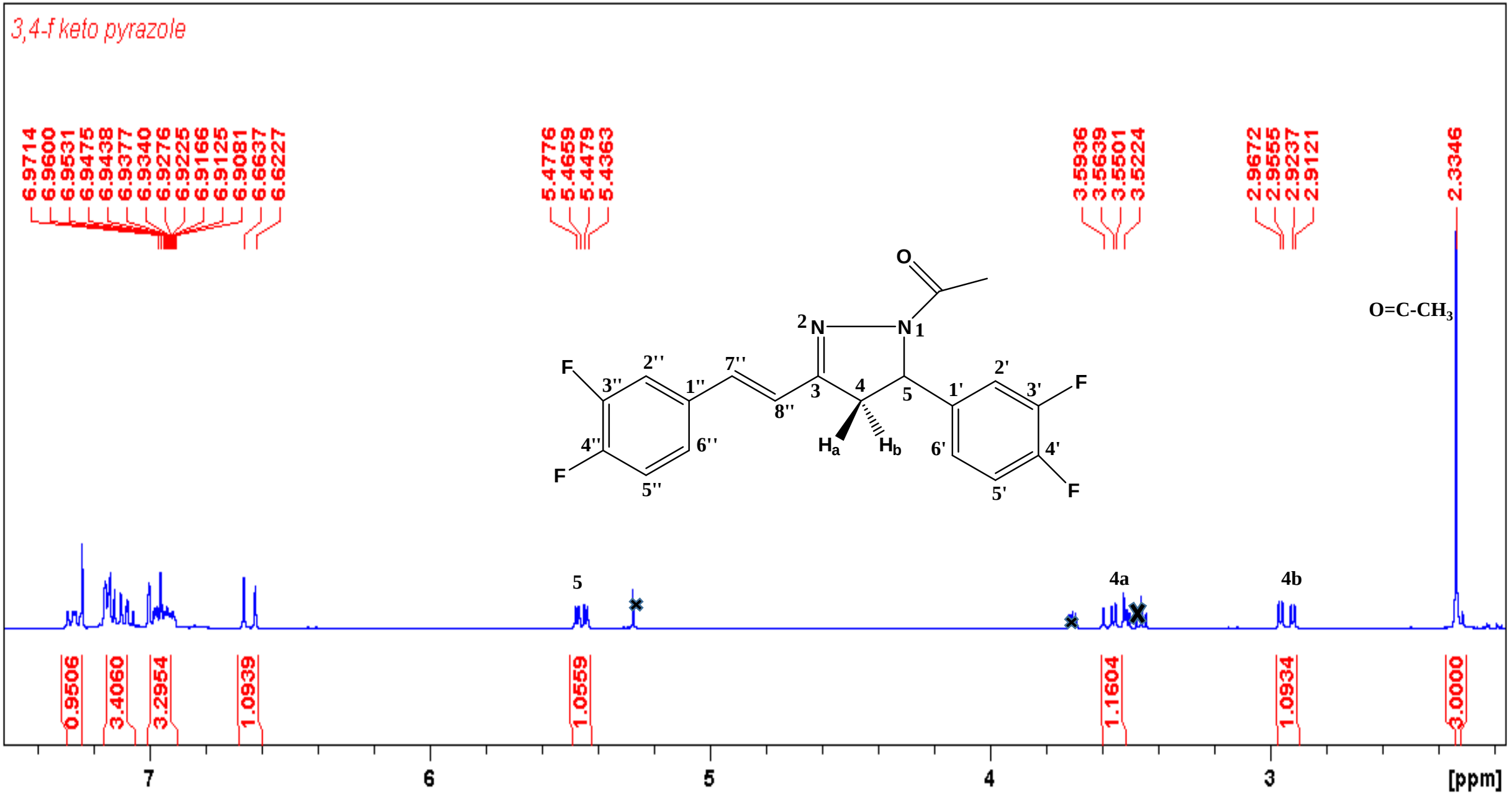
Monoisotopic Mass, Even Electron Ions
73 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 O: 0-1 F: 0-5 Na: 0-1

Compound 9 12 (0.371) Cm (1:61)
TOF MS ES+

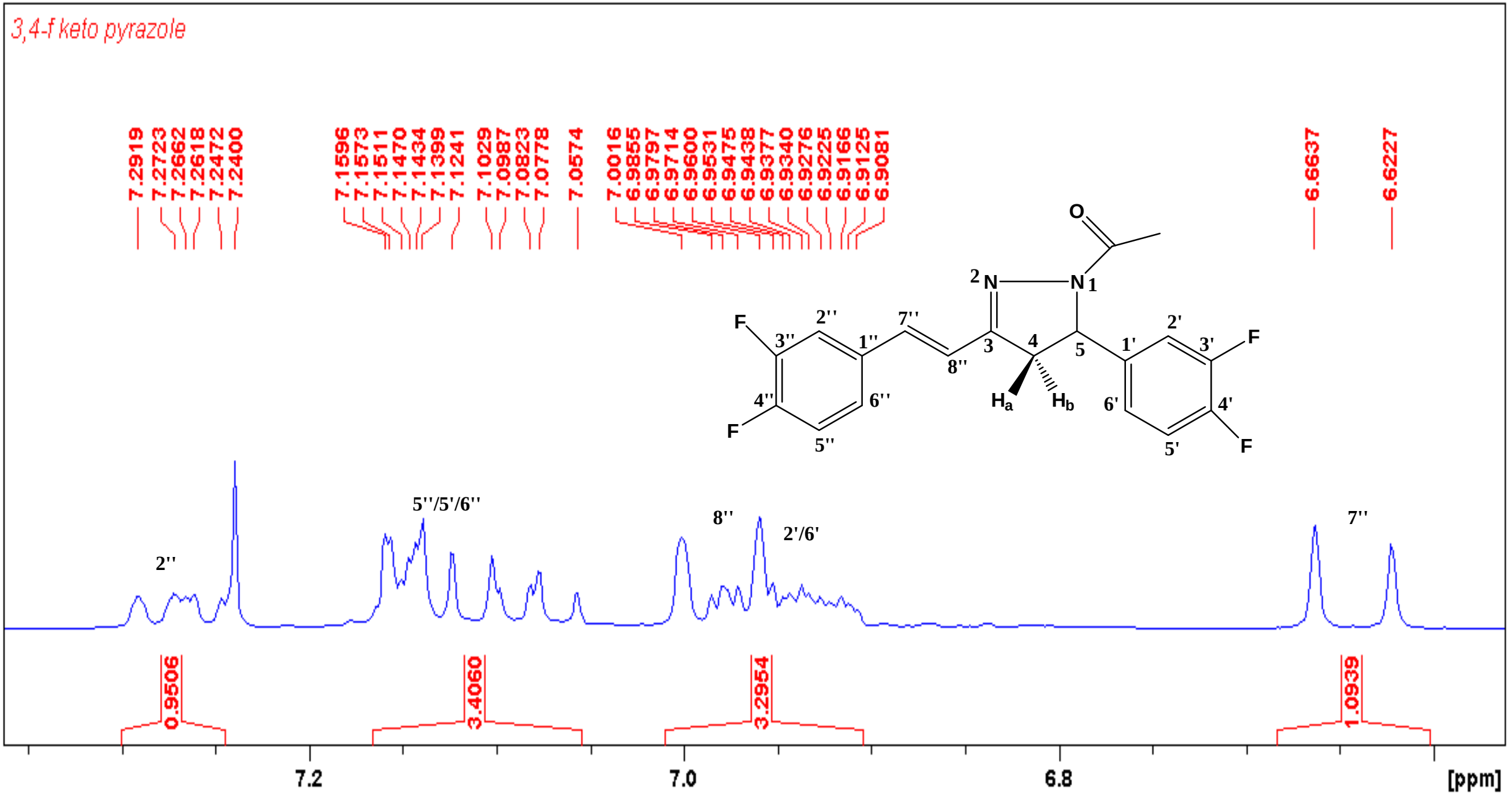


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
385.0929	385.0940	-1.1	-2.9	11.5	49.4	0.0	C19 H14 N2 O F4 Na

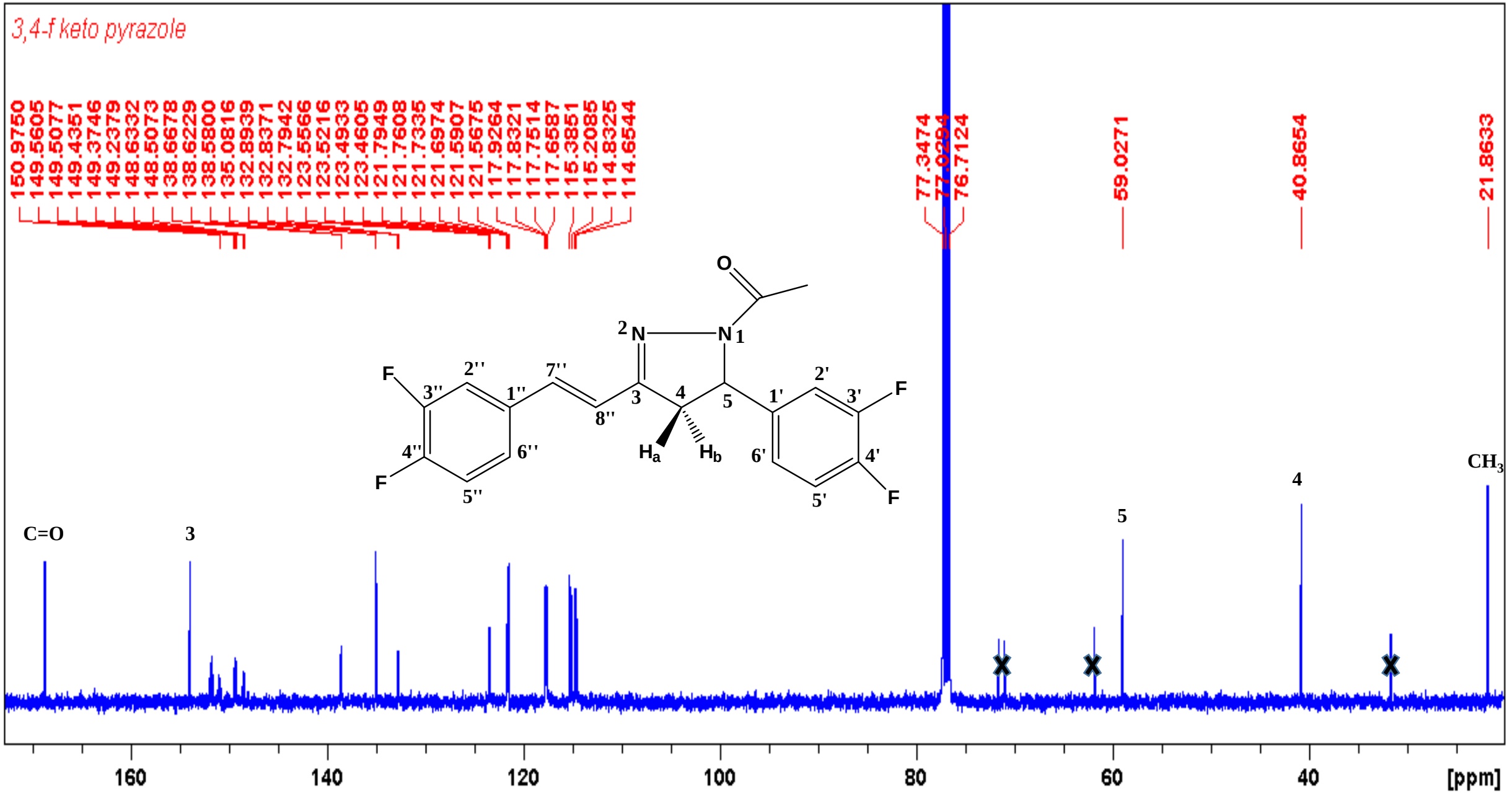
HRMS of Compound B-2i: (E)-1-(5-(2,4-difluorophenyl)-3-(2,4-difluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



^{1}H Spectrum of Compound B-2j: (E)-1-(5-(3,4-difluorophenyl)-3-(3,4-difluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

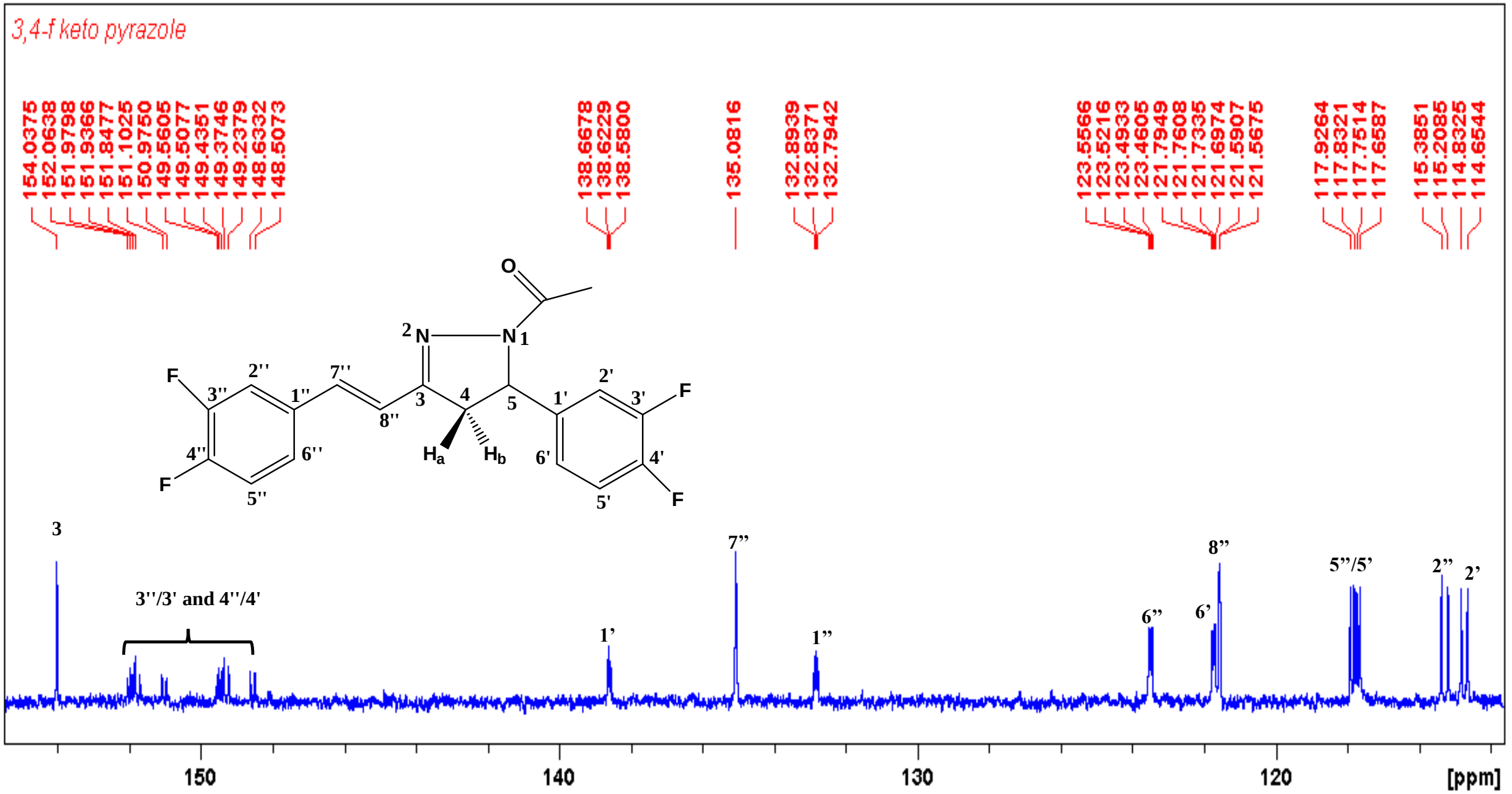


Expanded ^{1}H Spectrum of Compound B-2j: (E)-1-(5-(3,4-difluorophenyl)-3-(3,4-difluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



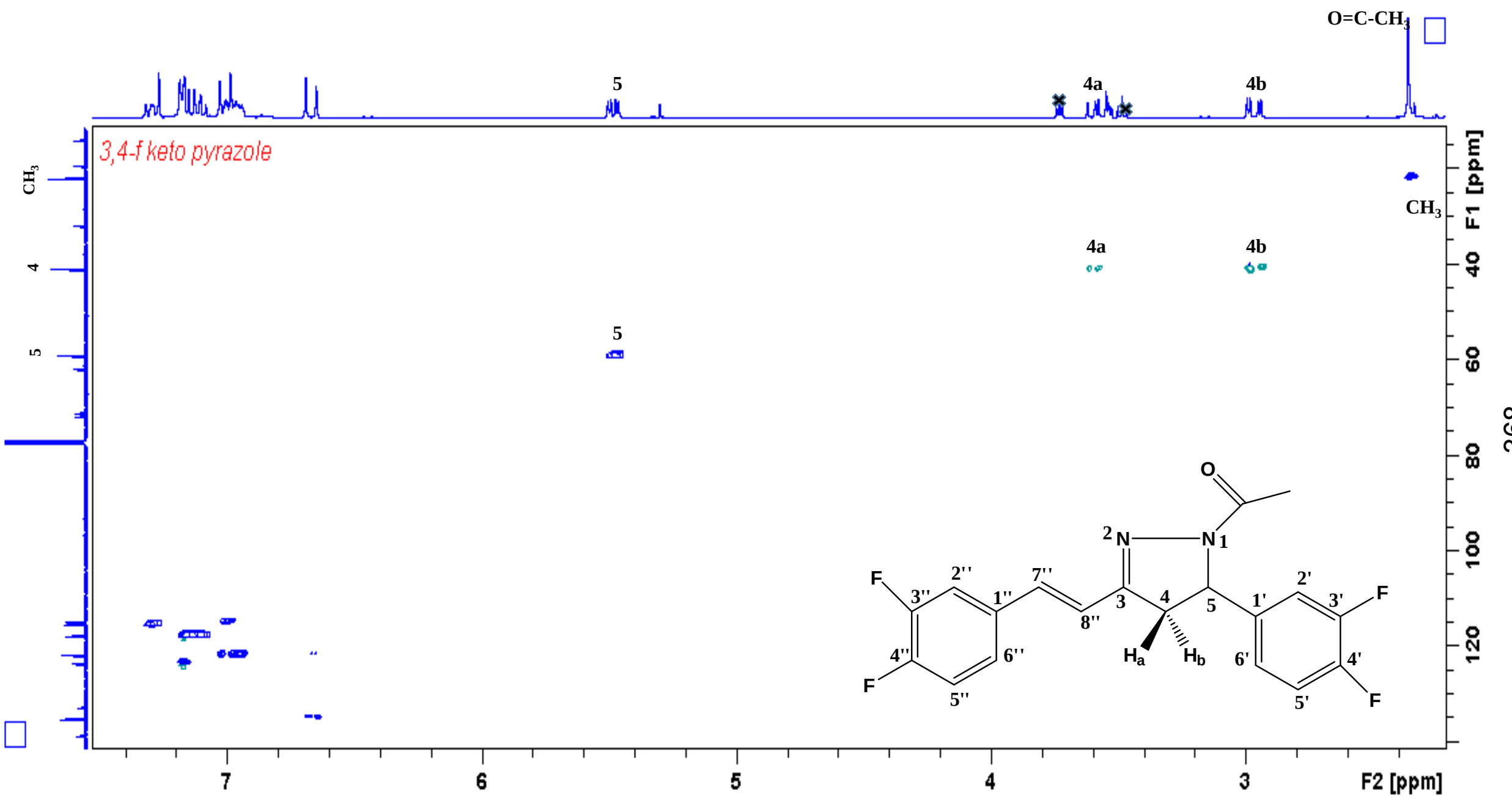
366

^{13}C Spectrum of Compound B-2j: (E)-1-(5-(3,4-difluorophenyl)-3-(3,4-difluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

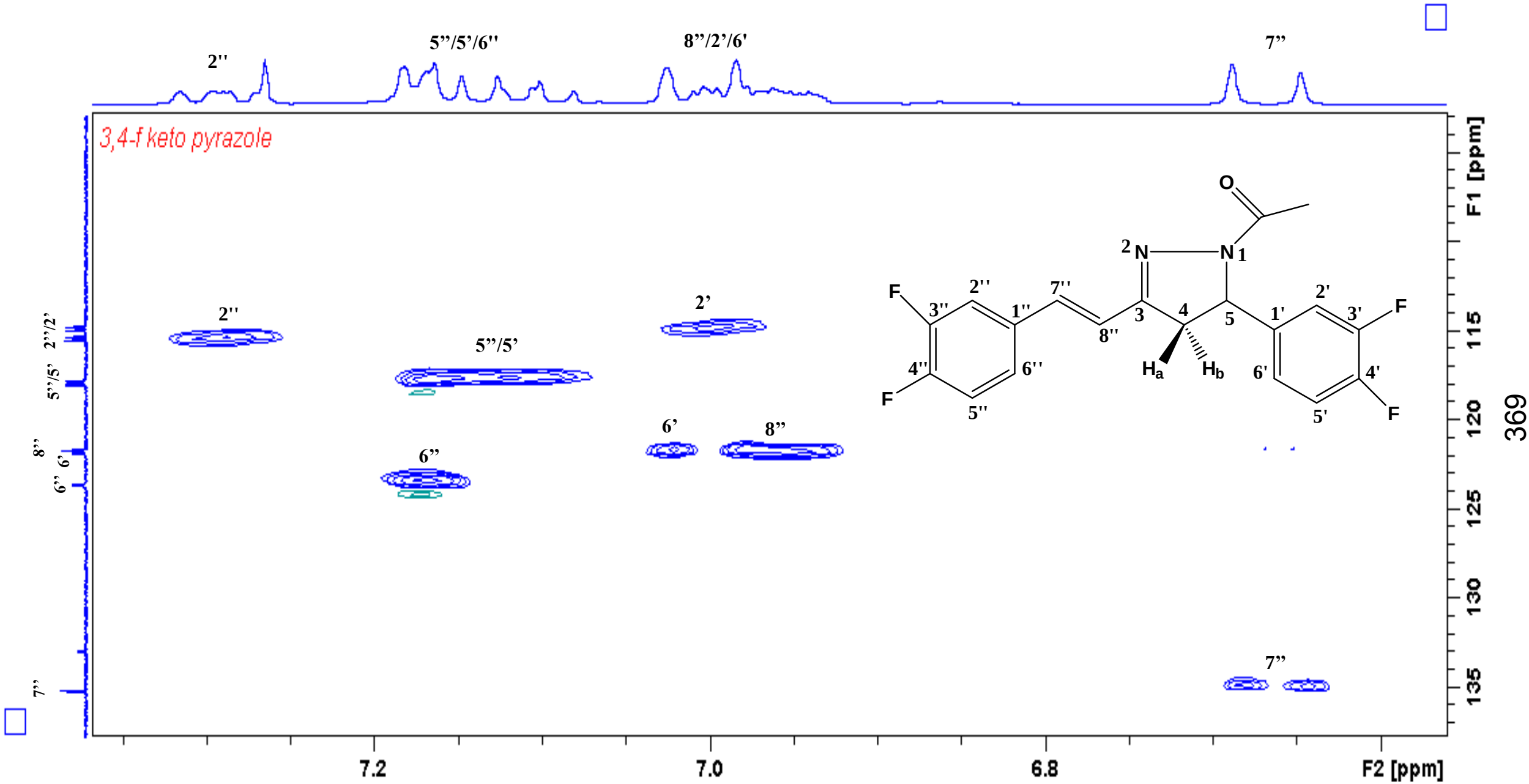


367

Expanded ^{13}C Spectrum of Compound B-2j: (E)-1-(5-(3,4-difluorophenyl)-3-(3,4-difluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

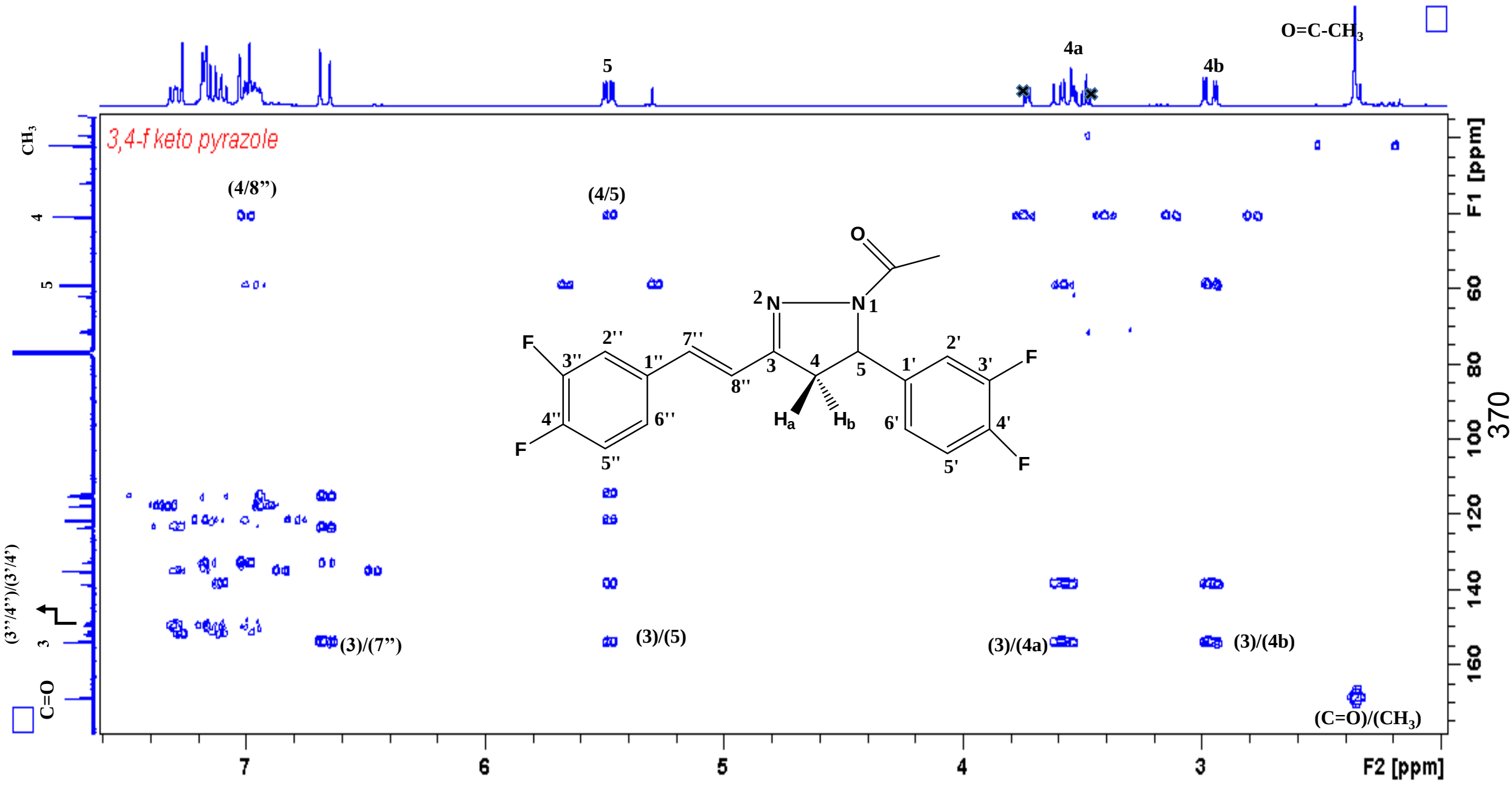


HSQC of Compound B-2j: (E)-1-(5-(3,4-difluorophenyl)-3-(3,4-difluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

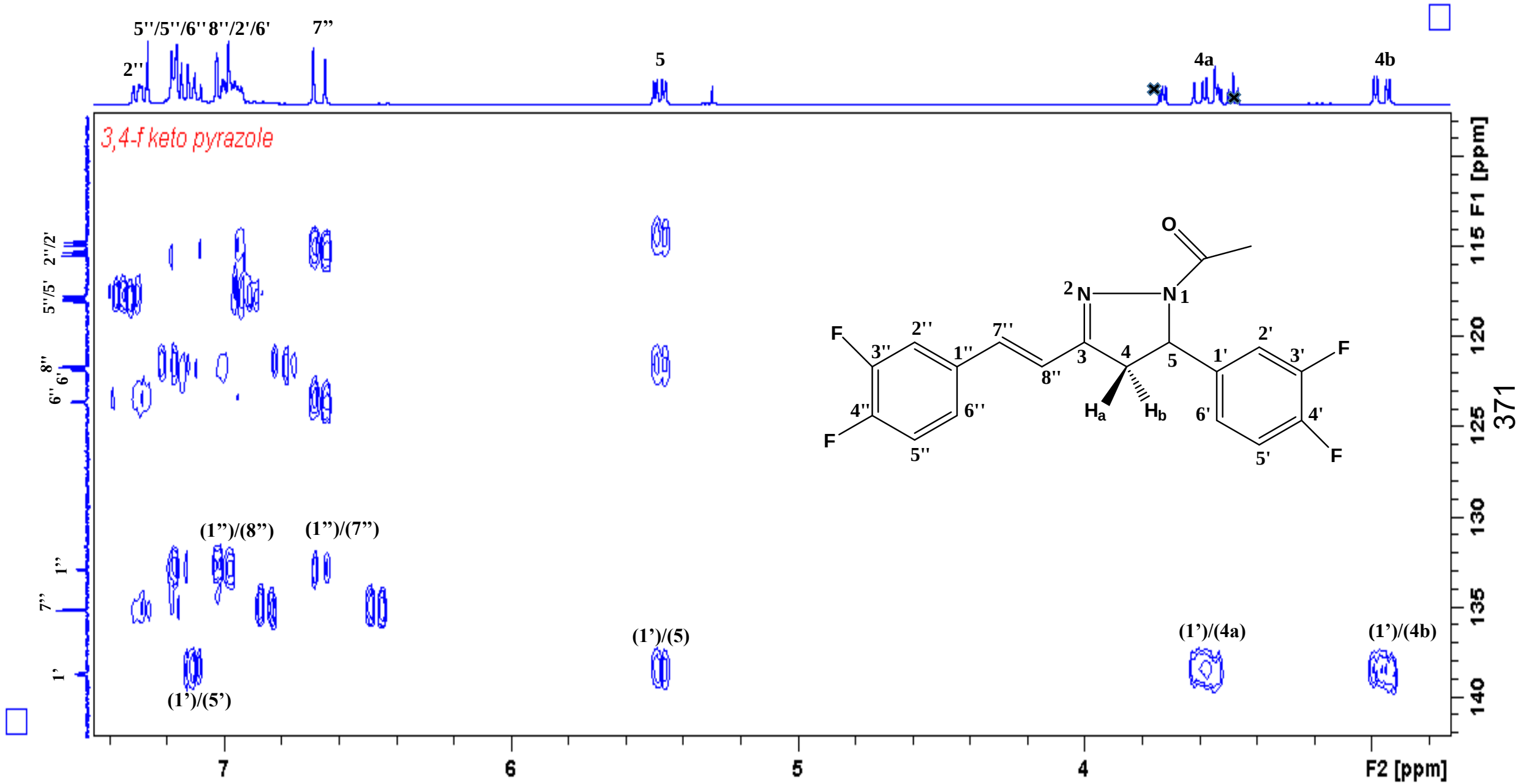


369

Expanded HSQC of Compound B-2j: (E)-1-(5-(3,4-difluorophenyl)-3-(3,4-difluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

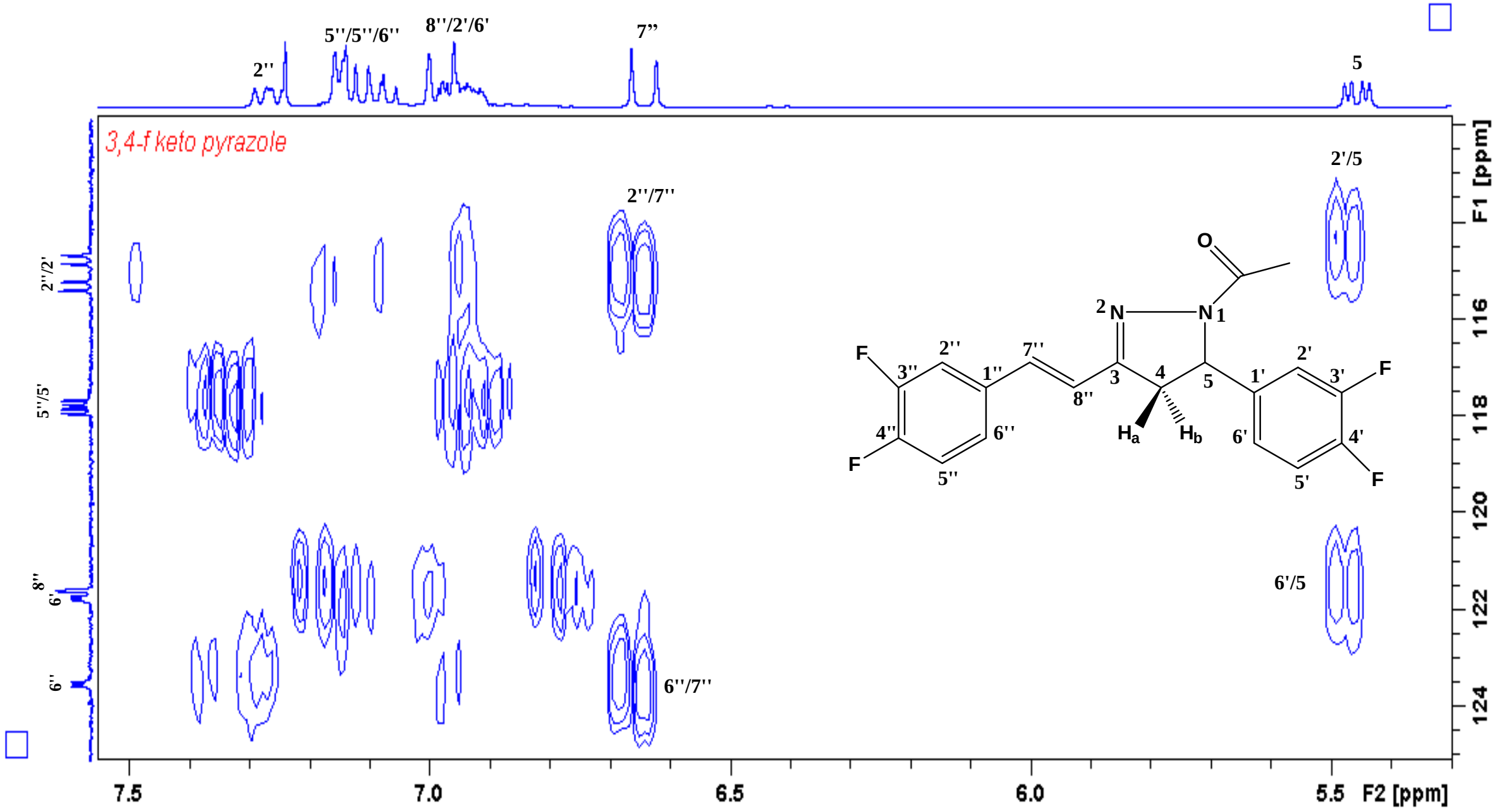


HMBC of Compound B-2j: (E)-1-(5-(3,4-difluorophenyl)-3-(3,4-difluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



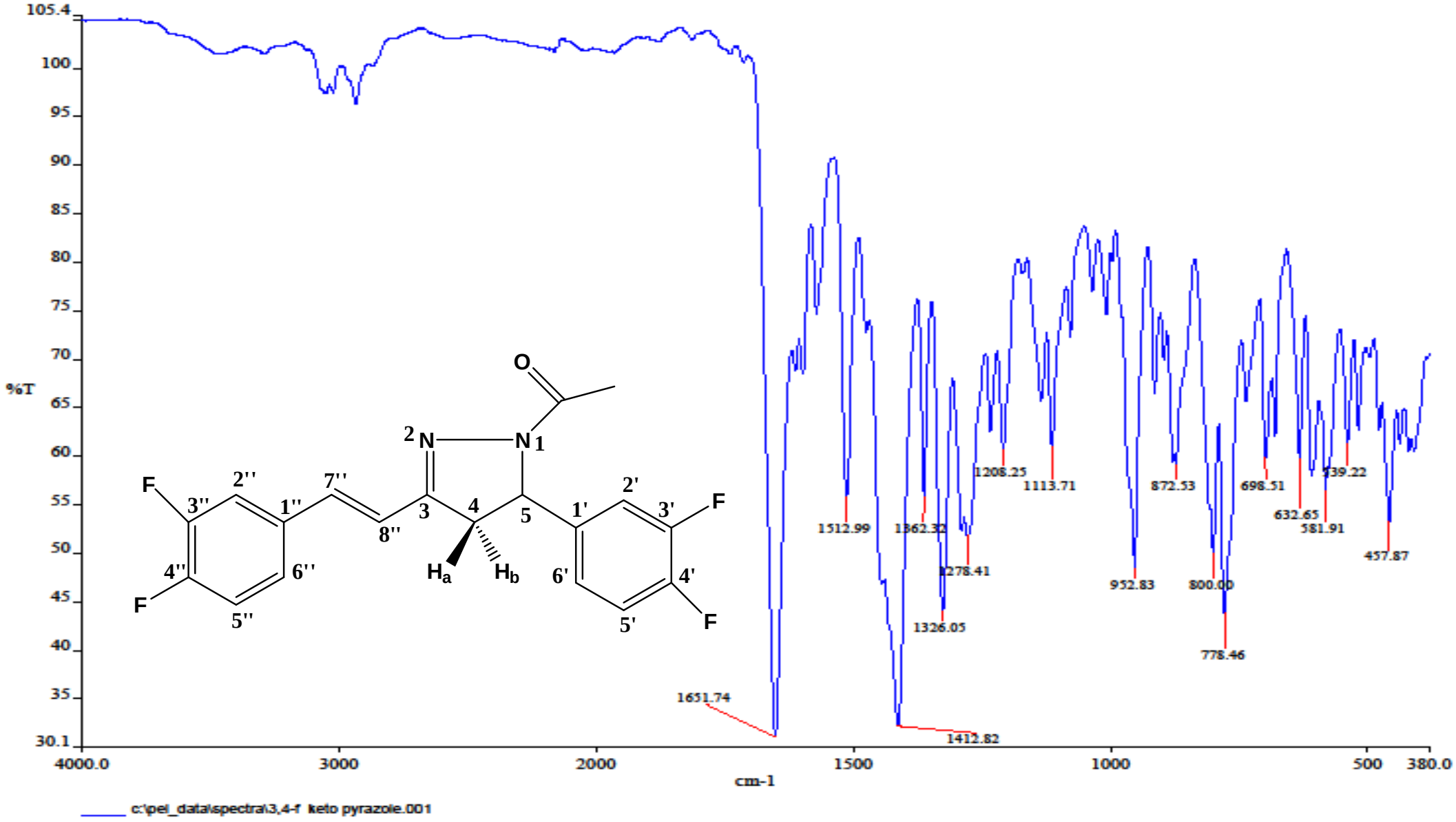
371

Expanded HMBC of Compound B-2j: (E)-1-(5-(3,4-difluorophenyl)-3-(3,4-difluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

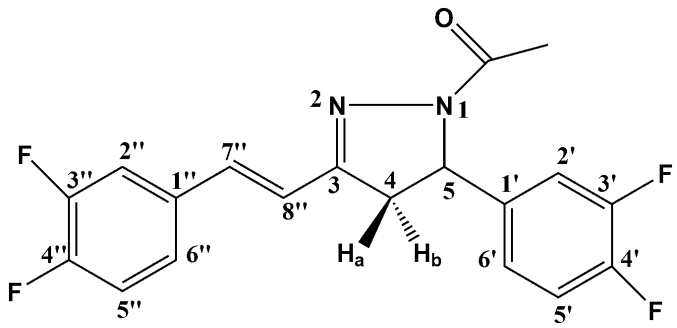


372

Expanded HMBC of Compound B-2j: (E)-1-(5-(3,4-difluorophenyl)-3-(3,4-difluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



Infrared Spectrum of Compound B-2j: (E)-1-(5-(3,4-difluorophenyl)-3-(3,4-difluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



Elemental Composition Report

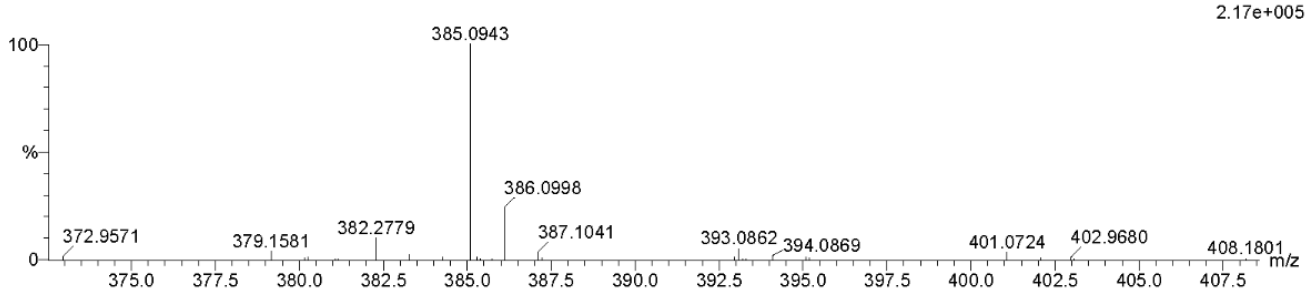
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
73 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 O: 0-1 F: 0-5 Na: 0-1

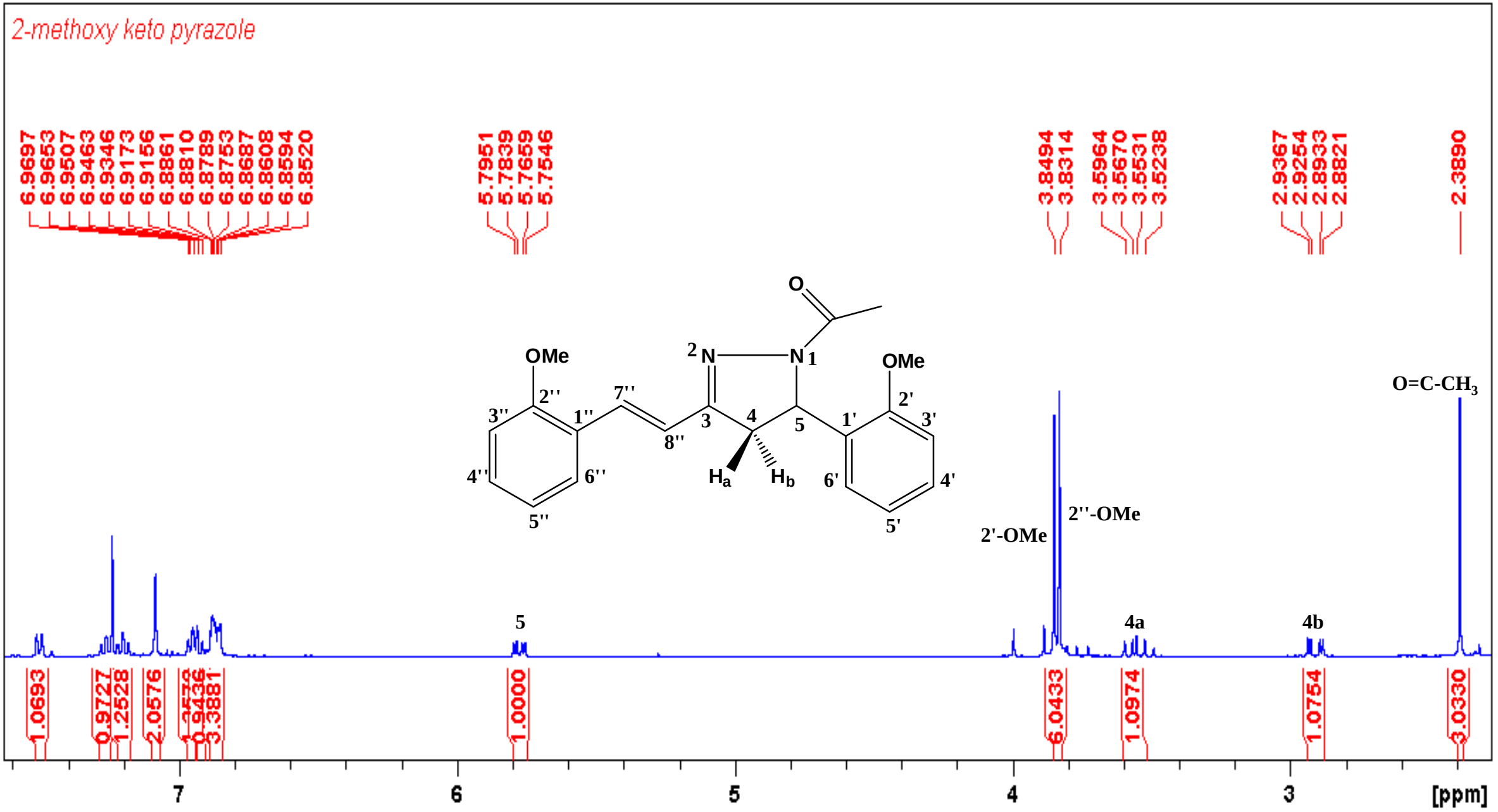
Compound 10 9 (0.303) Cm (1:14)
TOF MS ES+

2.17e+005



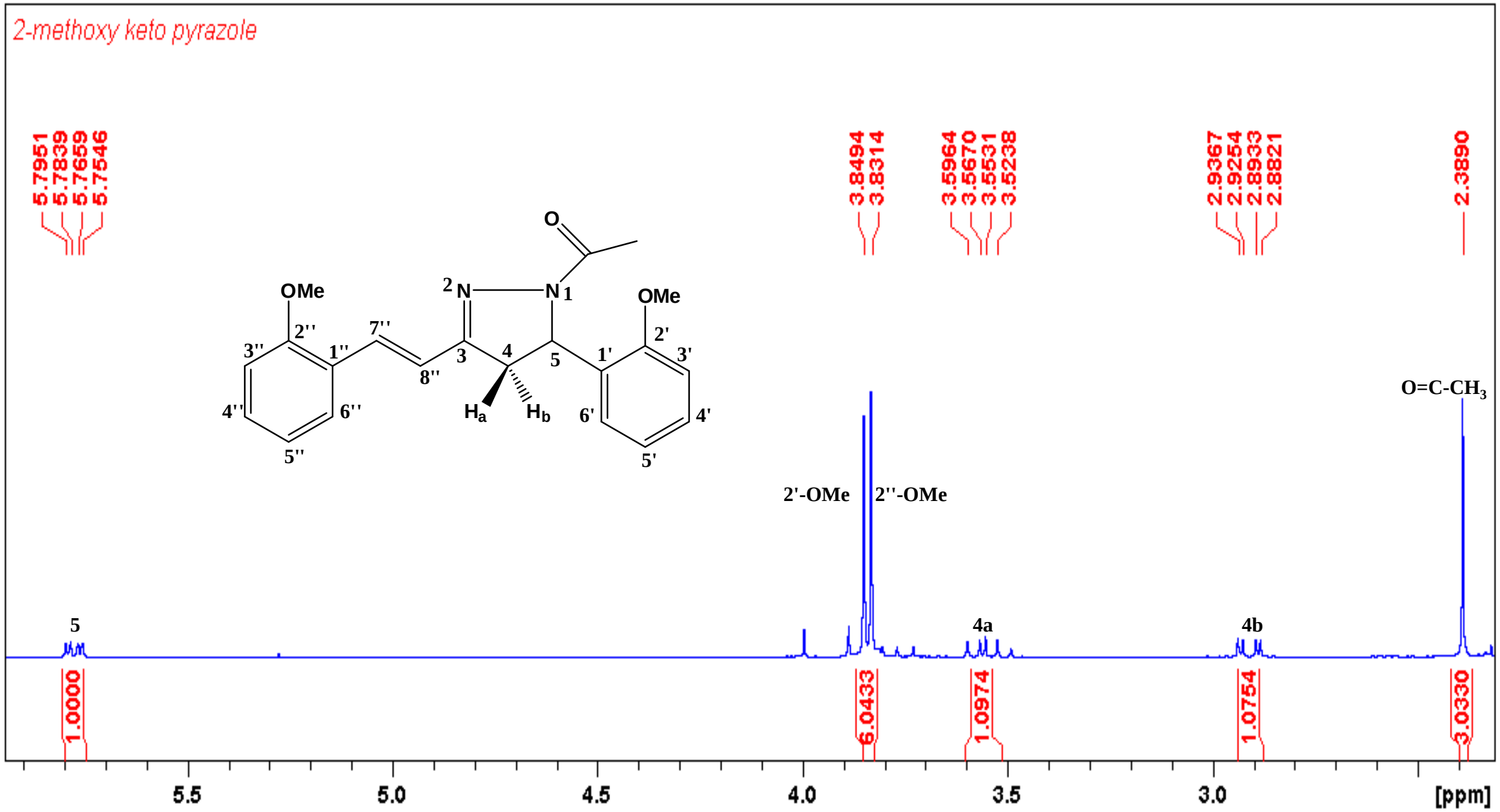
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
385.0943	385.0940	0.3	0.8	11.5	61.0	0.0	C19 H14 N2 O F4 Na

HRMS of Compound B-2j: (E)-1-(5-(3,4-difluorophenyl)-3-(3,4-difluorostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

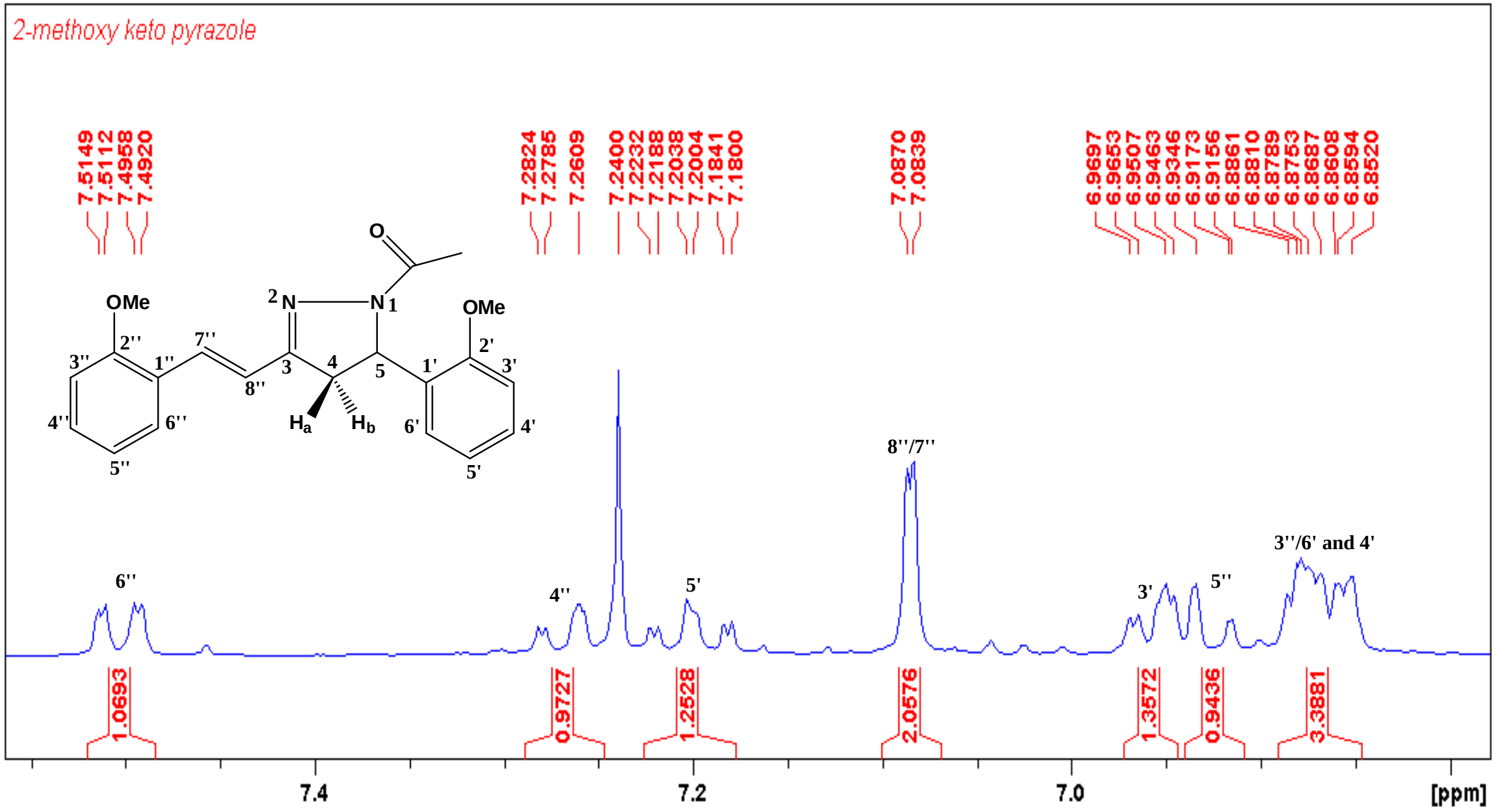


375

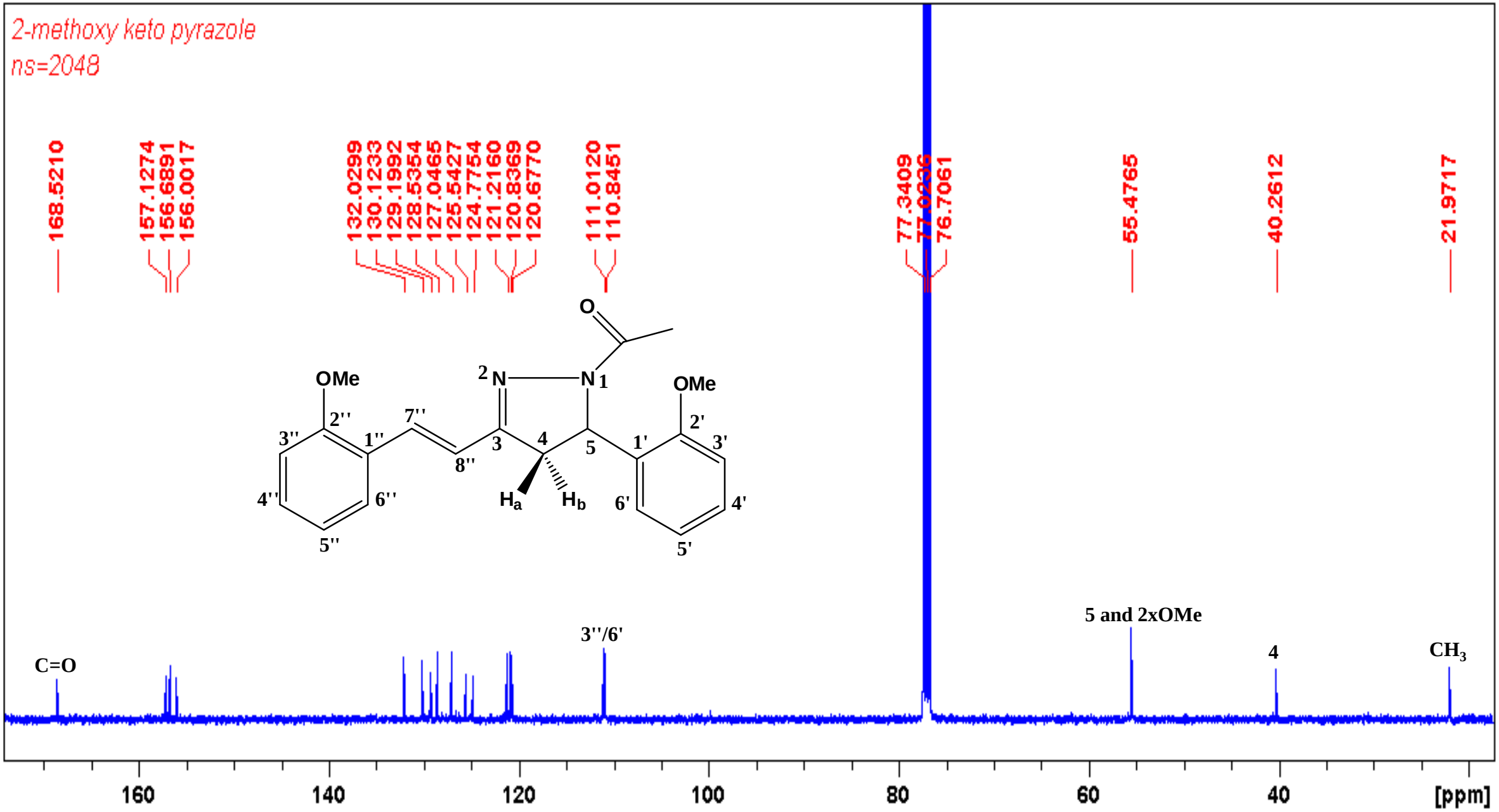
[1]H Spectrum of Compound B-2k: (E)-1-(5-(2-methoxyphenyl)-3-(2-methoxystyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



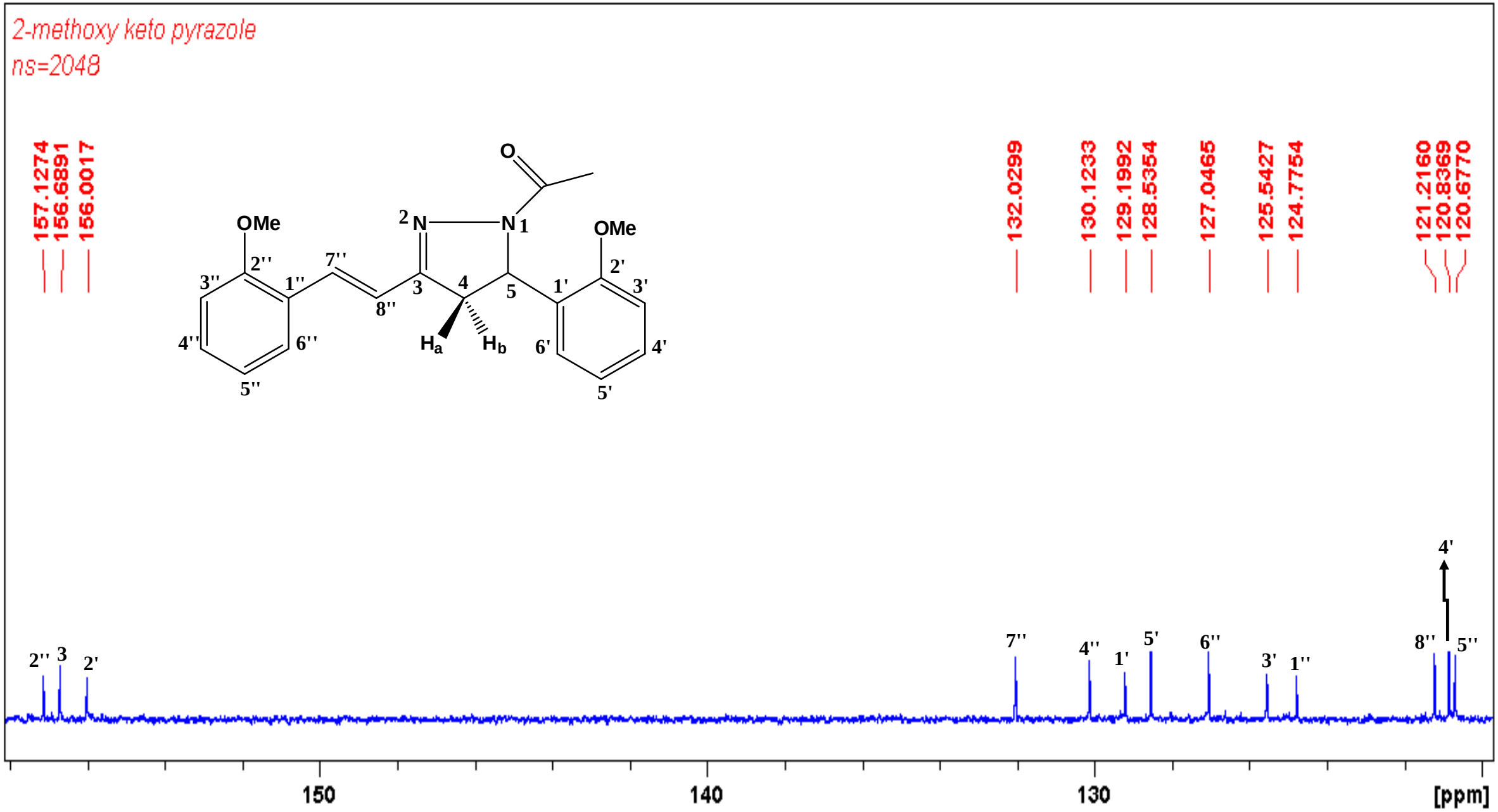
Expanded ^{1}H Spectrum of Compound B-2k: (E)-1-(5-(2-methoxyphenyl)-3-(2-methoxystyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



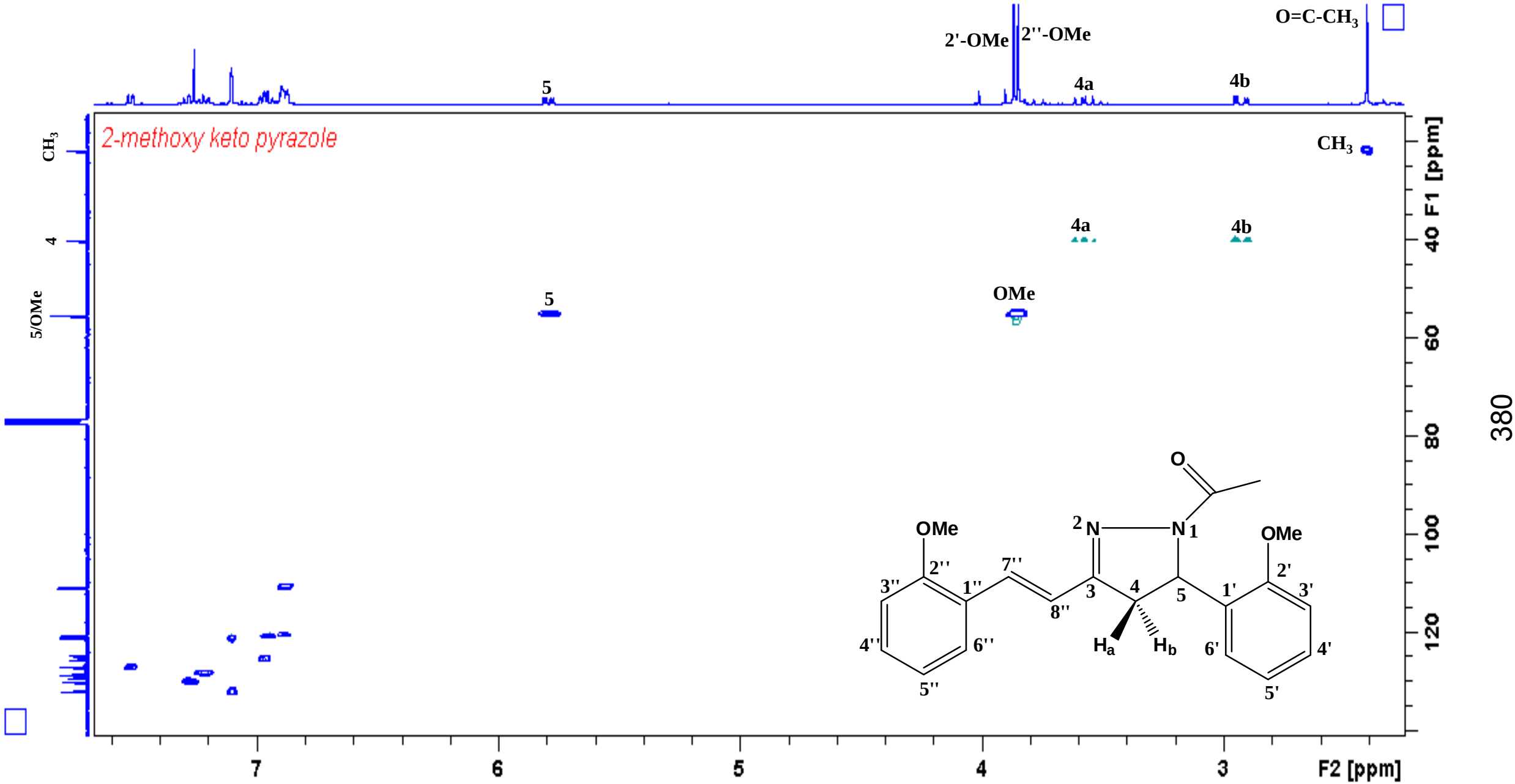
Expanded ^{1}H Spectrum of Compound B-2k: (E)-1-(5-(2-methoxyphenyl)-3-(2-methoxystyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



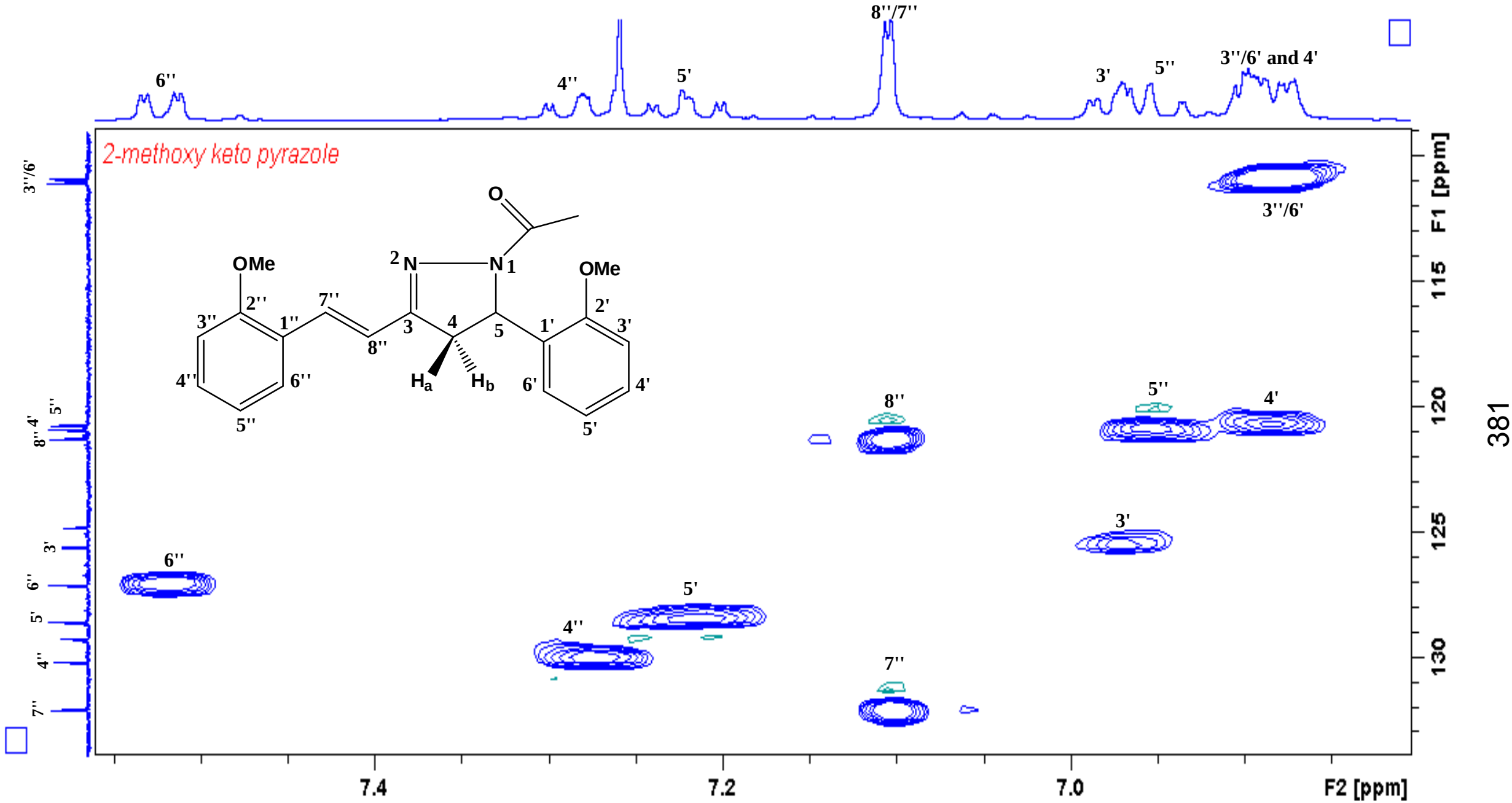
^{13}C Spectrum of Compound B-2k: (E)-1-(5-(2-methoxyphenyl)-3-(2-methoxystyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



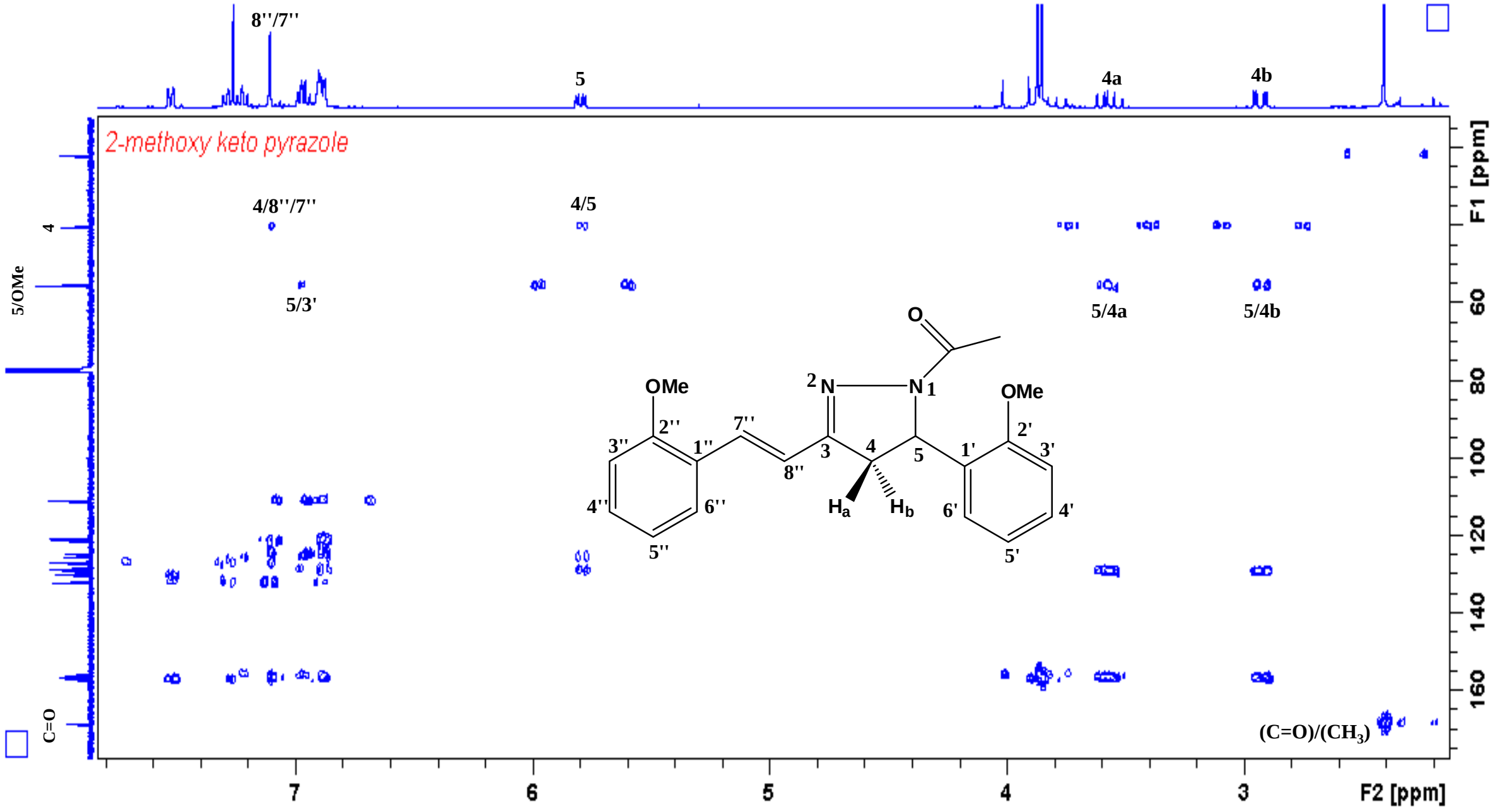
Expanded ^{13}C Spectrum of Compound B-2k: (E)-1-(5-(2-methoxyphenyl)-3-(2-methoxystyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



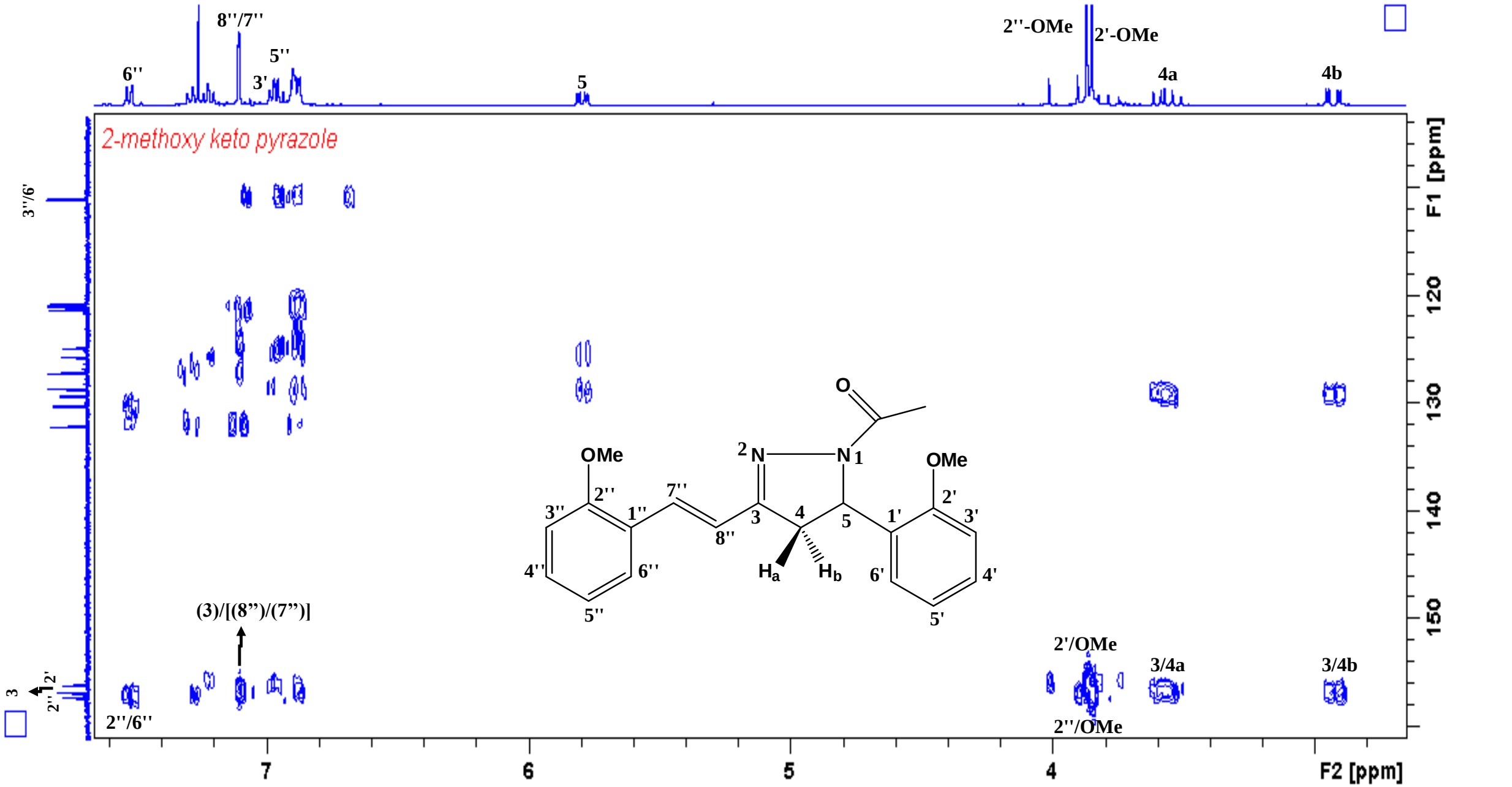
HSQC of Compound B-2k: (E)-1-(5-(2-methoxyphenyl)-3-(2-methoxystyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



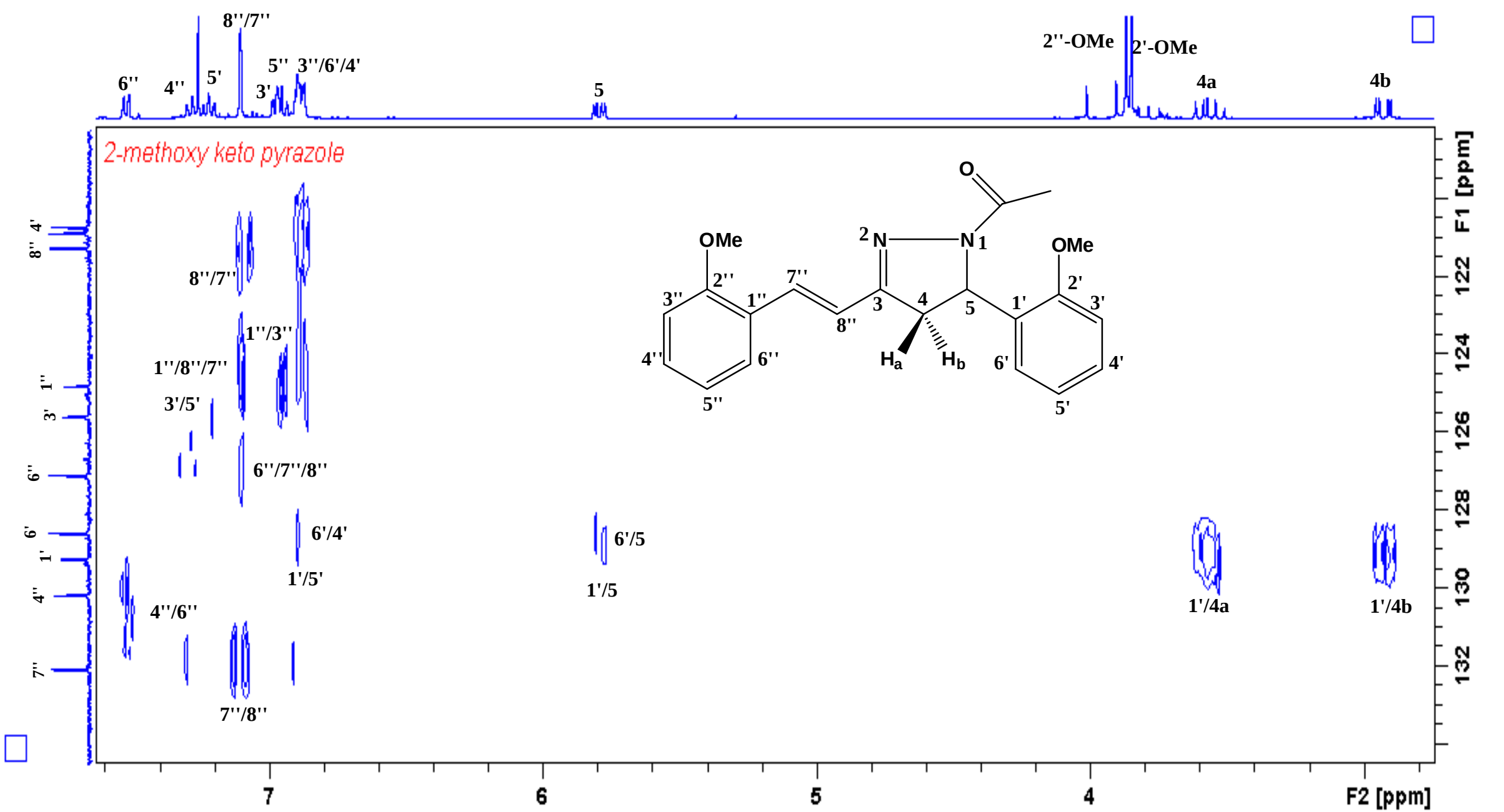
Expanded HSQC of Compound B-2k: (E)-1-(5-(2-methoxyphenyl)-3-(2-methoxystyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



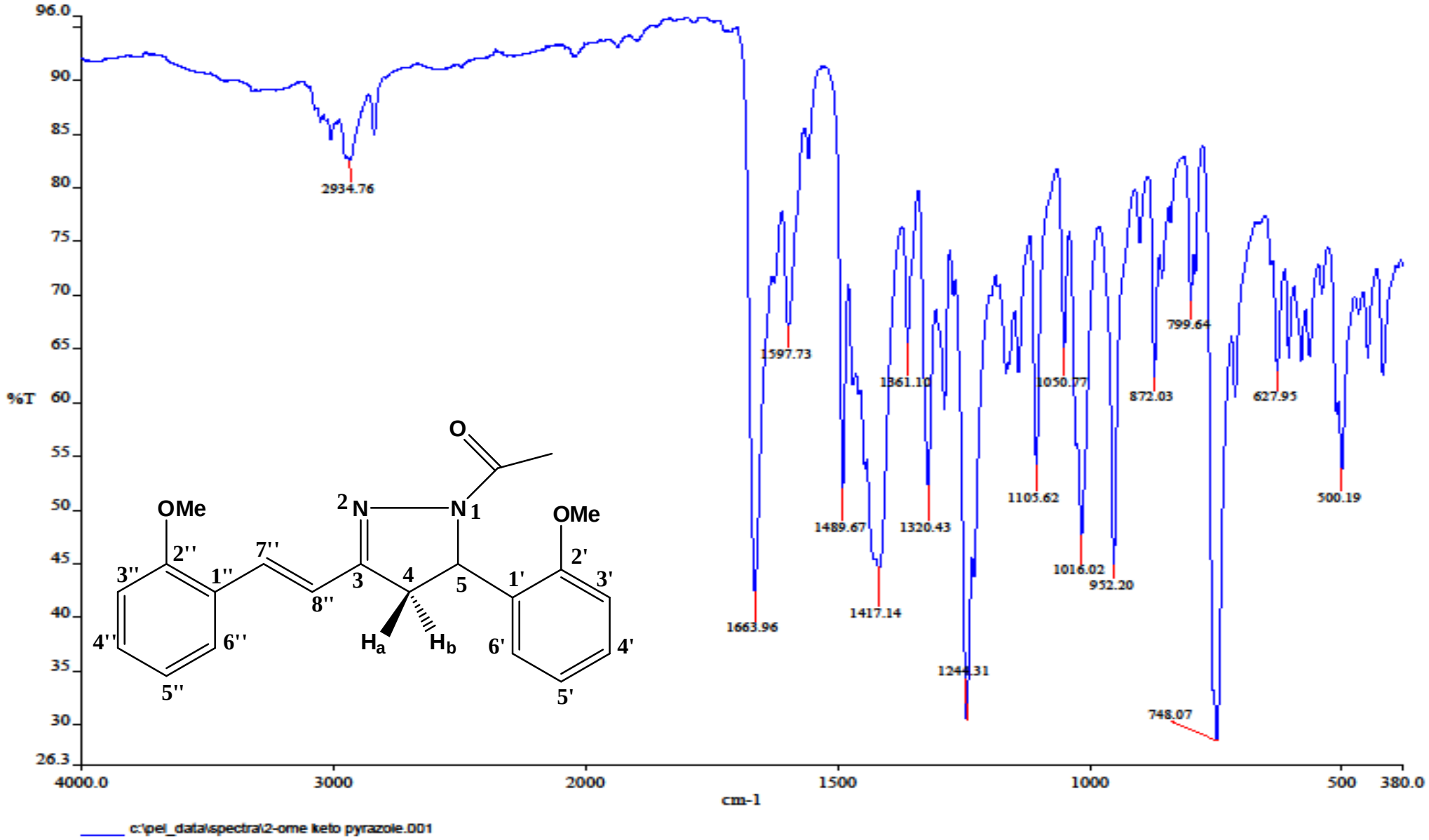
HMBC of Compound B-2k: (E)-1-(5-(2-methoxyphenyl)-3-(2-methoxystyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



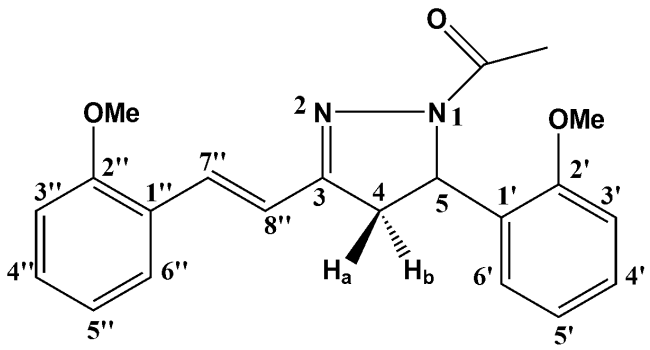
Expanded HMBC of Compound B-2k: (E)-1-(5-(2-methoxyphenyl)-3-(2-methoxystyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



Expanded HMBC of Compound B-2k: (E)-1-(5-(2-methoxyphenyl)-3-(2-methoxystyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



Infrared Spectrum of Compound B-2k: (E)-1-(5-(2-methoxyphenyl)-3-(2-methoxystyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

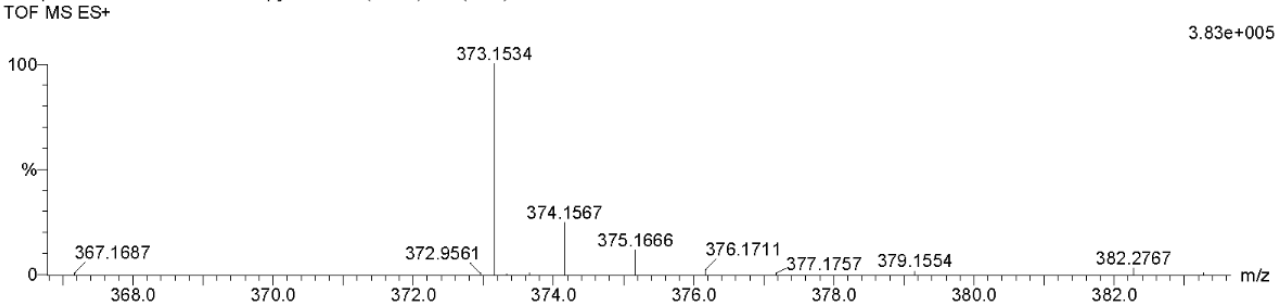


Elemental Composition Report

Single Mass Analysis

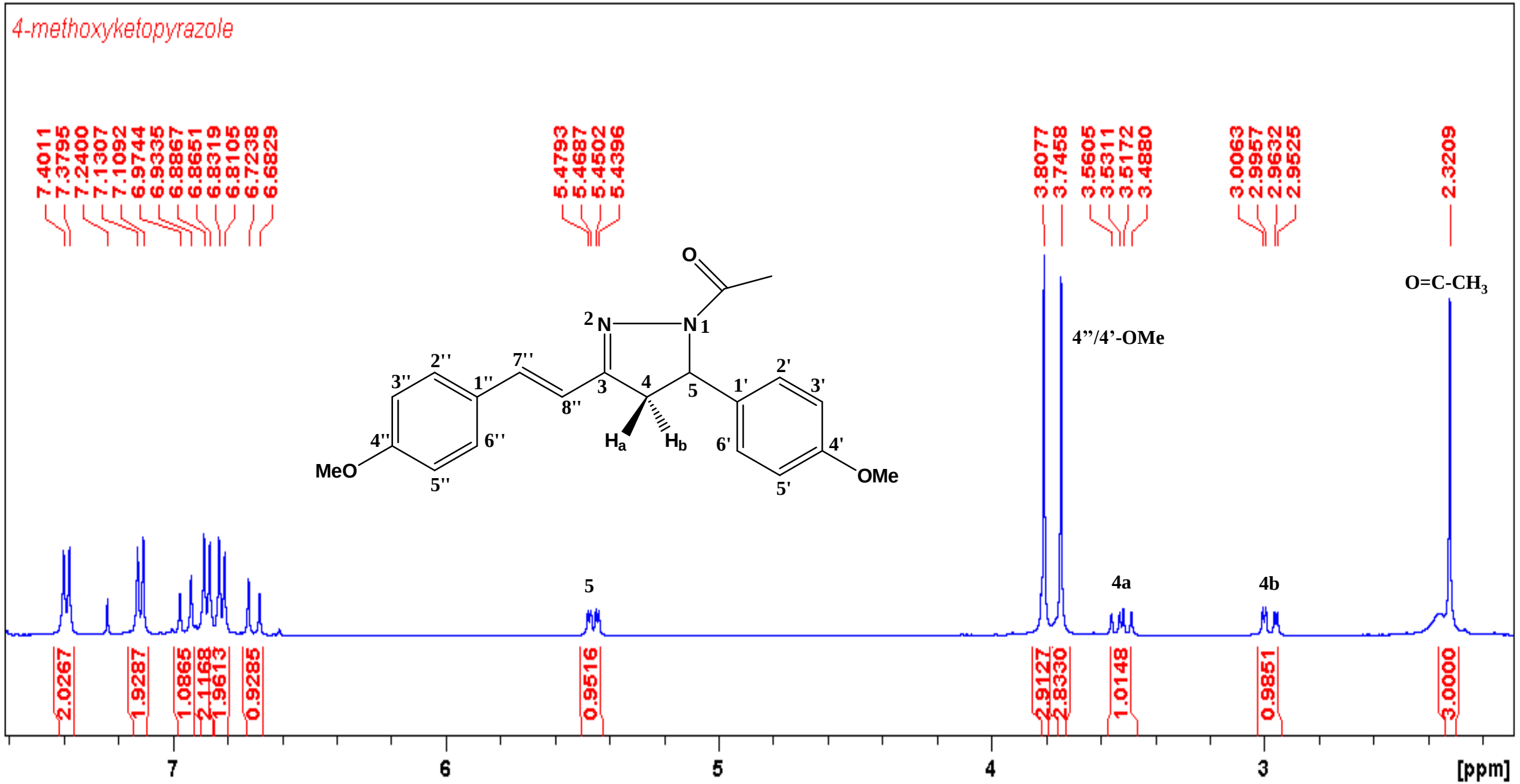
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
17 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 20-25 H: 20-25 N: 0-5 O: 0-5 Na: 1-1
Compound 11 2-OMe ketodiene pyrazole 32 (1.046) Cm (1:61)
TOF MS ES+

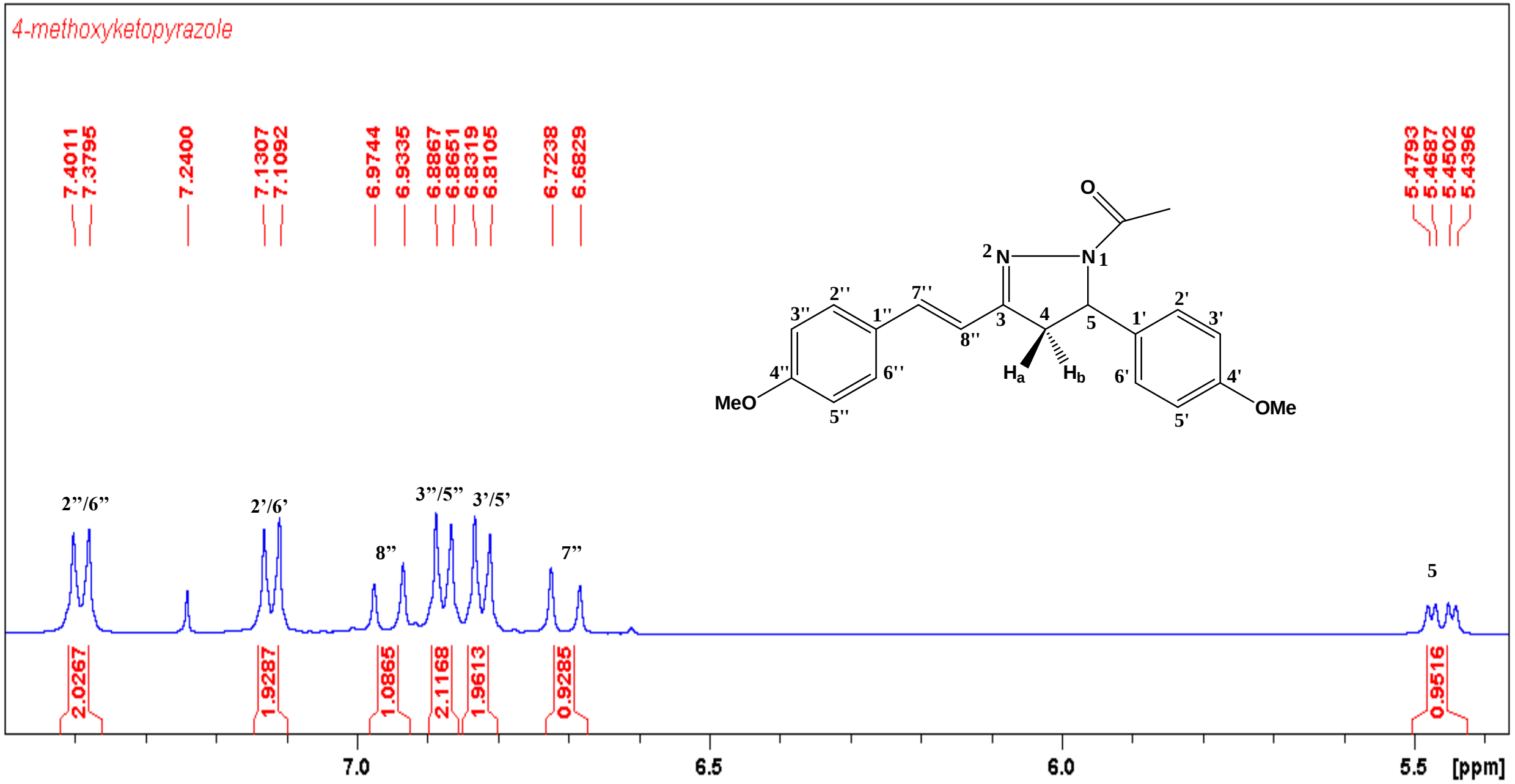


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
373.1534	373.1528	0.6	1.6	11.5	38.5	0.0	C21 H22 N2 O3 Na

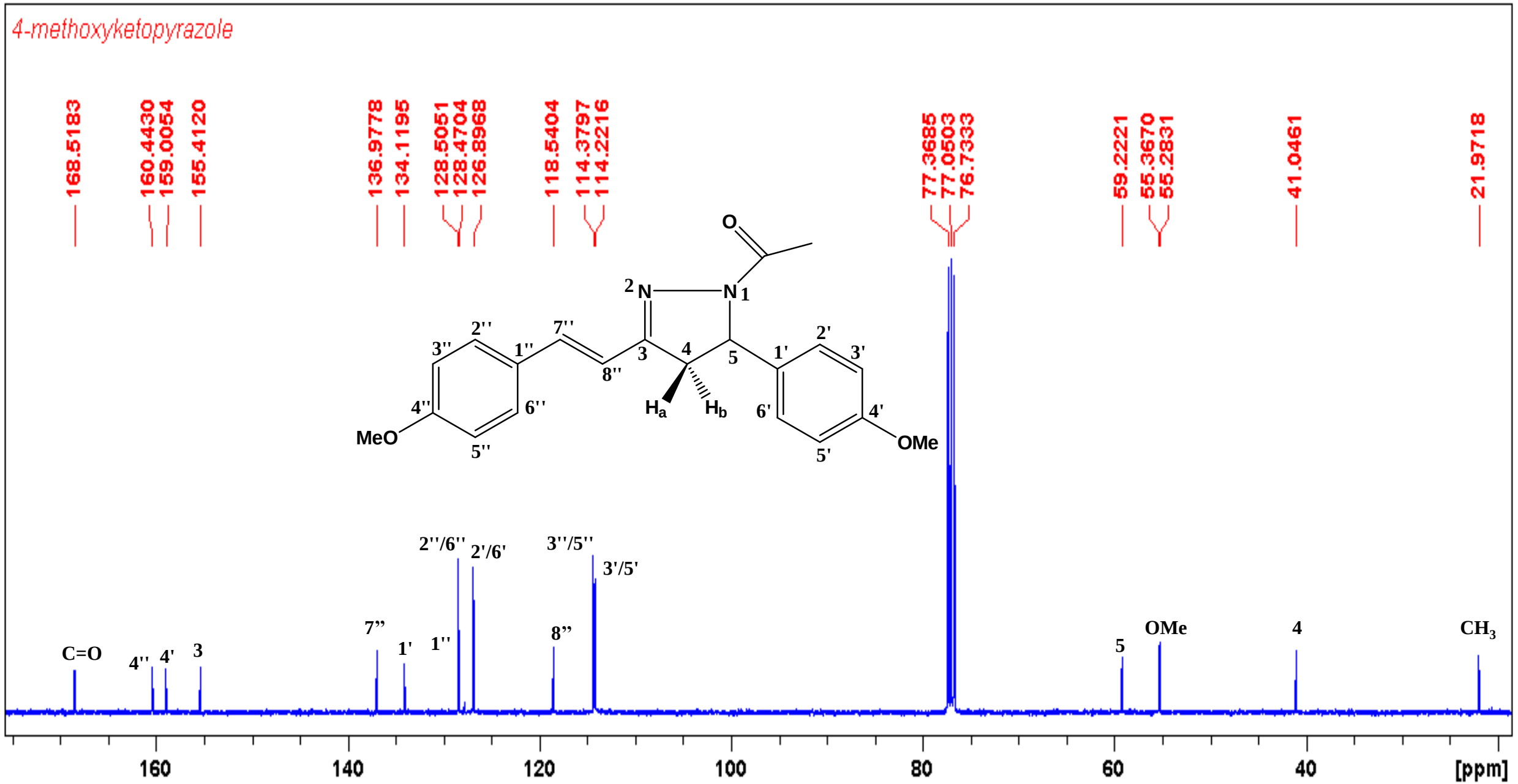
HRMS of Compound B-2k: (E)-1-(5-(2-methoxyphenyl)-3-(2-methoxystyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



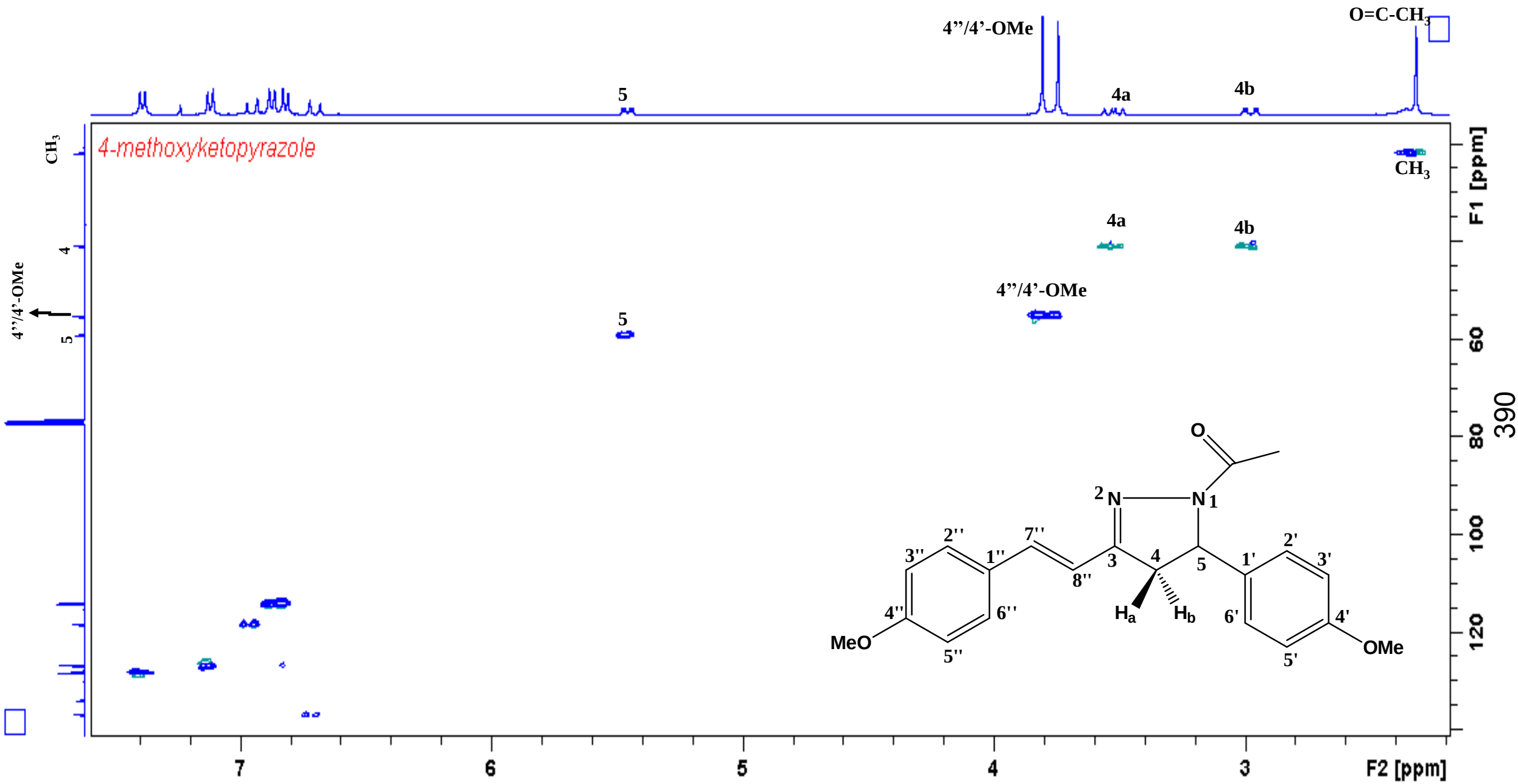
^{1}H Spectrum of Compound B-2l: (E)-1-(5-(4-methoxyphenyl)-3-(4-methoxystyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



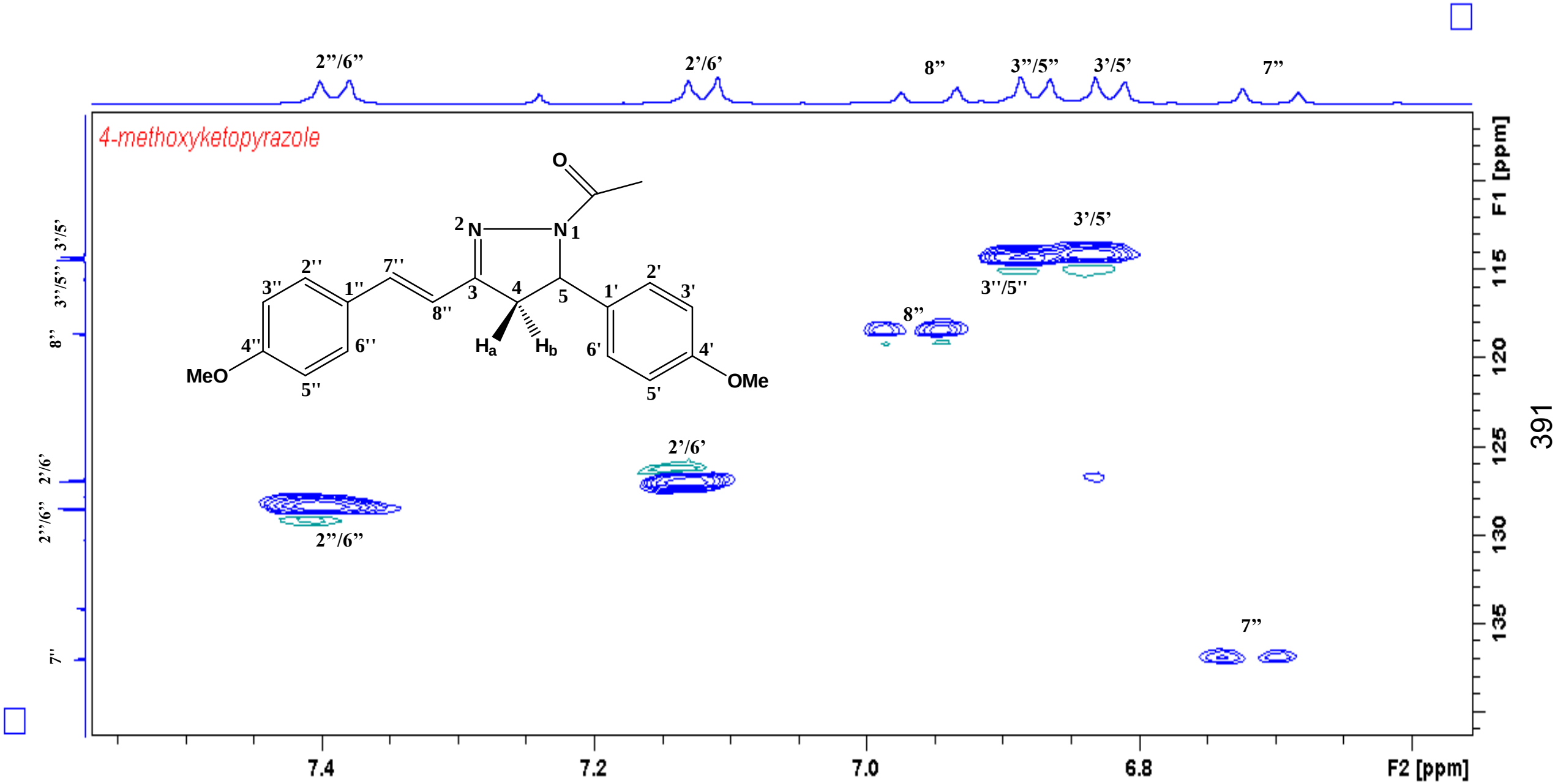
Expanded ^{1}H Spectrum of Compound B-2l: (E)-1-(5-(4-methoxyphenyl)-3-(4-methoxystyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



^{13}C Spectrum of Compound B-2l: (E)-1-(5-(4-methoxyphenyl)-3-(4-methoxystyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



HSQC of Compound B-2l: (E)-1-(5-(4-methoxyphenyl)-3-(4-methoxystyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



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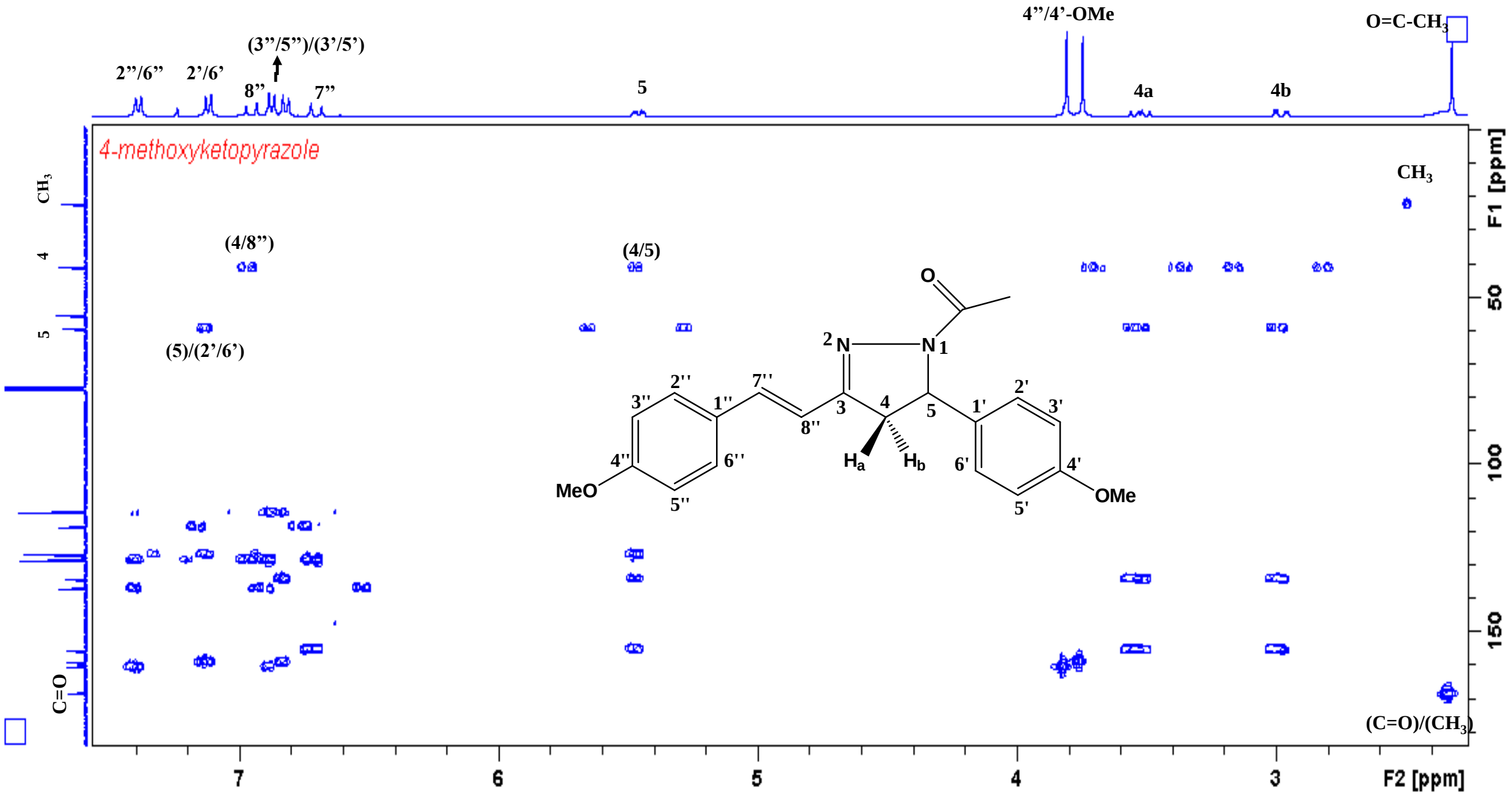
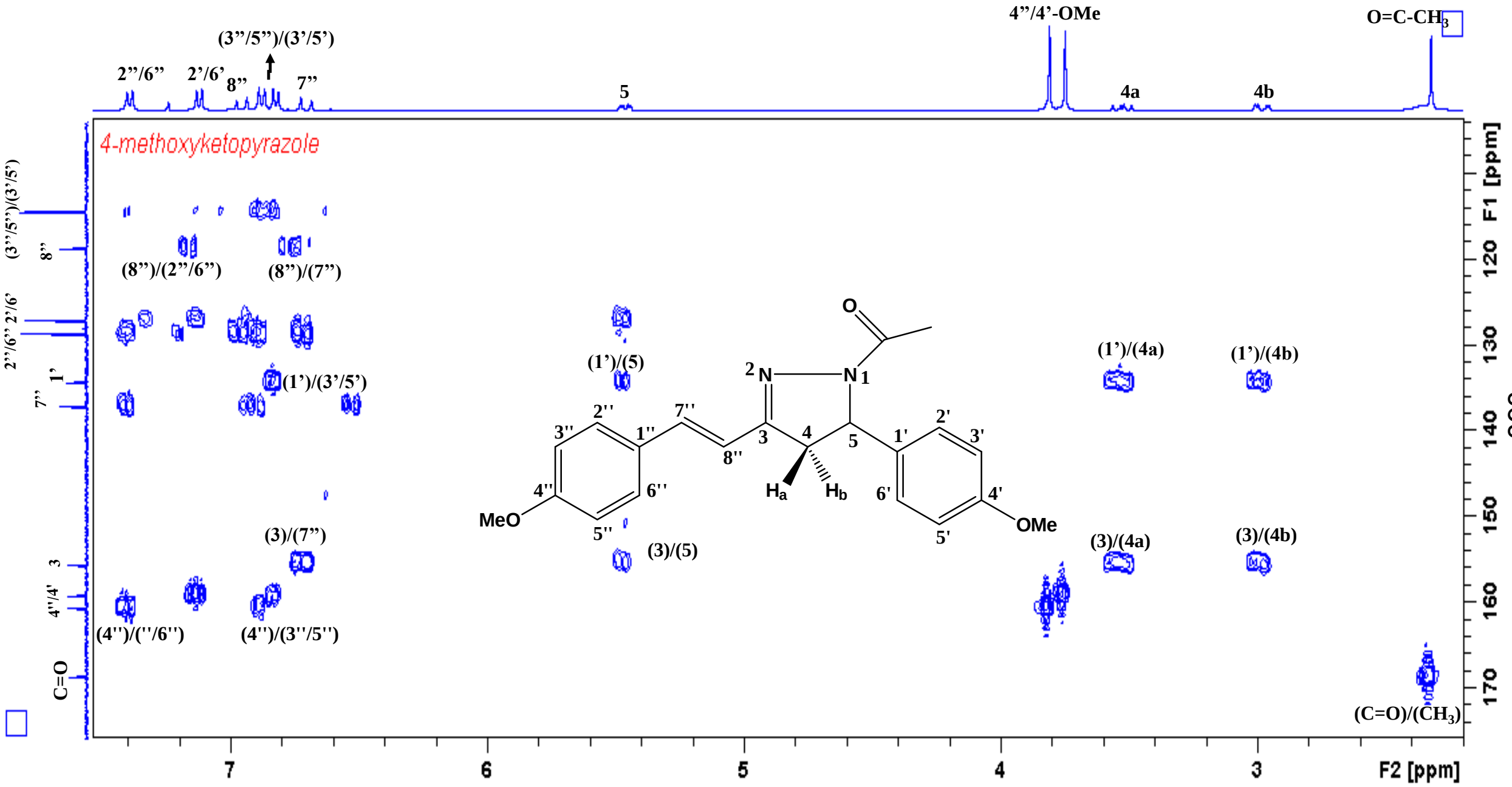
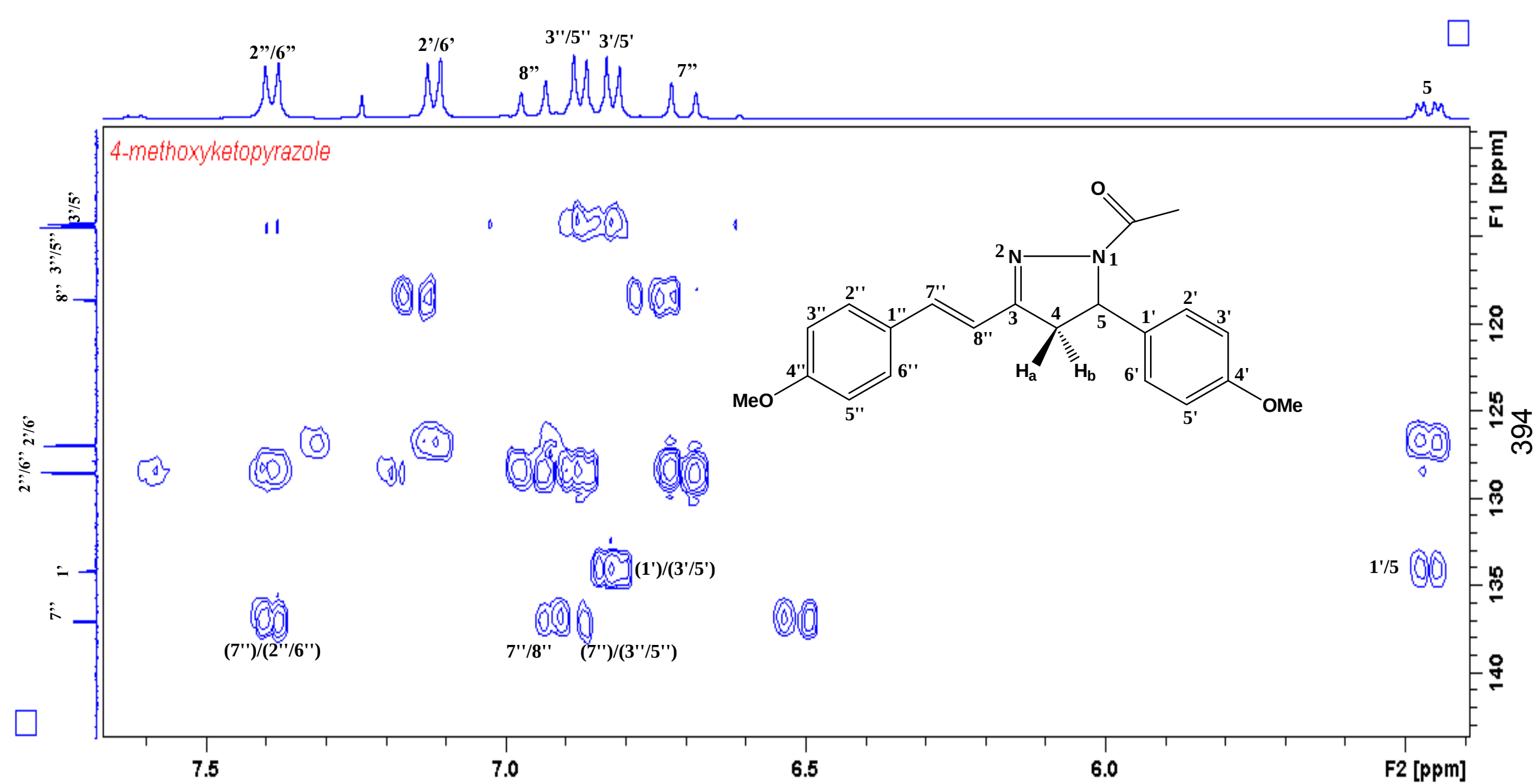
Expanded HSQC of Compound B-2l: (E)-1-(5-(4-methoxyphenyl)-3-(4-methoxystyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



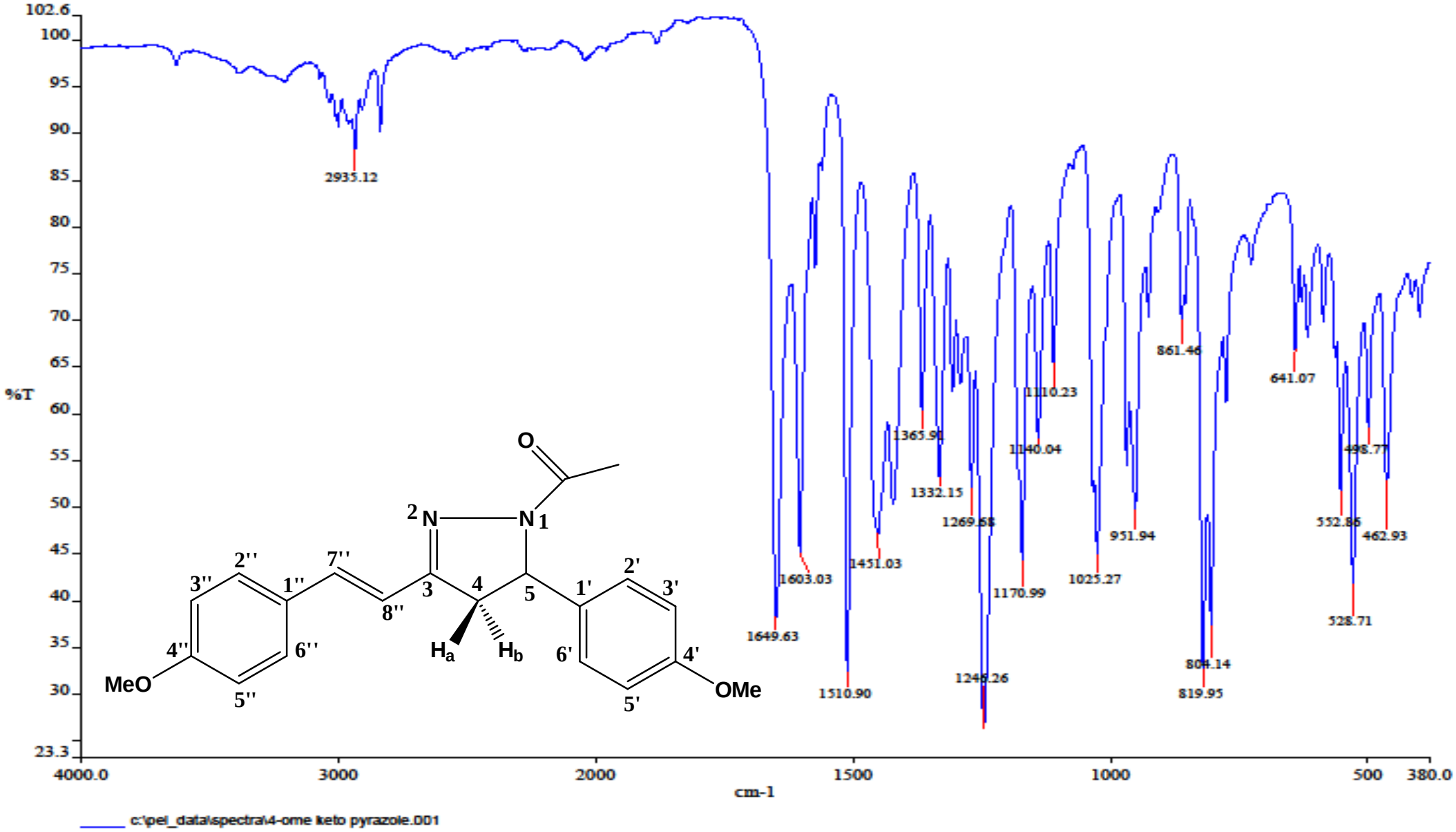
HMBC of Compound B-2l: (E)-1-(5-(4-methoxyphenyl)-3-(4-methoxystyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



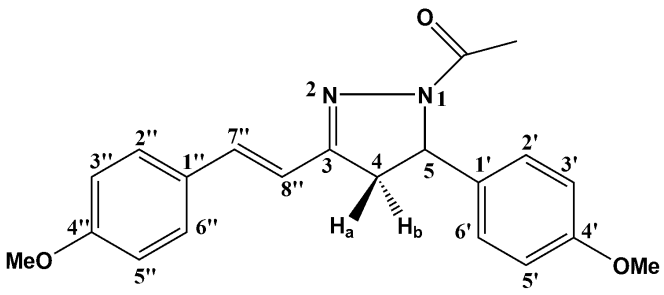
Expanded HMBC of Compound B-2l: (E)-1-(5-(4-methoxyphenyl)-3-(4-methoxystyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



Expanded HMBC of Compound B-2l: (E)-1-(5-(4-methoxyphenyl)-3-(4-methoxystyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



Infrared Spectrum of Compound B-2l: (E)-1-(5-(4-methoxyphenyl)-3-(4-methoxystyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



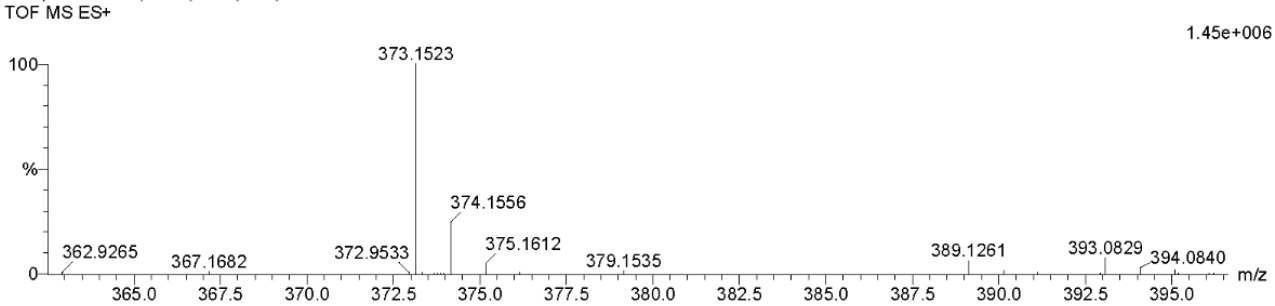
Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

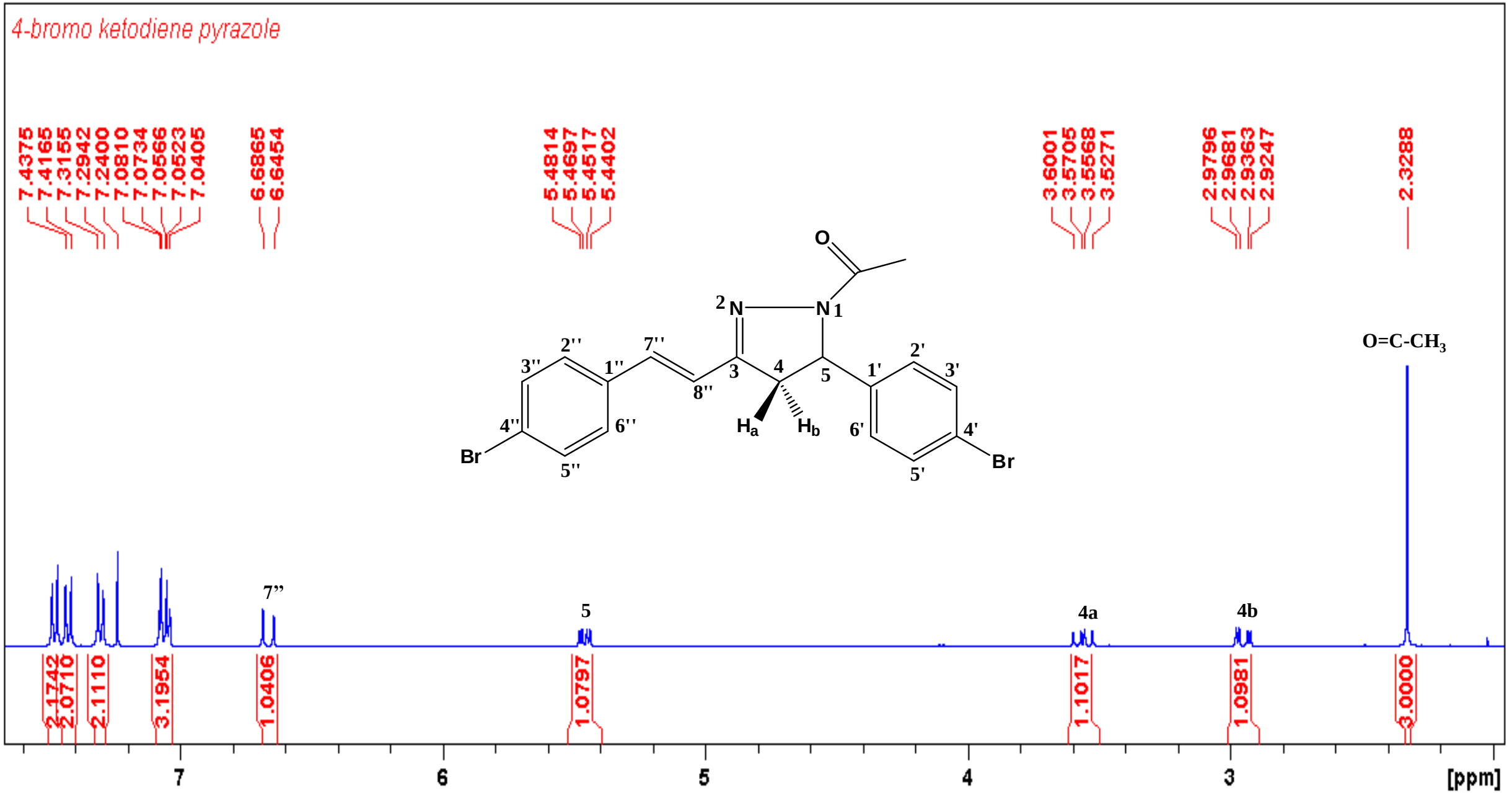
Monoisotopic Mass, Even Electron Ions
17 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 20-25 H: 20-25 N: 0-5 O: 0-5 Na: 1-1

Compound 12 9 (0.270) Cm (1:61)
TOF MS ES+



Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
373.1523	373.1528	-0.5	-1.3	11.5	97.1	0.0	C21 H22 N2 O3 Na

HRMS of Compound B-2l: (E)-1-(5-(4-methoxyphenyl)-3-(4-methoxystyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



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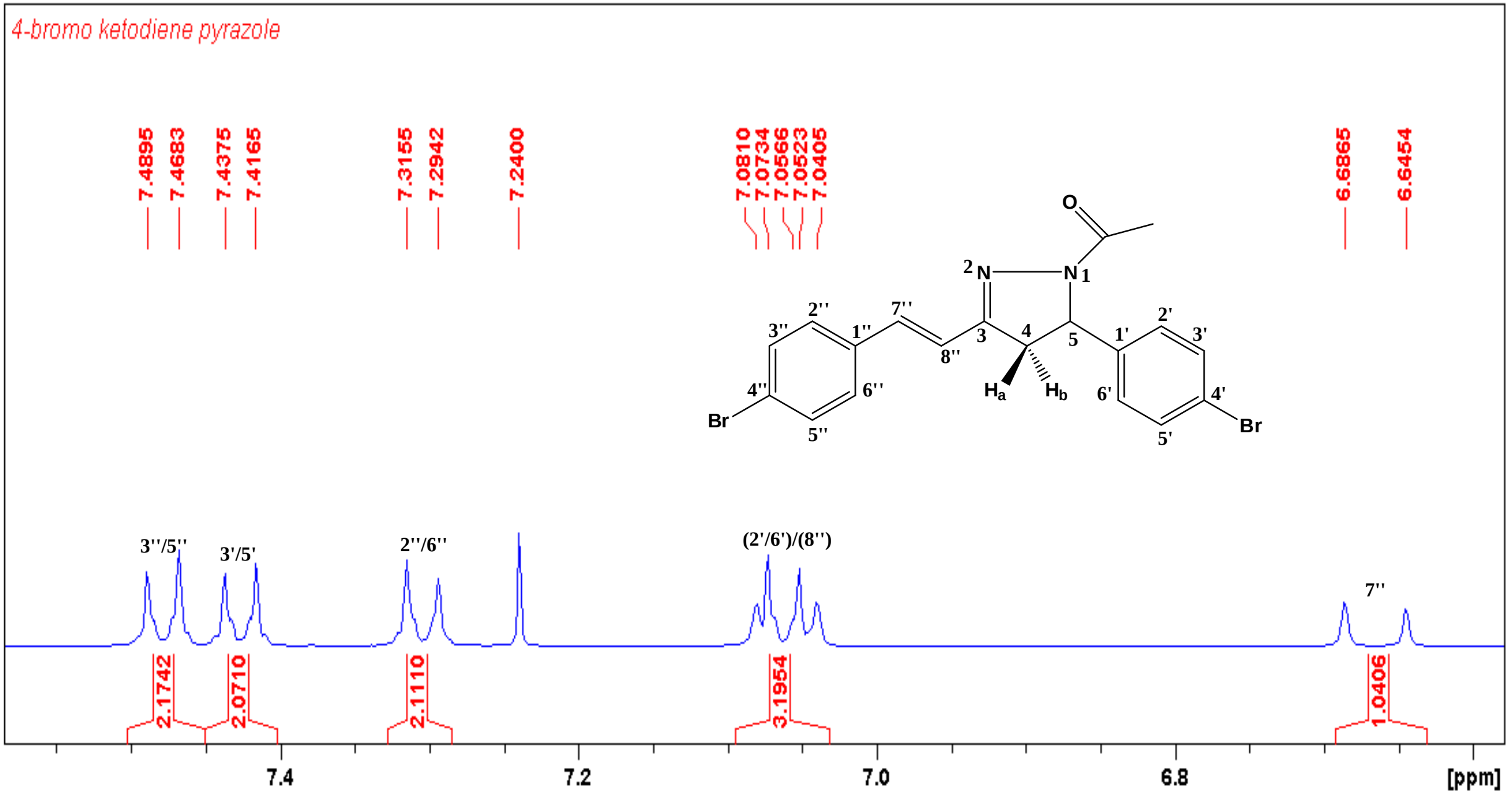
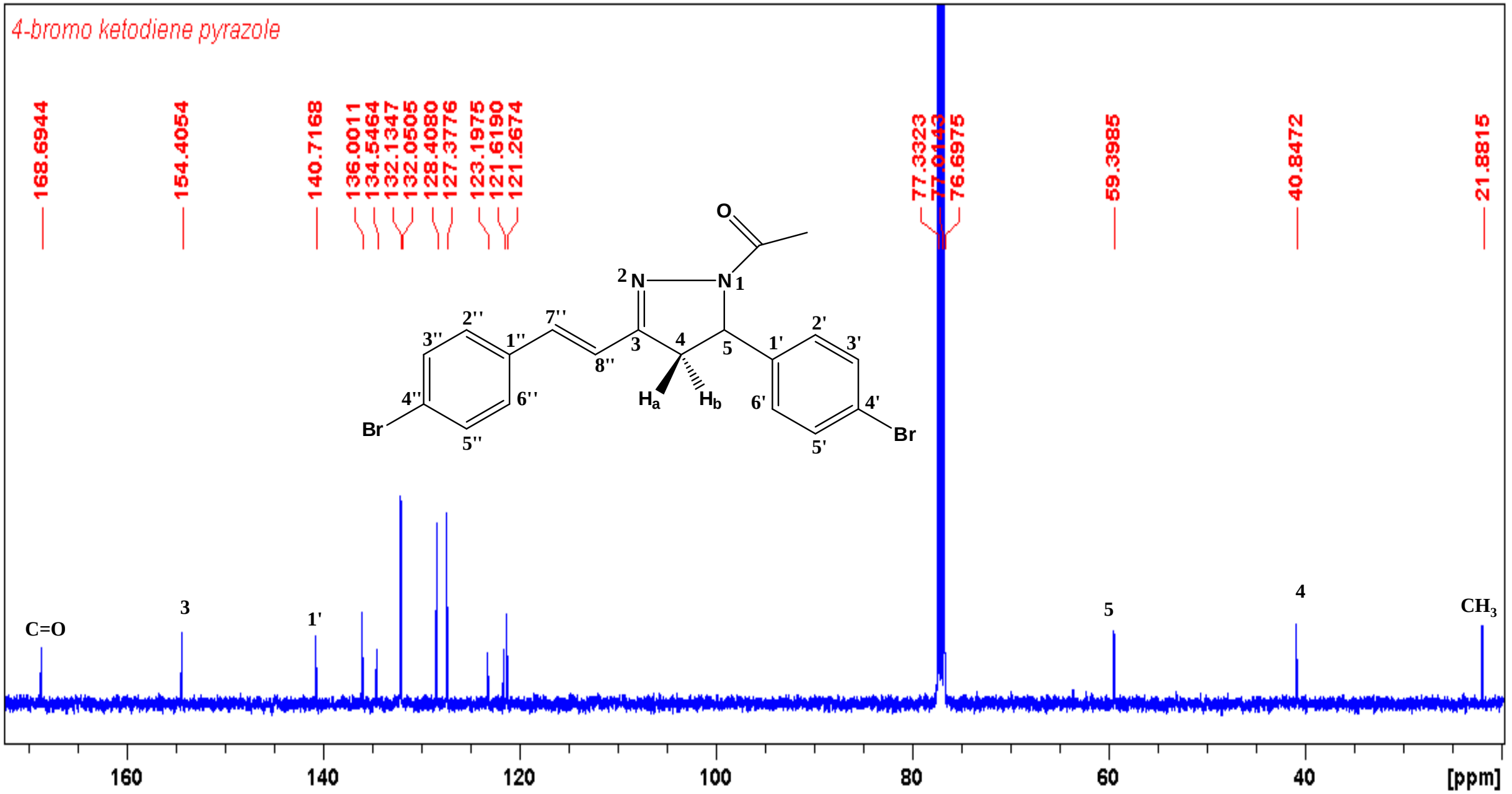
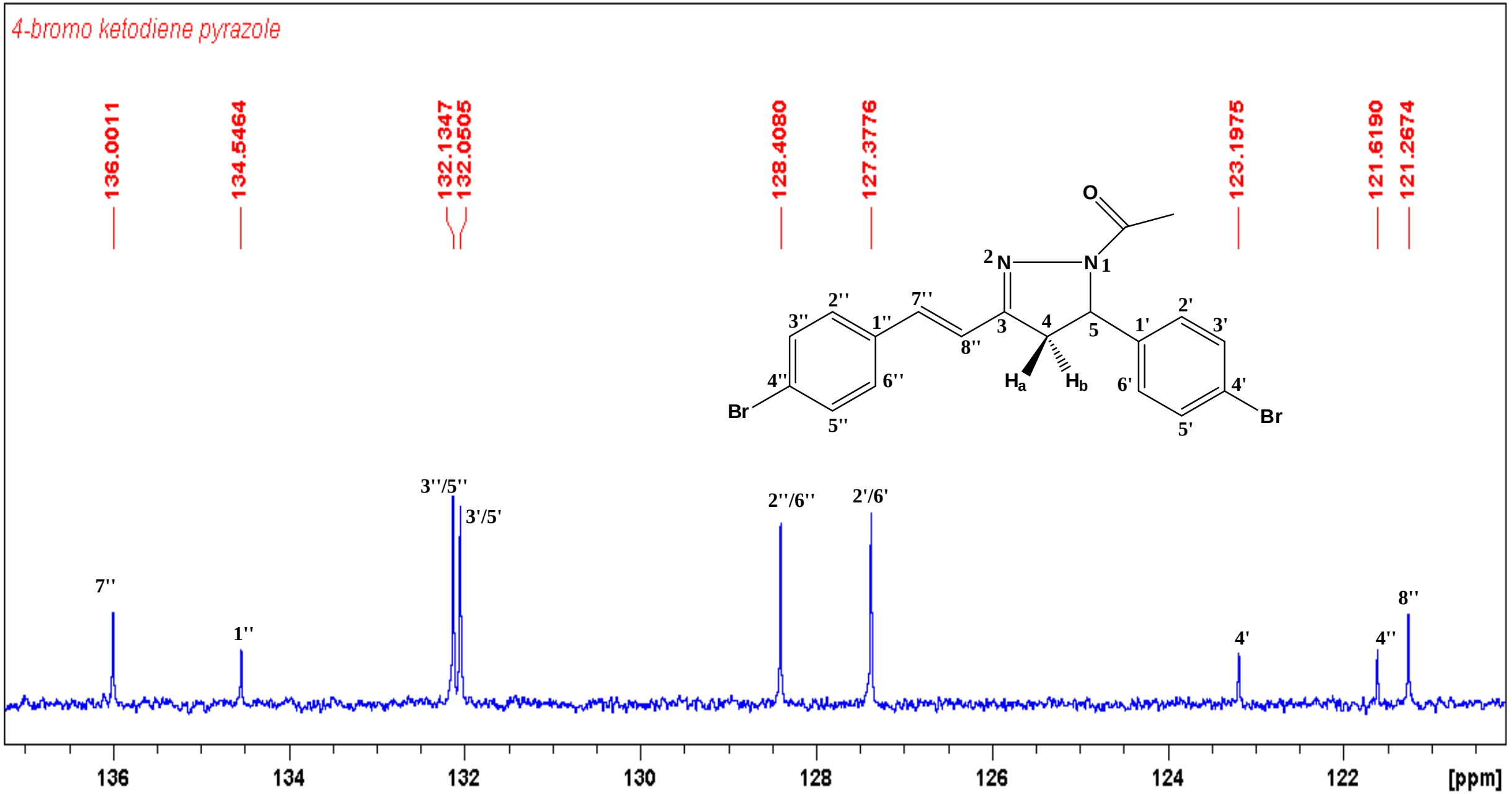
^{1}H Spectrum of Compound B-2n: (E)-1-(5-(4-bromophenyl)-3-(4-bromostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



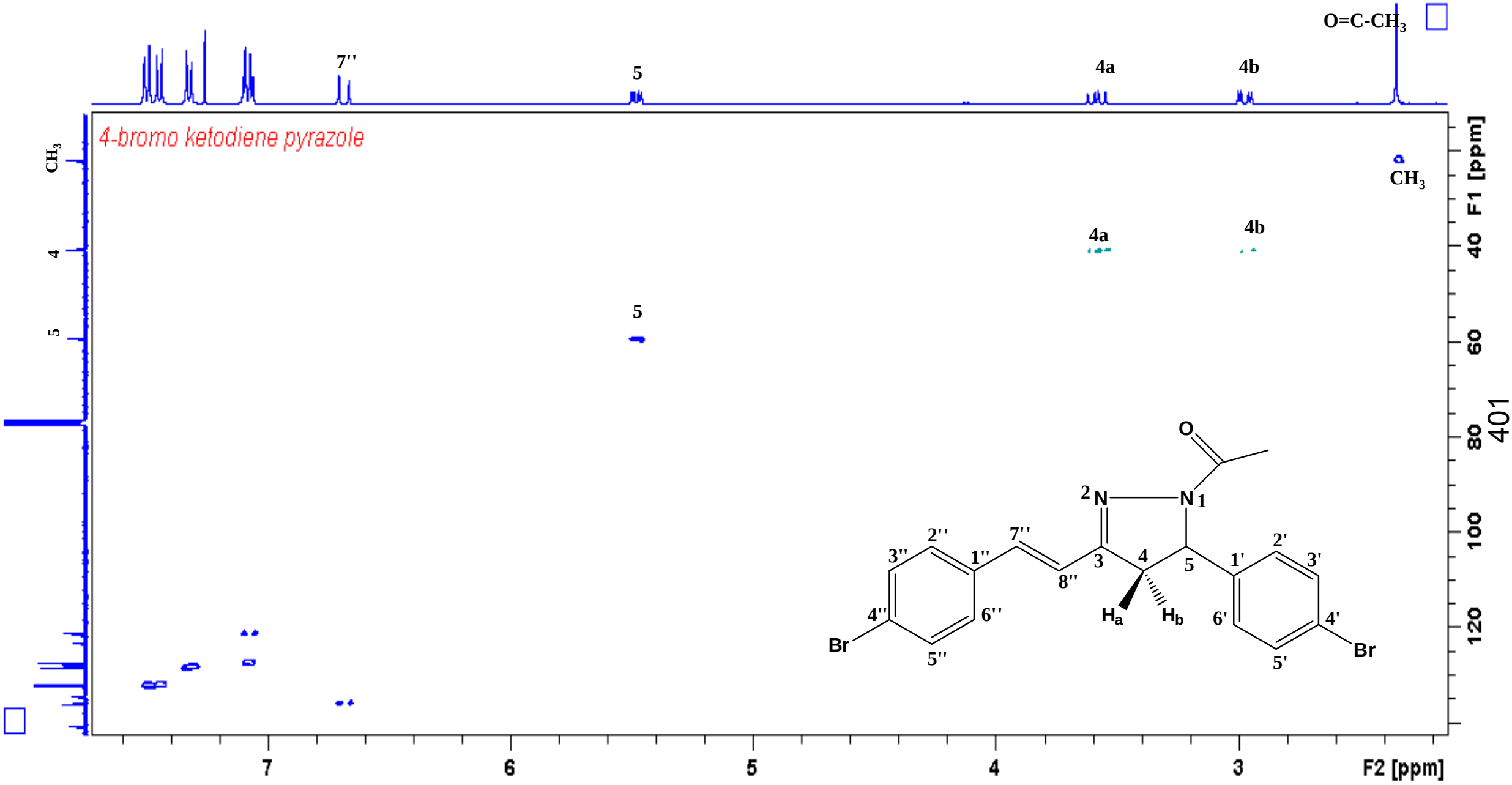
Expanded ^{1}H Spectrum of Compound B-2n: (E)-1-(5-(4-bromophenyl)-3-(4-bromostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



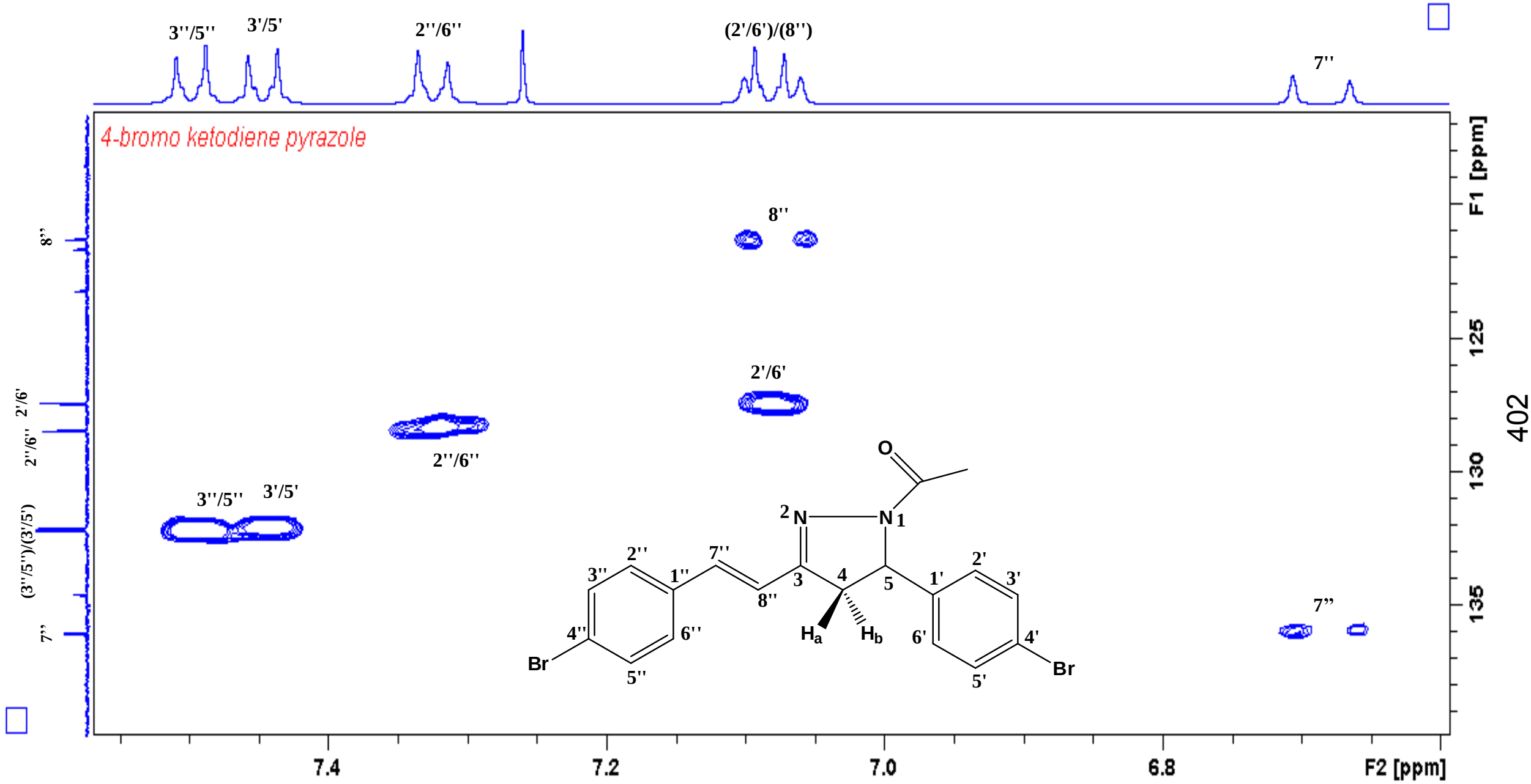
^{13}C Spectrum of Compound B-2n: (E)-1-(5-(4-bromophenyl)-3-(4-bromostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



Expanded ^{13}C Spectrum of Compound B-2n: (E)-1-(5-(4-bromophenyl)-3-(4-bromostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

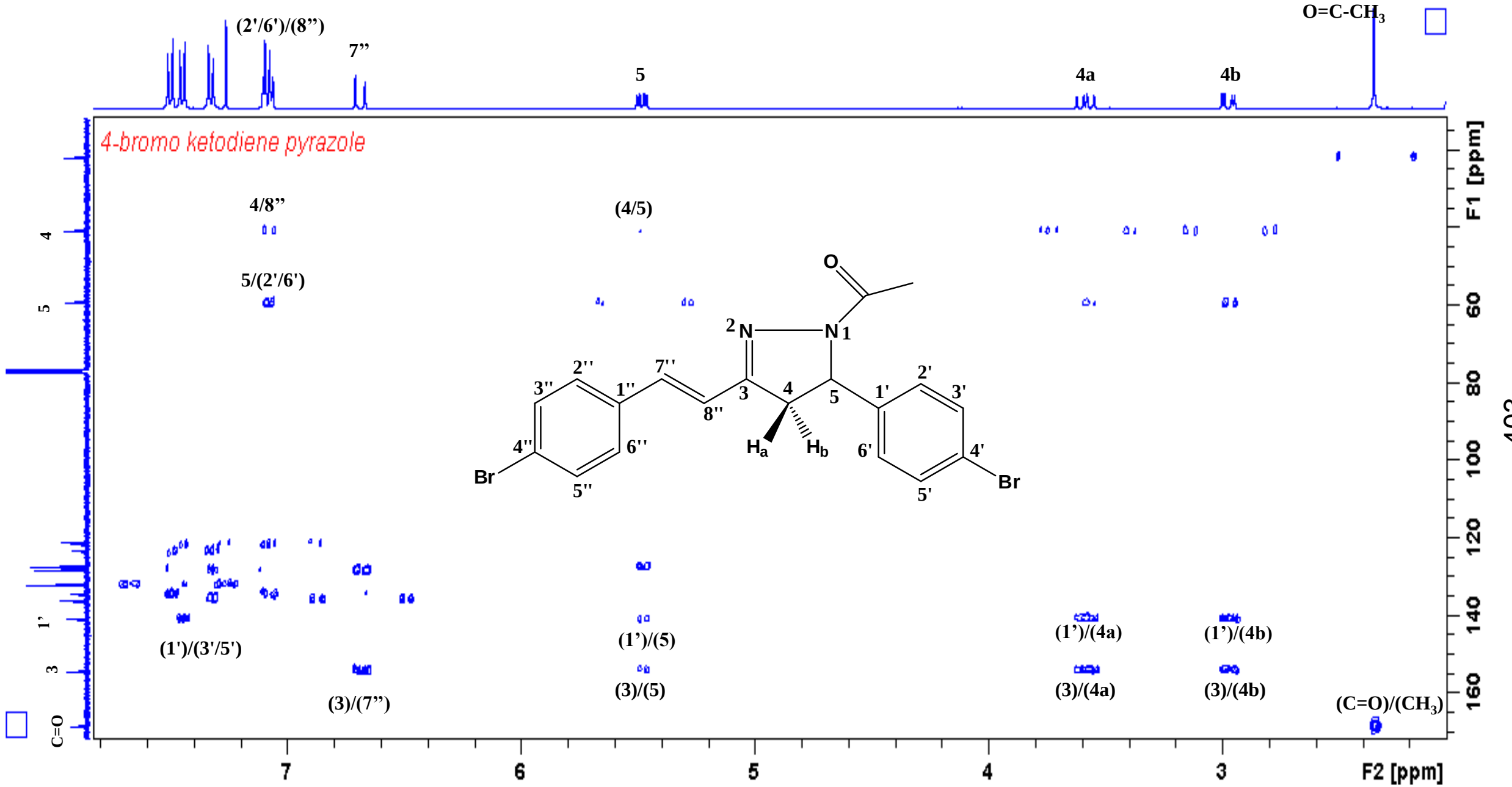


HSQC of Compound B-2n: (E)-1-(5-(4-bromophenyl)-3-(4-bromostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

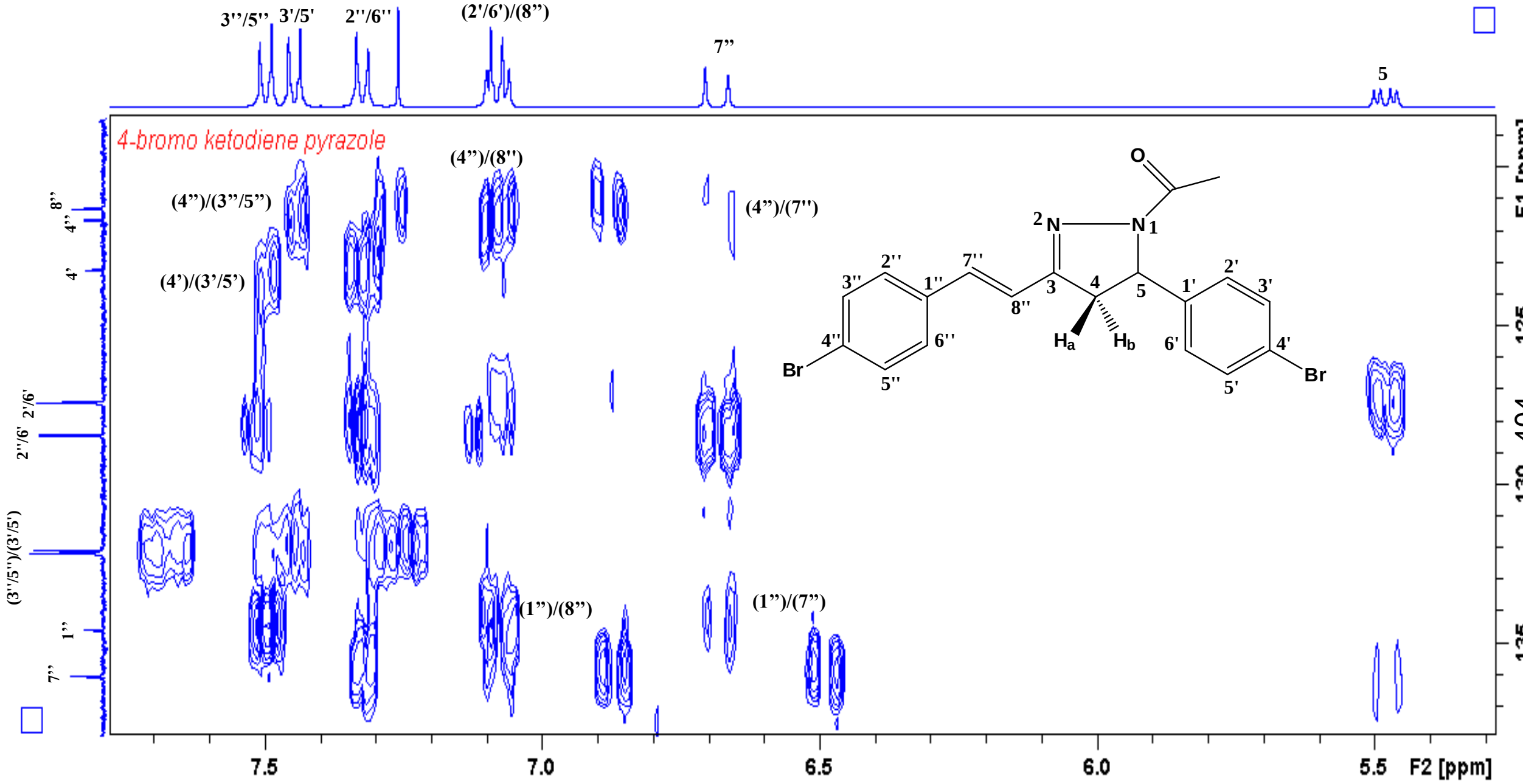


402

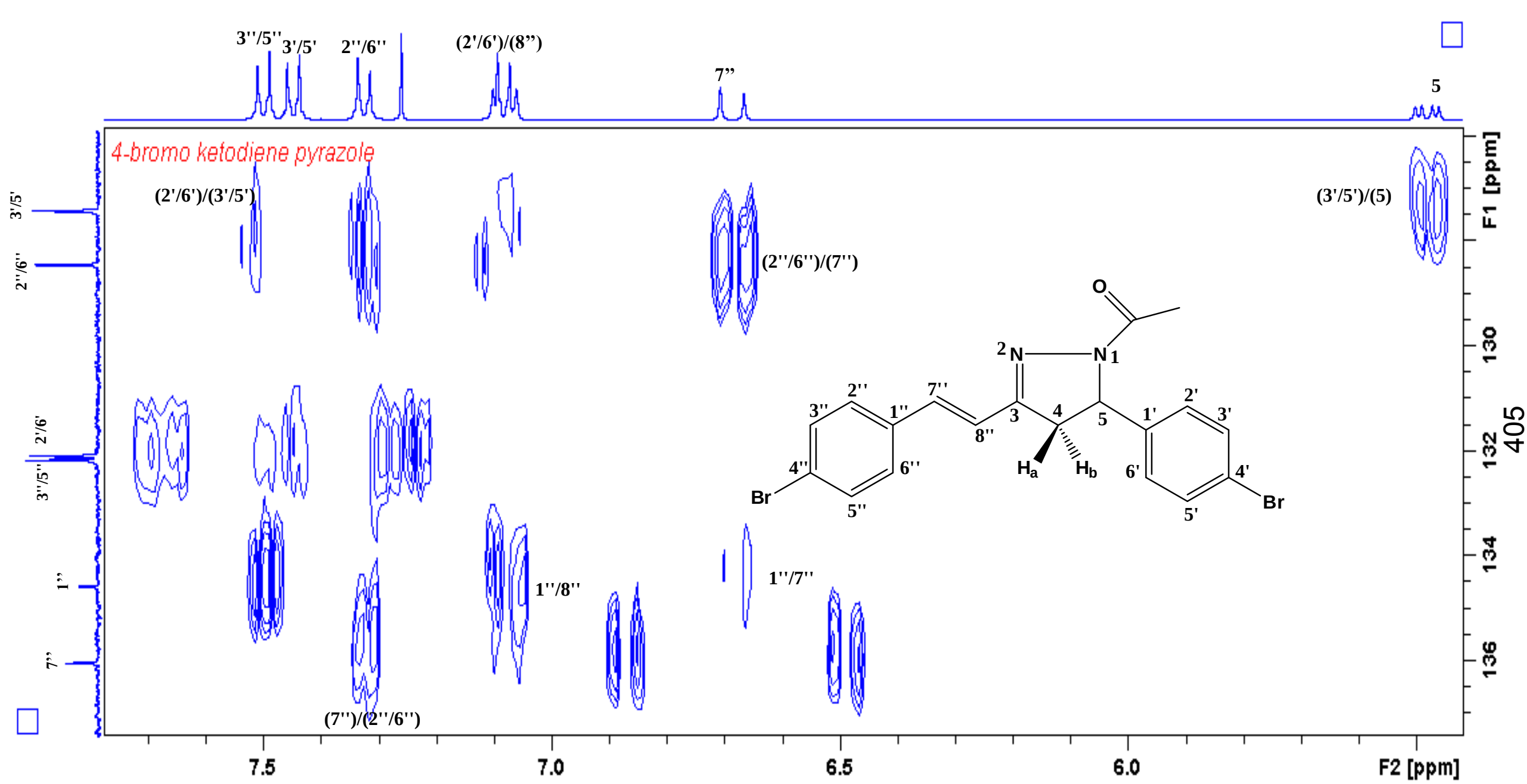
Expanded HSQC of Compound B-2n: (E)-1-(5-(4-bromophenyl)-3-(4-bromostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



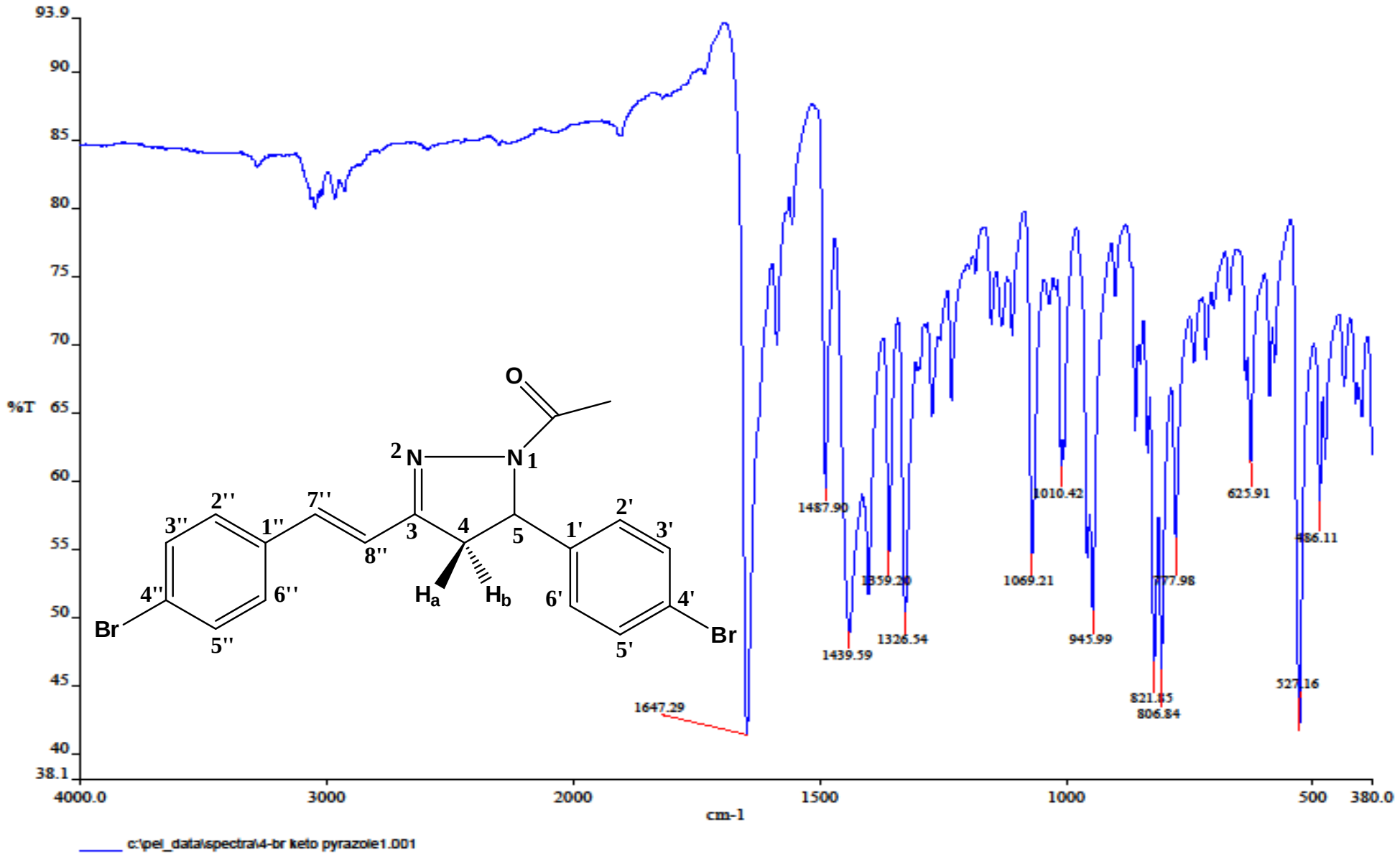
HMBC of Compound B-2n: (E)-1-(5-(4-bromophenyl)-3-(4-bromostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



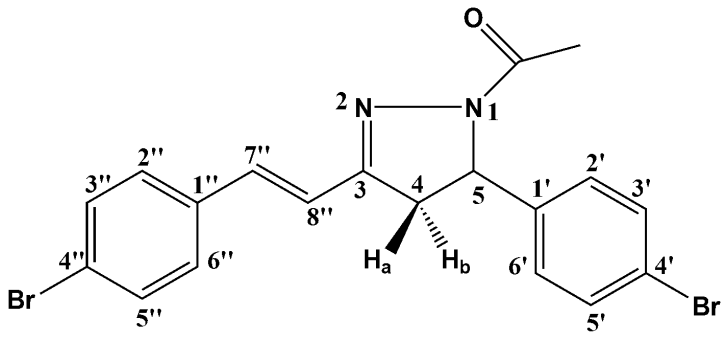
Expanded HMBC of Compound B-2n: (E)-1-(5-(4-bromophenyl)-3-(4-bromostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



Expanded HMBC of Compound B-2n: (E)-1-(5-(4-bromophenyl)-3-(4-bromostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



Infrared Spectrum of Compound B-2n: (E)-1-(5-(4-bromophenyl)-3-(4-bromostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



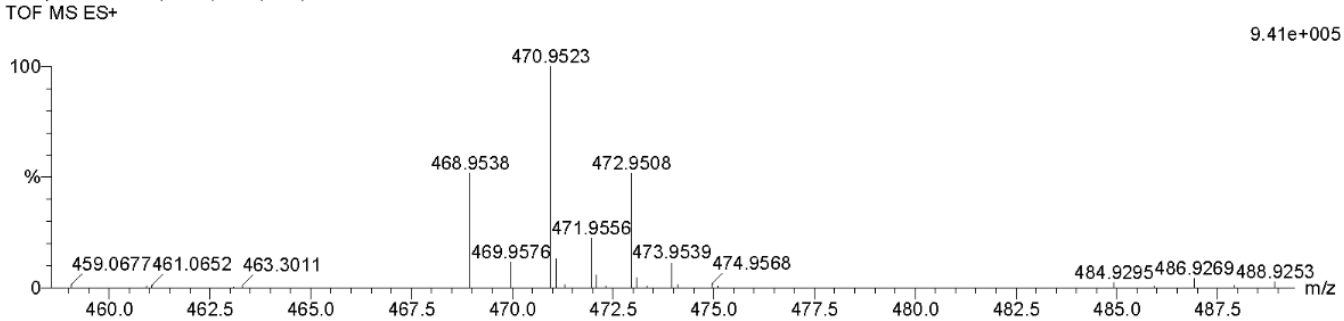
Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

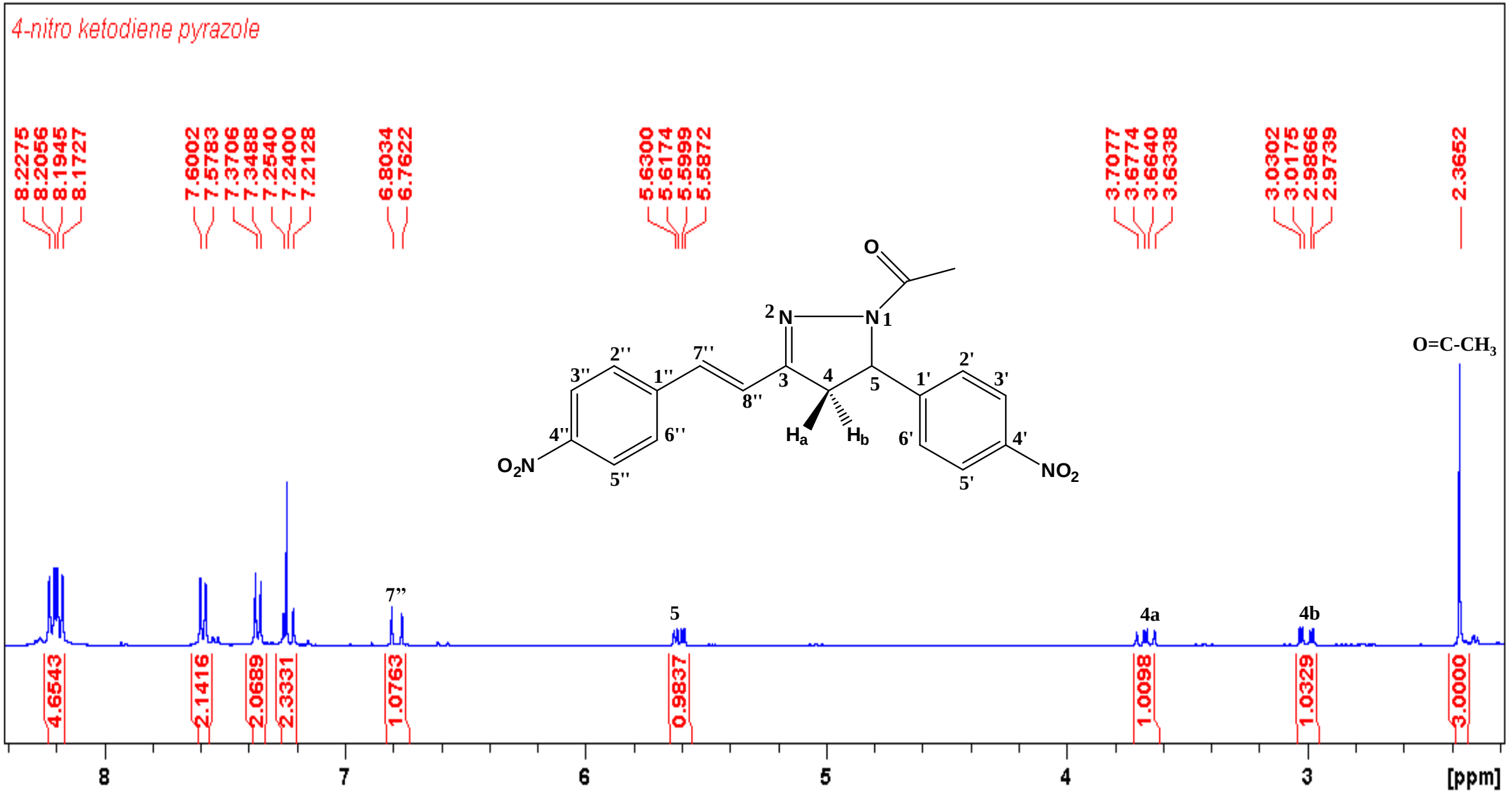
Monoisotopic Mass, Even Electron Ions
79 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 15-25 H: 15-25 N: 0-5 O: 0-5 Na: 1-1 Br: 0-2

Compound 13 12 (0.405) Cm (1:60)
TOF MS ES+

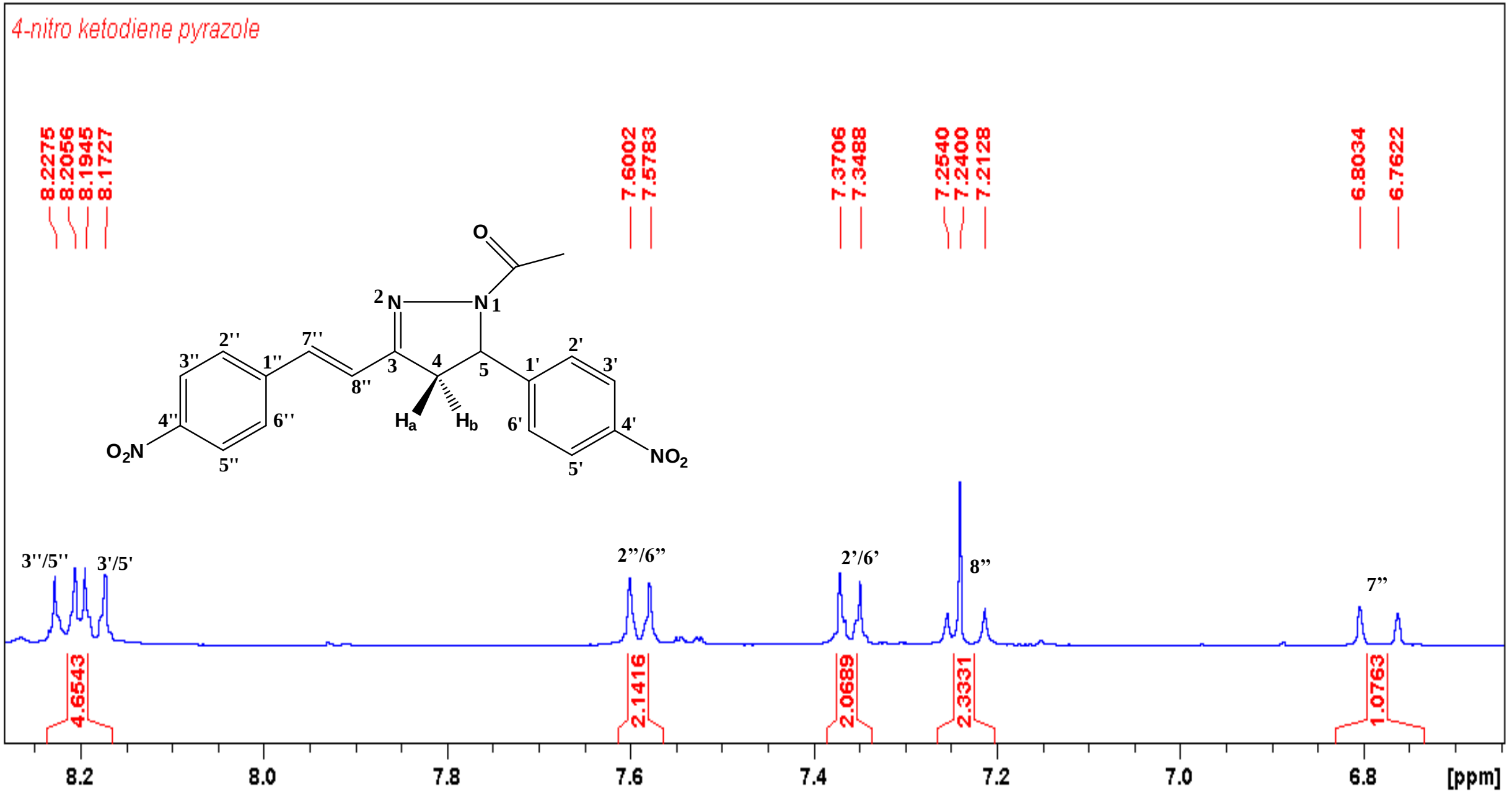


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
468.9538	468.9527	1.1	2.3	11.5	10.4	0.0	C19 H16 N2 O Na Br2

HRMS of Compound B-2n: (E)-1-(5-(4-bromophenyl)-3-(4-bromostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

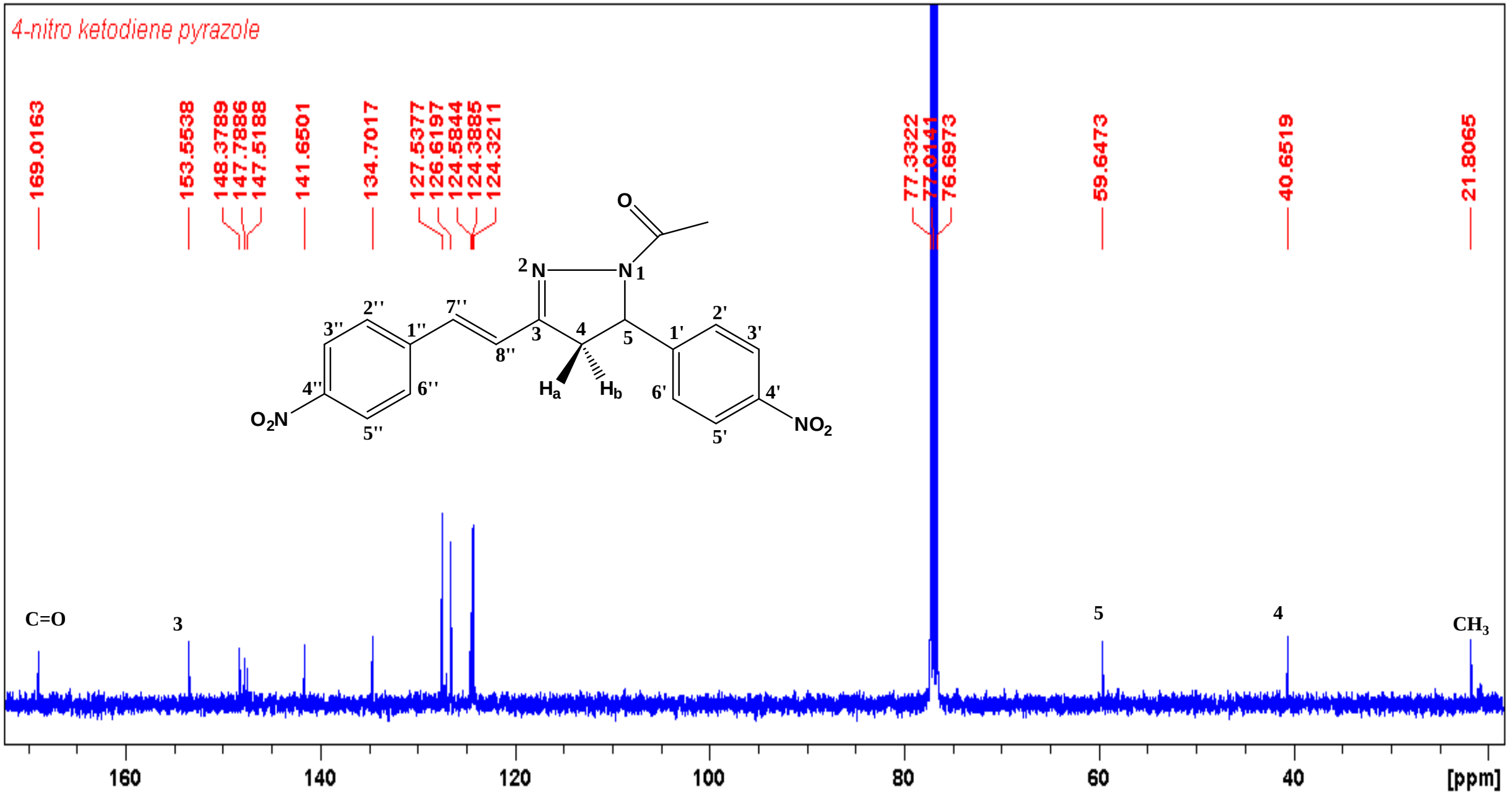


[1]H Spectrum of Compound B-2o: (E)-1-(5-(4-nitrophenyl)-3-(4-nitrostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

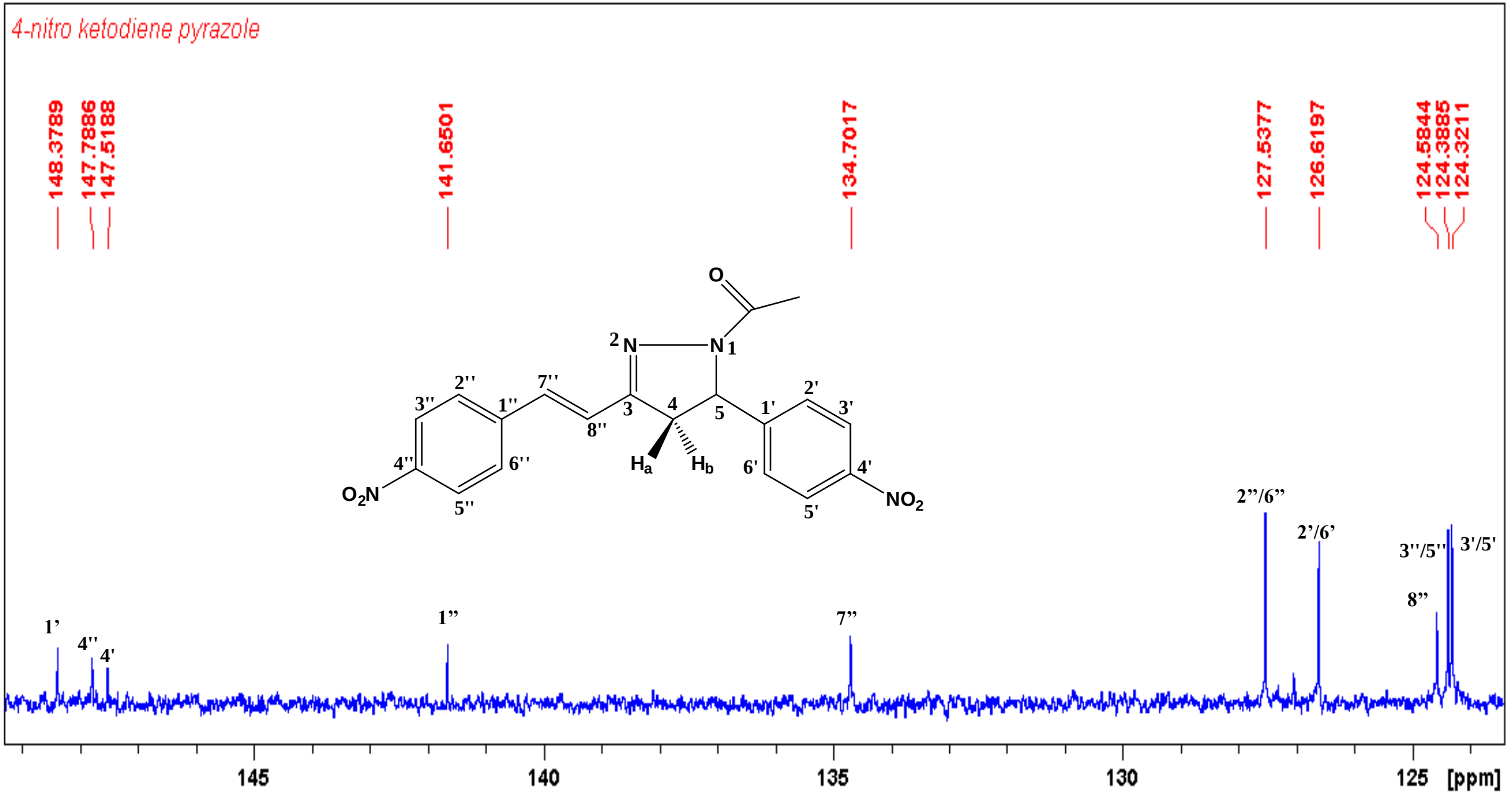


409

Expanded ^{1}H Spectrum of Compound B-2o: (E)-1-(5-(4-nitrophenyl)-3-(4-nitrostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

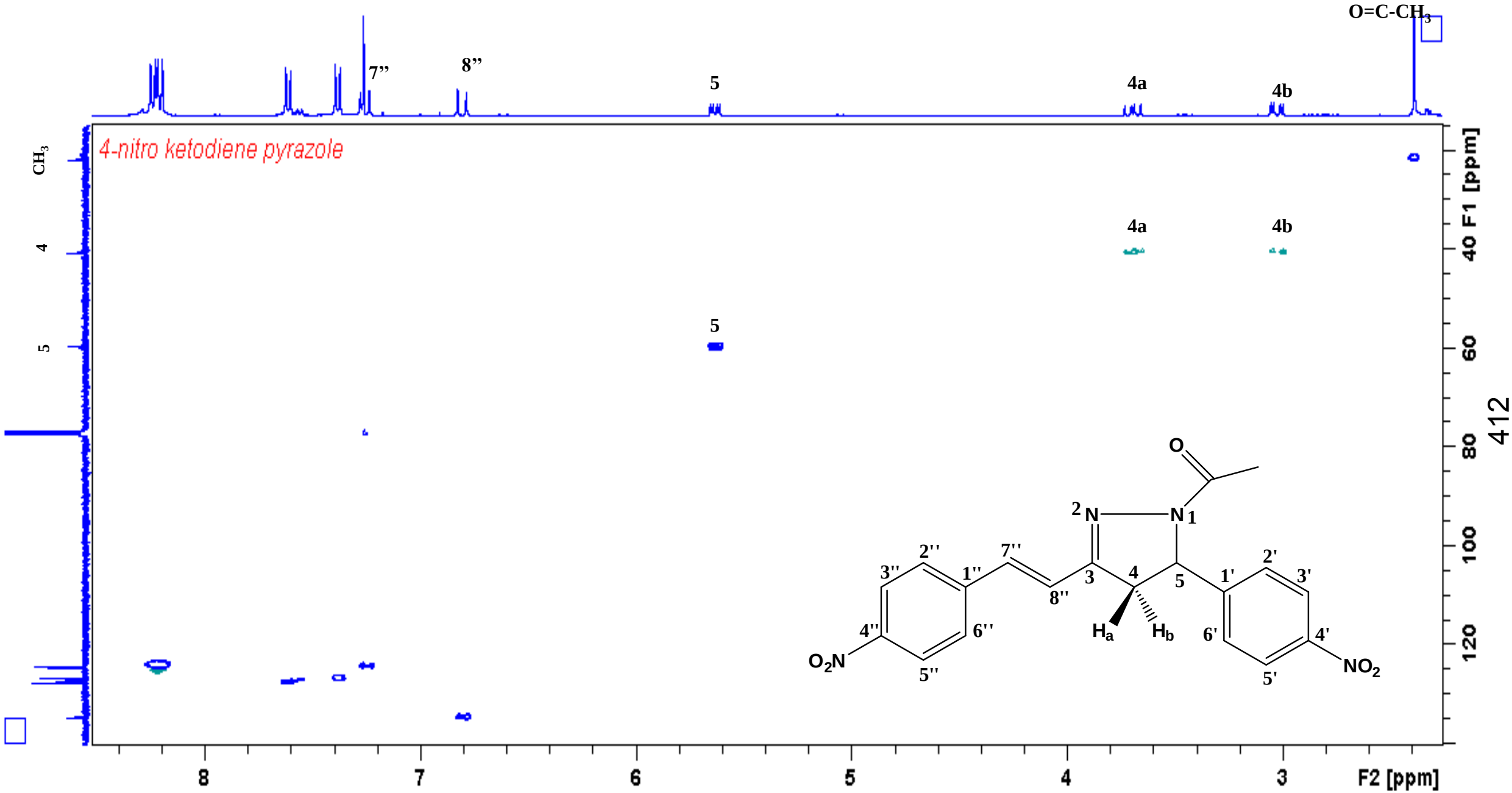


^{13}C Spectrum of Compound B-2o: (E)-1-(5-(4-nitrophenyl)-3-(4-nitrostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

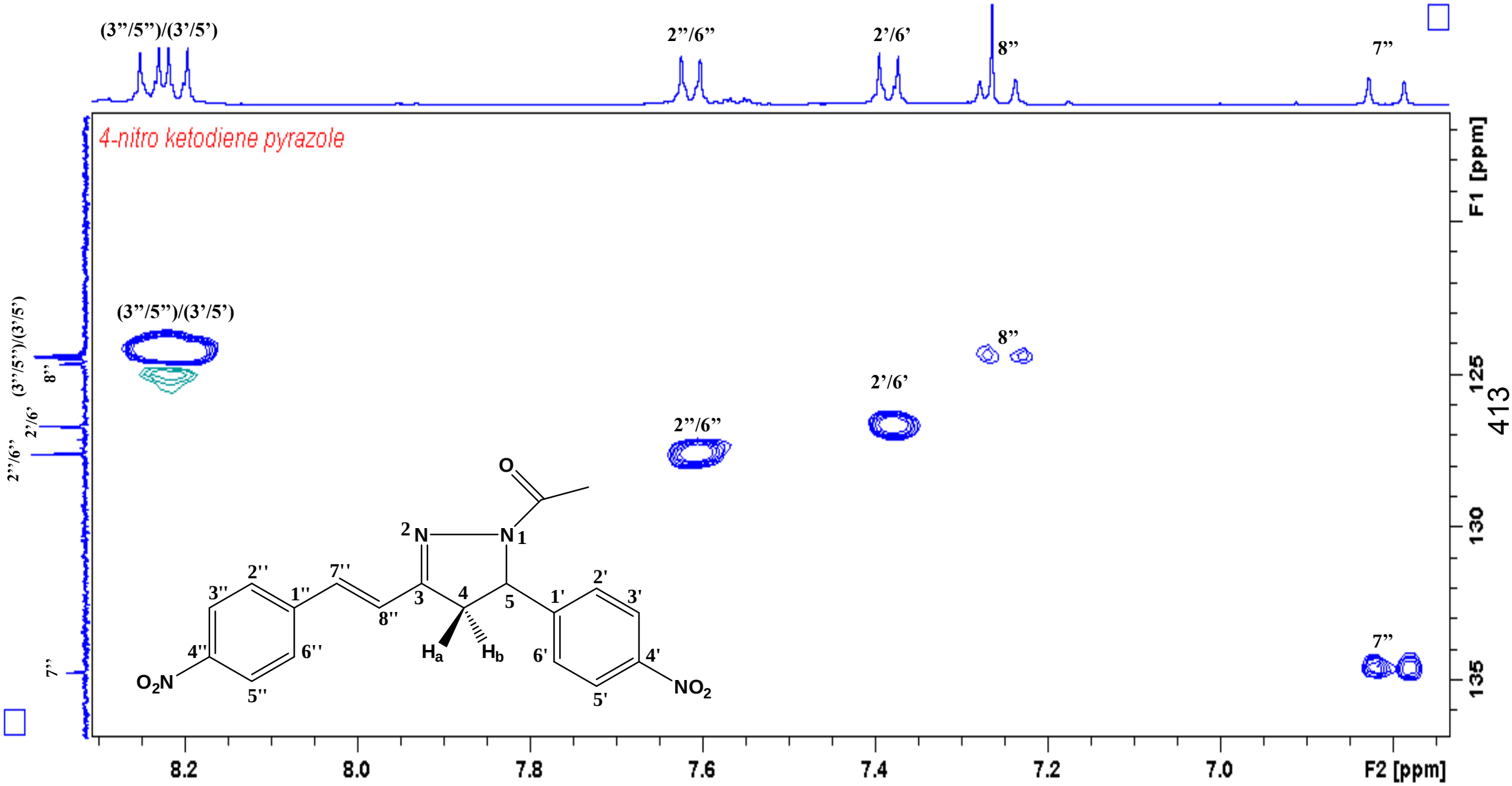


411

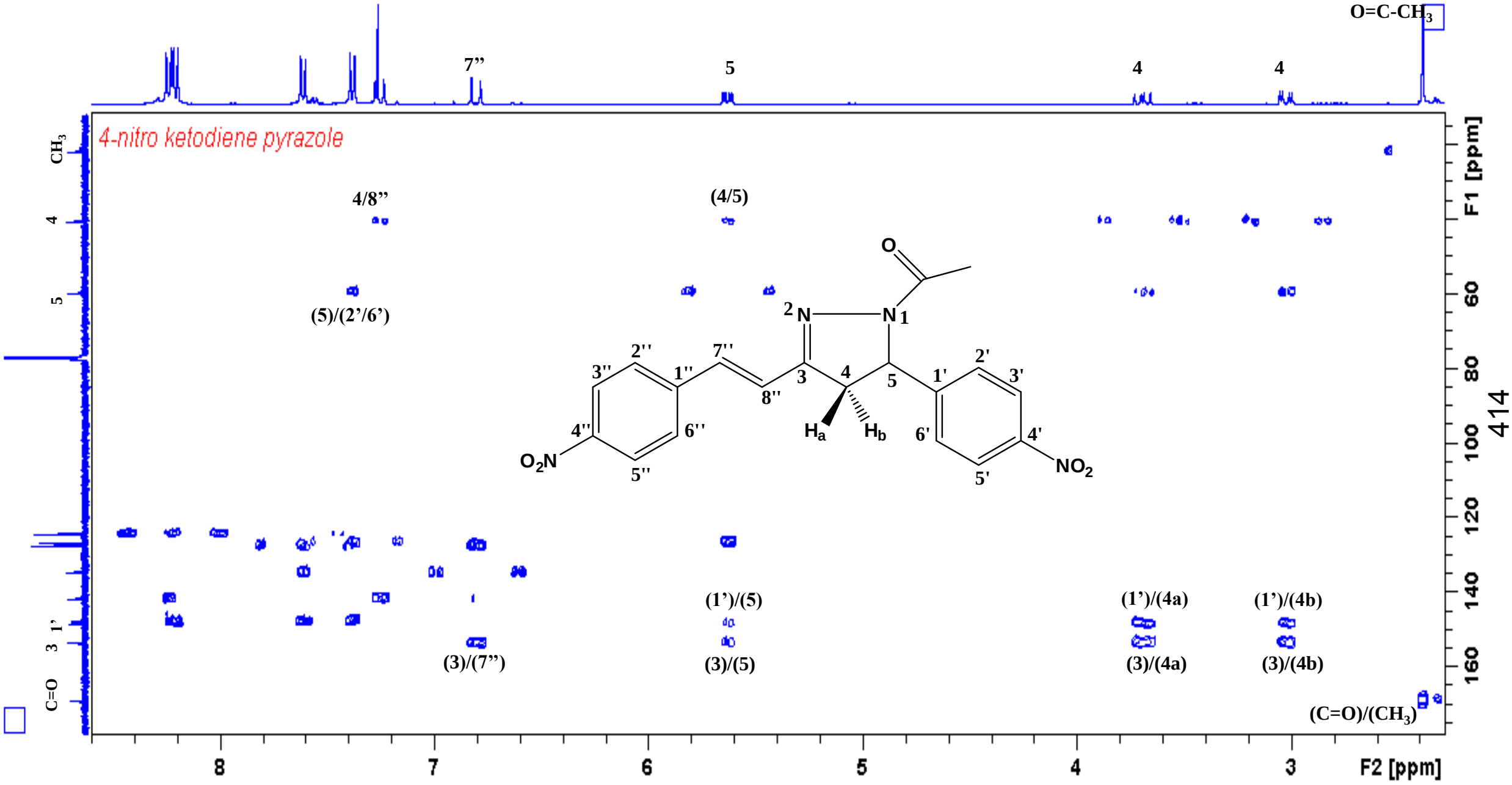
Expanded ^{13}C Spectrum of Compound B-2o: (E)-1-(5-(4-nitrophenyl)-3-(4-nitrostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



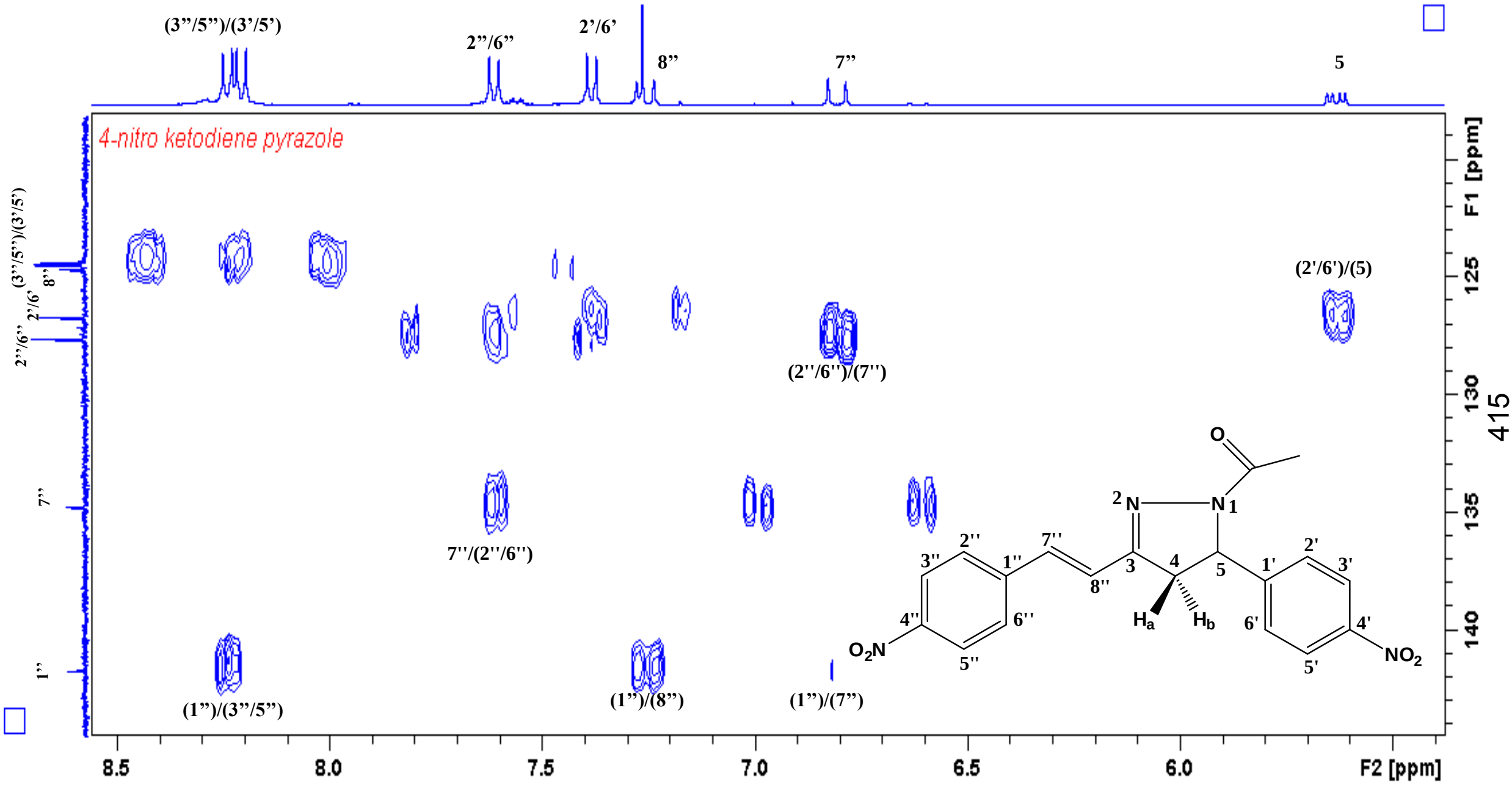
HSQC of Compound B-2o: (E)-1-(5-(4-nitrophenyl)-3-(4-nitrostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



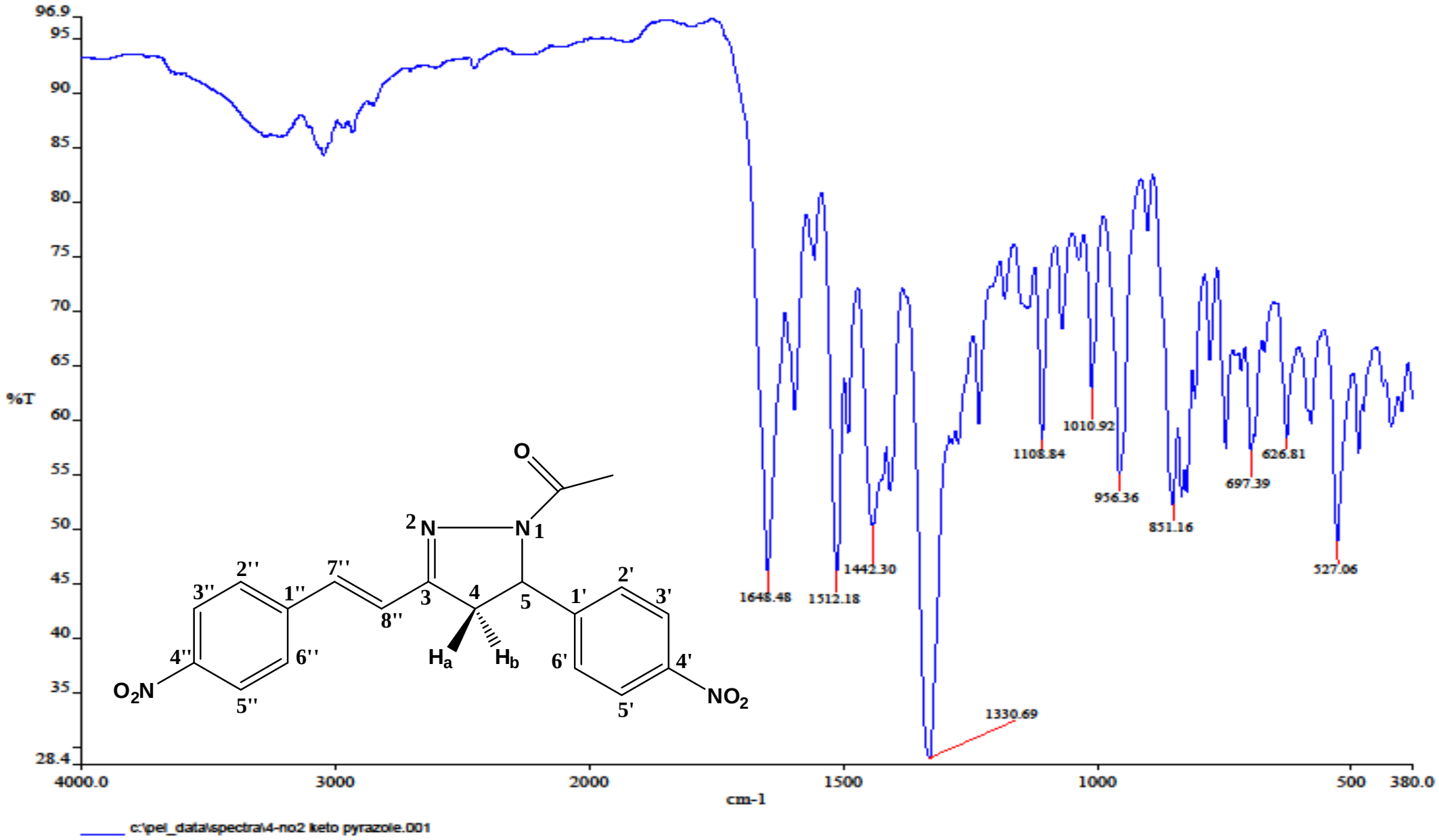
Expanded HSQC of Compound B-2o: (E)-1-(5-(4-nitrophenyl)-3-(4-nitrostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



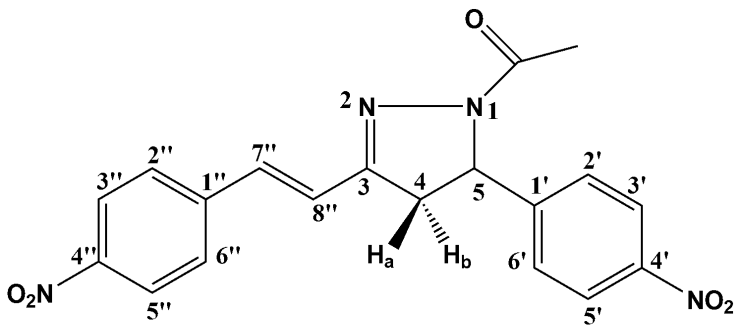
HMBC of Compound B-2o: (E)-1-(5-(4-nitrophenyl)-3-(4-nitrostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



Expanded HMBC of Compound B-2o: (E)-1-(5-(4-nitrophenyl)-3-(4-nitrostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



Infrared Spectrum of Compound B-2o: (E)-1-(5-(4-nitrophenyl)-3-(4-nitrostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone

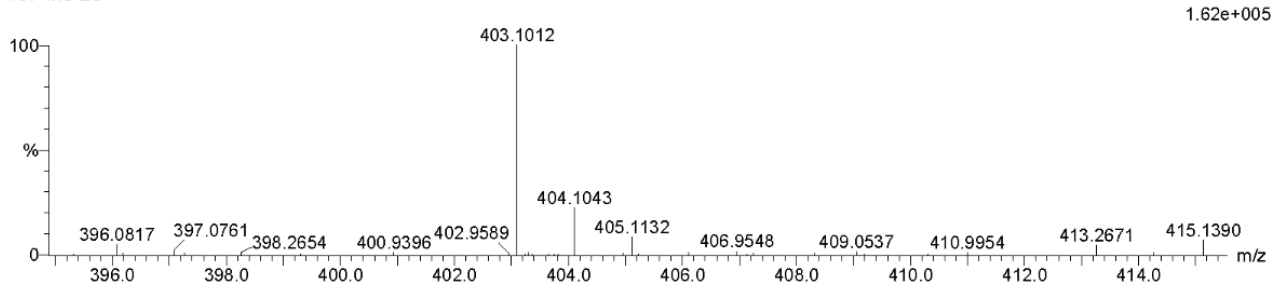


Elemental Composition Report

Single Mass Analysis

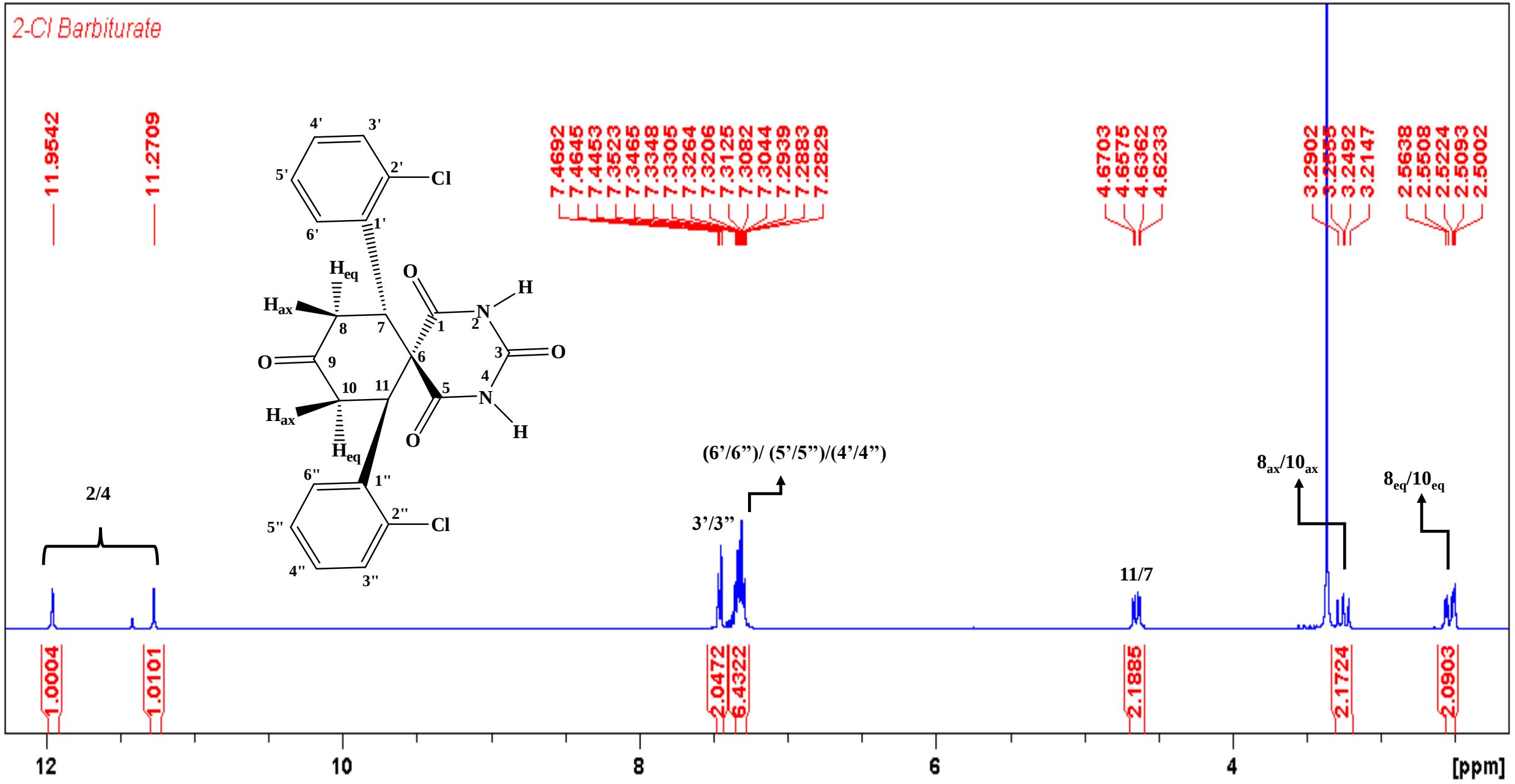
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
23 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 15-25 H: 15-25 N: 0-5 O: 0-5 Na: 1-1
Compound 14 37 (1.214) Cm (1:61)
TOF MS ES+

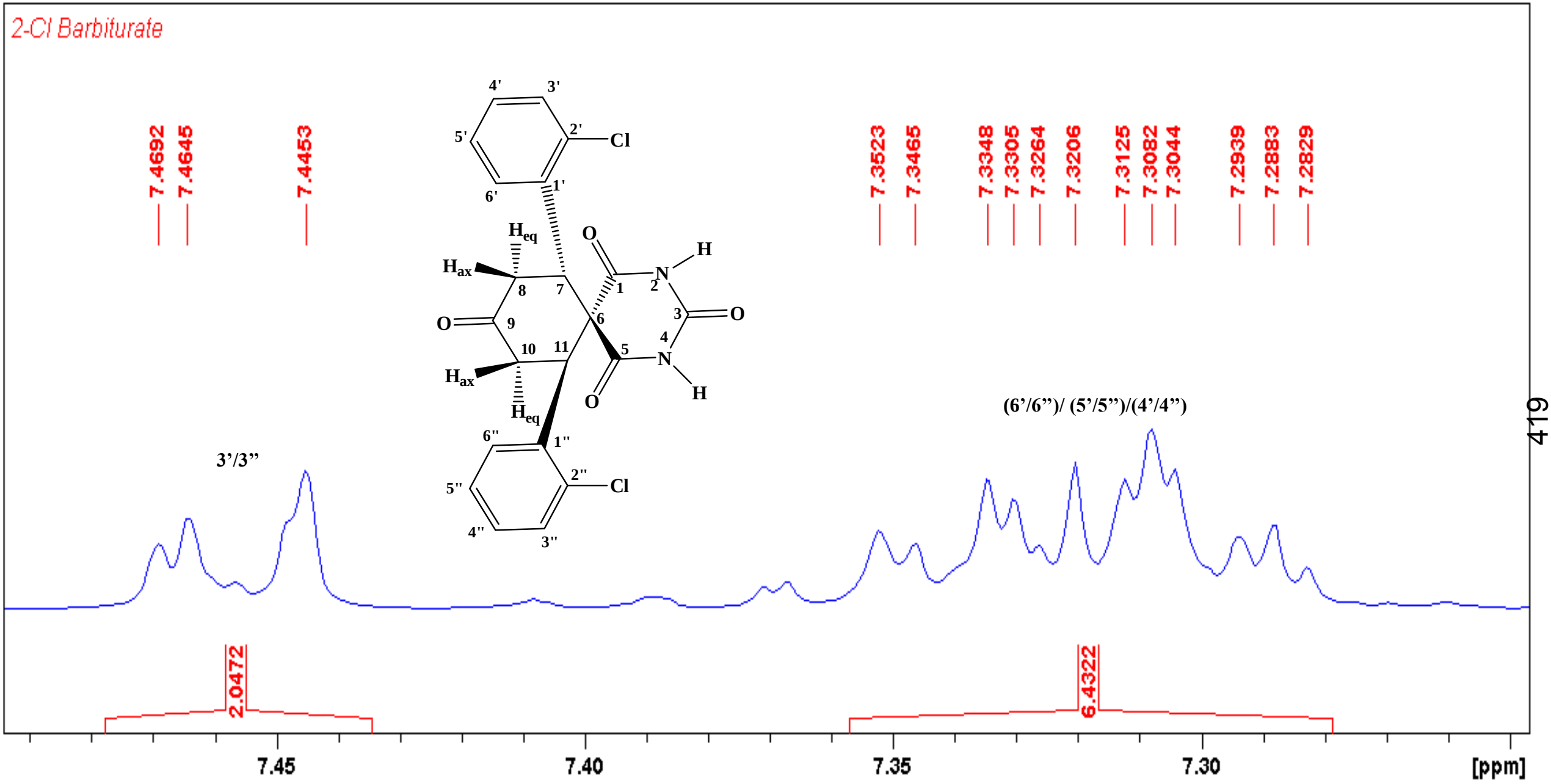


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
403.1012	403.1018	-0.6	-1.5	13.5	91.5	0.0	C19 H16 N4 O5 Na

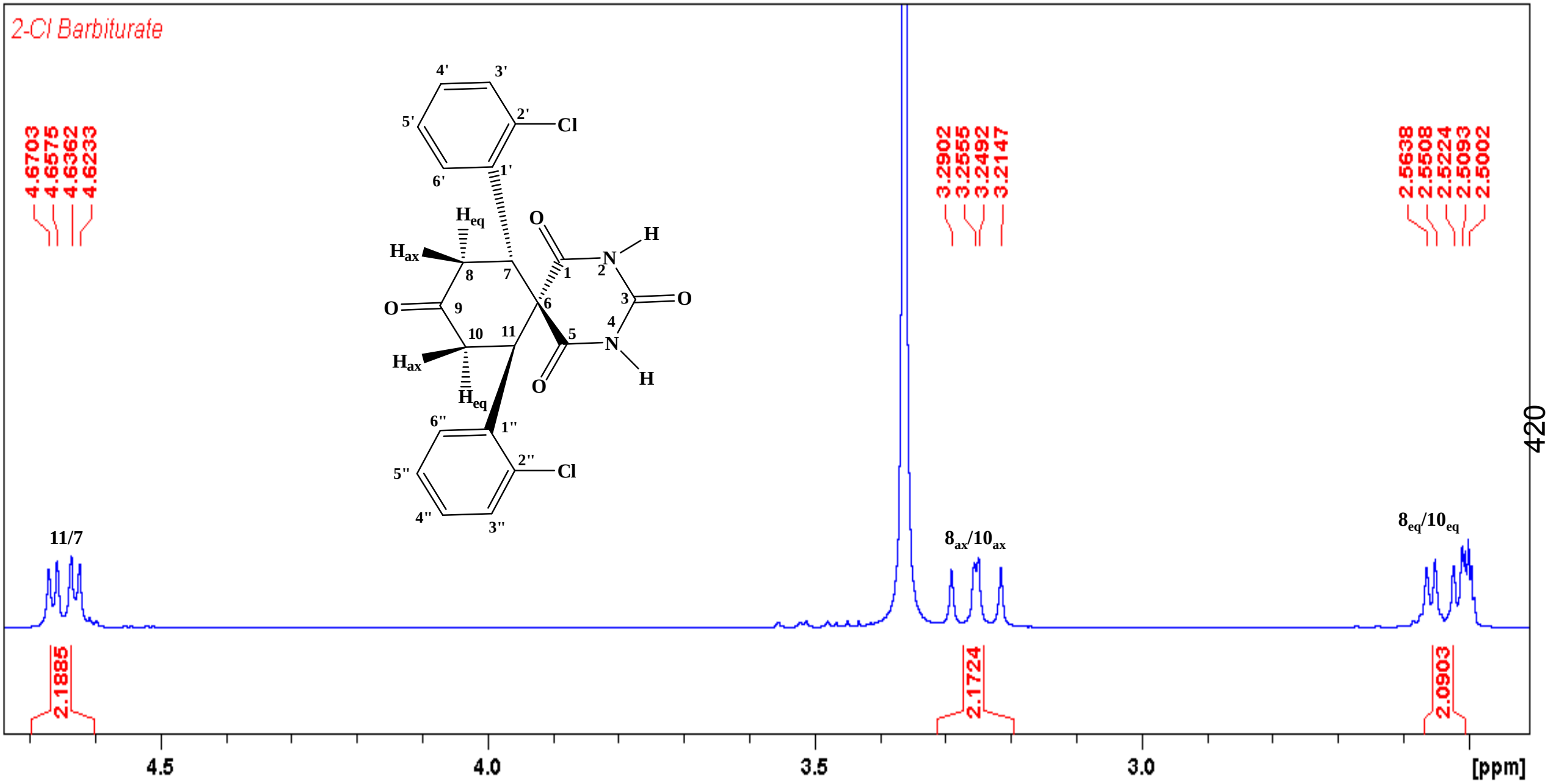
HRMS of Compound B-2o: (E)-1-(5-(4-nitrophenyl)-3-(4-nitrostyryl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone



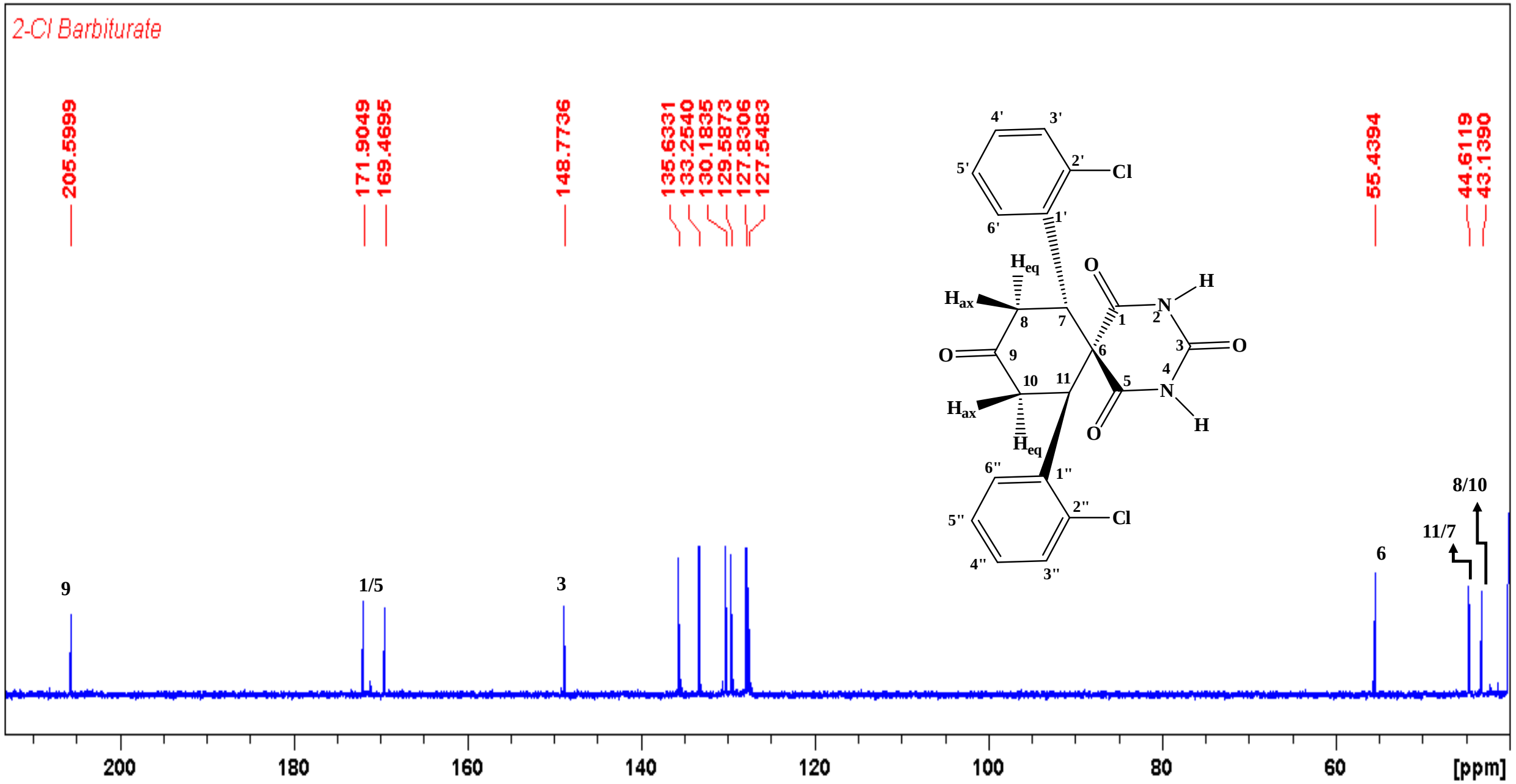
^{1}H Spectrum of Compound B-3a: 7,11-bis(2-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



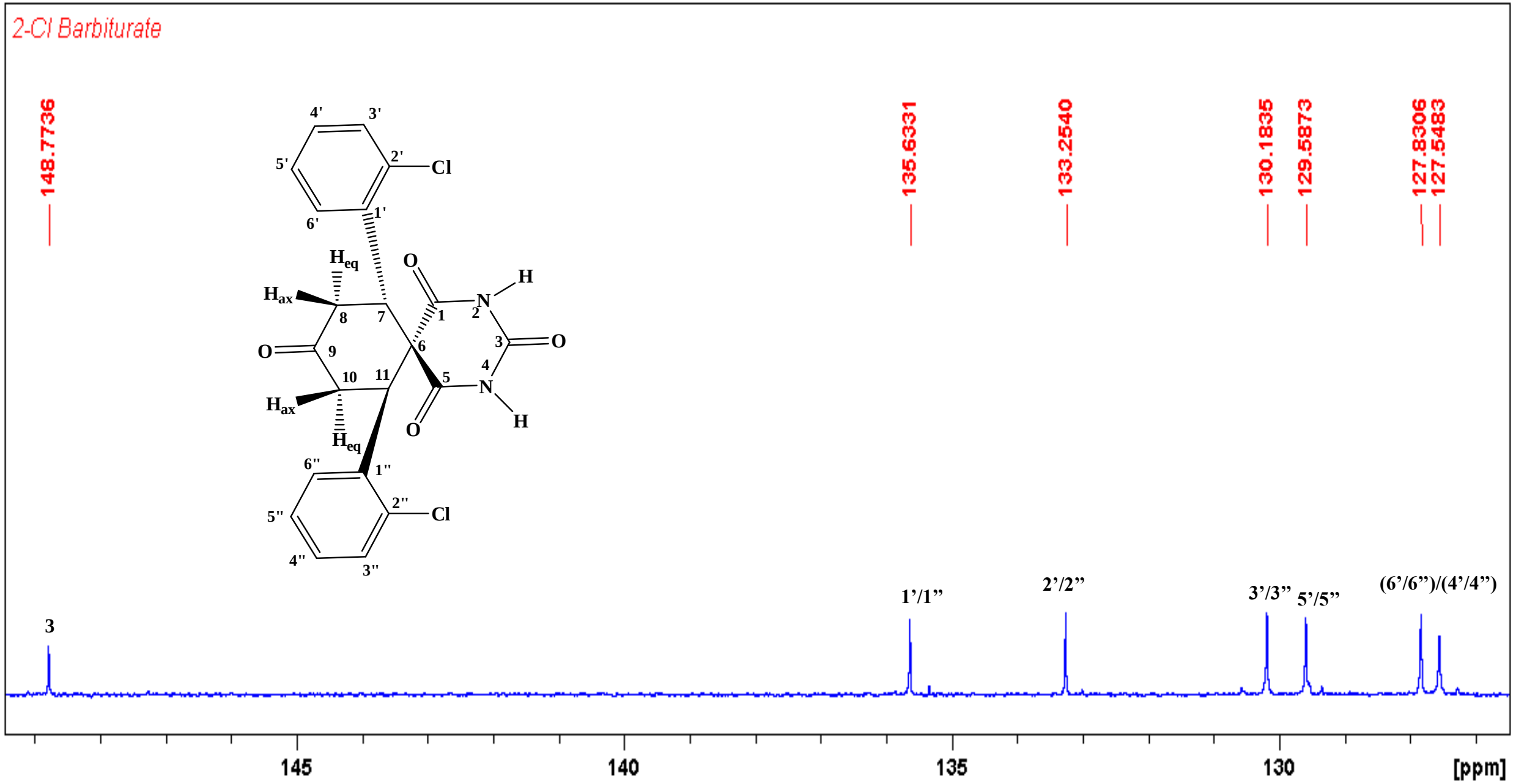
Expanded ^{1}H Spectrum of Compound B-3a: 7,11-bis(2-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



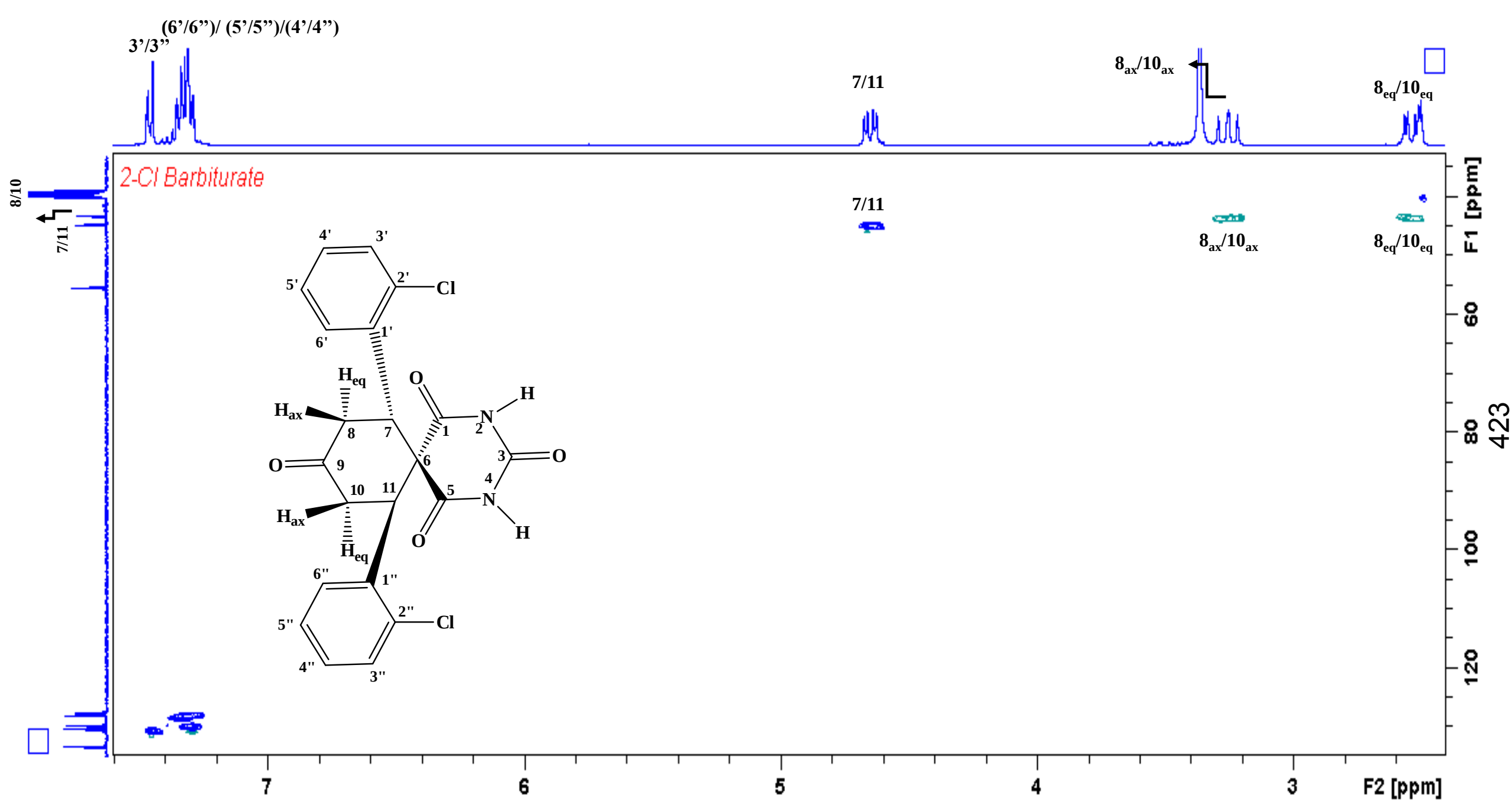
Expanded ^{1}H Spectrum of Compound B-3a: 7,11-bis(2-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



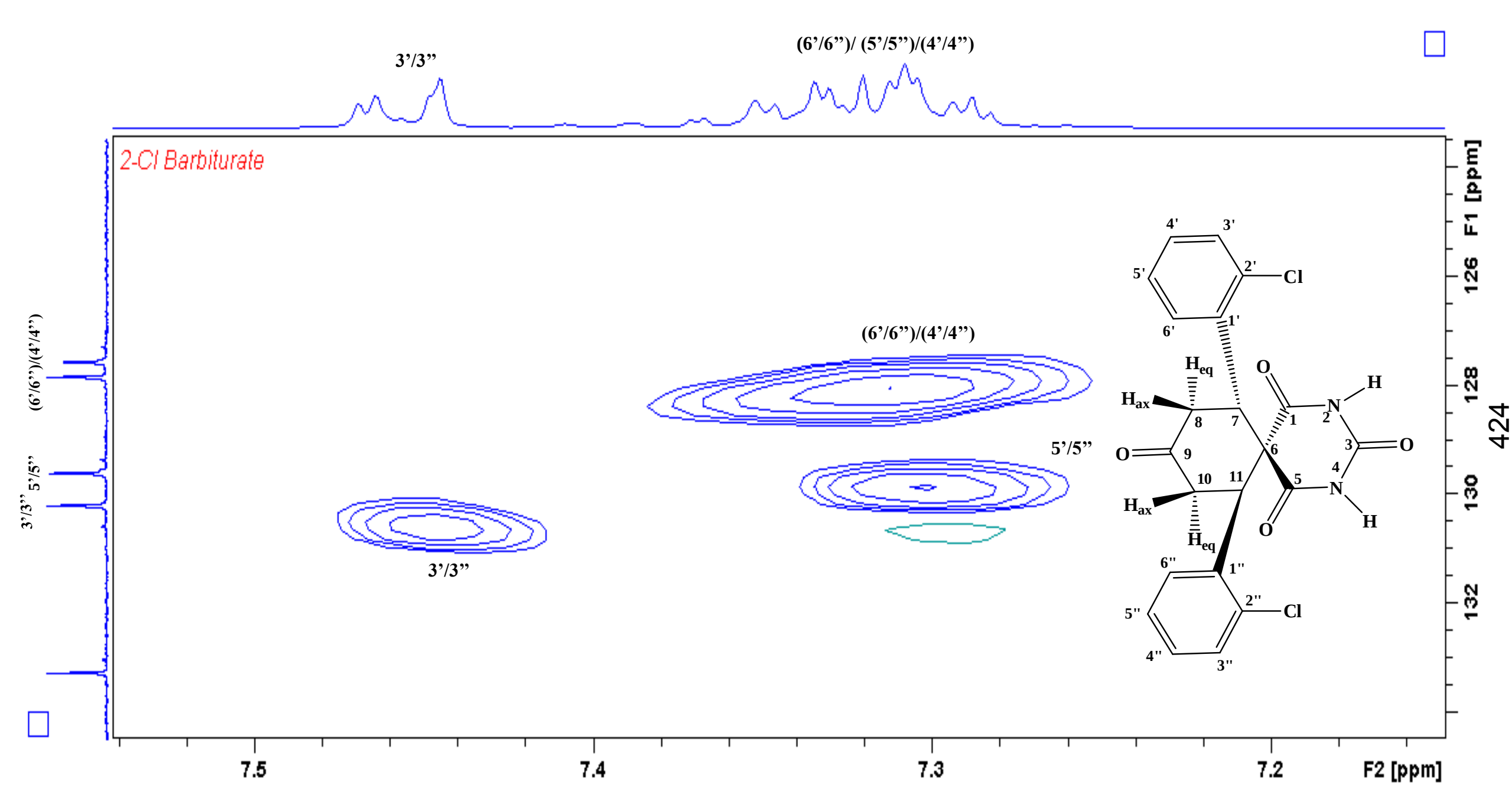
^{13}C Spectrum of Compound B-3a: 7,11-bis(2-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



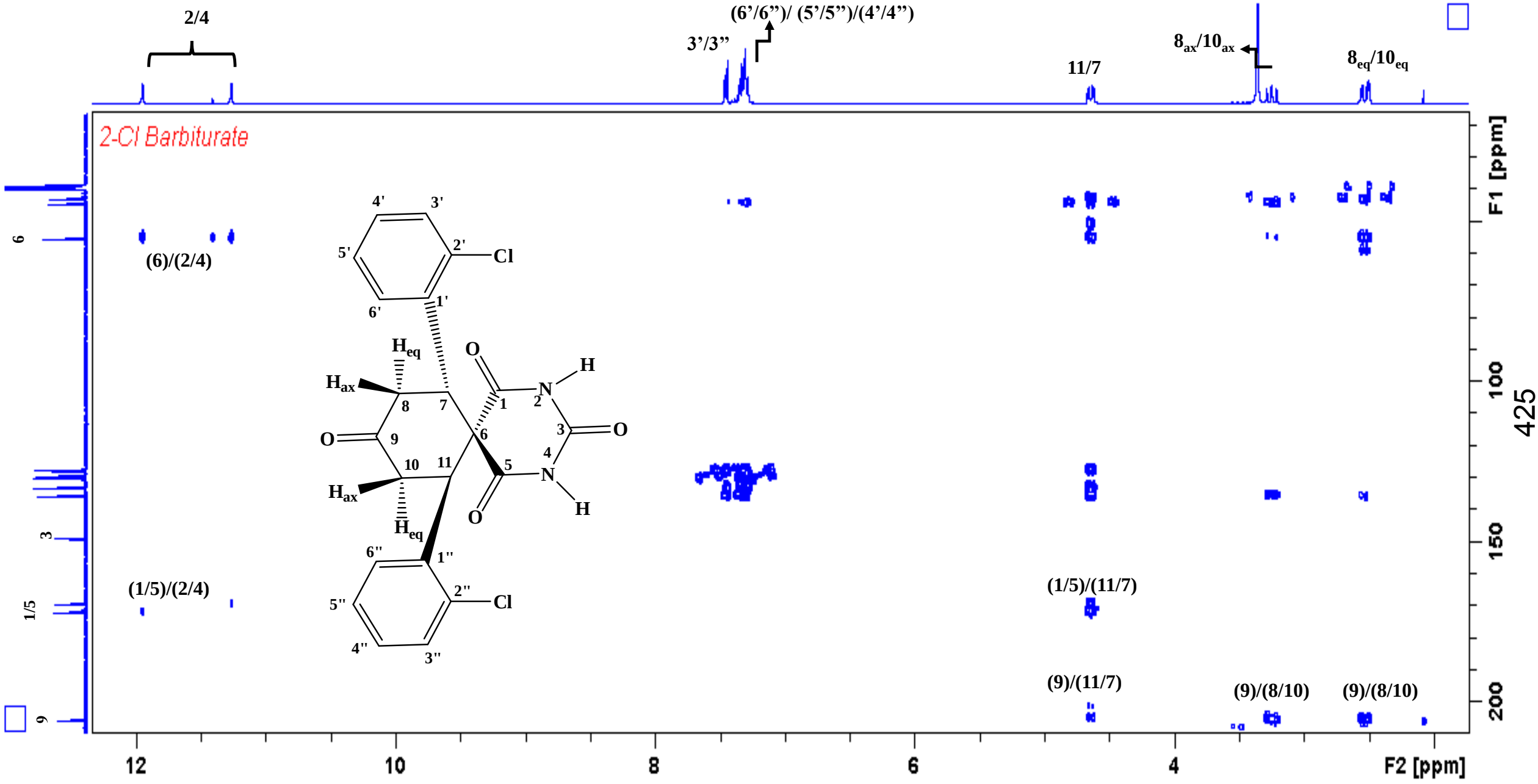
Expanded ^{13}C Spectrum of Compound B-3a: 7,11-bis(2-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



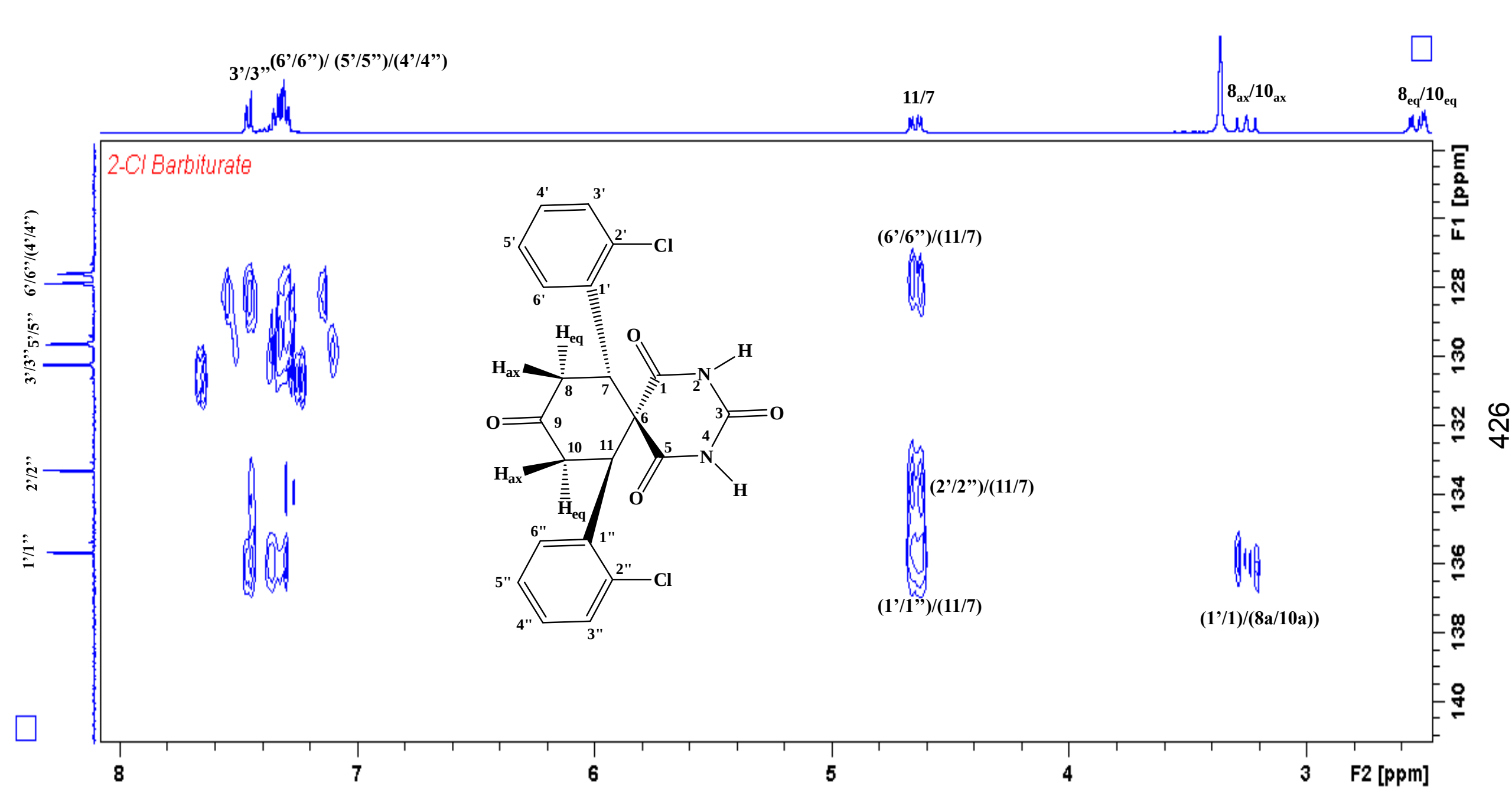
HSQC of Compound B-3a: 7,11-bis(2-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



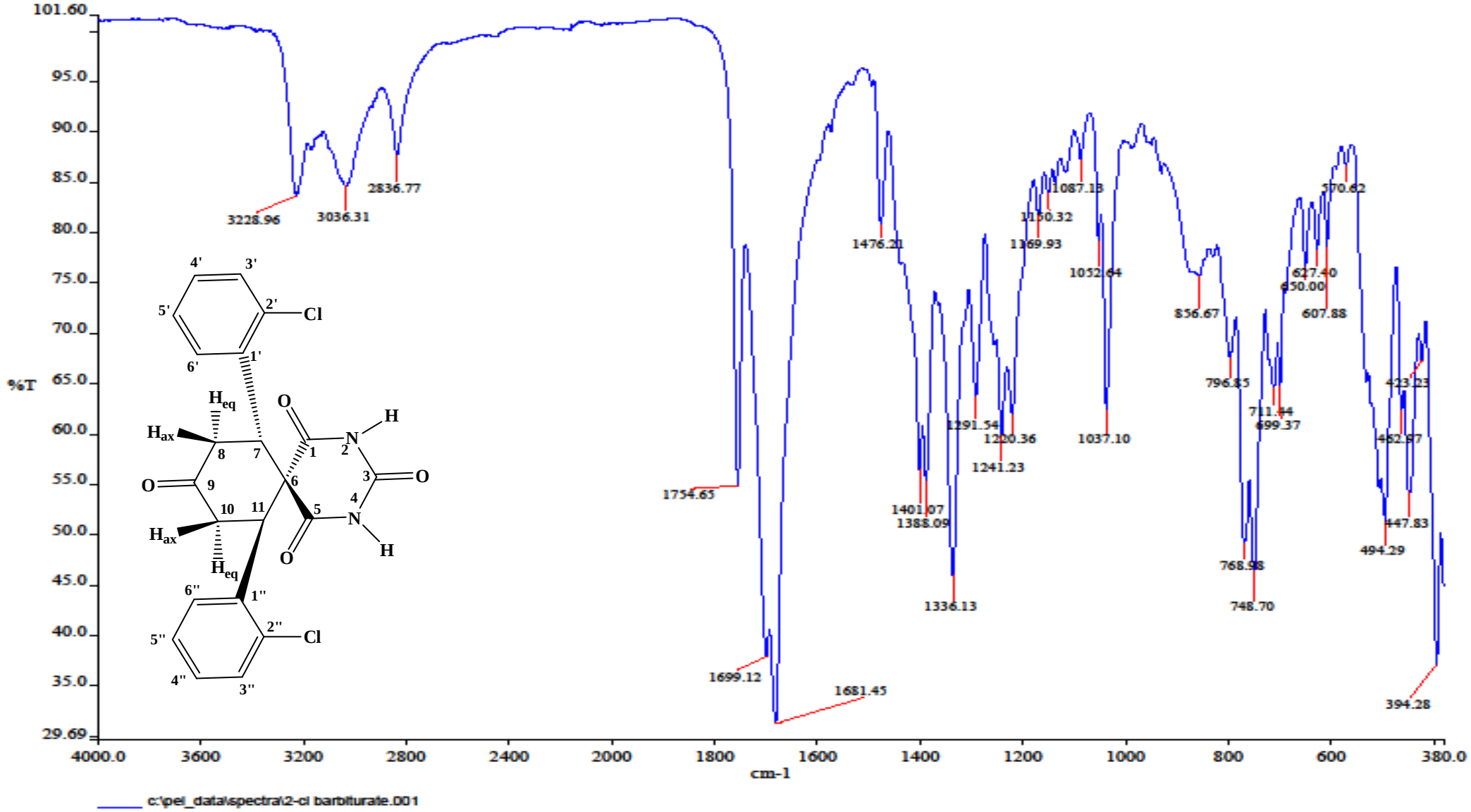
Expanded HSQC of Compound B-3a: 7,11-bis(2-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



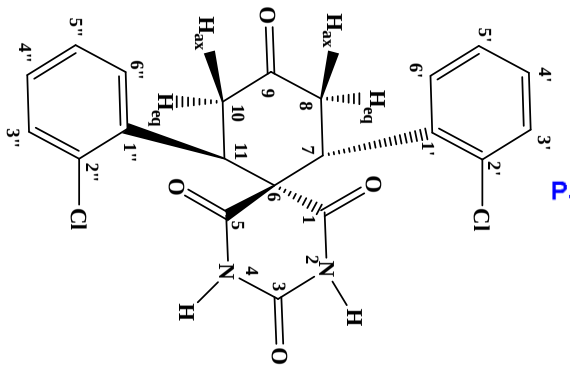
HMBC of Compound B-3a: 7,11-bis(2-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



Expanded HMBC of Compound B-3a: 7,11-bis(2-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



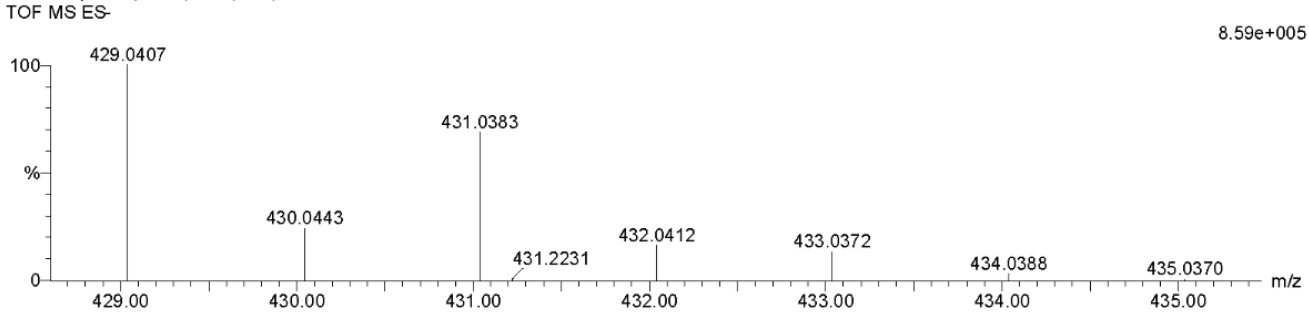
Infrared Spectrum of Compound B-3a: 7,11-bis(2-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



Elemental Composition Report **Page 1**

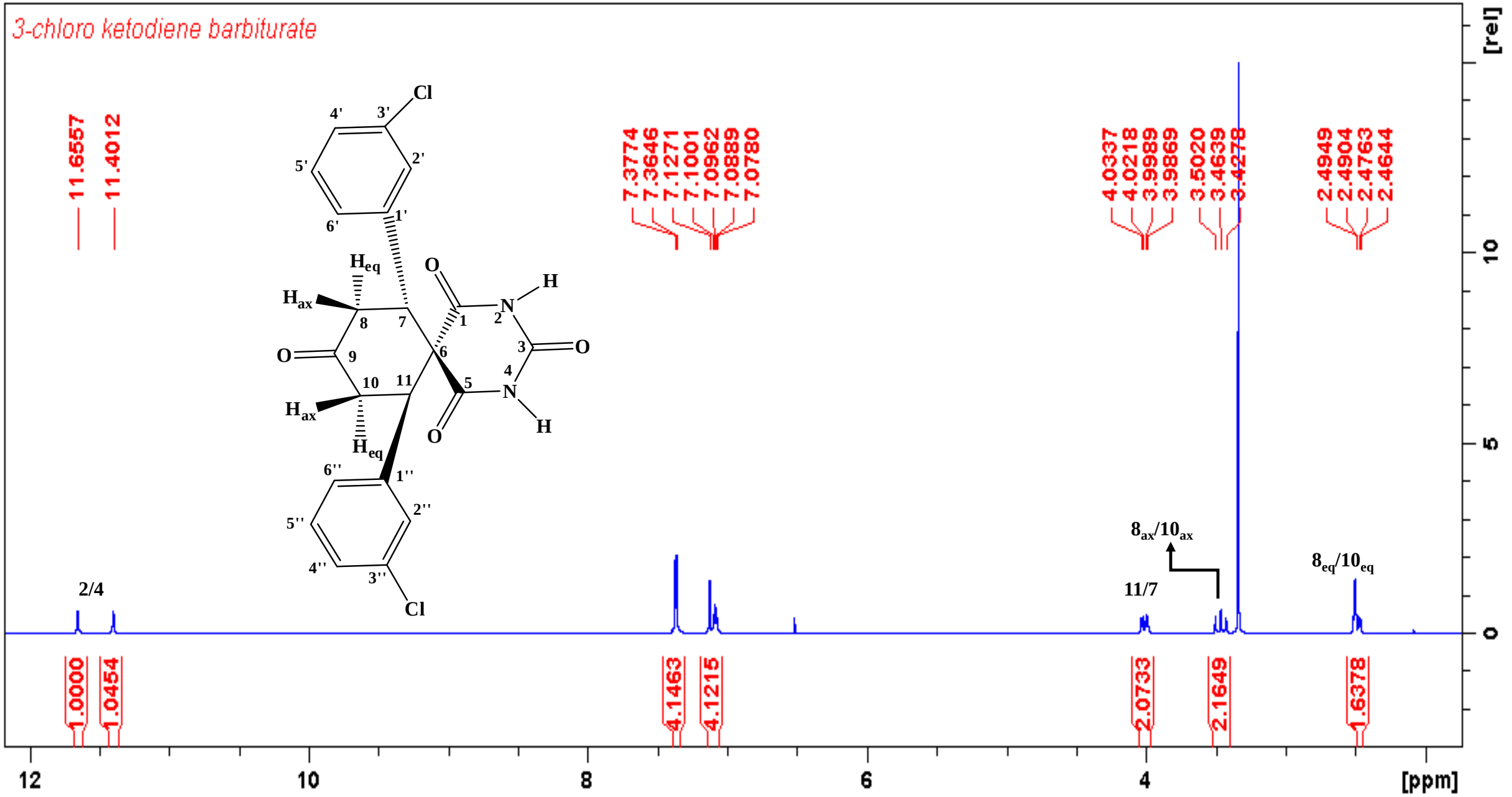
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
43 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 0-5 Cl: 0-2
14 2-Cl spiro 1 (0.034) Cm (1:60)
TOF MS ES-

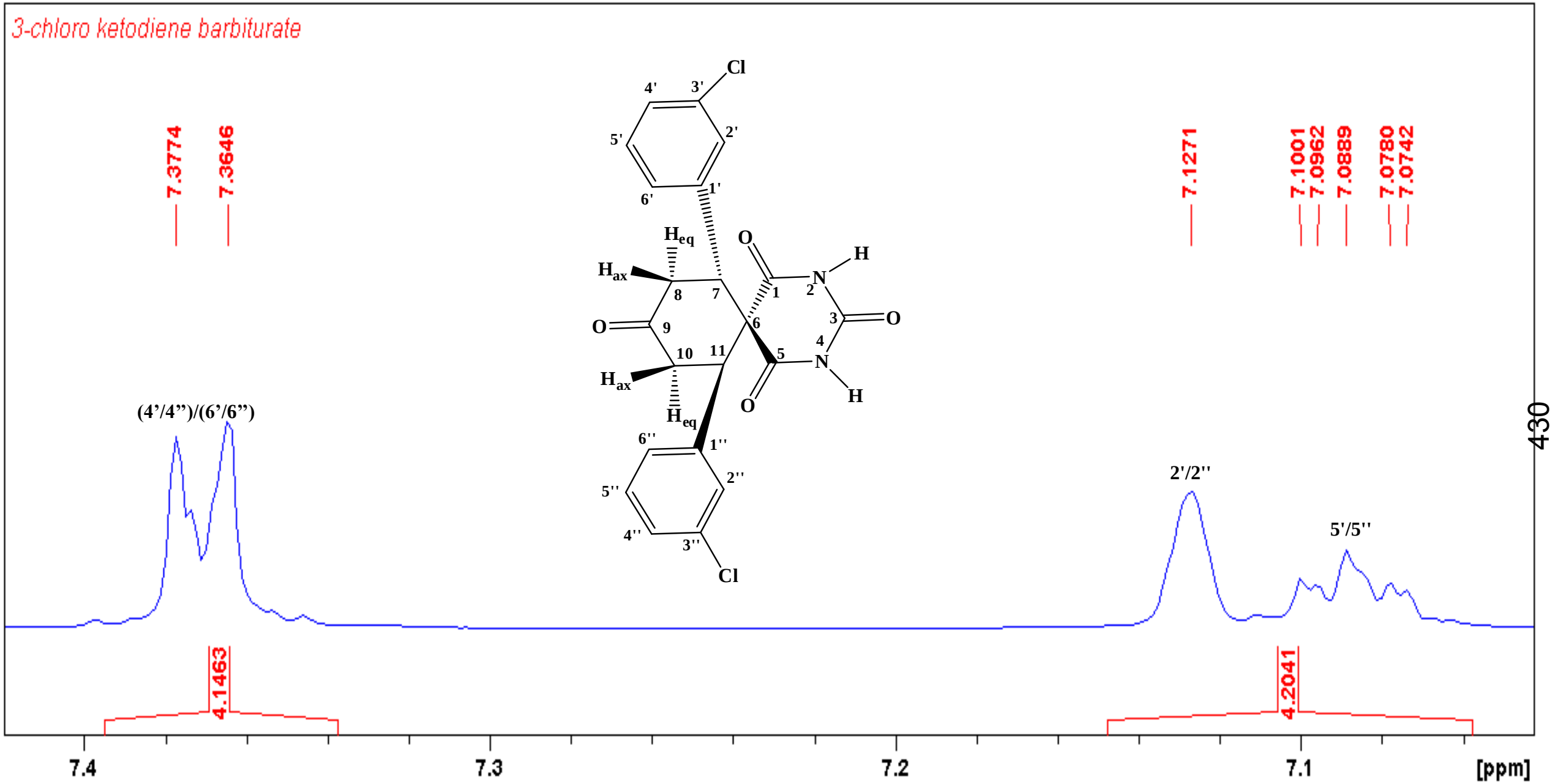


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
429.0407	429.0409	-0.2	-0.5	14.5	13.9	0.0	C21 H15 N2 O4 Cl2

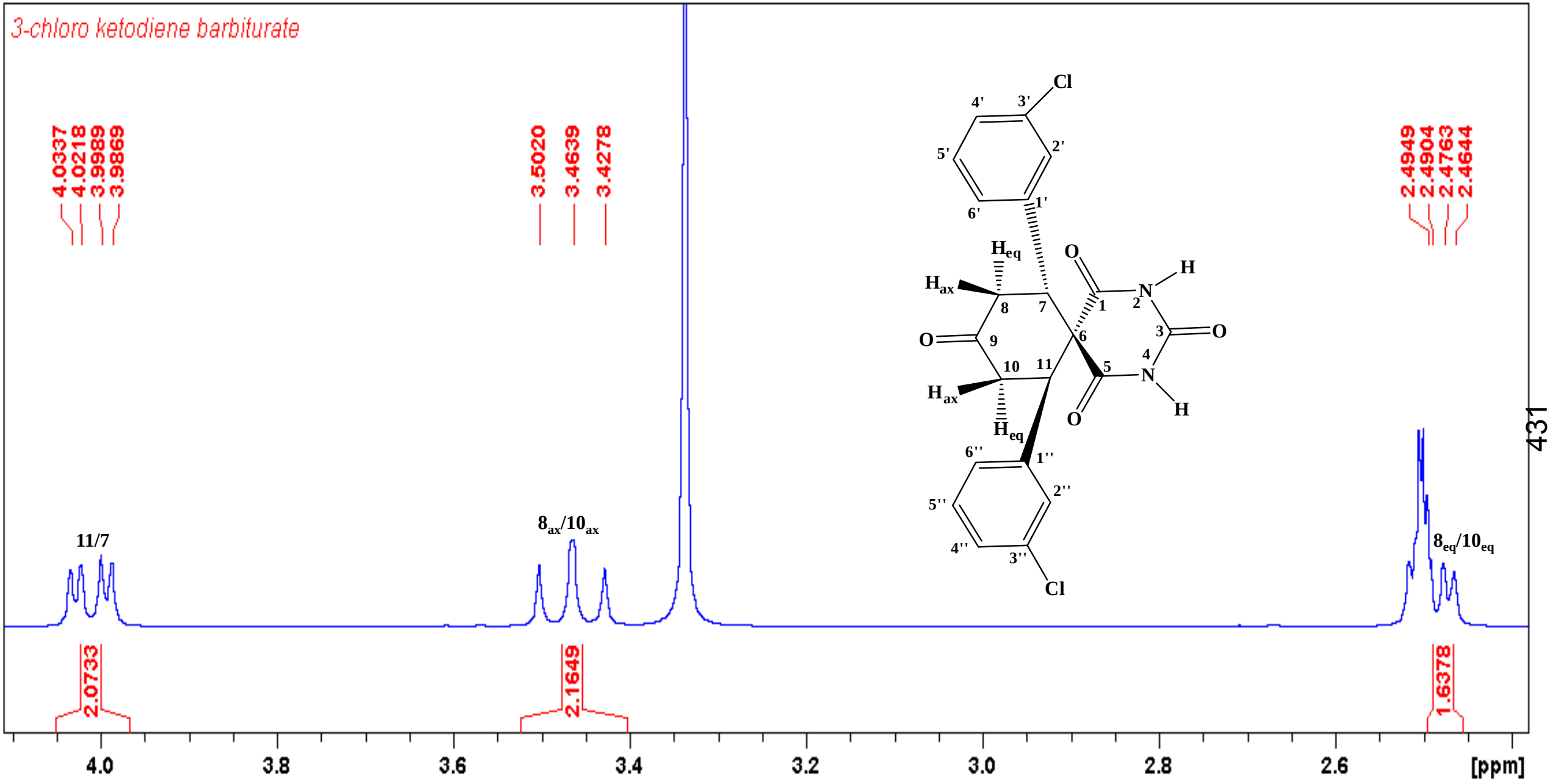
HRMS of Compound B-3a: 7,11-bis(2-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



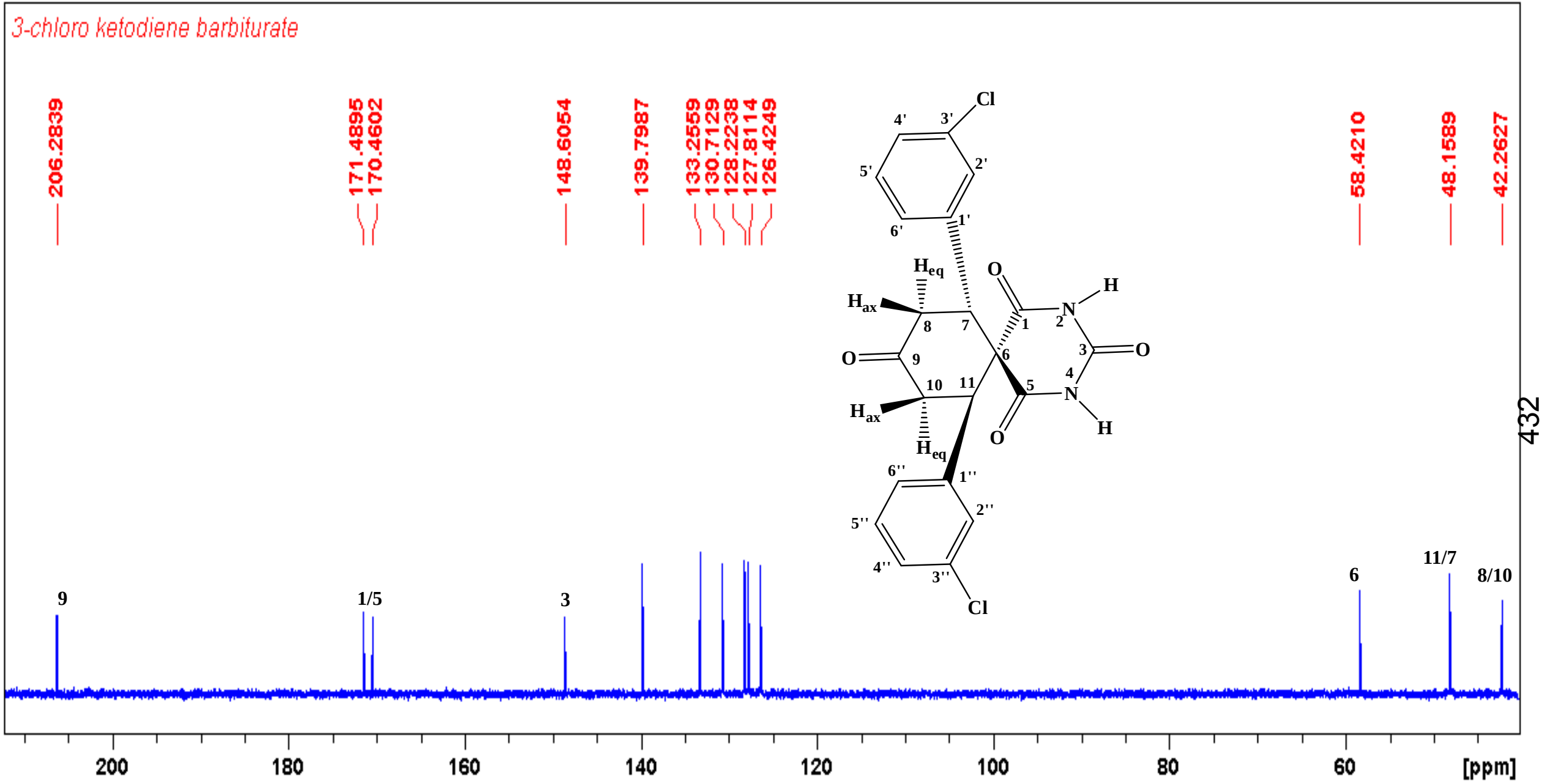
^{1}H Spectrum of Compound B-3b: 7,11-bis(3-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



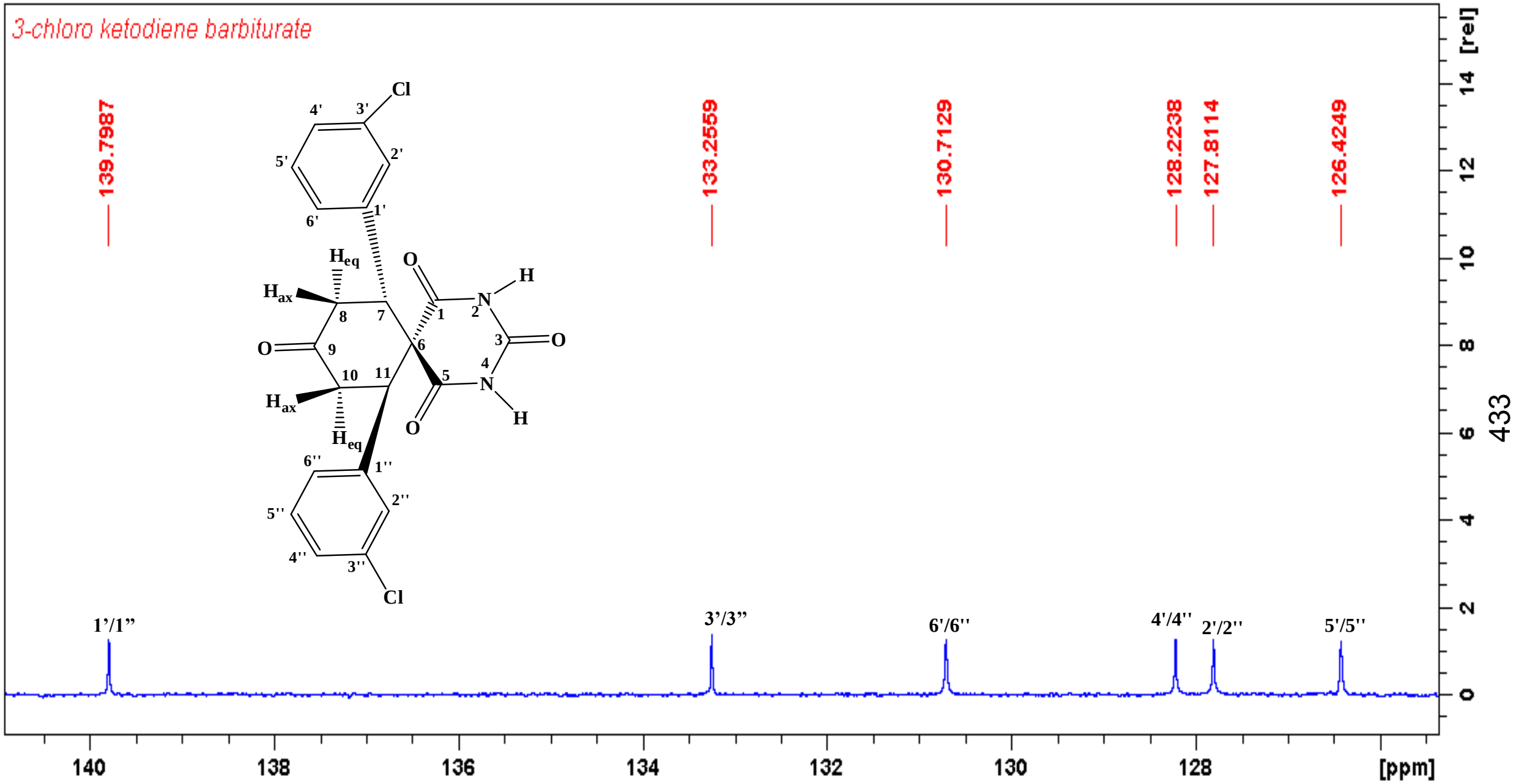
Expanded ^{1}H Spectrum of Compound B-3b: 7,11-bis(3-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



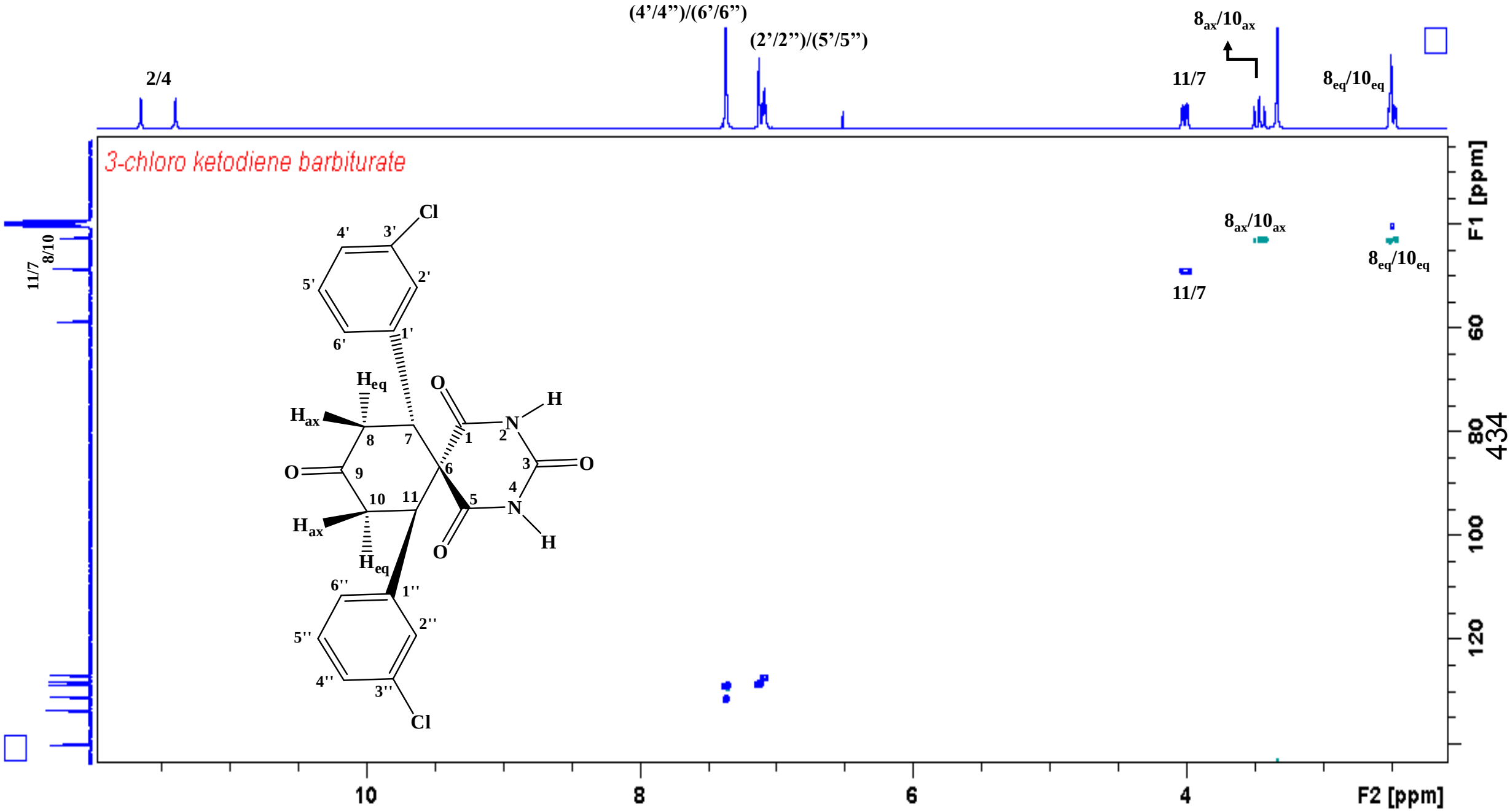
Expanded ^{1}H Spectrum of Compound B-3b: 7,11-bis(3-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



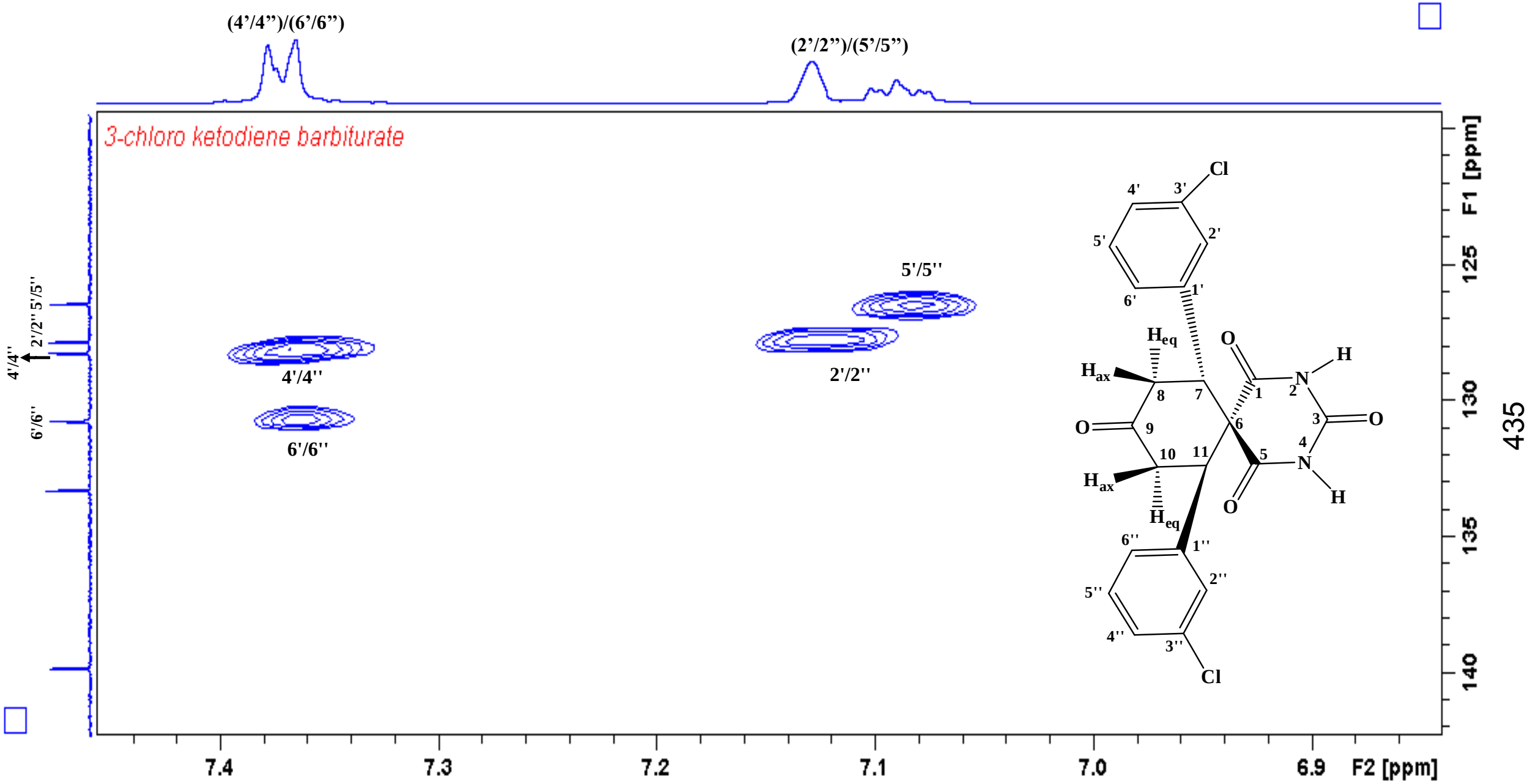
^{13}C Spectrum of Compound B-3b: 7,11-bis(3-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



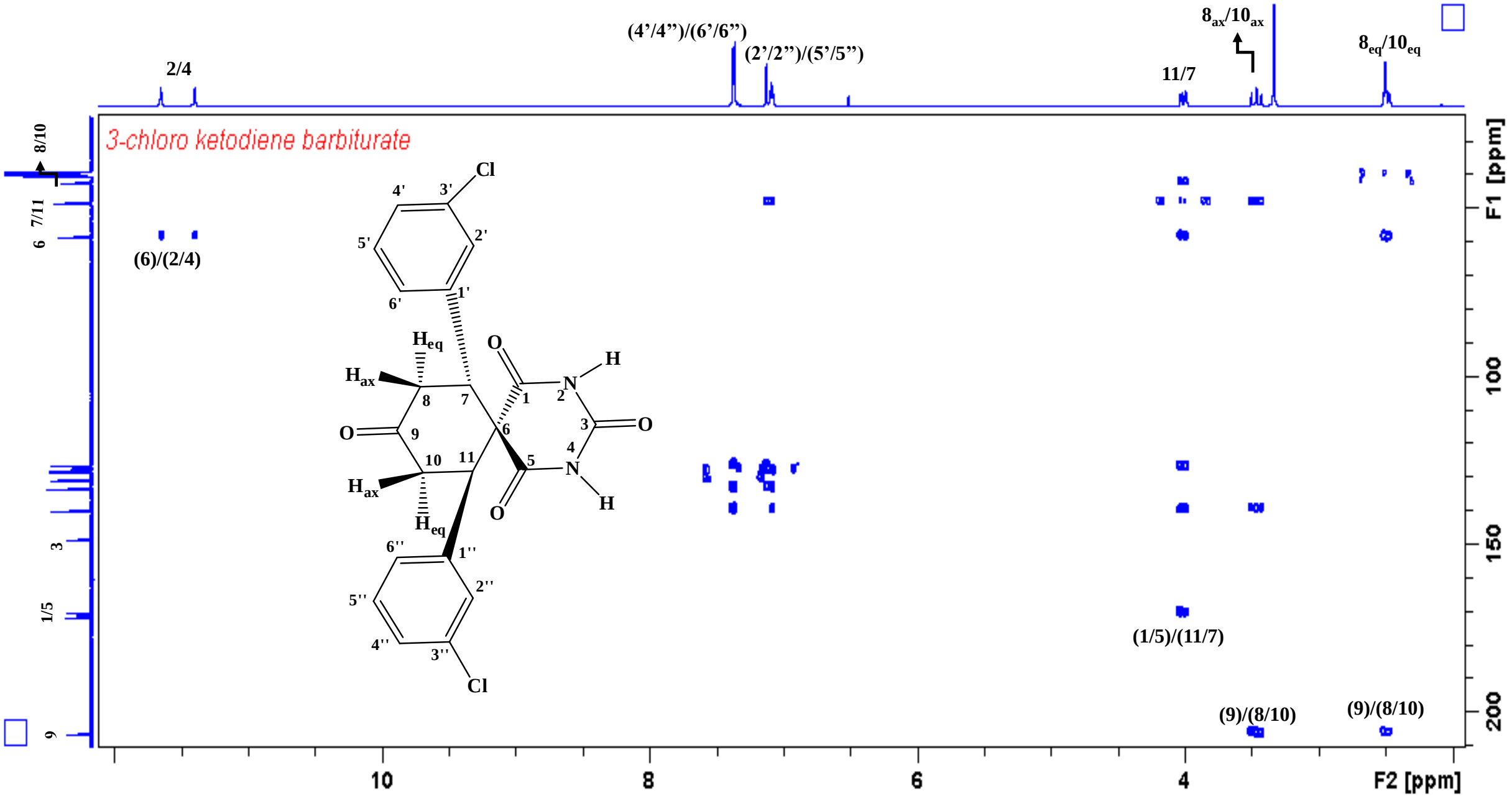
Expanded ^{13}C Spectrum of Compound B-3b: 7,11-bis(3-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone)



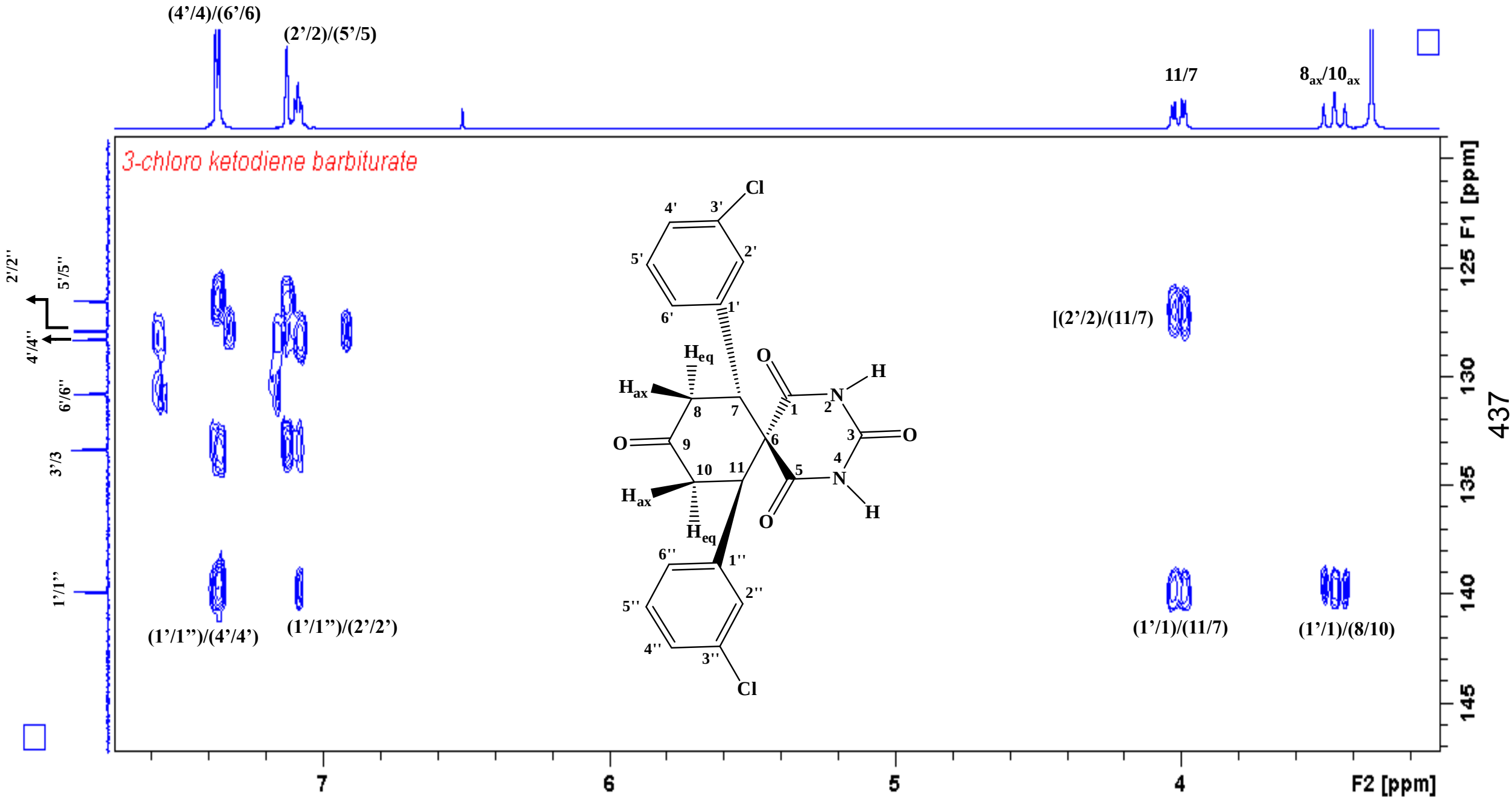
HSQC of Compound B-3b: 7,11-bis(3-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



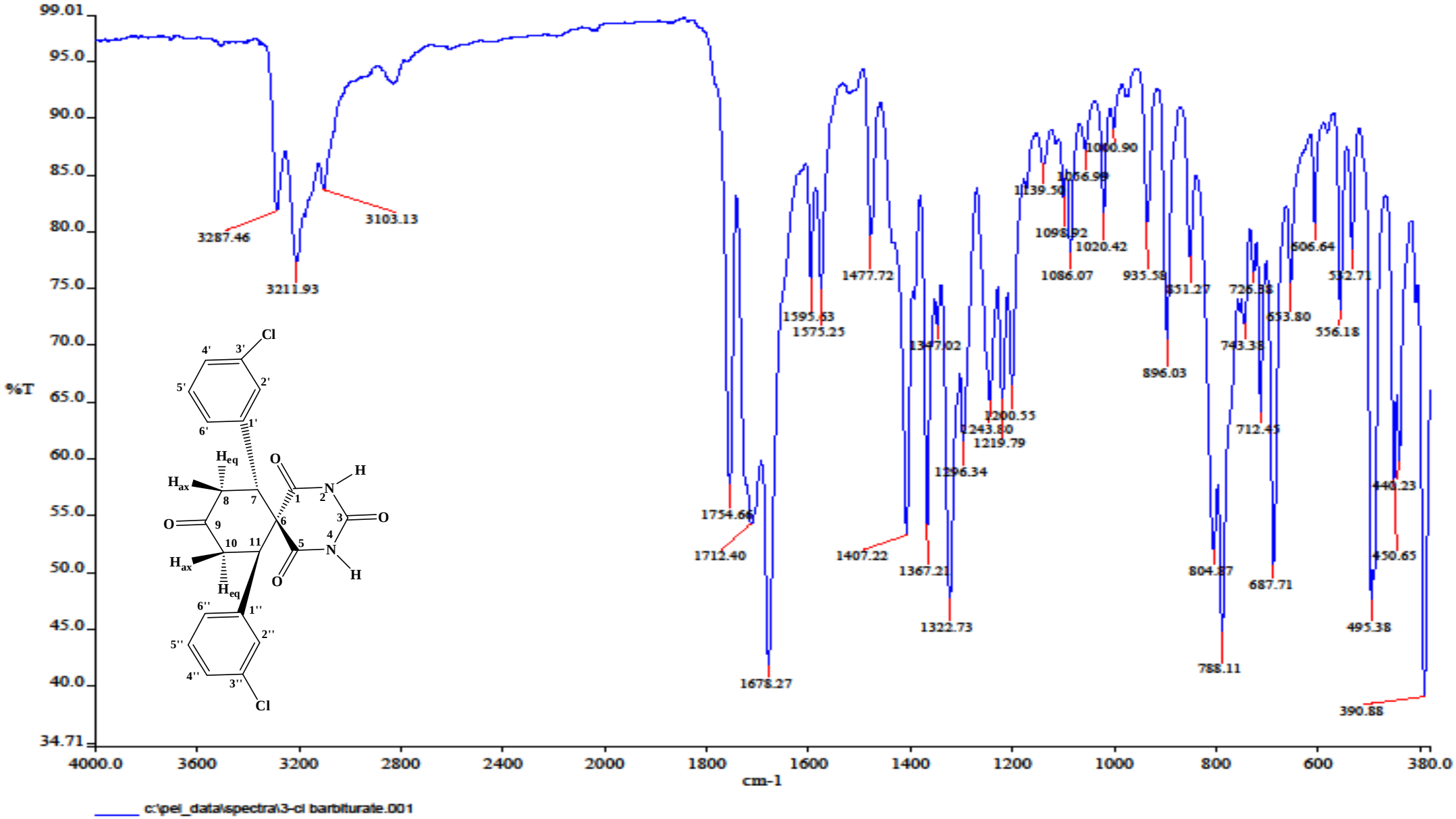
Expanded HSQC of Compound B-3b: 7,11-bis(3-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



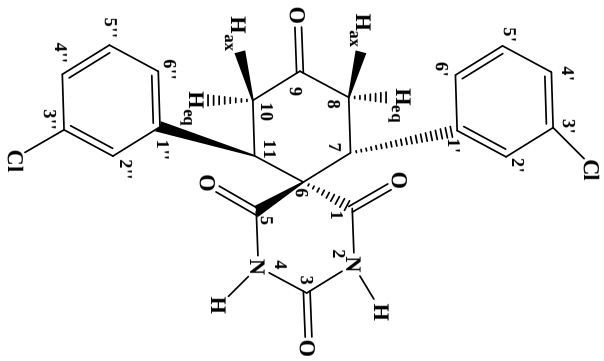
HMBC of Compound B-3b: 7,11-bis(3-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



Expanded HMBC of Compound B-3b: 7,11-bis(3-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



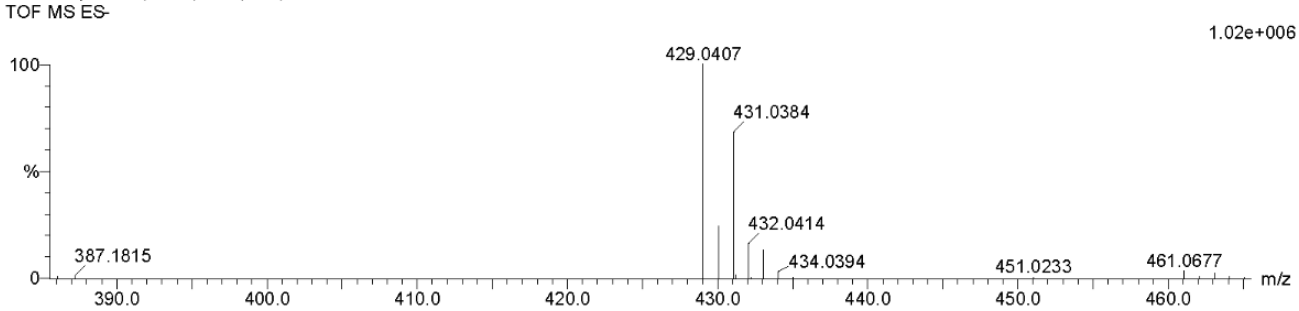
Infrared Spectrum of Compound B-3b: 7,11-bis(3-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



Elemental Composition Report **Page 1**

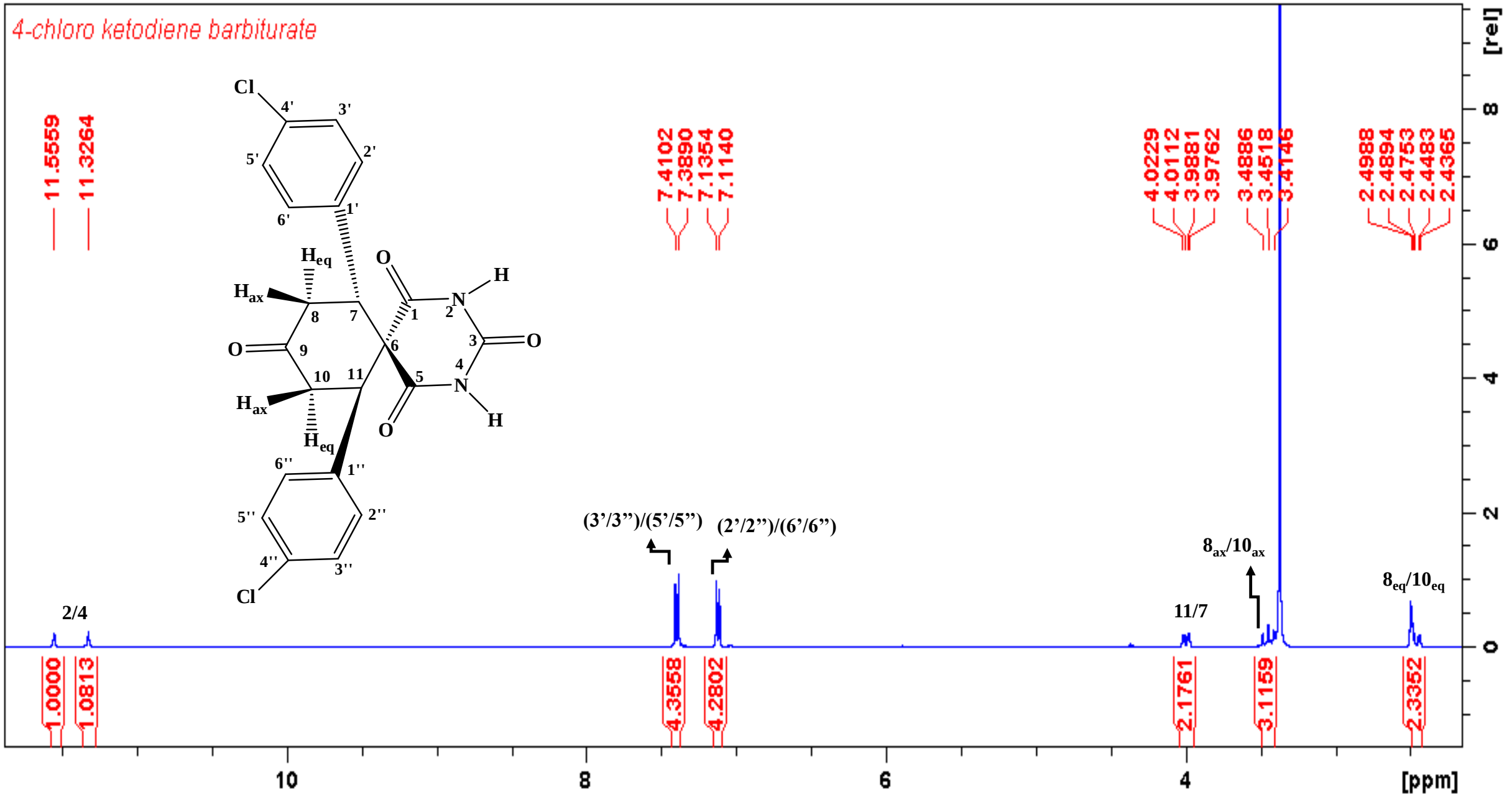
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
43 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 0-5 Cl: 0-2
15 3-Cl spiro 59 (1.957) Cm (1:61)
TOF MS ES-

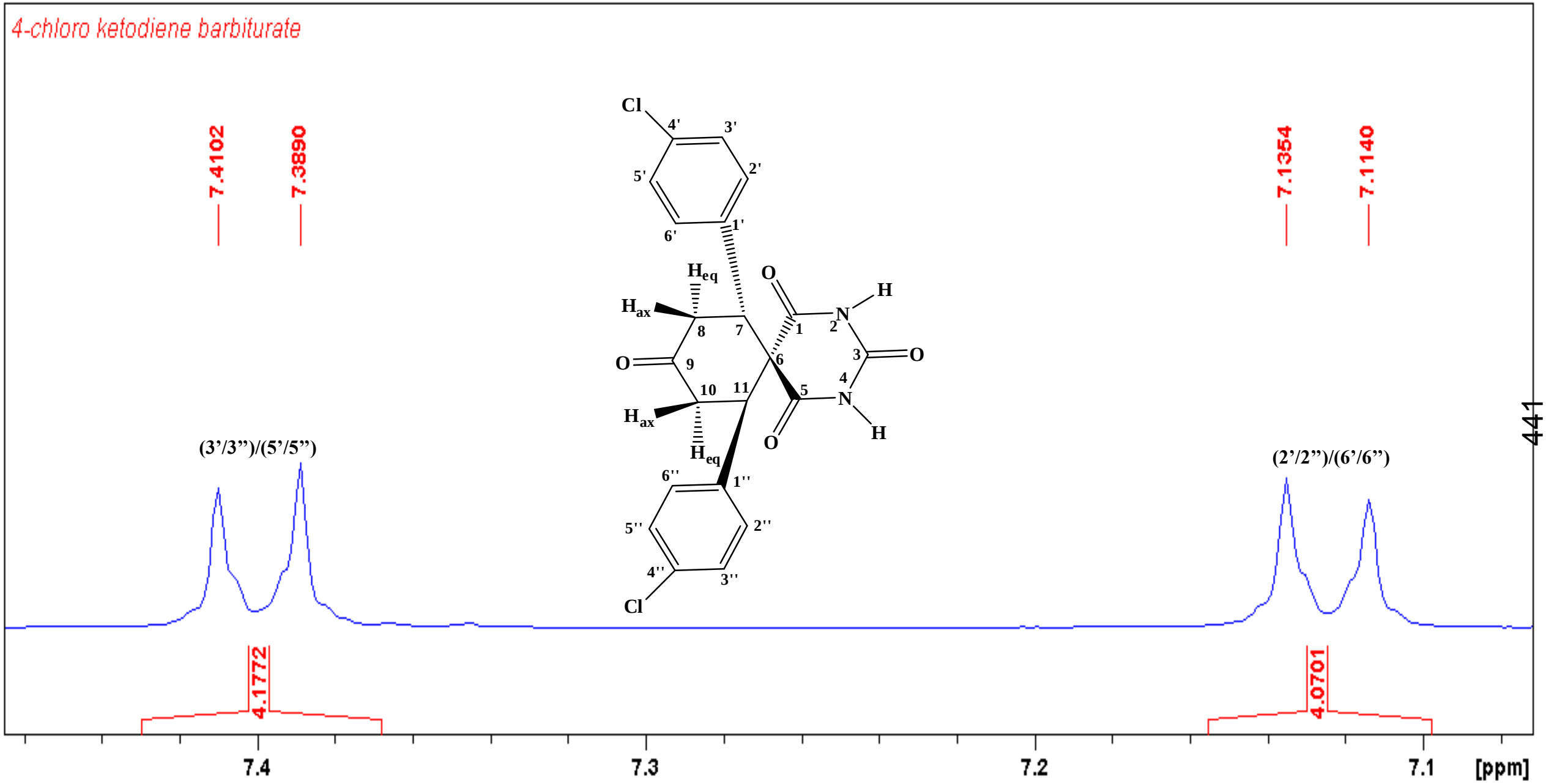


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
429.0407	429.0409	-0.2	-0.5	14.5	12.2	0.0	C21 H15 N2 O4 Cl2

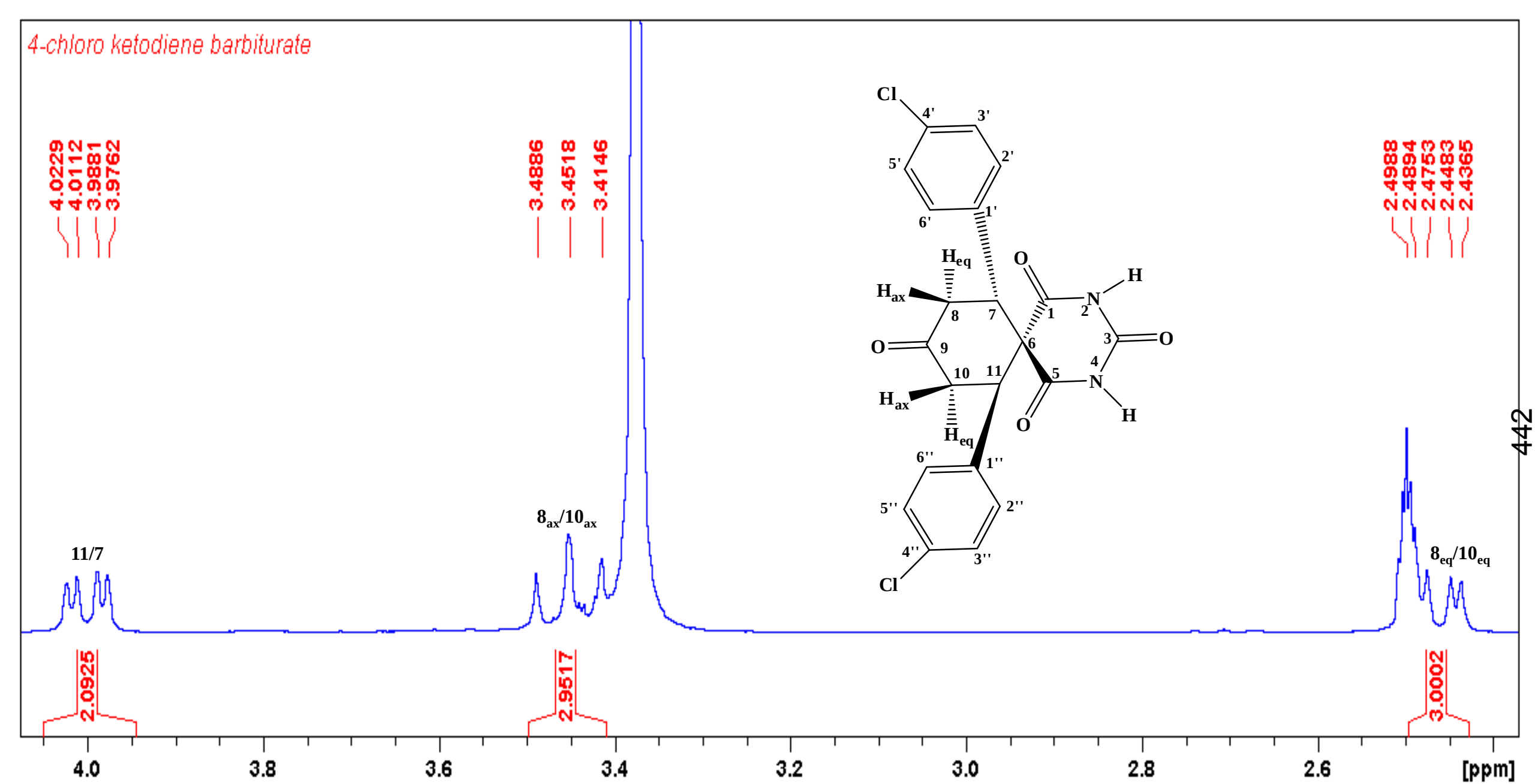
HRMS of Compound B-3b: 7,11-bis(3-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



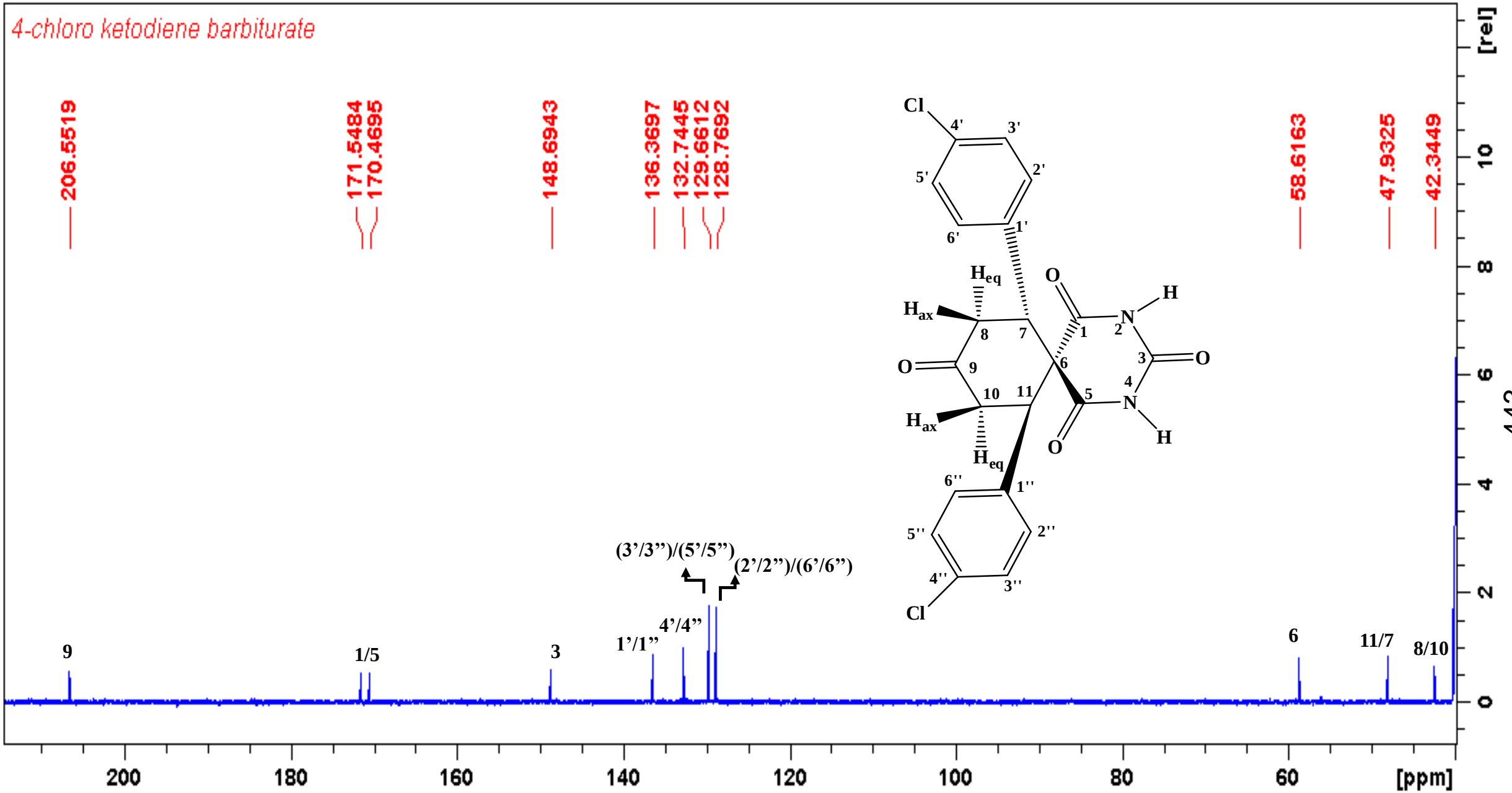
^{1}H Spectrum of Compound B-3c: 7,11-bis(4-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



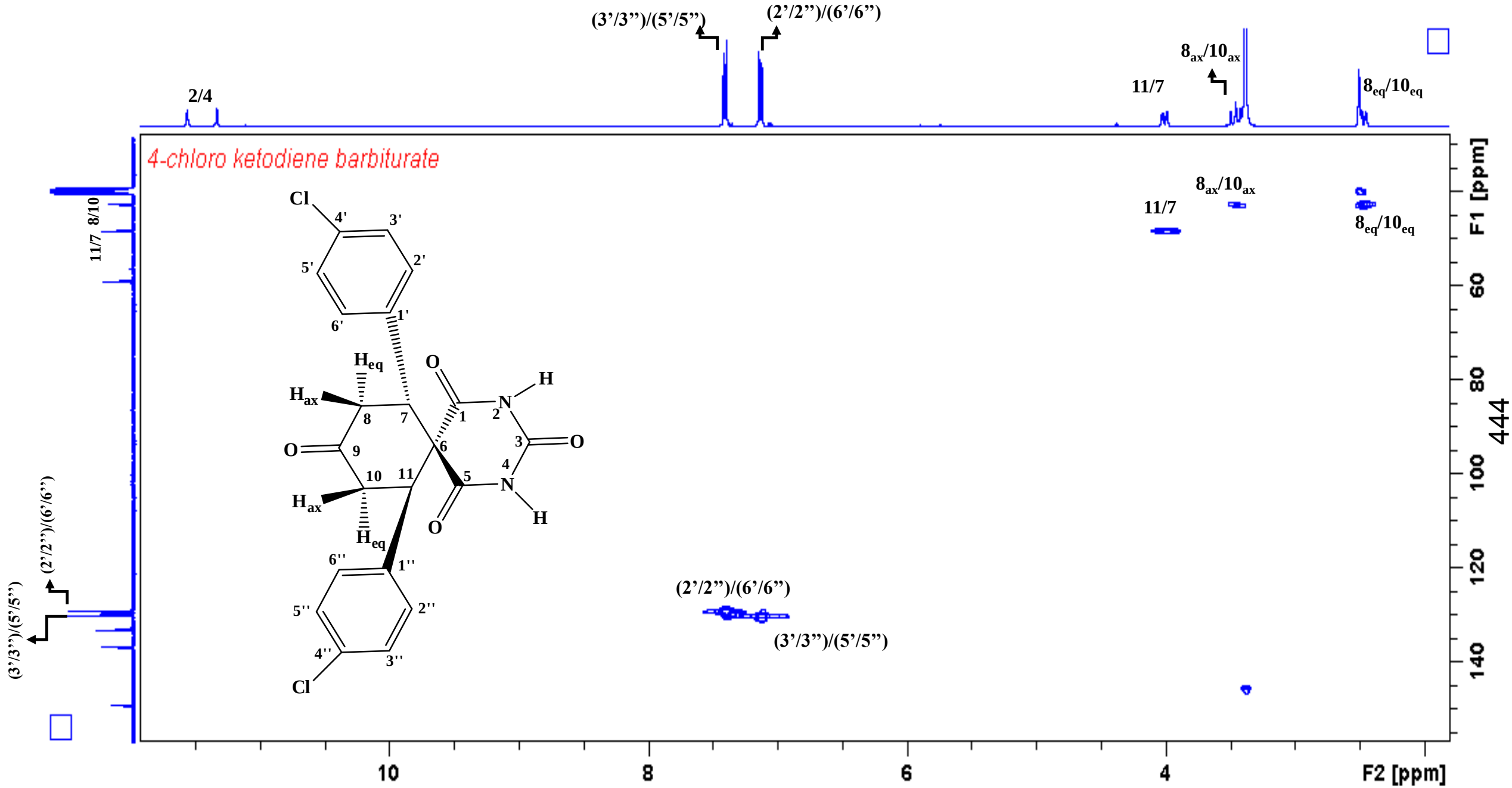
Expanded ^{1}H Spectrum of Compound B-3c: 7,11-bis(4-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



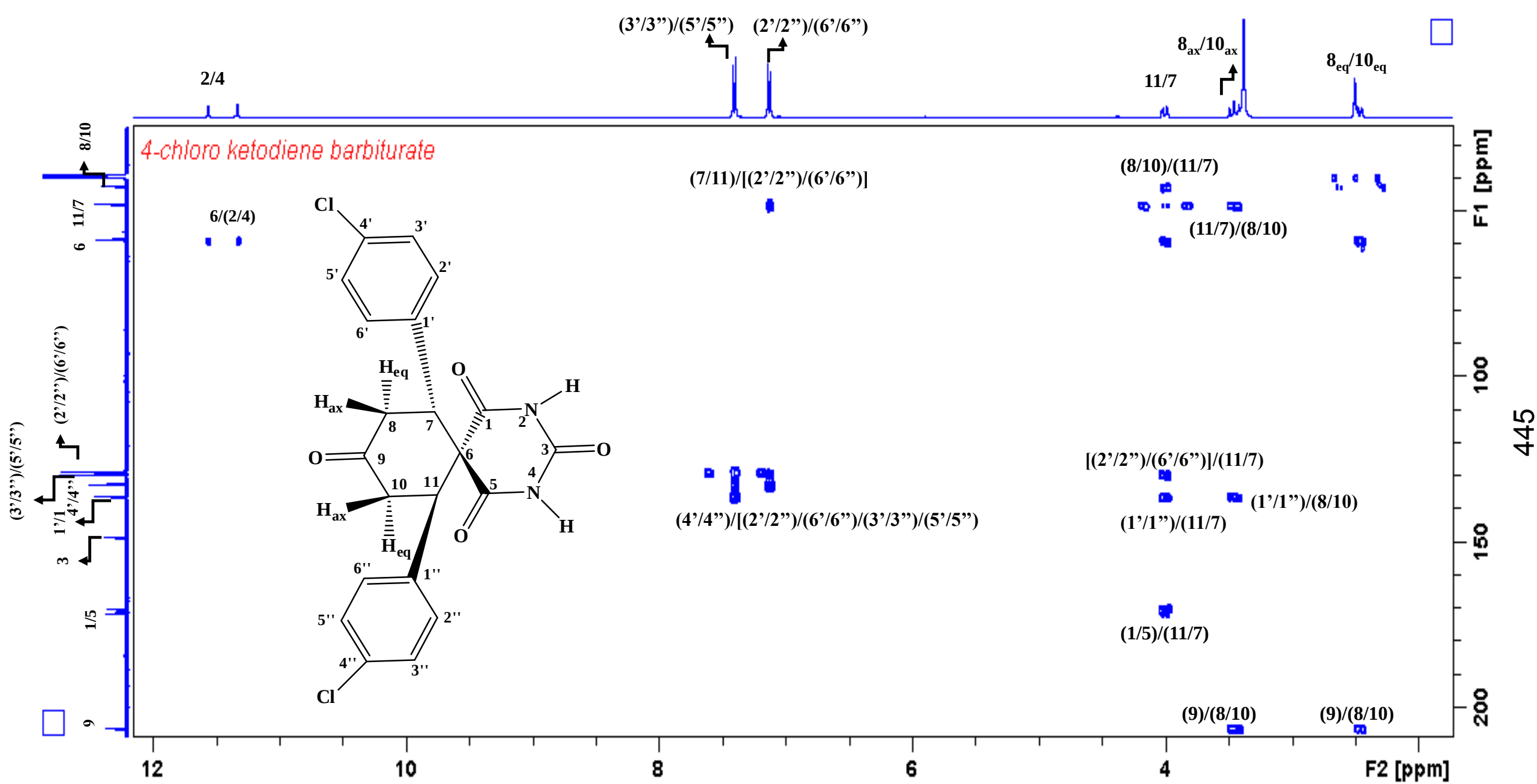
Expanded ^{1}H Spectrum of Compound B-3c: 7,11-bis(4-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



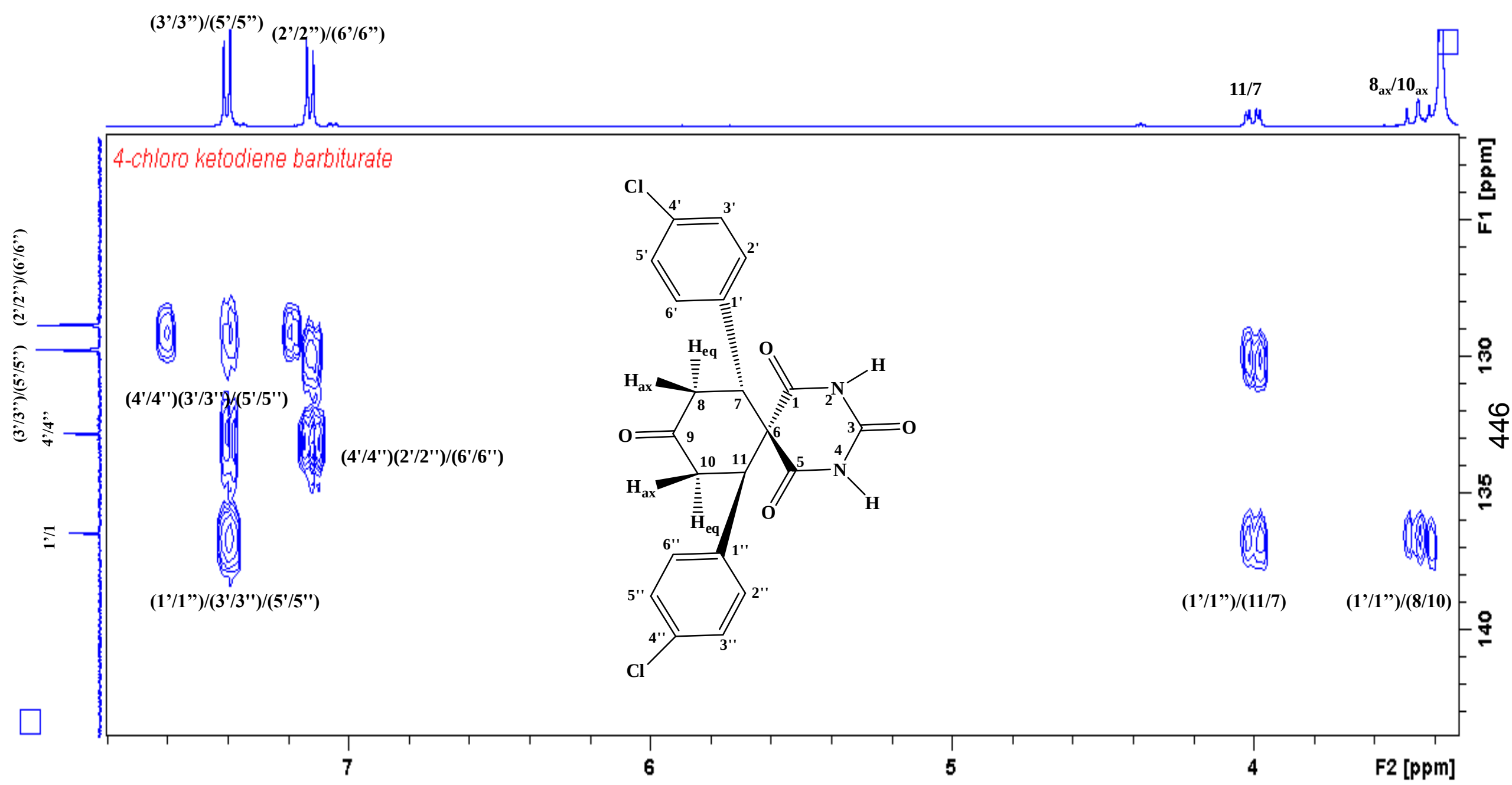
^{13}C Spectrum of Compound B-3c: 7,11-bis(4-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



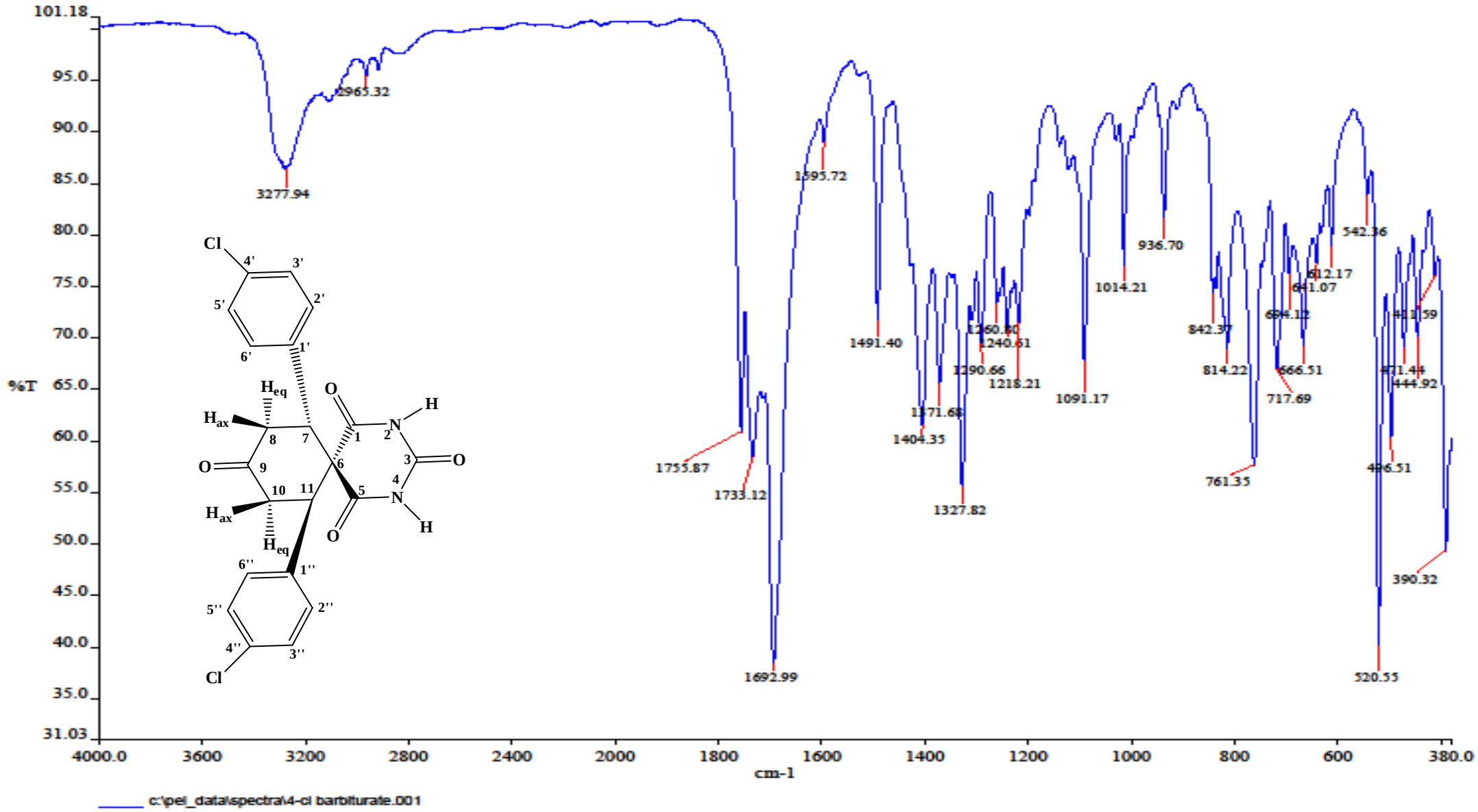
HSQC of Compound B-3c: 7,11-bis(4-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



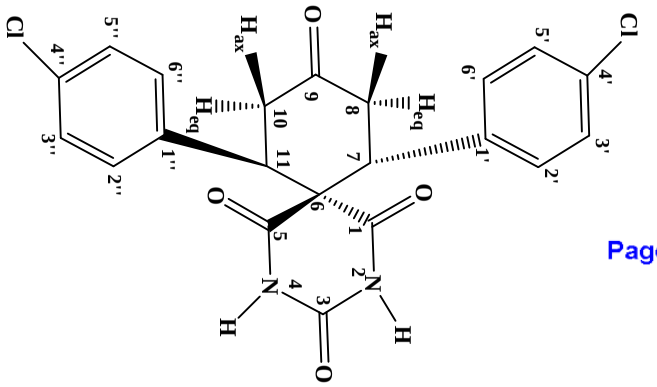
HMBC of Compound B-3c: 7,11-bis(4-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



Expanded HMBC of Compound B-3c: 7,11-bis(4-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



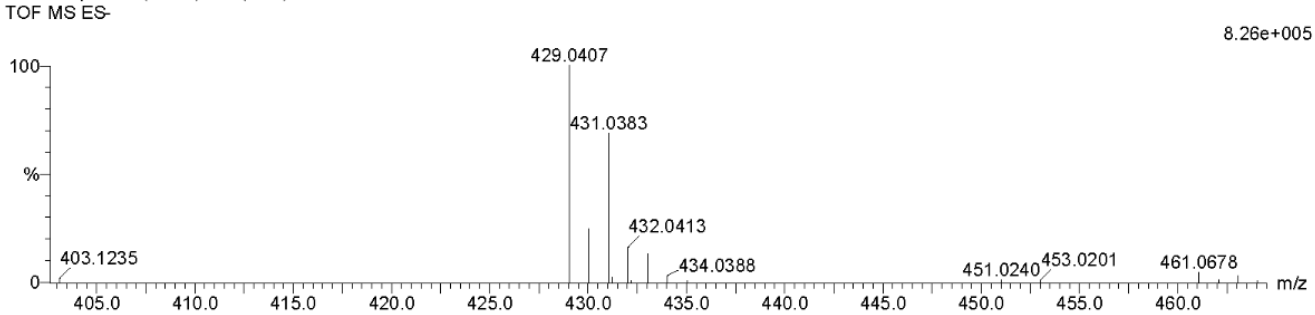
Infrared Spectrum of Compound B-3c: 7,11-bis(4-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



Elemental Composition Report

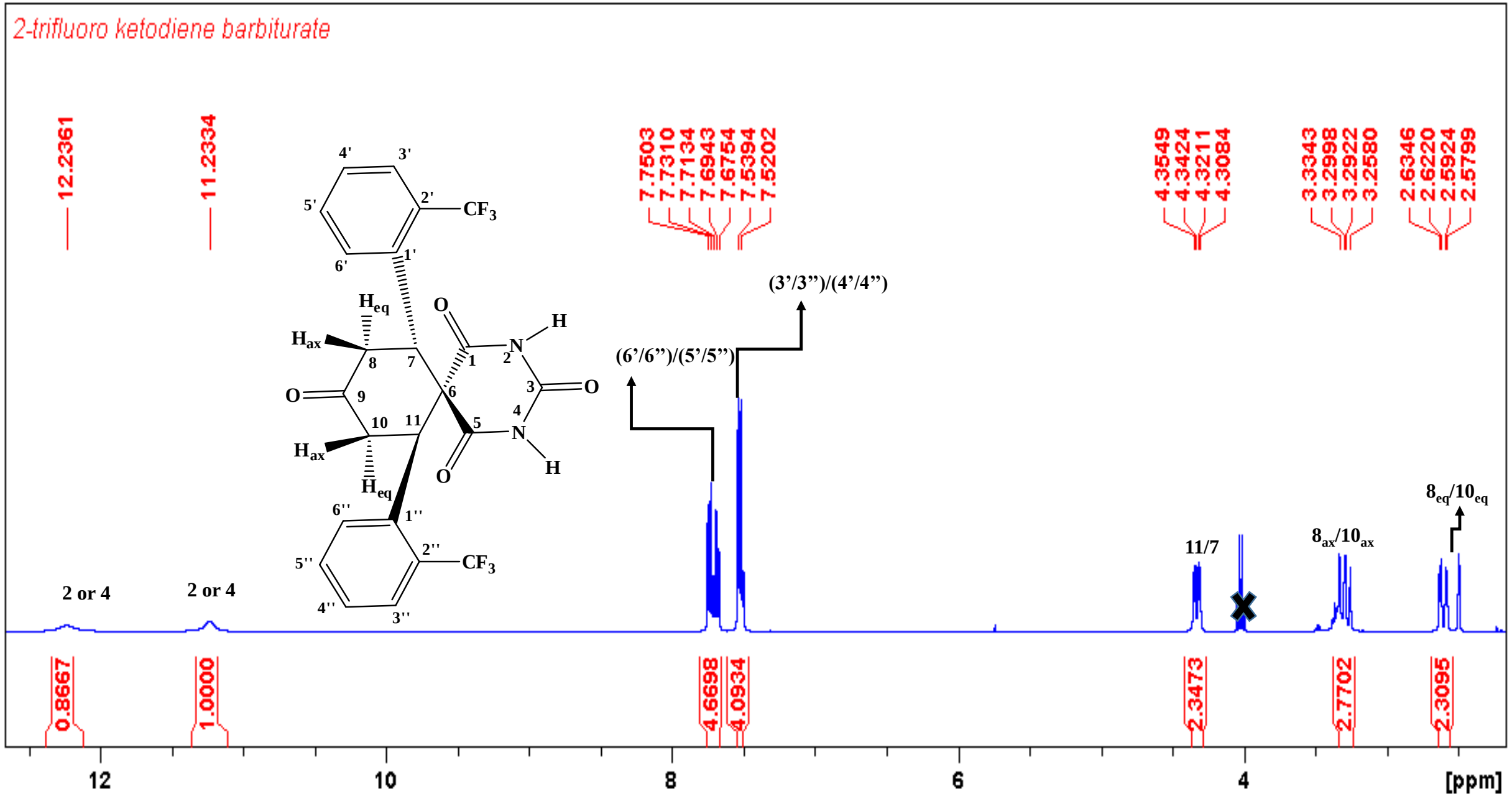
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
43 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 0-5 Cl: 0-2
16 4-Cl spiro 38 (1.248) Cm (1:61)
TOF MS ES-

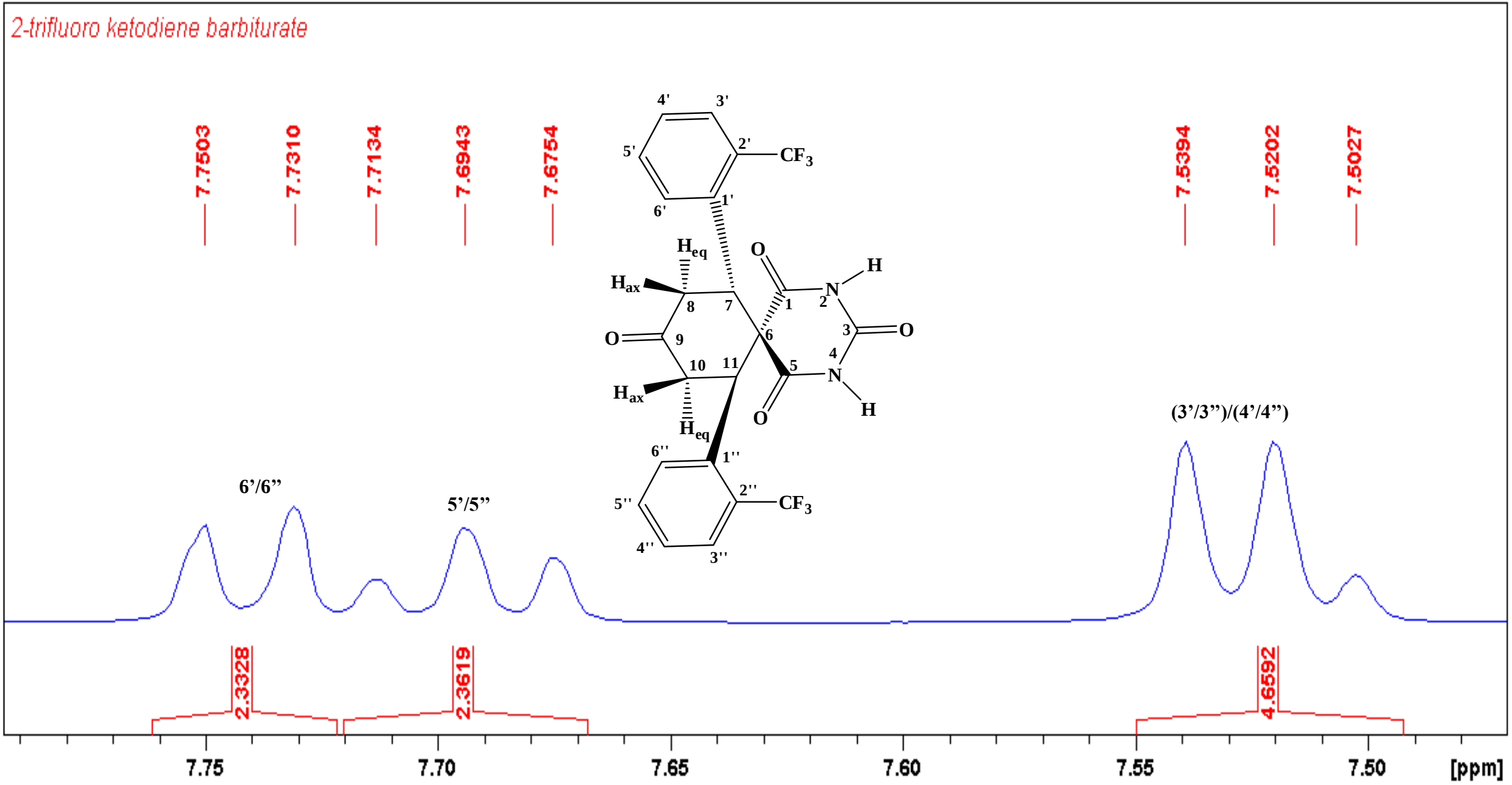


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
429.0407	429.0409	-0.2	-0.5	14.5	9.6	0.0	C21 H15 N2 O4 Cl2

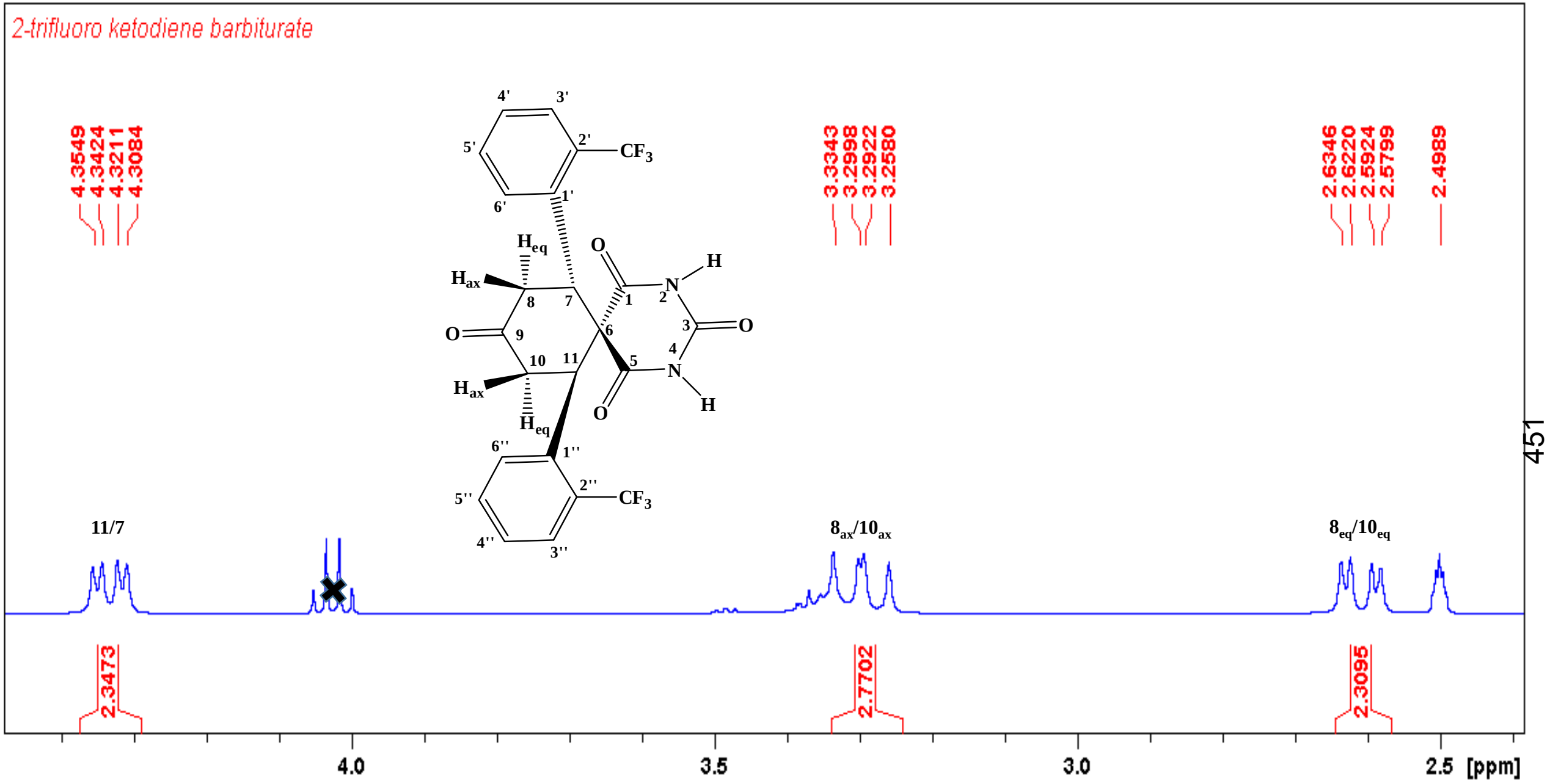
HRMS of Compound B-3c: 7,11-bis(4-chlorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



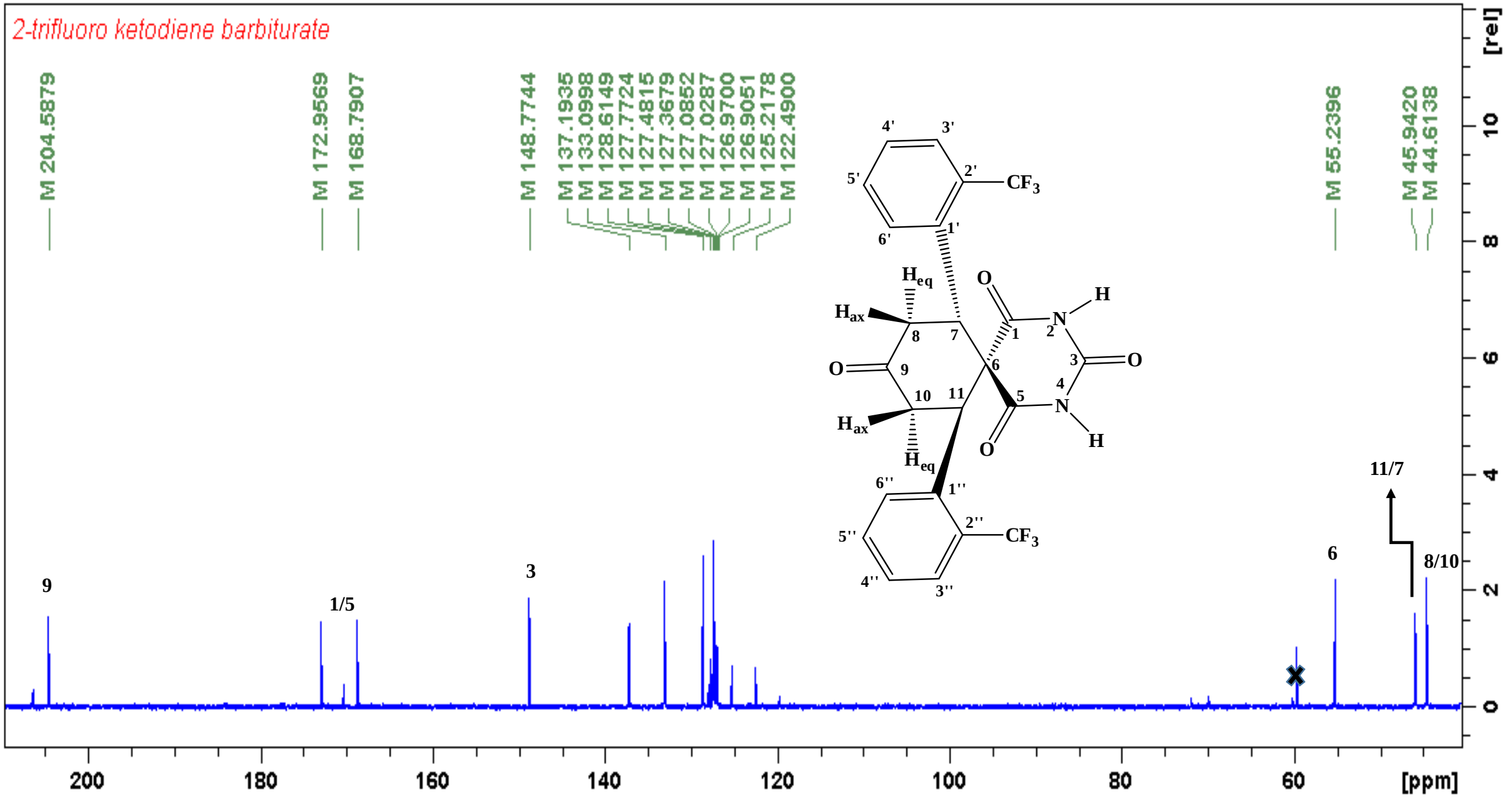
^{1}H Spectrum of Compound B-3d: 7,11-bis(2-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



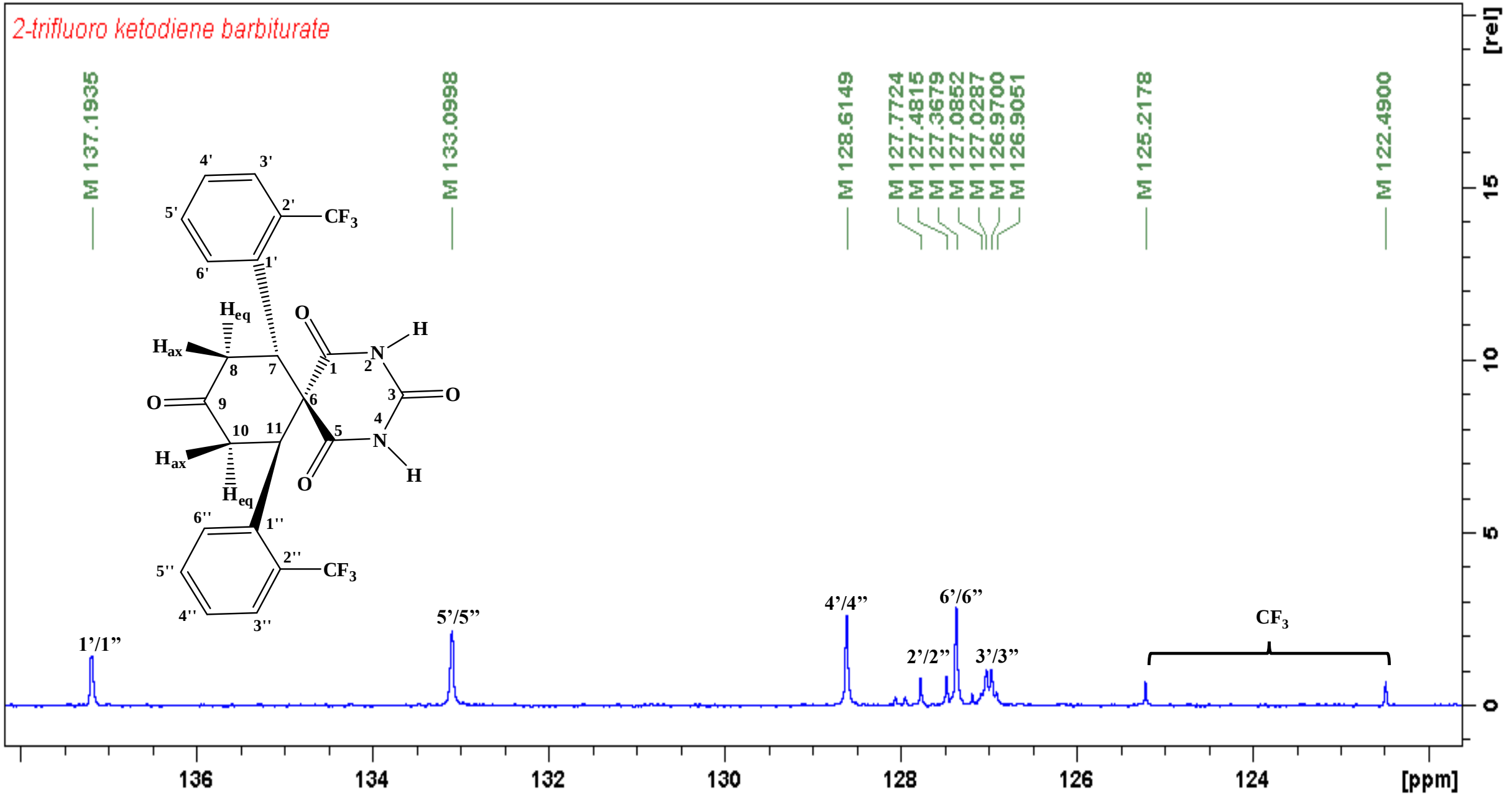
Expanded ^{1}H Spectrum of Compound B-3d: 7,11-bis(2-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



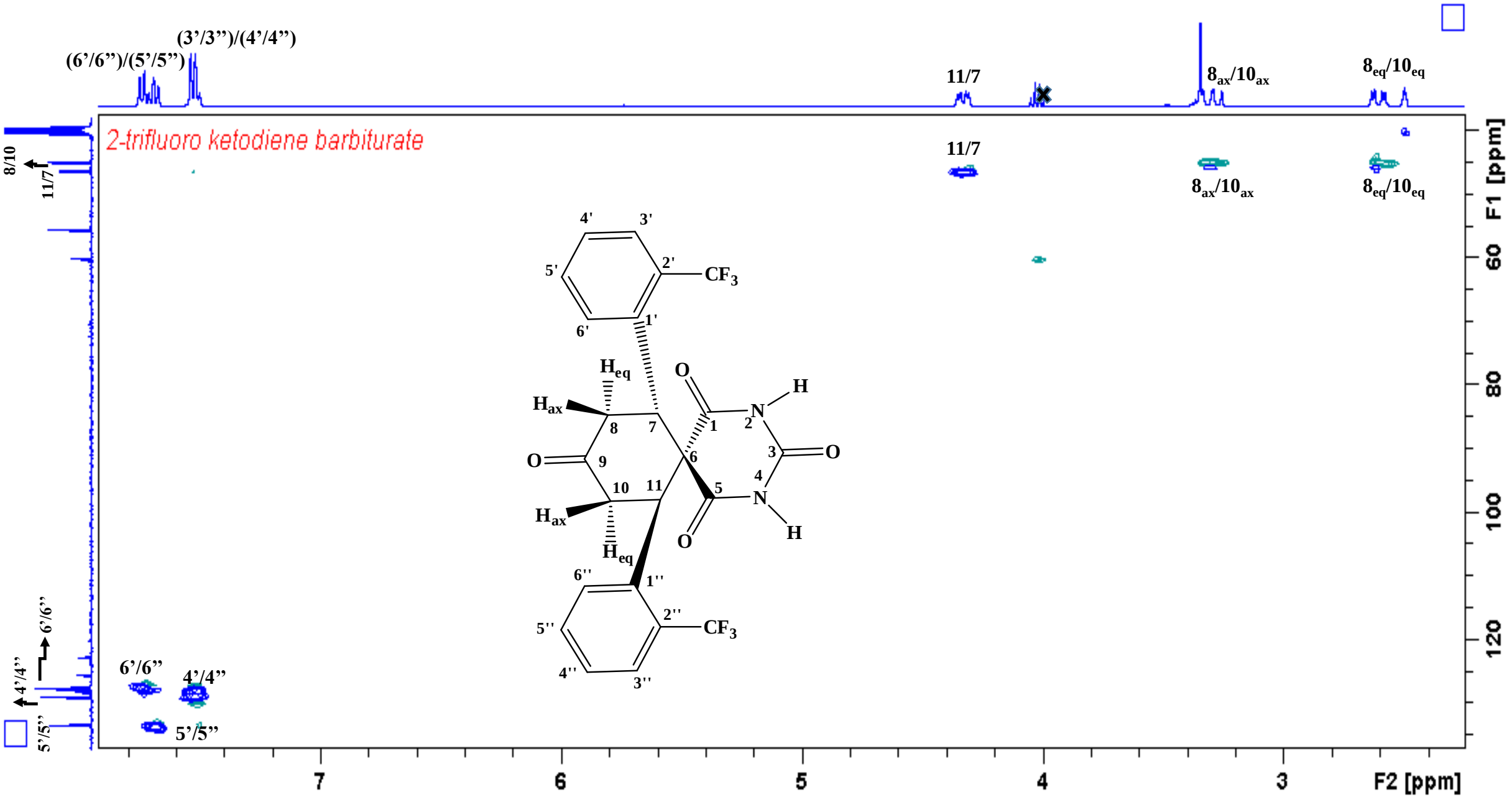
Expanded ^{1}H Spectrum of Compound B-3d: 7,11-bis(2-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



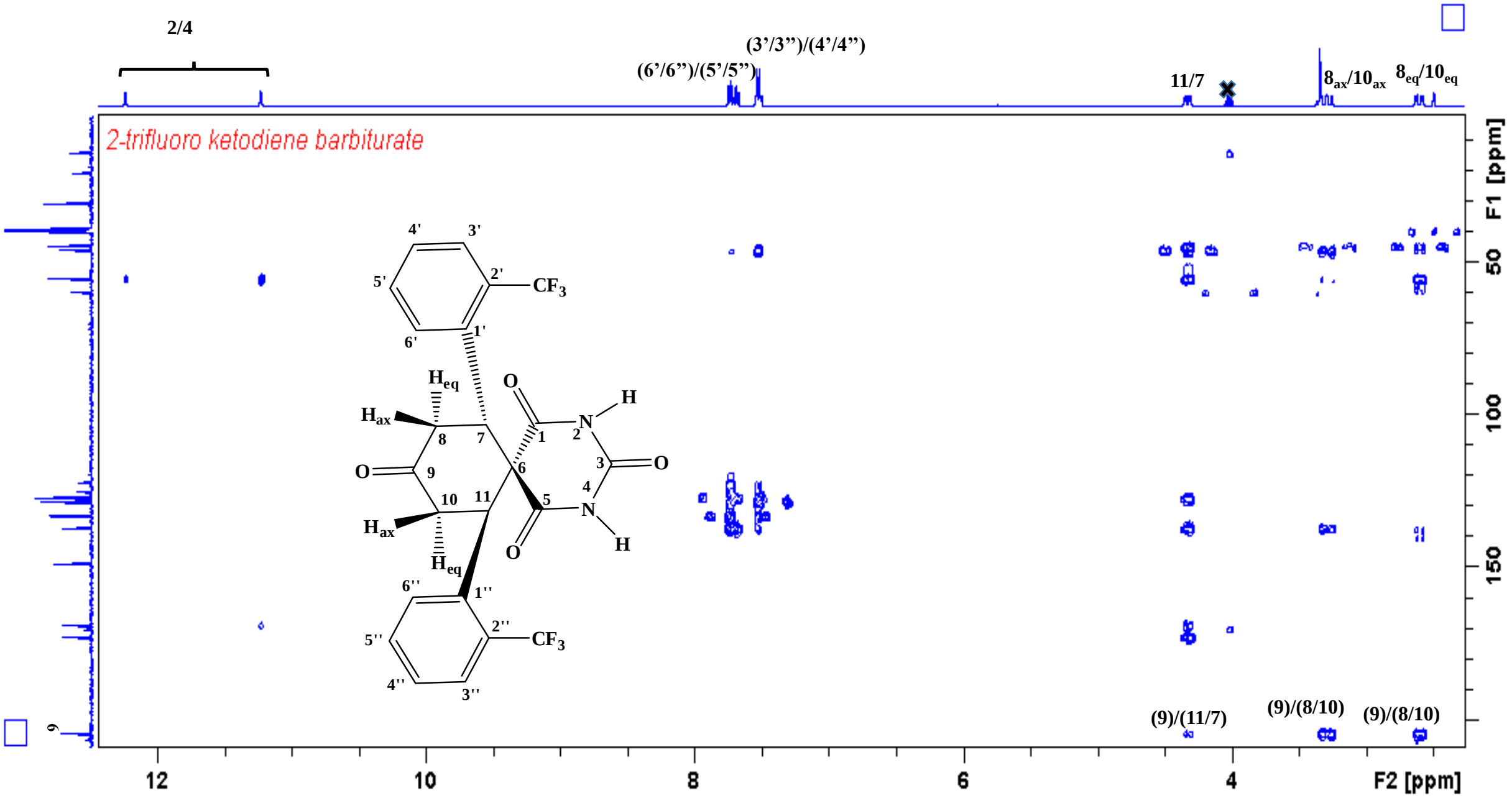
^{13}C Spectrum of Compound B-3d: 7,11-bis(2-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



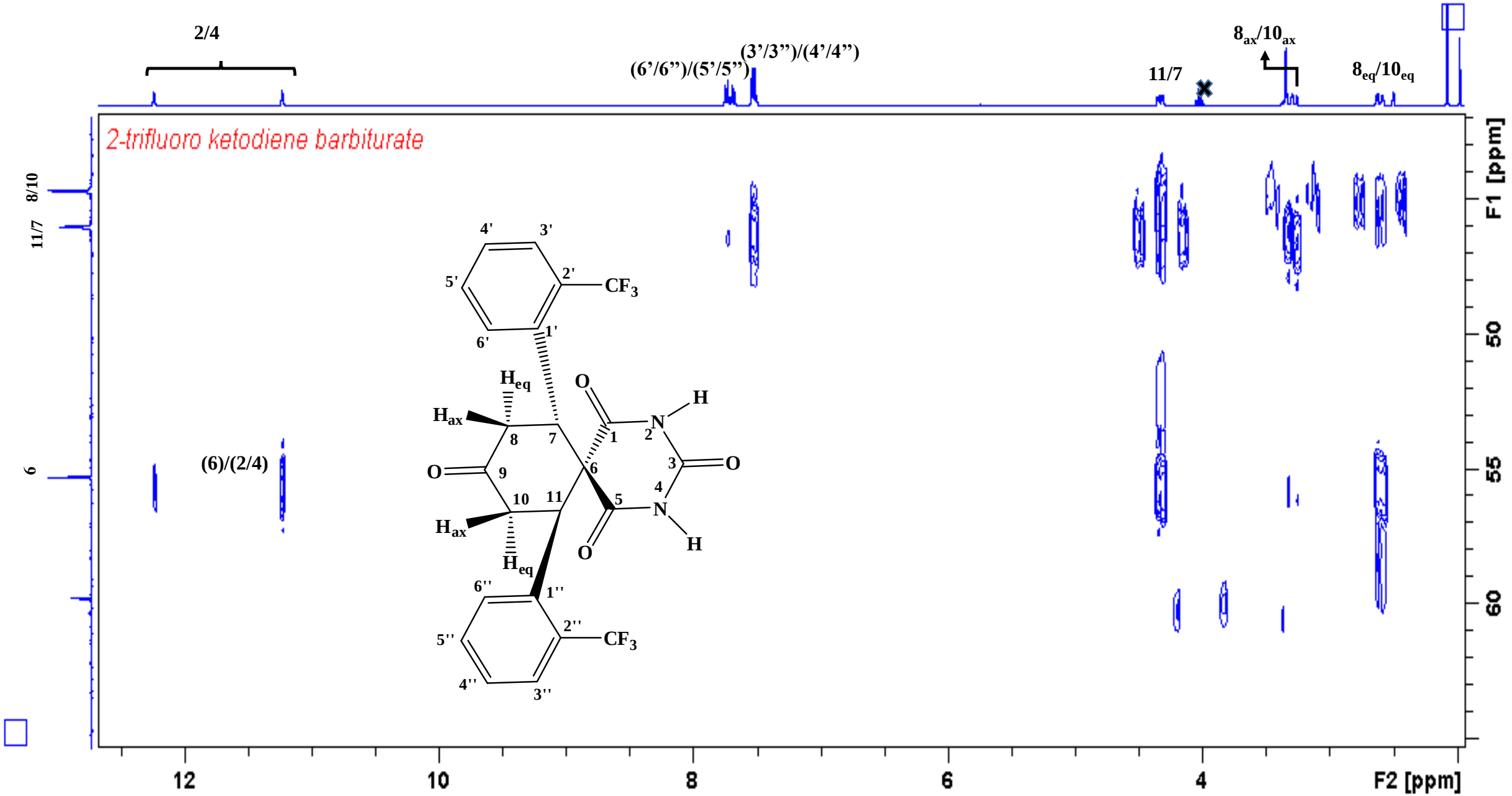
Expanded ^{13}C Spectrum of Compound B-3d: 7,11-bis(2-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



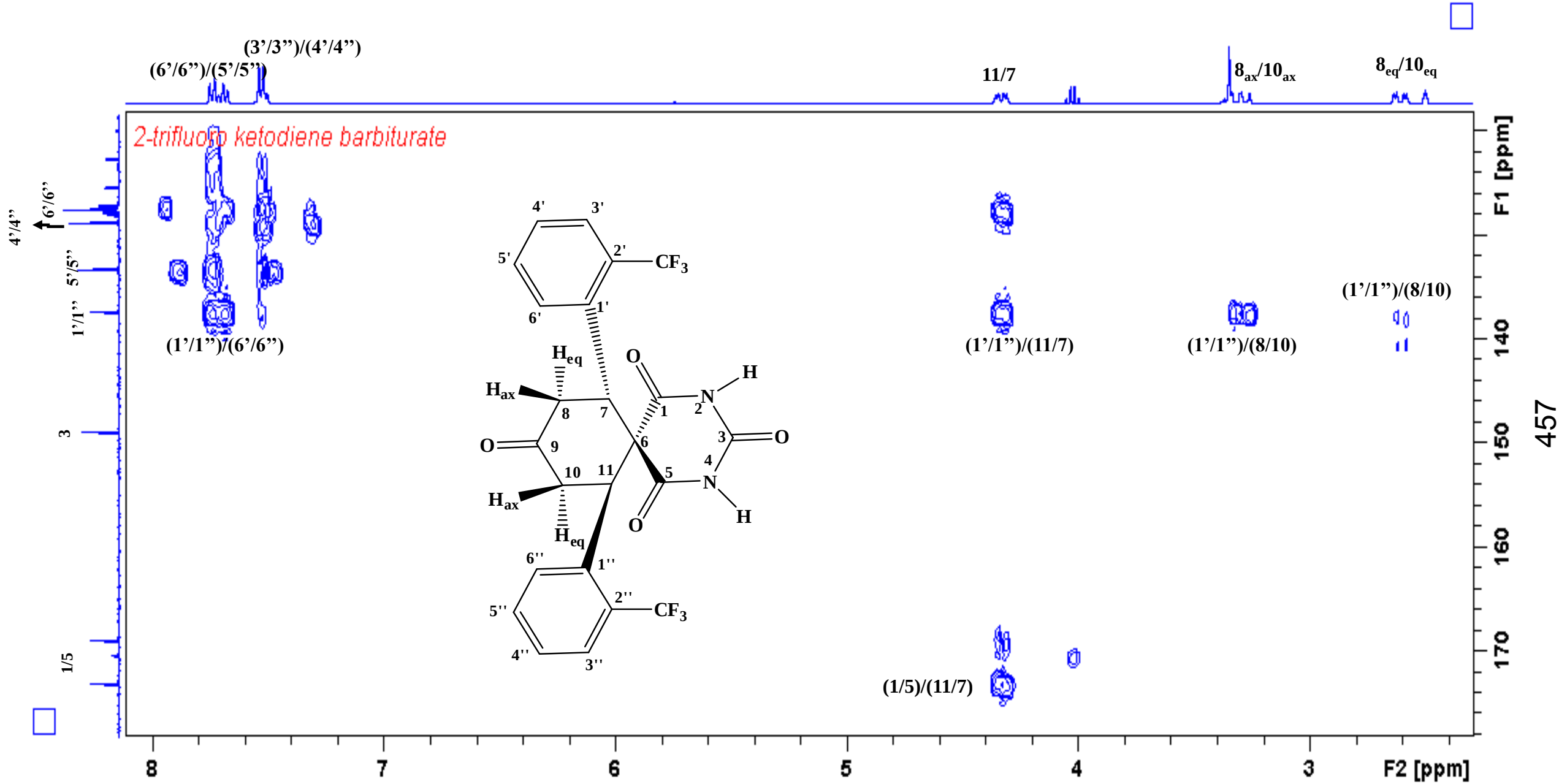
HSQC of Compound B-3d: 7,11-bis(2-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



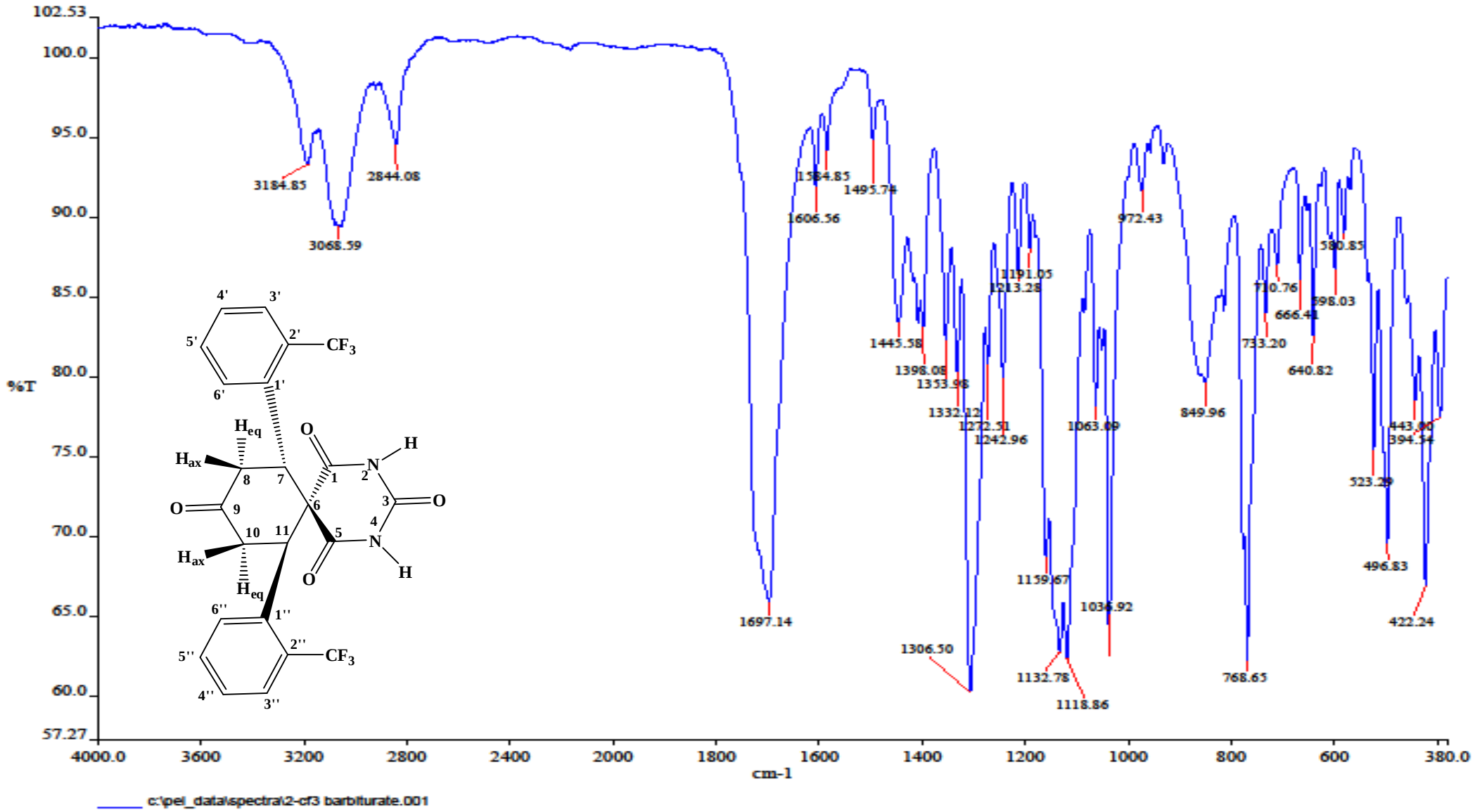
HMBC of Compound B-3d: 7,11-bis(2-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



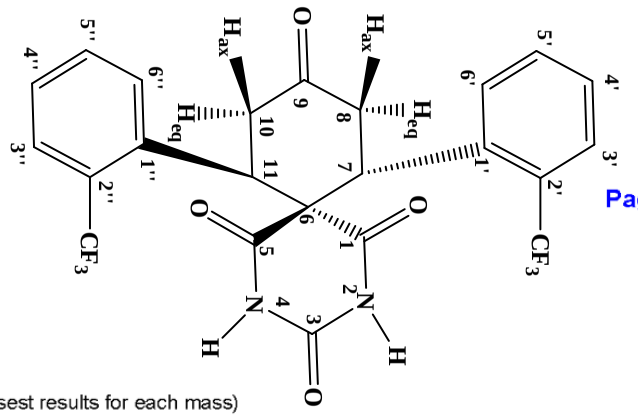
Expanded HMBC of Compound B-3d: 7,11-bis(2-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



Expanded HMBC of Compound B-3d: 7,11-bis(2-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



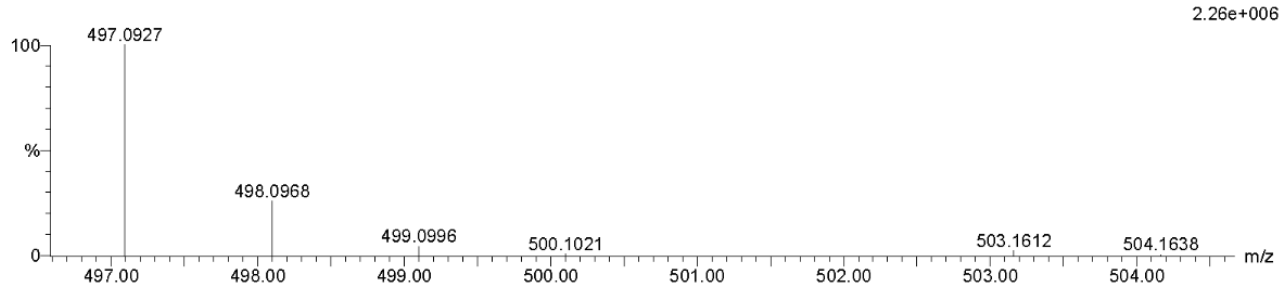
Infrared Spectrum of Compound B-3d: 7,11-bis(2-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



Elemental Composition Report

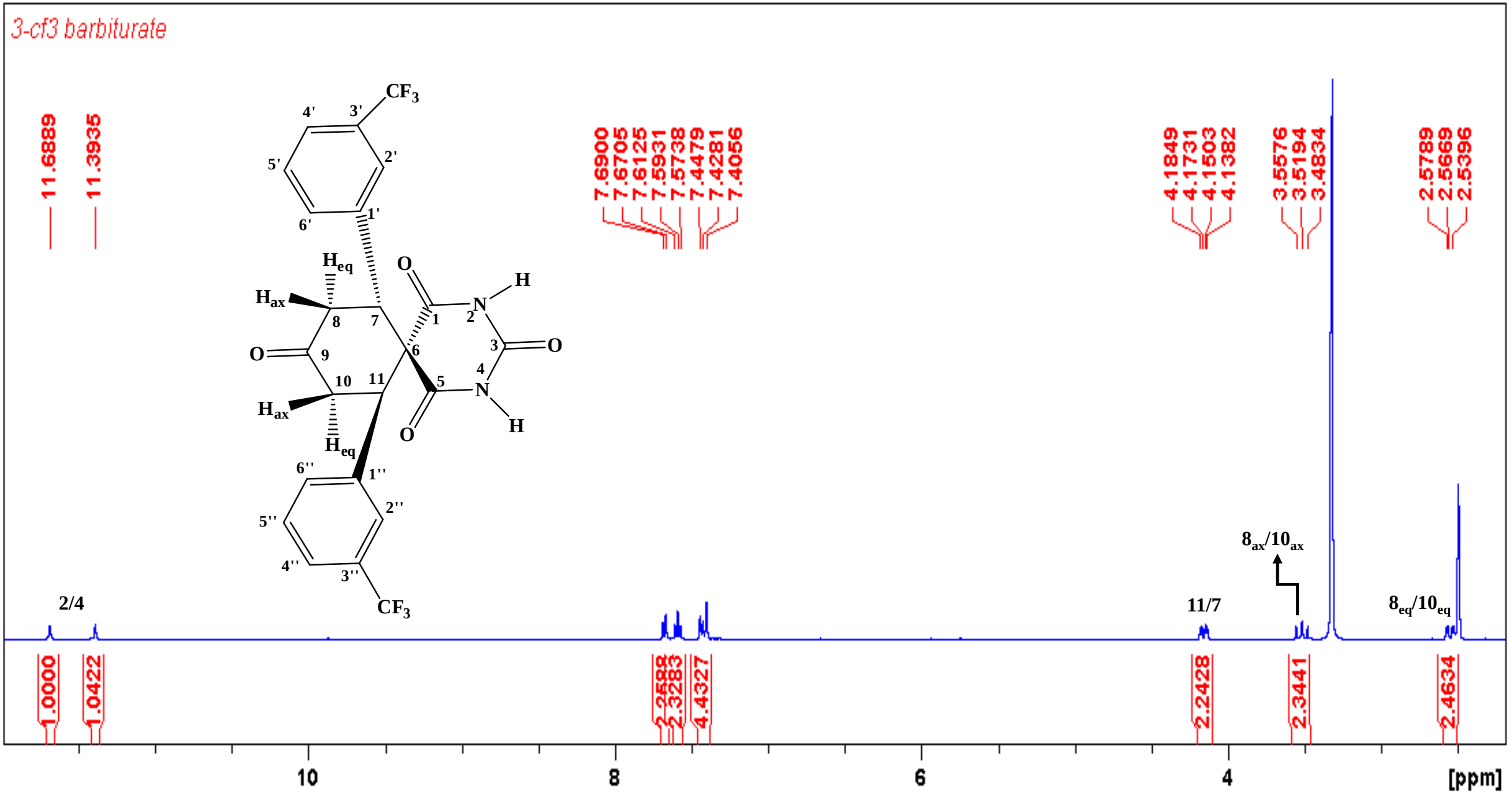
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
23 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 0-5 F: 5-6
17 2-CF3 spiro 61 (2.024) Cm (1:61)
TOF MS ES-

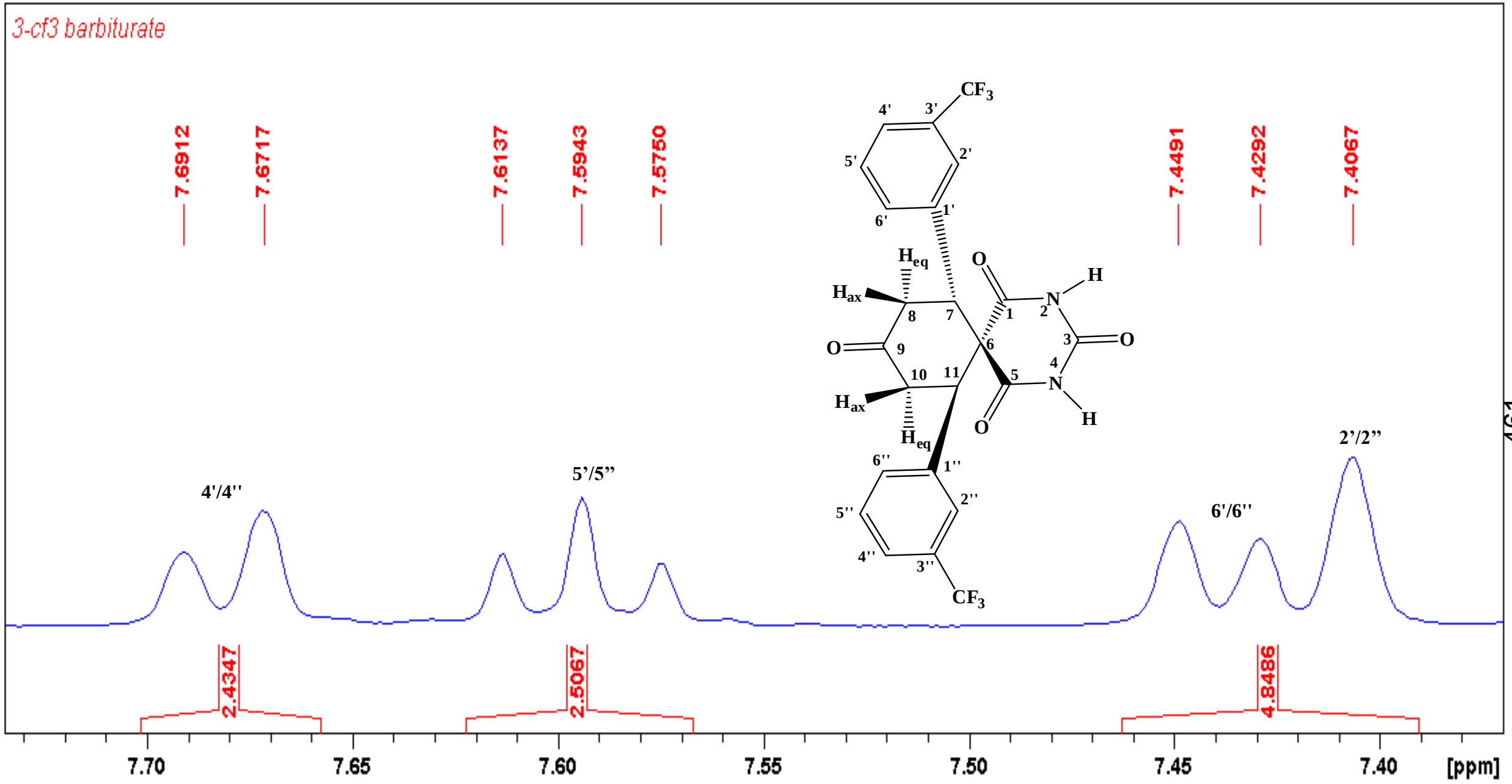


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
497.0927	497.0936	-0.9	-1.8	14.5	18.9	0.0	C23 H15 N2 O4 F6

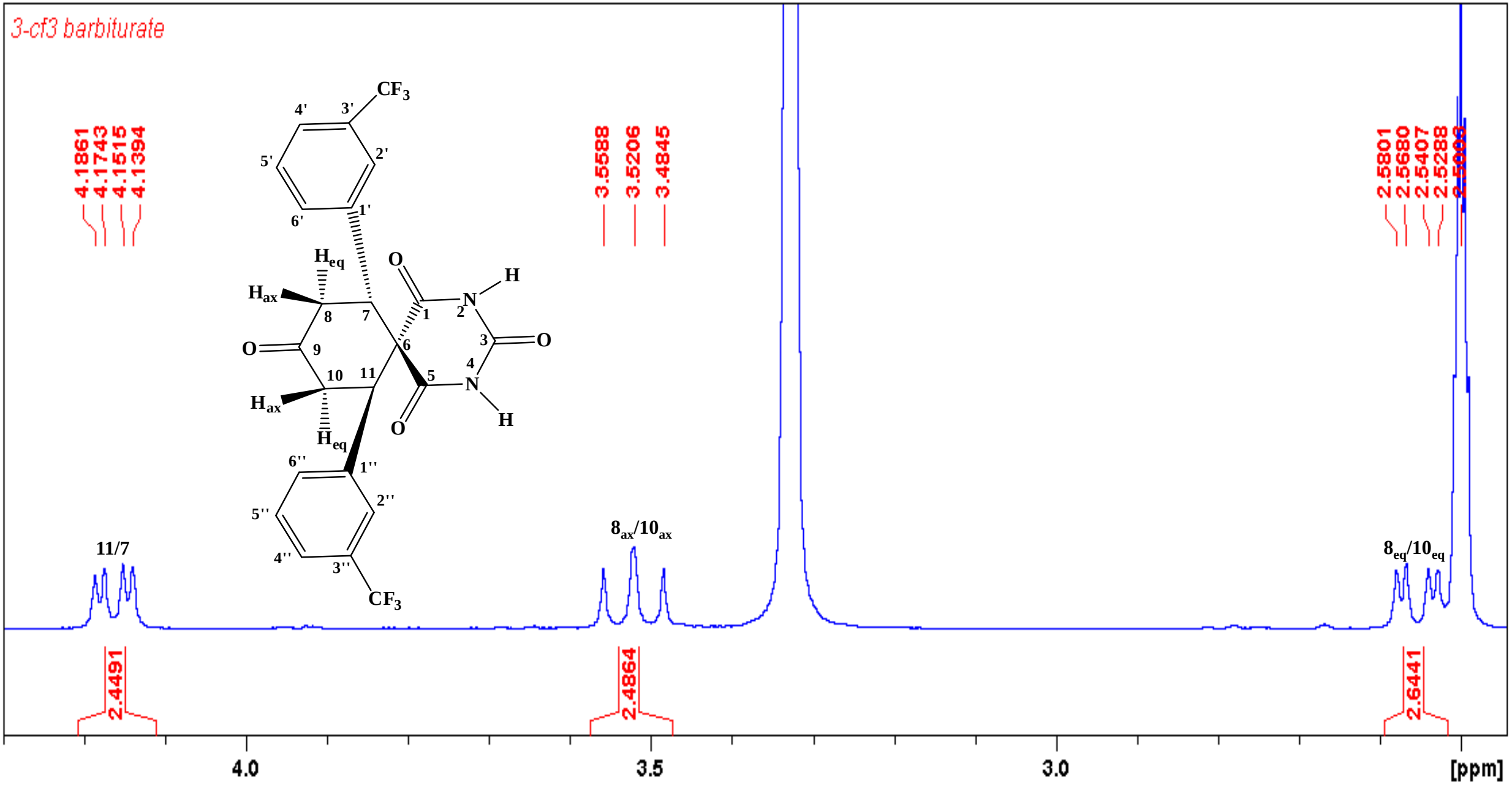
HRMS of Compound B-3d: 7,11-bis(2-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



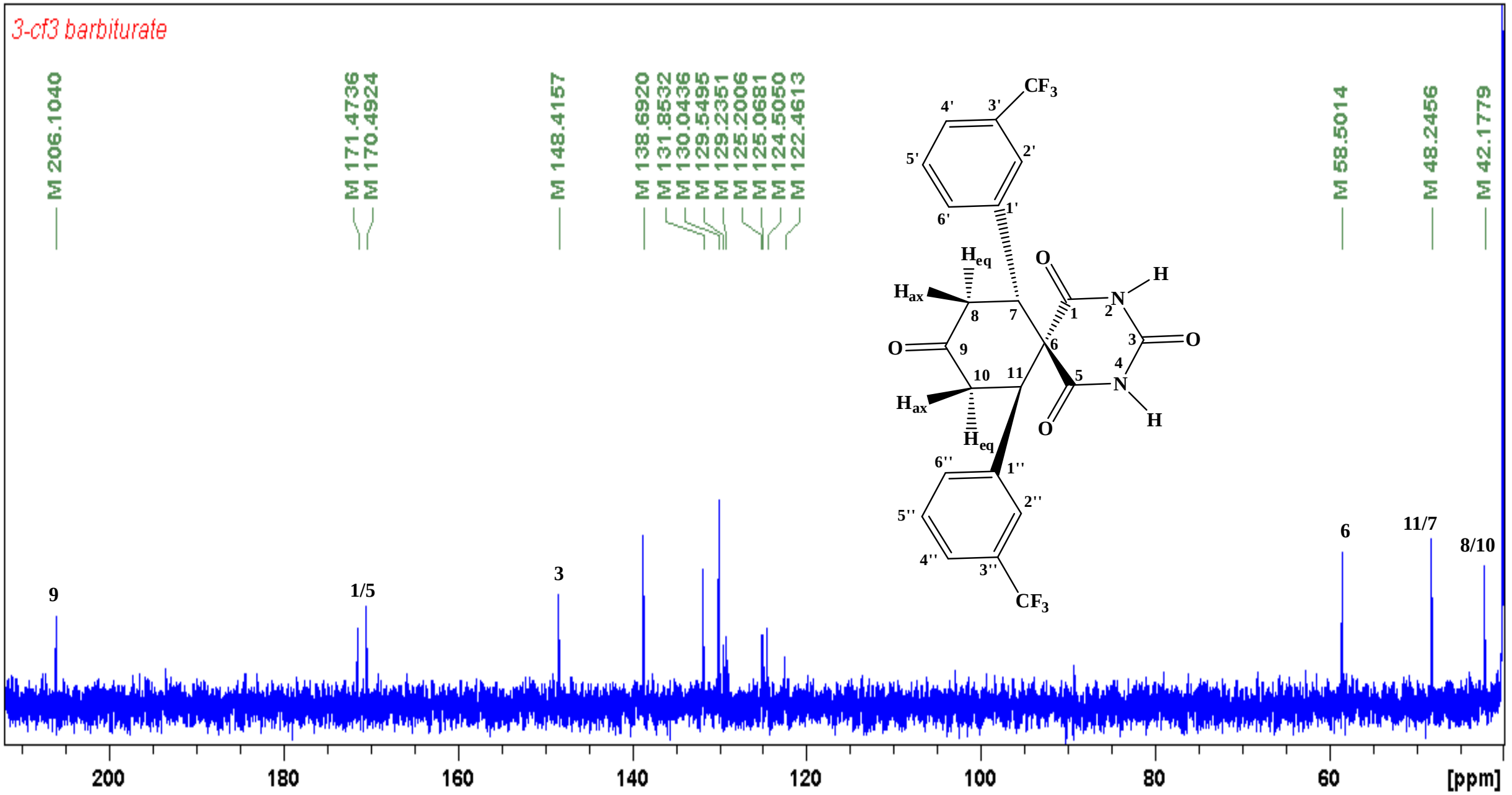
^{1}H Spectrum of Compound B-3e: 7,11-bis(3-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



Expanded ^{1}H Spectrum of Compound B-3e: 7,11-bis(3-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone

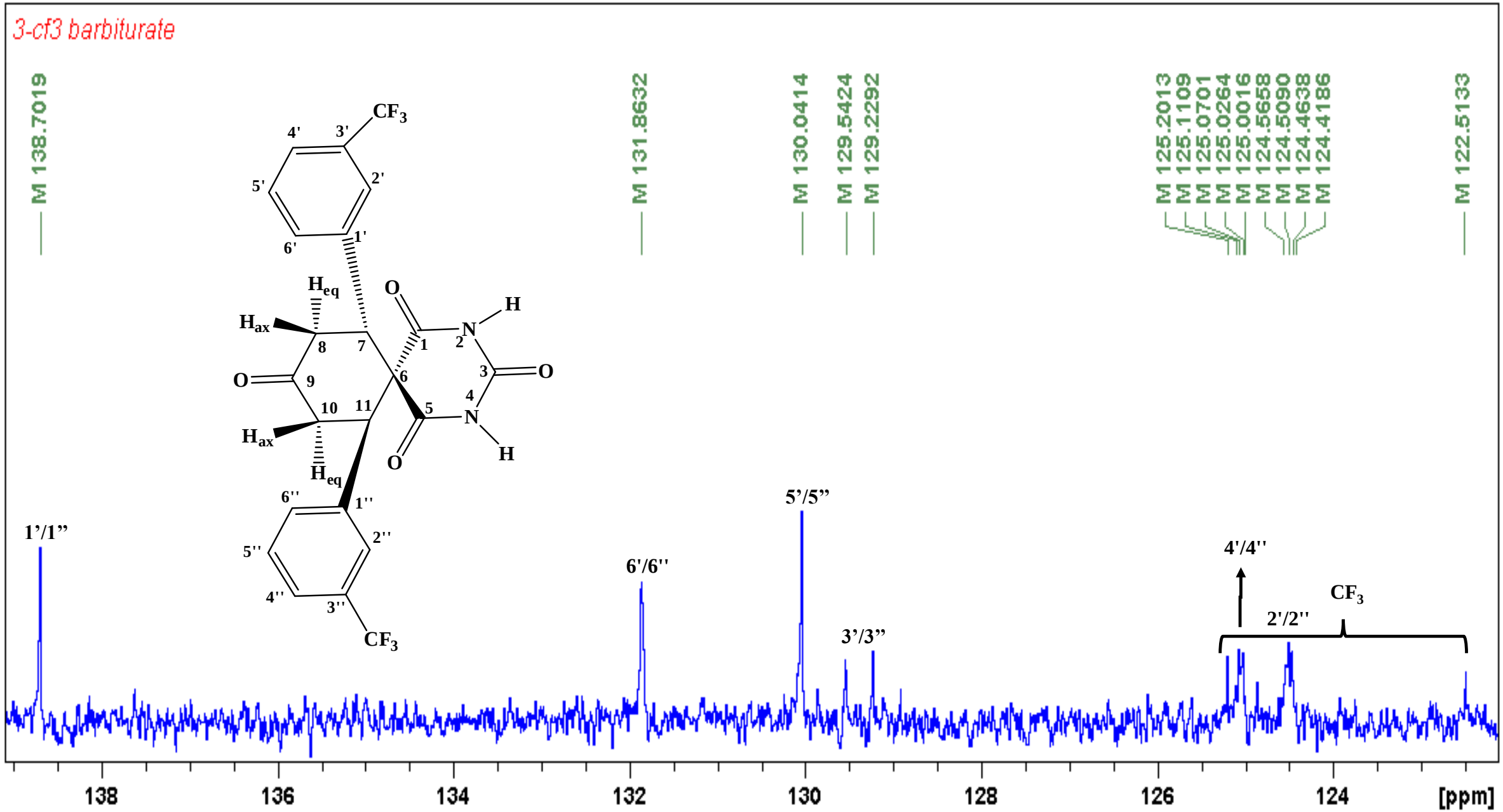


Expanded ^{1}H Spectrum of Compound B-3e: 7,11-bis(3-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone

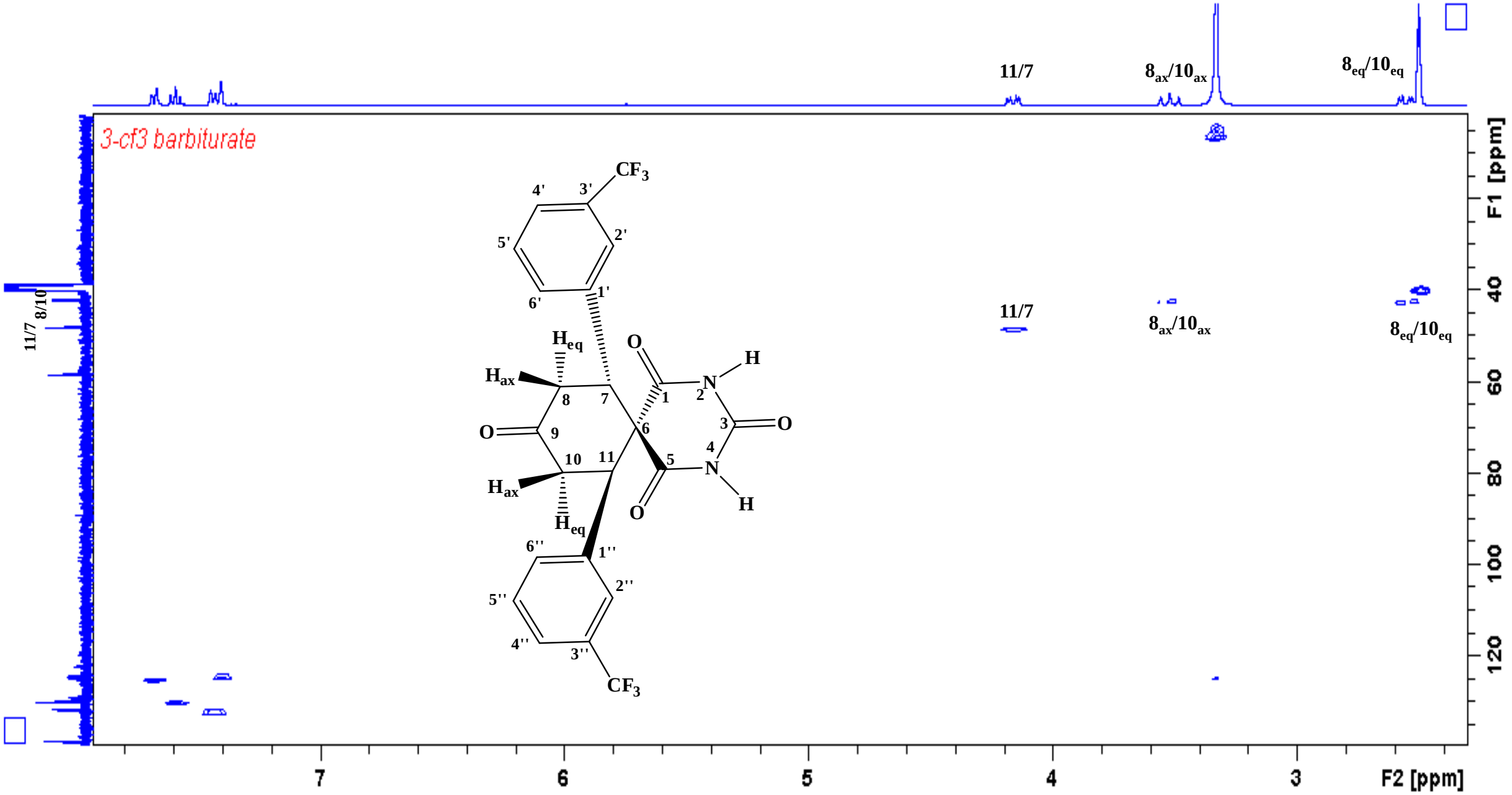


463

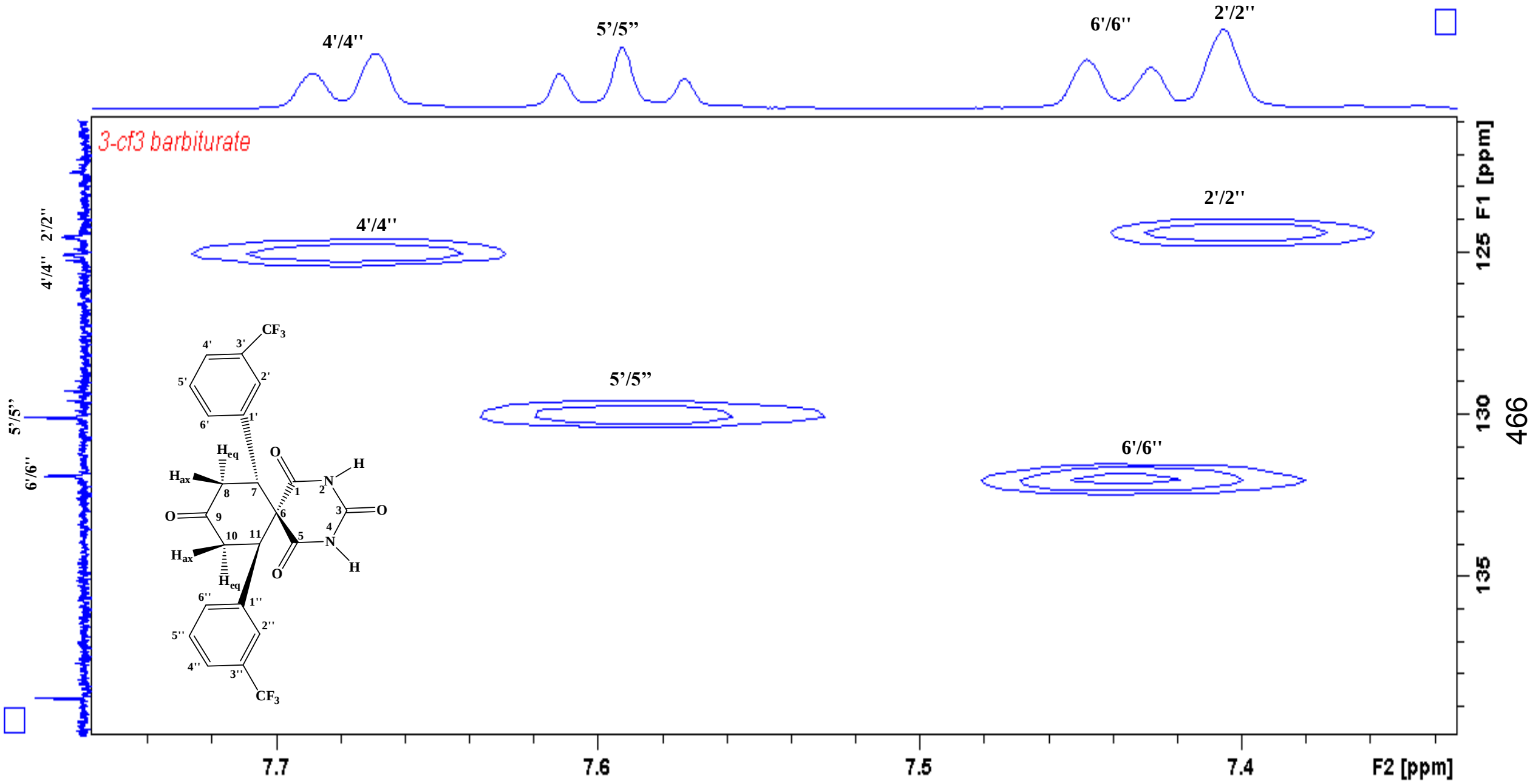
^{13}C Spectrum of Compound B-3e: 7,11-bis(3-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



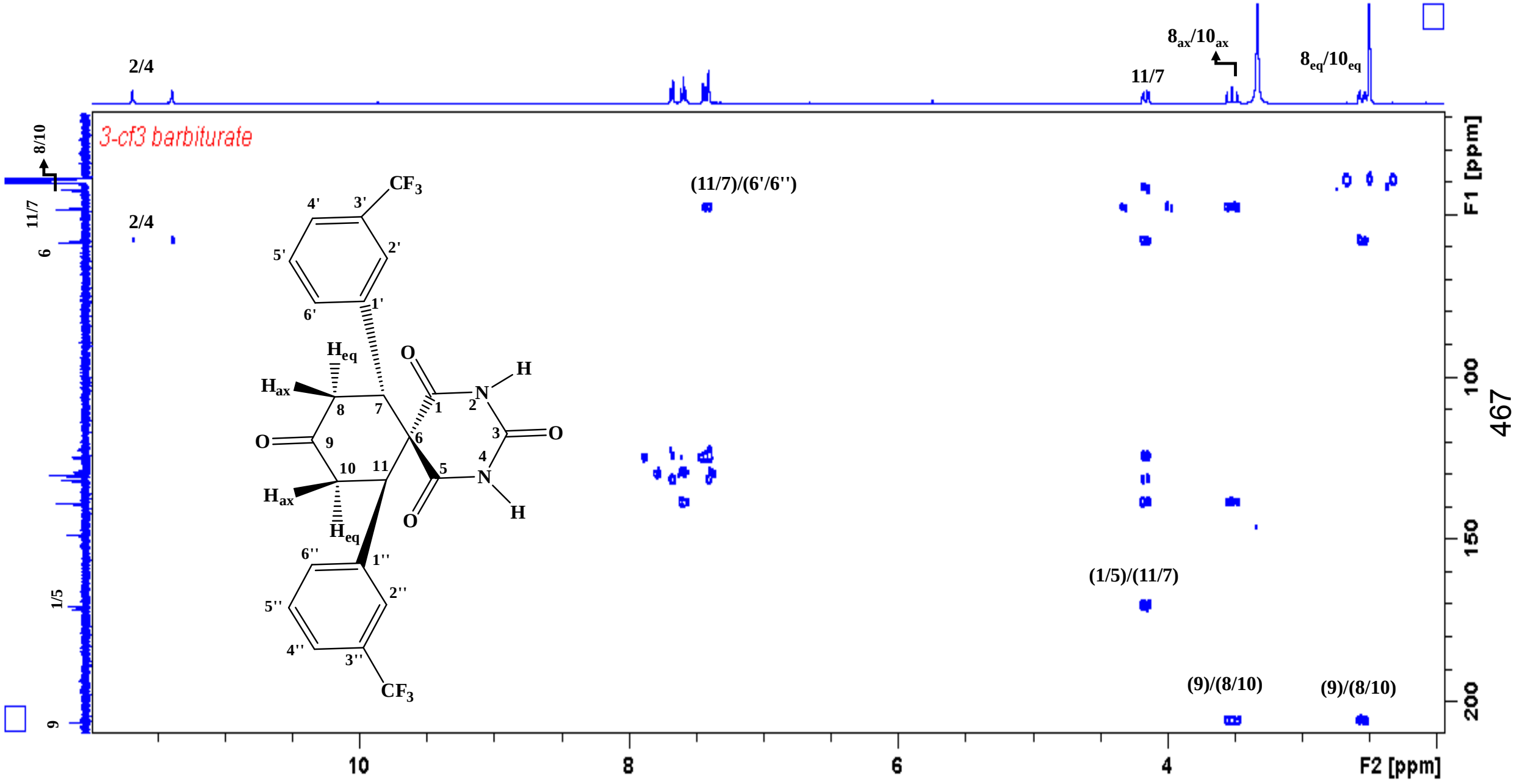
Expanded ^{13}C Spectrum of Compound B-3e: 7,11-bis(3-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



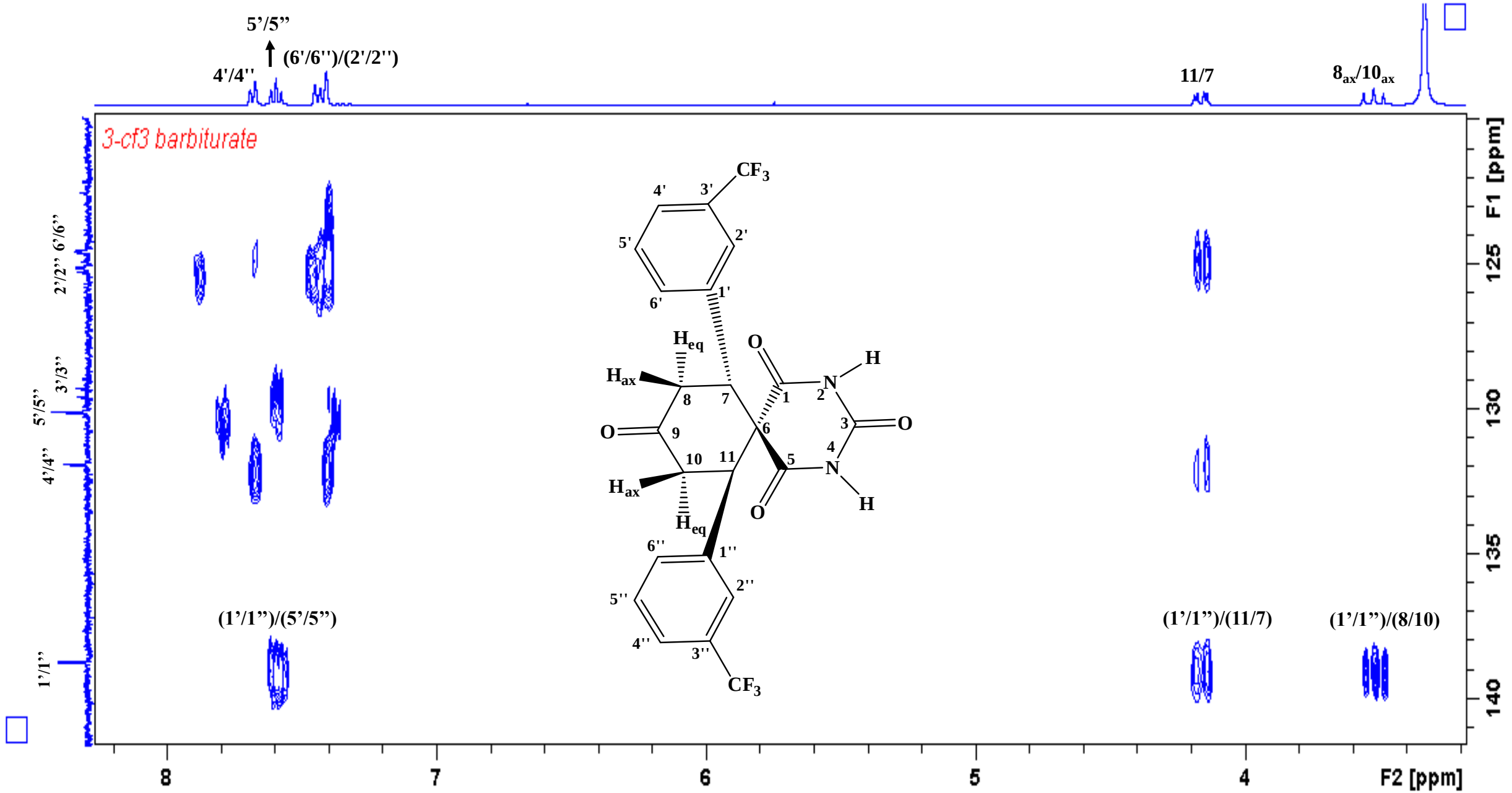
HSQC of Compound B-3e: 7,11-bis(3-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



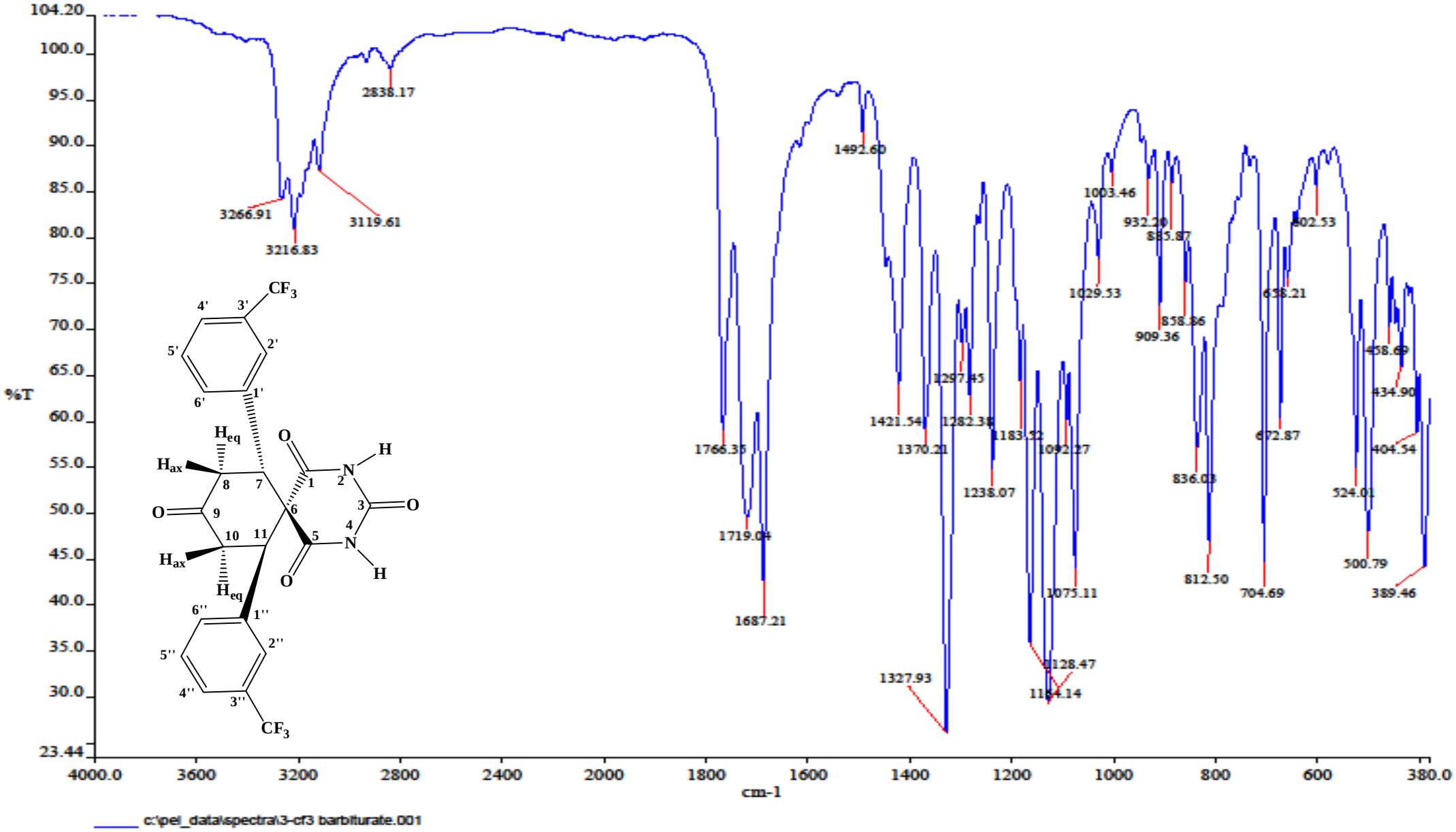
Expanded HSQC of Compound B-3e: 7,11-bis(3-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



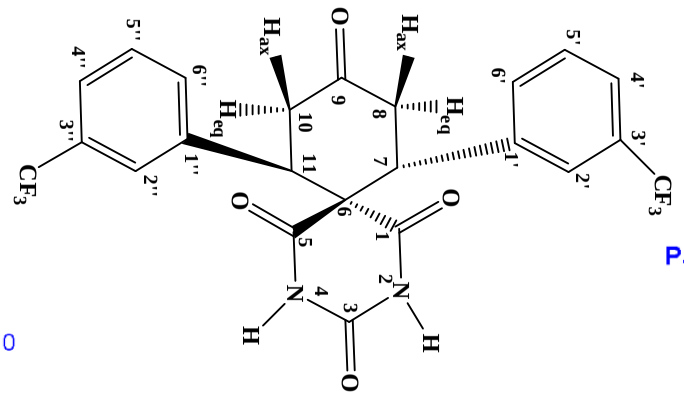
HMBC of Compound B-3e: 7,11-bis(3-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



Expanded HMBC of Compound B-3e: 7,11-bis(3-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



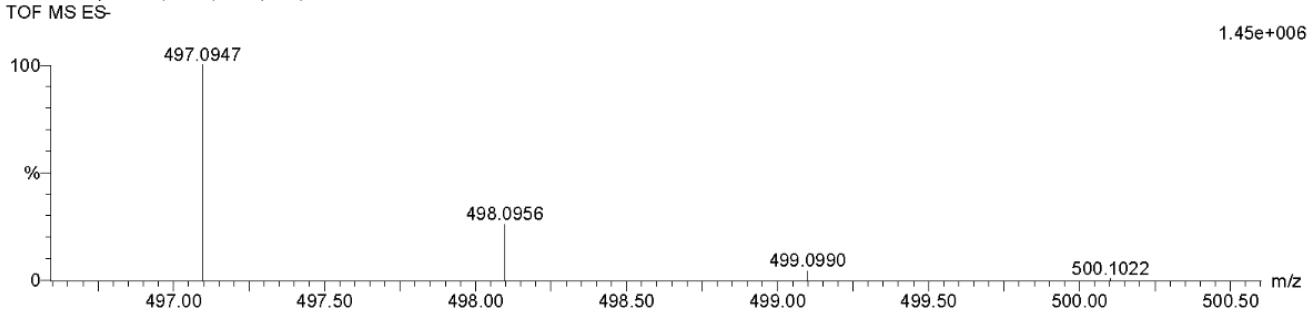
Infrared Spectrum of Compound B-3e: 7,11-bis(3-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



Elemental Composition Report **Page 1**

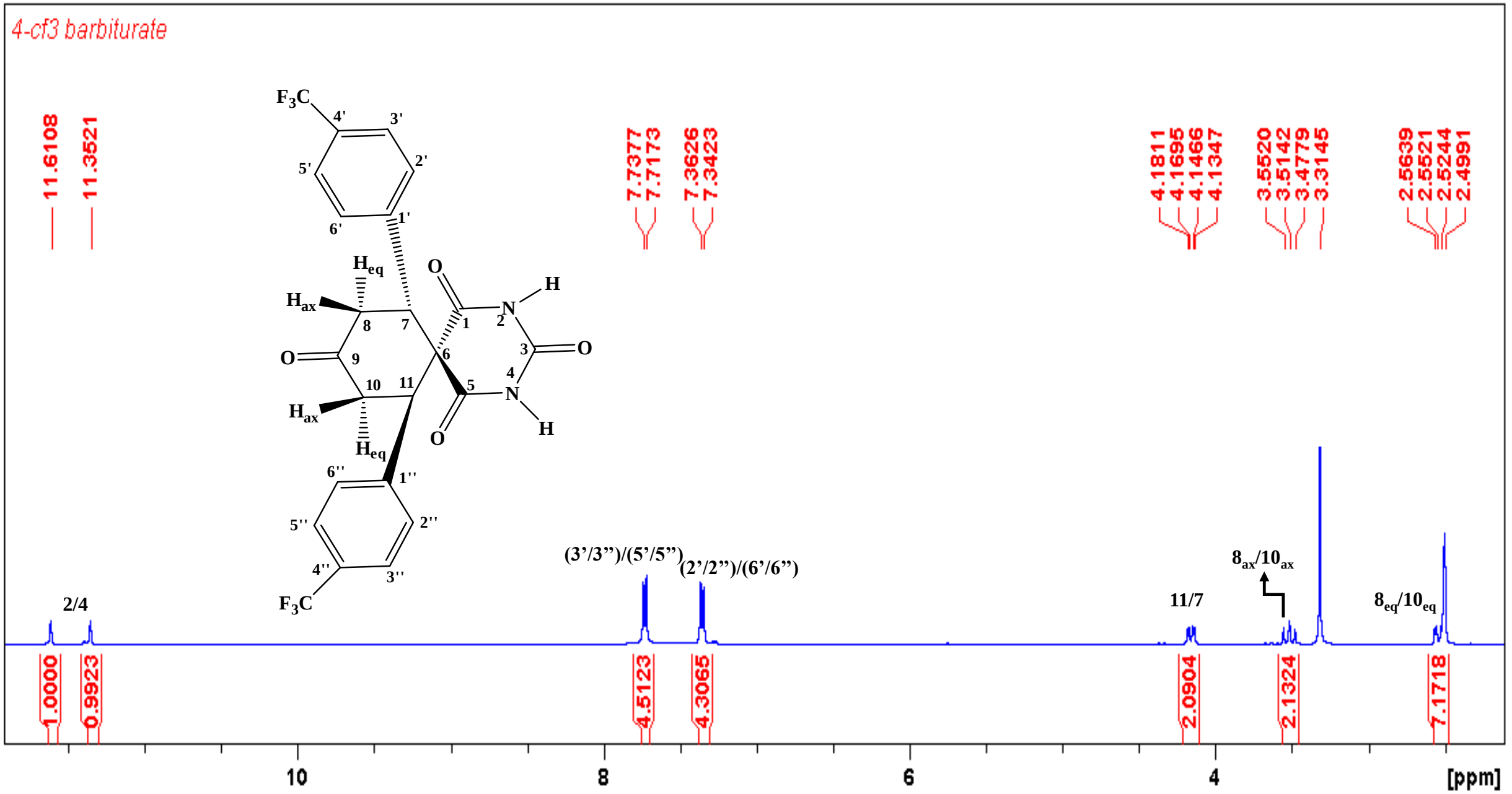
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
23 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 0-5 F: 5-6
18 3-CF3 spiro 60 (1.991) Cm (1:61)
TOF MS ES-



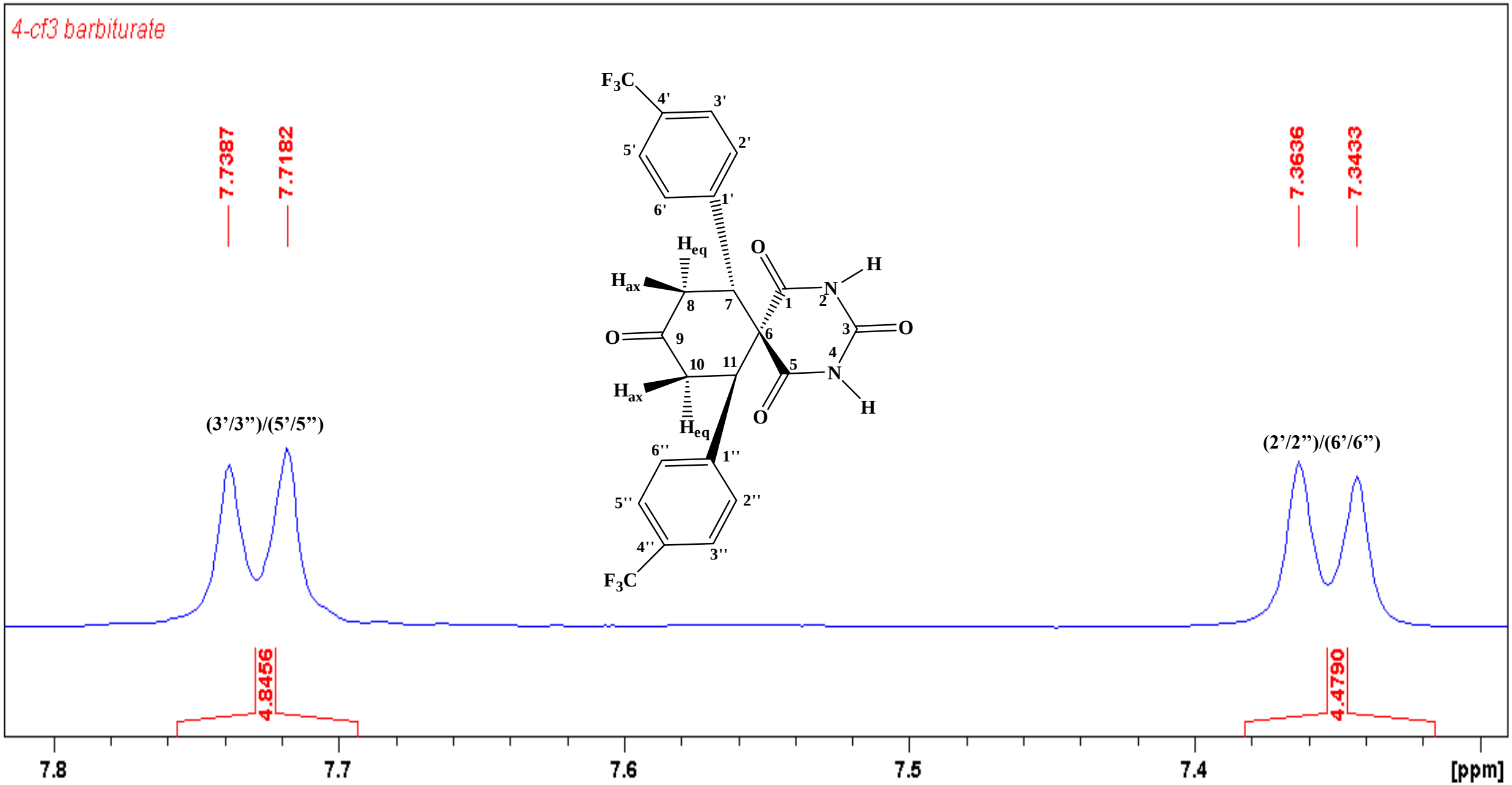
Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
497.0947	497.0936	1.1	2.2	14.5	19.7	0.0	C23 H15 N2 O4 F6

HRMS of Compound B-3e: 7,11-bis(3-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone

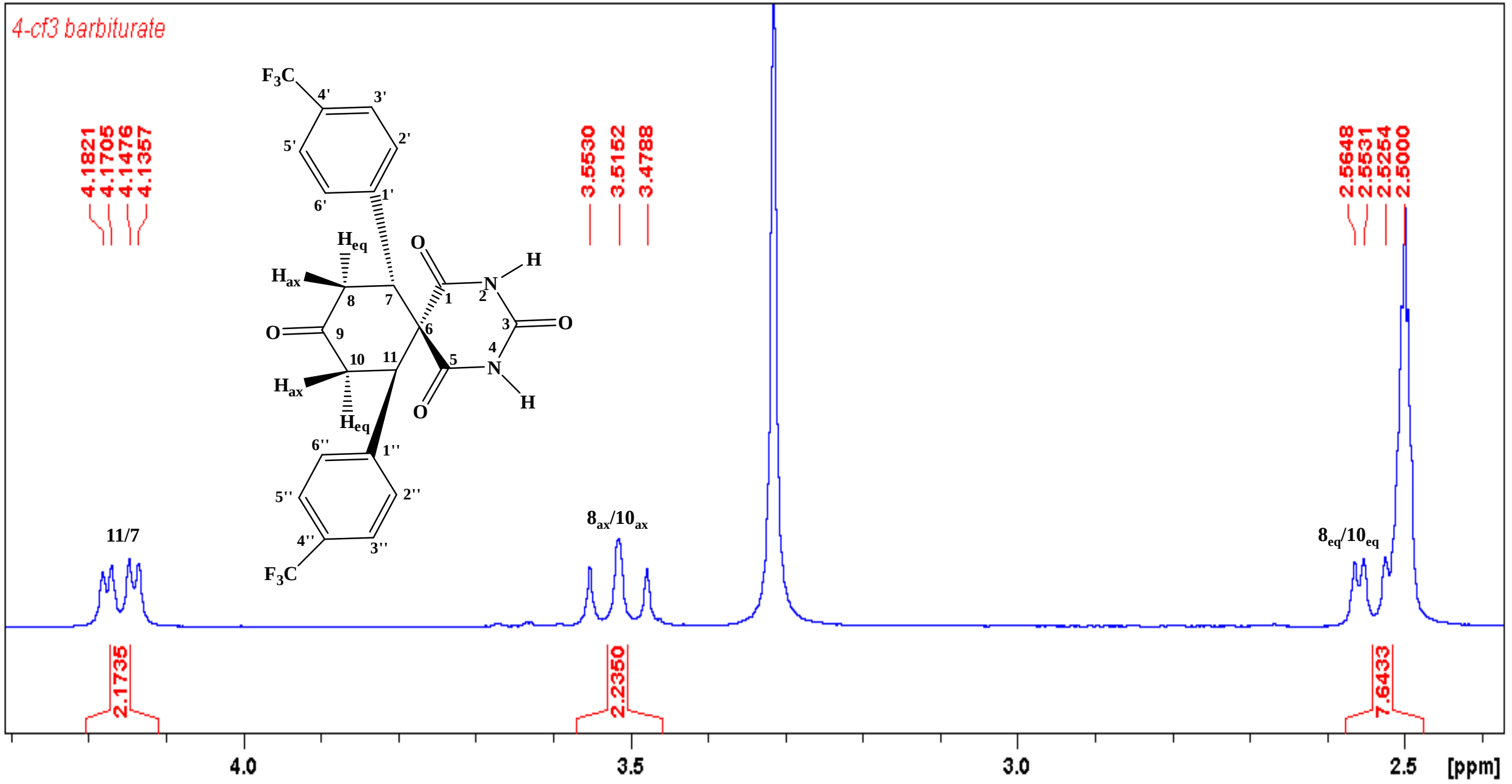


471

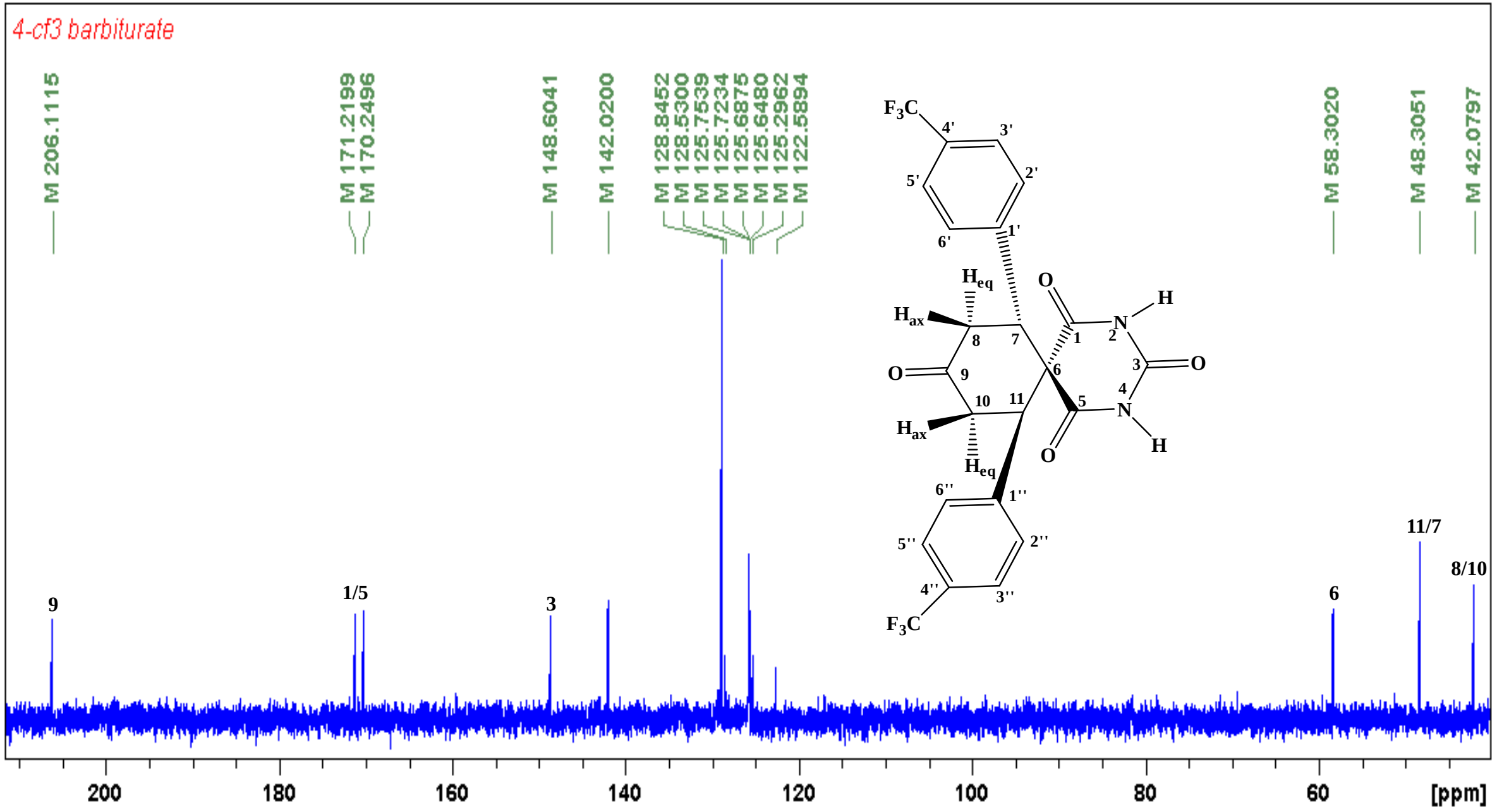
^{1}H Spectrum of Compound B-3f: 7,11-bis(4-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



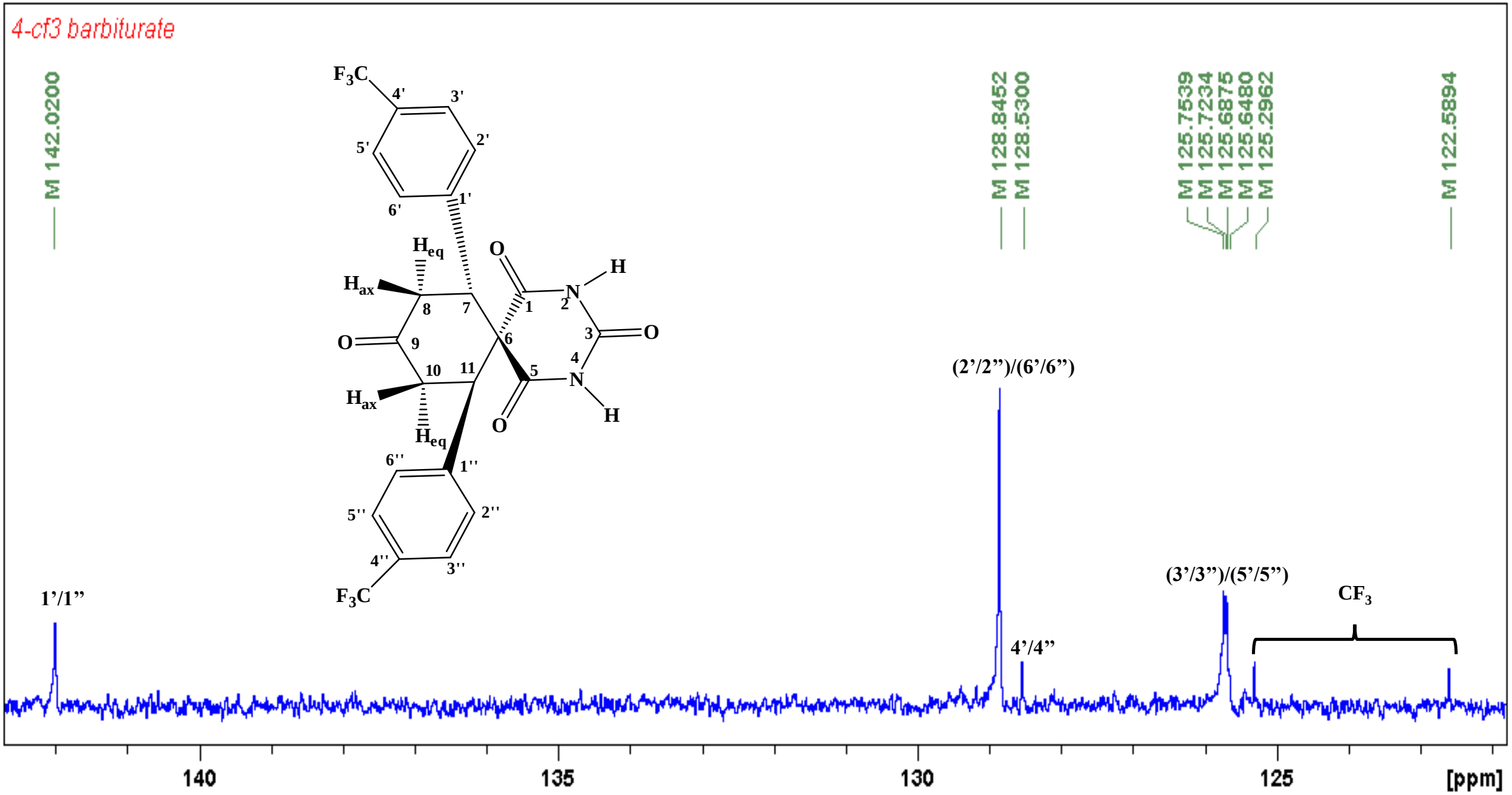
Expanded ^{1}H Spectrum of Compound B-3f: 7,11-bis(4-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



Expanded ^{1}H Spectrum of Compound B-3f: 7,11-bis(4-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone

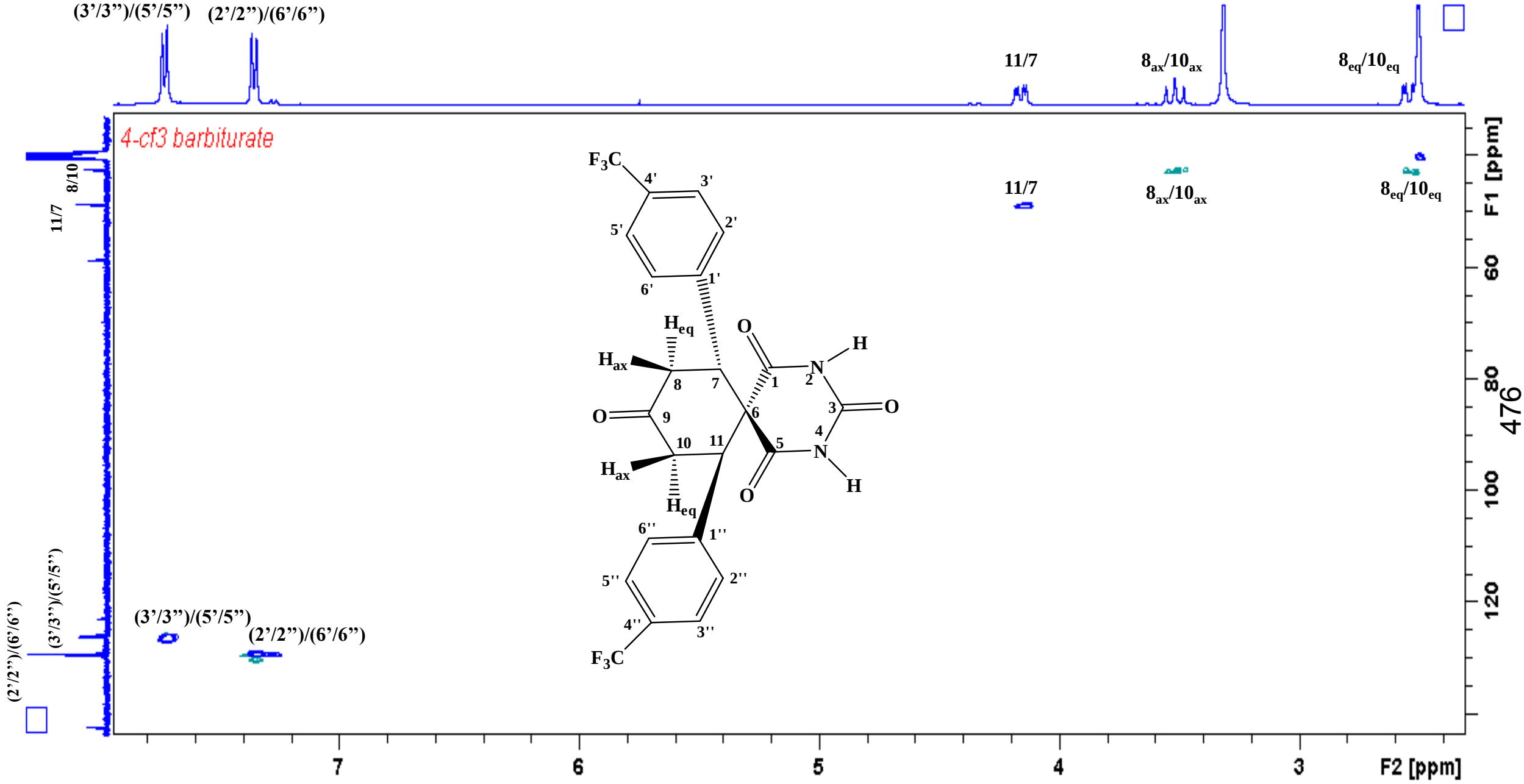


^{13}C Spectrum of Compound B-3f: 7,11-bis(4-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone

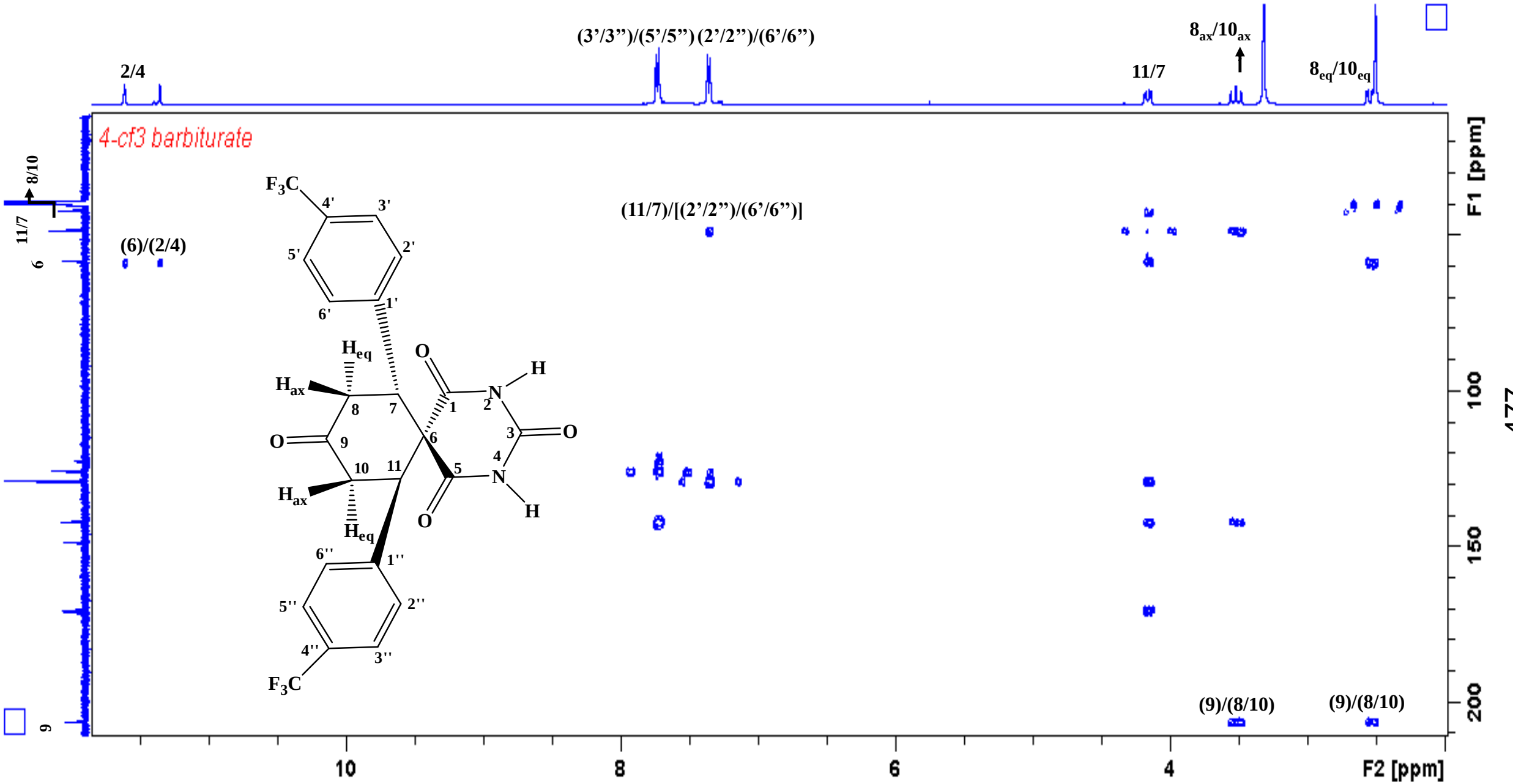


475

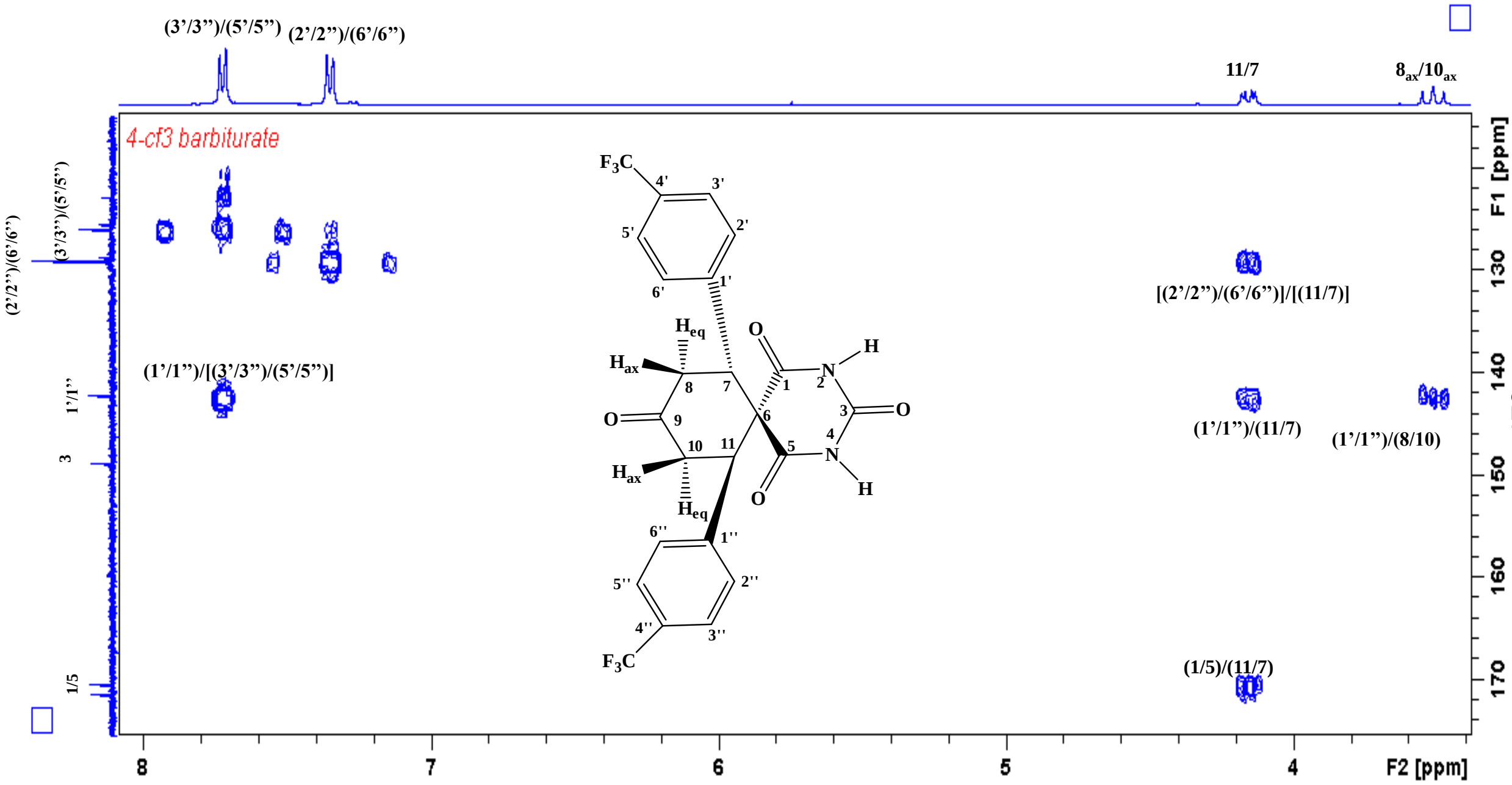
Expanded ^{13}C Spectrum of Compound B-3f: 7,11-bis(4-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



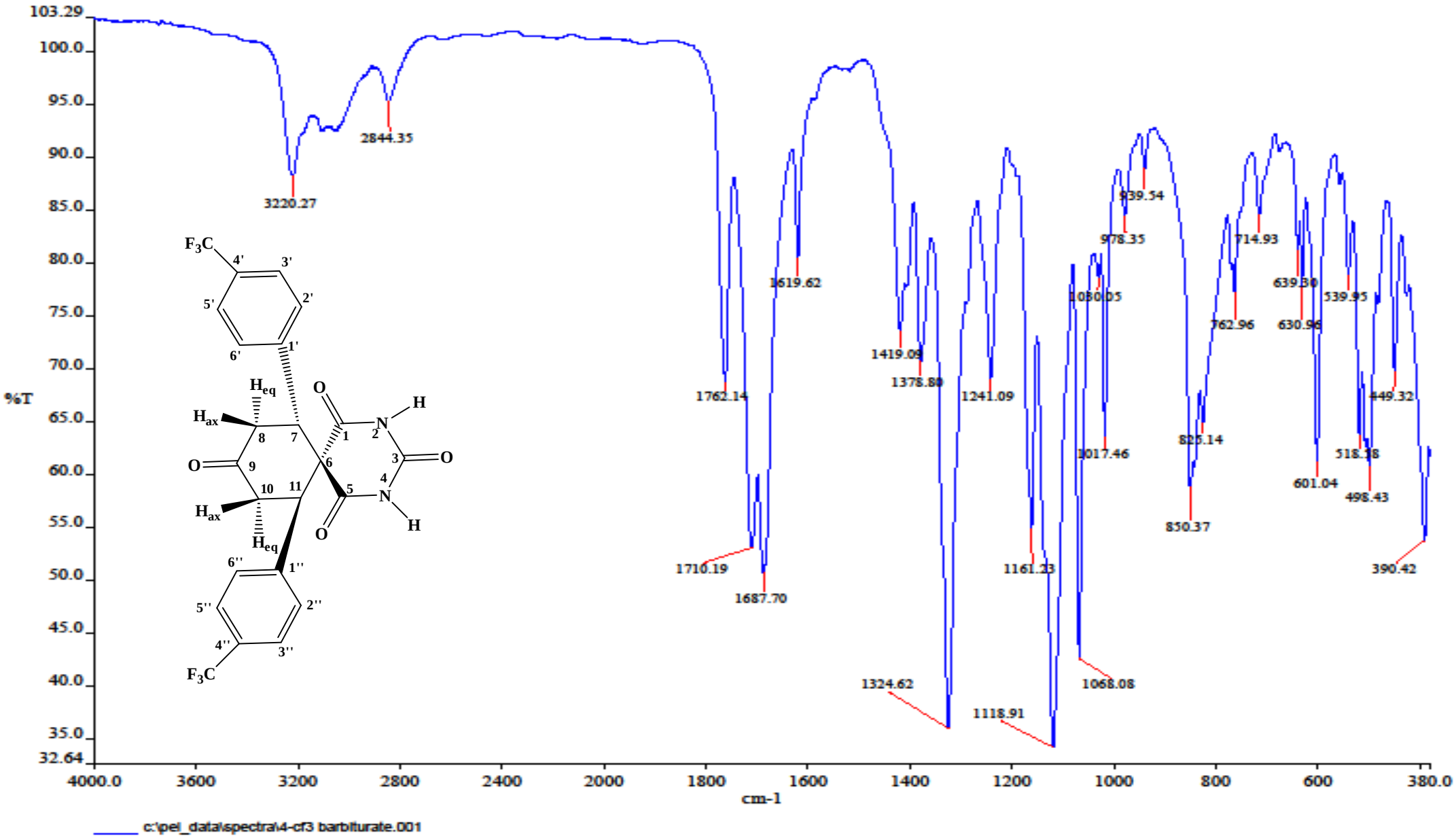
HSQC of Compound B-3f: 7,11-bis(4-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



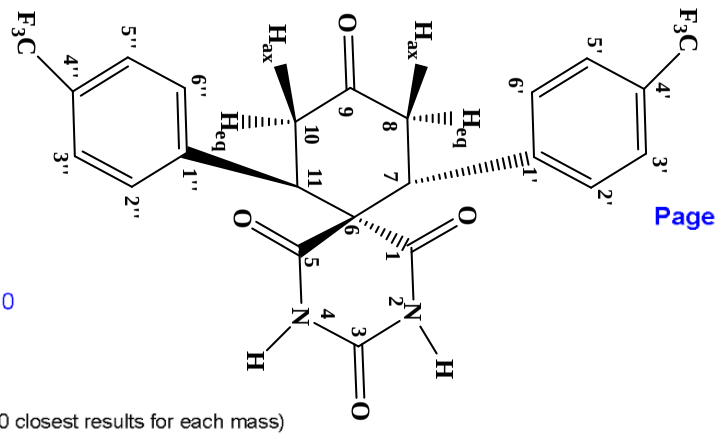
HMBC of Compound B-3f: 7,11-bis(4-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



Expanded HMBC of Compound B-3f: 7,11-bis(4-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



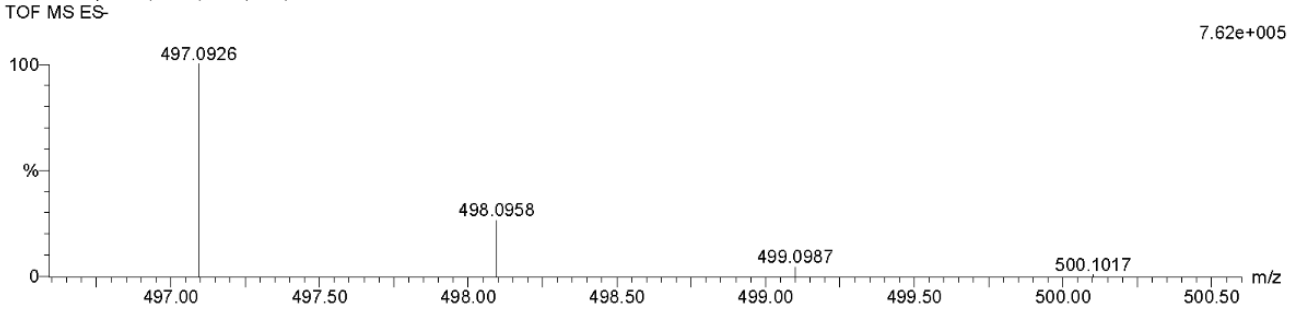
Infrared Spectrum of Compound B-3f: 7,11-bis(4-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



Elemental Composition Report **Page 1**

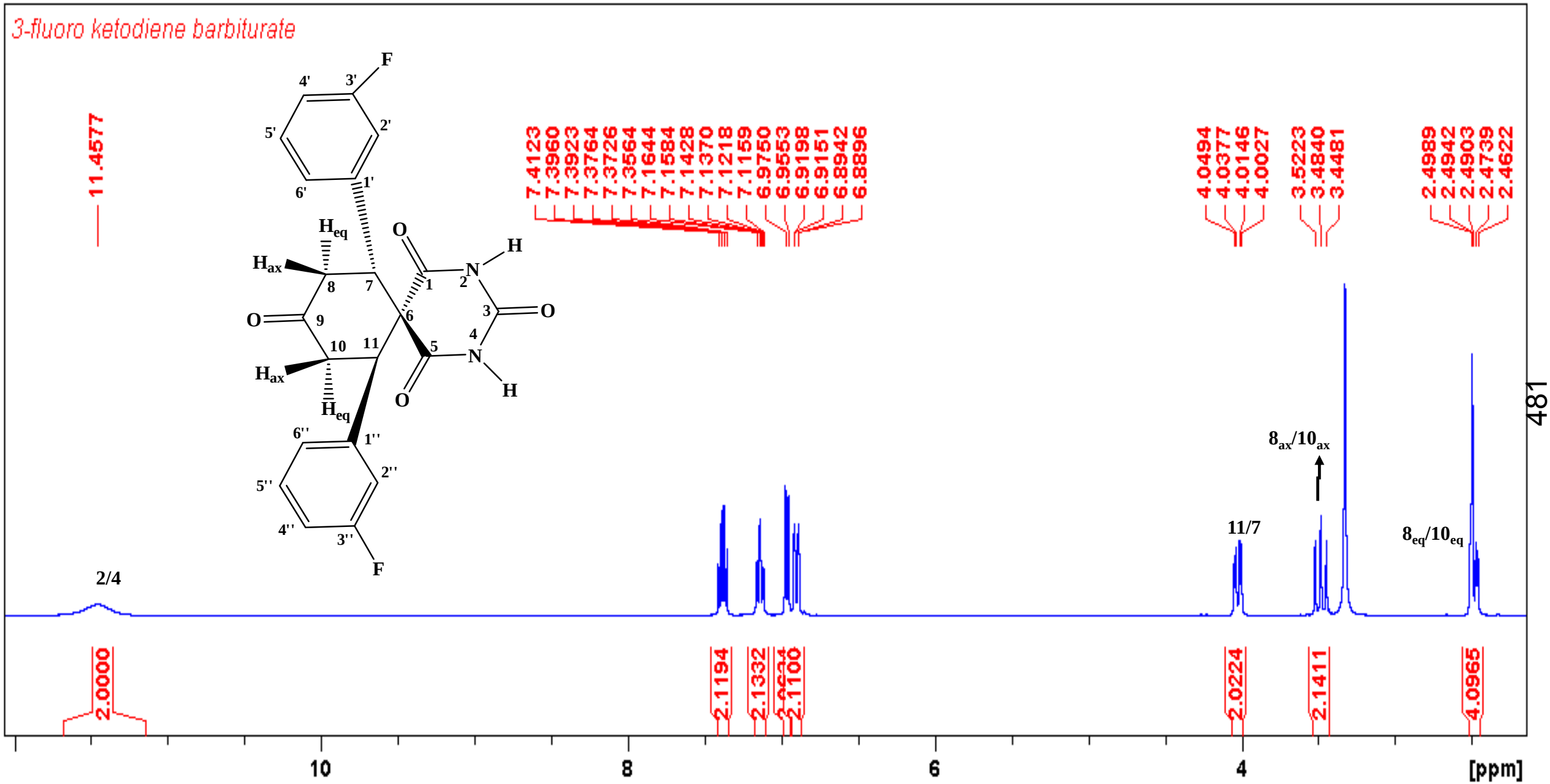
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
23 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 0-5 F: 5-6
19 4-CF3 spiro 8 (0.237) Cm (1:61)
TOF MS ES-

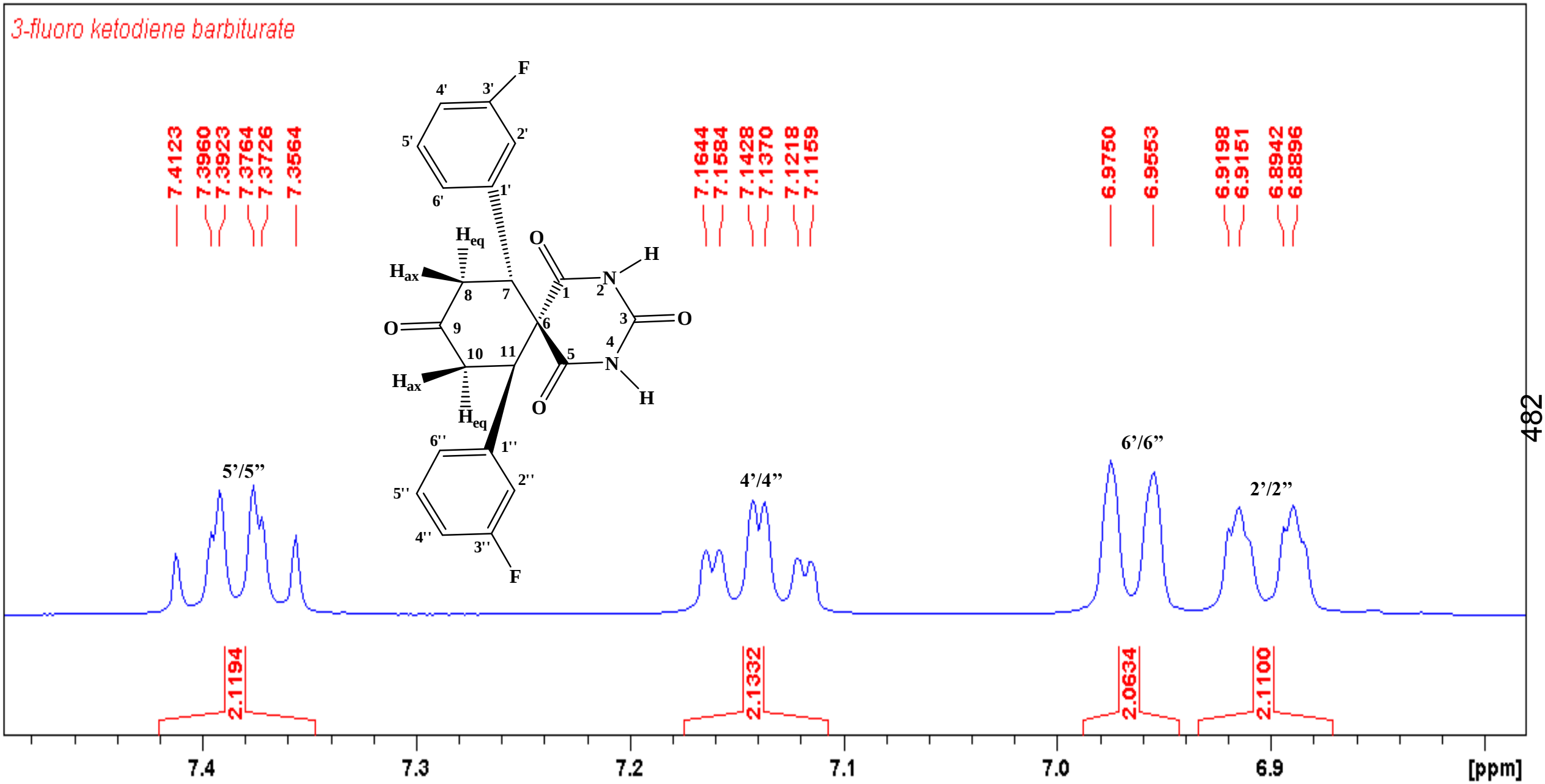


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
497.0926	497.0936	-1.0	-2.0	14.5	13.7	0.0	C23 H15 N2 O4 F6

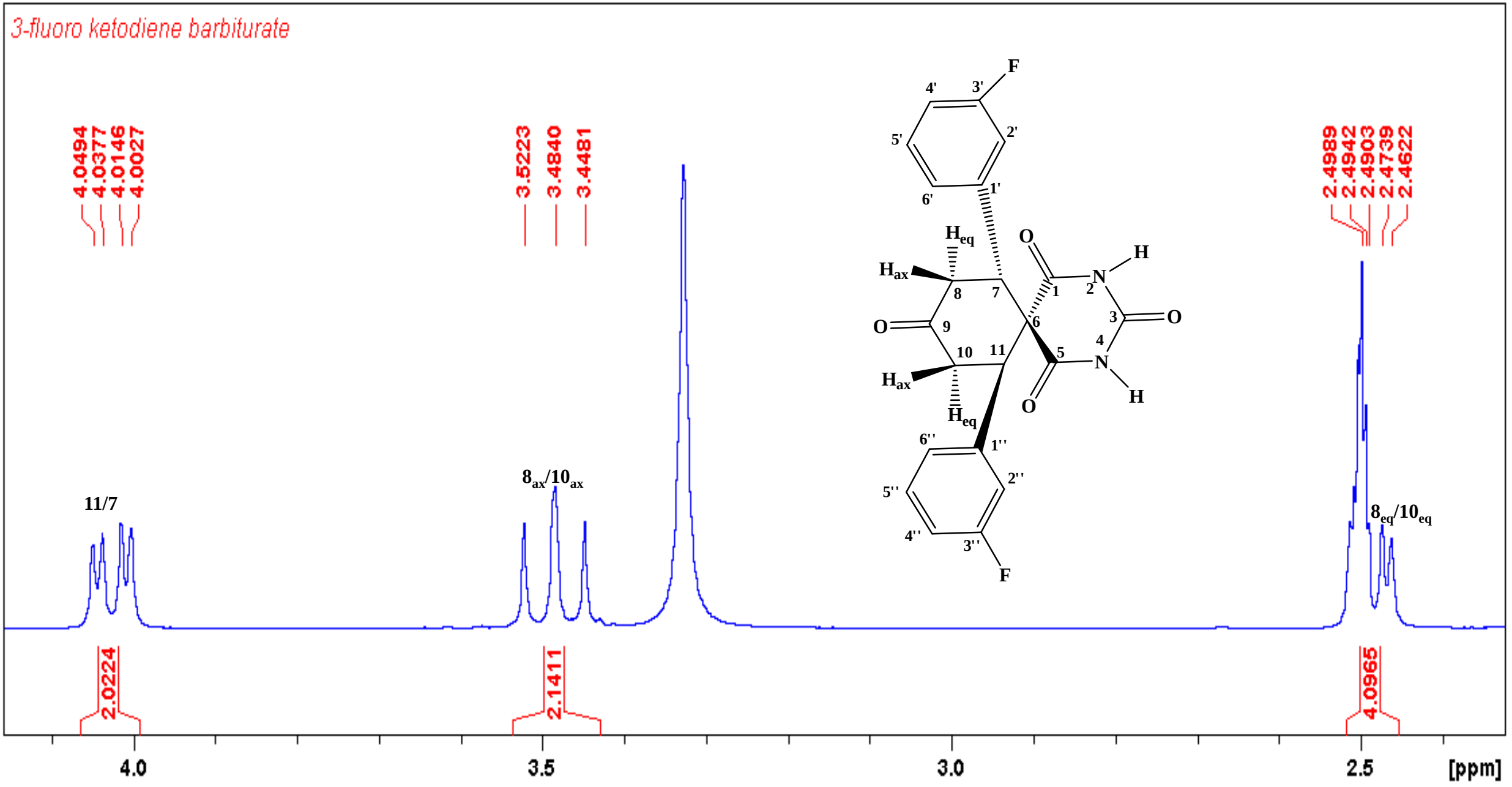
HRMS of Compound B-3f: 7,11-bis(4-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



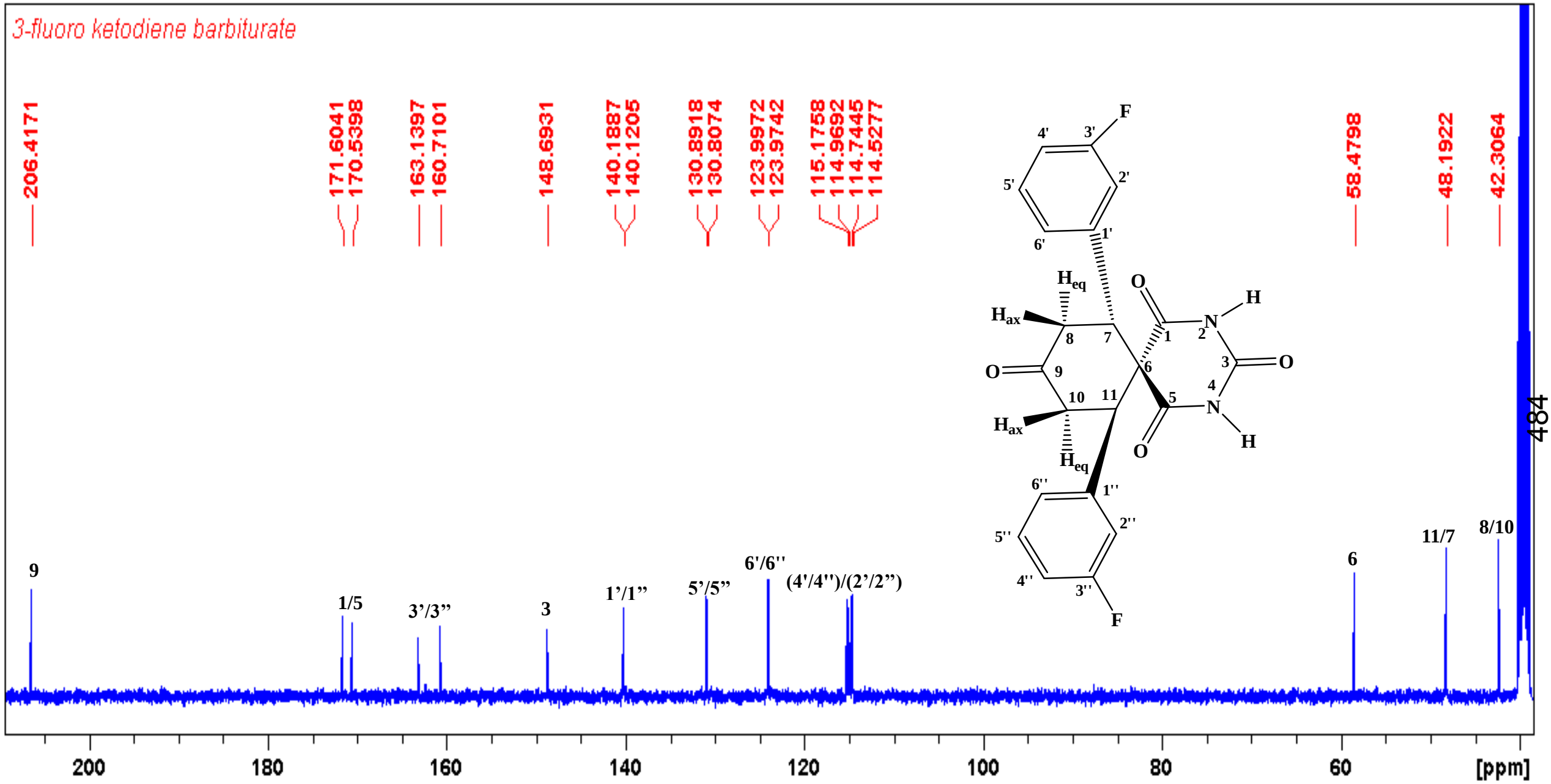
^{1}H Spectrum of Compound B-3g: 7,11-bis(3-fluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



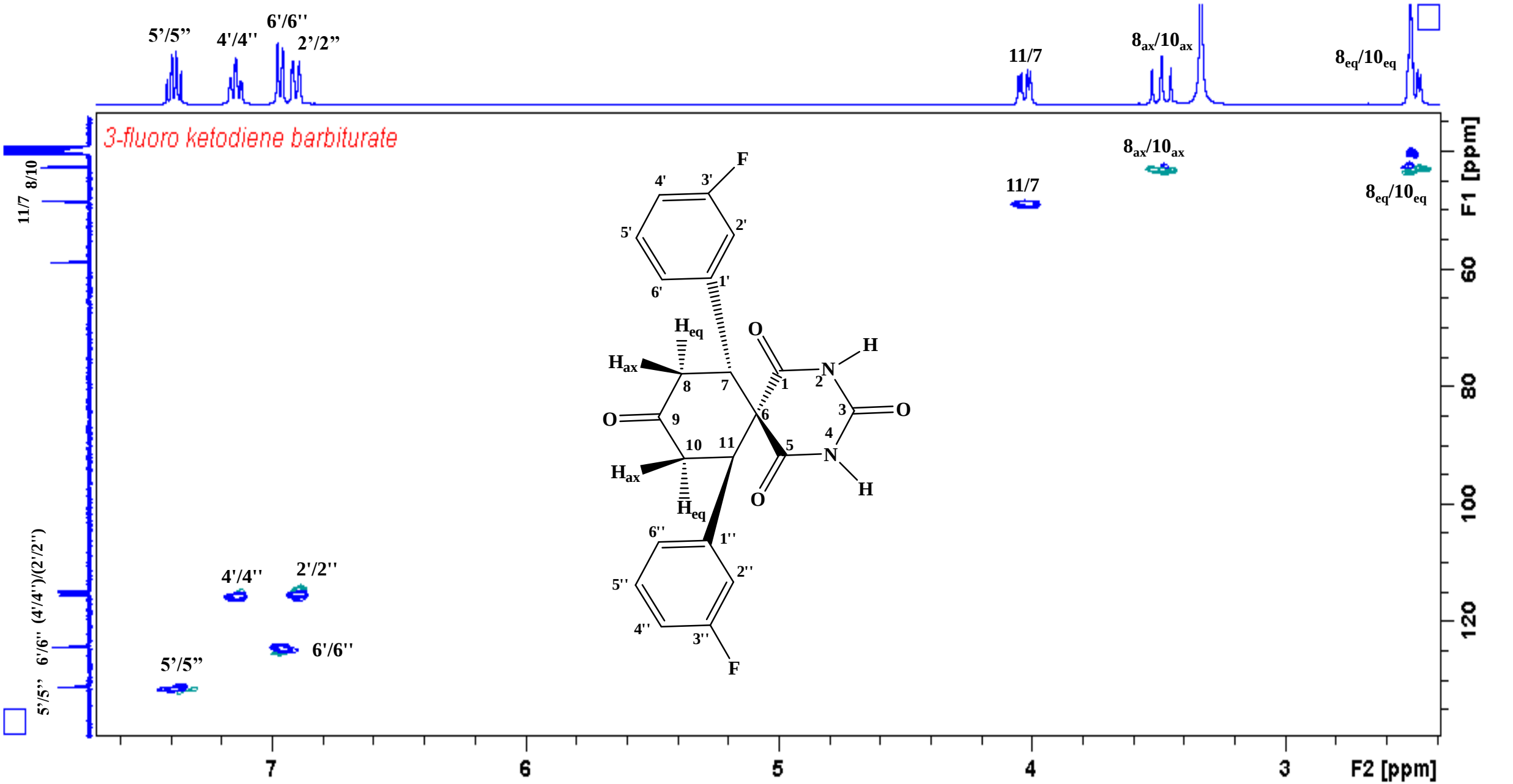
Expanded ^{1}H Spectrum of Compound B-3g: 7,11-bis(3-fluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



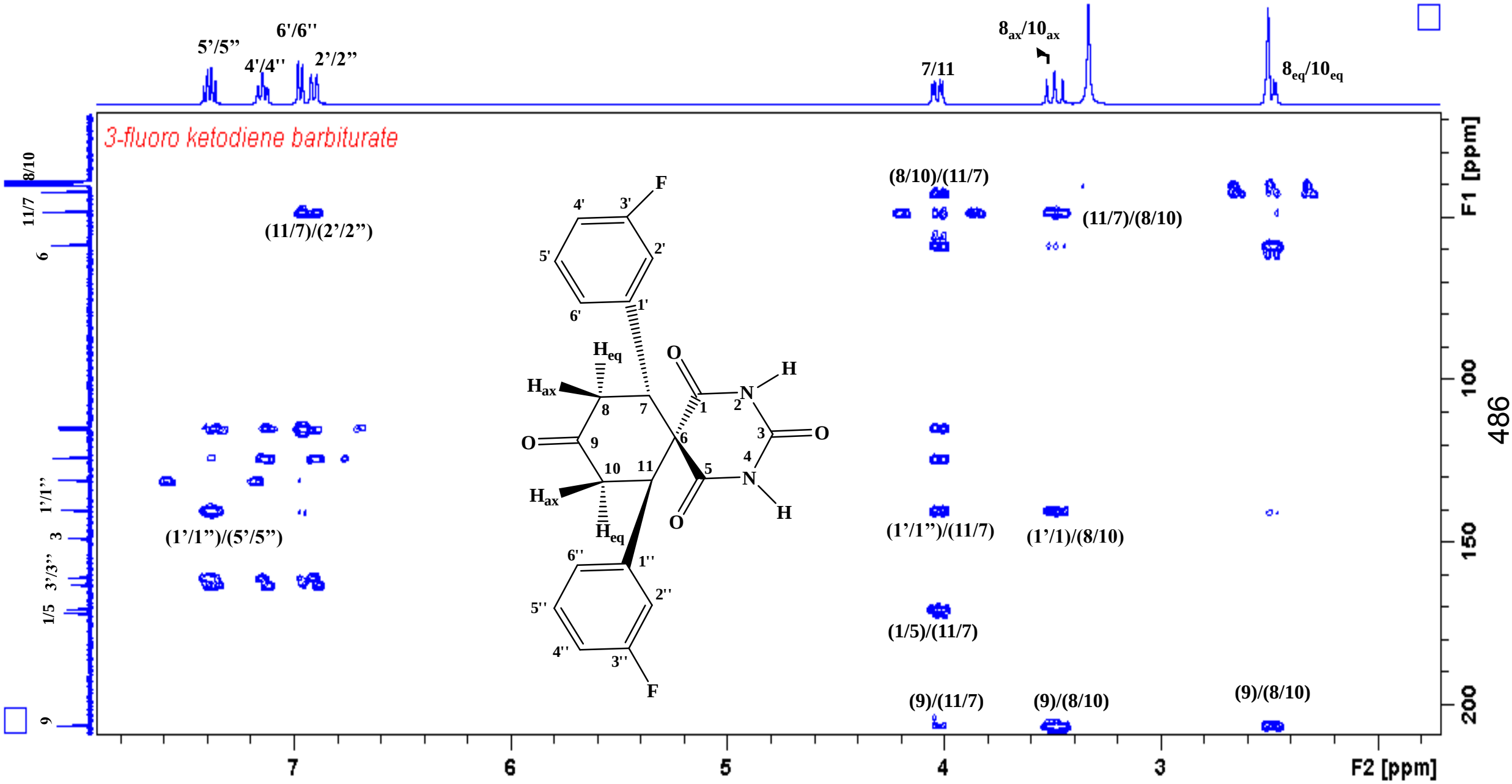
Expanded ^{1}H Spectrum of Compound B-3g: 7,11-bis(3-fluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



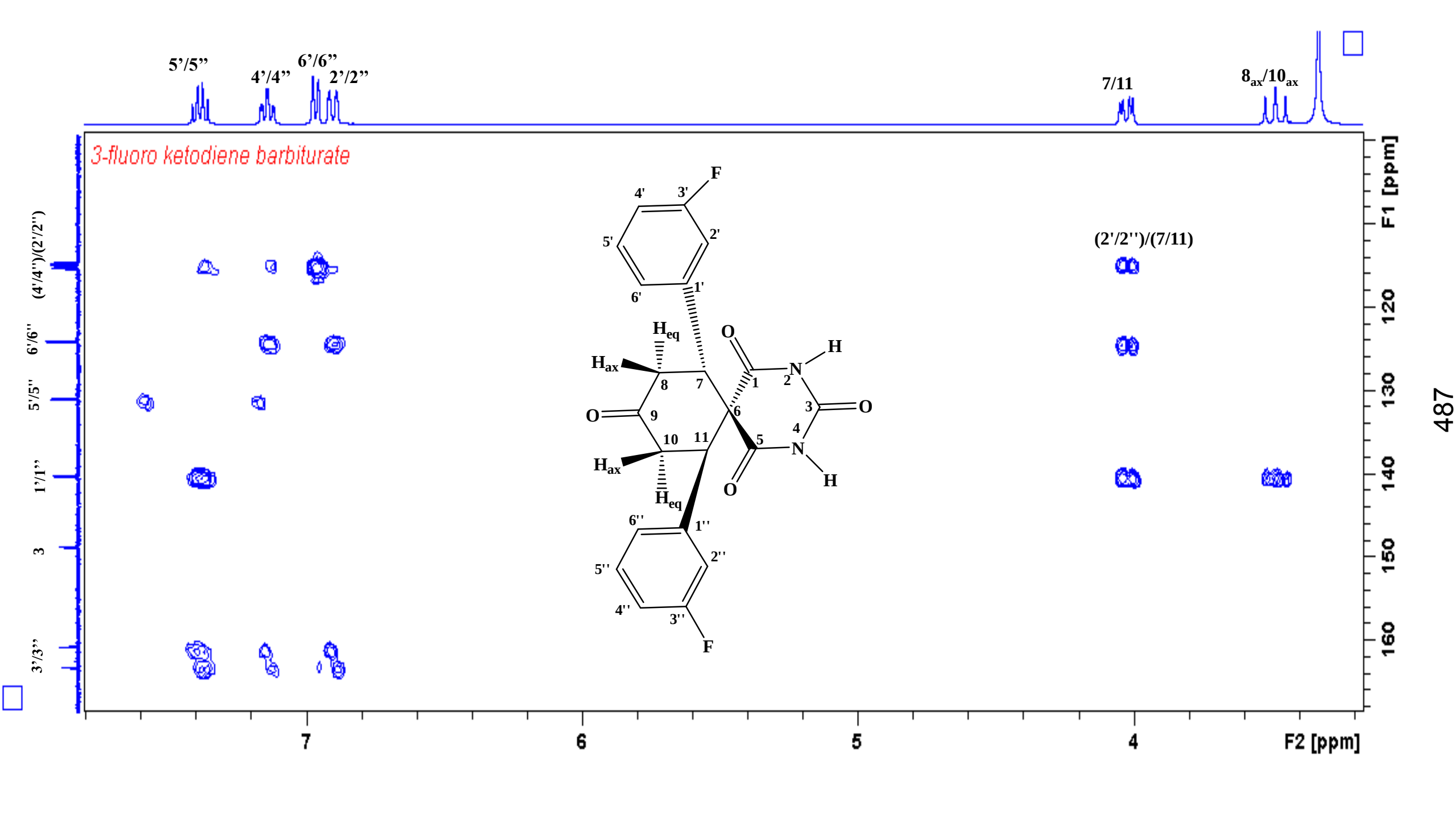
^{13}C Spectrum of Compound B-3g: 7,11-bis(3-fluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone

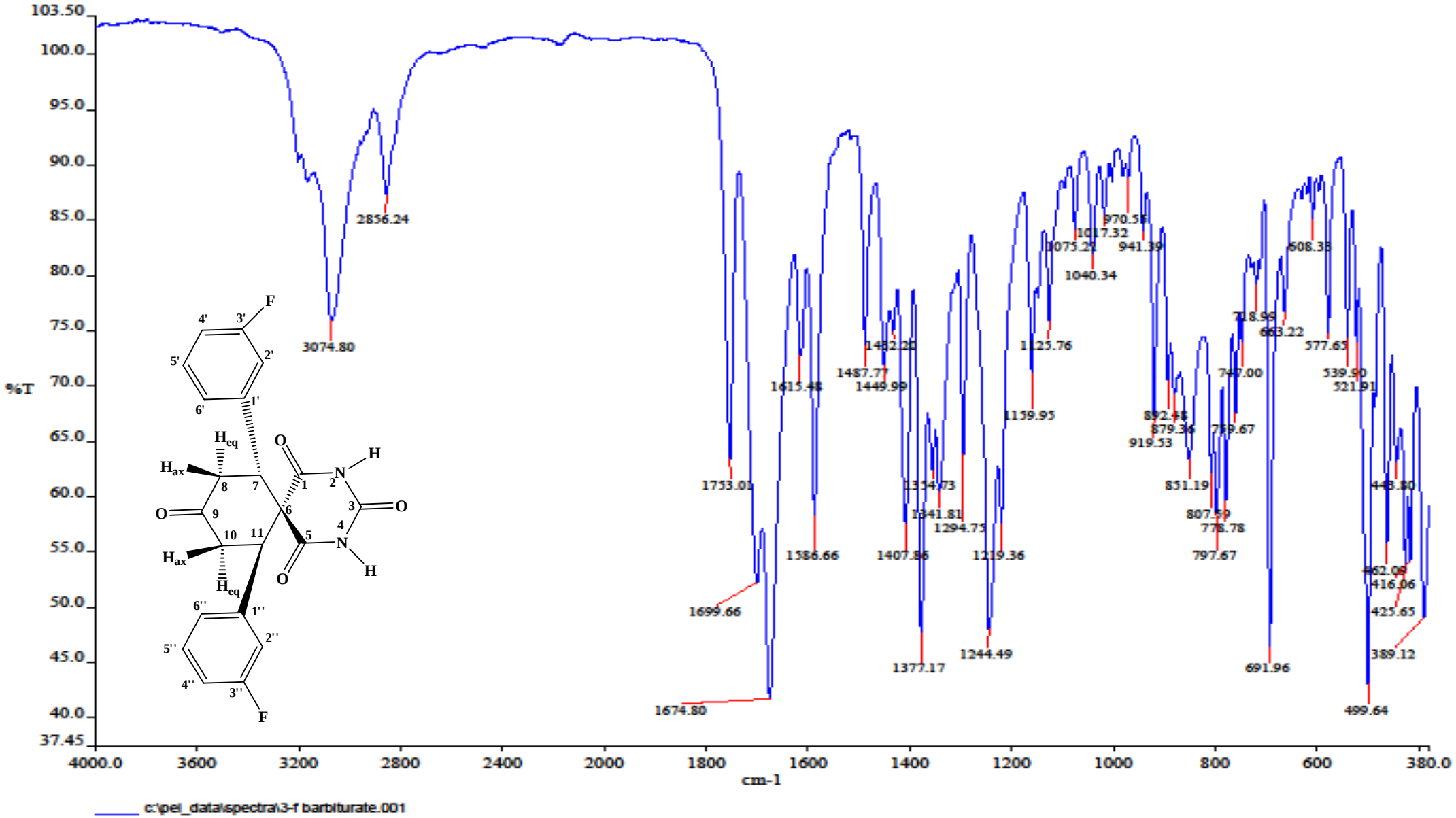


HSQC of Compound B-3g: 7,11-bis(3-fluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone

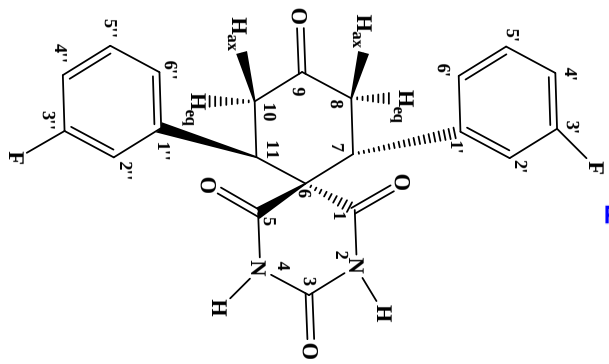


HMBC of Compound B-3g: 7,11-bis(3-fluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone


5'/5"
4'/4"
6'/6"
2'/2"
7/11
8ax/10ax
3-fluoro ketodiene barbiturate
(2'/2'')/(7/11)
(4'/4'')/(2'/2'')
6'/6''
5'/5''
1'/1''
3
3'/3''
F1 [ppm]
120
130
140
150
160
7
6
5
4
F2 [ppm]
487



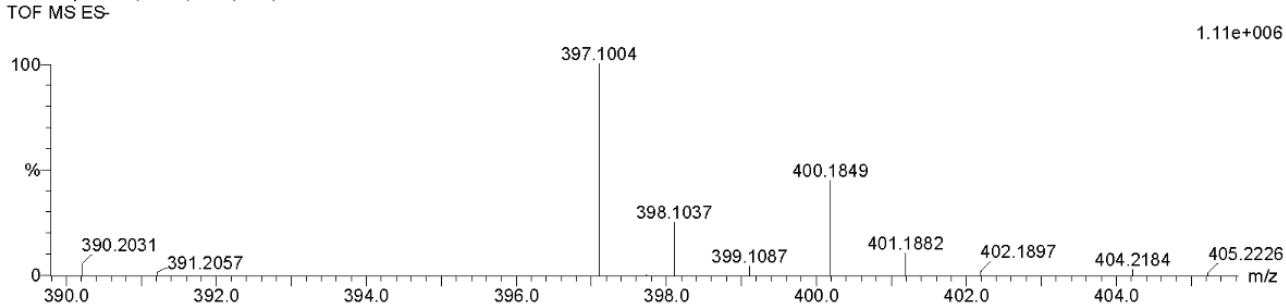
Infrared Spectrum of Compound B-3g: 7,11-bis(3-fluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



Elemental Composition Report **Page 1**

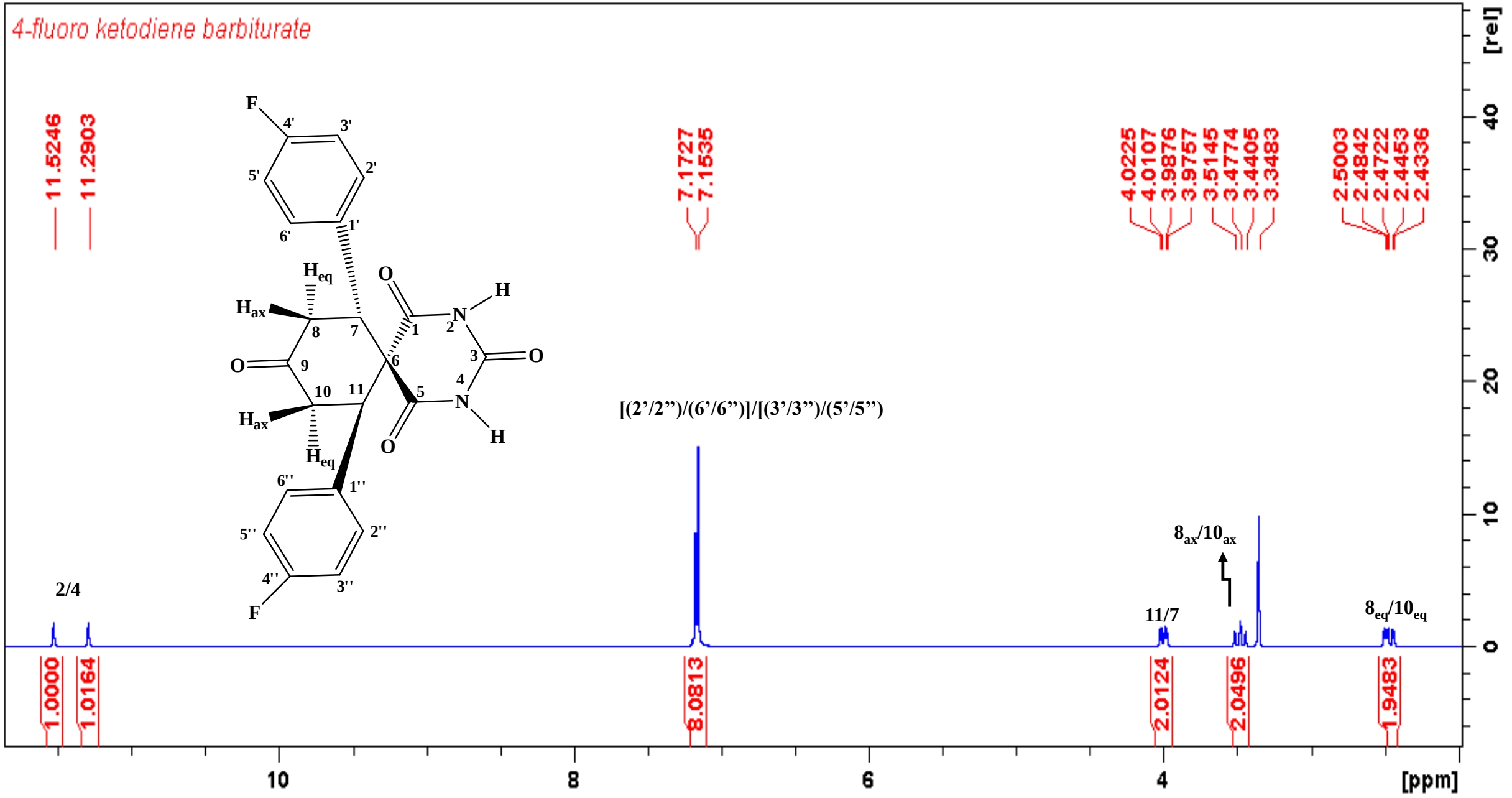
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
106 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 0-5 F: 0-5
20 3-F spiro 58 (1.923) Cm (1:61)
TOF MS ES-

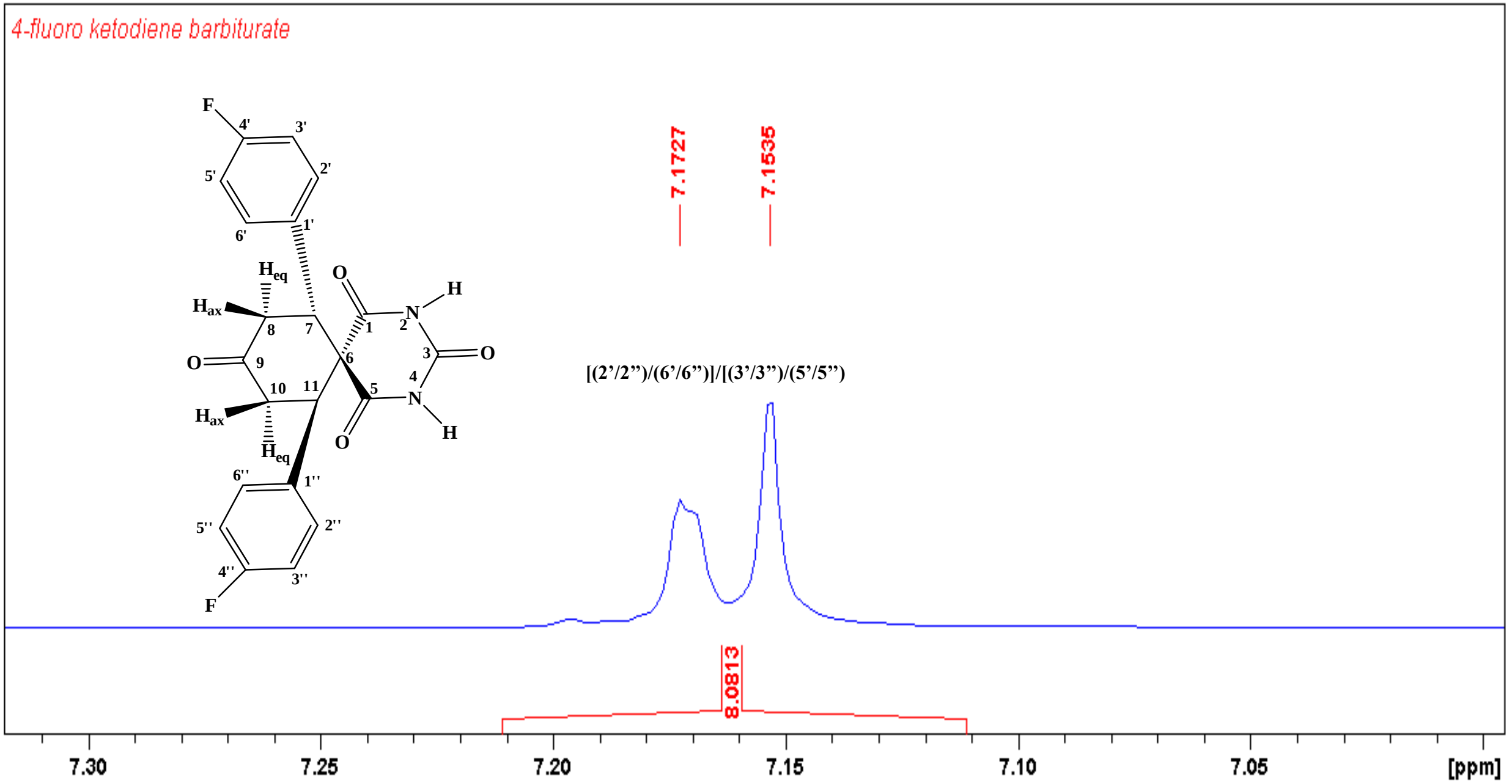


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
397.1004	397.1000	0.4	1.0	14.5	30.6	0.0	C21 H15 N2 O4 F2

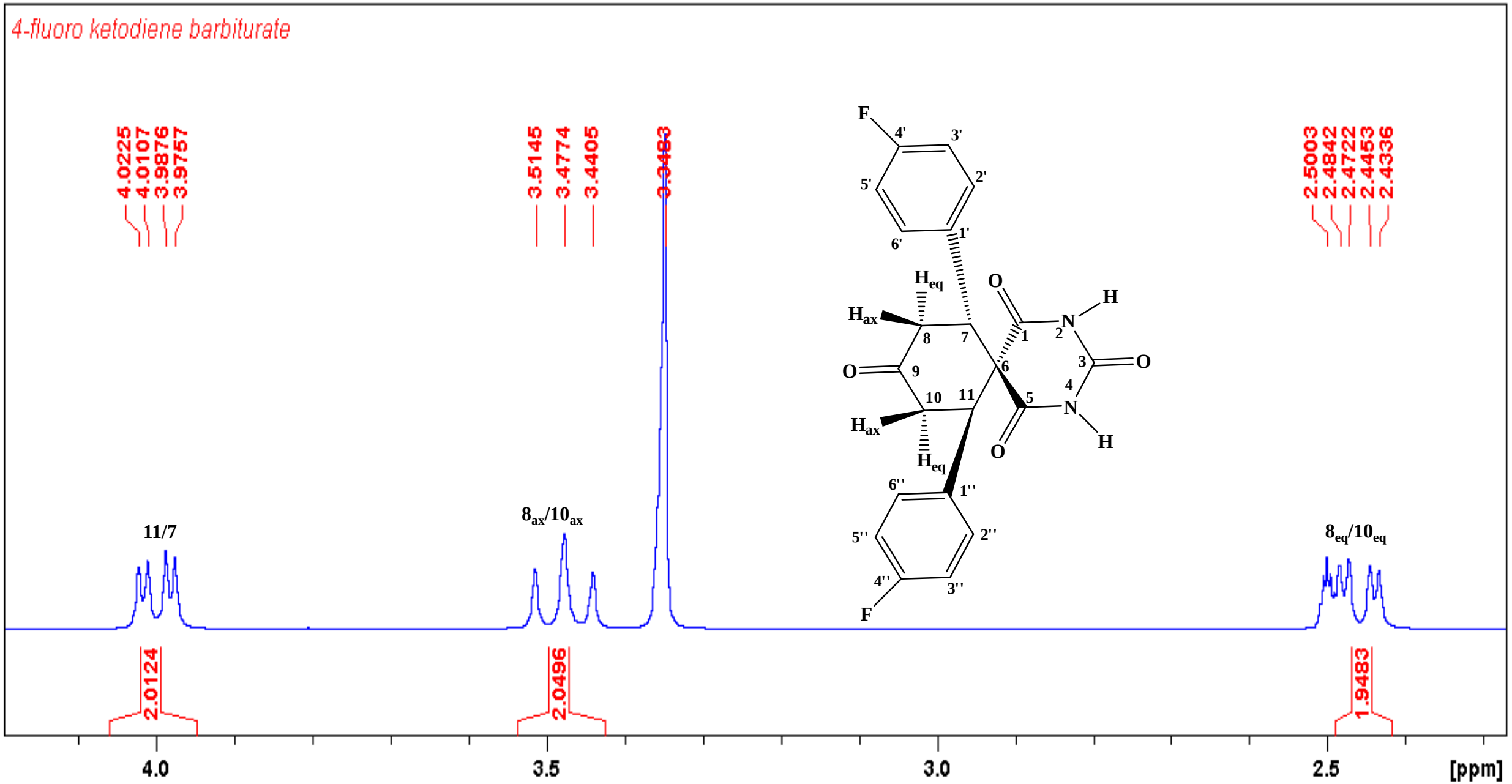
HRMS of Compound B-3g: 7,11-bis(3-fluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



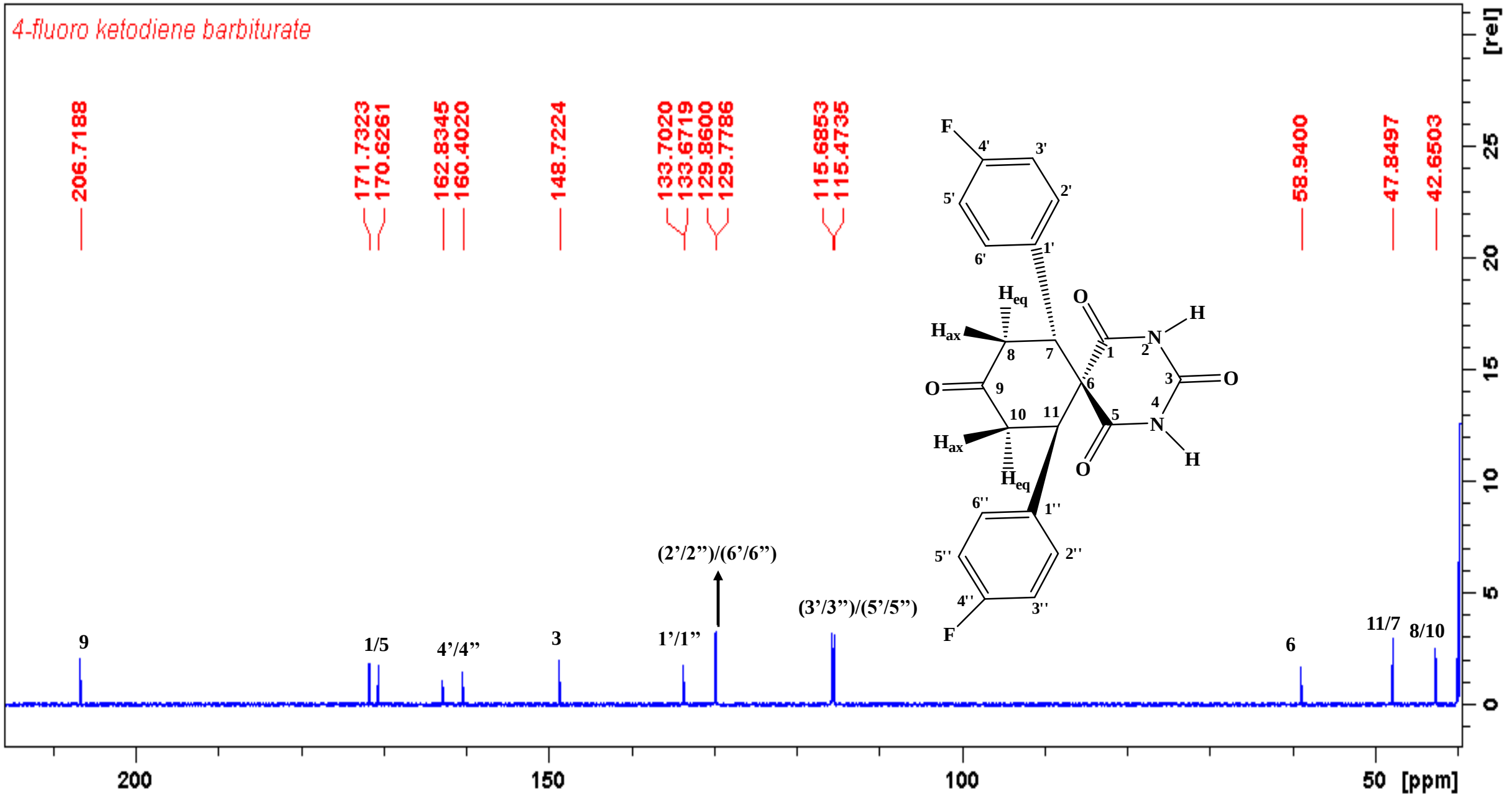
^{1}H Spectrum of Compound B-3h: 7,11-bis(4-fluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



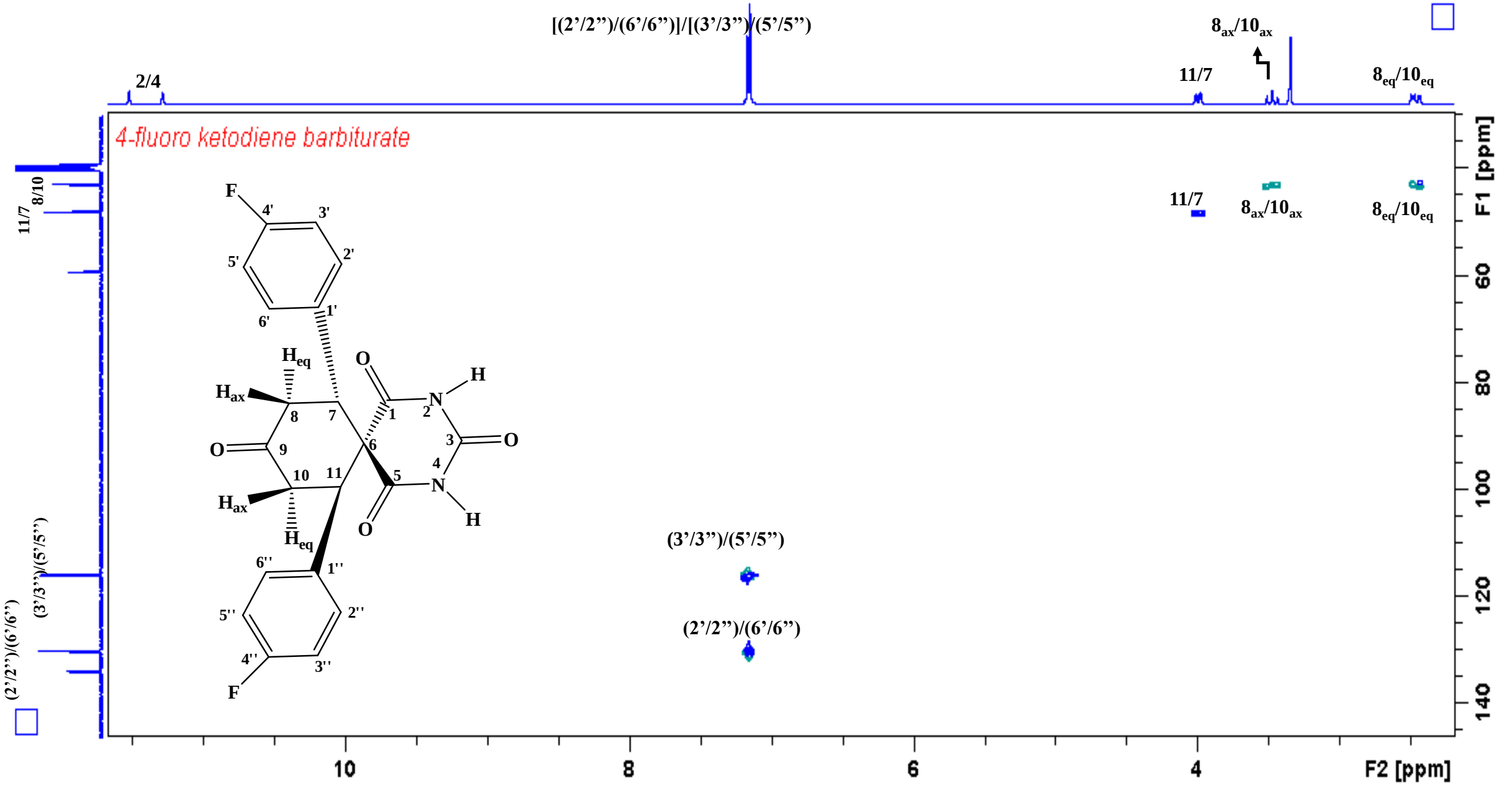
Expanded ^{1}H Spectrum of Compound B-3h: 7,11-bis(4-fluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



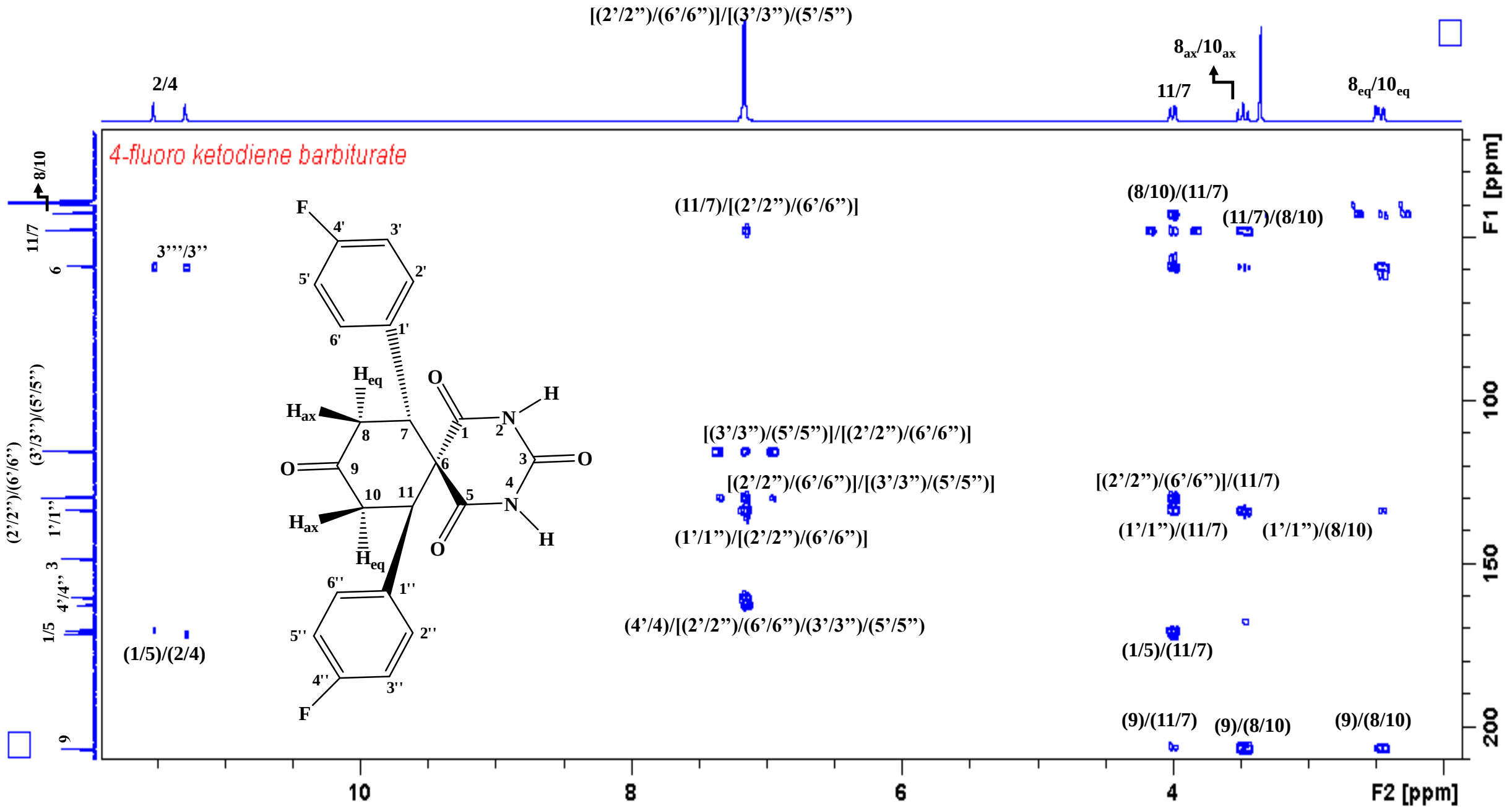
Expanded ^{1}H Spectrum of Compound B-3h: 7,11-bis(4-fluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



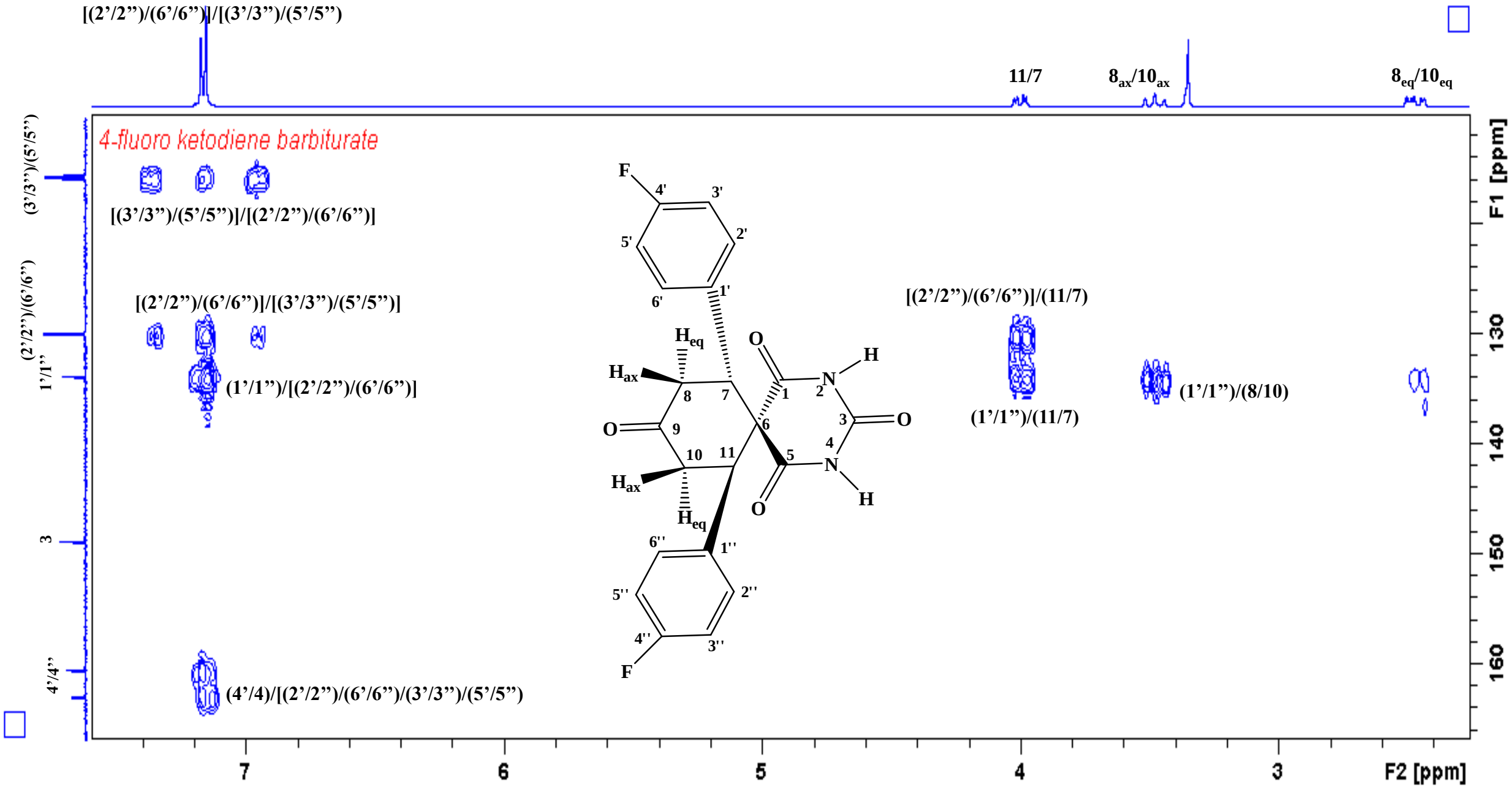
^{13}C Spectrum of Compound B-3h: 7,11-bis(4-fluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



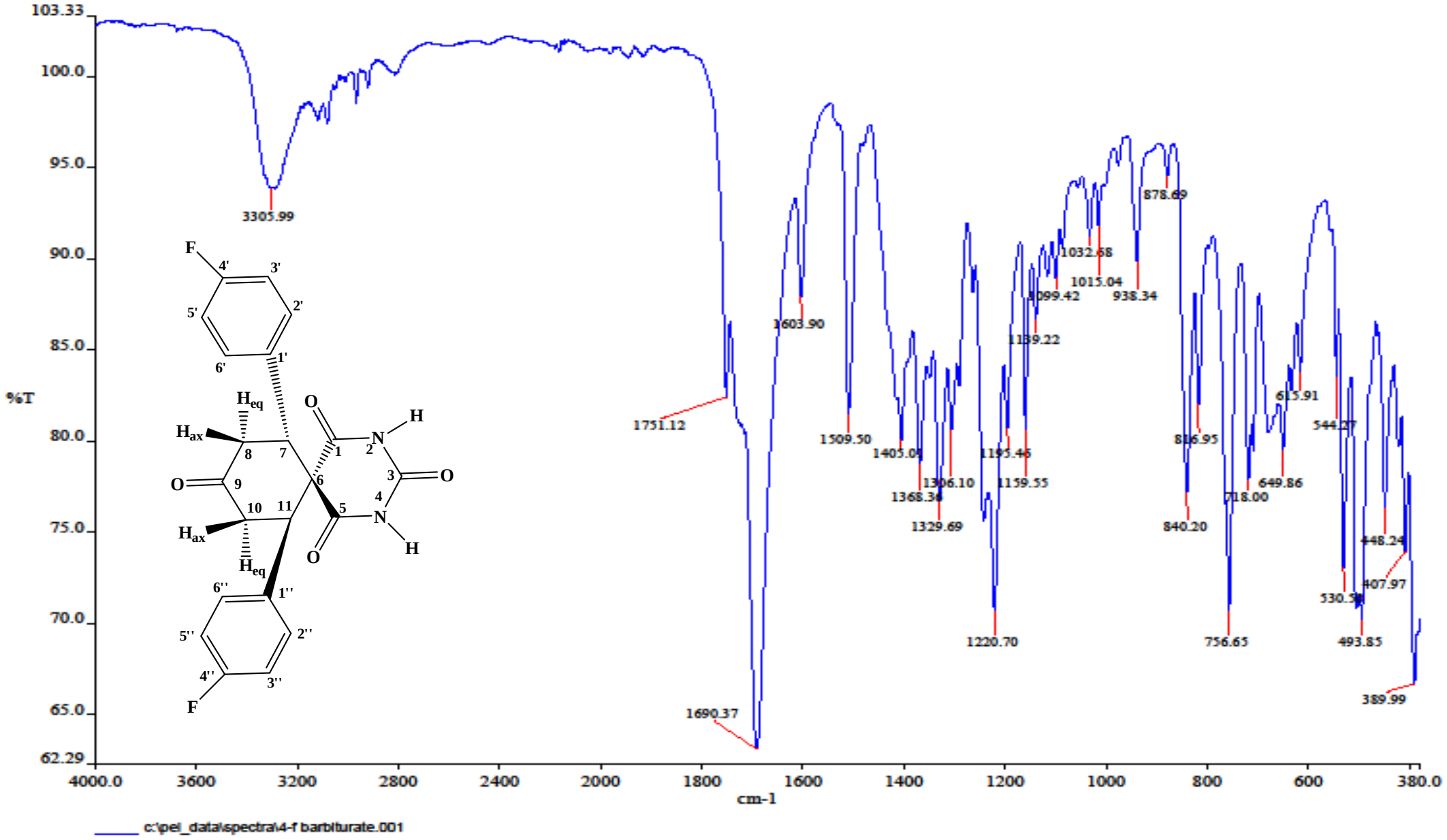
HSQC of Compound B-3h: 7,11-bis(4-fluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



HMBC of Compound B-3h: 7,11-bis(4-fluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone

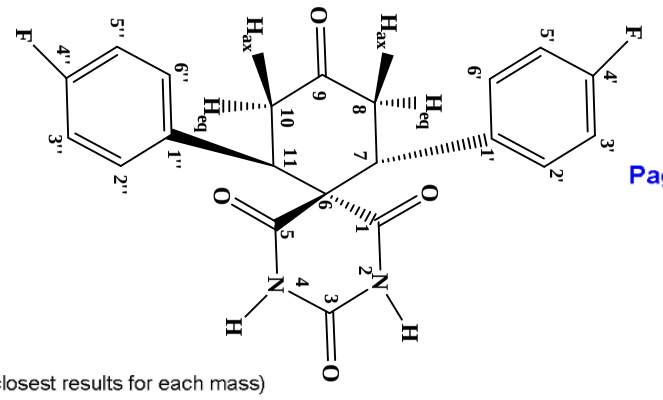


Expanded HMBC of Compound B-3h: 7,11-bis(4-fluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



c:\pel_data\spectra\4-f barbiturate.001

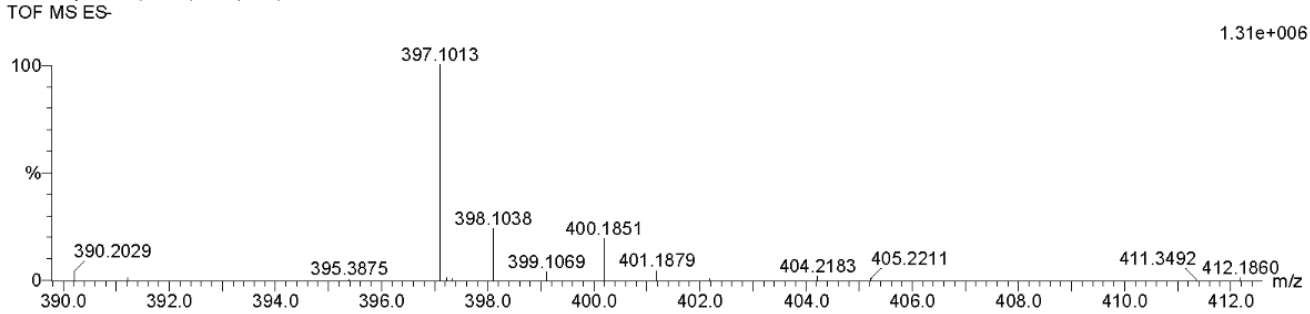
Infrared Spectrum of Compound B-3h: 7,11-bis(4-fluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



Elemental Composition Report **Page 1**

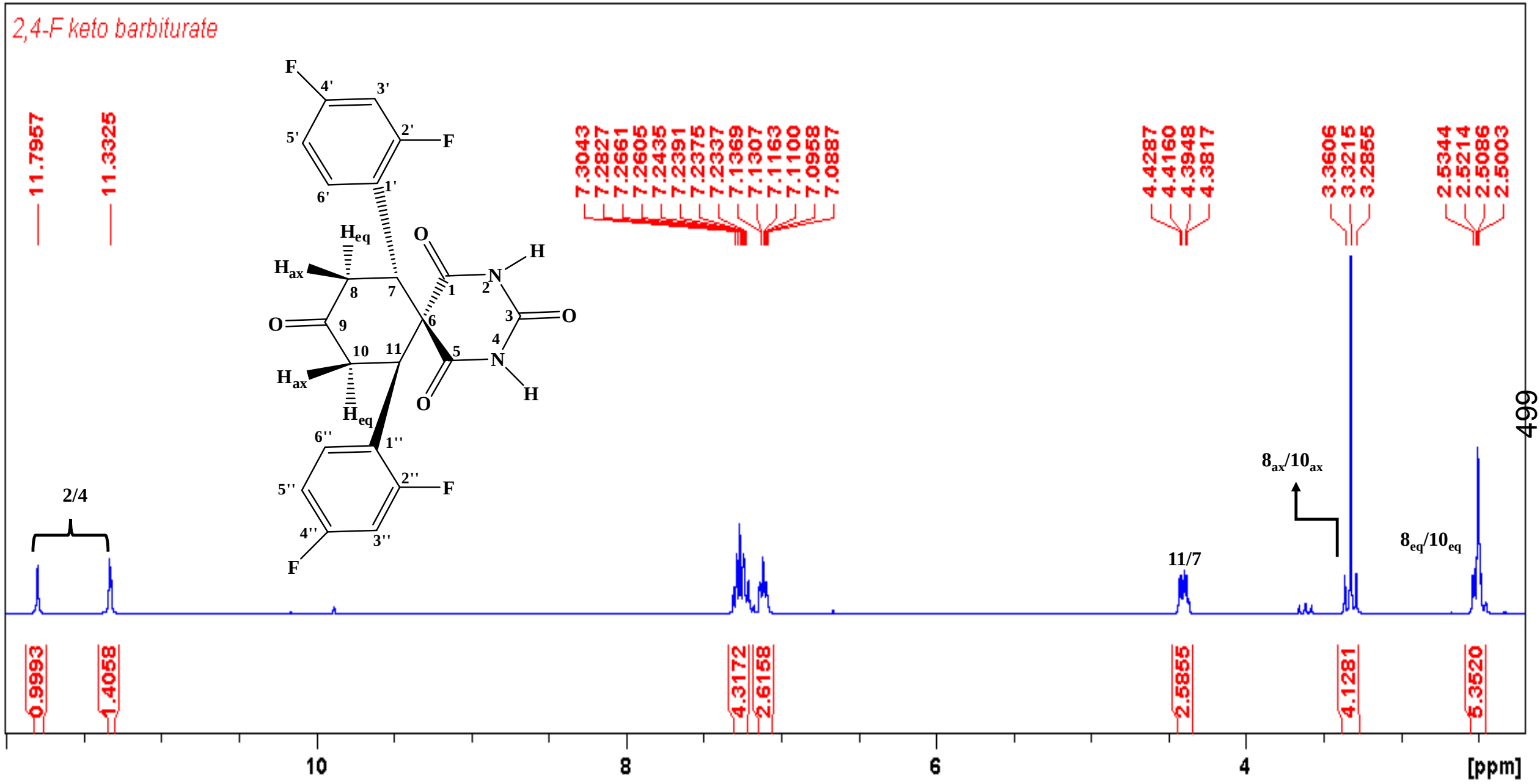
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
106 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 0-5 F: 0-5
21 4-F spiro 10 (0.304) Cm (1:61)
TOF MS ES-

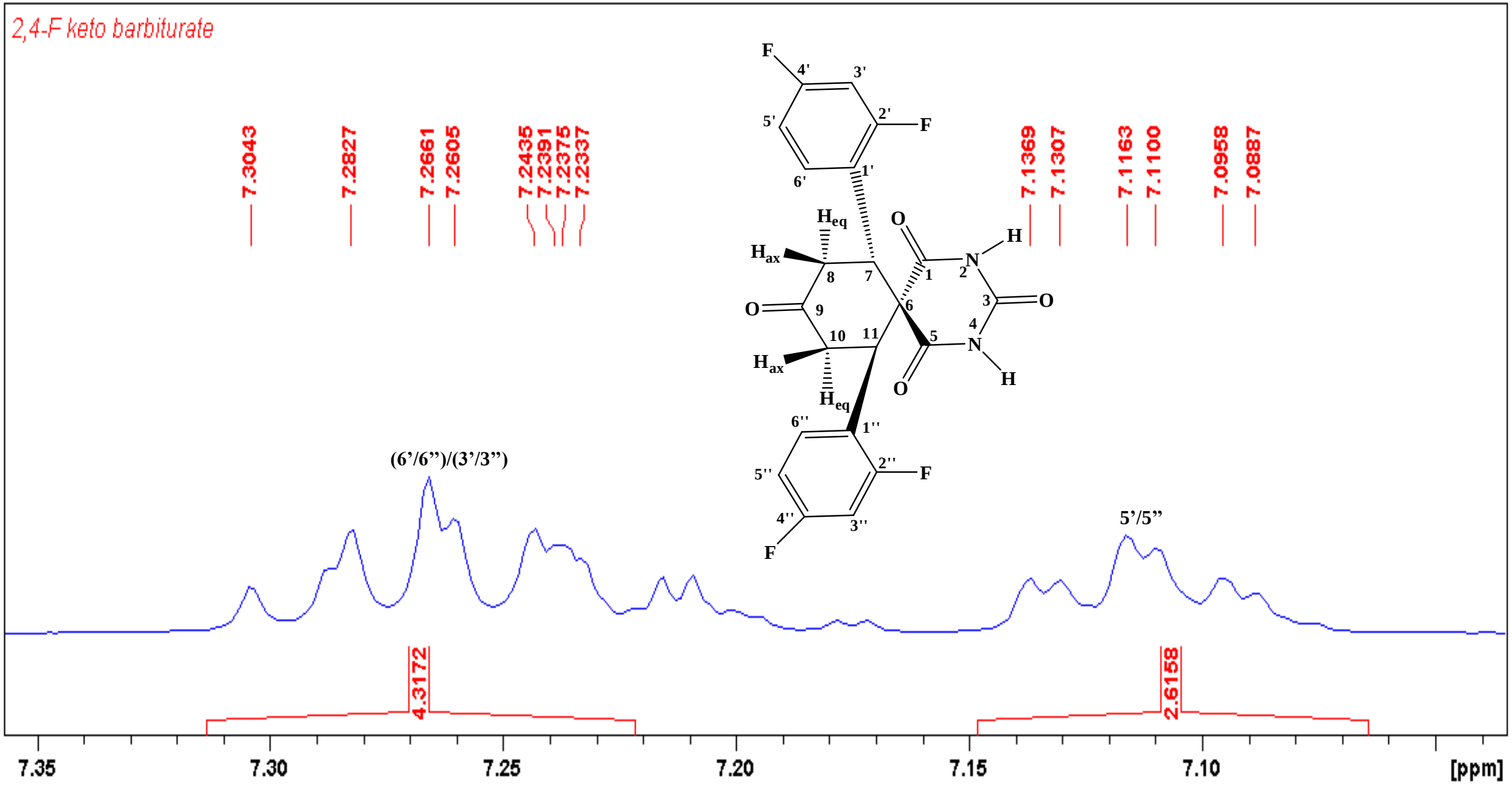


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
397.1013	397.1000	1.3	3.3	14.5	53.7	0.0	C21 H15 N2 O4 F2

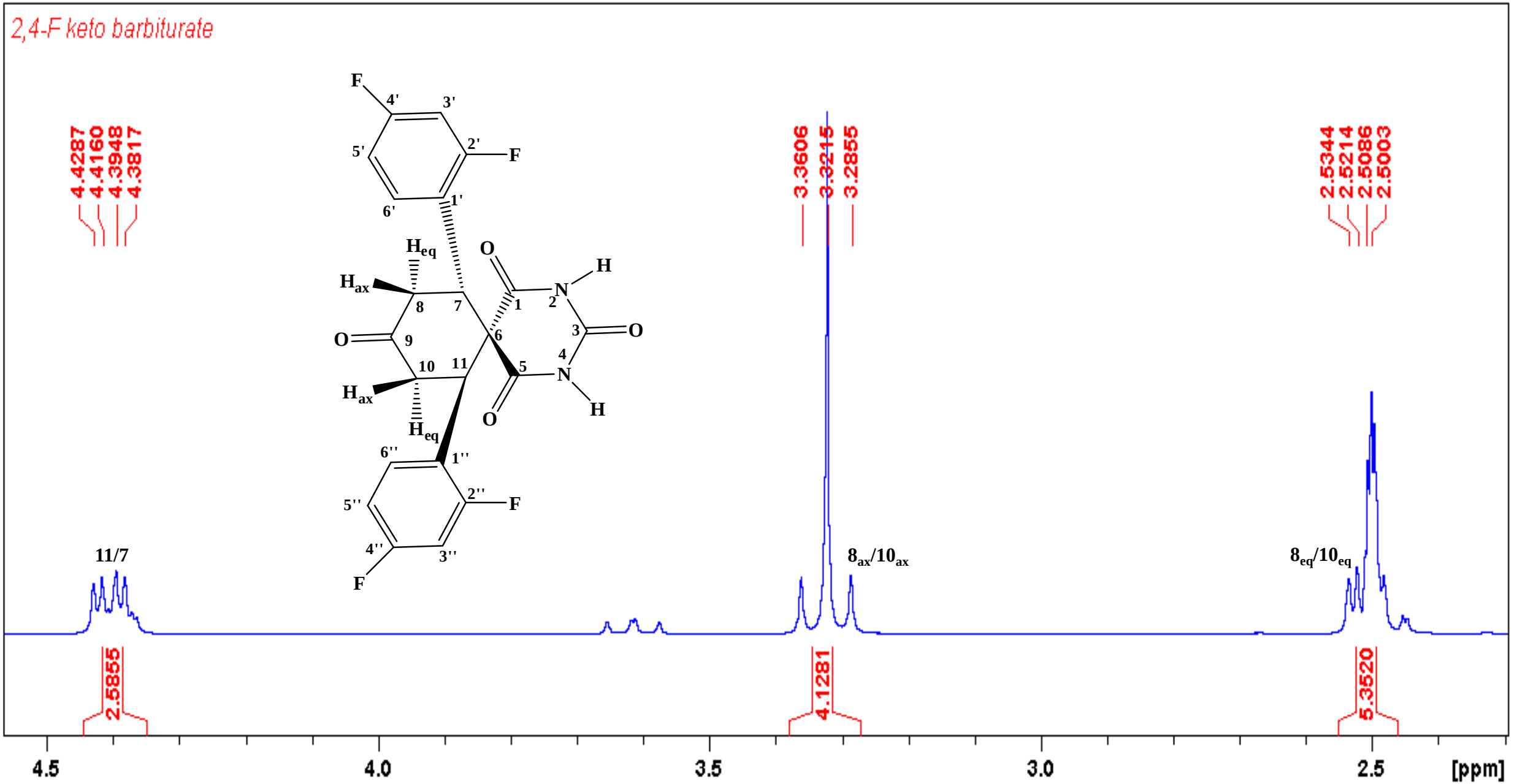
HRMS of Compound B-3h: 7,11-bis(4-fluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



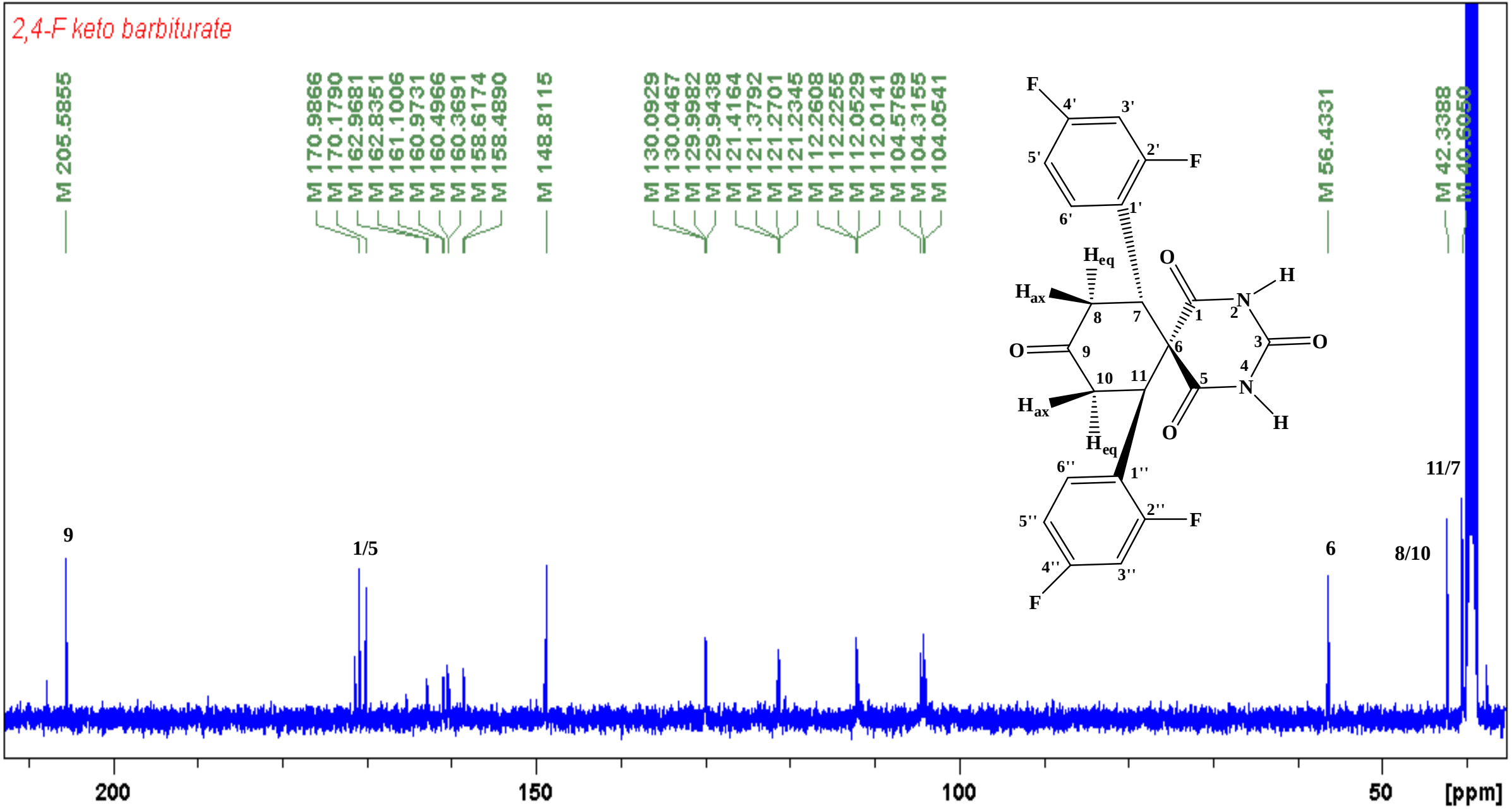
^{1}H Spectrum of Compound B-3i: 7,11-bis(2,4-difluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



Expanded ^{1}H Spectrum of Compound B-3i: 7,11-bis(2,4-difluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone

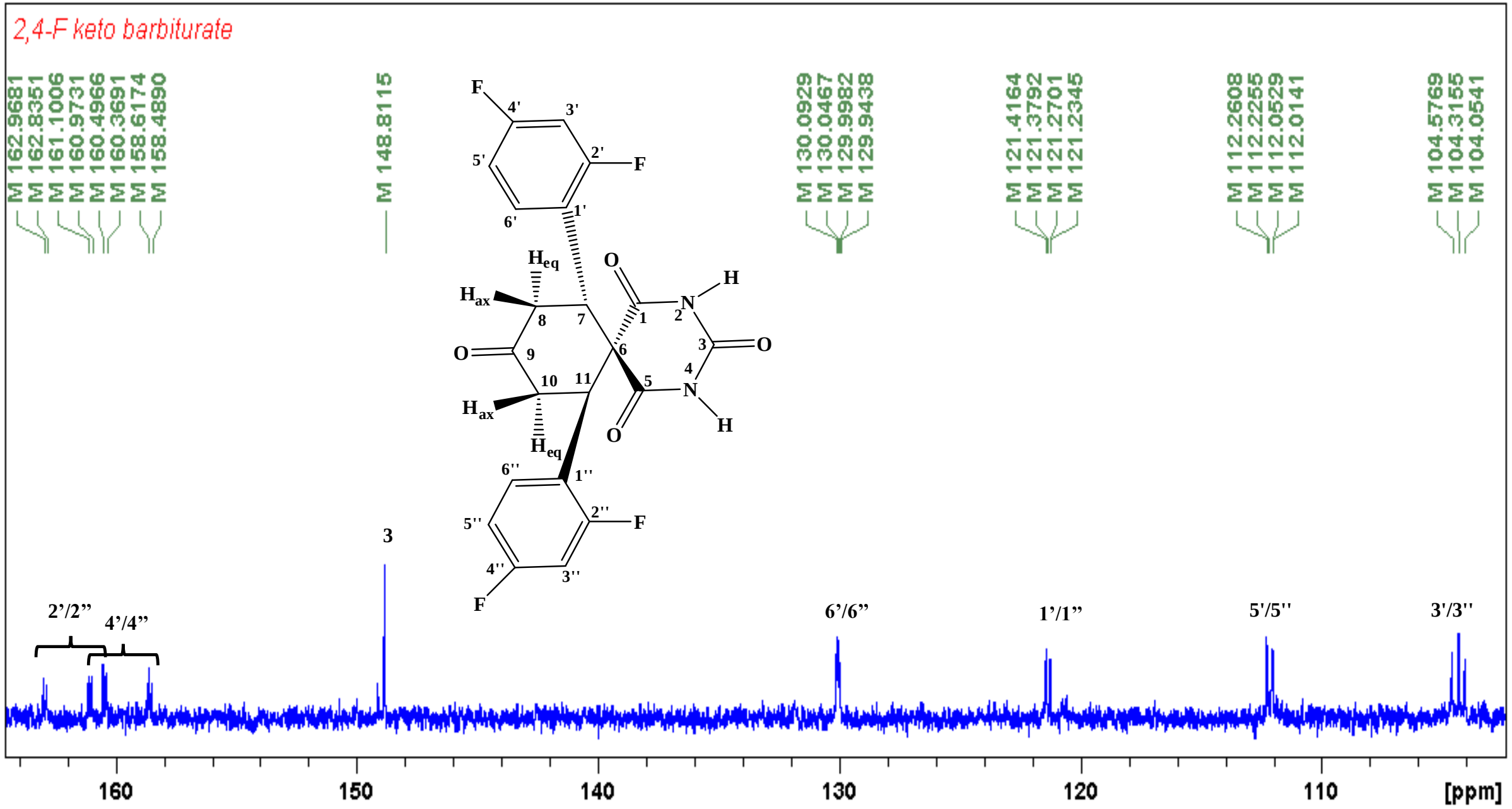


Expanded ^{1}H Spectrum of Compound B-3i: 7,11-bis(2,4-difluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



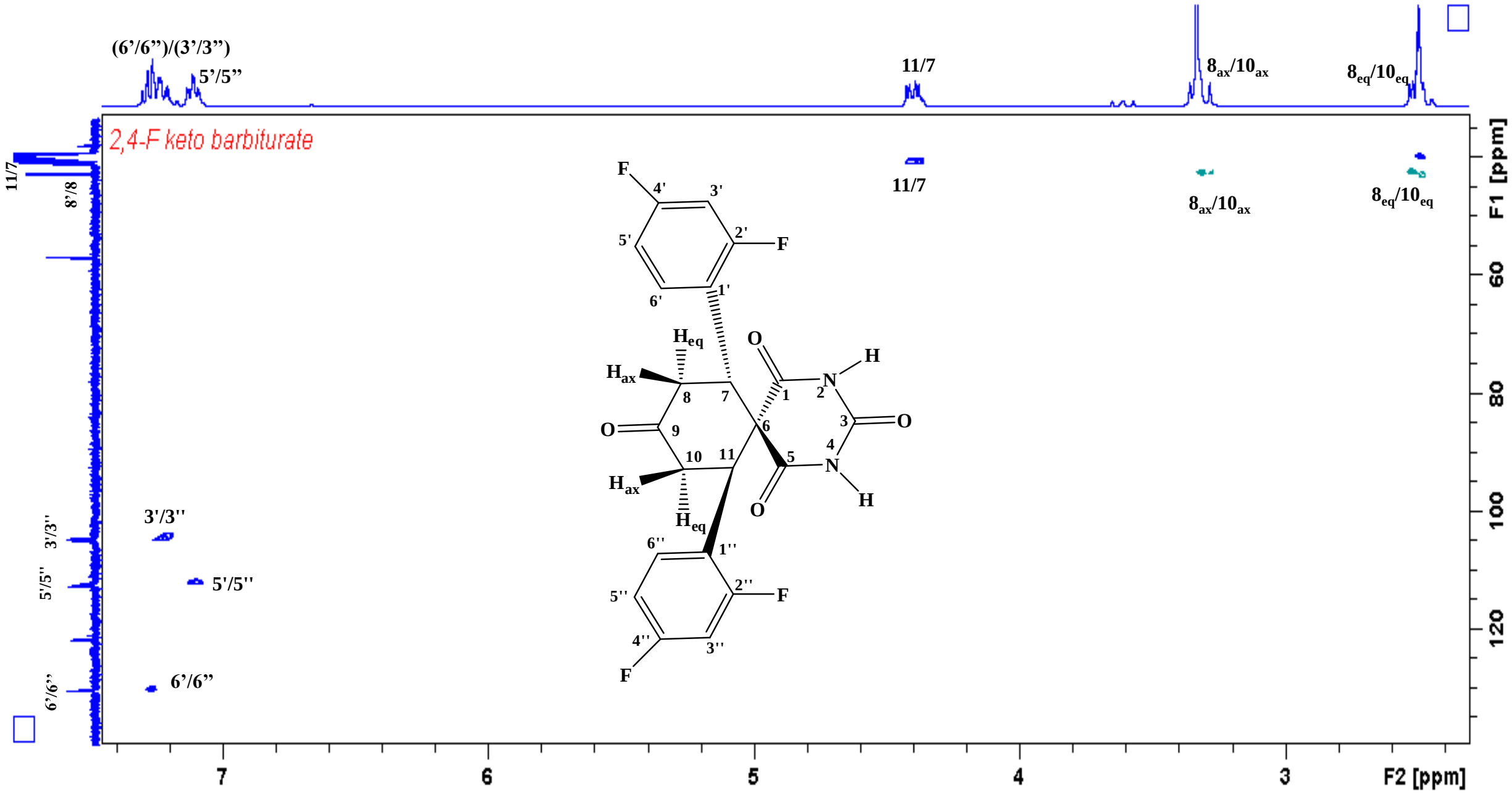
502

^{13}C Spectrum of Compound B-3i: 7,11-bis(2,4-difluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone

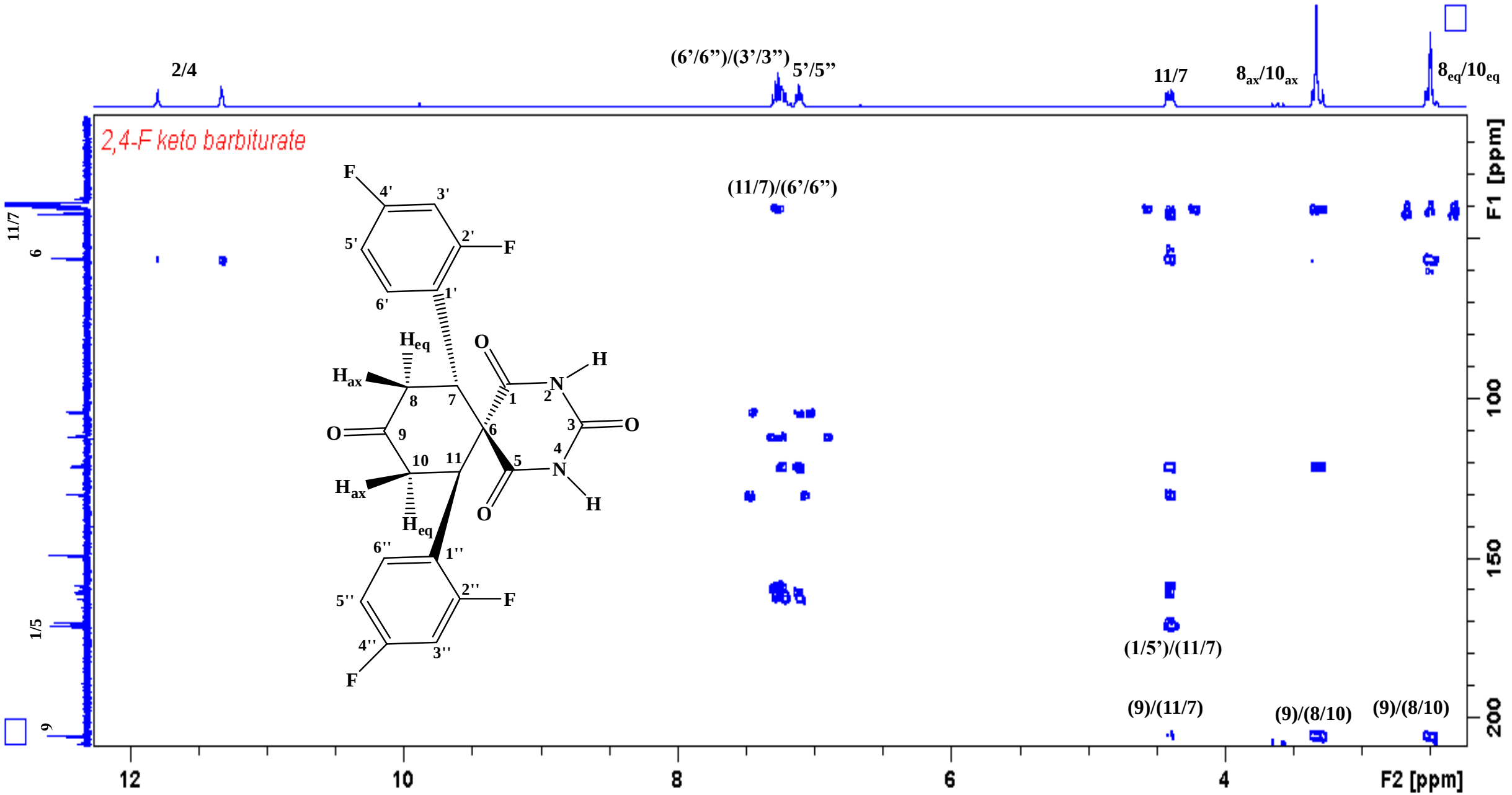


503

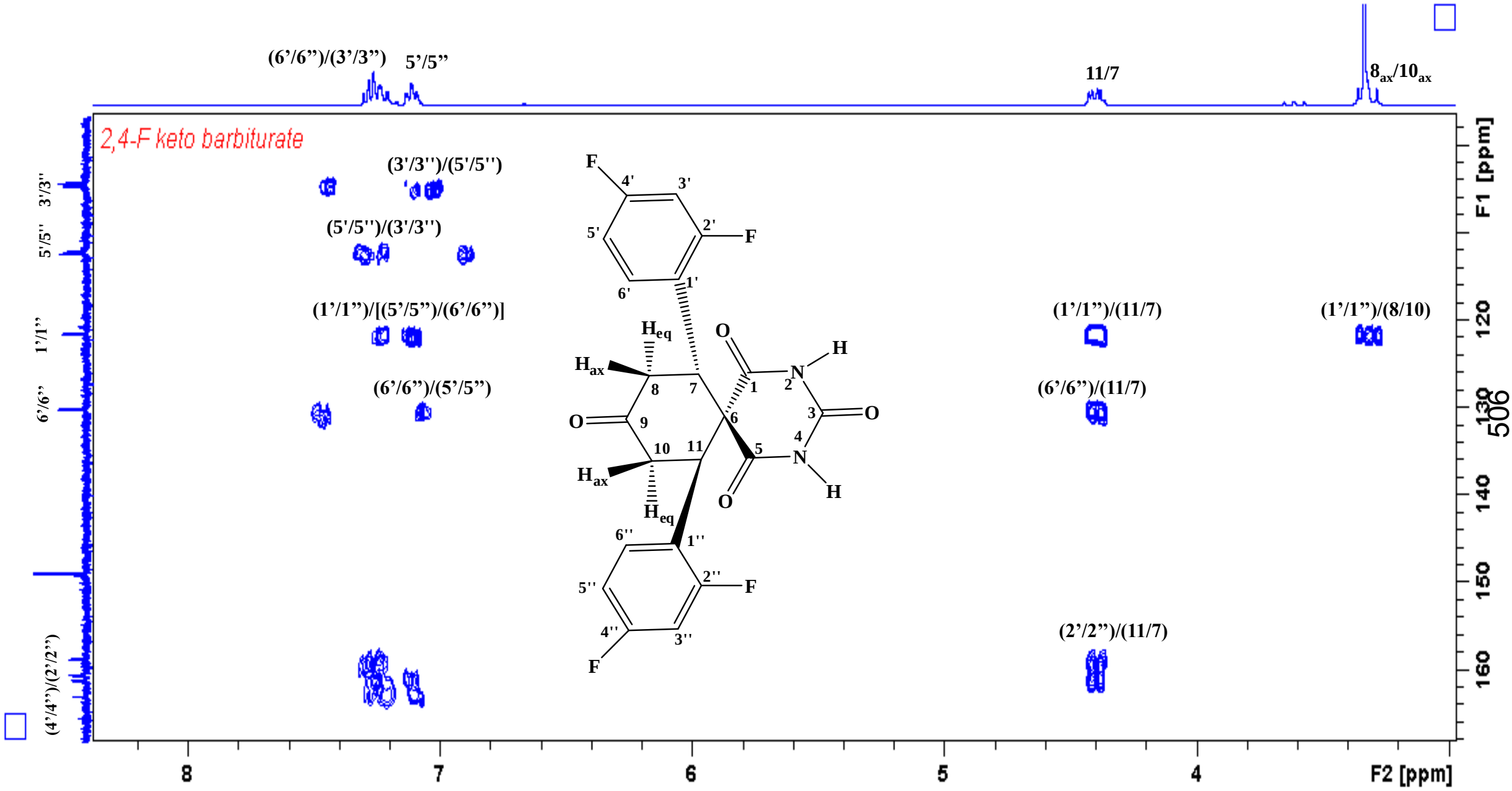
Expanded ^{13}C Spectrum of Compound B-3i: 7,11-bis(2,4-difluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



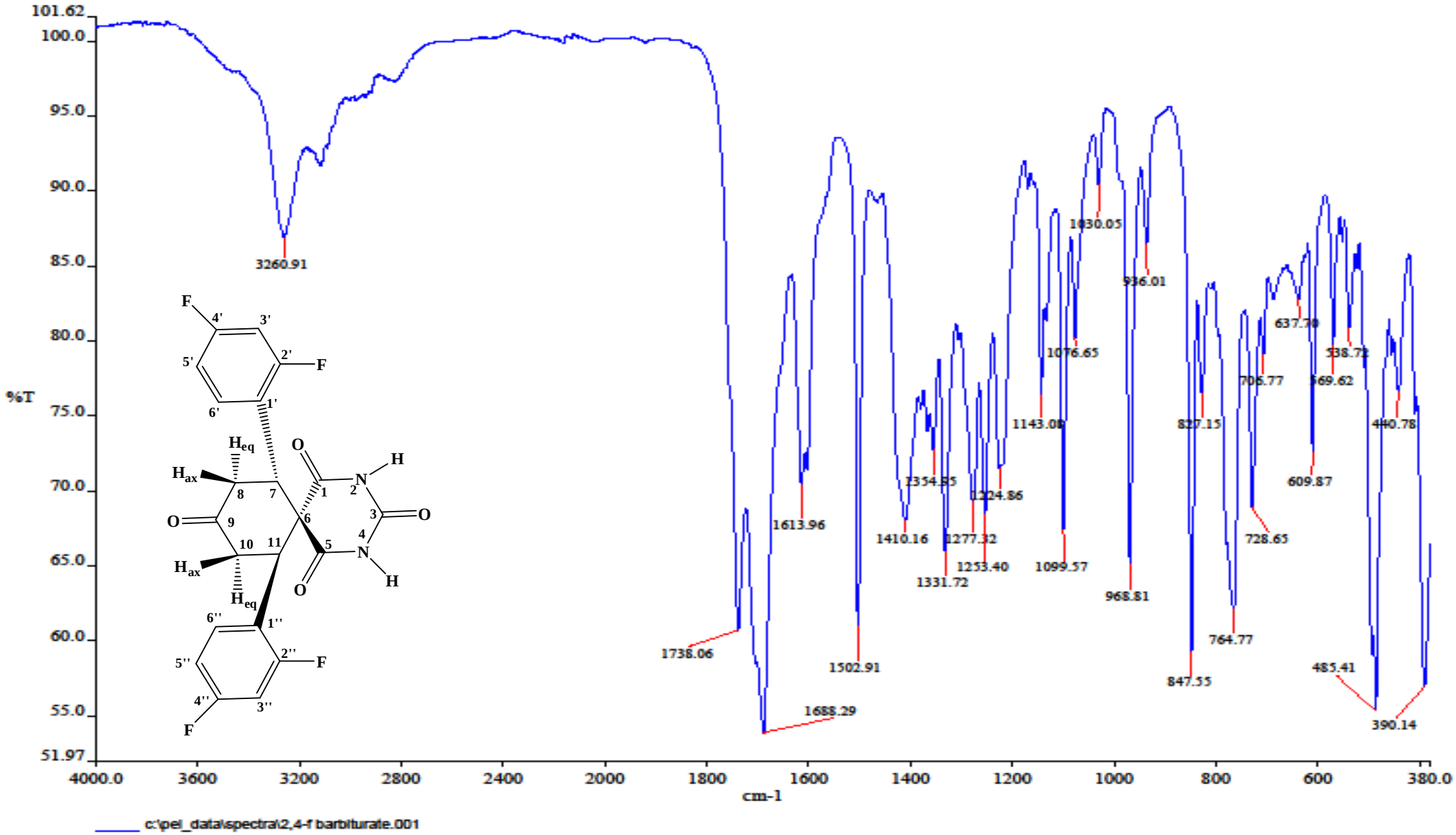
HSQC of Compound B-3i: 7,11-bis(2,4-difluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



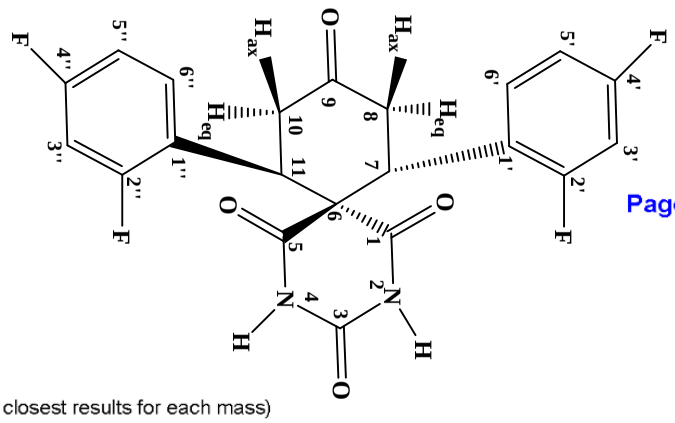
HMBC of Compound B-3i: 7,11-bis(2,4-difluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



Expanded HMBC of Compound B-3i: 7,11-bis(2,4-difluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



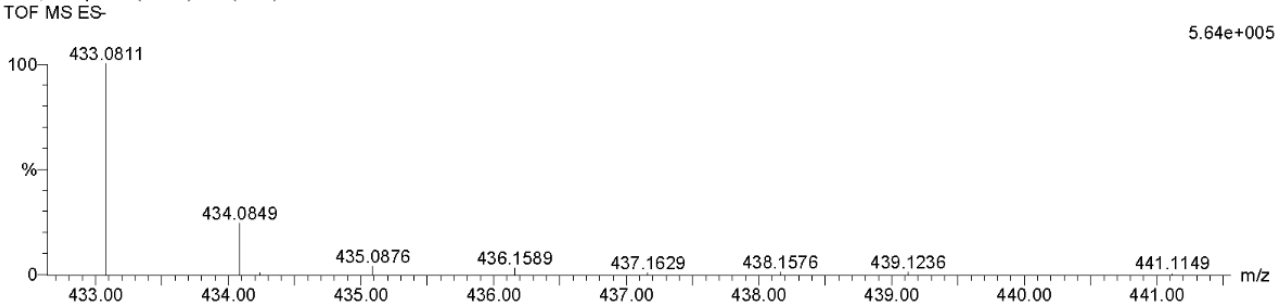
Infrared Spectrum of Compound B-3i: 7,11-bis(2,4-difluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



Elemental Composition Report **Page 1**

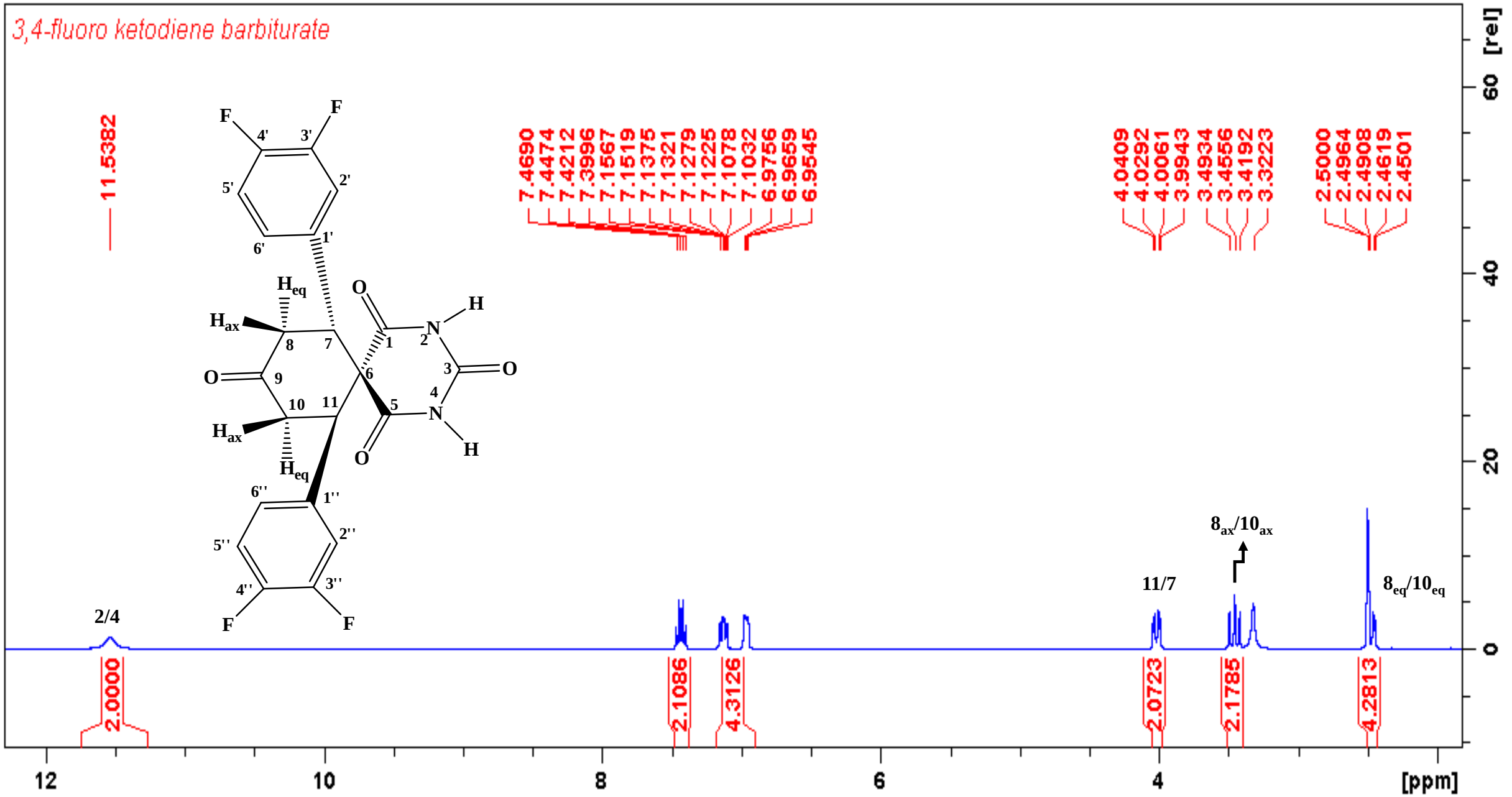
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
104 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-23 H: 10-15 N: 0-5 O: 0-5 F: 0-5
22 2,4-F spiro 8 (0.236) Cm (1:61)
TOF MS ES-

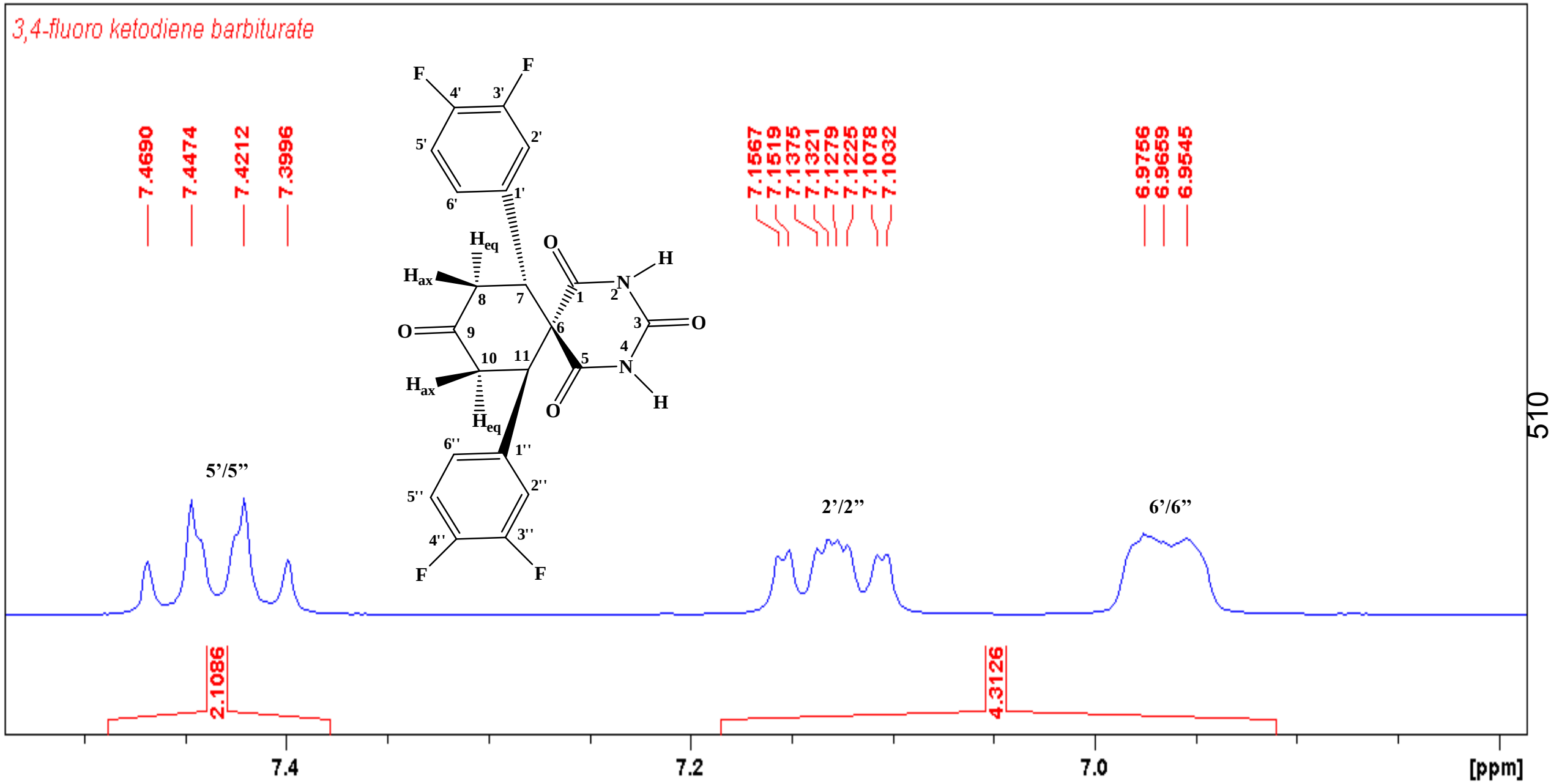


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
433.0811	433.0811	0.0	0.0	14.5	27.6	0.0	C21 H13 N2 O4 F4

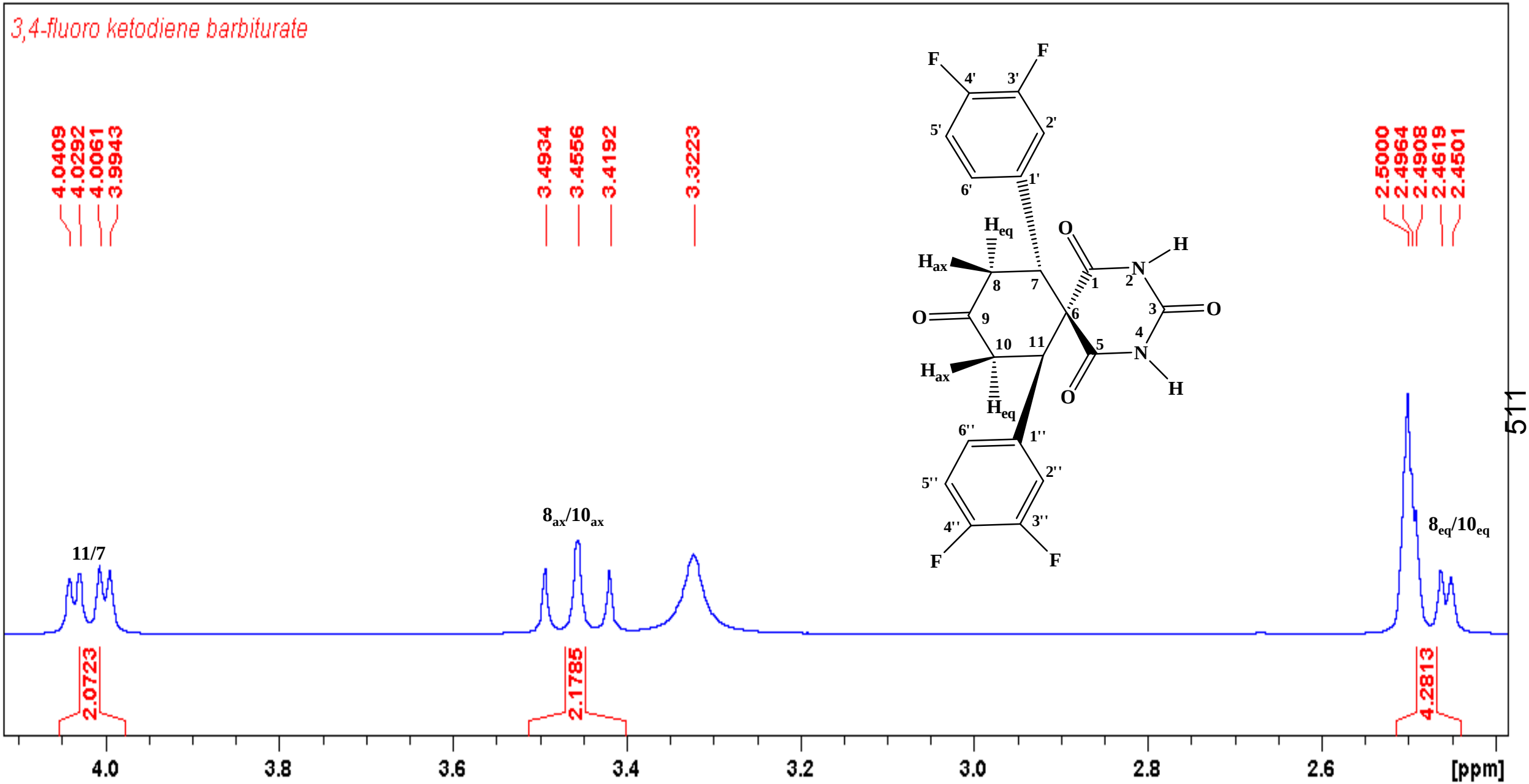
HRMS of Compound B-3i: 7,11-bis(2,4-difluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



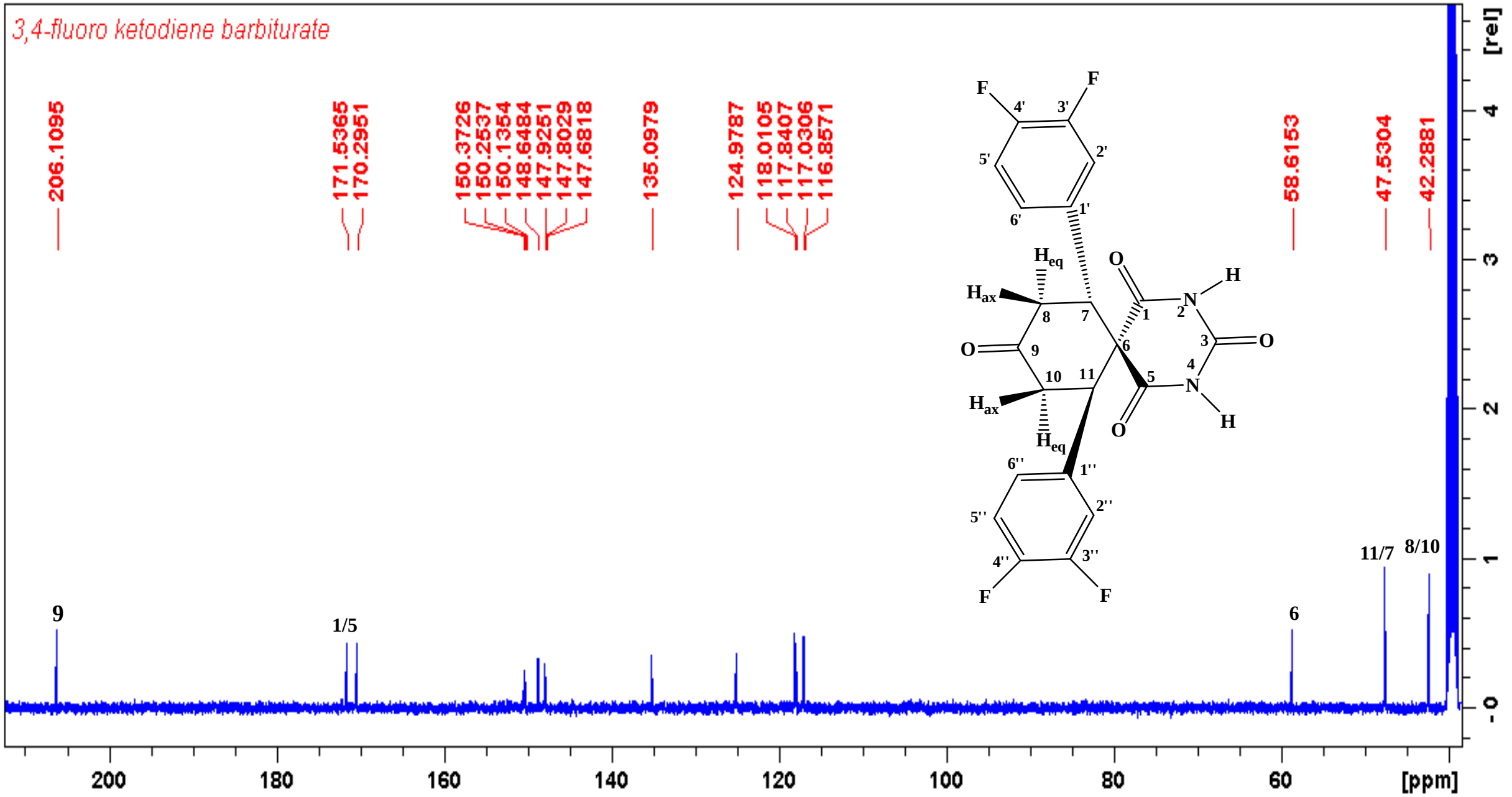
^{1}H Spectrum of Compound B-3j: 7,11-bis(3,4-difluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



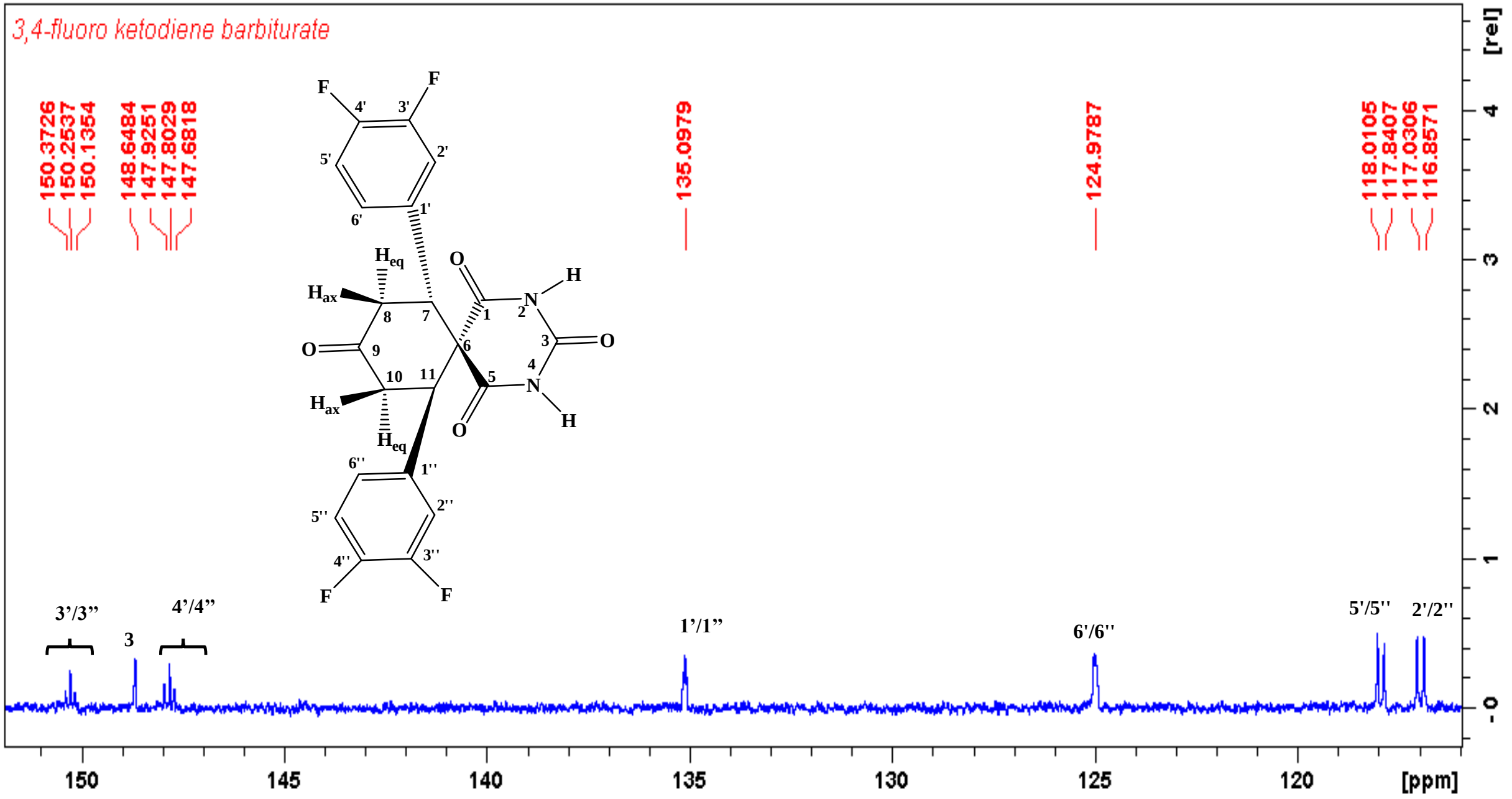
Expanded ^{1}H Spectrum of Compound B-3j: 7,11-bis(3,4-difluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



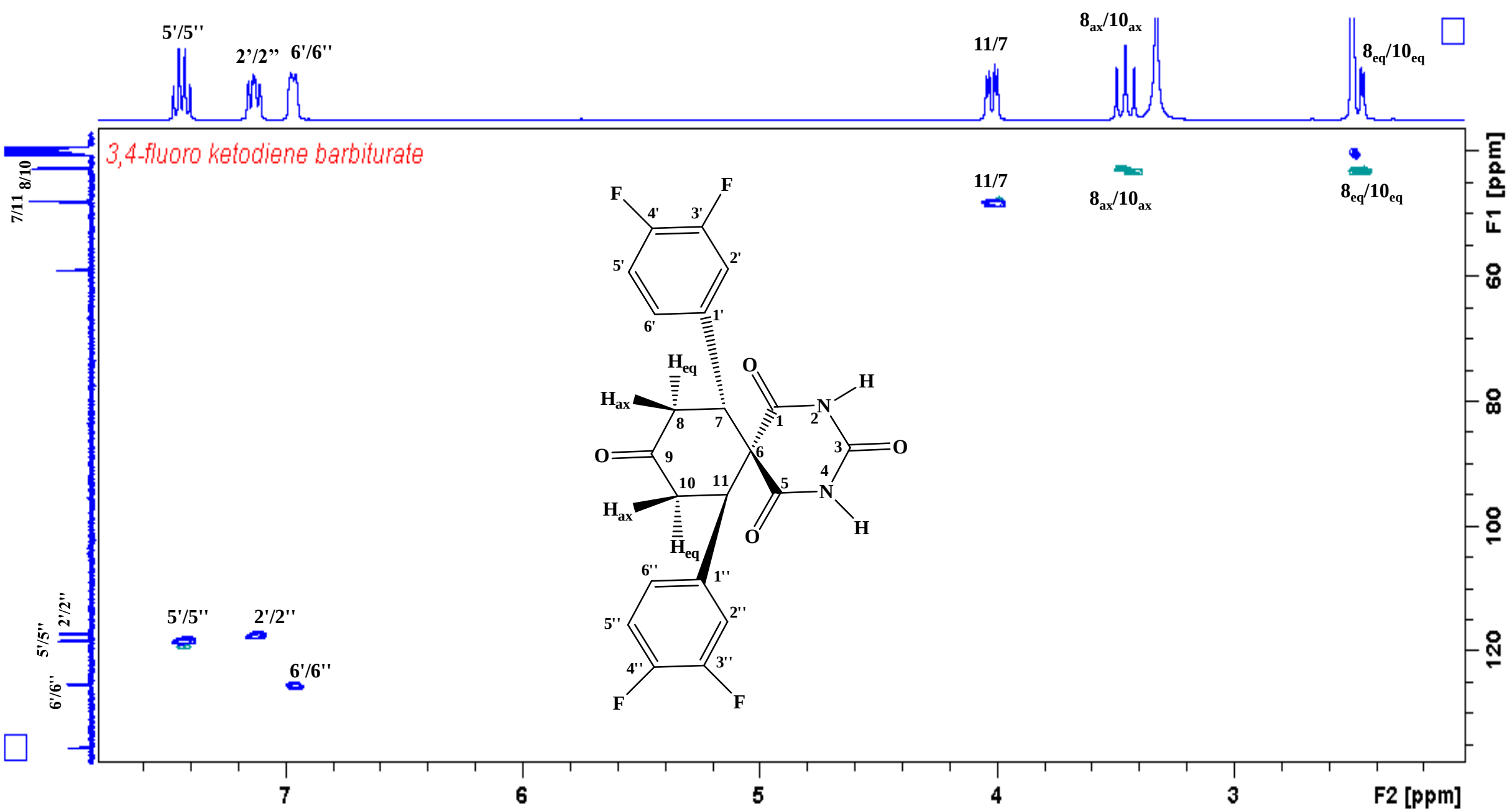
Expanded ^{1}H Spectrum of Compound B-3j: 7,11-bis(3,4-difluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



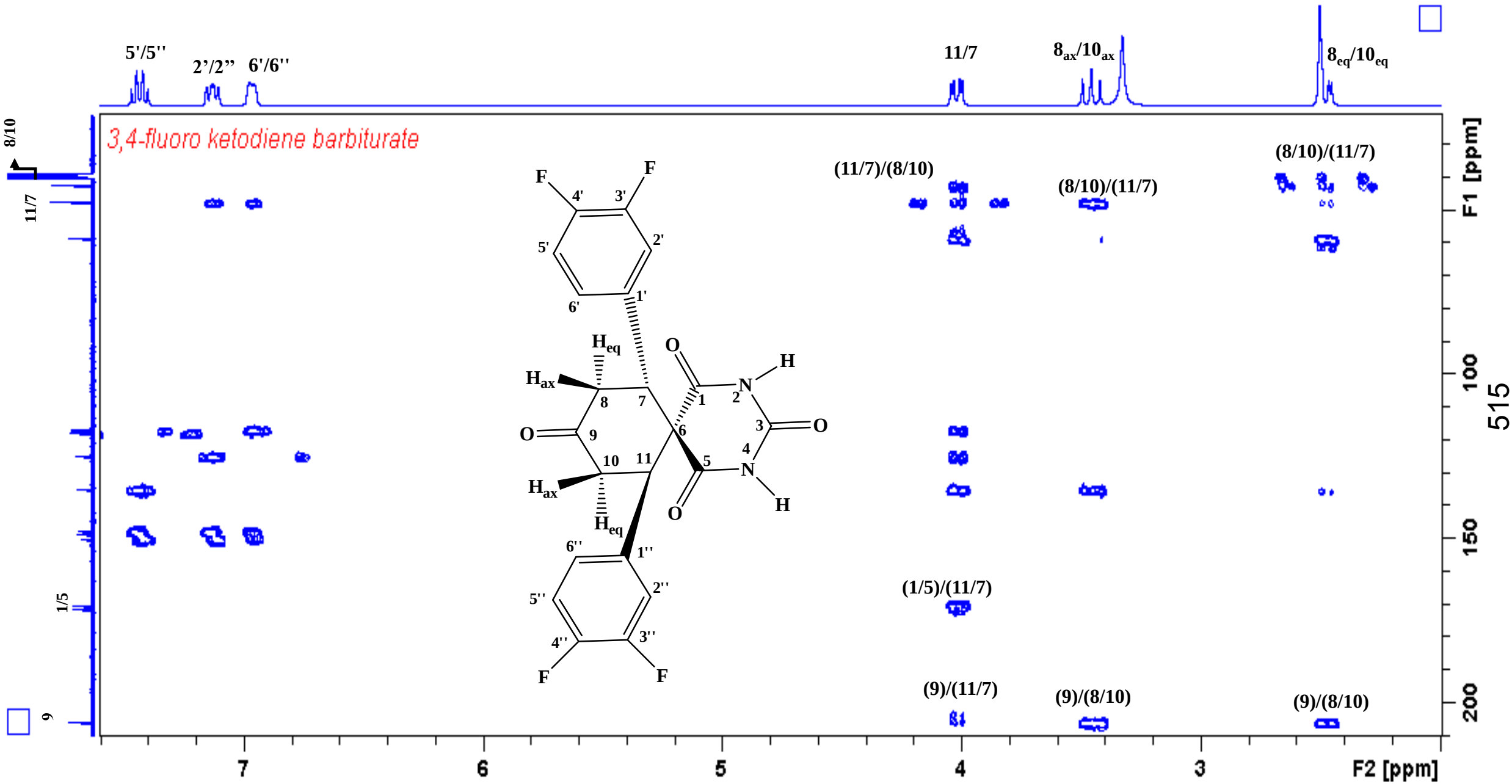
^{13}C Spectrum of Compound B-3j: 7,11-bis(3,4-difluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



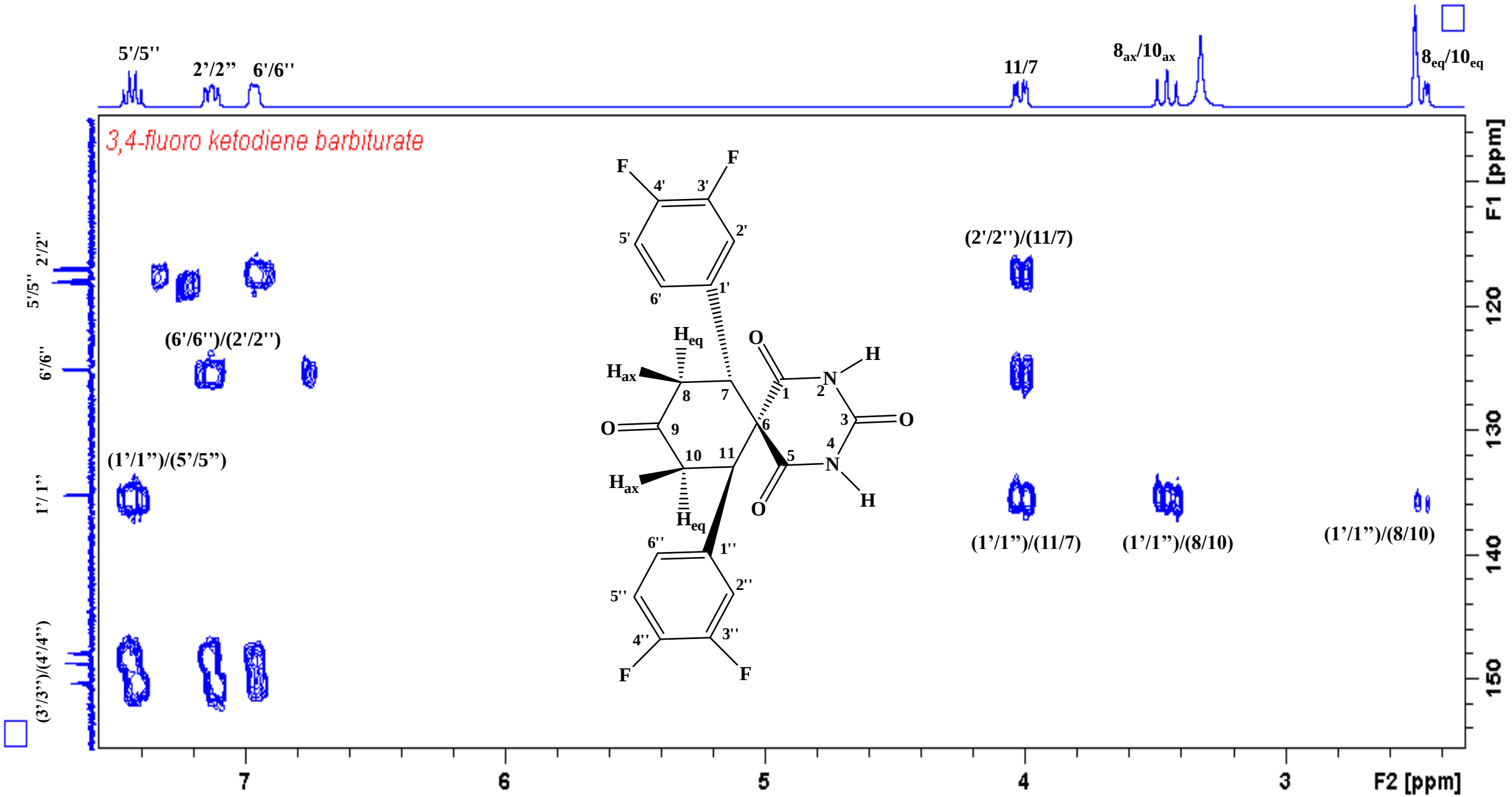
Expanded ^{13}C Spectrum of Compound B-3j: 7,11-bis(3,4-difluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



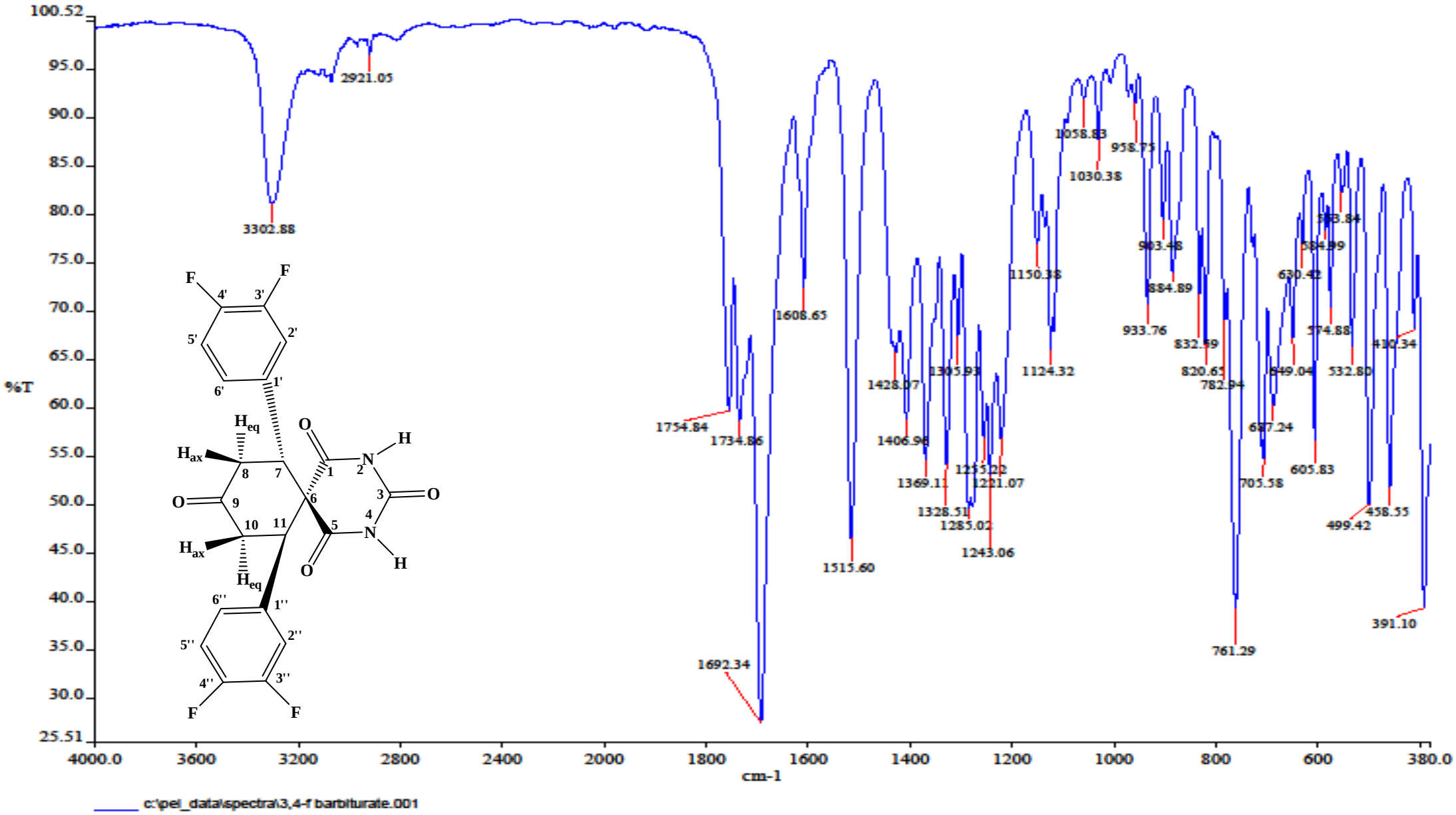
HSQC of Compound B-3j: 7,11-bis(3,4-difluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



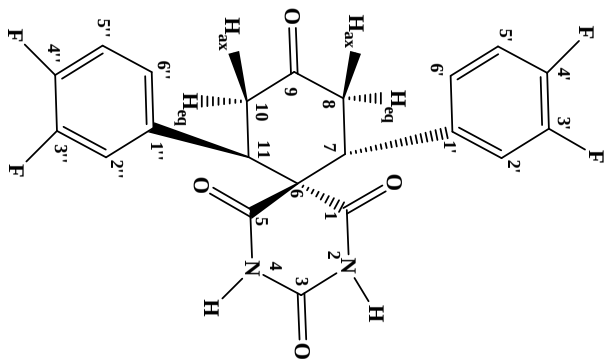
HMBC of Compound B-3j: 7,11-bis(3,4-difluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



Expanded HMBC of Compound B-3j: 7,11-bis(3,4-difluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



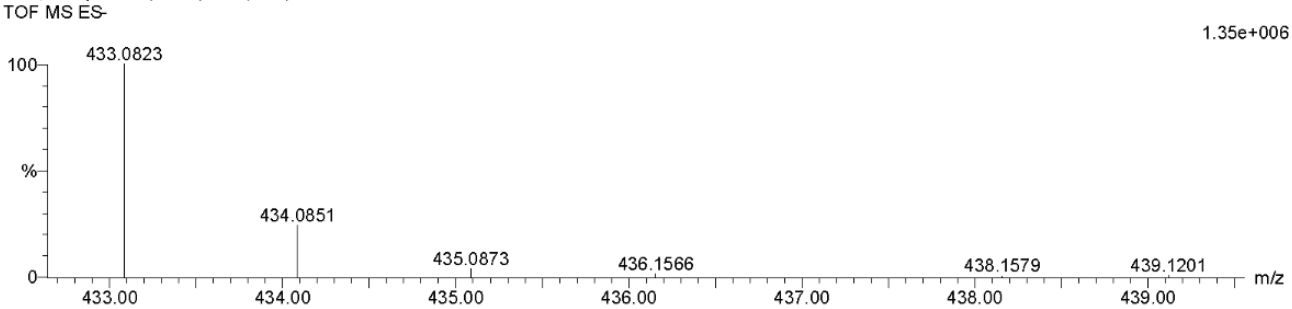
Infrared Spectrum of Compound B-3j: 7,11-bis(3,4-difluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



Elemental Composition Report **Page 1**

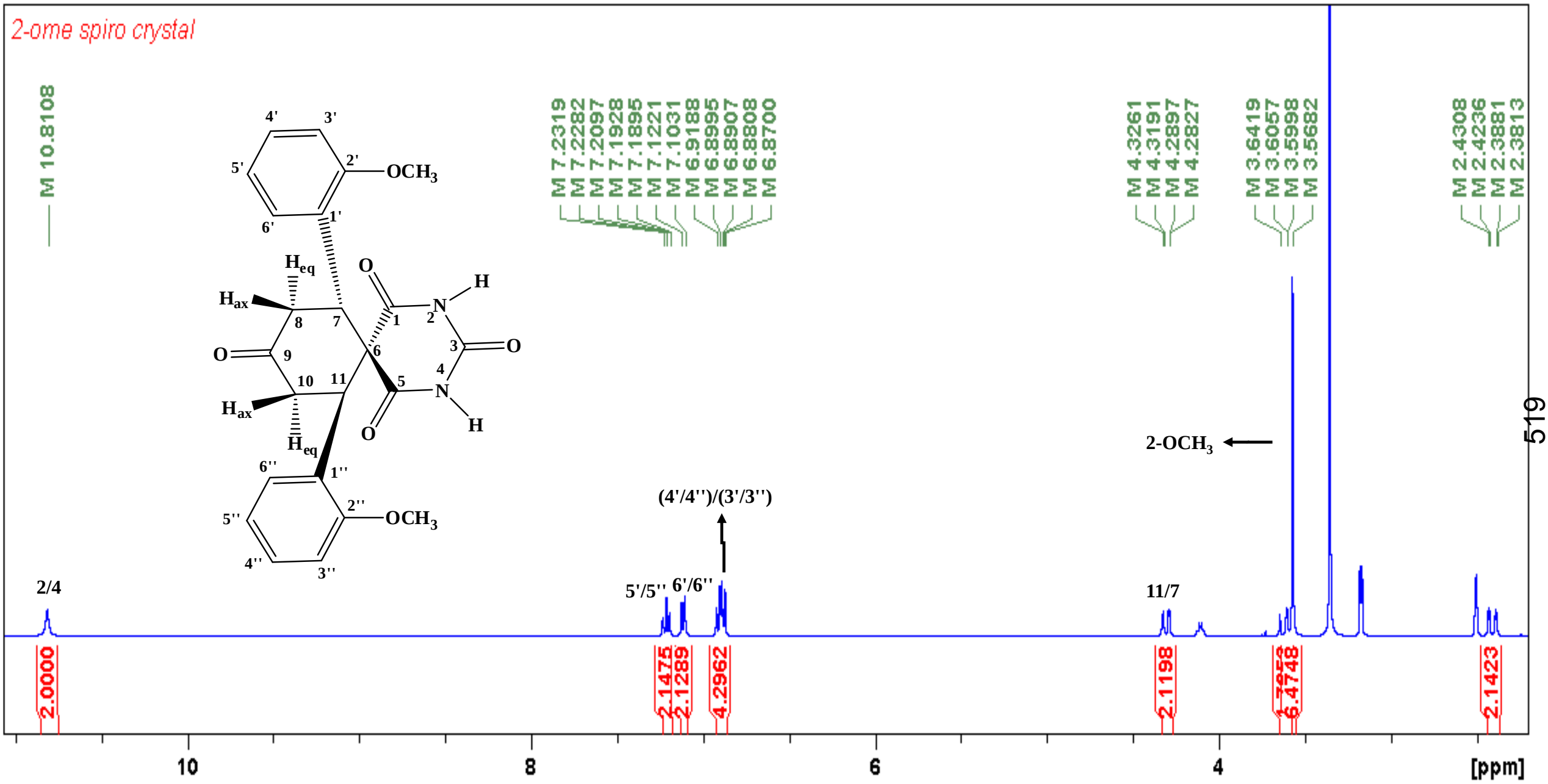
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
104 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-23 H: 10-15 N: 0-5 O: 0-5 F: 0-5
23 3,4-F spiro 48 (1.586) Cm (1:61)
TOF MS ES-

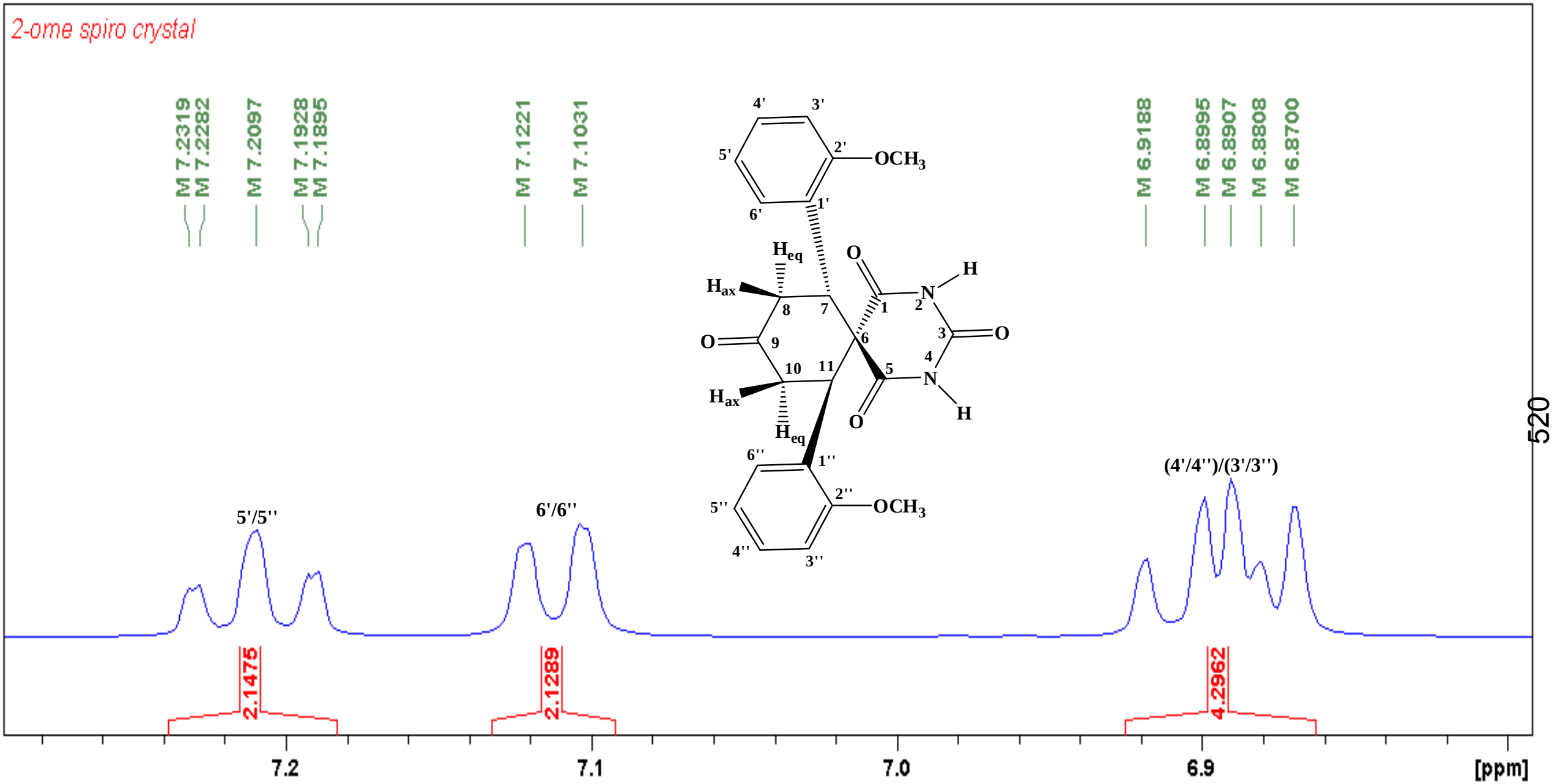


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
433.0823	433.0811	1.2	2.8	14.5	12.4	0.0	C21 H13 N2 O4 F4

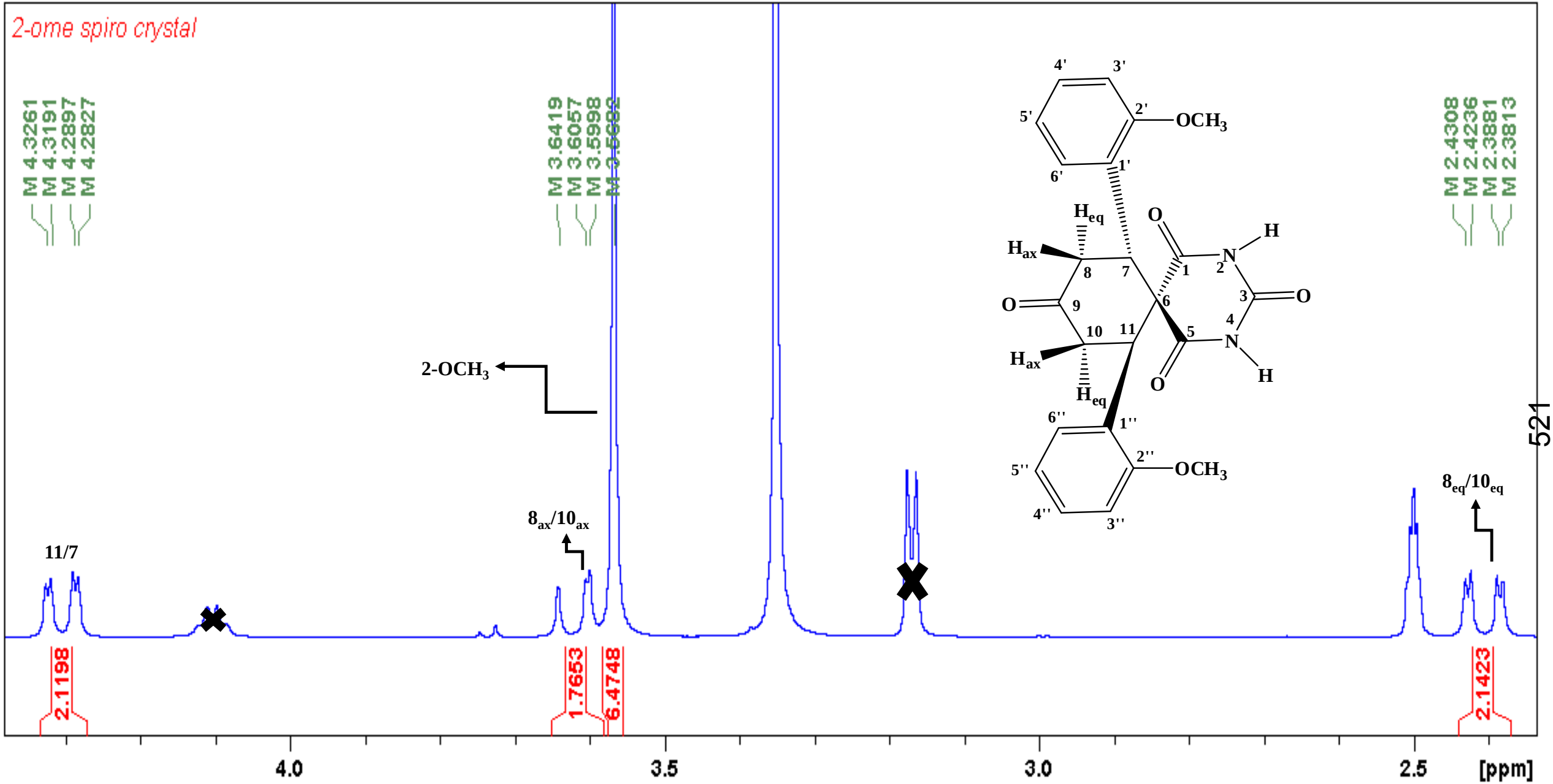
HRMS of Compound B-3j: 7,11-bis(3,4-difluorophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



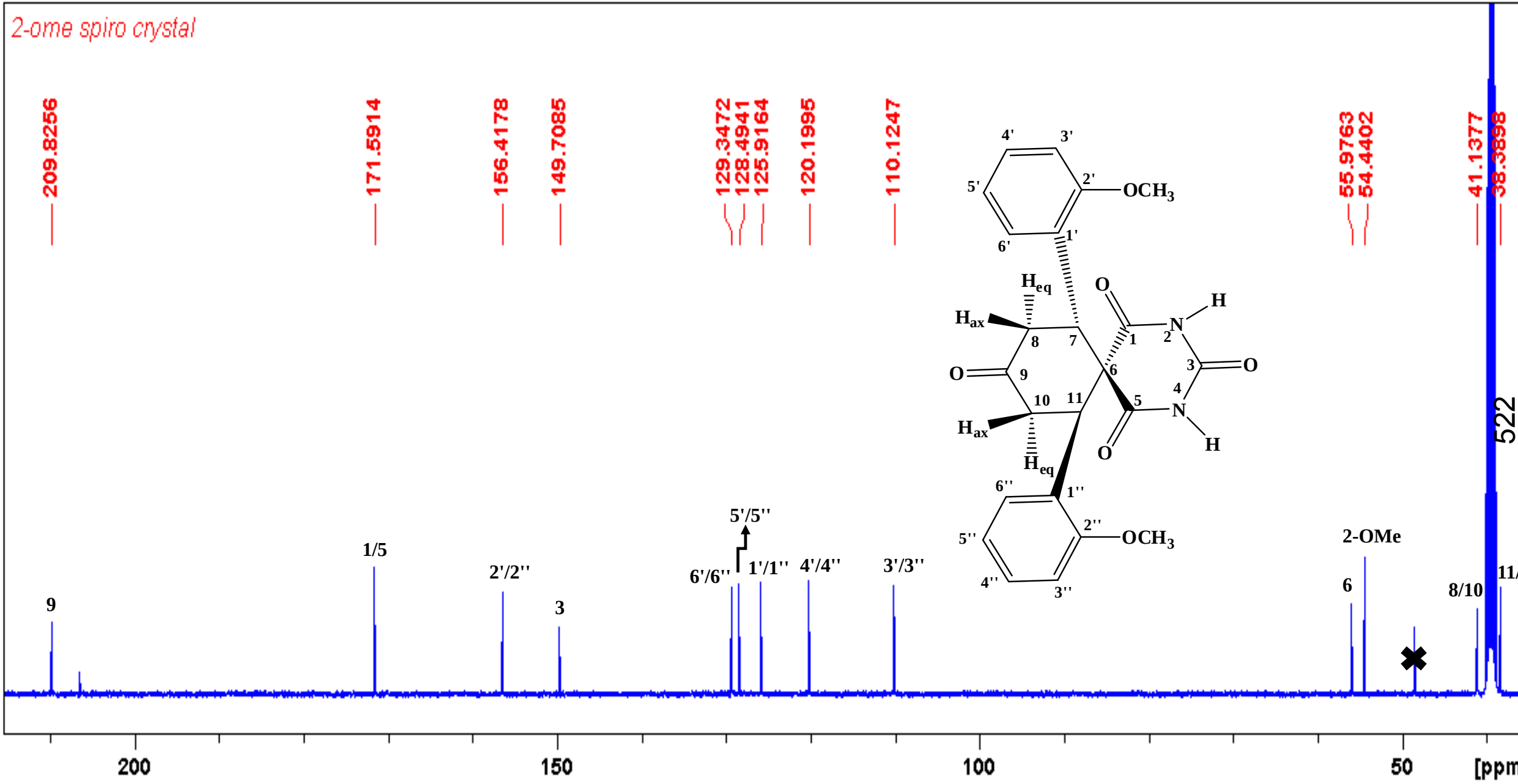
^{1}H Spectrum of Compound B-2k: 5,5'-(1,5-bis(2-methoxyphenyl)-3-oxopentane-1,5-diyl)bis(4,6-dihydroxypyrimidin-2(1H)-one)



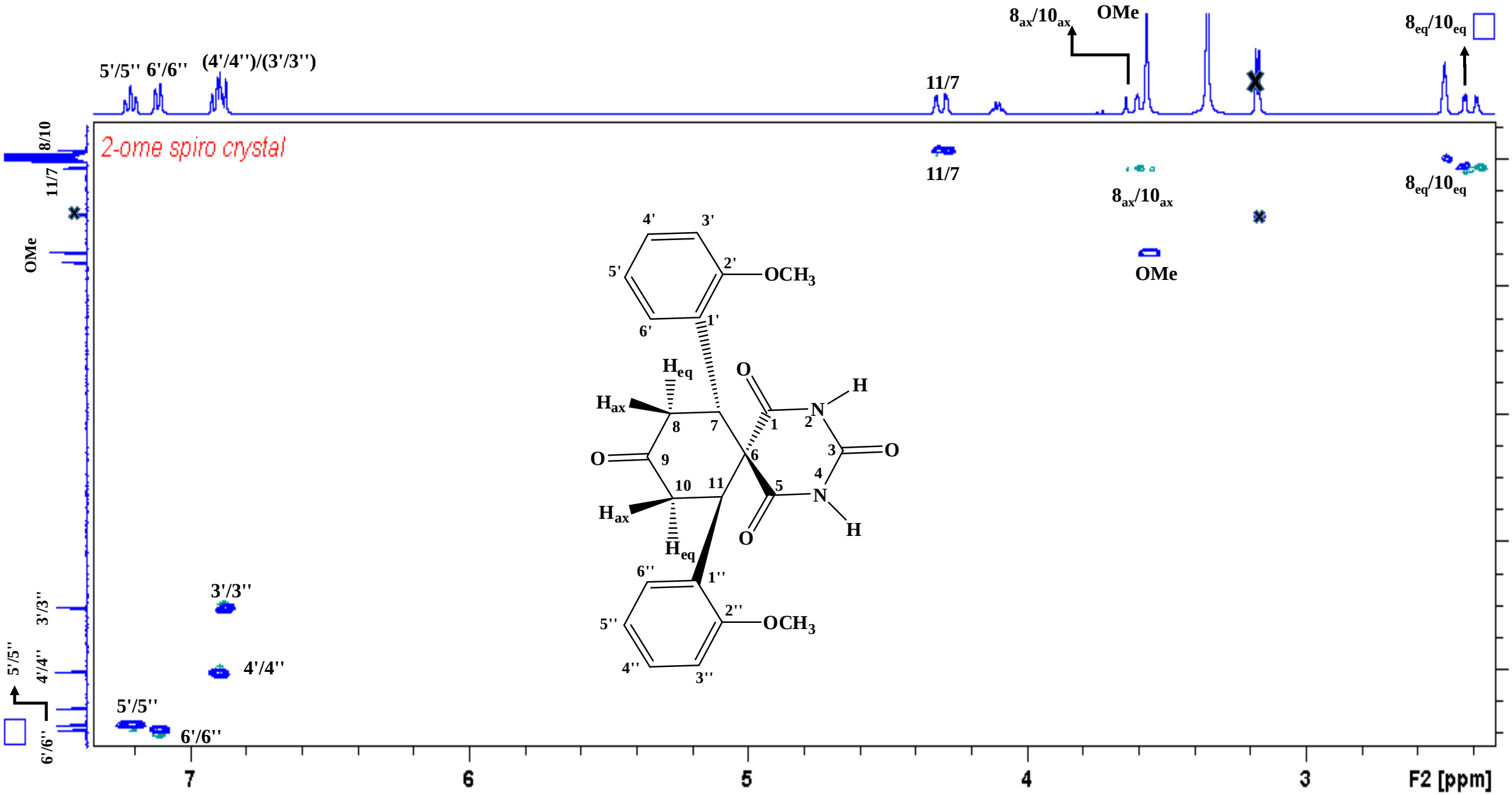
Expanded ^{1}H Spectrum of Compound B-2k: 5,5'-(1,5-bis(2-methoxyphenyl)-3-oxopentane-1,5-diyl)bis(4,6-dihydroxypyrimidin-2(1H)-one)



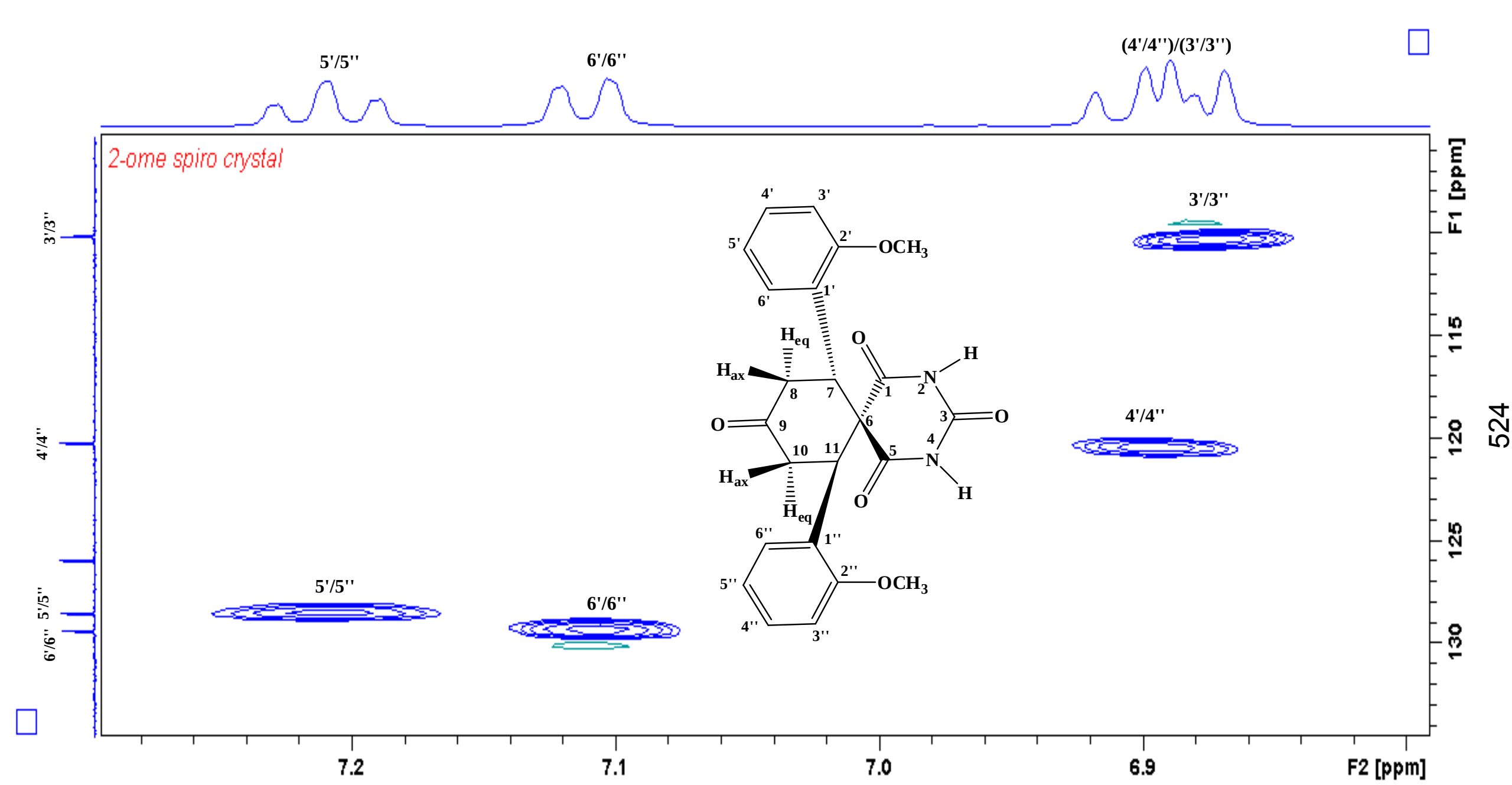
Expanded ^{1}H Spectrum of Compound B-2k: 5,5'-(1,5-bis(2-methoxyphenyl)-3-oxopentane-1,5-diyl)bis(4,6-dihydroxypyrimidin-2(1H)-one)



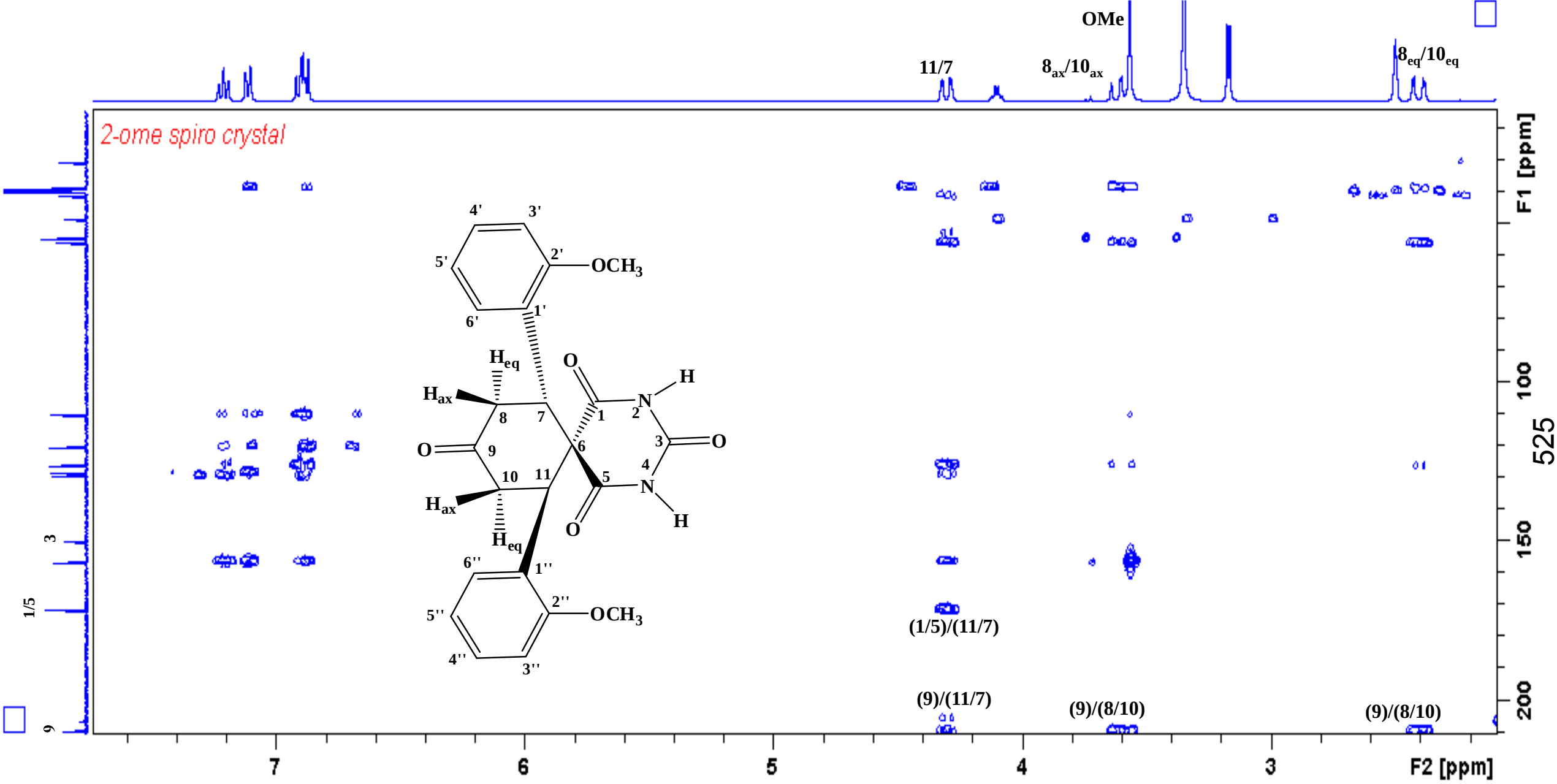
^{13}C Spectrum of Compound B-2k: 5,5'-(1,5-bis(2-methoxyphenyl)-3-oxopentane-1,5-diyl)bis(4,6-dihydroxypyrimidin-2(1H)-one)



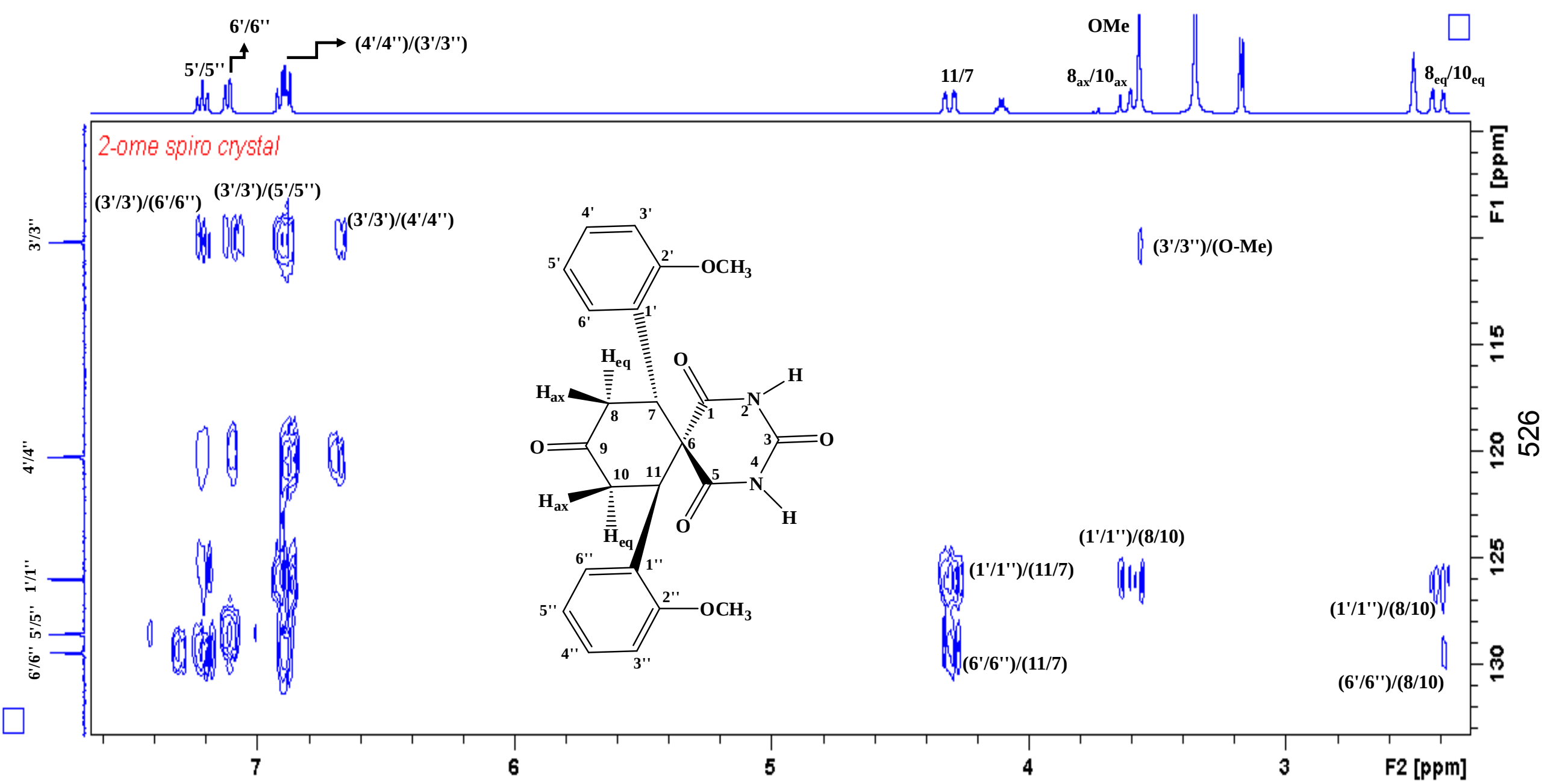
HSQC of Compound B-2k: 5,5'-(1,5-bis(2-methoxyphenyl)-3-oxopentane-1,5-diyl)bis(4,6-dihydroxypyrimidin-2(1H)-one)



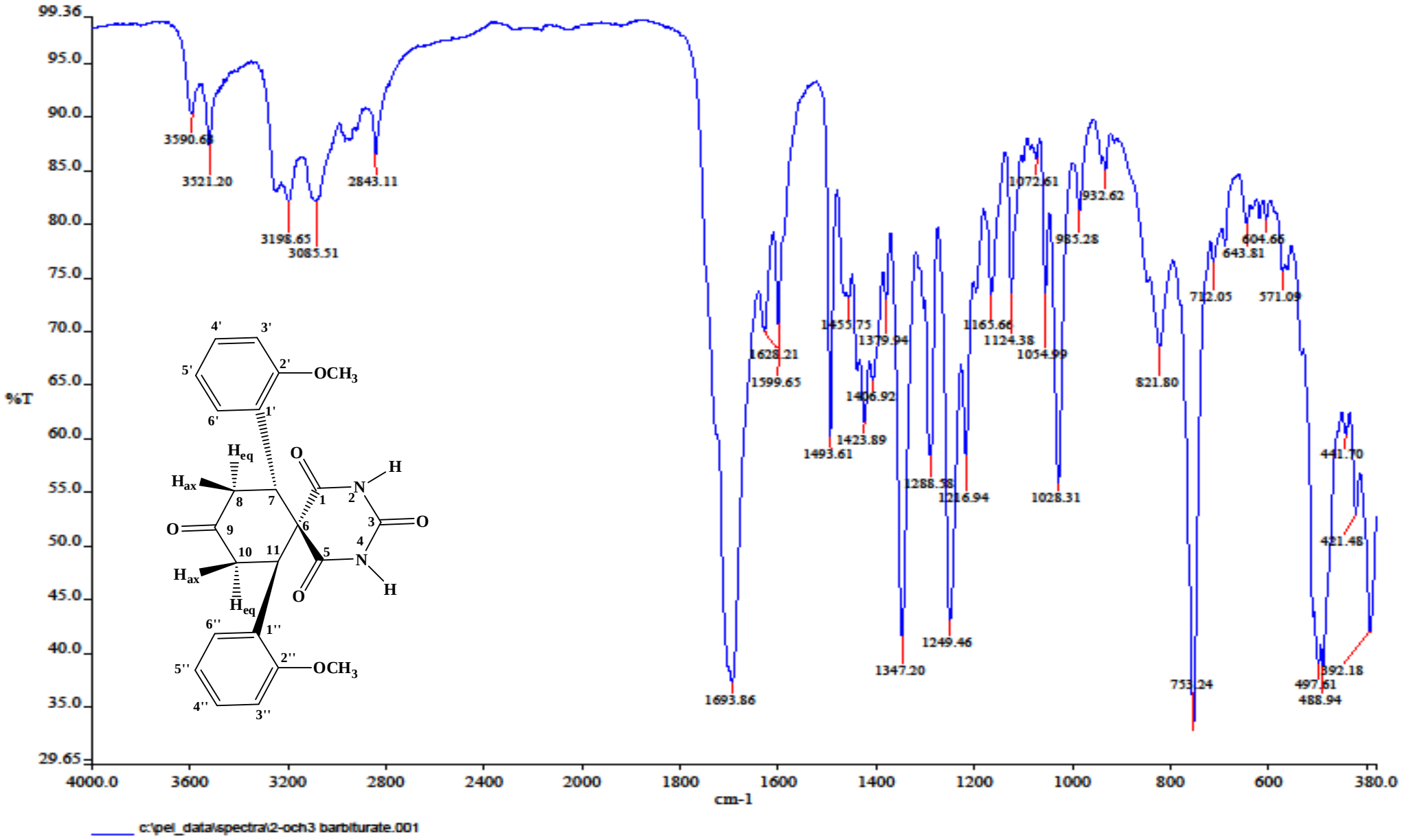
Expanded HSQC of Compound B-2k: 5,5'-(1,5-bis(2-methoxyphenyl)-3-oxopentane-1,5-diyl)bis(4,6-dihydroxypyrimidin-2(1H)-one)



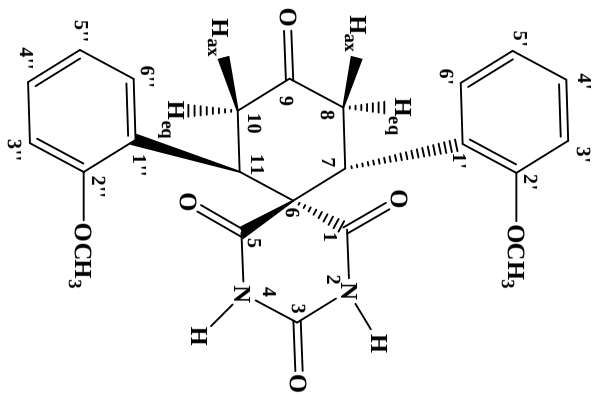
HMBC of Compound B-2k: 5,5'-(1,5-bis(2-methoxyphenyl)-3-oxopentane-1,5-diyl)bis(4,6-dihydroxypyrimidin-2(1H)-one)



Expanded HMBC of Compound B-2k: 5,5'-(1,5-bis(2-methoxyphenyl)-3-oxopentane-1,5-diyl)bis(4,6-dihydroxypyrimidin-2(1H)-one)



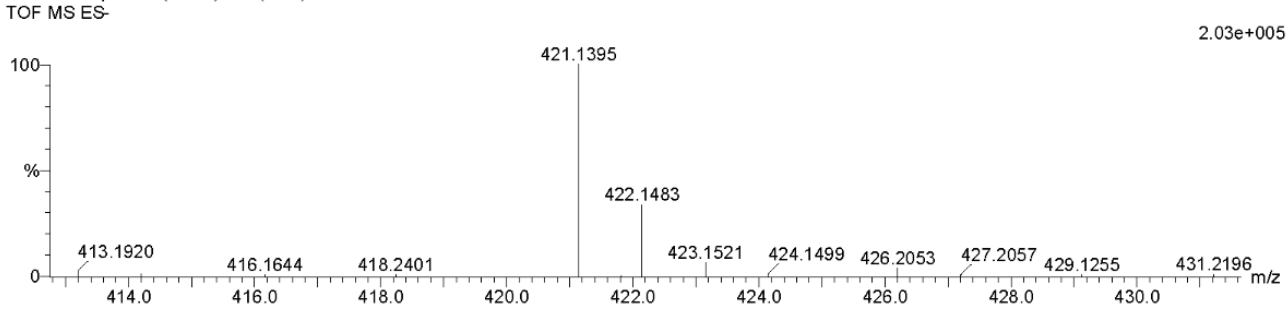
Infrared Spectrum of Compound B-2k: 5,5'-(1,5-bis(2-methoxyphenyl)-3-oxopentane-1,5-diyl)bis(4,6-dihydroxypyrimidin-2(1H)-one)



Elemental Composition Report **Page 1**

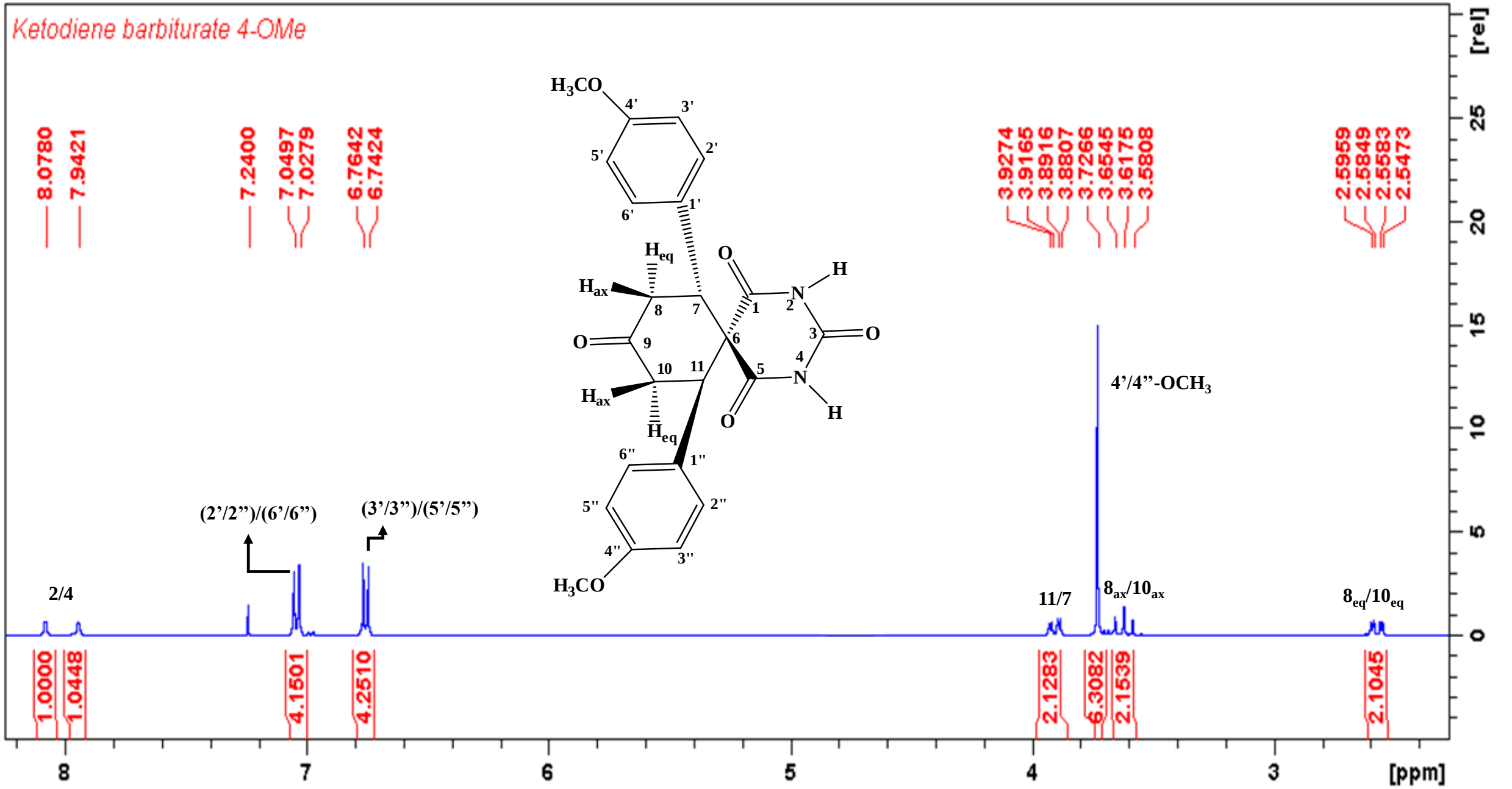
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
18 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 20-25 N: 0-5 O: 5-10
24 2-OMe spiro 32 (1.045) Cm (1:61)
TOF MS ES-

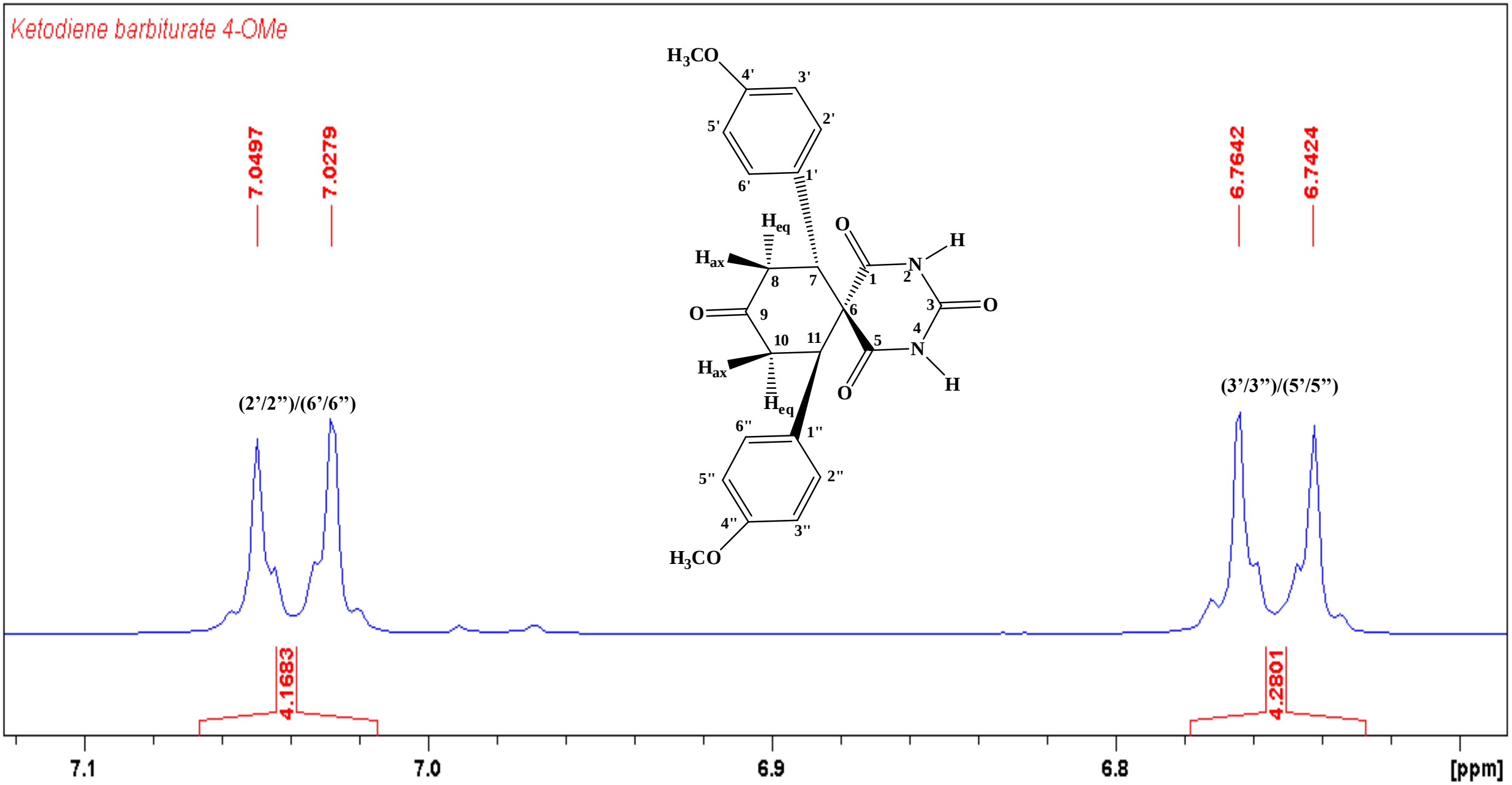


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
421.1395	421.1400	-0.5	-1.2	14.5	38.4	0.0	C23 H21 N2 O6

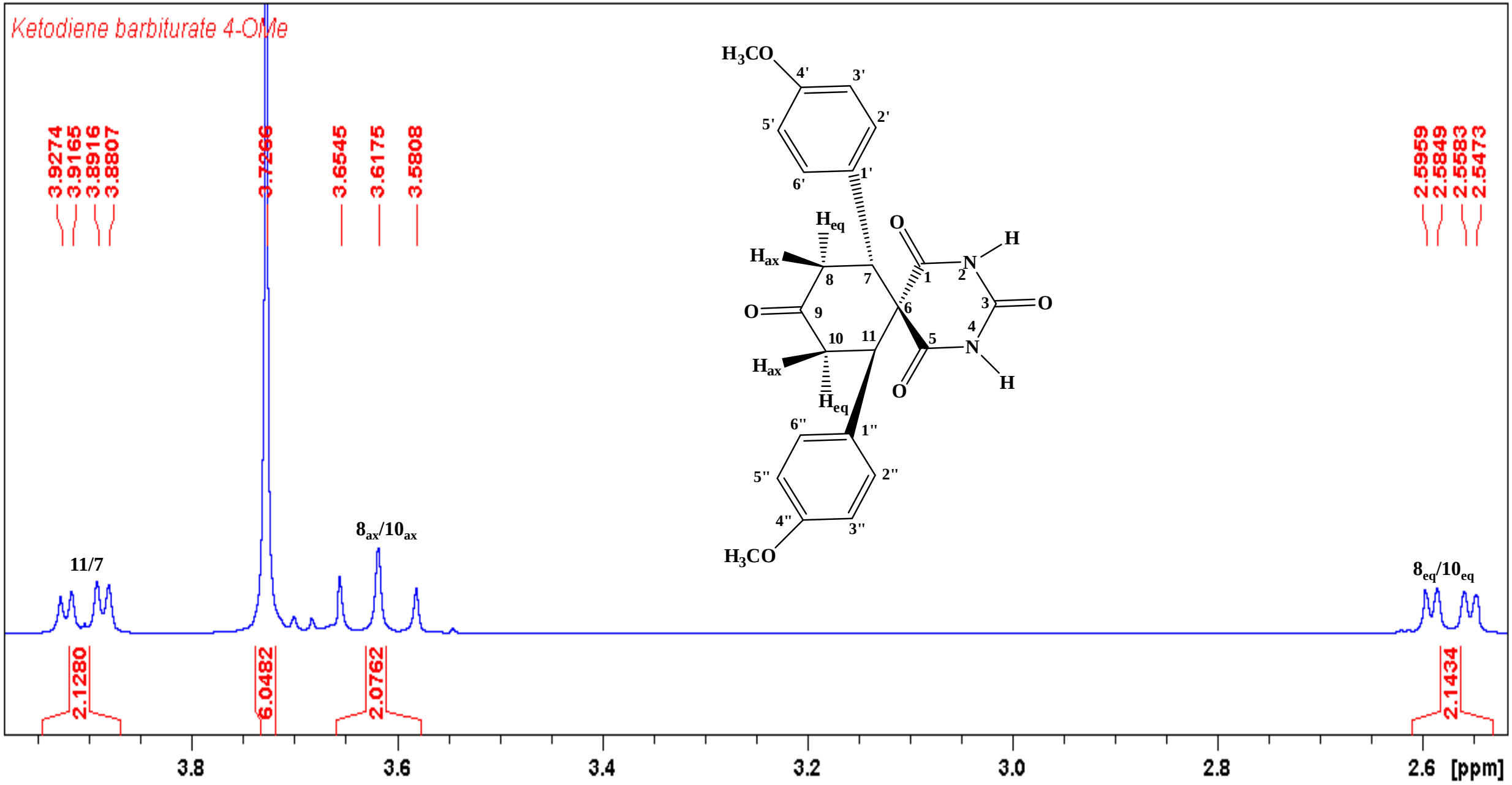
HRMS of Compound B-2k: 5,5'-(1,5-bis(2-methoxyphenyl)-3-oxopentane-1,5-diyl)bis(4,6-dihydroxypyrimidin-2(1H)-one)



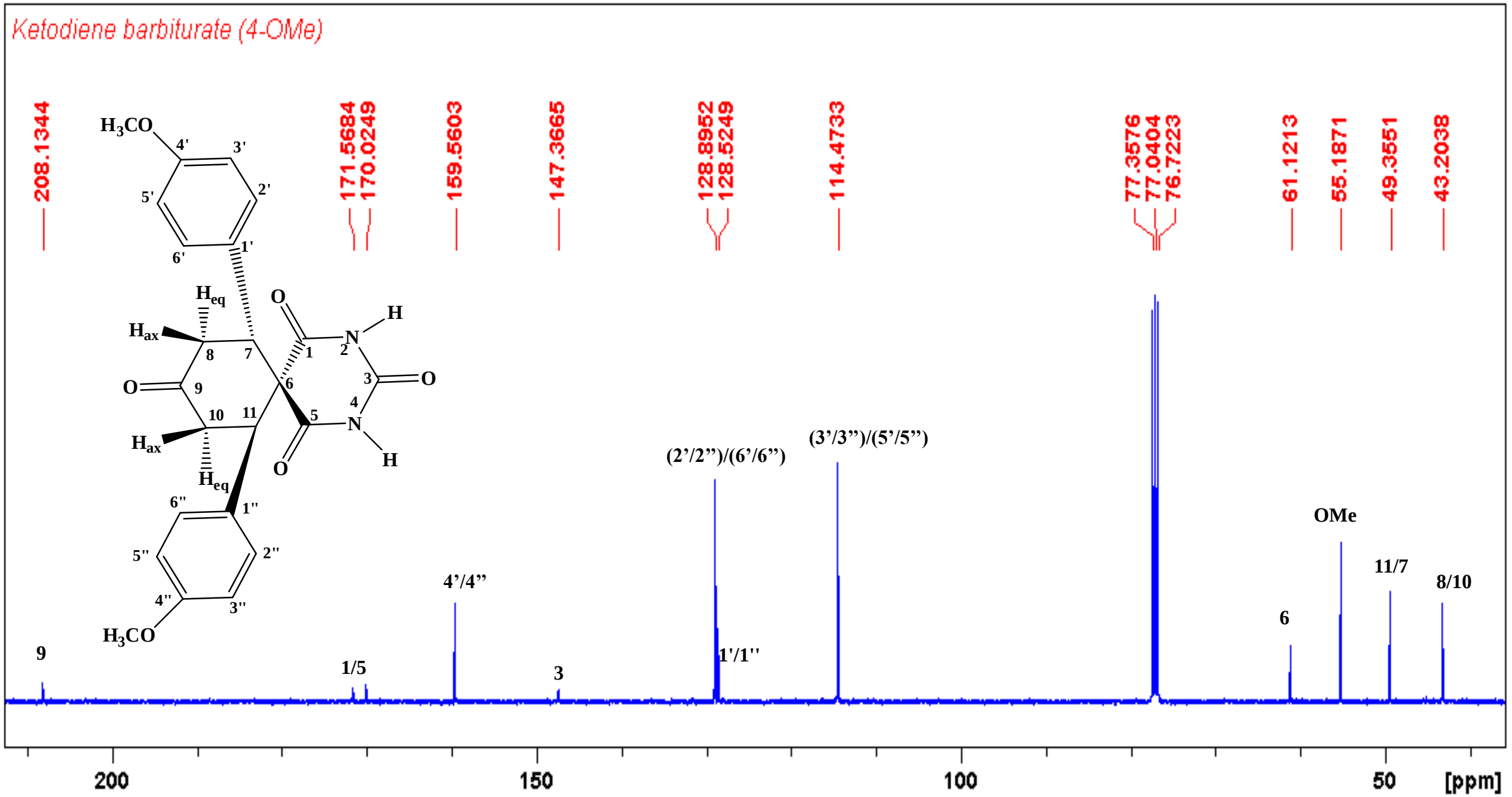
^{1}H Spectrum of Compound B-3l: 7,11-bis(4-methoxyphenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



Expanded ^{1}H Spectrum of Compound B-3l: 7,11-bis(4-methoxyphenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone

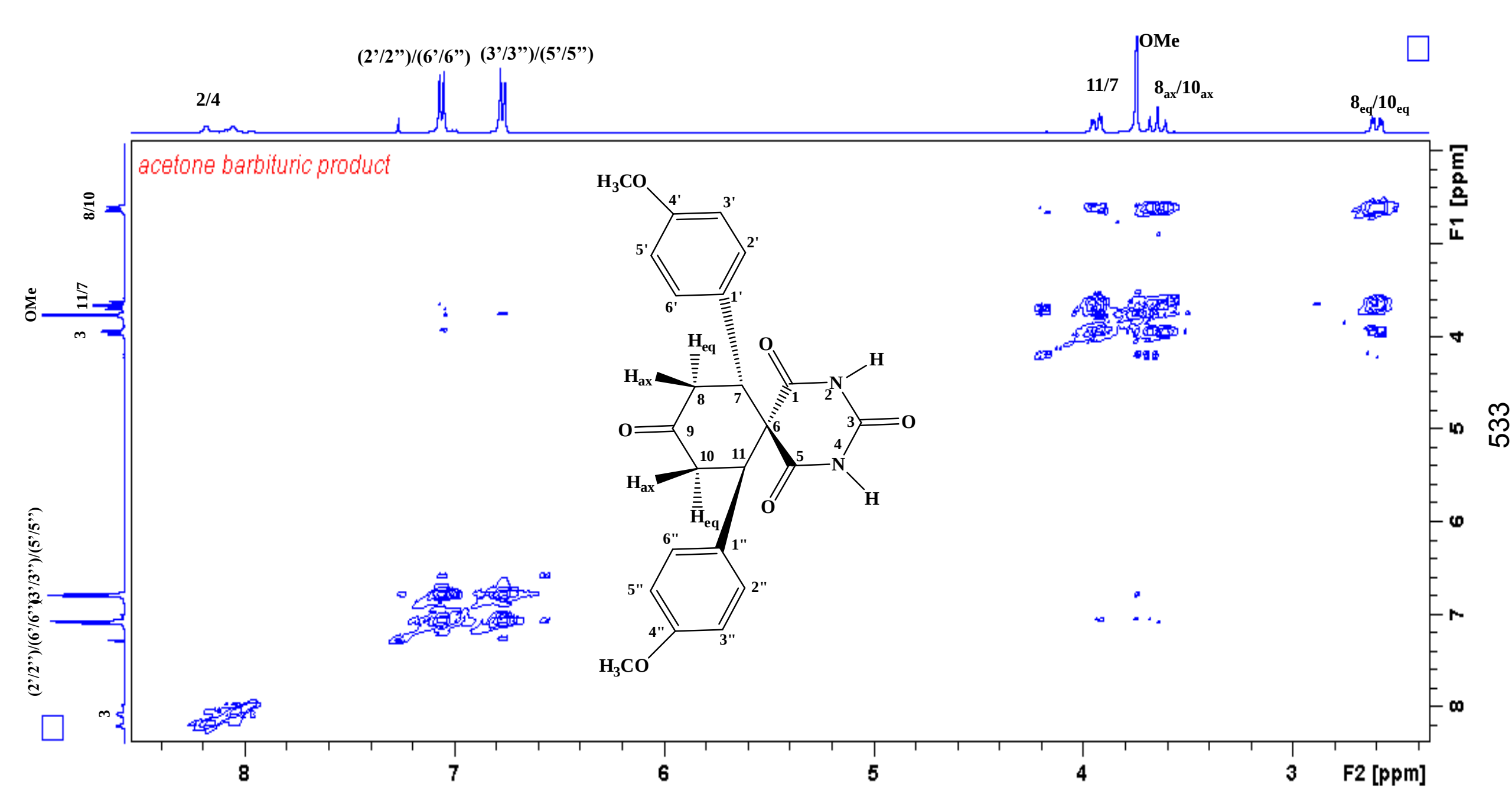


Expanded ^{1}H Spectrum of Compound B-3l: 7,11-bis(4-methoxyphenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone

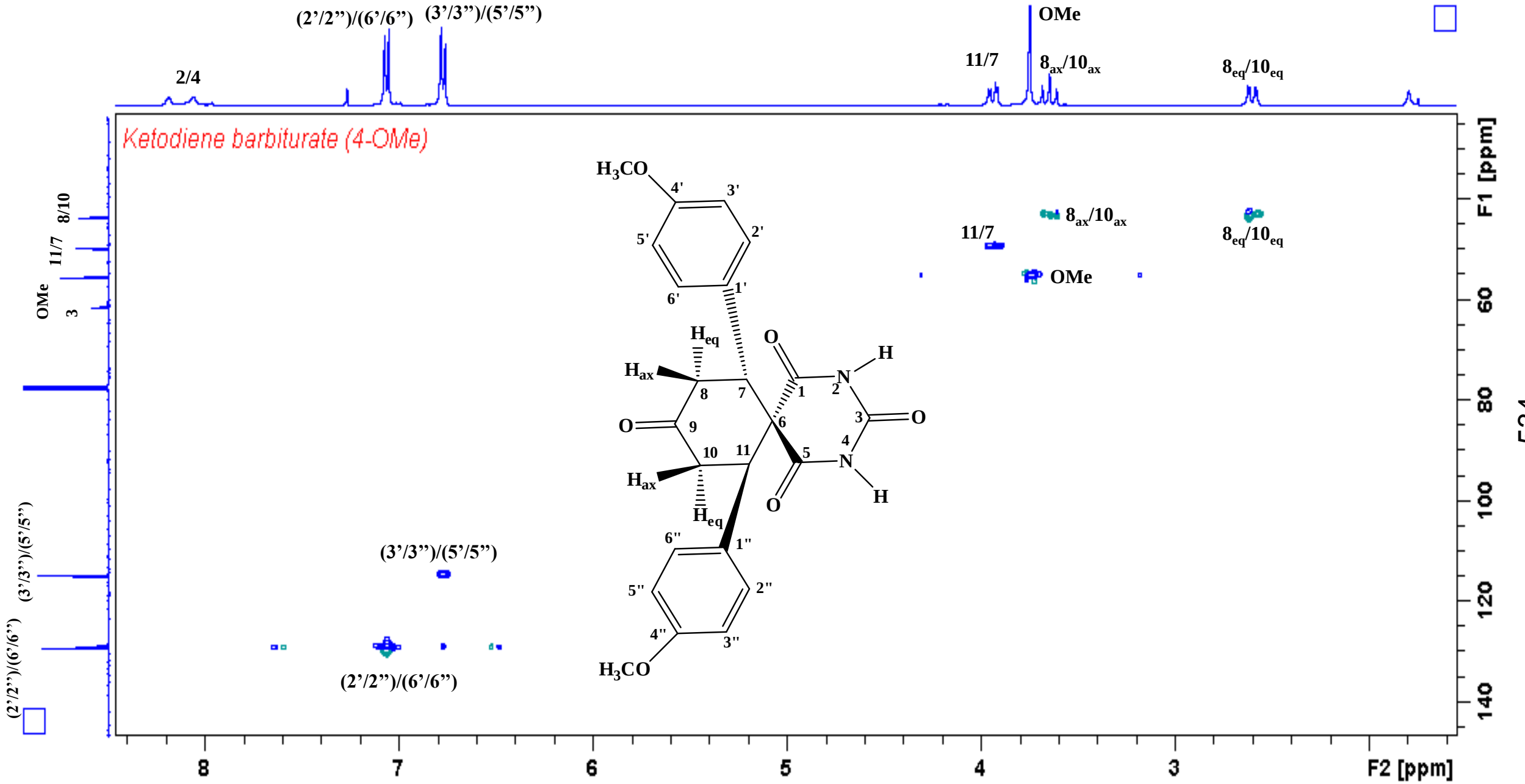


532

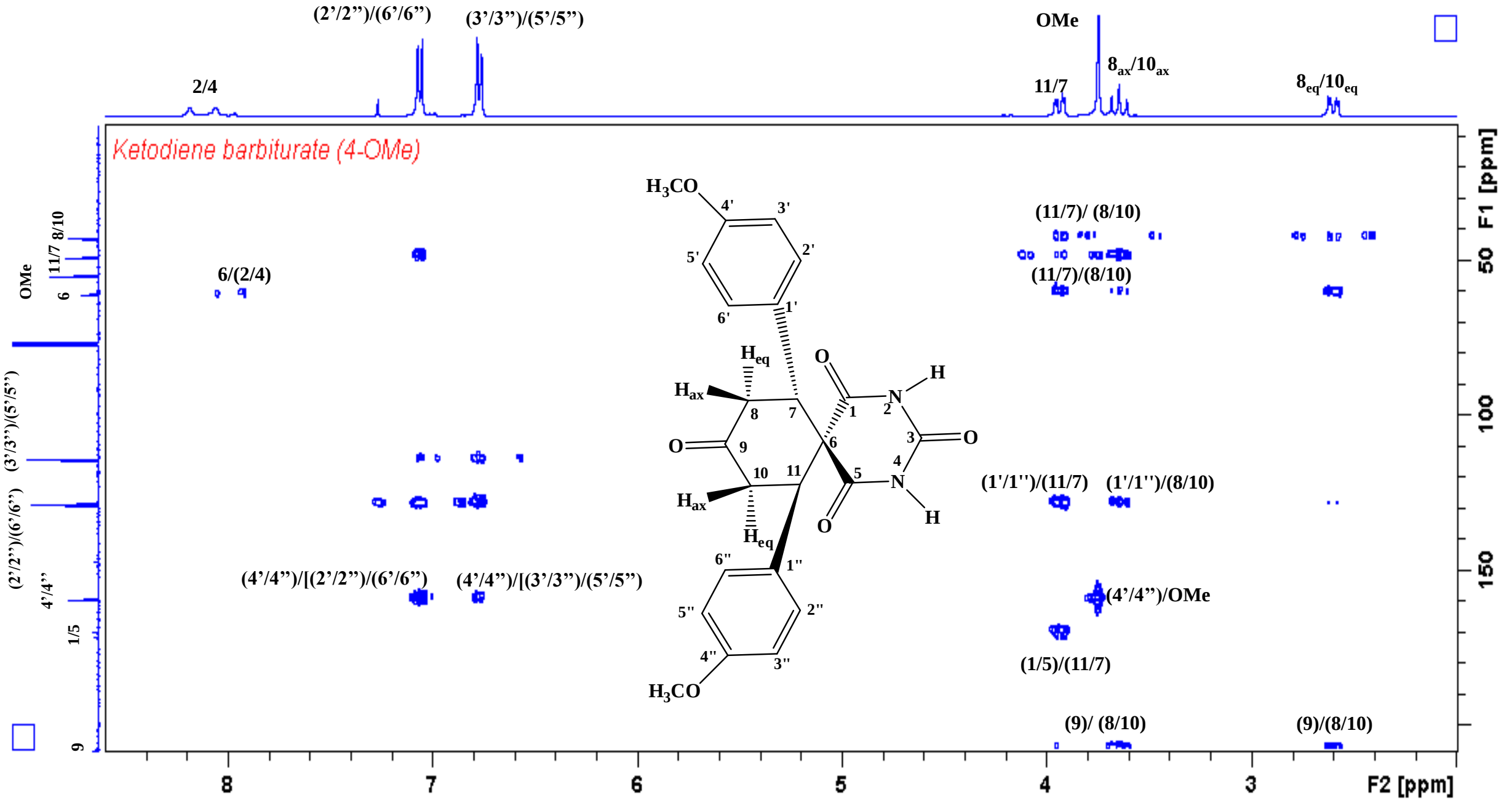
^{13}C Spectrum of Compound B-3l: 7,11-bis(4-methoxyphenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



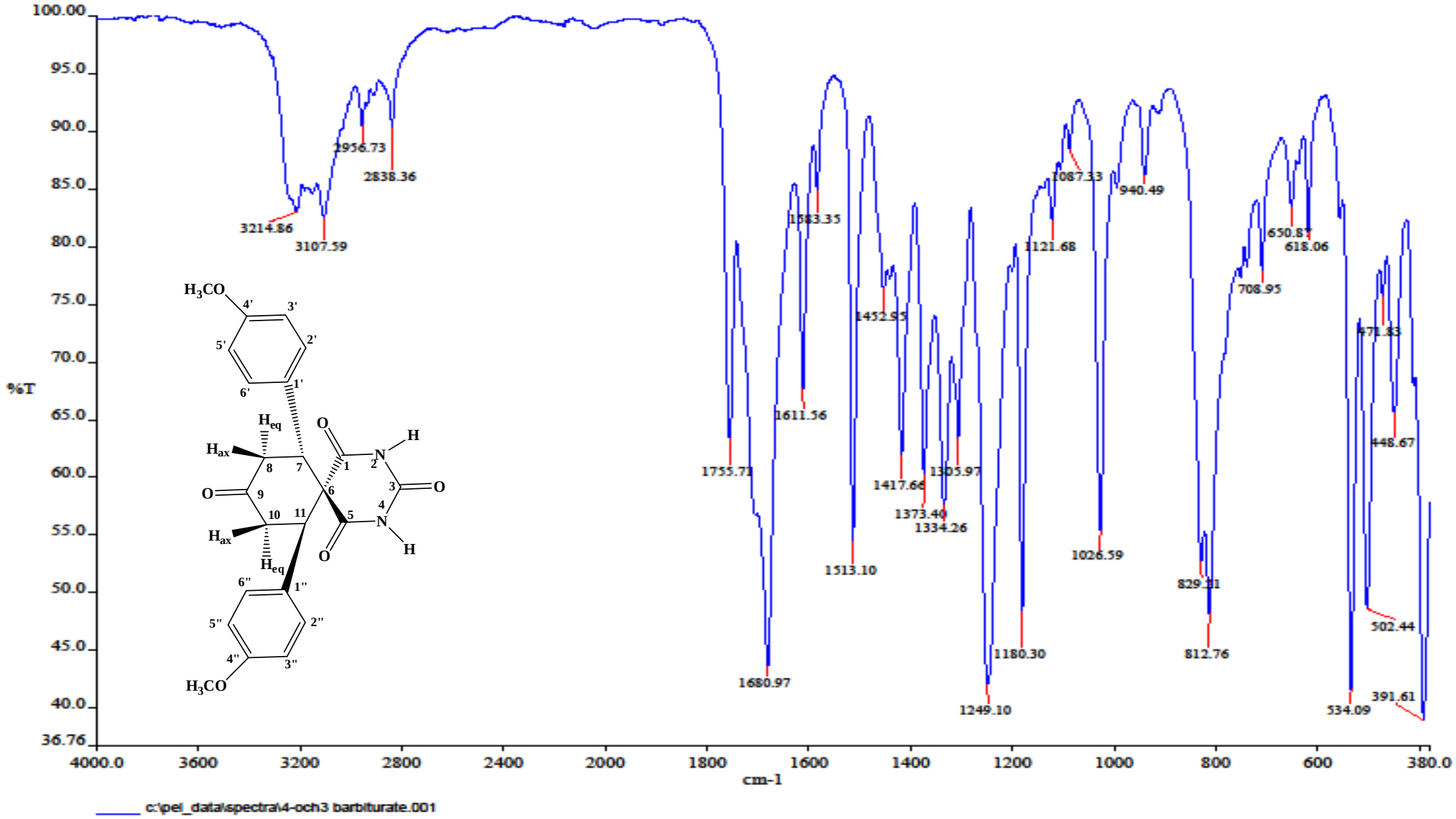
COSY of Compound B-3l: 7,11-bis(4-methoxyphenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



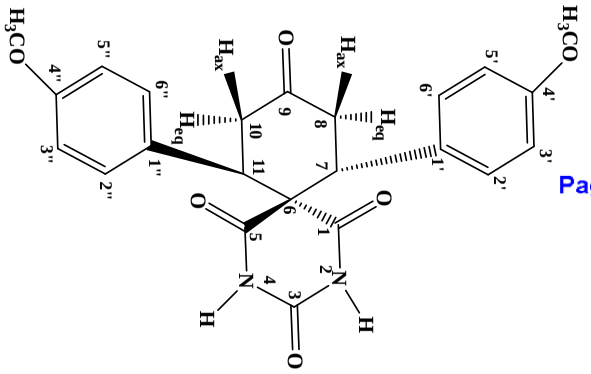
HSQC of Compound B-3l: 7,11-bis(4-methoxyphenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



HMBC of Compound B-3l: 7,11-bis(4-methoxyphenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



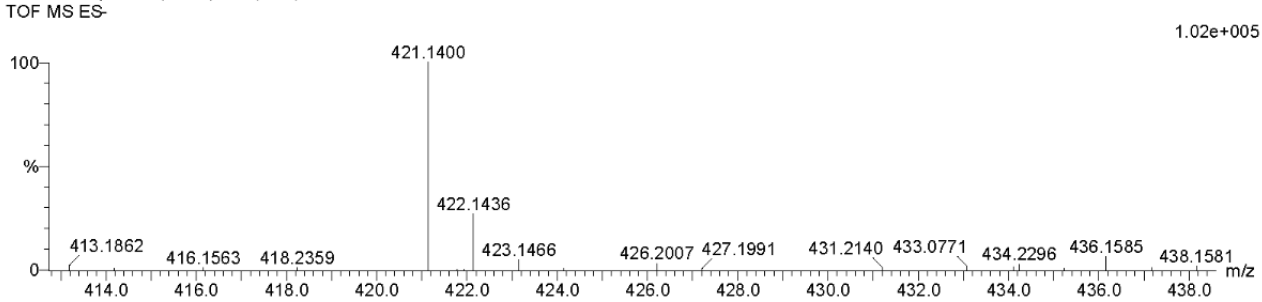
Infrared Spectrum of Compound B-3l: 7,11-bis(4-methoxyphenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



Elemental Composition Report **Page 1**

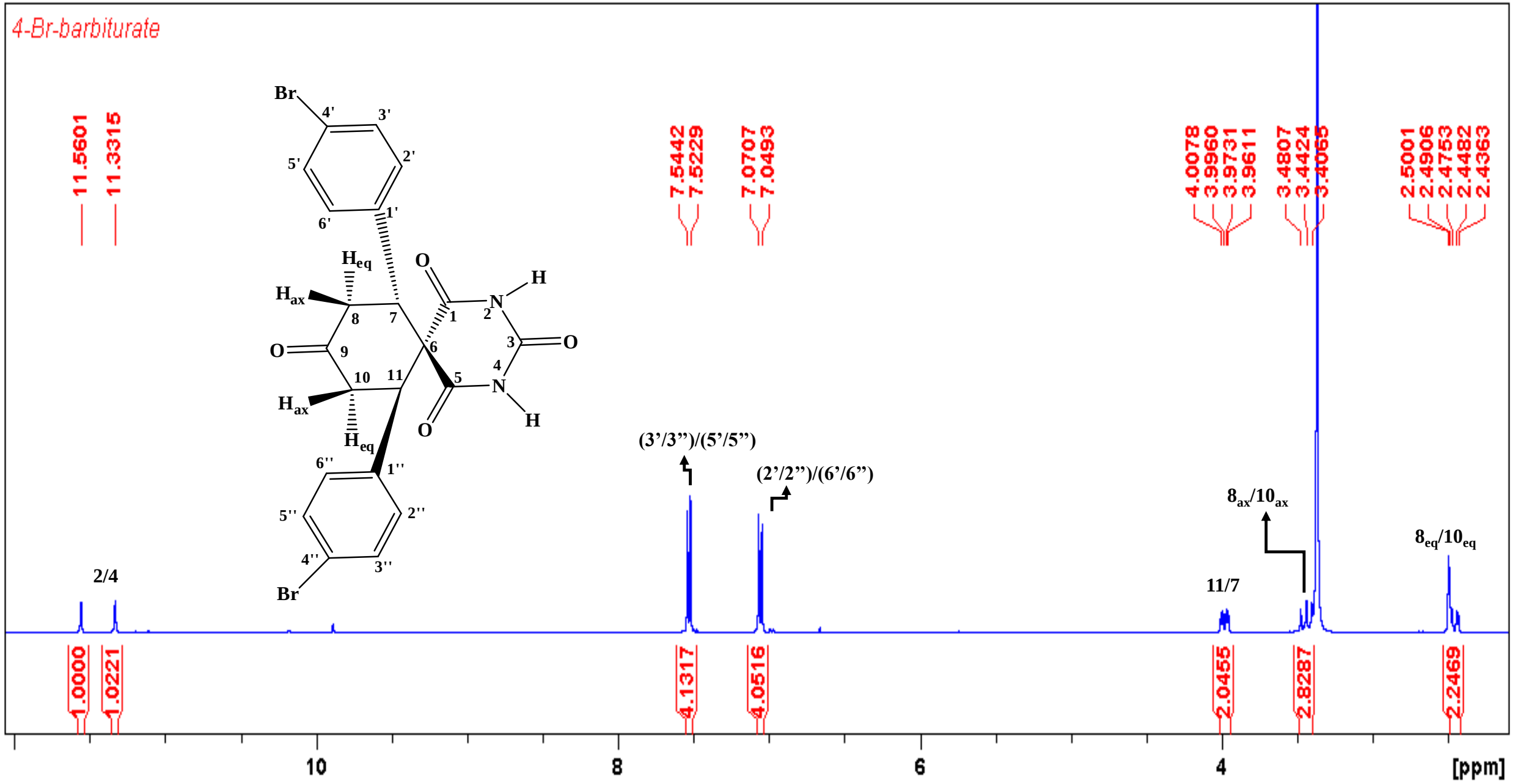
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
18 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 20-25 N: 0-5 O: 5-10
25 4-OMe spiro 26 (0.844) Cm (1:61)
TOF MS ES-

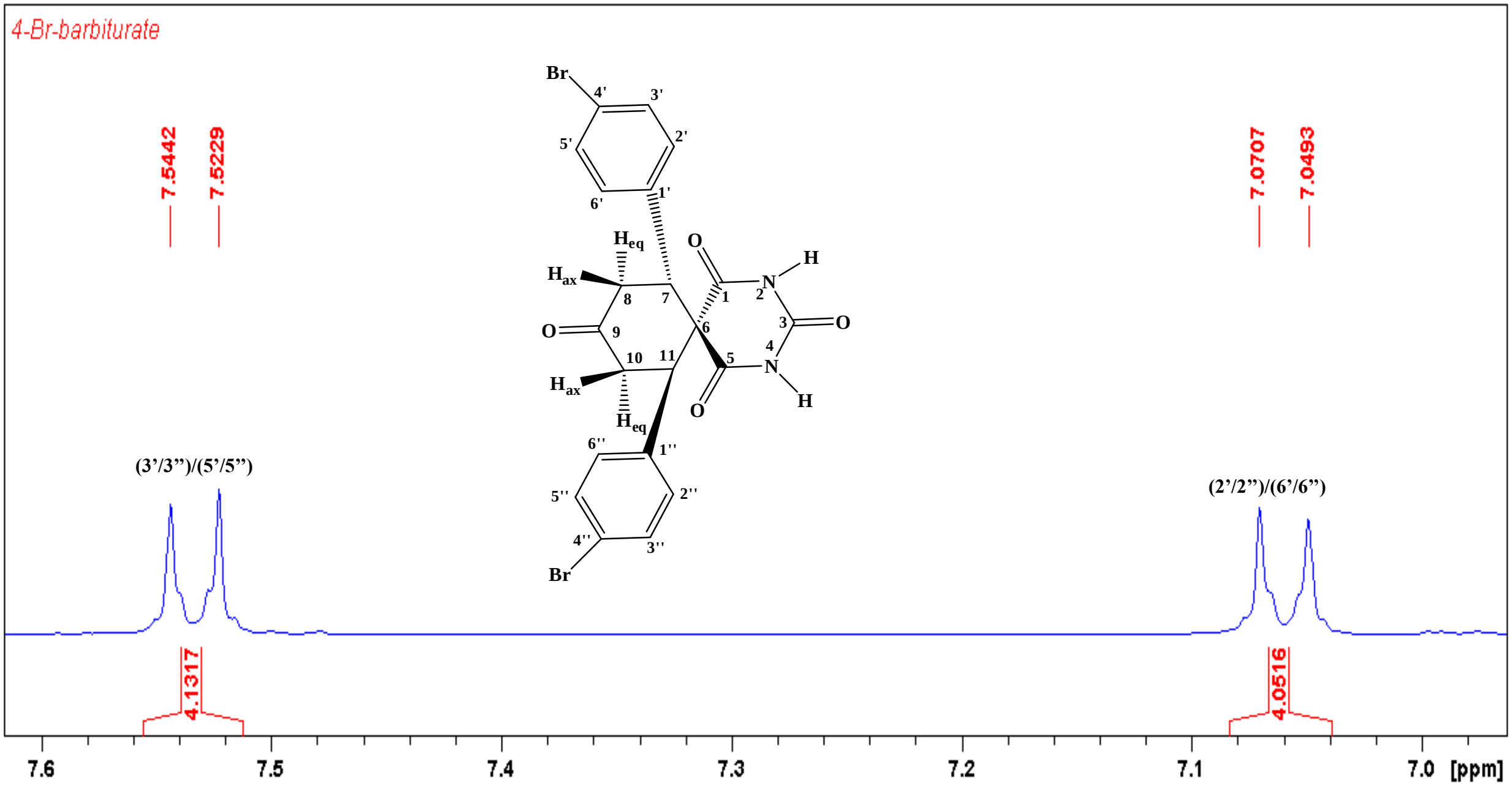


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
421.1400	421.1400	0.0	0.0	14.5	31.8	0.0	C23 H21 N2 O6

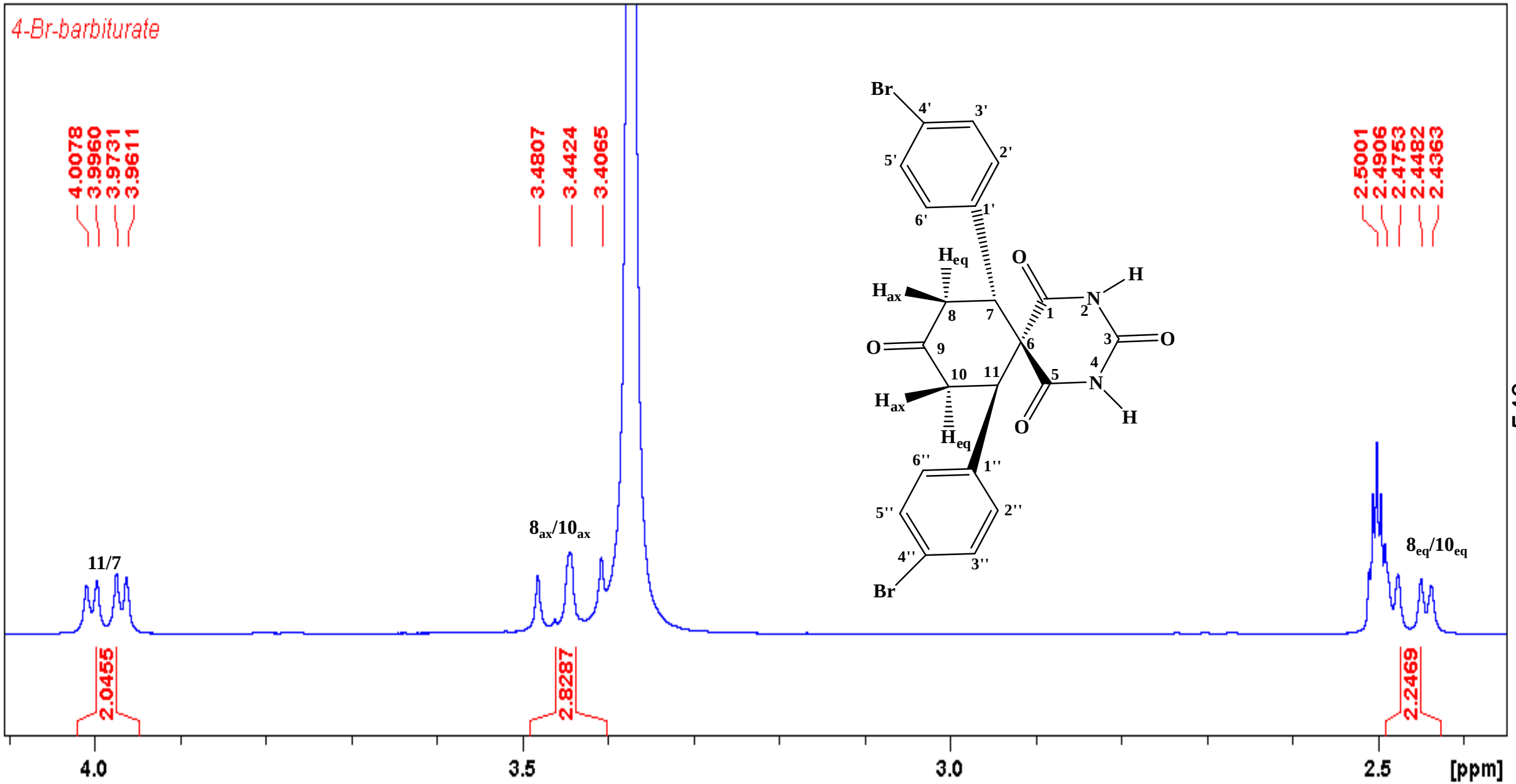
HRMS of Compound B-3l: 7,11-bis(4-methoxyphenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



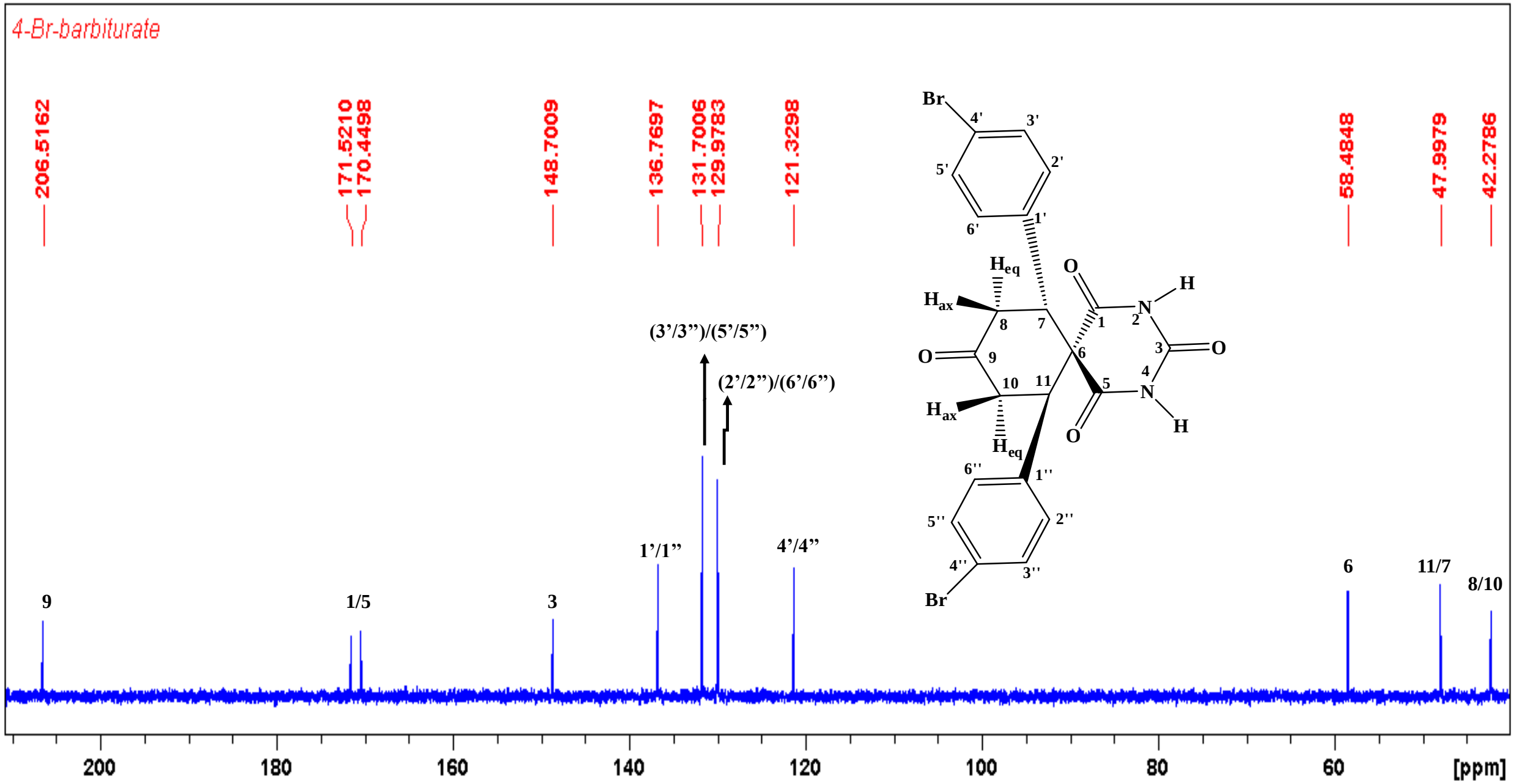
^{1}H Spectrum of Compound B-3n: 5,5'-(1,5-bis(4-bromophenyl)-3-oxopentane-1,5-diyl)bis(4,6-dihydroxypyrimidin-2(1H)-one)



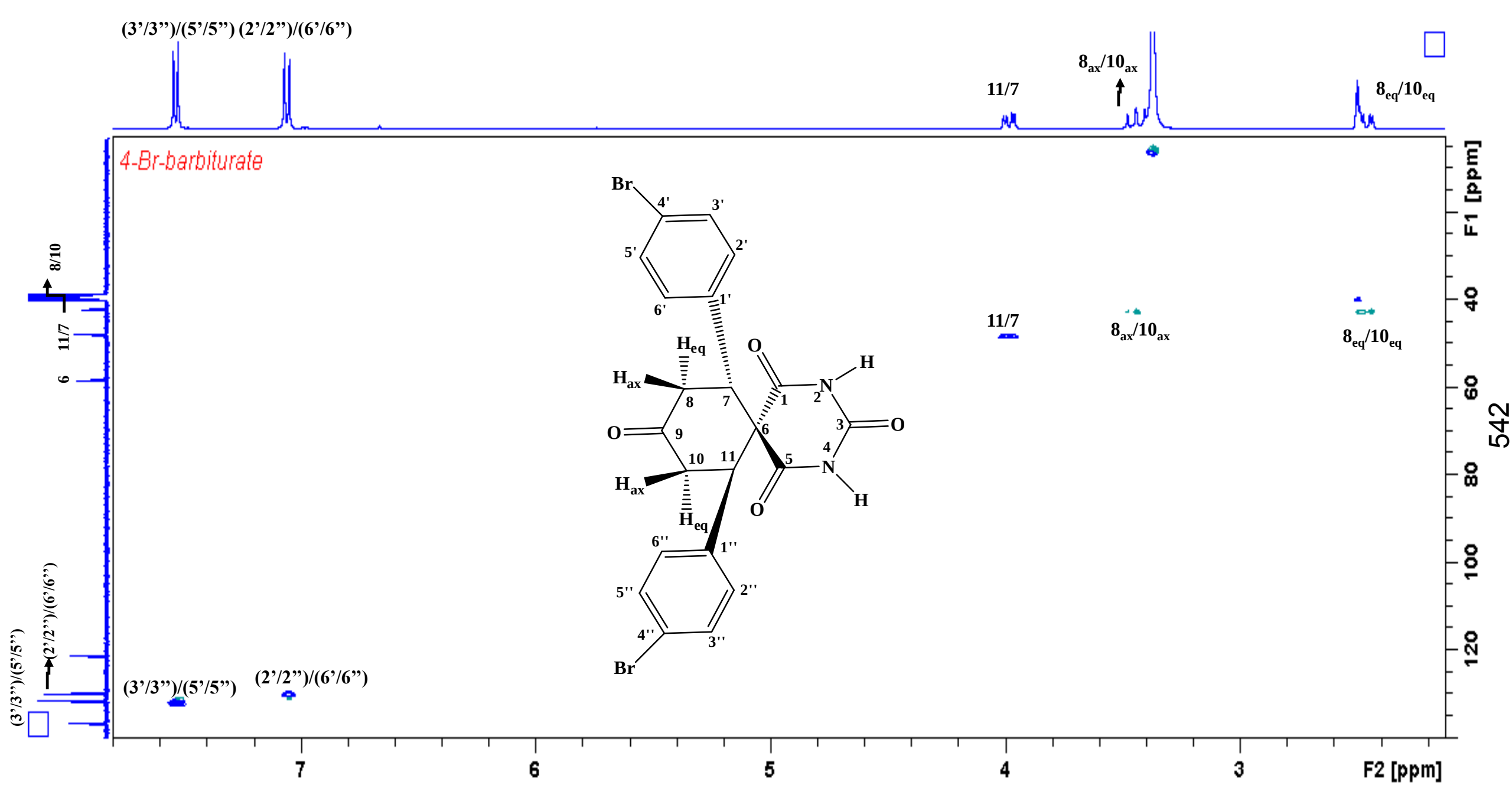
Expanded ^{1}H Spectrum of Compound B-3n: 5,5'-(1,5-bis(4-bromophenyl)-3-oxopentane-1,5-diyl)bis(4,6-dihydroxypyrimidin-2(1H)-one)



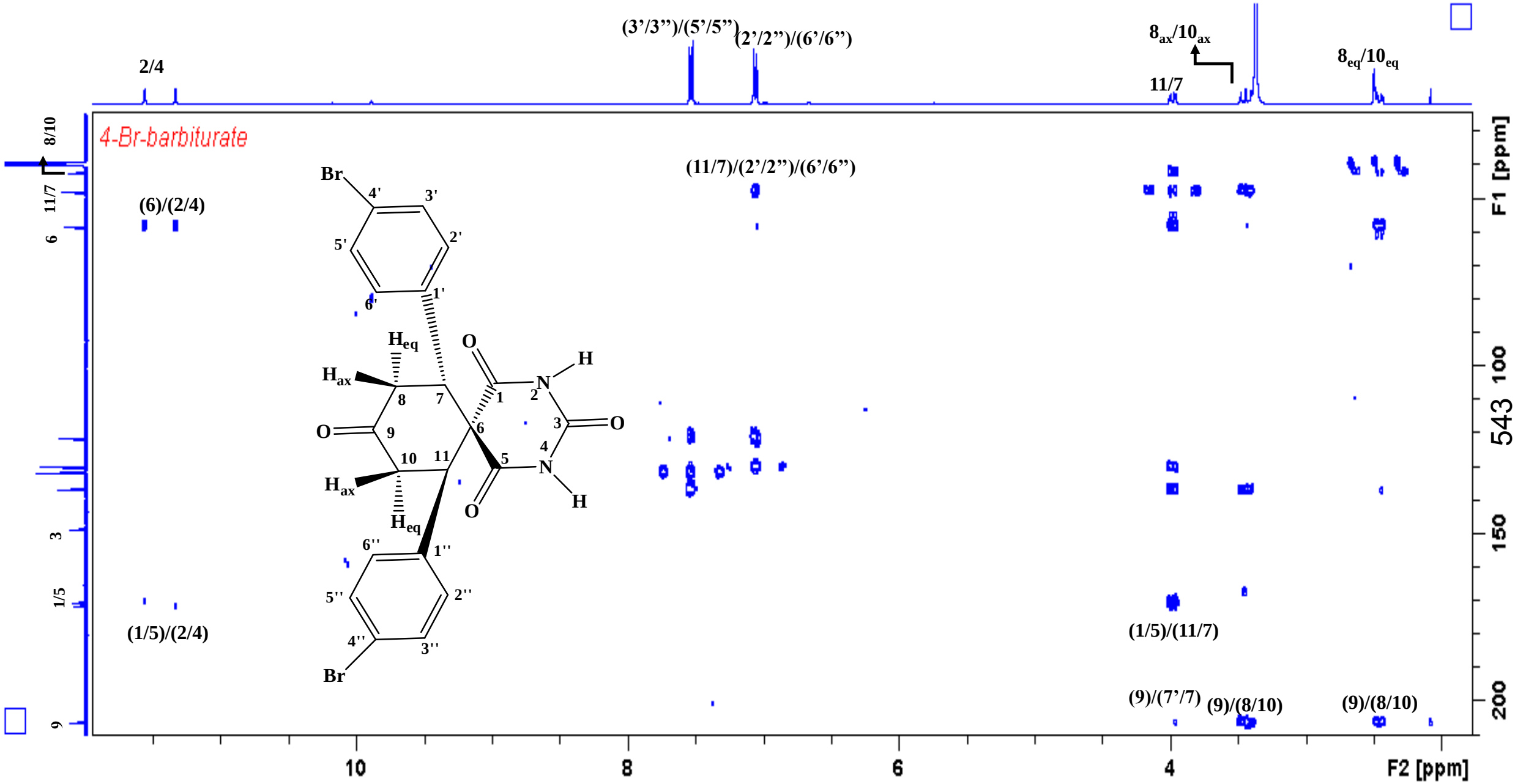
Expanded ^{1}H Spectrum of Compound B-3n: 5,5'-(1,5-bis(4-bromophenyl)-3-oxopentane-1,5-diyl)bis(4,6-dihydroxypyrimidin-2(1H)-one)



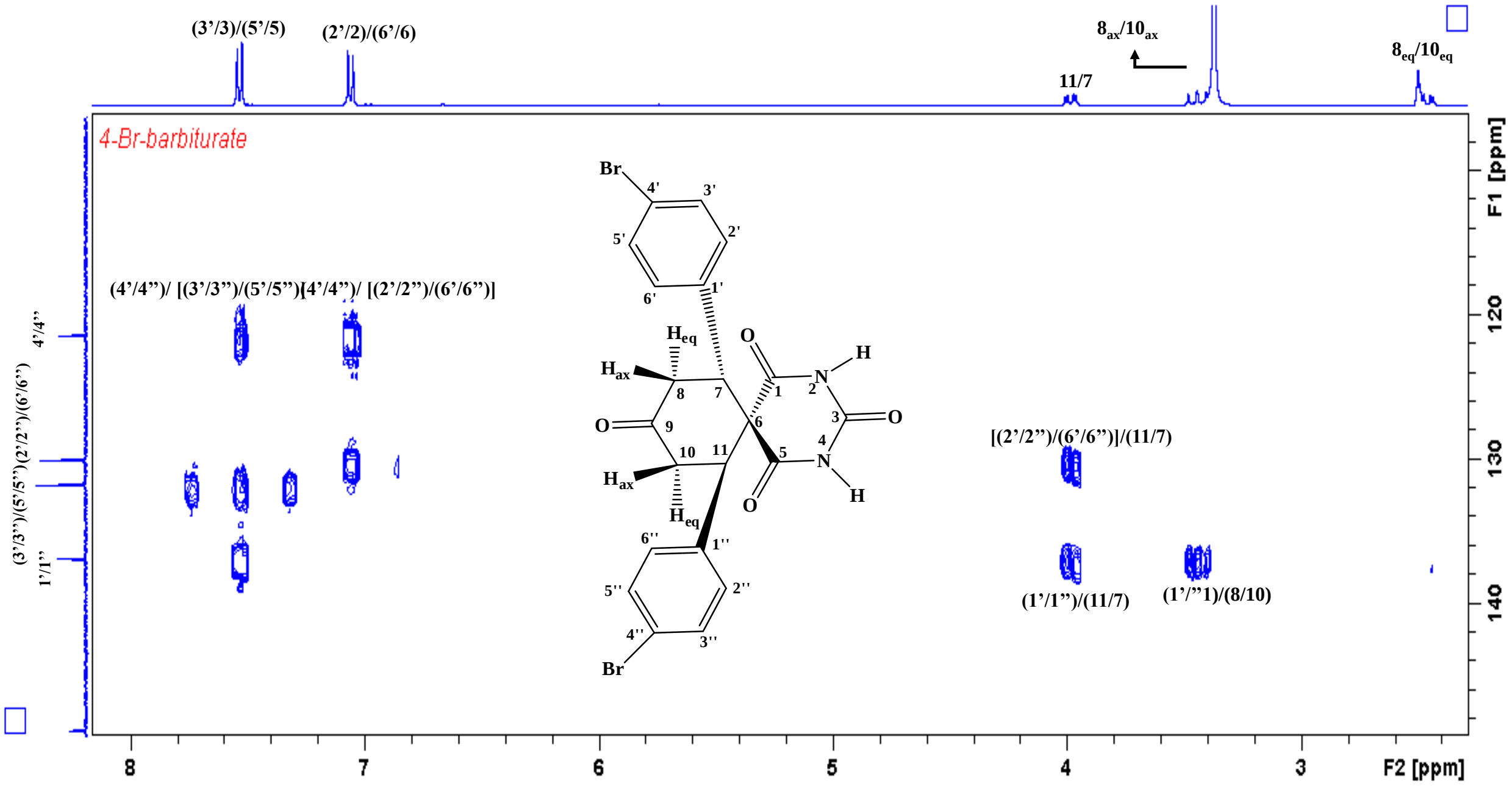
^{13}C Spectrum of Compound B-3n: 5,5'-(1,5-bis(4-bromophenyl)-3-oxopentane-1,5-diyl)bis(4,6-dihydroxypyrimidin-2(1H)-one)



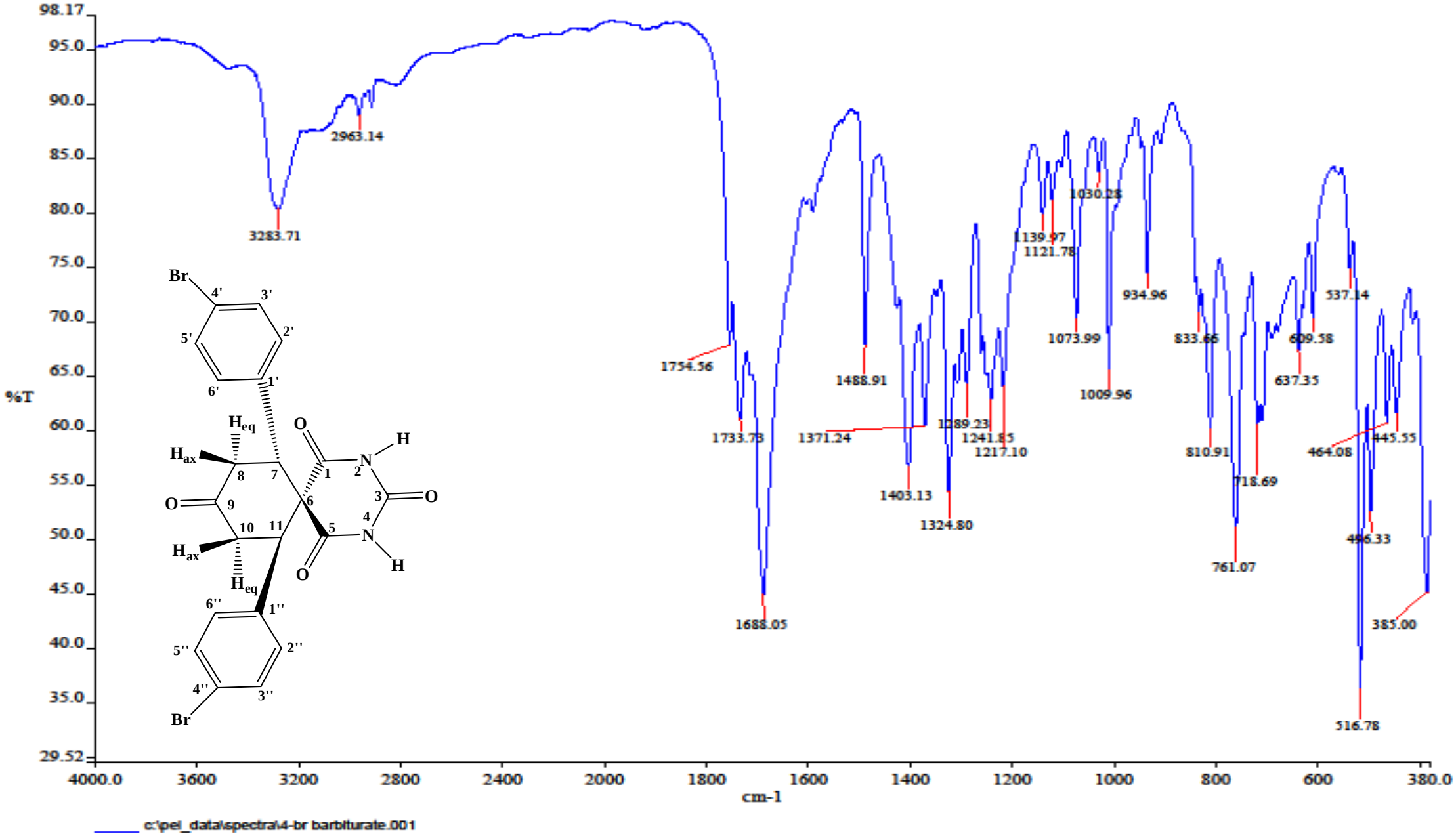
HSQC of Compound B-3n: 5,5'-(1,5-bis(4-bromophenyl)-3-oxopentane-1,5-diyl)bis(4,6-dihydroxypyrimidin-2(1H)-one)



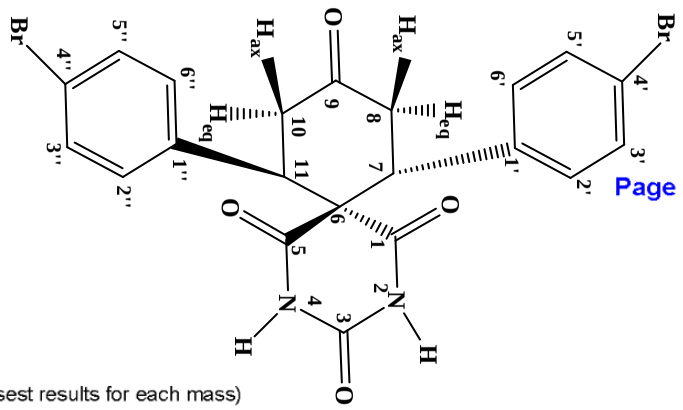
HMBC of Compound B-3n: 5,5'-(1,5-bis(4-bromophenyl)-3-oxopentane-1,5-diyl)bis(4,6-dihydroxypyrimidin-2(1H)-one)



Expanded HMBC of Compound B-3n: 5,5'-(1,5-bis(4-bromophenyl)-3-oxopentane-1,5-diyl)bis(4,6-dihydroxypyrimidin-2(1H)-one)



Infrared Spectrum of Compound B-3n: 5,5'-(1,5-bis(4-bromophenyl)-3-oxopentane-1,5-diyl)bis(4,6-dihydroxypyrimidin-2(1H)-one)



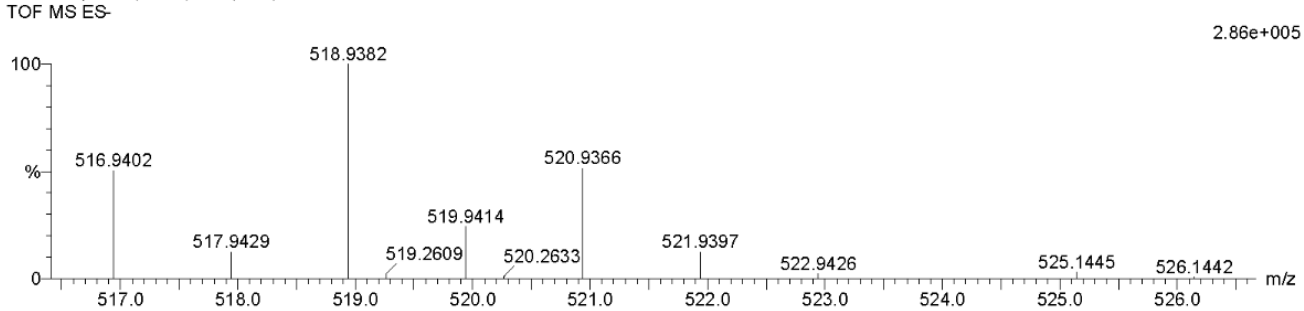
Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

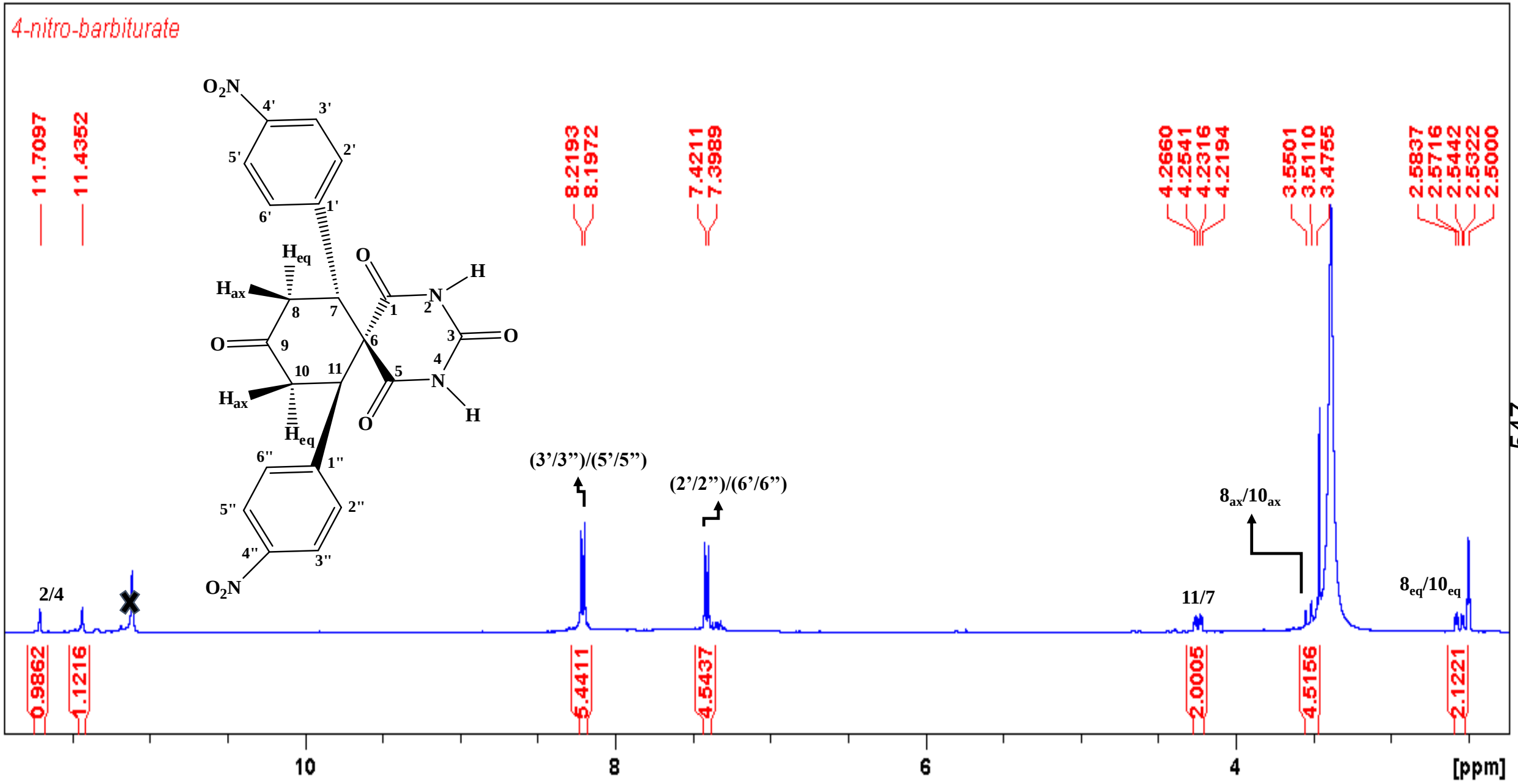
Monoisotopic Mass, Even Electron Ions
69 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 0-5 Br: 0-2

27 4-Br spiro 2 (0.034) Cm (1:61)
TOF MS ES-

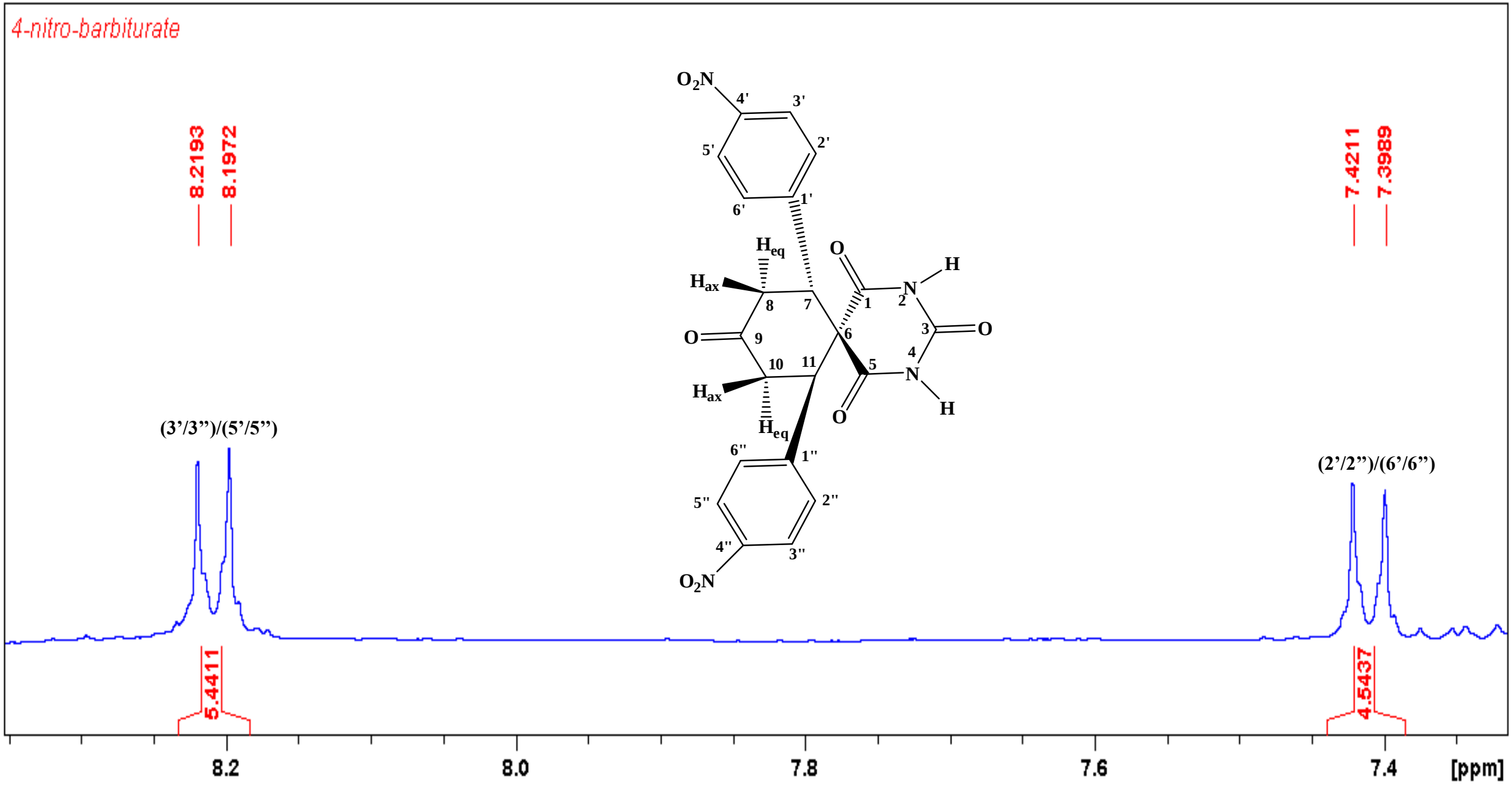


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
516.9402	516.9399	0.3	0.6	14.5	8.6	0.0	C21 H15 N2 O4 Br2

HRMS of Compound B-3n: 5,5'-(1,5-bis(4-bromophenyl)-3-oxopentane-1,5-diyl)bis(4,6-dihydroxypyrimidin-2(1H)-one)



^{1}H Spectrum of Compound B-3o: 7,11-bis(4-nitrophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



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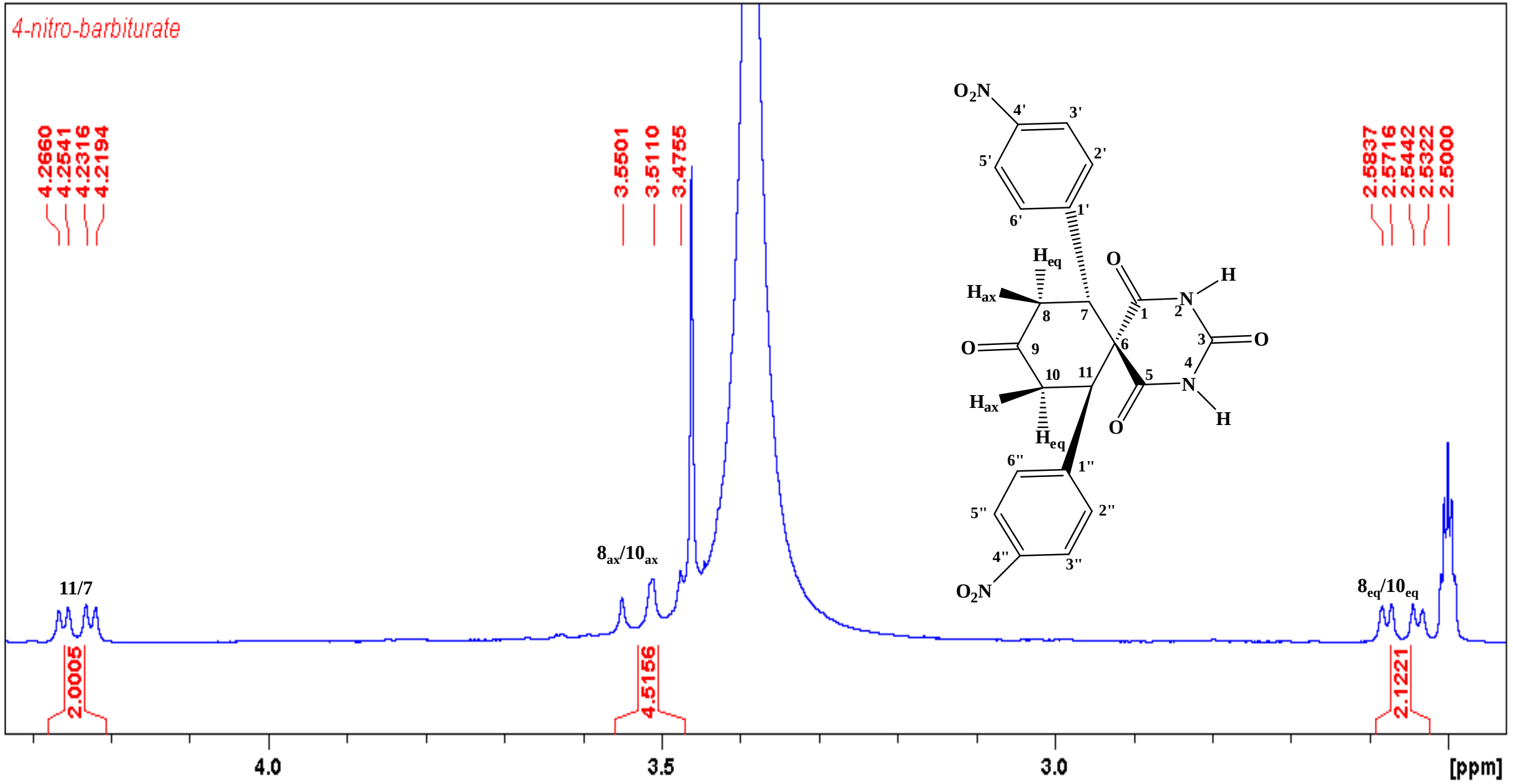
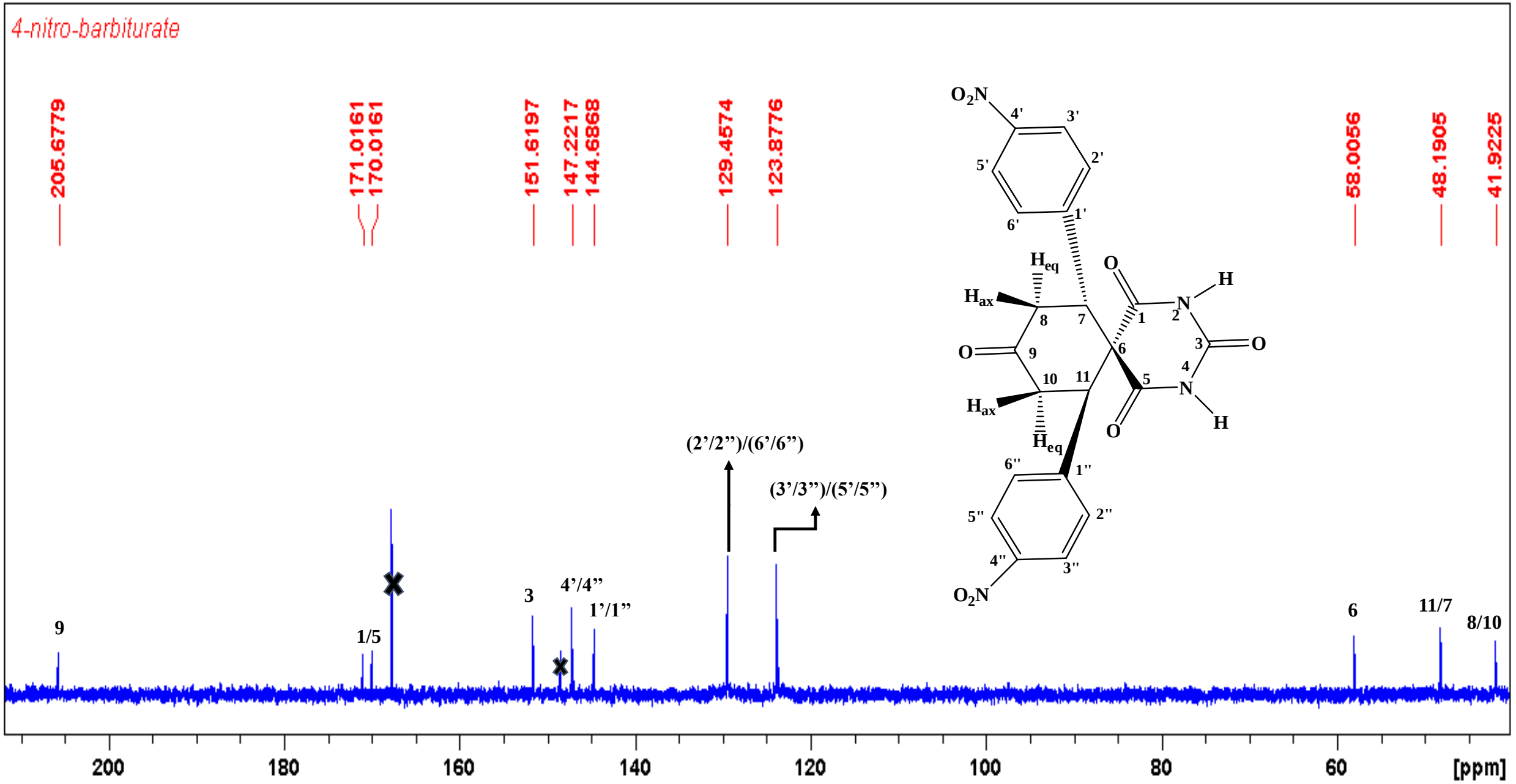
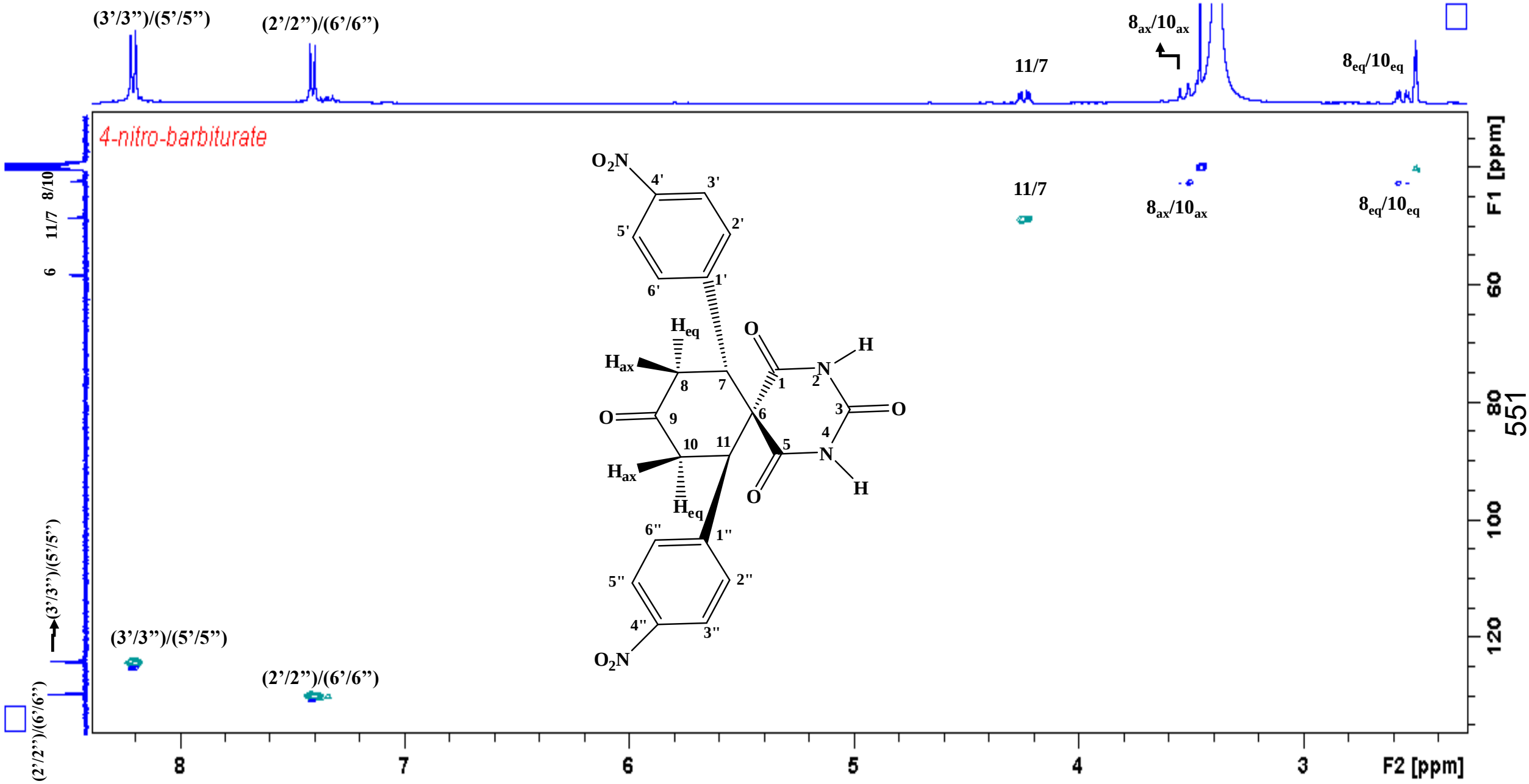
Expanded ^{1}H Spectrum of Compound B-3o: 7,11-bis(4-nitrophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



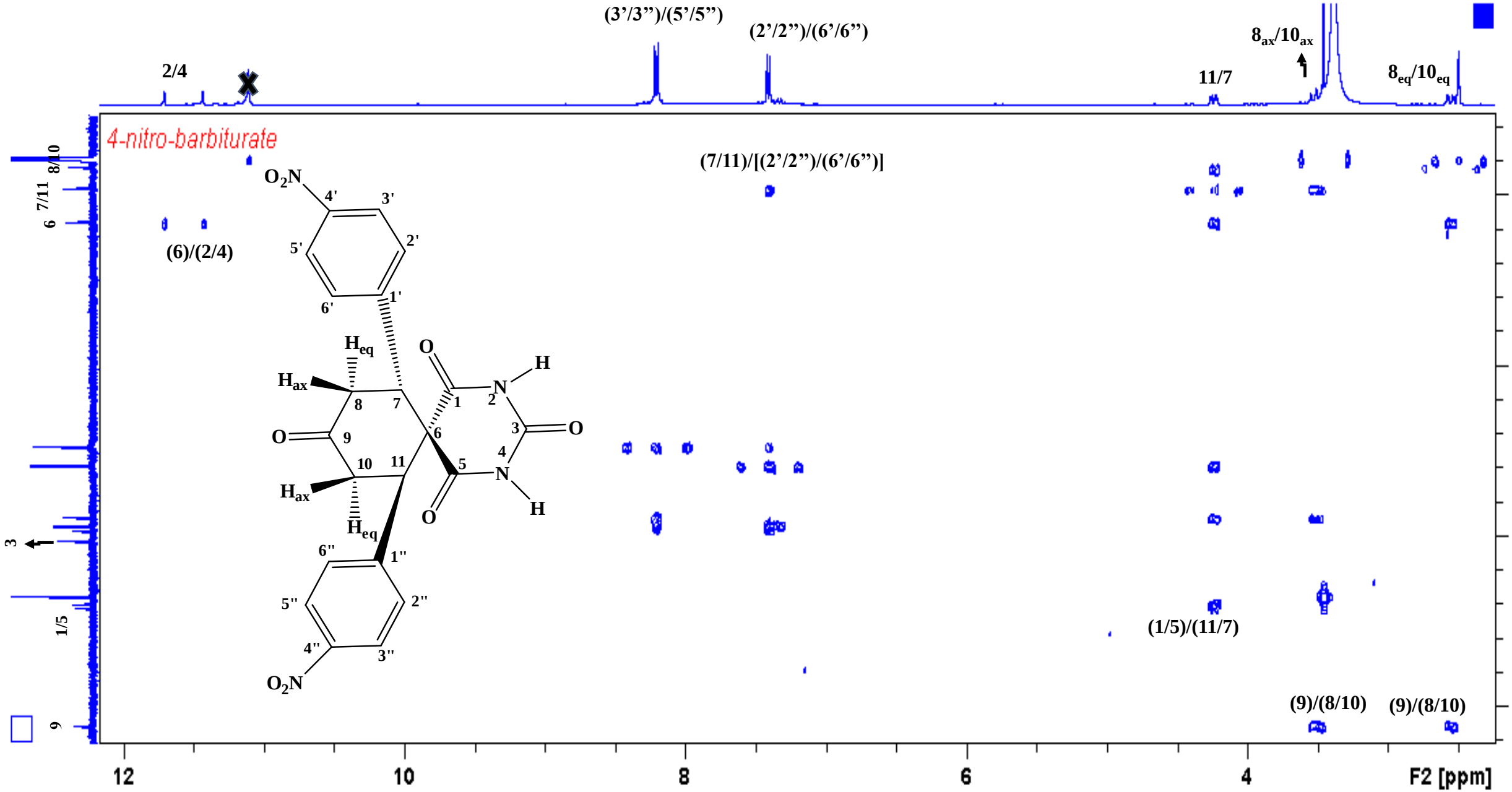
Expanded ^{1}H Spectrum of Compound B-3o: 7,11-bis(4-nitrophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



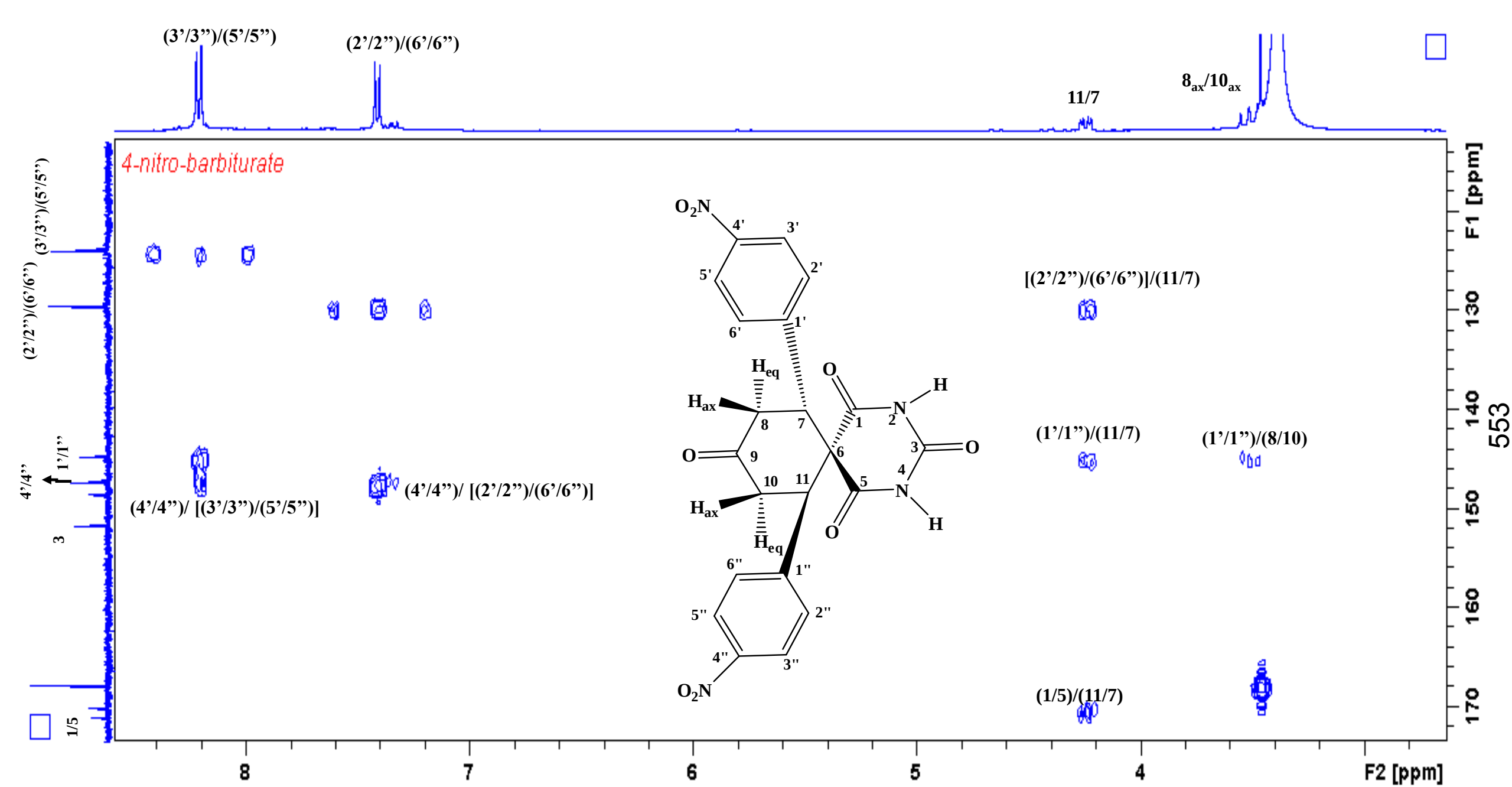
^{13}C Spectrum of Compound B-3o: 7,11-bis(4-nitrophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



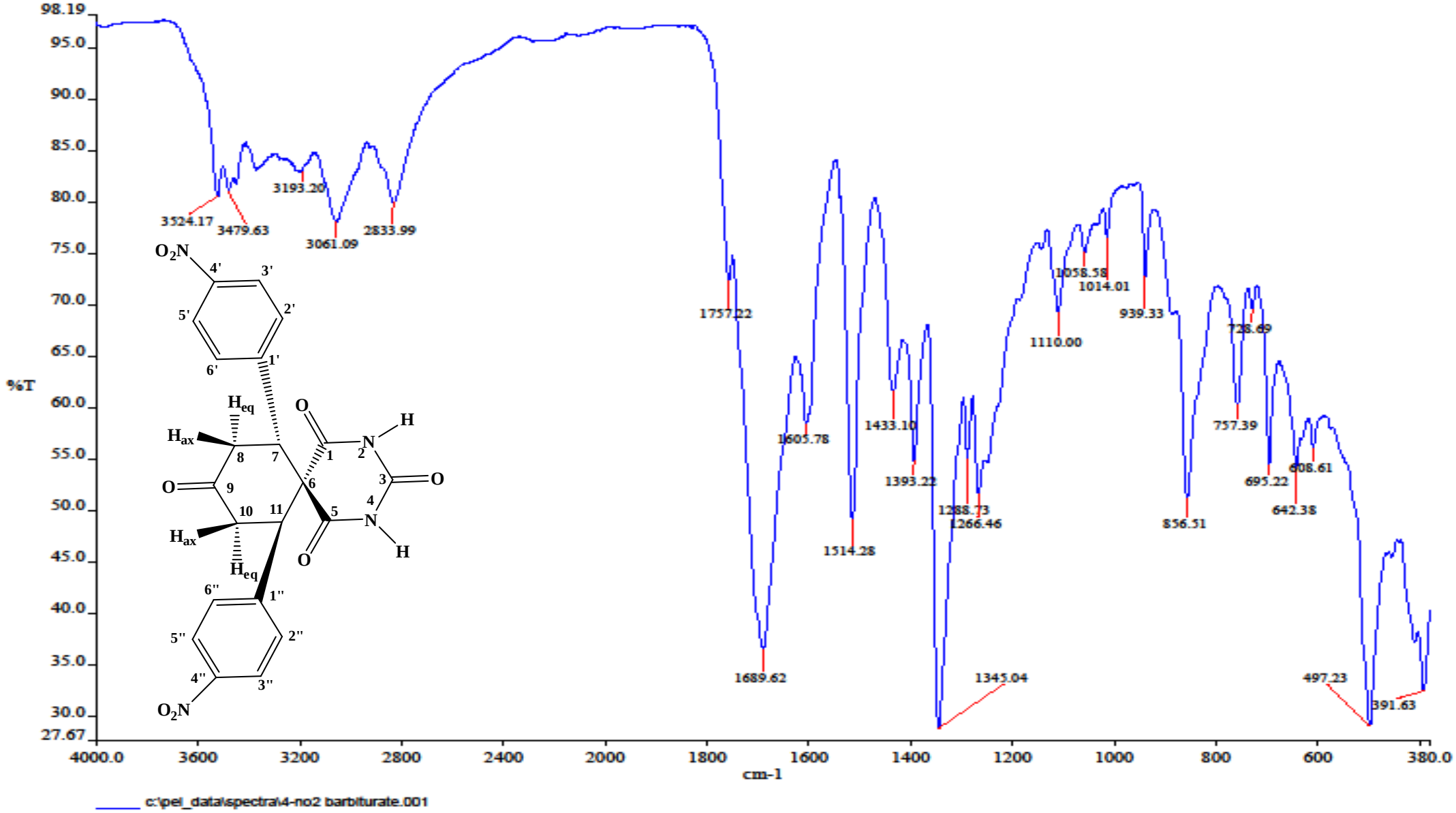
HSQC of Compound B-3o7,11-bis(4-nitrophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



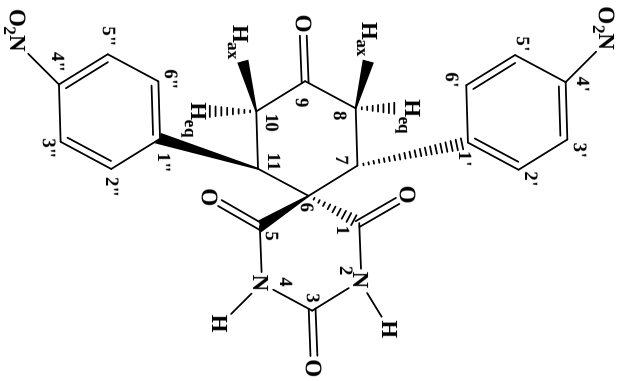
HMBC of Compound B-3o: 7,11-bis(4-nitrophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



Expanded HMBC of Compound B-3o: 7,11-bis(4-nitrophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



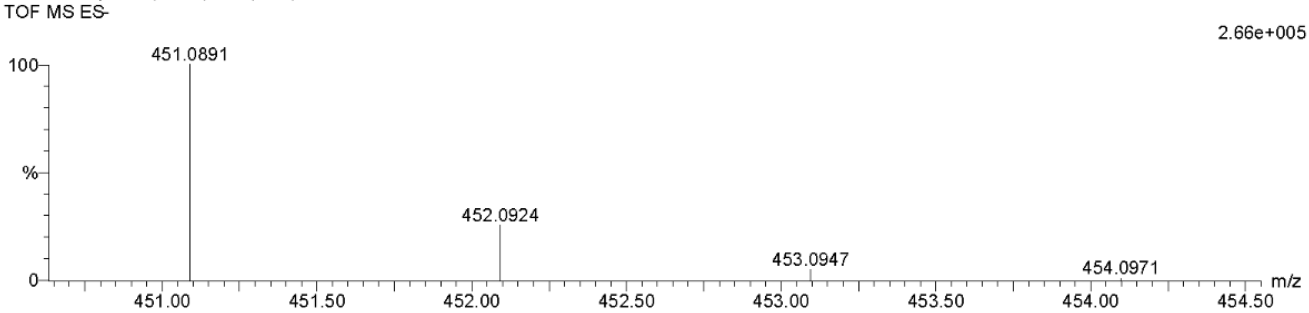
Infrared Spectrum of Compound B-3o: 7,11-bis(4-nitrophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone



Elemental Composition Report **Page 1**

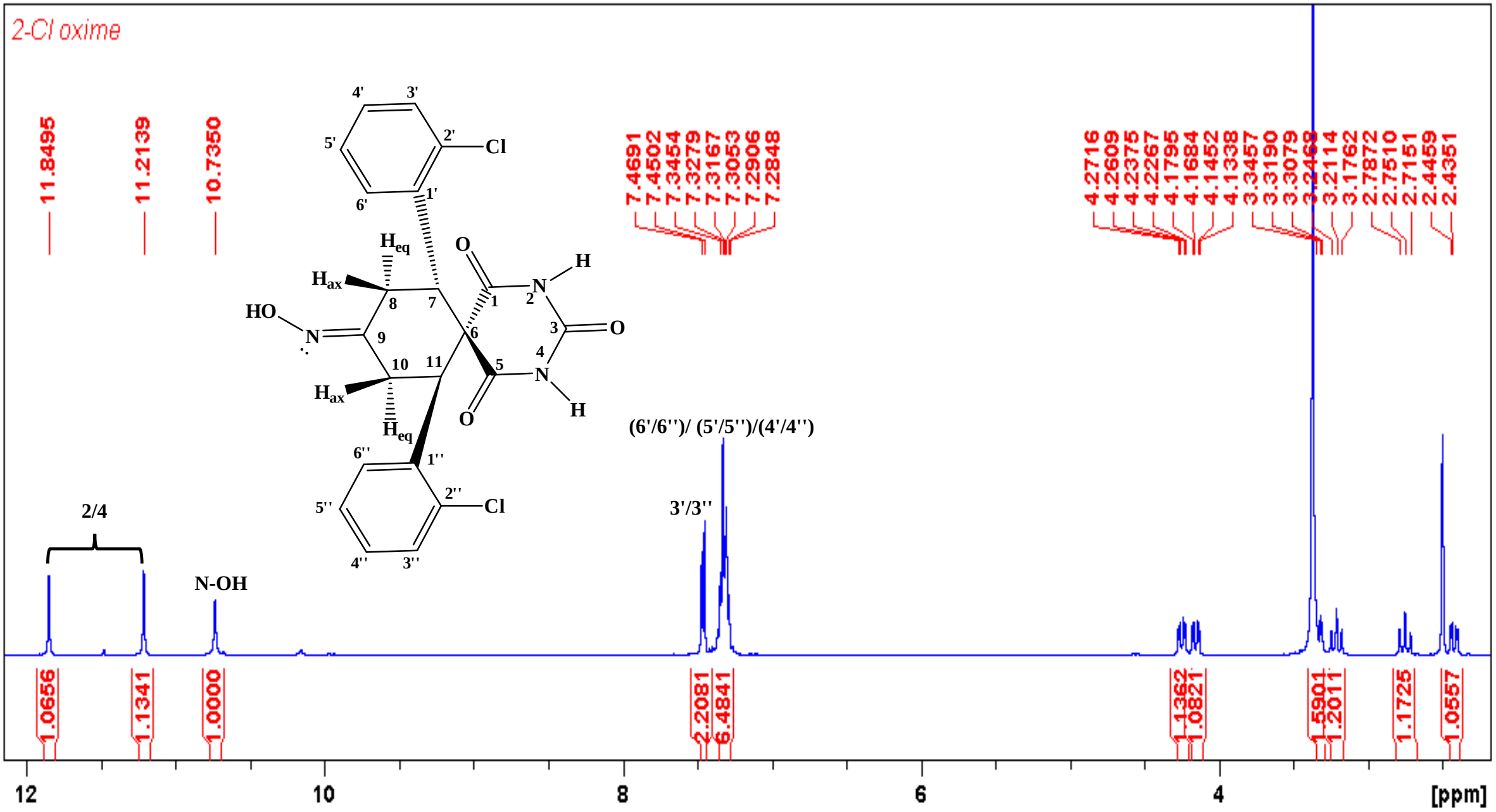
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
11 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 5-10
28 4-NO2 spiro 2 (0.034) Cm (1:61)
TOF MS ES-



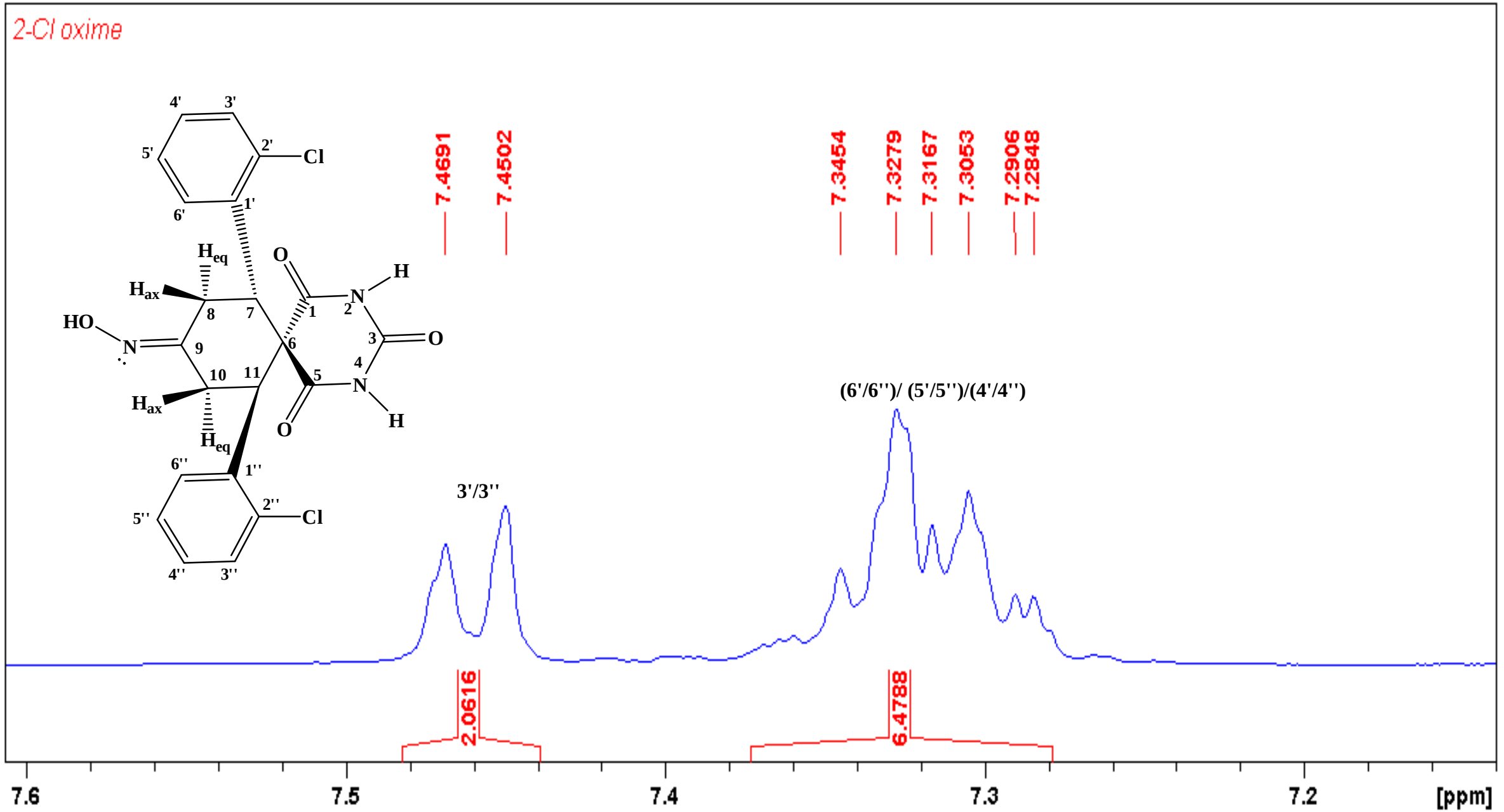
Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
451.0891	451.0890	0.1	0.2	16.5	8.7	0.0	C21 H15 N4 O8

HRMS of Compound B-3o: 7,11-bis(4-nitrophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5,9-tetraone

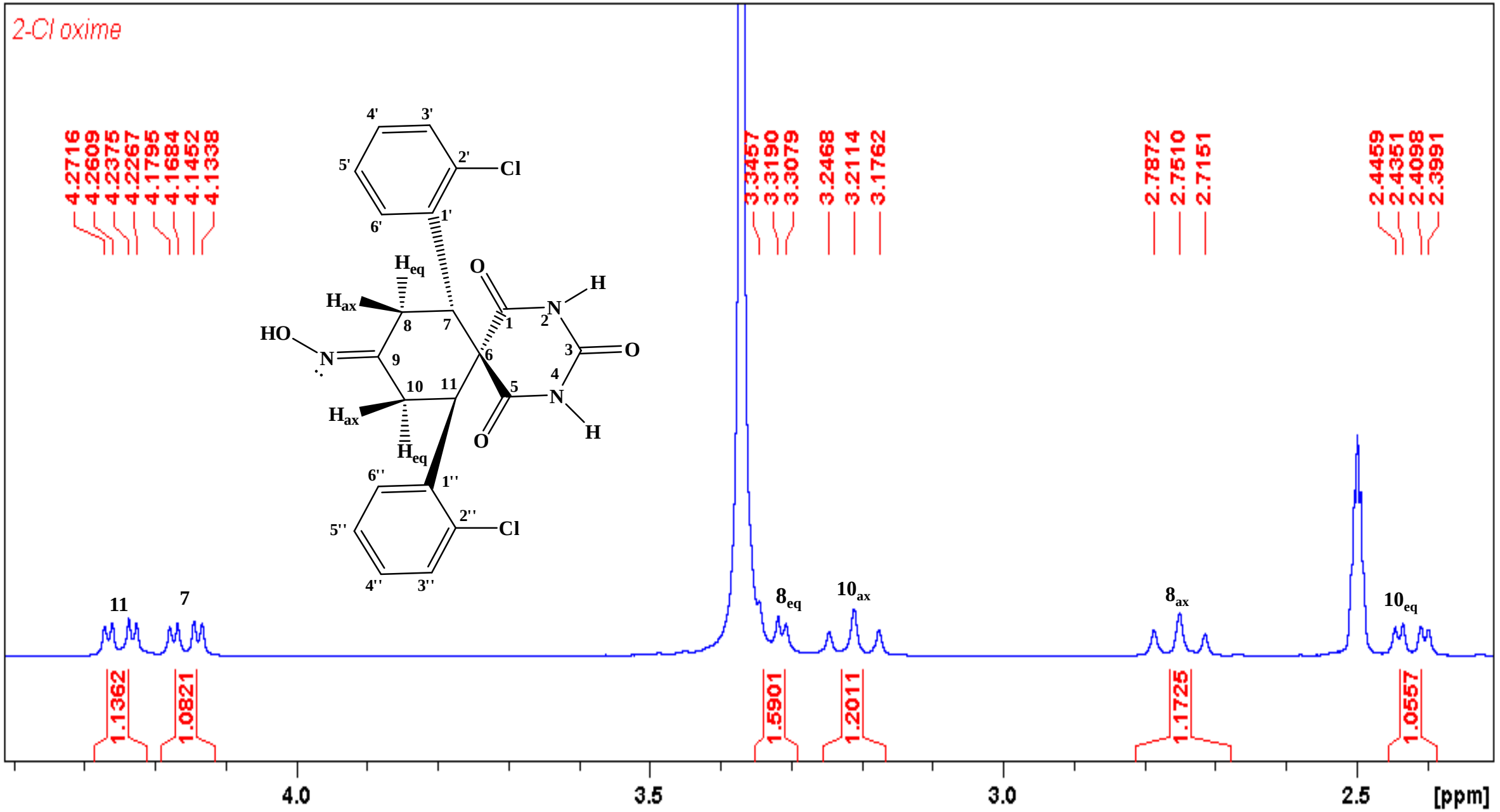


556

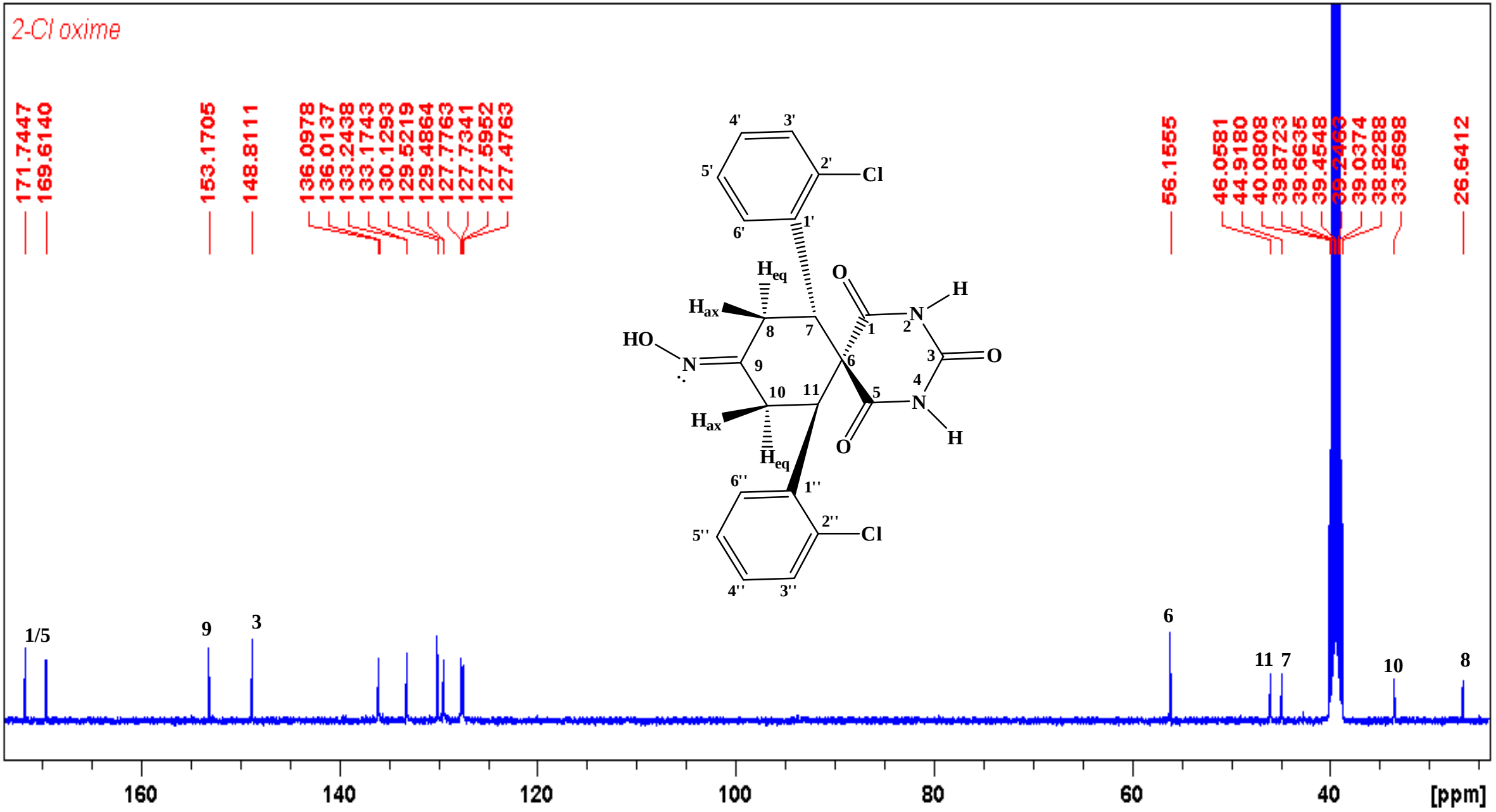
[1]H Spectrum of Compound B-4a: 7,11-bis(2-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Expanded ^{1}H Spectrum of Compound B-4a: 7,11-bis(2-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

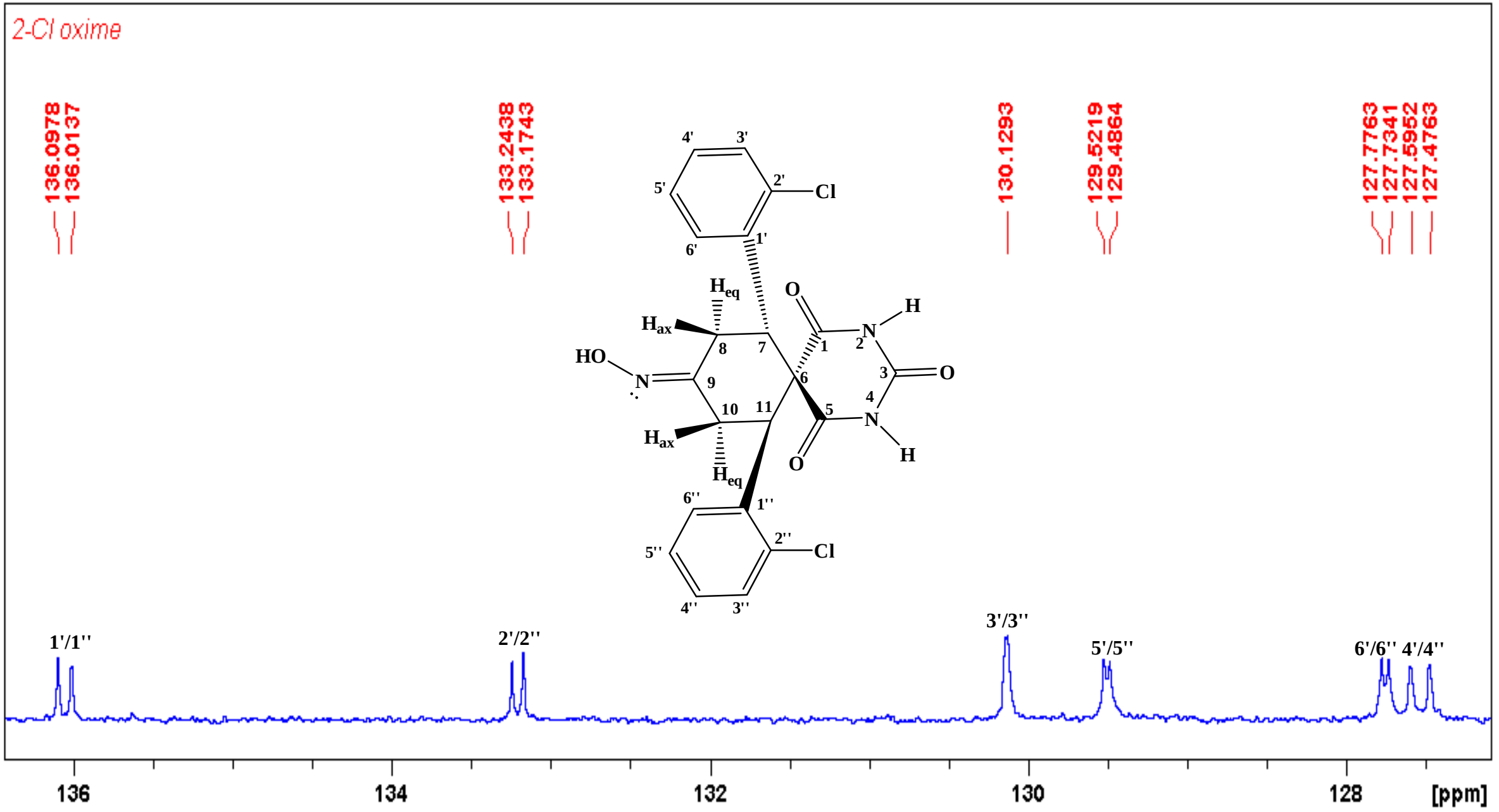


Expanded ^{1}H Spectrum of Compound B-4a: 7,11-bis(2-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



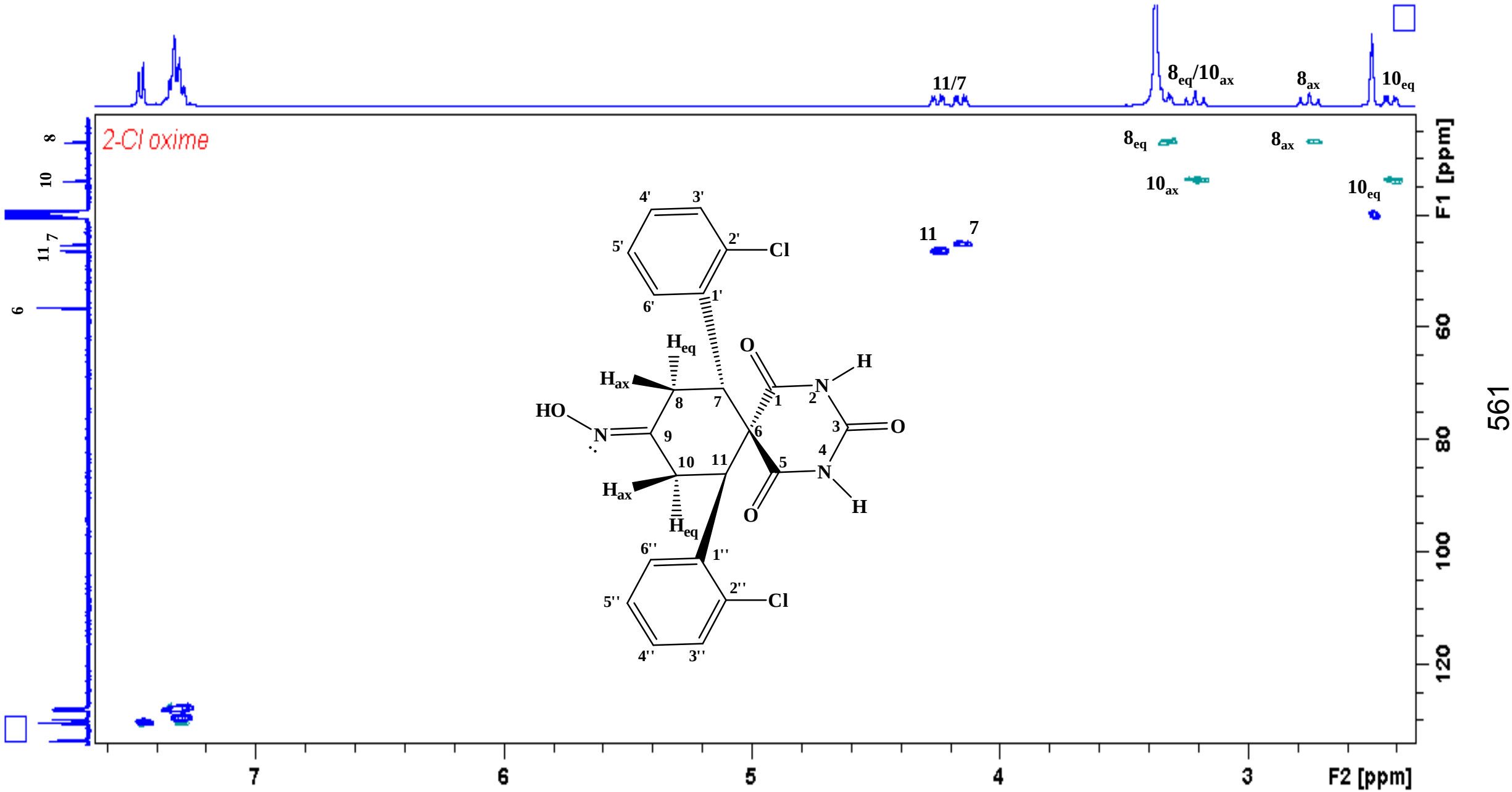
559

^{13}C Spectrum of Compound B-4a: 7,11-bis(2-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

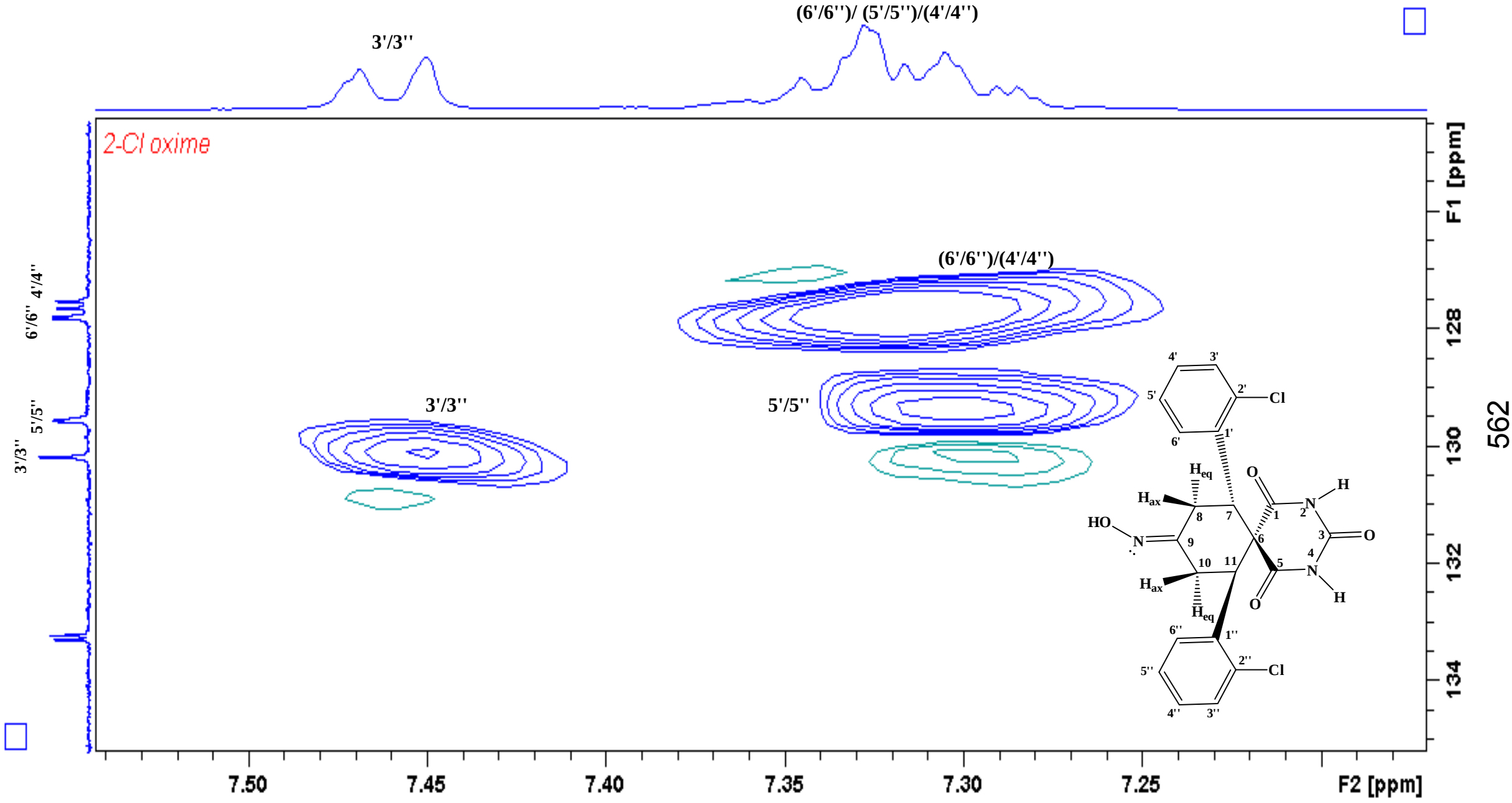


560

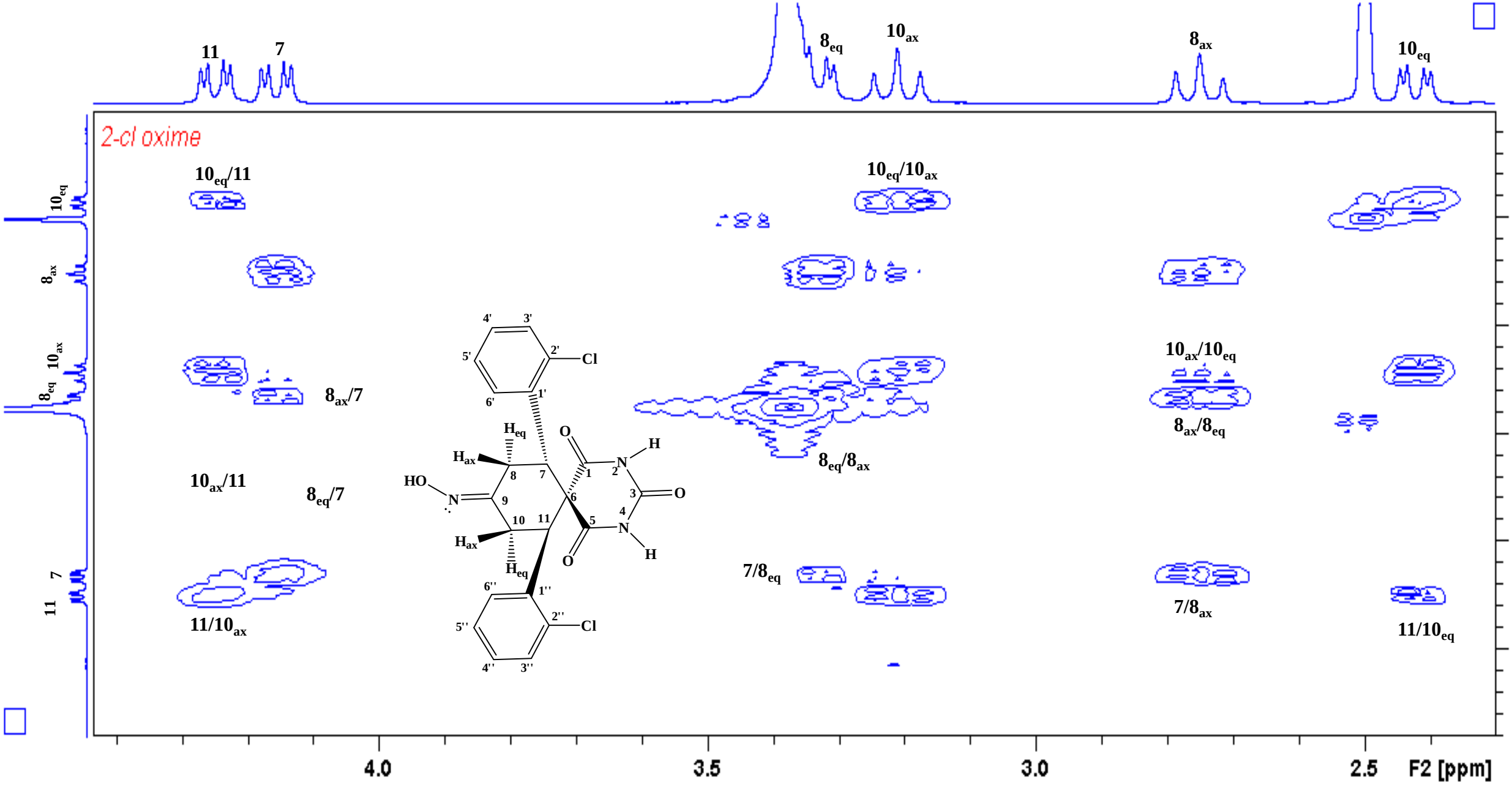
Expanded ^{13}C Spectrum of Compound B-4a: 7,11-bis(2-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



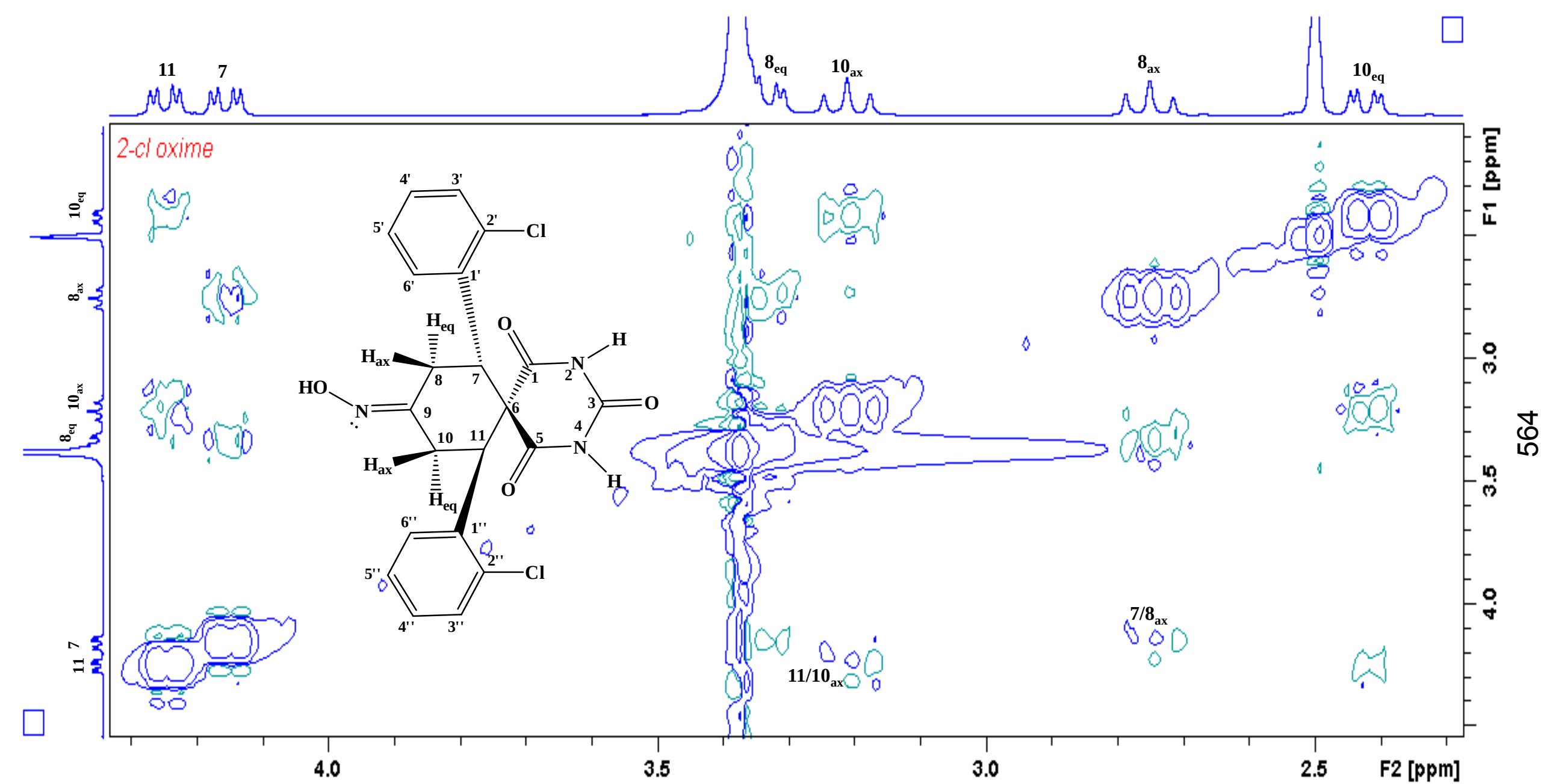
HSQC Spectrum of Compound B-4a: 7,11-bis(2-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



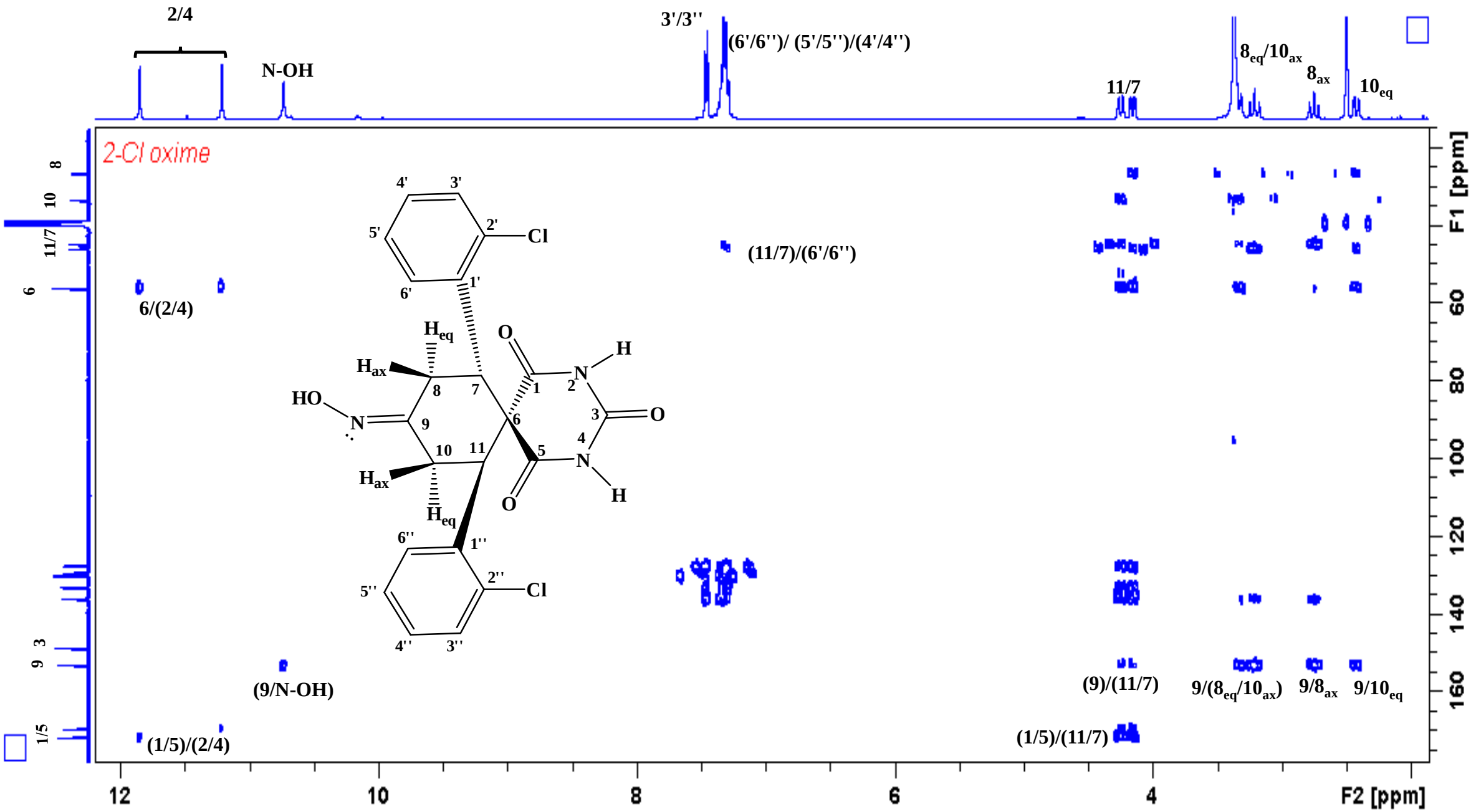
Expanded HSQC Spectrum of Compound B-4a: 7,11-bis(2-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



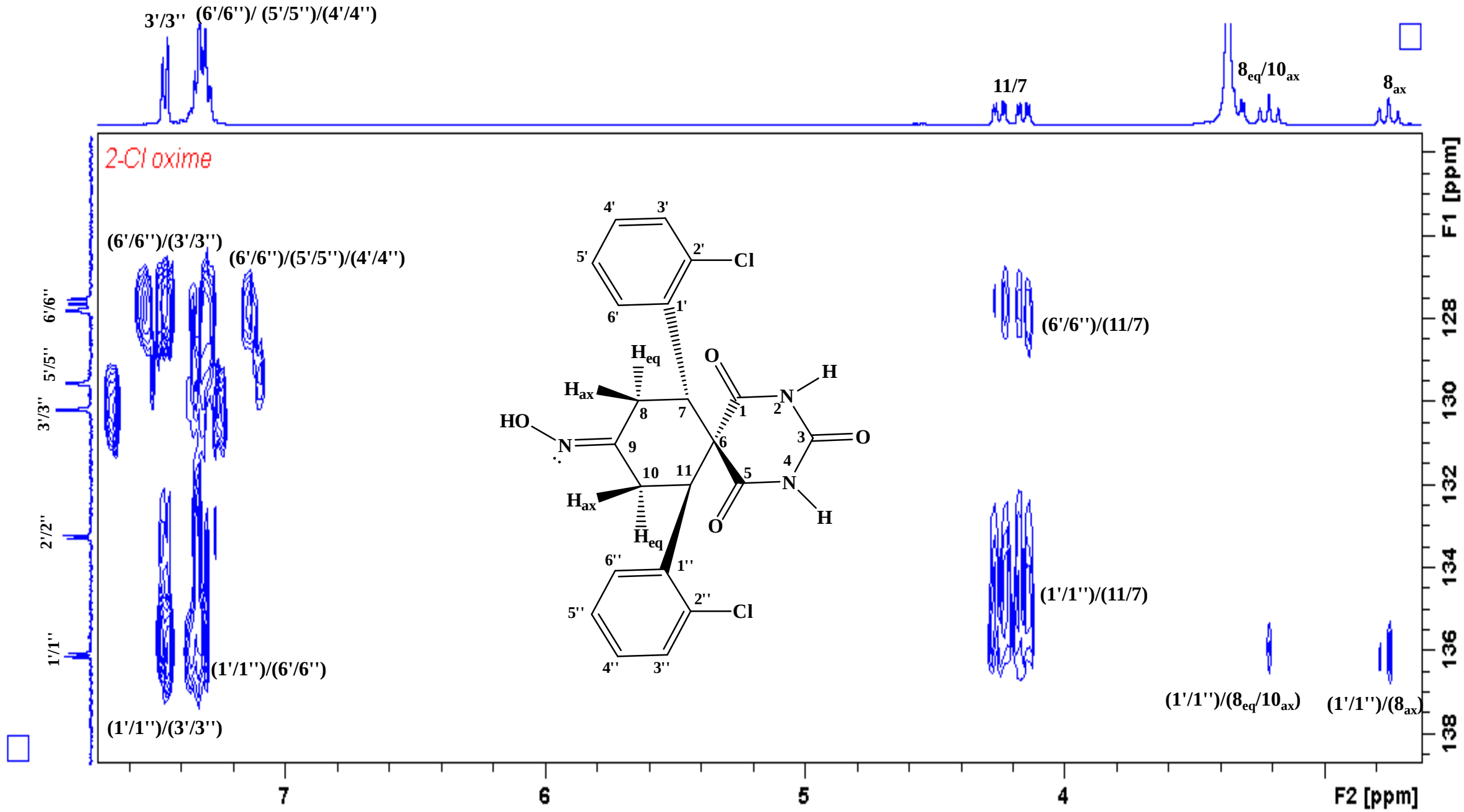
COSY Spectrum of Compound B-4a: 7,11-bis(2-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



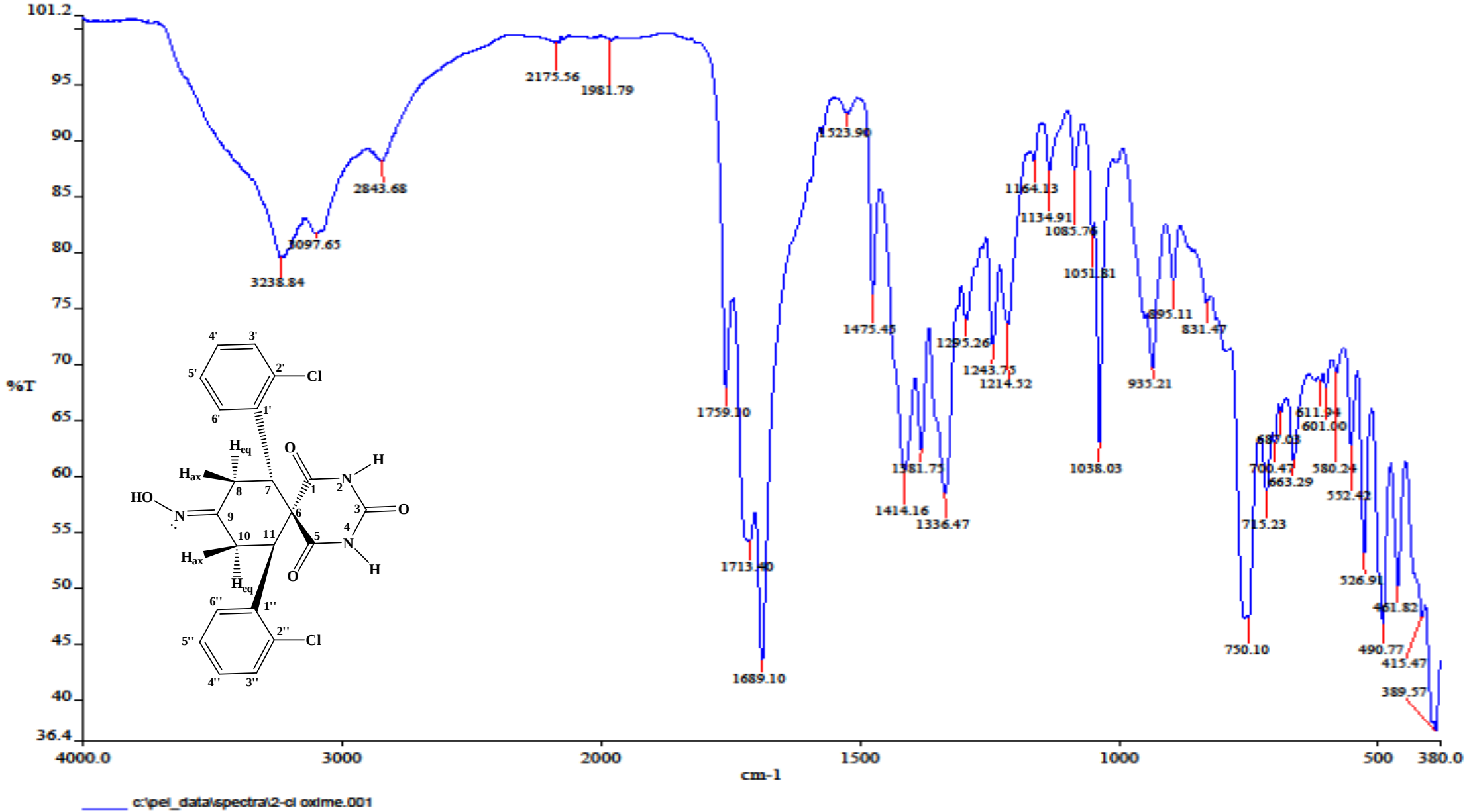
NOESY Spectrum of Compound B-4a: 7,11-bis(2-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



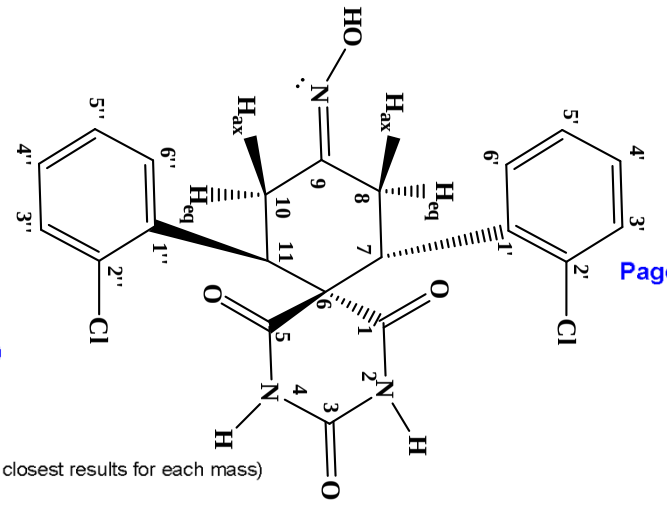
HMBC Spectrum of Compound B-4a: 7,11-bis(2-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Expanded HMBC Spectrum of Compound B-4a: 7,11-bis(2-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



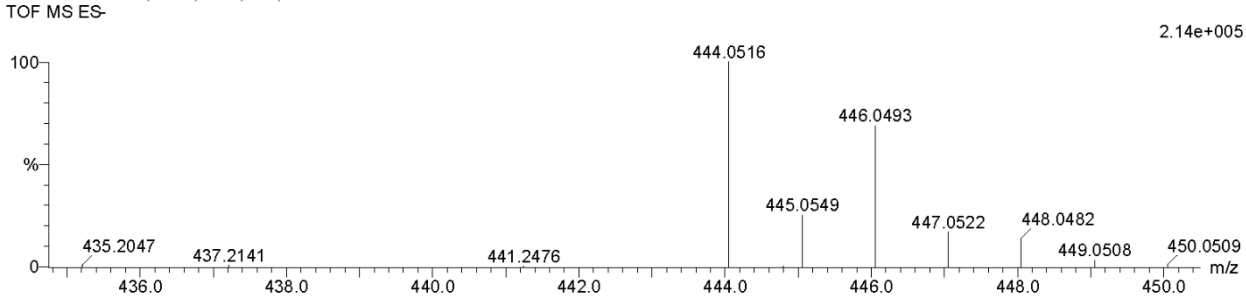
Infrared Spectrum of Compound B-4a: 7,11-bis(2-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Elemental Composition Report

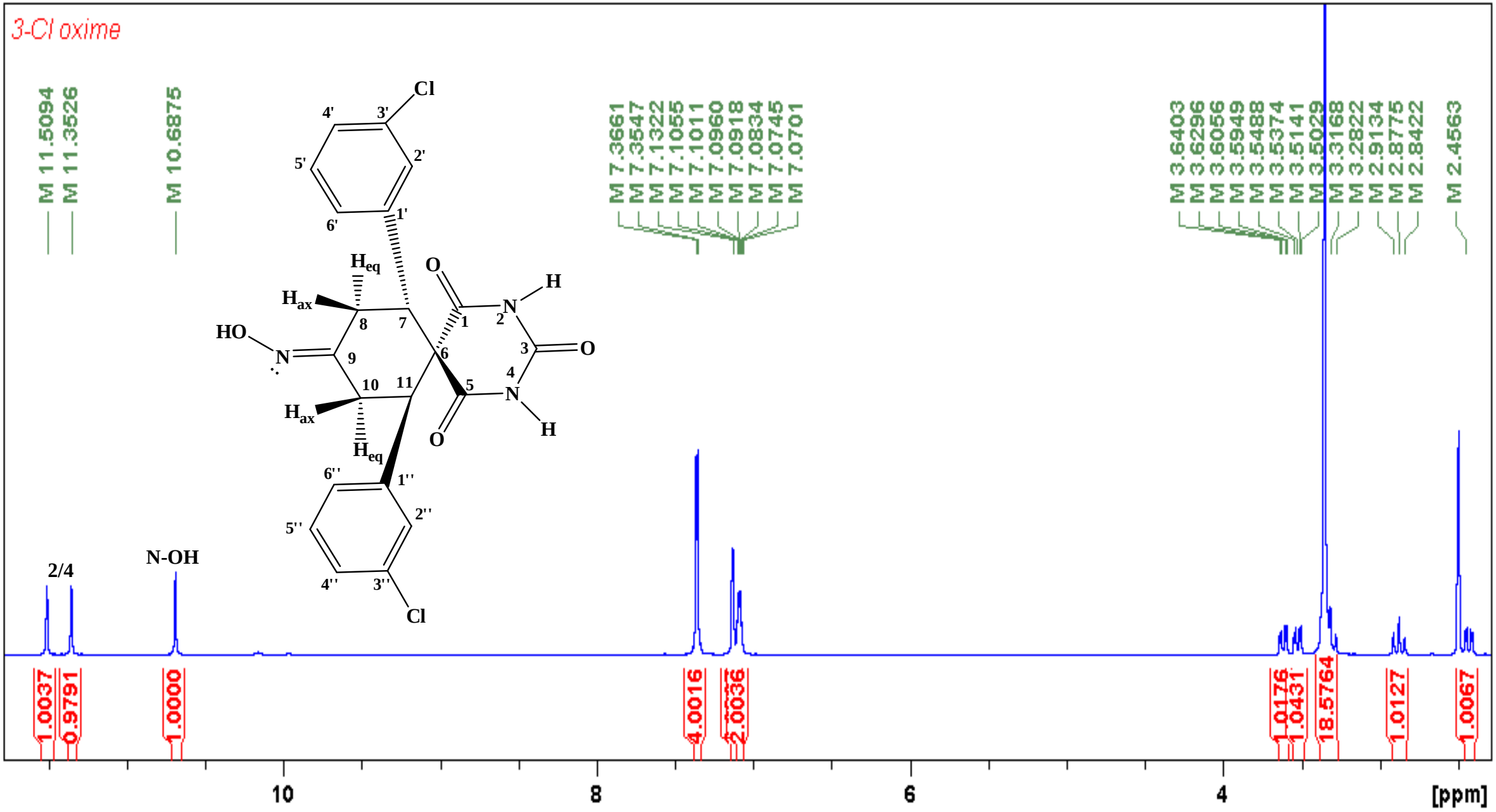
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
46 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 0-5 Cl: 0-2
29 2-Cl oxime 15 (0.473) Cm (1:61)
TOF MS ES-

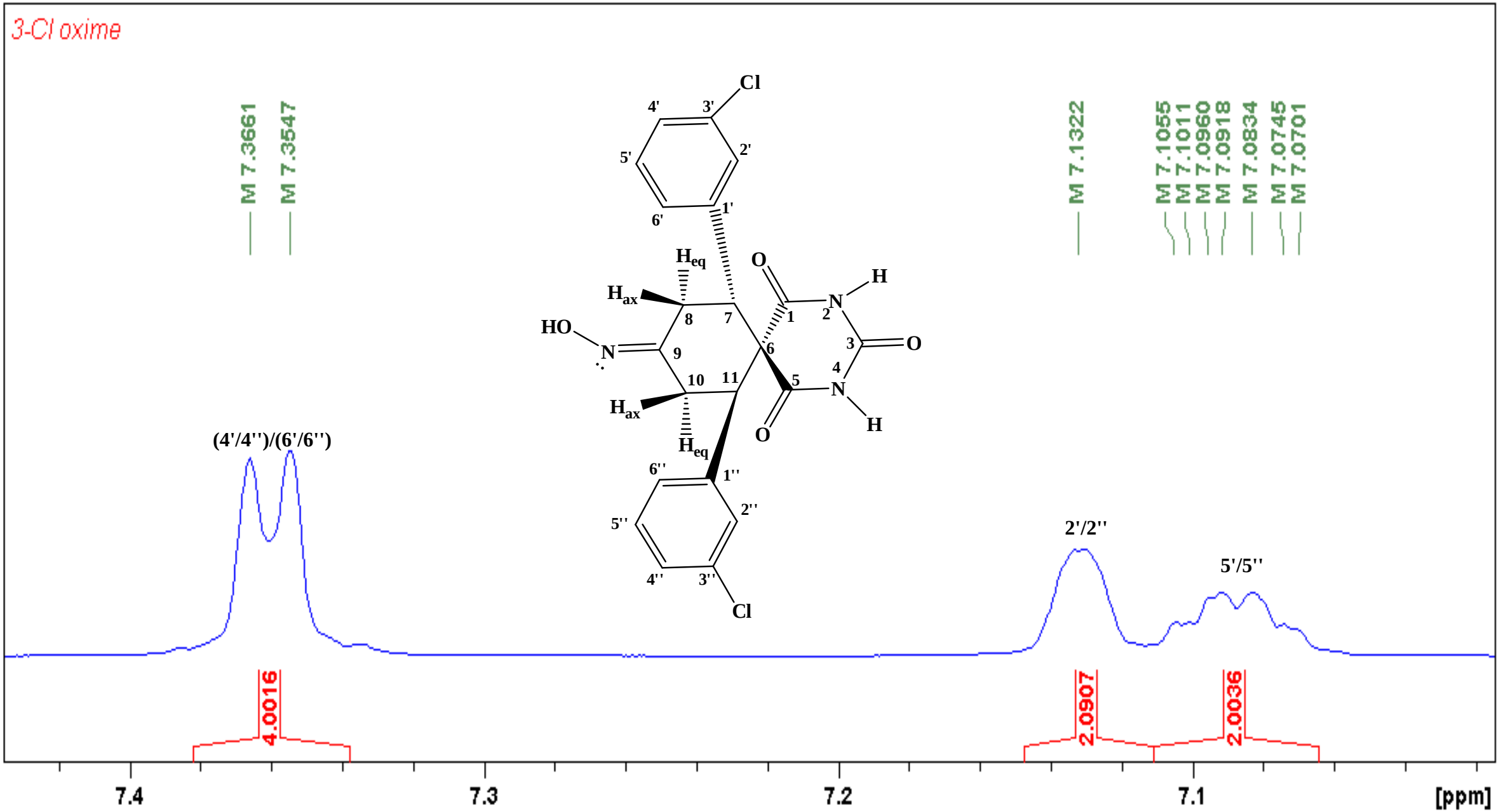


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
444.0516	444.0518	-0.2	-0.5	14.5	23.5	0.0	C21 H16 N3 O4 Cl2

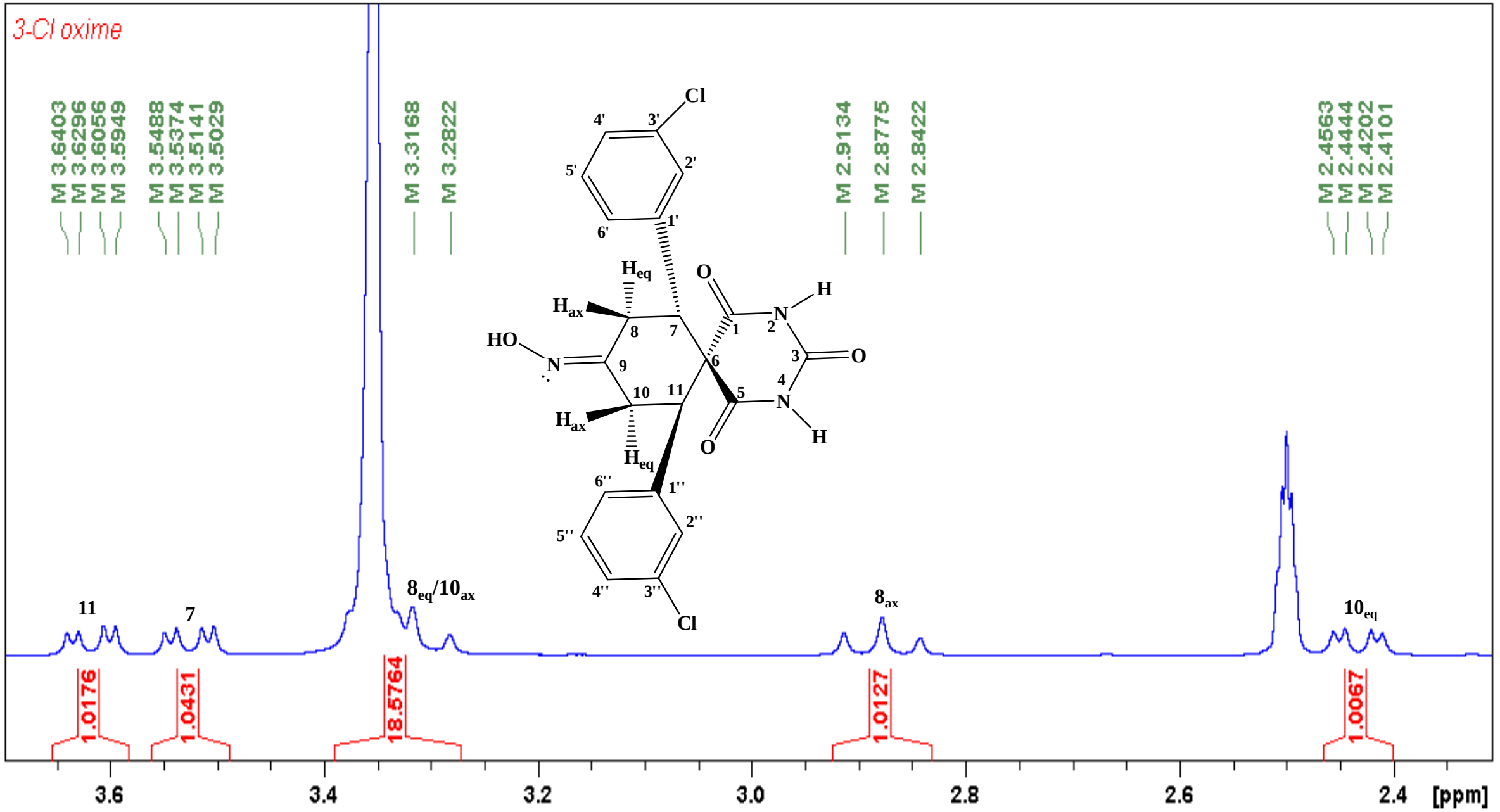
HRMS Spectrum of Compound B-4a: 7,11-bis(2-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



[1]H Spectrum of Compound B-4b: 7,11-bis(3-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

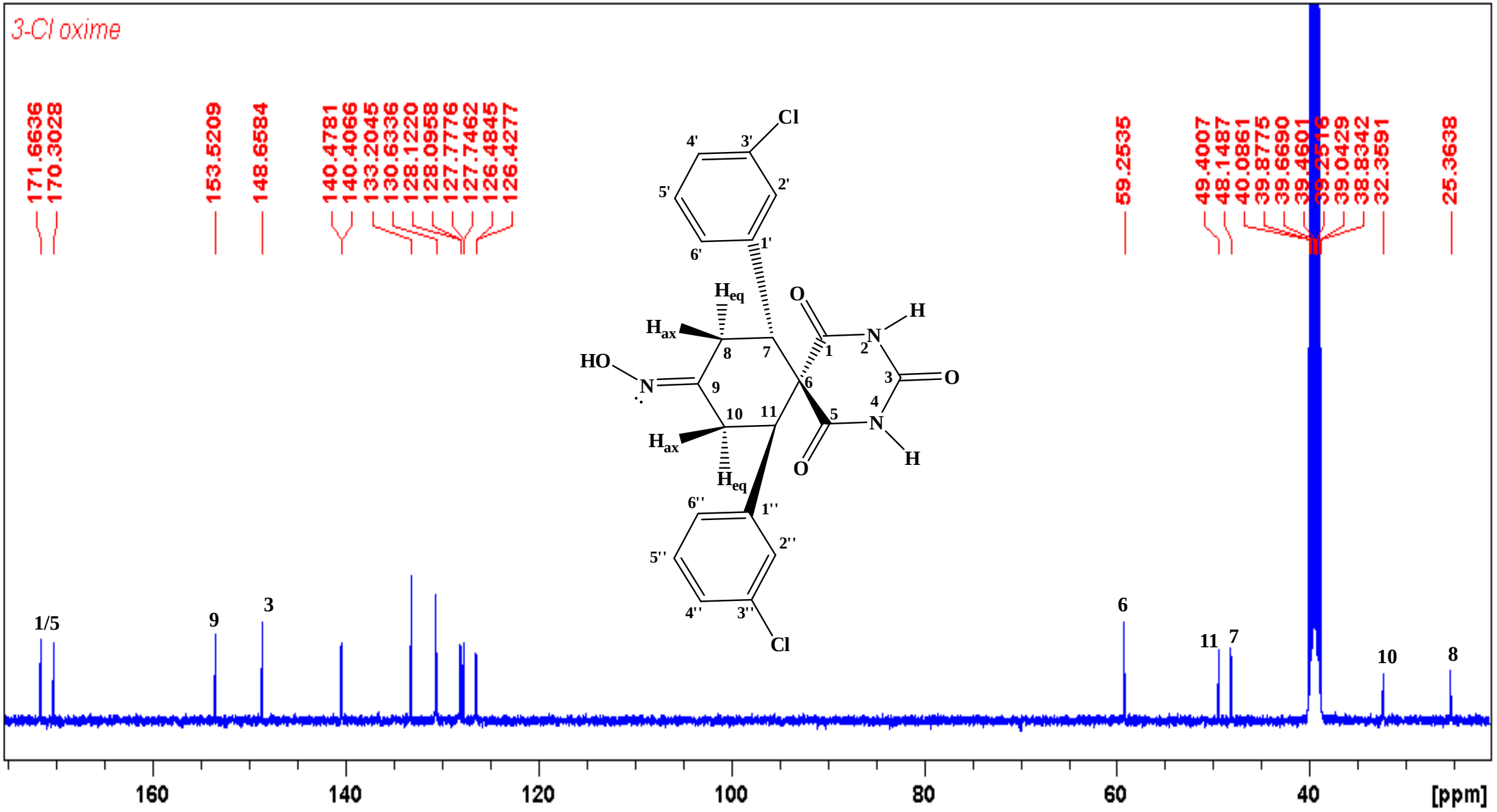


Expanded ^{1}H Spectrum of Compound B-4b: 7,11-bis(3-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



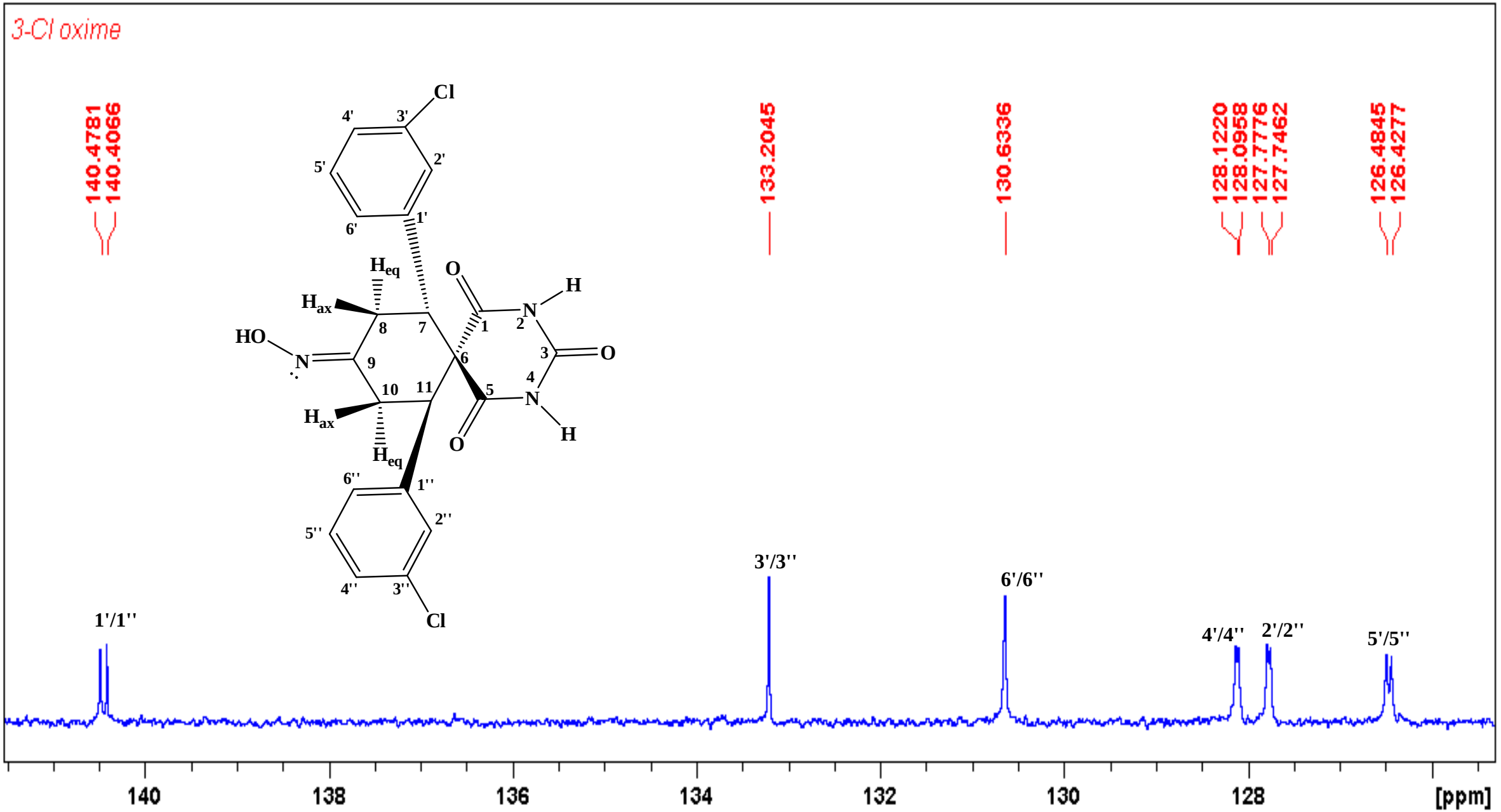
571

Expanded ^{1}H Spectrum of Compound B-4b: 7,11-bis(3-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

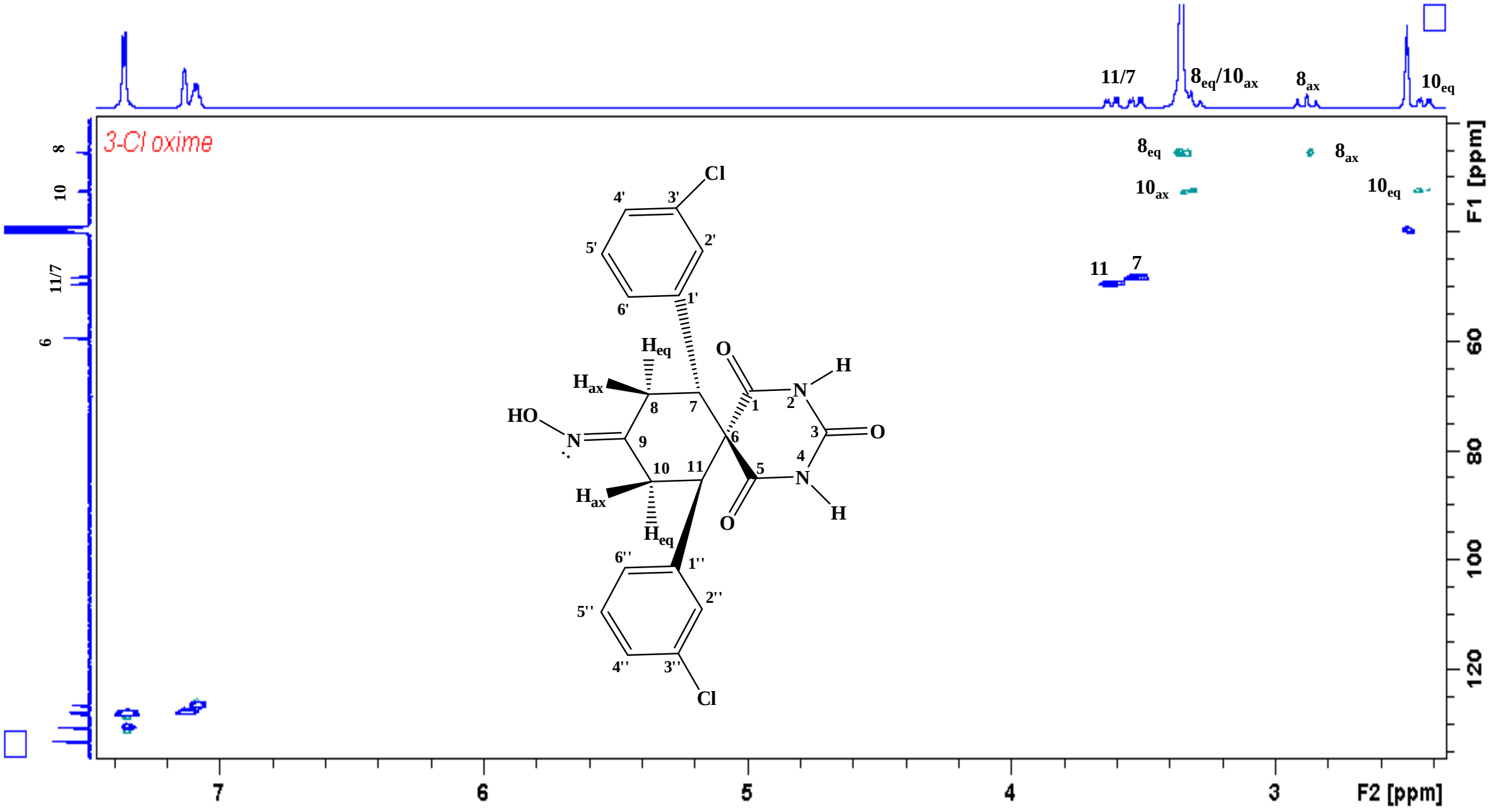


572

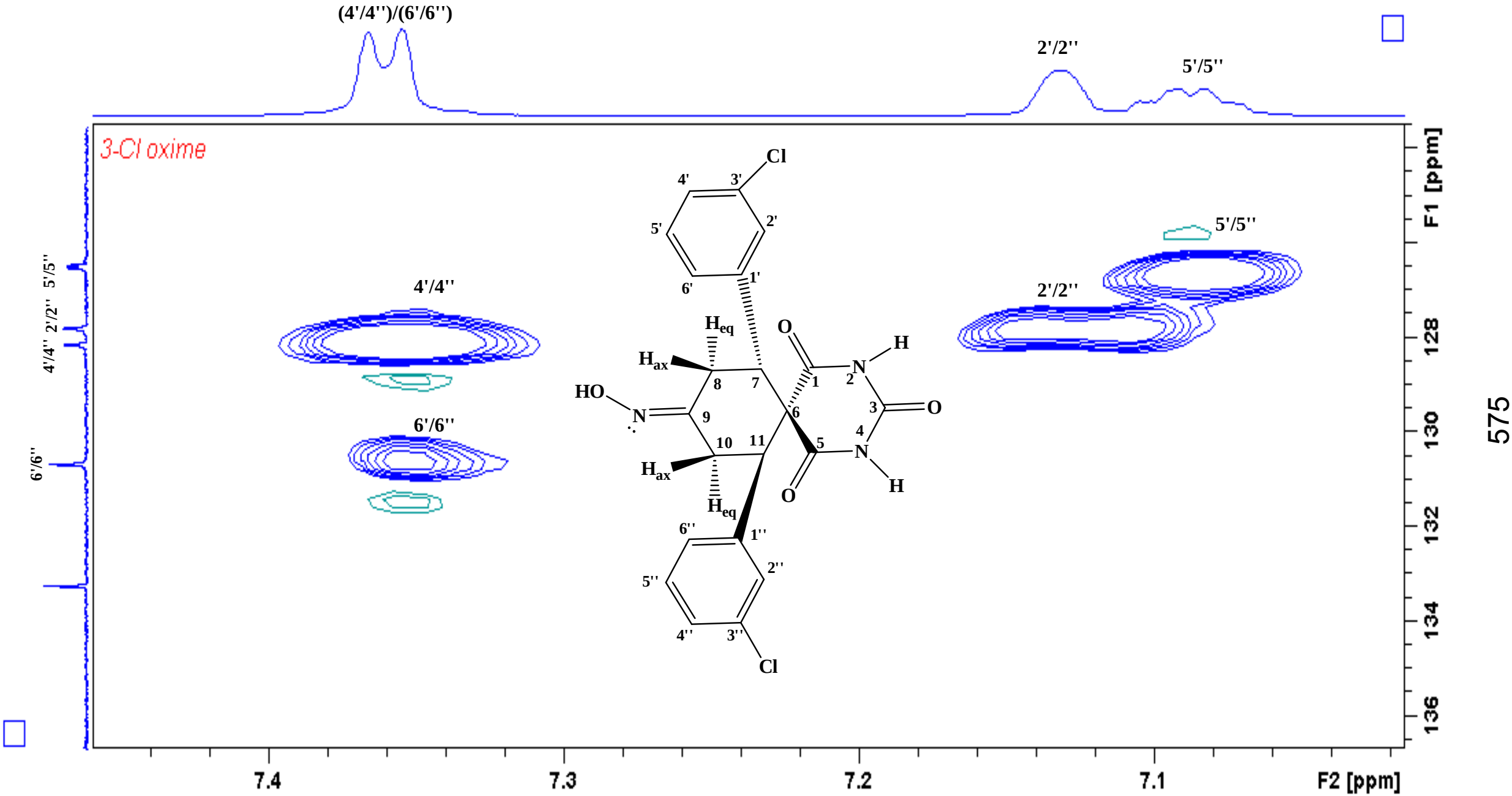
^{13}C Spectrum of Compound B-4b: 7,11-bis(3-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Expanded ^{13}C Spectrum of Compound B-4b: 7,11-bis(3-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

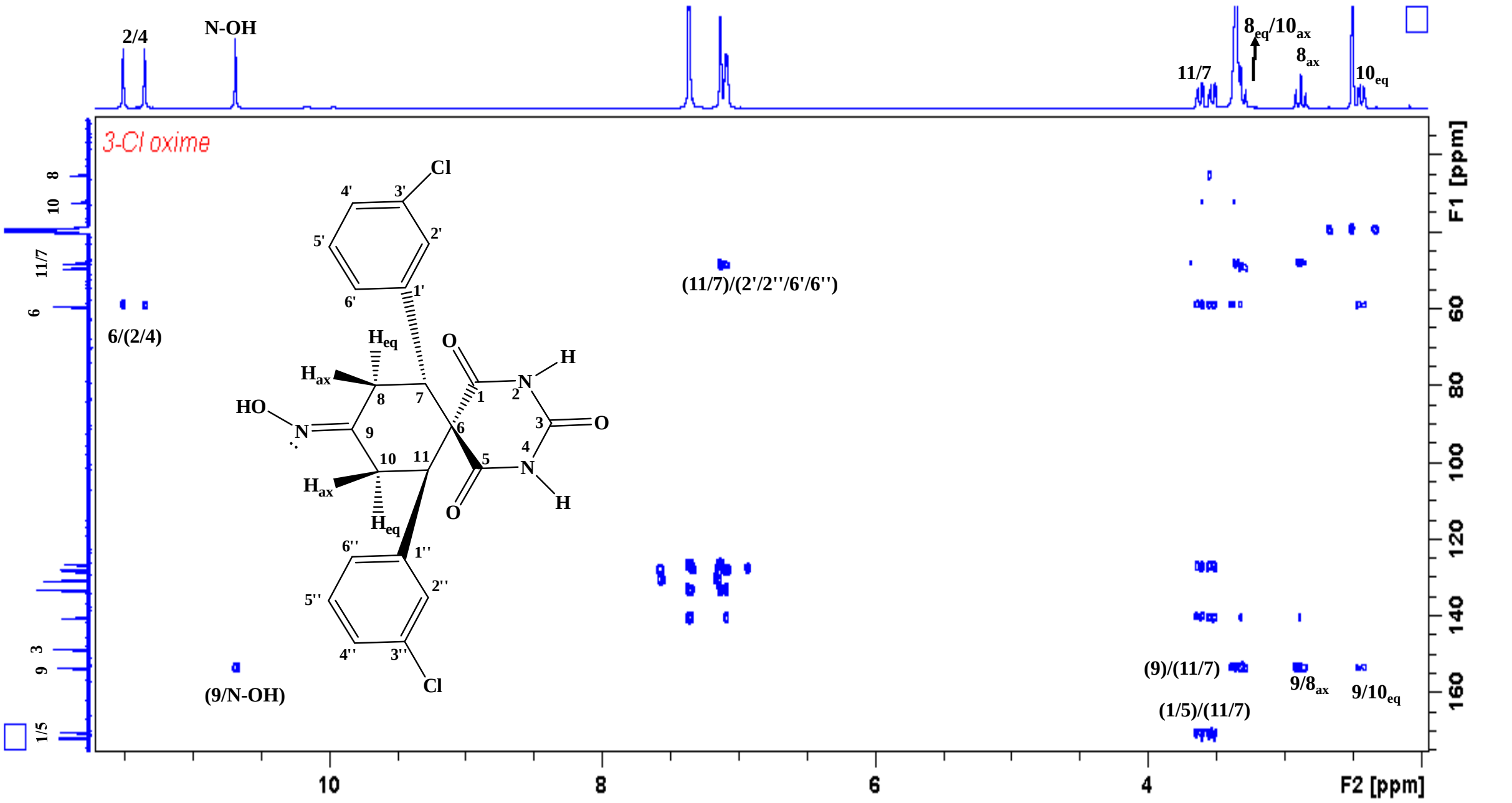


HSQC Spectrum of Compound B-4b: 7,11-bis(3-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

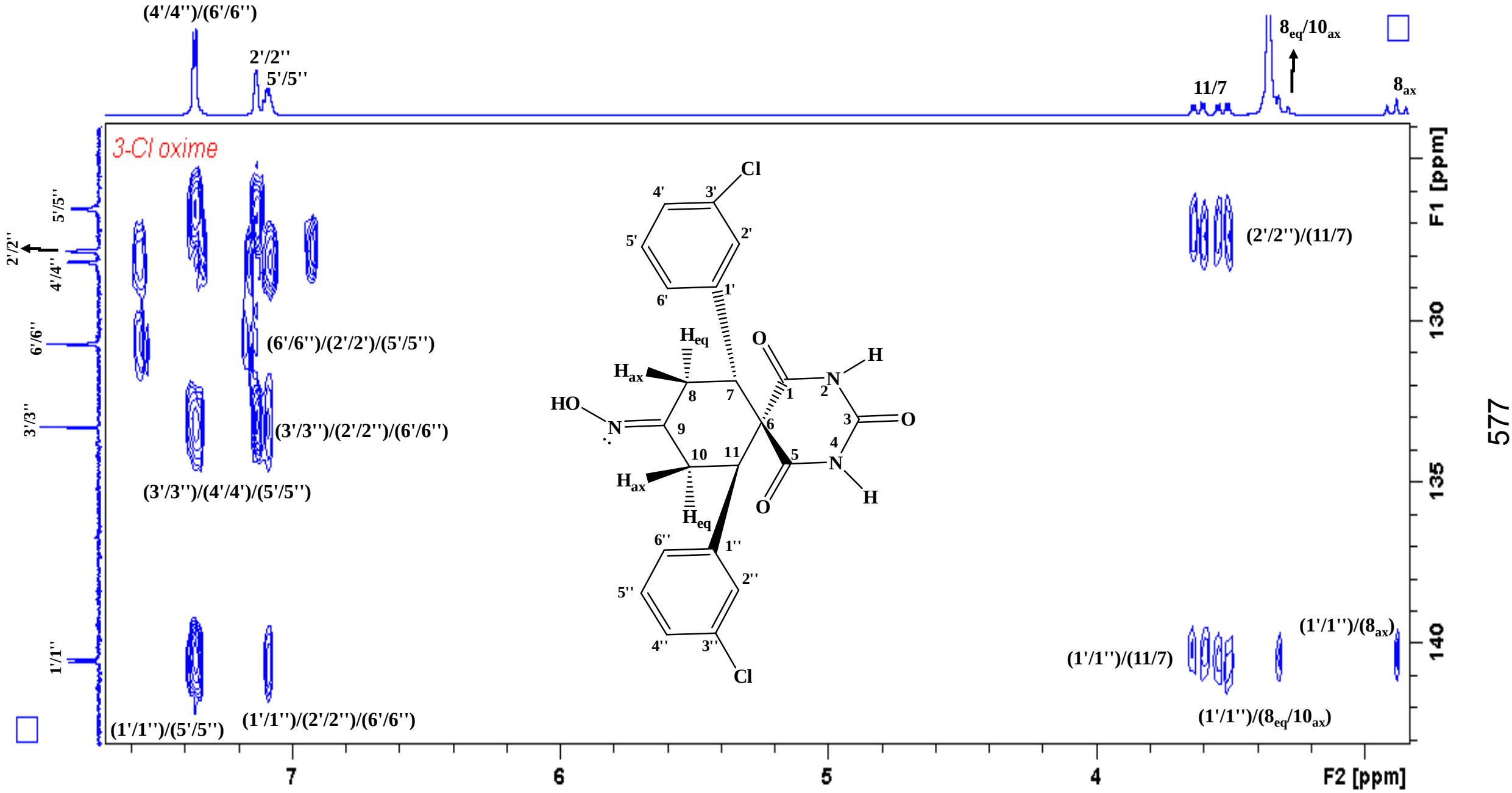


575

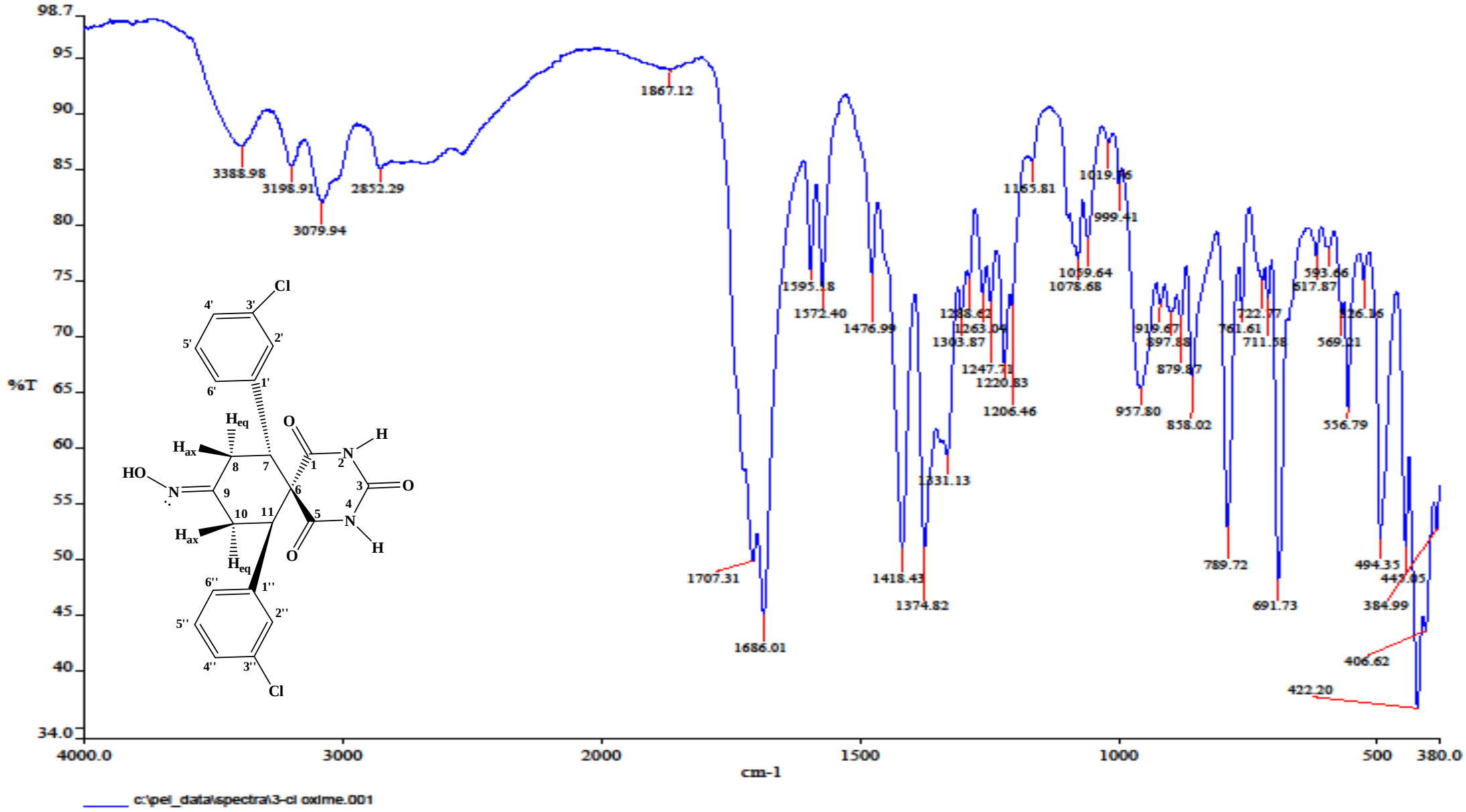
Expanded HSQC Spectrum of Compound B-4b: 7,11-bis(3-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



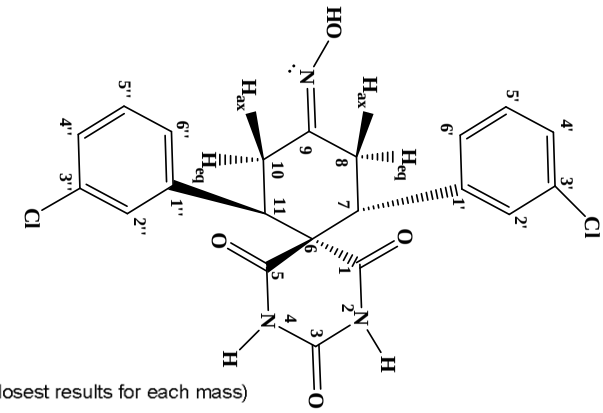
HMBC Spectrum of Compound B-4b: 7,11-bis(3-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Expanded HMBC Spectrum of Compound B-4b: 7,11-bis(3-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



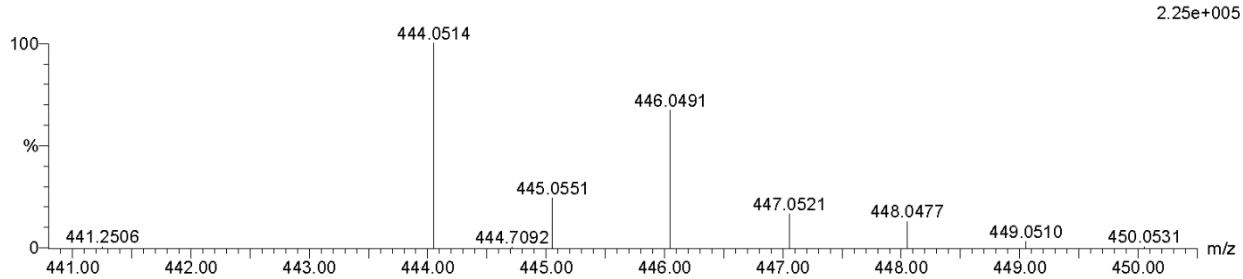
Infrared Spectrum of Compound B-4b: 7,11-bis(3-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Elemental Composition Report **Page 1**

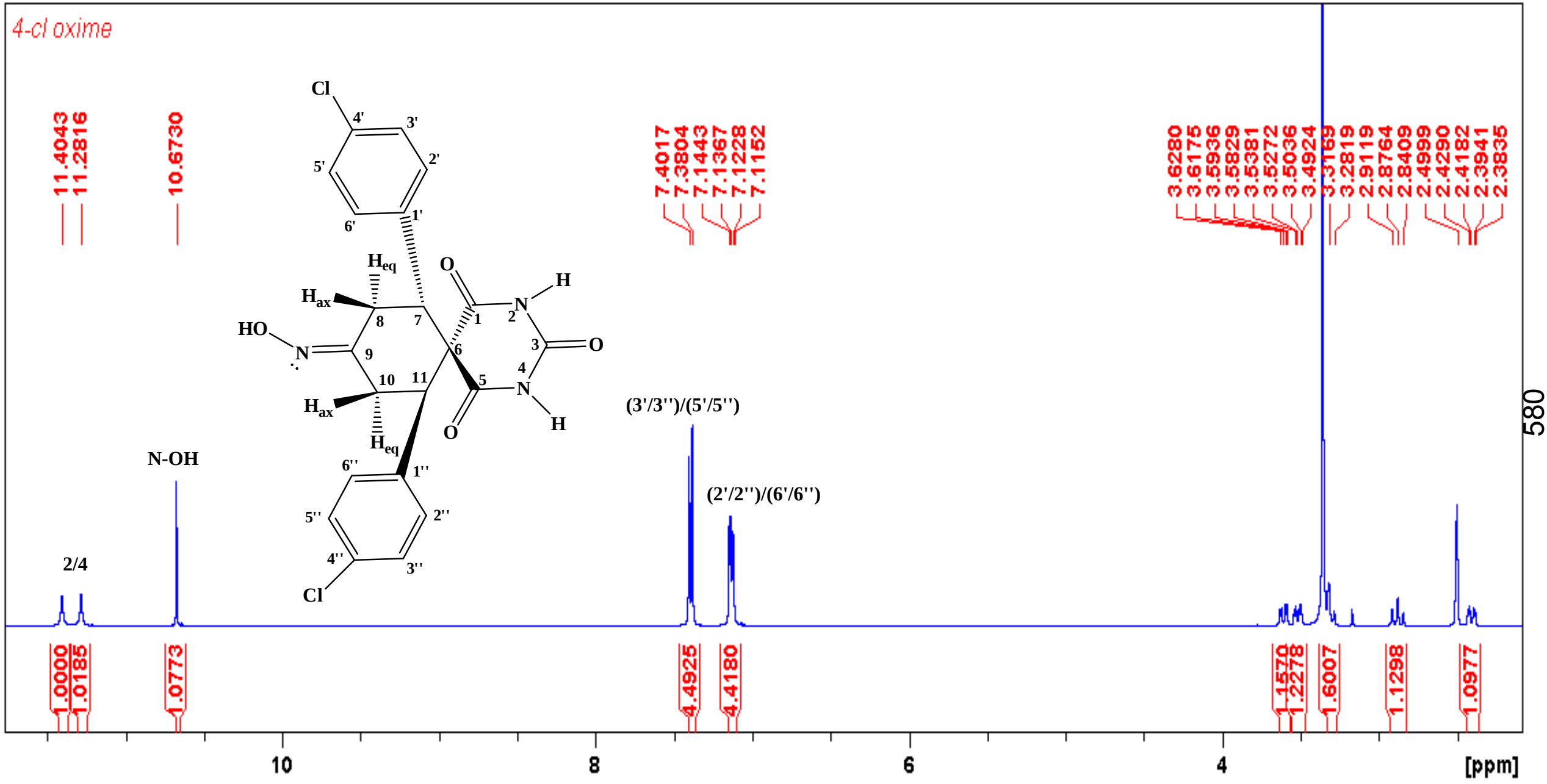
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
46 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 0-5 Cl: 0-2
30 3-Cl oxime 17 (0.540) Cm (1:61)
TOF MS ES-

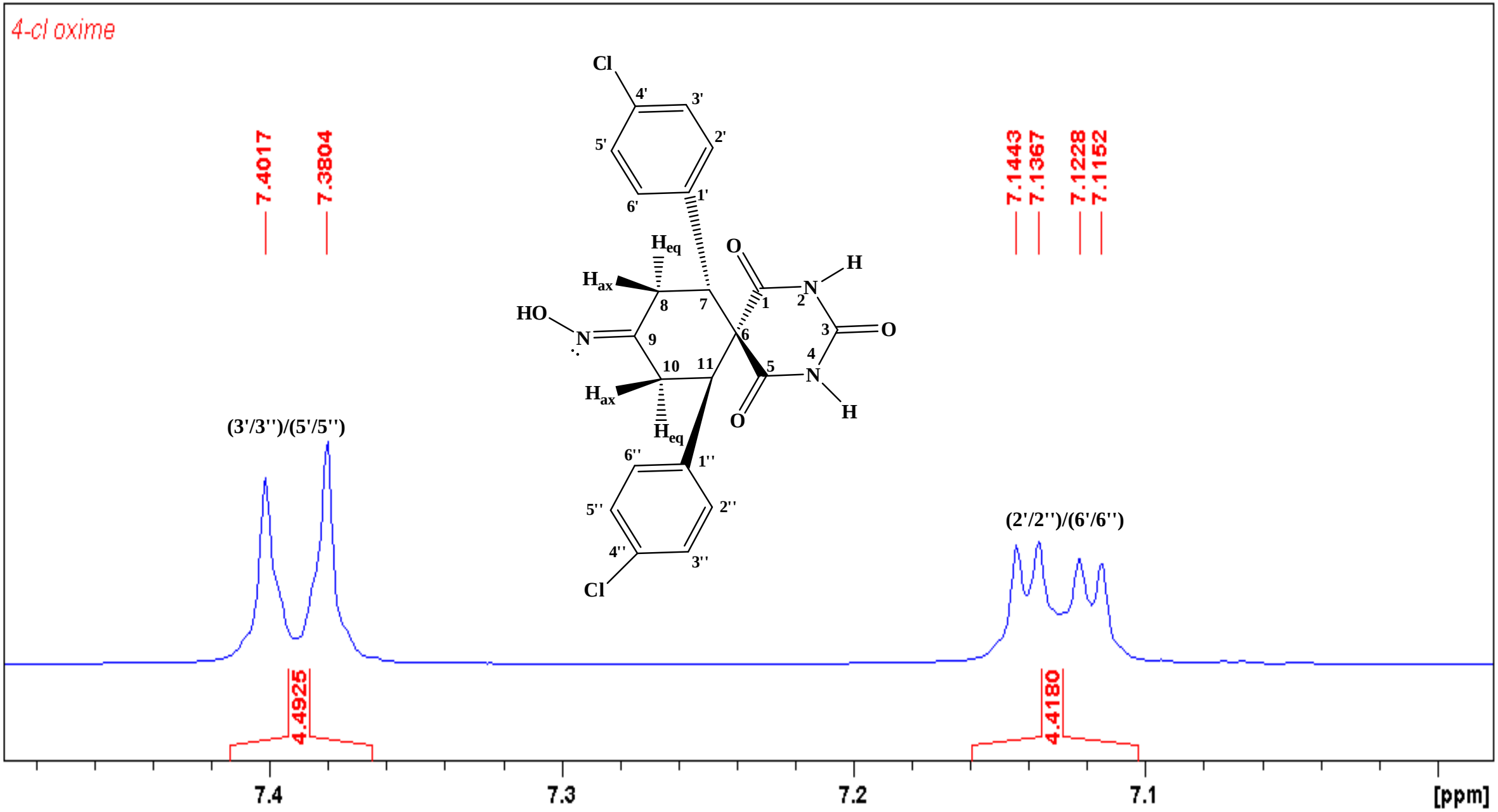


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
444.0514	444.0518	-0.4	-0.9	14.5	25.3	0.0	C21 H16 N3 O4 Cl2

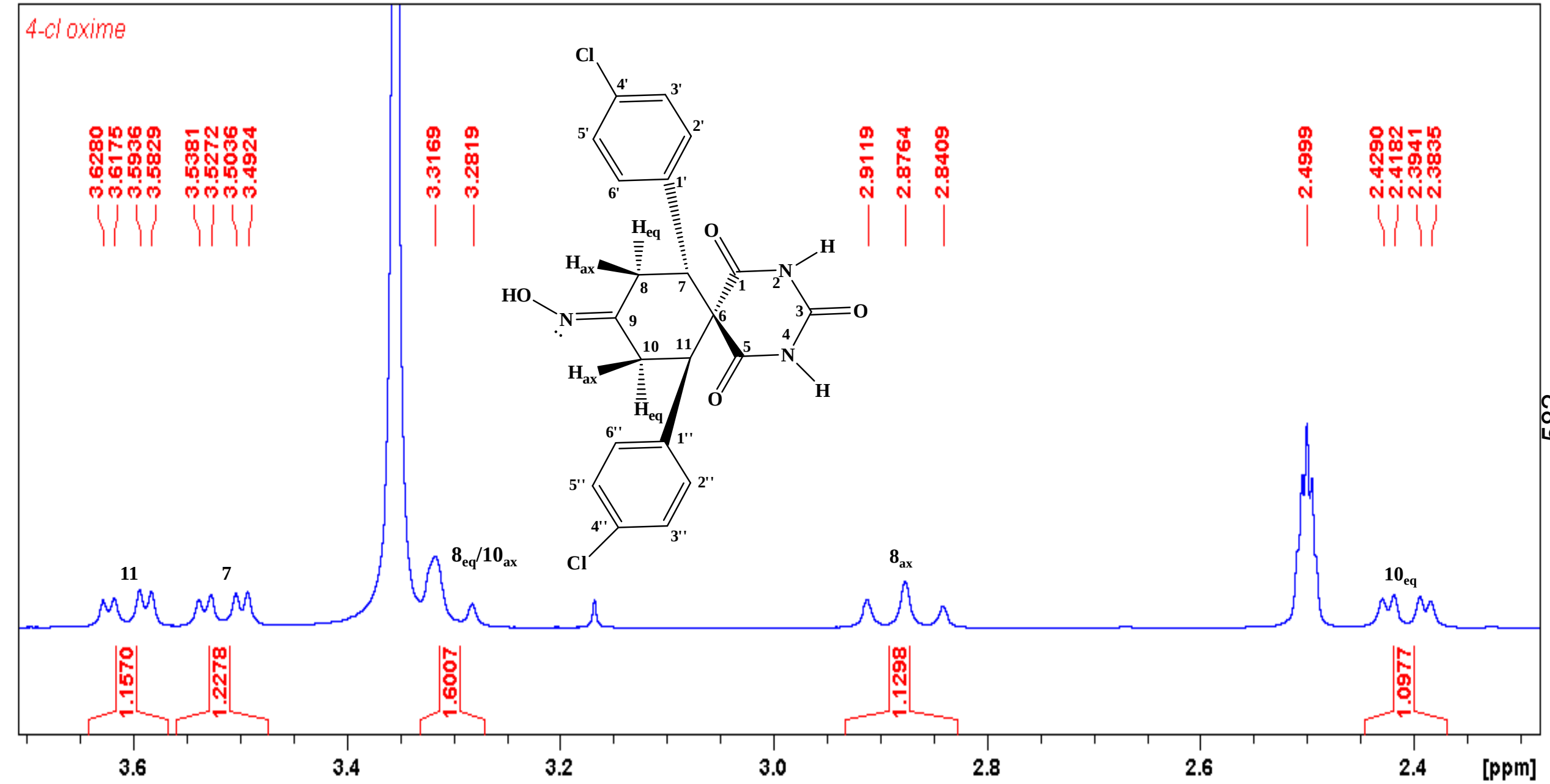
HRMS Spectrum of Compound B-4b: 7,11-bis(3-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



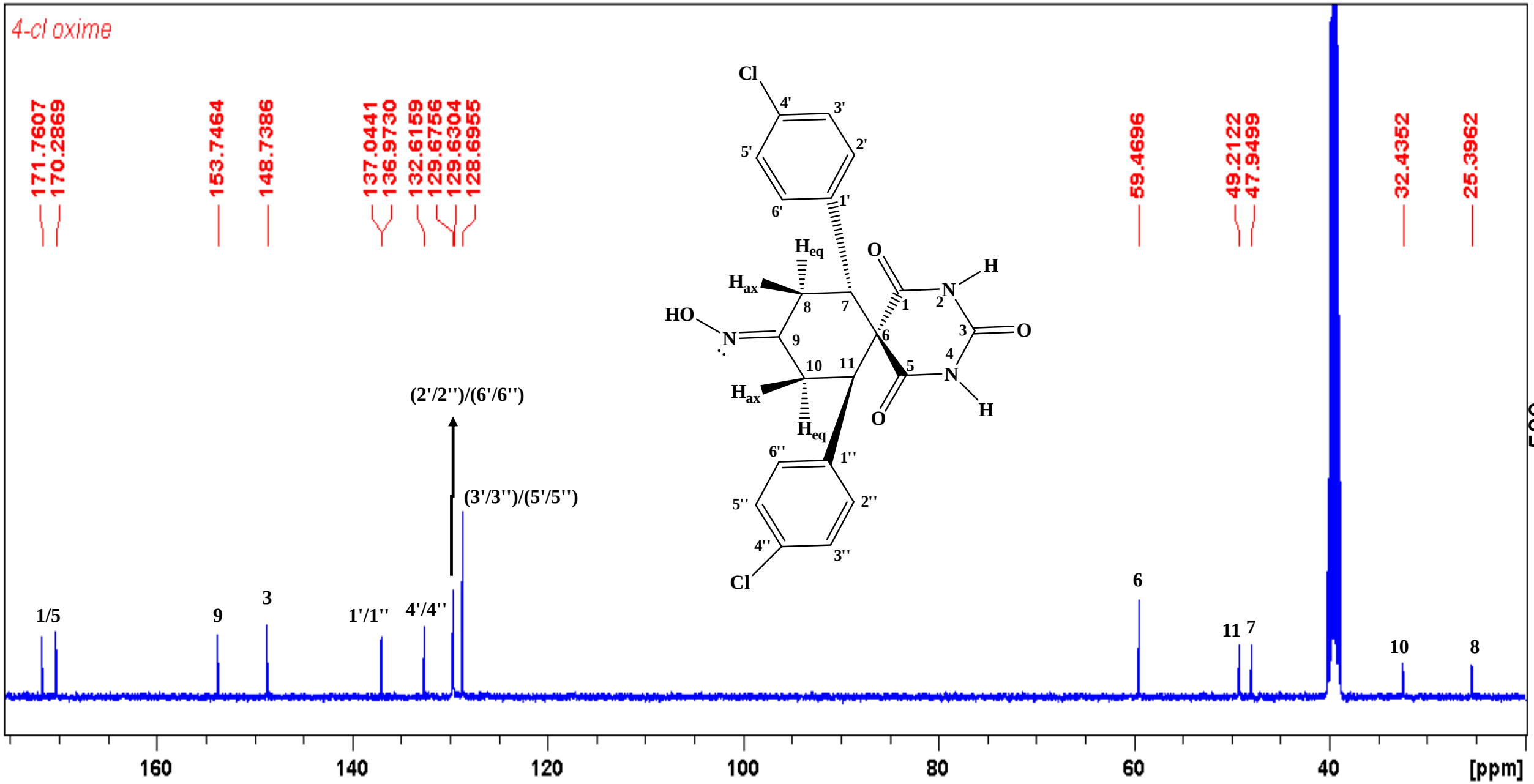
^{1}H Spectrum of Compound B-4c: 7,11-bis(4-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



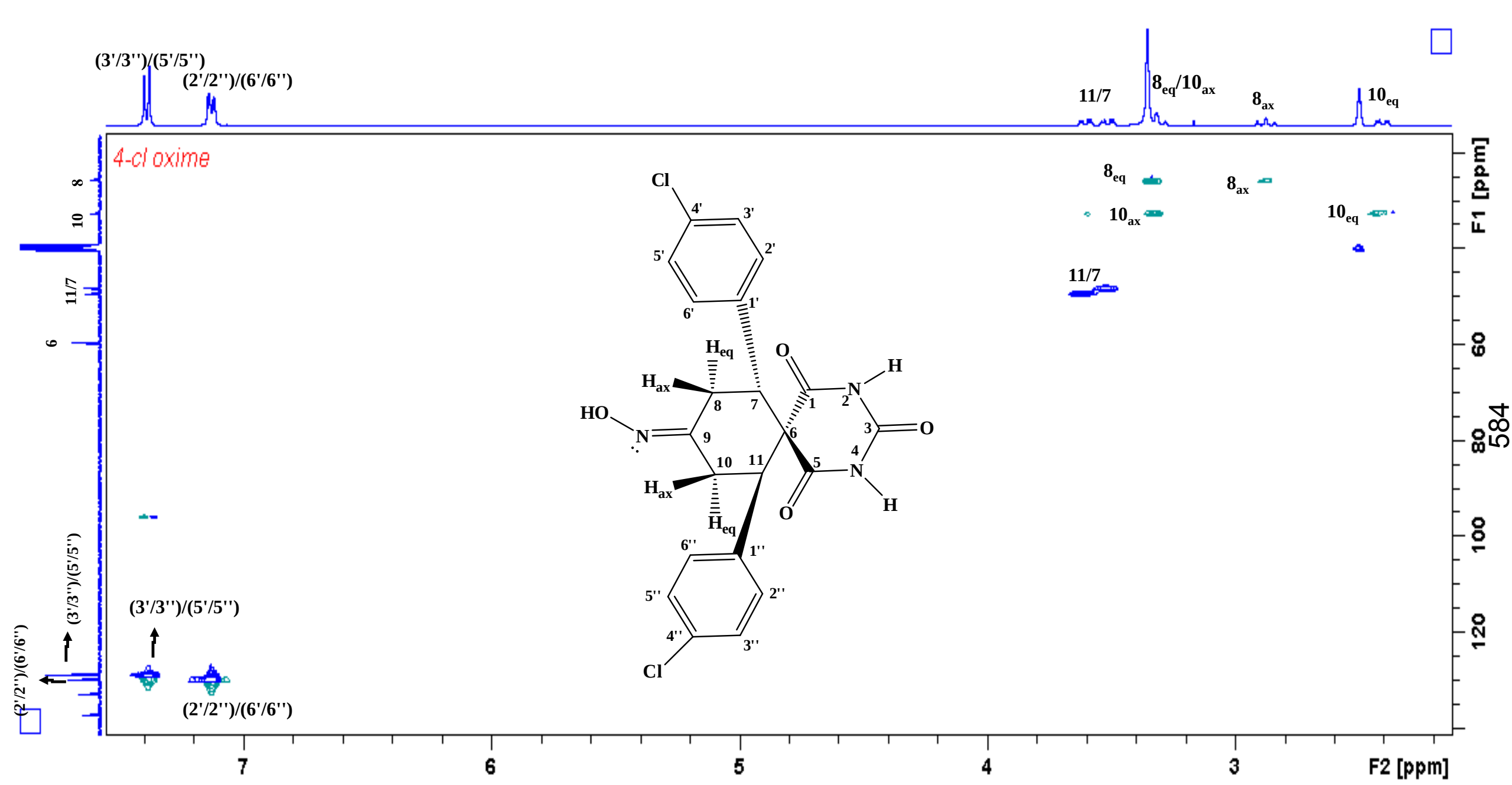
Expanded ^{1}H Spectrum of Compound B-4c: 7,11-bis(4-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



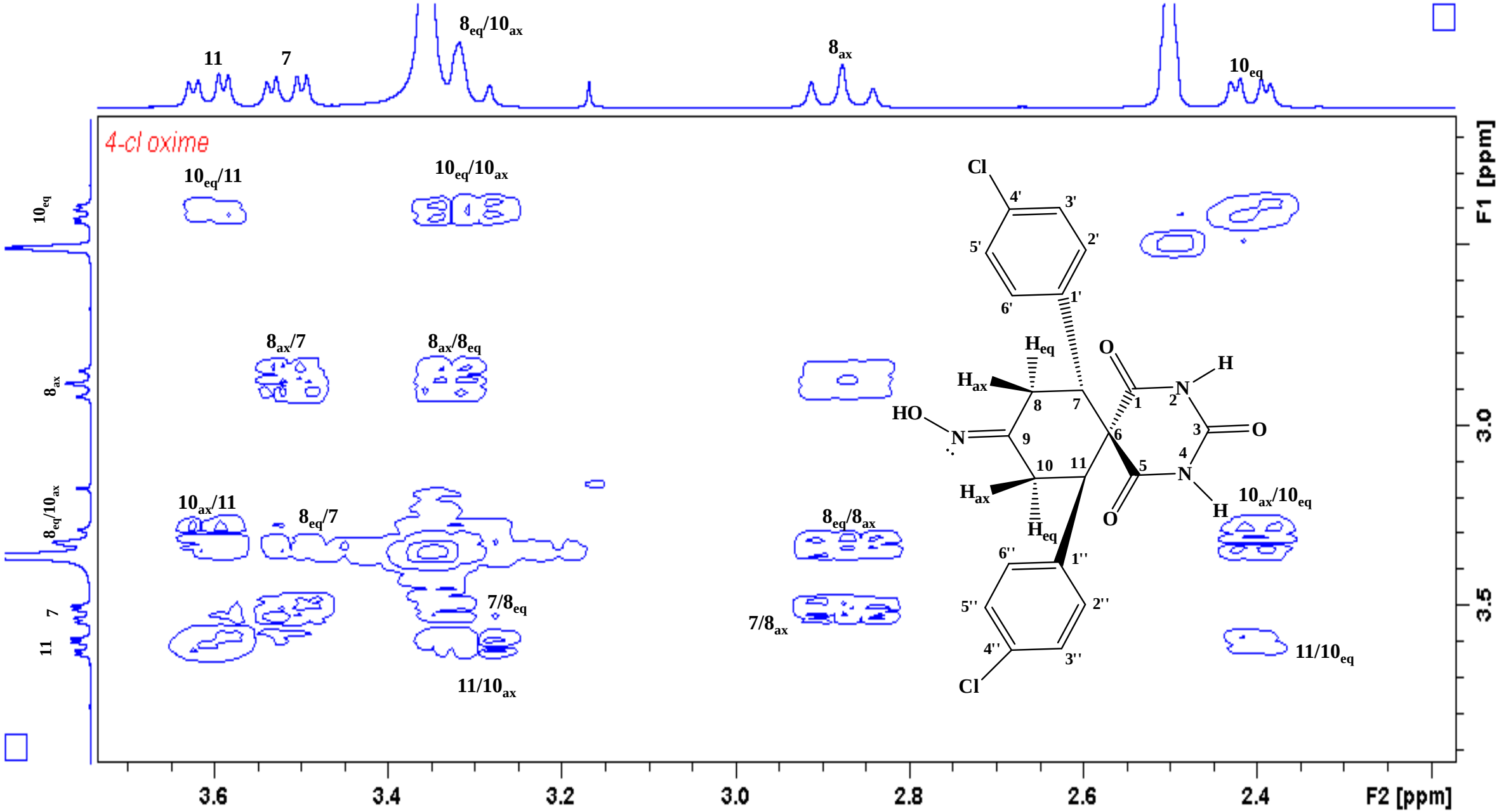
Expanded ^{1}H Spectrum of Compound B-4c: 7,11-bis(4-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



^{13}C Spectrum of Compound B-4c: 7,11-bis(4-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

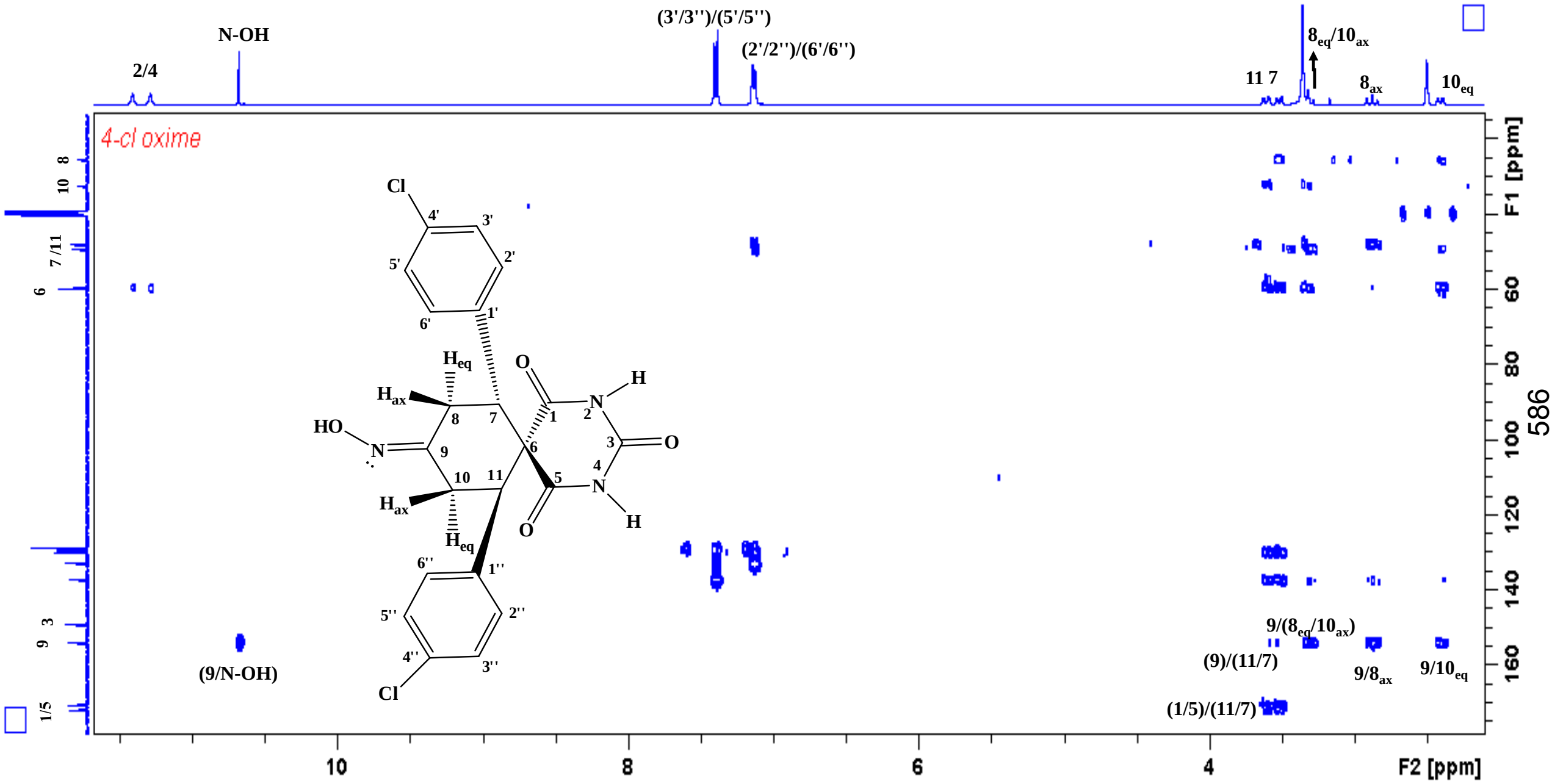


HSQC Spectrum of Compound B-4c: 7,11-bis(4-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

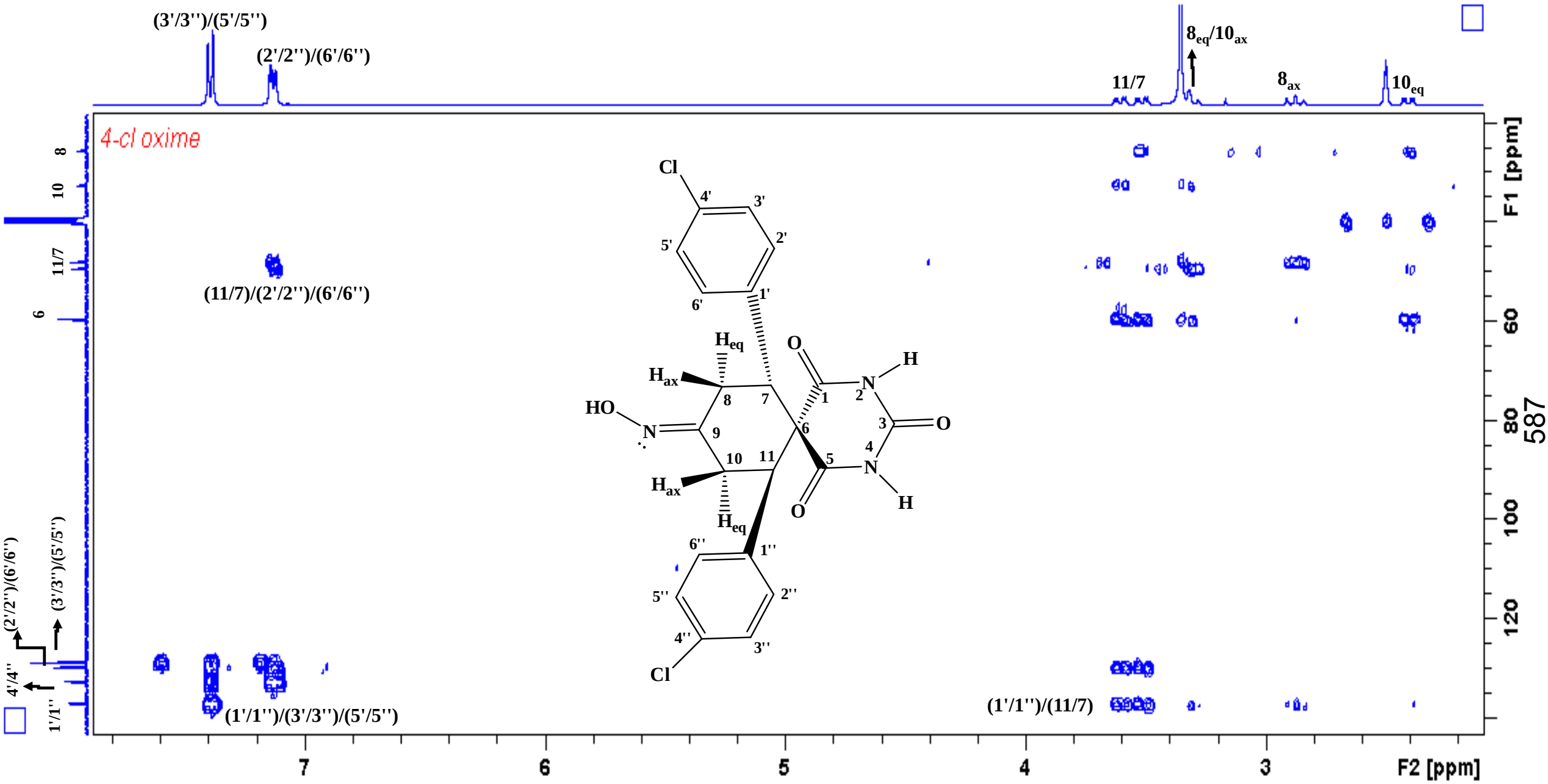


585

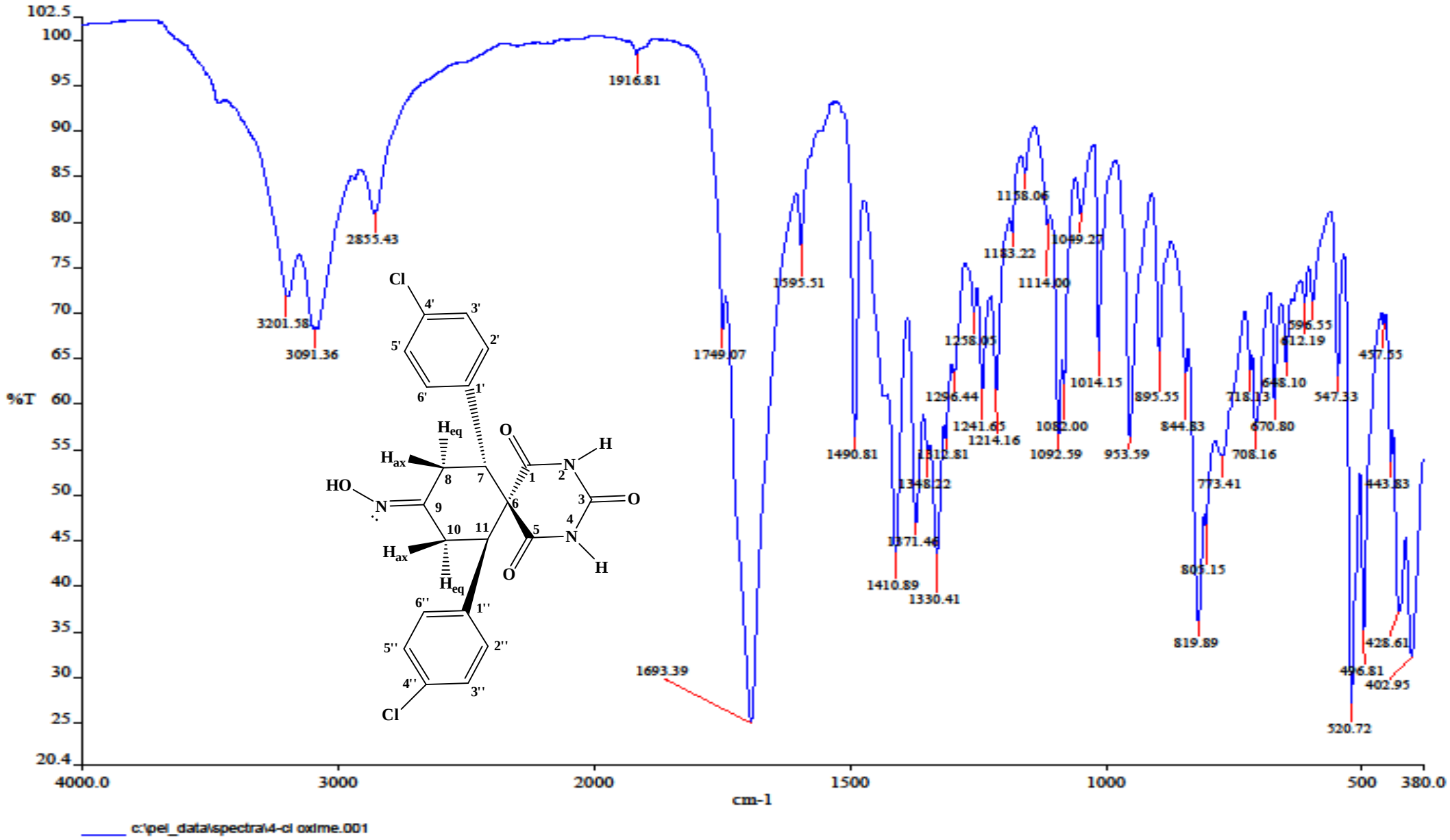
COSY Spectrum of Compound B-4c: 7,11-bis(4-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



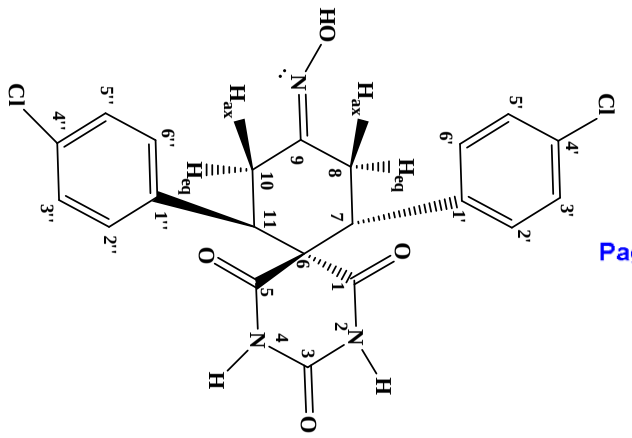
HMBC Spectrum of Compound B-4c: 7,11-bis(4-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Expanded HMBC Spectrum of Compound B-4c: 7,11-bis(4-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Infrared Spectrum of Compound B-4c: 7,11-bis(4-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

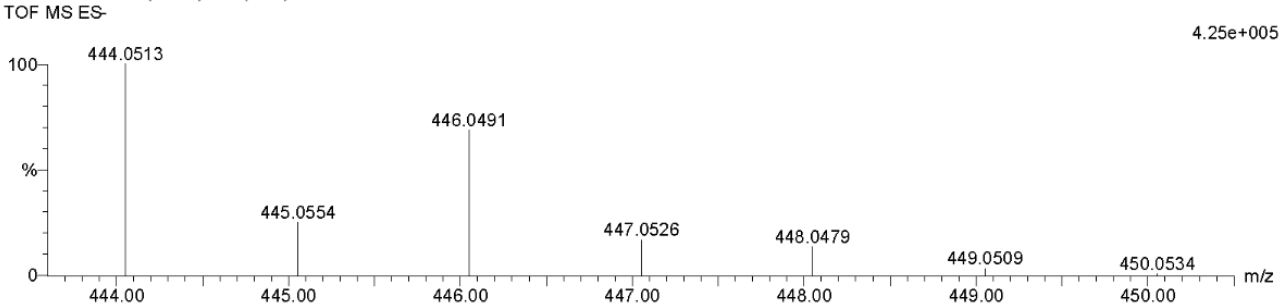


Elemental Composition Report

Single Mass Analysis

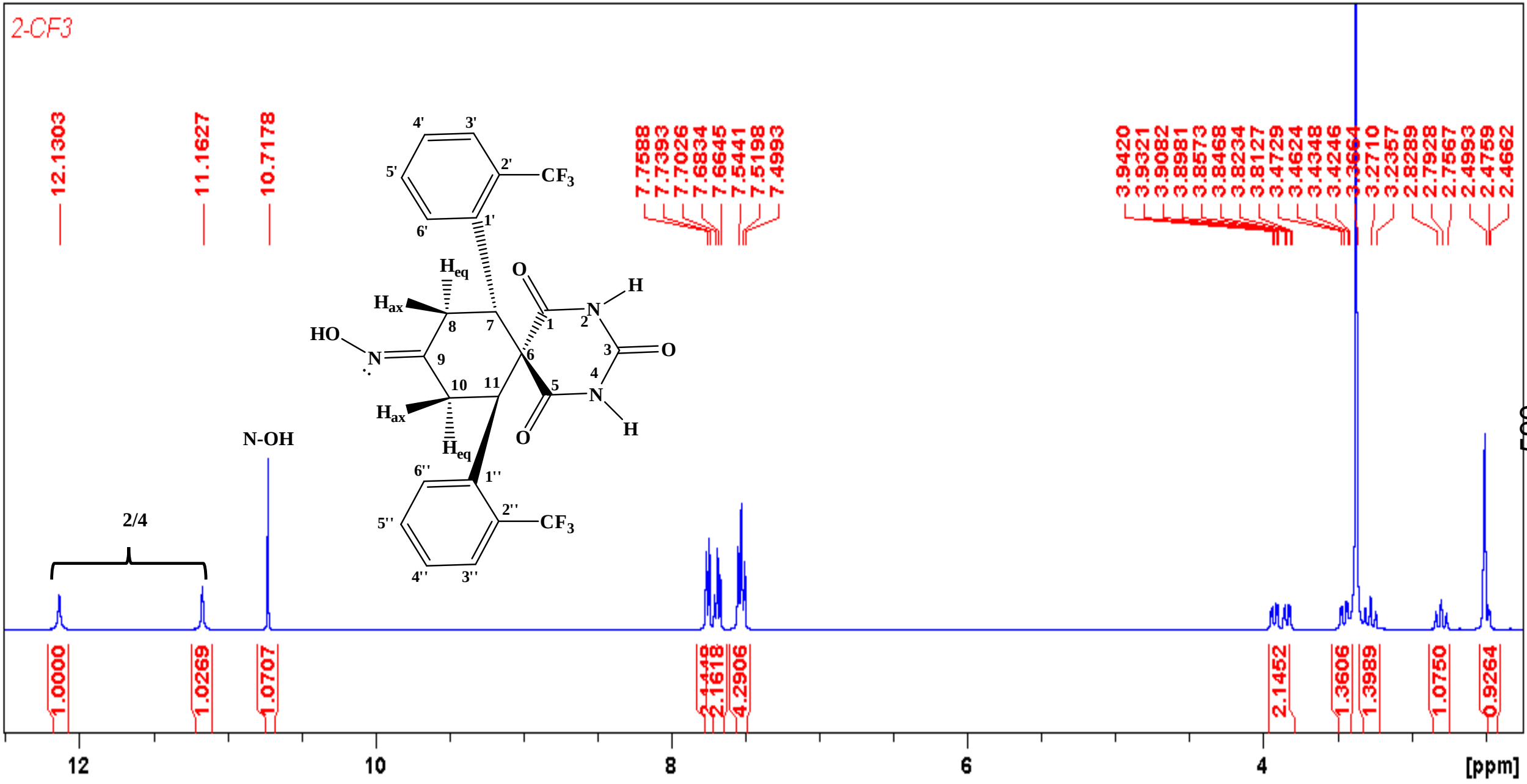
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
46 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 0-5 Cl: 0-2
31 4-Cl oxime 61 (2.024) Cm (1:61)
TOF MS ES-

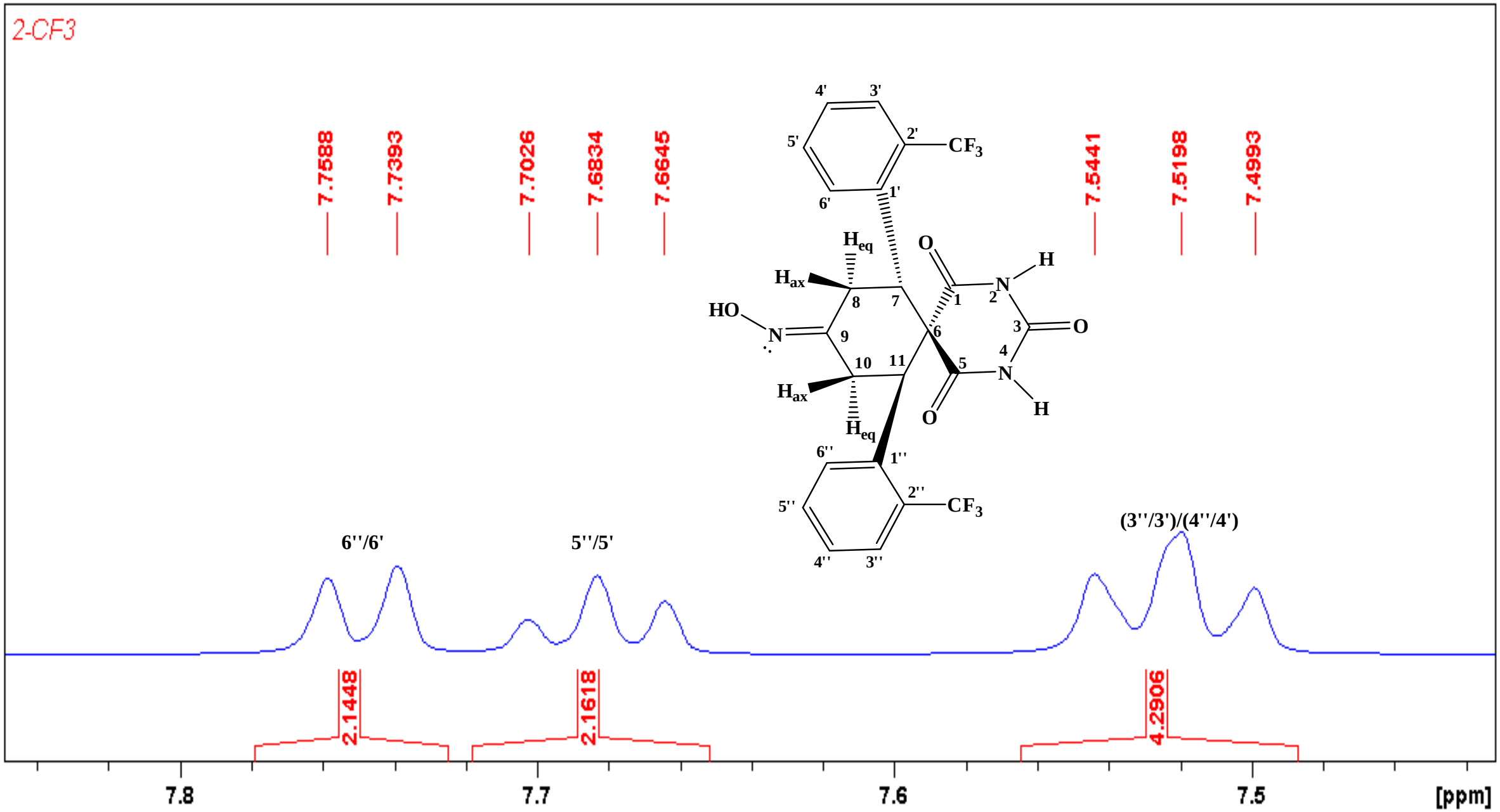


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
444.0513	444.0518	-0.5	-1.1	14.5	10.0	0.0	C21 H16 N3 O4 Cl2

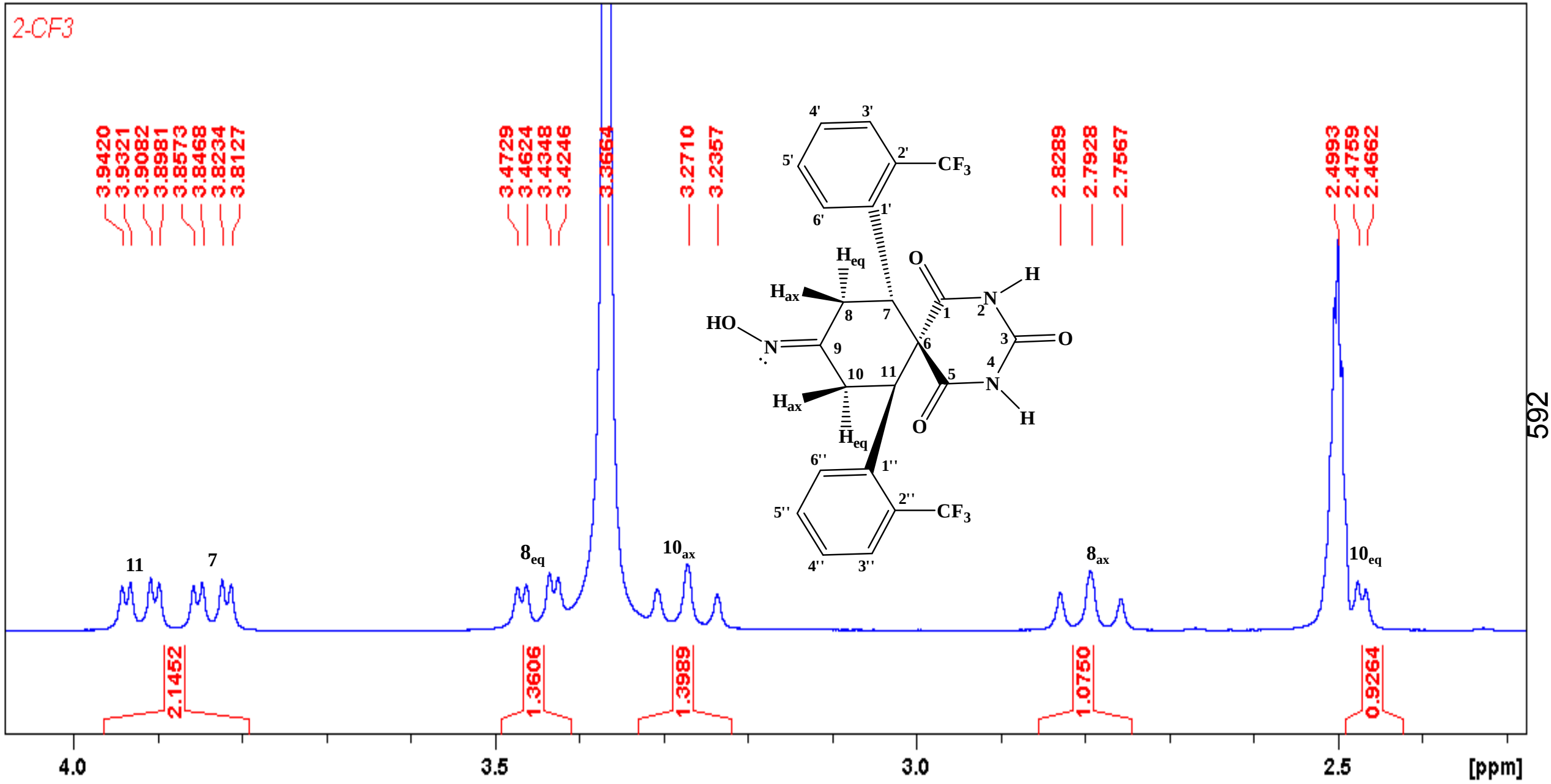
HRMS Spectrum of Compound B-4c: 7,11-bis(4-chlorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



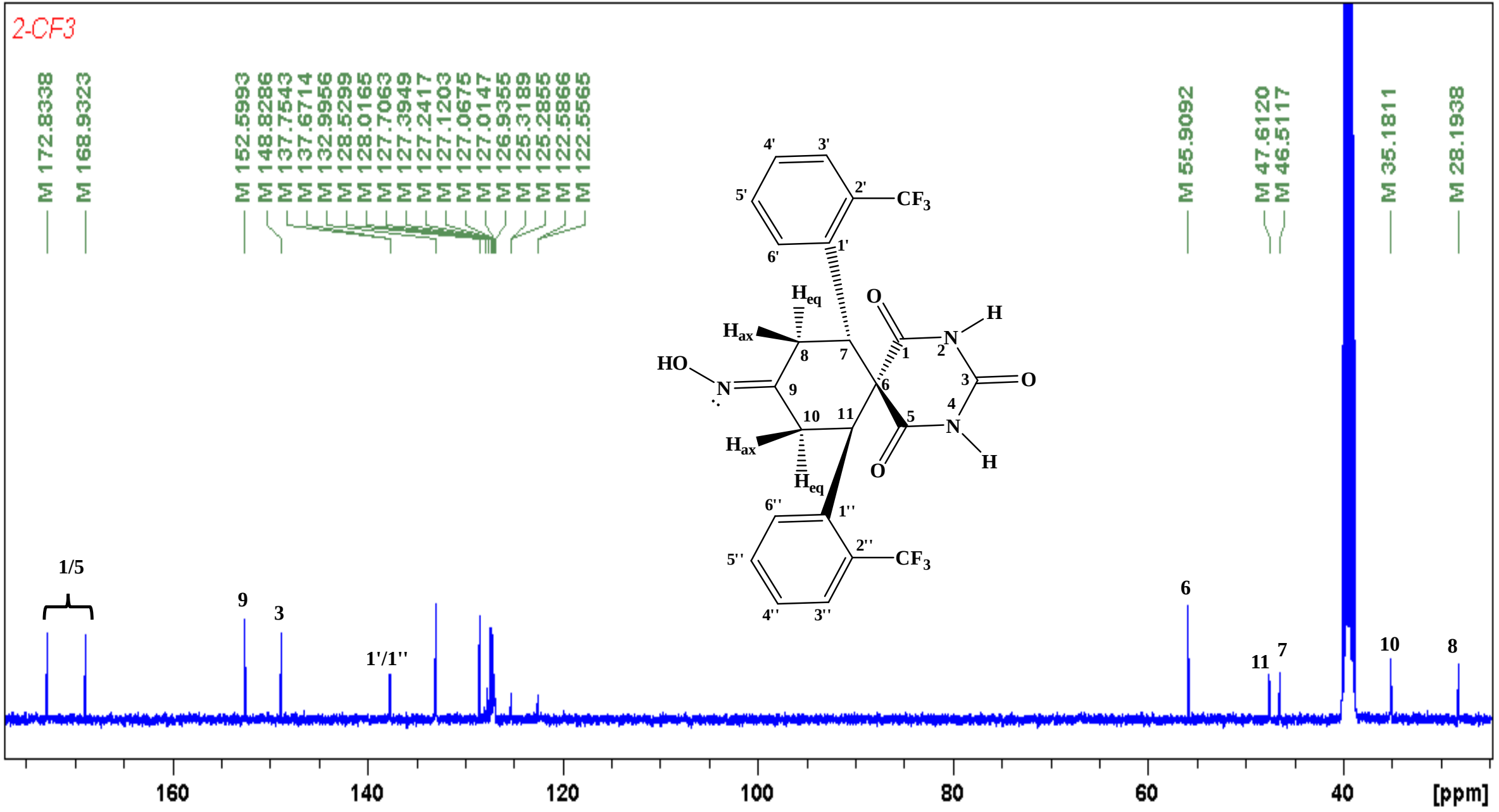
^{1}H Spectrum of Compound B-4d: 9-(hydroxyimino)-7,11-bis(2-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



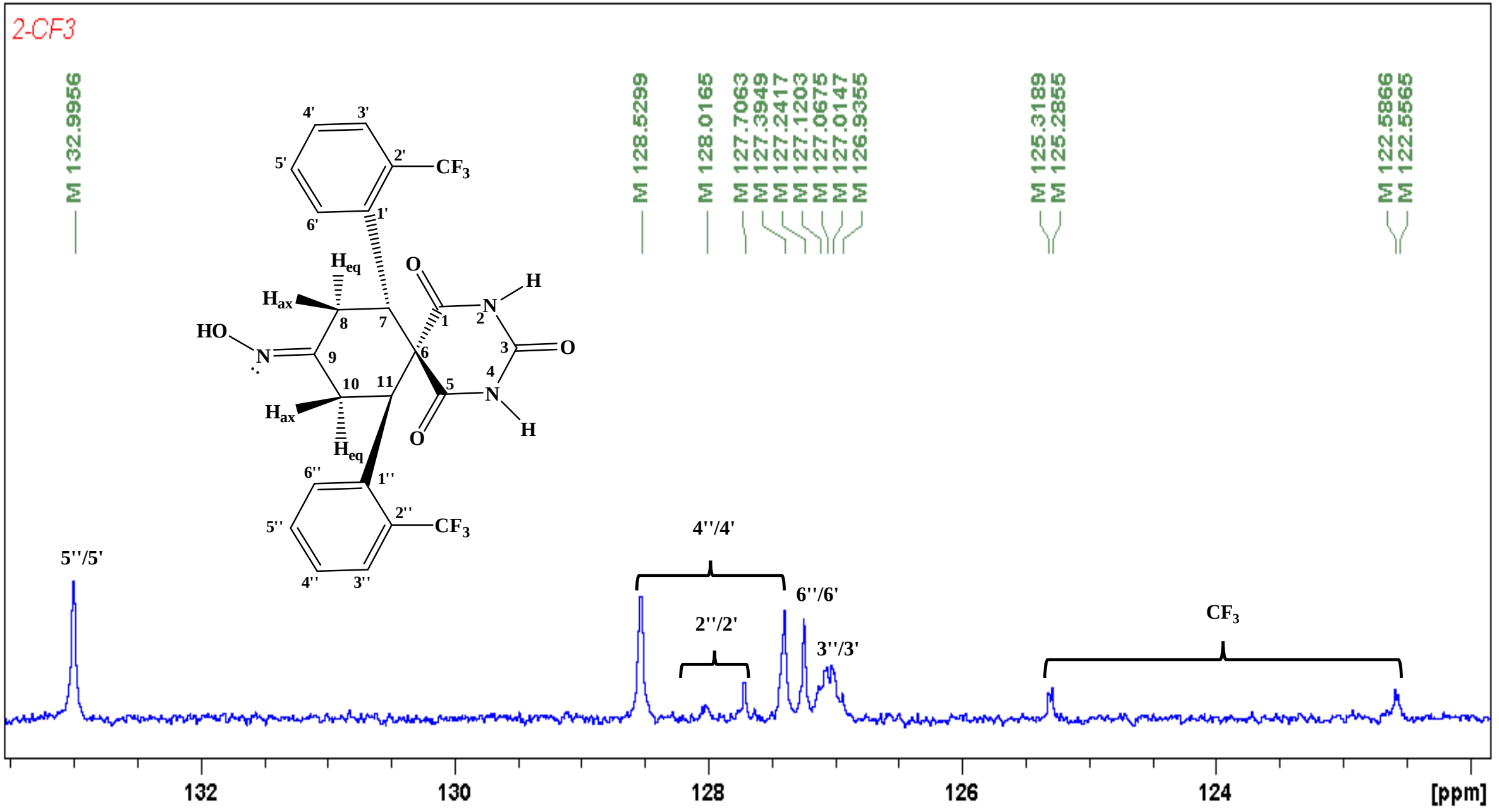
Expanded ^{1}H Spectrum of Compound B-4d: 9-(hydroxyimino)-7,11-bis(2-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



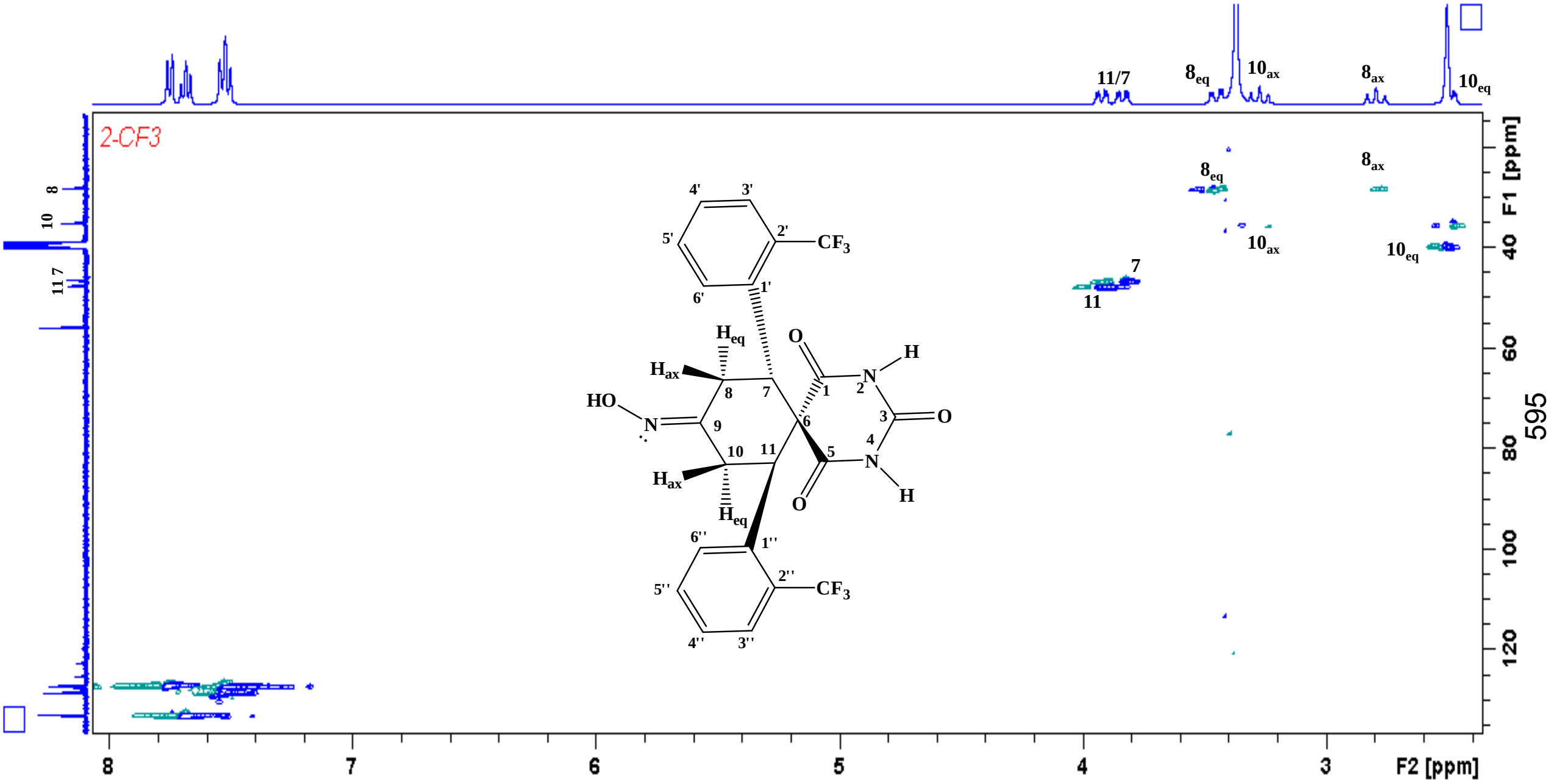
Expanded ^{1}H Spectrum of Compound B-4d: 9-(hydroxyimino)-7,11-bis(2-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



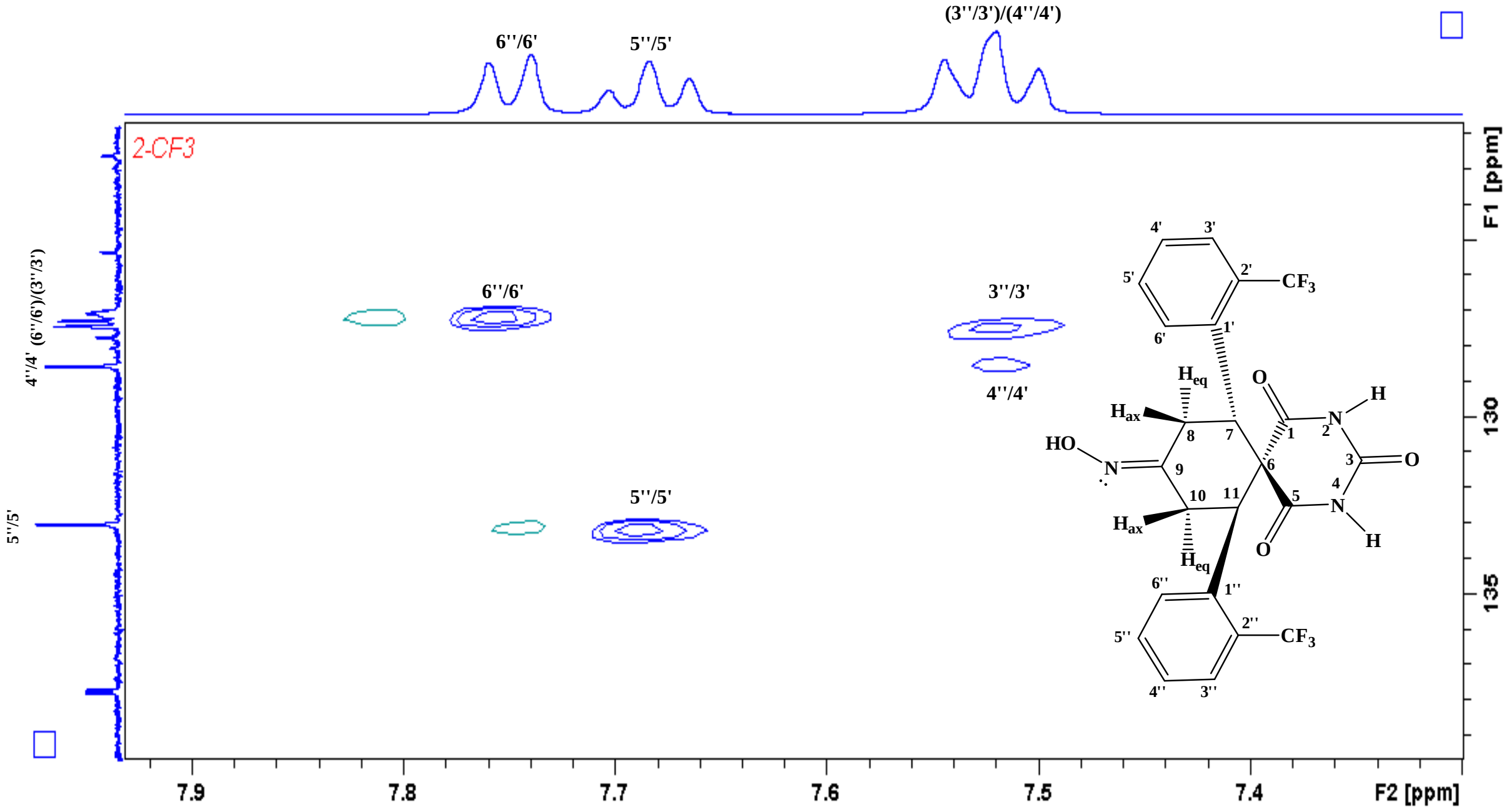
^{13}C Spectrum of Compound B-4d: 9-(hydroxyimino)-7,11-bis(2-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



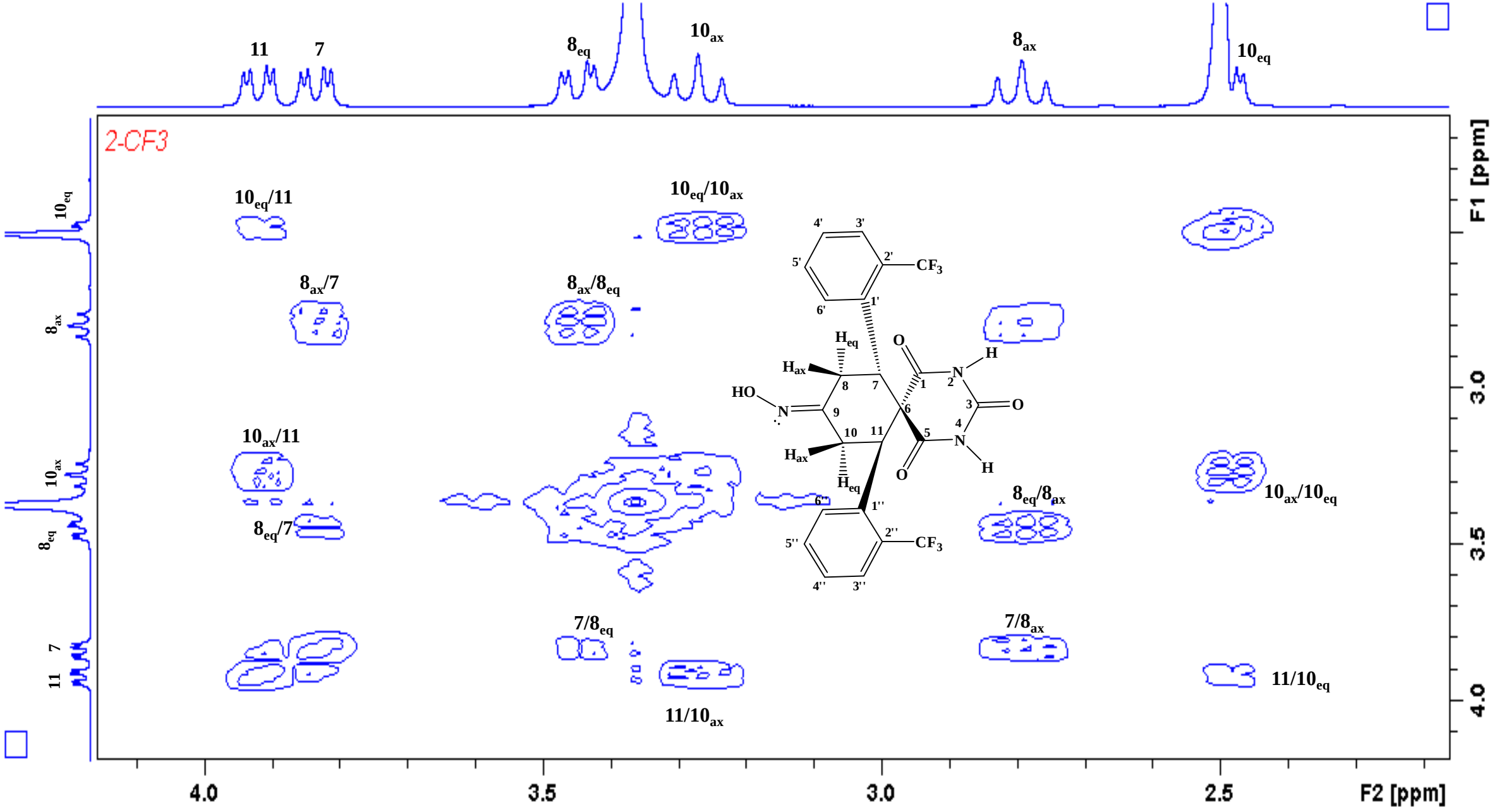
Expanded ^{13}C Spectrum of Compound B-4d: 9-(hydroxyimino)-7,11-bis(2-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



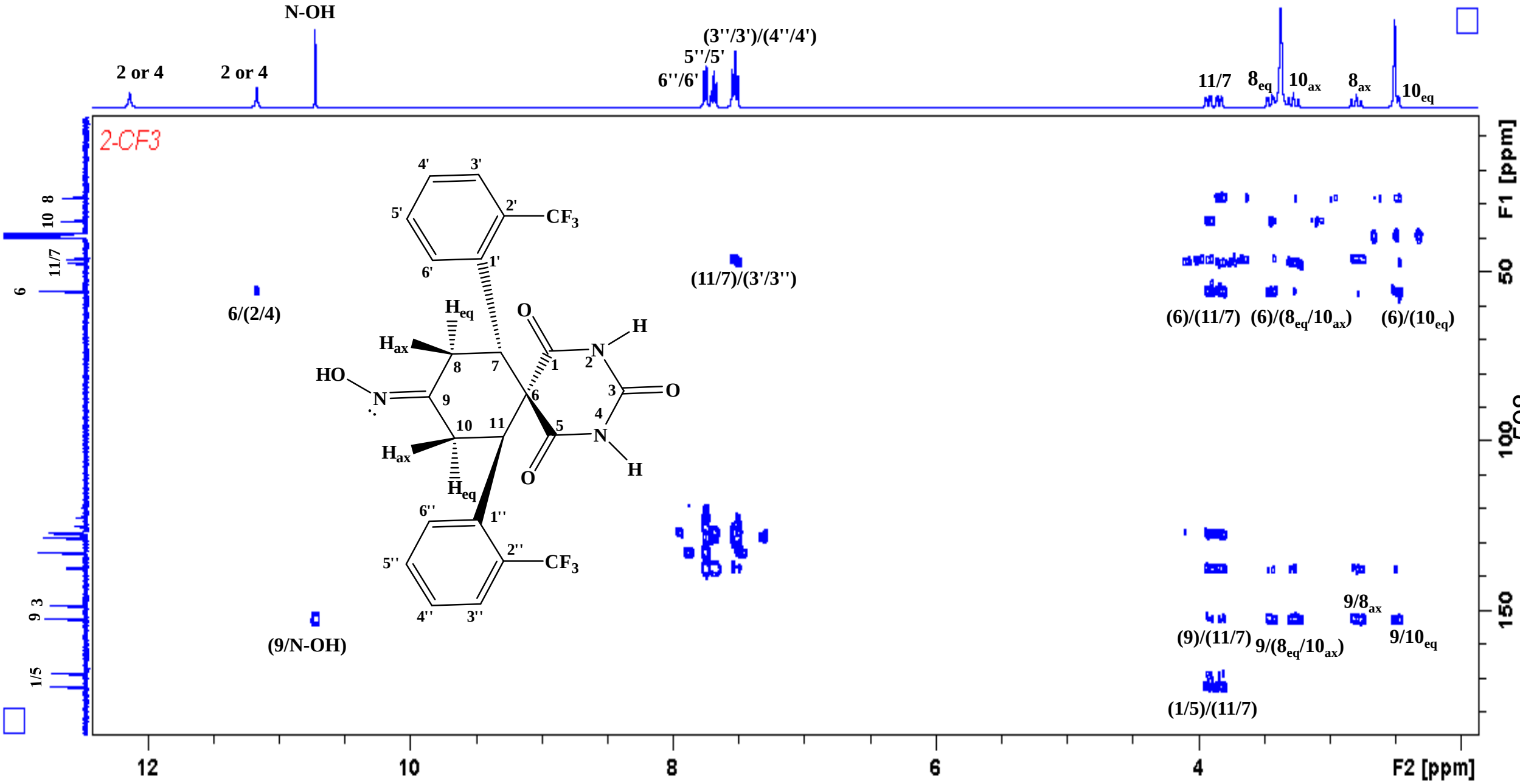
HSQC Spectrum of Compound B-4d: 9-(hydroxyimino)-7,11-bis(2-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



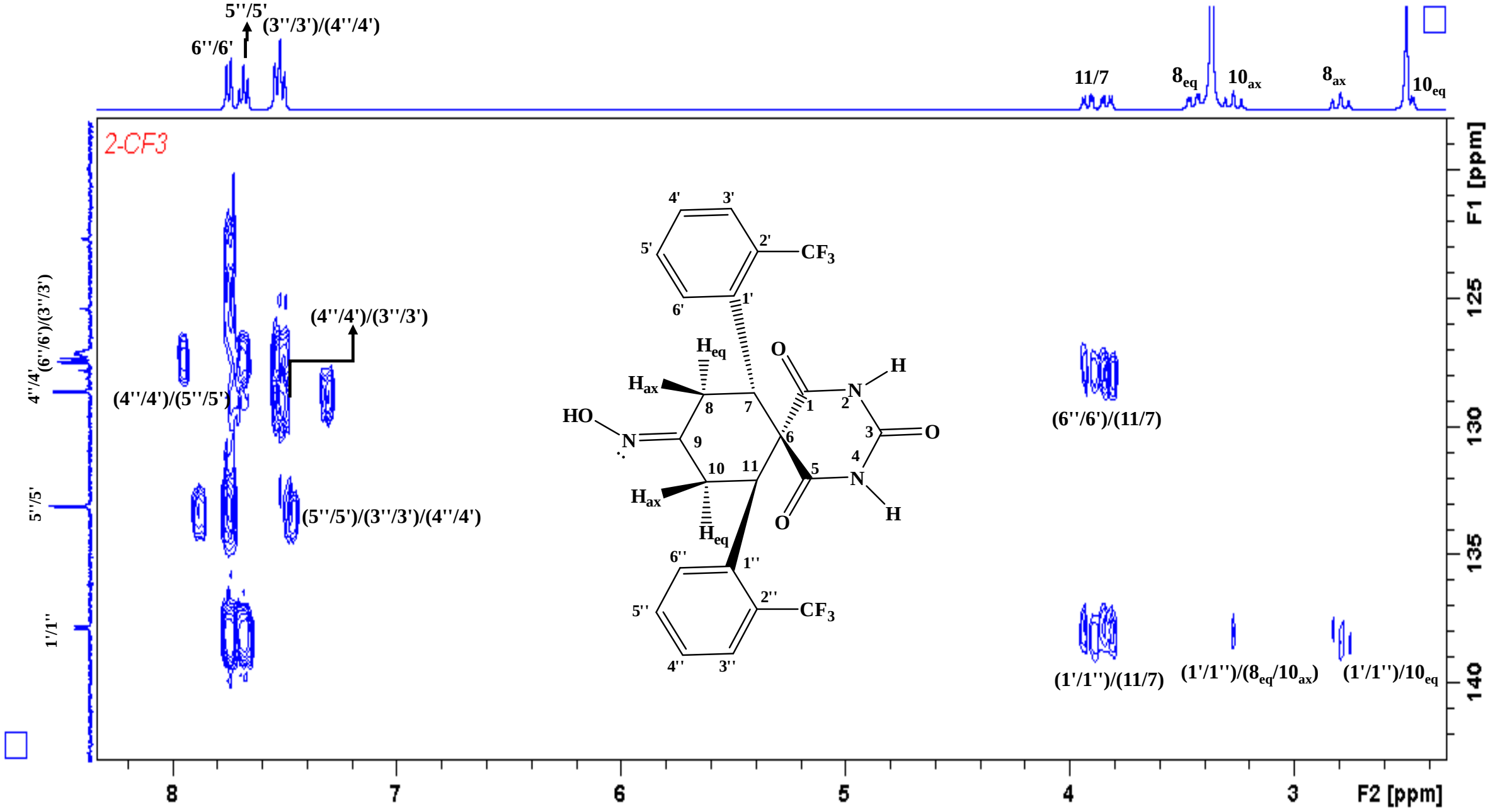
Expanded HSQC Spectrum of Compound B-4d: 9-(hydroxyimino)-7,11-bis(2-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



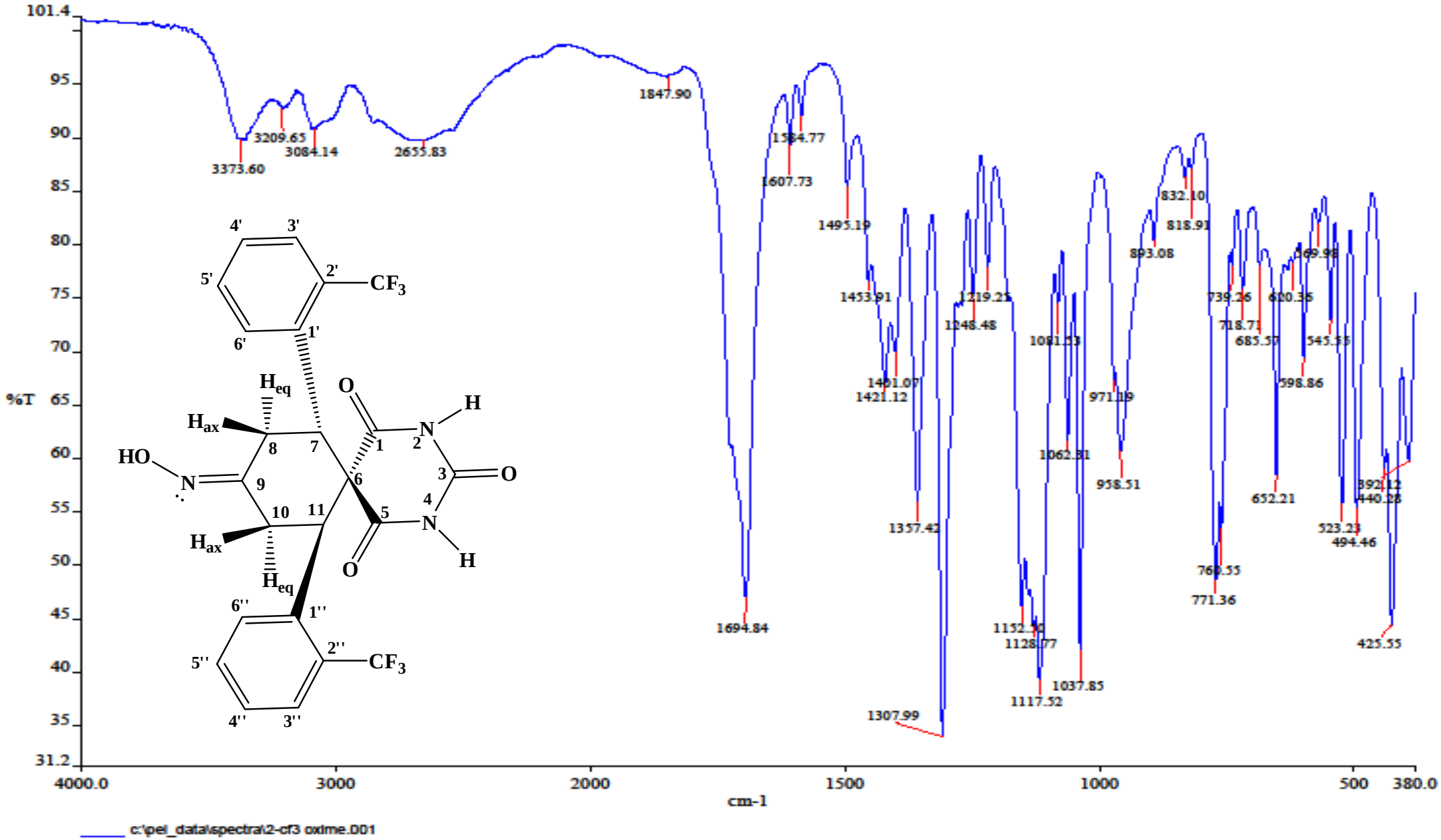
COSY Spectrum of Compound B-4d: 9-(hydroxyimino)-7,11-bis(2-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



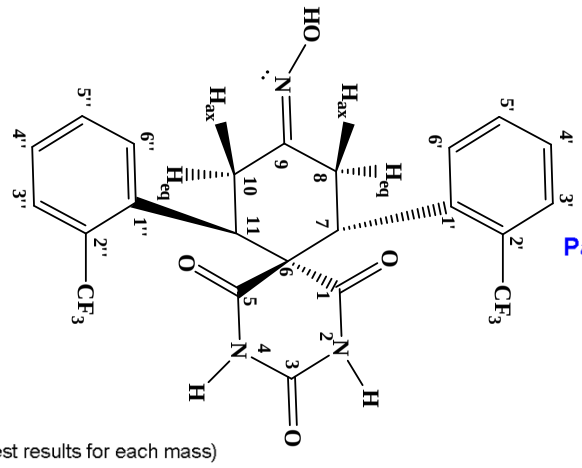
HMBC Spectrum of Compound B-4d: 9-(hydroxyimino)-7,11-bis(2-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Expanded HMBC Spectrum of Compound B-4d: 9-(hydroxyimino)-7,11-bis(2-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



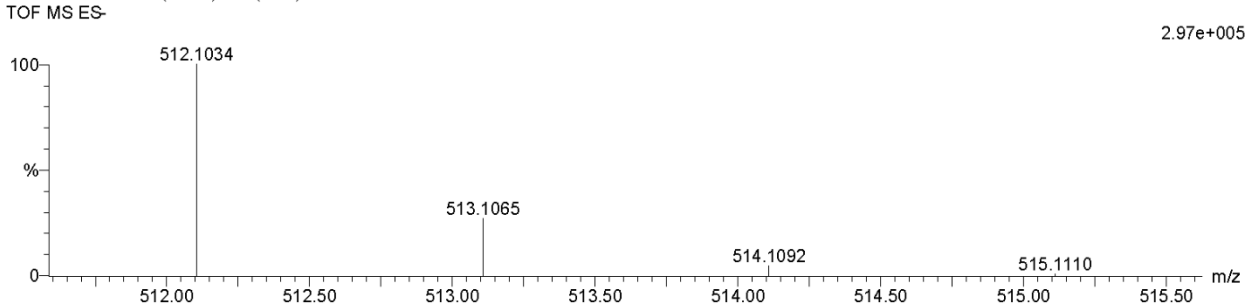
Infrared Spectrum of Compound B-4d: 9-(hydroxyimino)-7,11-bis(2-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Elemental Composition Report **Page 1**

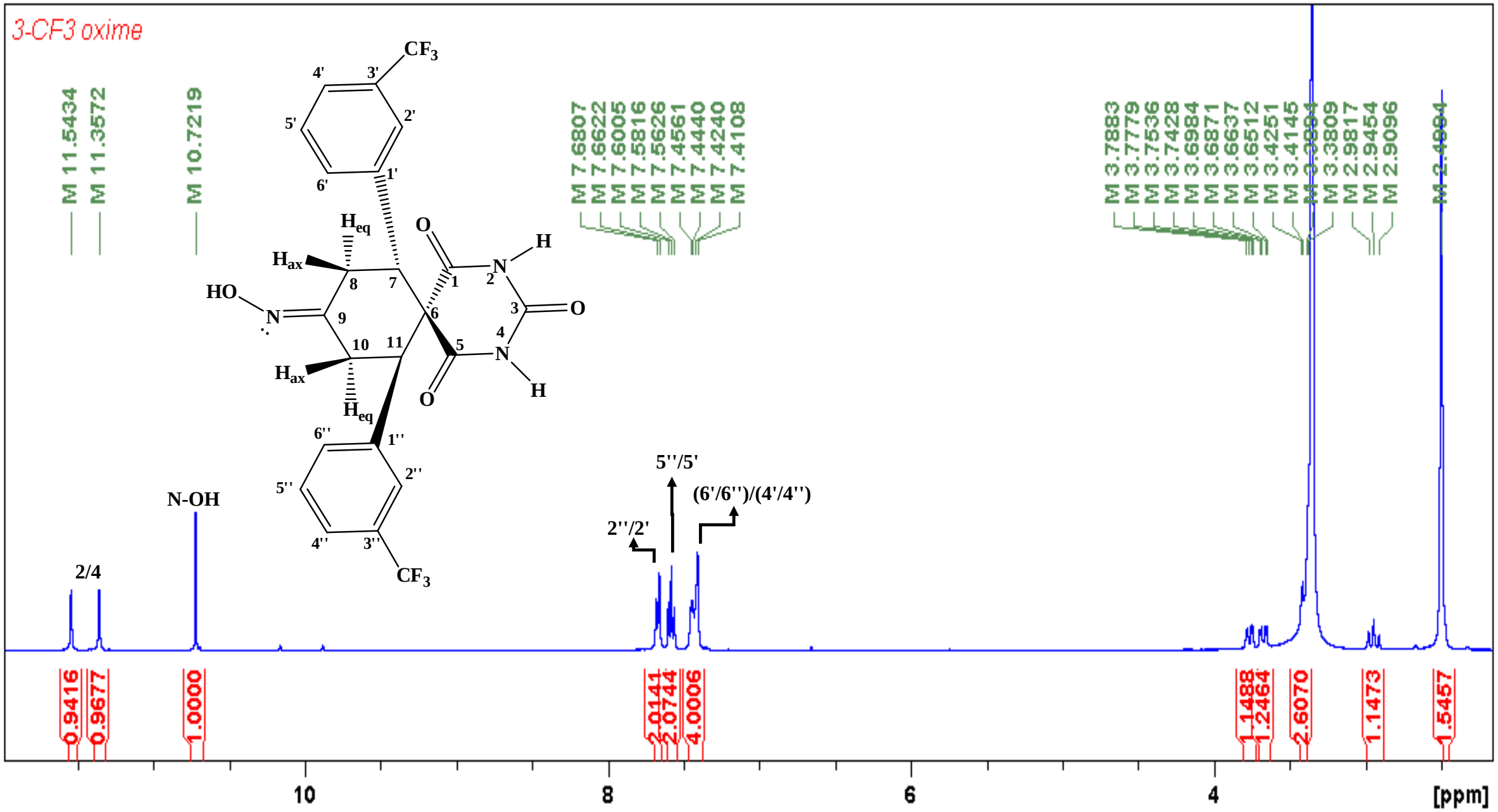
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
157 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 0-5 F: 0-6
32 2-CF3 oxime 59 (1.990) Cm (1:60)
TOF MS ES-



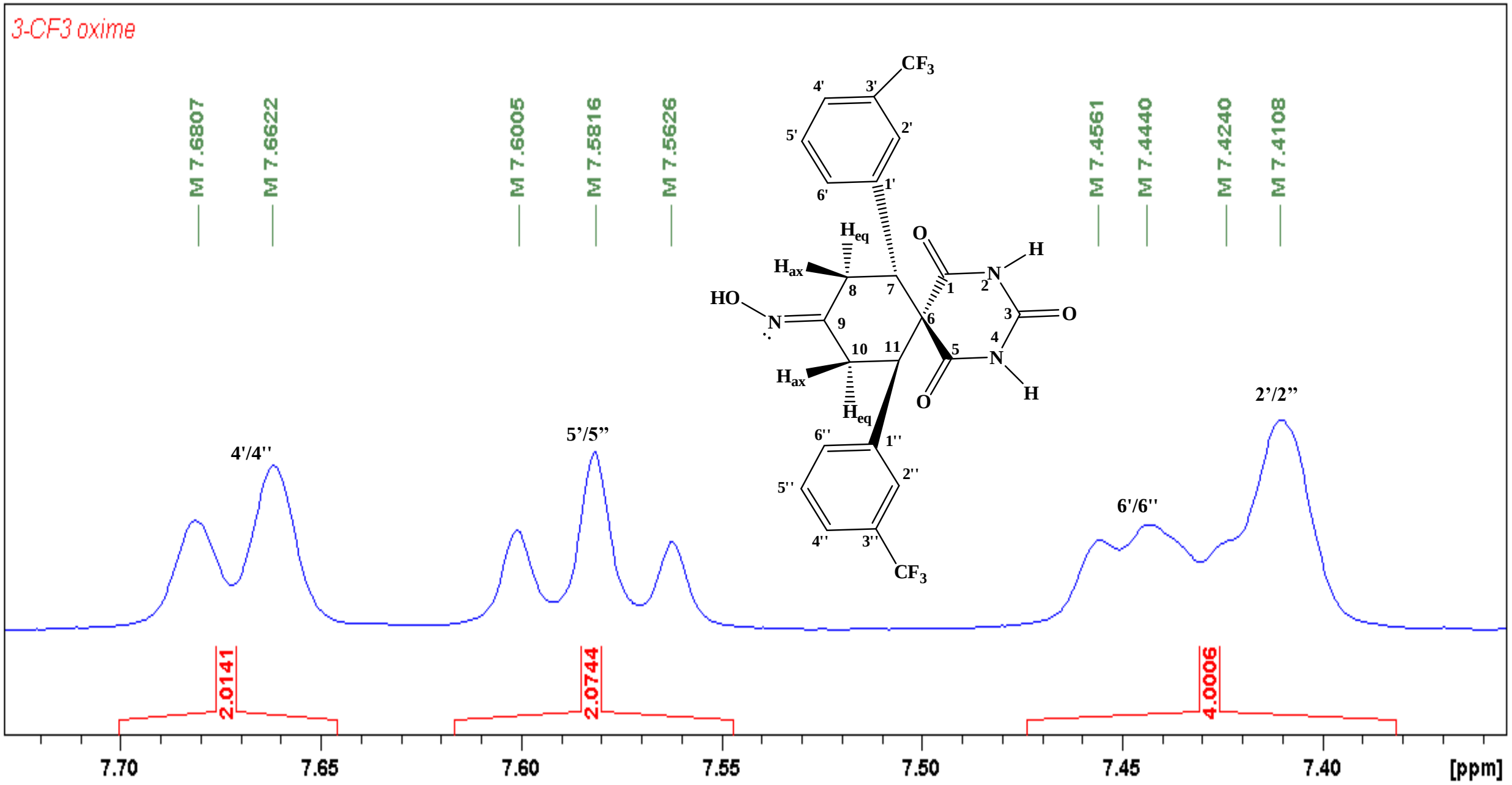
Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
512.1034	512.1045	-1.1	-2.1	14.5	10.1	0.0	C23 H16 N3 O4 F6

HRMS Spectrum of Compound B-4d: 9-(hydroxyimino)-7,11-bis(2-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

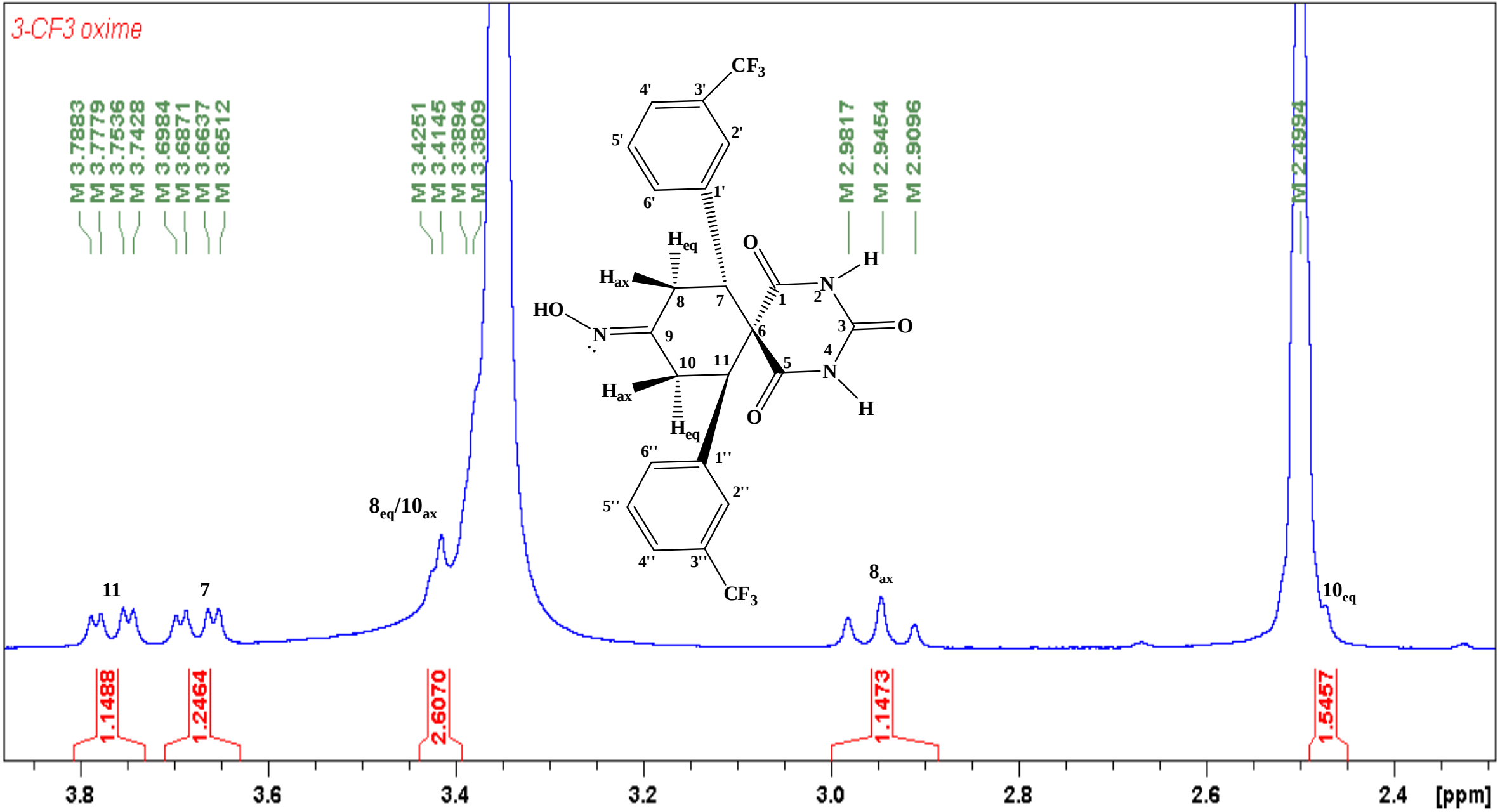


602

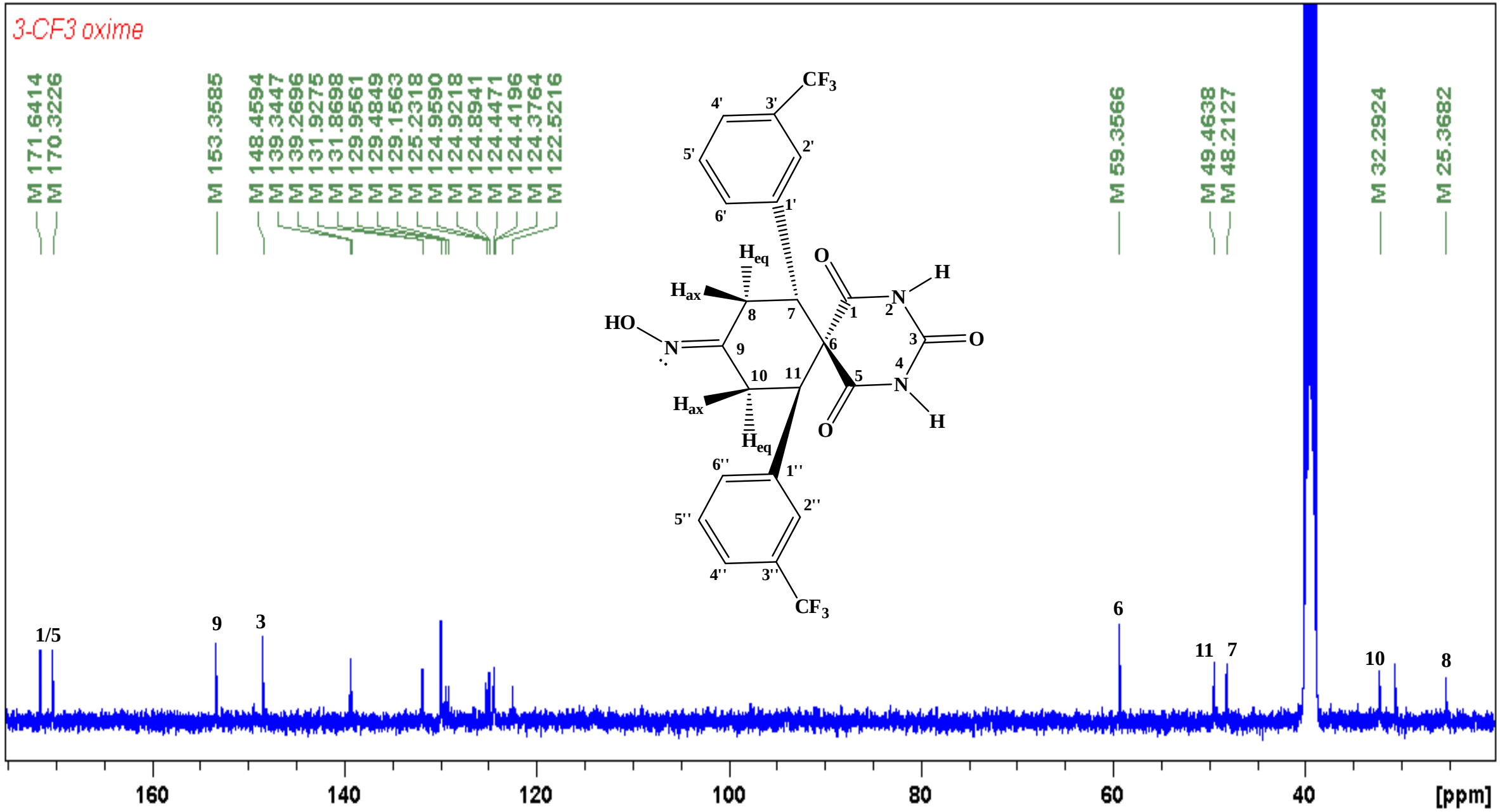
[1]H Spectrum of Compound B-4e: 9-(hydroxyimino)-7,11-bis(3-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Expanded ^{1}H Spectrum of Compound B-4e: 9-(hydroxyimino)-7,11-bis(3-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

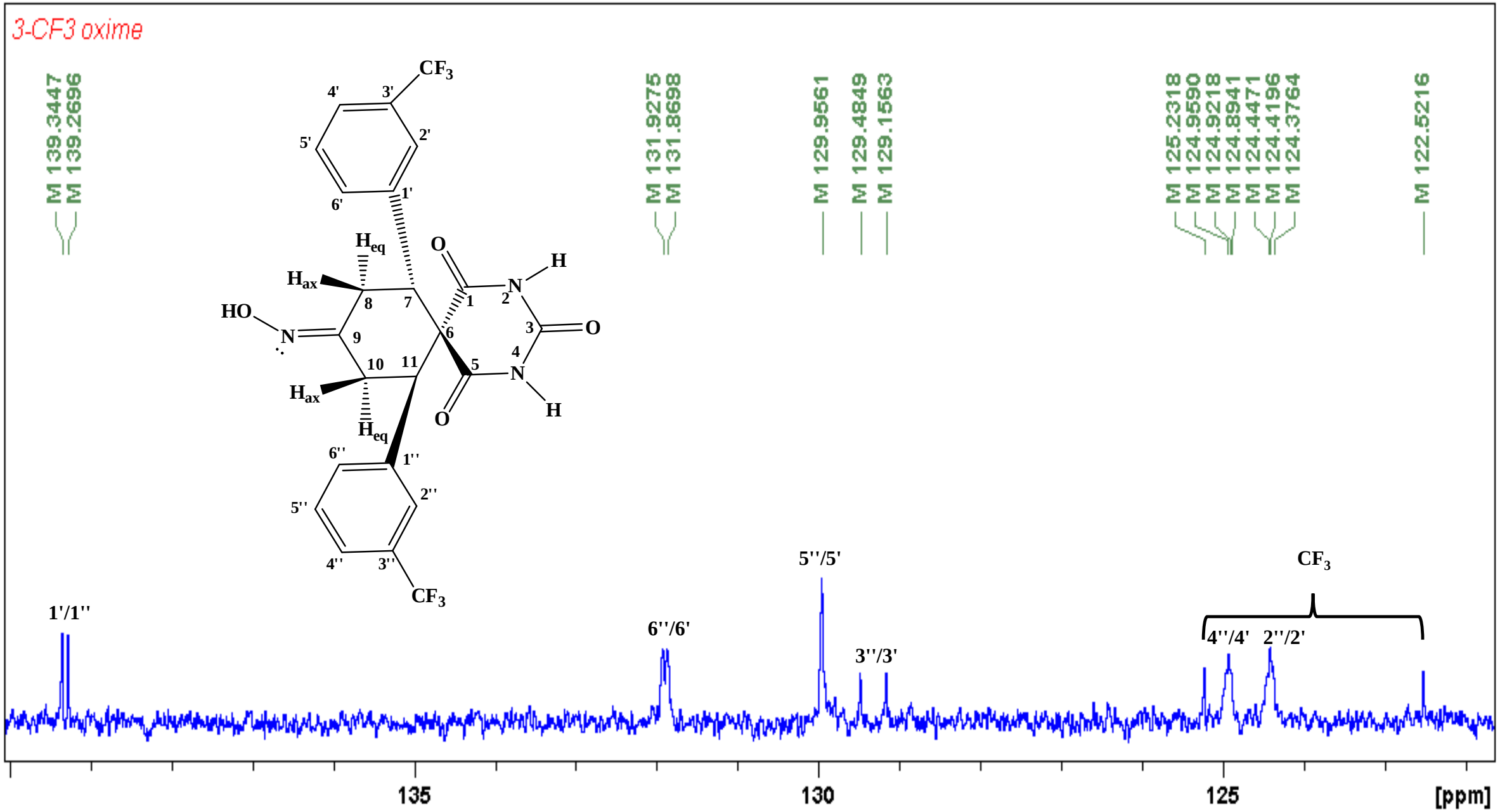


Expanded ^{1}H Spectrum of Compound B-4e: 9-(hydroxyimino)-7,11-bis(3-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



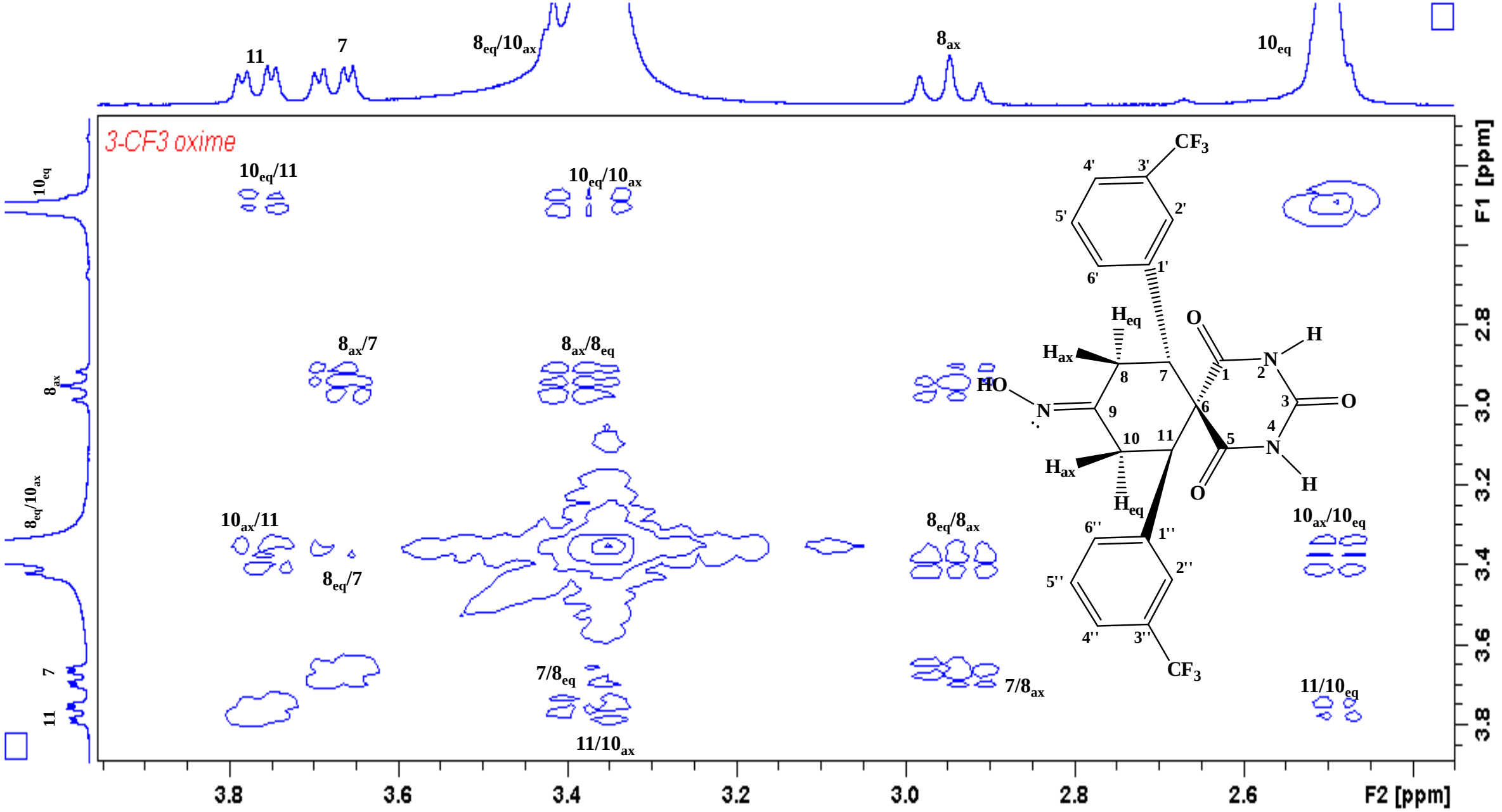
605

^{13}C Spectrum of Compound B-4e: 9-(hydroxyimino)-7,11-bis(3-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

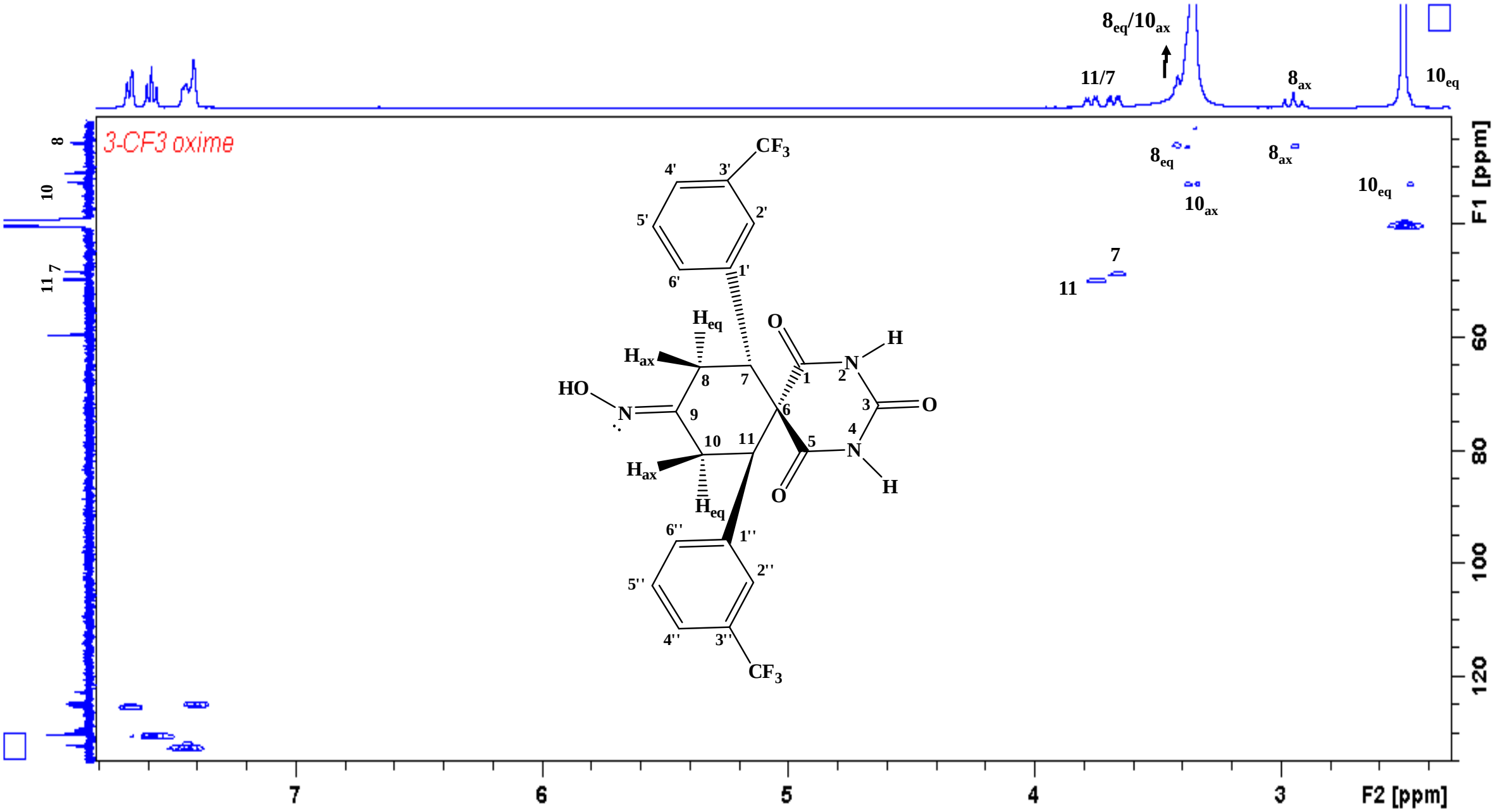


606

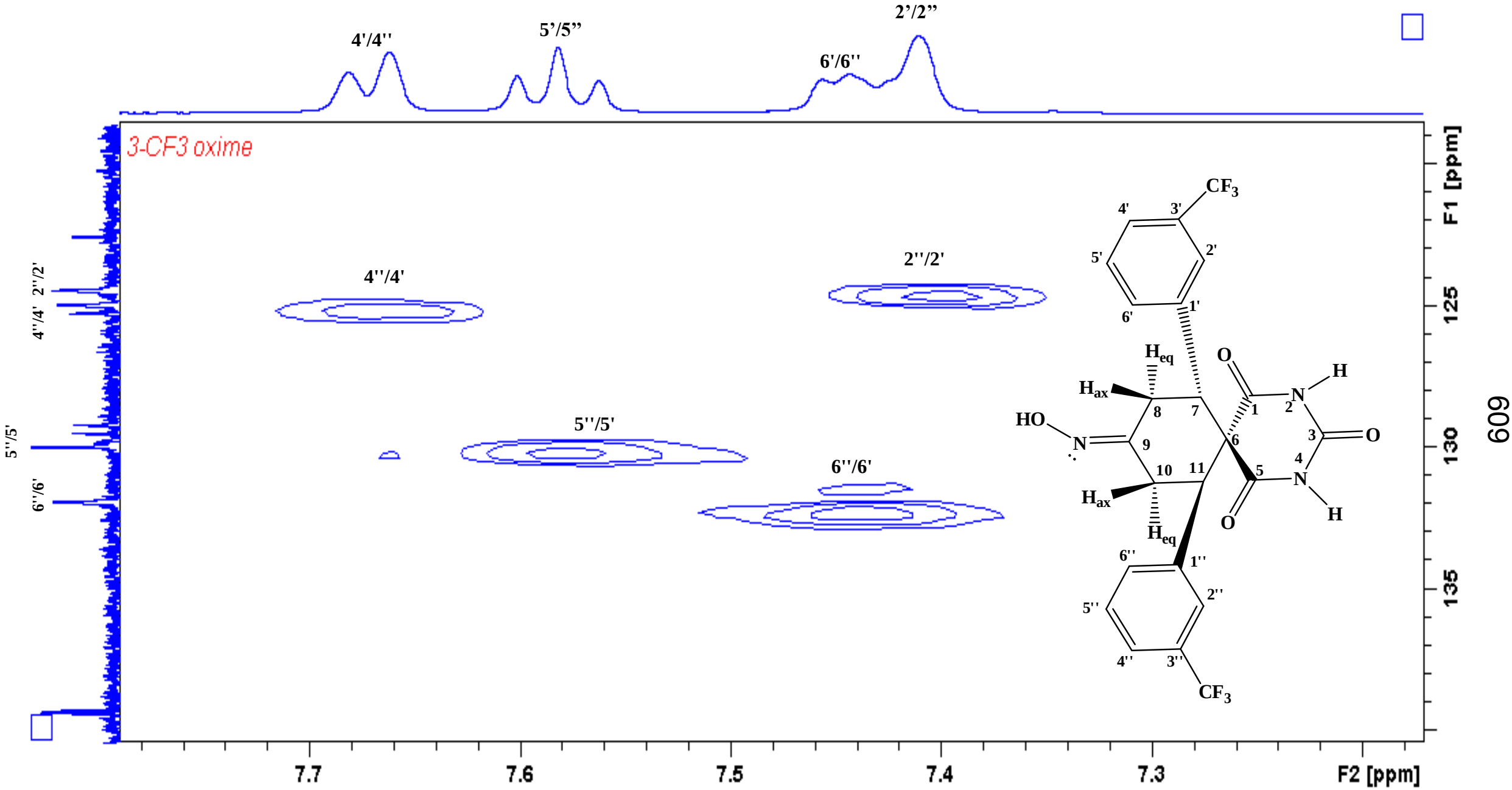
Expanded ^{13}C Spectrum of Compound B-4e: 9-(hydroxyimino)-7,11-bis(3-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



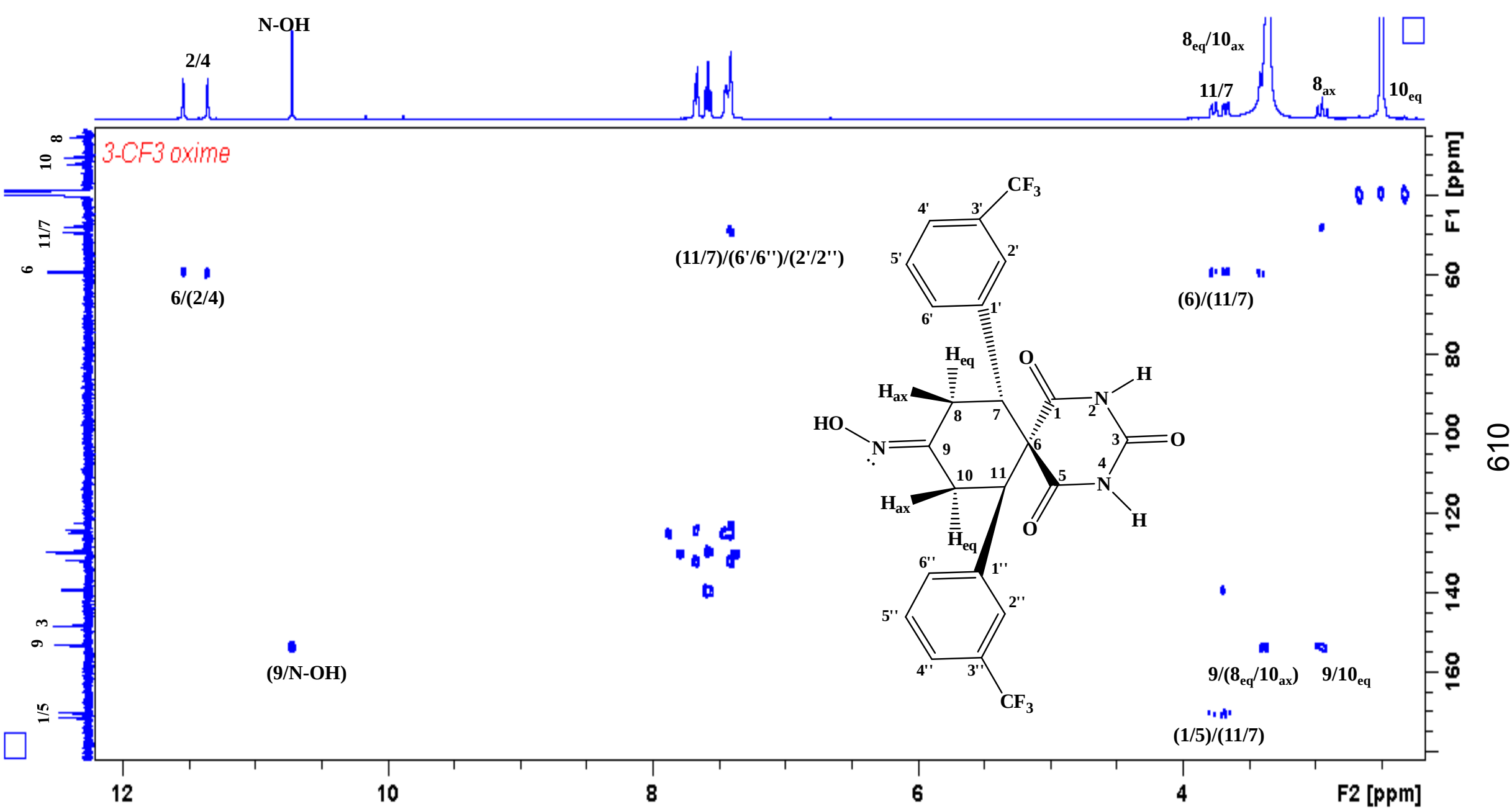
COSY Spectrum of Compound B-4e: 9-(hydroxyimino)-7,11-bis(3-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



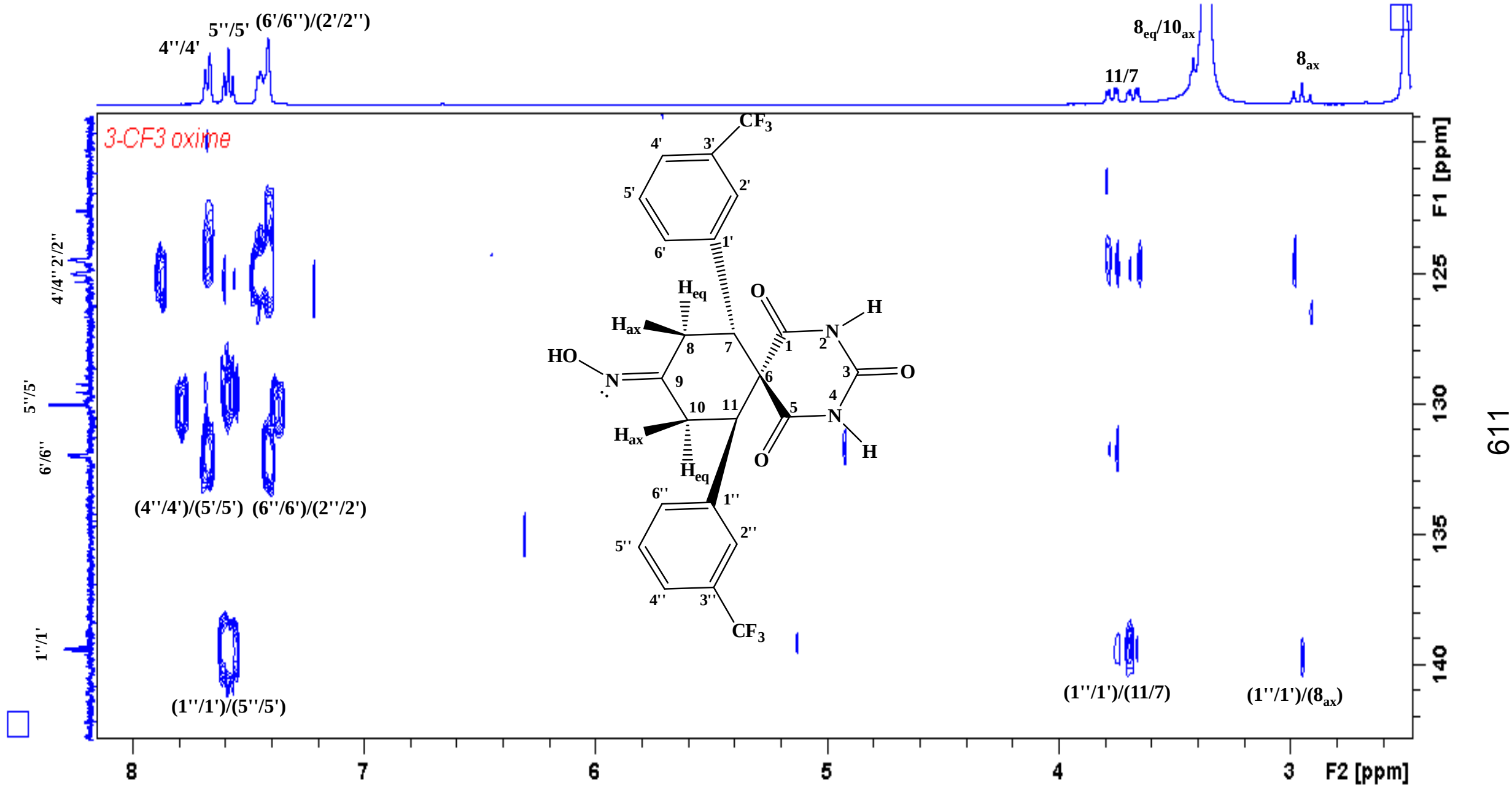
HSCQ Spectrum of Compound B-4e: 9-(hydroxyimino)-7,11-bis(3-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



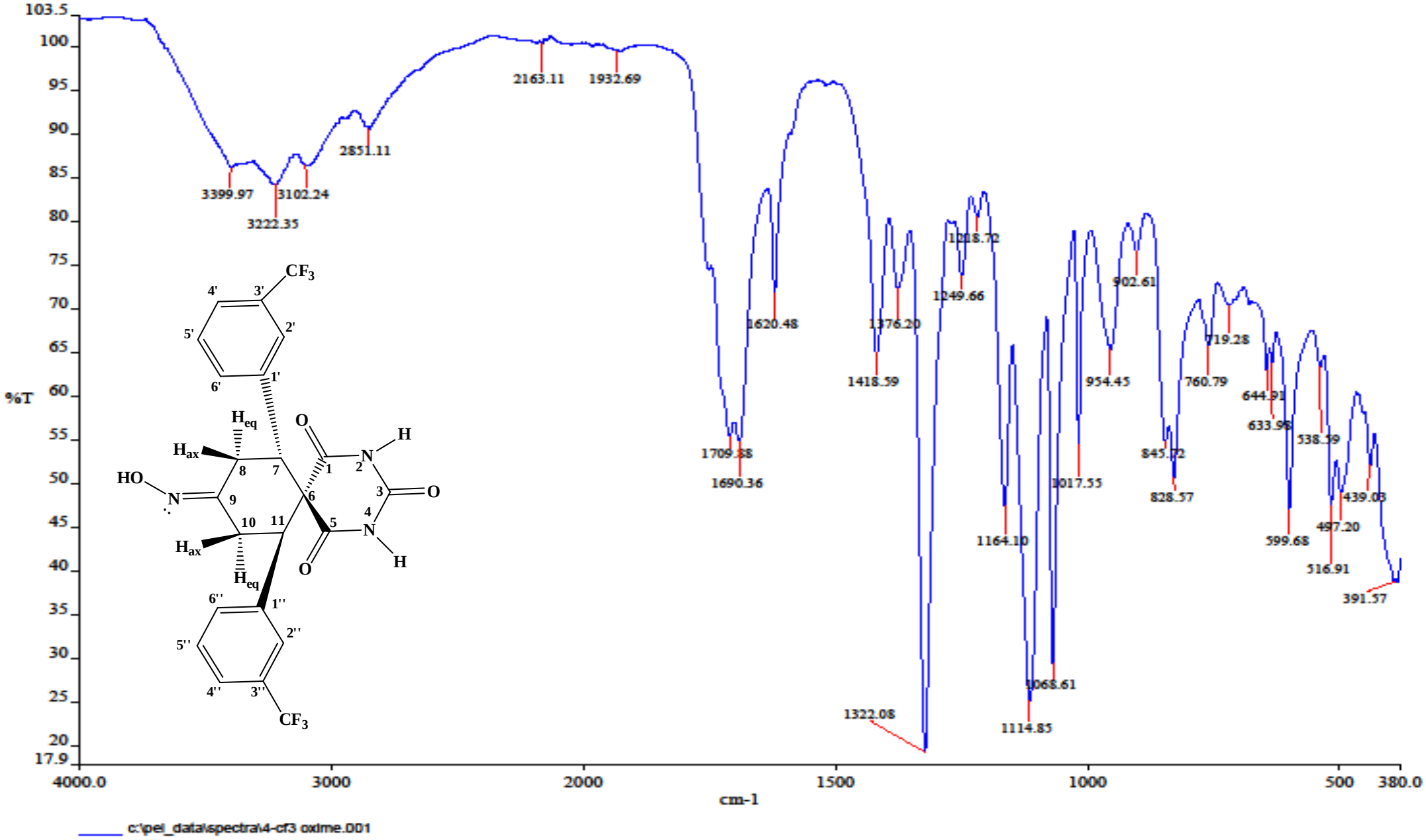
Expanded HSQC Spectrum of Compound B-4e: 9-(hydroxyimino)-7,11-bis(3-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



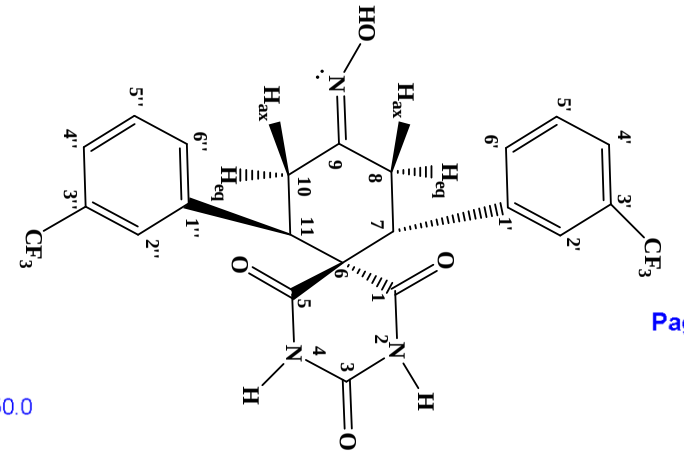
HMBC Spectrum of Compound B-4e: 9-(hydroxyimino)-7,11-bis(3-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Expanded HMBC Spectrum of Compound B-4e: 9-(hydroxyimino)-7,11-bis(3-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



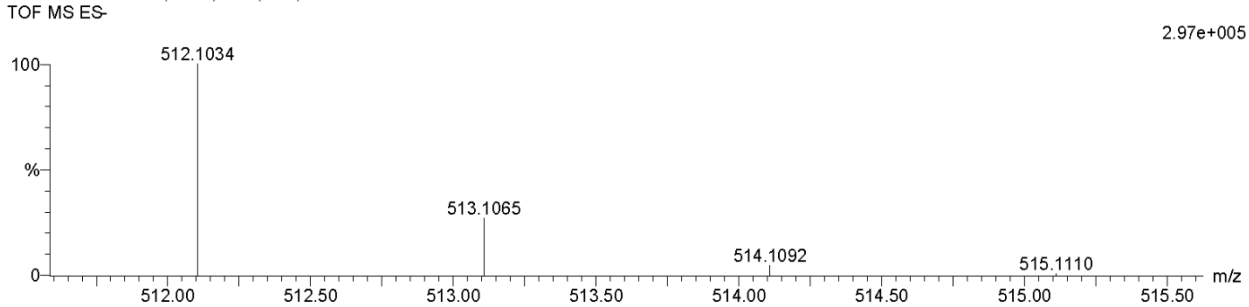
Infrared Spectrum of Compound B-4e: 9-(hydroxyimino)-7,11-bis(3-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Elemental Composition Report **Page 1**

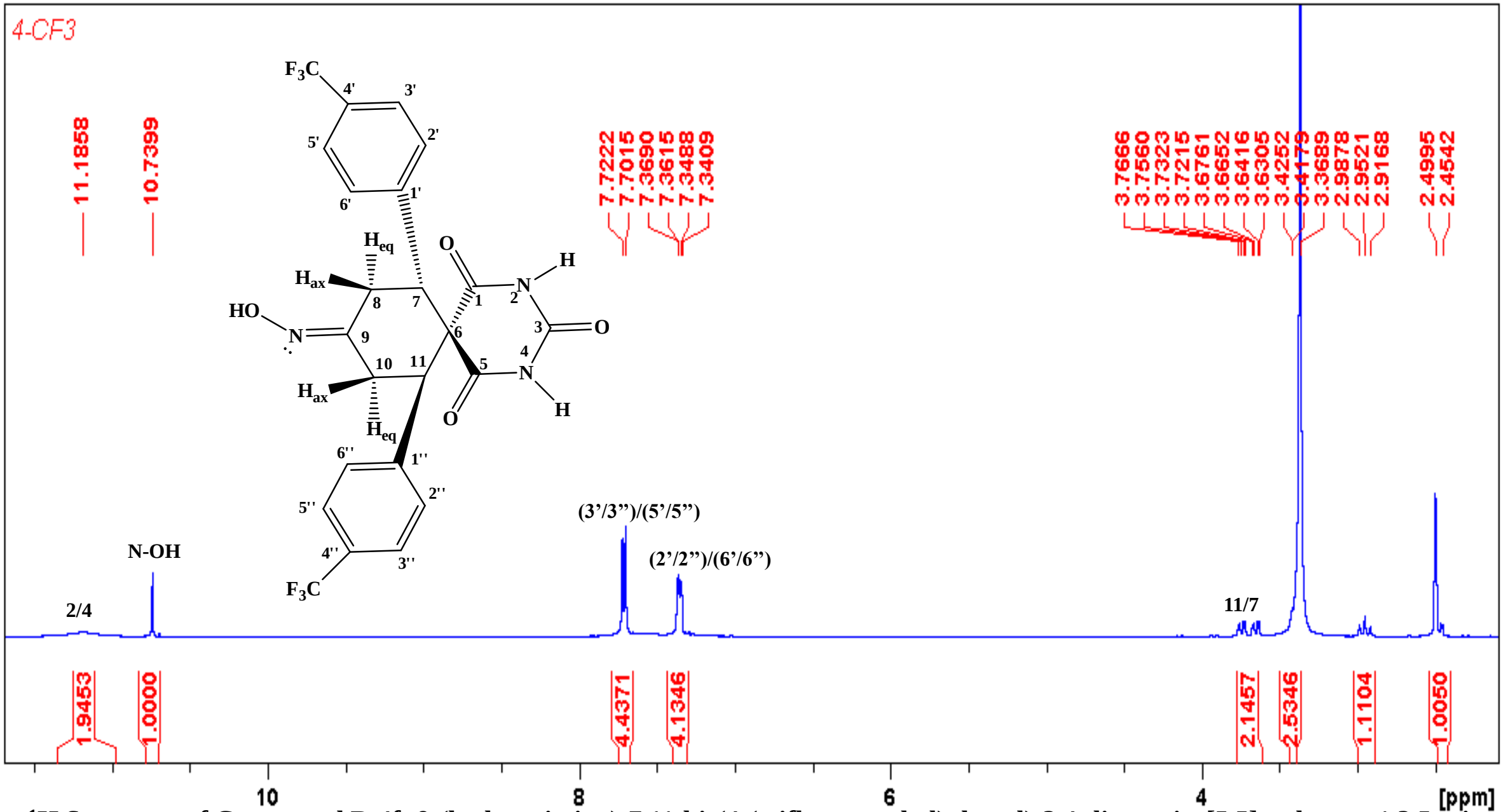
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
157 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 0-5 F: 0-6
32 2-CF3 oxime 59 (1.990) Cm (1:60)
TOF MS ES-

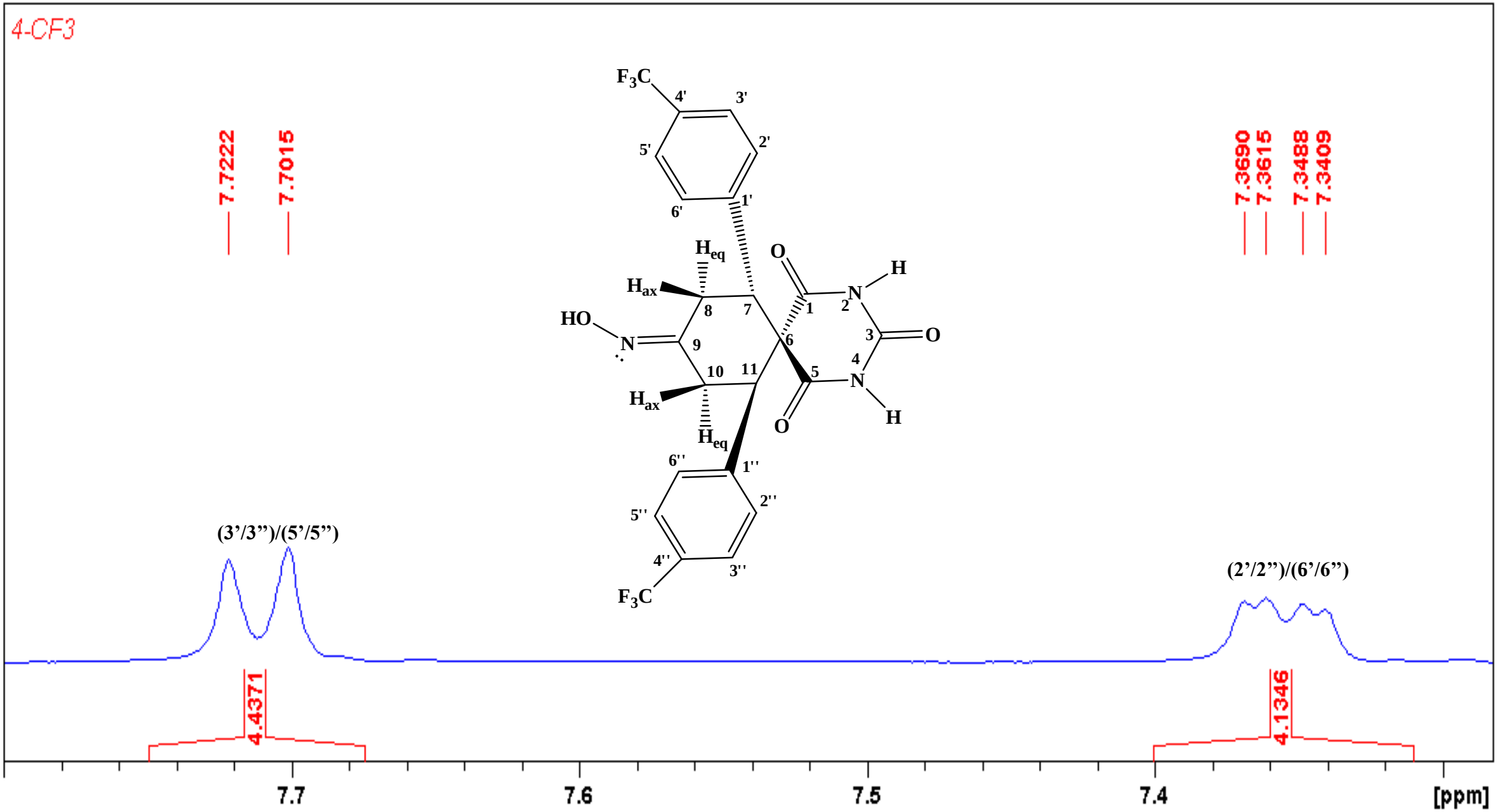


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
512.1034	512.1045	-1.1	-2.1	14.5	10.1	0.0	C23 H16 N3 O4 F6

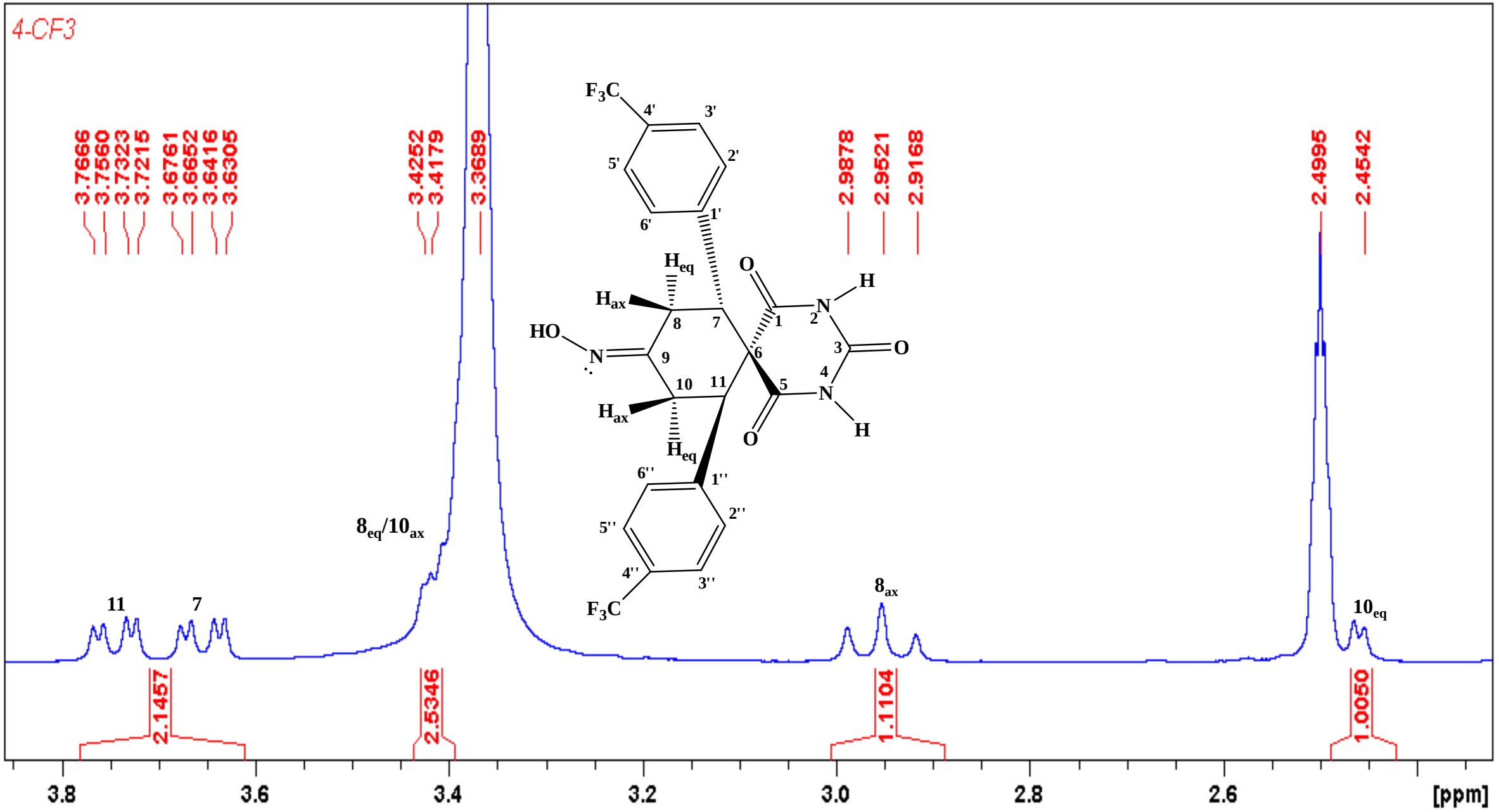
HRMS Spectrum of Compound B-4e: 9-(hydroxyimino)-7,11-bis(3-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



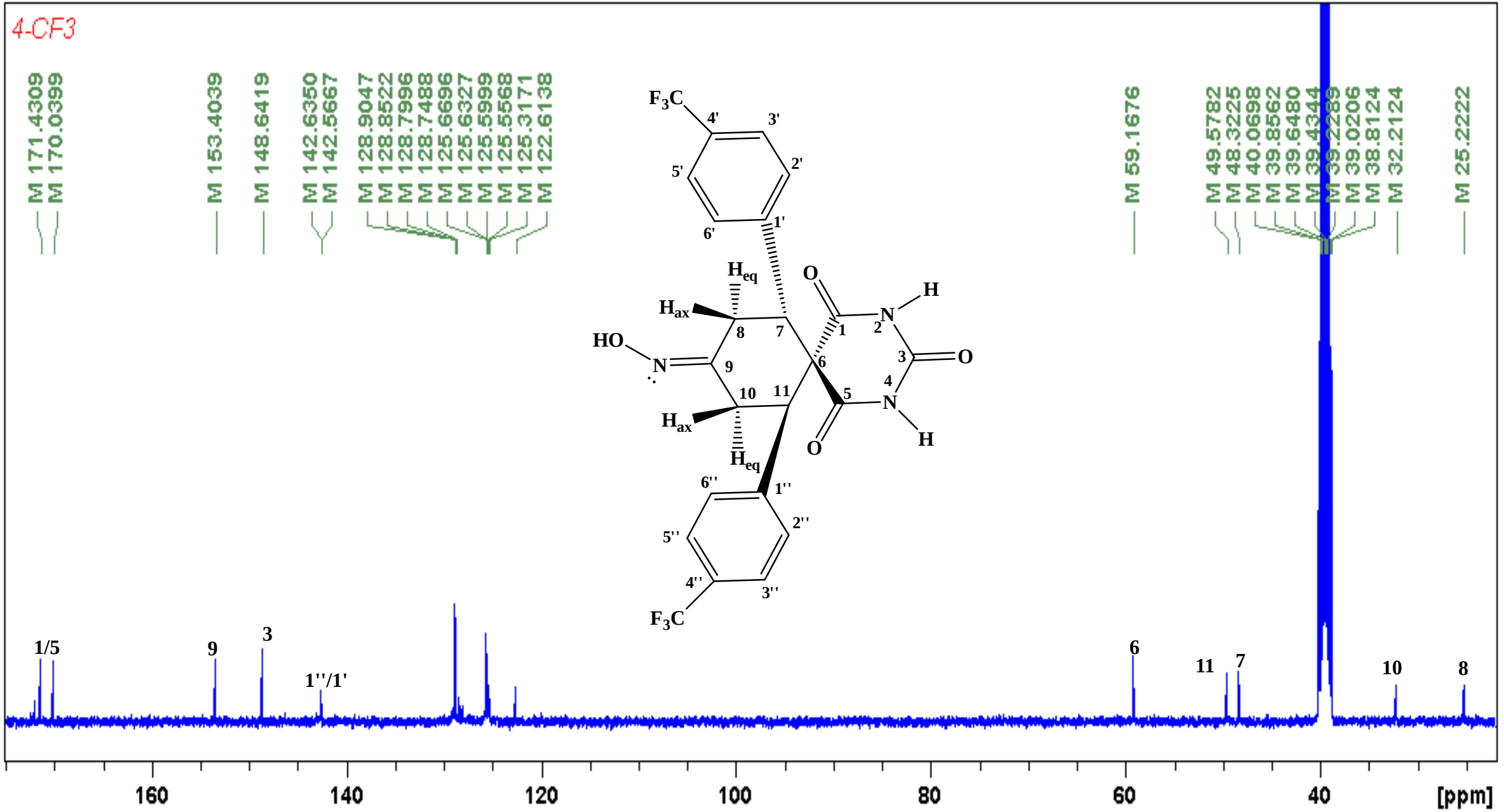
^{1}H Spectrum of Compound B-4f: 9-(hydroxyimino)-7,11-bis(4-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Expanded ^{1}H Spectrum of Compound B-4f: 9-(hydroxyimino)-7,11-bis(4-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

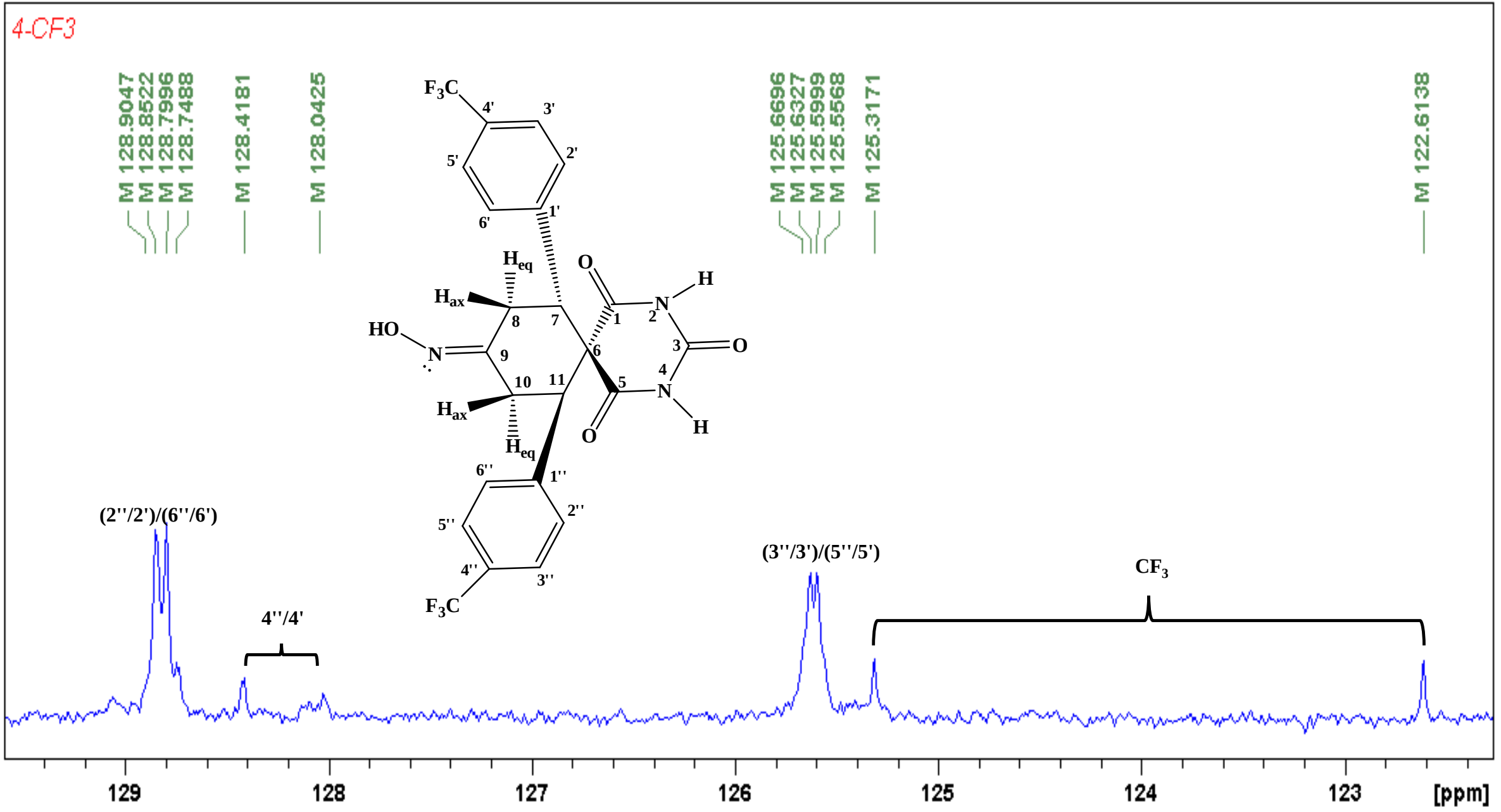


Expanded ^{1}H Spectrum of Compound B-4f: 9-(hydroxyimino)-7,11-bis(4-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



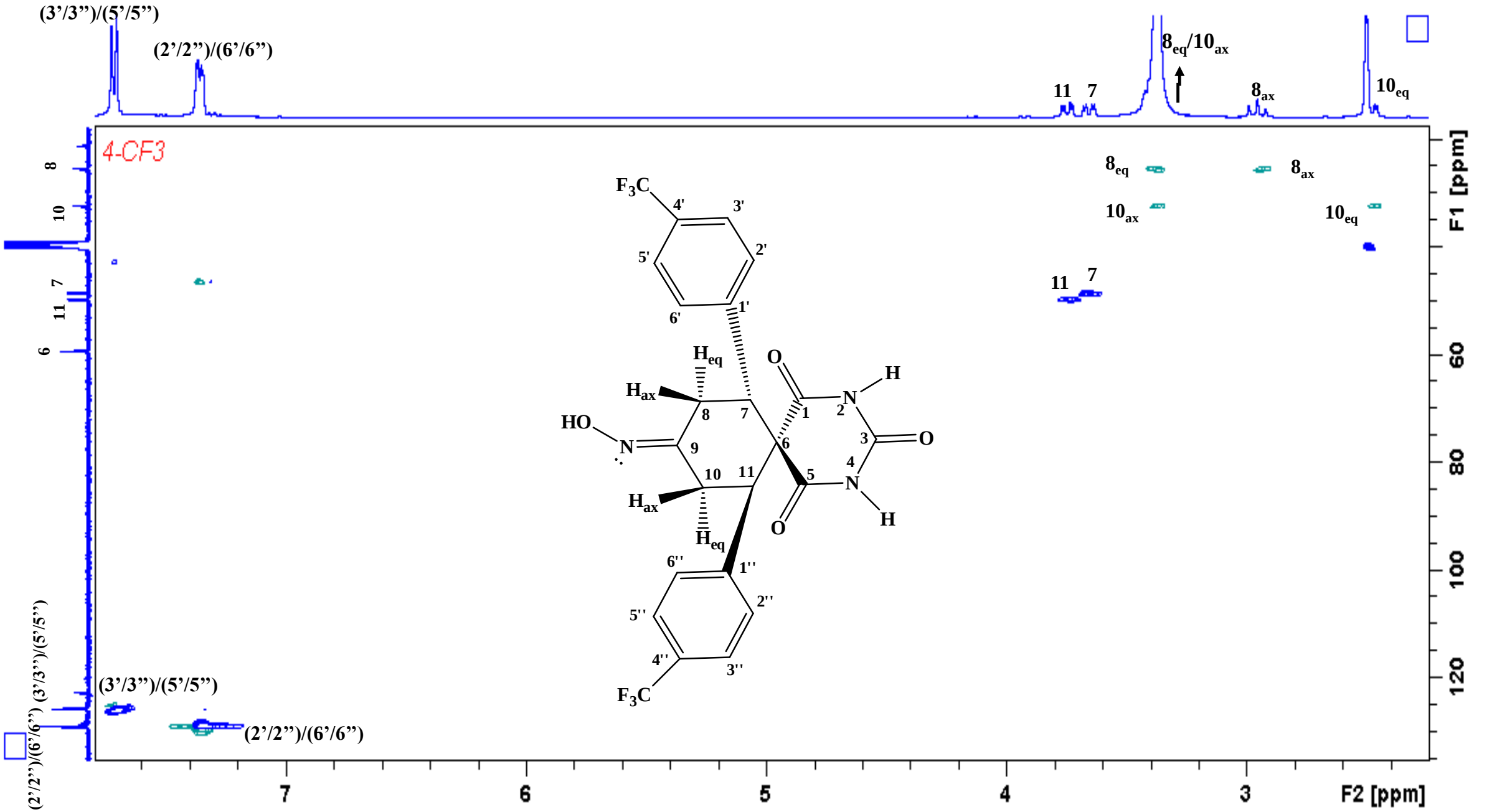
617

^{13}C Spectrum of Compound B-4f: 9-(hydroxyimino)-7,11-bis(4-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

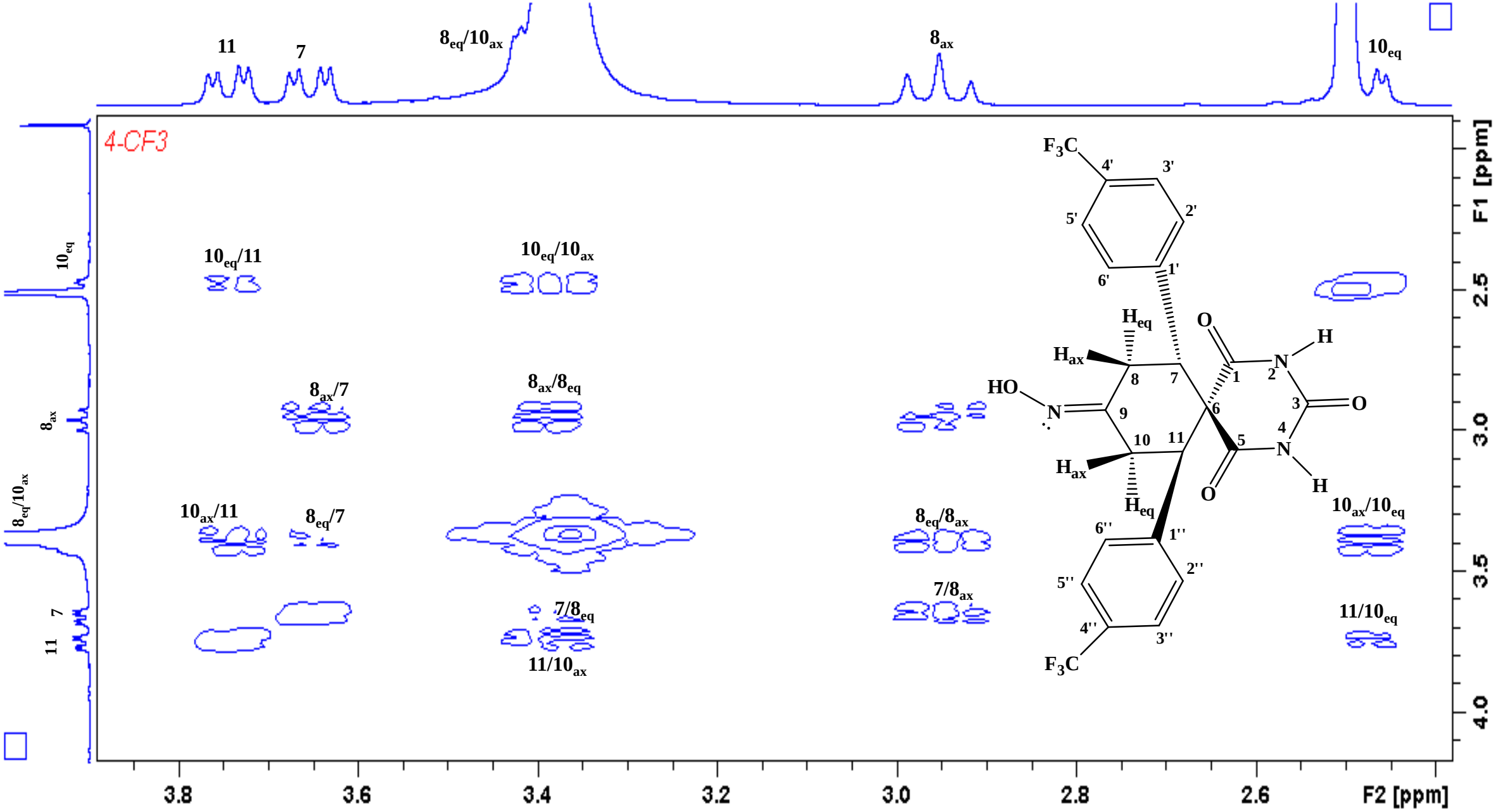


618

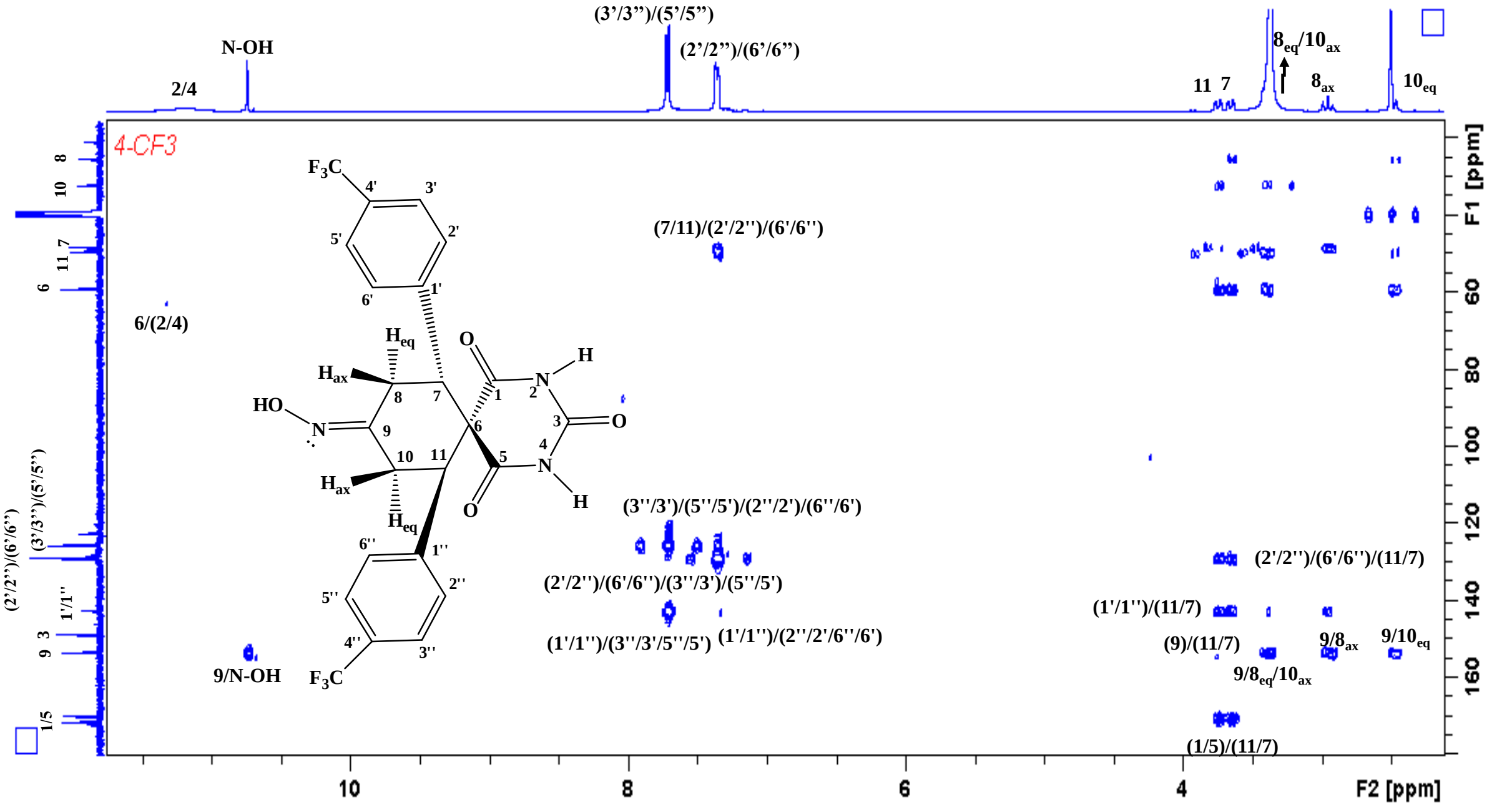
Expanded ^{13}C Spectrum of Compound B-4f: 9-(hydroxyimino)-7,11-bis(4-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



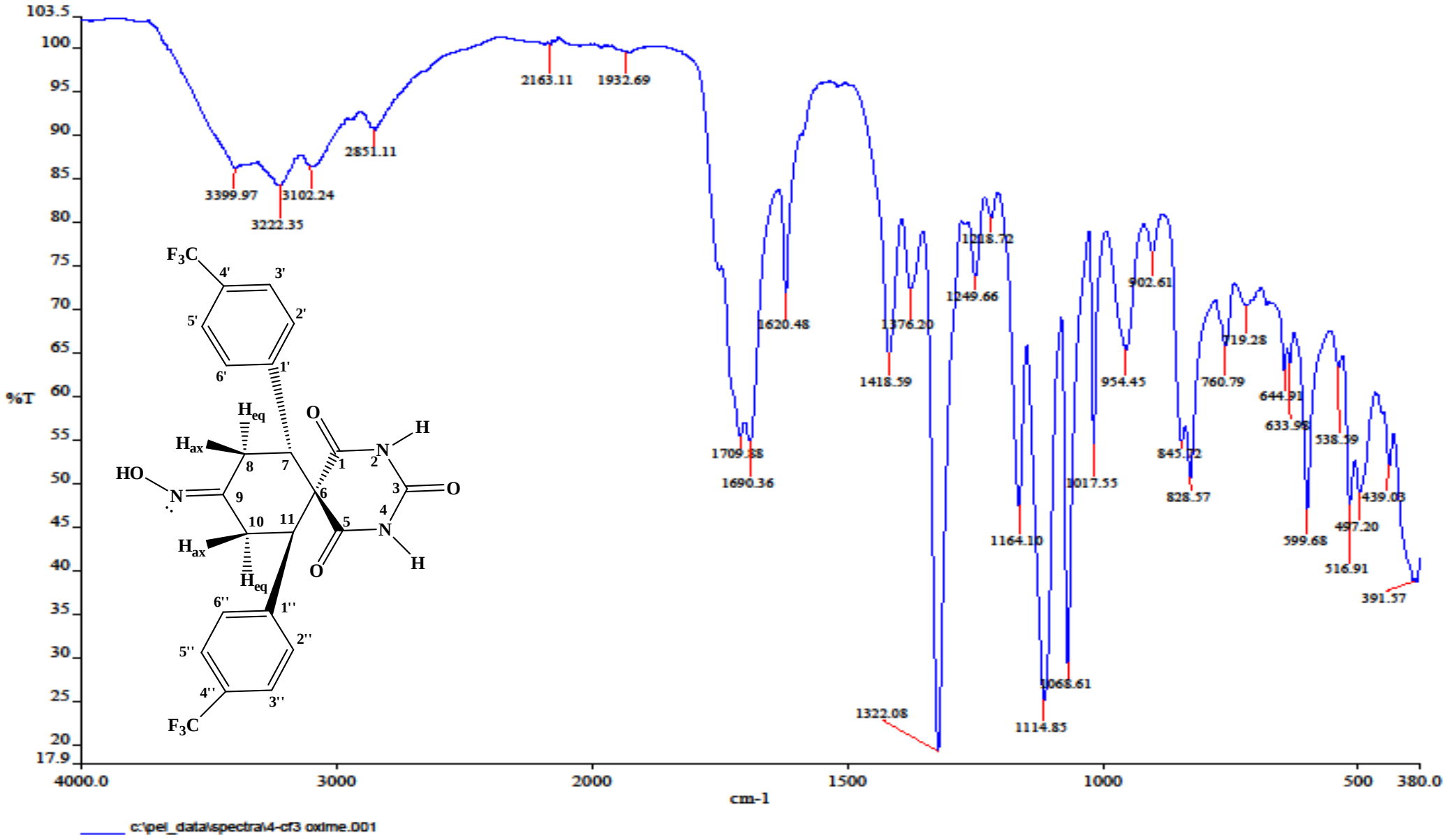
HSQC Spectrum of Compound B-4f: 9-(hydroxyimino)-7,11-bis(4-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



COSY Spectrum of Compound B-4f: 9-(hydroxyimino)-7,11-bis(4-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

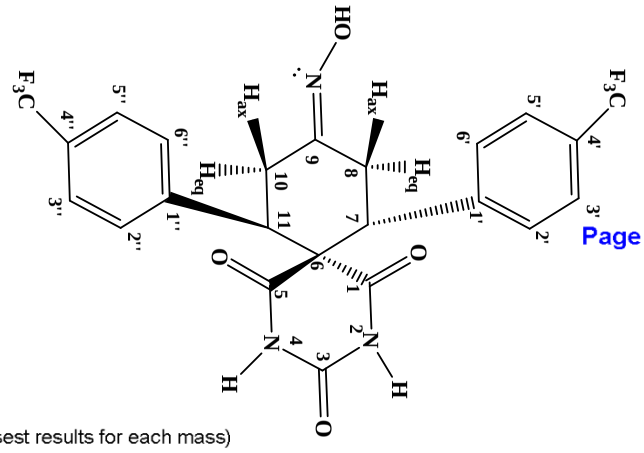


HMBC Spectrum of Compound B-4f: 9-(hydroxyimino)-7,11-bis(4-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



622

Infrared Spectrum of Compound B-4f: 9-(hydroxyimino)-7,11-bis(4-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



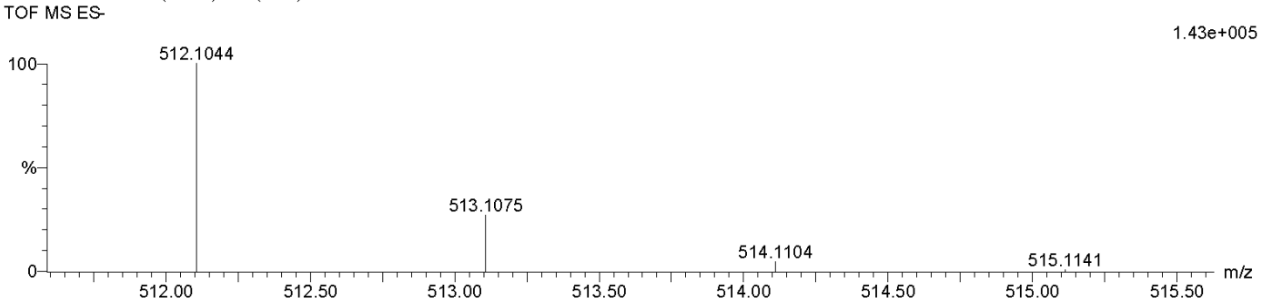
Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

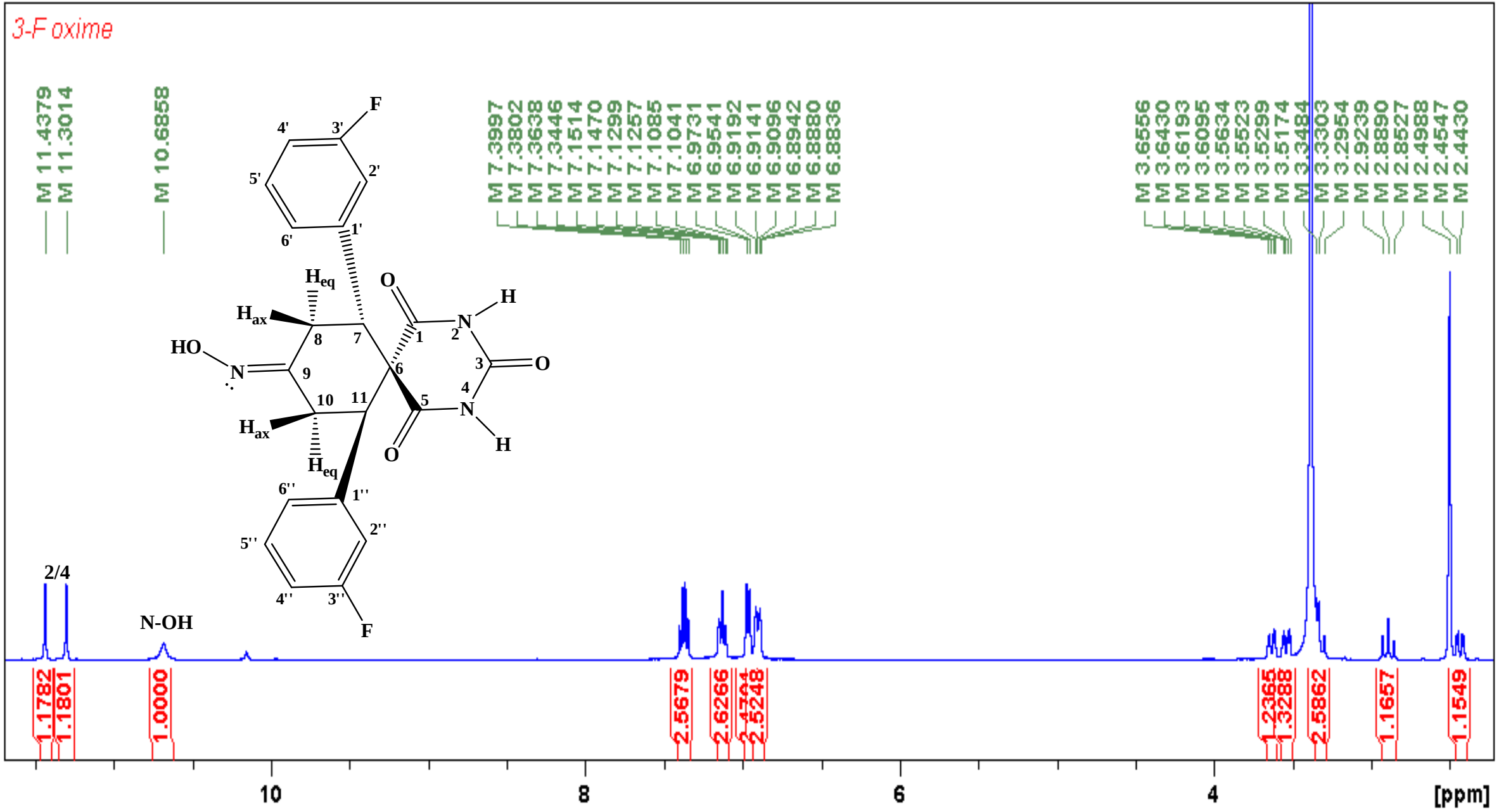
Monoisotopic Mass, Even Electron Ions
157 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 0-5 F: 0-6

33 4-CF3 oxime 38 (1.248) Cm (1:61)
TOF MS ES-



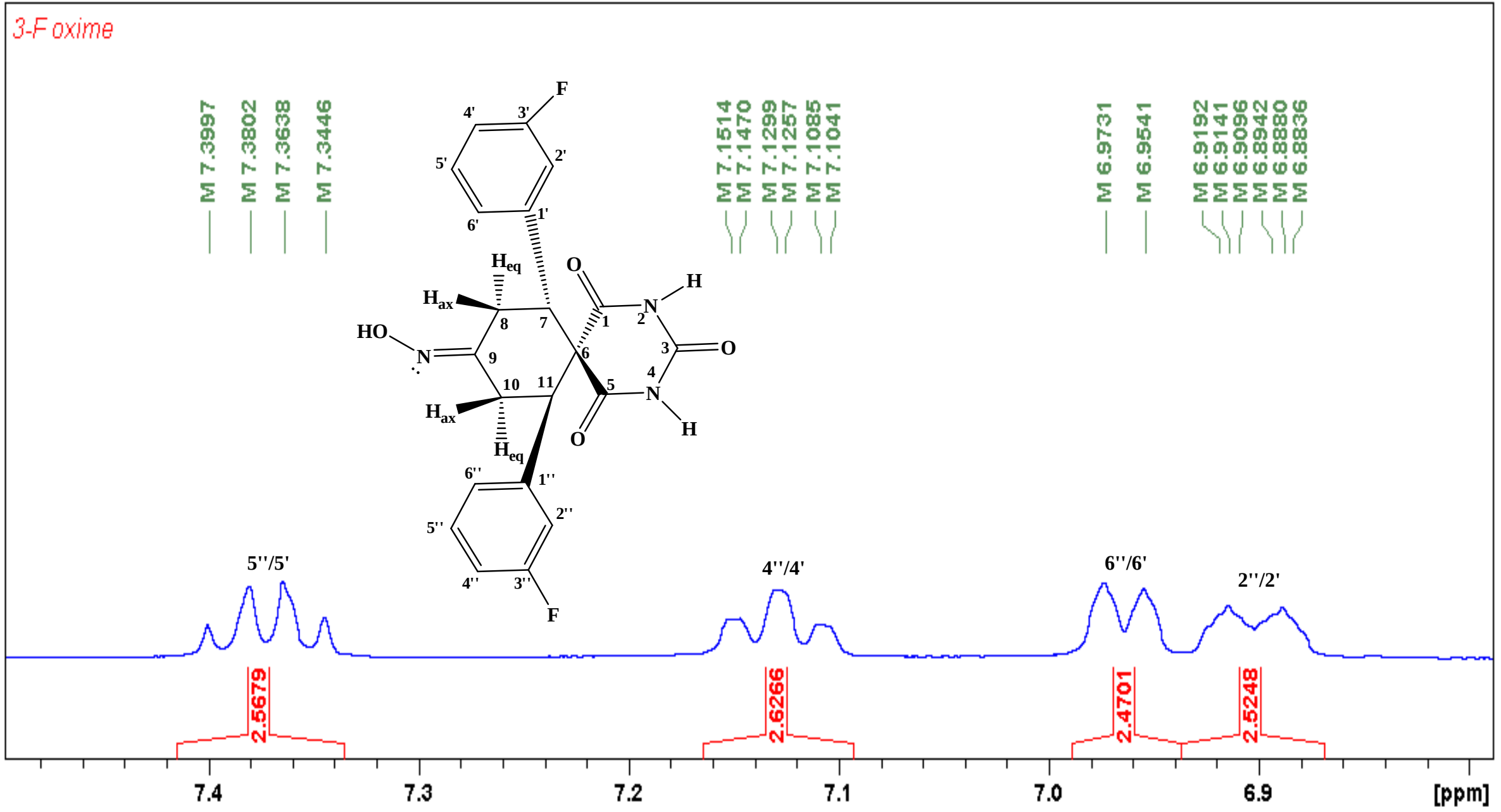
Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
512.1044	512.1045	-0.1	-0.2	14.5	8.5	0.0	C23 H16 N3 O4 F6

HRMS Spectrum of Compound B-4f: 9-(hydroxyimino)-7,11-bis(4-(trifluoromethyl)phenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

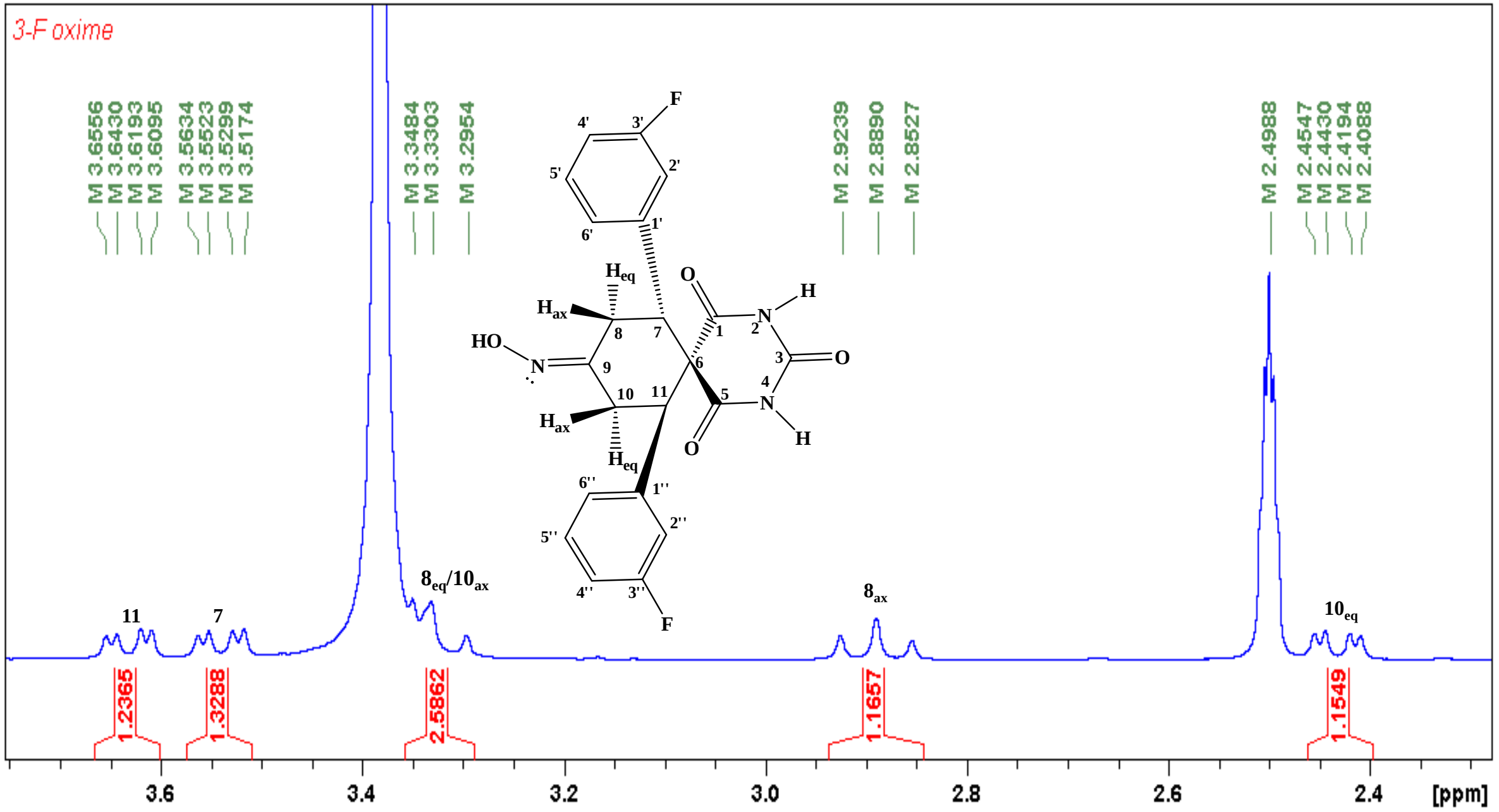


624

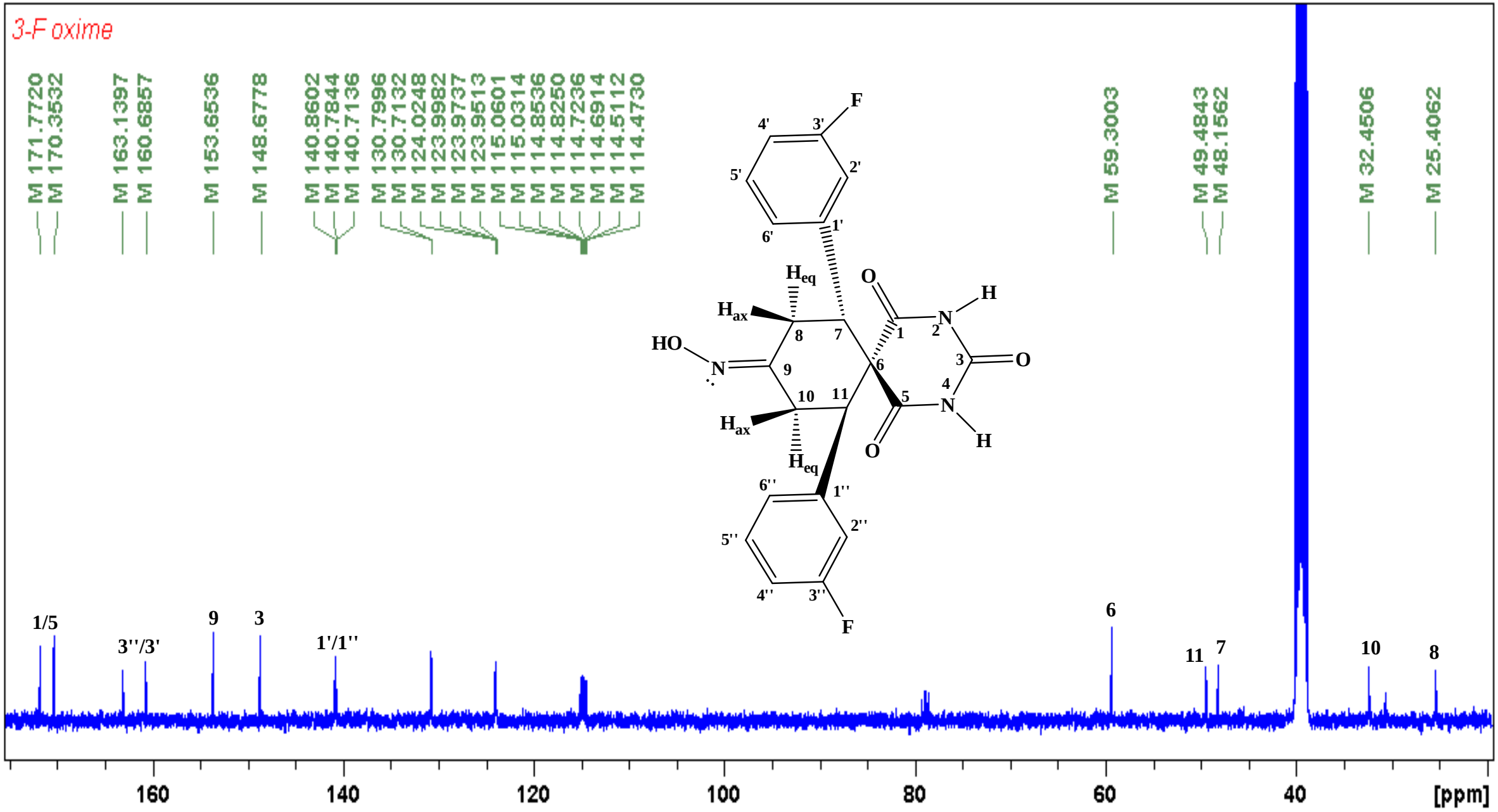
^{1}H Spectrum of Compound B-4g: 7,11-bis(3-fluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Expanded ^{1}H Spectrum of Compound B-4g: 7,11-bis(3-fluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

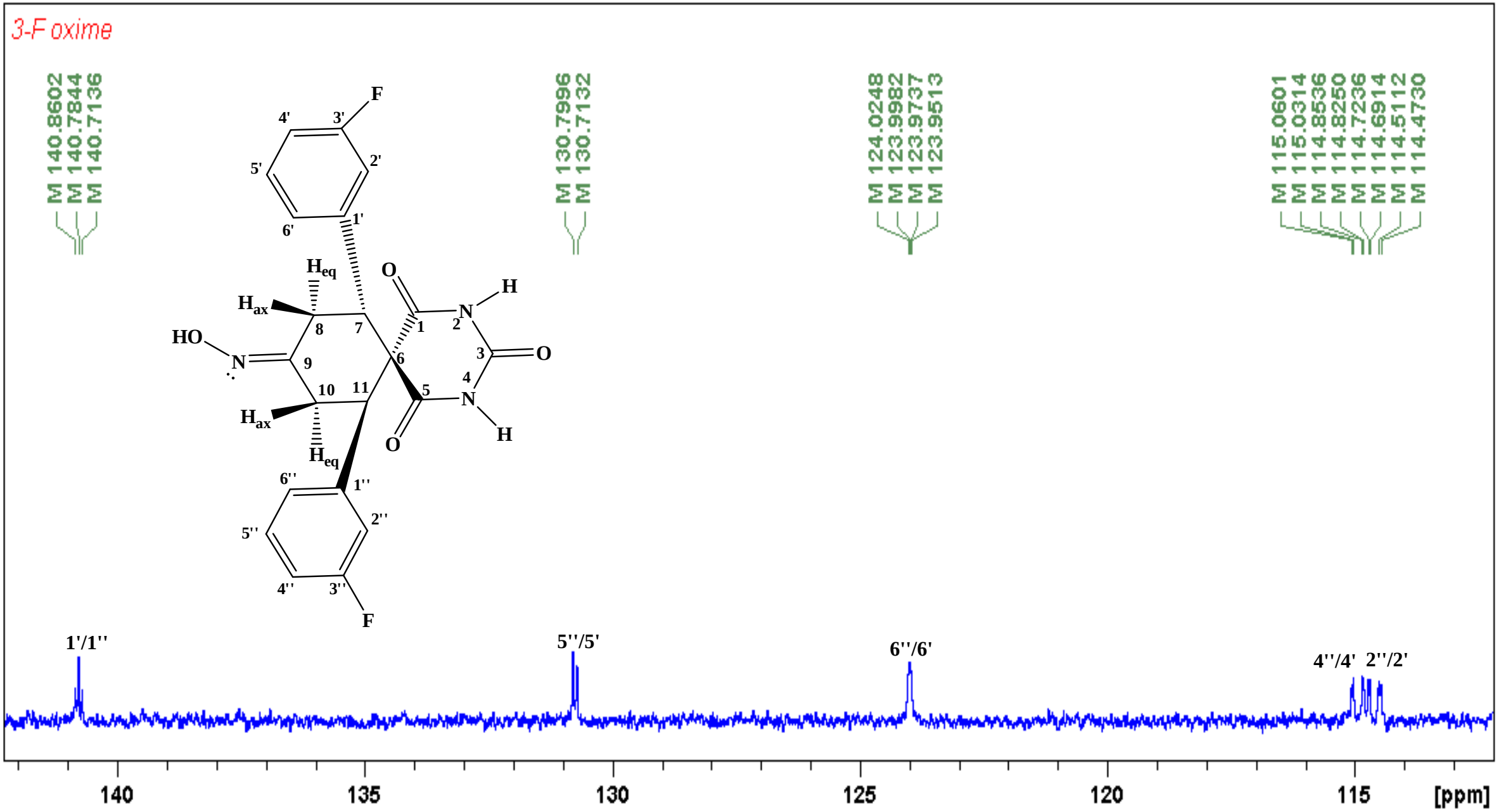


Expanded ^{1}H Spectrum of Compound B-4g: 7,11-bis(3-fluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



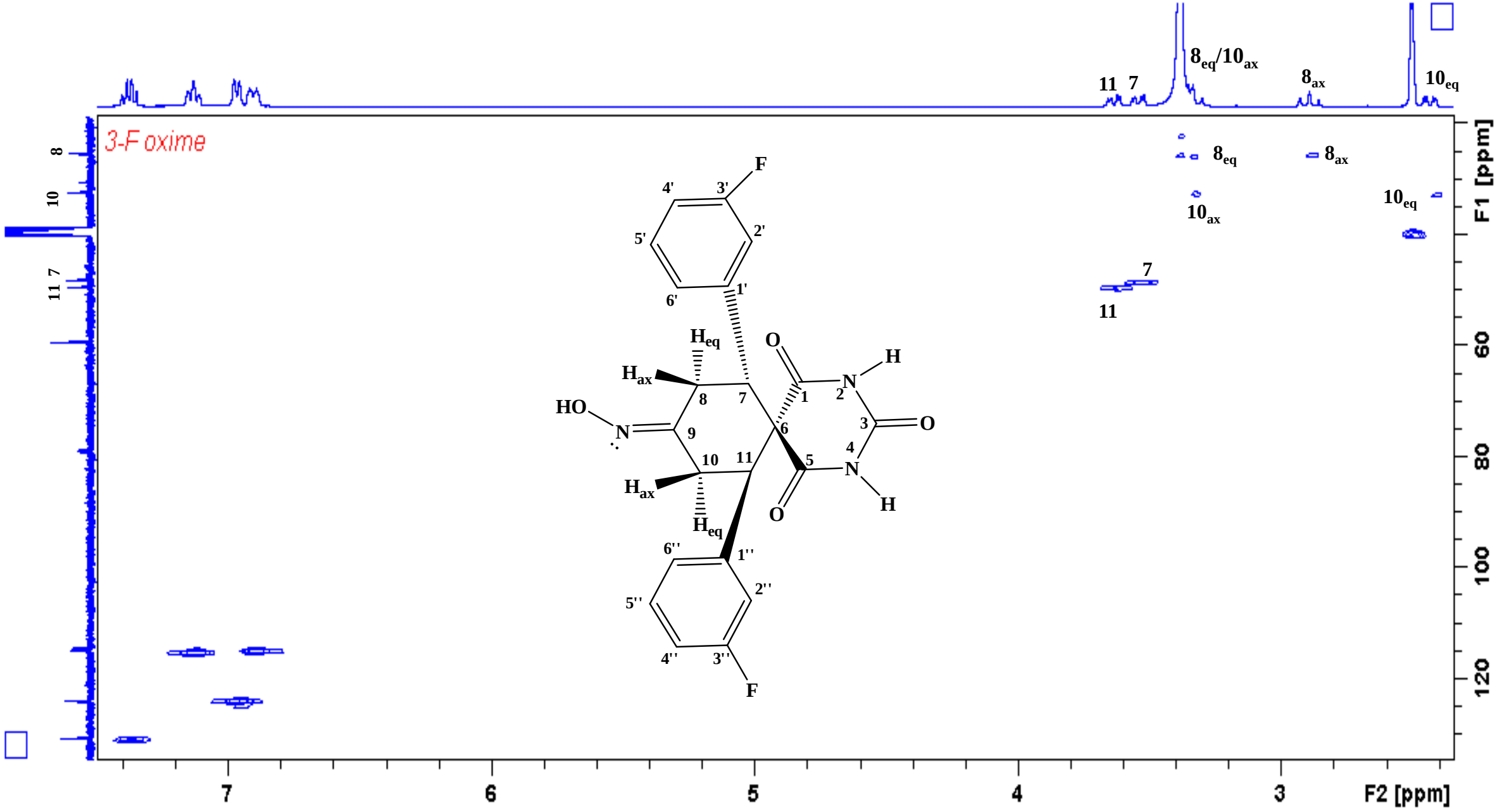
627

^{13}C Spectrum of Compound B-4g: 7,11-bis(3-fluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

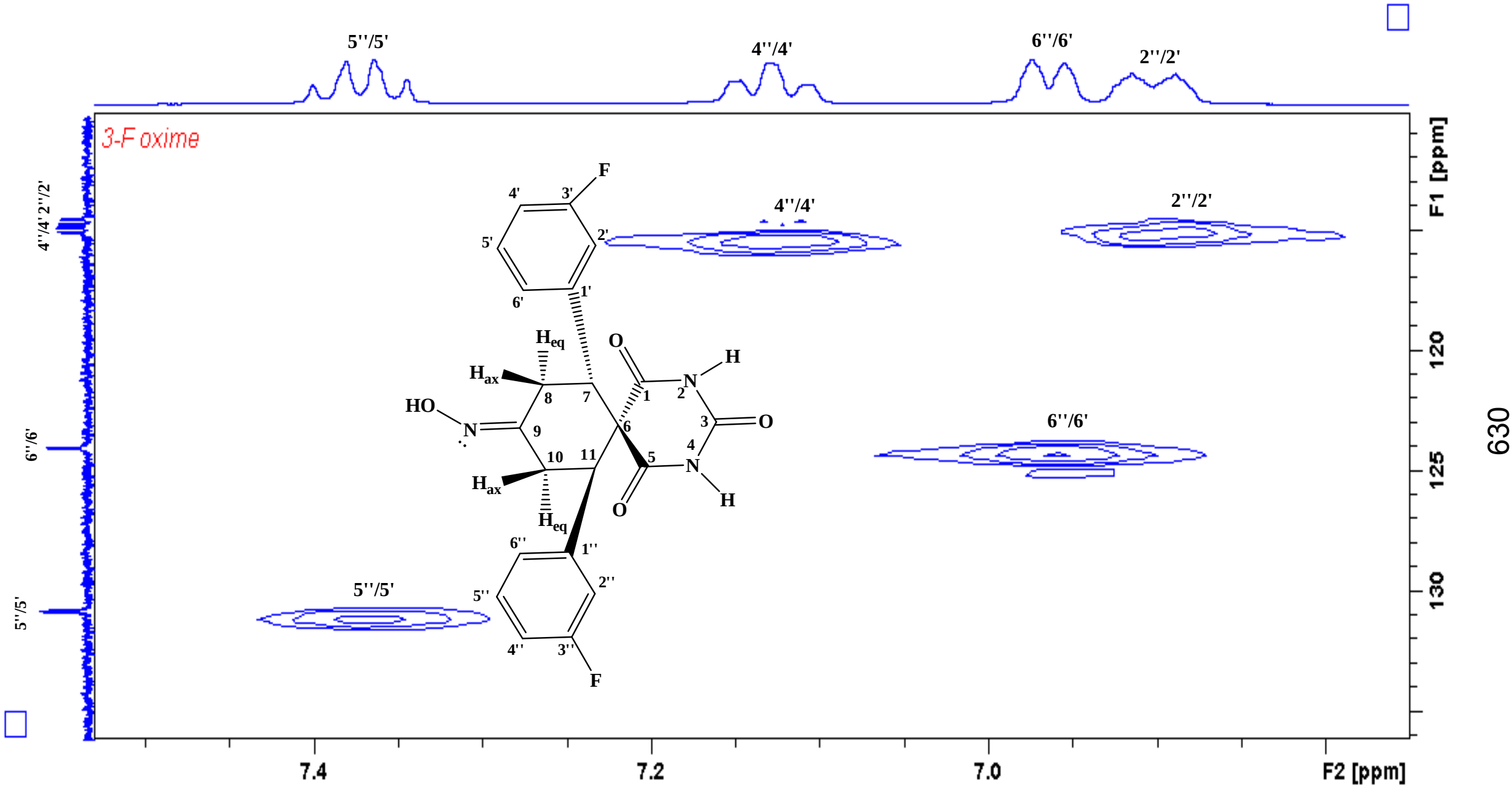


628

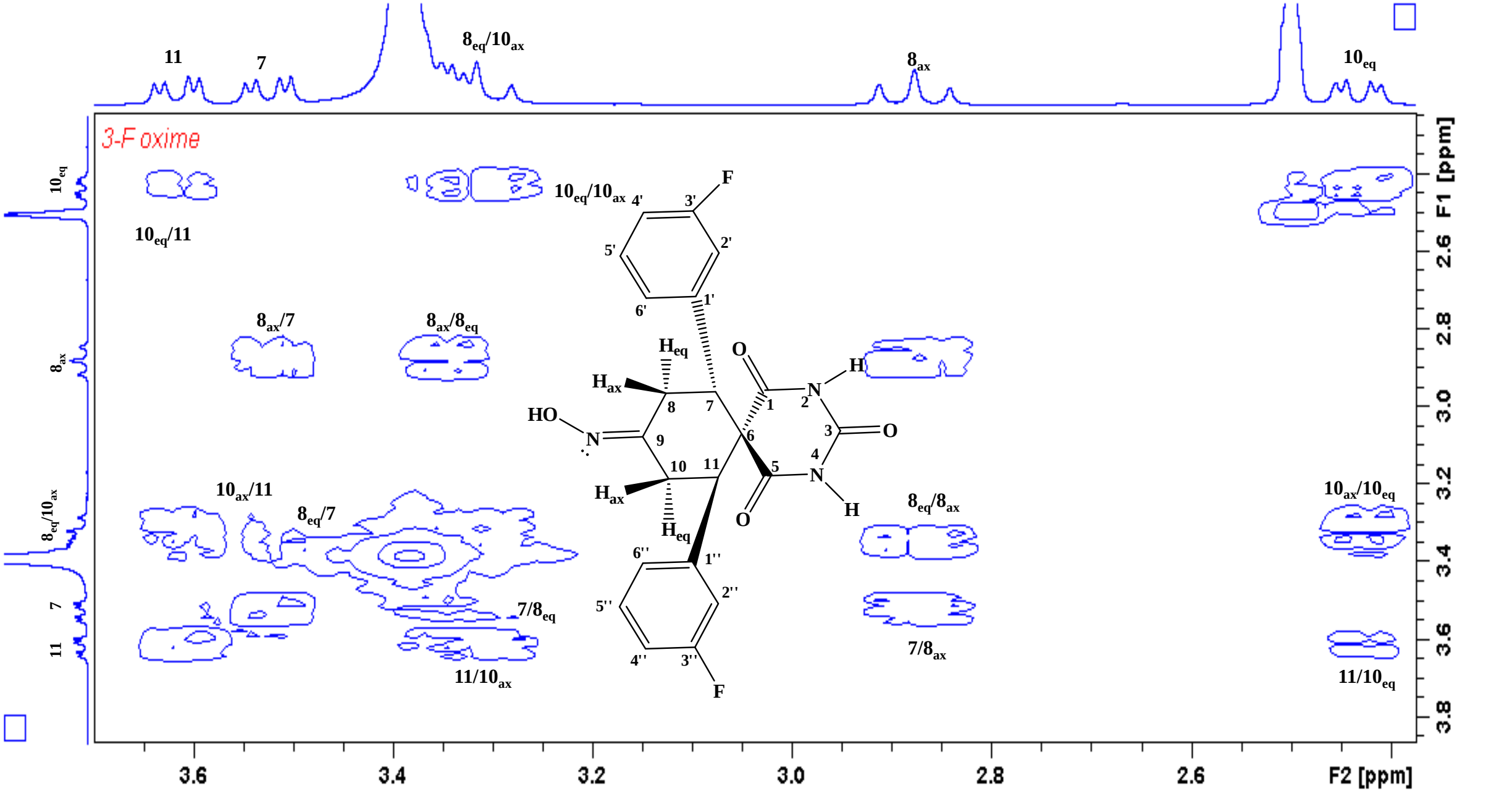
Expanded ^{13}C Spectrum of Compound B-4g: 7,11-bis(3-fluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



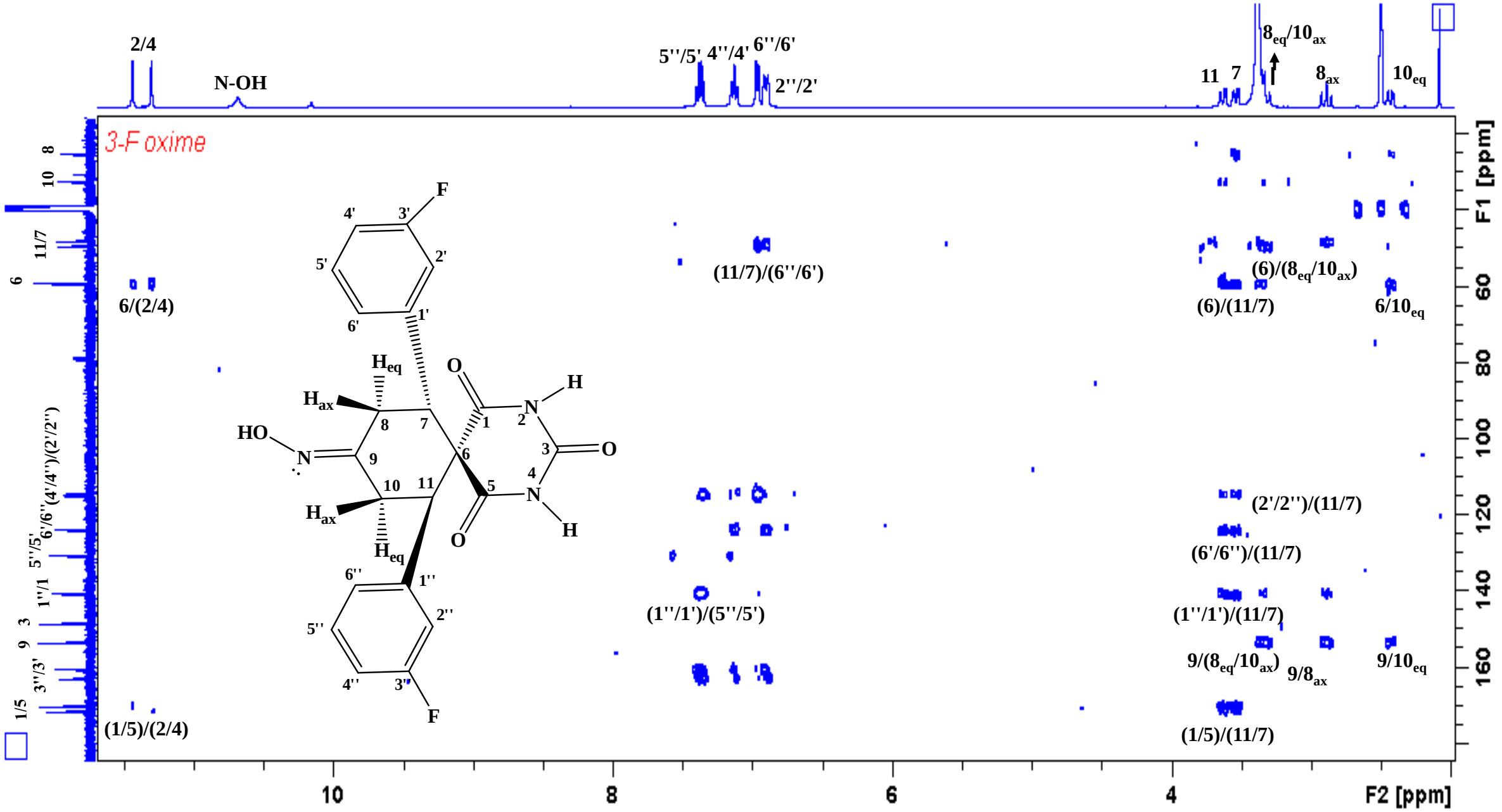
HSQC Spectrum of Compound B-4g: 7,11-bis(3-fluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



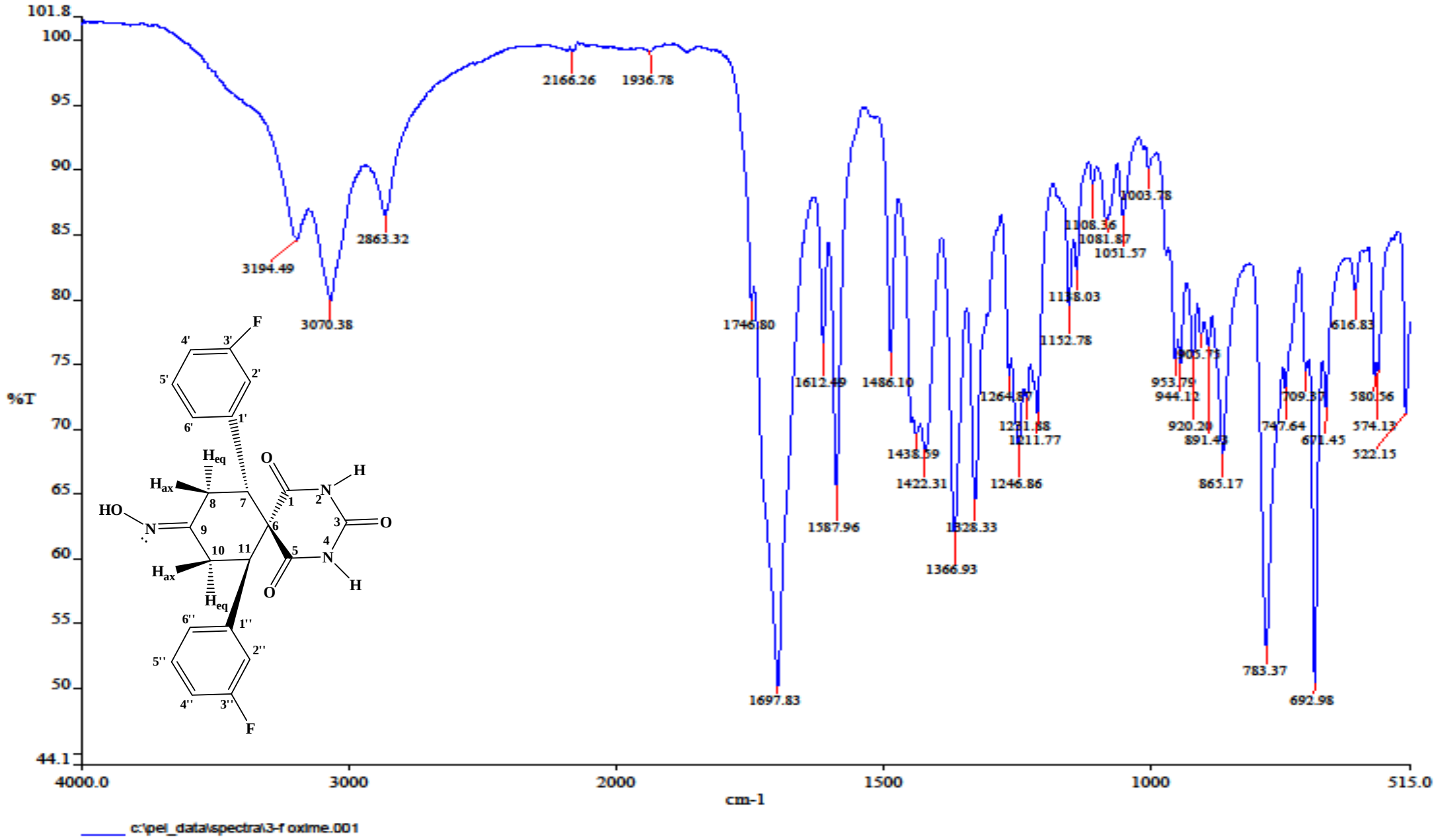
Expanded HSQC Spectrum of Compound B-4g: 7,11-bis(3-fluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



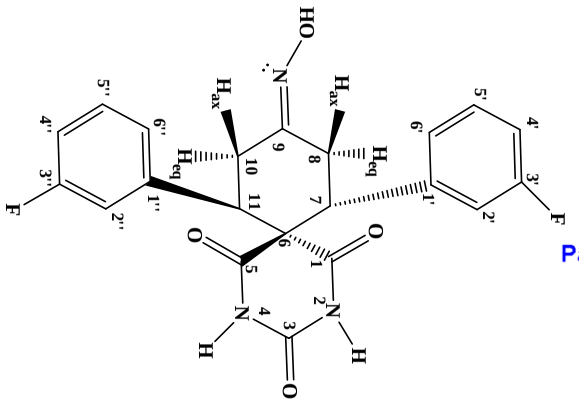
COSY Spectrum of Compound B-4g: 7,11-bis(3-fluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



HMBC Spectrum of Compound B-4g: 7,11-bis(3-fluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Infrared Spectrum of Compound B-4g: 7,11-bis(3-fluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

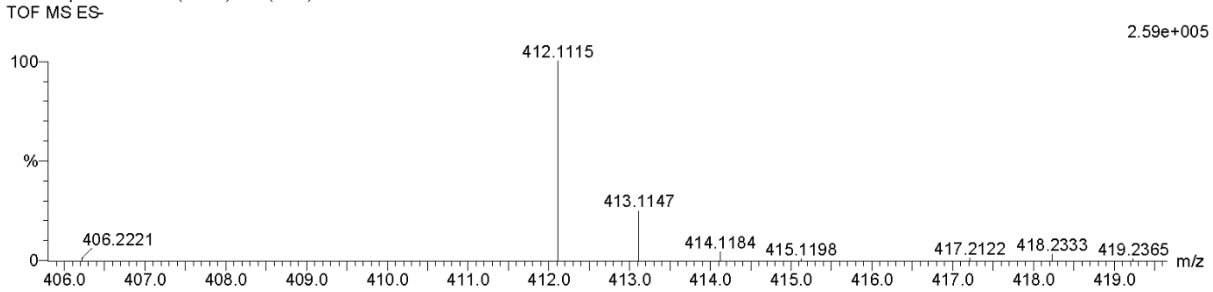


Elemental Composition Report

Single Mass Analysis

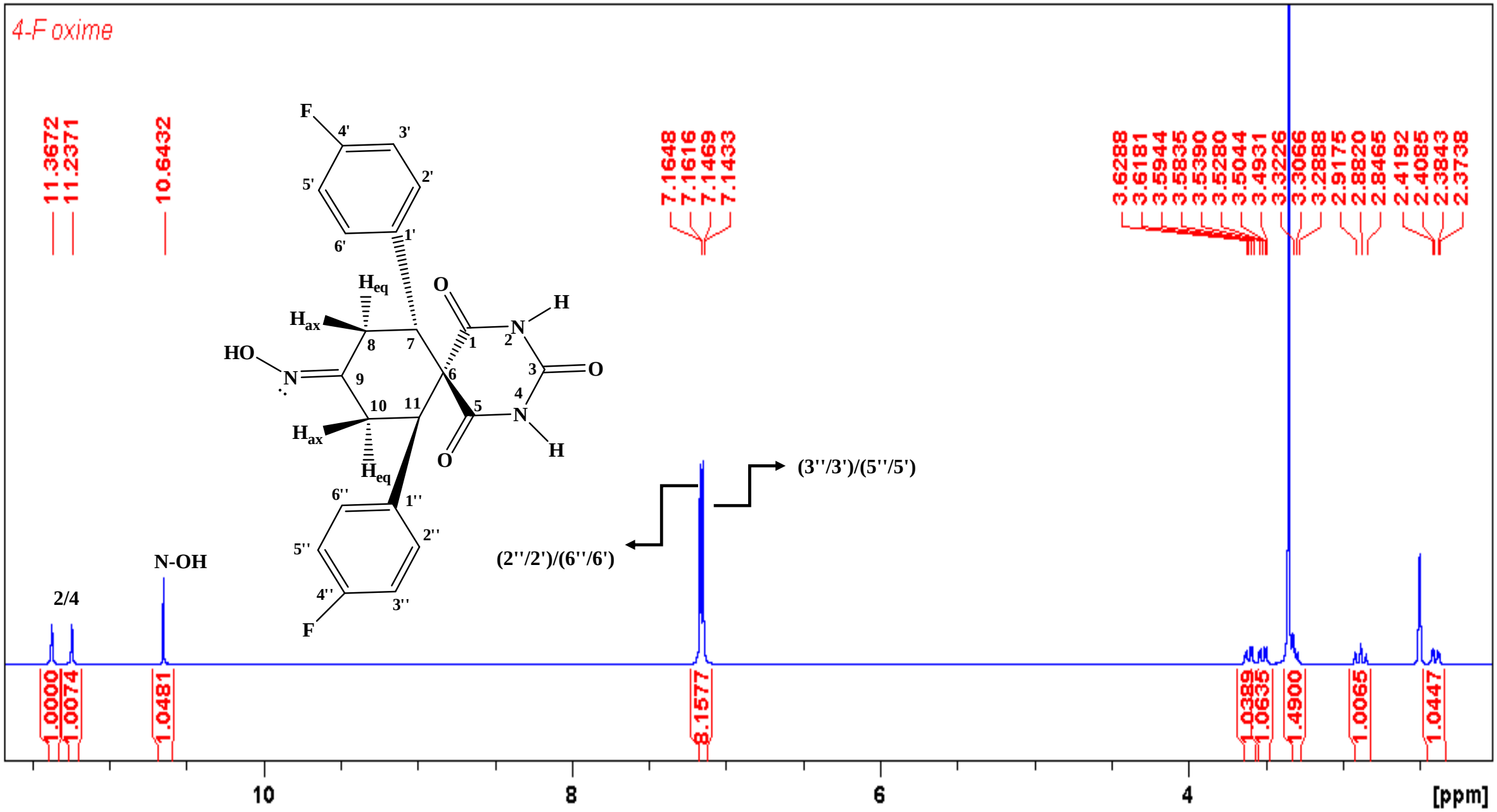
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
61 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 1-5 F: 2-5
34 3-F spiro oxime 43 (1.416) Cm (1:61)
TOF MS ES-

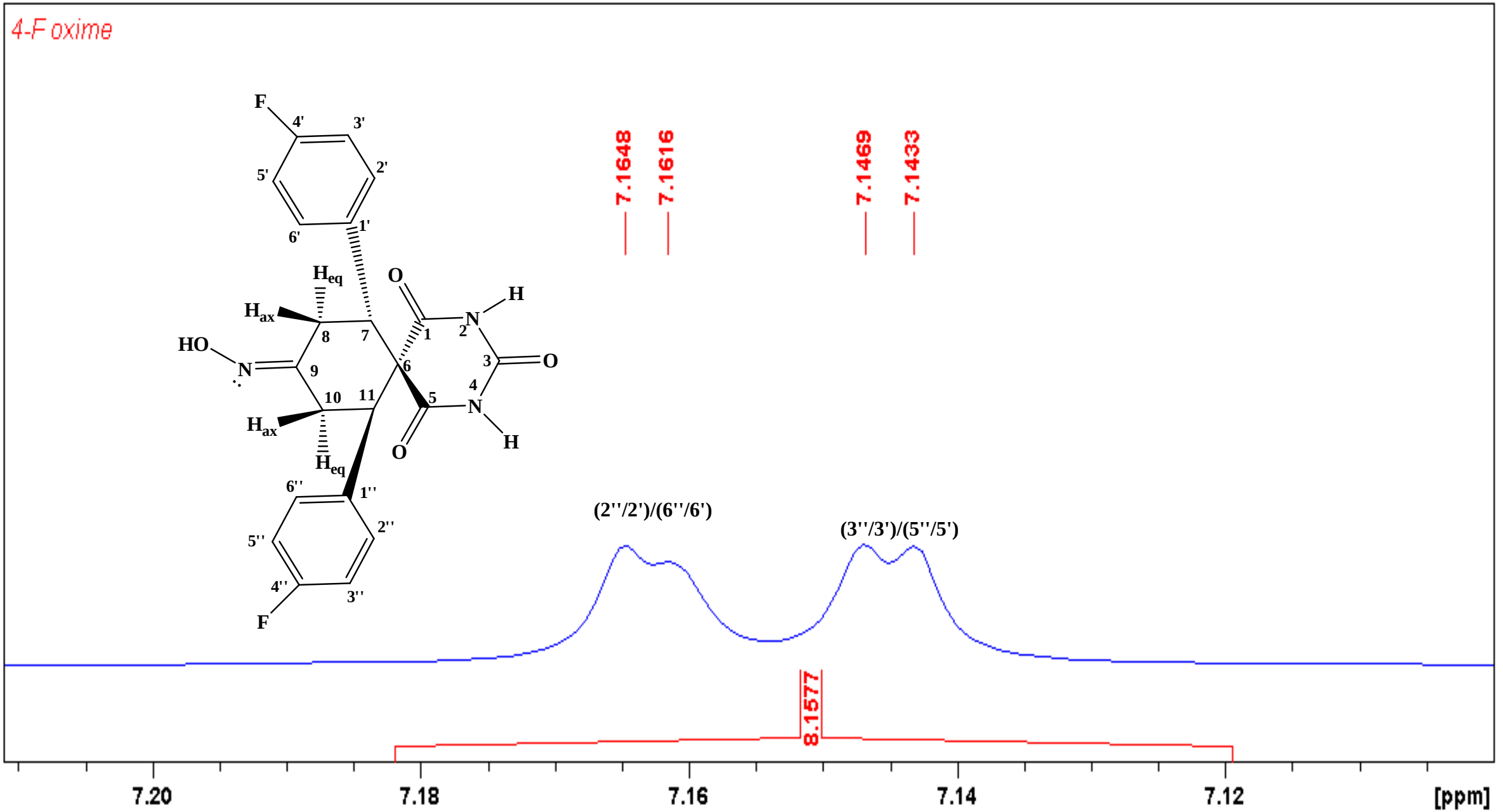


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
412.1115	412.1109	0.6	1.5	14.5	8.7	0.0	C21 H16 N3 O4 F2

HRMS Spectrum of Compound B-4g: 7,11-bis(3-fluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

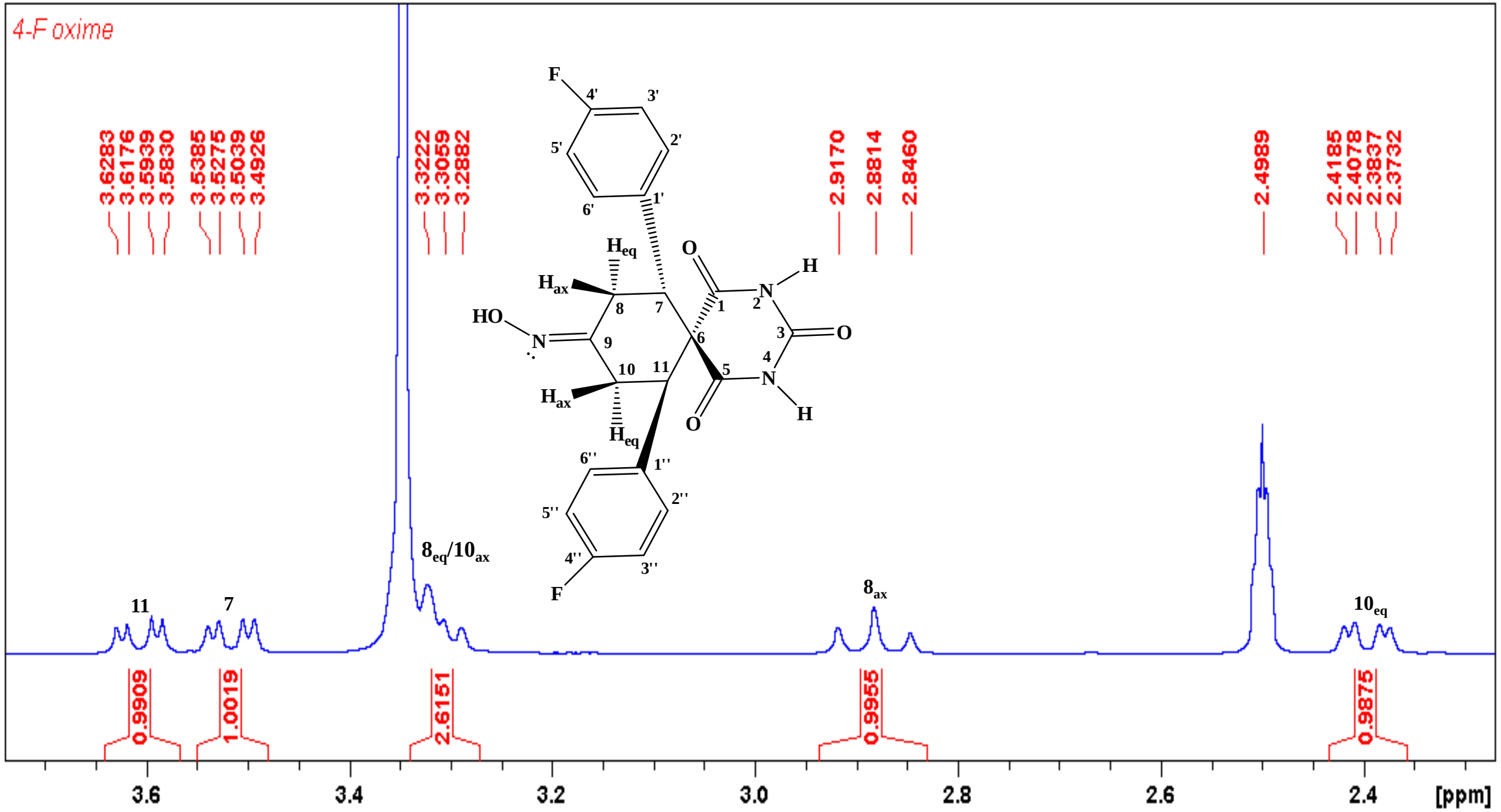


^{1}H Spectrum of Compound B-4h: 7,11-bis(4-fluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

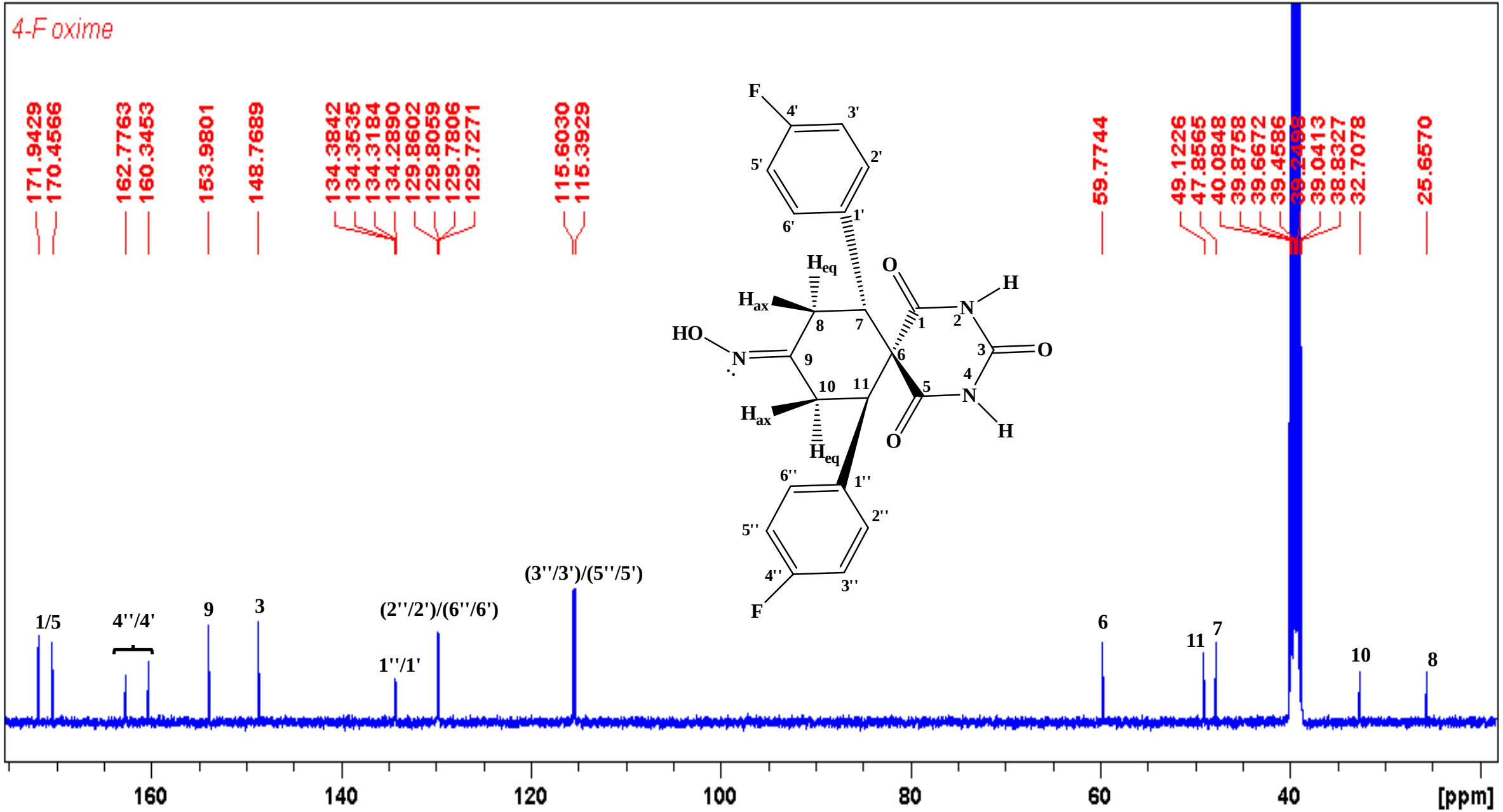


636

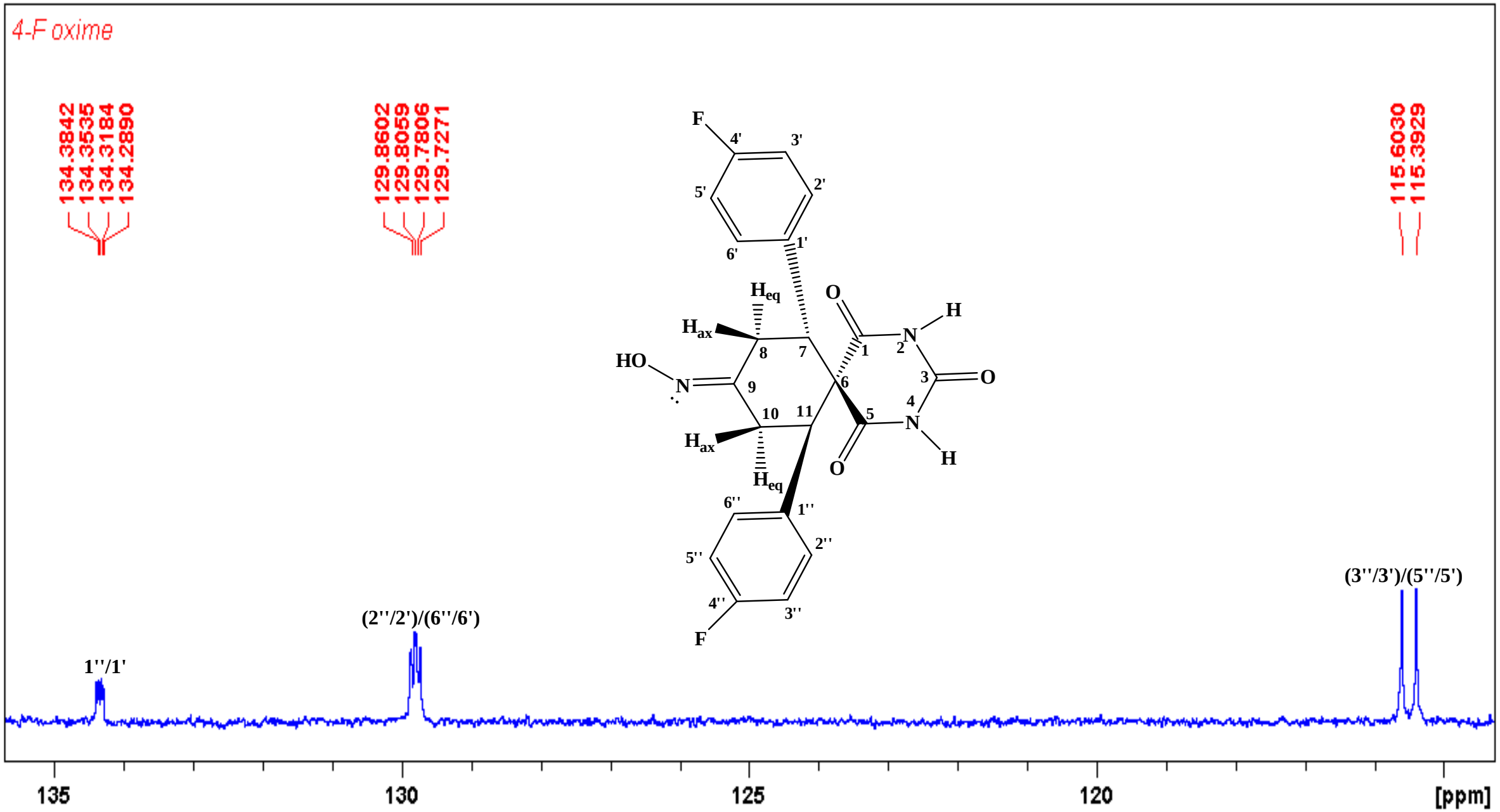
Expanded ^{1}H Spectrum of Compound B-4h: 7,11-bis(4-fluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



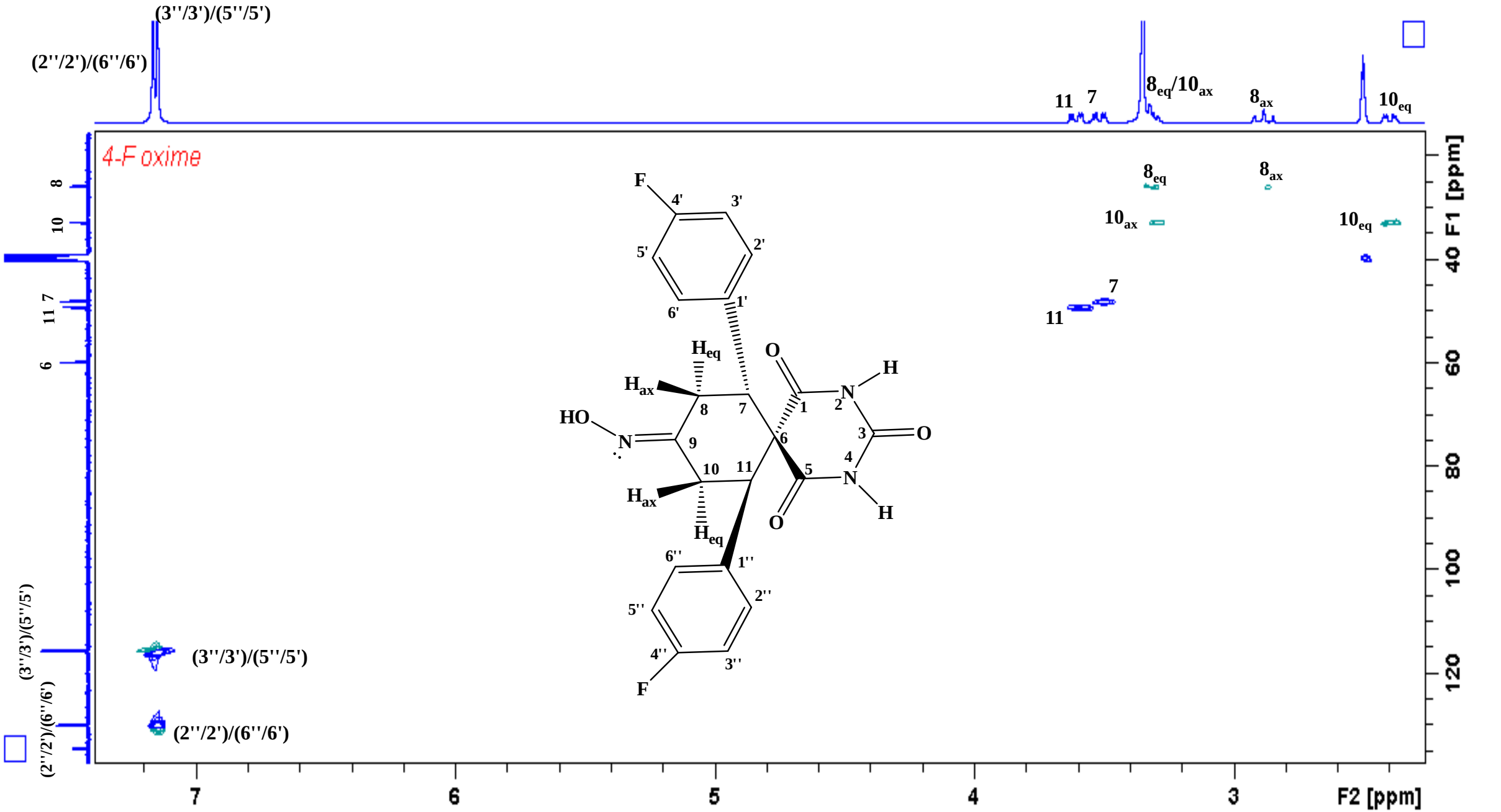
Expanded ^{1}H Spectrum of Compound B-4h: 7,11-bis(4-fluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



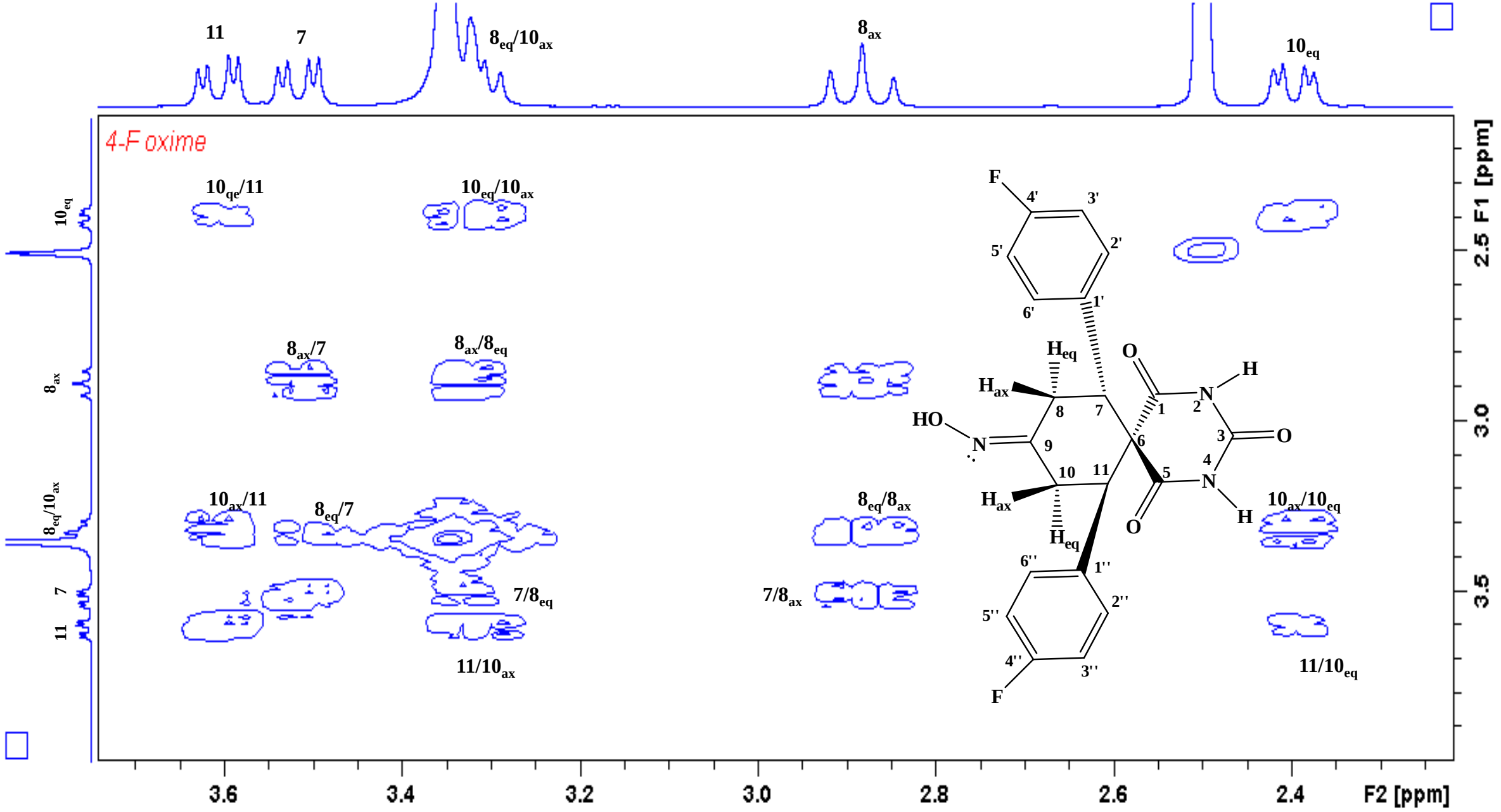
^{13}C Spectrum of Compound B-4h: 7,11-bis(4-fluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Expanded ^{13}C Spectrum of Compound B-4h: 7,11-bis(4-fluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



HSQC Spectrum of Compound B-4h: 7,11-bis(4-fluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



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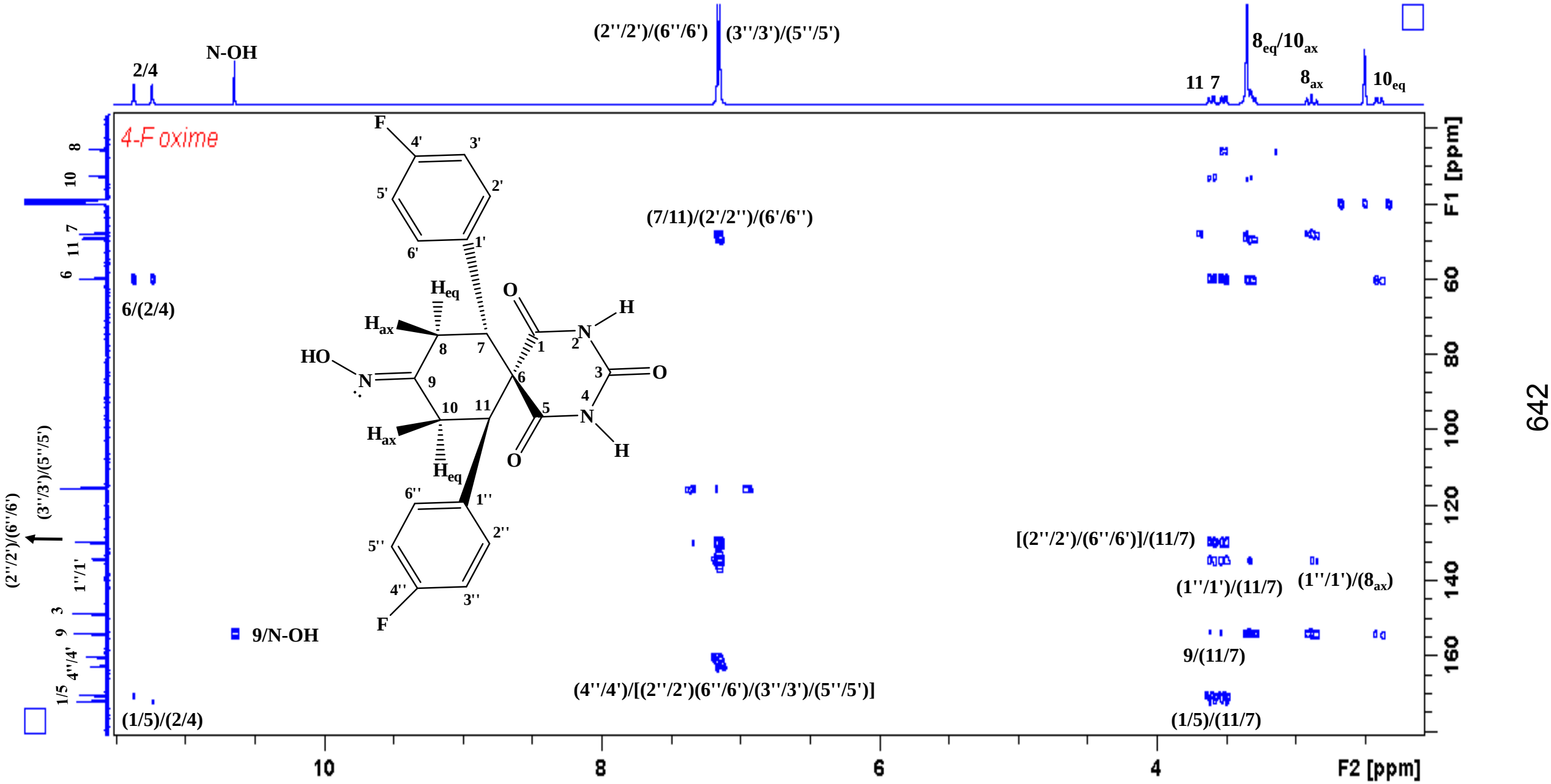
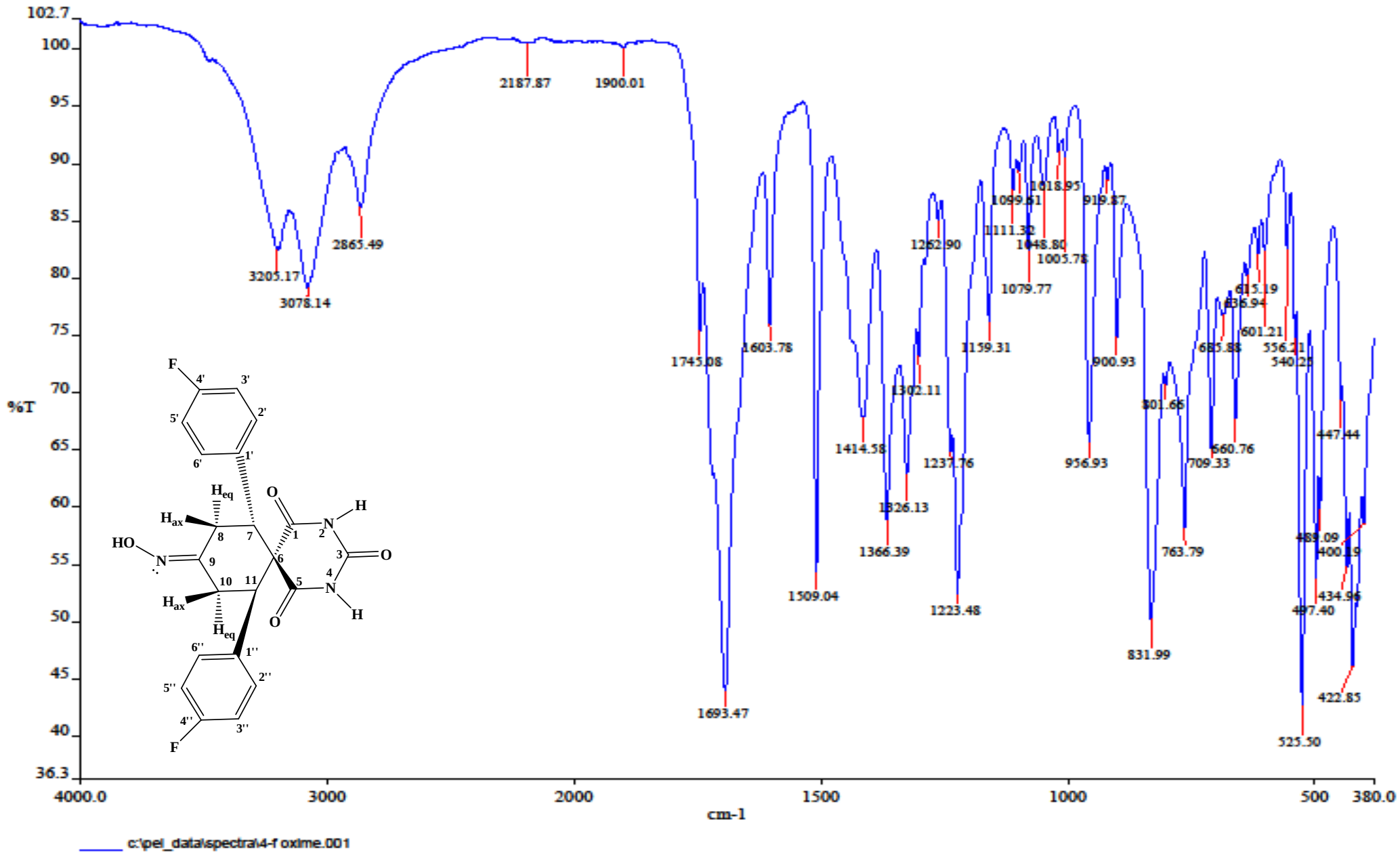
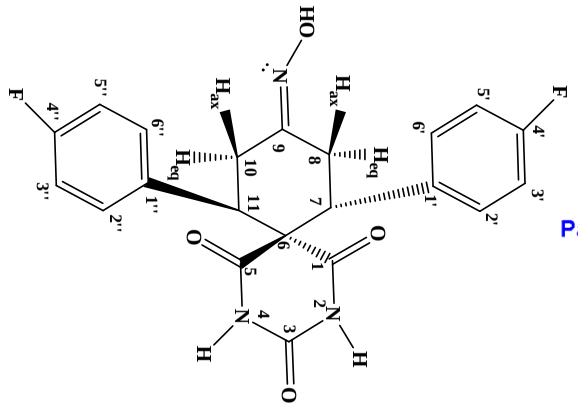
COSY Spectrum of Compound B-4h: 7,11-bis(4-fluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



HMBC Spectrum of Compound B-4h: 7,11-bis(4-fluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



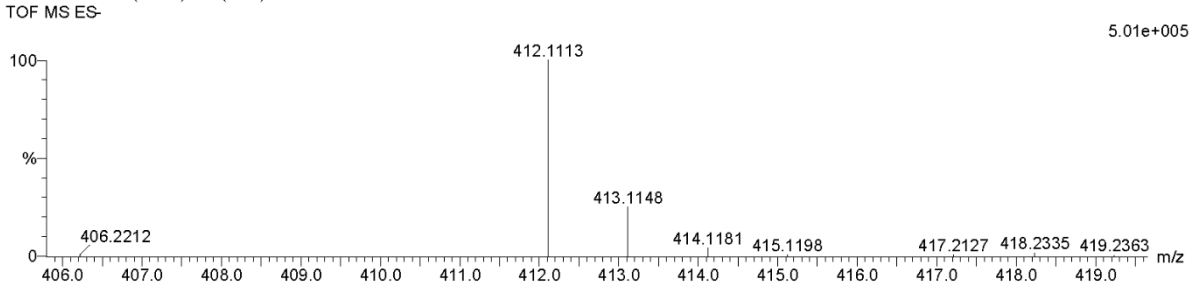
Infrared Spectrum of Compound B-4h: 7,11-bis(4-fluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Elemental Composition Report **Page 1**

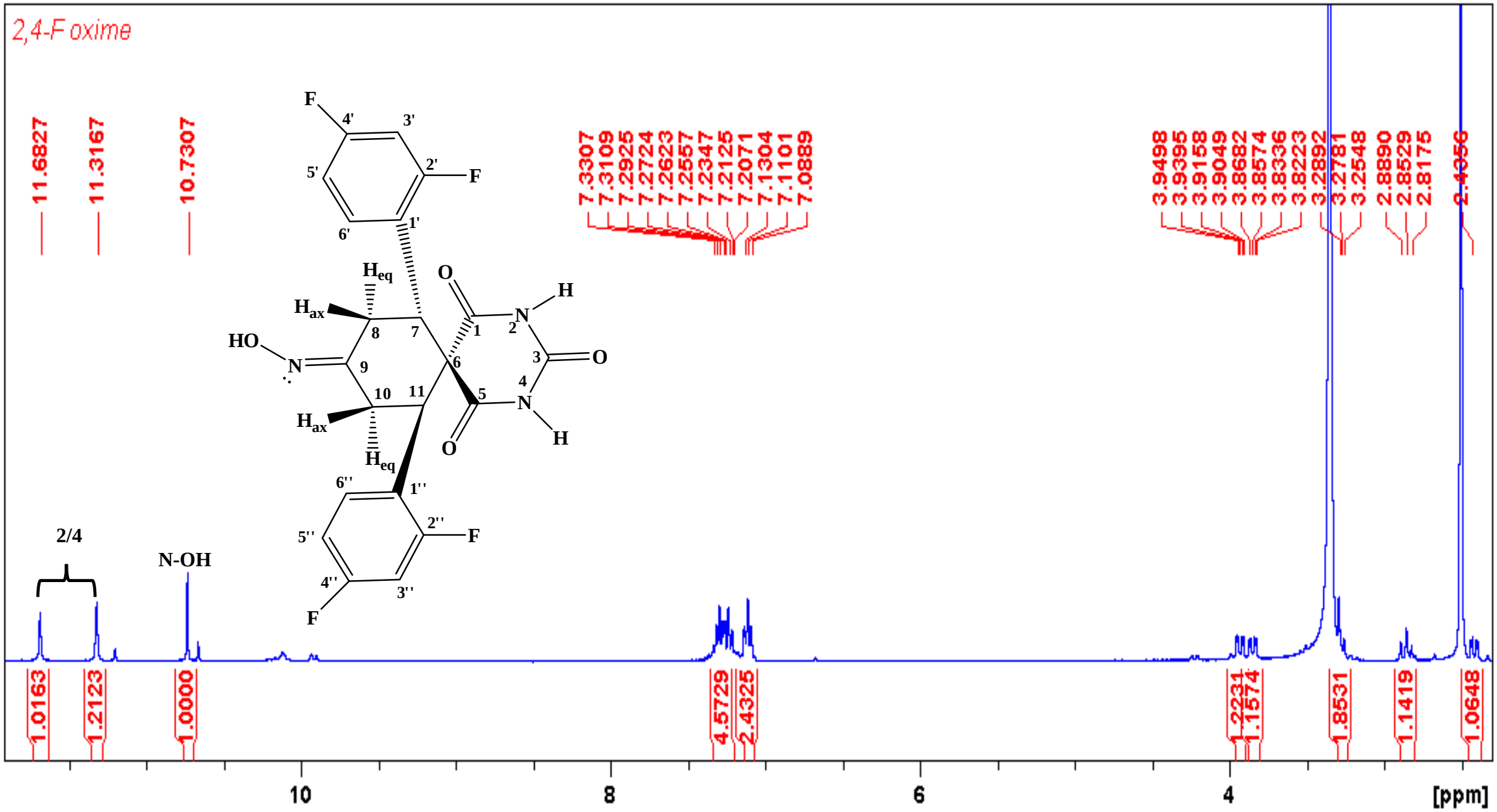
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
61 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 1-5 F: 2-5
35 4-F oxime 51 (1.687) Cm (1:61)
TOF MS ES-

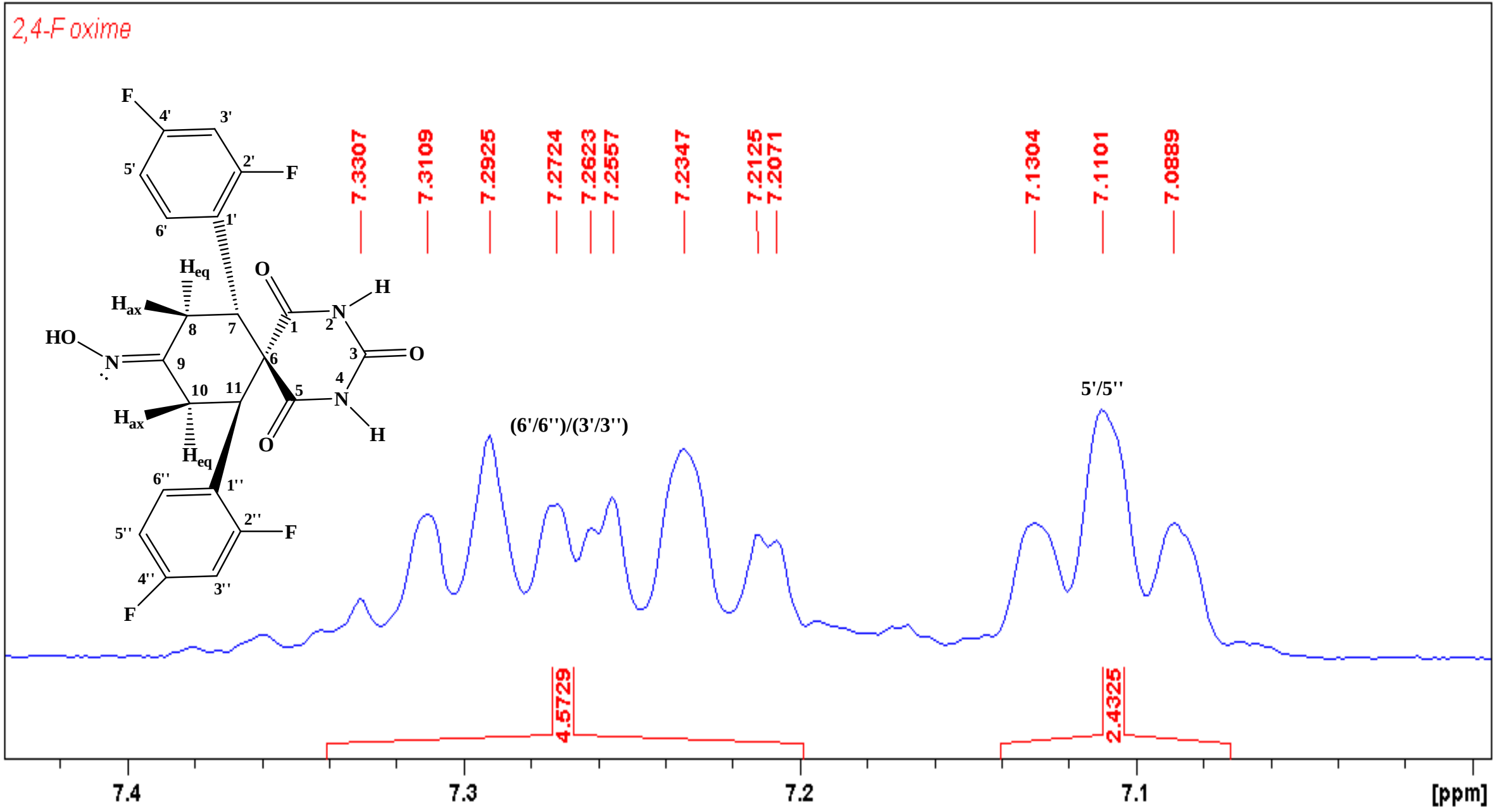


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
412.1113	412.1109	0.4	1.0	14.5	9.2	0.0	C21 H16 N3 O4 F2

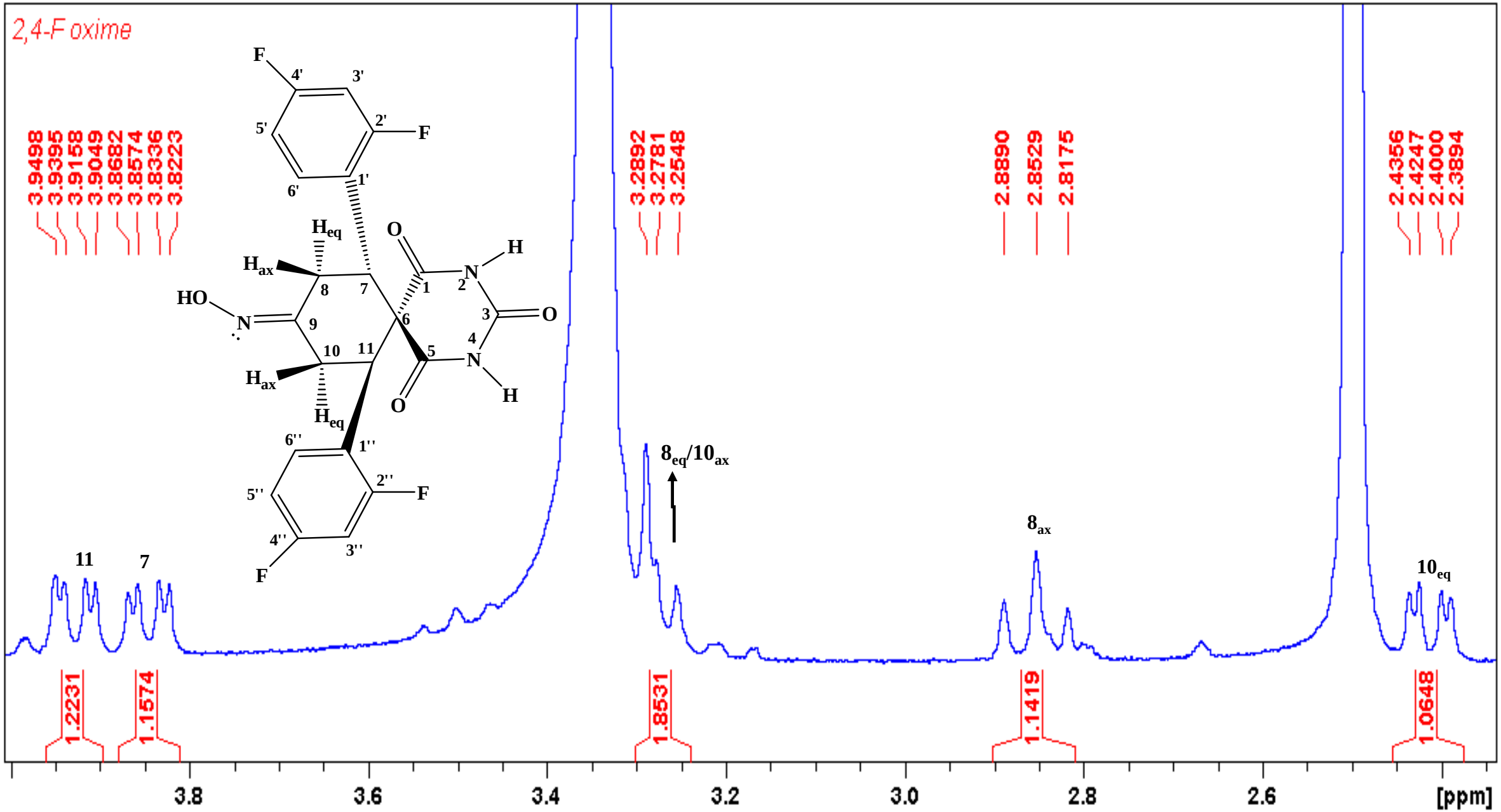
HRMS Spectrum of Compound B-4h: 7,11-bis(4-fluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



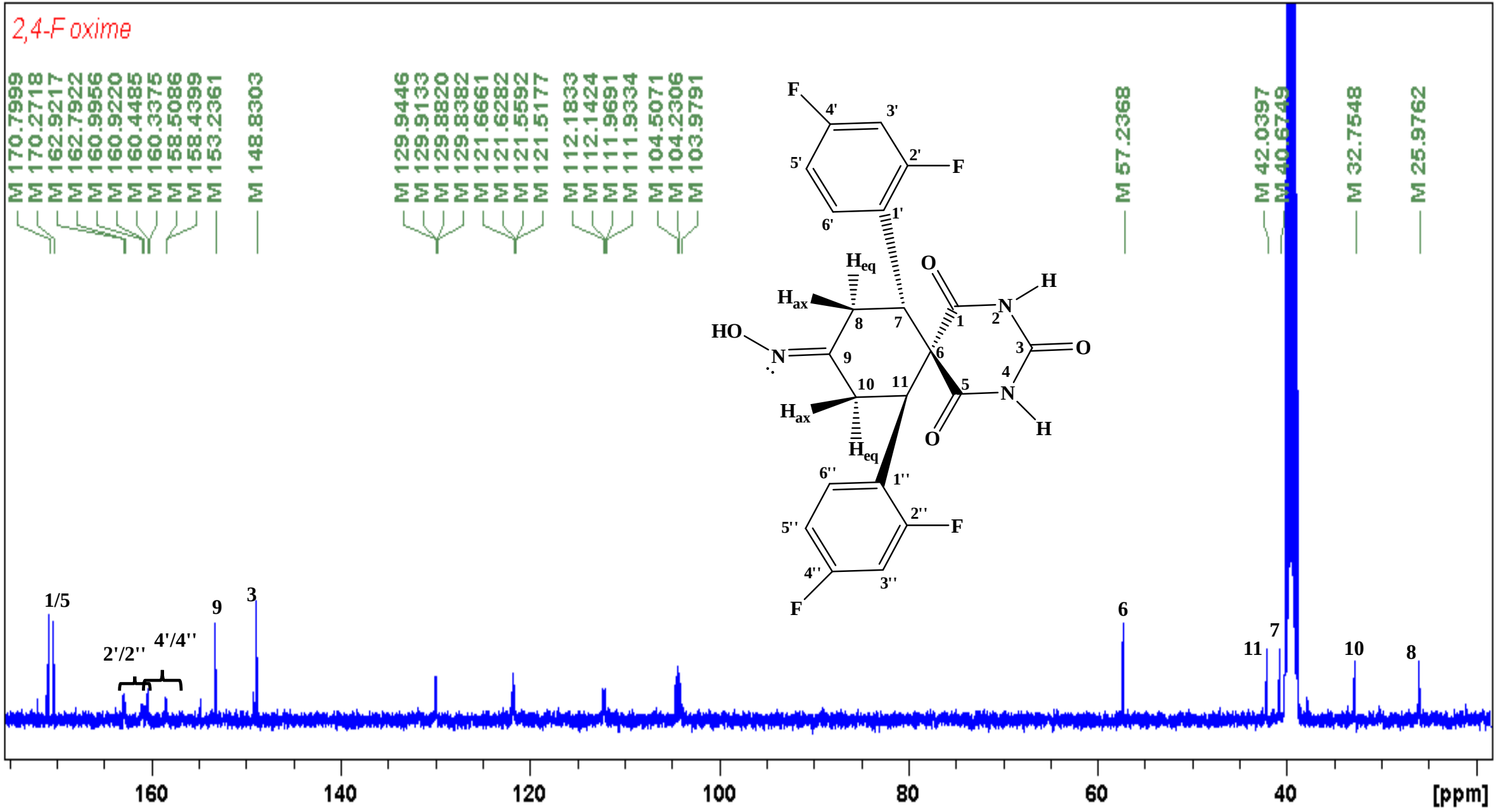
[1]H Spectrum of Compound B-4i: 7,11-bis(2,4-difluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



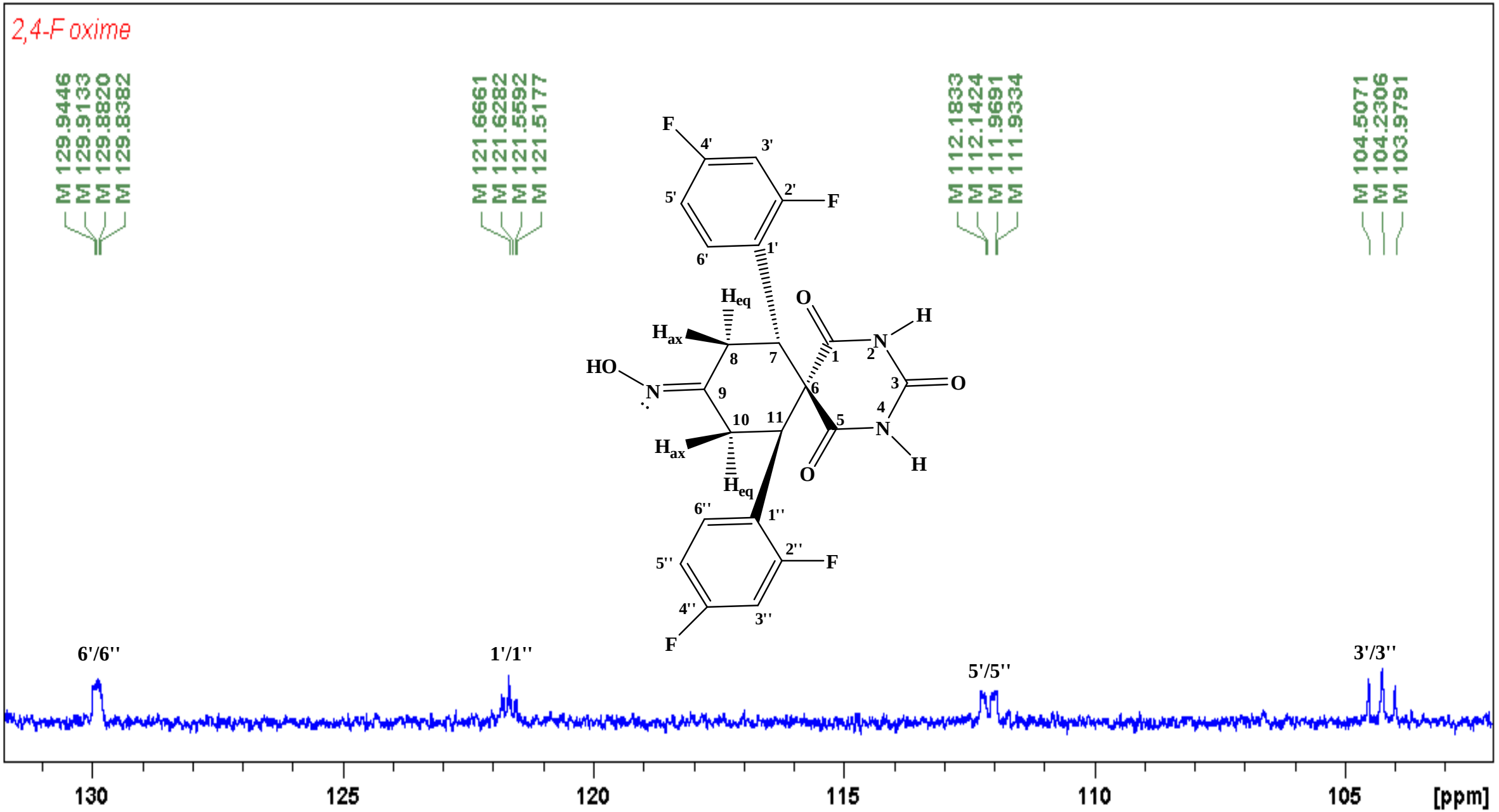
Expanded ^{1}H Spectrum of Compound B-4i: 7,11-bis(2,4-difluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



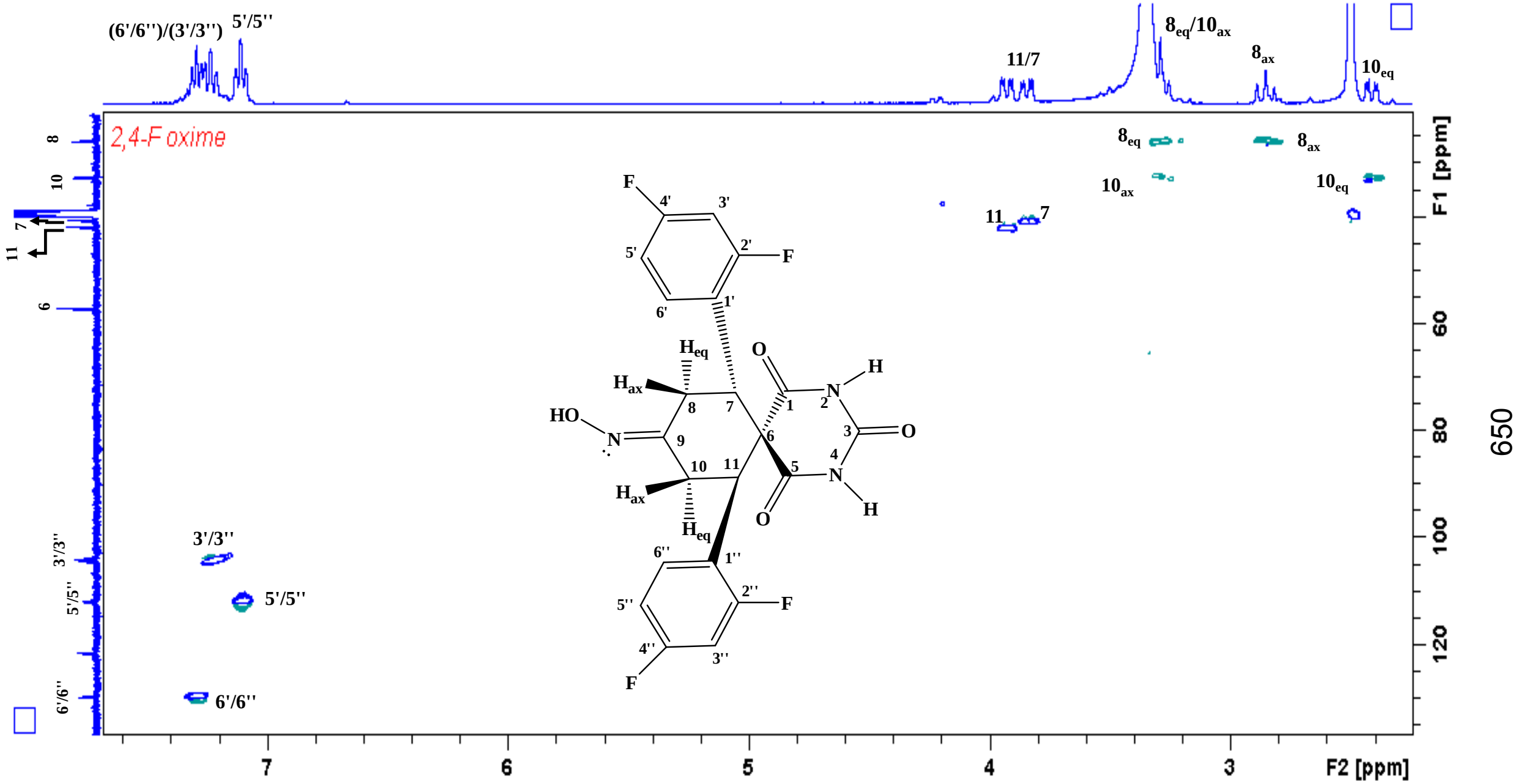
Expanded ^{1}H Spectrum of Compound B-4i: 7,11-bis(2,4-difluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



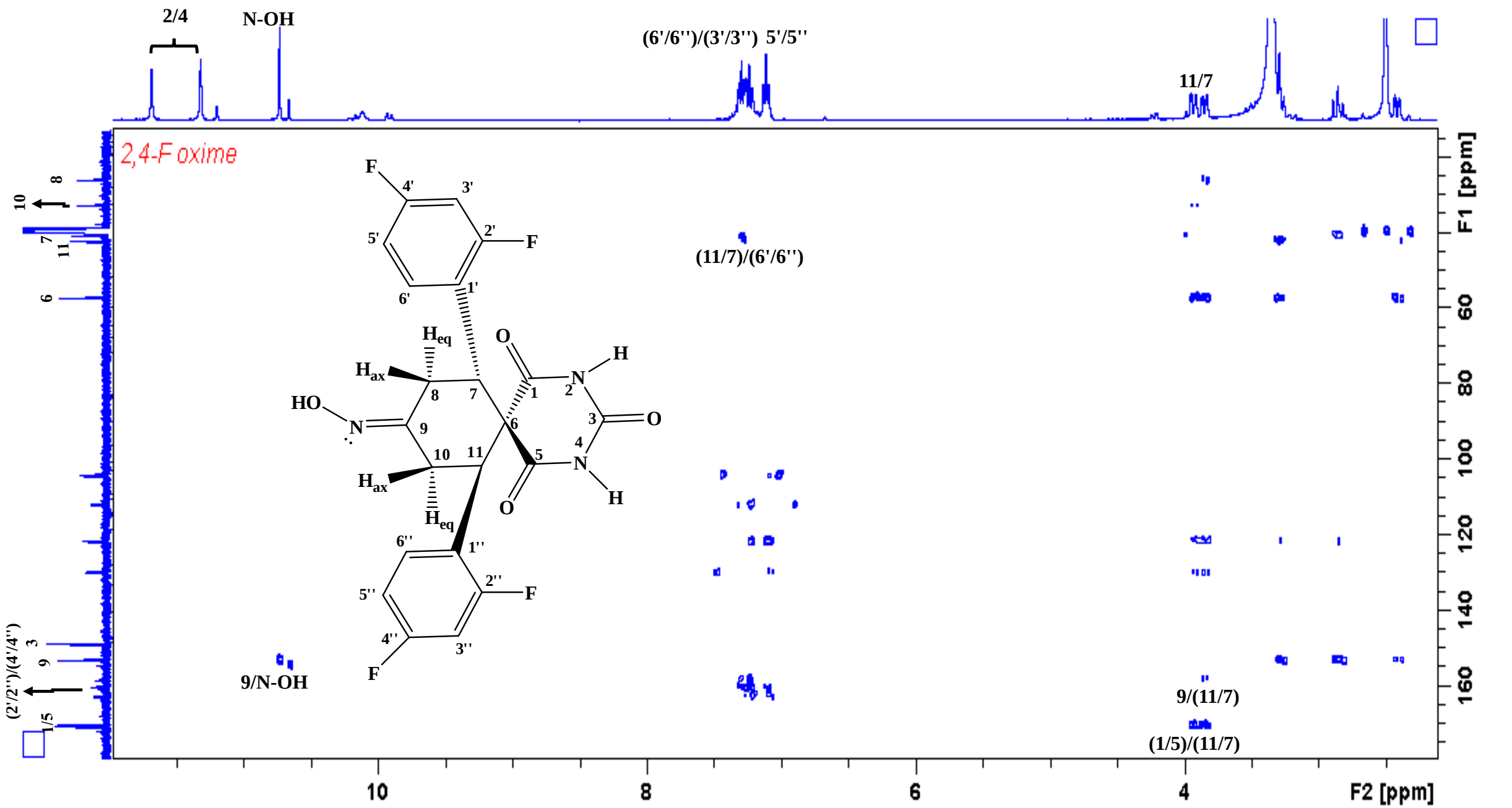
^{13}C Spectrum of Compound B-4i: 7,11-bis(2,4-difluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Expanded ^{13}C Spectrum of Compound B-4i: 7,11-bis(2,4-difluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

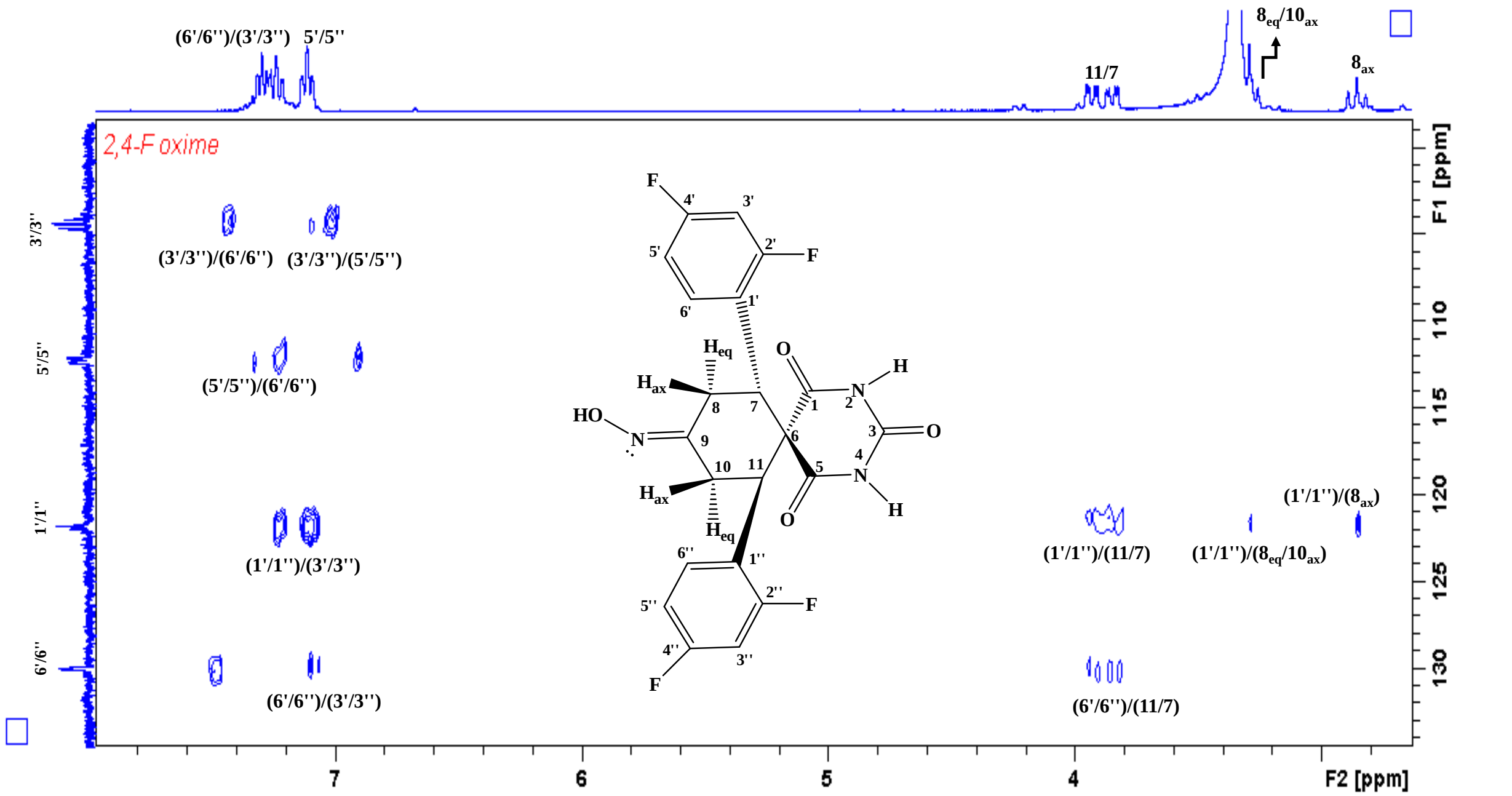


HSQC Spectrum of Compound B-4i: 7,11-bis(2,4-difluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

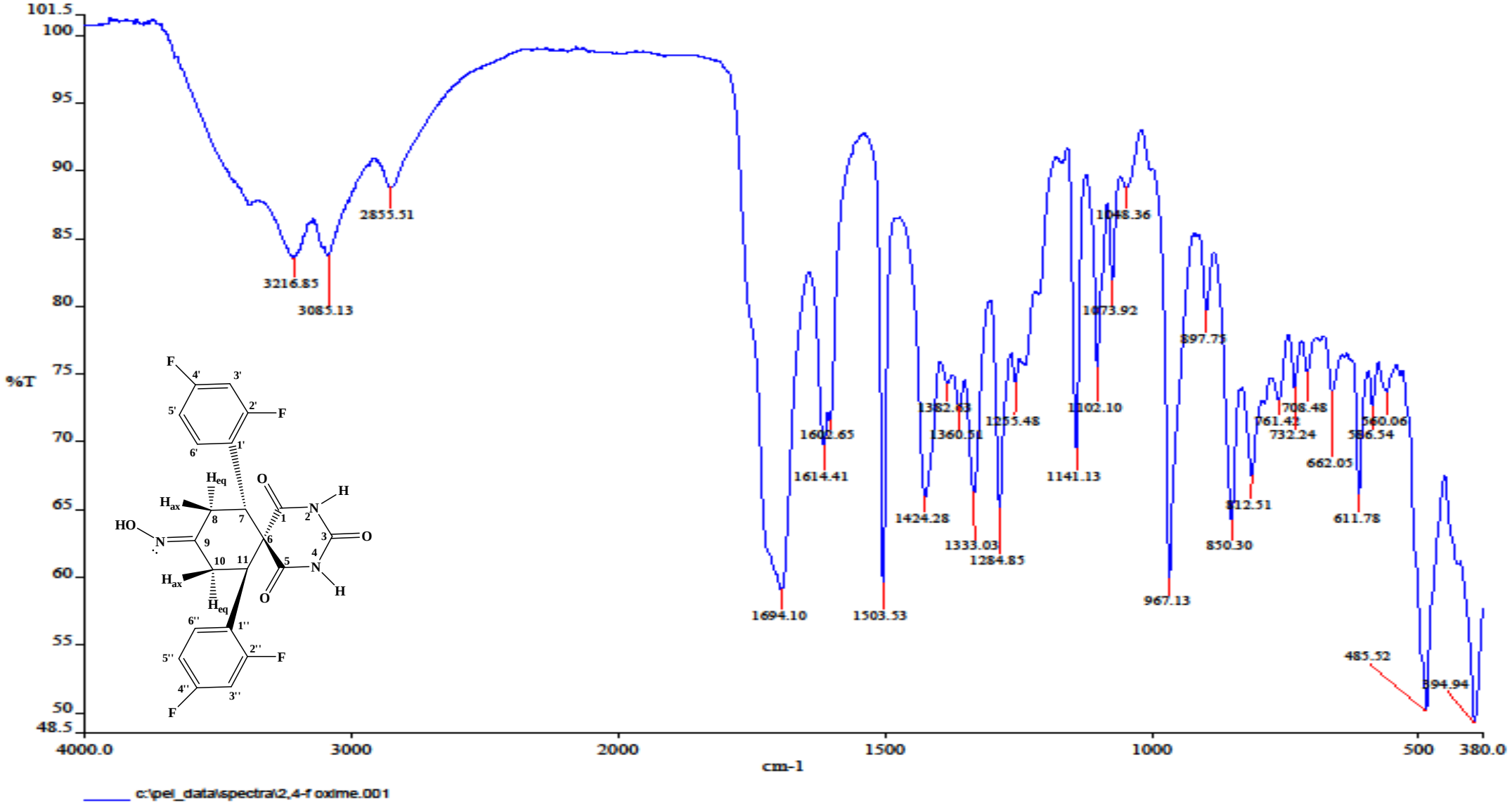


651

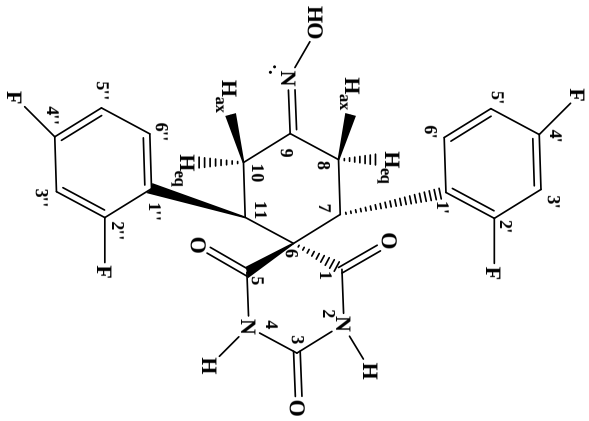
HMBC Spectrum of Compound B-4i: 7,11-bis(2,4-difluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Expanded HMBC Spectrum of Compound B-4i: 7,11-bis(2,4-difluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



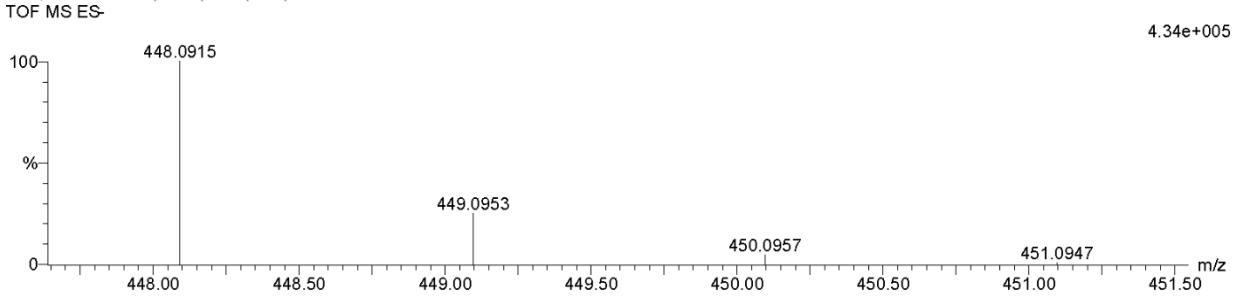
Infrared Spectrum of Compound B-4i: 7,11-bis(2,4-difluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Elemental Composition Report **Page 1**

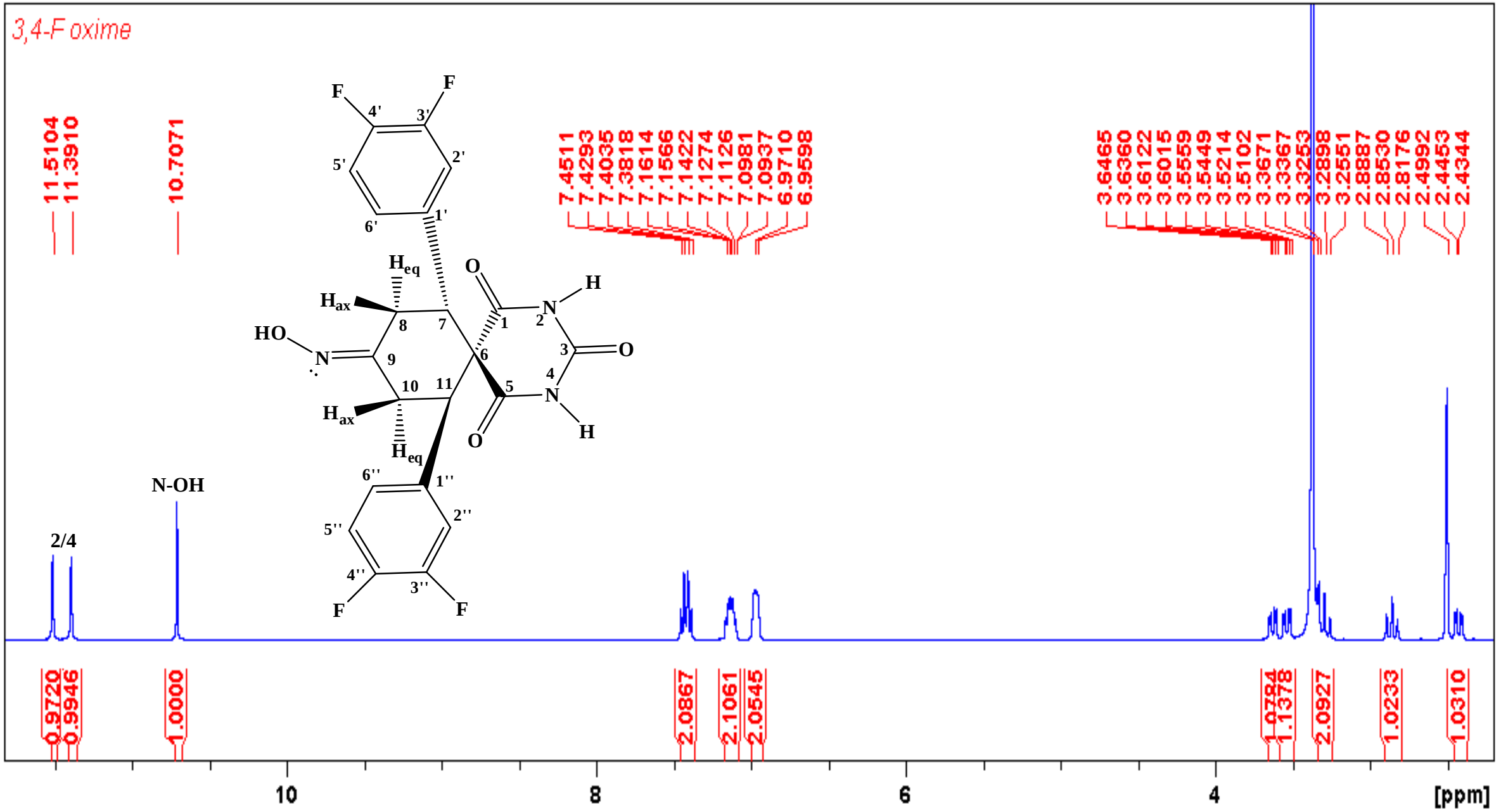
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
27 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 10-15 N: 0-5 O: 1-5 F: 4-5
36 2,4-F oxime 42 (1.383) Cm (1:61)
TOF MS ES-



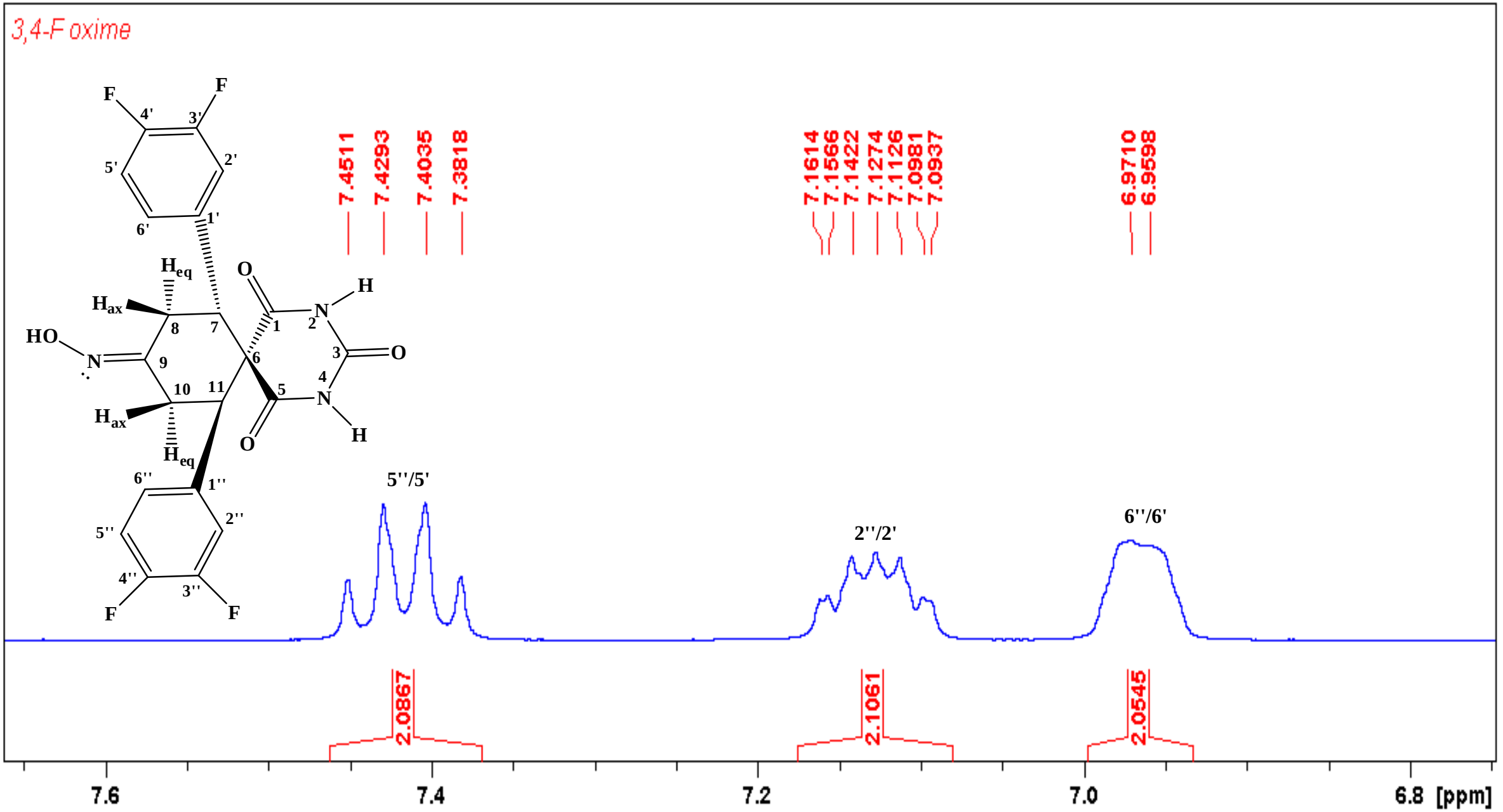
Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
448.0915	448.0920	-0.5	-1.1	14.5	11.3	0.0	C21 H14 N3 O4 F4

HRMS Spectrum of Compound B-4i: 7,11-bis(2,4-difluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

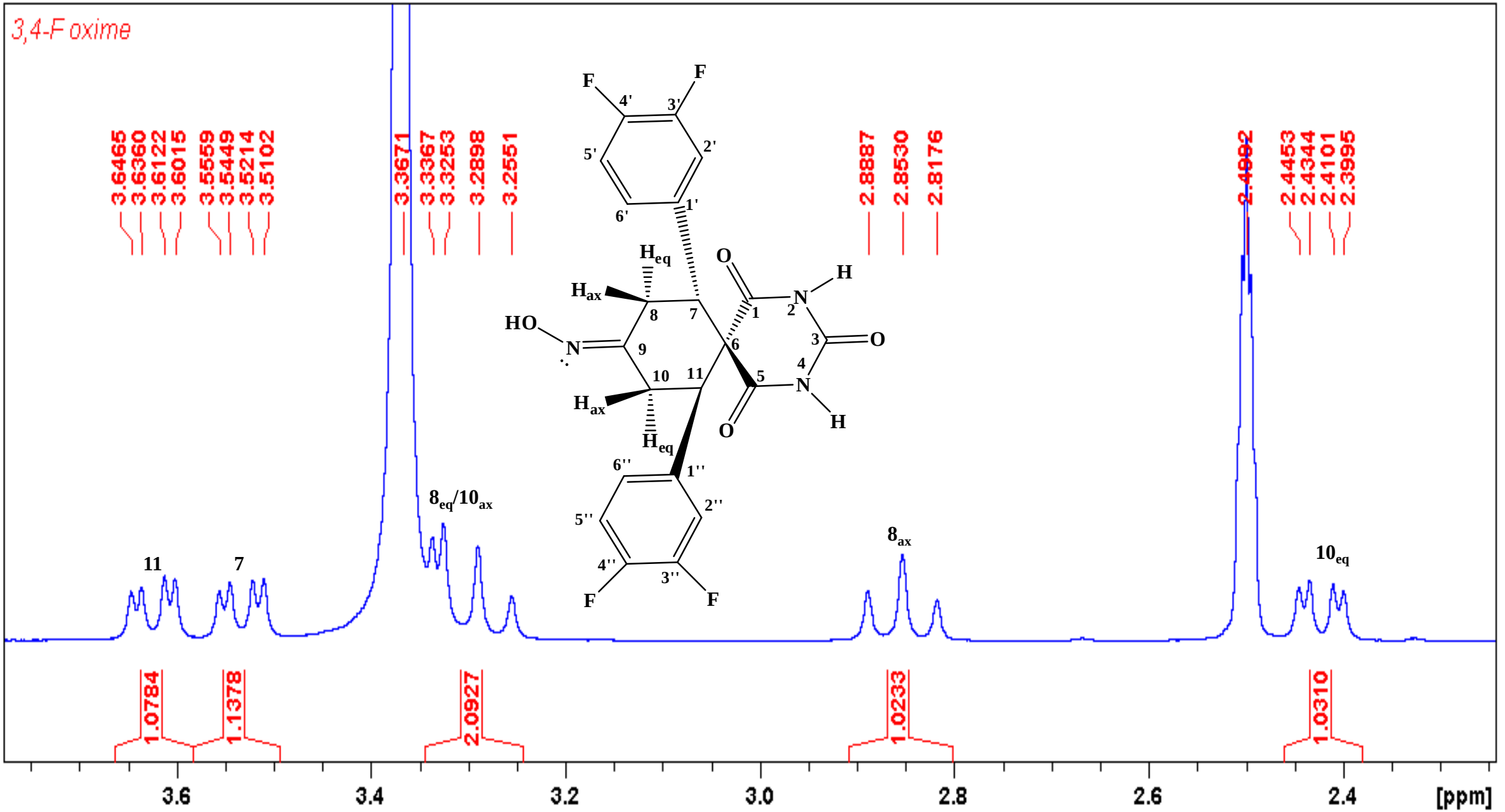


655

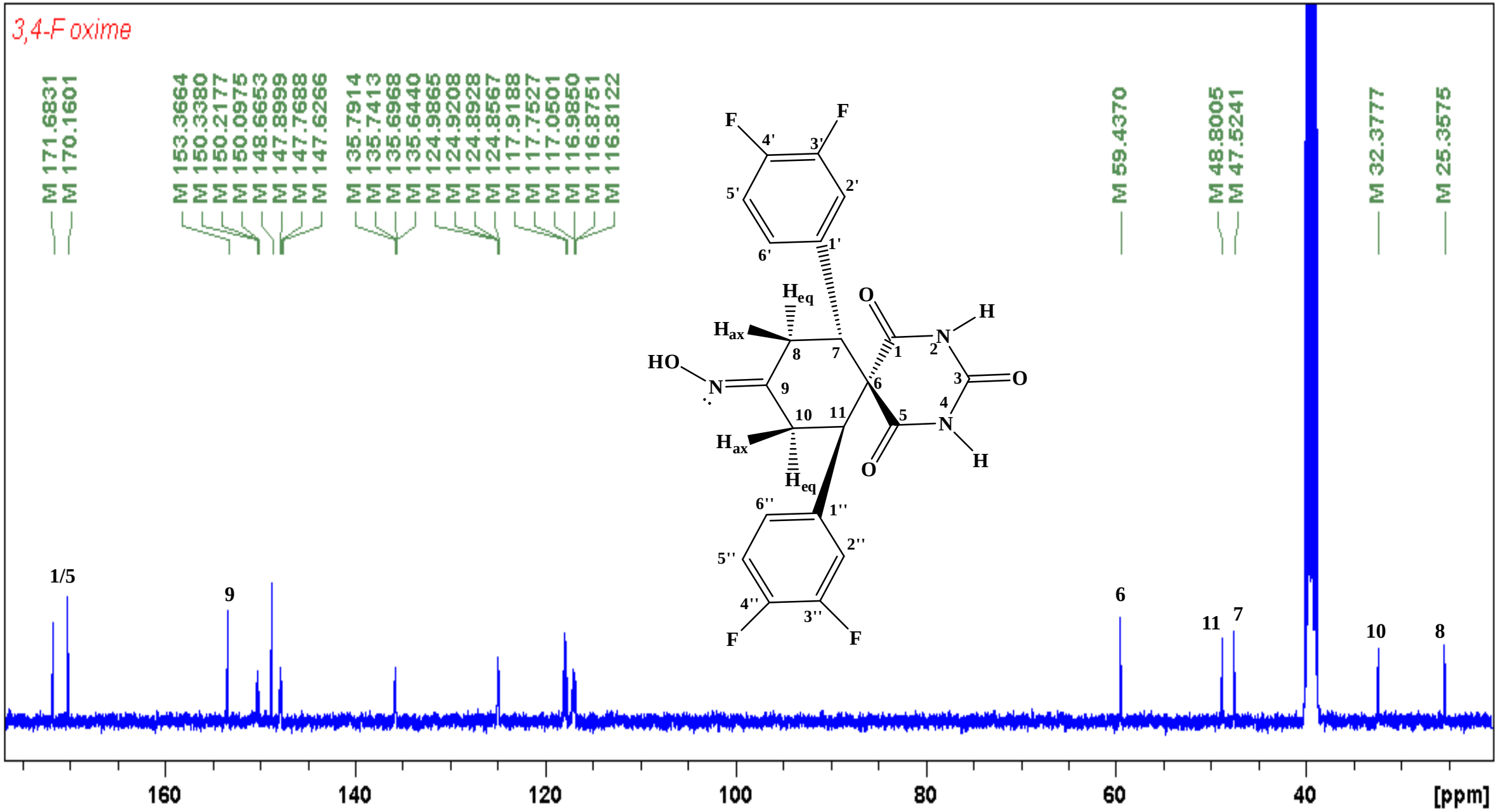
[1]H Spectrum of Compound B-4j: 7,11-bis(3,4-difluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



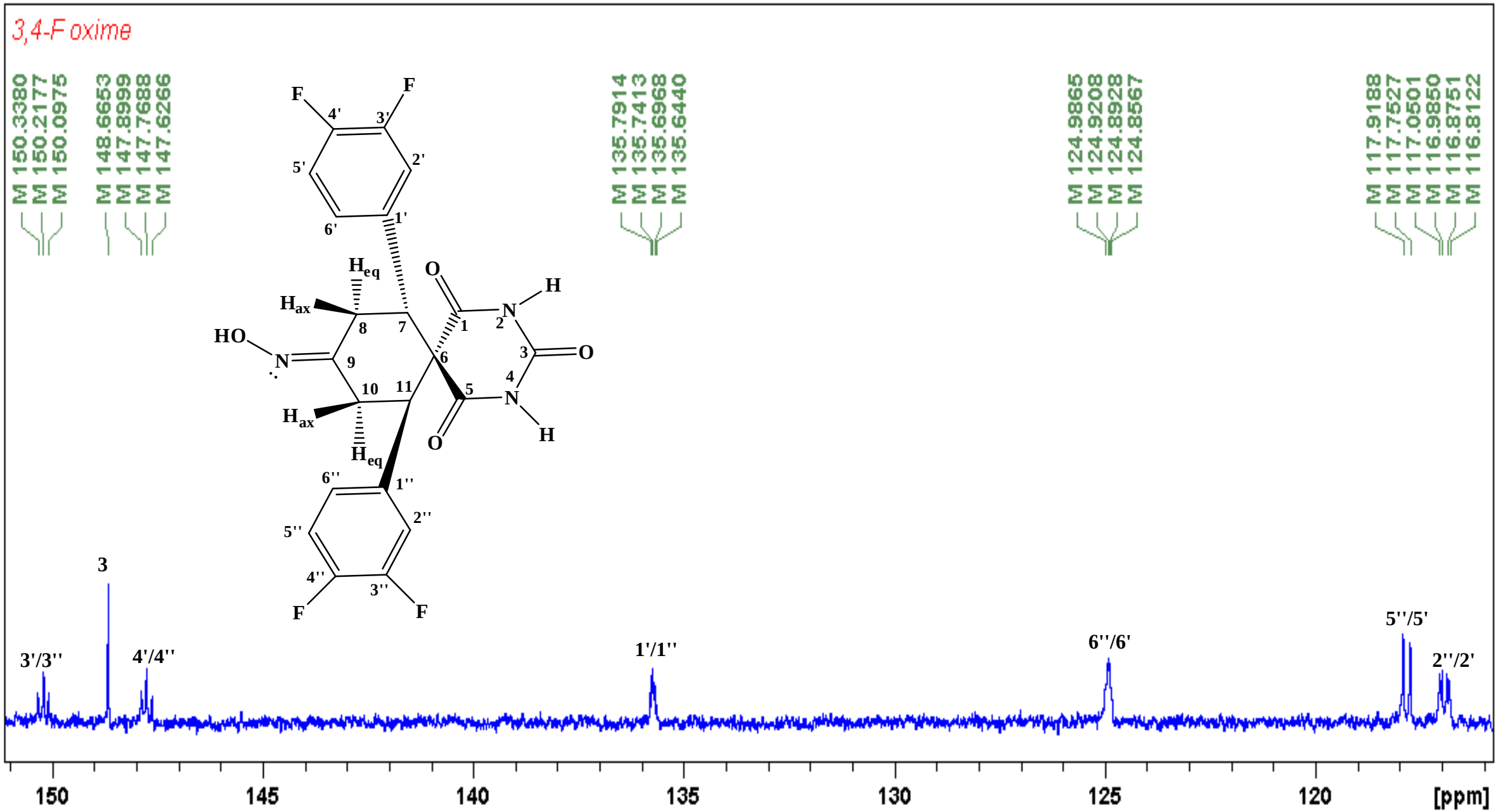
Expanded ^{1}H Spectrum of Compound B-4j: 7,11-bis(3,4-difluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



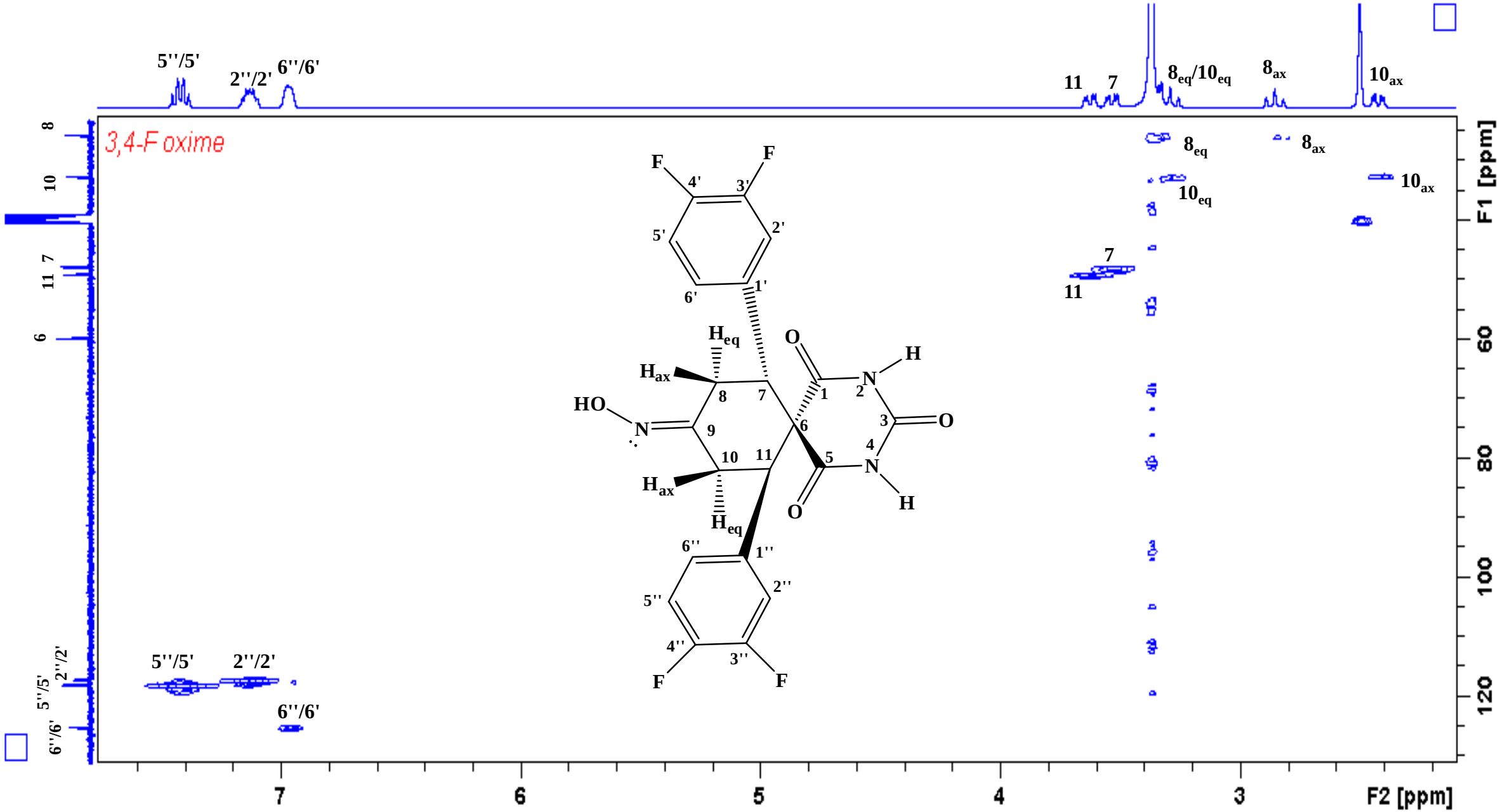
Expanded ^{1}H Spectrum of Compound B-4j: 7,11-bis(3,4-difluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



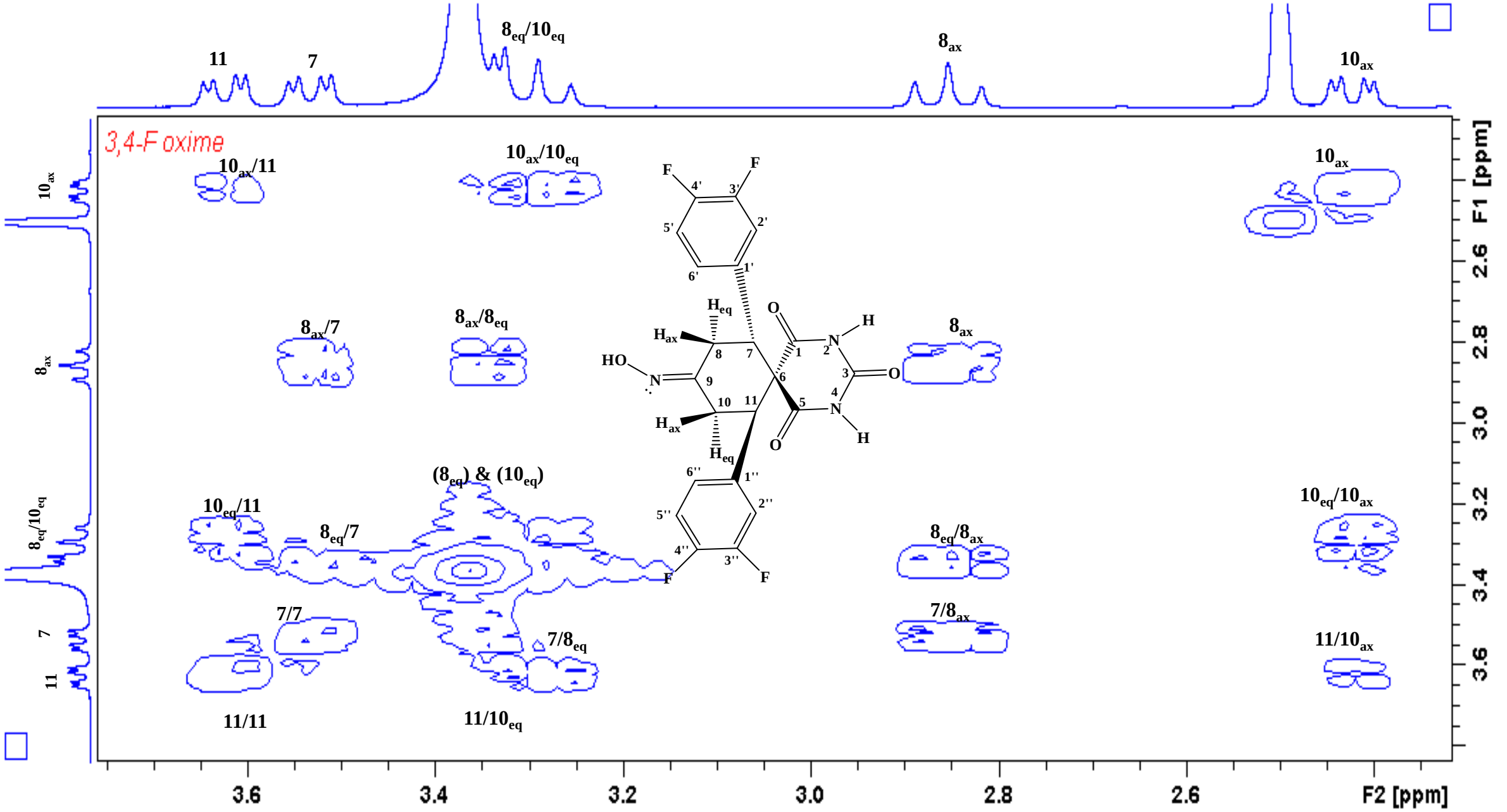
^{13}C Spectrum of Compound B-4j: 7,11-bis(3,4-difluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



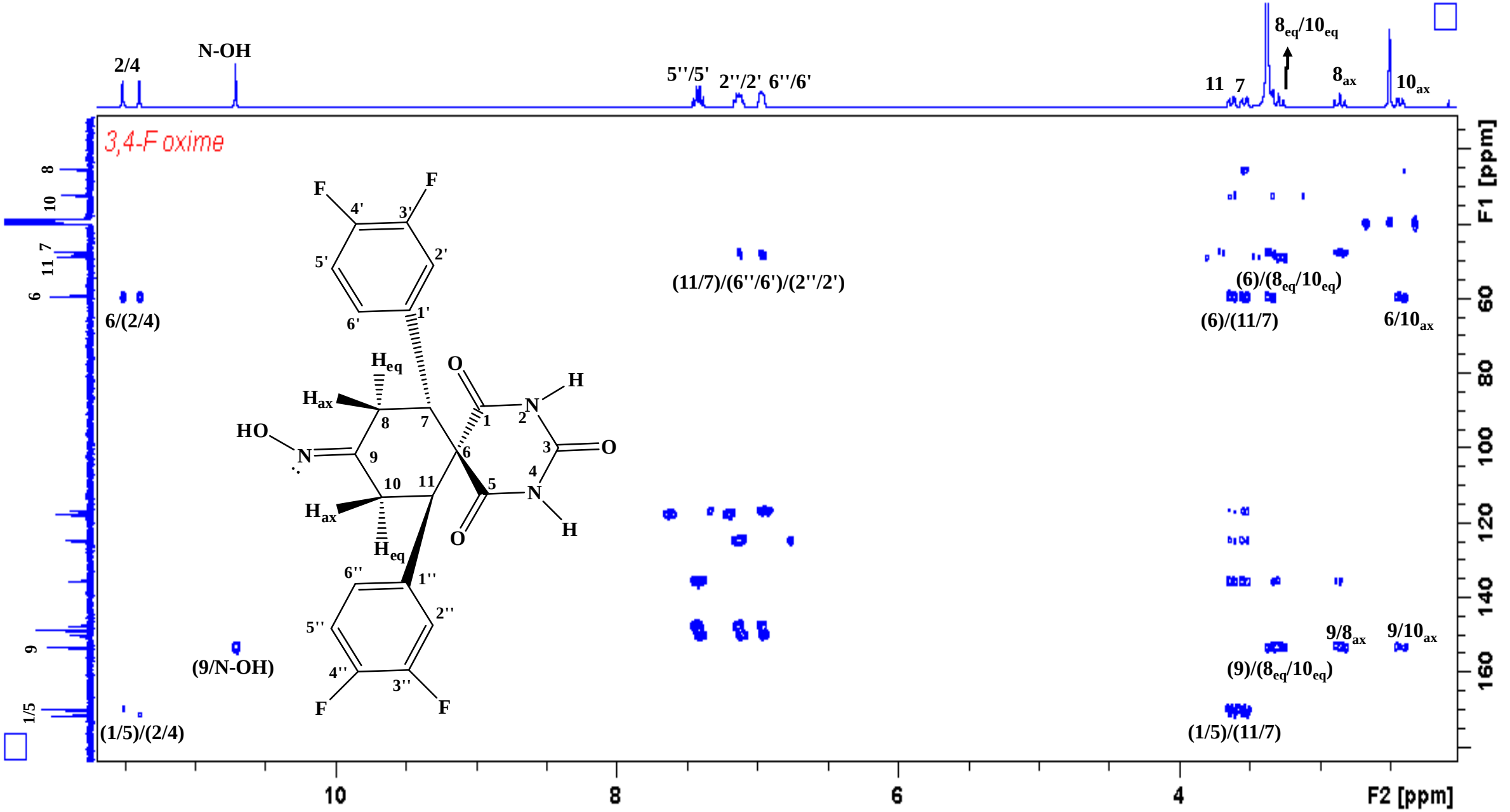
Expanded ^{13}C Spectrum of Compound B-4j: 7,11-bis(3,4-difluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



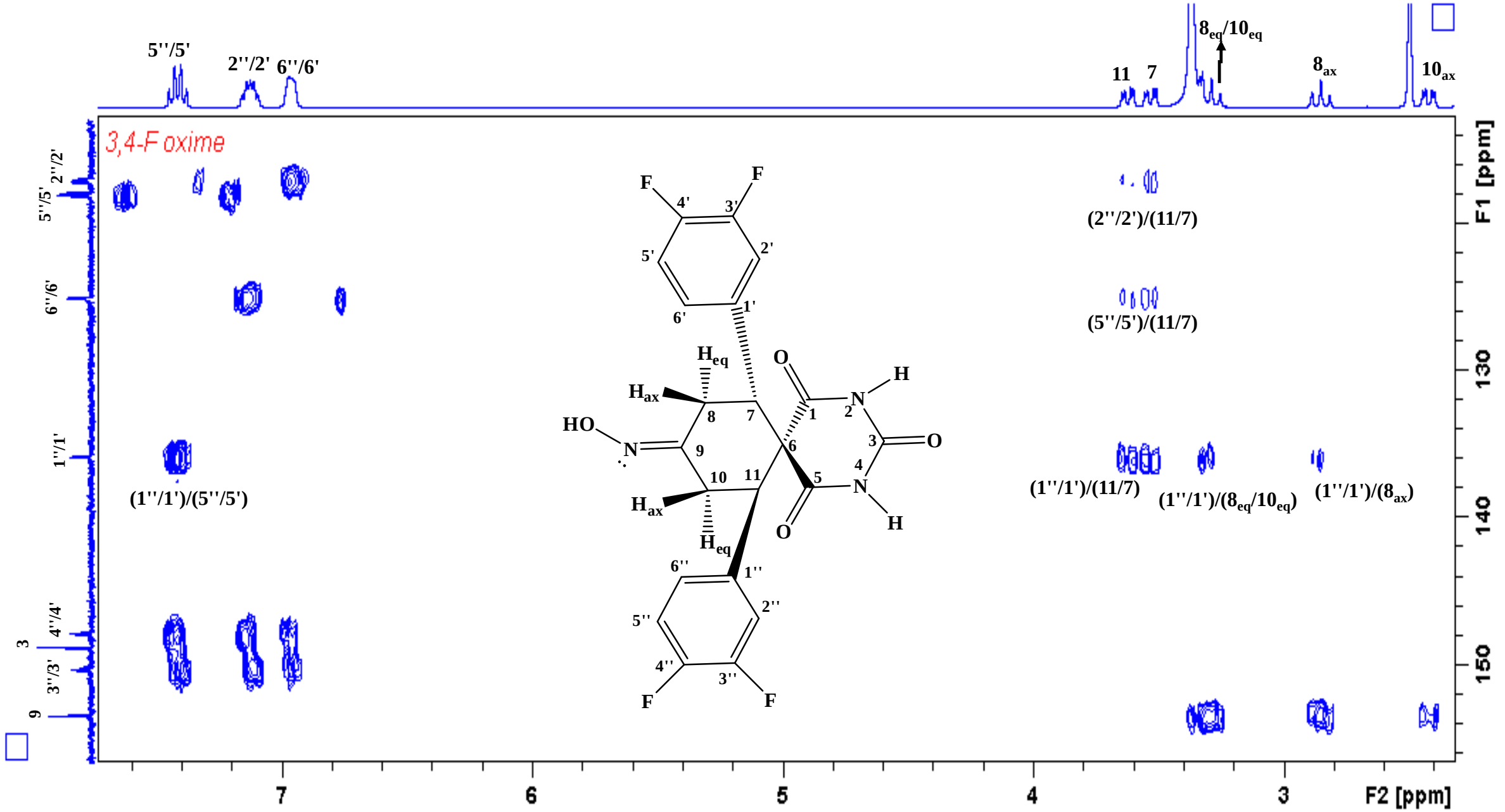
HSQC Spectrum of Compound B-4j: 7,11-bis(3,4-difluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



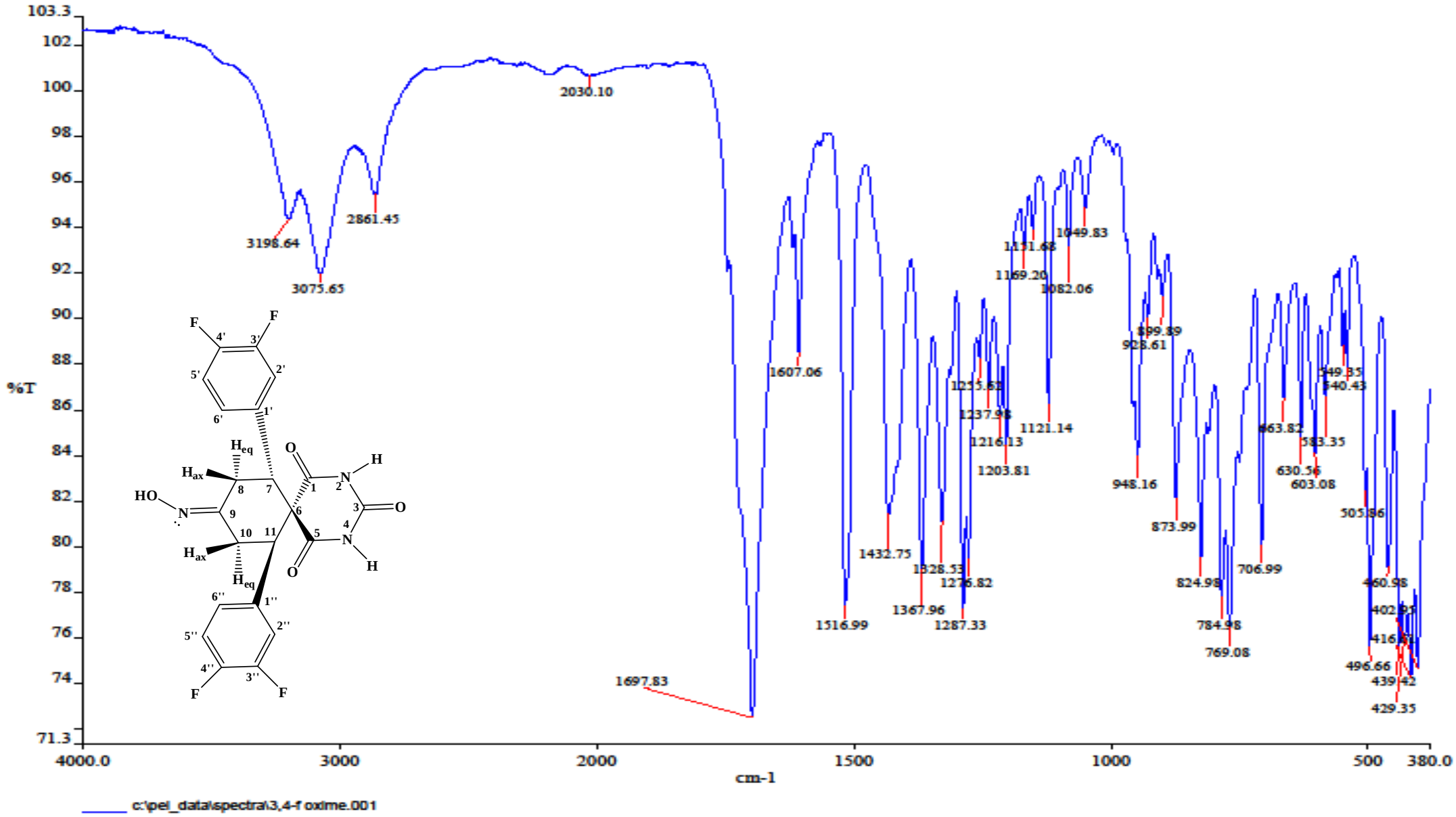
COSY Spectrum of Compound B-4j: 7,11-bis(3,4-difluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



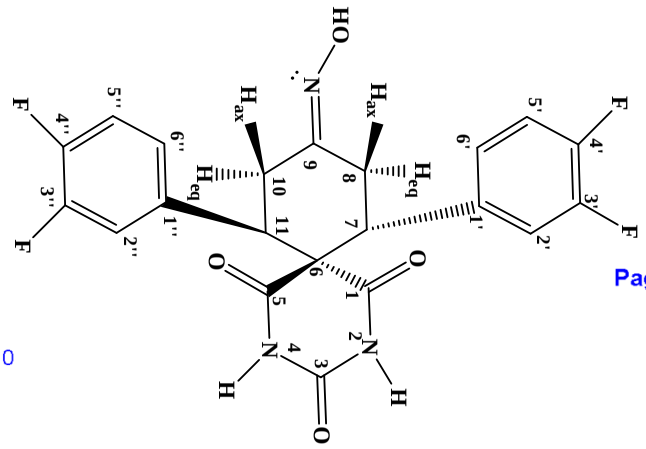
HMBC Spectrum of Compound B-4j: 7,11-bis(3,4-difluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Expanded HMBC Spectrum of Compound B-4j: 7,11-bis(3,4-difluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



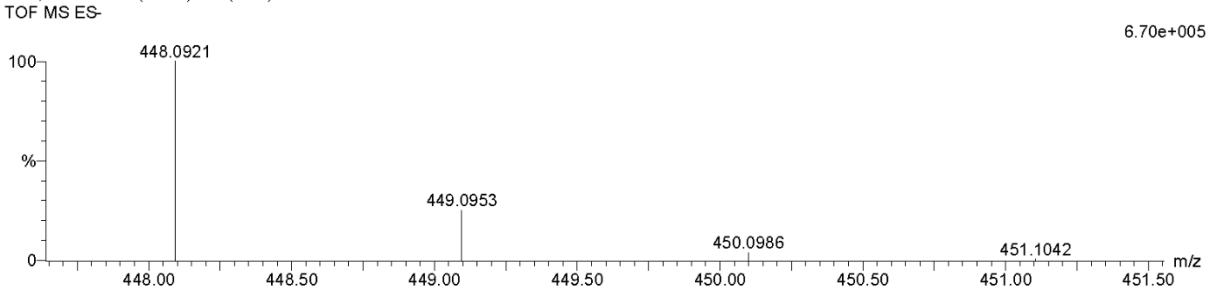
Infrared Spectrum of Compound B-4j: 7,11-bis(3,4-difluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Elemental Composition Report **Page 1**

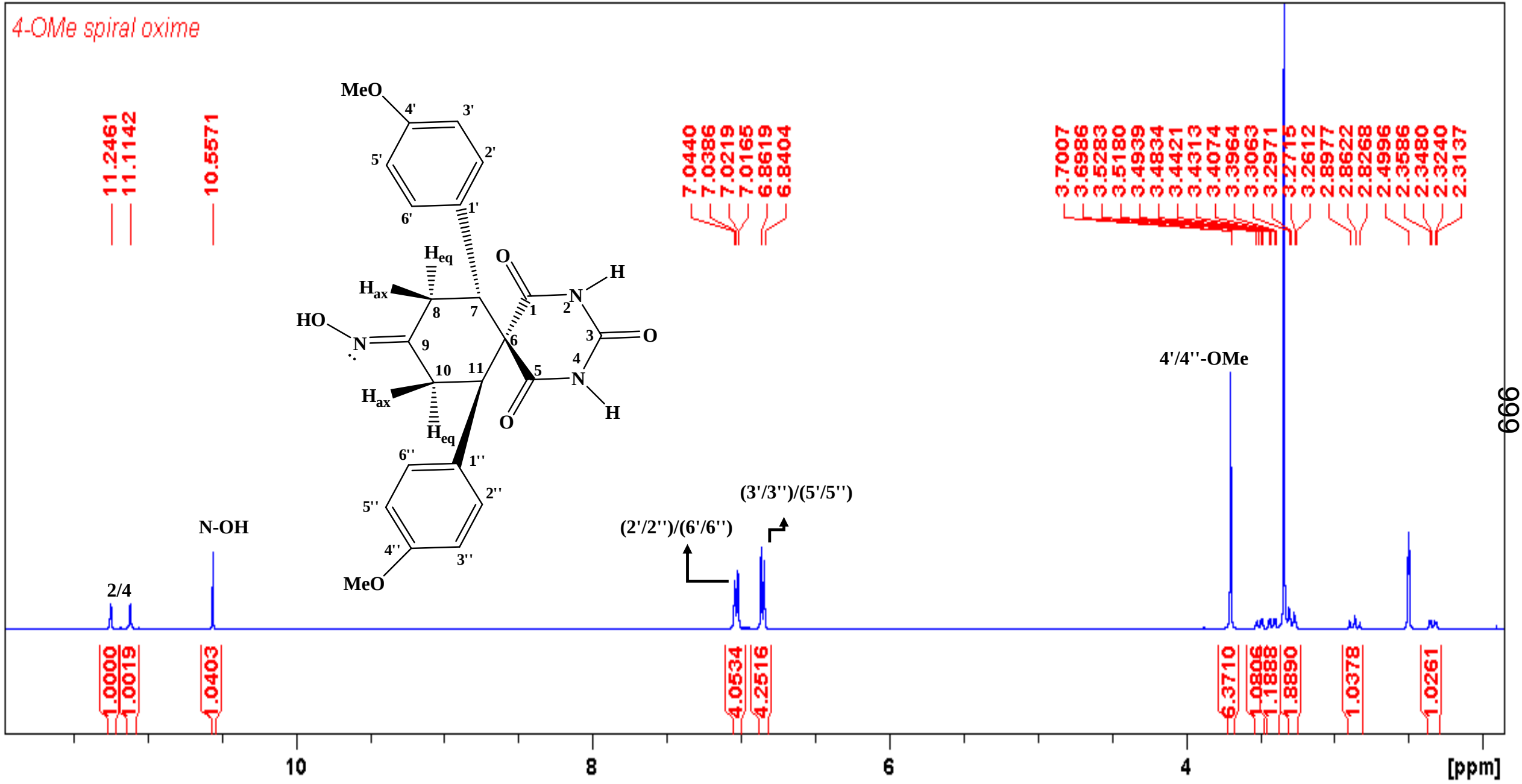
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
27 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 10-15 N: 0-5 O: 1-5 F: 4-5
37 3,4-F oxime 4 (0.102) Cm (1:61)
TOF MS ES-

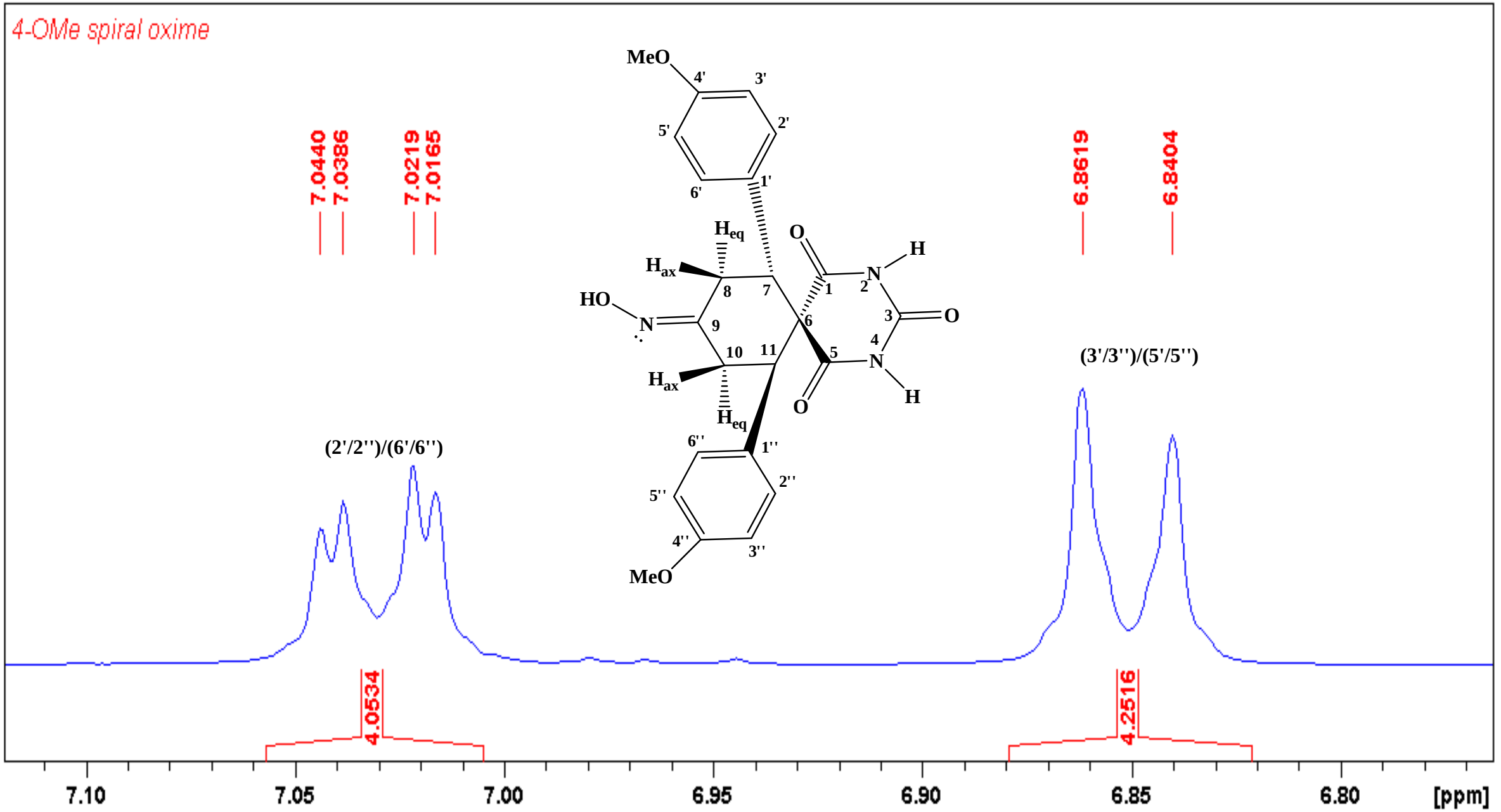


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
448.0921	448.0920	0.1	0.2	14.5	11.7	0.0	C21 H14 N3 O4 F4

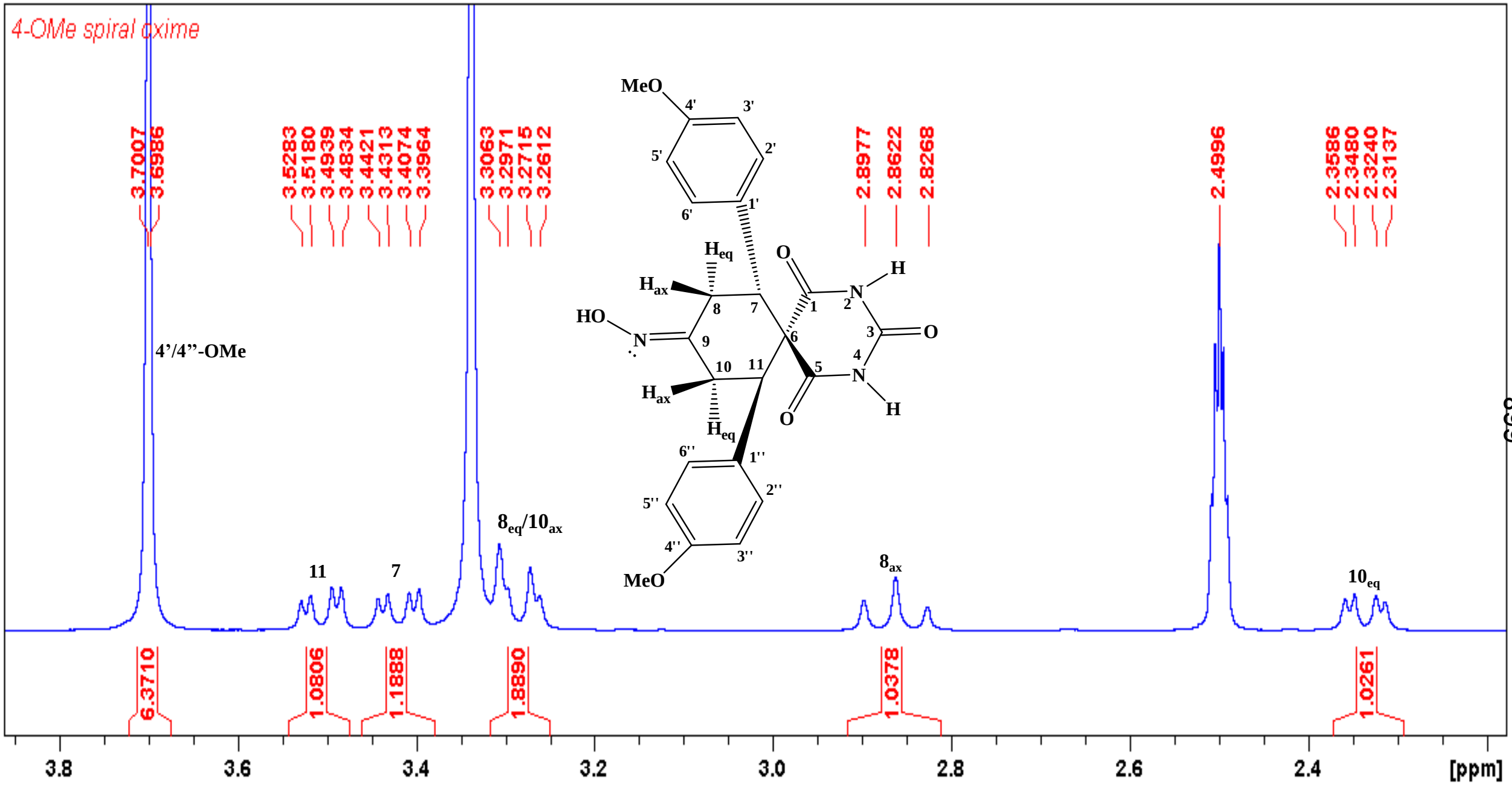
HRMS Spectrum of Compound B-4j: 7,11-bis(3,4-difluorophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



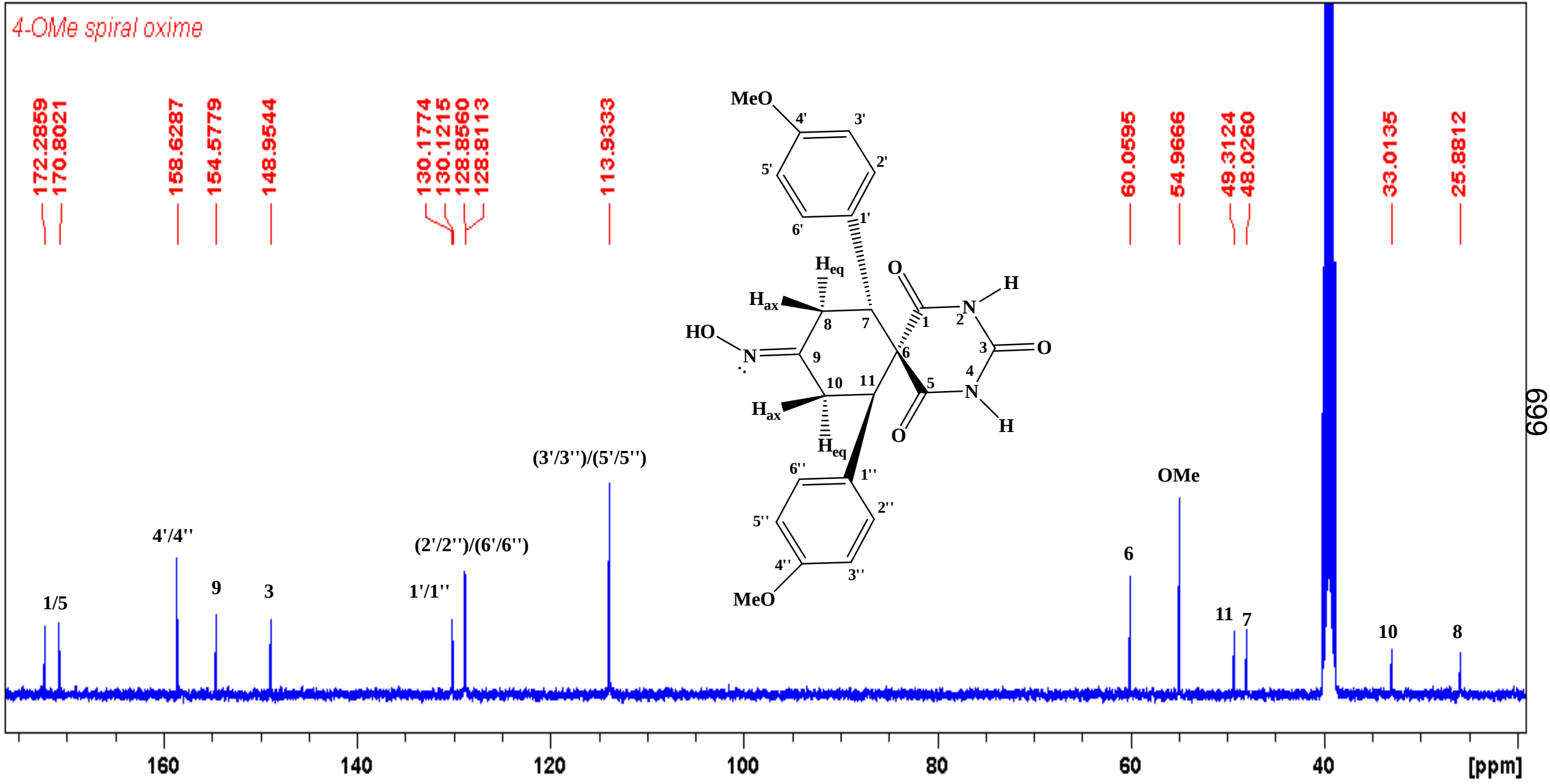
^{1}H Spectrum of Compound B-4l: 9-(hydroxyimino)-7,11-bis(4-methoxyphenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



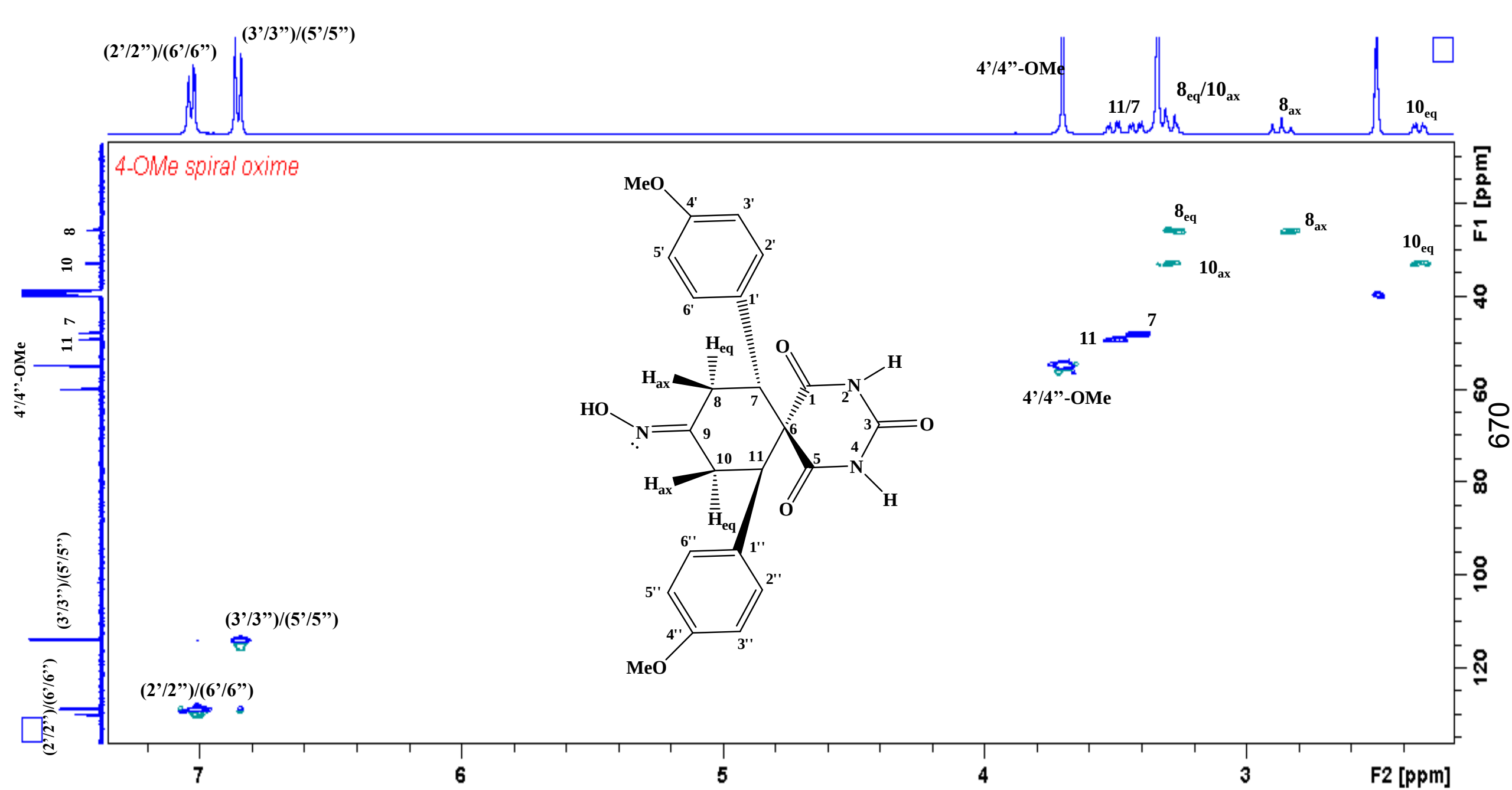
Expanded ^{1}H Spectrum of Compound B-4l: 9-(hydroxyimino)-7,11-bis(4-methoxyphenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



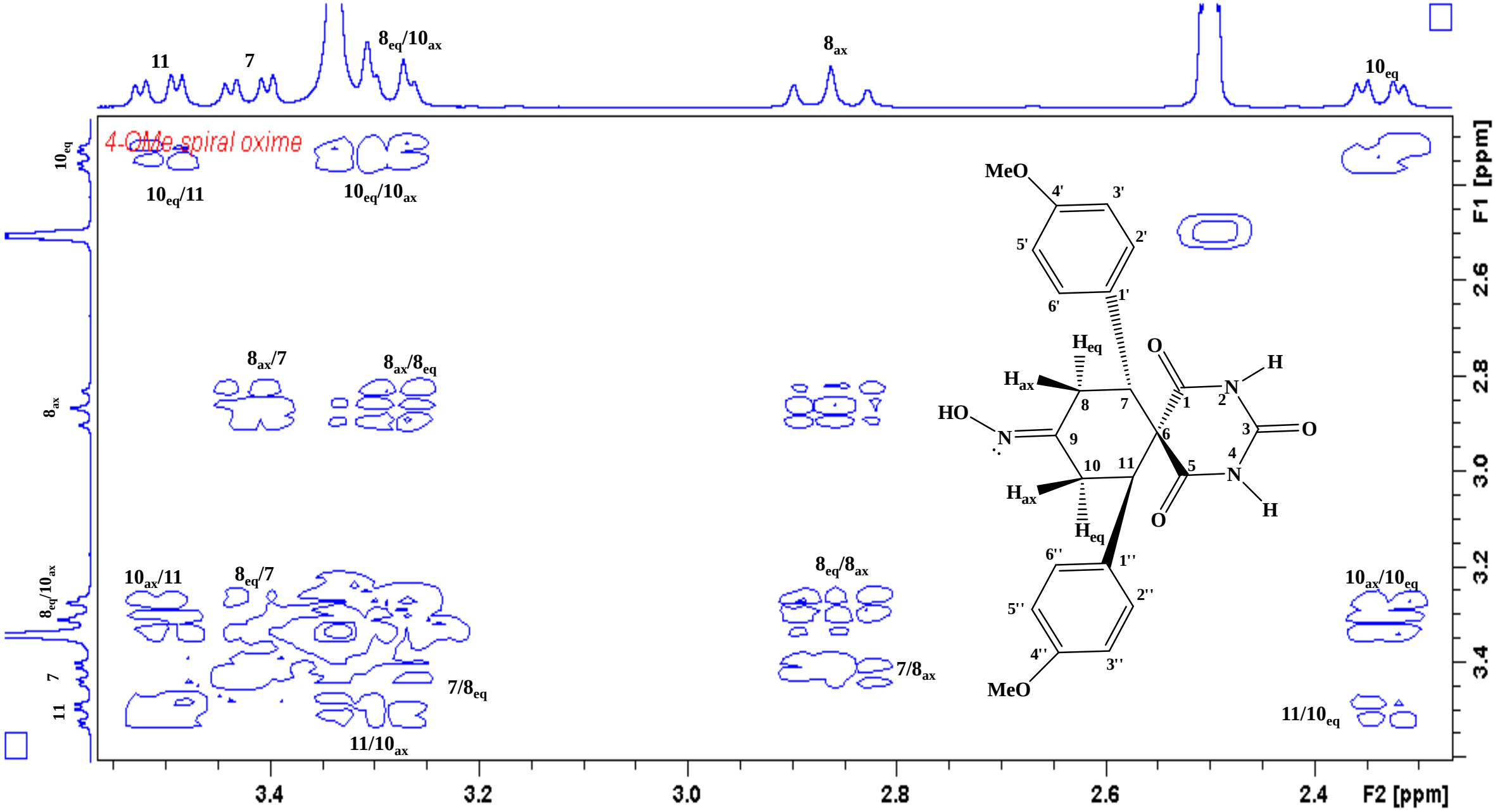
Expanded ^{1}H Spectrum of Compound B-4l: 9-(hydroxyimino)-7,11-bis(4-methoxyphenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



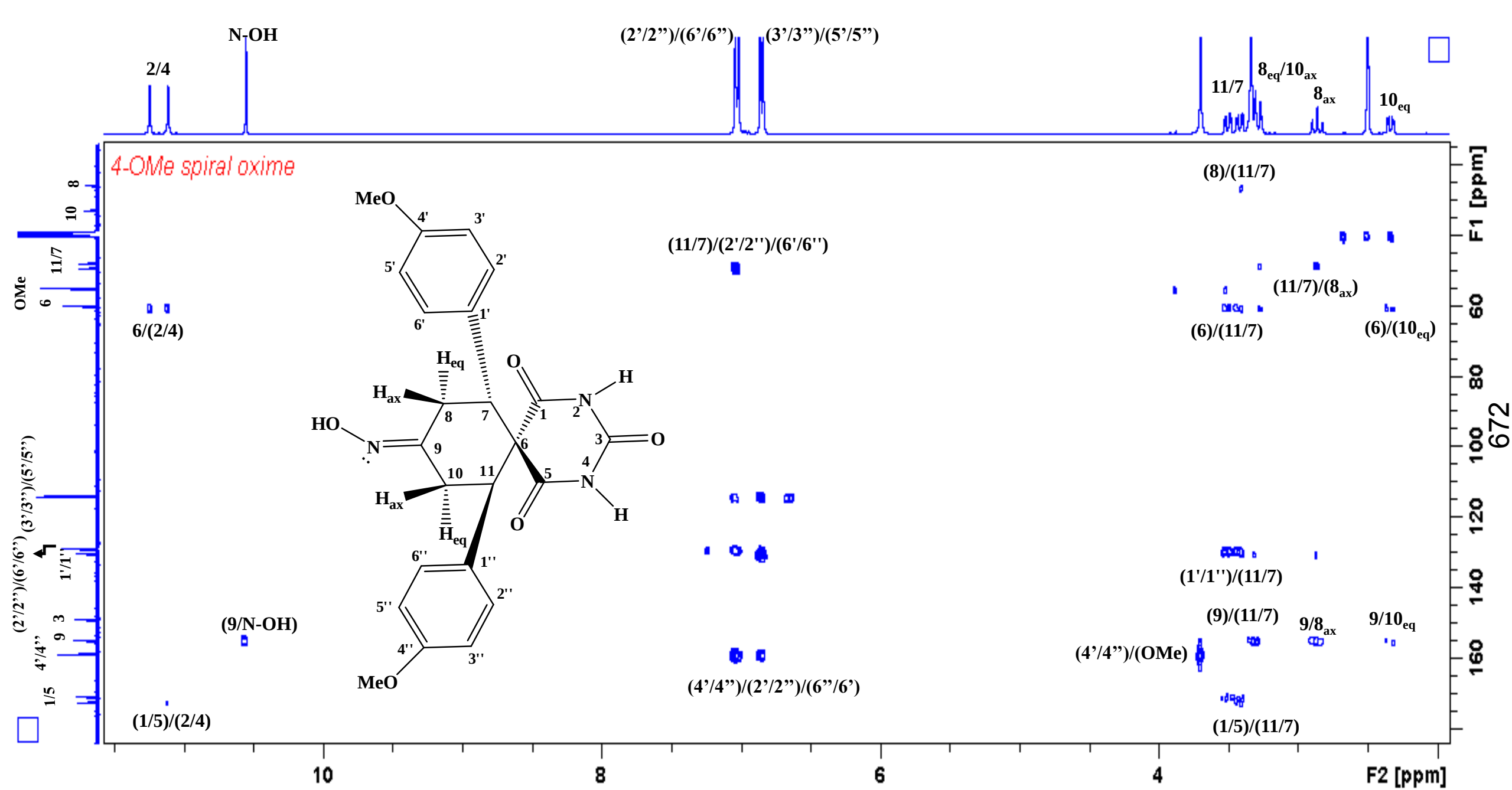
^{13}C Spectrum of Compound B-4l: 9-(hydroxyimino)-7,11-bis(4-methoxyphenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



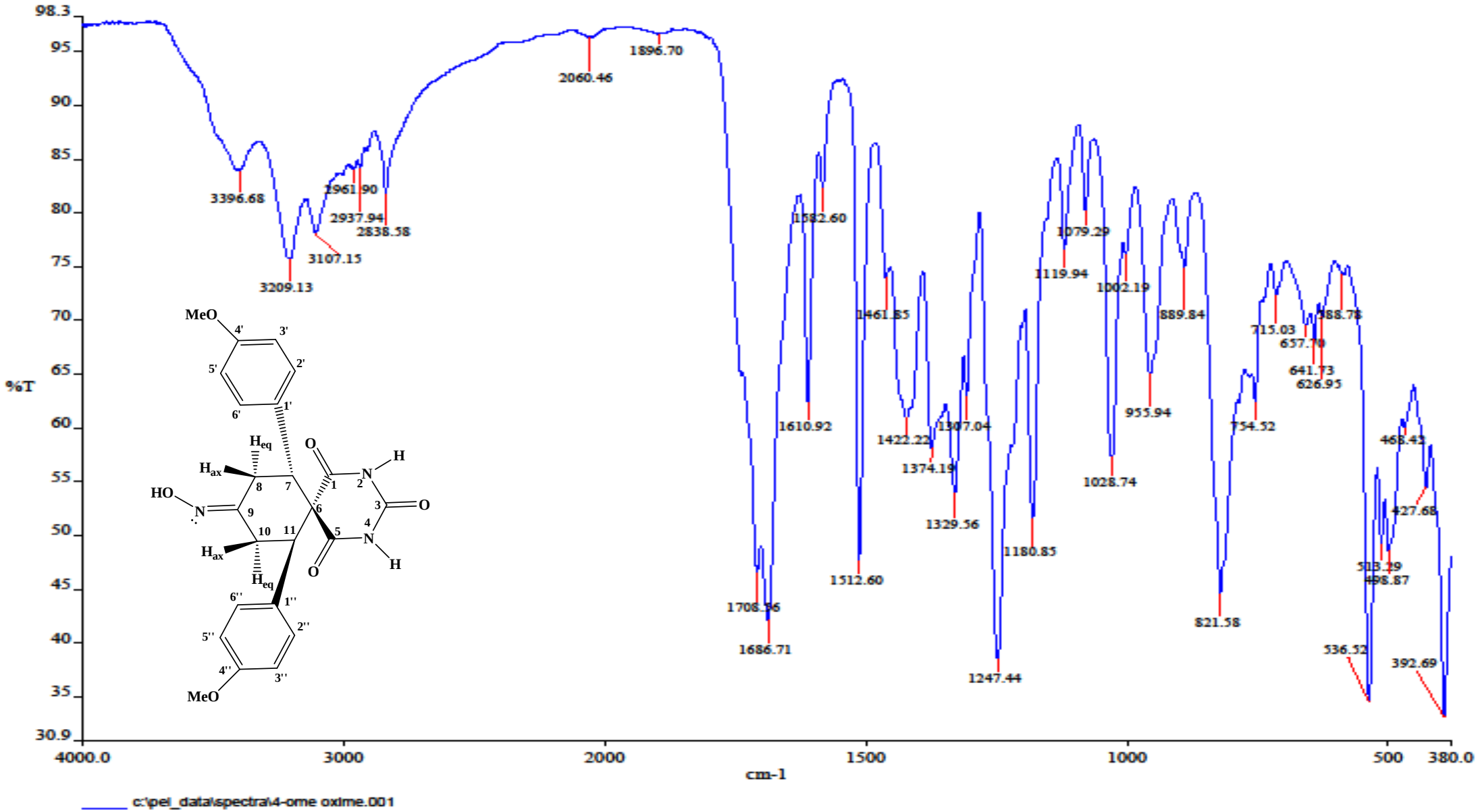
HSQC Spectrum of Compound B-4l: 9-(hydroxyimino)-7,11-bis(4-methoxyphenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



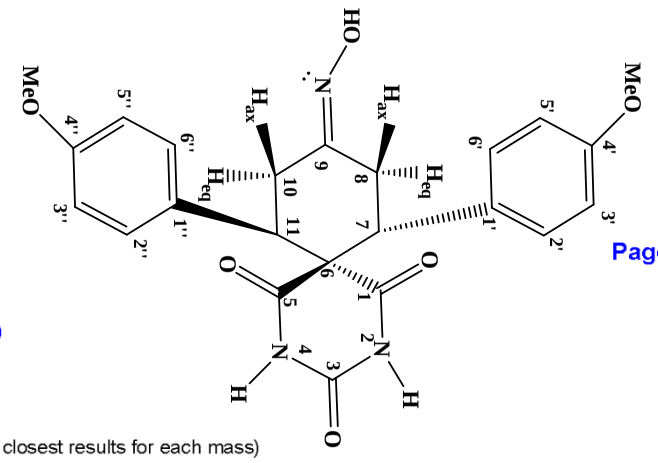
COSY Spectrum of Compound B-4l: 9-(hydroxyimino)-7,11-bis(4-methoxyphenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



HMBC Spectrum of Compound B-4l: 9-(hydroxyimino)-7,11-bis(4-methoxyphenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



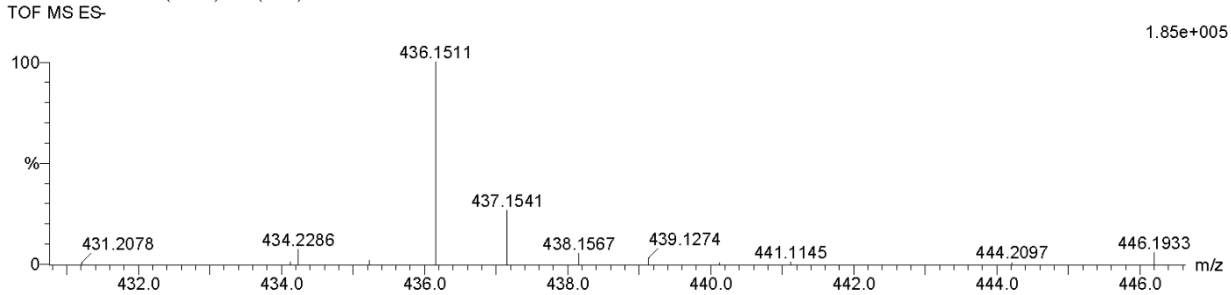
Infrared Spectrum of Compound B-4l: 9-(hydroxyimino)-7,11-bis(4-methoxyphenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Elemental Composition Report **Page 1**

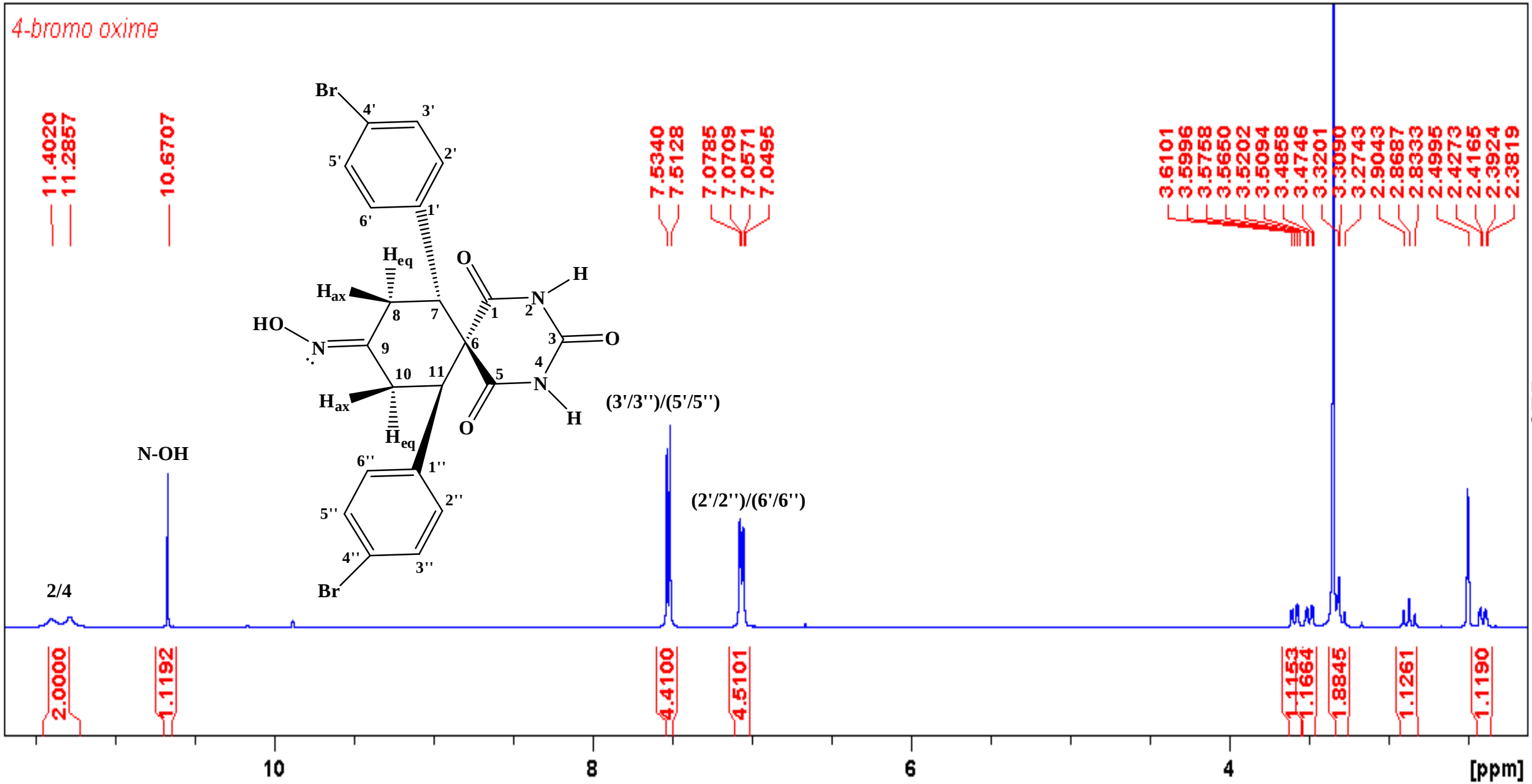
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
15 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 20-25 N: 0-5 O: 5-10
39 4-OMe oxime 60 (2.023) Cm (1:60)
TOF MS ES-

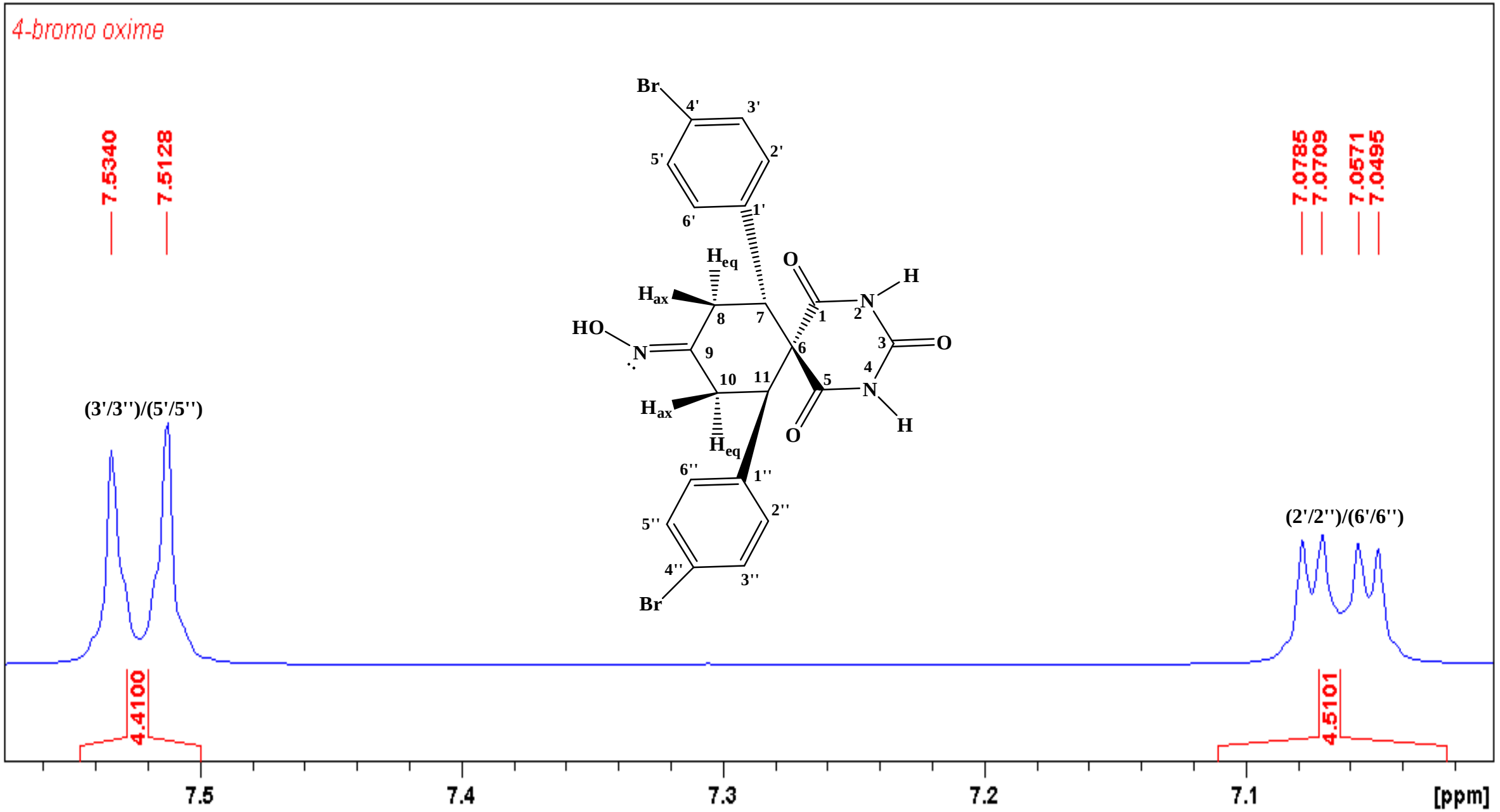


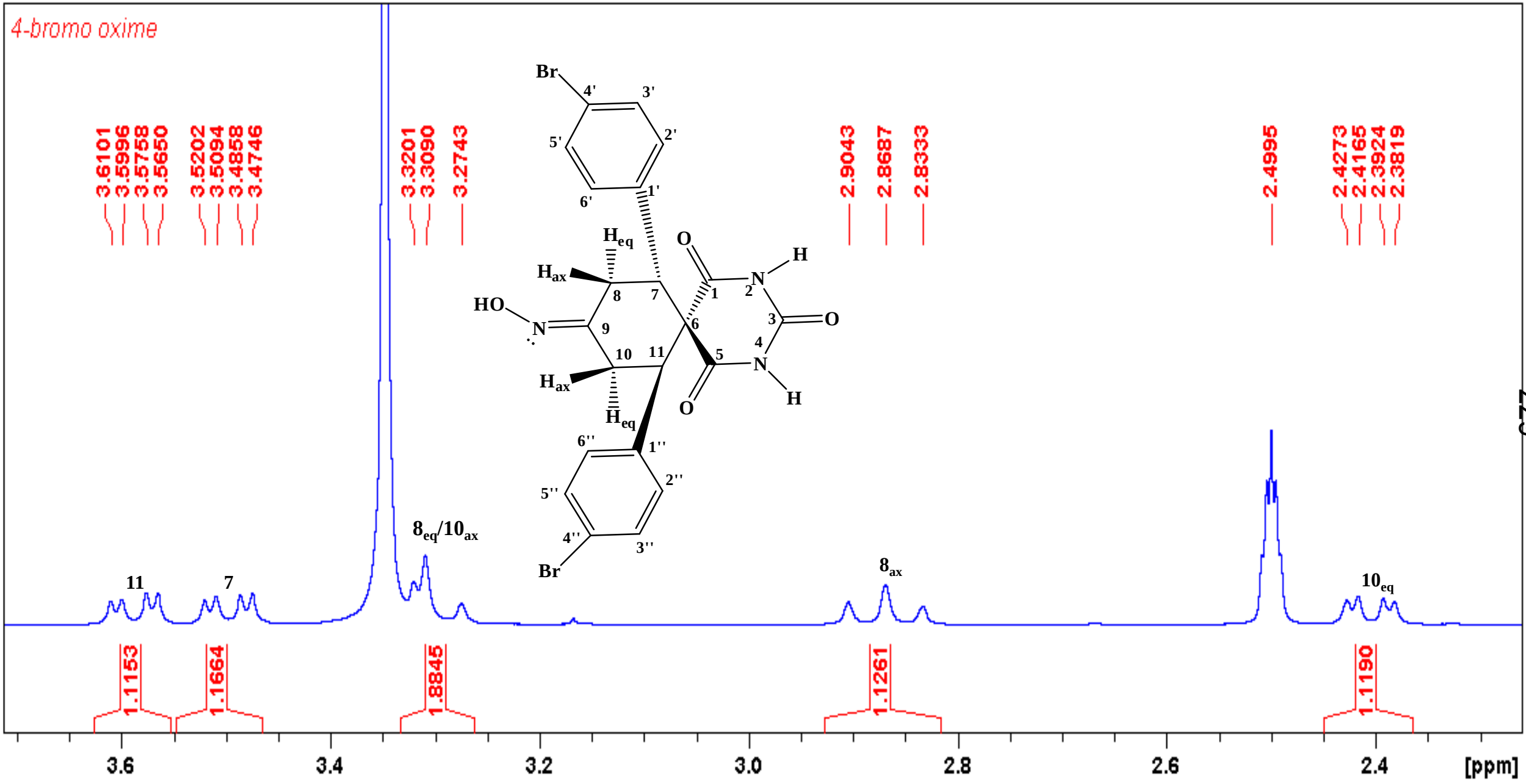
Minimum:				-1.5						
Maximum:		5.0	5.0	50.0						
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula			
436.1511	436.1509	0.2	0.5	14.5	11.7	0.0	C23	H22	N3	O6

HRMS Spectrum of Compound B-4l: 9-(hydroxyimino)-7,11-bis(4-methoxyphenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

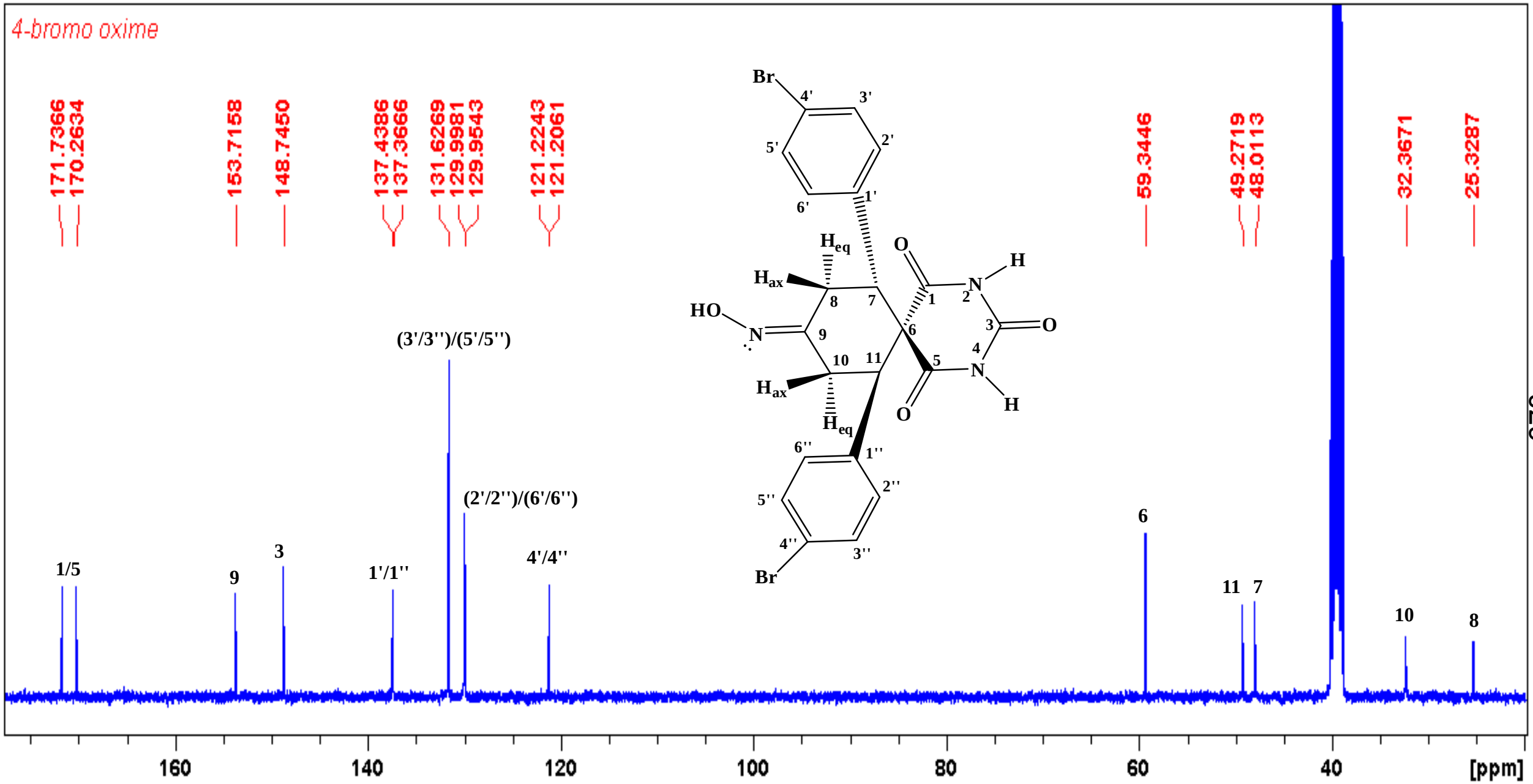


^{1}H Spectrum of Compound B-4n: 7,11-bis(4-bromophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

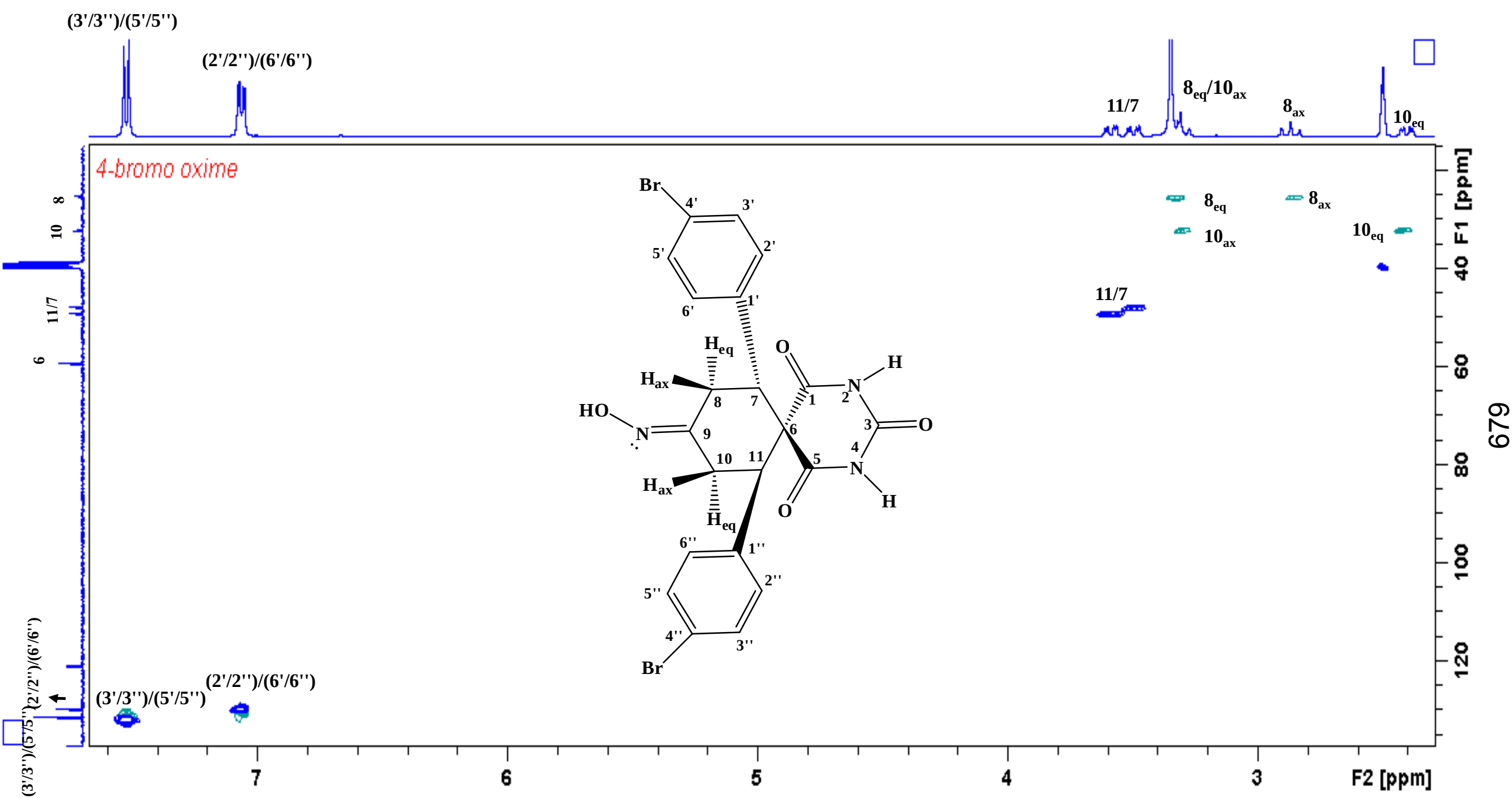

4-bromo oxime
7.5340
7.5128
7.0785
7.0709
7.0571
7.0495
(3'/3'')/(5'/5'')
(2'/2'')/(6'/6'')
4.4100
4.5101
7.5
7.4
7.3
7.2
7.1
[ppm]
Br
HO
N
O
H
Hax
Heq



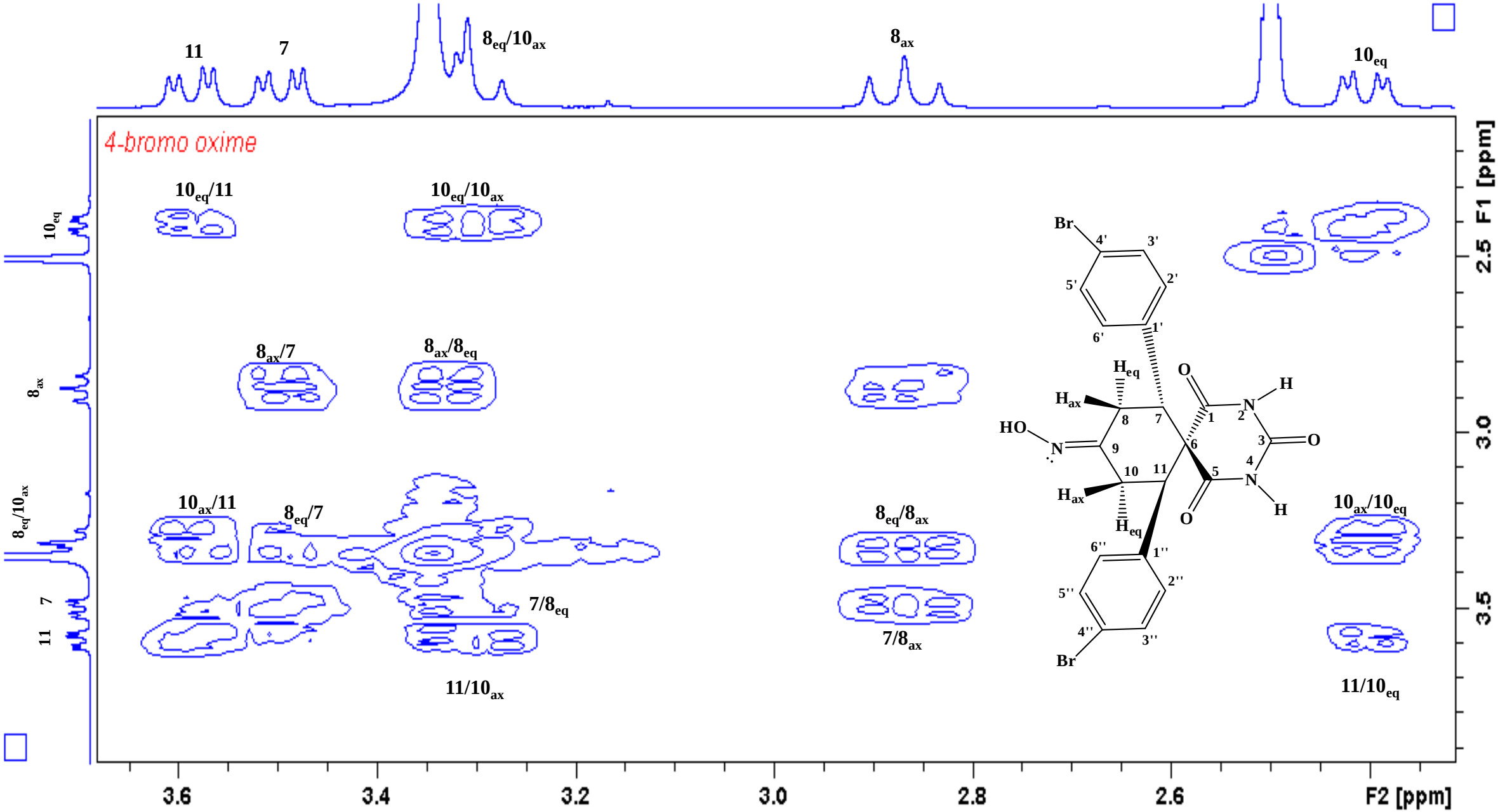
Expanded ^{1}H Spectrum of Compound B-4n: 7,11-bis(4-bromophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



^{13}C Spectrum of Compound B-4n: 7,11-bis(4-bromophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

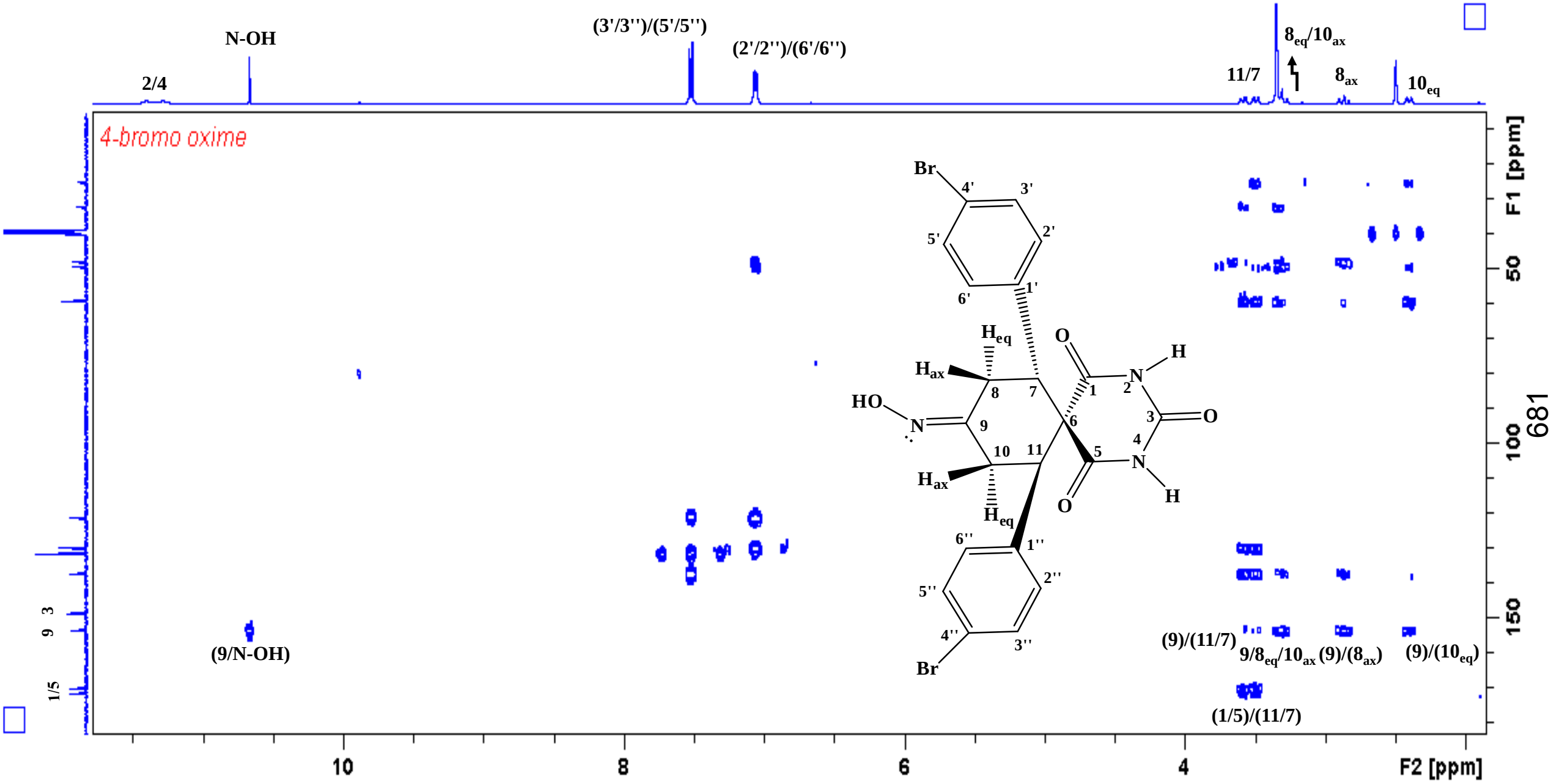


HSQC Spectrum of Compound B-4n: 7,11-bis(4-bromophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

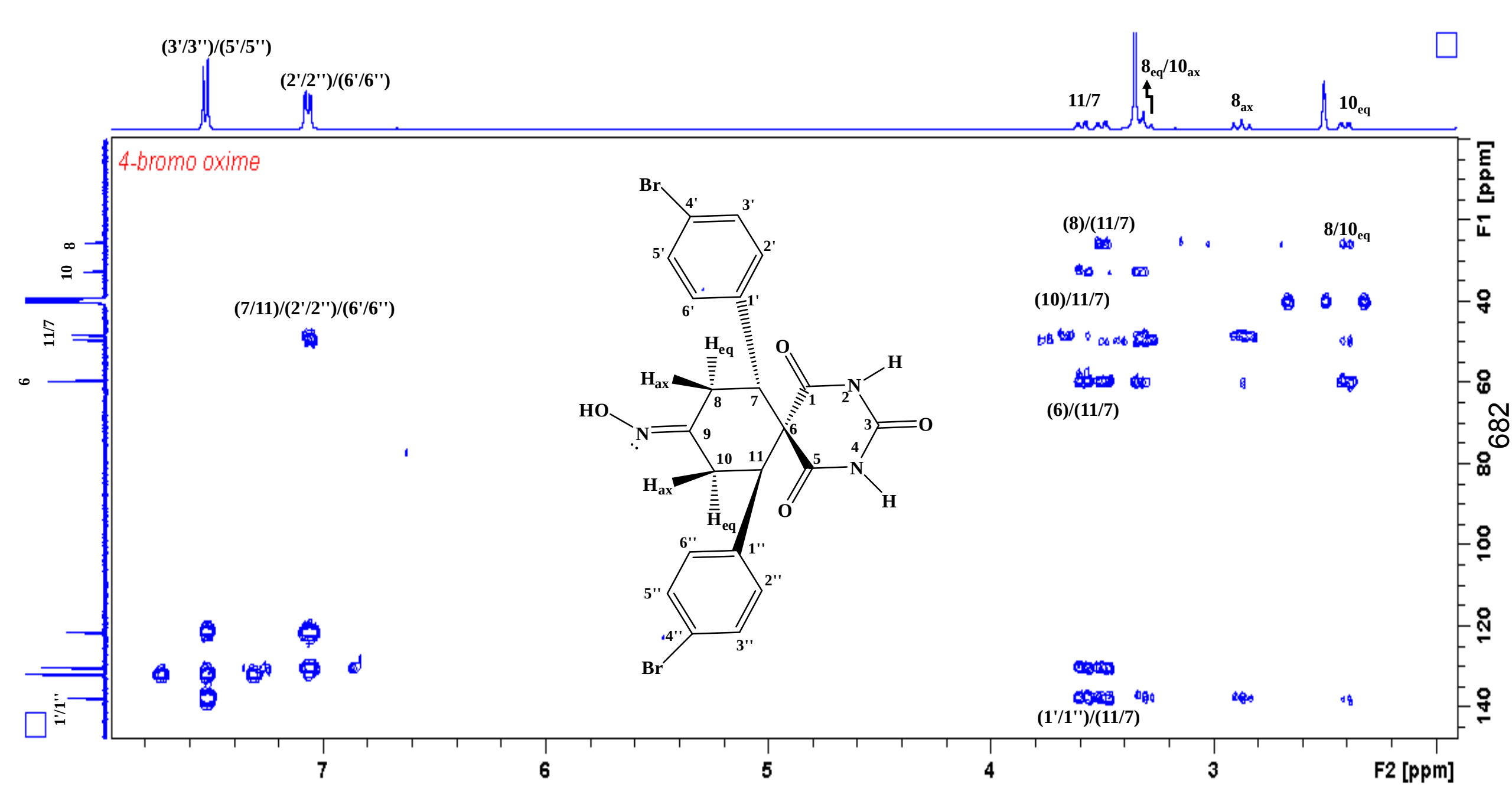


680

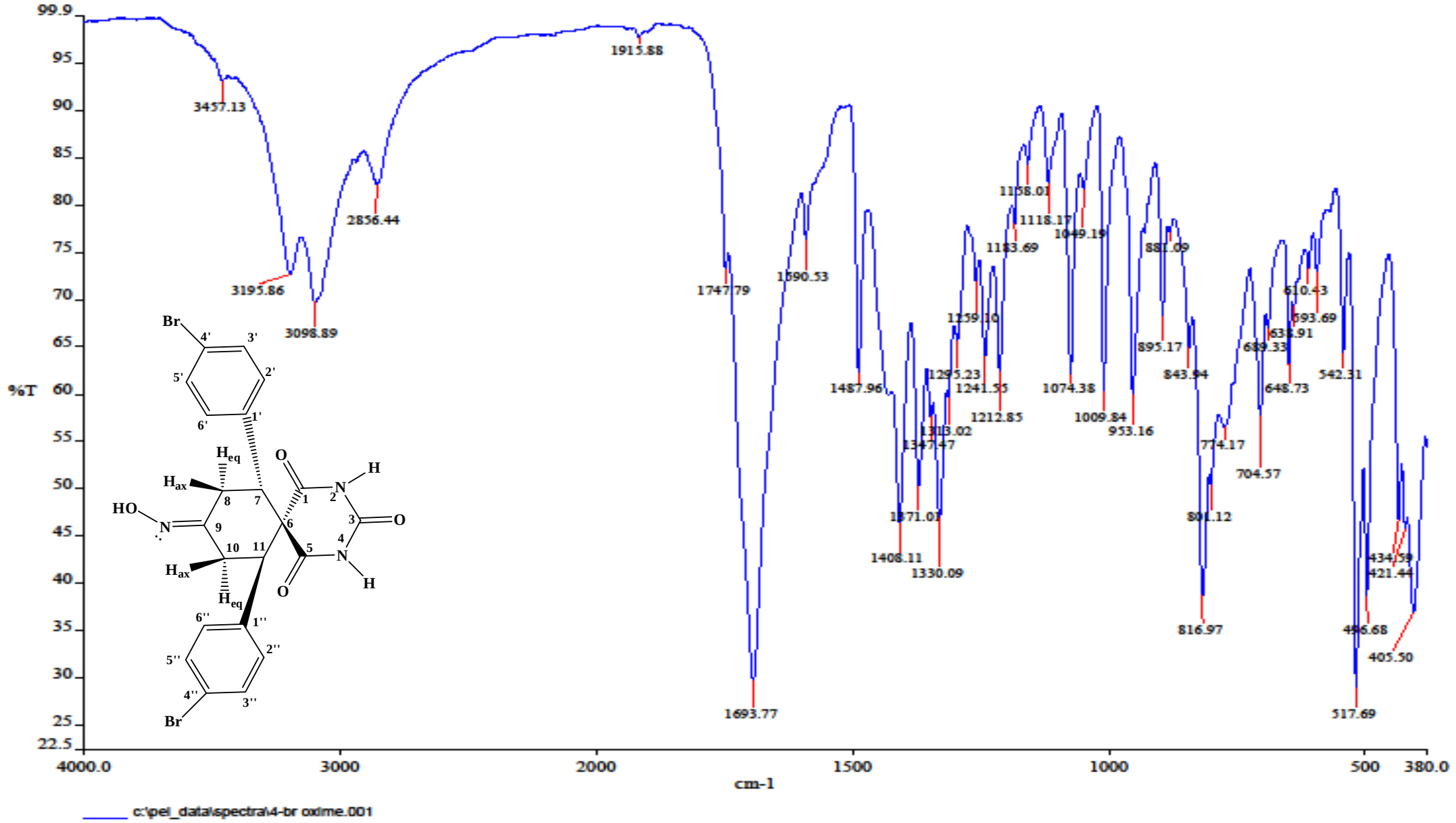
COSY Spectrum of Compound B-4n: 7,11-bis(4-bromophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



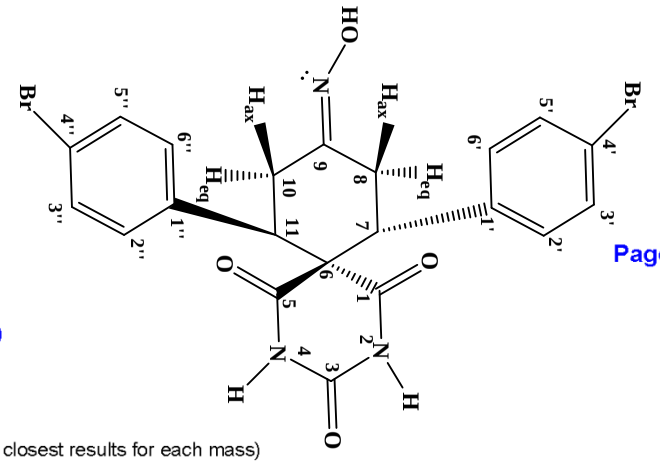
HMBC Spectrum of Compound B-4n: 7,11-bis(4-bromophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Expanded HMBC Spectrum of Compound B-4n: 7,11-bis(4-bromophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Infrared Spectrum of Compound B-4n: 7,11-bis(4-bromophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

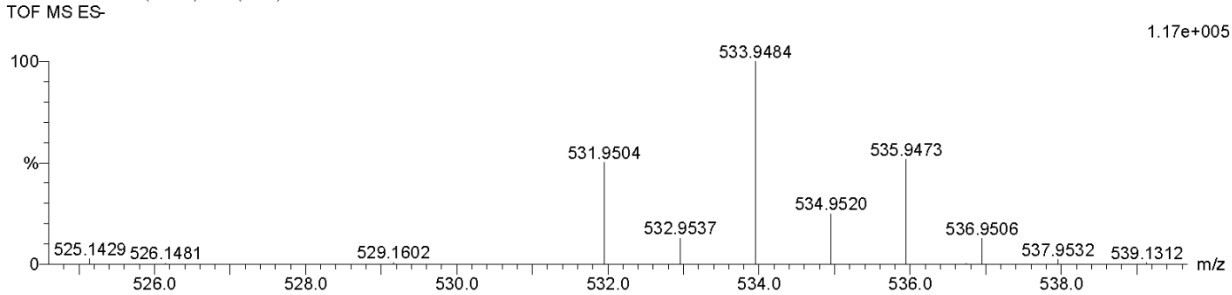


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

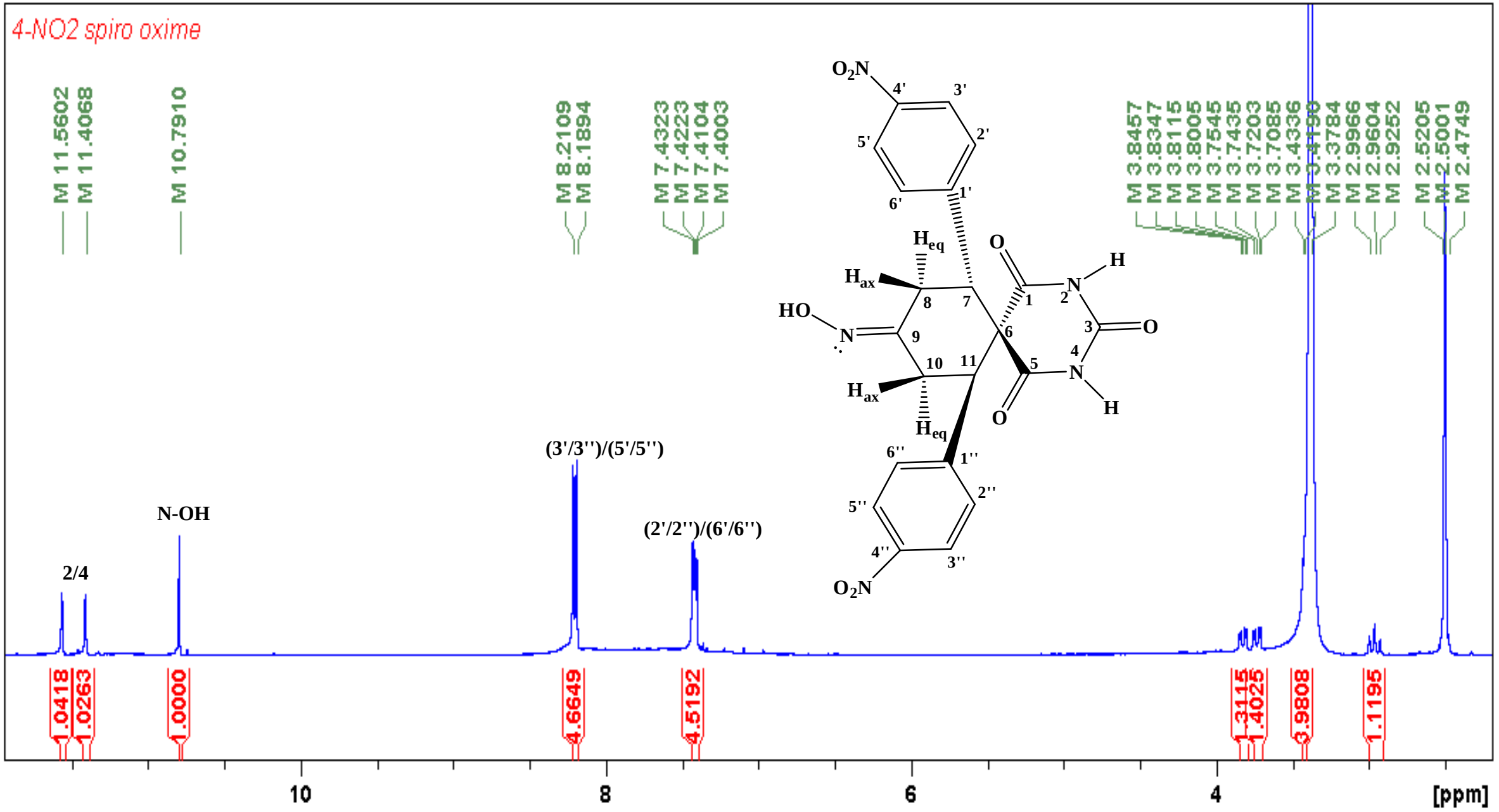
Monoisotopic Mass, Even Electron Ions
97 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 0-5 79Br: 0-1 81Br: 0-1

41 4-Br oxime 51 (1.686) Cm (1:61)
TOF MS ES-

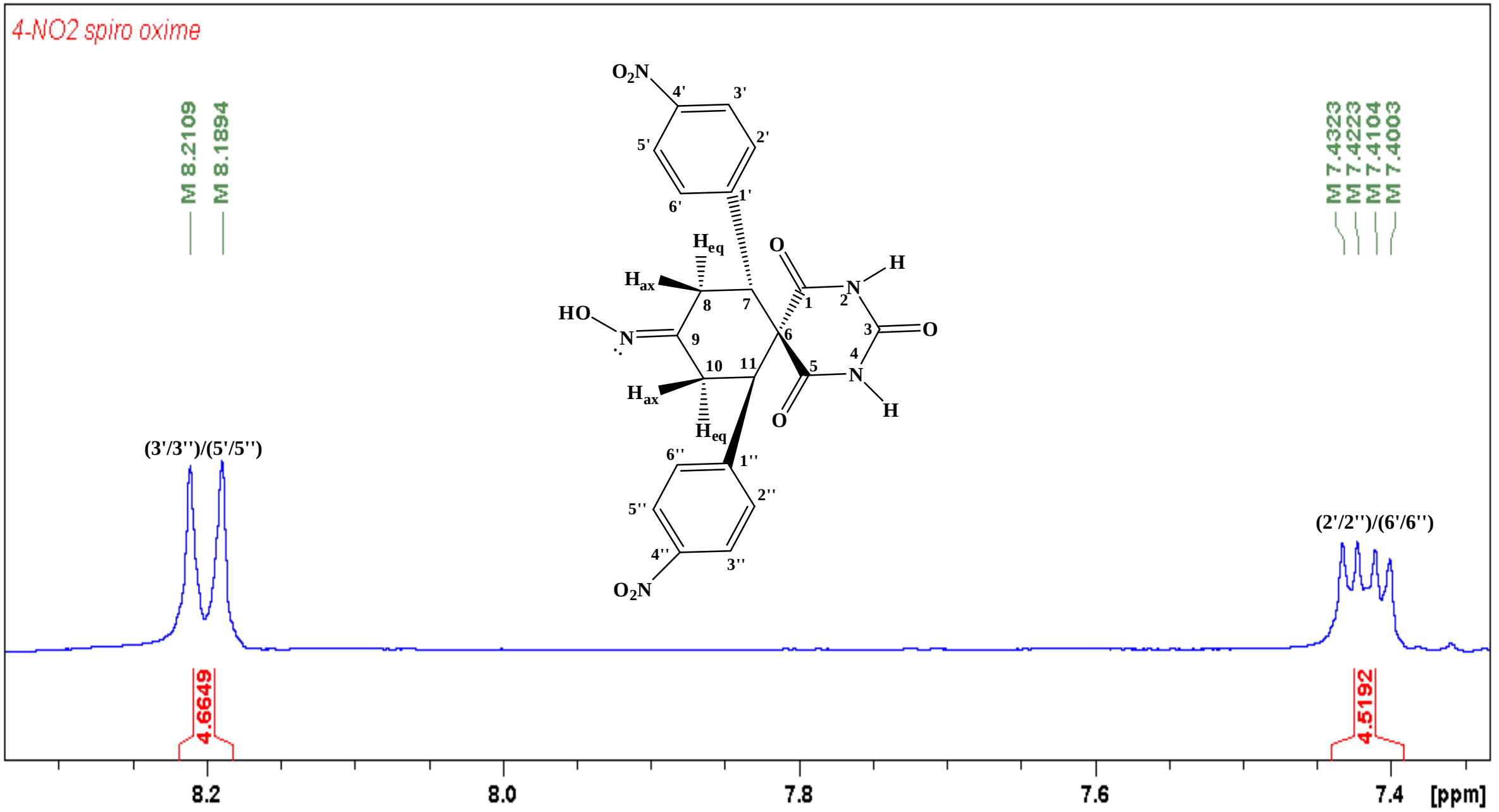


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
533.9484	533.9487	-0.3	-0.6	14.5	8.9	0.0	C21 H16 N3 O4 79Br 81Br

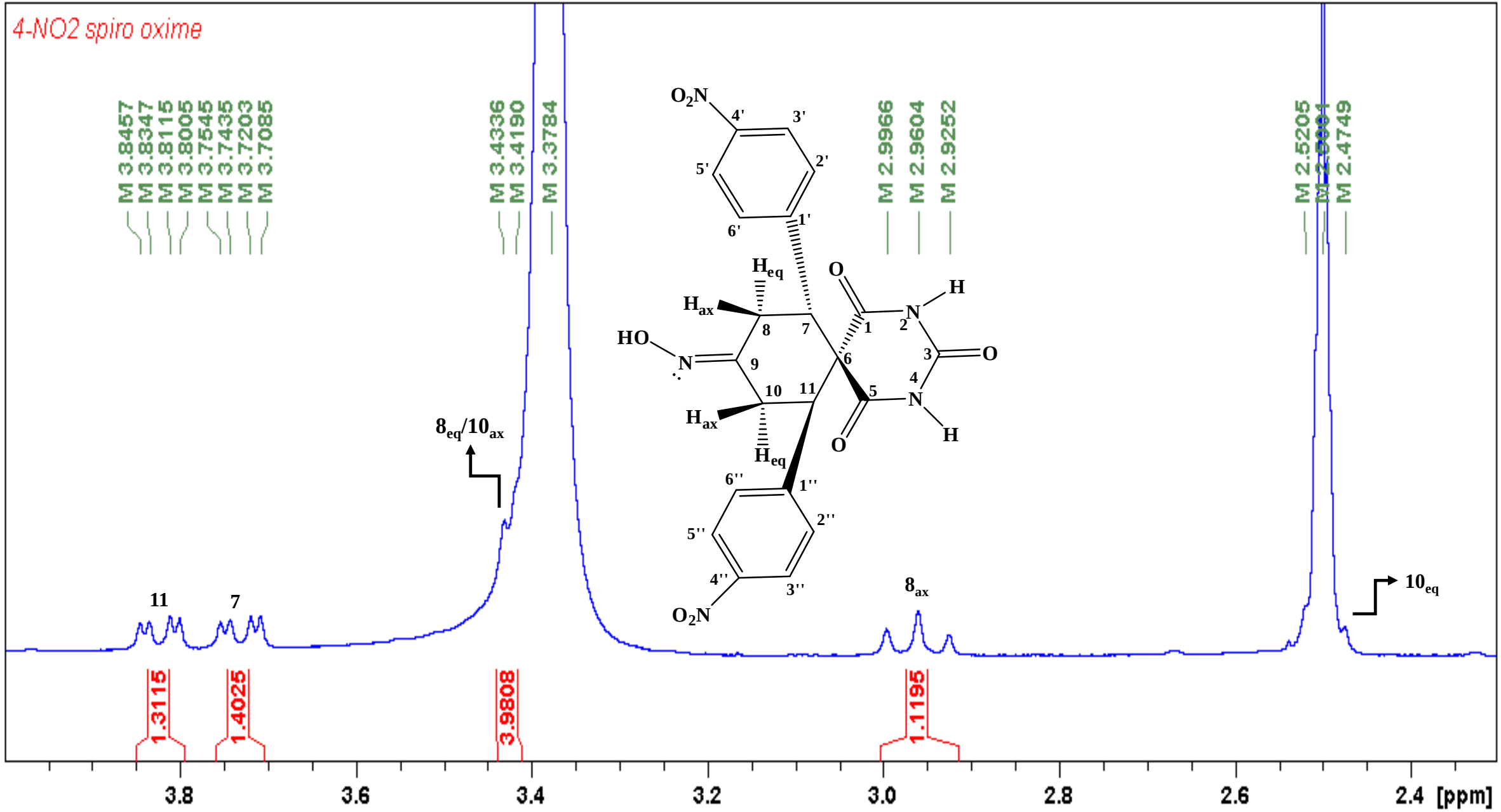
HRMS Spectrum of Compound B-4n: 7,11-bis(4-bromophenyl)-9-(hydroxyimino)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



[1]H Spectrum of Compound B-4o: 9-(hydroxyimino)-7,11-bis(4-nitrophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

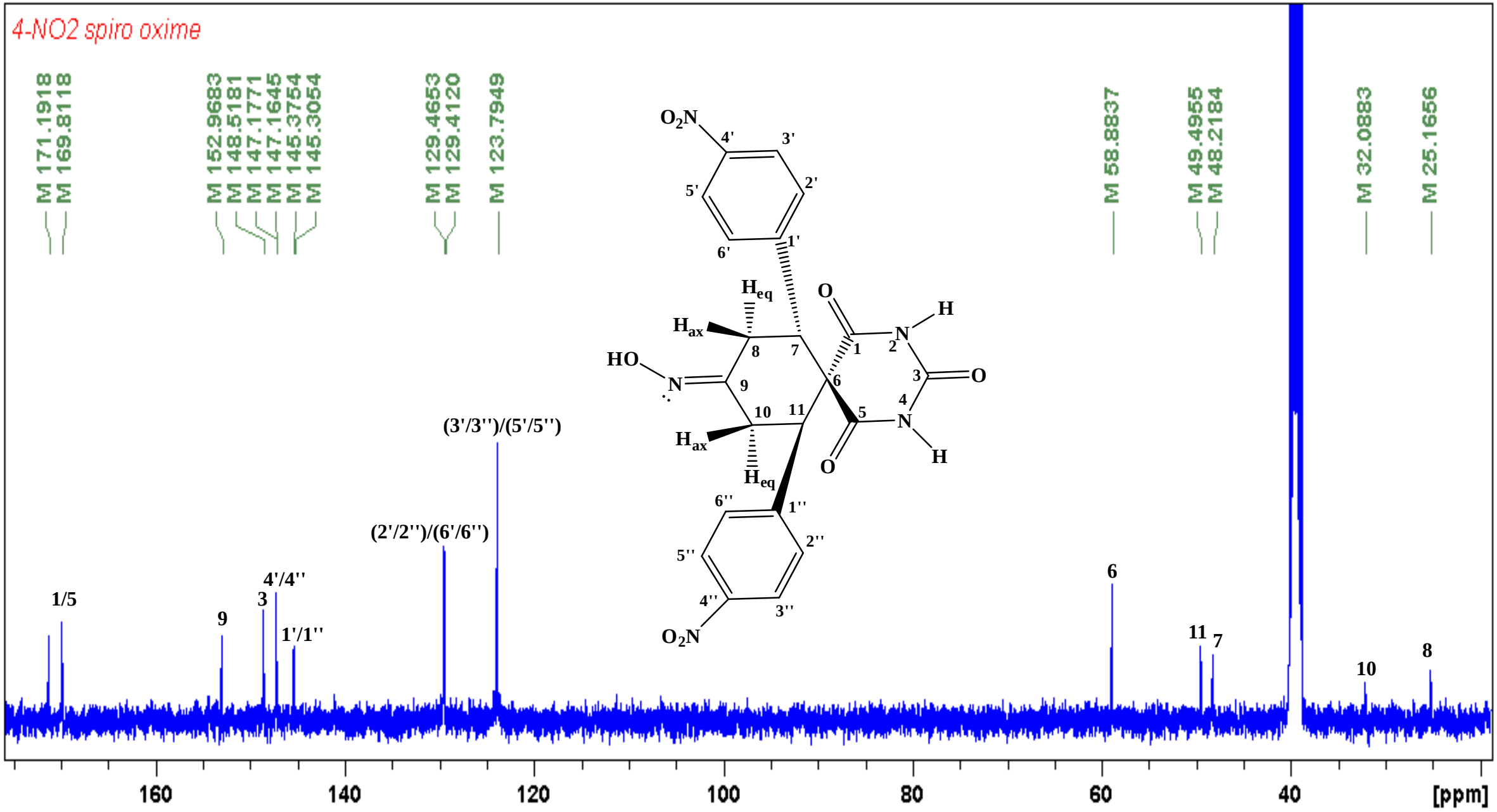


Expanded ^{1}H Spectrum of Compound B-4o: 9-(hydroxyimino)-7,11-bis(4-nitrophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



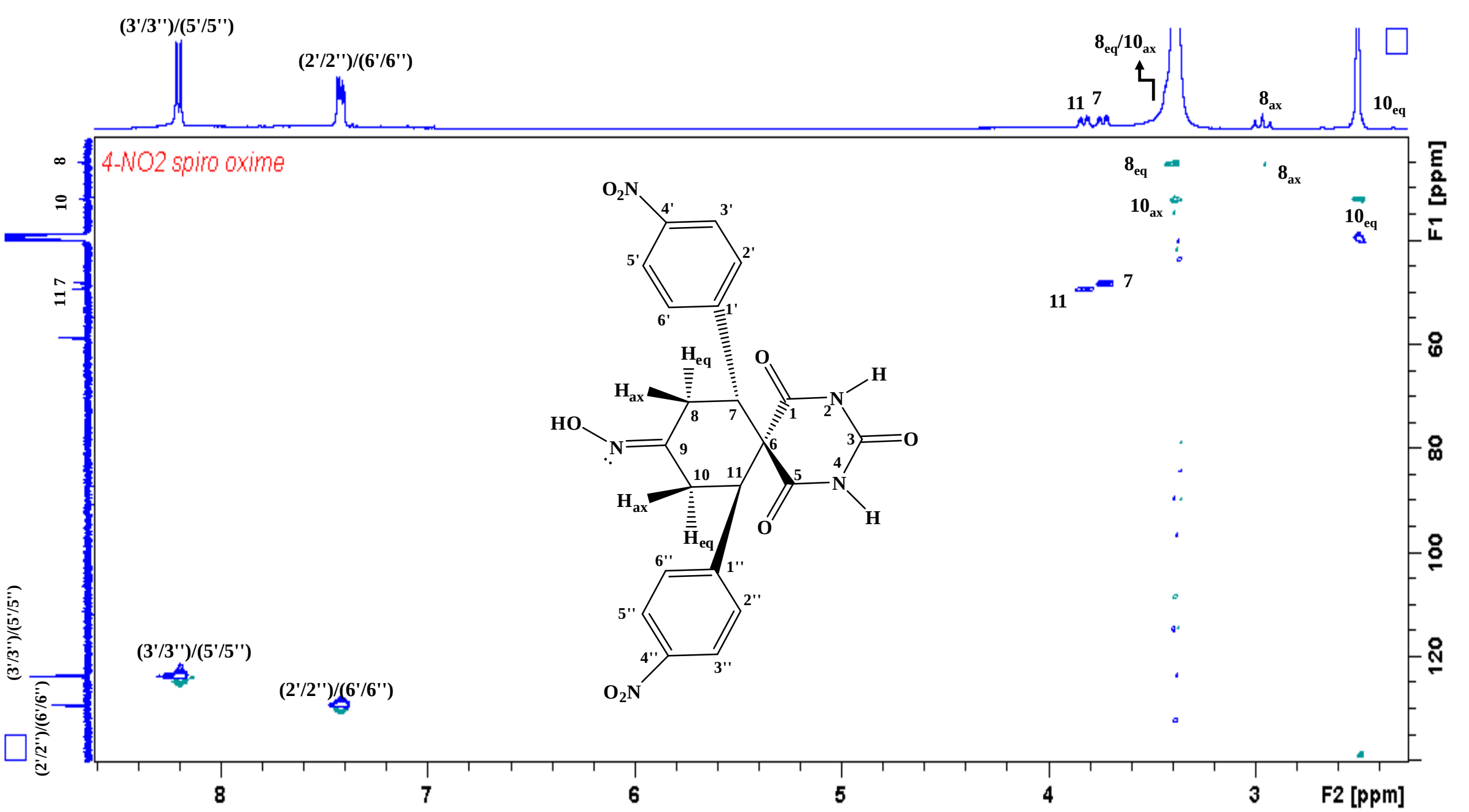
687

Expanded ^{1}H Spectrum of Compound B-4o: 9-(hydroxyimino)-7,11-bis(4-nitrophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

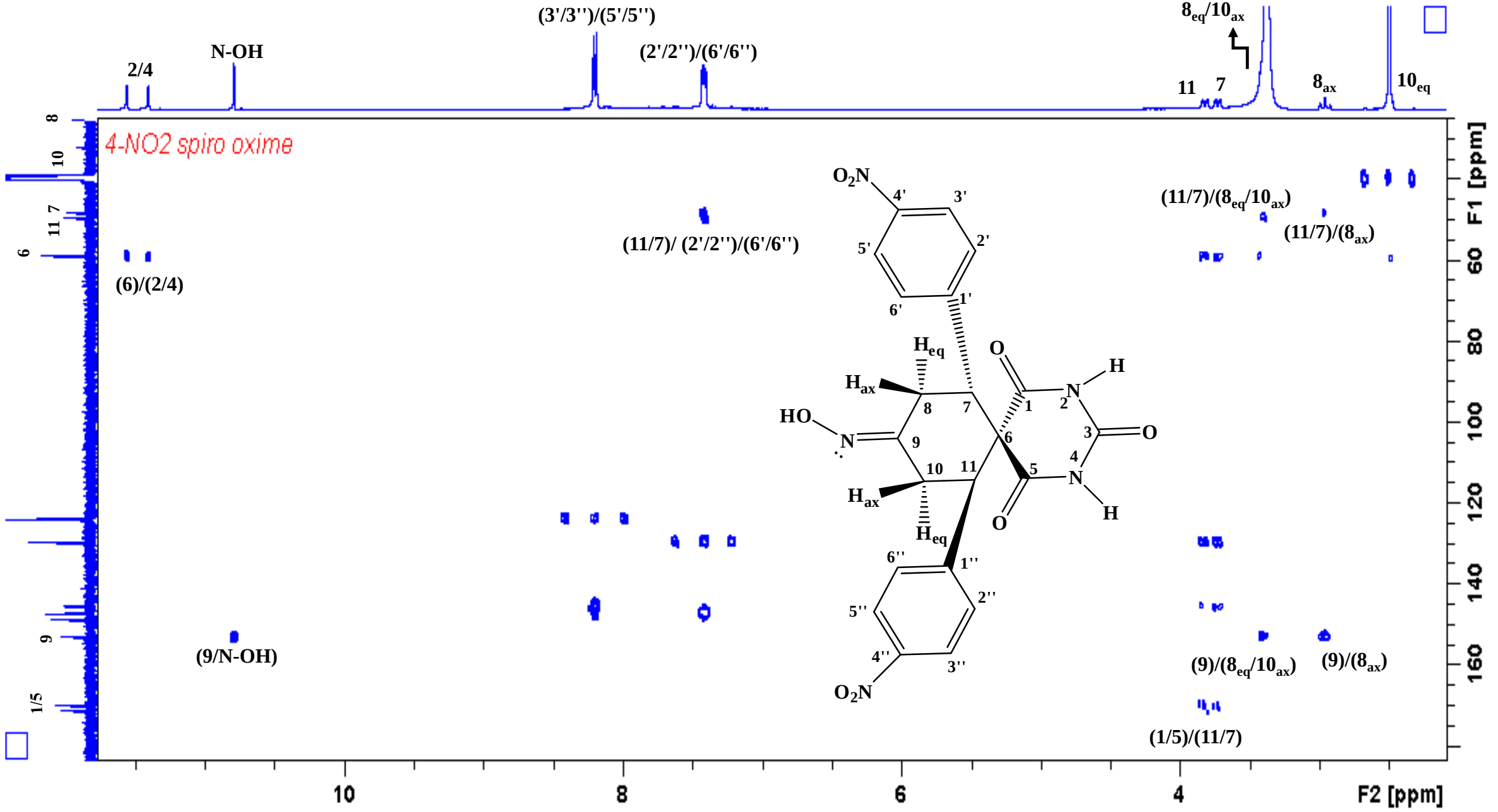


688

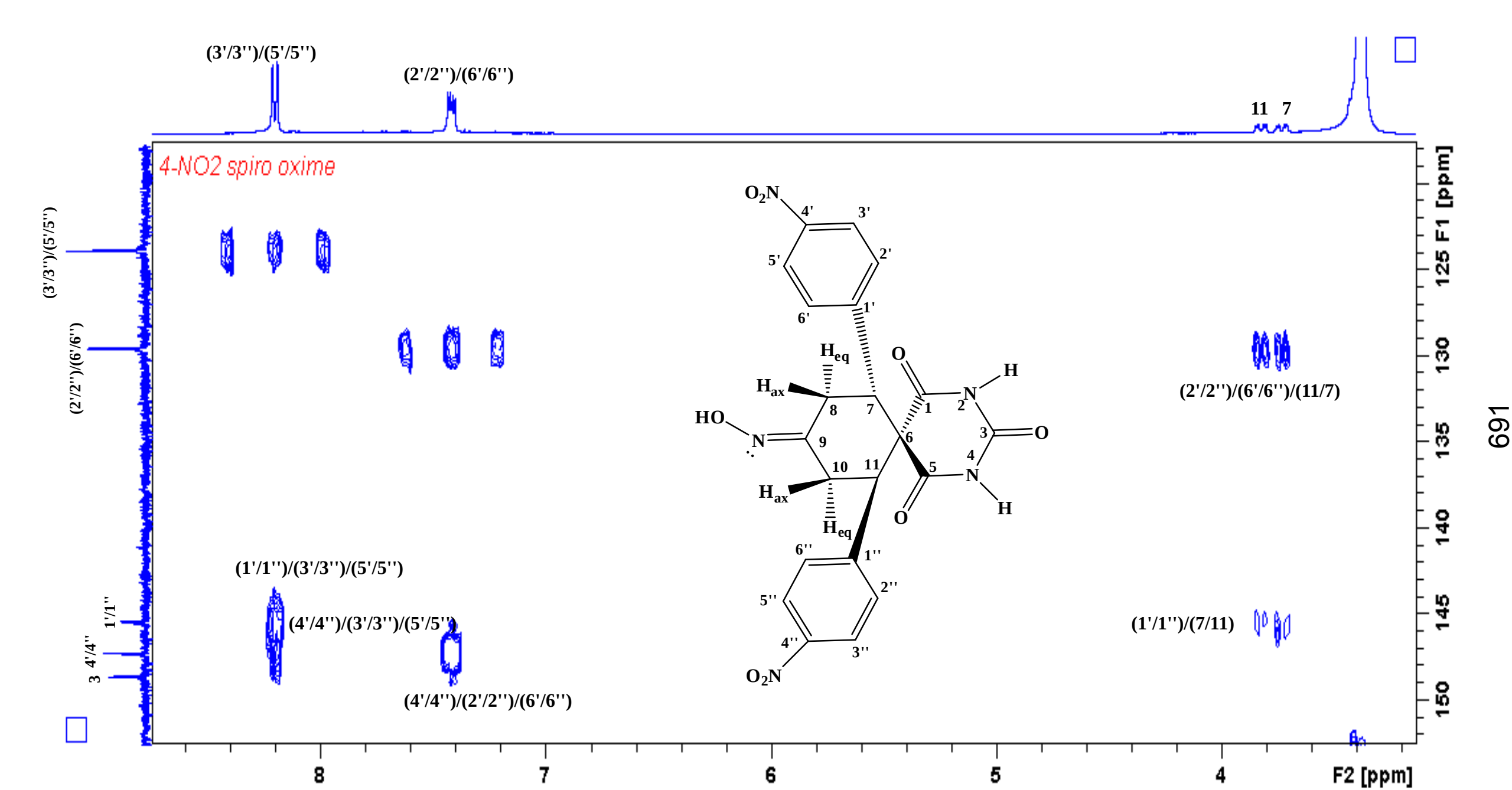
^{13}C Spectrum of Compound B-4o: 9-(hydroxyimino)-7,11-bis(4-nitrophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



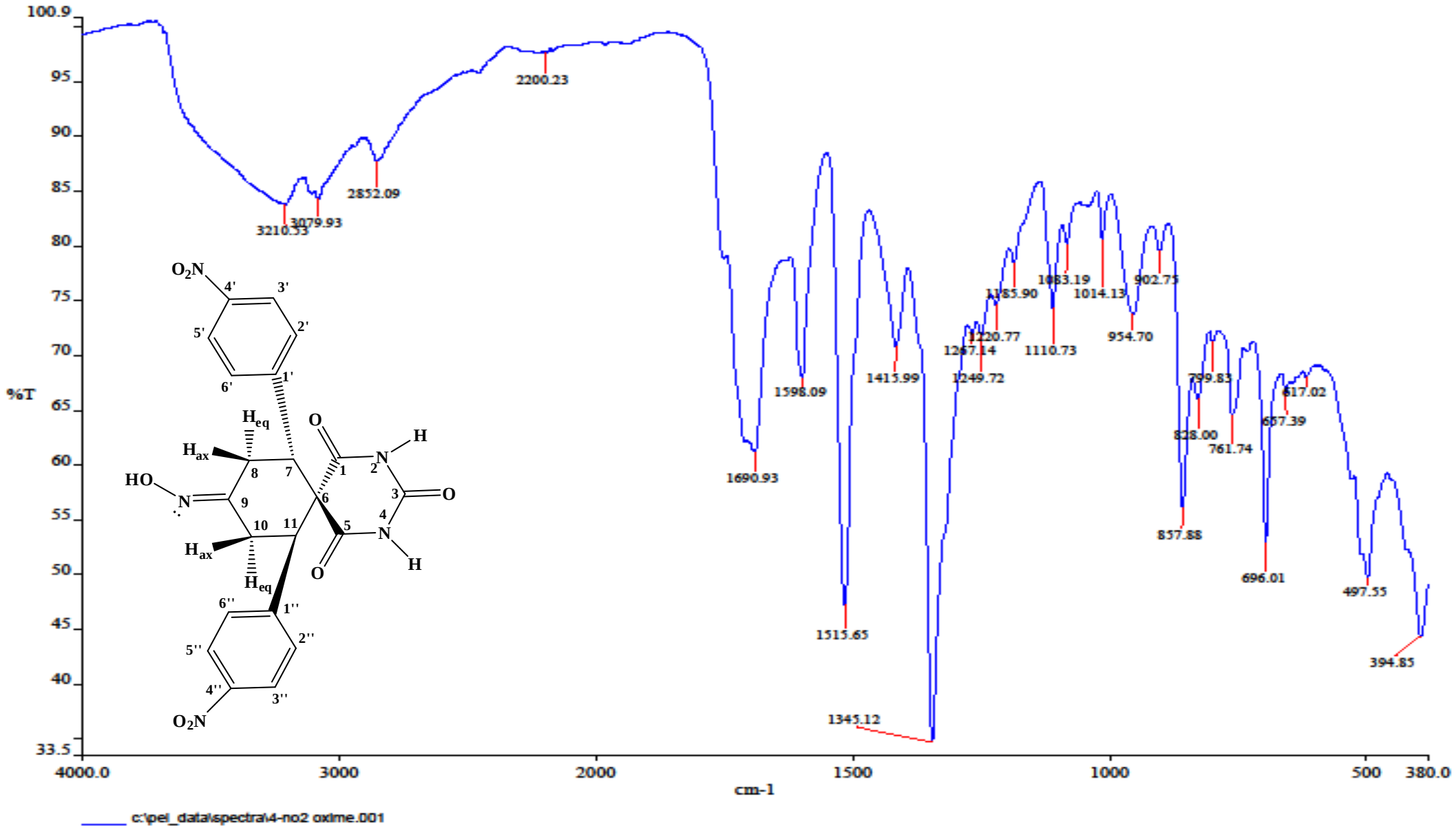
HSQC Spectrum of Compound B-4o: 9-(hydroxyimino)-7,11-bis(4-nitrophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



HMBC Spectrum of Compound B-4o: 9-(hydroxyimino)-7,11-bis(4-nitrophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

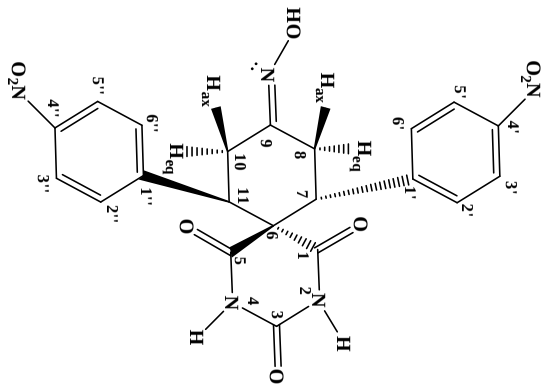


Expanded HMBC Spectrum of Compound B-4o: 9-(hydroxyimino)-7,11-bis(4-nitrophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



692

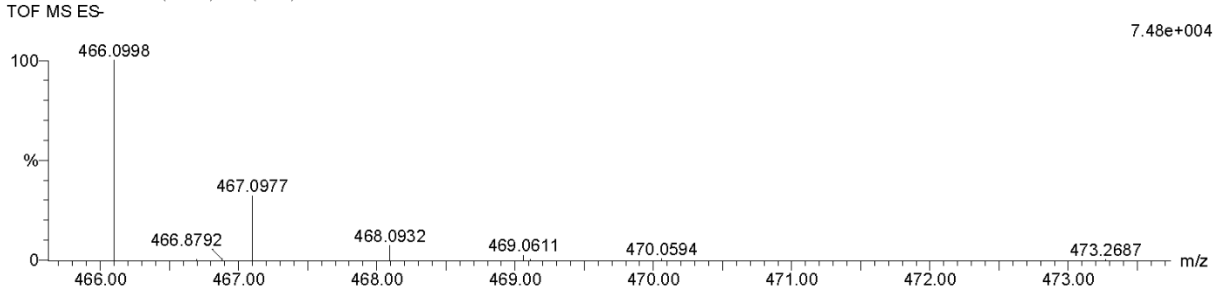
Infrared Spectrum of Compound B-4o: 9-(hydroxyimino)-7,11-bis(4-nitrophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione



Elemental Composition Report

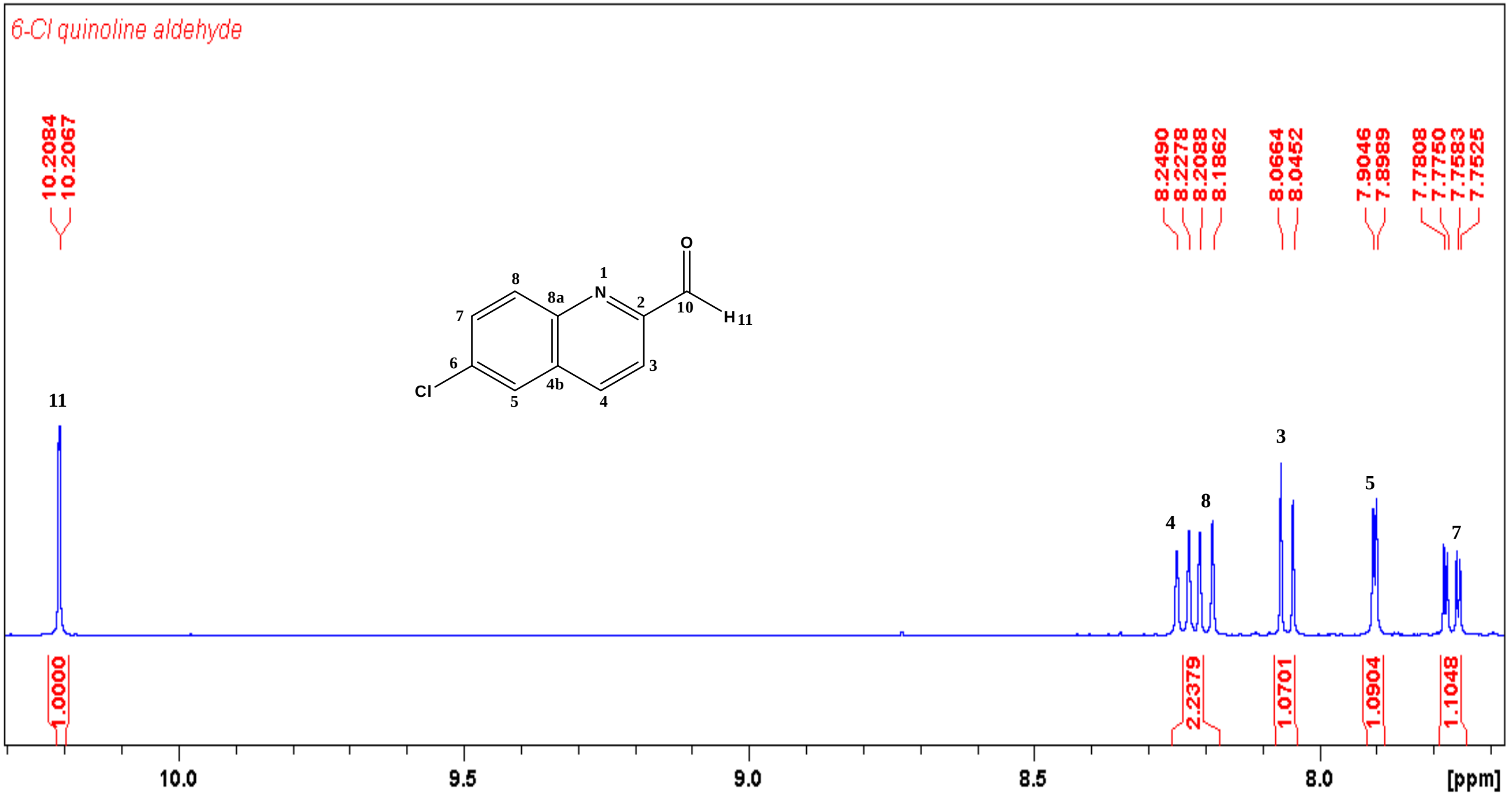
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
11 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 5-10
42 4-NO2 oxime 37 (1.213) Cm (1:61)
TOF MS ES-



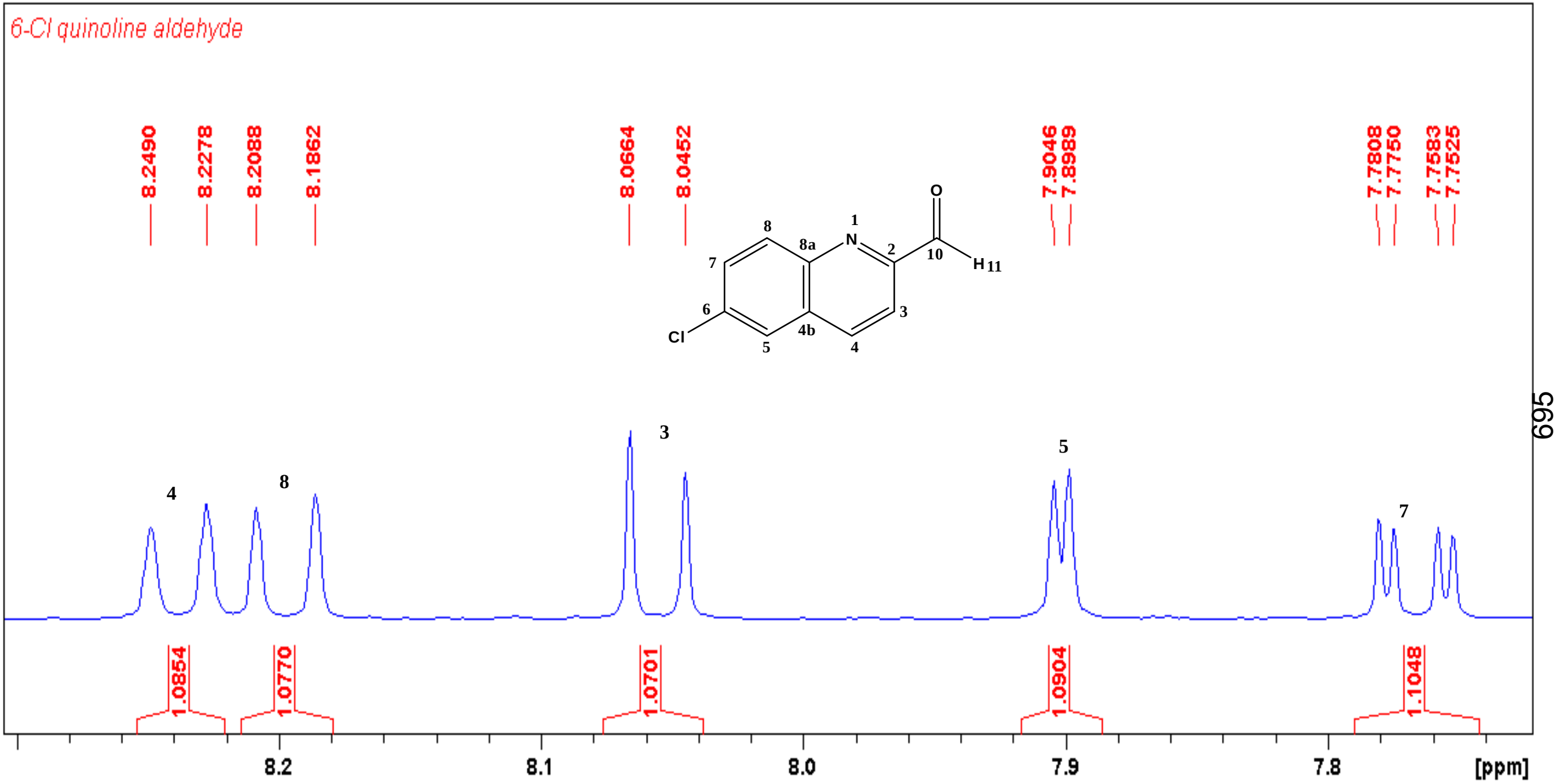
Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
466.0998	466.0999	-0.1	-0.2	16.5	46.0	0.0	C21 H16 N5 O8

HRMS Spectrum of Compound B-4o: 9-(hydroxyimino)-7,11-bis(4-nitrophenyl)-2,4-diazaspiro[5.5]undecane-1,3,5-trione

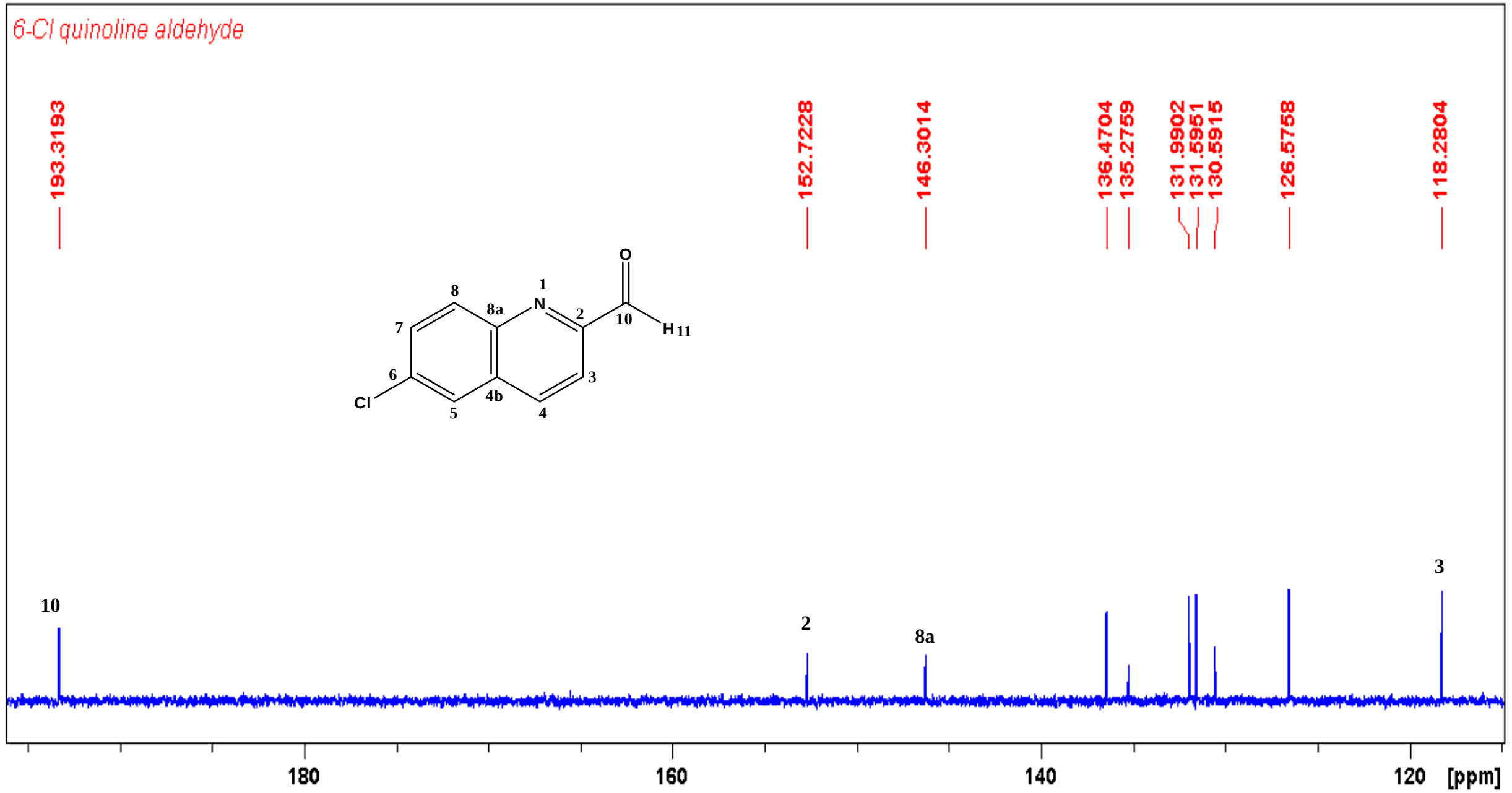


694

[1]H Spectrum of Compound C-1a: 6-chloroquinoline-2-carbaldehyde

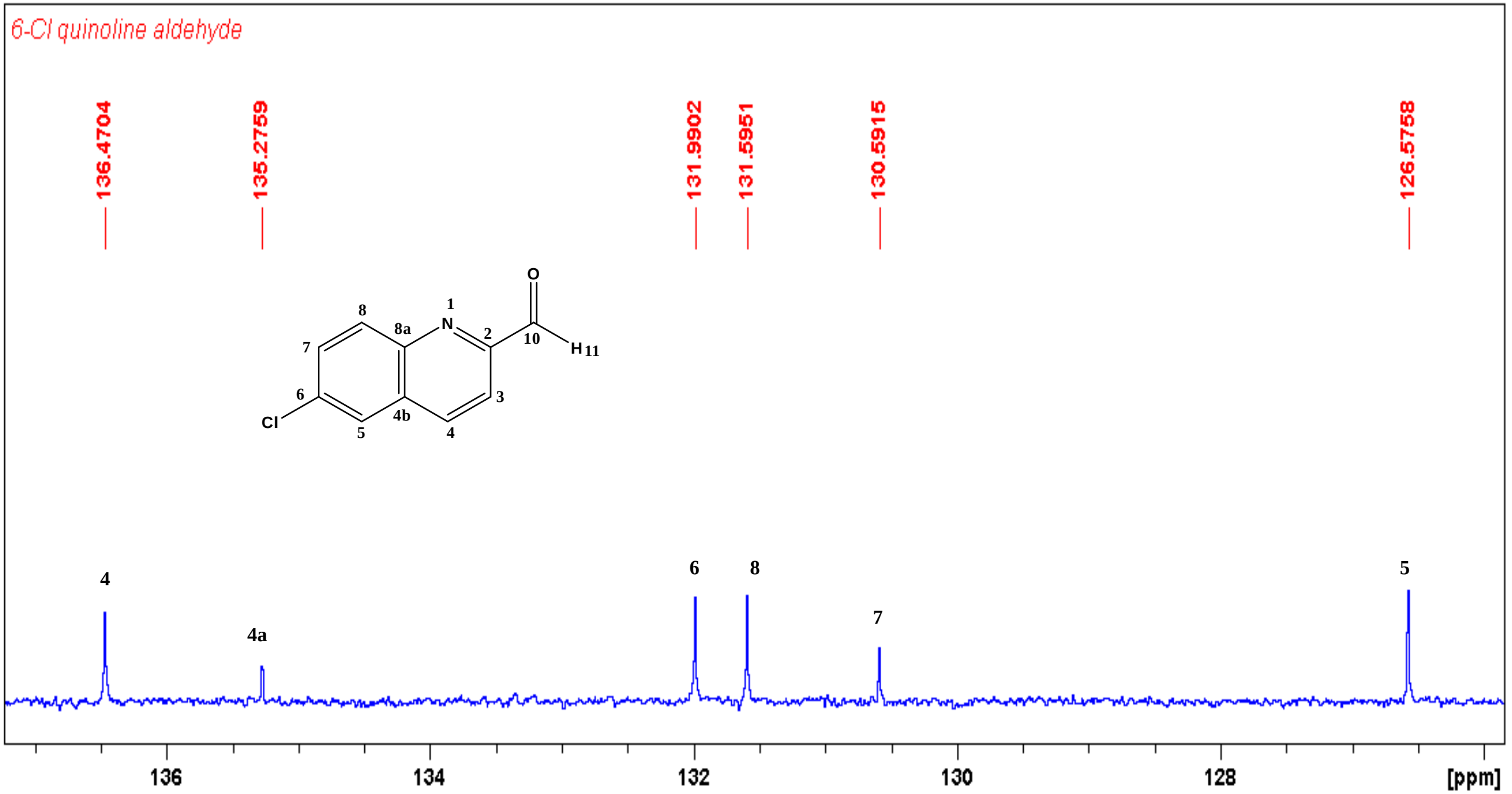


Expanded ^{1}H Spectrum of Compound C-1a: 6-chloroquinoline-2-carbaldehyde

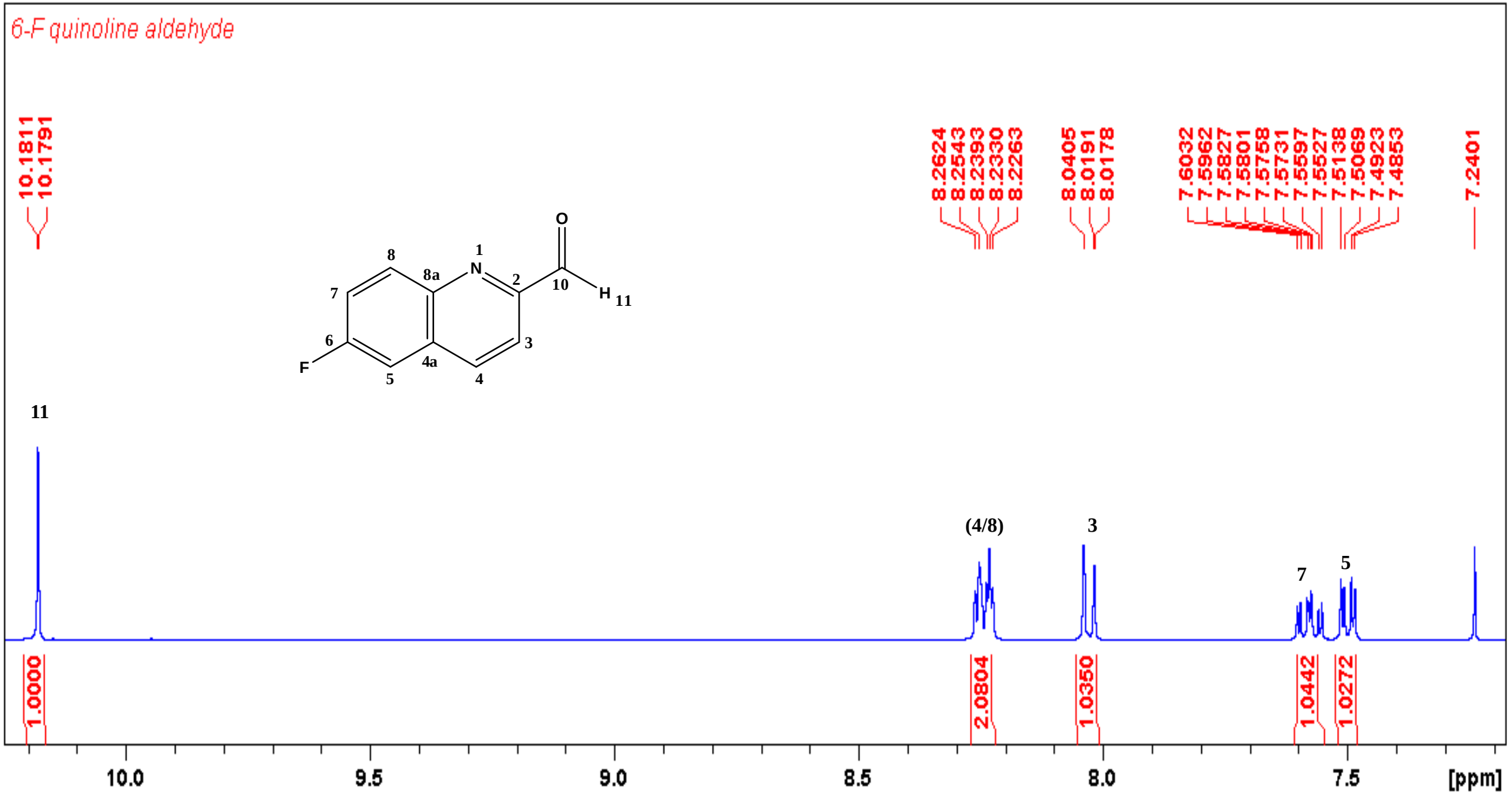


696

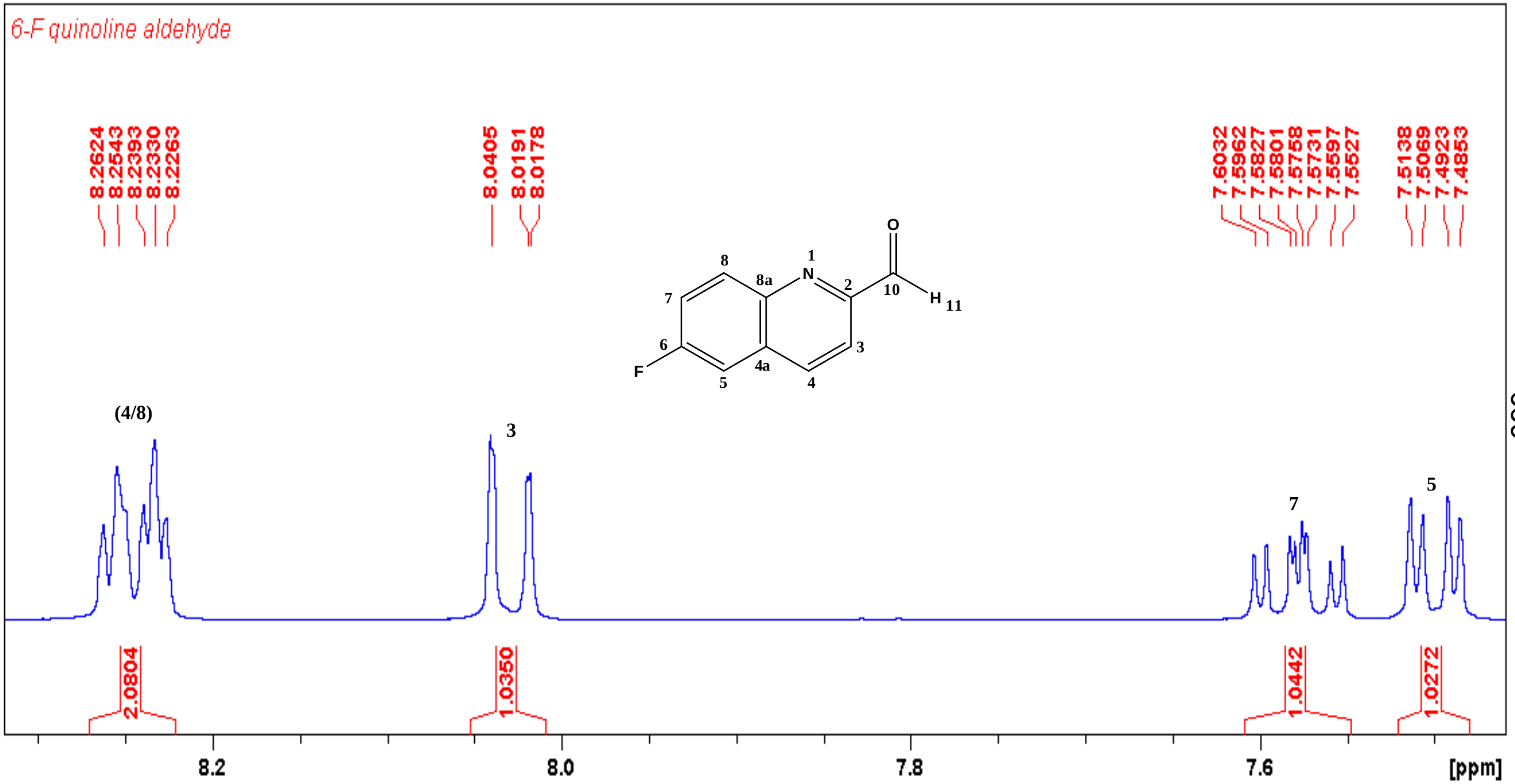
^{13}C Spectrum of Compound C-1a: 6-chloroquinoline-2-carbaldehyde



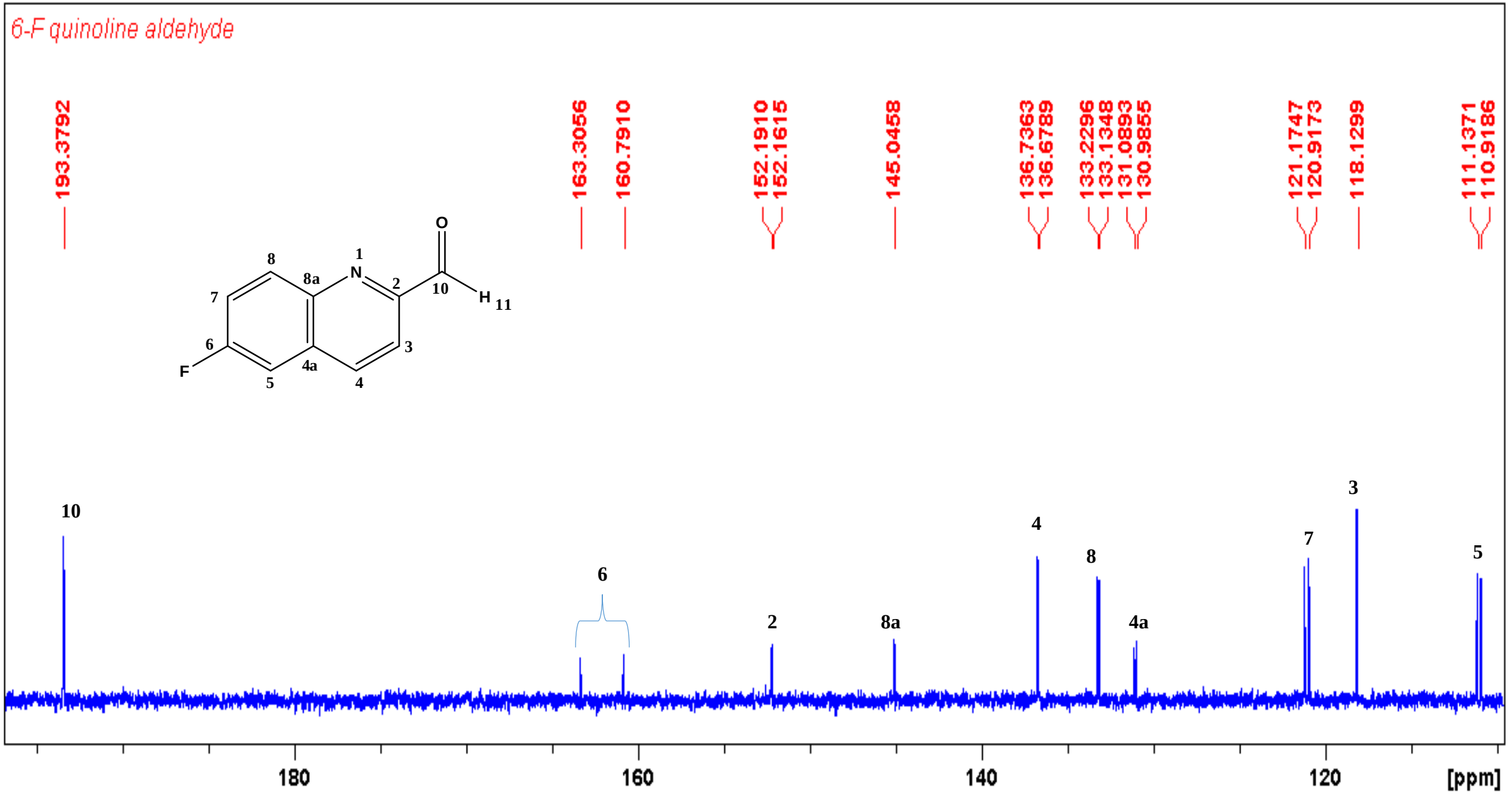
Expanded ^{13}C Spectrum of Compound C-1a: 6-chloroquinoline-2-carbaldehyde



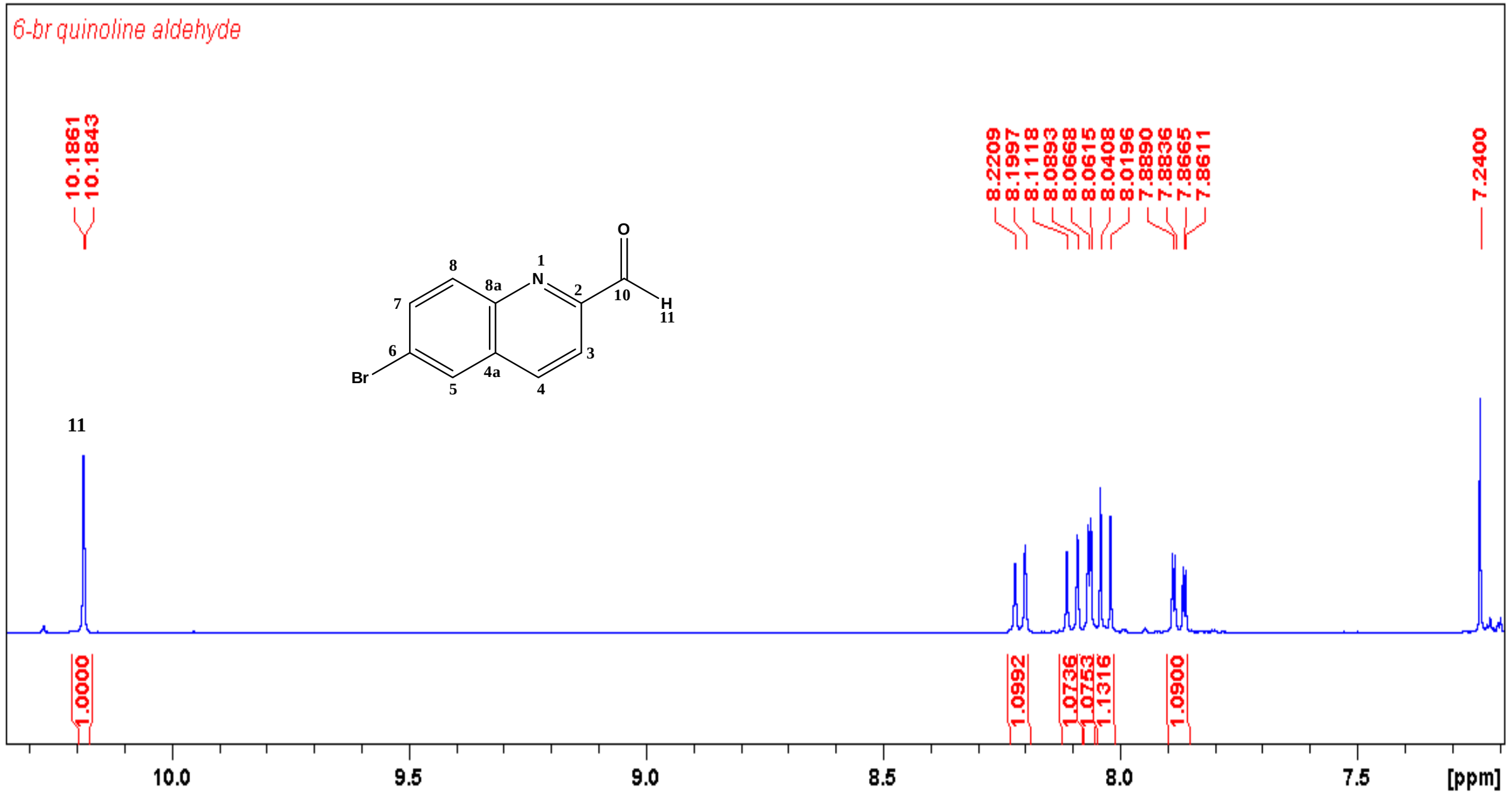
^{1}H Spectrum of Compound C-1b: 6-fluoroquinoline-2-carbaldehyde



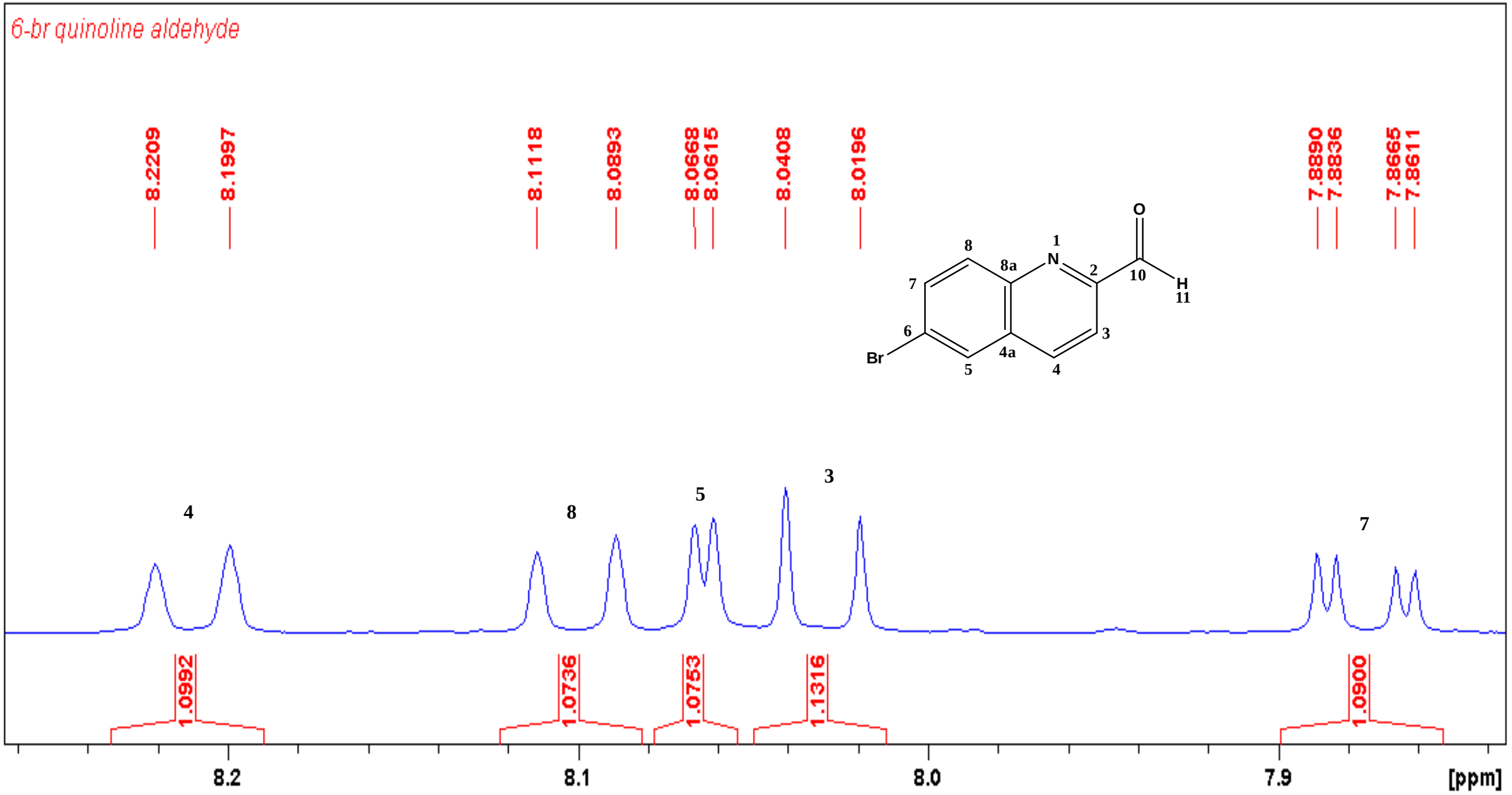
Expanded ^{1}H Spectrum of Compound C-1b: 6-fluoroquinoline-2-carbaldehyde



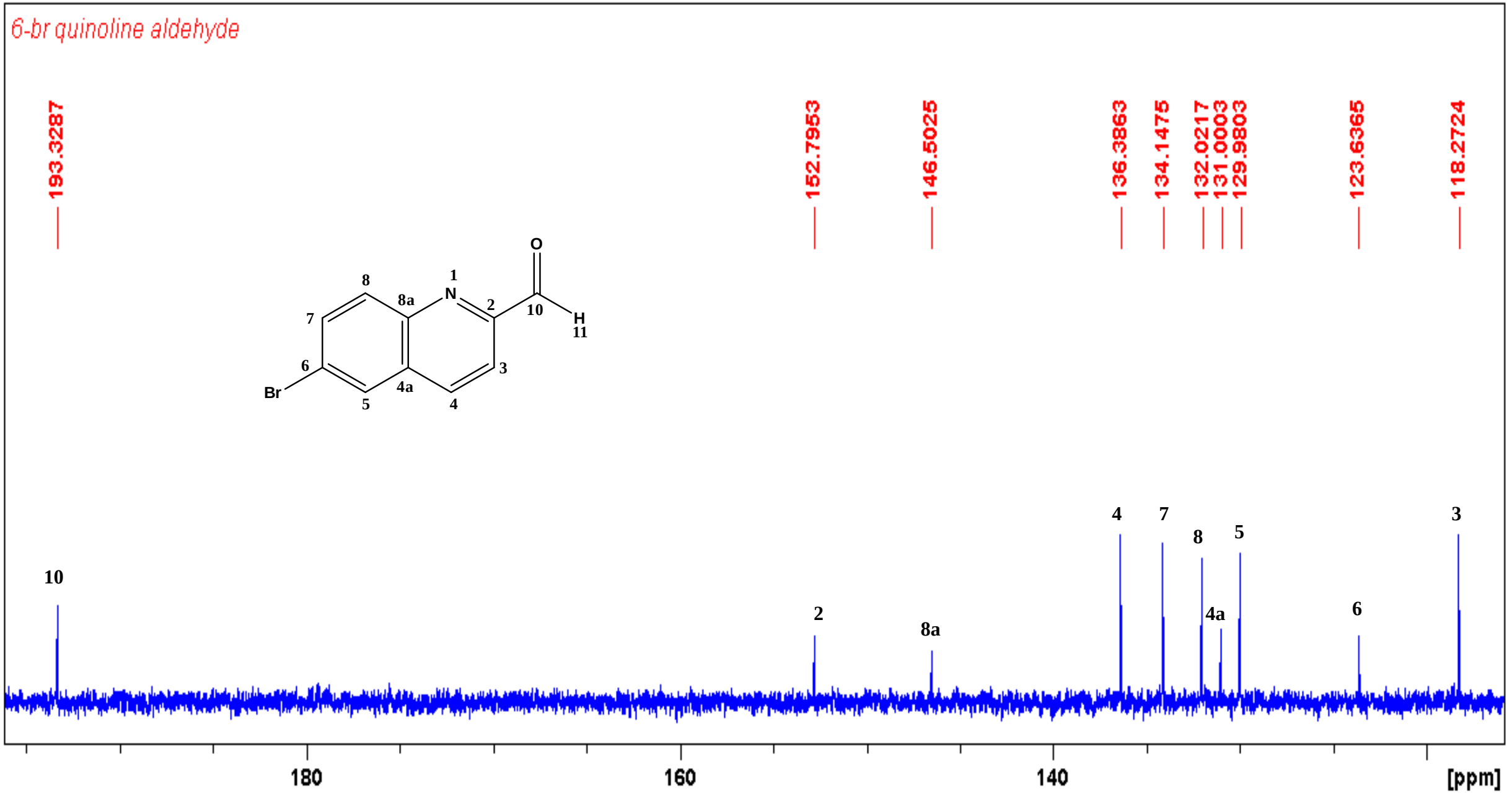
^{13}C Spectrum of Compound C-1b: 6-fluoroquinoline-2-carbaldehyde



^{1}H Spectrum of Compound C-1c: 6-bromoquinoline-2-carbaldehyde

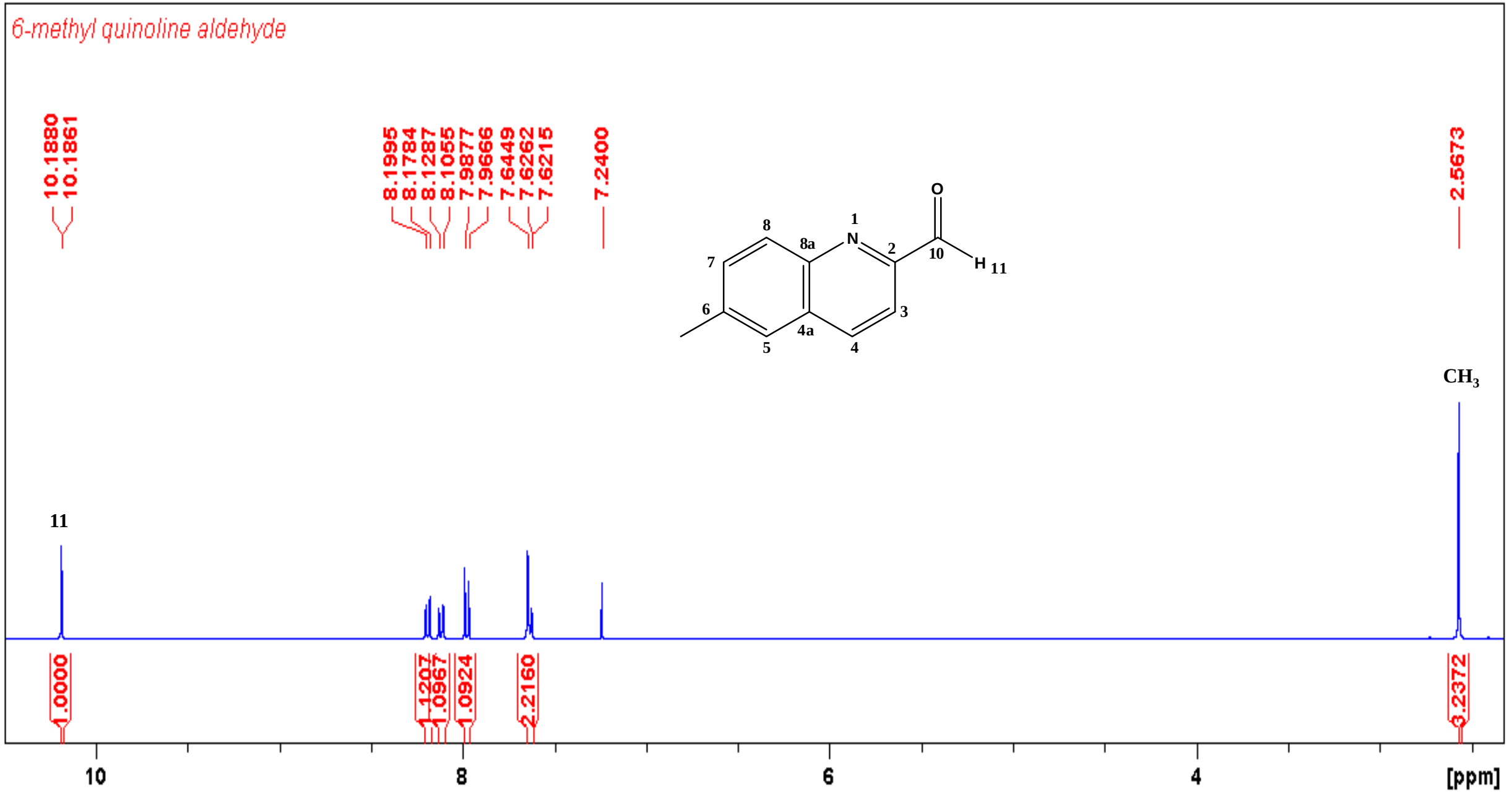


Expanded ^{1}H Spectrum of Compound C-1c: 6-bromoquinoline-2-carbaldehyde



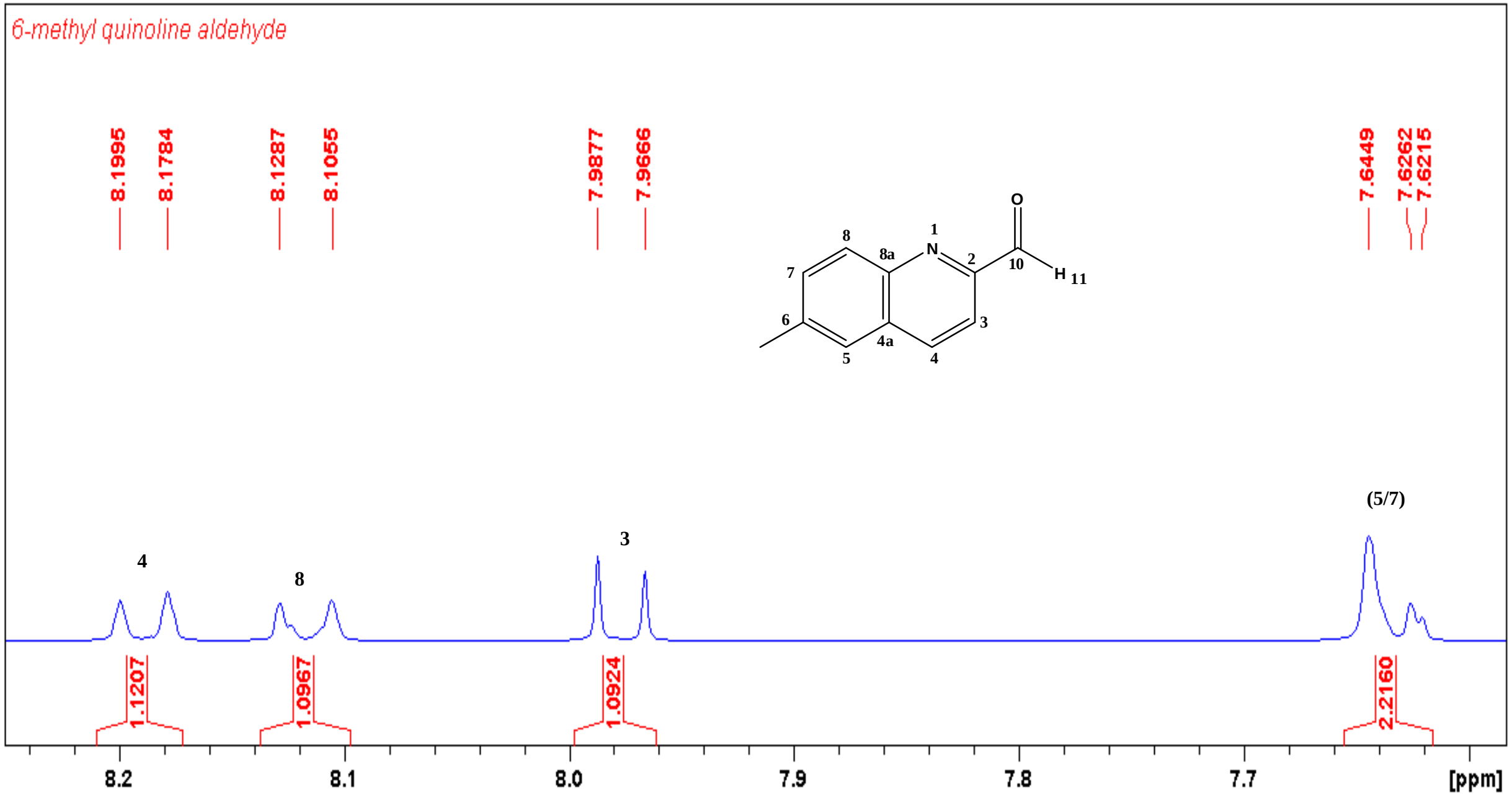
703

^{13}C Spectrum of Compound C-1c: 6-bromoquinoline-2-carbaldehyde

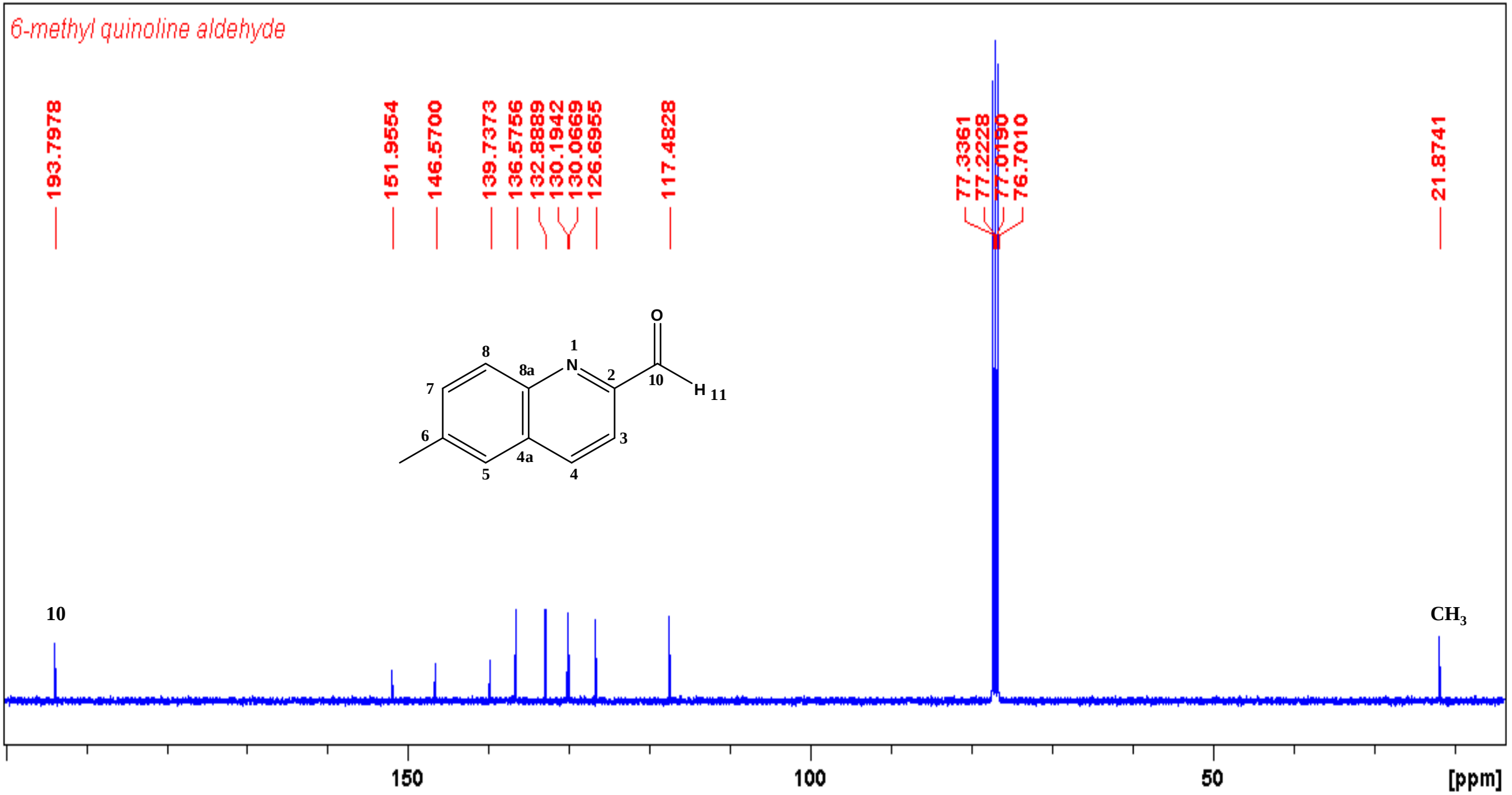


704

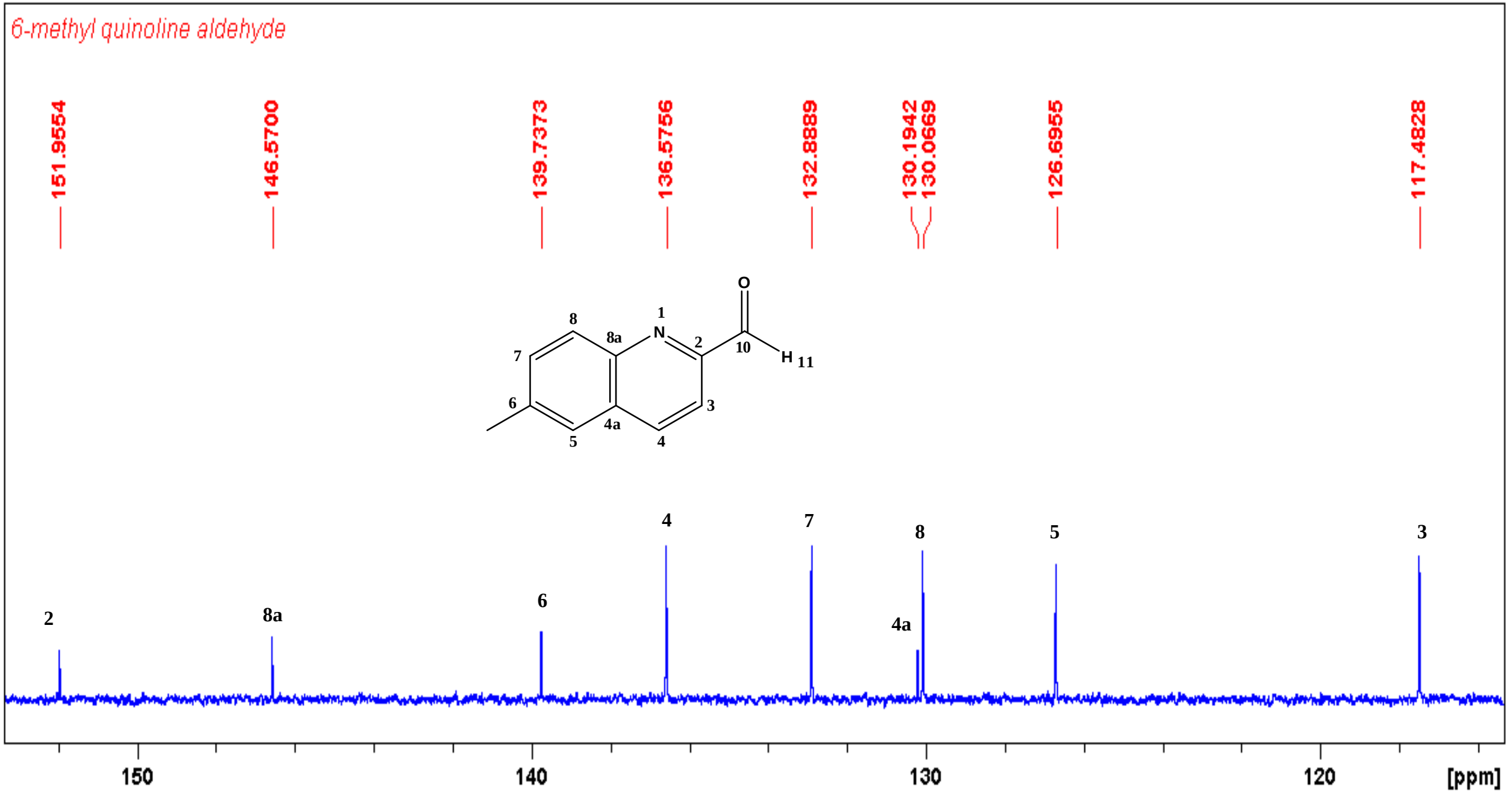
^{1}H Spectrum of Compound C-1d: 6-methylquinoline-2-carbaldehyde



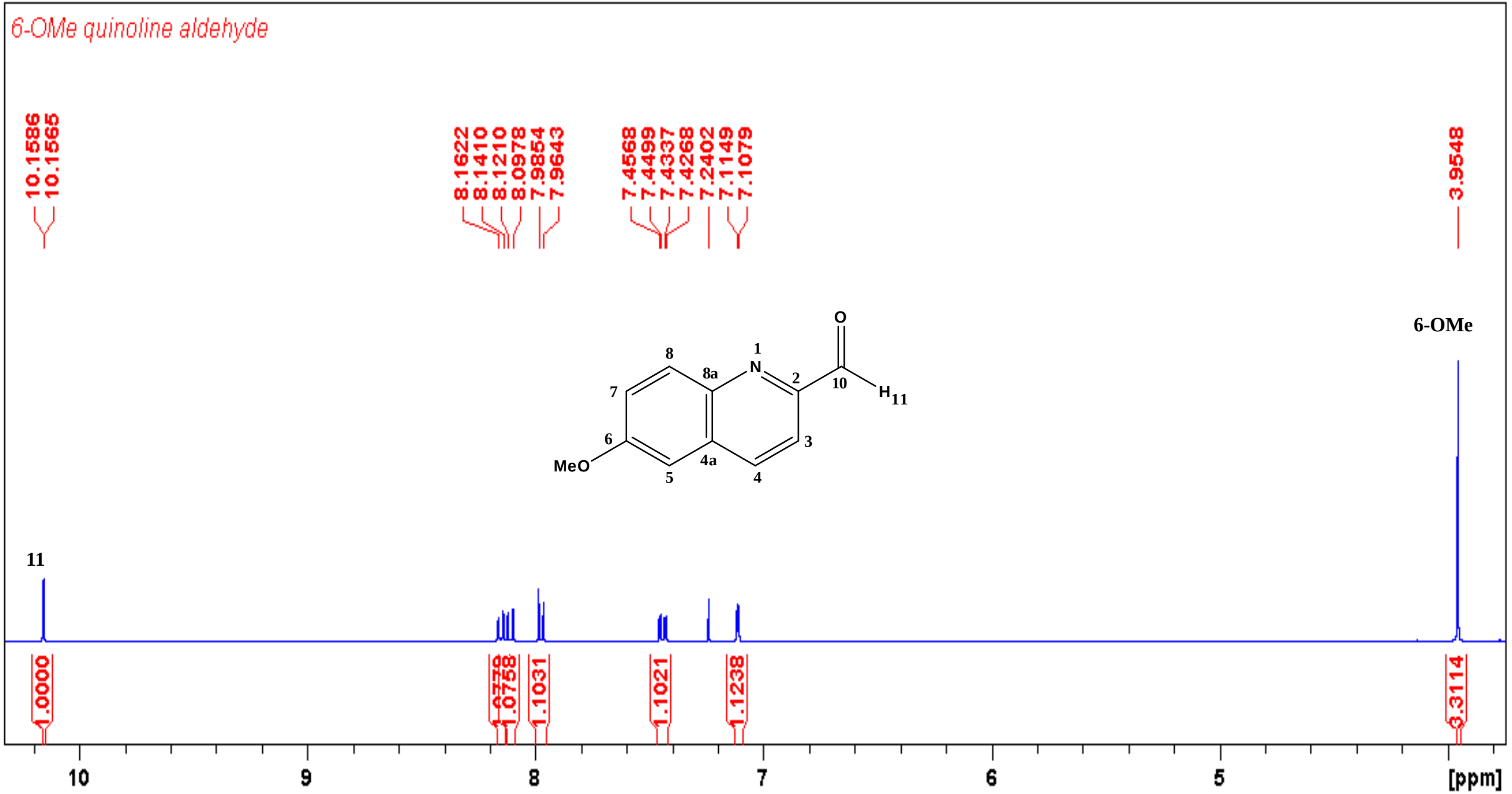
Expanded ^{1}H Spectrum of Compound C-1d: 6-methylquinoline-2-carbaldehyde



^{13}C Spectrum of Compound C-1d: 6-methylquinoline-2-carbaldehyde

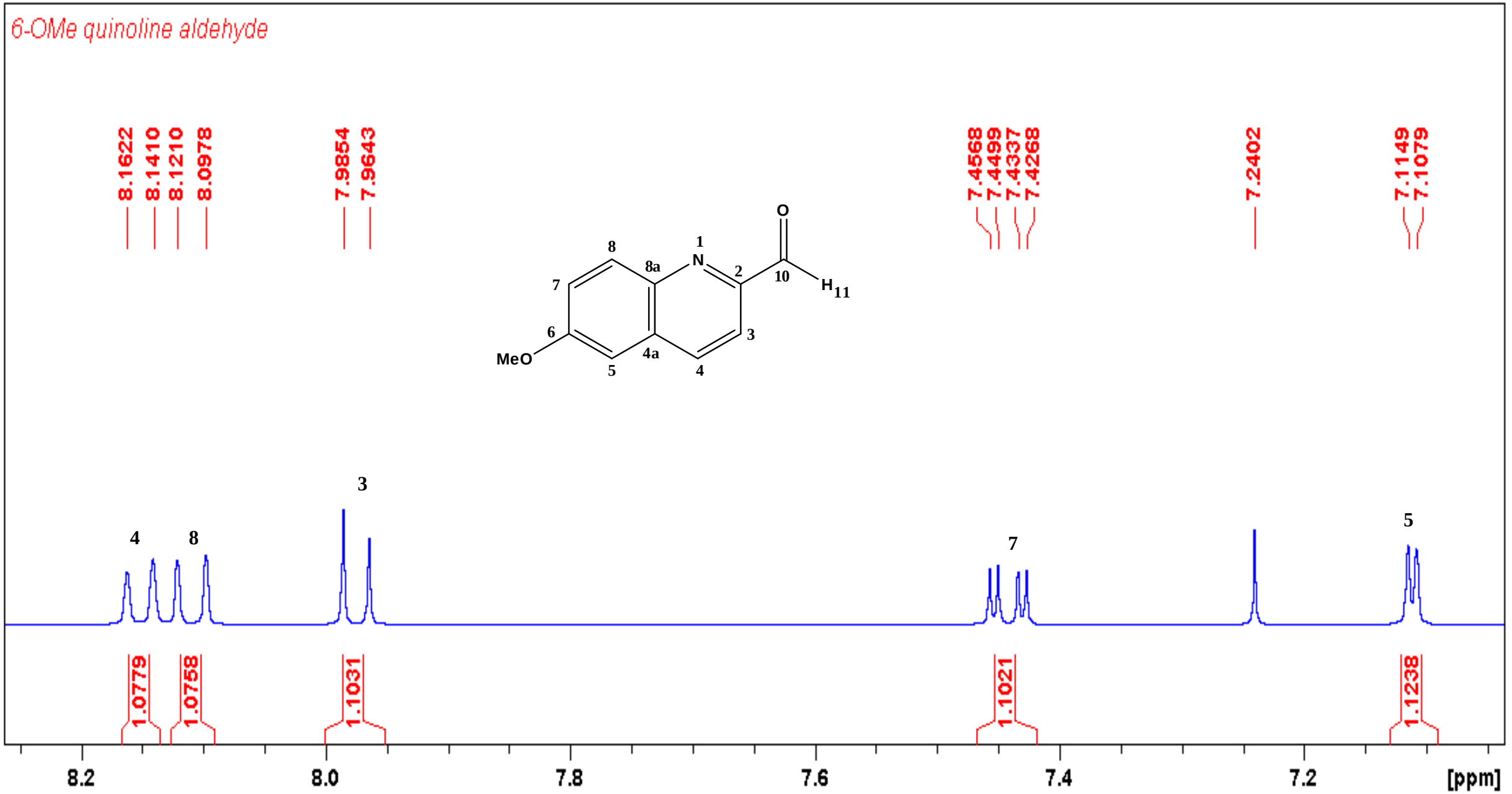


Expanded ^{13}C Spectrum of Compound C-1d: 6-methylquinoline-2-carbaldehyde

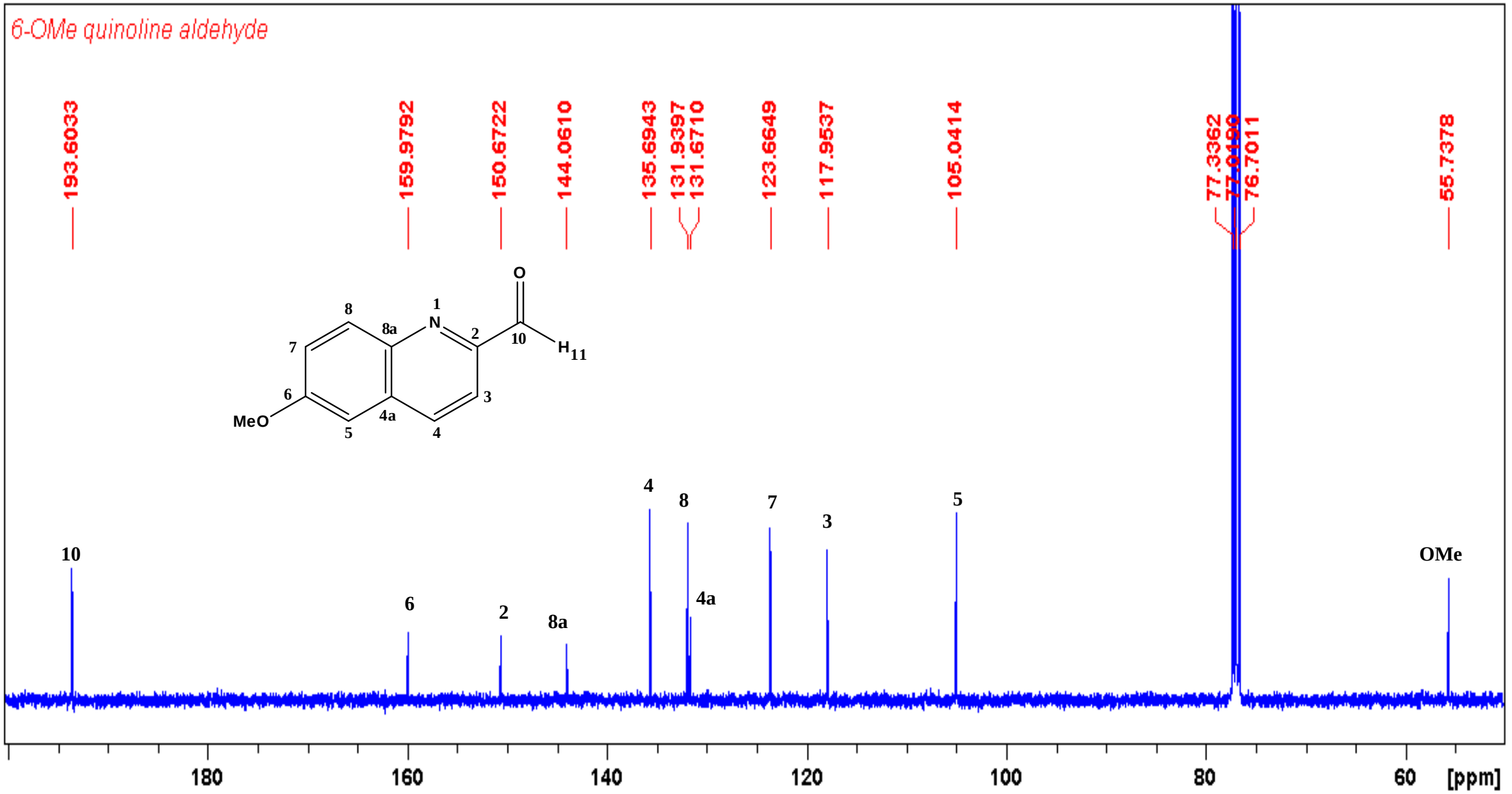


708

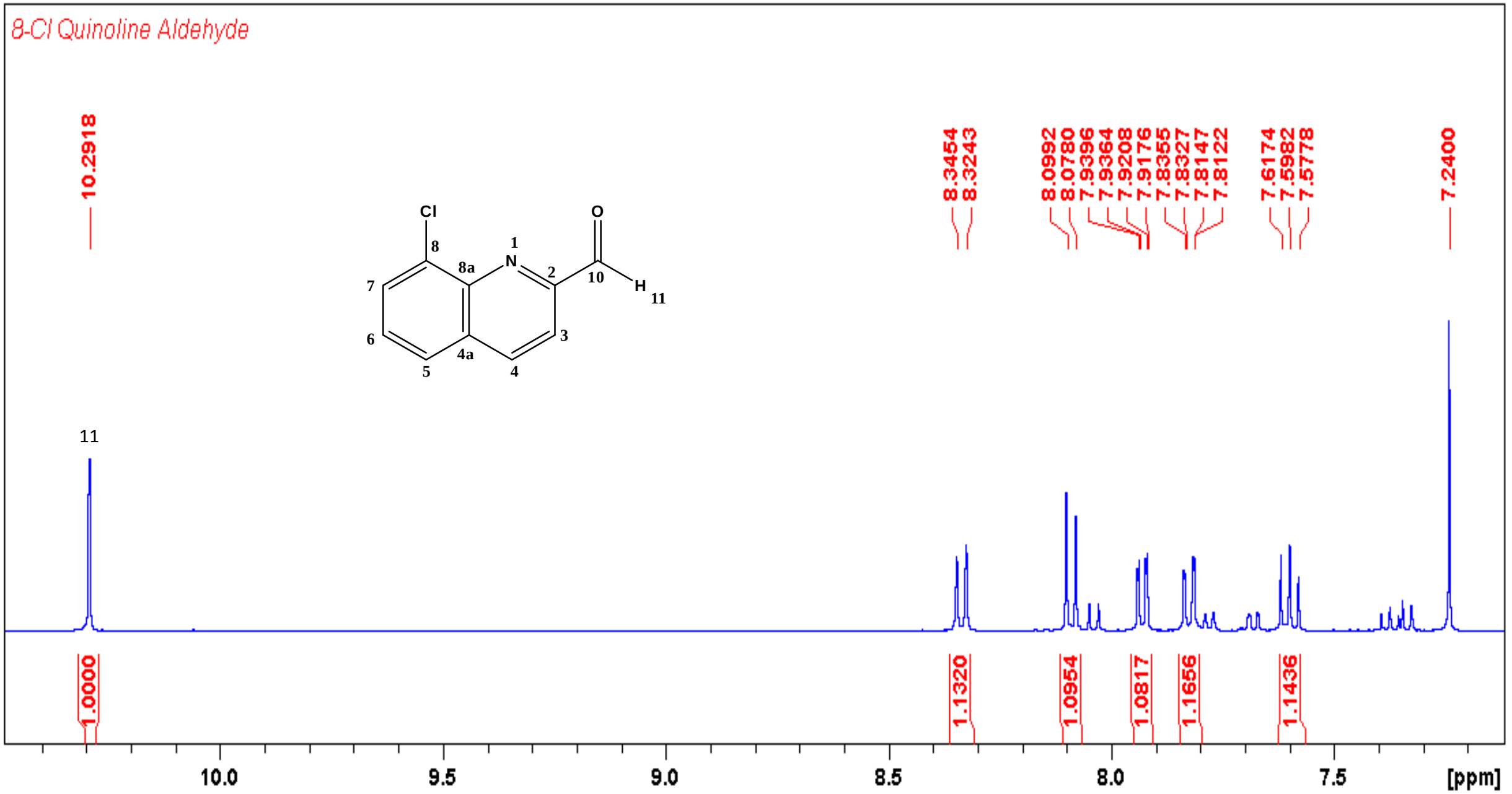
^{1}H Spectrum of Compound C-1e: 6-methoxyquinoline-2-carbaldehyde



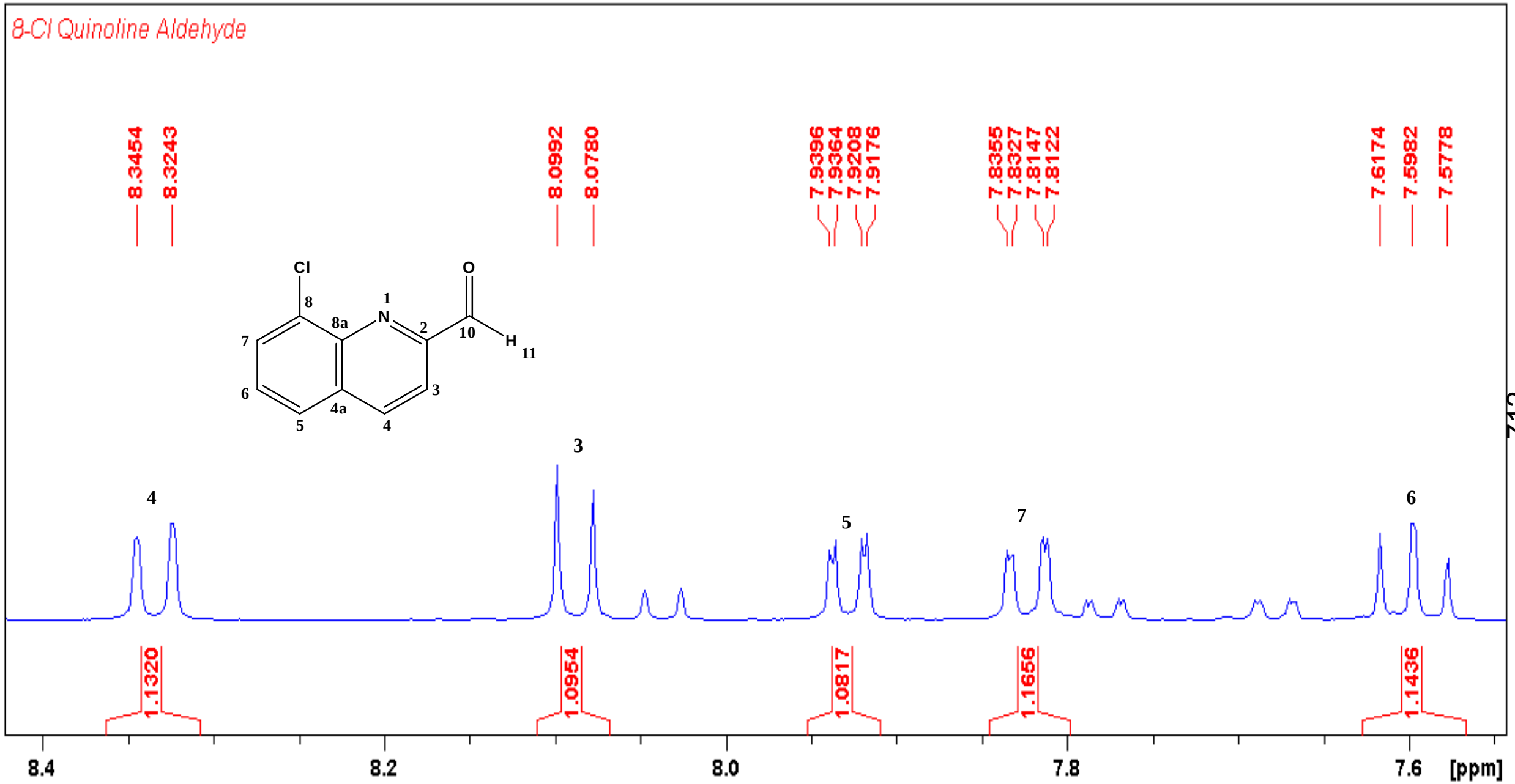
Expanded ^{1}H Spectrum of Compound C-1e: 6-methoxyquinoline-2-carbaldehyde



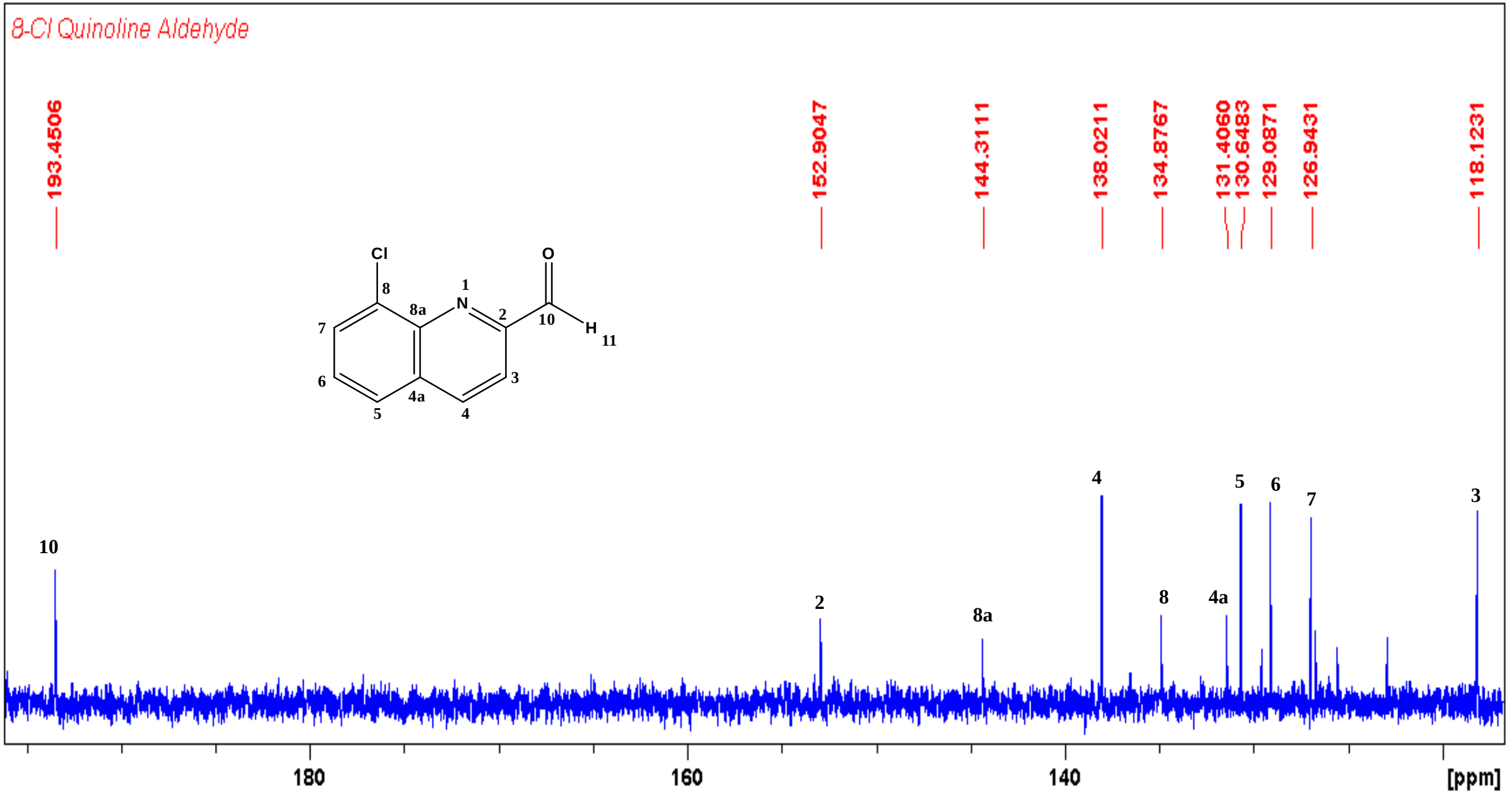
^{13}C Spectrum of Compound C-1e: 6-methoxyquinoline-2-carbaldehyde



^{1}H Spectrum of Compound C-1f: 8-chloroquinoline-2-carbaldehyde

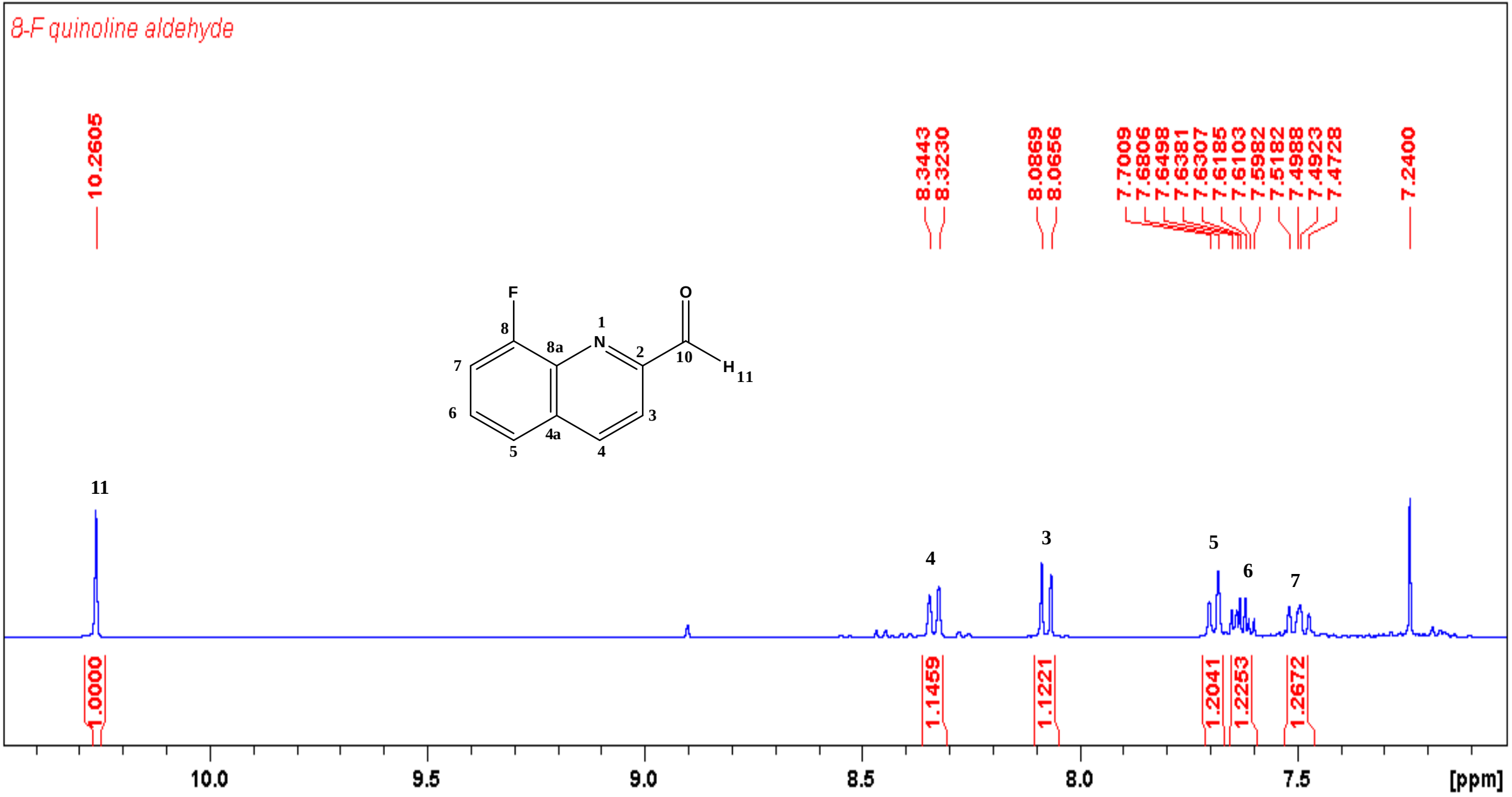


Expanded ^{1}H Spectrum of Compound C-1f: 8-chloroquinoline-2-carbaldehyde



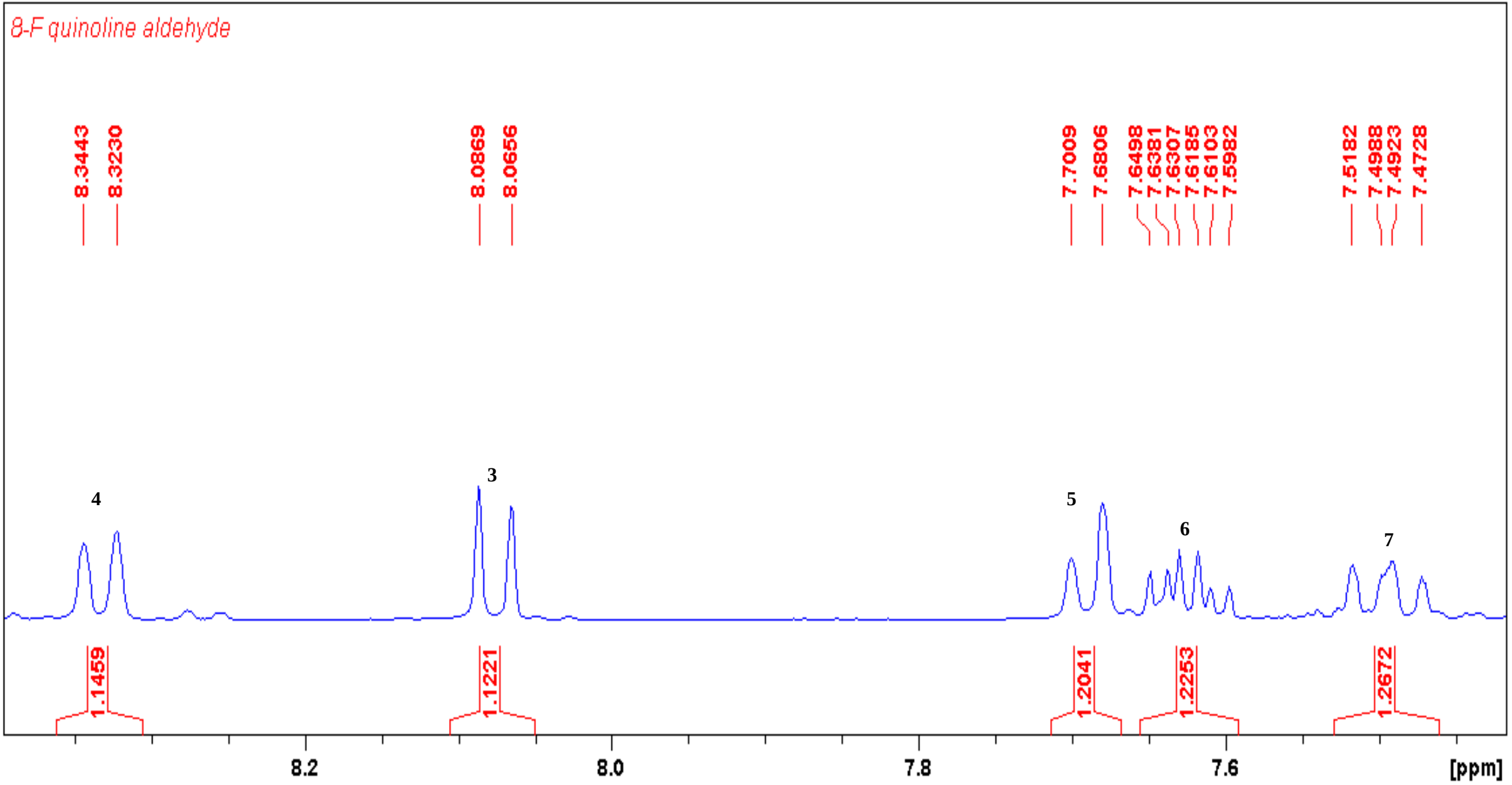
713

^{13}C Spectrum of Compound C-1f: 8-chloroquinoline-2-carbaldehyde

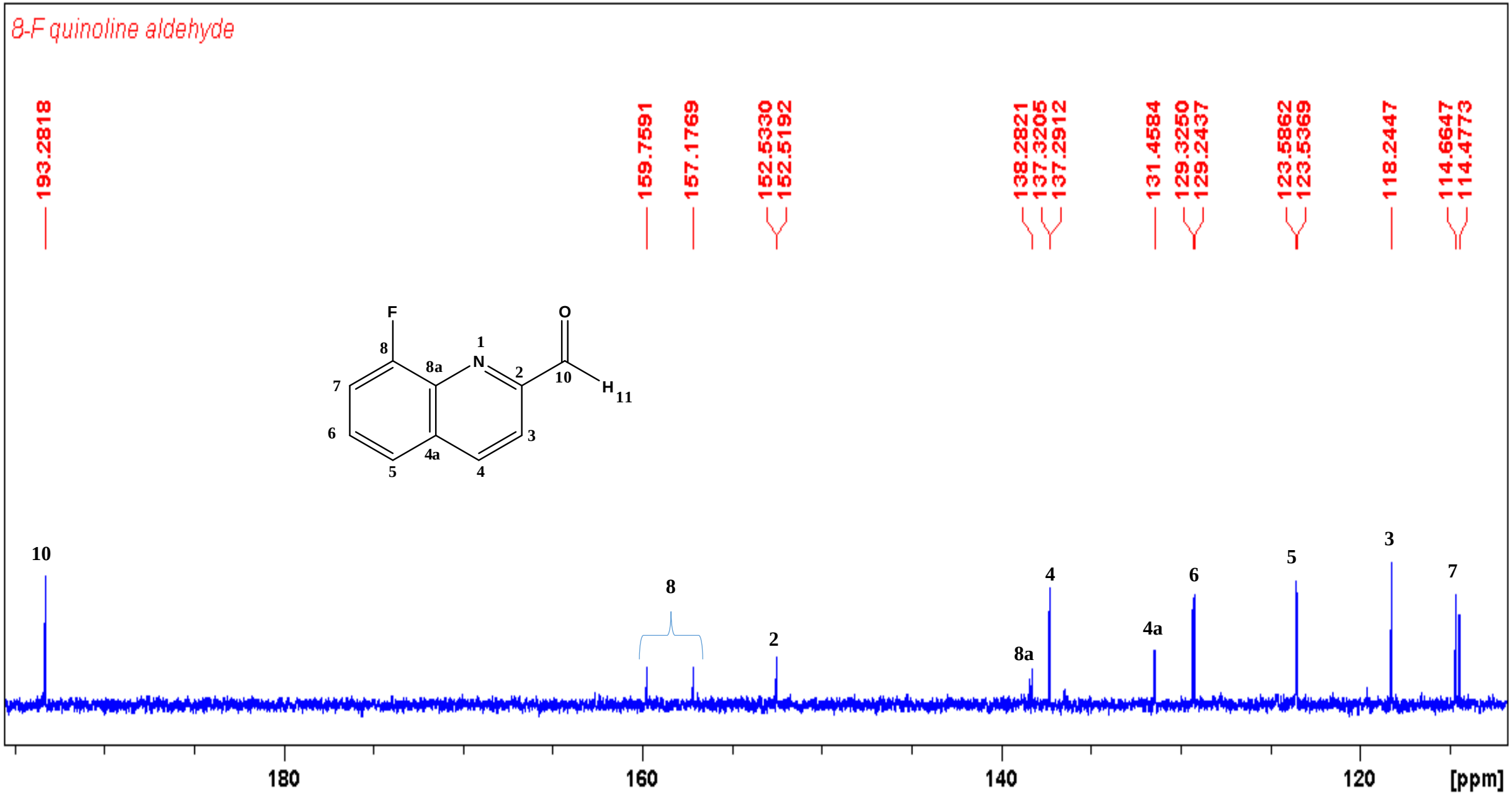


714

^{1}H Spectrum of Compound C-1g: 8-fluoroquinoline-2-carbaldehyde

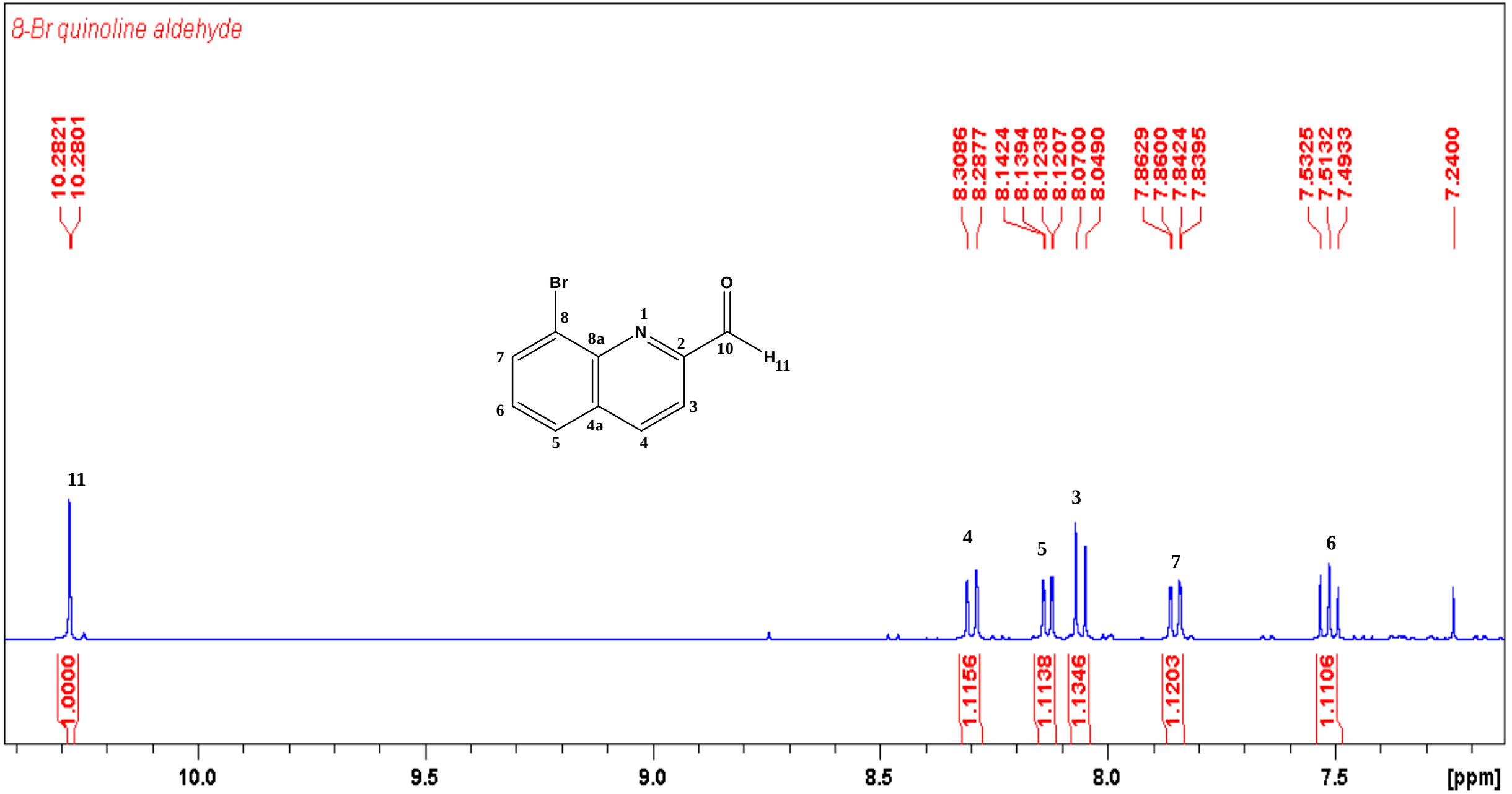


Expanded ^{1}H Spectrum of Compound C-1g: 8-fluoroquinoline-2-carbaldehyde

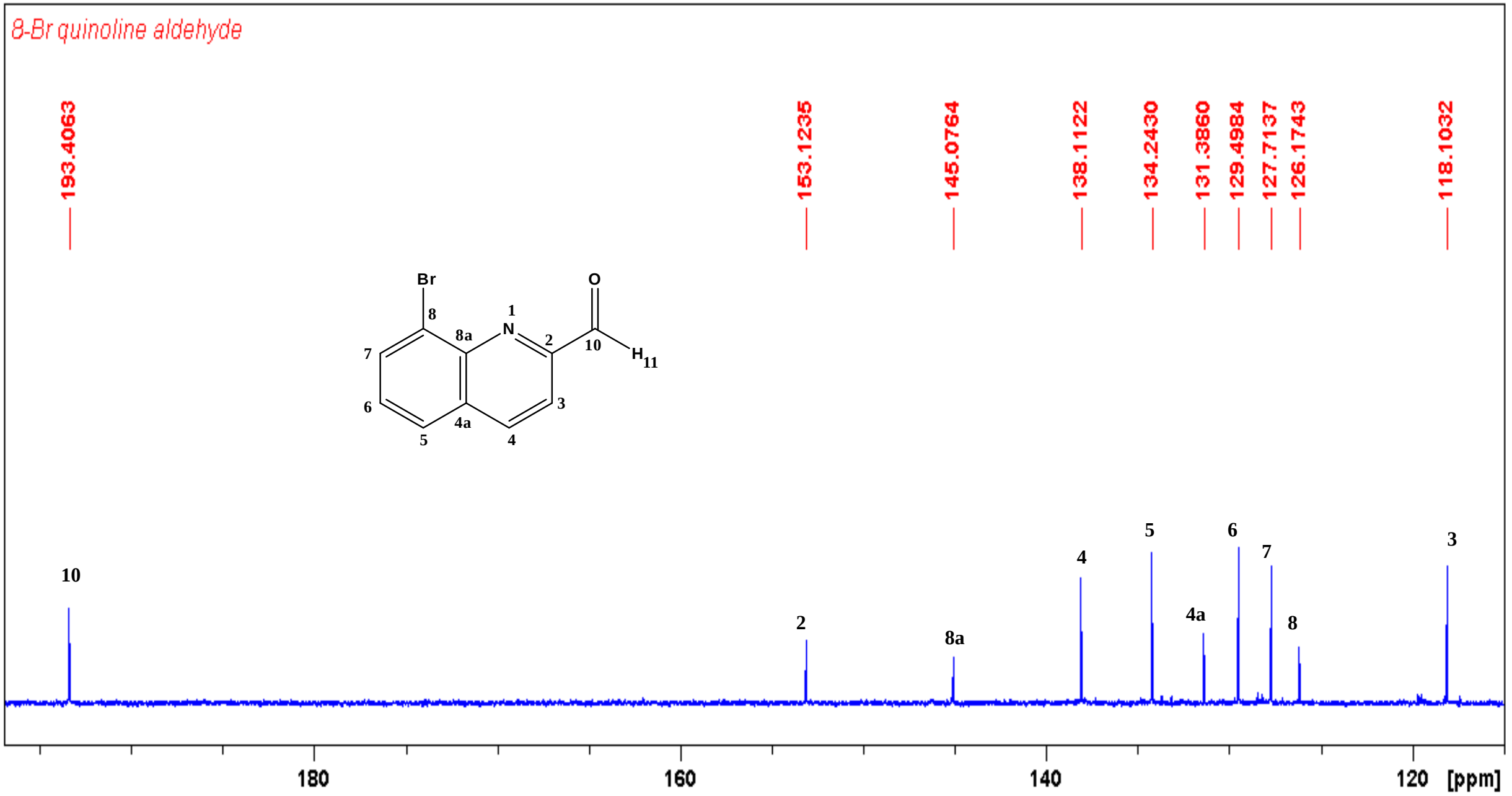


716

^{13}C Spectrum of Compound C-1g: 8-fluoroquinoline-2-carbaldehyde

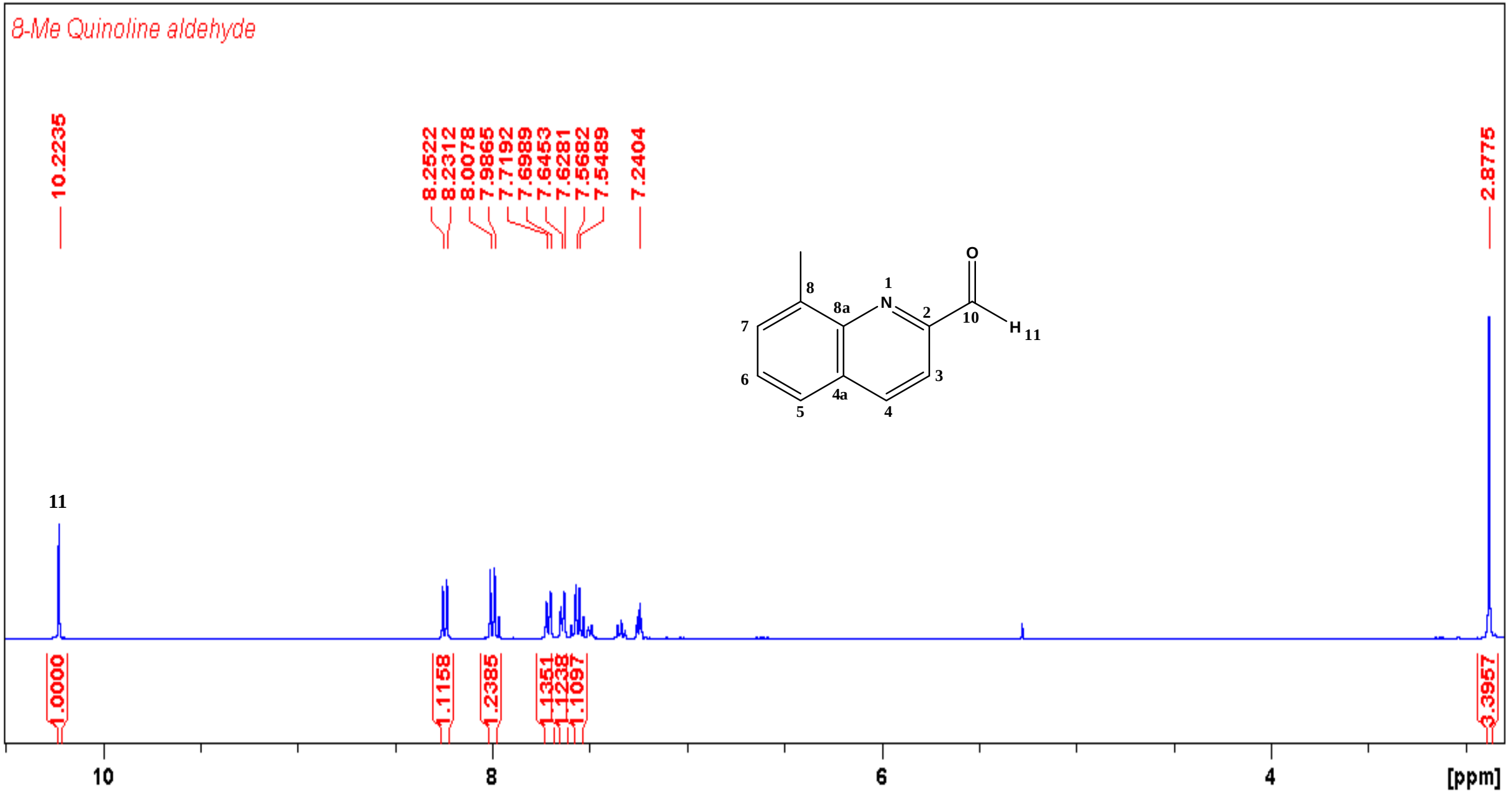


^{1}H Spectrum of Compound C-1h: 8-bromoquinoline-2-carbaldehyde

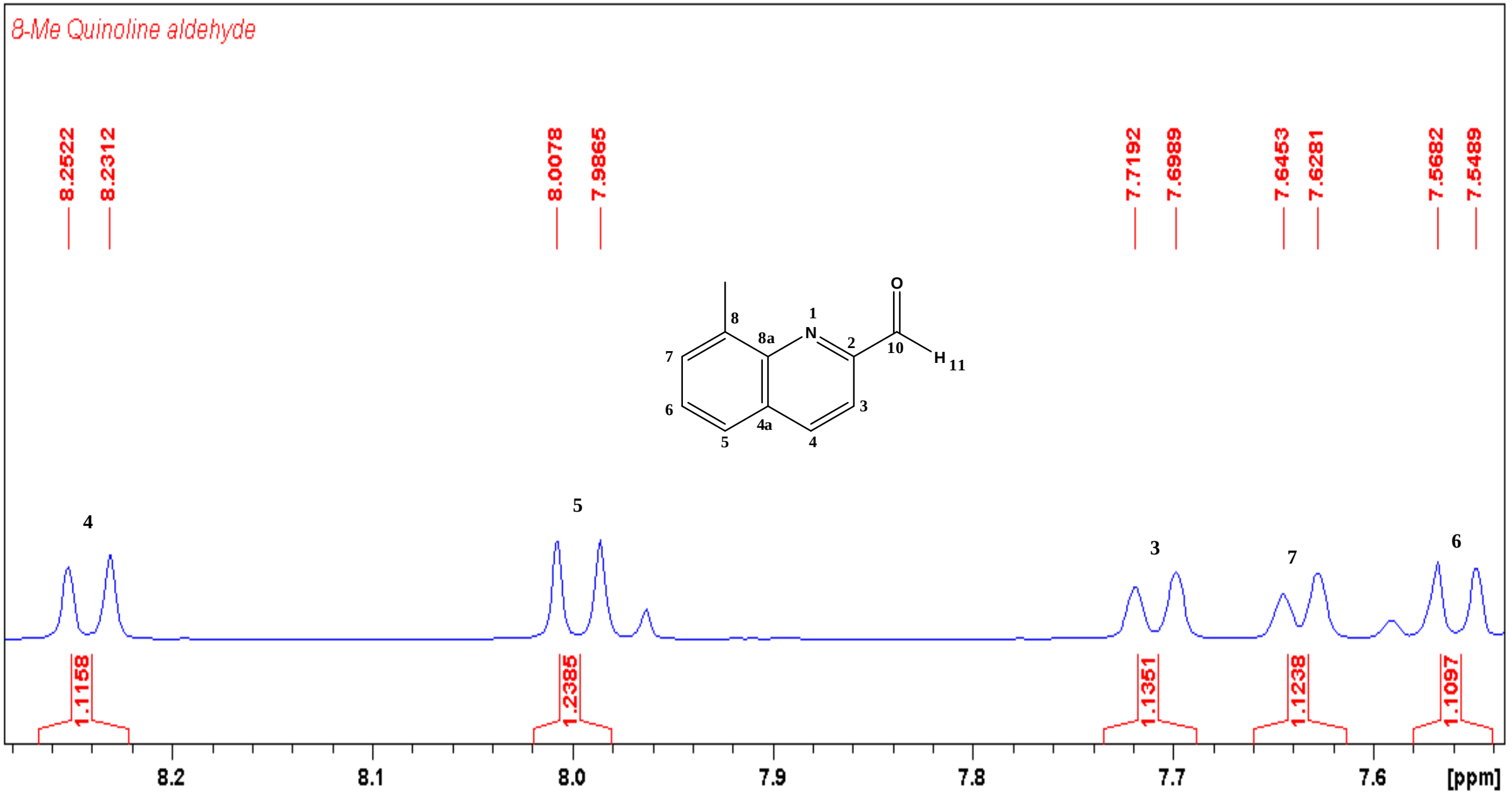


718

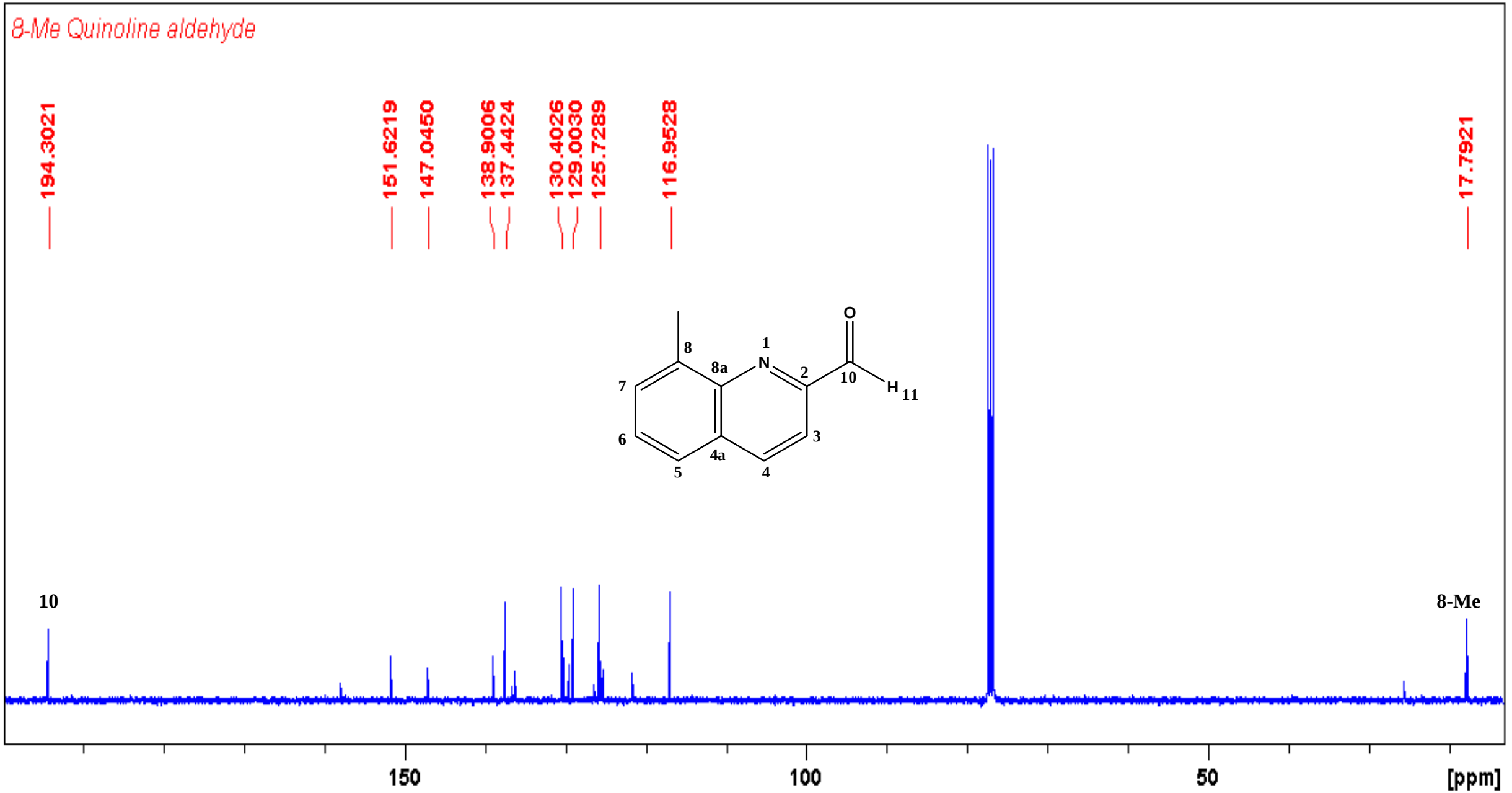
^{13}C Spectrum of Compound C-1h: 8-bromoquinoline-2-carbaldehyde



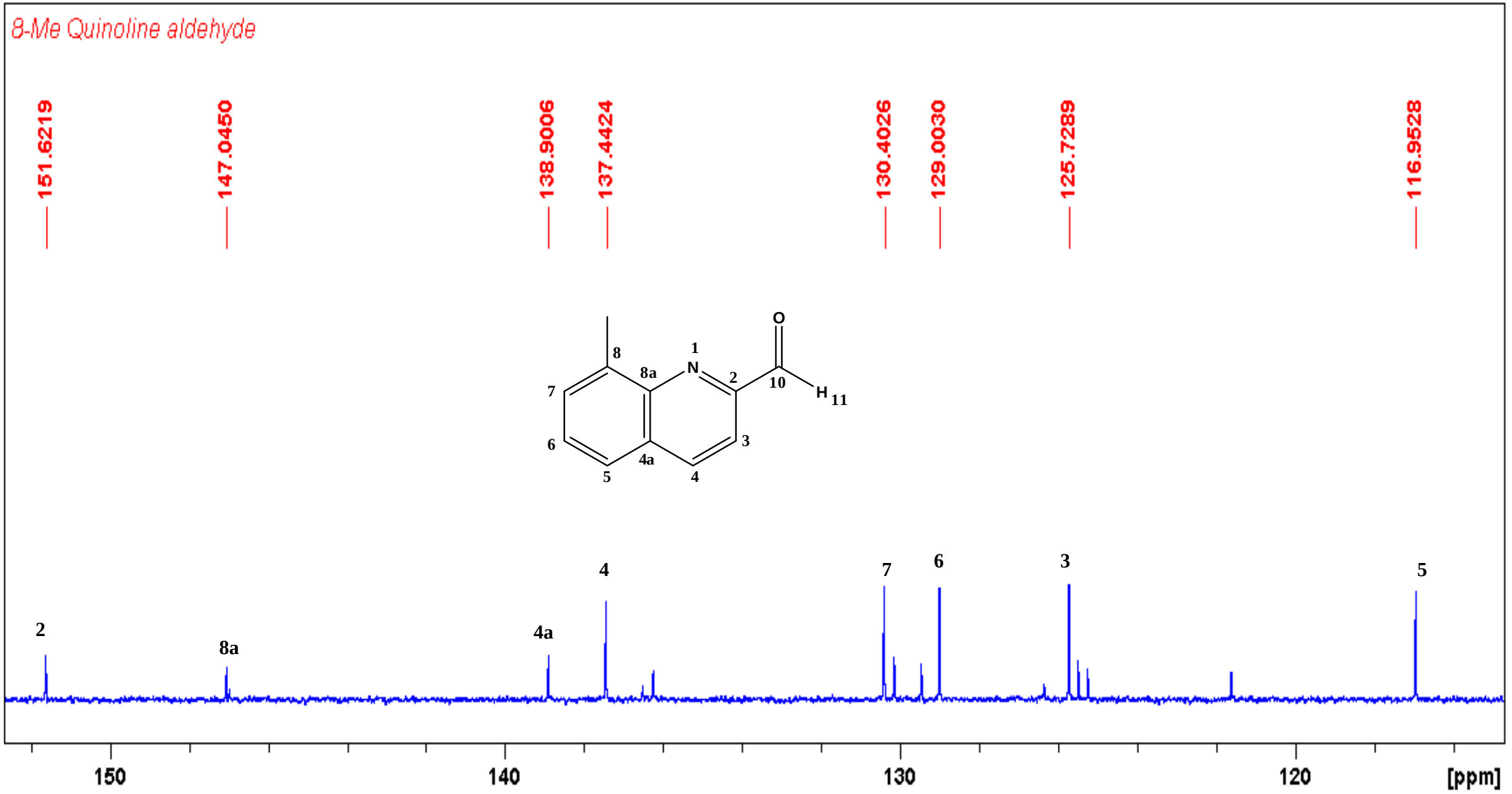
^{1}H Spectrum of Compound C-1i: 8-methylquinoline-2-carbaldehyde



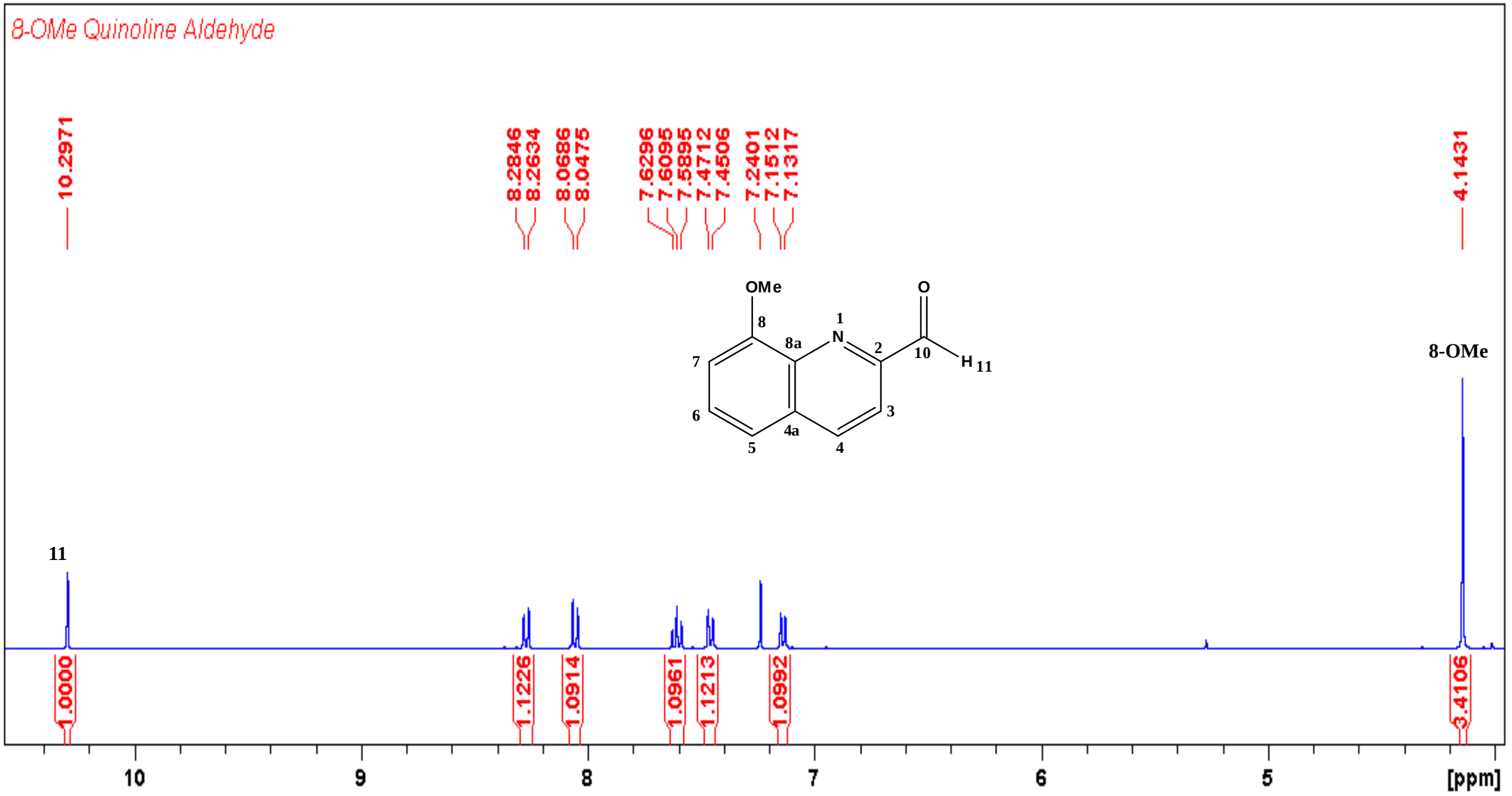
Expanded ^{1}H Spectrum of Compound C-1i: 8-methylquinoline-2-carbaldehyde



^{13}C Spectrum of Compound C-1i: 8-methylquinoline-2-carbaldehyde

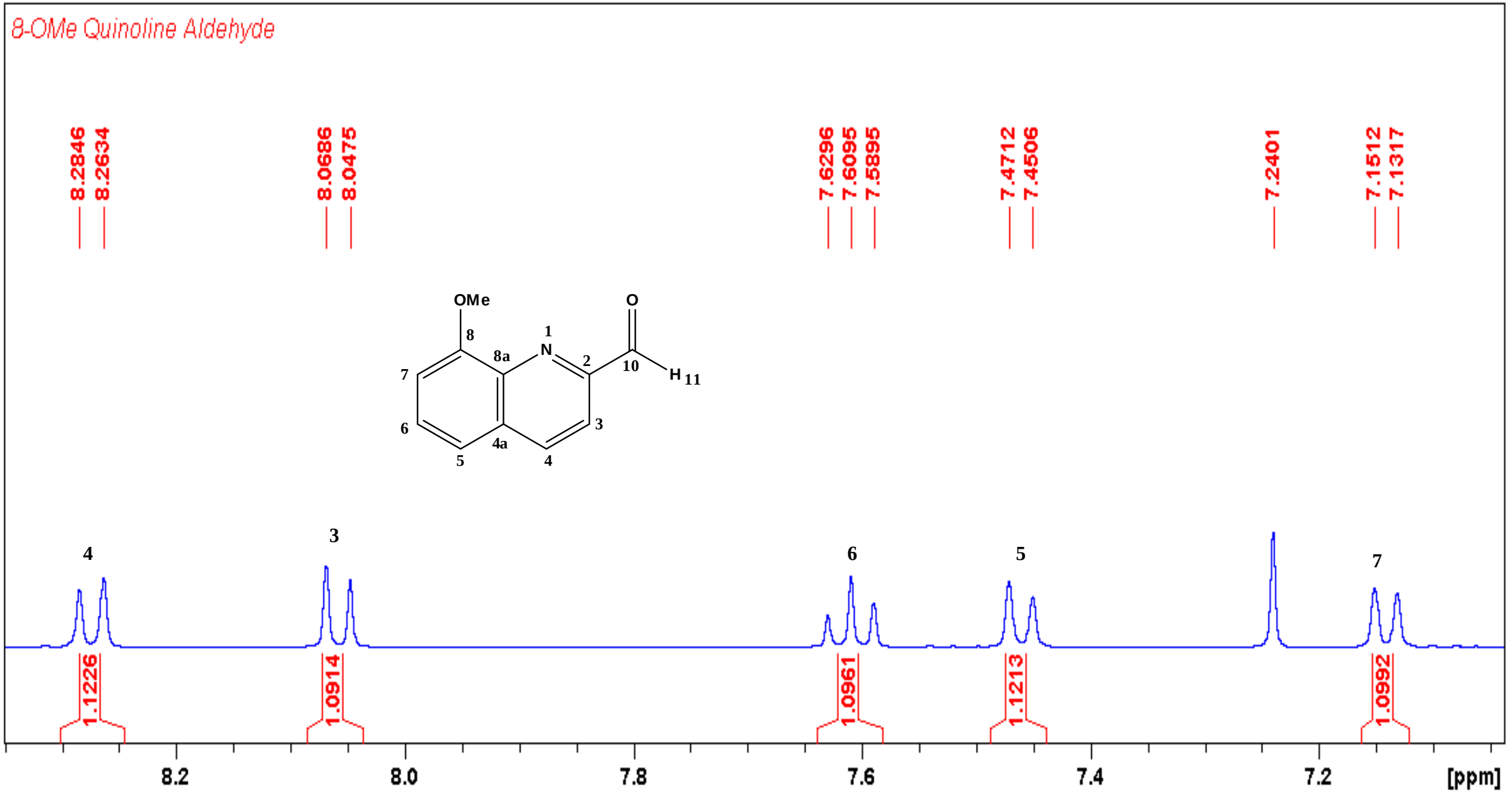


Expanded ^{13}C Spectrum of Compound C-1i: 8-methylquinoline-2-carbaldehyde

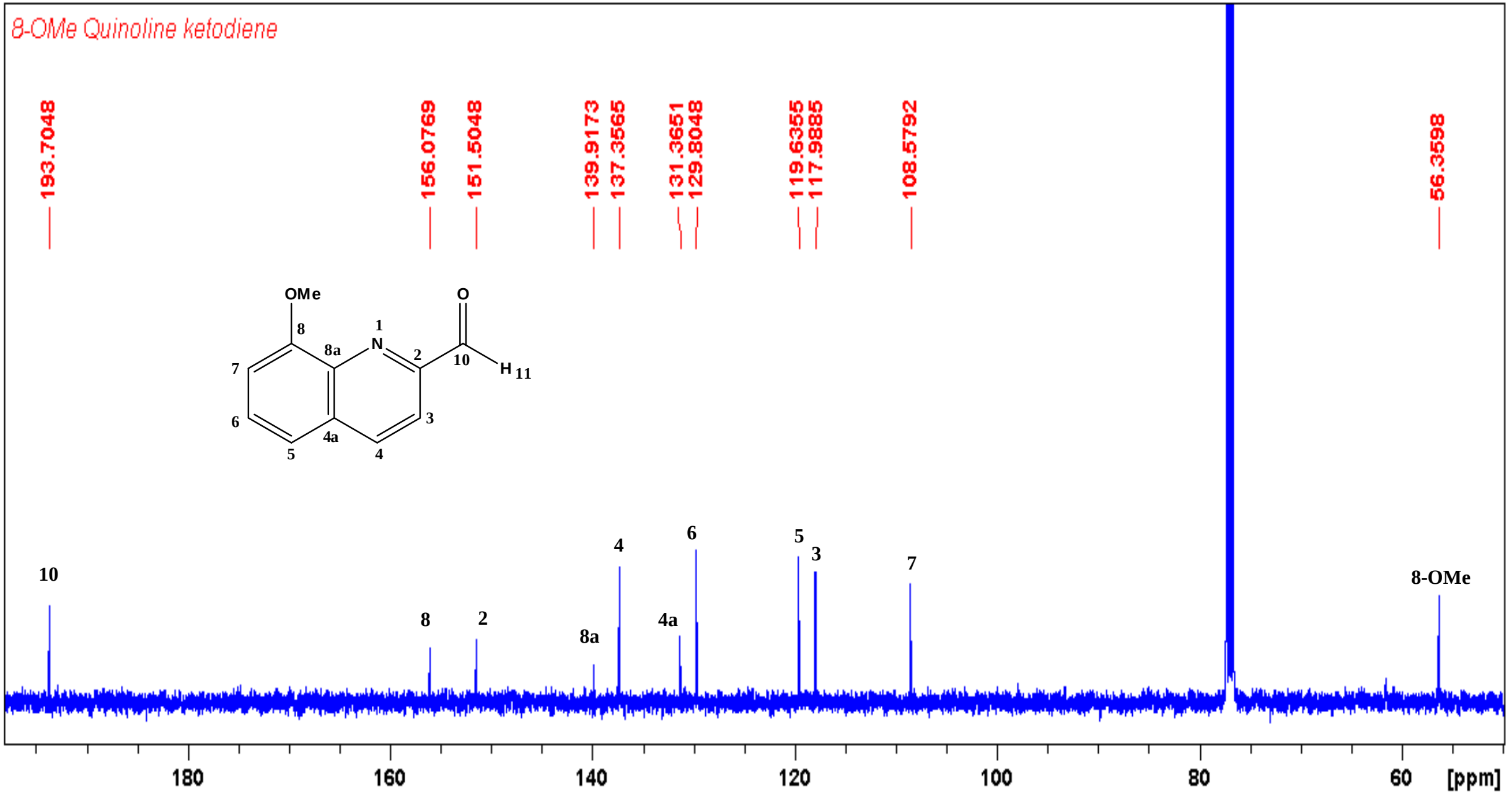


723

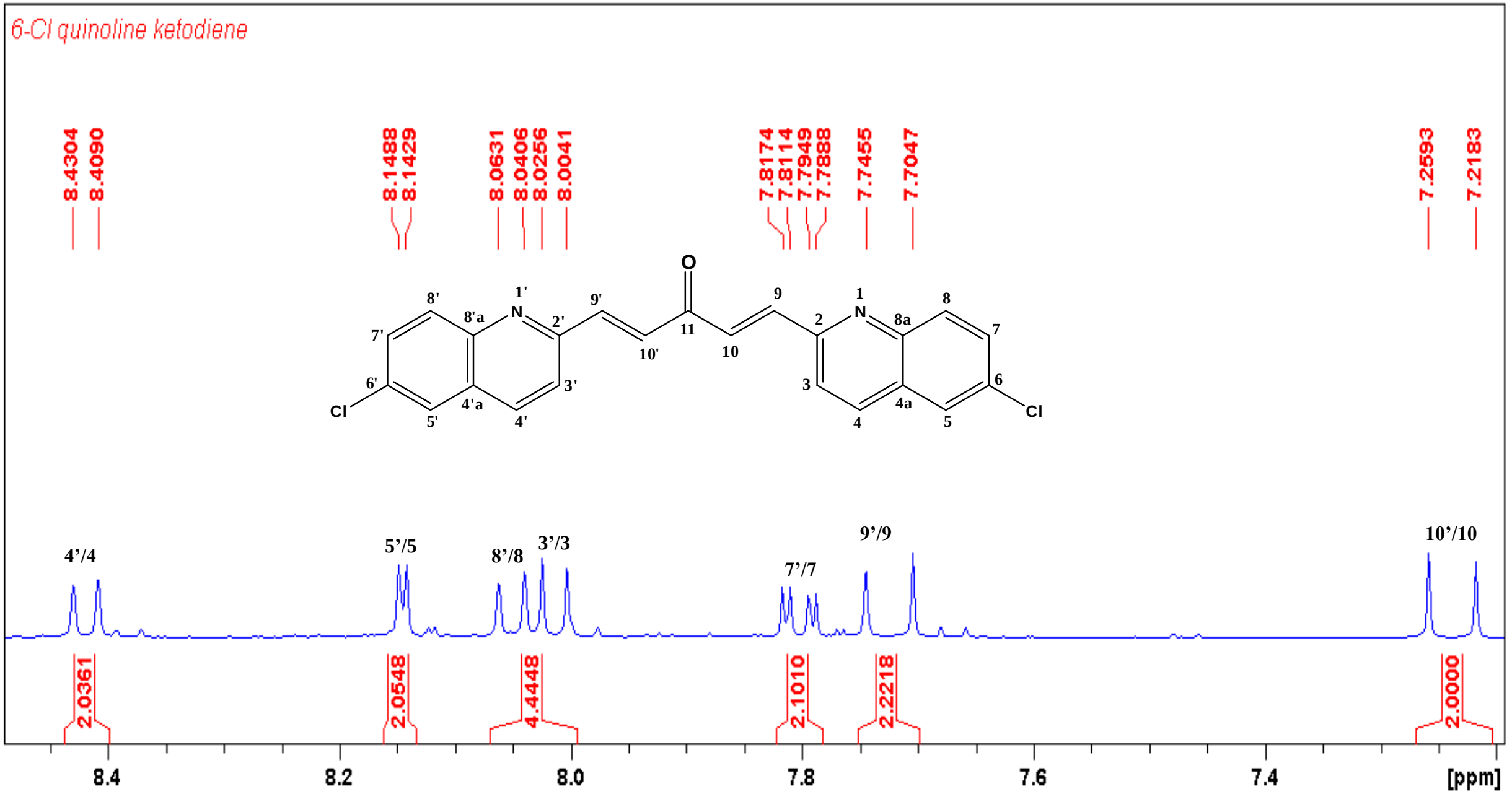
[1]H Spectrum of Compound C-1j: 8-methoxyquinoline-2-carbaldehyde



Expanded ^{1}H Spectrum of Compound C-1j: 8-methoxyquinoline-2-carbaldehyde

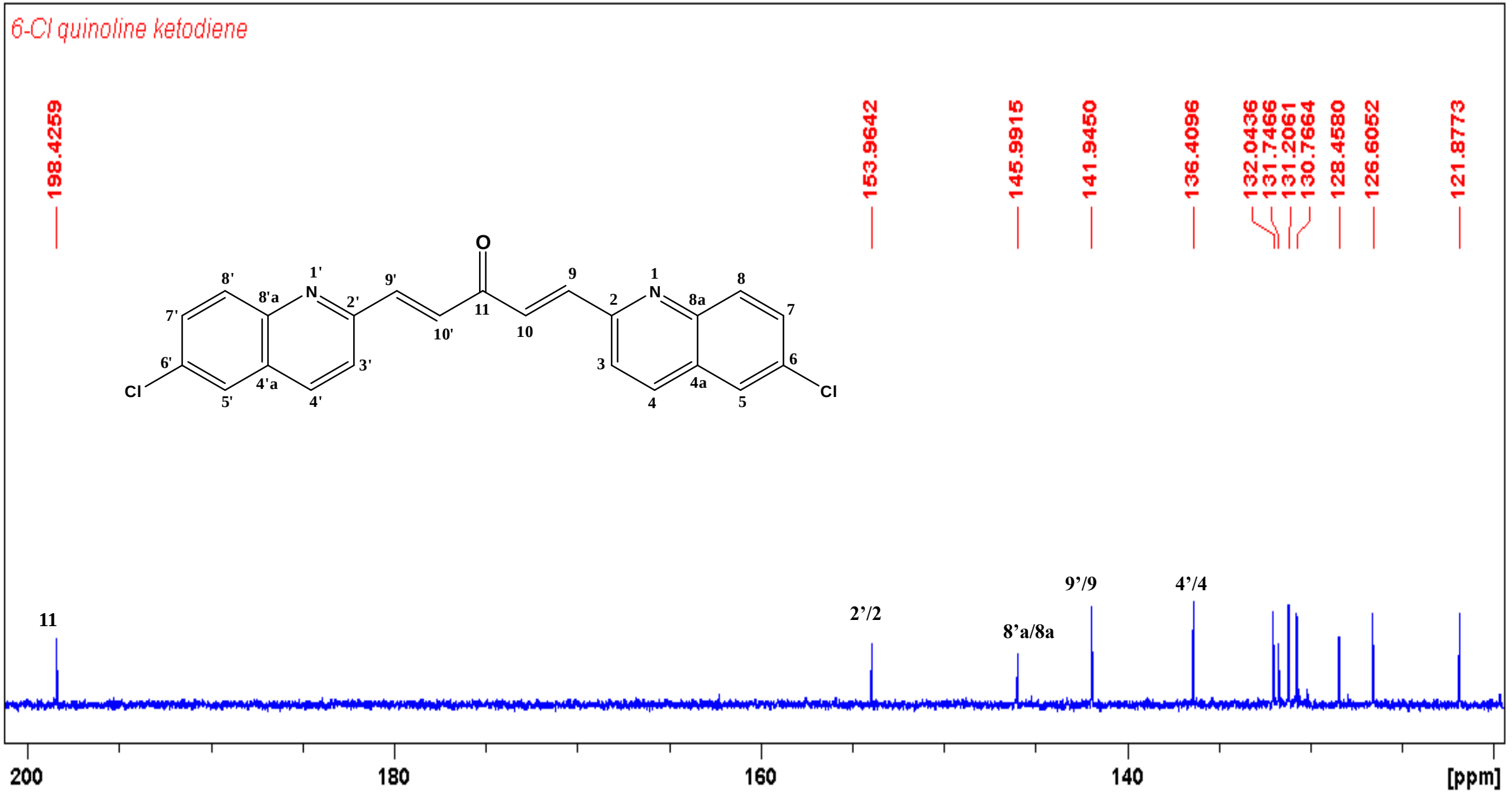


^{13}C Spectrum of Compound C-1j: 8-methoxyquinoline-2-carbaldehyde



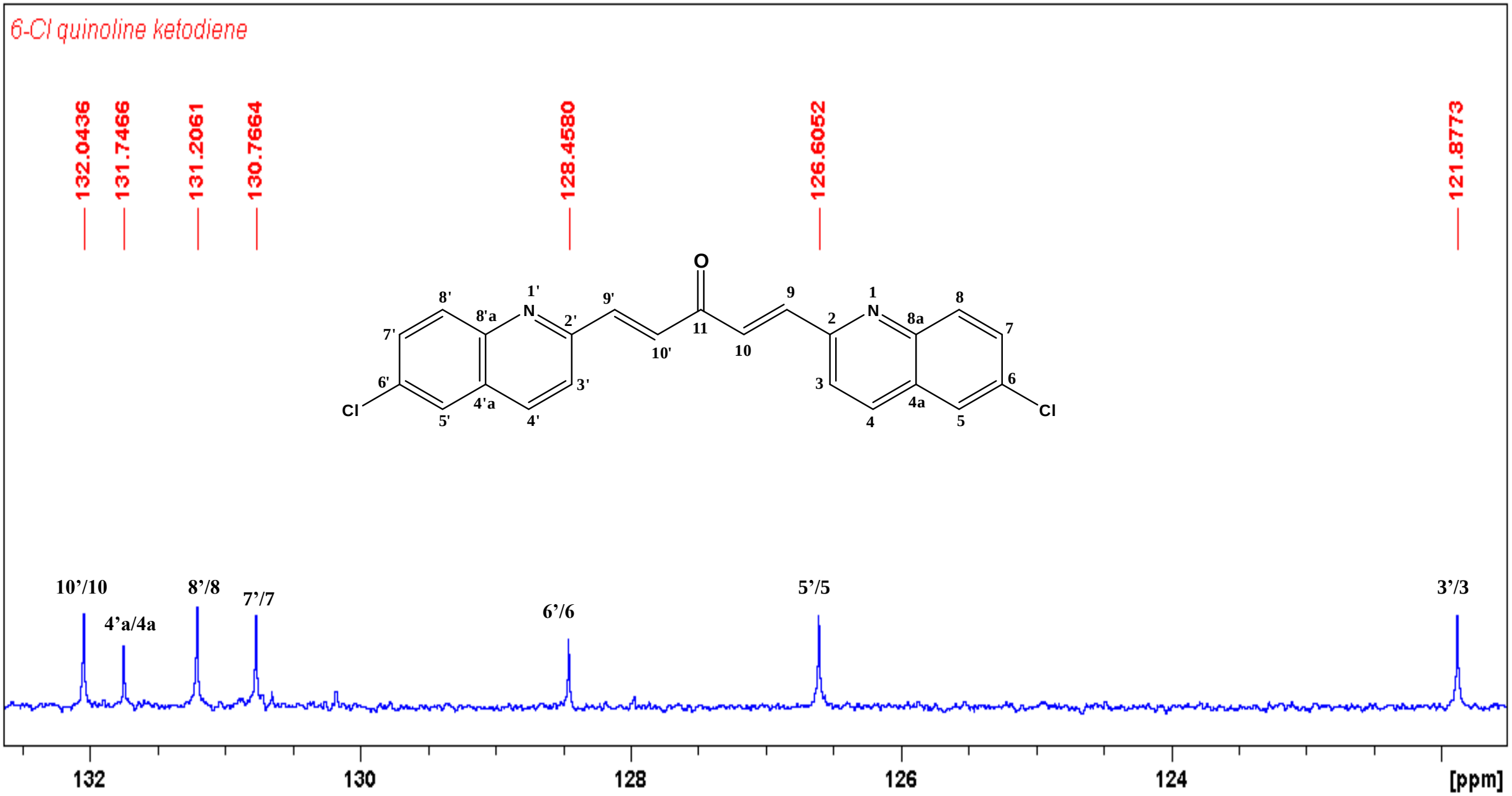
726

^{1}H Spectrum of Compound C-2a: (1E,4E)-1,5-bis(6-chloroquinolin-2-yl)penta-1,4-dien-3-one



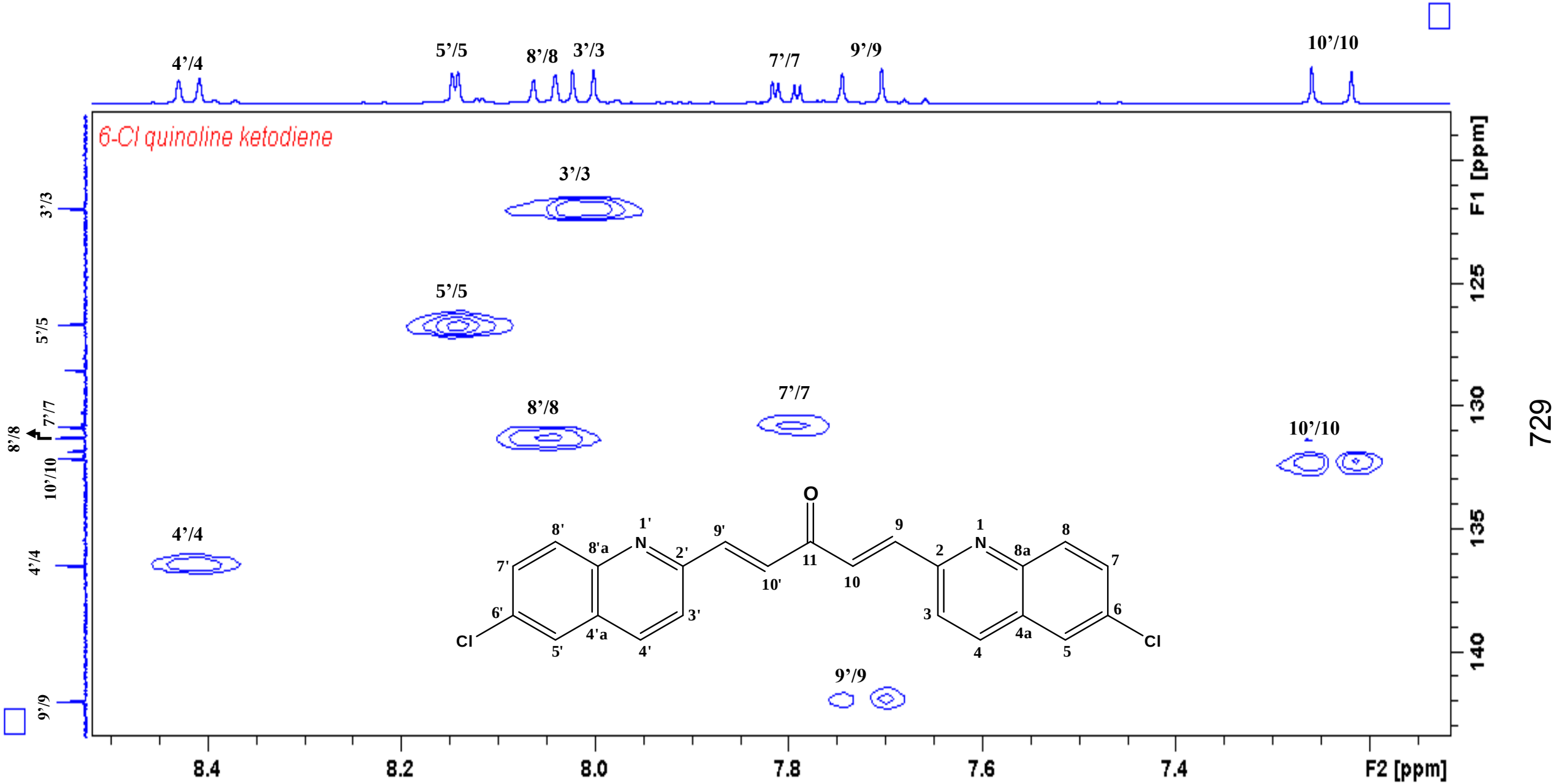
727

^{13}C Spectrum of Compound C-2a : (1E,4E)-1,5-bis(6-chloroquinolin-2-yl)penta-1,4-dien-3-one

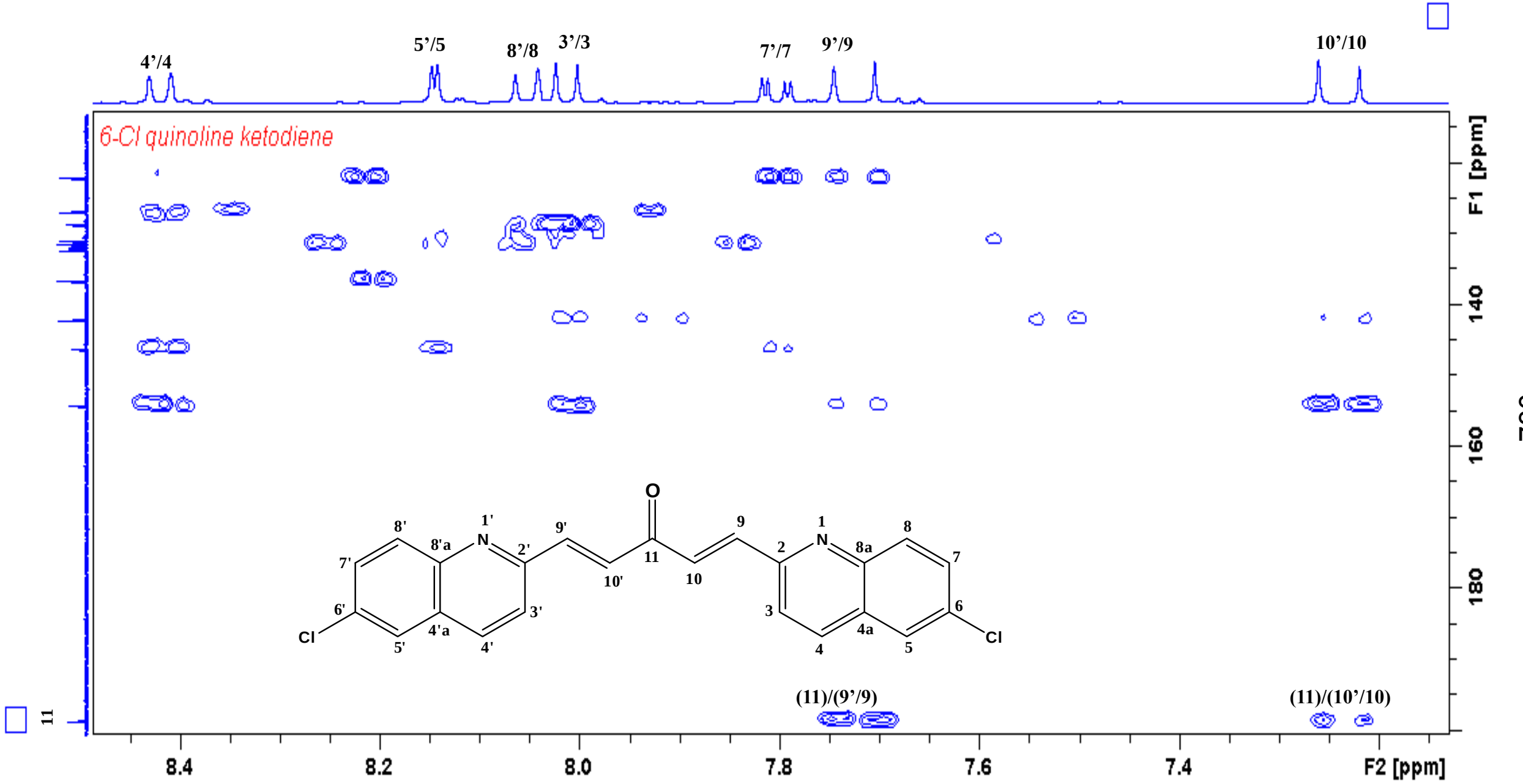


728

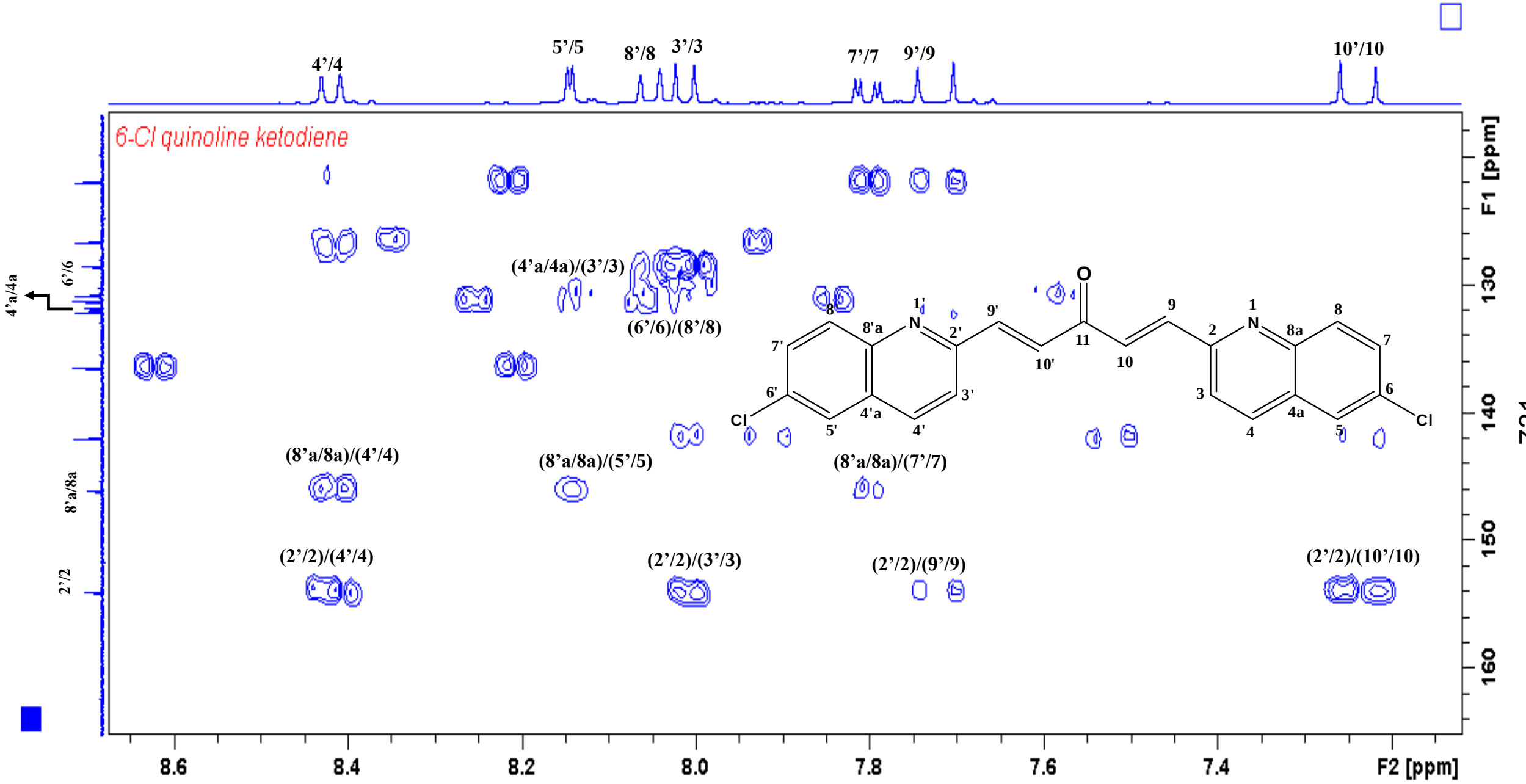
Expanded ^{13}C Spectrum of Compound C-2a : (1E,4E)-1,5-bis(6-chloroquinolin-2-yl)penta-1,4-dien-3-one



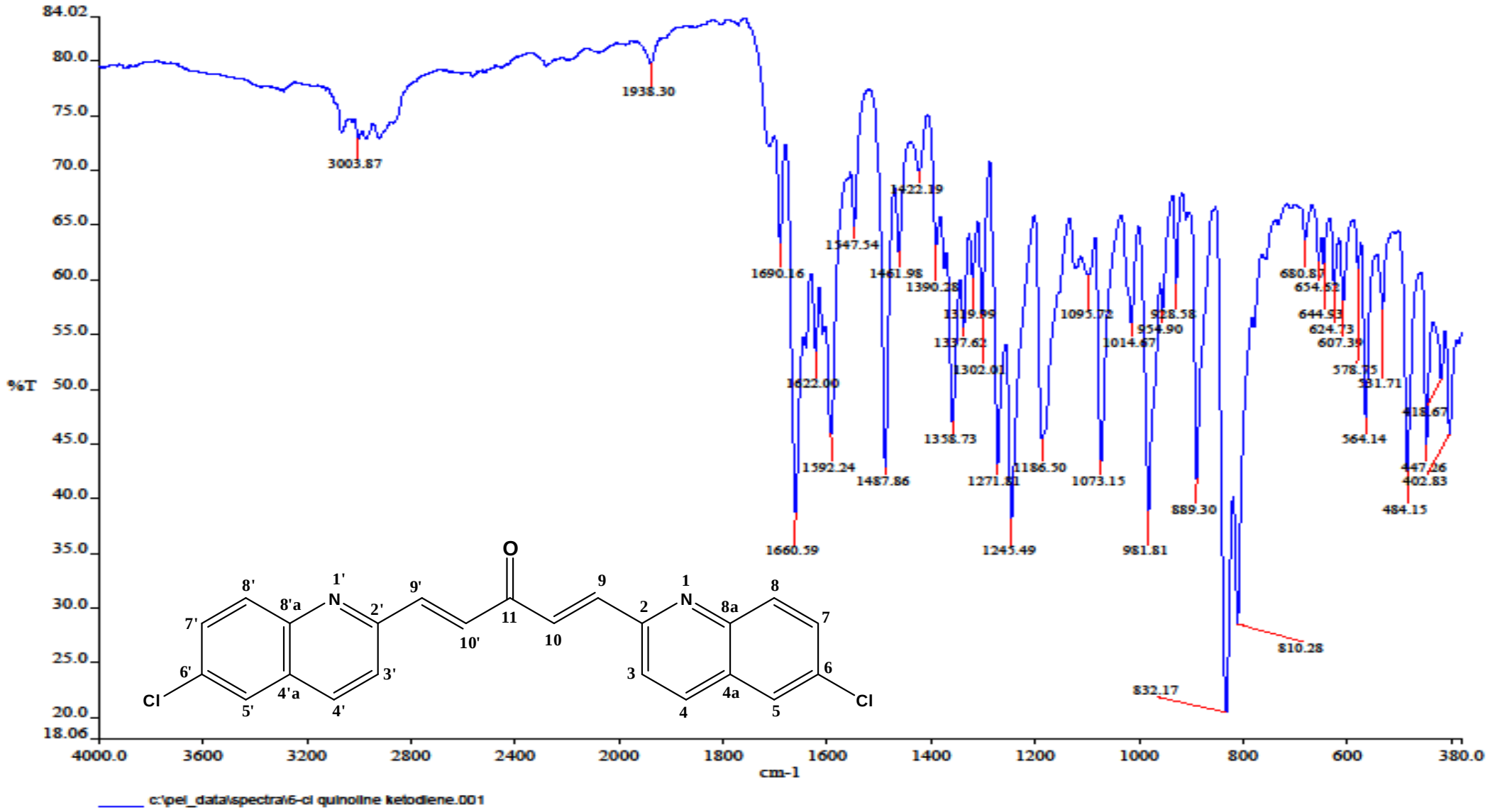
HSQC of Compound C-2a : (1E,4E)-1,5-bis(6-chloroquinolin-2-yl)penta-1,4-dien-3-one



HMBC of Compound C-2a: (1E,4E)-1,5-bis(6-chloroquinolin-2-yl)penta-1,4-dien-3-one



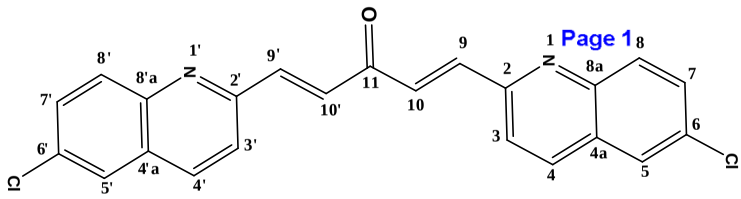
Expanded HMBC of Compound C-2a: (1E,4E)-1,5-bis(6-chloroquinolin-2-yl)penta-1,4-dien-3-one



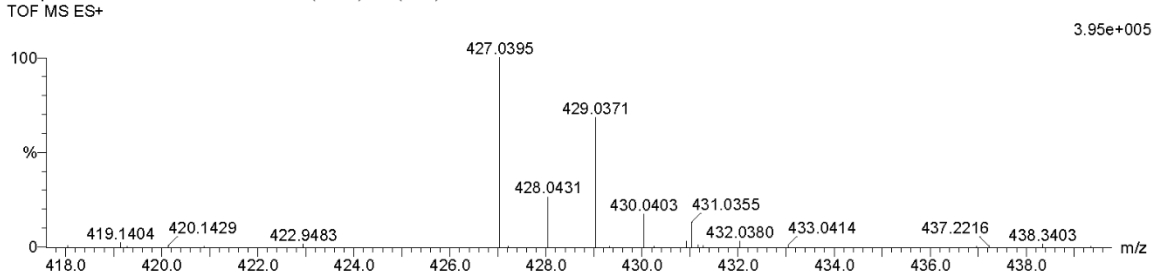
Infrared Spectrum of Compound C-2a: (1E,4E)-1,5-bis(6-chloroquinolin-2-yl)penta-1,4-dien-3-one

Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

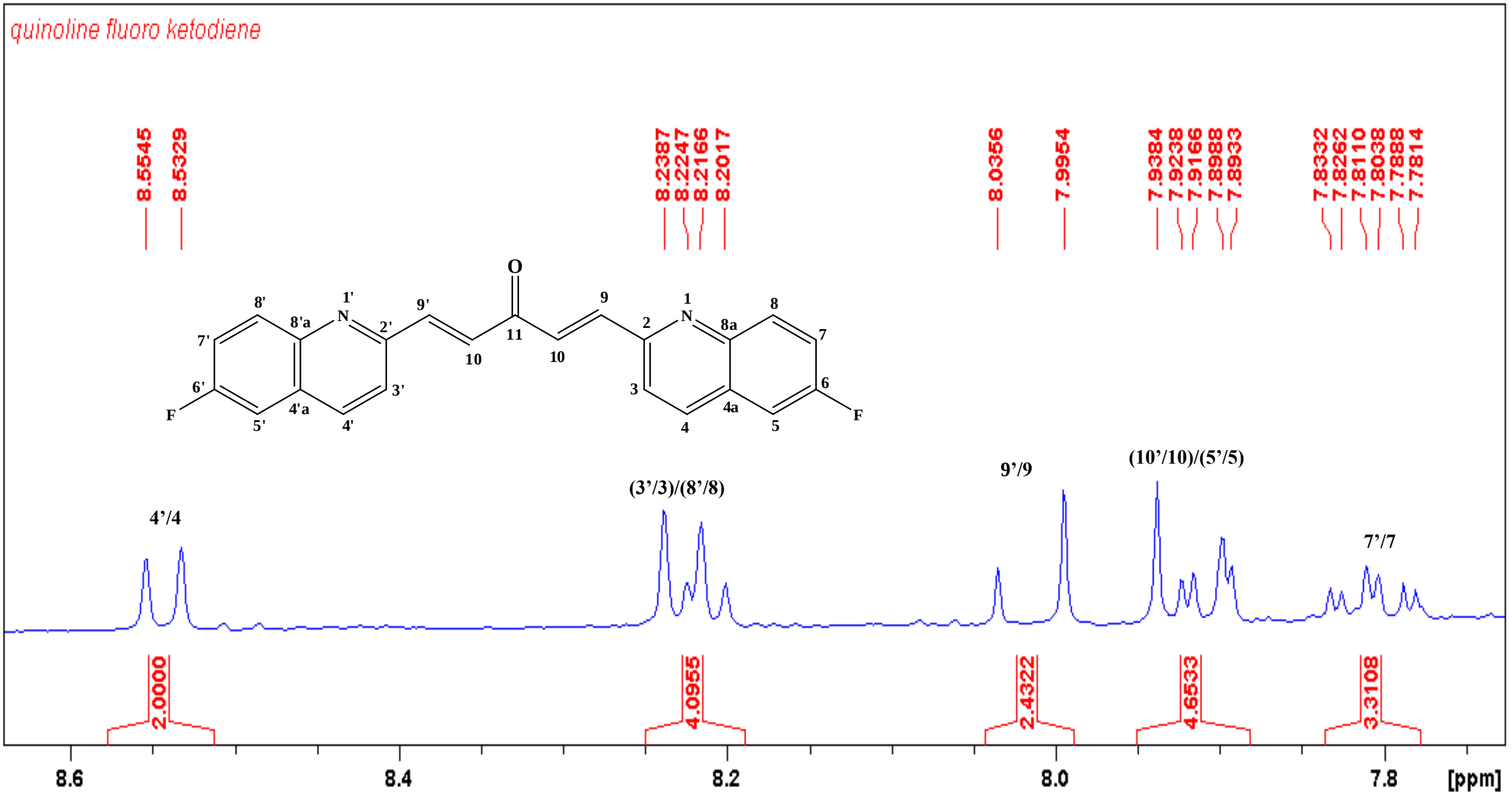


Monoisotopic Mass, Even Electron Ions
49 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 20-25 H: 10-15 N: 0-5 O: 0-5 Na: 1-1 Cl: 0-2
Compound 6 8-Cl Quinoline ketodiene 43 (1.416) Cm (1:61)
TOF MS ES+

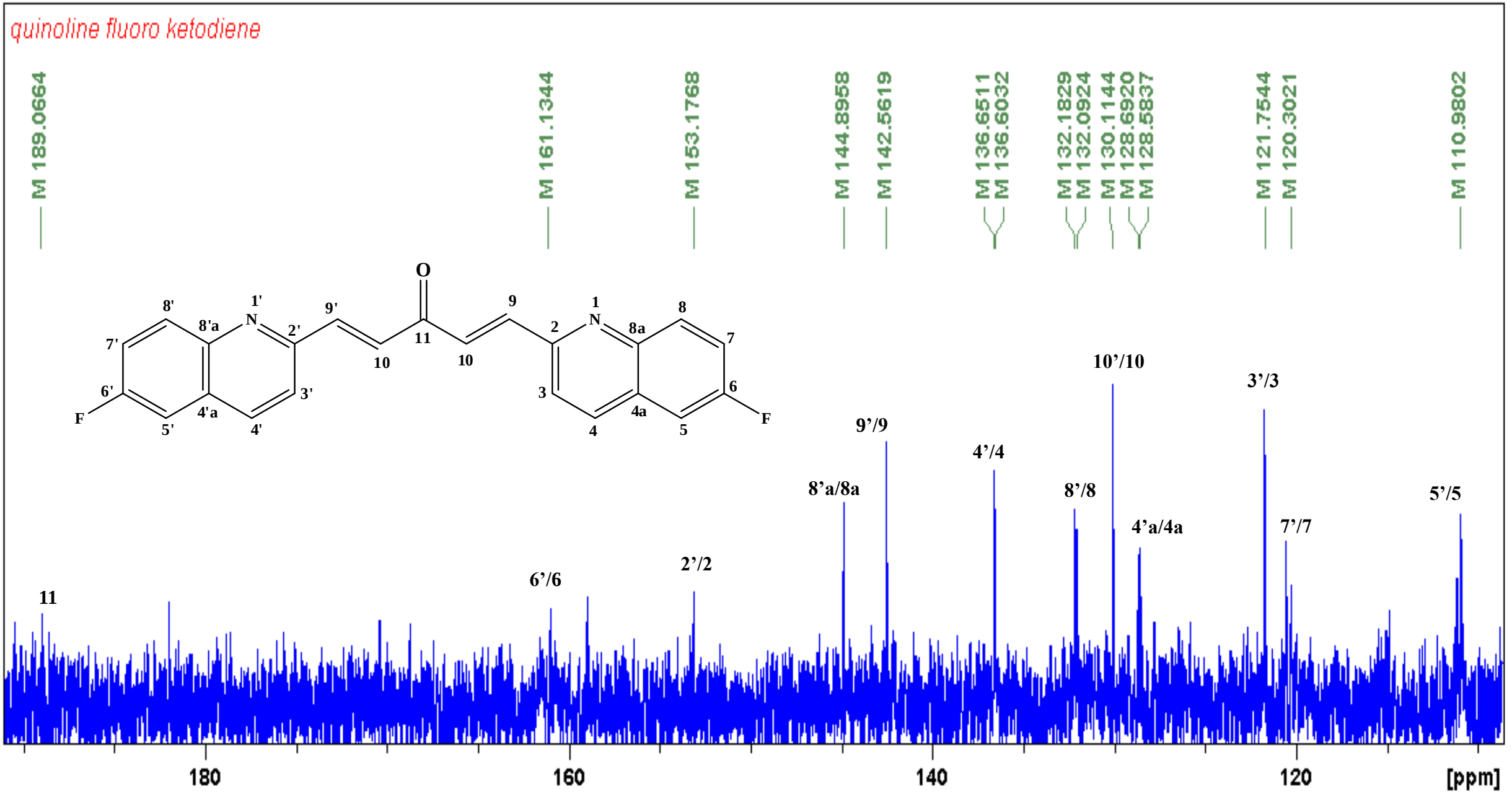


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
427.0395	427.0381	1.4	3.3	16.5	25.9	0.0	C23 H14 N2 O Na Cl2

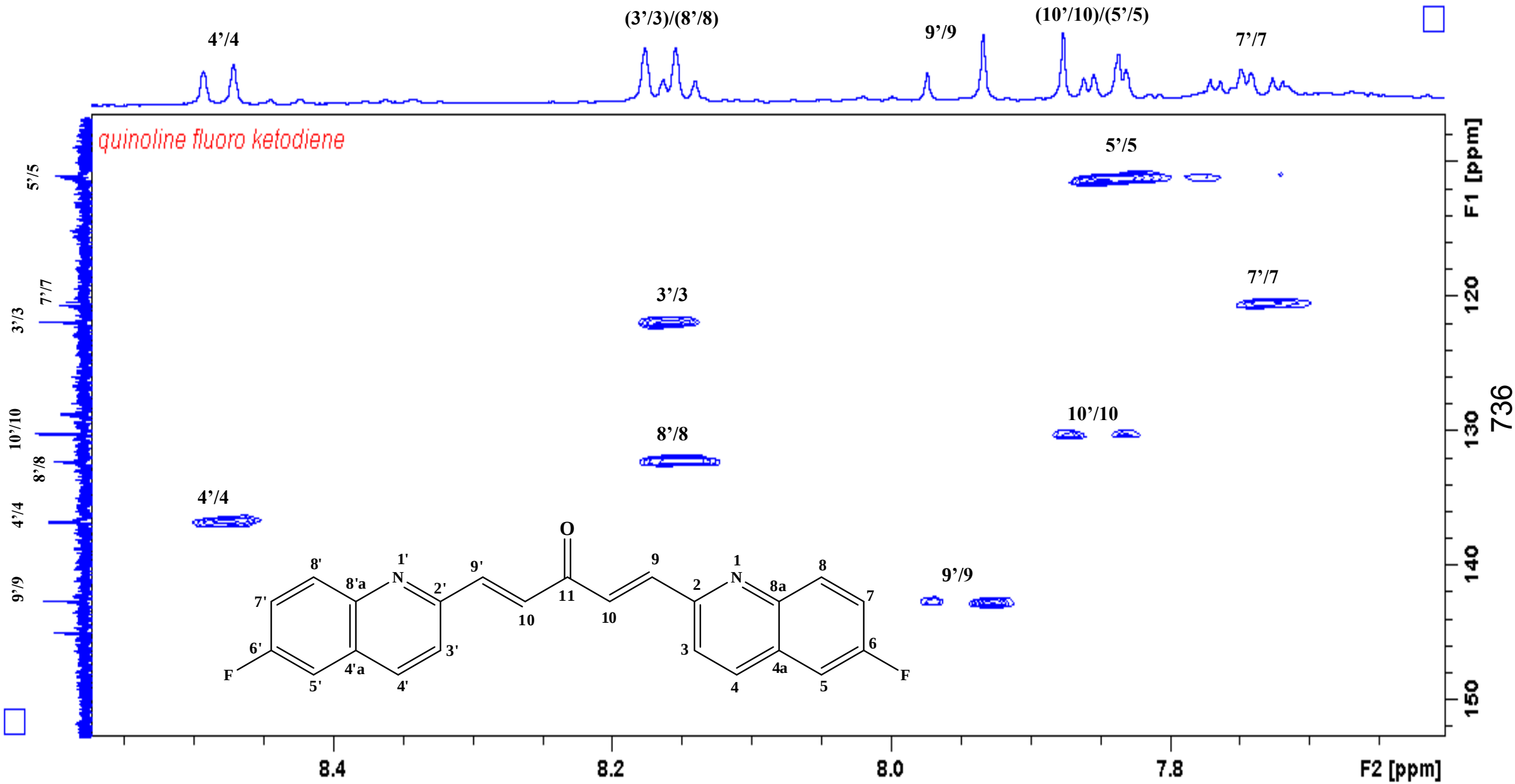
HRMS of Compound C-2a: (1E,4E)-1,5-bis(6-chloroquinolin-2-yl)penta-1,4-dien-3-one



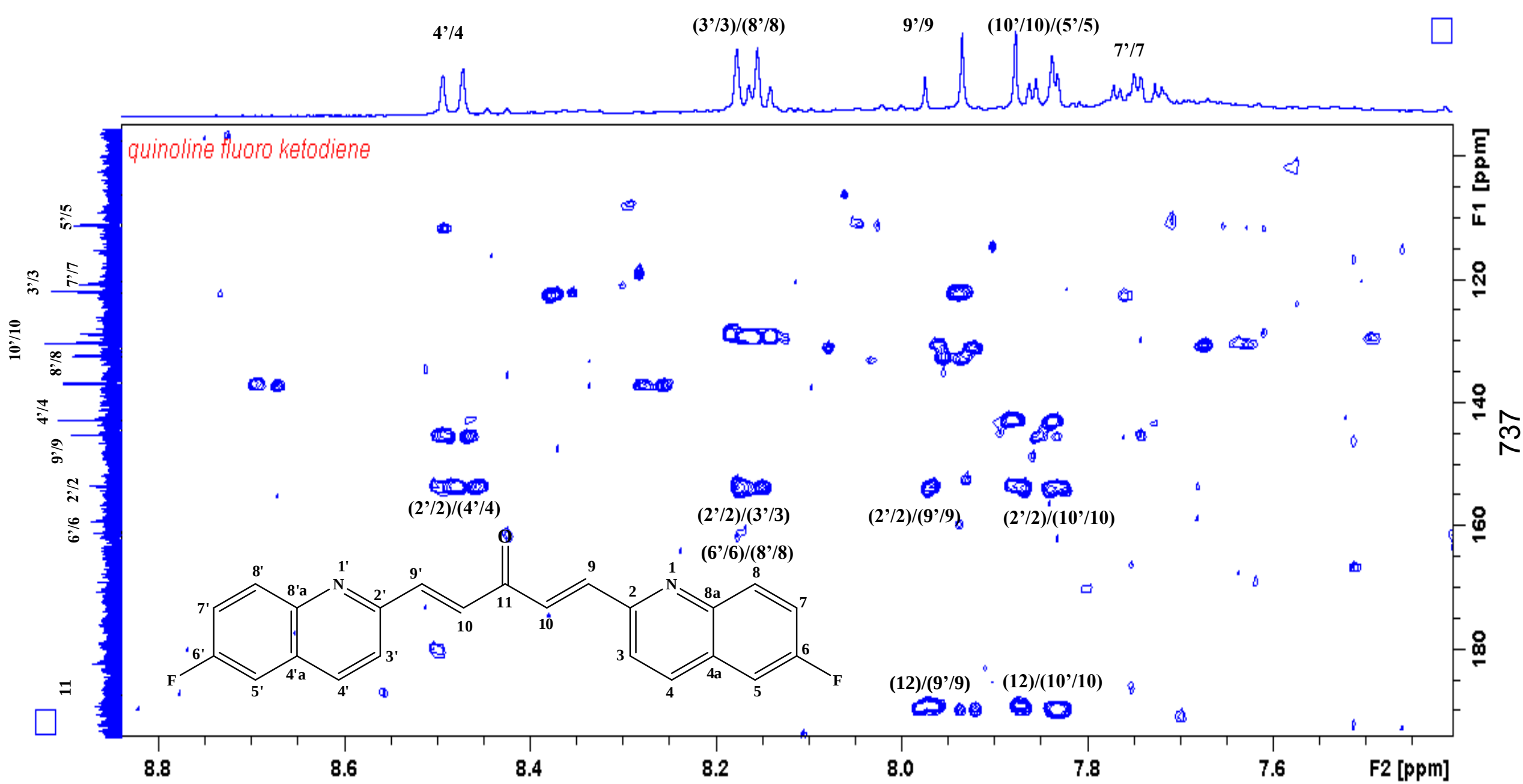
^{1}H Spectrum of Compound C-2b: (1E,4E)-1,5-bis(6-fluoroquinolin-2-yl)penta-1,4-dien-3-one



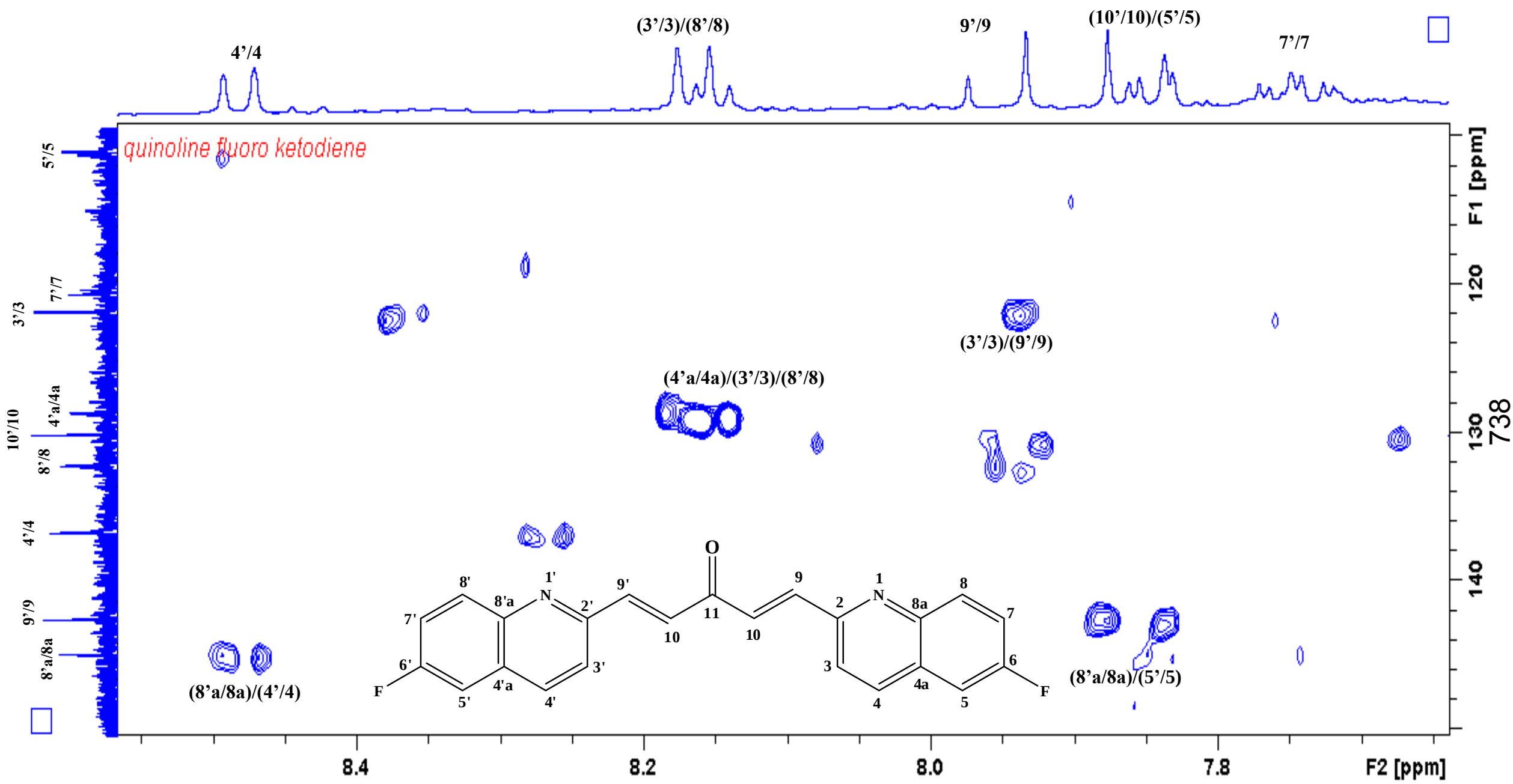
^{13}C Spectrum of Compound C-3b: (1E,4E)-1,5-bis(6-fluoroquinolin-2-yl)penta-1,4-dien-3-one



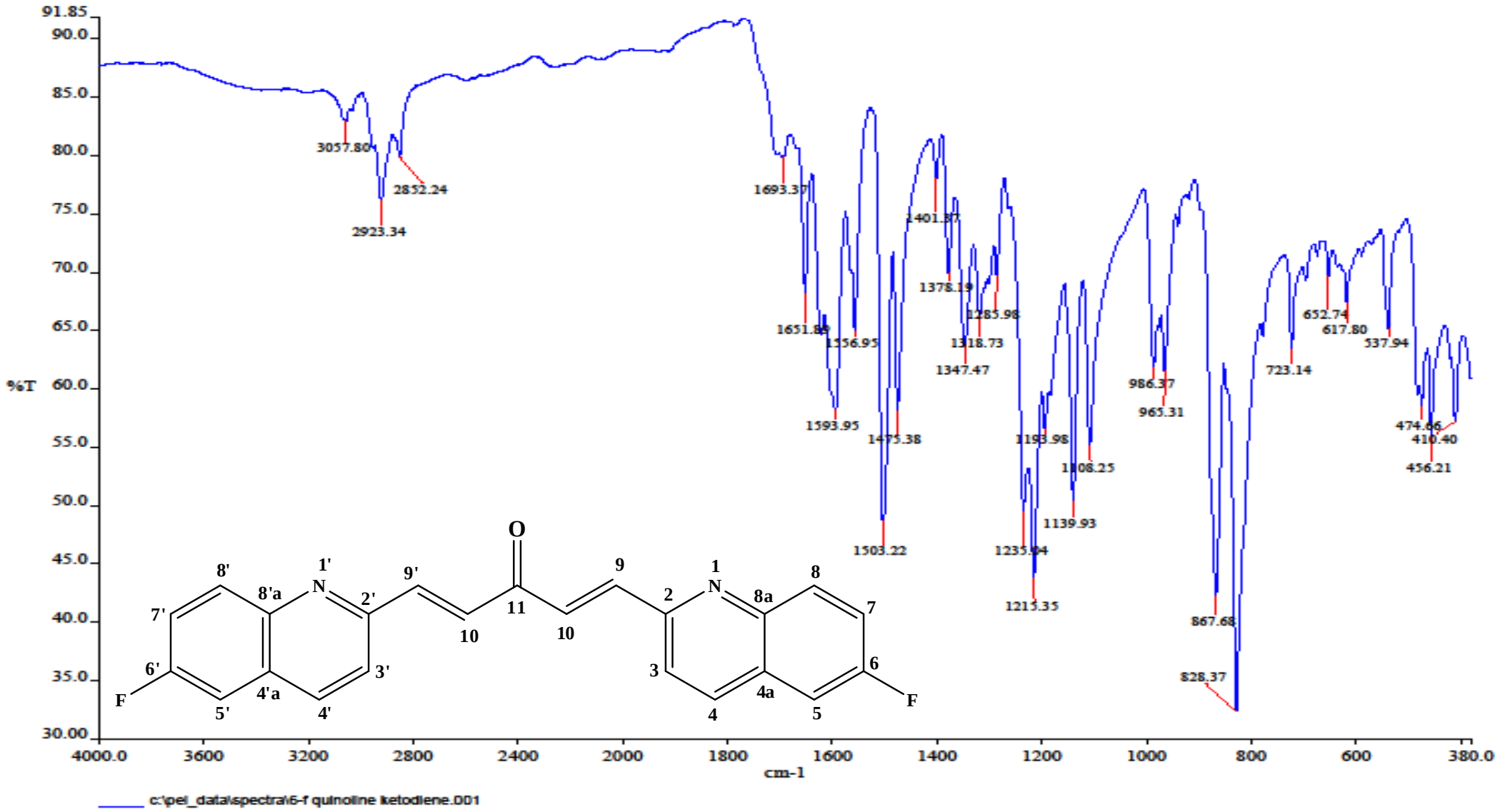
HSQC of Compound C-2b: (1E,4E)-1,5-bis(6-fluoroquinolin-2-yl)penta-1,4-dien-3-one



HMBC of Compound C-2b: (1E,4E)-1,5-bis(6-fluoroquinolin-2-yl)penta-1,4-dien-3-one



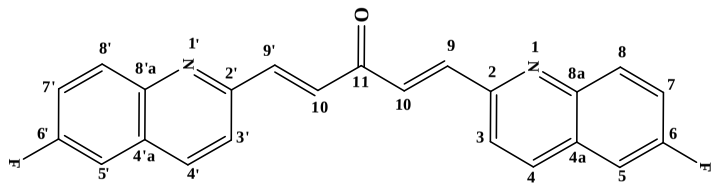
Expanded HMBC of Compound C-2b: (1E,4E)-1,5-bis(6-fluoroquinolin-2-yl)penta-1,4-dien-3-one



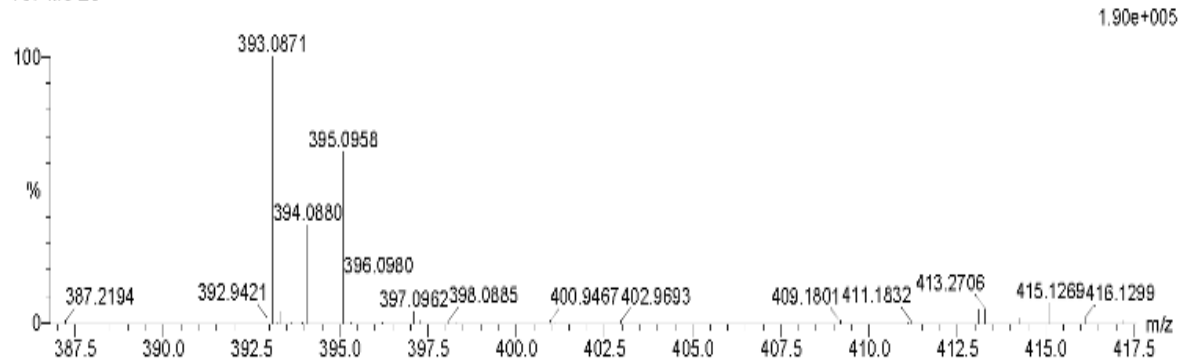
Infrared Spectrum of Compound C-2b: (1E,4E)-1,5-bis(6-fluoroquinolin-2-yl)penta-1,4-dien-3-one

Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

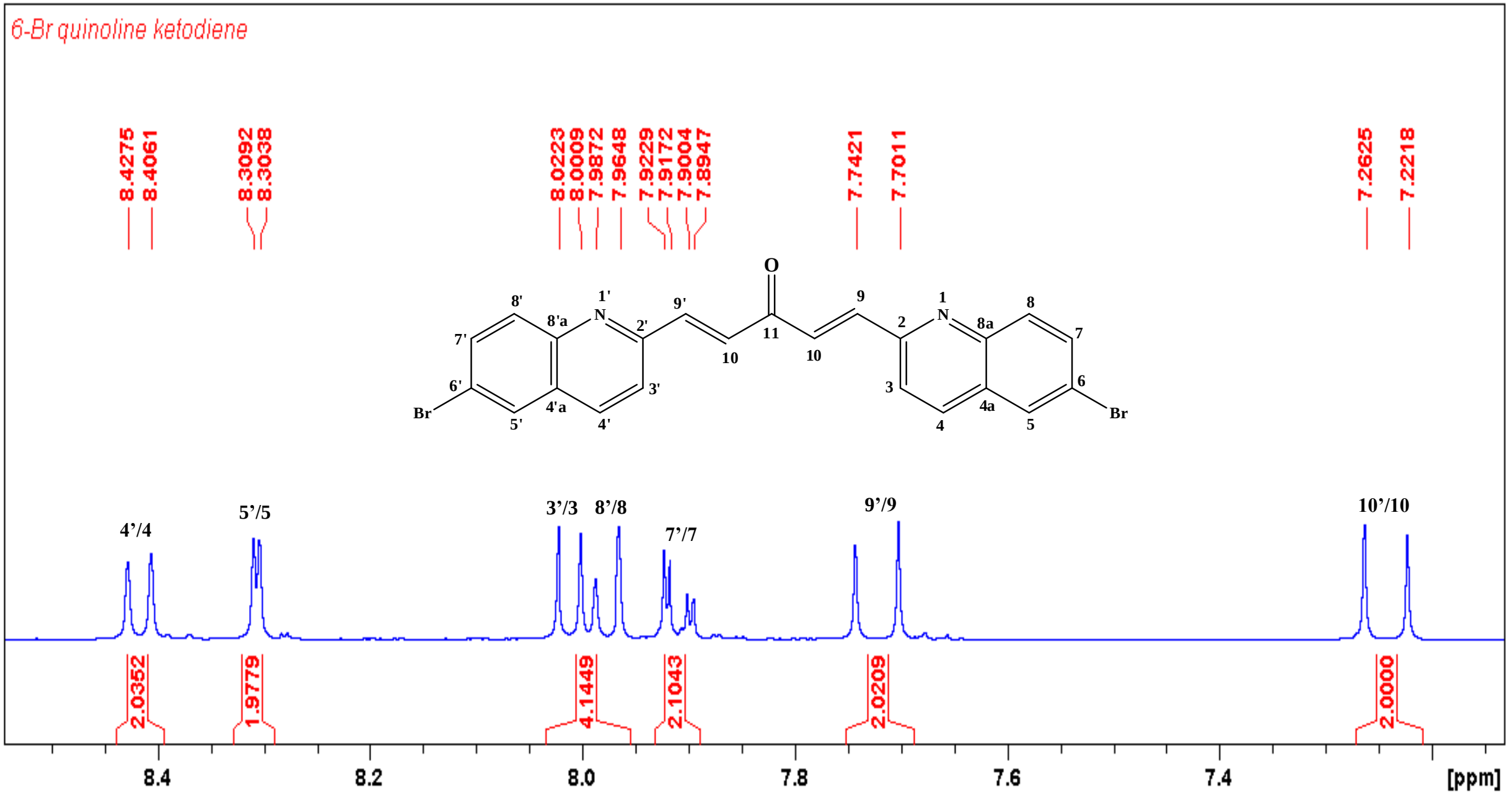


Monoisotopic Mass, Even Electron Ions
50 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 20-25 H: 10-15 N: 0-5 O: 0-5 F: 0-2 Na: 1-1
Compound 1 6-F Quinoline ketodiene 57 (1.889) Cm (1:61)
TOF MS ES+



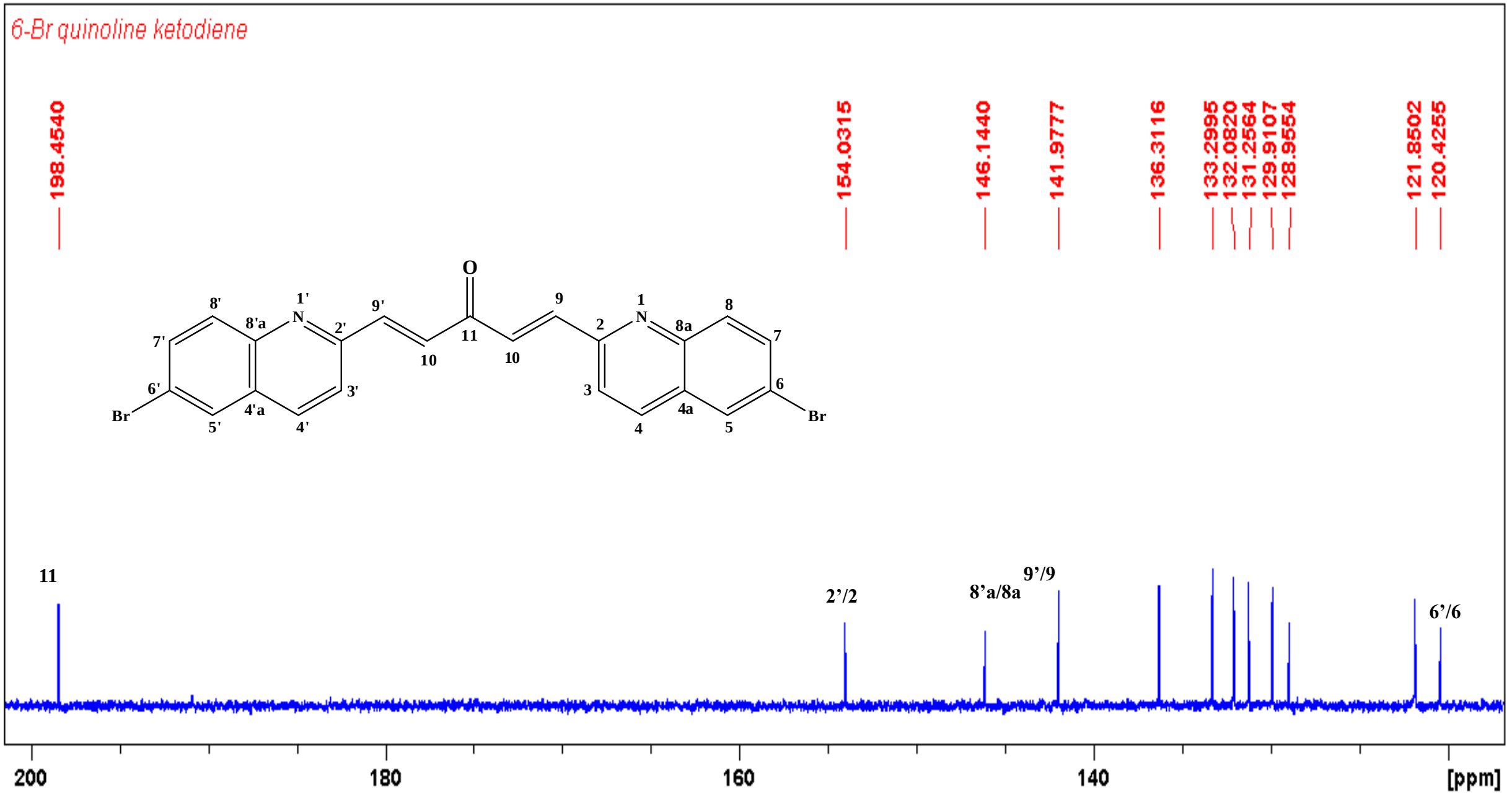
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
395.0958	395.0972	-1.4	-3.5	16.5	52.1	0.0	C23 H14 N2 O F2 Na

HRMS of Compound C-2b: (1E,4E)-1,5-bis(6-fluoroquinolin-2-yl)penta-1,4-dien-3-one

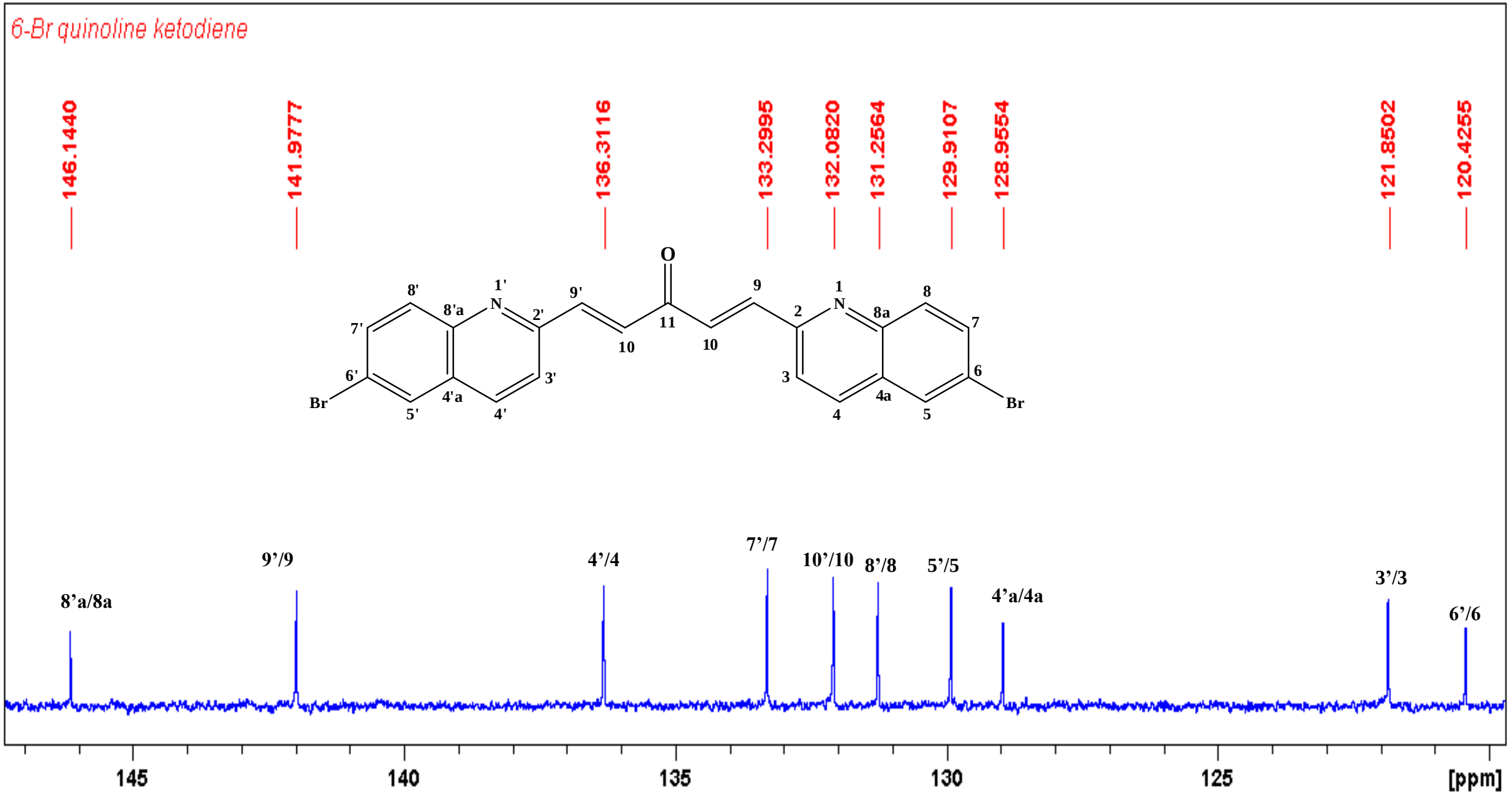


741

^{1}H Spectrum of Compound C-2c: (1E,4E)-1,5-bis(6-bromoquinolin-2-yl)penta-1,4-dien-3-one

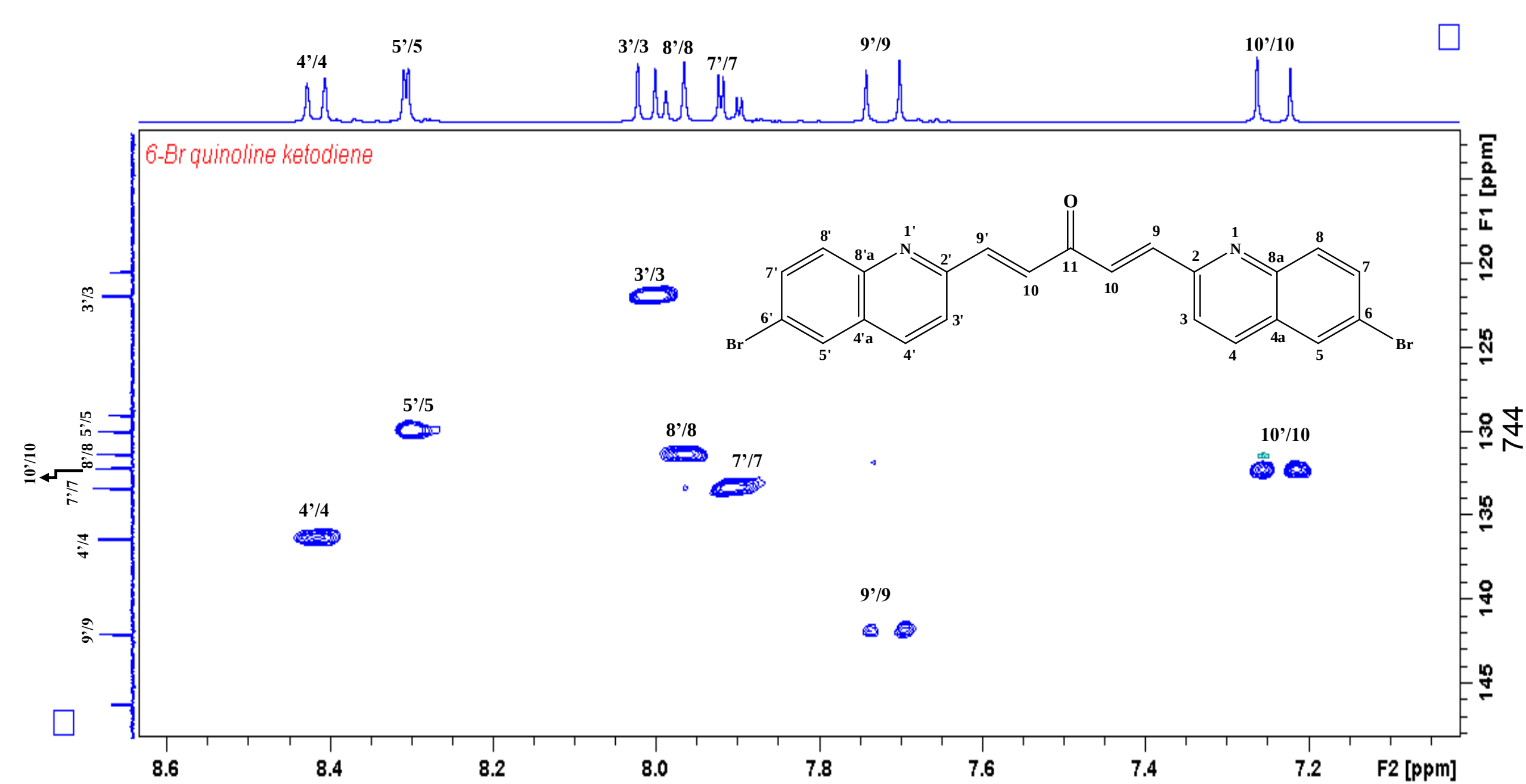


^{13}C Spectrum of Compound C-2c: (1E,4E)-1,5-bis(6-bromoquinolin-2-yl)penta-1,4-dien-3-one

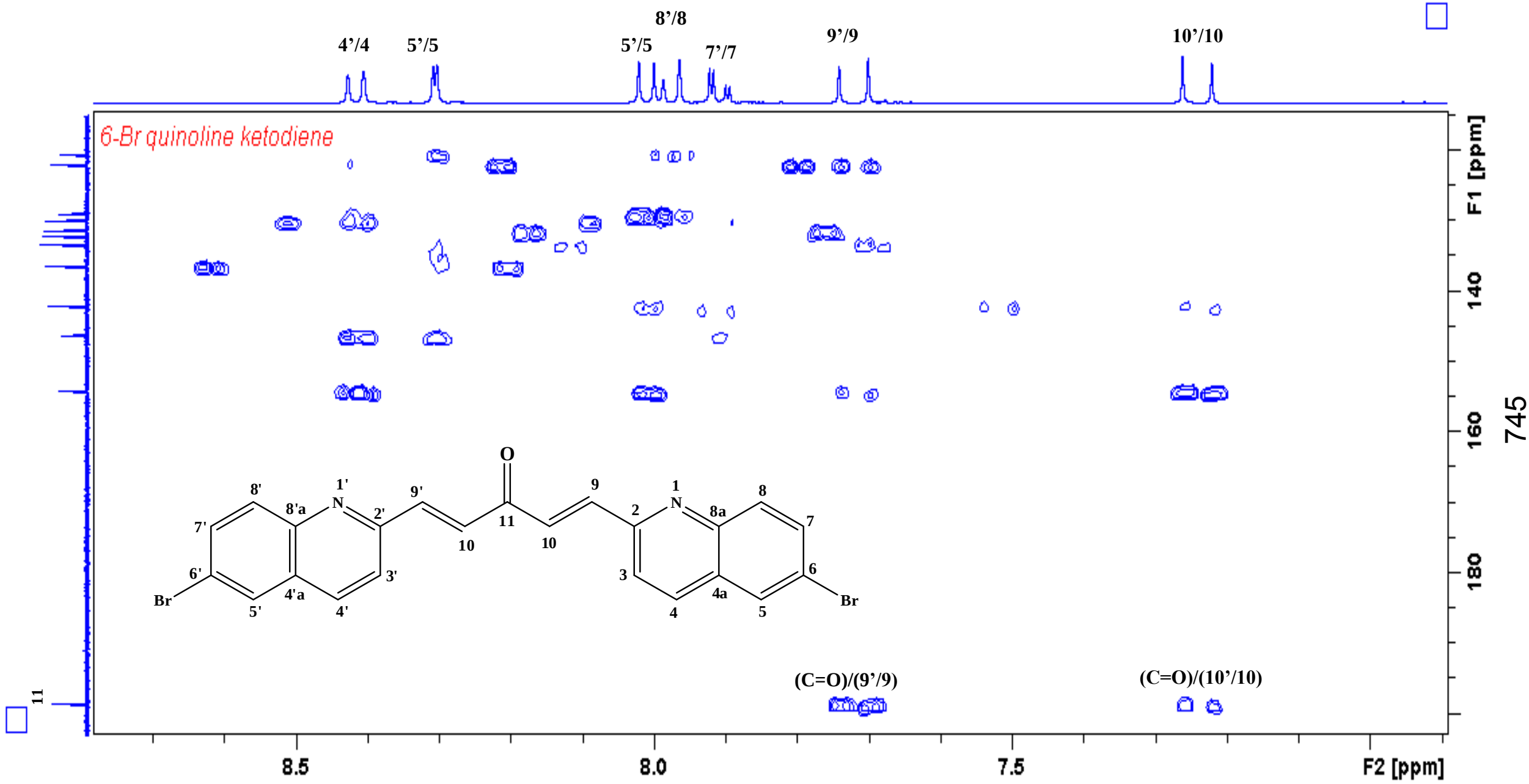


743

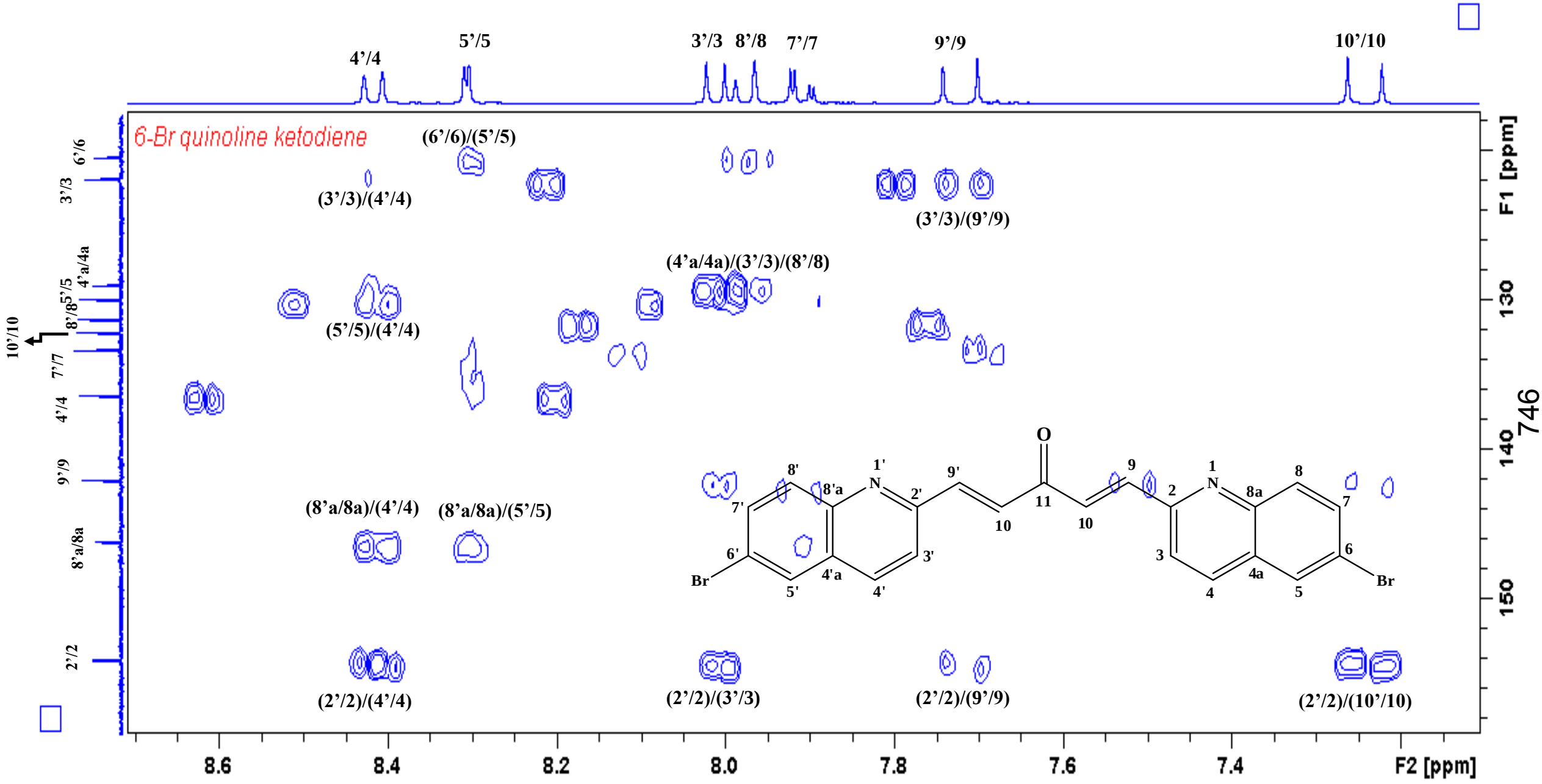
Expanded ^{13}C Spectrum of Compound C-2c: (1E,4E)-1,5-bis(6-bromoquinolin-2-yl)penta-1,4-dien-3-one



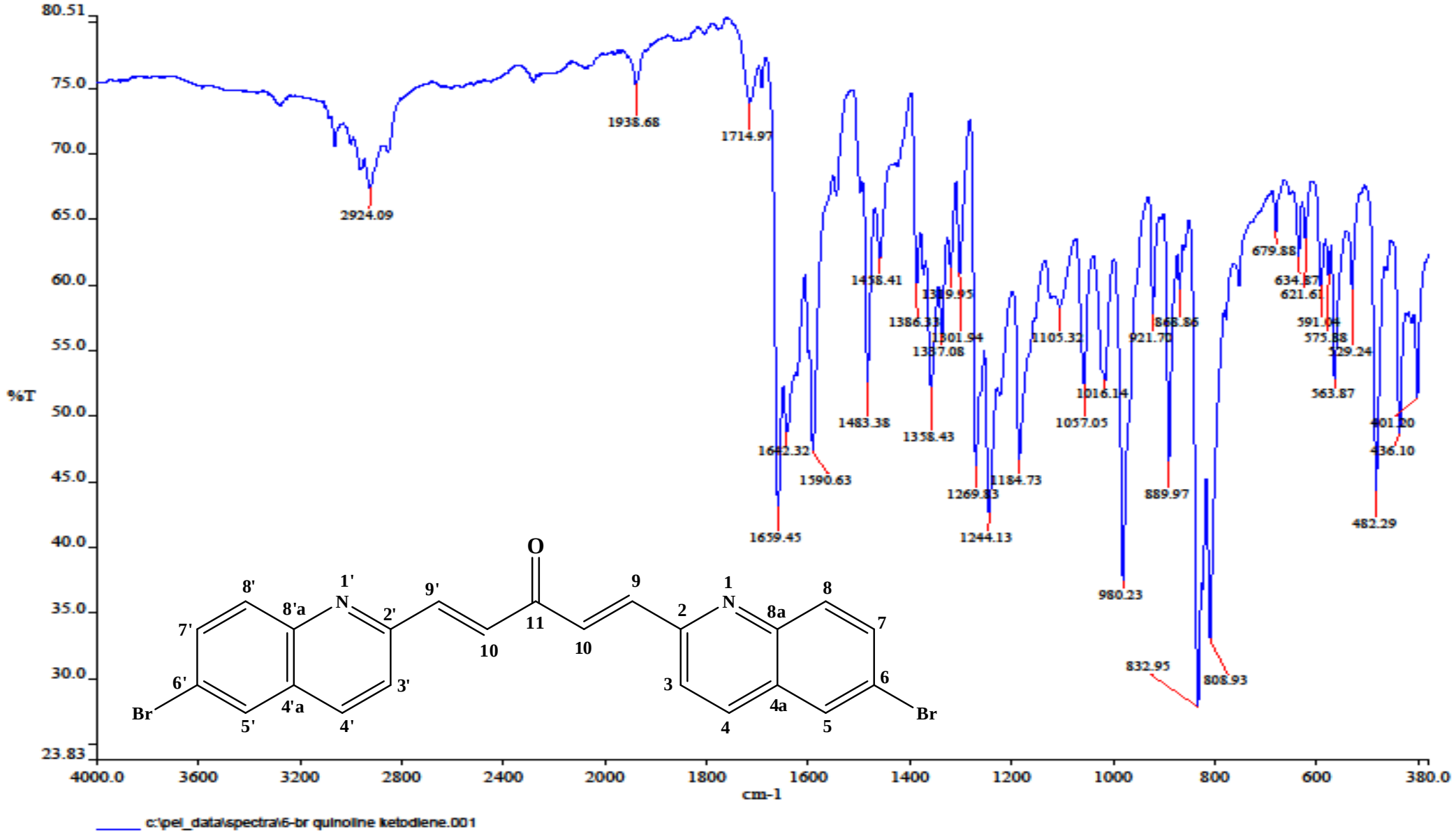
HSQC of Compound C-2c: (1E,4E)-1,5-bis(6-bromoquinolin-2-yl)penta-1,4-dien-3-one



HMBC of Compound C-2c: (1E,4E)-1,5-bis(6-bromoquinolin-2-yl)penta-1,4-dien-3-one



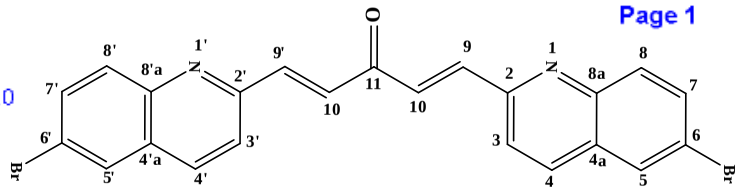
Expanded HMBC of Compound C-2c: (1E,4E)-1,5-bis(6-bromoquinolin-2-yl)penta-1,4-dien-3-one



Infrared Spectrum of Compound C-2c: (1E,4E)-1,5-bis(6-bromoquinolin-2-yl)penta-1,4-dien-3-one

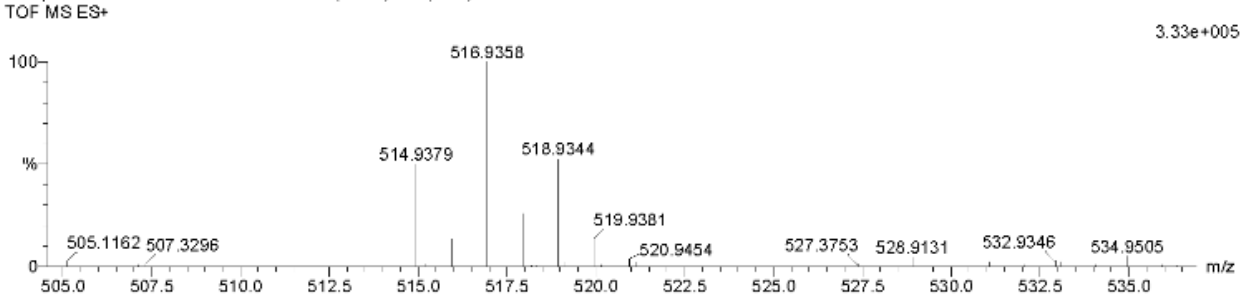
Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2



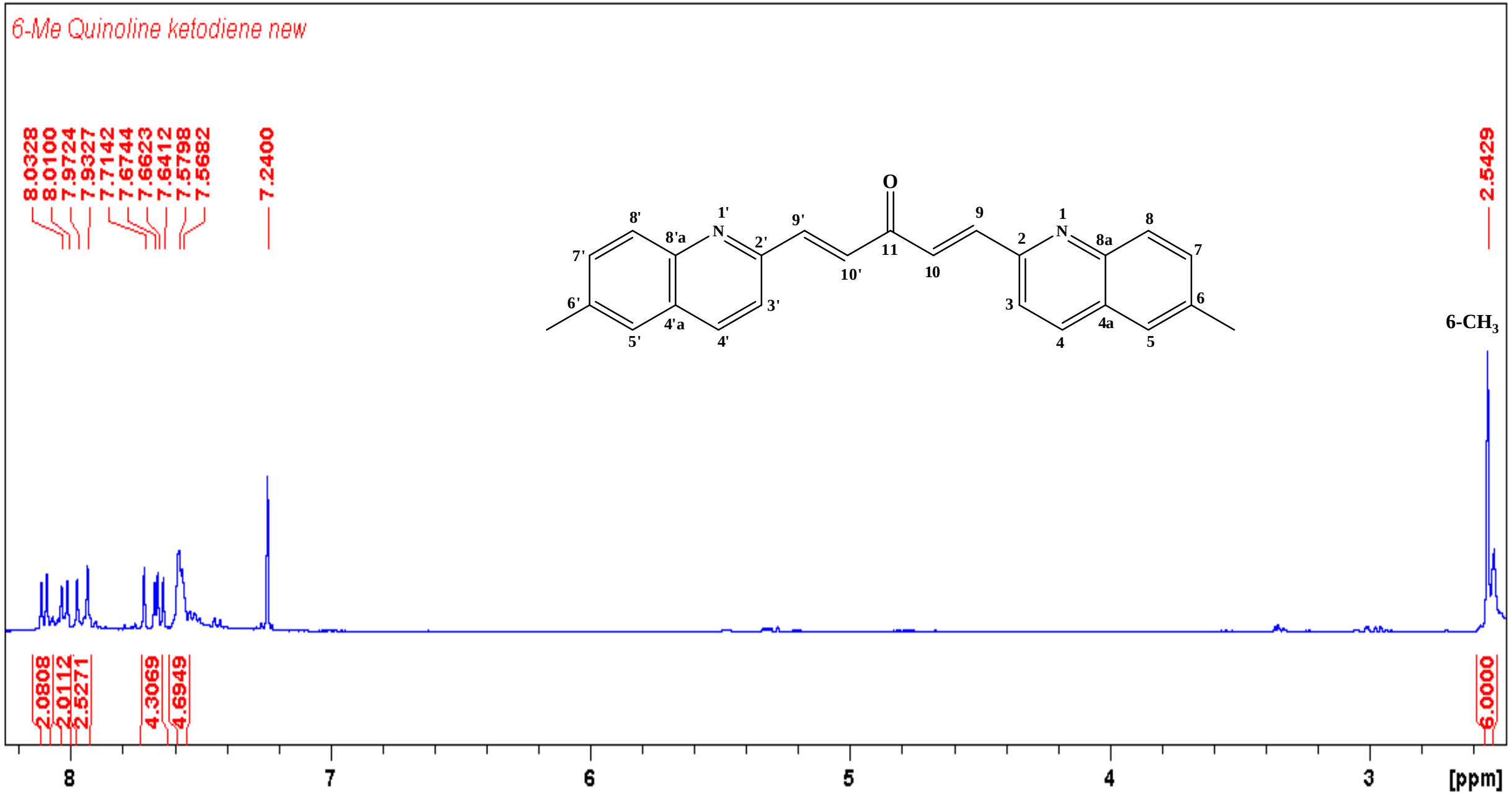
Monoisotopic Mass, Even Electron Ions
66 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 20-25 H: 10-15 N: 0-5 O: 0-5 Na: 1-1 Br: 0-2

Compound 8 8-Br Quinoline ketodiene 17 (0.540) Cm (1:61)
TOF MS ES+

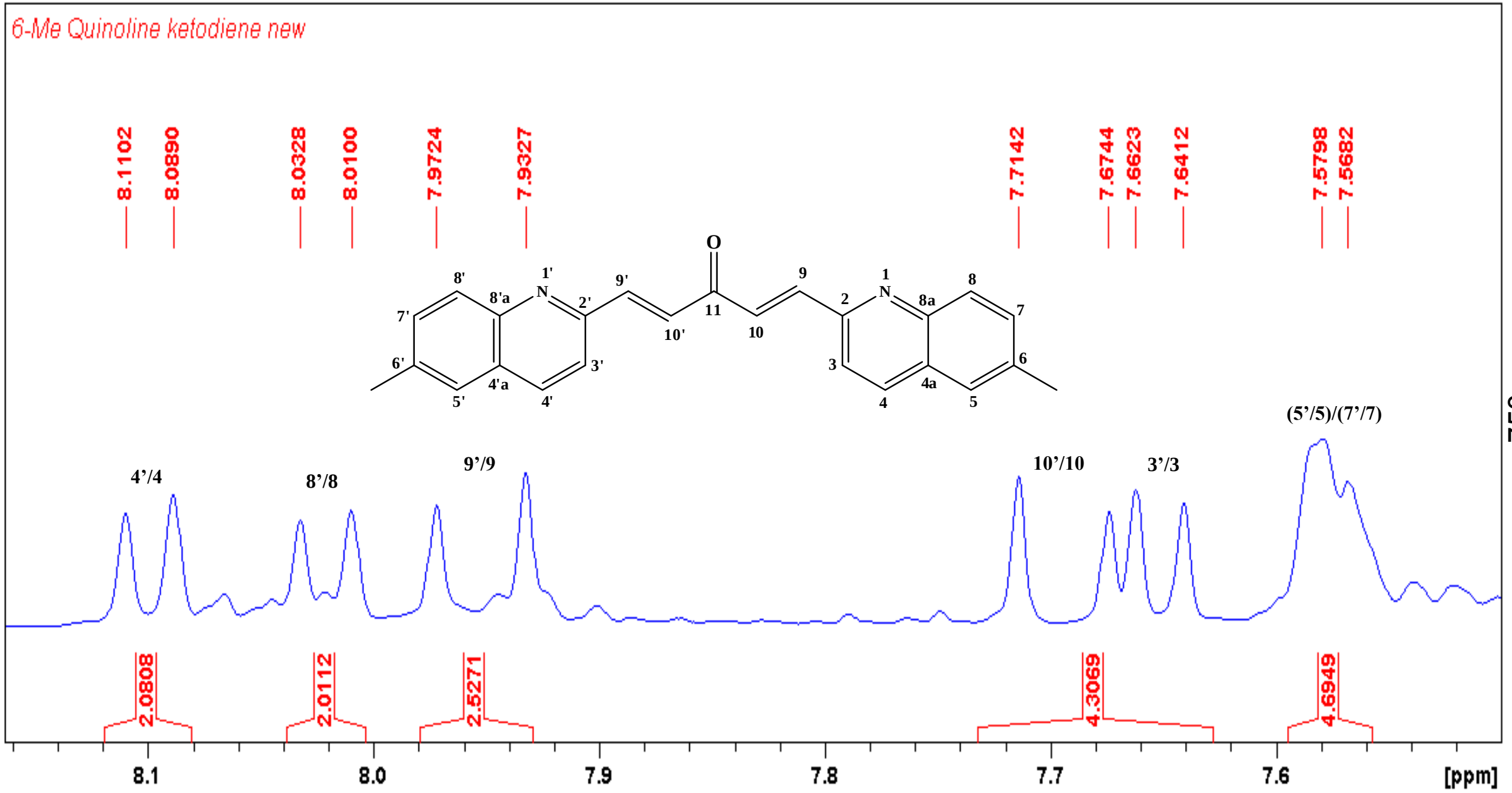


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
514.9379	514.9371	0.8	1.6	16.5	26.8	0.0	C23 H14 N2 O Na Br2

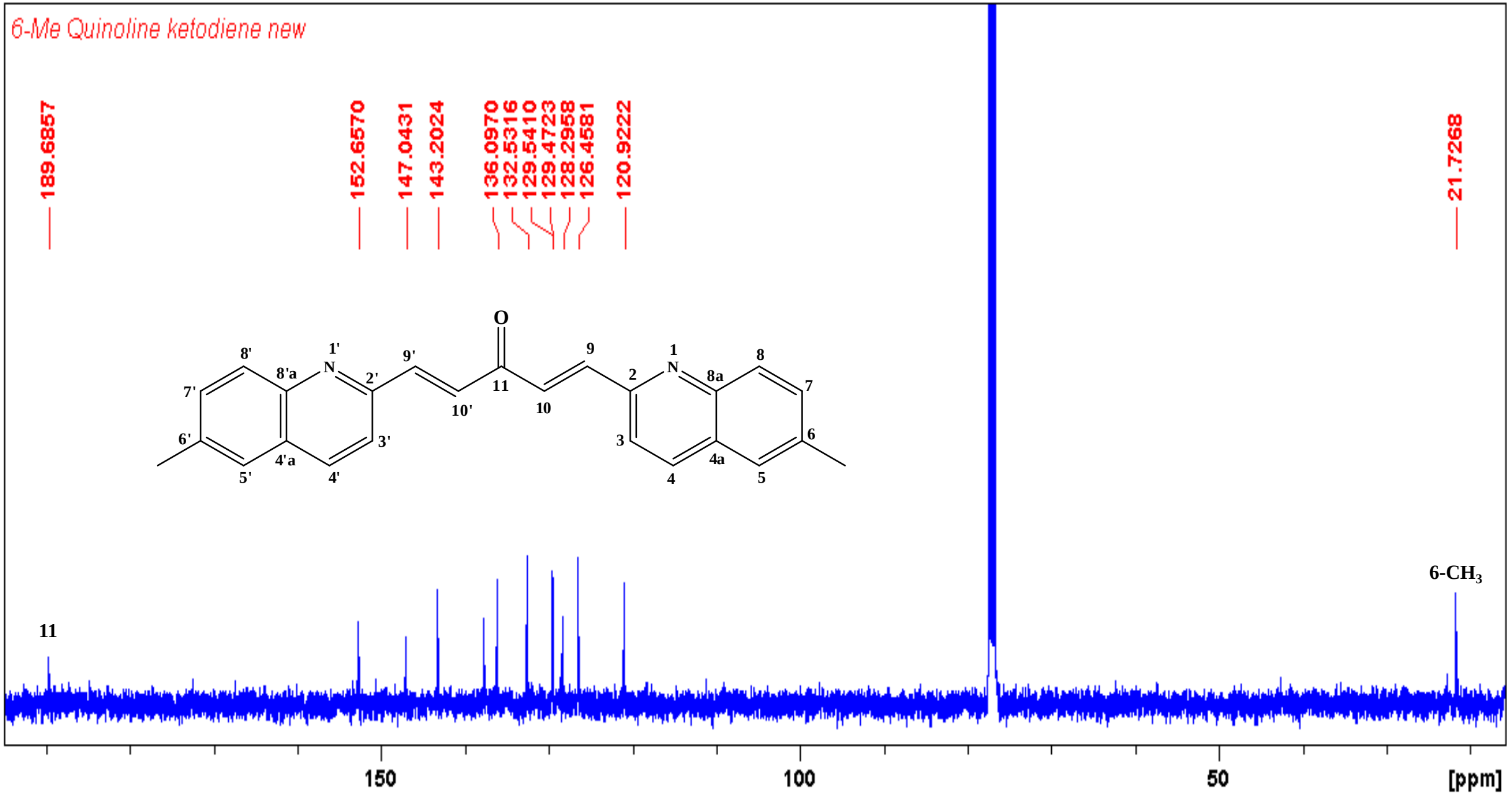
HRMS of Compound C-2c: (1E,4E)-1,5-bis(6-bromoquinolin-2-yl)penta-1,4-dien-3-one



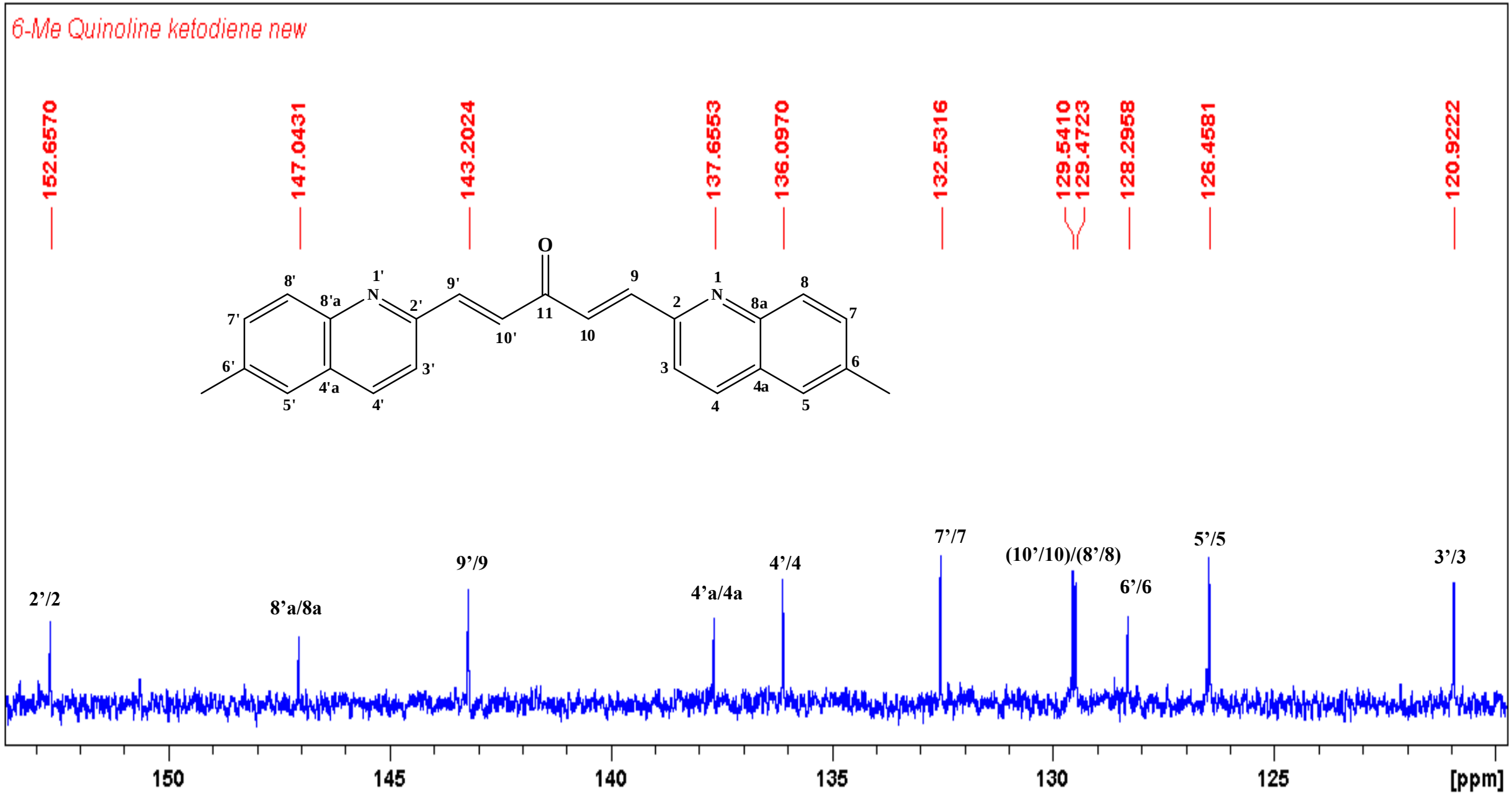
^{1}H Spectrum of Compound C-2d: (1E,4E)-1,5-bis(6-methylquinolin-2-yl)penta-1,4-dien-3-one



Expanded ^{1}H Spectrum of Compound C-2d: (1E,4E)-1,5-bis(6-methylquinolin-2-yl)penta-1,4-dien-3-one

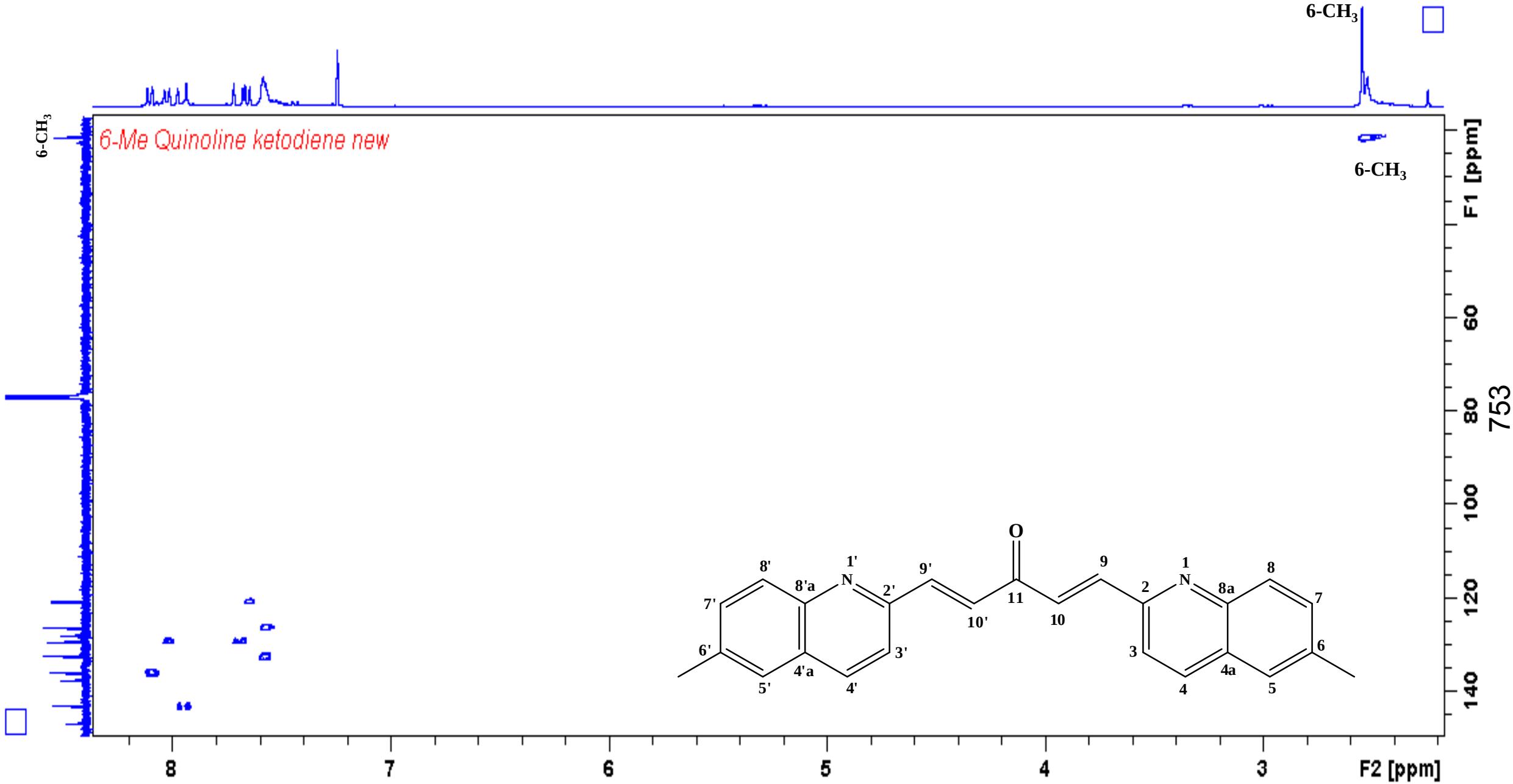


^{13}C Spectrum of Compound C-2d: (1E,4E)-1,5-bis(6-methylquinolin-2-yl)penta-1,4-dien-3-one

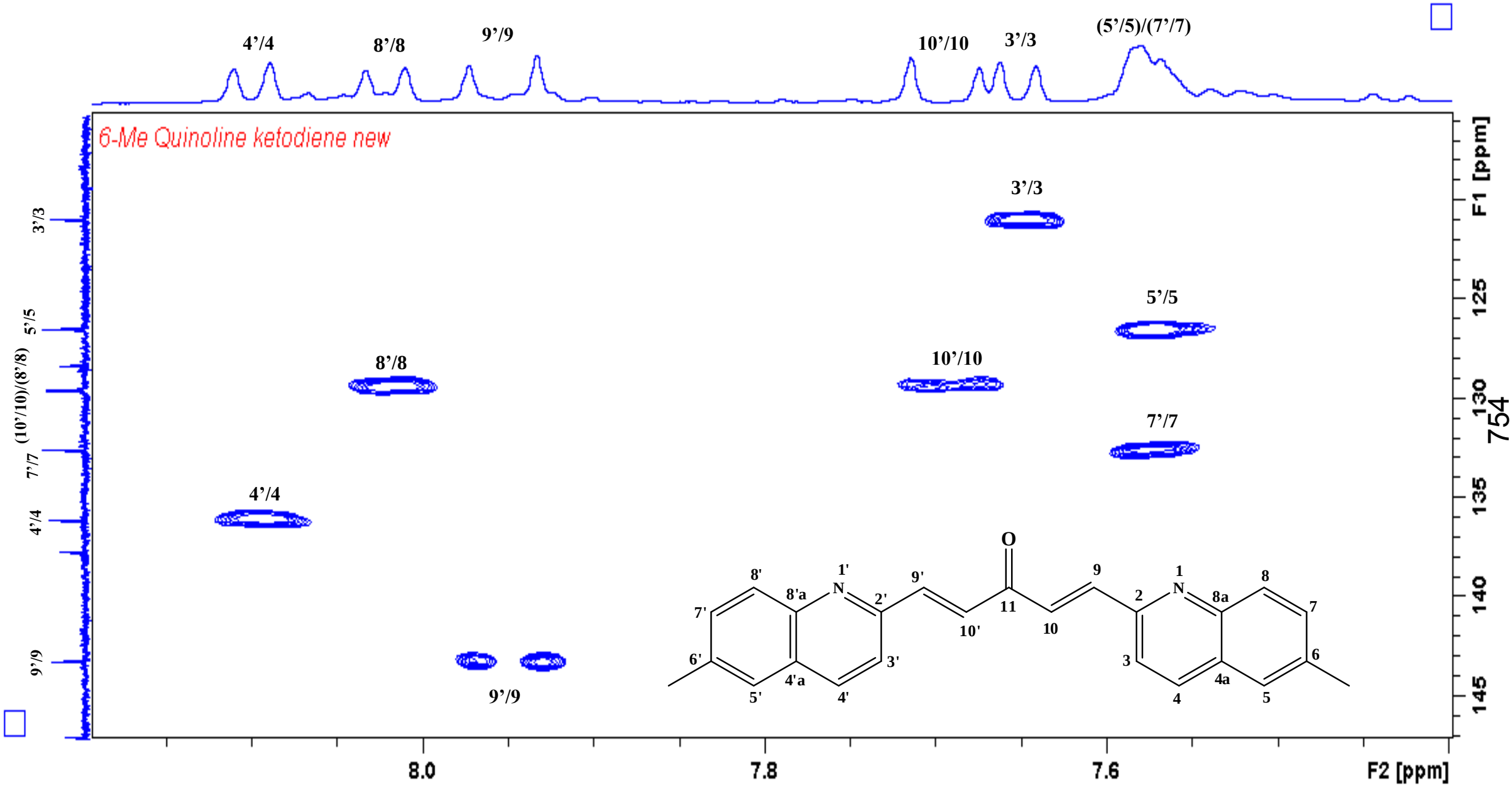


752

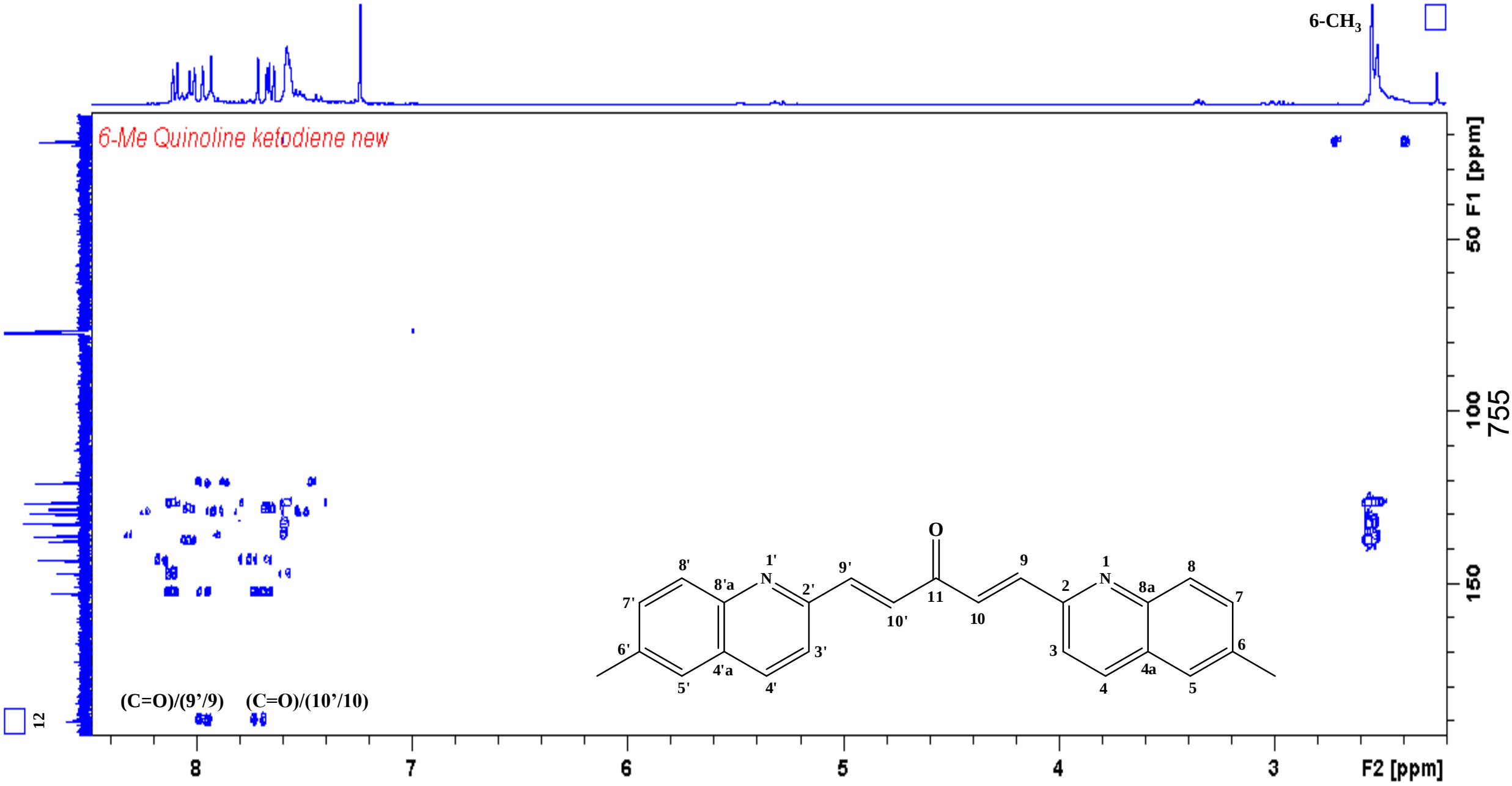
Expanded ^{13}C Spectrum of Compound C-2d: (1E,4E)-1,5-bis(6-methylquinolin-2-yl)penta-1,4-dien-3-one



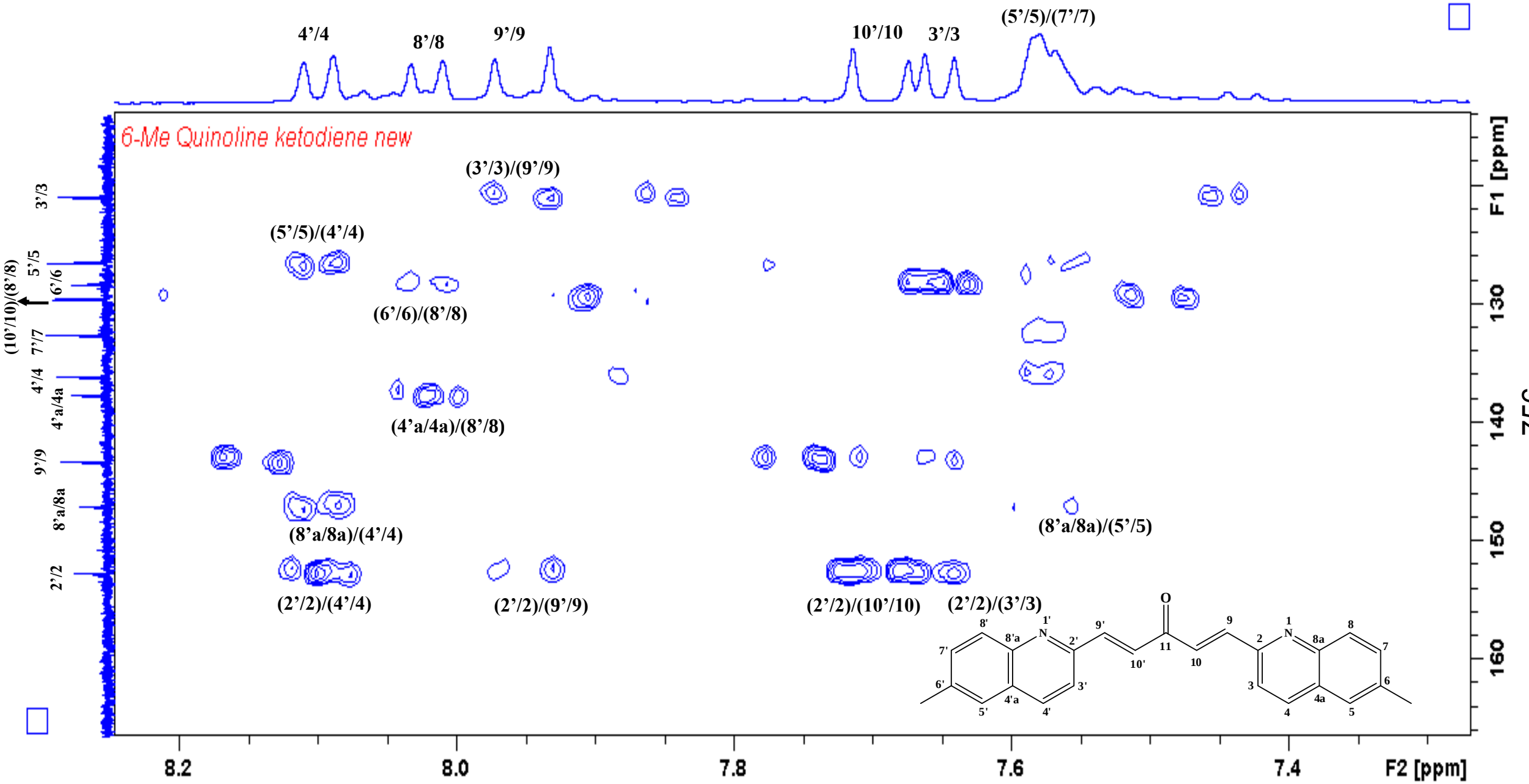
HSQC of Compound C-2d: (1E,4E)-1,5-bis(6-methylquinolin-2-yl)penta-1,4-dien-3-one



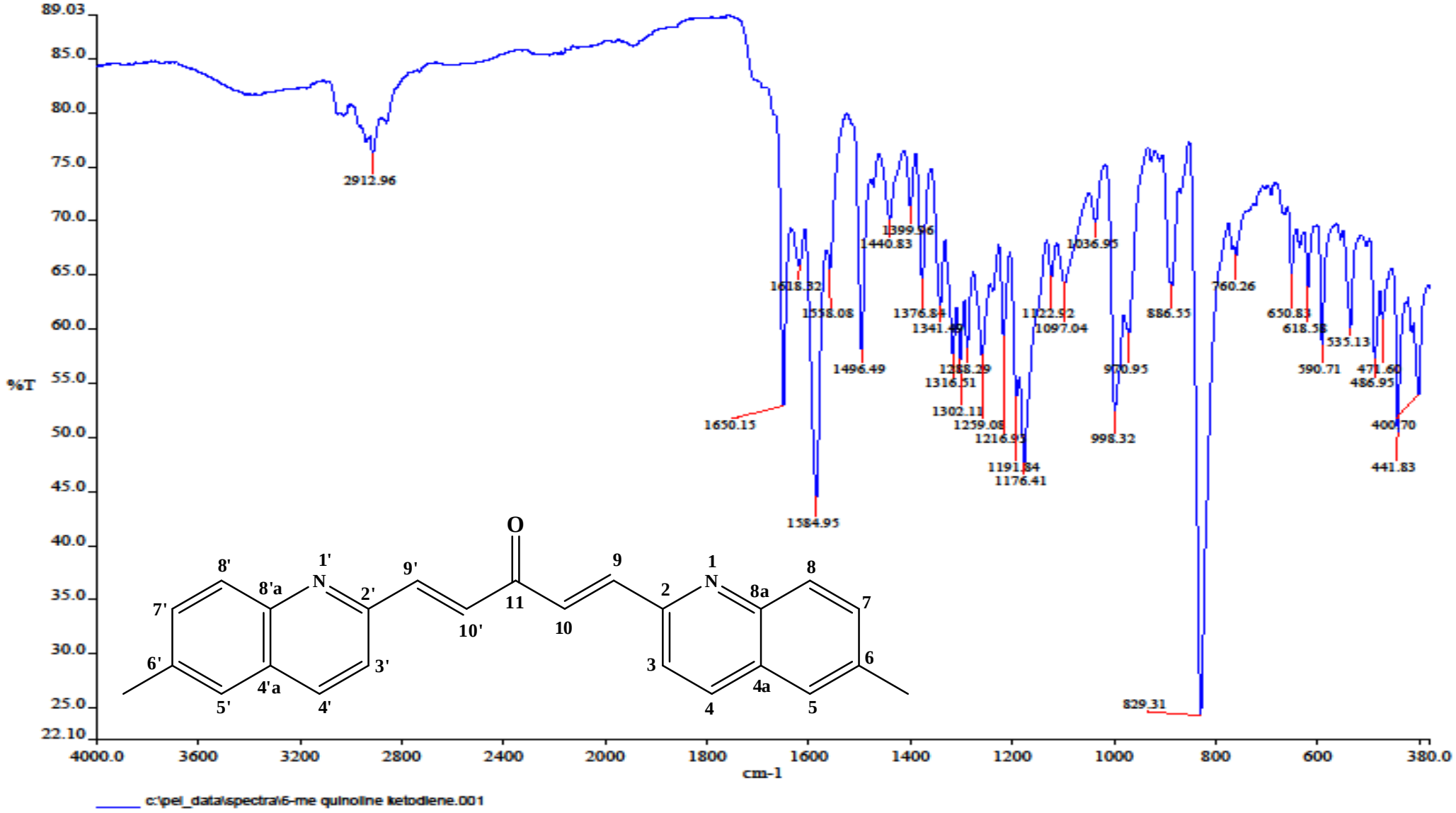
Expanded HSQC of Compound C-2d: (1E,4E)-1,5-bis(6-methylquinolin-2-yl)penta-1,4-dien-3-one



HMBC of Compound C-2d: (1E,4E)-1,5-bis(6-methylquinolin-2-yl)penta-1,4-dien-3-one



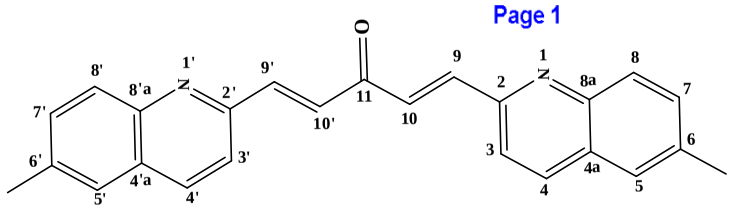
Expanded HMBC of Compound C-2d: (1E,4E)-1,5-bis(6-methylquinolin-2-yl)penta-1,4-dien-3-one



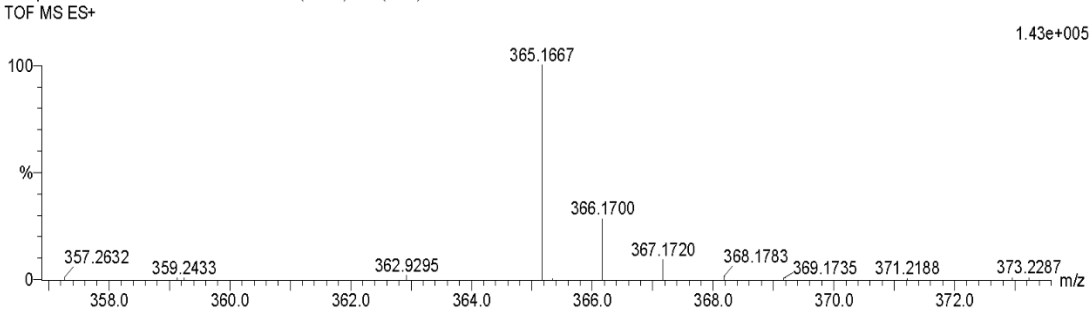
Infrared Spectrum of Compound C-2d: (1E,4E)-1,5-bis(6-methylquinolin-2-yl)penta-1,4-dien-3-one

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

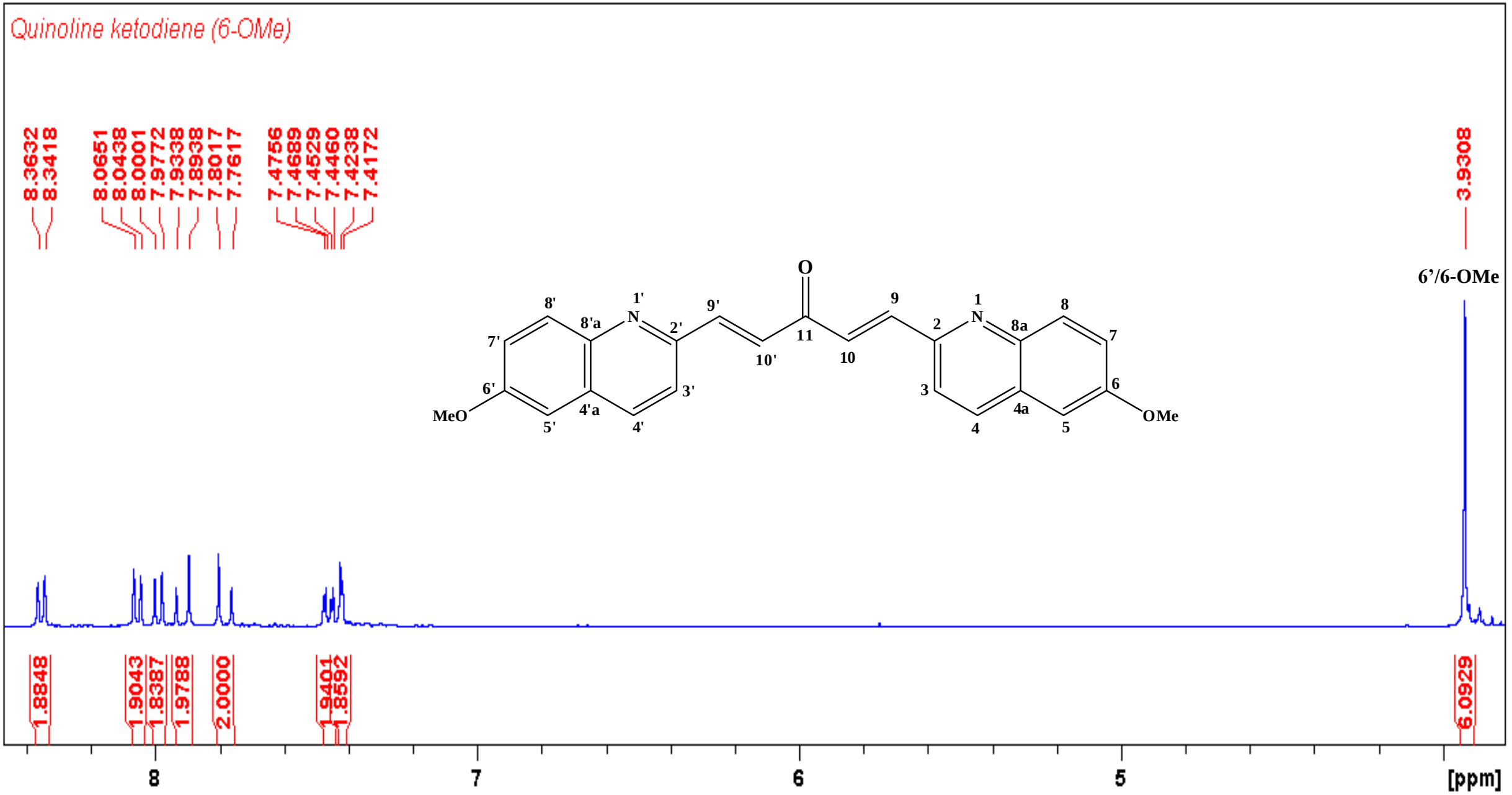


Monoisotopic Mass, Even Electron Ions
27 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 20-25 H: 15-25 N: 0-5 O: 0-5
Compound 4 6-Me Quinoline ketodiene 20 (0.641) Cm (1:61)
TOF MS ES+



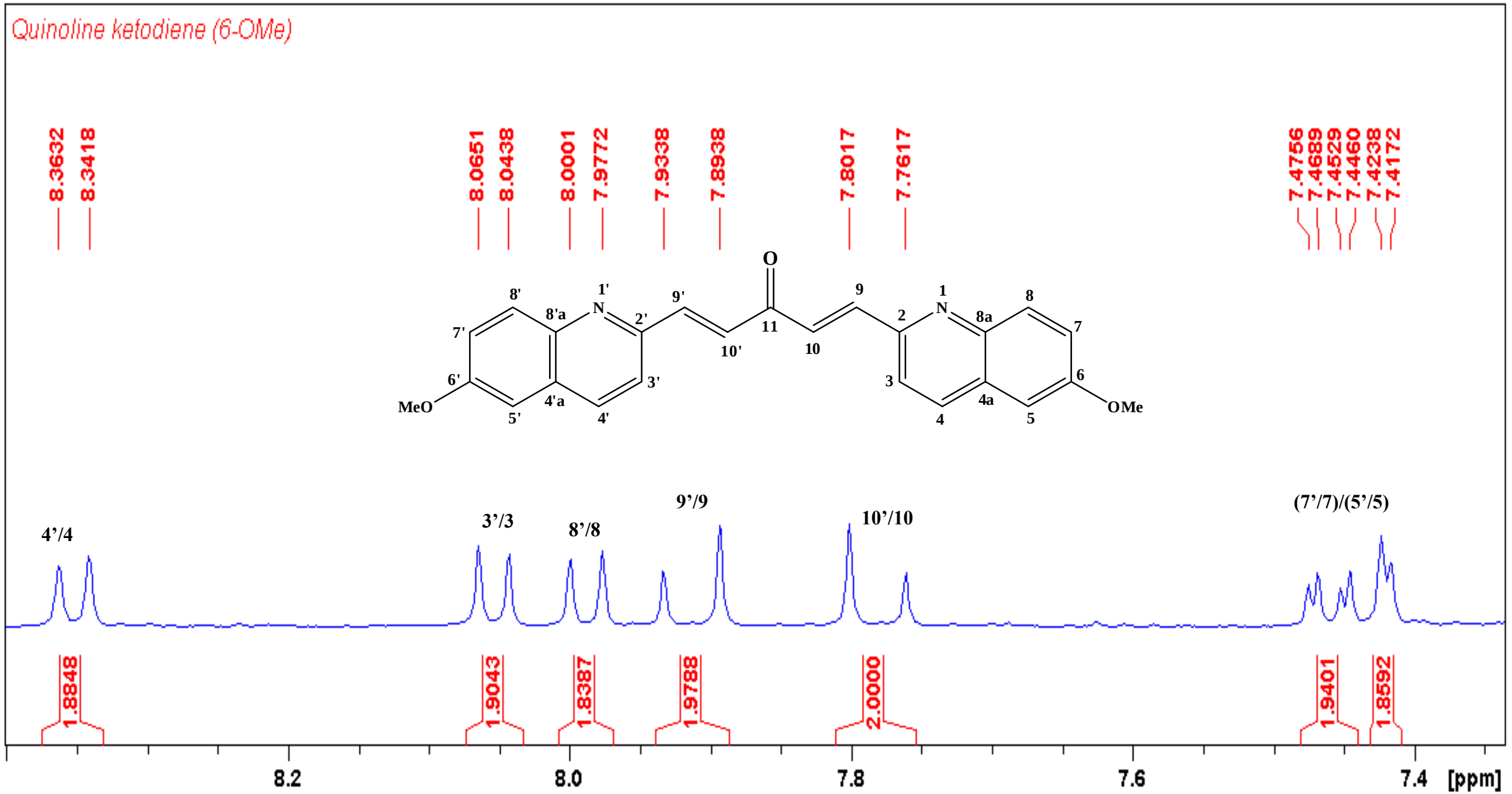
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
365.1667	365.1654	1.3	3.6	16.5	25.1	0.0	C25 H21 N2 O

HRMS of Compound C-2d: (1E,4E)-1,5-bis(6-methylquinolin-2-yl)penta-1,4-dien-3-one



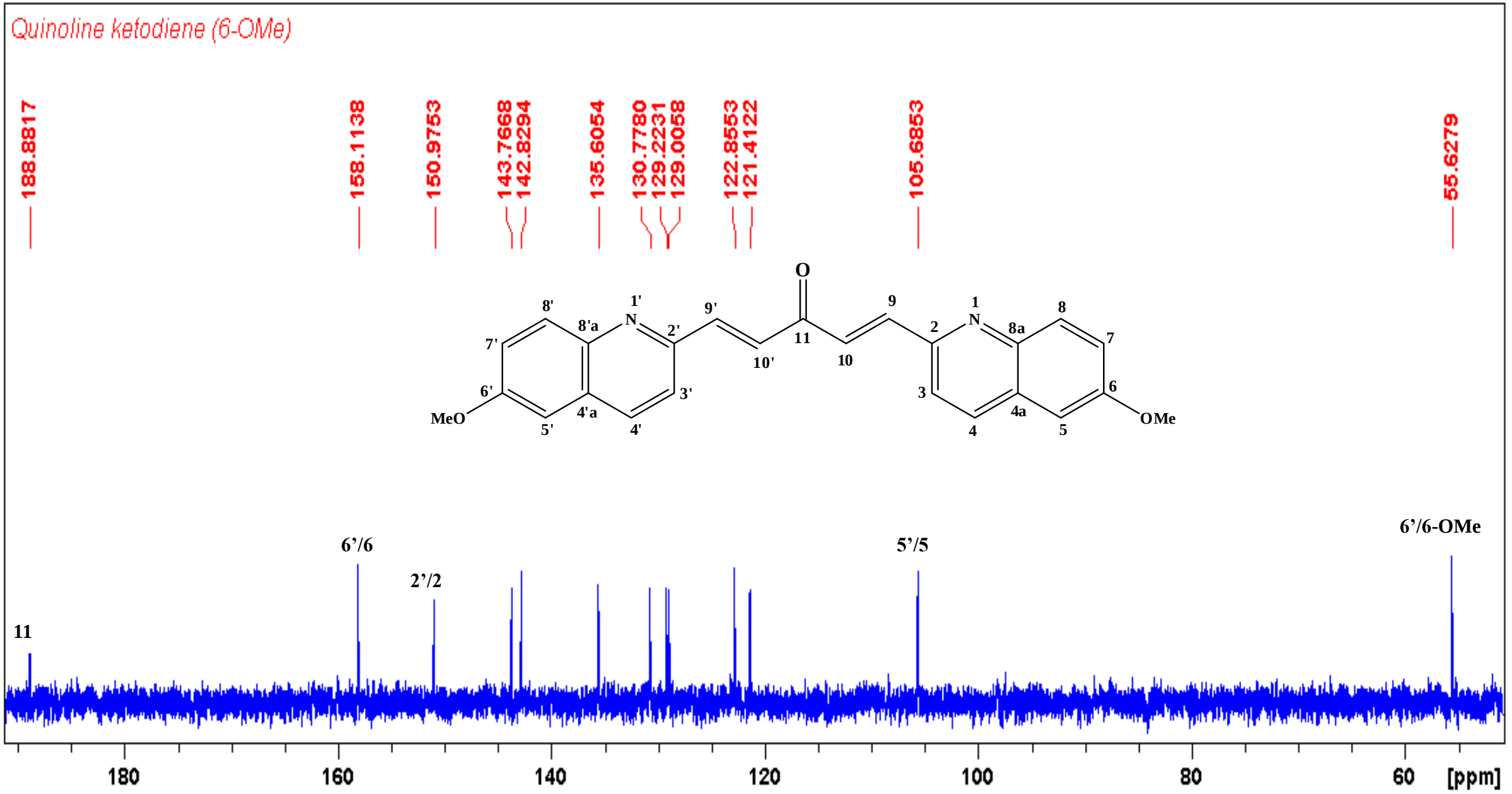
759

^{1}H Spectrum of Compound C-2e: (1E,4E)-1,5-bis(6-methoxyquinolin-2-yl)penta-1,4-dien-3-one



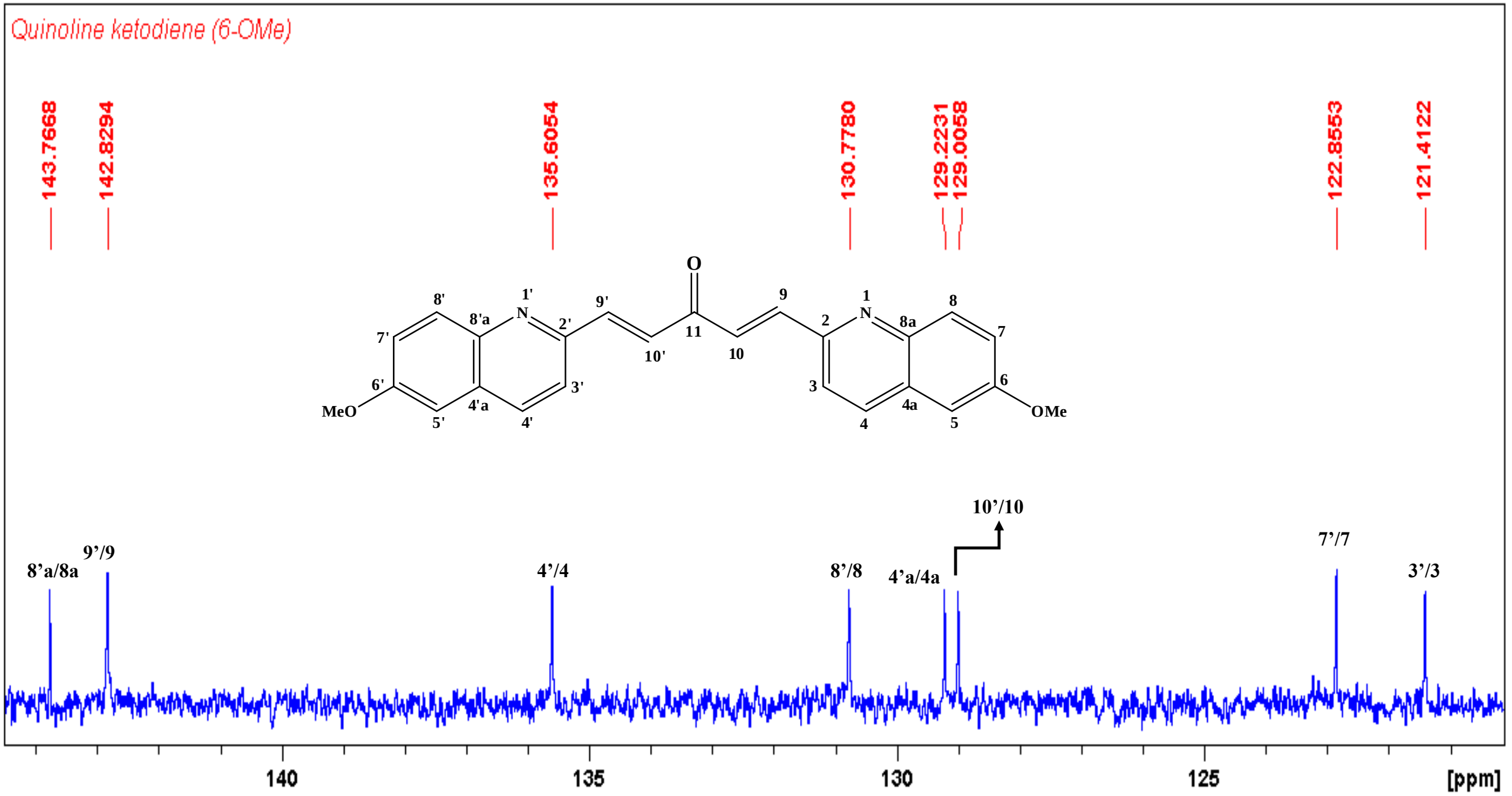
760

Expanded ^{1}H Spectrum of Compound C-2e: (1E,4E)-1,5-bis(6-methoxyquinolin-2-yl)penta-1,4-dien-3-one



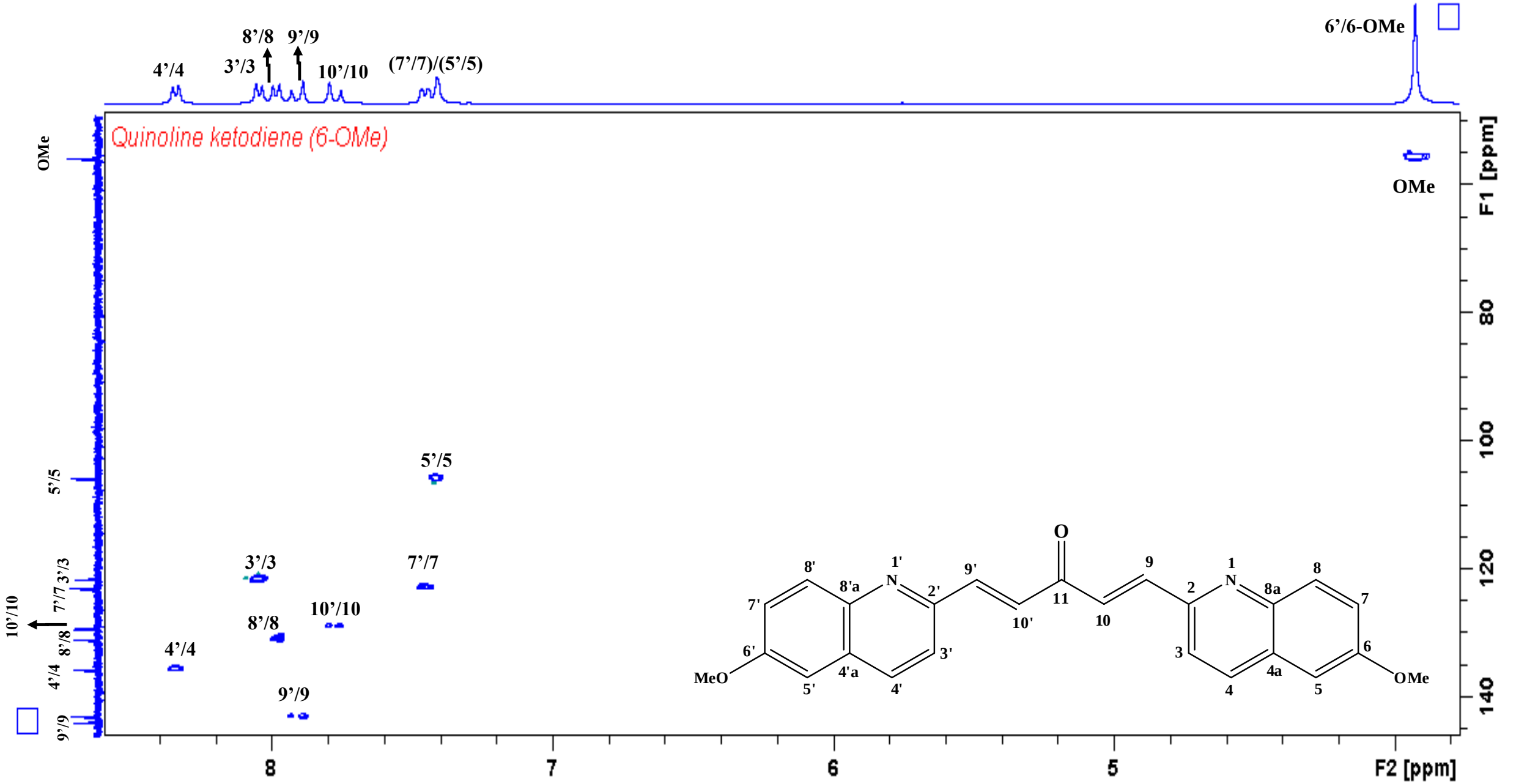
761

^{13}C Spectrum of Compound C-2e: (1E,4E)-1,5-bis(6-methoxyquinolin-2-yl)penta-1,4-dien-3-one

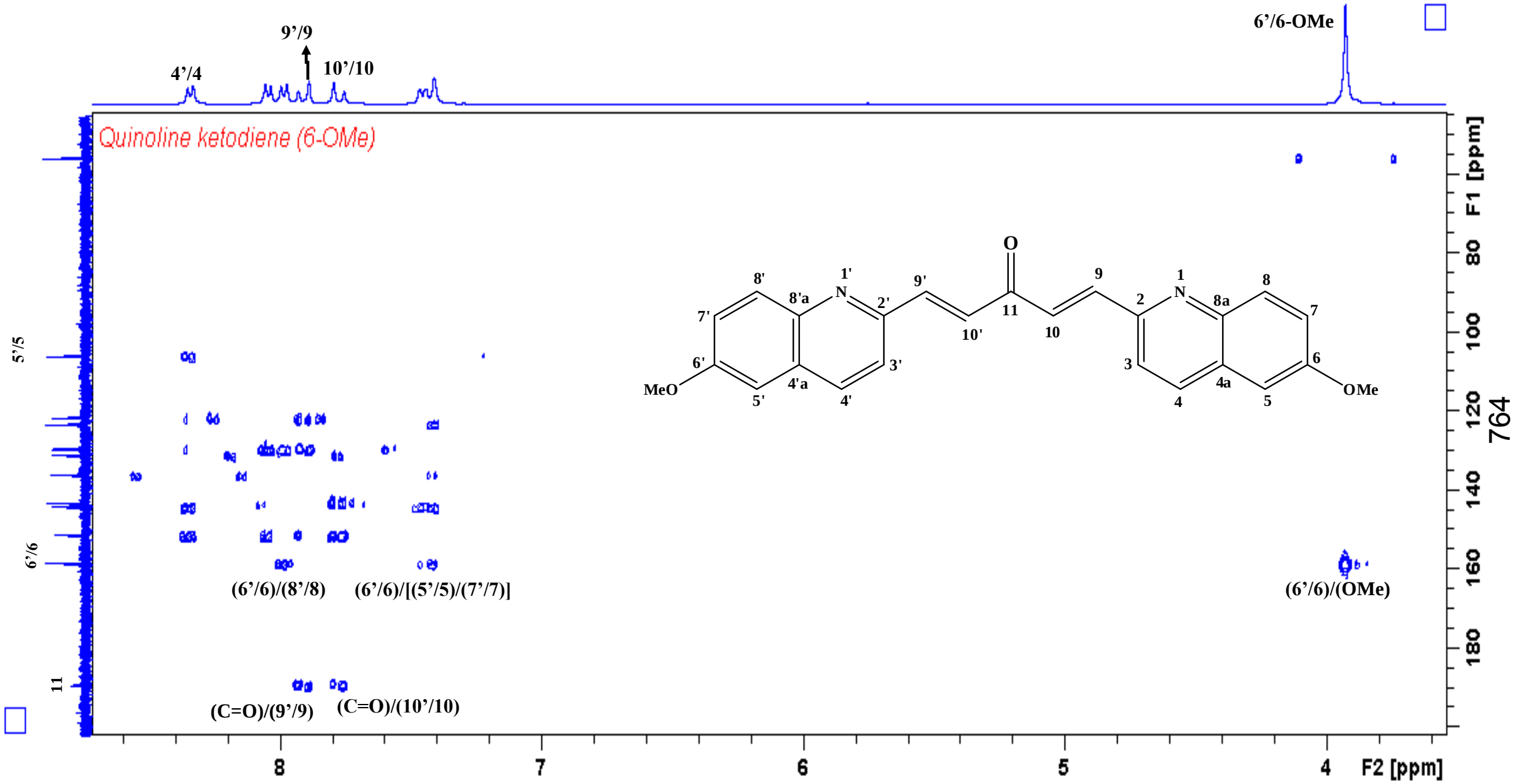


762

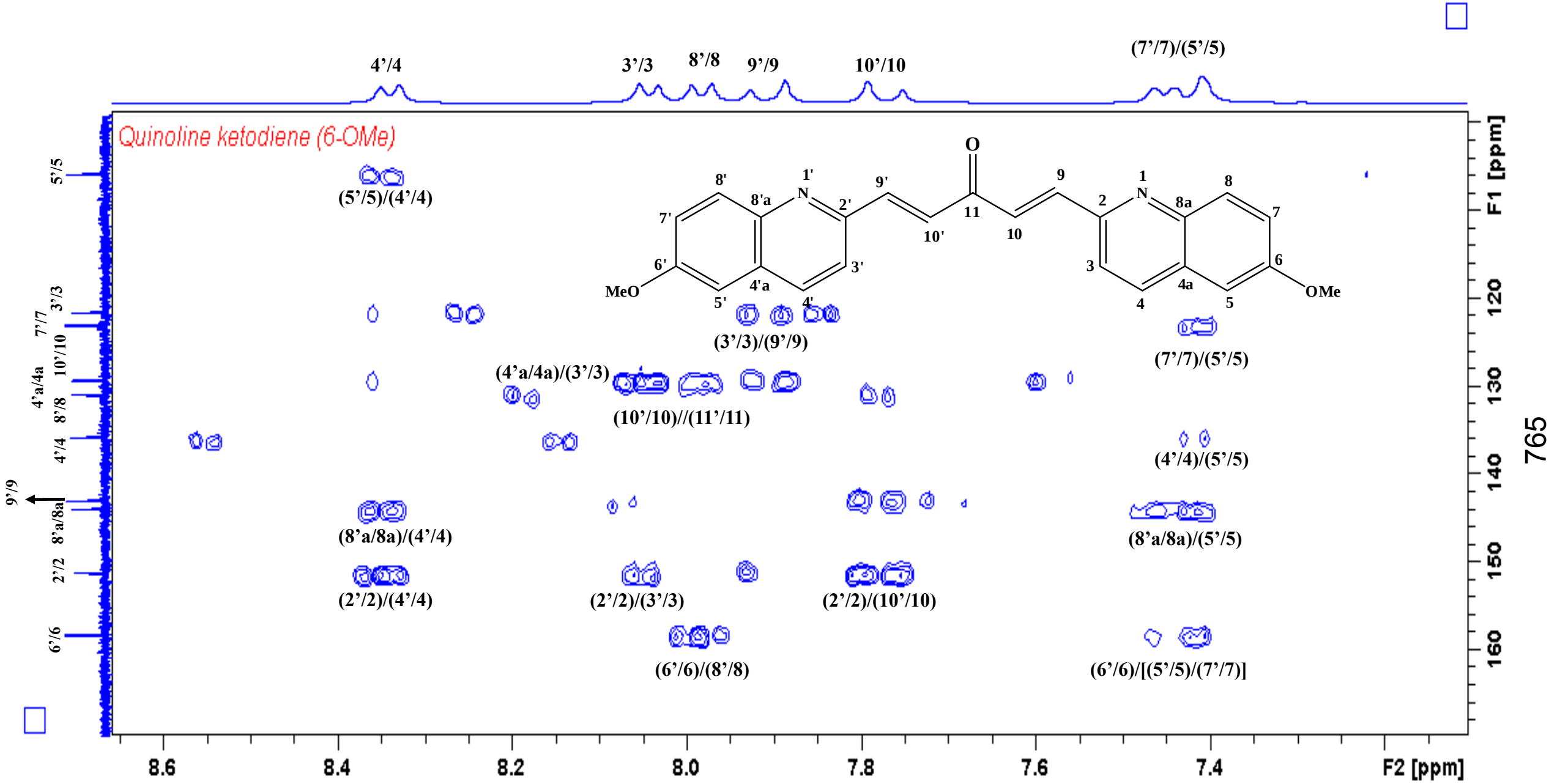
Expanded ^{13}C Spectrum of Compound C-2e: (1E,4E)-1,5-bis(6-methoxyquinolin-2-yl)penta-1,4-dien-3-one



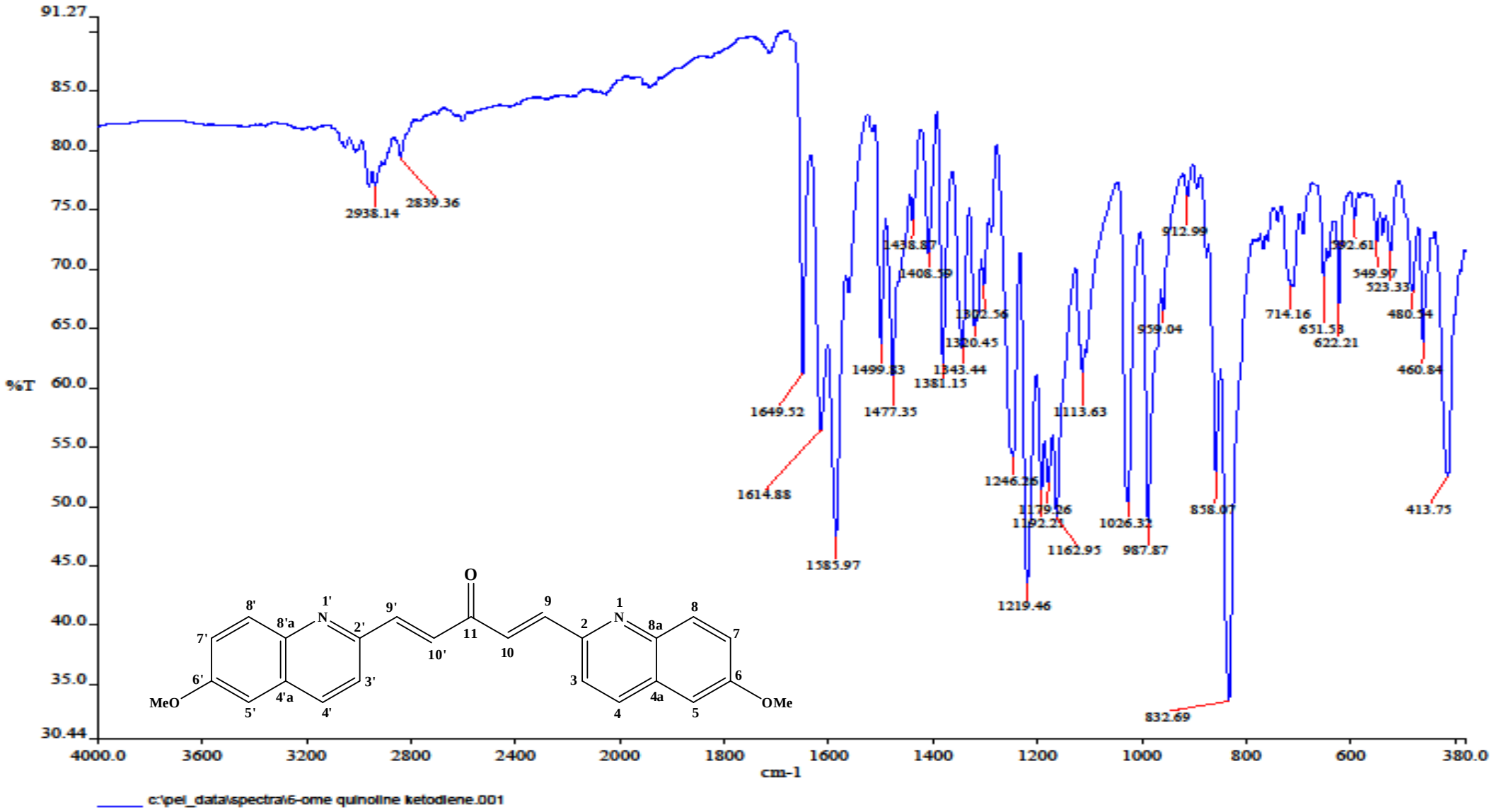
HSQC of Compound C-2e: (1E,4E)-1,5-bis(6-methoxyquinolin-2-yl)penta-1,4-dien-3-one



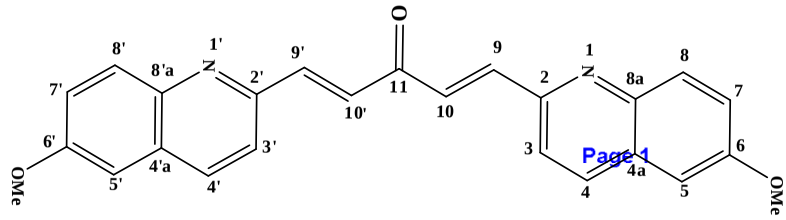
HMBC of Compound C-2e: (1E,4E)-1,5-bis(6-methoxyquinolin-2-yl)penta-1,4-dien-3-one



Expanded HMBC of Compound C-2e: (1E,4E)-1,5-bis(6-methoxyquinolin-2-yl)penta-1,4-dien-3-one



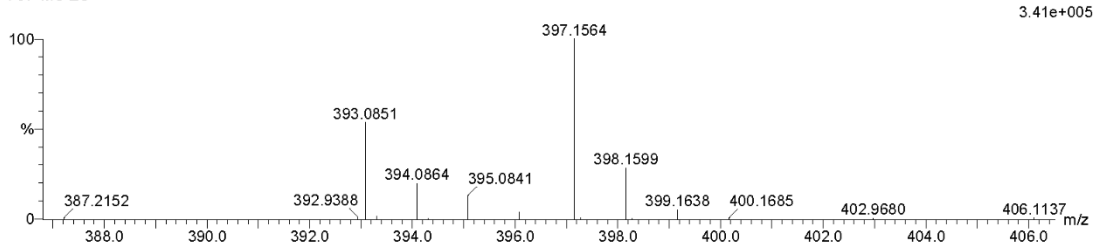
Infrared Spectrum of Compound C-2e: (1E,4E)-1,5-bis(6-methoxyquinolin-2-yl)penta-1,4-dien-3-one



Elemental Composition Report

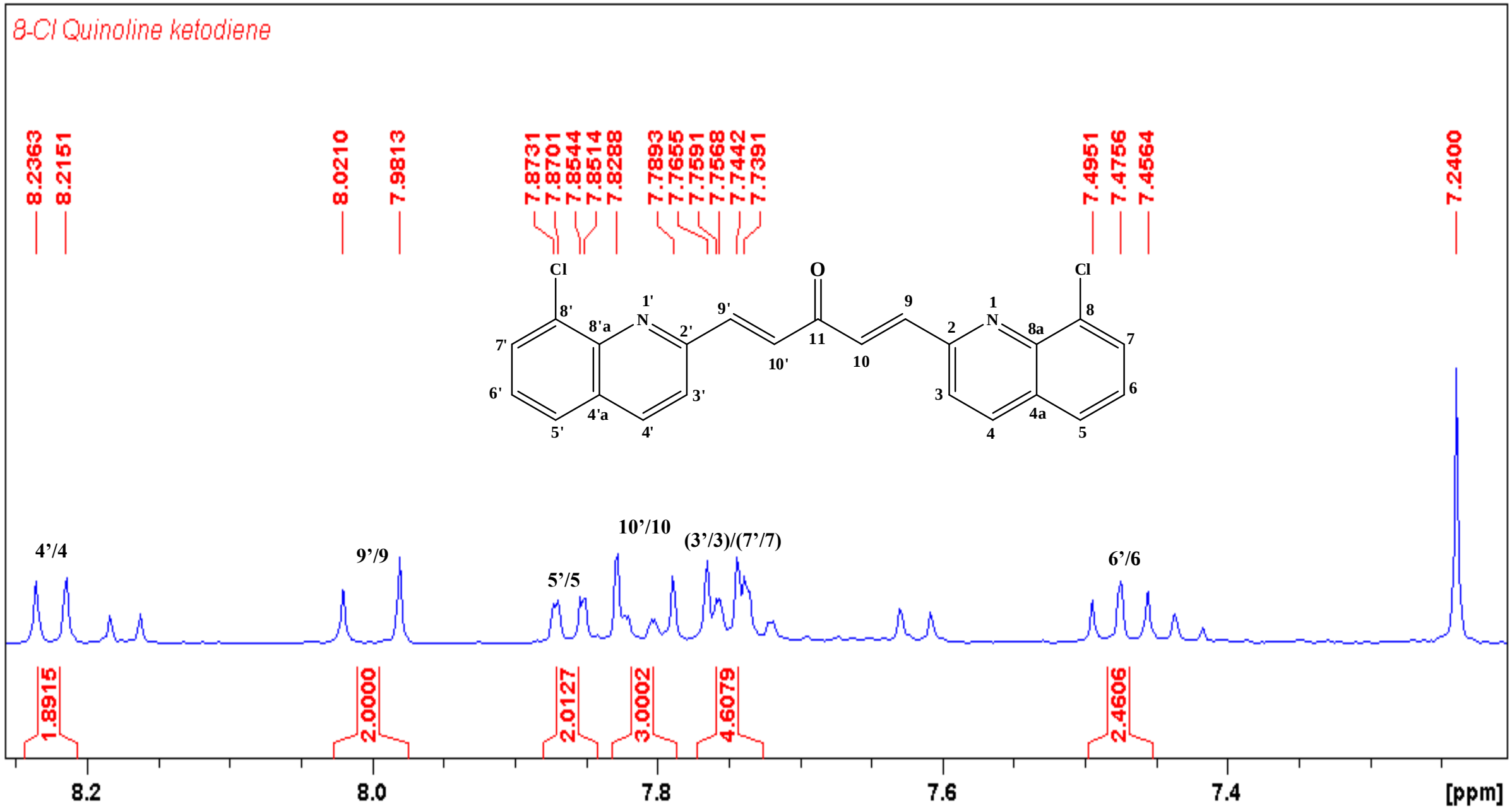
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
22 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 20-25 H: 15-25 N: 0-5 O: 0-5
Compound 5 6-OMe Quinoline ketodiene 39 (1.315) Cm (1:60)
TOF MS ES+

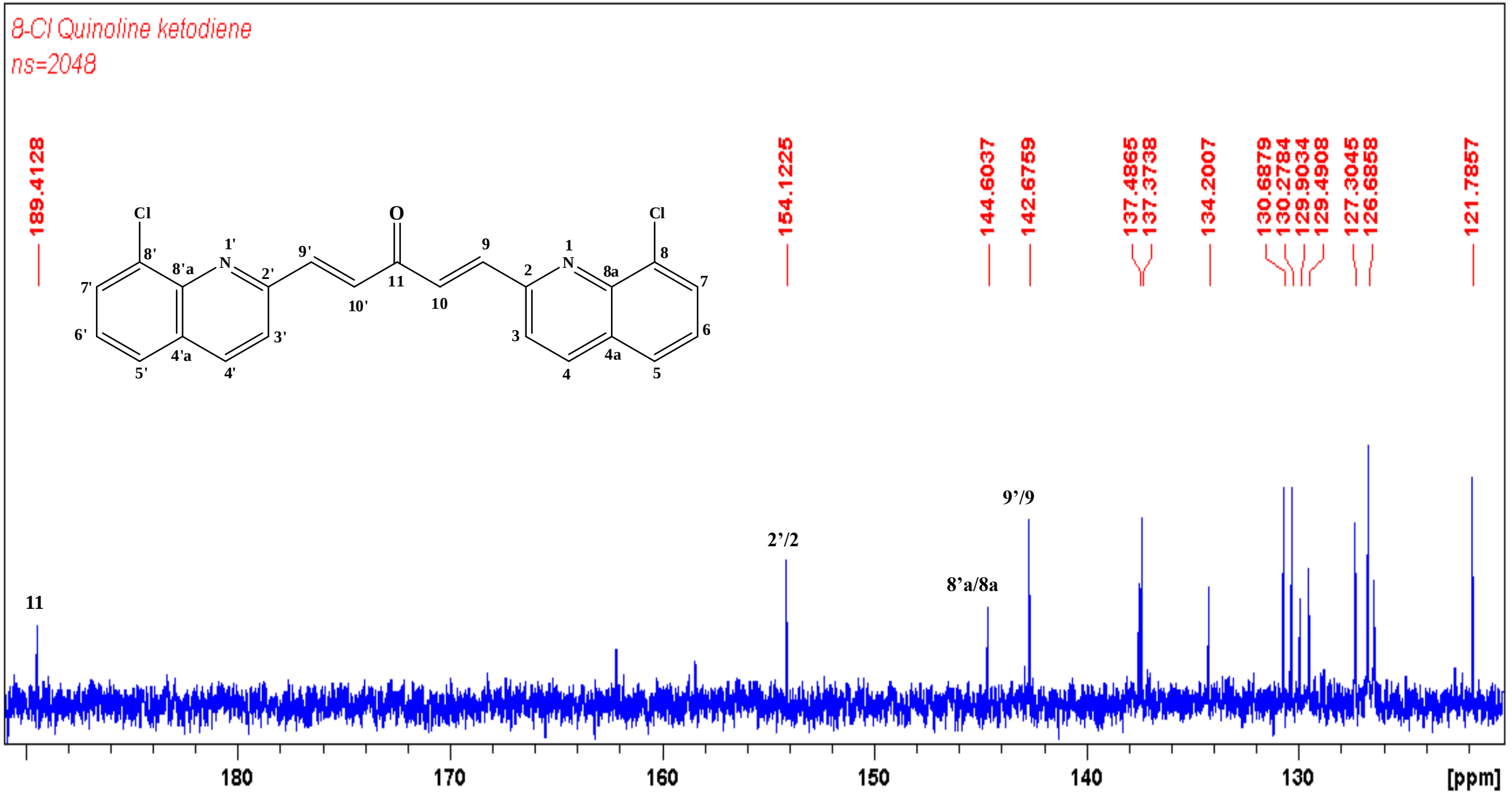


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
397.1564	397.1552	1.2	3.0	16.5	43.4	0.0	C25 H21 N2 O3

HRMS of Compound C-2e: (1E,4E)-1,5-bis(6-methoxyquinolin-2-yl)penta-1,4-dien-3-one

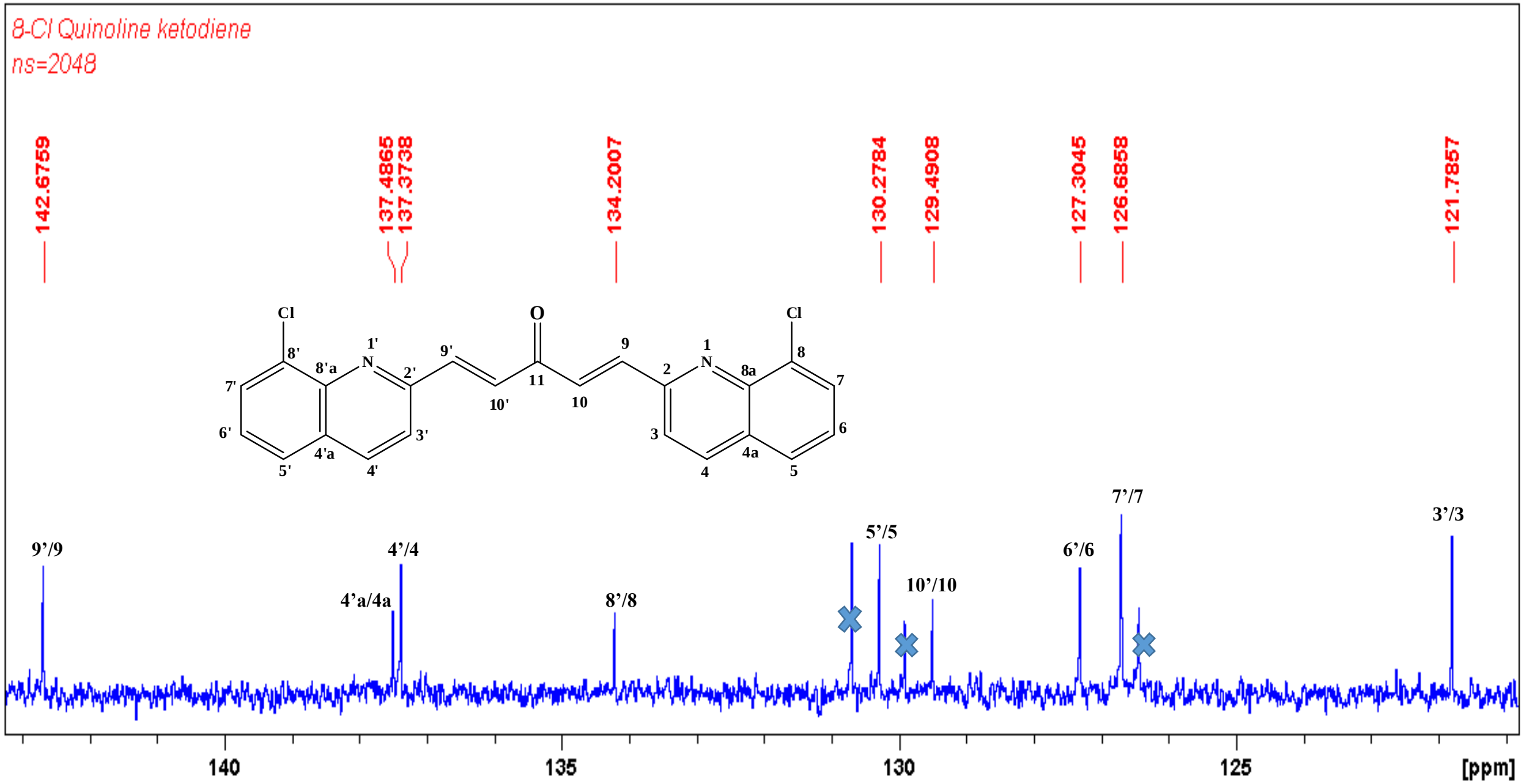


^{1}H Spectrum of Compound C-2f: (1E,4E)-1,5-bis(8-chloroquinolin-2-yl)penta-1,4-dien-3-one

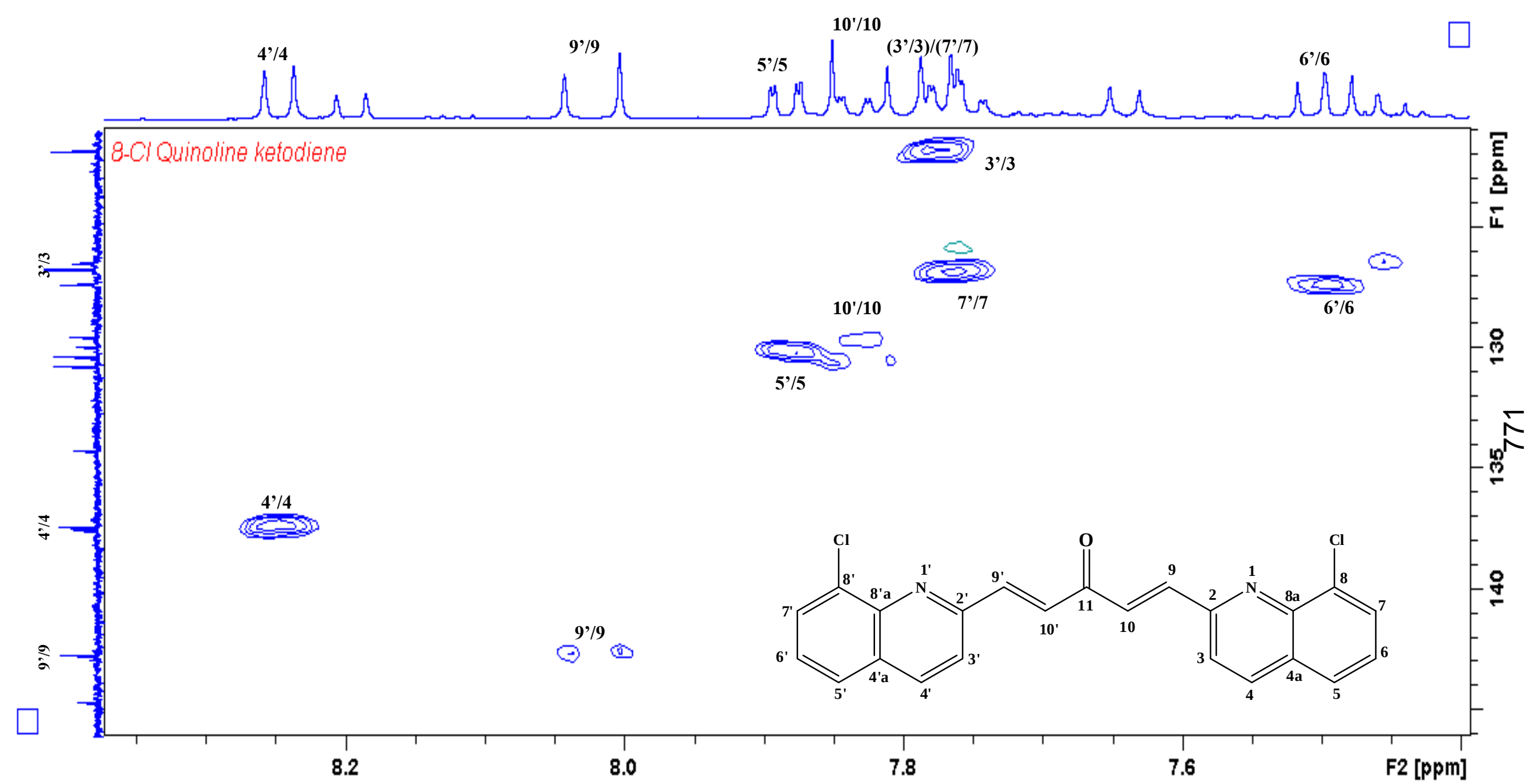


769

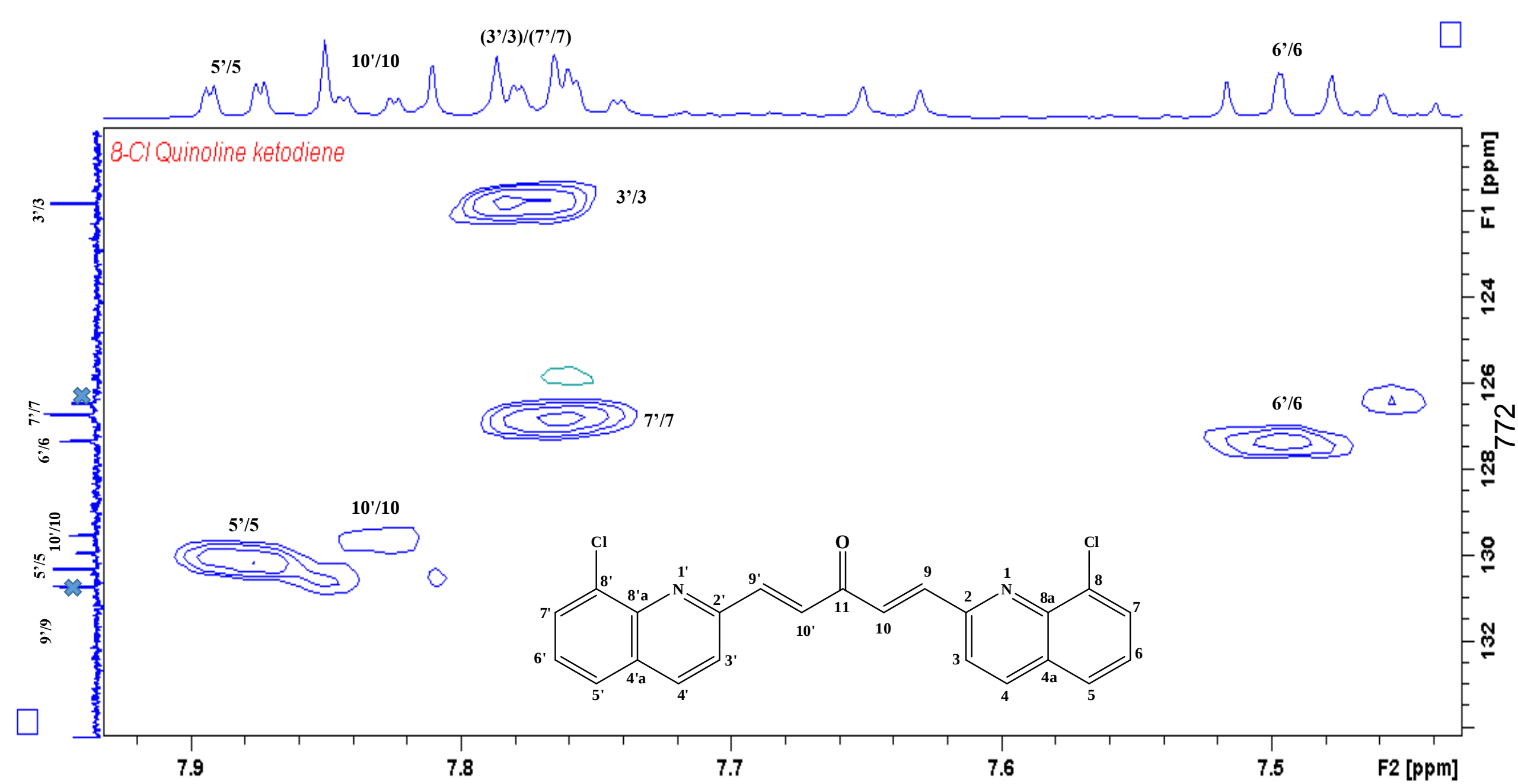
^{13}C Spectrum of Compound C-2f: (1E,4E)-1,5-bis(8-chloroquinolin-2-yl)penta-1,4-dien-3-one



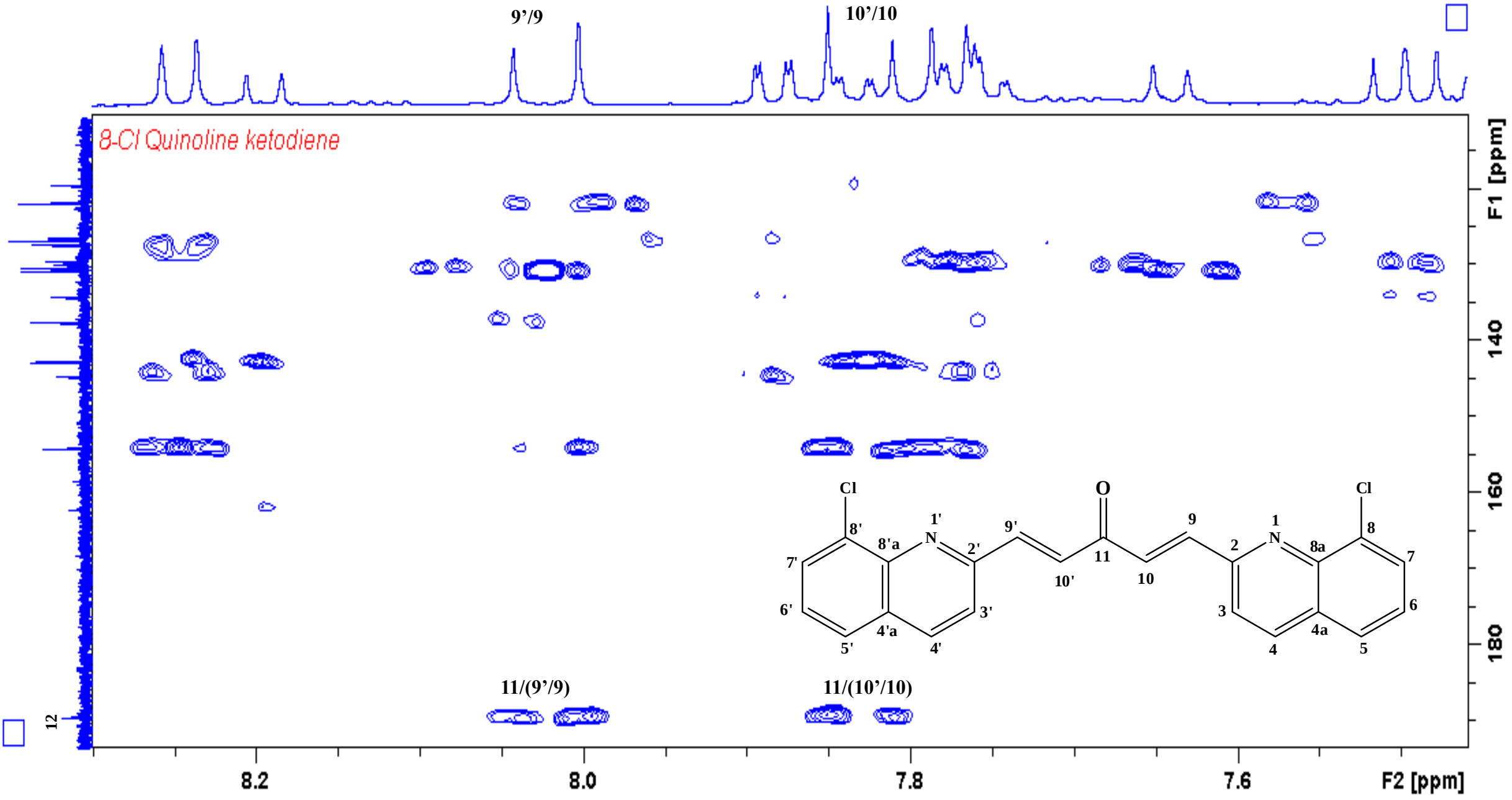
Expanded ^{13}C Spectrum of Compound C-2f: (1E,4E)-1,5-bis(8-chloroquinolin-2-yl)penta-1,4-dien-3-one



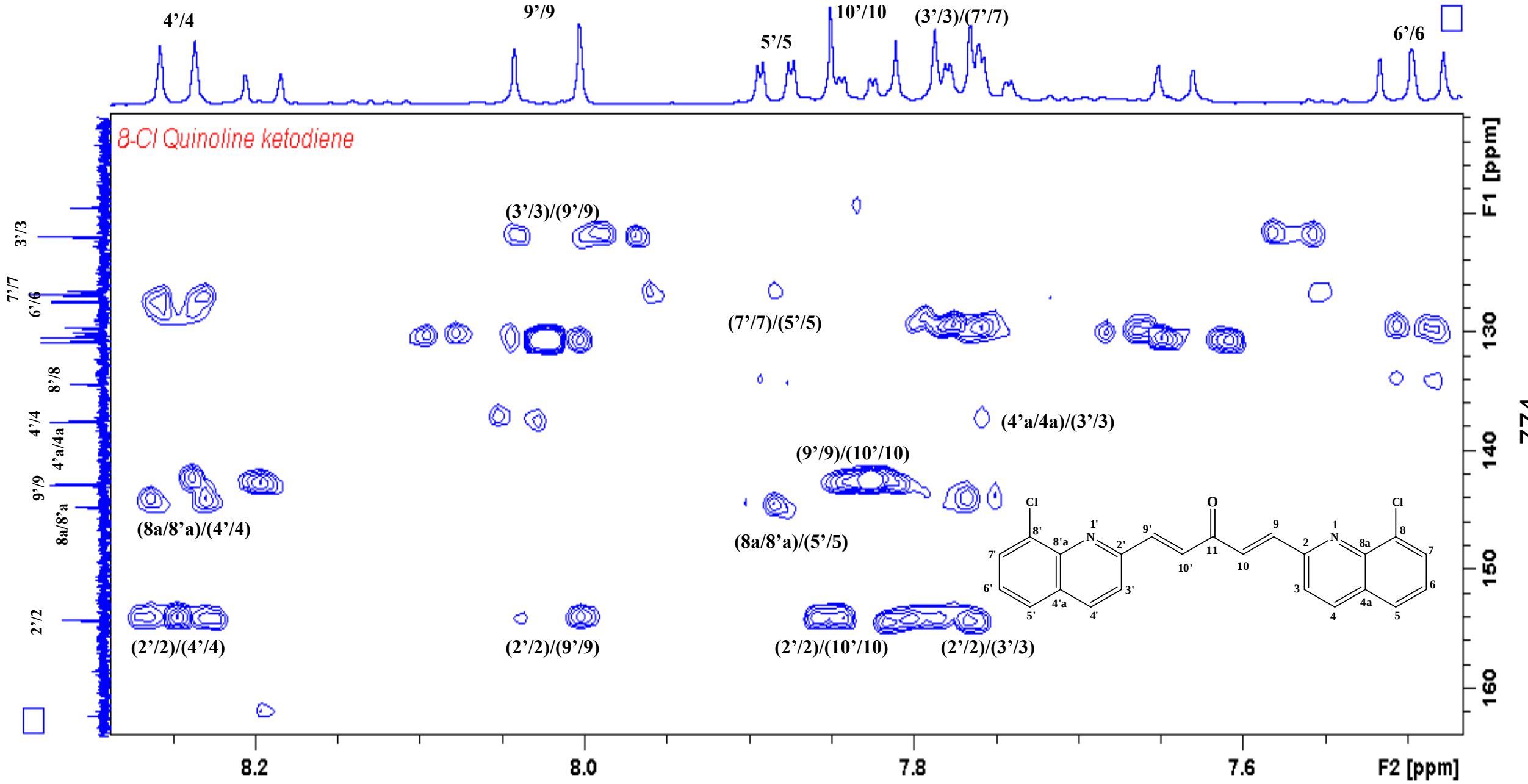
HSQC of Compound C-2f: (1E,4E)-1,5-bis(8-chloroquinolin-2-yl)penta-1,4-dien-3-one



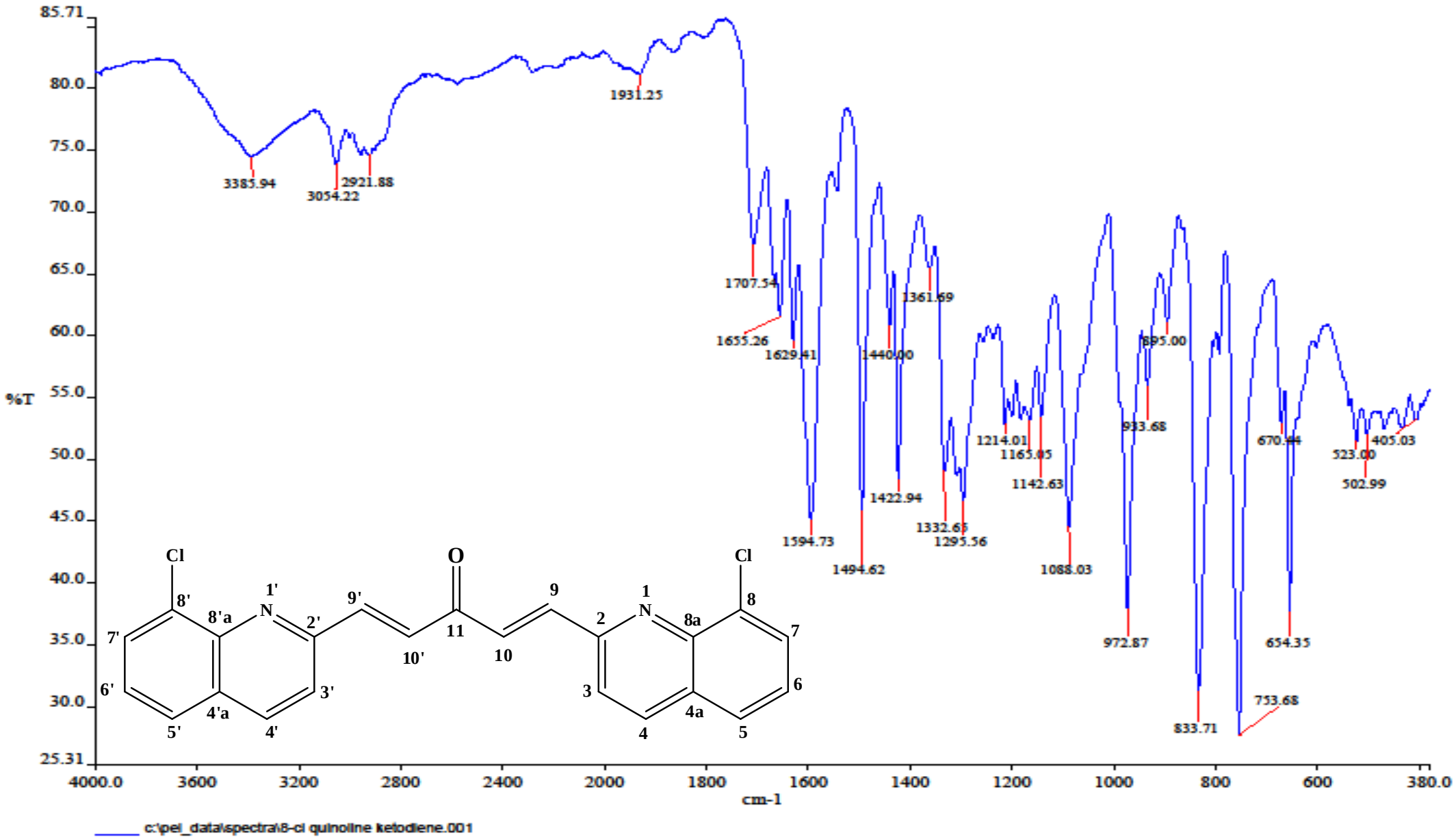
HSQC of Compound C-2f: (1E,4E)-1,5-bis(8-chloroquinolin-2-yl)penta-1,4-dien-3-one



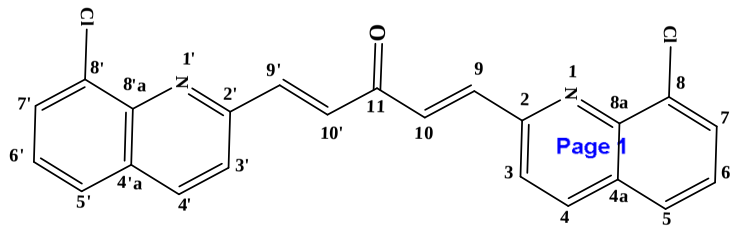
HMBC of Compound C-2f: (1E,4E)-1,5-bis(8-chloroquinolin-2-yl)penta-1,4-dien-3-one



Expanded HMBC of Compound C-2f: (1E,4E)-1,5-bis(8-chloroquinolin-2-yl)penta-1,4-dien-3-one



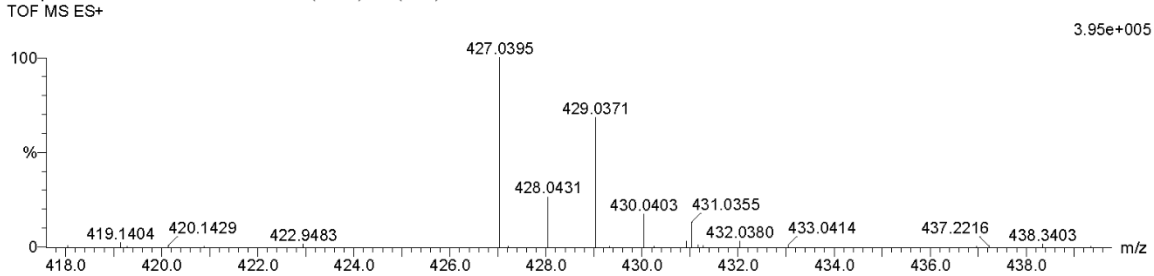
Infrared Spectrum of Compound C-2f: (1E,4E)-1,5-bis(8-chloroquinolin-2-yl)penta-1,4-dien-3-one



Elemental Composition Report Page 1

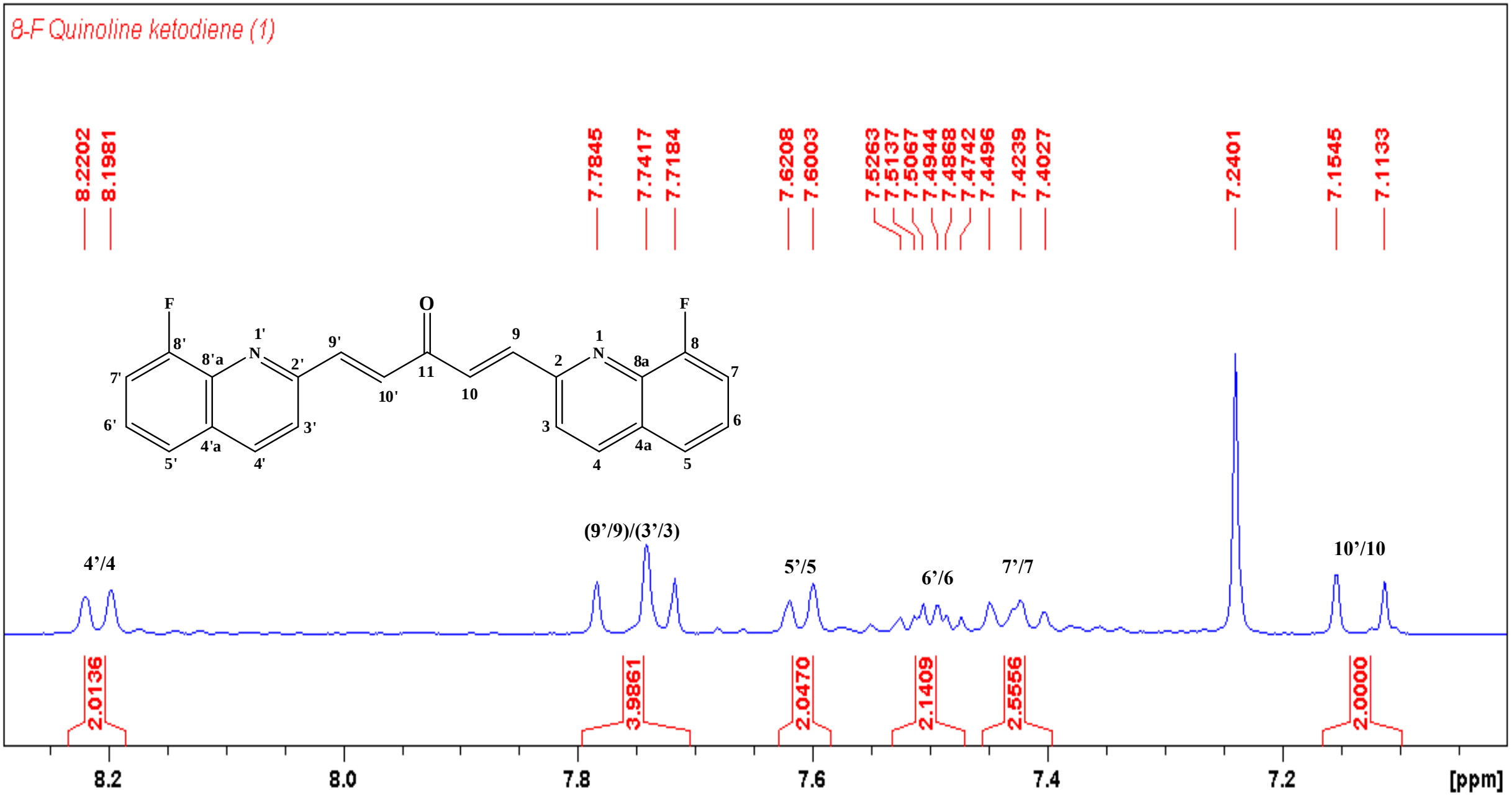
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
49 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 20-25 H: 10-15 N: 0-5 O: 0-5 Na: 1-1 Cl: 0-2
Compound 6 8-Cl Quinoline ketodiene 43 (1.416) Cm (1:61)
TOF MS ES+

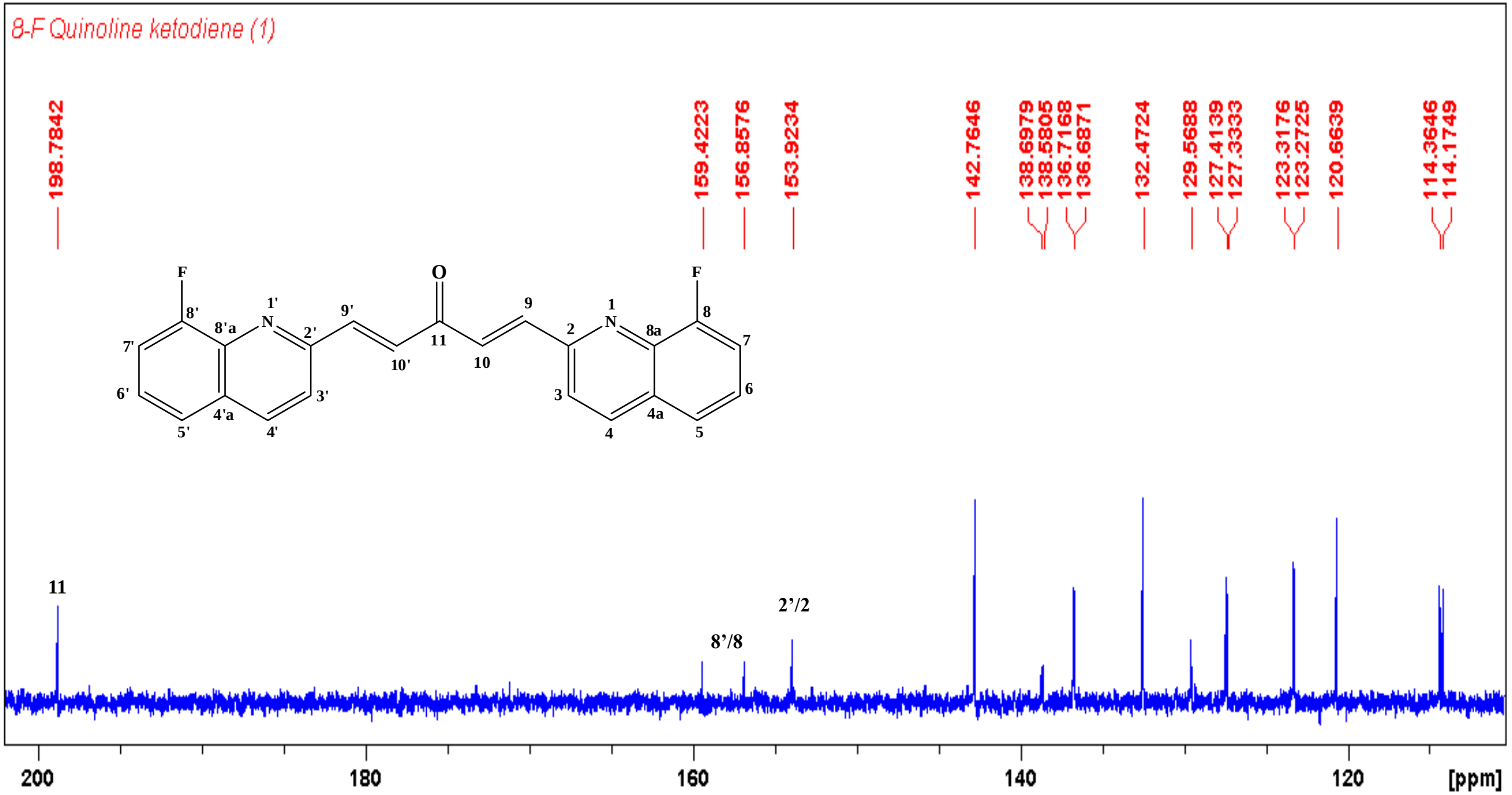


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
427.0395	427.0381	1.4	3.3	16.5	25.9	0.0	C23 H14 N2 O Na Cl2

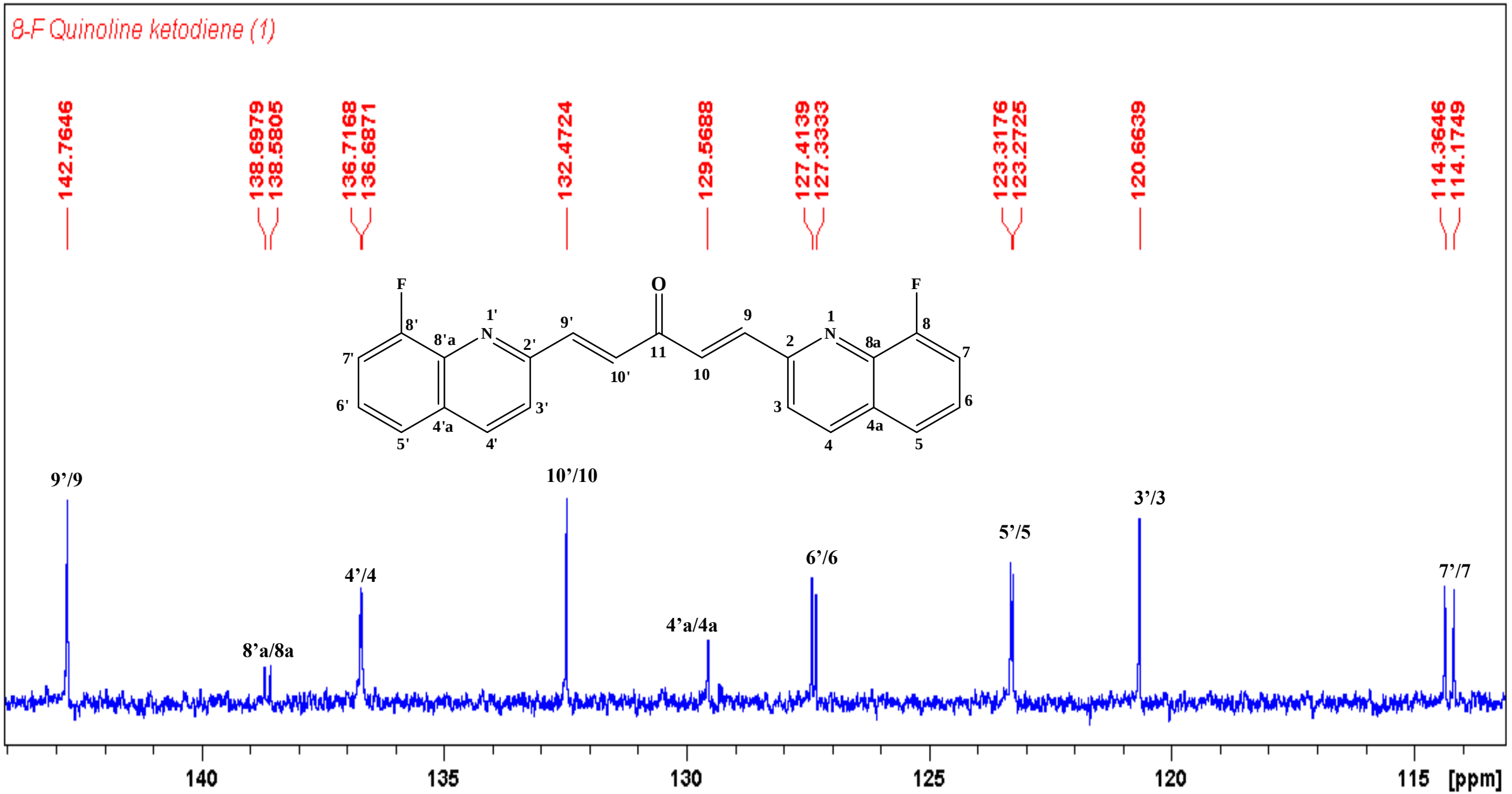
HRMS of Compound C-2f: (1E,4E)-1,5-bis(8-chloroquinolin-2-yl)penta-1,4-dien-3-one



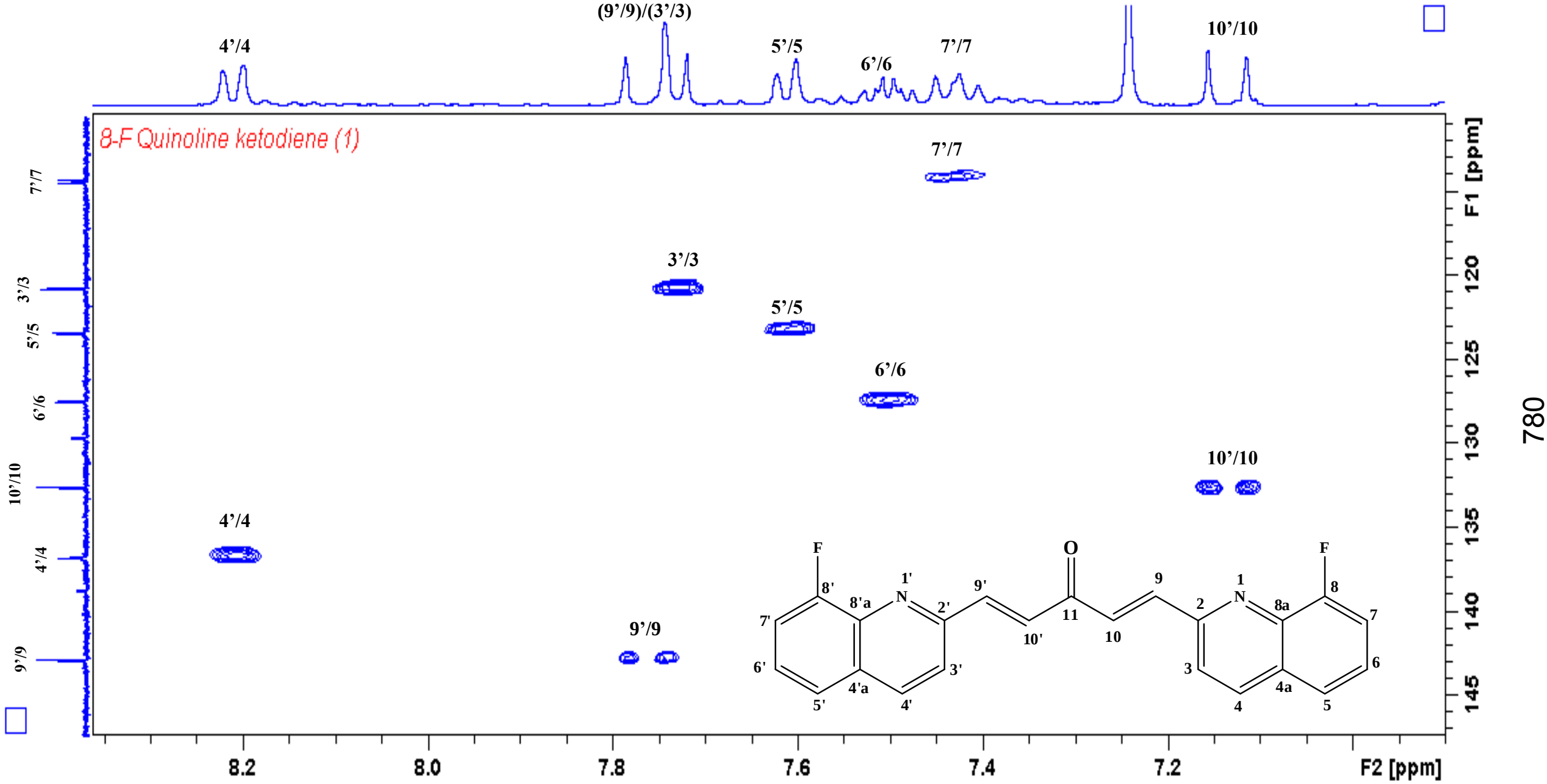
^{1}H Spectrum of Compound C-2g: (1E,4E)-1,5-bis(8-fluoroquinolin-2-yl)penta-1,4-dien-3-one



^{13}C Spectrum of Compound C-2g: (1E,4E)-1,5-bis(8-fluoroquinolin-2-yl)penta-1,4-dien-3-one

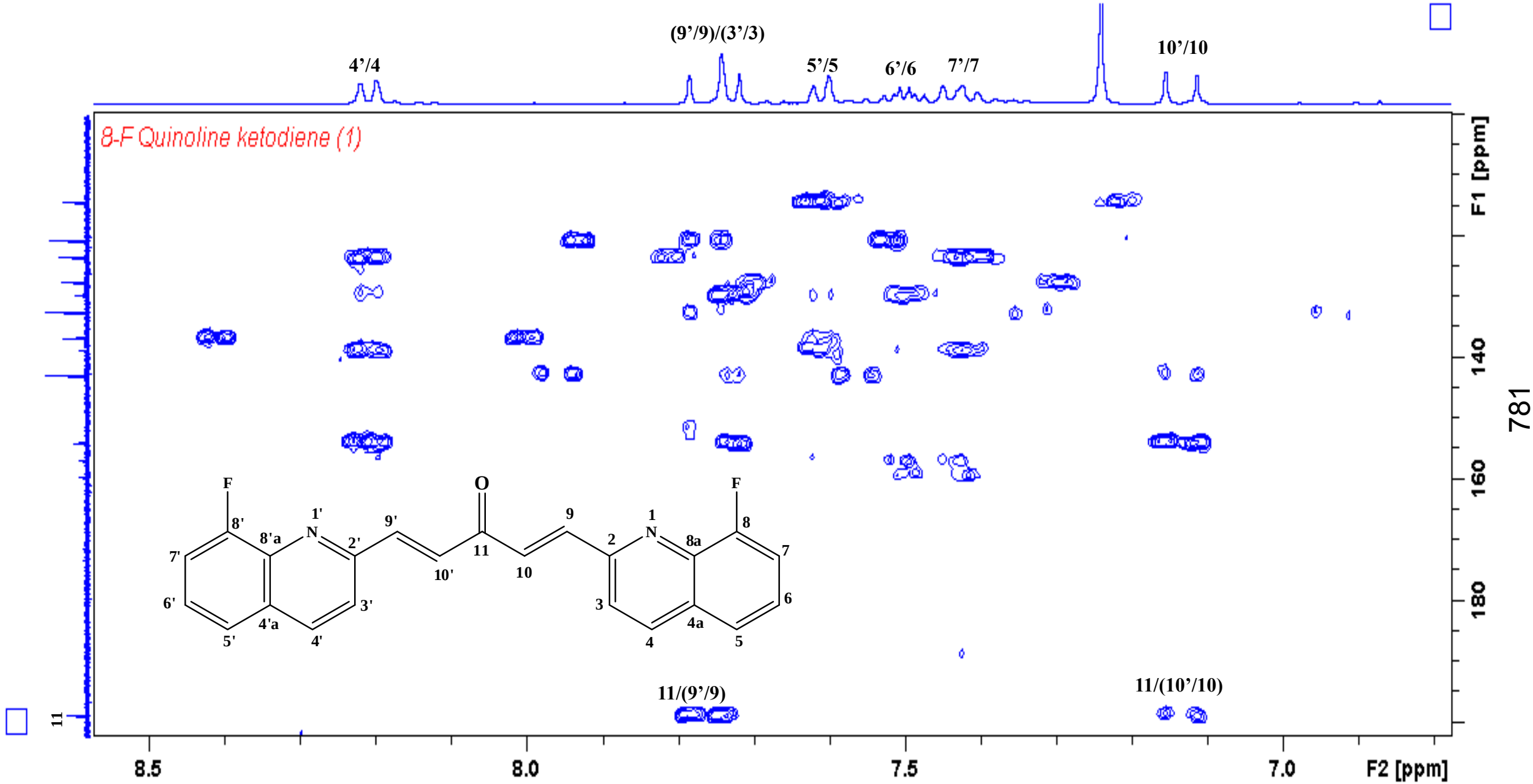


Expanded ^{13}C Spectrum of Compound C-2g: (1E,4E)-1,5-bis(8-fluoroquinolin-2-yl)penta-1,4-dien-3-one

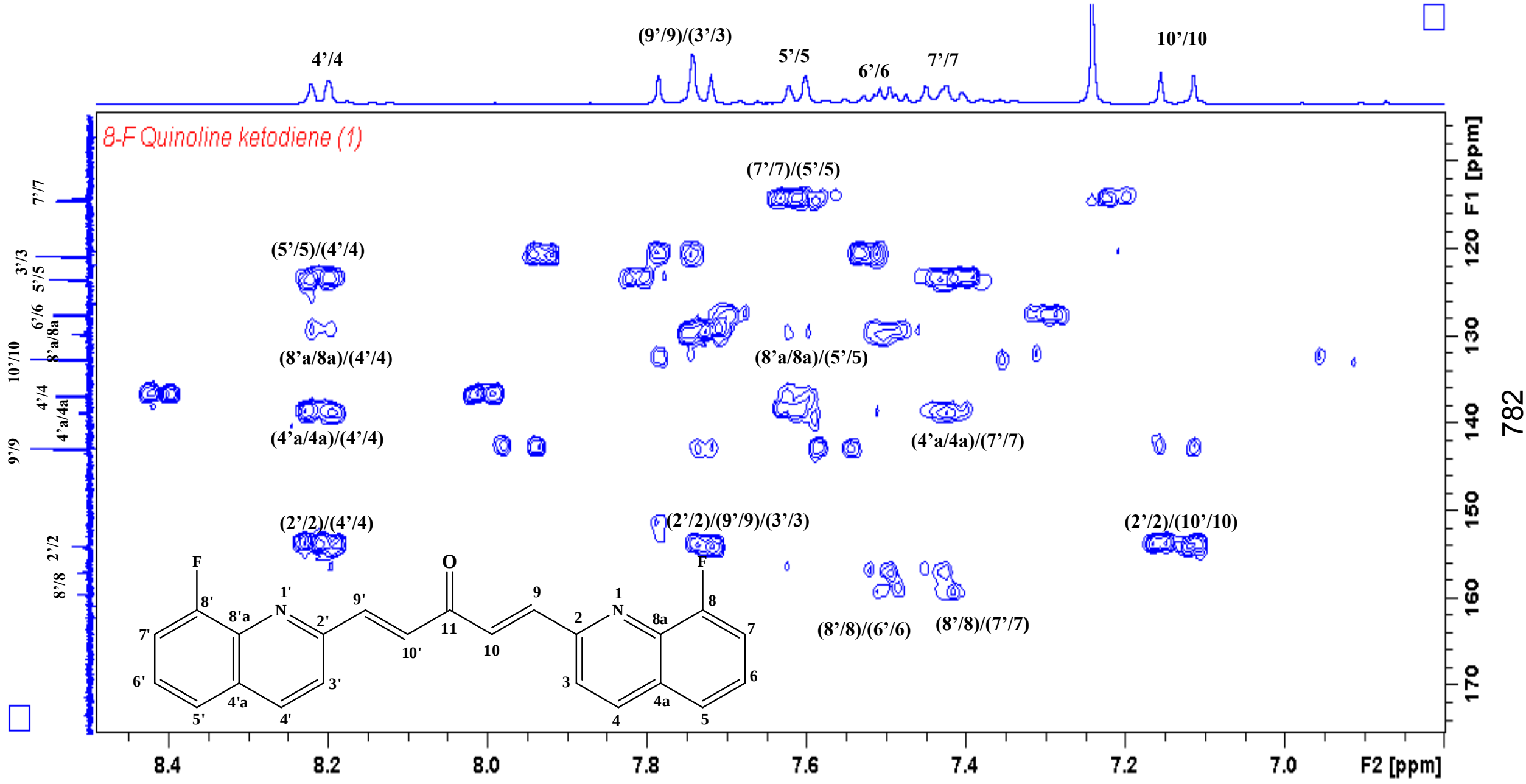


780

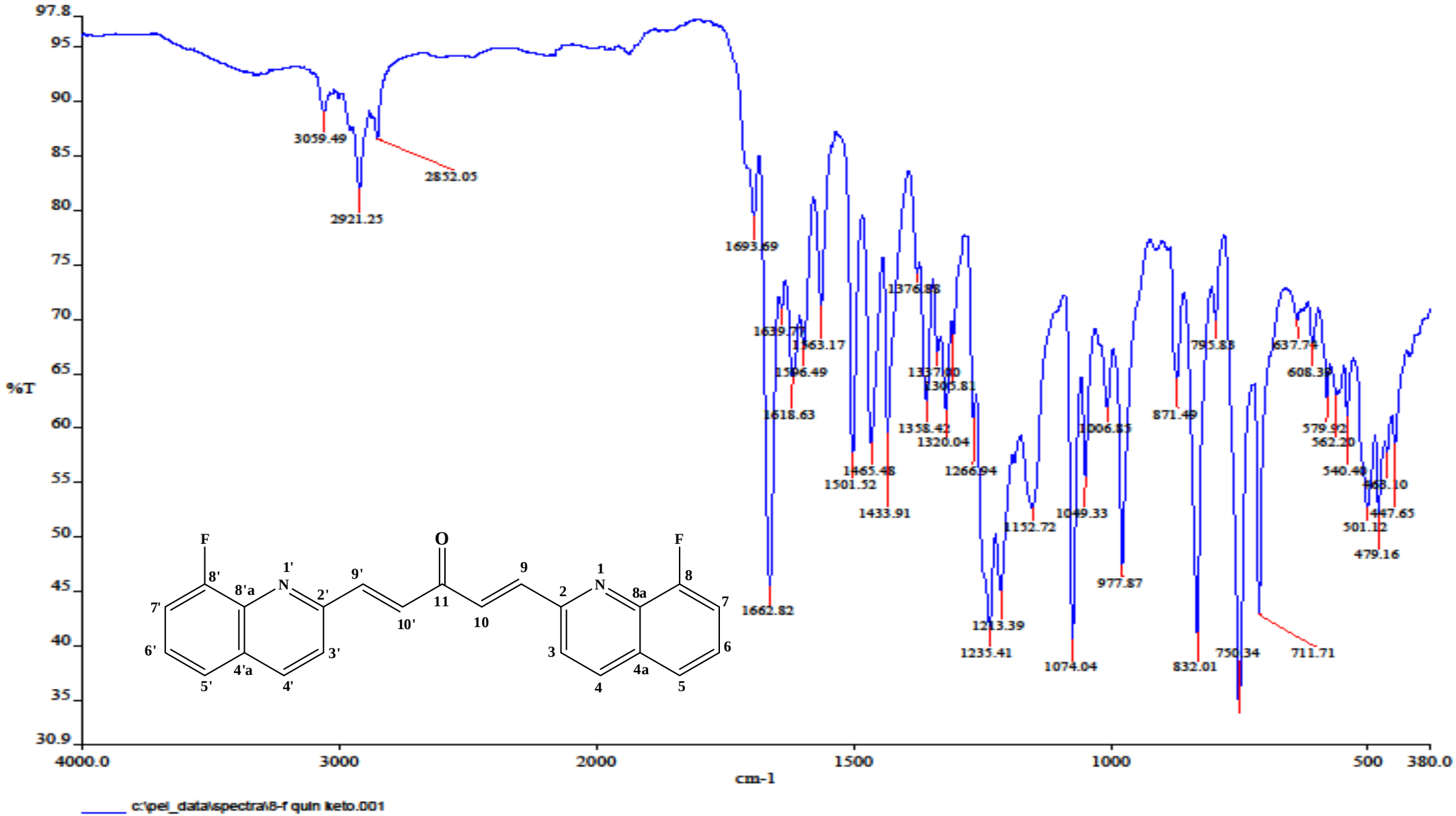
HSQC of Compound C-2g: (1E,4E)-1,5-bis(8-fluoroquinolin-2-yl)penta-1,4-dien-3-one



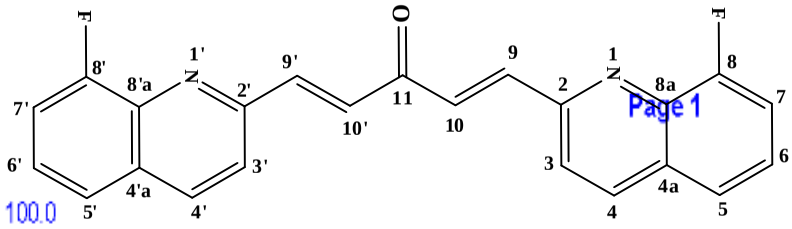
HMBC of Compound C-2g: (1E,4E)-1,5-bis(8-fluoroquinolin-2-yl)penta-1,4-dien-3-one



Expanded HMBC of Compound C-2g: (1E,4E)-1,5-bis(8-fluoroquinolin-2-yl)penta-1,4-dien-3-one



Ultraviolet Spectrum of Compound C-2g: (1E,4E)-1,5-bis(8-fluoroquinolin-2-yl)penta-1,4-dien-3-one



Elemental Composition Report Page 1

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions

50 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)

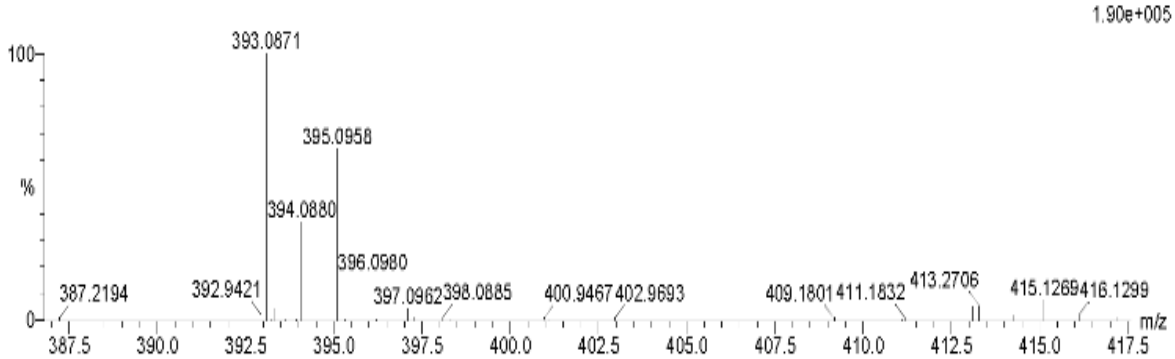
Elements Used:

C: 20-25 H: 10-15 N: 0-5 O: 0-5 F: 0-2 Na: 1-1

Compound 1 6-F Quinoline ketodiene 57 (1.889) Cm (1:61)

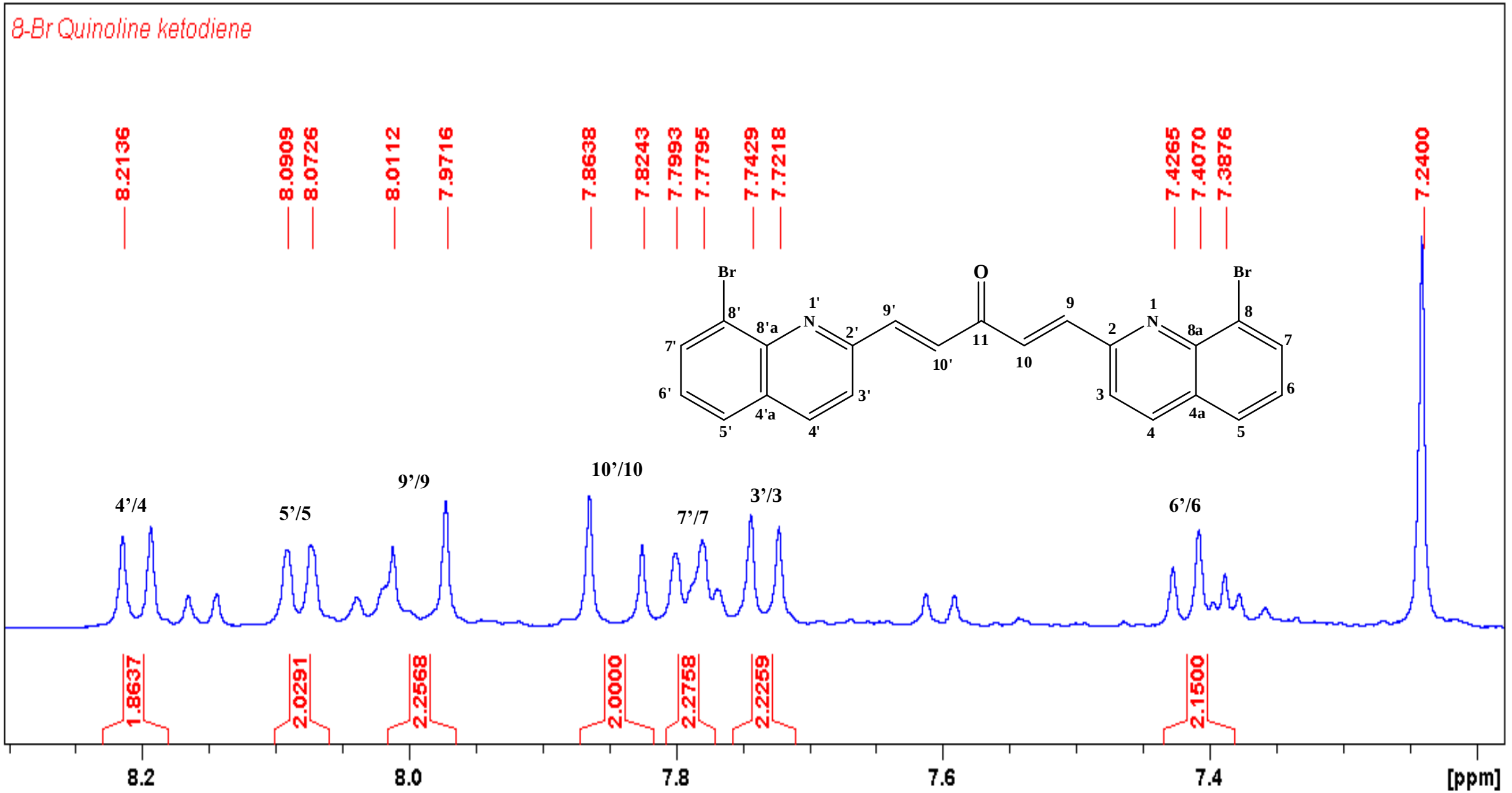
TOF MS ES+

1.90e+005



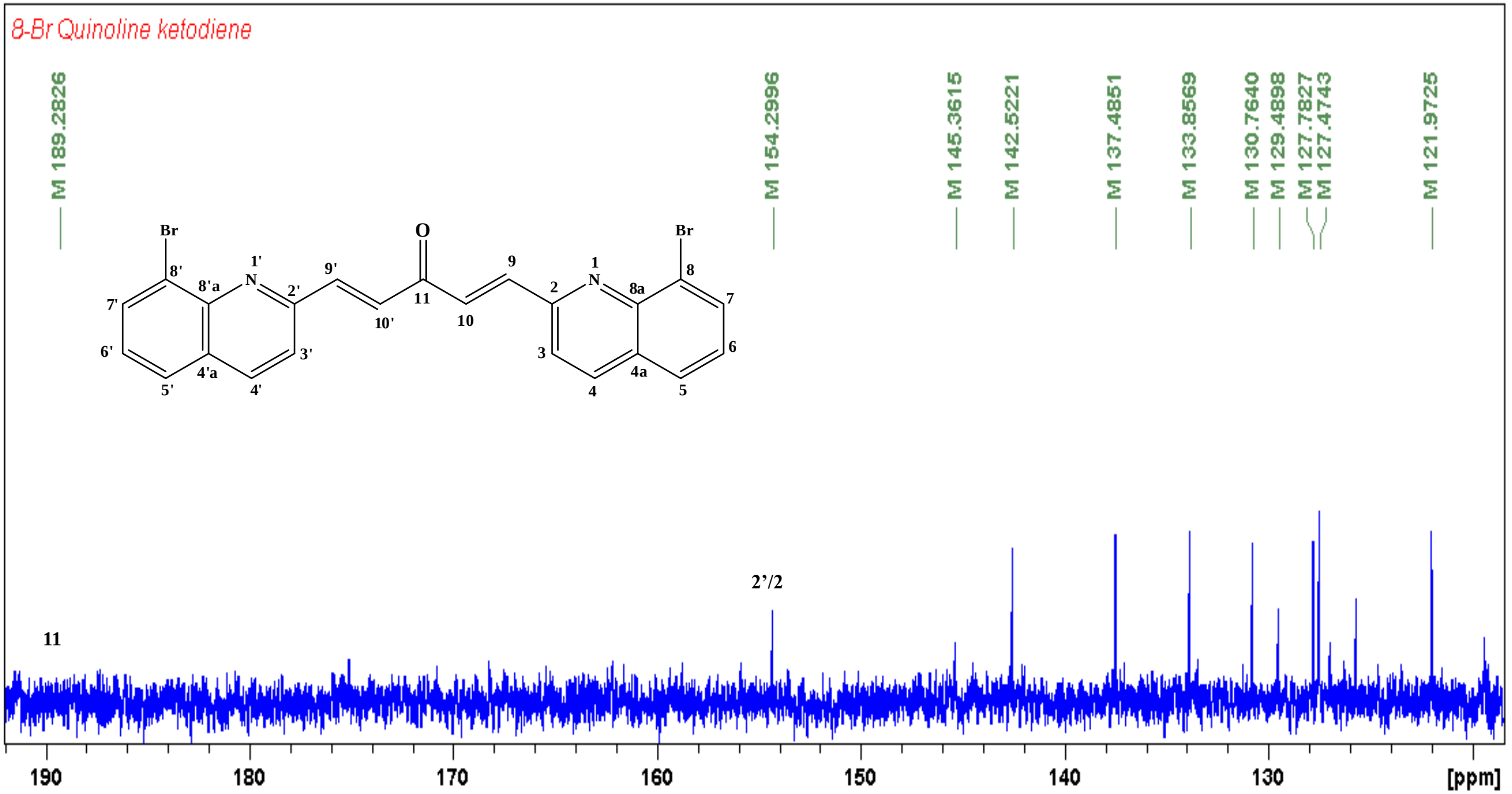
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
395.0958	395.0972	-1.4	-3.5	16.5	52.1	0.0	C23 H14 N2 O F2 Na

HRMS of Compound C-2g: (1E,4E)-1,5-bis(8-fluoroquinolin-2-yl)penta-1,4-dien-3-one



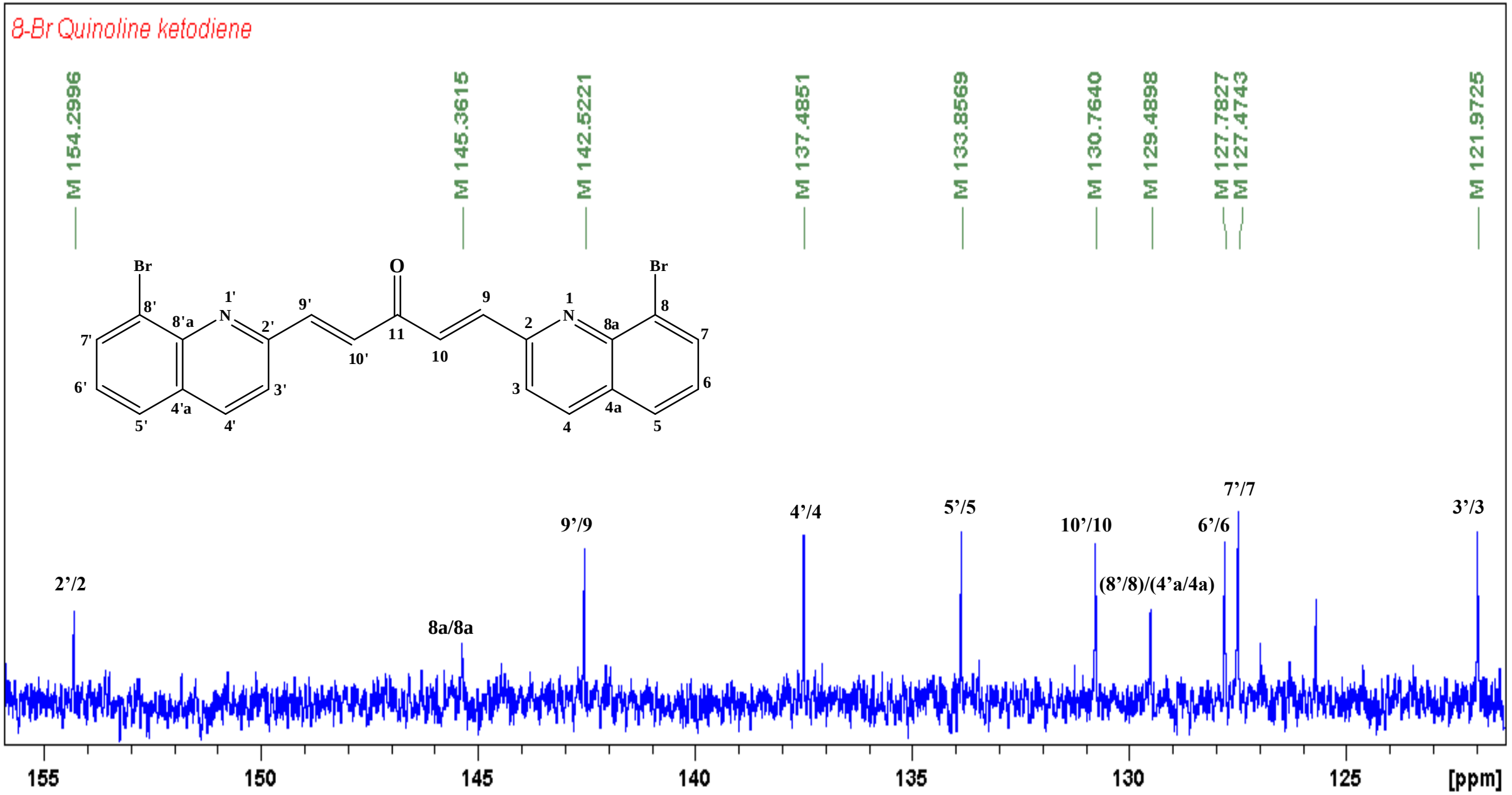
785

[1]H Spectrum of Compound C-2h: (1E,4E)-1,5-bis(8-bromoquinolin-2-yl)penta-1,4-dien-3-one

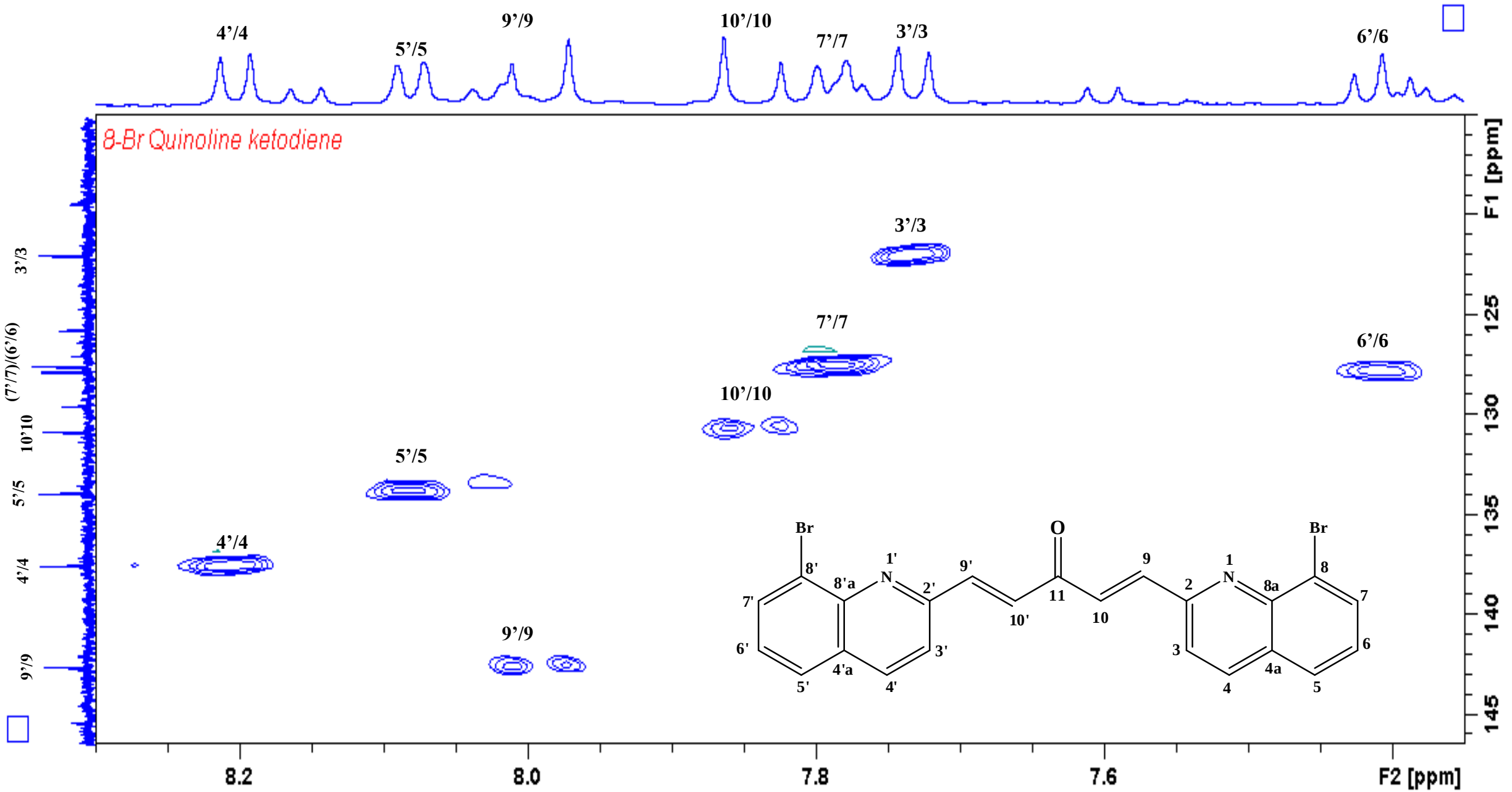


786

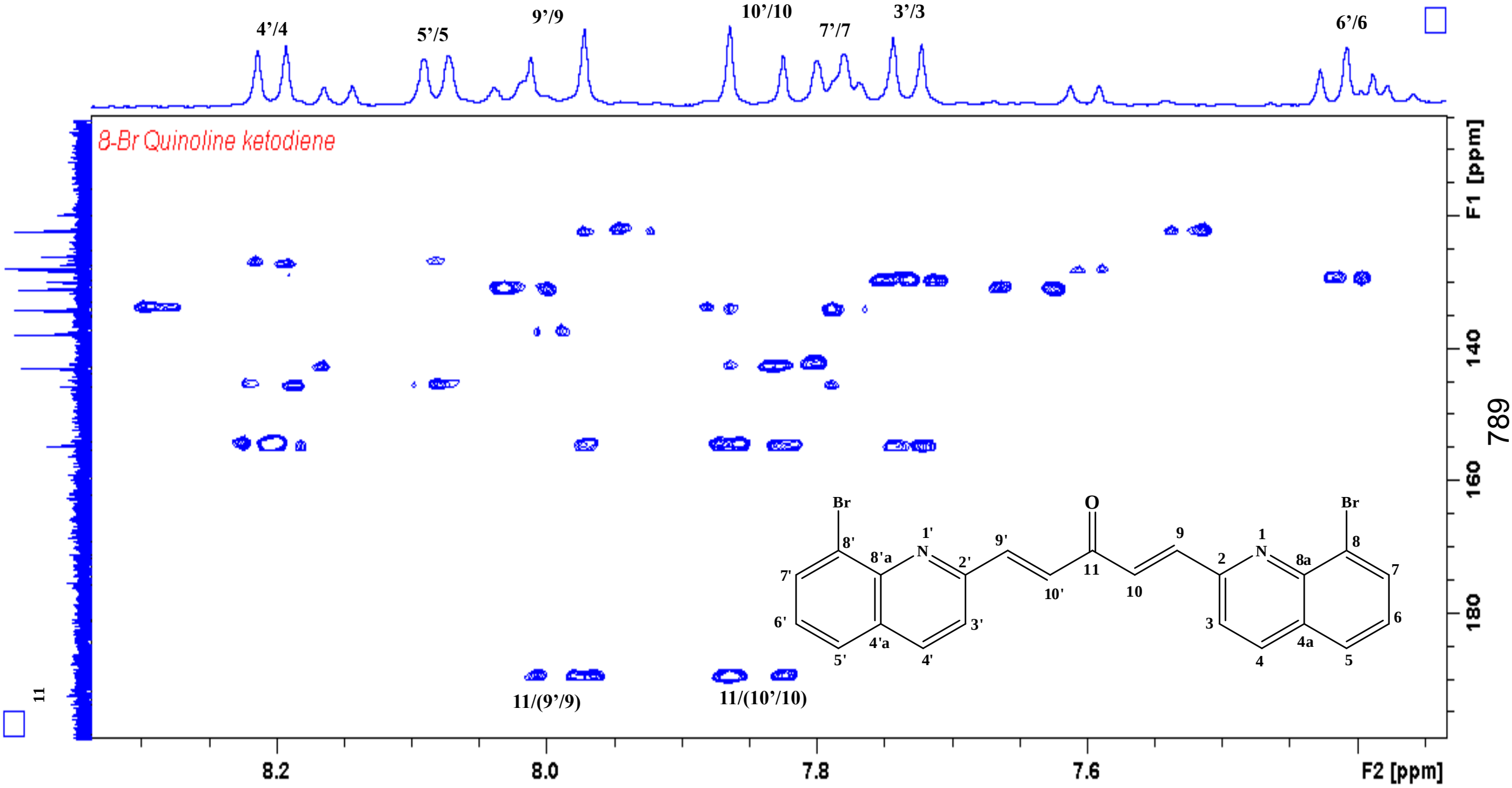
^{13}C Spectrum of Compound C-2h: (1E,4E)-1,5-bis(8-bromoquinolin-2-yl)penta-1,4-dien-3-one



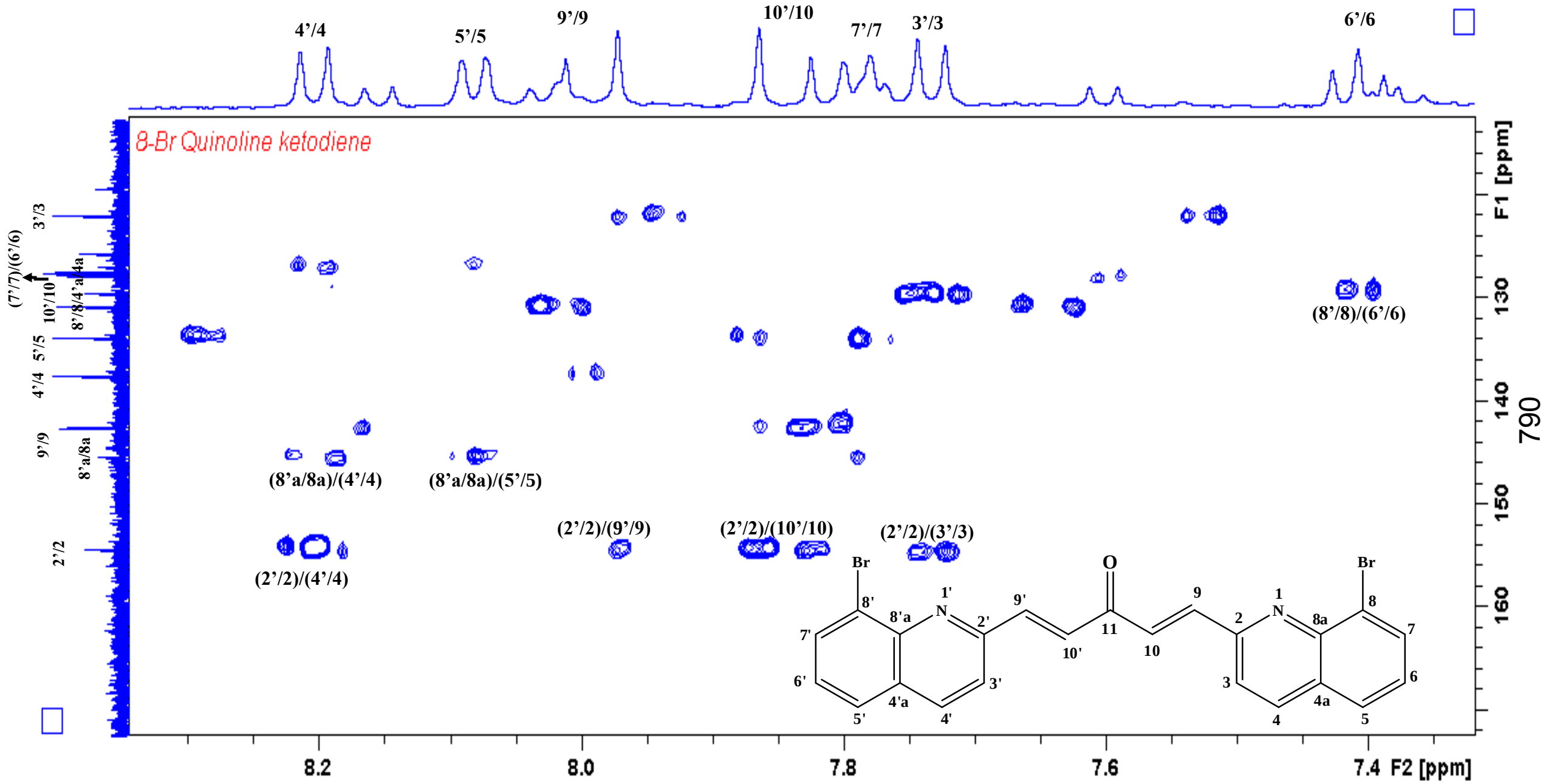
Expanded ^{13}C Spectrum of Compound C-2h: (1E,4E)-1,5-bis(8-bromoquinolin-2-yl)penta-1,4-dien-3-one



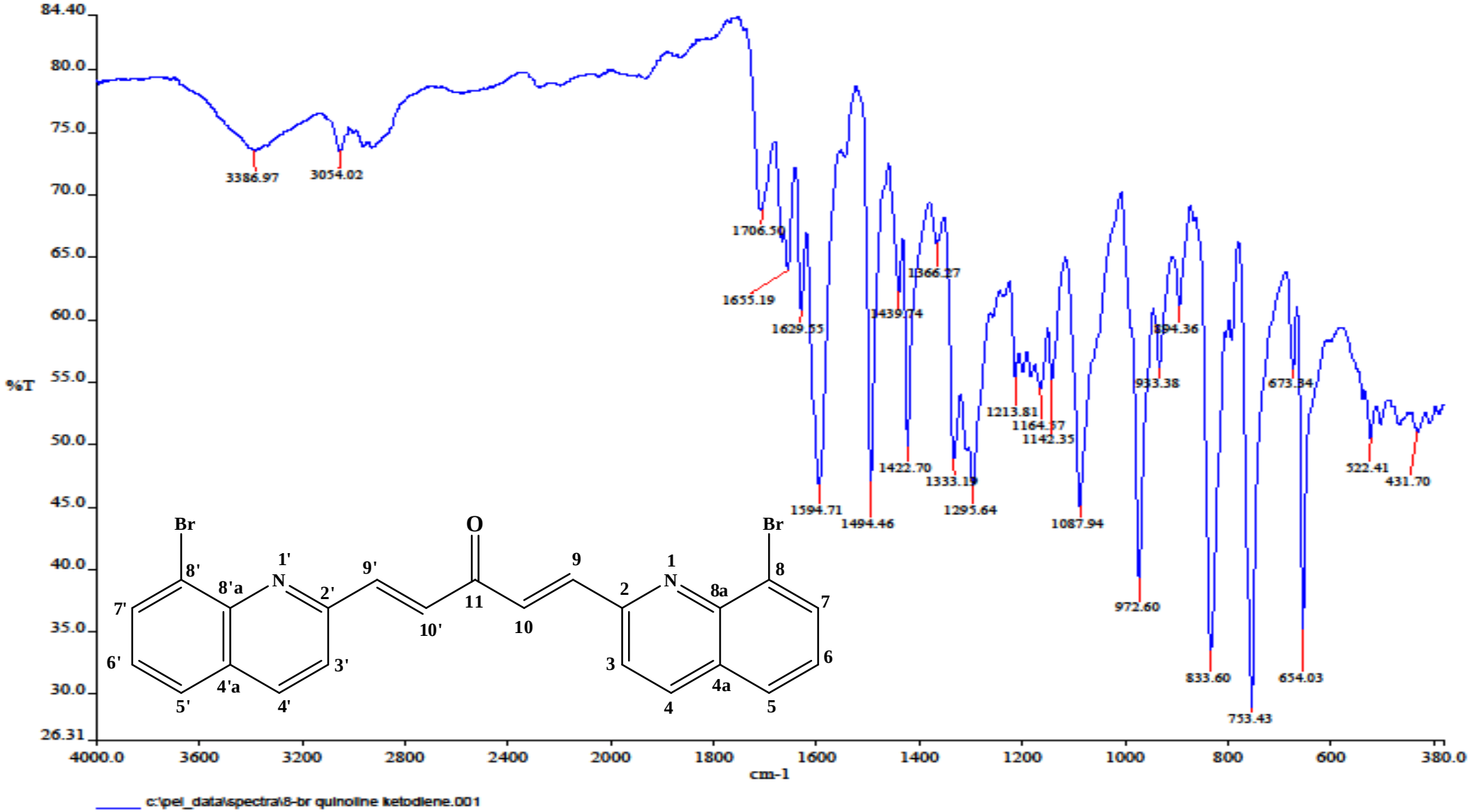
HSQC of Compound C-2h: (1E,4E)-1,5-bis(8-bromoquinolin-2-yl)penta-1,4-dien-3-one



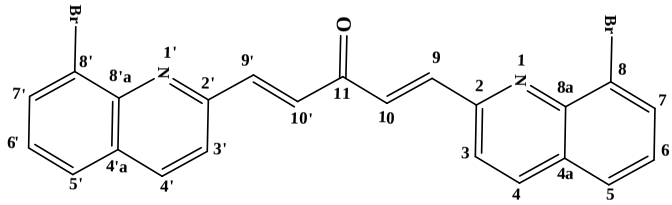
HMBC of Compound C-2h: (1E,4E)-1,5-bis(8-bromoquinolin-2-yl)penta-1,4-dien-3-one



Expanded HMBC of Compound C-2h: (1E,4E)-1,5-bis(8-bromoquinolin-2-yl)penta-1,4-dien-3-one



Infrared Spectrum of Compound C-2h: (1E,4E)-1,5-bis(8-bromoquinolin-2-yl)penta-1,4-dien-3-one

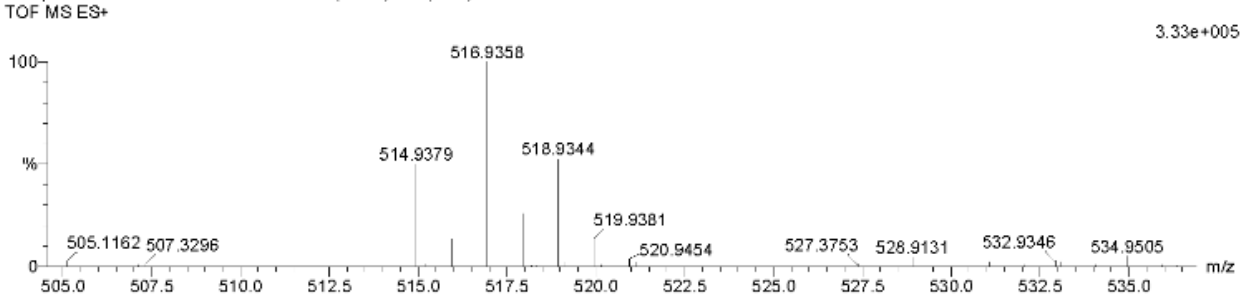


Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

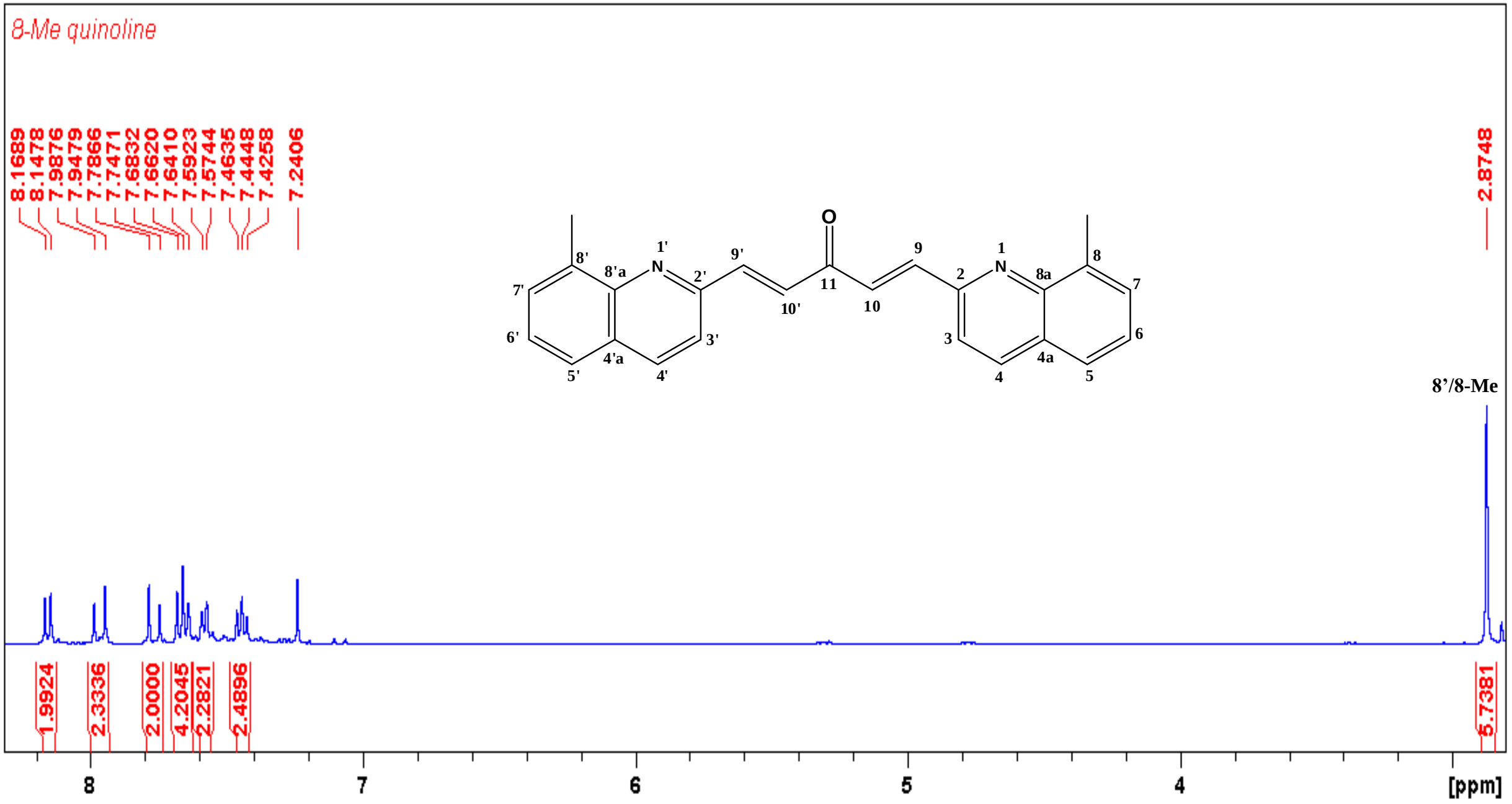
Monoisotopic Mass, Even Electron Ions
66 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 20-25 H: 10-15 N: 0-5 O: 0-5 Na: 1-1 Br: 0-2

Compound 8 8-Br Quinoline ketodiene 17 (0.540) Cm (1:61)
TOF MS ES+

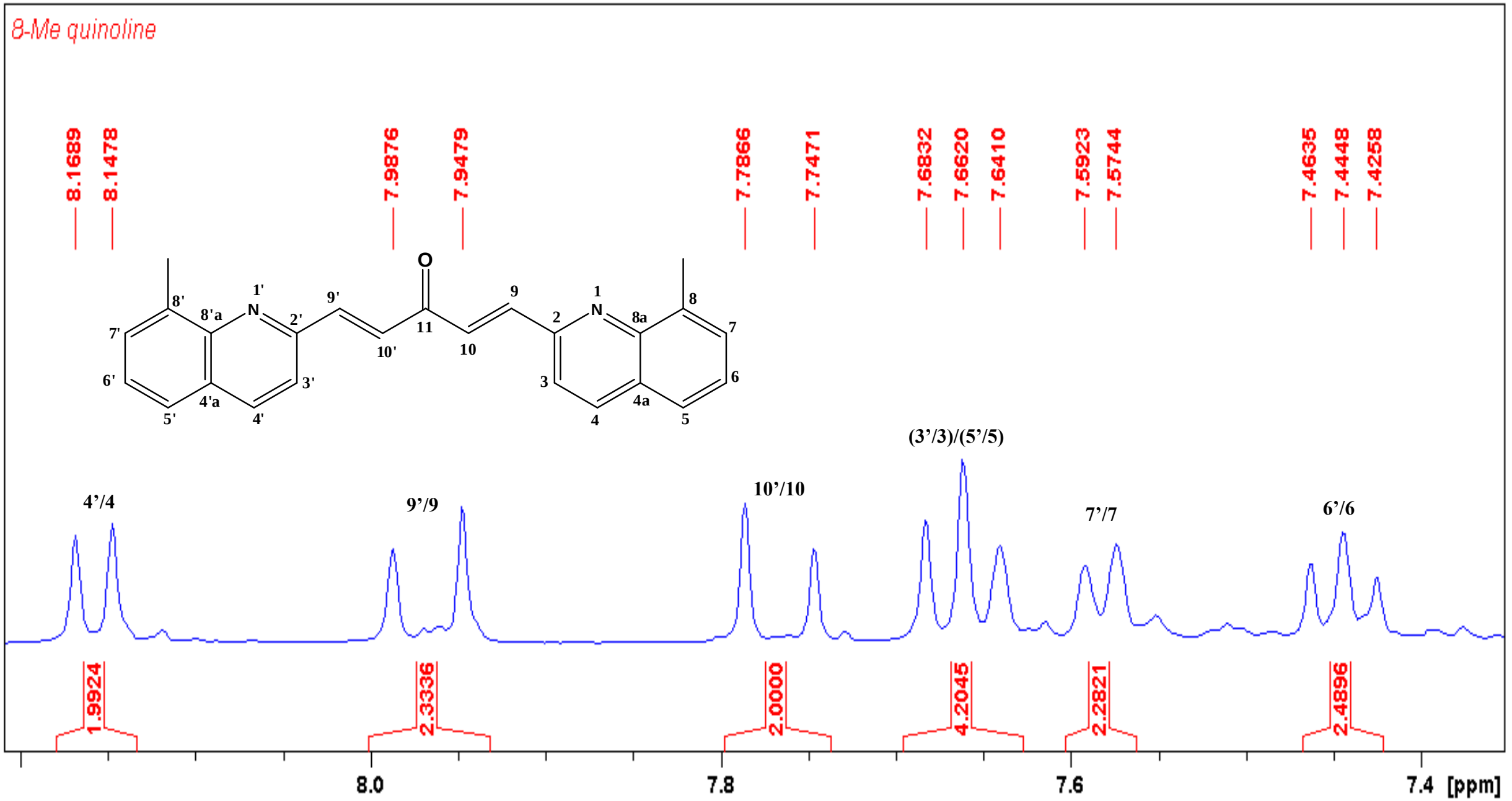


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
514.9379	514.9371	0.8	1.6	16.5	26.8	0.0	C23 H14 N2 O Na Br2

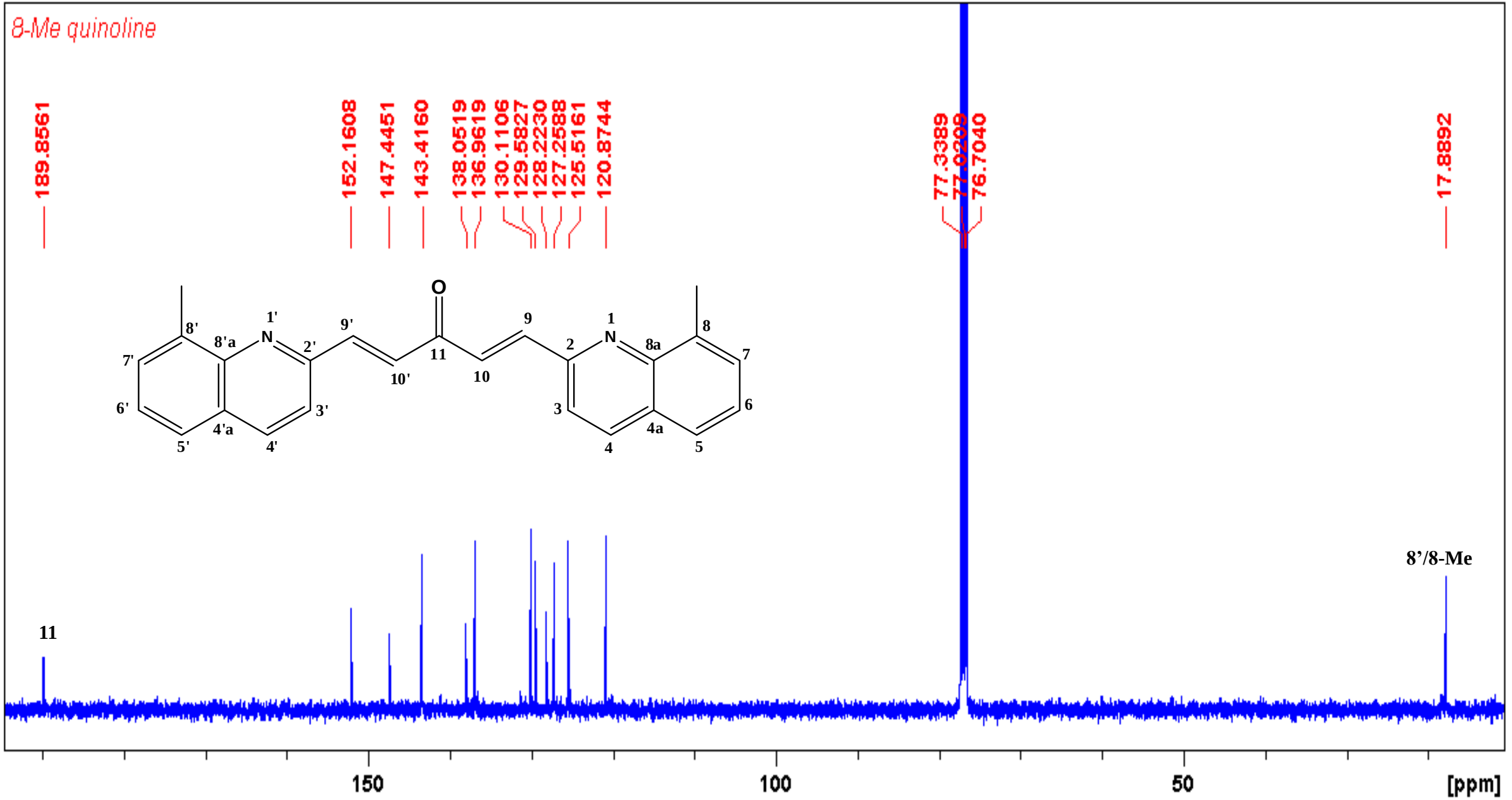
HRMS of Compound C-2h: (1E,4E)-1,5-bis(8-bromoquinolin-2-yl)penta-1,4-dien-3-one



^{1}H Spectrum of Compound C-2i: (1E,4E)-1,5-bis(8-methylquinolin-2-yl)penta-1,4-dien-3-one

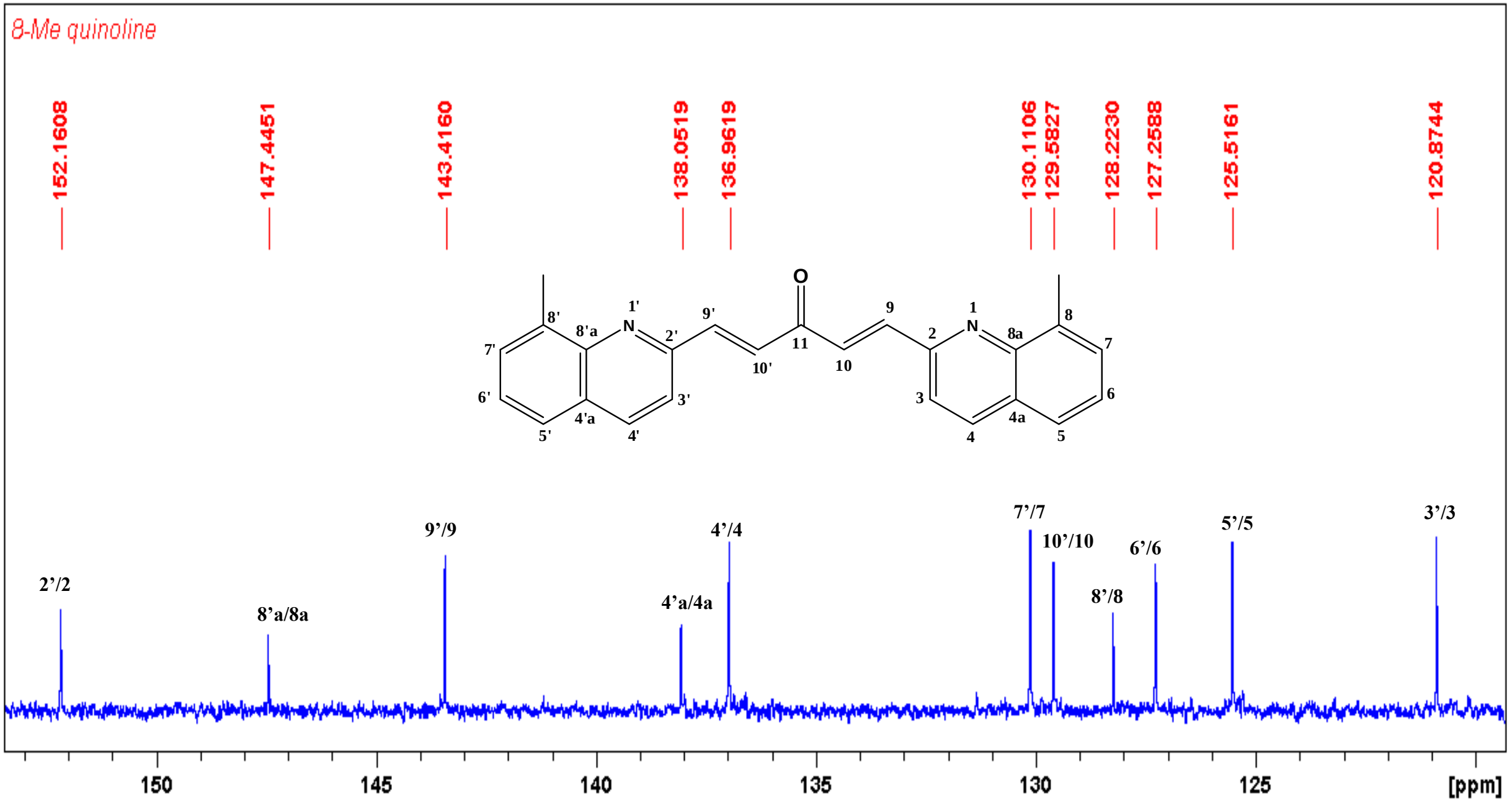


Expanded ^{1}H Spectrum of Compound C-2i: (1E,4E)-1,5-bis(8-methylquinolin-2-yl)penta-1,4-dien-3-one



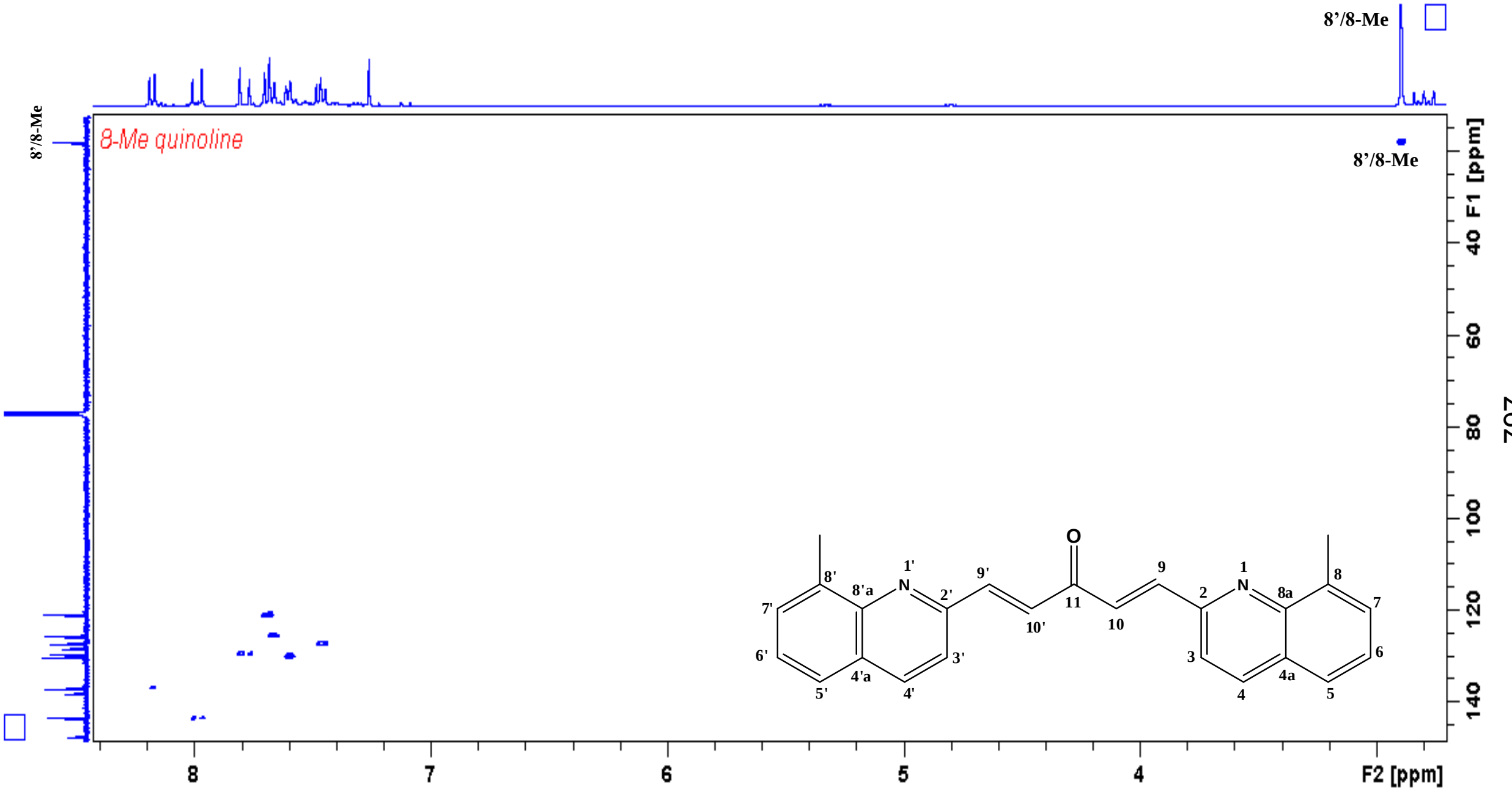
795

^{13}C Spectrum of Compound C-2i: (1E,4E)-1,5-bis(8-methylquinolin-2-yl)penta-1,4-dien-3-one

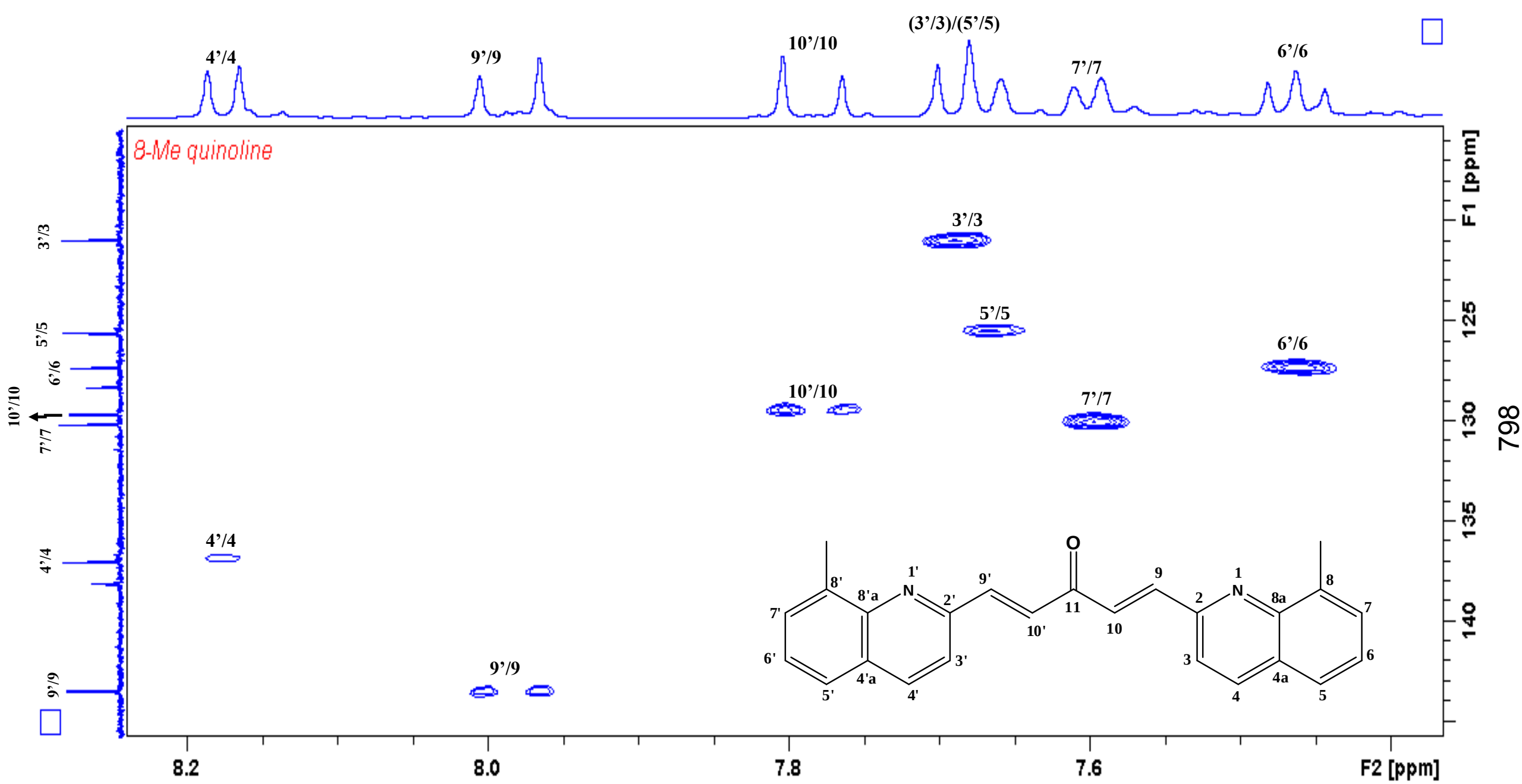


796

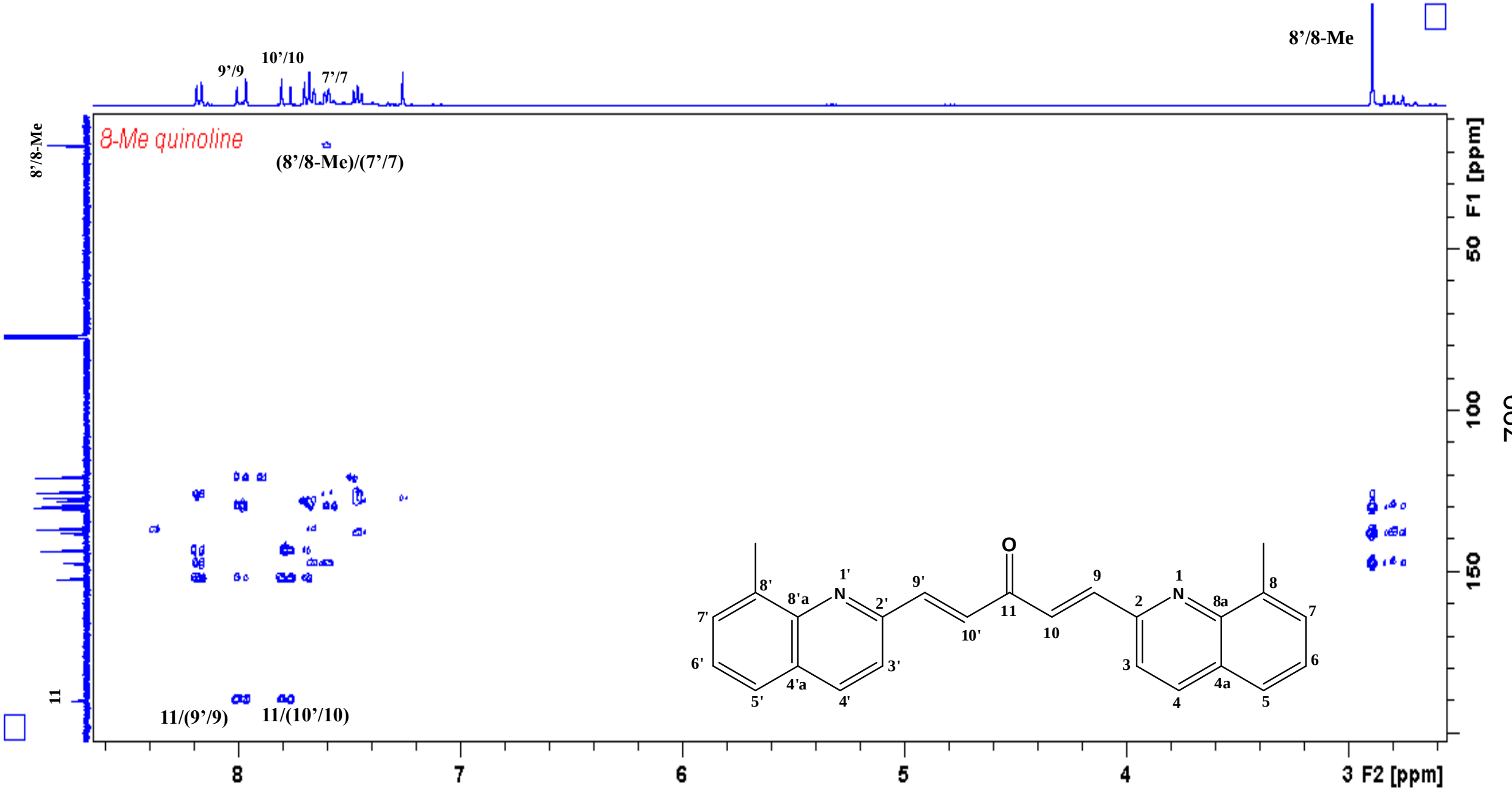
Expanded ^{13}C Spectrum of Compound C-2i: (1E,4E)-1,5-bis(8-methylquinolin-2-yl)penta-1,4-dien-3-one



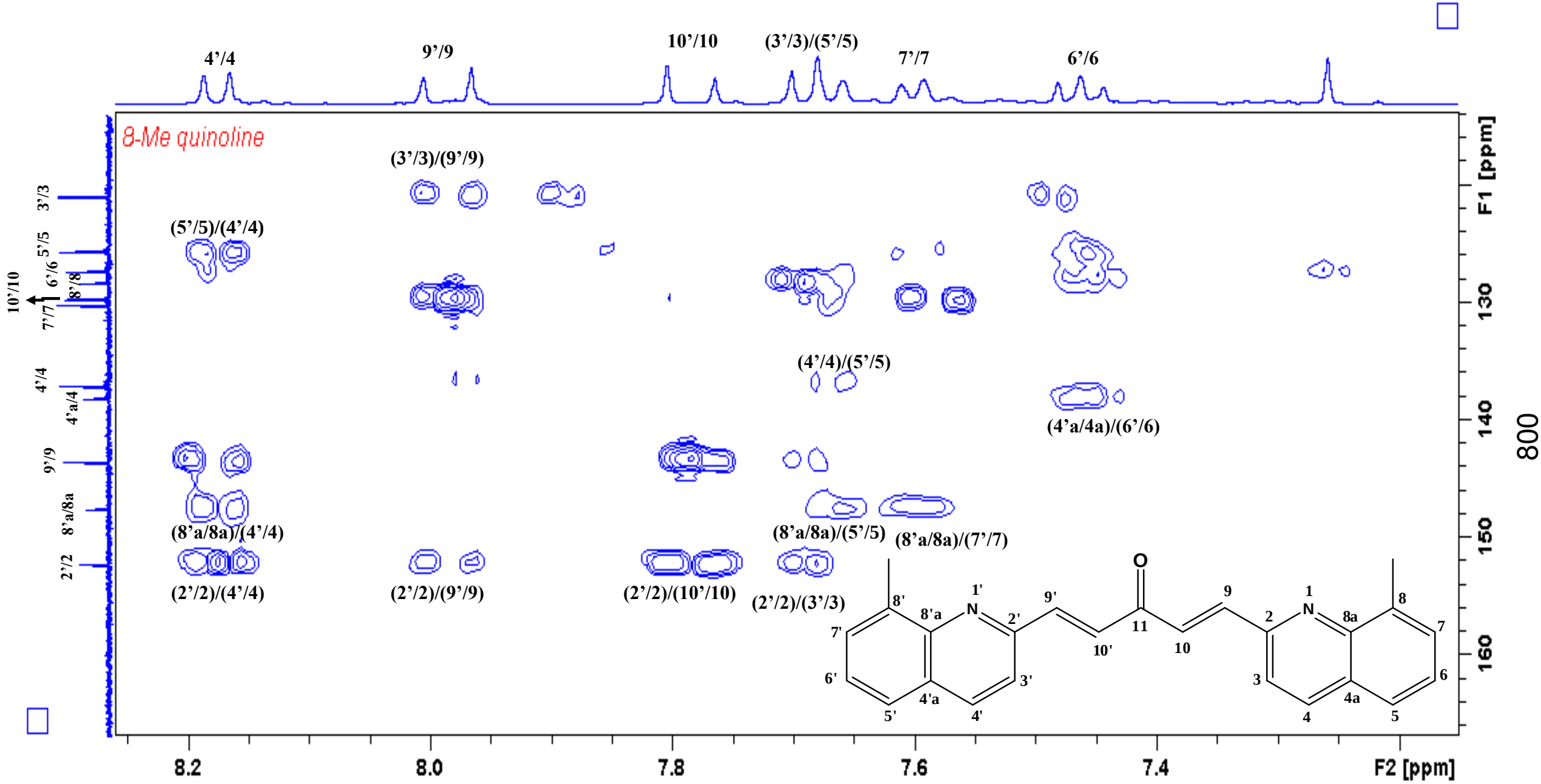
HSQC of Compound C-2i: (1E,4E)-1,5-bis(8-methylquinolin-2-yl)penta-1,4-dien-3-one



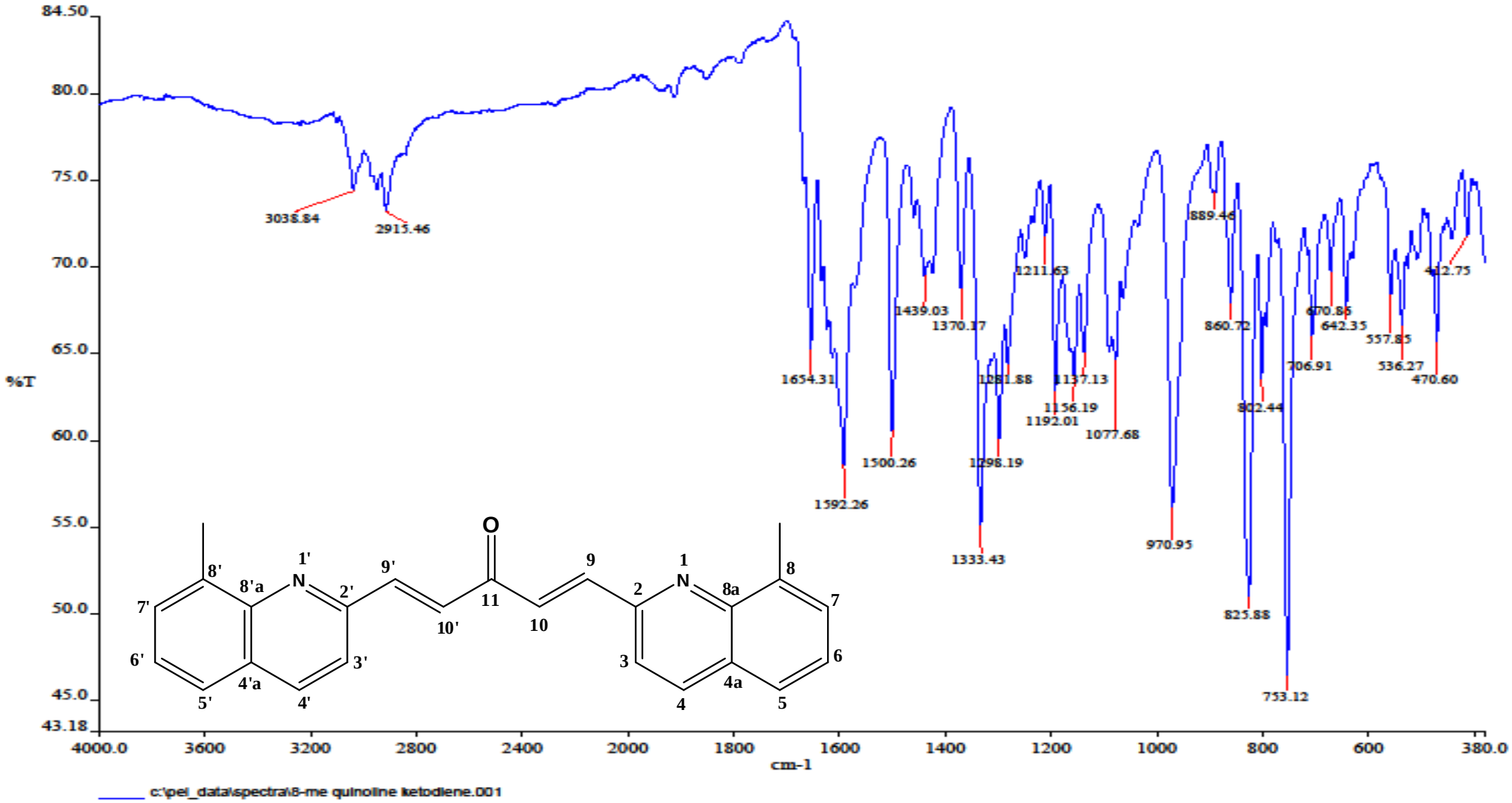
Expanded HSQC of Compound C-2i: (1E,4E)-1,5-bis(8-methylquinolin-2-yl)penta-1,4-dien-3-one



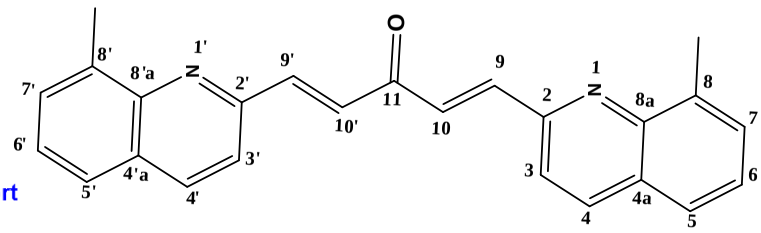
HMBC of Compound C-2i: (1E,4E)-1,5-bis(8-methylquinolin-2-yl)penta-1,4-dien-3-one



Expanded HMBC of Compound C-2i: (1E,4E)-1,5-bis(8-methylquinolin-2-yl)penta-1,4-dien-3-one

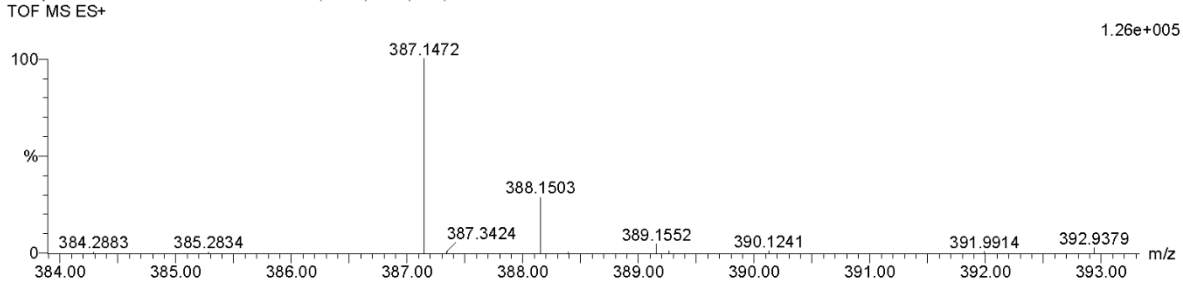


Infrared Spectrum of Compound C-2i: (1E,4E)-1,5-bis(8-methylquinolin-2-yl)penta-1,4-dien-3-one



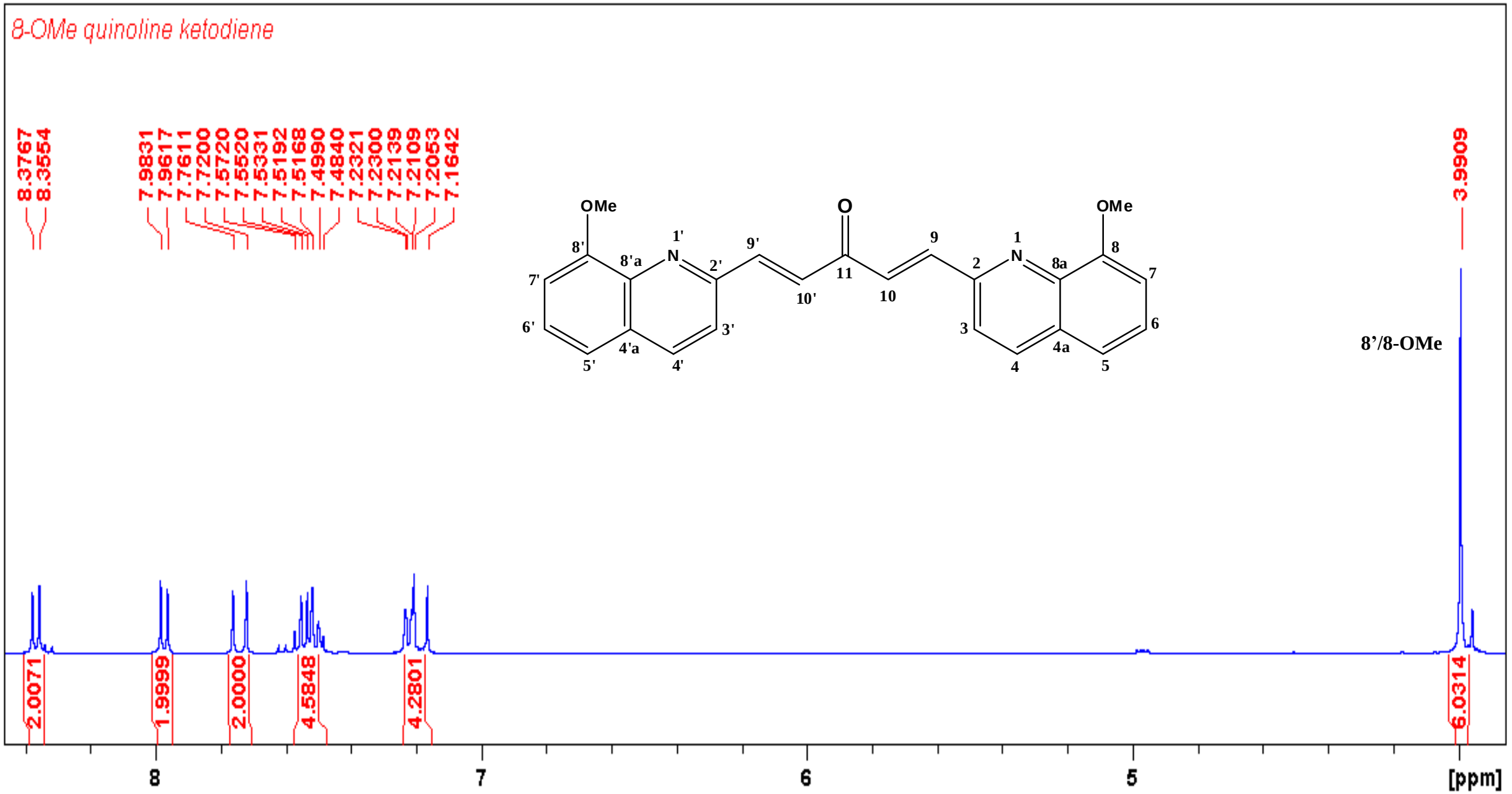
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
17 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 0-5 Na: 1-1
Compound 9 8-Me Quinoline ketodiene 3 (0.068) Cm (1:61)
TOF MS ES+



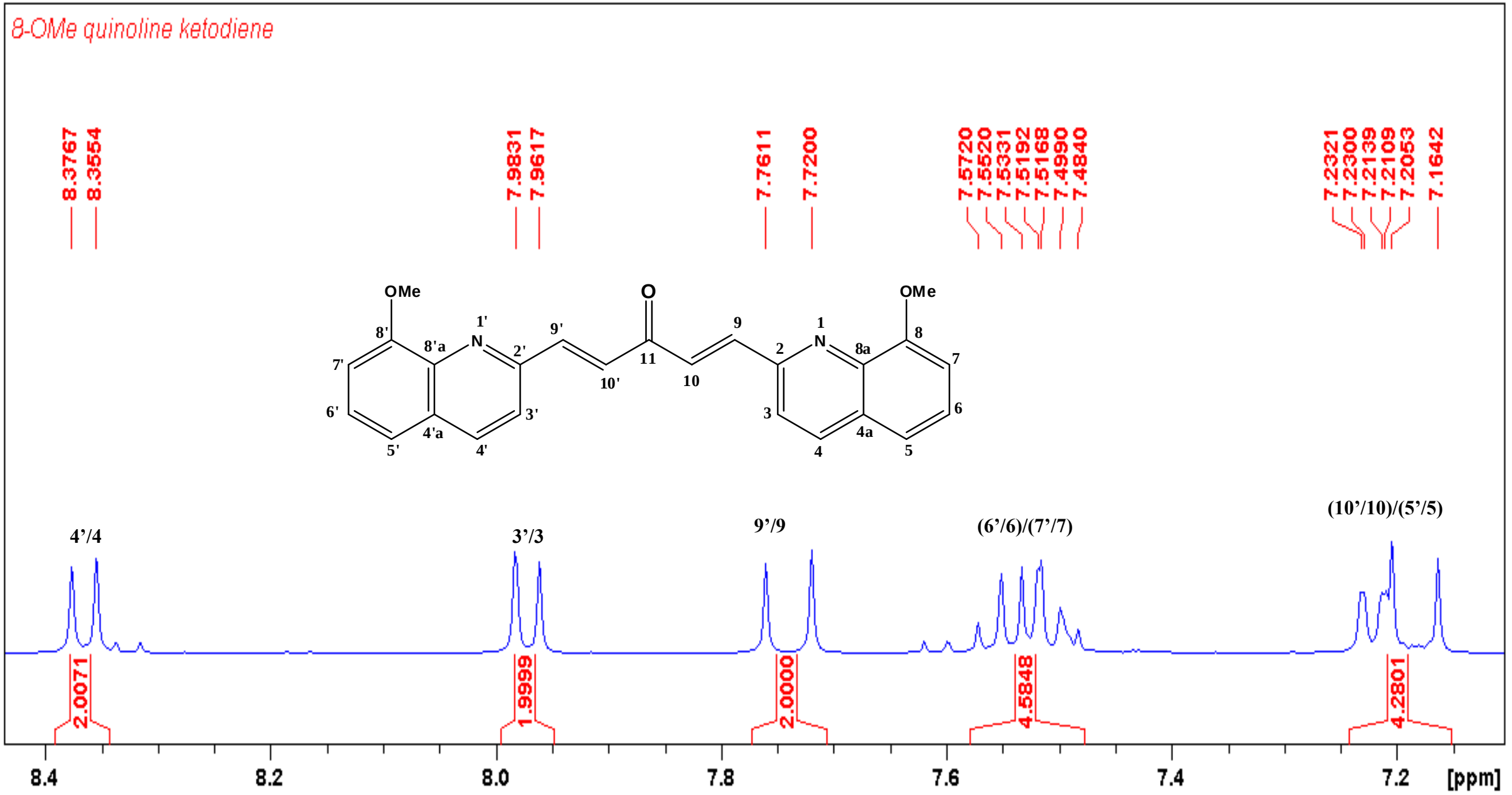
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
387.1472	387.1473	-0.1	-0.3	16.5	32.9	0.0	C25 H20 N2 O Na

Infrared Spectrum of Compound C-2i: (1E,4E)-1,5-bis(8-methylquinolin-2-yl)penta-1,4-dien-3-one



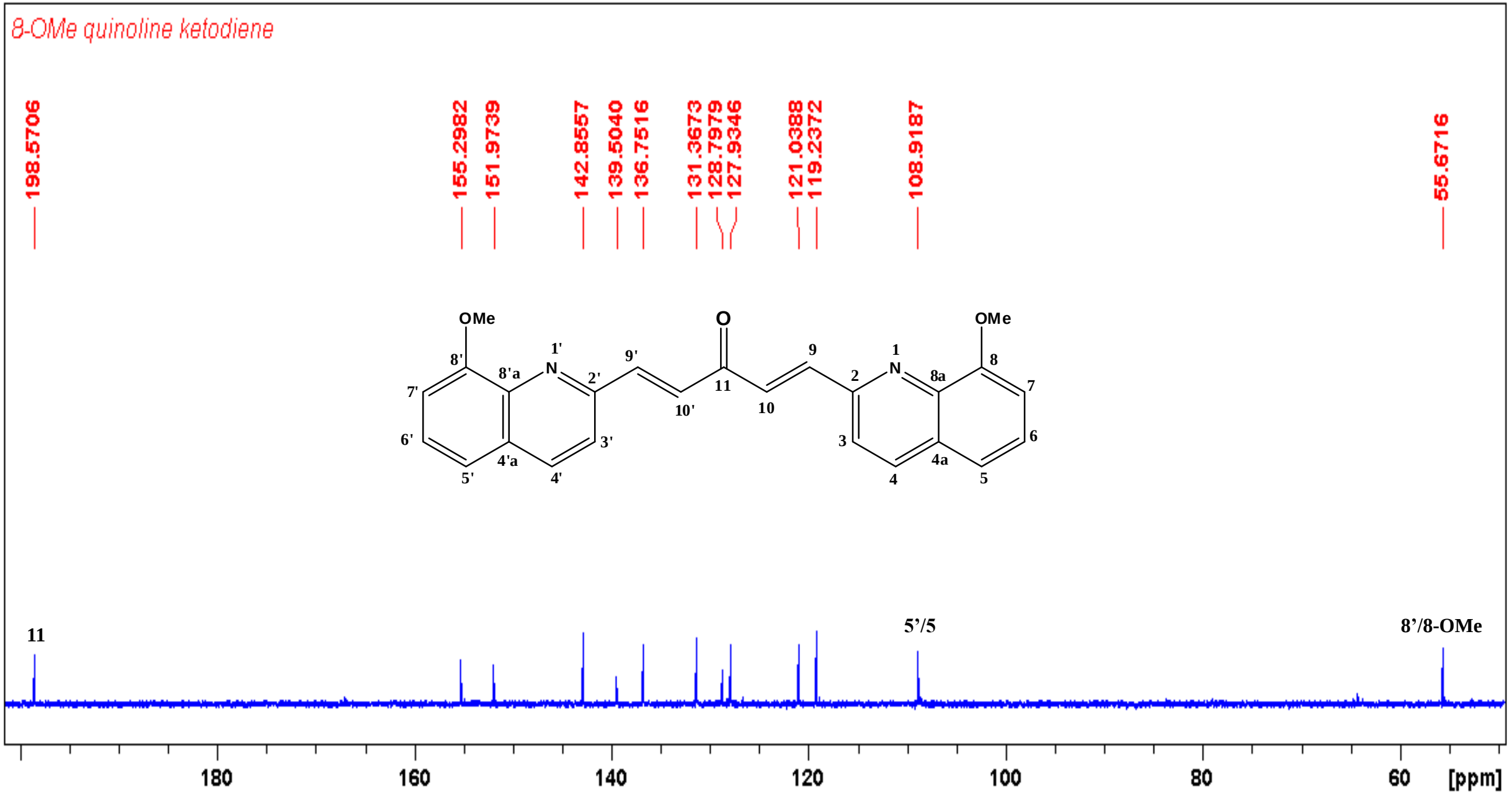
803

[1]H Spectrum of Compound C-2j: (1E,4E)-1,5-bis(8-methoxyquinolin-2-yl)penta-1,4-dien-3-one



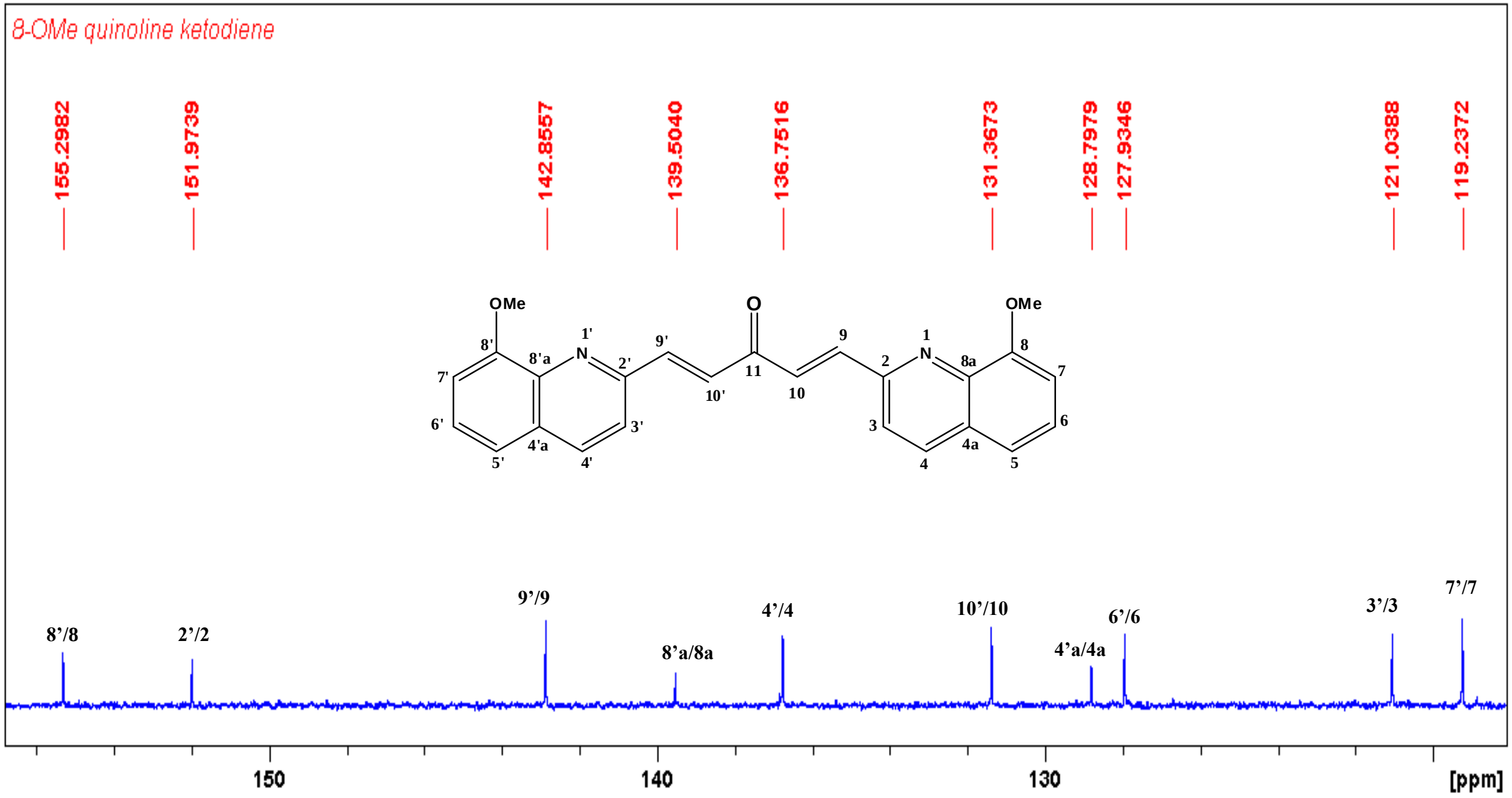
804

Expanded ^{1}H Spectrum of Compound C-2j: (1E,4E)-1,5-bis(8-methoxyquinolin-2-yl)penta-1,4-dien-3-one

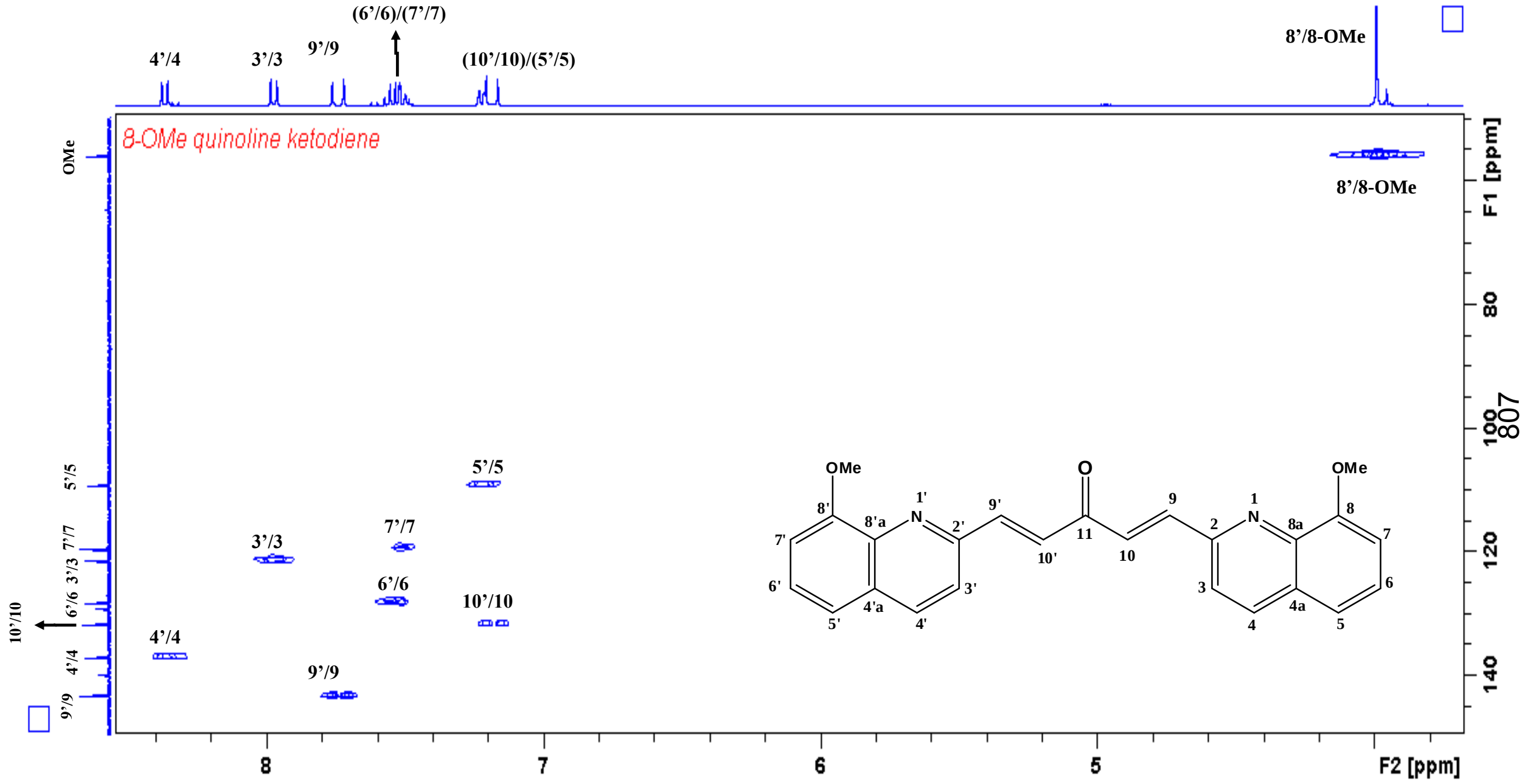


805

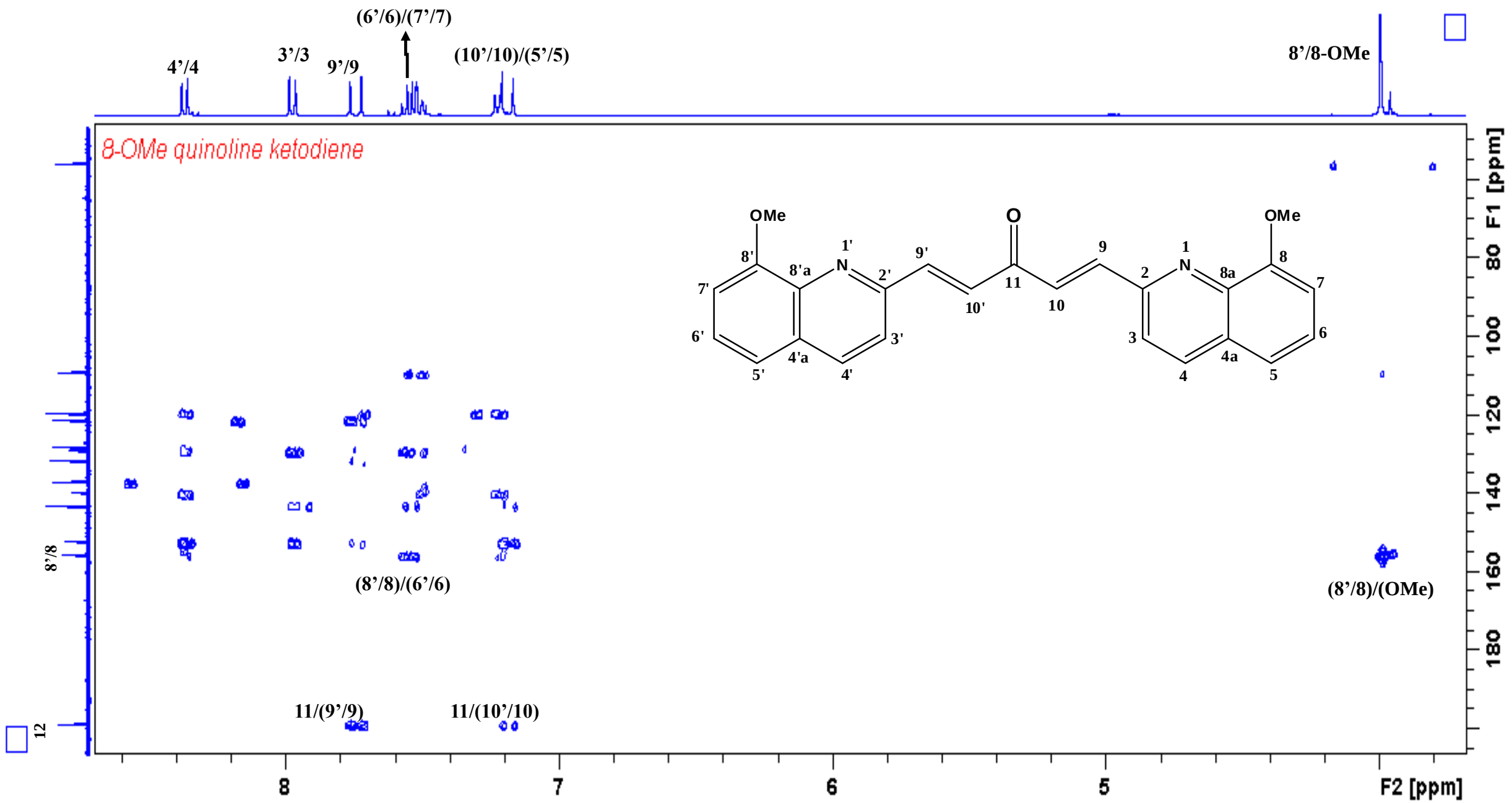
^{13}C Spectrum of Compound C-2j: (1E,4E)-1,5-bis(8-methoxyquinolin-2-yl)penta-1,4-dien-3-one



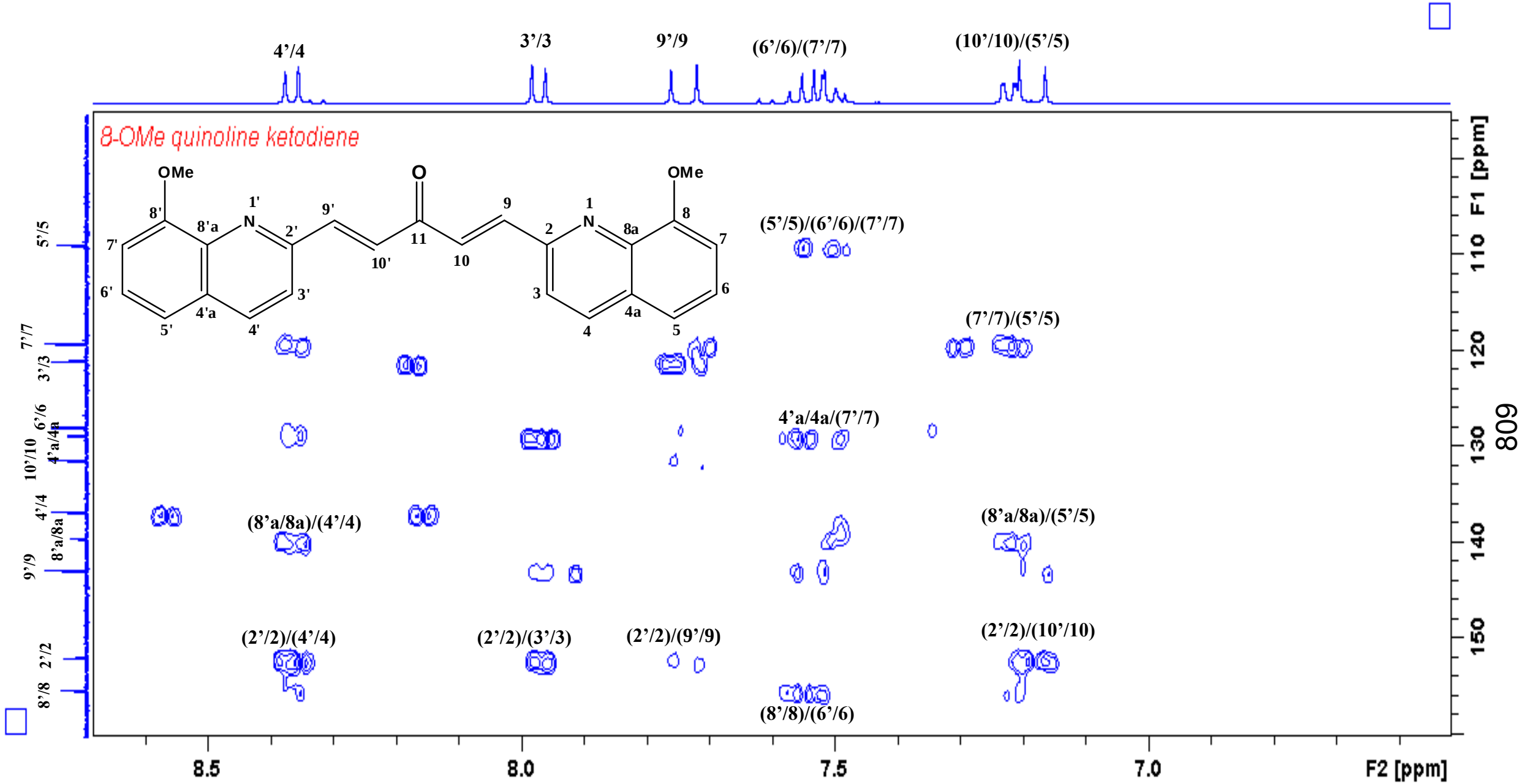
Expanded ^{13}C Spectrum of Compound C-2j: (1E,4E)-1,5-bis(8-methoxyquinolin-2-yl)penta-1,4-dien-3-one



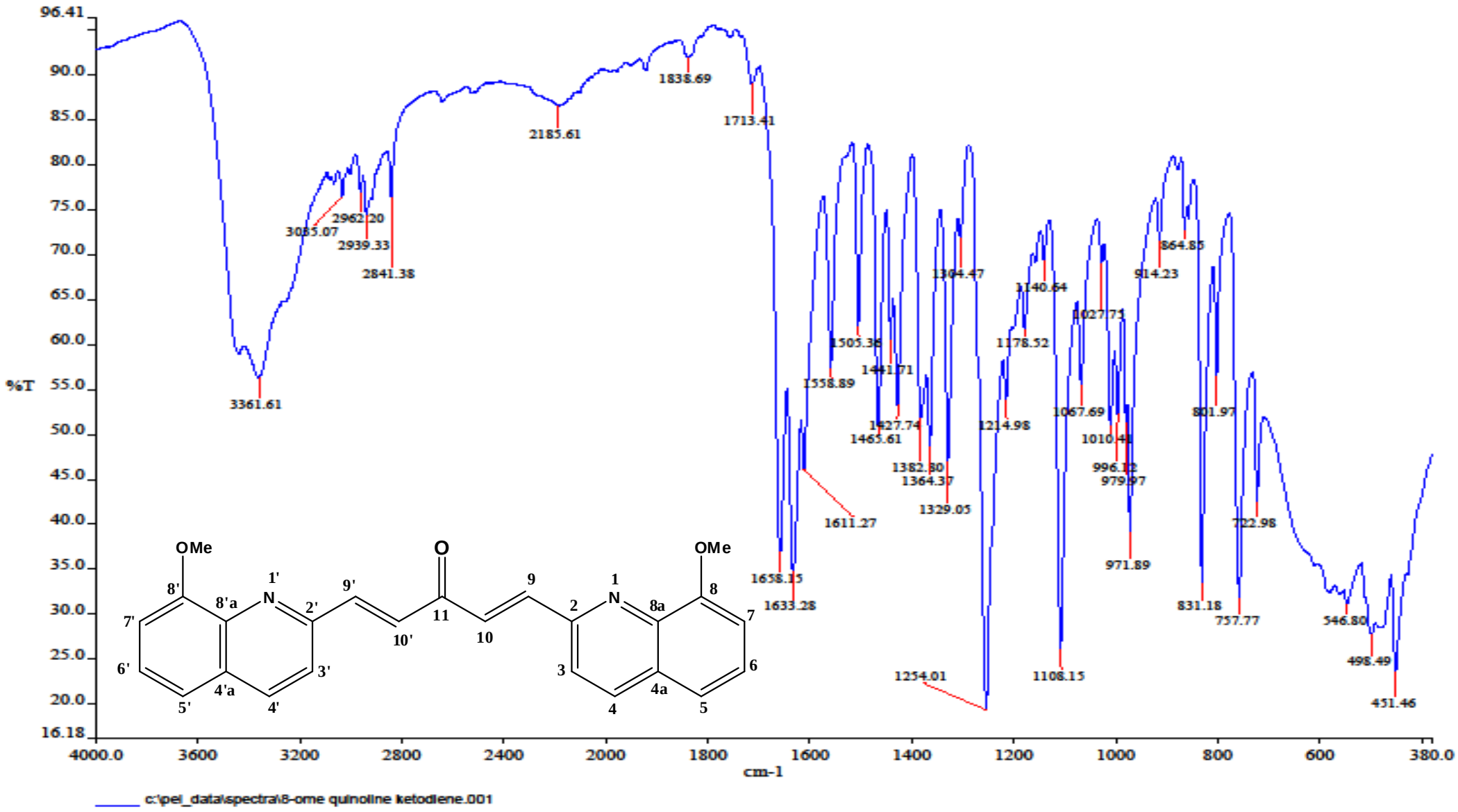
HSQC of Compound C-2j: (1E,4E)-1,5-bis(8-methoxyquinolin-2-yl)penta-1,4-dien-3-one



HMBC of Compound C-2j: (1E,4E)-1,5-bis(8-methoxyquinolin-2-yl)penta-1,4-dien-3-one



Expanded HMBC of Compound C-2j: (1E,4E)-1,5-bis(8-methoxyquinolin-2-yl)penta-1,4-dien-3-one

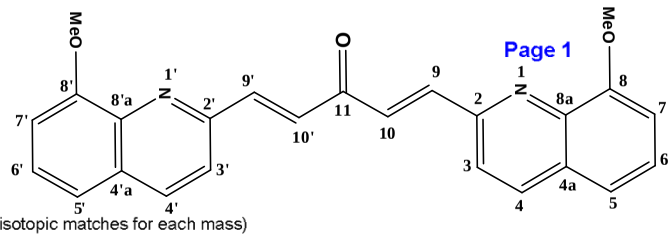


Infrared Spectrum of Compound C-2j: (1E,4E)-1,5-bis(8-methoxyquinolin-2-yl)penta-1,4-dien-3-one

Elemental Composition Report

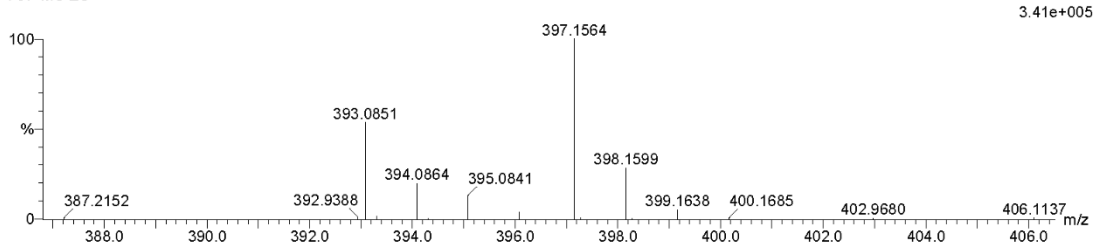
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2



Monoisotopic Mass, Even Electron Ions
22 formula(e) evaluated with 1 results within limits (up to 20 best isotopic matches for each mass)
Elements Used:
C: 20-25 H: 15-25 N: 0-5 O: 0-5

Compound 5 6-OMe Quinoline ketodiene 39 (1.315) Cm (1:60)
TOF MS ES+



Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
397.1564	397.1552	1.2	3.0	16.5	43.4	0.0	C25 H21 N2 O3

HRMS of Compound C-2j: (1E,4E)-1,5-bis(8-methoxyquinolin-2-yl)penta-1,4-dien-3-one

University of KwaZulu-Natal

Synthesis, Characterization and Antibacterial Activity of Curcumin and Curcumin-like derivatives

Appendix B: Crystal structure data

2018

Christina Kannigadu

List of Figures

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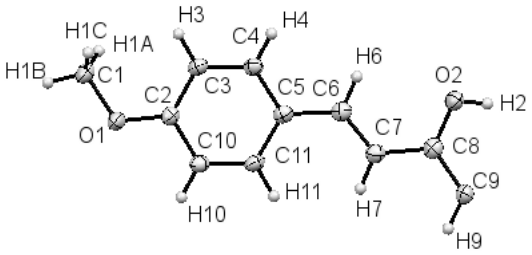

Figure 1: Ortep diagram of the asymmetric unit of 4-methoxycurcumin (**A-4m**)

Table 1: Crystal data and structure refinement for 4-methoxy curcumin (**A-4m**)

Identification code	**15mp_nk_ck1**	
Empirical formula	$C_{42}H_{20}BO_8$	
Formula weight	663.39	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal habit	intense yellow block	
Crystal system	Monoclinic	
Space group	C 1 2/c	
Unit cell dimensions	a = 11.6235(8) Å	α= 90°.
	b = 10.7759(8) Å	β= 99.082(2)°
	c = 13.6493(11) Å	γ = 90°.
Volume	1688.2(2) Å3	
Z	4	
Density (calculated)	2.610 Mg/cm3	
Absorption coefficient	0.181 mm-1	
F(000)	1364	
Theta range for data collection	2.59 to 28.60°	
Index ranges	-14<=h<=15, -11<=k<=14, -18<=l<=18	
Reflections collected	4298	
Independent reflections	1769 [R(int) = 0.0259]	
Absorption correction	multi-scan	
Structure solution program	SHELXS-97 (Sheldrick, 2008)	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	1769 / 0 / 116	
Goodness-of-fit on F^2	1.133	
Final R indices [I>2sigma(I)]	1474 data; I>2σ(I) R1 = 0.0467, wR2 = 0.1397	

	all data R1 = 0.0560, wR2 = 0.1513	
Largest diff. peak and hole	0.402 and -0.414 eÅ-3	
R.M.S. deviation from mean	0.065 eÅ-3	

Table 2: Atomic coordinates and equivalent isotropic displacement parameters ($Å^2$) for 4-methoxy curcumin (**A-4m**)

	X	**Y**	**Z**	**U(EQ)**
C1	0.45247(13)	0.87019(13)	0.92708(11)	0.0265(4)
C2	0.34303(12)	0.86720(12)	0.76320(10)	0.0221(4)
C3	0.35279(12)	0.99537(12)	0.75123(10)	0.0224(3)
C4	0.29735(12)	0.05124(12)	0.66450(10)	0.0232(4)
C5	0.23112(12)	0.98344(12)	0.58904(11)	0.0227(4)
C6	0.17318(12)	0.04787(13)	0.50043(11)	0.0245(4)
C7	0.10976(13)	0.99493(13)	0.42043(11)	0.0261(4)
C8	0.05321(12)	0.06409(14)	0.33439(10)	0.0243(4)
C9	0.0	0.99967(18)	0.25	0.0241(5)
C10	0.27903(13)	0.79709(12)	0.68725(11)	0.0252(4)
C11	0.22307(13)	0.85386(12)	0.60260(11)	0.0243(4)
O1	0.39210(10)	0.80197(9)	0.84483(7)	0.0286(3)
O2	0.05515(9)	0.18445(9)	0.33749(8)	0.0283(3)

Table 3: Bond lengths [Å] for 4-methoxy curcumin (**A-4m**)

C1-O1	**1.4298(17)**	**C1-H1A**	**0.98**
C1-H1B	0.98	C1-H1C	0.98
C2-O1	1.3638(17)	C2-C3	1.3975(18)
C2-C10	1.398(2)	C3-C4	1.392(2)
C3-H3	0.95	C4-C5	1.392(2)
C4-H4	0.95	C5-C11	1.4136(18)
C5-C6	1.463(2)	C6-C7	1.344(2)
C6-H6	0.95	C7-C8	1.457(2)
C9-H9	0.95	C10-C11	1.377(2)
C10-H10	0.95	C11-H11	0.95
O2-H2	0.84		

Symmetry transformations used to generate equivalent atoms: #1 -x, y, -z+1/2

Table 4: Bond angles [°] for 4-methoxy curcumin (**A-4m**)

O1-C1-H1A	**109.5**	**O1-C1-H1B**	**109.5**
H1A-C1-H1B	109.5	O1-C1-H1C	109.5
H1A-C1-H1C	109.5	H1B-C1-H1C	109.5
O1-C2-C3	125.01(13)	O1-C2-C10	115.61(12)
C3-C2-C10	119.39(13)	C4-C3-C2	119.44(13)
C4-C3-H3	120.3	C2-C3-H3	120.3
C5-C4-C3	122.00(12)	C5-C4-H4	119.0
C3-C4-H4	119.0	C4-C5-C11	117.51(13)
C4-C5-C6	119.42(12)	C11-C5-C6	123.06(13)
C7-C6-C5	126.29(13)	C7-C6-H6	116.9
C5-C6-H6	116.9	C6-C7-C8	123.88(14)
C6-C7-H7	118.1	C8-C7-H7	118.1
O2-C8-C9	121.65(13)	O2-C8-C7	118.79(13)
C9-C8-C7	119.55(14)	C8-C9-C8#1	120.65(18)
C8-C9-H9	119.7	C8#1-C9-H9	119.7
C11-C10-C2	120.52(12)	C11-C10-H10	119.7
C2-C10-H10	119.7	C10-C11-C5	121.11(13)
C10-C11-H11	119.4	C5-C11-H11	119.4
C2-O1-C1	117.81(11)	C8-O2-H2	109.5

Symmetry transformations used to generate equivalent atoms: #1 -x, y, -z+1/2

Table 5: Anisotropic displacement parameters ($Å^2$) for 4-methoxy curcumin (**A-4m**)

The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ \eta^2\alpha*^2Y^{11} + ... + 2\ h\ \kappa\ a*\ b*\ U^{12}\ ]$

	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
C1	0.0246(8)	0.0327(7)	0.0209(7)	0.0018(5)	0.0002(7)	0.0035(6)
C2	0.0214(8)	0.0251(7)	0.0202(7)	0.0006(5)	0.0045(7)	0.0021(5)
C3	0.0208(7)	0.0234(7)	0.0228(7)	0.0060(5)	0.0031(6)	0.0024(5)
C4	0.0236(8)	0.0205(6)	0.0255(7)	0.0038(5)	0.0038(7)	0.0004(5)
C5	0.0208(8)	0.0243(7)	0.0234(7)	0.0031(5)	0.0048(7)	0.0000(5)
C6	0.0216(8)	0.0246(7)	0.0279(8)	0.0028(5)	0.0058(7)	0.0007(5)
C7	0.0277(9)	0.0247(7)	0.0260(8)	0.0006(5)	0.0049(7)	0.0015(5)
C8	0.0203(8)	0.0274(7)	0.0257(8)	0.0008(5)	0.0055(7)	0.0007(5)
C9	0.0261(11)	0.0235(9)	0.0224(10)	0	0.0030(9)	0
C10	0.0301(9)	0.0217(6)	0.0243(7)	0.0016(5)	0.0054(7)	0.0065(5)
C11	0.0268(8)	0.0252(7)	0.0205(7)	0.0052(5)	0.0029(7)	0.0064(5)
O1	0.0351(7)	0.0260(5)	0.0223(6)	0.0005(4)	0.0032(6)	0.0054(4)
O2	0.0263(7)	0.0254(6)	0.0308(6)	0.0023(4)	0.0026(6)	0.0006(4)

Table 6: Hydrogen atomic coordinates and isotropic atomic displacement parameters ($Å^2$) for 4-methoxy curcumin (**A-4m**)

	x/a	y/b	z/c	U(eq)
H1A	0.5211	0.9103	0.9076	0.04
H1B	0.4771	0.8135	0.9825	0.04
H1C	0.4006	0.9336	0.9475	0.04
H3	0.3969	1.0440	0.8019	0.027
H4	0.3050	1.1383	0.6566	0.028
H6	0.1812	1.1356	0.4993	0.029
H7	0.1013	0.9072	0.4200	0.031
H9	0.0000	0.9115	0.2500	0.029
H10	0.2741	0.7096	0.6941	0.03
H11	0.1783	0.8051	0.5525	0.029
H2	0.0057	1.2128	0.2914	0.042

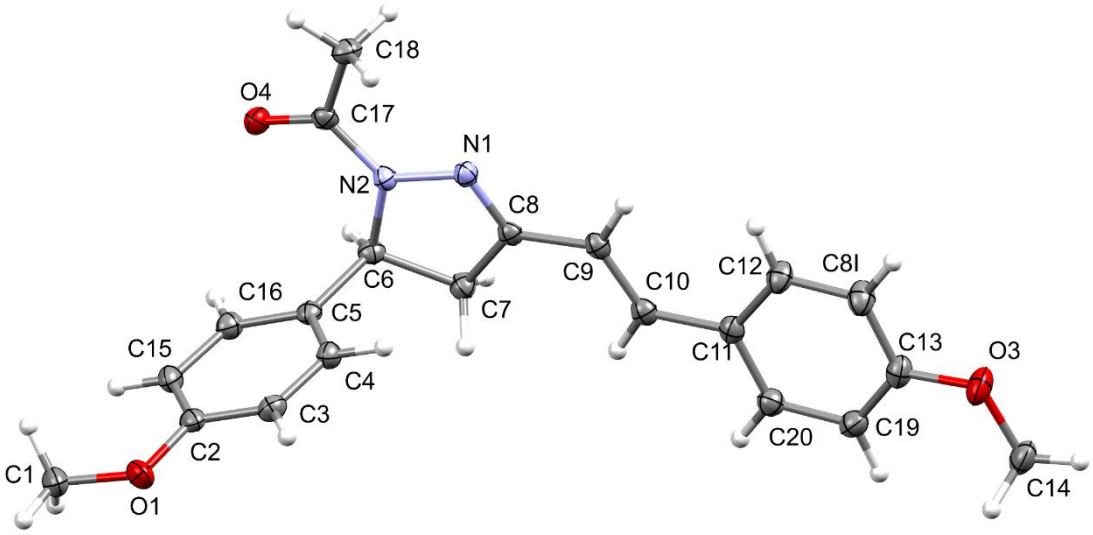

Figure 2: Ortep diagram of 4-methoxy ketopyrazole (**B-2l**)

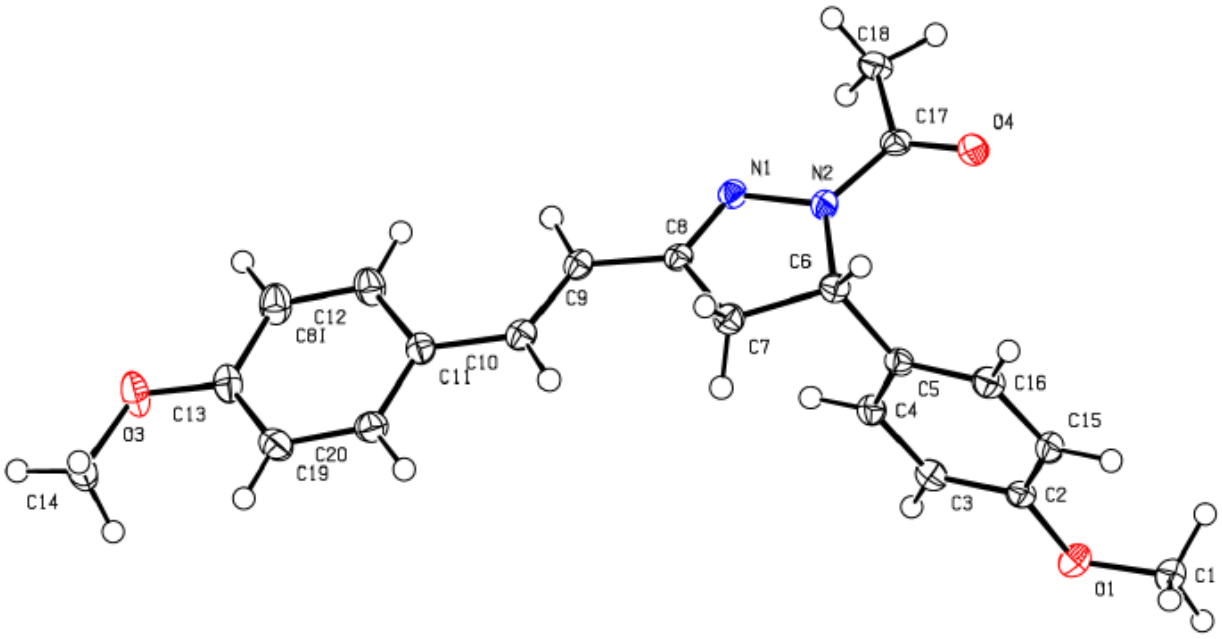

Figure 3: Olex diagram of 4-methoxy ketopyrazole (**B-2l**)

Table 7: Crystal data and structure refinement for 4-methoxy ketopyrazole(**B-2l**)

Identification code	**shelx**	
Empirical formula	C_{21} H_{22} N_2 O_3	
Formula weight	350.40	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 21/n	
Unit cell dimensions	a = 5.6830(10) Å	α= 90°.
	b = 11.936(2) Å	β= 92.464(2)°.
	c = 26.420(4) Å	γ = 90°.
Volume	1790.5(5) $Å^3$	
Z	4	
Density (calculated)	1.300 Mg/m^3	
Absorption coefficient	0.088 mm^{-1}	
F(000)	744	
Crystal size	0.340 x 0.210 x 0.150 mm^3	
Theta range for data collection	1.872 to 27.425°.	
Index ranges	-7<=h<=7, -15<=k<=15, -34<=l<=32	
Reflections collected	14402	
Independent reflections	4045 [R(int) = 0.0273]	
Completeness to theta = 25.242°	99.7 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.996 and 0.965	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4045 / 0 / 238	
Goodness-of-fit on F^2	1.024	
Final R indices [I>2sigma(I)]	R1 = 0.0407, wR2 = 0.1046	
R indices (all data)	R1 = 0.0580, wR2 = 0.1146	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.300 and -0.235 $e.Å^{-3}$	

Table 8: Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for 4-methoxy ketopyrazole (**B-2l**). U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor

	x	y	z	U(eq)
C(1)	8946(3)	674(1)	578(1)	28(1)
C(2)	7590(2)	1470(1)	1344(1)	20(1)
C(3)	5824(2)	1511(1)	1692(1)	21(1)
C(4)	5959(2)	2283(1)	2082(1)	19(1)
C(5)	7845(2)	3034(1)	2135(1)	16(1)
C(6)	7899(2)	3906(1)	2552(1)	17(1)
C(7)	7336(2)	3455(1)	3080(1)	19(1)
C(8)	4956(2)	3956(1)	3169(1)	15(1)
C(8I)	-634(3)	2768(1)	4895(1)	26(1)
C(9)	3569(2)	3711(1)	3601(1)	17(1)
C(10)	4132(2)	2910(1)	3943(1)	18(1)
C(11)	2762(2)	2592(1)	4375(1)	18(1)
C(12)	635(2)	3120(1)	4492(1)	23(1)
C(13)	173(2)	1880(1)	5203(1)	22(1)
C(14)	-417(3)	745(1)	5931(1)	29(1)
C(15)	9494(2)	2206(1)	1389(1)	21(1)
C(16)	9590(2)	2976(1)	1785(1)	20(1)
C(17)	5824(2)	5472(1)	2077(1)	17(1)
C(18)	3625(2)	6173(1)	2021(1)	21(1)
C(19)	2292(3)	1359(1)	5103(1)	23(1)
C(20)	3544(2)	1715(1)	4691(1)	21(1)
N(1)	4210(2)	4654(1)	2823(1)	17(1)
N(2)	5919(2)	4714(1)	2463(1)	16(1)
O(1)	7292(2)	670(1)	971(1)	26(1)
O(3)	-1252(2)	1598(1)	5585(1)	30(1)
O(4)	7490(2)	5558(1)	1796(1)	21(1)

Table 9: Bond lengths [Å] and angles [°] for 4-methoxy ketopyrazole (**B-2l**)

C(1)-O(1)	1.4303(17)
C(1)-H(1A)	0.9800
C(1)-H(1B)	0.9800
C(1)-H(1C)	0.9800
C(2)-O(1)	1.3773(16)
C(2)-C(3)	1.3914(19)
C(2)-C(15)	1.3945(19)
C(3)-C(4)	1.3812(19)
C(3)-H(3)	0.9500
C(4)-C(5)	1.3997(18)
C(4)-H(4)	0.9500
C(5)-C(16)	1.3864(19)
C(5)-C(6)	1.5132(18)
C(6)-N(2)	1.4930(16)
C(6)-C(7)	1.5418(18)
C(6)-H(6)	1.0000
C(7)-C(8)	1.5071(18)
C(7)-H(7A)	0.9900
C(7)-H(7B)	0.9900
C(8)-N(1)	1.2946(17)
C(8)-C(9)	1.4440(18)
C(8I)-C(12)	1.377(2)
C(8I)-C(13)	1.401(2)
C(8I)-H(8I)	0.9500
C(9)-C(10)	1.3448(18)
C(9)-H(9)	0.9500
C(10)-C(11)	1.4597(18)
C(10)-H(10)	0.9500
C(11)-C(20)	1.3980(19)
C(11)-C(12)	1.4095(19)
C(12)-H(12)	0.9500
C(13)-O(3)	1.3631(17)
C(13)-C(19)	1.391(2)
C(14)-O(3)	1.4350(18)
C(14)-H(14A)	0.9800
C(14)-H(14B)	0.9800
C(14)-H(14C)	0.9800
C(15)-C(16)	1.3936(19)
C(15)-H(15)	0.9500
C(16)-H(16)	0.9500

C(17)-O(4)	1.2326(16)
C(17)-N(2)	1.3620(17)
C(17)-C(18)	1.5059(18)
C(18)-H(18A)	0.9800
C(18)-H(18B)	0.9800
C(18)-H(18C)	0.9800
C(19)-C(20)	1.393(2)
C(19)-H(19)	0.9500
C(20)-H(20)	0.9500
N(1)-N(2)	1.3910(15)
O(1)-C(1)-H(1A)	109.5
O(1)-C(1)-H(1B)	109.5
H(1A)-C(1)-H(1B)	109.5
O(1)-C(1)-H(1C)	109.5
H(1A)-C(1)-H(1C)	109.5
H(1B)-C(1)-H(1C)	109.5
O(1)-C(2)-C(3)	115.25(12)
O(1)-C(2)-C(15)	124.66(13)
C(3)-C(2)-C(15)	120.09(12)
C(4)-C(3)-C(2)	119.79(13)
C(4)-C(3)-H(3)	120.1
C(2)-C(3)-H(3)	120.1
C(3)-C(4)-C(5)	121.40(13)
C(3)-C(4)-H(4)	119.3
C(5)-C(4)-H(4)	119.3
C(16)-C(5)-C(4)	117.85(12)
C(16)-C(5)-C(6)	121.74(12)
C(4)-C(5)-C(6)	120.36(12)
N(2)-C(6)-C(5)	109.79(10)
N(2)-C(6)-C(7)	100.59(10)
C(5)-C(6)-C(7)	114.76(11)
N(2)-C(6)-H(6)	110.4
C(5)-C(6)-H(6)	110.4
C(7)-C(6)-H(6)	110.4
C(8)-C(7)-C(6)	103.06(10)
C(8)-C(7)-H(7A)	111.2
C(6)-C(7)-H(7A)	111.2
C(8)-C(7)-H(7B)	111.2
C(6)-C(7)-H(7B)	111.2

H(7A)-C(7)-H(7B)	109.1
N(1)-C(8)-C(9)	121.00(12)
N(1)-C(8)-C(7)	114.40(11)
C(9)-C(8)-C(7)	124.60(11)
C(12)-C(8I)-C(13)	120.66(13)
C(12)-C(8I)-H(8I)	119.7
C(13)-C(8I)-H(8I)	119.7
C(10)-C(9)-C(8)	123.56(12)
H(7A)-C(7)-H(7B)	109.1
N(1)-C(8)-C(9)	121.00(12)
N(1)-C(8)-C(7)	114.40(11)
C(9)-C(8)-C(7)	124.60(11)
C(12)-C(8I)-C(13)	120.66(13)
C(12)-C(8I)-H(8I)	119.7
C(13)-C(8I)-H(8I)	119.7
C(10)-C(9)-C(8)	123.56(12)
C(10)-C(9)-H(9)	118.2
C(8)-C(9)-H(9)	118.2
C(9)-C(10)-C(11)	126.25(13)
C(9)-C(10)-H(10)	116.9
C(11)-C(10)-H(10)	116.9
C(20)-C(11)-C(12)	117.26(12)
C(20)-C(11)-C(10)	119.63(12)
C(12)-C(11)-C(10)	123.11(12)
C(8I)-C(12)-C(11)	121.03(13)
C(8I)-C(12)-H(12)	119.5
C(11)-C(12)-H(12)	119.5
O(3)-C(13)-C(19)	125.24(13)
O(3)-C(13)-C(8I)	115.22(13)
C(19)-C(13)-C(8I)	119.54(13)
O(3)-C(14)-H(14A)	109.5
O(3)-C(14)-H(14B)	109.5
H(14A)-C(14)-H(14B)	109.5
O(3)-C(14)-H(14C)	109.5
H(14A)-C(14)-H(14C)	109.5
H(14B)-C(14)-H(14C)	109.5
C(16)-C(15)-C(2)	118.99(13)
C(16)-C(15)-H(15)	120.5
C(2)-C(15)-H(15)	120.5
C(5)-C(16)-C(15)	121.87(13)

C(5)-C(16)-H(16)	119.1
C(15)-C(16)-H(16)	119.1
O(4)-C(17)-N(2)	119.96(12)
O(4)-C(17)-C(18)	123.36(12)
N(2)-C(17)-C(18)	116.68(12)
C(17)-C(18)-H(18A)	109.5
C(17)-C(18)-H(18B)	109.5
H(18A)-C(18)-H(18B)	109.5
C(17)-C(18)-H(18C)	109.5
H(18A)-C(18)-H(18C)	109.5
H(18B)-C(18)-H(18C)	109.5
C(13)-C(19)-C(20)	119.16(13)
C(13)-C(19)-H(19)	120.4
C(20)-C(19)-H(19)	120.4
C(19)-C(20)-C(11)	122.33(13)
C(19)-C(20)-H(20)	118.8
C(11)-C(20)-H(20)	118.8
C(8)-N(1)-N(2)	107.46(10)
C(17)-N(2)-N(1)	122.71(11)
C(17)-N(2)-C(6)	123.52(11)
N(1)-N(2)-C(6)	113.72(10)
C(2)-O(1)-C(1)	116.87(11)
C(13)-O(3)-C(14)	117.13(12)

Symmetry transformations used to generate equivalent atoms:

Table 10: Anisotropic displacement parameters ($Å^2 x\ 10^3$) for 4-methoxy ketopyrazole (**B-2l**).

The anisotropic displacement factor exponent takes the form: -

$$2\pi^2[\ \eta^2 \alpha^{*2} Y^{11} + ... + 2\ \eta\ \kappa\ \alpha^*\ \beta^*\ Y^{12}\ ]$$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(1)	43(1)	24(1)	19(1)	0(1)	8(1)	7(1)
C(2)	27(1)	16(1)	17(1)	2(1)	1(1)	5(1)
C(3)	22(1)	18(1)	22(1)	2(1)	1(1)	-2(1)
C(4)	19(1)	19(1)	20(1)	2(1)	4(1)	1(1)
C(5)	16(1)	16(1)	16(1)	3(1)	0(1)	3(1)
C(6)	15(1)	17(1)	19(1)	1(1)	-1(1)	1(1)
C(7)	19(1)	21(1)	17(1)	0(1)	0(1)	2(1)
C(8)	17(1)	14(1)	16(1)	-3(1)	-1(1)	-2(1)
C(8I)	20(1)	35(1)	22(1)	4(1)	2(1)	4(1)
C(9)	16(1)	18(1)	17(1)	-4(1)	0(1)	-2(1)
C(10)	18(1)	19(1)	18(1)	-3(1)	-1(1)	-2(1)
C(11)	20(1)	19(1)	16(1)	-2(1)	-1(1)	-5(1)
C(12)	21(1)	29(1)	19(1)	4(1)	-1(1)	1(1)
C(13)	22(1)	27(1)	17(1)	0(1)	1(1)	-6(1)
C(14)	39(1)	30(1)	19(1)	5(1)	4(1)	-6(1)
C(15)	22(1)	22(1)	20(1)	2(1)	7(1)	4(1)
C(16)	17(1)	19(1)	23(1)	2(1)	2(1)	0(1)
C(17)	21(1)	13(1)	18(1)	-3(1)	-1(1)	-3(1)
C(18)	22(1)	17(1)	25(1)	3(1)	1(1)	2(1)
C(19)	27(1)	20(1)	21(1)	2(1)	-2(1)	-2(1)
C(20)	21(1)	20(1)	22(1)	-1(1)	2(1)	0(1)
N(1)	17(1)	16(1)	17(1)	-1(1)	3(1)	-2(1)
N(2)	16(1)	17(1)	16(1)	0(1)	3(1)	1(1)
O(1)	37(1)	21(1)	20(1)	-5(1)	5(1)	1(1)
O(3)	29(1)	41(1)	21(1)	10(1)	6(1)	-1(1)
O(4)	22(1)	20(1)	22(1)	1(1)	5(1)	-2(1)

Table 11: Hydrogen coordinates (x 10^4) and isotropic displacement parameters (Å^{2}x 10^3) for 4-methoxy ketopyrazole (**B-2l**)

.	x	y	z	U(eq)
H(1A)	8926	1409	412	43
H(1B)	8515	94	329	43
H(1C)	10528	523	724	43
H(3)	4529	1008	1662	25
H(4)	4746	2306	2318	23
H(6)	9442	4309	2566	20
H(7A)	7268	2626	3080	23
H(7B)	8526	3706	3340	23
H(8I)	-2071	3132	4966	31
H(9)	2183	4138	3646	20
H(10)	5558	2513	3899	22
H(12)	68	3729	4290	27
H(14A)	-170	48	5744	44
H(14B)	-1585	619	6187	44
H(14C)	1073	985	6097	44
H(15)	10707	2182	1153	26
H(16)	10889	3476	1817	24
H(18A)	3612	6726	2294	32
H(18B)	2238	5688	2037	32
H(18C)	3592	6560	1693	32
H(19)	2879	767	5313	27
H(20)	4980	1350	4621	25

Table 12: Torsion angles [°] for 4-methoxy ketopyrazole (**B-2l**)

O(1)-C(2)-C(3)-C(4)	179.90(11)
C(15)-C(2)-C(3)-C(4)	0.2(2)
C(2)-C(3)-C(4)-C(5)	0.0(2)
C(3)-C(4)-C(5)-C(16)	-0.23(19)
C(3)-C(4)-C(5)-C(6)	177.33(12)
C(16)-C(5)-C(6)-N(2)	112.40(13)
C(4)-C(5)-C(6)-N(2)	-65.07(15)
C(16)-C(5)-C(6)-C(7)	-135.19(13)
C(4)-C(5)-C(6)-C(7)	47.33(16)
N(2)-C(6)-C(7)-C(8)	7.74(12)
C(5)-C(6)-C(7)-C(8)	-110.01(12)
C(6)-C(7)-C(8)-N(1)	-5.54(14)

C(6)-C(7)-C(8)-C(9)	174.85(11)
N(1)-C(8)-C(9)-C(10)	172.70(12)
C(7)-C(8)-C(9)-C(10)	-7.7(2)
C(8)-C(9)-C(10)-C(11)	-177.49(12)
C(9)-C(10)-C(11)-C(20)	178.09(12)
C(9)-C(10)-C(11)-C(12)	-1.1(2)
C(13)-C(8I)-C(12)-C(11)	0.6(2)
C(20)-C(11)-C(12)-C(8I)	-1.2(2)
C(10)-C(11)-C(12)-C(8I)	178.01(13)
C(12)-C(8I)-C(13)-O(3)	-179.06(13)
C(12)-C(8I)-C(13)-C(19)	0.9(2)
O(1)-C(2)-C(15)-C(16)	-179.76(12)
C(3)-C(2)-C(15)-C(16)	-0.09(19)
C(4)-C(5)-C(16)-C(15)	0.34(19)
C(6)-C(5)-C(16)-C(15)	-177.19(12)
C(2)-C(15)-C(16)-C(5)	-0.2(2)
O(3)-C(13)-C(19)-C(20)	178.37(13)
C(8I)-C(13)-C(19)-C(20)	-1.5(2)
C(13)-C(19)-C(20)-C(11)	0.9(2)
C(12)-C(11)-C(20)-C(19)	0.5(2)
C(10)-C(11)-C(20)-C(19)	-178.75(12)
C(9)-C(8)-N(1)-N(2)	179.88(11)
C(7)-C(8)-N(1)-N(2)	0.26(14)
O(4)-C(17)-N(2)-N(1)	173.32(11)
C(18)-C(17)-N(2)-N(1)	-6.62(17)
O(4)-C(17)-N(2)-C(6)	-4.05(19)
C(18)-C(17)-N(2)-C(6)	176.01(11)
C(8)-N(1)-N(2)-C(17)	-171.95(11)
C(8)-N(1)-N(2)-C(6)	5.66(14)
C(5)-C(6)-N(2)-C(17)	-69.69(15)
C(7)-C(6)-N(2)-C(17)	168.97(11)
C(5)-C(6)-N(2)-N(1)	112.73(12)
C(7)-C(6)-N(2)-N(1)	-8.61(13)
C(3)-C(2)-O(1)-C(1)	174.06(12)
C(15)-C(2)-O(1)-C(1)	-6.26(18)
C(19)-C(13)-O(3)-C(14)	4.3(2)
C(8I)-C(13)-O(3)-C(14)	-175.76(12)

Symmetry transformations used to generate equivalent atoms:

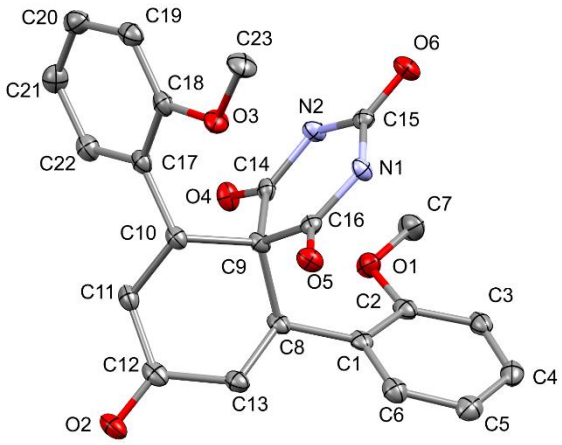

Figure 4: Ortep diagram of 2-methoxy spiro barbiturate (**B-3k**)

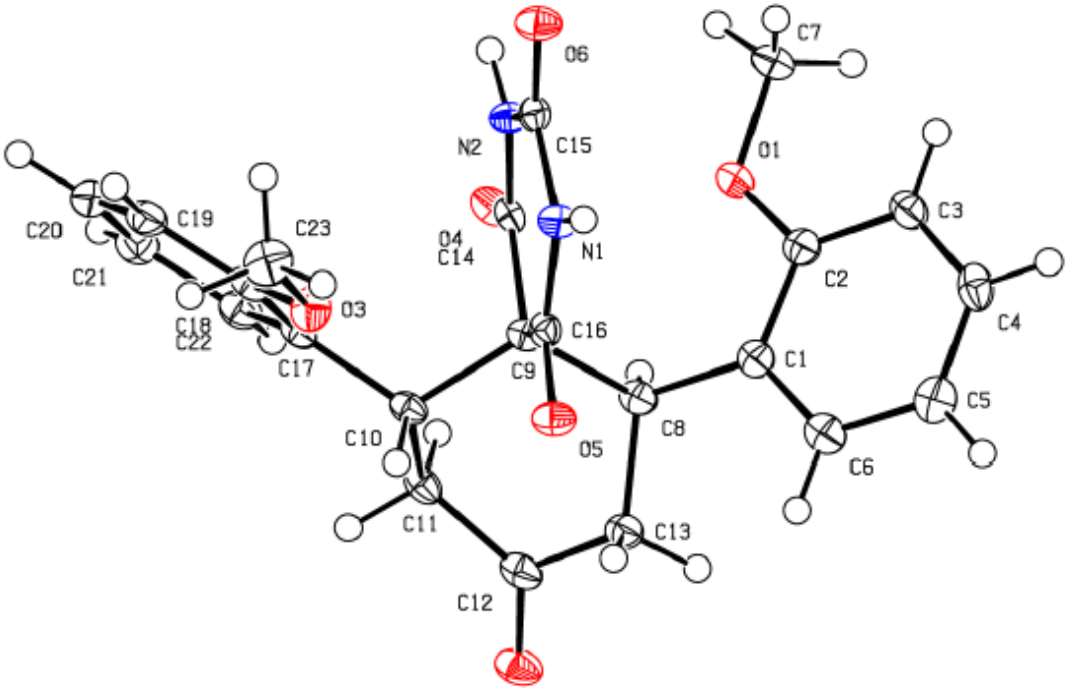

Figure 5: Olex diagram of 2-methoxy spiro barbiturate (**B-3k**)

Table 13: Crystal data and structure refinement for 2-methoxy spiro barbiturate (**B-3k**)

Identification code	2-OMe spiro
Formula	$C_{24}H_{26}N_2O_7$
$D_{calc.}$**/ g cm^{-3}**	1.405
μ**/mm^{-1}**	0.104
Formula Weight	454.47
Colour	clear colourless
Shape	block
Size/mm^3	0.30×0.22×0.14
***T*/K**	100(2)
Crystal System	monoclinic
Space Group	$P2_1/n$
***a*/Å**	12.2003(3)
***b*/Å**	10.2969(3)
***c*/Å**	17.6963(5)
α/°	90
β/°	104.8500(10)
γ/°	90
V/Å^3	2148.85(10)
Z	4
Z'	1
Wavelength/Å	0.71073
Radiation type	MoK_α
Θ_{min}/°	1.829
Θ_{max}/°	25.999
Measured Refl.	10698
Independent Refl.	4155
Reflections Used	3262
R_{int}	0.0255
Parameters	302
Restraints	0
Largest Peak	0.301
Deepest Hole	-0.288
GooF	1.032
wR_2 **(all data)**	0.0946
wR_2	0.0874
R_1 **(all data)**	0.0530
R_1	0.0380

Table 14: Fractional Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$) for 2-OMe spiro (**B-3k**). U_{eq} is defined as 1/3 of the trace of the orthogonalised U_{ij}

Atom	x	y	z	U_{eq}
C4	5498.2(13)	7411.8(17)	2792.7(10)	22.2(4)
C3	5527.7(13)	6133.5(17)	3043.2(10)	21.5(4)
C2	4655.9(13)	5294.1(16)	2688.1(9)	17.9(4)
C1	3772.8(12)	5689.1(15)	2053.4(9)	16.6(3)
C8	2827.4(12)	4747.6(16)	1676.5(9)	16.3(3)
C15	5543.6(12)	2838.1(15)	1325.4(9)	15.2(3)
C9	3196.0(12)	3632.5(15)	1168.0(9)	14.9(3)
C10	2113.5(12)	3002.5(15)	579.2(9)	15.9(3)
C17	2273.6(12)	1582.0(15)	395.7(9)	16.5(3)
C18	2974.4(12)	1254.8(16)	-89.3(9)	17.6(3)
C19	3111.9(13)	-18.6(16)	-299.6(10)	20.8(4)
C20	2548.2(13)	-1003.5(17)	-19.3(10)	23.4(4)
C23	4218.1(14)	2057.4(18)	-833.7(11)	25.9(4)
C21	1862.8(14)	-710.8(17)	471.3(10)	23.2(4)
C22	1731.8(13)	571.5(16)	673.6(10)	20.8(4)
C16	3947.6(12)	4256.1(15)	697.9(9)	14.9(3)
C14	3767.0(12)	2547.4(15)	1716.6(9)	14.4(3)
C13	1770.7(12)	5463.5(16)	1202.4(10)	18.6(3)
C12	771.7(13)	4577.4(17)	966.2(9)	18.9(4)
C11	1043.2(12)	3186.7(16)	870.9(10)	18.3(3)
C7	5486.0(14)	3559.5(17)	3544.6(10)	26.6(4)
C5	4610.0(14)	7846.4(17)	2194.4(10)	22.6(4)
C6	3769.9(13)	6977.5(16)	1823.8(10)	20.6(4)
C1S	7268.7(14)	602.8(19)	3054.0(12)	31.5(4)
N2	4855.1(10)	2207.0(13)	1707.0(8)	16.1(3)
N1	5066.2(10)	3882.9(12)	867.6(8)	15.3(3)
O3	3480.9(9)	2294.8(11)	-340.0(7)	21.5(3)
O5	3596.0(9)	5096.1(11)	210.3(6)	18.5(3)
O6	6514.7(8)	2513.8(11)	1381.8(7)	21.4(3)
O4	3263.3(9)	1954.9(11)	2113.4(6)	19.3(3)
O2	-202.5(9)	4964.1(11)	870.1(7)	23.8(3)
O1	4573.5(9)	4049.1(11)	2935.5(7)	22.1(3)
O1S	6067.2(9)	528.0(12)	2837.5(7)	24.4(3)

Table 15: Anisotropic Displacement Parameters ($\times 10^4$) 2-OMe spiro (**B-3k**). The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2} \times U_{11} + ... + 2hka^* \times b^* \times U_{12}]$

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
C4	20.3(8)	28(1)	20.5(9)	-8.1(8)	9.2(7)	-4.9(7)
C3	16.0(7)	29.5(10)	18.4(9)	-6.3(8)	3.1(7)	2.2(7)
C2	17.3(7)	22.2(9)	15.7(8)	-2.2(7)	6.8(7)	3.0(6)
C1	15.5(7)	22.5(9)	13.8(8)	-3.5(7)	7.5(7)	1.7(6)
C8	14.5(7)	23.2(9)	12.3(8)	-0.9(7)	5.4(6)	2.4(6)
C15	16.7(7)	15.9(8)	13.0(8)	-1.9(7)	3.9(6)	-0.3(6)
C9	13.2(7)	19.0(8)	13.0(8)	1.5(7)	4.4(6)	1.5(6)
C10	12.9(7)	22.8(9)	11.9(8)	0.8(7)	3.1(6)	1.1(6)
C17	13.0(7)	22.7(9)	12.2(8)	-0.1(7)	0.1(6)	0.6(6)
C18	13.2(7)	24.4(9)	13.1(8)	0.4(7)	-0.3(6)	-0.3(6)
C19	18.1(7)	27.0(9)	16.0(8)	-1.5(7)	2.2(7)	4.2(7)
C20	23.5(8)	21.4(9)	22.2(9)	-1.1(8)	0.4(7)	3.7(7)
C23	23.9(8)	32.4(10)	26.6(10)	-0.9(8)	16.2(8)	2.1(7)
C21	23.6(8)	23.0(9)	21.6(9)	4.4(8)	3.2(7)	-1.7(7)
C22	17.9(7)	27.5(9)	16.9(8)	1.0(8)	4.2(7)	0.0(7)
C16	16.5(7)	16.6(8)	11.8(8)	-2.6(7)	4.3(6)	-0.2(6)
C14	13.3(7)	17.7(8)	11.5(8)	-2.0(7)	1.7(6)	-2.7(6)
C13	17.0(7)	23.0(9)	15.8(8)	-1.1(7)	4.5(7)	3.8(6)
C12	16.6(7)	31.0(9)	9.7(8)	0.3(7)	4.5(6)	3.5(7)
C11	13.1(7)	26.7(9)	15.0(8)	-1.3(7)	3.4(6)	-2.9(6)
C7	23.1(8)	27.2(10)	23.4(10)	-0.6(8)	-5.2(8)	5.5(7)
C5	26.5(8)	24.3(9)	20.7(9)	-0.8(8)	13.0(7)	-2.0(7)
C6	19.3(8)	27.3(10)	16.6(8)	0.2(7)	7.0(7)	-0.1(7)
C1S	19.7(8)	37.6(11)	35.5(11)	8.2(9)	3.6(8)	-1.9(7)
N2	15.7(6)	16.1(7)	16.4(7)	4.4(6)	4.0(6)	2.6(5)
N1	12.9(6)	17.7(7)	16.2(7)	3.9(6)	5.6(5)	-0.1(5)
O3	21.3(6)	25.0(6)	22.0(6)	-3.4(5)	12.7(5)	-1.9(5)
O5	18.8(5)	21.9(6)	15.7(6)	5.5(5)	6.0(5)	3.6(4)
O6	15.3(5)	26.6(7)	23.4(6)	2.8(5)	6.9(5)	4.8(5)
O4	17.7(5)	25.6(6)	14.7(6)	5.2(5)	4.4(5)	-3.5(5)
O2	15.3(5)	35.8(7)	20.6(6)	0.0(5)	5.1(5)	6.1(5)
O1	21.2(6)	21.5(6)	19.0(6)	-1.0(5)	-3.3(5)	2.7(5)
O1S	18.4(5)	30.8(7)	24.3(7)	10.6(6)	6.0(5)	2.0(5)

Table 16: Bond Lengths in Å for 2-OMe spiro (**B-3k**)

Atom	Atom	Length/Å
C4	C3	1.386(2)
C4	C5	1.381(2)
C3	C2	1.389(2)
C2	C1	1.403(2)
C2	O1	1.367(2)
C1	C8	1.523(2)
C1	C6	1.387(2)
C8	C9	1.593(2)
C8	C13	1.535(2)
C15	N2	1.3692(19)
C15	N1	1.382(2)
C15	O6	1.2103(17)
C9	C10	1.595(2)
C9	C16	1.528(2)
C9	C14	1.526(2)
C10	C17	1.522(2)
C10	C11	1.534(2)
C17	C18	1.399(2)
C17	C22	1.389(2)
C18	C19	1.385(2)
C18	O3	1.3661(19)
C19	C20	1.386(2)
C20	C21	1.384(2)
C23	O3	1.4265(18)
C21	C22	1.388(2)
C16	N1	1.3749(18)
C16	O5	1.2195(19)
C14	N2	1.3773(18)
C14	O4	1.2100(18)
C13	C12	1.494(2)
C12	C11	1.489(2)
C12	O2	1.2233(18)
C7	O1	1.4284(19)
C5	C6	1.392(2)
C1S	O1S	1.4191(19)

Table 17: Bond Angles in ° for 2-OMe spiro (**B-3k**)

Atom	Atom	Atom	Angle/°
C5	C4	C3	120.24(15)
C4	C3	C2	119.47(15)
C3	C2	C1	121.58(15)
O1	C2	C3	123.32(15)
O1	C2	C1	115.09(13)
C2	C1	C8	120.41(14)
C6	C1	C2	117.10(14)
C6	C1	C8	122.38(14)
C1	C8	C9	114.47(12)
C1	C8	C13	111.68(13)
C13	C8	C9	110.90(12)
N2	C15	N1	116.03(13)
O6	C15	N2	122.67(14)
O6	C15	N1	121.30(14)
C8	C9	C10	110.83(11)
C16	C9	C8	107.47(12)
C16	C9	C10	109.09(12)
C14	C9	C8	108.50(12)
C14	C9	C10	106.51(12)
C14	C9	C16	114.46(12)
C17	C10	C9	113.59(12)
C17	C10	C11	110.95(12)
C11	C10	C9	111.32(12)
C18	C17	C10	119.64(14)
C22	C17	C10	123.08(14)
C22	C17	C18	117.27(15)
C19	C18	C17	121.87(15)
O3	C18	C17	114.13(14)
O3	C18	C19	123.99(14)
C18	C19	C20	119.41(15)
C21	C20	C19	120.00(16)
C20	C21	C22	119.80(16)
C21	C22	C17	121.65(15)
N1	C16	C9	117.65(13)
O5	C16	C9	122.13(13)
O5	C16	N1	120.15(13)
N2	C14	C9	117.56(13)
O4	C14	C9	121.42(13)
O4	C14	N2	120.89(14)
C12	C13	C8	111.75(13)
C11	C12	C13	115.46(13)
O2	C12	C13	122.28(15)
O2	C12	C11	122.25(15)
C12	C11	C10	112.97(13)
C4	C5	C6	119.43(16)
C1	C6	C5	122.05(16)
C15	N2	C14	126.89(13)
C16	N1	C15	126.23(13)
C18	O3	C23	118.25(12)
C2	O1	C7	117.76(12)

Table 18: Hydrogen Fractional Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$) for 2-OMe spiro (**B-3k**). U_{eq} is defined as 1/3 of the trace of the orthogonalised U_{ij}.

Atom	x	y	z	U_{eq}
H4	6091.18	7990.86	3033.51	27
H3	6138.94	5833.97	3454.27	26
H8	2599.18	4302.15	2114.68	20
H10	1988.67	3489.22	75.29	19
H19	3588.12	-215.57	-632.95	25
H20	2632.46	-1879.13	-163.86	28
H23A	3786.98	1661.86	-1323.61	39
H23B	4826.31	1467.39	-569.62	39
H23C	4547.49	2879.95	-947.13	39
H21	1483.21	-1385.59	668.89	28
H22	1259.63	762.57	1010.72	25
H13A	1587.62	6184.12	1519.79	22
H13B	1933.14	5842.87	728.61	22
H11A	393.44	2774.19	495.57	22
H11B	1148.33	2739.17	1379.69	22
H7A	6189.75	3586.47	3374.91	40
H7B	5323.51	2660.94	3662.95	40
H7C	5570.63	4095.01	4013.87	40
H5	4572.89	8730.89	2037.14	27
H6	3175.93	7275.95	1400.5	25
H1SA	7522.57	1168.02	2686.65	47
H1SB	7588.01	-267.86	3041.54	47
H1SC	7525.43	959.96	3583.35	47
H2	5137.83	1510.07	1974.56	19
H1	5521.92	4354.2	665.5	18
H1S	5835.1	335.44	3231.09	37