University Of KwaZulu-Natal

Synthesis, Characterization and Antibacterial Activity of Benzimidazole Derivatives

2018

Adele Cheddie

Synthesis, Characterization and Antibacterial Activity of Benzimidazole Derivatives

A THESIS

Submitted in fulfillment for the requirements

for the award of the degree of

DOCTOR OF PHILOSOPHY

in the

School of Chemistry and Physics

College of Agriculture, Engineering & Science,

By

Adele Cheddie

2018

Supervisor: Prof. N.A. Koorbanally

Synthesis, Characterization and Antibacterial Activity of Benzimidazole Derivatives

by

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2018

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.

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I hereby declare that the thesis entitled "**Synthesis, characterization and antibacterial activity of benzimidazole 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.

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Dedicated to

My Beloved Parents

Leka & Kishore

Acknowledgements

This doctoral study has been a long and arduous journey; it would not have been possible without the support of many individuals along the way. I hereby wish to extend my heartfelt appreciation for those who helped make this PhD possible.

I would like to thank my supervisor Professor Neil Koorbanally for your inspiring guidance, encouragement, unfailing attention and patience without which this work would not have come to this stage. Your rational and excellent scientific advice and the freedom with which I was allowed to undertake my research have contributed the most to my academic growth.

Thank you to Dr Chunderika Mocktar for your assistance with my antibacterial assays. My gratitude goes out to the technical staff of the School of Chemistry and Physics for their help especially Malini Padyachee, Greg Moodley, Dilip Jagjivan and Miler Nunkumar. You have always been willing to help me even with the smallest of tasks.

A very special thank you goes to Dr Suhas Shintre, Dr Pradneya Shintre,Dr Pramod Kadam, Pamela Joseph and Dr Patricia Govender for your support, encouragementand friendship. Dr Suhas, you have been an integral part of my work and I would not have been able to complete this degree without you. Your positive attitude and diligent work ethics have inspired me to never give up. I am grateful for all you have done for me.

I would like to thank my colleagues past and present of the Natural Products Research Laboratory with whom I had the pleasure to work with. You have made my time at NPRG enjoyable and I will always have fond memories of the good times shared. Thank you all for providing a supportive and cheerful atmosphere in the lab.

To my family, you are the people that mean the world to me. My mother Leka, Father Kishore, Brothers Ryan, Nikhiel and Shikaar, my uncle and aunt Anil Chetty and Anisha

Singh and grandmother Rani. Thank you for all the love, support and being there for me every step of the way. To my brother Ryan, thank you for being my best friend and helping me in any way you can.

The single largest contribution in shaping me comes from the faith, hope, encouragement and love from my parents Leka and Kishore. I dedicate this thesis to you; the debt of gratitude I owe you is beyond expression. Thank you for being such wonderful parents. You have given me the power to believe in myself and chase my dreams. Mum, you have been by my side at every stage of my life and you have selflessly sacrificed your life for your children.

The sum of all my past experiences and the help and support I have received has truly made me who I am today and has given me a deep appreciation and passion for life.

Thank you all for helping me find my way

Adele Cheddie

List of Abbreviations

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

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

°C - Degrees celsius

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

FT- IR - Fourier transform - infrared spectroscopy

HMBC - Heteronuclear multiple bond coherence

HPLC - High pressure liquid chromatography

HRMS - High resolution mass spectrometry

HSQC - Heteronuclear multiple quantum coherence

Hz - Hertz

m - Multiplet

MBC- Minimum bactericidal concentration

MS - Mass spectrometry

s - Singlet

t - Triplet

td - Triplet of doublets

TLC - Thin Layer Chromatography

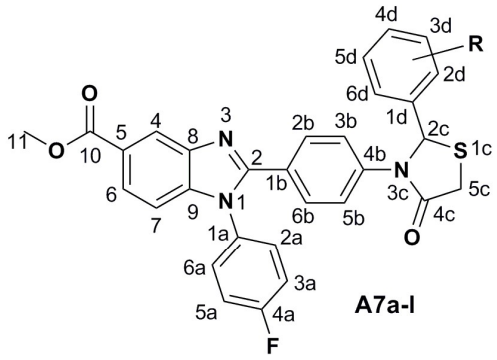
Abstract

Three series of benzimidazole hybrids were synthesised, two of which contained either thiazolidinone or amino acids tethered to the benzimidazole at C-2 by a phenyl linker and the third contained a thiazolidinone moiety at C-5 on the core structure of the benzimidazole. A total of 35 new compounds were synthesised. Variety was introduced into the molecules by using different benzaldehydes when forming the thiazolidinones or different amino acids substituted on the phenyl linker. Many of the reaction steps were carried out using microwave reactions and in one series, a comparative study was carried out between conventional synthesis and microwave irradiation. In all cases, the microwave methods had many advantages over conventional methods, having shorter reaction times, improved yields and use of green solvents. The synthesized compounds were characterised using mainly Nuclear Magnetic Resonance Spectroscopy and confirmed by High Resolution Mass Spectroscopy.

All compounds were tested for their antimicrobial properties against two Gram positive bacteria (*Staphylococcus aureus* ATCC 25923 and *Staphylococcus aureus* Rosenbach ATCC BAA-1683 (methicillin resistant *S. aureus*)) and four Gram negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 31488, *Escherichia coli* ATCC 25922 and *Salmonella typhimurium* ATCC 14026). A disc diffusion assay was used to first screen the compounds for bacterial activity, followed by the Minimum Bactericidal Concentration (MBC) assay. Among the three series, the thiazolidinones linked to the benzimidazole via the phenyl group at C-2 showed the lowest activity in the mM range. Both the amino acid derivatives linked to the phenyl group at C-2 and the thiazolidinone attached to C-5 of the benzimidazole showed antimicrobial activity in the μM range.

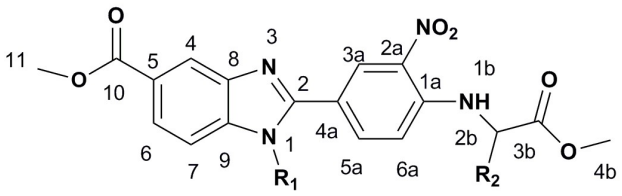
Although the benzimidazole amino acid hybrids were inactive against the Gram positive bacteria, **B7c** and **B7d** (the methionine derivatives) showed excellent inhibitory activity against S. *typhimurium* (MBC = 0.25 and 0.05 μM respectively), along with compounds **B7a** (valine derivative) and **B7j** (tryptophan derivative) which were active against *K. pneumoniae* with MBC values of 0.27 and 0.10 μM respectively. The benzimidazole–thiazolidinone hybrids, containing a trifluoromethyl group at C-2 and a thiazolidinone group at C-5, showed excellent activity with most compounds exhibiting activity ranging from 3 to over 100 fold higher than the standards. Among these, **C3d**, **C3f** and **C3j (**0.14-35.46 μM), containing bromine and nitro groups, displayed broad range activity against all strains tested. These findings are a major contribution and a good lead to developing new and better antimicrobial drugs.

Compounds Synthesized



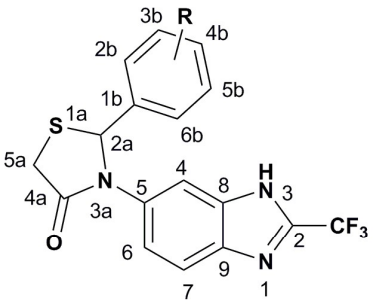
A7a-l

Compound	R
A7a	H
A7b	2'-F
A7c	4'-F
A7d	2'-CF3
A7e	4'-CF3
A7f	2'-NO2
A7g	4'-NO2
A7h	2'-Cl
A7i	4'-Cl
A7j	2'-OCH3
A7k	4'-OCH3
A7l	4'-SCH3



B7a-j

Compound	R_1	R_2 = amino acid side chain
B7a	Methyl	Valine
B7b	4'F-Ph	Valine
B7c	Methyl	Methionine
B7d	4'F-Ph	Methionine
B7e	Methyl	Leucine
B7f	4'F-Ph	Leucine
B7g	Methyl	Phenylalanine
B7h	4'F-Ph	Phenylalanine
B7i	Methyl	Tryptophan
B7j	4'F-Ph	Tryptophan



C3a-l

Compound	R
C3a	H
C3b	2'-F
C3c	2'-Cl
C3d	2'-Br
C3e	2'-CF3
C3f	2'-NO2
C3g	2'-OCH_3
C3h	4'-FI
C3i	4'-Cl
C3j	4'-Br
C3k	4'-CF_3
C3l	4'-NO_2

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Chapter 1 Introduction

In the mid 1800's, Louis Pasteur proved that microorganisms were the cause of diseases and infections, and went on to develop new ways to overcome these diseases. It wasn't until the early 1900s that the first antibiotic, penicillin, was discovered by Alexander Fleming. Currently there are hundreds of commercially available antimicrobial agents available for the treatment of diseases and infections. There is still however a high morbidity rate among infected individuals. Infectious diseases are an on-going health issue. Despite the progress in treating infectious diseases caused by bacteria, fungi and viruses, there has been rapid resistance to antimicrobial drugs.

Antibiotic resistance occurs when bacteria are no longer susceptible to currently available antibiotics. The emergence of new diseases and exponential rise in antibiotic resistance have posed serious global concerns. Resistant bacteria that infect humans and animals cause infections which are harder to treat, leading to higher medical costs, prolonged state of ill-health and increased mortality. Some current strains of resistant bacteria are, XDR and MDR *M.tuberculosis*, methicillin-resistant *S. aureus*, vancomycin-resistant *Enterococci*, ampicillin-resistant *E. aerogenes*, gentamycin-resistant *E. coli* and chloroquine-resistant *P. falciparum* (Prestinaci *et al*., 2015).

The Centre for Disease Dynamics, Economics & Policy's published a global report on the state of the world's antibiotics in 2015 (CDDEP, 2015). The overall picture is one of increasing resistance: *Staphalococcus* infections caused by MRSA is 90% resistant in Latin American countries. *E. coli* was found to be 80-100% resistant to third generation cephalosporins in European countries. The global mortality rate due to resistant bacteria is

overwhelming: In the USA and Europe, 99,000 and 25,000 deaths occur per year respectively while developing countries are hardest hit with 140,000 deaths each year.

Antibiotic resistance is a complex problem of global concern. Co-ordinated action is thus required to minimize the emergence and spread of resistance. Greater innovation and investment are required to develop new antimicrobial drugs. It is therefore highly imperative to continuously produce novel synthetic heterocyclic hit compounds that can be developed into drugs to counteract the emergence of resistant bacteria.

Aim: To design molecules with antibacterial activity that could be used as alternatives for currently used drugs when pathogenic bacteria become resistant to them.

Objectives

1. To design and synthesise new conjugate derivatives of benzimidazoles bearing α-amino acids and thiazolidinones.
2. To fully characterise all synthesised compounds using spectroscopic techniques, mainly NMR and HRMS.
3. To test these derivatives for their antibacterial activity and compare them to currently used antibiotics.

1.1 Hybrid molecules

Over the past few decades, the amalgamation of two or more chemical pharmacophores having different biological function into a single molecule has increased (Shaveta *et al*., 2016). This amalgamated molecules have been termed "hybrids". The appropriate definition is "A chemical entity with two or more different structural domains having different

biological functions" (Meunier, 2008). These molecules can act as two distinct pharmacophores. The advantage of hybrid molecules is their ability to target different bioactive sites. They have been known to increase therapeutic efficacy and reduce toxicity. Since more than one pharmacophore appears in a single molecule, multi-drug administration and drug to drug interference during combinatorial therapies is avoided (Choudhary *et al*., 2018).

1.2 Benzimidazoles

Benzimidazoles are aromatic organic compounds consisting of a six-membered benzene ring fused to a five-membered imidazole ring (Figure 1.1).

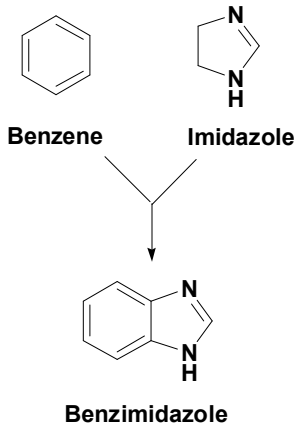


Figure 1.1 The structure of benzimidazole

Benzimidazole itself is a white solid with a melting point of 172°C and boiling point of 360°C. It is soluble in alcohols and organic solvents, but only slightly soluble in water. Benzimidazoles with hydrogen at N1 readily undergo tautomerisation (**Figure 1.2**).

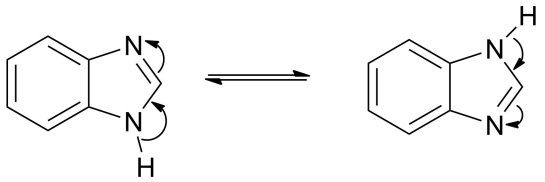
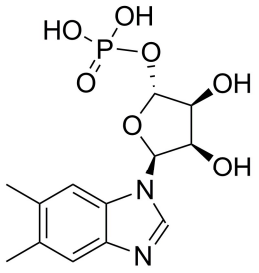


Figure 1.2 Tautomerism of benzimidazole

The core structure of benzimidazole occurs in nature and is found in *N*-ribosyl-dimethylbenzimidazole, an axial ligand in vitamin B12 (**Figure 1.3**).

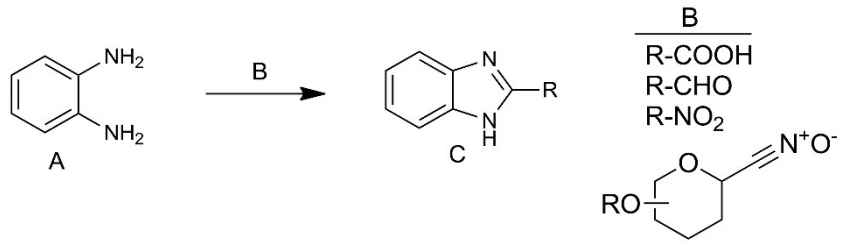


N-ribosyl-dimethylbenzimidazole

Figure 1.3 Bezimidazole in a naturally occurring ligand

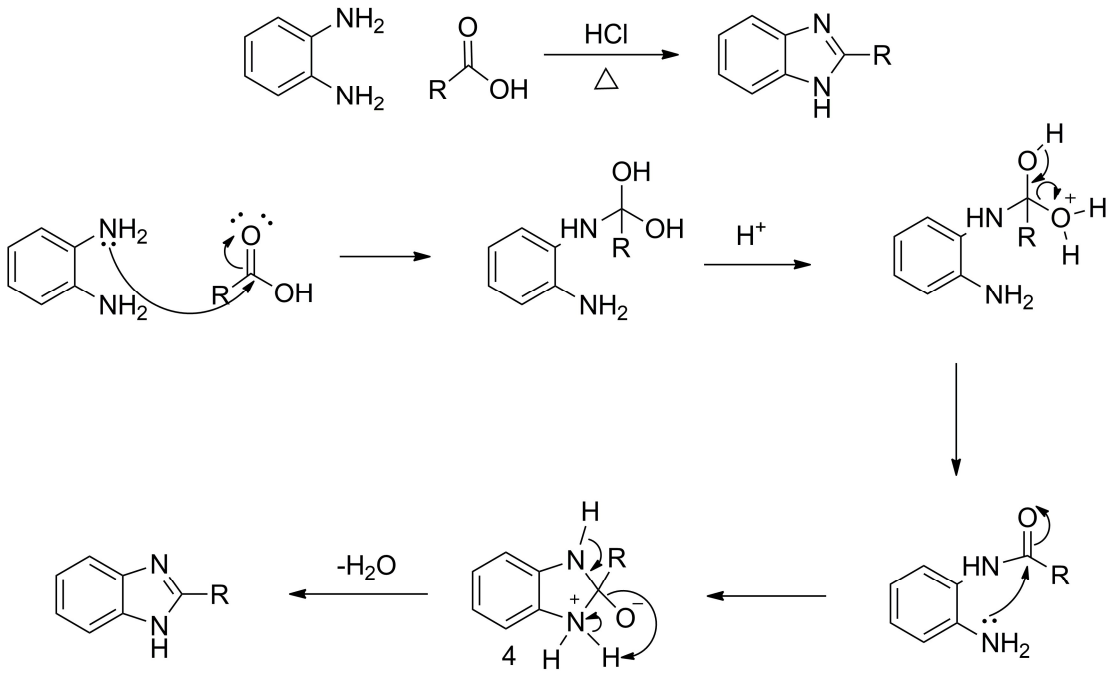
1.2.1 Synthesis of benzimidazoles

Ortho-phenylenediamine is a precursor in many different synthetic methods for the preparation of benzimidazoles and the conventional method for the synthesis of benzimidazoles is the condensation of substituted or unsubstituted *o*-phenylenediamine with carboxylic acids or its derivatives, aldehydes and nitro compounds under acidic conditions (**Scheme 1.1**) (Sugumaran and Kumar, 2012; Alaqeel, 2017).



Scheme 1.1 General scheme for the synthesis of benzimidazoles from *o*-phenylenediamine

Ortho-phenylenediamines react with most acids such as polyphosphoric acid, trifluoroacetic acid, carboxylic acid and formic acid to form 2-substituted benzimidazoles. The most commonly used method involves the condensation of *o*-phenyldiamines with the acid to produce an amide intermediate, which then undergoes cyclization under strong acidic or dehydrating conditions (**Scheme 1.2**). These reactions are carried out by heating under reflux as well as microwave assisted methods (Lin *et al*., 2006; Wang *et al*., 2006; Sugumaran and Kumar, 2012). These methods have several draw backs in that they use harsh conditions, toxic solvents and have low yields.

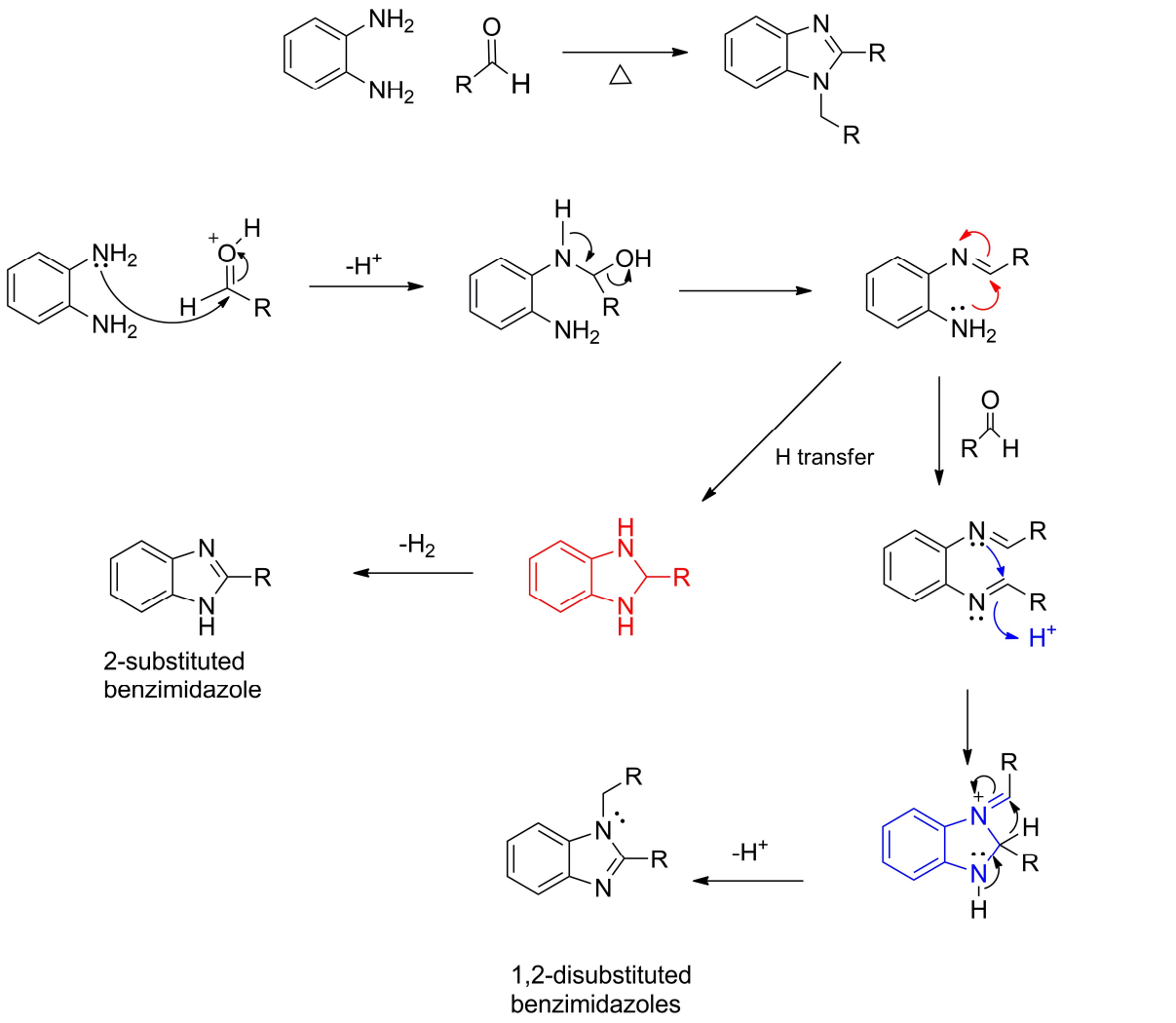


Scheme 1.2 Mechanism showing formation of benzimidazoles from acids

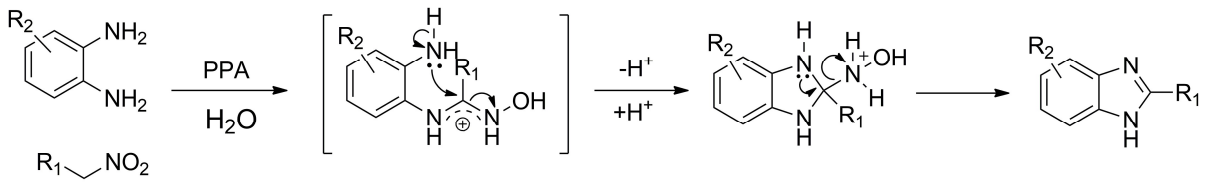
A common synthetic route involves the condensation of 1,2-phenyldiamines with aldehydes under oxidative conditions. This oxidation is brought about by catalysts such as $(NH_4)_2S_2O_8$ (Bahrami *et al*., 2010), MgI (Nagasawa *et al*., 2014), Pt/Al_2O_3 or Pt/TiO_2 (Chaudhari *et al*., 2015) to produce either 2-substituted or 1,2-disubstituted benzimidazole derivatives. The use of aldehydes is attractive in that there are numerous aldehyde derivatives leading to a larger more diverse library of benzimidazoles. This synthetic approach also uses milder reaction conditions with green solvents and lower temperatures.

In the synthesis of 2-substituted benzimidazoles shown in **Scheme 1.3**, substituted aldehydes are oxidised with catalysts leading to activation of the carbonyl carbon. This is then condensed with *o*-phenyldiamines affording imine imtermediates, which undergoes cyclisation to form benzimidazole derivatives. In the synthesis of 1,2-disubstituted benzimidazoles, the aldehyde reacts with *o*-phenyldiame to form imine intermediatesat both amino groups, followed by cyclisation and 1,3-hydride migration to form 1,2-disubstituted benzimidazoles.

Nitroalkanes were also used with *o*-phenyldiamine, together with a polyphosphoric acid (PPA) catalyst to yield benzimidazole derivatives. The PPA activates the α-carbon of the nitroalkanes making them electrophilic. The amino group then adds to this electrophilic centre to produce *N*-hydroxyimidamides followed by acid promoted cyclisation to form benzimidazoles (Aksenov *et al*., 2015) (**Scheme 1.4**).

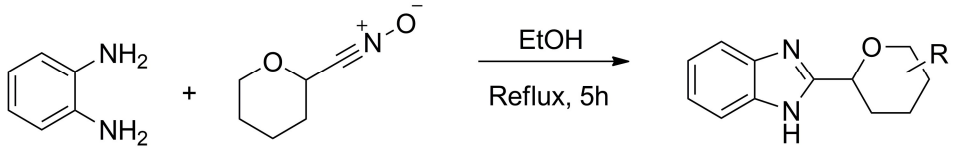


Scheme 1.3 Mechanism showing formation of benzimidazoles from aldehydes



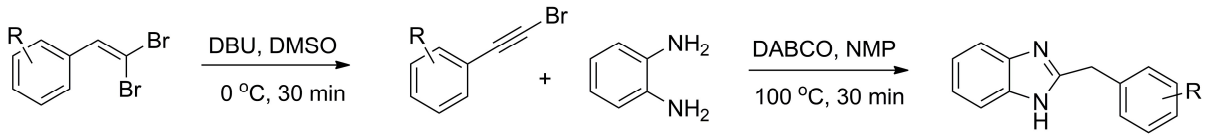
Scheme 1.4 Mechanism for cyclisation of benzimidazoles from nitroalkanes using PPA as catalyst

Benzimidazoles can also be prepared by reacting pyranosyl nitrile oxides with *o*-phenyldiamine in ethanol with 89% yield (Smellie *et al*., 2010) (**Scheme 1.5**).



Scheme 1.5 Reaction of nitrile oxides with *o*-phenyldiamine to form benzimidazoles

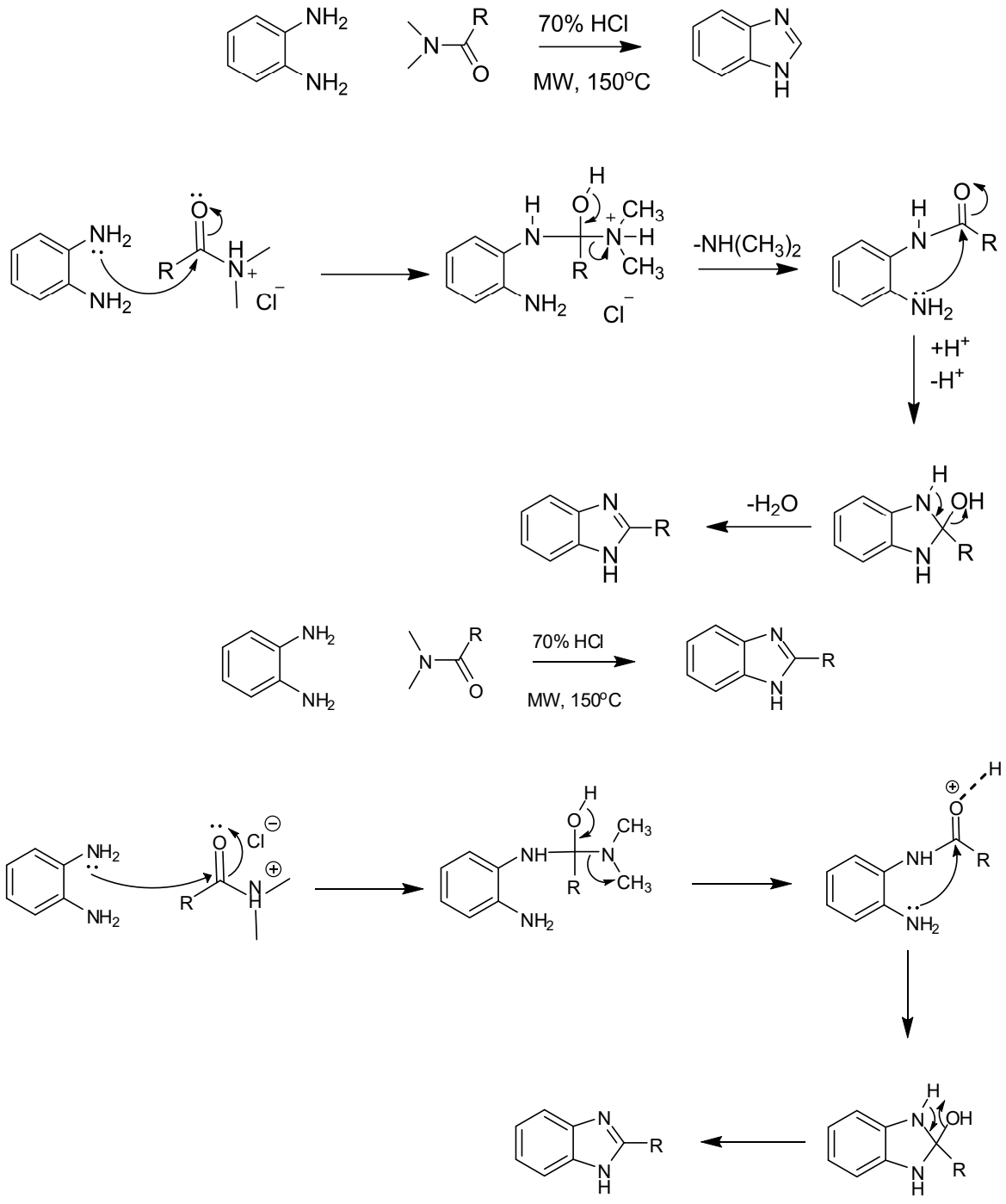
The use of brominated moieties has gained popularity due to their versatility as substrates for transition metal-catalyzed amination and condensation of *o*-phenylenediamines to benzimidazoles. Reactions with brominated groups also occur under milder reaction conditions. Wang *et al*. (2013) developed an efficient synthetic method for the synthesis of *N*,*N*-diarylbenzimidazoles using a Pd_2dba_3/BINAP catalytic system together with aryl bromides (**Scheme 1.6**). A mild and efficient method for the preparation of substituted benzimidazoles in good yields (53-86%) were also reported using *o*-phenylenediamines and 1,1-dibromoethenes using DABCO as a basic catalyst (Shen *et al*., 2008).



Scheme 1.6 Synthesis of diarylbenzimidazoles from aryl bromides and *o*-phenylenediamine

Another conventional synthetic approach to the benzimidazoles is the cyclisation of o-phenyldiamine using alternative carbon sources such as amides (Kattimani *et al*., 2015), dimethylformamide (DMF) (Zhu *et al*., 2017; Rasal and Yadav, 2018) and CO_2 (Yu *et al*., 2013; Vessally *et al*., 2017). Kattimani *et al*. (2015) investigated the reaction of various substituted amides with *o*-phenyldiamine, using DMF and acid at 150°C to synthesise benzimidazoles. The reaction follows a simple mechanism, whereby the acid protonates the nitrogen in the first step, activating the carbonyl group and allowing dimethylamine to be expelled in the second step. This is followed by nucleophilic attack by the second amino

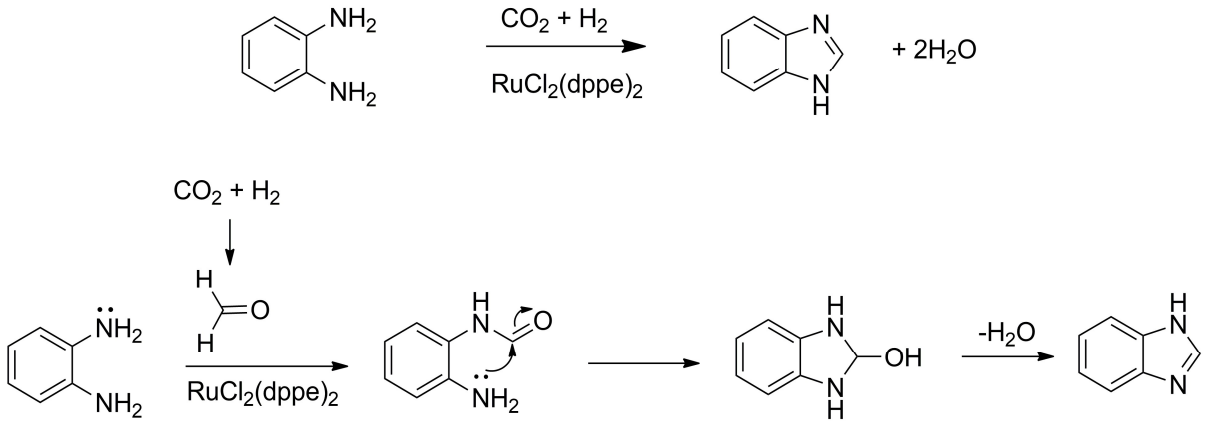
group on the carbonyl group, forming the benzimidazole ring (**Scheme 1.7**) (Kattimani *et al*., 2015).



Scheme 1.7 Mechanism showing formation of benzimidazoles from *o*-penyldiamine and DMF

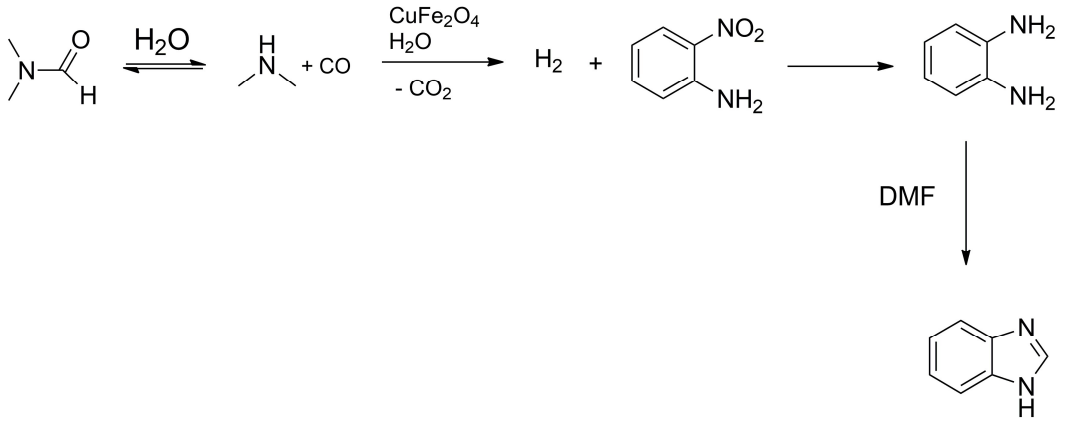
Ortho-phenyldiamines also undergo cyclisation with CO_2 to generate benzimidazole derivatives under mild conditions. Benzimidazoles were synthesised from *o*-phenyldiamines,

and CO_2 in the presence of H_2 using a $RuCl_2(dppe)_2$ catalyst (**Scheme 1.8**) (Yu *et al*., 2013). The reaction occurs by initially forming formic acid by hydrogenation of CO_2. The amino group on *o*-phenyldiamine then adds to the carbonyl group of formic acid by nucleophilic addition forming a formamide intermediate that undergoes a second nucleophilic addition by the other amino group resulting in cyclisation and dehydration to the benzimidazoles (Yu *et al*., 2013).



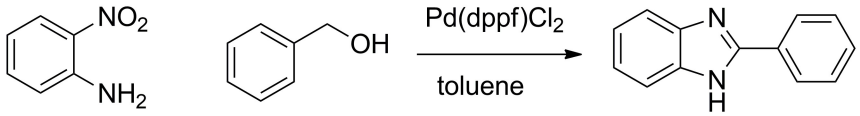
Scheme 1.8 Proposed mechanistic pathway for the cyclisation of benzimidazoles from CO_2 and H_2 with *o*-phenyldiamine

A simple one-pot method from *o*-nitroaniline using a $CuFe_2O_4$ catalyst and DMF was also used to synthesise benzimidazoles (**Scheme 1.9**). The reaction occurs by the initial decomposition of DMF in water into CO and dimethylamine. The CO then undergoes a water gas shift reaction to form H_2 and CO_2 using $CuFe_2O_4$. *Ortho*-nitroaniline is reduced by the H_2 to *o*-phenyldiamine, the precursor to the benzimidazoles. DMF is portable and a better alternative to CO gas (Rasal and Yadav, 2018).



Scheme 1.9 Synthesis of benzimidazoles using *o*-nitroaniline and DMF as carbon source

Recently, the use of alcohols as reducing agents has become an attractive method in the synthesis of benzimidazoles, due to their availability and low cost (Chaudhari *et al*., 2015). Li *et al*. (2016) have reported a reaction that utilises inexpensive benzyl alcohols, *o*-nitroanilines and a Pd(dppf)Cl_2 catalyst in a one-pot reaction to synthesise benzimidazoles in excellent yields (78-90%) (**Scheme 1.10**).



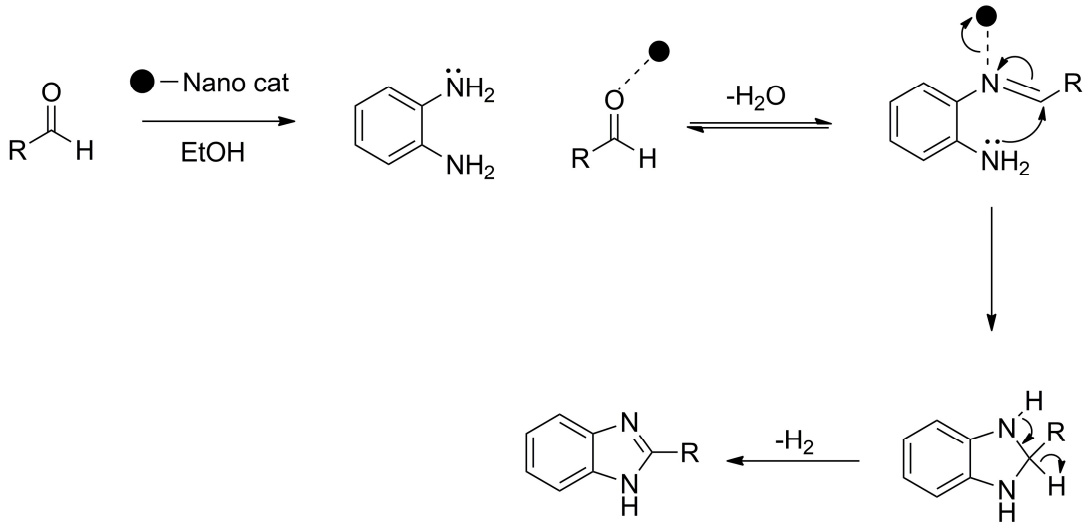
Scheme 1.10 Synthesis of benzimidazoles with *o*-nitroaniline and benzyl alcohol

The conventional methods listed herein often imply toxic reagents and harsh reaction conditions with strong acids, high temperatures, low yields, longer reaction times and tedious workup procedures.

New catalysts with improved performance in the synthesis of benzimidazoles have been developed. Nanocomposite materials is an example of this catalyst. They have become popular due to their low-cost manufacturing, recyclability and high efficiency (Saha *et al*.,

2009; Borade *et al*., 2018). A $Fe_3O_4SiO_2$/collagen nanocatalyst was used with *o*-phenyldiamine and aldehydes in the synthesis of benzimidazole derivatives. The reaction was carried out at room temperature for 12 minutes with yields of 78% and greater (**Scheme 1.11**).

The reported advantages of this nanocatalyst are easy separation, a facile synthetic procedure, high purity, increased yields and reduced reaction times (Ghafuri *et al*., 2016). Similar results were seen with magnetic polymer nanocomposites containing copper ions (Fe_3O_4@Cu-PMT), which was used to synthesise 2-phenyl-1*H*-benzimidazoles from benzaldehyde and *o*-phenyldiamine (Mobinikhaledi *et al*., 2018).

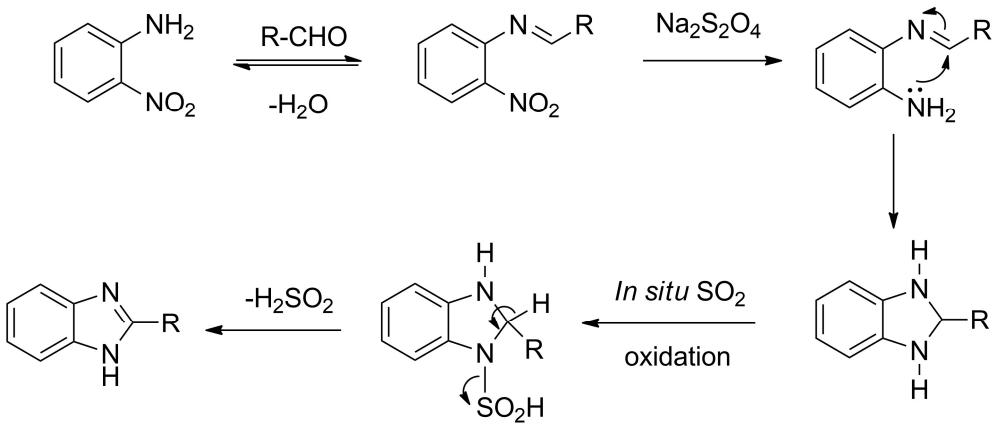


Scheme 1.11 Proposed mechanism for the synthesis of benzimidazoles using a nano catalyst

Microwave-assisted synthesis has been shown to be far superior to conventional heating. This technique offers clean reactions with high yields and shorter reaction times. It allows for rapid heating to extremely high temperatures, hence the decomposition of reagents and/or products are reduced (Mavandadi and Pilotti, 2006). A comparative study for the synthesis of 2-substituted aryl and alkyl benzimidazole derivatives between conventional and microwave-

assisted synthetic methods indicated that microwave-assisted methods reduced reaction times by 98% and increased yields up to 50% (Dubey *et al*., 2007).

Benzimidazoles substituted at C-2 were synthesised in a one-pot reaction from benzaldehydes and *o*-nitroaniline with sodium dithionite ($Na_2S_2O_4$) under microwave irradiation (**Scheme 1.12**). $Na_2S_2O_4$ acts as a reducing agent in converting the nitro group to an amine. The $Na_2S_2O_4$ then decomposes into SO_2 in the presence of water and oxygen, which oxidizes the dihydrobenzimidazole intermediate to form the target molecules. Advantages of this procedure are high yields (90-97%), short reaction times (4-11 min), cost effective reagents and mild reaction conditions (Naeimi and Alishahi, 2014).



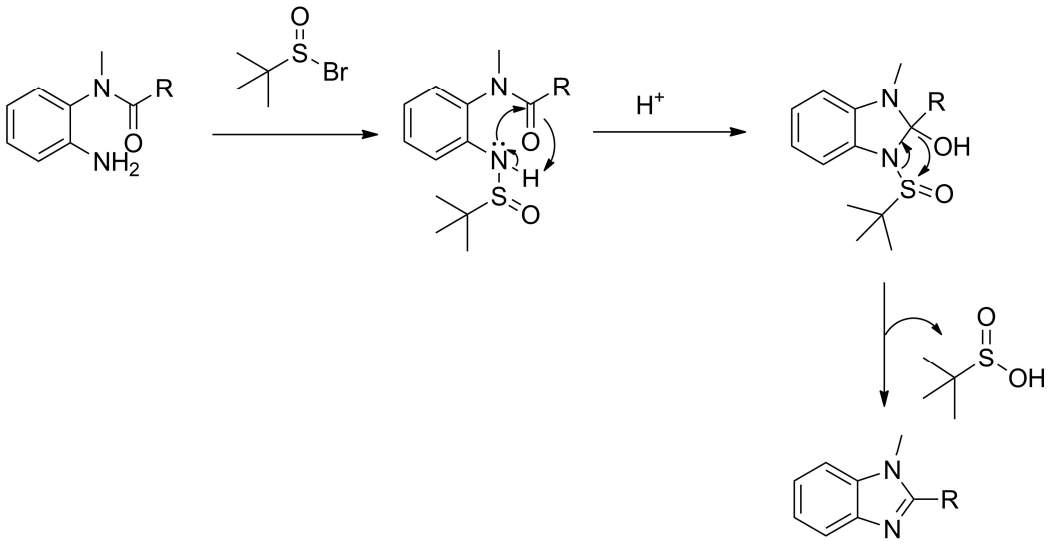
Scheme 1.12 Probable mechanism for the formation of 2-substituted benzimidazoles with sodium dithionate under microwave conditions

1.2.2 Synthesis of 1,2-disubstituted benzimidazoles

1,2-Disubstituted benzimidazoles allow for a more comprehensive library of compounds to be generated having different substituents at position N1 and C2. Conventional methods reported include the condensation of aldehydes with *o*-phenyldiamine and intramolecular cyclisation using transition metals (Brasche and Buchwald, 2008, Cho *et al*., 2011;

Bandyopadhyay *et al*., 2011). However, these methods employ harsh conditions with costly catalysts.

The synthesis of 1,2-disubstituted benzimidazoles from commercially available *o*-phenylaminoamides, benzaldehydes, *tert*-butanesulfoxide and NBS occur via an aza-Wittig-equivalent process (**Scheme 1.13**). This process makes use of mild conditions with good yields (73-80%) (Chen *et al*., 2017).

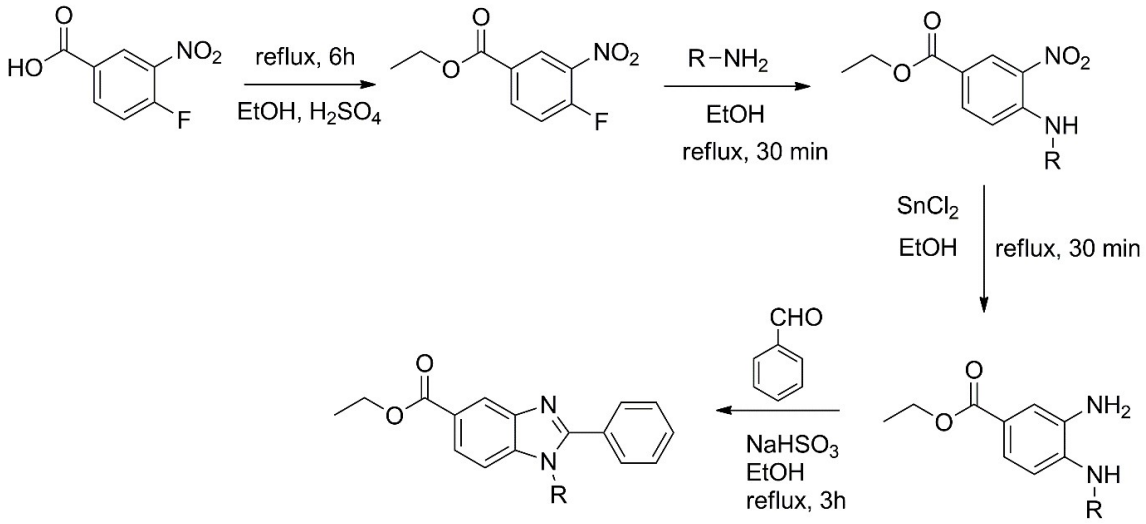


Scheme 1.13 Synthesis of 1,2-substituted benzimidazole using the aza-Wittig reaction

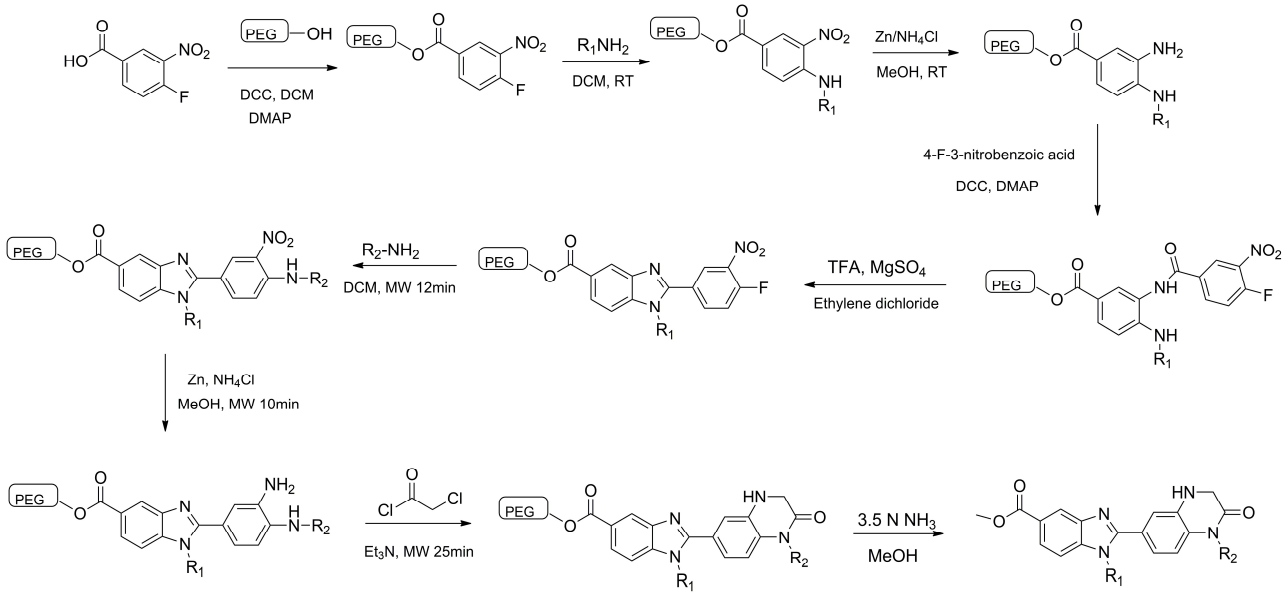
A one-pot facile method to synthesise 1,2-disubstituted bezimidazole derivatives was carried out using 4-fluoro-3-nitrobenzoic acid, where after esterification, the fluorine was substituted by various amines by formation of a Meisenheimer adduct in ethanol (Yeong *et al*., 2014). The nitro group was reduced with stannous chloride andcyclised with benzaldehyde forming disubstituted derivatives (**Scheme 1.14**). This procedure eliminated the need for costly metal catalysts, and purification is only required at the final stage.

A synthetic route using polymer support and microwave irradiation to develop benzimidazole linked quinoxalinones was reported (Chou et al., 2011). *Ortho*-phenyldiamine was

immobilised on polyethylene glycol (PEG-4000) and condensed with 4-fluoro-3-nitrobenzoic acid by DCC activation and subsequently cyclised into benzimidazoles using trifluoroacetic acid in 1,2-dichloroethane. This scheme employs simple reactions combining two well-known techniques and is advantageous with short reaction times, increased purity, yields and efficiency of the overall process (**Scheme 1.15**) (Chou *et al*., 2011).



Scheme 1.14 Synthetic scheme for the synthesis of 1,2-disubstituted benzimidazoles from 4-fluoro-3-nitrobenzoic acid



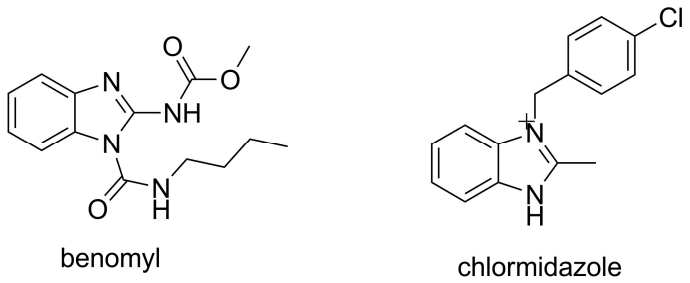
Scheme 1.15 Synthetic route to benzimidazole linked quinoxalines

1.2.3 Bioactivity of benzimidazoles

The use of benzimidazoles as potential chemotherapeutic agents was first described in the 1950s. A few years later, in 1961, the discovery of thiabendazole, an anthelmintic drug, sparked research around the design and synthesis of benzimidazole molecules as potential drugs or intermediates in organic reactions. Benzimidazoles have since shown to possess antimicrobial (Hosamani *et al*., 2009; Ajani *et al*., 2017), antihypertensive (Narasimhan *et al*., 2012), anticancer (Refaat, 2010; Zhao *et al*., 2015), anthelmintic (Gutiérrez *et al*., 2018), anti-inflammatory (Gaba *et al*., 2014), antidiabetic (Vijayakumar and Ahamed, 2010), antiviral (Xu *et al*., 2014; Tonelli *et al*., 2018) and anti-ulcer activity (Yadav and Ganguly, 2015). Due to its wide range of applications, the benzimidazole core nucleus has become an important pharmacophore in drug discovery and is a key focus area in medicinal research.

1.2.4 Antimicrobial activity

A number of benzimidazole derivatives are commercially available for the treatment of microbial infections. Some examples are benomyl and chlormidazole (**Figure 1.4**).



benomyl chlormidazole

Figure 1.4 Structure of some antimicrobial drugs containing the benzimidazole nucleus

5,6-Dichloro-2-(4-fluorophenyl)-1*H*-benzimidazole was seen to exhibit potent antibacterial activity against *S. aureus* with an MIC of 3.12 μg mL^{-1} (Tunçbilek *et al*., 2009). Broad spectrum activity (MIC = 15.63 – 250.0 μg mL^{-1}) was seen by a library of mono and

disubstituted derivatives of benzimidazole with substituted phenyl, benzyl or 1,3 dinotrophenyl groups at C-2 and substituted phenyl groups at N-1, with the highest activity being exhibited by 2-(1*H*-benzimizadol-2-yl)aniline (MIC of 15.63µg mL^{-1} against *S. aureus*), and 2-benzyl-1-(phenylsulfonyl)-1*H*-benzimidazole (MIC of 15.63 µg mL^{-1} against *P. aeruginosa*) (**Figure 1.5**) (Ajani *et al*., 2017).

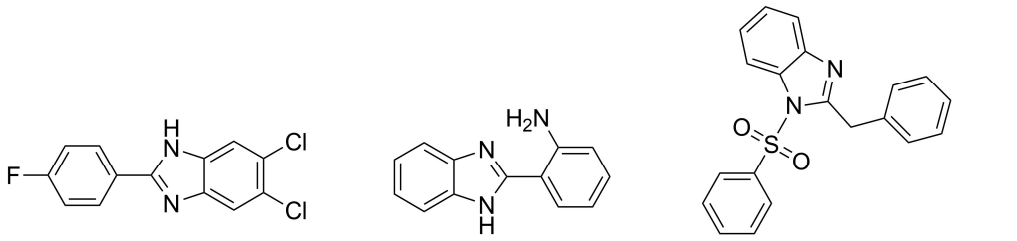


Figure 1.5 Structures of antibacterial benzimidazole derivatives with substituted benzyl and phenyl groups

1.2.5 Anti-inflammatory activity

2-(1-(Biphenyl-4-ylmethyl)-5-nitro-benzimidazole)isoindoline-1,3-dione (**Figure 1.6**) is a potent antagonist having multiple inhibitory effects against inflammation-released targets, for example COX and LOX enzymes (COX-1 IC_{50} = 9.85µM; COX-2 IC_{50} = 1.00µM; 5-LOX IC_{50} = 0.32 µM and 15-LOX IC_{50} = 1.02 µM) (Kaur and Silakari, 2018).

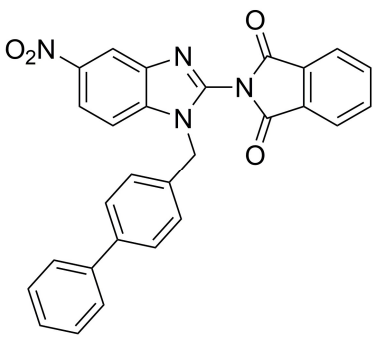


Figure 1.6 Structure of 2-(1-(biphenyl-4-ylmethyl)-5-nitro-benzimidazole)isoindoline-1,3-dione

1.2.6 Antiviral activity

Maribavir (**Figure 1.7**) is a commercial available antiviral drug with a benzimidazole core used for the treatment of Human Herpes virus-5 (HHV-5) which affects almost everyone, but can be fatal for immune-compromised individuals. 2-Phenylbenzimidazole derivatives were also shown to exhibit selective and high activity against 10 RNA and DNA viruses (Tonelli *et al.*, 2010). Simple compounds such as 5,6-dichloro-2-(4-nitrophenyl)benzimidazole were 110 times more potent than 6-azauridine (a currently marketed drug) with an EC_{50} value of 0.1 μM against Vaccinia Virus, and *N*-(4-(5-nitro-benzimidazol-2-yl)phenyl)acetamide (**Figure 1.7**) exhibited high activity (EC_{50} = 0.8 μM) against Bovine Viral Diarrhoea Virus (Tonelli *et al.*, 2010).

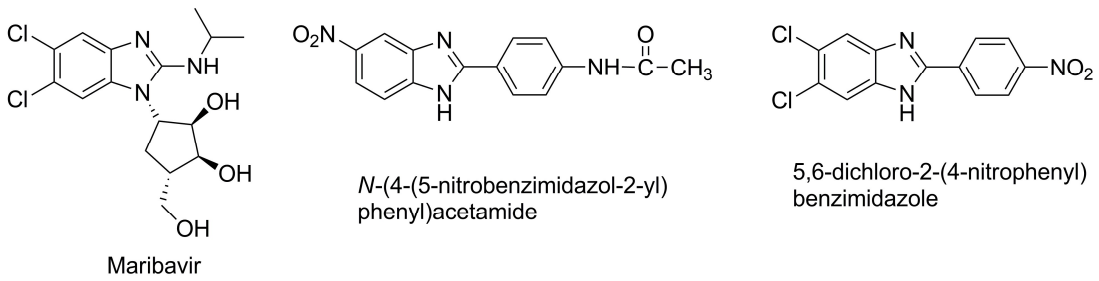


Figure 1.7 Structures of some antiviral benzimidazole derivatives

1.2.7 Anticancer activity

Current anticancer drugs containing a benzimidazole core structure include bendamustine, veliparib and nocodazole (**Figure 1.8**). Benzimidazoles containing thiazolidinedione moieties were found to be good anticancer agents against prostrate (Pc-3), cervical (HeLa), lung (A549) and bone (HT1080) cancer cells. Derivatives of 3-benzyl-5-((2-phenylbenzimidazol-5-yl)methylene)thiazolidine-2,4-dione (**Figure 1.8**) was found to be most cytotoxic with an IC_{50} value in the range of 0.096-0.63 μM (Sharma *et al.*, 2017).

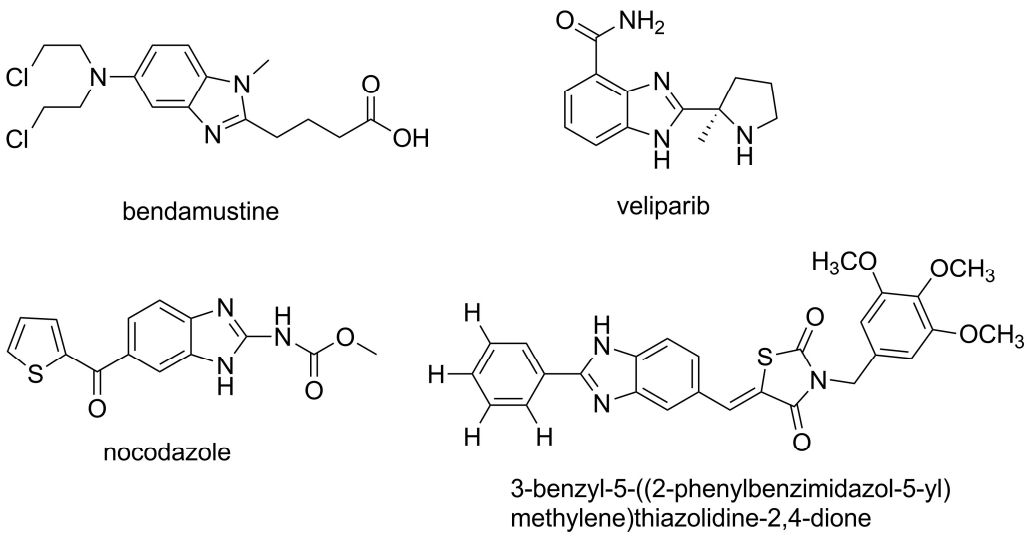


Figure 1.8 Structure of Bendamustine and anticancer derivative

1.2.8 Anti-ulcer

Benzimidazole derivatives bearing sulfoxide and methylene groups are known to inhibit H^+/K^+-ATPase and block gastric acid secretion preventing gastric ulcers and reflux-oesophagitis lesions. Omeprazole and its *S*-isomer esomeprazole, pantoprazole and lansoprazole (**Figure 1.9**) are current agents against gastric acid production. Methoxybenzyl-sulfonyl-benzimidazole derivatives were shown to be effective H^+/K^+-ATPase inhibitors. Inhibitory activity of 95% was achieved with 2-(4-((4-(3-methoxypropoxy)-3-methylpyridin-2-yl)methoxy)-3-methoxybenzyl sulfonyl)-5-(difluoromethoxy)-1*H*-benzo[d]imidazole (**Figure 1.9**) higher than omeprazole (80%) (Rajesh *et al.*, 2017).

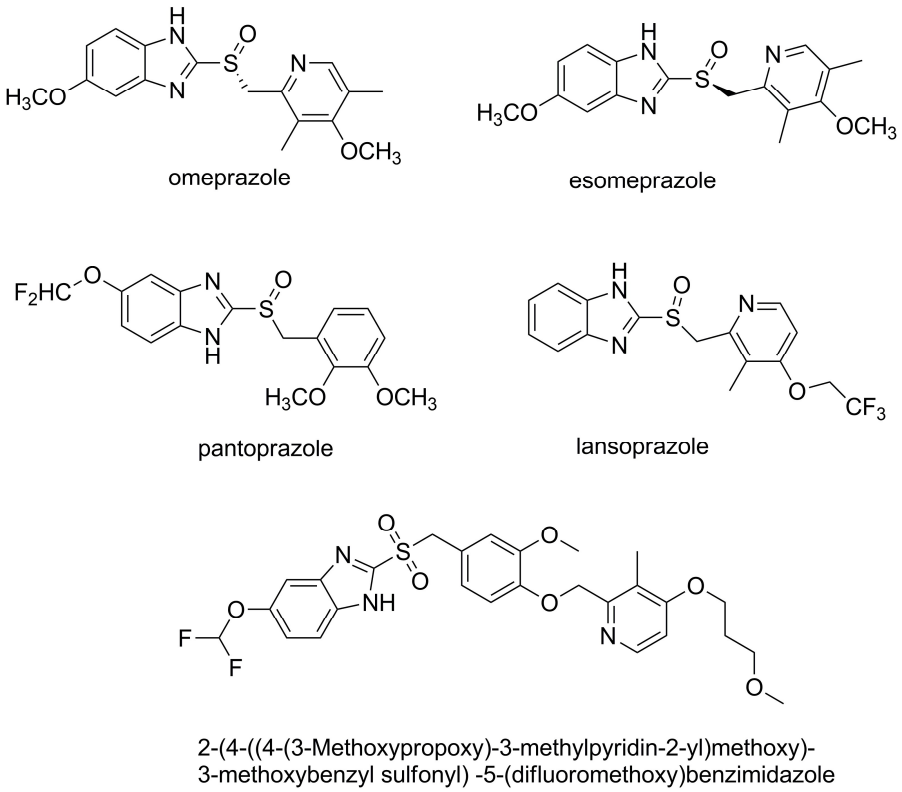


Figure 1.9 Structures of some anti-ulcer drugs

1.2.9 Antidiabetic activity

Rivoglitazone (**Figure 1.10**) is a benzimidazole-thiazolidinone drug used for the treatment of type-II-diabetes. It acts by increasing the interaction of peroxisome proliferator-activated receptorsin fat cells and the hormone insulin. A series of benzimidazole derivatives, also showed antidiabetic activity by *α*-glucosidase inhibition. Benzimidazoles containing a hydrazide and oxadiazole (**Figure 1.10**) demonstrated IC_{50} values of 10.49 µM and 26.93 µM respectively with 99% inhibition (Özil *et al.,* 2016).

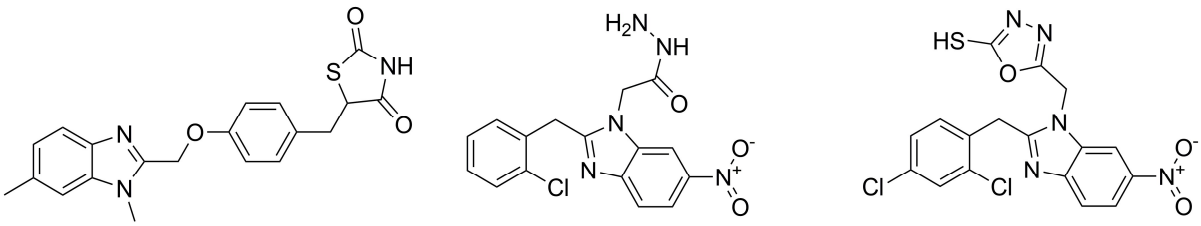


Figure 1.10 Structures of antidiabetic benzimidazoles

1.2.10 Antihypertensive activity

Azilsartan and candesartan (**Figure 1.11**) are benzimidazole containing drugs used in hypersensitive treatments acting as angiotension-II receptor antagonists. Other *N*-aryl substituted benzimidazoles (**Figure 1.11**) were also shown to be potent angiotension-II-AT_1 receptor antagonists by significantly reducing blood pressure by up to 36.2 mmHg (Shah *et al*., 2008).

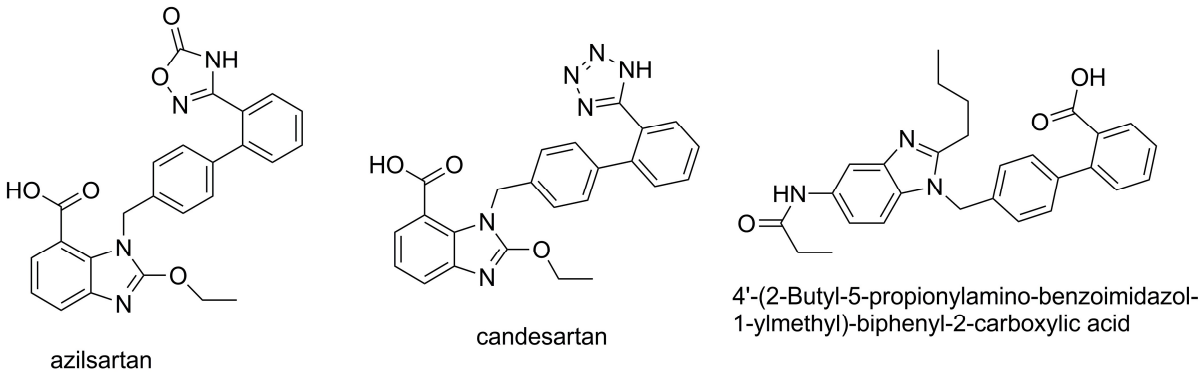


Figure 1.11 Structures of some antihypertensive agents

1.2.11 Anthelmintic activity

Benzimidazoles are known to be highly effective anthelmintic agents, with thiabendazole and mebendazole acting against nematode infections. Albendazole (**Figure 1.12**) is another

example, which is effective against tapeworms and roundworms. Benzimidazole-chalcone hybrids were shown to exhibit significant *in vitro* anthelmintic activity against *Pheretimaposthuma.* Structure-activity relationship studies showed that polar electron donating groups (OH, OCH_3) and halogens at the *para* position increased activity, while non-polar electron donating groups (NCH_3) reduced activity (Babu and Selvakumar, 2013).

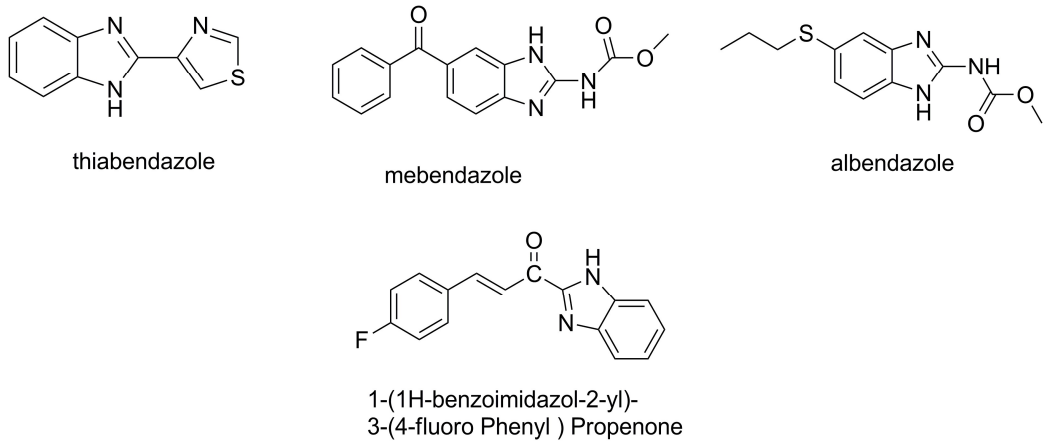


Figure 1.12 Anthelmintic benzimidazoles

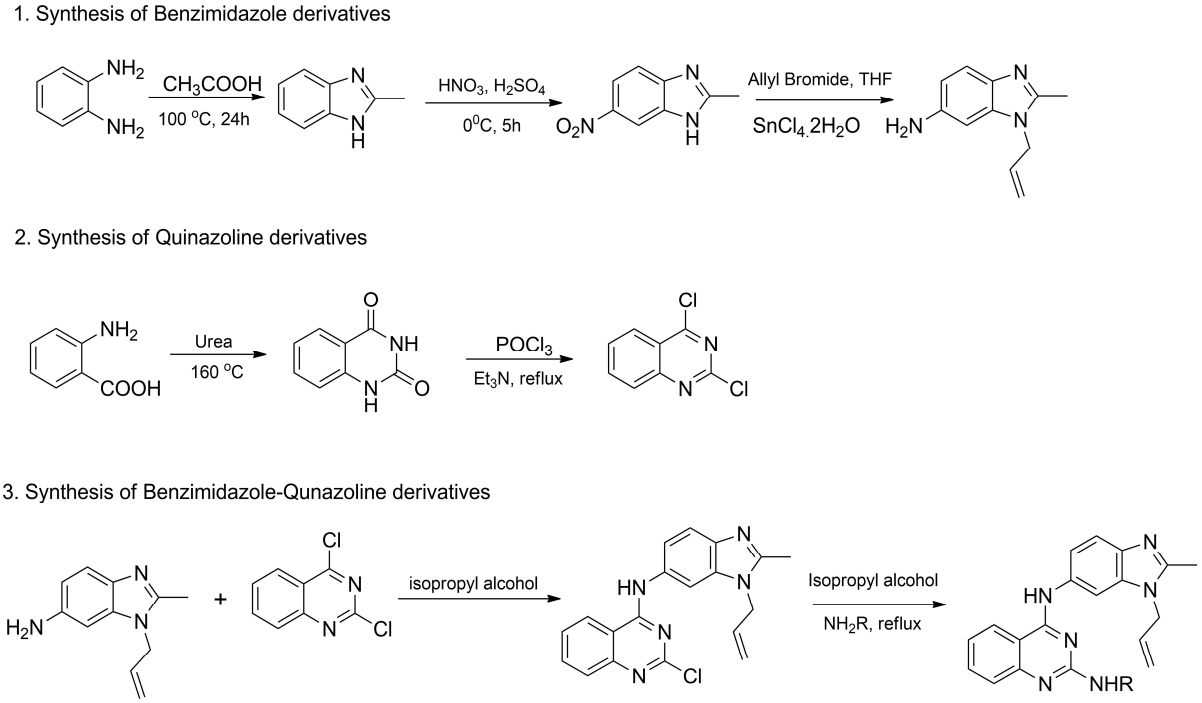
1.3 Benzimidazole hybrids

Benzimidazoles are excellent scaffolds to which other pharmacophores can be attached, since other frameworks can be added to the nitrogen atoms, C-2 or functional groups present on the aromatic ring.

Benzimidazole-quinazoline hybrids were synthesised in a two step process where the benzimidazoles were first synthesized from *o*-phenyldiamines and carboxylic acid. These compounds were further nitrated producing 5-nitrobenzimidazole which then underwent nucleophilic substitution with aryl bromide to yield 5-nitro-1-allyl-2-methylbenzimidazole and finally reduced to amine derivatives. The quinazoilines were synthesised from aminobenzoic acid and urea and subsequently treated with phosphoryl chloride to form 2,4-

dichloroquinazoline derivatives. The benzimidazoles were then coupled with 2,4-dichloro quinazolines in the presence of isopropyl alcohol to afford the final hybrid molecules (**Scheme 1.16**).

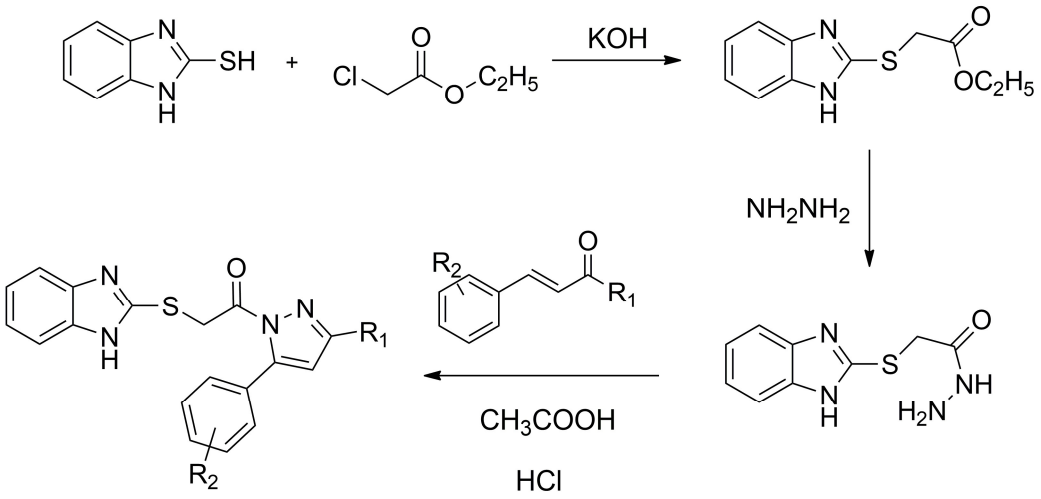
These compounds were shown to have antitumor activity against 60 tumour cell lines (Sharma *et al*., 2013). On their own, quinazolines have been shown to effect critical phases in the cancer cell cycle by modulating aurora kinase (Bebbington *et al*., 2009) activity or inducing apoptosis (Sirisoma *et al*., 2008) and benzimidazoles are known to inhibit cell proliferation by acting on cyclin dependant kinase (Snow *et al*., 2007). Molecular docking predicted the mode of action of the benzimidazole-quinazoline hybrids, showing binding in the Topo I and Topo II active site, suggesting topoisomerase enzyme inhibition.



Scheme 1.16 Synthetic route for the preparation of benzimidazole-quinazoline hybrids

A series of benzimidazole-pyrazole hybrids were synthesised by Noor *et al*. (2017) by a three step reaction process where 2-mercaptobenzimidazole was treated with ethyl chloroacetate to form ethyl 2-(benzimidazolythio) acetate, which was further condensed with hydrazine hydrate to yield hydrazides. The hydrazides were further reacted with chalcones in the presence of acid catalysts to form pyrazole derivatives (**Scheme 1.17**).

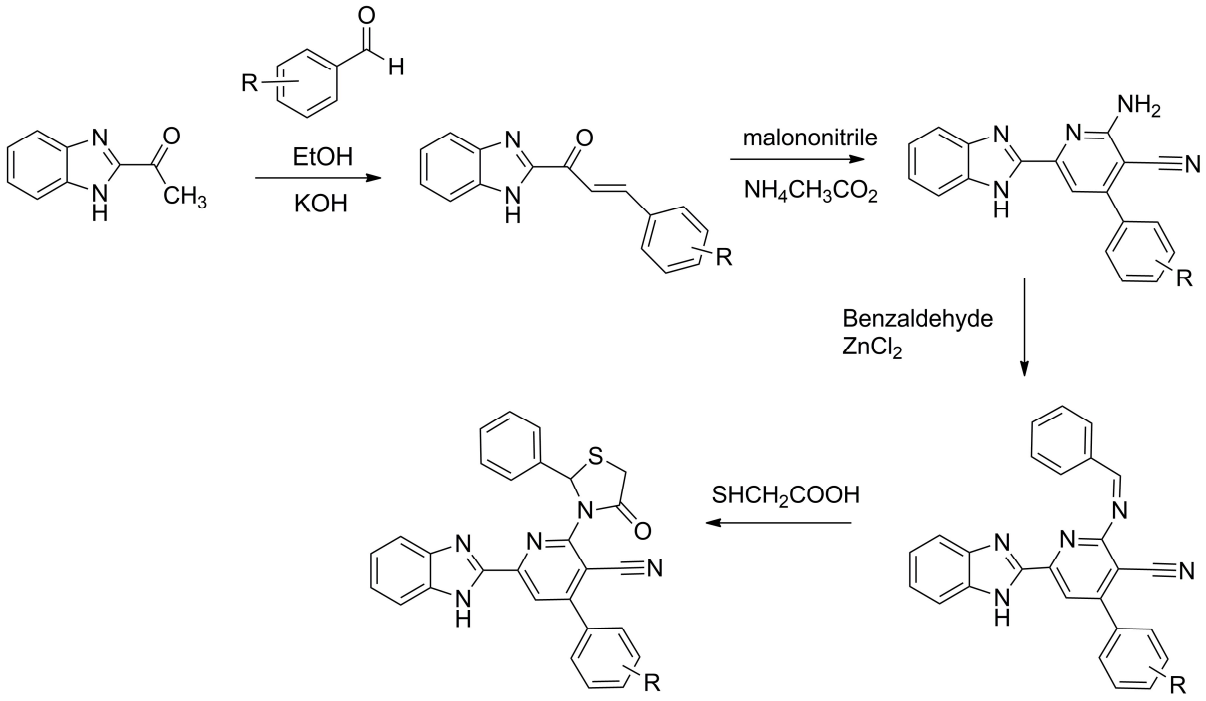
The anti-ulcer activity of these hybrids was tested *in vivo* by the ethanol-induced gastric ulcer model in rats. All compounds were found to exhibit anti-ulcer activity. Pyrazoles have been previously reported to inhibit the bacterium *Helicobacter pylori*, the causative agent of gastritis and gastric ulcers (Haque *et al*., 2002). The hybridization of these two pharmacophores showed comparable or higher anti-ulcer activity than omeprazole (Noor *et al*., 2017).



Scheme 1.17 Synthetic procedure for benzimidazole-pyrazole hybrids

A series of thiazolidinone and cyanopyridine based benzimidazoles were synthesised from benzimidazoyl ethanone and converted to chalcones with substituted benzaldehydes under basic conditions (**Scheme 1.18**). The chalcone intermediates then reacted in a Knoevenagel

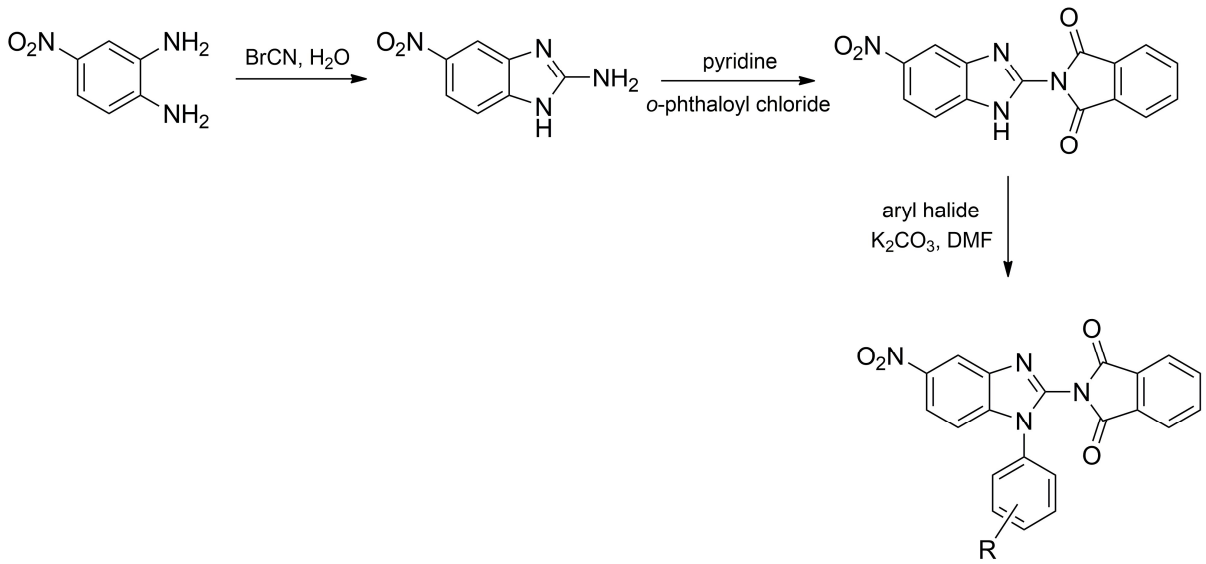
condensation with malanonitrile and ammonium acetate to produce 2-aminobenzimidazole-cyanopyridines. This was followed by condensation of the amine group with benzaldehydes yielding an imine intermediate, which was converted to thiazolidinones with thioglycolic acid (**Scheme 1.18**). The synthesized compounds showed antibacterial activity comparable to chloramphenicol, with compounds containing electron withdrawing groups at the *ortho* and *para* positions having the highest activity (Desai *et al*., 2014).



Scheme 1.18 Synthesis of benzimidazole-thiazolidinone-cyanopyridine hybrids

Since benzimidazoles are well-known cyclooxygenase (COX) and lipoxygenase (LOX) enzyme inhibitors (Gaba *et al*., 2014; Carvalho *et al*., 2015; Rathore *et al*., 2015) and phthalimide, a subunit of thalidomide was reported as TNF-*α* inhibitors (Deng *et al*., 2003; Casal *et al*., 2016), a hybrid molecule was designed, which incorporated both pharmacophores (**Scheme 1.19**) (Kaur and Silakari, 2018). This hybridised molecule showed

a reduction of inflammation at sub-micromolar concentrations (COX-2 IC_{50} = 100 μM, 5-LOX IC_{50}= 0.32 μM).



Scheme 1.19 Synthetic scheme to benzimidazole-thalidomide hybrids

1.4 Thiazolidinones

Thiazolidinones belong to a class of heterocyclic compounds containing sulphur, nitrogen and a carbonyl group in a five membered ring. When these molecules have a carbonyl group at position 4, they are called 4-thiazolidinone derivatives (**Figure 1.13**). Substitutions at positions 2, 3 and 5 can lead to a large number of new derivatives. The 4-thiazolidinones occur as a yellow odourless solid, soluble in water and ethanol.

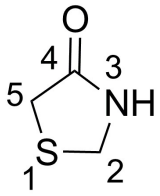
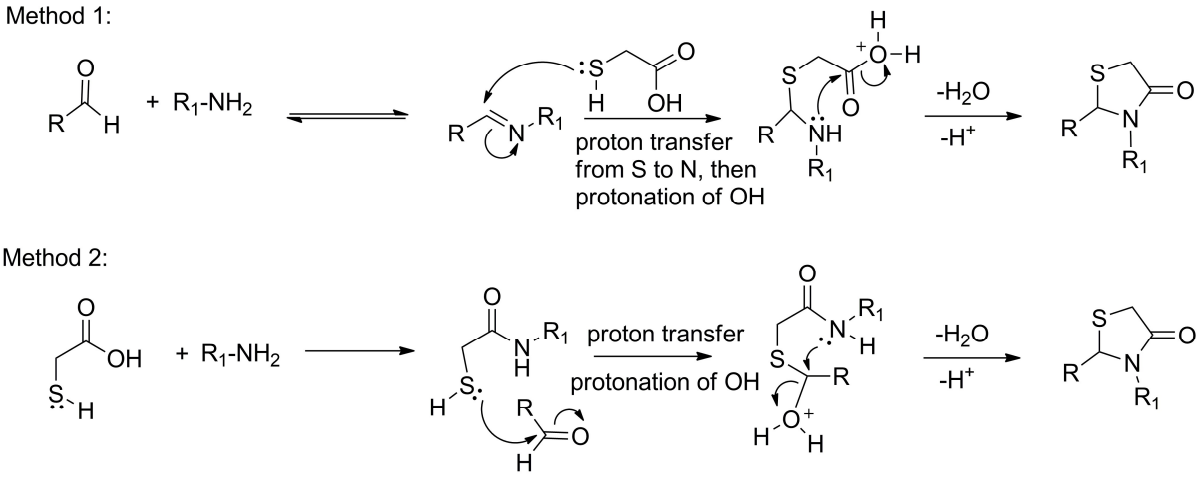


Figure 1.13 Structure of 4-thiazolidinone

1.4.1 Synthesis

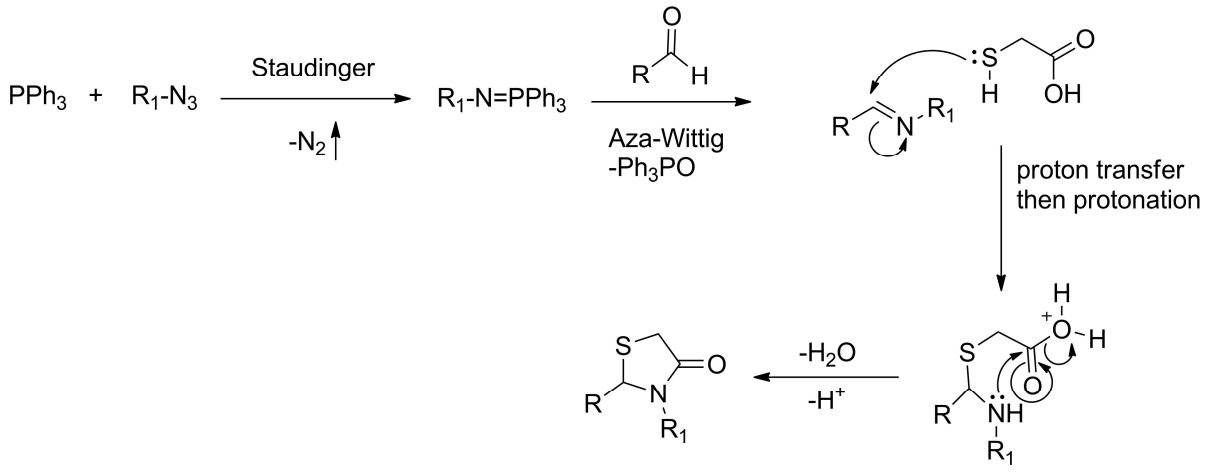
Thiazolidinones and their derivatives are synthesised from various methods using different substrates and reagents. Substitutions occur at the 2, 3 and 5 positions. They are commonly synthesised by the cyclisation of aliphatic acyclic compounds using thioglycolic acid (TGA) and thiourea (Brown, 1961; Yusuf and Jain, 2014; Manjal *et al*., 2017). TGA reacts with Schiff bases giving rise to 2-substituted-4-thiazolidinones.

The reaction mechanism is well documented (**Scheme 1.20**) (Bolognese *et al*., 2004; Kumar *et al*., 2013). Two mechanisms are proposed. The first proceeds by the formation of an imine from an aldehyde and amine. Thioglycolic acid then adds to the imine through the sulfur and cyclises in the following step by nucleophilic substitution of the amide at the carbonyl group of the acid. The second approach is the condensation of the amine with TGA, forming a thioamide, which is followed by condensation of the aldehyde with the thiol group of the thioamide resulting in the formation of the thiazolidinone.



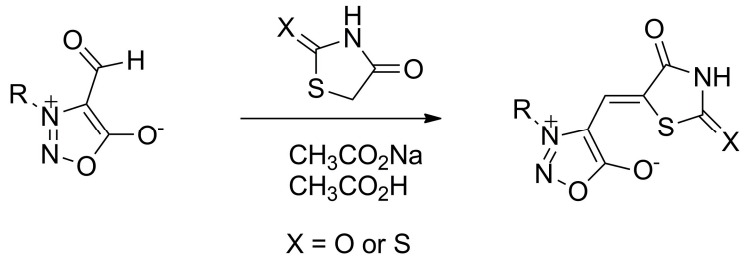
Scheme 1.20 Mechanism showing cyclisation of thiazolidinone

Alternative synthetic approaches have also been documented. A solvent free, one-pot synthetic route using azides, triphenylphosphine, aldehydes and TGA in a Staudinger/aza-Wittig reaction was also successful under microwave conditions (**Scheme 1.21**) (Shanmugavelan et al., 2014). This reaction commences with the *in situ* generation of phosphazenes, which give rise to imines when reacted with aldehydes. This is followed by nucleophilic attack of TGA on the imines and proceeds to intramolecular cyclisation as in the previous mechanism (Shanmugavelan *et al*., 2014).



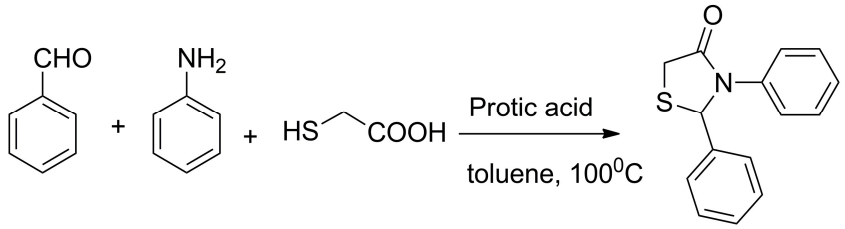
Scheme 1.21 Mechanism showing the synthesis of thiazolidinones using the aza-Wittig reaction

A sydnonyl group was substituted onto the thiazolidinone by a modified Knoevenagel condensation (**Scheme 1.22**). This reaction was carried out with 3-aryl-4-formylsydnones and condensed with thiazolidine-2,4-dione or 2-thioxo-thiazolidinone in a piperidine/glacial acetic acid buffer system using ethanol (Shih *et al*., 2015).



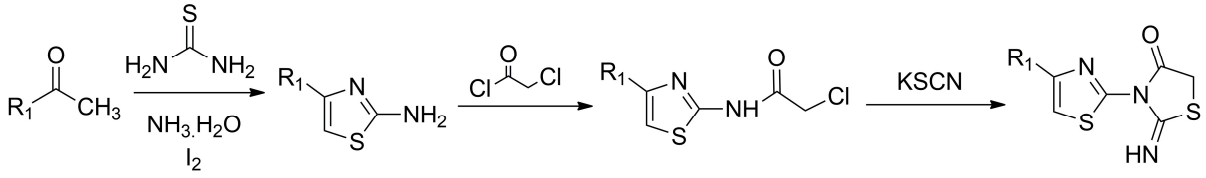
Scheme 1.22 Synthesis of sydnonyl-thiazolidinone derivatives

Thiazolidinones substituted at the 2- and 3- positions were synthesised in a one-pot reaction from aromatic aldehydes, anilines and thioglycolic acid using various protic acids (TfOH, H_2SO_4, *p*-TsOH, TFA) as catalysts (**Scheme 1.23**) (Kumar *et al*., 2013).



Scheme 1.23 Protic acid catalysed synthesis of thiazolidinone derivatives

A series of 2-imino-3-(4-arylthiazol-2-yl)thiazolidin-4-ones were synthesised via a 2-amino-4-arylthiazole intermediate, which was reacted with chloroacetyl chloride to produce 2-chloroacetamido-4-arylthiazoles. This was then treated with potassium thiocyanate under reflux with acetone to give rise to the imino thiazolidinone derivatives (**Scheme 1.24**) (Liu *et al*., 2000).



Scheme 1.24 Synthesis of 2-imino-3-(4-arylthiazol-2-yl)-thiazolidinones

1.4.2 Biological activity

Thiazolidinones are attractive pharmacophores in the field of medicinal chemistry. They occur in nature as a substructure of penicillin, and in the antibiotic actithiazic acid (**Figure 1.14**), isolated from *Actinomyces* and *Streptomyces spp*. (Schenck and De Rose, 1952). This is a versatile scaffold found in a number of clinically used drugs such as pioglitazone (antidiabetic), etozoline (antihypertensive), ralitoline (anticonvulsant) and thiazolidomycin (antibacterial) (**Figure 1.14**).

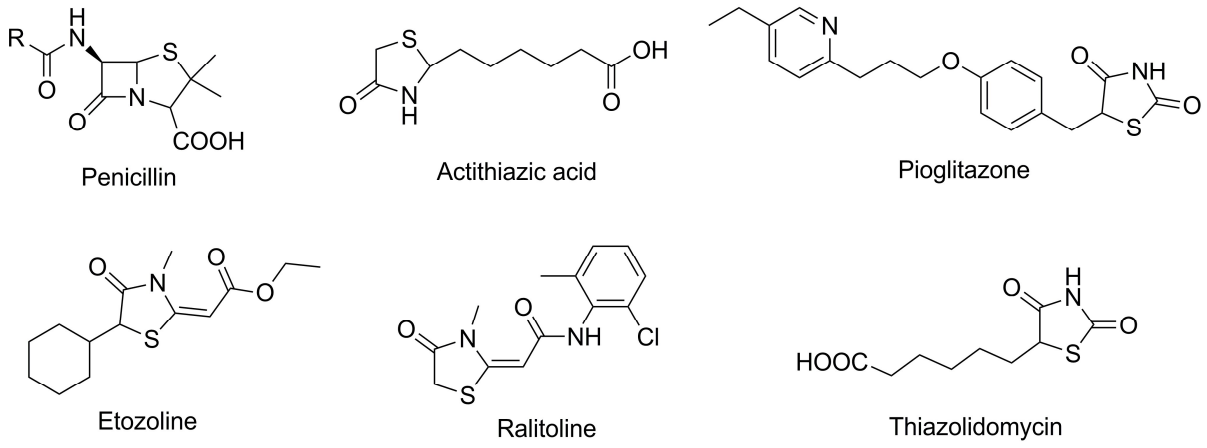
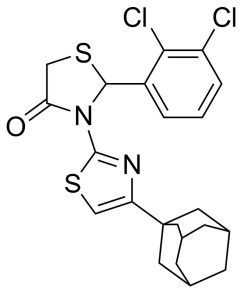


Figure 1.14 Structures of thiazolidinone drugs

Thiazolidinone derivatives are known to exhibit antibacterial (Andres *et al*., 2000; Mahmoud *et al*., 2013), anticancer (Angapelly *et al*., 2017; Sharma *et al*., 2017), anti-tubercular, anti-inflammatory (Abdellatif *et al*., 2016), anticonvulsant, antidiabetic (Nanjan *et al*., 2018), FSH receptor agonist (Jain *et al*., 2012), anti-HIV (Suryawanshi *et al*., 2017), antimalarial (Jain *et al*., 2018), antifungal (Carradori *et al*., 2017), COX-2 inhibitory (Manjal *et al*., 2017) and antihypertensive activity (Bhalgat *et al*., 2014).

Thiazolidinine-thiazole-adamantine hybrid molecules showed broad spectrum activity against Gram +ve and Gram –ve bacteria, and fungi. Their MIC activity was between 4 to 14 times

more active than the reference drugs ampicillin and streptomycin. These molecules also showed excellent antifungal activity with MICs as low as 0.21 μM. The derivative with a dichlorophenyl substituted thiazolidinone moiety (**Figure 1.15**), recorded the best overall antibacterial (MIC 9.0 – 62.5 μM) and antifungal activity (MIC 0.21- 0.42 μM) (Pitta *et al*., 2015). The compounds showed no activity against HIV-1 reverse transcriptase.



3-(4-(adamantan-1-yl)thiazol-2-
(2,3-dichlorophenyl)thiazolidinone

Figure 1.15 3-(4-(adamantan-1-yl)thiazol-2-(2,3-dichlorophenyl)thiazolidinone

Pyridine-thiazolidinone derivatives were shown to possess anticancer activity by carbonic anhydrase IX (CAIX) inhibition (Ansari *et al*., 2018). CAIX is a protein responsible for cell survival by regulating cellular pH levels (Sedlakova *et al*., 2014). It is also responsible for the progression of cancer by stimulating cancer cell migration and adhesion (Kato *et al*., 2013). Derivatives of 2-(phenylimino)-3-(pyridine-4-ylmethyl)thiazolidin-4-one (**Figure 1.16**) were the most effective with an IC_{50} value of 1.61 and 1.84 μM against MCF-7 (breast) and HepG-2 (liver) cancer cell lines.

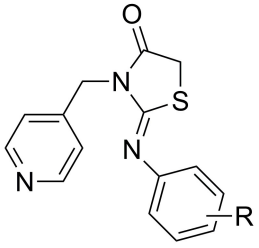
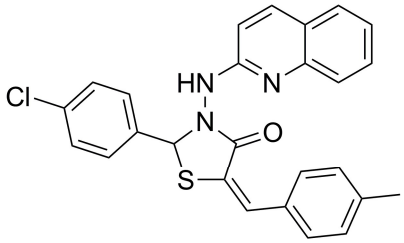


Figure 1.16 The structure of 2-(phenylimino)-3-(pyridine-4-ylmethyl)thiazolidin-4-one

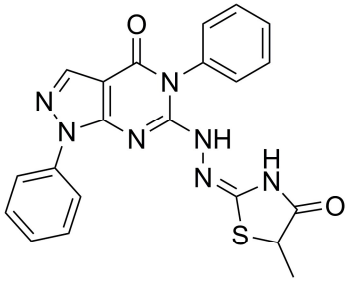
A series of quinoline-thiazolidinone hybrids (**Figure 1.17**) showed good *in vitro* antimalarial activity with EC_{50}values of 0.42-2.62 and 0.82-11.41 μg mL^{-1} against 3D7 and RKL-9 strains of *Plasmodium falciparum* respectively. The most potent activity was shown by the chloro derivative (Figure 21) with an EC_{50} of 0.42 and 0.82 μg mL^{-1} against 3D7 and RKL-9 respectively (Jain *et al*., 2018).



5-(4-methylbenzylidene)-2-(4-chlorophenyl)-3-(4-methylquinolin-2-ylamino) thiazolidin-4-one

Figure 1.17 Antimalarial thiazolidinone hybrid molecules

Pyrazolo[3,4-d]pyrimidine-thiazolidinone derivatives (**Figure 1.18**) displayed higher *in vitro* COX-1 and COX-2 anti-inflammatory activity (IC_{50} = 2.87-5.27 μM) than the reference drug celecoxib (IC_{50} = 5.64 μM). The compounds were further tested for their *in vivo* anti-inflamatory activity using the formalin-induced paw edema bioassay. The activity (AI) of the compounds (%AI of 48.6-85.7) was also higher than celecoxib (%AI = 46.4) (Tageldin *et al*., 2018).



5-methyl-2-(2-(4-oxo-1,5-diphenyl-4,5-dihydropyrazolo [3,4-d]pyrimidin-6-yl)hydrazono)thiazolidin-4-one

Figure 1.18 Pyrazolo[3,4-d]pyrimidine-thiazolidinone derivatives with anti-inflammatory activity

1.5 Amino Acids

Amino acids are naturally occurring organic compounds and the building blocks of proteins and enzymes. They contain a carboxyl group (COOH), amino group (NH_2) and a variable side chain at the α-carbon (**Figure 1.19**). Amino acids exist as neutral or zwitterions that make them partially soluble in water. Naturally occurring *α*-amino acids possess a stereogenic centre, but are common and commercially available.

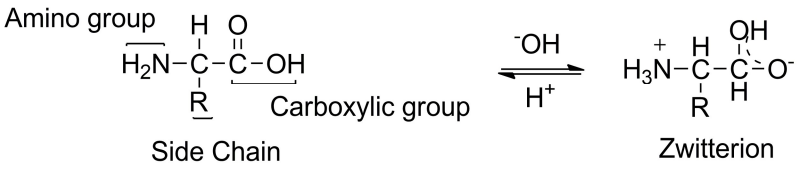


Figure 1.19 General structure of *α*-amino acid

Amino acids play a significant role in drug discovery and occur in antibiotics such as bacitracin and vancomycin. They are becoming prominent features in new drugs due to having two orthogonal functional groups, which are easily modified by conventional chemistry, for example acylation and amidation. They also have a wide variety of side chains attached to a chiral centre.

Conjugation of heterocycles with amino acids and peptides is an important branch of biomedical research. Amino acids are found naturally in bacterial cells and conjugating an amino acid to another pharmacophore aids in facilitating the passage of the conjugated drug across cell membranes for release into the cytosol (Beck, 2012). They also bind specifically to their *in vivo* targets, increasing the potency of the drug. Other advantages of peptide drugs include lower toxicity, broad range of targets and low accumulation in tissues (Craik, 2013).

A study of 1,3-benzodioxole-amino acid hybrids (**Figure 1.20**) on antitumor activity *in vivo* indicated that compounds linked to leucine, valine, glutamic acid and glycine inhibited tumour mass in mice by 66-83% (Leite *et al*., 2004).

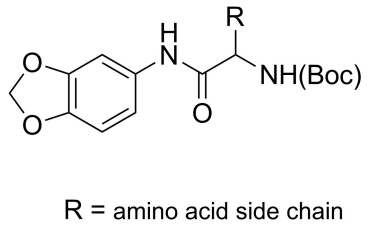


Figure 1.20 General structure of a 1,3-benzodioxole-amino acid derivative

N-glycoconjugates of D-glucose linked with amino acids (**Figure 1.21**) exhibited good activity against Gram +ve and –ve bacterial strains, with the highest activity shown by the tryptophan derivative against *E. coli* and the isoleucine derivative against *K. pneumoniae*, both with a MIC of 16 μg mL^{-1} (Baig *et al*., 2012).

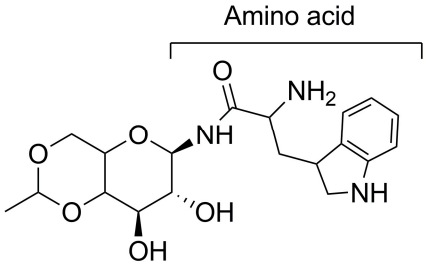
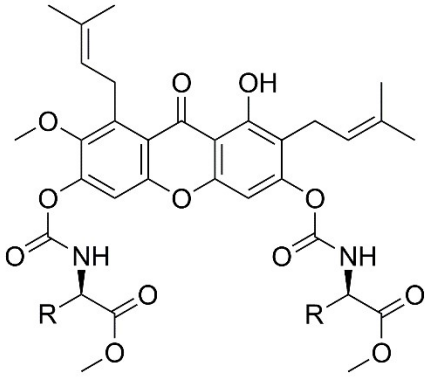


Figure 1.21 Structure of D-glucose linked-amino acids

Xanthone conjugated amino acids modified with cationic amino acids and lipophilic chains (**Figure 1.22**) displayed promising antimicrobial activity against multidrug-resistant Gram +ve MRSA and VRE (MIC = 0.5-3.0 μg mL^{-1})(Koh et al., 2015). Cationic amino acids/peptides are components of the innate immune system and act by disrupting the bacterial membrane (Bai *et al*., 2012).

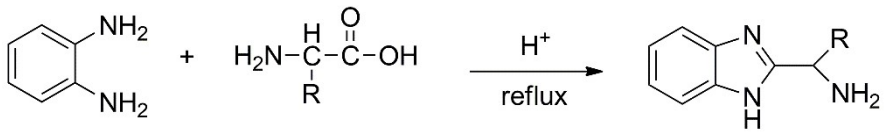


R= cationic amino acid side chain

Figure 1.22 Structure of xanthone conjugated amino acids

1.6 Synthesis involving amino acids and benzimidazoles

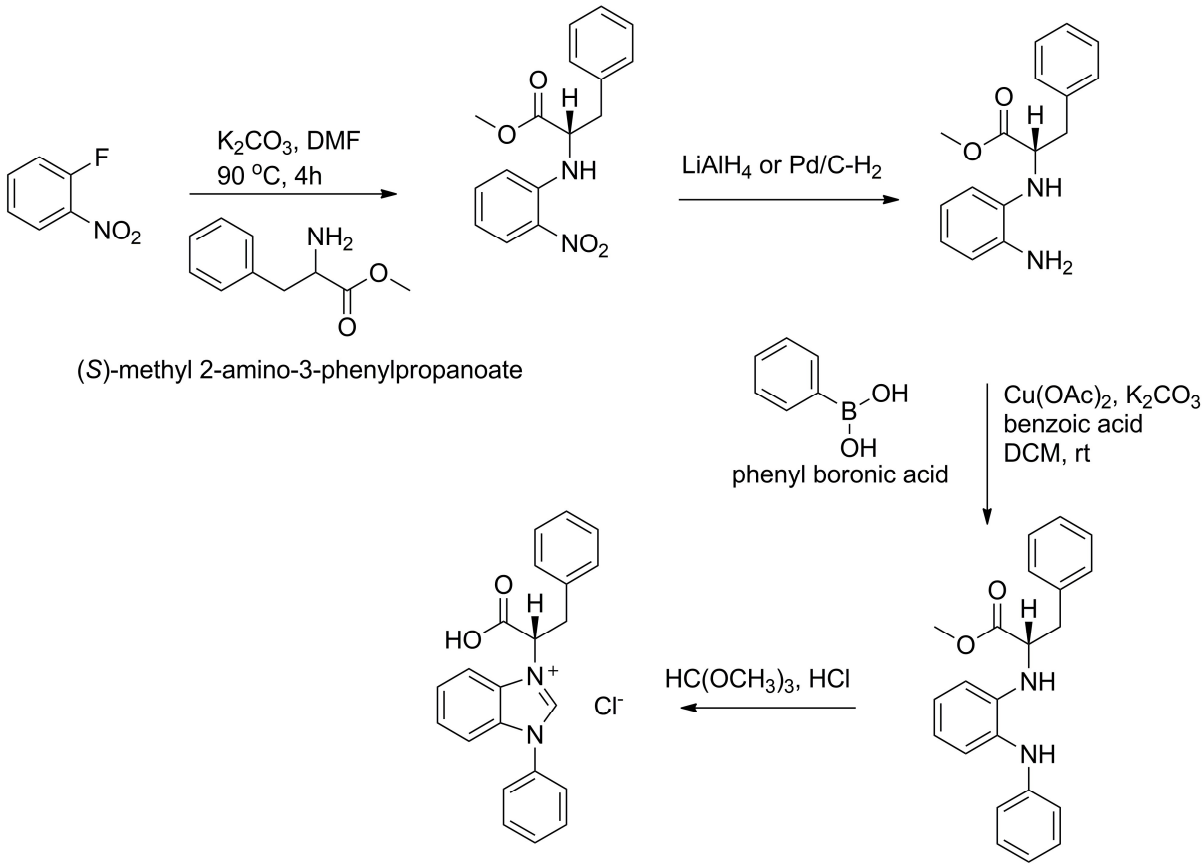
There are few reports in literature for the synthesis of benzimidazoles coupled with amino acid residues, but numerous reports on amino acid conjugated heterocycles. A commonly reported reaction is cyclisation of *ortho* phenylenediamine with *α*-amino acids (**Scheme 1.25**) to synthesise benzimidazoles with chiral substituents at C-2 possessing an amino acid side chain (Zhang *et al*., 2009; Xu *et al*., 2012; Ajani *et al*., 2016; Sabithakala *et al*., 2016).



Scheme 1.25 General scheme involving amino acids to synthesise benzimidazoles

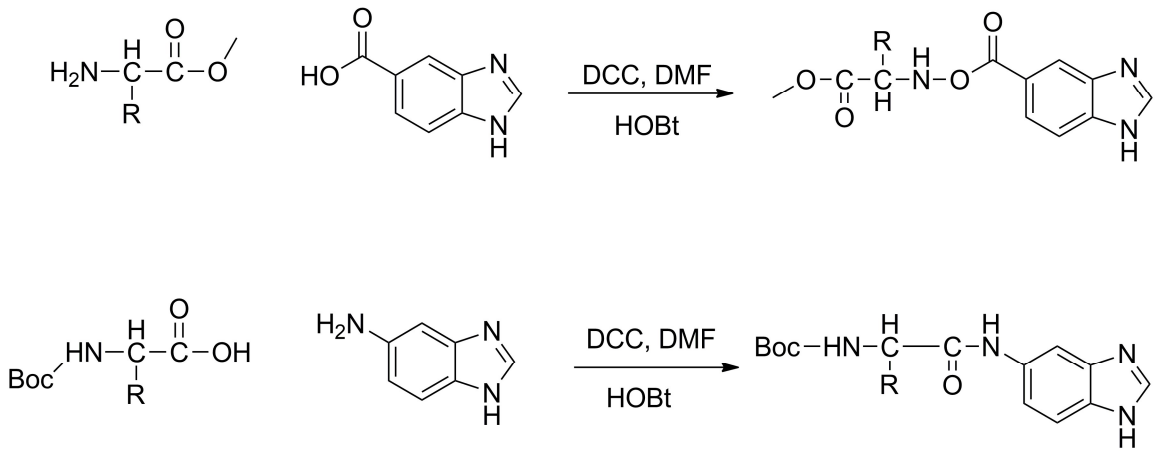
Amino acids do not easily undergo standard chemical conversions due to their acid/base character and low solubility in organic solvents. It is therefore necessary to protect the amine and/or carboxylic acid functional groups. The carboxylic acid is easily converted to an ester and the amine is protected with protecting groups such as *t*-butoxycarbonyl (Boc) and benzyloxycarbonyl (Cbz).

In an alternate synthetic procedure to benzimidazoles synthesised from amino acids, the amino acids protected at the carboxyl end as esters were substituted with the fluorine in *ortho* nitrofluorobenzene in the presence of a base to form nitrobenzylamines. The nitro group was subsequently reduced and the resultant amino group arylated by a Chan-Lam cross coupling reaction before the molecule was cyclised to benzimidazole salts with trimethoxymethane (**Scheme 1.26**) (Zhou *et al*., 2016).



Scheme 1.26 Synthesis of chiral benzimidazoles from an *o*-fluoronitrobenzene precursor

α-Amino acids were also coupled to benzimidazoles with either carboxylic acid or amino groups using esterified or Boc protected amino acids respectively. This coupling was carried out with the coupling reagents, dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole (HOBt) (**Scheme 1.27**) (Leite *et al*., 2004; Kim *et al*., 2009; Koh *et al*., 2014).



Scheme 1.27 Amino acids coupled to the benzimidazole framework by a carboxyl or amino group

1.7 References

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Chapter 2 Conventional and Microwave-Assisted Synthesis of Benzimidazole-Thiazolidinone Derivatives and their Antibacterial Activity

***** 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 **7c** is referred to as **A-7c**.

Abstract

A series of benzimidazole thiazolidinone derivatives were designed and synthesized using conventional and microwave-assisted techniques. The hybrid molecules were prepared in a six step synthesis, where a comparison was made in steps 1, 2 and 4 between the conventional methods and using microwave irradiation. The reduction steps 3 and 5 were only possible by the conventional method and step 6, formation of the thiazolidinone ring only worked with microwave irradiation. Those steps where a comparison was made resulted in a significant reduction in reaction times and improvement in yields using microwave irradiation. All synthesized compounds **7a-l** were evaluated for their *in vitro* antibacterial activity against two Gram +ve bacteria, *Staphylococcus aureus* and MRSA and four Gram – ve bacteria, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli* and *Salmonella typhimurium*, using the Minimum Bactericidal Concentration assay. Compounds **7f** (2'-nitro derivative) and **7i** (4'-chloro derivative) were found to be most active with MBC values of 1.9 and 2.0 mM respectively against *P. aeruginosa*. All other compounds showed activity between 3.9 and 8.3 mM.

Keywords: Benzimidazole, thiazolidinone, microwave irradiation, antibacterial

2.1 Introduction

In recent years, molecular hybridisation has gained much attention. The combination of two or more active scaffolds, producing hybrid molecules provides a possible synergistic effect to improving activity while reducing the risk of side effects (Tripathi *et al.*, 2014). Benzimidazoles are an attractive moiety due to their broad range of biological applications (Campbell, 1990; Nájera and Yus, 2015; Sreerama *et al*., 2017). Synthetic benzimidazoles are widely used as molecular scaffolds in numerous drugs such ascandesertan (antihypertensive), astemizole (antihistamine) and bendamustine (anticancer) (Akhtar *et al*., 2017). Benzimidazole scaffolds are reported to show various activities such as anthelmintic (Lingala *et al*., 2011; Fraga *et al*., 2017), anticancer (Nofal *et al*., 2014), antiviral (Tonelli*et al*., 2008; Xu*et al*., 2014), antibacterial (Negi*et al*., 2017; Olayinka *et al*., 2017), anti-inflammatory (Pan *et al*., 2017; Sharma *et al*., 2017a) and antimycobacterial (Umaa *et al*., 2009; Yadav *et al*., 2015).

On the other hand, thiazolidinones are bioactive molecules that occur in natural products and pharmaceutical compounds (Shanmugavelan *et al*., 2014). Thiazolidinone derivatives are shown to posses significant antimicrobial bioactivities (Adhikari *et al*., 2012; Pitta *et al*., 2015) due to their ability to prevent bacterial cell wall production by inhibiting the Mur B bacterial enzyme (Andres *et al*., 2000). The synergistic combination of benzimidazoles with thiazolidinones have the possible advantages of increasing the antimicrobial spectrum, dosage reduction, and minimizing drug resistance and adverse side effects (Shanmugapandiyan *et al*., 2010; Mahmoud *et al*., 2013; Desai *et al*., 2014).

The conventional approach to the synthesis of 1,2-disubstituted benzimidazoles is the condensation of an aldehyde with *o*-phenylenediamine (Yeong *et al.*, 2014). Different

aldehydes lead to different substituents at the 2-position, while substitution on the amino groups of the diamine lead to different substitution patterns on the nitrogen. Thiazolidinones are synthesized by various methods depending on their substitution patterns. The synthesis of unsubstituted thiazolidinones is conducted by refluxing chloroacetic acid with thiourea. Disubstituted thiazolidinones are formed by one-pot three component synthesis, involving the reaction between aldehydes, amines and thioglycolic acid in the presence of tetrahydrofuran (Manjal *et al*., 2017).

Microwave irradiation (MW) is a rapidly growing area in organic synthesis and has become popularised due to its short reaction times and higher yields (Ravichandran and Karthikeyan, 2011; Santagada *et al*., 2004). Several benzimidazole compounds and their derivatives have been synthesised using microwave irradiation (VanVliet *et al*., 2005; Bui *et al*., 2016; Sharma *et al*., 2017b). Simple benzimidazoles were prepared from *o*-nitrobenzazldehyde, tin chloride and simple carboxylic acids in a one-pot reaction using microwave irradiation (VanVliet *et al*., 2005). 4-Oxo-4*H*-quinolizinecarbaldehyde or naphthalenecarbaldehyde were used together with *o*-phenylenediamines, *o*-nitroaniline or 2,3-pyridinediamine using either sodium hydrosufite or sodium metabisulfite under microwave conditions to synthesise 2-quinolizinylbenzimidazoles and 2-naphthalylbenzimidazoles (Bui *et al*., 2016). Most recently, benzimidazoles were made in a similar manner to that reported in Bui *et al*. (2016), but used the esters of *o*-phenylenediamine in order to link a thiazolidine moiety to the framework of the benzimidazole (Sharma *et al*., 2017b).

We herein report the synthesis of new benzimidazole-thiazolidinone hybrid molecules and comparison of several steps using the conventional and microwave methods with

phenylenediamine derivatives, and nitrobenzaldehyde. We also report on the antibacterial activity of the synthesised compounds.

2.2 Experimental

2.2.1 Chemistry

Chemicals and reagent grade solvents were purchased from Sigma Aldrich and Merck, South Africa. Thin layer chromatography was performed on silica gel 60 F_{254} plates (Merck). For column chromatography, silica gel (60-120 mesh, Merck) and varying ratios of ethyl acetate: hexane was used as the stationary and mobile phases respectively. Infrared spectra were obtained on a Perkin Elmer Spectrum 100 FTIR spectrometer with universal attenuated total reflectance sampling accessory. Melting points were determined on a Stuart Smart Scientific melting point instrument. NMR spectra were recorded on a Bruker Avance 400 MHz instrument using DMSO-d_6 and CD_3OD as solvents and referenced to the solvent peaks at δ 2.50 and δ39.51 for ^1H and ^{13}C NMR respectively for DMSO-d_6 and δ 4.78 (^1H) and δ 49.15 (^{13}C) for CD_3OD relative to the internal standard tetramethylsilane (TMS). High Resolution Mass Spectrometry was carried out on a Bruker microTOF-Q II ESI instrument. Microwave assisted reactions were performed using a CEM Discover, Explorer-12 Hybrid microwave.

Esterification of 4-fluoro-3-nitrobenzoic acid

Conventional method

4-Fluoro-3-nitrobenzoic acid (1.0 g, 5.5 mmol) was dissolved in 20 mL methanol. Concentrated sulfuric acid (0.5 mL) was then added dropwise at 0-5 °C and the reaction mixture refluxed for 4 h. On completion, the reaction was basified to pH 8 using $NaHCO_3$ and extracted with ethyl acetate (3 x 15 mL). The organic layer was dried over anhydrous

Na_2SO_4 and concentrated under reduced pressure to yield **2** as a cream coloured solid in a yield of 93%.

Microwave irradiation

4-Fluoro-3-nitrobenzoic acid (0.5 g, 2.7 mmol) was dissolved with 5 mL methanol in a 10 mL microwave process vial and 2-3 drops concentrated sulphuric acid added. The reaction mixture was heated with stirring for 10 minutes at 80 ºC using microwave irradiation. On completion the reaction was worked up as above producing a yield of 95%.

Synthesis of methyl-4-(4-fluorophenylamino)-3-nitrobenzoate (3)

Conventional method

Methyl-4-fluoro-3-nitrobenzoate **2** (1.0 g, 5.0 mmol) and 4-fluorophenylaniline (0.65 mL, 5.0 mmol) were dissolved in DMF (10 mL) and stirred at room temperature for 12 h whilst being monitored by TLC. Upon completion, the reaction mixture was dissolved in 20 mL water and 10% Na_2CO_3 (10 mL) and extracted with ethyl acetate (2 x 20 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford a solid product, which was further purified by column chromatography (ethyl acetate: hexane, 30:70) to yield a reddish solid **3** in 80% yield.

Microwave irradiation

Methyl-4-fluoro-3-nitrobenzoate **2** (0.5 g, 2.5 mmol) and 4-fluorophenylaniline (0.32 mL, 2.5 mmol) were dissolved in 5 mL ethanol and heated under microwave irradiation for 5 min at 120 ºC. The reaction mixture was worked up as above to obtain a reddish solid **3** in good yields of 90%.

Reduction of methyl-4-(4-fluorophenylamino)-3-nitrobenzoate (4)

Methyl 4-(4-fluorophenylamino)-3-nitrobenzoate **3** (1.0 g, 1 mmol) and activated palladium on carbon was added to a two necked round bottom flask and dissolved in dry methanol (10 mL). The flask was evacuated and hydrogen gas slowly introduced to the flask. This was repeated three times. The reaction was stirred under hydrogen atmosphere for four hours until completion (monitored by TLC), where the reaction was filtered through celite 545 to remove the Pd/C. The filtrate was evaporated under reduced pressure and the crude product purified by column chromatography (ethyl acetate:hexane, 30:70) to yield a brown solid with a yield of 78%.

Synthesis of methyl-1-(4-fluorophenyl)-2-(4-nitrophenyl)-1*H*-benzimidazole-5-carboxylate (5)

Conventional method

Methyl-3-amino-4-(4-fluorophenylamino)benzoate **4** (1.0 g, 4.0 mmol), 4-nitrobenzaldehyde 0.5 g, 4.0 mmol) and 20 mol% $Na_2S_2O_5$ was refluxed for 12 h in ethanol, after which the solvent was evaporated under reduced pressure. The crude product was dissolved in 20 mL water, extracted with ethyl acetate (3 x 15 mL) and the organic layer dried over anhydrous Na_2SO_4 before being concentrated under reduced pressure. The solid was purified by column chromatography (ethyl acetate:hexane, 30:70) to yield a brown solid **5** with a yield of 81%.

Microwave irradiation

Methyl-3-amino-4-(4-fluorophenylamino) benzoate **4** (1.0 g, 4.0 mmol) and 4-nitrobenzaldehyde (0.5 g, 4.0 mmol) was dissolved in 5 mL ethanol and subject to microwave irradiation for 10 min at 110 °C. The reaction was worked up as above and purified by

column chromatography (ethyl acetate:hexane, 30:70) to yield a brown solid **5** with a yield of 85%.

General procedure for the preparation of methyl-2-(4-aminophenyl)-1-(4-fluorophenyl)-1*H*-benzimidazole-5-carboxylate (6)

Stannous chloride (7.5 mmol) and methyl-1-(4-fluorophenyl)-2-(4-nitrophenyl)-1*H*-benzimidazole-5-carboxylate **5** (2.5 mmol) was refluxed in ethanol (20 mL) for 8h. On completion the solvent was evaporated under reduced pressure and the crude product basified (pH 8) with $NaHCO_3$ and extracted with ethyl acetate (3 x 15 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The solid was purified using column chromatography (ethyl acetate:hexane, 30:70) to yield a brown solid **6** with a yield of 85%.

General procedure for the preparation of methyl-1-(4-fluorophenyl)-2-(4-(4-oxo-2-phenylthiazolidinyl)phenyl)-benzimidazole-5-carboxylates (7a-l)

Methyl-2-(4-aminophenyl)-1-(4-fluorophenyl)-1*H*-benzimidazole-5-carboxylates **6** (0.5 g, 1.4 mmol) and various substituted benzaldehydes (2.8 mmol) were mixed in a 10 mL microwave process vial with 3-5 mL ethanol. Thioglycolic acid (5.6 mmol; 0.5 mL) and 3-5 drops glacial acetic acid was added dropwise to the mixture before being subject to microwave irradiation for 30 min at 110°C. On completion, the reaction was basified (pH 8) using $NaHCO_3$, extracted with ethyl acetate (3 x 10 mL) and the organic layer dried over anhydrous Na_2SO_4, concentrated under reduced pressure and purified by column chromatography (ethyl acetate:hexane, 30:70) to obtain **7a-l** in yields of 74-88%.

Methyl 1-(4-fluorophenyl)-2-(4-(4-oxo-2-phenylthiazolidin-3-yl)phenyl)-1*H*-benzimidazole-5-carboxylate (**7a**); cream solid; 82% yield; mp 165-166 °C; IR (KBr) υ_{max}: 1693 (C=O); ^{1}H NMR (DMSO-*d*$_6$, 400 MHz) δ8.32 (1H, d, *J* = 1.2 Hz, H-4), 7.89 (1H, dd, *J* = 8.5, 1.4 Hz, H-6), 7.49 (2H, dd, *J* = 8.9, 5.0 Hz, H-2a/6a), 7.43 (2H, d, *J* = 8.7 Hz, H-2b/6b), 7.39 (4H, d, *J* = 8.8 Hz, H- 3a/5a, H-2d/6d),7.35 (2H, d, *J* = 8.7 Hz, H-3b/5b), 7.26-7.23 (3H, m, H-3d, H-4d, H-5d), 7.23 (1H, d, *J* = 8.5 Hz, H-7), 6.55 (1H, s, H-2c), 4.01 (1H, d, *J* = 15.7 Hz, H-5ci), 3.88 (1H, d, *J* = 15.7 Hz, H-5cii), 3.88 (3H, s, H-11); ^{13}C NMR (DMSO-*d*$_6$, 100 MHz) δ 170.6 (C-4c), 166.5 (C-10), 161.9 (d, *J* = 245.5 Hz, C-4a), 153.2 (C-2), 141.9 (C-8), 140.4 (C-9), 139.6 (C-1d), 139.0 (C-4b), 132.1 (d, *J* = 2.8 Hz, C-1a),129.9 (d, *J* = 9.1 Hz, C-2a/6a), 129.2 (C-2b/6b), 128.6 (C-3d/5d), 128.5 (C-4d), 126.9 (C-3b/5b), 126.5 (C-1b), 124.6 (C-2d/6d), 124.5 (C-6), 124.4 (C-5), 120.8 (C-4), 117.0 (d, *J* = 23.0 Hz, C-3a/5a), 110.6 (C-7), 63.0 (C-2c), 52.1 (C-11), 32.8 (C-5c). HRMS (*m/z*): [M+Na] 546.1270 (calculated for $C_{30}H_{22}FN_3O_3SNa$, 546.1264).

Methyl 1-(4-fluorophenyl)-2-(4-(2-(2-fluorophenyl)-4-oxothiazolidin-3-yl)phenyl)-1*H*-benzimidazole-5-carboxylate (**7b**); cream solid; 85% yield; mp 210-212 °C; IR (KBr) υ_{max}:1685 (C=O); ^{1}H NMR (DMSO-*d*$_6$, 400 MHz) δ 8.32 (1H, d, *J* = 1.2 Hz, H-4), 7.89 (1H, dd, *J* = 8.5, 1.5 Hz, H-6), 7.50 (2H, dd, *J* = 8.9, 5.0 Hz, H-2a/6a), 7.44 (2H, d, *J* = 8.8 Hz, H-2b/6b), 7.42 (2H, t, *J* = 8.8 Hz, H-4d, H-6d),7.40 (2H, t, *J* = 8.7 Hz, H-3a/5a), 7.24 (1H, d, *J* = 8.6 Hz, H-7), 7.09 (2H, t, *J* = 8.8 Hz, H-3d, H-5d), 6.57 (1H, s, H-2c), 4.02 (1H, dd, *J* =15.8 Hz, H-5ci), 3.89 (1H, d, *J* = 15.8 Hz, H-5cii), 3.88 (3H, s, H-11); ^{13}C NMR (DMSO-*d*$_6$, 100 MHz) δ 170.4 (C-4c), 166.5 (C-10), 161.9 (d, *J* = 245.5 Hz, C-4a, C-2d), 153.2 (C-2), 141.9 (C-8), 140.4 (C-9), 138.8 (C-4b), 135.7 (d, *J* = 3.5 Hz, C-1d), 132.0 (d, *J* = 3.5 Hz, C-1a), 129.9 (d, *J* = 9.0 Hz, C-2a/6a), 129.5 (C-2b/6b), 129.3 (d, *J* = 8.3 Hz, C-4d, C-6d), 126.6 (C-1b), 124.7 (C-3b/5b), 124.5 (C-6), 124.3 (C-5), 120.8 (C-4), 116.9 (d, *J* = 22.8 Hz, C-

3a/5a), 115.5 (d, *J* = 21.7 Hz, C-3d, C-5d), 110.6 (C-7), 62.3 (C-2c), 52.1 (C-11), 32.8 (C-5c). HRMS (*m/z*): [M+Na] 564.1169 (calculated for $C_{30}H_{21}F_2N_3O_3SNa$, 564.1169).

Methyl 1-(4-fluorophenyl)-2-(4-(2-(4-fluorophenyl)-4-oxothiazolidin-3-yl)phenyl)-1*H*-benzimidazole-5-carboxylate (**7c**); pale yellow solid; 83% yield; mp 191-192 °C; IR (KBr) υ_{max}:1693 (C=O); ^{1}H NMR (DMSO-*d*$_6$, 400 MHz) δ8.32 (1H, d, *J* = 1.4 Hz, H-4), 7.89 (1H, dd, *J* = 8.5, 1.4 Hz, H-6), 7.48 (2H, dd, *J* = 8.8, 5.0 Hz, H-2a/6a), 7.45 (2H, d, *J* = 8.6 Hz, H-2b/6b),7.39 (2H, d, *J* = 8.6 Hz, H-3b/5b),7.38 (2H, t, *J* = 8.8 Hz, H-3a/5a), 7.30 (2H, ddd, *J* = 7.5, 5.6, 2.1 Hz, H-2d/6d), 7.24 (1H, d, *J*= 8.5 Hz, H-7), 7.10 (2H, t, *J* = 7.5 Hz, H-3d/5d), 6.73 (1H, s, H-2c), 3.99 (1H, dd, *J* =16.6, 1.2 Hz, H-5ci), 3.89 (1H, d, *J* = 16.6 Hz, H-5cii), 3.87 (3H, s, H-11); ^{13}C NMR (DMSO-*d*$_6$, 100 MHz) δ170.6 (C-4c), 166.7 (C-10), 162.1 (d, *J* = 245.4 Hz, C-4a), 159.9 (d, *J* = 246.0 Hz, C-4d), 153.3 (C-2), 141.9 (C-8), 140.4 (C-9), 138.9 (C-4b, C-1d), 132.1 (d, *J* = 2.8 Hz, C-1a), 130.9 (d, *J* = 8.2Hz, C-2d/6d),130.0 (d, *J* = 9.0 Hz, C-2a/6a), 129.8 (C-2b/6b),126.9 (C-1b), 124.69 (C-6), 124.60 (C-3b/5b), 124.56 (C-5), 120.9 (C-4), 117.2 (d, *J* = 22.9 Hz, C-3a/5a), 116.0 (d, *J* = 20.2 Hz, C-3d/5d), 110.8 (C-7), 58.1 (C-2c), 52.3 (C-11), 32.9 (C-5c). HRMS (*m/z*): [M+Na] 564.1170 (calculated for $C_{30}H_{21}F_2N_3O_3SNa$, 564.1169).

Methyl 1-(4-fluorophenyl)-2-(4-(4-oxo-2-(2-(trifluoromethyl)phenyl)thiazolidin-3-yl)phenyl)-1*H*-benzimidazole-5-carboxylate (**7d**); yellow solid; 82% yield; mp 215-217 °C, IR (KBr) υ_{max}:1694 (C=O); ^{1}H NMR (DMSO-*d*$_6$, 400 MHz) δ8.31 (1H, d, *J* = 1.5 Hz, H-4), 7.89 (1H, dd, *J* = 8.5, 1.5 Hz, H-6), 7.76 (1H, d, *J* = 8.0 Hz, H-6d), 7.64 (2H, d, *J* = 7.8 Hz, H-4d, H-5d), 7.49 (2H, dd, *J* = 8.8, 4.9 Hz, H-2a/6a), 7.44 (2H, d, *J* = 8.8 Hz, H-3b/5b), 7.42 (1H, m, H-3d),7.37 (2H, t, *J* = 8.8 Hz, H-3a/5a),7.36 (2H, d, *J* = 8.8 Hz, H-3b/5b),7.24 (1H, d, *J* = 8.5 Hz, H-7), 6.70 (1H, s, H-2c), 4.05 (1H, dd, *J* =15.7, 1.2 Hz, H-5ci), 3.92 (1H, d, *J* =

15.7 Hz, H-5cii), 3.87 (3H, s, H-11); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 170.7 (C-4c), 166.5 (C-10), 161.9 (d, J = 245.3 Hz, C-4a), 153.2 (C-2), 141.9 (C-8), 140.3 (C-9), 138.7 (C-4b), 138.4 (C-1d), 133.4* (C-4d), 131.9 (C-1a), 129.9 (d, J = 8.9 Hz, C-2a/6a), 129.7 (C-2b/6b),128.9* (C-5d),128.5 (C-6d), 127.2 (C-1b), 125.7 (C-3d), 125.3 (C-3b/5b), 124.5 (C-6), 124.3 (C-5), 120.8 (C-4), 116.9 (d, J = 22.7 Hz, C-3a/5a), 110.6 (C-7), 59.1 (C-2c), 52.1 (C-11), 32.5 (C-5c). HRMS (*m/z*): [M+Na] 614.1152 (calculated for $C_{31}H_{21}F_4N_3O_3SNa$, 614.1137).

* assignments are interchangeable; C-2d not observed.

Methyl 1-(4-fluorophenyl)-2-(4-(4-oxo-2-(4-(trifluoromethyl)phenyl)thiazolidin-3-yl)phenyl)-1*H*-benzimidazole-5-carboxylate (**7e**); yellow solid; 83% yield; mp 205-207 °C; IR (KBr) υ_{max}: 1694 (C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.31 (1H, d, J = 1.5 Hz, H-4), 7.89 (1H, dd, J = 8.5, 1.5 Hz, H-6), 7.74 (2H, d, J = 7.6 Hz, H-2d/6d),7.64 (2H, d, J = 7.6 Hz, H-3d/5d),7.49 (2H, dd, J = 8.9, 4.9 Hz, H-2a/6a), 7.44 (2H, d, J = 8.7 Hz, H-2b/6b), 7.37 (2H, t, J = 8.7 Hz, H-3a/5a), 7.36 (2H, d, J = 8.7 Hz, H-3b/5b), 7.24 (1H, d, J = 8.5 Hz, H-7), 6.70 (1H, s, H-2c), 4.05 (1H, dd, J = 15.8, 1.2 Hz, H-5ci), 3.92 (1H, d, J = 15.8 Hz, H-5cii), 3.87 (3H, s, H-11); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 170.8 (C-4c), 166.5 (C-10), 161.8 (d, J = 249.0 Hz, C-4a), 153.3 (C-2), 141.9 (C-8), 140.4 (C-9), 138.8 (C-4b), 138.5 (C-1d), 132.1 (d, J = 3.0 Hz, C-1a), 130.0 (d, J = 9.0 Hz, C-2a/6a), 129.7 (C-2b/6b), 128.6 (q, J = 2.2 Hz, C-2d/6d), 128.5 (q, J = 4.8 Hz, C-3d/5d), 127.2 (C-1b), 125.4 (C-3b/5b), 124.5 (C-6), 124.4 (C-5), 120.9 (C-4), 117.0 (d, J = 22.9 Hz, C-3a/5a), 110.7 (C-7), 59.1 (C-2c), 52.2 (C-11), 32.6 (C-5c). HRMS(*m/z*): [M+Na] 614.1143 (calculated for $C_{31}H_{21}F_4N_3O_3SNa$, 614.1137).

Methyl 1-(4-fluorophenyl)-2-(4-(2-(2-nitrophenyl)-4-oxothiazolidin-3-yl)phenyl)-1*H*-benzimidazole-5-carboxylate (**7f**); yellow solid; 74% yield; mp 170-172 °C; IR (KBr) υ_{max}:

1709 (C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ8.32 (1H, d, J = 1.5 Hz, H-4), 8.04 (1H, dd, J = 8.2, 1.0 Hz, H-3d), 7.89 (1H, dd, J = 8.5, 1.5 Hz, H-6), 7.71-7.67 (1H, *t*, J = 8.0 Hz, H-5d), 7.60 (1H, dd, J = 8.0, 2.0 Hz, H-6d), 7.53 (2H, d, J = 8.8 Hz, H-2b/6b), 7.55-7.50 (3H, m, H-2a/6a, 4d), 7.46 (2H, d, J = 8.8 Hz, H-3b/5b), 7.39 (2H, t, J = 8.6 Hz, H-3a/5a), 7.24 (1H, d, J = 8.5 Hz, H-7), 6.85 (1H, s, H-2c), 3.99 (1H, dd, J = 16.0, 1.2 Hz, H-5ci), 3.84 (1H, d, J = 16.0 Hz, H-5cii), 3.88 (3H, s, H-11); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.0 (C-4c), 166.5 (C-10), 161.9 (d, J = 245.5 Hz, C-4a), 153.2 (C-2), 146.8 (C-2d), 141.9 (C-8), 140.4 (C-9), 138.6 (C-4b), 135.1 (C-1b), 134.5 (5d), 132.1 (d, J =2.8 Hz, C-1a), 129.9 (d, J = 8.9 Hz, C-2a/6a), 129.7 (C-2b/6b), 129.6 (C-6d), 127.3 (C-4d), 126.6 (C-1d), 125.2 (C-3d), 124.4 (C-6), 124.3 (C-5), 124.0 (C-3b/5b), 120.8 (C-4), 117.0 (d, J = 22.8 Hz, C-3a/5a), 110.6 (C-7), 59.0 (C-2c), 52.1 (C-11), 31.8 (C-5c). HRMS (*m/z*): [M+Na] 591.1106 (calculated for $C_{30}H_{21}F_4N_4O_5SNa$, 591.1114).

Methyl 1-(4-fluorophenyl)-2-(4-(2-(4-nitrophenyl)-4-oxothiazolidin-3-yl)phenyl)-1*H*-benzimidazole-5-carboxylate (**7g**); pale yellow solid; 79% yield; mp 230-231 °C; IR (KBr) υ_{max}: 1689 (C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.31 (1H, d, J = 1.5 Hz, H-4), 8.13 (2H, d, J = 8.8 Hz, H-3d/5d), 7.89 (1H, dd, J = 8.5, 1.5 Hz, H-6), 7.67 (2H, d, J = 8.8 Hz, H-2d/6d), 7.50 (2H, dd, J = 8.9, 5.0 Hz, H-2a/6a), 7.46 (2H, d, J = 8.9 Hz, H-2b/6b), 7.41 (2H, d, J = 8.9 Hz, H-3b/5b), 7.37 (2H, t, J = 8.7 Hz, H-3a/5a), 7.23 (1H, d, J = 8.5 Hz, H-7), 6.74 (1H, s, H-2c), 4.07 (1H, dd, J = 16.0, 1.2 Hz, H-5ci), 3.93 (1H, d, J = 16.0 Hz, H-5cii), 3.87 (3H, s, H-11); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 170.6 (C-4c), 166.5 (C-10), 160.7 (d, J = 247.2 Hz, C-4a), 153.2 (C-2), 147.4* (C-4d), 147.3* (C-1d), 141.9 (C-8), 140.4 (C-9), 138.7 (C-4b), 132.1 (d, J = 3.2 Hz, C-1a), 129.9 (d, J = 8.8 Hz, C-2a/6a), 129.7 (C-2b/6b), 128.2 (C-2d/6d), 126.8 (C-1b), 124.5 (C-6), 124.4 (C-3b/5b), 123.9 (C-3d/5d), 120.9 (C-4), 116.9 (d, J = 23.0 Hz, C-3a/5a), 110.7 (C-7), 61.8 (C-2c), 52.1 (C-11), 32.6 (C-5c). HRMS (*m/z*):

[M+Na] 591.1110 (calculated for $C_{30}H_{21}FN_4O_5SNa$, 591.1114). C-5 could not be observed. * Assignments are interchangeable.

Methyl 2-(4-(2-(2-chlorophenyl)-4-oxothiazolidin-3-yl)phenyl)-1-(4-fluorophenyl)-1*H*-benzimidazole-5-carboxylate (**7h**); yellow solid; 83% yield; mp 180-181 °C; IR (KBr) υ_{max}:1700 (C=O); ^{1}H NMR (DMSO-*d*$_6$, 400 MHz) δ 8.32 (1H, d, *J* = 1.5 Hz, H-4), 7.90 (1H, dd, *J* = 8.5, 1.5 Hz, H-6); 7.50 (2H, dd, *J* = 9.0, 5.0 Hz, H-2a/6a), 7.45 (2H, d, *J* = 8.7 Hz, H-2b/6b),7.41-7.36 (6H, m, H-3a/5a, 3d-6d), 7.32 (2H, d, *J* = 8.7 Hz, H-3b/5b), 7.24 (1H, d, *J* = 8.5 Hz, H-7), 6.58 (1H, s, H-2c), 4.03 (1H, dd, *J* = 16.0, 1.0 Hz, H-5ci), 3.89 (1H, d, *J* = 16.0 Hz, H-5cii), 3.87 (3H, s, H-11); ^{13}C NMR (DMSO-*d*$_6$, 100 MHz) δ 170.5 (C-4c); 166.5 (C-10), 161.9 (d, *J* = 245.4 Hz, C-4a), 153.2 (C-2), 141.9 (C-8), 140.4 (C-9), 138.8 (C-4b), 138.7 (C-1d), 133.0 (C-2d), 132.1 (d, *J* = 2.8 Hz, C-1a), 129.9 (d, *J* = 9.0 Hz, C-2a/6a), 129.6 (C-2b/6b), 128.9* (C-3d, 4d), 128.7* (C-5d, 6d), 126.7 (C-1b), 124.6 (C-3b/5b), 124.5 (C-6), 124.4 (C-5), 120.8 (C-4), 116.9 (d, *J* = 22.8 Hz, C-3a/5a), 110.7 (C-7), 60.2 (C-2c), 52.1 (C-11), 32.7 (C-5c). HRMS (*m/z*): [M+Na] 580.0873 (calculated for $C_{30}H_{21}ClFN_3O_3SNa$, 580.0874). *Assignments overlap and may be interchanged.

Methyl 2-(4-(2-(4-chlorophenyl)-4-oxothiazolidin-3-yl)phenyl)-1-(4-fluorophenyl)-1*H*-benzimidazole-5-carboxylate (**7i**); yellow solid; 79% yield; mp 201-202 °C; IR (KBr) υ_{max}:1693 (C=O); ^{1}H NMR (DMSO-*d*$_6$, 400 MHz) δ8.32 (1H, d, *J* = 1.5 Hz, H-4), 7.90 (1H, dd, *J* = 8.5, 1.5 Hz, H-6), 7.51 (2H, d, *J* = 8.9, 4.9 Hz, H-2a/6a), 7.48 (2H, d, *J* = 8.3 Hz, H-2b/6b), 7.43 (2H, d, *J* = 8.3 Hz, H-3b/5b), 7.42-7.37 (4H, m, H-3a/5a, H-2d/6d), 7.30-7.26 (2H, m, H-3d/5d), 7.24 (1H, d, *J* = 8.5 Hz, H-7), 6.55 (1H, s, H-2c), 3.99 (1H, dd, *J* =16.0, 1.2 Hz, H-5ci), 3.89 (1H, d, *J* = 16.0 Hz, H-5cii), 3.87 (3H, s, H-11); ^{13}C NMR (DMSO-*d*$_6$, 100 MHz) δ 170.7 (C-4c), 166.5 (C-10), 161.9 (d, *J* = 245.5 Hz, C-4a), 153.2 (C-2), 141.9

(C-8), 140.4 (C-9), 138.8 (C-4b), 136.4 (C-1d), 132.1 (d, *J* = 3.4 Hz, C-1a), 131.6 (C-4d), 130.1 (C-2d/6d), 129.9 (d, *J* = 8.8 Hz, C-2a/6a), 129.6 (C-2b/6b), 127.7 (C-3d/5d), 126.7 (C-1b), 124.5 (C-6), 124.4 (C-5), 124.1 (C-3b/5b), 120.8 (C-4), 116.9 (d, *J* = 22.7 Hz, C-3a/5a), 110.6 (C-7), 60.4 (C-2c), 52.1 (C-11), 32.5 (C-5c). HRMS (*m/z*): [M+Na] 580.0873 (calculated for $C_{30}H_{22}ClFN_3O_3SNa$, 580.0874).

Methyl 1-(4-fluorophenyl)-2-(4-(2-(2-methoxyphenyl)-4-oxothiazolidin-3-yl)phenyl)-1*H*-benzimidazole-5-carboxylate (**7j**); yellow solid; 76% yield; mp 205-206 °C; IR (KBr) υ_{max}: 1689 (C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.32 (1H, d, *J* = 1.5 Hz, H-4), 7.89 (1H, dd, *J* = 8.5, 1.5 Hz, H-6), 7.52 (2H, dd, *J* = 8.9, 5.0 Hz, H-2a/6a), 7.46 (2H, d, *J* = 8.8 Hz, H-2b/6b), 7.42 (2H, t, *J* = 8.9 Hz, H-3a/5a), 7.40 (2H, d, *J* = 8.8 Hz, H-3b/5b), 7.24 (1H, d, *J* = 8.5 Hz, H-7), 7.22 (1H, m, H-5d), 7.16 (1H, d, *J* = 7.6, 1.5 Hz, H-6d), 6.98 (1H, d, *J* = 8.1 Hz, H-3d), 6.83 (1H, t, *J* =8.1 Hz, H-4d), 6.63 (1H, s, H-2c), 3.93 (1H, dd, *J* = 15.8, 1.3 Hz, H-5ci), 3.82 (1H, d, *J* = 15.8 Hz, H-5cii), 3.87 (3H, s, H-11), 3.80 (3H, s, OCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ170.8 (C-4c), 166.5 (C-10), 161.9 (d, *J* = 245.3 Hz, C-4a), 156.4 (C-2d), 153.2 (C-2), 141.9 (C-8), 140.4 (C-9), 139.1 (C-4b), 132.1 (d, *J* = 2.9 Hz, C-1a), 129.9 (d, *J* = 9.0 Hz, C-2a/6a), 129.7 (C-6d), 129.5 (C-2b/6b), 127.1 (C-1d), 127.0 (C-5d), 126.3 (C-1b), 124.4 (C-6), 124.3 (C-5), 123.9 (C-3b/5b), 120.8 (C-4), 120.4 (C-4d), 117.0 (d, *J* = 22.8 Hz, C-3a/5a), 111.4 (C-3d), 110.6 (C-7), 58.8 (C-2c), 55.7 (OCH_3), 52.1 (C-11), 32.7 (C-5c). HRMS (*m/z*): [M+Na] 576.1365 (calculated for $C_{31}H_{24}FN_3O_4SNa$, 576.1369).

Methyl 1-(4-fluorophenyl)-2-(4-(2-(4-methoxyphenyl)-4-oxothiazolidin-3-yl)phenyl)-1*H*-benzimidazole-5-carboxylate (**7k**); yellow solid; 78% yield; mp 105-106 °C; IR (KBr) υ_{max}:1693 (C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.32 (1H, s, H-4), 7.90 (1H, d, *J* = 8.5 Hz, H-6), 7.51 (2H, dd, *J* = 8.8, 5.0 Hz, H-2a/6a), 7.43 (2H, d, *J* = 8.8 Hz, H-2b/6b), 7.39

(2H, t, *J* = 8.8 Hz, H-3a/5a), 7.34 (2H, d, *J* = 8.8 Hz, H-3b/5b), 7.29 (2H, d, *J* = 8.8 Hz, H-2d/6d), 7.24 (1H, d, *J* = 8.4 Hz, H-7), 6.81 (2H, d, *J* = 8.6 Hz, H-3d/5d), 6.50 (1H, s, H-2c), 3.98 (1H, d, *J* = 16.0 Hz, H-5ci), 3.87 (1H, d, *J* = 16.0 Hz, H-5cii), 3.89 (3H, s, H-11), 3.68 (3H, s, OCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 170.4 (C-4c), 166.5 (C-10), 161.9 (d, *J* = 245.5 Hz, C-4a), 159.2 (C-4d), 153.2 (C-2), 141.9 (C-8), 140.3 (C-9), 139.0 (C-4b), 132.1 (d, *J* = 3.0 Hz, C-1a), 131.0 (C-1d), 129.9 (d, *J* = 8.8 Hz, C-2a/6a), 129.4 (C-2b/6b), 128.5 (C-2d/6d), 126.4 (C-1b), 124.8 (C-3b/5b), 124.4 (C-6), 124.3 (C-5), 120.8 (C-4), 117.0 (d, *J* = 22.9 Hz, C-3a/5a), 113.9 (C-3d/5d), 110.6 (C-7), 62.8 (C-2c), 55.0 (OCH_3), 52.1 (C-11), 32.8 (C-5c). HRMS (*m/z*): [M+Na] 546.1270 (calculated for $C_{30}H_{22}FN_3O_3SNa$, 546.1264).

Methyl 1-(4-fluorophenyl)-2-(4-(2-(4-(methylthio)phenyl)-4-oxothiazolidin-3-yl)phenyl)-1*H*-benzimidazole-5-carboxylate (**7l**); yellow solid; 88% yield; mp 105-107 °C; IR (KBr) υ_{max}: 1688 (C=O); ^1H NMR (DMSO-d_6, 400 MHz) δ 8.32 (1H, d, *J* = 1.5 Hz, H-4), 7.89 (1H, dd, *J* = 8.5, 1.5 Hz, H-6), 7.50 (2H, dd, *J* = 8.8, 5.1 Hz, H-2a/6a), 7.45 (2H, d, *J* = 8.8, H-2b/6b), 7.39 (2H, t, *J* = 8.8 Hz, H-3a/5a), 7.37 (2H, d, *J* = 8.8 Hz, H-3b/5b), 7.29 (2H, d, *J* = 8.8 Hz, H-2d/6d), 7.24 (1H, d, *J* = 8.5 Hz, H-7), 7.12 (2H, d, *J* = 8.4 Hz, H-3d/5d), 6.53 (1H, s, H-2c), 4.00 (1H, dd, *J* = 16.0, 1.0 Hz, H-5ci), 3.88 (1H, d, *J* = 16.0 Hz, H-5cii), 3.87 (3H, s, H-11), 2.40 (3H, s, SCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 170.5 (C-4c), 166.5 (C-10), 161.9 (d, *J*= 245.3 Hz, C-4a), 153.2 (C-2), 141.9 (C-8), 140.3 (C-9), 139.0 (C-4b), 138.8 (C-1d), 135.8 (C-4d),132.1 (d, *J* = 3.2 Hz, C-1a), 129.9 (d, *J* = 8.8 Hz, C-2a/6a), 129.5 (C-2b/6b), 127.6 (C-2d/6d), 126.5 (C-1b), 125.6 (C-3d/5d), 124.6 (C-3b/5b), 124.4 (C-6), 124.3 (C-5), 120.8 (C-4), 116.9 (d, *J* = 22.9 Hz, C-3a/5a), 110.6 (C-7), 32.7 (C-2c), 52.0 (C-11), 32.7 (5c), 14.1 (SCH_3). HRMS (*m/z*): [M+Na] 592.1143 (calculated for $C_{31}H_{24}FN_3O_4S_2Na$, 592.1141).

2.2.2 Antibacterial assays

Benzimidazole-thiazolidinones **7a-l** were screened for their antimicrobial activity using the disc diffusion assay against two Gram +ve bacteria, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus aureus* Rosenbach ATCC BAA-1683 (methicillin resistant *S. aureus)* and four Gram –ve bacteria, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 31488*, Escherichia coli* ATCC 25922 and *Salmonella typhimurium* ATCC 14026.

All microbial cultures were grown on Mueller-Hinton agar (21.0 g L^{-1}) at 37 °C in a CO_2 incubator for 24 h and adjusted to a 0.5 McFarland standard in saline solution. Mueller-Hinton agar plates were prepared using 38 g of agar in 1 L of water, dispensed into sterile petri dishes and set at room temperature.

Disc diffusion assay

Preliminary screening was conducted using the disc diffusion assay. Mueller-Hinton agar plates were lawn inoculated with the required strain of bacteria. A volume of 5 µL of each compound (10 mg mL^{-1} in DMSO) was inoculated onto 6 mm assay discs, placed onto the MHA plates and incubated for 24 h at 37 °C. Zones of inhibition around the spotted compound indicated activity.

Minimum bactericidal concentration (MBC) assay

Compounds showing activity in the disc diffusion assay were further analysed for their MBC values using the broth dilution assay with Levofloxacin as the control. The broth dilution method was performed in Mueller Hinton broth prepared as above. Test compounds (10 mg) were dissolved in 1 mL DMSO and serially diluted over five dilutions, which were inoculated with 20 µL of bacterial culture and incubated for 24 h at 37 °C. After incubation, 10 µL of

each dilution was spotted on MHA plates and further incubated for 24 h at 37 °C to determine the MBC value. The MBC was determined to be the lowest concentration that showed no growth of bacteria. All assays were performed in duplicate and were reproducible.

2.2.3 Single Crystal X-ray Diffraction Analysis

Crystals were grown by slow evaporation in dimethyl sulfoxide at room temperature. The crystal structure was determined on a Bruker Smart APEX II diffractometer with Mo Kα radiation. Data reduction was carried out using System Administrator's Integrated Network Tool (SAINT+) and the structure was solved and refined using SHELXS. All hydrogen atoms were positioned geometrically and refined isotropically. Crystallographic images were prepared using Ortep-3.

Crystallographic data has been deposited with the Cambridge Crystallographic Data Centre, CCDC, 12 Union Road, Cambridge CB21EZ, UK. Copies of the data can be obtained free of charge on quoting the depository number CCDC-1860248 (Fax: +44-1223-336-033; E-Mail: deposit@ccdc.cam.ac.uk, http://www.ccdc.cam.ac.uk).

2.3 Results and Discussion

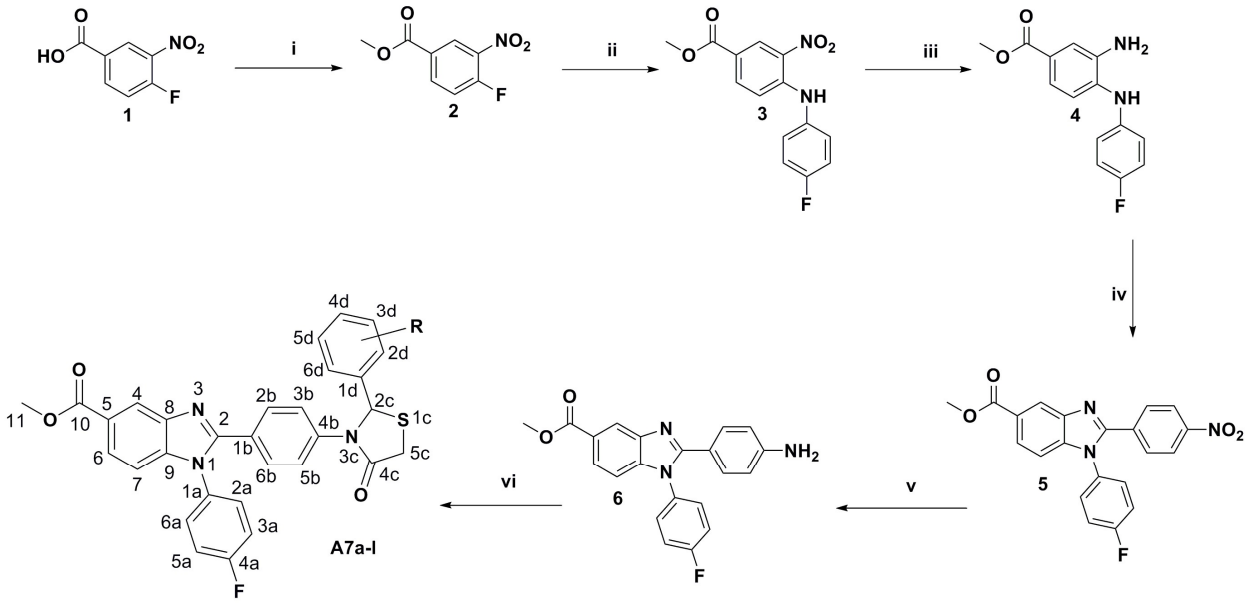
2.3.1 Chemistry

The project was designed to produce hybrid molecules with a benzimidazole core structure to which phenyl and thiazolidinone moieties were attached. This was done to introduce more functionality into the benzimidazole core structure to improve bioactivity. Variation was introduced on the phenyl group of the thiazolidinone moiety to produce a series of 12 compounds (**7a-l**). This was easily done by introducing various benzaldehydes, which were *meta* or *para* substituted with electron withdrawing groups (F, Cl, CF_3 and NO_2) or electron

donating groups (OCH_3 and SCH_3). The ester functionality at C-5 and the 4-fluorophenyl group at *N*-1 were also unique to this set of benzimidazoles.

The benzimidazole-thiazolidinone hybrids were synthesized in a six step reaction (**Scheme 2.1**). The synthesis began with esterification of 4-fluoro-3-nitrobenzoic acid, followed by a substitution reaction where 4-fluoroaniline substituted the fluoro group at the *para* position of the ester. This step was thought to proceed by the formation of a Meisenheimer adduct (Yeong *et al*., 2014) generated by intermolecular hydrogen bonding of the fluorine atom, amine and ethanol. The fluorine atom forms a strong hydrogen bond with ethanol, and the carboxyl group withdraws electron density and facilitates *N*-arylation resulting in **3** (**Scheme 2.2**).

Reduction of the nitro group was carried out with Pd/C in methanol under hydrogen atmosphere forming the amino benzoate precursor **4** to which *para*-nitrobenzaldehyde was added to form the benzimidazole. The nitro group on this phenyl ring was then reduced by stannous chloride resulting in the amino phenyl precursor **6.** Stannous chloride was used since reduction with Pd/C led to more side products being formed, resulting in an extra purification step. The reaction with stannous chloride needed no purification. In the final step of the reaction, the thiazolidinone ring was formed with substituted benzaldehydes resulting in Schiff bases with the primary amino group on the benzimidazole intermediate and further cyclisation with thioglycolic acid to produce the final compounds **7a-l**. The reaction proceeded nicely with F, Cl, CF_3, NO_2, OCH_3 and SCH_3 benzaldehydes, but was unsuccessful with hydroxybenzaldehyde precursors, probably due to the acidity of the phenolic proton, rendering a phenoxide ion and preventing imine formation from occurring.

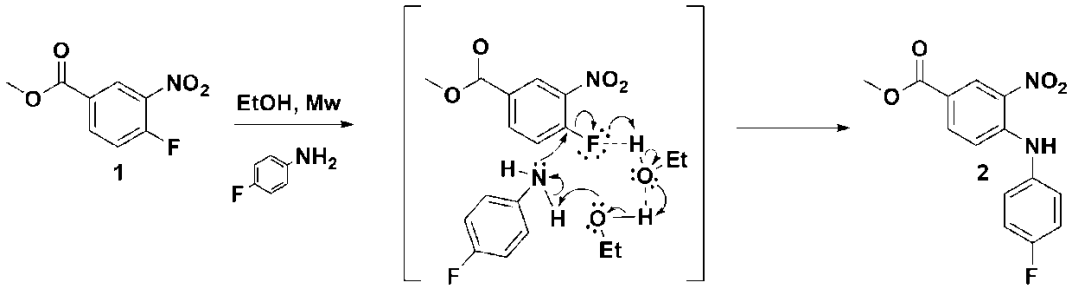


Compound	R
7a	H
7b	2'F
7c	4'F
7d	2'CF_3
7e	4'CF_3
7f	2'NO_2
7g	4'NO_2
7h	2'Cl
7i	4'Cl
7j	2'OCH_3
7k	4'OCH_3
7l	4'SCH_3

Scheme 2.1 Synthetic scheme to benzimidazole-thiazolidinone derivatives **A7a-l**. (i) MeOH, H_2SO_4, A: reflux, 12h, B: Mw 80°C, 10 min; (ii) 4-F-phenylaniline, A: DMF, RT, 12h; B: Mw 120°C, EtOH, 15 min; (iii) 10% Pd/C, EtOH, H_2, RT, 4h; (iv) 4-NO_2-benzaldehyde, A: $Na_2S_2O_5$ (20 mol%), EtOH, reflux, 12h; B: Mw 110°C, EtOH, 15min; (v) $SnCl_2.2H_2O$, EtOH, reflux, 8h; (vi) R-CHO, TGA, EtOH, Mw 110°C, 30min.

Several steps in the reaction scheme were carried out by both the conventional reflux and microwave methods. The conventional methods occurred with reaction times of 4-12 hours, which was drastically reduced using microwave synthesis, which occurred between 10-30 minutes. There were no notable differences in yields between the two methods, however the yield was slightly higher for the microwave synthesis over the conventional method. In step 2, the aniline substitution, DMF was used in the conventional reflux method as the boiling

point of ethanol was too low for the reflux conditions required, but under microwave irradiation, the greener ethanol worked well. Furthermore, in step 4, the sodium metabisulfite catalyst was not needed under microwave conditions. The major advantage of microwave reactions over the conventional synthesis was the time needed to carry out the reactions. For steps 1, 2 and 4 the time was reduced from 12 hours to 15 minutes or less with slightly better yields (**Table 2.1**).



Scheme 2.2 *N*-arylation and hydrogen bonding by a Meisenheimer reaction (Yeong *et al*., 2014).

Table 2.1 Comparison of yields and time for steps 1, 2 and 4 between microwave and conventional synthesis

Comp.	Microwave synthesis		Conventional synthesis	
	Time (min)	Yield (%)	Time (hrs)	Yield (%)
2	10	95	12	93
3	15	90	12	80
5	15	85	12	81

2.3.2 Structural elucidation

The structures of compounds **7a-l** were established based on their FTIR, HRMS and NMR analysis. Using **7j**, the 2-methoxy derivative as an example, H-4, H-6 and H-7 on the benzimidazole core occurred at δ 8.32 (d, J = 1.2 Hz), 7.89 (dd, J =8.5, 1.5 Hz) and 7.24 (d, J = 8.5 Hz) respectively. The methyl resonance on the ester (H-11) and the methyl on the

methoxy group both occurred as singlets at δ 3.87 and δ 3.80 respectively. The aromatic resonances, H-3d, H-4d and H-6d on the phenyl ring attached to the thiazolidinone group could be seen as a doublet at δ 6.98 (J = 8.1 Hz), a triplet at δ 6.83 (J = 8.1 Hz) and a double doublet at δ 7.16 (J = 7.6, 1.5 Hz) respectively. The remaining proton on this ring, H-5d overlapped with the H-7 resonance at δ 7.24. The aromatic resonances of the fluorinated aromatic ring attached to *N*-1 and the phenyl ring attached to the benzimidazole moiety all appeared between δ 7.38 to δ 7.53. The H-2a/6a and H-3a/5a resonances of the fluorinated ring had characteristic splitting patterns of a double doublet for H-2a/6a at δ 7.52 with J = 8.9 and 5.0 Hz and a triplet for H-3a/5a at δ 7.42 with J = 8.9 Hz. The H-2b/6b and H-3b/5b doublet resonances occurred at δ 7.46 and δ 7.40 (J = 8.8 Hz), H-3b/5b coinciding with H-3a/5a. The diastereotopic methylene protons H-5ci and H-5cii occurred at δ 3.93 and 3.82 (J = 15.8 Hz) and H-2c on the thiazolidine ring appeared at δ 6.63 as a singlet.

The aromatic carbon resonances on the fluorinated ring were easy to identify, since they showed characteristic coupling constants for *ortho* and *meta* coupled protons: C-2a/6a at δ 129.9 (d, J = 8.9 Hz) and C-3a/5a at δ 117.0 (d, J = 22.8 Hz). Two doublets with very different coupling constants were observed for C-4a at δ 161.9 (J = 245.3 Hz) and C-1a at δ 132.1 (J = 2.9 Hz). The ester carbonyl (C-10) was seen at δ 166.5 and distinguished from the thiazolidine carbonyl group at δ 170.9 by a HMBC correlation to H-11. The C-4c resonance was confirmed by a HMBC correlation to H-5ci and H-5cii. The aromatic quaternary resonances C-8 and C-9 occurred at δ 141.9 and 140.4 respectively and were assigned due to HMBC correlations of C-9 to H-6 and H-4 and C-8 to H-7. The other aromatic carbon resonance in this region at δ 139.2 was assigned to C-4b by its HMBC correlation to H-2b/6b.

The C-2 and C-5 resonances on the imidazole moiety were observed at δ 153.2 and 124.3 respectively. C-2 was confirmed by its HMBC correlations with H-2b/6b.

2.3.3 Crystal structure

The crystal structure of methyl 1-(4-fluorophenyl)-2-(4-(2-(2-methoxyphenyl)-4-oxothiazolidin-3-yl) phenyl)-1*H*-benzimidazole-5-carboxylate (**7j**) was solved in the P21/n space group with 4 molecules in the unit cell. The structure consisted of a planar benzimidazole ring with the phenyl ring attached to N3 pointing away from the benzimidazole ring twisted out of the plane of the benzimidazole ring with an angle of 72.69°. The aromatic ring at C-9 also pointed away from the benzimidazole ring, twisted slightly out of plane with an angle of 29.98°. The phenyl ring at C-4 was almost orthogonal to the thiazolidinone ring with anangle of 84.33°. The configuration at C-4 was also found to be *S*. An ortep diagram of **7j** is shown in **Figure 2.1**.

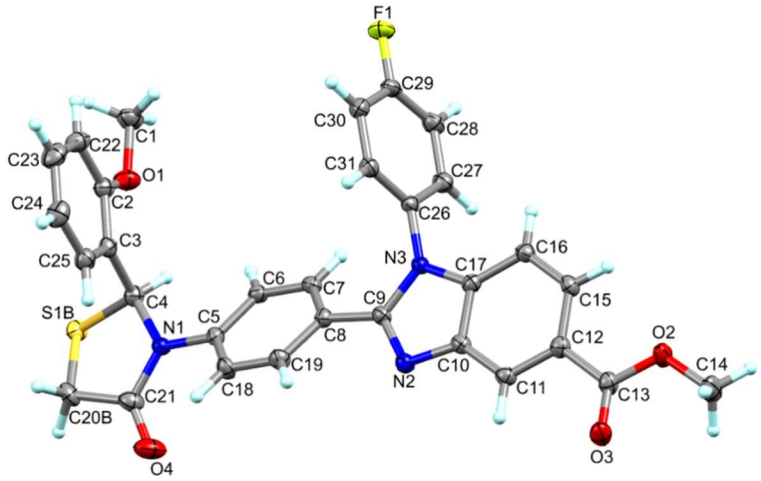


Figure 2.1 Crystal structure of methyl-1-(4-fluorophenyl)-2-(4-(2-(2-methoxyphenyl)-4-oxothiazolidin-3-yl)phenyl)-1*H*-benzimidazole-5-carboxylate (**7j**).

2.3.4 Antibacterial activity

Six compounds showed activity in the preliminary disc diffusion assay and their MBC values determined. All six compounds indicated moderate activity against the test organisms, being active against four of the six bacterial strains tested against with MBC values between 1.9 to 8.3 mM (**Table 2.2**). Compounds **7f** and **7i** exhibited the best activity, being active at 1.9 and 2.0 mM, and 3.9 and 4.0 mM against *P. aeruginosa* and *S. aureus* respectively. Compound **7f**, with a 2-NO_2 substituted on the phenyl ring of the thiazolidinone moiety showed the best activity of all the compounds, being active at < 4.0 mM in all four bacterial species (*S. aureus*, *K. pneumoniae*, *P. aeruginosa* and *S. typhimurium*).

Table 2.2 Minimum Bactericidal Concentration (MBC, mM) of benzimidazole-thiazolidinone derivatives**.**

Compound	R	*S. aureus*	*K. pneumonia*	*P. aeruginosa*	*S. Typhimurium*
7b	2-F	8.3	8.3	4.1	8.3
7e	4-CF_3	3.8	7.6	3.8	7.6
7f	2-NO_2	3.9	3.9	1.9	3.9
7h	2-Cl	8.0	8.0	4.4	8.0
7i	4-Cl	4.0	8.0	2.0	8.0
7l	4-SCH_3	7.5	7.5	4.1	7.5
Levofloxacin		0.0216	0.0216	0.345	0.0216

2.4 Conclusion

A practical protocol for the synthesis of a library of benzimidazole-thiazolidinone derivatives was demonstrated, where microwave irradiation for several of the steps reduced the time of reaction drastically from 12 hours to 15 minutes or less in slightly better yields than the conventional reflux methods. Compounds **7a-l** demonstrated moderate to low activity against Gram +ve and –ve bacteria.

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Chapter 3 Synthesis, Characterisation and Antibacterial Activity of Benzimidazole-amino Acid Hybrids

***** The compounds referred to in the chapter are referred to in the Abstract, Conclusion and Appendices with **B** preceding the number of the compound.For example **7g** is referred to as **B-7g**.

Abstract

A series of new benzimidazole-amino acids were synthesized using an efficient and rapid microwave irradiation method, which reduces the time of four of the six steps from 12-16 hours to under 30 minutes. The biological evaluation revealed that some target compounds could be potential antibacterial agents as they exhibited excellent strain specific activity in comparison to ciprofloxacin and levofloxacin. Compound **7g** with a methyl group at *N*-1 and a phenylalanine moiety incorporated into the structure at the phenyl ring showed excellent broad spectrum activity against *S. typhimurium* (4.00 μM), *E. coli* (4.00 μM) and *K. pneumoniae* (2.00 μM).

Keywords: benzimidazoles, amino acids, antibacterial activity, hybrid compounds

3.1 Introduction

Benzimidazoles are highly versatile heterocyclic compounds exhibiting a broad range of applications. They possess antimicrobial (Yadav and Ganguly, 2015), antitumor (Yadav*et al*., 2018), antiviral (Tonelli*et al*., 2014), anthelmintic (Gutiérrez*et al*., 2018), antidiabetic (Abdullah *et al*., 2016), analgesic, anti-inflammatory (Gaba*et al*., 2014) and many other diverse medical applications. Benzimidazoles are starting materials for the synthesis of other bioactive structures, and numerous pharmaceutical drugs such as albendazole, bendamustine and benomyl incorporate this moiety into their structure (Akhtar *et al*., 2017).

Benzimidazole analogues bearing amino acid side chains have shown significant antimicrobial properties (Sindhe *et al.*, 2016). α-Amino acids are naturally occurring organic molecules and considered prerequisites for the genesis of life. Their asymmetric centres in combination with suitable heterocyclic compounds render very interesting optically active compounds, which generally possess biological activity and are routinely used in medicinal chemistry (Khose *et al*., 2017; Perin *et al*., 2017).

Construction of heterocyclic hybrids is one of the most significant areas in synthetic organic chemistry. Benzimidazoles and amino acids are popular scaffolds used in organic synthesis. The amino acids are popular due to their low toxicity and biocompatibility (Teno *et al*., 2016). Currently, there is huge tendency to conjugate amino acid/peptide residues with small bioactive heterocyclic scaffolds in the field of biomedical research.

Benzimidazoles are normally synthesised by heterocyclization of acids or aldehydes and *ortho*-phenylenediamine (Alaqeel, 2017). Amino acids, having both a carboxyl and amino terminus is a popular unit to tag onto other pharmacophores. There are a few reports on

benzimidazole derivatives coupled to amino acid residues, mostly at C2 (Ajani *et al*., 2016; Sabithakala *et al*., 2016). Conjugation of amino acids to heterocyclic molecules has shown successful results in their applications, by increasing solubility, potency and cell membrane permeability as well as reducing toxicity making them attractive pharmacological agents. (Pinazo *et al.*, 2016; Wang *et al*., 2018). Based on this, we anticipate that amino acid-benzimidazole conjugates may result in enhanced antimicrobial activity, and possible new lead compounds.

This work reports on the synthesis of benzimidazole-amino acid derivatives and testing them for their antibacterial potential.

3.2 Experimental

3.2.1 Chemistry

Chemicals and reagent grade solvents were purchased from Sigma Aldrich and Merck, South Africa and used without purification. Open capillary tubes were used to measure melting points on a Stuart Smart Scientific melting point instrument. Silica gel 60 F_{254} plates (Merck) was used to perform Thin layer chromatography. When necessary, compounds were purified by column chromatography using silica gel (60-120 mesh) and a mobile phase of varying ratios of ethyl acetate:hexane. A Perkin Elmer Spectrum 100 FTIR spectrometer with universal attenuated total reflectance sampling accessory was used to record Infrared spectra. NMR spectra were recorded on a Bruker Avance 400 MHz instrument. ^{1}H-NMR spectra were recorded at 400 MHz, and ^{13}C NMR spectra at 100 MHz in deuterated DMSO-d_6 and referenced to the solvent peaks at δ 2.50 for ^{1}H and δ 39.51 for ^{13}C. High Resolution Mass Spectrometry was carried out on a Bruker micro TOF-Q II ESI instrument. Microwave assisted reactions were performed using a CEM Discover, Explorer-12 Hybrid microwave.

General method for the preparation of 4-substituted 3,4-diamino methyl benzoates 4a-b

Esterification of 4-fluoro-3-nitrobenzoic acid (2.7 mmol, 0.5g) was carried out in methanol with a catalytic amount of sulphuric acid under microwave irradiation (80°C, 10 min). Methylamine or 4-fluoro-3-nitroaniline (2.5 mmol) dissolved in ethanol (5mL) was added to this mixture and subject to microwave irradiation for 15 minutes to obtain the 4-substituted-3-nitrobenzoate esters **3a-b**. The nitrobenzoate esters (1.0 g) were then reduced to its amines **4a-b** by adding 10% Pd/C (0.01 g) to the reaction mixture under hydrogen atmosphere and stirring the contents at room temperature for 4 h. The diamine was then purified by column chromatography on silica gel with varying concentrations of ethyl acetate and hexane.

Synthesis of methyl 2-(4-fluoro-3-nitrophenyl)-*N*-substituted benzimidazole-5-carboxylates (6a-b)

The diamine **4a-b** (2.0 mmol, 0.5 g), *N*,*N*-dicyclohexylcarbodiimide (DCC) (3.0 mmol, 0.6 g) and a catalytic amount (3 mg) of *N*,*N*-dimethylaminopyridine (DMAP) added to a solution of 4-fluoro-3-nitrobenzoic acid (3.0 mmol, 0.5 g) in dichloromethane (5 mL) and the contents irradiated under microwave conditions for 20 minutes at 40 °C. Upon completion, the dicyclohexyl urea was filtered off, and the reaction mixture extracted with ethyl acetate (2 x 5 mL). The organic layer was concentrated under reduced pressure to obtain the amide conjugate **5a-b** and used without purification for the next step.

To a solution of glacial acetic acid (2.4 mmol, 0.5 mL) and $MgSO_4$ (1.8 mmol, 0.2 g) in 1,2 dichloroethane (5 mL), 1.2 mmol of **5a-b** was added and reacted for 30 min at 75 °C under microwave irradiation. The $MgSO_4$ was then filtered off and the reaction mixture neutralized with sodium hydrogen bicarbonate and extracted with ethyl acetate (2 x 5 mL). The organic

layer was concentrated and purified by column chromatography using ethyl acetate:hexane (30:70) to afford the benzimidazole carboxylates **6a-b**.

Synthesis of methyl 2-(4-amino acid substituted-3-nitrophenyl)-*N*-substituted-benzimidazole-5-carboxylates (7a-j)

Various amino acids (0.1 mol, 1.0g) were esterified by the addition of thionyl chloride (0.3 mol; 0.5 mL) in methanol and stirring at room temperature for 4 h. The reaction mixture was reduced under pressure to remove the methanol and thionyl chloride. The amino acid esters (2.4 mmol) were then added to the 2-(4-fluoro-3-nitro) benzimidazole-5-carboxylates (**6a-b**) (1.2 mmol) in THF (10 mL). Triethylamine (2.4 mmol, 0.3 mL) was then added to the reaction mixture, which was then heated under reflux at 70 °C for 16 h. Upon completion, the solution was washed with water and brine, and extracted with ethyl acetate. The final compounds were purified using column chromatography with ethyl acetate: hexane (30:70) to afford the benzimidazole amino acid hybrids **7a-j** in yields of 62–73%.

Methyl 2-(4-(1-methoxy-3-methyl-1-oxobutan-2-ylamino)-3-nitrophenyl)-1-methyl-1*H*-benzimidazole-5-carboxylate (**7a**); yellow solid; IR (KBr) υ_{max}: 1704 cm^{-1}(C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.61 (1H, d, J = 2.0 Hz, H-4), 8.53 (1H, d, J = 8.2 Hz, H-1b), 8.25 (1H, d, J = 1.5 Hz, H-3a), 8.07 (1H, dd, J = 8.5, 2.0 Hz, H-6), 7.91 (1H, dd, J = 8.5, 1.5 Hz, H-5a), 7.71 (1H, d, J = 8.5 Hz, H-7), 7.24 (1H, d, J = 8.5 Hz, H-6a), 4.63 (1H, dd, J = 8.2, 5.0 Hz, H-2b), 3.94 (3H, s, NCH_3), 3.87 (3H, s, H-11), 3.74 (3H, s, H-4b), 2.36-2.28 (1H, m, H-5b), 1.04 (3H, d, J = 7.0 Hz, H-6b), 0.99 (3H, d, J = 7.0 Hz, H-7b); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.6 (C-3b), 166.9 (C-10), 153.5 (C-2), 144.9 (C-1a), 141.9 (C-8), 140.0 (C-9), 136.9 (C-5a), 131.5 (C-2a), 127.5 (C-3a), 123.6 (C-5), 123.5 (C-6), 120.5 (C-4), 117.4 (C-4a), 115.4 (C-6a), 110.8 (C-7), 60.0 (C-2b), 52.2 (C-4b), 52.1 (C-11), 32.1 (NCH_3), 30.9 (C-

5b), 18.5 (C-6b), 17.9 (C-7b); HRMS (*m/z*): [M-H] 439.1614 (calculated for $C_{22}H_{23}N_4O_6$, 439.1618).

Methyl 1-(4-fluorophenyl)-2-(4-(1-methoxy-3-methyl-1-oxobutan-2-ylamino)-3-nitrophenyl)-1*H*-benzimidazole-5-carboxylate (**7b**); yellow solid; IR (KBr) υ_{max}:1709 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ8.48 (1H, d, *J* = 8.5 Hz, H-1b), 8.35 (1H, d, *J* = 1.0 Hz, H-4), 8.25 (1H, d, *J* = 2.0 Hz, H-3a), 7.90 (1H, dd, *J* = 8.5, 1.0 Hz, H-6), 7.72 (1H, dd, *J* = 9.0, 2.0 Hz, H-5a), 7.64 (2H, dd, *J* = 8.6, 4.9 Hz, H-2c/6c), 7.49 (2H, t, *J* = 8.6 Hz, H-3c/5c), 7.24 (1H, d, *J* = 8.5 Hz, H-7), 7.09 (1H, d, *J* = 9.0 Hz, H-6a), 4.56 (1H, dd, *J* = 8.2, 5.0 Hz, H-2b), 3.89 (3H, s, H-11), 3.71 (3H, s, H-4b), 2.30-2.22 (1H, m, H-5b), 1.00 (3H, d, *J* = 6.8 Hz, H-6b), 0.94 (3H, d, *J* = 6.8 Hz, H-7b); ^{13}C NMR (DMSO-d_6, 100 MHz) δ171.4 (C-3b), 166.5 (C-10), 162.2 (d, *J* = 247.5 Hz, C-4c), 152.1 (C-2), 144.7 (C-1a), 142.0 (C-8), 140.5 (C-9), 136.4 (C-5a), 132.2 (d, *J* = 1.9 Hz, C-1c), 131.0 (C-2a), 130.1 (d, *J* = 8.8 Hz, C-2c/6c), 127.2 (C-3a), 124.4 (C-6), 120.6 (C-4), 117.4 (d, *J* = 23.1 Hz, C-3c/5c), 116.8 (C-5), 115.1 (C-6a), 110.5 (C-7), 59.8 (C-2b), 52.4 (C-4b), 52.1 (C-11), 30.8 (C-5b), 18.4 (C-6b), 17.8 (C-7b); HRMS (*m/z*): [M-H] 519.1666 (calculated for $C_{27}H_{24}N_4O_6F$, 519.1680).

Methyl 2-(4-(1-methoxy-4-(methylthio)-1-oxobutan-2-ylamino)-3-nitrophenyl)-1-methyl-1*H*-benzimidazole-5-carboxylate (**7c**) yellow solid; IR (KBr) υ_{max}: 1706 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ8.61 (1H, d, *J* = 2.1 Hz, H-4), 8.49 (1H, d, *J* = 8.0 Hz, H-1b), 8.25 (1H, d, *J* = 1.5, H-3a), 8.08 (1H, dd, *J* = 8.9, 2.1 Hz, H-6), 7.92 (1H, dd, *J* = 8.5, 1.5 Hz, H-5a), 7.72 (1H, d, *J* = 8.9 Hz, H-7), 7.24 (1H, d, *J* = 8.5 Hz, H-6a), 4.86-4.81 (1H, m, H-2b), 3.94 (3H, s, NCH_3), 3.87 (3H, s, H-11), 3.75 (3H, s, H-4b), 2.60-2.56 (2H, m, H-6b), 2.26-2.20 (2H, m, H-5b), 2.10 (3H, s, H-7b); ^{13}C NMR (DMSO-d_6, 100 MHz) δ171.8 (C-3b), 166.8 (C-10), 153.5 (C-2), 144.4 (C-1a), 141.9 (C-8), 140.0 (C-9), 136.7 (C-5a), 131.8 (C-

2a), 127.5 (C-3a), 123.6 (C-5), 123.5(C-6), 120.5 (C-4), 117.4 (C4a), 115.3 (C-6a), 110.8 (C-7), 54.0 (C-2b), 52.9 (C-4b), 52.9 (C-11), 32.1 (NCH_3), 30.9 (C-5b), 29.7 (C-6b), 14.7 (C-7b); HRMS (*m/z*): [M-H] 471.1340 (calculated for $C_{22}H_{23}N_4O_6S$, 471.1338).

Methyl 1-(4-fluorophenyl)-2-(4-(1-methoxy-4-(methylthio)-1-oxobutan-2-ylamino)-3-nitrophenyl)-1*H*-benzimidazole-5-carboxylate (**7d**); yellow solid; IR (KBr) υ_{max}: 1707 cm^{-1} (C=O); ^1H NMR (DMSO-d_6, 400 MHz) δ 8.44 (1H, d, *J* = 8.0 Hz, H-1b), 8.34 (1H, d, *J* = 1.5 Hz, H-4), 8.23 (1H, d, *J* = 2.2 Hz, H-3a), 7.90 (1H, dd, *J* = 8.5, 1.5 Hz, H-6), 7.72 (1H, dd, *J* = 8.0, 2.2 Hz, H-5a), 7.64 (2H, dd, *J* = 8.8, 4.9 Hz, H-2c/6c), 7.48 (2H, t, *J* = 8.8 Hz, H-3c/5c), 7.24 (1H, d, *J* = 8.5 Hz, H-7), 7.09 (1H, d, *J* = 8.0 Hz, H-6a), 4.81-4.63 (1H, m, H-2b), 3.88 (3H, s, H-11), 3.71 (3H, s, H-4b), 2.56-2.52 (2H, m, H-6b), 2.19-2.15 (2H, m, H-5b), 2.01 (3H, s, H-7b); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.7 (C-3b), 166.6 (C-10), 162.2 (d, *J* = 245.4 Hz, C-4c), 152.2 (C-2), 144.3 (C-1a), 142.0 (C-8), 140.6 (C-9), 136.3 (C-5a), 132.2 (d, *J* = 4.5 Hz, C-1c), 131.3 (C-2a), 130.1 (d, *J* = 9.0 Hz, C-2c/6c), 127.2 (C-3a), 124.4 (C-6), 120.6 (C-4), 117.3 (d, *J* = 22.8 Hz, C-3c/5c), 116.7 (C-4a), 115.1 (C-6a), 110.5 (C-7), 53.9 (C-2b), 52.7 (C-4b), 52.2 (C-11), 30.8 (C-5b), 29.0 (C-6b), 14.6 (C-7b); HRMS (*m/z*): [M-H] 551.1386 (calculated for $C_{27}H_{24}N_4O_6SF$, 551.1401).

Methyl 2-(4-(1-methoxy-4-methyl-1-oxopentan-2-ylamino)-3-nitrophenyl)-1-methyl-1*H*-benzimidazole-5-carboxylate (**7e**); yellow solid; IR (KBr) υ_{max}: 1706 cm^{-1} (C=O); ^1H NMR (DMSO-d_6, 400 MHz) δ 8.61 (1H, d, *J* = 2.0, H-3a), 8.35 (1H, d, *J* = 8.0 Hz, H-1b), 8.26 (1H, d, *J* = 1.5 Hz, H-4), 8.08 (1H, dd, *J* = 9.0, 2.0 Hz, H-5a), 7.92 (1H, dd, 8.5, 1.5 Hz, H-6), 7.72 (1H, d, *J* = 8.5 Hz, H-7), 7.21 (1H, d, *J* = 9.0 Hz, H-6a), 4.68 (1H, dd, *J* = 8.0, 5.5 Hz, H-2b), 3.95 (3H, s, NCH_3), 3.88 (3H, s, H-11), 3.72 (3H, s, H-4b), 1.93-1.82 (2H, m, H-5b), 1.80-1.72 (1H, m, H-6b), 0.97 (3H, d, *J* = 6.4 Hz, H-7b), 0.92 (3H, d, *J* = 6.4 Hz, H-8b); ^{13}C NMR

(DMSO-d_6, 100 MHz) δ 172.5 (C-3b), 167.09 (C-10), 153.4 (C-2), 144.6 (C-1a), 141.9 (C-8), 140.0 (C-9), 136.8 (C-5a), 131.5 (C-2a), 127.4 (C-3a), 123.6 (C-5), 123.4 (C-6), 120.4 (C-4), 117.4 (C-4a), 115.2 (C-6a), 110.7 (C-7), 53.7 (C-2b), 52.2 (C-11), 52.0 (C-4b), 40.4 (C-5b), 32.1 (NCH_3), 24.6 (C-6b), 22.6 (C-7b), 21.9 (C-8b); HRMS (*m/z*): [M-H] 453.1764 (calculated for $C_{23}H_{25}N_4O_6$, 453.1774).

Methyl 1-(4-fluorophenyl)-2-(4-(1-methoxy-4-methyl-1-oxopentan-2-ylamino)-3-nitrophenyl)-1*H*-benzimidazole-5-carboxylate (**7f**); yellow solid; IR (KBr) υ_{max}: 1710 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.34 (1H, d, *J* = 1.0 Hz, H-4), 8.29 (1H, d, *J* = 8.0 Hz, H-1b), 8.22 (1H, d, *J* = 2.0 Hz, H-3a), 7.90 (1H, dd, *J* = 8.5, 1.0 Hz, H-6), 7.73 (1H, dd, *J* = 9.0, 2.0 Hz, H-5a), 7.64 (2H, dd, *J* = 8.8, 4.9 Hz, H-2c/6c), 7.49 (2H, t, *J* = 8.8 Hz, H-3c/5c), 7.24 (1H, d, *J* = 8.5 Hz, H-7), 7.06 (1H, d, *J* = 9.0 Hz, H-6a), 4.59 (1H, dd, *J* = 7.7, 5.5 Hz, H-2b), 3.88 (3H, s, H-11), 3.68 (3H, s, H-4b), 1.87-1.76 (2H, m, H-5b), 1.64-1.58 (1H, m, H-6b), 0.93 (3H, d, *J* = 6.4 Hz, H-7b), 0.87 (3H, d, *J* = 6.4 Hz, H-8b); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 172.3 (C-3b), 166.6 (C-10), 162.2 (d, *J* = 245.2 Hz, C-4c), 152.1 (C-2), 144.5 (C-1a), 142.0 (C-8), 140.6 (C-9), 136.4 (C-5a), 132.2 (d, *J* = 3.4 Hz, C-1c), 131.1 (C-2a), 130.1 (d, *J* = 8.8 Hz, C-2c/6c), 127.1 (C-3a), 124.4 (C-6), 120.6 (C-4), 117.3 (d, *J* = 22.7 Hz, C-3c/5c), 116.8 (C-4a), 115.1 (C-6a), 110.5 (C-7), 53.6 (C-2b), 52.5 (C-4b), 52.2 (C-11), 40.4 (C-5b), 24.6 (C-6b), 22.6 (C-7b), 21.9 (C-8b); HRMS (*m/z*): [M-H] 533.1821 (calculated for $C_{28}H_{26}N_4O_6F$, 533.1836).

Methyl 2-(4-(1-methoxy-1-oxo-3-phenylpropan-2-ylamino)-3-nitrophenyl)-1-methyl-1*H*-benzimidazole-5-carboxylate (**7g**); yellow solid; IR (KBr) υ_{max}: 1704 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.58 (1H, d, *J* = 2.0 Hz, H-3a), 8.40 (1H, d, *J* = 8.0 Hz, H-1b), 8.25 (1H, s, H-4), 8.05 (1H, dd, *J* = 9.0, 2.0 Hz, H-5a), 7.92 (1H, dd, *J* = 8.5, 1.2 Hz, H-6), 7.72

(1H, d, *J* = 8.5 Hz, H-7), 7.32-7.28 (1H, m, H-9b), 7.30 (1H, d, *J* = 9.0 Hz, H-6a), 7.25-7.20 (4H, m, H-7b, 8b, 10b, 11b), 5.10-5.05 (1H, m, H-2b), 3.93 (3H, s, NCH_3), 3.87 (3H, s, H-11), 3.73 (3H, s, H-4b), 3.30-3.27 (2H, m, H-5b); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.3 (C-3b), 166.8 (C-10), 153.4 (C-2), 144.2 (C-1a), 141.9 (C-8), 139.4 (C-9), 136.6 (C-5a), 135.9 (C-2a), 131.4 (C-6b), 129.3 (C-7b/11b), 128.5 (C-8b/10b), 127.3 (C-3a), 123.6 (C-5), 123.4 (C-6), 120.4 (C-4), 117.4 (C-4a), 115.5 (C-6a), 110.7 (C-7), 55.9 (C-2b), 52.5 (C-11), 52.0 (C-4b), 37.1 (C-5b), 32.0 (NCH_3); HRMS (*m/z*): [M-H] 487.1608 (calculated for $C_{26}H_{23}N_4O_6$, 487.1618).

Methyl 1-(4-fluorophenyl)-2-(4-(1-methoxy-1-oxo-3-phenylpropan-2-ylamino)-3-nitrophenyl)-1*H*-benzimidazole-5-carboxylate (**7h**); yellow solid; IR (KBr) υ_{max}:1712 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 100 MHz) δ 8.36 (1H, s, H-1b), 8.34 (1H, d, *J* = 1.5 Hz, H-4), 8.20 (1H, d, *J* = 2.2 Hz, H-3a), 7.89 (1H, dd, *J* = 8.5, 1.5 Hz, H-6), 7.66 (1H, dd, *J* = 9.0, 2.0 Hz, H-5a), 7.62 (2H, dd, *J* = 8.8, 4.9 Hz, H-2c/6c), 7.48 (2H, t, *J* = 8.8 Hz, H-3c/5c), 7.27-7.21 (4H, m, H-7, H-8b, H-9b, H-10b), 7.16 (2H, d, *J* = 8.6 Hz, H-7b/11b), 7.04 (1H, d, *J* = 9.0 Hz, H-6a), 5.00-4.95 (1H, m, H-2b), 3.88 (3H, s, H-11), 3.69 (3H, s, H-4b), 3.22 (2H, d, *J* = 6.7 Hz, H-5b); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.2 (C-3b), 166.6 (C-10), 162.2 (d, *J* = 245.6 Hz, C-4c), 152.1 (C-2), 144.2 (C-1a), 142.0 (C-8), 140.5 (C-9), 136.2 (C-5a), 135.8 (C-6b), 132.2 (d, *J* = 2.9 Hz, C-1c), 131.0 (C-2a), 130.1 (d, *J* = 8.7 Hz, C-2c/6c), 129.3 (C-7b/11b), 128.5 (C-8b/10b), 127.13 (C-9b), 127.06 (C-3a), 124.4 (C-6), 124.4 (C-5), 120.6 (C-4), 117.3 (d, *J* = 23.0 Hz, C-3c/5c), 116.8 (C-4a), 115.4 (C-6a), 110.5 (C-7), 55.9 (C-2b), 52.5 (C-4b), 52.2 (C-11), 37.1 (C-5b); HRMS (*m/z*): [M-H] 567.1678 (calculated for $C_{31}H_{24}N_4O_6F$, 567.1680).

Methyl 2-(4-(3-(1*H*-indol-3-yl)-1-methoxy-1-oxopropan-2-ylamino)-3-nitrophenyl)-1-methyl-1*H*-benzimidazole-5-carboxylate (**7i**); yellow solid; IR (KBr) υ_{max}:1704 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.00 (1H, s, H-1b'), 8.56 (1H, d, *J* = 2.0 Hz, H-3a), 8.48 (1H, d, *J* = 7.7 Hz, H-1b), 8.24 (1H, d, *J* = 1.5 Hz, H-4), 8.02 (1H, dd, *J* = 9.0, 2.0 Hz, H-5a), 7.90 (1H, dd, *J* = 8.5, 1.5 Hz, H-6), 7.69 (1H, d, *J* = 8.5 Hz, H-7), 7.38 (1H, d, *J* = 8.0 Hz, H-4b'), 7.21 (1H, d, *J* = 8.0 Hz, H-7b'), 7.22-7.20 (2H, m, H-6a, H-2b'), 7.06 (1H, t, *J* = 7.8 Hz, H-6b'), 6.95 (1H, t, *J* = 7.8 Hz, H-5b'), 5.03 (1H, dd, *J* = 7.8, 5.2 Hz, H-2b), 3.91 (3H, s, NCH_3), 3.87 (3H, s, H-11), 3.70 (3H, s, H-4b), 3.44 (2H, d, *J* = 5.3 Hz, H-5b); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.6 (C-3b), 166.8 (C-10), 153.4 (C-2), 144.3 (C-1a), 141.9 (C-8), 140.0 (C-9), 136.5 (C-5a), 136.1 (C-9b'), 131.3 (C-2a), 127.3 (C-3a), 127.1 (C-8b'), 124.4 (C-2b'), 123.5 (C-5), 123.3 (C-6), 121.1 (C-6b'), 120.4 (C-4), 118.6 (C-5b'), 117.9 (C-4b'), 117.2 (C-4a), 115.4 (C-6a), 111.5 (C-7b'), 110.6 (C-7), 107.8 (C-3b'), 55.5 (C-2b), 52.5 (C-4b), 52.0 (C-11), 32.0 (NCH_3), 27.3 (C-5b); HRMS (*m/z*): [M-H] 526.1743 (calculated for $C_{28}H_{24}N_5O_6$, 526.1727).

Methyl 2-(4-(3-(1*H*-indol-3-yl)-1-methoxy-1-oxopropan-2-ylamino)-3-nitrophenyl)-1-(4-fluorophenyl)-1*H*-benzimidazole-5-carboxylate (**7j**); yellow solid; IR (KBr) υ_{max}:1711 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.97 (1H, s, H-1b'), 8.41 (1H, d, *J* = 8.0 Hz, H-1b), 8.34 (1H, d, *J* = 1.5 Hz, H-4), 8.17 (1H, d, *J* = 2.0Hz, H-3a), 7.90 (1H, dd, *J* = 8.5, 1.5 Hz, H-6), 7.70 (1H, dd, *J* = 9.0, 2.0 Hz, H-5a), 7.61 (2H, dd, *J* = 8.8, 5.0 Hz, H-2c/6c), 7.47 (2H, t, *J* = 8.6 Hz, H- 3c/5c), 7.32 (1H, d, *J* = 8.1 Hz, H-4b'), 7.29 (1H, d, *J* = 8.1 Hz, H-7b'), 7.24 (1H, d, *J* = 8.5 Hz, H-7), 7.15 (1H, d, *J* = 2.0 Hz, H-2b'), 7.08 (1H, d, *J* = 9.0 Hz, H-6a), 7.06 (1H, t, *J* = 7.8 Hz, H-6b'), 6.91 (1H, t, *J* = 7.8 Hz, H-5b'), 4.95 (1H, dd, *J* = 7.7, 5.2 Hz, H-2b), 3.88 (3H, s, H-11), 3.66 (3H, s, H-4b), 3.38-3.36 (2H, m, H-5b); ^{13}C NMR (DMSO-d_6, 100 MHz) δ171.6 (C-3b), 166.7 (C-10), 162.2 (d, *J* = 245.6 Hz, C-4c), 152.2 (C-2), 144.3

(C-1a), 142.0 (C-8), 140.6 (C-9), 136.4 (C-5a), 136.2 (C-9b'), 132.2 (d, *J* = 2.7 Hz, C-1c), 131.0 (C-2a), 130.1 (d, *J* = 8.9 Hz, C-2c/6c), 127.2 (C-3a), 124.52 (C-6), 124.50 (C-5), 124.4 (C-2b'), 121.3 (C-6b'), 120.7 (C-4), 118.6 (C-5b'), 117.9 (C-4b'), 117.4 (d, *J* = 23.0 Hz, C-3c/5c), 116.6 (C-4a), 115.5 (C-6a), 111.6 (C-7b'), 110.6 (C-7), 107.8 (C-3b'), 55.5 (C-2b), 52.6 (C-4b), 52.2 (C-11), 27.2 (C-5b); HRMS (*m/z*): [M-H] 606.1768 (calculated for $C_{33}H_{25}N_5O_6F$, 606.1789).

3.2.2 Antibacterial assays

Initial screening using the disc diffusion assay

The synthesised compounds were tested against two Gram positive bacteria, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus aureus* Rosenbach ATCC BAA-1683 (methicillin resistant *S. aureus*) and four Gram negative bacteria, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 31488, *Escherichia coli* ATCC 25922 and *Salmonella typhimurium* ATCC 14026. A suspension of the microorganisms (0.5 McFarland barium sulphate standard) were inoculated onto Mueller-Hinton agar (MHA) plates by placing a small amount onto the agar and streaking it evenly over the agar surface.

The test compounds **7a-j** were dissolved in dimethyl sulphoxide (DMSO) to a concentration of 10.0 mg mL^{-1} and 5 µL loaded onto the lawn inoculated agar plates and incubated at 37 °C for 24 h. Compounds showing a zone of inhibition around the place where the compound was spotted were tested further using the minimum bactericidal concentration (MBC) assay.

Minimum Bactericidal Concentration (MBC) Assay

Those compounds which showed activity in the disc diffusion assay were serially diluted with DMSO to prepare a series of concentrations from 1000 µg mL^{-1} to 0.015 µg mL^{-1}. Each

concentration was spotted onto MHA plates prepared as above and incubated for 24 h at 37 °C. The smallest concentration that showed a zone of inhibition around the compound was recorded as the minimum bactericidal concentration. Experiments were performed in triplicate and ciprofloxacin and levofloxacin used as positive controls, while DMSO was used as the negative control. The DMSO control showed no inhibition across all bacterial strains.

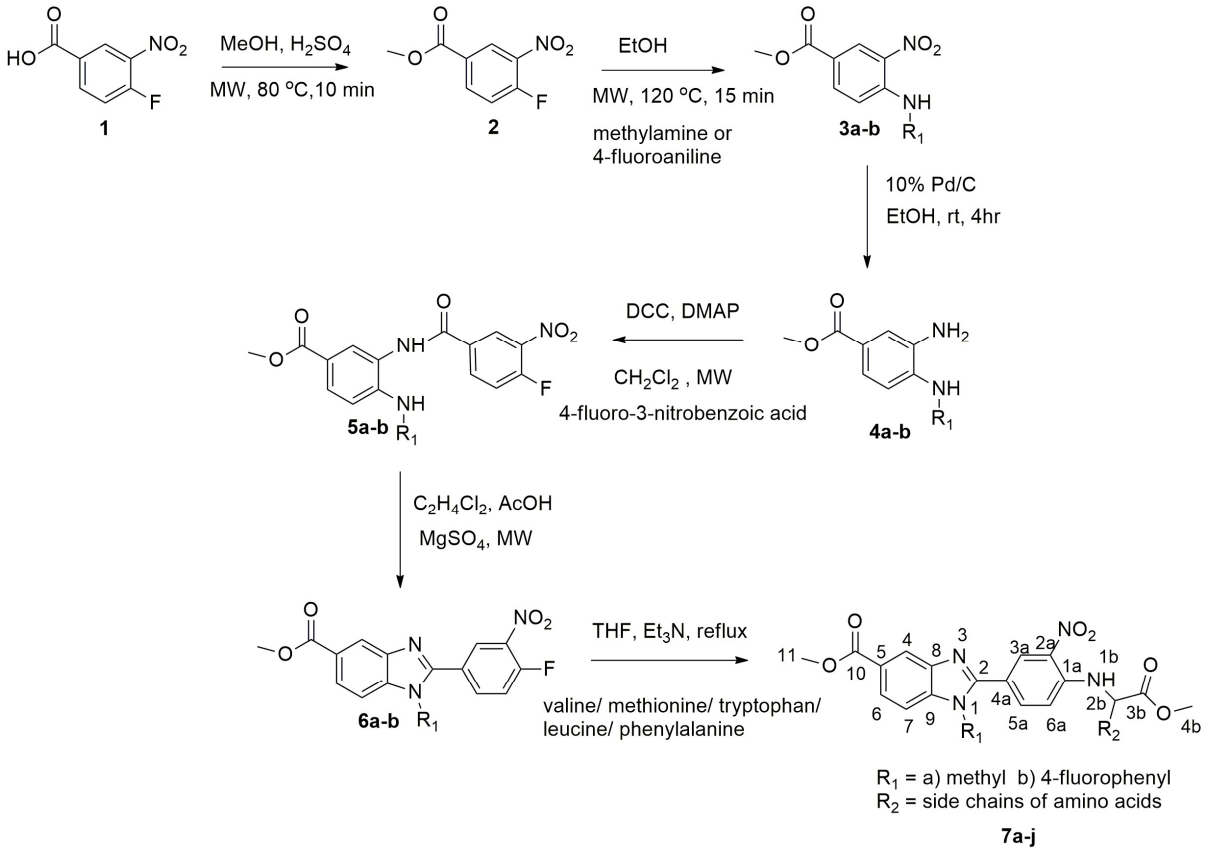
3.3 Results

3.3.1 Chemistry

The novel compounds synthesized contained the benzimidazole core scaffold. These compounds were diversified by substituting either a methyl or 4-fluorophenyl group onto one of the benzimidazole nitrogen atoms and by incorporating different amino acids into the structure. Valine, leucine, methionine, phenylalanine and tryptophan were substituted onto the nitrophenyl ring present at C-2. The amino acids were chosen for their structural diversity. Tryptophan contains an indole side chain that is non-polar and hydrophobic while phenylalanine contains a hydrophobic phenyl ring. Valine and leucine are also non-polar and hydrophobic amino acids containing aliphatic isopropyl groups and methionine has a sulphur containing hydrophobic, aliphatic side chain (Ullah *et al*., 2018).

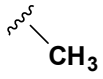
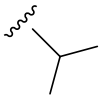
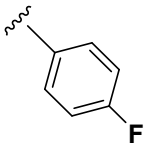
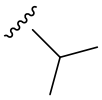
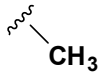
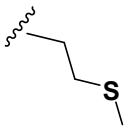
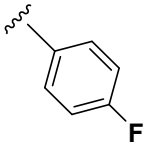
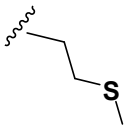
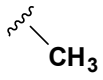
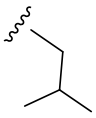
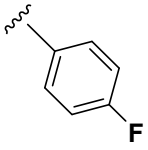
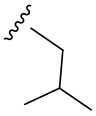
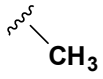
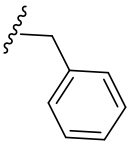
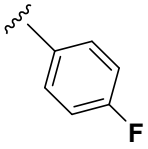
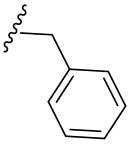
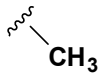
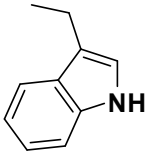
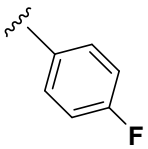
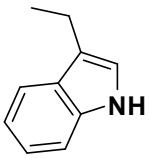
The synthesis of the amino acid linked benzimidazole derivatives is a six step process shown in **Scheme 3.1**. The reaction starts with the esterification of 4-fluoro-3-nitrobenzoic acid to protect the acid group, followed by substitution of the 4-fluoro group with either methylamine or 4-fluoroaniline, introducing the first amino group. Reduction of the nitro group using Pd/C in the presence of H_2 introduced the second amino group. This primary amino group was then coupled with 4-fluoro-3-nitrobenzoic acid through an activated ester generated by dicyclohexyl carbodiimide (DCC) and dimethylaminopyridine (DMAP) to form

an amide with an *ortho* amino group **5**. The next step was cyclization of the benzimidazole under acidic conditions. Amino acids protected as methyl esters were then substituted onto the fluorinated phenyl ring, introducing this functionality to the molecule. The final compounds were synthesised in yields of between 62-73% (**Table 3.1**). Amino acid substitution was also attempted with the esters of alanine, glycine and histidine without any success. We are quite puzzled by this and cannot offer an explanation as to why the reaction was unsuccessful with these amino acids.



Scheme 3.1 Synthetic scheme for the preparation of benzimidazole amino acid hybrids

Table 3.1 Yield (%) and mp (°C) for the final benzimidazole-amino acid compounds

Compound	R_1	R_2	Yield (%)	Mp (°C)
7a			65	150-151
7b			68	122-123
7c			62	167-169
7d			65	152-153
7e			65	120-122
7f			66	110-111
7g			73	155-156
7h			72	127-128
7i			70	193-194
7j			72	184-185

A comparison between microwave reactions and conventional reactions were carried out for this reaction sequence. Apart from step 3 (reduction of the nitro group) and the final step (nucleophilic substitution of the amino acid onto the phenyl group), all other intermediates

were synthesised under microwave conditions. In fact, microwave irradiation resulted in slightly higher yields than the conventional methods in a fraction of the time. The microwave reactions were all 30 minutes or less in yields above 80%, while in the conventional methods, the duration of these reactions were between 12-16 hours with similar yields (**Table 3.2**).

Table 3.2 Comparison of yields and duration of reaction for the intermediates in Scheme 3.1using microwave and conventional synthesis

Intermediate	Microwave synthesis		Conventional synthesis	
	Time (min)	Yield (%)	Time (h)	Yield (%)
2	10	95	12	93
3	15	90	12	80
5	20	85	12	81
6	30	81	16	75

The final substitution reaction to produce **7a-j** required a weak basic catalyst such as triethylamine (Et_3N) and a non-protic solvent such as tetrahydrofuran (THF) in order to proceed. However, with one equivalent of Et_3N at temperatures of 70 °C, only a 25% yield was achieved. Changing the solvent to increase the temperature of the reaction resulted in a lower yield (10%) with dimethylformamide (DMF) and no product with dimethyl sulfoxide (DMSO). Other weak bases such as $NaHCO_3$ and K_2CO_3 did not increase yields with THF and did not yield products with solvents other than THF (**Table 3.3**). Thus, the optimal reagents were THF as a solvent and Et_3N as a base. Increasing the equivalents of Et_3N up to 4 revealed that the optimum basic condition was two equivalents of Et_3N, which resulted in a yield of 65% (**Table 3.4**).

Table 3.3 Yields of the final product 7a at different temperatures and with different solvents and bases.

Entry	Solvent	Base	Temperature (°C)	% Yield
1	THF	Et_3N	25	5
2	THF	Et_3N	70	25
3	THF	$NaHCO_3$	25	5
4	THF	$NaHCO_3$	70	5
5	THF	K_2CO_3	25	10
6	THF	K_2CO_3	70	10
7	DMF	Et_3N	25	10
8	DMF	Et_3N	160	10
9	DMF	K_2CO_3	25	NR
10	DMF	K_2CO_3	160	NR
11	DMF	$NaHCO_3$	25	NR
12	DMF	$NaHCO_3$	160	NR
13	DMSO	$NaHCO_3$	25	NR
14	DMSO	$NaHCO_3$	190	NR
15	DMSO	Et_3N	25	NR
16	DMSO	Et_3N	190	NR
17	DMSO	K_2CO_3	25	NR
18	DMSO	K_2CO_3	190	NR

Compound **7a** was prepared from methyl-benzimidazole-fluoronitrobenzene (**6a**, 0.05mmol) and valine ester hydrochloride salt (0.1 mmol), duration of reaction (16 hours), NR = No reaction.

Table 3.4 Yield produced for the formation of the final compound **7a**with different amounts of Et_3N used in the reaction

Et_3N equivalents	**% Yield**
1	25
1.5	45
2	65
2.5	60
3	60
4	40

Compound **7a** prepared from methyl-benzimidazole-fluoronitrobenzene (**6a**, 0.05 mmol) and valine ester hydrochloride salt (0.1 mmol), reflux (16h).

3.3.2 Structural elucidation

The structures of the synthesised amino acid benzimidazole hybrids were confirmed by NMR and mass spectral analysis. Using **7d,** the methionine derivative as a typical example, the benzimidazole proton resonances of H-4, H-6 and H-7 were seen at δ 8.34 (d, J = 1.5 Hz),δ7.90 (dd, J = 8.5, 1.5 Hz) and δ7.24 (d, J = 8.5 Hz) respectively. The methyl peaks of the two ester groups, H-11 and H-4b were seen as singlets at δ 3.89 and 3.72 respectively. These were differentiated by H-11 showing a HMBC correlation to C-10, which in turn showed HMBC correlations to H-4 and H-6 on the benzimidazole ring (Figure 1). The aromatic protons of the nitrophenyl ring had a similar splitting pattern to that of the benzimidazole moiety with a doublet, double doublet and doublet observed for H-3a, H-5a and H-6a at δ 8.23, 7.72 and 7.10 with $J_{3a,5a}$= 2.2 Hz and $J_{5a,6a}$ = 8.0 Hz. The HMBC correlation between H-3a and C-2 supports the assignment of the aromatic protons on the nitrophenyl ring (**Figure 3.1**).

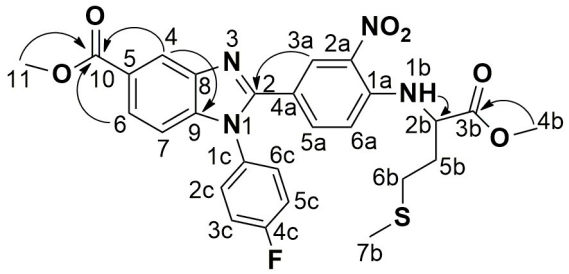


Figure 3.1 Selected HMBC correlations for **7d**

The most deshielded carbon resonances at δ 171.7 and 166.6 were attributed to the carbonyl groups, C-3b and C-10, followed by the resonance of the carbon directly bonded to the fluorine (C-4c), typical at δ 162.2 with a J value of 254.4 Hz. Four other characteristic carbon resonances for this core structure were C-2 at δ 152.2, C-1a at δ 144.3 and C-8 and C-9 at δ 142.0 and 140.6 respectively.

The proton resonances of the *para* disubstituted fluorophenyl moiety attached to N-1, were seen at δ7.65 as a double doublet (J = 5.0 Hz, H-2c/6c) and at δ 7.49 as a triplet (J = 8.6 Hz, H-3c/5c). These resonances were confirmed by their corresponding doublet carbon resonances at δ 130.1 (J = 9.0 Hz, C-2c/5c) and δ117.3 (J = 22.8 Hz, C-3c/5c), typical of carbon resonances *meta* and *ortho* to the fluorine atom. The carbon *para* to the fluorine, C-1c was present at δ132.2 (J = 4.5 Hz). The chiral proton of the methionine side chain H-2b was present as a multiplet at δ4.64-4.82 and the two methylene proton resonances as multiplets at δ 2.16-2.19 (H-5b) and 2.53-2.56 (H-6b). The methyl resonance of the S-CH_3 group occurred as a singlet at δ 2.02 and the N-H proton of the amino acid moiety occurred at δ 8.44 as a doublet (J = 8.0 Hz).

3.3.3 Antibacterial activity

Compounds **7a-j** were evaluated for their antibacterial activity against two Gram +ve bacteria, *S. aureus* (ATCC 25923) and methicillin resistant *S. aureus* (MRSA) (ATCC BAA-1683) and four Gram -ve bacterial strains, *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 31488), *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella typhimurium* (ATCC 14026). The compounds were initially screened for activity using the disc diffusion assay and thereafter, the minimum bactericidal concentration (MBC) of all compounds showing activity was determined.

The compounds were inactive against the Gram +ve bacteria, but showed excellent strain specific activity, more than the ciprofloxacin and levofloxacin against the Gram –ve bacteria. Several compounds were active at concentrations < 1 μM against specific strains of bacteria. Compound **7g**, with a methyl substituent at *N*-1 and a phenylalanine moiety on the phenyl

group showed the broadest spectrum of activity, being active against *S. typhimurium* (4.00 μM), *E. coli* (4.00 μM) and *K. pneumoniae* (2.00 μM). *K. pneumonia* was the most susceptible to these types of compounds, being susceptible to 6 out of the 10 derivatives synthesised. Both compounds with the methionine group (**7c** and **7d**) were very active against *S. typhimurium* only (0.25 and 0.05 μM respectively), while *E. coli* was only susceptible to both compounds with a phenylalanine group (**7g** and **7h**). The MBC values are reported in**Table 3.5**.

Table 3.5 Minimum Bactericidal Concentration of compounds **7a-j** (MBC in μM)

Compound	R_1	R_2	*S. typhimurium*	*K. pneumoniae*	*E. coli*	*P. aeruginosa*
7a	**CH_3**	**Val**	-	0.27	-	-
7b	**4-F phenyl**	**Val**	-	0.46	-	7.51
7c	**CH_3**	**Met**	0.25	-	-	-
7d	**4-F phenyl**	**Met**	0.05	-	-	-
7e	**CH_3**	**Leu**	-	-	-	-
7f	**4-F phenyl**	**Leu**	0.92	-	-	0.45
7g	**CH_3**	**Phe**	4.00	2.00	4.00	-
7h	**4-F phenyl**	**Phe**	-	0.42	1.73	-
7i	**CH_3**	**Trp**	-	0.46	-	-
7j	**4-F phenyl**	**Trp**	-	0.10	-	12.87
	Ciprofloxacin		2.95	2.95	2.95	188.6
	Levofloxacin		21.6	21.6	0.34	345.0

"-" indicates activity greater than 500 μM

3.4 Conclusion

The current synthetic method is an efficient and faster route for the synthesis of new derivatives of benzimidazole-amino acid hybrids using microwave irradiation. The reduction of the reaction time from 12-16 hours to less than 30 minutes for four of the six steps in the synthesis makes this an attractive synthetic method. In addition, the synthesized compounds showed excellent strain specific activity against the Gram –ve bacteria tested against, with the

broadest spectrum of activity shown by **7g**, with a methyl group at *N*-1 and a phenylalanine group on the phenyl portion of the molecule.

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Chapter 4 Synthesis, Characterisation and Antibacterial Activity of a Series of 2-Trifluoromethylbenzimidazole-thiazolidinone Derivatives

***** The compounds referred to in the chapter are referred to in the Abstract, Conclusion and Appendices with **C** preceding the number of the compound.For example **3d** is referred to as **C-3d**.

Abstract

A series of 2-phenyl-3-(2-(trifluoromethyl)-1*H*-benzoimidazol-6-yl)thiazolidin-4-one derivatives were synthesized from *p*-nitro-*o*-phenylenediamine in a three step reaction. The structures of the compounds were elucidated on the basis of NMR and Mass spectral data. The synthesised compounds showed excellent antibacterial activity ranging from three to greater than 100 fold higher than the standards, ciprofloxacin and levofloxacin. Compounds **3d** (2'-Br), **3j** (4'-Br) and **3l** (4'-NO_2) displayed a broad spectrum of activity against the strains tested against (0.14 – 38.33 μM). The brominated derivatives **3d** and **3j** showed excellent activity against the Gram positive bacterial strains (MBC between 0.12 and 35.46 μM), while the nitro derivative **3l** showed excellent activity against all four Gram negative strains tested against (MBC between 0.15 and 9.58 μM).

Keywords: benzimidazoles, thiazolidines, antibacterial activity, hybrid molecules

4.1 Introduction

Bacterial infections are a significant health threat, and responsible for the majority of hospital-acquired infections, leading to extensive mortality and high burden on healthcare systems. The emergence of new virulent forms of bacteria with different levels of resistance to current therapeutic treatments, such as methicillin-resistant *Staphylococcus aureus* (MRSA) (Hassoun *et al*., 2017), multi drug resistant tuberculosis (MDR-TB) (Almahdawi et al., 2018), extended-spectrum *β*-lactamase (ESBL)-producing Enterobacteriaceae and *Escherichia coli* have emerged (Mulisa *et al*., 2016). There is now urgency for new and more effective drug development against both drug resistant and drug sensitive pathogens.

Recently, much attention has focussed on the development of heterocyclic scaffolds for the discovery of new drugs (Taylor *et al*., 2016; Campbell *et al*., 2018). Benzimidazoles are five membered aromatic heterocyclic compounds containing two nitrogen atoms. They are structural isoteres of nucleotides and used extensively in medicinal chemistry with the core moiety being present in a number of well established drugs such as albendazole and tridabendazole (anthelmintic), pantoprazol and omeprazole (proton pump inhibitors), bendamustine (anticancer), carbendazim and fuberidazole (antimicrobials) and telmisartan (antihypertensive) (Akhtar *et al*., 2016).

Thiazolidinones are derivatives of thiazoles containing nitrogen, sulfur and a carbonyl group. They are considered as privileged scaffolds in medicinal chemistry due to its various biological activities such as antimicrobial (Gilani *et al*., 2016; Carradori *et al*., 2017), anti-inflammatory (Abdellatif *et al*., 2016), anticancer (Sharma *et al*., 2016, Szychowski *et al*., 2017), anti-HIV (Suryawanshi *et al*., 2017), antihypertensive (Bhalgat *et al*., 2014) and antidiabetic activities (Nanjan *et al*., 2018).

Hybrid molecules have recently been developed as new antimicobacterial agents (Shaveta *et al*., 2016) and are obtained by the amalgamation of two or more different pharmacophores. Hybrid molecules have also demonstrated good bioactivity as antimicrobial agents (Mahmoud *et al*., 2013; Desai *et al*., 2014; Singh *et al*., 2017). The promising activity exhibited by these molecules prompted us to explore new conjugates by combining benzimidazole and thiazolidinones to search for possible hybrid molecules with antimicrobial activity.

Microwave assisted synthesis has recently been shown to have a number of advantages over conventional methods for organic reactions. They were shown to reduce the duration of the reactions, and improve quality and yields of the products (Dubbey and Moorthy; 2007). It has been popularly used for the synthesis of benzimidazole derivatives with different solvents and catalysts (Khajavi *et al*., 1999; Hosamani *et al*., 2009; Naeimi and Alishahi, 2014; Sharma *et al*., 2017). Although the benzimidazole core has been synthesised using microwave irradiation, there have been relatively few reports on the thiazolidinone moiety being synthesised under microwave conditions (Dandia *et al*., 2006; Patil *et al*., 2011; Sharma *et al*., 2017).

In the present investigation, the synthesis of benzimidazole-thiazolidinone hybrid molecules were synthesised using microwave synthesis and the antimicrobial activity of the compounds determined.

4.2 Experimental

4.2.1 Chemistry

All reagents and chemicals were purchased from Sigma Aldrich (Germany) and used without purification. Reactions were monitored using thin layer chromatography (silica gel 60-F_{254} aluminium coated plates) and visualised under UV light. Column chromatography was carried out using silica gel (60-120 mesh) as the stationary phase and varying ratios of organic solvents as the mobile phase. Melting points were obtained using a Stuart Smart Scientific melting point instrument and are uncorrected. Microwave reactions were performed using a CEM Discover, Explorer-12 Hybrid microwave. ^{1}H, ^{13}C and 2D NMR analysis were acquired on a Bruker Avance 400 MHz spectrometer using tetramethylsilane (TMS) as the internal standard. Chemical shifts were reported in ppm in DMSO-d_6 (δ 2.50 for ^{1}H and 39.51 for ^{13}C NMR) or CD_3OD (δ 3.35 for ^{1}H and 49.15 for ^{13}C NMR). Coupling constants (J) are reported in hertz (Hz). High Resolution Mass Spectrometry (HRMS) was recorded with a Bruker micro TOF-Q II ESI instrument. All column chromatography was performed using silica gel (60-120 mesh).

General procedure for the synthesis of 2-phenyl-3-(2-(trifluoromethyl)-1*H*-benzo[d]imidazol-6-yl)thiazolidin-4-ones

4-Nitro-1,2-phenyldiamine (1.00 g, 0.65 mol) was stirred at room temperature in trifluoro acetic acid (10 mL) for two hours. The reaction was monitored to completion by TLC, after which the trifluoroacetic acid was evaporated under pressure. The crude 6-nitro-2-trifluoromethylbenzimidazole, was dissolved in ethyl acetate (15 mL), basified with sodium hydrogen carbonate (pH 8.5) and partitioned with water (15 mL). The organic layer was evaporated under reduced pressure, to yield 6-nitro-2-trifluoromethyl-benzimidazole **1** as a pale white solid (86% yield). Pd/C (10%) was then added to **1** (0.5 g, 2.2 mmol) dissolved in

methanol and the mixture stirred at room temperature for 4h under hydrogen atmosphere. On completion, the Pd was filtered off under vacuum using celite 545 and the filtrate reduced under pressure and purified by column chromatography using ethyl acetate and hexane (30:70) to yield 2-trifluoromethylbenzimidazol-6-amine **2** as a brown solid (70% yield).

Compound **2** (0.2 g, 1.0 mmol) and substituted benzaldehydes (1.5 mmol) was dissolved in ethanol (3 mL) and thioglycolic acid (0.2 mL, 3.0 mmol) and glacial acetic acid (3-5 drops) added dropwise to the mixture. The mixture then underwent microwave irradiation for 30 min at 110 °C. The reaction was then basified using sodium bicarbonate until just alkaline and extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (hexane:ethyl acetate, 7:3) to yield cream solids **3a-l** in yields of between (75 – 83%)%.

2-Phenyl-3-(2-(trifluoromethyl)-1*H*-benzimidazol-5-yl)thiazolidin-4-one (**3a**); cream solid; 82% yield; mp 117-118 °C; IR (KBr) υ_{max}:1631 (C=O);^{1}H NMR (DMSO-d_6, 400 MHz)δ 13.99 (1H, s, H-NH), 7.70 (1H, bs, H-4), 7.60 (1H, bs, H-7), 7.41 (2H, d, J = 7.5 Hz, H-2b/6b), 7.32 (1H, bs, H-6), 7.25 (2H, t, J = 7.5 Hz, H-3b/5b), 7.20 (1H, t, J = 7.5 Hz, H-4b), 6.55 (1H, s, H-2a), 4.05 (1H, d, J = 15.6 Hz, 5ai), 3.92 (1H, d, J = 15.6 Hz, 5aii); ^{13}C NMR (DMSO-d_6, 100 MHz)δ 170.7 (C-4a), 140.9 (q, J = 39.1 Hz, C-2), 139.9 (C-1b), 128.6 (C-3b/5b), 128.5 (C-4b), 127.2 (C-2b/6b), 118.9 (q, J = 270.7, CF_3), 64.0 (C-2a), 32.7 (C-5a); HRMS (*m/z*) (neg): [M-H] 362.0583 (calculated for $C_{17}H_{11}N_3OF_3S$, 362.0575).

2-(2-Fluorophenyl)-3-(2-(trifluoromethyl)-1*H*-benzimidazol-5-yl)thiazolidin-4-one (**3b**); cream solid; 81% yield; mp 200-202 °C; IR (KBr) υ_{max}:1631 (C=O); ^{1}H NMR (DMSO-d_6,

400 MHz) δ 7.69 (2H, bs, H-4,7), 7.49 (1H, t, *J* = 8.0 Hz, H-6b), 7.35 (1H, d, *J* = 8.0 Hz, H-6), 7.26 (1H, m, H-4b),7.10 (2H, m, H-3b/5b), 6.74 (1H, s, H-2a), 4.03 (1H, d, *J* = 15.6 Hz, H-5ai), 3.93 (1H, d, *J* = 15.6 Hz, H-5aii); ^{13}C NMR (DMSO-d_6, 100 MHz)δ 170.6 (C-4a), 159.9 (d, *J* = 245.8 Hz, C-2b), 141.2 (q, *J* = 39.3 Hz, C-2), 130.8 (d, *J* = 8.5 Hz, C-4b), 129.3 (d, *J* = 2.7 Hz, C-6b), 126.9 (d, *J* = 10.8, 1b), 124.9 (d, *J* = 3.2 Hz, C-5b), 118.9 (q, *J* = 271.7 Hz, CF_3), 115.9 (d, *J* = 20.8 Hz, C-3b), 58.9 (d, *J* = 2.9 Hz, C-2a), 32.8 (C-5a); HRMS (*m/z*) (neg): [M-H] 380.0484 (calculated for $C_{17}H_{10}N_3OF_4S$, 380.0481).

2-(2-Chlorophenyl)-3-(2-(trifluoromethyl)-1*H*-benzimidazol-5-yl)thiazolidin-4-one (**3c**); cream solid; 76% yield; mp 162-163 °C; IR (KBr) υ_{max}:1630 (C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ14.02 (NH), 7.82 (1H, bs, H-4), 7.72 (1H, bs, H-7), 7.59 (1H, bs, H-3b),7.43 (1H, bs, H-6), 7.36 (1H, d, *J* = 7.8 Hz, H-6b),7.29-7.21 (2H, m, H-4b, H-5b), 6.87 (1H, s, H-2a), 4.03 (1H, d, *J* = 15.5 Hz, H-5ai), 3.94 (1H, d, *J* = 15.5 Hz, H-5aii); ^{13}C NMR (DMSO-d_6, 100 MHz)δ 170.8 (C-4a), 141.5 (q, *J* = 38.9 Hz, C-2), 136.7 (C-2b), 131.6 (C-1b), 130.0* (2C, C-3b, 5b), 127.7* (2C, C-4b, C-6b), 118.8 (q, *J* = 270.7 Hz, CF_3), 61.9 (C-2a), 32.62 (C-5a); HRMS (*m/z*) (neg): [M-H] 396.0174 (calculated for $C_{17}H_{10}N_3OF_3SCl$, 396.0185). * assignments may be interchanged.

2-(2-Bromophenyl)-3-(2-(trifluoromethyl)-1*H*-benzimidazol-5-yl)thiazolidin-4-one (**3d**); cream solid; 81% yield; mp 191-192 °C; IR (KBr) υ_{max}: 1679 (C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.75 (1H, bs, H-4), 7.65 (1H, bs, H-6b), 7.59 (1H, bs, H-7), 7.53 (1H, d, *J* = 7.8 Hz, H-3b), 7.42 (1H, d, *J* = 8.4 Hz, H-6), 7.32 (1H, t, *J* = 7.8 Hz, H-5b), 7.15 (1H, dt, *J* = 7.8, 1.6 Hz, H-4b), 6.79 (1H, s, H-2a), 4.02 (1H, dd, *J* = 15.6, 1.3 Hz, H-5ai), 3.93 (1H, d, *J* = 15.6 Hz, H-5aii); ^{13}C NMR (DMSO-d_6, 100 MHz)δ 170.9 (C-4a), 141.3 (q, *J* = 39.4 Hz, C-2), 140.9 (C-2b), 130.3 (C-3b), 128.3 (2C, C-5b, C-6b), 121.7 (C-1b), 118.8 (q, *J* = 270.1

Hz,CF_3), 63.5 (C-2a), 31.3 (C-5a); HRMS (*m/z*) (neg): [M-H] 439.9683 (calculated for $C_{17}H_{10}N_3OF_3SBr$, 439.9680).

3-(2-(trifluoromethyl)-1*H*-benzimidazol-5-yl)-2-(2-(trifluoromethyl)phenyl)thiazolidin-4-one (**3e**); cream solid; 79% yield; mp 194-196 °C;IR (KBr) υ_{max}: 1667 (C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.96 (1H, d, *J* = 7.8 Hz, H-6b), 7.71 (1H, bs, H-4), 7.65 (2H, m, H-4b, H-6), 7.57 (1H, d, *J* = 7.8 Hz, H-3b), 7.40 (2H, m, H-5b, H-7), 6.74 (1H, s, H-2a), 4.07 (1H, d, *J* = 15.5 Hz, H-5ai), 3.95 (1H, d, *J* = 15.5 Hz, H-5aii); ^{13}C NMR (DMSO-d_6, 100 MHz)δ 171.0 (C-4a), 141.6 (q, *J* = 39.5 Hz, C-2), 138.4 (C-1b), 133.5 (C-4b), 129.2 (C-5b), 129.0 (C-6b), 125.6 (q, *J* = 45.6 Hz, C-2b), 125.4 (q, *J* = 5.7 Hz, C-3b), 118.8 (q, *J* = 270.3 Hz, CF_3), 59.8 (C-2a), 32.6 (C-5a); HRMS (*m/z*): [M-H] 430.0445 (calculated for $C_{18}H_{10}N_3OF_6S$, 430.0449).

2-(2-Nitrophenyl)-3-(2-(trifluoromethyl)-1*H*-benzimidazol-5-yl)thiazolidin-4-one (**3f**); cream solid; 76% yield; mp 149-150 °C; IR (KBr) υ_{max}:1667 (C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.94 (1H, dd, *J* = 8.0, 1.2 Hz, H-3b), 7.88 (1H, dd, *J* = 8.0, 1.2 Hz, H-6b), 7.83 (1H, bs, H-4), 7.71 (1H, dt, *J* = 8.0, 1.2 Hz, H-5b), 7.64 (1H, d, *J* = 8.8 Hz, H-7), 7.50 (1H, dd, *J* = 8.8, 1.5 Hz, H-6),7.47 (1H, dt, *J* = 8.0, 1.2 Hz, H-4b), 6.85 (1H, s, H-2a), 4.05 (1H, dd, *J* = 15.8, 1.5 Hz, H-5ai), 3.88 (1H, d, *J* = 15.8 Hz, H-5aii); ^{13}C NMR (DMSO-d_6, 100 MHz)δ 171.0 (C-4a), 147.2 (C-2b), 141.1 (q, *J* = 39.3 Hz, C-2), 135.2 (C-1b), 134.4 (C-5b), 129.5 (C-4b), 128.1 (C-6b), 124.8 (C-3b), 59.6 (C-2a), 31.9 (C-5a); HRMS (*m/z*) (neg): [M-H] 407.0420 (calculated for $C_{17}H_{10}N_4O_3F_3S$, 407.0426).

2-(2-Methoxyphenyl)-3-(2-(trifluoromethyl)-1*H*-benzimidazol-5-yl)thiazolidin-4-one (**3g**); cream solid; 75% yield; mp 170-172 °C; IR (KBr) υ_{max}: 1676 (C=O); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.97 (1H, d, *J* = 7.4 Hz, H-6), 7.63 (1H, d, *J* = 8.6 Hz, H-3b), 7.20 (1H, dt, *J* = 8.0,

1.5 Hz, H-5b), 7.02-6.96 (3H, m, H-4b, H-4, H-7), 6.78 (1H, d, *J* = 8.0 Hz, H-6b), 6.24 (1H, bs, H-2a), 3.40 (1H, d, *J* = 16.0 Hz, H-5ai), 3.38 (3H, s, OCH_3), 2.81 (1H, d, *J* = 16.0 Hz, H-5aii); ^{13}C NMR (CD_3OD-d_4, 100 MHz) δ 173.8 (C-4a), 158.4 (C-2b), 130.4 (C-3b), 129.6 (C-5b), 121.0 (C-4b), 111.6 (C-6b), 55.6 (OCH_3), 31.9 (C-5a); HRMS (*m/z*) (neg): [M-H] 392.0674 (calculated for $C_{18}H_{13}N_3O_2F_3S$, 392.0681).

2-(4-Fluorophenyl)-3-(2-(trifluoromethyl)-1*H*-benzimidazol-5-yl)thiazolidin-4-one (**3h**); cream solid; 83% yield; mp 212-213 °C; IR (KBr) υ_{max}: 1632 (C=O); ^1H NMR (DMSO-d_6, 400 MHz) δ 7.62 (2H, bs, H-4, H-7), 7.47 (2H, dd, *J* = 8.8, 5.4 Hz, H-2b/6b), 7.29 (1H, d, *J* = 8.6 Hz, H-6), 7.07 (2H, t, *J* = 8.8 Hz, H-3b/5b), 6.55 (1H, s, H-2a), 4.04 (1H, d, *J* = 15.6 Hz, H-5ai), 3.92 (1H, d, *J* = 15.6 Hz, H-5aii); ^{13}C NMR (DMSO-d_6, 100 MHz)δ 170.7 (C-4a), 162.1 (d, *J* = 243.7 Hz, C-4b), 141.1 (q, *J* = 39.9, C-2), 136.1 (d, *J* = 2.8 Hz, C-1b), 129.5 (d, *J* = 9.2 Hz, C-2b/6b), 118.8 (q, *J* = 271.2 Hz, CF_3), 115.5 (d, *J* = 21.1 Hz, C-3b/5b), 63.4 (C-2a), 32.8 (C-5a); HRMS (*m/z*) (neg): [M-H] 380.0484 (calculated for $C_{17}H_{10}N_3OF_4S$, 380.0481).

2-(4-Chlorophenyl)-3-(2-(trifluoromethyl)-1*H*-benzimidazol-5-yl)thiazolidin-4-one (**3i**); cream solid; 78% yield; mp 188-190 °C; IR (KBr) υ_{max}: 1656 (C=O); ^1H NMR (DMSO-d_6, 400 MHz) δ 14.00 (1H, bs, NH), 7.71 (1H, bs, H-4), 7.58 (1H, bs, H-7), 7.46 (2H, d, *J* = 8.4 Hz, H-2b/6b), 7.31 (1H, bs, H-6*), 7.30 (2H, d, *J* = 8.4 Hz, 3b/5b*), 6.58 (1H, s, H-2a), 4.06 (1H, d, *J* = 15.7 Hz, H-5ai), 3.93 (1H, d, *J* = 15.7 Hz, H-5aii); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 170.5 (C-4a), 141.2 (q, *J* = 39.8 Hz, C-2), 139.0 (C-1b), 132.9 (C-4b), 129.2 (C-2b/6b), 128.6 (C-3b/5b), 118.8 (q, *J* = 270.2 Hz, CF_3), 63.1 (C-2a), 32.7 (C-5a); HRMS (*m/z*) (neg): [M-H] 396.0179 (calculated for $C_{17}H_{10}N_3OF_3SCl$, 396.0185). *overlapping resonances

2-(4-Bromophenyl)-3-(2-(trifluoromethyl)-1*H*-benzimidazol-5-yl)thiazolidin-4-one (**3j**); cream solid; 83% yield; mp 200-201 °C; IR (KBr) υ_{max}:1634 (C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.64 (2H, bs, H-4, H-7), 7.45 (2H, d, J = 8.5 Hz, H- 3b/5b), 7.40 (2H, d, J = 8.5 Hz, H-2b/6b), 7.31 (1H, d, J = 8.8 Hz, H-6), 6.56 (1H, s, H-2a), 4.06 (1H, dd, J = 15.7, 1.4 Hz, H-5ai), 3.93 (1H, d, J = 15.7 Hz, H-5aii); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 170.6 (C-4a), 141.2 (q, J = 39.5 Hz, C-2), 139.5 (C-1b), 131.5 (C-3b/5b), 129.5 (C-2b/6b), 121.6 (C-4b), 118.8 (q, J = 271.2 Hz, C-CF_3), 63.2 (C-2a), 37.7 (C-5a); HRMS (*m/z*) (neg): [M-H] 439.9682 (calculated for $C_{17}H_{10}N_3OF_3SBr$, 439.9680).

3-(2-(Trifluoromethyl)-1*H*-benzimidazol-5-yl)-2-(4-(trifluoromethyl)phenyl)thiazolidin-4-one (**3k**); cream solid; 82% yield; mp 183-184 °C; IR (KBr) υ_{max}: 1637 (C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.71 (1H, s, H-4), 7.67 (3H, d, J = 8.3 Hz, H-2b/6b, H-7*), 7.61 (2H, d, J = 8.3 Hz, H-3b/5b), 7.35 (1H, d, J = 8.7 Hz, H-6), 6.68 (1H, s, H-2a), 4.10 (1H, d, J = 15.6 Hz, H-5ai), 3.95 (1H, d, J = 15.6 Hz, H-5aii); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 170.8 (C-4a), 144.9 (C-1b), 141.4 (q, J = 40.0 Hz, C-2),128.9 (q, J = 31.3 Hz, C-4b),128.0 (C-2b/6b), 125.7 (d, J = 3.7 Hz, C-3b/5b), 124.7 (q, J = 270.4 Hz, Ph$\underline{C}F_3$),118.8 (q, J = 270.4 Hz, CF_3), 63.2 (C-2a), 32.7 (C-5a); HRMS (*m/z*) (neg): [M-H] 480.0448 (calculated for $C_{18}H_{10}N_3OF_6S$, 430.0449). *H-7 is underneath the H-2b/6b resonance.

2-(4-Nitrophenyl)-3-(2-(trifluoromethyl)-1*H*-benzimidazol-5-yl)thiazolidin-4-one (**3l**); cream solid; 78% yield; mp 153-154 °C; IR (KBr) υ_{max}: 1671 (C=O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.07 (2H, d, J = 8.8 Hz, H-3b/5b), 7.68 (3H, d, J = 8.8, H-2b/6b, H-4*), 7.61 (1H, d, J = 8.8 Hz, H-7), 7.33 (1H, dd, J = 8.8, 1.9 Hz, H-6), 6.64 (1H, s, H-2a), 4.07 (1H, dd, J = 15.8, 1.5 Hz, H-5ai), 3.92 (1H, d, J = 15.8 Hz, H-5aii); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.5 (C-4a), 147.9 (C-1b), 141.9 (q, J = 40.0 Hz, C-2), 133.9 (C-4b), 129.0 (C-2b/6b), 124.3

(C-3b/5b), 119.1 (q, J = 271.6 Hz, CF_3), 63.5 (C-2a), 33.2 (C-5a); HRMS (*m/z*): [M-H] 407.0431 (calculated for $C_{17}H_{10}N_4O_3F_3S$, 407.0426). * H-4 overlaps with H-2b/6b.

Note: C-4, C-6 and C-7 for all compounds were not present in the ^{13}C NMR spectra due to degenerated tautomerism of the NH proton, resulting in the resonances becoming magnetically equivalent due to rapid proton exchange between N1 and N3 (Nieto *et al*., 2014). This is also responsible for broadening of the ^{1}H resonances for H-4, H-6 and H-7 resulting in the theoretical splitting patterns being absent. HRMS confirmed the structures of all compounds.

4.2.2 Antibacterial assays

The antibacterial activity of the synthesized compounds were tested against two Gram positive bacteria, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus aureus* Rosenbach ATCC BAA-1683 (methicillin resistant *S. aureus*) and four Gram negative bacteria, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 31488, *Escherichia coli* ATCC 25922 and *Salmonella typhimurium* ATCC 14026.

Sterilized Mueller-Hinton agar plates were lawn inoculated with different bacterial strains. Stock solutions of each test compound (1 mg mL^{-1} in DMSO) were prepared and 5 µl of each compound placed onto the plates and incubated at 37 °C for 24 h, after which the compounds that inhibited bacterial growth were further tested using the minimum bactericidal concentration (MBC) assay.

Minimum Bactericidal Concentration Assay

All compounds showing any activity in the initial disc diffusion screening assay were tested for their antibacterial activity by the MBC assay. They were dissolved in DMSO and two fold serially diluted to give a range from 1000 µg mL^{-1} to 0.03 µg mL^{-1}. Mueller-Hinton agar plates lawn inoculated with the different bacterial strains were impregnated with 5 µl of each concentration of compound and incubated for 24 h at 37 °C. Ciprofloxacin and Levofloxacin were used as positive controls while DMSO was used as a negative control. The lowest concentration that inhibited the growth of bacteria was taken to be the MBC value. The assay was performed in triplicate and an average of the readings taken.

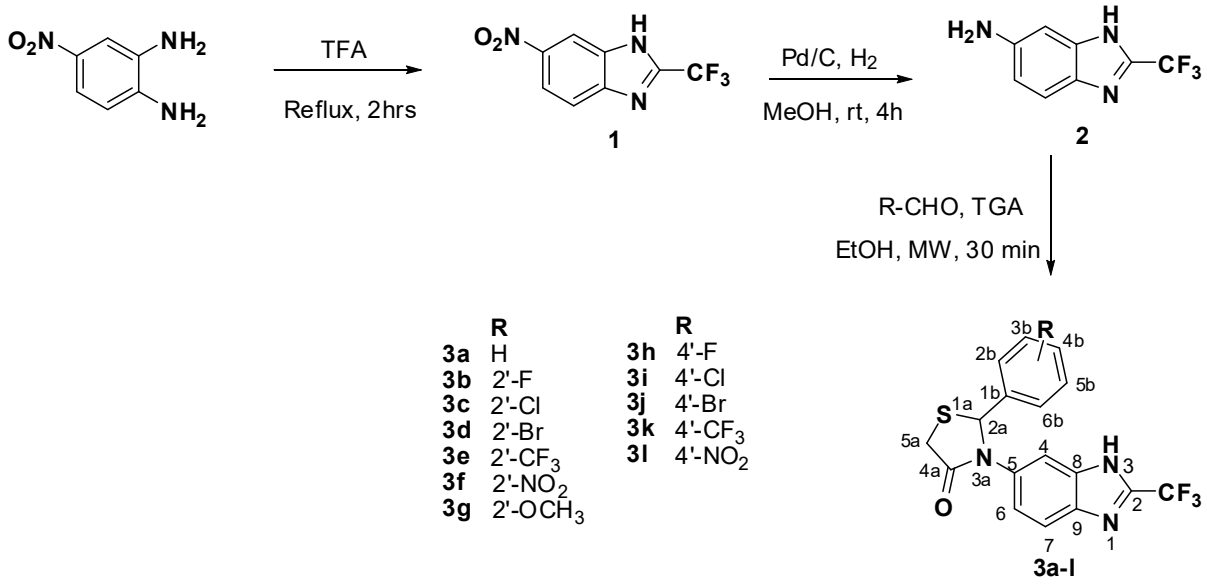
4.3 Results and Discussion

4.3.1 Chemistry

An appropriate synthetic strategy was selected for the synthesis of benzimidazole-thiazolidinone hybrids **3a-l** (**Scheme 4.1**). We have successfully synthesized 12 new derivatives using microwave-assisted irradiation in the final step. The 2-trifluoromethyl benzimidazoles **2** were prepared from 4-nitro-1,2-phenyldiamine and trifluoroacetic acid. The nitro group was then reduced to the amine by catalytic (Pd/C) hydrogenation and thiazolidinones formed using a one-pot technique by employing the Knovenagel condensation of the amino group (of the benzimidazole), substituted benzaldehydes and thioglycolic acid under microwave conditions. The final benzimidazole-thiazolidinones **3a-l** was synthesized as racemic mixtures in yields of 75-83%.

Although forming thiazolidines with primary amines, aldehydes and thioglycolic acid is common, conventional methods use harsh solvents such as DMF, dioxane and toluene with long reaction times (16 h) (Dandia *etal*., 2006; Saini *et al*. 2018) or microwave methods with

shorter reaction times (5-10 min) with toluene as the solvent (Sharma et al., 2017). We have successfully synthesised the thiazolidonones from benzimidazole precursors using the eco-friendly ethanol as solvent under microwave irradation. This is a simple, attractive and greener method for preparing benzimidazole-thiazolidinone hybrid molecules.



Scheme 4.1 Synthesis of substituted 2-phenyl-3-(2-(trifluoromethyl)-1*H*-benzimidazol-6-yl)thiazolidin-4-ones

The structures of the synthesised compounds were established by NMR spectroscopy and High Resolution Mass Spectrometry (HRMS). HRMS was particularly important since the benzimidazole carbon resonances were not observed in the ^{13}C NMR spectra due to degenerated tautomerism of the NH proton, resulting in the resonances becoming magnetically equivalent due to the rapid proton exchange between N1 and N3 (Nieto *et al.*, 2014). This is also responsible for the broadening of the H-4, H-6 and H-7 resonances, which should have appeared as a doublet ($J \sim 2$ Hz), double doublet ($J \sim 8$ and 2 Hz) and doublet ($J \sim 8$ Hz) respectively, but appeared as broadened singlets for H-4 and H-7. H-6 appeared as a doublet (not a double doublet) in many instances and only in the case of **3l**, the *para* nitro derivative, did it appear with its correct splitting pattern, a double doublet. In **3l**, H-4

coincided with the H-2b/6b doublet at δ 7.68, appearing underneath this resonance, and H-7 and H-6 appeared at δ 7.61 and δ 7.33 as a doublet and double doublet with $J_{H6,H7}$ = 8.8 Hz and $J_{H6,H4}$ = 1.9 Hz. The H-3b/5b doublet appeared at δ 8.07. The H-2a proton was present at δ 6.64 and the diastereotopic protons H-5ai and H-5aii as a double doublet and doublet respectively at δ4.07 andδ 3.92 with $J_{H5ai,H5aii}$ = 15.8Hz and $J_{H5ai,H2a}$ = 1.5 Hz. These coupling constants were clearly seen, even though the resonances coincided with the solvent peak. In other compounds however, H-5ai and H-5aii were separated from the solvent peak and are clearly observed.

As mentioned previously, due to the degenerated tautomerism of the NH proton, the benzimidazole carbon resonances C-4 to C-9 were not observed in the ^{13}C NMR spectra, however C-2 and the CF_3 carbon were present in almost all compounds. These occurred as two quartets at δ141.9 (J = 40.0 Hz) and δ119.1 (J = 271.6 Hz) in **3l.** The three thiazolidinone carbon resonances were present at δ 171.5 (C-4a), 63.5 (C-2a) and 33.2 (C-5a). The nitrophenyl carbon resonances were present at δ 147.9 (C-1b), 129.0 (C-2b/6b), 124.3 (C-3b/5b) and 133.9 (C-4b).

4.3.2 Antimicrobial Activity

The synthesized compounds were evaluated for their *in-vitro* antibacterial activity against four Gram negative bacteria *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli* and *Salmonella typhimurium* and two Gram positive bacteria, *Staphylococcus aureus* and *Staphylococcus aureus* Rosenbach (methicillin resistant *S. aureus* (MRSA)). These are causative agents for urinary tract, gastrointestinal, nosocomial infections as well as pneumonia and bronchitis.

Varying electron donating and withdrawing substituents did not show any appreciable differences in activity (**Table 4.1**). Overall, the compounds demonstrated excellent activity when compared to the known antibiotics, ciprofloxacin and levofloxacin. The Gram negative *K. Pneumonia* and Gram positive *S. aureus* were most susceptible to all the synthesised derivatives with MBC values of between 0.14-5.28 μM and 0.12-38.33 μM respectively. Compounds **3d**, **3j** and **3l**, containing bromine and nitro groups, displayed broad range activity against all test strains. The 2-Br (**3d**) and 4-Br (**3j**) derivatives showed excellent activity, higher than ciprofloxacin, against both the Gram positive strains (*S. aureus* = 94.3 μM and MRSA = 188.6 μM). The 4-NO_2 (**3l**) derivative showed excellent activity against the Gram negative strains with MBC values of 0.15 μM against *S. typhimurium*, *K. pneumonia* and *E. coli* and 9.58 μM against *P. aeruginosa*.

Similar benzimidazoles with a phenyl group instead of a trifluoromethyl group also showed good antimicrobial activity, comparable to ciprofloxacin (Verma *et al*., 2014) and 2,3-disubstituted thiazolidinone-benzimidazole hybrids with a methylthio group instead of the trifluromethyl at C-2 and a thialzolidinone group attached to a thiosemicarbazone at N-1, showed broad spectrum antibacterial activity similar to ampicillin (Mahmoud *et al*., 2013). 2-Mercaptobenzimidazole-4-phenylthiazolidinone derivatives, with a thiol group at C-2 instead of a trifluoromethyl group showed up to four-fold better activity against strain specific bacteria than ciprofloxacin (Saini *et al*., 2018). Our results, although having similar activity to those previously reported have shown a broader spectrum of activity than those previously reported and has furthermore shown the potential of the trifluoromethyl group to be a useful moiety to tag onto C-2 of benzimidazoles to produce small molecules with good antibacterial activity.

Table 4.1 Minimum Bactericidal Concentration (MBC in μM) of compounds **3a-l**

		Gram –ve			Gram +ve		
No.	**R**	***S.t.***	***K.p.***	***E.c.***	***P.a.***	***S.a.***	**MRSA**
3a	**H**	-	-	0.66	-	0.66	-
3b	**2-F**	-	0.32	-	0.16	0.16	-
3c	**2-Cl**	-	5.28	0.65	0.16	0.16	-
3d	**2-Br**	-	0.14	0.54	0.27	35.46	17.73
3e	**2-CF_3**	-	0.14	-	0.28	9.07	-
3f	**2-NO_2**	0.15	0.15	4.79	-	4.79	-
3g	**2-OCH_3**	-	0.15	79.71	-	0.15	-
3h	**4-F**	-	0.32	-	0.16	0.16	-
3i	**4-Cl**	-	0.61	0.61	0.61	0.30	-
3j	**4-Br**	17.73	0.14	0.27	0.55	0.27	35.46
3k	**4-CF_3**	-	0.25	-	-	0.12	-
3l	**4-NO_2**	0.15	0.15	0.15	9.58	38.33	>250
Cip		2.95	11.8	2.95	188.6	94.3	188.6
Lev		21.6	21.6	0.34	345.0	21.6	86.5

S.t. = S. typhimurium, *K.p. = Klebsiella pneumonia*, *E.c. = E. coli*, *P.a. = P. aeruginosa*, *S.a. = S. aureus*, Cip = Ciprofloxacin, Lev = Levofloxacin

4.4 Conclusion

A series of new benzimidazole-thiazolidinone hybrids were synthesized by a simple and attractive method using 4-nitro-1,2-phenyldiamine, trifluoroacetic acid, thioglycolic acid and a series of benzaldehydes. Microwave synthesis using ethanol as the solvent was successfully employed to synthesise the thiazolidinones in the final step with good yields in a more eco-friendly method. This is a rapid method of adding a thiazolidinone moiety to a core structure with a free amino group, providing an avenue to increase its bioactivity. The synthesised compounds showed excellent antibacterial activity against a series of Gram positive and negative bacterial strains. Among them, the bromo and nitro derivatives **3d**, **3j**and **3l** showed a broad spectrum of activity being active in all of the strains tested against with MBC values between 0.14 and 38.33 μM. These compounds are good hits that can be developed into lead compounds for new antibiotics.

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Chapter 5 Conclusion

The emergence of drug resistant bacteria and the increase of bacterial infections and diseases threaten millions of people and every effort to facilitate and support studies to develop novel antibacterial agents is beneficial. This thesis focuses on the synthesis of benzimidazole hybrid derivatives containing thiazolidinone and amino acid motifs and their potential as antibacterial agents.

The synthesis of twenty-four benzimidazole-thiazolidinone derivatives (**A7a-l and C3a-l**) and ten benzimidazole-amino acid hybrids (**B7a-j**) was successfully carried out using multi-step reactions preceding from substituted *o*-phenylenediamines. Various synthetic methods were used and in every case the most practical methods were selected, which were simple and cost effective. These steps were performed under conventional methods and microwave-assisted methods with yields in excess of 60%. Microwave-assisted synthesis reduced the reaction times drastically from 12-16 hours to 15-30 minutes with slightly better yields than the conventional reflux methods. All compounds synthesized were characterized by NMR, HRMS and IR spectroscopy.

Derivatives have been designed with structural variations in each series to identify potential hit antibacterial agents. The initial findings of benzimidazole derivatives as antibacterial compounds was based on *in vitro* screening using the minimum bactericidal concentration (MBC) assay which was used instead of the minimum inhibitory concentration (MIC) assay due to insolubility of compounds in bacterial growth medium.

Of the three class of compounds synthesized, the best activity was seen by trifluoromethyl-benzimidazole-thiazolidinone derivatives (**C3a-l**). These compounds showed broad spectrum

activity against *K. pneumoniae* and *S. aureus* with MBC values < 40 μM. The best activity was seen by **C3k**,the 4'-trifluoromethyl derivative with a MBC value of 0.12 μM against *S. aureus*. The bromo and nitro derivatives **C3d**, **C3j** and **C3l** showed a broad spectrum of activity being active in all of the strains tested against with MBC values between 0.14 and 38.33 μM. The other class of benzimidazole-thiazolidinone derivatives (**A7a-j**) exhibited moderate to low antibacterial activity. Compounds **A7f** and **A7i**, the 2'-NO2 and 4'-Cl derivatives exhibited the best activity of the series, being active at 1.9 and 2.0 mM, and 3.9 and 4.0 mM against *P. aeruginosa* and *S. aureus* respectively. The 2'-NO_2 derivative **A7f** showed the best overall activity within the series with MBC values < 4.0 mM.

Literature showed that compounds bearing chiral *α*-amino acid residues especially those tethered to benzimidazole moieties are a relatively untapped part of organic synthesis. We have synthesised benzimidazoles bearing amino acid side chains (**B7a-j**) with either a fluorophenyl or methyl group attached to *N*-1. Since the nitro groups were the most active in previously synthesized compounds, we decided to attach the amino acid to a nitrophenyl motif. However, this class of compounds did not exhibit a broad spectrum of activity. The compounds that did show activity displayed MBC values < 13 μM for selected strains, with **B7d**, the 4'-fluorophenyl derivative with a methionine side chain having the best activity (MBC of 0.05 μM against *S. typhimurium*). Compound **B7g**, with a methyl substituent at *N*-1 and a phenylalanine moiety on the phenyl group showed the broadest spectrum of activity, being active against *S. typhimurium* (4.00 μM), *E. coli* (4.00 μM) and *K. pneumoniae* (2.00 μM). *K. pneumonia* was the most susceptible to these types of compounds, being susceptible to 6 out of the 10 derivatives synthesised.

The 2-trifluromethyl-benzimidazole-thiazolidinone derivatives (**C3a-l**), the most active of the three series, showed two fold or greater activity than the standards ciprofloxacin and levofloxicin, with compounds **C3d-f** and **C3j-l** carrying bromo, nitro and triflouromethyl substituents having the best results. These findings were also corroborated by literature, in which a series of 2-mercapto-benzimidazole-thiazolidinone compounds showed three fold higher activity than ciprofloxacin, with compounds having nitro, cyano and trifluoromethyl substituents on the benzene ring of thiazolidinone showing the highest activity (Saini *et al*., 2018). Benzimidazole-thiazolidinone compounds showed very interesting results, as very small structural modifications affected the antibacterial activity considerably. The 2-trifluromethyl-benzimidazole-thiazolidinone scaffold is an interesting subject for future studies as their high antibacterial activity make them a good scaffold to build on.

Other future work will be to investigate other motifs on the trifluoromethyl-benzimidazoles, such as quinolines, *β*-lactams and Schiff bases to determine their synergistic effect on antibacterial activity. The antibacterial structure activity relationship of the synthesized benzimidazole derivatives differ considerably and their mechanism of action remain elusive.Further studies should be conducted to clarify these. Cytotoxity assays on the most active compounds also need to be carried out as these assays were not available during this study.

In conclusion, our study involved the design, synthesis and characterization of new benzimidazole, thiazolidinone and amino acid derivatives and evaluation of their antibacterial properties. The optimised synthetic methods developed would be highly useful to future researchers. The study found that triflouromethyl-benzimidazole-thiazolidinone derivatives

could be an attractive template for the identification of novel and potential antibacterial agents.

5.1 References

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University Of Kwazulu-Natal

Synthesis, Characterization And Antibacterial Activity Of Benzimidazole Derivatives

Appendix A: Characterization data

2018

Adele Cheddie

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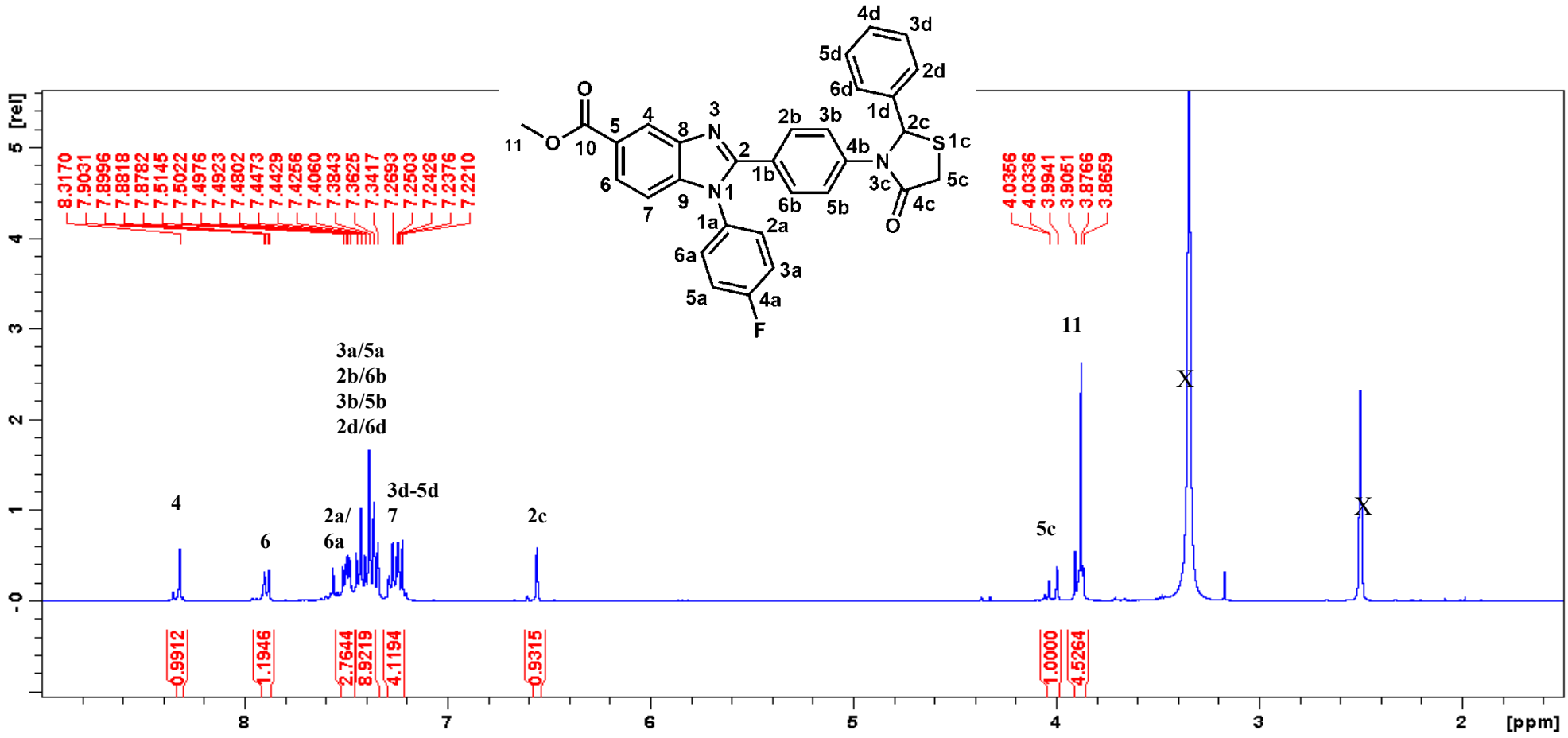

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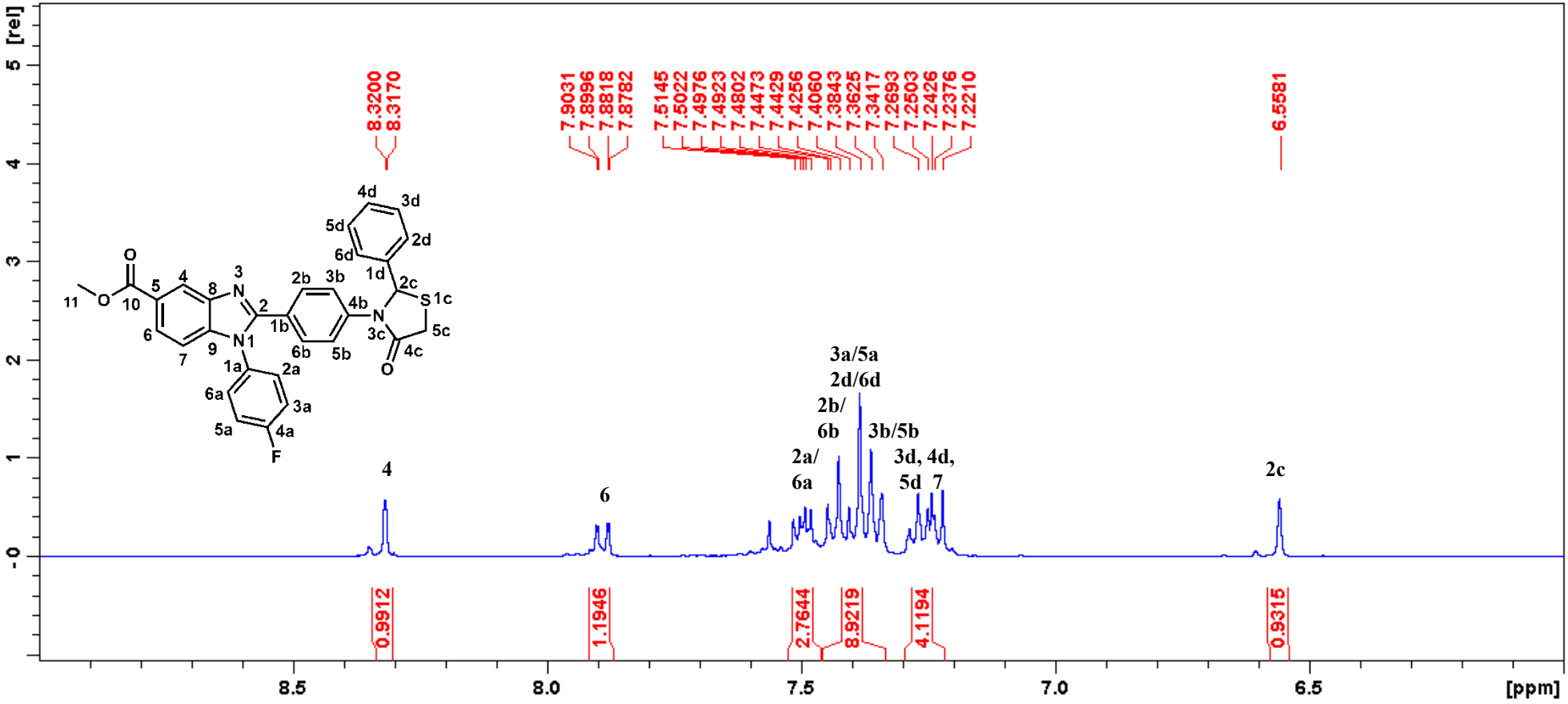

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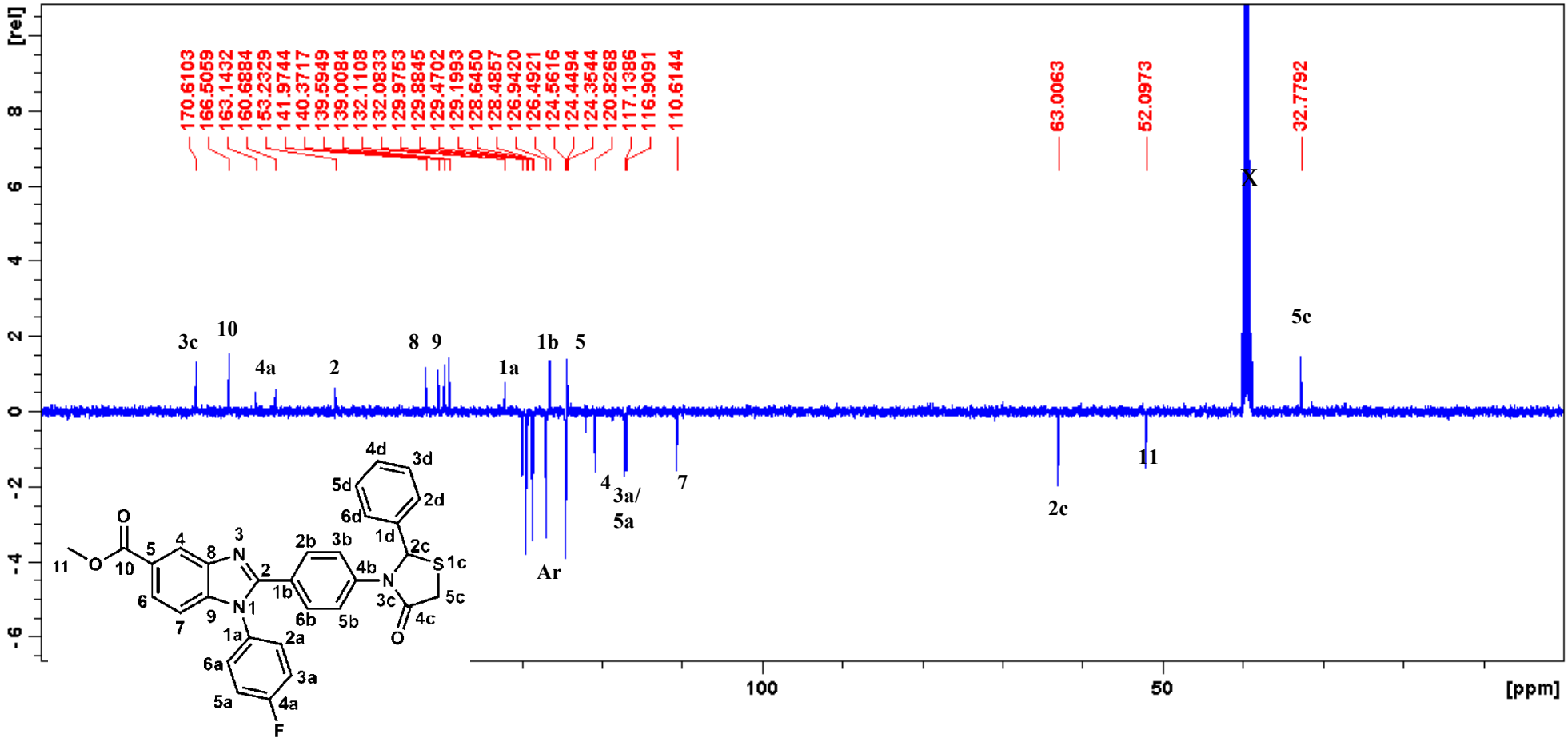

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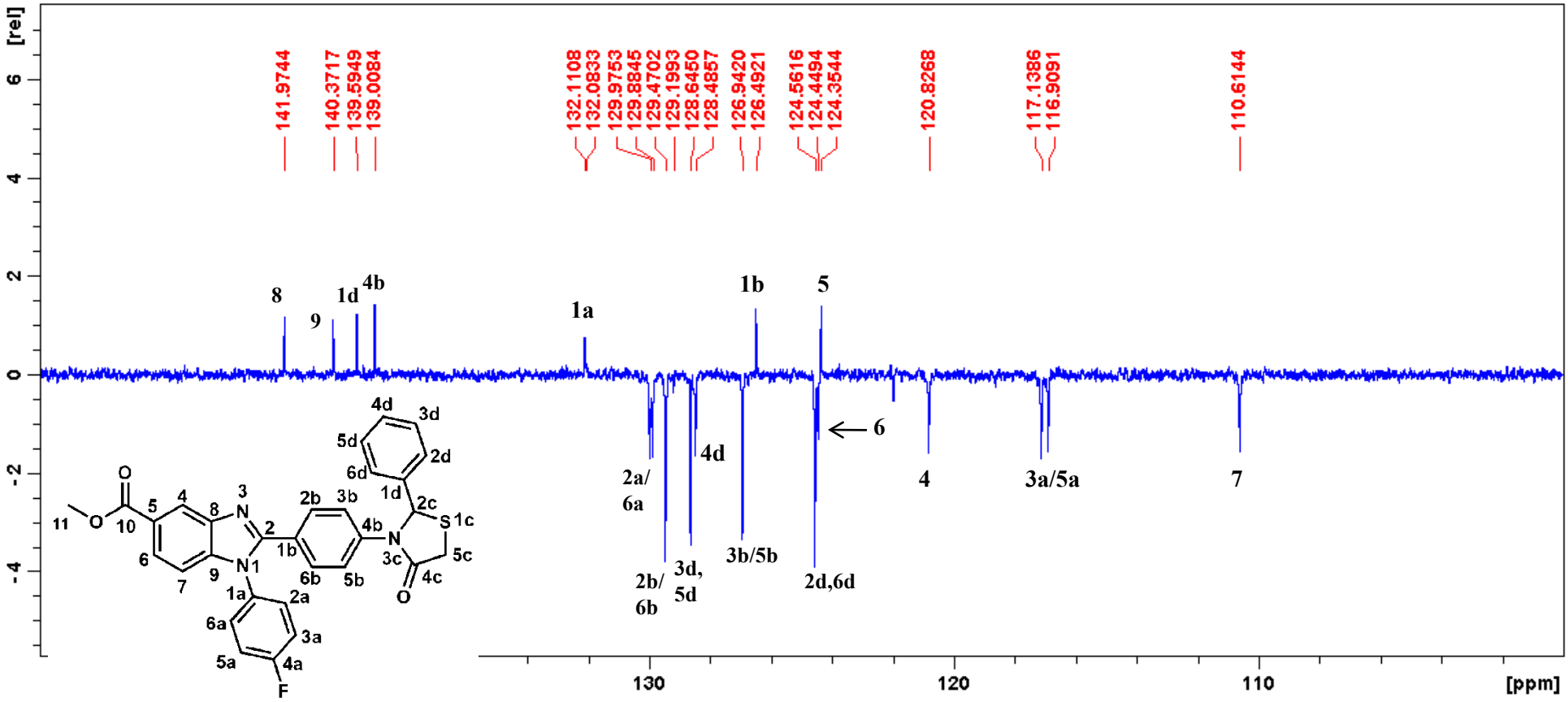

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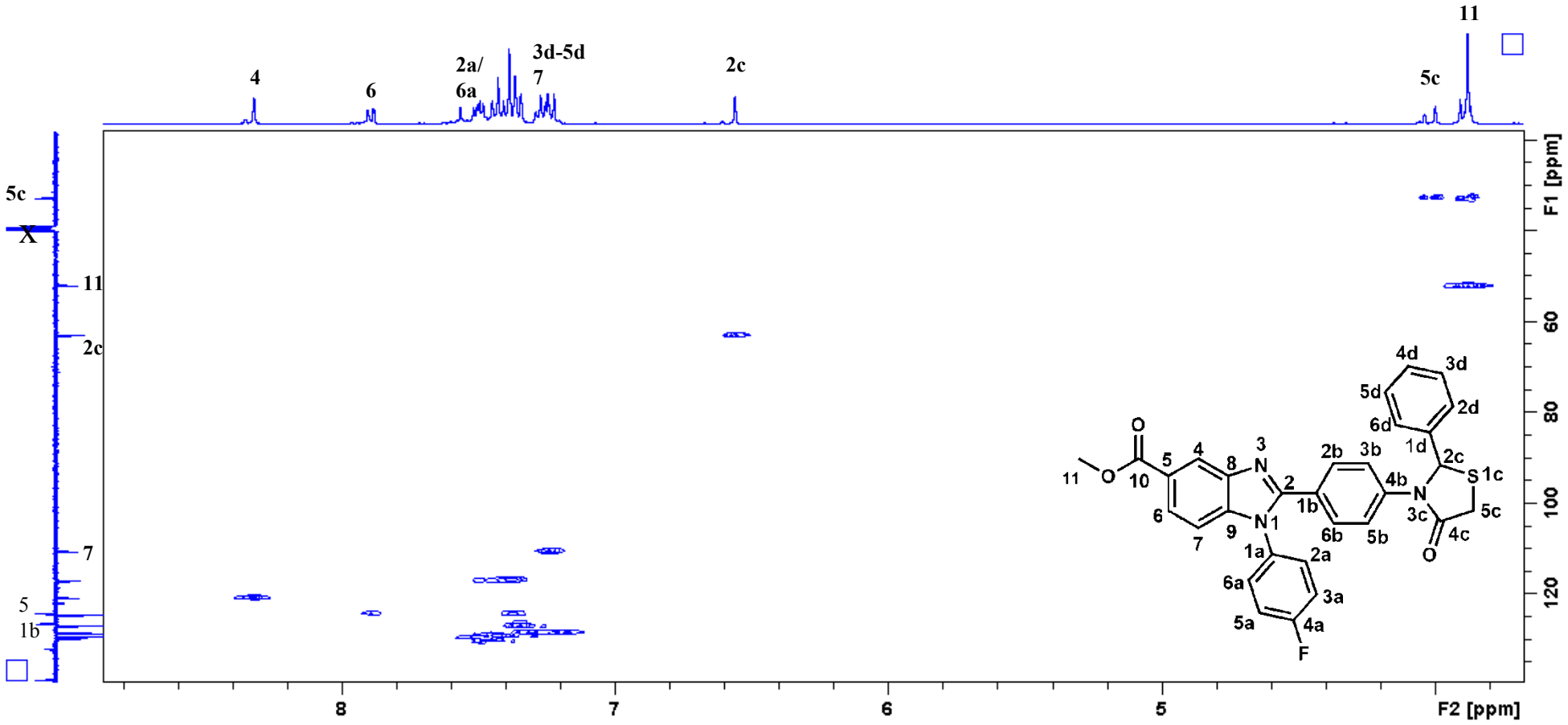

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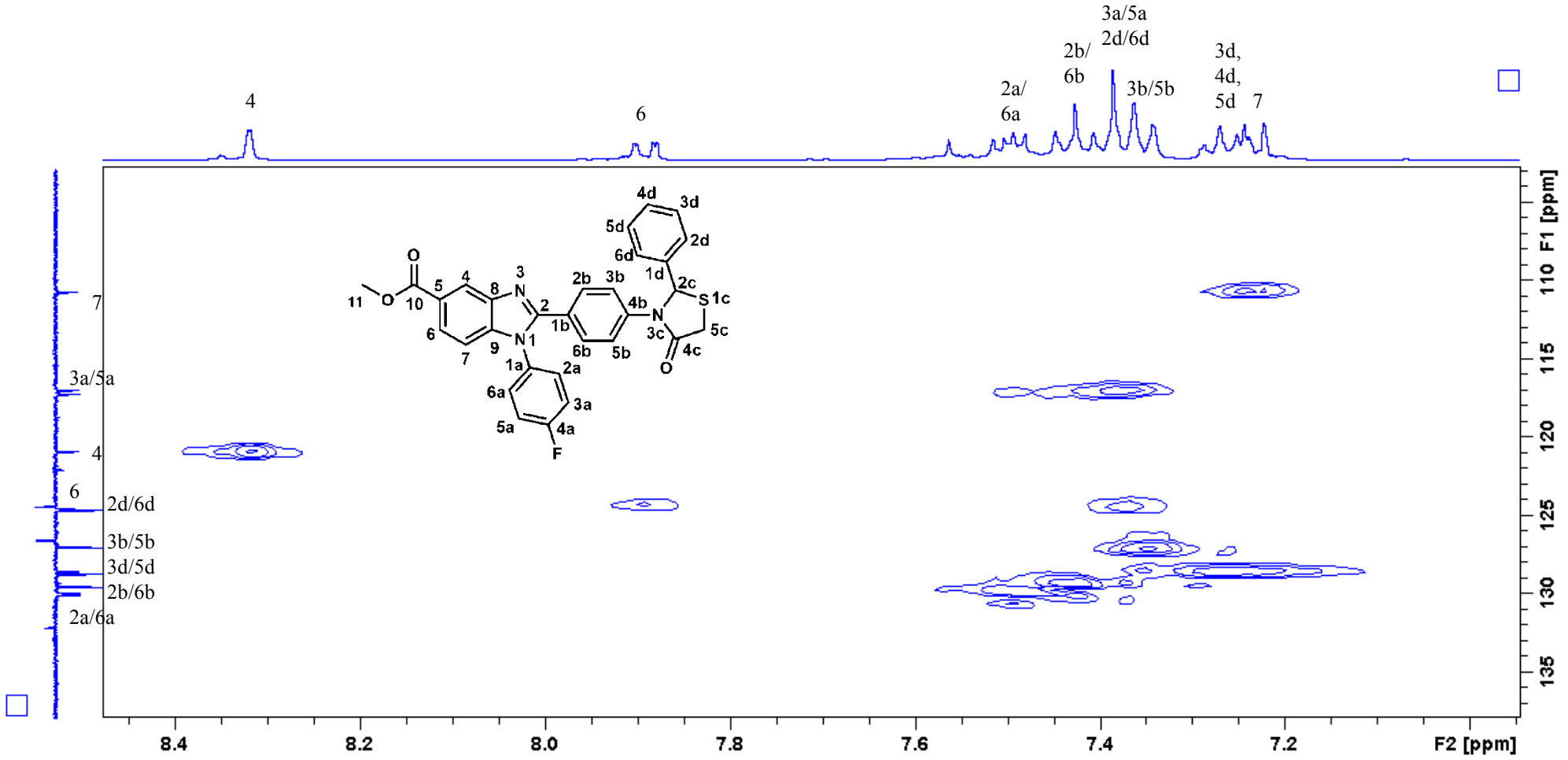

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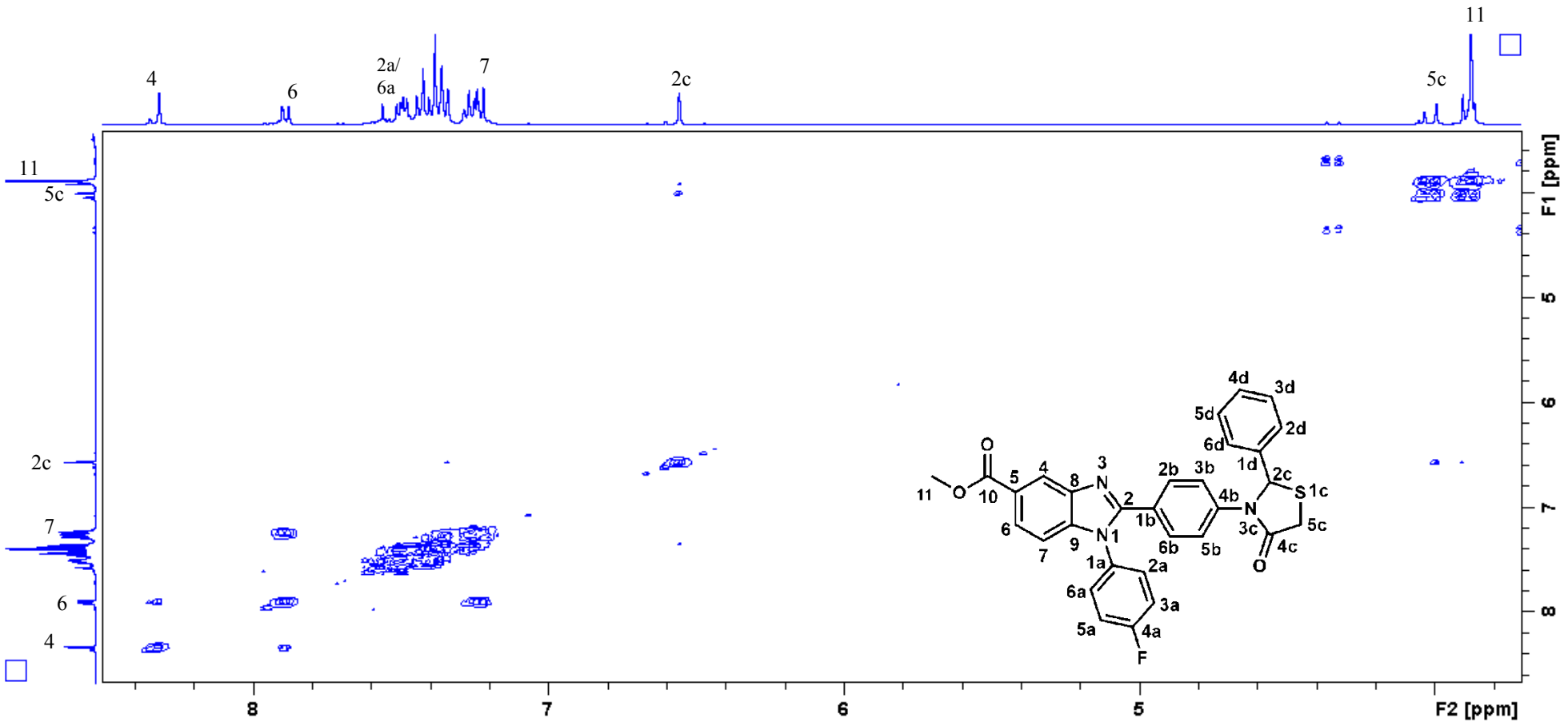

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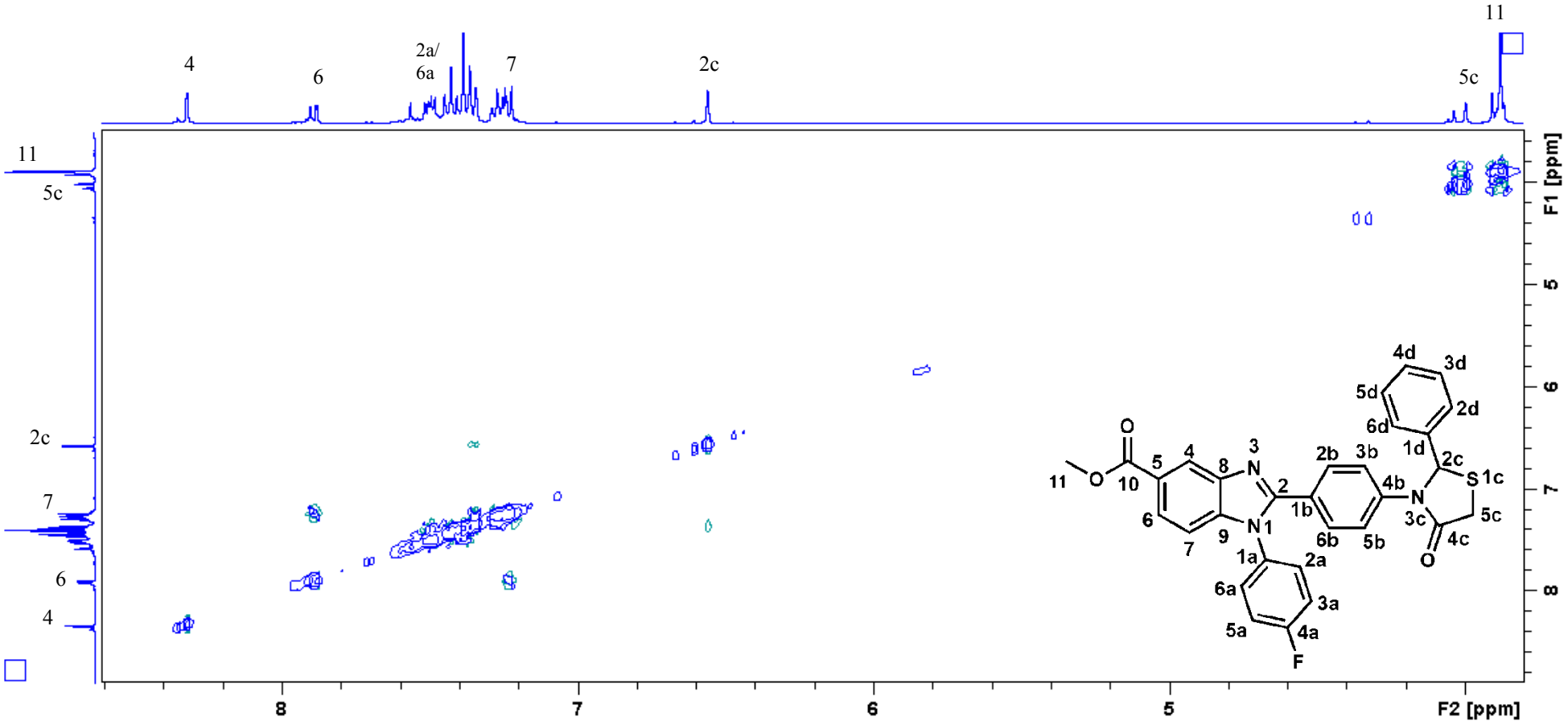

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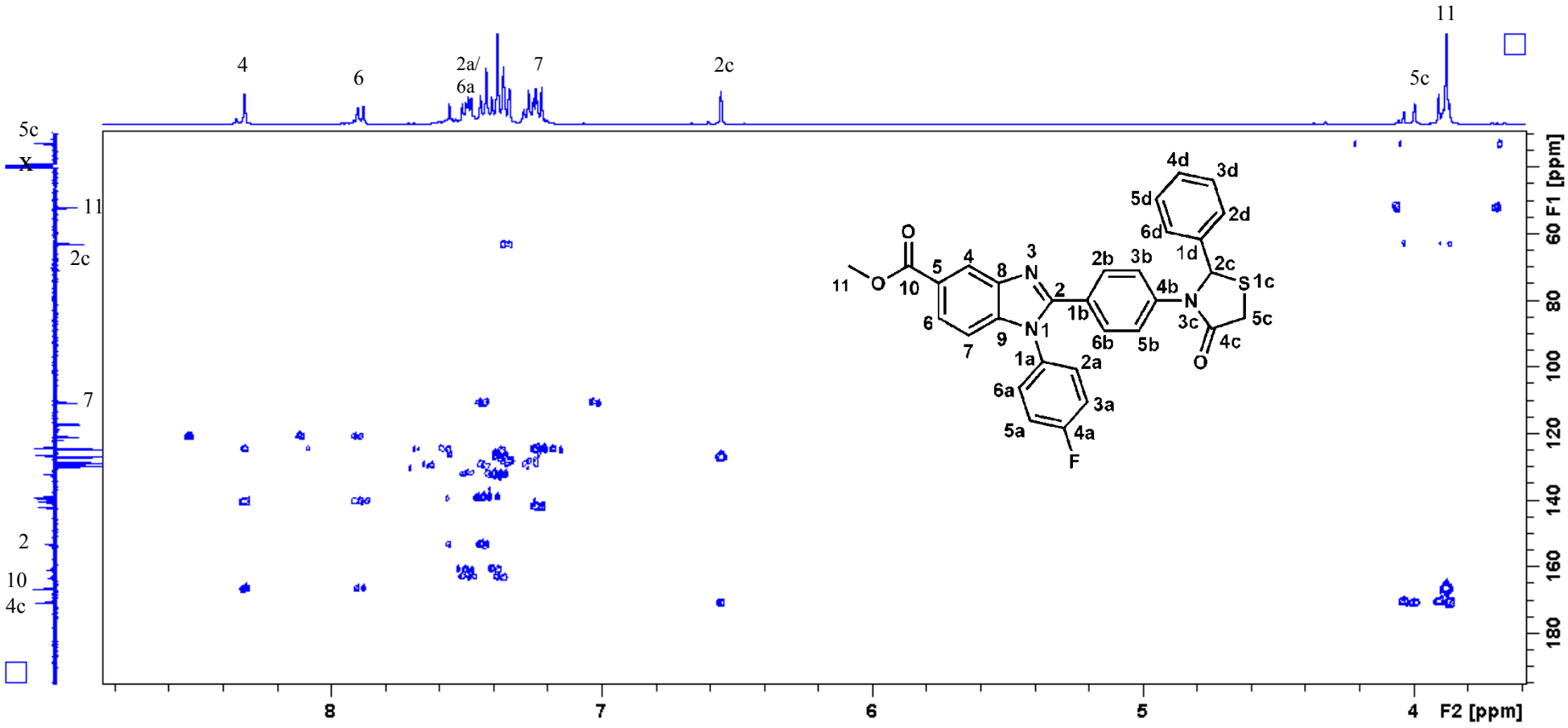

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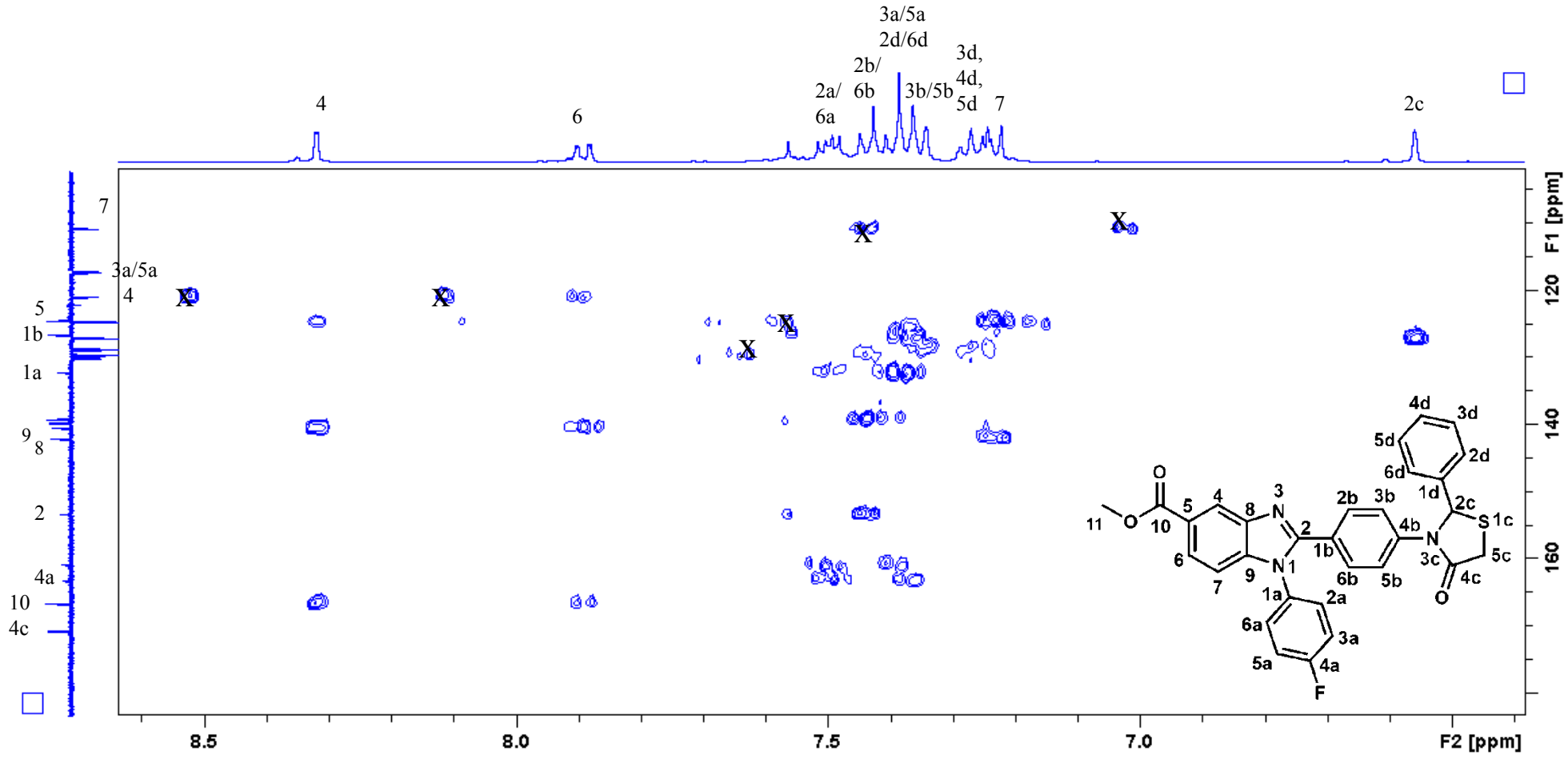
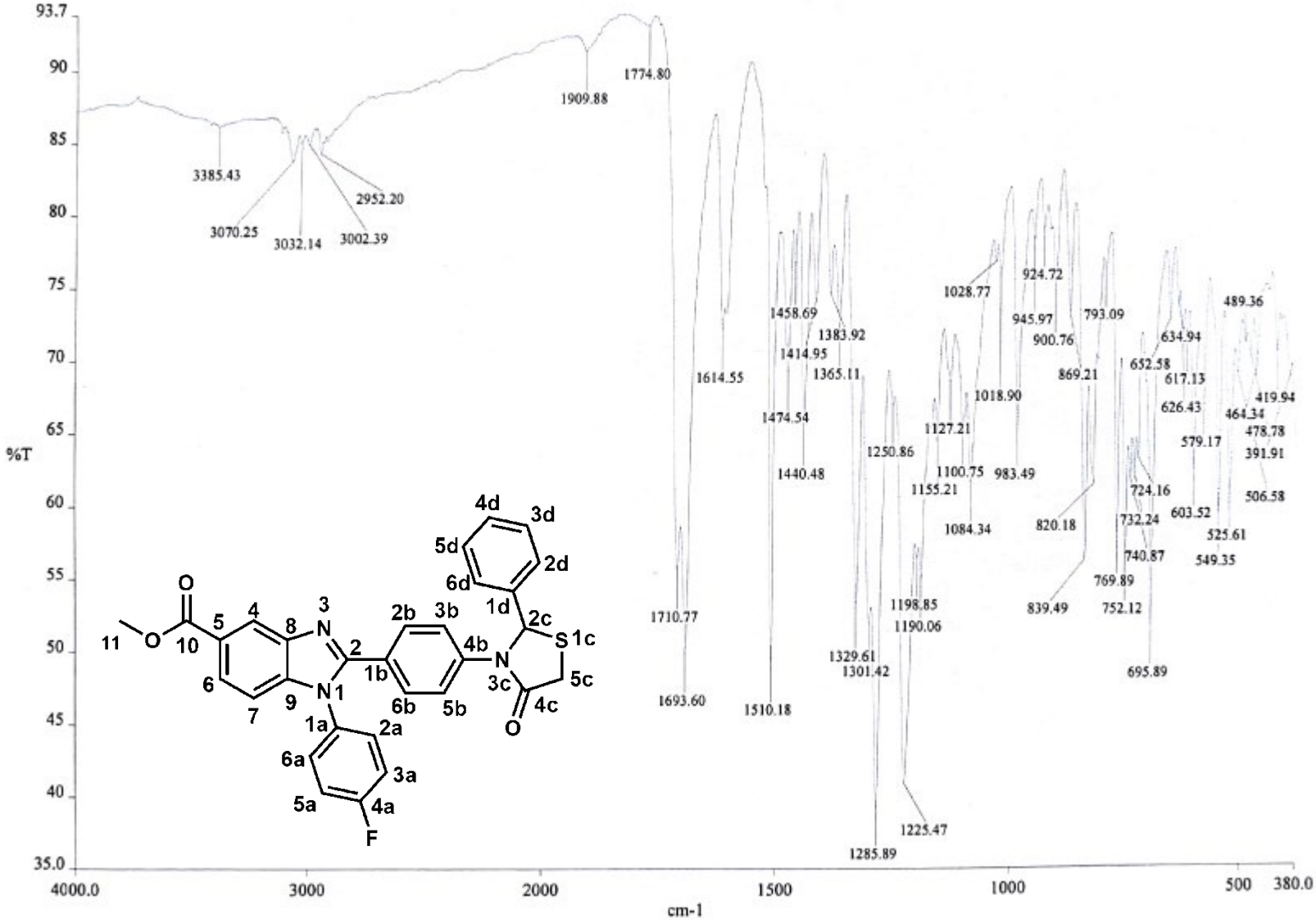


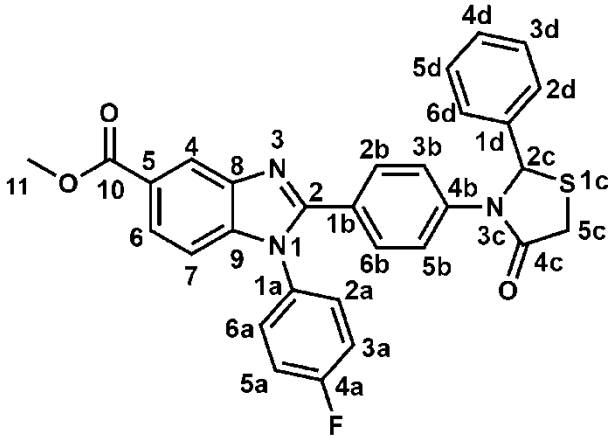
Expanded HMBC Spectrum of Compound A-7a

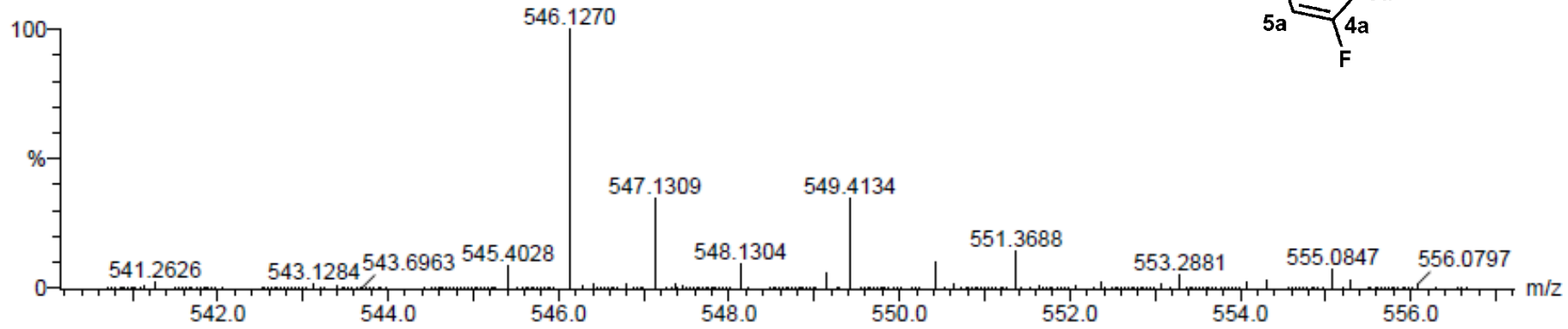


Infrared Spectrum of Compound A-7a

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 = 3

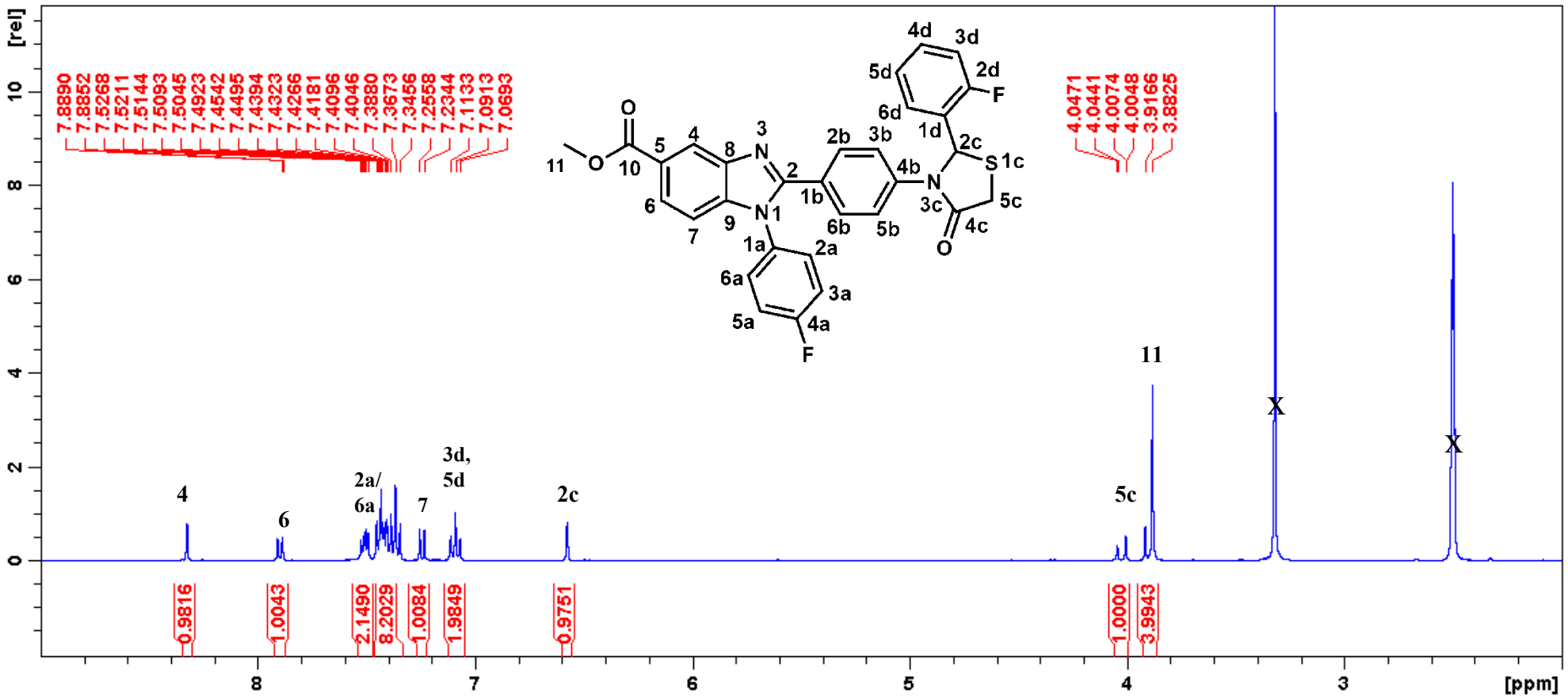
Monoisotopic Mass, Even Electron Ions
57 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 25-30 H: 20-25 N: 0-5 O: 0-5 F: 0-1 Na: 1-1 S: 0-1
BI 1 20 (0.641) Cm (1:61)
TOF MS ES+





Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
546.1270	546.1264	0.6	1.1	20.5	449.3	0.0	C30 H22 N3 O3 F Na S

HRMS Spectrum of Compound A-7a



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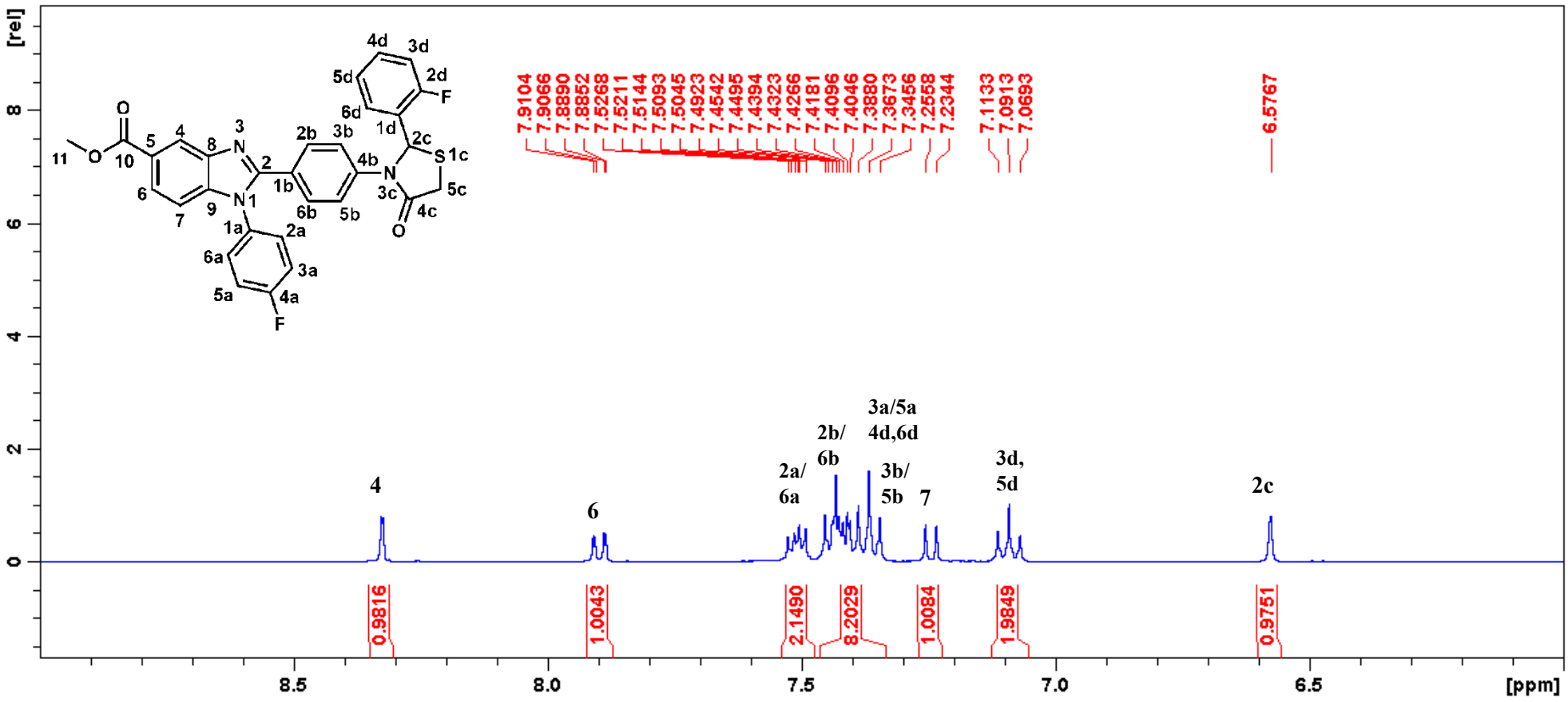
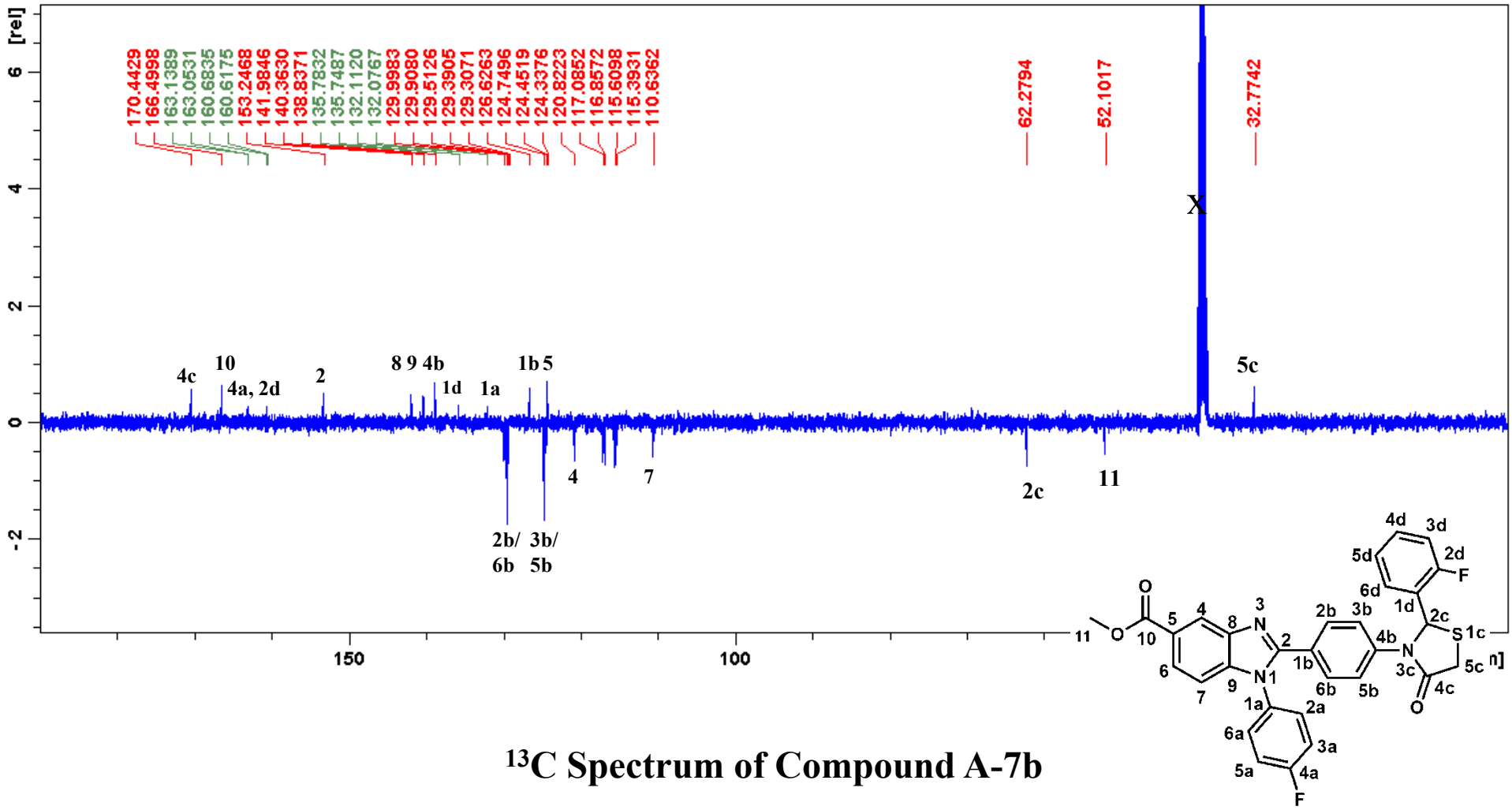


Expanded ^{1}H Spectrum of Compound A-7b



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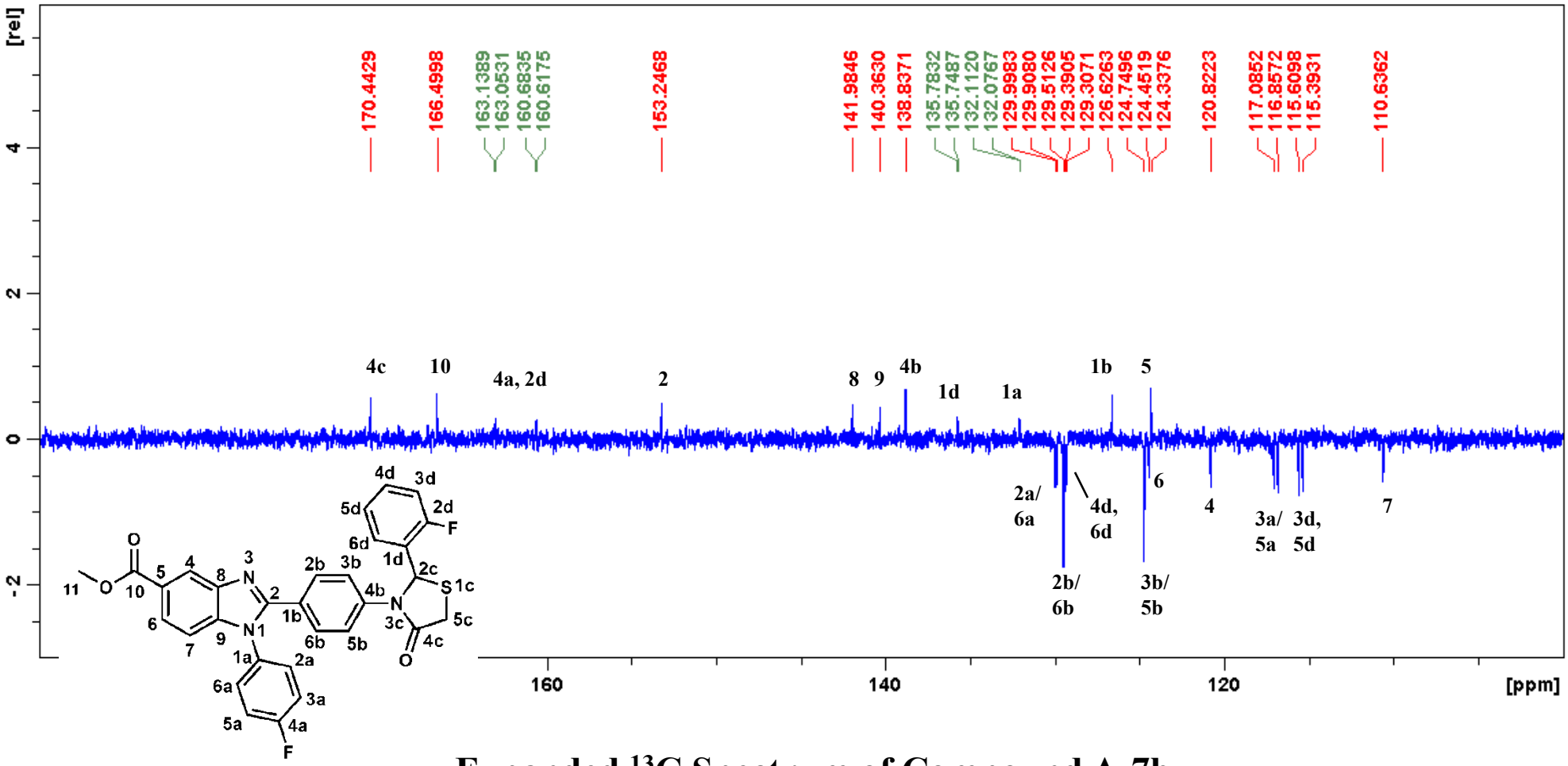

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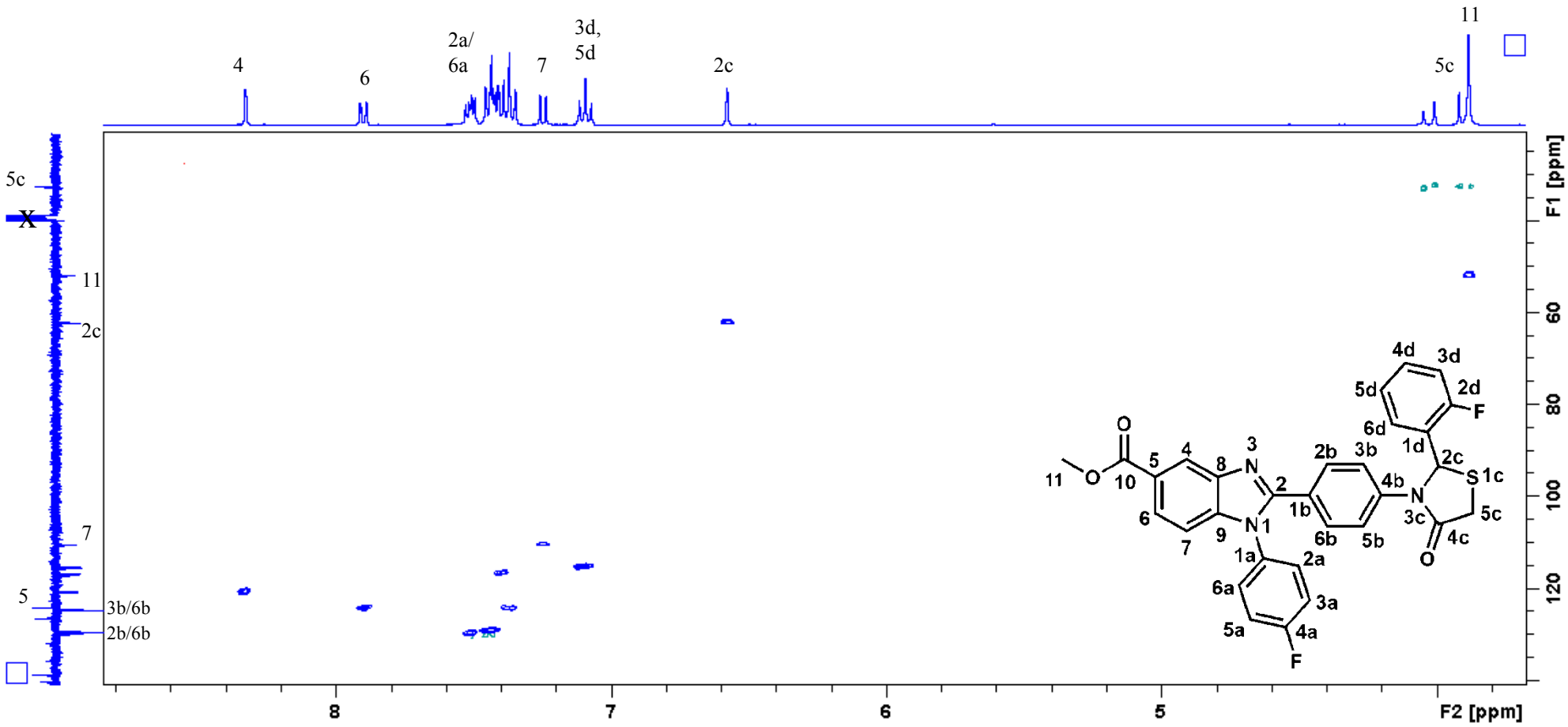

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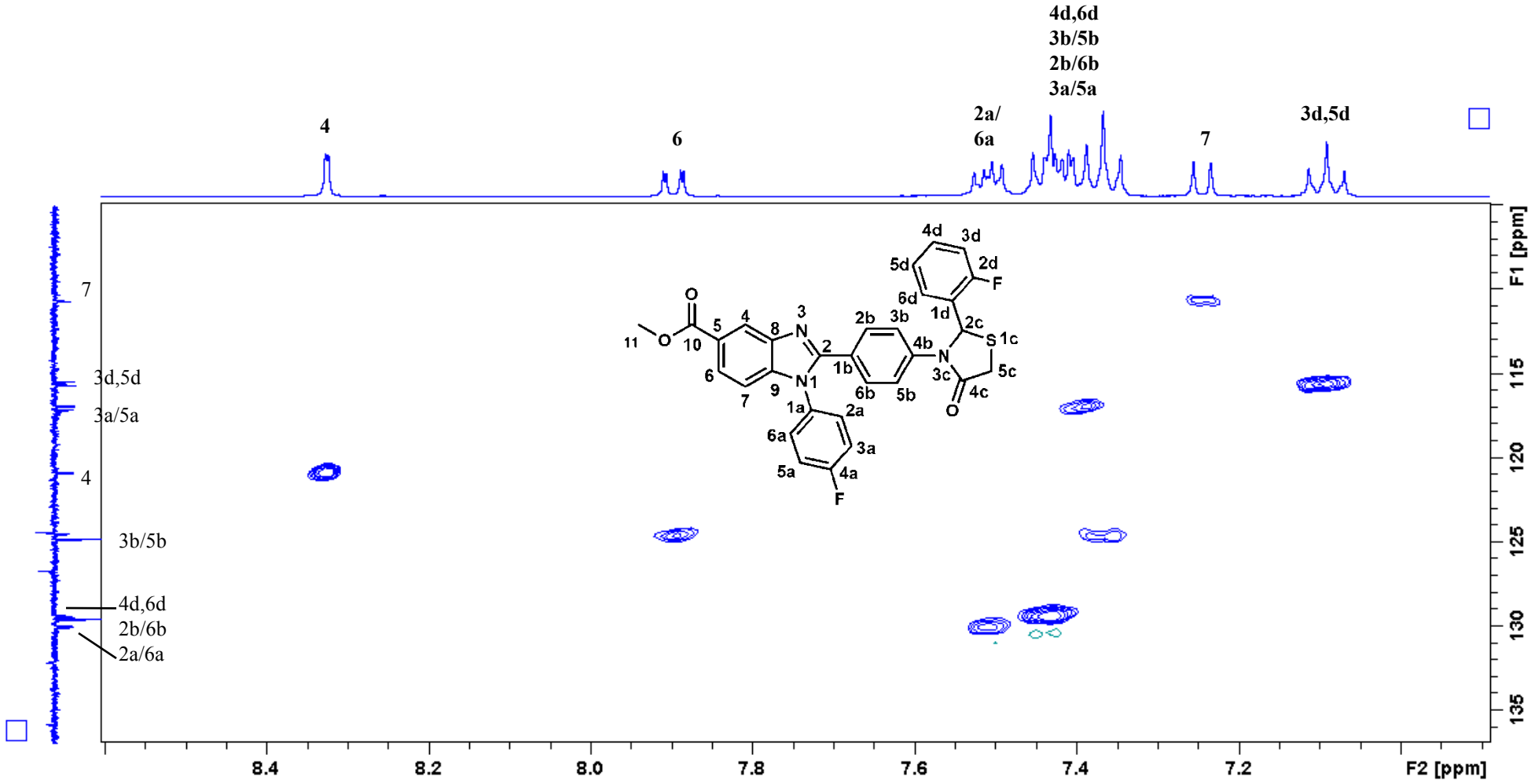

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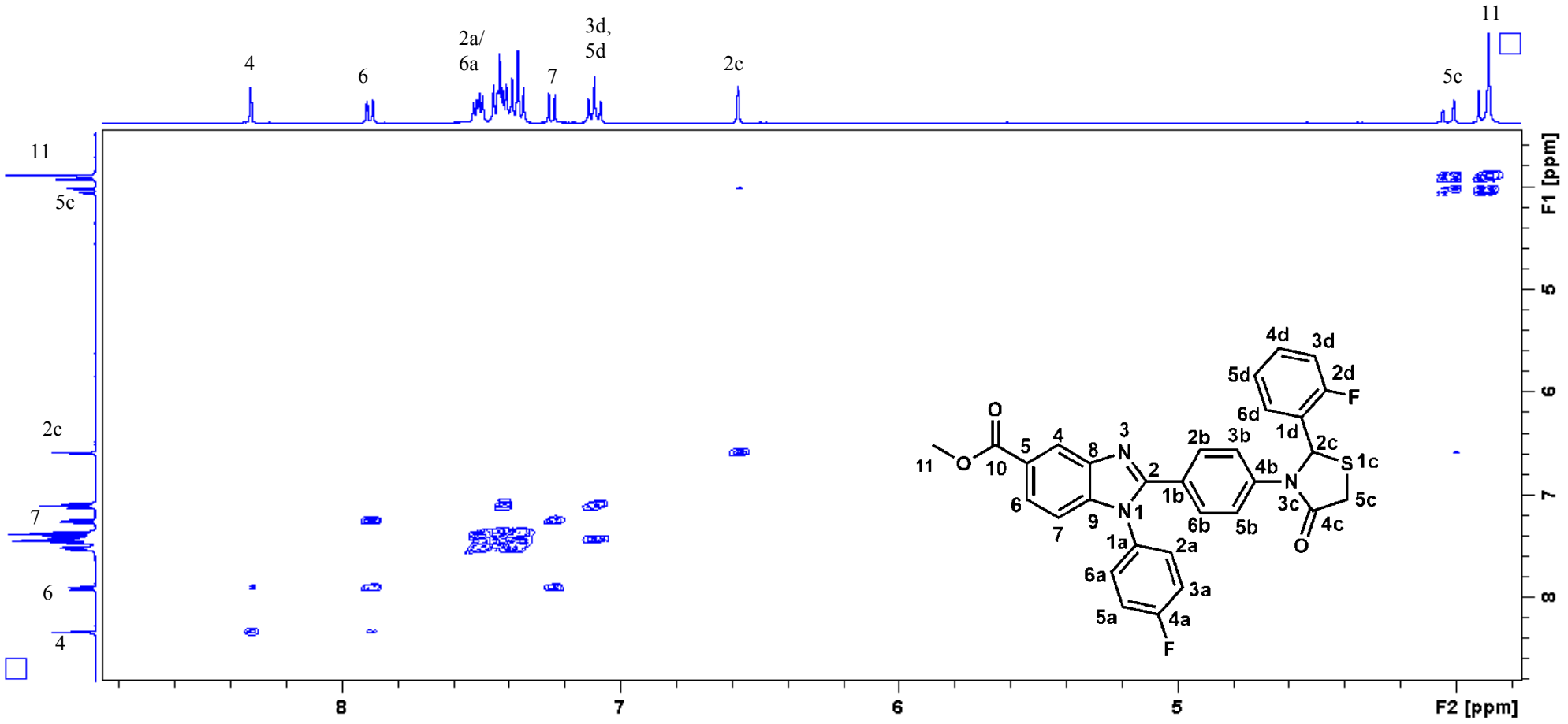
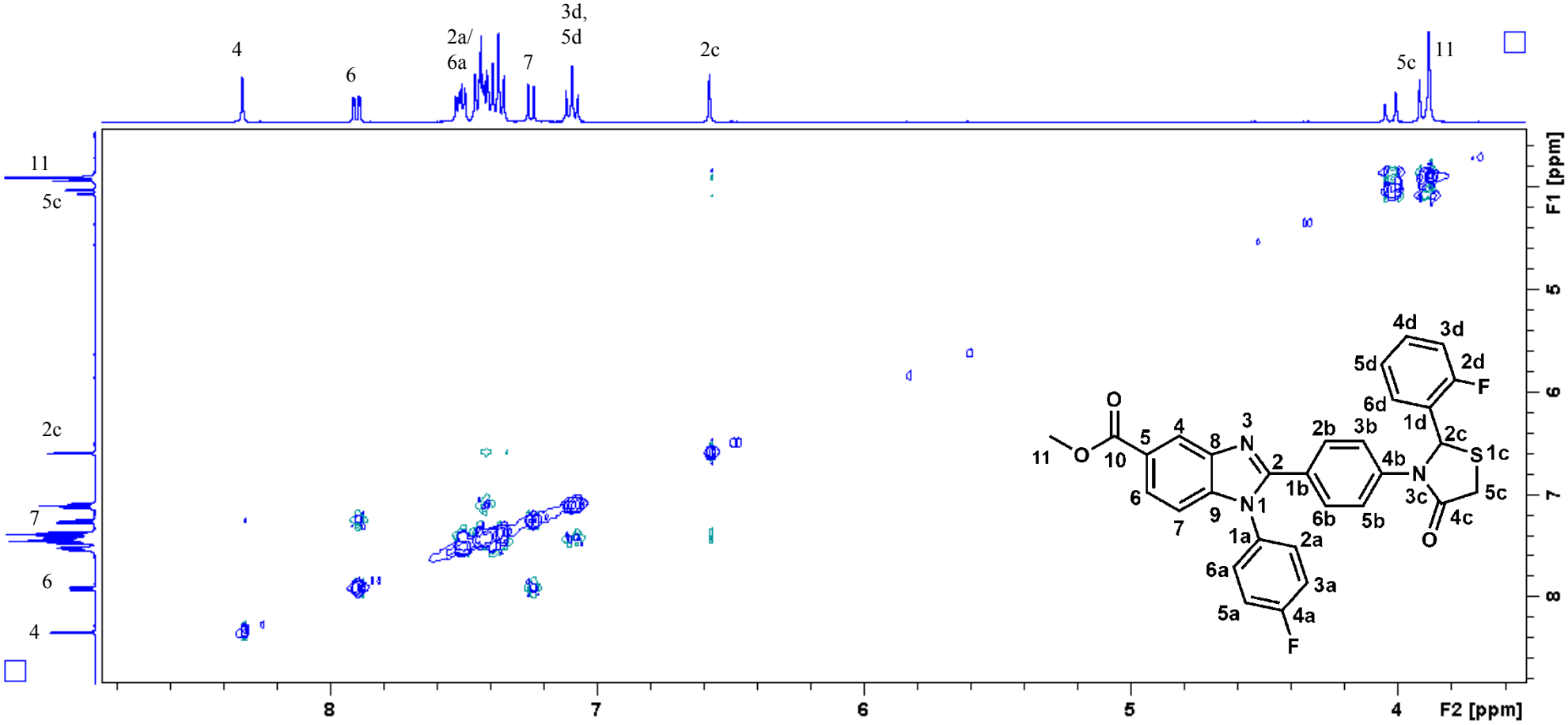


COSY Spectrum of Compound A-7b



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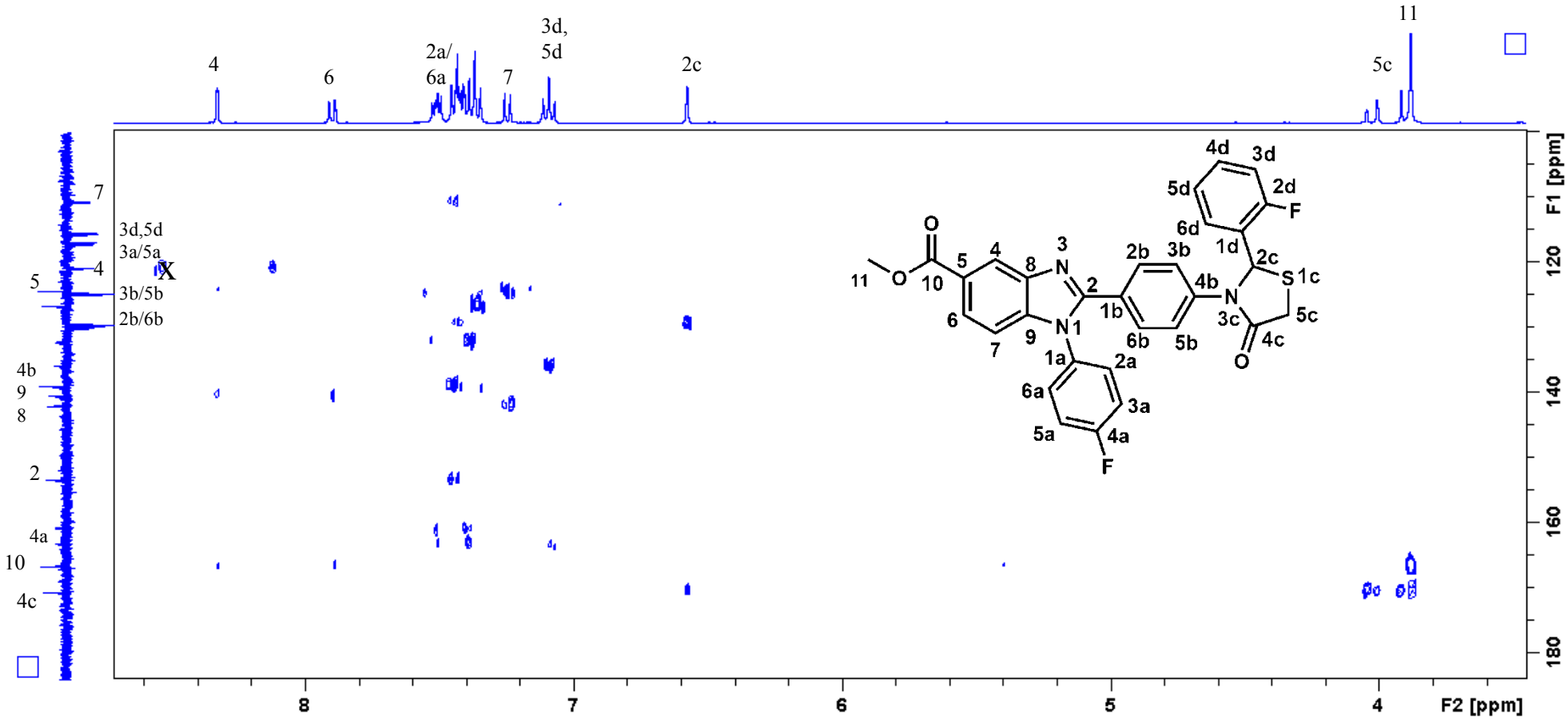
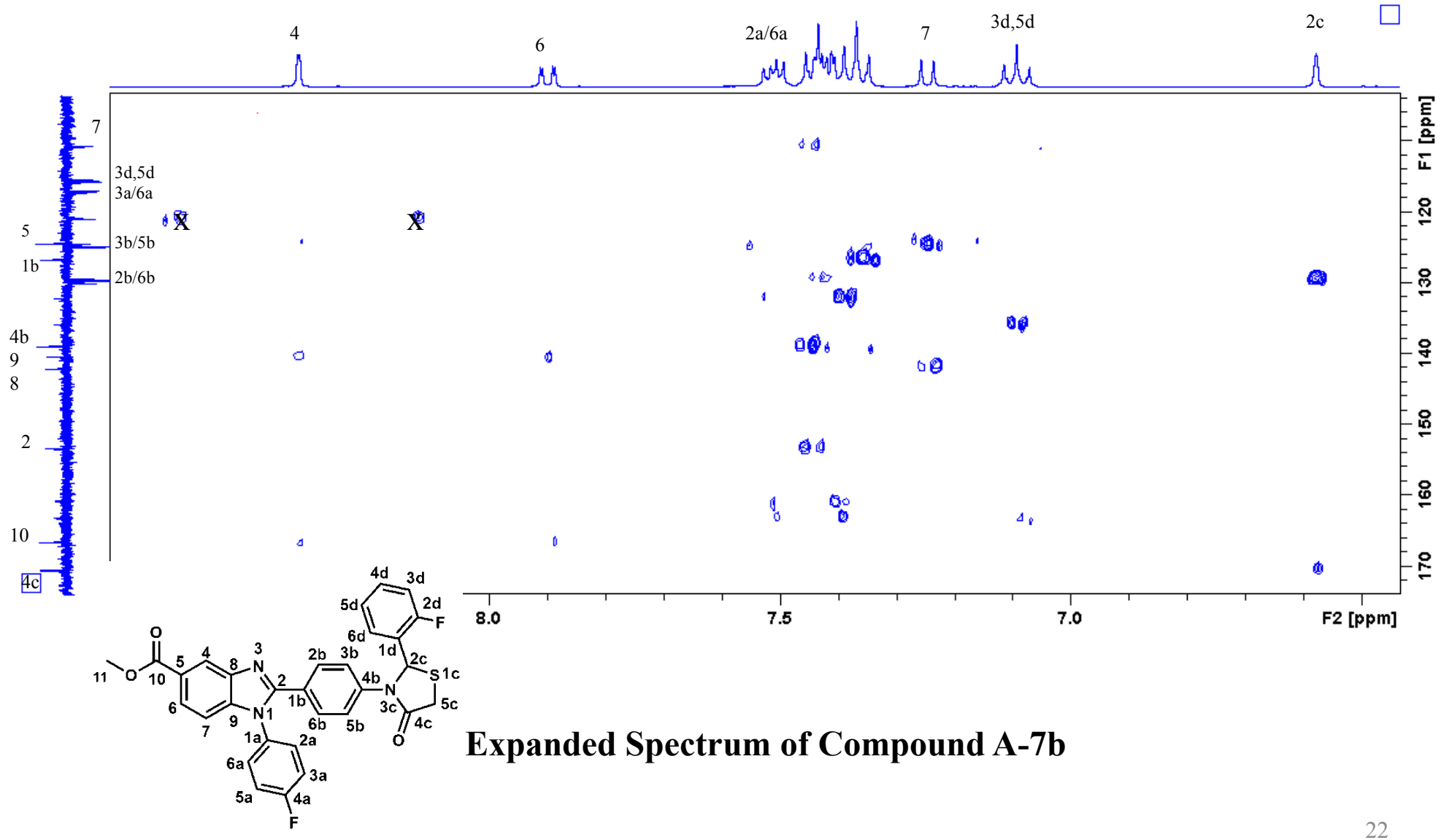
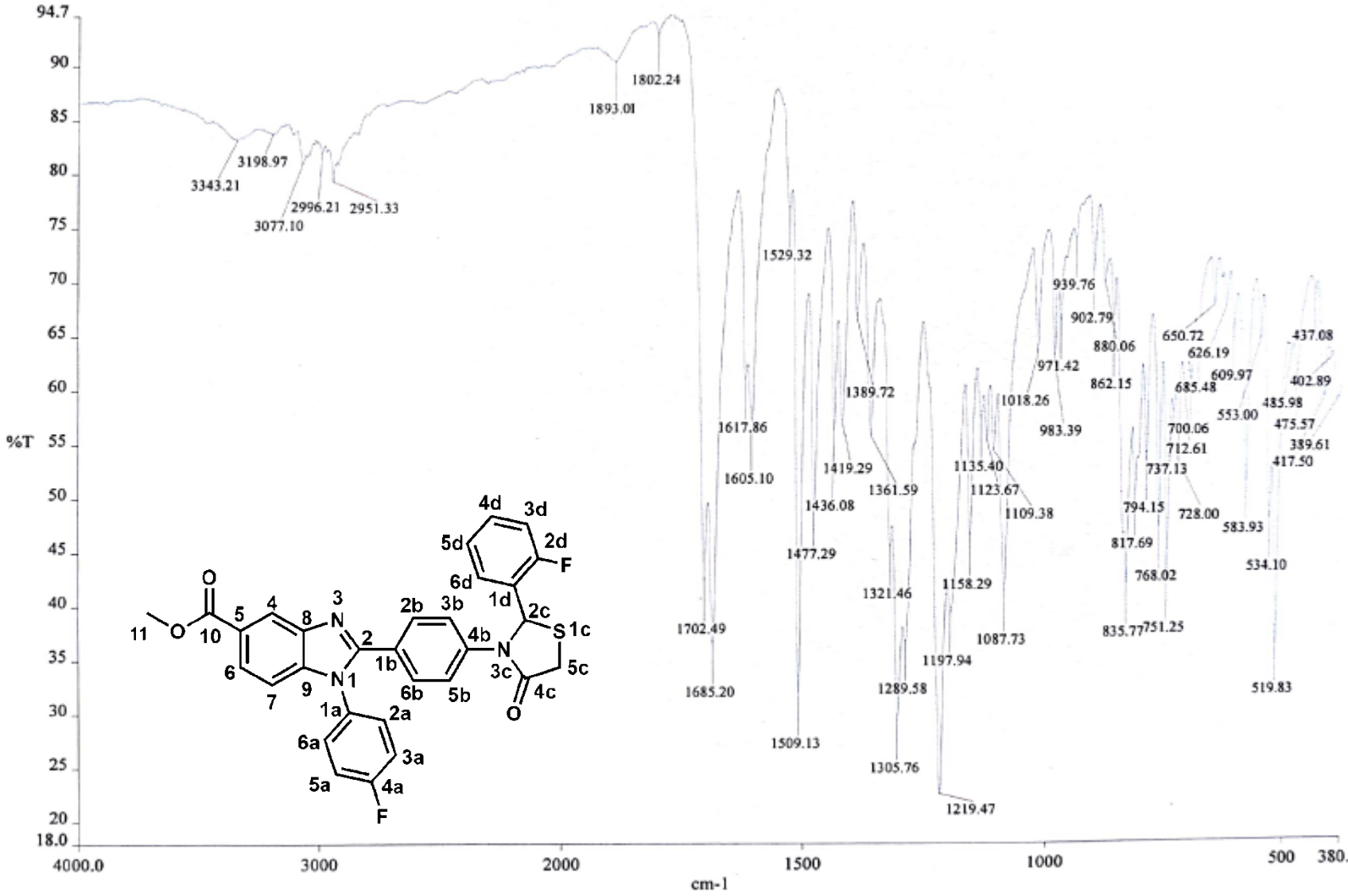


HMBC Spectrum of Compound A-7b



Expanded Spectrum of Compound A-7b



Infrared Spectrum of Compound A-7b

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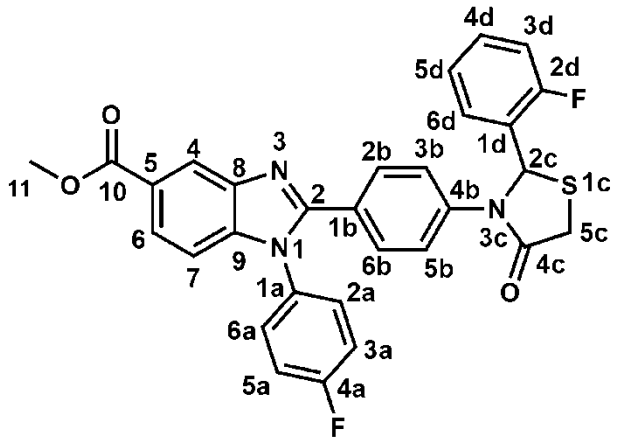
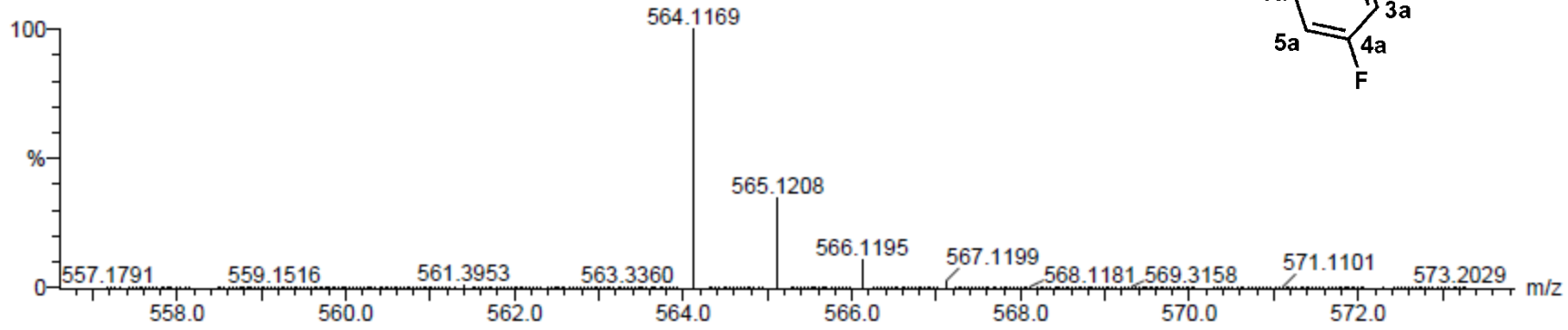
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

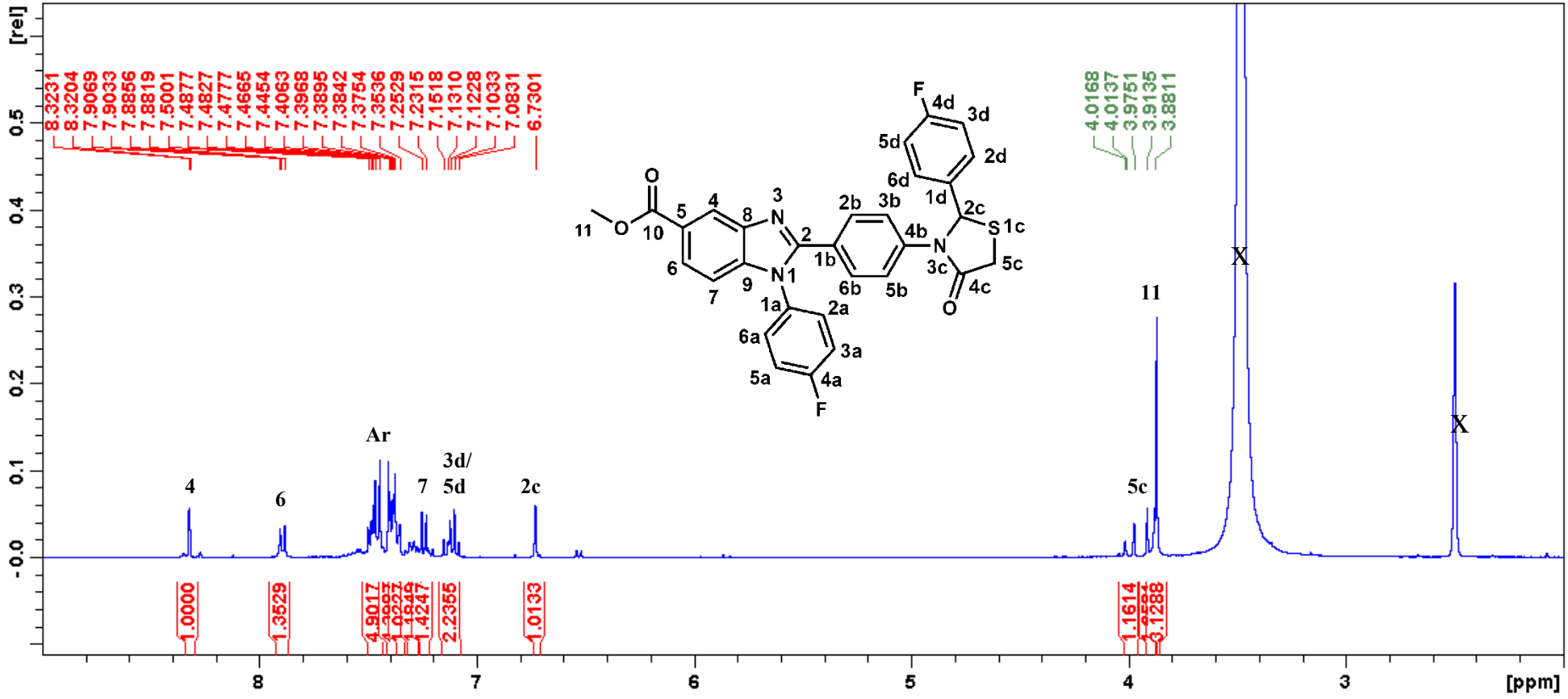
Monoisotopic Mass, Even Electron Ions
97 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 25-30 H: 20-25 N: 0-5 O: 0-5 F: 0-2 Na: 1-1 S: 0-1
BI 2 41 (1.349) Cm (1:61)
TOF MS ES+



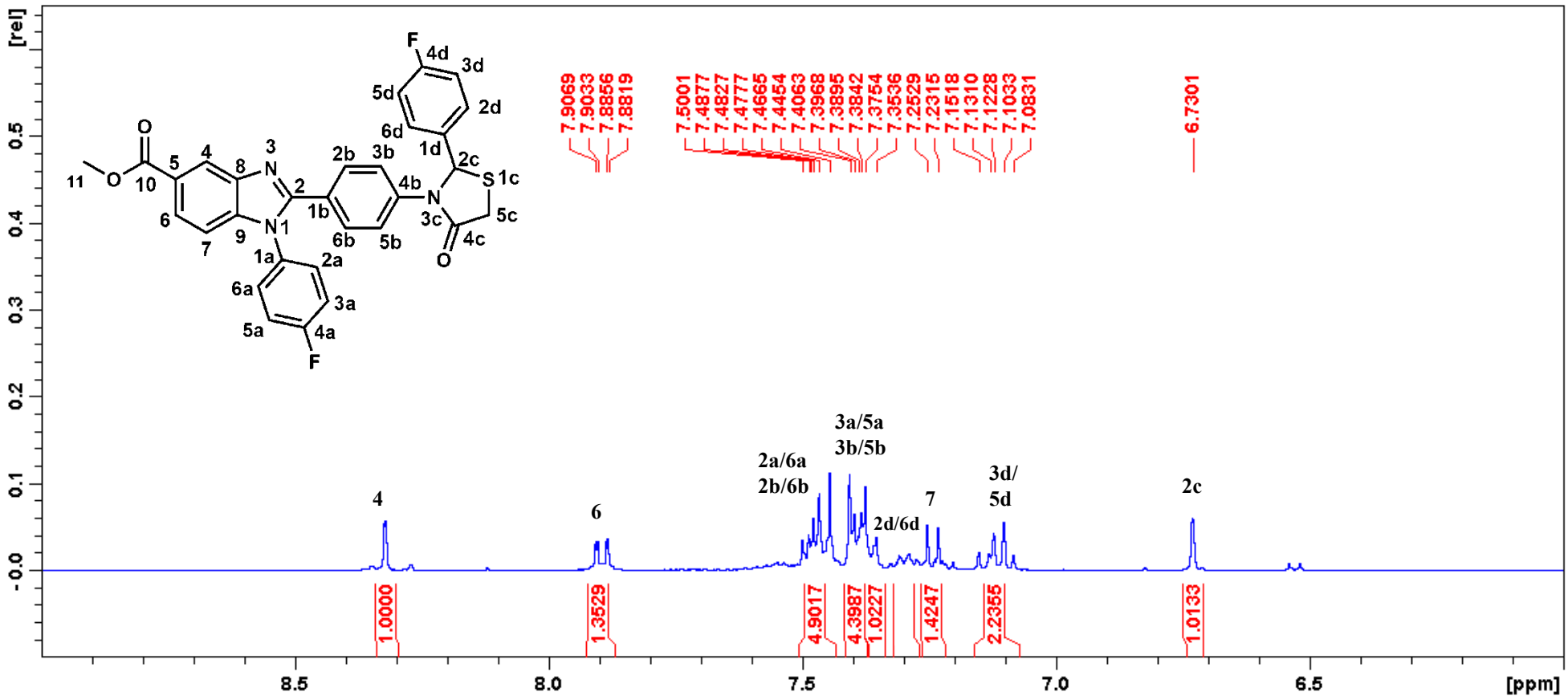


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
564.1169	564.1169	0.0	0.0	20.5	558.2	0.0	C30 H21 N3 O3 F2 Na S

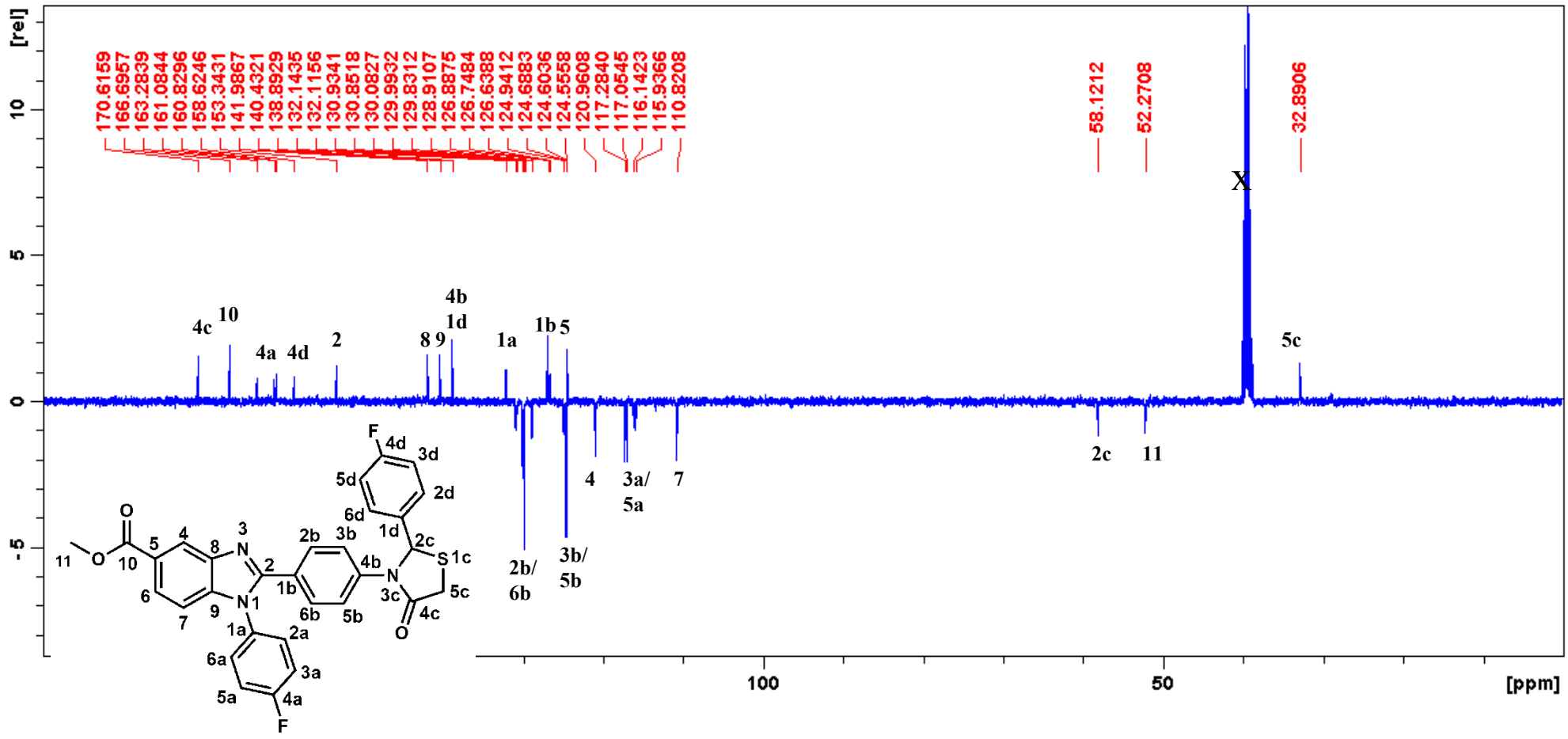
HRMS Spectrum of Compound A-7b



^{1}H Spectrum of Compound A-7c



Expanded ^{1}H Spectrum of Compound A-7c



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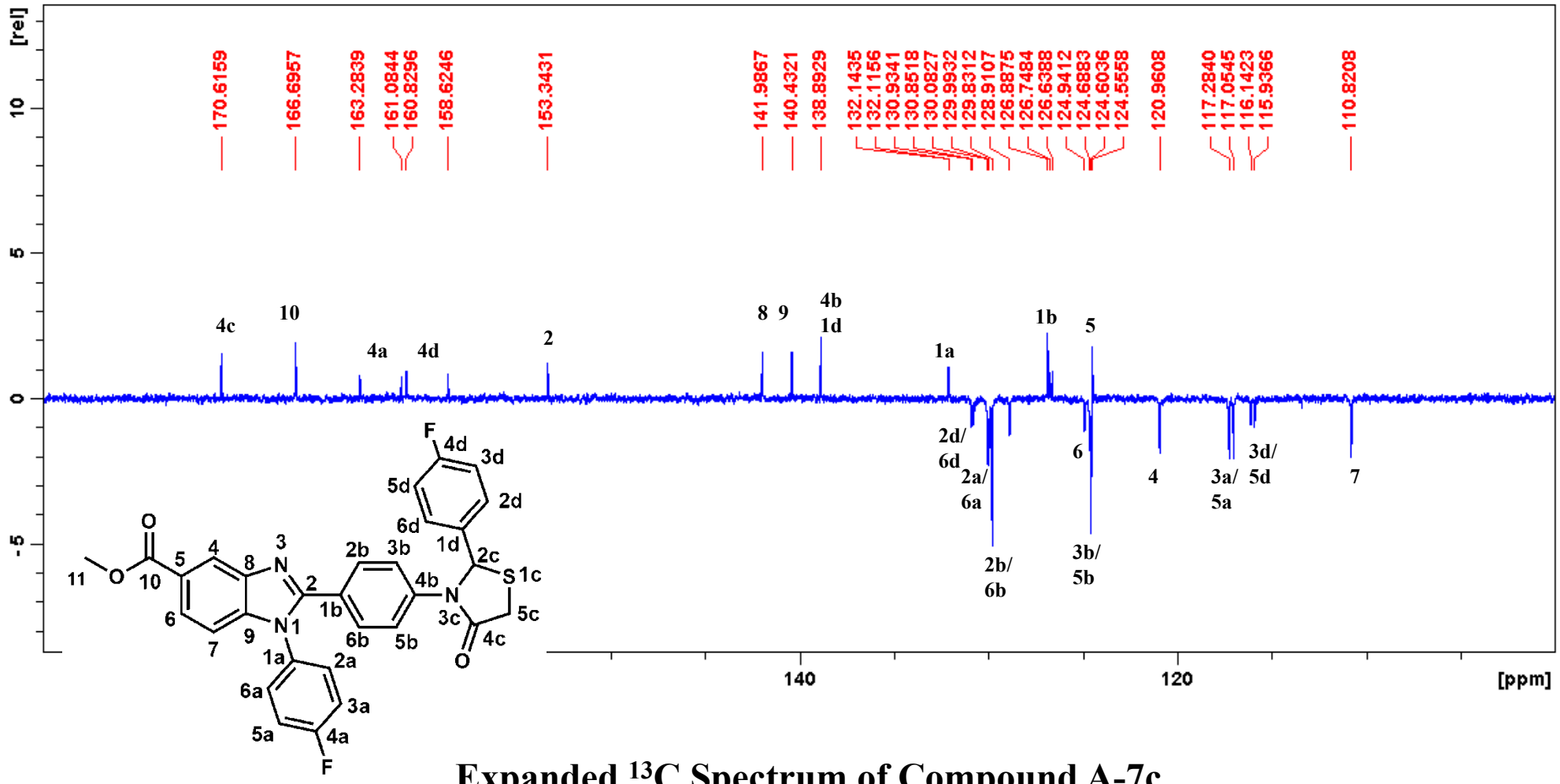

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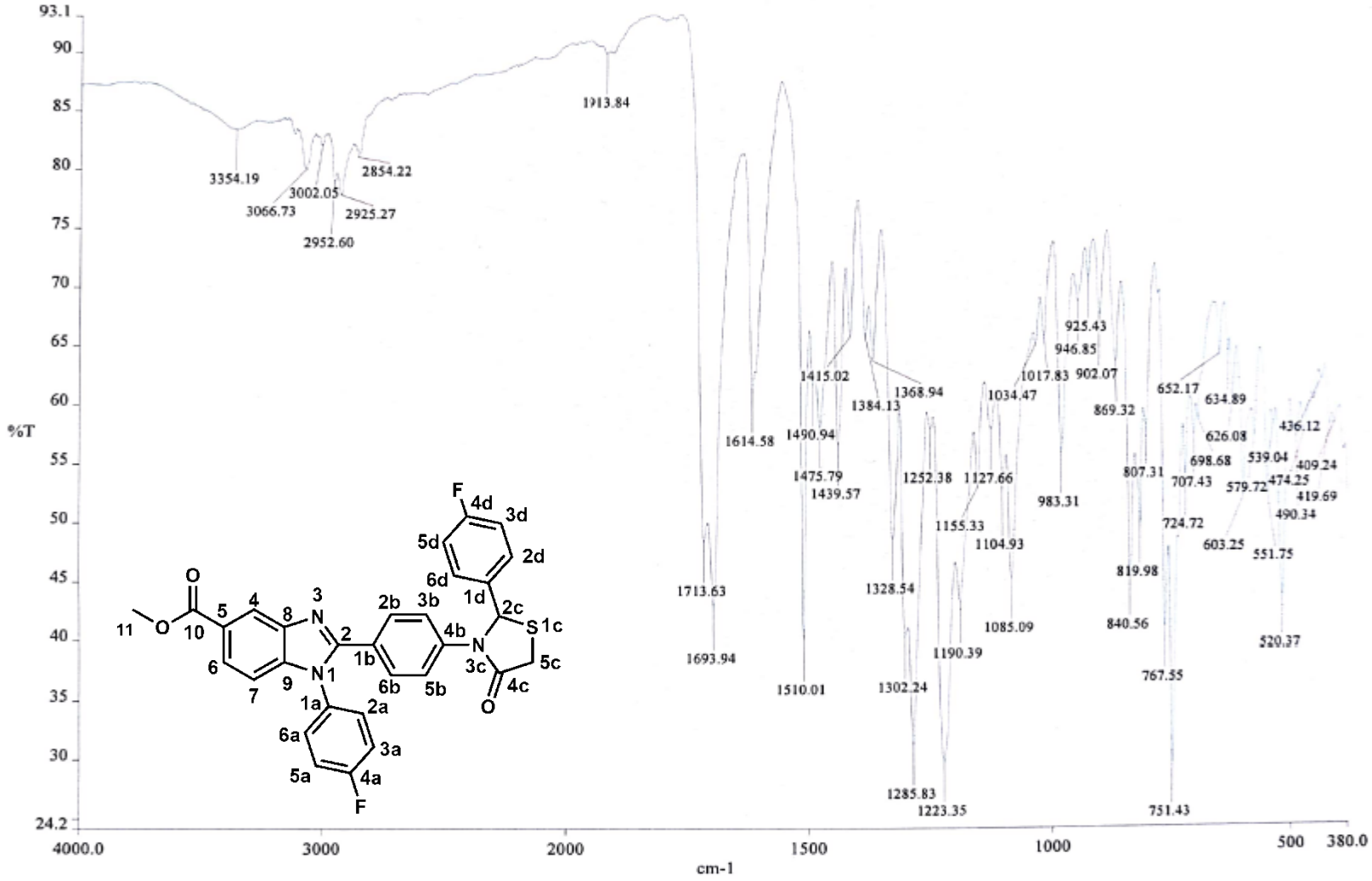

Infrared Spectrum of Compound A-7c

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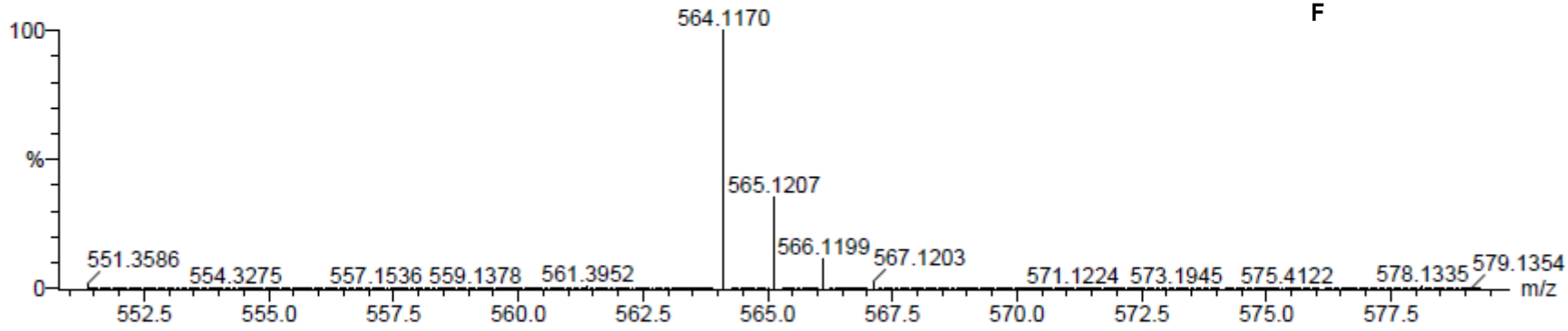
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

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: 25-30 H: 20-25 N: 0-5 O: 0-5 F: 0-2 Na: 1-1 S: 0-1
BI 3 21 (0.676) 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
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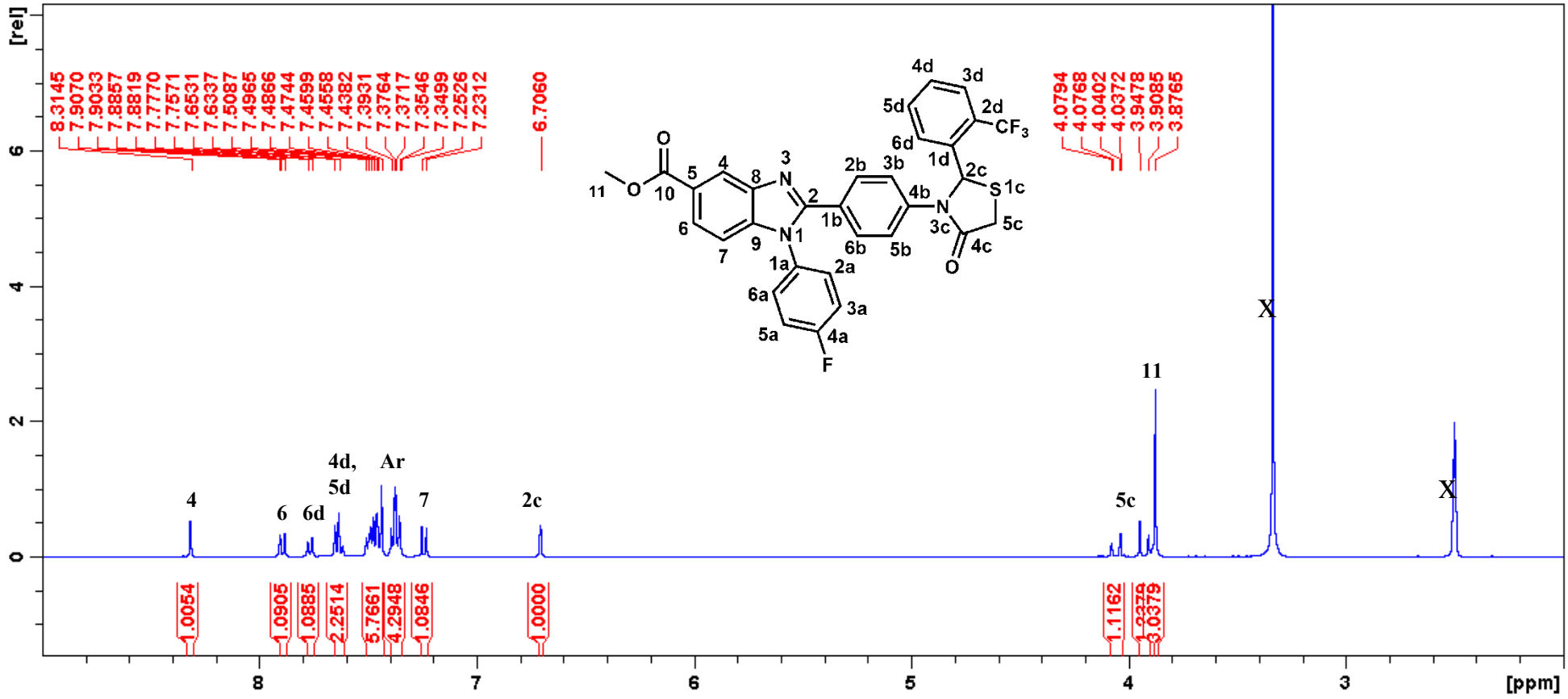
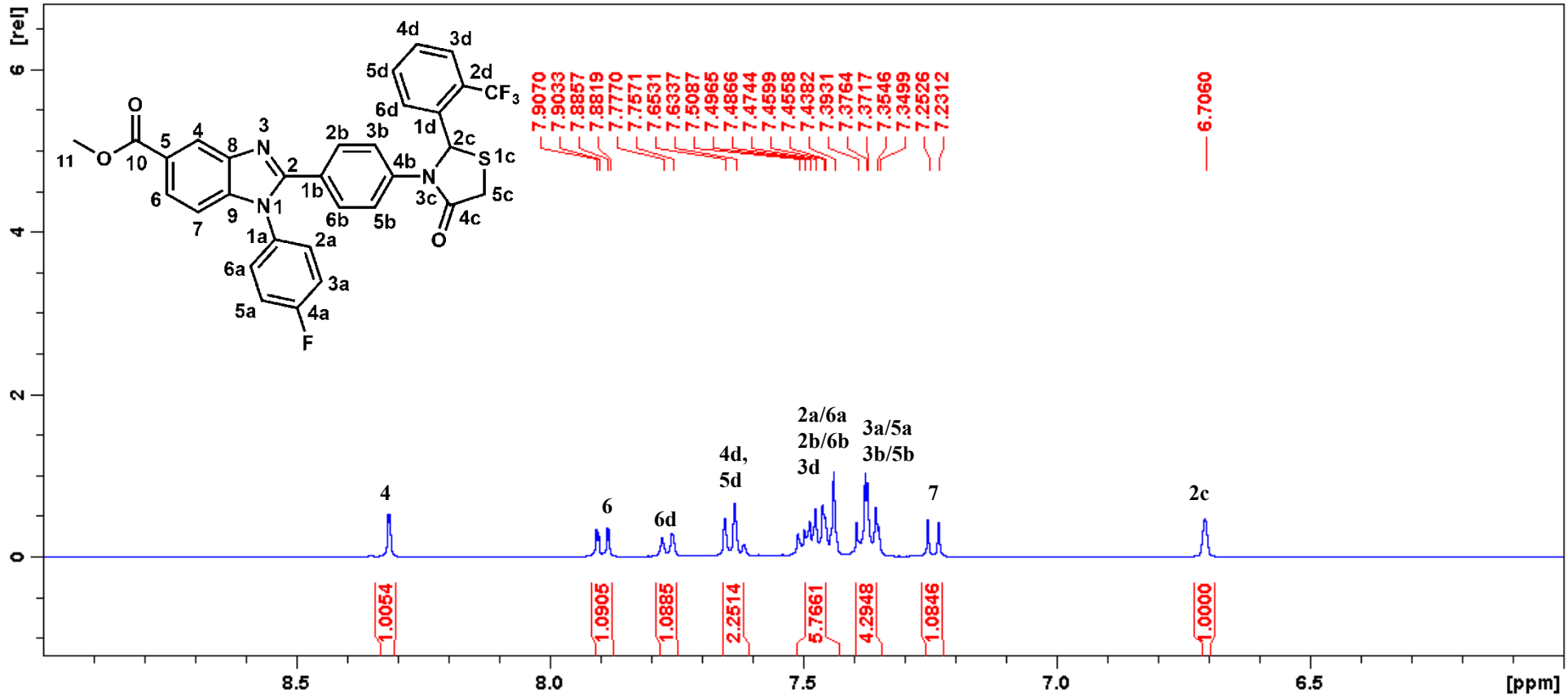
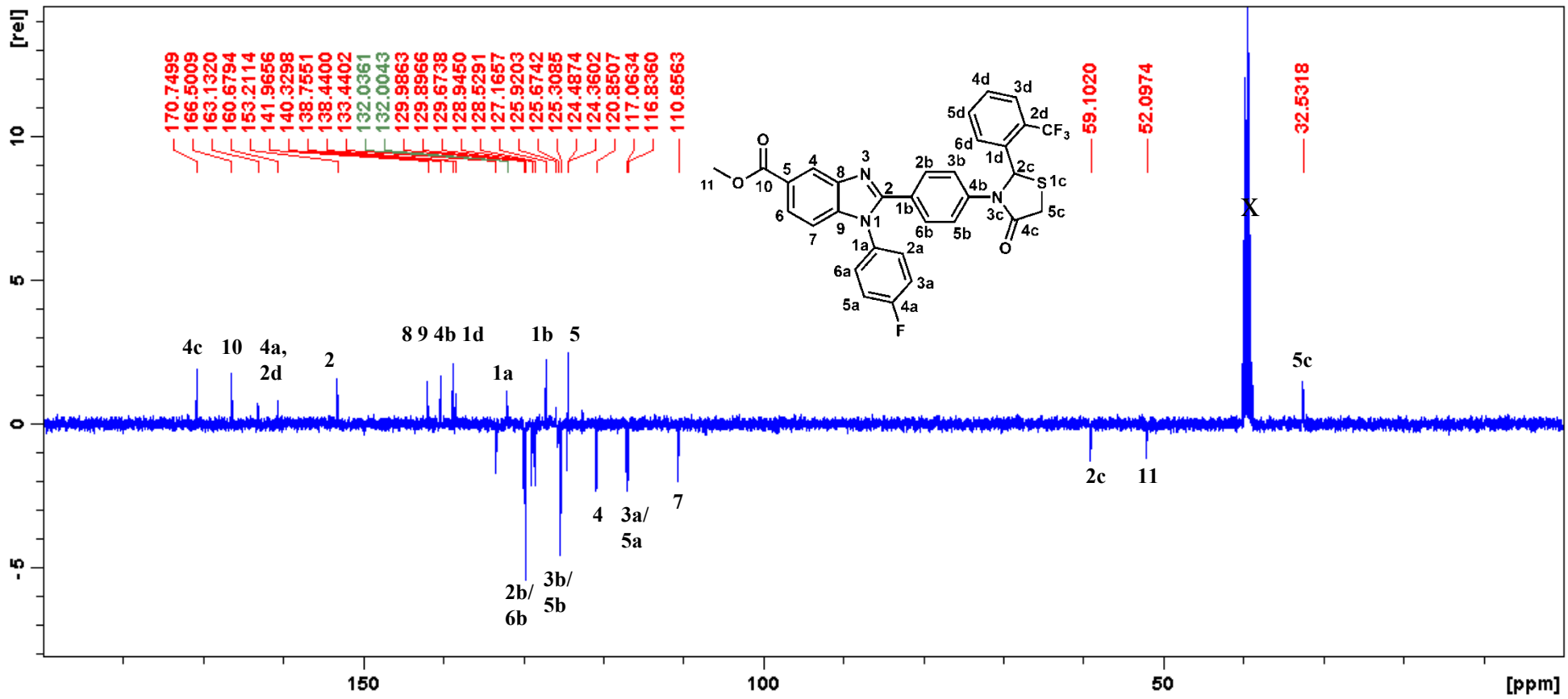
HRMS Spectrum of Compound A-7c



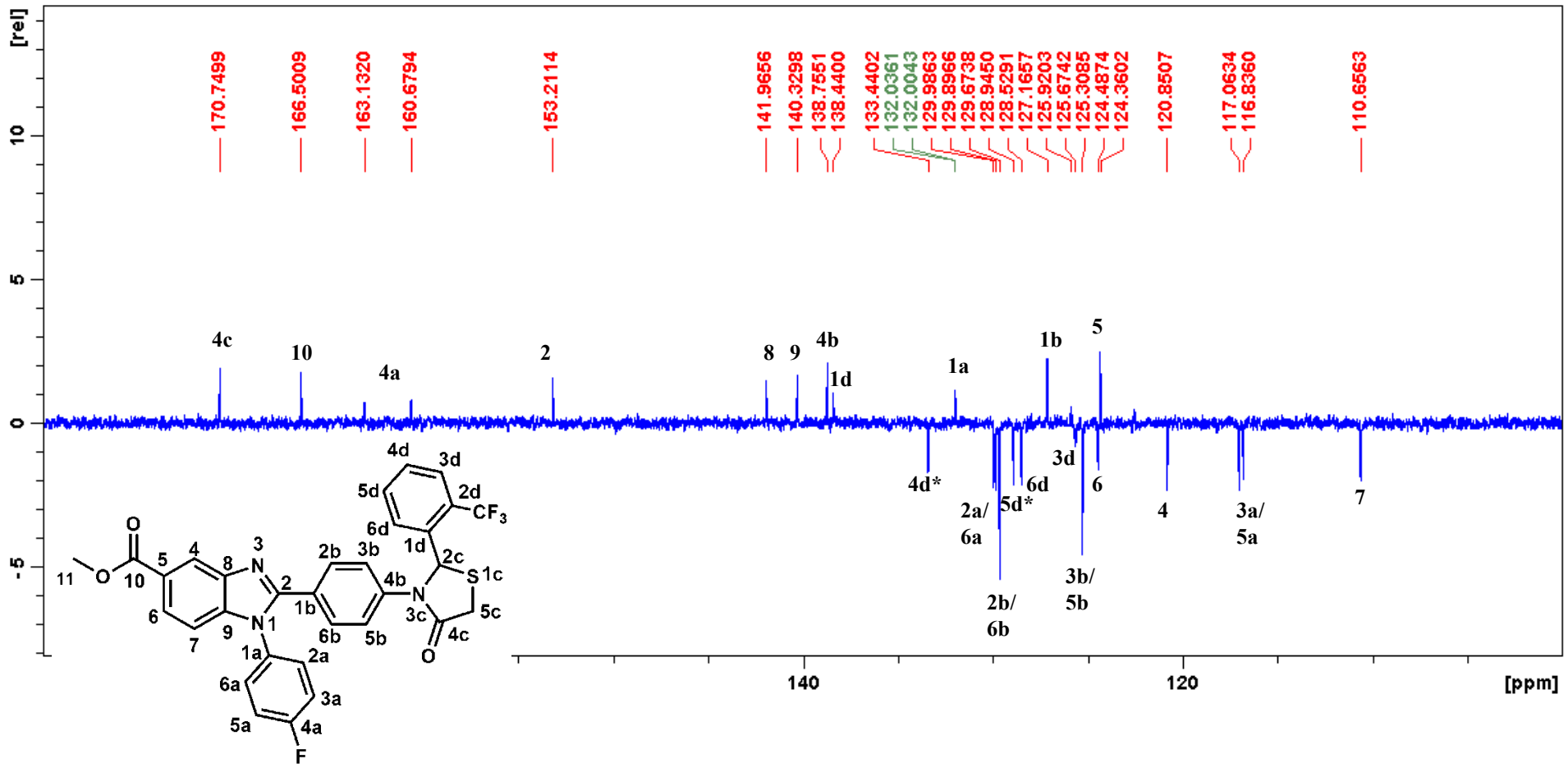
^{1}H Spectrum of Compound A-7d



Expanded ^{1}H Spectrum of Compound A-7d

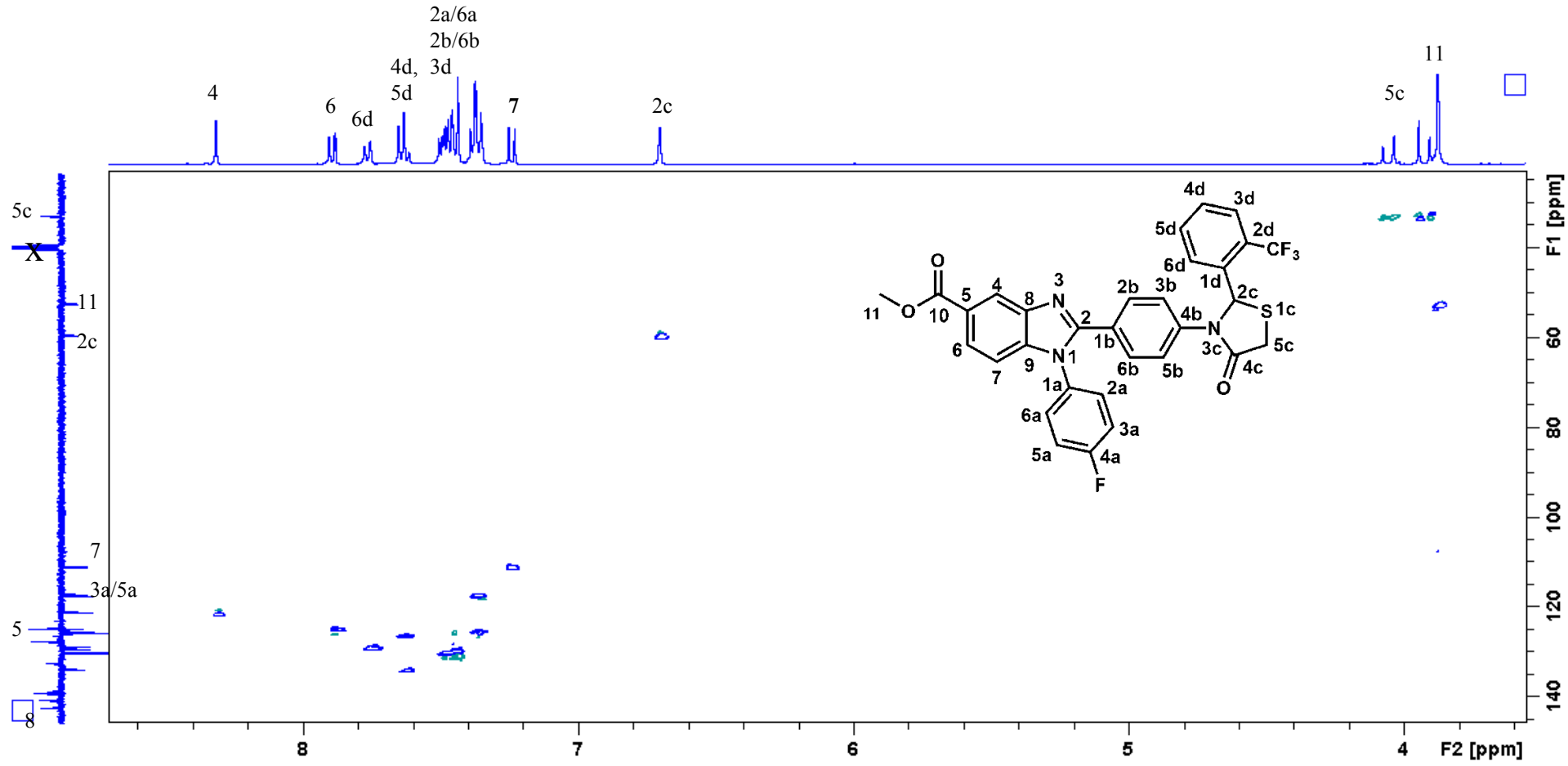


^{13}C Spectrum of Compound A-7d

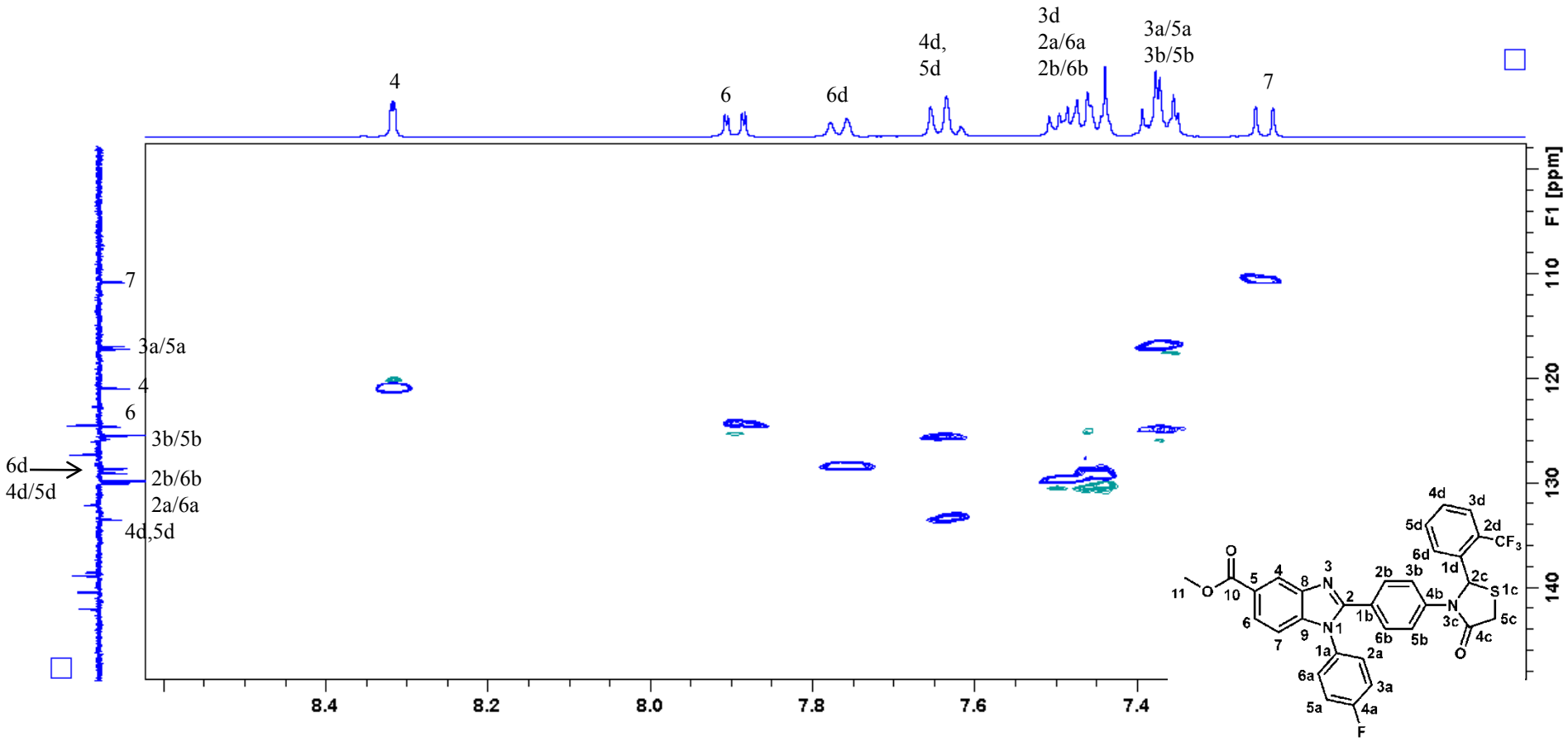


Expanded ^{13}C Spectrum of Compound A-7d

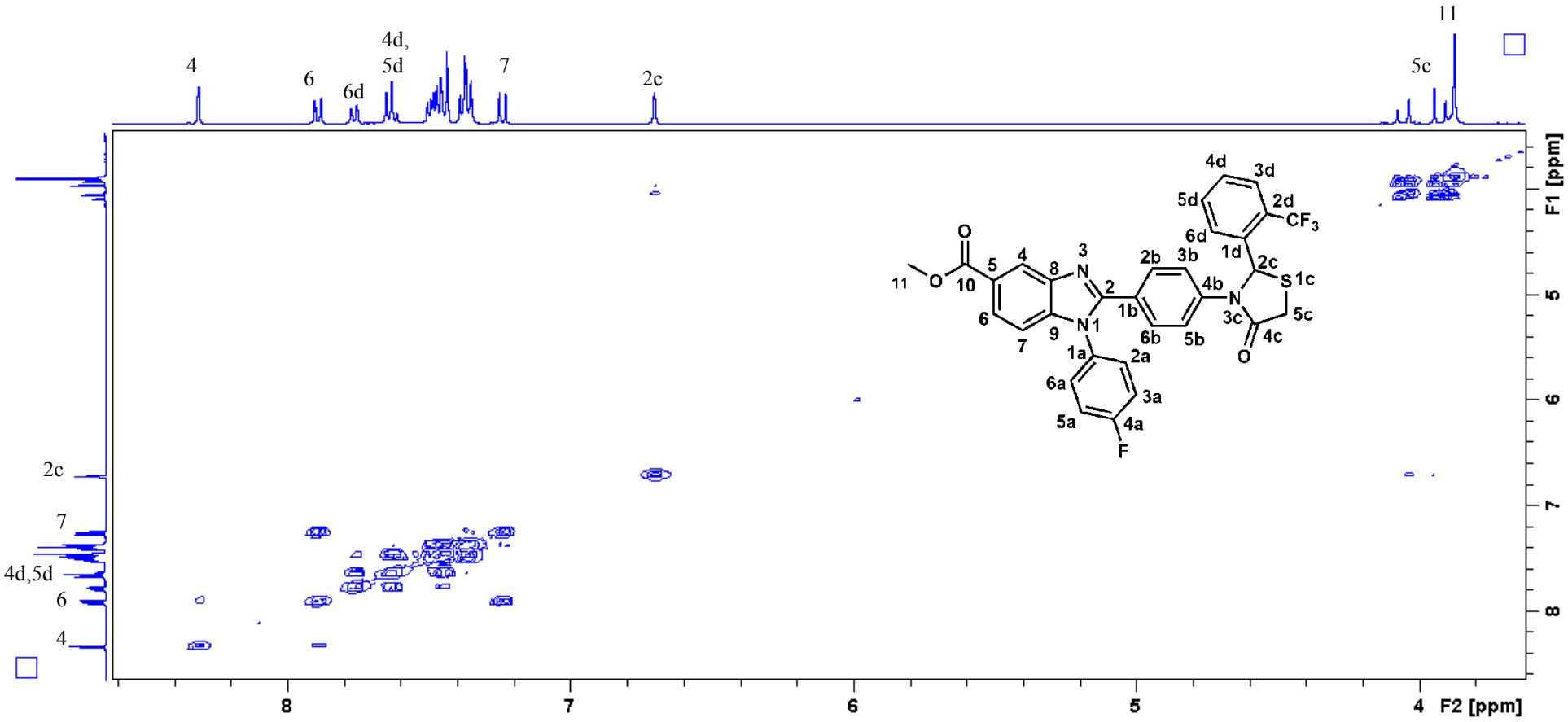
* Assignments are interchangeable



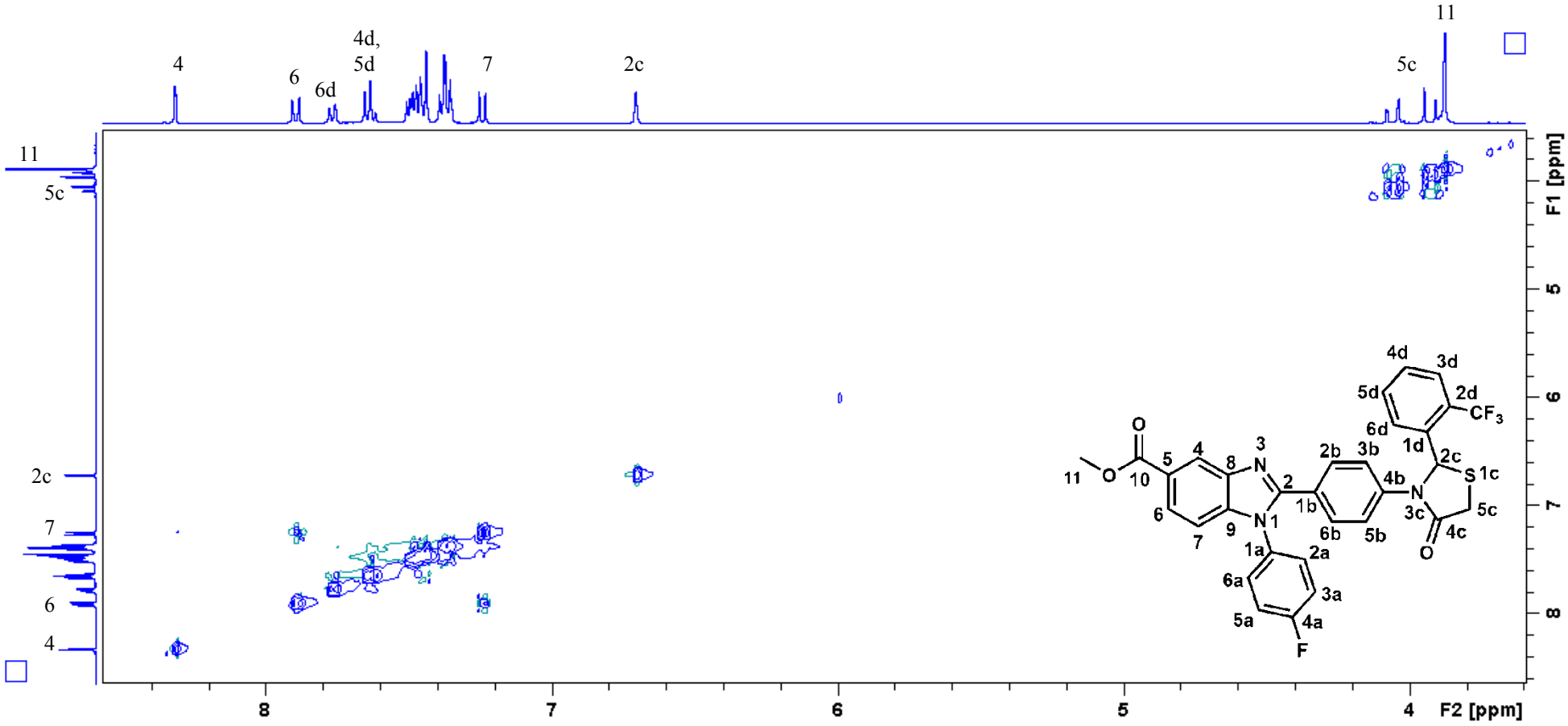
HSQC Spectrum of Compound A-7d



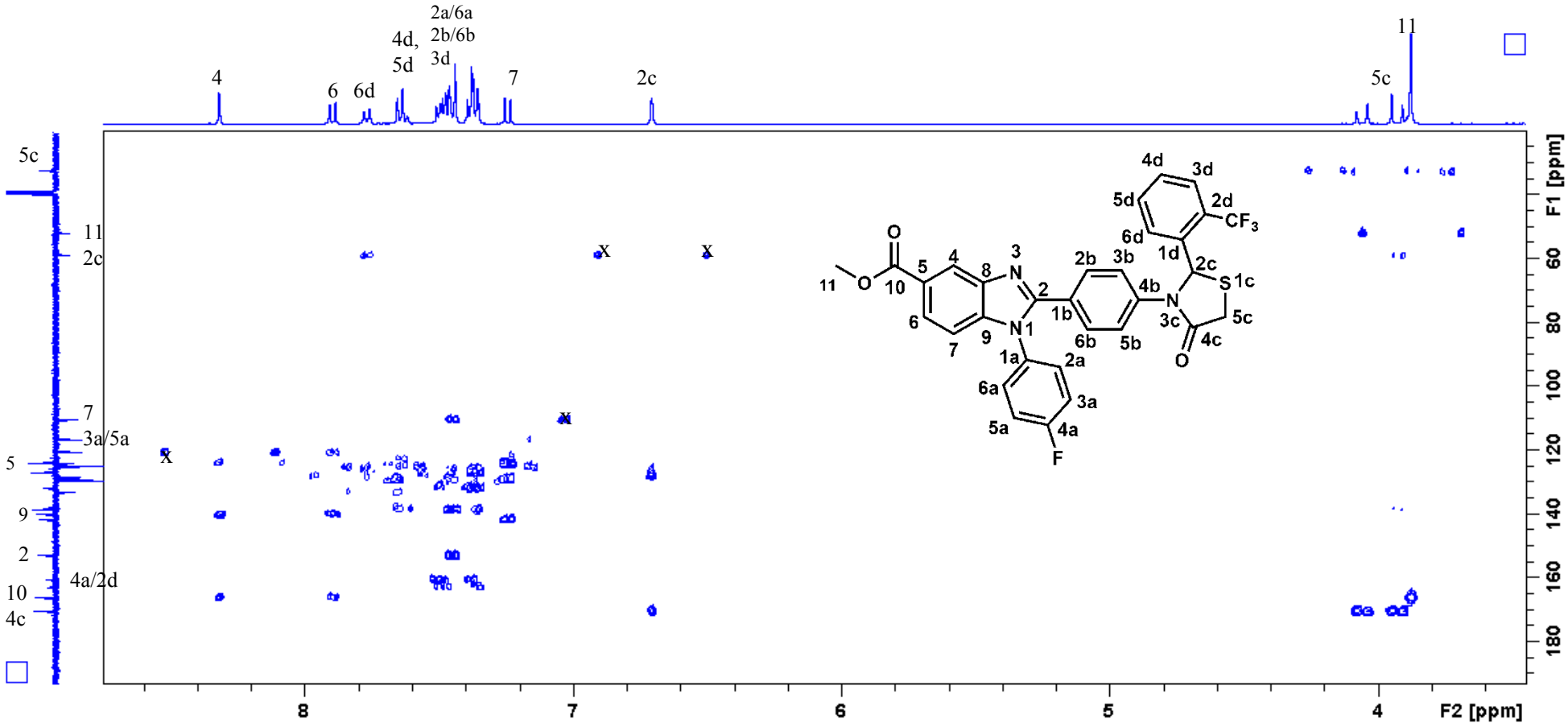
Expanded HSQC Spectrum of Compound A7d



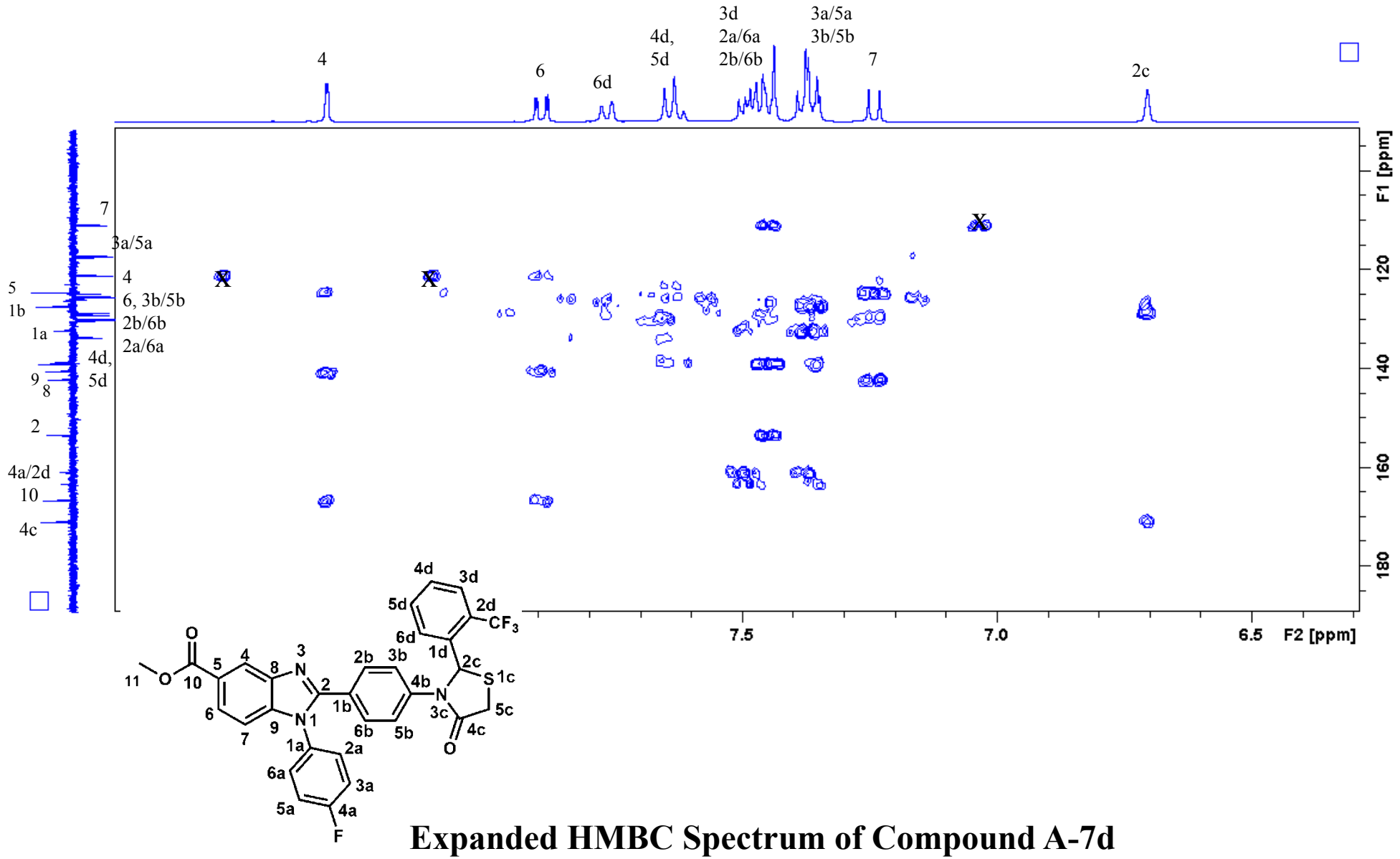
COSY Spectrum of Compound A7d



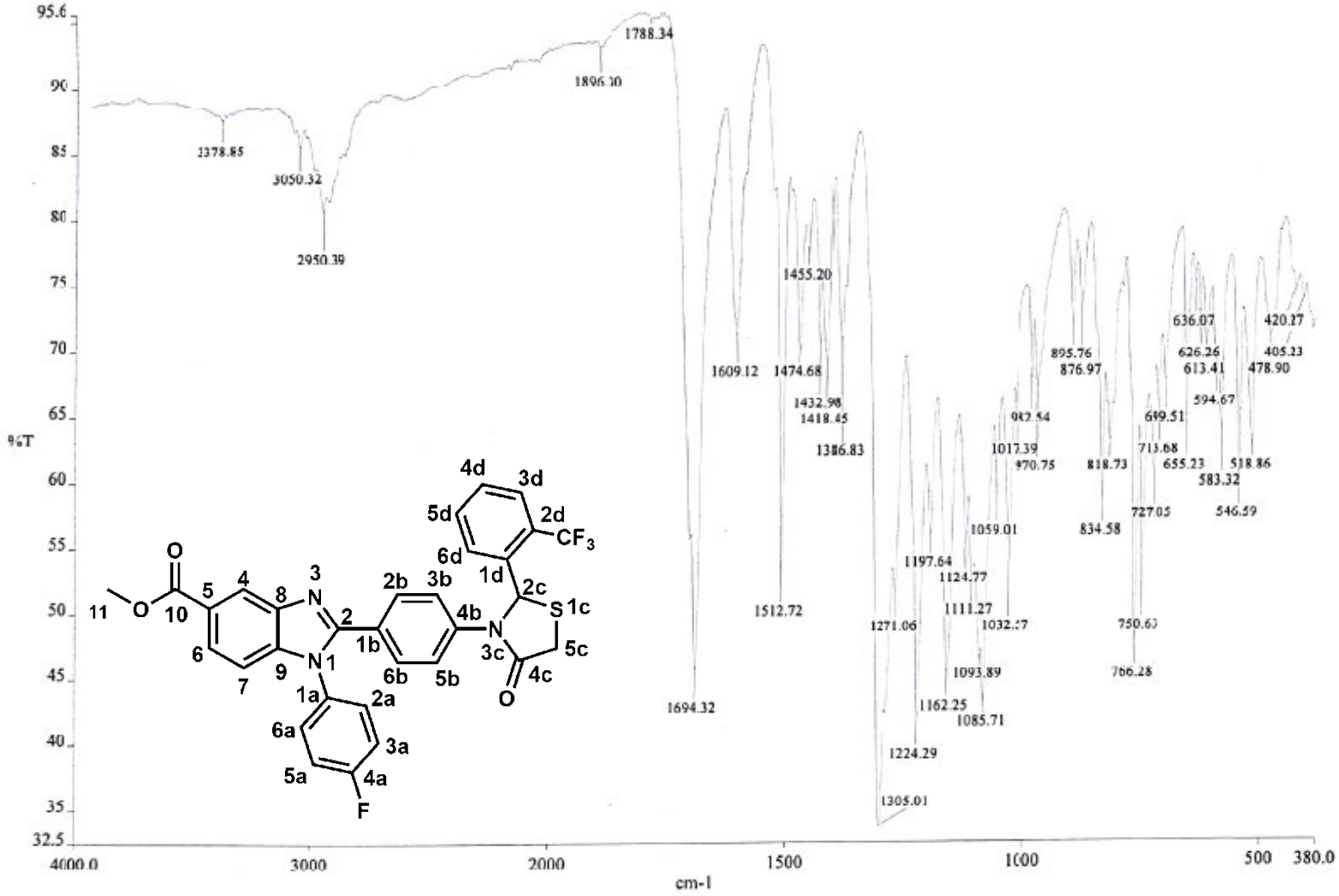
NOESY Spectrum of Compound A7d



HMBC Spectrum of Compound A7d



Expanded HMBC Spectrum of Compound A-7d

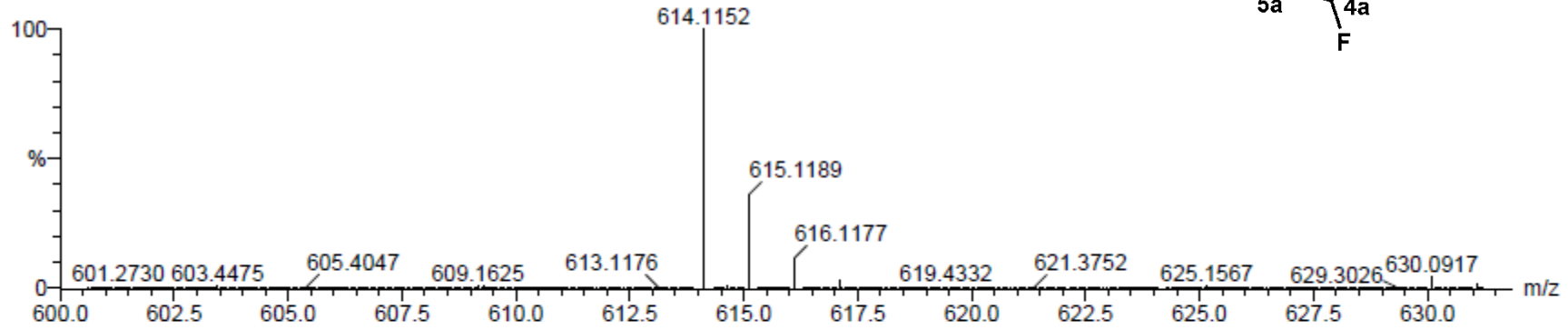


Infrared Spectrum of Compound A-7d

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 = 3

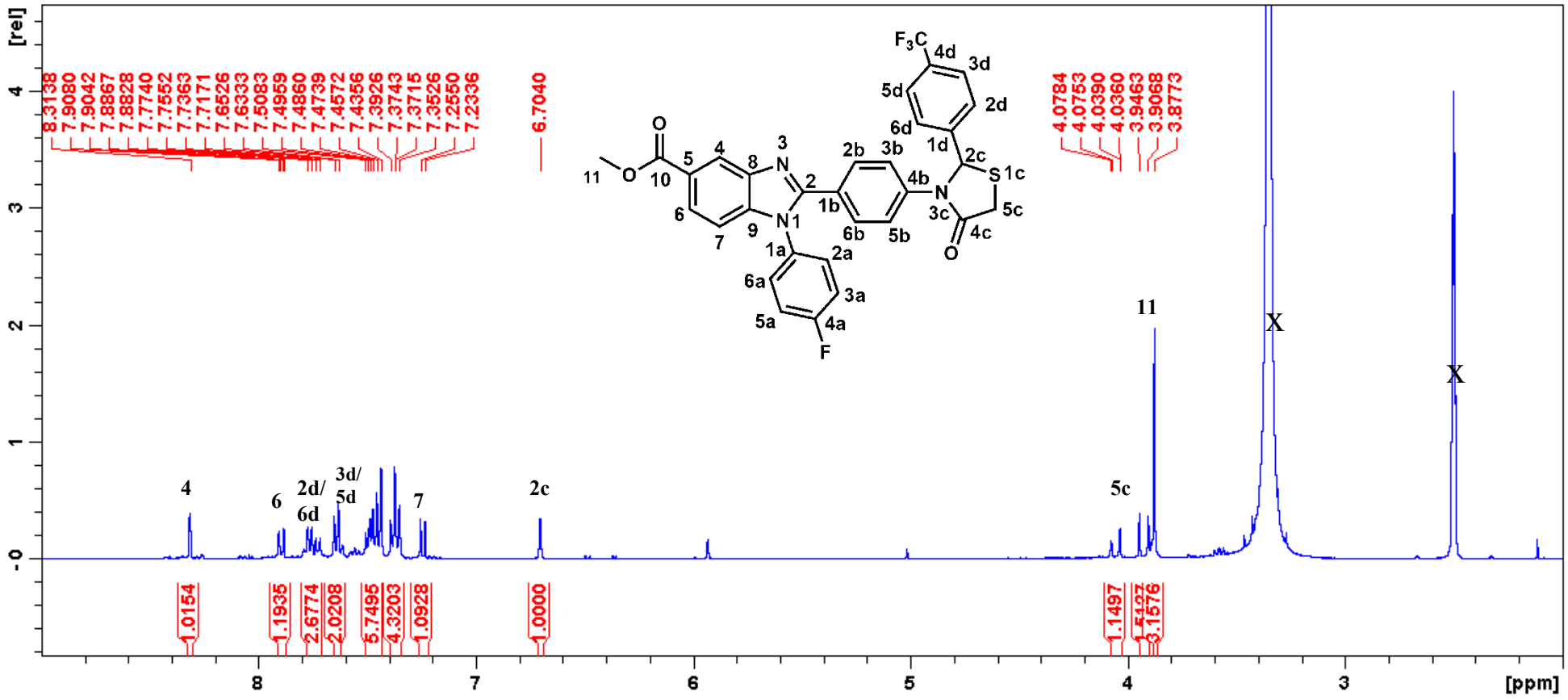
Monoisotopic Mass, Even Electron Ions
190 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 30-32 H: 20-25 N: 0-5 O: 0-5 F: 0-5 Na: 1-1 S: 0-1

BI 6 37 (1.216) Cm (1:61)
TOF MS ES+

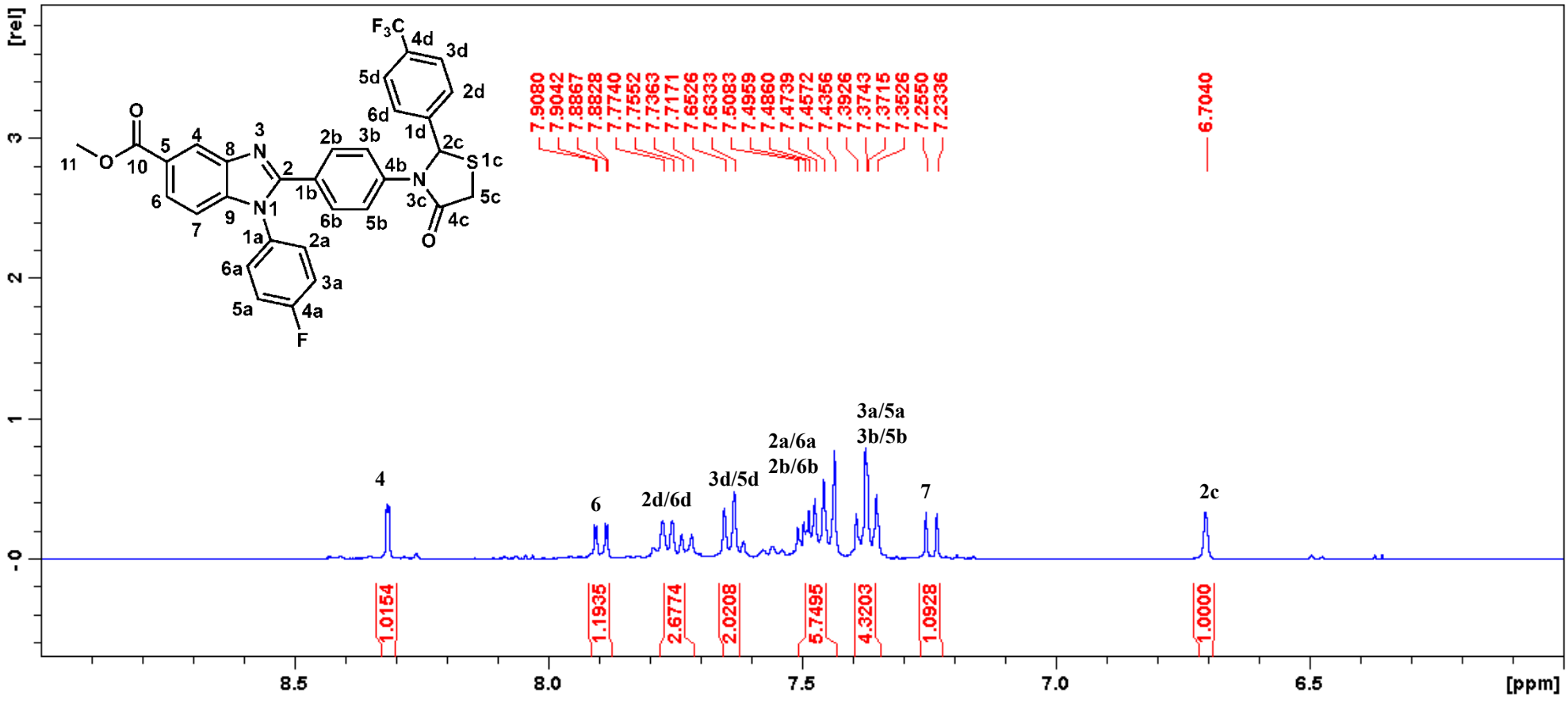


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
614.1152	614.1137	1.5	2.4	20.5	459.5	0.0	C31 H21 N3 O3 F4 Na S

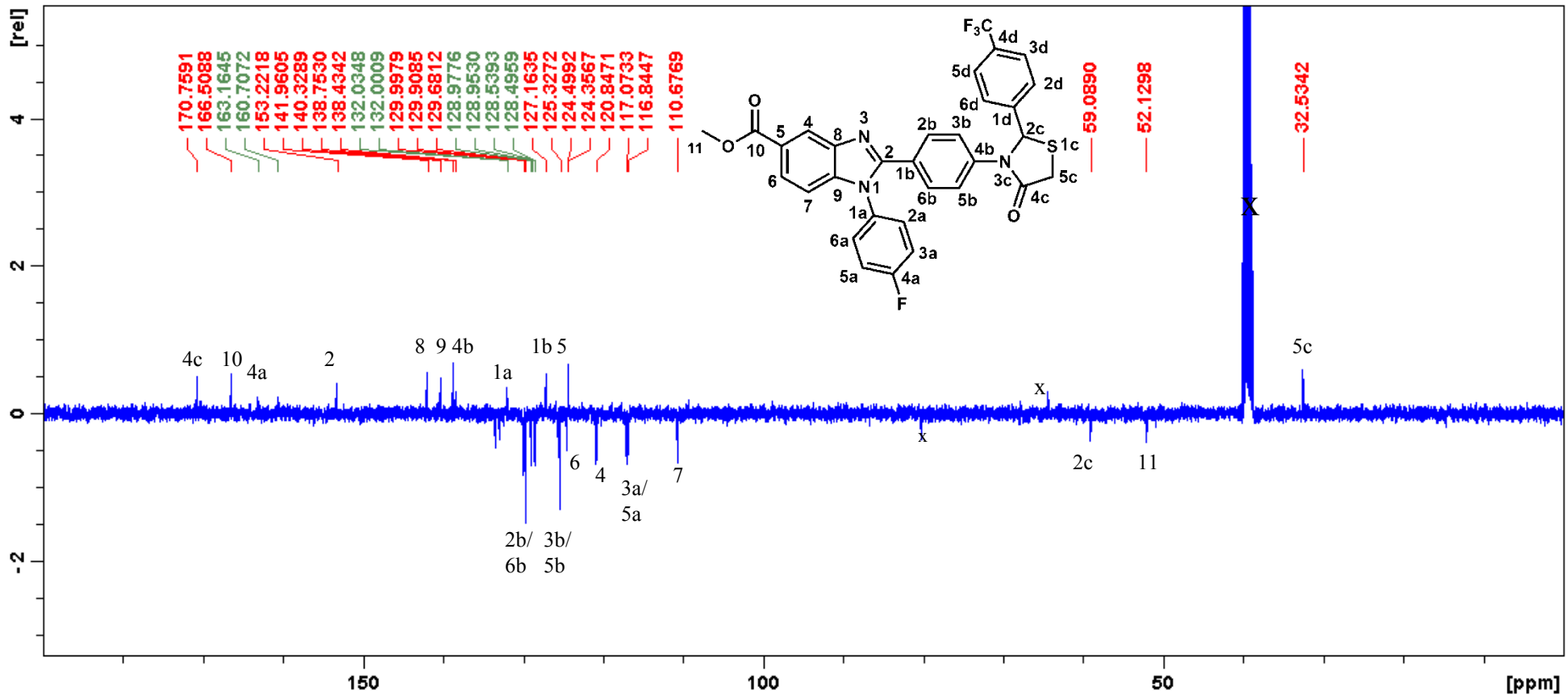
HRMS Spectrum of Compound A-7d



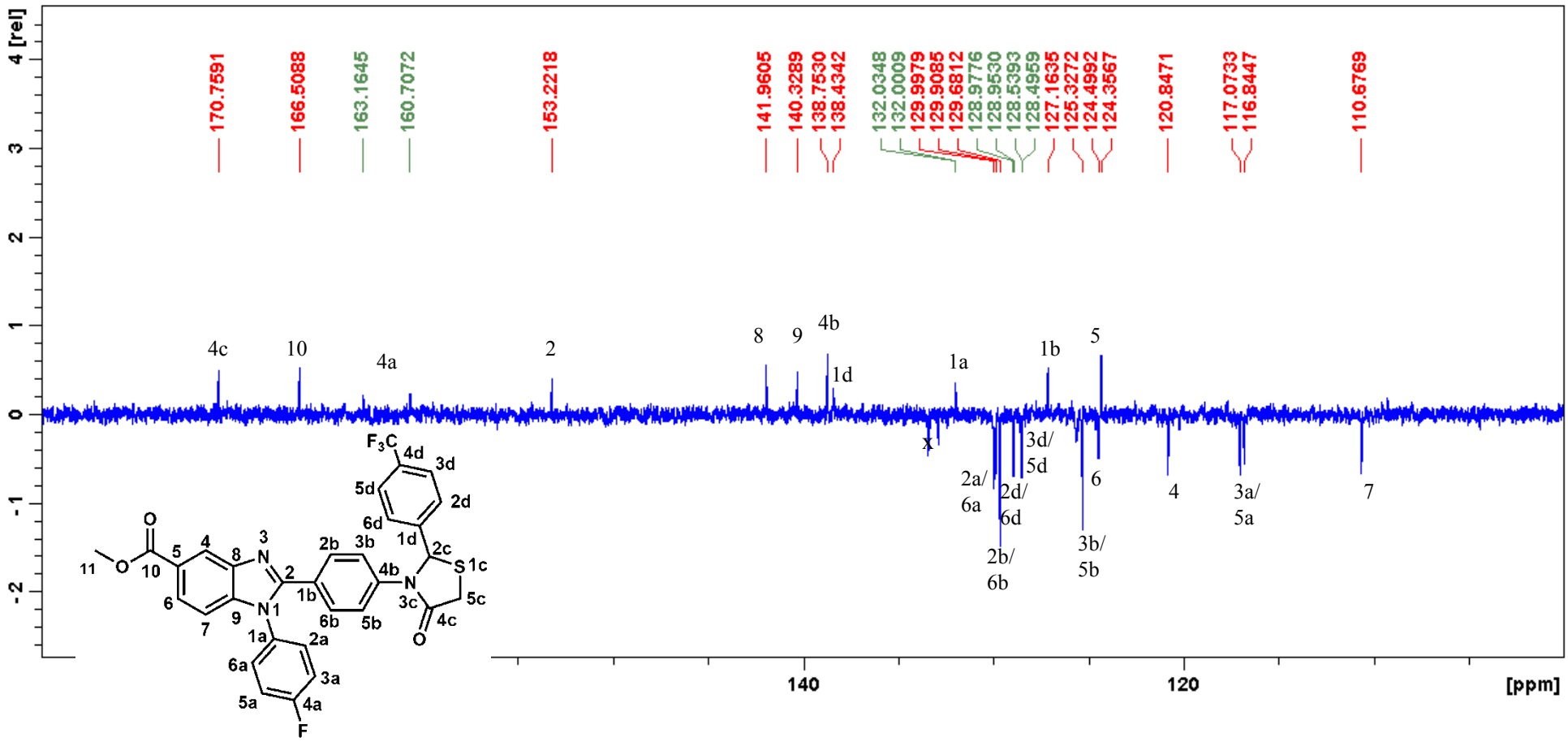
^{1}H Spectrum of Compound A-7e



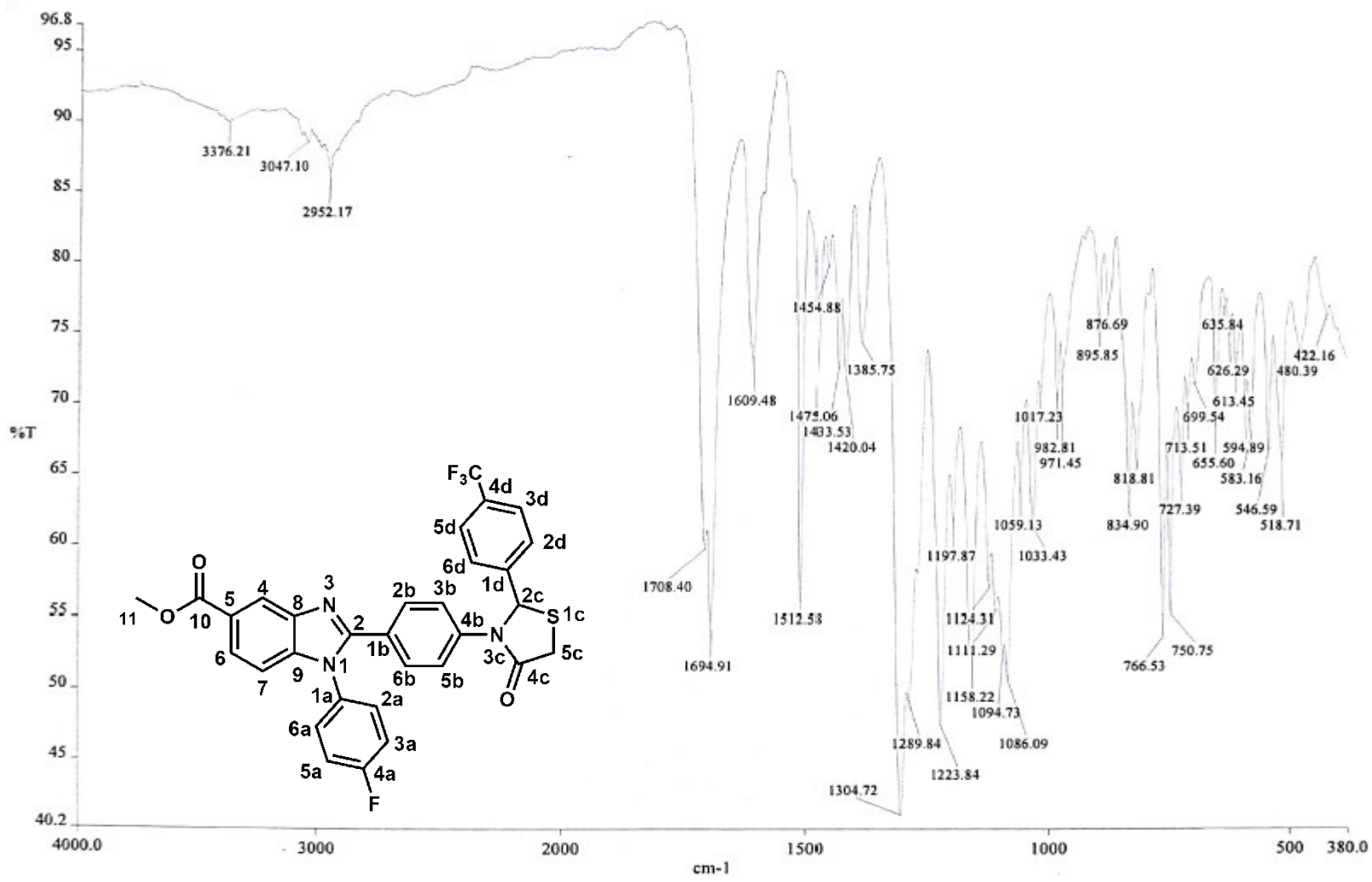
Expanded ^{1}H Spectrum of Compound A-7e



^{13}C Spectrum of Compound A-7e



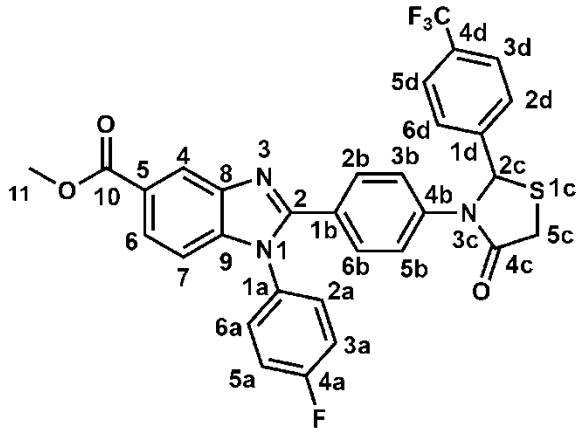
Expanded ^{13}C Spectrum of Compound A-7e

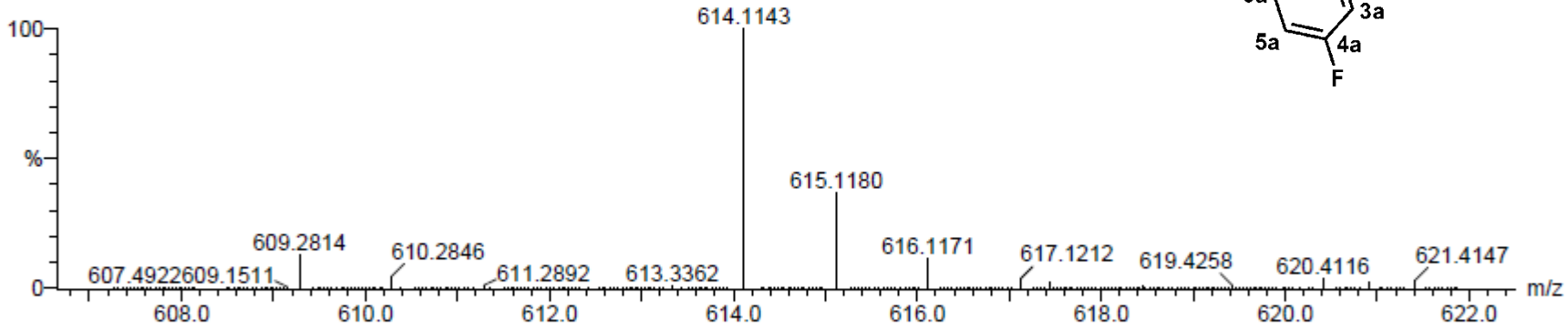


Infrared Spectrum of Compound A-7e

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 = 3

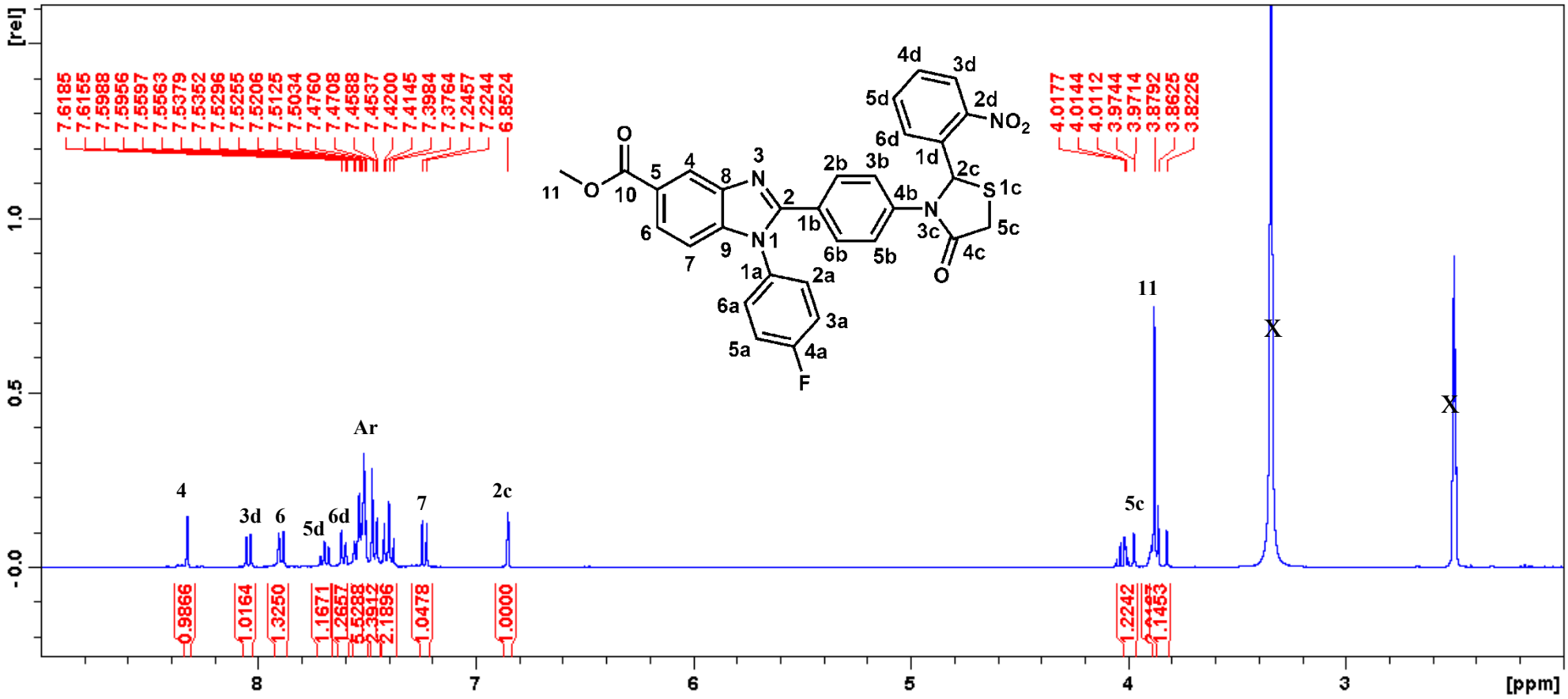
Monoisotopic Mass, Even Electron Ions
190 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 30-32 H: 20-25 N: 0-5 O: 0-5 F: 0-5 Na: 1-1 S: 0-1
BI 5 42 (1.417) Cm (1:60)
TOF MS ES+



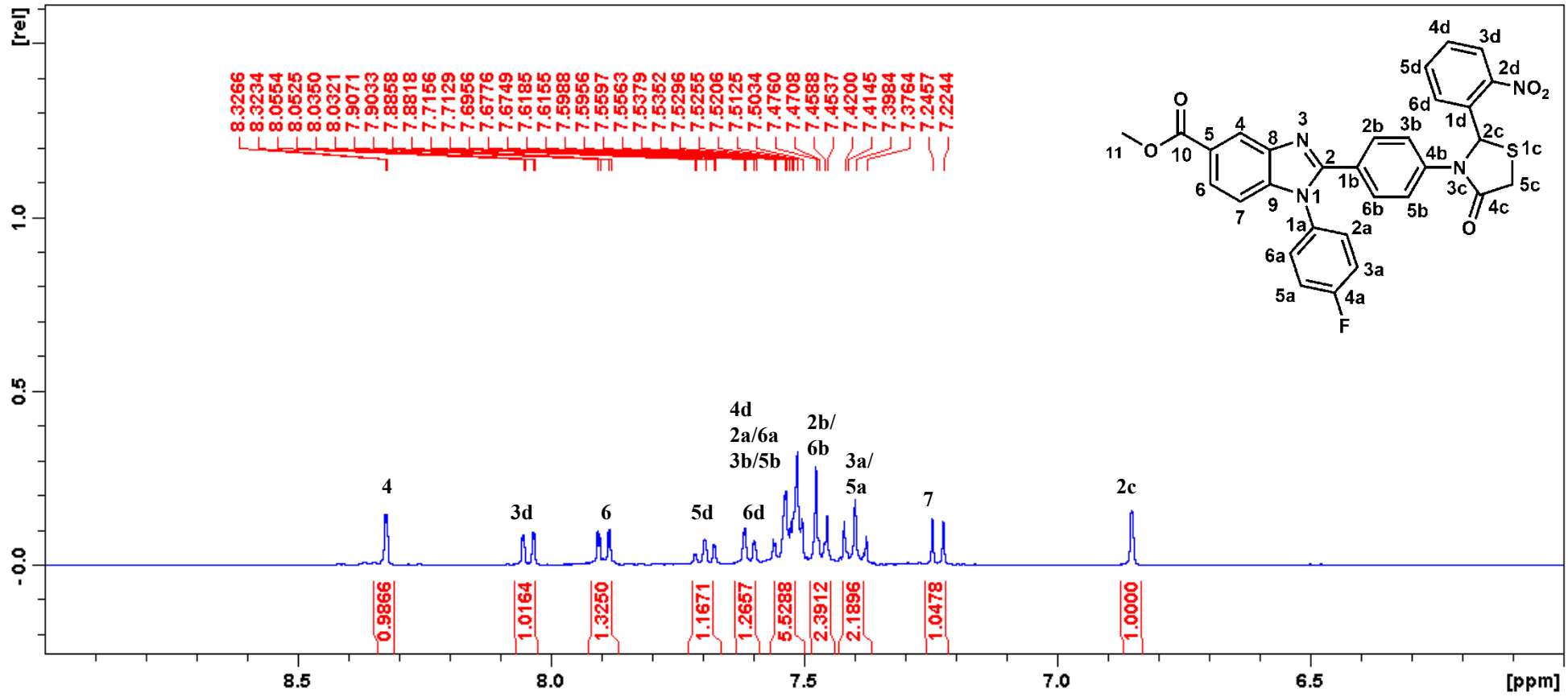


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
614.1143	614.1137	0.6	1.0	20.5	461.1	0.0	C31 H21 N3 O3 F4 Na S

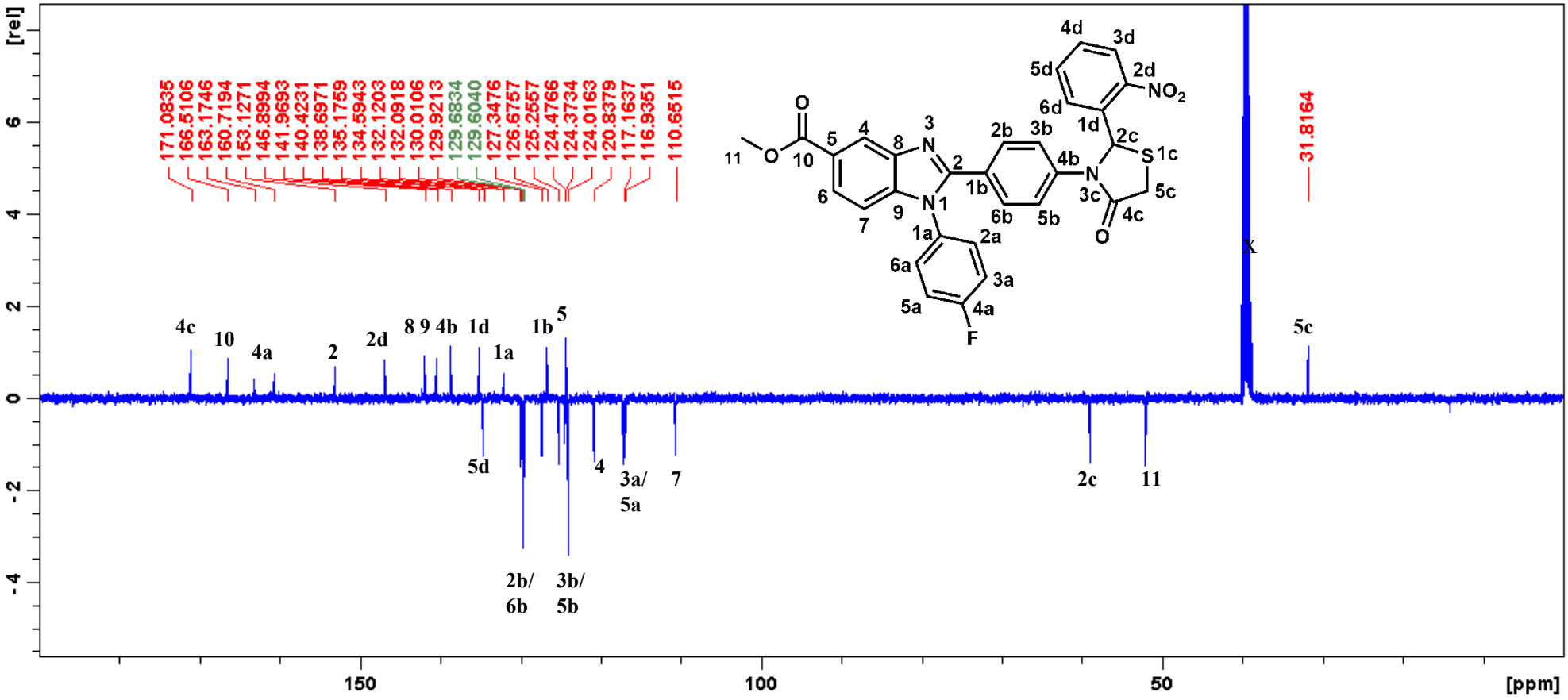
HRMS Spectrum of Compound A-7e



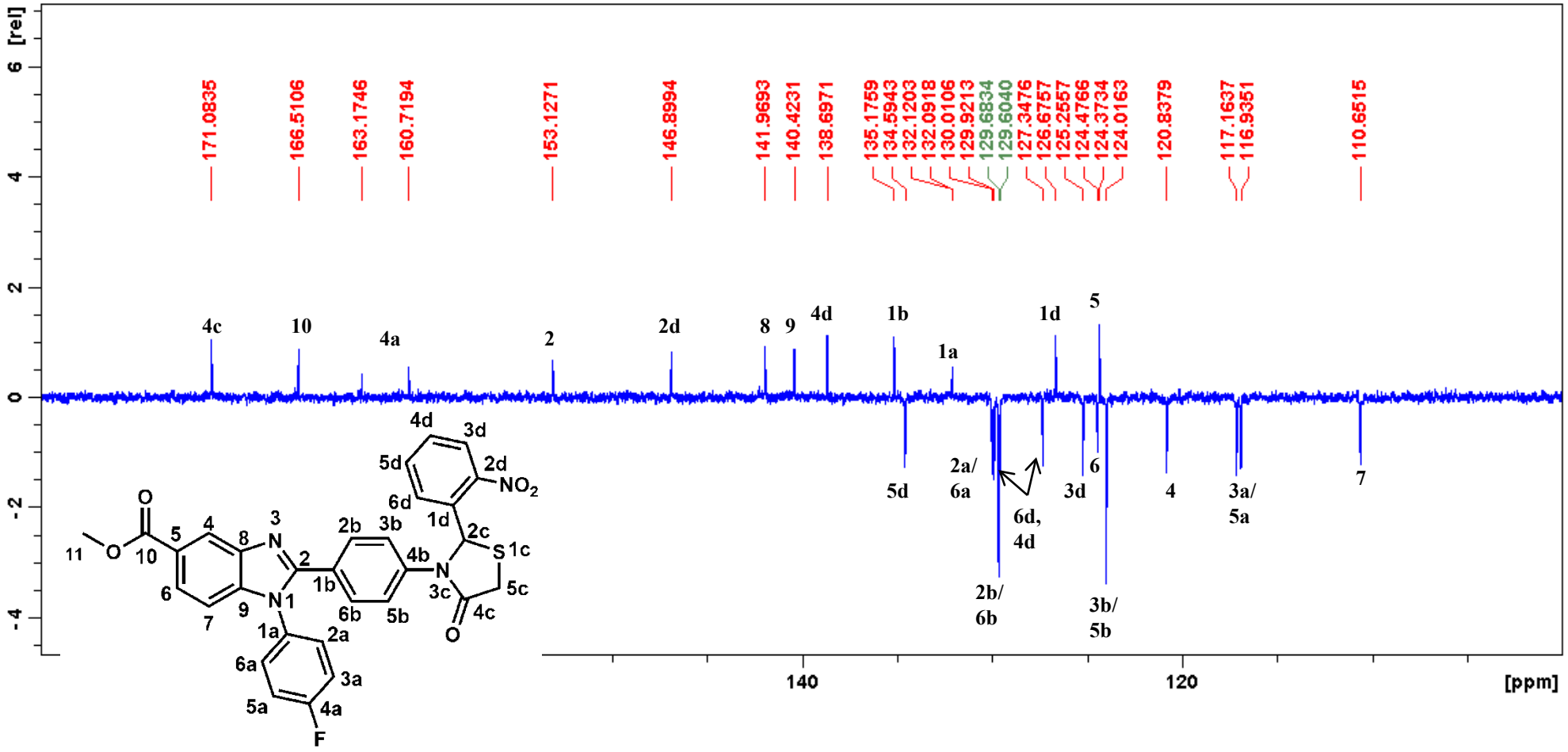
^{1}H Spectrum of Compound A-7f



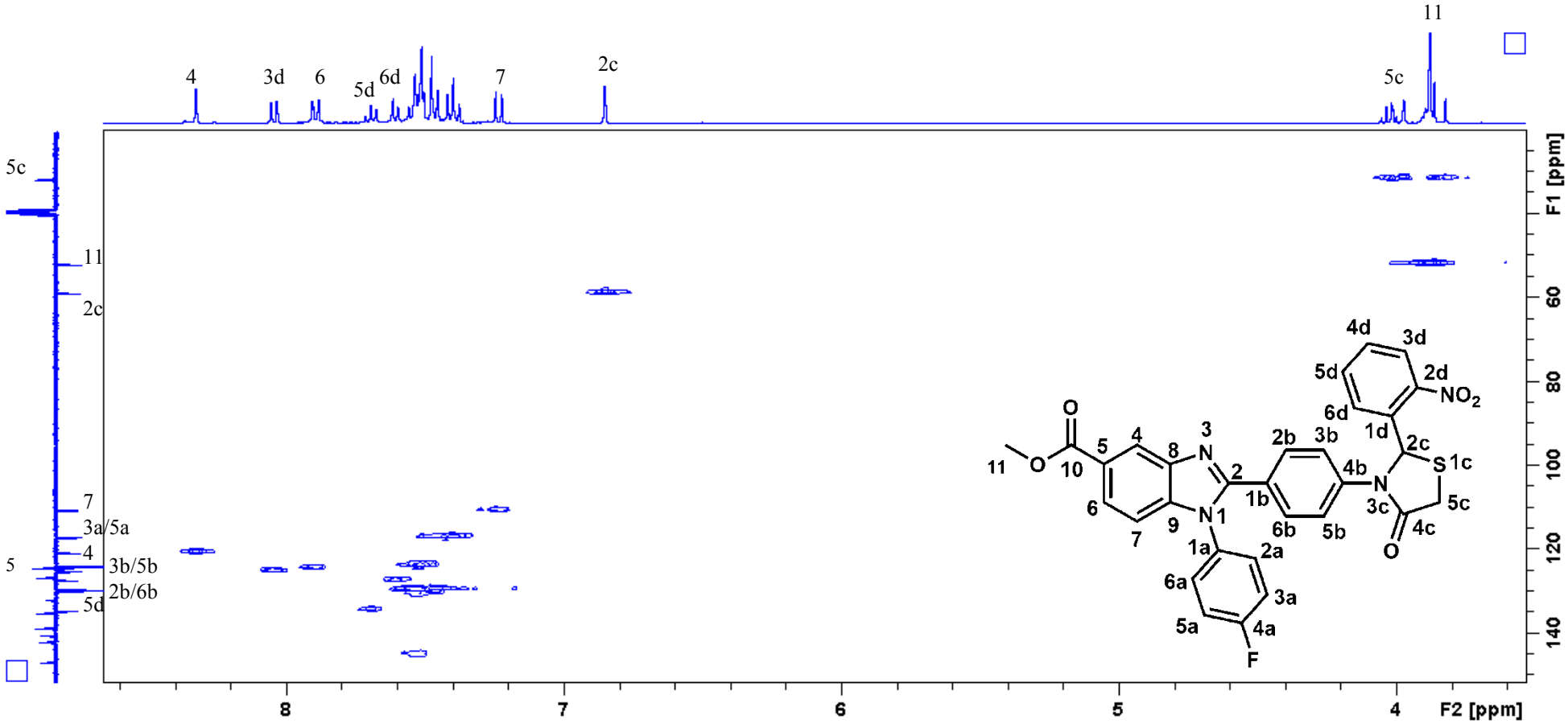
Expanded ^{1}H Spectrum of Compound A-7f



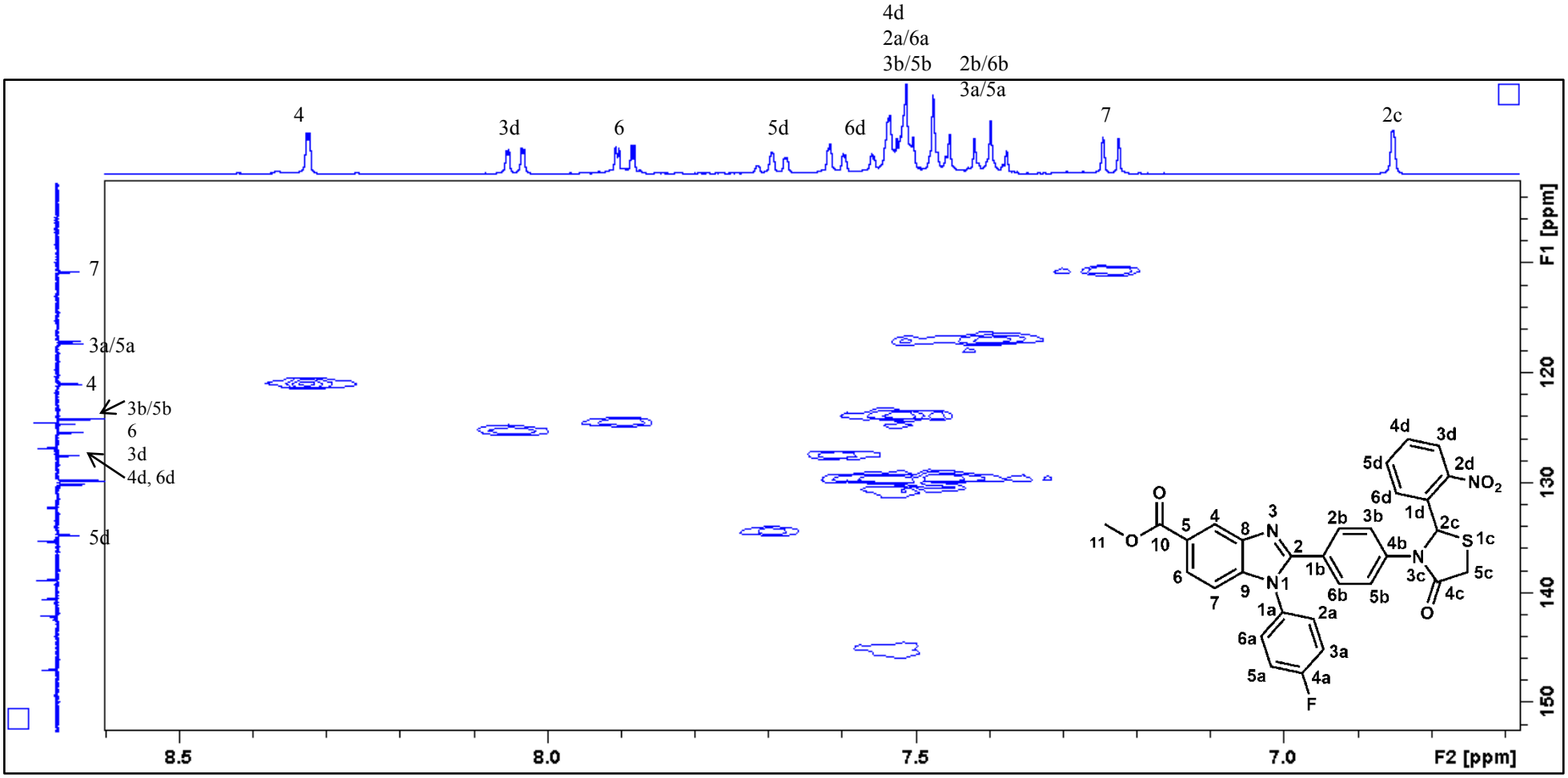
^{13}C Spectrum of Compound A-7f



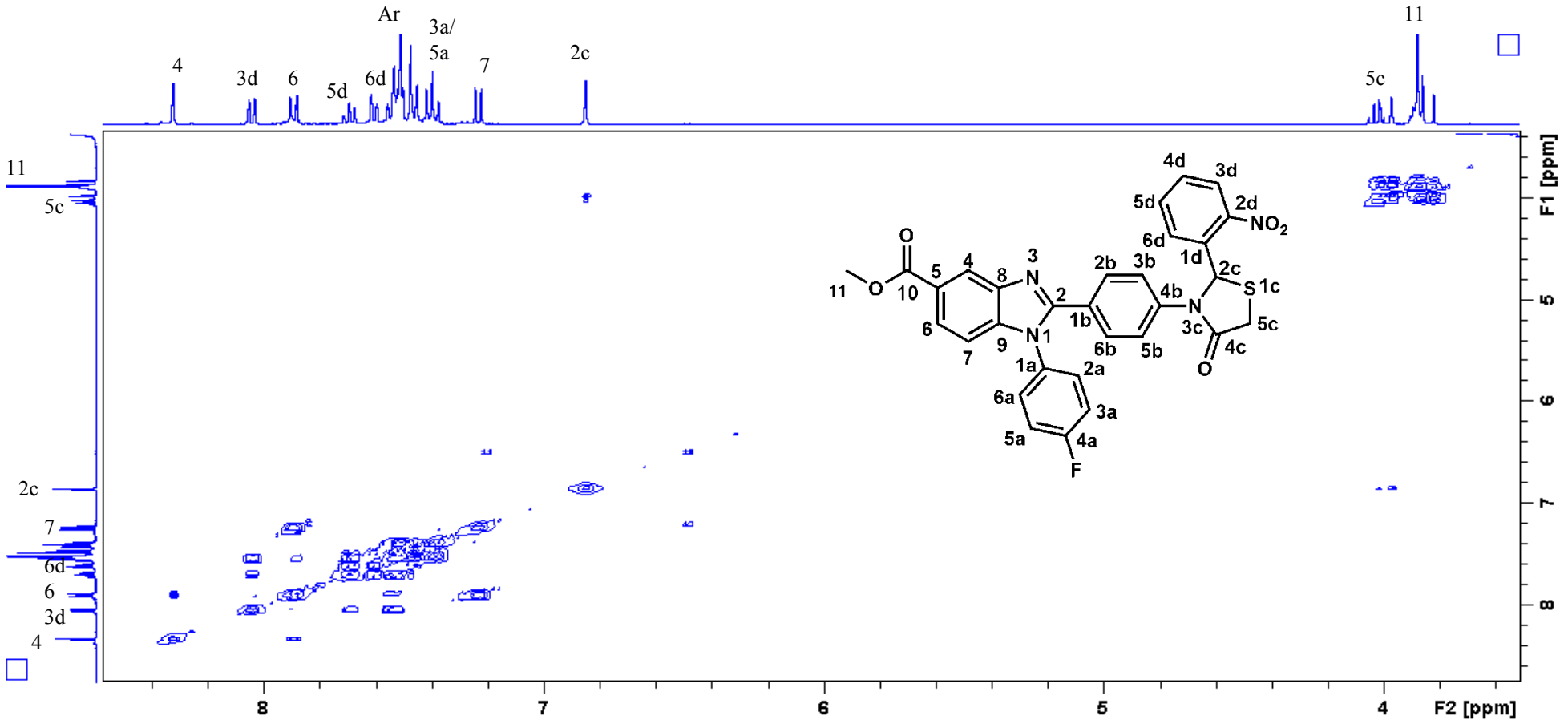
Expanded ^{13}C Spectrum of Compound A-7f



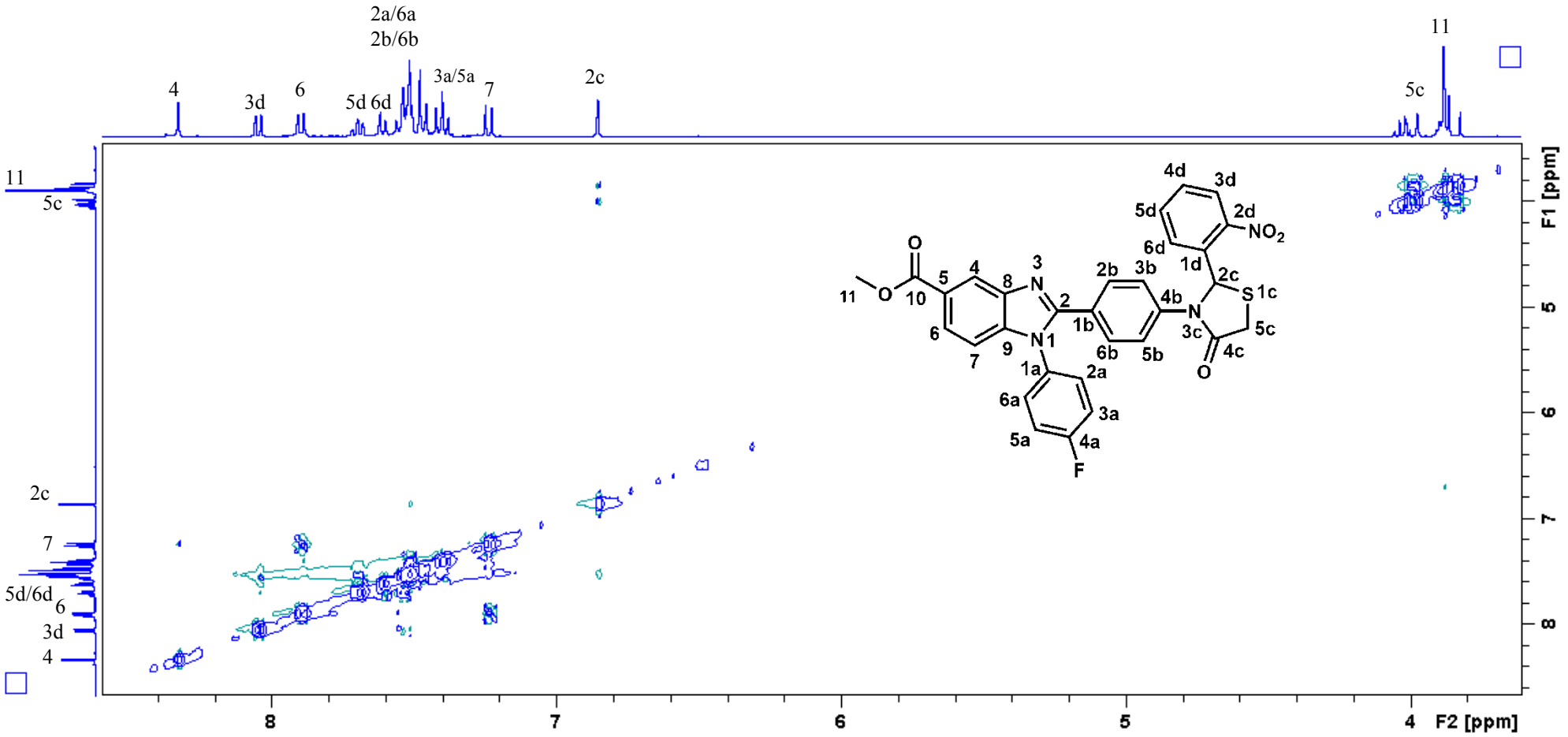
HSQC Spectrum of Compound A-7f



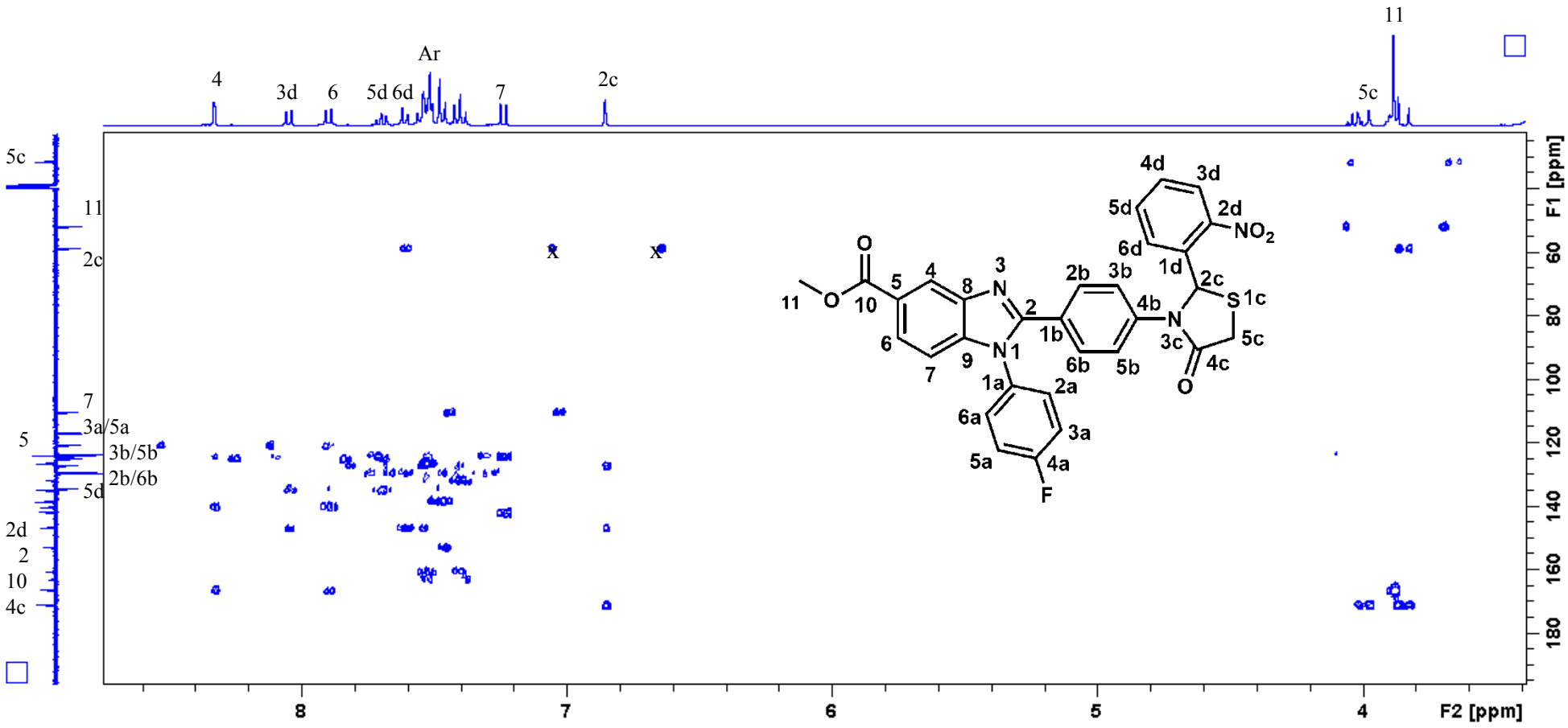
Expanded HSQC Spectrum of Compound A-7f



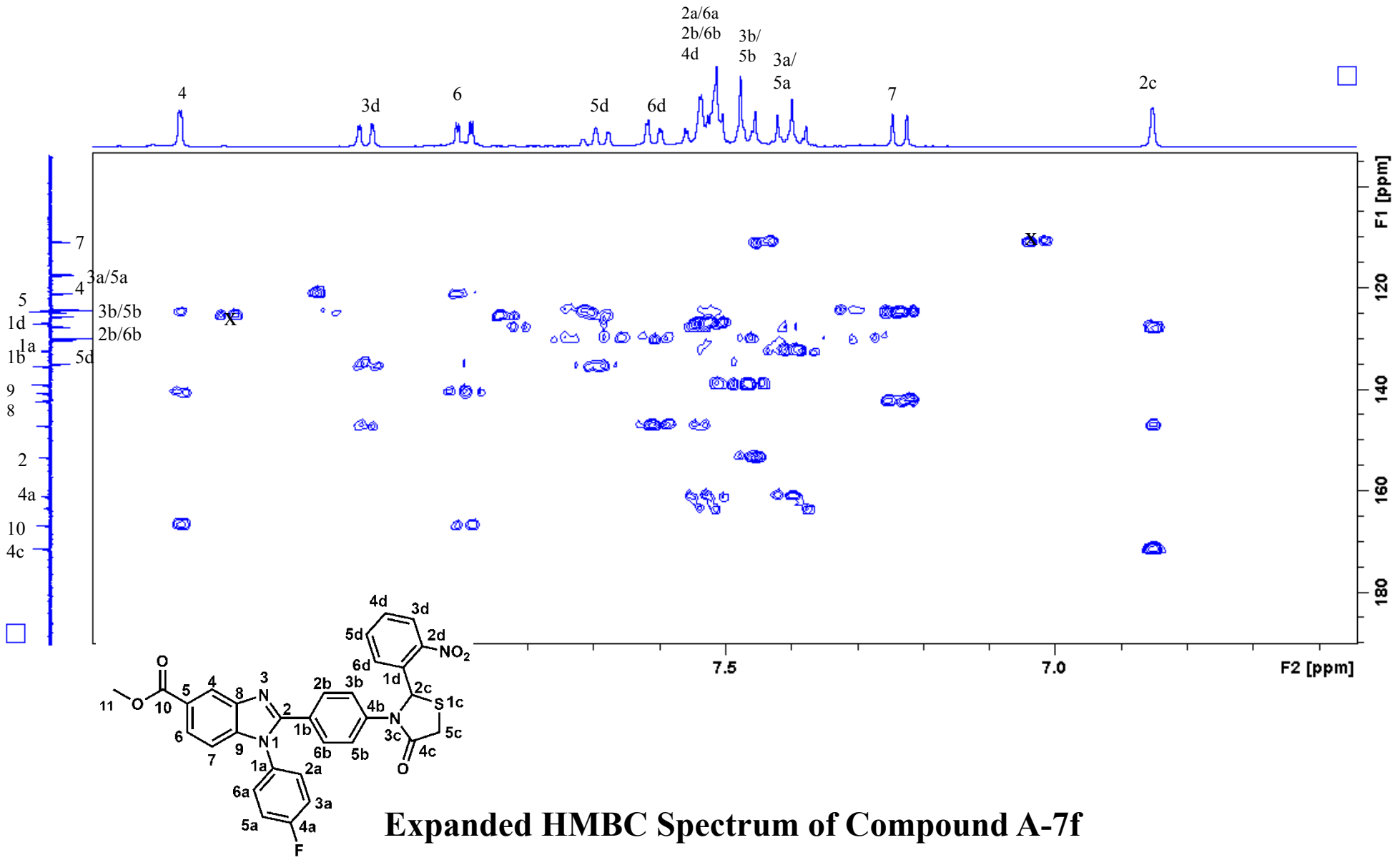
COSY Spectrum of Compound A-7f



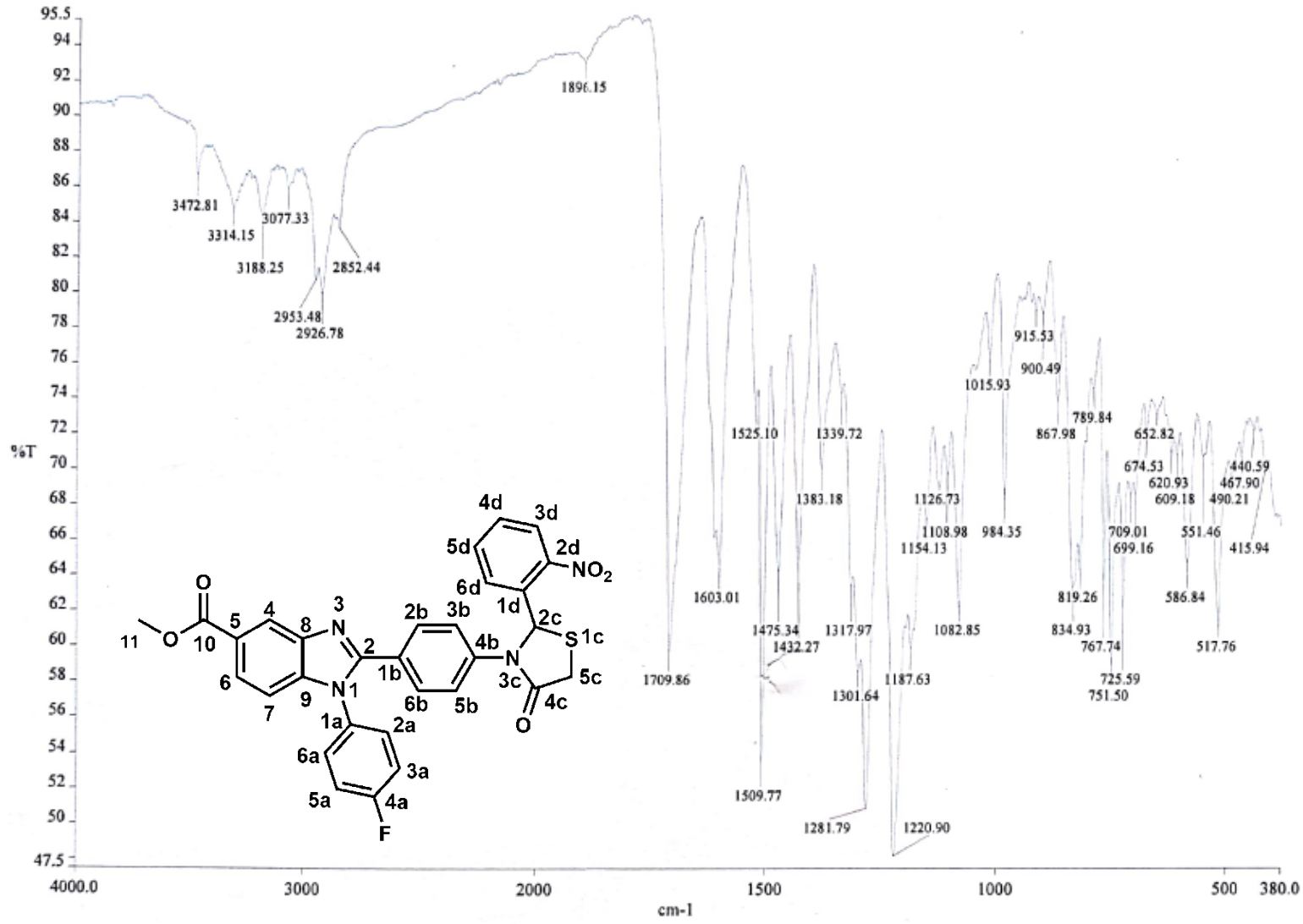
NOESY Spectrum of Compound A-7f



HMBC Spectrum of Compound A-7f



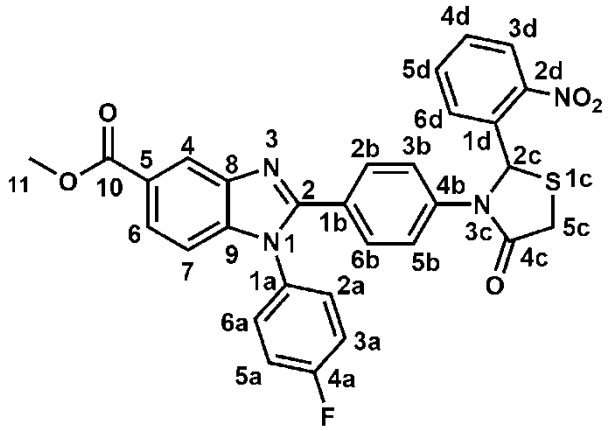
Expanded HMBC Spectrum of Compound A-7f

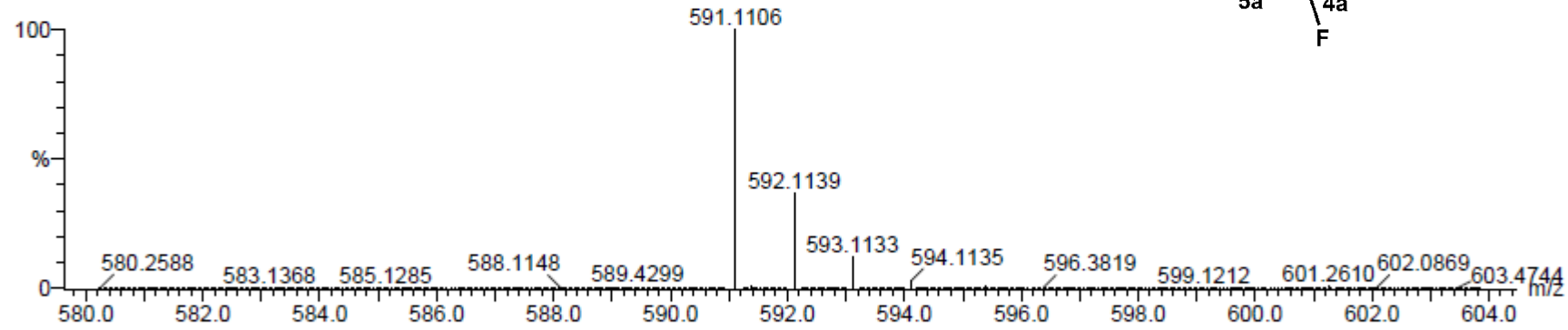


Infrared Spectrum of Compound A-7f

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 = 3

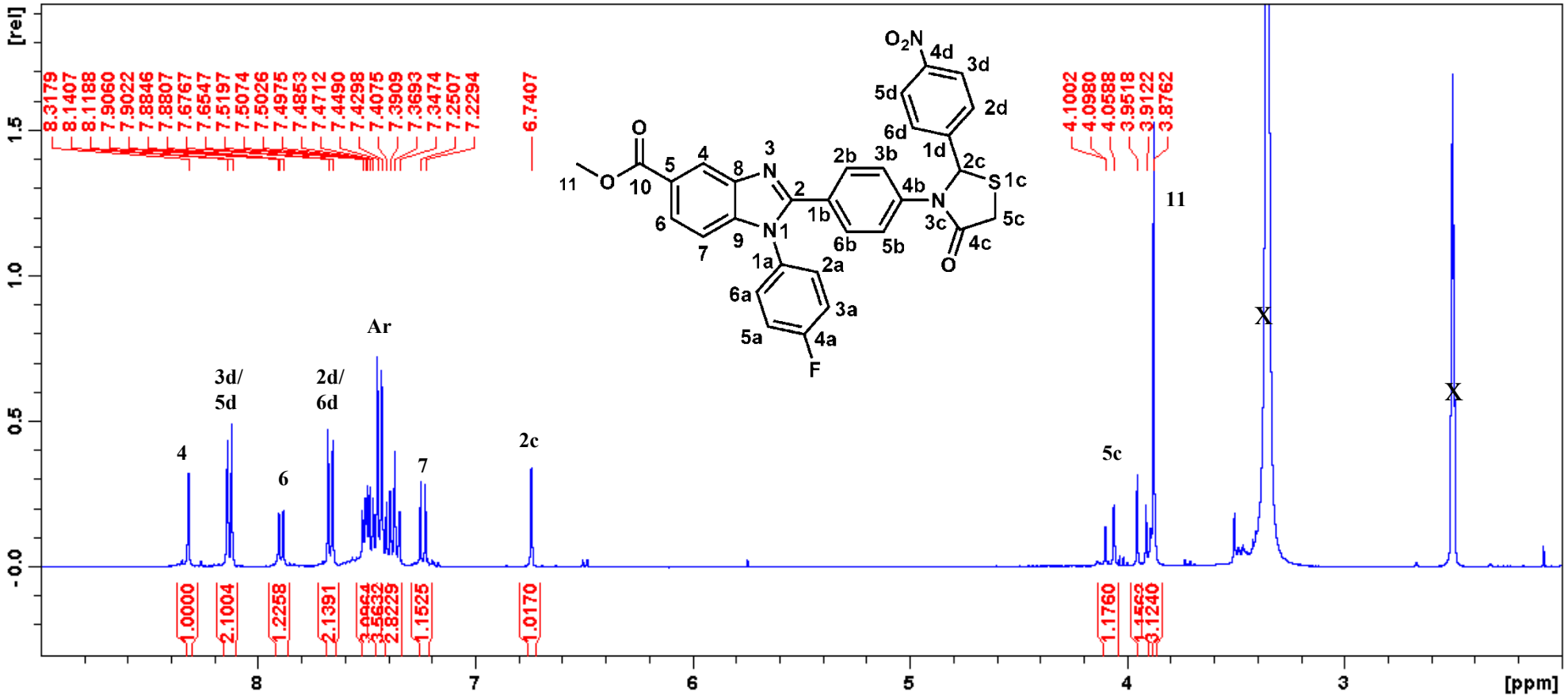
Monoisotopic Mass, Even Electron Ions
20 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 30-35 H: 20-25 N: 0-5 O: 0-5 F: 1-1 Na: 1-1 S: 0-1
BI 7 52 (1.722) Cm (1:61)
TOF MS ES+



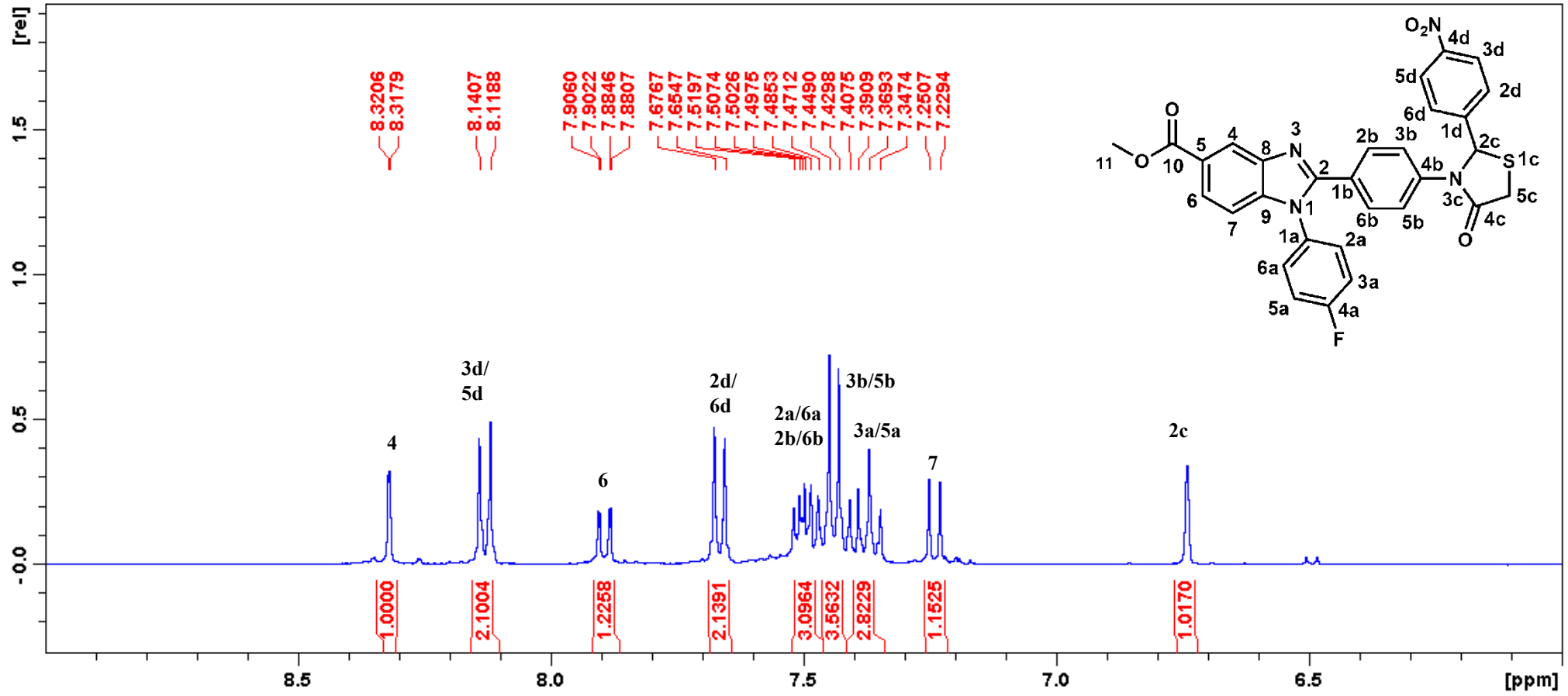


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
591.1106	591.1114	-0.8	-1.4	21.5	487.8	0.0	C30 H21 N4 O5 F Na S

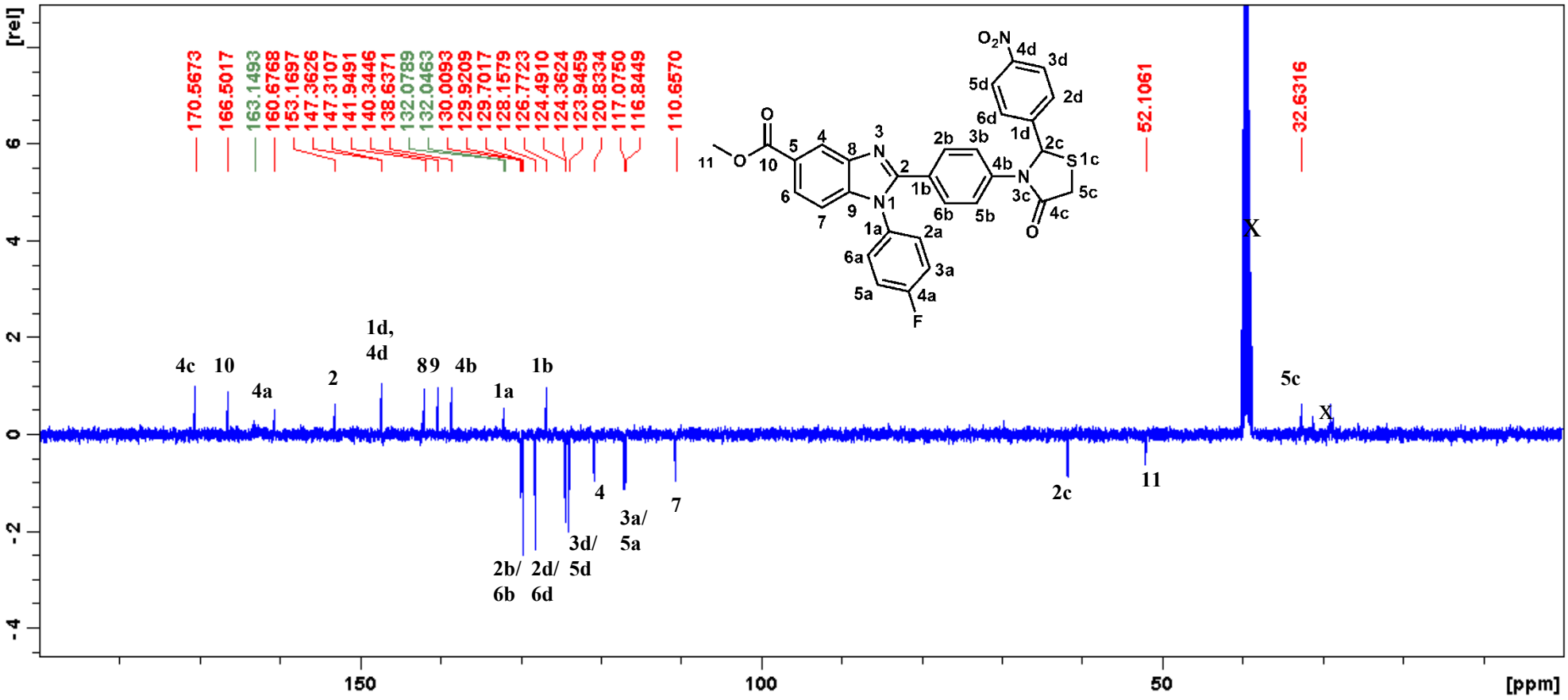
HRMS Spectrum of Compound A-7f



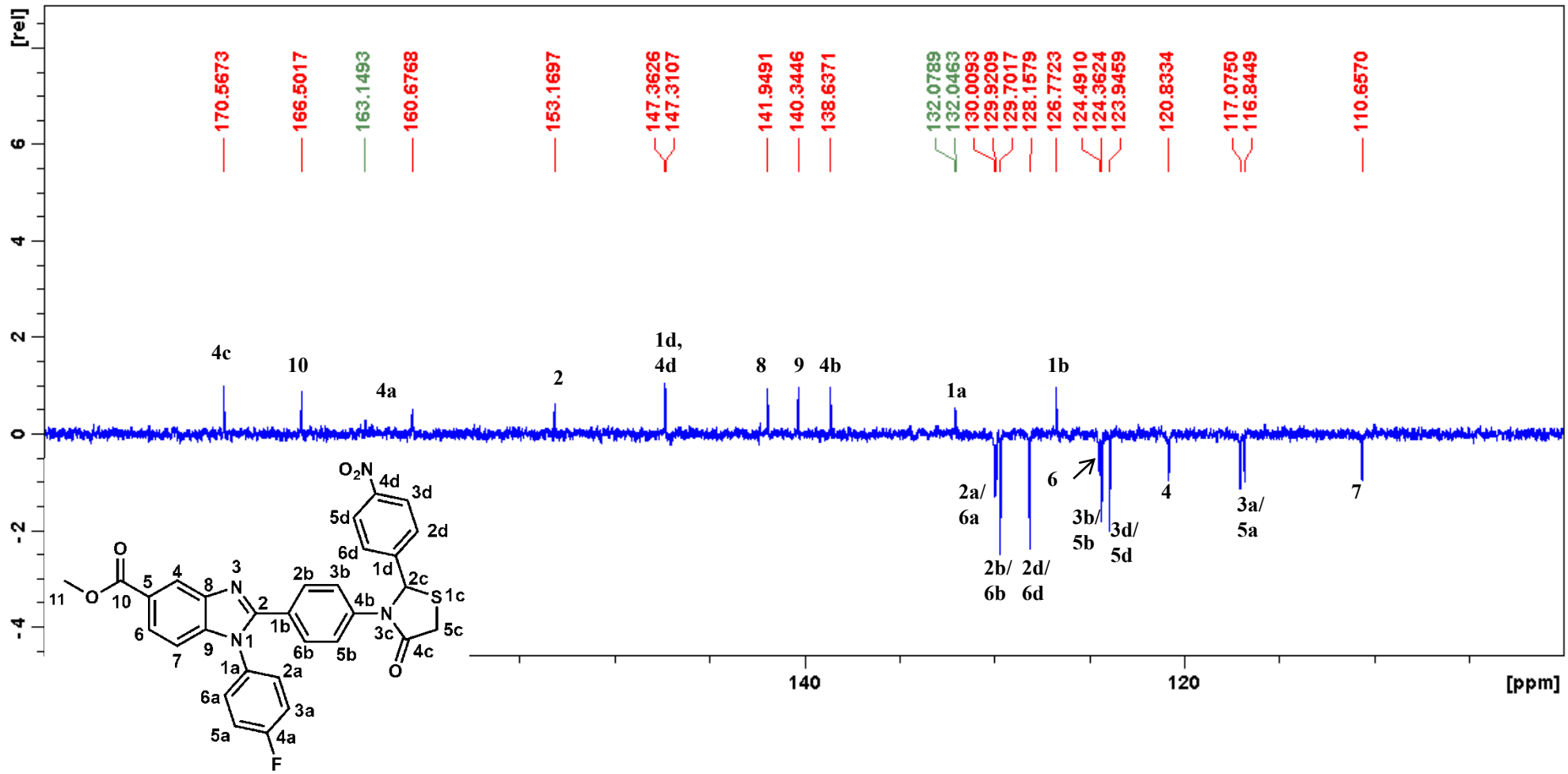
^{1}H Spectrum of Compound A-7g



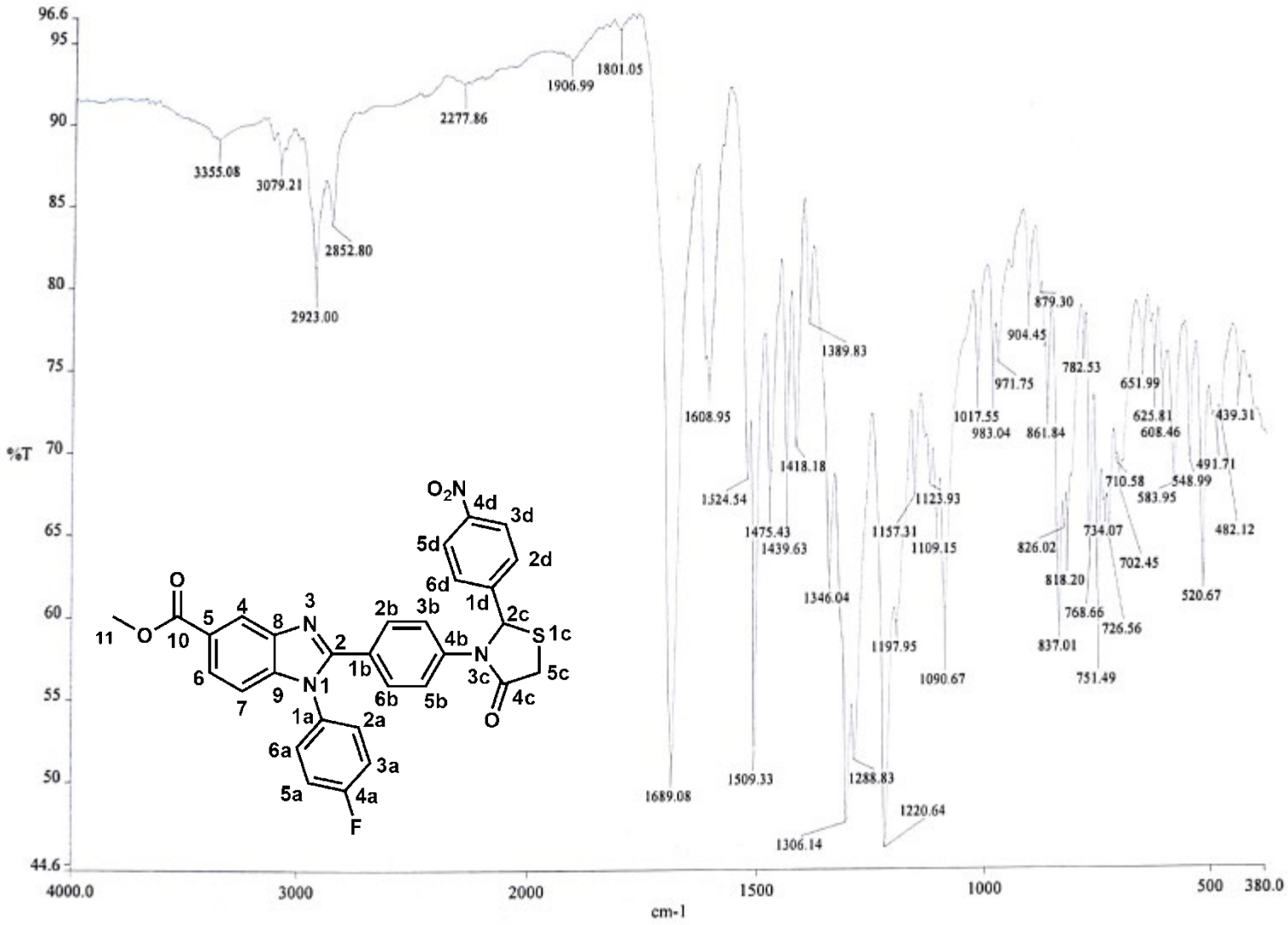
Expanded ^{1}H Spectrum of Compound A-7g



^{13}C Spectrum of Compound A-7g



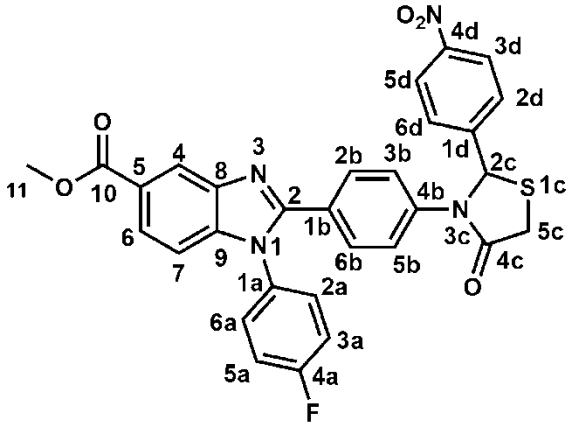
Expanded ^{13}C Spectrum of Compound A-7g

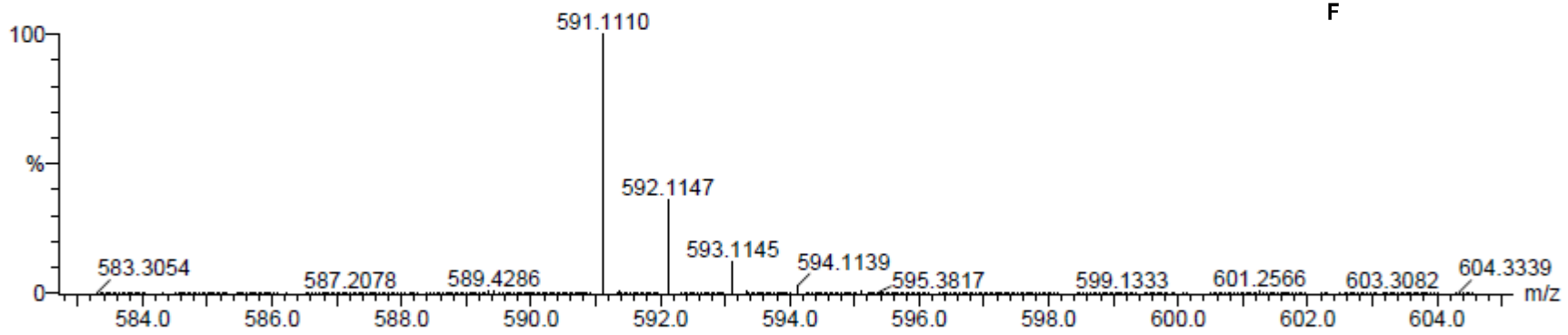


Infrared Spectrum of Compound A-7g

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

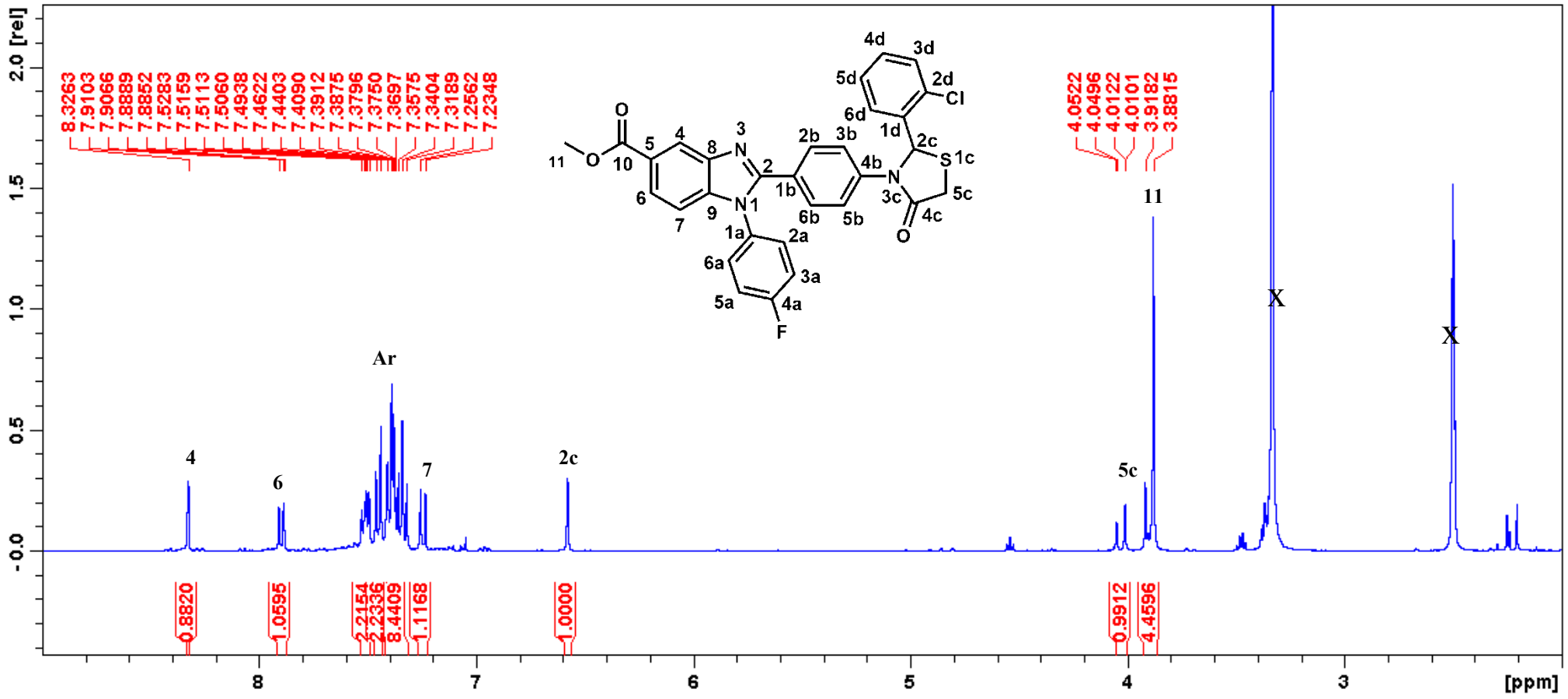
Monoisotopic Mass, Even Electron Ions
98 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 25-30 H: 20-25 N: 0-5 O: 0-5 F: 0-1 Na: 1-1 S: 0-1
BI 4 55 (1.822) Cm (1:61)
TOF MS ES+



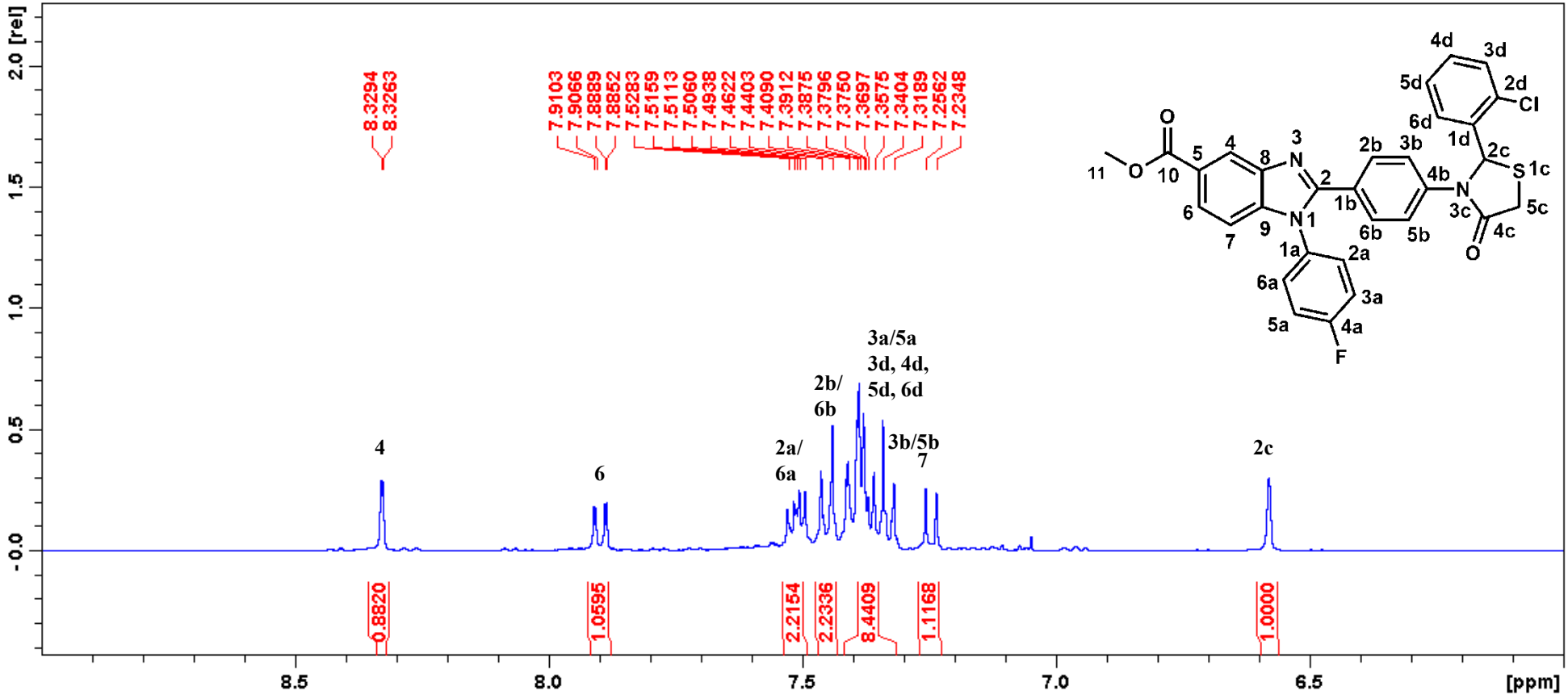


Minimum:				-1.5			
Maximum:		5.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
591.1110	591.1114	-0.4	-0.7	21.5	513.1	0.0	C30 H21 N4 O5 F Na S

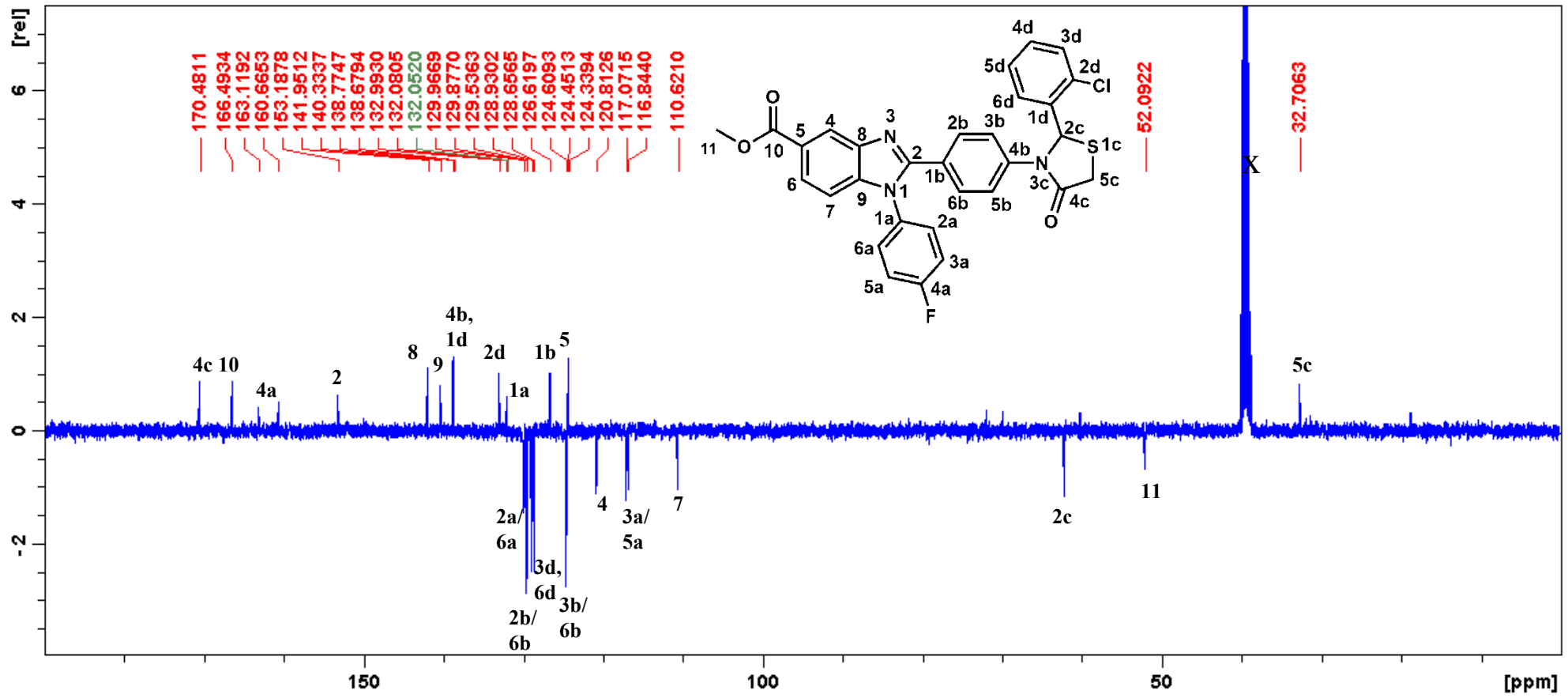
HRMS Spectrum of Compound A-7g



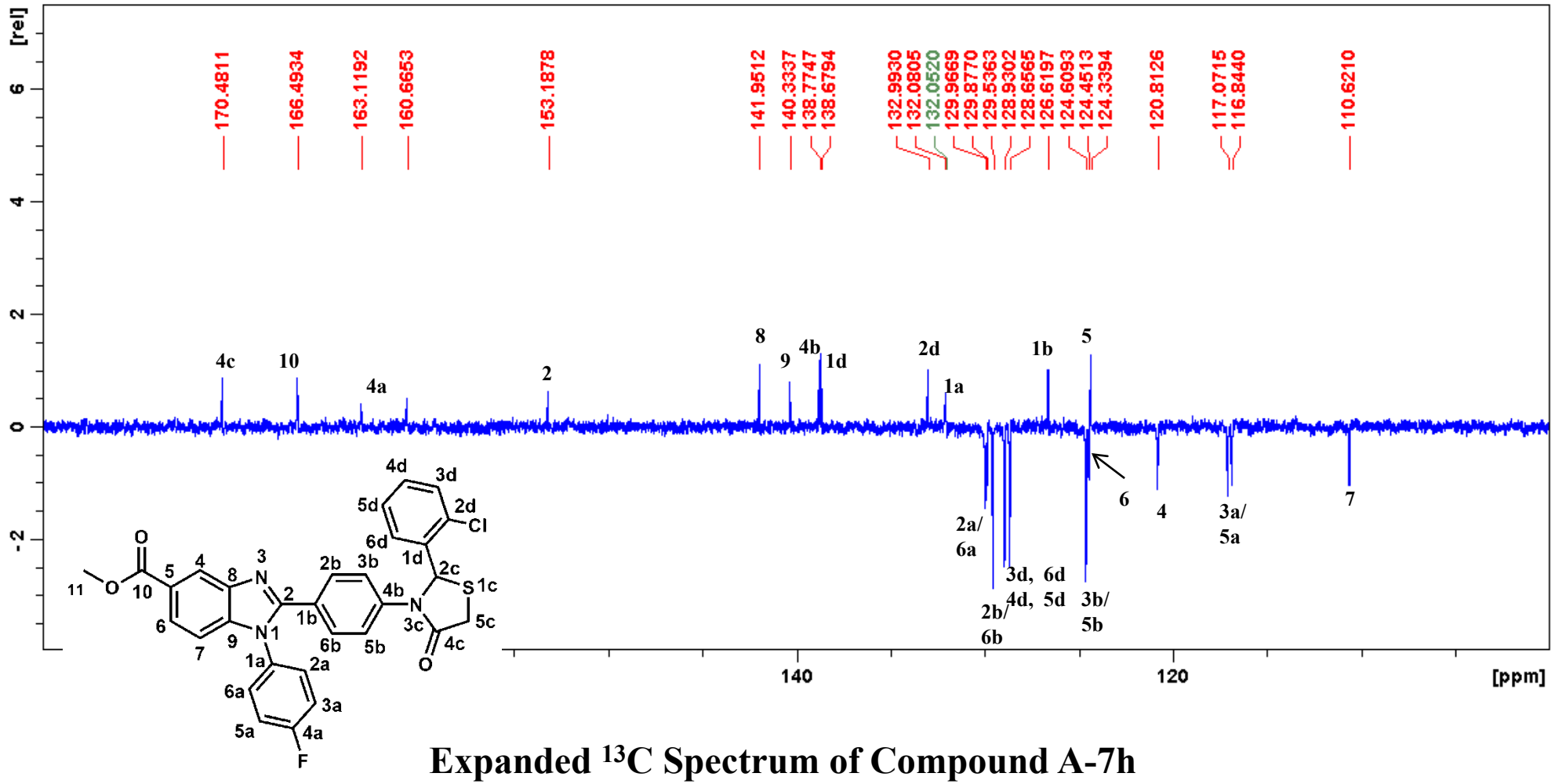
^{1}H Spectrum of Compound A-7h



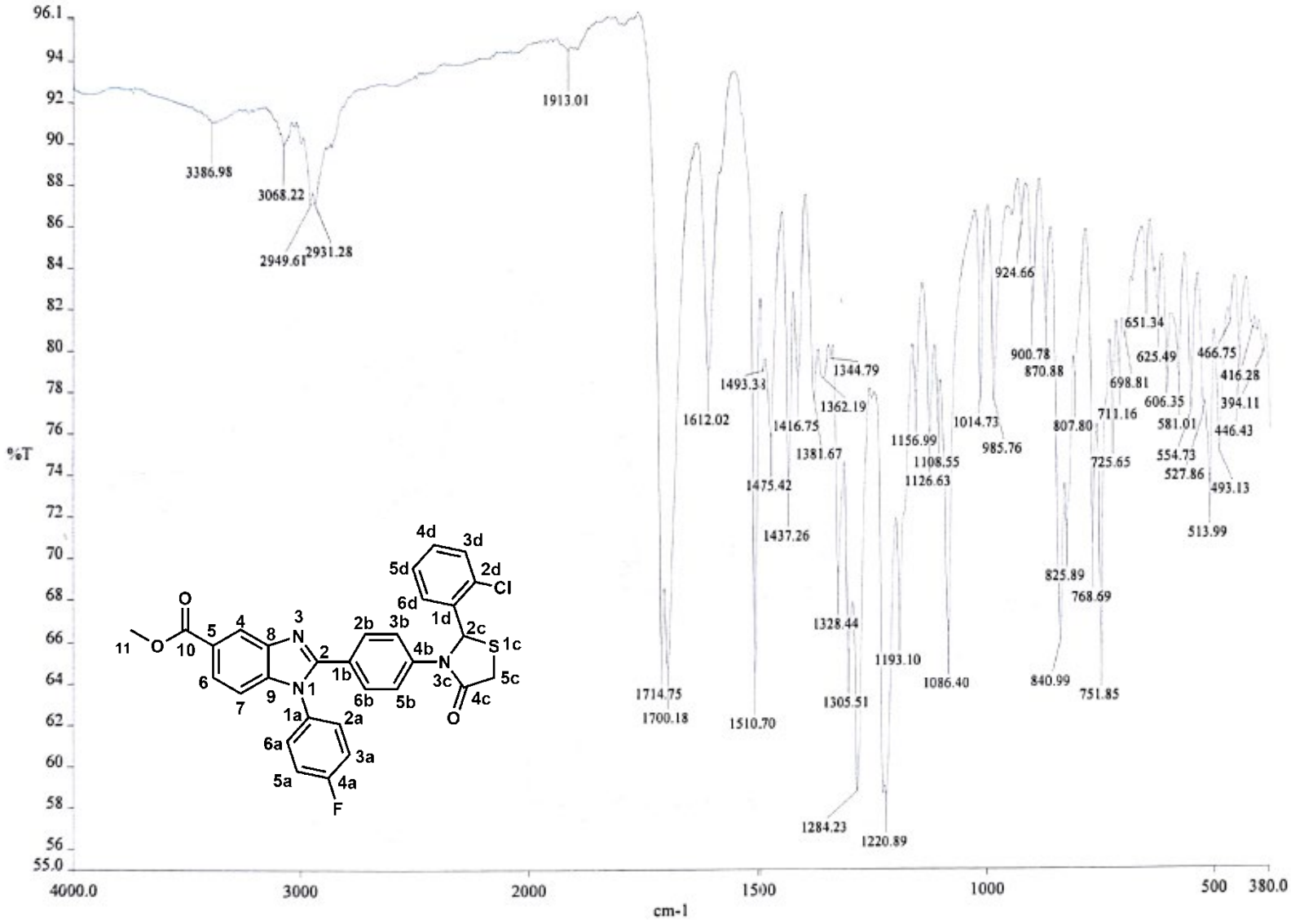
Expanded ^{1}H Spectrum of Compound A-7h



^{13}C Spectrum of Compound A-7h



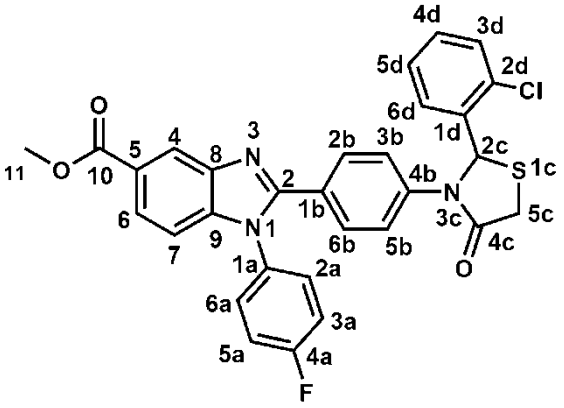
Expanded ^{13}C Spectrum of Compound A-7h

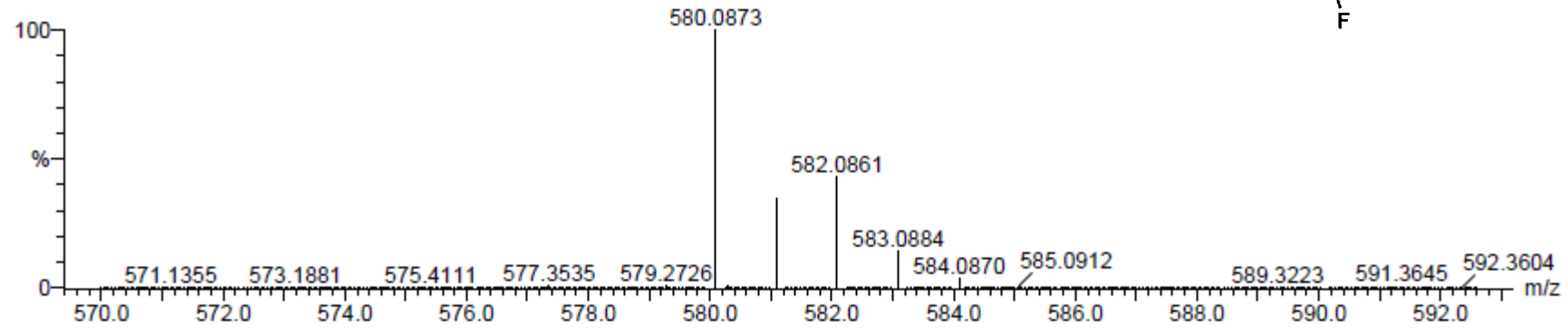


Infrared Spectrum of Compound A-7h

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 = 3

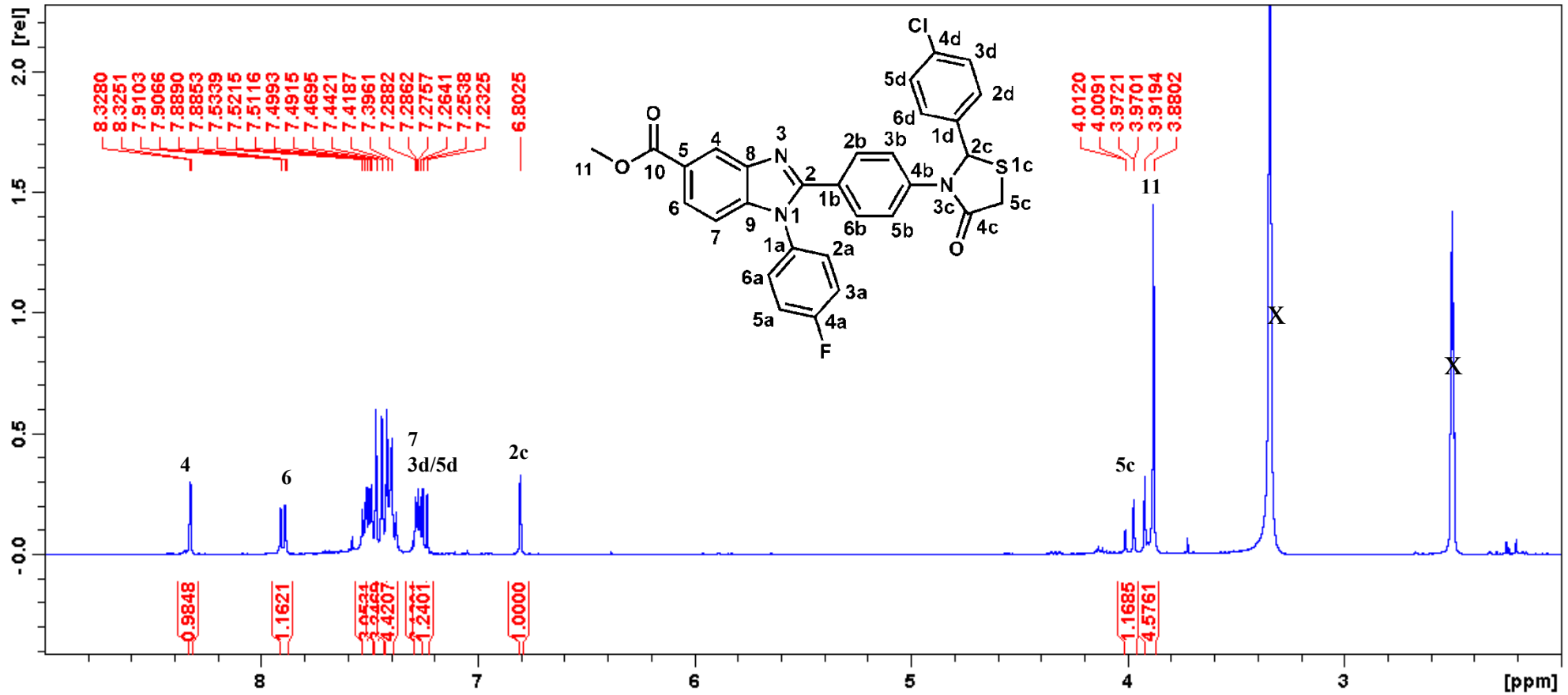
Monoisotopic Mass, Even Electron Ions
51 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 30-35 H: 20-25 N: 0-5 O: 0-5 F: 1-1 Na: 1-1 S: 0-1 Cl: 0-1
BI 8 59 (1.958) Cm (1:61)
TOF MS ES+



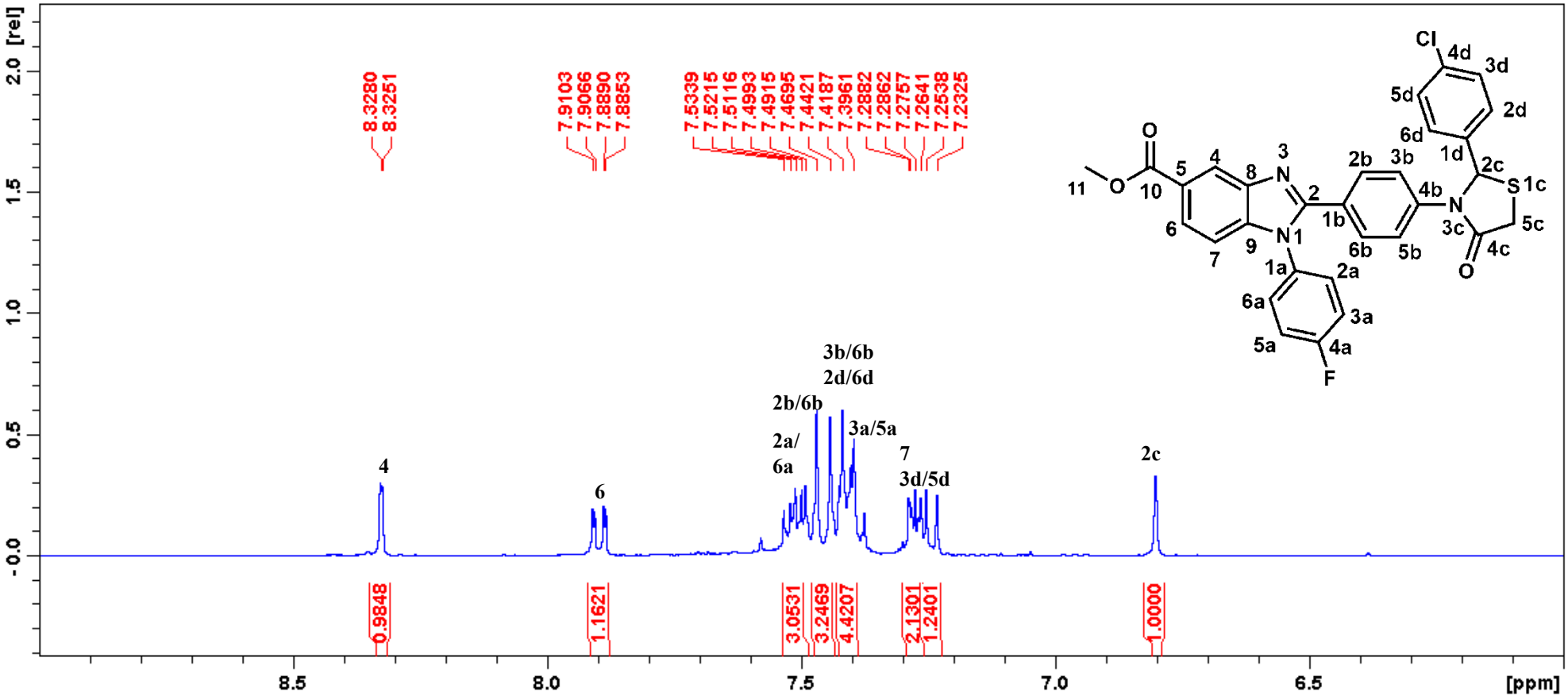


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
580.0873	580.0874	-0.1	-0.2	20.5	457.9	0.0	C30 H21 N3 O3 F Na S Cl

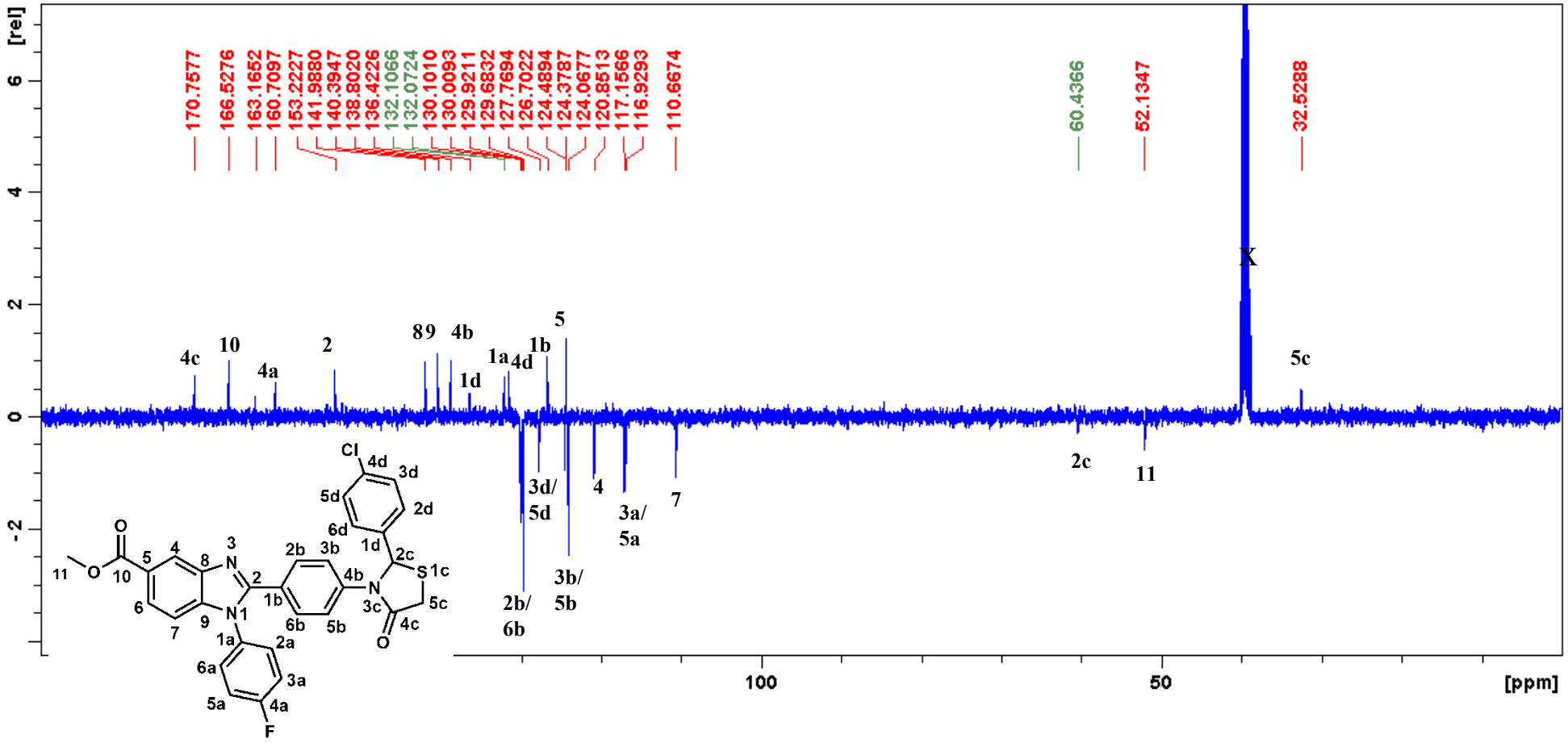
HRMS Spectrum of Compound A-7h



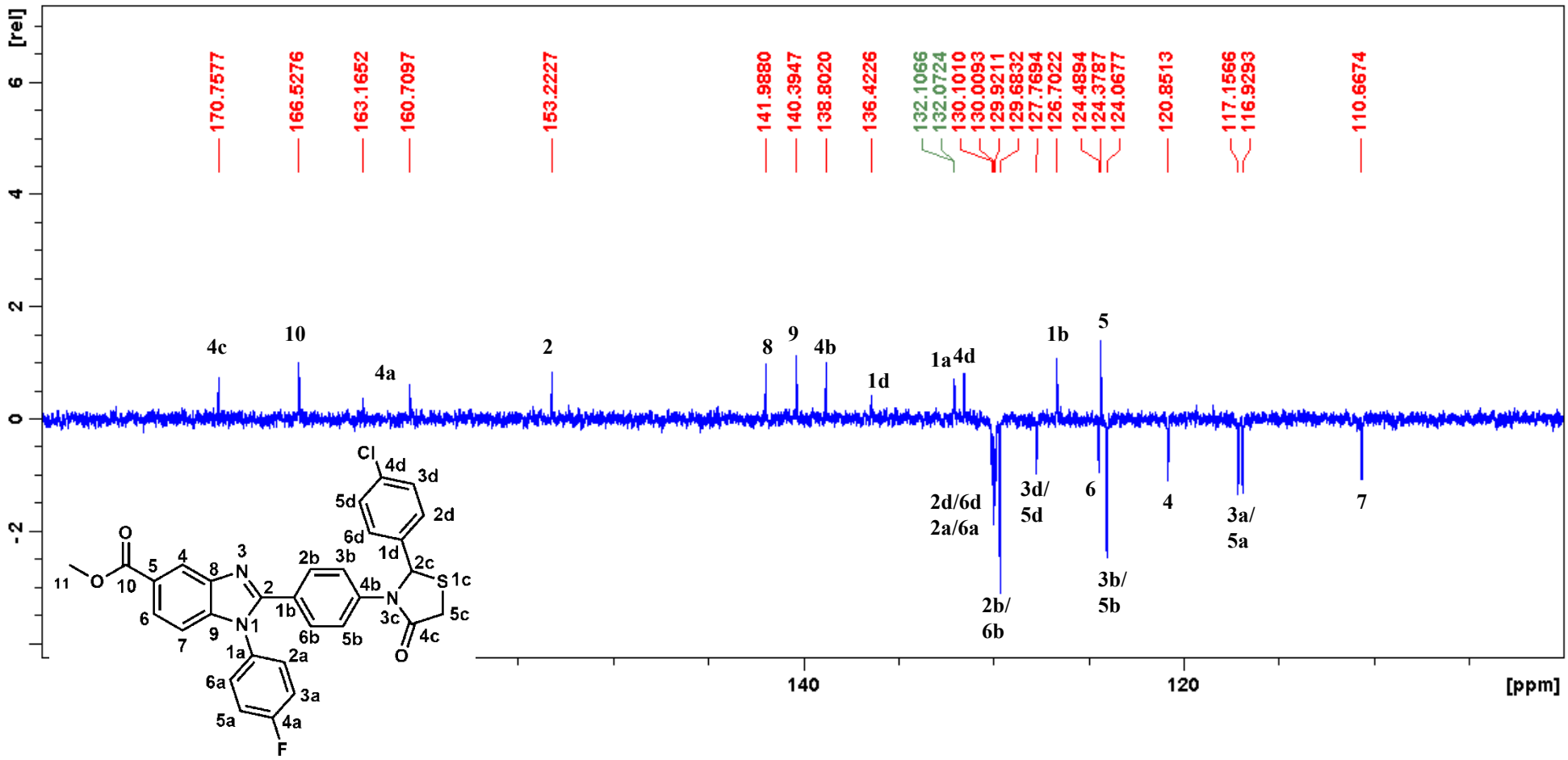
^{1}H Spectrum of Compound A-7i



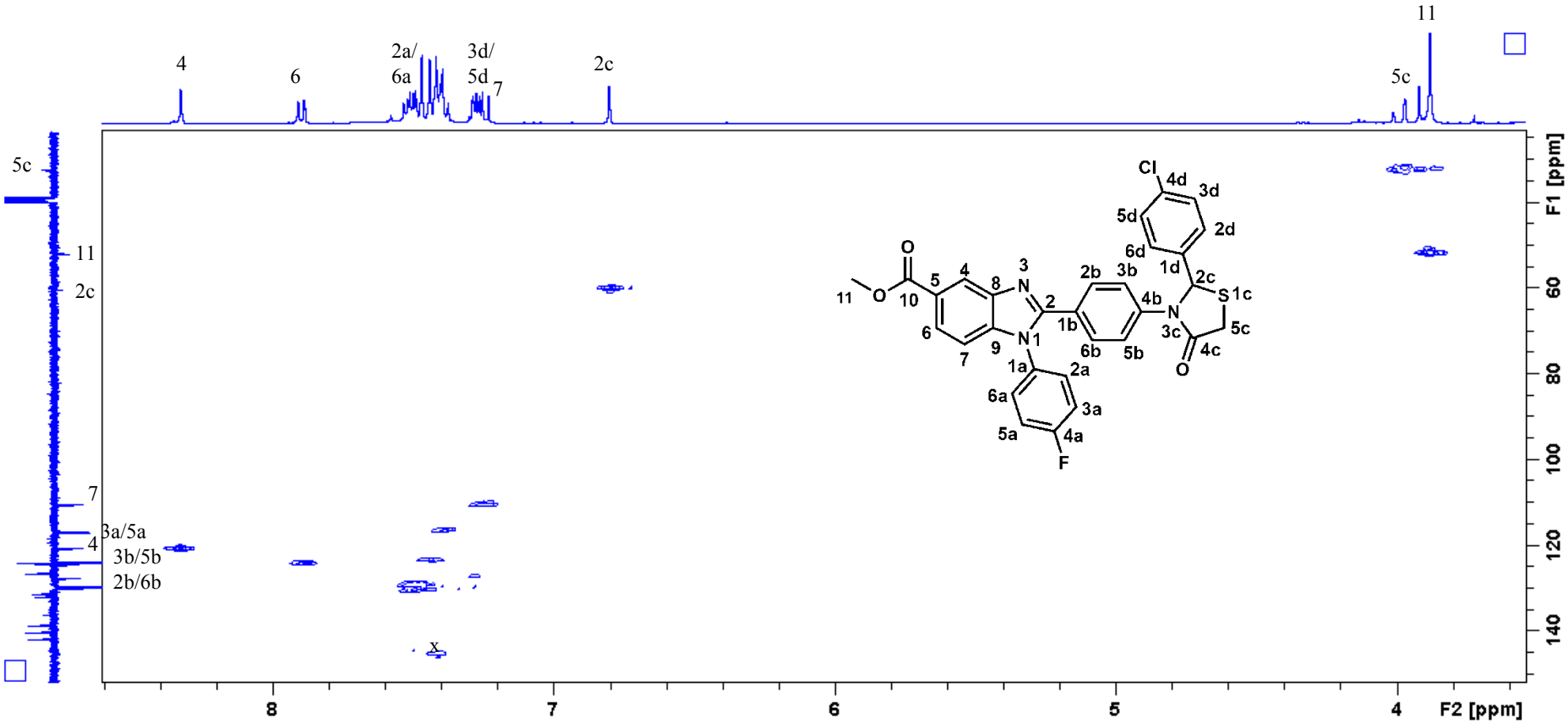
Expanded ^{1}H Spectrum of Compound A-7i



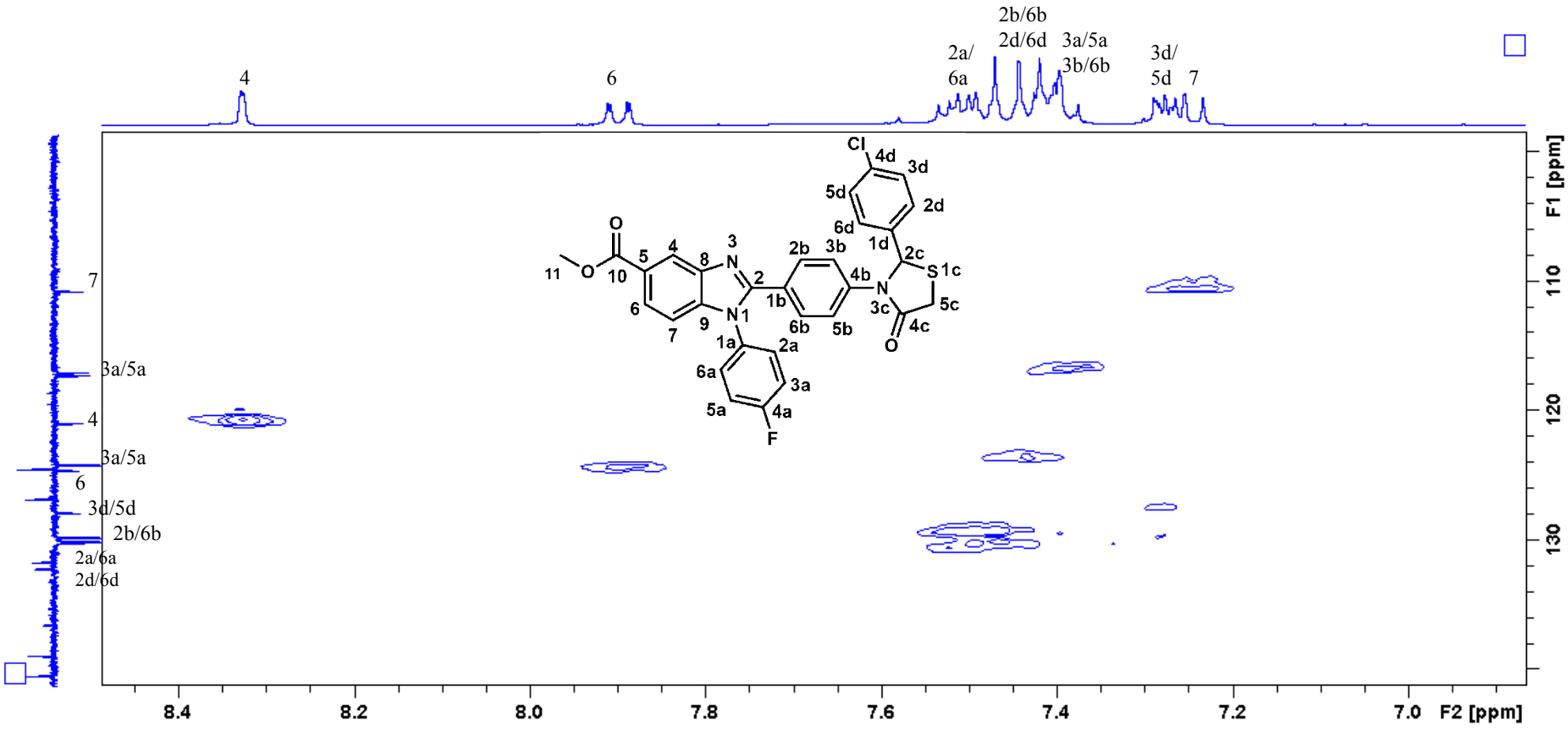
^{13}C Spectrum of Compound A-7i



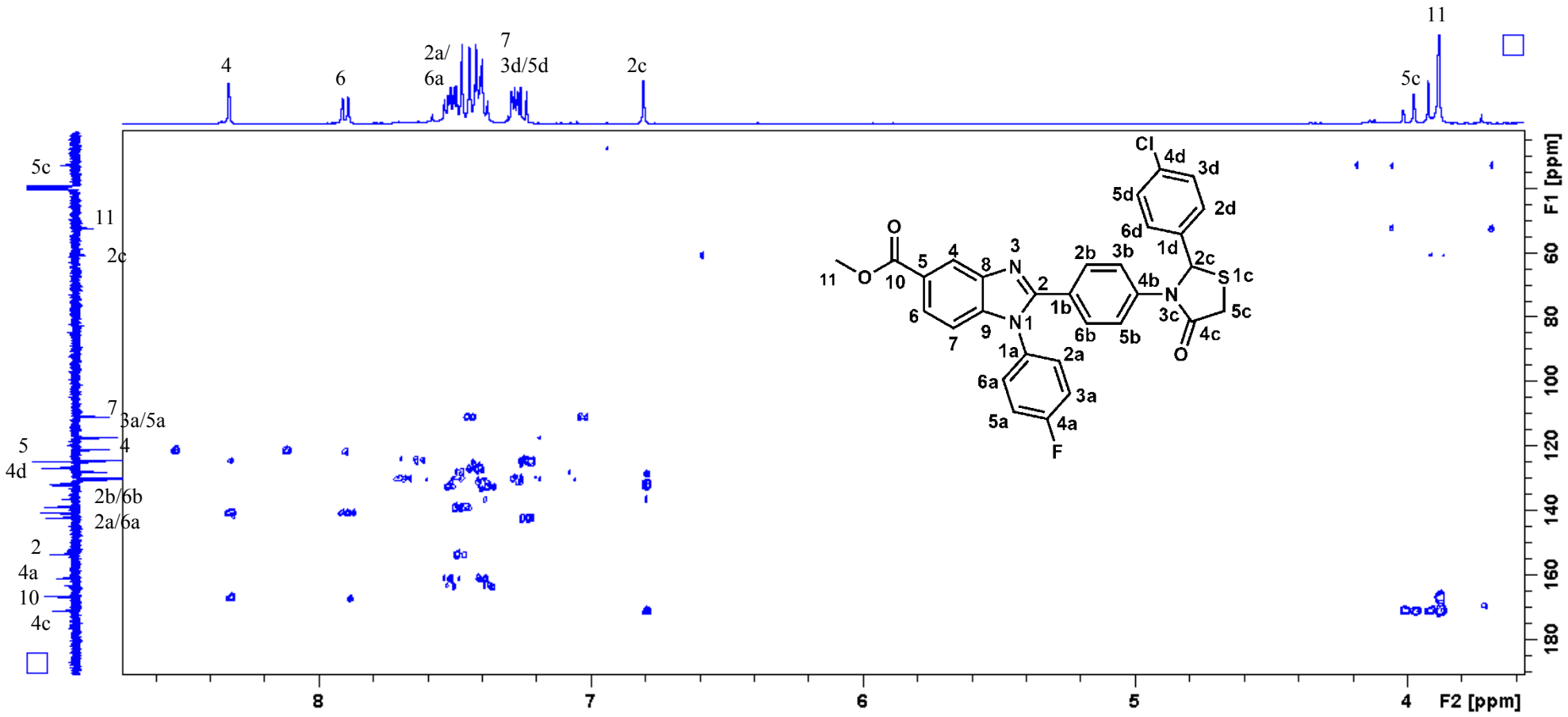
Expanded ^{13}C Spectrum of Compound A-7i



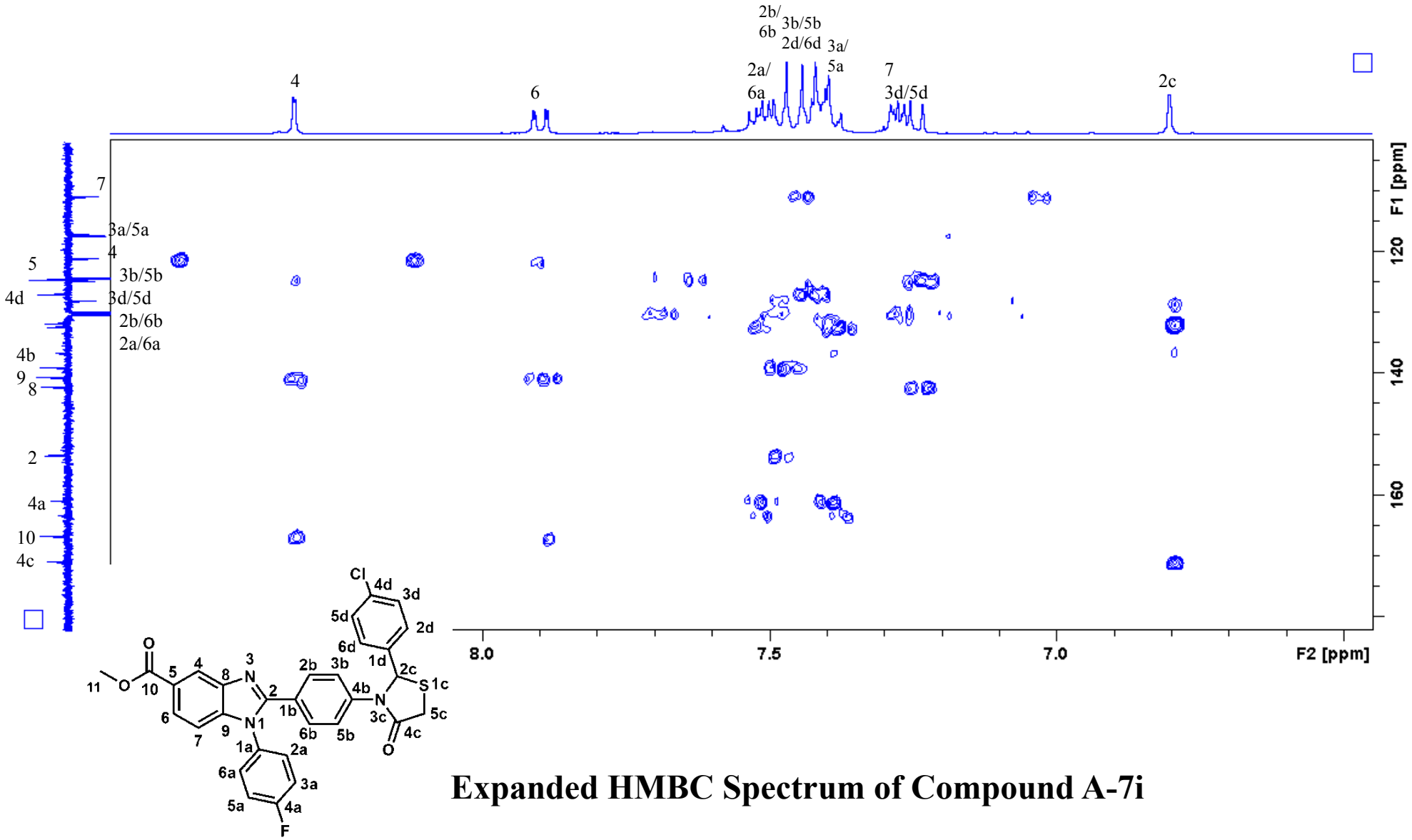
HSQC Spectrum of Compound A-7i



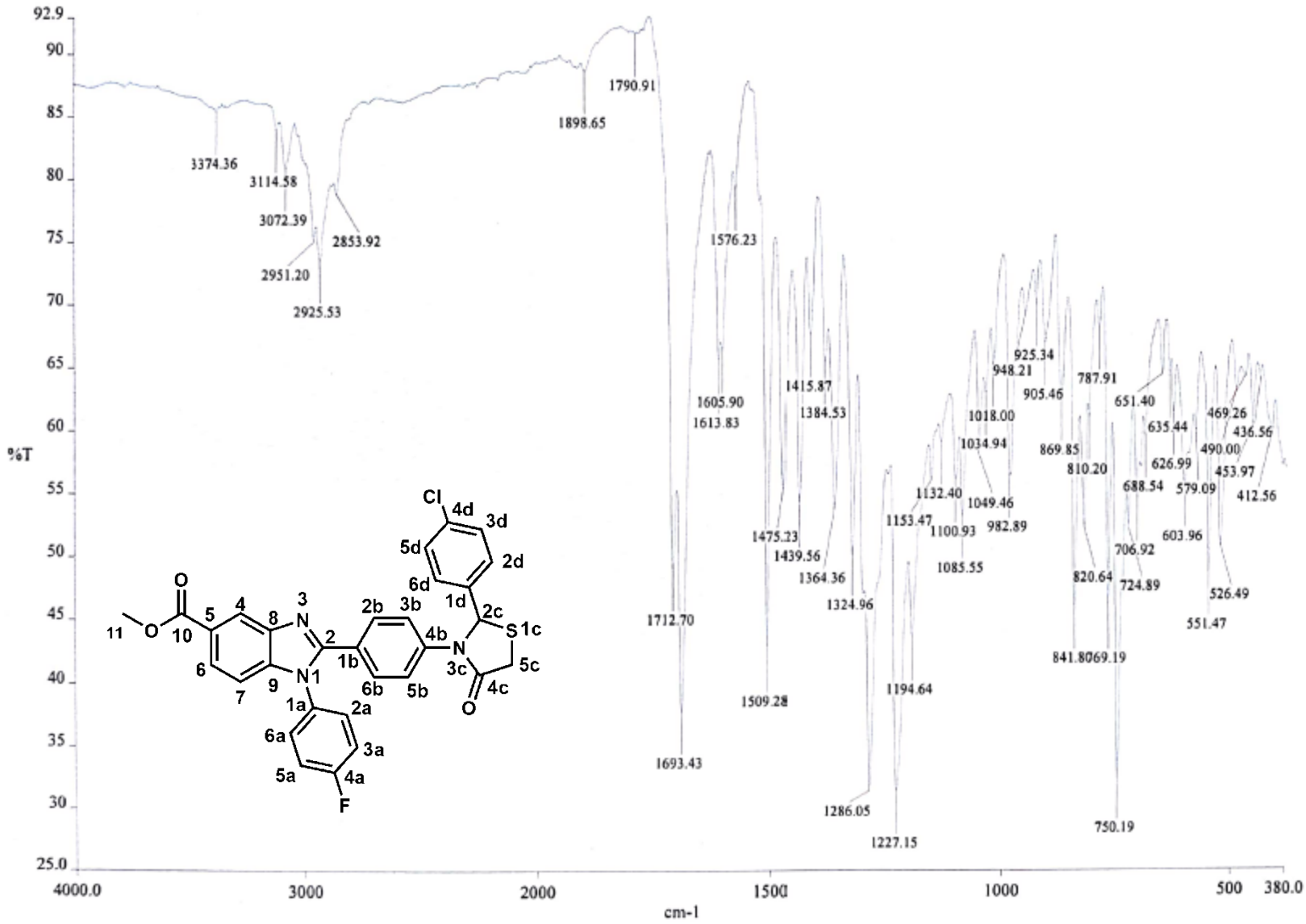
Expanded HSQC Spectrum of Compound A-7i



HMBC Spectrum of Compound A-7i



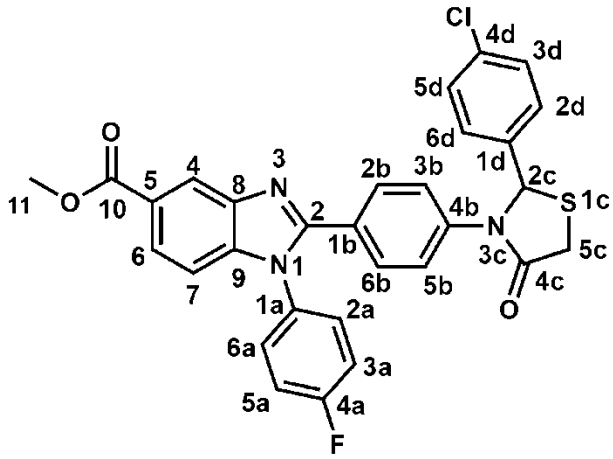
Expanded HMBC Spectrum of Compound A-7i

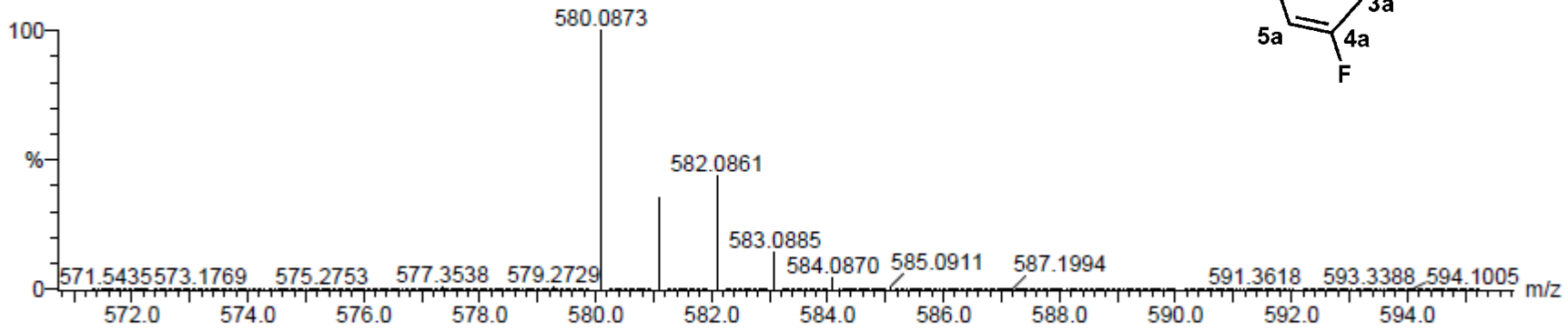


Infrared Spectrum of Compound A-7i

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 = 3

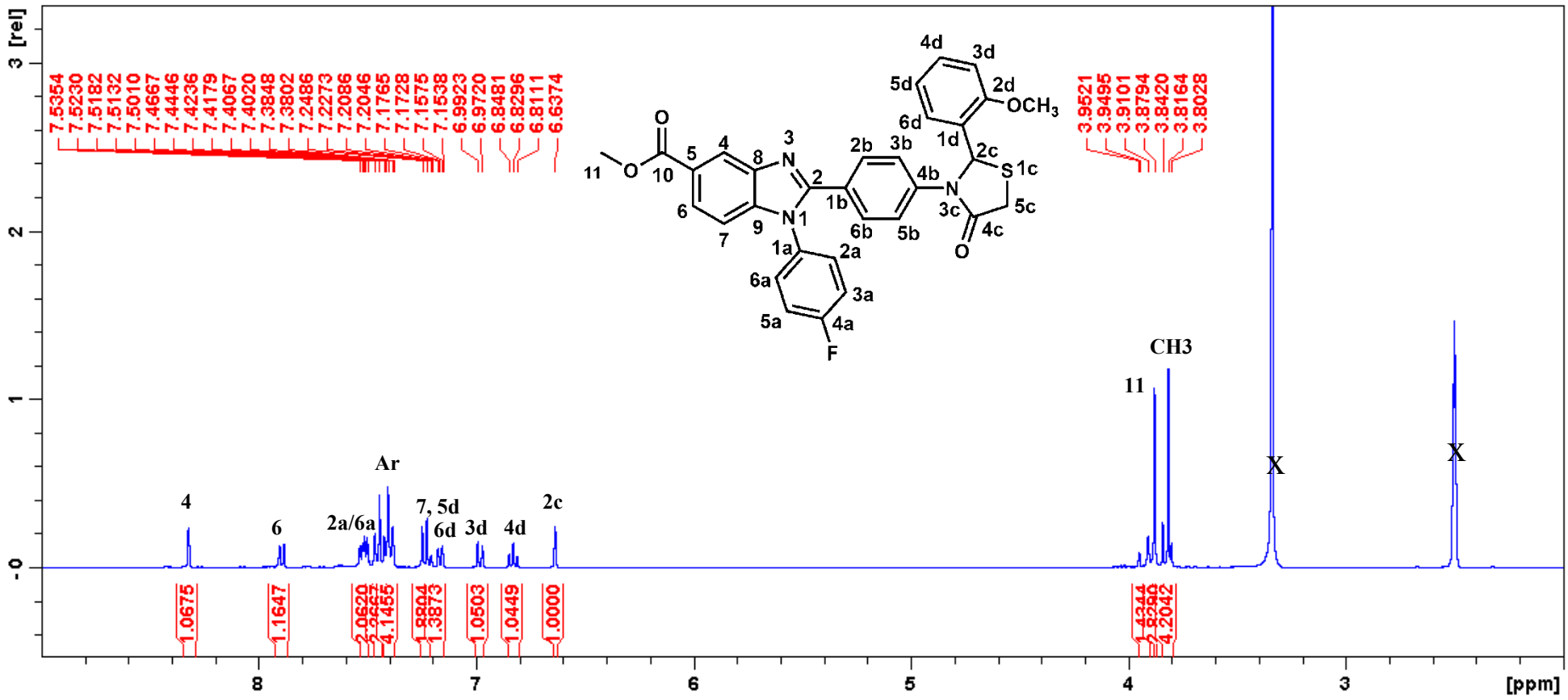
Monoisotopic Mass, Even Electron Ions
51 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 30-35 H: 20-25 N: 0-5 O: 0-5 F: 1-1 Na: 1-1 S: 0-1 Cl: 0-1
BI 9 6 (0.169) Cm (1:61)
TOF MS ES+



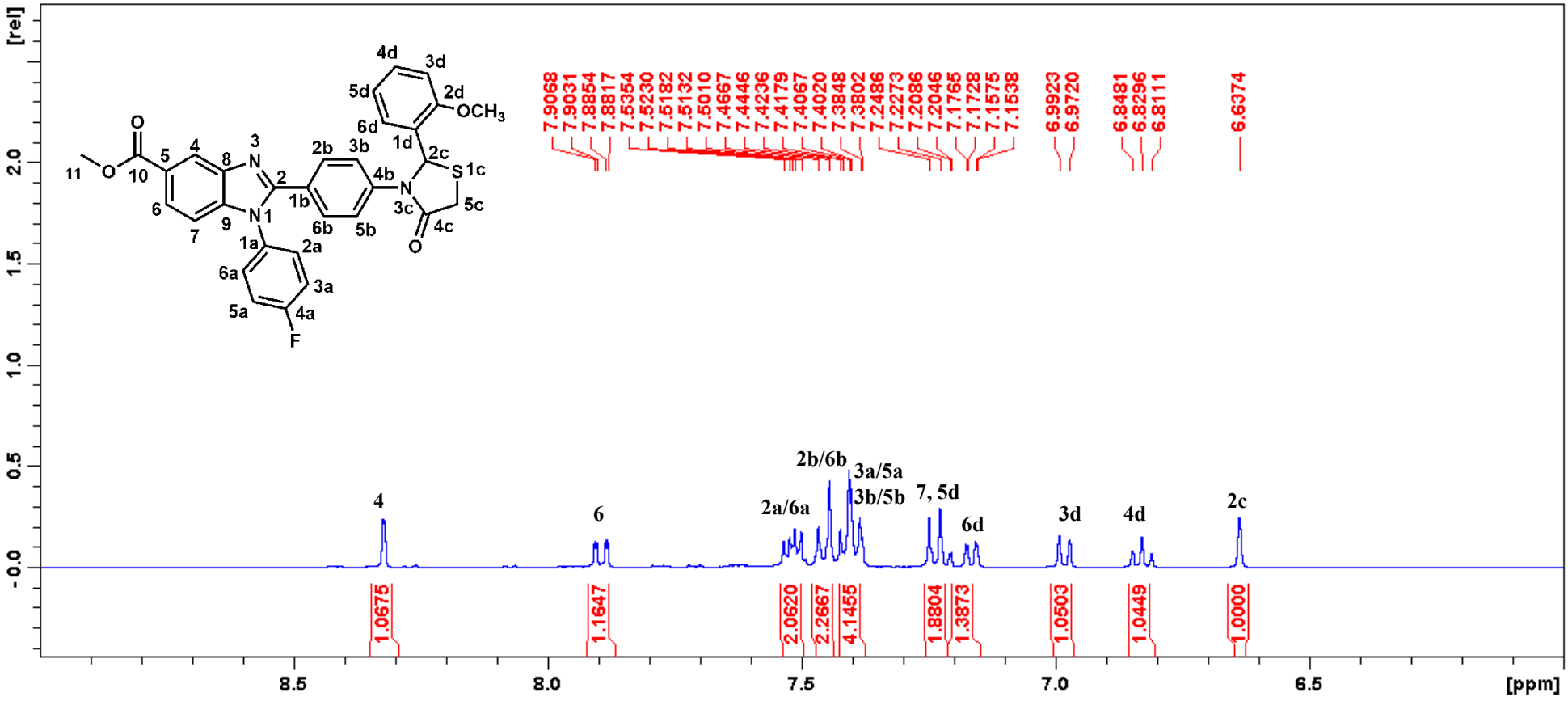


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
580.0873	580.0874	-0.1	-0.2	20.5	483.1	0.0	C30 H21 N3 O3 F Na S Cl

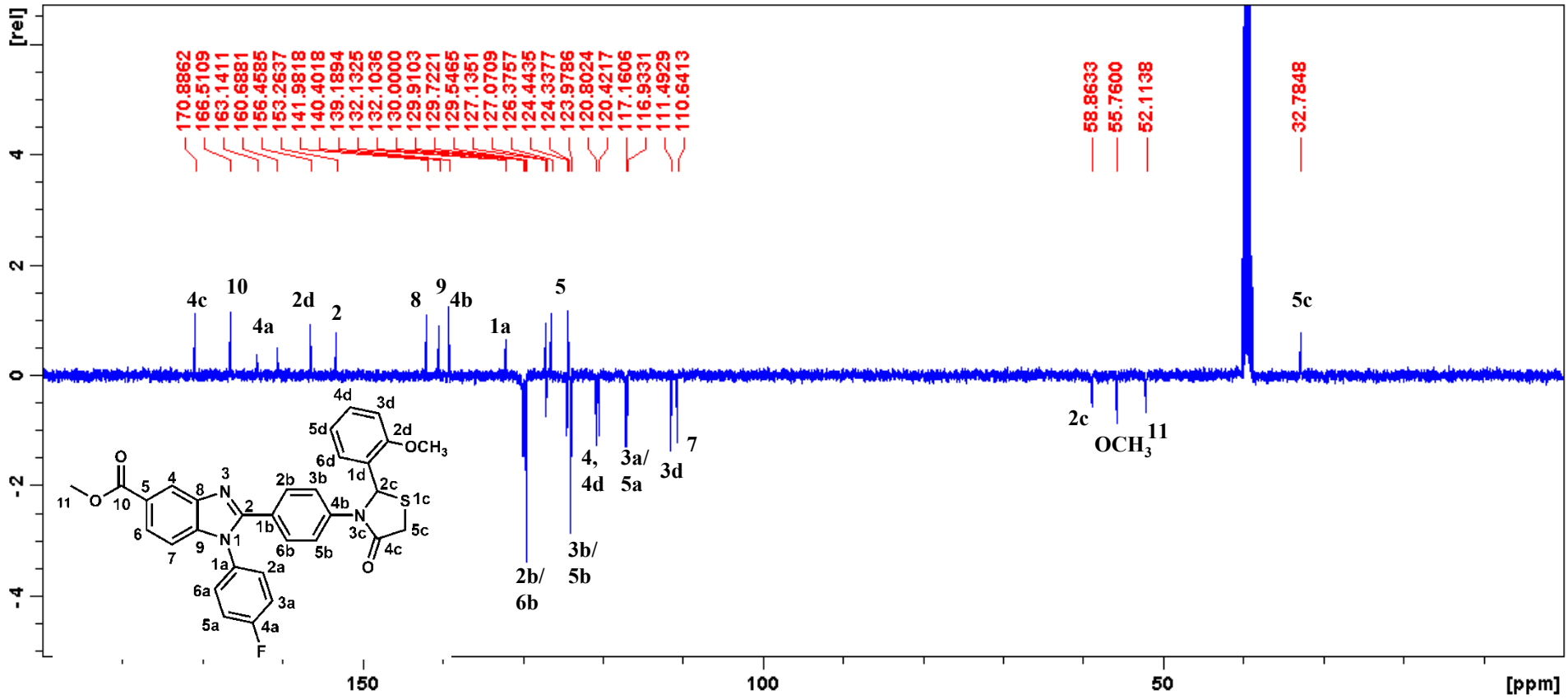
HRMS Spectrum of Compound A-7i



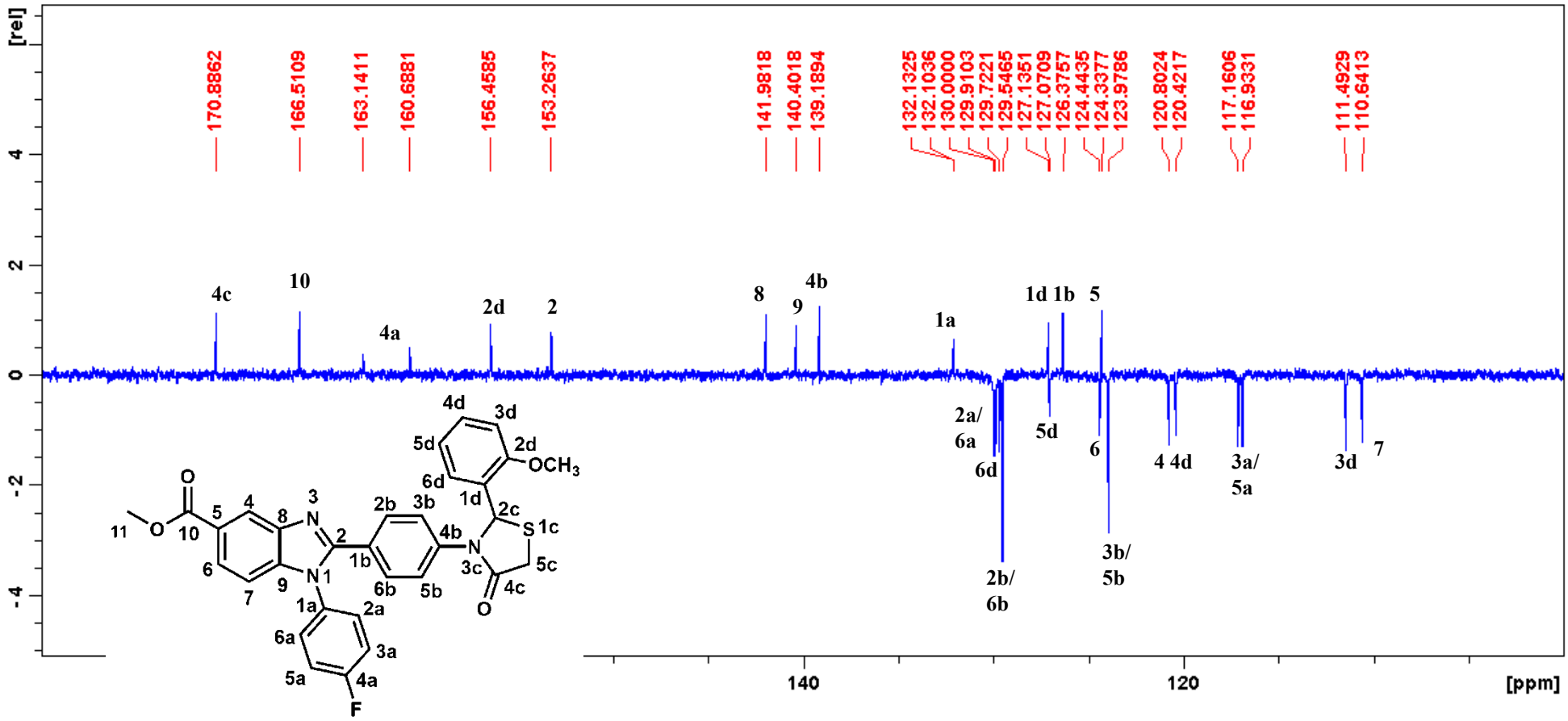
^{1}H Spectrum of Compound A-7j



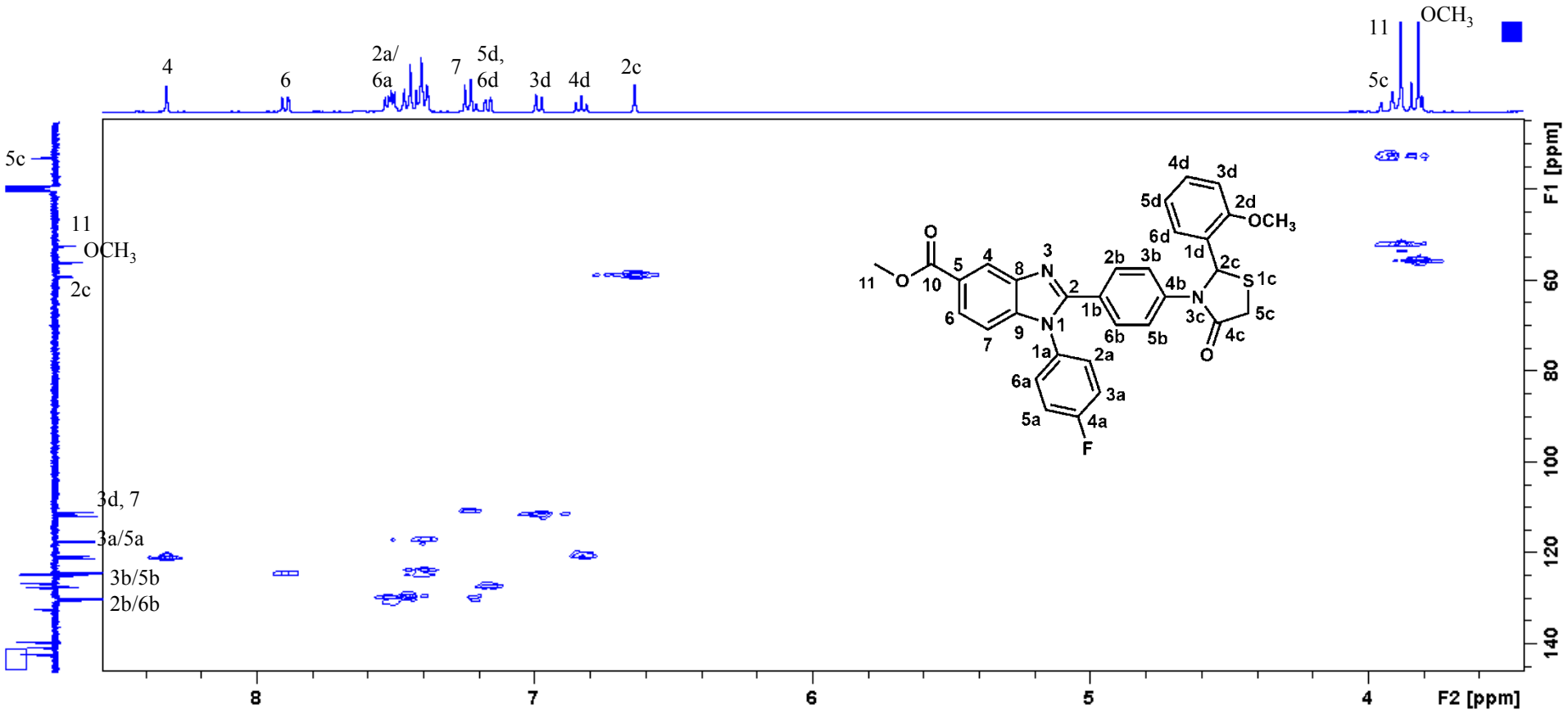
Expanded ^{1}H Spectrum of Compound A-7j



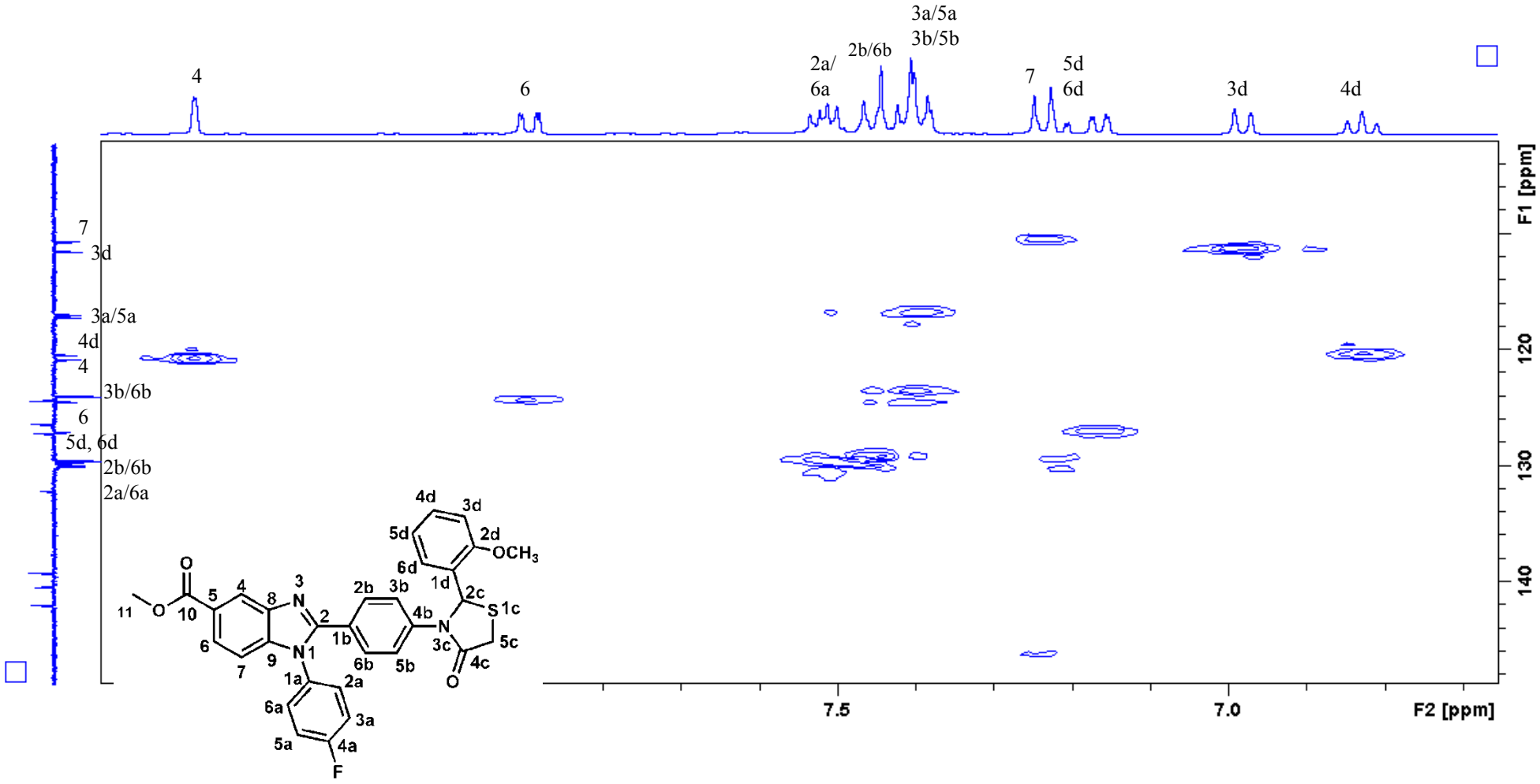
^{13}C Spectrum of Compound A-7j



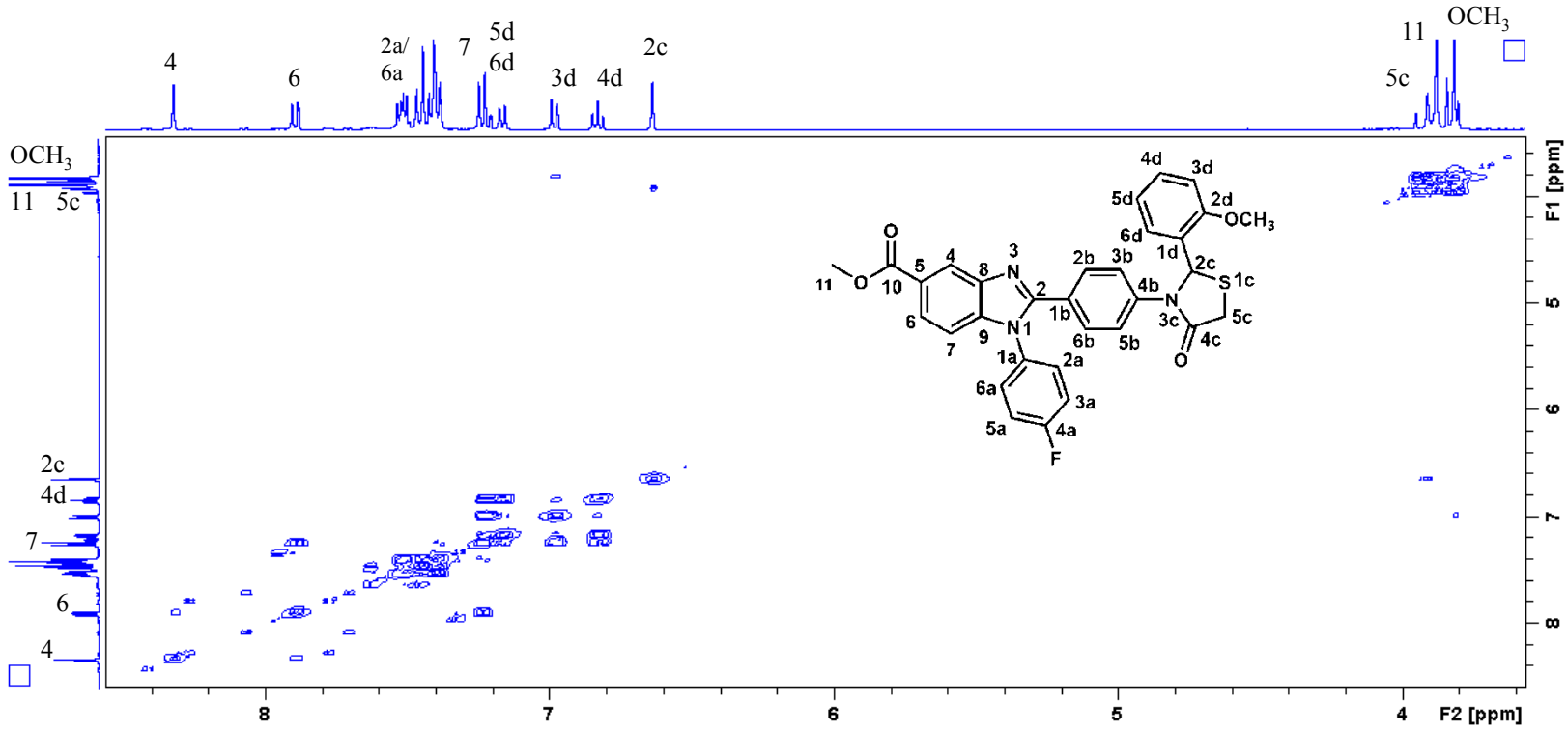
Expanded ^{13}C Spectrum of Compound A-7j



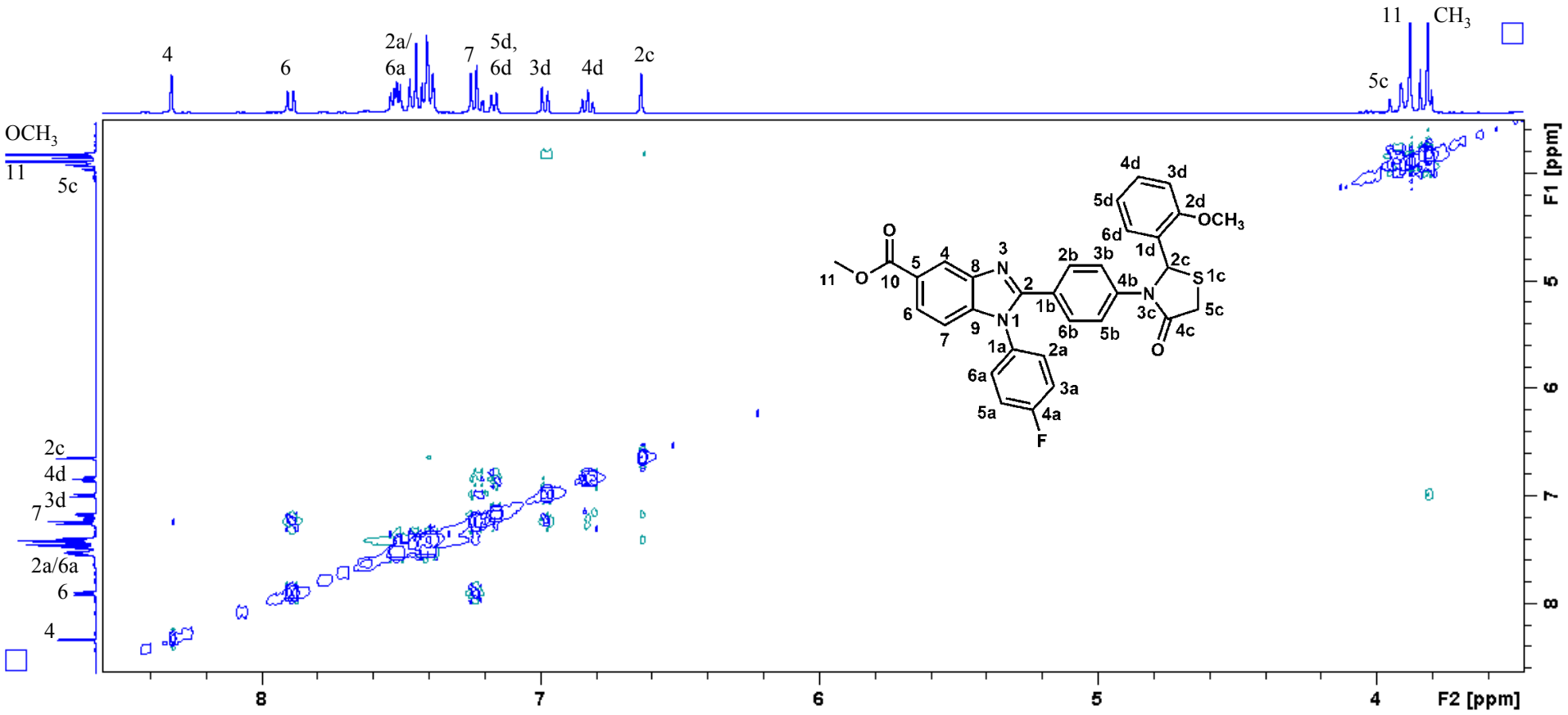
HSQC Spectrum of Compound A-7j



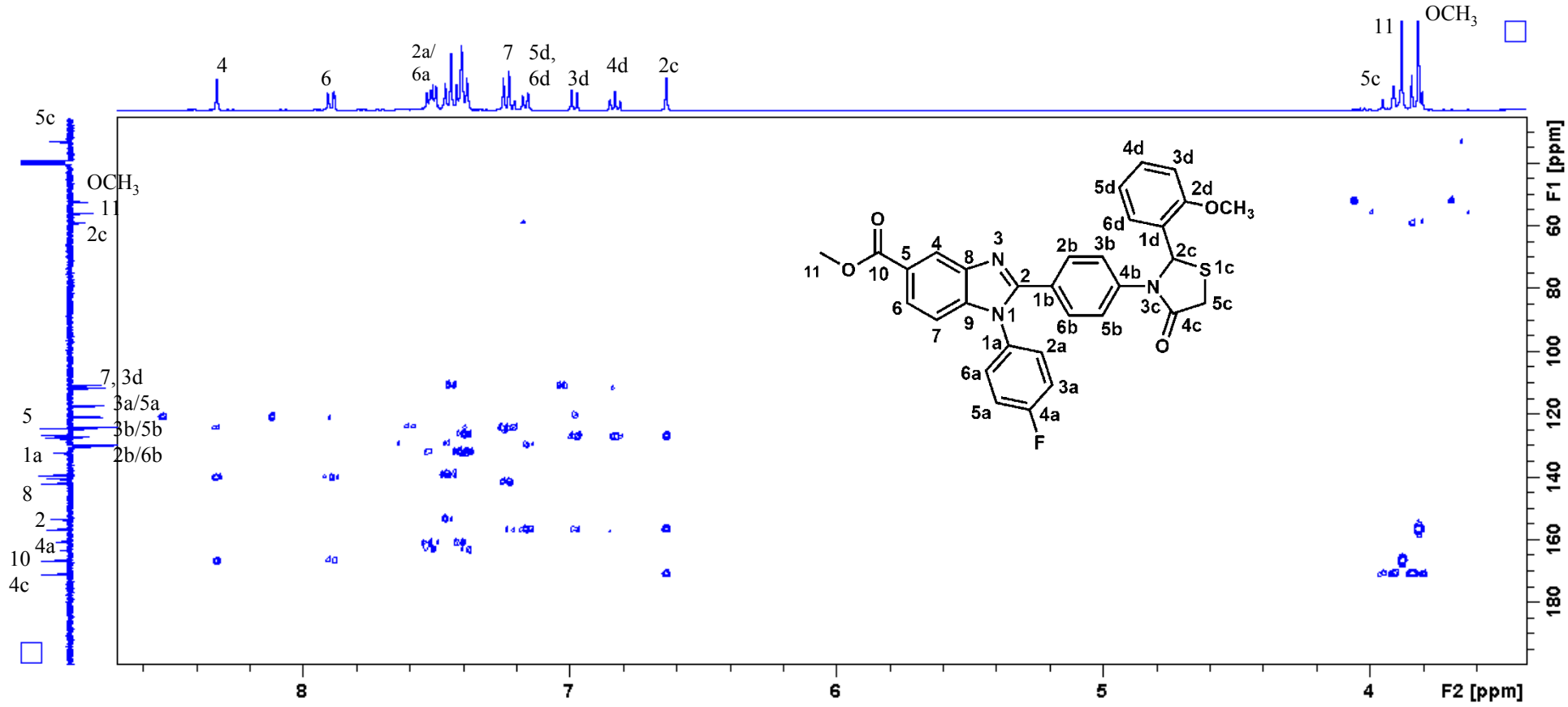
Expanded HSQC Spectrum of Compound A-7j



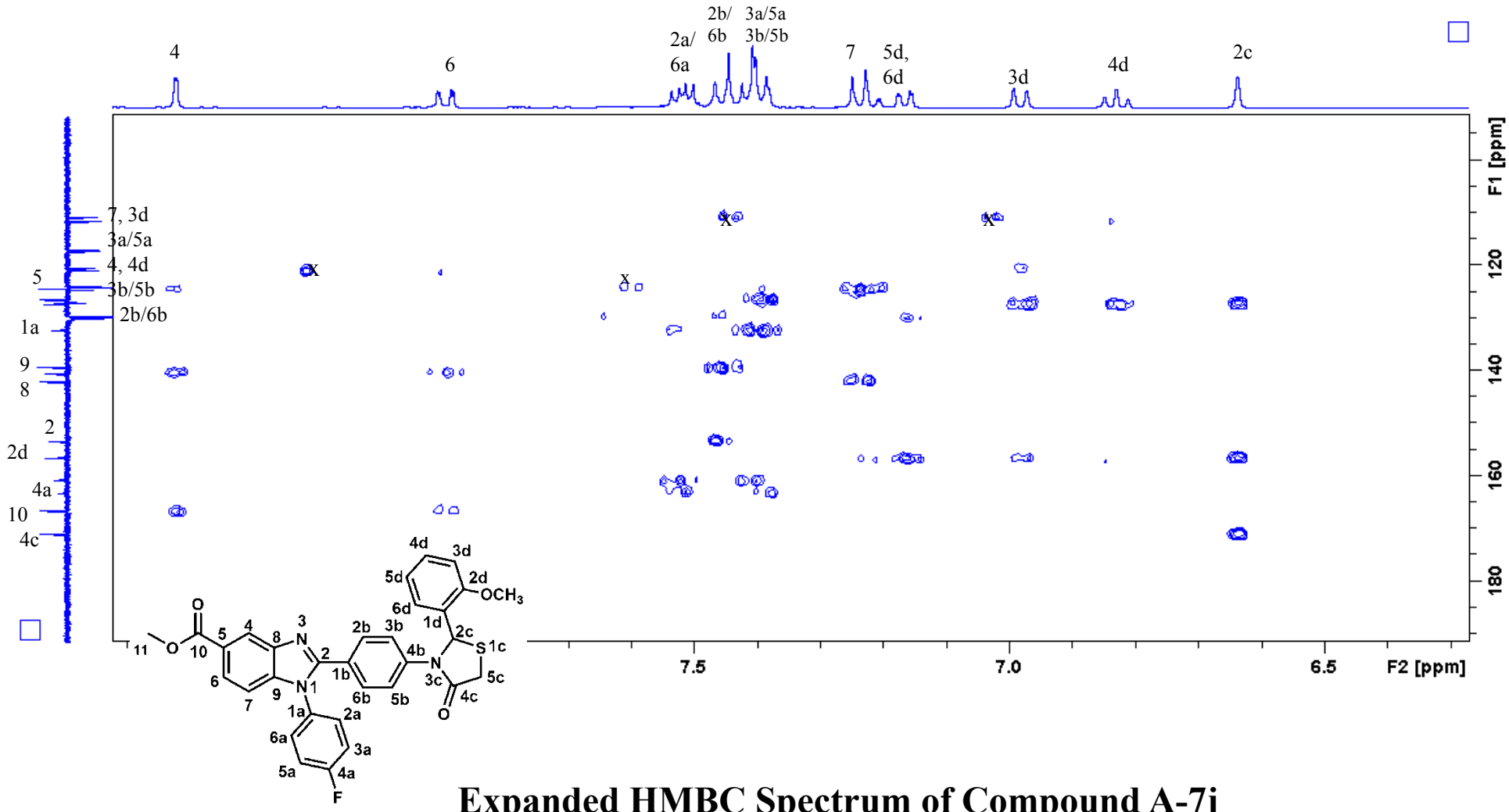
COSY Spectrum of Compound A-7j



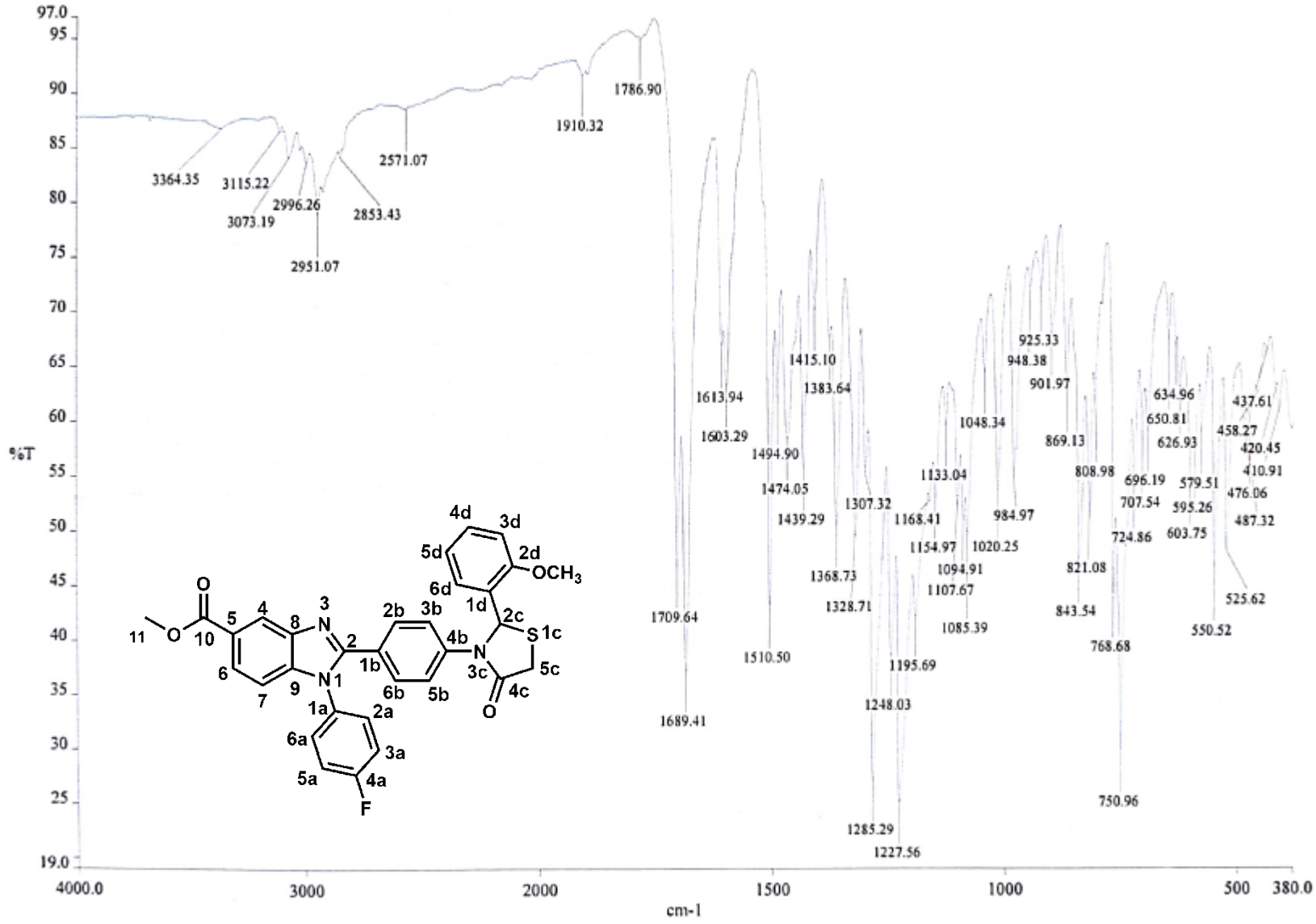
NOESY Spectrum of Compound A-7j



HMBC Spectrum of Compound A-7j



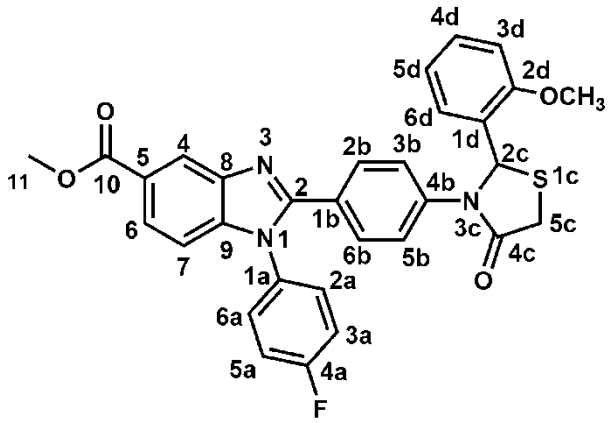
Expanded HMBC Spectrum of Compound A-7j

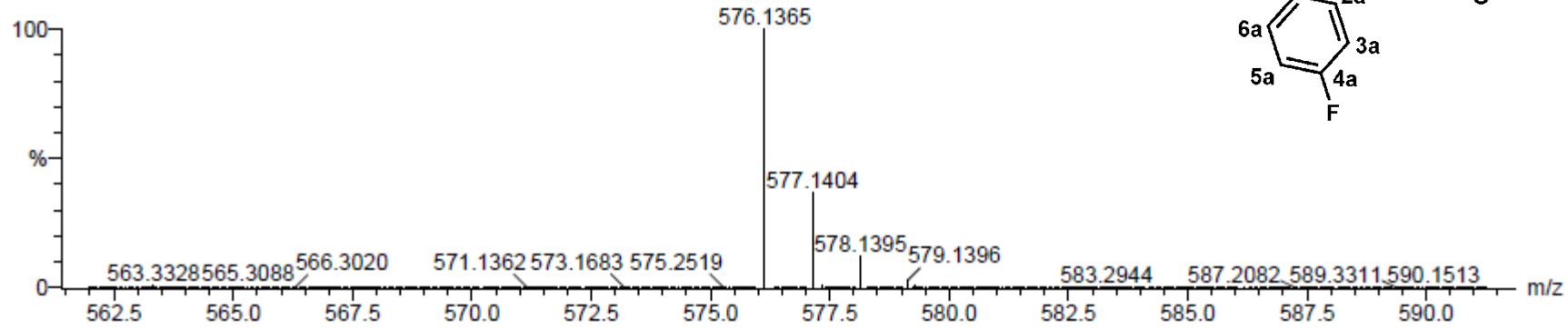


Infrared Spectrum of Compound A-7j

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 = 3

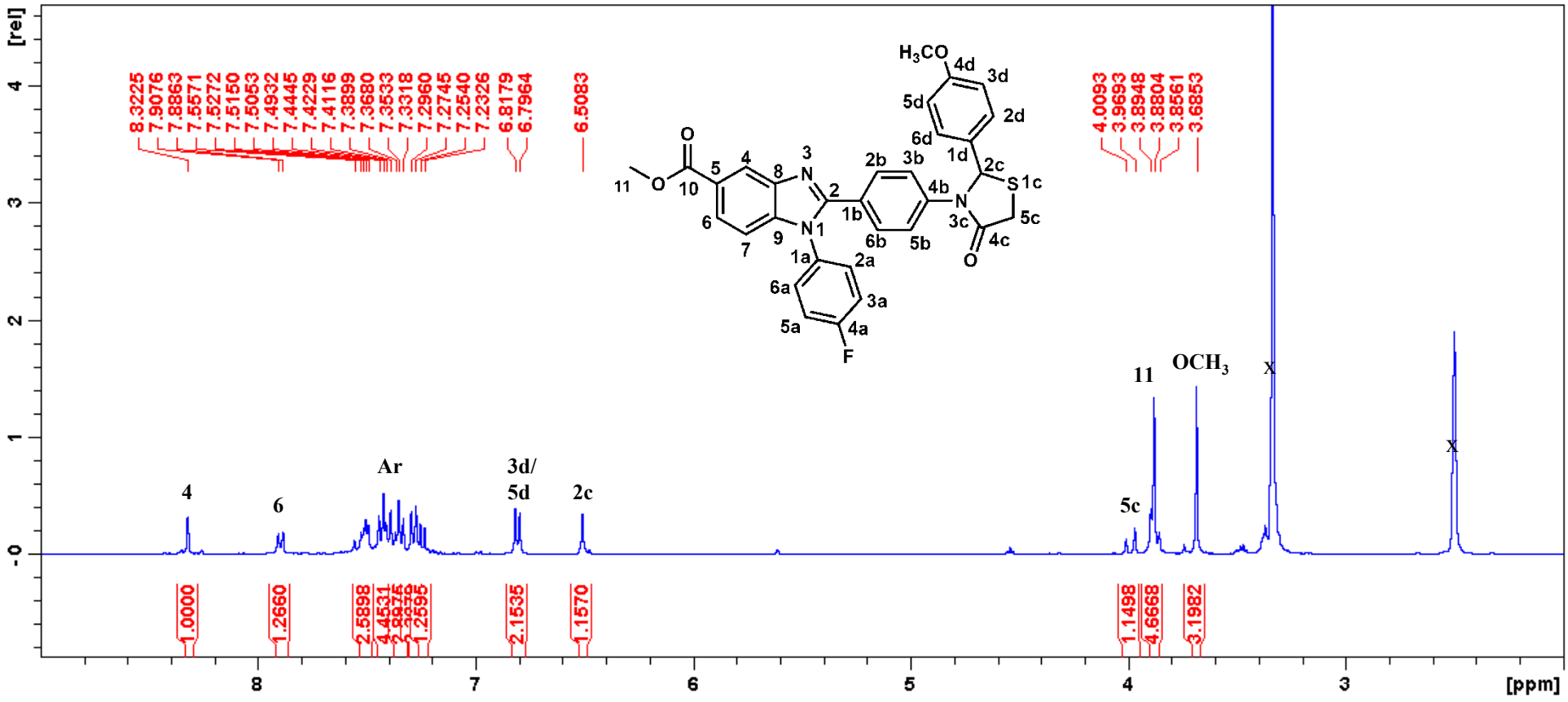
Monoisotopic Mass, Even Electron Ions
25 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 30-35 H: 20-25 N: 0-5 O: 0-5 F: 1-1 Na: 1-1 S: 0-1
BI 11 3 (0.068) Cm (1:61)
TOF MS ES+



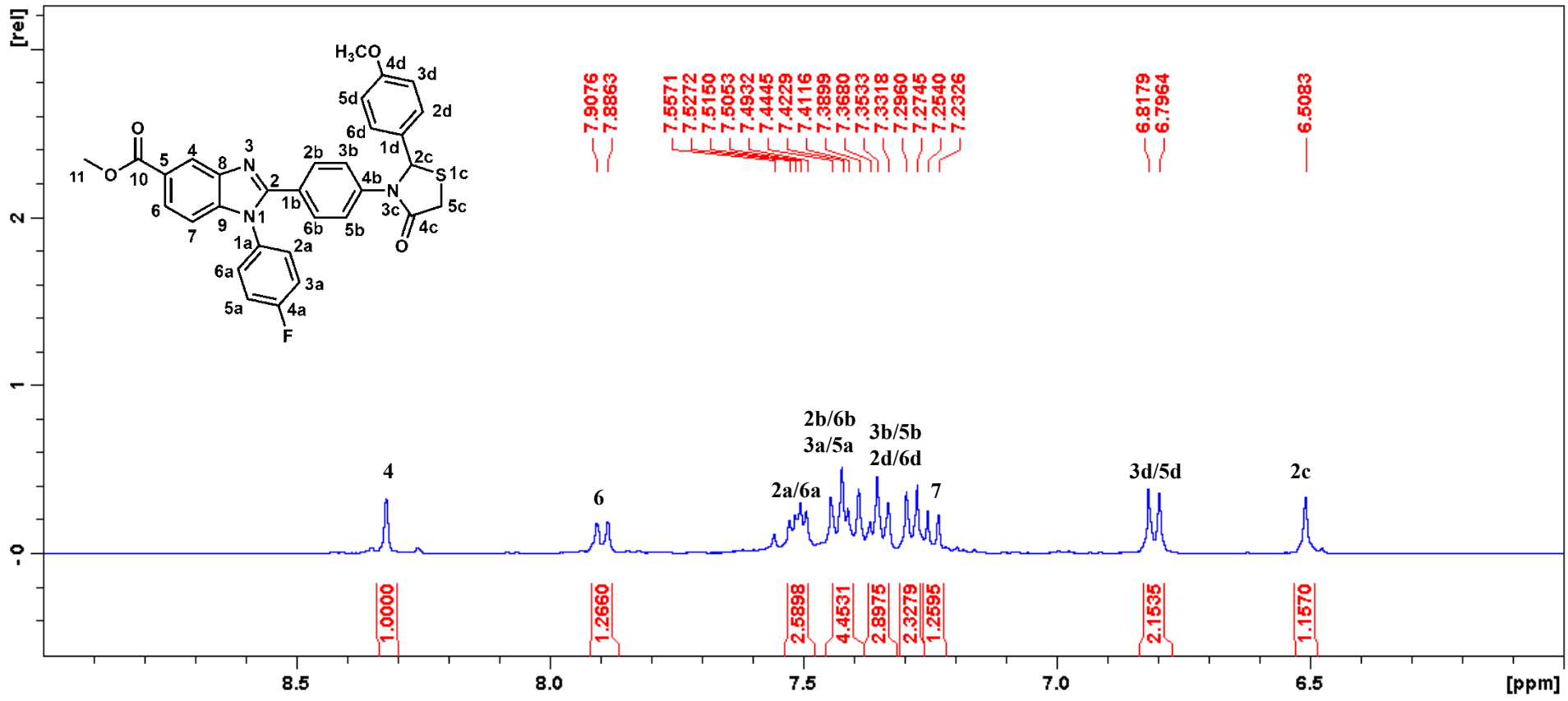


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
576.1365	576.1369	-0.4	-0.7	20.5	520.1	0.0	C31 H24 N3 O4 F Na S

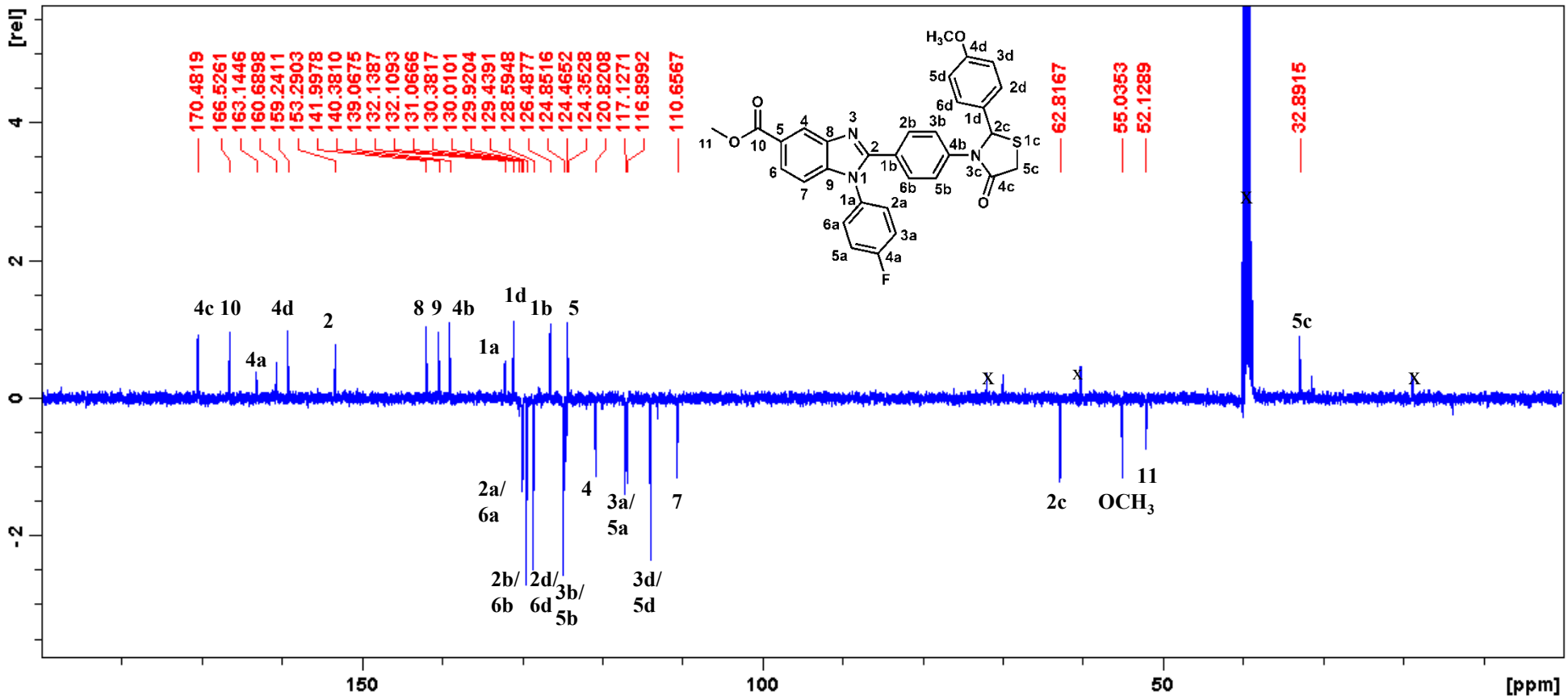
HRMS Spectrum of Compound A-7j



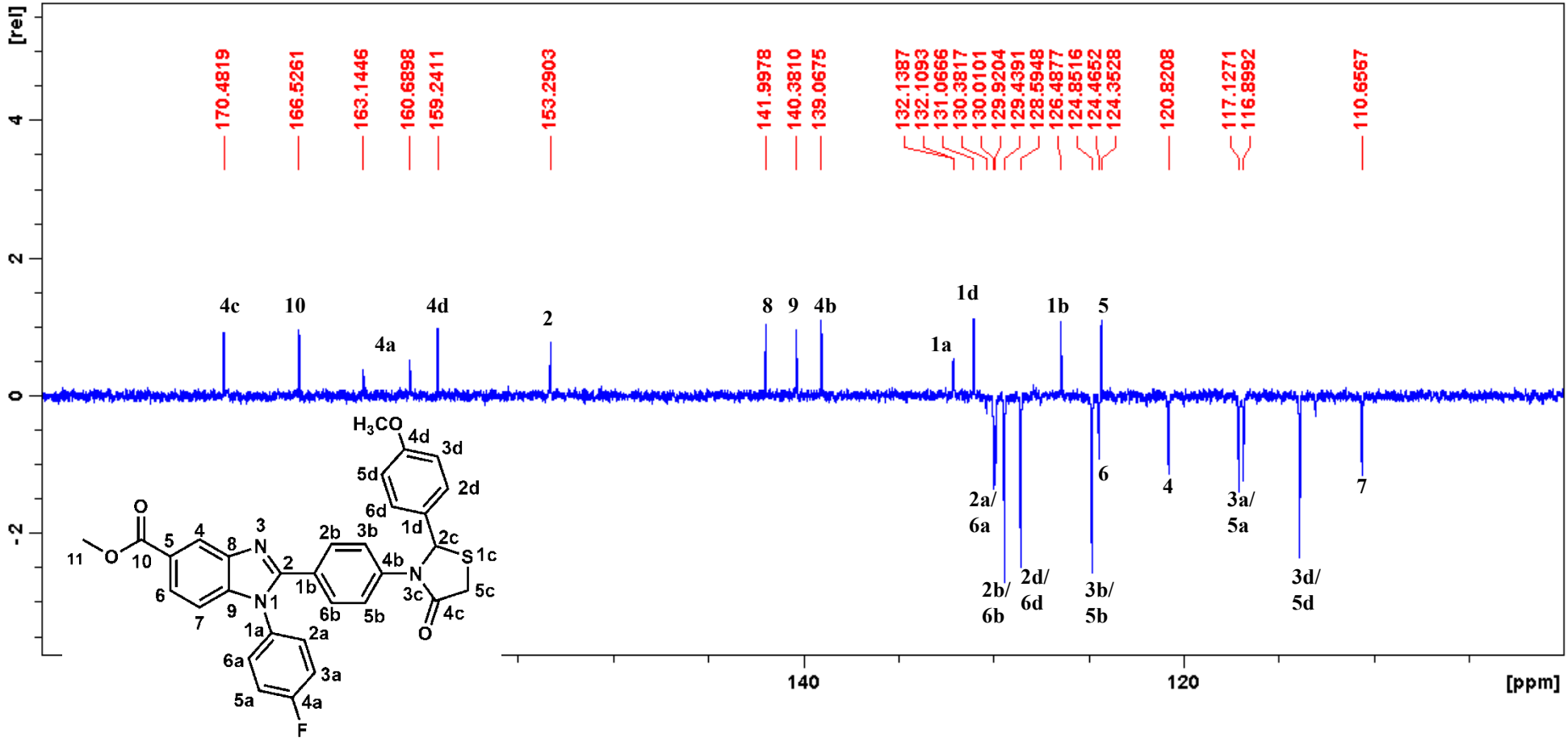
^{1}H Spectrum of Compound A-7k



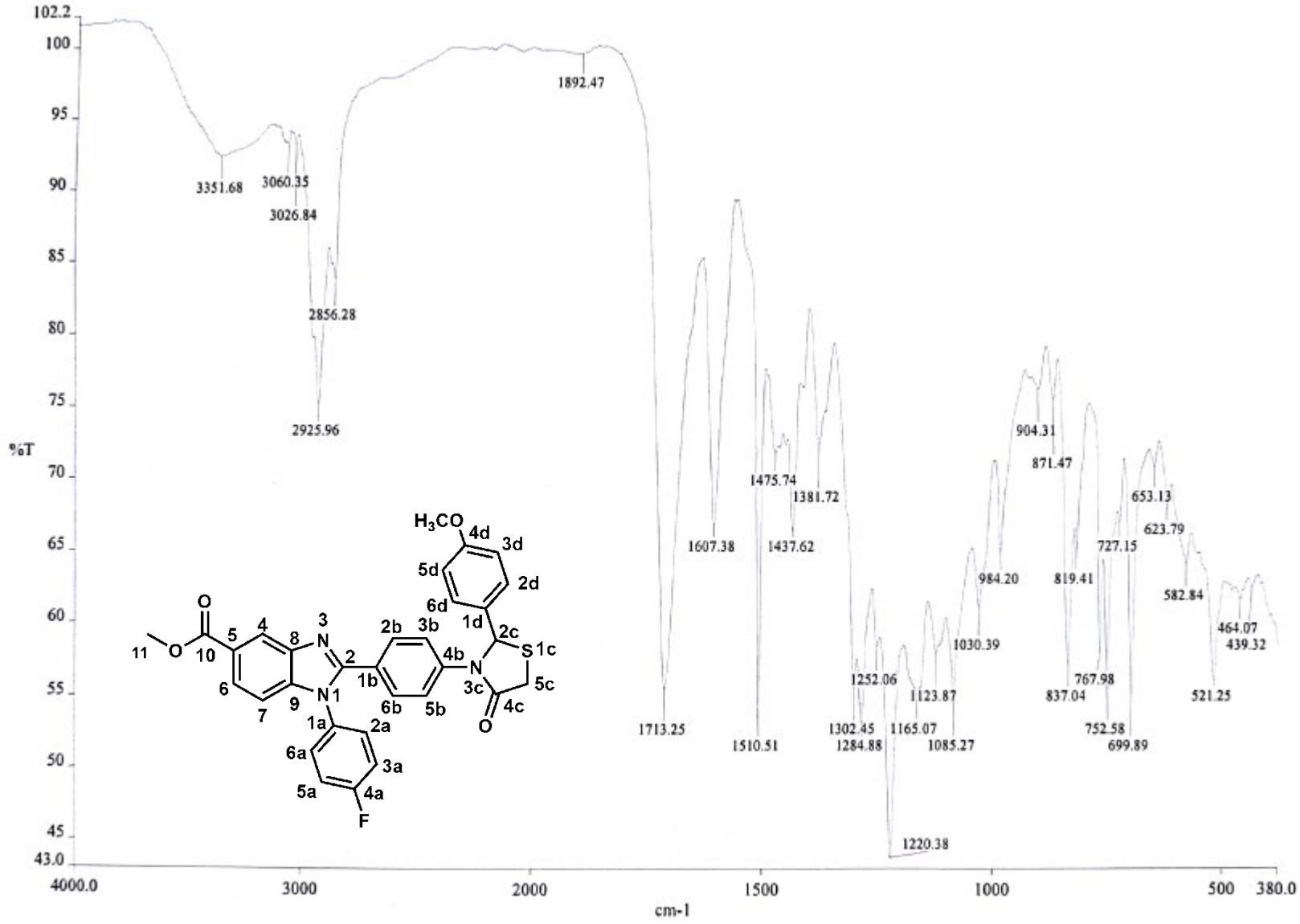
Expanded ^{1}H Spectrum of Compound A-7k



^{13}C Spectrum of Compound A-7k



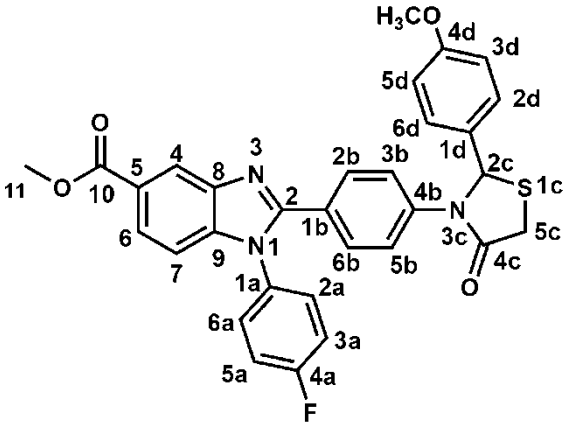
^{13}C Spectrum of Compound A-7k

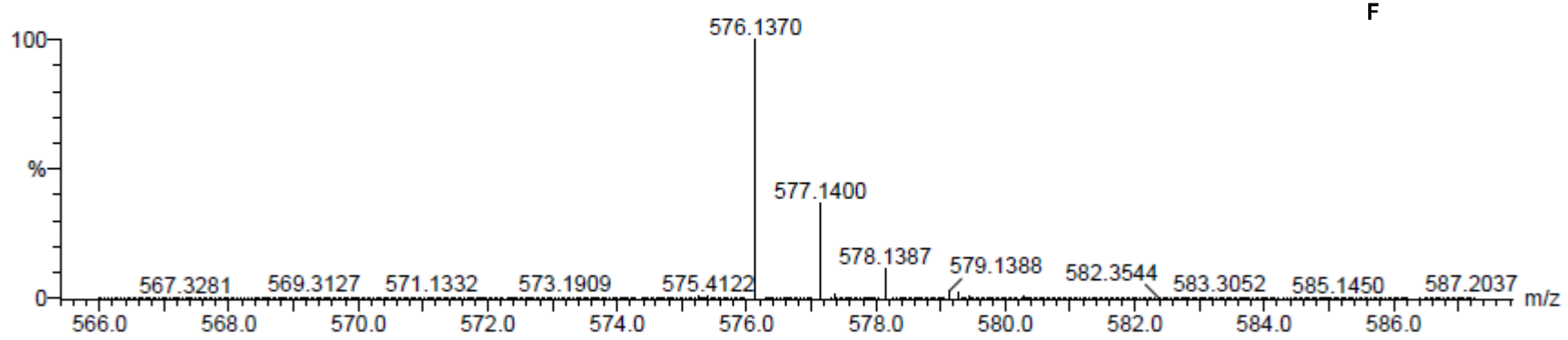


Infrared Spectrum of Compound A-7k

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 = 3

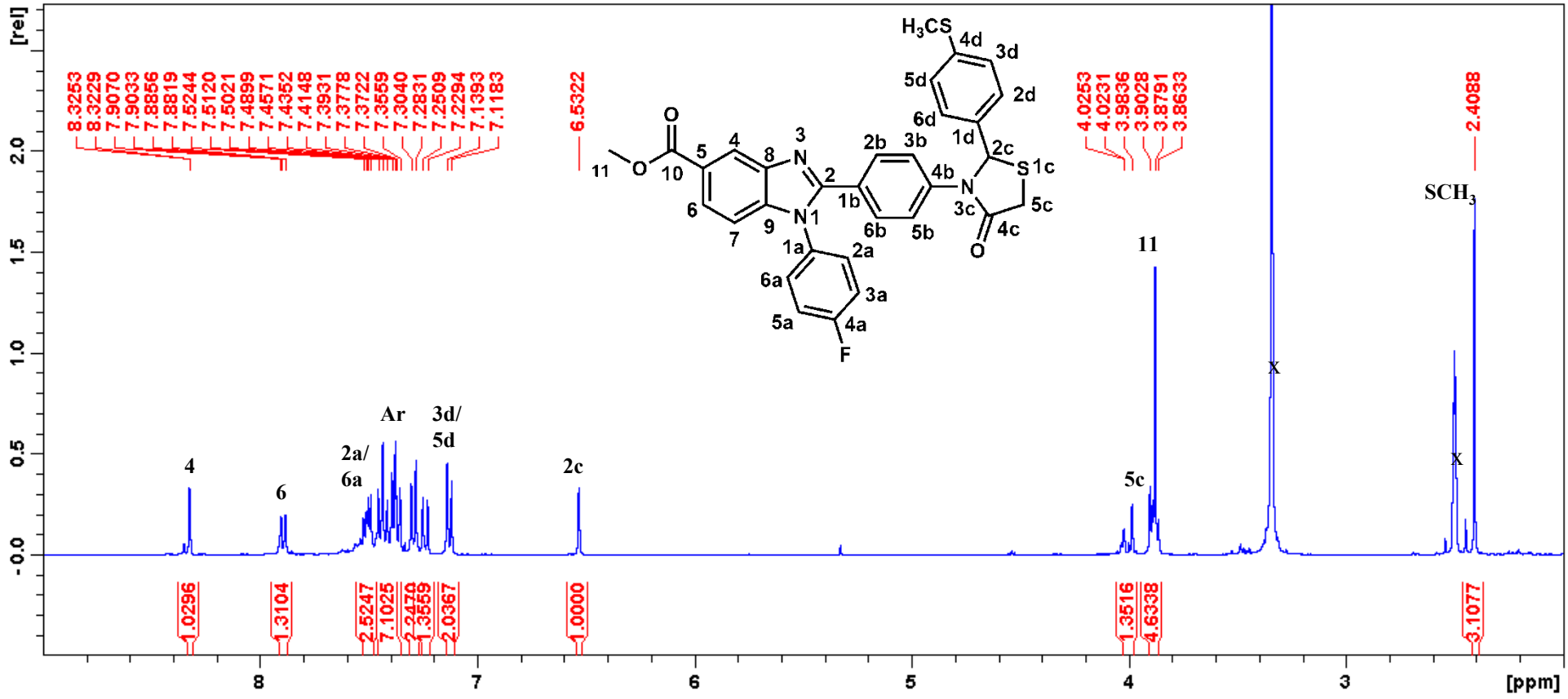
Monoisotopic Mass, Even Electron Ions
25 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 30-35 H: 20-25 N: 0-5 O: 0-5 F: 1-1 Na: 1-1 S: 0-1
BI 10 43 (1.419) Cm (1:61)
TOF MS ES+



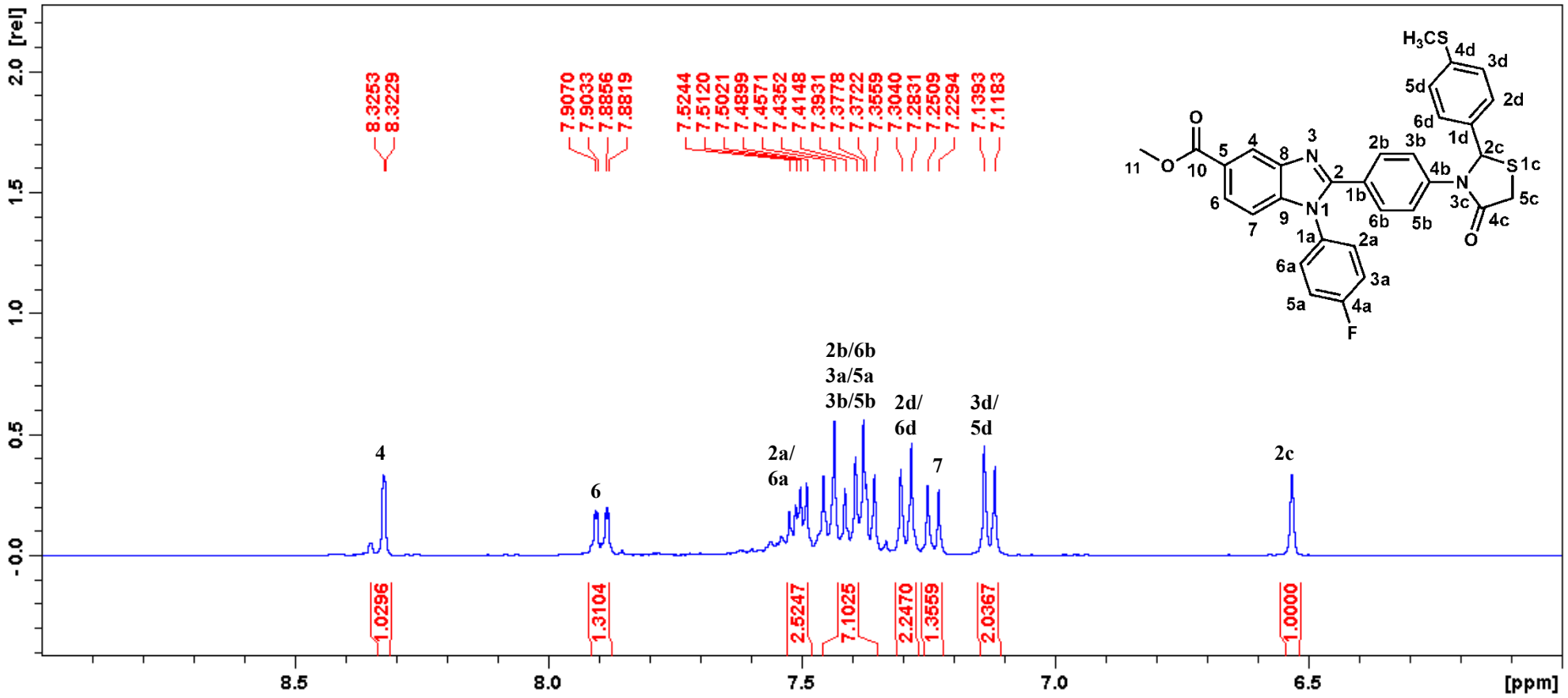


Minimum:				-1.5								
Maximum:		5.0	5.0	100.0								
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula					
576.1370	576.1369	0.1	0.2	20.5	475.0	0.0	C31	H24	N3	O4	F	Na	S

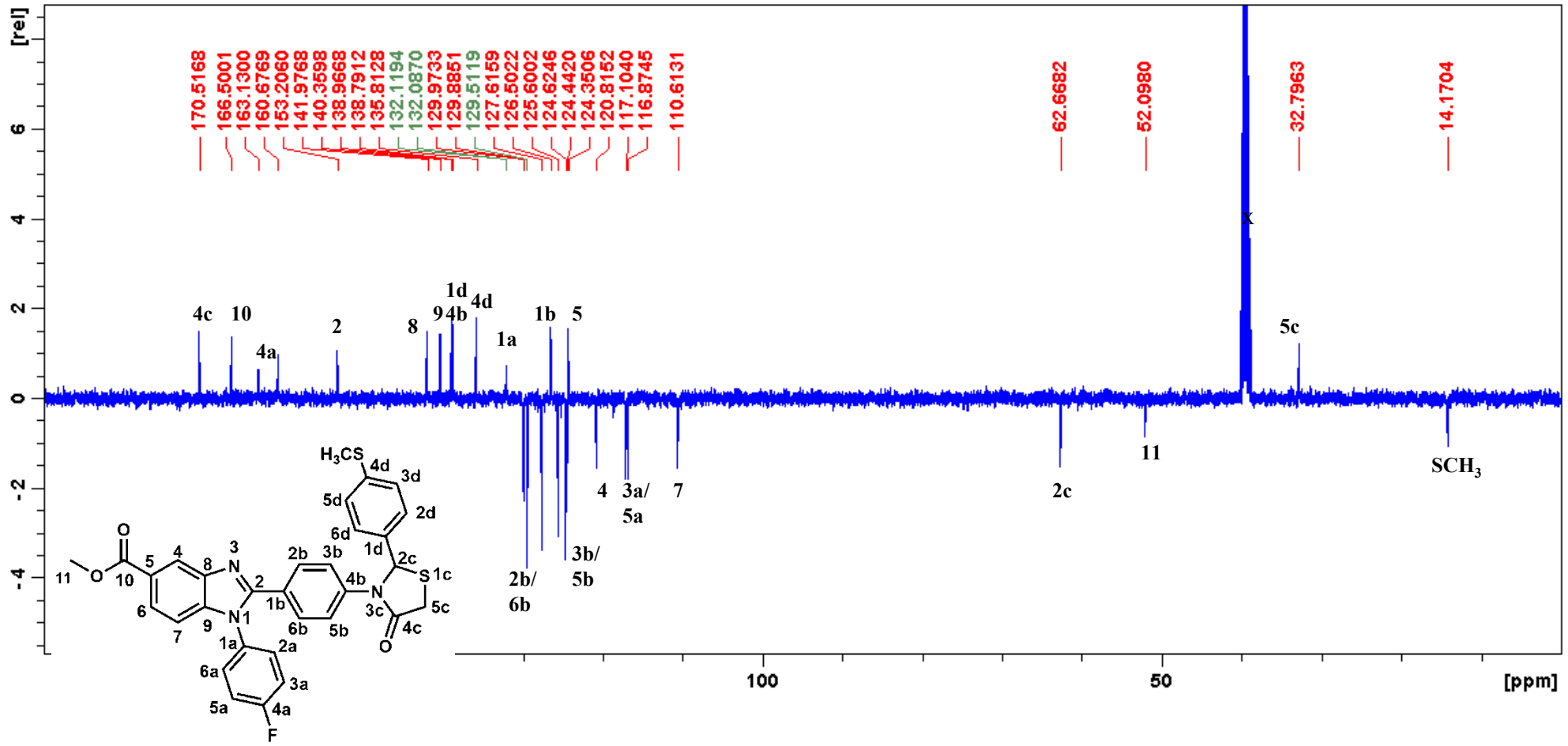
HRMS Spectrum of Compound A-7k



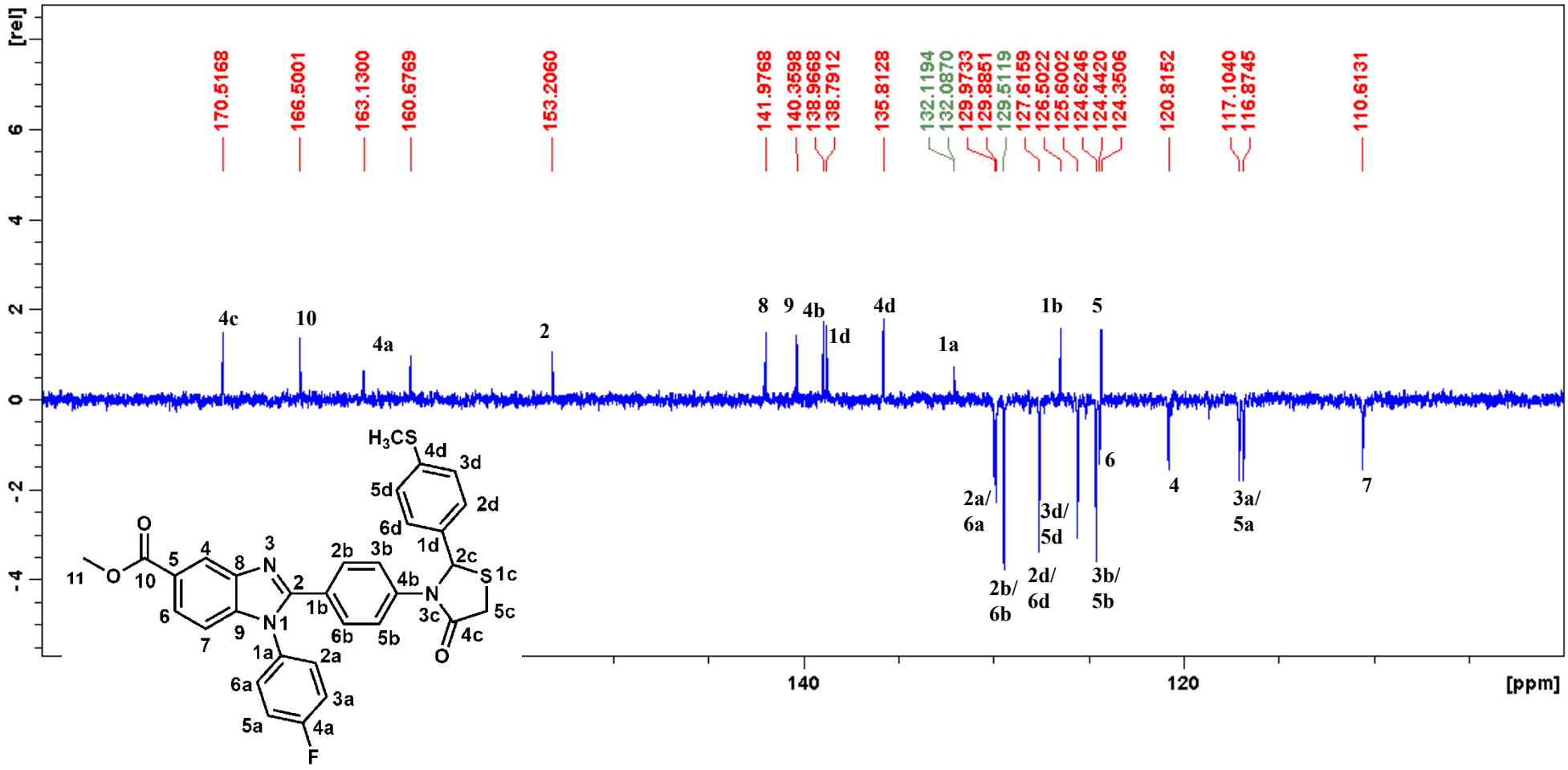
^{1}H Spectrum of Compound A-7l



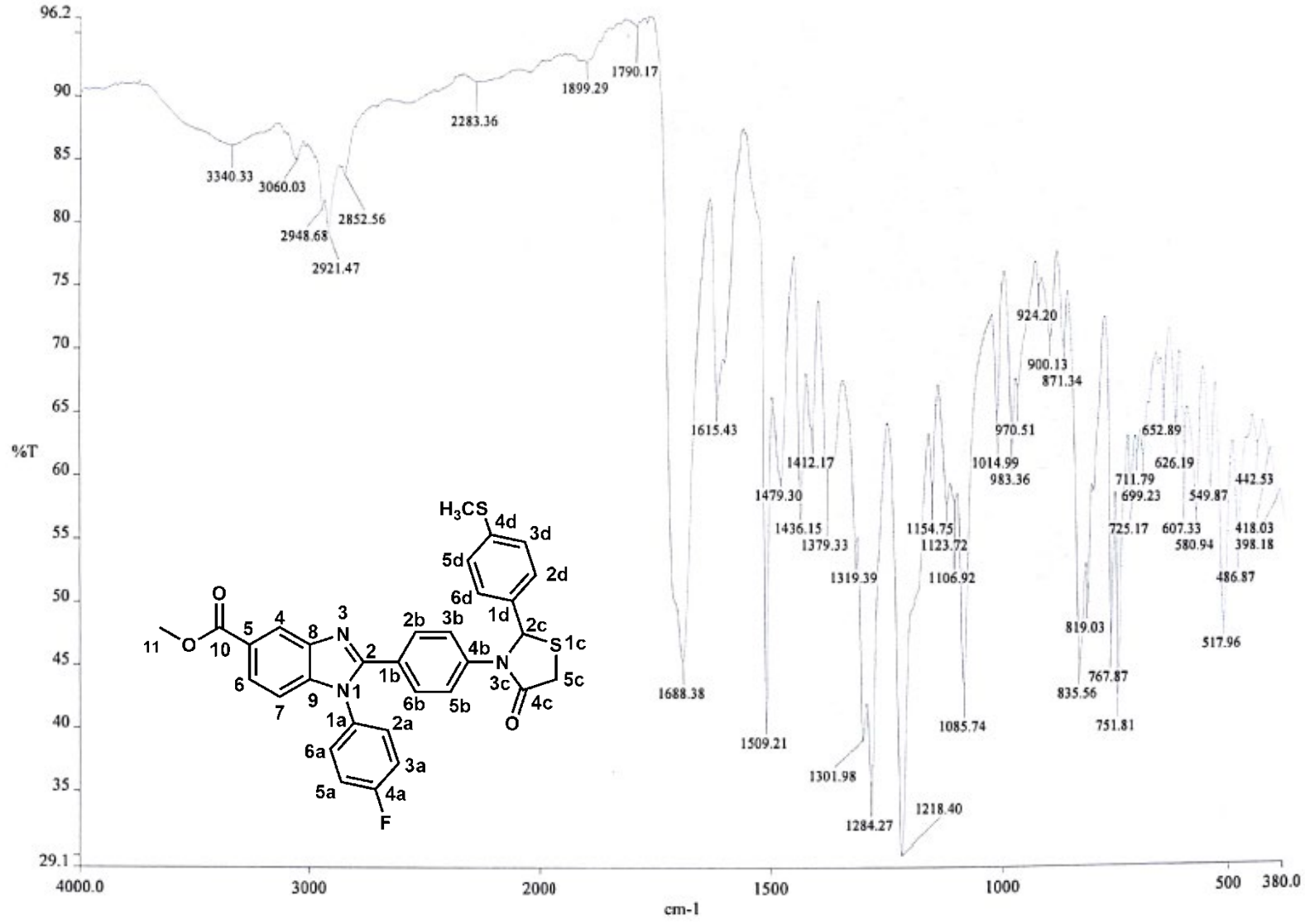
Expanded ^{1}H Spectrum of Compound A-7l



^{13}C Spectrum of Compound A-7l



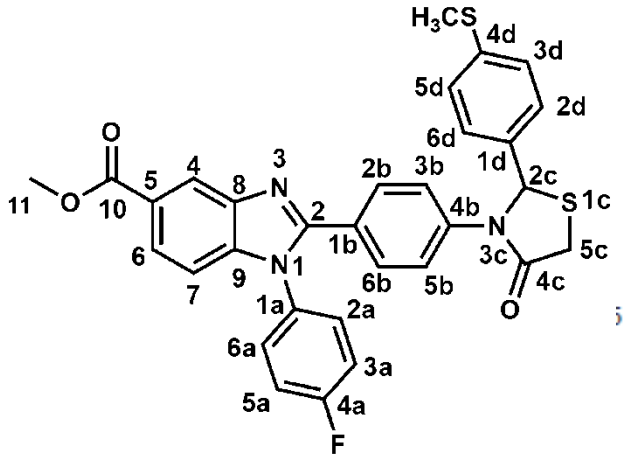
Expanded ^{13}C Spectrum of Compound A-7l

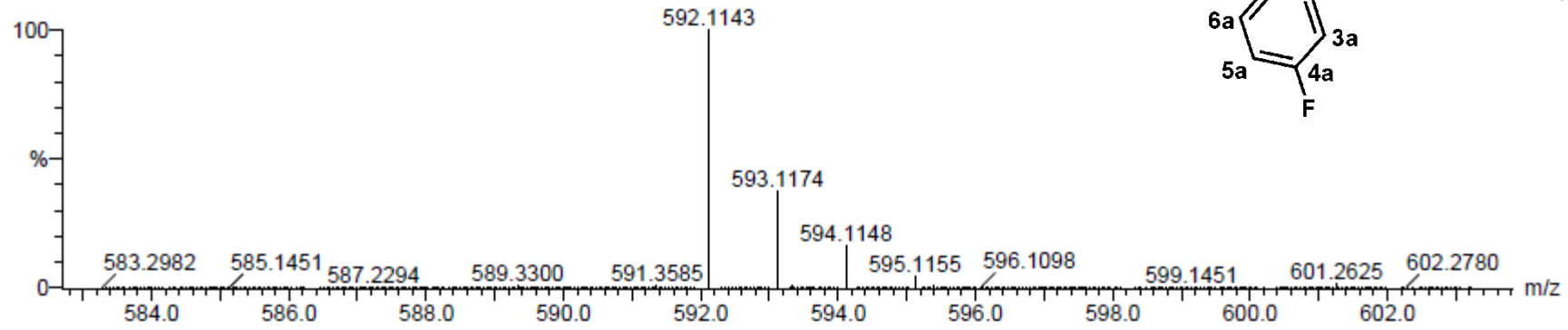


Infrared Spectrum of Compound A-7l

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 = 3

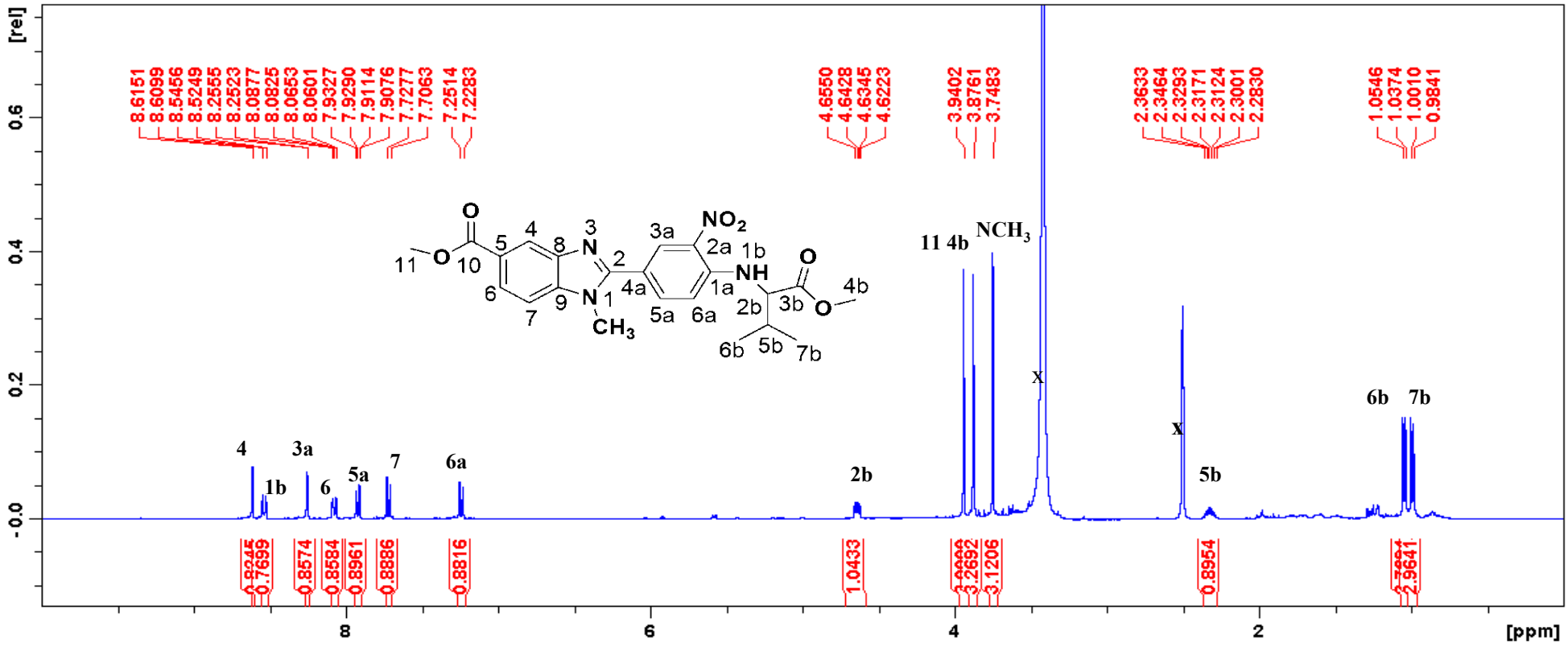
Monoisotopic Mass, Even Electron Ions
20 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 25-35 H: 20-30 N: 0-3 O: 0-3 F: 1-1 Na: 1-1 S: 0-2
BI 12 5 (0.135) Cm (1:61)
TOF MS ES+



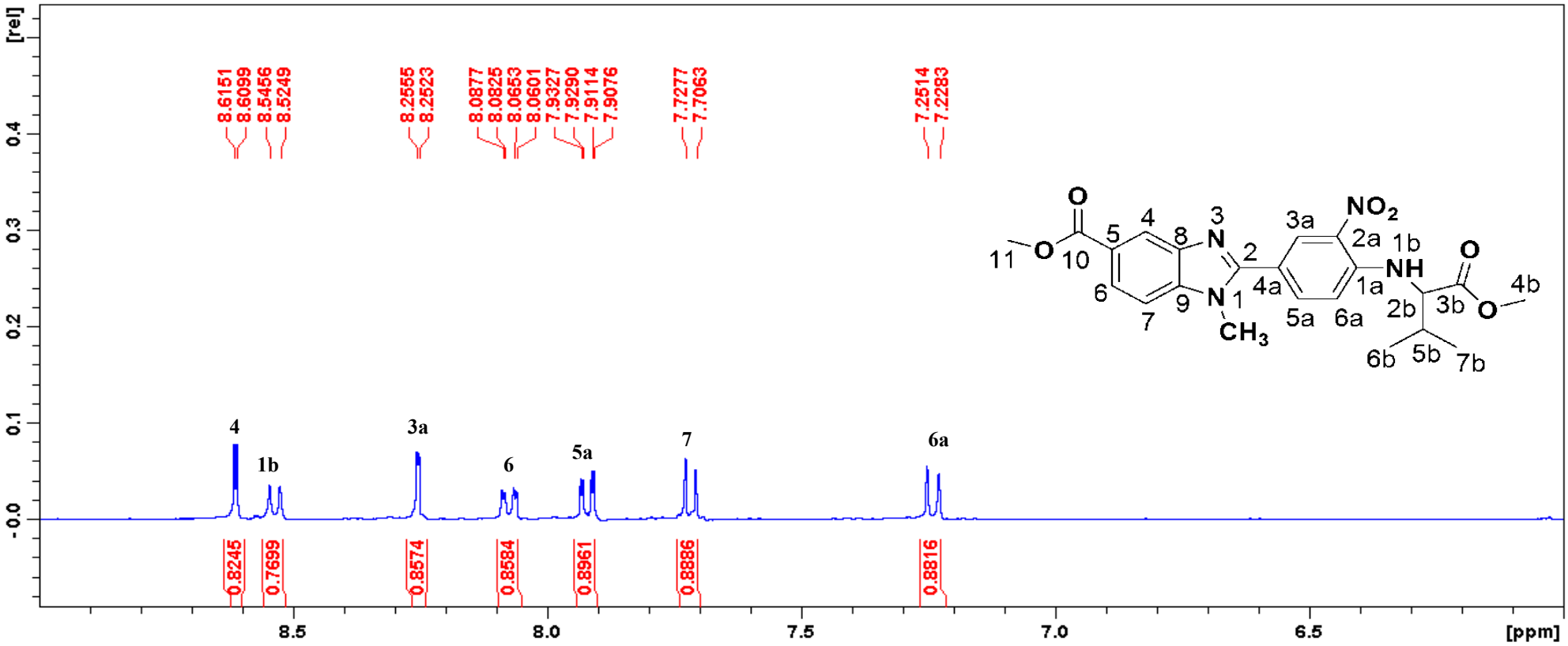


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
592.1143	592.1141	0.2	0.3	20.5	451.8	0.0	C31 H24 N3 O3 F Na S2

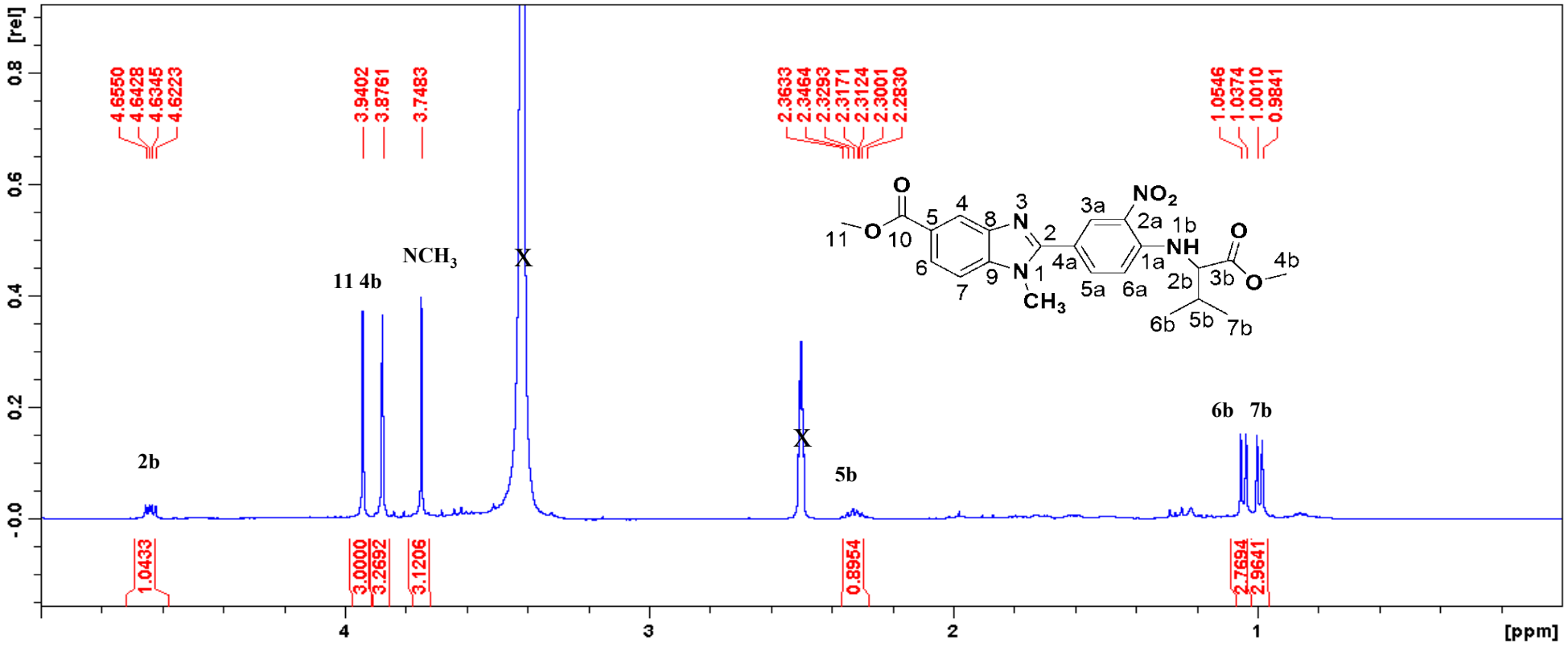
HRMS Spectrum of Compound A-7l



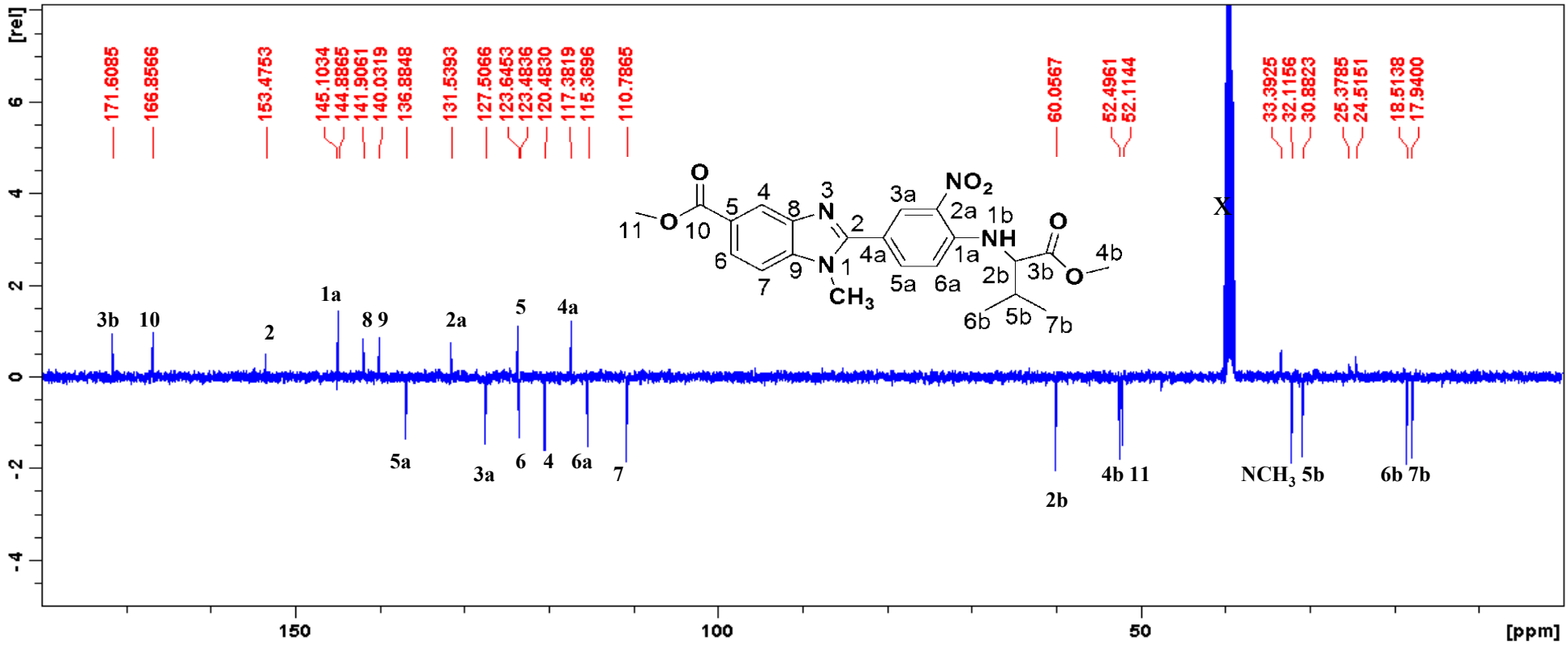
^{1}H Spectrum of Compound B-7a



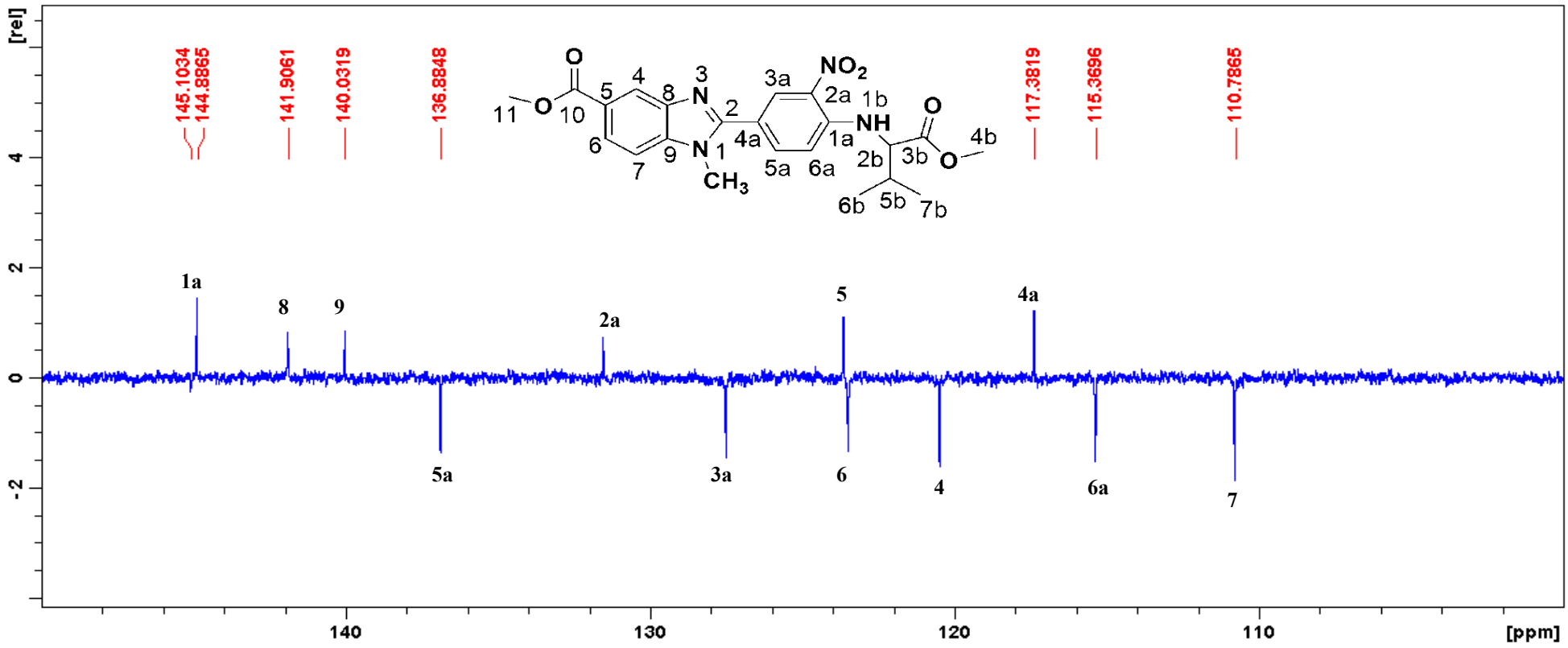
Expanded ^{1}H Spectrum of Compound B-7a



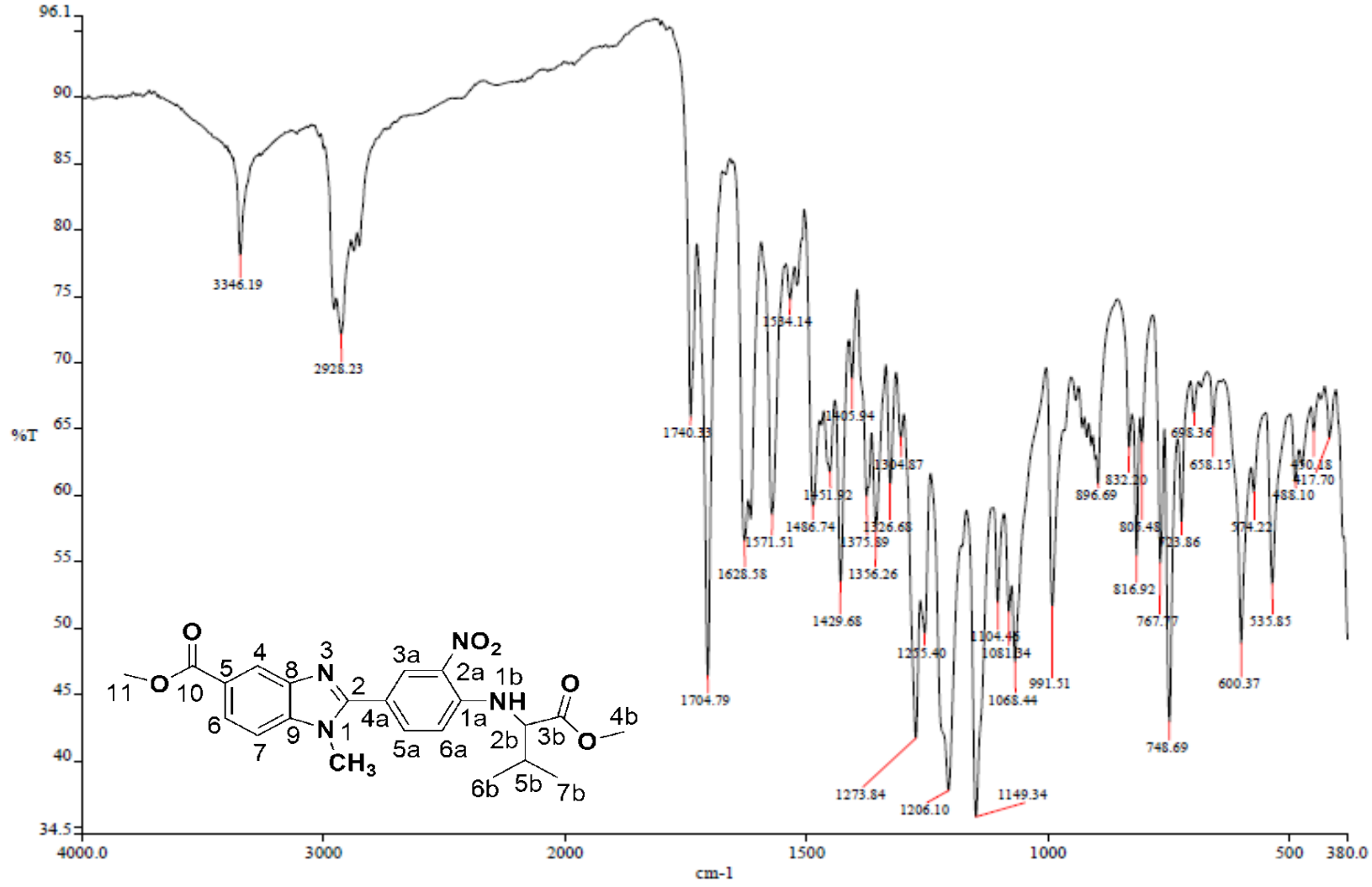
Expanded ^{1}H Spectrum of Compound B-7a



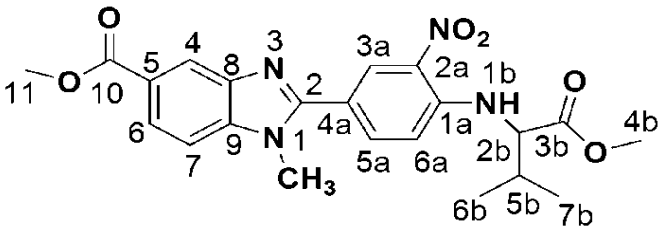
^{13}C Spectrum of Compound B-7a



Expanded ^{13}C spectrum of Compound B-7a



Infrared Spectrum of Compound B-7a

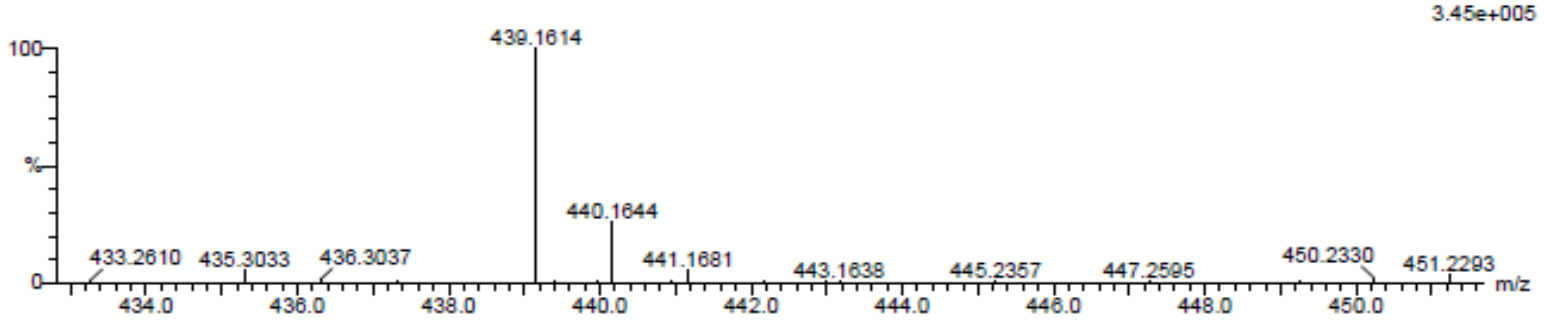


Elemental Composition Report

Single Mass Analysis

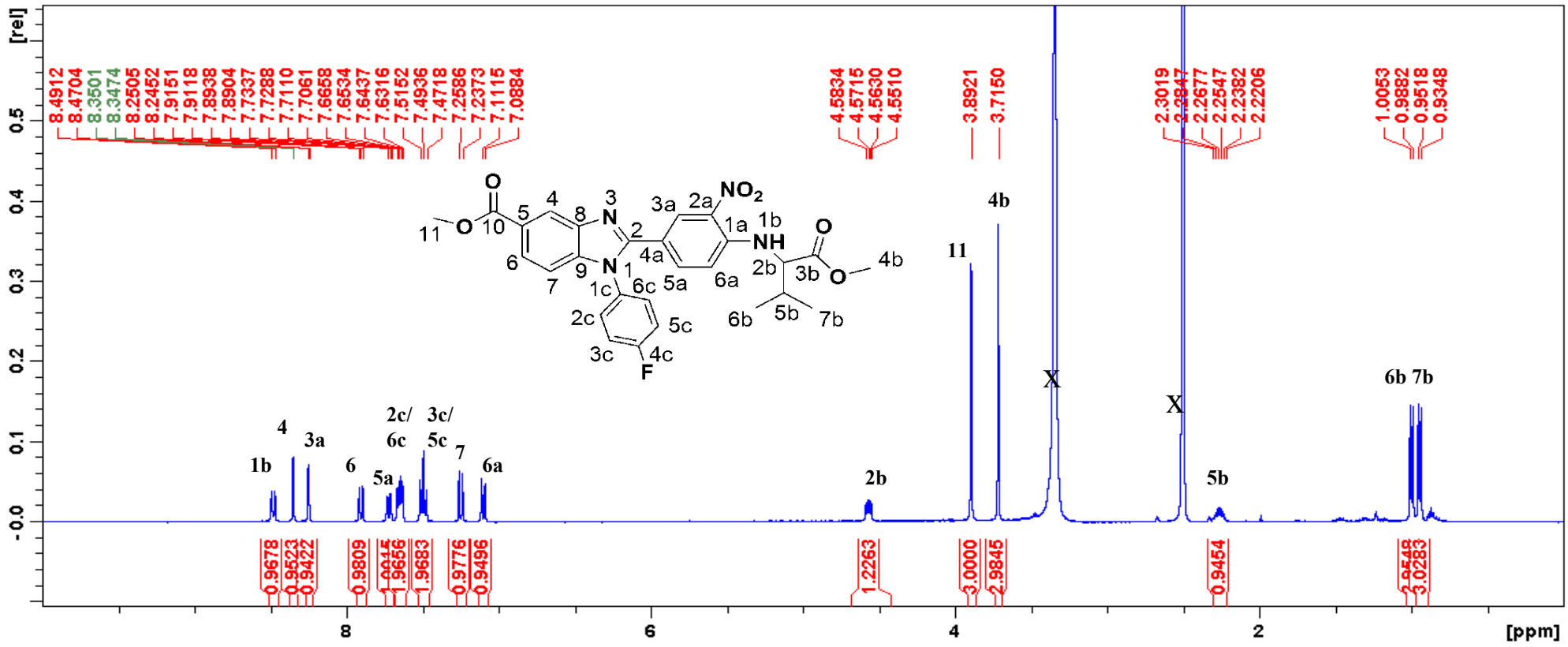
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
27 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: 0-10
BA 4 10 (0.304) Cm (1:61)
TOF MS ES-

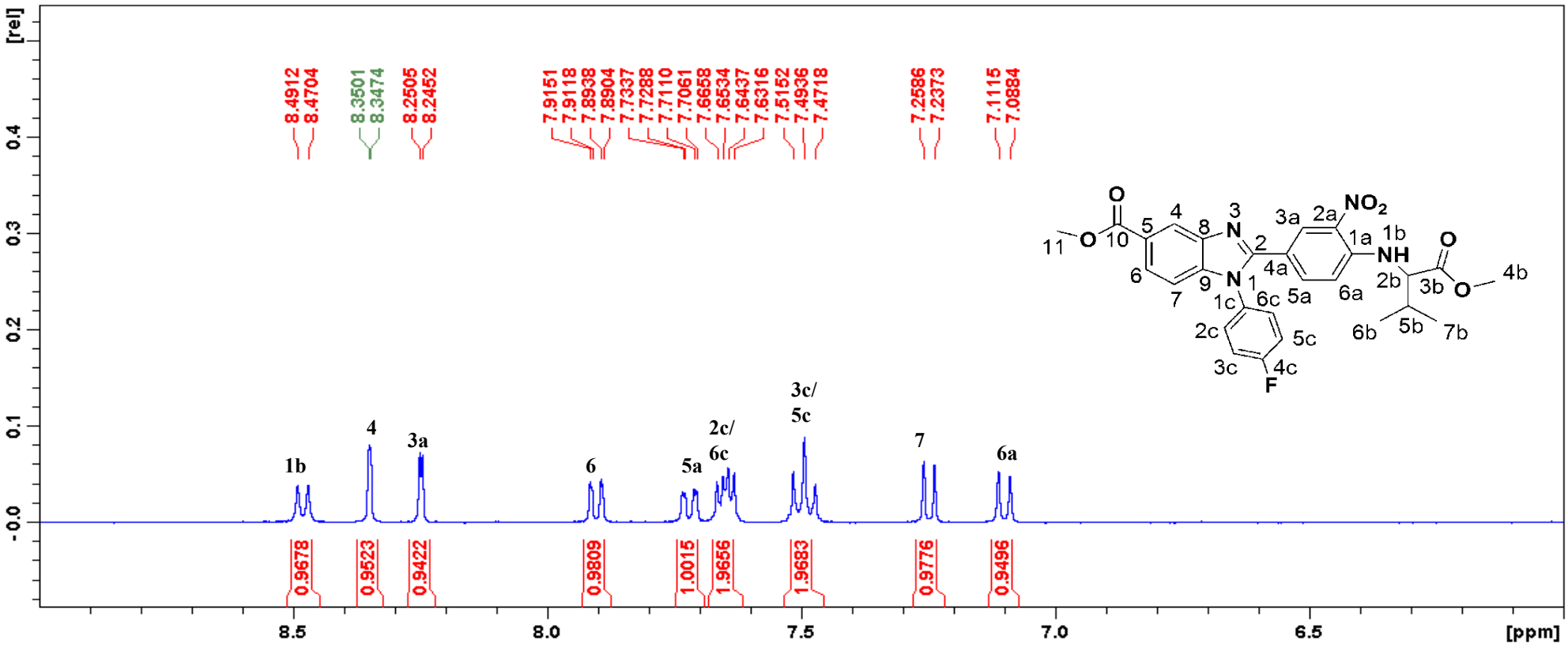


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
439.1614	439.1618	-0.4	-0.9	13.5	41.5	0.0	C22 H23 N4 O6

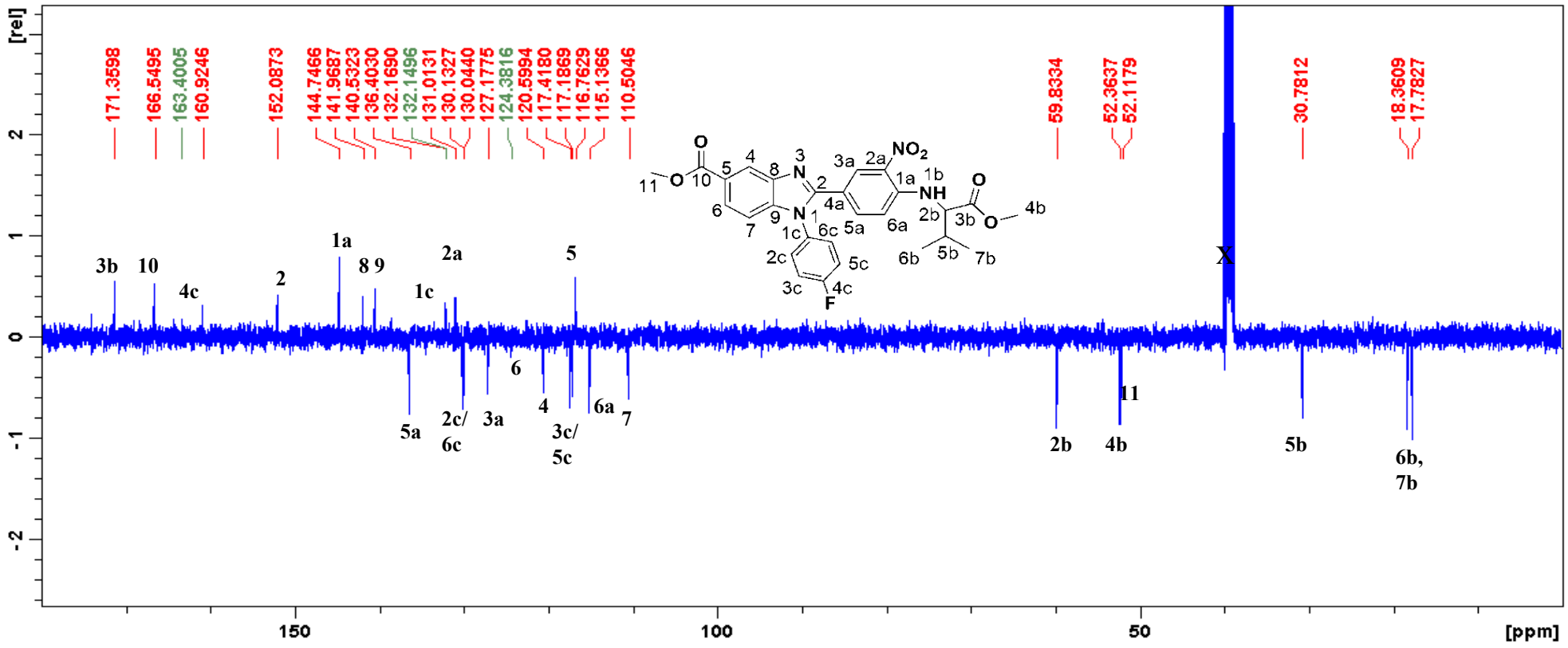
HRMS Spectrum of Compound B-7a



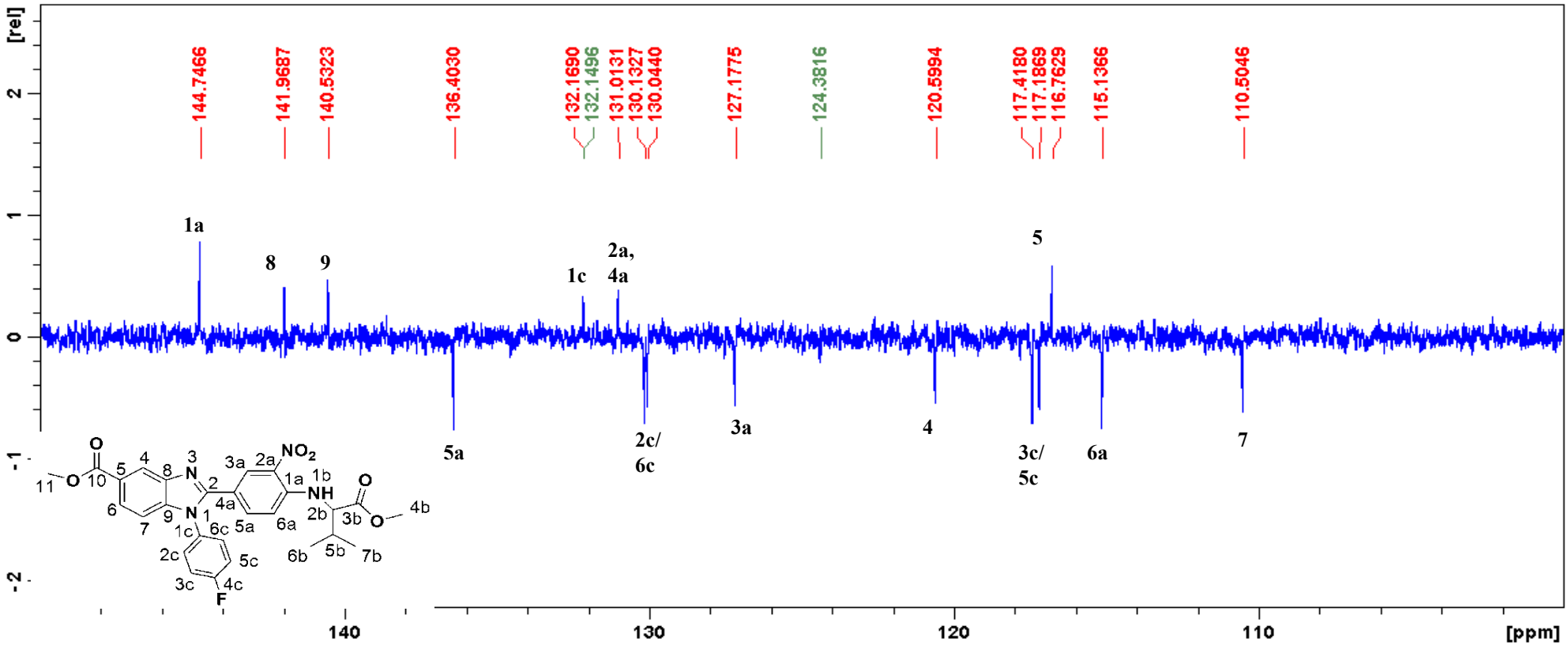
^{1}H Spectrum of Compound B-7b



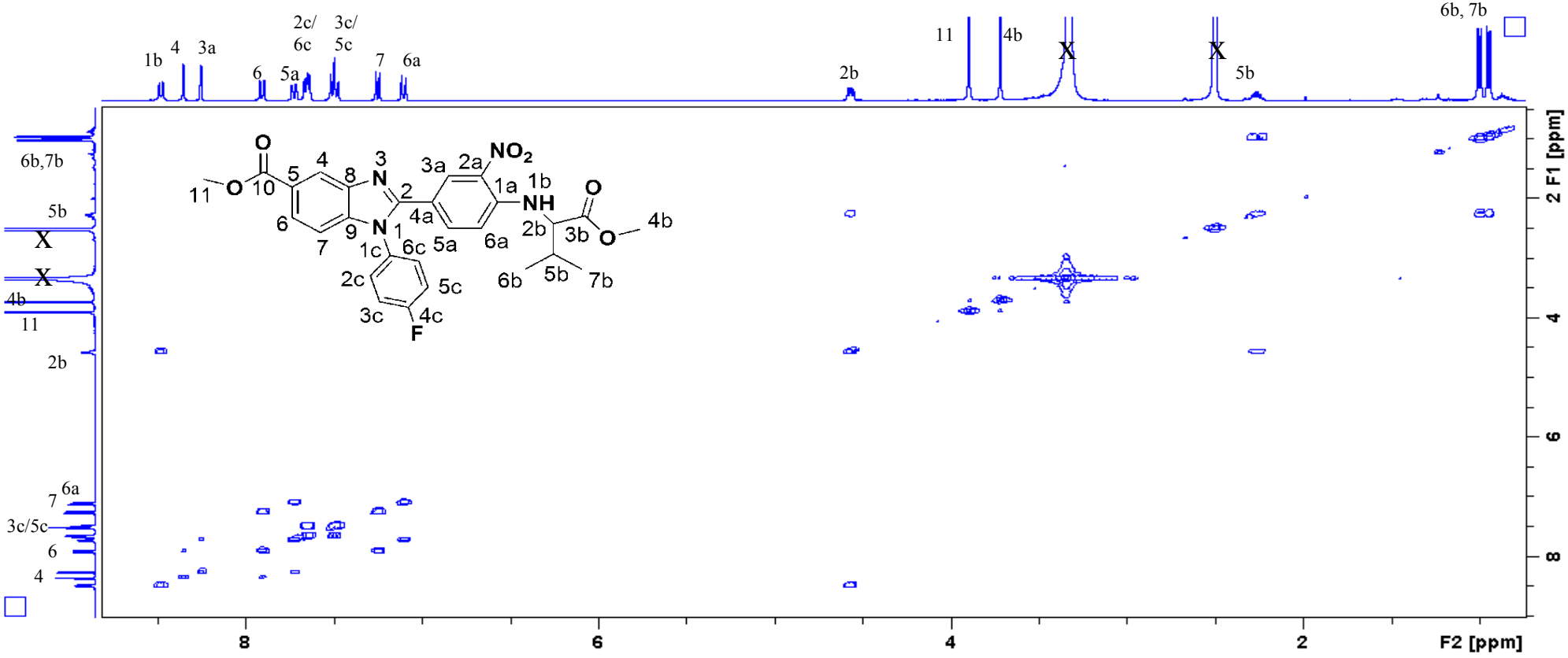
Expanded ^{1}H Spectrum of Compound B-7b



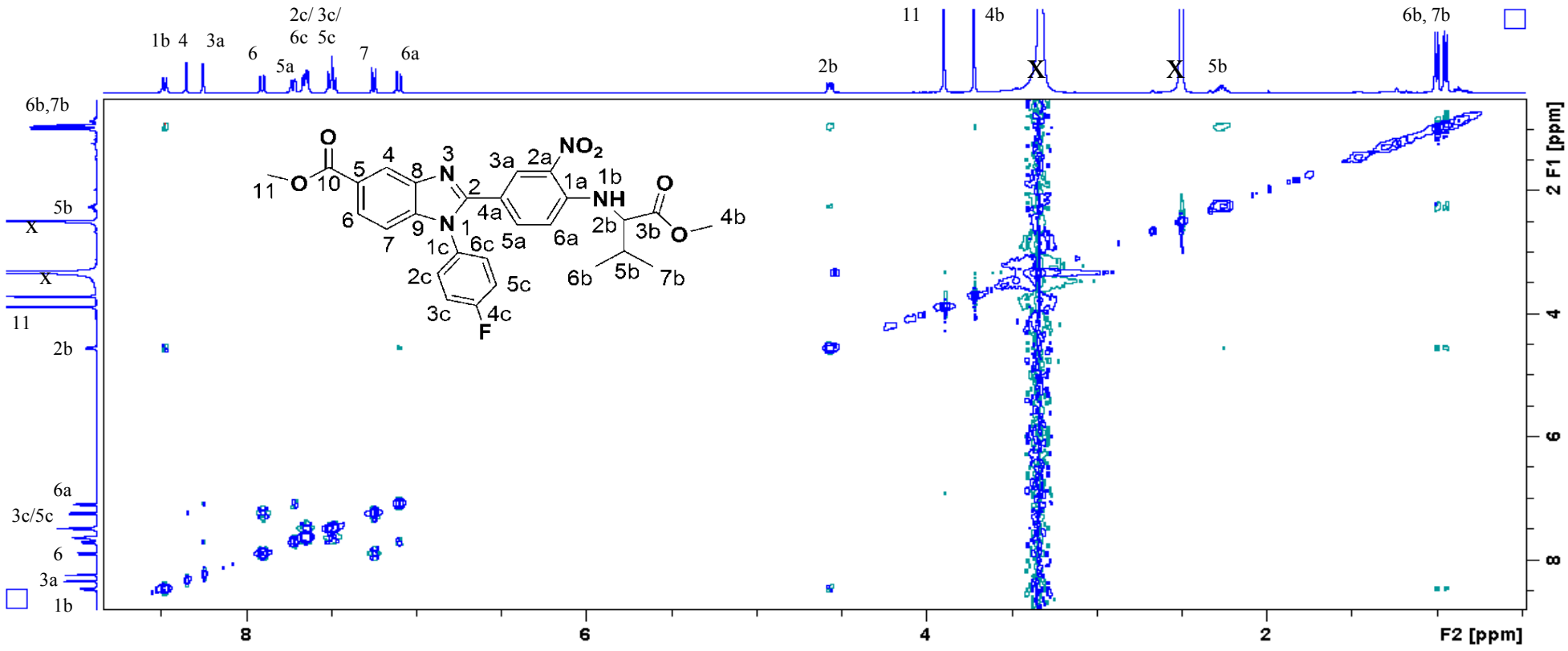
^{13}C Spectrum of Compound B-7b



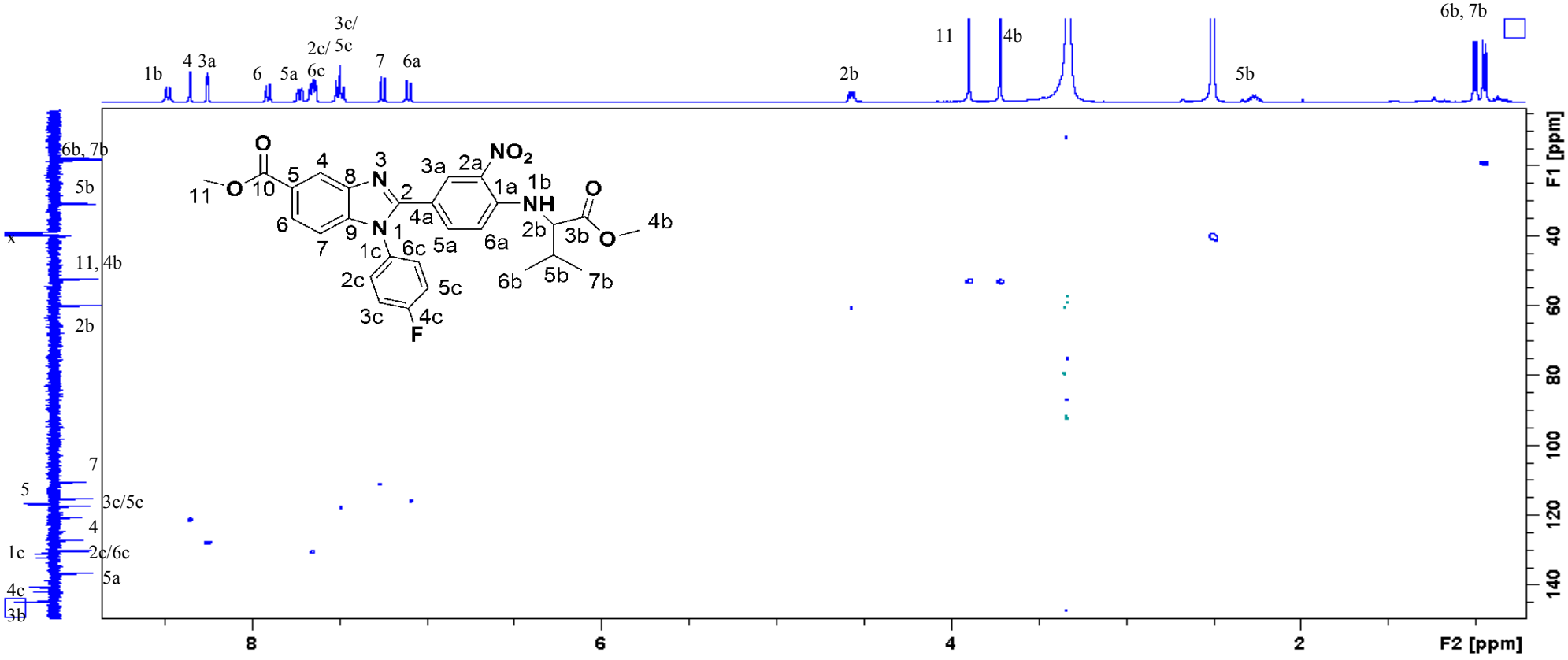
Expanded ^{13}C Spectrum of Compound B-7b



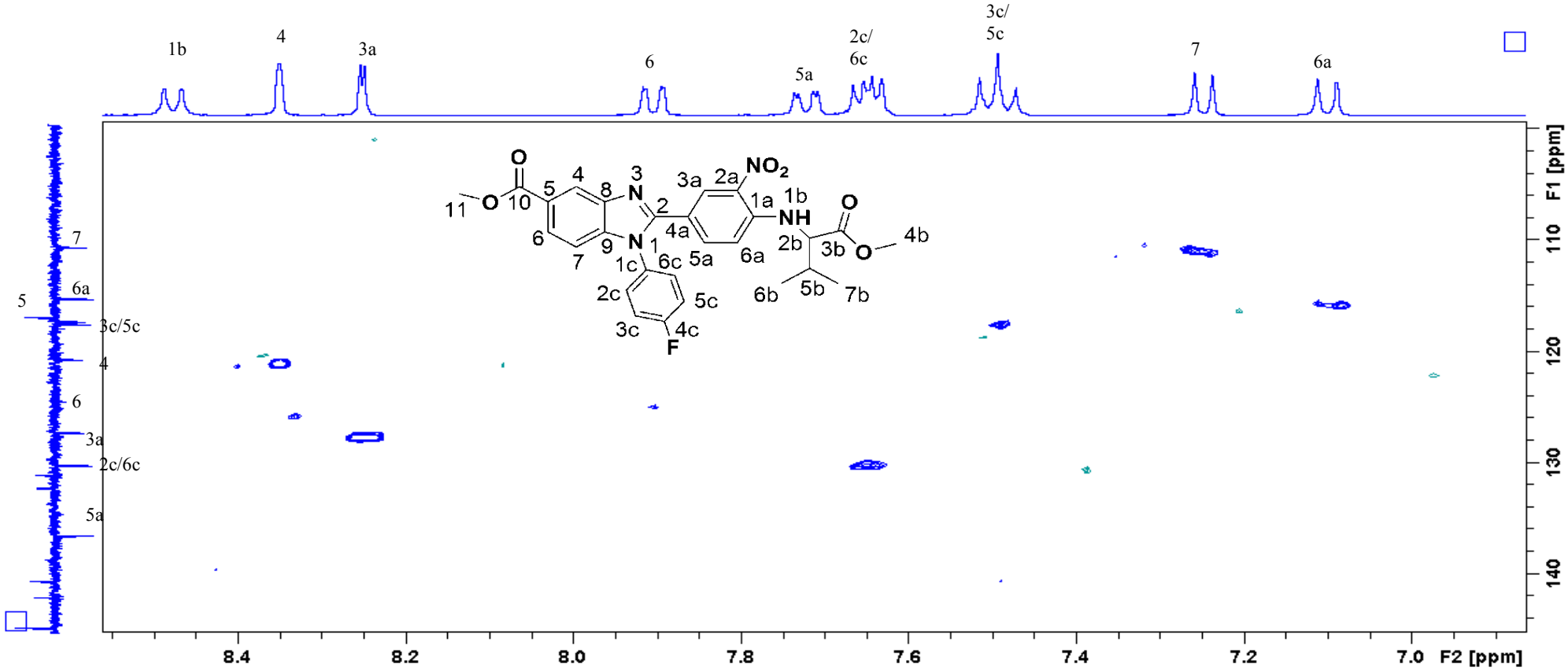
COSY Spectrum of Compound B-7b



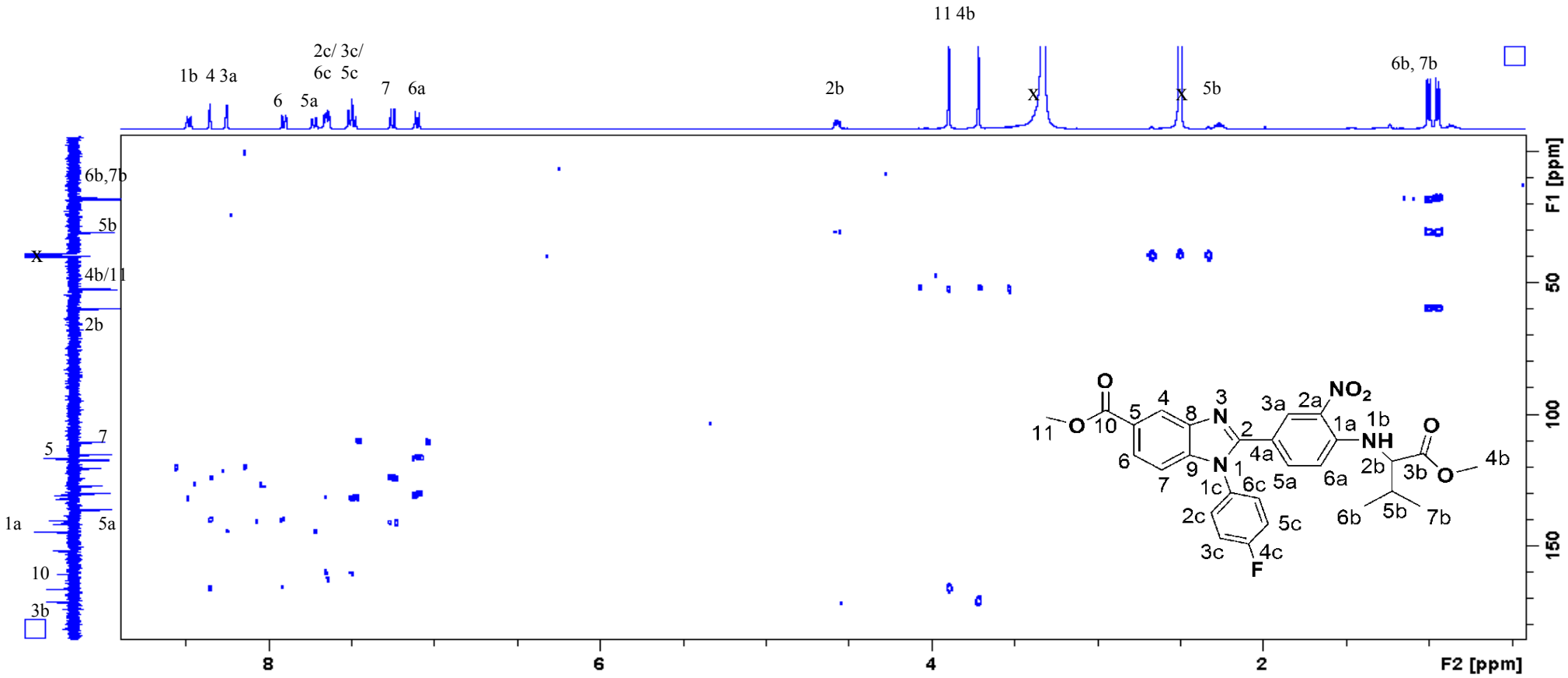
NOESY Spectrum of Compound B-7b



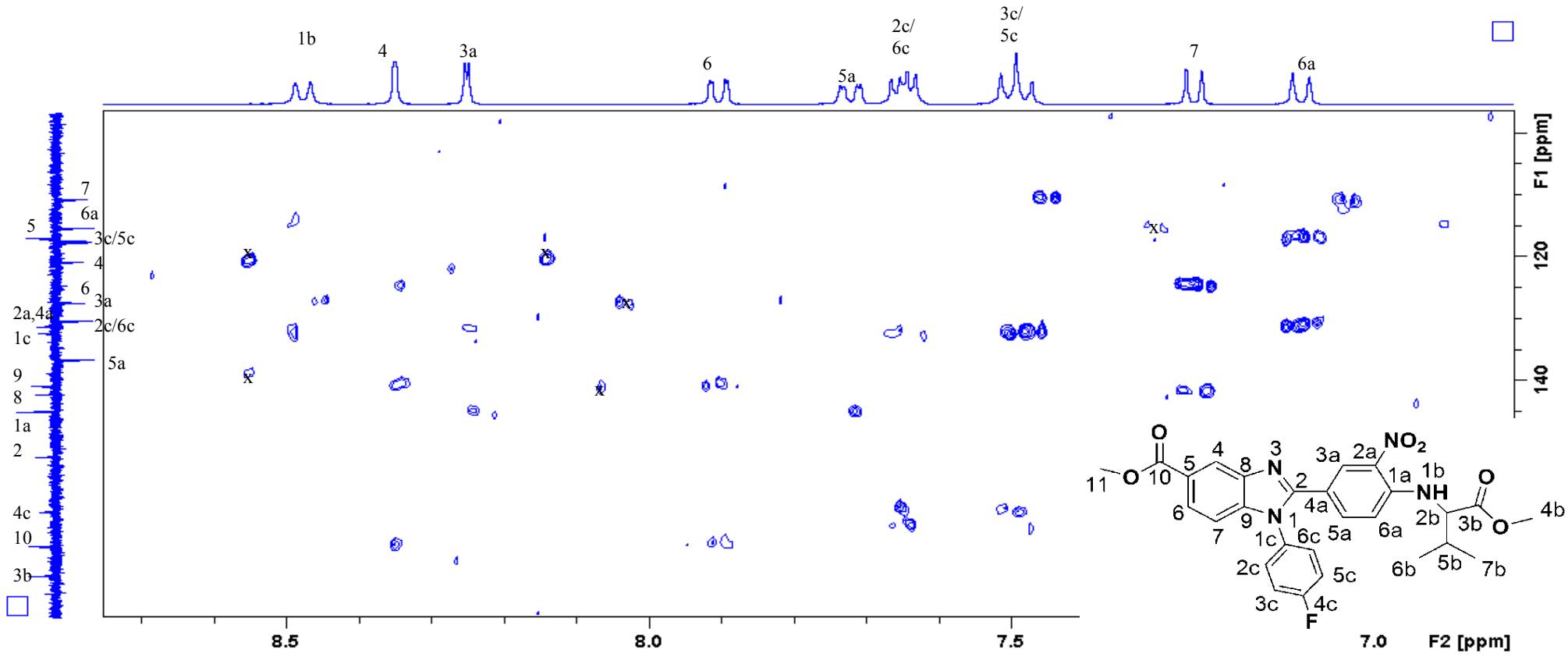
HSQC Spectrum for Compound B-7b



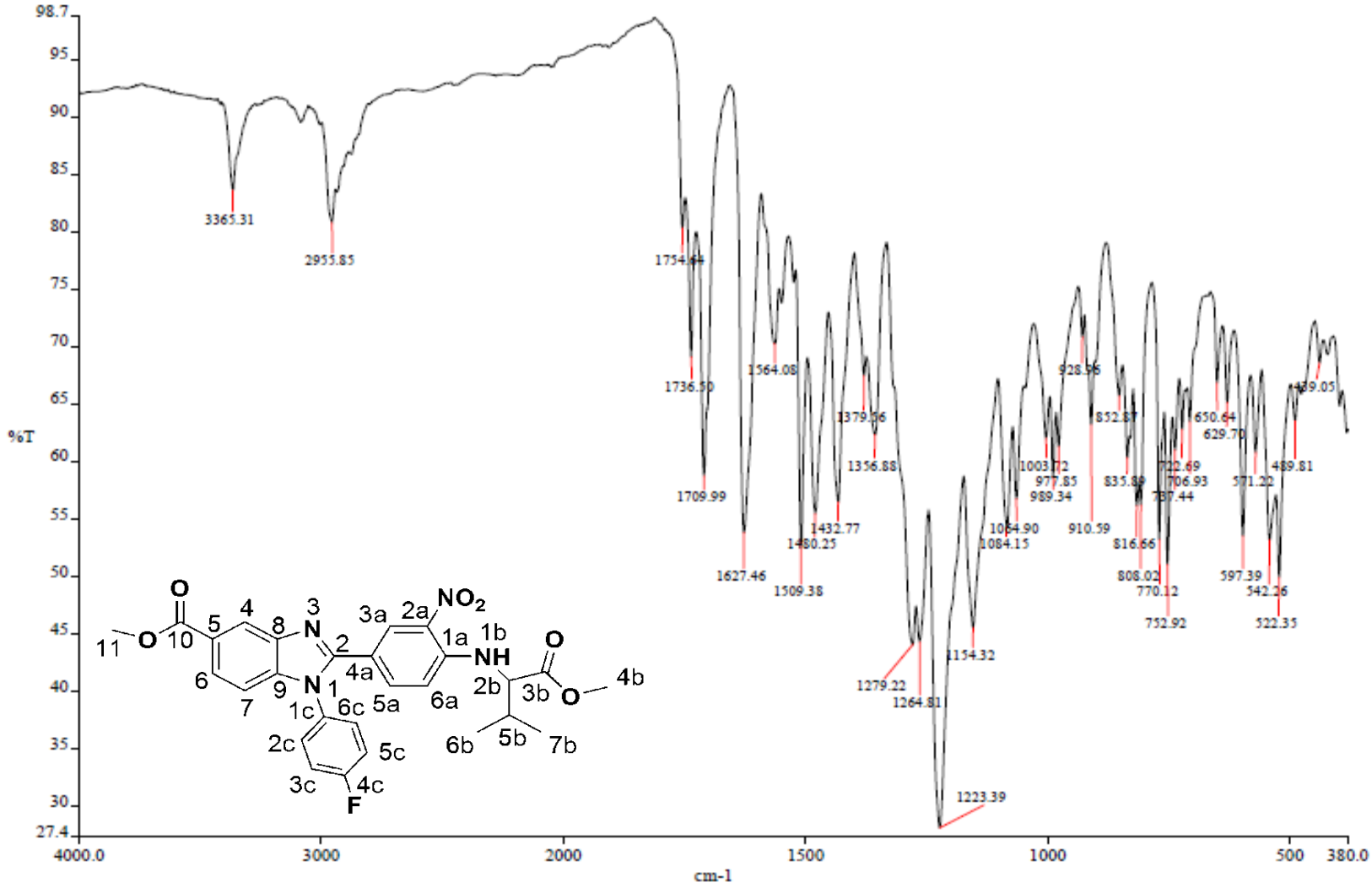
Expanded HSQC Spectrum of Compound B-7b



HMBC Spectrum of Compound B-7b



Expanded HMBC Spectrum of Compound B-7b



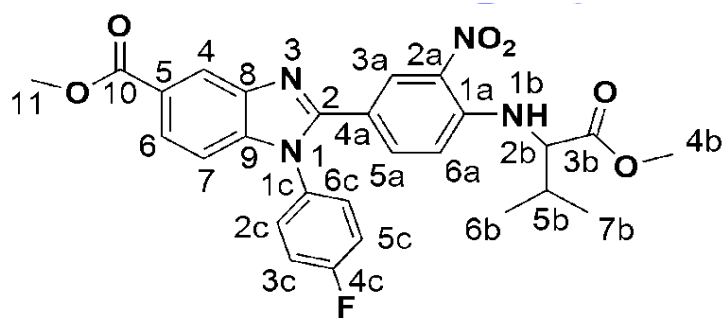
Infrared Spectrum of Compound B-7b

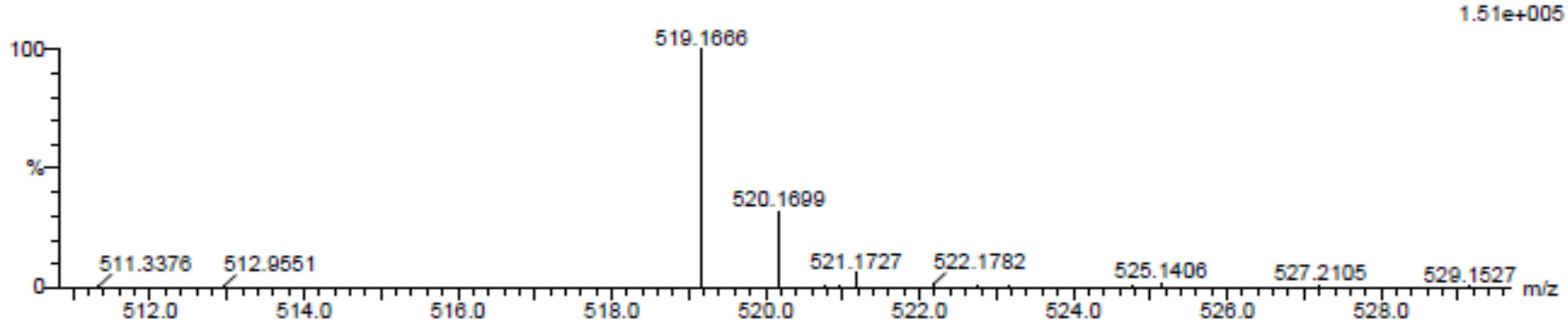
Elemental Composition Report

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

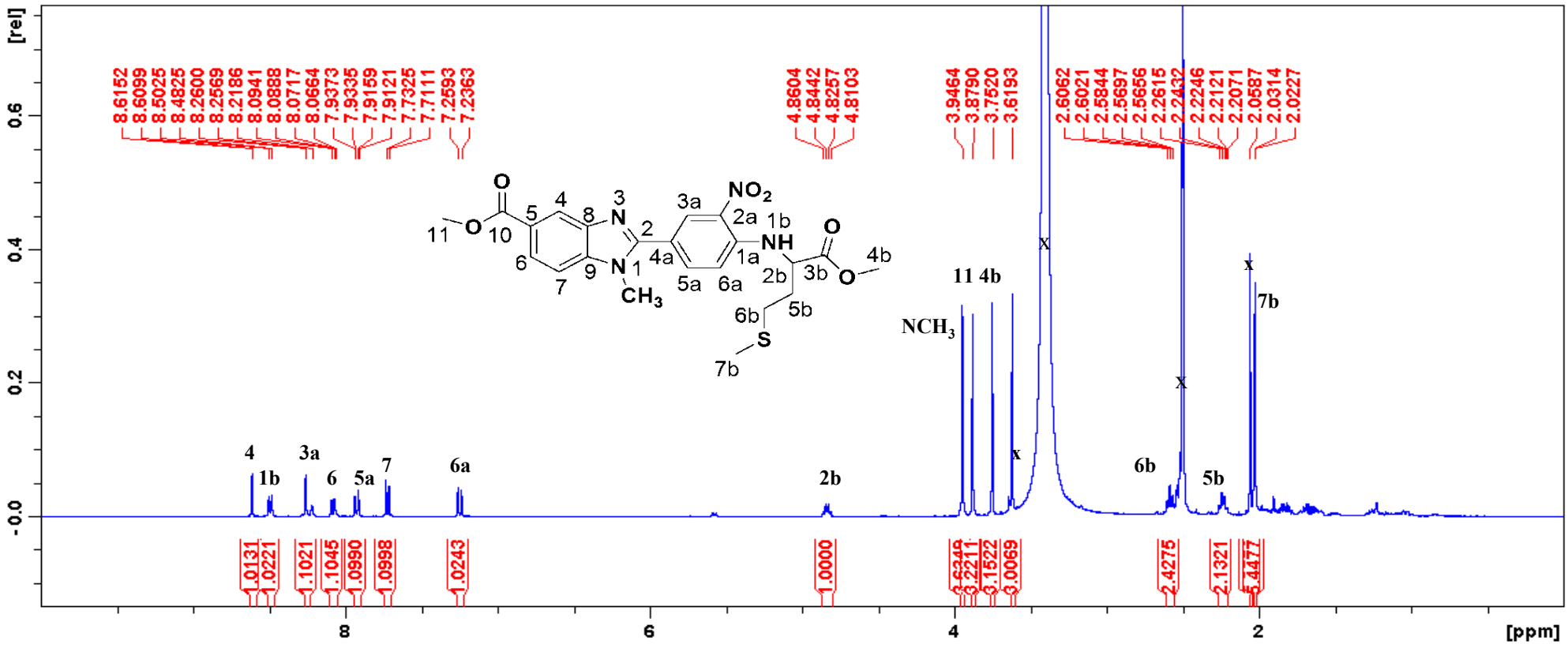
Monoisotopic Mass, Even Electron Ions
24 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 25-30 H: 20-25 N: 0-5 O: 0-10 F: 1-1
BA 3 51 (1.720) Cm (1:60)
TOF MS ES-



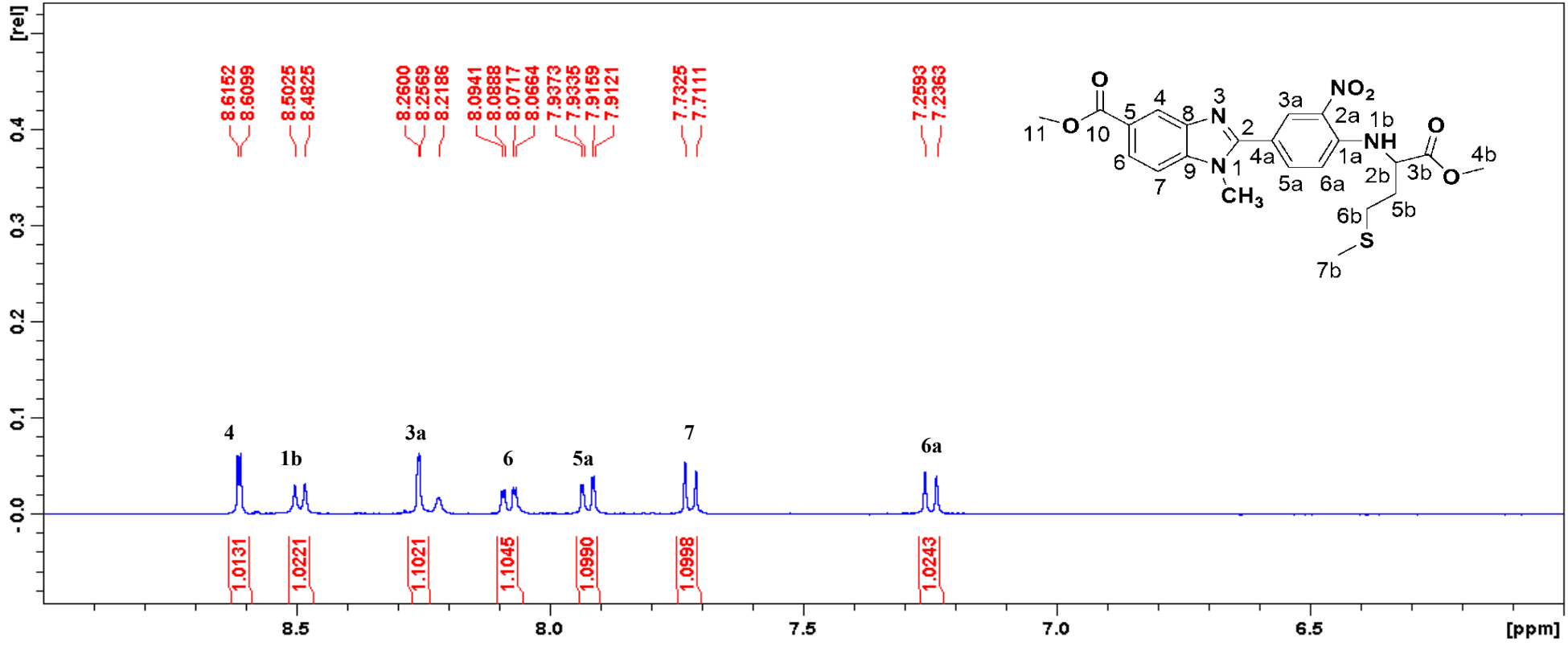


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
519.1666	519.1680	-1.4	-2.7	17.5	10.2	0.0	C27 H24 N4 O6 F

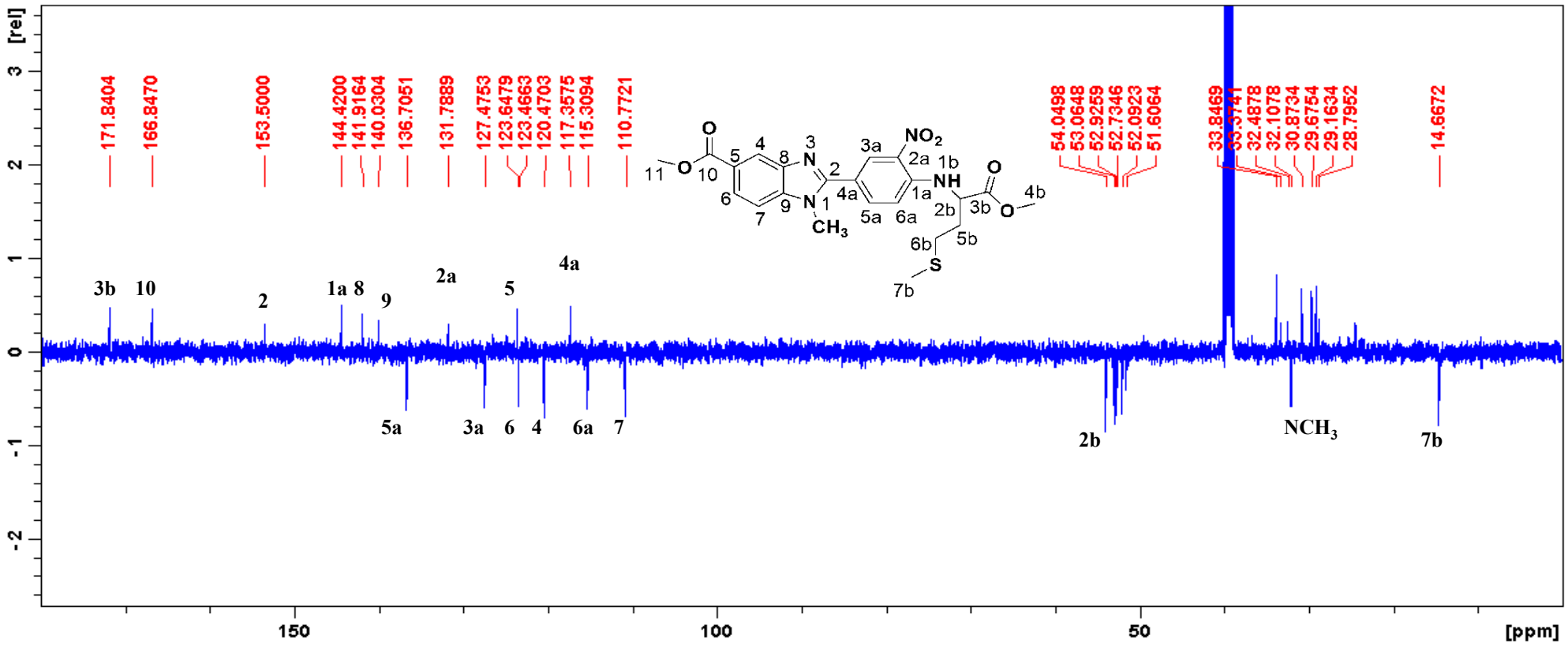
HRMS Spectrum of Compound B-7b



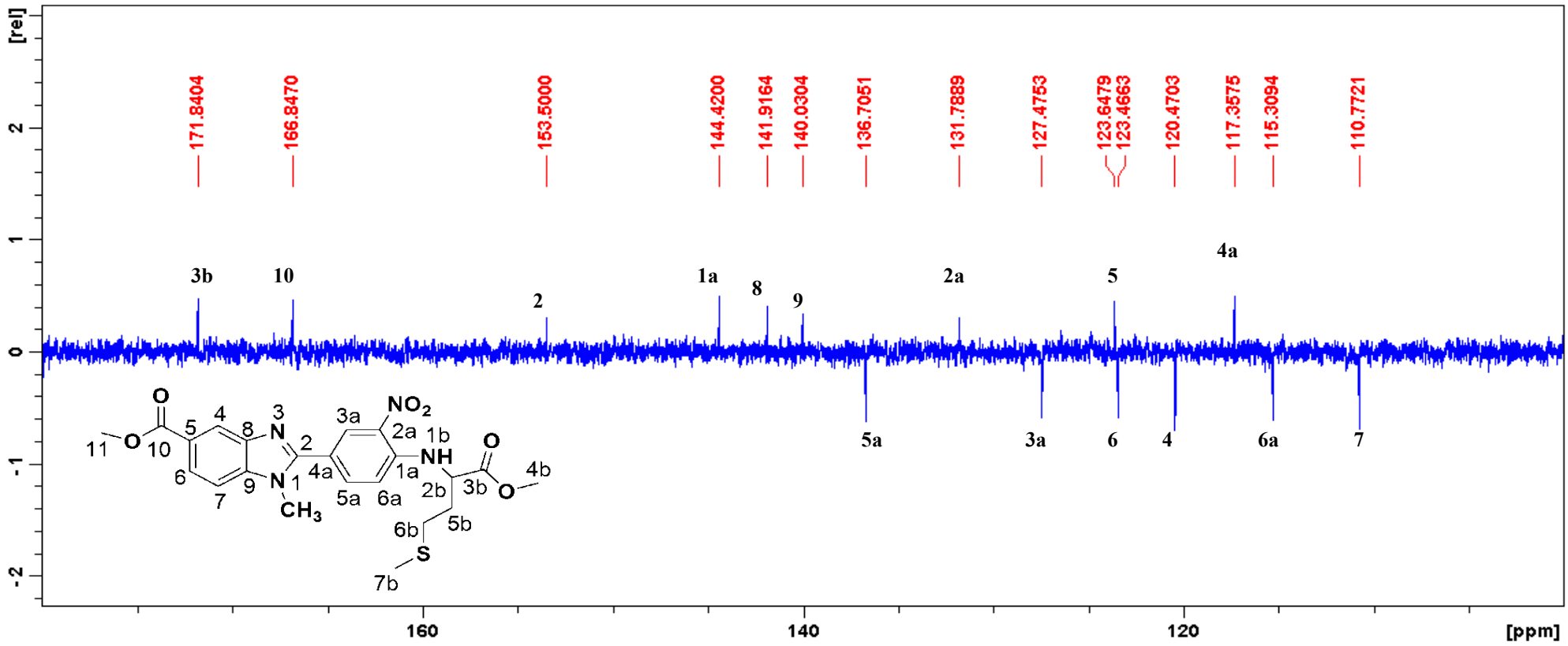
^{1}H Spectrum of Compound B-7c



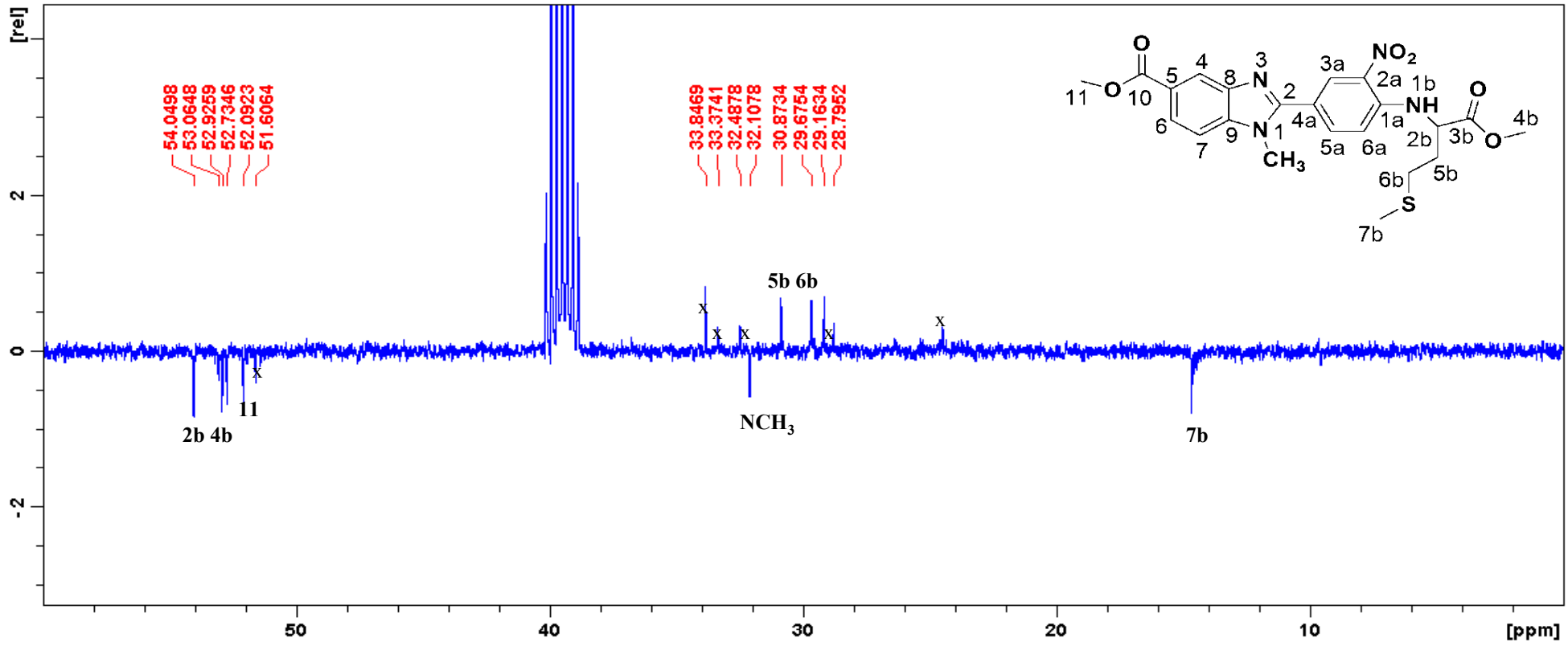
Expanded ^{1}H Spectrum of Compound B-7c



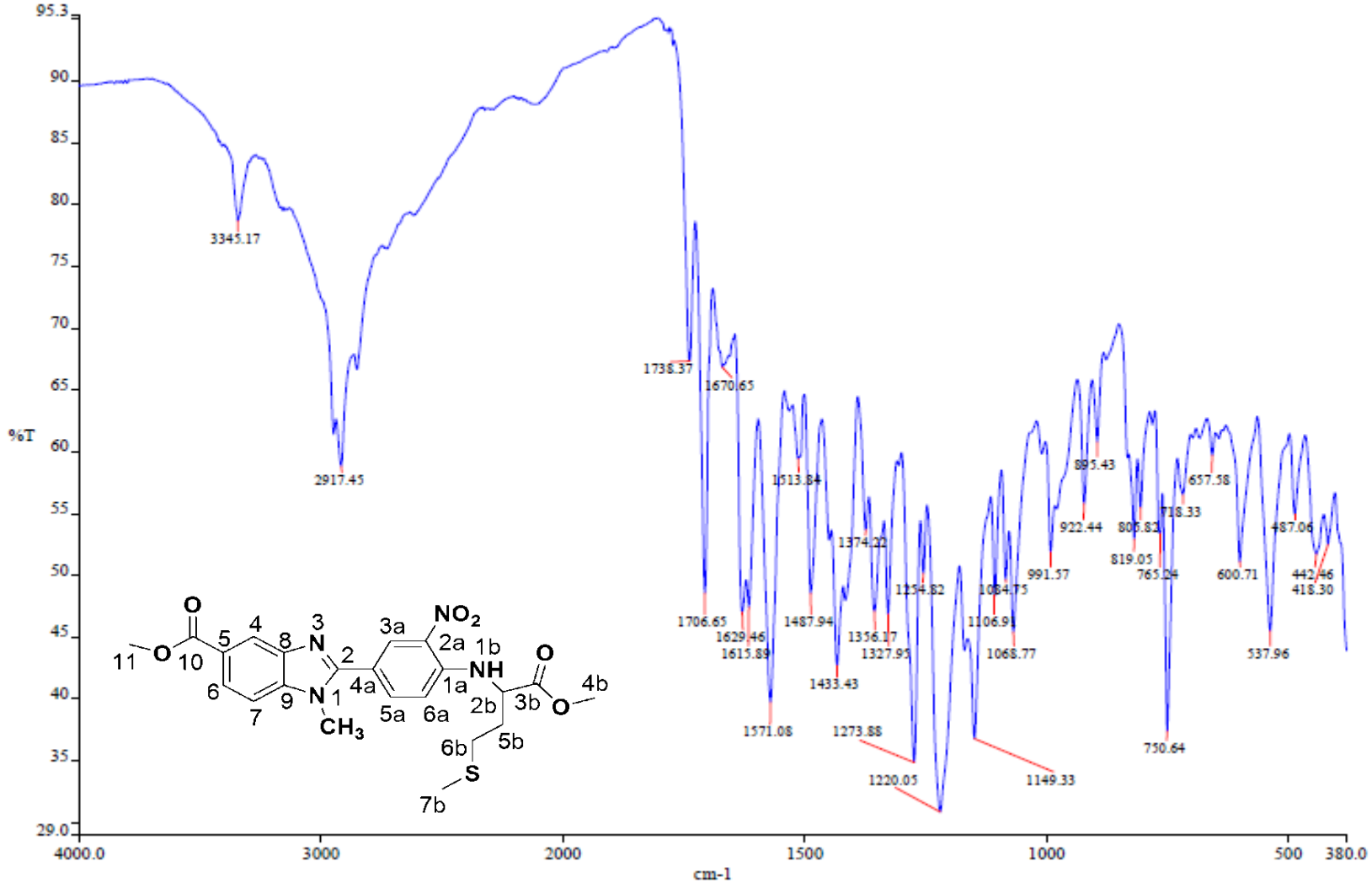
^{13}C Spectrum of Compound B-7c



Expanded ^{13}C Spectrum of Compound B-7c

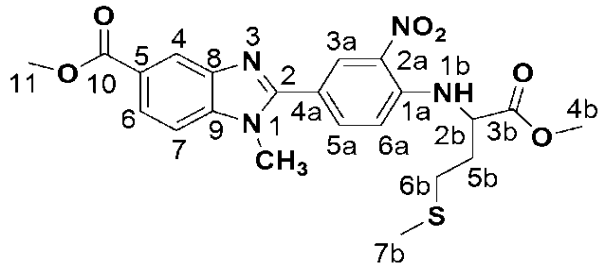


Expanded ^{13}C Spectrum of Compound B-7c



Infrared Spectrum of Compound B-7c

Elemental Composition Report

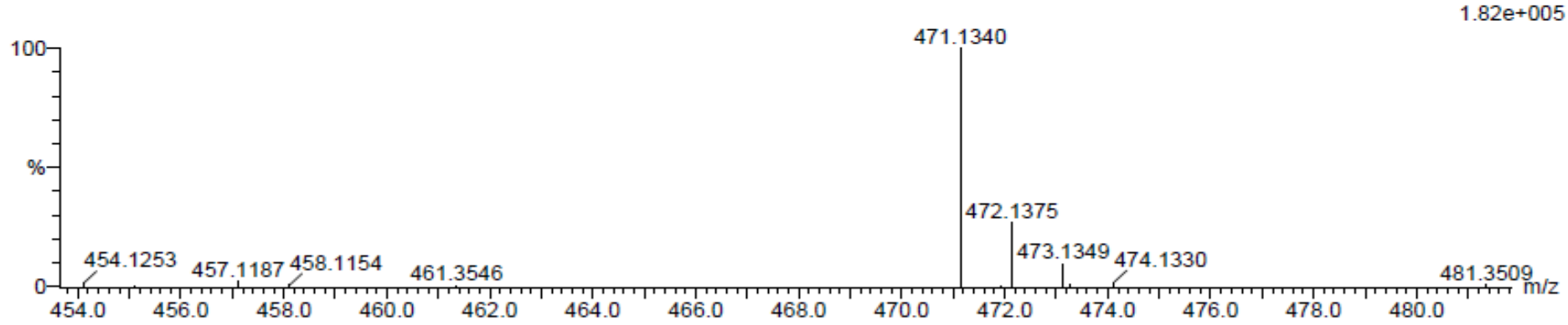


Single Mass Analysis

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

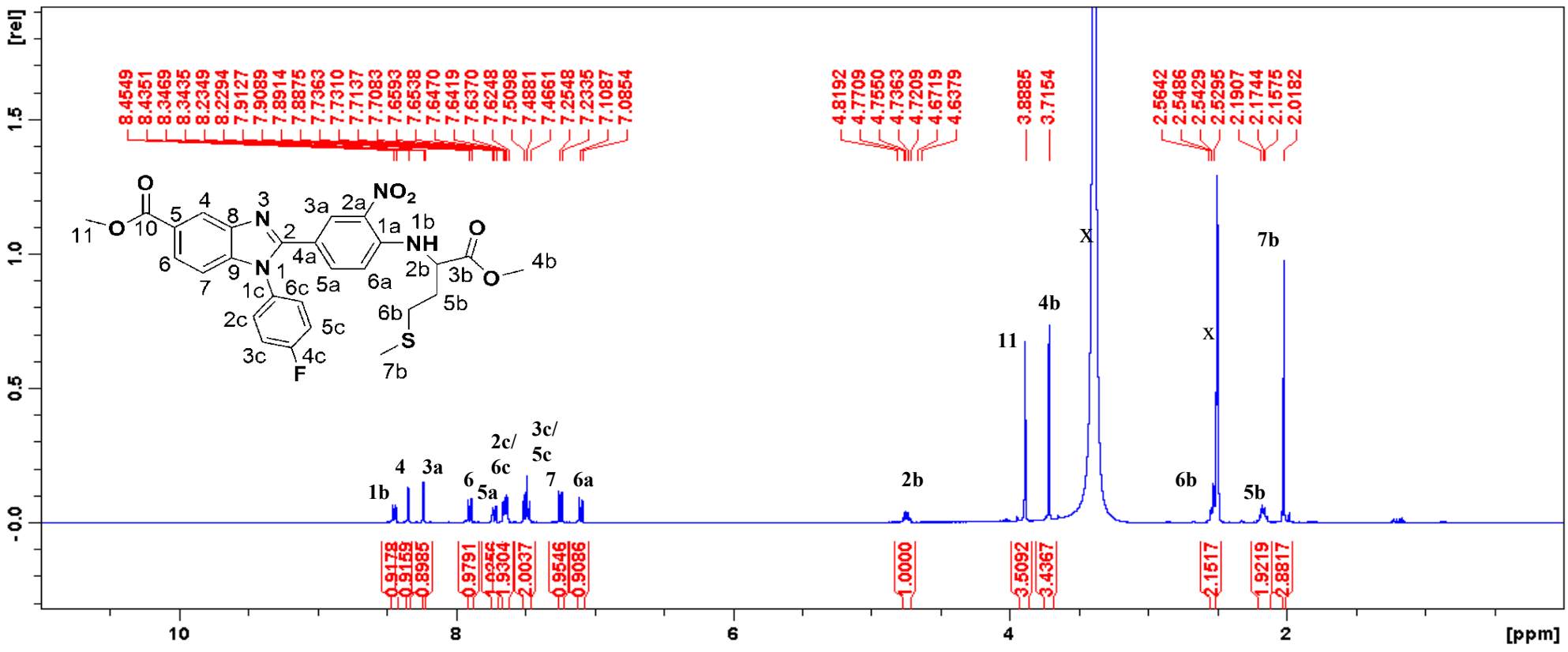
Monoisotopic Mass, Even Electron Ions
27 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 20-25 N: 0-5 O: 0-10 S: 1-1

BA 6 52 (1.720) Cm (1:61)
TOF MS ES-

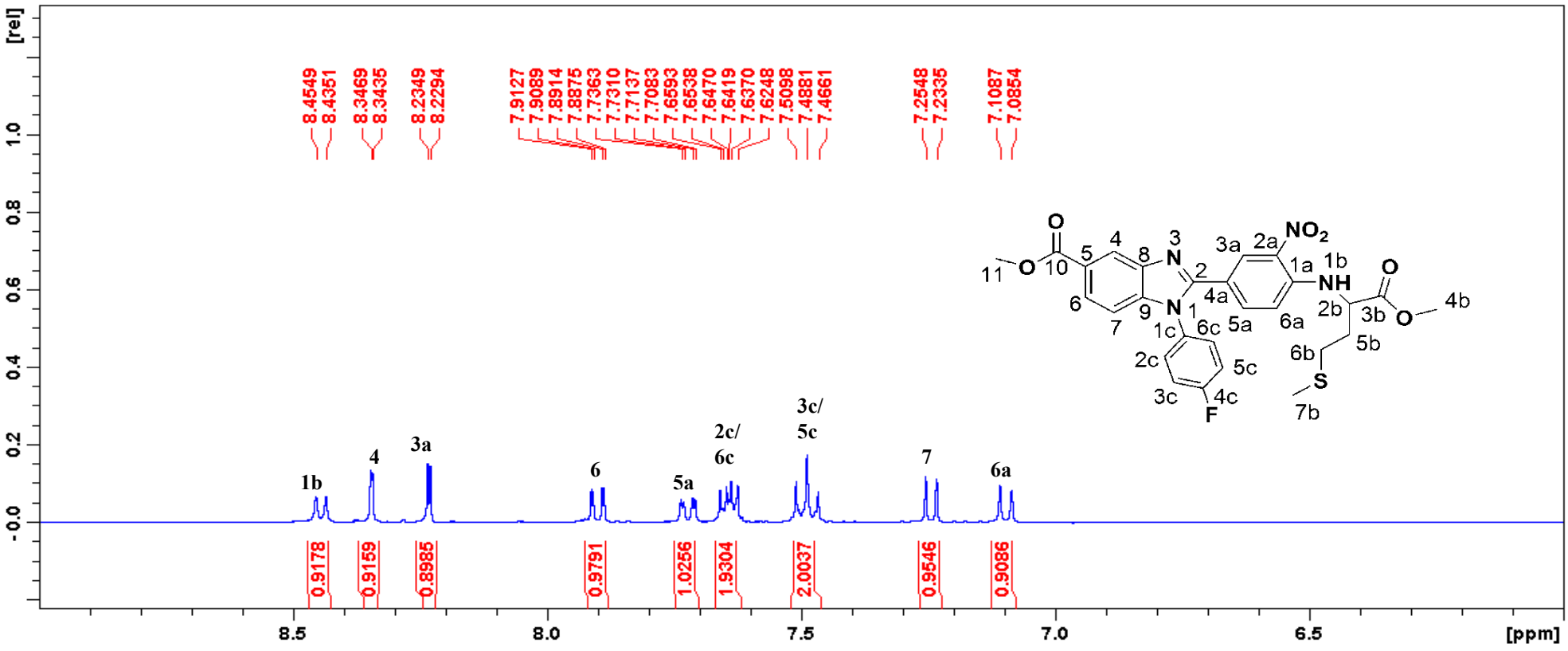


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
471.1340	471.1338	0.2	0.4	13.5	23.1	0.0	C22 H23 N4 O6 S

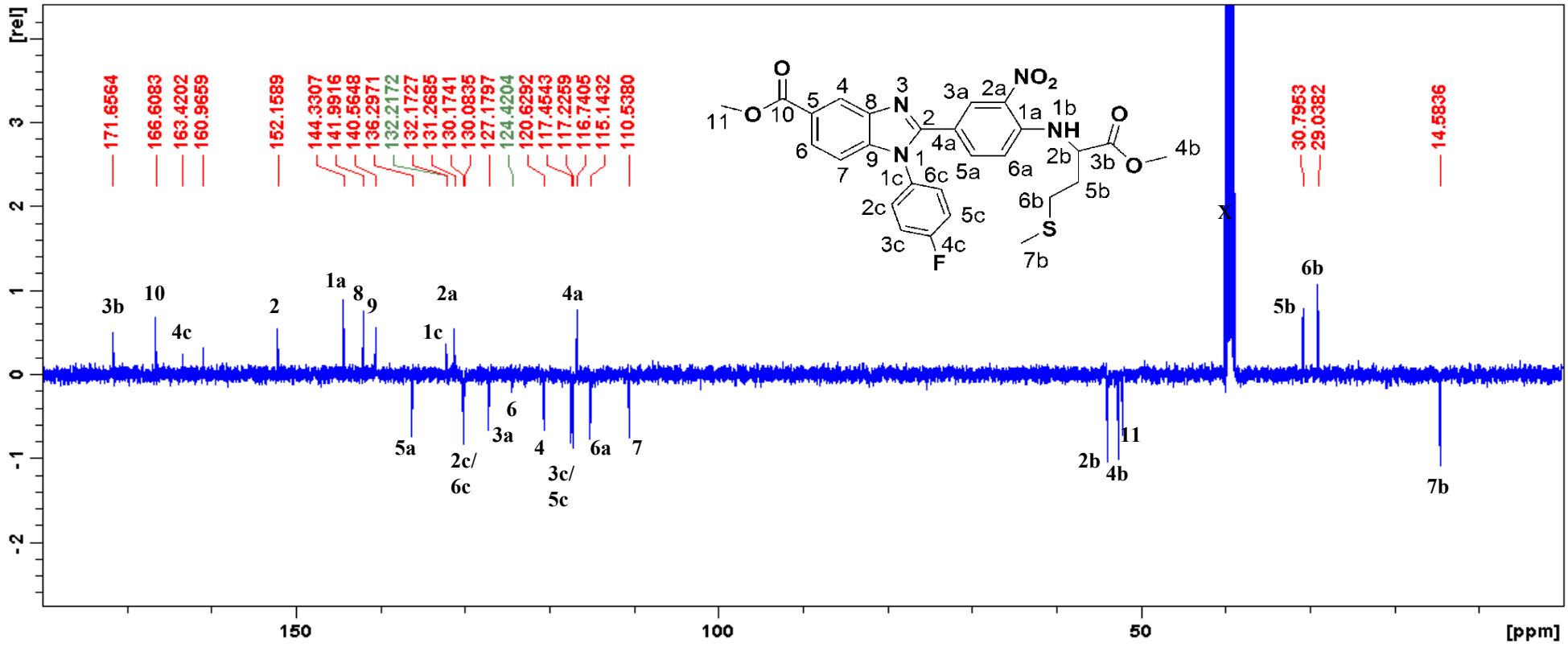
HRMS Spectrum of Compound B-7c



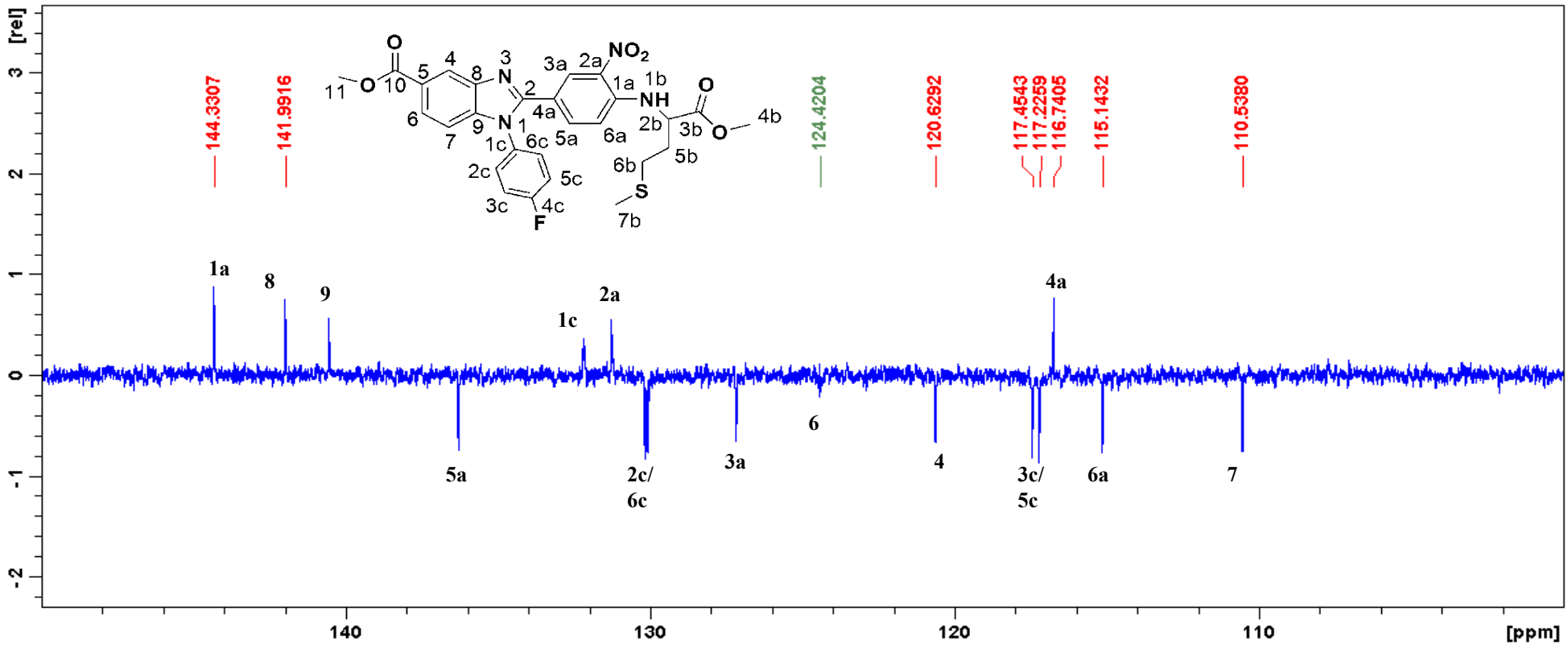
^{1}H Spectrum of Compound B-7d



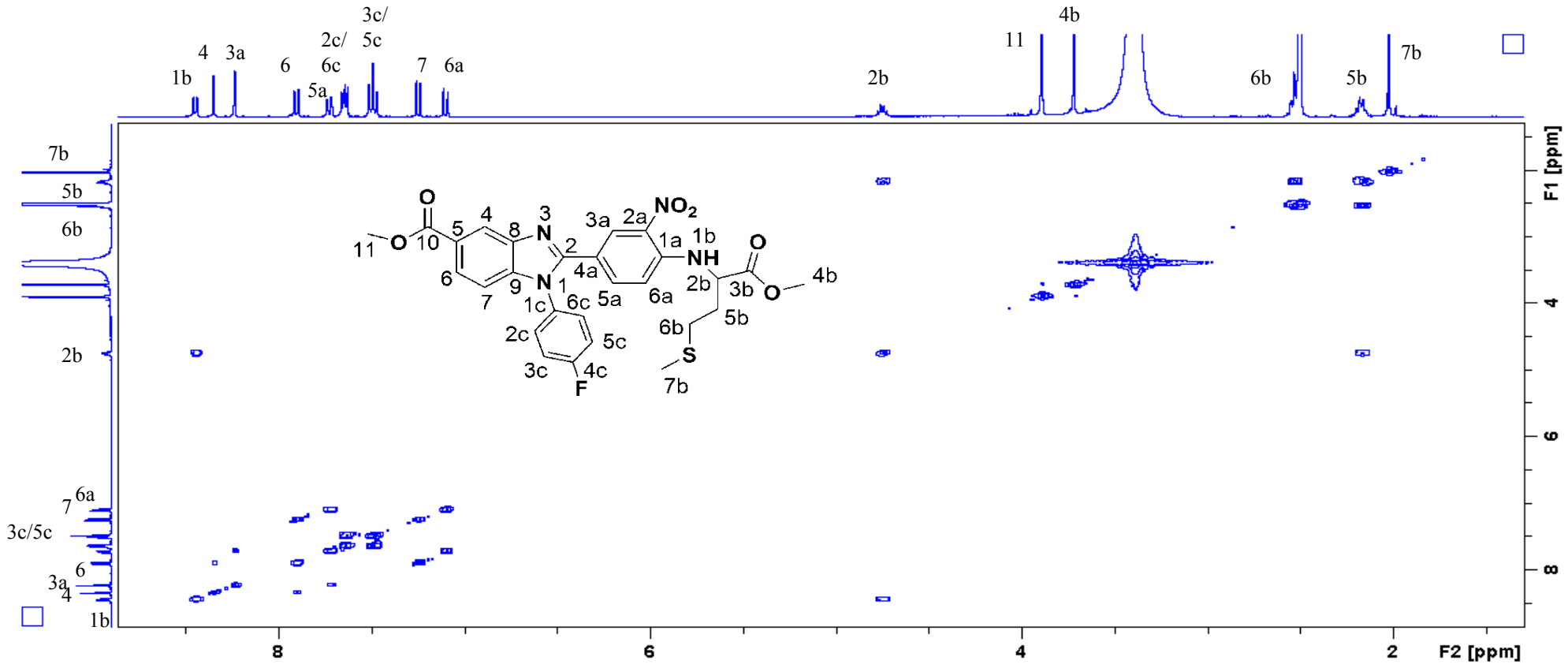
Expended ^{1}H Spectrum of Compound B-7d



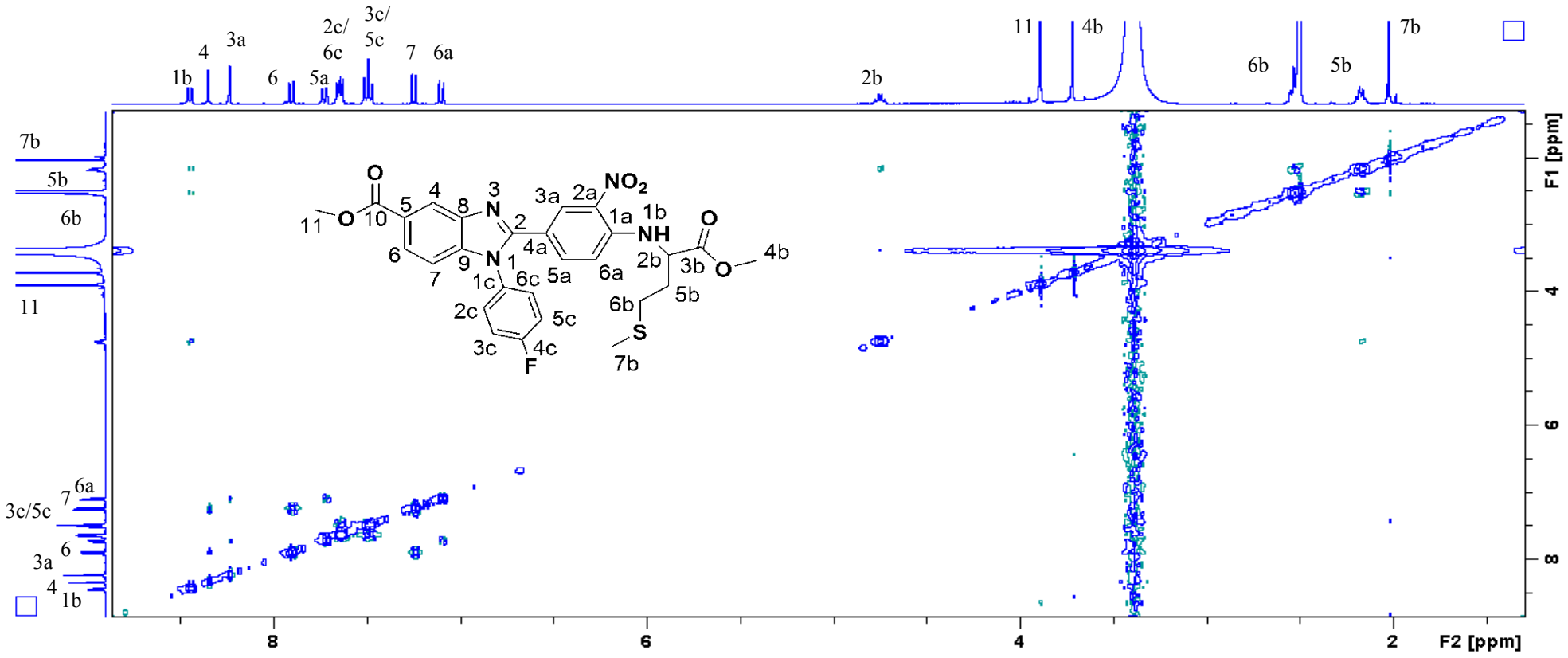
^{13} C Spectrum of Compound B-7d



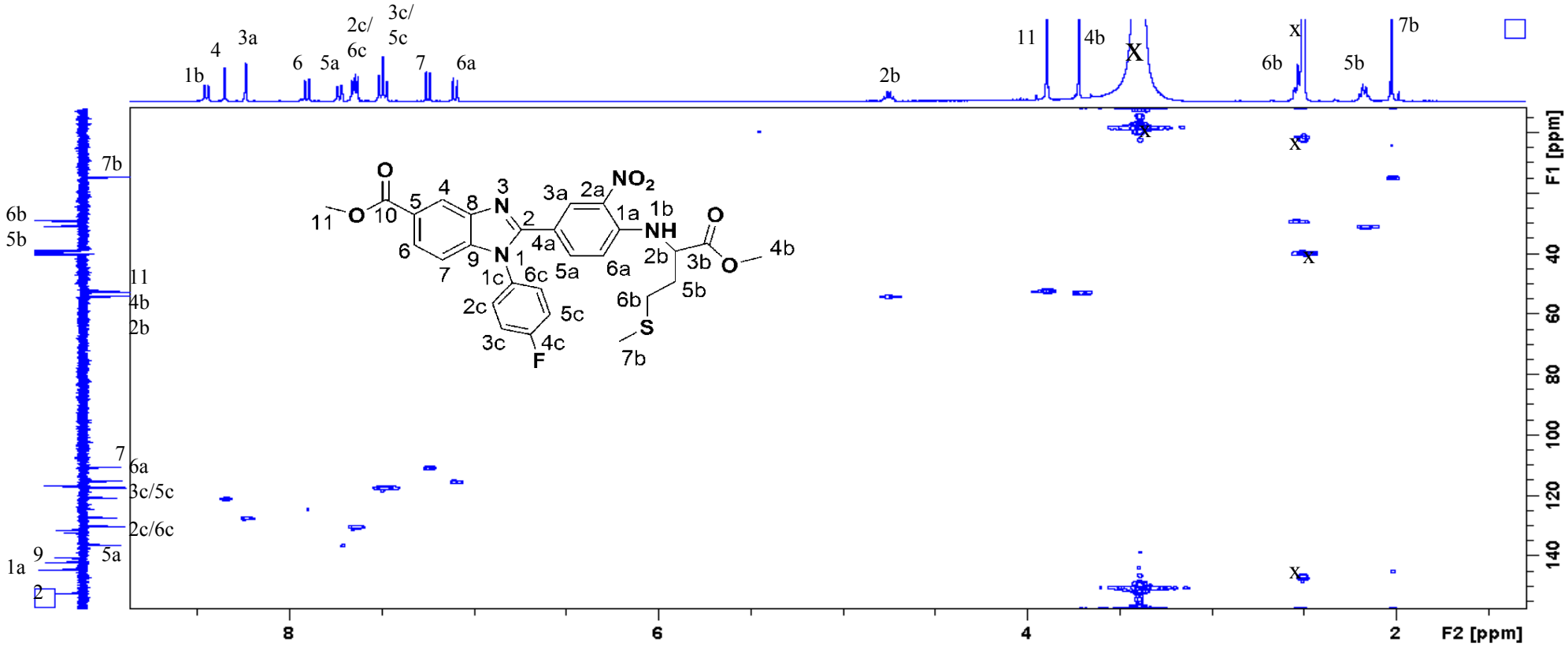
Expanded ^{13}C Spectrum of Compound B-7d



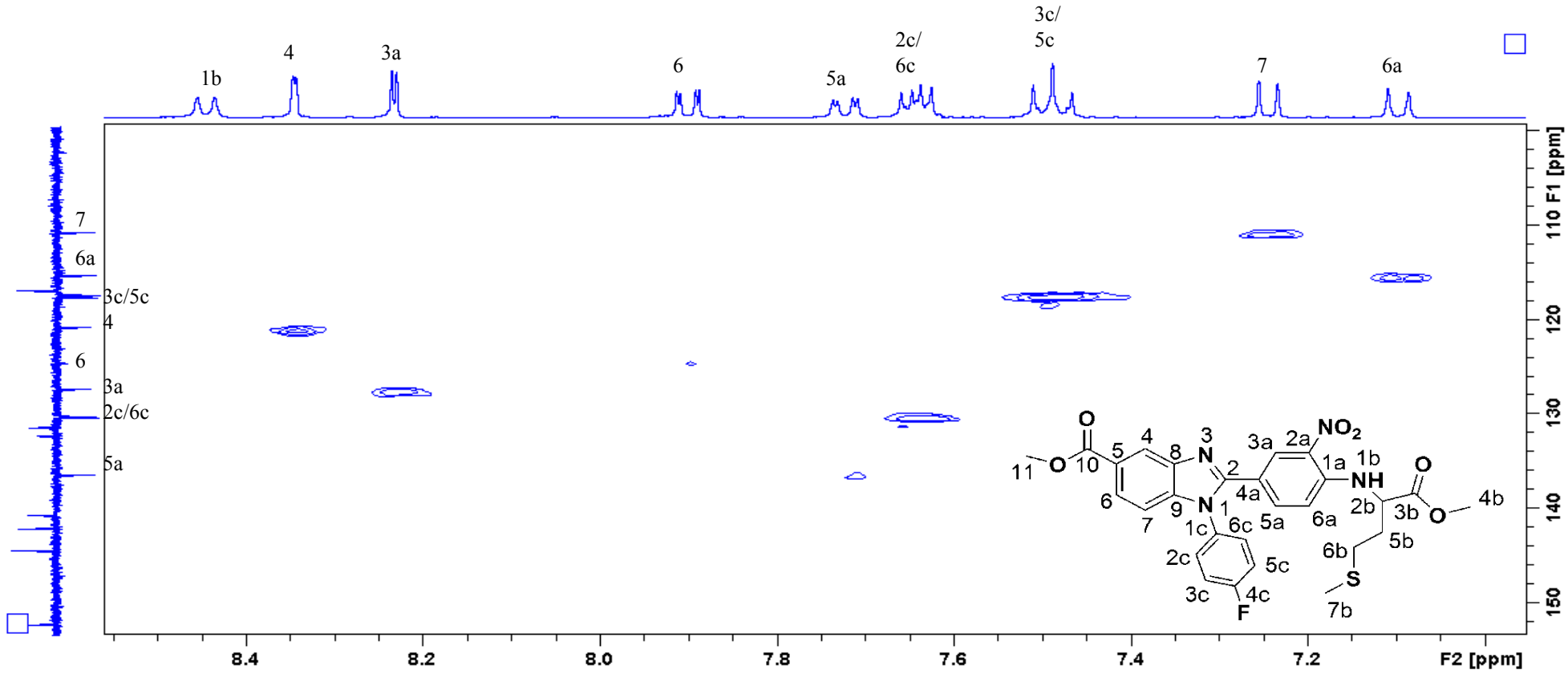
COSY Spectrum of Compound B-7d



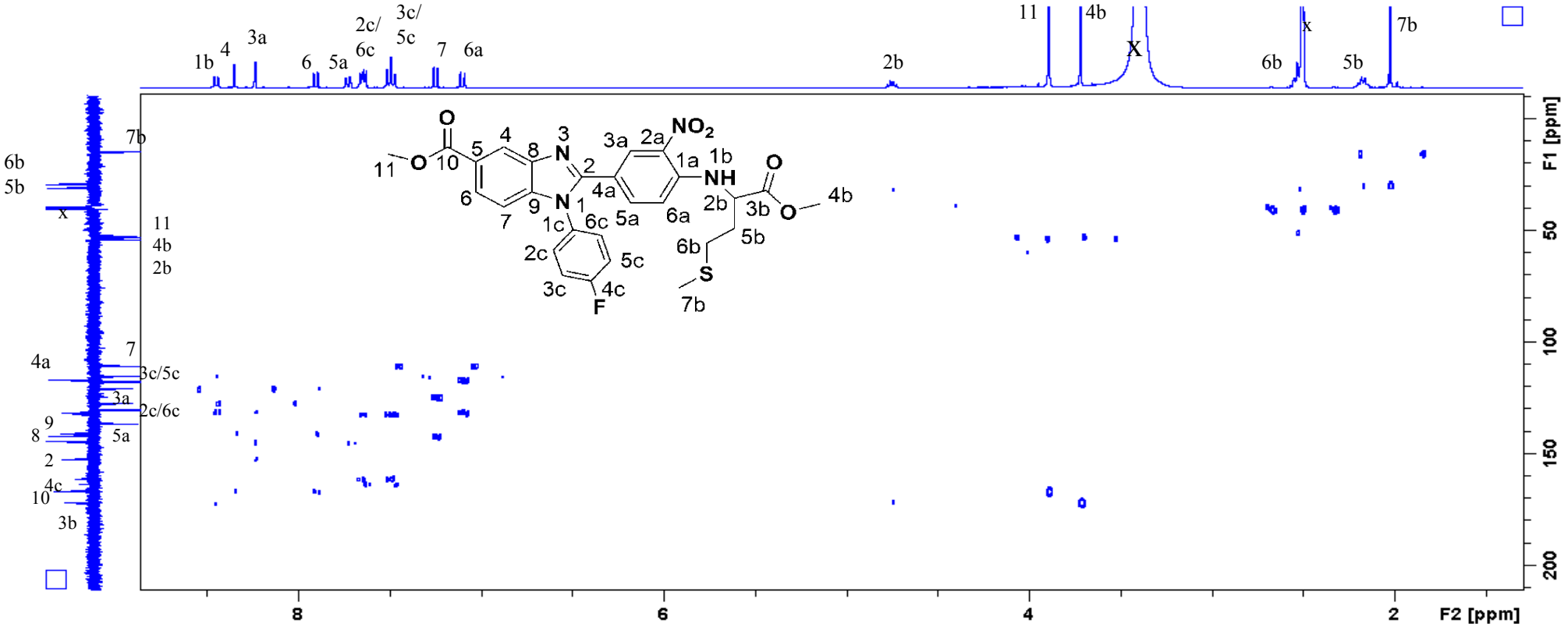
NOESY Spectrum of Compound B-7d



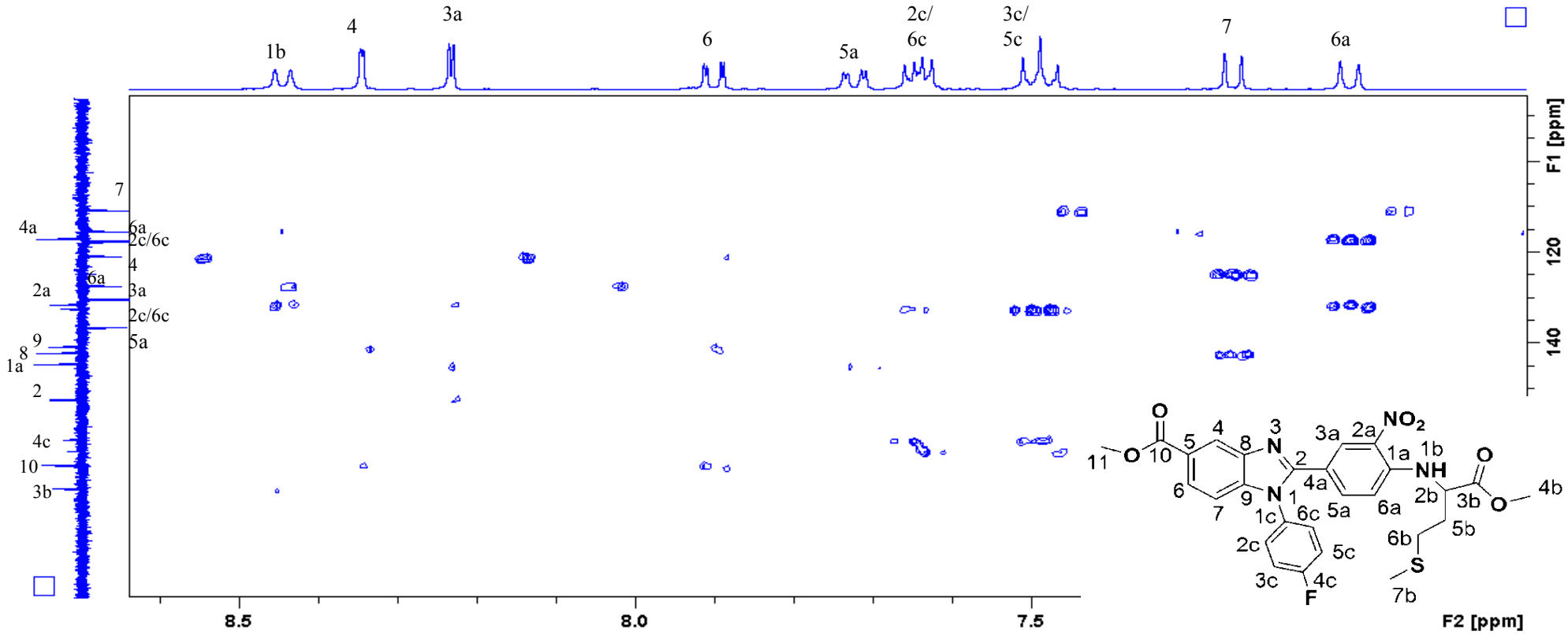
HSQC Spectrum of Compound B-7d



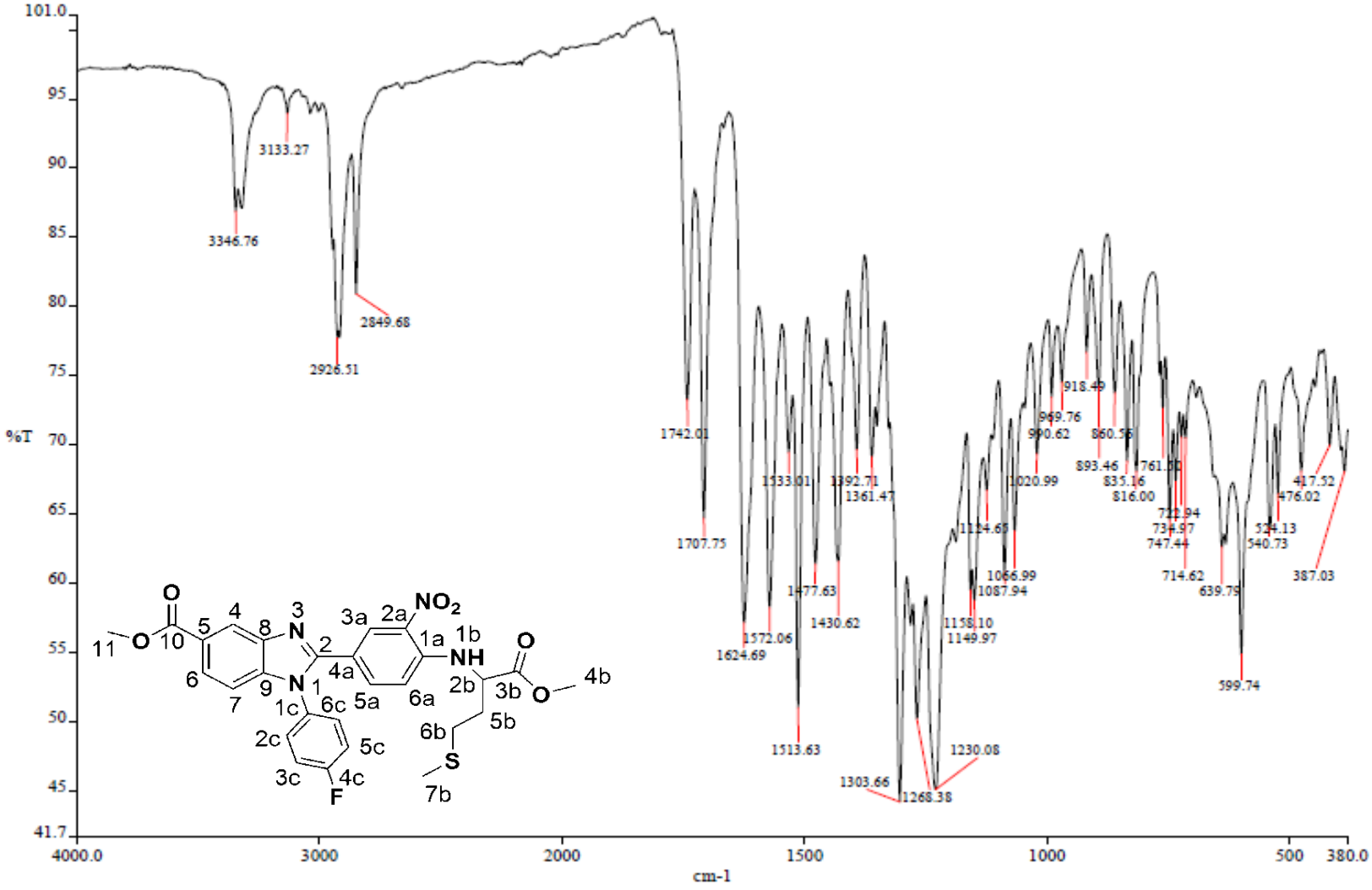
Expanded HSQC Spectrum of Compound B-7d



HMBC Spectrum of Compound B-7d

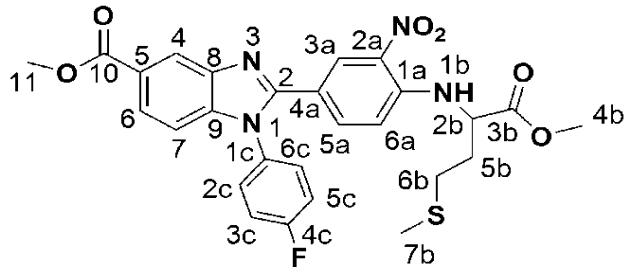


Expanded HMBC Spectrum of Compound B-7d



Infrared Spectrum of Compound B-7d

Elemental Composition Report

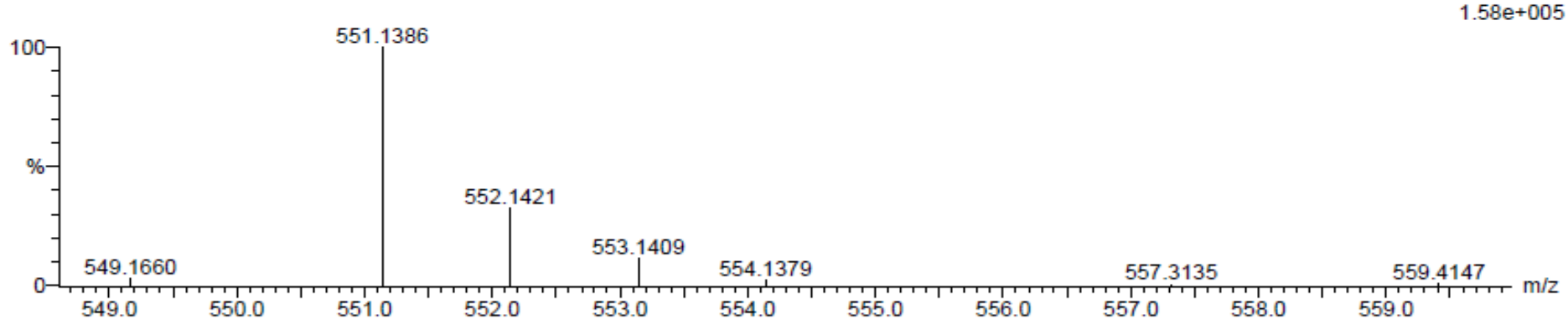


Single Mass Analysis

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

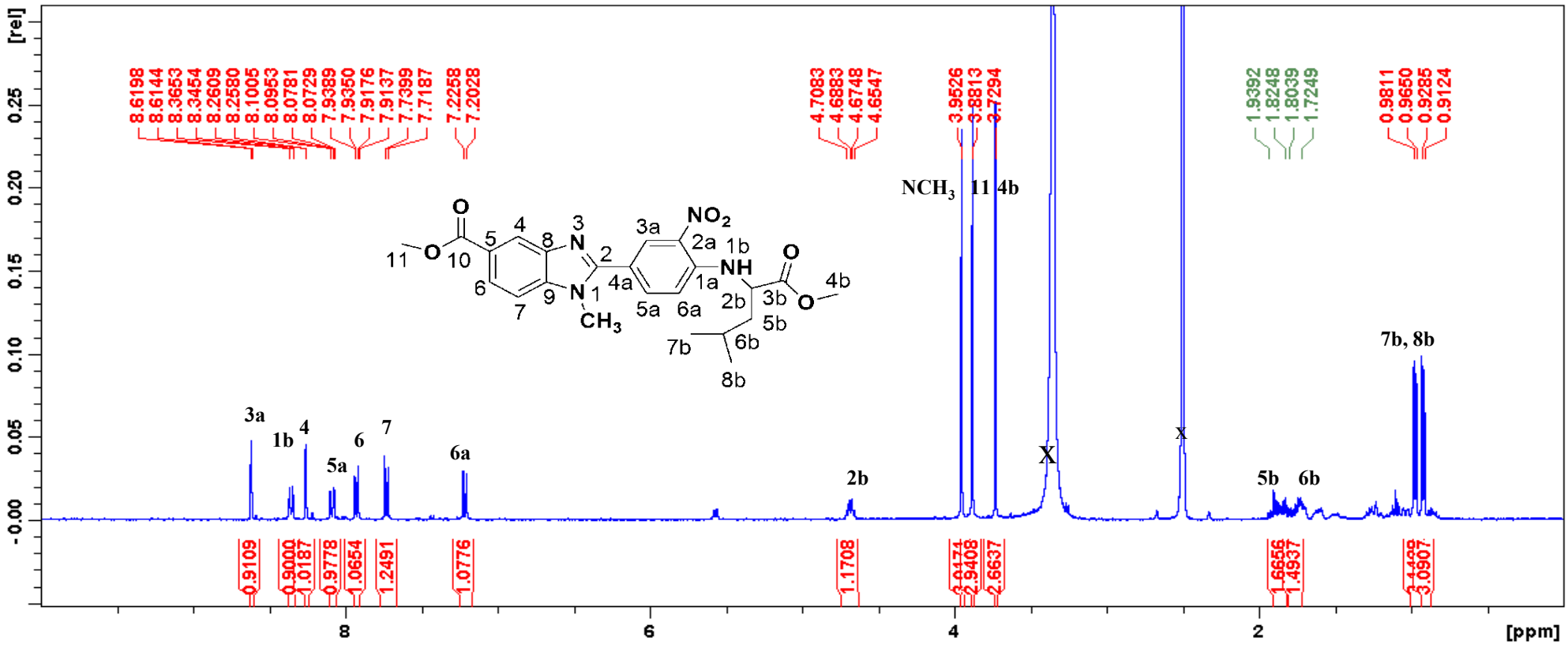
Monoisotopic Mass, Even Electron Ions
24 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 25-30 H: 20-25 N: 0-5 O: 0-10 F: 1-1 S: 1-1

BA 5 30 (0.978) Cm (1:61)
TOF MS ES-

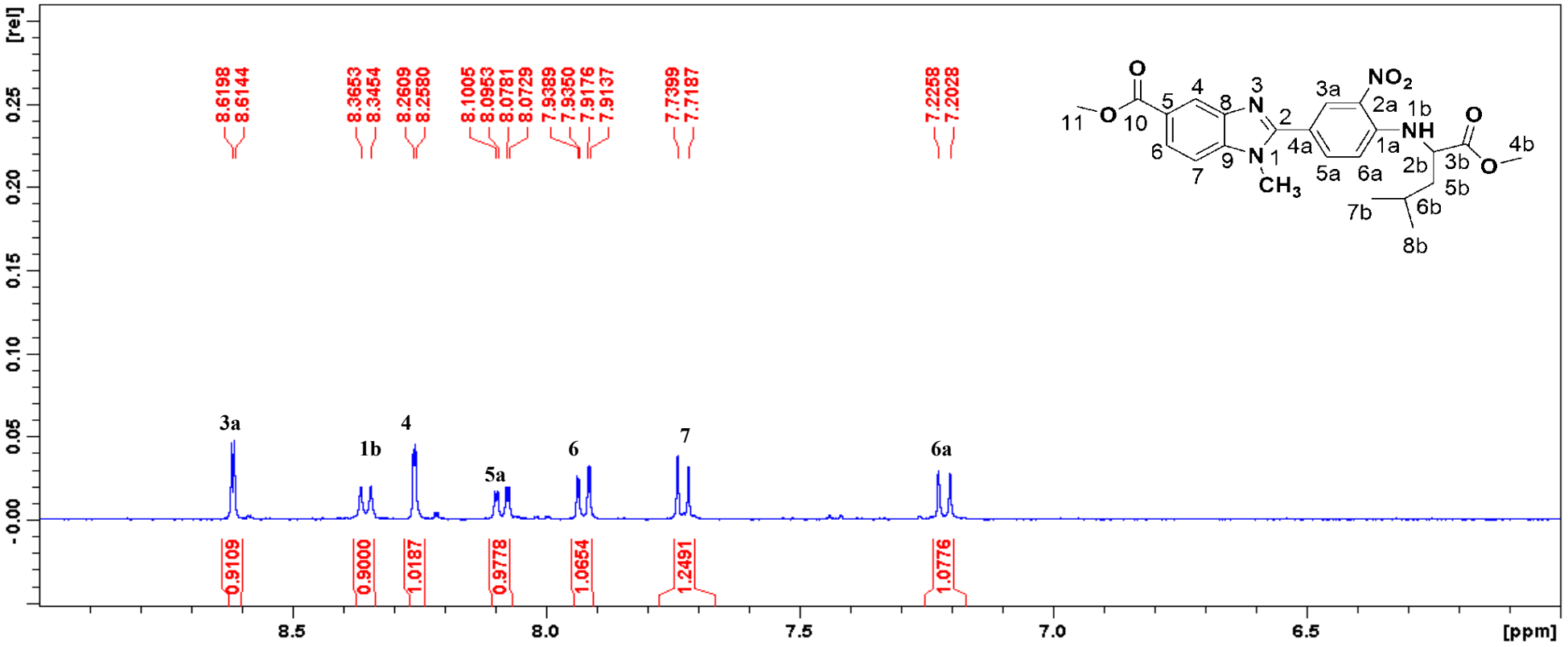


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
551.1386	551.1401	-1.5	-2.7	17.5	10.6	0.0	C27 H24 N4 O6 F S

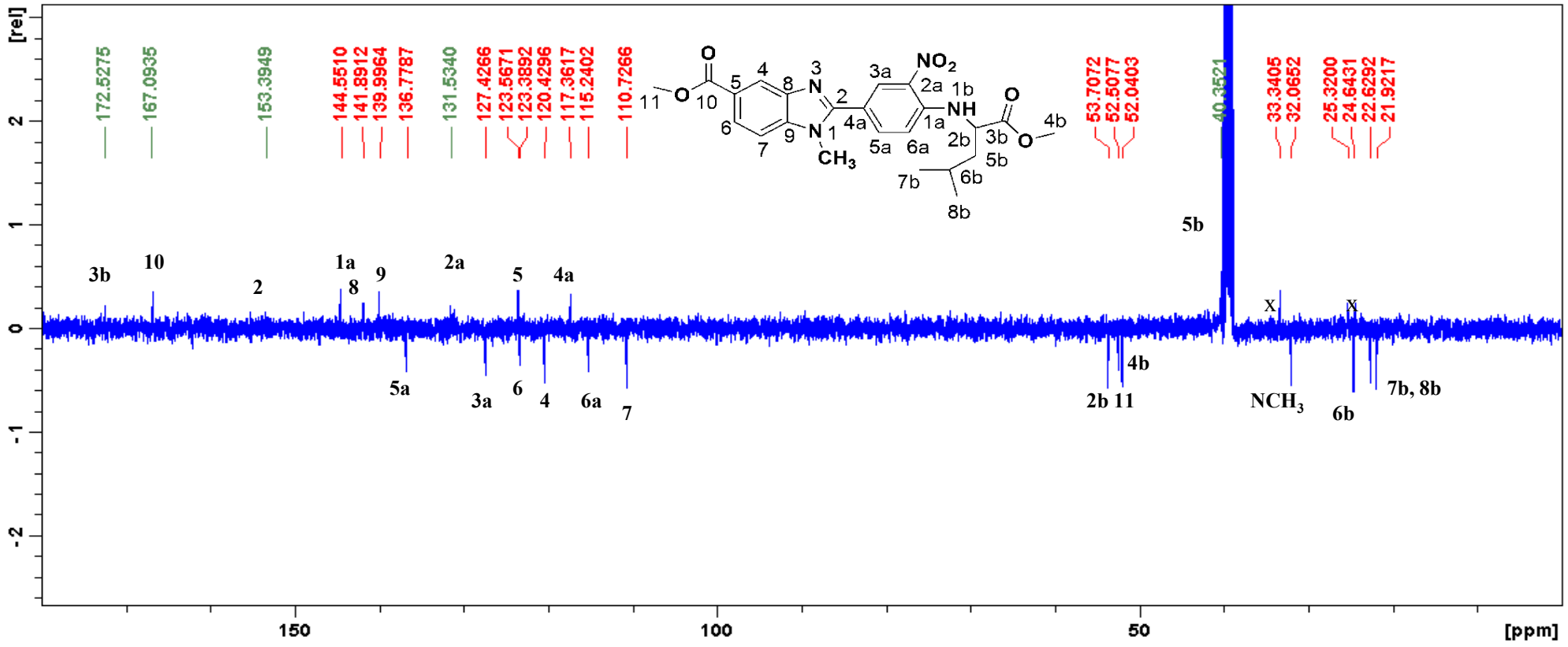
HRMS Spectrum of Compound B-7d



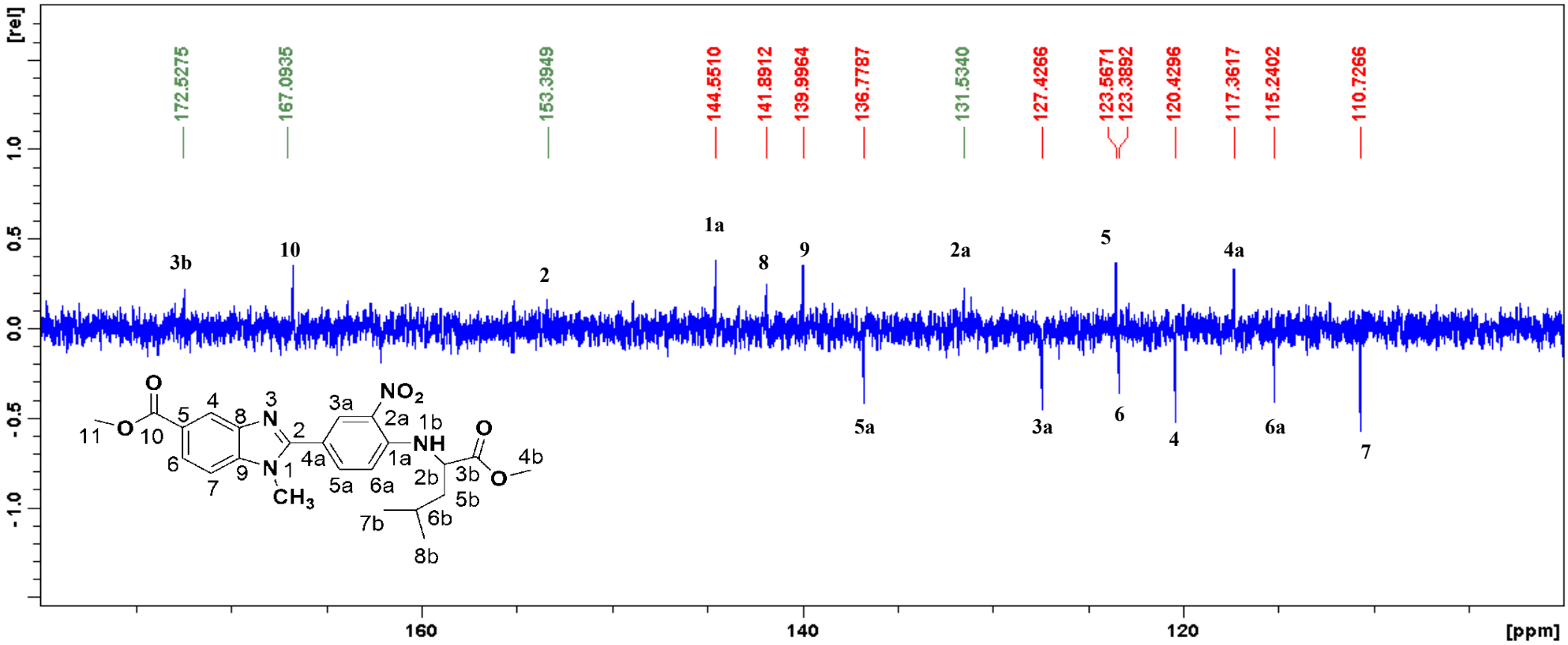
^{1}H Spectrum of Compound B-7e



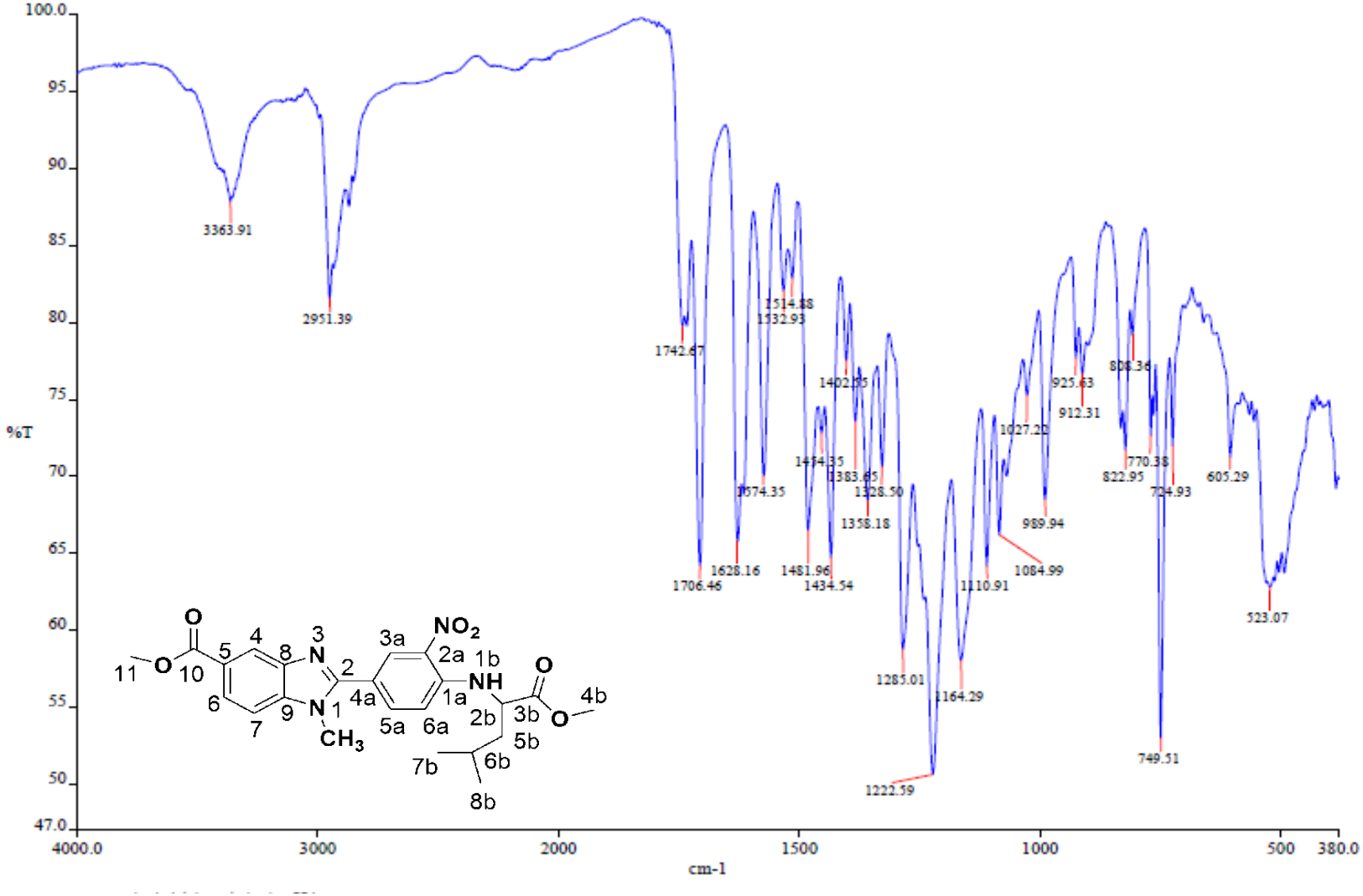
Expanded ^{1}H Spectrum of Compound B-7e



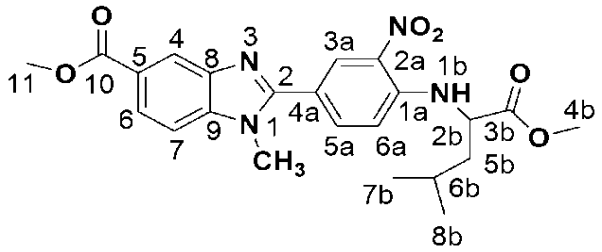
^{13}C Spectrum of Compound B-7e



Expanded ^{13}C Spectrum of Compound B-7e



Infrared Spectrum of Compound B-7e

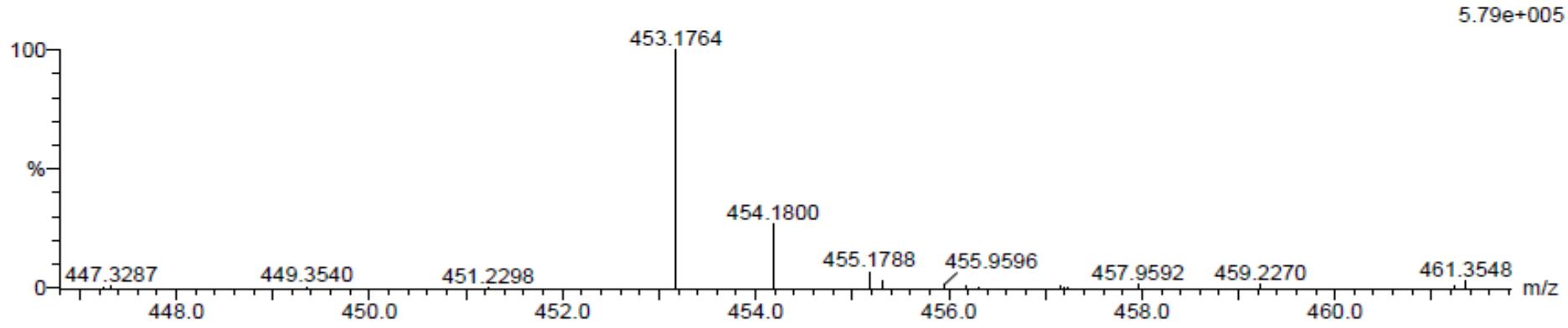


Elemental Composition Report

Single Mass Analysis

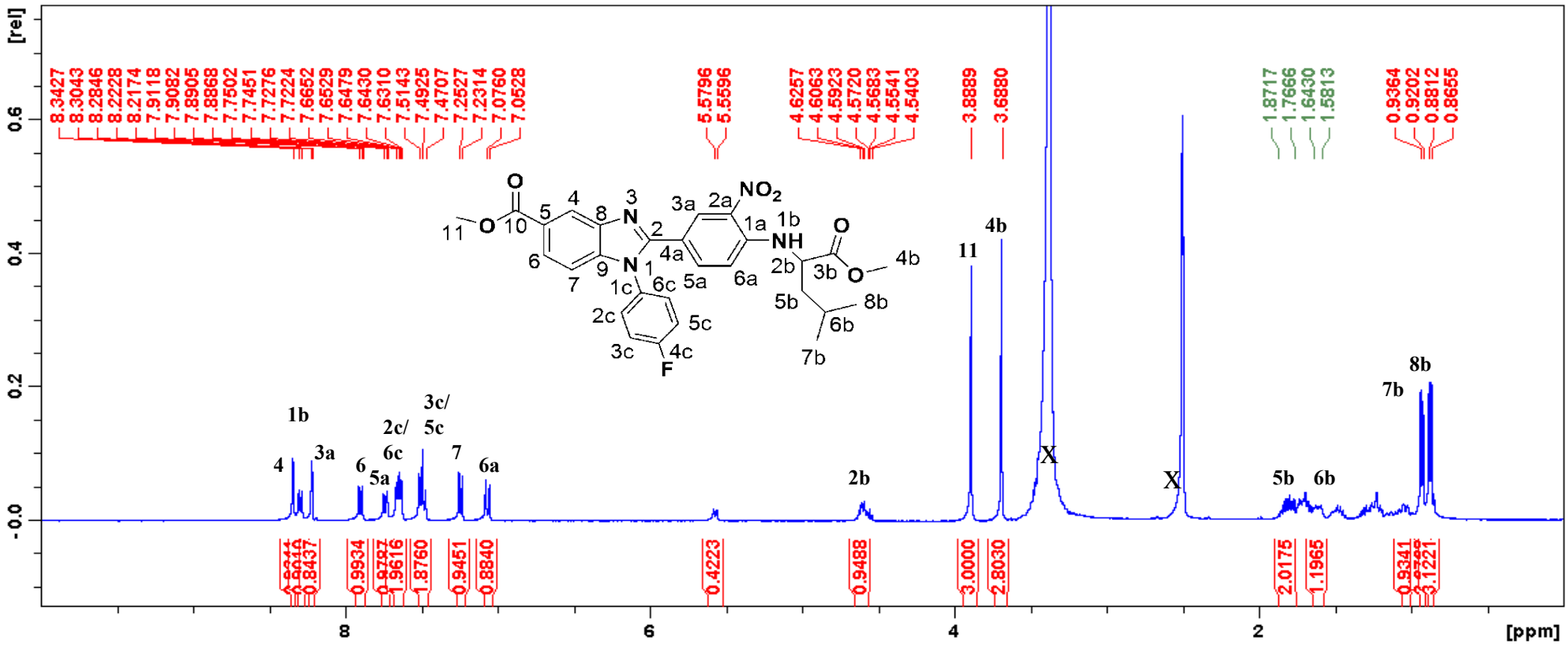
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
25 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 25-30 N: 0-5 O: 0-8
BA 10 24 (0.776) Cm (1:61)
TOF MS ES-

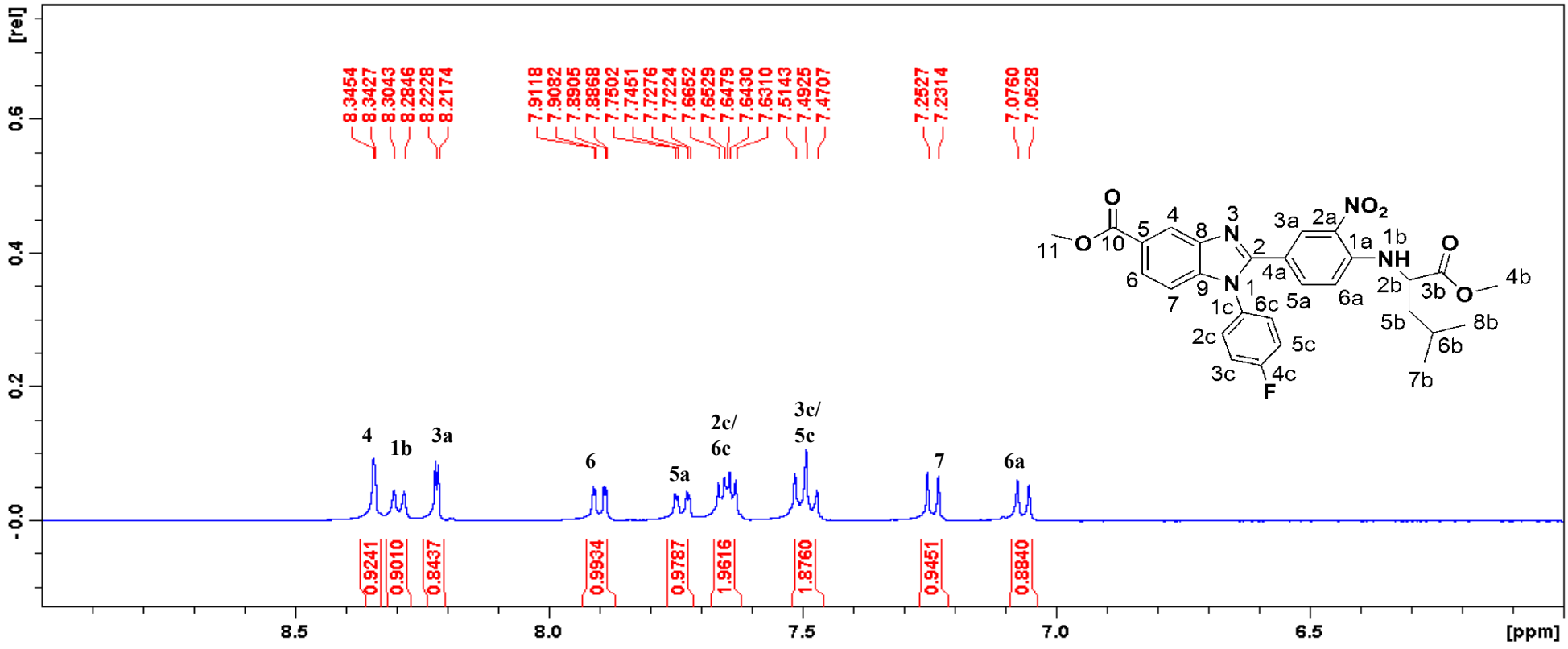


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
453.1764	453.1774	-1.0	-2.2	13.5	14.0	0.0	C23 H25 N4 O6

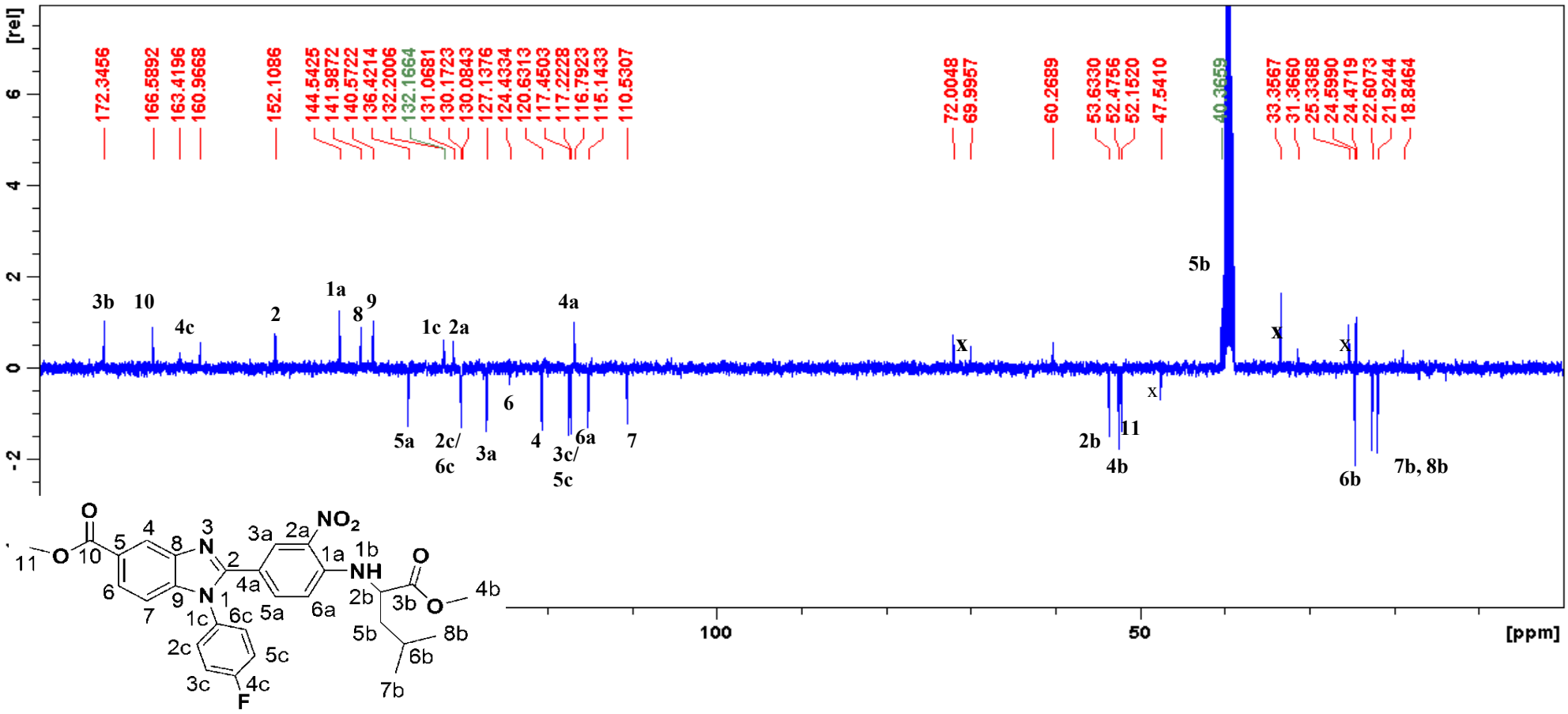
HRMS Spectrum of Compound B-7e



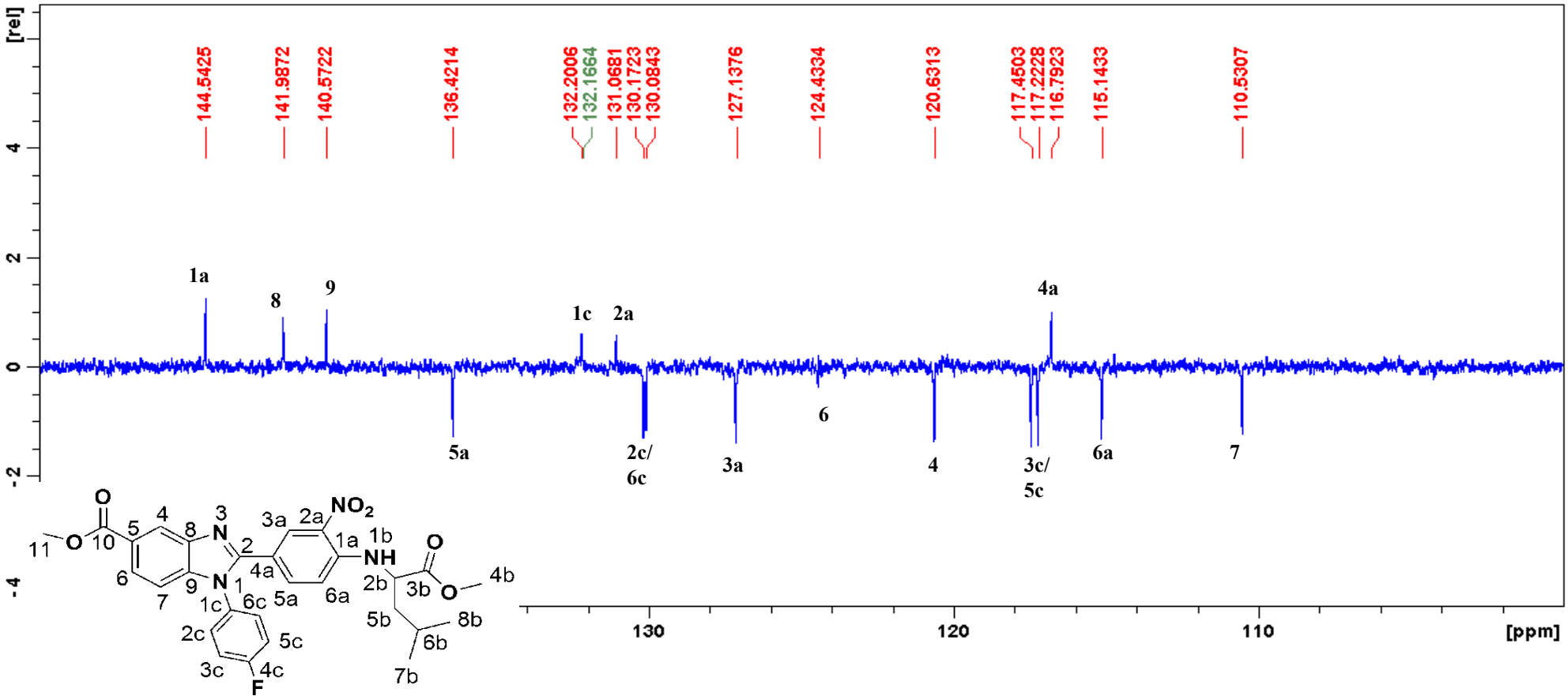
^{1}H Spectrum of Compound B-7f



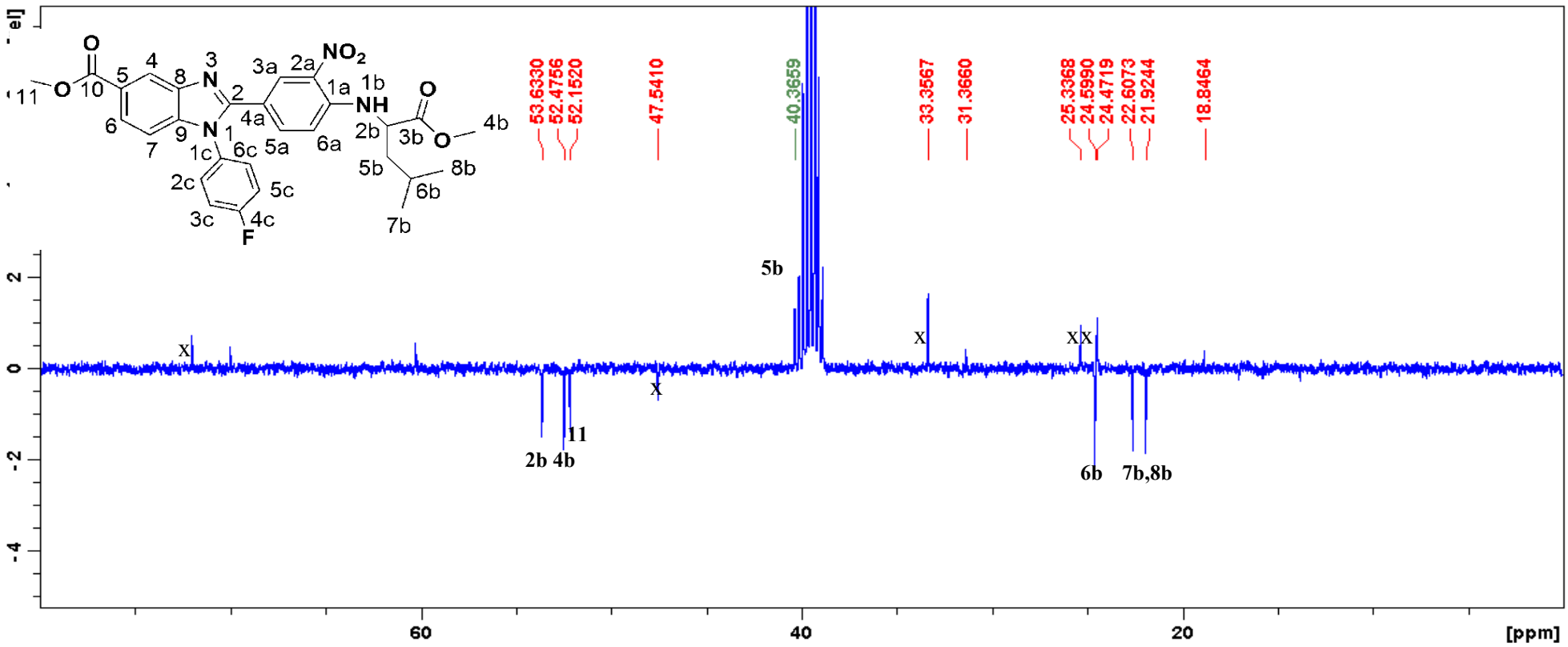
Expanded ^{1}H Spectrum of Compound B-7f



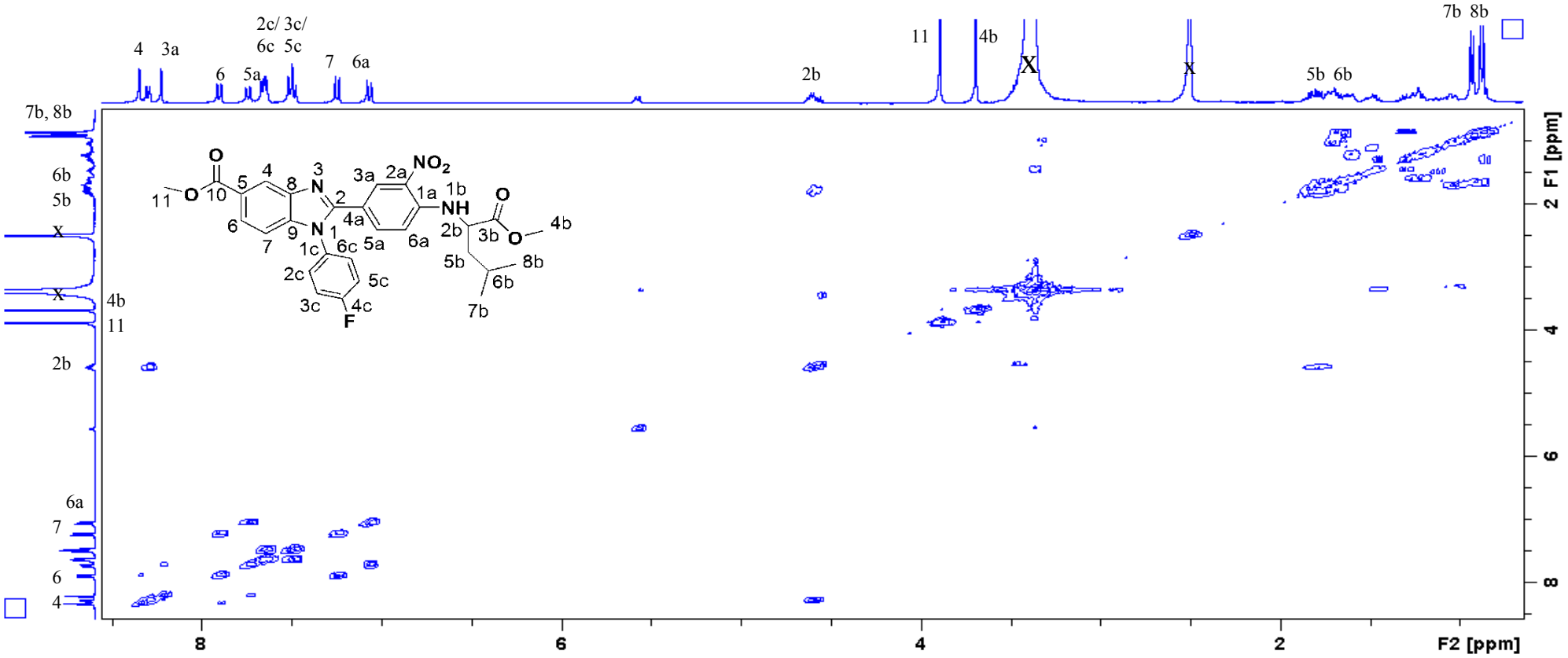
^{13}C Spectrum of Compound B-7f



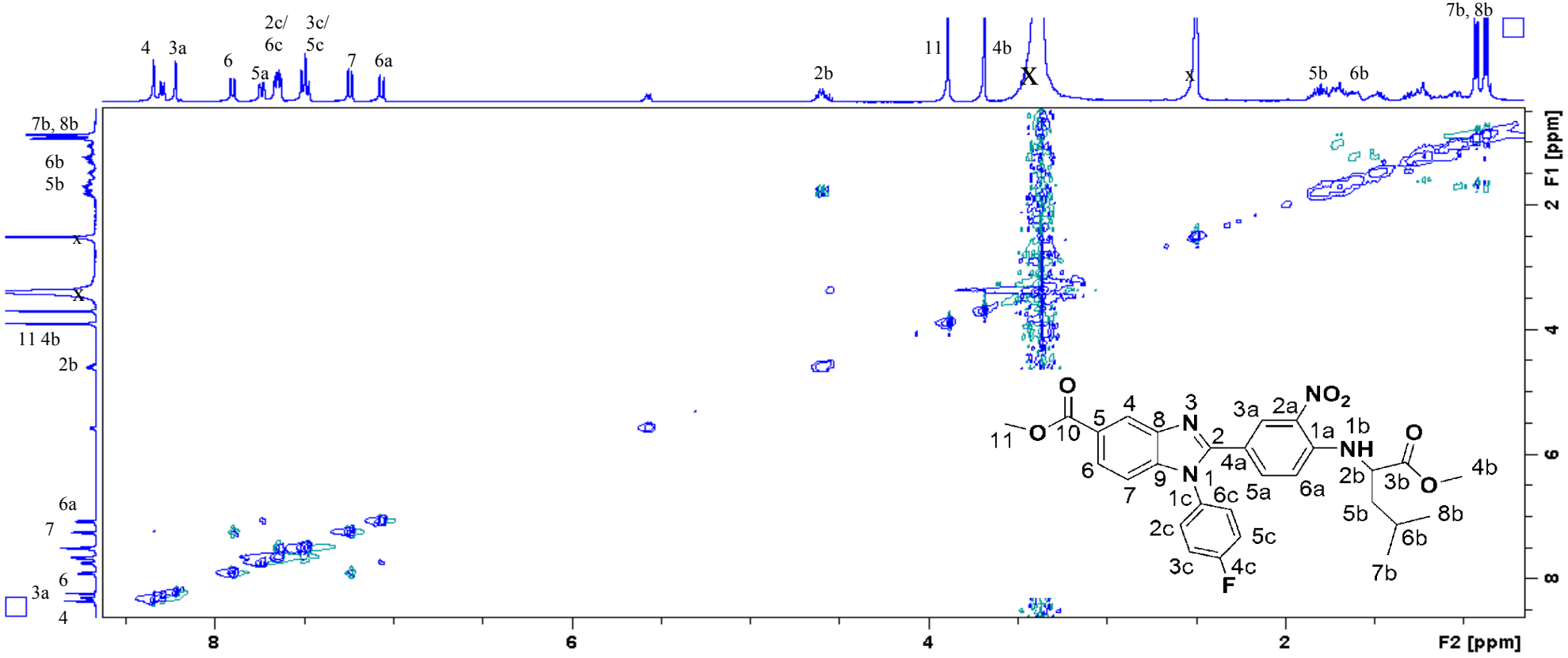
Expanded ^{13}C Spectrum of Compound B-7f



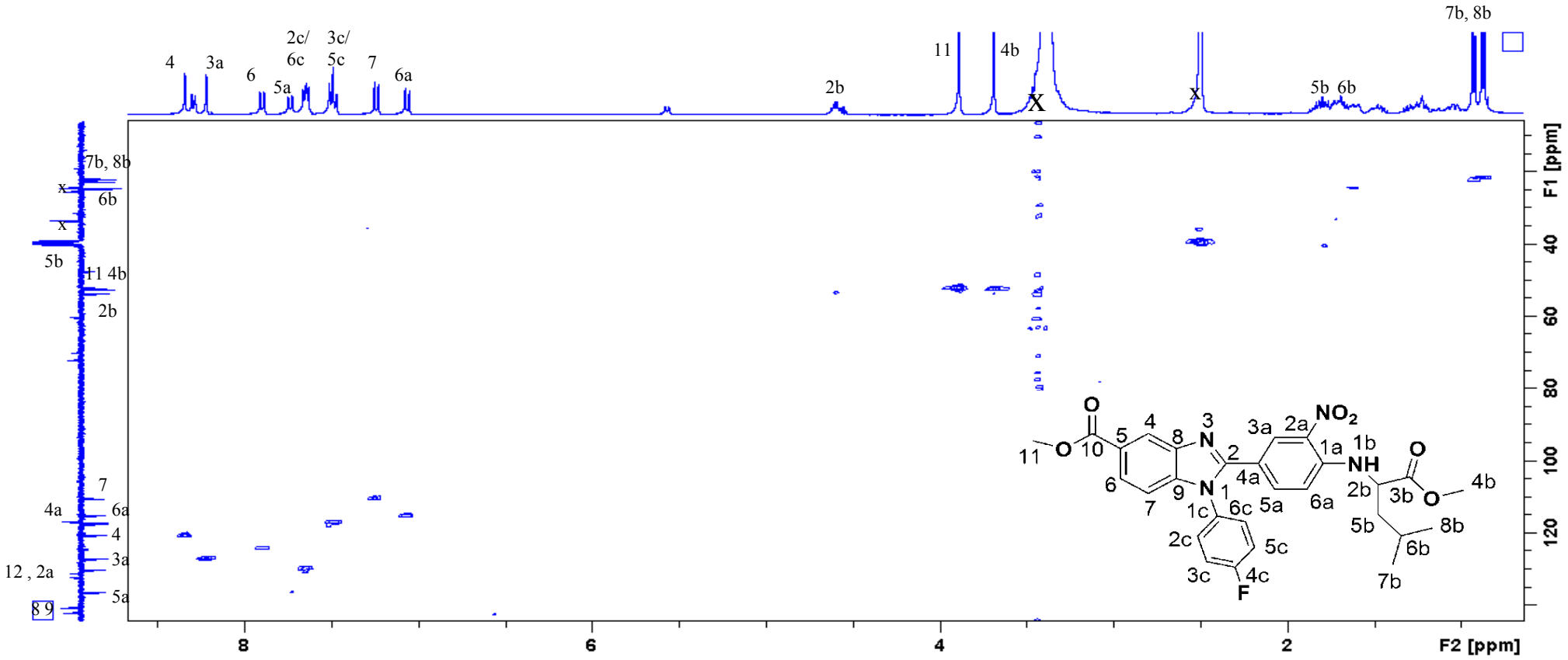
Expanded ^{13}C Spectrum of Compound B-7f



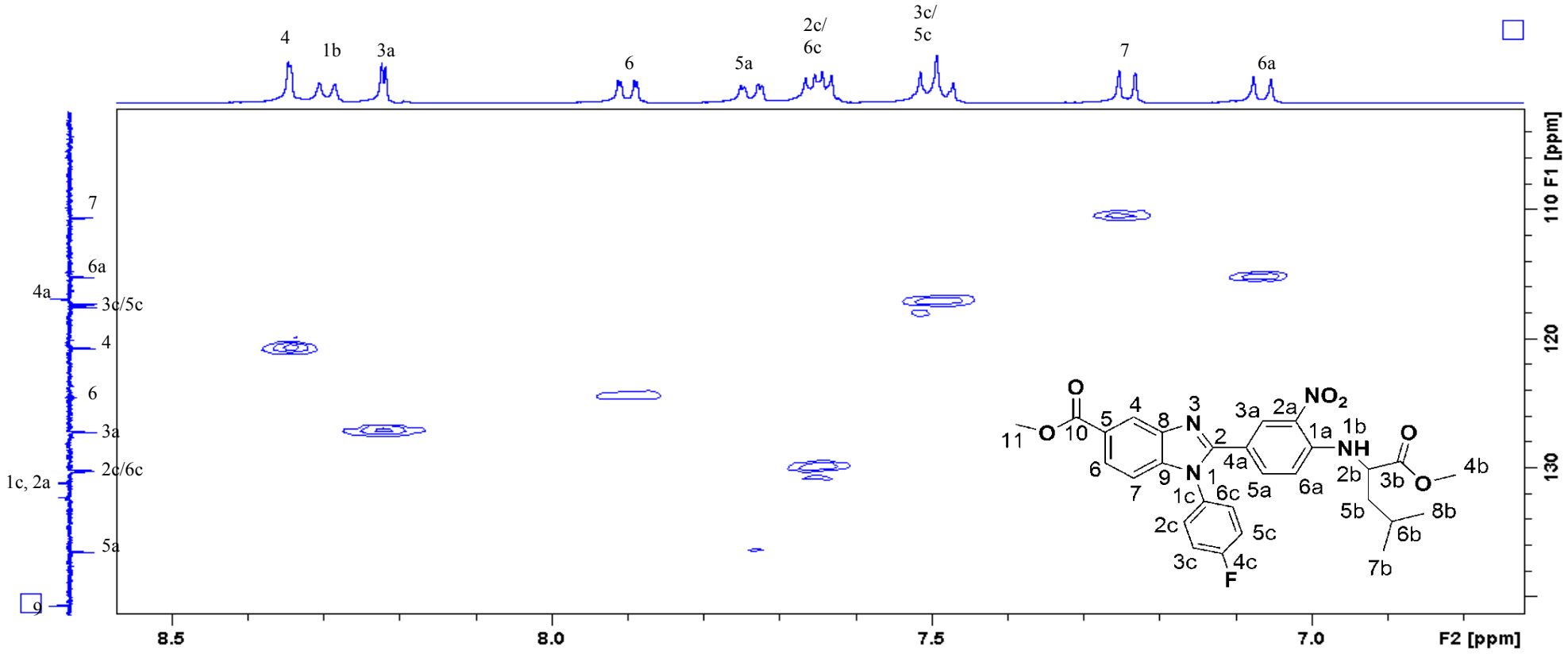
COSY Spectrum of Compound B-7f



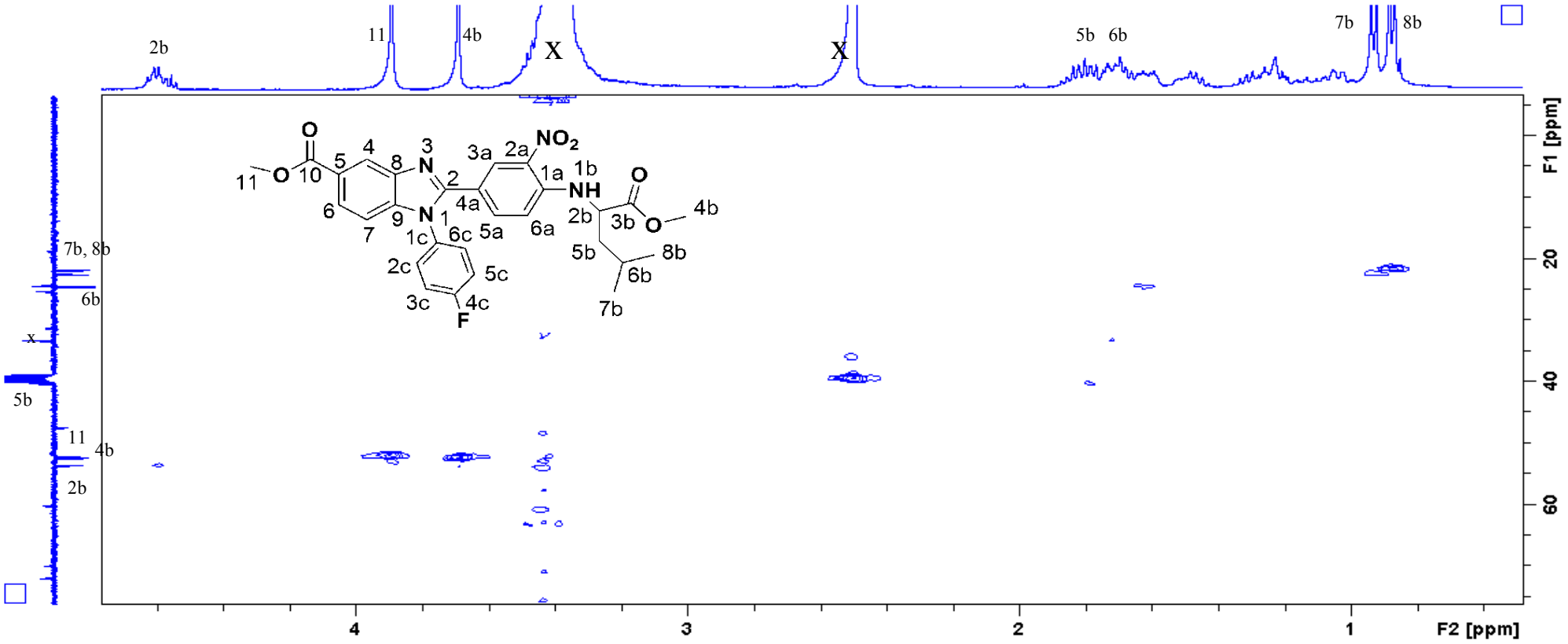
NOESY Spectrum of Compound B-7f



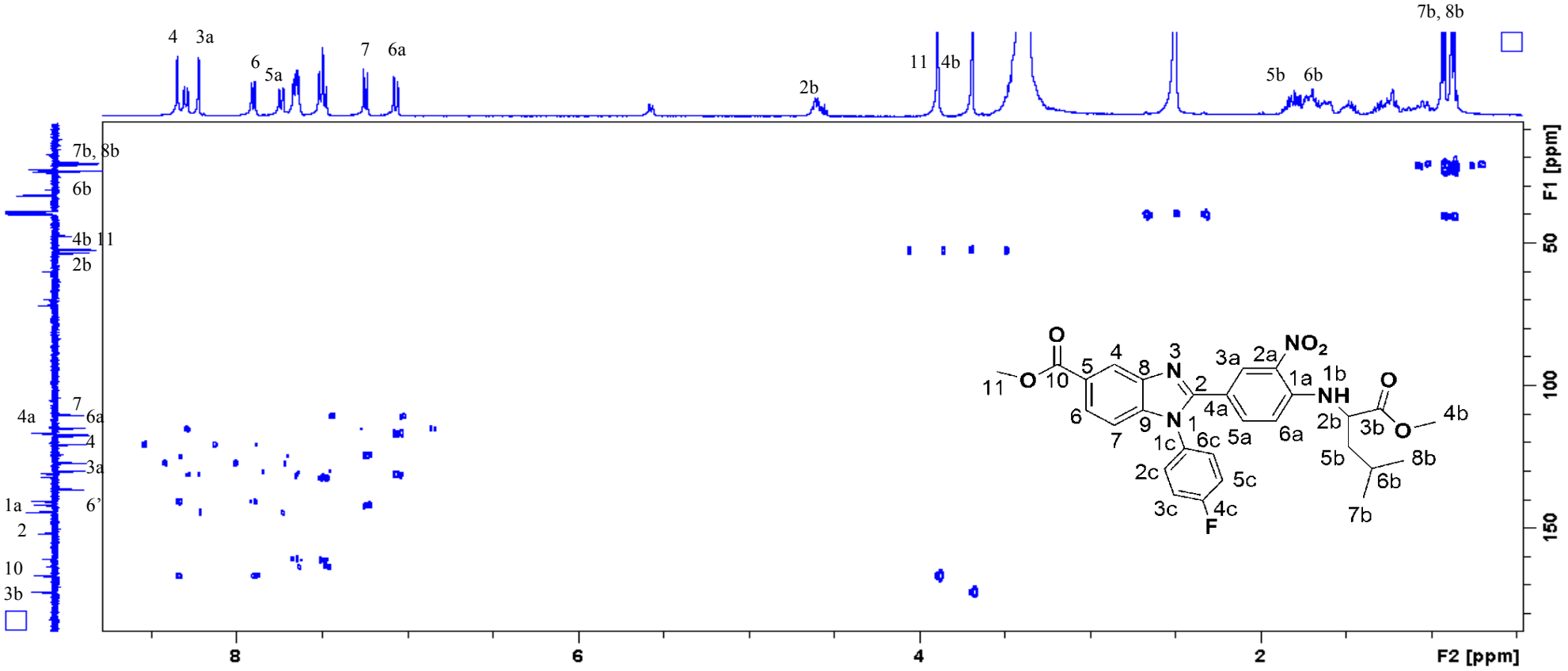
HSQC Spectrum of Compound B7-f



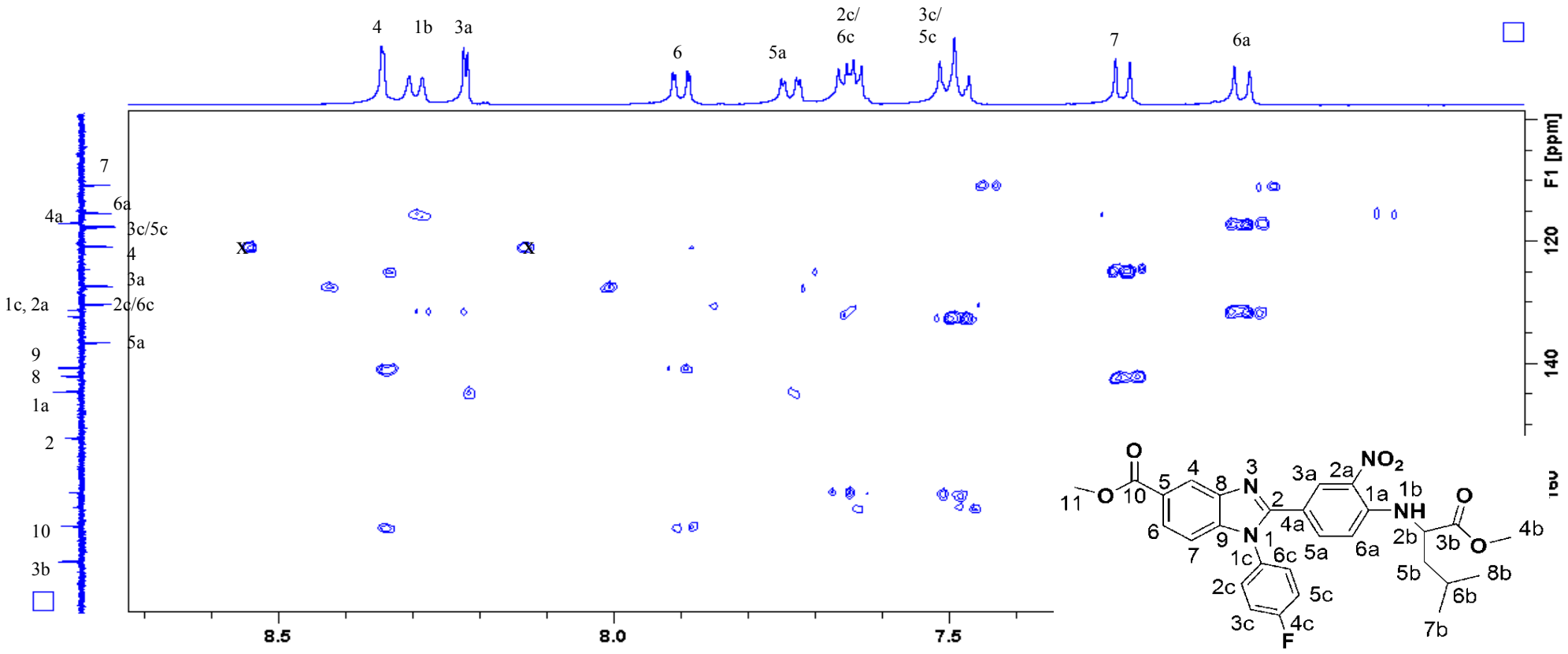
Expanded HSQC Spectrum of Compound B-7F



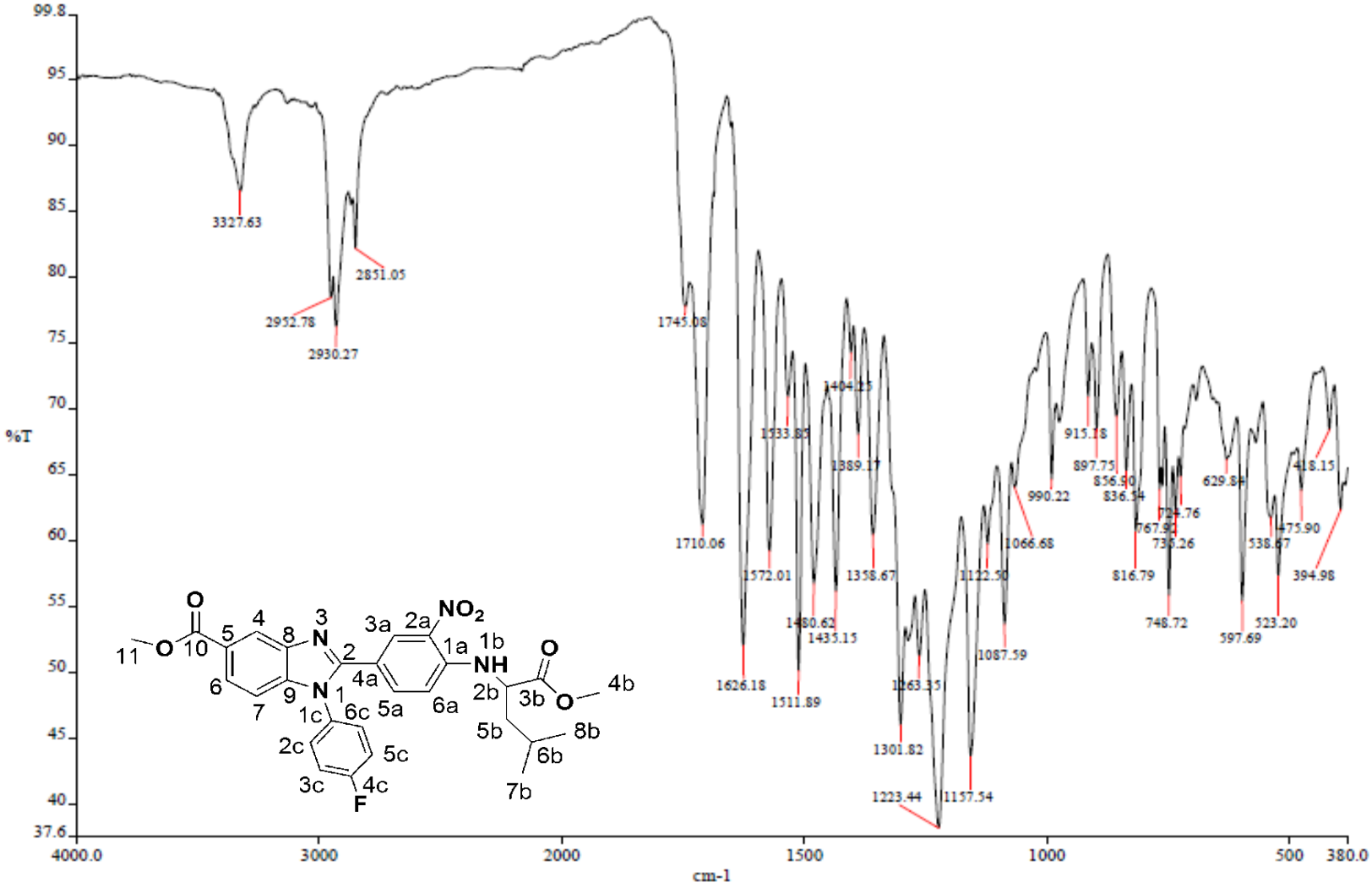
Expanded HSQC Spectrum of Compound B-7f



HMBC Spectrum of Compound B-7f

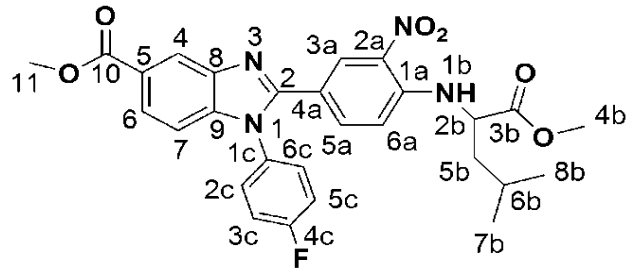


Expanded HMBC Spectrum of Compound B-7f



Infrared Spectrum of Compound B-7f

Elemental Composition Report

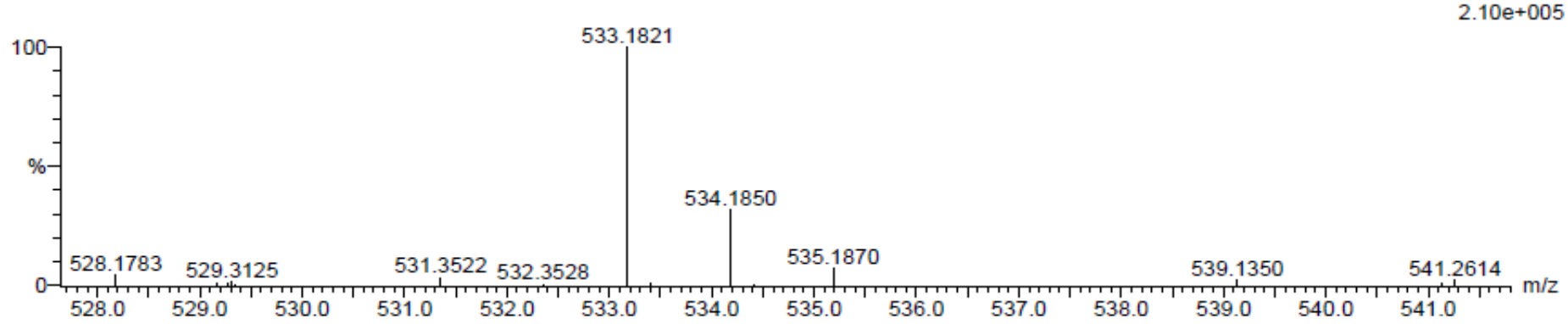


Single Mass Analysis

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

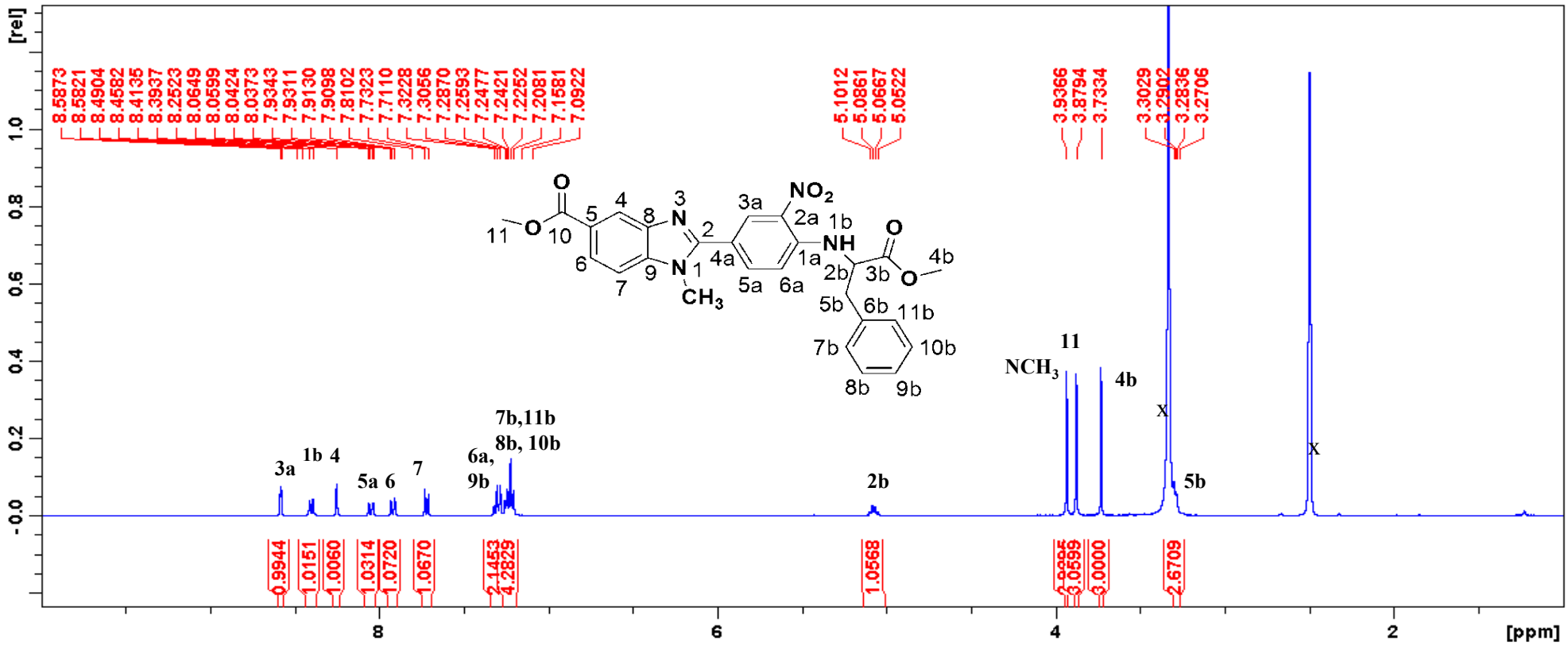
Monoisotopic Mass, Even Electron Ions
22 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 25-30 H: 25-30 N: 0-5 O: 0-8 F: 1-1

BA 9 44 (1.451) Cm (1:61)
TOF MS ES-

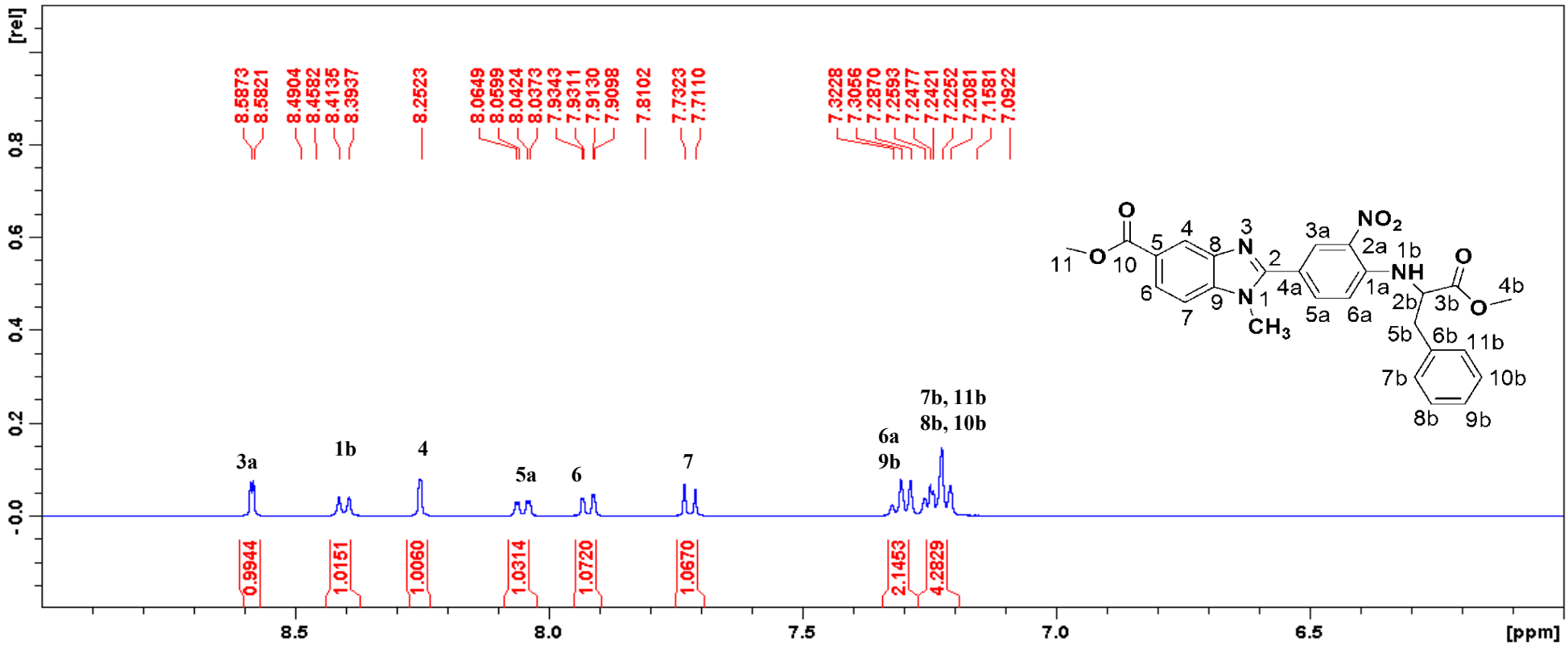


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
533.1821	533.1836	-1.5	-2.8	17.5	43.4	0.0	C28 H26 N4 O6 F

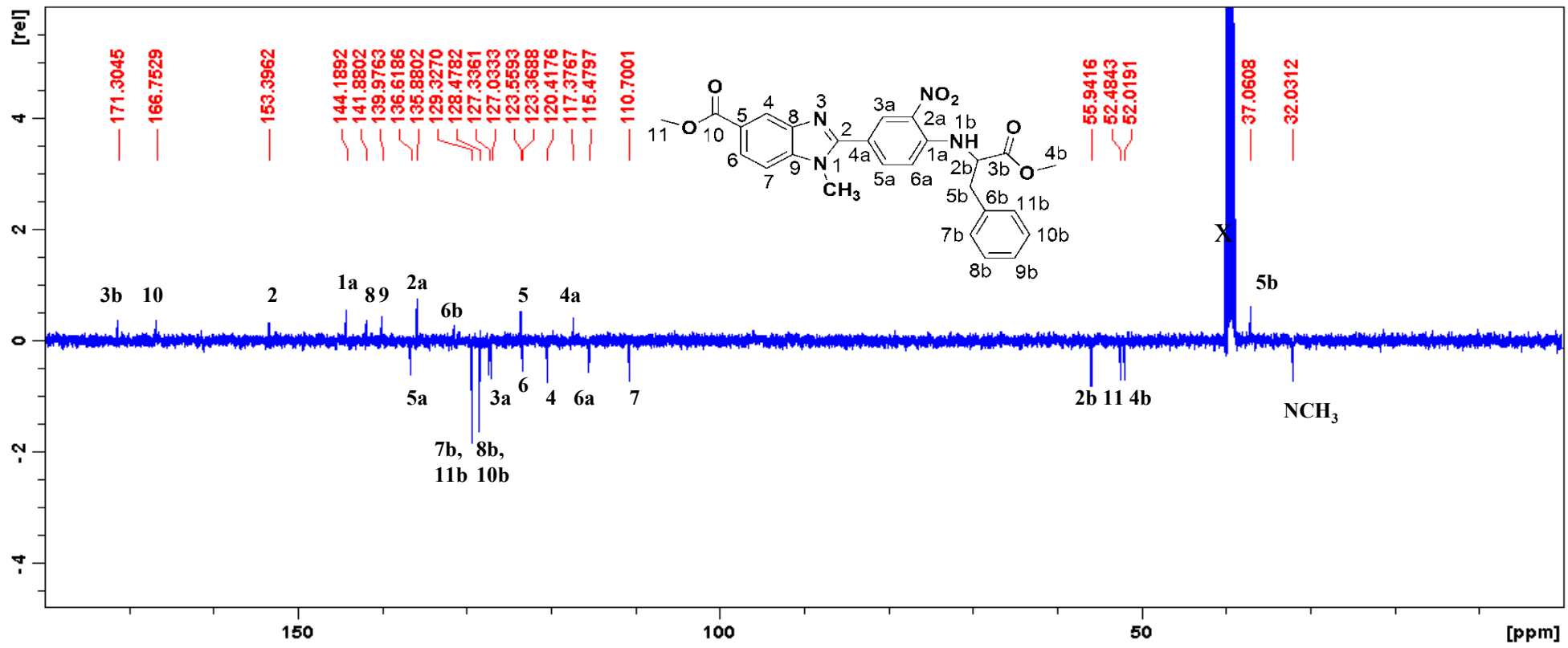
HRMS Spectrum of Compound B-7f



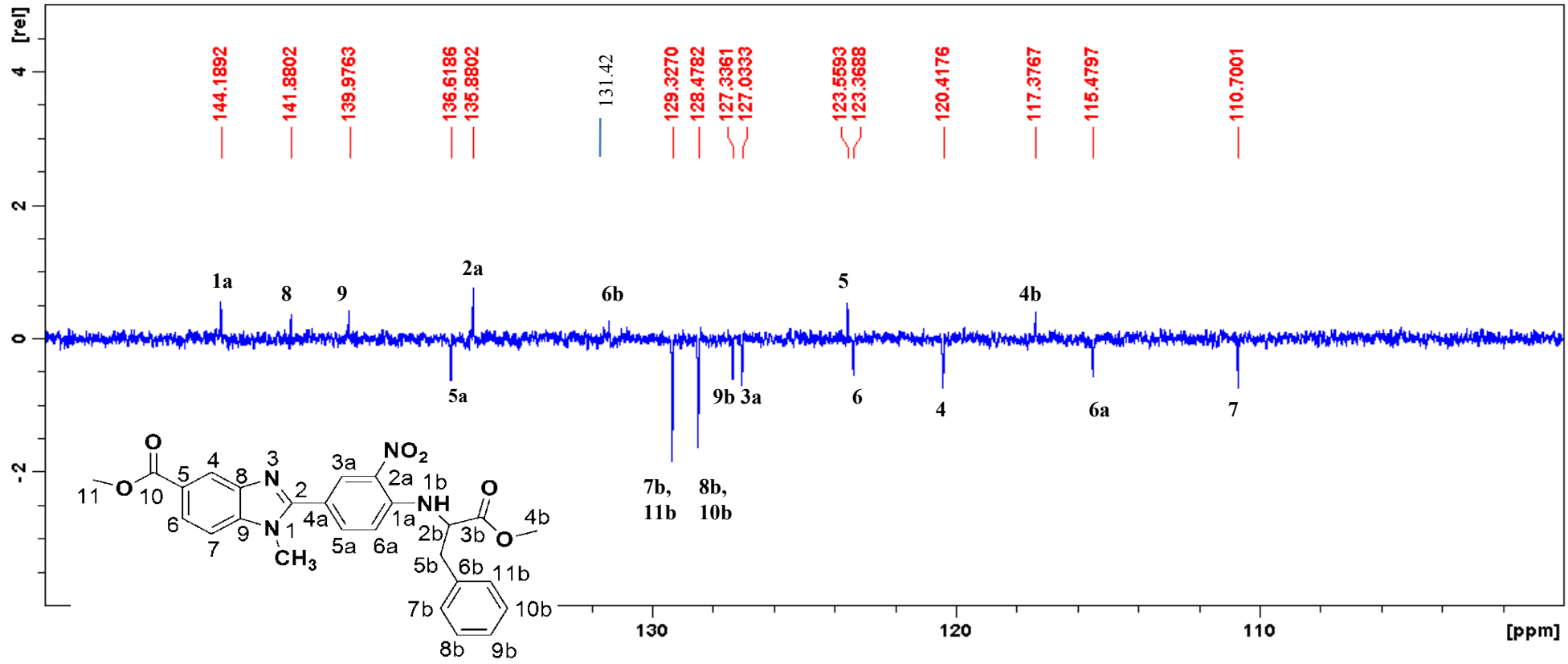
^{1}H Spectrum of Compound B-7g



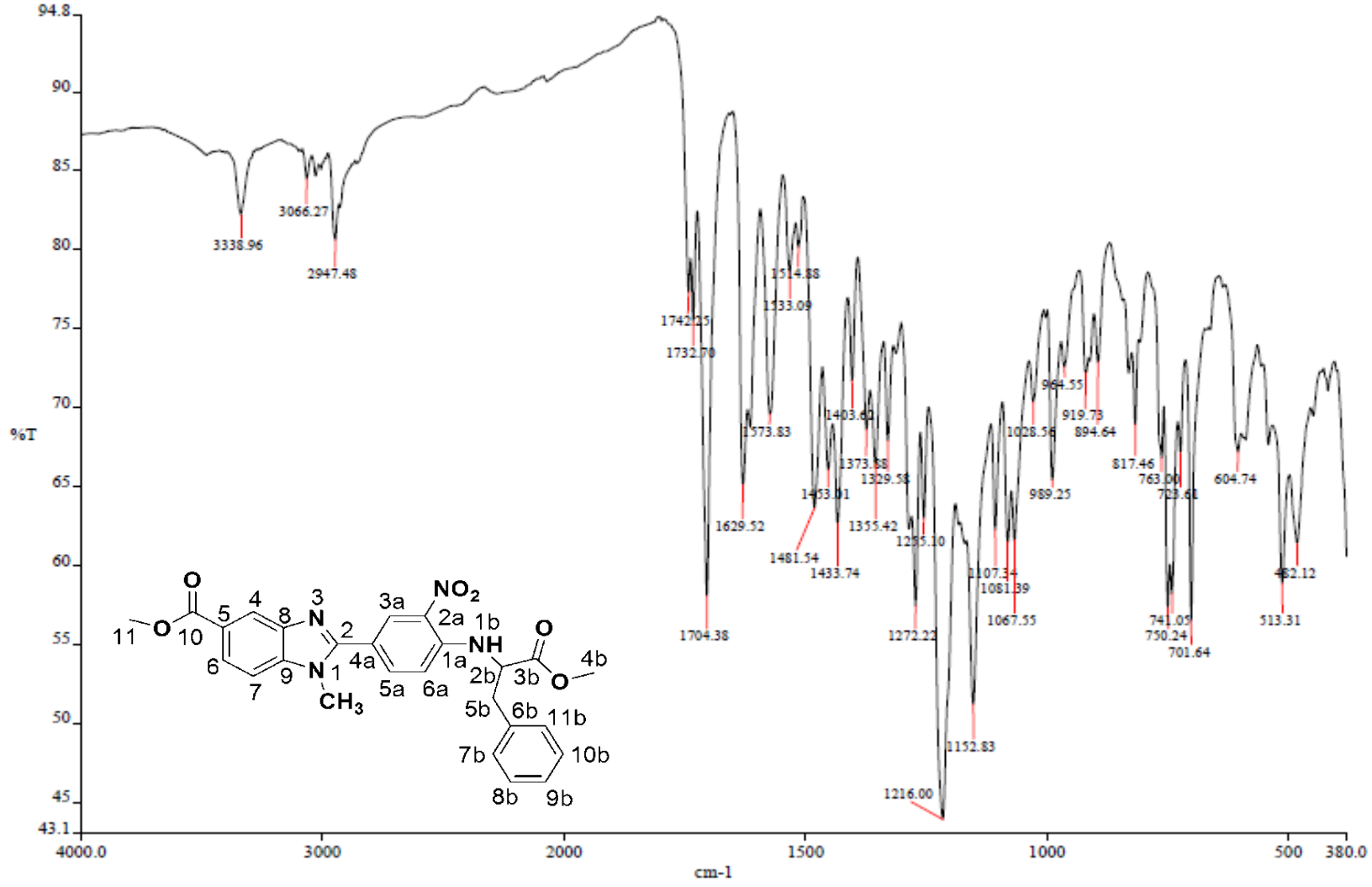
Expanded ^{1}H Spectrum of Compound B-7g



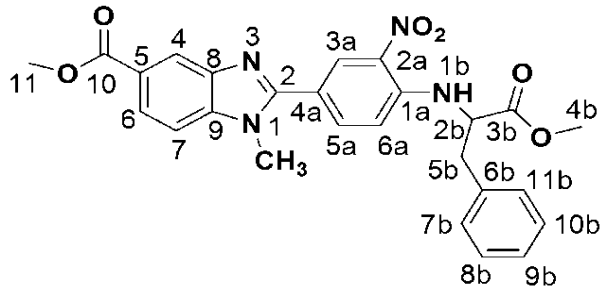
^{13}C Spectrum of Compound B-7g



Expanded ^{13}C Spectrum of Compound B-7g



Infrared Spectrum of Compound B-7g

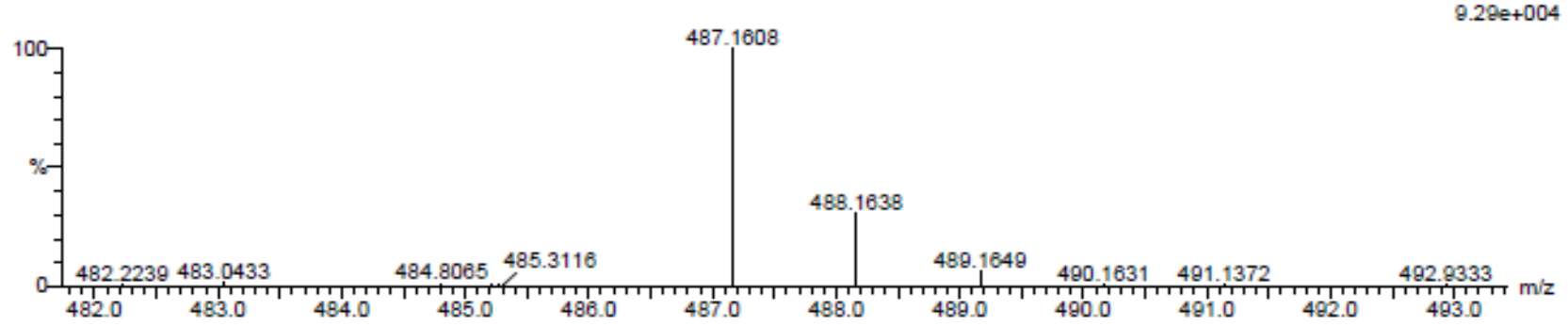


Elemental Composition Report

Single Mass Analysis

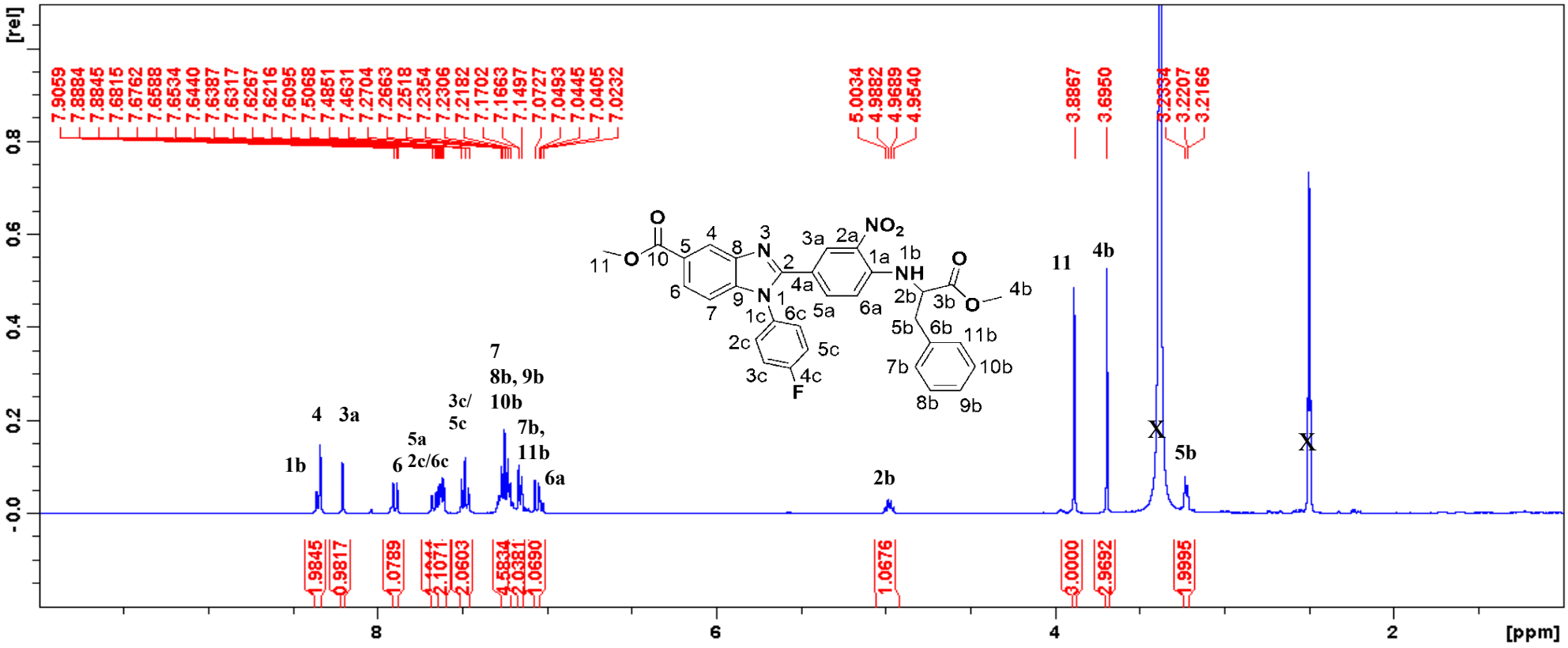
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
25 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 25-30 H: 20-25 N: 0-5 O: 0-10
BA 2 2 (0.034) Cm (1:61)
TOF MS ES-

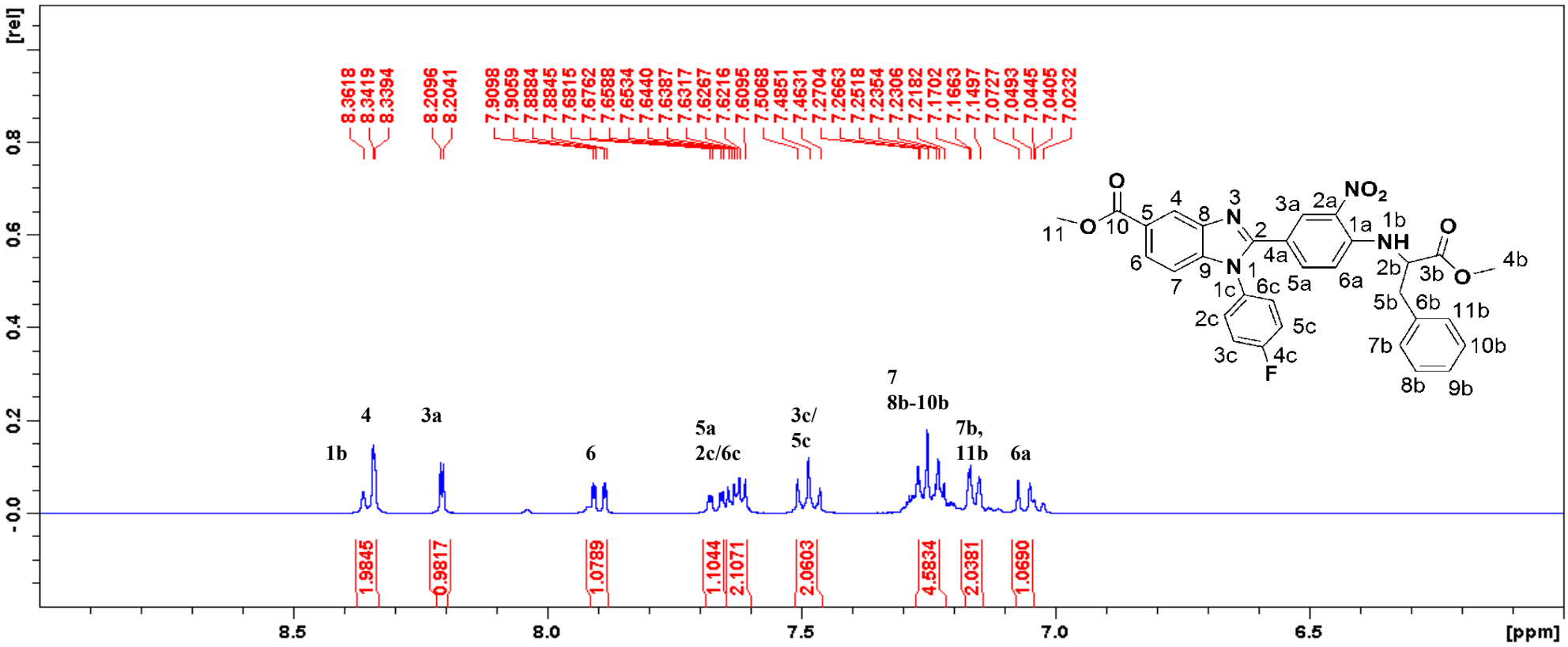


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
487.1608	487.1618	-1.0	-2.1	17.5	11.1	0.0	C26 H23 N4 O6

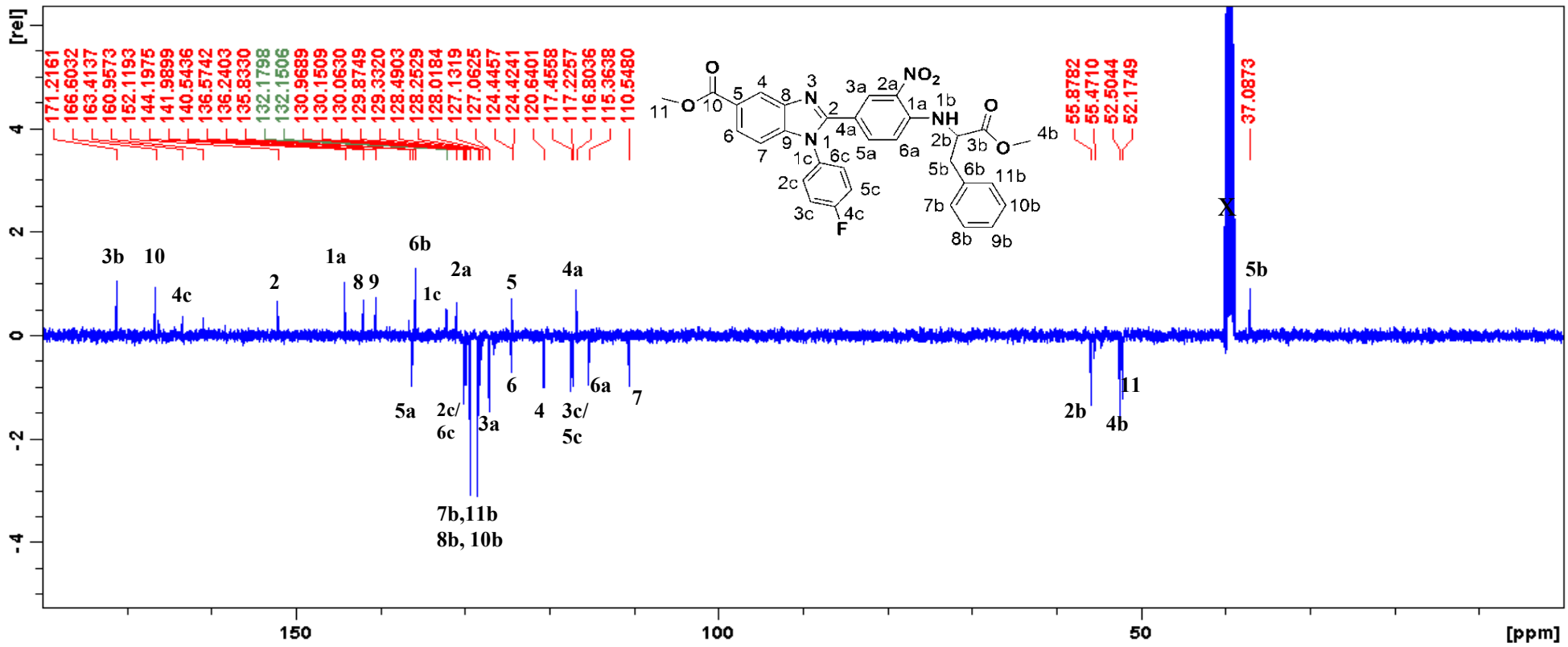
HRMS Spectrum of Compound B-7g



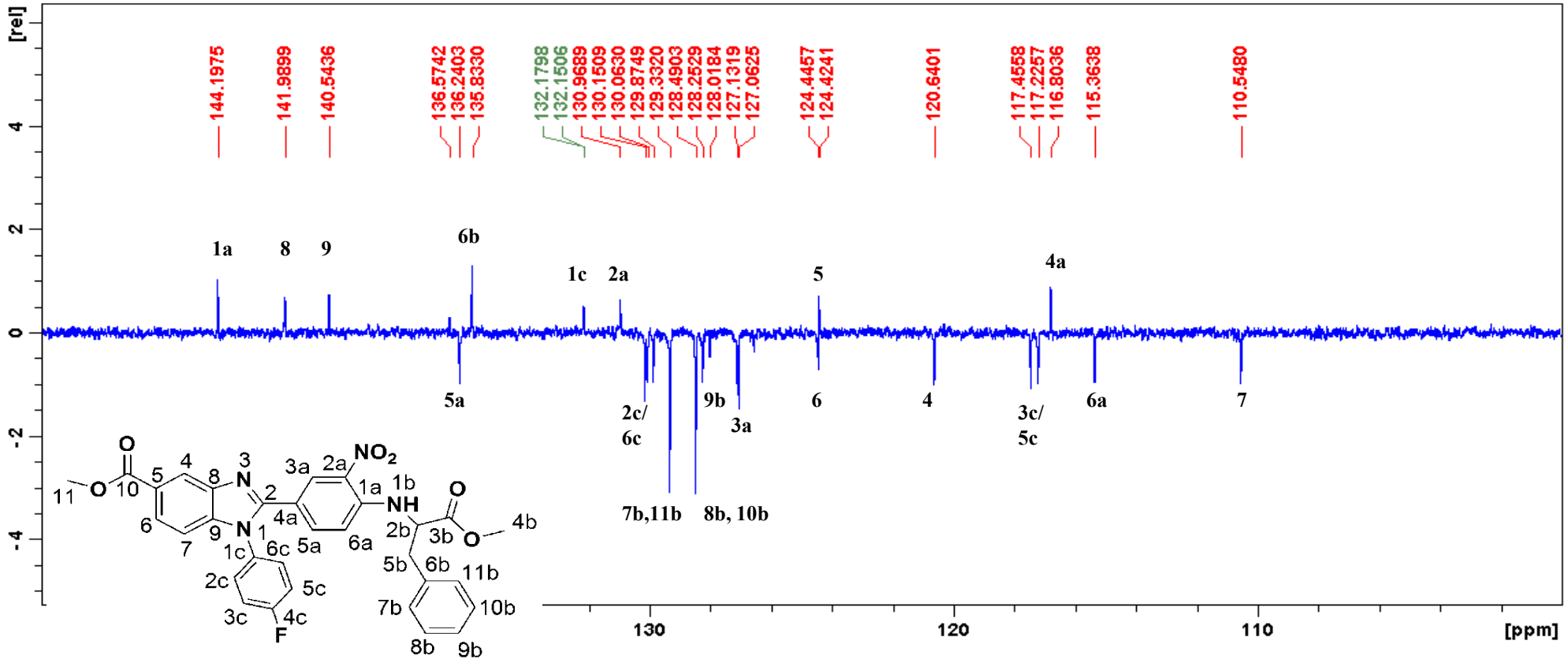
^{1}H Spectrum of Compound B-7h



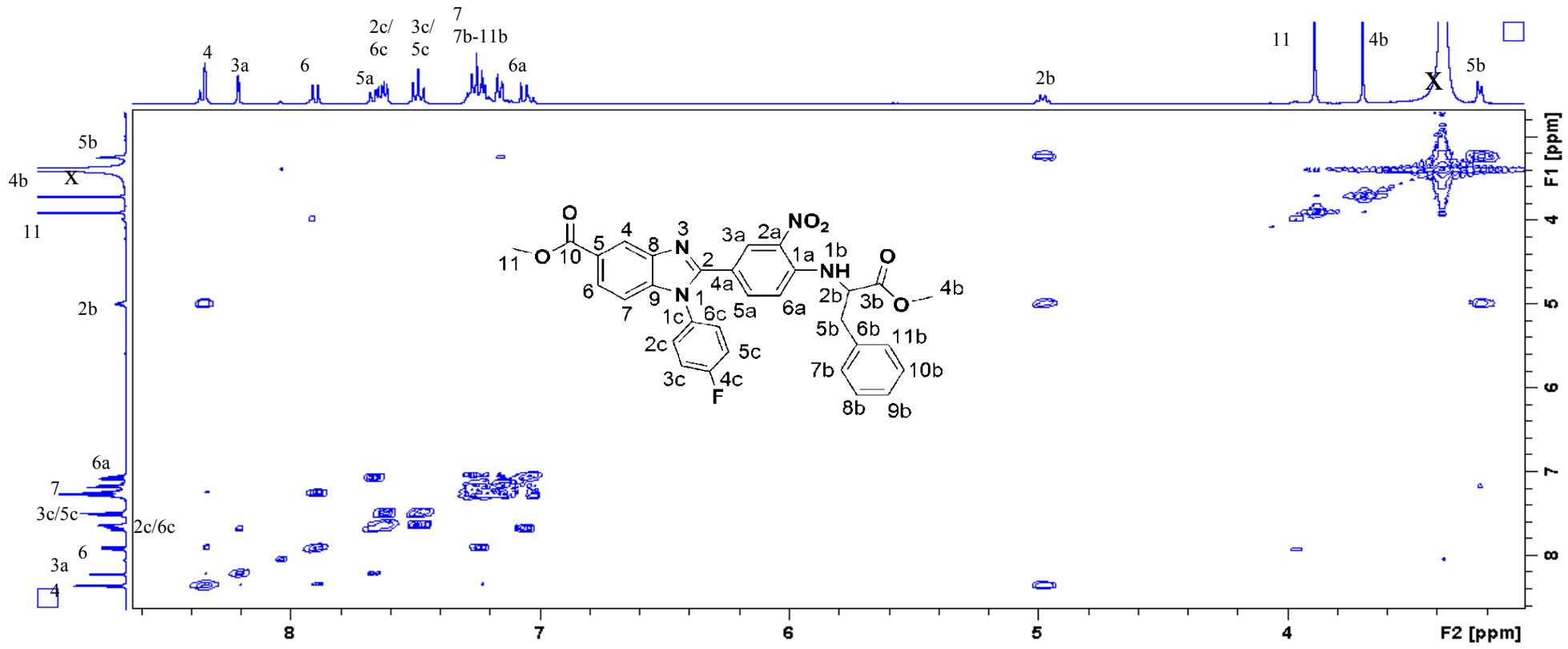
Expanded ^{1}H Spectrum of Compound B-7h



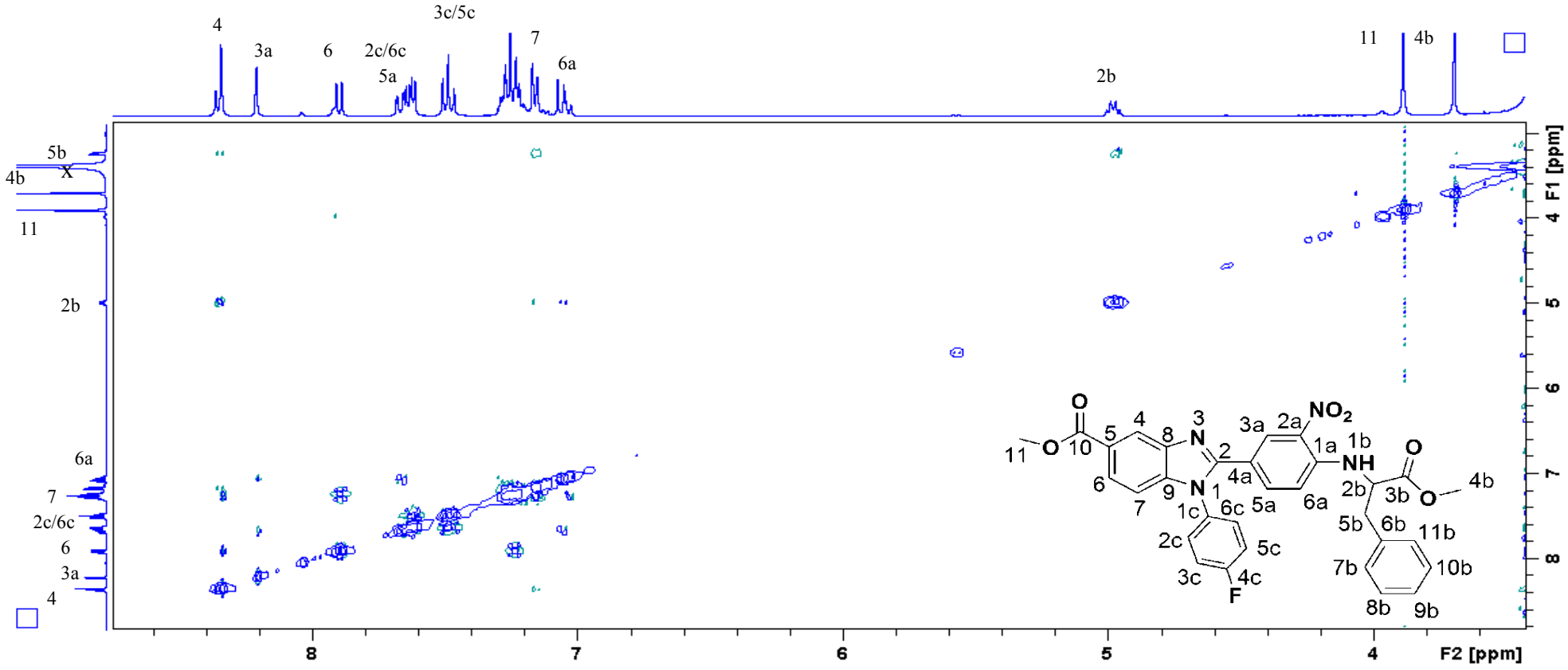
^{13}C Spectrum of Compound B-7h



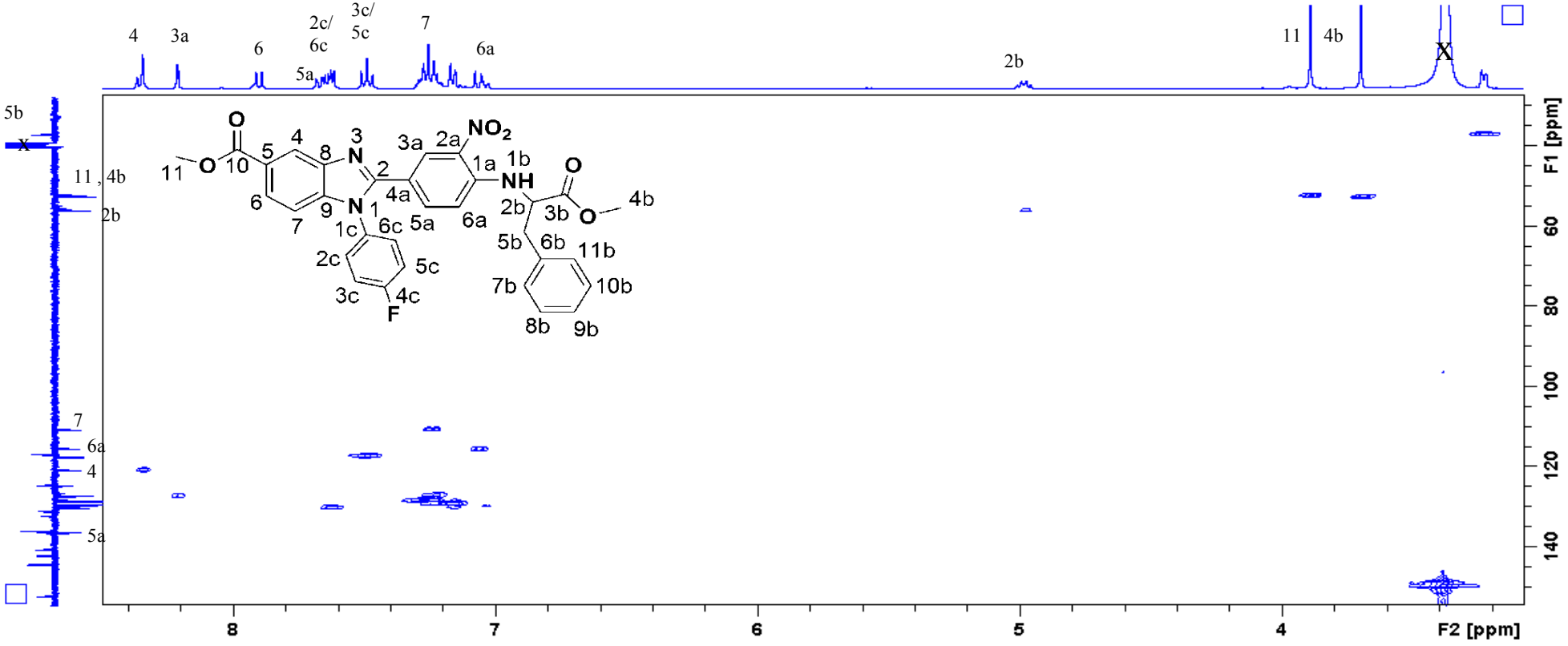
Expanded ^{13}C spectrum of Compound B-7h



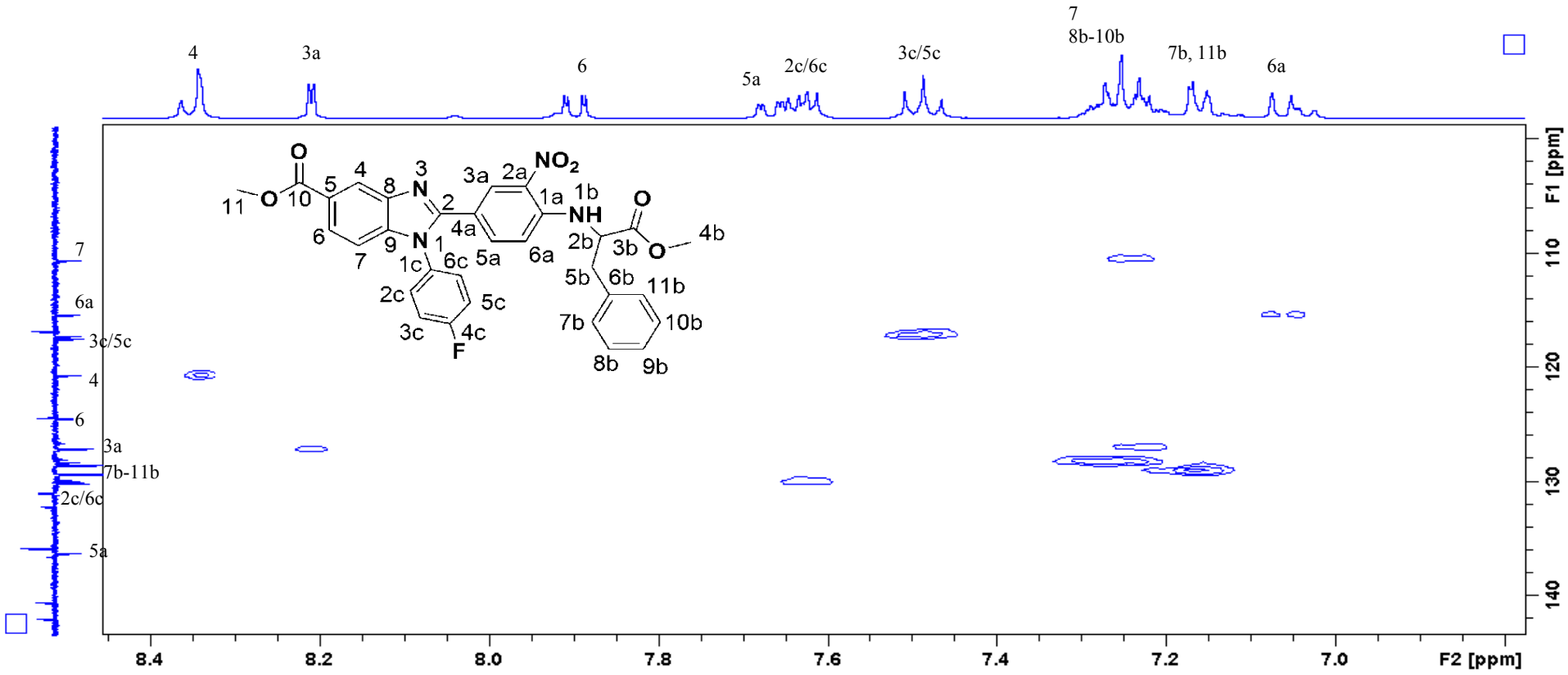
COSY Spectrum of Compound B-7h



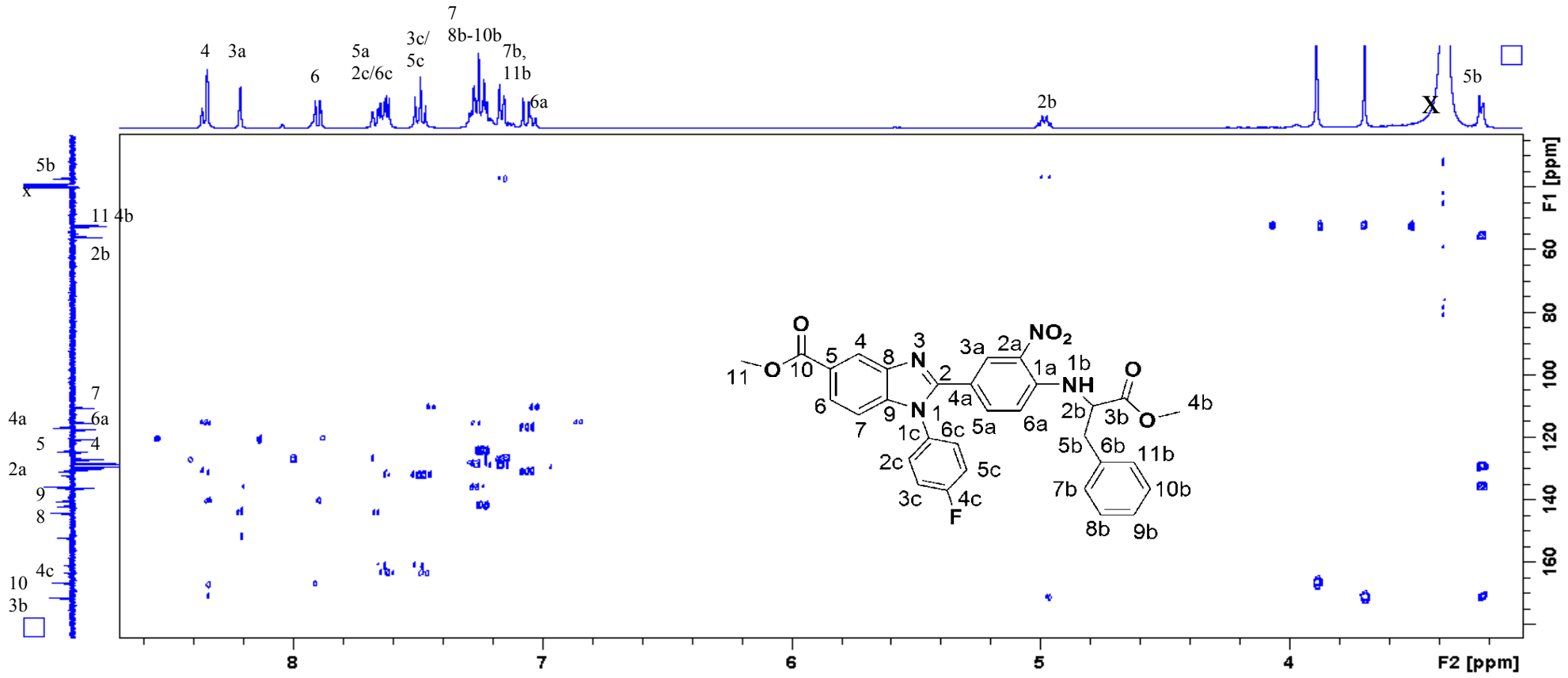
NOESY Spectrum of Compound B-7h



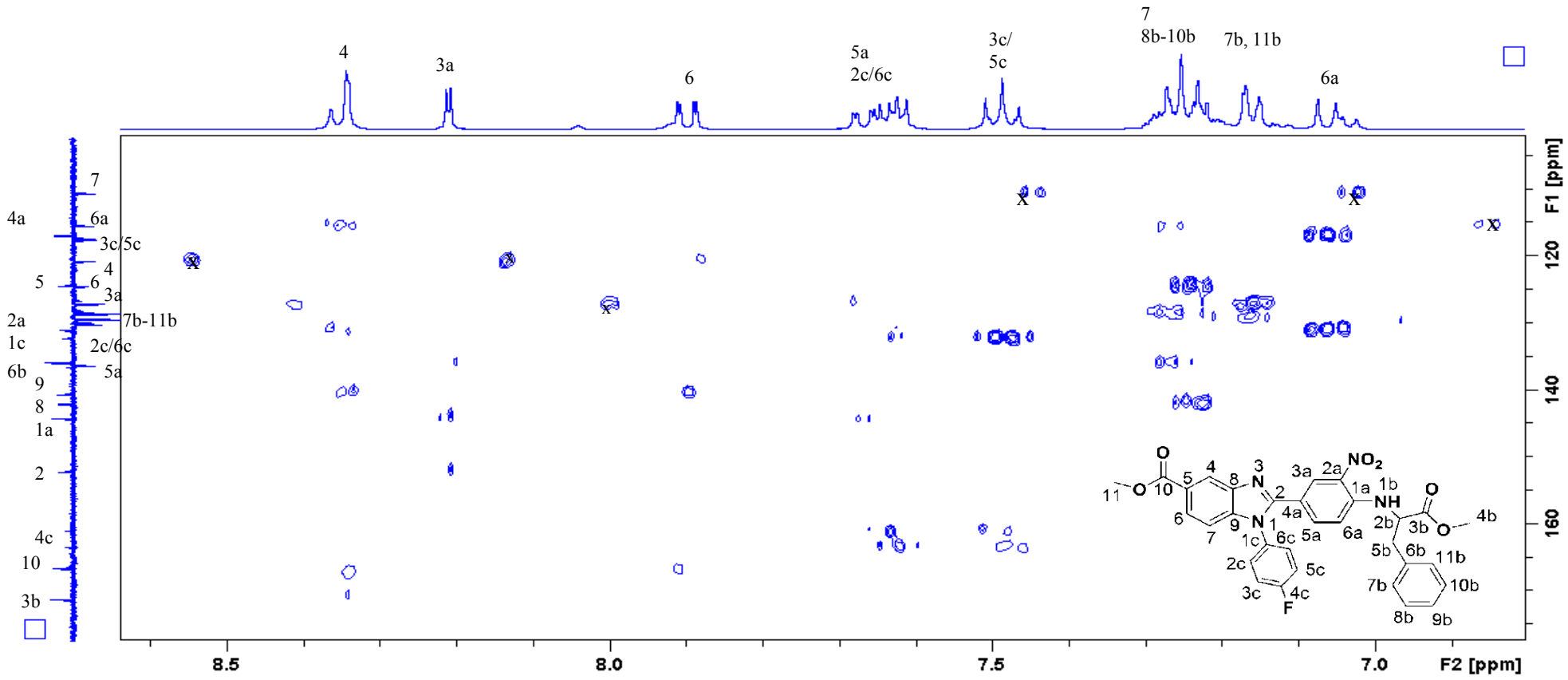
HSQC Spectrum of Compound B-7h



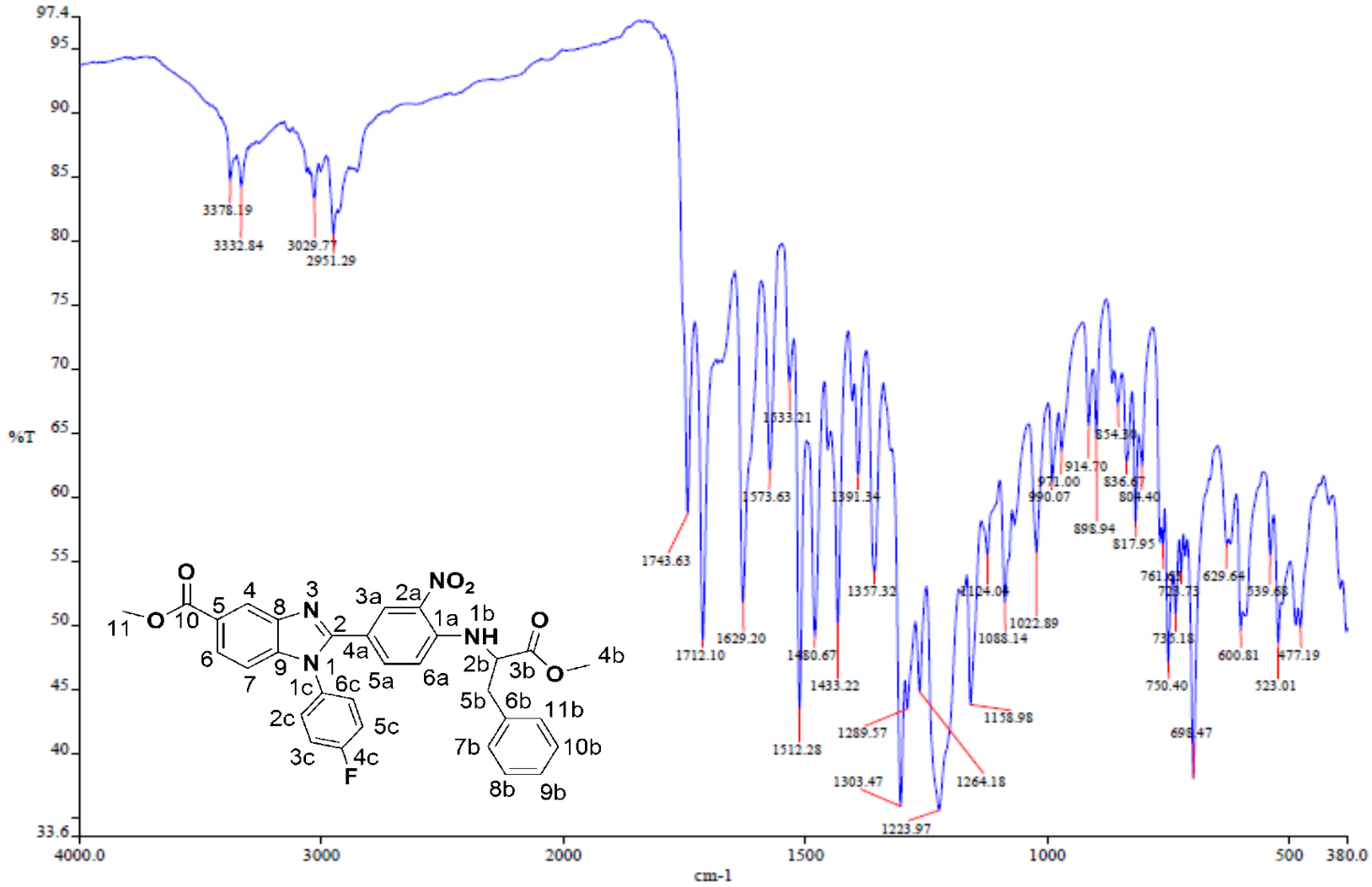
Expanded HSQC Spectrum of Compound B-7h



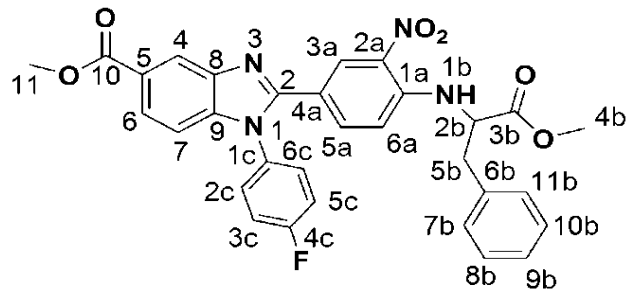
HMBC Spectrum of Compound B-7h



Expanded HMBC Spectrum of Compound B-7h



Infrared Spectrum of Compound B-7h



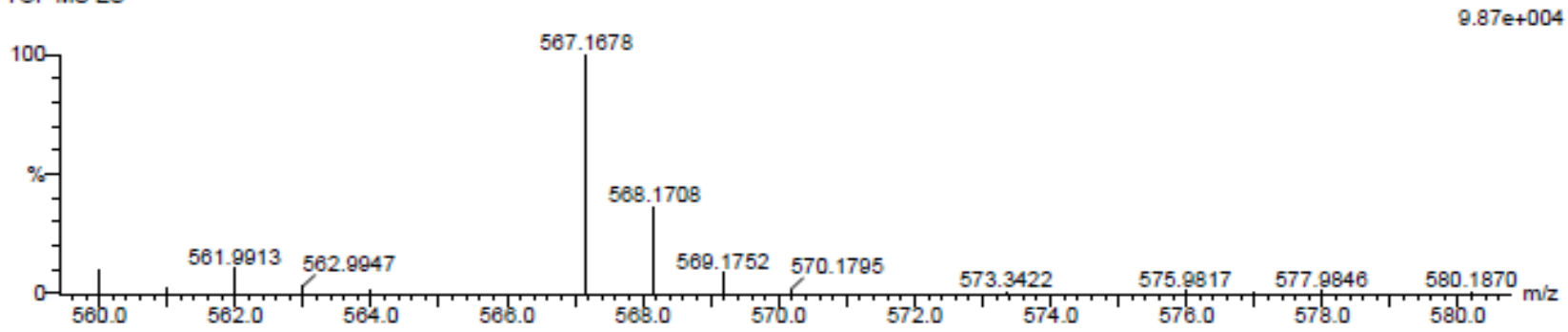
Elemental Composition Report

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

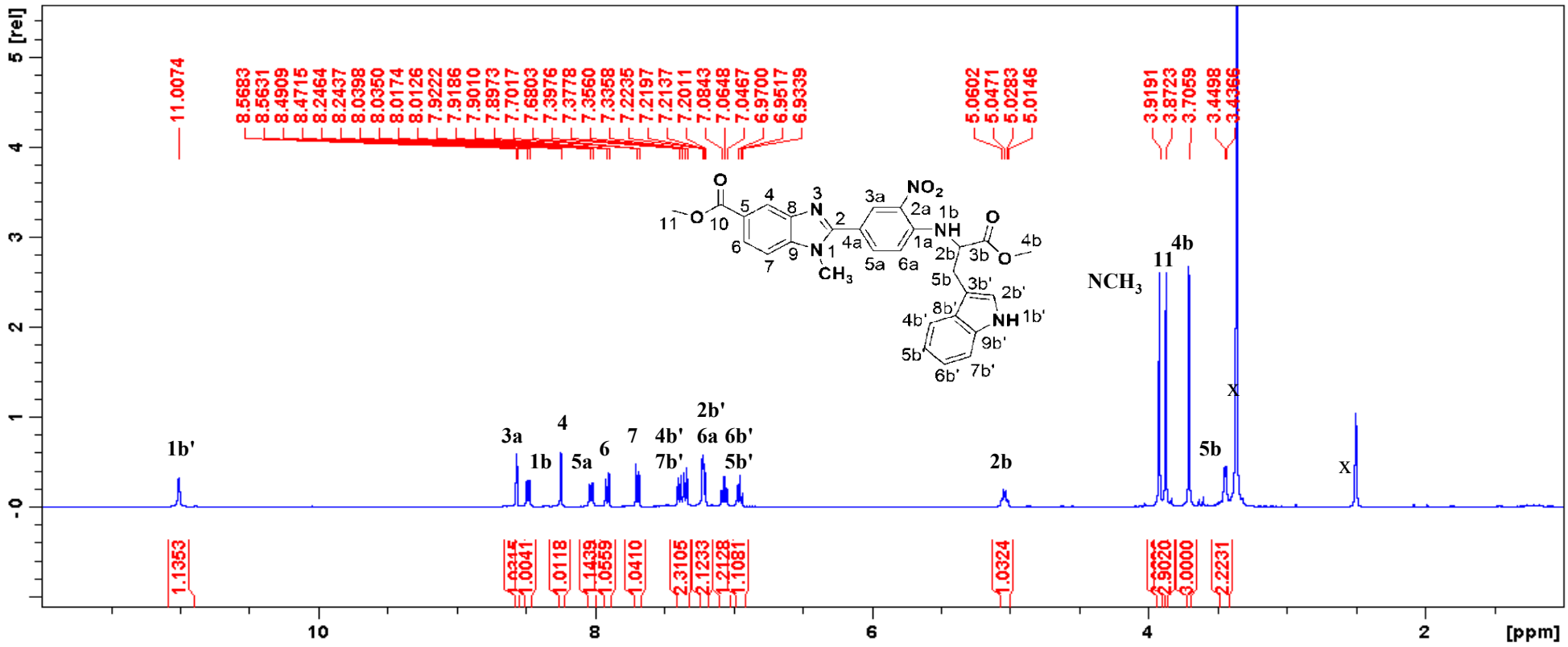
Monoisotopic Mass, Even Electron Ions
115 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 30-35 H: 20-25 N: 0-5 O: 5-10 F: 1-5

BA 1 24 (0.776) Cm (1:61)
TOF MS ES-

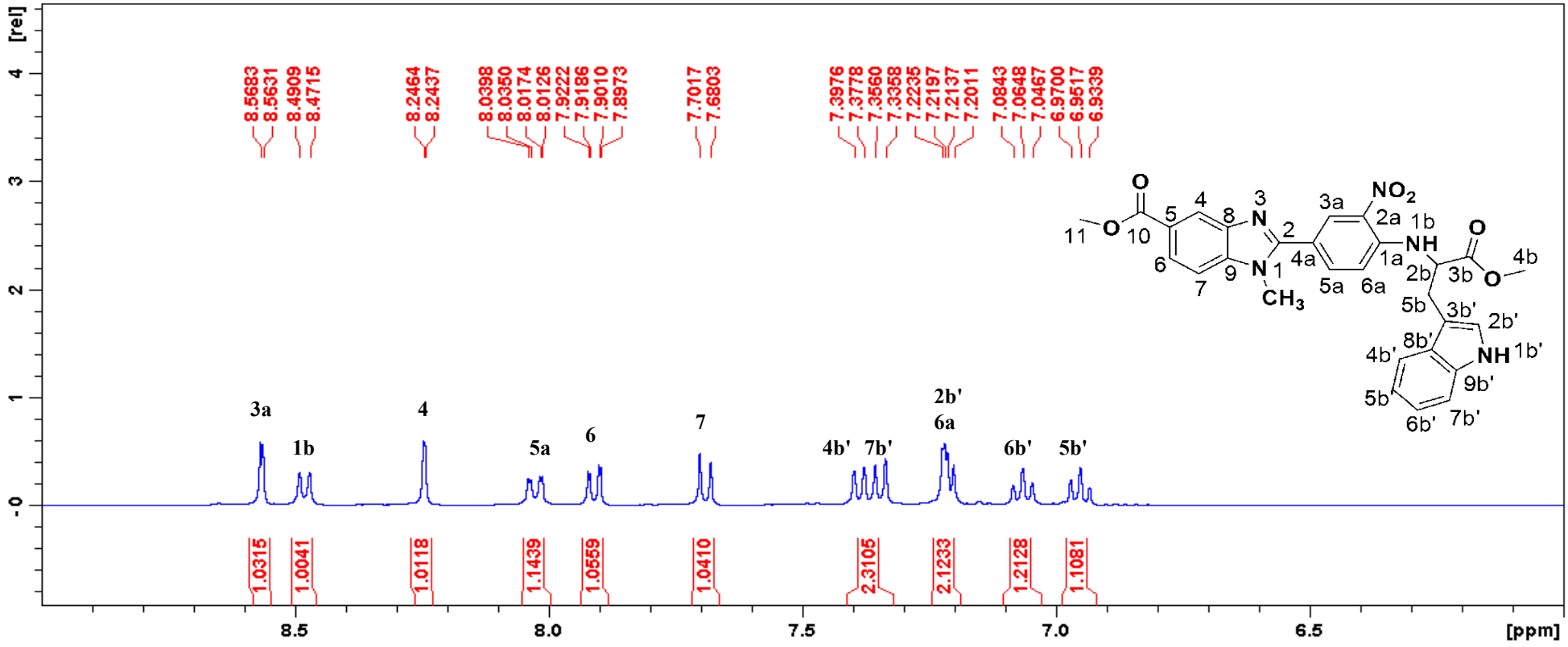


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
567.1678	567.1680	-0.2	-0.4	21.5	9.9	0.0	C31 H24 N4 O6 F

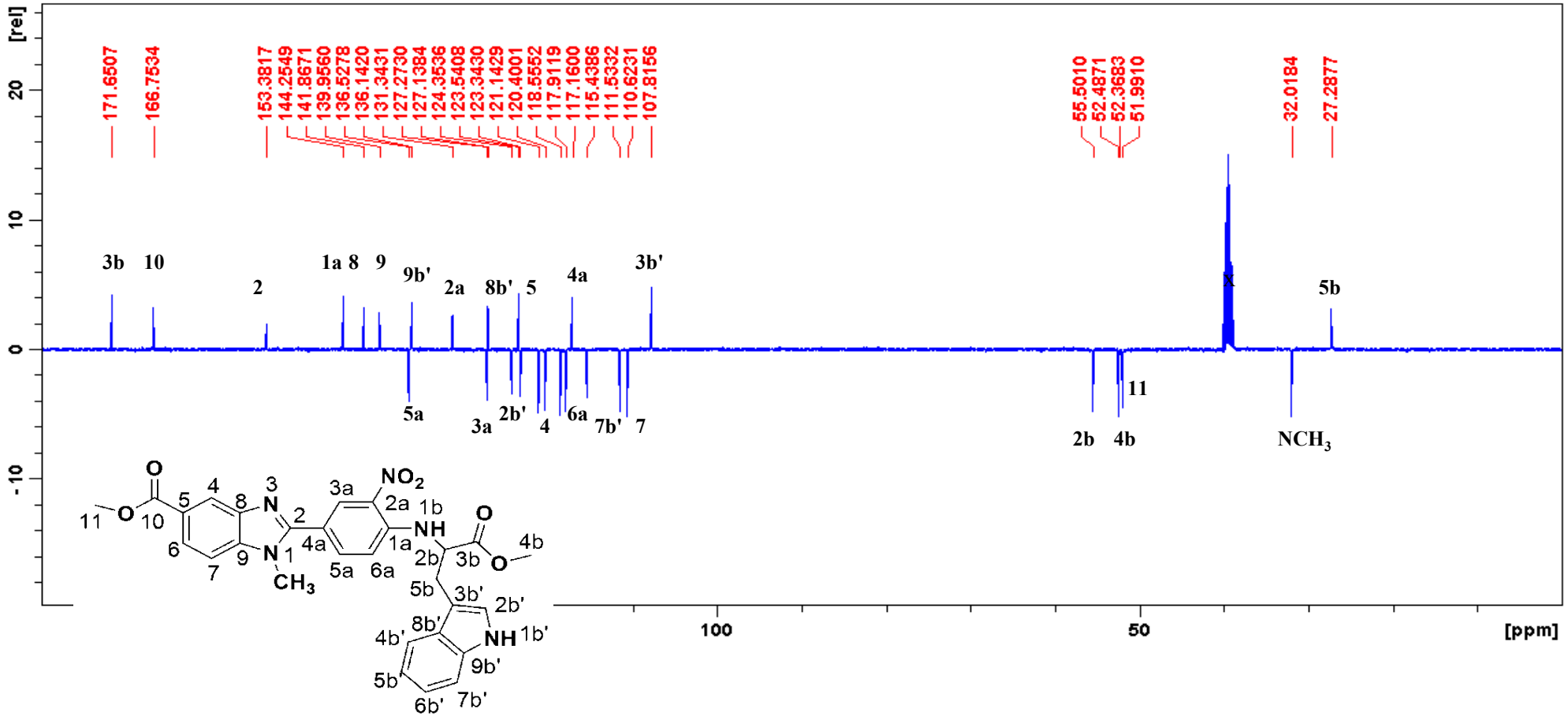
HRMS Spectrum of Compound B-7h



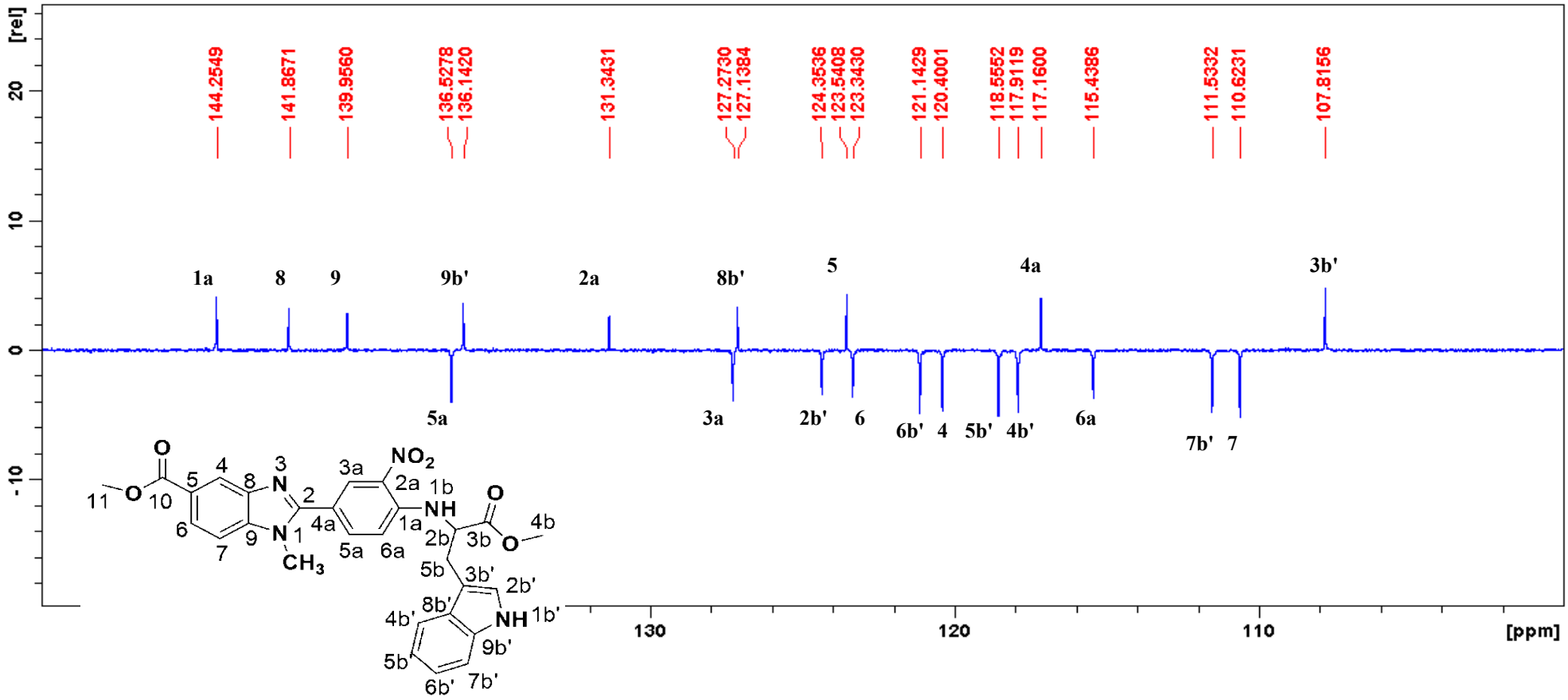
^{1}H Spectrum of Compound B-7i



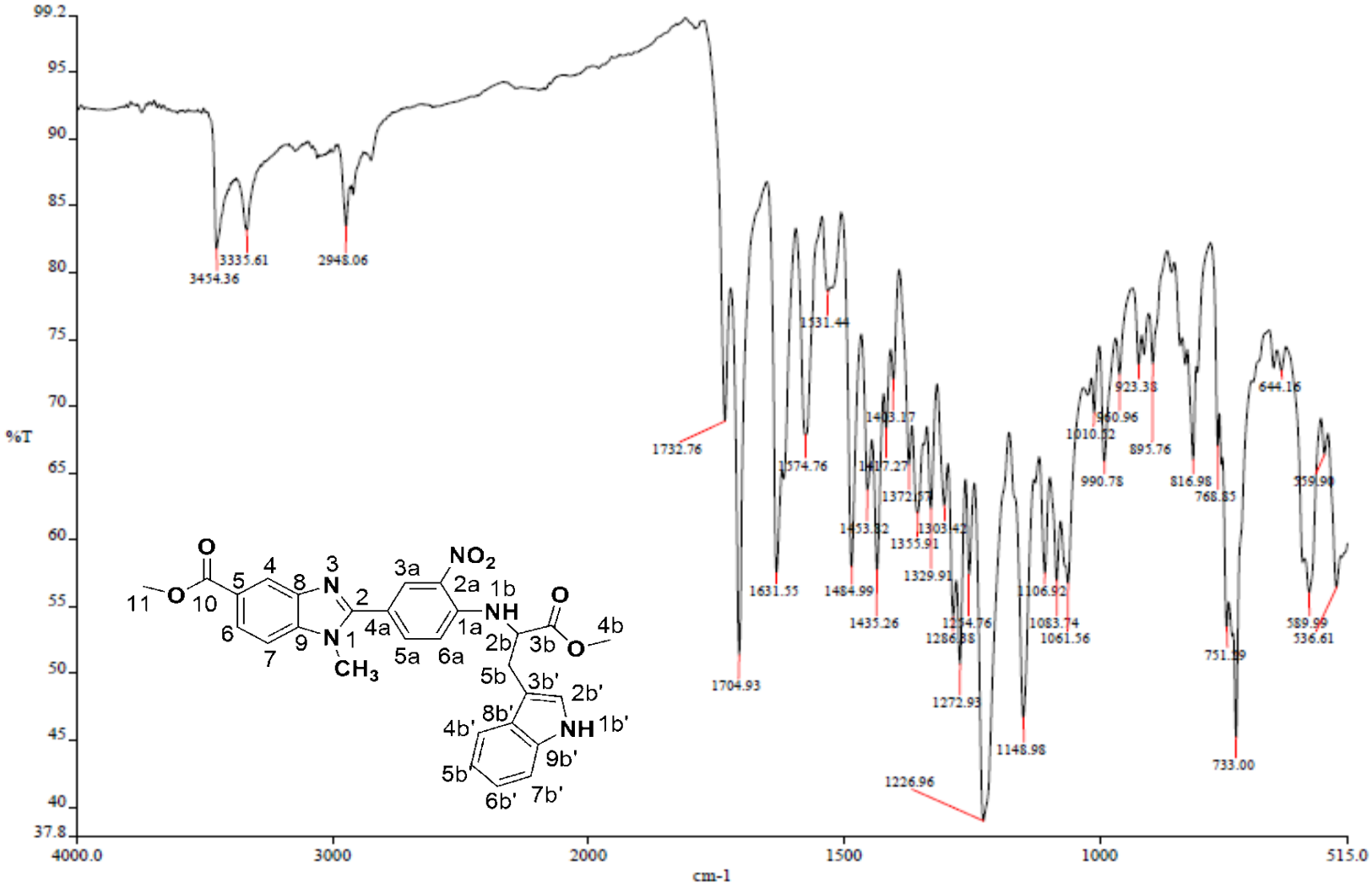
Expanded ^{1}H Spectrum of Compound B-7i



^{13}C Spectrum of Compound B-7i

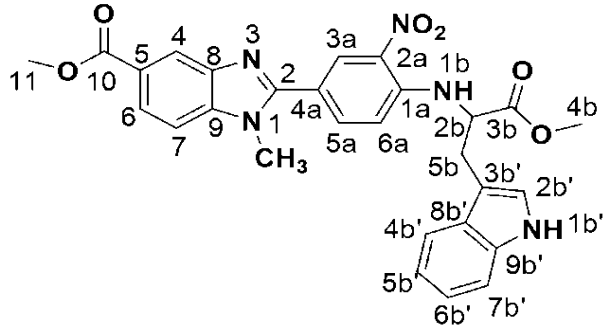


Expanded ^{13}C Spectrum of Compound B-7i



Infrared Spectrum of Compound B-7i

Elemental Composition Report

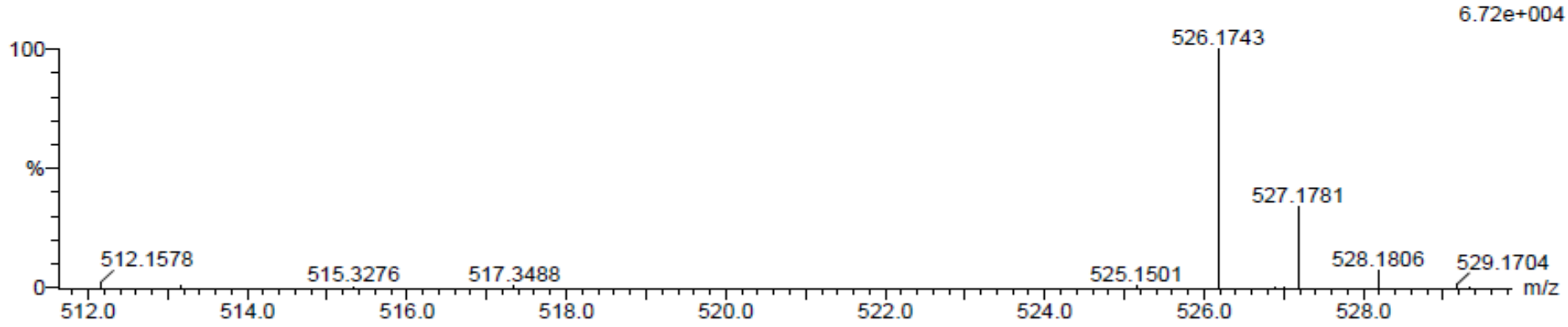


Single Mass Analysis

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

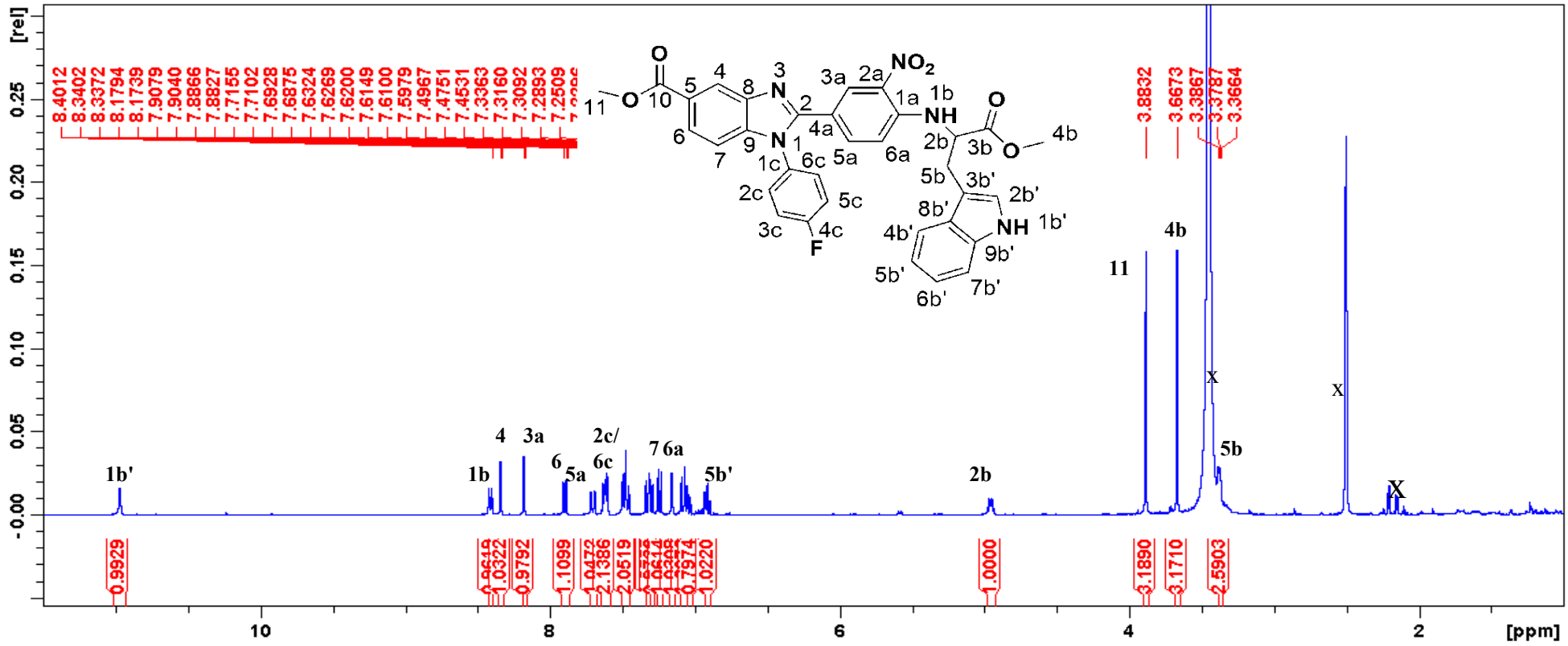
Monoisotopic Mass, Even Electron Ions
27 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 25-30 H: 20-25 N: 0-5 O: 0-8

BA 8 60 (1.991) Cm (1:61)
TOF MS ES-

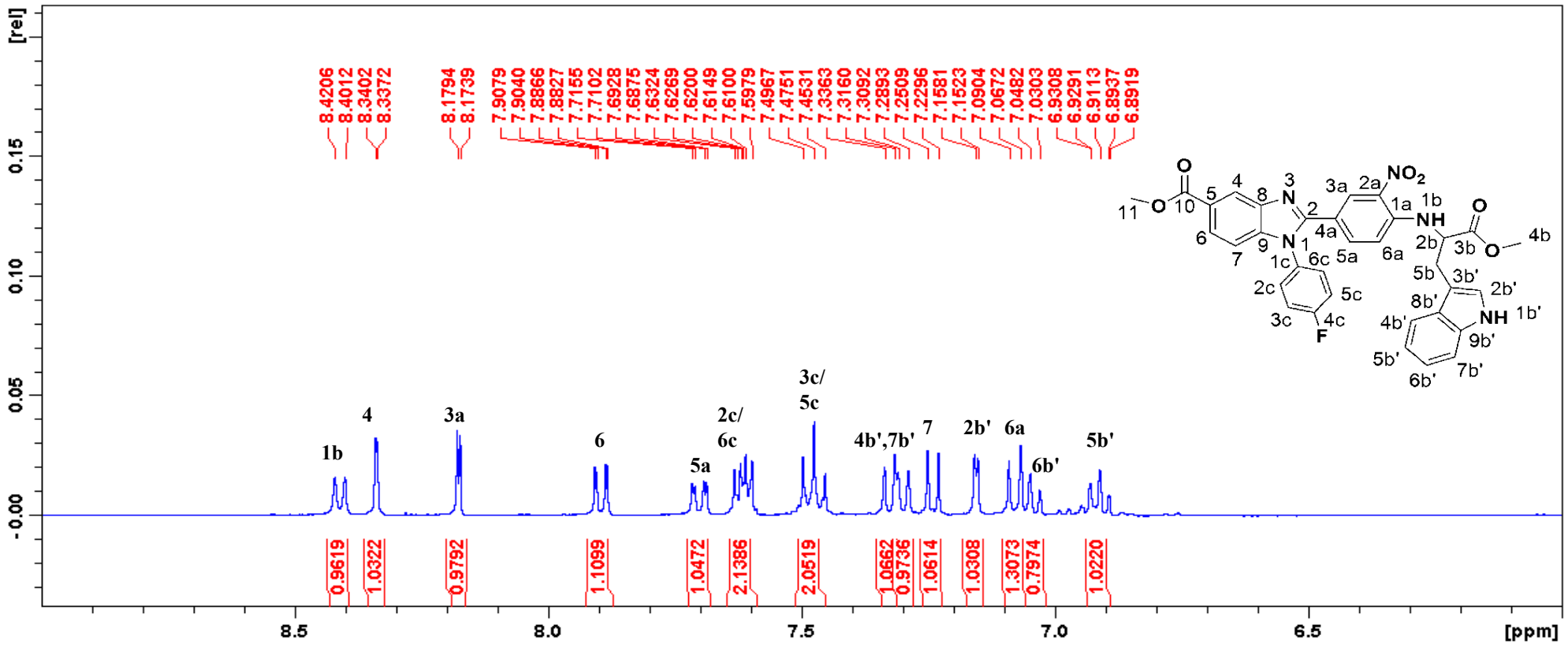


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
526.1743	526.1727	1.6	3.0	19.5	32.3	0.0	C28 H24 N5 O6

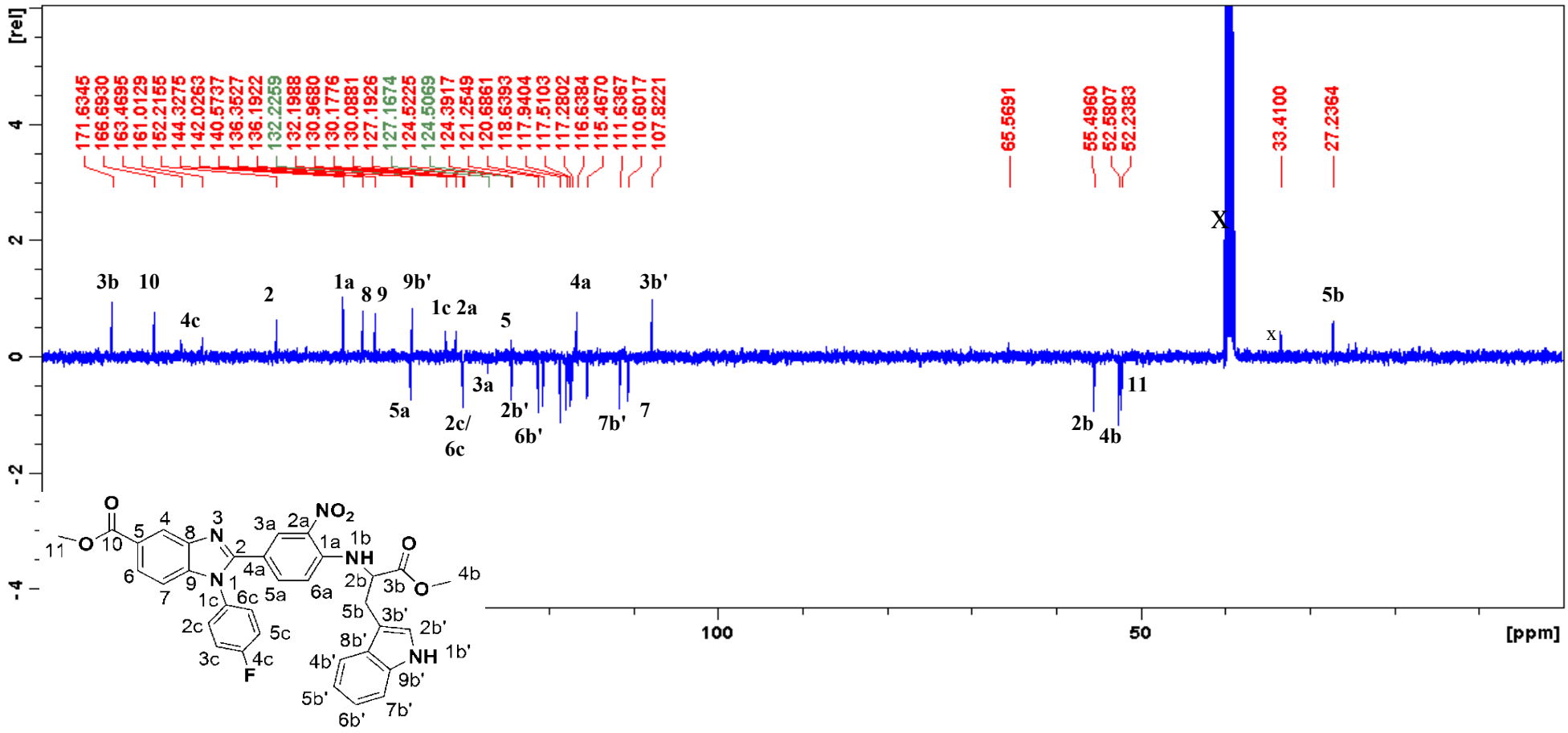
HRMS Spectrum of Compound B-7i



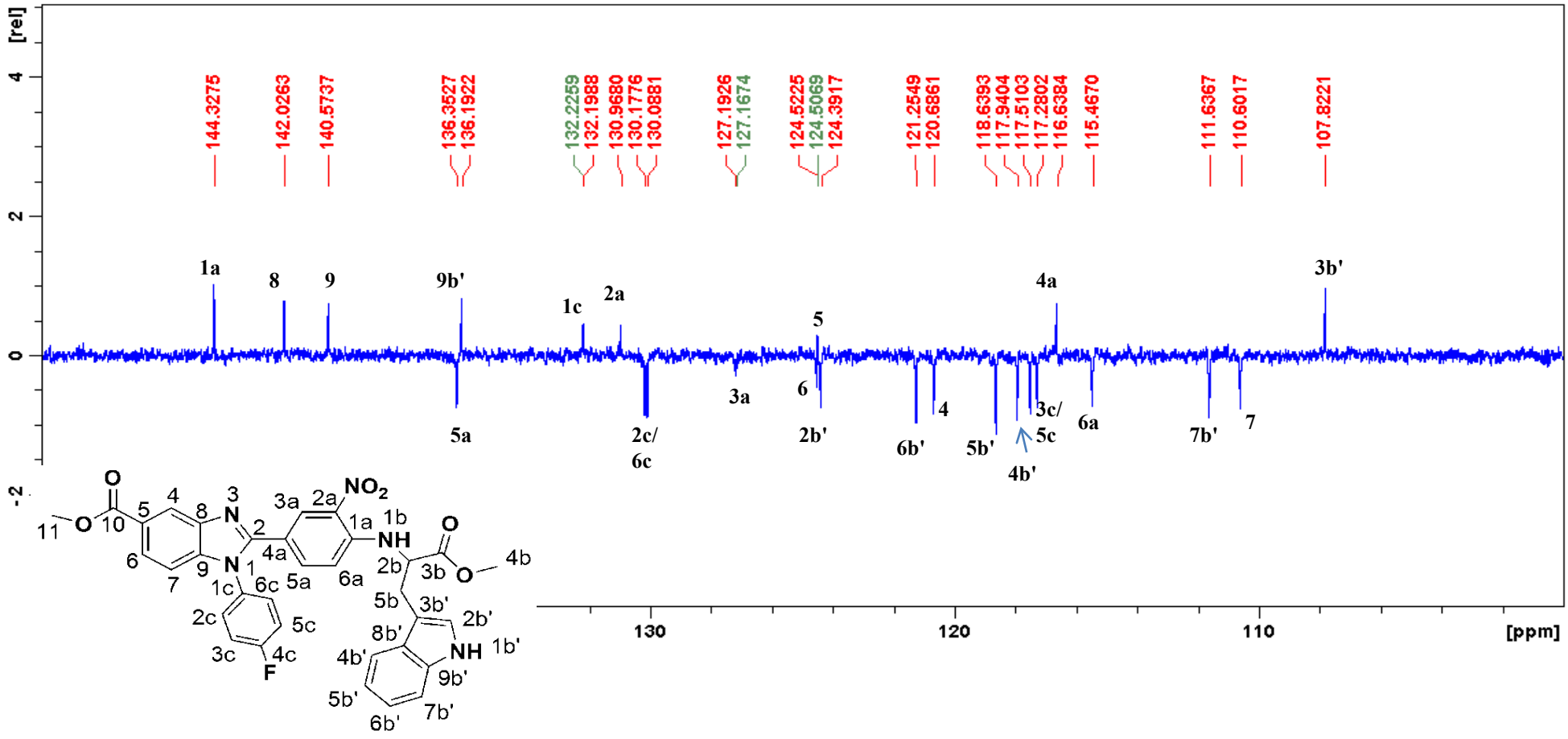
^{1}H Spectrum of Compound B-7j



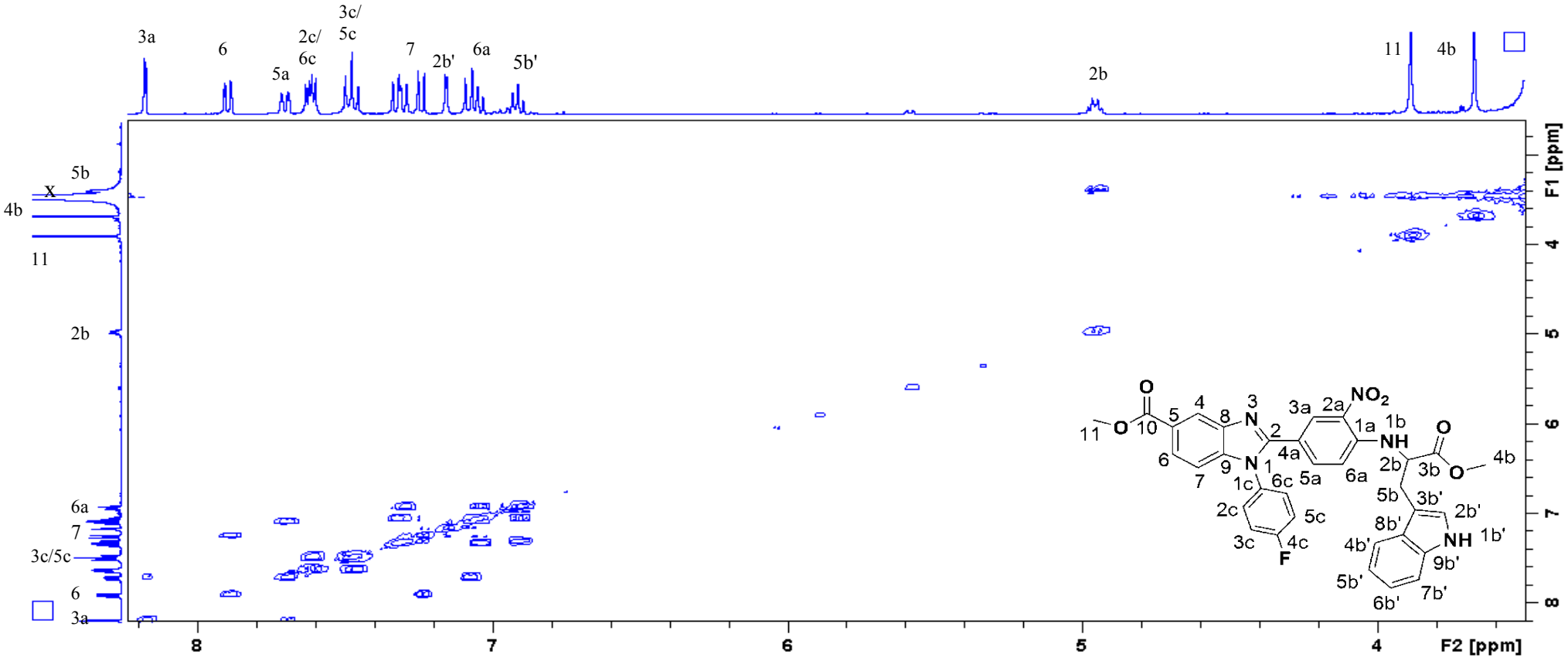
Expanded ^{1}H Spectrum of Compound B-7j



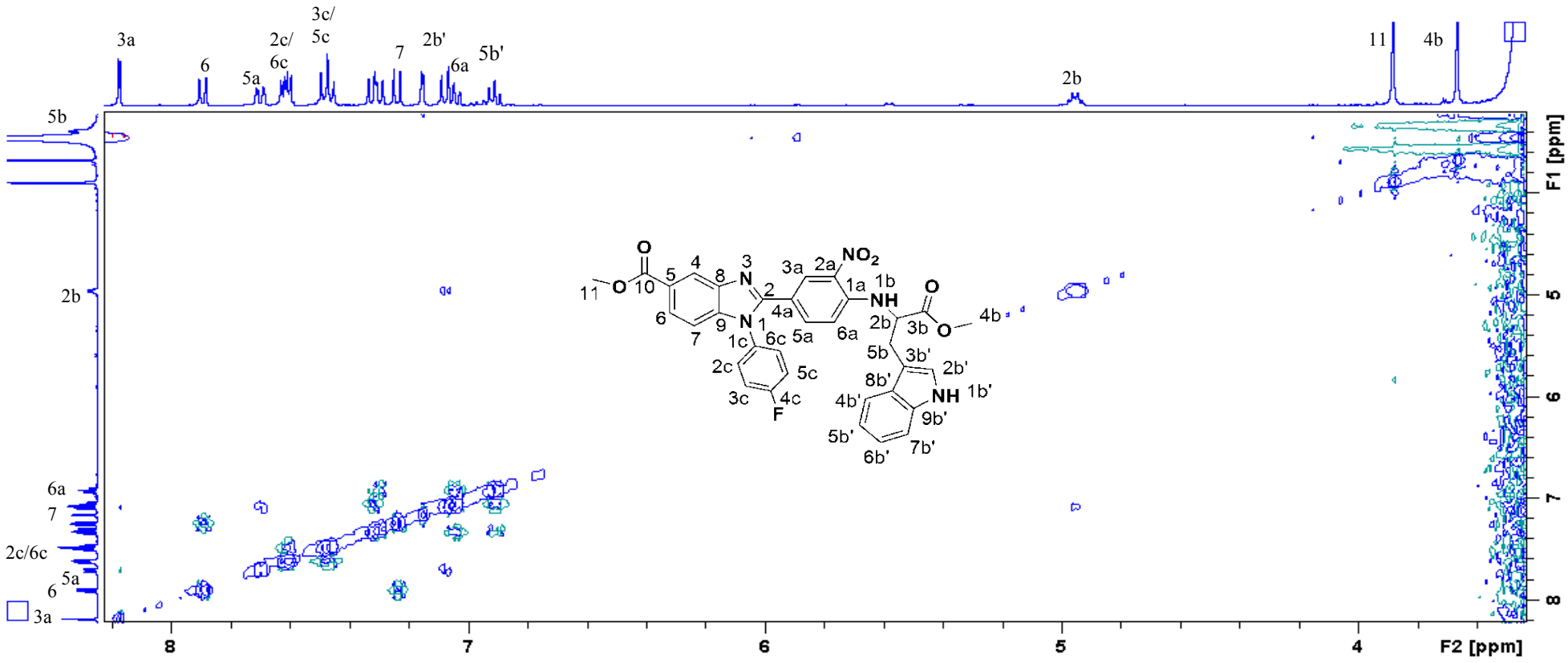
^{13}C Spectrum of Compound B-7j



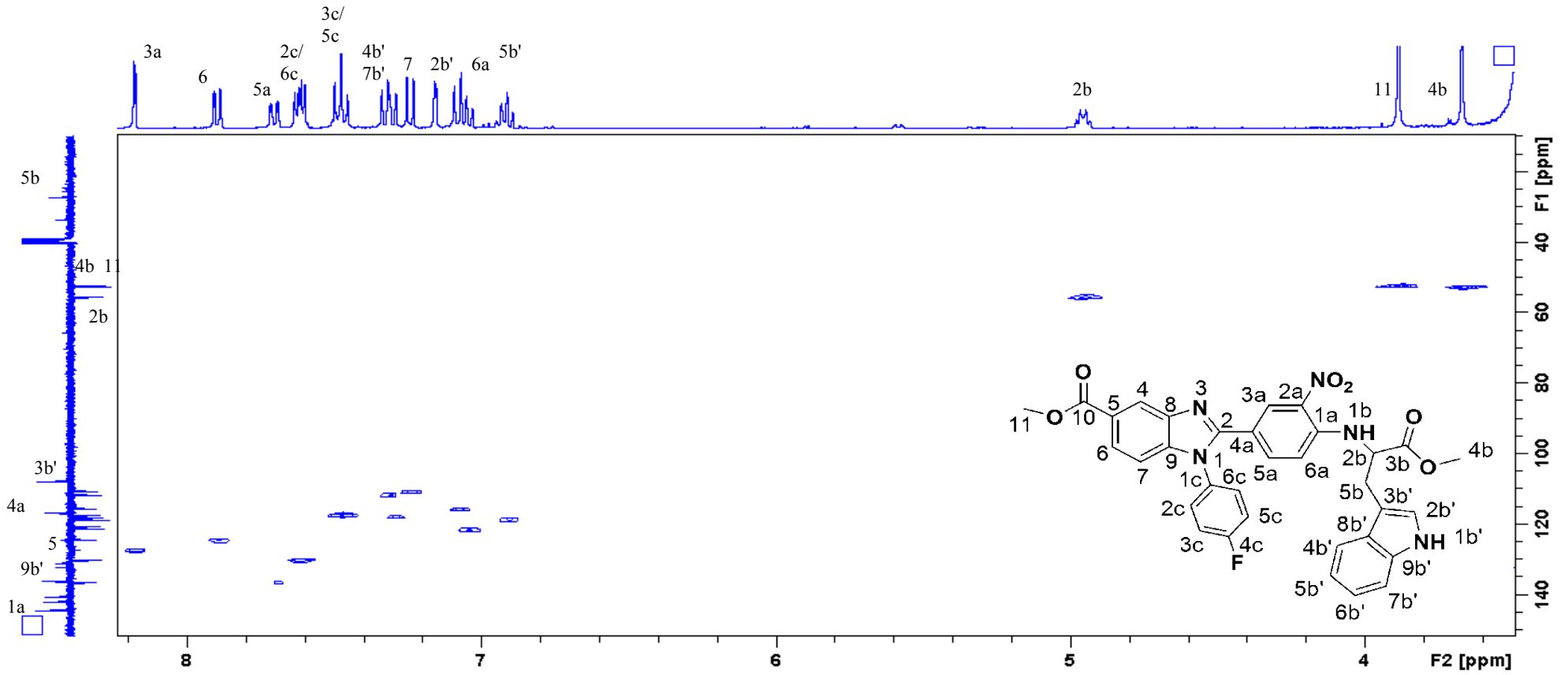
Expanded ^{13}C Spectrum of Compound B-7j



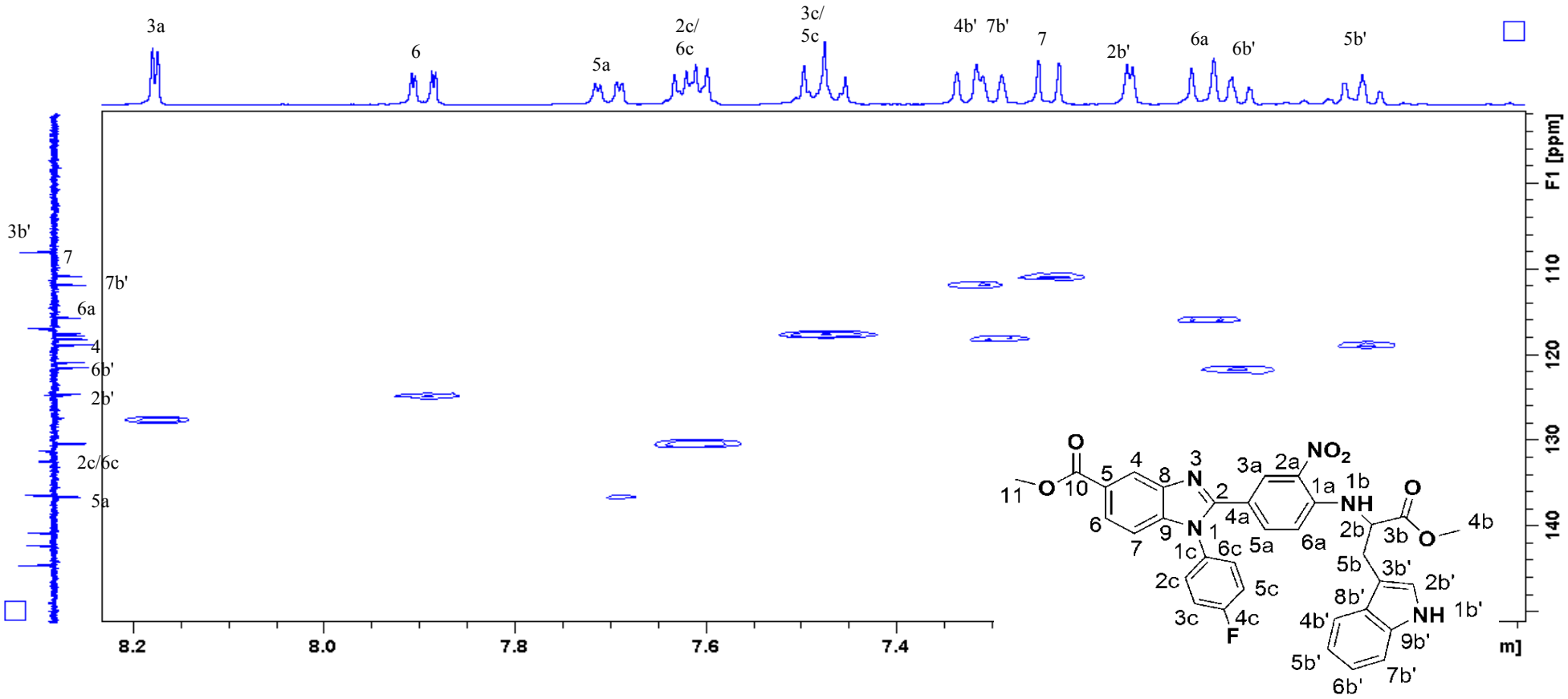
COSY Spectrum of Compound B-7j



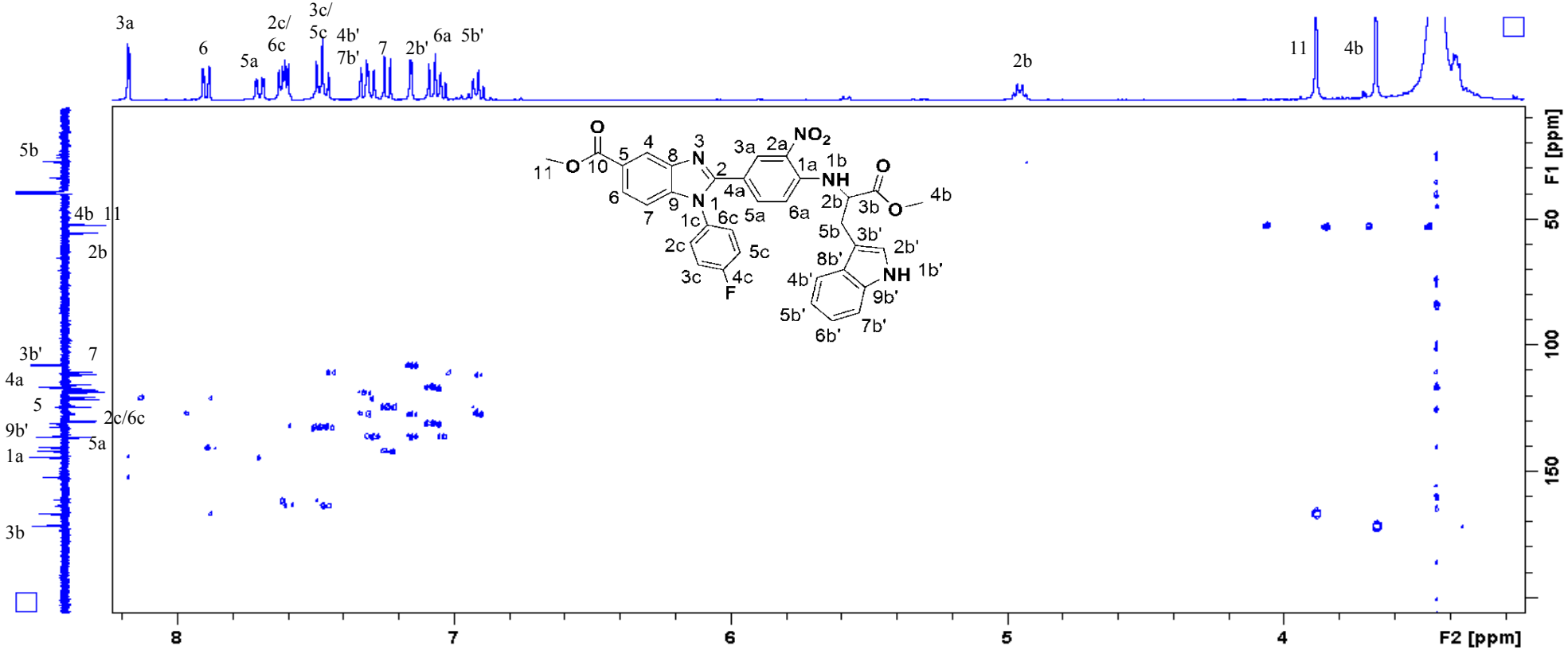
NOESY Spectrum of Compound B-7j



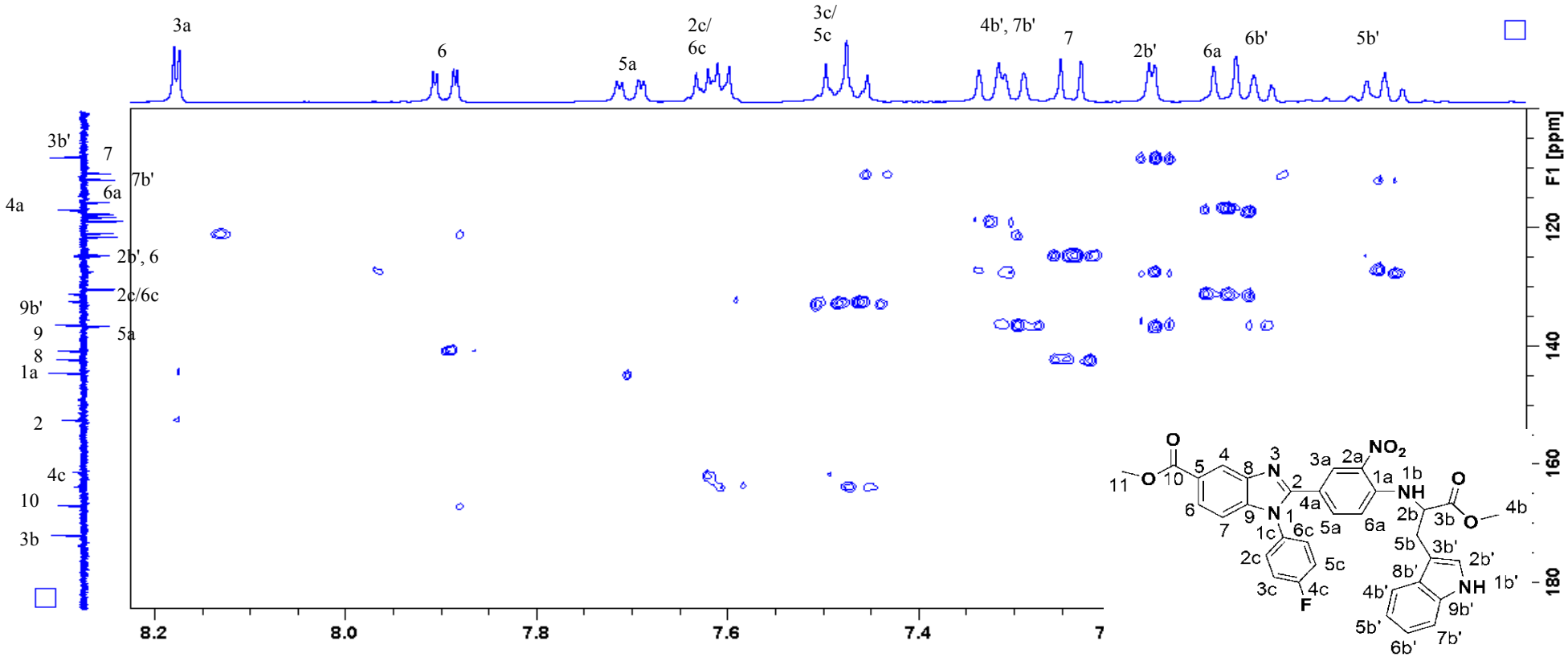
HSQC Spectrum of Compound B-7j



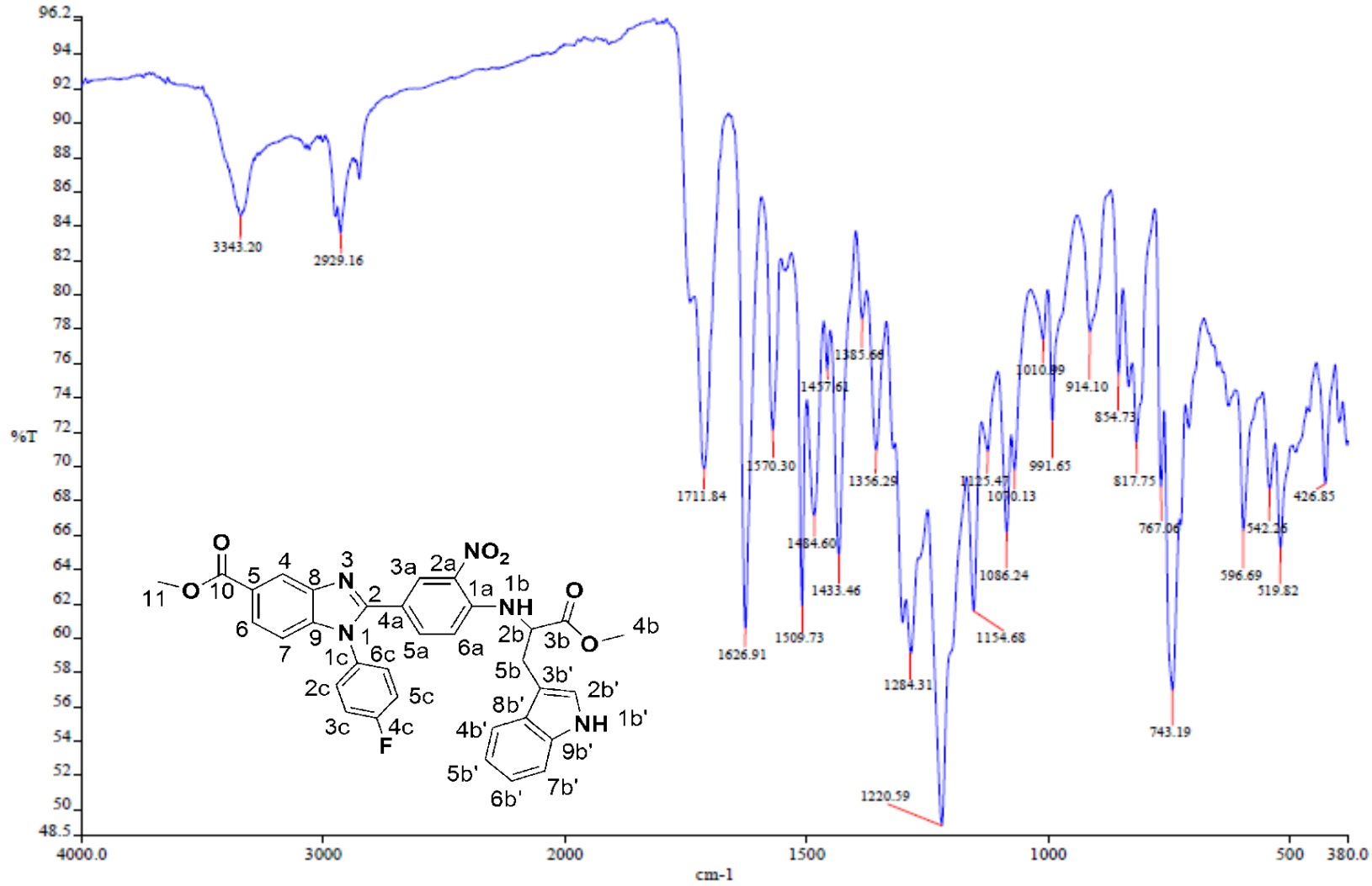
Expanded HSQC Spectrum of Compound B-7j



HMBC Spectrum of Compound B-7j

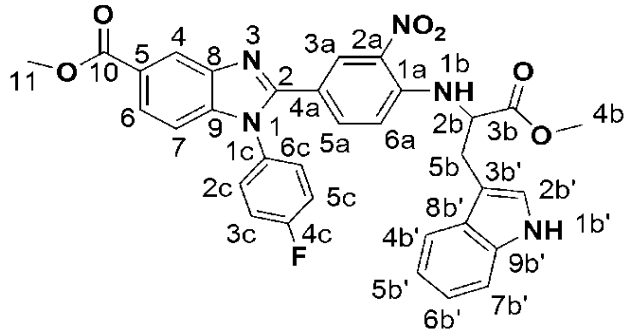


Expanded HMBC Spectrum of Compound B-7j



Infrared Spectrum of Compound B-7j

Elemental Composition Report

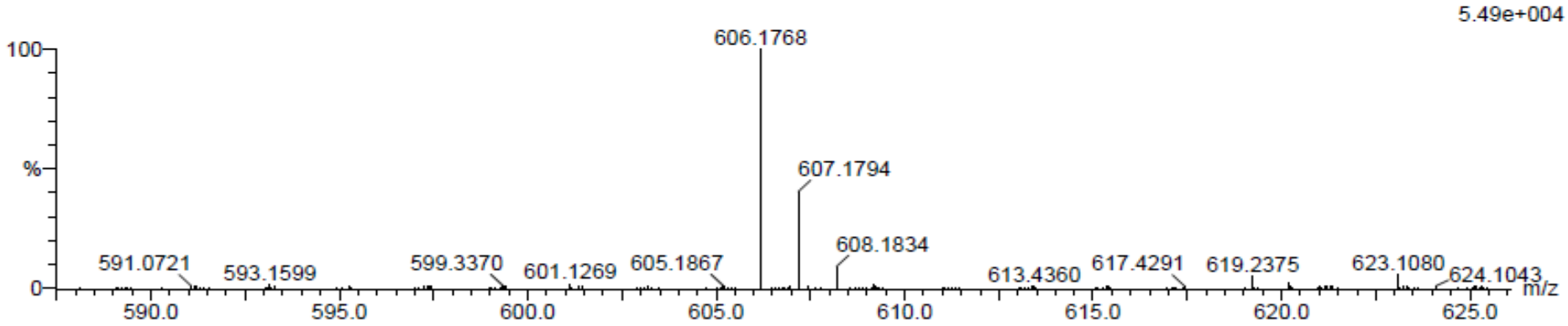


Single Mass Analysis

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

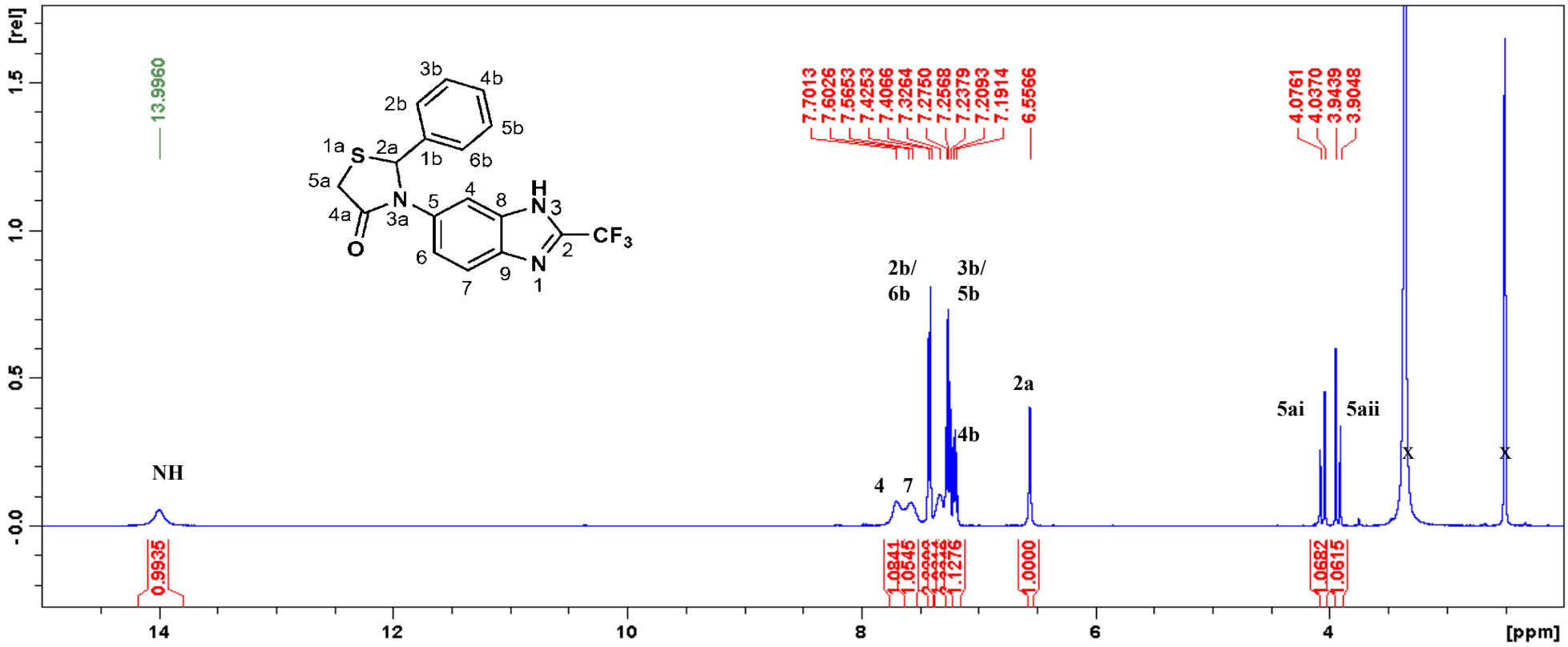
Monoisotopic Mass, Even Electron Ions
23 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 30-35 H: 25-30 N: 0-5 O: 0-8 F: 1-1

BA 7 53 (1.754) Cm (1:61)
TOF MS ES-

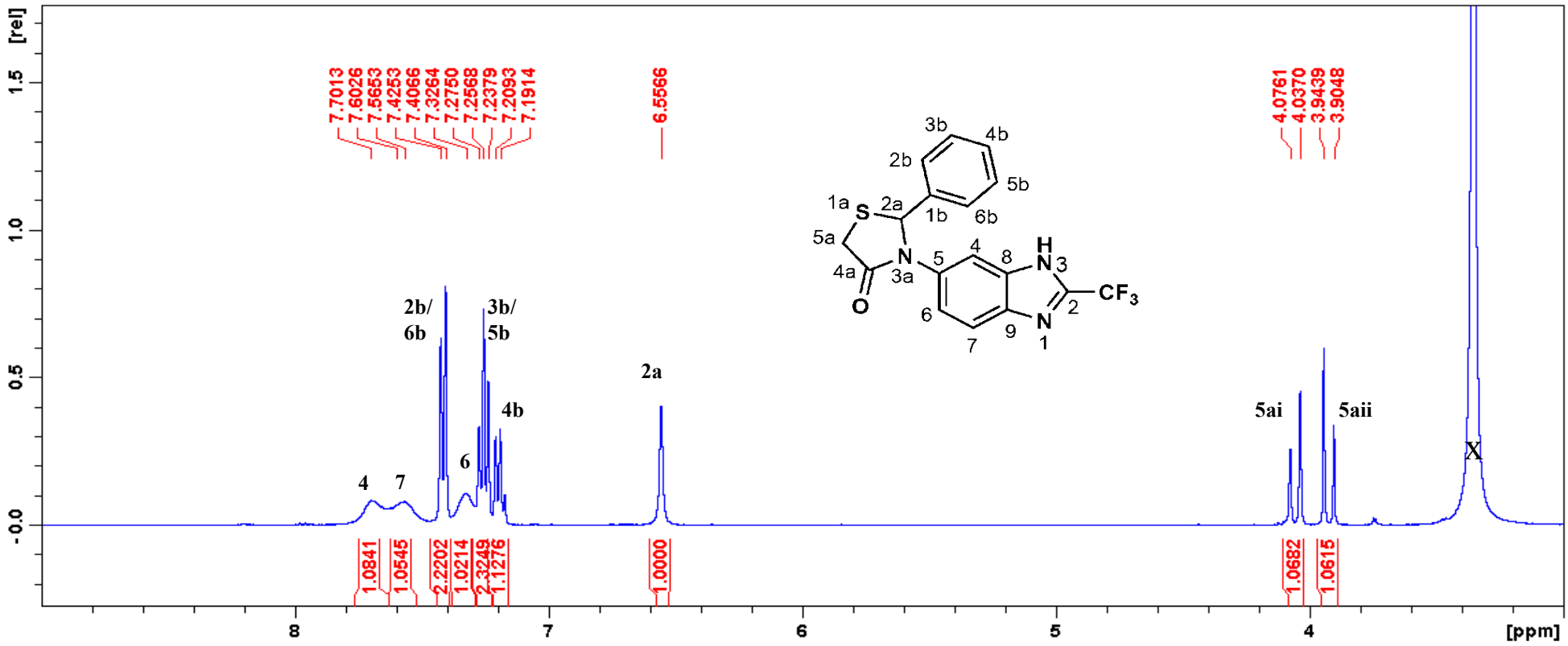


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
606.1768	606.1789	-2.1	-3.5	23.5	110.3	0.0	C33 H25 N5 O6 F

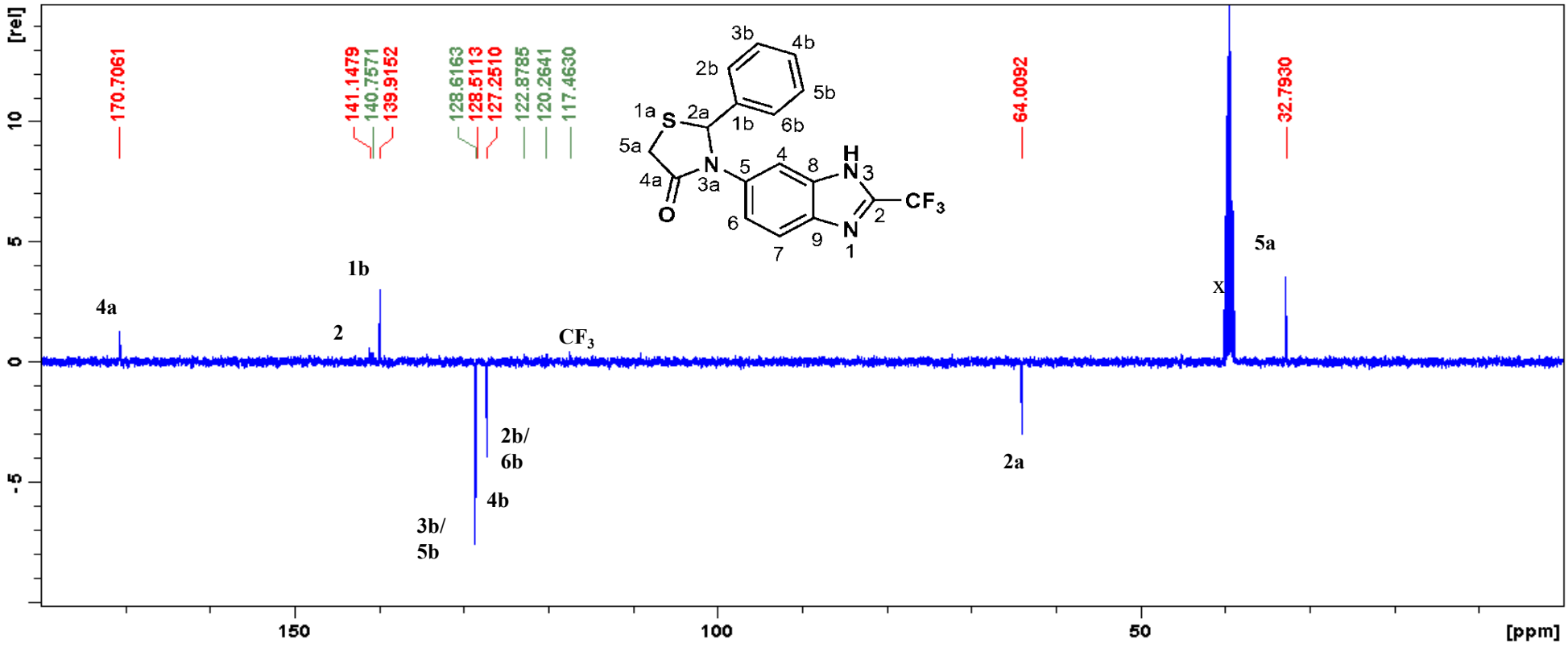
HRMS Spectrum of Compound B-7j



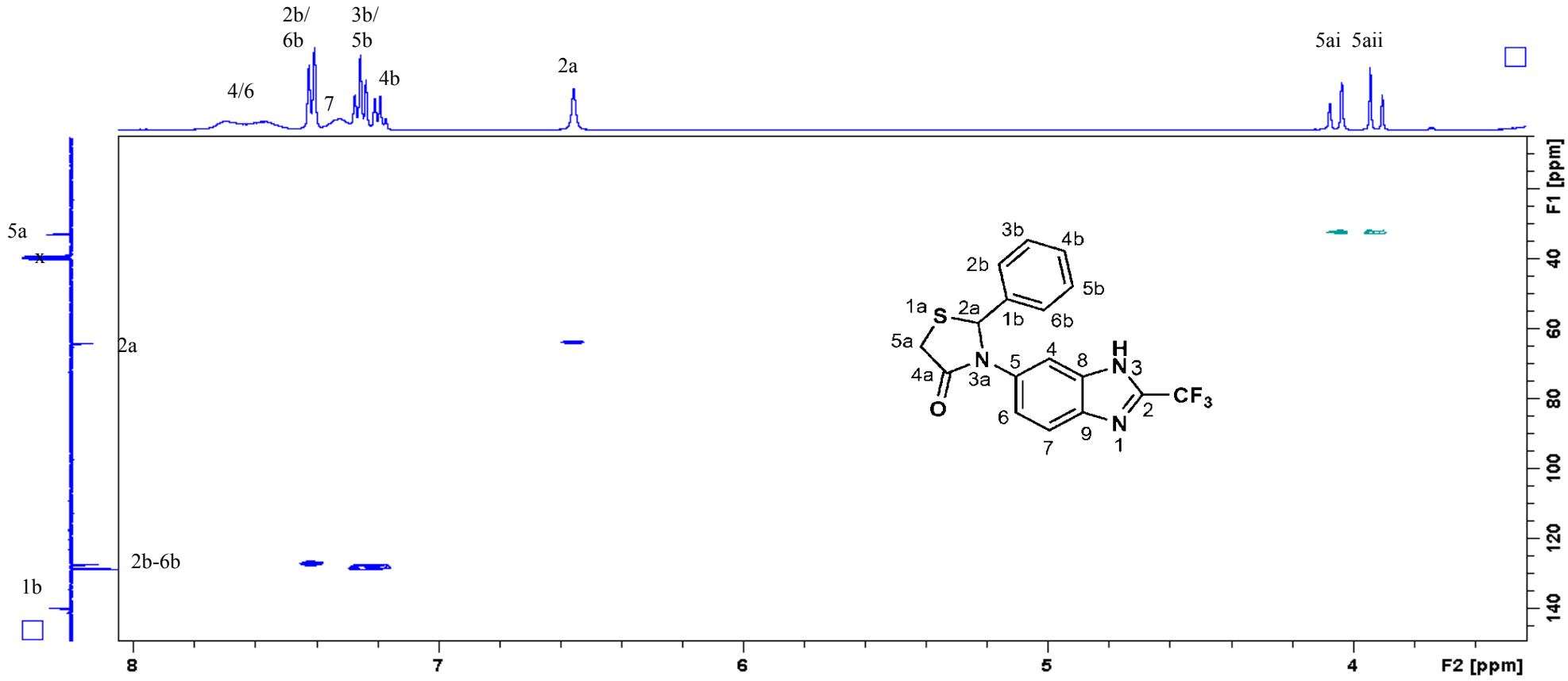
^{1}H NMR Spectrum of C-3a



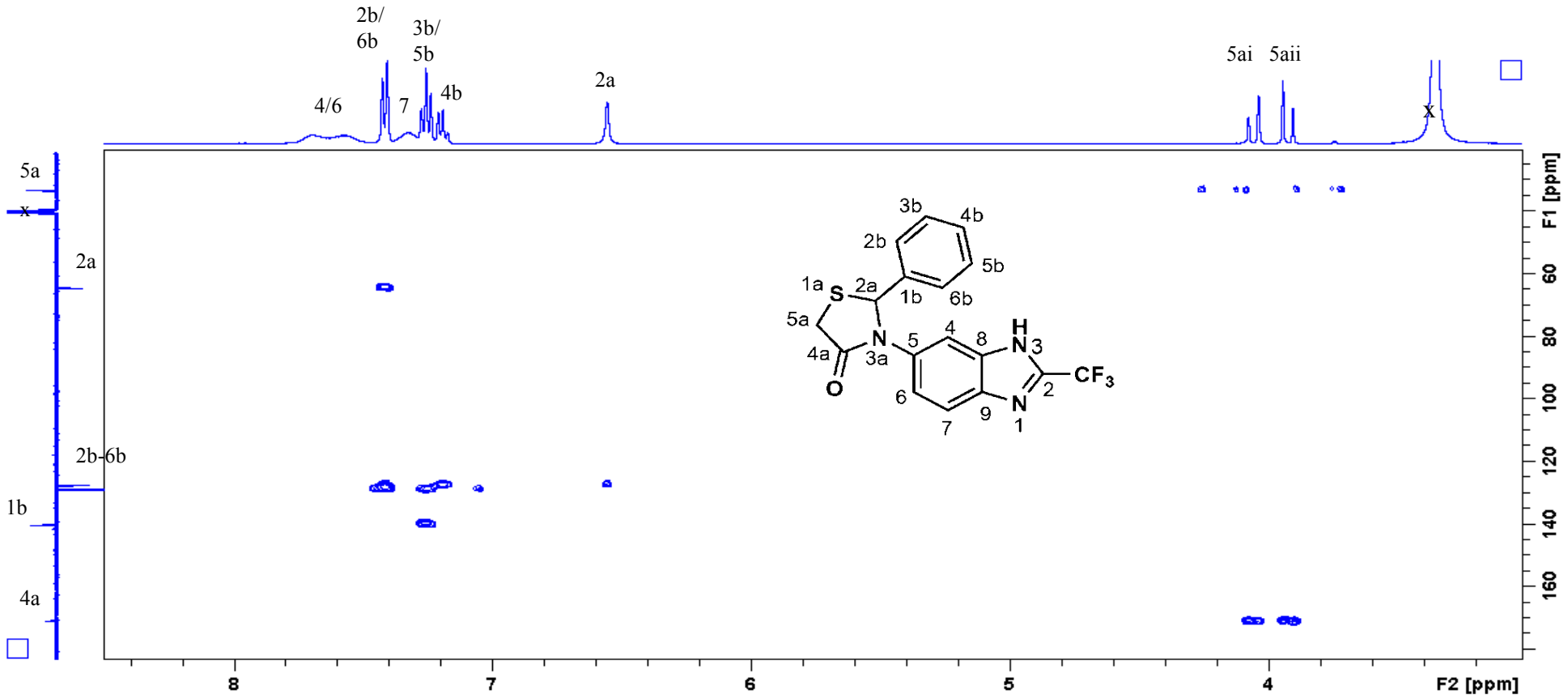
Expanded ^{1}H NMR Spectrum of C-3a



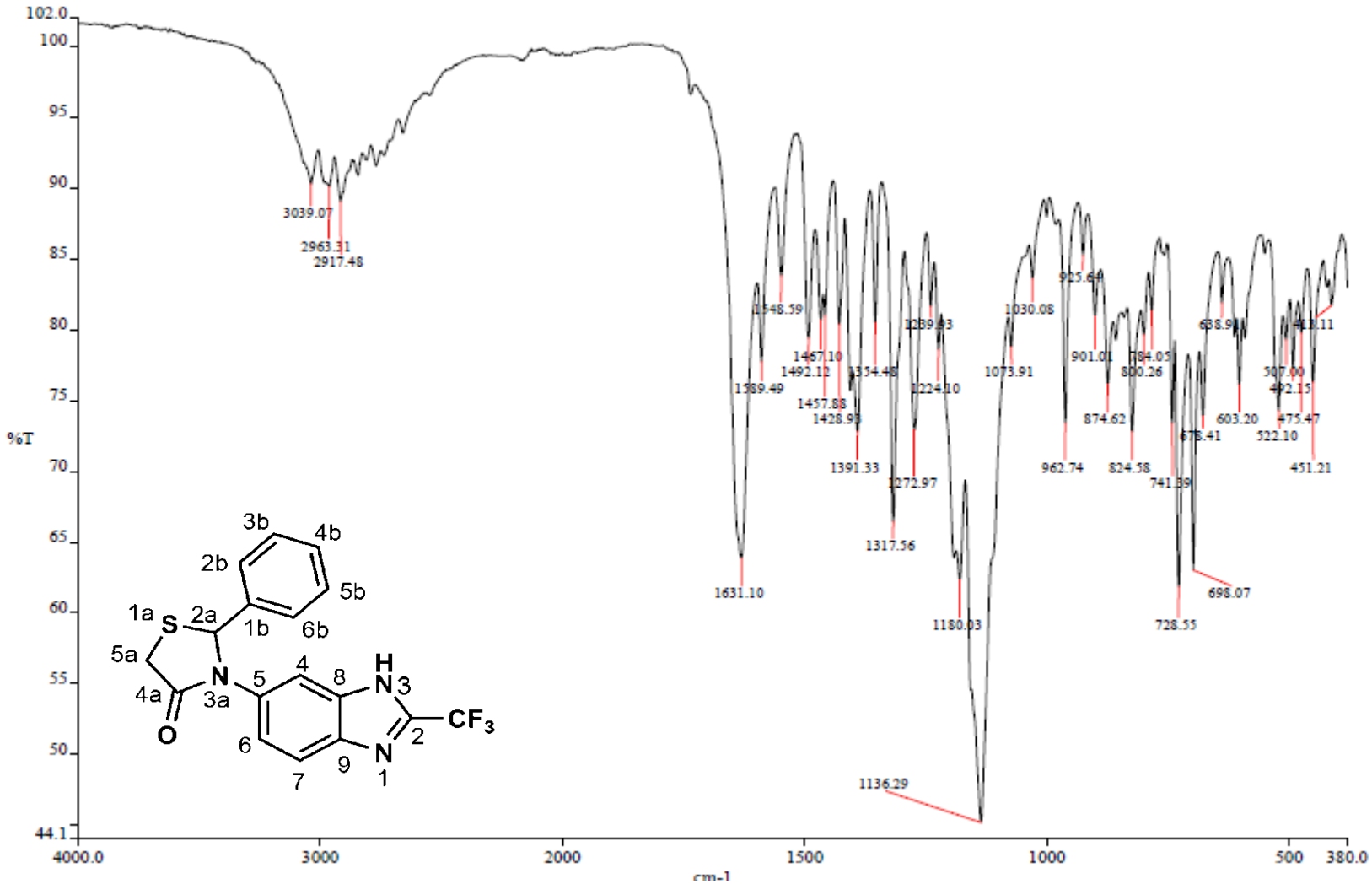
^{13}C NMR Spectrum of C-3a



HSQC Spectrum of C-3a



HMBC Spectrum of C-3a



Infrared Spectrum of C-3a

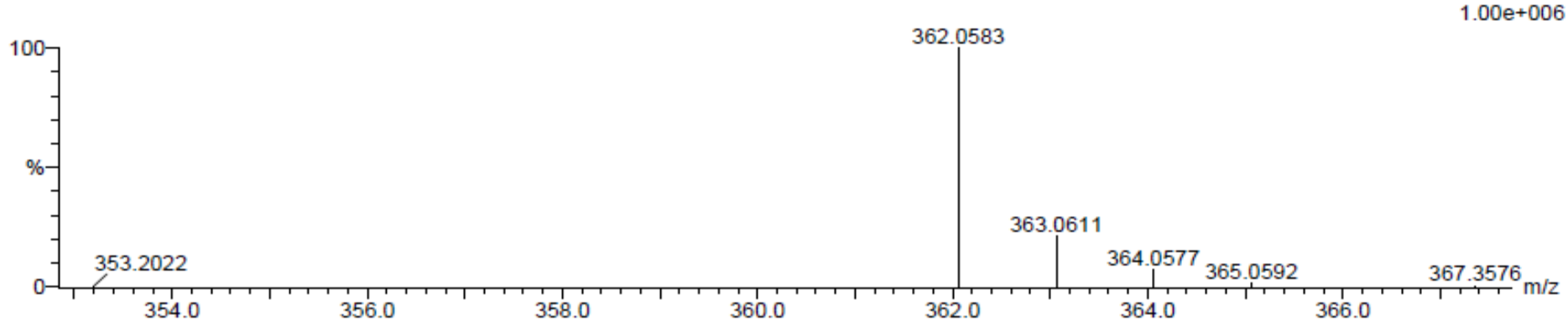
Elemental Composition Report

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

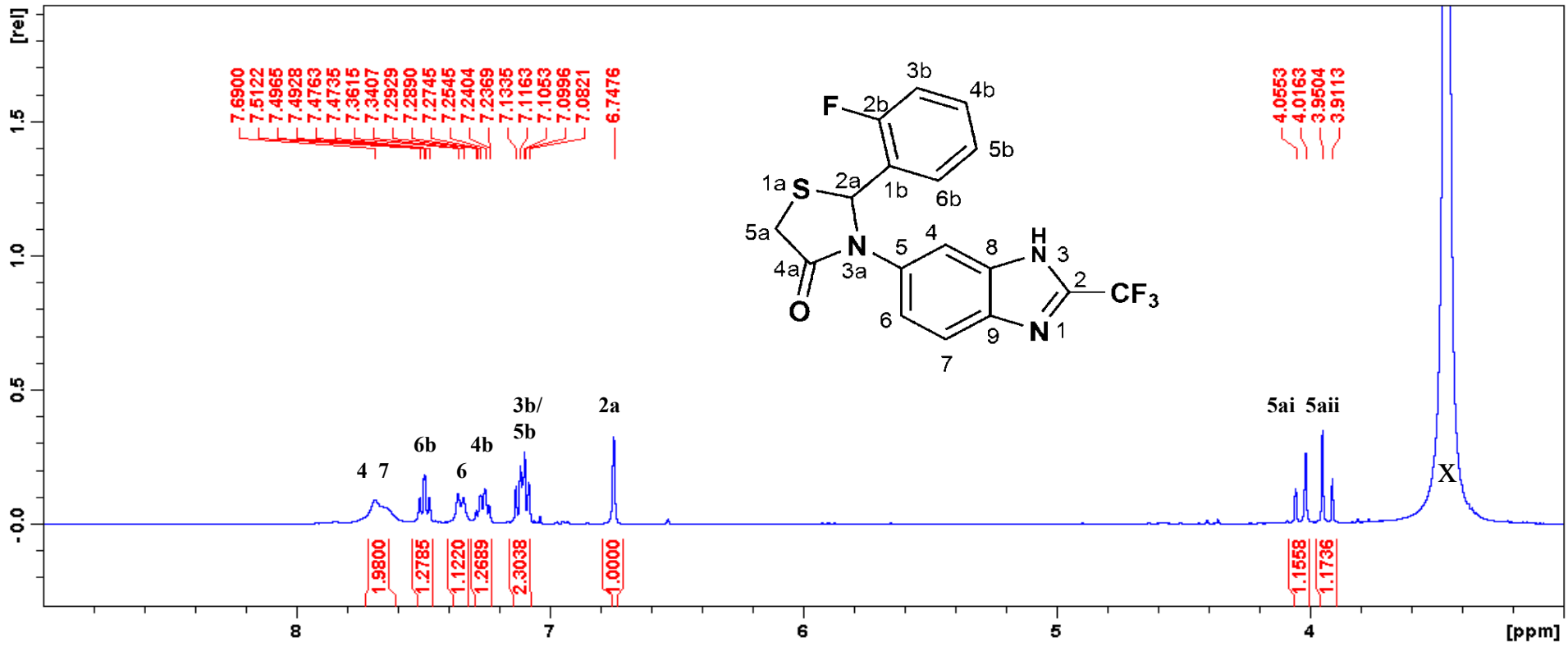
Monoisotopic Mass, Even Electron Ions
102 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 O: 0-5 F: 1-5 S: 1-1

CF 1 29 (0.945) Cm (1:61)
TOF MS ES-

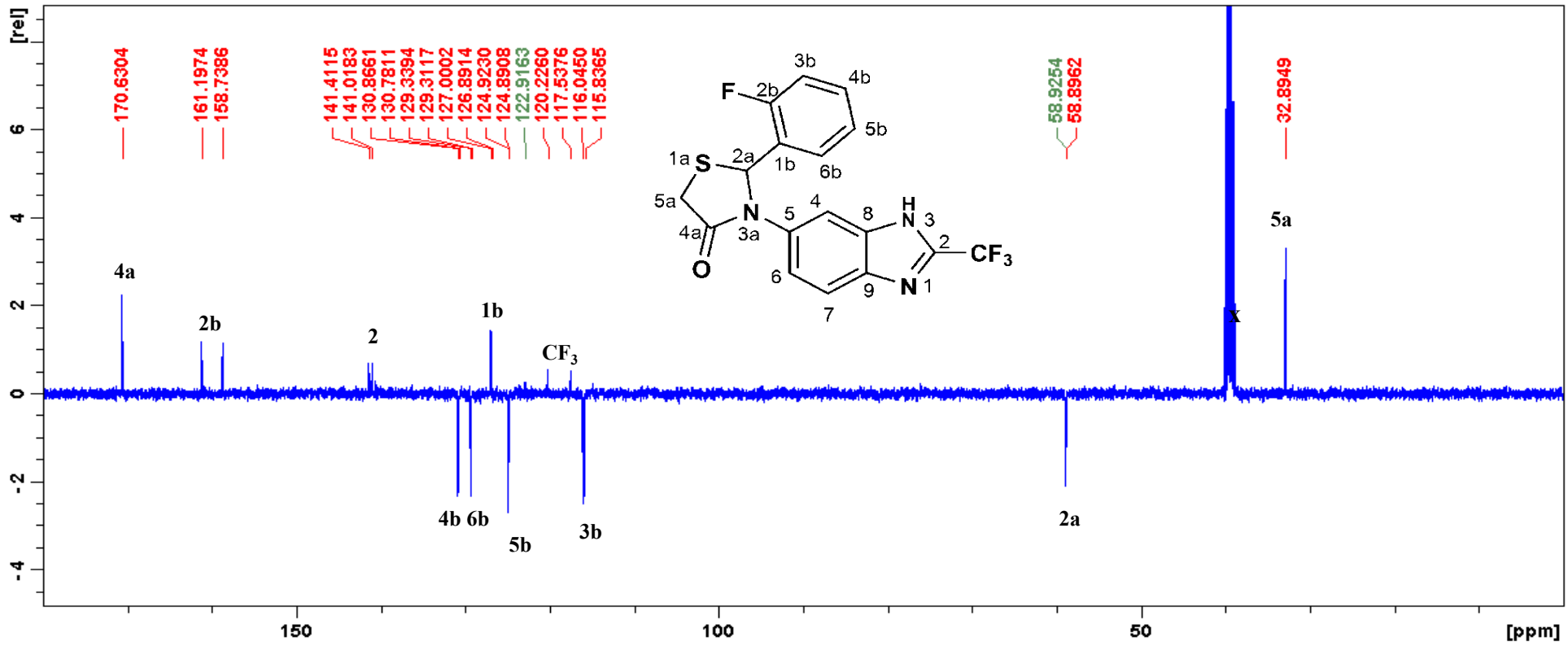


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
362.0583	362.0575	0.8	2.2	12.5	9.2	0.0	C17 H11 N3 O F3 S

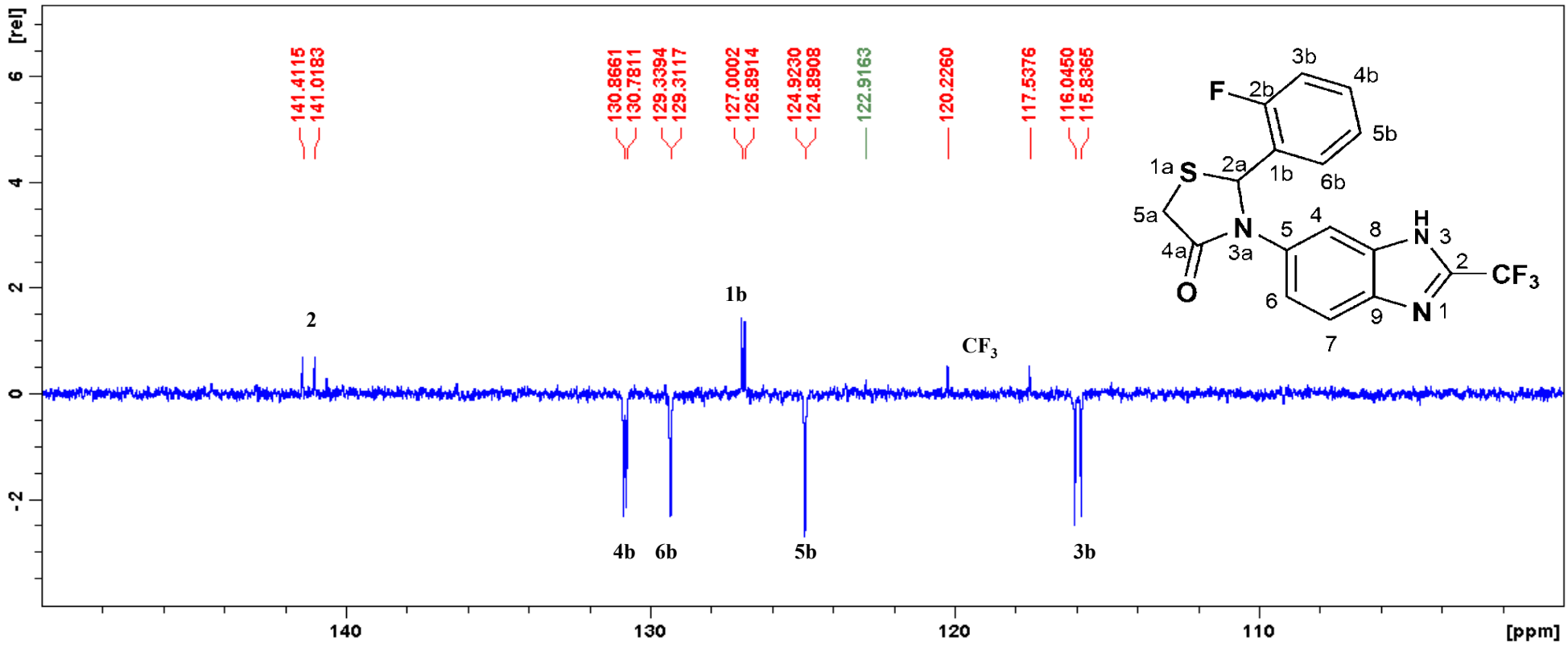
HRMS Spectrum of C-3a



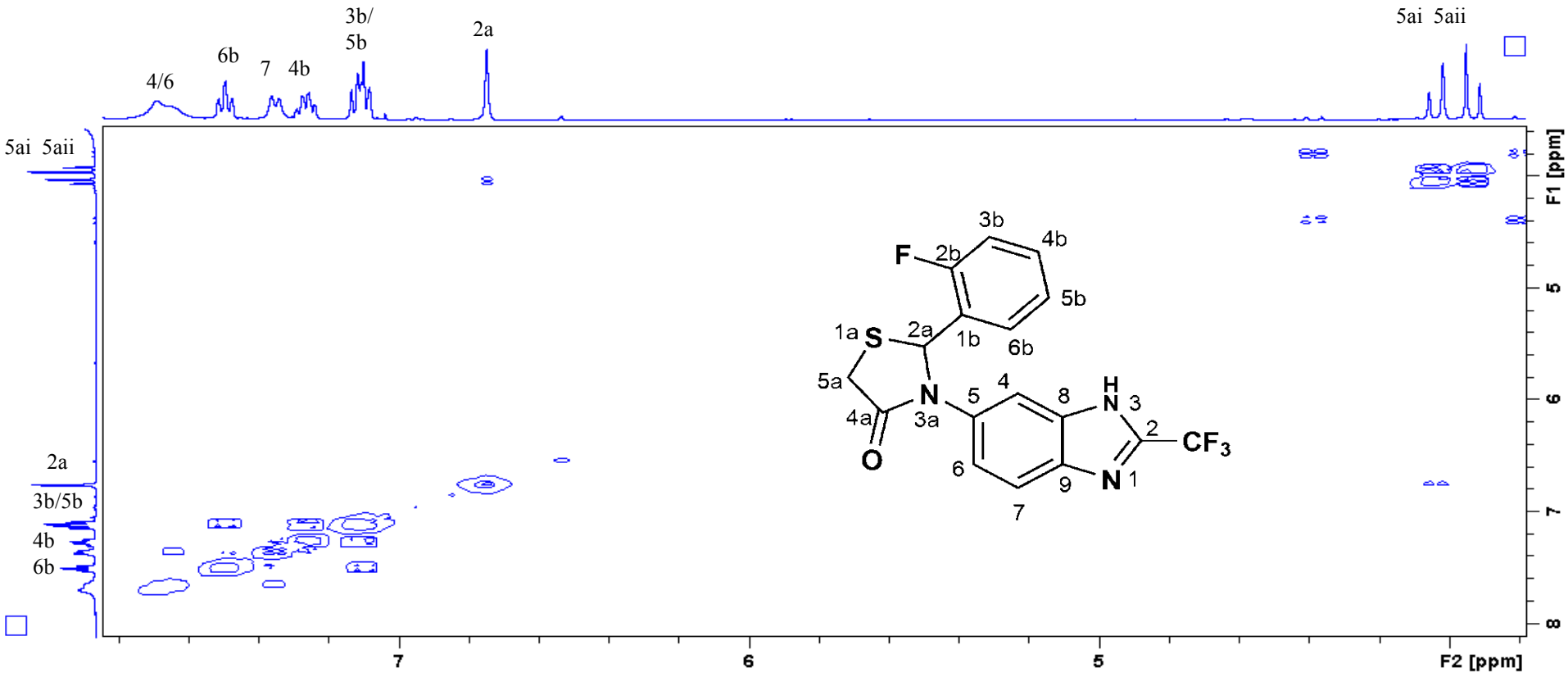
^{1}H NMR Spectrum of C-3b



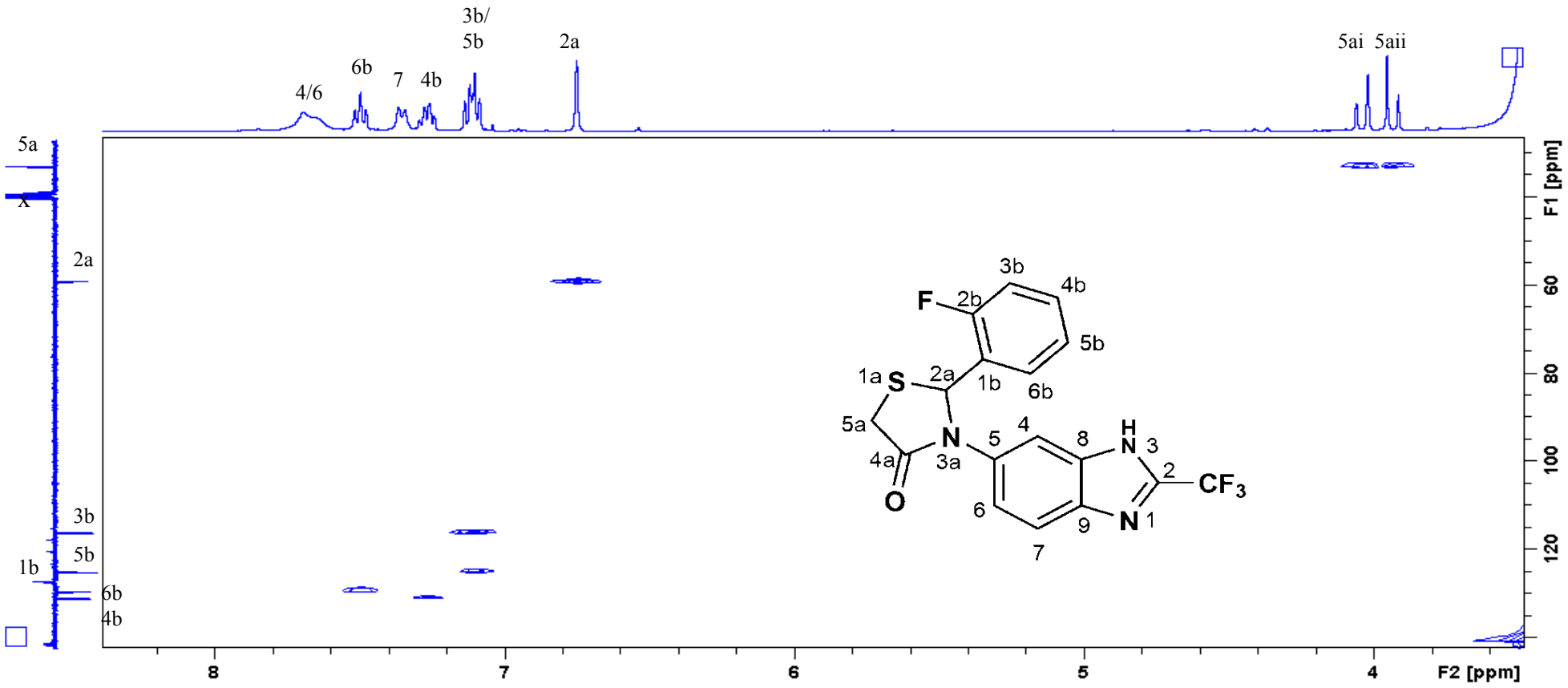
^{13}C NMR Spectrum of C-3b



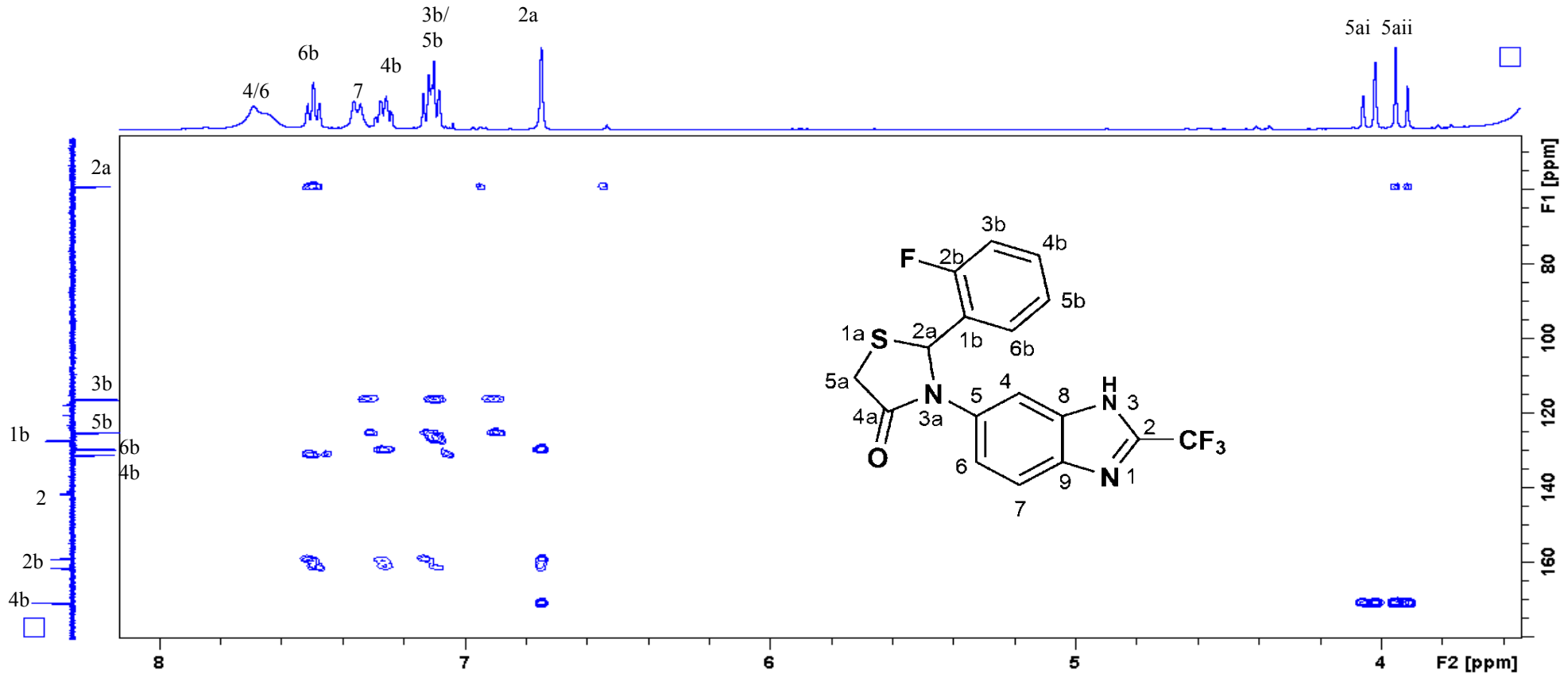
Expanded ^{13}C NMR Spectrum of C-3b



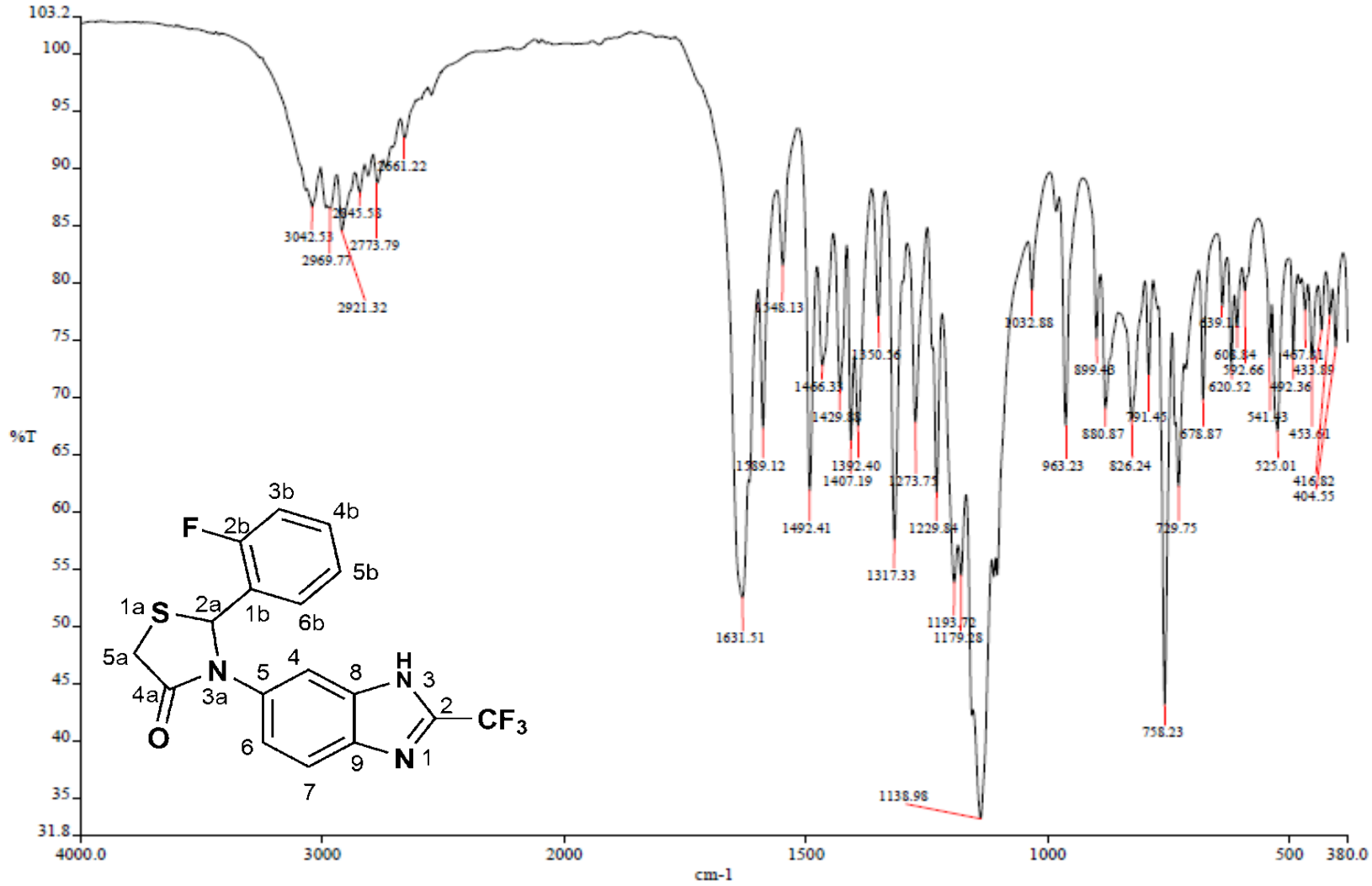
COSY Spectrum of C-3b



HSQC Spectrum of C-3b



HMBC Spectrum of C-3b



Infrared Spectrum of C-3b

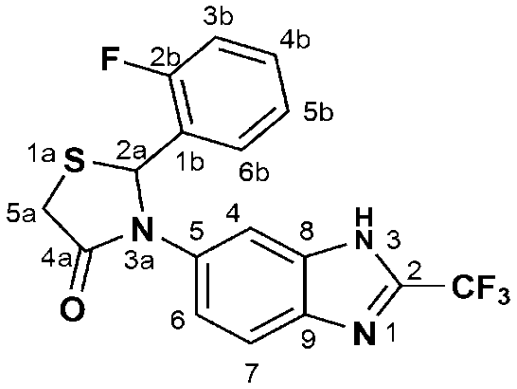
Elemental Composition Report

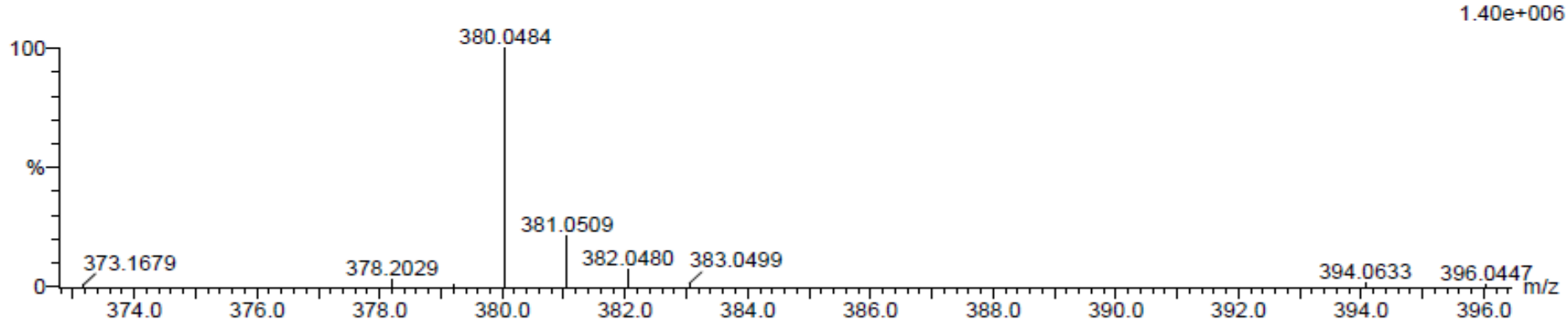
Single Mass Analysis

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
88 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 O: 0-5 F: 1-5 S: 1-1

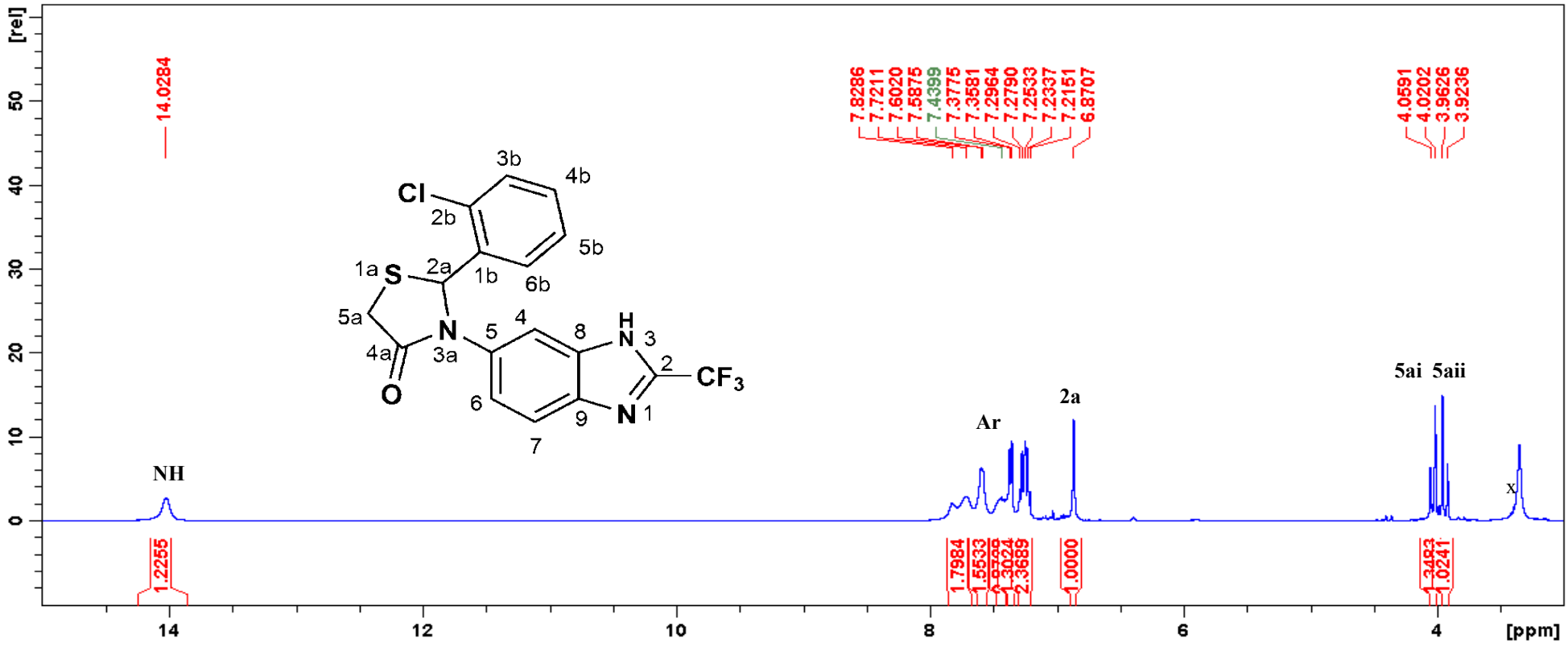
CF 32 (1.047) Cm (1:61)
TOF MS ES-



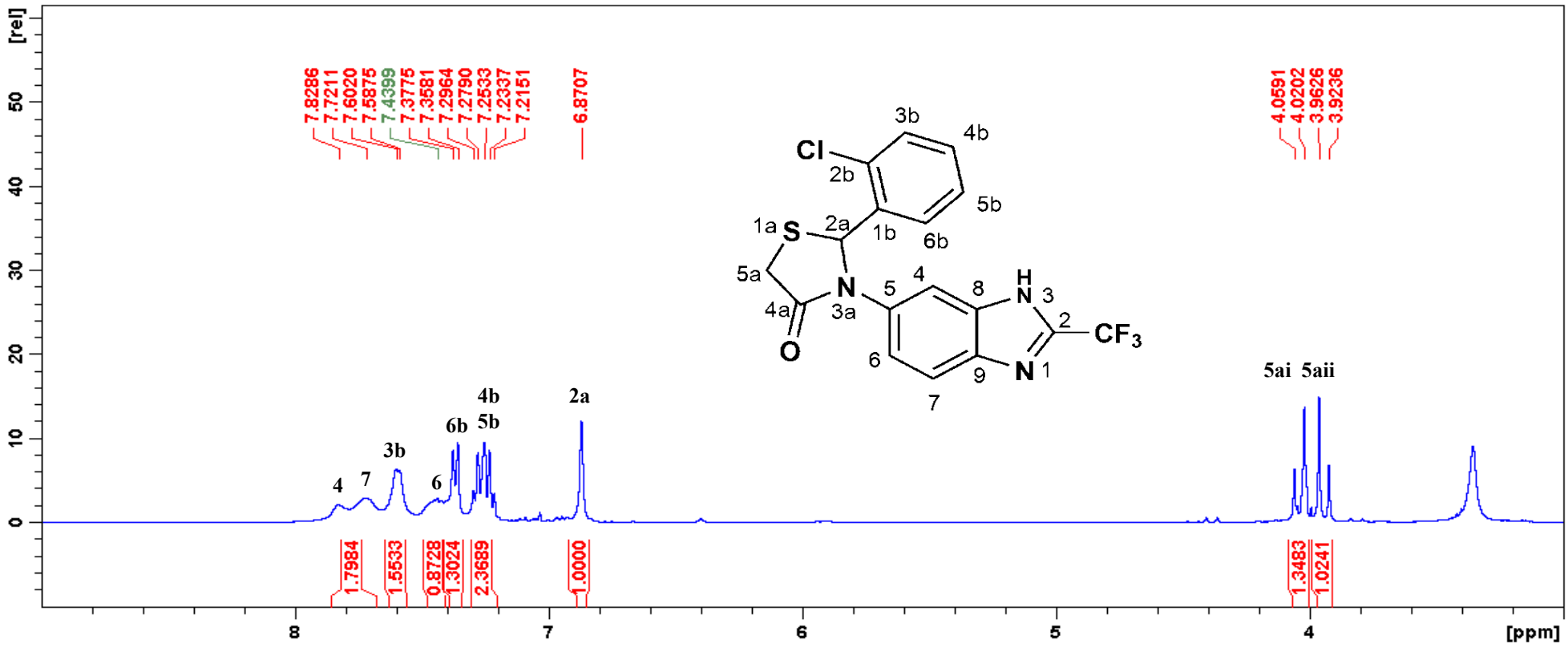


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
380.0484	380.0481	0.3	0.8	12.5	11.5	0.0	C17 H10 N3 O F4 S

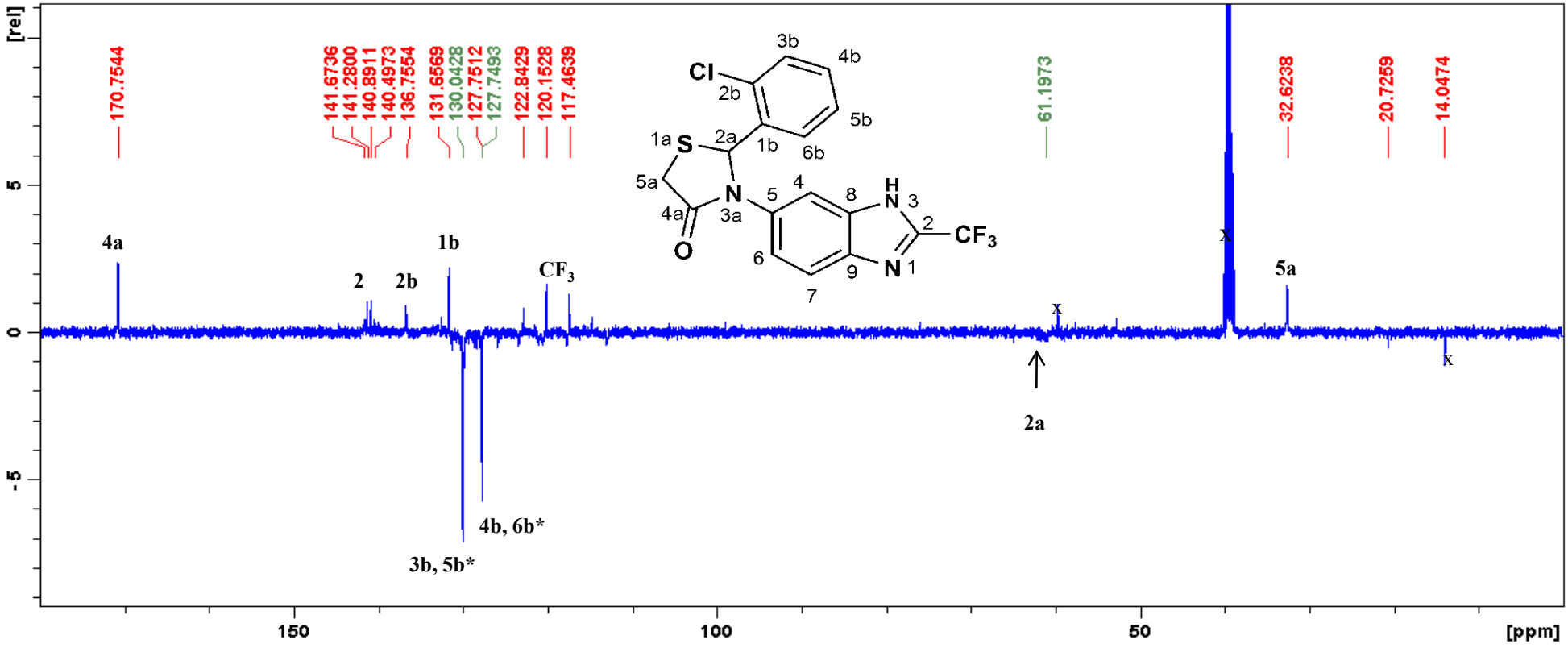
HRMS Spectrum of C-3b



^{1}H NMR Spectrum of C-3c

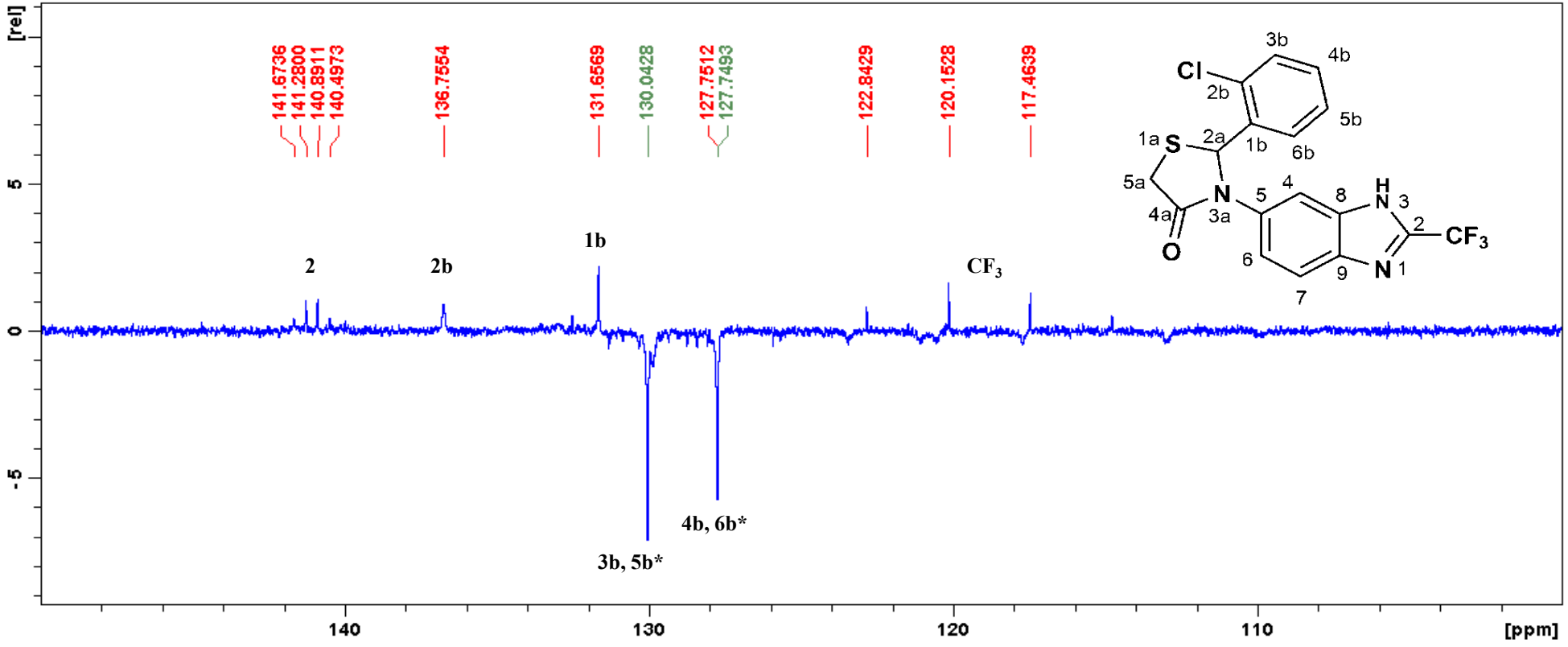


Expanded ^{1}H NMR Spectrum of C-3c



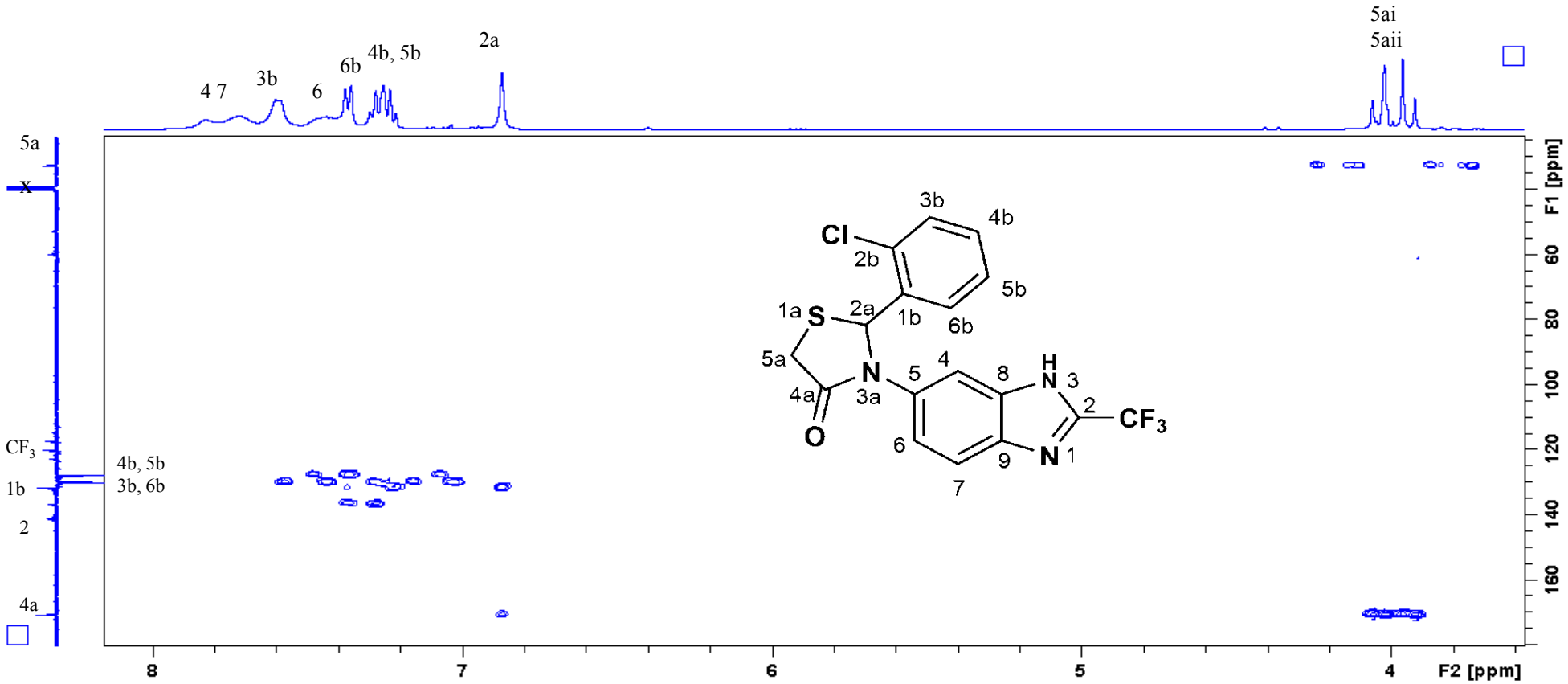
* Assignments may be interchanged

^{13}C NMR Spectrum of C-3c

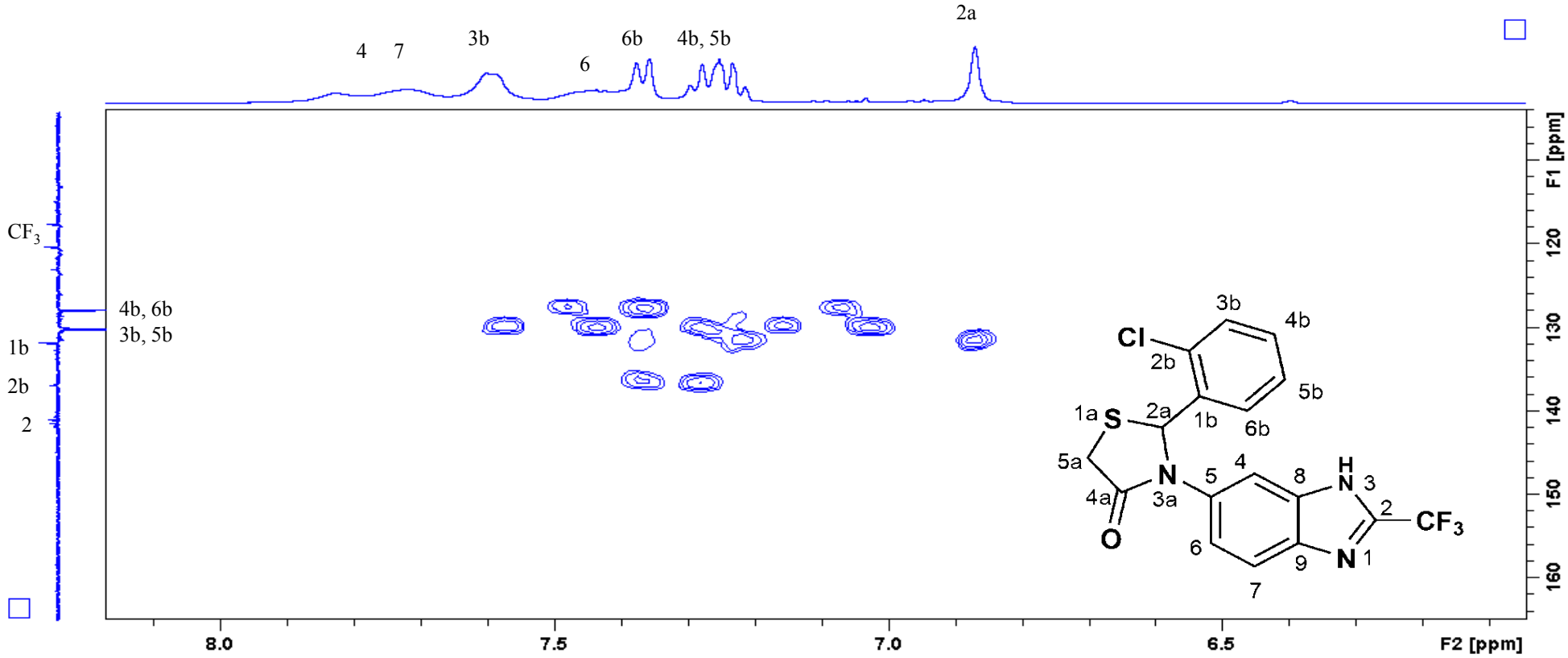


*** Assignments may be interchanged**

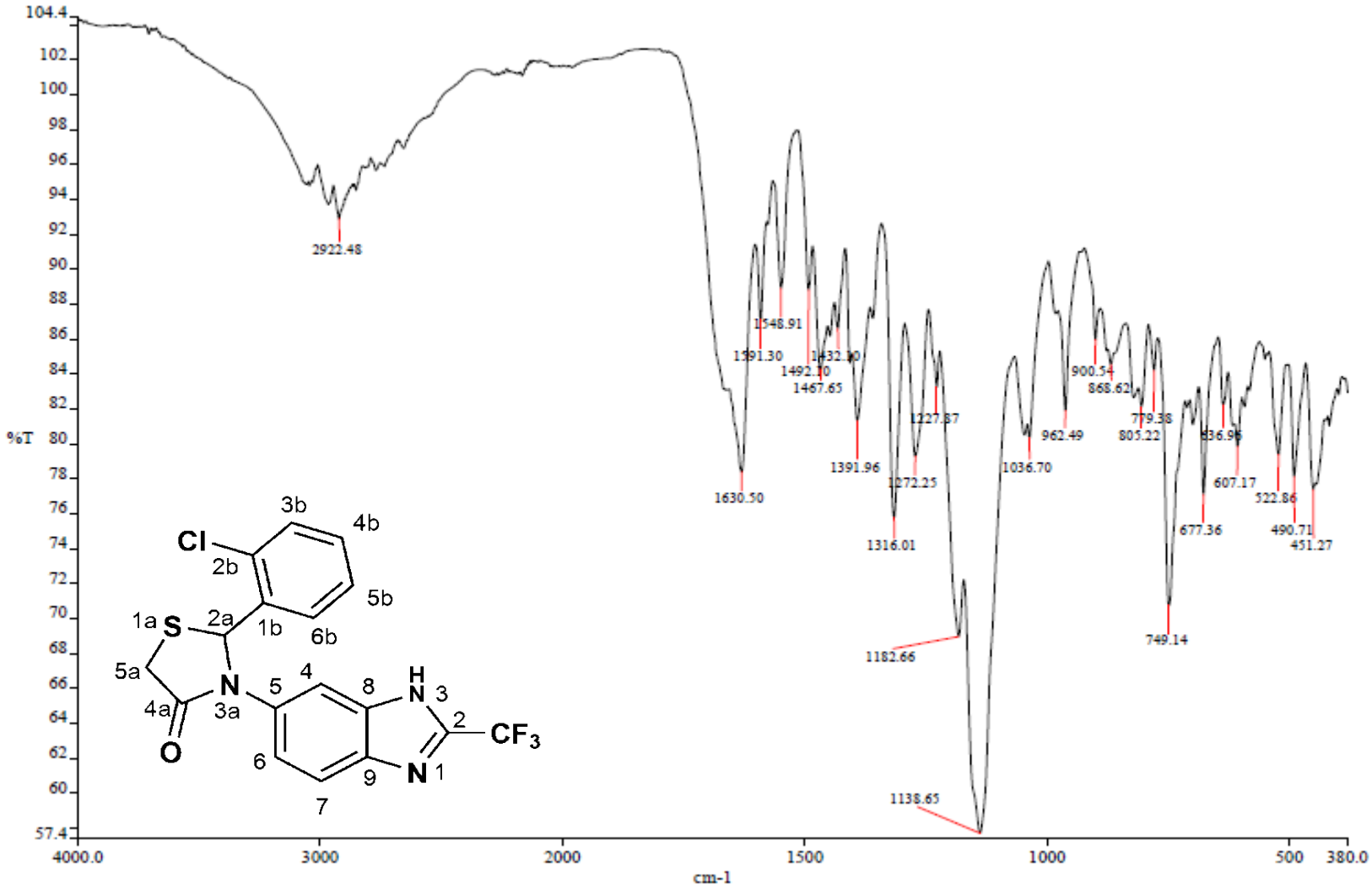
Expanded ^{13}C NMR Spectrum of C-3c



HMBC Spectrum of C-3c



Expanded HMBC Spectrum of C-3c



Infrared Spectrum of C-3c

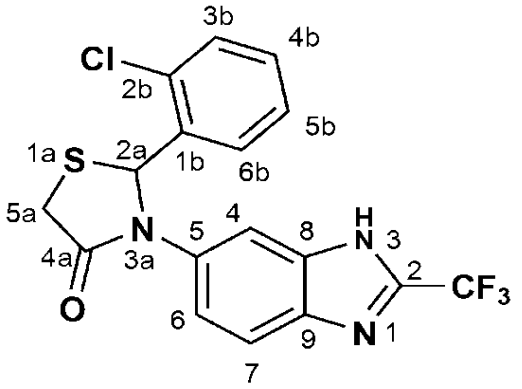
Elemental Composition Report

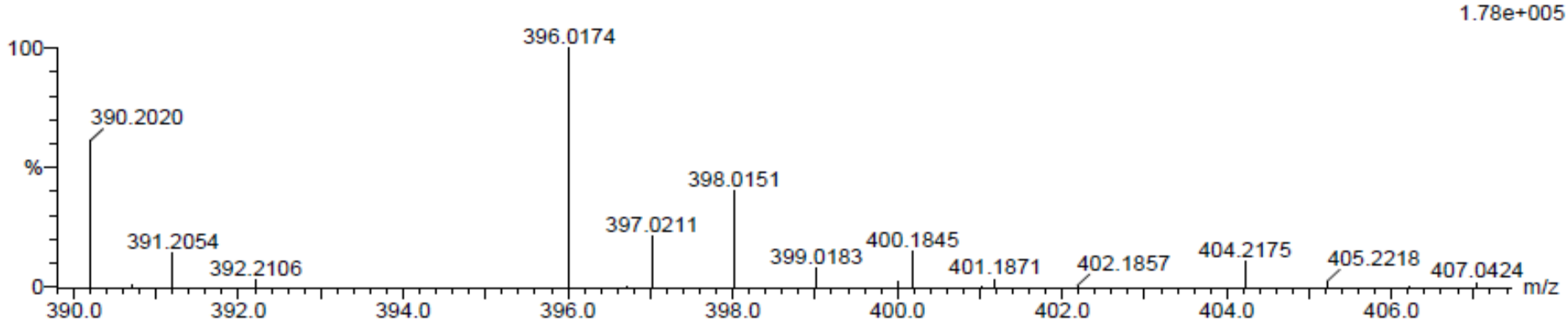
Single Mass Analysis

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
183 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 O: 0-5 F: 1-5 S: 1-1 Cl: 0-1

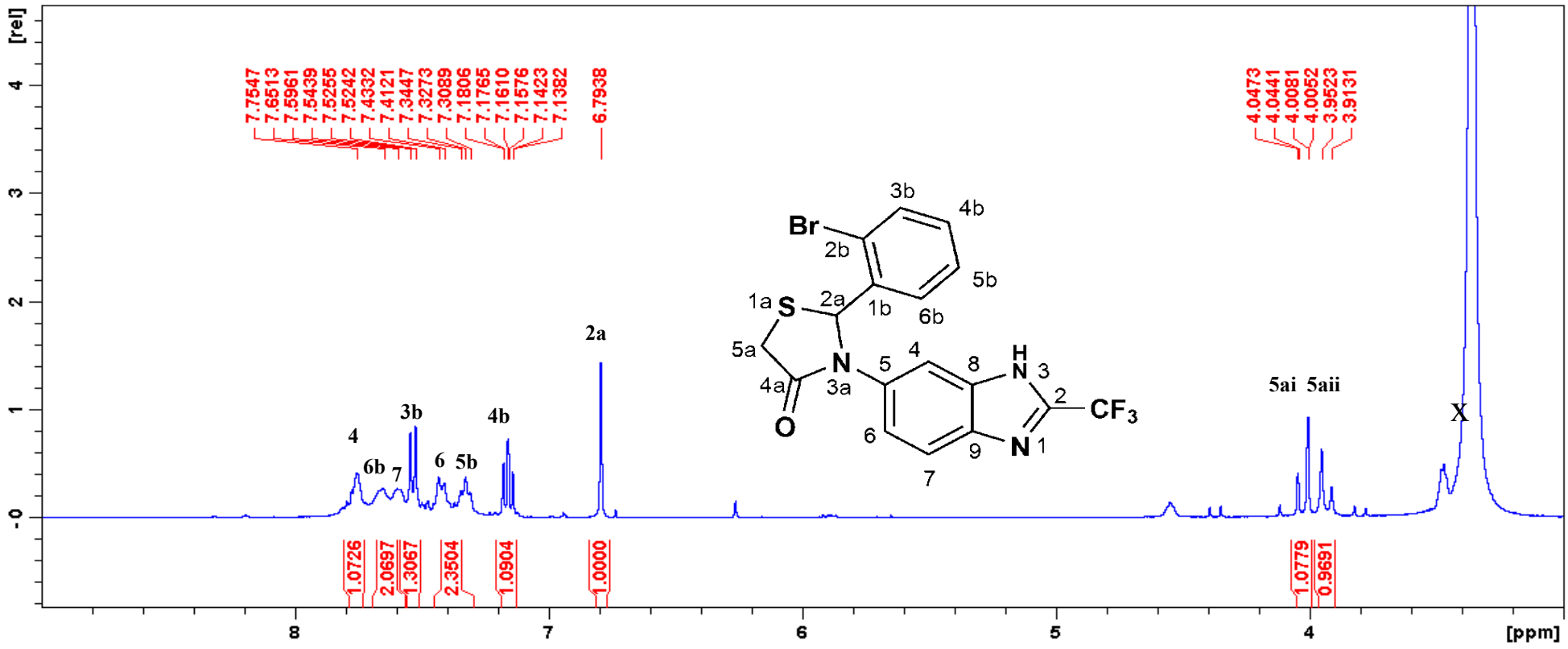
CF 10 46 (1.517) Cm (1:61)
TOF MS ES-



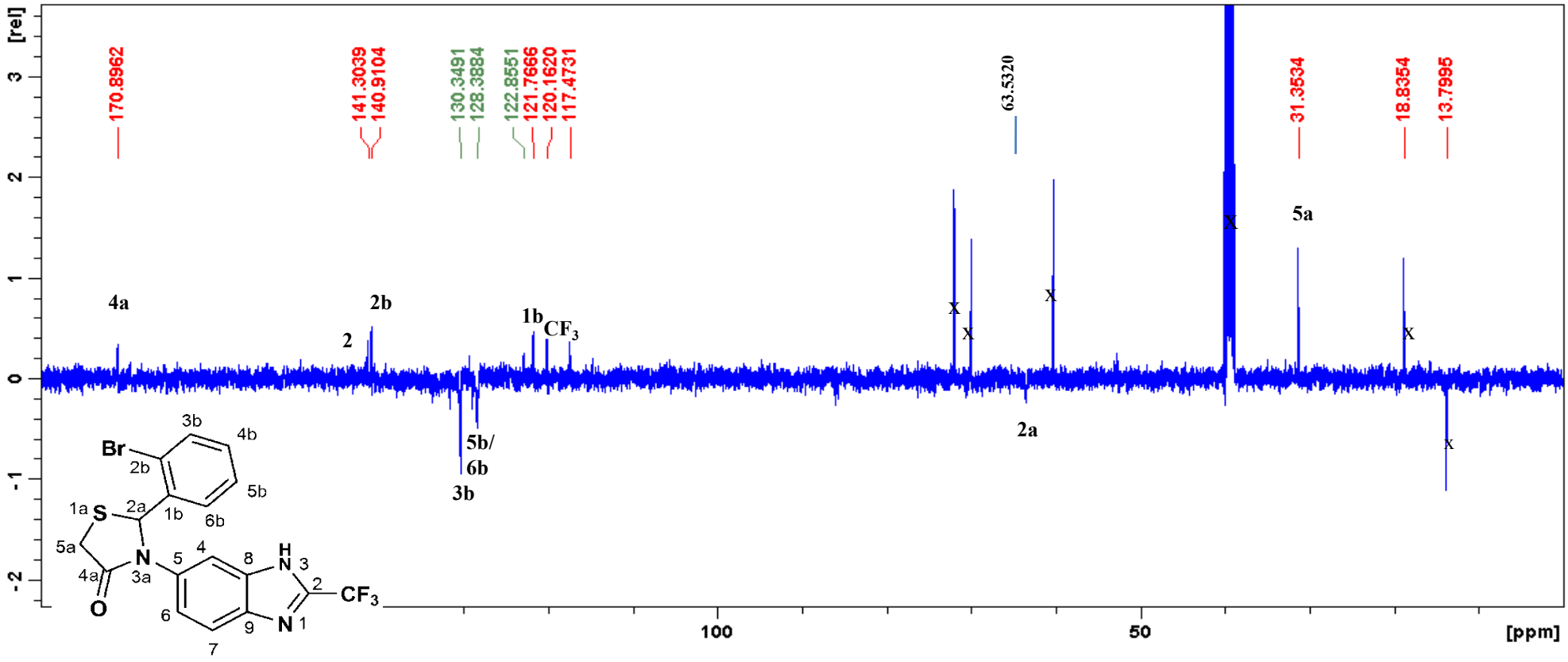


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
396.0174	396.0185	-1.1	-2.8	12.5	23.3	0.0	C17 H10 N3 O F3 S Cl

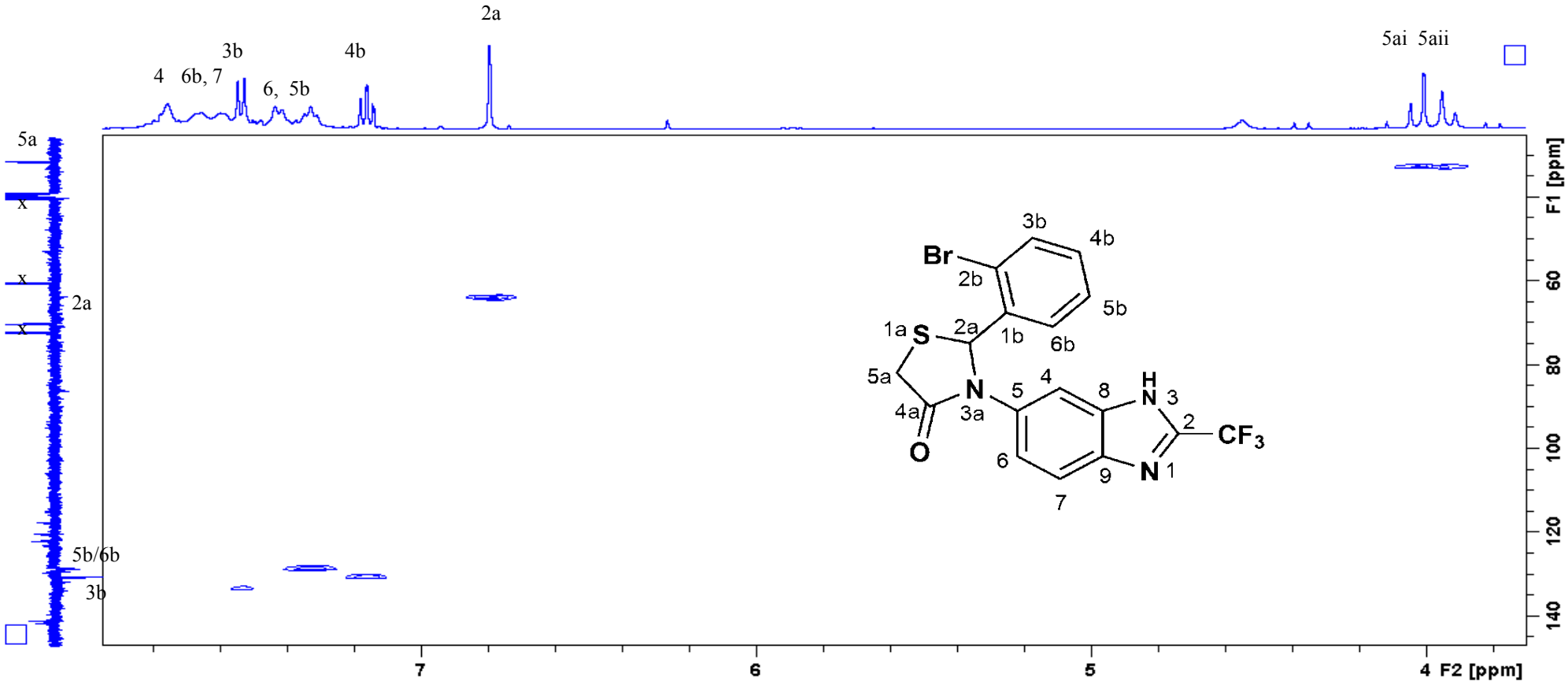
HRMS Spectrum of C-3c



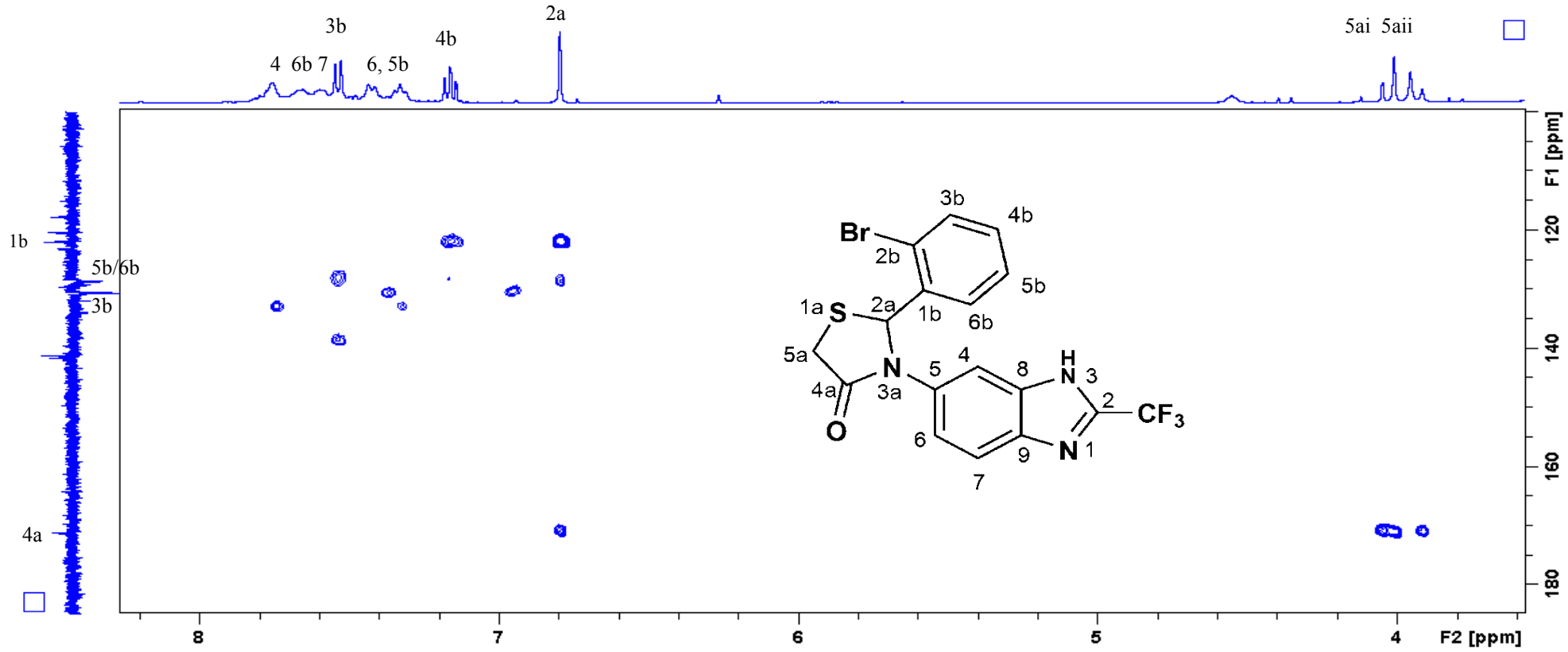
^{1}H NMR Spectrum of C-3d



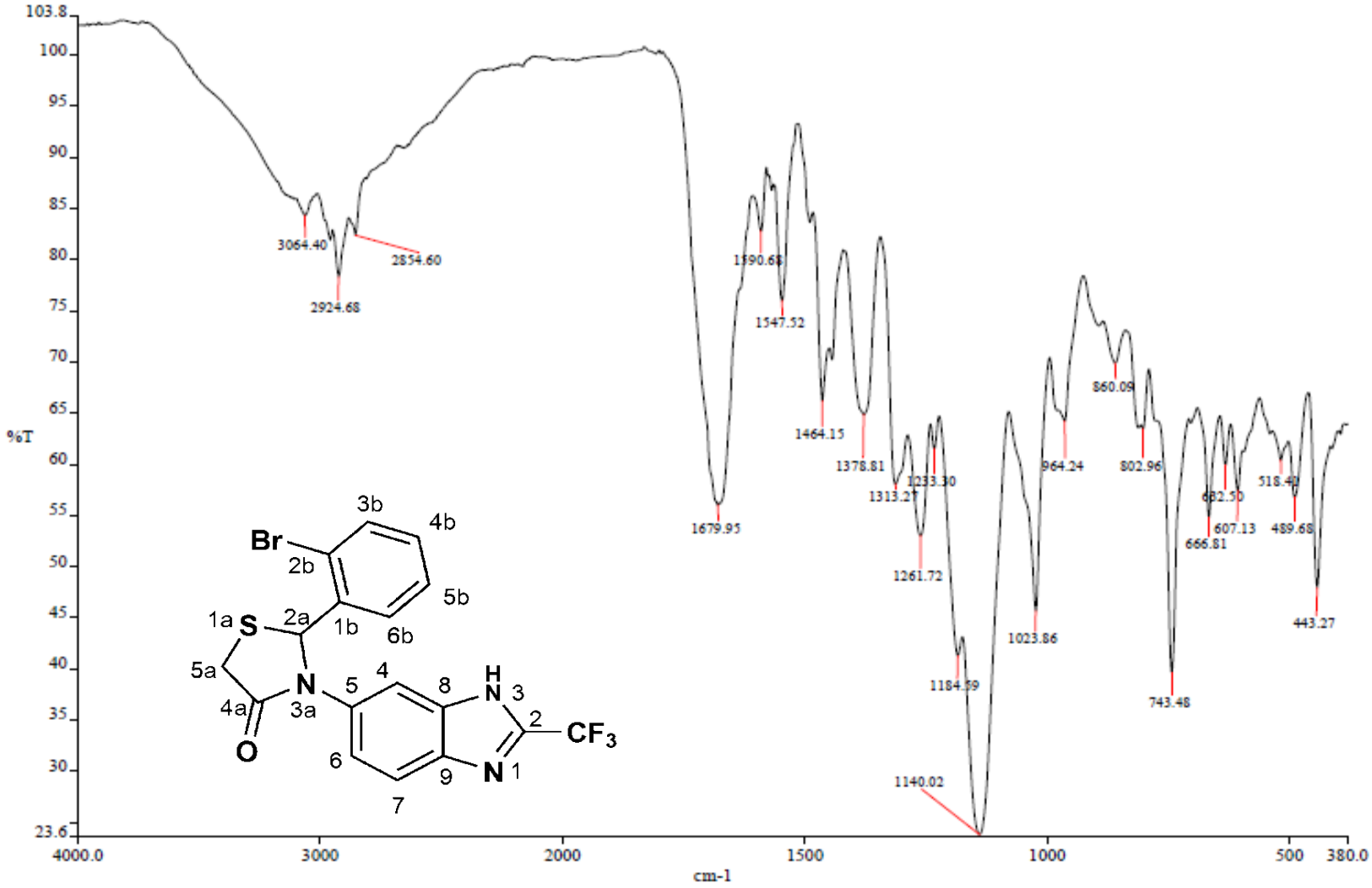
^{13}C NMR Spectrum of C-3d



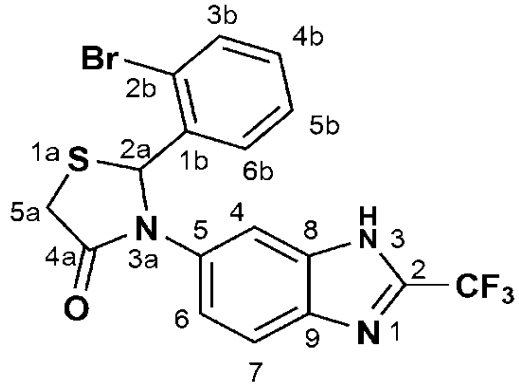
HSQC Spectrum of C-3d



HMBC Spectrum of C-3d



Infrared Spectrum of C-3d



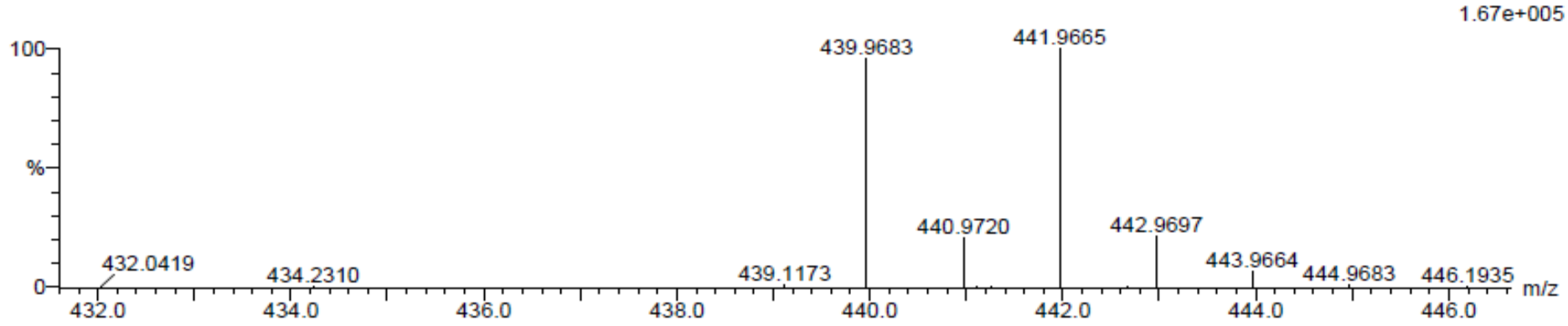
Elemental Composition Report

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

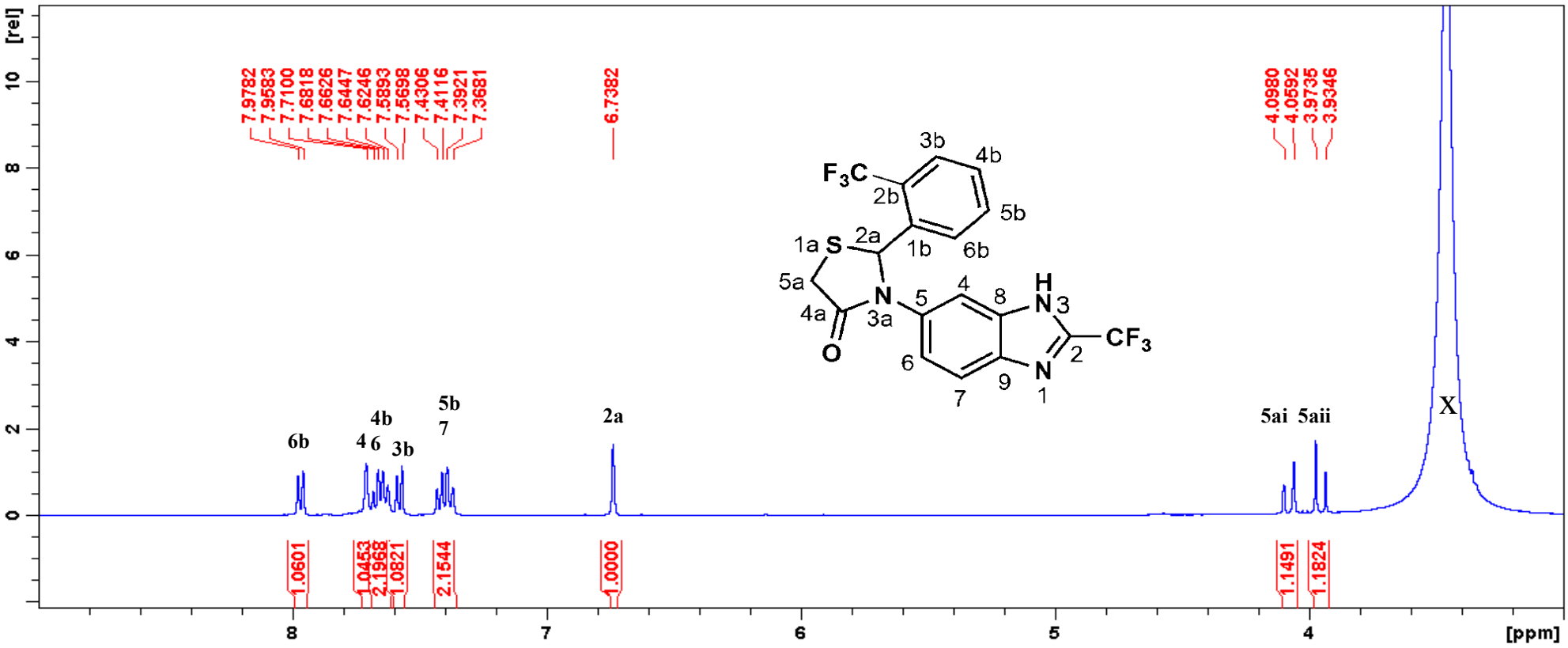
Monoisotopic Mass, Even Electron Ions
84 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 O: 0-1 F: 0-5 S: 1-1 Br: 0-1

CF 6 3 (0.068) Cm (1:61)
TOF MS ES-

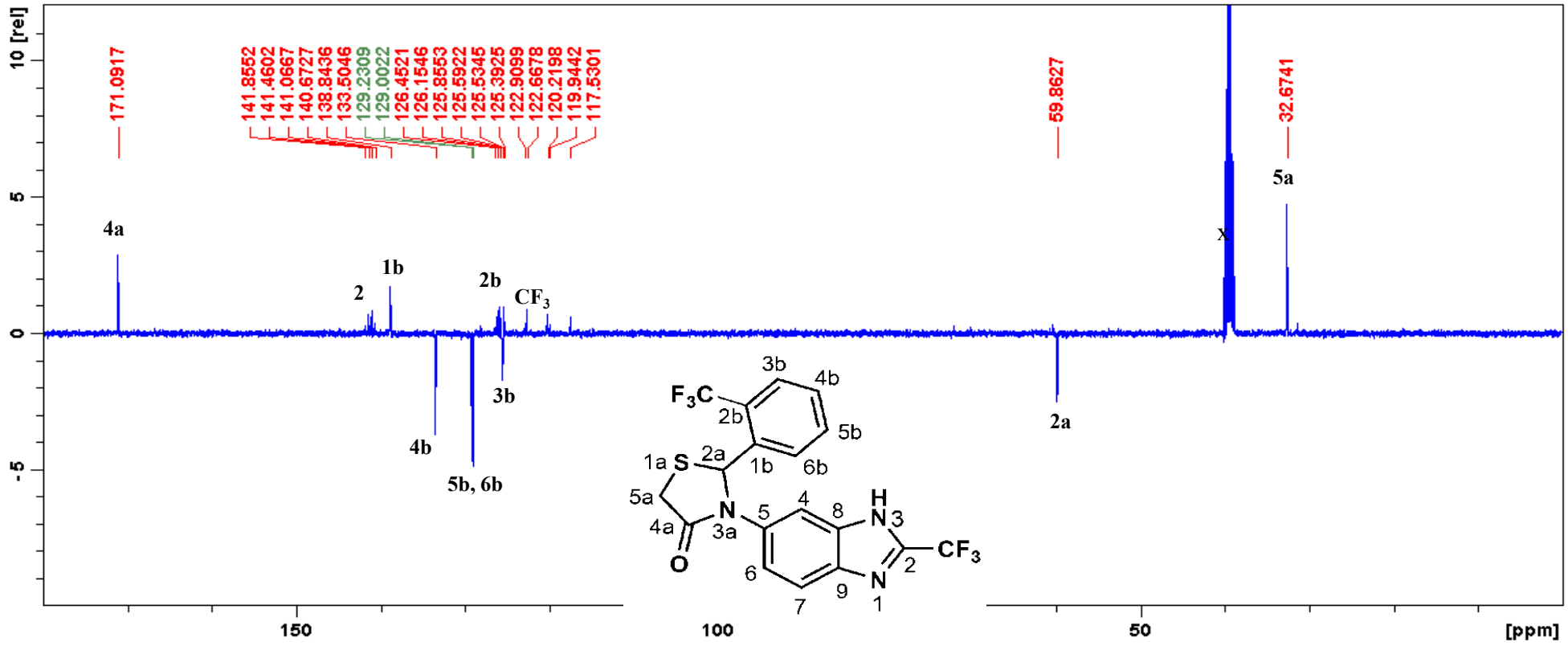


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
439.9683	439.9680	0.3	0.7	12.5	36.2	0.0	C17 H10 N3 O F3 S Br

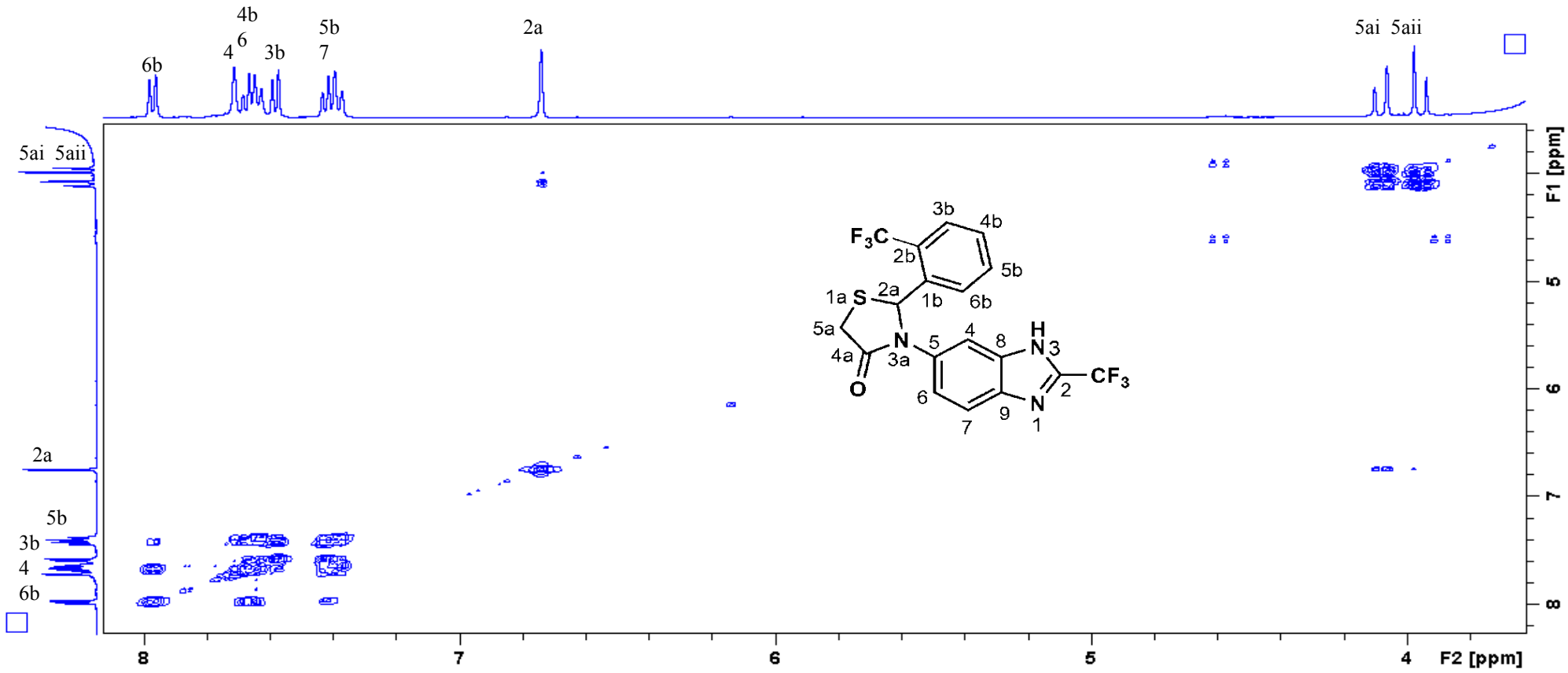
HRMS Spectrum of C-3d



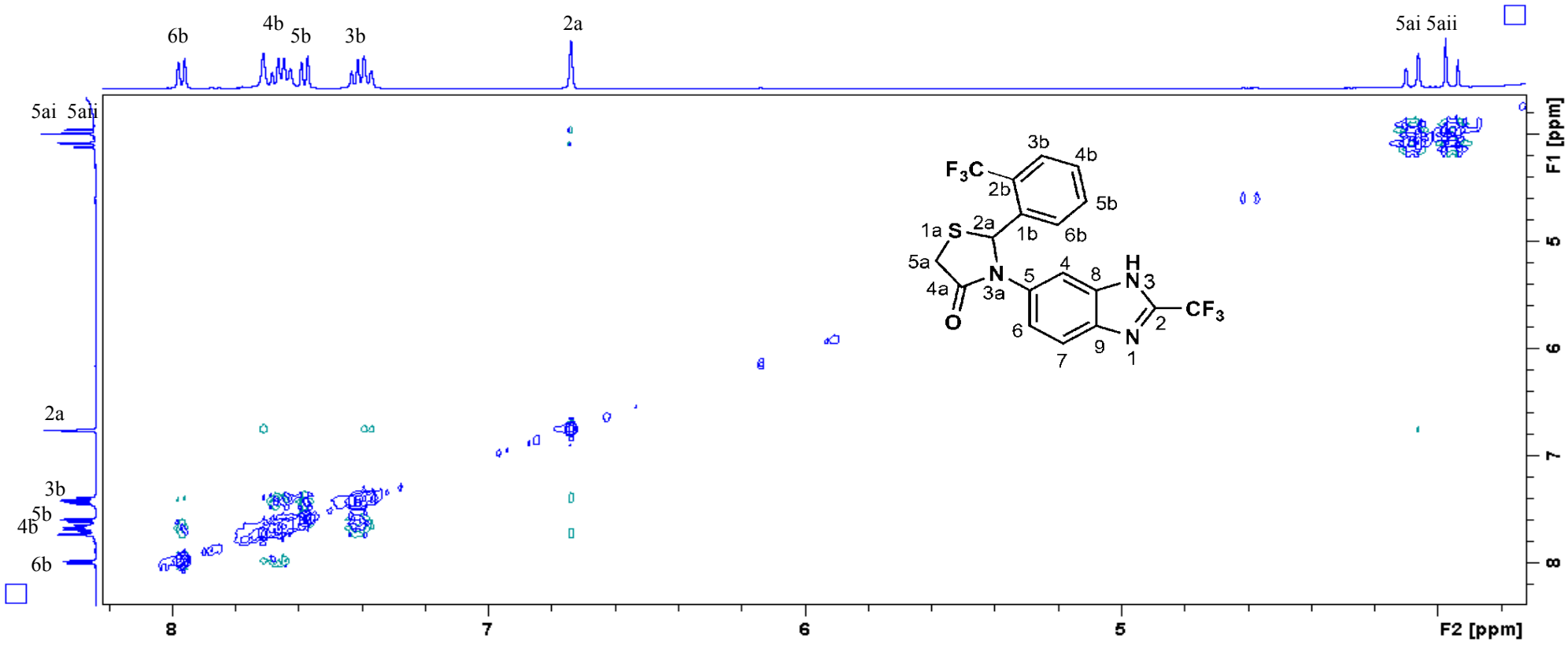
^{1}H NMR Spectrum of C-3e



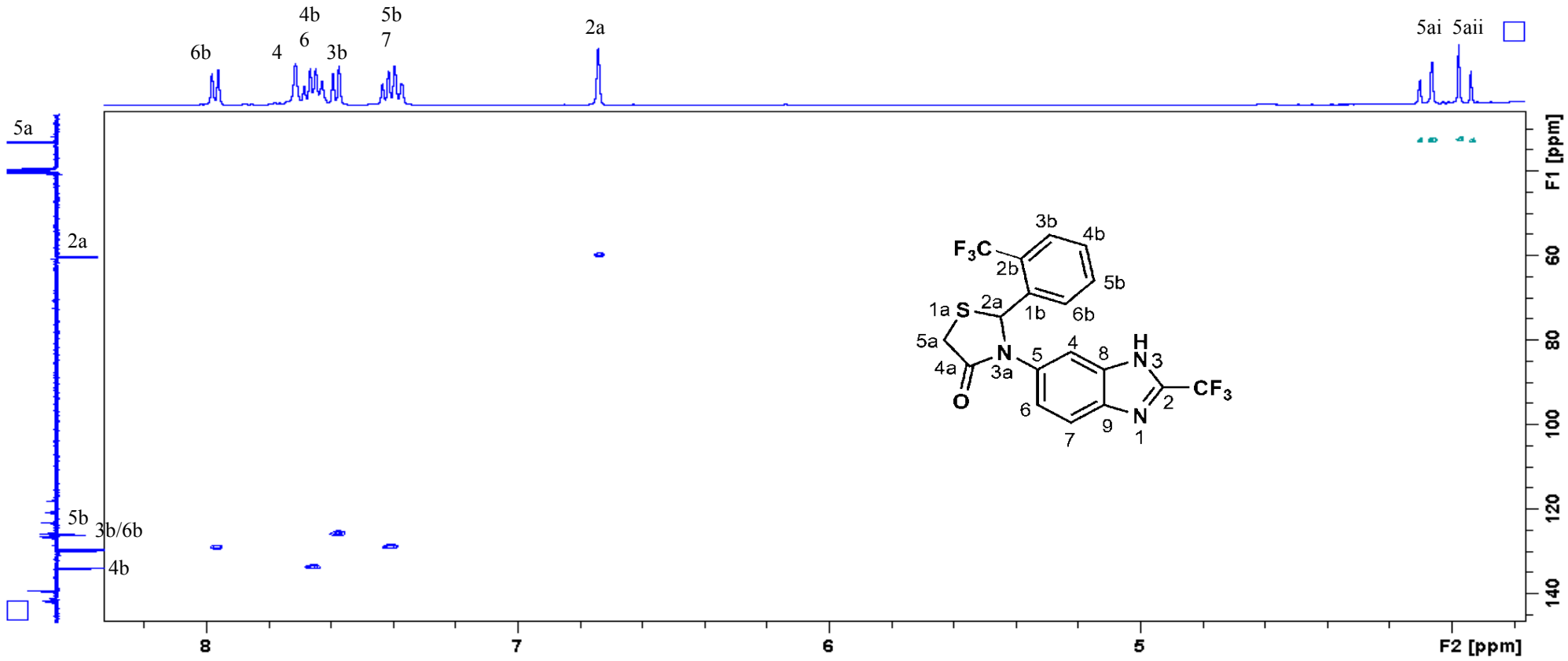
^{13}C NMR Spectrum of C-3e



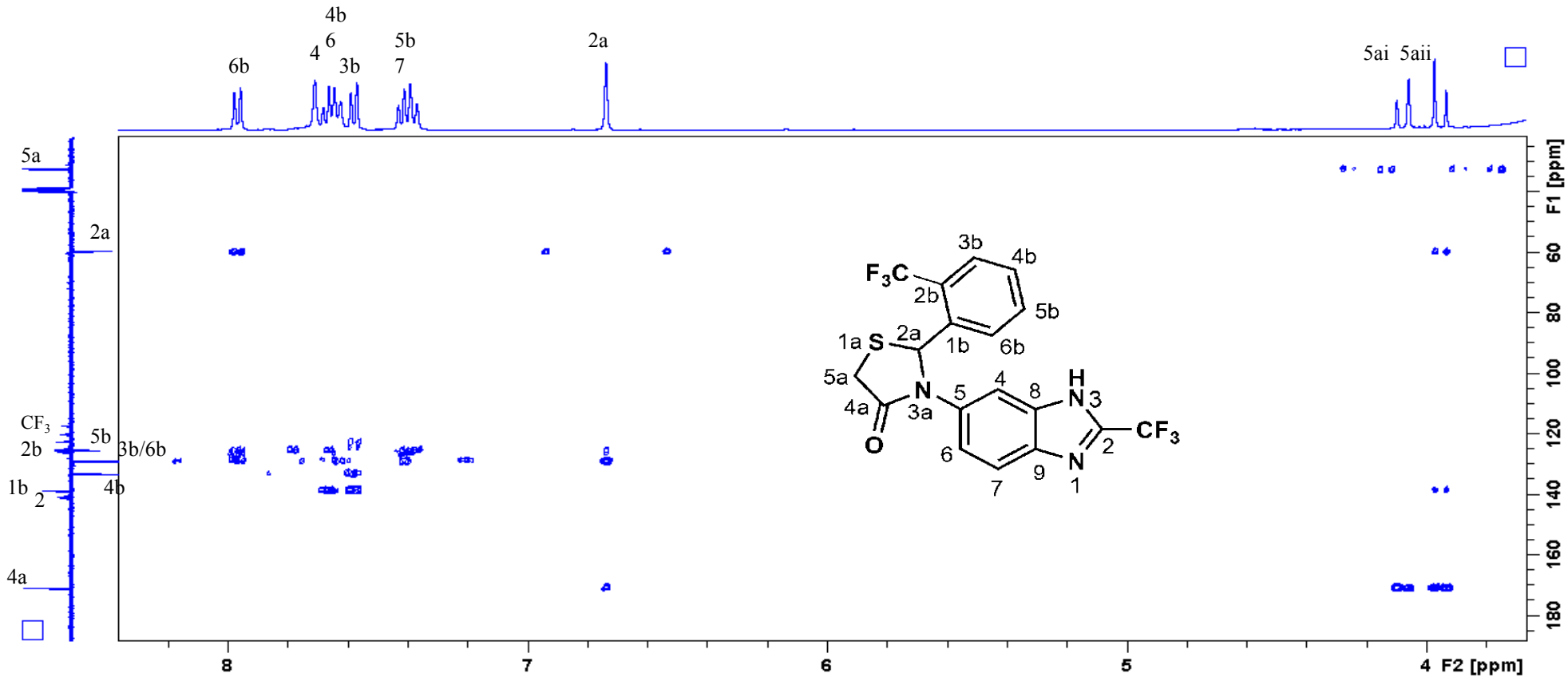
COSY Spectrum of C-3e



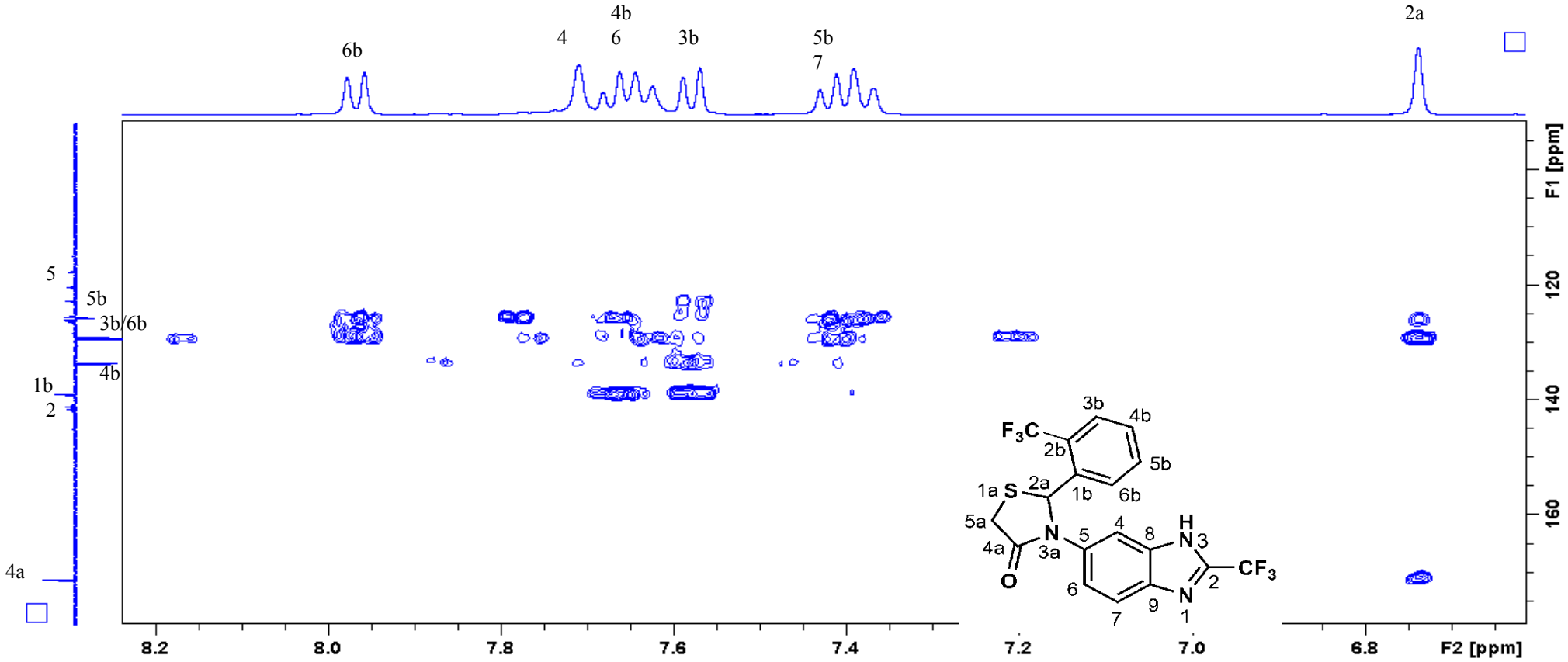
NOESY Spectrum of C-3e



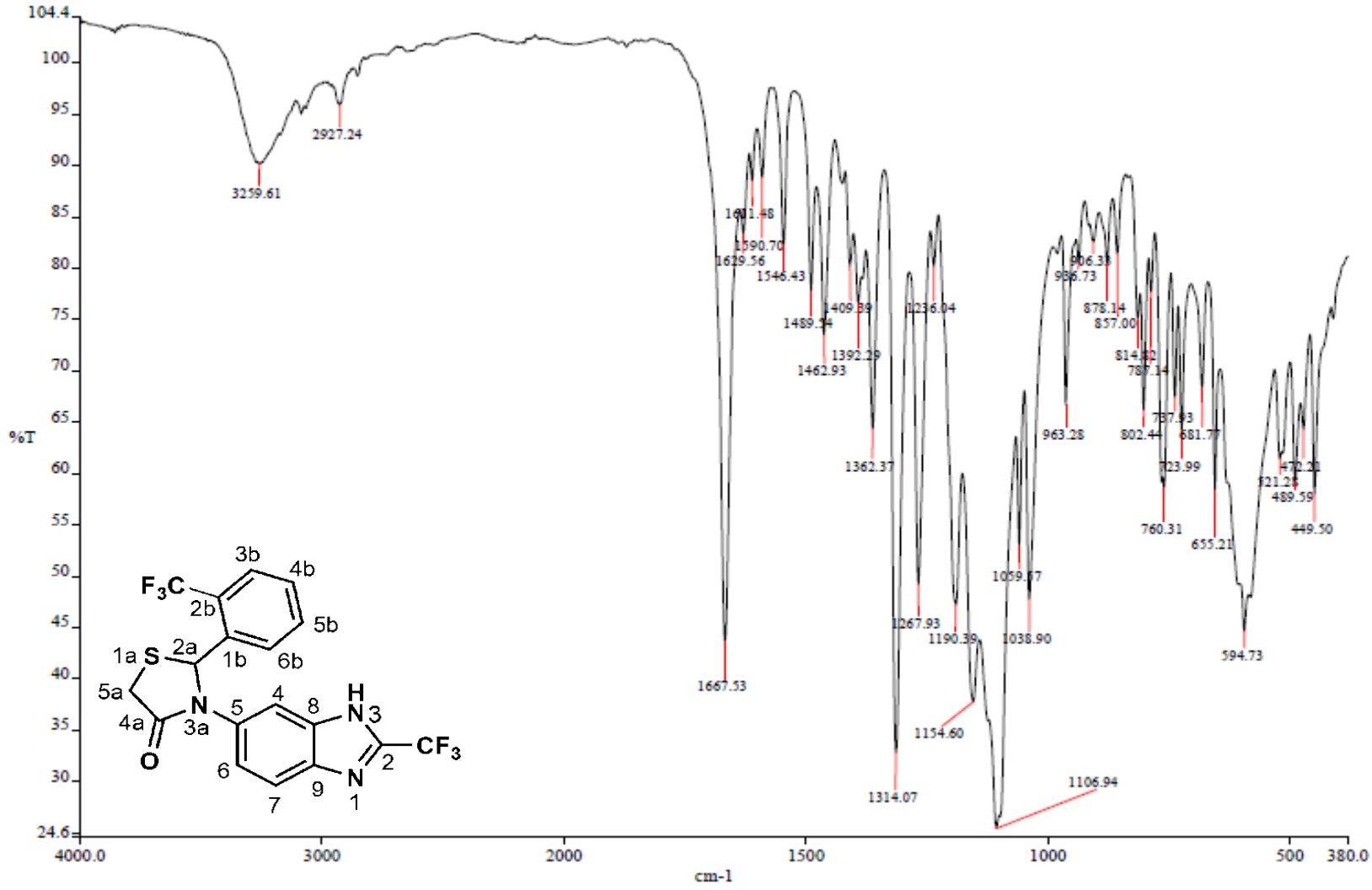
HSQC Spectrum of C-3e



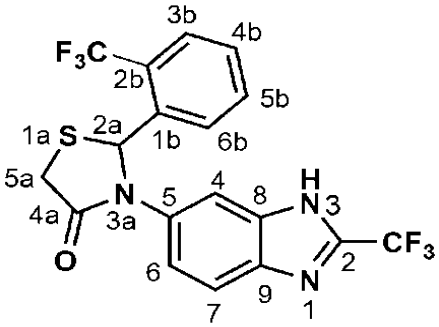
HMBC Spectrum of C-3e



Expanded HMBC Spectrum of C-3e



Infrared Spectrum of C-3e

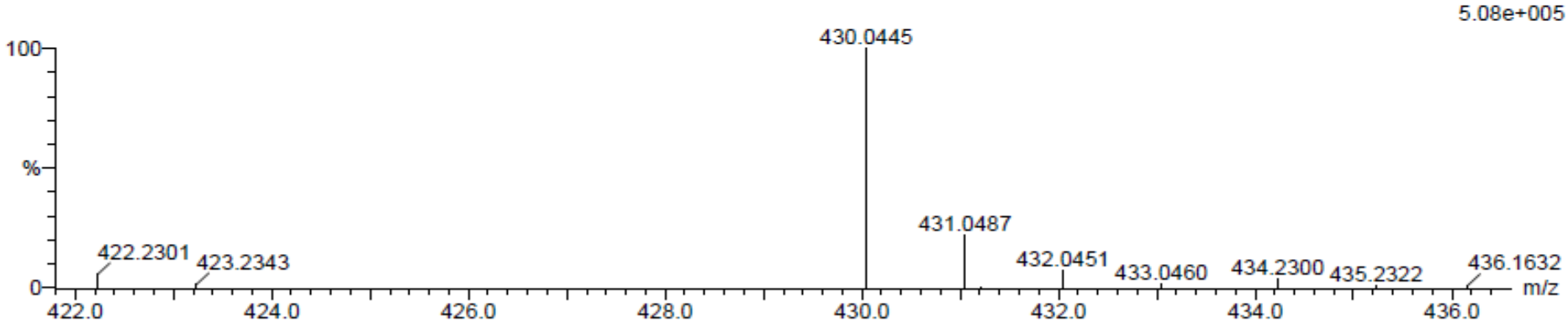


Elemental Composition Report

Single Mass Analysis

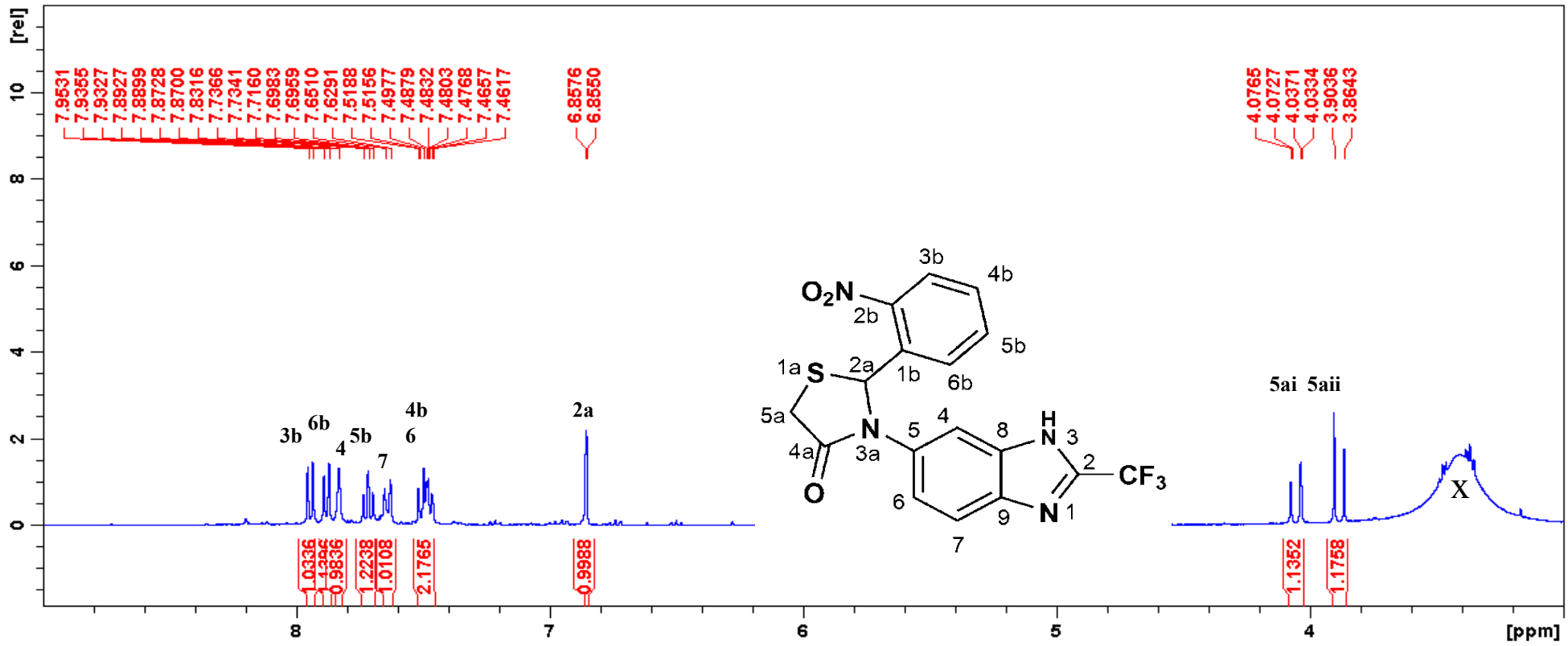
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
35 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 O: 0-1 F: 5-10 S: 1-1
CF 4 18 (0.574) Cm (1:61)
TOF MS ES-

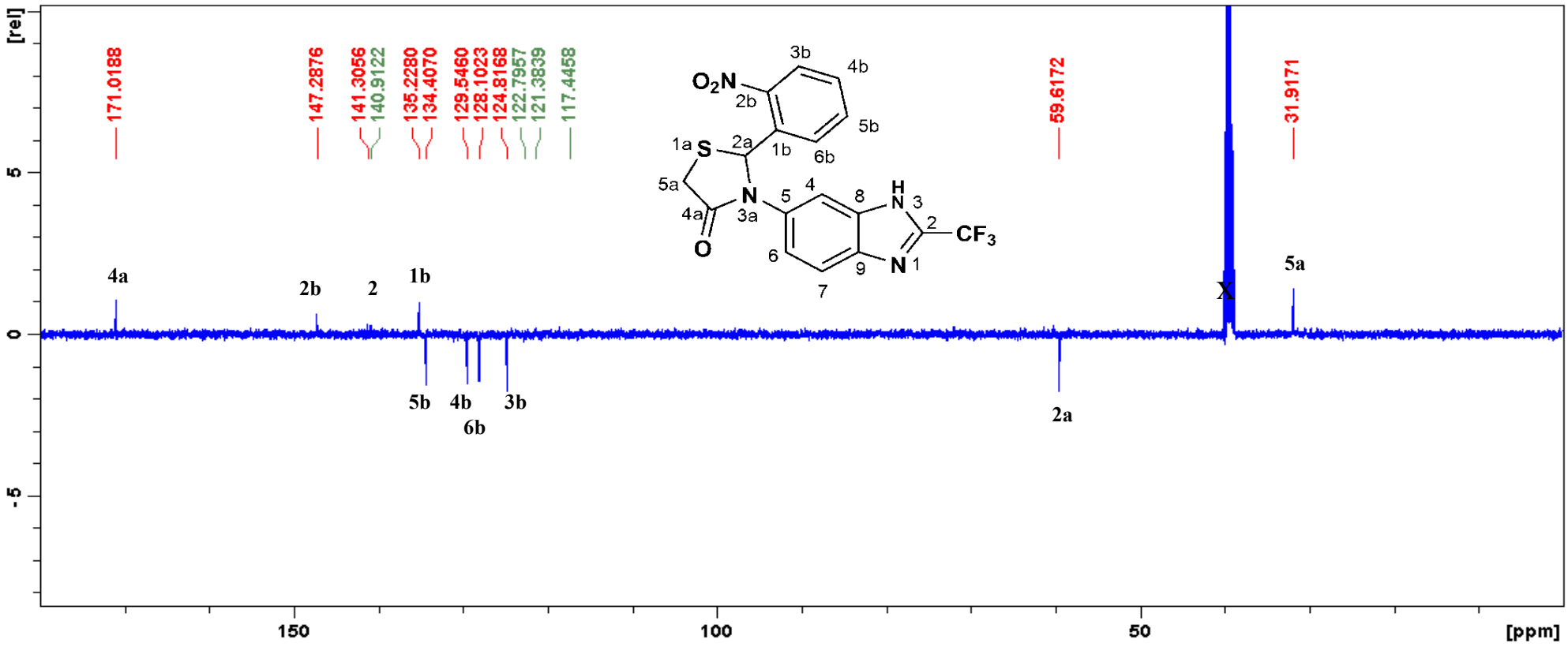


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
430.0445	430.0449	-0.4	-0.9	12.5	26.9	0.0	C18 H10 N3 O F6 S

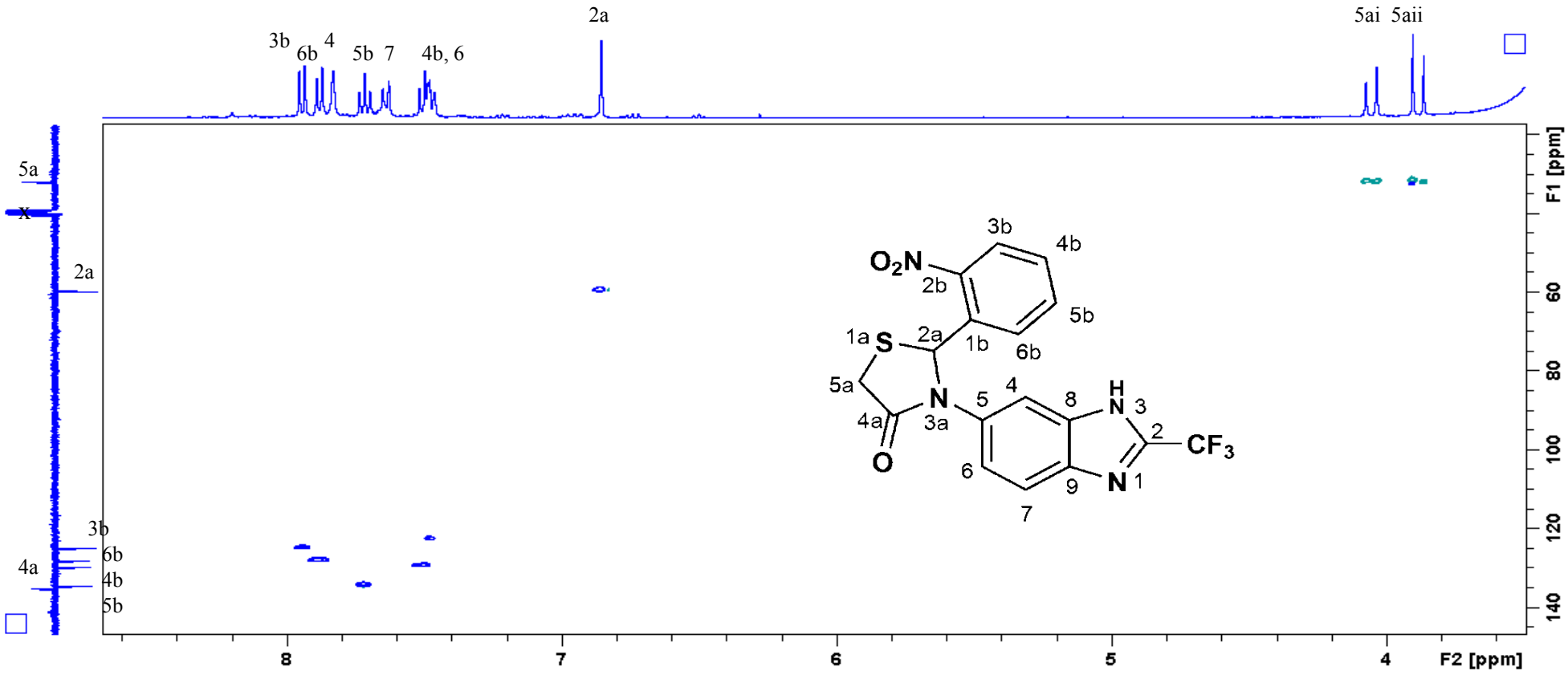
HRMS Spectrum of C-3e



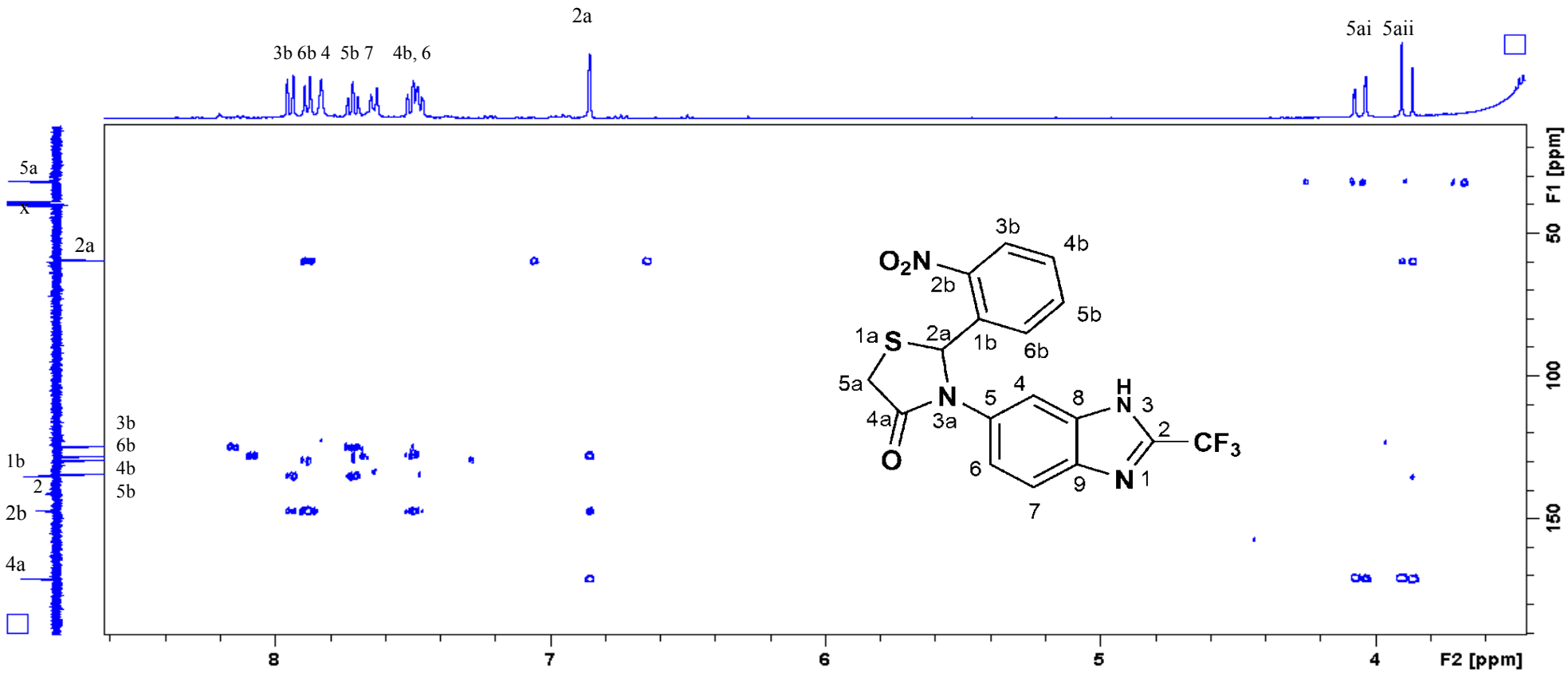
^{1}H NMR Spectrum of C-3f



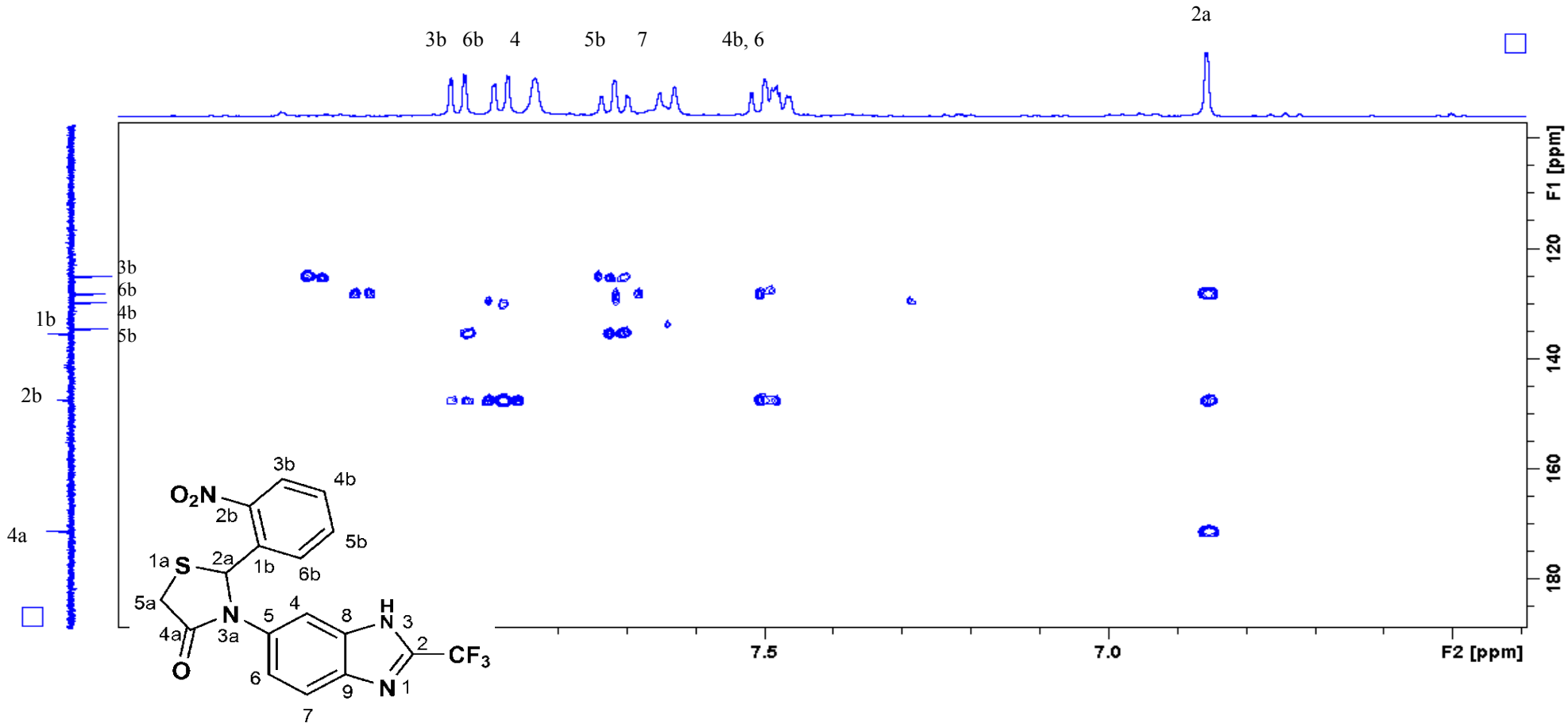
^{13}C NMR Spectrum of C-3f



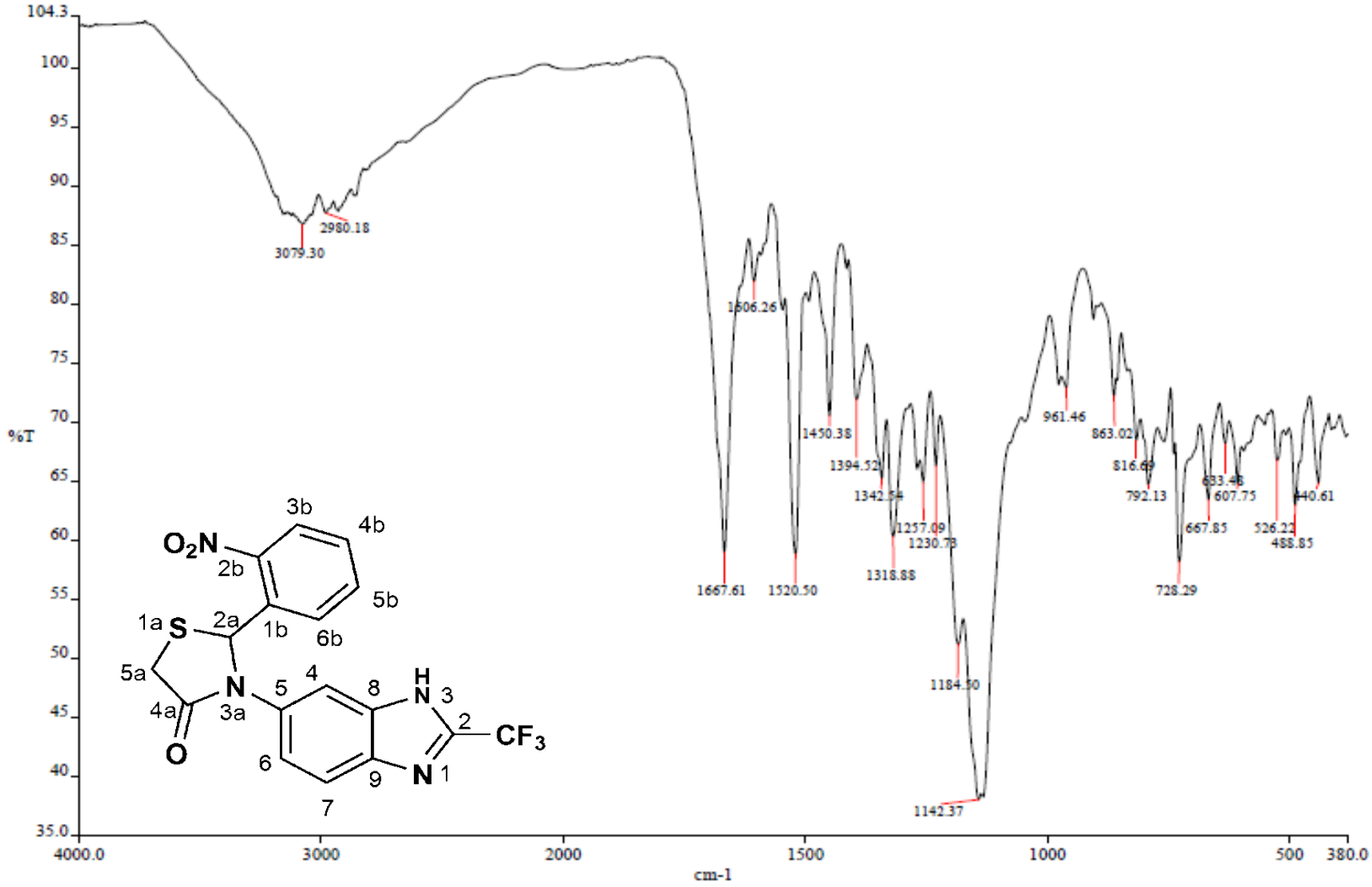
HSQC Spectrum of C-3f



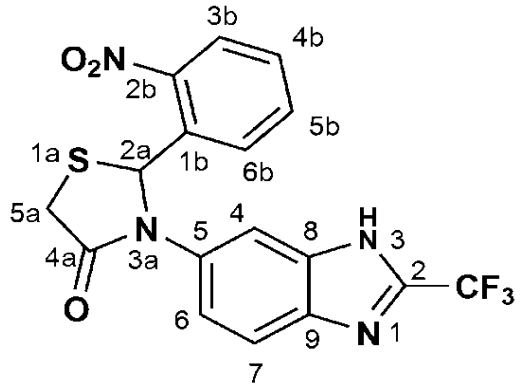
HMBC Spectrum of C-3f



Expanded HMBC Spectrum of C-3f



Infrared Spectrum of C-3f

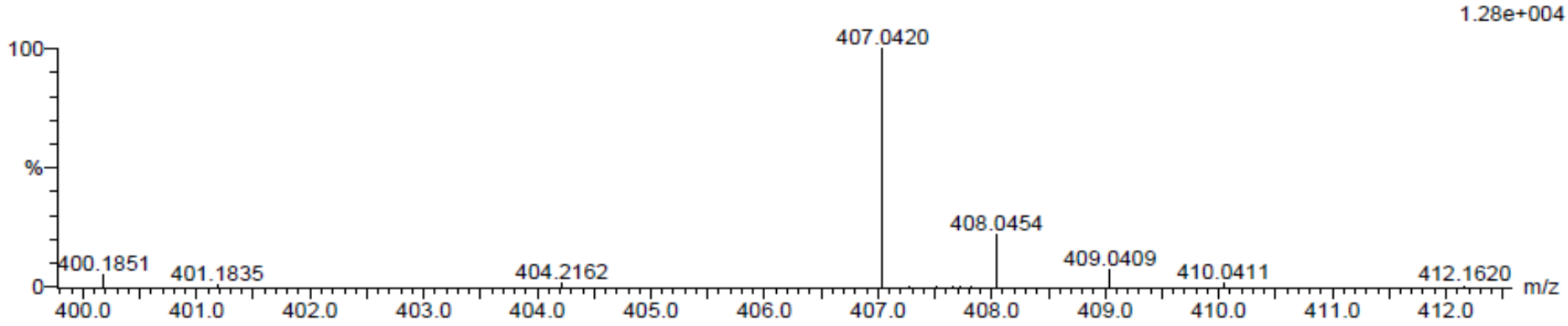


Elemental Composition Report

Single Mass Analysis

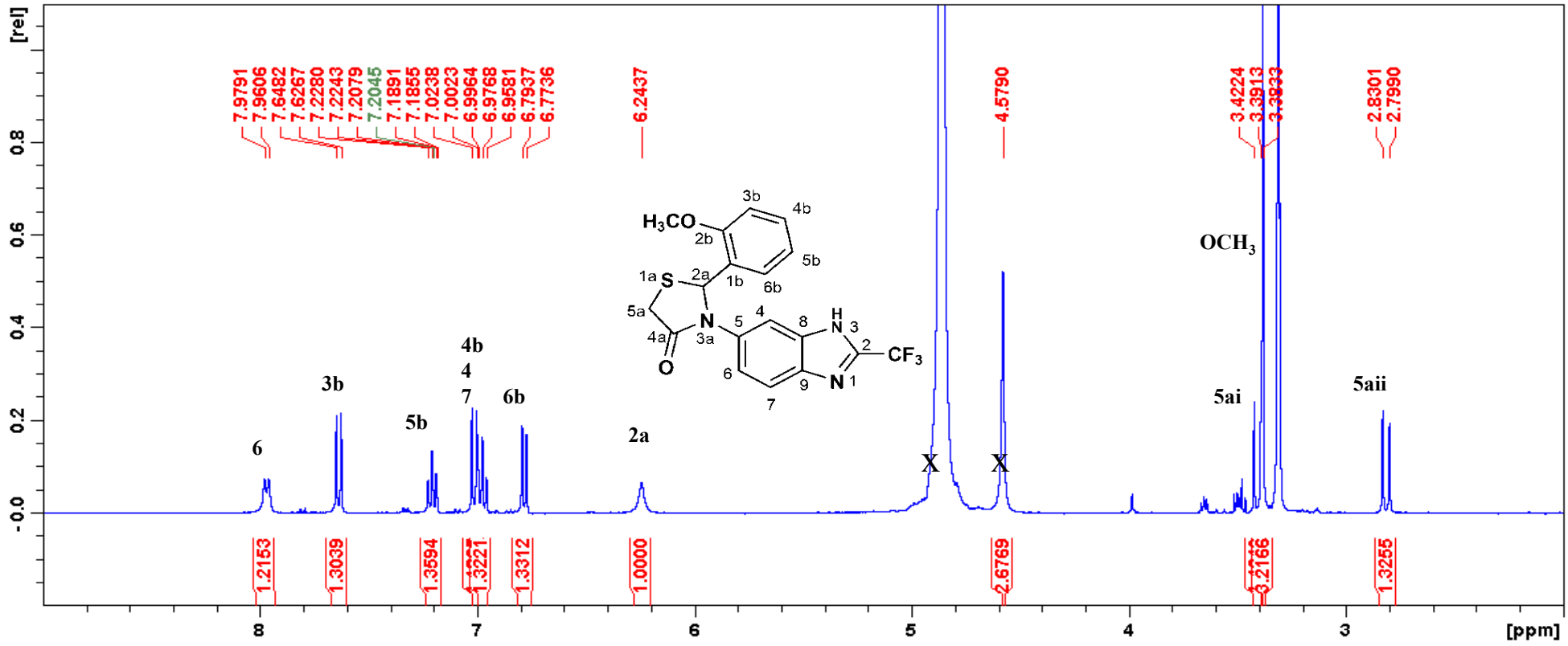
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
103 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 O: 0-5 F: 0-5 S: 1-1
CF 8 3 (0.068) Cm (1:17)
TOF MS ES-

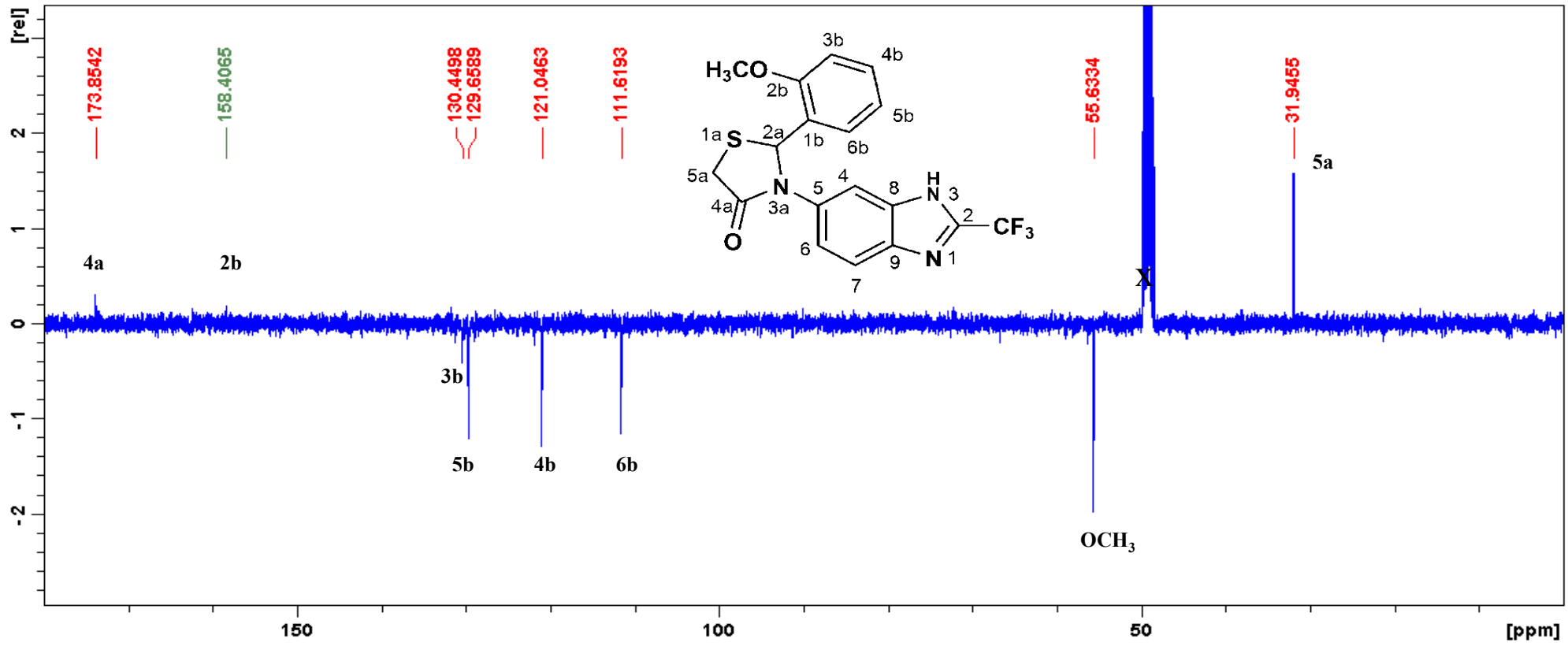


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
407.0420	407.0426	-0.6	-1.5	13.5	45.8	0.0	C17 H10 N4 O3 F3 S

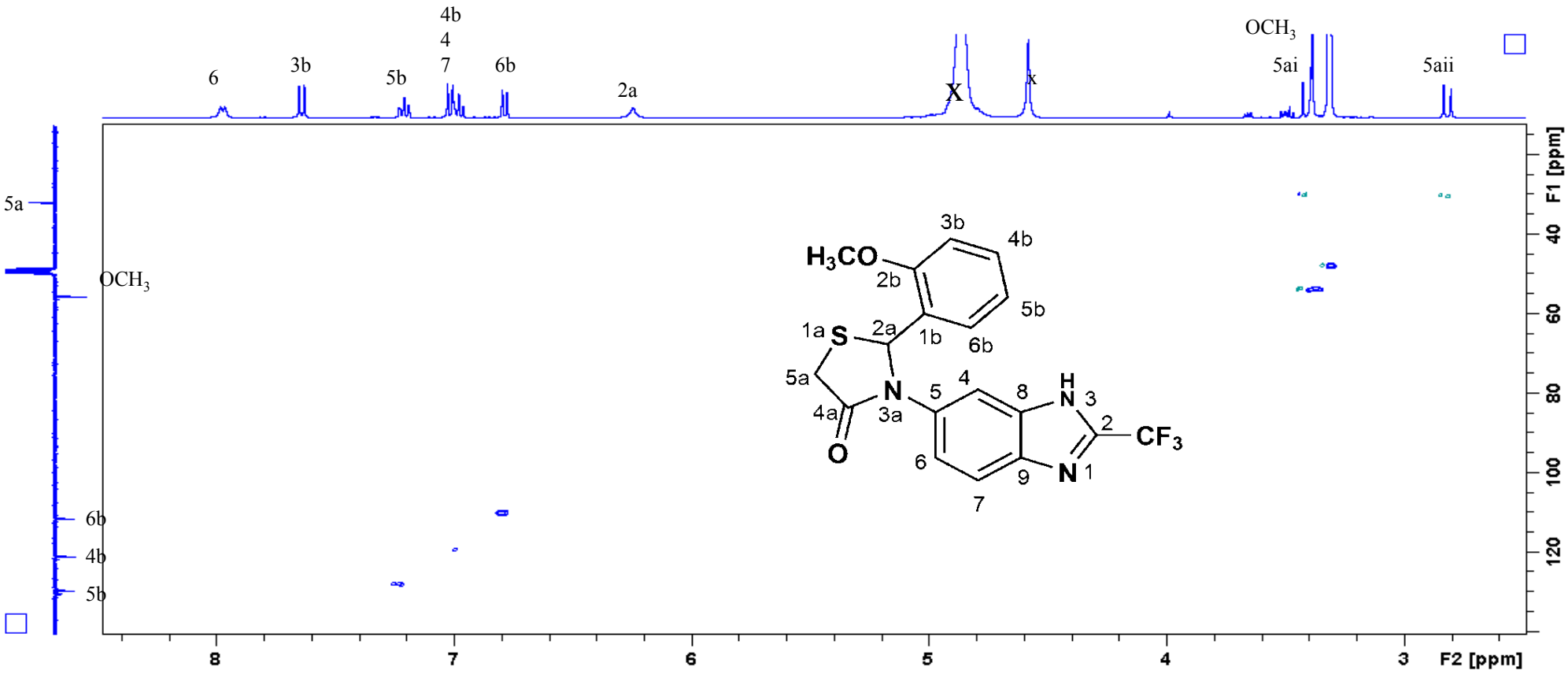
HRMS Spectrum of C-3f



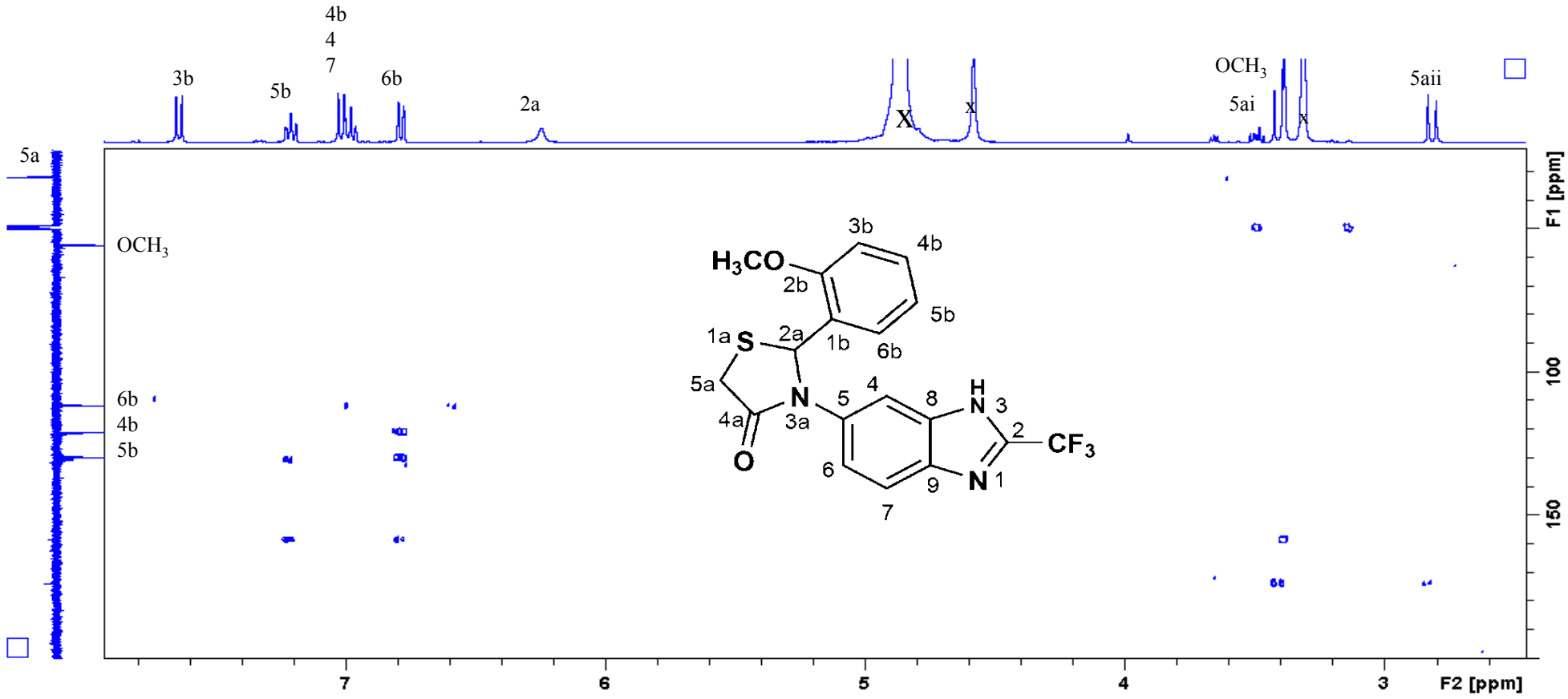
^{1}H NMR Spectrum of C-3g



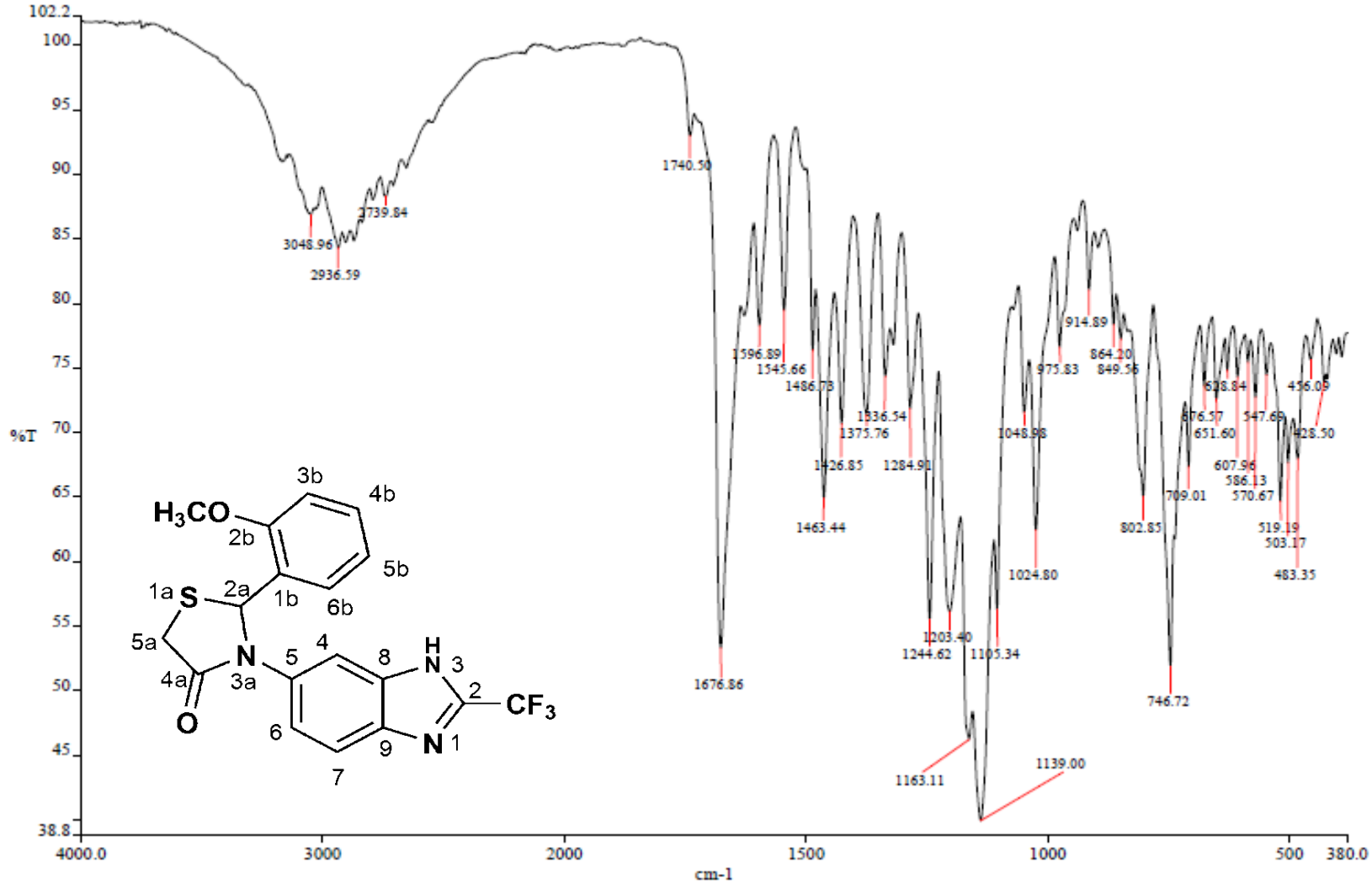
^{13}C NMR Spectrum of C-3g



HSQC Spectrum of C-3g



HMBC Spectrum of C-3g



Infrared Spectrum of C-3g

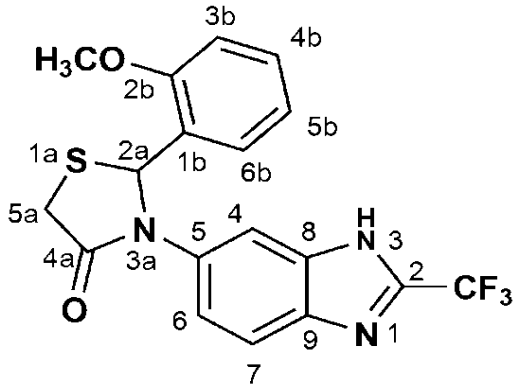
Elemental Composition Report

Single Mass Analysis

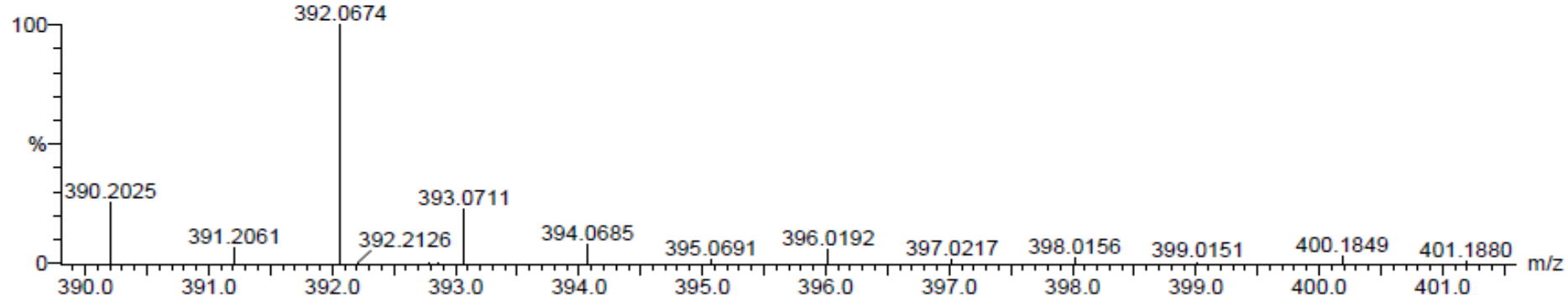
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
43 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 O: 0-5 F: 1-3 S: 1-1

CF 12 44 (1.450) Cm (1:61)
TOF MS ES-

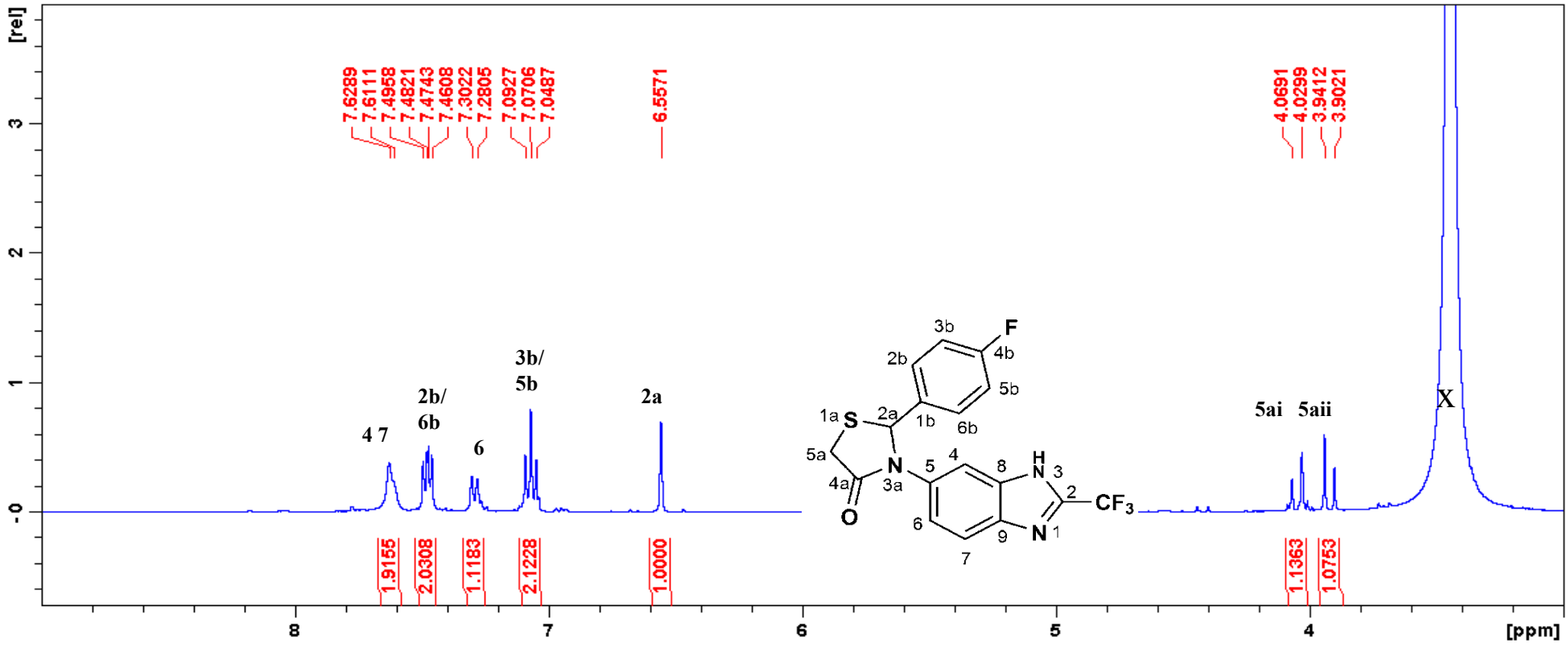


2.67e+005

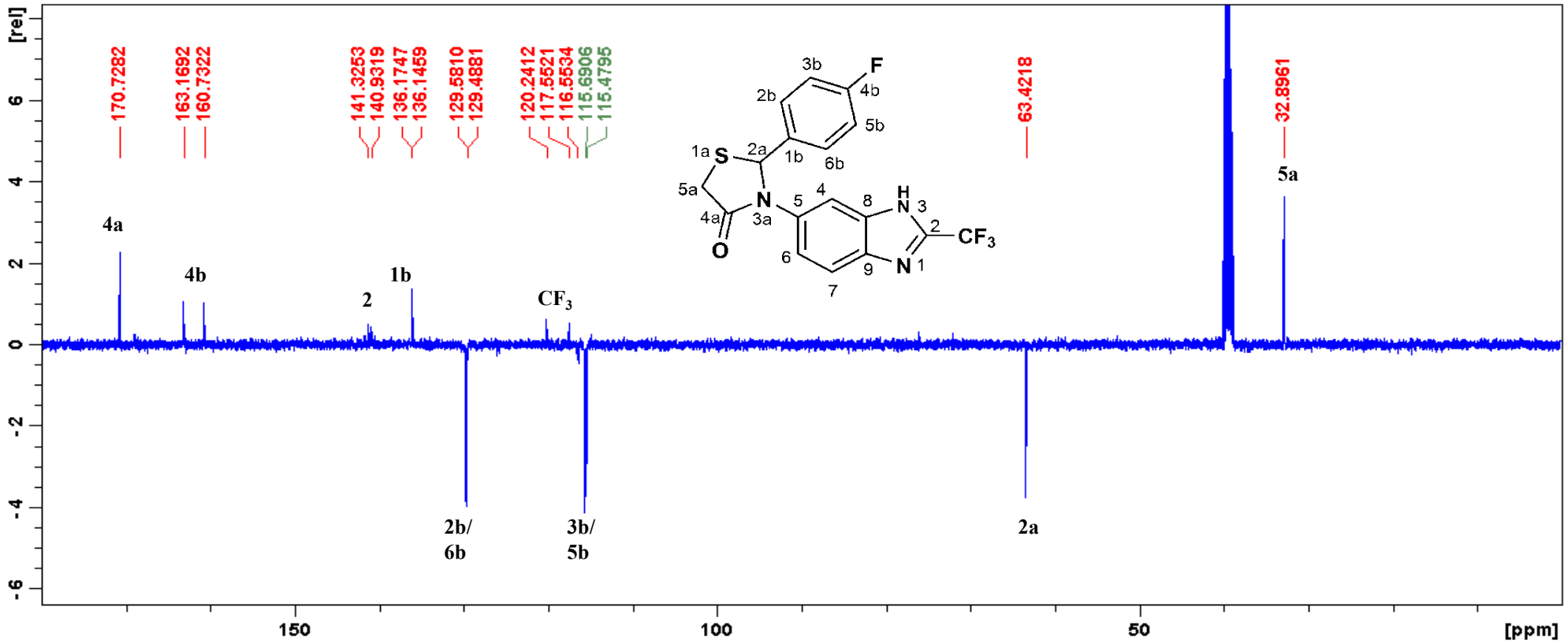


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
392.0674	392.0681	-0.7	-1.8	12.5	52.4	0.0	C18 H13 N3 O2 F3 S

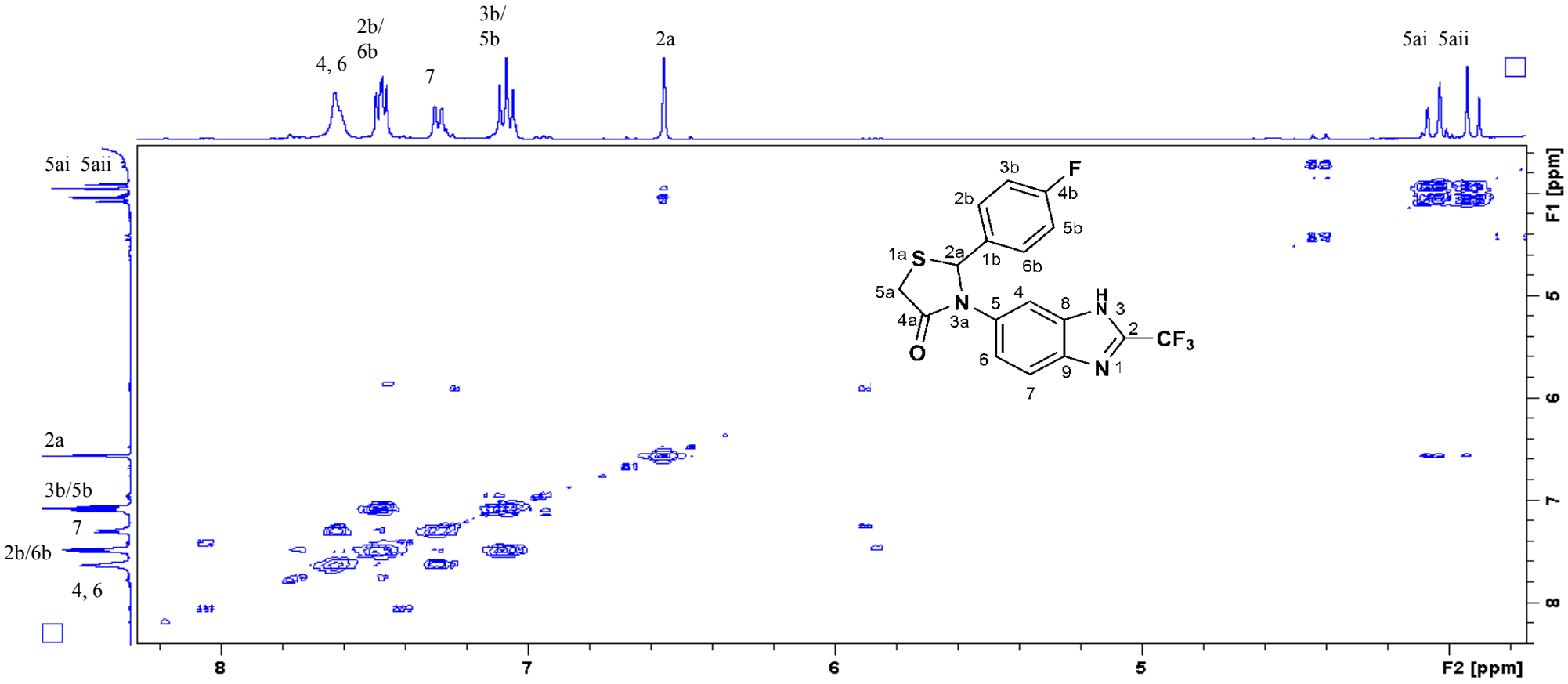
HRMS Spectrum of C-3g



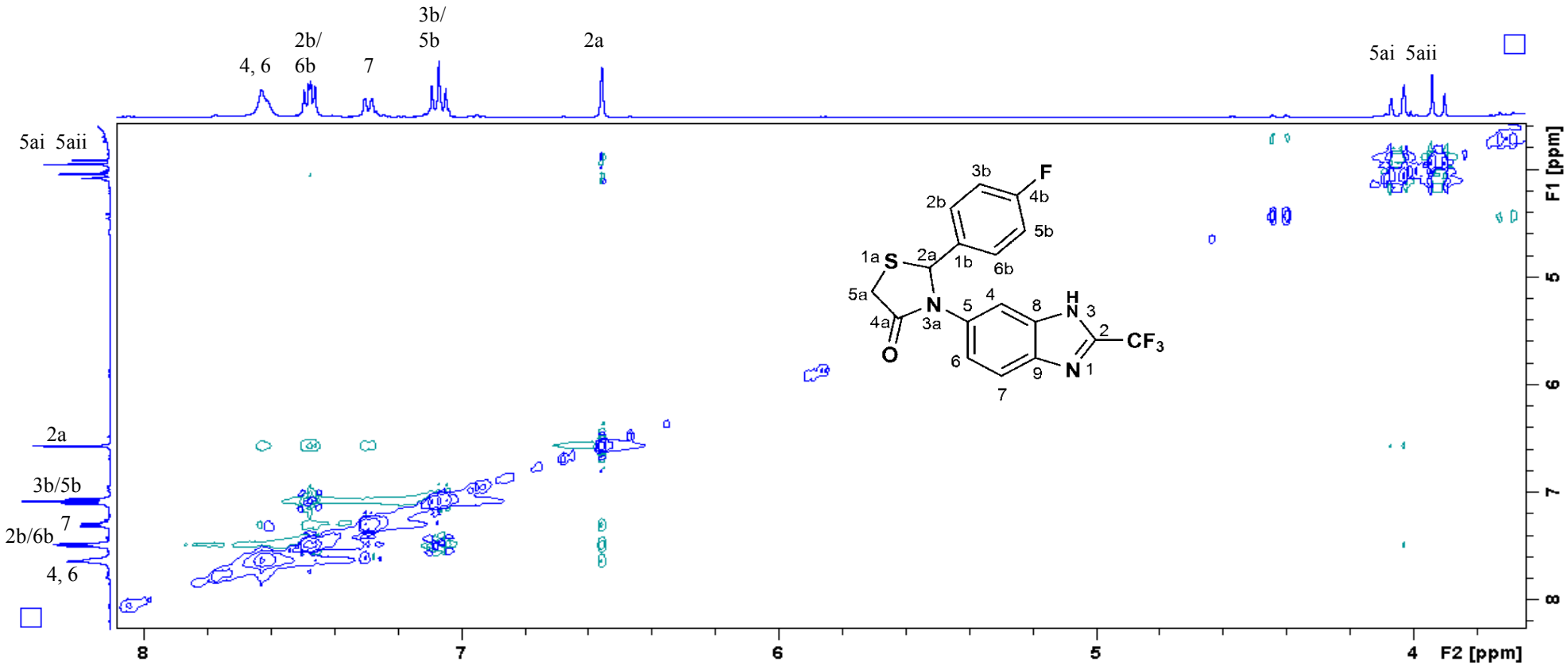
^{1}H Spectrum of C-3h



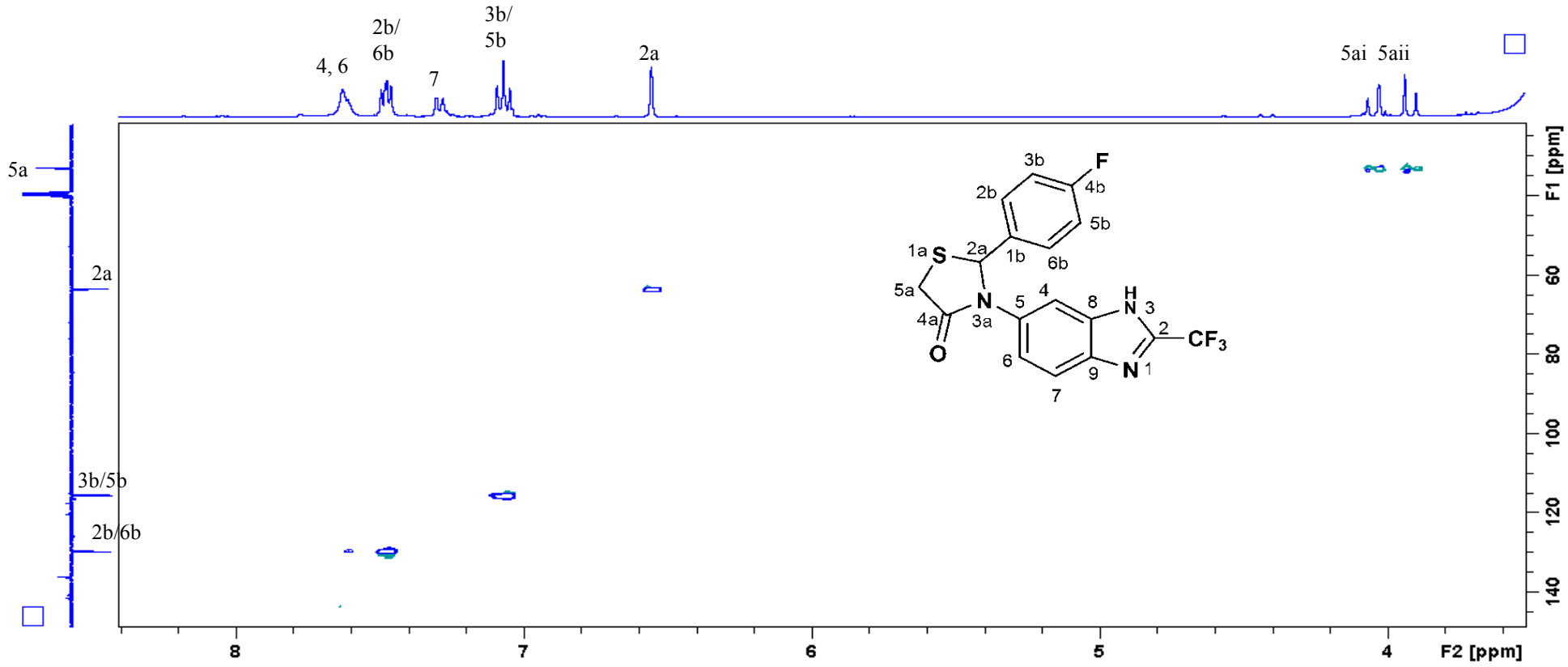
^{13}C Spectrum of C-3h



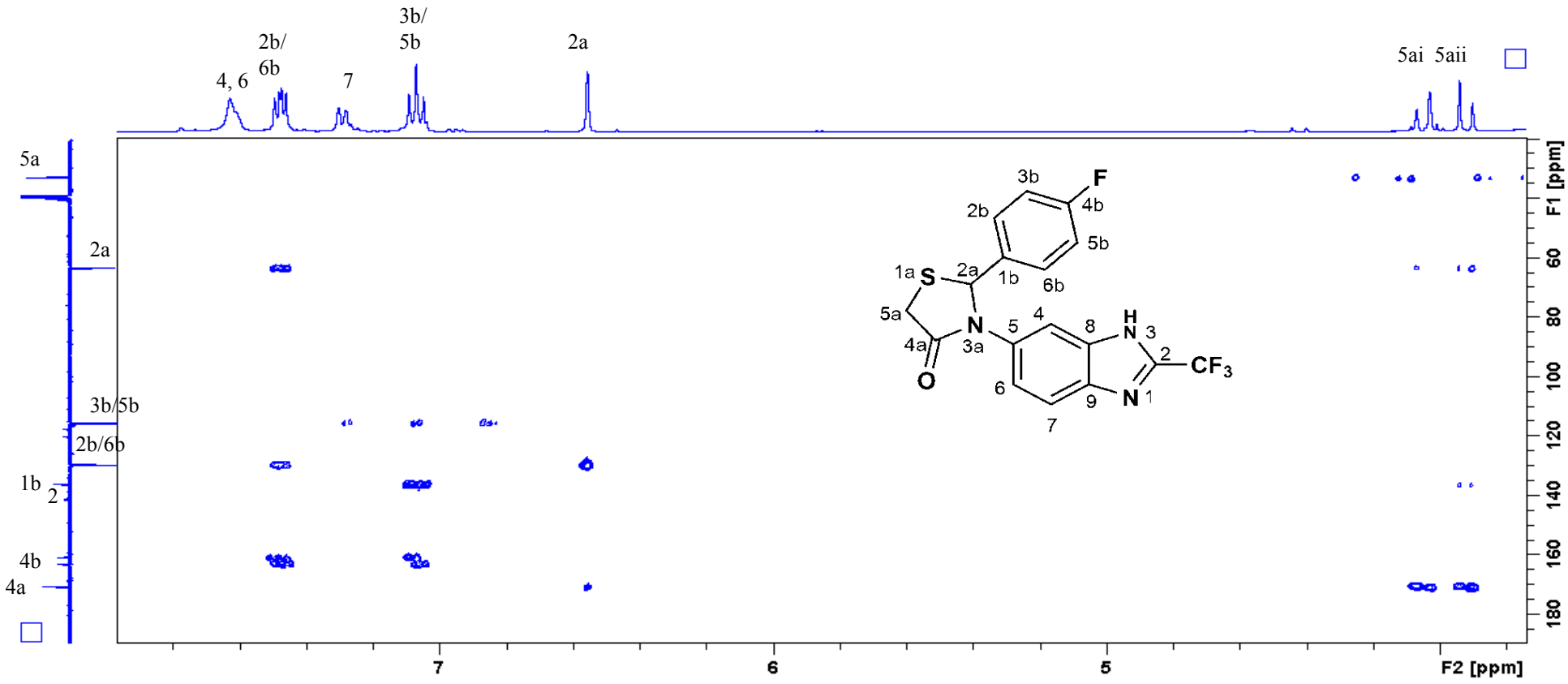
COSY Spectrum of C-3h



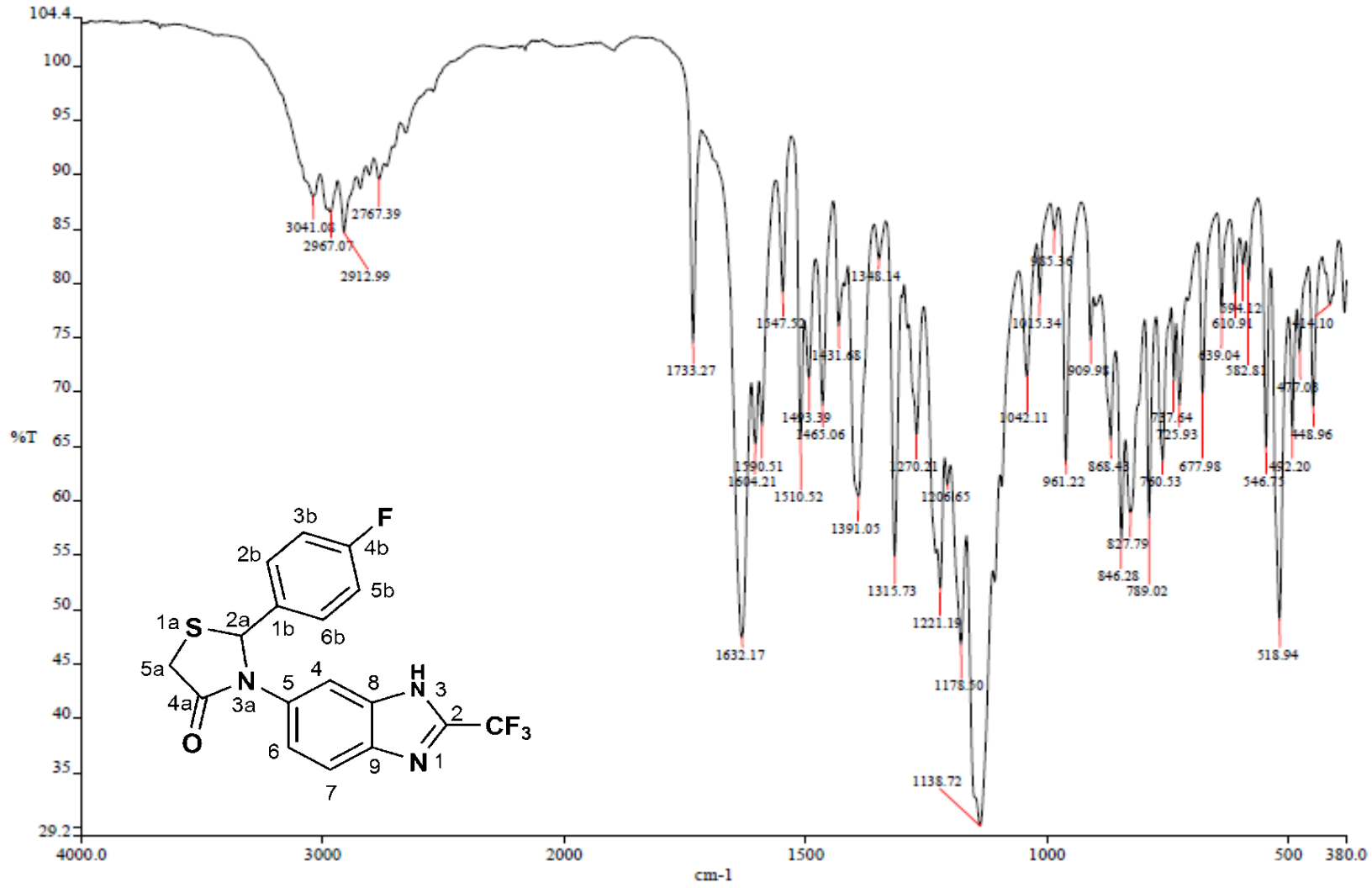
NOESY Spectrum of C-3h



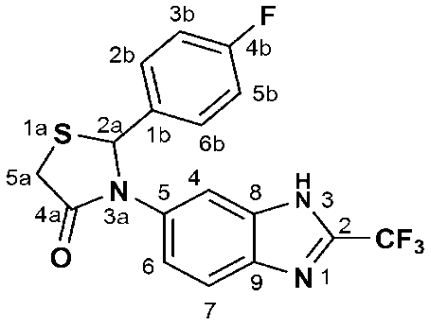
HSQC Spectrum of C-3h



HMBC Spectrum of C-3h



Infrared Spectrum of C-3h

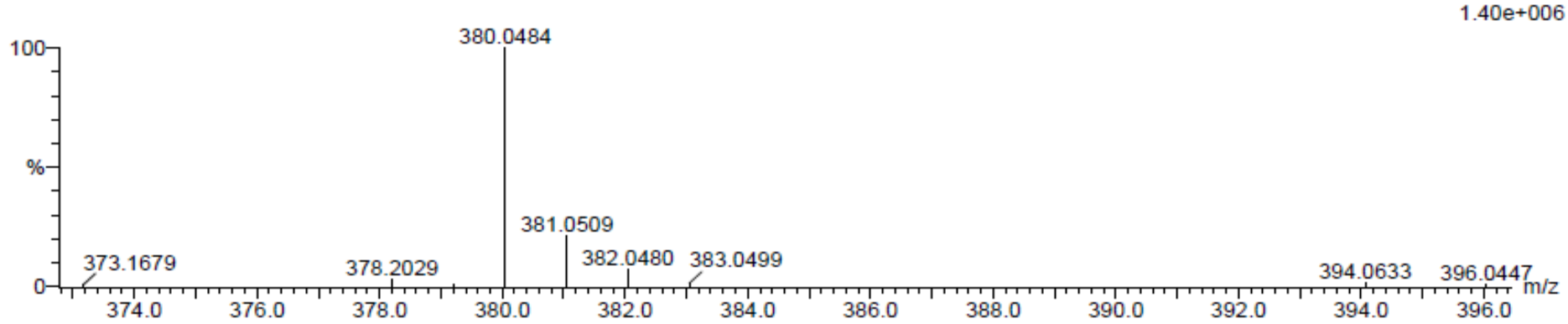


Elemental Composition Report

Single Mass Analysis

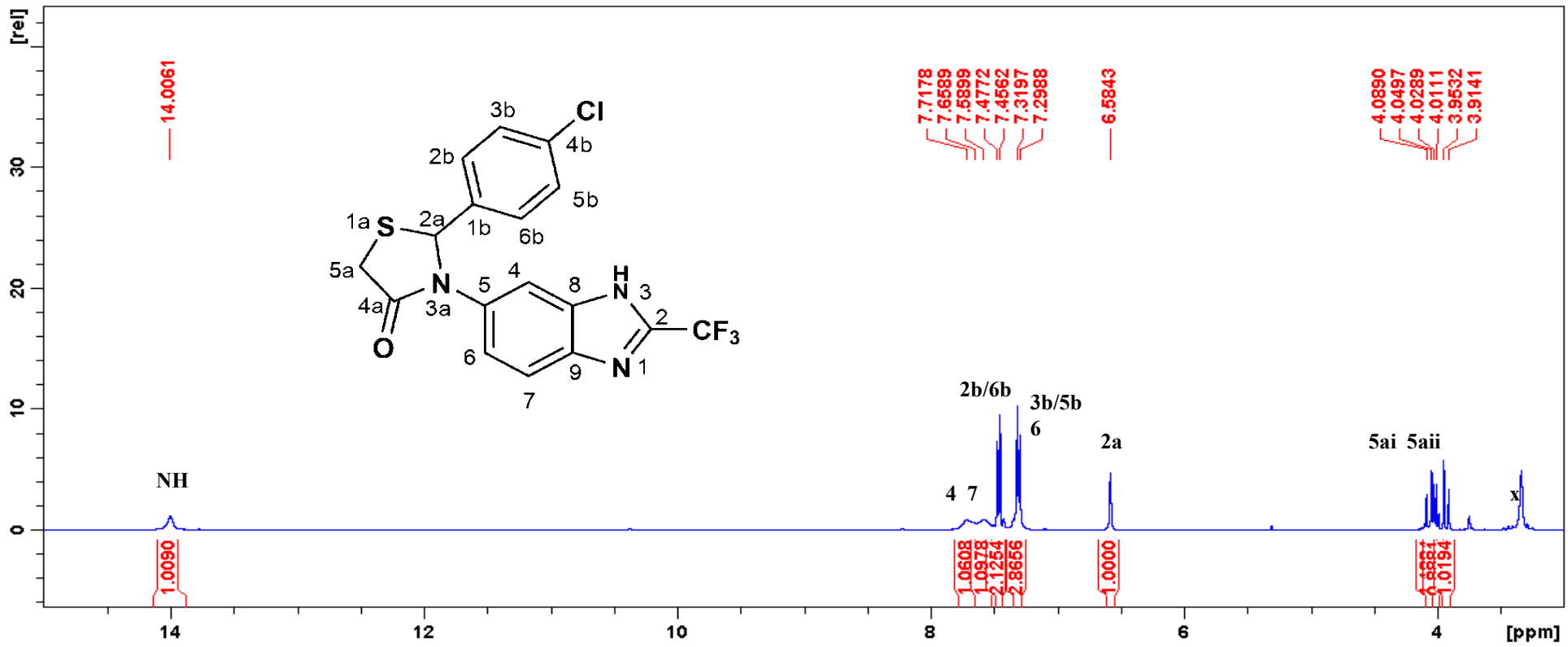
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
88 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 O: 0-5 F: 1-5 S: 1-1
CF 32 (1.047) Cm (1:61)
TOF MS ES-

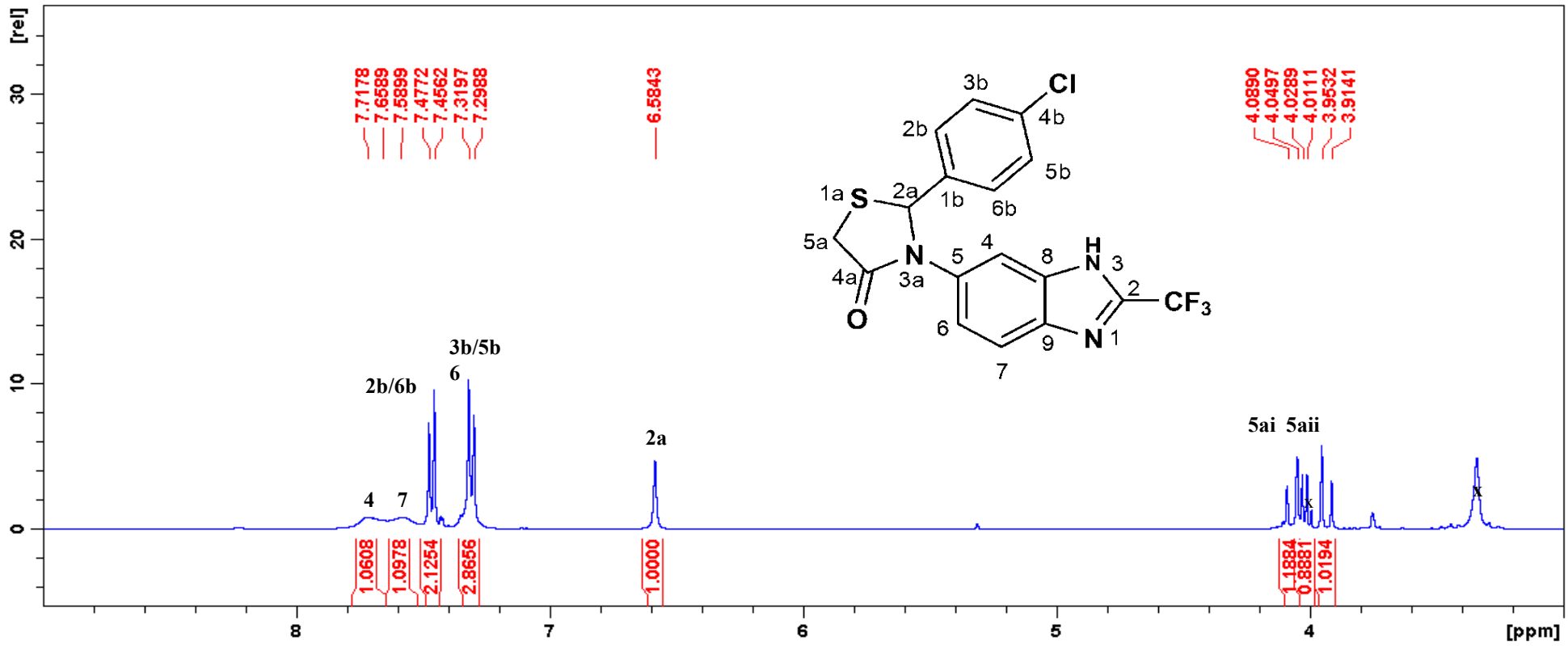


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
380.0484	380.0481	0.3	0.8	12.5	11.5	0.0	C17 H10 N3 O F4 S

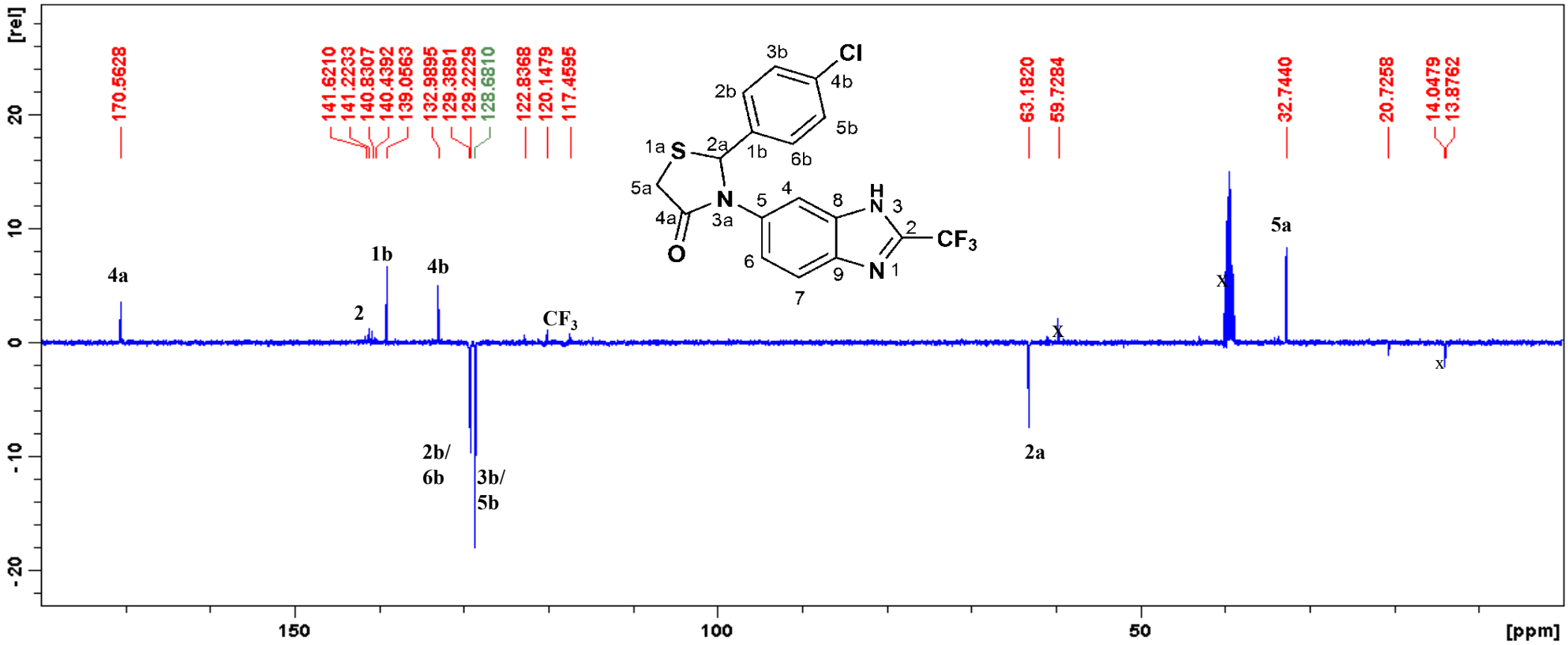
HRMS Spectrum of C-3h



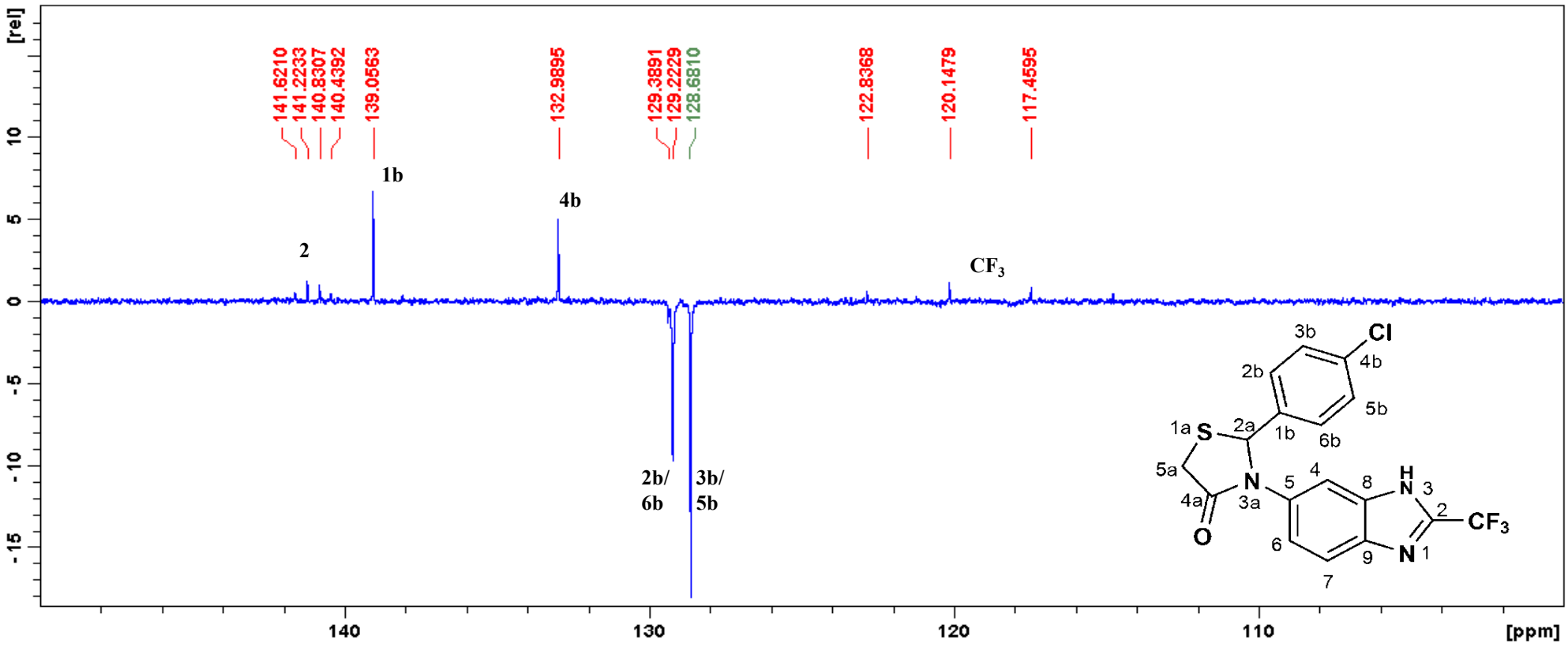
^{1}H Spectrum of C-3i



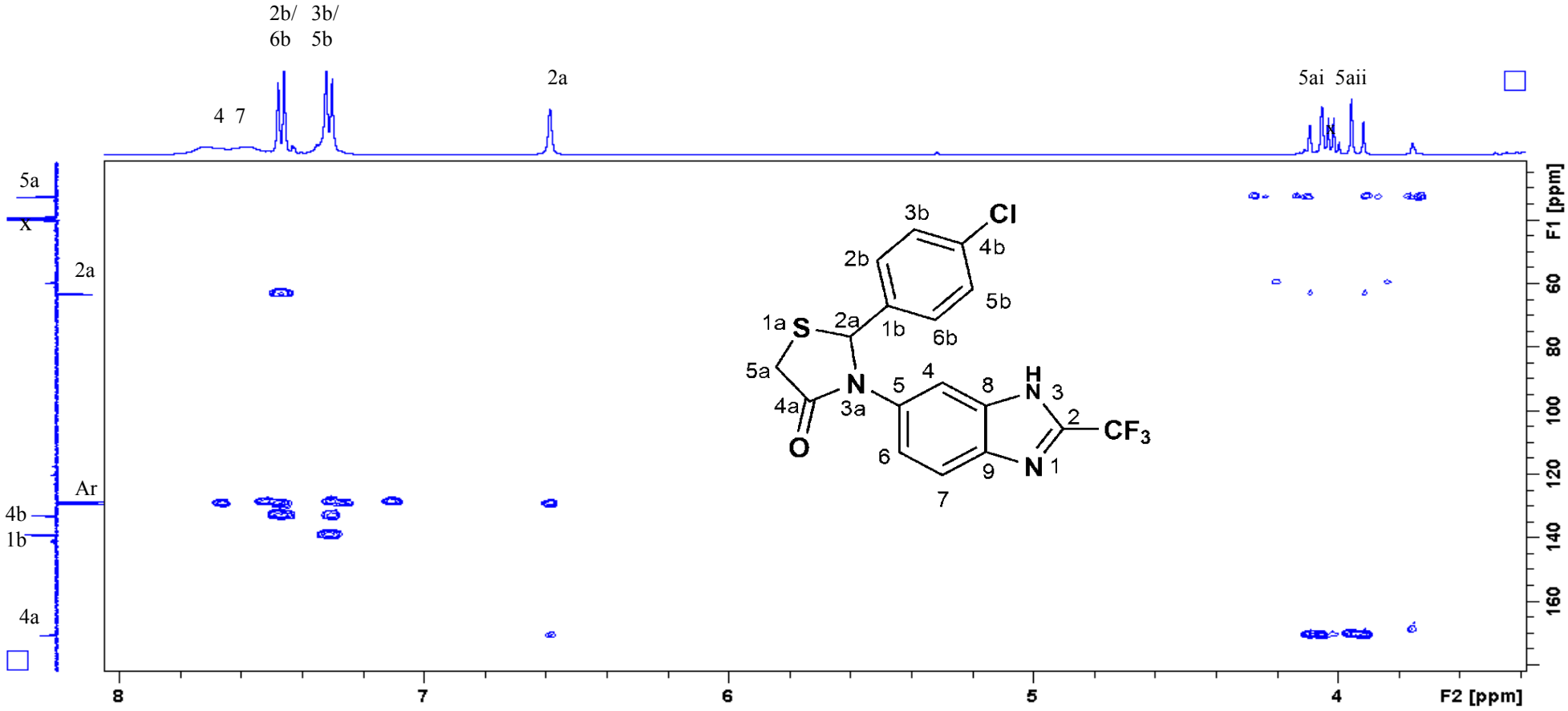
Expanded ^{1}H Spectrum of C-3i



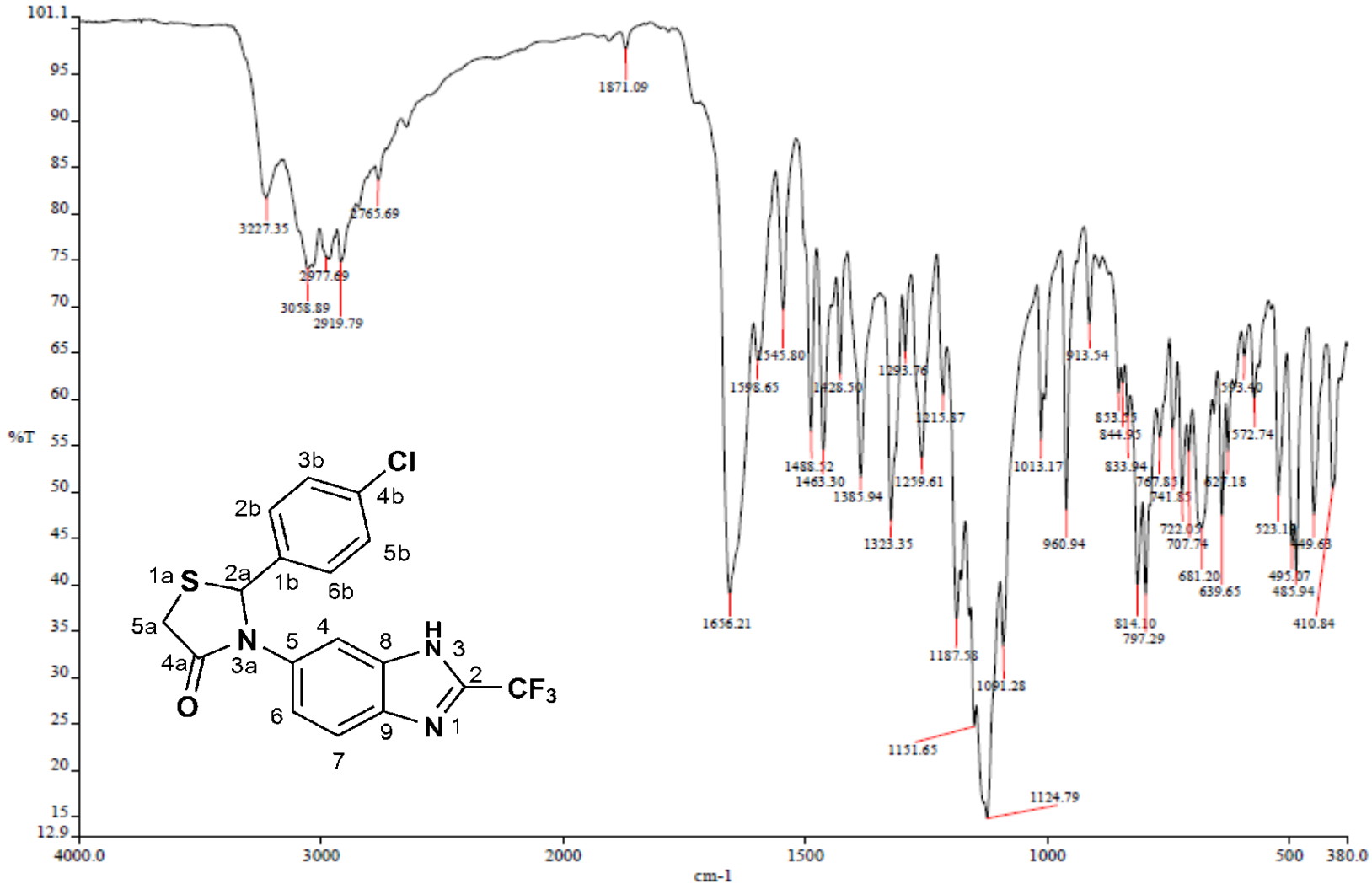
^{13}C Spectrum of C-3i



Expanded ^{13}C Spectrum of C-3i



HMBC Spectrum of C-3i



Infrared Spectrum of C-3i

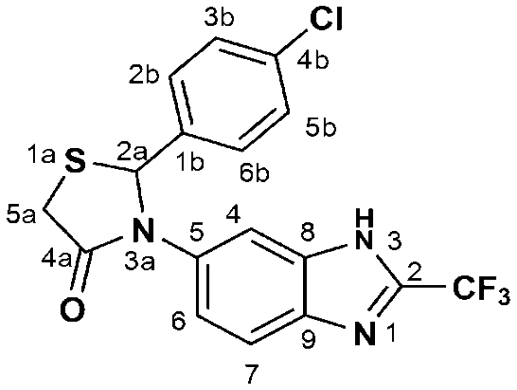
Elemental Composition Report

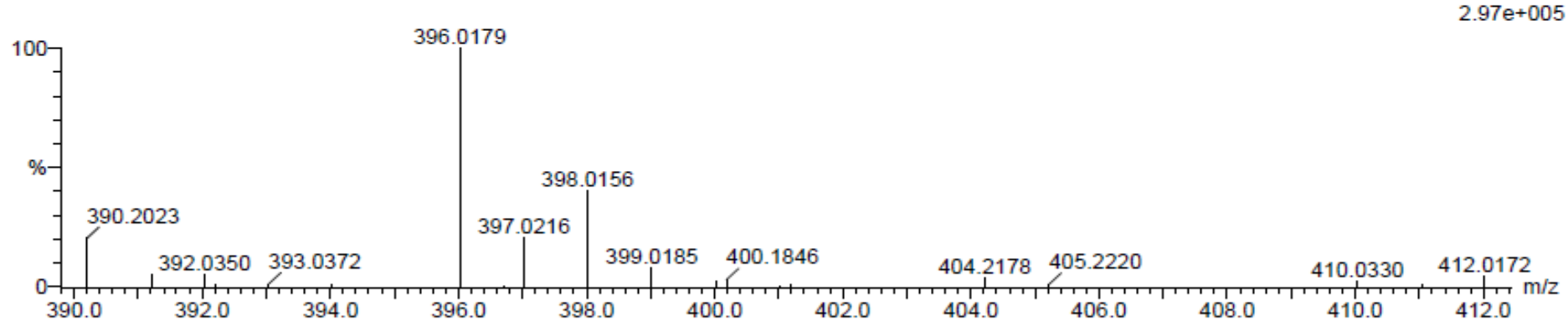
Single Mass Analysis

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
183 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 O: 0-5 F: 1-5 S: 1-1 Cl: 0-1

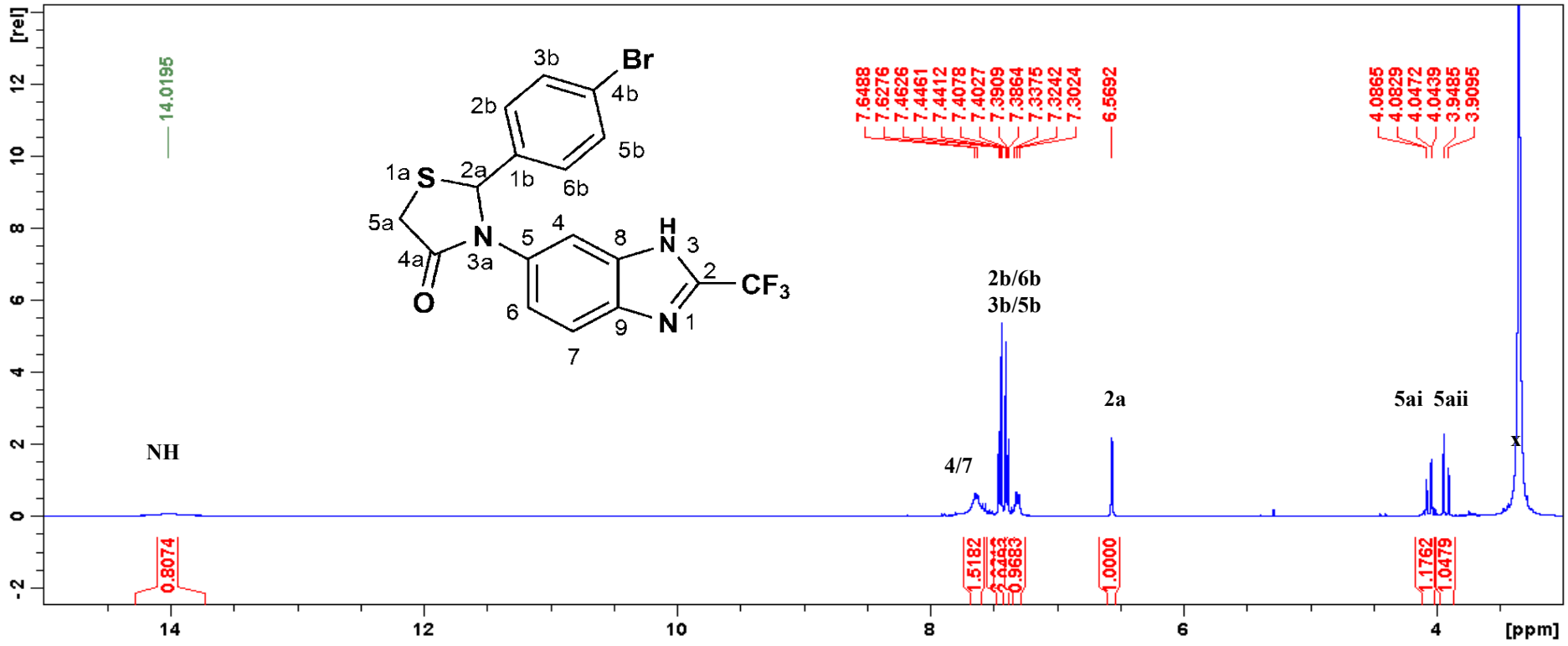
CF 11 55 (1.821) Cm (1:61)
TOF MS ES-



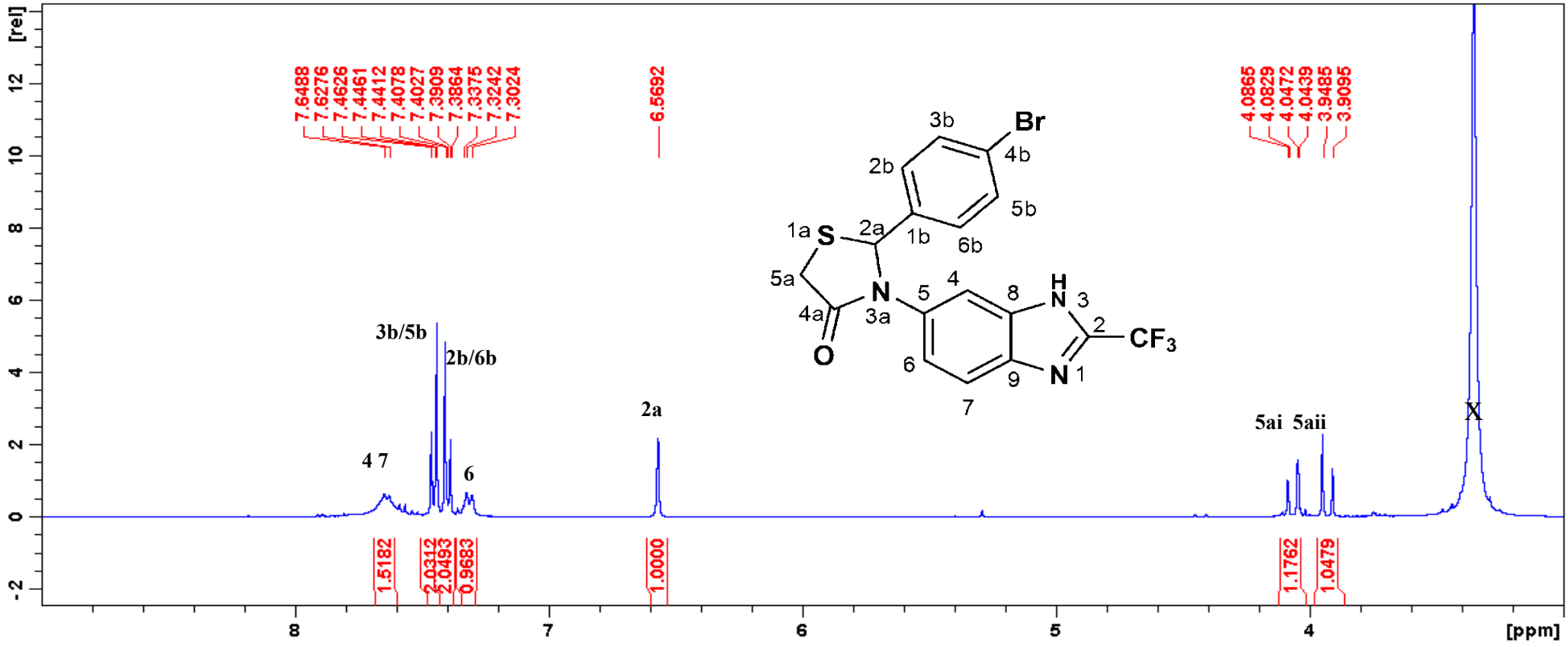


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
396.0179	396.0185	-0.6	-1.5	12.5	24.5	0.0	C17 H10 N3 O F3 S Cl

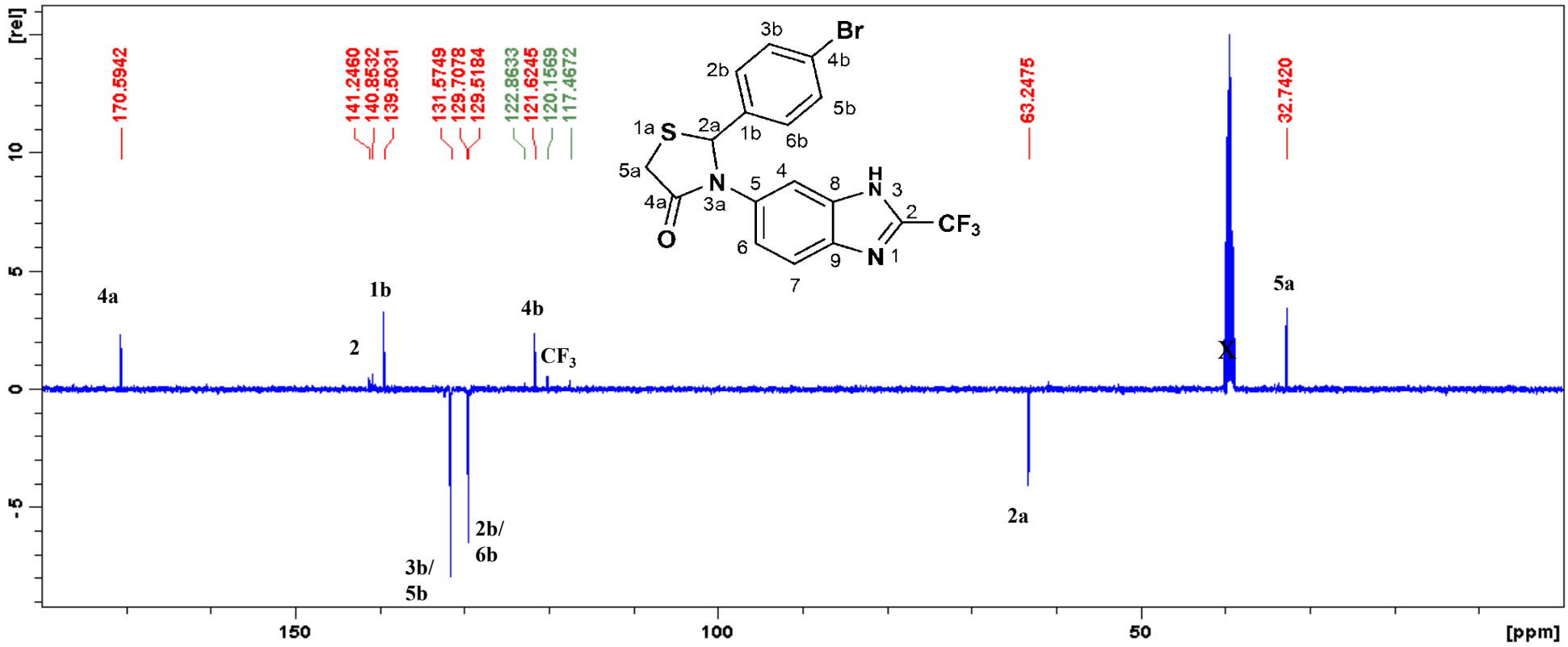
HRMS Spectrum of C-3i



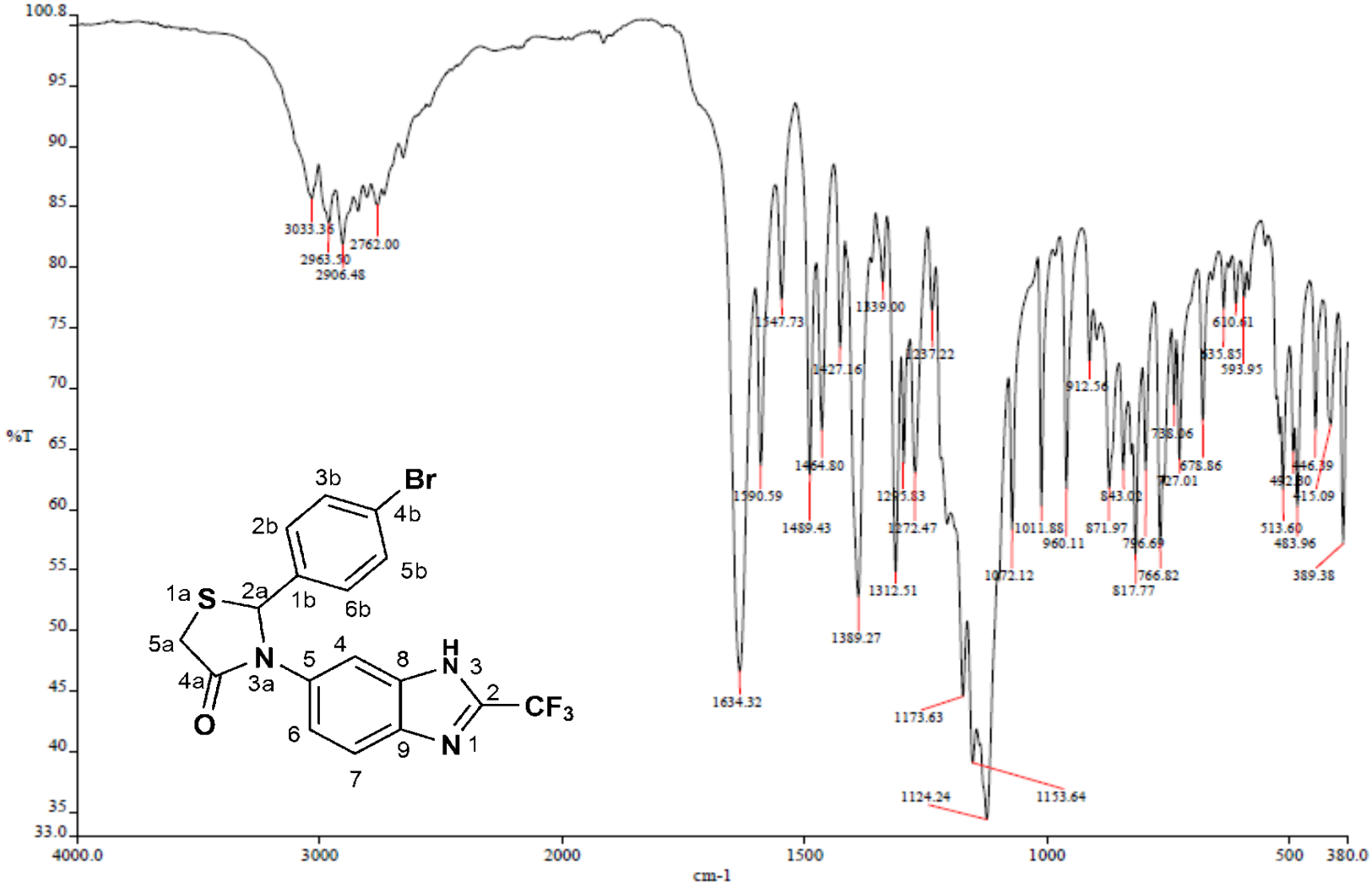
^{1}H Spectrum of C-3j



Expanded ^{1}H Spectrum of C-3j



^{13}C Spectrum of C-3j



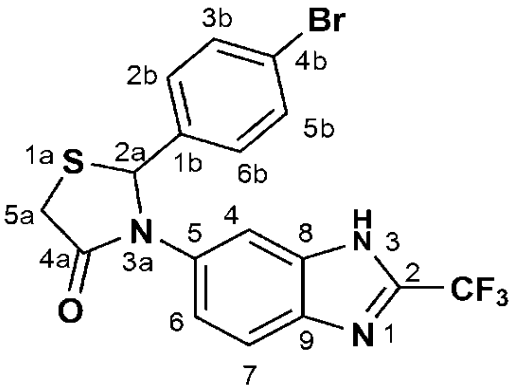
Infrared Spectrum of C-3j

Elemental Composition Report

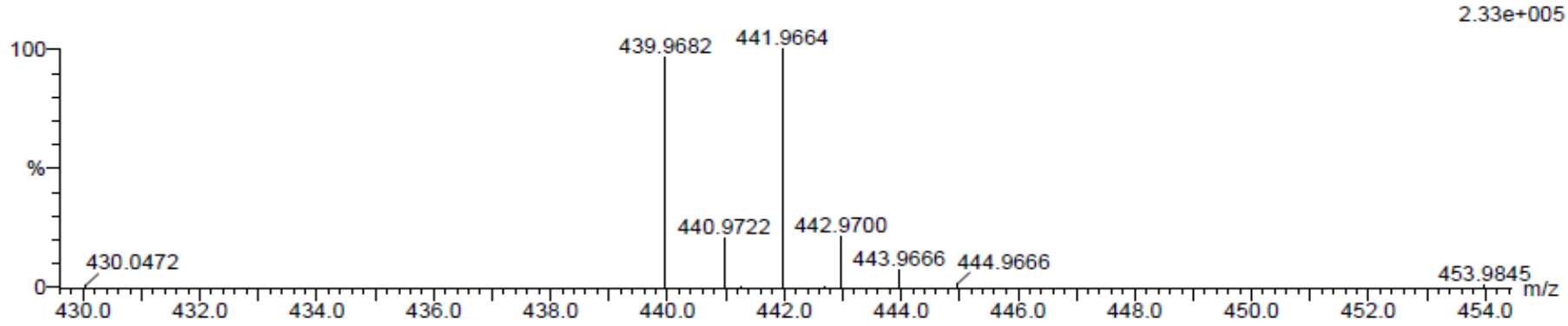
Single Mass Analysis

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
84 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 O: 0-1 F: 0-5 S: 1-1 Br: 0-1

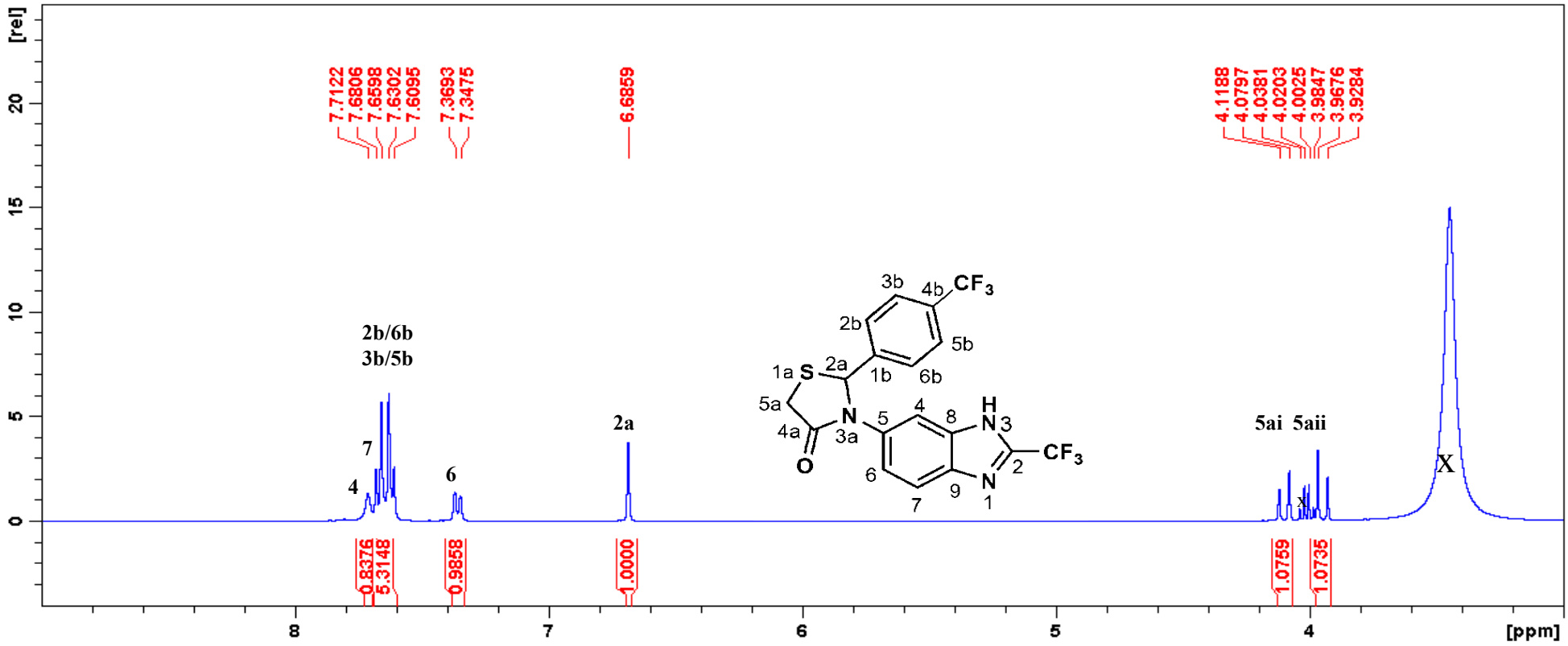


CF 7 44 (1.450) Cm (1:61)
TOF MS ES-

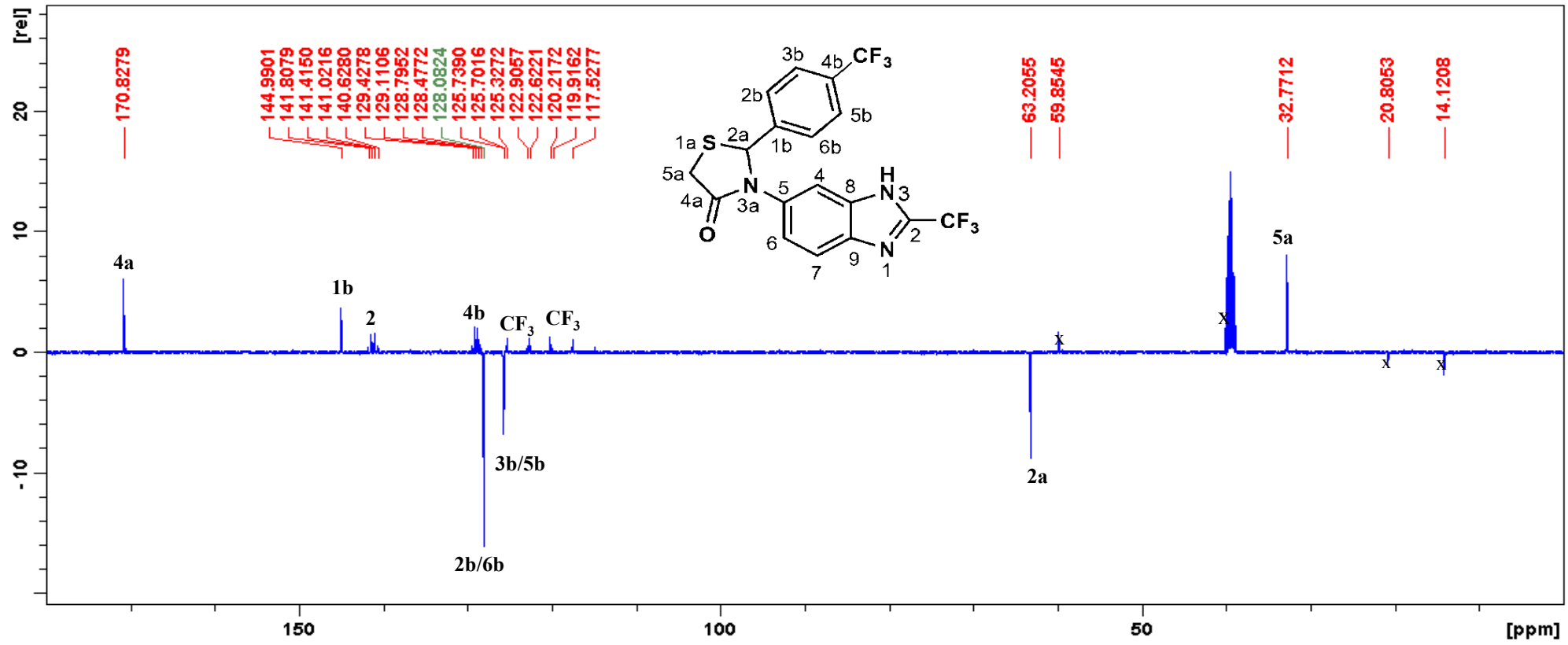


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
439.9682	439.9680	0.2	0.5	12.5	27.3	0.0	C17 H10 N3 O F3 S Br

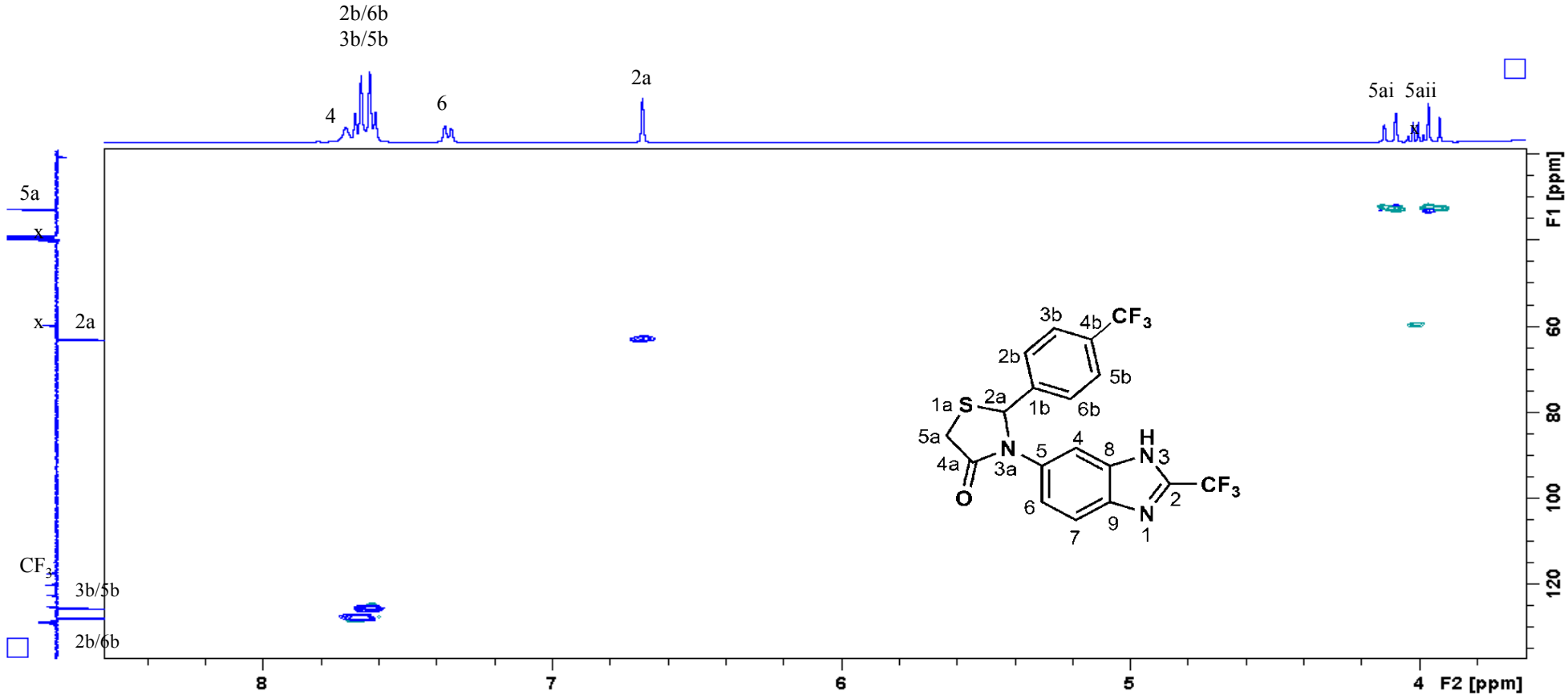
HRMS Spectrum of C-3j



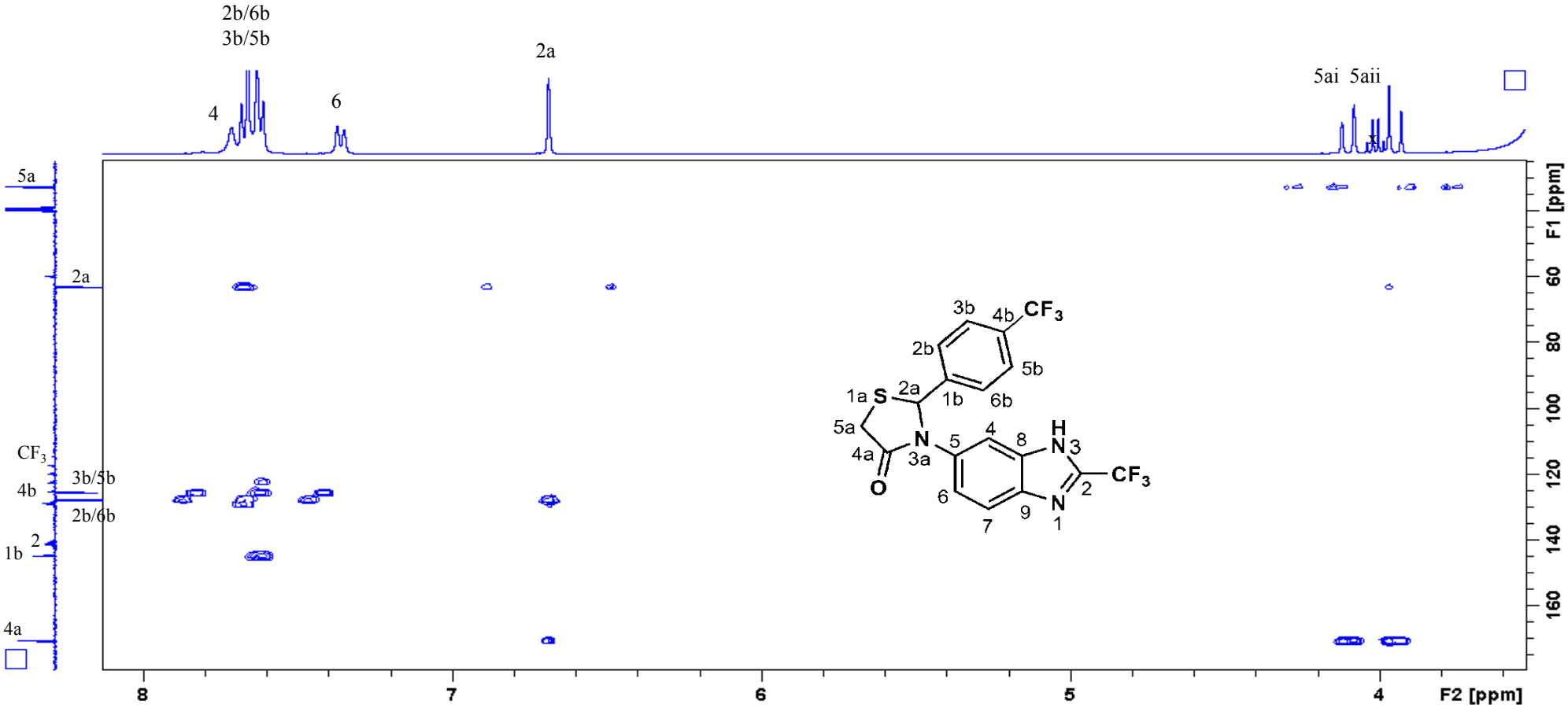
^{1}H Spectrum of C-3k



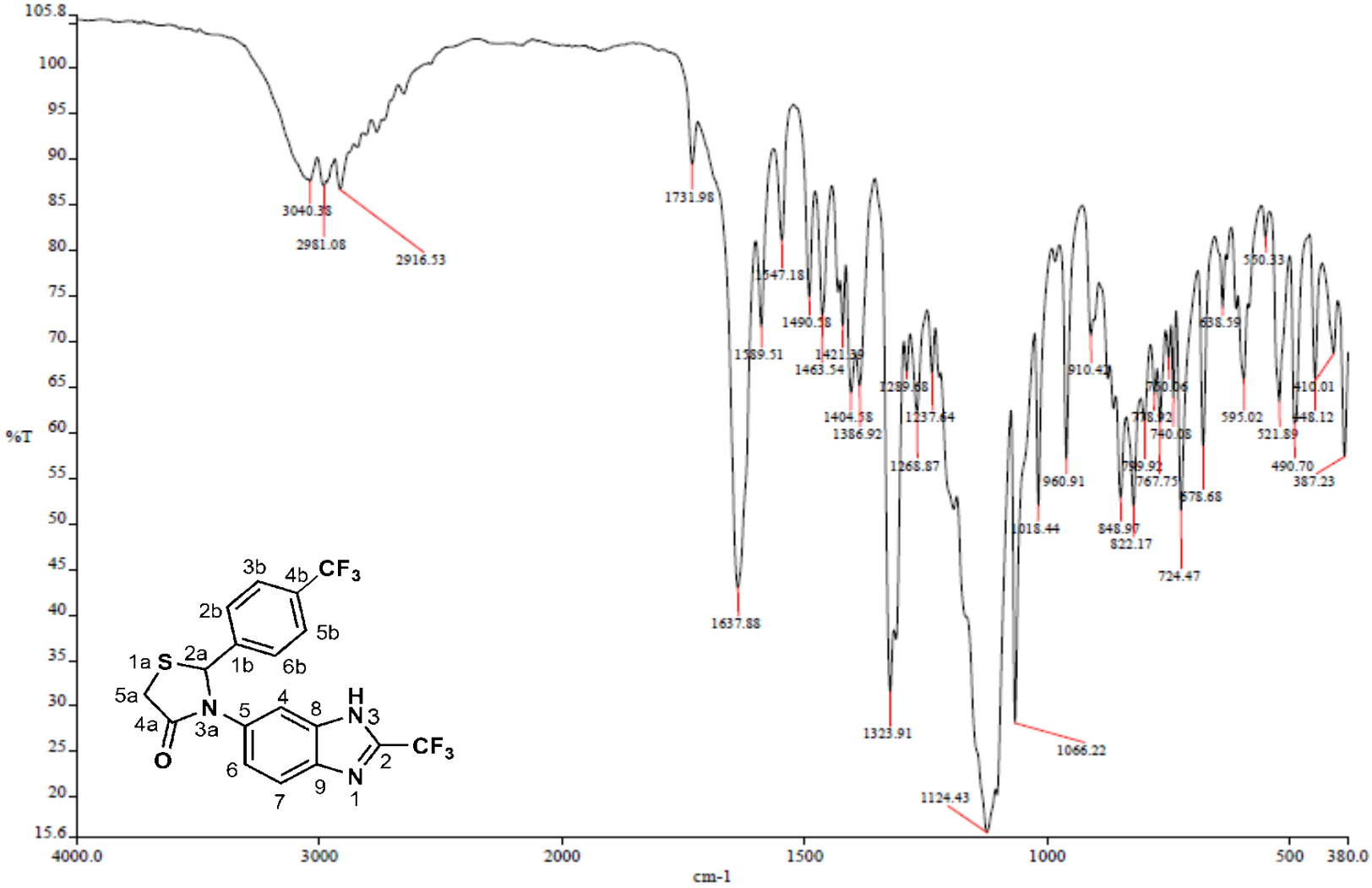
^{13}C Spectrum of C-3k



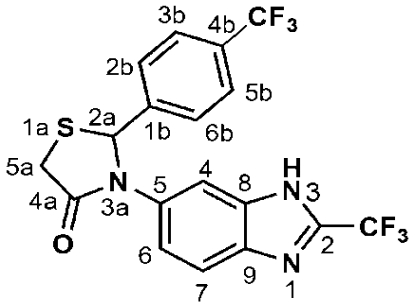
HSQC Spectrum of C-3k



HMBC Spectrum of C-3k



Infrared Spectrum of C-3k



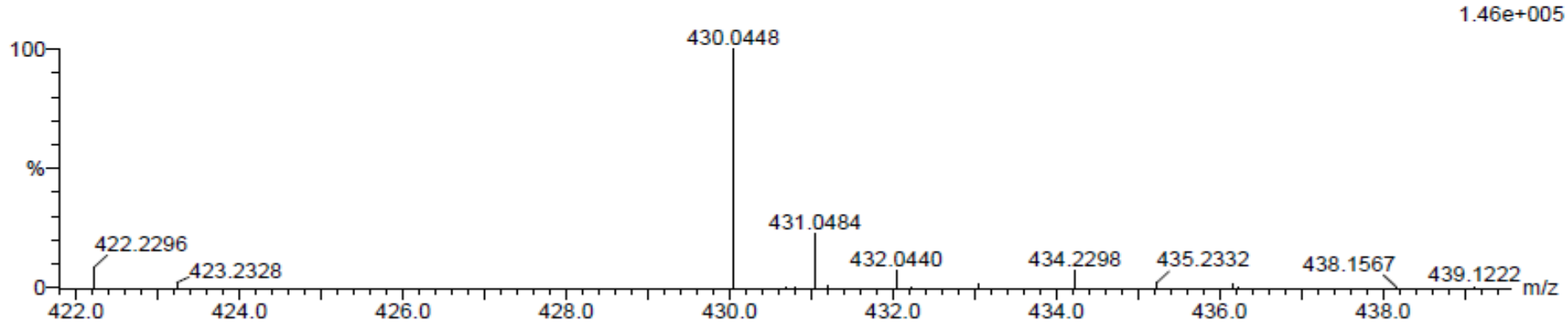
Elemental Composition Report

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

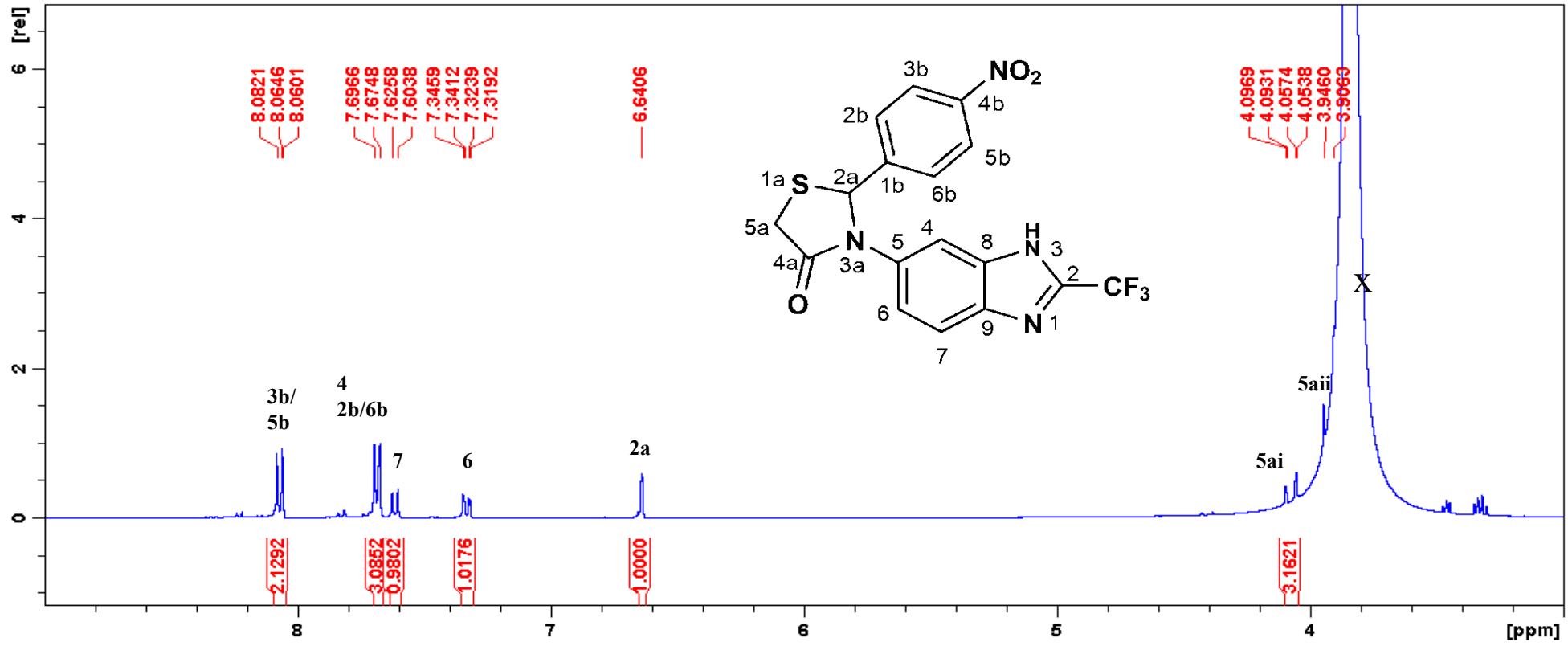
Monoisotopic Mass, Even Electron Ions
35 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 O: 0-1 F: 5-10 S: 1-1

CF 5 26 (0.843) Cm (1:61)
TOF MS ES-

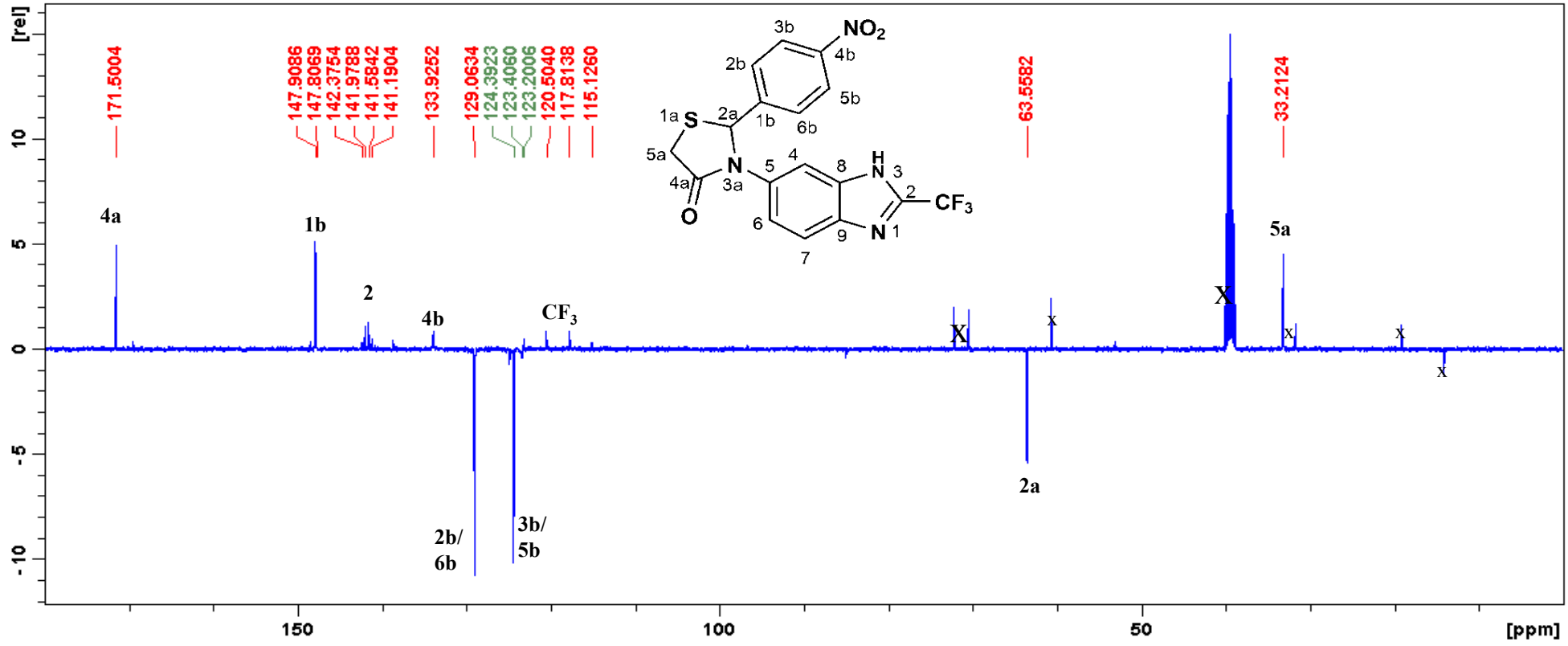


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
430.0448	430.0449	-0.1	-0.2	12.5	48.3	0.0	C18 H10 N3 O F6 S

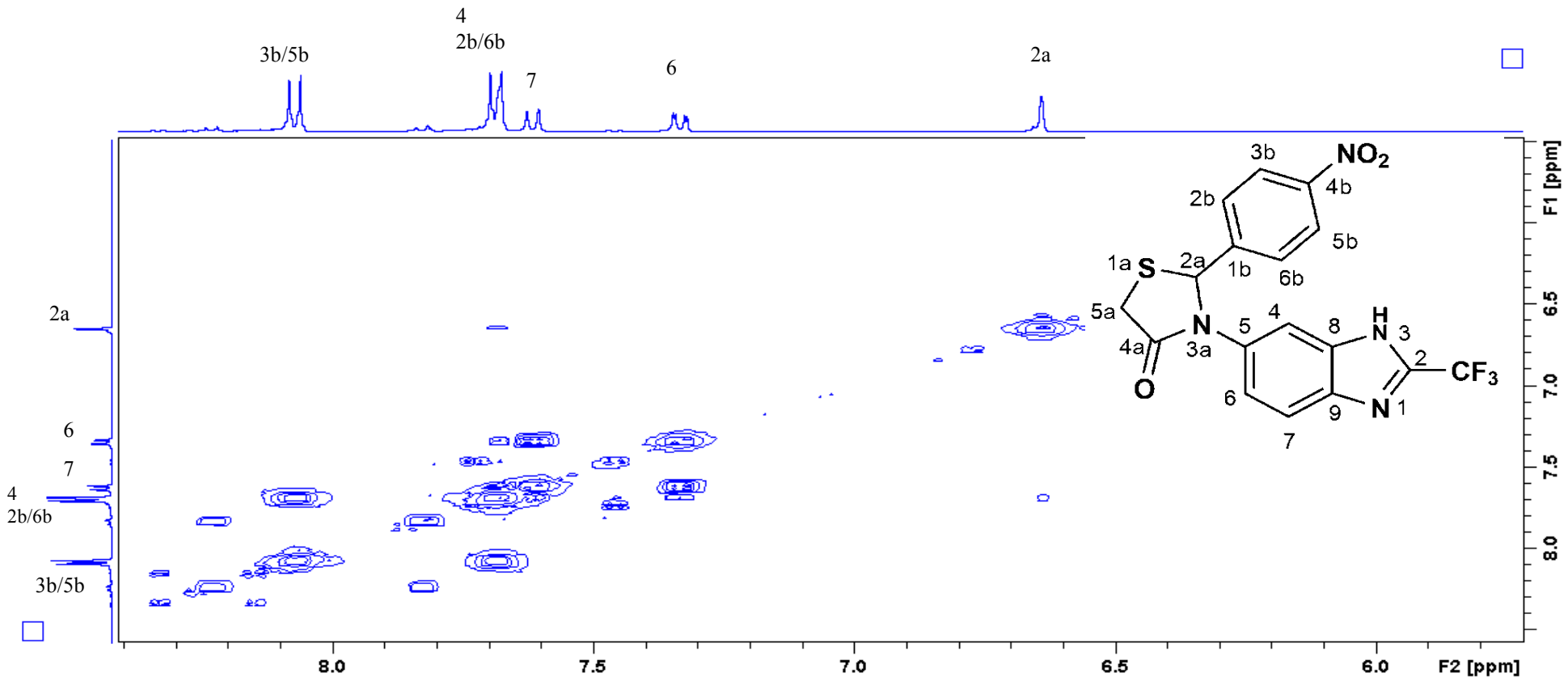
HRMS Spectrum of C-3k



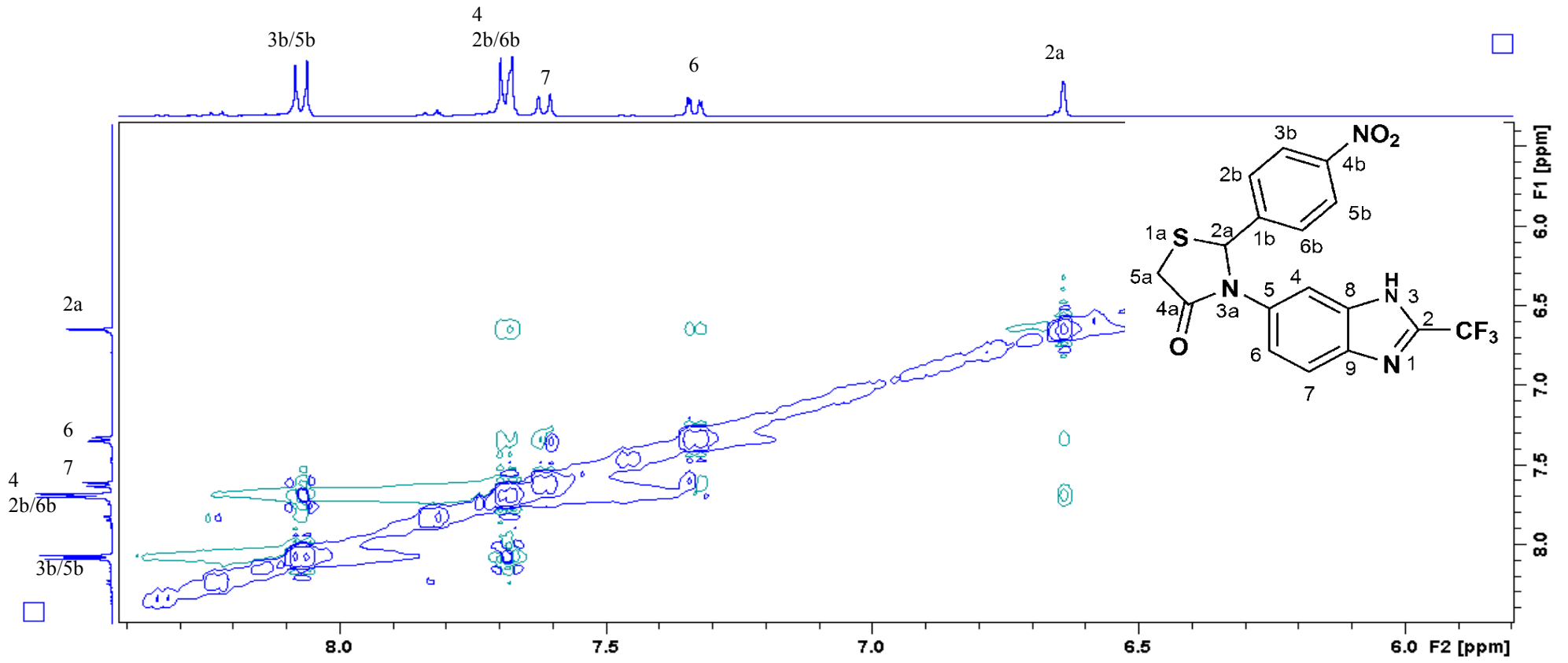
^{1}H Spectrum of C-3l



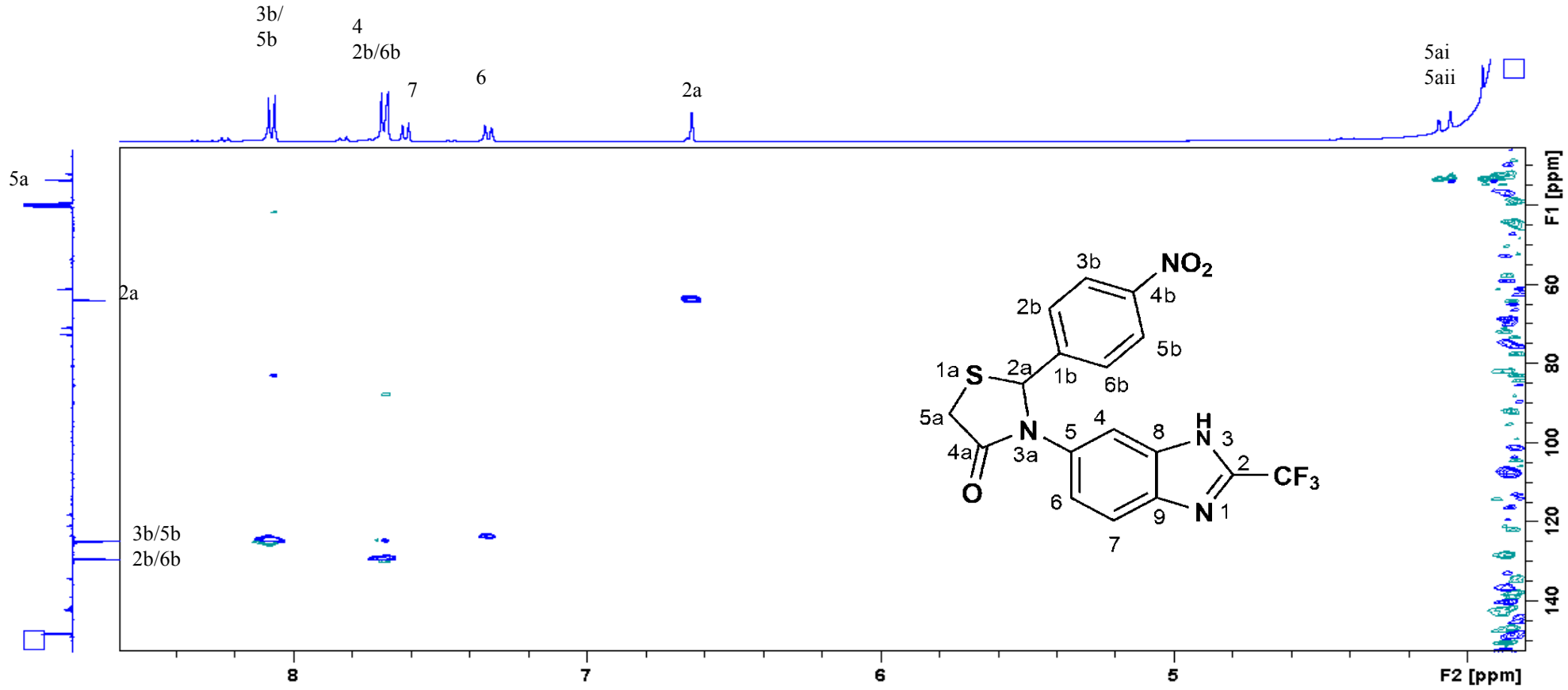
^{13}C Spectrum of C-3l



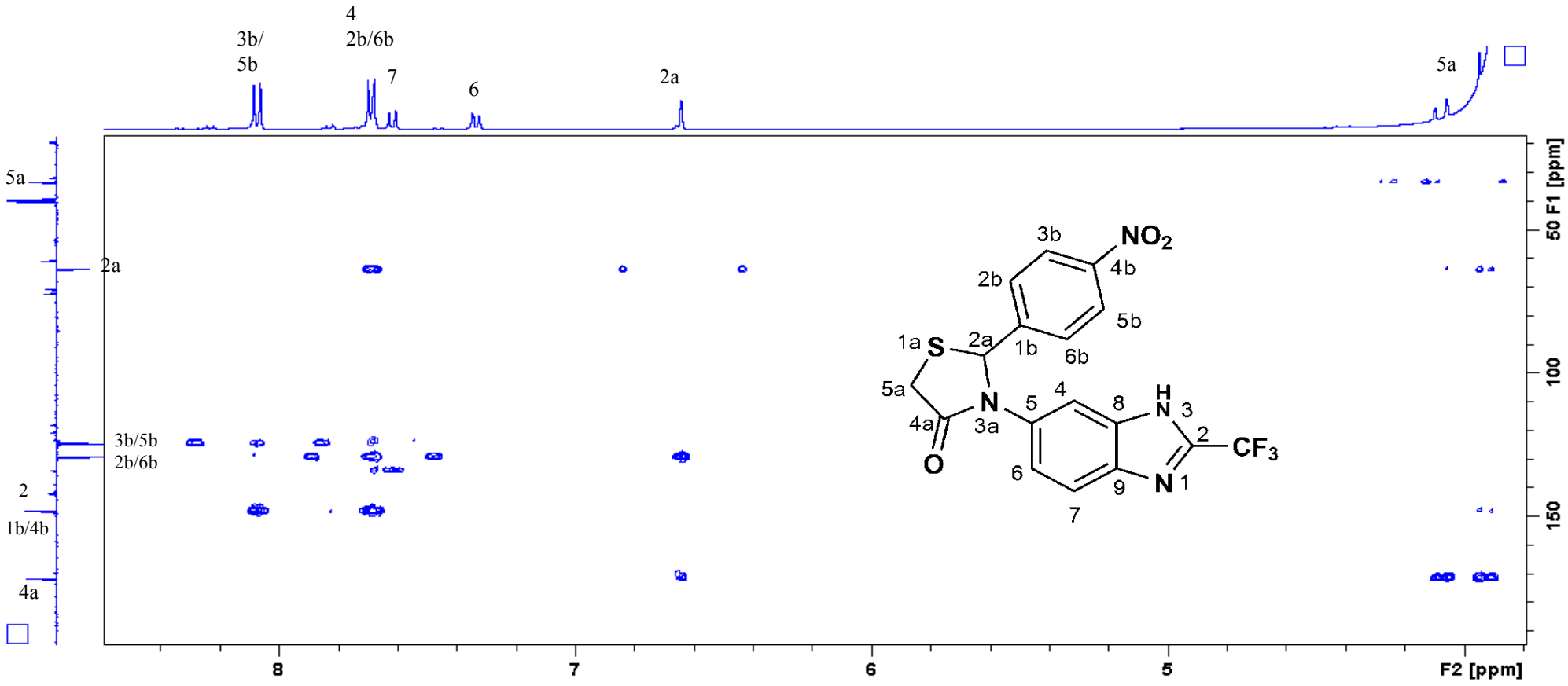
COSY Spectrum of C-3l



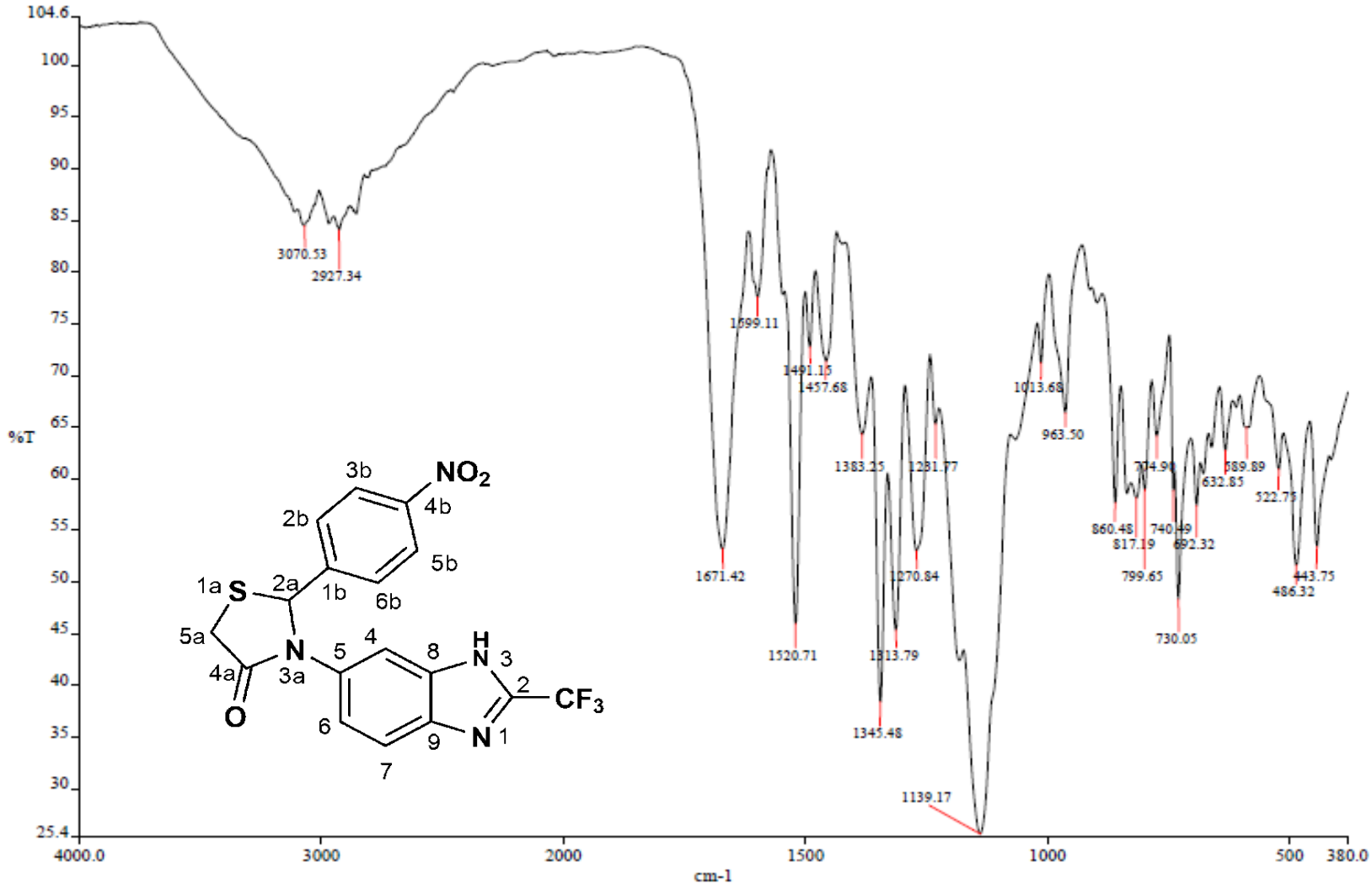
NOESY Spectrum of C-3l



HSQC Spectrum of C-3l



HMBC Spectrum of C-3l



Infrared Spectrum of C-4k

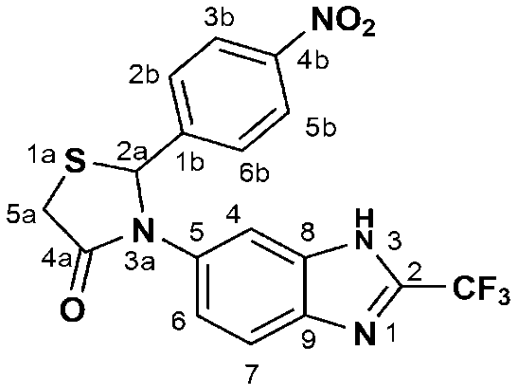
Elemental Composition Report

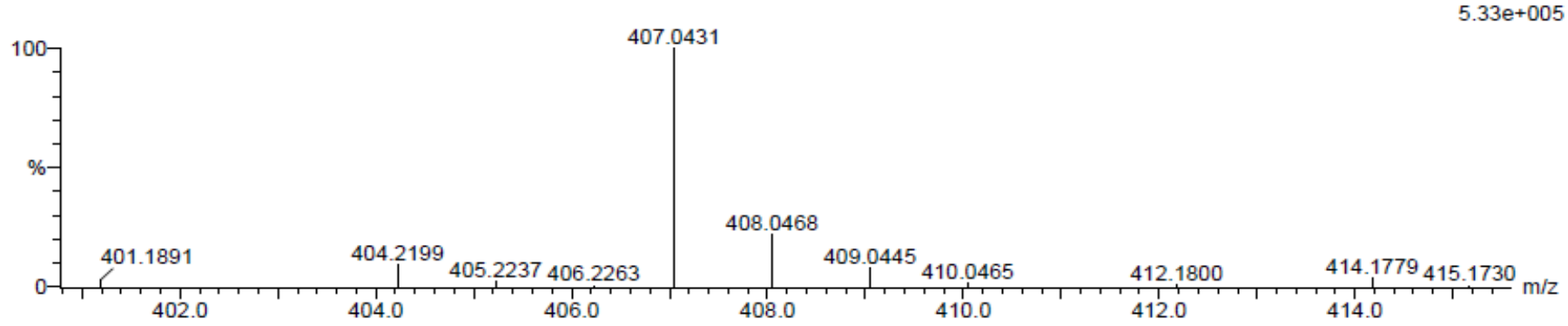
Single Mass Analysis

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
80 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 O: 0-5 F: 1-5 S: 1-1

CF 9 26 (0.843) 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
407.0431	407.0426	0.5	1.2	13.5	15.0	0.0	C17 H10 N4 O3 F3 S

HRMS Spectrum of C-4k

University Of KwaZulu-Natal

Synthesis, Characterization and Antibacterial Activity of Benzimidazole Derivatives

Appendix B: Data for Crystal Structure

2018

Adele Cheddie

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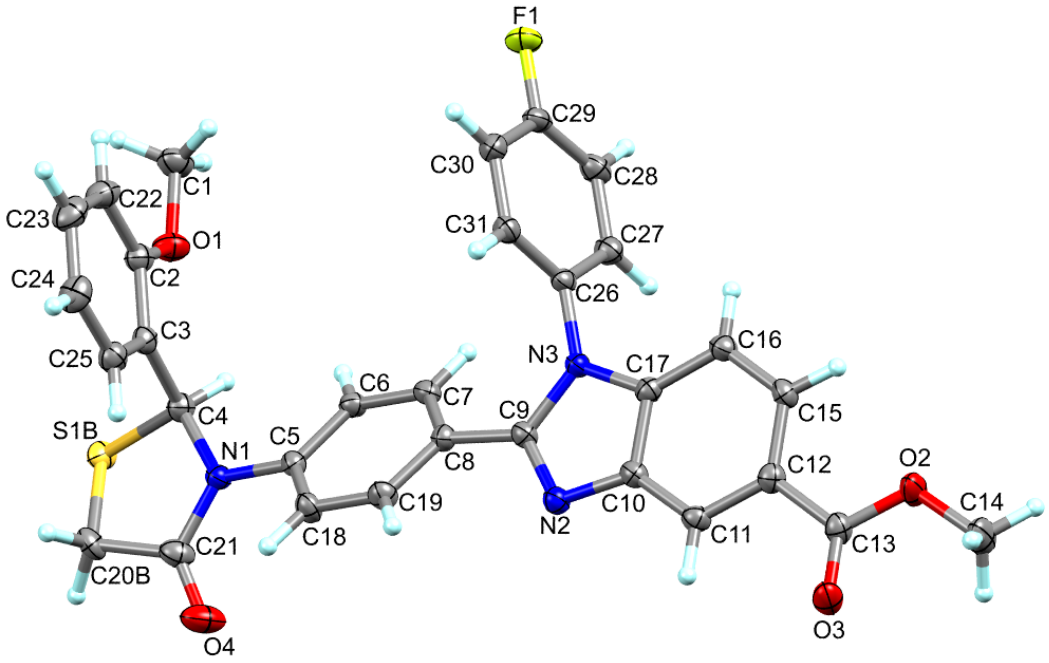

Figure 1: Ortep diagram of methyl-1-(4-fluorophenyl)-2-(4-(2-(2-methoxyphenyl)-4-oxothiazolidin-3-yl)phenyl)-1*H*-benzimidazole-5-carboxylate (A-**7j)**

Table 1: Crystal data and structural refinement for **A-7j**

Identification code	shelx	
Empirical formula	C31 H24 F N3 O4 S	
Formula weight	553.59	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 21/n	
Unit cell dimensions	a = 10.3568(9) Å	α= 90°.
	b = 19.6476(19) Å	β= 100.901(4)°.
	c = 12.7632(12) Å	γ = 90°.
Volume	2550.3(4) $Å^3$	
Z	4	
Density (calculated)	1.442 Mg/m^3	
Absorption coefficient	0.179 mm^{-1}	
F(000)	1152	
Crystal size	0.280 x 0.150 x 0.120 mm^3	

Theta range for data collection	1.927 to 28.360°.
Index ranges	-13<=h<=13, -24<=k<=26, -17<=l<=17
Reflections collected	32002
Independent reflections	6343 [R(int) = 0.0259]
Completeness to theta = 25.242°	100.0 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.989 and 0.945
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	6343 / 13 / 382
Goodness-of-fit on F^2	1.026
Final R indices [I>2sigma(I)]	R1 = 0.0418, wR2 = 0.1047
R indices (all data)	R1 = 0.0538, wR2 = 0.1115
Extinction coefficient	n/a
Largest diff. peak and hole	0.659 and -0.342 e.$Å^{-3}$

Table 2: . Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for **A-7j**.

	X	Y	Z	U(EQ)
F(1)	4160(1)	12359(1)	1788(1)	33(1)
O(1)	6907(1)	9336(1)	155(1)	28(1)
O(2)	121(1)	11308(1)	8512(1)	29(1)
O(3)	-292(1)	10187(1)	8417(1)	29(1)
O(4)	5104(1)	7071(1)	3020(1)	38(1)
N(1)	5444(1)	8066(1)	2160(1)	19(1)
N(2)	2094(1)	9525(1)	5285(1)	20(1)
N(3)	2607(1)	10508(1)	4552(1)	19(1)
C(1)	7349(2)	9908(1)	-375(1)	31(1)
C(2)	5661(2)	9103(1)	-231(1)	23(1)
C(3)	5214(1)	8597(1)	375(1)	21(1)
C(4)	6131(1)	8340(1)	1352(1)	21(1)
C(5)	4729(1)	8514(1)	2720(1)	19(1)
C(6)	5147(1)	9184(1)	2928(1)	19(1)
C(7)	4462(1)	9618(1)	3487(1)	19(1)
C(8)	3342(1)	9394(1)	3851(1)	19(1)

C(9)	2660(1)	9801(1)	4547(1)	19(1)
C(10)	1642(1)	10068(1)	5805(1)	19(1)
C(11)	1009(1)	10073(1)	6680(1)	20(1)
C(12)	741(1)	10700(1)	7092(1)	20(1)
C(13)	129(1)	10693(1)	8059(1)	22(1)
C(14)	-441(2)	11329(1)	9468(2)	38(1)
C(15)	1058(1)	11315(1)	6633(1)	21(1)
C(16)	1667(1)	11318(1)	5756(1)	21(1)
C(17)	1958(1)	10686(1)	5364(1)	19(1)
C(18)	3589(1)	8292(1)	3056(1)	23(1)
C(19)	2914(1)	8726(1)	3618(1)	22(1)
C(21)	5518(2)	7376(1)	2323(1)	26(1)
C(22)	4876(2)	9334(1)	-1169(1)	29(1)
C(23)	3642(2)	9045(1)	-1505(1)	33(1)
C(24)	3181(2)	8545(1)	-909(1)	30(1)
C(25)	3966(2)	8323(1)	32(1)	25(1)
C(26)	3013(1)	10989(1)	3830(1)	19(1)
C(27)	4082(1)	11409(1)	4190(1)	23(1)
C(28)	4476(2)	11874(1)	3500(1)	26(1)
C(29)	3776(2)	11907(1)	2469(1)	24(1)
C(30)	2701(2)	11507(1)	2104(1)	24(1)
C(31)	2308(1)	11040(1)	2797(1)	22(1)
S(1B)	7195(1)	7656(1)	1053(1)	23(1)
C(20B)	6135(2)	7026(1)	1476(2)	26(1)
S(1A)	6576(13)	7455(4)	713(7)	86(3)
C(20A)	6630(30)	7049(16)	1830(20)	91(10)

Table 3: Bond lengths [Å] and angles [°] for **A-7j**

F(1)-C(29)	1.3533(17)
O(1)-C(2)	1.3701(19)
O(1)-C(1)	1.4305(19)
O(2)-C(13)	1.3401(19)
O(2)-C(14)	1.4484(19)
O(3)-C(13)	1.2088(19)
O(4)-C(21)	1.216(2)
N(1)-C(21)	1.371(2)

N(1)-C(5)	1.4255(18)
N(1)-C(4)	1.4631(18)
N(2)-C(9)	1.3169(18)
N(2)-C(10)	1.3827(18)
N(3)-C(17)	1.3821(18)
N(3)-C(9)	1.3905(19)
N(3)-C(26)	1.4380(18)
C(1)-H(1A)	0.9800
C(1)-H(1B)	0.9800
C(1)-H(1C)	0.9800
C(2)-C(22)	1.390(2)
C(2)-C(3)	1.392(2)
C(3)-C(25)	1.392(2)
C(3)-C(4)	1.5041(19)
C(4)-S(1B)	1.8231(15)
C(4)-S(1A)	2.010(7)
C(4)-H(4A)	1.0000
C(5)-C(6)	1.396(2)
C(5)-C(18)	1.400(2)
C(6)-C(7)	1.390(2)
C(6)-H(6)	0.9500
C(7)-C(8)	1.399(2)
C(7)-H(7)	0.9500
C(8)-C(19)	1.400(2)
C(8)-C(9)	1.472(2)
C(10)-C(11)	1.397(2)
C(10)-C(17)	1.404(2)
C(11)-C(12)	1.388(2)
C(11)-H(11)	0.9500
C(12)-C(15)	1.409(2)
C(12)-C(13)	1.490(2)
C(14)-H(14A)	0.9800
C(14)-H(14B)	0.9800
C(14)-H(14C)	0.9800
C(15)-C(16)	1.386(2)
C(15)-H(15)	0.9500
C(16)-C(17)	1.393(2)
C(16)-H(16)	0.9500

C(18)-C(19)	1.385(2)
C(18)-H(18)	0.9500
C(19)-H(19)	0.9500
C(21)-C(20B)	1.519(3)
C(21)-C(20A)	1.55(3)
C(22)-C(23)	1.391(2)
C(22)-H(22)	0.9500
C(23)-C(24)	1.381(2)
C(23)-H(23)	0.9500
C(24)-C(25)	1.388(2)
C(24)-H(24)	0.9500
C(25)-H(25)	0.9500
C(26)-C(31)	1.385(2)
C(26)-C(27)	1.387(2)
C(27)-C(28)	1.383(2)
C(27)-H(27)	0.9500
C(28)-C(29)	1.379(2)
C(28)-H(28)	0.9500
C(29)-C(30)	1.372(2)
C(30)-C(31)	1.387(2)
C(30)-H(30)	0.9500
C(31)-H(31)	0.9500
S(1B)-C(20B)	1.803(2)
C(20B)-H(20C)	0.9900
C(20B)-H(20D)	0.9900
S(1A)-C(20A)	1.63(3)
C(20A)-H(20A)	0.9900
C(20A)-H(20B)	0.9900
C(2)-O(1)-C(1)	117.19(12)
C(13)-O(2)-C(14)	115.11(13)
C(21)-N(1)-C(5)	123.65(12)
C(21)-N(1)-C(4)	116.77(12)
C(5)-N(1)-C(4)	119.57(11)
C(9)-N(2)-C(10)	105.22(12)
C(17)-N(3)-C(9)	106.34(12)
C(17)-N(3)-C(26)	123.83(12)
C(9)-N(3)-C(26)	129.62(12)
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(22)	123.91(14)
O(1)-C(2)-C(3)	115.40(13)
C(22)-C(2)-C(3)	120.68(14)
C(2)-C(3)-C(25)	119.22(13)
C(2)-C(3)-C(4)	118.41(13)
C(25)-C(3)-C(4)	122.29(14)
N(1)-C(4)-C(3)	113.11(12)
N(1)-C(4)-S(1B)	105.62(10)
C(3)-C(4)-S(1B)	112.69(10)
N(1)-C(4)-S(1A)	98.43(19)
C(3)-C(4)-S(1A)	96.4(3)
N(1)-C(4)-H(4A)	108.4
C(3)-C(4)-H(4A)	108.4
S(1B)-C(4)-H(4A)	108.4
C(6)-C(5)-C(18)	118.94(13)
C(6)-C(5)-N(1)	120.41(12)
C(18)-C(5)-N(1)	120.64(13)
C(7)-C(6)-C(5)	120.43(13)
C(7)-C(6)-H(6)	119.8
C(5)-C(6)-H(6)	119.8
C(6)-C(7)-C(8)	120.90(13)
C(6)-C(7)-H(7)	119.5
C(8)-C(7)-H(7)	119.5
C(7)-C(8)-C(19)	118.24(13)
C(7)-C(8)-C(9)	123.57(13)
C(19)-C(8)-C(9)	117.95(13)
N(2)-C(9)-N(3)	112.62(12)
N(2)-C(9)-C(8)	122.58(13)
N(3)-C(9)-C(8)	124.68(13)
N(2)-C(10)-C(11)	129.92(13)
N(2)-C(10)-C(17)	110.45(12)
C(11)-C(10)-C(17)	119.57(13)
C(12)-C(11)-C(10)	117.93(13)

C(12)-C(11)-H(11)	121.0
C(10)-C(11)-H(11)	121.0
C(11)-C(12)-C(15)	121.61(13)
C(11)-C(12)-C(13)	116.97(13)
C(15)-C(12)-C(13)	121.41(13)
O(3)-C(13)-O(2)	123.12(14)
O(3)-C(13)-C(12)	124.08(14)
O(2)-C(13)-C(12)	112.78(13)
O(2)-C(14)-H(14A)	109.5
O(2)-C(14)-H(14B)	109.5
H(14A)-C(14)-H(14B)	109.5
O(2)-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(12)	121.12(13)
C(16)-C(15)-H(15)	119.4
C(12)-C(15)-H(15)	119.4
C(15)-C(16)-C(17)	116.72(13)
C(15)-C(16)-H(16)	121.6
C(17)-C(16)-H(16)	121.6
N(3)-C(17)-C(16)	131.57(13)
N(3)-C(17)-C(10)	105.36(12)
C(16)-C(17)-C(10)	123.03(13)
C(19)-C(18)-C(5)	120.38(14)
C(19)-C(18)-H(18)	119.8
C(5)-C(18)-H(18)	119.8
C(18)-C(19)-C(8)	121.06(13)
C(18)-C(19)-H(19)	119.5
C(8)-C(19)-H(19)	119.5
O(4)-C(21)-N(1)	125.44(15)
O(4)-C(21)-C(20B)	123.53(16)
N(1)-C(21)-C(20B)	110.98(14)
O(4)-C(21)-C(20A)	119.0(12)
N(1)-C(21)-C(20A)	111.9(12)
C(2)-C(22)-C(23)	119.09(15)
C(2)-C(22)-H(22)	120.5
C(23)-C(22)-H(22)	120.5
C(24)-C(23)-C(22)	120.90(15)

C(24)-C(23)-H(23)	119.6
C(22)-C(23)-H(23)	119.6
C(23)-C(24)-C(25)	119.59(15)
C(23)-C(24)-H(24)	120.2
C(25)-C(24)-H(24)	120.2
C(24)-C(25)-C(3)	120.51(15)
C(24)-C(25)-H(25)	119.7
C(3)-C(25)-H(25)	119.7
C(31)-C(26)-C(27)	121.14(13)
C(31)-C(26)-N(3)	119.43(13)
C(27)-C(26)-N(3)	119.40(13)
C(28)-C(27)-C(26)	119.68(14)
C(28)-C(27)-H(27)	120.2
C(26)-C(27)-H(27)	120.2
C(29)-C(28)-C(27)	118.26(14)
C(29)-C(28)-H(28)	120.9
C(27)-C(28)-H(28)	120.9
F(1)-C(29)-C(30)	118.51(14)
F(1)-C(29)-C(28)	118.56(14)
C(30)-C(29)-C(28)	122.93(14)
C(29)-C(30)-C(31)	118.72(14)
C(29)-C(30)-H(30)	120.6
C(31)-C(30)-H(30)	120.6
C(26)-C(31)-C(30)	119.25(13)
C(26)-C(31)-H(31)	120.4
C(30)-C(31)-H(31)	120.4
C(20B)-S(1B)-C(4)	90.99(8)
C(21)-C(20B)-S(1B)	105.34(13)
C(21)-C(20B)-H(20C)	110.7
S(1B)-C(20B)-H(20C)	110.7
C(21)-C(20B)-H(20D)	110.7
S(1B)-C(20B)-H(20D)	110.7
H(20C)-C(20B)-H(20D)	108.8
C(20A)-S(1A)-C(4)	92.3(11)
C(21)-C(20A)-S(1A)	104.3(19)
C(21)-C(20A)-H(20A)	110.9
S(1A)-C(20A)-H(20A)	110.9
C(21)-C(20A)-H(20B)	110.9

S(1A)-C(20A)-H(20B)	110.9
H(20A)-C(20A)-H(20B)	108.9

Table 4: Anisotropic displacement parameters ($Å^2 x\ 10^3$) for **A-7j**

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
F(1)	44(1)	24(1)	33(1)	7(1)	16(1)	-7(1)
O(1)	24(1)	27(1)	33(1)	7(1)	9(1)	1(1)
O(2)	39(1)	24(1)	31(1)	-6(1)	21(1)	-4(1)
O(3)	33(1)	26(1)	32(1)	-5(1)	17(1)	-8(1)
O(4)	37(1)	29(1)	51(1)	17(1)	19(1)	8(1)
N(1)	21(1)	16(1)	20(1)	1(1)	7(1)	1(1)
N(2)	21(1)	18(1)	23(1)	0(1)	7(1)	0(1)
N(3)	23(1)	15(1)	20(1)	0(1)	7(1)	1(1)
C(1)	35(1)	25(1)	37(1)	4(1)	17(1)	-2(1)
C(2)	24(1)	22(1)	25(1)	1(1)	9(1)	4(1)
C(3)	23(1)	20(1)	20(1)	-1(1)	6(1)	5(1)
C(4)	20(1)	22(1)	23(1)	1(1)	7(1)	2(1)
C(5)	21(1)	18(1)	18(1)	0(1)	4(1)	2(1)
C(6)	19(1)	18(1)	20(1)	2(1)	6(1)	0(1)
C(7)	23(1)	15(1)	20(1)	2(1)	4(1)	0(1)
C(8)	20(1)	18(1)	18(1)	0(1)	5(1)	1(1)
C(9)	19(1)	16(1)	21(1)	0(1)	4(1)	-1(1)
C(10)	18(1)	17(1)	22(1)	0(1)	4(1)	0(1)
C(11)	19(1)	19(1)	23(1)	1(1)	5(1)	-1(1)
C(12)	17(1)	23(1)	21(1)	-1(1)	5(1)	0(1)
C(13)	19(1)	23(1)	25(1)	-4(1)	7(1)	-1(1)
C(14)	55(1)	31(1)	37(1)	-7(1)	30(1)	-4(1)
C(15)	22(1)	18(1)	24(1)	-2(1)	5(1)	2(1)
C(16)	23(1)	18(1)	23(1)	2(1)	5(1)	1(1)
C(17)	19(1)	20(1)	18(1)	1(1)	4(1)	0(1)
C(18)	23(1)	18(1)	27(1)	-4(1)	7(1)	-4(1)
C(19)	21(1)	21(1)	26(1)	-1(1)	9(1)	-3(1)
C(21)	24(1)	22(1)	34(1)	5(1)	8(1)	4(1)
C(22)	32(1)	32(1)	26(1)	9(1)	11(1)	8(1)

C(23)	33(1)	42(1)	25(1)	5(1)	2(1)	9(1)
C(24)	24(1)	40(1)	27(1)	-2(1)	2(1)	3(1)
C(25)	25(1)	25(1)	25(1)	-1(1)	8(1)	0(1)
C(26)	23(1)	14(1)	22(1)	1(1)	9(1)	2(1)
C(27)	24(1)	21(1)	24(1)	0(1)	3(1)	-1(1)
C(28)	25(1)	20(1)	32(1)	-1(1)	7(1)	-4(1)
C(29)	31(1)	15(1)	27(1)	3(1)	15(1)	1(1)
C(30)	32(1)	22(1)	20(1)	0(1)	7(1)	1(1)
C(31)	25(1)	18(1)	23(1)	-2(1)	6(1)	-2(1)
S(1B)	22(1)	23(1)	25(1)	3(1)	9(1)	9(1)
C(20B)	27(1)	17(1)	33(1)	-5(1)	7(1)	4(1)
S(1A)	119(7)	65(4)	81(4)	-18(3)	35(5)	32(4)
C(20A)	99(13)	85(12)	91(12)	-11(9)	20(9)	0(9)

Table 5: Hydrogen coordinates (x 10^4) and isotropic displacement parameters for **A-7j**.

	X	**Y**	**Z**	**U(eq)**
H(1A)	7400	9782	-1109	47
H(1B)	8221	10048	5	47
H(1C)	6730	10286	-384	47
H(4A)	6698	8727	1674	25
H(6)	5904	9344	2685	23
H(7)	4758	10072	3624	23
H(11)	770	9661	6982	24
H(14A)	-1390	11247	9280	57
H(14B)	-281	11777	9805	57
H(14C)	-32	10976	9966	57
H(15)	851	11735	6931	25
H(16)	1876	11730	5437	25
H(18)	3277	7842	2897	27
H(19)	2148	8567	3849	27
H(22)	5179	9684	-1575	35
H(23)	3108	9193	-2154	40
H(24)	2332	8354	-1142	36

H(25)	3650	7981	446	30
H(27)	4541	11377	4907	28
H(28)	5210	12164	3731	31
H(30)	2234	11548	1391	29
H(31)	1564	10758	2565	26
H(20C)	5449	6879	869	31
H(20D)	6647	6623	1776	31
H(20A)	6467	6557	1702	109
H(20B)	7494	7110	2308	109

Table 6: Torsion angles [°] for **A-7j**.

C(1)-O(1)-C(2)-C(22)	7.9(2)
C(1)-O(1)-C(2)-C(3)	-173.09(13)
O(1)-C(2)-C(3)-C(25)	-179.04(13)
C(22)-C(2)-C(3)-C(25)	0.0(2)
O(1)-C(2)-C(3)-C(4)	-2.2(2)
C(22)-C(2)-C(3)-C(4)	176.83(14)
C(21)-N(1)-C(4)-C(3)	110.57(15)
C(5)-N(1)-C(4)-C(3)	-68.59(16)
C(21)-N(1)-C(4)-S(1B)	-13.13(15)
C(5)-N(1)-C(4)-S(1B)	167.71(10)
C(21)-N(1)-C(4)-S(1A)	9.9(4)
C(5)-N(1)-C(4)-S(1A)	-169.3(4)
C(2)-C(3)-C(4)-N(1)	153.88(13)
C(25)-C(3)-C(4)-N(1)	-29.39(19)
C(2)-C(3)-C(4)-S(1B)	-86.40(15)
C(25)-C(3)-C(4)-S(1B)	90.33(15)
C(2)-C(3)-C(4)-S(1A)	-104.1(3)
C(25)-C(3)-C(4)-S(1A)	72.6(3)
C(21)-N(1)-C(5)-C(6)	149.22(14)
C(4)-N(1)-C(5)-C(6)	-31.68(18)
C(21)-N(1)-C(5)-C(18)	-31.7(2)
C(4)-N(1)-C(5)-C(18)	147.42(13)
C(18)-C(5)-C(6)-C(7)	1.9(2)
N(1)-C(5)-C(6)-C(7)	-179.03(12)

C(5)-C(6)-C(7)-C(8)	-0.1(2)
C(6)-C(7)-C(8)-C(19)	-1.3(2)
C(6)-C(7)-C(8)-C(9)	172.90(13)
C(10)-N(2)-C(9)-N(3)	0.02(15)
C(10)-N(2)-C(9)-C(8)	176.36(12)
C(17)-N(3)-C(9)-N(2)	0.29(16)
C(26)-N(3)-C(9)-N(2)	-174.58(13)
C(17)-N(3)-C(9)-C(8)	-175.96(13)
C(26)-N(3)-C(9)-C(8)	9.2(2)
C(7)-C(8)-C(9)-N(2)	-145.45(14)
C(19)-C(8)-C(9)-N(2)	28.7(2)
C(7)-C(8)-C(9)-N(3)	30.4(2)
C(19)-C(8)-C(9)-N(3)	-155.37(14)
C(9)-N(2)-C(10)-C(11)	-177.38(14)
C(9)-N(2)-C(10)-C(17)	-0.32(15)
N(2)-C(10)-C(11)-C(12)	175.54(14)
C(17)-C(10)-C(11)-C(12)	-1.3(2)
C(10)-C(11)-C(12)-C(15)	1.8(2)
C(10)-C(11)-C(12)-C(13)	-176.92(12)
C(14)-O(2)-C(13)-O(3)	-0.4(2)
C(14)-O(2)-C(13)-C(12)	-178.88(14)
C(11)-C(12)-C(13)-O(3)	-9.7(2)
C(15)-C(12)-C(13)-O(3)	171.64(14)
C(11)-C(12)-C(13)-O(2)	168.77(13)
C(15)-C(12)-C(13)-O(2)	-9.9(2)
C(11)-C(12)-C(15)-C(16)	-0.8(2)
C(13)-C(12)-C(15)-C(16)	177.83(13)
C(12)-C(15)-C(16)-C(17)	-0.7(2)
C(9)-N(3)-C(17)-C(16)	177.35(15)
C(26)-N(3)-C(17)-C(16)	-7.4(2)
C(9)-N(3)-C(17)-C(10)	-0.45(14)
C(26)-N(3)-C(17)-C(10)	174.79(12)
C(15)-C(16)-C(17)-N(3)	-176.35(14)
C(15)-C(16)-C(17)-C(10)	1.1(2)
N(2)-C(10)-C(17)-N(3)	0.49(15)
C(11)-C(10)-C(17)-N(3)	177.90(12)
N(2)-C(10)-C(17)-C(16)	-177.56(13)
C(11)-C(10)-C(17)-C(16)	-0.1(2)

C(6)-C(5)-C(18)-C(19)	-2.2(2)
N(1)-C(5)-C(18)-C(19)	178.68(13)
C(5)-C(18)-C(19)-C(8)	0.8(2)
C(7)-C(8)-C(19)-C(18)	0.9(2)
C(9)-C(8)-C(19)-C(18)	-173.59(13)
C(5)-N(1)-C(21)-O(4)	-7.5(2)
C(4)-N(1)-C(21)-O(4)	173.38(15)
C(5)-N(1)-C(21)-C(20B)	169.73(14)
C(4)-N(1)-C(21)-C(20B)	-9.40(19)
C(5)-N(1)-C(21)-C(20A)	-165.3(14)
C(4)-N(1)-C(21)-C(20A)	15.5(14)
O(1)-C(2)-C(22)-C(23)	177.92(15)
C(3)-C(2)-C(22)-C(23)	-1.0(2)
C(2)-C(22)-C(23)-C(24)	1.4(3)
C(22)-C(23)-C(24)-C(25)	-0.7(3)
C(23)-C(24)-C(25)-C(3)	-0.4(2)
C(2)-C(3)-C(25)-C(24)	0.7(2)
C(4)-C(3)-C(25)-C(24)	-175.99(14)
C(17)-N(3)-C(26)-C(31)	-104.29(16)
C(9)-N(3)-C(26)-C(31)	69.8(2)
C(17)-N(3)-C(26)-C(27)	73.83(18)
C(9)-N(3)-C(26)-C(27)	-112.10(17)
C(31)-C(26)-C(27)-C(28)	-1.7(2)
N(3)-C(26)-C(27)-C(28)	-179.84(13)
C(26)-C(27)-C(28)-C(29)	0.5(2)
C(27)-C(28)-C(29)-F(1)	-179.69(13)
C(27)-C(28)-C(29)-C(30)	0.9(2)
F(1)-C(29)-C(30)-C(31)	179.60(13)
C(28)-C(29)-C(30)-C(31)	-1.0(2)
C(27)-C(26)-C(31)-C(30)	1.7(2)
N(3)-C(26)-C(31)-C(30)	179.74(13)
C(29)-C(30)-C(31)-C(26)	-0.3(2)
N(1)-C(4)-S(1B)-C(20B)	24.84(12)
C(3)-C(4)-S(1B)-C(20B)	-99.13(13)
S(1A)-C(4)-S(1B)-C(20B)	-50.1(4)
O(4)-C(21)-C(20B)-S(1B)	-155.17(15)
N(1)-C(21)-C(20B)-S(1B)	27.55(18)
C(20A)-C(21)-C(20B)-S(1B)	-69(3)

C(4)-S(1B)-C(20B)-C(21)	-29.62(14)
O(4)-C(21)-C(20A)-S(1A)	161.8(12)
N(1)-C(21)-C(20A)-S(1A)	-39(2)
C(20B)-C(21)-C(20A)-S(1A)	54(2)
C(4)-S(1A)-C(20A)-C(21)	36.6(19)