UNIVERSITY OF KWAZULU-NATAL

SYNTHESIS, CHARACTERISATION AND BIOLOGICAL STUDIES OF NOVEL PYRIMIDINE DERIVATIVES

2016

OLUWOLE SAMUEL AREMU

SYNTHESIS, CHARACTERISATION AND BIOLOGICAL STUDIES OF NOVEL PYRIMIDINE DERIVATIVES

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2016

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

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

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

Preface

I hereby declare that the thesis entitled "Synthesis, characterization and biological studies of novel pyrimidine derivatives" submitted to the University of KwaZulu-Natal for the award of the 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 in the School of Chemistry and Physics, University of KwaZulu-Natal, Westville campus, Durban, South Africa.

Signed: _____

Oluwole Samuel Aremu, M. Sc

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

Signed: _____ Date: _____

Professor Neil A. Koorbanally, PhD (Natal)

DECLARATIONS

DECLARATION 1 – PLAGIARISM

I, Oluwole Samuel Aremu declare that

1. The research reported in this thesis is my original research, except where otherwise indicated.

2. This thesis has not been submitted for any degree or examination at any other university.

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

    a. Their words have been re-written but the general information attributed to them have been referenced

    b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.

5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

Signed ……

DECLARATION 2-PUBLICATIONS

DETAILS OF CONTRIBUTIONS TO PUBLICATIONS that form part of and/or include research presented in this thesis (including publications in preparation, submitted, in press or published and giving details of the contributions of each author to the experimental work and writing of each publication).

Publication 1

Oluwole S. Aremu, Kaalin Gopaul, Pramod Kadam, Parvesh Singh, Moganavelli Singh, Chunderika Mocktar and Neil A. Koorbanally, Synthesis, characterization, anticancer and antibacterial activity of some novel pyrano[2,3-*d*]pyrimidinone carbonitrile derivatives. Paper accepted 8th August 2016 in Anticancer Agents in Medicinal Chemistry

Publication 2

Oluwole S. Aremu, Parvesh Singh, Moganavelli Singh, Chunderika Mocktar and Neil A. Koorbanally, Synthesis of chloro, fluoro and nitro derivatives of 7-amino-5-aryl-6-cyano-5*H*-pyrano pyrimidin-2,4-diones using organic catalysts and their antimicrobial and anticancer activities. To be submitted to Medicinal Chemistry Research.

Publication 3

Oluwole S. Aremu, Parvesh Singh, Moganavelli Singh, Chunderika Mocktar and Neil A. Koorbanally, Facile one-pot synthesis of novel pyridodipyrimidine derivatives: Their anticancer activity against a HeLa human cervical cancer cell line. To be submitted to Bioorganic & Medicinal Chemistry Letters.

My role in all the publications mentioned above included carrying out all the experimental work and writing of the publications. My supervisor guided me and assisted me with writing the results for publication. The other co-authors contribution was mainly in the bioactivity and checking whether the results were correctly interpreted.

Signed:

ACKNOWLEDGEMENTS

This dissertation would not have been possible without the guidance and the help of several individuals who in one way or another contributed and extended their valuable assistance in the preparation and completion of this study.

First and foremost, I would like to express my deepest gratitude to the Almighty God for source of life and His Grace to see the end of the PhD work and my supervisor and mentor, Prof. Neil Anthony Koorbanally for his excellent guidance, caring, patience, support and providing me with an excellent atmosphere throughout my years of study on both an academic and a personal level. His passion and humility has always encouraged me to give my best to my studies. His guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my Ph.D. study.

My thanks also go to Dr Chunderika Mocktar for guiding me through the antibacterial assays and Dr Moganavelli Singh for her help in carrying out the anticancer and cytotoxicity assays. I also acknowledge Dr Parvesh Singh for his guidance through the computational studies of the work in this thesis.

I would also like to thank all my colleagues in the Natural Products Group for the good working relationship in the laboratory and for their help whenever called upon. I appreciate the time and moments spent with you all. I am pleased to express my gratitude to all faculty and staff members of the School of Chemistry and Physics, University of KwaZulu Natal, for their valuable co-operation. I am grateful to Mr Dilip Jagjivan for assisting me with NMR analysis, and Neal Broomhead and Anita Naidoo for help in the instrument laboratory. I also thank Mr Gregory Moodley and Mr Raj Somaru who have assisted me in a number of ways.

My special thanks go to the Nigerian community here at the University of KwaZulu-Natal. I am also indebted to all my friends for their invaluable support, day in and day out during all these years.

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Finally, I would like to thank my wife, Dr Rhoda Aremu. She always stood by me through the good times and bad with great patience at all times and also my jewels Favour, Mercy and Grace Aremu for their understanding and prayers during the course of this programme. Thank you with all my heart!

I thank all those who have helped me directly or indirectly in the successful completion of my thesis. Anyone missed in this acknowledgement are also thanked.

ABBREVIATIONS

^{13}C NMR	(C-13) nuclear magnetic resonance spectroscopy
^{1}H NMR	proton (H-1) nuclear magnetic resonance spectroscopy
^{19}F NMR	fluorine-19 (F-19) nuclear magnetic resonance spectroscopy
Ac	acetate
EtOH	ethanol
MeOH	methanol
aq	aqueous
br	broad
c	concentration
cc	column chromatography
CD_3OD	deuterated methanol
$CDCl_3$	deuterated chloroform
DMSO-d_6	deuterated dimethyl sulfoxide
D_2O	deuterated water
COSY	correlated spectroscopy
d	doublet
dd	double of doublets
DABCO	1,4-diazabicyclo[2.2.2]octane
DBA	dibutylamine
DEPT	distortionless enhancement by polarization transfer
DNA	deoxyribonucleic acid
DNP	dictionary of natural products
EIMS	electron impact mass spectroscopy
HMBC	heteronuclear multiple bond coherence
HPLC	high pressure liquid chromatography
HREIMS	high resolution electron impact mass spectroscopy
HSQC	heteronuclear single quantum coherence
Hz	hertz
IC_{50}	The concentration of an inhibitor where the response is reduced by half
IR	infrared
m	multiplet
Me	methyl
Mp	melting point

MS	mass spectroscopy
NOESY	nuclear overhauser effect spectroscopy
RSA	radical scavenging activity
s	singlet
t	triplet
td	triplet of doublets
TCA	trichloroacetic acid
TGI	total growth inhibition
TLC	thin layer chromatography
UV	ultraviolet
MIC	minimum inhibitory concentration

ABSTRACT

Pyrimidine derivatives have shown antitubercular, analgesic, anti-inflammatory, antibacterial, antimalarial, antifungal, antitumour and anti-HIV activity. There has therefore been an increasing number of molecules based on the pyrimidine scaffold that have been synthesised in the last decade. This was done in order to identify lead compounds that could be developed and used as pharmaceuticals.

In this work, two types of molecules were synthesised, both of which contained the pyrimidine scaffold in their structure. The first type was 7-amino-5-aryl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-diones (set A and set B) and the second was 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) derivatives (set C). In set A, oxygenated derivatives (hydroxy and methoxy) were synthesised and in set B, chloro, nitro and fluoro derivatives were synthesised. Since the oxygenated compounds showed good activity against a HeLa cancer cell line, the second set (set B) of pyranopyrimidines with deactivating halogens and nitro groups was synthesized.

The 7-amino-5-aryl-6-cyano-5*H*-pyrano [2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-diones (**A1-7** and **B1-11**) were synthesised from substituted aromatic benzaldehydes (ArCHO), malononitrile, barbituric acid and DABCO in aqueous ethanol in yields of 90-96% in a one pot reaction. Compounds **A3**, **A7** and **B11** were synthesised for the first time in this work. Several catalysts were also investigated during the optimisation of the reaction including 1,4-diazabicyclo[2.2.2]octane (DABCO), triethylamine, dibutylamine (DBA), K_2CO_3 and L-proline. Among these, DABCO was found to be the best catalyst, forming the product in 38 minutes with a yield of 94%.

The 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) derivatives (**C1-13**) were synthesized by the condensation of 6-amino-2-thiouracil and benzaldehydes derivatives under reflux in glacial acetic acid in yields of 68-96%. Nine compounds (**C3**, **C5** and **C7-13**) were synthesized for the first time in this work. All compounds were purified either by recrystallization or by column chromatography and their structures confirmed by 1D and 2D NMR and mass spectroscopy.

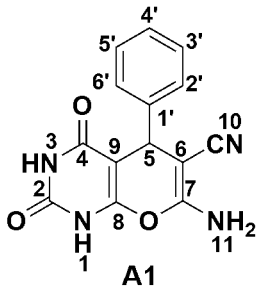
All of the derivatives were screened for anti-bacterial activities against five different bacterial strains; two Gram-positive strains, *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* (MRSA) as well as three Gram-negative strains, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. Compound **A6** (2',3'-dimethoxy derivative) showed broad spectrum activity amongst the strains tested. In particular, **A6** was active against *S. aureus* at 45.6 μM and against *E. coli* at 91.3 μM. None of the chloro, fluoro and nitro derivatives of 7-amino-5-aryl-6-cyano-5*H*-pyrano [2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-diones (**B2-11**) showed appreciable antibacterial activity. The 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) derivatives (**C1-13**) did not show any antibacterial activity in preliminary disk diffusion assays.

All the synthesised pyrimidines were also tested for their anticancer activity. Set A was tested against three cell lines, HeLa (cervical cancer cells), Caco-2 (colorectal adenocarcinoma cells) and HEK293 (human embryonic kidney cells). Compounds **A2**, **A5** and **A7** had IC_{50} values ten times lower (3.46, 4.36 and 4.44 μM) than 5-FU (41.85 μM) in the HeLa cell line. All compounds **A1-A7** were comparable (IC_{50} of 166-297 μM) to 5-FU (170 μM) in the Caco-2 cell line and better than 5-FU in the HEK293 cell line (137-333 μM as opposed to 505 μM for 5-FU). The remaining two sets of compounds were tested only

against a HeLa cell line, where **B1-11** showed comparable activity to 5-FU in the same assay and **C1-13** showed 3-8 times better activity than 5-FU (IC_{50} of 10.20-26.02 μM as opposed to 92.81 μM for 5-FU).

To support our anticancer results, the synthesised compounds were docked computationally into the active site of the Eg5 protein (a human kinesin molecular motor protein essential in mitosis), which is a therapeutic target for anticancer treatment. The pyranopyrimidines **A1**, **A7**, **B4** and **B8** showed calculated binding energies of -180.25, -140.9, -184.4 and -150.1 kcal mol^{-1}, better than 5-FU with a binding energy of -116.7 kcal mol^{-1} using the same parameters. All the 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) derivatives C1-13 were also docked into the active site of Eg5 but using an implicit solvent model. With the exception of **C9** (BE = -5.0 kcal mol^{-1}), all the compounds showed better binding energies (-12.4 to -26.7 kcal mol^{-1}) than 5-FU using the same parameters. In addition, the compounds were seen to form hydrogen bonds between their protonated NH groups and CN groups with amino acid residues in the protein. Electrostatic interactions and hydrophobic forces with amino acid residues were also observed.

Structures of synthesised compounds reported in Chapter 2



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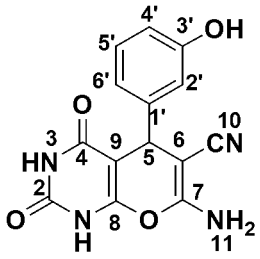

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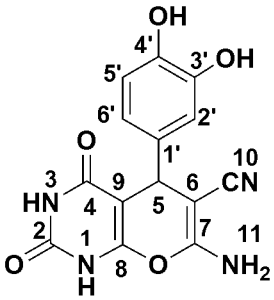

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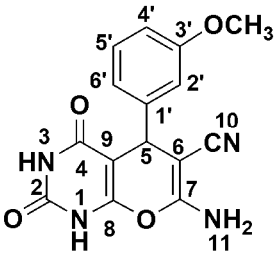

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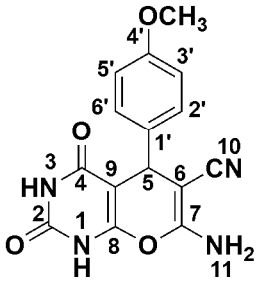

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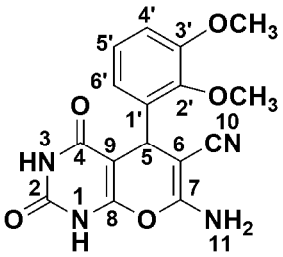

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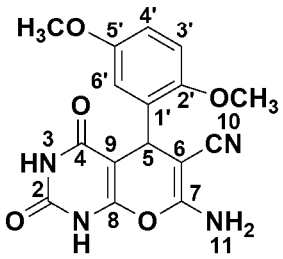

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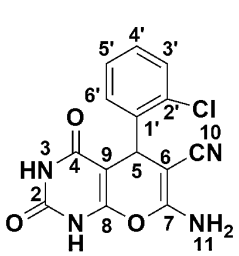
Structures of synthesised compounds reported in Chapter 3



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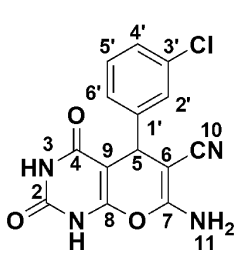

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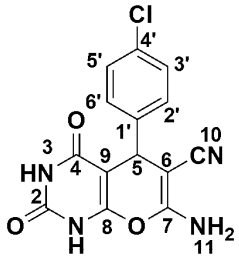

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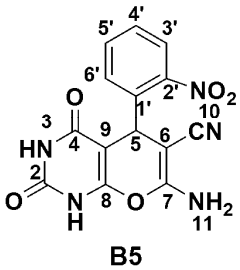

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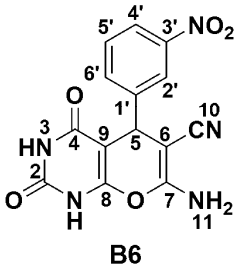

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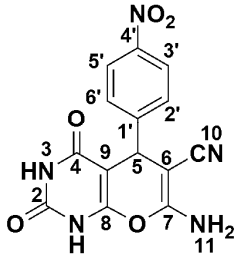

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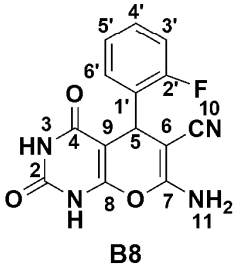

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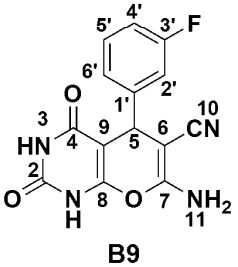

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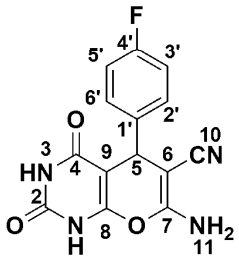

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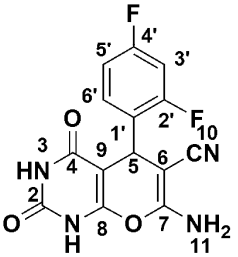

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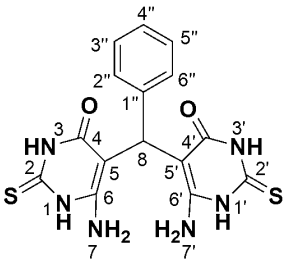
Structures of synthesised compounds reported in Chapter 4



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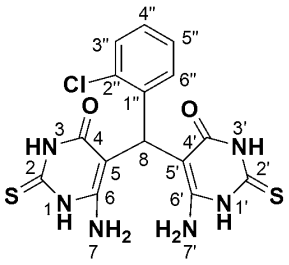

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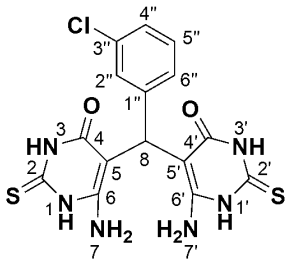

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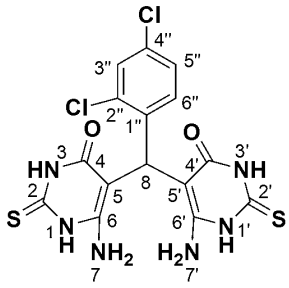

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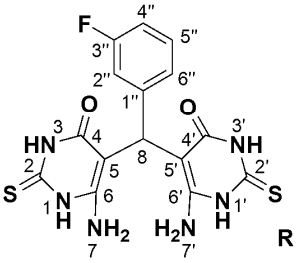

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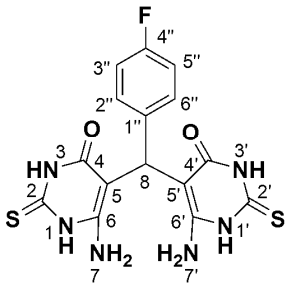

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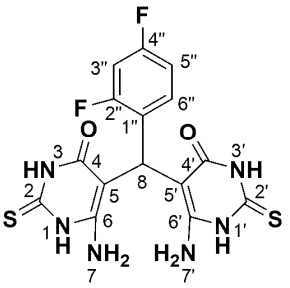

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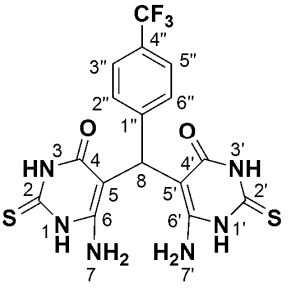

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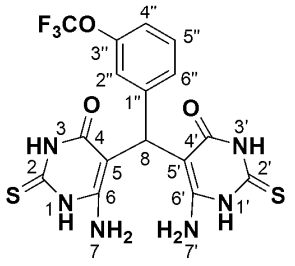

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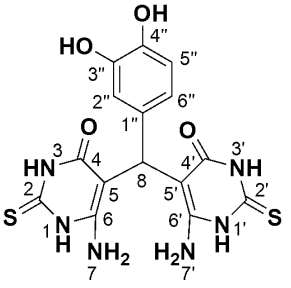

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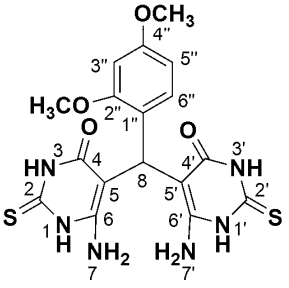

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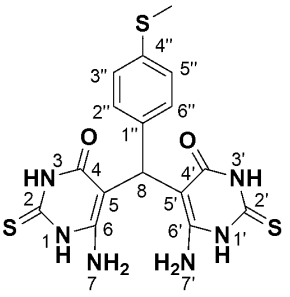

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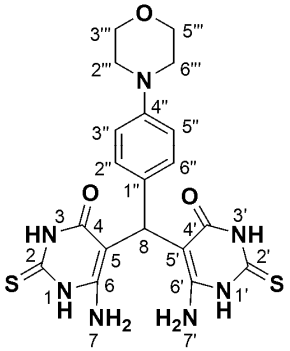

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Chapter 1. Introduction

Pyrimidine (1,3-diazine) (**1**) is a six-membered heterocyclic aromatic organic compound containing two nitrogen atoms at positions 1 and 3. The common name pyrimidine was approved by the International Union of Pure and Applied Chemistry (IUPAC) for this heterocyclic ring system. Several hydro and oxo derivatives of pyrimidine such as 2,4-(1*H*, 3*H*)-pyrimidinedione (**2**) (uracil), 5-methyluracil (thymine) (**3**) and 4-aminopyrimidine-2-(1*H*)-one (cytosine) (**4**) are important in biological systems being the nucleotide bases in nucleic acids (Loudon and Parise, 2016) (**Figure 1-1**). Pyrimidines are involved in attaining the three-dimensional structure of RNA and DNA (Loudon and Parise, 2016).

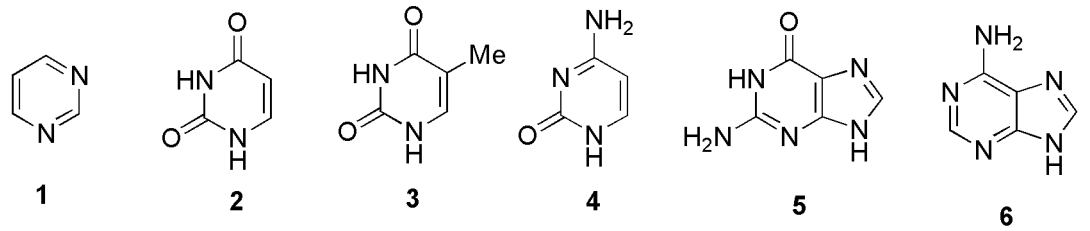

Figure 1-1 Structures with a pyrimidine nucleus: pyrimidine (**1**), uracil (**2**), thymine (**3**), cytosine (**4**), guanine (**5**) and adenine (**6**)

Pyrimidine is a water-soluble, colourless, hygroscopic liquid (m.p. 22.5 °C, b.p. 124 °C) with a characteristic odour, similar to pyridine and is rarely found in nature in the free state (Verma et al., 2012). The pyrimidine moiety in biological molecules such as nucleic acids, amino acids, vitamins, and their metabolism and biosynthesis is discussed in Lagoja (2005). It is a constituent of the purines, guanine (**5**) and adenine (**6**) (Loudon and Parise, 2016), thiamine (vitamin B_1) (**7**) (Müller et al., 2009), antibiotics such as bacimethrin (**8**) (Reddick et al., 2001), bleomycin (**9**) (Sugiyama and Kumagai, 2002) and sparsomycin (**10**) (Wiley and MacKellar, 1976) and products of metabolism such as uric acid (**11**) (Bruice, 2011) and toxins such as cylindrospermopsin (**12**) (Banker et al., 2001) (**Figure 1-2**).

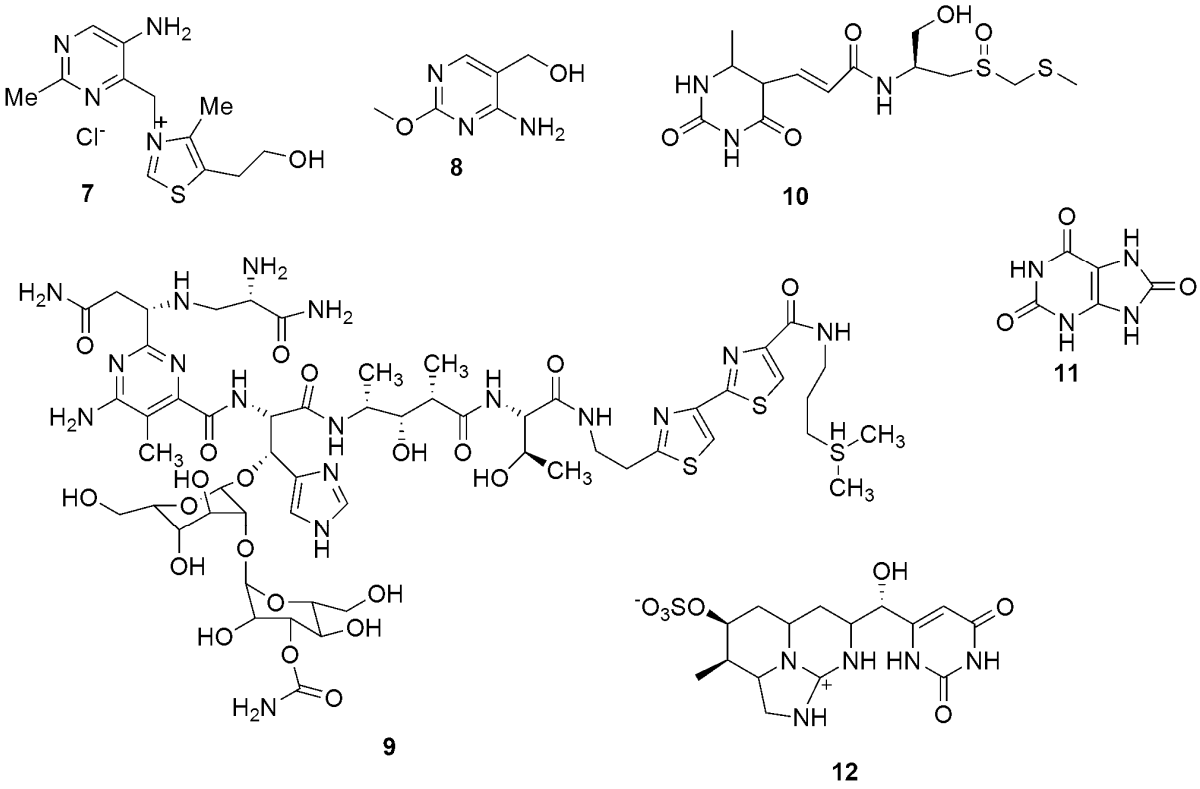

Figure 1-2 Some naturally occurring pyrimidine derivatives

Pyrimidine derivatives have also been known to possess biological activities such as antidiabetic (Panahi et al., 2013; Yousefi et al., 2015), antibacterial (Paliwal et al., 2013), anti-inflammatory and analgesic (Kadry, 2014), antitumor (Mohamed et al., 2007), antimycobacterial (Read et al., 2010), antimalarial (Joshi et al., 2005), and anti HIV (Malik et al., 2006).

The pyrimidine scaffold is found in many chemotherapeutic agents. Sulfadiazine (**13**) (Oprea et al., 2013), trimethoprim (**14**) (Bushby and Hitchings, 1968) and piritrexim (**15**) (Kovacs et al., 1988) are well known antibiotics with a pyrimidine scaffold (**Figure 1-3**).

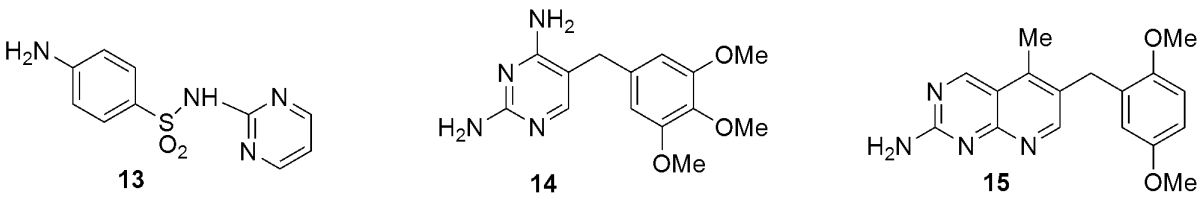

Figure 1-3 Some synthetic pyrimidine chemotherapeutic agents

Some pyrimidine derivatives such as the methylsulfonylpiperidine pyrimidine methanone (**16**) (Chu et al., 2006) and tetrasubstituted purine derivatives, for example *N*-(4-aminocyclohexyl)-*N*-(3-chlorophenyl)-9-isopropyl-9H-purine-2,6-diamine (**17**) (Moravec et al., 2003) have been reported as inhibitors of cyclin dependent kinase with significant *in vivo* antitumour activity. Pyrimidine carboxamides such as compound **18** were shown to have anti-inflammatory and anti-immunosuppressive activity (Sullivan et al., 1998), the indole thienopyrimidine (**19**) was shown to have anti-tumour activity (Munchhof et al., 2004) and the pyrazolo thiazolo pyrimidines such as compound **20** were shown to be adenosine enzyme inhibitors (Baraldi et al., 2001). 5-Fluorouracil (5-FU) (**21**) and its derivatives (**Figure 1-4**), were shown to have anticancer activity (Diaso and Harris, 1989; Jain et al., 2006). 5-FU itself has been used for a number of years as an efficient cancer treatment (Diaso and Harris, 1989).

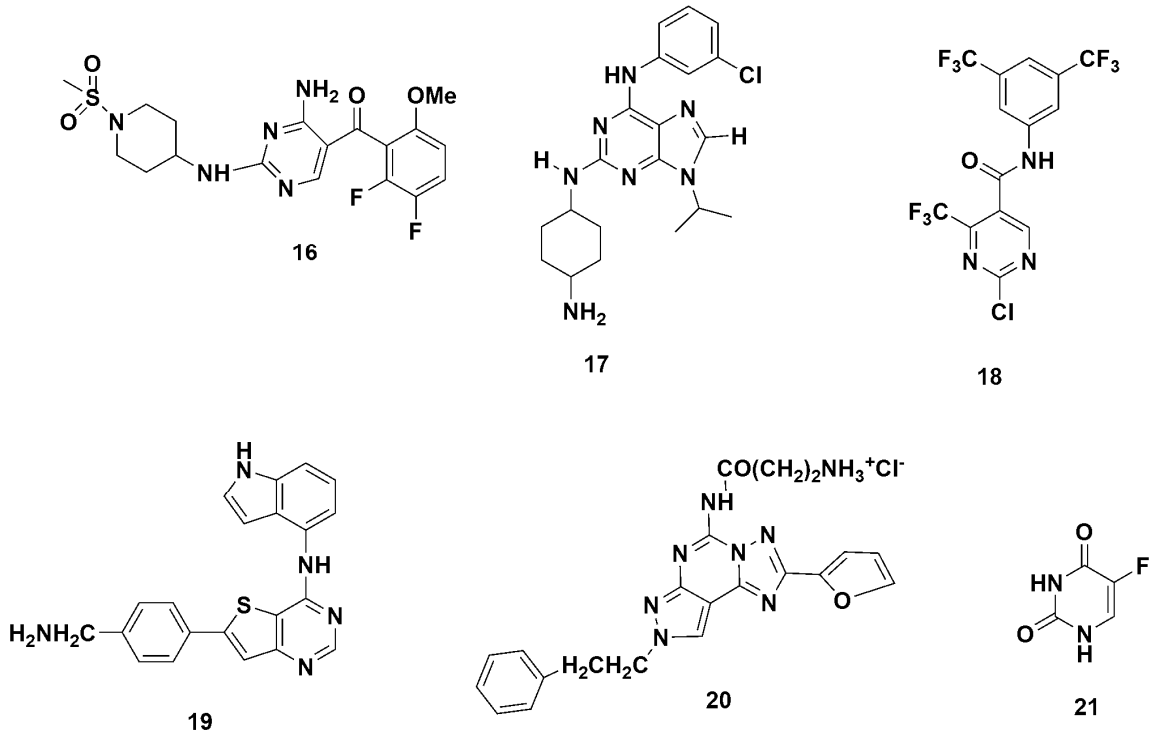

Figure 1-4 Structures of pyrimidine compounds with anticancer, anti-inflammatory, anti-immunosuppressive and CDK and adenosine enzyme inhibition

Pyrrolo-pyrimidine nucleoside derivatives such as toyocamycin (**22**) (Varaprasad et al., 2007) and valopicitabine (**23**) (Coelmont et al., 2006) were shown to act as anti-Hepatitis C (HCV) agents and thieno aminopyrimidines such as compound **24** (**Figure 1-5**) were shown to be potent phosphodiesterase (PDE) inhibitors (Crespo et al., 1998).

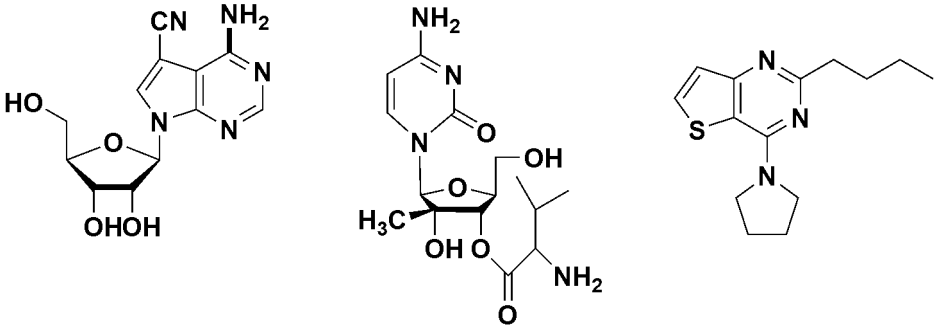

Figure 1-5 Structures of anti-HCV and PDE pyrimidine inhibitors

Zidovudine or azidothymidine (AZT) (**25**), an anti HIV drug (Darbyshire et al., 2000), the general anaesthetic, sodium pentothal (**26**) (Paul and Harris, 1970), anti-epileptic mephobarbital (**27**) (Aikens et al., 2000), and anti-hypertensive minoxidil (**28**) (**Figure 1-6**) (Gottlieb et al., 1972) are further examples of drugs containing a pyrimidine scaffold.

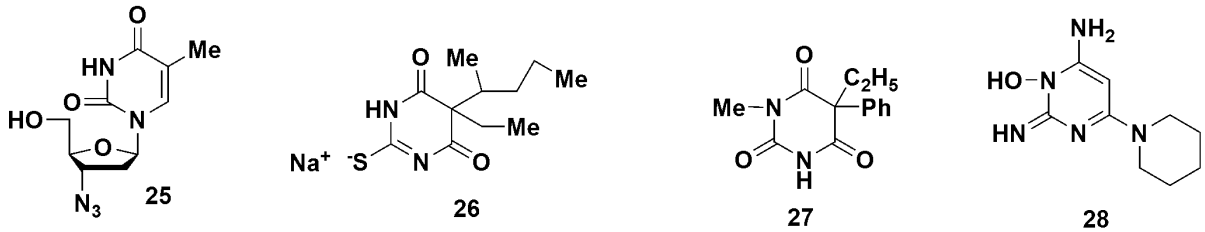

Figure 1-6 Structures of pyrimidine drugs with anti-HIV, anaesthetic, anti-epileptic and anti-hypertensive activities

1.1 Pyranopyrimidines

Pyrano [2,3-*d*]pyrimidine-2,4-diones consist of an unsaturated *N*-heterocyclic skeleton containing pyran and pyrimidine rings fused together. They are generally fused in a [2,3-*d*] fashion (**Figure 1-7**), the "2,3" referring to the position of the fused side in relation to the oxygen of the pyran ring and the "d" referring the position of the fused side in relation to the pyrimidine ring (Moss, 1998). The numbering system used for pyrano[2,3-*d*]pyrimidines and pyrano[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones are given in **Figure 1-7** below.

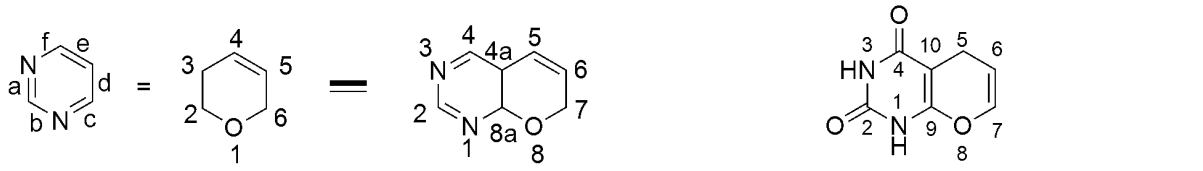

Figure 1-7 Nomenclature of pyranopyrimidines

Synthesis

Pyranopyrimidines are synthesised most commonly from aldehydes, barbituric acid and malonitrile. They are also synthesised from arylidene malonitriles and barbituric acid as well as several other methods employing other reagents. These are discussed below.

Pyranopyrimidines from aldehydes, barbituric acid and malonitrile

Pyrano [2,3-*d*]pyrimidines are generally synthesized by multicomponent reactions (Armstrong et al., 1996). The most common procedure for the preparation of pyrano[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones is the reaction of barbituric acid or thiobarbituric acid, malononitrile and aldehydes (**Figure 1-8**). This reaction is usually carried out in a one pot reaction under traditional thermal conditions (Heravi et al., 2010; Mobinikhaledi et al., 2010; Khurana et al., 2014). Other conditions under which the reaction is carried out include

microwave irradiation, which was done using methylcyanoaceate instead of malonitrile (Bhat et al., 2015), ultrasonic irradiation (Yadav and Quraishi, 2014), solvent free (Ziarani et al., 2013; Khazaei et al., 2015) or catalyst free conditions (Safaei et al., 2012). The catalysts employed for these reactions include amino acids such as L-proline (Barjanian et al., 2009) and nanocatalysts (Kidwai et al., 2012; Sadeghi et al., 2015).

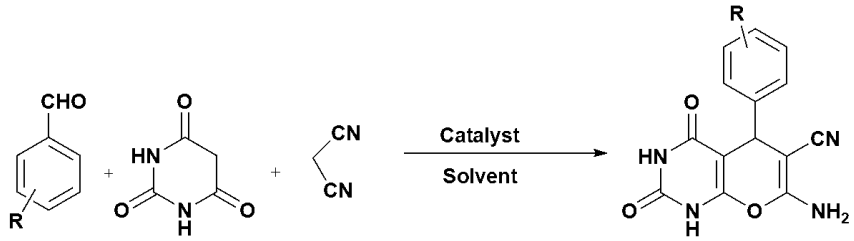

Figure 1-8 General reaction of pyranopyrimidines synthesis by the three component reaction of aldehydes, barbituric acid and malonitrile

The mechanism for the reaction involving malonitrile, aldehydes and barbituric acid is discussed in detail in Chapter 3.

Most of the syntheses are carried out using ethanol, water, or a mixture of the two as a solvent (Albadi et al., 2014; Maddila et al., 2015; Sadeghi et al., 2015). The reaction time varies between 30 minutes to 3 hours and the yield varies between 72 to 97% depending on the type of catalysts used in the reaction (Bararjanian et al., 2009; Mobinikhaledi and Fard, 2010; Safaei et al., 2012).

With base catalysts such as 1,4-diazabicyclo[2.2.2]octane (DABCO), dibutylamine (DBA), diammonium hydrogen phosphate or Zn[(L) proline]$_2$, the product is formed in less than an hour with a yield of 90 percent or more (Balalaie et al., 2008; Heravi et al., 2010; Azizian et al., 2012; Jain et al., 2014; Bhat et al., 2014; 2016). When this reaction is performed under microwave irradiation the product is formed in less than 7 minutes in catalyst free conditions

with yields of 78-94% (Bhat et al., 2015). Yadav and Quraishi (2014) compared the conventional reaction with ultrasonic irradiation and found that the although the yields were still the same under both conditions (42-98%), the duration of the reaction was slightly shorter for ultrasound irradiation (for example, <2 minutes as opposed to 5 minutes). They also found that triethanolamine is a good, cost effective base and that ethanol is a good solvent for the reaction.

Ziarani et al. (2013) made use of a SBA-Pr-SO_3H nanocatalyst without a solvent to produce pyranopyrimidines in yields of between 30-90% in 45 minutes or less, whilst Khazaei et al. (2015) used a $ZnFe_2O_4$ nanocatalyst to achieve yields of 86-96% in under 30 minutes, also under solvent free conditions. Pyranopyridimidines were also formed under catalyst free conditions using glycerol as a solvent in yields of 90-95% in reaction times of between one to three and a half hours (Safaei et al., 2012).

Pyranopyrimidines from arylidinemalonitriles and barbituric acid

Another common method for forming pyranopyrimidines involves reacting arylidenemalonitriles with barbituric acid or thiobarbituric acid (**Figure 1-9**).

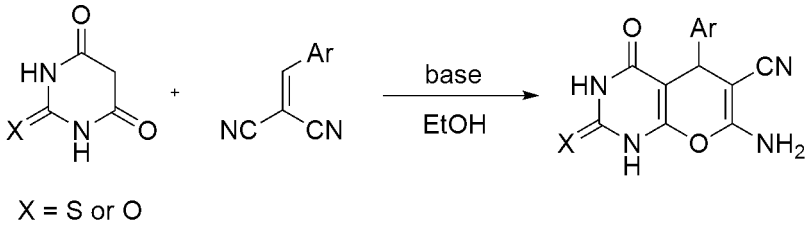

Figure 1-9 General reaction of pyranopyrimidines synthesised by a two component reaction of arylidenemalonitrile and barbituric acid

These reactions were carried out under reflux for 3 hours using triethylamine with ethanol resulting in yields of 80% and above (Aly and Kamal, 2012). They were also formed by dissolving the two components in ionic liquids and heating them to 90 ºC for 3 hours (Yu and Wang, 2005). Here, the pyridine and imidazole ionic liquids acted as a solvent and catalyst in one. Instead of conventional heating and reflux conditions, these reactions were also successful under microwave conditions in 3-5 minutes, producing excellent yields of between 86-94% (Gao et al., 2004).

Miscellaneous methods

Pyranopyrimidines were also formed by reacting aldehydes, malonitrile and ethyl acetoacetate to first form 2-amino-4-phenyl-4*H*-chromen-3-carbonitrile derivatives, which are subsequently converted to pyranopyrimidines in yields of between 50-90% by cyclising them with acetic anhydrides in the presence of a silica sulphuric acid catalyst for 1-3 hours (**Figure 1-10**) (El-Bayouki et al., 2013) or under reflux in acetic anhydride with a catalytic amount of concentrated sulphuric acid (Shahi et al., 2015). A base catalyst was not needed for this reaction as discovered by Litvinov and Shestopalov (2008), who obtained a tar like substance when heating the 6-methyl-5-carboxylate derivative of 2-amino-4-phenyl-4*H*-chromen-3-carbonitriles with piperidine, but obtained the pyranopyrimidine without the base catalyst in 10 hours with a yield of 51%. On the contrary, catalytic amounts of sulfuric acid led to high selectivity and increased yields of various pyranopyrimidine derivatives (42-75%).

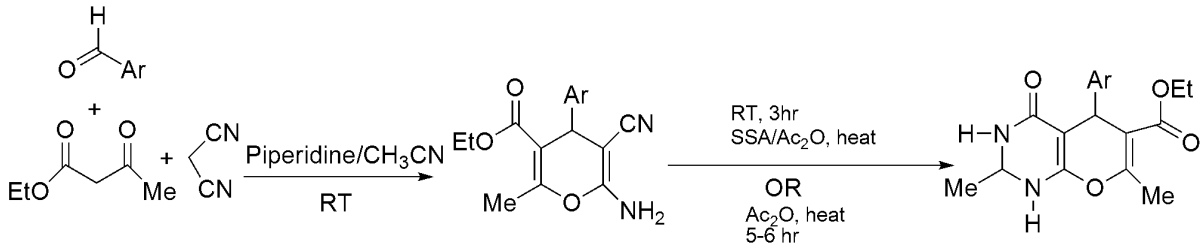

Figure 1-10 Pyranopyrimidines from 2-amino-4-phenyl-4*H*-chromen-3-carbonitrile derivatives and acetic anhydride

Quintela et al. (1995) used arylidenemalonitriles to form 2-amino-4-phenyl-4*H*-chromen-3-carbonitriles with ethyl acetoacetate and then convert these products with a phosgeneiminium salt in 1,2-dichloroethane under reflux for 1 hour followed by passing through a stream of dry hydrogen chloride through the mixture to produce the pyranopyrimidines in yields of 80-95% (**Figure 1-11**).

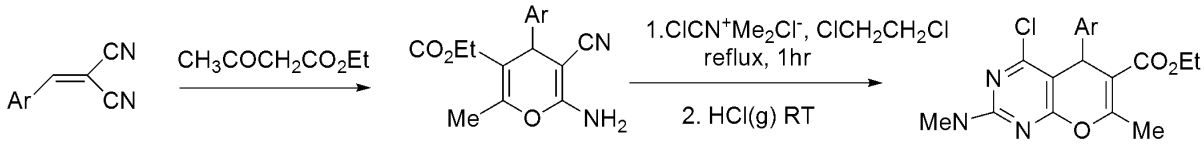

Figure 1-11 Conversion of 2-amino-4-phenyl-4*H*-chromen-3-carbonitriles with a phosgeneiminium salt and dry HCl

Abd El-Wahab et al. (2012) first formed 2-amino-3-cyano-4-arylnaphthopyrans from arylidene malonitriles and 6-methoxy-2-naphthol to produce 2-amino-3-cyano-4-arylnaphthopyran derivatives, which were then converted to three different pyranopyrimidine derivatives using either acetic anyhdride as above, benzoyl chloride or formamide (**Figure 1-12**).

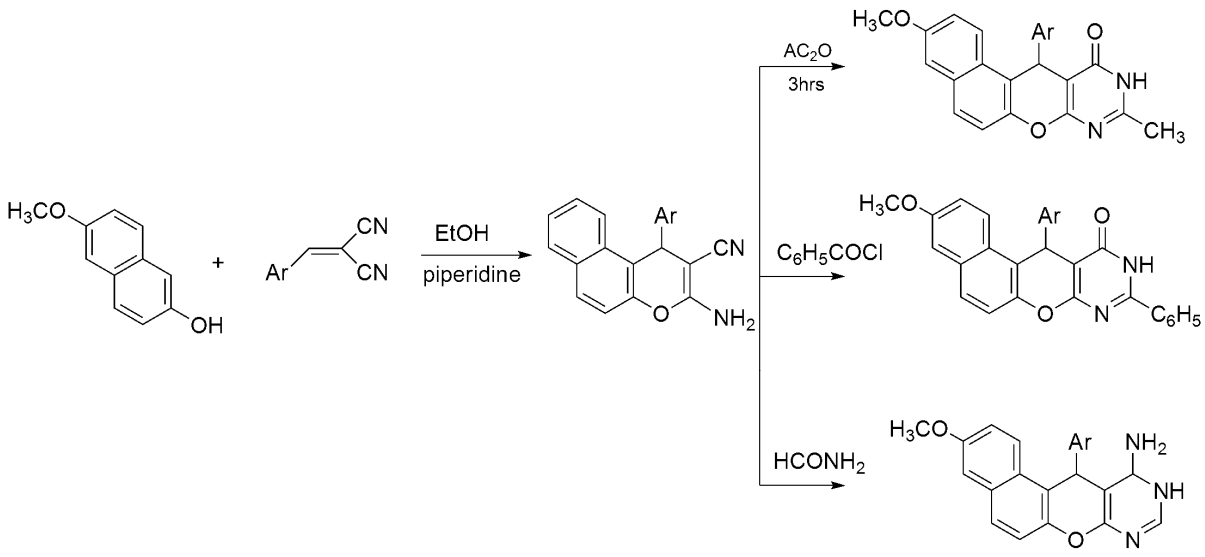

Figure 1-12 Synthesis of three different pyranopyrimidines from 2-amino-3-cyano-4-arylnaphtho-4H-pyrans using various reagents

The 2-amino-4-aryl-3-cyano-4*H*-pyran intermediates were also converted to pyranopyrimidines using *N*,*N*-dimethylacetaldehyde dimethyl acetal and aromatic amines in the presence of an imidazole ionic liquid to produce pyranopyrimidines in 87-94% yield in 2-4 hours at 80 ºC (**Figure 1-13**) (Suresh et al., 2015).

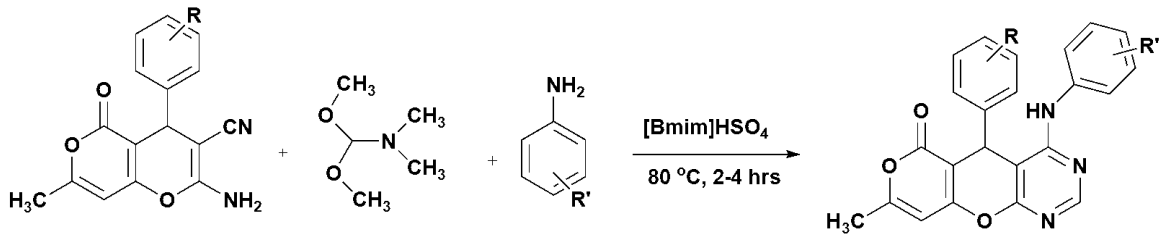

Figure 1-13 Synthesis of pyranopyrimidines using *N*,*N*-dimethylacetaldehyde dimethyl acetal, aromatic amines in an ionic liquid

When the 2-amino-4-phenyl-4*H*-chromen-3-carbonitrile derivatives were first treated with triethyl orthoformate, they converted the primary amino group to an ethoxymethyleneimino group, which was subsequently cyclised to imino pyranopyrimidines in 76% yield with hydrazine hydrate in ethanol in 45 minutes (**Figure 1-14**) (El-Agrody et al., 2001). A similar

reaction was used to synthesise pyrazolopyranopyrimidines from pyrazolo 4-aryl-3-cyano-2-ethoxymethyleneimino-4*H*-pyran derivatives (El-Assiery et al., 2004).

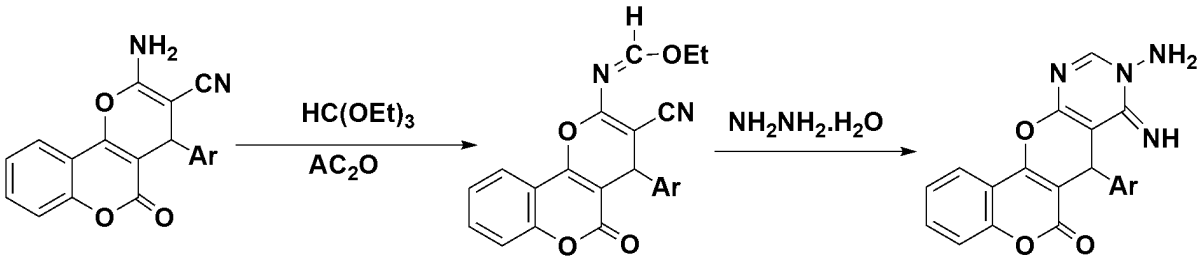

Figure 1-14 Synthesis of pyranopyrimidines from 2-ethoxymethyleneimino-4-phenyl-4*H*-chromen-3-carbonitrile derivatives

β-aroylacrylic acids were first converted to 4-carboxyl-2-ethoxymethyleneimino-4*H*-chromene-3-carbonitrile derivatives using either malonitrile and ammonium acetate or malonitrile and piperidine. This intermediate was then converted to various pyranopyrimidines using sodium hydrogen sulphide, hydrazine, phenylhydrazine, ammonia and alkyl and aryl amines (**Figure 1-15**) (El-Hashash et al., 2009).

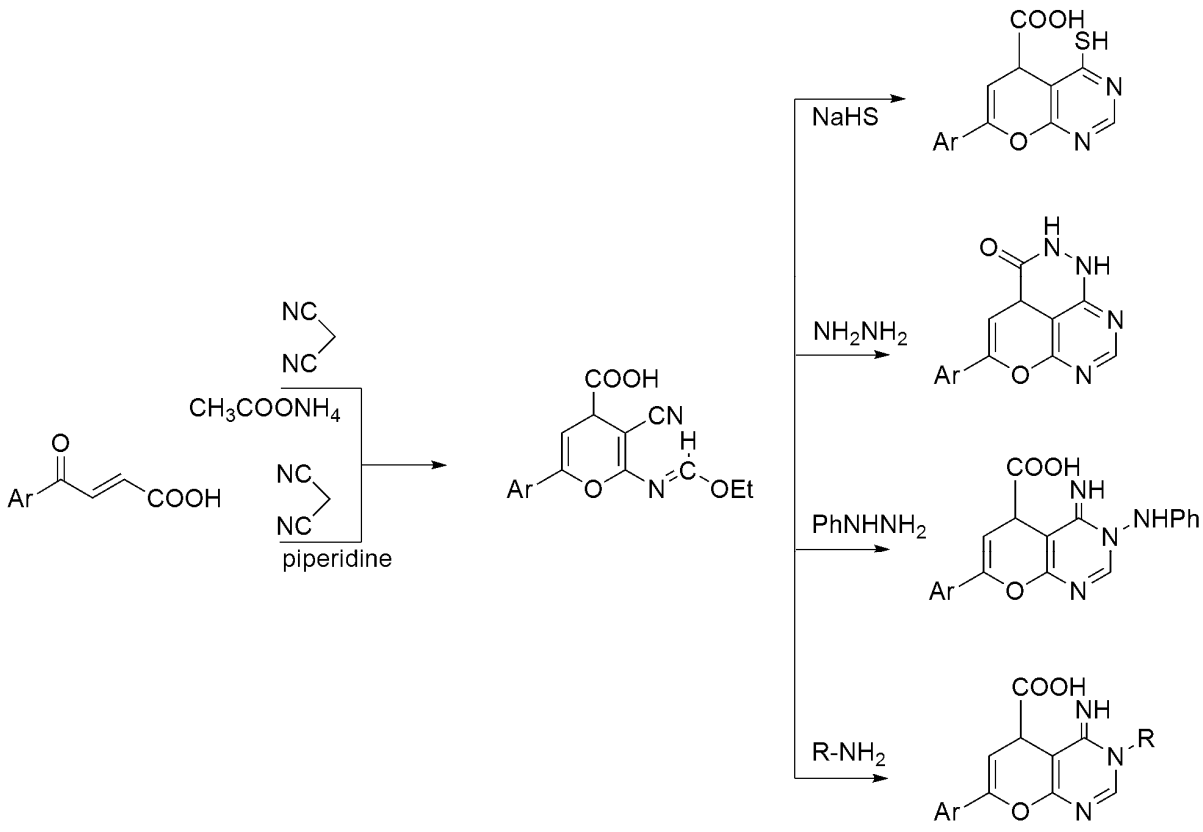

Figure 1-15 The synthesis of pyranopyrimidines from 4-carboxyl-2-ethoxymethyleneimino-4*H*-chromene-3-carbonitrile derivatives using various reagents

Chalcones and barbituric acids were shown to produce pyranopyrimidines in the presence of phosphorus pentoxide and acetic acid under reflux at 135-140 ºC for 6-8.5 hours in yields of 52-86% (**Figure 1-16**) (Rahman et al., 2013).

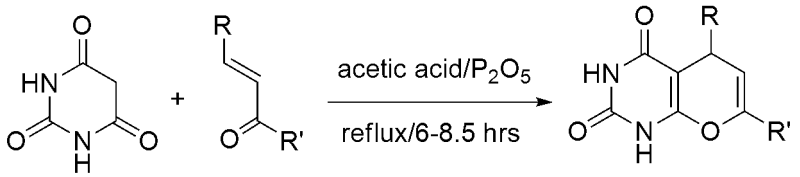

Figure 1-16 Synthesis of pyranopyrimidines from chalcones and barbituric acid

Yousefi et al. (2015) used curcumin instead of malonitrile together with barbituric acid and aldehdyes to form pyranopyrimidine derivatives in yields of between 80-87% using a para-toluene sulphonic acid catalyst (PTSA) under reflux in ethanol for 8 hours (**Figure 1-17**).

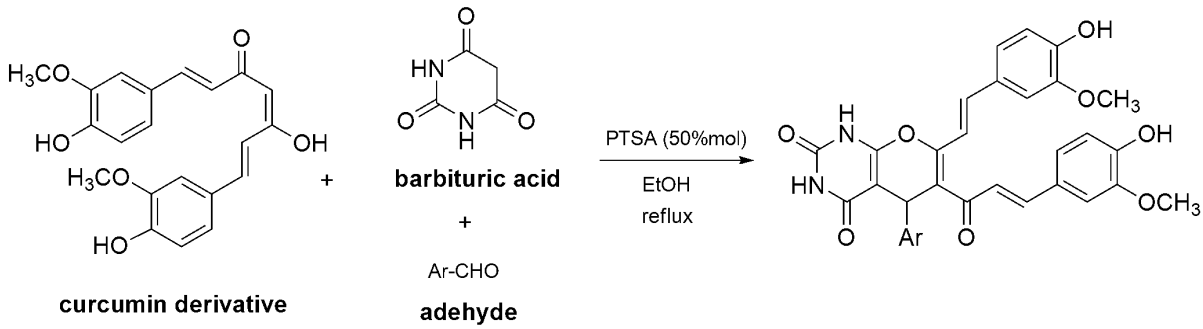

Figure 1-17 Synthesis of curcumin derivatives of pyranopyrimidine

Tetrahydro-2*H*-pyran-2-one was used instead of barbituric acid with different nitriles in the presence of trifluoromethanesulfonic anhydride to produce 2,4 disubstituted 6,7-dihydro-5H-pyrano[2,3-d]pyrimidines (**Figure 1-18**) (Herrera et al., 2006). The reagents were cooled to -78 ºC, added and stirred at º0 C for 4 days to produce yields of 40-90%.

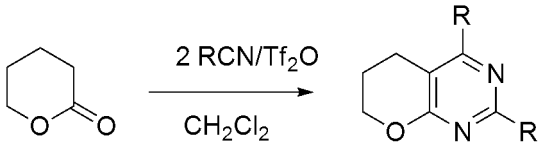

Figure 1-18 Synthesis of pyranopyrimidines from tetrahydro-2*H*-pyran-2-one derivatives and acetonitriles

A mixture of 5-ethoxycarbonyl-2H-pyran-2-one, substituted amidine hydrochlorides and DBU (as a base) in ethanol produced the corresponding pyranopyrimidine under microwave irradiation in 120 minutes at 110 ºC in yields of 56% (Hren et al., 2009). Under the same conditions, but with sodium carbonate as a base, the didehydro amino acid derivatives could be prepared, which could be converted to the corresponding pyranopyrimidines in 92% yield.

Thus, the two step reaction resulted in better yields (78% overall) than the 56% yield in the one step reaction (**Figure 1-19**) (Hren et al., 2009).

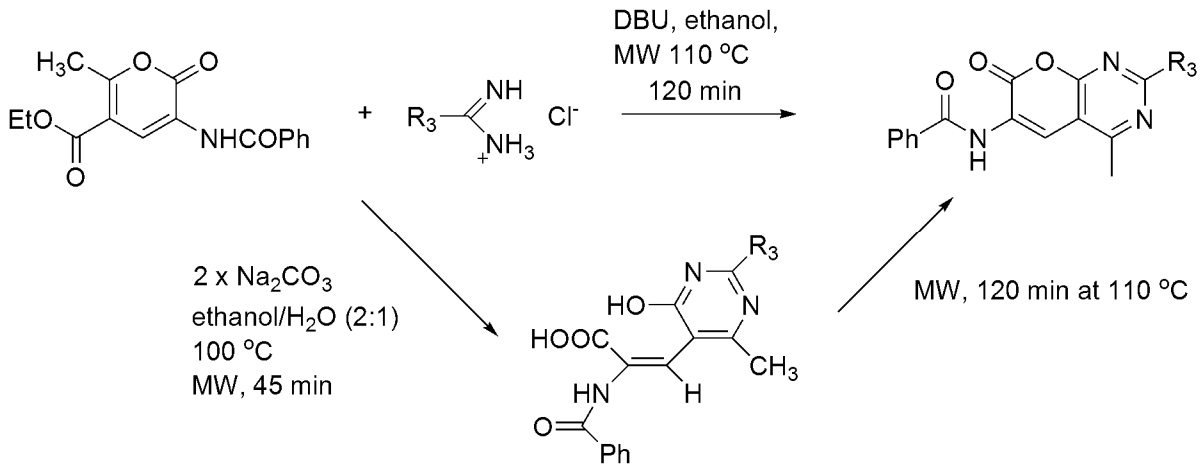

Figure 1-19 Synthesis of pyranopyrimidine from 5-ethoxycarbonyl-2*H*-pyran-2-one under microwave conditions

Pyranopyrimidines were also formed in yields of 56-63% by the reaction of 5-formyl-6-hydroxyuracils and Meldrum's acid with a catalytic amount of piperidine in ethanol under reflux for 20 hours (**Figure 1-20**) (Deb and Bhuyan, 2006).

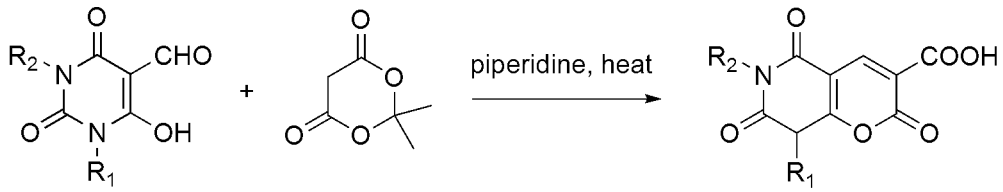

Figure 1-20 Synthesis of pyranopyrimidines from 5-formyl-6-hydroxyuracils and Meldrum's acid

The same starting material (5-formyl-6-hydroxyuracil) also formed pyranopyrimidines with maleimide or phenylisocyanate under microwave conditions at 120 ºC for 5 minutes, resulting in yields of 80-90% (**Figure 1-21**) (Devi et al., 2004).

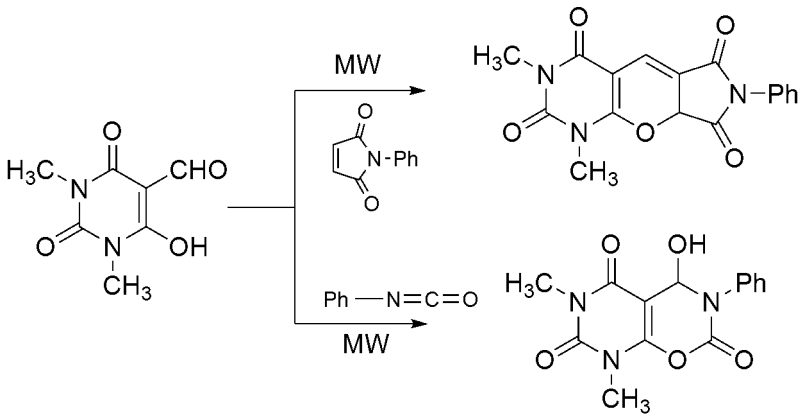

Figure 1-21 Pyranopyrimidines from 5-formyl-6-hydroxyuracil and either maleimide or phenylisocyanate

Kidwai et al. (2000) reported the synthesis of functionalized pyrano[2,3-*d*]pyrimidines from 1,3-diarylthiobarbituric acids, hippuric acid and triethylorthoacetate on basic alumina in ethanol under microwave conditions for 1 minute, which yielded the products in yields of 92-95% (**Figure 1-22**).

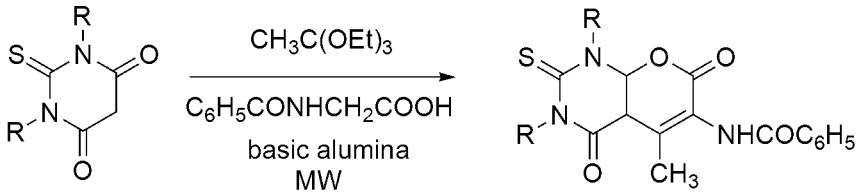

Figure 1-22 Pyranopyrimidines synthesised from thiobarbituric acid, hippuric acid and triethylorthoacetate

1.2 5,5'-(Phenylmethylene)bis(6-amino-2-thiouracil) derivatives

5,5'-(Phenylmethylene)bis(6-amino-2-thiouracil) consists of two 6-amino-2-thiouracil units, a phenyl unit and a hydrogen atom all attached to a central carbon atom (**Figure 1-23**).

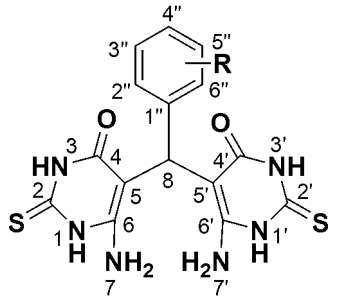

Figure 1-23 The basic structure of 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) derivatives

The first reported synthesis of 6-amino-2-thiouracil with formaldehyde was reported by Pfleiderer et al. (1966) (**Figure 1-24**). The mechanism for this reaction is discussed in detail in Chapter 4.

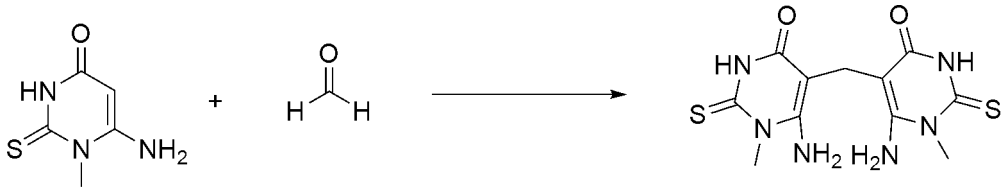

Figure 1-24 The first reported reaction of 6-amino-1-methyl-2-thiouracil

Chebanov et al. (2005) prepared the methyl, chloro and fluoro phenyl derivatives of 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) derivatives whilst trying to find alternate methods to form pyrido[2,3-*d*]pyrimidine derivatives. Instead of reacting 6-amino-2-thiouracil with chalcones, he reacted them with chalcone precursors of acetophenone and benzaldehyde. To their surprise, the reaction proceeded without the participation of acetophenone and formed 5,5'-(phenylmethylene)bis(6-amino-2-thiouracils) (**Figure 1-25**).

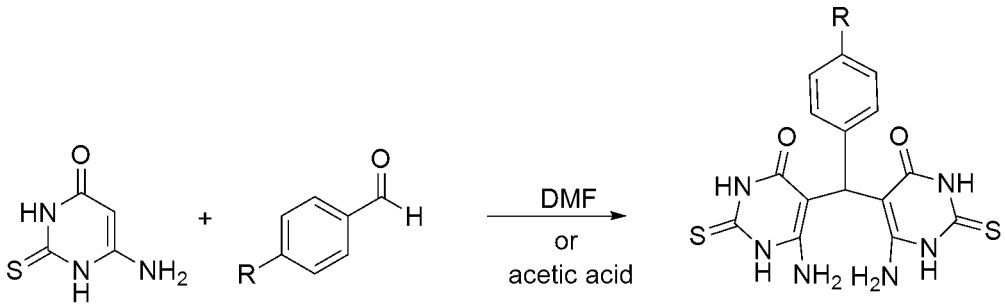

Figure 1-25 Reaction of 6-amino-2-thiouracils with *para* substituted benzaldehydes

6-Amino-1-methyl-2-thiouracil reacted with aromatic aldehydes in absolute ethanol in the presence of HCl at room temperature for one and a half hours or glacial acetic acid under reflux for 1 hour to produce 5,5'-(phenylmethylene)bis(6-amino-1-methyl-2-thiouracil) derivatives (**Figure 1-26**) (El-kalyoubi et al., 2015). The duration of the reflux is critical as they achieved pyrido[2,3-*d*]pyrimidines upon refluxing for 4-6 hours. Consequently, refluxing the bis compounds for 1 hour in an equal mixture of acetic acid and HCl also resulted in pyrido[2,3-*d*]pyrimidines.

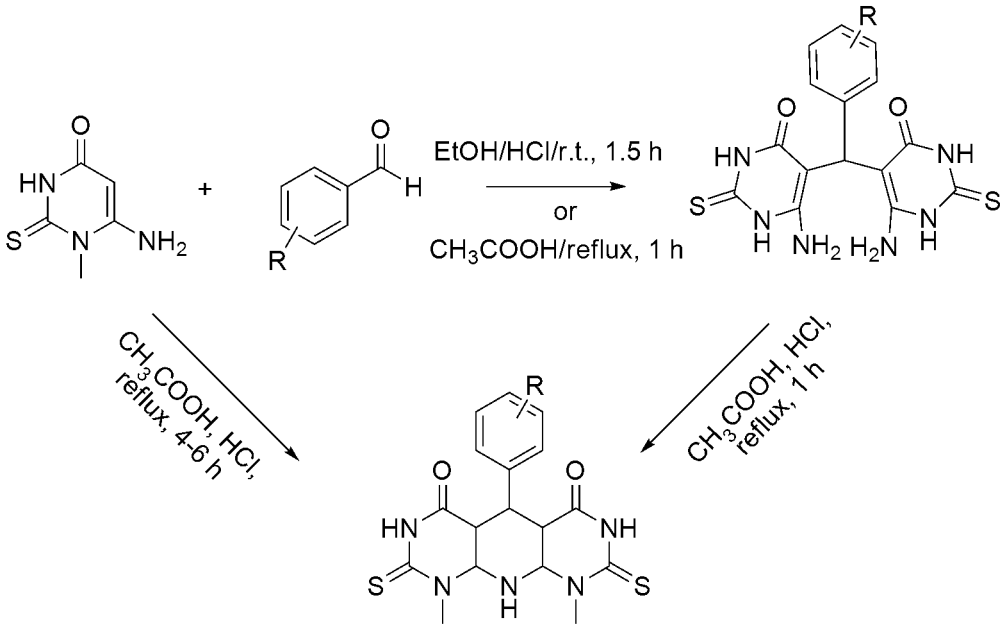

Figure 1-26 Formation of 5,5'-(phenylmethylene)bis(6-amino-1-methyl-2-thiouracil) with acetic acid

Youssif et al. (2003) reported a similar reaction with 6-amino-2-thiouracil and formalin in ethanol under reflux for 4 hours forming the bis products. Upon heating these bis products under reflux with acetic acid and a catalytic amount of HCl, pyridodipyrimidines were formed (**Figure 1-27**). In 2008, Youssif and Mohamed repeated the reaction, but with substituted benzaldehydes, where the bis (6-amino-2-thiouracils) were formed in 1.5-2.5 hours at room temperature, but the pyridopyrimidines were formed in 2-7 hours under reflux (Youssif and Mohamed, 2008).

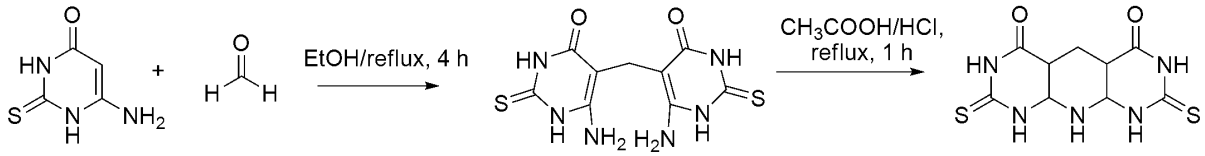

Figure 1-27 Synthesis of methylene bis(6-amino-2-thiouracil) and its conversion to pyridopyrimidines

To the best of our knowledge, 5,5'-(phenylmethylene)bis(6-amino-2-thiouracils) have not been extensively tested for their bioactivity. El-Khayoubi et al. (2015), tested several derivatives for anitviral and antibacterial activity. Only the *para* fluoro derivative (**Figure 1-28**) showed broad spectrum antibacterial activity. None of the other compounds tested showed appreciable antibacterial activity. They also showed poor antiviral activity in the same study. Youssif et al. (2008) also reported poor antimicrobial activity of the 5,5'-(phenylmethylene)bis(6-amino-2-thiouracils) they synthesised.

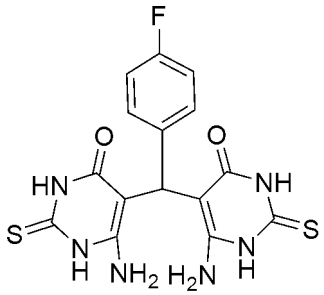

Figure 1-28 5,'5-(4-phenylmethylene)bis(6-amino-2-thiouracil) showing broad spectrum antibacterial activity

Although not much biological studies were carried out on 5,5'-(phenylmethylene)bis(6-amino-2-thiouracils), there have been biological studies on other 6-amino-2-thiouracil derivatives.

S-benzylated analogues of pyrimidine-4-ones, structurally similar to the 6-amino-2-thiouracils (**Figure 1-29**) (El-Moghazy et al., 2011), the pyrrolopyrido 2-thiouracil derivative (**Figure 1-30**) (Gomha et al., 2014) and some benzenesulfonamide derivatives of 6-amino-2-thiouracil (**Figure 1-31**) (Awad et al., 2015) have shown promising anticancer activity.

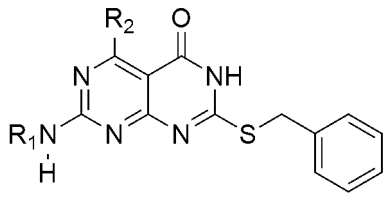

Figure 1-29 *S*-benzylated analogues of 6-amino-2-thiouracils with promising anticancer activity

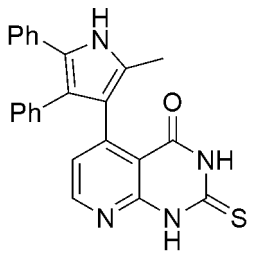

Figure 1-30 A pyrrolopyrido 2-thiouracil derivative with anticancer activity

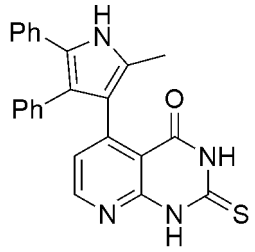

Figure 1-31 Some benzenesulfonamide derivatives of 6-amino-2-thiouracil with promising anticancer activity

1.3 Hypothesis

It is hypothesized that since pyrimidine derivatives have found application in a wide variety of pharmaceutical applications, including anticancer and antibacterial activity, novel compounds with similar backbones and scaffolds may have enhanced chemotherapeutic applications.

1.4 Aims

The main aim of this project is to synthesise two small libraries of pyrimidine molecules and test them for their anticancer and antibacterial activity in order to identify lead compounds, which could be developed further into anticancer and antibacterial agents.

Specific aims

1. To synthesise two classes of pyrimidine derivatives, which vary at one point in their scaffold.
2. To characterize the synthesised compounds using NMR and Mass spectroscopy to verify that these compounds had indeed been formed.
3. To test the synthesised compounds for their anticancer activity.
4. To test the synthesised compounds for their antibacterial activity.

1.5 References

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Chapter 2. Synthesis, characterization, anticancer and antibacterial activity of some novel pyrano[2,3-*d*]pyrimidinone carbonitrile derivatives

* The compounds referred to in this chapter are referred to in the abstract, conclusion and appendices or elsewhere in the thesis with an **A** preceding the number of the compound. For example, **1** and **2** are referred to as **A1** and **A2** elsewhere in the thesis.

ABSTRACT

A series of oxygenated substituted 7-amino-5-aryl-6-cyano-5*H*-pyrano pyrimidin-2,4-dione derivatives were synthesized in a one-pot reaction by reacting malononitrile and barbituric acid with several aromatic aldehydes in the presence of 1,4-diazabicyclo[2.2.2]octane (DABCO) in aqueous medium to produce the title compounds. The 3,4-dihydroxyaryl (**3**) and the 2,5-dimethoxy (**7**) derivatives were novel. The structures of the synthesized compounds were elucidated by means of ^{1}H, ^{13}C and 2D NMR spectroscopy. Compounds **3** (3',4'-dihydroxy derivative), **5** (4'-methoxy derivative) and **6** (2',3'-dimethoxy derivative) showed antibacterial activity comparable to or better than the standard ampicillin. Compound **6** is an excellent candidate for a broad spectrum antibiotic with MBCs up to five times better (45.6-365.2 μM) than ampicillin, while both **3** and **6** have the potential to be developed into an antibiotic against MRSA, with MBCs of 183-199 μM, five times better than ampicillin. All the test compounds **1-7** showed good anticancer activity. The IC_{50} values ranged from 3.46 to 37.13 μM (HeLa); 136.78 to 297.05 μM (Caco-2) and 137.84 to 333.81 μM (HEK293). The best activity was seen in the HeLa cell line when compared to the standard 5FU (5-Fluorouracil IC_{50} of 41.85 μM), with **1**, **2**, **5** and **7** having IC_{50} values of 10.64, 3.46, 4.36 and 4.44 μM respectively. Additionally, two representative compounds (**1** and **7**) found to be potent against the two cell lines (HeLa and HEK 293) were docked into the binding site of human kinesin Eg5 with the aim of predicting their binding propensities and to

establish their mechanism of action. This one pot reaction is carried out in aqueous ethanol and therefore the procedure is considered green. The reaction occurs under mild conditions producing high yields of products.

Keywords: Pyrano pyrimidinedione, malononitrile, barbituric acid, DABCO, MRSA.

2.1 Introduction

The heterocyclic 7-amino-5-aryl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-diones are annelated pyrimidine derivatives, containing pyrimidine, pyrano and aryl functionalities within a single molecule. Pyrimidines have widespread pharmacological activity such as anticancer (Babu et al., 2004), antiviral (Chern et al., 2004), antimalarial (Joshi et al., 2005) and antibacterial properties (Mishra et al., 2008) and therefore synthesis of molecules containing pyrimidine in its core structure is always of interest.

The 7-amino-5-aryl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-diones have been previously synthesised under microwave conditions without a catalyst from arylidene malonitrile and barbituric acid (3-5 minutes) (Gao et al., 2004) and from benzaldehydes, alkyl nitriles and barbituric acid (4-8 minutes) (Devi et al., 2003). Various catalysts such as diammonium hydrogen phosphate (Balalaie et al., 2008), Zn[(L) proline]$_2$ (Heravi et al., 2010) and a nanoporous SBA-Pr-SO_3H nanocatalyst (Ziarini et al., 2013) were also used to synthesise these compounds from benzaldehydes, malonitrile and barbituric acid in good yields. The reaction was also carried out with arylidene malonitrile and barbituric acid dissolved in ionic liquids (Yu and Wang, 2005). Triethylamine was used to catalyse the reaction of 2-thioxodihydropyrimidin-4,6(1*H*,5*H*)-dione (instead of barbituric acid) with benzylidene malonitrile to synthesise 2-thioxo derivatives of these compounds (Aly et al.,

2012). Other potential catalysts for this reaction are salts of Li, which although not used to synthesise these molecules, have been used to catalyse the synthesis of chromene derivatives from benzaldehydes, nitriles and a cyclic 1,3-dicarbonyl compound (Sun et al., 2010).

In this work, we have used DABCO (1,4-diazabicyclo[2.2.2]octane), reported to be a mild and efficient catalytic system for the synthesis of pyrano[2,3-*d*]pyrimidinones in good yields (Jain et al., 2014; Bhat et al., 2014) to synthesise a small library of 7-amino-5-aryl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-diones and test them for their antibacterial and anticancer activity.

2.2 Results and Discussion

Chemistry

The title compounds were synthesized in a one pot reaction from substituted aromatic benzaldehydes (ArCHO), malononitrile, barbituric acid and DABCO (10% mol ratio) in aqueous ethanol (**Figure 2-1**) to produce 7-amino-5-aryl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-diones (**1-7**) in yields of between 90-96%. The structures of the products were confirmed from their ^{1}H and ^{13}C NMR spectra (**Table 2-1** and **Table 2-2**) and the resonances assigned with the aid of 2D HSQC and HMBC spectra. We have chosen oxygenated derivatives such as hydroxy and methoxy derivatives to investigate the effect these moieties have on the pyrano[2,3-*d*]pyrimidinones in anticancer and antibacterial assays.

The reaction occurs via proton removal from the methylene group of malonitrile, which subsequently forms an arylidene derivative with substituted benzaldehydes via a Knoevenagel condensation. This arylidene derivative then undergoes a Michael reaction with

barbituric acid and subsequent cyclisation results in the pyrano[2,3-*d*]pyrimidinones (Kaupp et al., 2003).

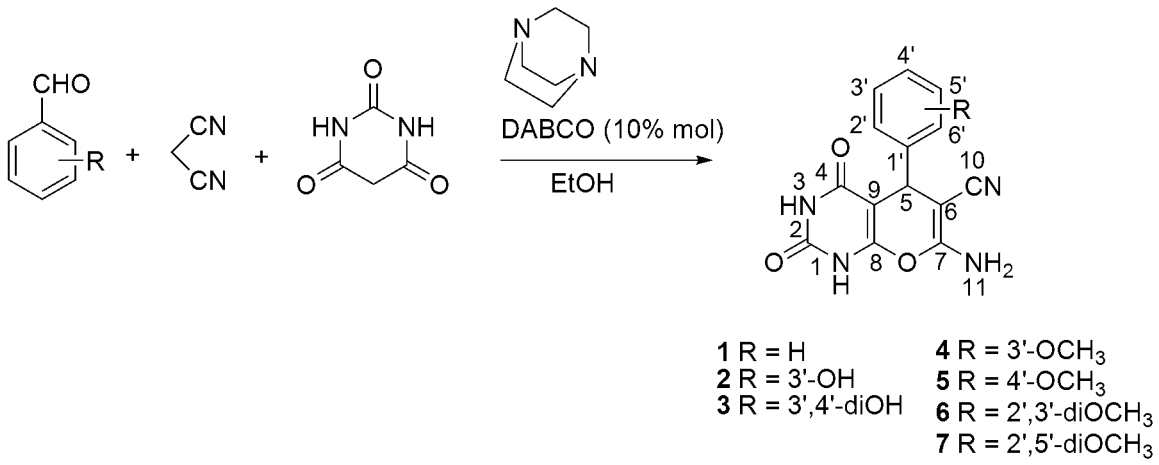


Figure 2-1 DABCO catalyzed reaction of pyrano pyrimidine derivatives (**1-7**)

Table 2-1 ^{1}H NMR data of 7-amino-5-aryl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-diones in DMSO-d_6 (400 MHz)

Pos.	**1**	**2**	**3**	**4**	**5**	**6**	**7**
5	4.26 s	4.11 s	4.01 s	4.20 s	4.16 s	4.47 s	4.42 s
NH-1	12.07br s	12.04 br s	11.94 s	12.04 s	12.00 br s	11.97 br s	11.98 br s
NH-3	11.08 s	11.05 s	11.04 s	11.05 s	11.02 s	10.97 s	10.99 s
NH_2-11	7.14 s	7.03-7.07	7.01 s	7.08 s	7.04 s	6.98 s	6.95 s
2'	7.18-7.22 m	6.54-6.61 m	6.55 d (2.0)	6.72-6.76 m	7.10 d (8.6)	-	-
3'	7.29-7.32 m	-	-	-	6.83 d (8.6)	-	6.89 d (8.9)
4'	7.18-7.22 m	6.54-6.61 m	-	6.72-6.76 m	-	6.87 d (7.6)	6.75 dd (8.9,3.1)
5'	7.29-7.32 m	7.03-7.07 m	6.62 d (8.1)	7.20 dd (7.9, 7.9)	6.83 d (8.6)	6.94 dd (7.6, 7.6)	-
6'	7.18-7.22 m	6.54-6.61 m	6.45 dd (8.1, 2.0)	6.72-6.76 m	7.10 d (8.6)	6.65 dd (7.6, 1.2)	6.59 d (3.1)
OH		9.30 s	8.69				
OH			8.81				
OCH_3				3.72 s	3.72 s	3.77 s (3'-OCH_3)	3.68 s (2'-OCH_3)
OCH_3						3.72 s (2'-OCH_3)	3.66 s (5'-OCH_3)

Table 2-2 ^{13}C NMR data of 7-amino-5-aryl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-diones in DMSO-d_6 (100 MHz)

Pos.	1	2	3	4	5	6	7
2	149.4	149.5	149.6	149.8	149.4	149.5	149.5
4	162.4	162.4	162.4	162.4	162.4	162.3	162.4
5	35.6	35.5	34.8	35.6	34.8	30.7	31.1
6	58.7	58.9	59.6	58.6	59.1	58.3	57.7
7	157.7	157.6	157.6	157.6	157.5	157.9	158.2
8	152.4	152.2	152.0	152.7	152.0	152.2	153.0
9	88.5	88.6	89.4	88.3	88.7	88.4	87.6
10	119.1	119.2	119.5	119.1	119.2	119.2	119.2
1'	144.2	145.6	135.2	145.9	136.2	136.8	132.8
2'	129.4	113.7	114.6	113.7	128.3	146.5	151.4
3'	128.2	157.2	145.1	159.2	113.6	152.4	112.9
4'	127.4	113.9	144.2	112.0	158.0	111.3	111.6
5'	128.2	129.2	115.4	129.2	113.6	123.5	152.7
6'	129.4	117.8	118.0	119.3	128.3	120.8	115.3
OCH_3				54.9	55.0	59.8 (2'-OCH_3)	55.2 (5'-OCH_3)
OCH_3						55.4 (3'-OCH_3)	56.4 (2'-OCH_3)

In order to identify the best catalyst for the reaction, five basic catalysts were used independently under reflux conditions at 80 ºC with equimolar quantities of benzaldehyde, malonitrile and barbituric acid in a mixture of 1:1 ethanol and water to identify the catalyst that produced the greatest yield in the shortest time. The catalysts investigated were L-proline, K_2CO_3, dibutylamine (DBA), triethylamine (Et_3N) and 1,4-diazabicyclo[2.2.2]octane (DABCO). Of these, DBA, Et_3N and DABCO resulted in yields of over 70% in approximately one hour (**Table 2-3**). DABCO performed the best, producing yields of 94% in 38 minutes.

Table 2-3 The effect of different catalysts under optimized reaction conditions[a]

Catalyst	Time (mins)	Yield %
No catalyst	1080 (18h)	5
L-Proline	180 (3h)	45
K_2CO_3	110	57
DBA[b]	54	74
Et_3N	70	81
DABCO[c]	38	94

[a]Reaction conditions: barbituric acid (1 mmol), benzaldehyde (1 mmol), malononitrile (1.1 mmol) and EtOH:water (1:1 v/v, 10 mL), R.T; [b]dibutylamine; [c]1,4-diazabicyclo[2.2.2]octane

The ^1H and ^{13}C NMR spectra were similar for all seven compounds and the only difference in the spectra were brought about by differences in the aryl moiety of the molecule substituted at C-5. Using compound **1** (the pyrimidine with the unsubstituted aryl ring) as an example, a characteristic H-5 resonance was seen as a singlet at δ 4.26, which was an indication that the pyrimidines had formed. Due to the lack of protonated carbons on the core pyrimidine skeleton, the only other proton indicators were the protons on the 1° and 2° nitrogen atoms. NH_2-11 occurred as a two proton singlet together with the aromatic protons at δ 7.14, NH-1 occurred as a singlet at δ 11.08 and NH-2 occurred as a broadened singlet at δ 12.07. The aromatic resonances occurred as a three-proton and two proton multiplet at δ 7.18-7.22 (H-2'/4'/6') and δ 7.29-7.32 (H-3'/5').

For **2** (3'-hydroxy derivative), H-2'/4'/6' occurred as a three-proton multiplet at δ 6.54-6.61 and H-5' overlapped at δ 7.03-7.07 with NH_2-11 as a multiplet. The 3',4'-dihydroxy derivative (**3**) showed the presence of two doublets and a double doublet for H-5', H-2' and H-6' at δ 6.62 (J = 8.1 Hz), δ 6.55 (J = 2.0 Hz) and δ 6.45 (J = 8.1, 2.0 Hz) respectively. In the 3'-methoxy derivative (**4**), the H-5' double doublet occurred as a triplet due to the same coupling constants at δ 7.20 (J = 7.9, 7.9 Hz) downfield from the other three aromatic proton resonances, which occurred at δ 6.72-6.76 as a multiplet. The 3'-OCH_3 resonance appeared as an intense singlet at δ 3.72. In compound **5**, the phenyl ring at C-5 was *para* disubstituted

with a methoxy group at C-4'. This methoxy group was seen at δ 3.72 as a singlet. A pair of doublets at δ 7.10 and δ 6.83, each with J = 8.6 Hz, were assigned to H-2'/6' and H-3'/5' respectively. These assignments were made due to H-3'/5' being more electron dense than H-2'/6' due to resonance electron donation from the methoxy group and was confirmed by an HMBC correlation from H-2'/6' to C-5.

In the 2',3'-dimethoxy derivative (**6**), H-5' partially overlaps with H-11, but can still be observed as a double doublet (appearing as triplet) at δ 6.95 (J = 7.6, 7.6 Hz) and H-4' and H-5' are observed as a doublet and double doublet at δ 6.87 (J = 7.6 Hz) and δ 6.65 (J = 7.6, 1.2 Hz) respectively. The 2'-methoxy resonance was assigned to δ 3.72 since it shows an HMBC correlation to C-2', which in turn was identified by an HMBC correlation to H-5. The 3'-methoxy group was present at δ 3.77. In the 2',5'-dimethoxy derivative (**7**), H-3' and H-6' occurred as doublets at δ 6.89 (J = 8.9 Hz) and δ 6.59 (J = 3.1 Hz) respectively and H-4' occurred as a double doublet at δ 6.75 (J = 8.9, 3.1 Hz). The two methoxy group resonances were present at δ 3.68 (2'-OCH_3) and δ 3.66 (5'-OCH_3) and were identified in a similar manner to **6**. C-2' showed a HMBC correlation to both H-5 and the 2'-OCH_3 resonance.

The ^{13}C NMR spectrum of the 7-amino-5-aryl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-2,4-dione core has characteristic carbon resonances. There are five downfield carbon resonances at δ 162.4 (C-4), 157.7 (C-7), 152.4 (C-8), 149.4 (C-2) and 144.2 (C-1'), which consisted of the two carbonyl groups, the two olefinic C-O resonances and the fully substituted aromatic carbon (C-1') of the phenyl ring. Four other characteristic carbon resonances, consisting of the carbonitrile carbon (C-10, δ 119.1), the olefinic carbon on the pyrimidine ring (C-9, δ 88.5), the olefinic carbon attached to the nitrile group (C-6, δ 58.7) and the methine carbon

on the pyran ring (C-5, δ 35.6) resonated more upfield. The HMBC correlations of the H-5 proton resonance were instrumental in assigning the carbon resonances in the core structure (**Figure 2-2**). H-5 showed HMBC correlations to C-8, C-9 and C-4 of the pyrimidine ring, C-6 and C-7 of the pyran ring, C-1', C-2' and C-6' of the phenyl ring and the cyano carbon C-10. The C-7 carbon resonance at δ 157.7 was distinguished from C-4 and C-8 due to an HMBC correlation to the NH_2 proton resonance at δ 7.14. For the 2' substituted pyrimidinones **6** (2',3'-dimethoxy) and **7** (2',5'-dimethoxy), a HMBC correlation from H-5 to C-2' and then from C-2' to the 2'-OCH_3 proton resonance was used to assign the methoxy group to the 2'-position. The remaining resonance was then assigned to C-3' (in **6**) and C-5' (in **7**).

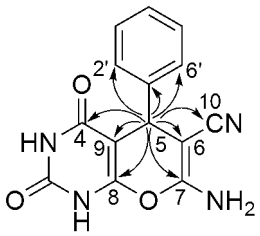


Figure 2-2 HMBC correlations from H-5 to the surrounding carbon atoms in **1**

Antibacterial activity

Compounds **3**, **5** and **6** showed promising antibacterial activity from preliminary disc diffusion assays and were selected to determine their MBC values using the broth microdilution method. They were tested against the Gram positive *Staphylococcus aureus* ATCC 25923 and methicillin resistant *Staphylococcus aureus* ATCC BAA-1683 (MRSA) as well as three Gram negative strains, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumonia* ATCC 31488. Compound **6** showed excellent activity showing fivefold better activity than ampicillin against MRSA (at 183 μM) and *E. coli* (91.3 μM) and 2.5 fold better activity than *K. pneumonia* (183 μM) (**Table 2-4**).

Compound **3** also showed excellent activity against MRSA at 199 μM and the activity of **5** was twofold higher than that of ampicillin (100 μM).

These results show that compound **6** is a potential broad spectrum antibiotic since it shows better activity than ampicillin and excellent activity against Gram negative bacteria. Compound **3** can also be considered a lead compound to be developed into an antibiotic against MRSA and **5** a potential antibiotic for *Staphylococcal* infections.

Table 2-4 Minimum bactericidal concentration (MBC in μM) of **3**, **5** and **6**

Bacterial strains	3	5	6	Amp[a]	Cip[b]
S. aureus	398	100	45.6	55.9	1.84
MRSA	199	801	183	894	7.36
E. coli	1591	801	91.3	447	1.84
P. aeruginosa	1591	400	365.2	1788	1.84
K. pneumoniae	796	801	183	447	1.84

[a]ampicillin; [b]ciprofloxacin

Anticancer activity

The *in vitro* cytotoxicity levels of compounds **1-7** in the three cell lines, viz. HeLa, Caco-2 and HEK293 are summarized in **Table 2-5**, which shows the IC_{50} values of the 7 compounds. These IC_{50} values were calculated from experiments carried out at concentrations ranging from 10-100 μg mL^{-1}. All results show that almost all compounds were active from the lowest concentration tested (10 μg mL^{-1}). The IC_{50} values ranged from 3.46 to 37.13 μM in the HeLa cells; 136.78 to 297.05 μM in the Caco-2 cells and 137.84 to 333.81 μM in the HEK293 cells. A typical dose dependent cytotoxicity was seen in the Caco-2 and HEK293 cells. The levels of cytotoxicity of all seven compounds were very similar to that of the standard 5FU, however in comparison to the standard drug 5FU (IC_{50} of 41.85 μM) in the HeLa cells, compounds **2**, **5** and **7** were ten times more active with IC_{50} values of 3.46, 4.36 and 4.44 μM respectively. There was no real trend between the position and nature of the

substituent on the aromatic ring in relation to the activity. Compound **2** has a *meta* hydroxy group, compound **5** has a *para* methoxy group and compound 7 has methoxy groups at the *ortho* and *meta* positions (2' and 5'). The activity of these compounds could be specific to these substituents at those particular positions on the aromatic rings as changing these positions (compound **6** vs compound **7** and compound **4** vs compound **5**) resulted in decreased activity.

Compound **1** was also quite active with an IC_{50} of 10.64 µM. This is considerably lower than that of the standard cancer drug (5FU), suggesting that these compounds are active at a much lower dose which indicates potential for future use as possible anti-cancer agents. However, this does not discount the other compounds (**3**, **4** and **6**) tested which also produced lower IC_{50} values. In the Caco-2 cells, although all 7 compounds had IC_{50} values higher than that of 5FU, they still produced significant cytotoxicity with compound **3** containing hydroxy groups at the *meta* and *para* positions, eliciting the most activity. In the case of the normal cell line HEK293, there was reduced cytotoxicity levels compared to that in the cancer cell lines suggesting cell specific cytotoxicity. The methoxy compounds **5** (4'-OCH_3) and **6** (2',3'-diOCH_3) least affected the HEK293 cells and should be more closely examined in future studies. Further optimization and testing of all compounds are hence warranted.

Table 2-5 *In vitro* cytotoxic effect of synthesized compounds **1-7** (IC_{50} µM)

No	HeLa	Caco-2	HEK293
1	10.64±0.02^{b}	166.28±0.03^{b}	193.97±0.02^{c}
2	3.46±0.01^{a}	287.42±0.03^{g}	243.59±0.02^{d}
3	26. 02±0.0^{d}	136.78±0.02^{a}	177.42±0.02^{b}
4	20.35±0.07^{c}	175.13±0.02^{d}	184.23±0.02^{b}
5	4.36±0.07^{a}	185.45±0.01^{e}	333.81±0.01^{e}
6	37.13±0.05^{e}	297.05±0.01^{h}	293.80±0.02^{e}
7	4.44±0.05^{a}	235.00±0.02^{f}	137.84±0.02^{a}
5FU*	41.85±0.06^{f}	170.77±0.2^{c}	505.15±0.02^{f}

*5-Fluorouracil

Data are presented as the mean ± SD values of triplicate determinations. $^{a-f}$ Different superscript letters for a given value within a column are significantly different from each other (Tukey's-HSD multiple range *post hoc* test, $p < 0.05$), ND, Not determined.

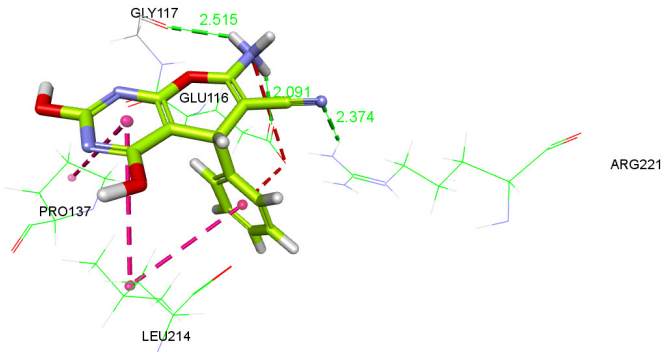
Computational results

Human kinesin, Eg5 is an important target protein for the design of new anti-cancer agents owing to its overexpression in various cancers such as breast, uterus, ovary and lung cancer (Garcia-Saez et al., 2007). Several pyrimidine scaffolds inhibiting Eg5 are reported in the literature (Yan et al., 2004). This motivated us to assess the tendency of our synthesized compounds to inhibit this enzyme with the aim of establishing the mechanism for their anti-cancer activity observed under *in vitro* conditions. First, the X-ray structure of Eg5 co-crystallized with the native ligand (KZ91367) was downloaded from the protein data bank database, and processed according to the procedure set out in the experimental. Two compounds (**1** and **7**), potent against both HeLa and HEK 295 cancer cell lines, were docked along with the reference drug (5-FU) into the binding site of Eg5, using the CDocker algorithm (Wu et al., 2003) embedded in Discovery Studio (DS).

The computed binding energy (BE) data suggested good binding affinity of the compounds with Eg5. Both the synthesized molecules, **1** (BE= -180.25 kcal mol^{-1}) and **7** (BE = -140.9 kcal mol^{-1}) were found to be stronger inhibitors of Eg5 than 5-FU, supporting a good correlation between the binding affinity and anti-cancer activity, as both compounds were found to be more potent than 5-FU experimentally. Compound **1** (**Figure 2-3a**) formed three hydrogen bonds; two concurrent with Glu116 (2.09Å) and Gly117 (2.51Å) through its protonated $–NH_2$ (proton donor) group and one with Arg221 (2.37Å) *via* its –CN (proton acceptor) functionality. Additionally, an electrostatic interaction (with Glu116) and hydrophobic forces (with Pro137 and Leu214) were also observed. Compound **7** (**Figure 2-3b**), displayed predominantly hydrophobic interactions with Eg5 residues (Glu116, Pro137, Leu214, Tyr211 and Arg119), although a hydrogen bond with Glu116 (2.49Å), a non-

conventional hydrogen bond with Glu118 (2.88Å) and an electrostatic force with Glu116 were also present.

(a)



(b)

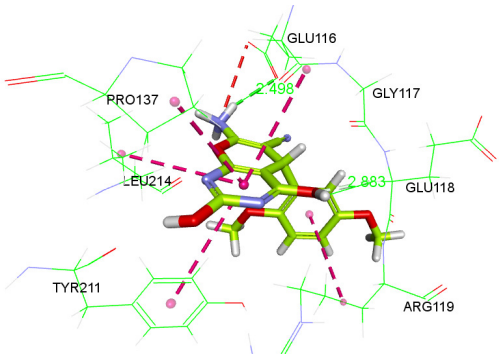


Figure 2-3 Docked poses of **1** (a) and **7** (b) in the binding site of Eg5. Both ligands are shown in lemon sticks whereas the interacting amino acids of Eg5 are depicted in green lines. Hydrogen bonds (conventional) as green, non-conventional hydrogen bonds as grey, hydrophobic as magenta and electrostatic interactions are shown as red dotted lines.

The reference drug, 5-FU (**Figure 2-4**) displayed only few non-conventional hydrogen bond interactions with Glu118 (3.04Å), Gly117 (2.81Å) and Ala133 (2.54Å), and a hydrophobic force with Pro137 of Eg5. The presence of weaker hydrogen bonds and absence of other non-bonded forces (hydrophobic and electrostatic) probably account for the lower binding energy of 5-FU in comparison to the other two compounds (**1** and **7**). Overall, the docking results suggest that the pyrimidine ring and $–NH_2$ are very significant components of the synthesized

compounds and play very important roles in locking their structures in the binding site of Eg5.

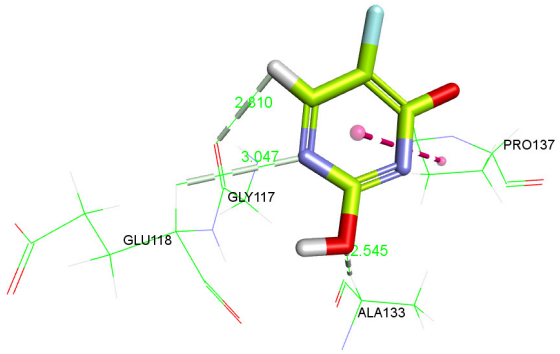


Figure 2-4 Docked pose of 5-FU (in lemon sticks) in the binding site of Eg5. The interacting amino acids of Eg5 are depicted in green lines. Non-conventional hydrogen bonds and hydrophobic interactions are shown as grey and magenta dotted lines.

Finally, both compounds (**1** and **7**) were subjected to the Lipinski rule of five to determine their potential of being an oral drug in humans (Ertl et al., 2000; Lipinski et al., 2012). This rule states that for any compound to become an orally active drug the following criteria needs to be satisfied; molecular weight (MW) less than 500 daltons, logP (an octanol-water partition coefficient) value less than 5, number of hydrogen bond donors (HBDs) less than 5 and number of hydrogen bond acceptors (HBAs) less than 10. The "Filter by Lipinski and Veber rule" algorithm in DS was used for these predictions. The computed parameters: MW, 283 (for **1**) and 343 (for **7**); AlogP, 1.2 (for **1**) and 1.16 (for **7**); HBDs, 5 (for both **1** and **7**) and HBAs, 7 (for **1**) and 9 (for **7**) were in agreement with the Lipinski rules, and suggested their potential of being orally active drugs. Finally, the polar surface area (PSA), a parameter that is very useful to assess the cell membrane permeability, of both compounds was computed using the same algorithm. Generally, a PSA value < 140 $Å^2$ indicates efficient membrane permeation (Ertl et al., 2000). The computed PSA values for **1** (PSA= 126.9) and **7** (PSA= 145.3) favored the drug likeness of the former over the latter.

2.3 Material and Methods

General Experimental Procedures

Reagents and chemicals used in this study were purchased from Sigma Aldrich via Capital Laboratories, South Africa, and were reagent grade. All organic solvents were redistilled and dried according to standard procedures. NMR spectra were recorded using a Bruker AvanceIII 400 MHz spectrometer at room temperature with chemical shifts (δ) recorded against the internal standard, tetramethylsilane (TMS). ^{1}H, ^{13}C and 2D NMR spectroscopy (HSQC and HMBC) were used for the structural elucidation of the synthesized compounds. IR spectra were recorded on a Perkin Elmer Spectrum 100 FTIR spectrometer with universal ATR sampling accessory. For GC-MS analyses, the samples were analysed on an Agilent GC-MSD apparatus equipped with a DB-5SIL MS (30m × 0.25mm) fused-silica capillary column. Helium (at 2 mL min^{-1}) was used as a carrier gas. The MS was operated in the EI mode at 70 eV. Optical rotation was recorded on a Perkin Elmer, Model 341 Polarimeter. Melting points were recorded on an Ernst Leitz Wetzlar micro hotstage melting point apparatus.

General Procedure for the synthesis of aromatic substituted pyrano[2,3-*d*]pyrimidiones (1-7)

Substituted aromatic benzaldehydes (ArCHO), malononitrile, barbituric acid (1.0 mmol each) and 10% DABCO were dissolved in 20 mL of aqueous ethanol and stirred for 1 hour. The progress of the reaction was monitored by TLC. The products were then filtered, recrystallized in ethanol and dried under vacuum to produce **1-7** in good yields of between 90-96%. Optical rotation of the synthesized compounds indicated that all the compounds are racemic mixtures.

7-amino-5-phenyl-6-cyano-5H-pyrano[2,3-d]pyrimidin-(1H,3H)-2,4-dione ***(1)*** white powder, yield 92%; mp 205-208 ºC; IR (KBr) υ_{max} (cm^{-1}): 3184 (NH_2), 2194 (CN), 1718 (C=O); EIMS (*m/z*, rel. int.) 282 [M^+] (38), 207 (62), 193 (25), 154 (58), 127 (100). Anal. calc. for CHN: C: 59.57, H: 3.57, N: 19.85, O: 0.17, Found: C: 59.17, H: 3.46, N: 19.20, O: 0.17.

7-amino-5-(3-hydroxyphenyl)-6-cyano-5H-pyrano[2,3-d]pyrimidin-(1H,3H)-2,4-dione ***(2)*** white powder (90%); mp 230-232 ºC; IR (KBr) υ_{max} (cm^{-1}): 3424 (NH_2), 3176 (NH), 2828 (CH), 2205 (CN), 1676 (C=O), 1459 (C=C); EIMS (*m/z*, rel. int.): 298 [M^+] (10), 281 (96), 208 (50), 207 (100), 193 (43), 191 (71); Anal. calc. for CHN: C: 56.38, H: 3.38, N: 18.78, O: 21.46, Found: C: 56.12, H: 3.01, N: 17.76, O: 20.98.

7-amino-5-(3,4-dihydroxyphenyl)-6-cyano-5H-pyrano[2,3-d]pyrimidin-(1H,3H)-2,4-dione ***(3)*** Light yellow powder (96%); mp 190-192 ºC; IR (KBr) υ_{max} (cm^{-1}): 3424 (NH_2), 3176 (NH), 2828 (CH), 2205 (CN), 1676 (C=O), 1459(C=C); EIMS (*m/z*, rel. int.): 314 [M^+] (38), 301 (21), 271 (28), 223 (27), 216 (53), 207 (100), 131 (92); Anal. calc. for CHN: C: 53.51, H: 3.21, N: 17.83, O: 25.46, Found: C: 53.31, H: 2.99, N: 17.64, O: 25.28.

7-amino-5-(3-methoxyphenyl)-6-cyano-5H-pyrano[2,3-d]pyrimidin-(1H,3H)-2,4-dione ***(4)*** White powder (90%); mp 285-288 ºC; IR (KBr) υ_{max} (cm^{-1}): 3392 (NH_2), 3172 (NH), 3049 (CH), 2193 (CN), 1676 (C=O), 1462 (C=C); EIMS (*m/z*, rel. int.): 312 [M^+] (34), 282 (25), 207 (100), 189 (27), 176 (19), 125 (42); Anal. calc. for CHN: C: 57.69, H: 3.87, N: 17.94, O: 20.94, Found: C: 57.45, H: 3.67, N: 17.39, O: 19.96.

7-amino-5-(4-methoxyphenyl)-6-cyano-5H-pyrano[2,3-d]pyrimidin-(1H,3H)-2,4-dione ***(5)*** Yellow powder (95%); mp 287-289 ºC; IR (KBr) υ_{max} (cm^{-1}): 3186 (NH_2), 3071 (NH), 2832 (CH), 2194 (CN), 1714 (C=O), 1439 (C=C); EIMS (*m/z*, rel. int.): 312 [M^+] (12), 294 (64),

281 (43), 207 (100), 191 (82); Anal. calc. for CHN: C: 57.69, H: 3.87, N: 17.94, O: 20.49, Found C: 56.99, H: 3.12, N: 17.52, O: 20.27.

7-amino-5-(2,3-dimethoxyphenyl)-6-cyano-5H-pyrano[2,3-d]pyrimidin-(1H,3H)-2,4-dione ***(6)*** White powder (94%); mp > 300 ºC; IR (KBr) υ_{max} (cm^{-1}): 3420 (NH_2), 3261 (NH), 3162 (CH), 2190 (CN), 1716 (C=O), 1479 (C=C); EIMS (*m/z*, rel. int.): 342 [M^+] (8), 327 (16), 281(16), 207 (100); Anal. calc. for CHN: C: 56.14, H:14.12, N: 16.37, O: 23.37, Found: C: 56.05, H: 13.88, N: 16.08, O: 23.15.

7-amino-5-(2,5-dimethoxyphenyl)-6-cyano-5H-pyrano[2,3-d]pyrimidin-(1H,3H)-2,4-diones ***(7)*** Light yellow powder (95%); mp > 300 ºC; IR (KBr) υ_{max} (cm^{-1}): 3284 (NH_2), 3171 (CH), 2198 (CN), 1706 (C=O), 1462 (C=C); EIMS (*m/z*, rel. int.) 342 [M^+] (8), 327 (8), 281 (16), 244 (100), 229 (72), 207 (52), 201 (71); Anal. calc. for CHN: C: 56.14, H: 4.12, N: 16.37, O: 23.37 Found: C: 56.07, H: 3.98, N: 16.07, O: 23.27.

Antimicrobial Susceptibility Testing

Antibacterial Assay

The antimicrobial activity of the synthesized compounds (**1-7**) were tested against the Gram positive *Staphylococcus aureus* ATCC 25923 and methicillin resistant *Staphylococcus aureus* ATCC BAA-1683 (MRSA), and three Gram negative strains, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumonia* ATCC 31488. The standard antibiotics Ciprofloxacin and Ampicillin were used as controls for comparison. Mueller Hilton agar (Biolab, South Africa) was prepared (38 g in 1 L of water), poured into sterile pre-labelled petri dishes and allowed to set and dry at room temperature. Bacterial cultures were standardized using a 0.5 McFarland standard turbidity and then swabbed onto agar plates. Paper discs were loaded with sample (20 µL) of the synthesised compounds and

placed onto the prepared agar plates, which were inverted and incubated at 35-37°C for 24 hours. The diameter of the zone of inhibition was measured in mm. Compounds showing an inhibition zone of > 9 mm were selected to determine their MBC values using the broth dilution assay with Ampicillin and Ciprofloxacin as the controls following the method in Andrews (2001). Compounds **3, 5** and **6** were chosen for the broth dilution method to determine their MBCs.

The compounds were dissolved in DMSO (10 mg mL^{-1}), serially diluted with Mueller-Hinton broth (Oxoid, England), inoculated with the respective bacterial cultures and incubated at 37ºC for 18 hours. This was performed in duplicate. Thereafter, 10 µL from each concentration was placed on Mueller-Hinton plates and incubated at 37ºC for 18 hours to determine the MBC. The MBC was the lowest concentration which showed no bacterial growth in the area in which the sample was placed.

Cytotoxicity tests by MTT assays

Human cervical cancer cells (HeLa) and human colon adenocarcinoma cells (Caco-2) were purchased from Highveld Biological (Pty) Ltd. (Lyndhurst, RSA). HEK293 cells (human embryonic kidney) were obtained from the Medical School, University of Witwatersrand (Gauteng, South Africa). All cells were grown to semi-confluency in 25 cm^2 tissue culture flasks in EMEM (Eagle's Minimum Essential Medium, Lonza BioWhittaker, Verviers, Belgium) supplemented with 10% fetal bovine serum and antibiotics (100 U mL^{-1} penicillin, 100 µg mL^{-1} streptomycin). For the MTT assay, cells were seeded at a density of 1.8 $x10^3$ cells per well in a 96 well plate containing 100 µL of medium. The cells were then incubated for 24 h at 37 ºC in 5% CO_2, after which the medium was removed and 100 µL fresh medium was added. Thereafter, compounds **1-7** at concentrations of 10, 25, 50 and 100 µg mL^{-1} was

added in triplicate to the cells and incubated for 48 h at 37 ºC. 5FU was used as a standard positive control.

The MTT assay was adapted from Mossman (1983). It measures the metabolic activity of cells by the reduction of MTT to formazan using the succinate-tetrazolium reductase system. After 48 h of incubation, the spent medium was replaced with 100 µL fresh medium and 100 µL of MTT (5 mg mL^{-1} in PBS), and cells were incubated for 4 h at 37 ºC. Thereafter, the medium and MTT were removed, and 200 µL of DMSO added to each well to dissolve the formazan salt. The absorbance of the resulting purple solution was read in a Mindray 96A microplate reader (Vacutec, Hamburg, Germany) at 570 nm (detection l) and 630 nm (reference l for nonspecific signals) wavelengths. Cell viability (%) was directly correlated to absorbance and calculated in comparison to the untreated control as follows:

$[(OD_{570}\text{Treated} - OD_{630}\text{Treated}) / (OD_{570}\text{Control} - OD_{630}\text{Control}) \times 100)]$

Tests were conducted in triplicate and calculation of the concentration at which 50% cell death (IC_{50}) was achieved by plotting the data on graphs using Microsoft Excel 2010™.

Docking Methodology

Three dimensional co-ordinates of human kinesin Eg5 were obtained from its X-ray structure (pdb id: 2x7c) co-crystallized with its native ligand (KZ91367) in the protein data bank database (www.rcsb.org). The duplicate chain and water molecules of the protein were removed, and protonation of different amino acids determined based on their pKa values using “Prepare Protein” algorithm in DS. Different isomers of the RCs and 5-FU were generated using the “Prepare ligands” module, and minimized. The isomer with the lowest CHARMm energy was selected for docking. A binding sphere covering the binding site residues of Eg5 was generated using the “Define and edit binding site” module. Docking of

all compounds was performed using the CDocker Algorithm (Wu et al., 2003), a CHARMm force field based program. The conformational space of each ligand, during docking, was explored using molecular dynamics method and refined by grid-based simulated annealing method. The top 10 poses were arranged according to the -CDocker energy, and the one with lowest energy was used for binding energy calculations.

2.4 Conclusion

In conclusion we have reported and described an eco-compatible synthesis of pyrano[2,3-*d*]pyrimidinone derivatives using a green and efficient catalyst, DABCO, which has the advantage of operational simplicity, mild reaction conditions and high yields of the final product. A structural elucidation including ^{1}H and ^{13}C NMR assignments are also made and will aid in identifying similar compounds synthesized. Compounds **3**, **5** and **6** showed promising antibacterial activity, with **6** showing excellent potential as a lead antibiotic. Both compounds **3** and **6** have the potential to be developed into an antibiotic against MRSA. Compounds **1**, **2**, **5** and **7** showed excellent activity against HeLa cells with **2**, **5** and **7** being approximately ten times more active than the standard 5FU. These compounds are therefore good lead compounds for anticancer agents. Additionally, the docking simulations suggested good binding affinity of the compounds with Eg5 and indicated their anti-cancer action, at least partially, through its inhibition. The predicted Lipinski descriptors also indicated the potential of these compounds to be an orally active drug.

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Chapter 3. Synthesis of chloro, fluoro and nitro derivatives of 7-amino-5-aryl-6-cyano-5*H*-pyrano pyrimidin-2,4-diones using organic catalysts and their antimicrobial and anticancer activities

* The compounds referred to in this chapter are referred to in the abstract, conclusion and appendices or elsewhere in the thesis with a **B** preceding the number of the compound, with the exception of compound **1**, which is the same as **A1**. Compounds **2** and **3** for example are referred to as **B2** and **B3** elsewhere in the thesis.

ABSTRACT

A range of substituted 7-amino-5-aryl-6-cyano-5*H*-pyrano pyrimidin-2,4-dione derivatives were synthesized in a one-pot reaction by reacting malononitrile and barbituric acid with aromatic aldehydes in the presence of 1,4-diazabicyclo[2,2,2]octane (DABCO) in aqueous medium. This is the first report of these compounds being synthesized with DABCO as a catalyst, which produced the compounds in yields in excess of 90%. The 2,4-difluoro (**11**) derivative was novel. The structures of the synthesized compounds were elucidated by means of ^{1}H, ^{13}C NMR and 2D NMR spectroscopy. Compound **2** (2-Cl derivative) had MBC values of < 200 µM against both *S. aureus* and MRSA and the 2-nitro derivative **5** had an MBC of 191 µM against the Gram –ve *E. coli*. The synthesized compounds were also tested for their anticancer activity against a HeLa cell line, where all the compounds showed better activity (IC_{50} values ranging from 129 to 340 µM) than 5-fluorouracil, a commonly known anticancer drug.

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Keywords: Pyrano pyrimidinone, malononitrile, barbituric acid, HeLa, anticancer.

3.1 Introduction

Pyrimidines and its analogues are an important class of heterocyclic compounds and their structural skeleton is a key constituent of nucleic acids, alkaloids and numerous other pharmacophores with a variety of potent biological activities (Ajani et al., 2015). Pyrano[2,3-*d*]pyrimidines consists of a pyran ring fused together with pyrimidine. As such, their basic framework consists of both nitrogen and oxygen in their carbocyclic structure. This pyrimidine annulated derivative is known to possess good pharmacological activity such as antioxidant, antidiabetic (Yousefi et al., 2015), antimicrobial (Paliwal et al., 2013; Mohamed et al., 2007), anti-inflammatory, analgesic (Kadry, 2014), moderate anticancer (Mohamed et al., 2007), antimycobacterial (Read et al., 2010) and antimalarial activity (Joshi et al., 2005).

Pyrimidines are generally synthesized by a Biginelli multicomponent reaction (MCR), a method considered green since they are fast, solvent free, produces good yields and avoids exposing the environment to environmentally harmful intermediates (Gore and Rajput, 2013). Pyrano[2,3-*d*]pyrimidines are commonly formed from barbituric acid, malonitrile and aldehdyes with a catalyst such as the organic catalyst 1,4-diazabicyclo[2,2,2]octane (DABCO) (Jain et al., 2014), a mesoporous solid acid catalyst (SBA-15-Pr-SO_3H) (Ziarini et al., 2013), a $ZnFe_2O_4$ nanocatalyst (Khazaei et al., 2015), a nano-sawdust-OSO_3H catalyst (Sadeghi et al., 2015), a Mn doped ZrO_2 catalyst (Maddila et al., 2015) or triethylammonium acetate (a green catalyst) (Paliwal et al., 2013). The same compounds were made using an arylidenemalonitrile with barbituric acid under microwave irradiation without a catalyst (Gao et al., 2004) or using ionic liquid catalysts such as *N*-butyl-*N*-methyl imidazole tetrafluoroborate [BMIm]BF_4, *N*-ethyl-*N*-methyl imidazole tetrafluoroborate [EMIm]BF_4 or *N*-butyl pyridinium tetrafluoroborate [BPy]BF_4 (Yu and Wang, 2005). The resultant

pyrimidine diones usually have a substituted phenyl group at position 5 and reactive nitrile and amino groups at positions 6 and 7 on the pyrano[2,3-*d*]pyrimidine skeleton.

By reacting ethyl 2-cyanoacetate with barbituric acid and substituted benzaldehydes in the presence of DABCO, an ethyl ester group was placed at C-6 instead of the nitrile group (Bhat et al., 2014). Pyrano[2,3-*d*]pyrimidines with phenyl groups at both positions 5 and 7 were synthesized by the reaction of chalcones with barbituric acid in the presence of acetic acid and P_2O_5 as a catalyst (Rahman et al., 2013).

Other pyrimidines with substituted phenyl groups were synthesized in a one-pot reaction with 1-nitroguanidine, malonitrile and substituted benzaldehydes under basic conditions in a one pot reaction with short reaction times, mild reaction conditions and excellent yields (Xia et al., 2012) or with guanidine nitrate, ethylcyanoacetate and aldehydes using piperidine as a catalyst (Bhatewara et al., 2012). Spiropyrimidinones were synthesized using urea instead of malonitrile together with barbituric acid and benzaldehydes in the presence of a nanoporous solid acid catalyst (SBA-Pr-SO_3H) (Ziarini et al., 2015). A totally different synthesis to pyrano[2,3-*d*]pyrimidines was achieved using a one-pot three component reaction of 2-amino-7-methyl-5-oxo-4-phenyl-4,5-dihydropyrano[4,3-*b*]pyran-3-carbonitriles, *N*,*N*-dimethylacetaldehyde dimethyl acetal and aromatic amines in the presence of 1-butyl-3-methylimidazole hydrogen sulphate (an ionic liquid) as a catalyst (Suresh et al., 2015). Another synthesis reports the use of *N*,*N*-dimethyl-5-formylbarbituric acid with maleimide and phenyl isocyanate under microwave irradiation to afford pyrano[2,3-*d*]pyrimidines in good yields (Devi et al., 2004).

We herein report the synthesis of a small library of chloro, nitro and fluoro 7-amino-5-aryl-6-cyano-5*H*-pyrano [2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-diones using DABCO as a catalyst together with their antibacterial and anticancer activity.

3.2 Results and Discussion

Chemistry

The title compounds were synthesized in a one pot reaction from substituted chloro, fluoro and nitro aromatic benzaldehydes (ArCHO), malononitrile, barbituric acid and DABCO in aqueous ethanol (**Figure 3-1**) to produce 7-amino-5-aryl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-diones (**1-11**) in yields of between 90-96% (**Table 3-1**). The structures of the products were confirmed from their ^{1}H and ^{13}C NMR spectra and the resonances assigned with the aid of 2D HSQC and HMBC spectra.

With the exception of the novel, 2,4-difluoro derivative **11**, all the compounds were synthesised previously in comparable yields using a variety of methods or catalysts (**Table 3-1**) including microwave synthesis without the use of a catalyst (Gao et al., 2004), with the ionic liquids 1-n-butyl-3-methylimidazolium tetrafluoroborate and 1-butylpyridinium tetrafluoroborate (Yu and Wang, 2005), a Zn [(L) proline] catalyst (Heravi et al., 2010), with glycerol without the use of a catalyst (Safaei et al., 2012), nanocatalysts Fe_3O_4 (Kidwai et al., 2012) and SBA-15-Pr-SO_3H (a mesoporous solid acid catalyst) (Ziarini et al., 2013) and ethyl cyanoacetate (Sharanin and Klokol, 1984). DABCO has been used previously for the synthesis of furan-2-yl, pyrrol-2-yl and thiophen-2-yl derivatives of pyrano pyrimidin-2,4-diones in a one pot reaction, but there are no reports of this catalyst being used in the synthesis of substituted phenyl derivatives.

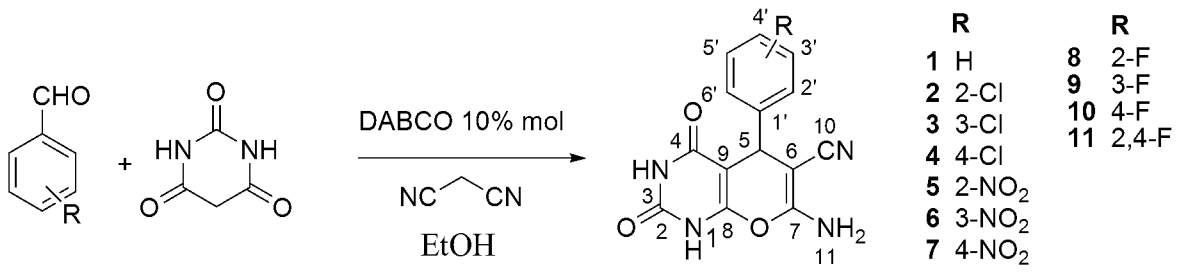

Figure 3-1 DABCO catalyzed reaction of pyrano pyrimidine derivatives (**1-11**)

This reaction occurs via proton removal from the methylene group of malonitrile, which subsequently forms an arylidene derivative via a Knoevenagel condensation. This arylidene derivative then undergoes a Michael reaction with barbituric acid and subsequent cyclization results in the pyrano [2,3-*d*] pyrimidinones. Plausible mechanisms are provided in Bhat et al. (2004) and Jain et al. (2014), however these mechanisms are either not quite clear or misses out some steps. A more comprehensive mechanism is presented in **Figure 3-2**.

Four basic organic catalysts and K_2CO_3 were investigated for this one-pot reaction under reflux conditions at 80 ºC with equimolar quantities of benzaldehyde, malonitrile and barbituric acid in a mixture of 1:1 ethanol and water to identify the catalyst among them that produced the greatest yield in the shortest amount of time. The organic catalysts investigated were L-proline, dibutylamine (DBA), triethylamine (Et_3N) and 1,4-diazabicyclo [2.2.2] octane (DABCO). Of these, DBA, Et_3N and DABCO resulted in yields of over 70% in approximately one hour (**Table 3-2**). DABCO performed the best, producing yields of 94% in 38 minutes. DBA and Et_3N also produced acceptable yields in approximately one hour, Et_3N performing slightly better than DBA.

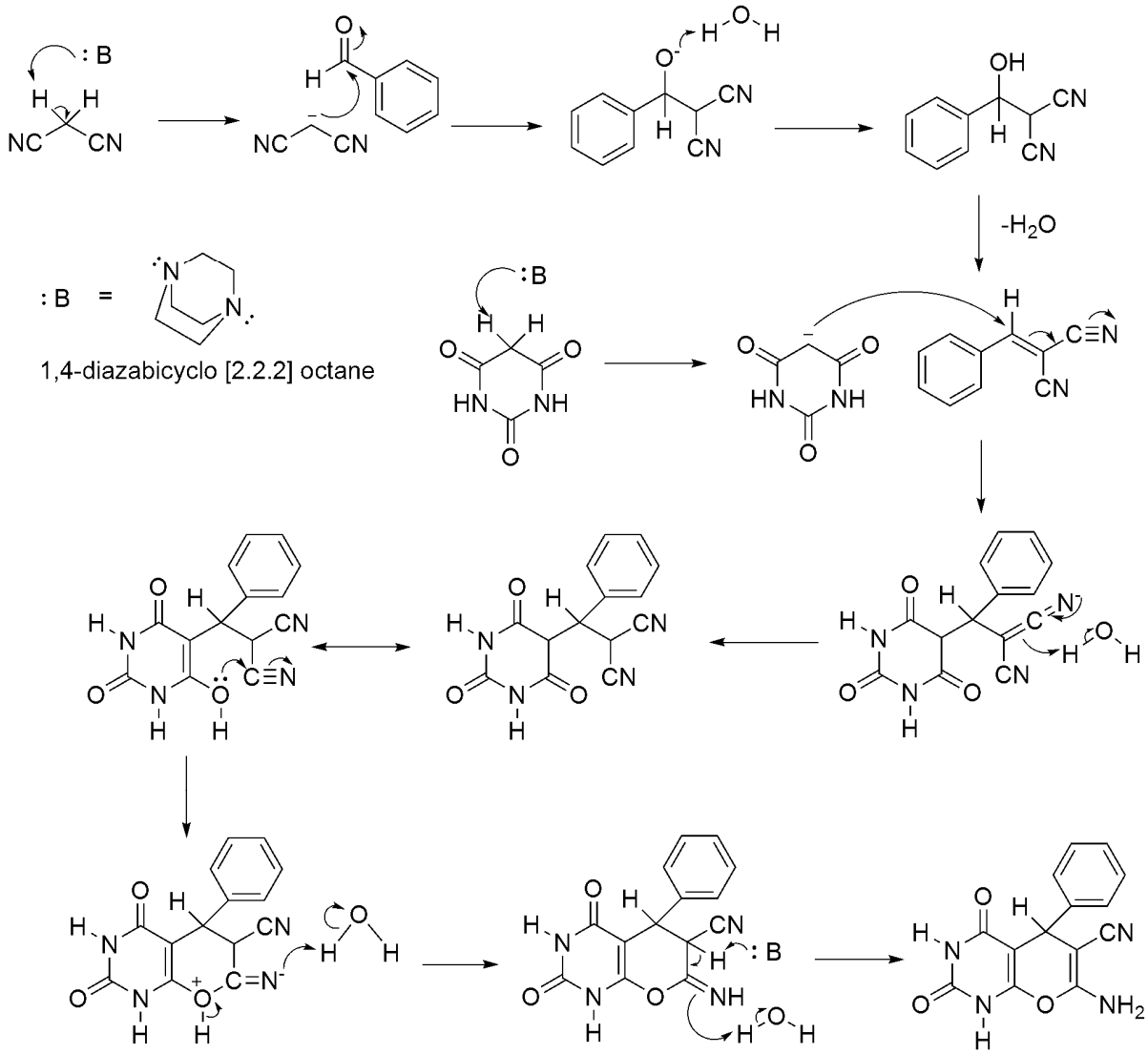

Figure 3-2 A plausible mechanism for the one pot reaction of malonitrile, barbituric acid and benzaldehydes in the presence of DABCO.

Table 3-1 Yields and melting points of aromatic pyrano [2,3-*d*] pyrimidines catalyzed by DABCO[a]

Entry	R	Yield	mp (°C)	Reference	Yield	Catalyst/method
1	H	90	205-208	Gao et al. (2004)	92	Microwave[d]
				Yu and Wang (2005)	84	ILs[e]
				Heravi et al. (2010)	85	Zn [(L) proline]
				Ziarani et al. (2013)	65	SBA-Pr-SO_3H[f]
2	2-Cl	93	212-215	Gao et al. (2004)	89	Microwave[d]
				Yu and Wang (2005)	94	ILs[e]
				Safaei et al. (2012)	90	Glycerol[d]
3	3-Cl	91	223-225	Safaei et al. (2012)	89	Glycerol[d]
				Kidwai et al. (2012)	94	Fe_3O_4[f]
4	4-Cl	93	235-237	Gao et al. (2004)	92	Microwave[d]
				Yu and Wang (2005)	92	ILs[e]
				Heravi et al. (2010)	90	Zn [(L) proline]
				Ziarani et al. (2013)	30	SBA-Pr-SO_3H[f]
				Safaei et al. (2012)	91	Glycerol[d]
				Kidwai et al. (2012)	97	Fe_3O_4[f]
5	2-NO_2	96	258-259	Safaei et al. (2012)	92	Glycerol[d]
6	3-NO_2	92	256-258	Heravi et al. (2010)	90	Zn [(L) proline]
				Ziarani et al. (2013)	80	SBA-Pr-SO_3H[f]
				Safaei et al. (2012)	94	Glycerol[d]
7	4-NO_2	90	225-228	Gao et al. (2004)	86	Microwave[d]
				Heravi et al. (2010)	92	Zn [(L) proline]
				Ziarani et al. (2013)	90	SBA-Pr-SO_3H[f]
				Safaei et al. (2012)	95	Glycerol[d]
				Kidwai et al. (2012)	95	Fe_3O_4[f]
8	2-F	92	226-229	Commercially available[b]		
9	3-F	94	224-226	Sharanin and Klokol (1984)	90	Ethyl cyanoacetate
10	4-F	90	228-230	Gao et al. (2004)	91	Microwave[d]
				Yu and Wang (2005)	90	ILs[e]
11	2,4-diF	93	250-252	novel		

[a]Reaction conditions: barbituric acid (1.0 mmol), substituted benzaldehydes (1.0 mmol) and malononitrile (1.1 mmol), EtOH:water (1:1 v/v, 10 mL), room temp.; [b]Akos Consulting and Solutions; Sigma Aldrich; [c]The 7-indolyl group replaces the substituted aromatic group; [d]no catalyst; [e]ionic liquids – 1-n-butyl-3-methylimidazolium tetrafluoroborate and 1-butylpyridinium tetrafluoroborate; [f]nanocatalyst

Table 3-2 Yields and reaction times for the one-pot reaction of pyranopyrimidinones with different catalysts[a]

Catalyst	Time (min)	Yield %
No catalyst	1080 (18h)	5
L-Proline	180 (3h)	45
K_2CO_3	110	57
DBA	54	74
Et_3N	70	81
DABCO	38	94

[a] Reagents and reaction conditions: barbituric acid (1.0 mmol), benzaldehyde (1.0 mmol) and malononitrile (1.1 mmol), EtOH:water (1:1 v/v, 10 mL), room temp.

The ^{1}H and ^{13}C NMR spectra were similar for all compounds and the only difference in the spectra were brought about by differences in the aryl moiety of the molecule substituted at C-5. Using compound **1** (the pyrimidine with the unsubstituted aryl ring) as an example, a characteristic H-5 resonance was seen at δ 4.26. This was an indication that the pyranopyrimidines had formed. The only other protons on the core pyrimidine skeleton, were the 1º and 2º amino and amide protons. The NH_2-11 resonance occurred at δ 7.14 as a two proton singlet together with the aromatic protons at δ 7.18-7.22 (H-2',4',6'), and δ 7.29-7.32 (H-3',5'). NH_2-11 showed HMBC correlations with both C-6 and C-7, supporting this assignment. The NH-1 and NH-3 proton resonances occurred at δ 11.08 (sharp singlet) and δ 12.07 (broadened singlet), however, these resonances could not be assigned unequivocally and are interchangeable.

The core structure of the pyrano[2,3-*d*]pyrimidines synthesized in this work have very characteristic carbon resonances. The nitrile carbon C-10, pyrimidine carbon C-9, pyran carbon C-6 to which the nitrile group is attached and the methine carbon C-5 of the pyran ring to which the aromatic group is attached all appear very distinctly at δ 119.1 (C-10), 88.5

(C-9), 58.7 (C-6) and 35.6 (C-5). There are five downfield carbon resonances. The most shielded resonance at δ 144.2 was assigned to the aromatic carbon C-1' due to a HMBC correlation with H-3',5' and C-7 was assigned to δ 157.7 due to a HMBC correlation with NH_2-11. The other three carbon resonances in this region at δ 149.4 (C-2), 152.4 (C-8) and 162.4 (C-4) were characteristic of the pyrimidinone carbon resonances. The HMBC correlations of H-5 were instrumental in assigning the carbon resonances on the core structure (**Figure 3-3**). H-5 showed HMBC correlations to C-8, C-9 and C-4 of the pyrimidine ring, C-6 and C-7 of the pyran ring, C-1', C-2' and C-6' of the phenyl ring and the cyano carbon C-10.

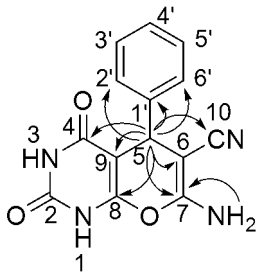

Figure 3-3 Selected HMBC correlations for compound **1**

Antimicrobial activity

The synthesized compounds were tested for their antimicrobial activity against two Gram +ve and three Gram –ve strains of bacteria. The compounds were initially screened for their antimicrobial potential using the disk diffusion assay, where compounds showing a mean inhibition zone of greater than 9 mm were selected to determine their minimum bactericidal concentrations (MBCs). In general, the compounds were more active against the Gram +ve *Staphylococcus aureus* and MRSA than the Gram –ve strains (**Table 3-3**). Compound **2**, the 2-chloro derivative was active at < 200 μM for both the Gram +ve strains. Several of the compounds were also active at < 200 μM against MRSA, showing better activity than

ampicillin. Only compound **5**, the 2-nitro derivative showed activity < 200 µM against *E. coli*. None of the other compounds showed any appreciable activity to the Gram –ve strains.

Table 3-3 Antibacterial activity of the synthesized compounds

Bacterial strains	**Minimum Bactericidal Concentration (µM)**							
Compounds	**2**	**3**	**4**	**5**	**8**	**11**	**AMP**	**CIP**
Gram +ve								
S. aureus	197	394	394	382	208	196	56	1.84
MRSA	197	197	394	191	1665	393	894	7.36
Gram -ve								
E. coli	1579	1579	789	191	1665	786	447	1.84
P. aeruginosa	1579	1579	789	1528	1665	1571	1788	1.84
K. pneumoniae	394	789	1579	382	1665	1571	447	3.68

Data is reported as the average of duplicate readings

MTT cytotoxicity assay

The *in vitro* cytotoxicity levels of compounds **1-11** in the HeLa cells are summarized in **Table 3-4** below. Most of the compounds tested showed a typical dose dependent cytotoxicity profile. Based on the IC_{50} values it can be deduced that all compounds are most active at concentrations greater 100 µg mL^{-1}. The activities of all compounds were better than 5-fluorouracil (5FU), a commonly used anticancer drug, with IC_{50} values ranging from 129 to 340 µM. 5FU had an IC_{50} in the same assay at 480 µM. The activities of the compounds could be due to the pyranopyrimidinone core structure, with aryl groups at C-5 to which electron withdrawing groups were attached. Compounds **3**, **4**, **7** and **8** were active at a much lower dose, which augurs well for their future use as possible anti-cancer agents. Further studies and testing of high activity compounds are hence warranted.

Table 3-4 Viabilities (%) of the HeLa cell lines at different concentrations of compounds **1-11**

Cpd	10 µg mL^{-1}	25 µg mL^{-1}	50 µg mL^{-1}	100 µg mL^{-1}	IC_{50} µg mL^{-1}	IC_{50} µM
1	95.58±0.02^{c}	85.82±0.0005^{b,c}	70.10±0.01^{b}	41.35±0.02^{a}	84.92	301
2	96.72±0.01^{d}	83.06±0.01^{c}	65.40±0.02^{b}	40.48±0.01^{a}	81.34	257
3	79.11±0.01^{d}	65.64±0.001^{c}	53.20±0.02^{b}	43.33±0.04^{a}	74.02	234
4	72.08±0.02^{d}	52.13±0.02^{c}	37.99±0.02^{b}	27.84±0.02^{a}	40.67	129
5	98.22±0.03^{d}	82.46±0.01^{c}	69.79±0.02^{b}	46.68±0.02^{a}	90.86	278
6	95.81±0.02^{d}	78.36±0.01^{c}	69.31±0.02^{b}	57.15±0.02^{a}	111.27	340
7	86.65±0.02^{d}	73.82±0.02^{c}	62.80±0.01^{b}	40.64±0.02^{a}	78.81	241
8	81.04±0.02^{d}	68.92±0.01^{c}	50.87±0.03^{a}	42.54±0.03^{a}	72.54	242
9	87.88±0.02^{c}	83.25±0.01^{c}	69.91±0.01^{b}	50.91±0.03^{a}	101.11	337
10	88.55±0.02^{d}	69.79±0.01^{c}	57.86±0.01^{b}	41.19±0.01^{a}	96.51	322
11	84.79±0.01^{c}	77.37±0.02^{c}	63.67±0.02^{b}	52.41±0.02^{a}	101.2	318
12	90.28±0.01^{d}	85.15±0.01^{c}	71.56±0.01^{a}	71.56±0.02^{a}	108.75	339
5FU	78.40±0.03^{d}	58.89±0.03^{b}	50.47±0.02^{b}	38.00±0.02^{a}	62.41	480

Data are presented as the mean ± SD value of triplicate determinations. $^{a-d}$ Different superscript letters for a given value within a column are significantly different from each other (Tukey's-HSD multiple range *post hoc* test, $p < 0.05$).

Molecular docking studies

In order to support the experimental anti-cancer activity of the synthesized compounds and to predict their mechanism of action, two representative compounds (RCs) **4** (4-Cl) and **8** (2F) were docked and observed to be potent under *in vitro* conditions, into the binding site of human kinesin protein, Eg5. The CDocker docking method embedded in the Discovery Studio (DS) was used for all docking simulations. The docking results obtained suggested both compounds to be strong inhibitors of Eg5 based on the computed binding energy (BE) data. The most active compound **4** with BE of value -184.4 kcal mol^{-1} exhibited a stronger interaction than its structural analogue **8** (BE = -150.1 kcal mol^{-1}). Both the RCs exhibited stronger binding affinity for Eg5 relative to the standard drug, 5FU (BE = -116.7 kcal mol^{-1}).

To understand the host-guest relationship between ligand and receptor, the docked complexes of both RCs were further visualized using DS visualizer, and are diagrammatically represented in **Figure 3-4** and **Figure 3-5**. Compound **4** (**Figure 3-4**) exhibited two concurrent hydrogen bond interactions through its protonated amine functionality (NH_2) with

Glu116 (1.81 Å) and Gly117 (1.98 Å) amino acid residues of Eg5. Additionally, an electrostatic interaction between **4** and Glu116 including several hydrophobic forces (with Pro137, Trp127, Arg119, Tyr211 and Ala133) were also observed. Similarly, **8** (**Figure 3-5**) interacted with Eg5 through two hydrogen bonds; one conventional with Arg221 (1.17Å) through its nitrile group and another non-conventional with Arg119 (2.80 Å) through the nitrogen atom of the pyrimidine ring. In addition, two electrostatic interactions (with Glu116 and Arg221) and hydrophobic forces (with Tyr211, Ala218 and Pro137) were also observed.

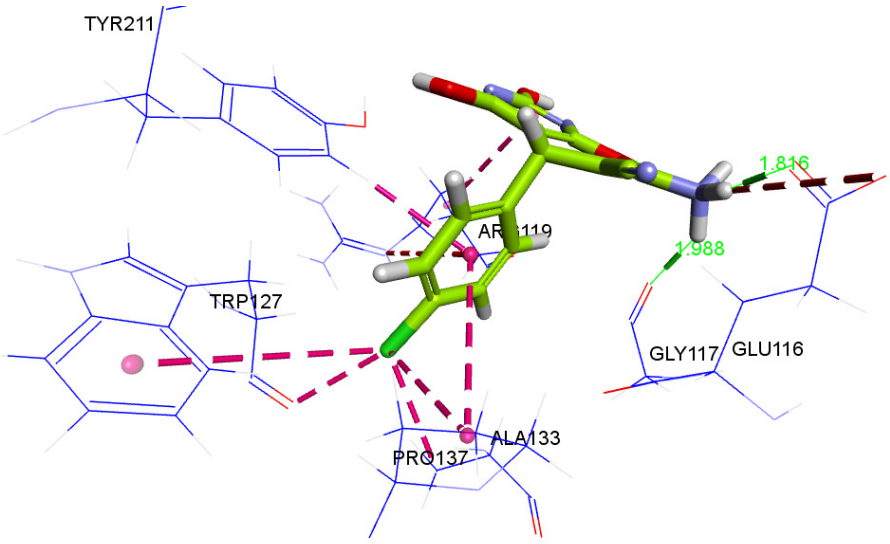

Figure 3-4 Docked pose of **4** in the binding site of Eg5. Hydrophobic and electrostatic interactions are shown as magenta and red dotted lines respectively. Conventional hydrogen bonds are shown as green.

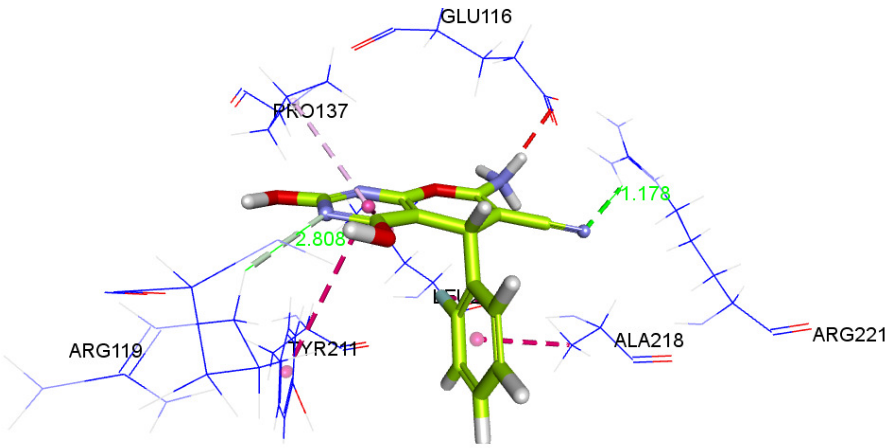

Figure 3-5 Docked pose **8** in the binding site of Eg5. Hydrophobic and electrostatic interactions are shown as magenta and red dotted lines respectively. Conventional hydrogen bonds are shown as green and non-conventional hydrogen bonds are shown as grey.

3.3 Materials and Methods

General Experimental Procedures

Reagents and chemicals used in this study were purchased from Sigma Aldrich via Capital Laboratories, South Africa, and were reagent grade. All organic solvents were redistilled and dried according to standard procedures. NMR spectra were recorded using a Bruker AvanceIII 400 MHz spectrometer at room temperature with chemical shifts (δ) recorded against the internal standard, tetramethylsilane (TMS). 2D NMR spectroscopy (HSQC, and HMBC) were used for the structural elucidation of the synthesized compounds. IR spectra were recorded on a Perkin Elmer Spectrum 100 FTIR spectrometer with universal ATR sampling accessory. For GC-MS analyses, the samples were analyzed on an Agilent GC-MSD apparatus equipped with a DB-5SIL MS (30 m × 0.25 mm) fused-silica capillary column. Helium (at 2 mL min^{-1}) was used as a carrier gas. The MS was operated in the EI mode at 70 eV. Optical rotation was recorded using a Perkin Elmer, Model 341 Polarimeter. Melting points were recorded on an ErnstLeitzWetzlar micro hot stage melting point apparatus and are uncorrected.

General procedure for the synthesis of aromatic substituted pyrano [2,3-*d*] pyrimidinones (1-11)

Substituted aromatic benzaldehydes (1.0 mmol each), malononitrile (396 mg, 1.0 mmol), barbituric acid (640 mg, 1.0 mmol each) and 10 mol % DABCO (30.45 mg, 0.271 mmol) were added to 20 mL of aqueous ethanol (**Figure 3-1**) and the reaction mixture stirred for 1 hour at room temperature. The progress of the reaction was monitored by TLC. The products were filtered, recrystallized in ethanol and dried under vacuum.

(1) *7-amino-5-phenyl-6-cyano-5H-pyrano[2,3-d]pyrimidin-(1H,3H)-2,4-dione*, white powder (92% yield); mp 205-208 ºC; UV λ_{max} (DMSO) nm (log ϵ) 312 (2.66); IR (KBr) ν_{max}: 3184 (NH_2), 2194 (CN), 1718 (C=O), 1674 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ_H 12.07 (brs, NH) 11.08 (s, NH), 7.29-7.32 (2H, m, H-3',5'), 7.18-7.22 (3H, m, H-2',4′,6'), 7.14 (s, NH_2-11), 4.26 (1H, s, H-5); ^{13}C NMR (100 MHz, DMSO) δ_C 162.4 (C-4), 157.7 (C-7), 152.4 (C-8), 149.4 (C-2), 144.2 (C-1'), 129.4 (2CH, C-2',6'), 128.2 (2CH, C-3',5'), 127.4 (CH, C-4'), 119.1 (C-10), 88.5 (C-9), 58.7 (C-6), 35.6 (CH, C-5); EIMS (*m/z*, rel. int.) 282 [M^+] (38), 207 (62), 193 (25), 154 (58), 127 (100); Anal. calc. for CHN: C: 59.57, H: 3.57, N: 19.85, Found: C: 59.17, H: 3.46, N: 19.20.

(2) *7-amino-5-(2-chlorophenyl)-6-cyano-5H-pyrano[2,3-d]pyrimidin-(1H,3H)-2,4-dione*, white powder, (93% yield); mp 212-215 ºC; UV λ_{max} (DMSO) nm (log ϵ) 259 (2.74); IR (KBr) ν_{max}: 3013 (NH), 2192 (CN), 1714 (C=O), 1673 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO) 12.07 (brs, NH) 11.04 (s, NH), 7.36 (1H, d, *J* = 8.0 Hz, H-6'), 7.21-7.27 (3H, m, H-3',4',5'), 7.12 (s, NH_2-11), 4.72 (1H, s, H-5); ^{13}C NMR (100 MHz, DMSO) δ_C 162.2 (C-4), 157.8 (C-7), 152.7 (C-8), 149.5 (C-2), 140.7 (C-1'), 132.2 (C-2'), (130.4, 129.5, 128.4, 127.4 (4CH, C-3',4',5',6')), 118.7 (C-10), 87.5 (C-9), 56.0 (C-6), 30.6 (CH, C-5); EIMS (*m/z*, rel. int.) 316 (M^+) (14), 281 (31), 207 (100), 273 (14), 189 (15); Anal. calc. for CHN: C: 53.09, H: 2.86, N: 17.69, Found: C: 53.00, H: 2.79, N: 17.60.

(3) *7-amino-5-(3-chlorophenyl)-6-cyano-5H-pyrano[2,3-d]pyrimidin-(1H,3H)-2,4-dione*, white powder, (95% yield); mp 223-225 ºC; UV λ_{max} (DMSO) nm (log ϵ) 259 (2.69); IR (KBr) ν_{max}: 3417 (NH), 3317 (NH), 2192 (CN), 1706 (C=O), 1660 (C=O)cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ_H 12.07 (brs, NH), 11.07 (s, NH), 7.32 (1H, t, *J* = 8.4 Hz, H-5'), 7.26-7.28 (2H, m, H-4',6'), 7.19 (1H, s, H-2'), 7.16 (s, NH_2-11), 4.26 (1H, s, H-5); ^{13}C NMR (100 MHz, DMSO) δ_C 162.4 (C-4), 157.6 (C-7), 152.4 (C-8), 149.5 (C-2), 146.6 (C-1'), 132.8 (C-3'), 130.1 (C-4'), 127.2 (CH, C-6'), 126.7 (CH, C-2'), 126.1 (CH, C-5'), 118.9 (C-10), 87.7 (C-9), 58.1 (C-6), 35.4 (CH, C-5); EIMS (*m/z*, rel. int.) 316 (M^+) (6), 281 (11), 207 (100), 188 (22), 153 (33); Anal. calc for CHN: C: 53.09, H: 2.86, N: 17.69, Found: C: 53.10, H: 2.59, N: 17.70.

(4) *7-amino-5-(4-chlorophenyl)-6-cyano-5H-pyrano[2,3-d]pyrimidin-(1H,3H)-2,4-dione*, white powder, (94% yield); mp 235-237 ºC; UV λ_{max} (DMSO) nm (log ϵ) 258 (2.68); IR (KBr) υ_{max}: 3383 (NH_2), 3186 (NH), 2197 (CN), 1717 (C=O), 1672 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ_H 12.06 (brs, NH), 11.05 (s, NH), 7.33 (2H, d *J* = 8.4 Hz, H-3',5'), 7.23 (2H, d, *J* = 8.4 Hz, H-2',6'), 7.13 (s, NH_2-11), 4.24 (1H, s, H-5); ^{13}C NMR (100 MHz, DMSO) δ_C 162.4 (C-4), 157.6 (C-7), 152.3 (C-8), 149.5 (C-2), 143.1 (C-1'), 131.2 (C-4'), 129.3 (2CH, C-2',6'), 128.2 (2CH, C-3',5'), 119.0 (C-10), 88.0 (C-9), 58.3 (C-6), 35.1 (CH, C-5); EIMS (*m/z*, rel. int.) 316 (M^+) (15), 281 (33), 207 (100), 188 (25), 153 (23); Anal. calc. for CHN: C: 53.09, H: 2.66, N: 17.65, Found: C: 53.09, H: 2.86, N: 17.69.

(5) *7-amino-5-(2-nitrophenyl)-6-cyano-5H-pyrano[2,3-d]pyrimidin-(1H,3H)-2,4-dione*, off white powder, (96% yield); mp 258-259 ºC; UV λ_{max} (DMSO) nm (log ϵ) 260 (2.71); IR (KBr) υ_{max} $^{-1}$: 3365 (NH_2), 2198 (CN), 1697 (C=O), 1618 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ_H 12.14 (brs, NH) 11.06 (s, NH), 7.82 (1H, d, *J* = 8.3 Hz, H-6'), 7.65 (1H, dd, *J* = 8.1, 7.6 Hz, H-4'), 7.49 (1H, d, *J* = 7.6 Hz, H-3'), 7.45 (1H, dd, *J* = 8.3, 8.1 Hz, H-5'), 7.27 (s, NH_2-11), 5.04 (1H, s, H-5); ^{13}C NMR (100 MHz, DMSO) δ_C 162.4 (C-4), 158.4 (C-7),

152.3 (C-8), 149.4 (C-2), 149.2 (C-2'), 138.1 (C-1'), 133.3 (CH, C-6'), 130.7 (CH, C-4'), 128.0 (CH, C-3'), 123.7 (CH, C-5'), 118.6 (C-10), 87.9 (C-9), 56.7 (C-6), 30.3 (CH, C-5); EIMS (*m/z*, rel. int.) 327 (M^+) (14), 298 (32), 207 (100); Anal. calc. for CHN: C: 51.38, H: 2.77, N: 21.40, Found: C: 51.04, H: 2.47, N: 21.36.

(6) *7-amino-5-(3-nitrophenyl)-6-cyano-5H-pyrano[2,3-d]pyrimidin-(1H,3H)-2,4-dione*, off white powder, (90% yield); mp 256-258 ºC; UV λ_{max} (DMSO) nm (log ϵ) 262 (2.77); IR (KBr) υ_{max}: 3414 (NH_2), 3202 (NH), 2192 (CN), 1707 (C=O), 1687 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO) δ_H 12.15 (brs, NH), 11.09 (s, NH), 8.08 (1H, d, *J* = 8.1 Hz, H-6'), 8.06 (1H, brs, H-2'), 7.74 (1H, d, *J* = 7.7, H-4'), 7.60 (1H, dd, *J* = 8.1, 7.7 Hz, H-5'), 7.26 (s, NH_2-11), 4.47 (1H, s, H-5); ^{13}C NMR (100 MHz, DMSO) δ_C 162.5 (C-4), 157.8 (C-7), 152.5 (C-8), 149.5 (C-2), 147.7 (C-1'), 146.4 (C-3'), 134.7 (C-2'), 129.8 (CH, C-6'), 122.0 (CH, C-4'), 121.9 (CH, C-5'), 118.8 (C-10), 87.4 (C-9), 57.6 (C-6), 35.4 (CH, C-5); EIMS (*m/z*, rel. int.): 327 (M^+) (15), 281 (40), 207 (100); Anal. calc. for CHN: C: 51.38, H: 2.77, N: 21.40, Found: C: 51.01, H: 2.46, N: 21.24.

(7) *7-amino-5-(4-nitrophenyl)-6-cyano-5H-pyrano[2,3-d]pyrimidin-(1H,3H)-2,4-dione*, white powder, (93% yield); mp 225-228 ºC; UV λ_{max} (DMSO) nm (log ϵ) 260 (2.84); IR (KBr) υ_{max} (cm^{-1}): 3186 (NH), 2196 (CN), 1720 (C=O), 1671 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO) δ_H 12.15 (brs, NH), 11.10 (s, NH), 8.15 (2H, d, *J* = 8.7 Hz, H-3',5'), 7.52 (2H, d, *J* = 8.7 Hz, H-2',6'), 7.25 (s, NH_2-11), 4.41 (1H, s, H-5); ^{13}C NMR (100 MHz, DMSO) δ_C 162.4 (C-4), 157.7 (C-7), 152.6 (C-8), 151.7 (C-4'), 149.5 (C-2), 146.4 (C-1'), 128.8 (2CH, C-2',6'), 123.5 (2CH, C-3',5'), 118.8 (C-10), 87.4 (C-9), 57.4 (C-6), 35.6 (CH, C-5); EIMS (*m/z*, rel. int.): 327 (M^+) (27), 207 (100); Anal. calc. for CHN: C: 51.38, H: 2.77, N: 21.40, Found: C: 50.93, H: 2.37, N: 21.16.

(8) *7-amino-5-(2-fluorophenyl)-6-cyano-5H-pyrano[2,3-d]pyrimidin-(1H,3H)-2,4-dione*, white powder; (90% yield); mp 226-229 ºC; UV λ_{max} (DMSO) nm (log ϵ) 260 (2.93); IR

(KBr) υ_{max} cm^{-1}: 3421 (NH), 3303 (NH), 2203 (CN), 1716 (C=O), 1692 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ_H 12.11 (brs, NH), 11.07 (s, NH), 7.23-7.28 (2H, m, H- 4',6'), 7.14 (2H, s, NH_2-11), 7.07-7.12 (2H, m H-3',5'), 4.50 (1H, s, H-5); ^{13}C NMR (100 MHz, DMSO) δ_C 162.3 (C-4), 160.1 (d, *J* = 243.8 Hz, C-2'), 157.9 (C-7), 152.6 (C-8), 149.5 (C-2), 130.6 (d, *J* = 11.8 Hz, CH, C-4'), 129.8 (d, *J* = 3.8 Hz, C-1'), 128.7 (d, *J* = 8.4 Hz, CH, C-6'), 124.4 (d, *J* = 3.0 Hz, CH, C-5'), 118.9 (C-10), 115.3 (d, *J* = 21.7 Hz, CH, C-3'), 87.2 (C-9), 57.3 (C-6), 30.6 (CH, C-5); EIMS (*m/z*, rel. int.): 300 (M^+) (25), 281 (35), 226 (35), 207 (100), 167 (40), 159 (42); Anal. calc. for CHN: C: 56.00, H: 3.02, N: 18.66, Found: C: 56.10, H: 2.97, N: 18.02.

(9) *7-amino-5-(3-fluorophenyl)-6-cyano-5H-pyrano[2,3-d]pyrimidin-(1H,3H)-2,4-dione*, white powder; (96% yield); mp 224-226 ºC; UV λ_{max} (DMSO) nm (log ϵ) 270 (2.93); IR (KBr) υ_{max}: 3376 (NH), 3186 (NH), 2197 (CN), 1717 (C=O), 1674 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ_H 12.06 (brs, NH), 11.06 (s, NH), 7.30-7.36 (1H, m, H-5'), 7.14 (s, NH_2), 7.01-7.07 (3H, m H-2',4',6'), 4.27 (1H, s, H-5); ^{13}C NMR (100 MHz, DMSO) δ_C 162.4 (C-4), 162.1 (d, *J* = 241.9 Hz, C-3'), 157.6 (C-7), 152.4 (C-8), 149.5 (C-2), 147.0 (d, *J* = 6.3 Hz, C-1'), 130.1 (d, *J* = 8.1 Hz, CH, C-5'), 123.4 (CH, C-6'), 119.0 (C-10), 114.0 (d, *J* = 21.5 Hz, CH, C-2'), 113.5 (d, *J* = 20.9 Hz, CH, C-4'), 87.8 (C-9), 58.2 (C-6), 35.4 (CH, C-5); EIMS (*m/z*, rel. int.): 300 (M^+) (18), 281 (41), 207 (100), 172 (74); Anal. calc. for CHN: C: 56.00, H: 3.02, N: 18.66, Found: C: 55.97, H: 2.85, N: 18.39.

(10) *7-amino-5-(4-fluorophenyl)-6-cyano-5H-pyrano[2,3-d]pyrimidin-(1H,3H)-2,4-dione*, white powder, (92% yield); mp 228-230 ºC; UV λ_{max} (DMSO) nm (log ϵ) 260 (2.93); IR (KBr) υ_{max}: 3194 (NH), 2197 (CN), 1723 (C=O), 1677 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ_H 12.06 (brs, NH), 11.05 (s, NH), 7.25 (2H, dd, *J* = 8.6, 5.6 Hz, H-2',6'), 7.10 (2H, dd, *J* = 8.6, 8.6 Hz, H-3',5'), 7.10 (s, NH), 4.26 (1H, s, H-5); ^{13}C NMR (100 MHz, DMSO) δ_C 162.4 (C-4), 161.0 (d, *J* = 240.8 Hz, C-4'), 157.5 (C-7), 152.2 (C-8), 149.5 (C-2), 140.1 (d, *J*

= 2.8 Hz, C-1'), 129.1 (d, *J* = 8.1 Hz, 2CH, C-2',6'), 119.1 (C-10), 114.8 (d, *J* = 21.2 Hz, 2CH, C-3',5'), 88.3 (C-9), 58.7 (C-6), 35.0 (CH, C-5); EIMS (*m/z*, rel. int.): 300 (M^+) (10), 281 (13), 266 (19), 233 (19), 207 (100); Anal. calc. for CHN: C: 56.00, H: 3.02, N: 18.66, Found C: 56.22, H: 2.65, N: 18.14.

(11) *7-amino-5-(2,4-difluorophenyl)-6-cyano-5H-pyrano[2,3-d]pyrimidin-(1H,3H)-2,4-dione*, white powder; (92% yield); mp 250-252 ºC; UV λ_{max} (DMSO) nm (log ϵ) 265 (2.98); IR (KBr) υ_{max}: 3395 (NH), 3306 (NH), 2195 (CN), 1718 (C=O), 1674 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO) δ_H 12.10 (brs, NH), 11.08 (s, NH), 7.32 (ddd, *J* = 8.6, 8.6, 6.3, H-6'), 7.15 (1H, ddd, *J* = 11.8, 9.4, 2.6, H-3'), 6.99 (1H, ddd, *J* = 8.4, 8.4, 2.4 Hz, H-5'), 7.18 (s, NH_2), 4.49 (s, H-5); ^{13}C NMR (100 MHz, DMSO) δ_C 162.3 (C-4), 161.1 (dd, *J* = 243.0, 12.4 Hz, C-2'*), 160.1 (dd, *J* = 246.5, 12.3 Hz, C-4'*), 157.9 (C-7), 152.6 (C-8), 149.5 (C-2), 131.1(dd, *J* = 9.6, 5.6 Hz, C-6'), 127.1 (dd, *J* = 12.3, 3.6 Hz, C-1'), 118.9 (C-10), 111.4 (d, *J* = 20.8 Hz, CH, C-5'), 103.6 (t, *J* = 25.9 Hz, CH, C-3') 87.0 (C-9), 57.0 (C-6), 29.6 (CH, C-5); EIMS (*m/z*, rel. int.): 318 (M^+) (15), 207 (100); Anal. calc. for CHN: C: 52.84, H: 2.53, N: 17.61, Found: C: 51.98, H: 2.39, N: 17.47. * assignments can be interchanged.

Antibacterial Assay

The antimicrobial activity of the synthesized compounds (**1-11**) were tested against the Gram +ve *Staphylococcus aureus* ATCC 25923 and methicillin resistant *S. aureus* ATCC BAA-1683 (MRSA), and three Gram -ve strains, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumonia* ATCC 314588. The standard antibiotics ciprofloxacin and ampicillin were used as controls for comparison.

Mueller Hilton agar (Biolab, South Africa) was prepared (38 g in 1 L of water), poured into sterile pre-labelled petri dishes and allowed to set and dry at room temperature. Bacterial

cultures were standardized using a 0.5 McFarland standard turbidity and swabbed onto agar plates. Paper discs were loaded with 20 µL of the synthesised compounds and placed onto the prepared agar plates, inverted and incubated at 35-37°C for 24 hours. The diameter of the zone of inhibition was measured in mm. Compounds showing an inhibition zones of > 9 mm were selected to determine their minimum bactericidal concentration (MBC) values using the broth dilution assay with ampicillin and ciprofloxacin as the controls following the method in Andrews (2001). Compounds **2-5**, **8** and **11** showed zones of inhibition of > 9 mm and were tested further.

To calculate the MBC values, the compounds were dissolved in DMSO (4.0 mg mL^{-1}) and serially diluted in Mueller-Hinton broth (Oxoid, England) in Eppendorf tubes. Each Eppendorf containing 180 µL of compound at different concentrations was inoculated with 20 µL of the respective bacterial cultures and incubated at 37 ^{o}C for 18 hours. This was performed in duplicate. Thereafter, 10 µL from each concentration was spotted onto Mueller-Hinton plates and incubated at 37 ^{o}C for 18 hours to determine the MBC. The MBC was the lowest concentration which showed no bacterial growth in the area in which the sample was placed.

Cytotoxicity tests by the MTT assay

Human cervical cancer (HeLa) cells were purchased from Highveld Biological (Pty) Ltd. (Lyndhurst, RSA). Cells were grown to semi-confluency in 25 cm^2 tissue culture flasks in EMEM (Eagle's Minimum Essential Medium, Lonza BioWhittaker, Verviers, Belgium) supplemented with 10% fetal bovine serum and antibiotics (100 U mL^{-1} penicillin, 100 µg mL^{-1} streptomycin). For the MTT assay, cells were seeded at a density of 1.8 x10^3 cells per

well in a 96 well plate containing 100 µL of medium. The cells were then incubated for 24 h at 37ºC in 5% CO_2, after which the medium was removed and 100 µL fresh medium added.

Compounds **1-11** (initially dissolved in DMSO at a concentration of 1 mg mL^{-1}) were then added in triplicate to the cells (containing 100 µL of fresh medium) to a final concentration of 10, 25, 50 and 100 µg mL^{-1} and incubated for 48 h at 37 ºC. 5FU was used as a standard positive control.

The MTT assay was adapted from Mosmann et al. (1983) and measures the metabolic activity of cells by the reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to formazan using the succinate-tetrazolium reductase system. After 48 h of incubation, the spent medium was replaced with 100 µL fresh medium and 100 µL of MTT (5 mg mL^{-1} in PBS), and cells were incubated for 4 h at 37ºC. Thereafter, the medium and MTT were removed, and 200 µL of DMSO was added to each well to dissolve the formazan salt. The absorbance of the resulting purple solution was read on a Mindray 96A microplate reader (Vacutec, Hamburg, Germany) at 570 nm (detection l) and 630 nm (reference l for nonspecific signals) wavelengths. Cell viability (%) was directly correlated to absorbance and calculated in comparison to the untreated control as follows:

[(OD_{570}Treated - OD_{630}Treated) / (OD_{570}Control - OD_{630}Control) x 100)]

Tests were conducted in triplicate and the IC_{50} values (concentration at which 50% cell death was achieved) were determined using Microsoft Excel 2010™.

Molecular Docking

Different isomers of the representative compounds at physiological pH were generated using "Prepare Ligands" and energetically minimized in DS using CHARMm force field. The

isomer with the lowest CHARMm energy was selected for docking. The crystal structure of Human EG5 protein (pdb id: 2X7C) was downloaded from the protein data bank (http://www.rcsb.org). Only the B-chain of protein was considered while the native ligand, (s)-Enastron (KZ91367) and associated water molecules were removed. Initially, the protonation state of the protein was determined at physiological pH followed by its minimization. The "Prepare Protein" module in DS was used to build any missing loops/chains and determine the protonation state of each amino acid of the protein.

The shake algorithm was used to constrain the hydrogen atoms of the protein during minimization. Before docking, a binding sphere (diameter 6.34 Å) with co-ordinates 16.8 (X), 14.4 (Y) and -30.9 (Z), was generated using DS. Docking simulations were conducted using the CDocker docking program (Wu et al., 2003) by keeping the position of protein fixed while allowing the ligand to flex. A total of 10 poses were generated for each compound and ranked according to the scoring function (-CDocker energy). The best pose was selected for binding energy calculations.

3.4 Conclusion

An environmentally friendly synthesis of pyrano [2,3-*d*] pyrimidinone derivatives in a one pot reaction using an organic catalyst, DABCO, which has the advantage of mild reaction conditions producing high yields of the final product (>90%) was reported. The synthesized compounds showed good anticancer activity against HeLa cells. The most active compounds also showed good binding affinity to the human kinesin protein, Eg5, which supported our experimental findings.

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3.5 References

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Chapter 4. Synthesis, molecular docking and anticancer activity of 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) derivatives

* The compounds referred to in this chapter are referred to in the abstract, conclusion and appendices or elsewhere in the thesis with a **C** preceding the number of the compound. For example, **1** and **2** are referred to as **C1** and **C2** elsewhere in the thesis.

ABSRACT

A series of 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) derivatives (**1-13**) were synthesized by the condensation of 6-amino-2-thiouracil and benzaldehydes derivatives under reflux in glacial acetic acid. Nine compounds (**3**, **5** and **7-13**) were novel. Good binding affinity (BE) in the active site of Eg5 was shown by all compounds, which ranged between -5.0 kcal mol^{-1} to -26.7 kcal mol^{-1}. Most compounds in the series exhibited stronger interaction than 5-FU (BE = -8.0 kcal mol^{-1}) with Eg5. The docking results were supported by cytotoxicity studies of the synthesized compounds against HeLa (cervical cancer) cell lines, where all compounds exhibited better anti-cancer activity than 5-fluorouracil (IC_{50} = 12.08 µg mL^{-1}) at the lowest concentration (10 µg mL^{-1}) with IC_{50} values ranging from 4.18 to 10.20 µg mL^{-1}.

Keywords: 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil), 6-amino-2-thiouracil, HeLa, anticancer

4.1 Introduction

5-Fluorouracil (5-FU) is a well-known anticancer drug used since the last century for the treatment of colon, breast and skin cancer either alone or in combination with other drugs (Diaso and Harris, 1989). In the last few decades, it has been the major chemotherapeutic treatment for colorectal carcinoma (Adjei, 1999). It is incorporated into RNA leading to interferences with maturation of RNA (Pinedo and Peters, 1988). Thiouracils or mercaptouracil derivatives have also been known to possess good bioactivities, showing antiplatelet aggregating activity, anti-inflammatory, antiarrhythmic and antihyperlipidemic activity (Ranise et al., 1994). Pyrimidine thioethers have also shown to be HIV-1 reverse transcriptase inhibitors (Nugent et al., 1998; Navrotskii, 2005; Li et al., 2010; Wu et al., 2013). Fused uracil derivatives synthesized in a one-pot reaction also demonstrated binding, chelation and fragmentation of DNA (Mousa et al., 2012; 2015).

6-Aminouracils and 6-amino-2-thiouracils (6-amino-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one) are generally the precursors for the synthesis of pyridodipyrimidines, usually formed by the formylation of uracil precursors (Youssif et al., 1999; Shaker et al., 2009; Mousa et al., 2012; Abdel-Aziem et al., 2012; Bhat and Dongre, 2015). Youssif et al. (2003) reports the synthesis of (methylene)bis(6-amino-2-thiouracil) derivatives as an intermediate prior to the formation of the dipyrimidinopyrimidines, which was carried out with glacial acetic acid and a catalytic amount of hydrochloric acid. Quinolines such as 7-chloro-5,8-quinolinedione was also reacted with 2,6-diamino-5-mercapto-4(3*H*)-pyrimidinone, similar to 6-amino-2-thiouracil to synthesise triazaphenothiazine derivatives (Ezema, 2009). Furochromone pyrimidine derivatives with anti-inflammatory and analgesic activity were also prepared by the reaction of furochromones with 6-amino-2-thiouracil (Abu-Hashem et al., 2011).

The synthesis of 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) was first reported in 1966 by Pfleiderer and co-workers (Pfleiderer et al., 1966) by condensing 6-amino-2-thiouracil with benzaldehydes under reflux conditions. The 4-methyl, 4-chloro and 4-fluorophenyl derivatives of 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) were prepared by chance on investigating the reaction of 6-amino-2-thiouracil with chalcones to produce pyrido[2,3-*d*]pyrimidine derivatives (Chebanov et al., 2005). Instead of using the chalcones themselves, they decided to use the chalcone precursors, benzaldehydes and acetophenone with 6-amino-2-thiouracil and found that condensation took place with benzaldehydes only and that acetophenone took no part in the reaction. In addition, El-kalyoubi et al. (2015) synthesised the 4-chloro, 3-nitro, 4-nitro, 4-bromo and 2-hydroxyphenyl derivatives of 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) in a similar way before forming the pyridodipyrimidines by reflux with acetic acid and HCl under reflux. They also report that the pyridodipyrimidines form without the isolation of the methylene bis(thiouracils) with longer reflux times.

To the best of our knowledge, 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) has not been investigated for their anticancer activity. We therefore carried out a simple condensation reaction of various substituted benzaldehydes with 6-amino-2-thiouracils under reflux to form the titled compounds and test them for their anticancer activity.

4.2 Results and Discussion

Synthesis

Thirteen 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) derivatives (**1-13**) were synthesized from 6-amino-2-thiouracil and substituted benzaldehydes in the presence of an acetic acid catalyst in yields of between 68-96% after a 4 hour reflux (**Figure 4-1**). Nine of

the compounds (**3**, **5** and **7-13**) were to the best of our knowledge novel. The structures of the synthesized compounds were confirmed by ^{1}H and ^{13}C NMR spectroscopy. Compounds **2** and **4**, the 2-chloro and 2,4-dichloro derivatives are commercially available and **1** and **6**, the unsubstituted and the 4-fluoro derivative were previously reported (Pfleiderer et al., 1966; Chebanov et al., 2005).

Acetic acid was used in the reaction to activate the benzaldehydes in the reaction before it was attacked by the electrons from the double bond in 6-amino-2-thiouracil, forming a hydroxy intermediate (I). Elimination of water then occurs, probably due to the conjugated system that results when water is eliminated resulting in (II). A second molecule of 6-amino-2-thiouracil then adds to the olefinic carbon in II, by a Michael addition, resulting in a 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) after abstraction of a proton from III by the acetate ion (**Figure 4-2**).

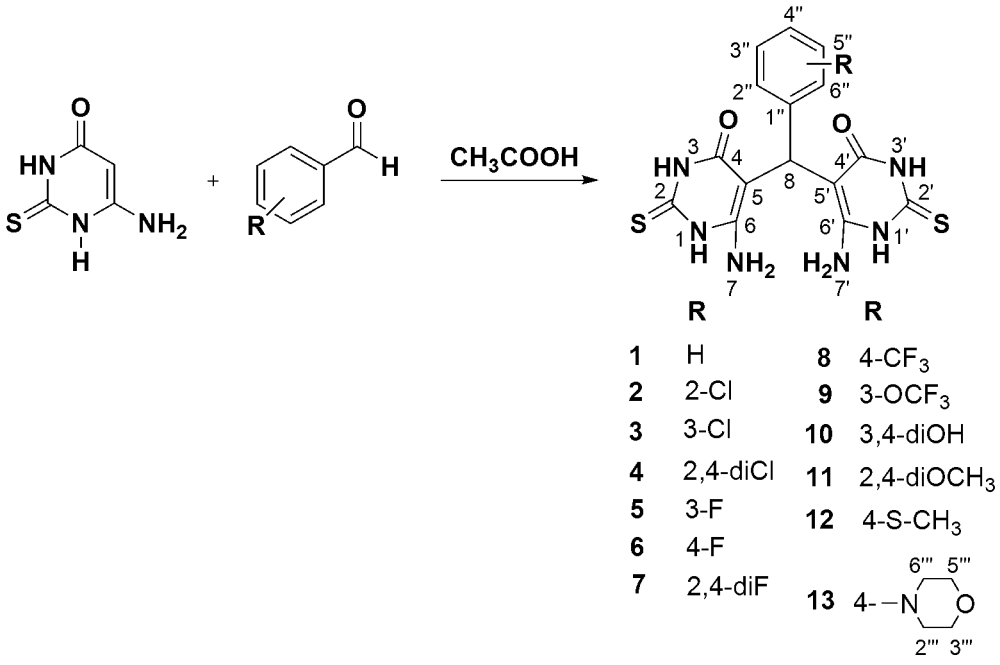

Figure 4-1 Synthesis of 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) derivatives (**1-13**)

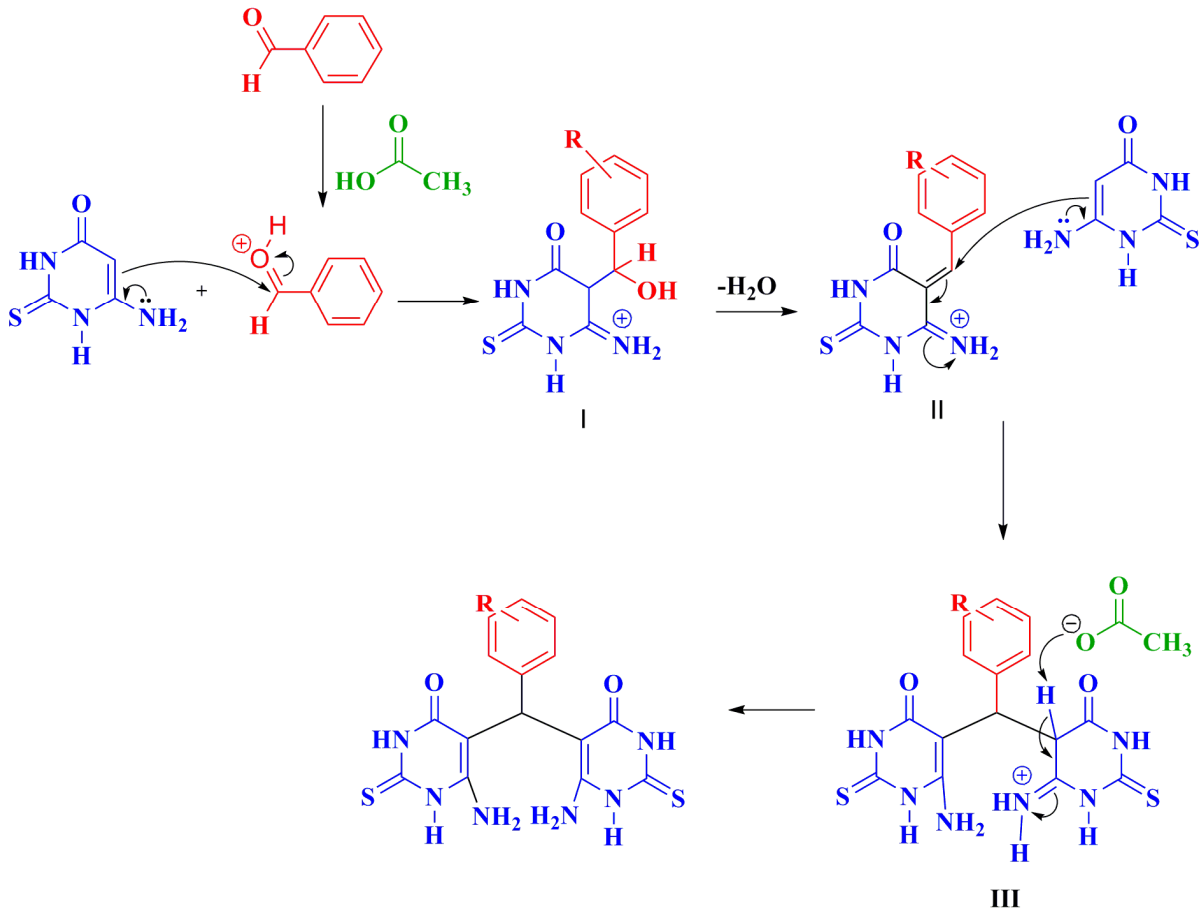

Figure 4-2 Plausible mechanism for the formation of 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) derivatives (**1-13**)

The structures of the novel compounds were elucidated using 1D ^{1}H, ^{13}C and 2D NMR spectroscopy along with mass spectrometry. Condensation of two molecules of 6-amino-2-thiouracil with benzaldeyde was indicated by HMBC correlations between H-8 (occurring as a singlet at δ 5.34 in **1**) and C-4/4' (δ 162.9), C-5/5' (δ 90.2), C-6/6' (δ 153.4), C-1" (δ 137.9) and C-2"/6" (δ 126.4). The NH protons in 2-thiouracil occurred as a sharp singlet at δ 12.03 and broad singlet at δ 11.82, each integrating to two protons relative to the H-8 resonance. The resonances of the NH_2 groups occurred at δ 6.77 as a broad singlet and the resonances of the phenyl ring appeared between δ 7.06 and δ 7.24.

Molecular docking

Human Kinesin Eg5, a member of the kinesin superfamily, plays an important role in cellular mitotic spindle formation (Blangy et al., 1995). The inhibition of this protein has been reported to cause arrest of cells in mitosis and thus serves as an excellent target for the discovery of new cancer chemotherapeutics. The efficiency of the docking protocol was checked by redocking the X-ray bound drug (KZ91367) into the binding site of Eg5, using the CDocker docking algorithm (Wu et al., 2003) embedded in Discovery Studio (DS). The results obtained revealed the interaction of the ligand with similar amino acids (Glu118, Gly117, Pro137 and Ala133) as observed in the X-ray structure. The computed all atoms root mean square deviation (RMSD) between the predicted ligand pose and its X-ray structure was found to be 0.76 Å (**Figure 4-3**), validating the accuracy of the docking method.

All the synthesized compounds (**1-13**) were subsequently docked into the binding site of Eg5 using the same docking procedure. The predicted binding affinity (BE) data for compounds **1-13** ranged between -5.0 kcal mol^{-1} to -26.7 kcal mol^{-1} (**Table 4-1**), indicating good affinity by all compounds in the active site of Eg5. In addition, a good correlation between the predicted BEs and experimental biological activity data was observed. With the exception of **9**, all compounds of the series exhibited stronger interaction than 5-FU (BE = -8.0 kcal mol^{-1}) with Eg5.

The complexes of the two most potent compounds (**4** and **7**) with Eg5 were subsequently visualized (**Figure 4-4** and **Figure 4-5**) in order to get a deeper understanding of their binding modes in the active site of the protein. It could be seen that both the compounds interacted with almost similar amino acid residues of Eg5 through hydrogen bonding and electrostatic and hydrophobic modes of interaction. Compound **7** (2,4-difluoro derivative),

the most active compound, penetrated deep into the active site of Eg5 (**Figure 4-6**), and exhibited a hydrogen bond (2.7 Å) with Gly117 through its nitrogen atom (NH_2 group) in addition to two electrostatic interactions with Arg119 and Glu116. Additionally, hydrogen bond interactions (non-conventional) with Pro137, Glu215 and Glu116 were also observed along with a few hydrophobic interactions with Trp117, Pro137 and Ala218 (**Figure 4-4**).

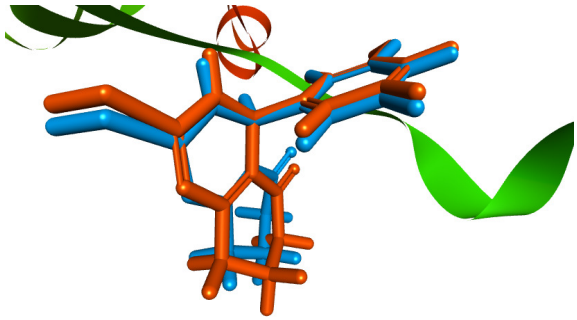

Figure 4-3 Overlay of the predicted pose of the native ligand, KZ91367 (light blue) obtained from docking with its X-ray structure (orange). RMSD (all atoms) is approximately 0.8 Å.

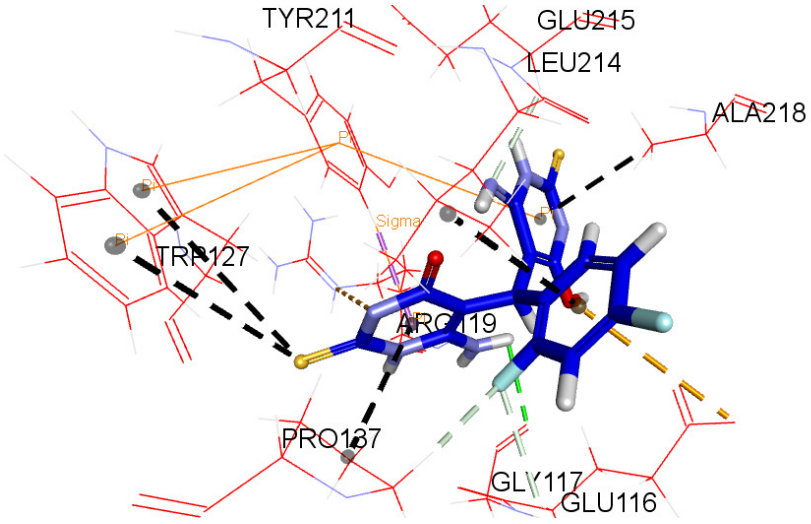

Figure 4-4 Complex showing important interactions of Eg5 amino acid residues (line format) with **7** (blue sticks). Hydrogen bonds (conventional – green; non-conventional - grey), electrostatic (gold) and hydrophobic (black) interactions are depicted**.**

Compound **4** (2,4-dichloro derivative) interacted in a similar fashion with Eg5 showing two concurrent hydrogen bonds (2.5 and 2.8 Å) through its amine (NH_2) functionalities as depicted in **Figure 4-5**. In addition, hydrophobic (Leu160, Leu214, Ala218, Arg119 and Ala133) and electrostatic interactions (Arg119, Glu116 and Trp127) were also observed.

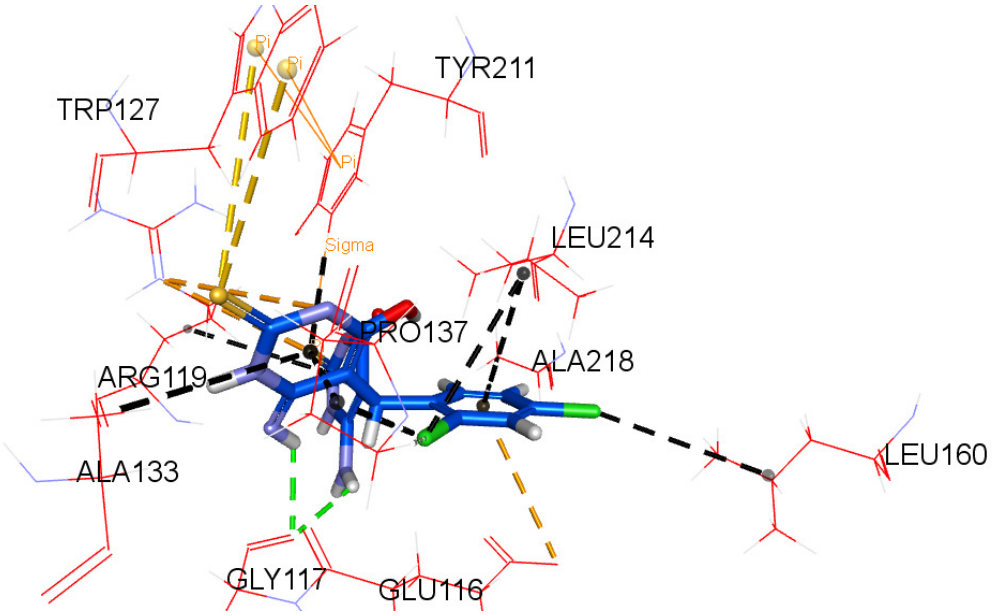

Figure 4-5 Complex showing important interactions of Eg5 amino acid residues (line format) with **4** (blue sticks). Hydrogen bonds (green dashed lines), electrostatic (golden dashed) and hydrophobic interactions (black dashed) are depicted.

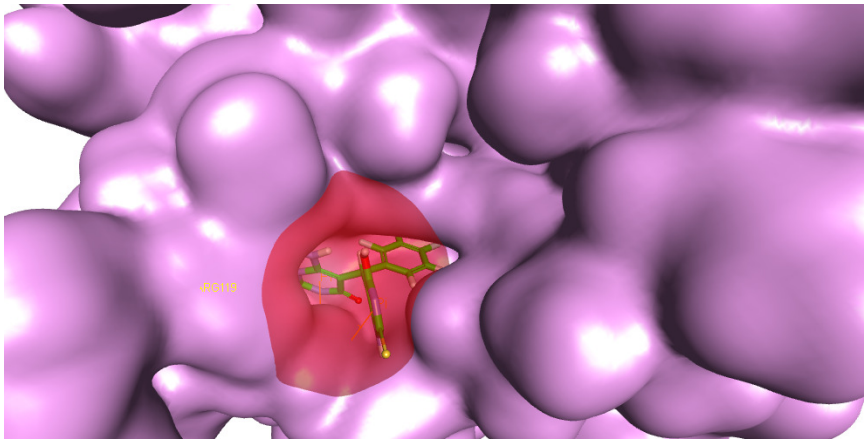

Figure 4-6 Surface representation of Eg5 showing deep penetration of **7** (green sticks) into the binding site cavity (pink sphere).

Anticancer activity

Based on the predicted results from molecular docking, the synthesized compound were tested for their anticancer activity against a cervical cancerous cell line (HeLa) using the MTT assay. The *in vitro* cytotoxicity levels of compounds **1-13** using HeLa cells are summarized in **Table 4-1**. The results indicated that almost all compounds were active at the lowest concentration (10 µg mL^{-1}), with equally low IC_{50} values, ranging from 4.18 to 10.20 µg mL^{-1}, however no real trend was seen between concentration and activity. All 13 compounds showed better IC_{50} values compared to 5FU (12.08 µg mL^{-1}) and can be considered lead compounds in cancer treatment. Further studies and testing of high activity compounds are hence warranted.

Table 4-1 Viabilities (%) of the HeLa cell lines at different concentration of compounds **1-13** including their binding affinity (BE)

Cpd	10 µg mL^{-1}	25 µg mL^{-1}	50 µg mL^{-1}	100 µg mL^{-1}	IC_{50} µg mL^{-1}	IC_{50} µM	BE[a]
1	8.28±0.04^{a}	23.12±0.13^{b,c}	24.12±0.08^{c}	19.7±0.11^{b}	5.83	15.58	-26.7
2	13.89±0.12^{b,c}	15.79±0.02^{c}	9.9±0.05^{a}	11.56±0.10^{a,b}	6.50	15.93	-17.2
3	24.93±0.05^{c}	10.42±0.10^{b}	2.24±0.04^{a}	7.85±0.01^{b}	7.80	19.12	-20.6
4	11.23±0.10^{b}	8.61±0.01^{a}	31.78±0.27^{c}	14.37±0.09^{b}	4.89	11.10	-22.6
5	5.85±0.05	8.04±0.07	6.57±0.03	7.14±0.02	7.23	18.44	-23.6
6	64.3±0.19^{c}	45.6±012^{a}	42.6±0.33^{a}	51.2±0.55^{b}	10.20	26.02	-25.3
7	10.7±0.06^{a,b}	17.13±0.05^{c}	6.28±0.11^{a}	11.23±0.06^{b}	4.18	10.20	-20.1
8	16.79±0.17^{b}	14.32±0.01^{a,b}	10.99±0.03^{a}	20.08±0.36^{c}	8.20	18.55	-15.8
9	9.37±0.03^{a}	28.5±0.05^{c}	24.45±0.02^{c}	17.17±0.05^{b}	5.83	12.73	-5.0
10	34.68±0.21^{c}	6.14±0.06^{a}	4.71±0.03^{a}	10.23±0.01^{b}	7.17	17.66	-21.1
11	14.61±0.05^{b}	6.23±0.06^{a}	12.75±0.05^{b}	11.09±0.11^{b}	5.83	13.43	-16.6
12	22.93±0.18^{b}	12.94±0.03^{a}	16.41±0.03^{a}	13.32±0.11^{a}	6.50	15.48	-22.9
13	31.59±0.05^{c}	13.32±010^{b}	6.28±0.04^{a}	11.85±0.06^{b}	5.17	11.26	-12.4
5FU	74.46±0.04^{a}	71.360.08^{a}	73.92±0.07^{a}	78.63±0.02^{b}	12.08	92.81	-8.0

[a]BE = [($BE_{complex}$-BE_{ligand}+$BE_{protein}$)\

Data are presented as the mean ± SD values of triplicate determinations. [a-c] Different superscript letters for a given value within a column are significantly different from each other (Tukey's-HSD multiple range *post hoc* test, $p < 0.05$).

4.3 Experimental

General procedure for the synthesis of 5,5'(phenylmethylene)bis(6-amino-2-thiouracil) derivatives

A solution of 6-amino-2-thiouracil (484 mg; 3.4 mmol) in glacial acetic acid (15 mL) and the appropriate aromatic aldehyde (1.5 mmol) was heated under reflux for 4 h. The reaction mixture was diluted with water and allowed to cool to room temperature. The crude product was filtered and recrystallized from ethanol to obtain the 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) derivatives in yields of between 68-96%.

(1) 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil), white powder (80% yield); mp 168-170 ºC; IR (KBr) ν_{max}: 3394 (NH), 3054, 2894, 1630 (C=O), 1597, 1548 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 12.03 (2H, bs, 2NH), 11.80 (2H, bs, 2NH), 7.21 (2H, t, *J* = 7.5 Hz, H-3",5"), 7.11 (1H, t, *J* = 7.5 Hz, H-4"), 7.07 (2H, d, *J* = 7.5 Hz, H-2",6"), 6.76 (4H, bs, $2NH_2$), 5.34 (1H, s, H-8); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 172.7 (2C, C-2,2'), 162.9 (2C, C-4,4'), 153.4 (2C, C-6,6'), 137.9 (C-1"), 127.8 (2CH, C-3",5"), 126.4 (2CH, C-2",6"), 125.3 (CH, C-4"), 90.2 (2C, C-5,5'), 32.4 (CH, C-8); HRMS (*m/z*): 373.0547 (M-H) (calculated for $C_{15}H_{13}N_6O_2S_2$, 373.0541).

(2) 5,5'-((2-chlorophenyl)methylene)bis(6-amino-2-thiouracil), white powder (78% yield); IR (KBr) ν_{max}: 3484 (NH), 2879, 1636 (C=O), 1611, 1545 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 12.02 (2H, bs, 2NH), 11.89 (2H, bs, 2NH), 7.16-7.31 (4H, m, H-3"-6"), 6.57 (4H, bs, $2NH_2$), 5.30 (1H, s, H-8); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 172.7 (2C, C-2,2'), 162.5 (2C, C-4,4'), 153.1 (2C, C-6,6'), 136.9 (C-2"), 132.3 (C-1"), 129.5 (C-3"), 128.9 (C-5"), 127.5 (C-4"), 126.6 (C-6"), 89.7 (2C, C-5,5'), 31.9 (CH, C-8); HRMS (*m/z*): 409.0300 (M+H) (calculated for $C_{15}H_{14}ClN_6O_2S_2$, 409.0308).

(3) 5,5'-((3-chlorophenyl)methylene)bis(6-amino-2-thiouracil), white powder (83% yield); IR (KBr) ν_{max}: 3397 (NH), 1635 (C=O), 1605 cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ_H 12.08 (2H, s, 2NH), 11.86 (2H, s, 2NH), 7.25 (1H, t, *J* = 7.8 Hz, H-5''), 7.18 (1H, d, *J* = 7.8 Hz, H-4''), 7.07 (1H, s, H-2''), 7.06 (1H, d, *J* = 7.8 Hz, H-6''), 6.78 (4H, bs, $2NH_2$), 5.34 (1H, s, H-8); ^{13}C NMR (100 MHz, DMSO) δ_C 172.8 (C-2/2'), 162.9 (C-4/4'), 153.4 (C-6/6'), 140.9 (C-1''), 132.7 (C-3''), 129.6 (CH, C-5''), 126.3 (CH, C-2''), 125.4 (CH, C-4''), 125.3 (CH, C-6''), 89.7 (C-5/5'), 32.3 (CH, C-8); HRMS (*m/z*): 409.0303 (M+H) (calculated for $C_{15}H_{14}ClN_6O_2S_2$, 409.0308).

(4) 5,5'-((2,4-dichlorophenyl)methylene)bis(6-amino-2-thiouracil), white powder (96% yield); IR (KBr) ν_{max}: 3383 (NH), 3358 (NH), 2877, 1649 (C=O), 1633, 1610, 1585, 1544 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 12.06 (2H, s, 2NH), 11.91 (2H, s, 2NH), 7.45 (1H, d, *J* = 2.1 Hz, H-3''), 7.31 (1H, dd, *J* = 8.5, 2.1 Hz, H-5''), 7.26 (1H, d, *J* = 8.5 Hz, H-6''), 6.55 (4H, bs, $2NH_2$), 5.25 (1H, s, H-8); ^{13}C NMR (100 MHz, DMSO) δ_C 172.7 (C-2/2'), 162.5 (C-4/4'), 153.0 (C-6/6'), 136.3 (C-2''), 133.1 (C-4''), 131.1 (C-1''), 130.3 (CH, C-6''), 128.8 (CH, C-3''), 126.7 (CH, C-5''), 89.3 (C-5/5'), 31.6 (CH, C-8); HRMS (*m/z*): 442.9912 (M+H) (calculated for $C_{15}H_{13}Cl_2N_6O_2S_2$, 442.9918).

(5) 5,5'-((3-fluorophenyl)methylene)bis(6-amino-2-thiouracil), white powder (92% yield); IR (KBr) ν_{max}: 3399 (NH), 2890, 1693 (C=O), 1605, 1555 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 12.07 (2H, s, 2NH), 11.85 (2H, bs, 2NH), 7.27 (1H, ddd*, *J* = 7.9, 7.9, 7.9 Hz, H-5''), 6.94 (1H, dd, *J* = 8.7, 2.0 Hz, H-2''), 6.92 (1H, d, *J* = 7.6 Hz, H-6''), 6.86 (1H, d, *J* = 11.0 Hz, H-4''), 6.77 (4H, bs, $2NH_2$), 5.34 (1H, s, H-8); ^{13}C NMR (100 MHz, DMSO) δ_C 172.8 (C-2/2'), 162.4 (d, *J* = 242.6 Hz, C-3''), 162.9 (C-4/4'), 153.4 (C-6/6'), 141.4, (d, *J* = 7.1 Hz, C-1''), 129.5 (CH, C-5''), 122.5 (CH, C-6''), 113.4, (CH, d, *J* = 21.4 Hz, C-4''), 112.2 (CH, d, *J* = 20.9 Hz, C-2''), 89.9 (C-5/5'), 32.4 (CH, C-8); HRMS (*m/z*): 393.0603 (M+H) (calculated for $C_{15}H_{14}FN_6O_2S_2$, 393.0604). * resonance appears as a quartet due to signal overlap.

(6) 5,5'-((4-fluorophenyl)methylene)bis(6-amino-2-thiouracil), white powder (90% yield); IR (KBr) ν_{max}: 3396 (NH), 1631 (C=O), 1601, 1550 cm^{-1}; ^{1}H NMR (400 MHz) δ_H 12.07 (2H, s, 2NH), 11.84 (2H, s, 2NH), 7.08 (2H, dd, *J* = 8.5, 5.8 Hz, H-2"/6"), 7.02 (2H, t, *J* = 8.8 Hz, H-3"/5"), 6.77 (4H, bs, $2NH_2$), 5.31 (1H, s, H-8); ^{13}C NMR (100 MHz, DMSO) δ_C 173.3 (C-2/2'), 163.4 (C-4/4'), 160.6 (d, *J* = 191.7 Hz, C-4"), 153.9 (C-6/6'), 134.4 (C-1"), 128.8 (2CH, d, *J* = 6.4 Hz, C-2"/6"), 114.8 (2CH, d, *J* = 16.9 Hz, C-3"/5"), 90.7 (C-5/5'), 32.4 (CH, C-8); HRMS (*m/z*): 393.0598 (M+H) (calculated for $C_{15}H_{14}FN_6O_2S_2$, 393.0604).

(7) 5,5'-((2,4-difluorophenyl)methylene)bis(6-amino-2-thiouracil), white powder (86% yield); IR (KBr) ν_{max}: 3385 (NH), 3052, 2897, 1634 (C=O), 1601, 1549 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 12.03 (2H, s, 2NH), 11.85 (2H, bs, 2NH), 7.12 (1H, ddd*, *J* = 8.9, 8.9, 8.9 Hz, H-6"), 7.01 (1H, ddd, *J* = 11.5, 9.1, 2.5 Hz, H-3"), 6.91 (1H, td, *J* = 8.4, 2.4 Hz, H-5"), 6.58 (4H, bs, $2NH_2$), 5.30 (1H, s, H-8); ^{13}C NMR (100 MHz, DMSO) δ_C 172.7 (C-2/2'), 162.7 (C-4/4'), 160.7 (dd, *J* = 242.6, 12.2 Hz, C-2"), 160.4 (dd, *J* = 246.7, 12.0 Hz, C-4"), 152.8 (C-6/6'), 129.5 (CH, C-6"), 122.0 (dd, *J* = 12.5, 3.6 Hz, C-1"), 110.3 (CH, d, *J* = 20.7 Hz, C-5"), 103.4 (CH, t, *J* = 25.9 Hz, C-3"), 89.5 (C-5/5'), 28.2 (CH, C-8); A mass spectrum could not be detected for this molecule.

(8) 5,5'-((4-trifluoromethylphenyl)methylene)bis(6-amino-2-thiouracil), white powder (84% yield); IR (KBr) ν_{max}: 3396 (NH), 3052, 2896, 1634 (C=O), 1601, 1550; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 12.09 (2H, s, 2NH), 11.88 (2H, s, 2NH), 7.56 (2H, d, *J* = 8.2 Hz, H-3"/5"), 7.30 (d, *J* = 8.2 Hz, H-2"/6"), 6.77 (4H, bs, $2NH_2$), 5.40 (1H, s, H-8); ^{13}C NMR (100 MHz, DMSO) δ_C 172.9 (C-2/2'), 162.9 (C-4/4'), 153.4 (C-6/6'), 143.3 (C-1"), 127.4 (C-2"/6"), 126.1 (q, *J* = 31.5 Hz, C-4"), 124.5 (q, *J* = 270.2 Hz, $\underline{C}F_3$), 124.6 (C-3"/5"), 89.7 (C-5/5'), 32.6 (CH, C-8); HRMS (*m/z*): 443.0562 (M+H) (calculated for $C_{16}H_{14}F_3N_6O_2S_2$, 443.0572).

(9) 5,5'-((3-trifluoromethoxyphenyl)methylene)bis(6-amino-2-thiouracil), white powder (72% yield); IR (KBr) ν_{max}: 3400 (NH), 2855, 1610, 1546 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 12.12 (2H, s, 2NH), 11.90 (2H, bs, 2NH), 7.39 (1H, t, *J* = 8.0 Hz, H-5''), 7.15 (2H, d*, *J* = 8.0 Hz, H-4''/6''), 7.04 (1H, s, H-2''), 6.81 (4H, bs, $2NH_2$), 5.40 (1H, s, H-8); ^{13}C NMR (100 MHz, DMSO) δ_C 172.8 (C-2/2'), 172.0 (C-3''), 162.9 (C-4/4'), 153.4 (C-6/6'), 141.2 (C-1''), 129.5 (CH, C-5''), 125.7 (CH, C-6''), 120.1 (q, *J* = 254.3 Hz, O<u>C</u>F_3), 119.3 (CH, C-2''), 117.7 (CH, C-4''), 89.7 (C-5/5'), 32.4 (CH, C-8); HRMS (*m/z*): 457.0364 (M^+-H) (calculated for $C_{16}H_{12}F_3N_6O_3S_2$, 457.0364). *overlapping doublets.

(10) 5,5'-((3,4-dihydroxyphenyl)methylene)bis(6-amino-2-thiouracil), yellow powder (92% yield); IR (KBr) ν_{max}: 3171, 1650 (C=O), 1581, 1533 cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ_H 11.97 (2H, s, 2NH), 11.76 (2H, bs, 2NH), 8.59 (1H, s, 4'-O<u>H</u>), 8.42 (1H, s, 3'-O<u>H</u>), 6.72 (4H, bs, $2NH_2$), 6.52 (1H, d, *J* = 8.2 Hz, H-5''), 6.43 (1H, d, *J* = 1.2 Hz, H-2''), 6.26 (1H, dd, *J* = 8.2, 1.2 Hz, H-6''), 5.17 (1H, s, H-8); ^{13}C NMR (100 MHz, DMSO) δ_C 172.6 (C-2/2'), 162.9 (C-4/4'), 153.3 (C-6/6'), 144.7 (C-4''), 142.8 (C-3''), 128.4 (C-1''), 117.1 (CH, C-6''), 115.1 (CH, C-5''), 113.9 (CH, C-2''), 90.7 (C-5/5'), 31.7 (CH, C-8); HRMS (*m/z*): 407.0573 (M+H) (calculated for $C_{15}H_{15}N_6O_4S_2$, 407.0596).

(11) 5,5'-((2,4-dimethoxyphenyl)methylene)bis(6-amino-2-thiouracil), yellow powder (68% yield); IR (KBr) ν_{max}: 3398 (NH), 3314 2885, 1634 (C=O), 1610, 1547 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 11.90 (2H, s, 2NH), 11.77 (2H, bs, 2NH), 6.90 (1H, d, *J* = 8.4 Hz, H-6''), 6.51 (4H, bs, $2NH_2$), 6.42 (1H, d, *J* = 2.4 Hz, H-3''), 6.39 (1H, dd, *J* = 8.4, 2.4 Hz, H-5''), 5.18 (1H, s, H-8), 3.71(3H, s, 2''-OCH_3), 3.62 (3H, s, 4''-OCH_3); ^{13}C NMR (100 MHz, DMSO) δ_C 172.5 (C-2/2'), 162.7 (C-4/4'), 158.9 (C-2''), 158.2 (C-4''), 152.7 (C-6/6'), 127.8 (CH, C-6''), 119.1 (C-1''), 104.0 (CH, C-5''), 98.4 (CH, C-3''), 91.1 (C-5/5'), 55.7 (2''-O<u>C</u>H_3), 55.1 (4''-O<u>C</u>H_3), 28.7 (CH, C-8); HRMS (*m/z*): 457.0721 (M+Na) (calculated for $C_{17}H_{18}N_6O_4S_2Na$, 457.0729).

(12) 5,5'-((4-methylthiophenyl)methylene)bis(6-amino-2-thiouracil), yellow powder (89% yield); IR (KBr) ν_{max}: 3393 (NH), 3060, 2895, 1632 (C=O), 1599, 1550 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 12.03 (2H, s, 2NH), 11.80 (2H, bs, 2NH), 7.10 (2H, d, *J* = 8.2 Hz, H-3''/5''), 6.99 (2H, d, *J* = 8.2 Hz, H-2''/6''), 6.75 (4H, bs, $2NH_2$), 5.28 (1H, s, H-8), 2.41 (3H, s, S-C$\underline{H}_3$); ^{13}C NMR (100 MHz, DMSO) δ_C 172.7 (C-2/2'), 162.9 (C-4/4'), 153.3 (C-6/6'), 134.8 (C-1''), 134.3 (C-4''), 127.2 (2CH, C-2''/6''), 125.8 (2CH, C-3''/5''), 90.1 (C-5/5'), 31.8 (CH, C-8), 15.0 (S-$\underline{C}H_3$); HRMS (*m/z*): 421.0571 (M+H) (calculated for $C_{16}H_{17}N_6O_2S_3$, 421.0575).

(13) 5,5'-((4-morpholinophenyl)methylene)bis(6-amino-2-thiouracil), yellow powder (90% yield); IR (KBr) ν_{max}: 3422 (C=O), 3310, 3180, 2894, 1635 (C=O), 1539 cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ_H 12.01 (2H, s, 2NH), 11.80 (2H, s, 2NH), 6.91 (2H, d, *J* = 8.5 Hz, H-2''/6''), 6.80 (2H, d, *J* = 8.5 Hz, H-3''/5''), 6.79 (4H, bs, $2NH_2$), 5.27 (1H, s, H-8), 3.71 (4H, t, *J* = 4.4 Hz, H-3'''/5'''), 3.03 (4H, t, *J* = 4.4 Hz, H-2'''/6'''); ^{13}C NMR (100 MHz, DMSO) δ_C 172.7 (C-2/2'), 162.9 (C-4/4'), 153.3 (C-6/6'), 148.7 (C-4''), 128.4 (C-1''), 127.0 (2CH, C-2''/6''), 114.8 (2CH, C-3''/5''), 90.5 (C-5/5'), 66.1 (C-3'''/5'''), 48.7 (C-2'''/6'''), 31.6 (CH, C-8); HRMS (*m/z*): 482.1065 (M+Na) (calculated for $C_{19}H_{21}N_7O_3S_2Na$, 482.1045).

Cytotoxicity tests

Human cervical cancer (HeLa) cells were purchased from Highveld Biological (Pty) Ltd. (Lyndhurst, RSA). Cells were grown to semi-confluency in 25 cm^2 tissue culture flasks in EMEM (Eagle's Minimum Essential Medium, Lonza BioWhittaker, Verviers, Belgium) supplemented with 10% fetal bovine serum and antibiotics (100 U mL^{-1} penicillin, 100 µg mL^{-1} streptomycin). For the MTT assay, cells were seeded at a density of 1.8 $x10^3$ cells per well in a 96 well plate containing 100 µL of medium. The cells were then incubated for 24 h at 37°C in 5% CO_2, after which the medium was removed and 100 µL fresh medium was added.

Compounds **1-13** at concentrations of 10 µg mL^{-1}, 25 µg mL^{-1}, 50 µg mL^{-1} and 100 µg mL^{-1}, were then added in triplicate to the cells and incubated for 48 h at 37 ºC. 5FU (5-fluorouracil) was used as a standard positive control.

The MTT assay was adapted from that of Mosmann et al. (1983) and measured the metabolic activity of cells by the reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to formazan using the succinate-tetrazolium reductase system. After 48 h of incubation, the spent medium was replaced with 100 µL fresh medium and 100 µL of MTT (5 mg mL^{-1} in PBS), and the cells incubated for 4 h at 37 ºC. Thereafter, the medium and MTT were removed, and 200 µL of DMSO was added to each well to dissolve the formazan salt. The absorbance of the resulting purple solution was read in a Mindray 96A microplate reader (Vacutec, Hamburg, Germany) at 570 nm (detection l) and 630 nm (reference l for nonspecific signals) wavelengths. Cell viability (%) was directly correlated to absorbance and calculated in comparison to the untreated control as follows:

$[(OD_{570}\text{Treated} - OD_{630}\text{Treated}) / (OD_{570}\text{Control} - OD_{630}\text{Control}) \times 100)]$

Tests were conducted in triplicate and calculation of the concentration at which 50% cell death was achieved (IC_{50}) was carried out using Microsoft Excel 2010™.

Molecular docking

The atomic co-ordinates of Human EG5 protein (pdb id: 2X7C) were retrieved from the protein data bank (http://www.rcsb.org). All chains with the exception of the B-chain with the native ligand, (s)-Enastron (KZ91367) were removed using DS visualizer. Initially, the protonation state of protein was determined at physiological pH followed by its minimization using the conjugate gradient algorithm with the CHARMm force field, using the Prepare Protein algorithm in DS. All hydrogen atoms of the protein were constrained during the

minimization process by applying the shake algorithm. A sphere of diameter dimensions 6.34 Å with co-ordinates 16.8 (X), 14.4 (Y) and -30.9 (Z), covering all the active site residues was generated using the Define and Edit Binding site module embedded in DS. The 3D structure of each synthesized compound was subjected to "Prepare Ligands" in DS to generate its isomers at physiological pH. The isomers obtained were further minimized and the isomer with the lowest CHARMm energy selected for docking. The CDocker docking program available in DS was used to dock compounds in the binding site of the protein (Wu et al., 2003). This program samples different conformations of the ligand during docking simulation by keeping the protein conformation fixed. A total of 10 poses of each ligand were ranked on the basis of their scoring function (-CDocker energy).

4.4 Conclusion

A series of 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) derivatives were synthesized in good yields from 6-amino-2-thiouracil and benzaldehyde derivatives. All the synthesised compounds showed better activity than 5-FU in a cancerous HeLa cell line. Molecular docking indicated that most compounds displayed stronger interactions than 5-FU with Eg5. This series of compounds may be good lead compounds in cancer therapy.

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Chapter 5. Conclusion

Two types of pyrimidine derivatives were successfully synthesised and characterised. The first type was of the 7-amino-5-aryl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-diones and the second was of the 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) type. Various substituted aryl derivatives of 7-amino-5-aryl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-diones with hydroxy, methoxy, chloro, nitro and fluoro substituents on the phenyl ring were synthesised in excellent yields (90-96%) in a one-pot reaction from substituted aromatic benzaldehydes, malonitrile and barbituric acid using DABCO as a catalyst in aqueous ethanol in 1 hour at room temperature. An investigation into several catalysts including DABCO, DBA, triethylamine, K_2CO_3 and L-proline indicated that DABCO was the best catalyst for the reaction. This reaction using DABCO as a catalyst is therefore a fast, easy route for the synthesis of pyranopyrimidines from aldehydes, malonitrile and barbituric acid. Variation can be introduced into the molecule using various substituted aldehydes and small libraries of these types of compounds can quickly be synthesised and tested for their bioactivity.

A total of thirteen 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) derivatives were successfully synthesised by a condensation reaction between 6-amino-2-thiouracil and benzaldehyde derivatives in glacial acetic acid under reflux with yields of 68-96%. The duration of the reaction was 4 hours. There are variable reports in the literature which indicate that refluxing for 4-6 hours (El-kalyoubi et al., 2015) or 2-7 hours (Youssif and Mohamed, 2008) results in pyridodipyrimidines, however we did not synthesise pyridopyrimidines in our work. This was confirmed by High Resolution Mass Spectroscopy. This method represents a relatively fast and easy method for condensing benzaldehydes and 6-amino-2-thiouracils to form the target compounds. The products must be characterised by

Mass spectrometry to detect whether or not the pyridopyrimidines or the 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) derivatives formed.

All the synthesised compounds were easily purified either by recrystallization or column chromatography and characterised by 1D and 2D NMR and Mass spectroscopy. A total of 12 compounds, three from the pyranopyrimidine series (**A3**, **A7** and **B11)** and 9 from the 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) series (**C3**, **C5** and **C7-13**) were novel derivatives.

The synthesized compounds were tested for their antibacterial and anticancer activity since pyrimidines have been known to be active in antibacterial and anticancer assays. The compounds were tested against five bacterial strains, two Gram +ve, *Staphylococcus aureus* and MRSA and three Gram –ve strains, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. The oxygenated pyranopyrimidines performed better in the antibacterial assays than the chloro, fluoro and nitro derivatives. In particular, **A6** (2',3'-dimethoxy derivative) showed broad spectrum activity amongst the strains tested having IC_{50} values of 45.6 µM and 91.3 µM against *S. aureus* and *E. coli* respectively. The 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) derivatives did not show any antibacterial activity in preliminary disk diffusion assays carried out. The results indicated that even though **A6** showed some good antibacterial activity, these classes of compounds in general were not suitable as antibacterial agents.

In general, all the synthesised compounds showed good activity in the anticancer assays carried out. This could be due to their structural similarity with known anticancer agents such as 5-FU, which was used as a standard for comparison in this work. Compounds **A1-A7**

were tested against three cell lines, HeLa (cervical cancer), Caco-2 (colorectal adenocarcinoma) and HEK293 (human embryonic kidney). All the compounds were comparable to 5-FU in all three cell lines, however three compounds, **A2**, **A5** and **A7** had IC_{50} values ten times lower than 5-FU in the HeLa cell line (3.46, 4.36 and 4.44 μM respectively opposed to 41.85 μM for 5-FU) and could be excellent lead compounds for anticancer treatment. The other two sets of compounds, **B1-11** and **C1-13** were tested only in the HeLa cell line, since the first set of compounds were most active in this cell line. The anticancer activity of **B1-11** was comparable to 5-FU in the same assay and that of **C1-13** was 3-8 times better than 5-FU. The three most active compounds of this series were the 2,4-difluoro derivative (**7**) (IC_{50} 10.20 μM), the 2,4-dichloro derivative (**4**) (IC_{50} 11.10 μM) and the morpholine derivative (**13**) (IC_{50} 11.26 μM). Thus, the 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) could also be good leads for anticancer treatment and need to be explored further.

In order to provide a possible explanation for the anticancer activity shown by the pyrimidines synthesised in this work, two representative compounds from each of the series A and B, **A1**, **A7**, **B4** and **B8** were docked computationally into the active site of a human kinesin molecular motor protein essential in mitosis, Eg5. This enzyme is a common target for anticancer treatment. All four compounds had better calculated binding energies than 5-FU (-180.25, -140.9, -184.4 and -150.1 kcal mol^{-1} respectively as opposed to -116.7 kcal mol^{-1} for 5-FU). For the 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) series **C1-13**, all compounds were docked with Eg5 computationally using an implicit solvent model. All the compounds in this series, except for **C-9** (-5.0 kcal mol^{-1}) showed better binding energies than 5-FU (-8.0 kcal mol^{-1}). Their binding energies ranged from -12.4 to 26.7 kcal mol^{-1}. The molecular docking studies thus indicate that a possible mechanism for the anticancer

activity could be due to the synthesised compounds binding with the Eg5 protein, altering its structure slightly, causing the enzyme to lose its function.

Future Work

There are three good leads for anticancer treatment from the first set of compounds **A2**, **A5** and **A7** and all of 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) derivatives **C1-13** are also good leads for cancer therapy. These compounds need to be explored further and tested *in vivo* to see whether or not they could be used in anticancer treatment. They also need to be tested in other cancerous cell lines to see whether they can be used for the treatment of other cancerous cells as well.

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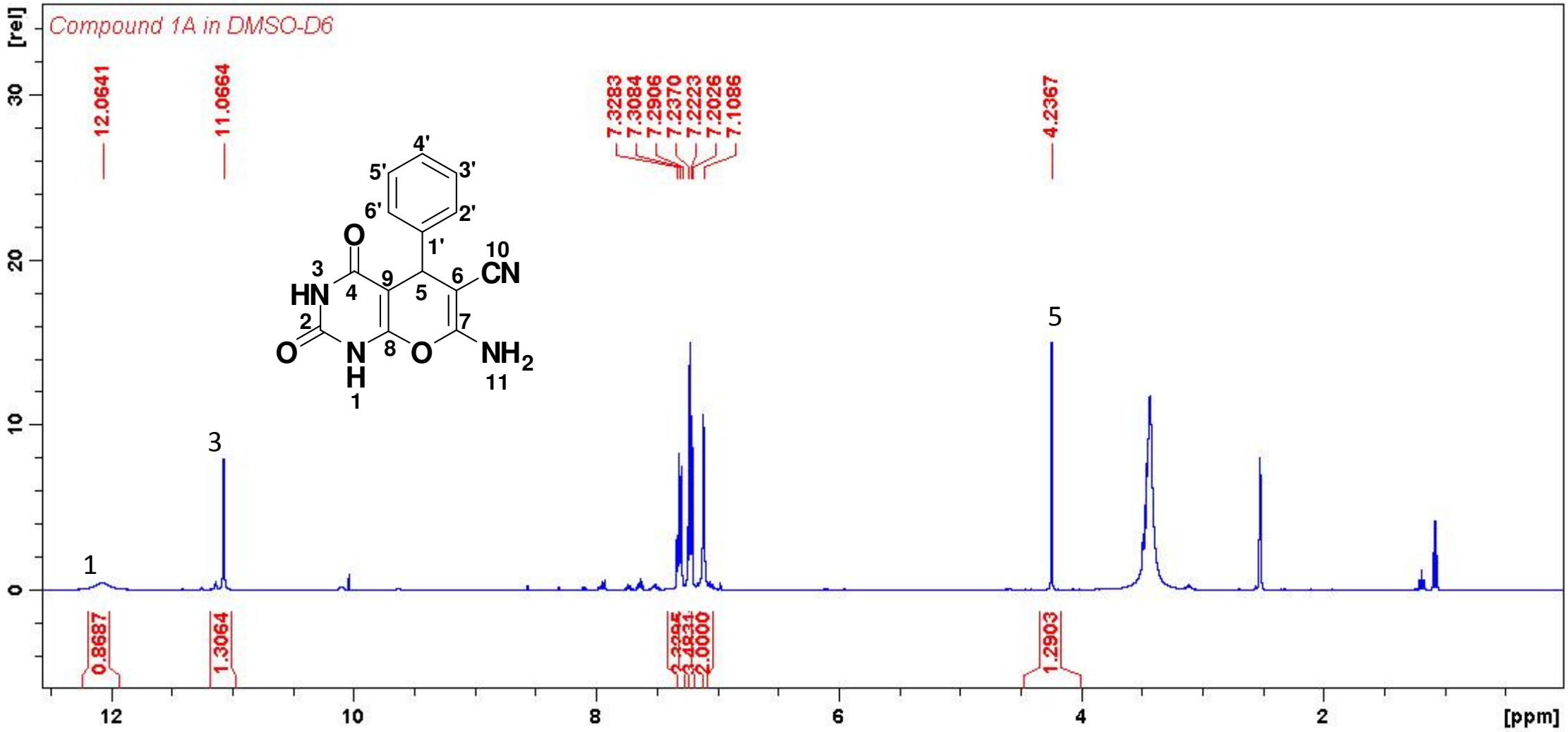

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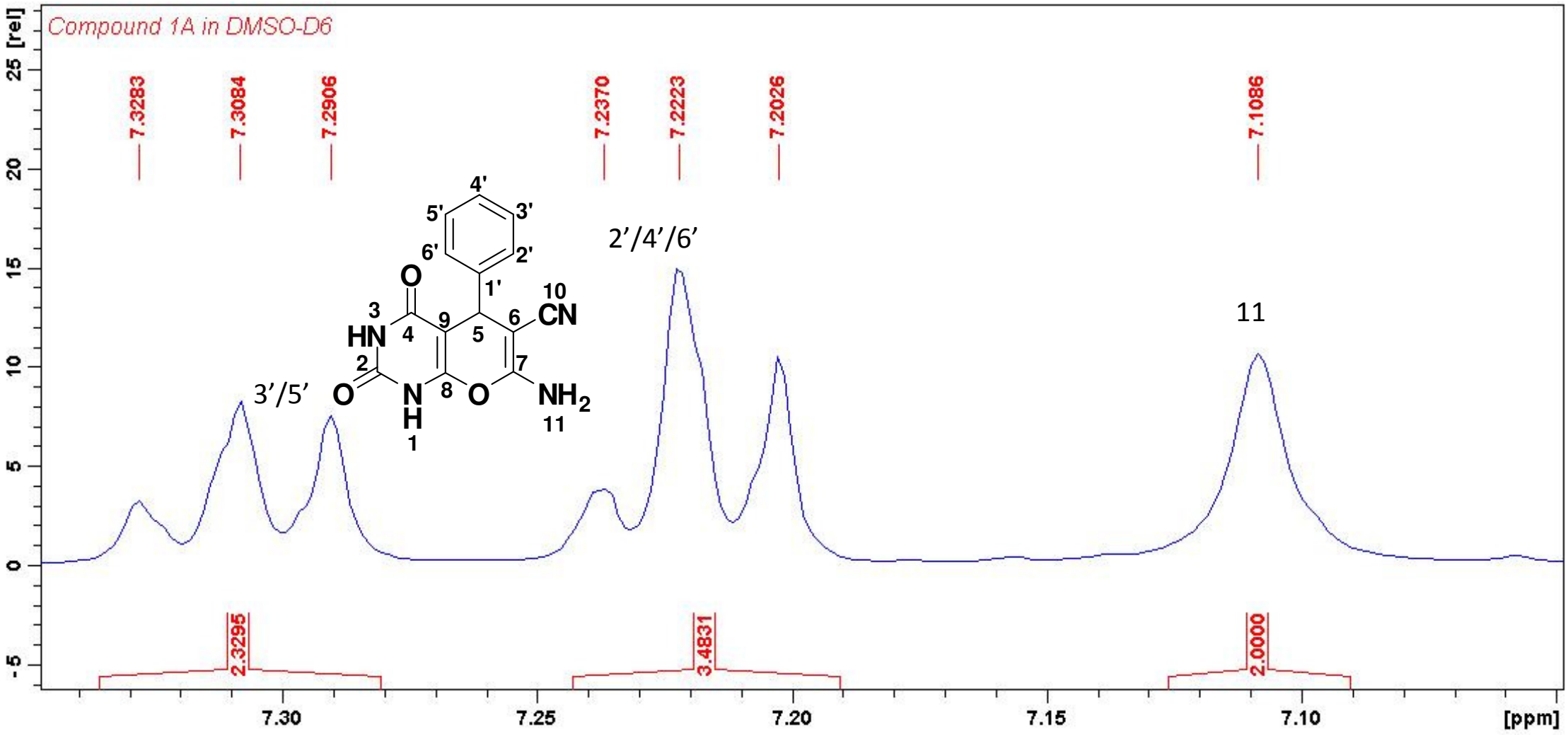
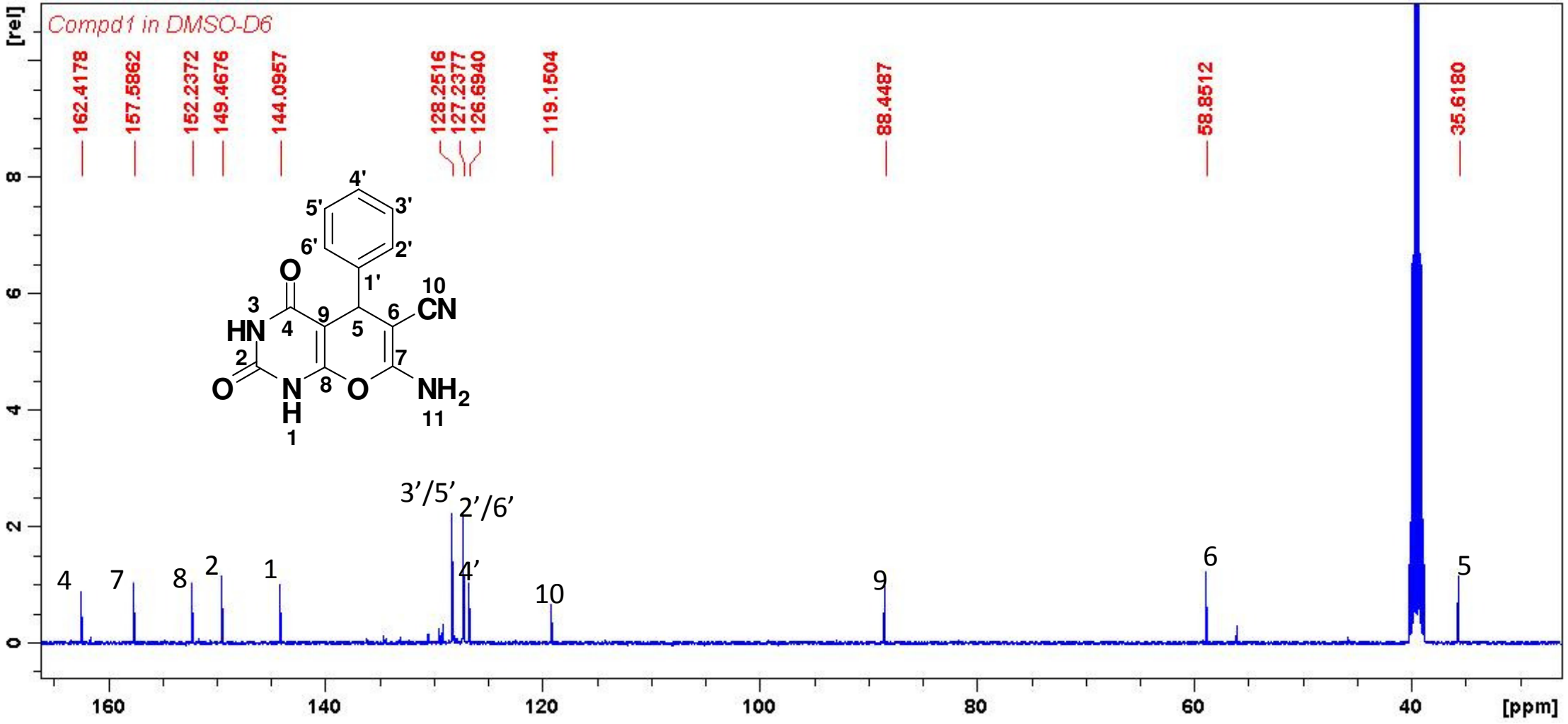
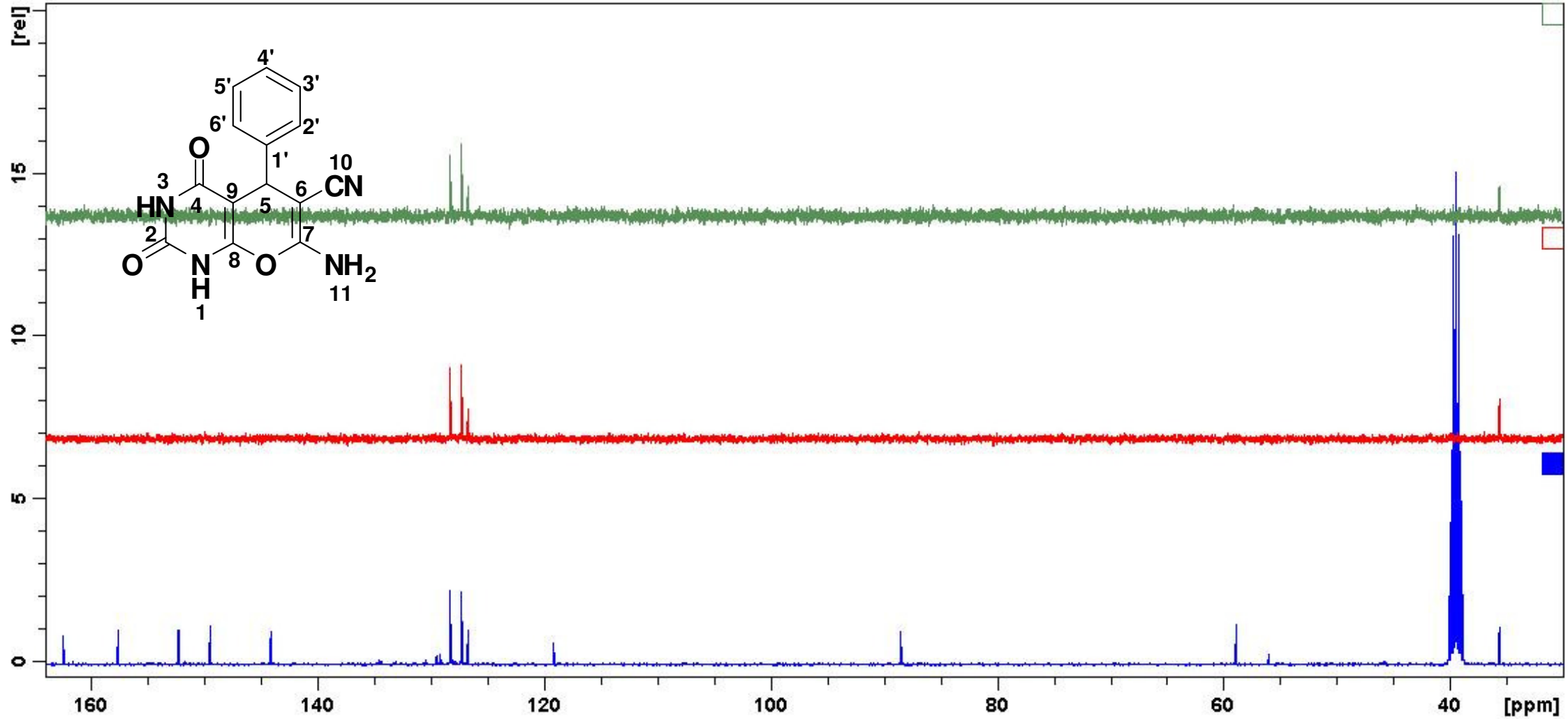


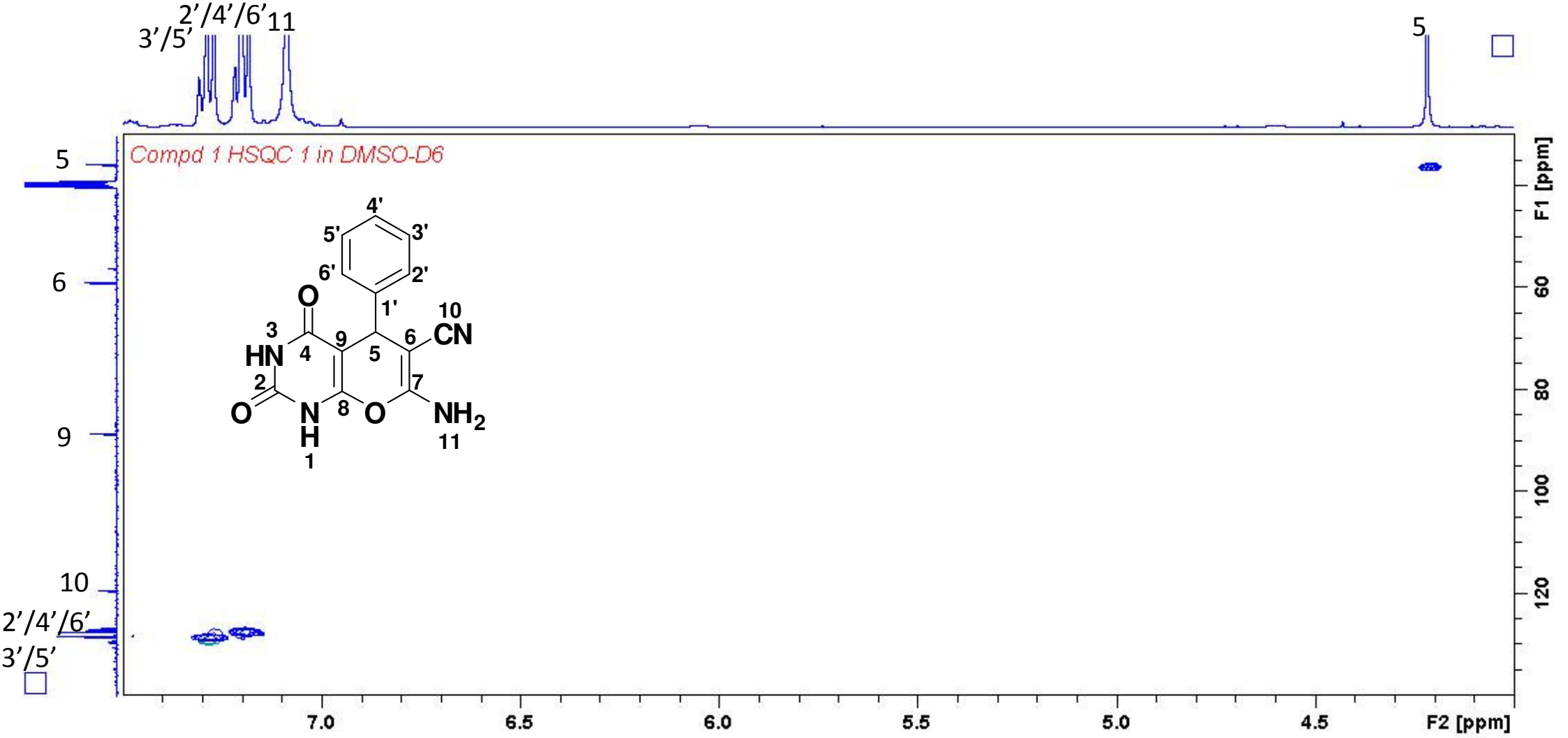
^{1}H NMR spectrum of 7-amino-5-phenyl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A1)



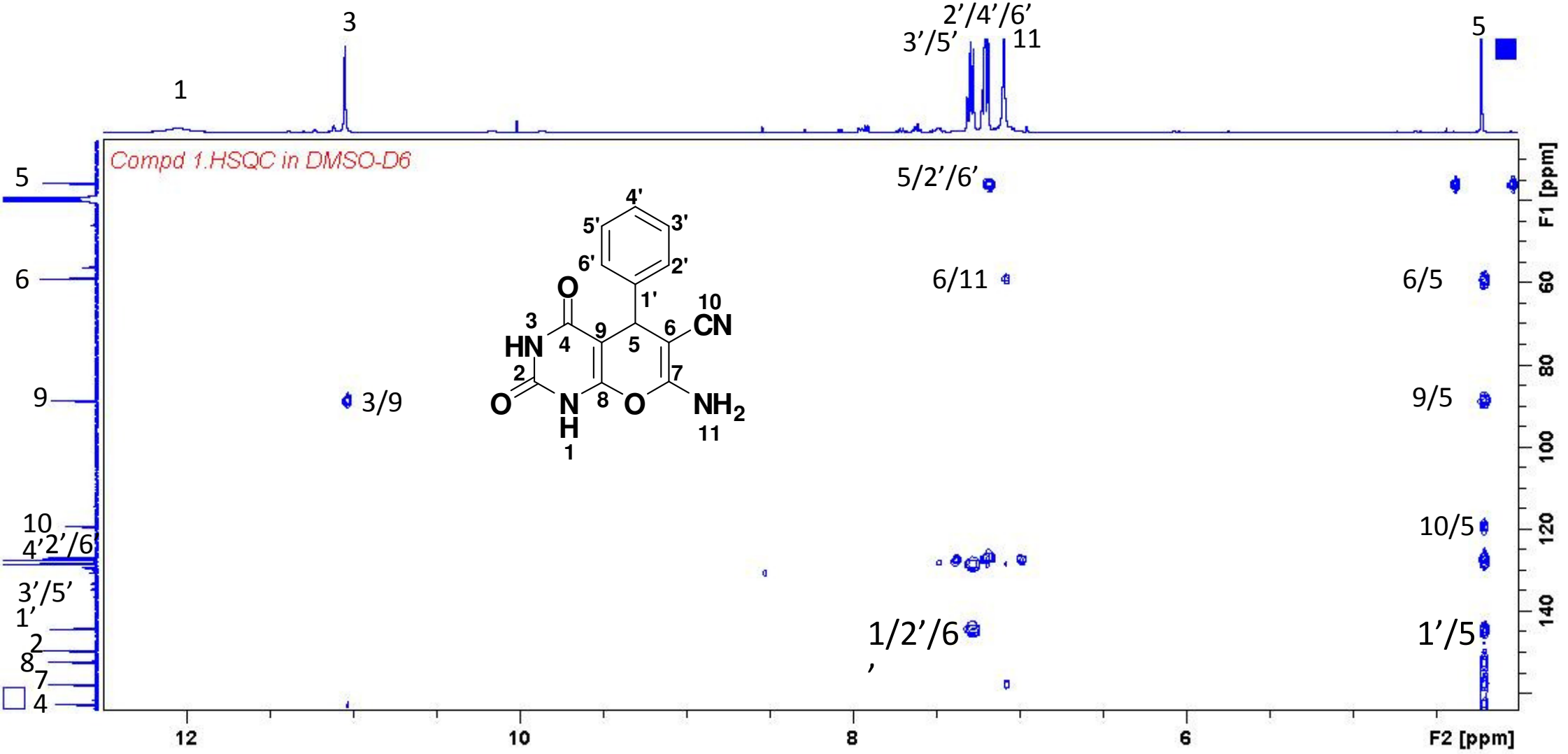
^{13} C NMR spectrum of 7-amino-5-phenyl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A1)



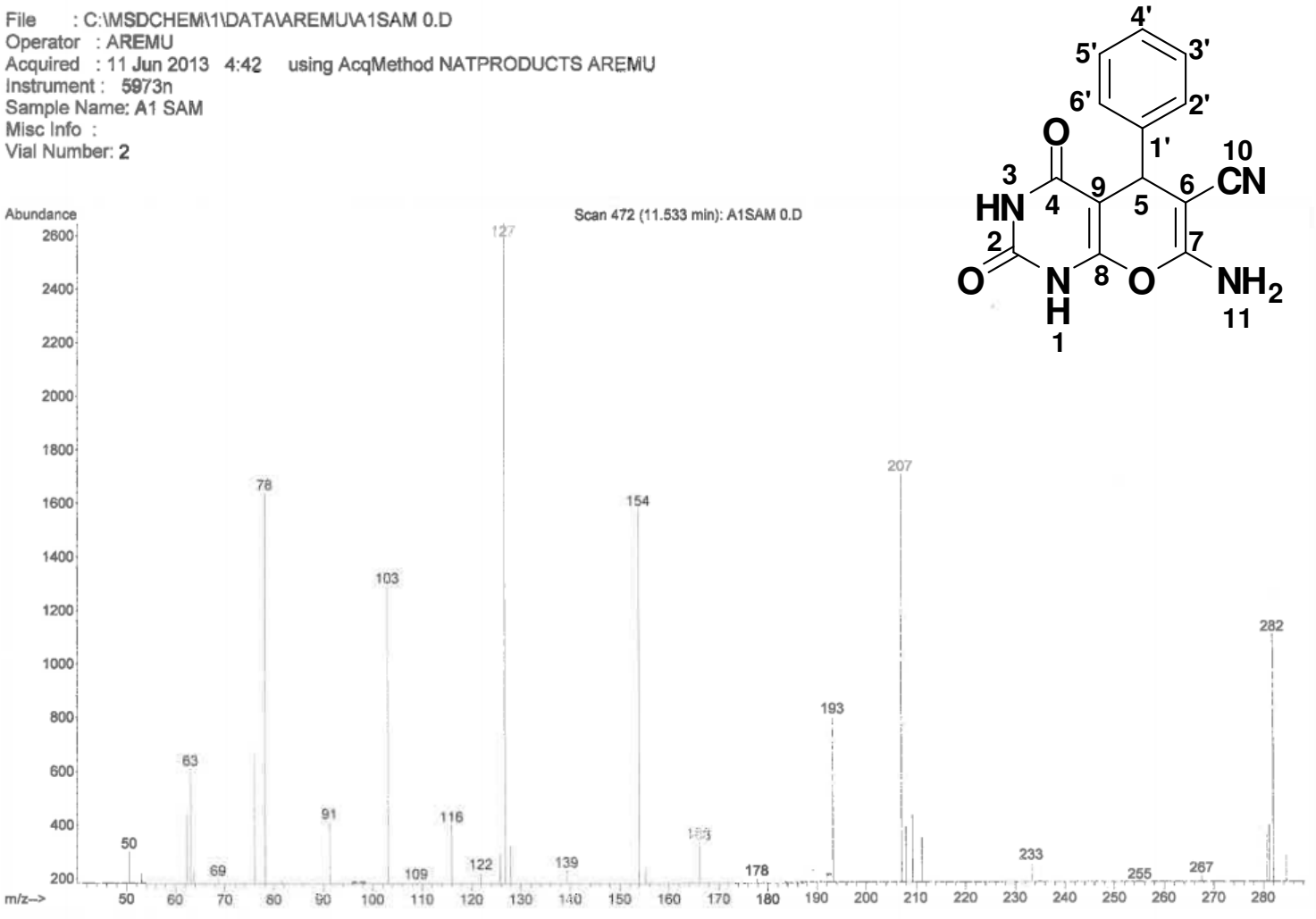
DEPT spectrum of 7-amino-5-phenyl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A1)



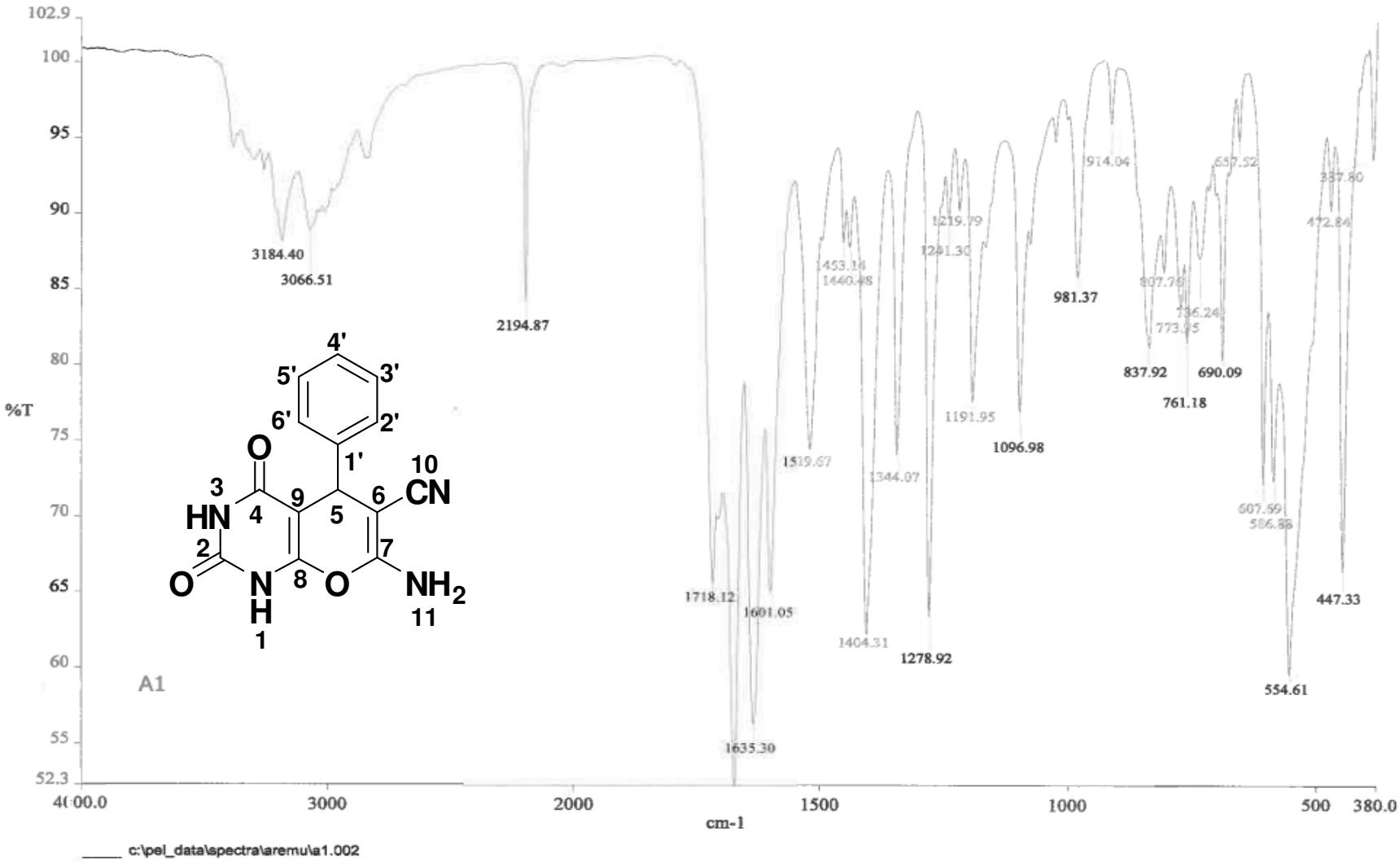
HSQC spectrum of 7-amino-5-phenyl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A1)



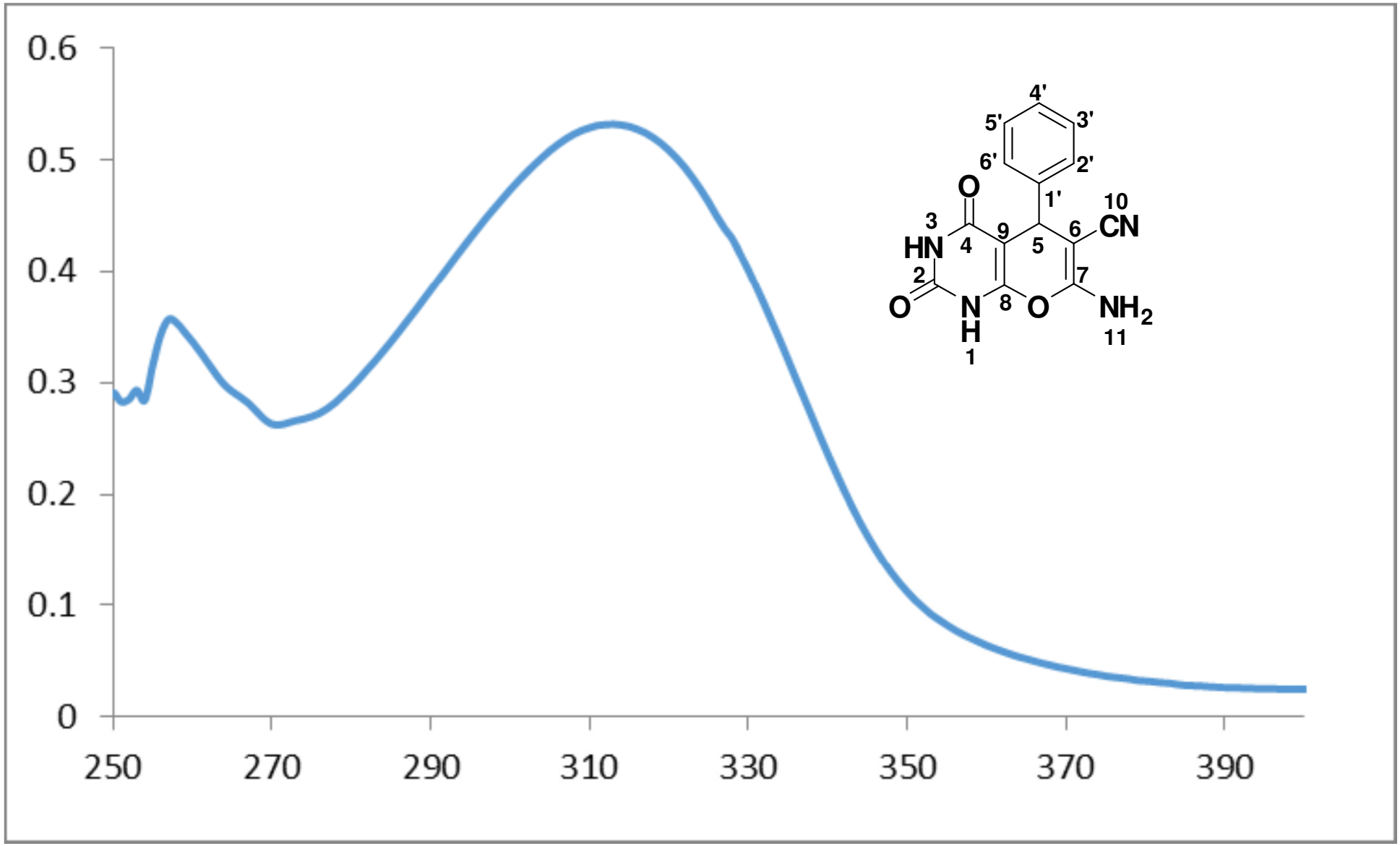
HMBC spectrum of 7-amino-5-phenyl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A1)



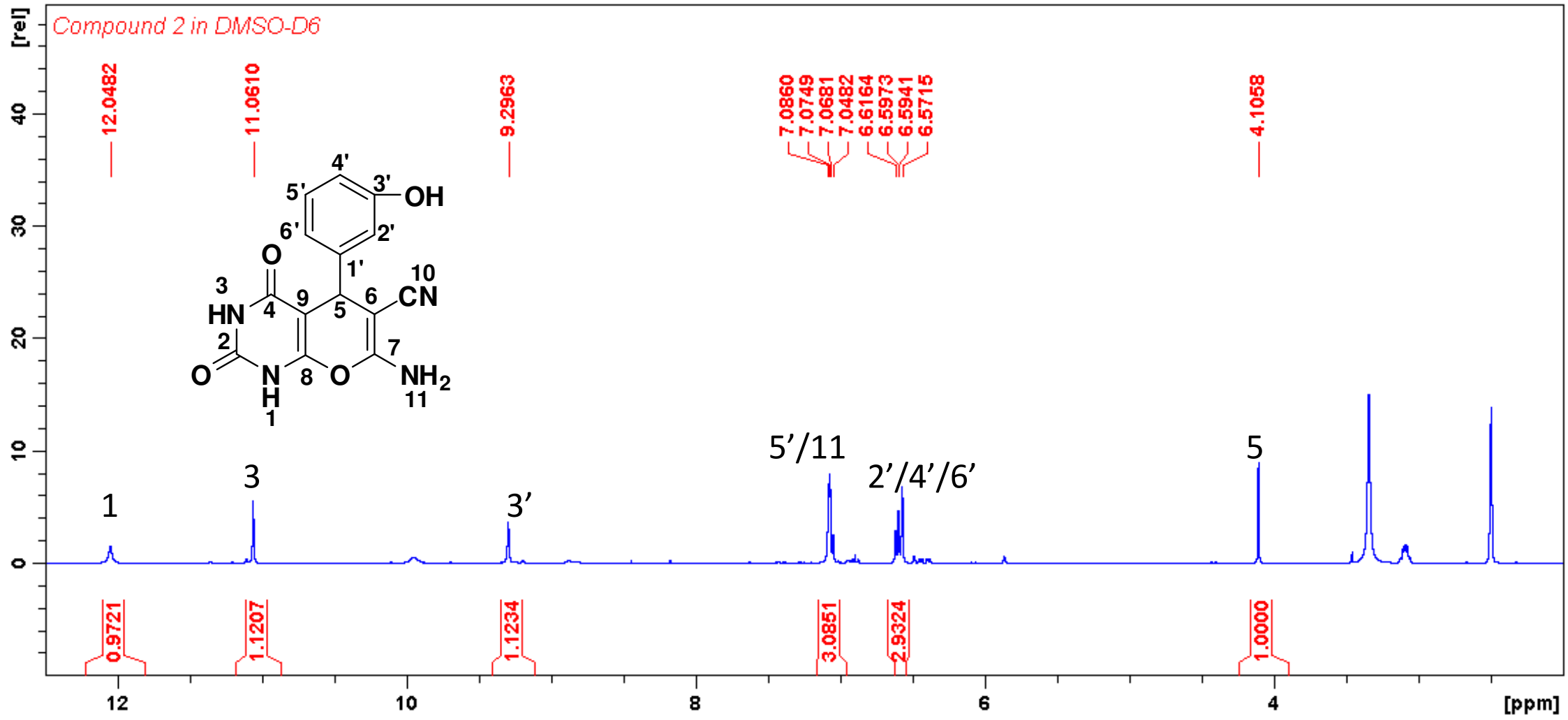
Mass spectrum of 7-amino-5-phenyl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A1)



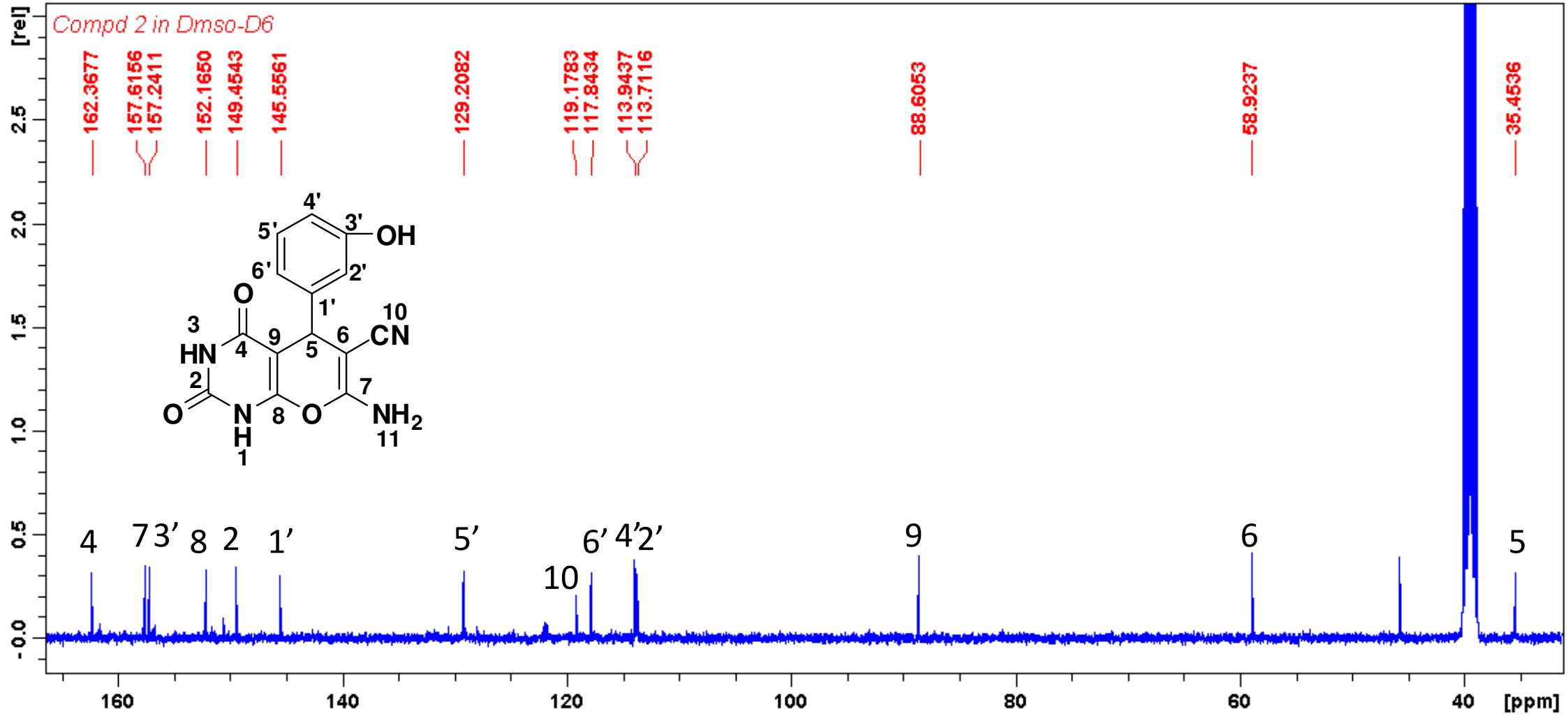
Infra red spectrum of 7-amino-5-phenyl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A1)



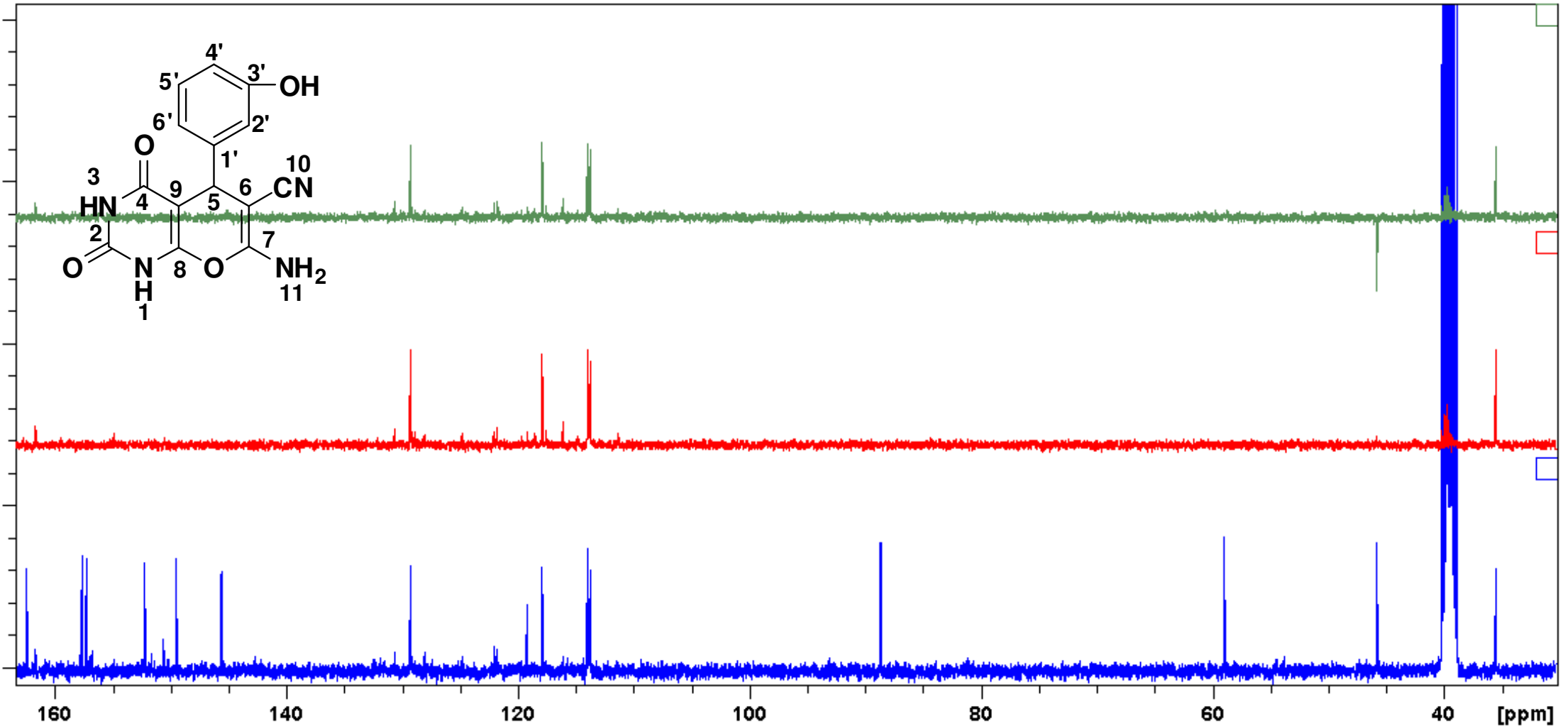
UV spectrum of 7-amino-5-phenyl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-diones (A1)



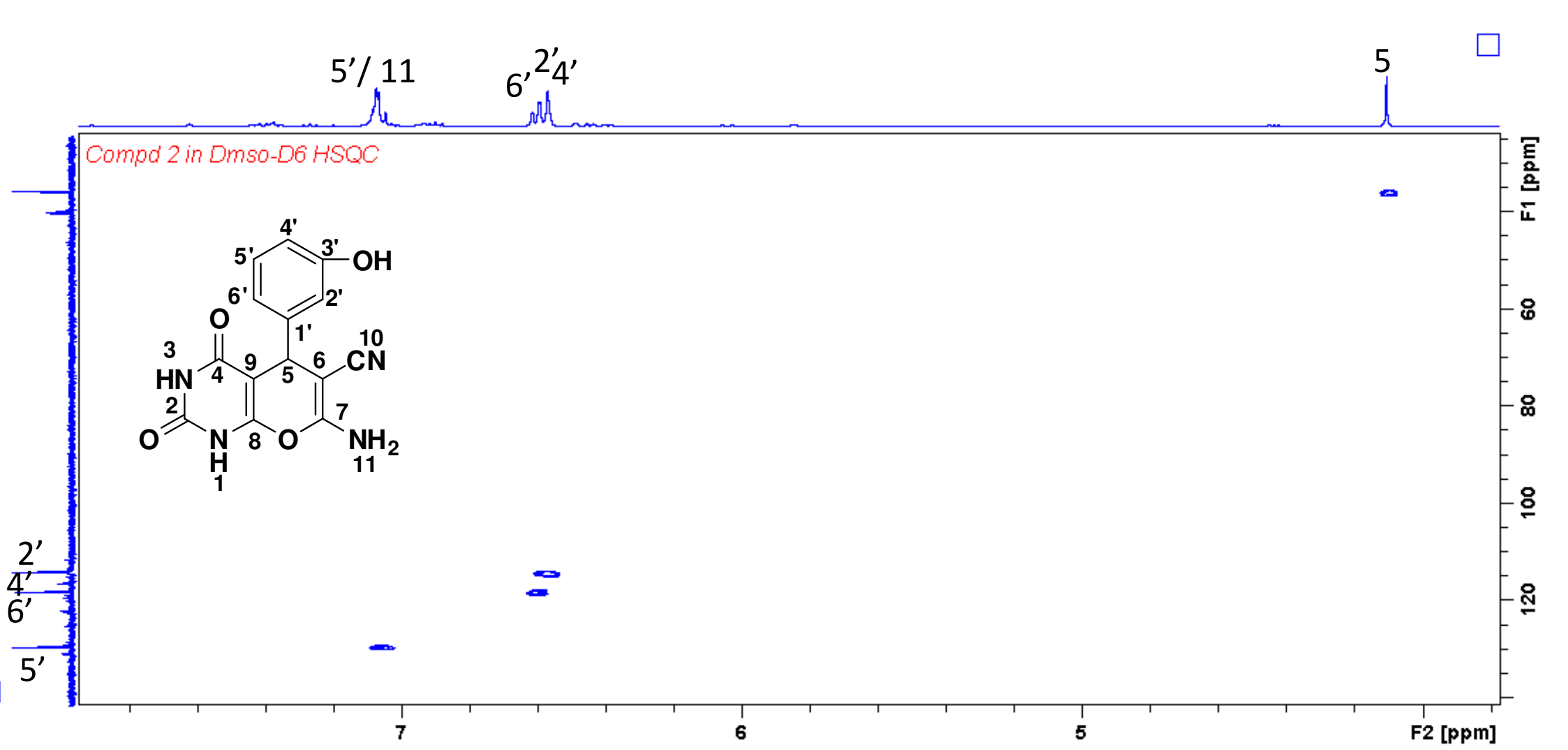
^{1}H NMR spectrum of 7-amino-5-(3-hydroxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A2)



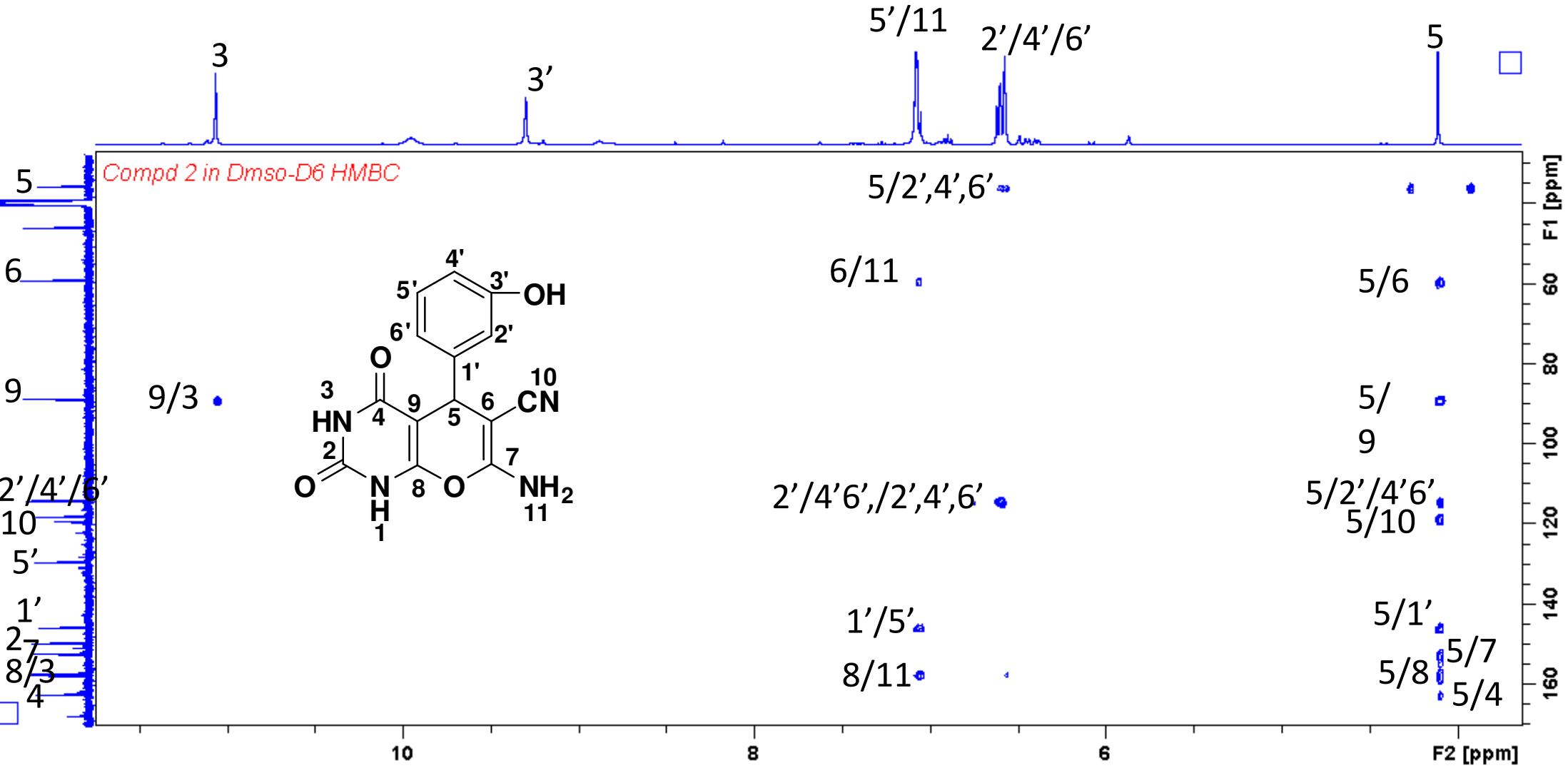
^{13}C NMR spectrum of 7-amino-5-(3-hydroxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A2)



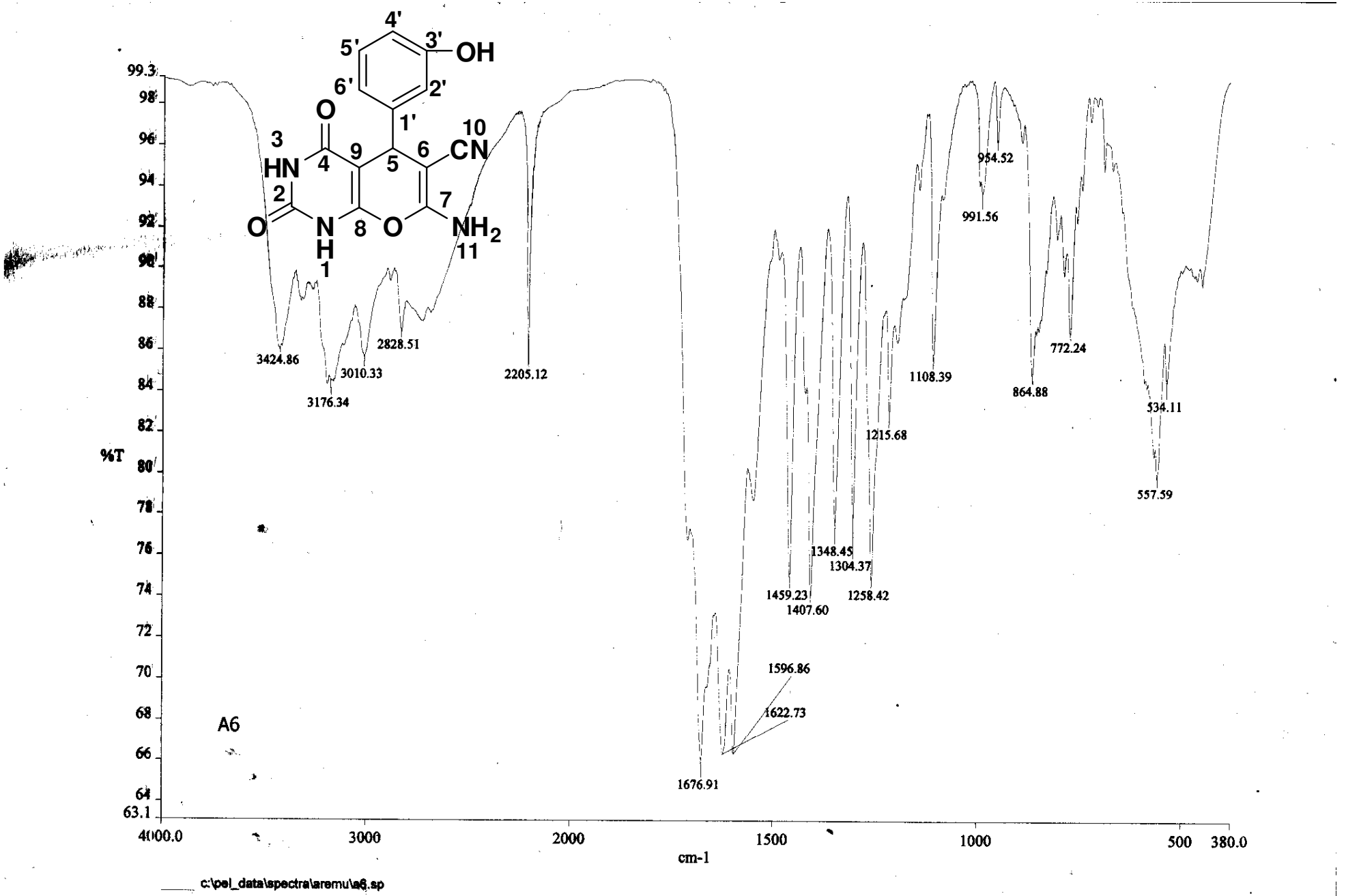
DEPT spectrum of 7-amino-5-(3-hydroxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A2)



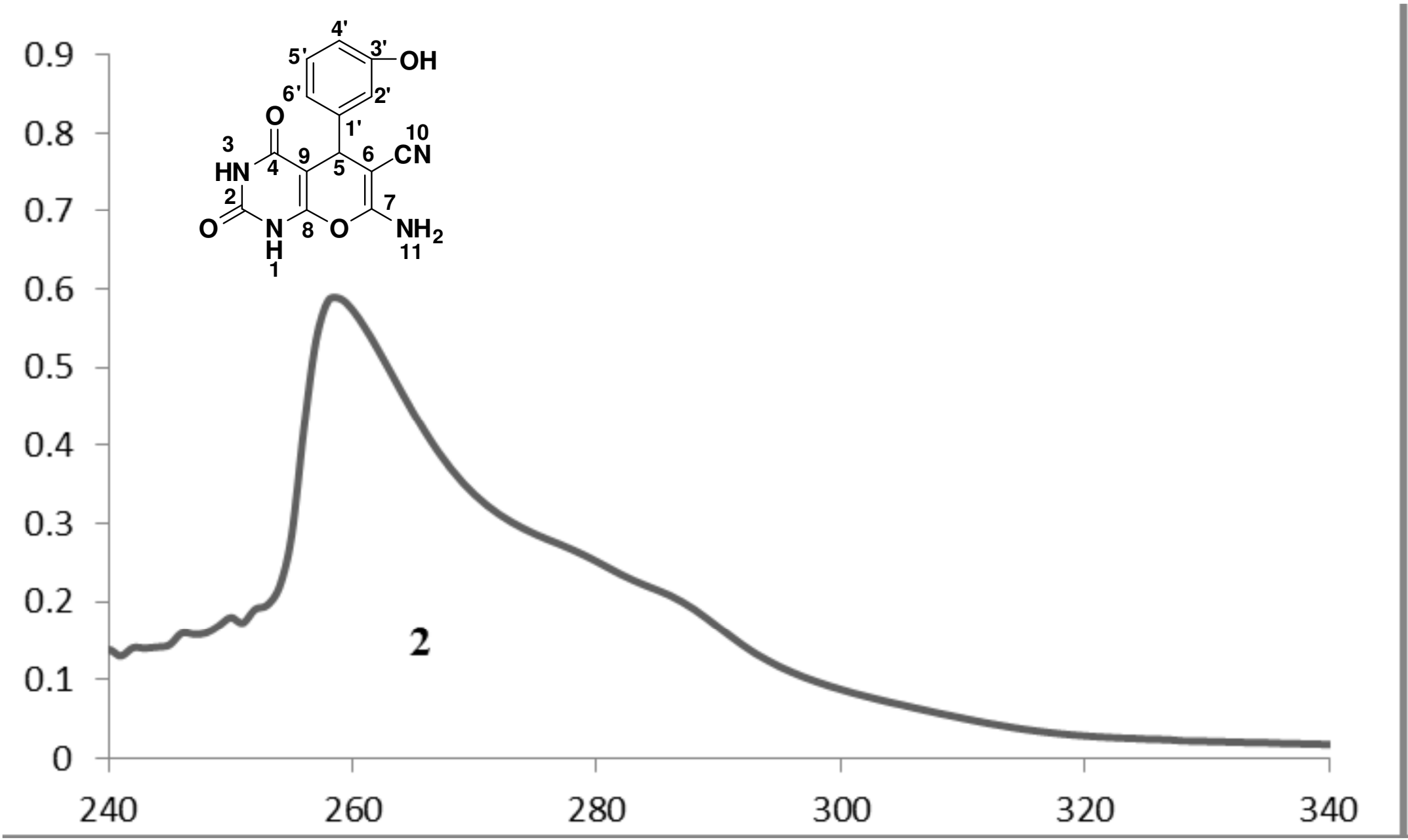
HSQC spectrum of 7-amino-5-(3-hydroxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A2)



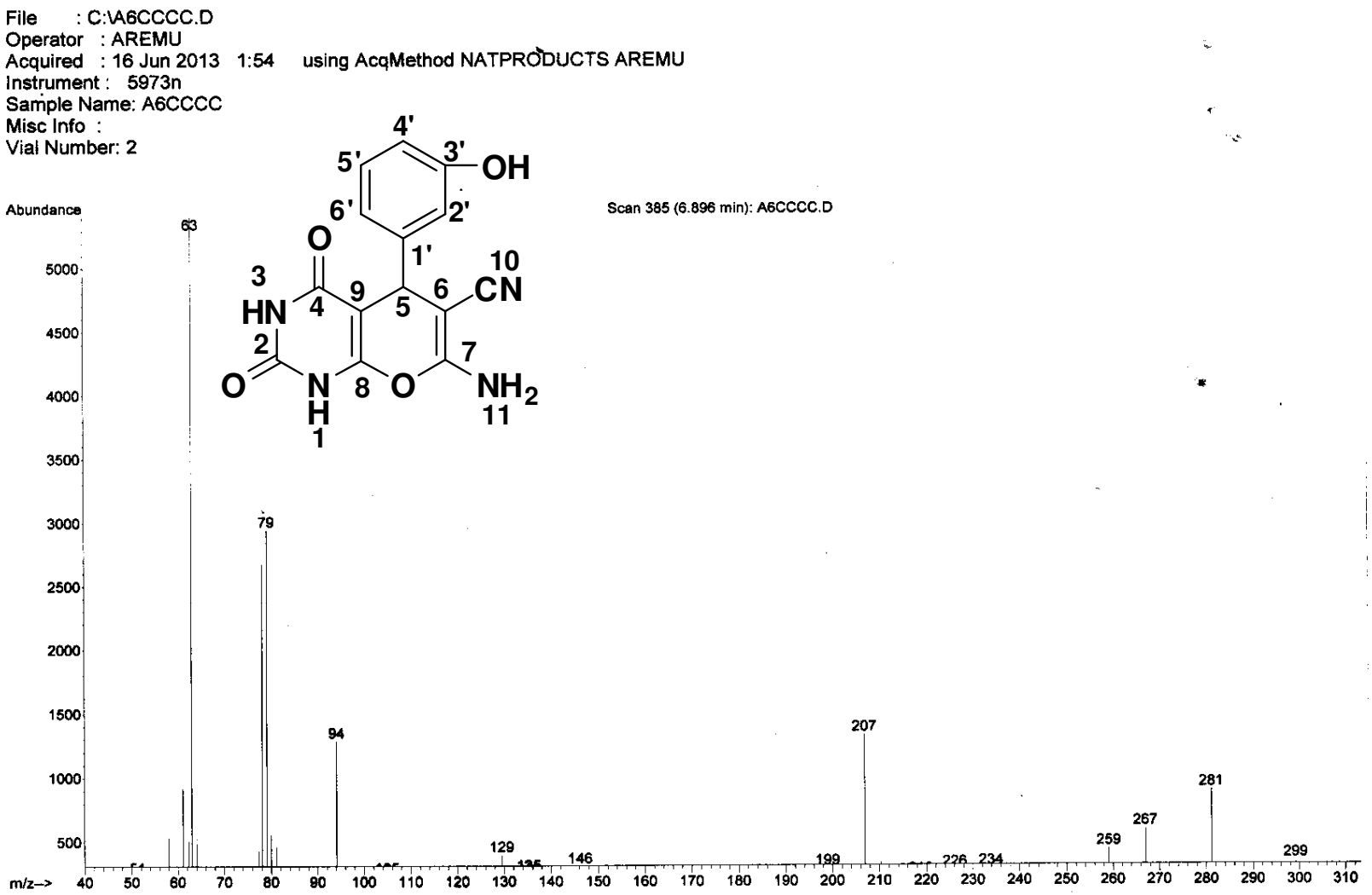
HMBC spectrum of 7-amino-5-(3-hydroxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A2)



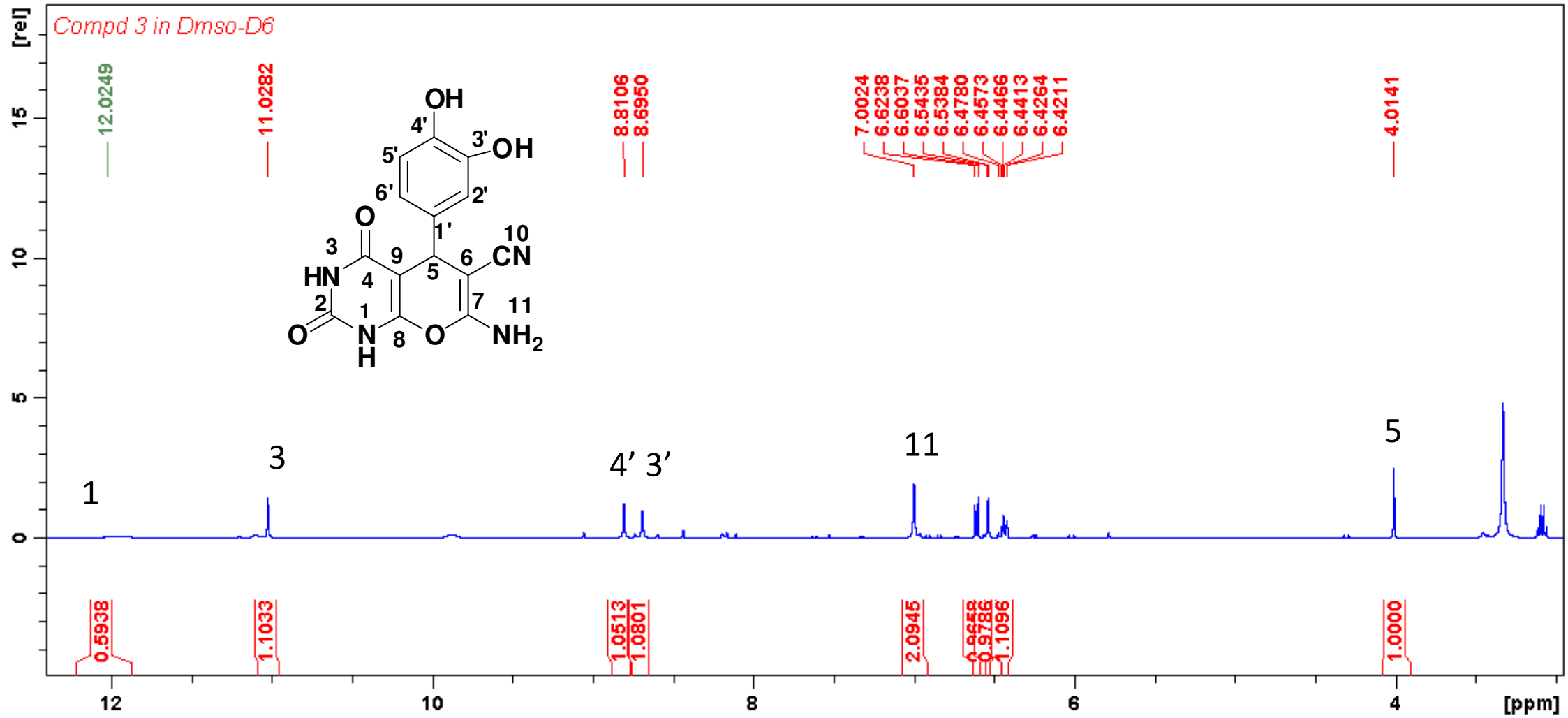
IR spectrum of 7-amino-5-(3-hydroxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A2)



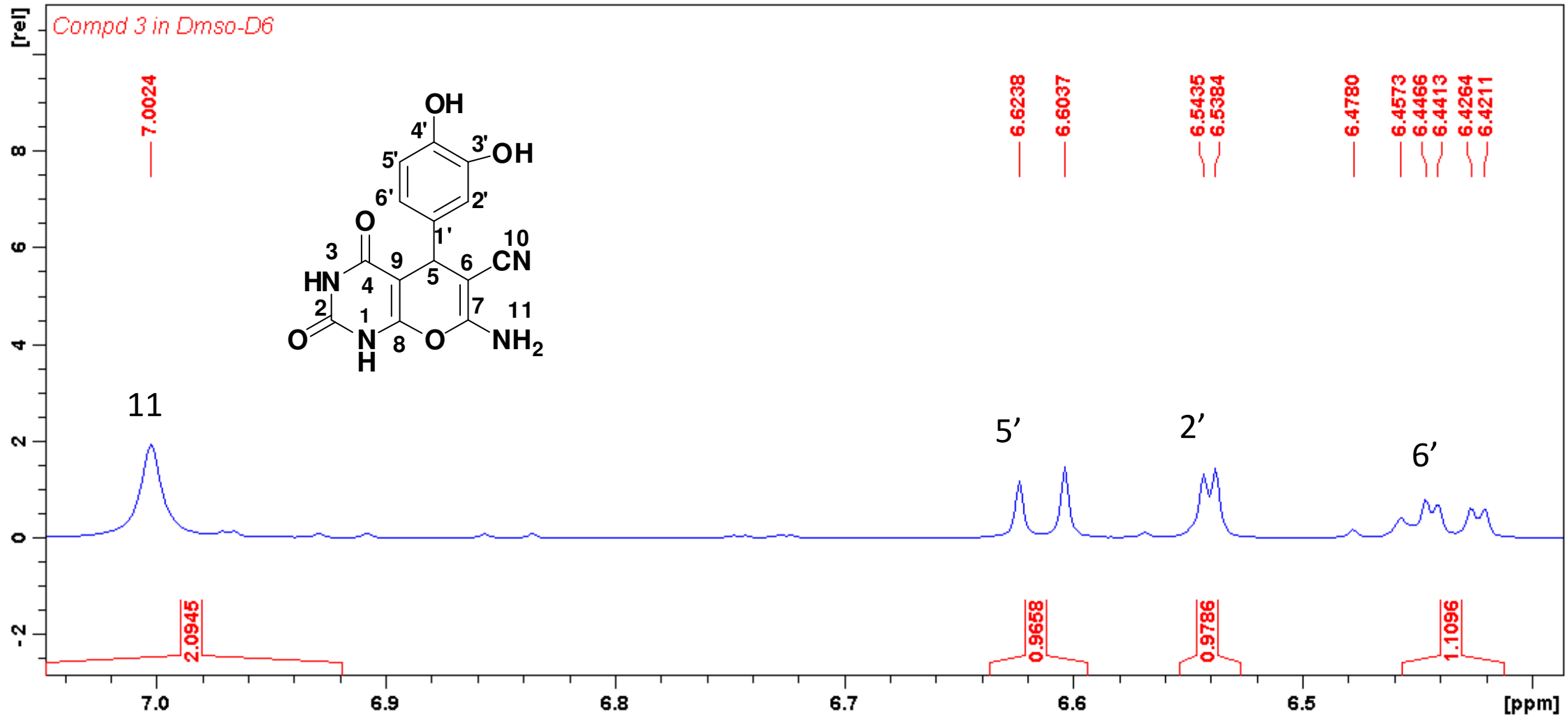
UV spectrum of 7-amino-5-(3-hydroxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A2)



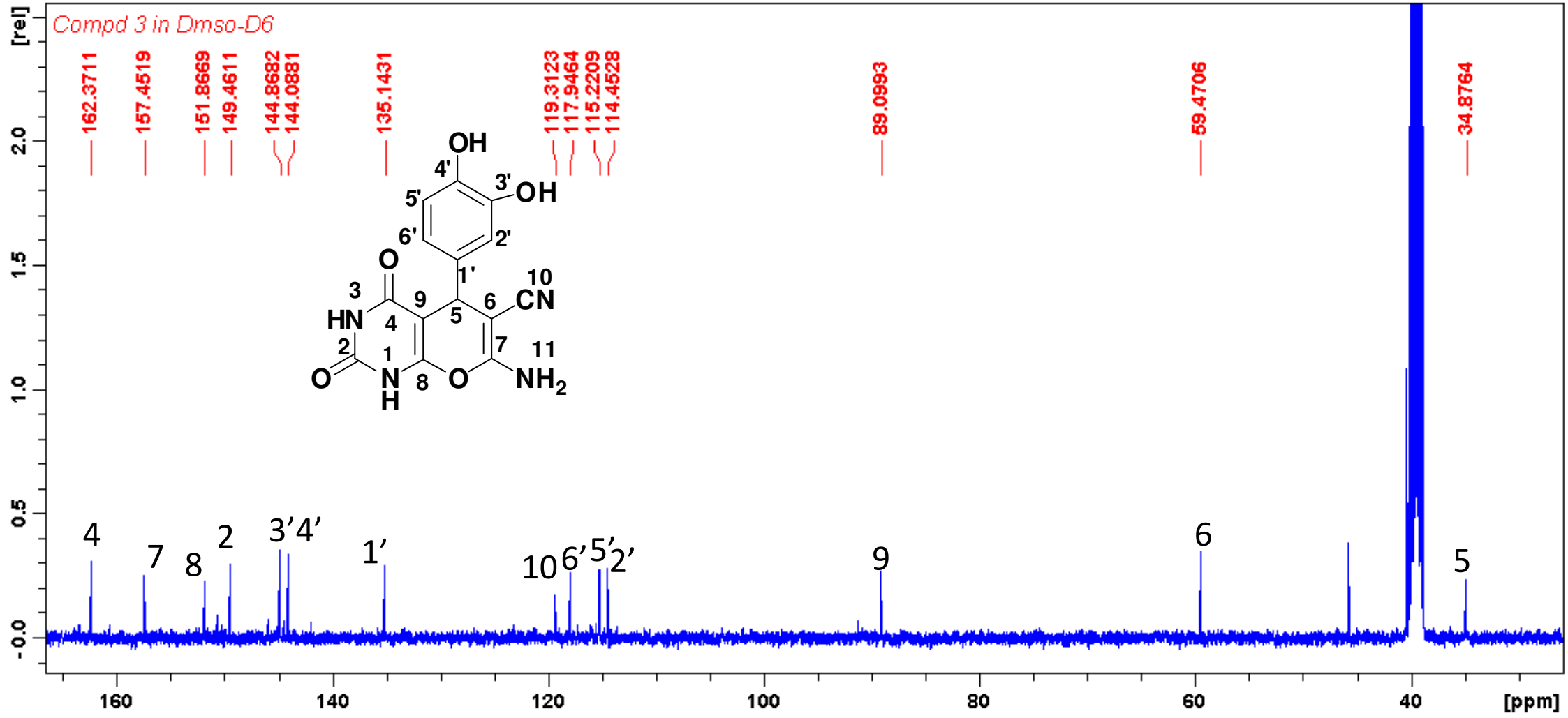
MS spectrum of 7-amino-5-(3-hydroxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A2)



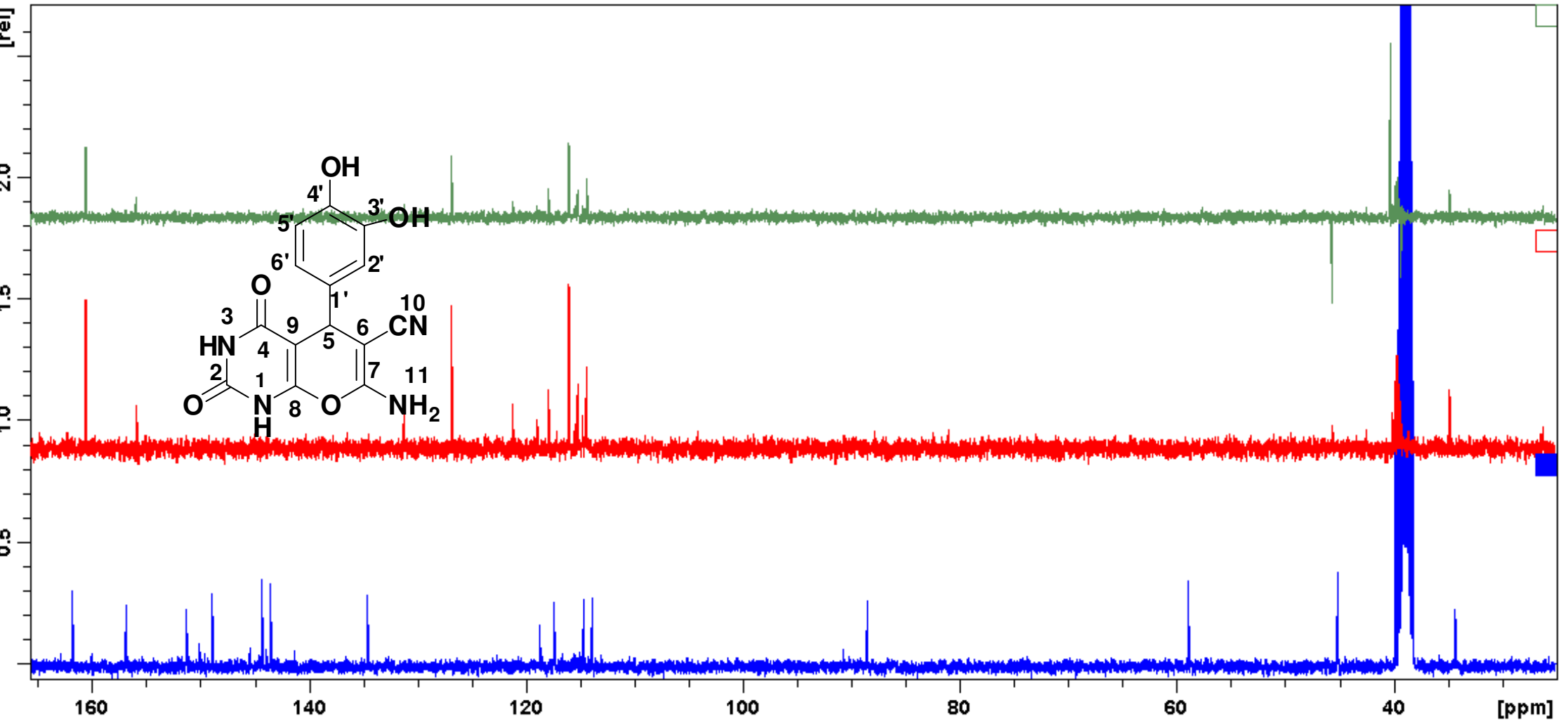
^{1}H NMR spectrum of 7-amino-5-(3,4-dihydroxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A3)



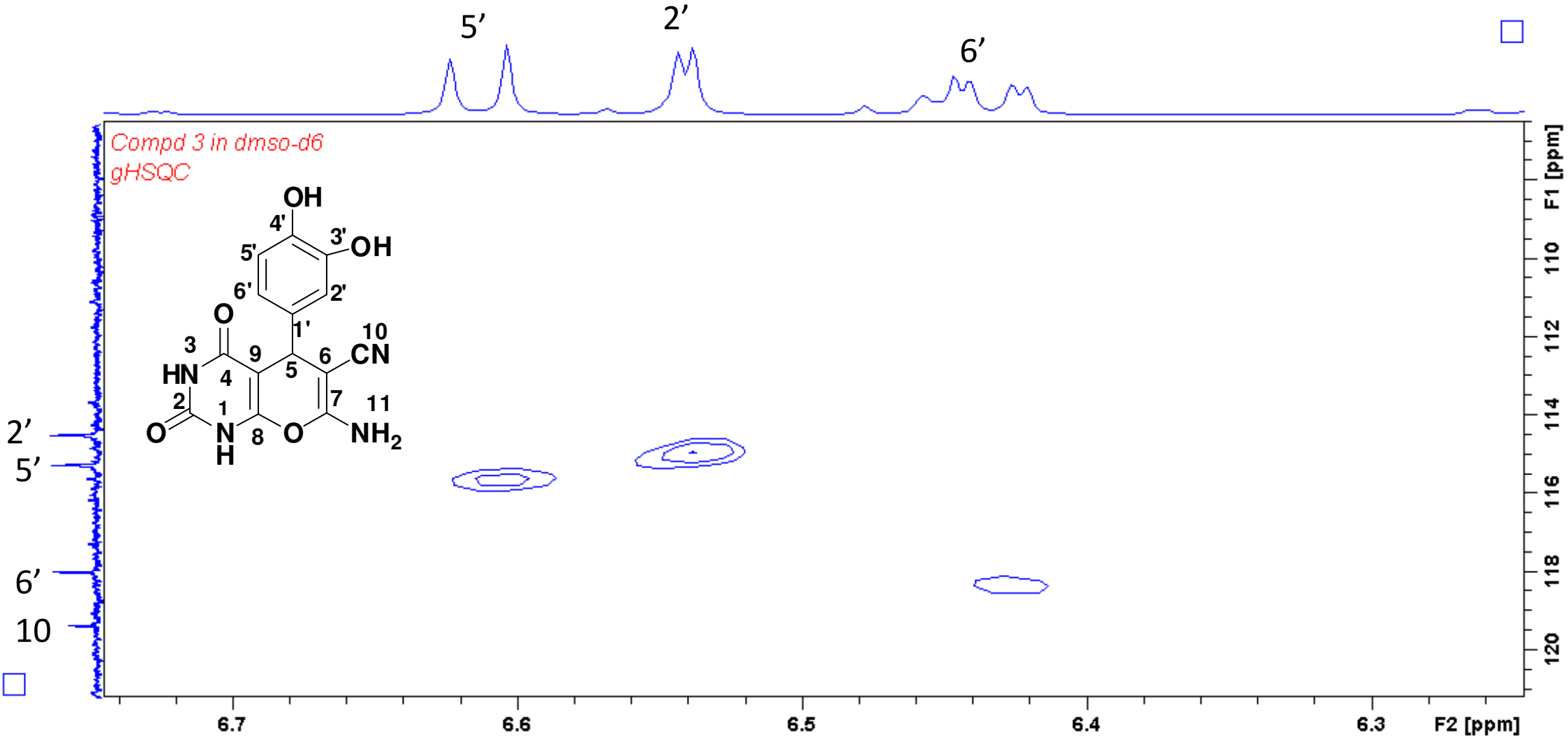
^{1}H NMR spectrum of 7-amino-5-(3,4-dihydroxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A3)



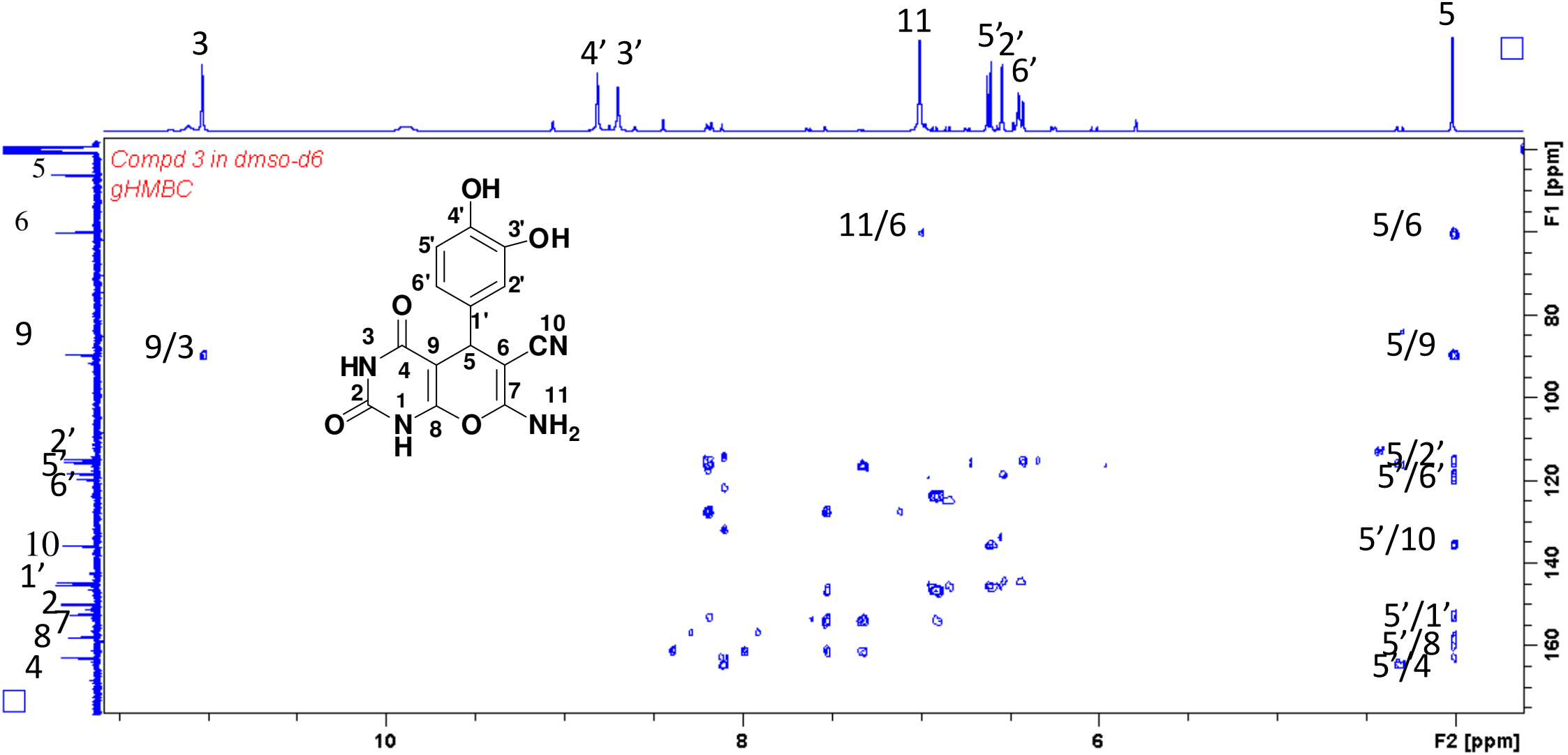
^{13}C NMR spectrum of 7-amino-5-(3,4-dihydroxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A3)



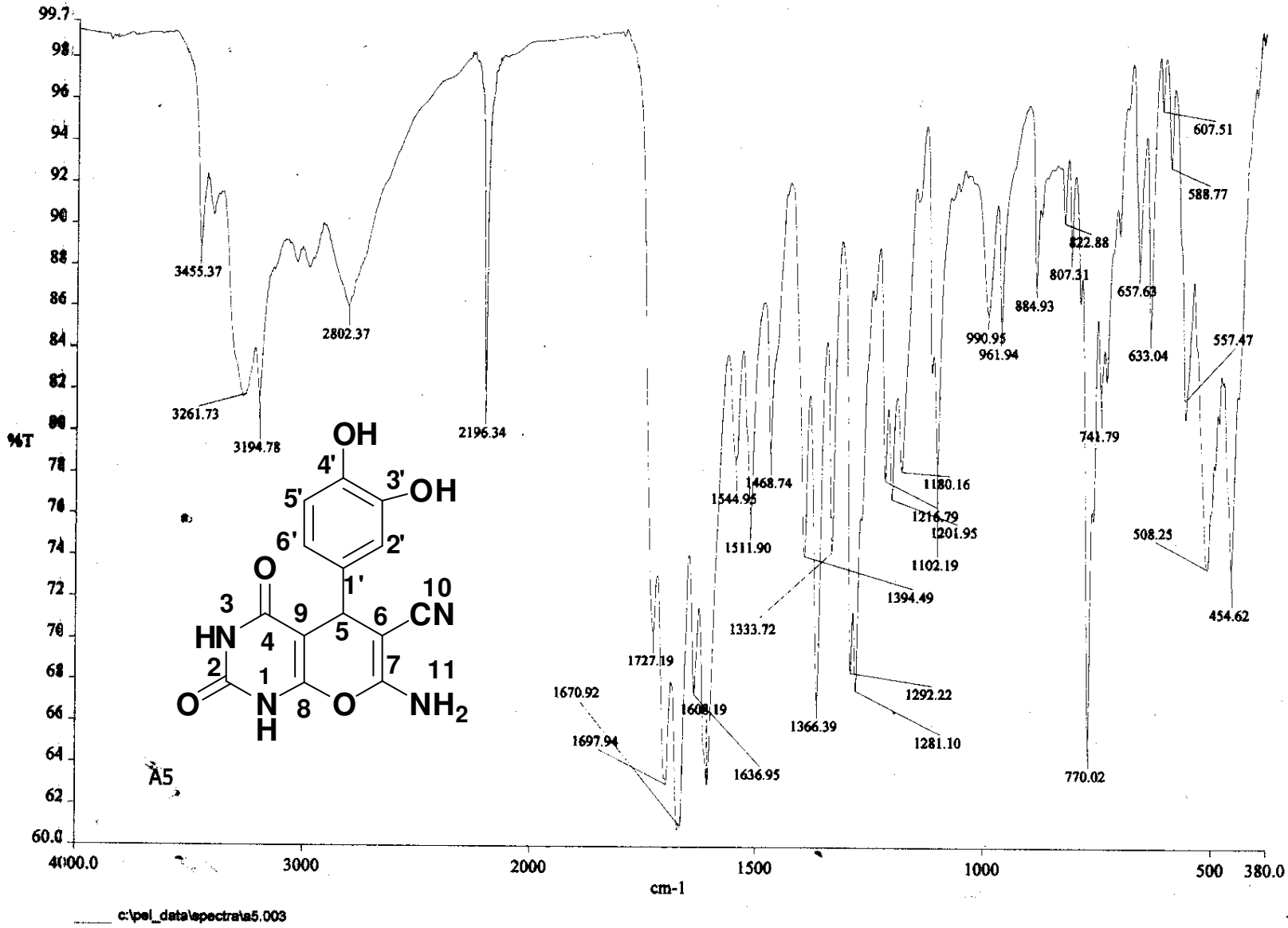
DEPT spectrum of 7-amino-5-(3,4-dihydroxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A3)



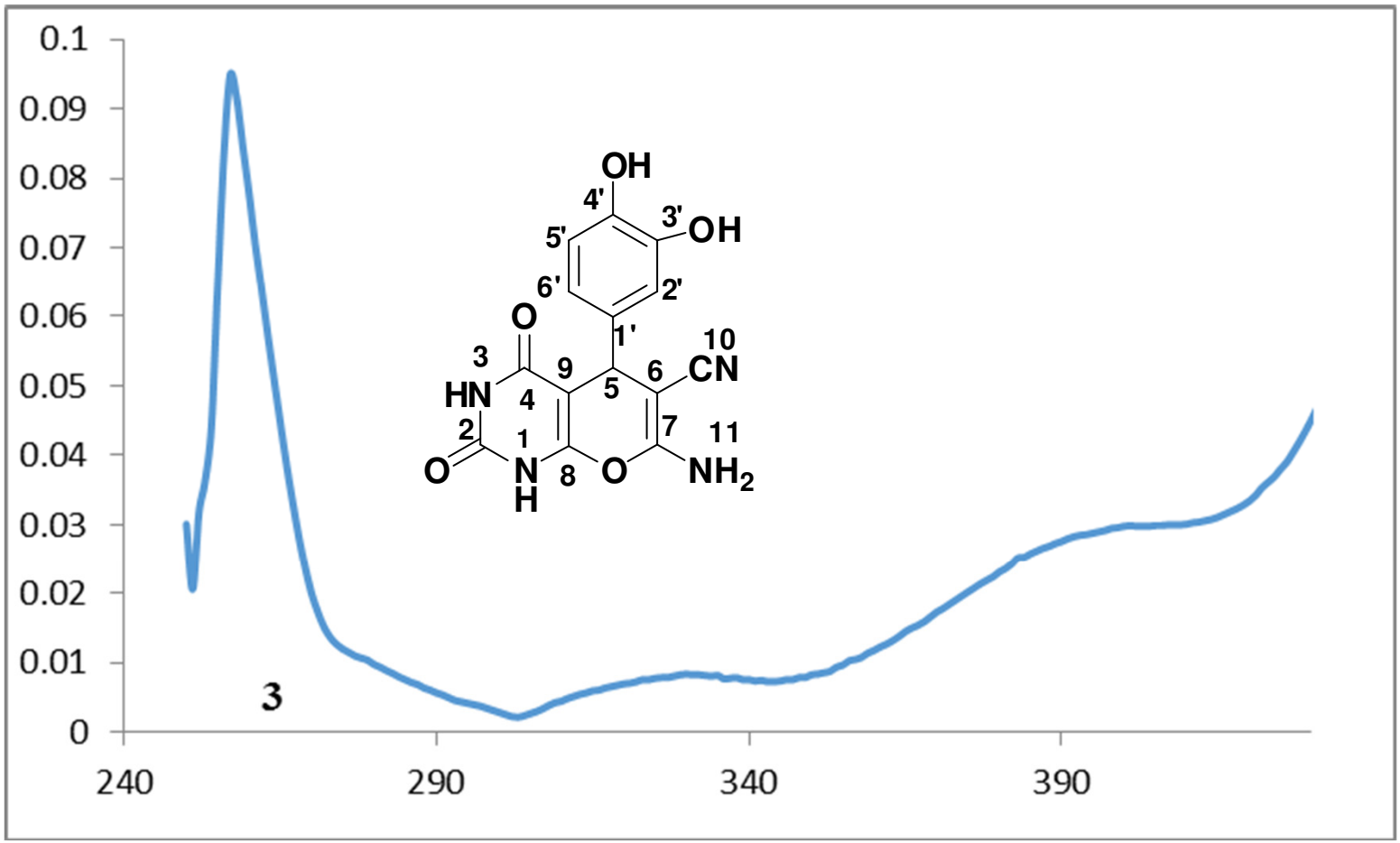
HSQC spectrum of 7-amino-5-(3,4-dihydroxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A3)



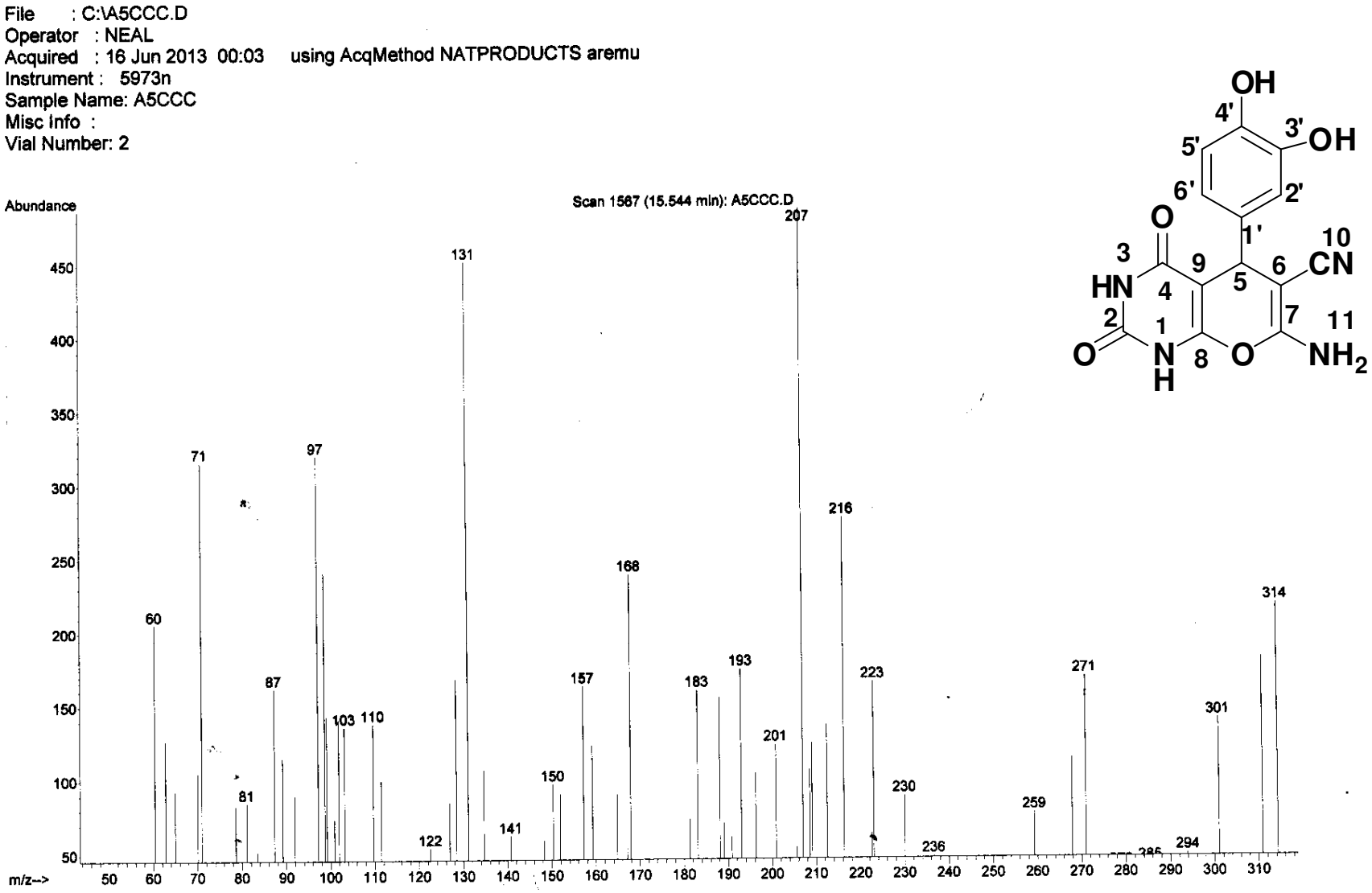
HMBC spectrum of 7-amino-5-(3,4-dihydroxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A3)



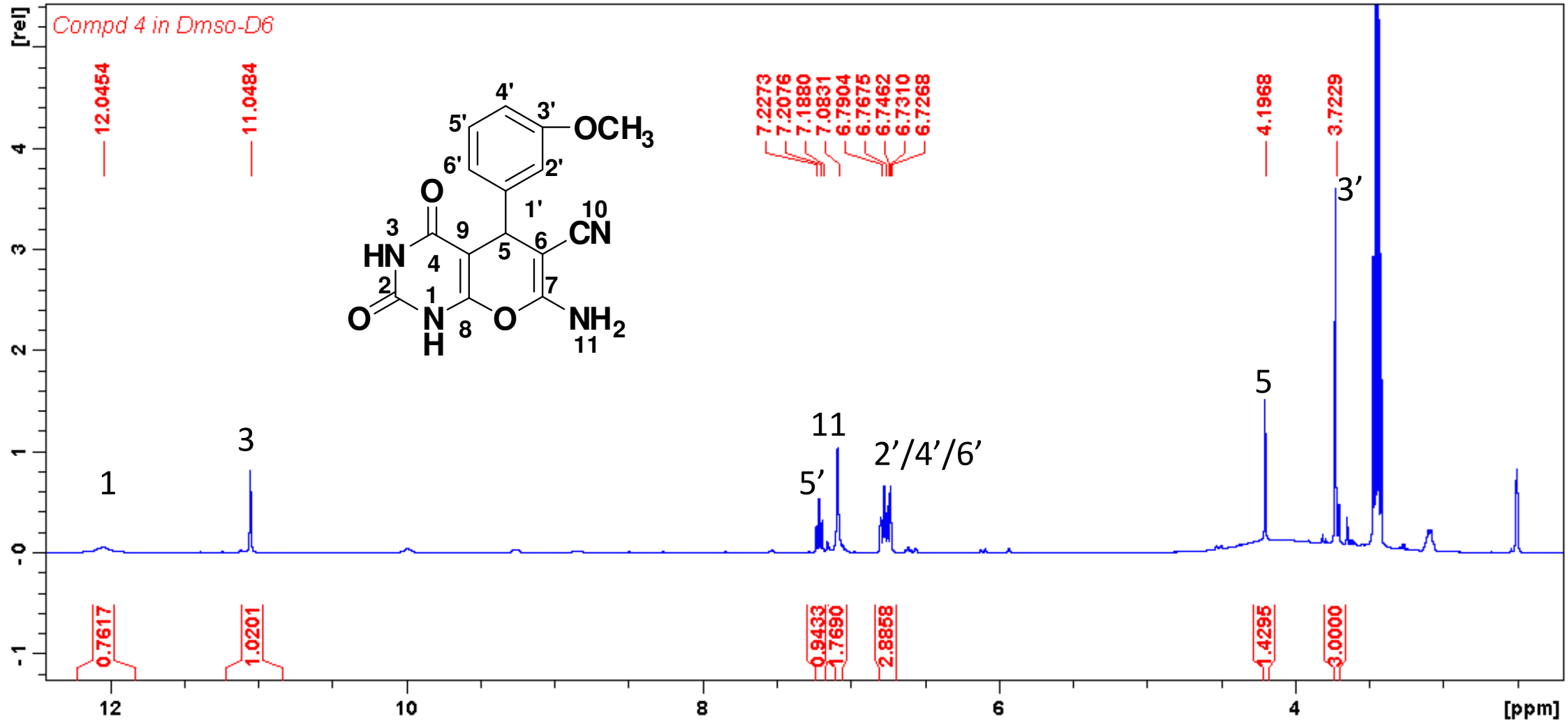
IR spectrum of 7-amino-5-(3,4-dihydroxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A3)



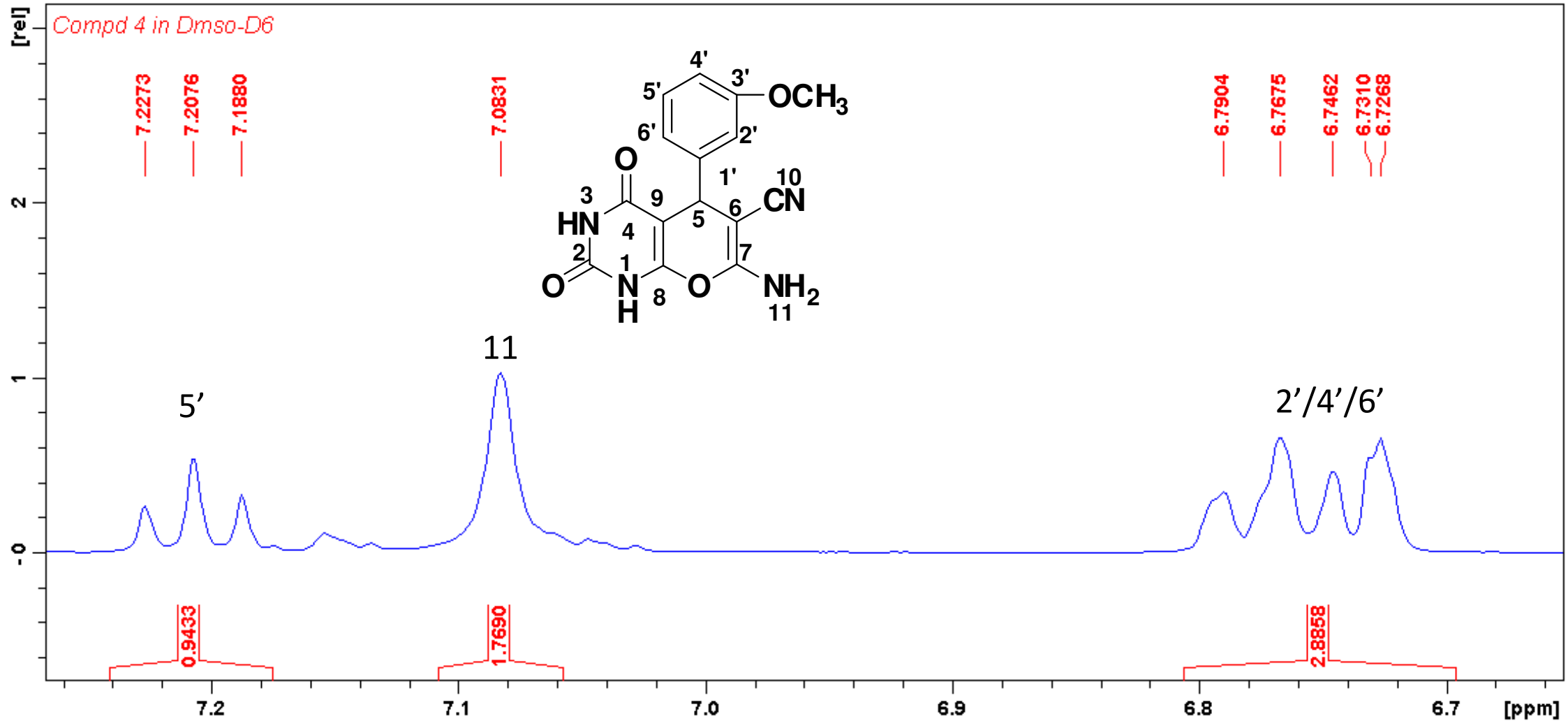
UV spectrum of 7-amino-5-(3,4-dihydroxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A3)



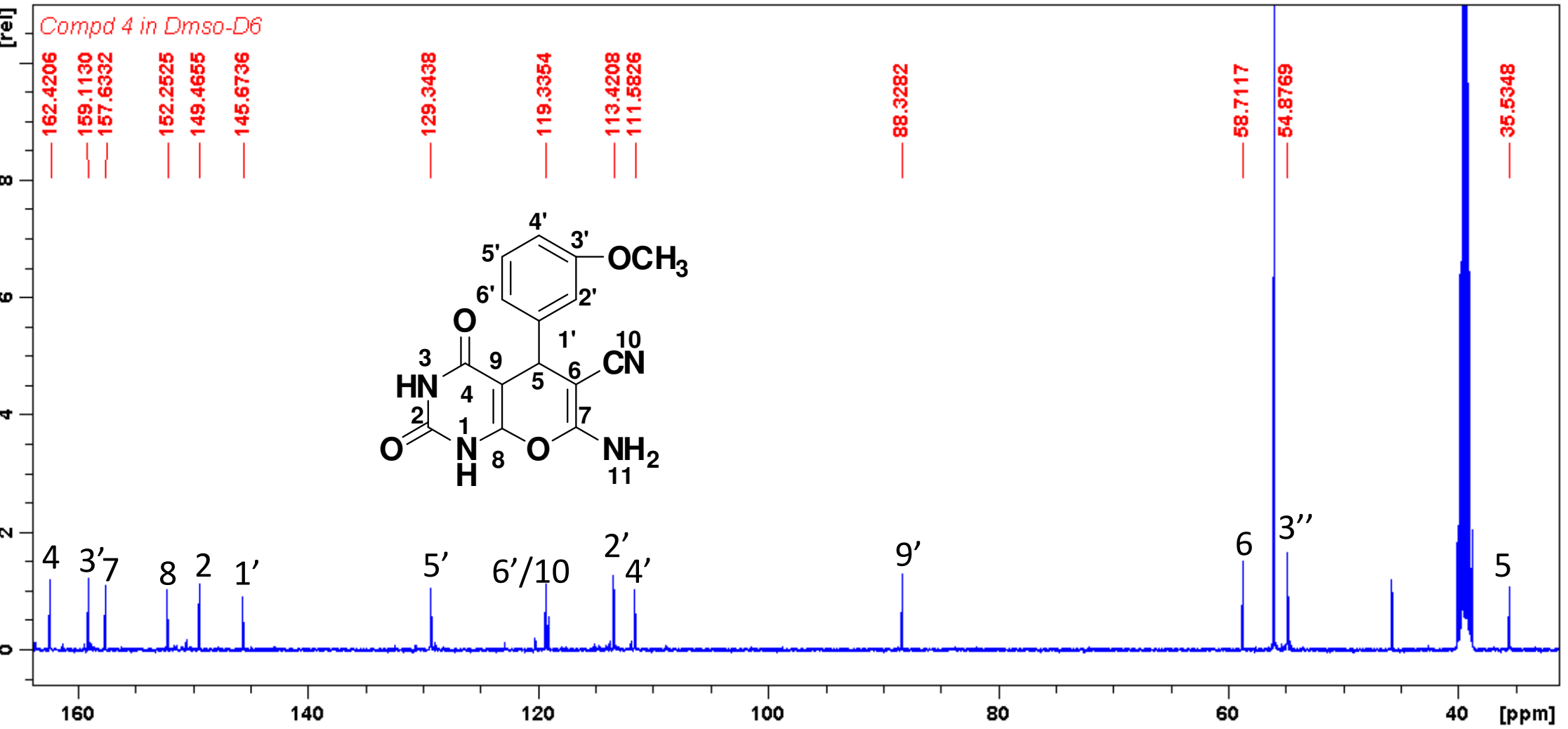
Mass spectrum of 7-amino-5-(3,4-dihydroxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A3)



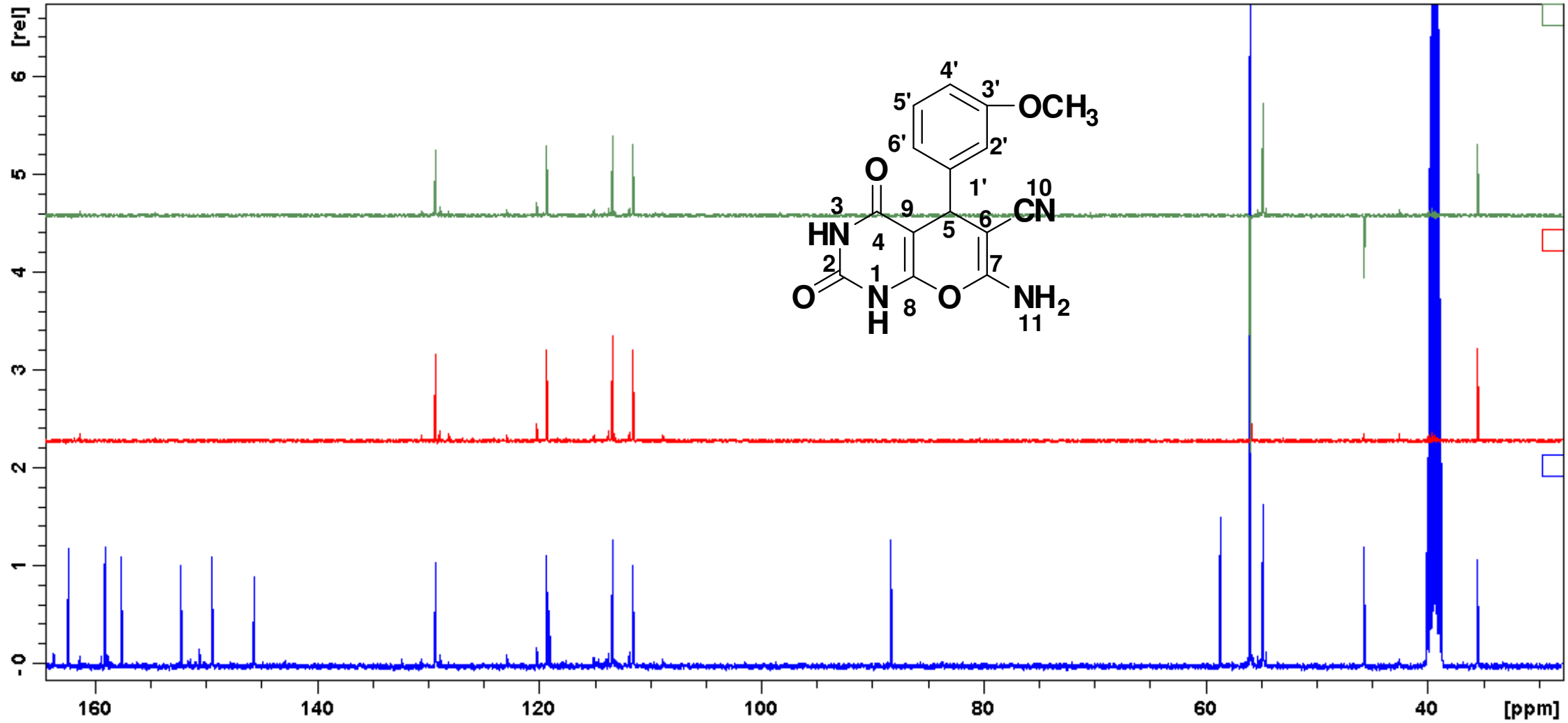
^{1}H NMR spectrum of 7-amino-5-(3-methoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A4)



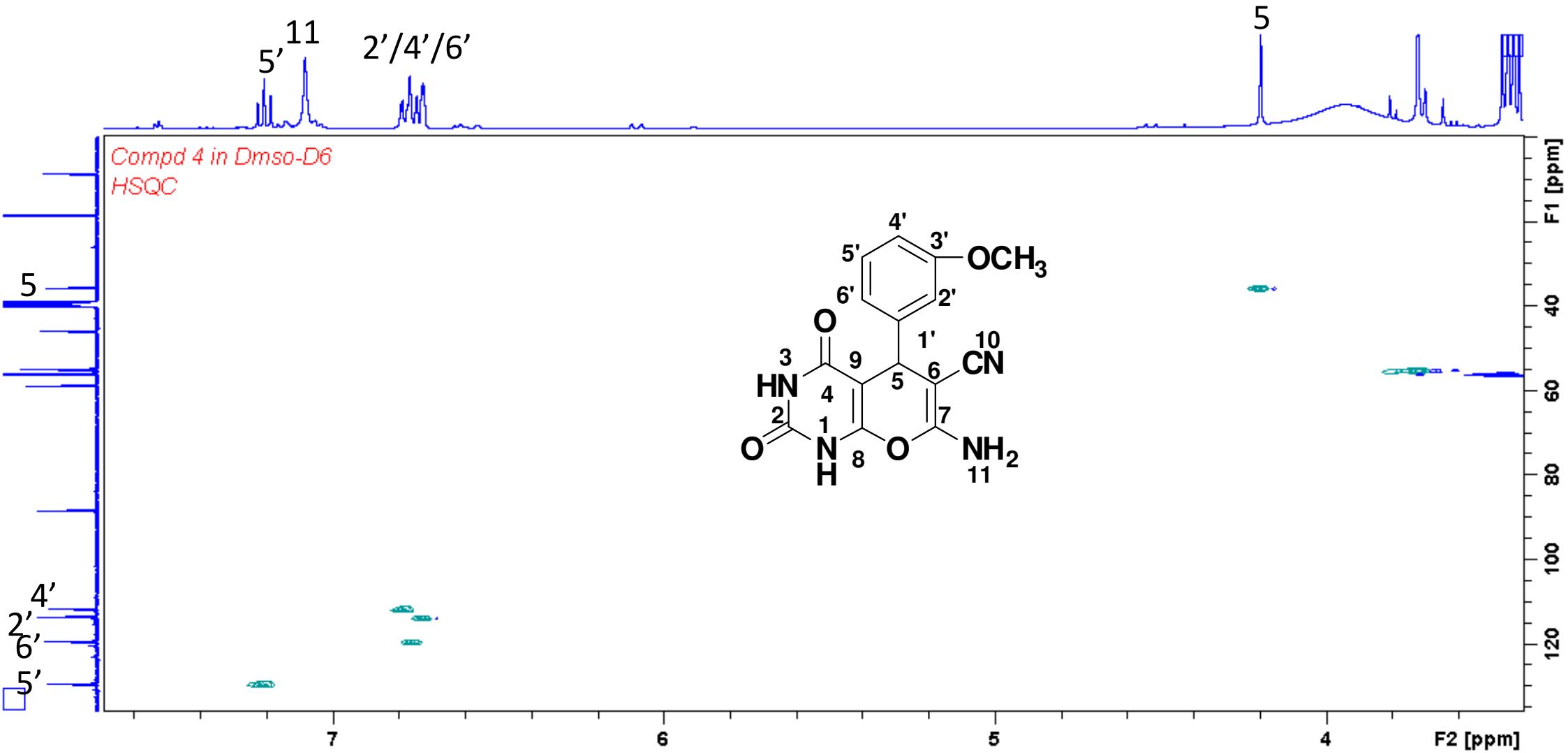
^{1}H NMR spectrum of 7-amino-5-(3-methoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A4)



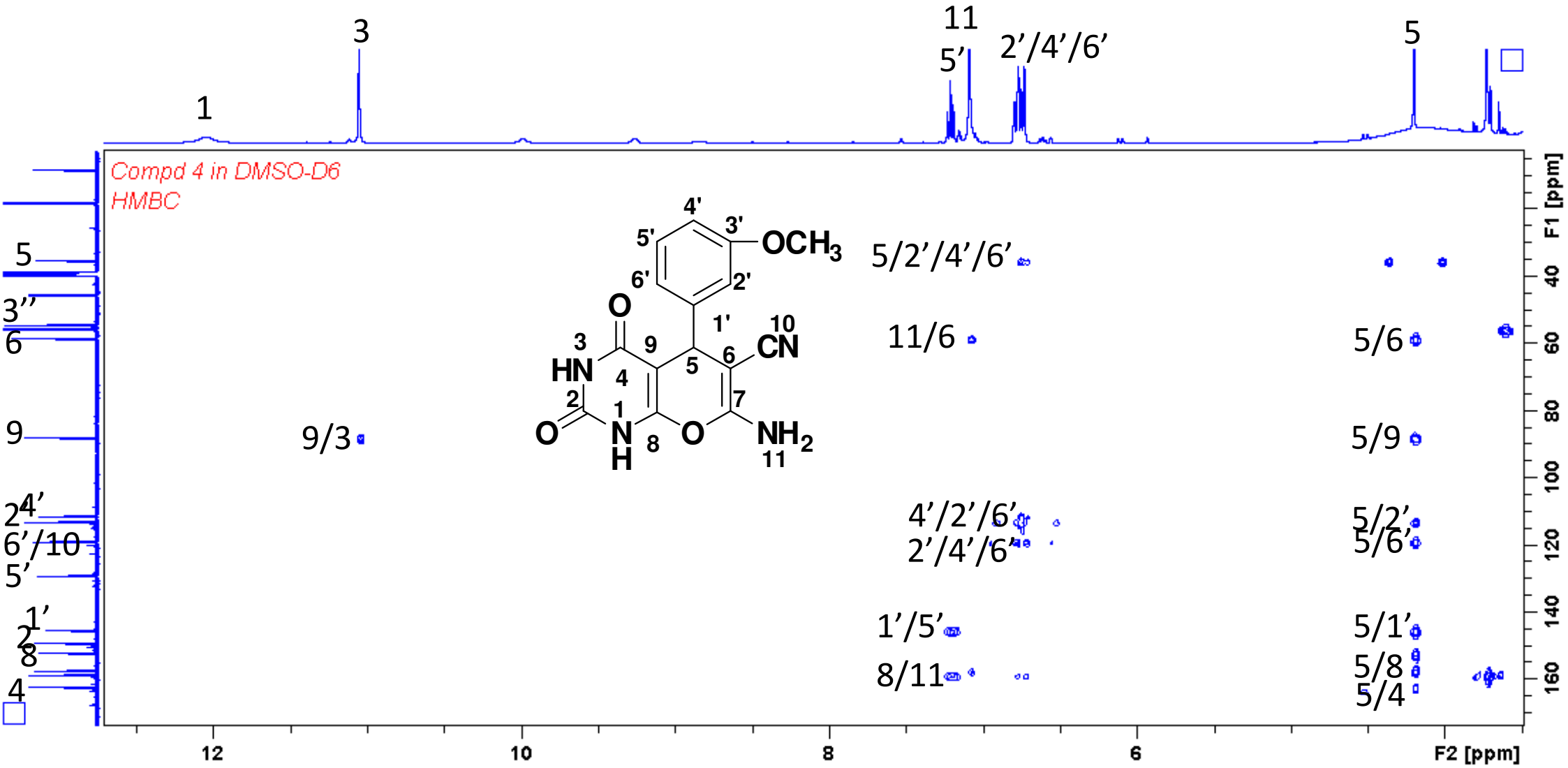
^{13}C NMR spectrum of 7-amino-5-(3-methoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A4)



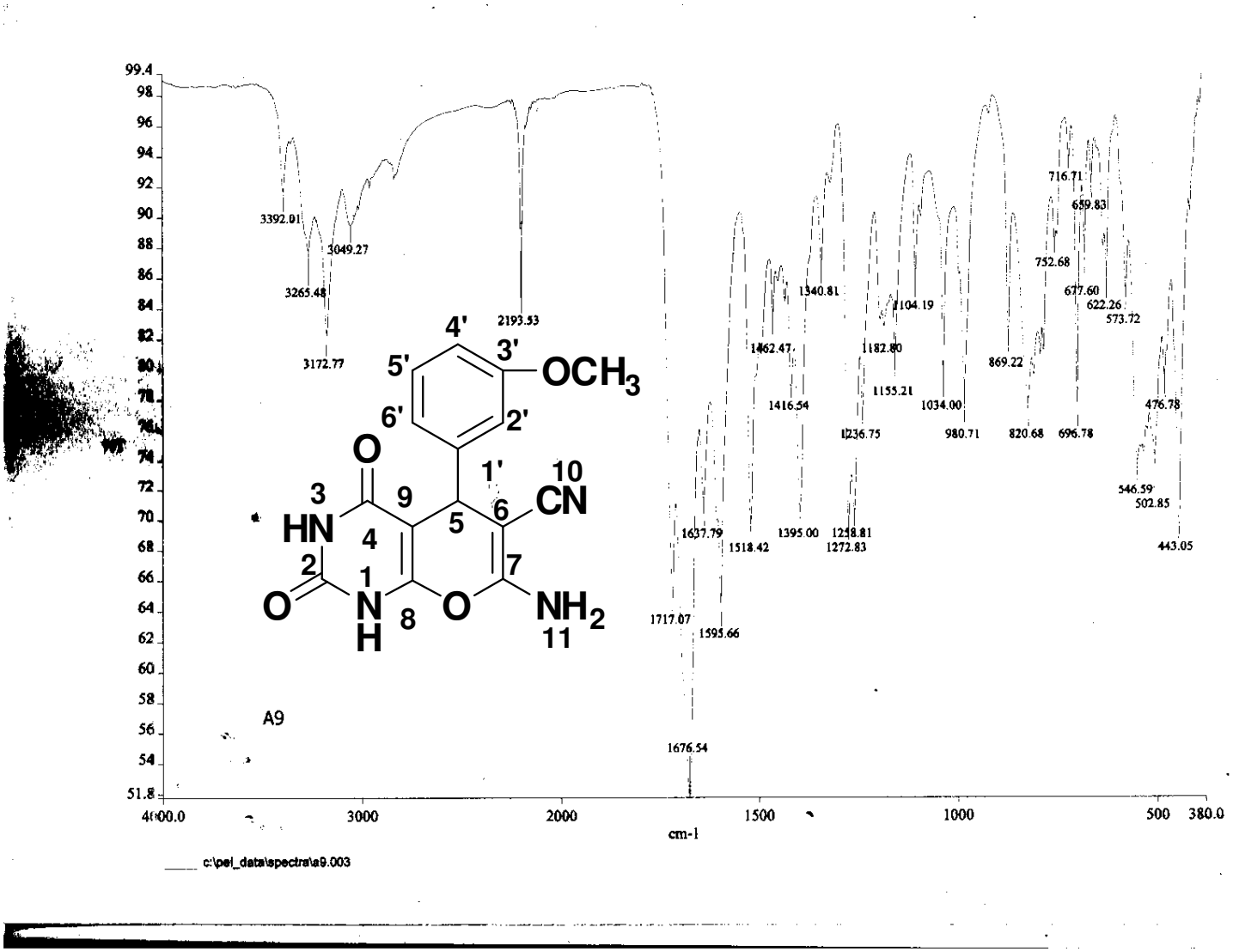
DEPT spectrum of 7-amino-5-(3-methoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A4)



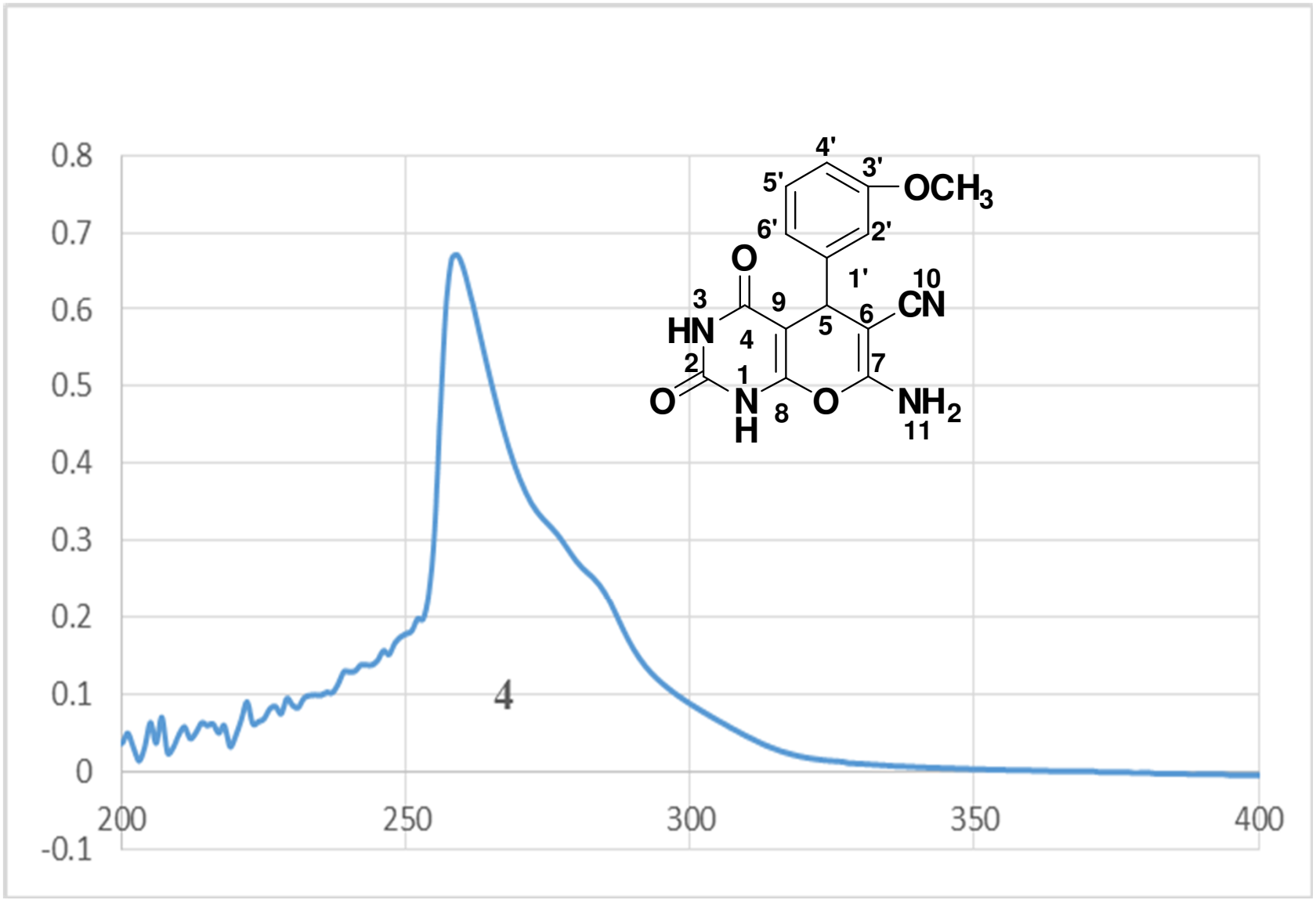
HSQC spectrum of 7-amino-5-(3-methoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A4)



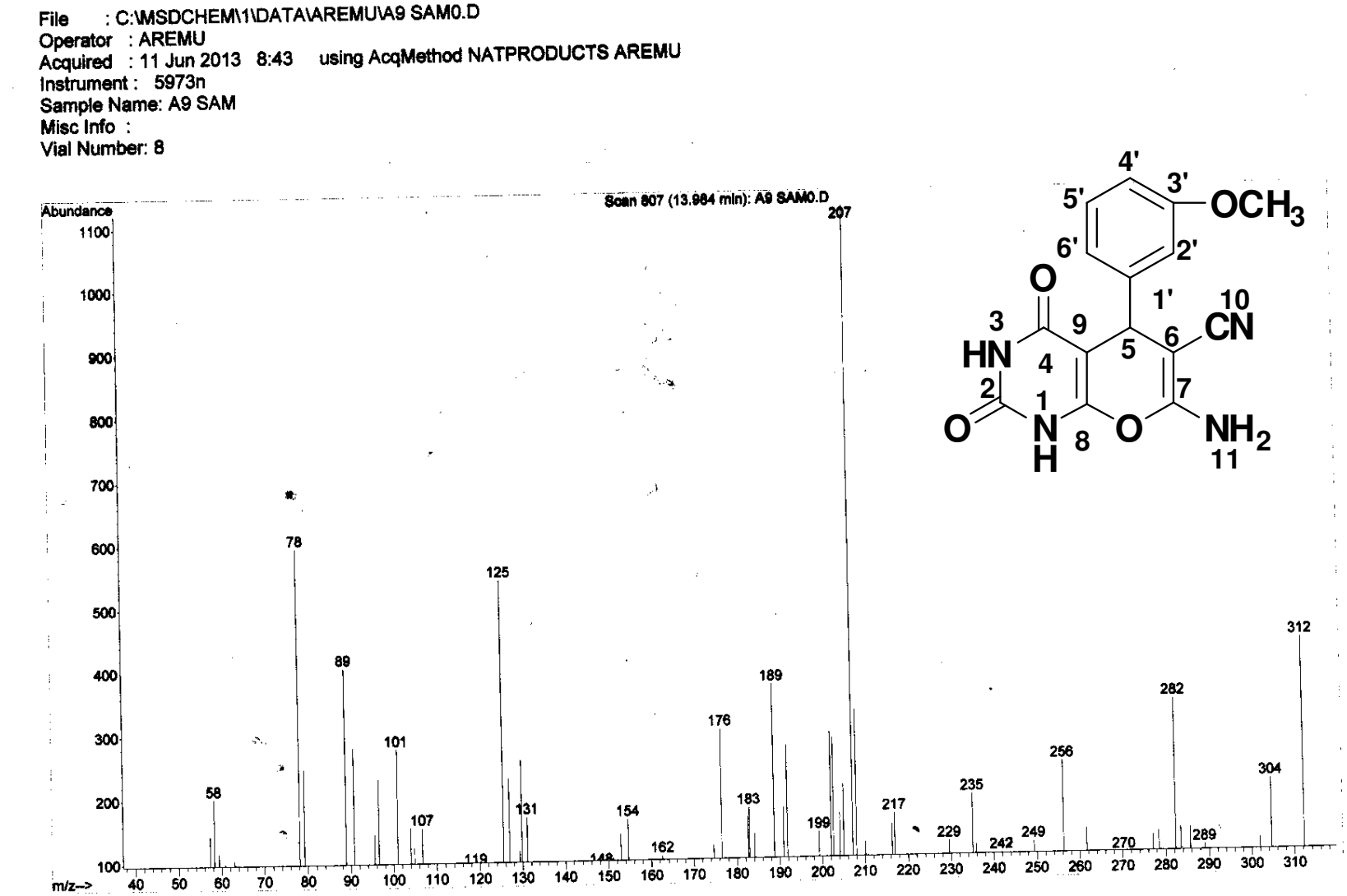
HMBC spectrum of 7-amino-5-(3-methoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A4)



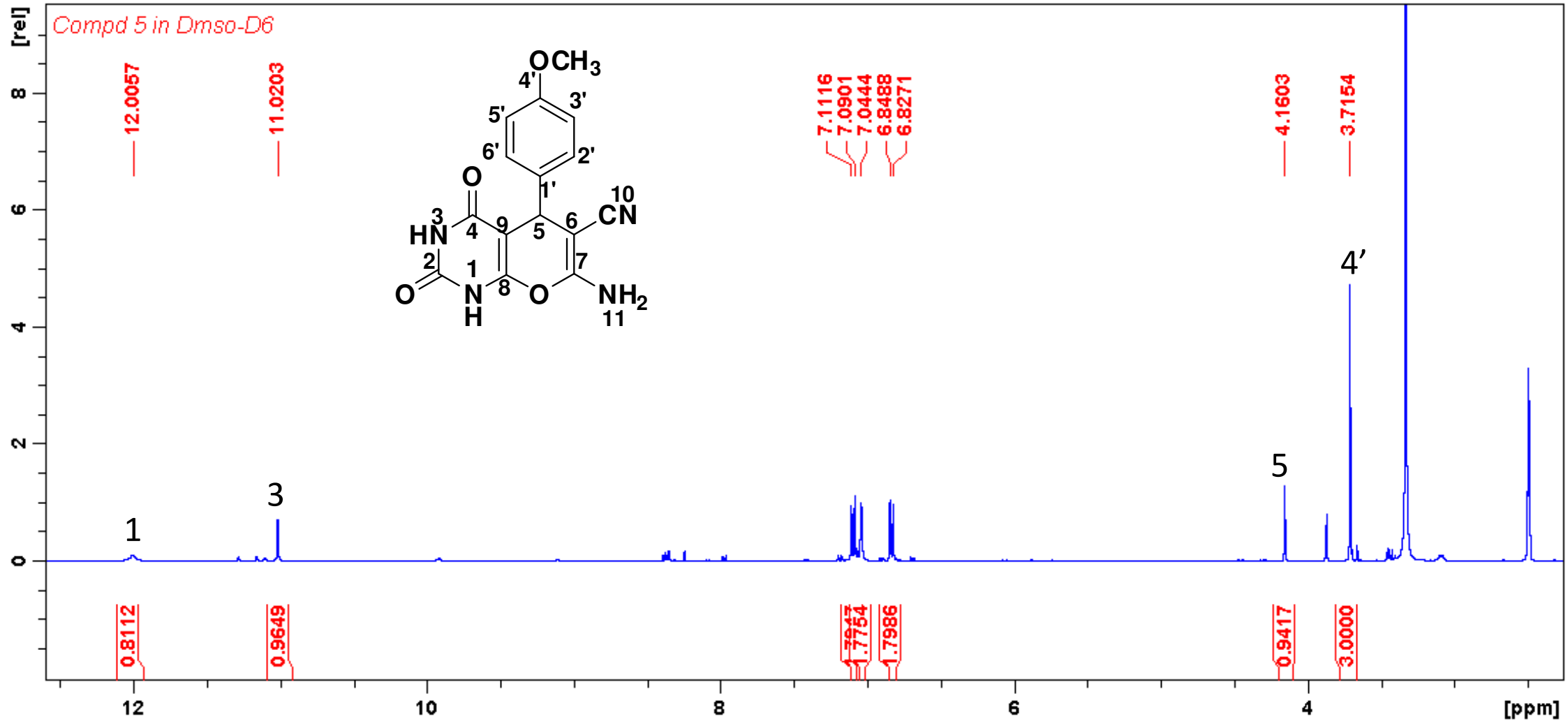
IR spectrum of 7-amino-5-(3-methoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A4)



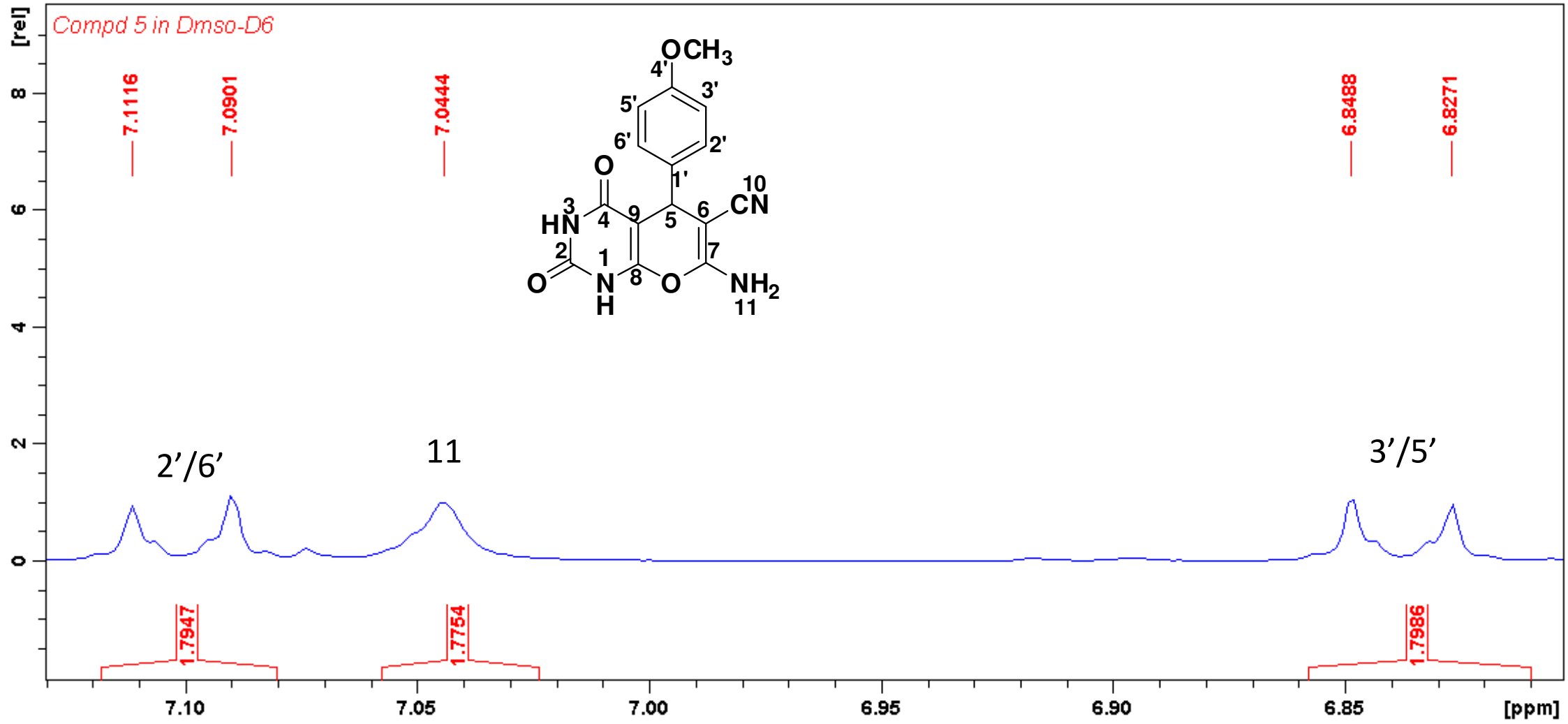
UV spectrum of 7-amino-5-(3-methoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A4)



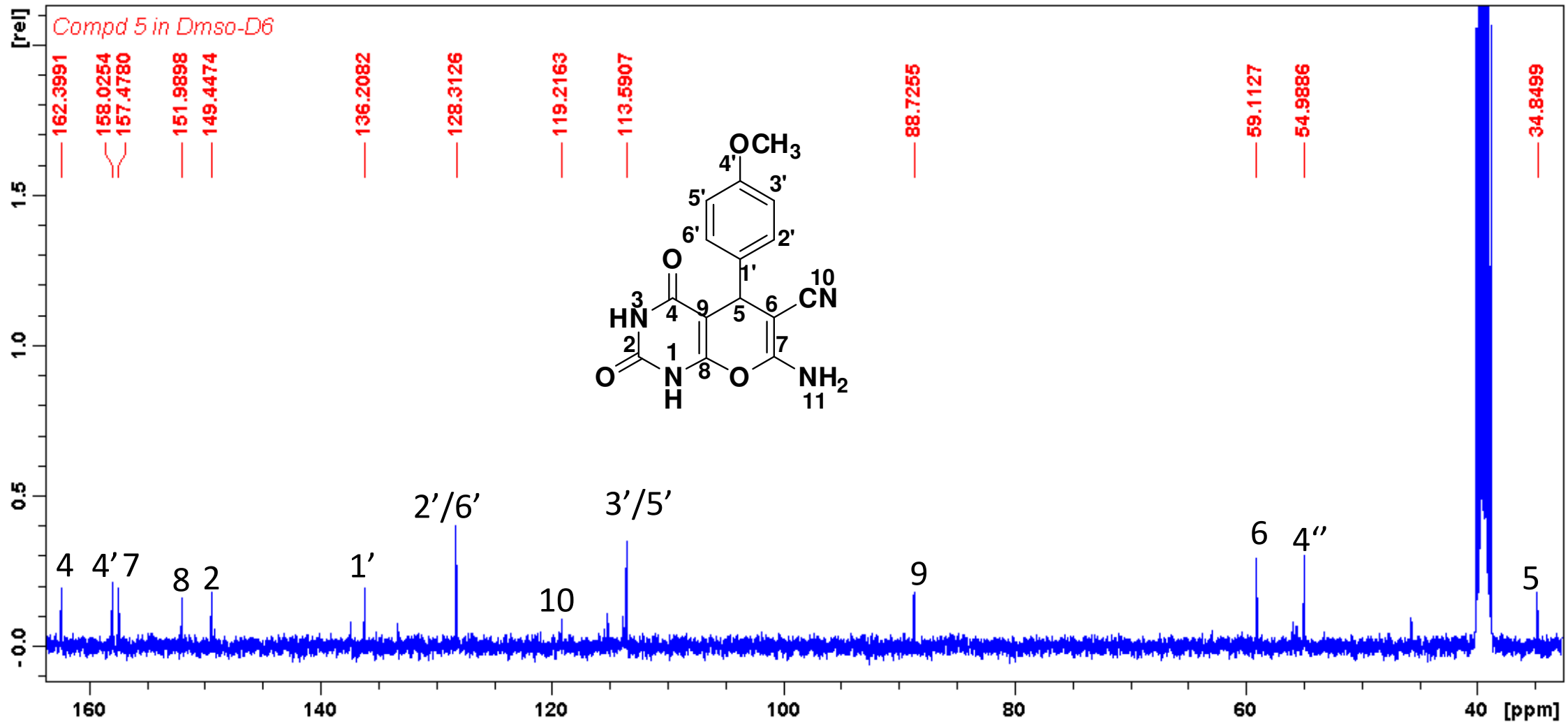
Mass spectrum of 7-amino-5-(3-methoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A4)



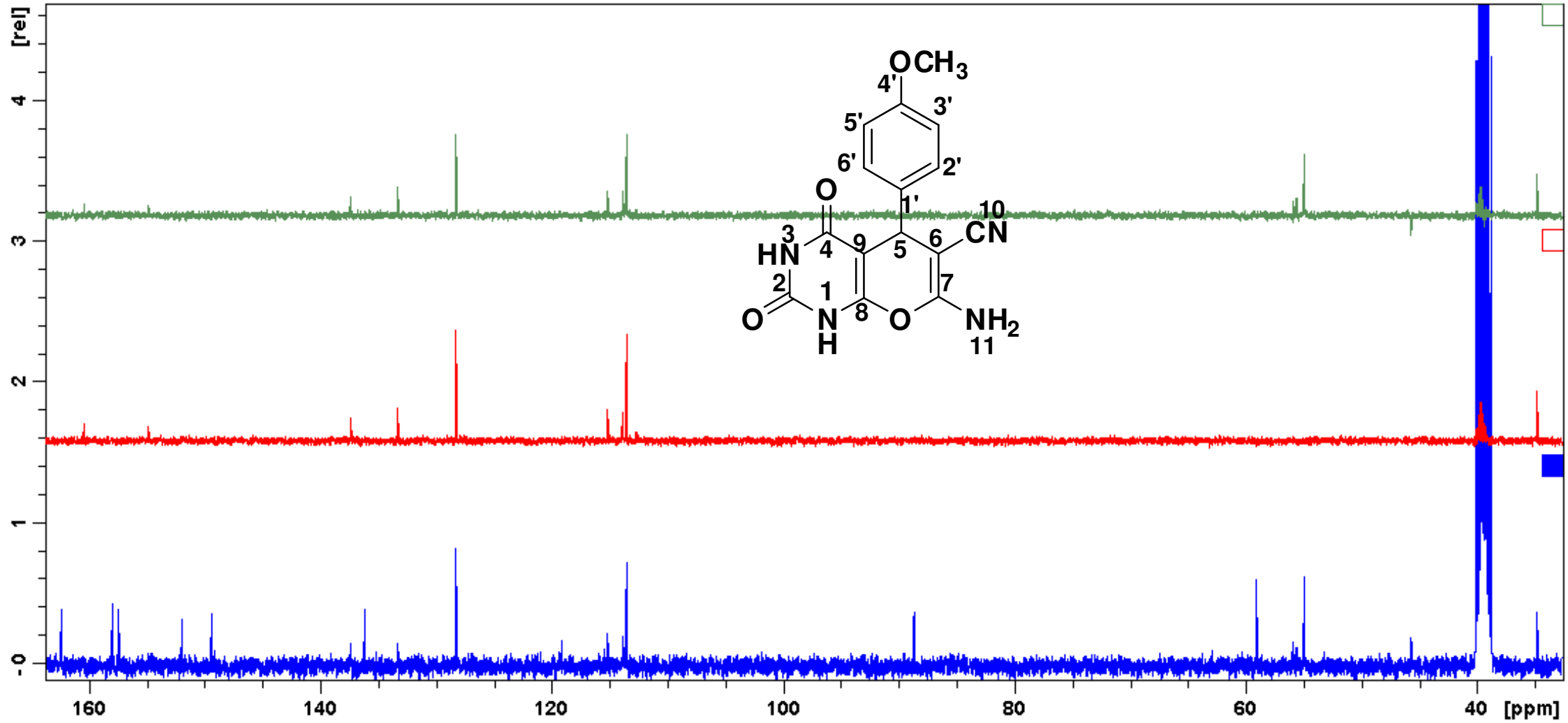
^{1}H NMR spectrum of 7-amino-5-(4-methoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A5)



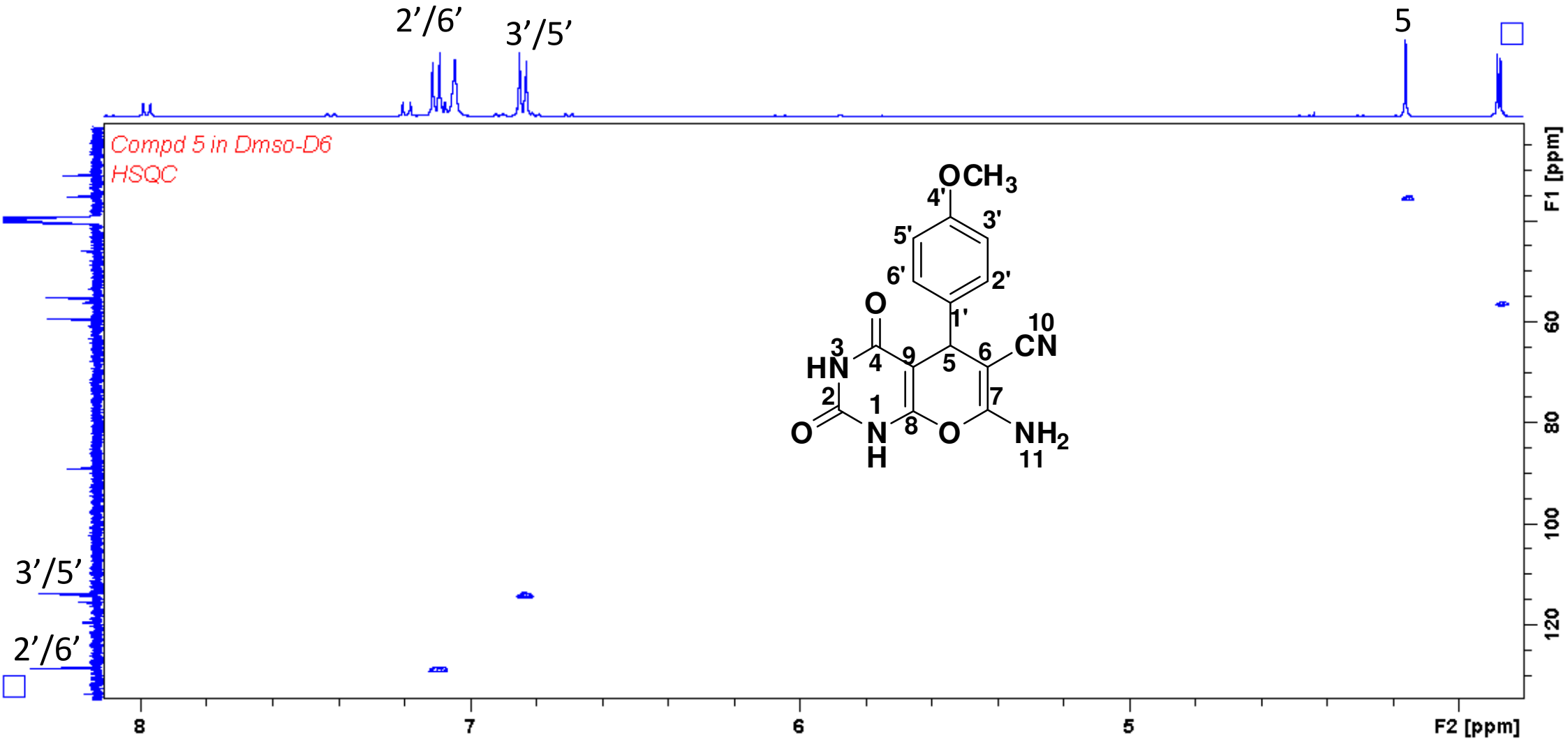
^{1}H NMR spectrum of 7-amino-5-(4-methoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A5)



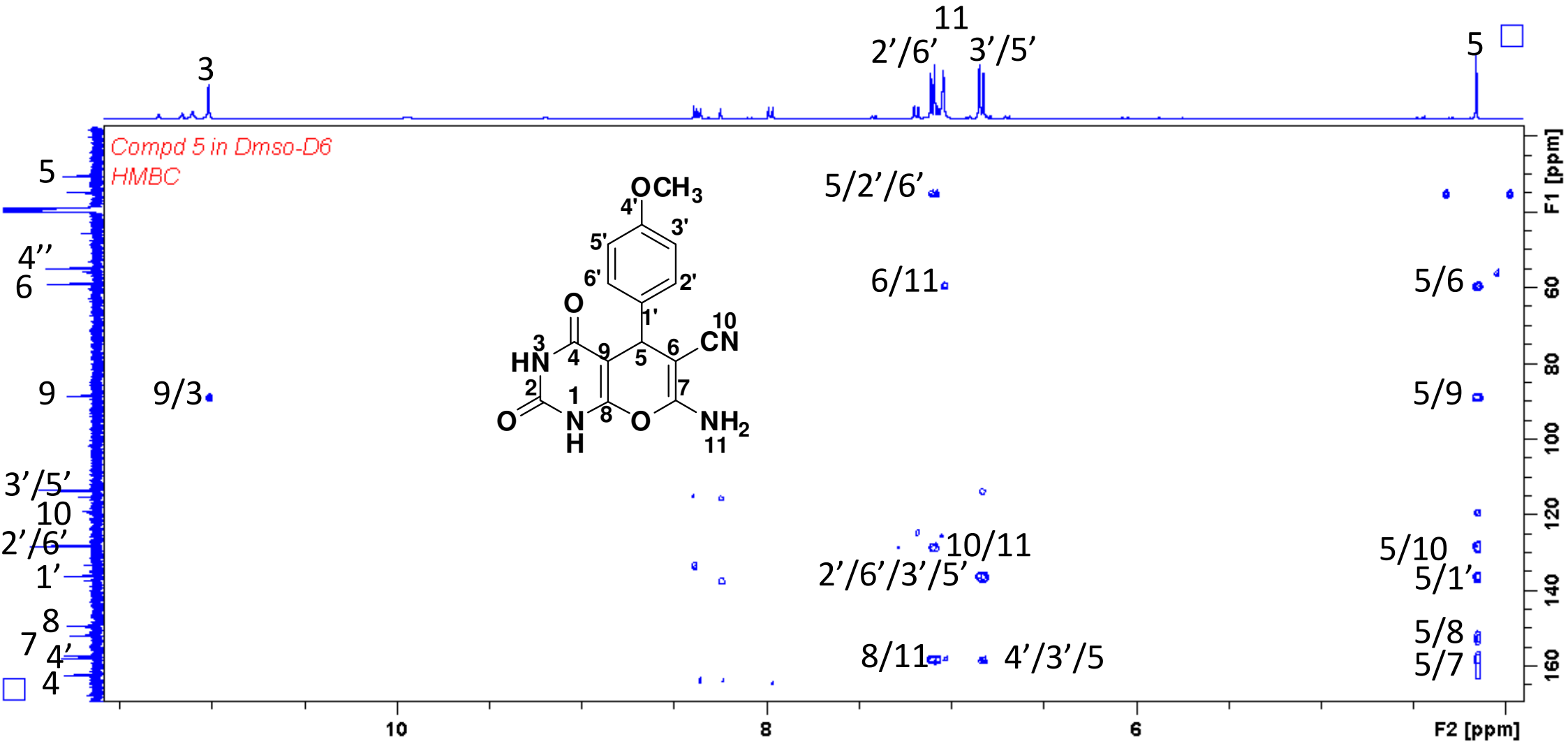
^{13}C NMR spectrum of 7-amino-5-(4-methoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A5)



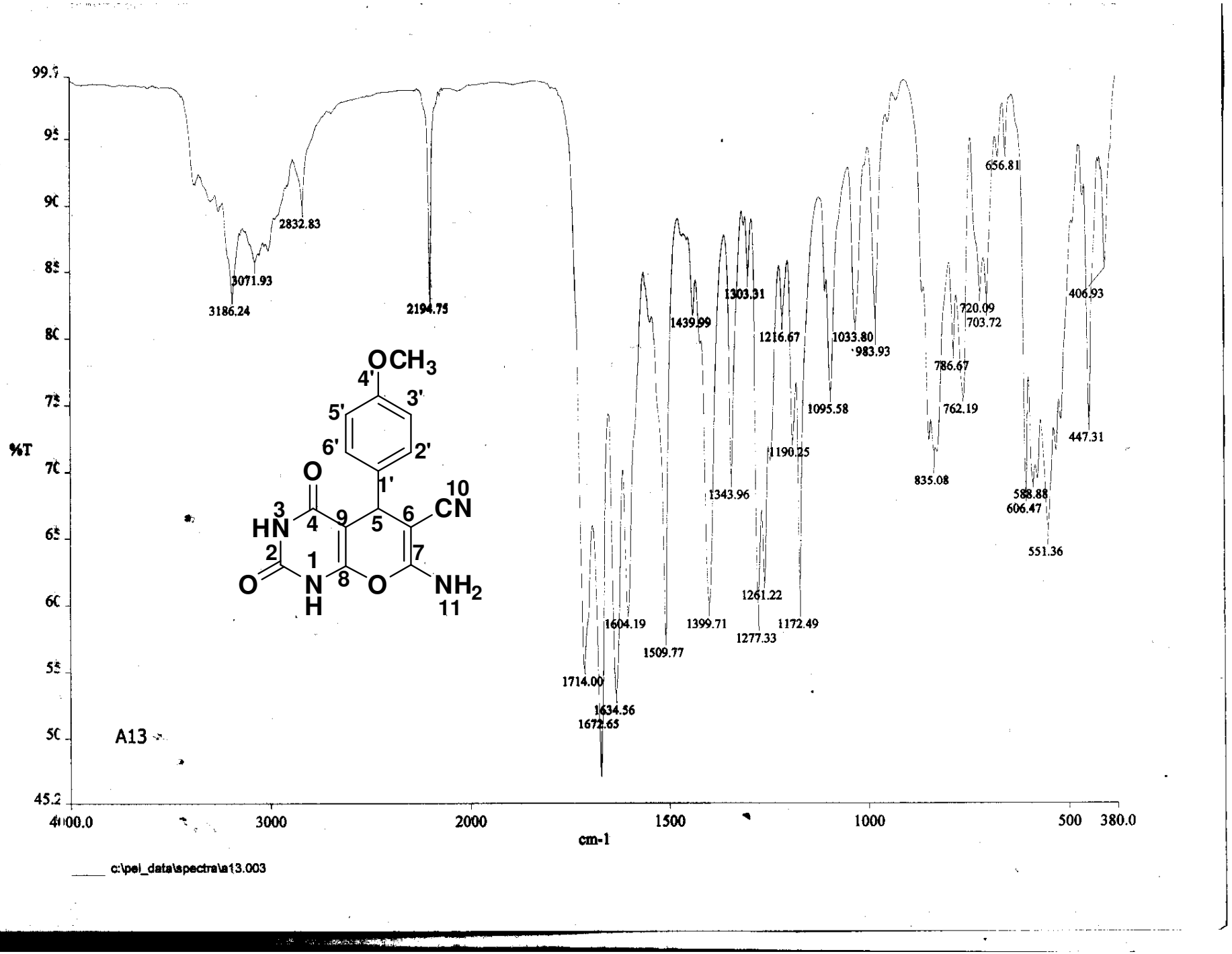
DEPT spectrum of 7-amino-5-(4-methoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A5)



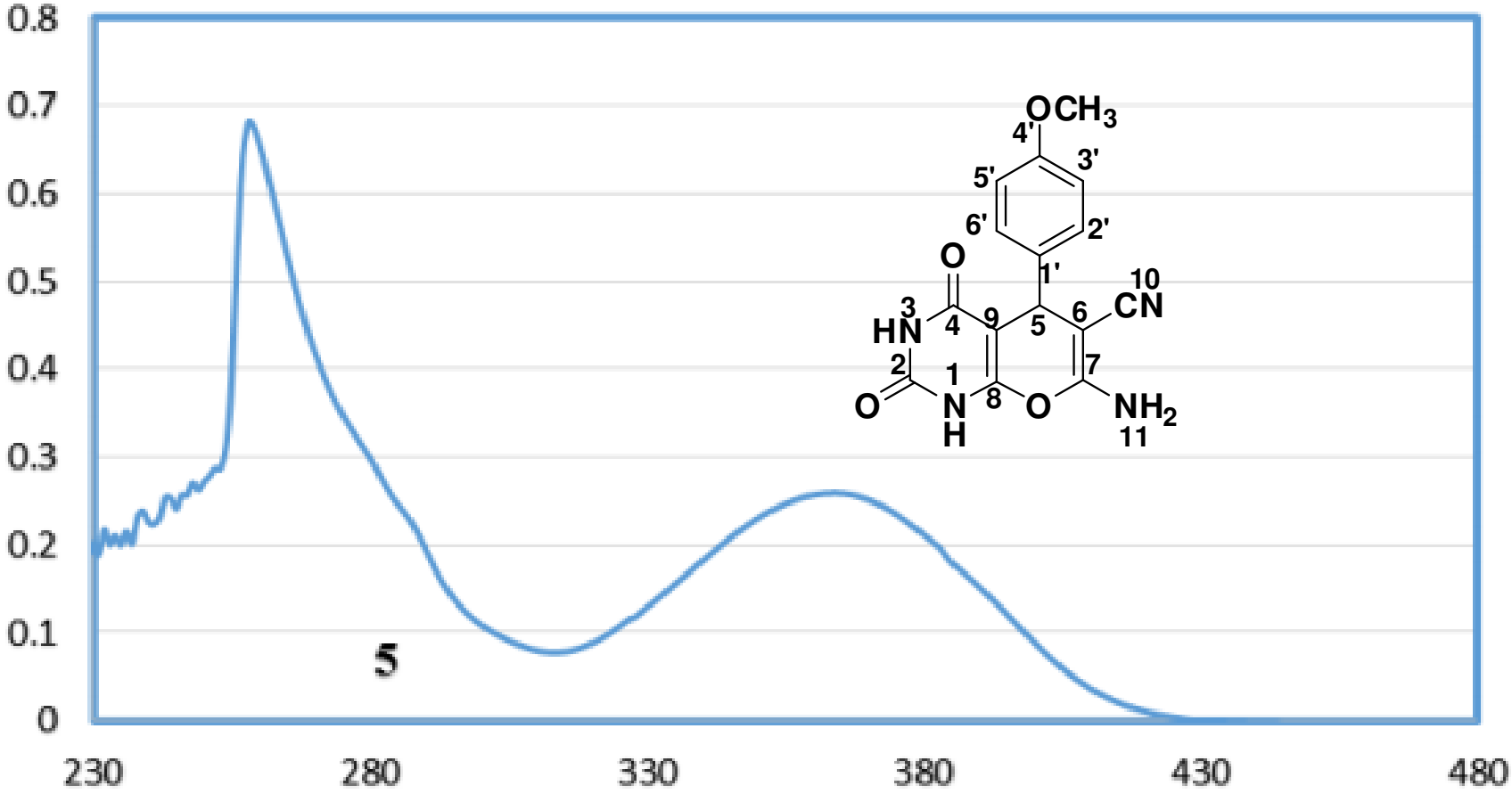
HSQC spectrum of 7-amino-5-(4-methoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A5)



HMBC spectrum of 7-amino-5-(4-methoxylphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A5)

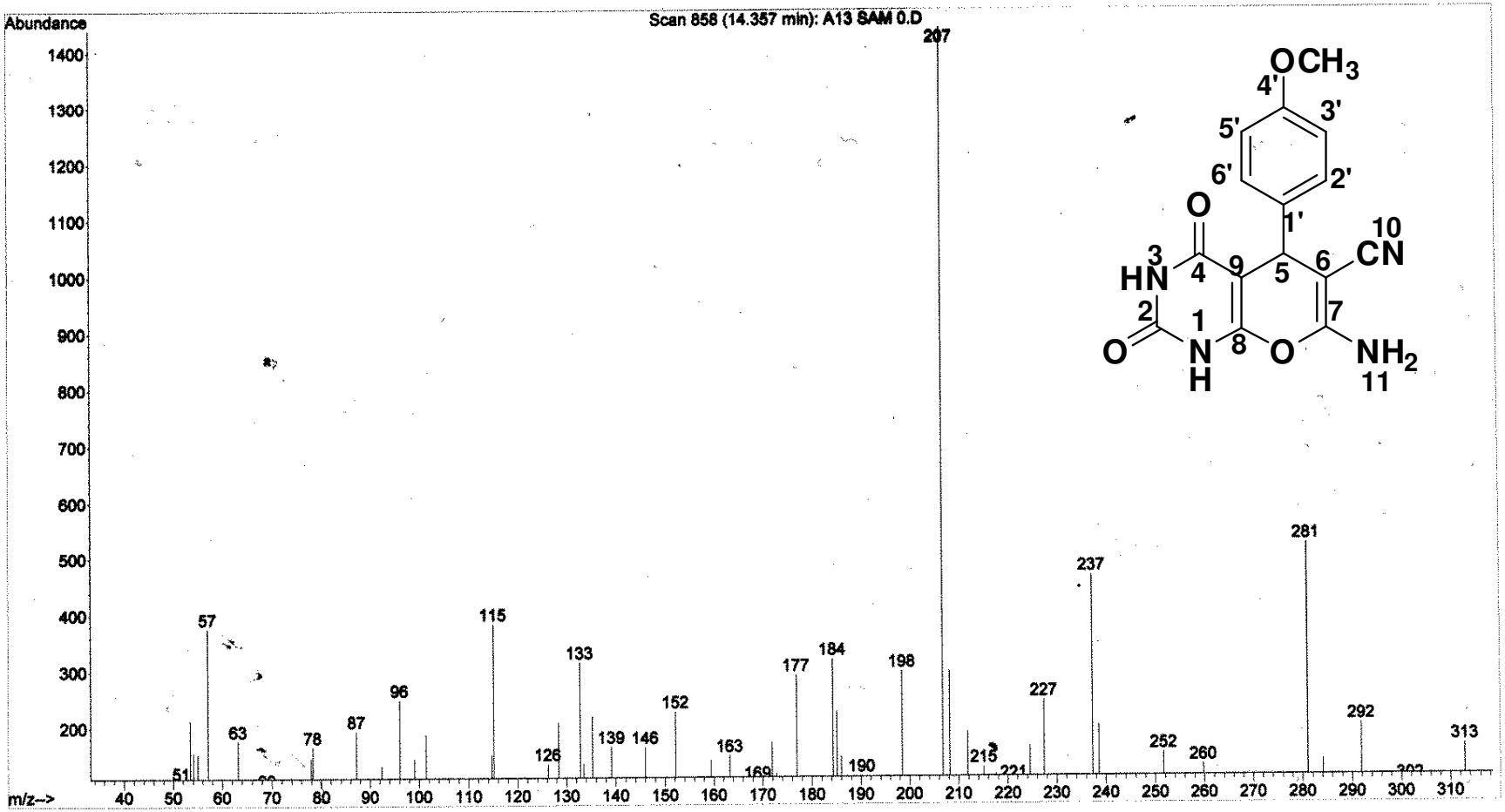


IR spectrum of 7-amino-5-(4-methoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A5)

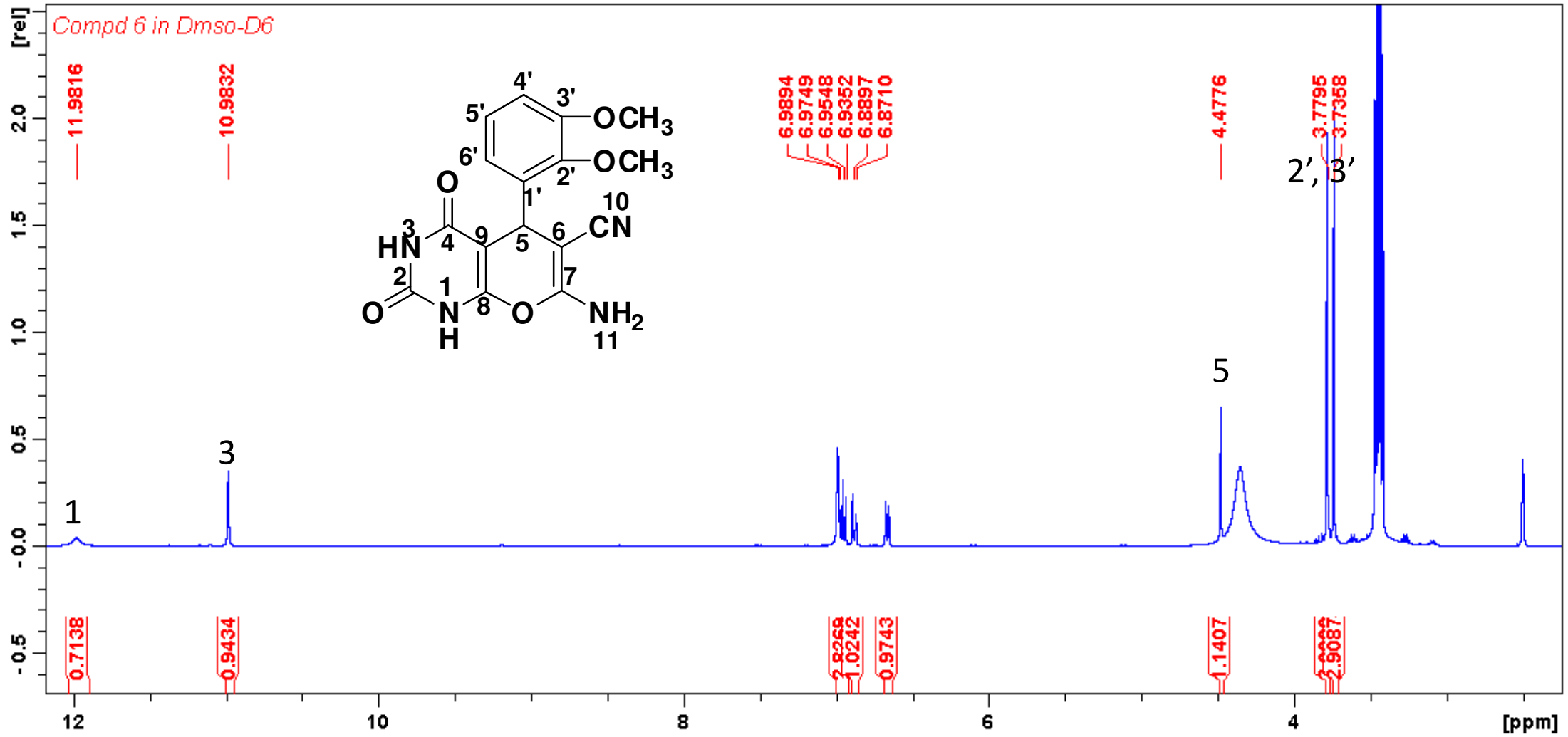


UV spectrum of 7-amino-5-(4-methoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A5)

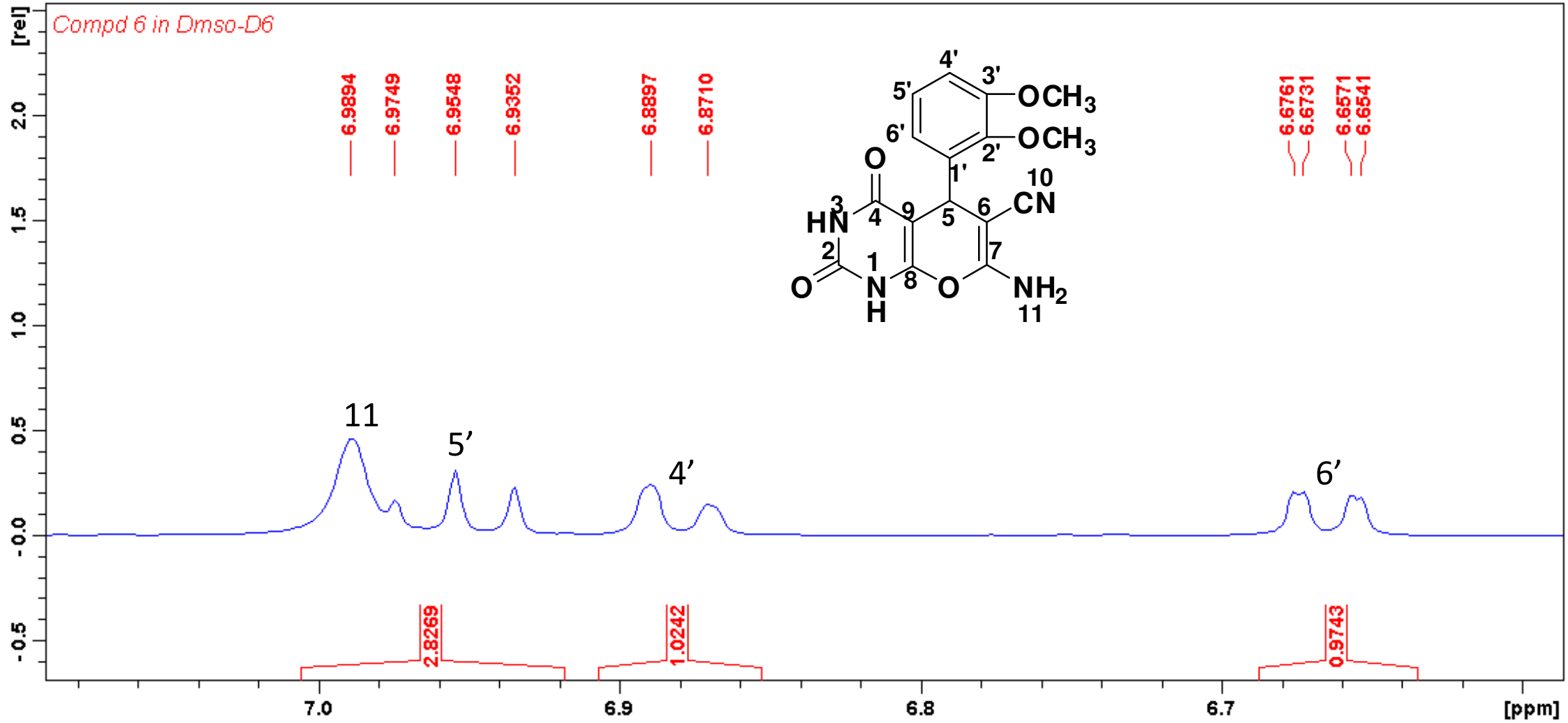
File : C:\MSDCHEM\1\DATA\AREMU\A13 SAM 0.D
Operator : AREMU
Acquired : 11 Jun 2013 11:36 using AcqMethod NATPRODUCTS AREMU
Instrument : 5973n
Sample Name: A13 SAM
Misc Info :
Vial Number: 12



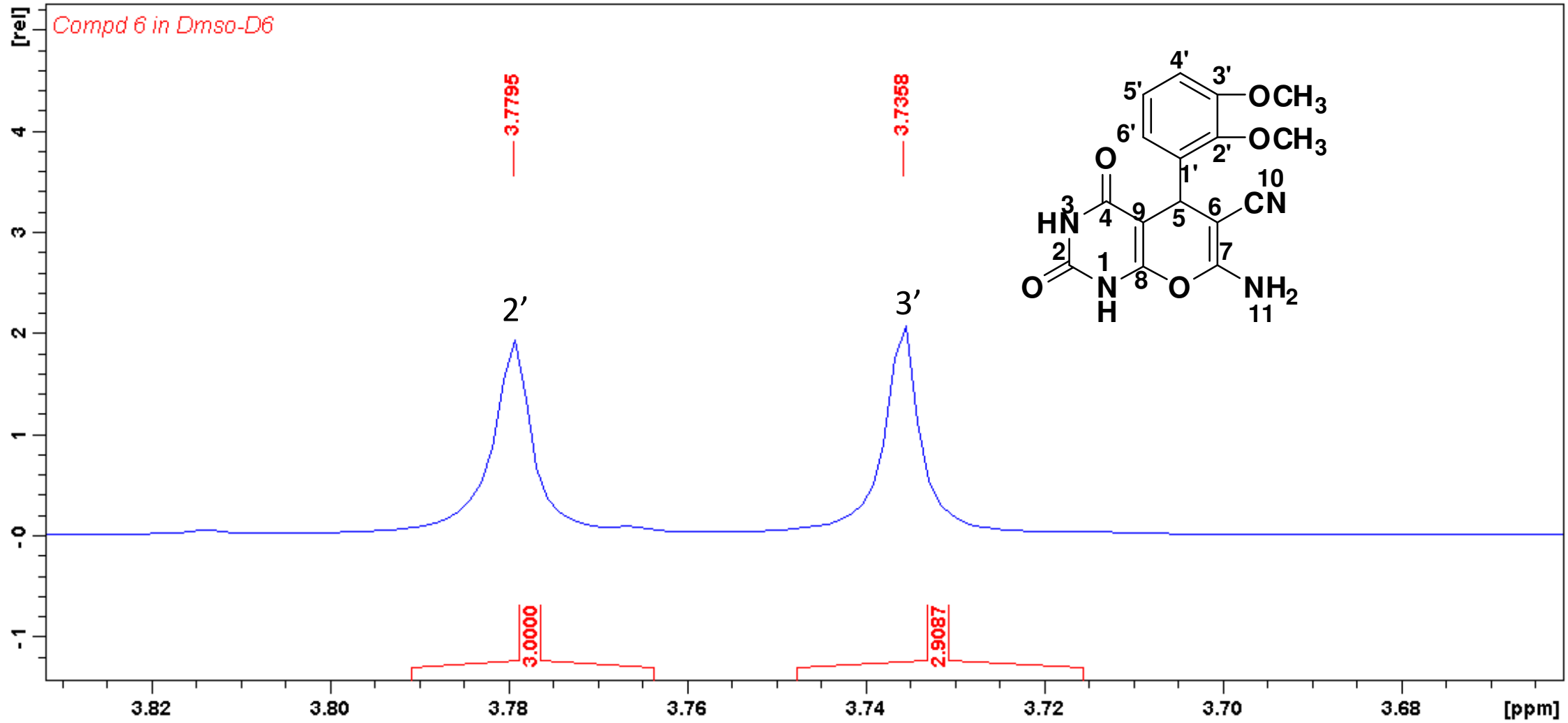
Mass spectrum of 7-amino-5-(4-methoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A5)



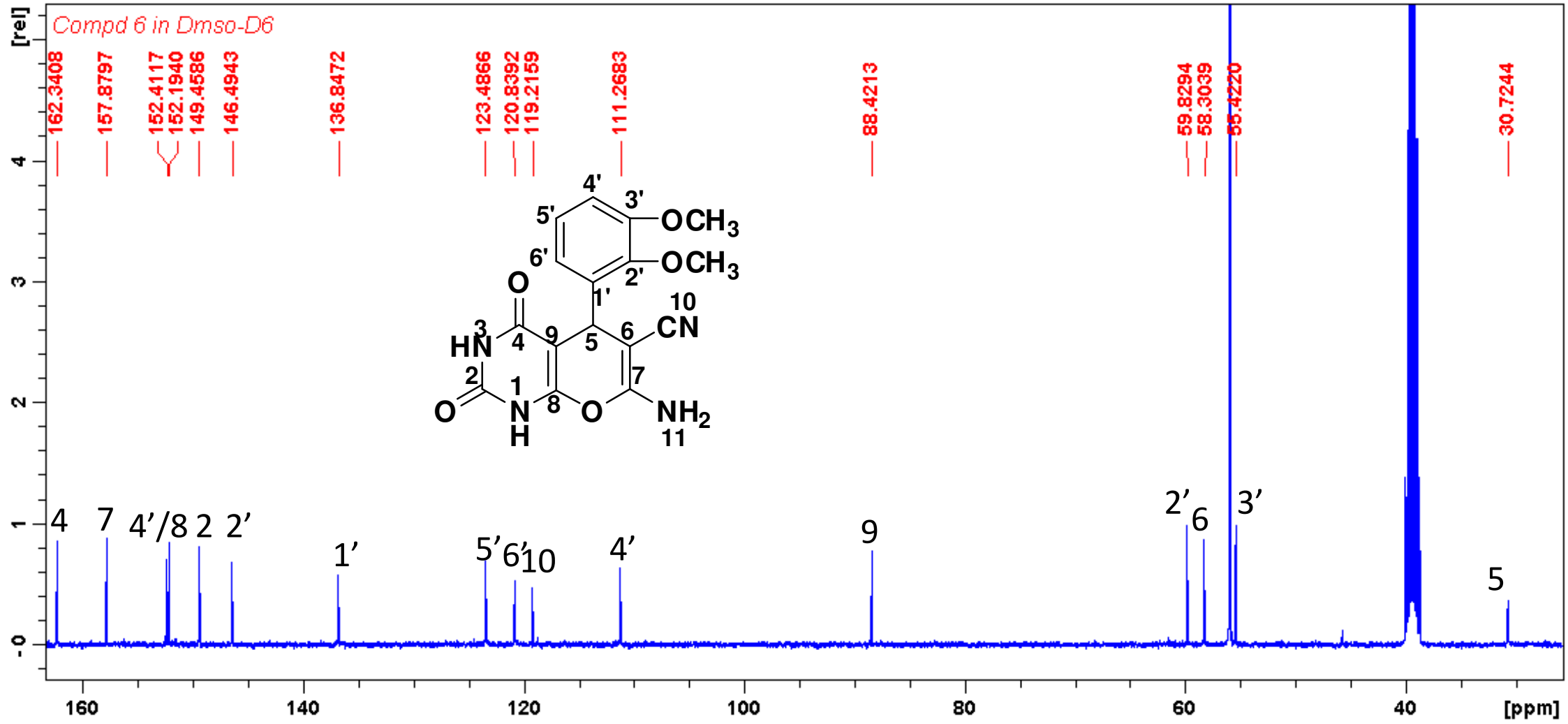
^{1}H NMR spectrum of 7-amino-5-(2,3-dimethoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A6)



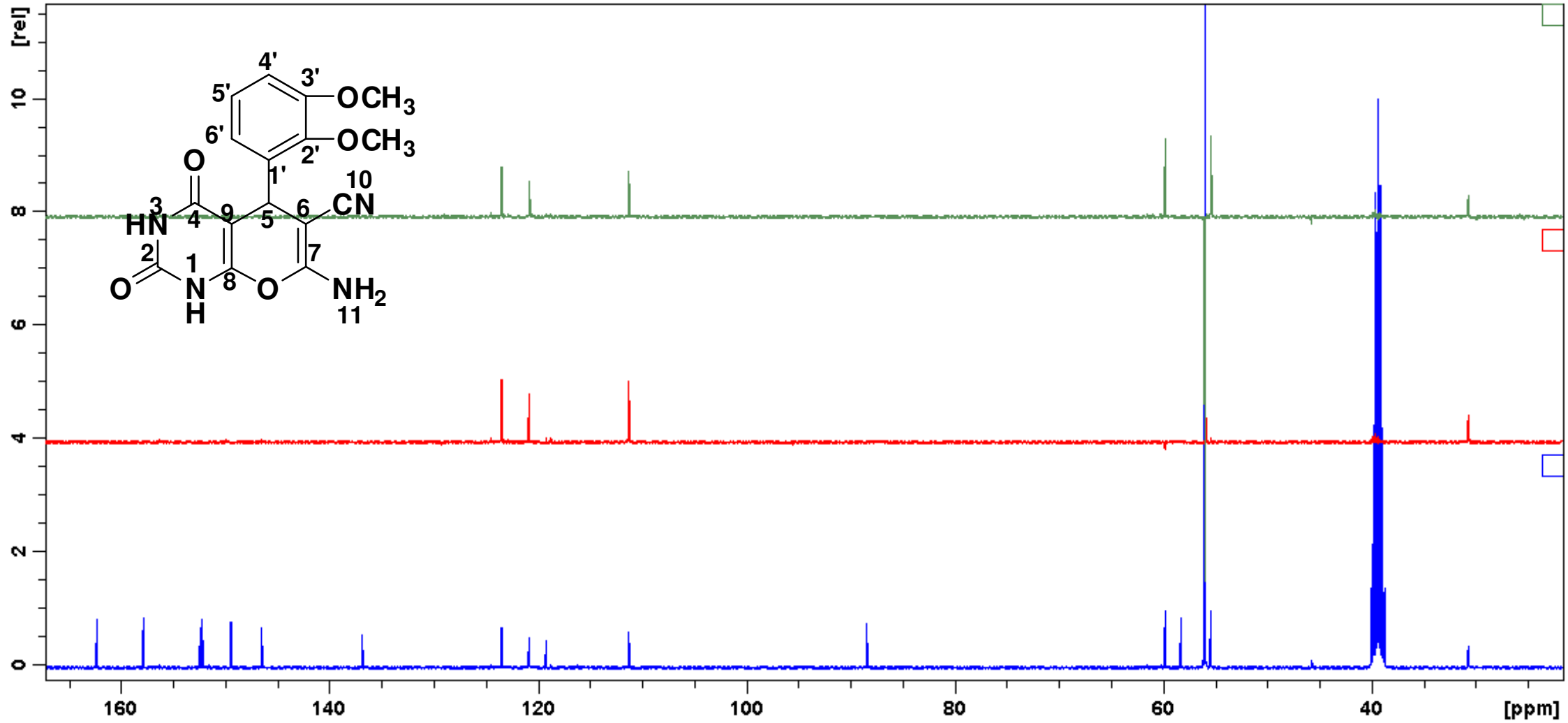
^{1}H NMR spectrum of 7-amino-5-(2,3-dimethoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A6) expanded



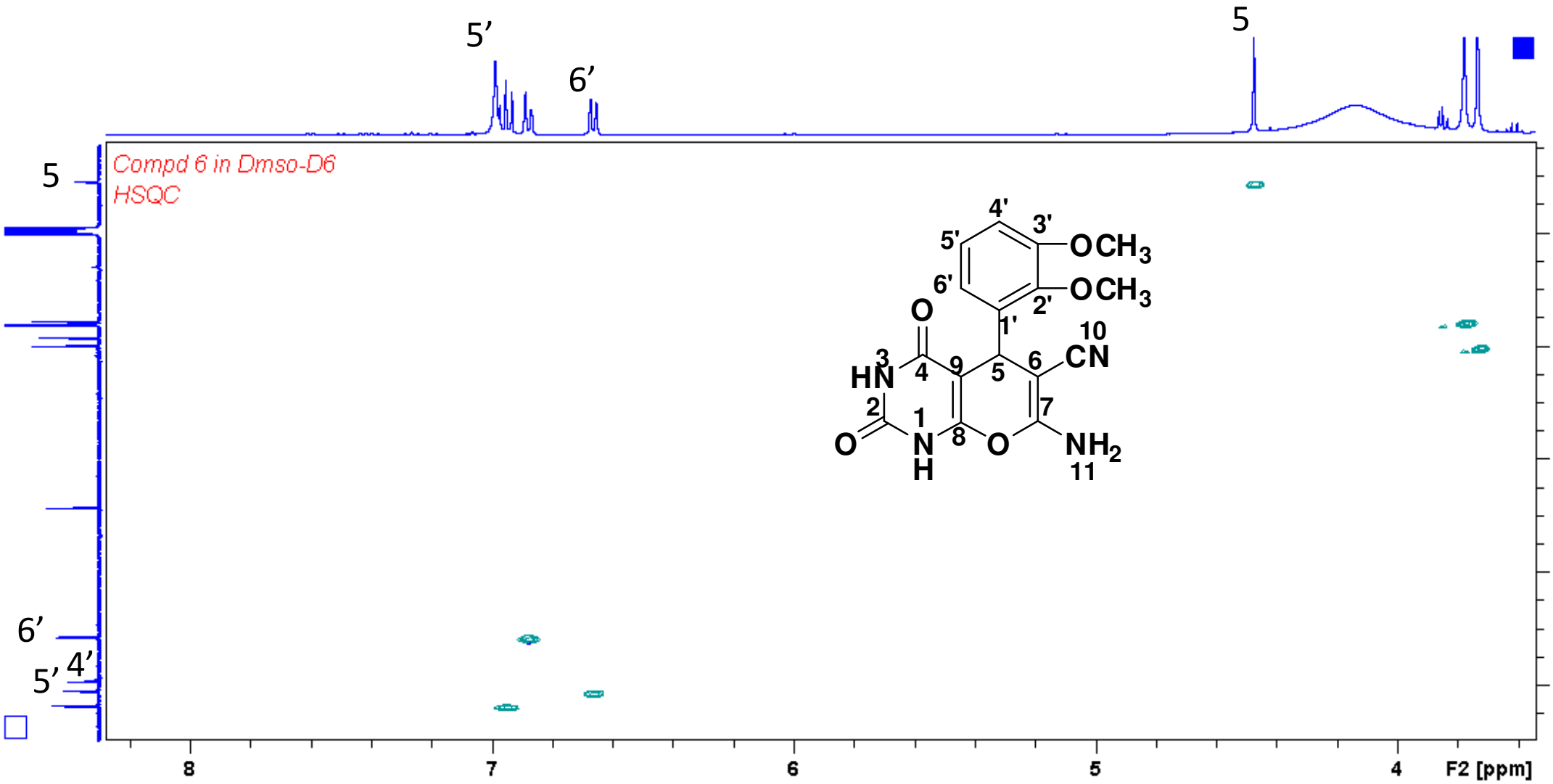
^{1}H NMR spectrum of 7-amino-5-(2,3-dimethoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A6) expanded



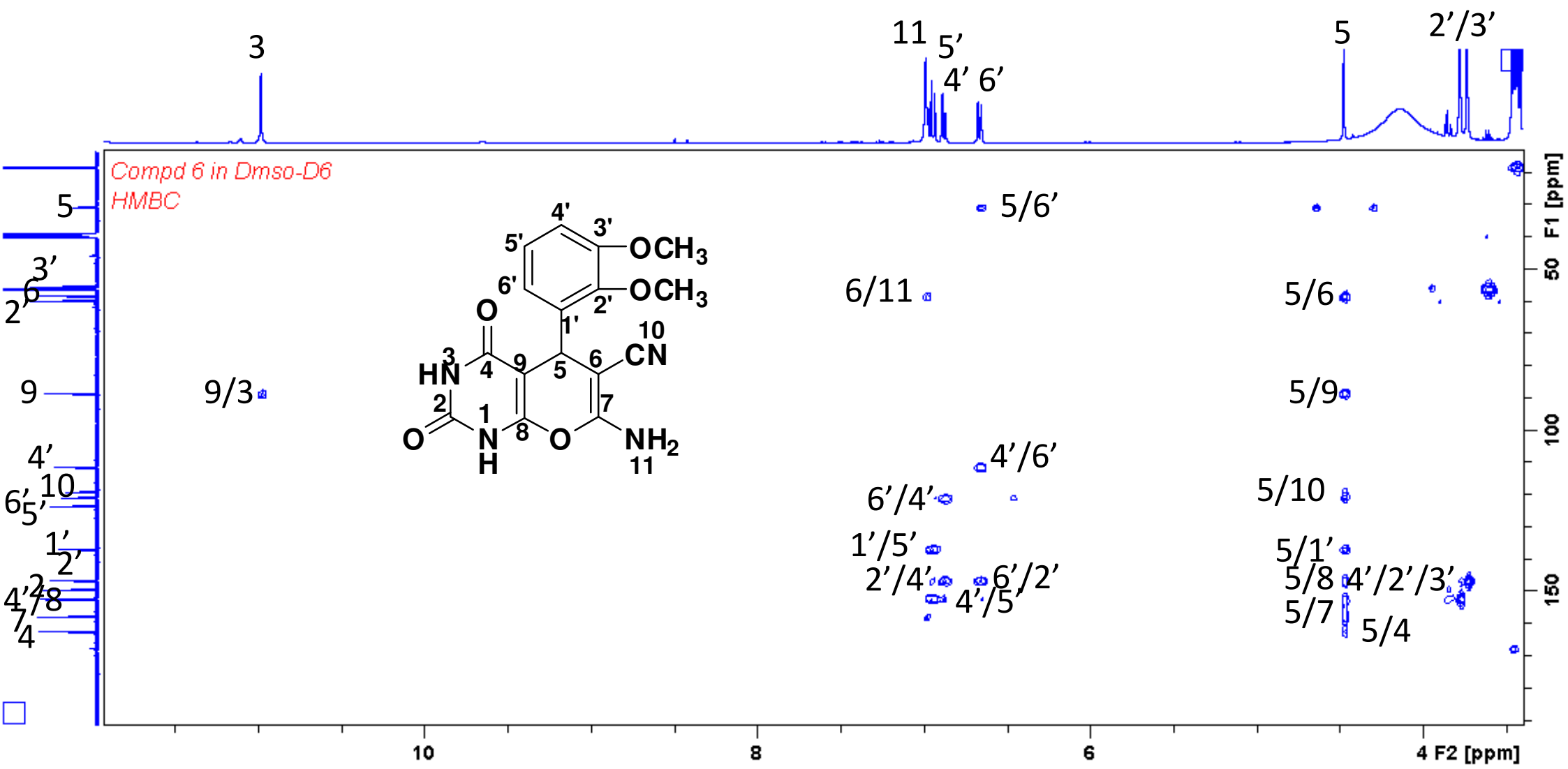
^{13}C NMR spectrum of 7-amino-5-(2,3-dimethoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A6)



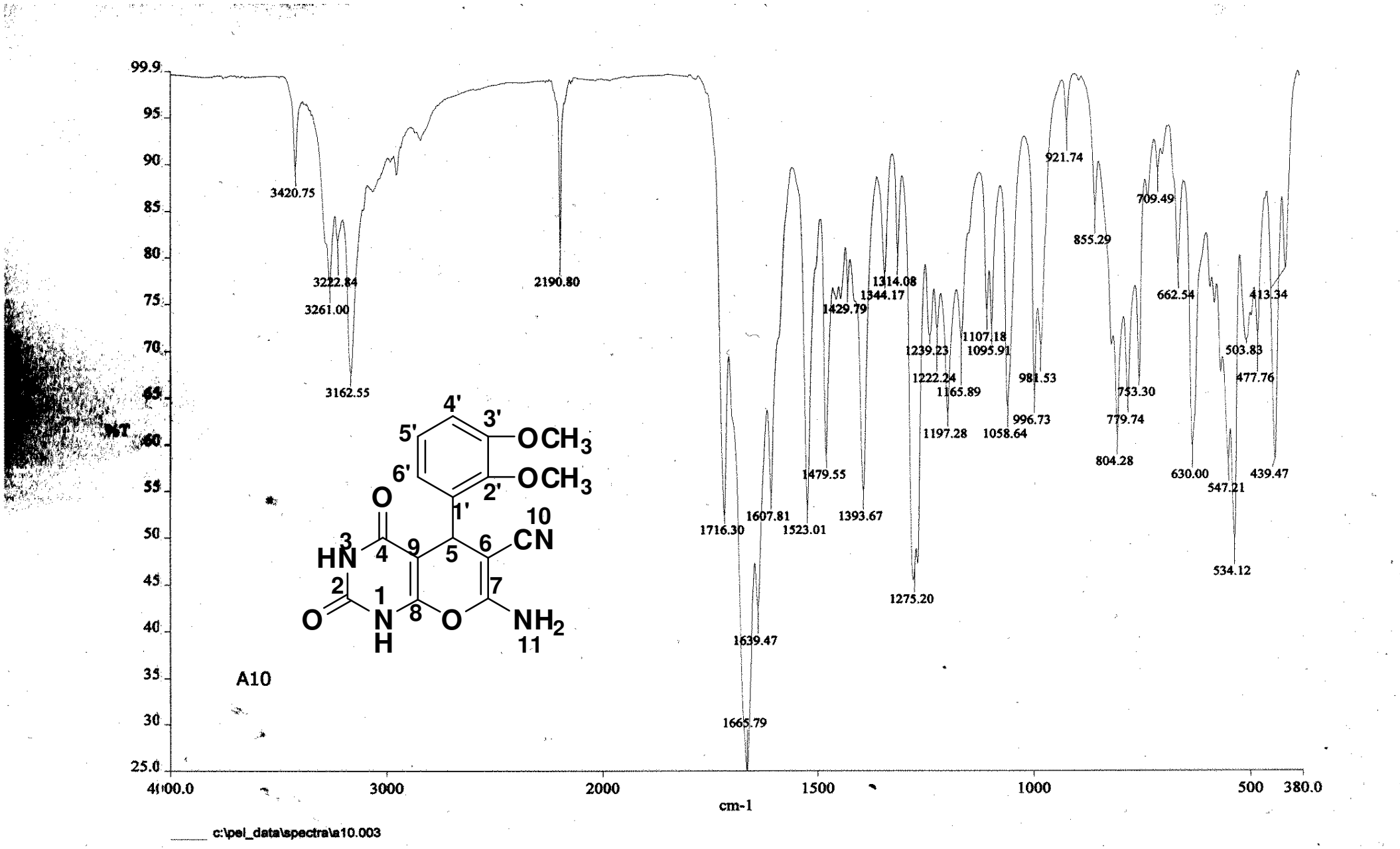
DEPT spectrum of 7-amino-5-(2,3-dimethoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A6)



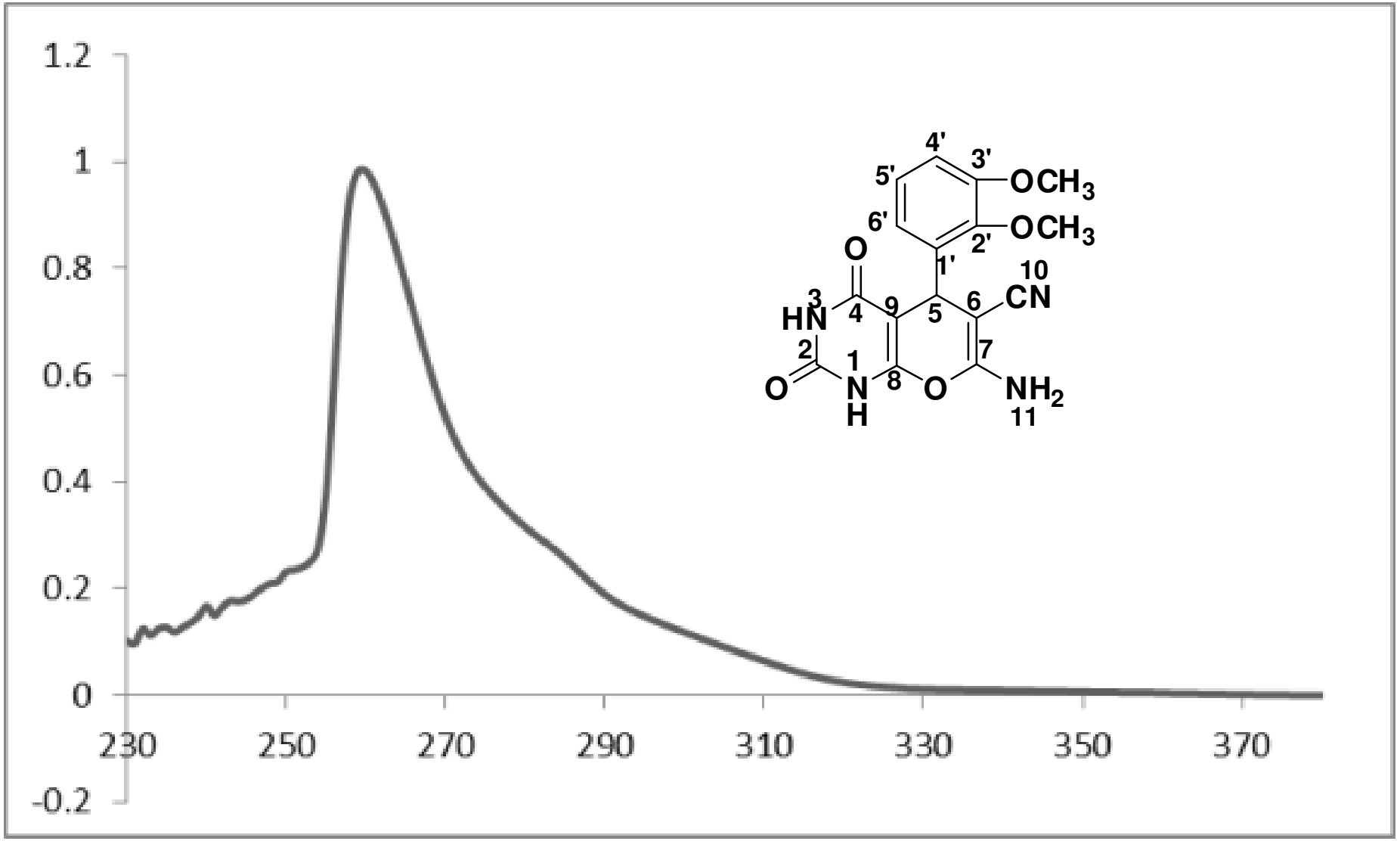
HSQC spectrum of 7-amino-5-(2,3-dimethoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A6)



HMBC spectrum of 7-amino-5-(2,3-dimethoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A6)



IR spectrum of 7-amino-5-(2,3-dimethoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A6)



UV spectrum of 7-amino-5-(2,3-dimethoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A6)

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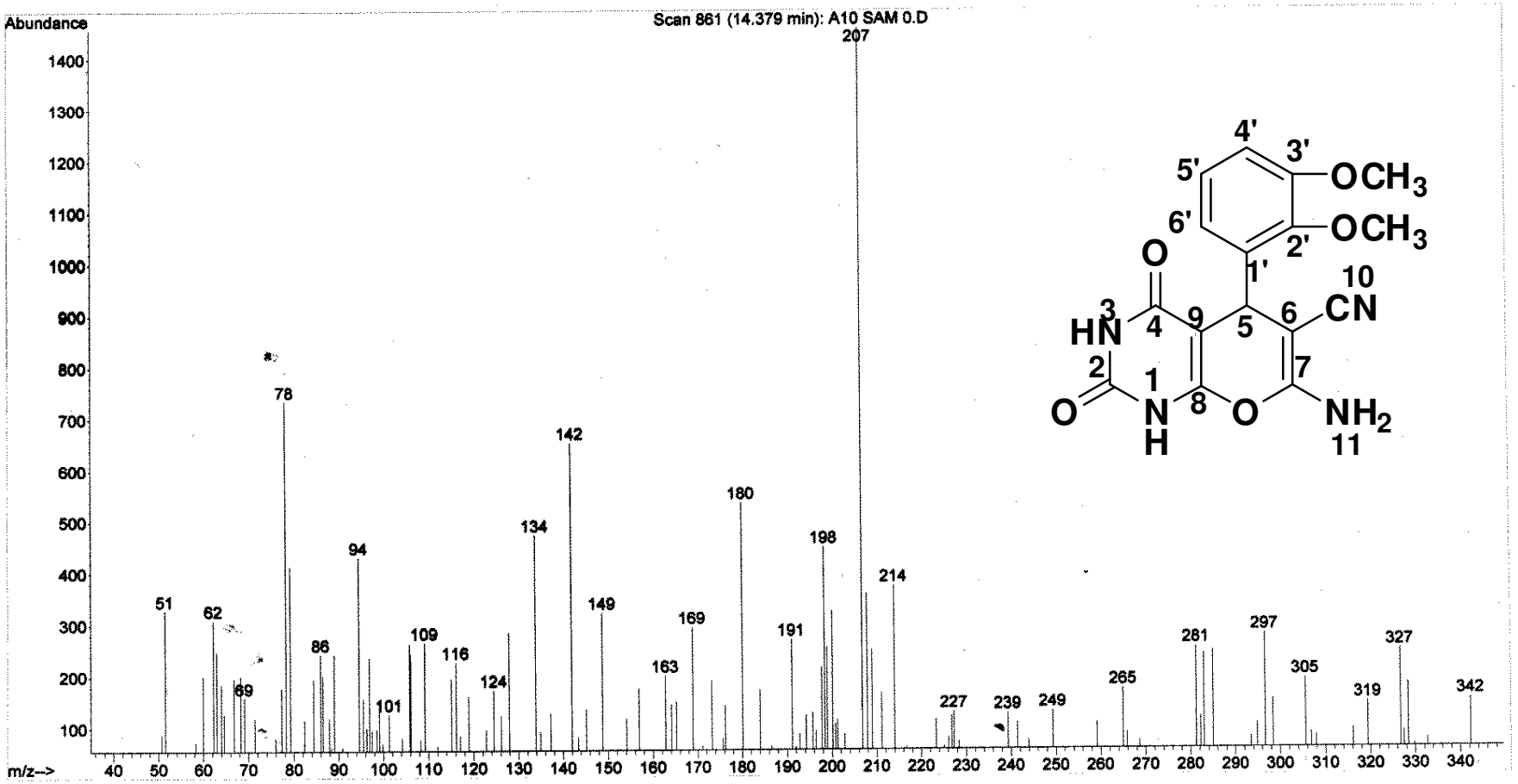
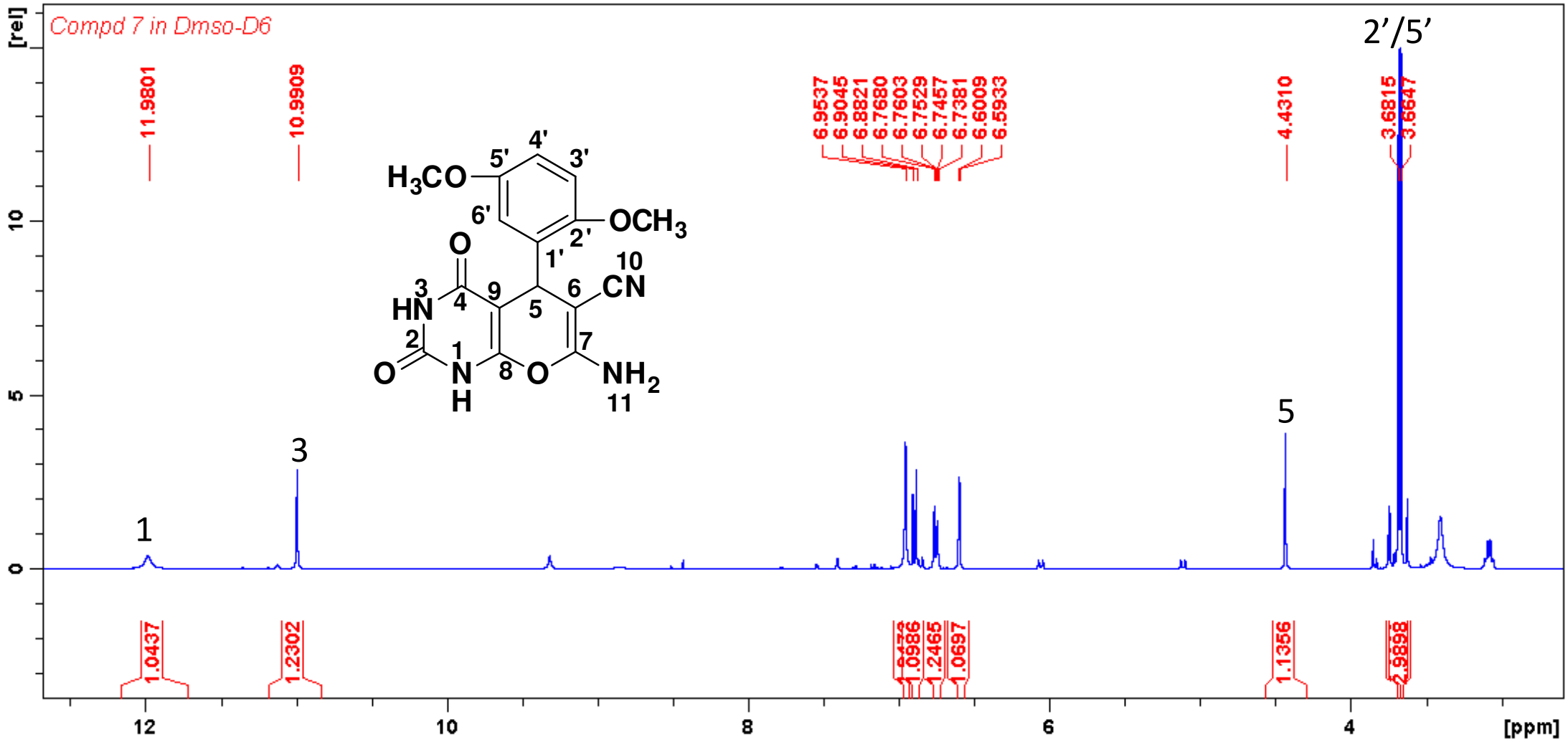
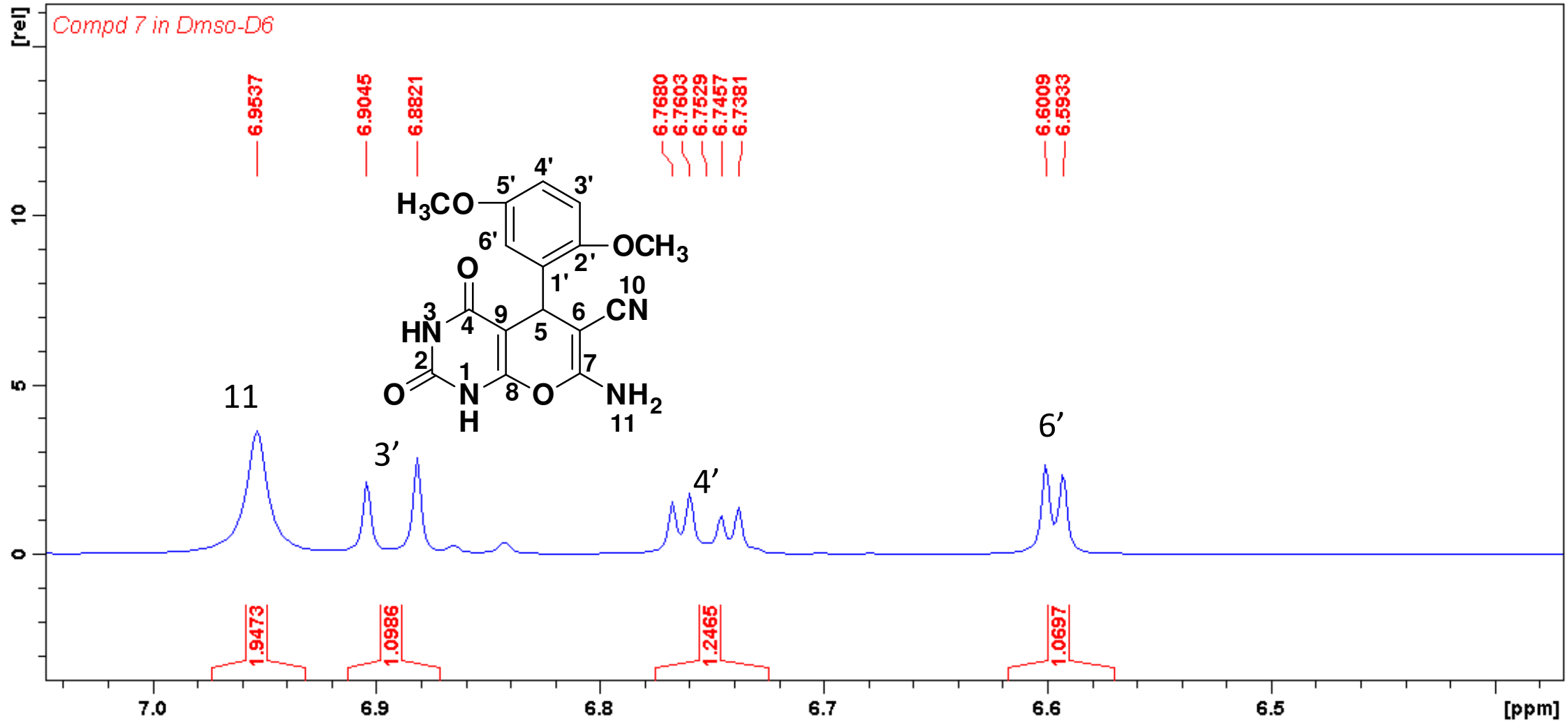
Operator : AREMU
Acquired : 11 Jun 2013 9:18 using AcqMethod NATPRODUCTS AREMU
Instrument : 5973n
Sample Name: A10 SAM
Misc Info :
Vial Number: 9



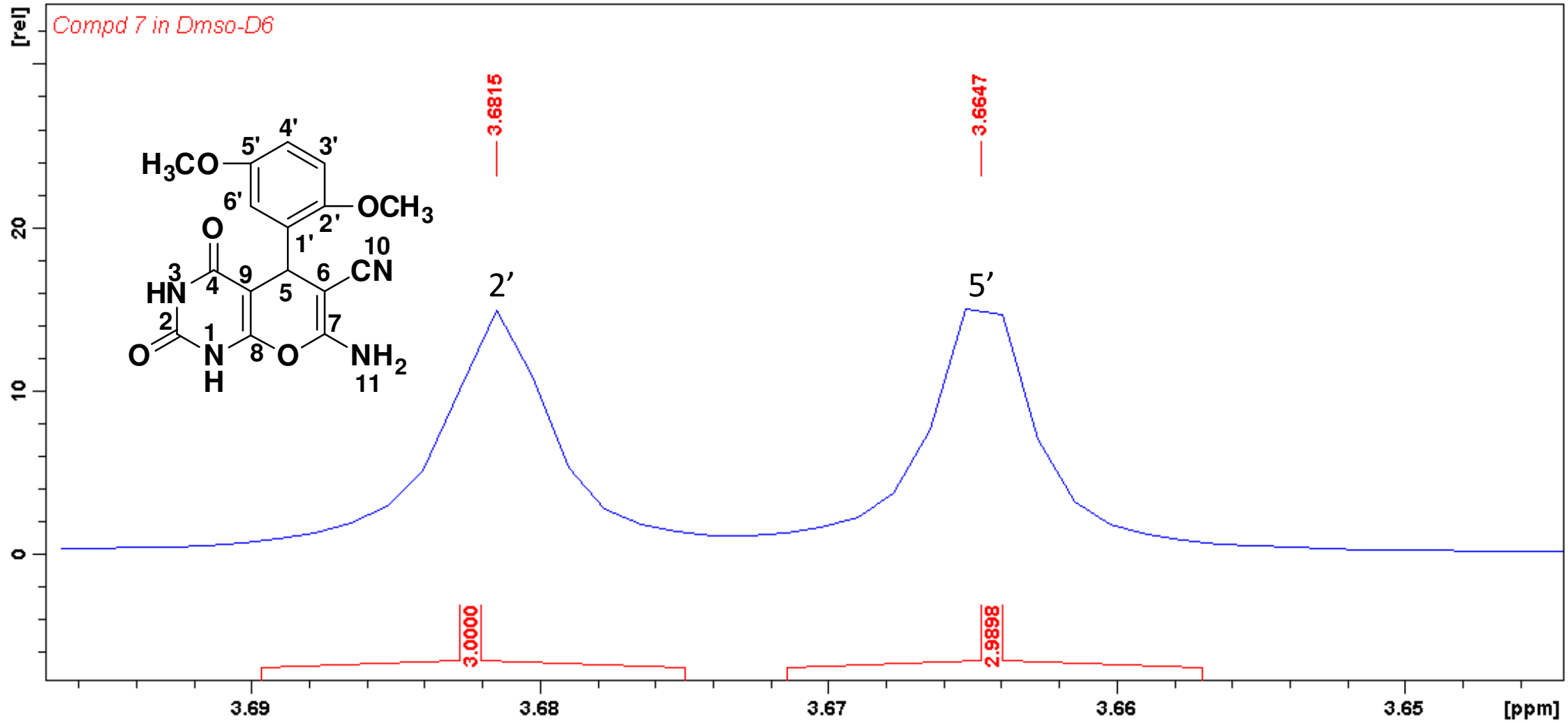
Mass spectrum of 7-amino-5-(2,3-dimethoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A6)



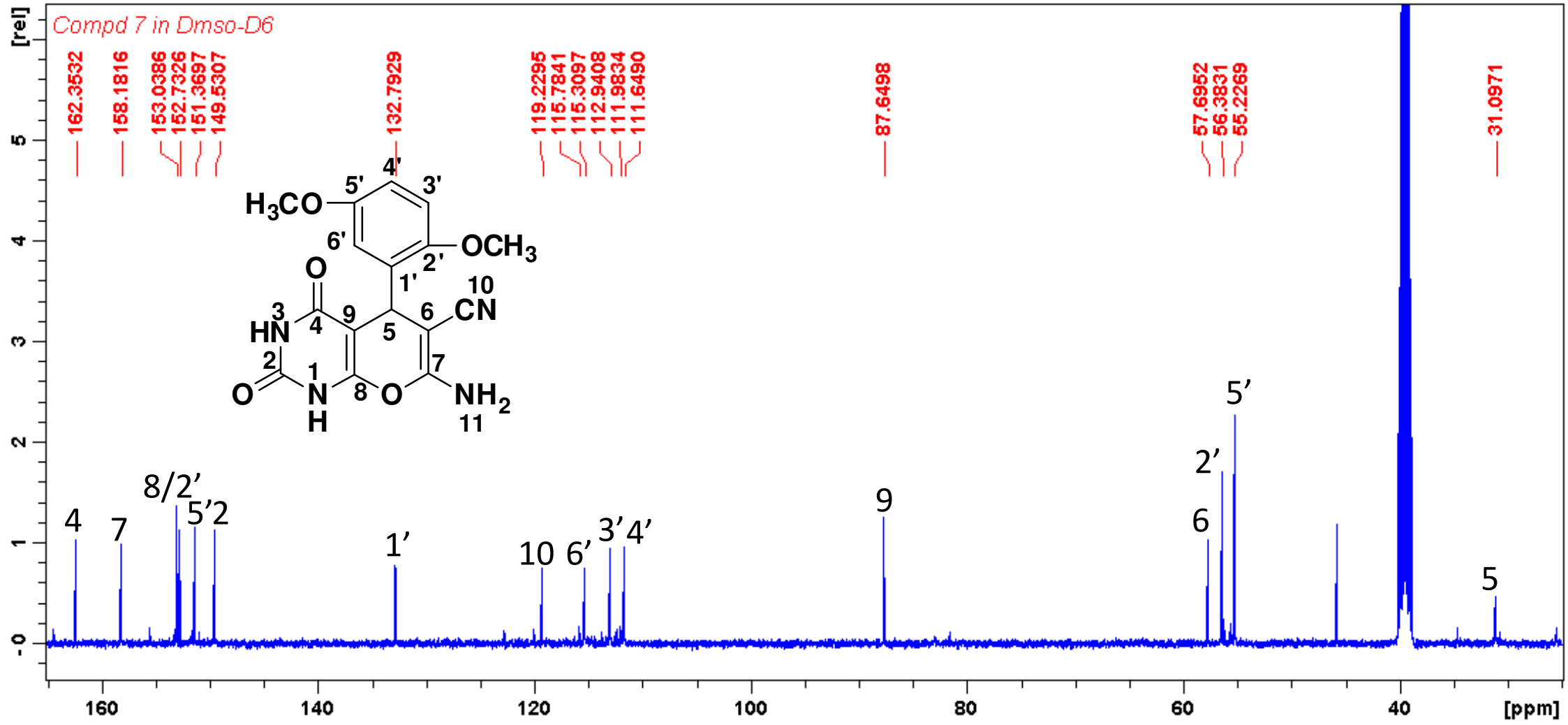
^{1}H NMR spectrum of 7-amino-5-(2,5-dimethoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A7)



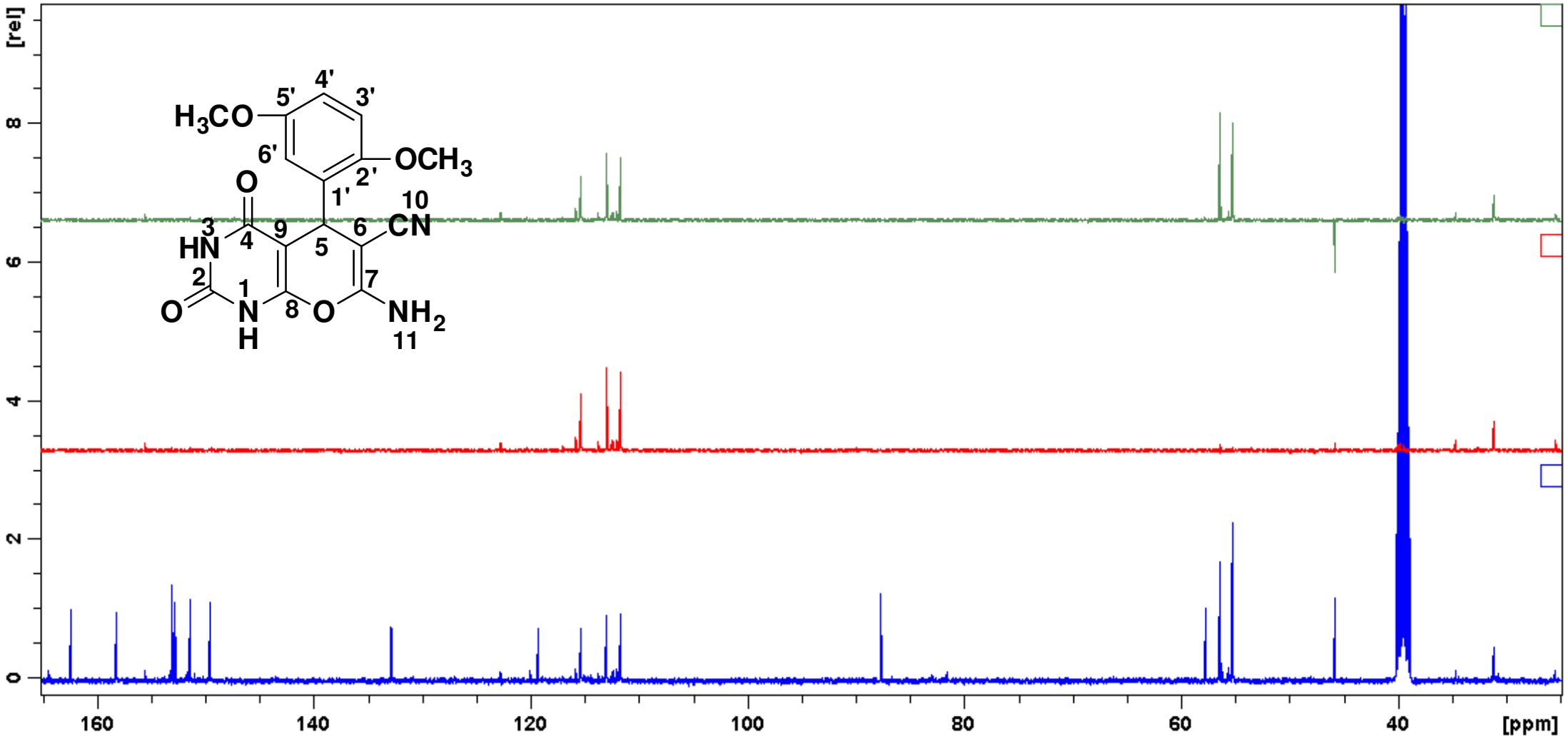
^{1}H NMR spectrum of 7-amino-5-(2,5-dimethoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A7) expanded



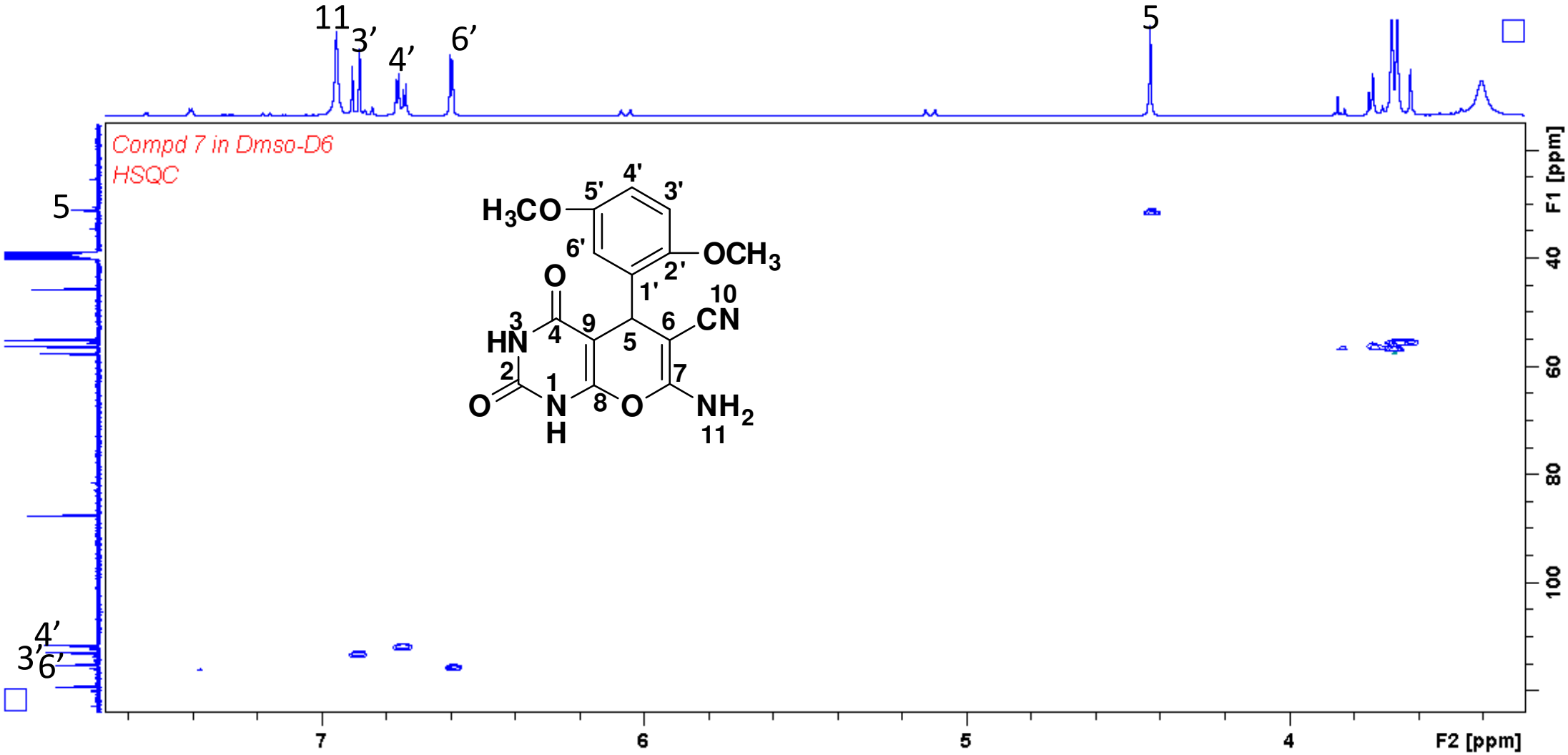
^{1}H NMR spectrum of 7-amino-5-(2,5-dimethoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A7) expanded



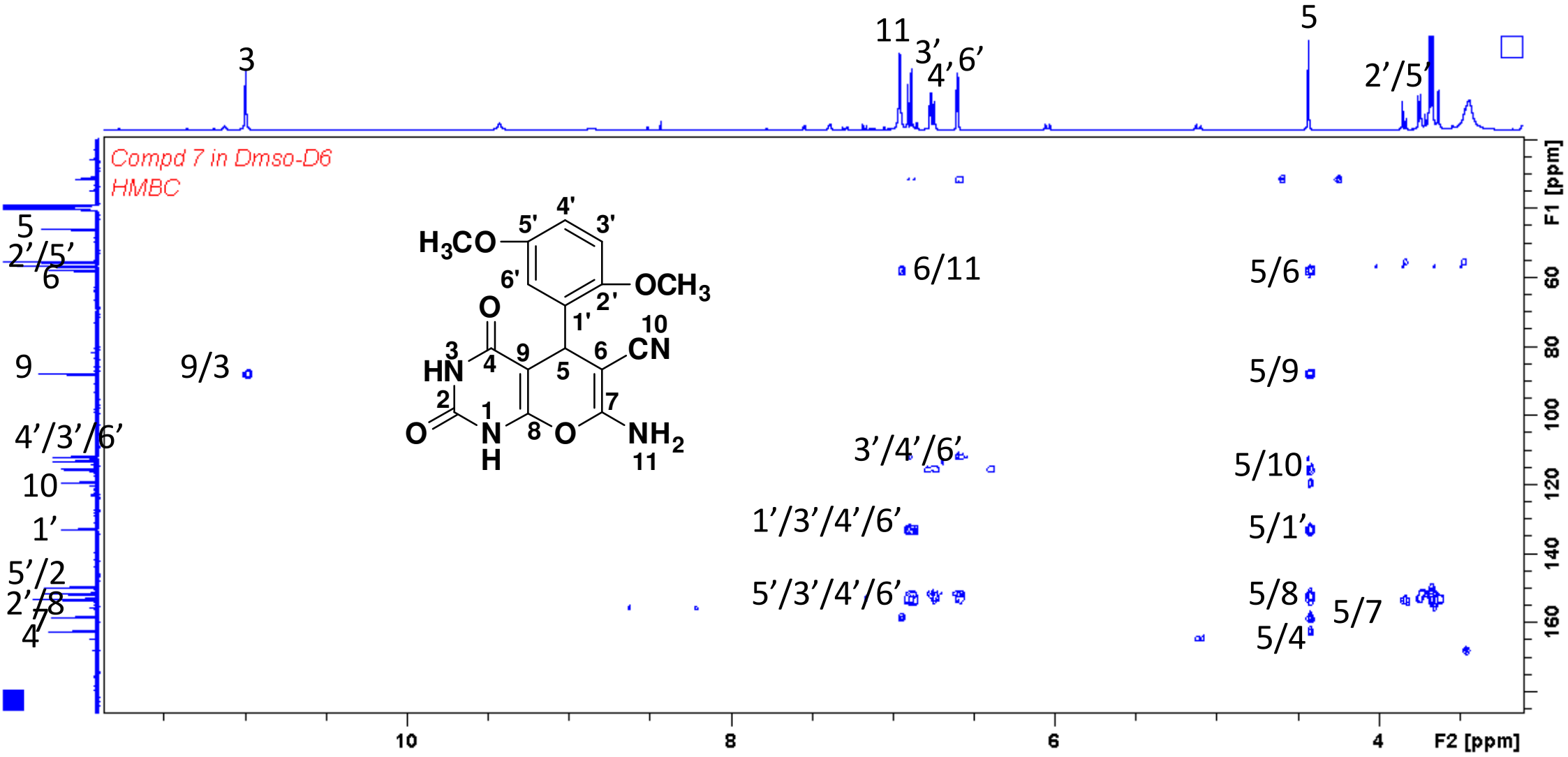
^{13}C NMR spectrum of 7-amino-5-(2,5-dimethoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A7)



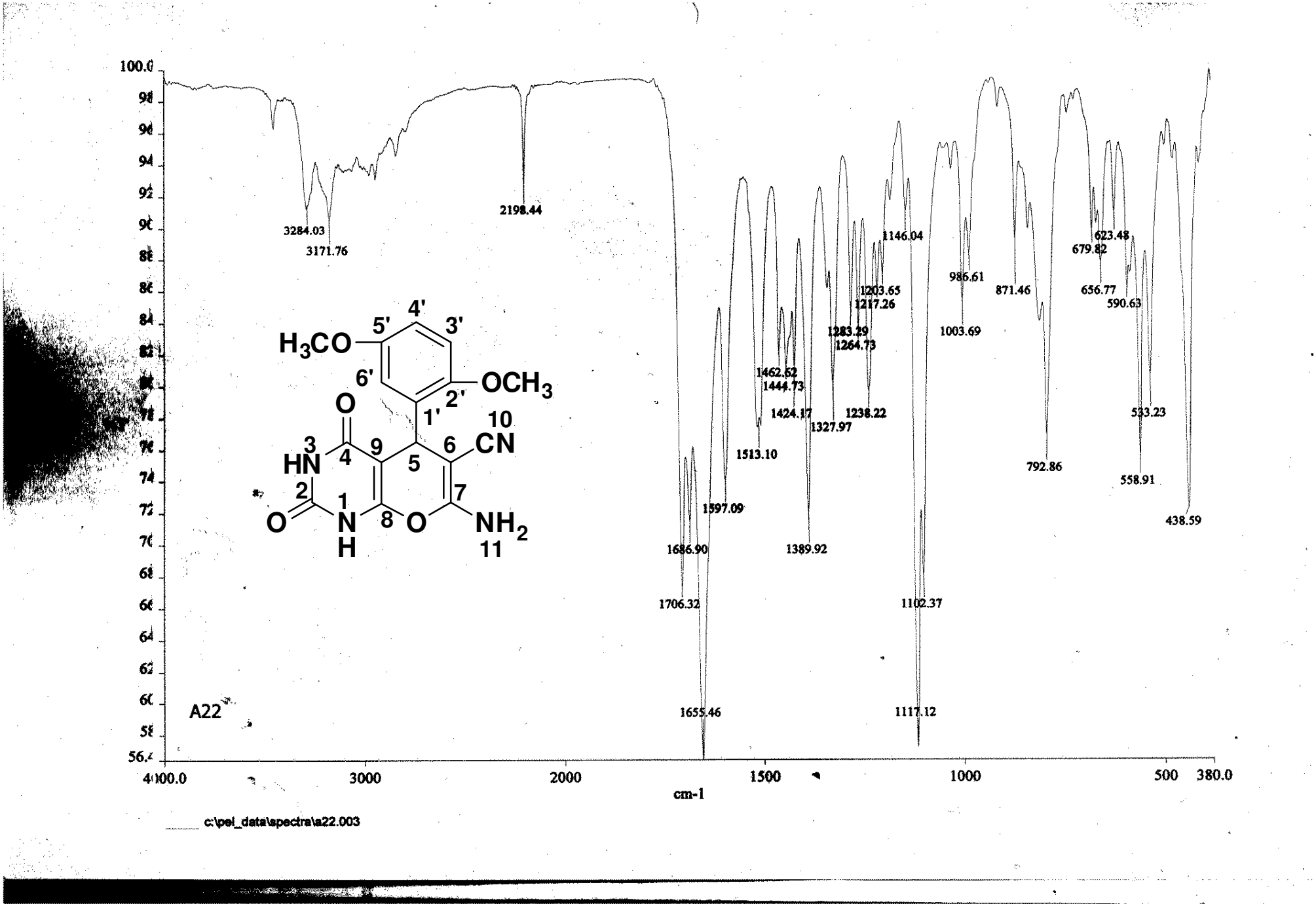
DEPT spectrum of 7-amino-5-(2,5-dimethoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A7)



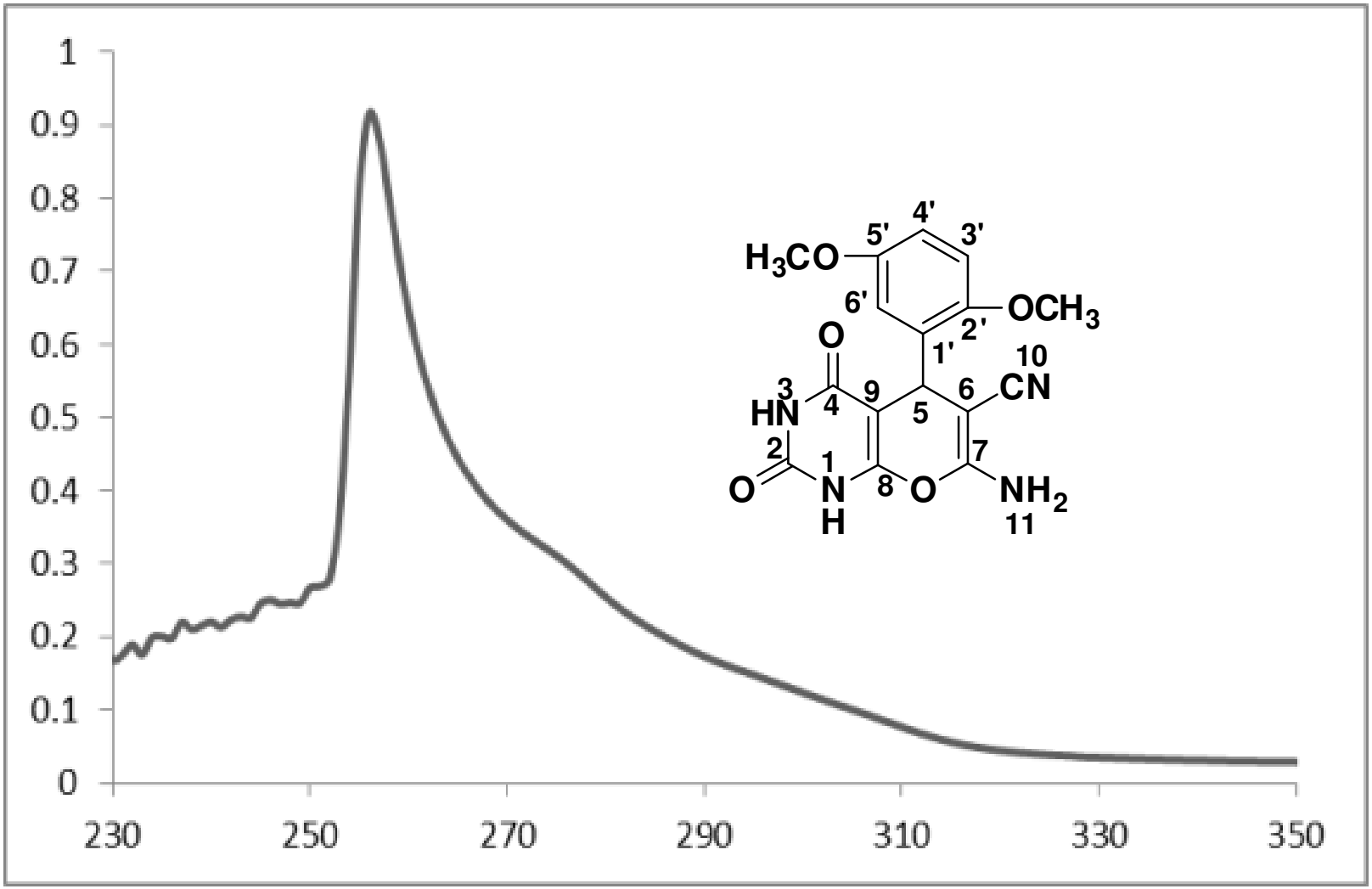
HSQC spectrum of 7-amino-5-(2,5-dimethoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A7)



HMBC spectrum of 7-amino-5-(2,5-dimethoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A7)



IR spectrum of 7-amino-5-(2,5-dimethoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A7)



UV spectrum of 7-amino-5-(2,5-dimethoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A7)

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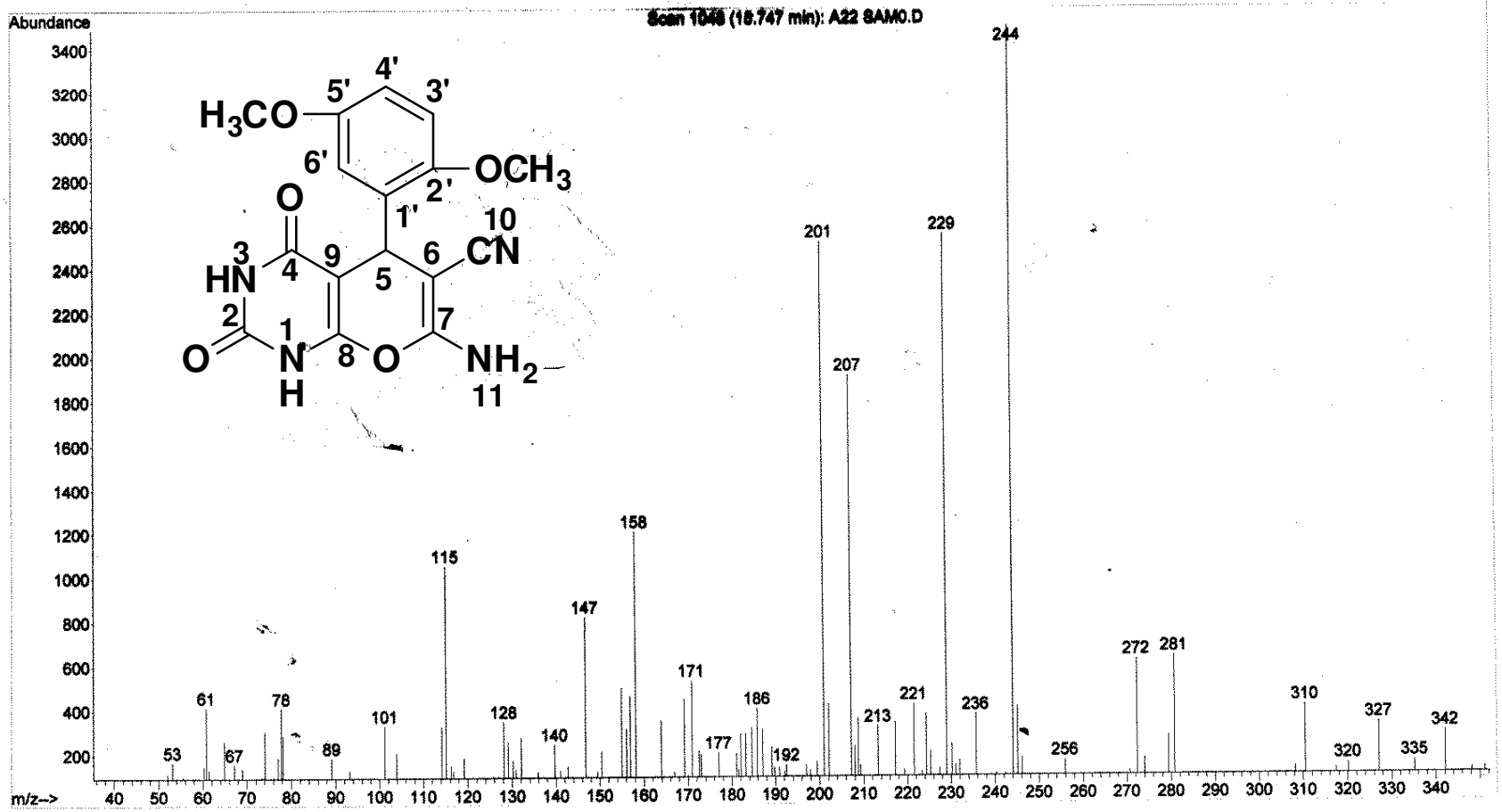
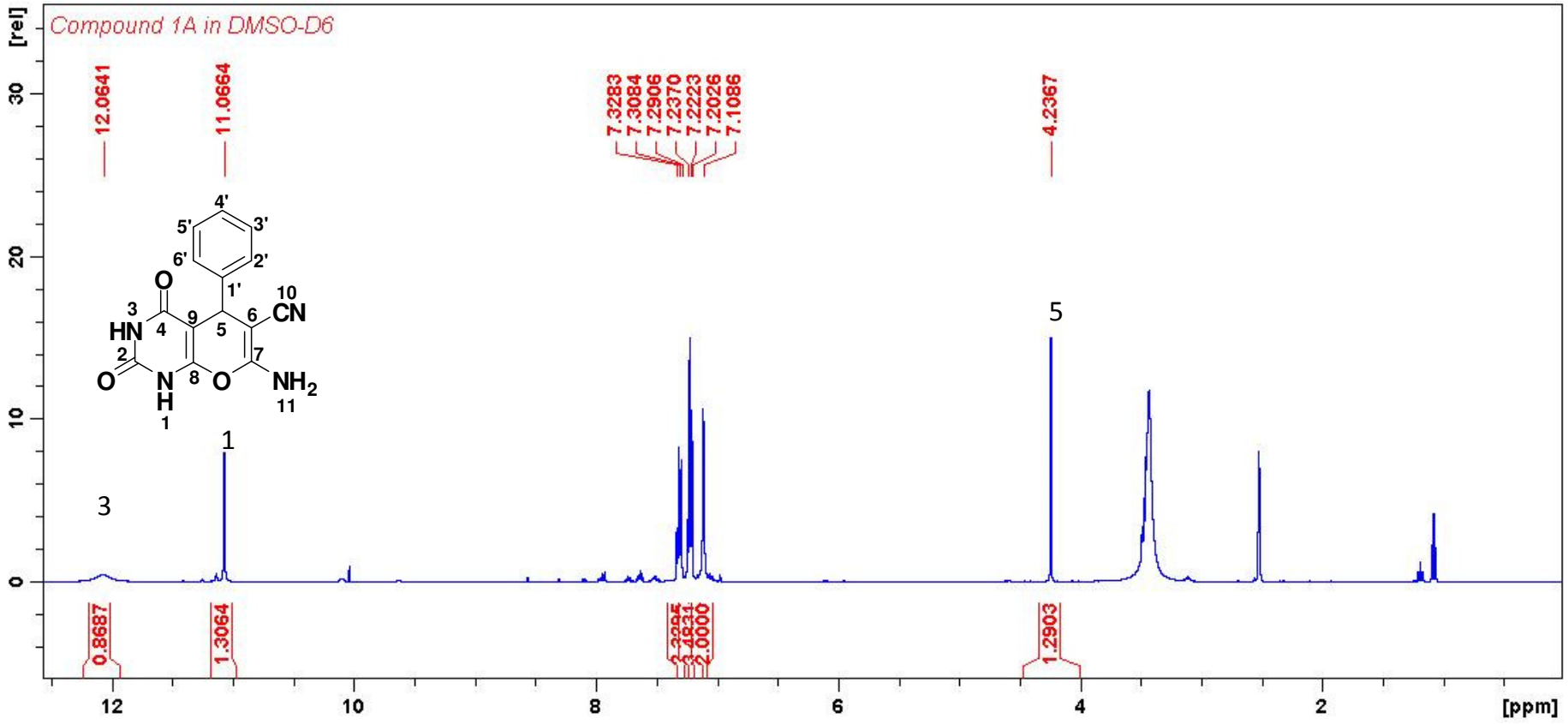
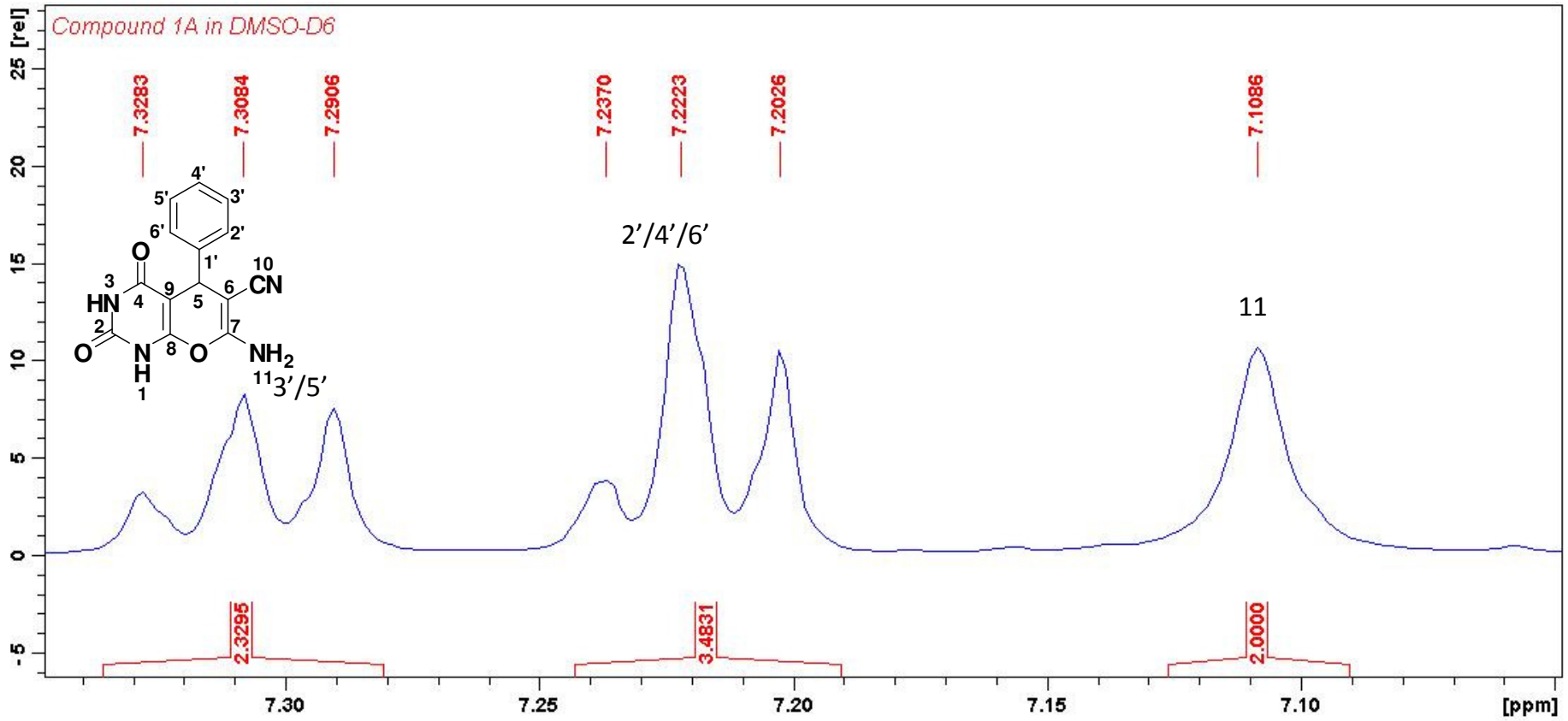
Operator : AREMU
Acquired : 11 Jun 2013 22:19 using AcqMethod NATPRODUCTS AREMU
Instrument : 5973n
Sample Name: A22 SAM
Misc Info :
Vial Number: 8



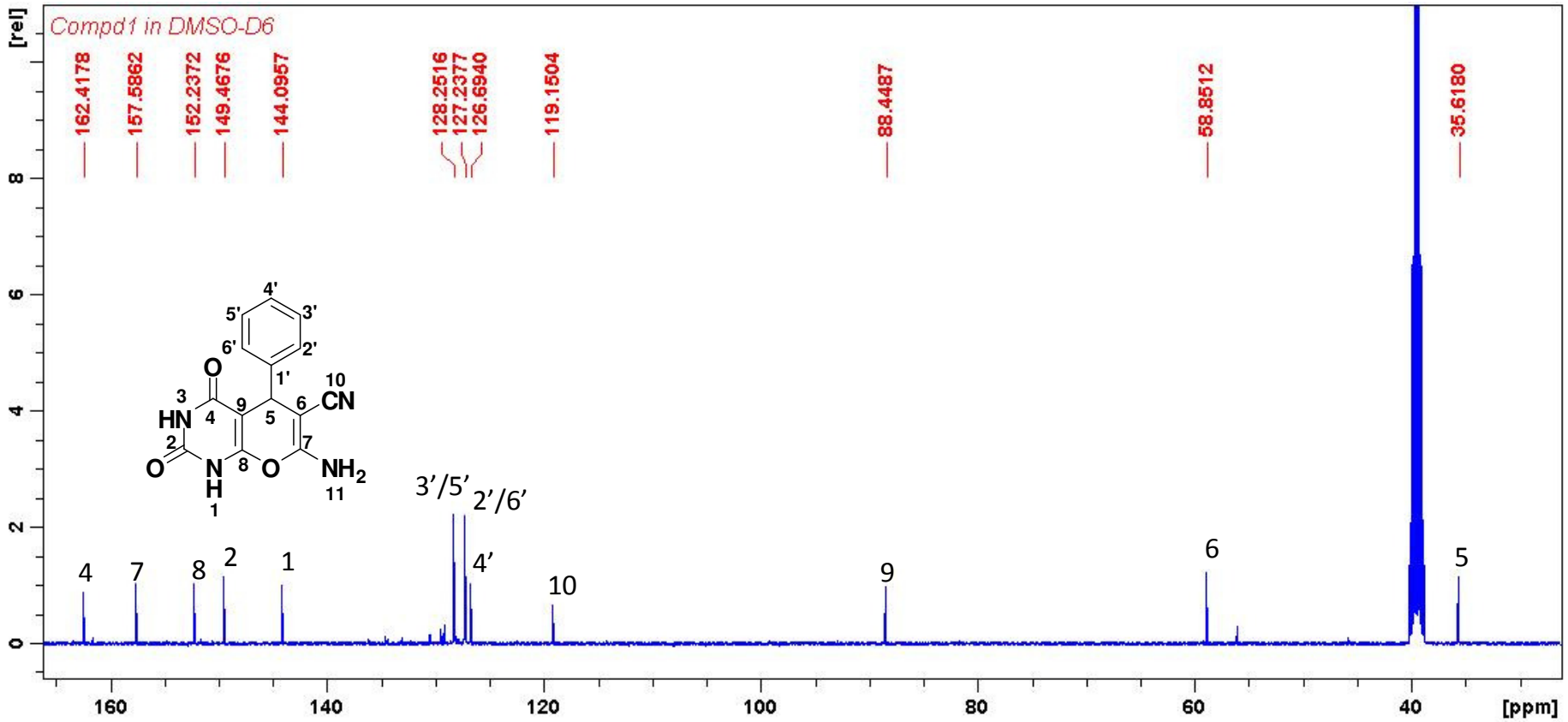
Mass spectrum of 7-amino-5-(2,5-dimethoxyphenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (A7)



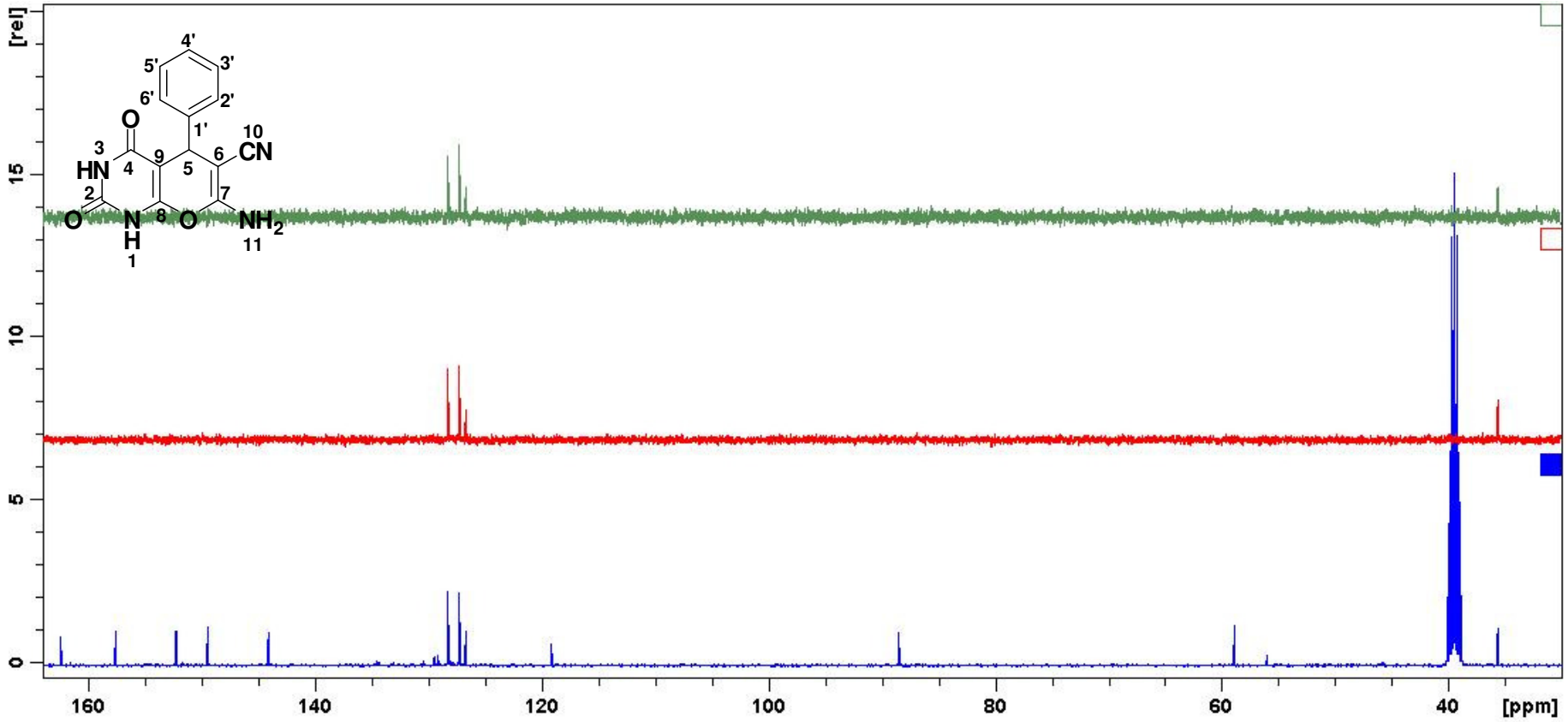
^{1}H NMR spectrum of 7-amino-5-phenyl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B1)



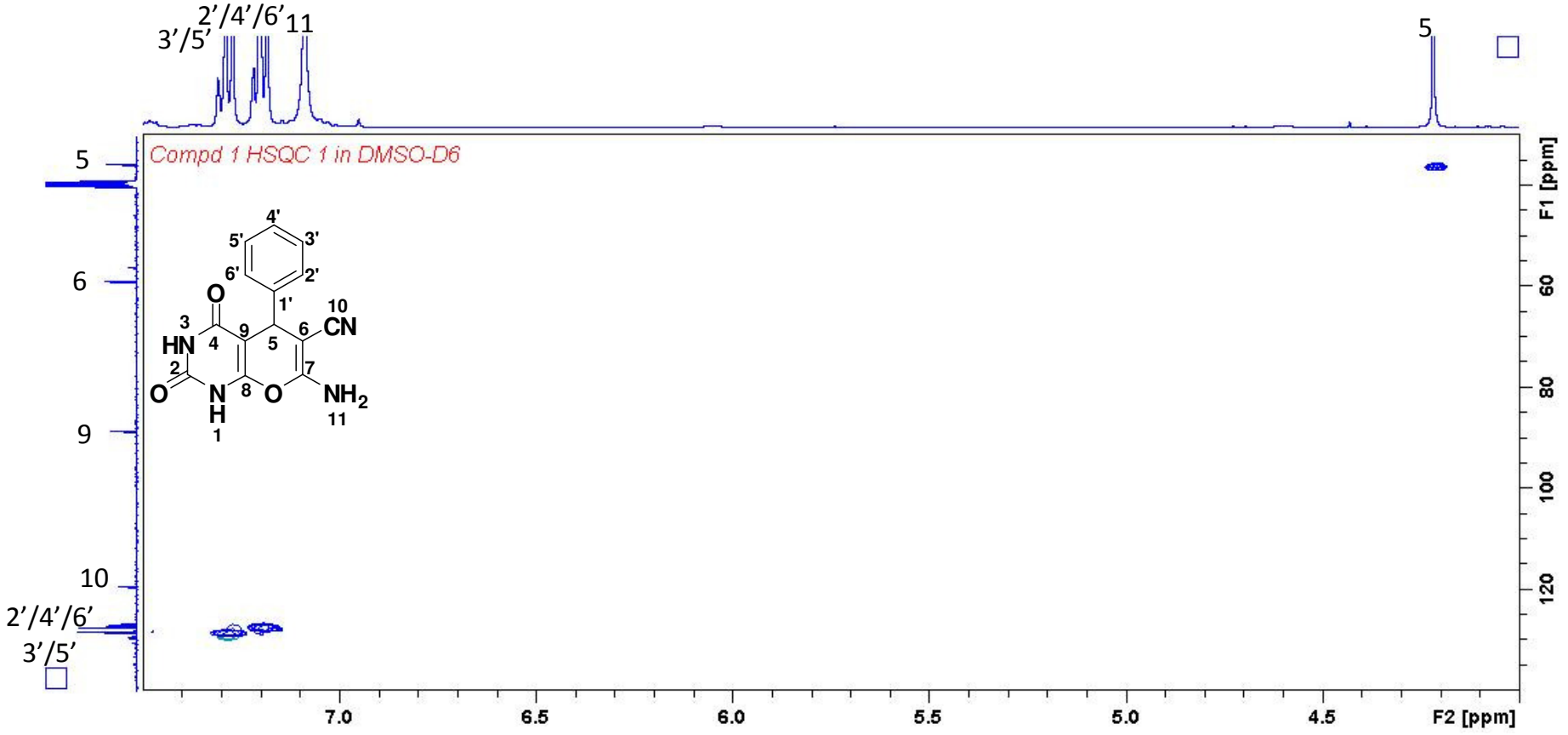
^{1}H NMR spectrum of 7-amino-5-phenyl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B1)



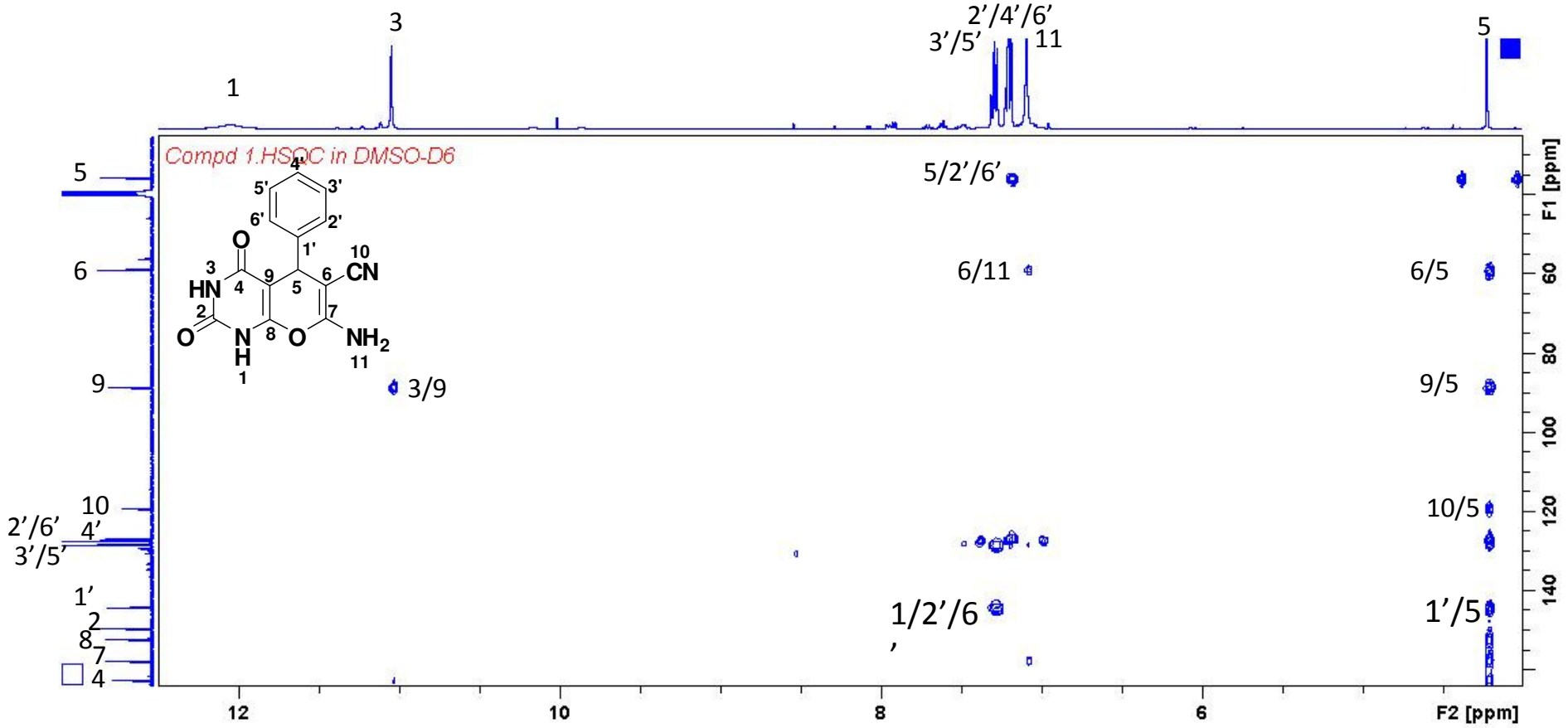
^{13} C NMR spectrum of 7-amino-5-phenyl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B1)



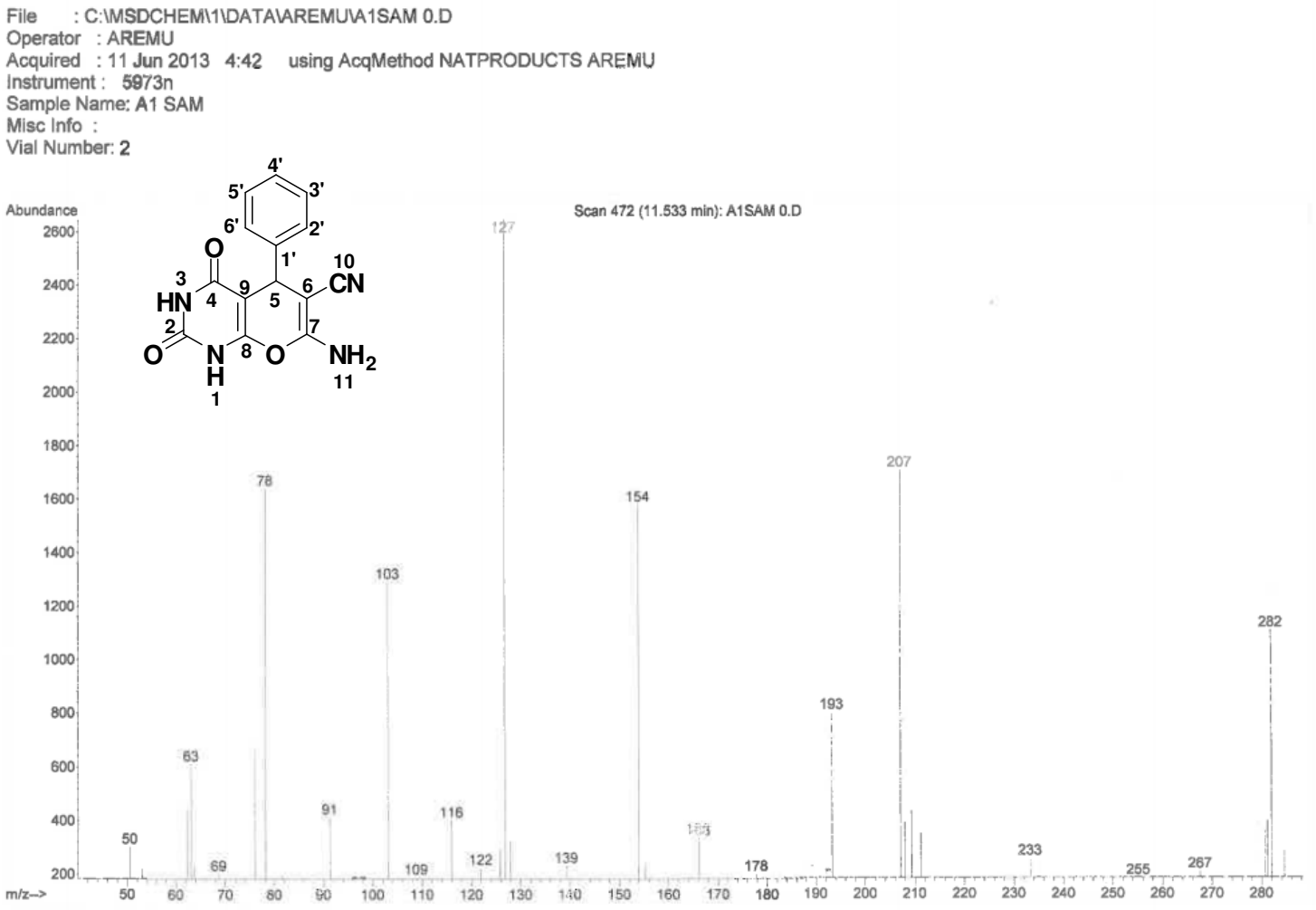
DEPT spectrum of 7-amino-5-phenyl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B1)



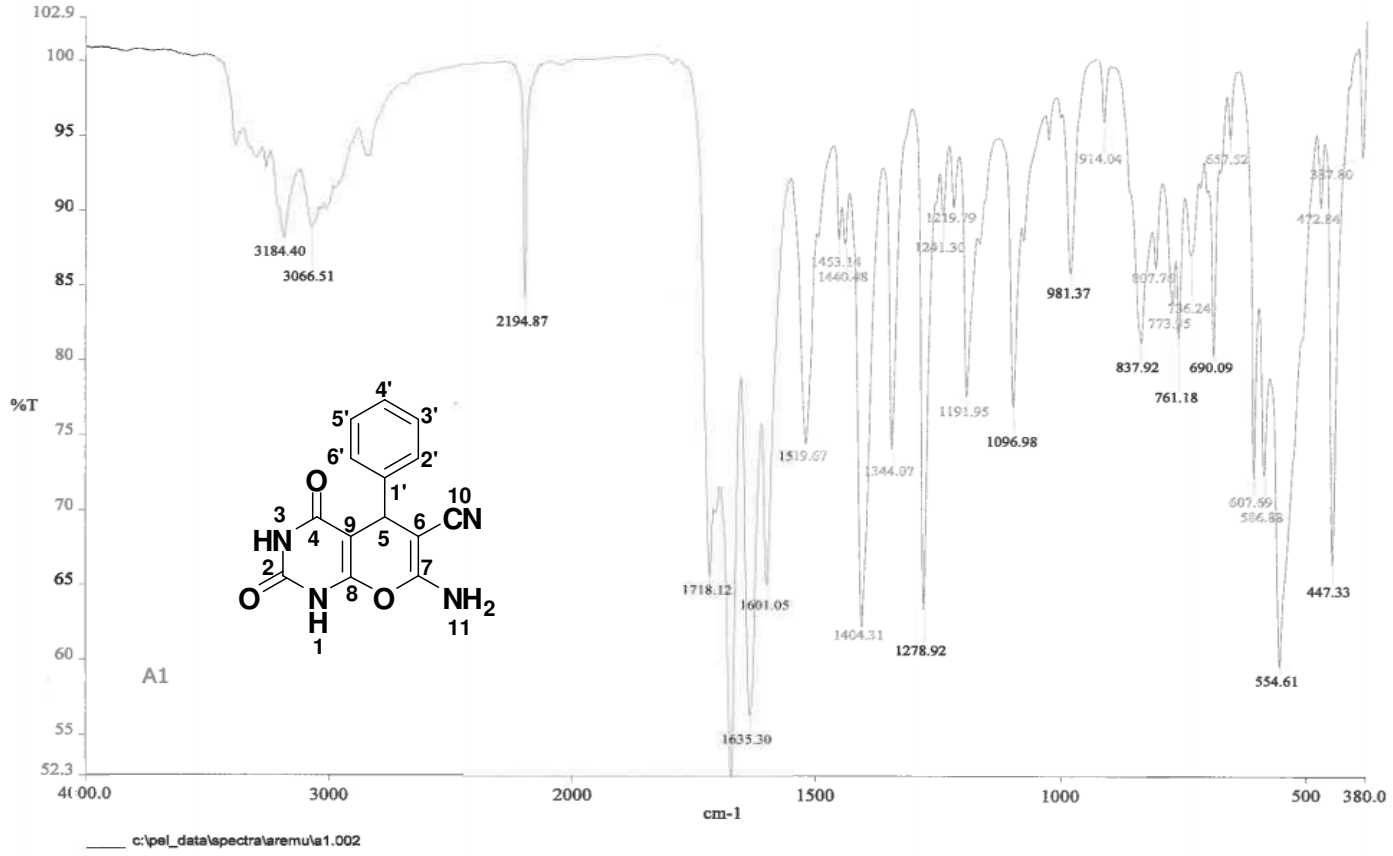
HSQC spectrum of 7-amino-5-phenyl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B1)



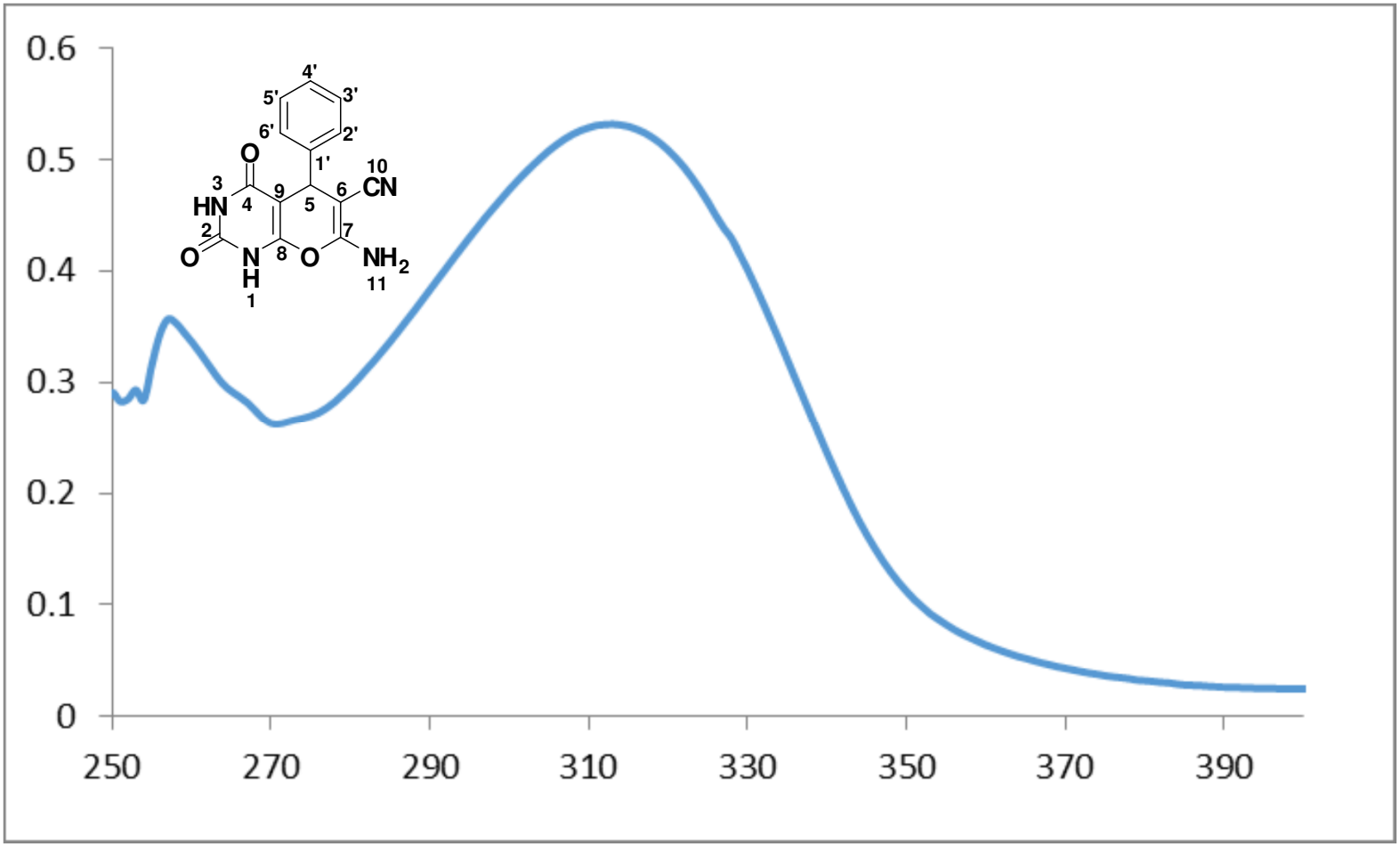
HMBC spectrum of 7-amino-5-phenyl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B1)



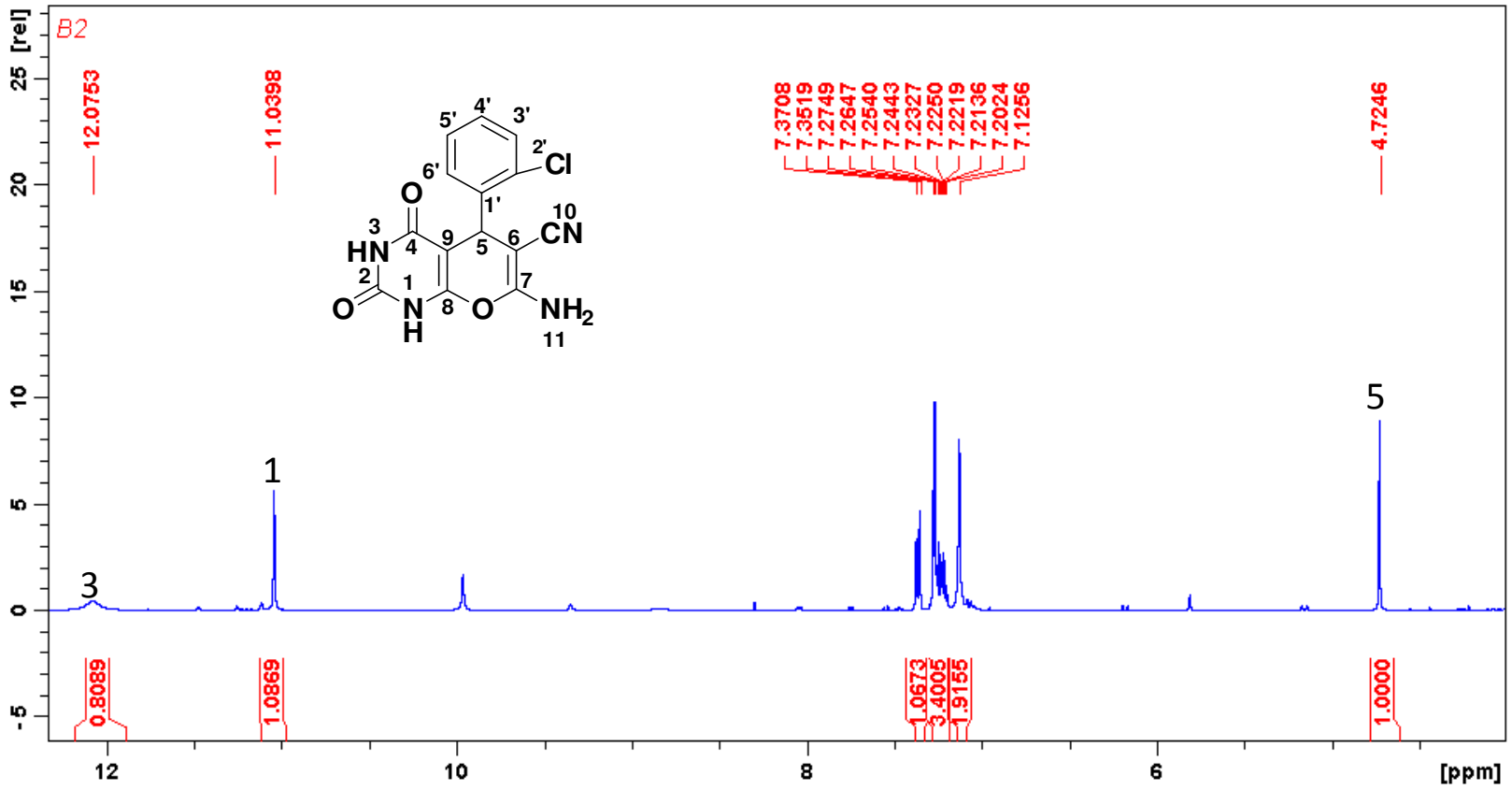
Mass spectrum of 7-amino-5-phenyl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B1)



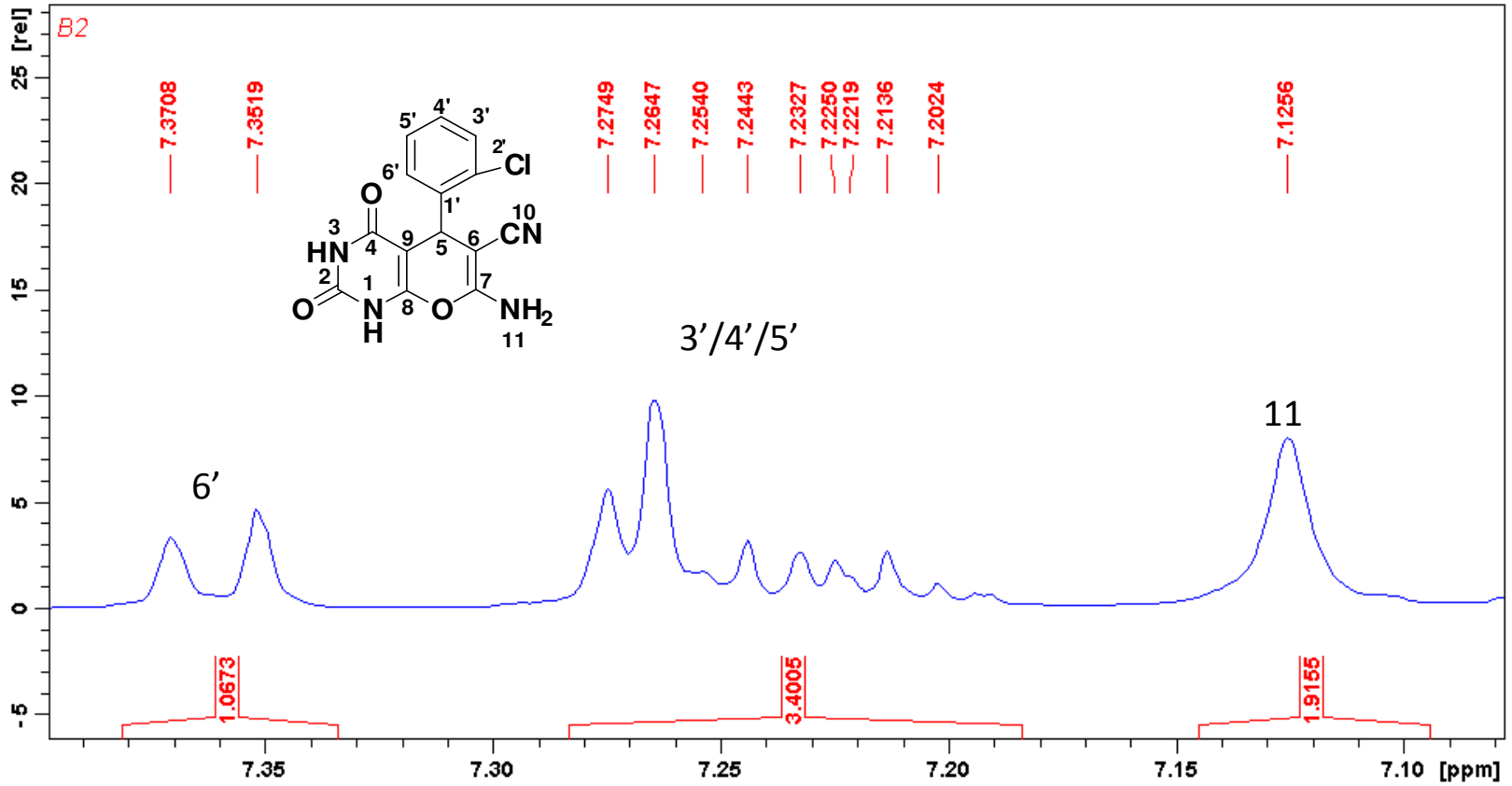
Infra red spectrum of 7-amino-5-phenyl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B1)



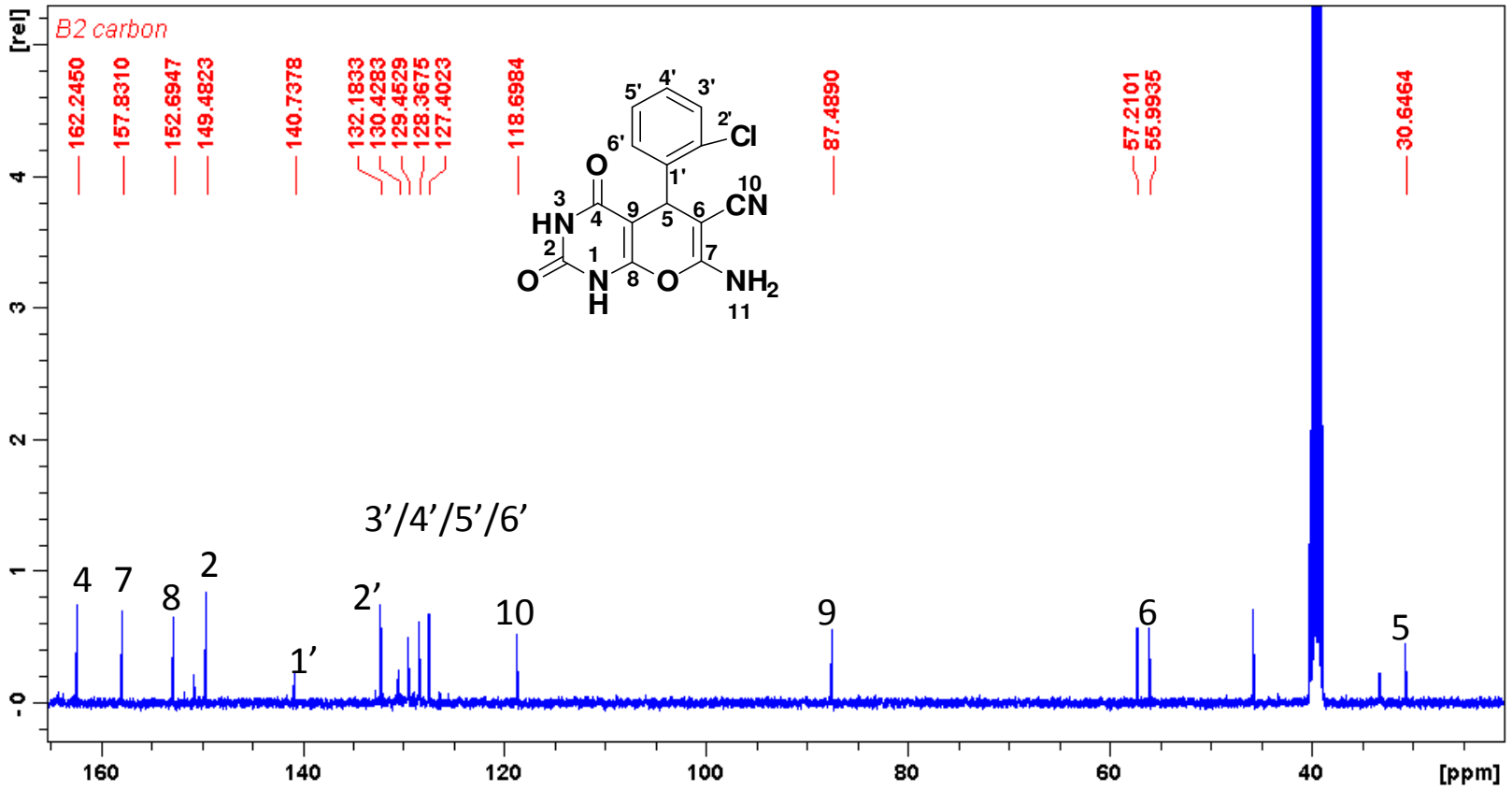
UV spectrum of 7-amino-5-phenyl-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B1)



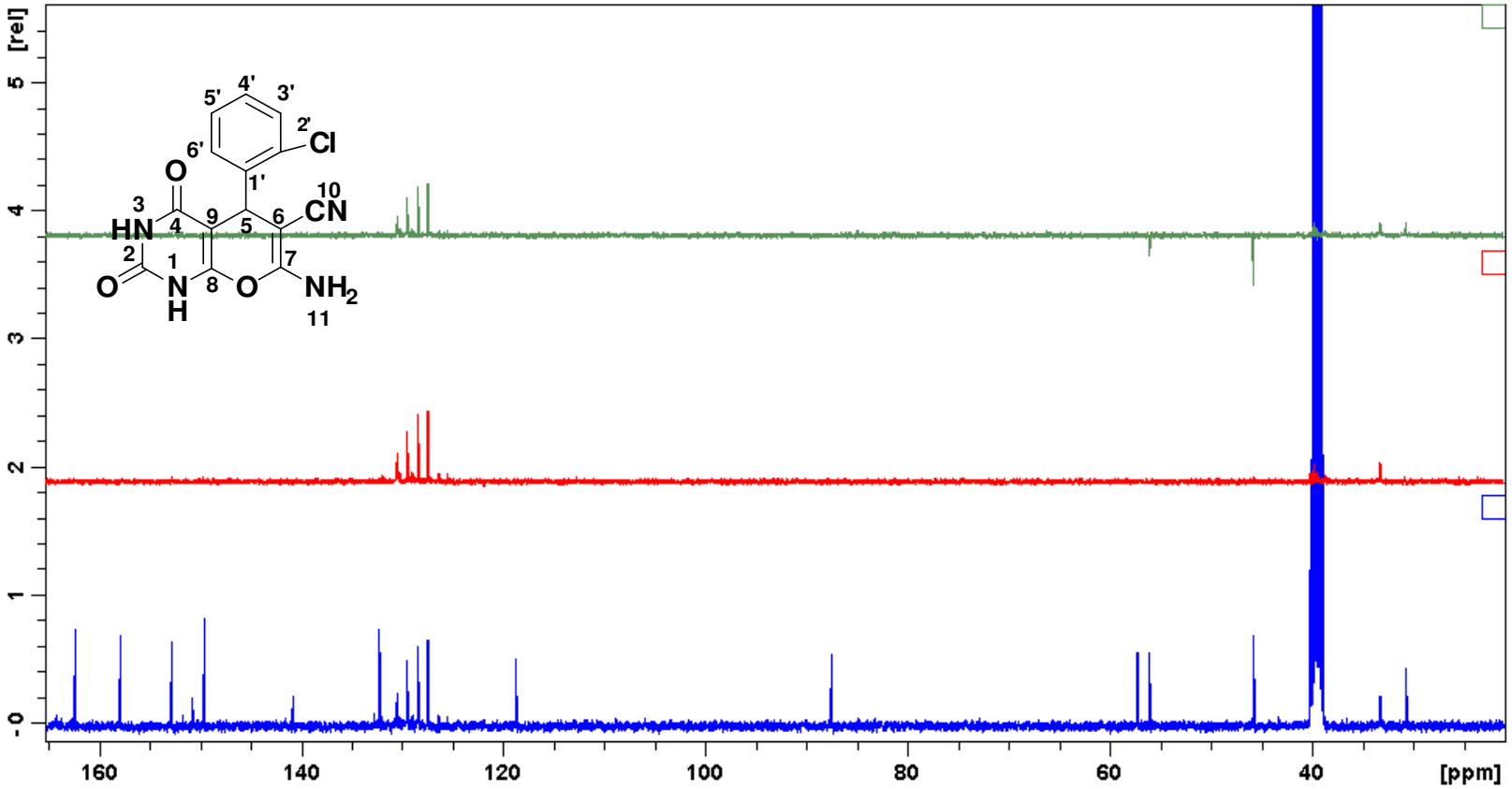
^{1}H NMR spectrum of 7-amino-5-(2-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B2)



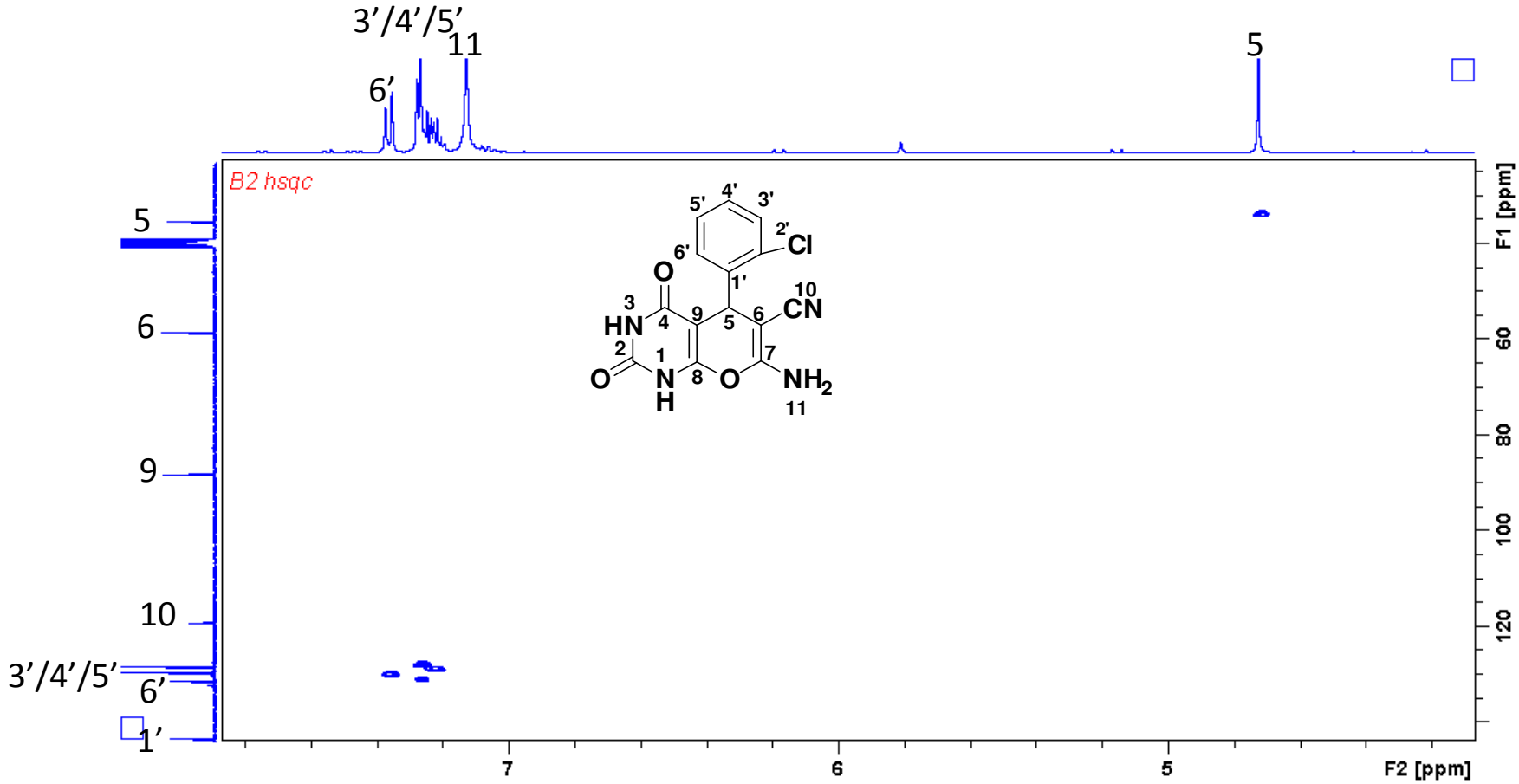
[1]H NMR spectrum of 7-amino-5-(2-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B2) (Expanded)



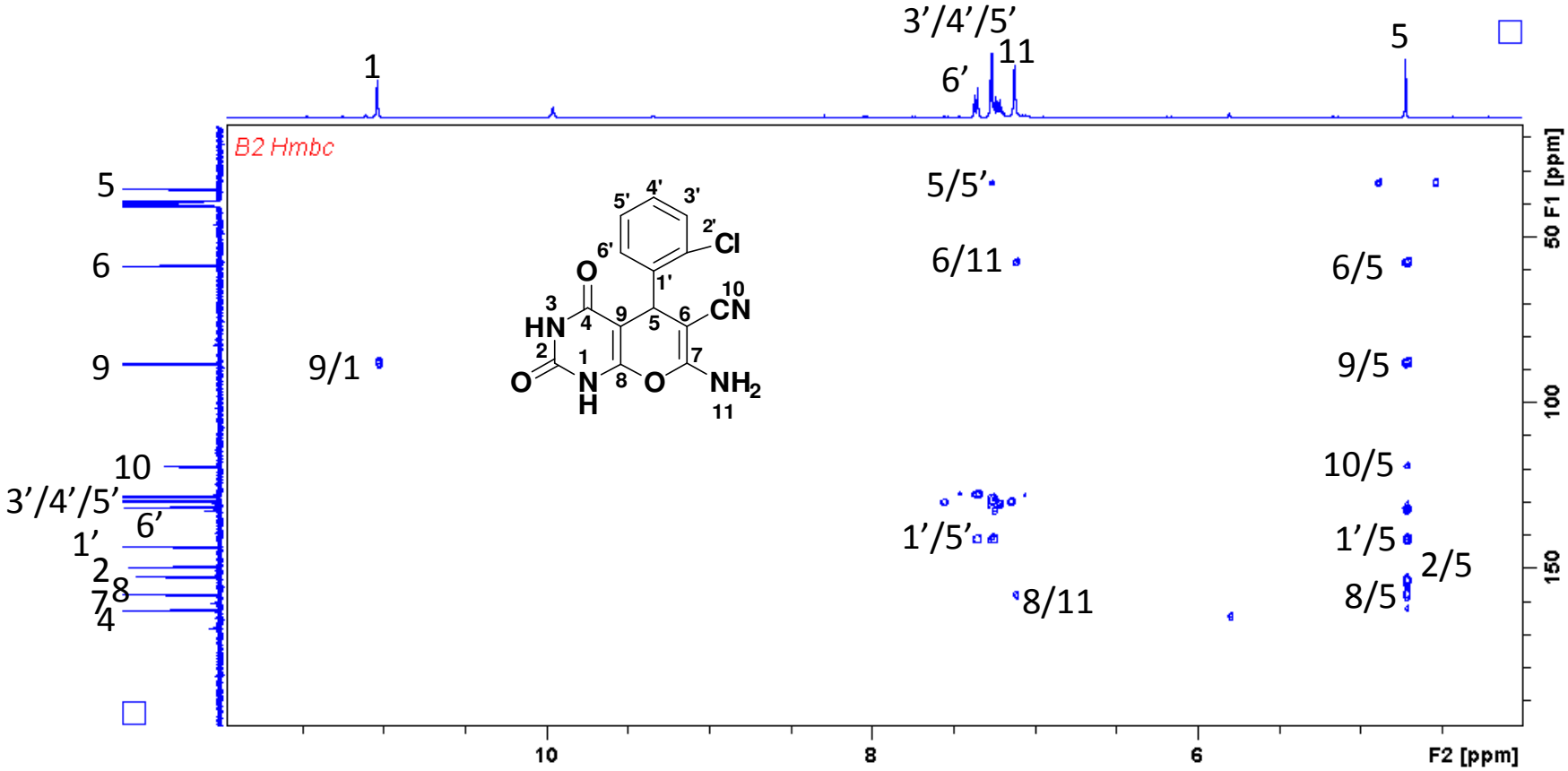
^{13}C NMR spectrum of 7-amino-5-(2-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B2)



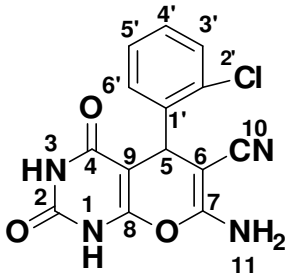
DEPT spectrum of 7-amino-5-(2-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B2)



HSQC spectrum of 7-amino-5-(2-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B2)



HMBC spectrum of 7-amino-5-(2-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B2)



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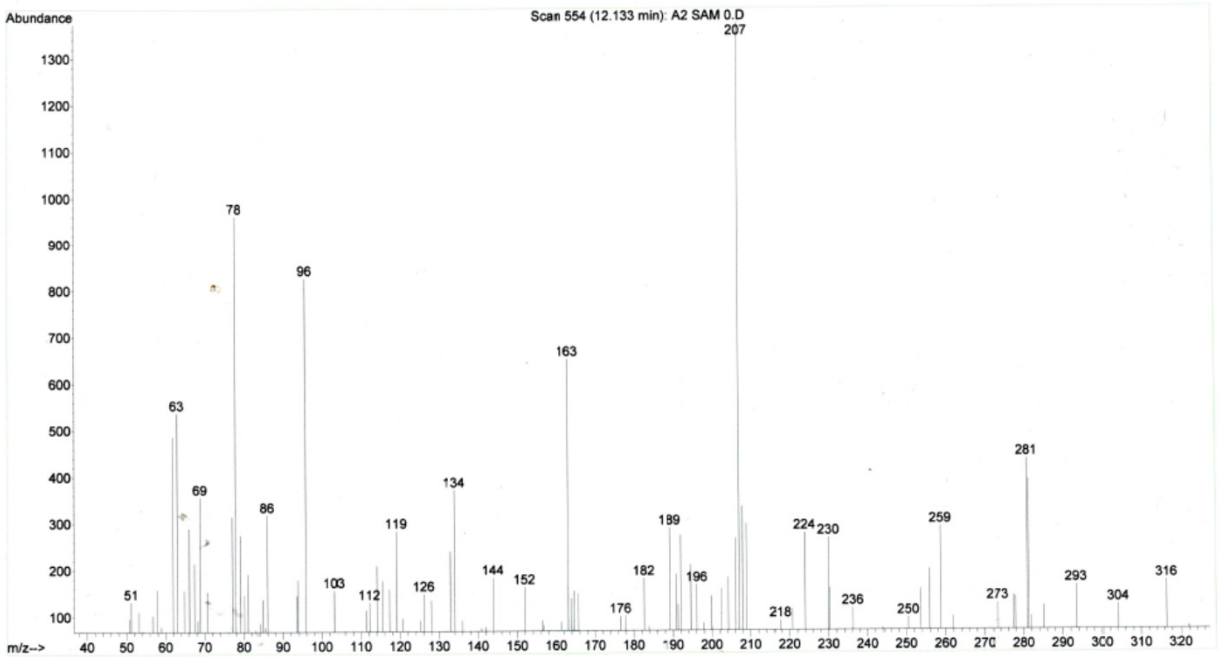
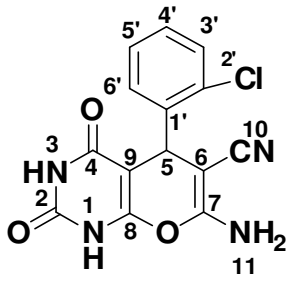
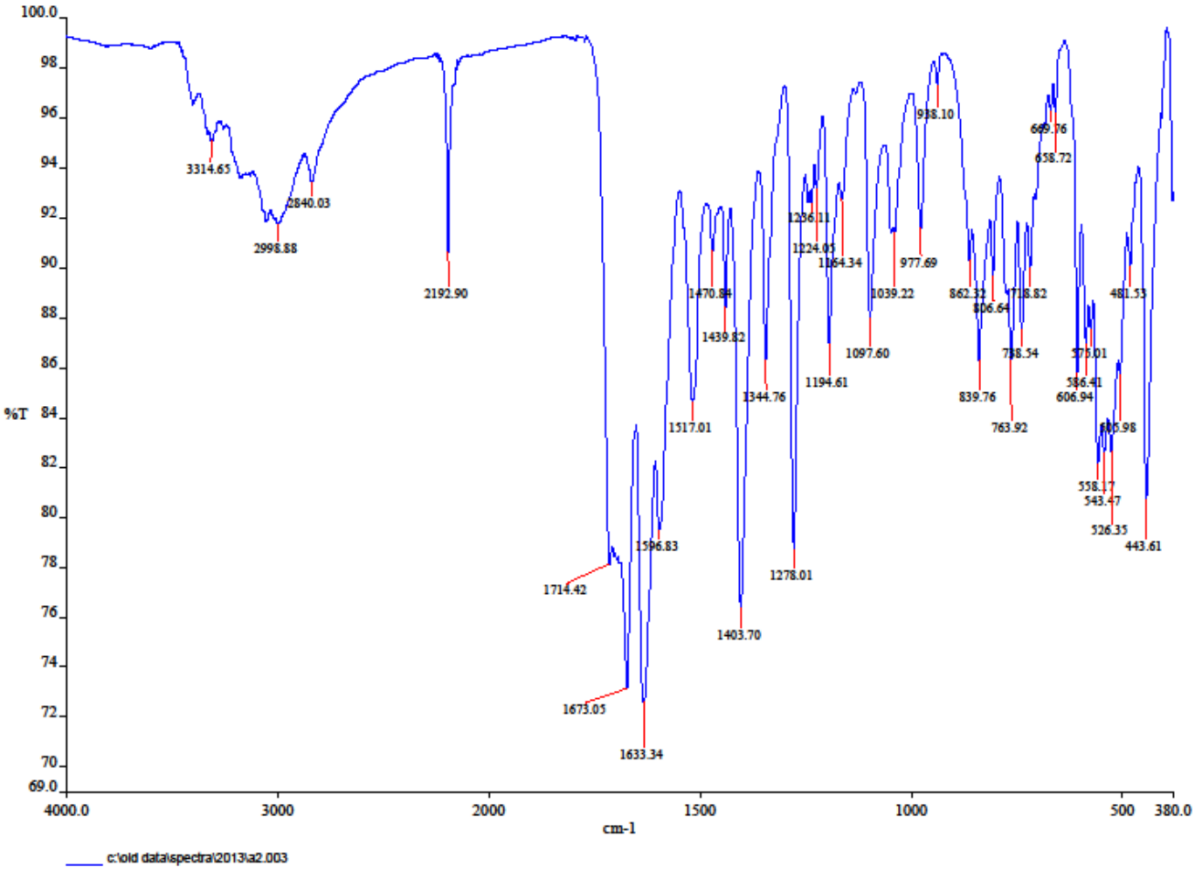
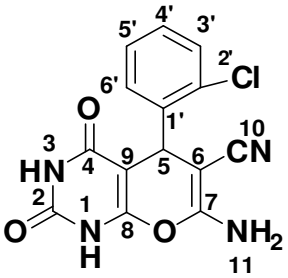


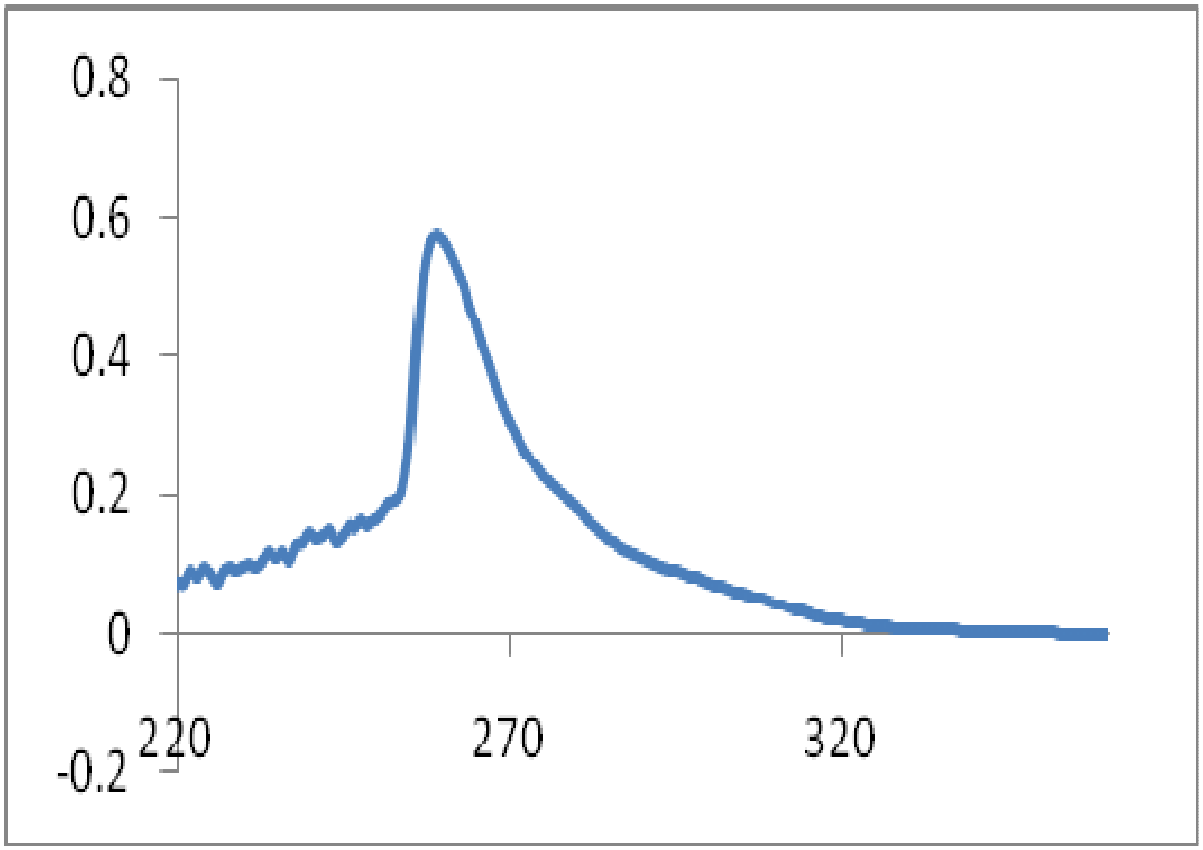
Mass Spectrum of 7-amino-5-(2-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B2)



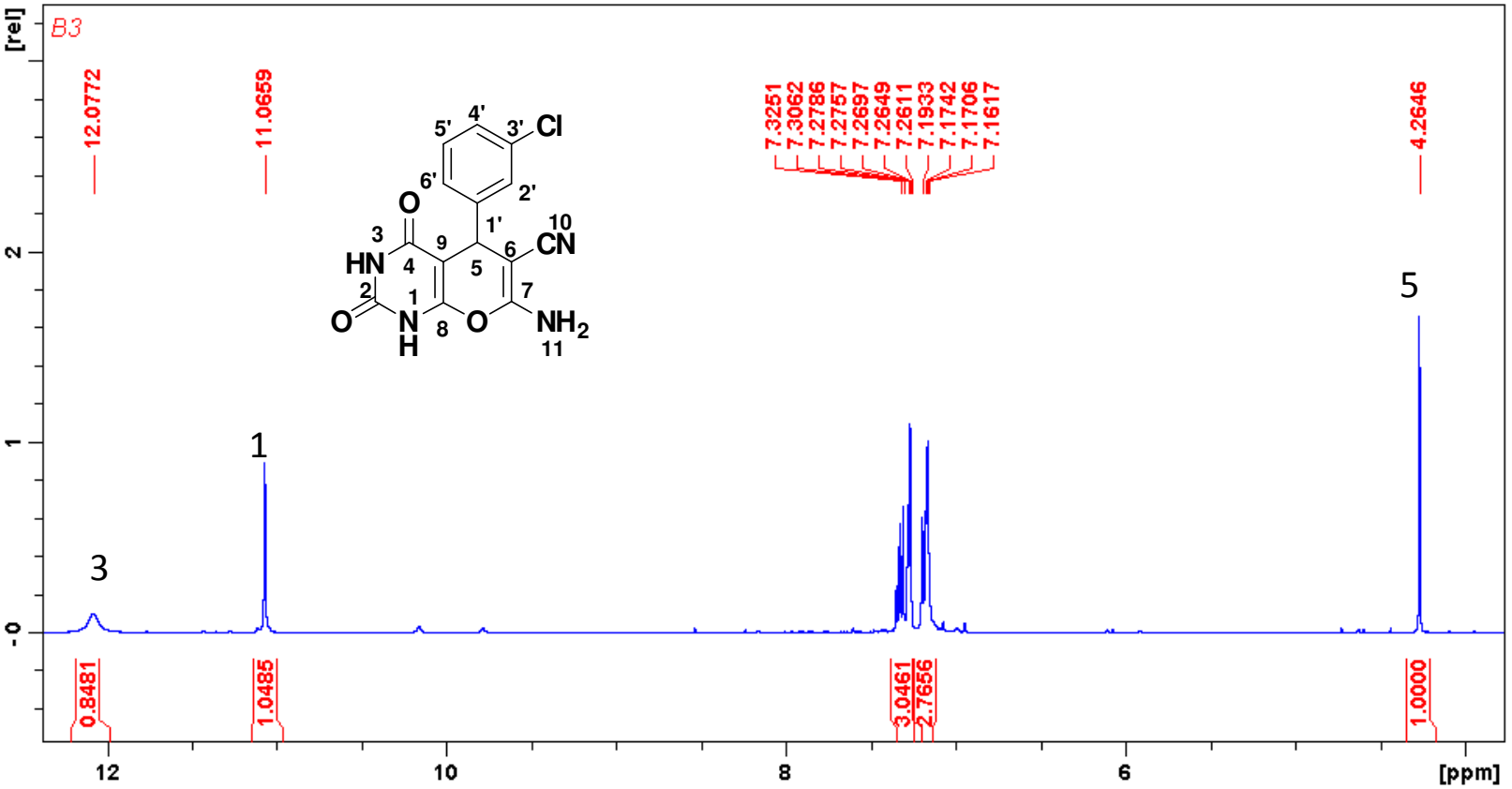


IR spectrum of 7-amino-5-(2-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B2)

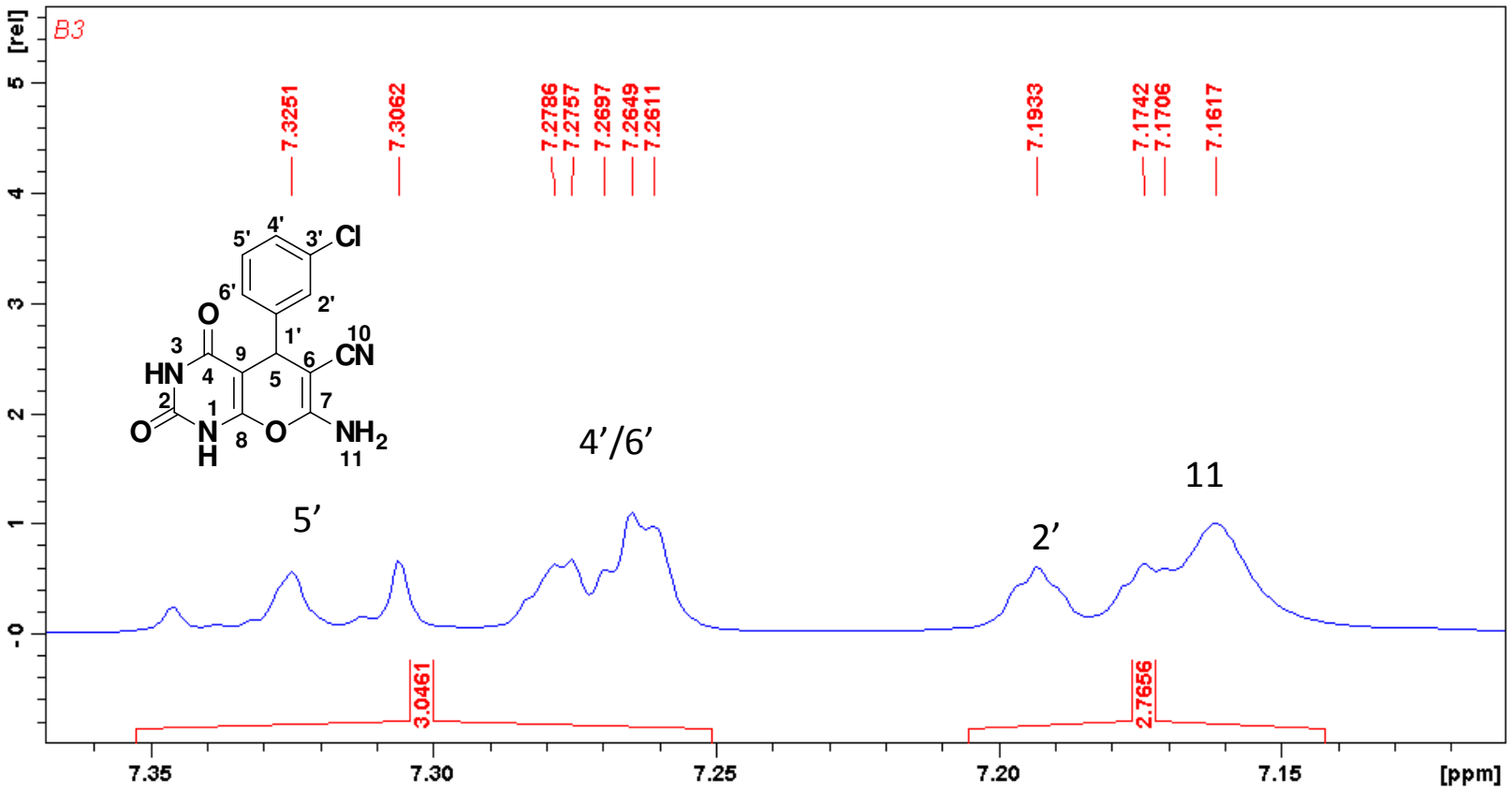




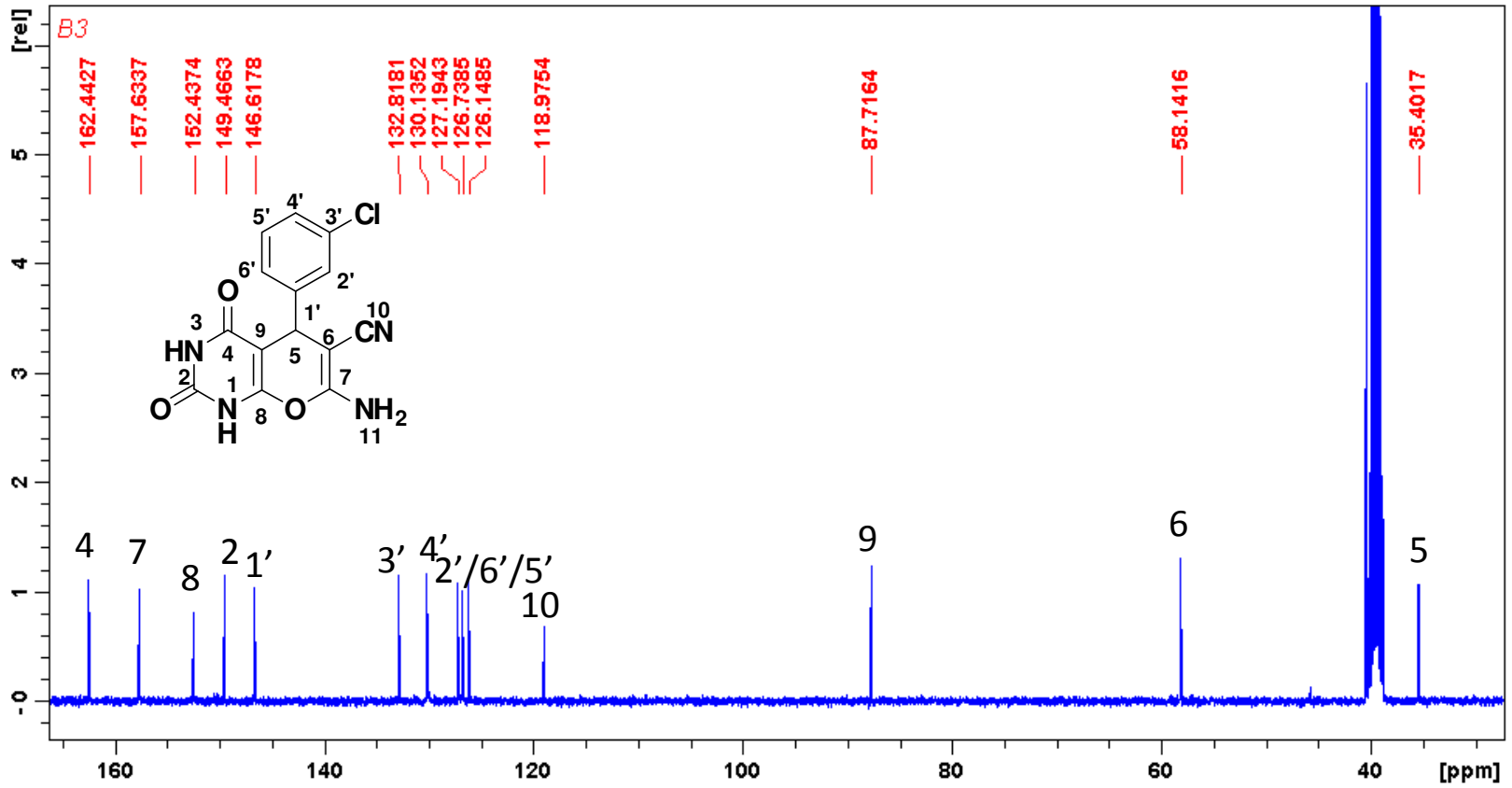
UV spectrum of 7-amino-5-(2-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B2)



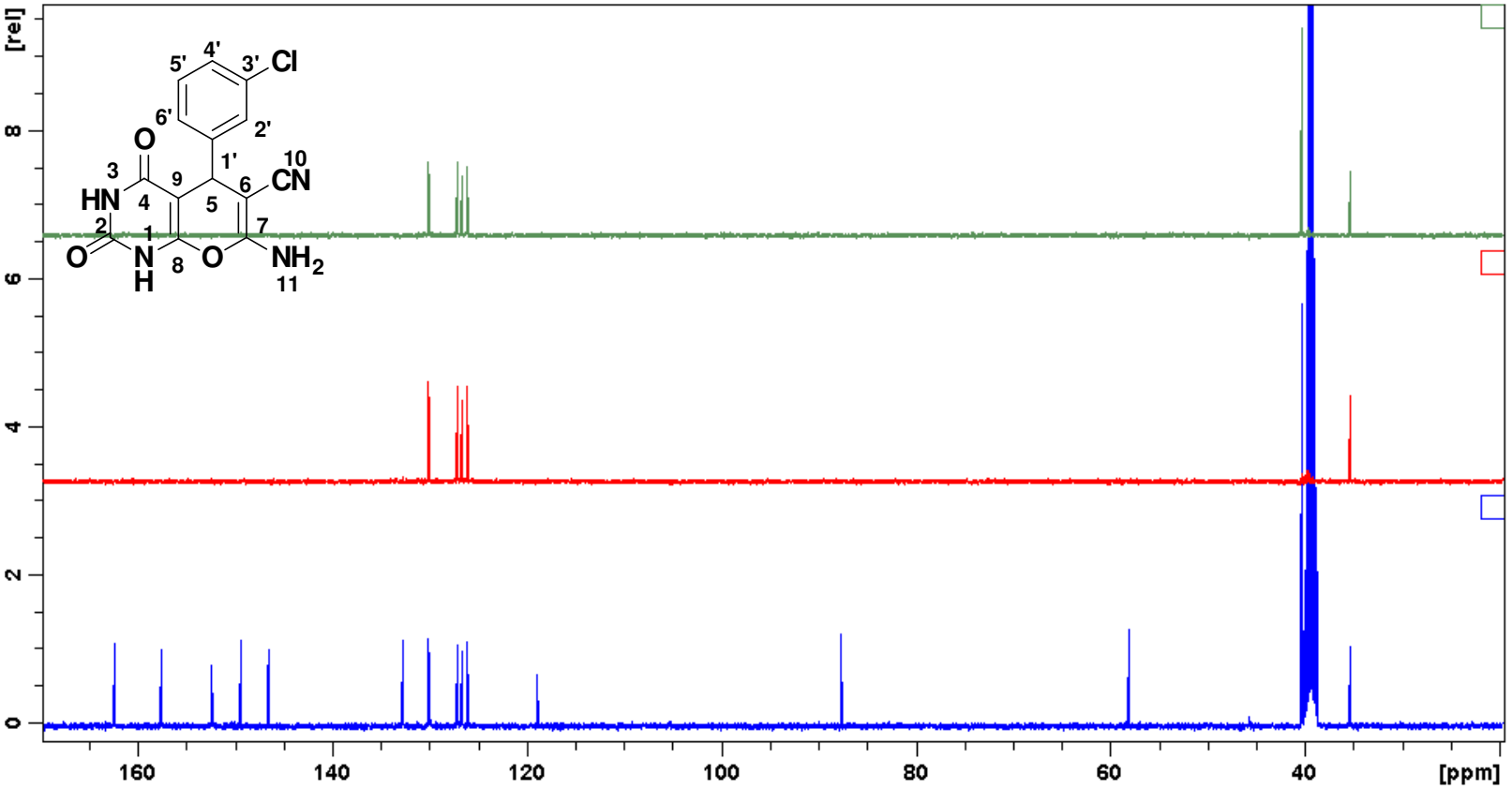
^{1}H NMR spectrum of 7-amino-5-(3-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B3)



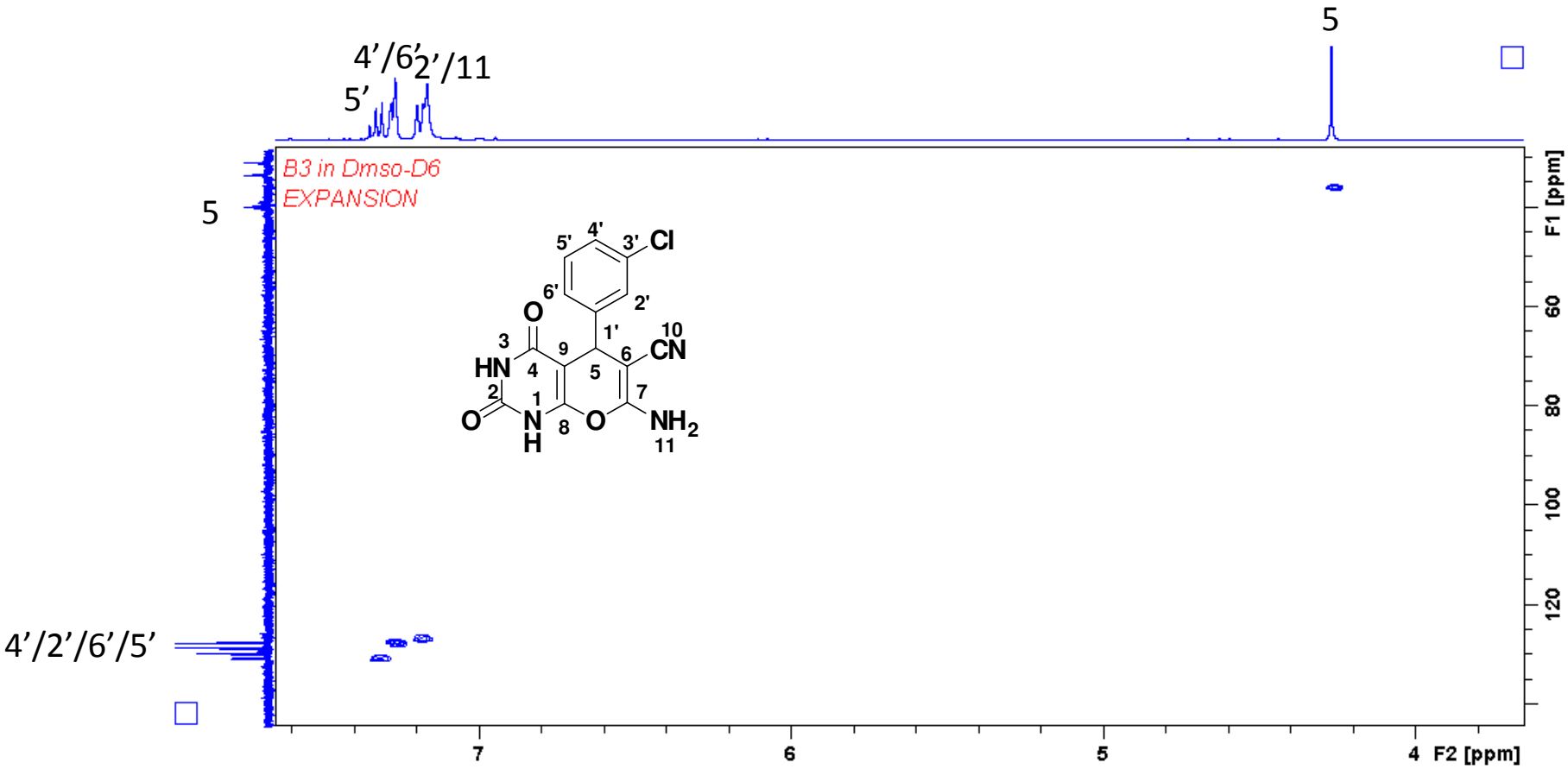
^{1}H NMR spectrum of 7-amino-5-(3-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B3) expanded



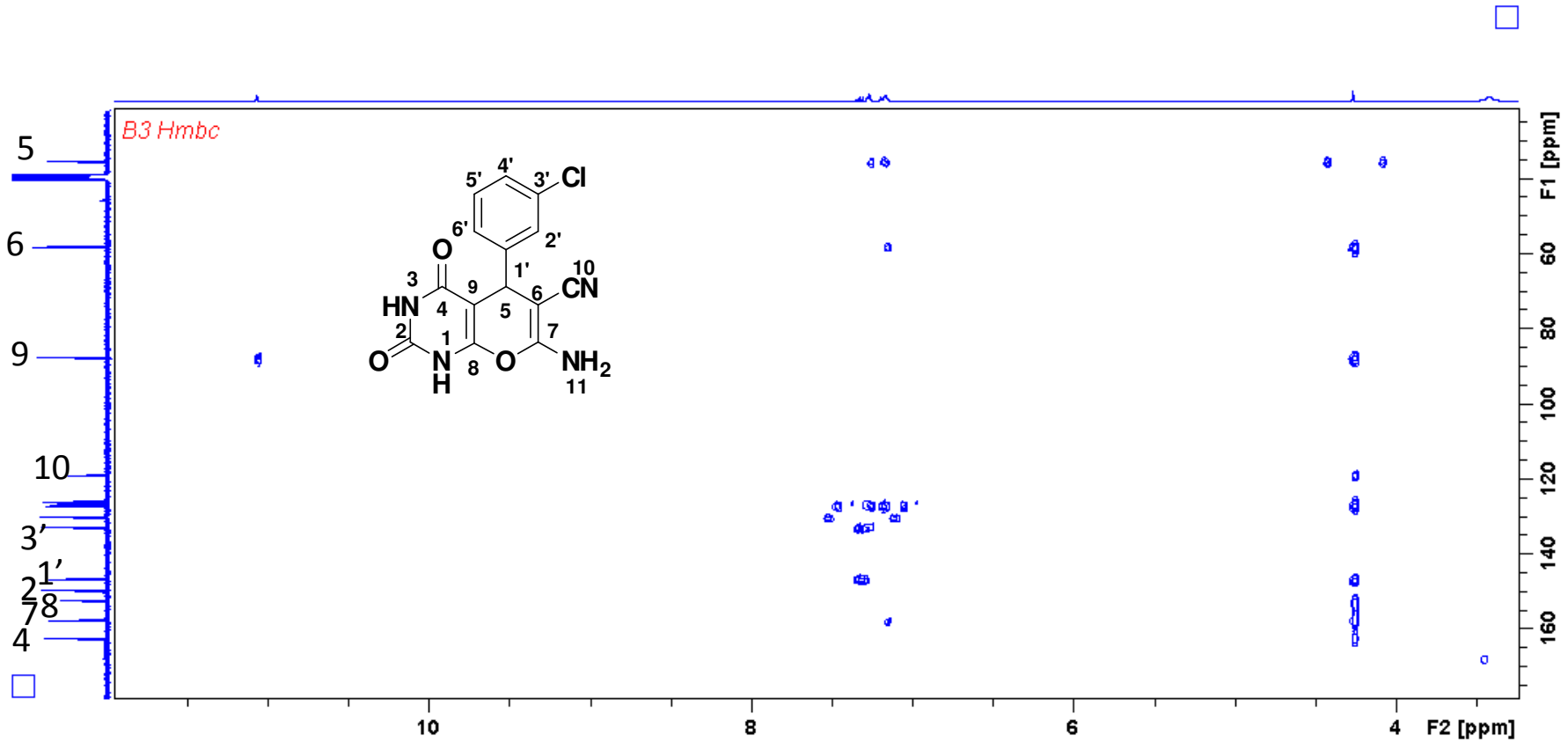
^{13}C NMR spectrum of 7-amino-5-(3-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B3)



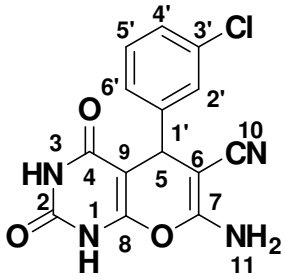
DEPT spectrum of 7-amino-5-(3-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B3)



HSQC spectrum of 7-amino-5-(3-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B3)



HMBC spectrum of 7-amino-5-(3-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B3)



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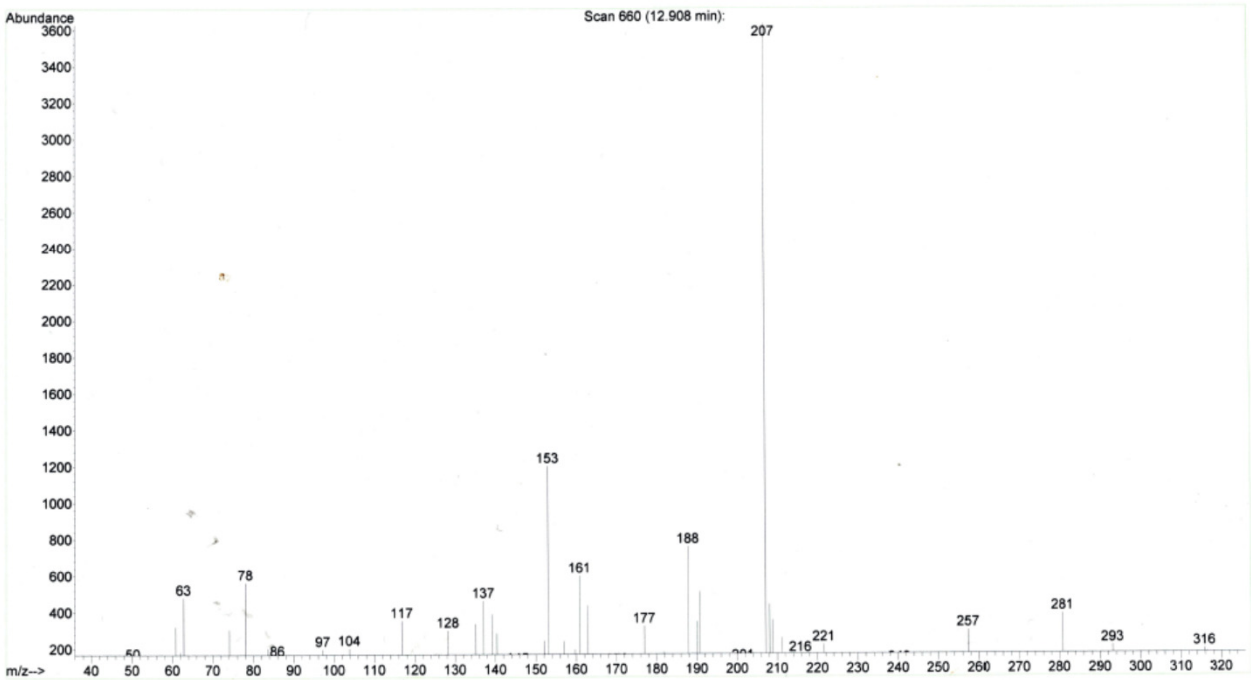
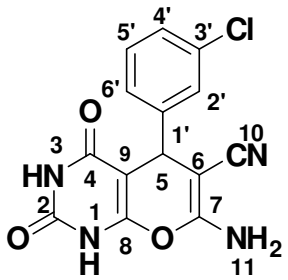
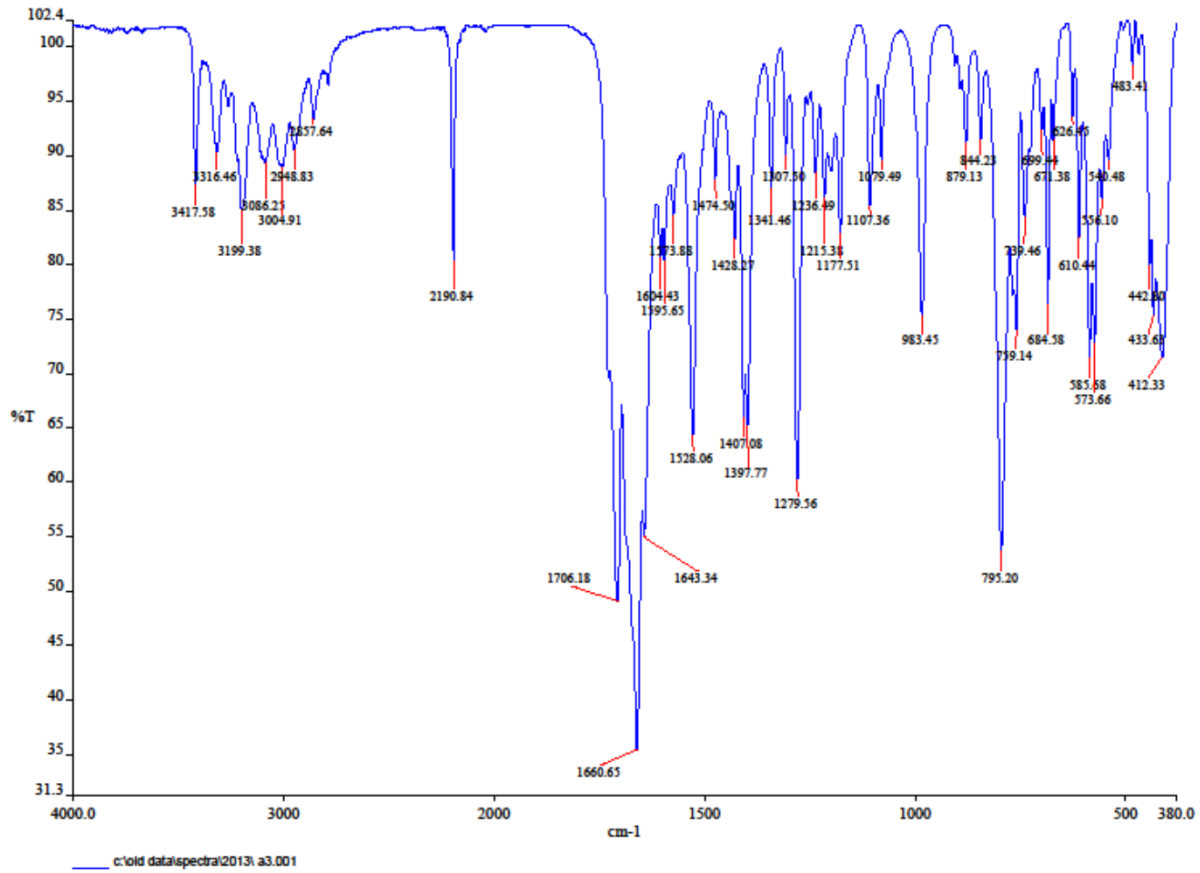
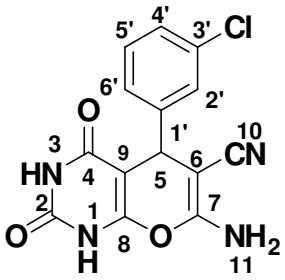


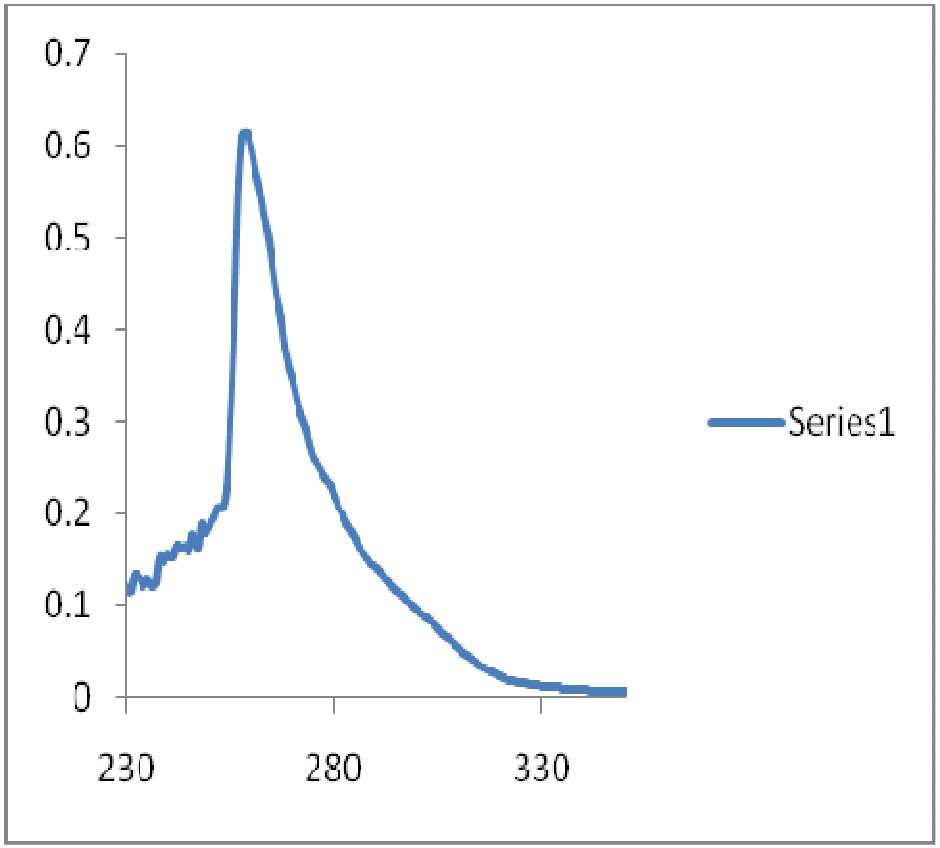
Mass spectrum of 7-amino-5-(3-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B3)



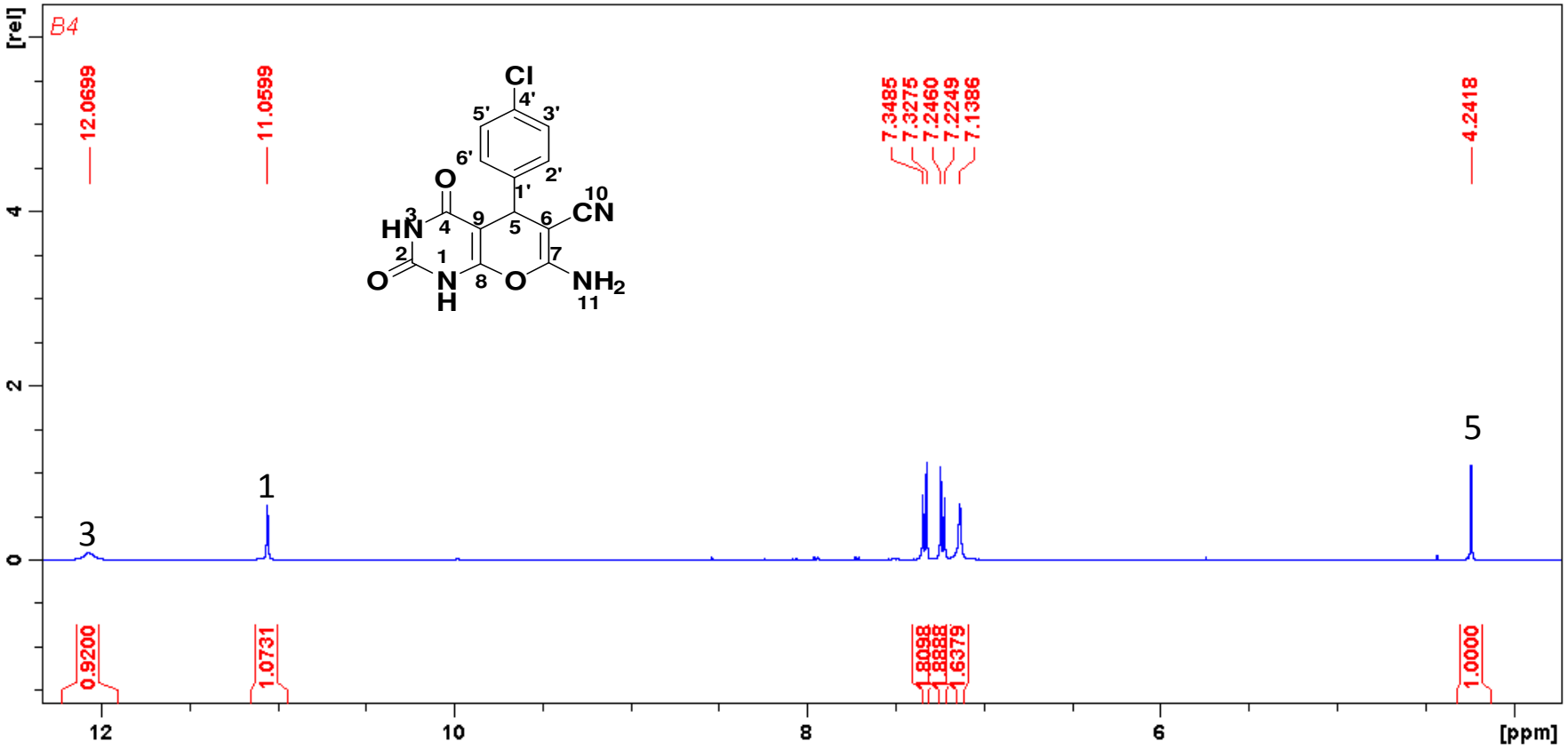


IR spectrum of 7-amino-5-(3-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B3)

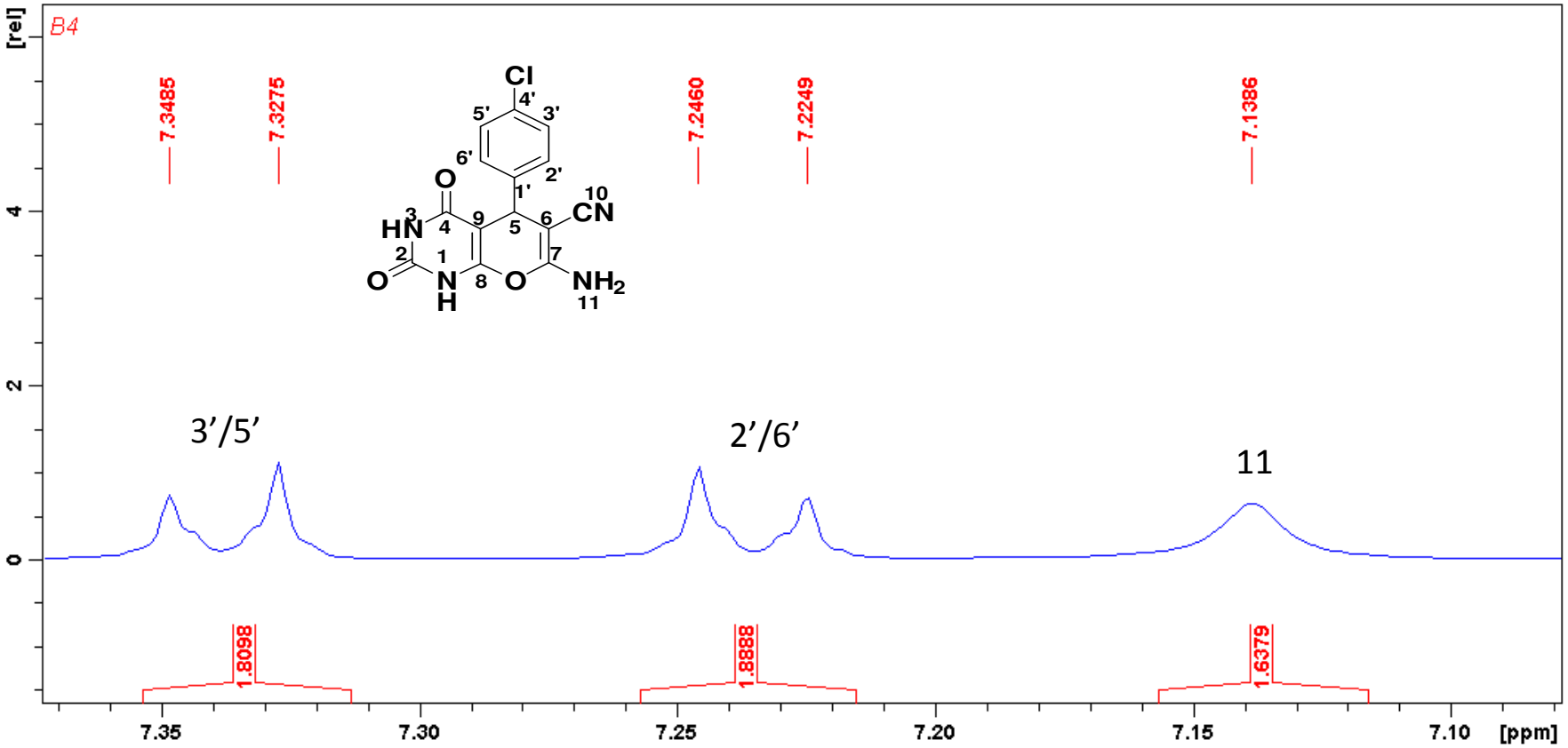




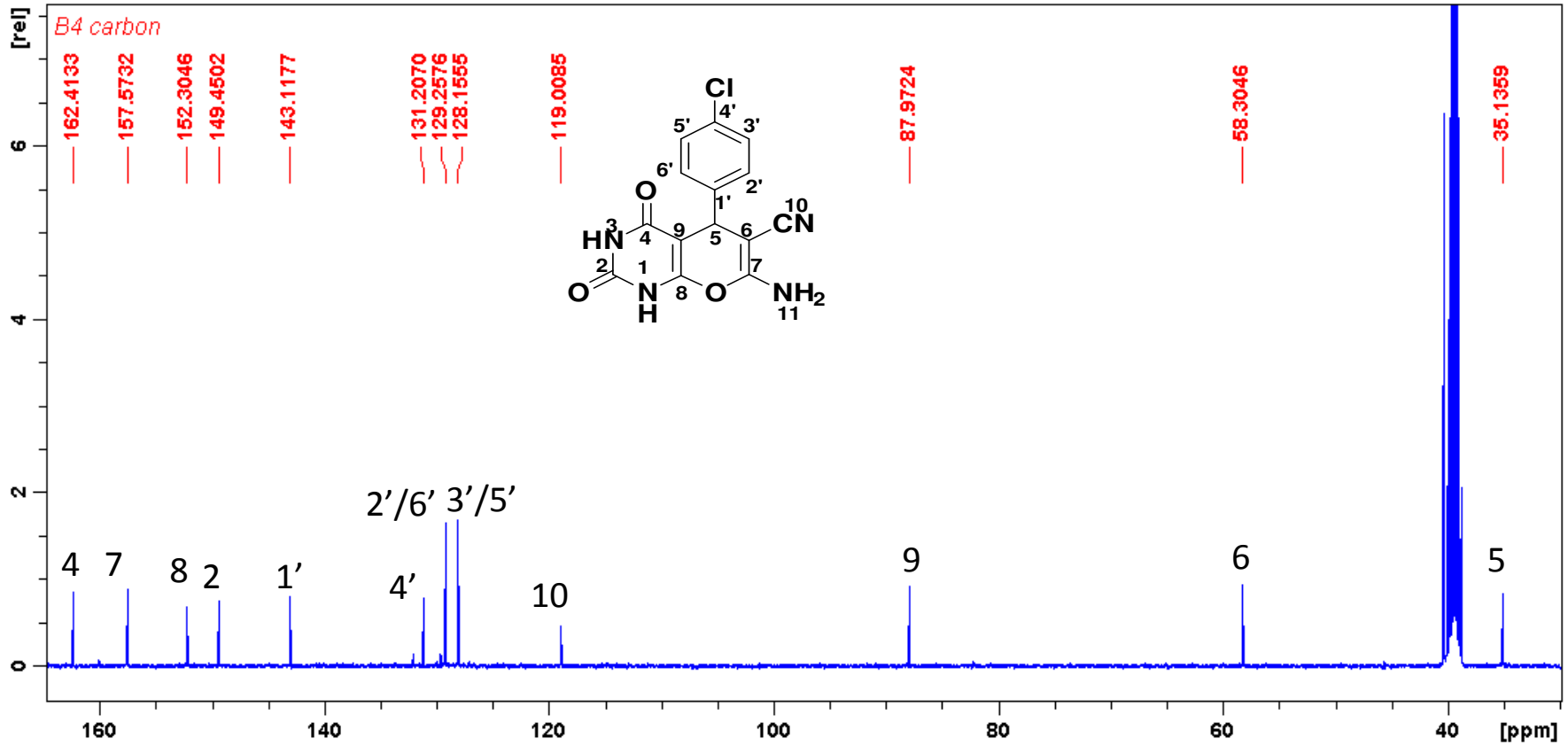
UV spectrum of 7-amino-5-(3-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B3)



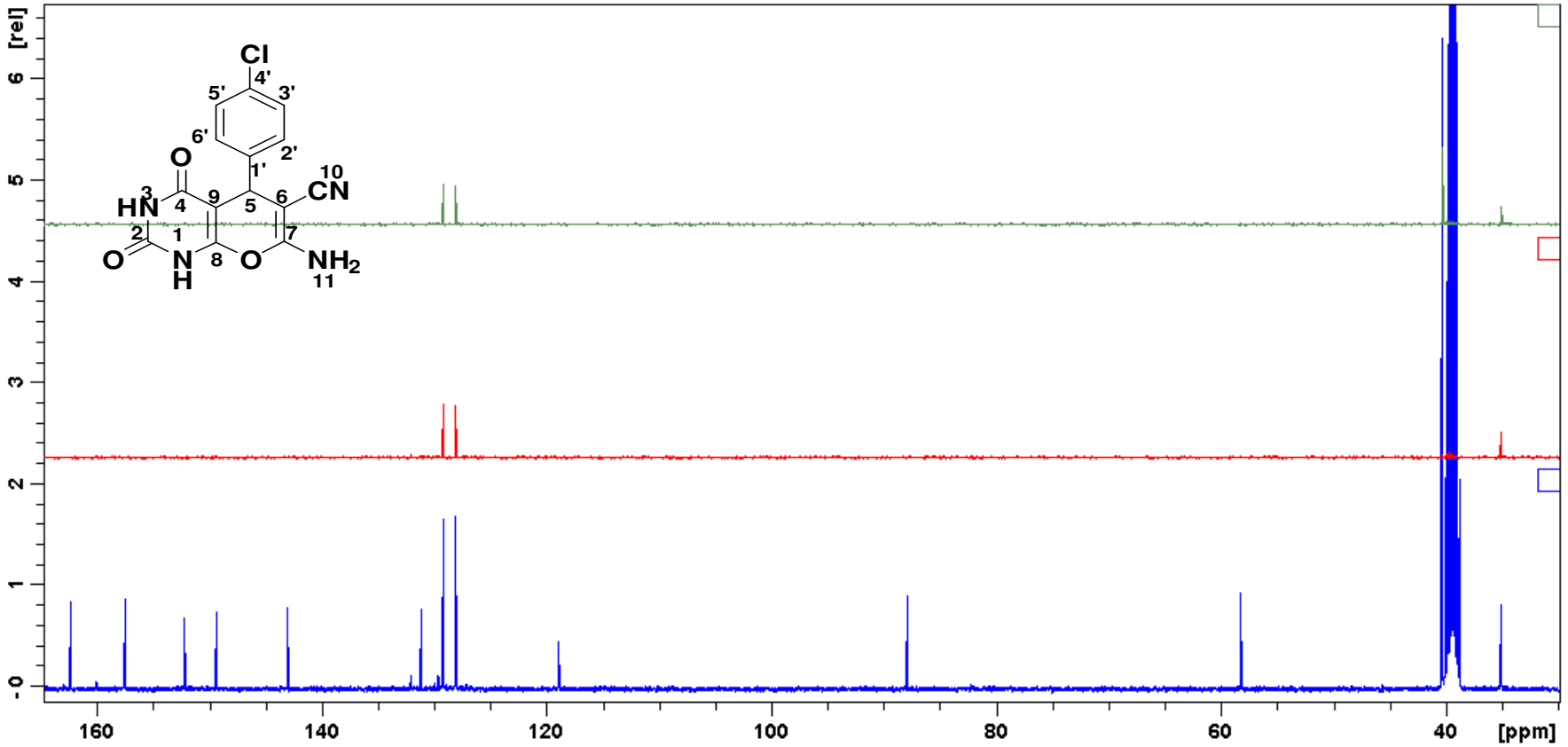
^{1}H NMR spectrum of 7-amino-5-(4-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B4)



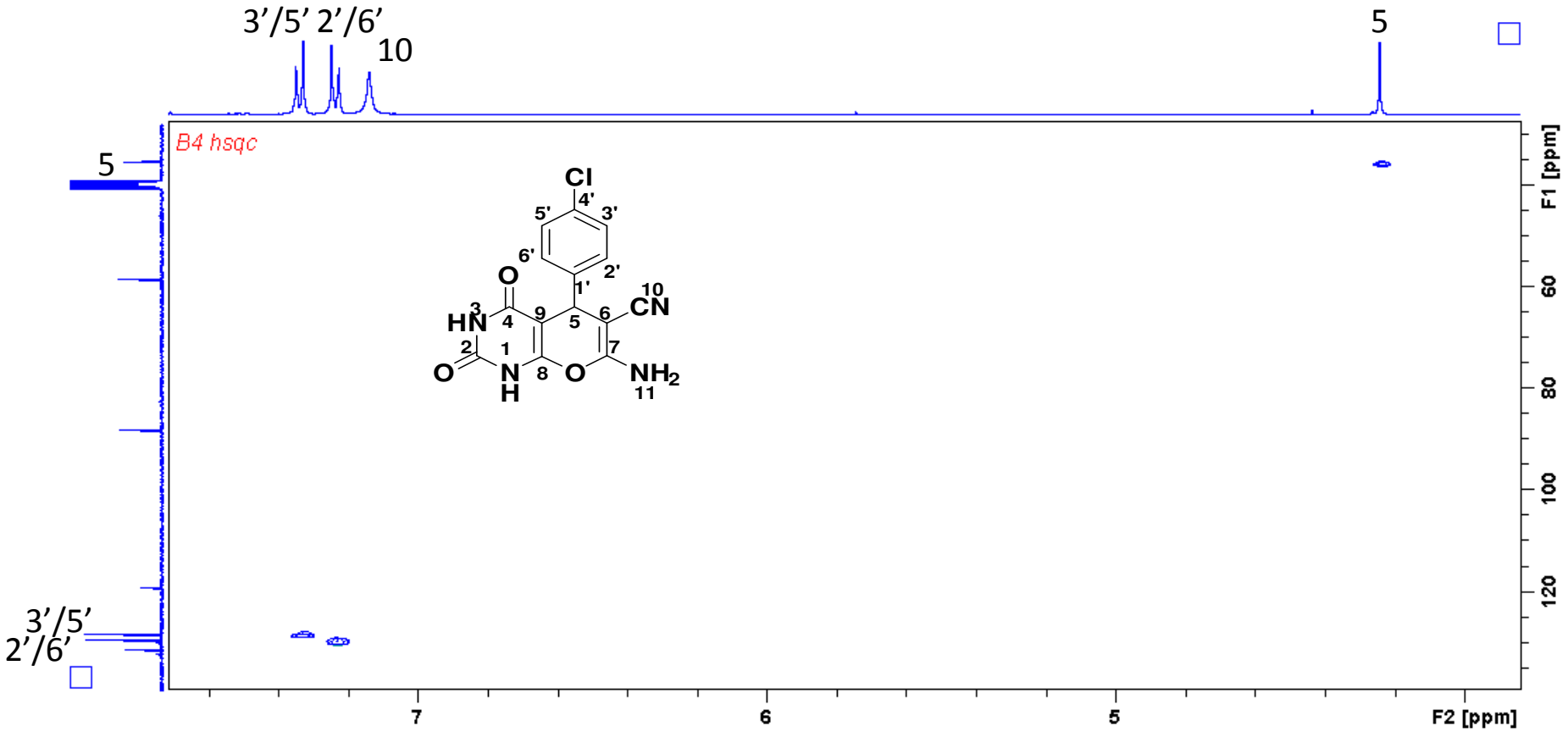
^{1}H NMR spectrum of 7-amino-5-(4-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B4) expanded



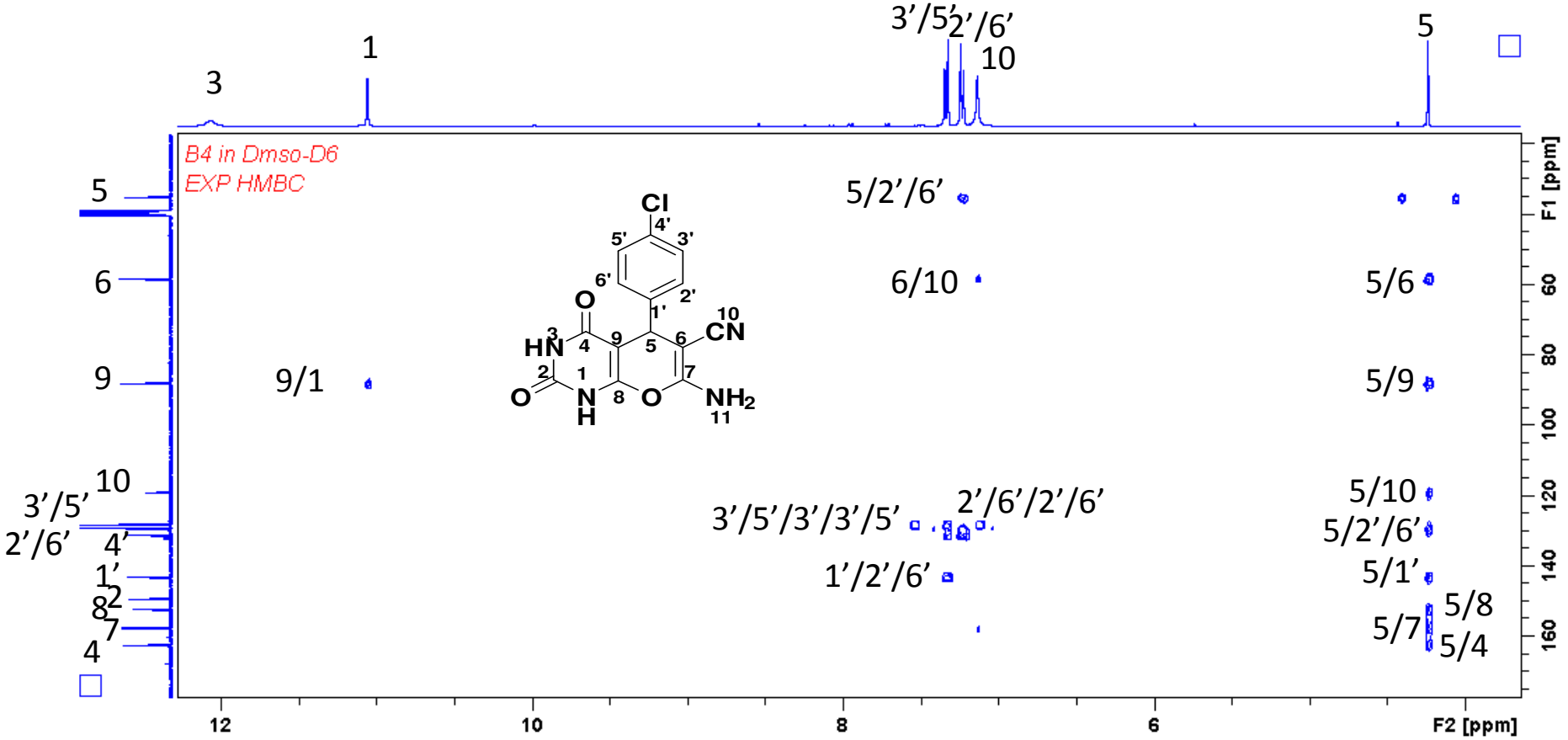
^{13}C NMR spectrum of 7-amino-5-(4-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B4)



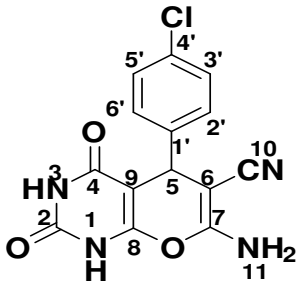
DEPT spectrum of 7-amino-5-(4-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B4)

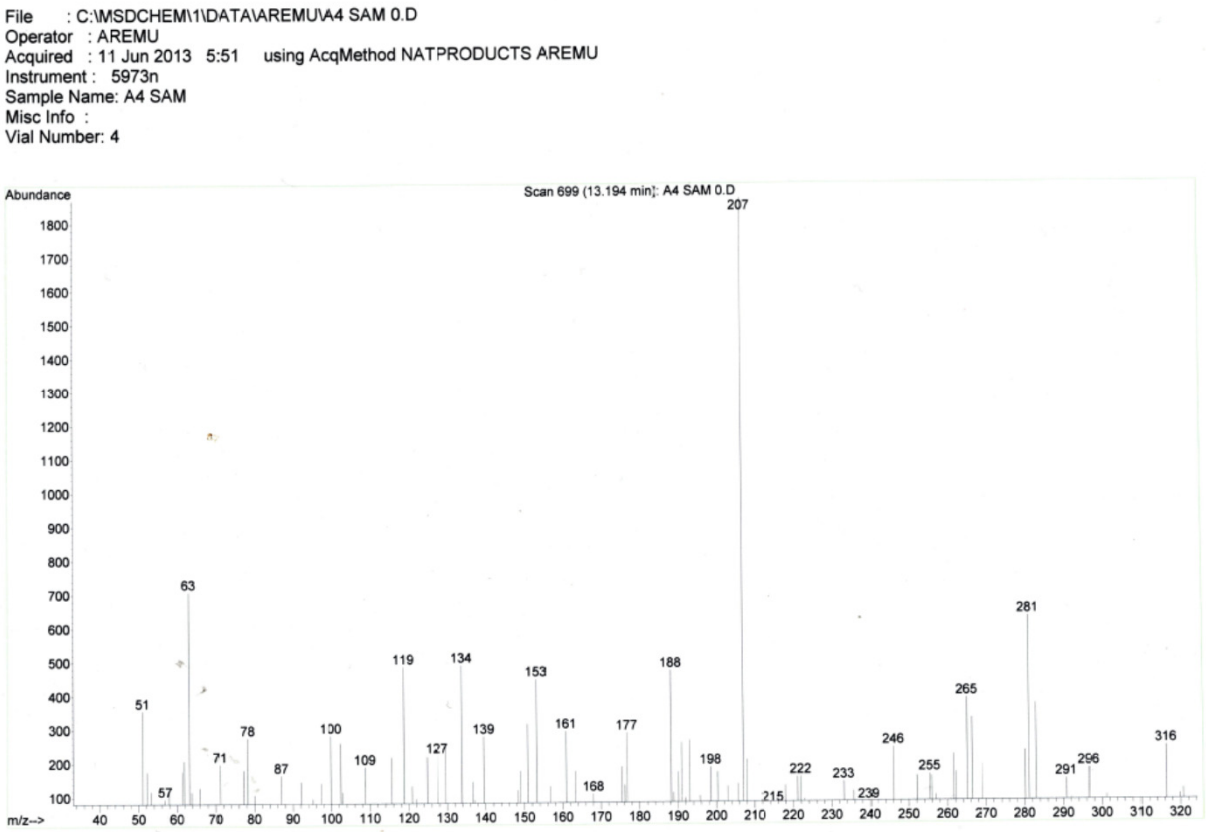


HSQC spectrum of 7-amino-5-(4-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B4)

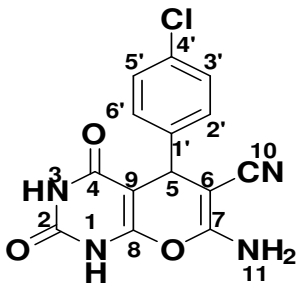


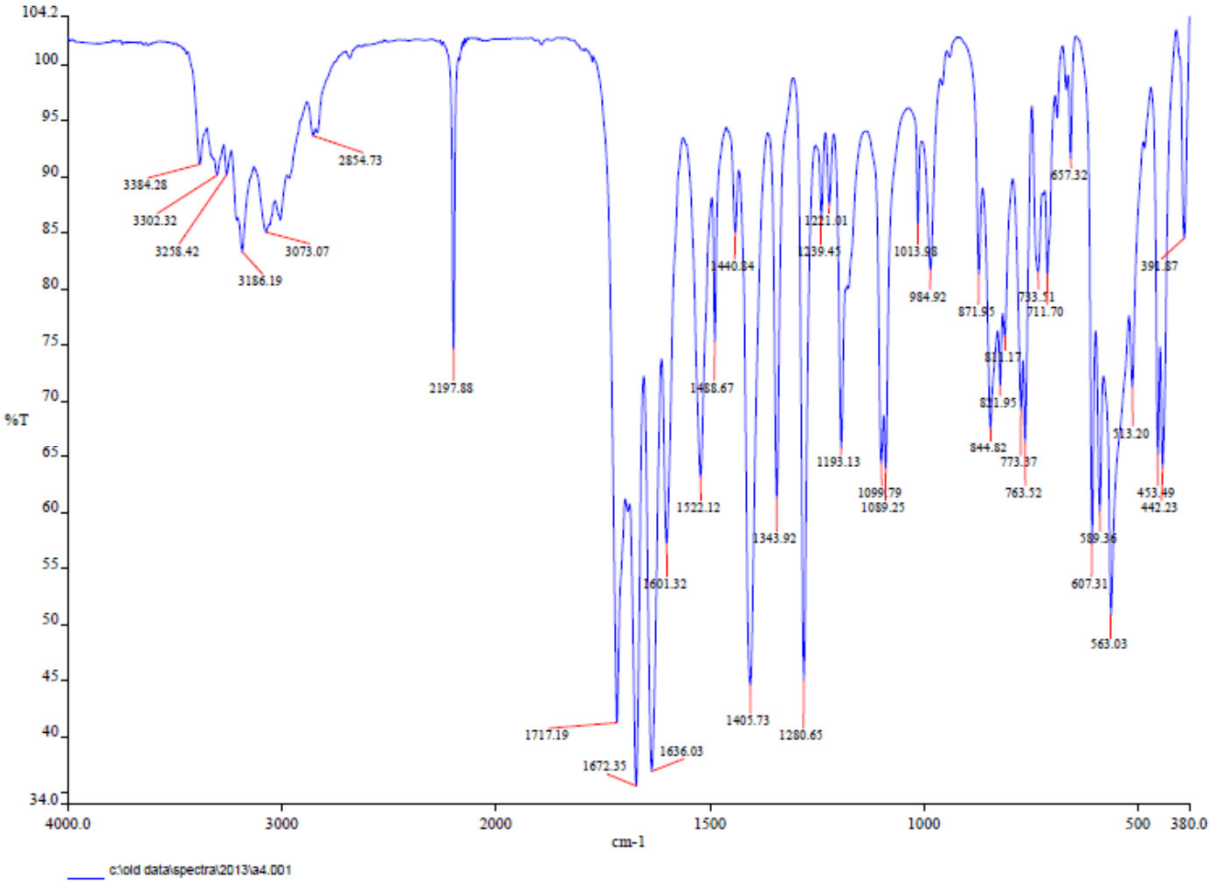
HMBC spectrum of 7-amino-5-(4-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B4)



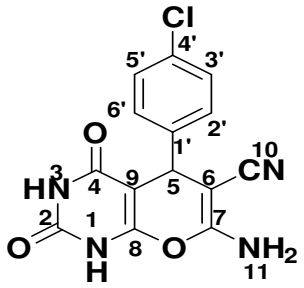


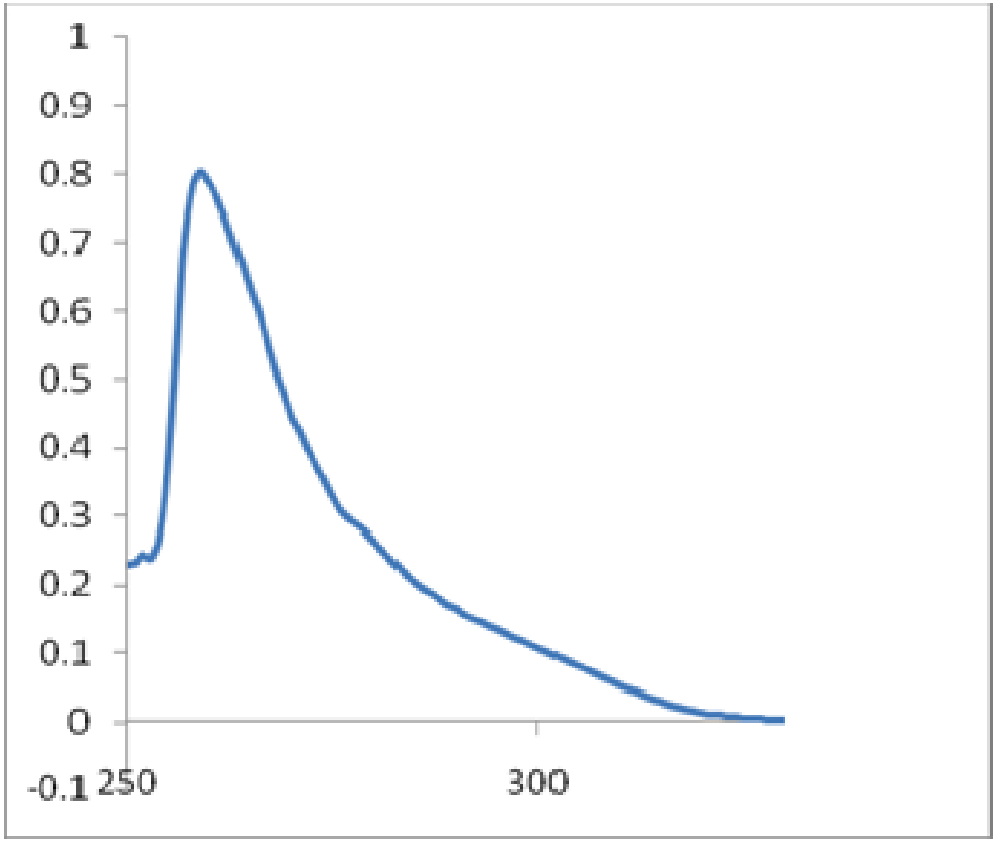
Mass spectrum of 7-amino-5-(4-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B4)



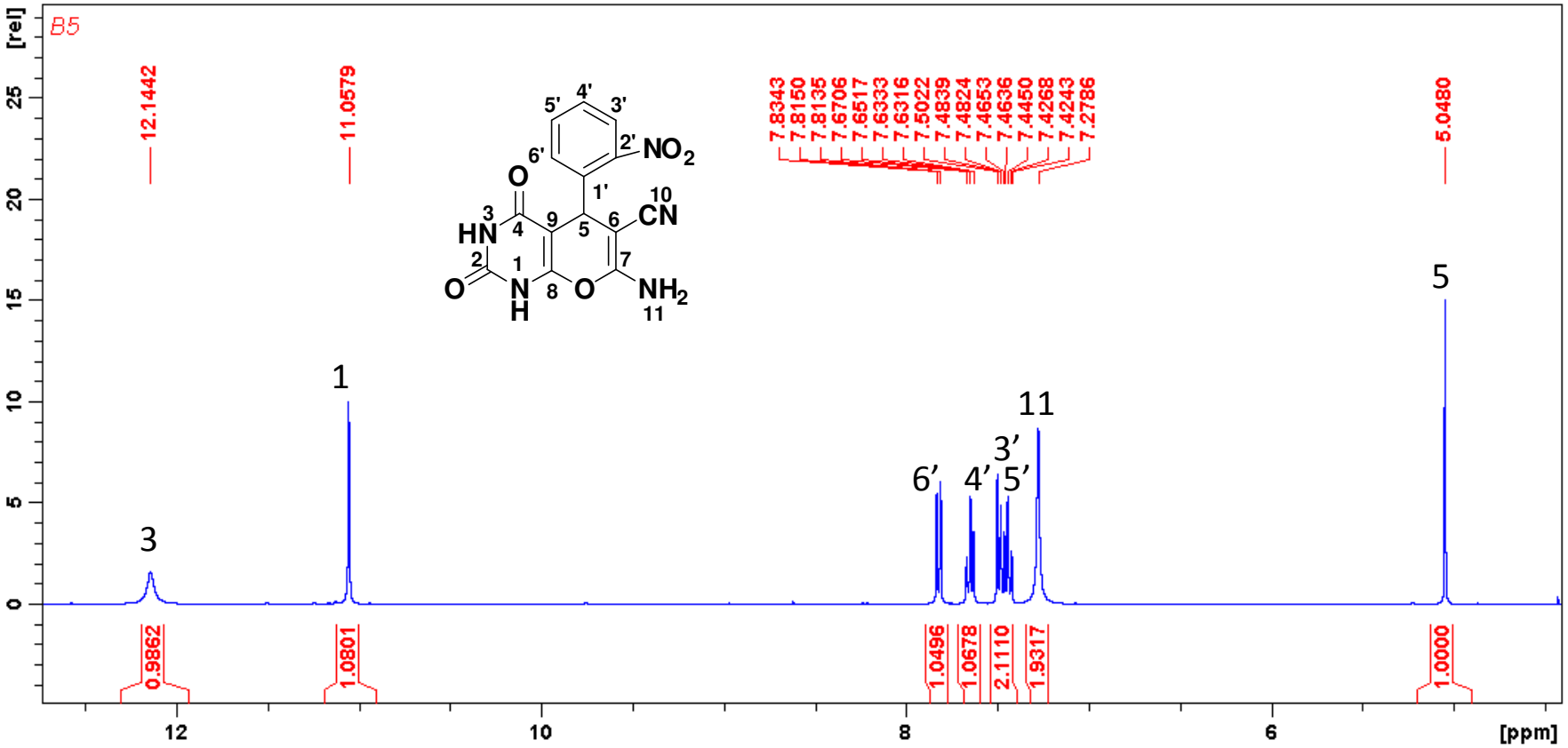


IR spectrum of 7-amino-5-(4-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B4)

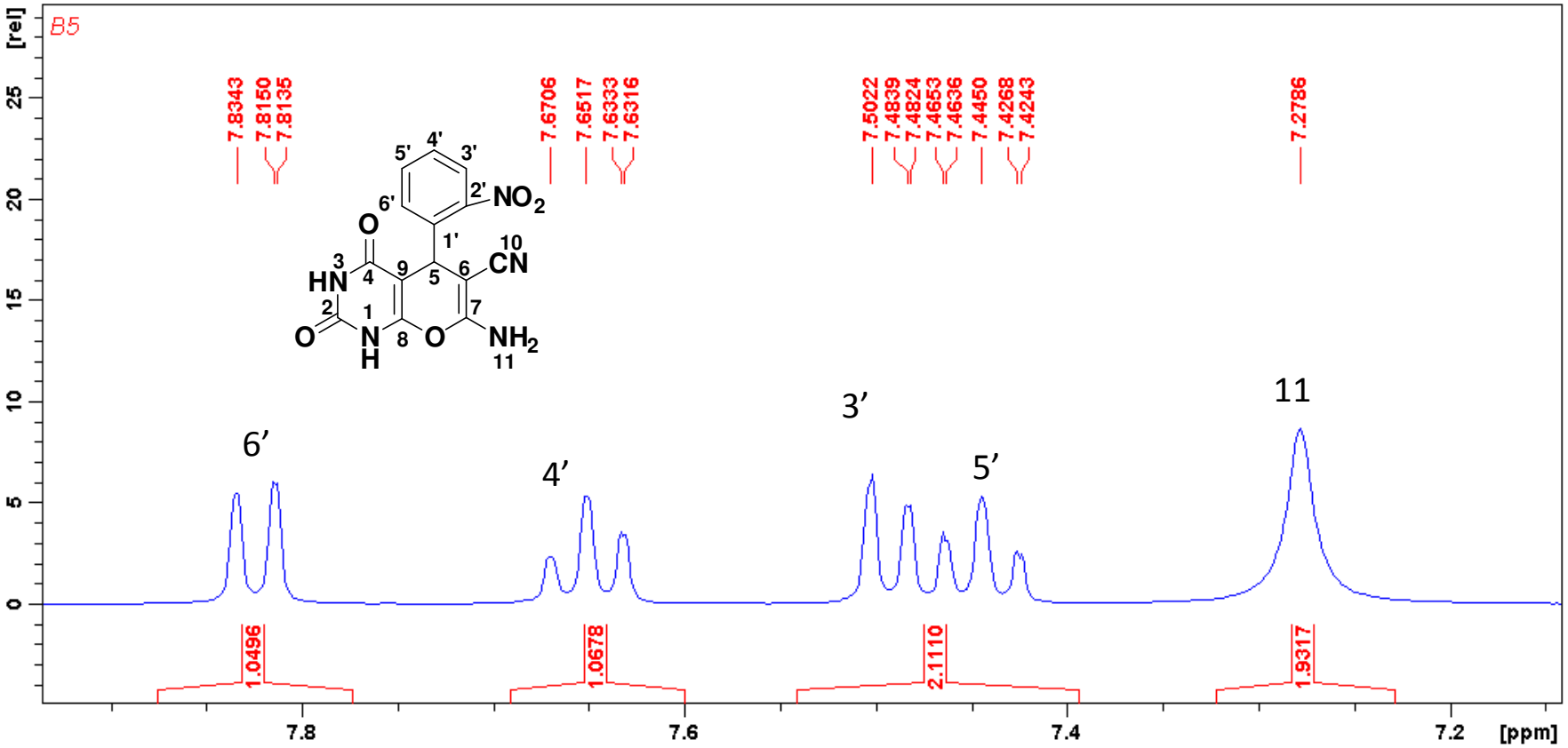




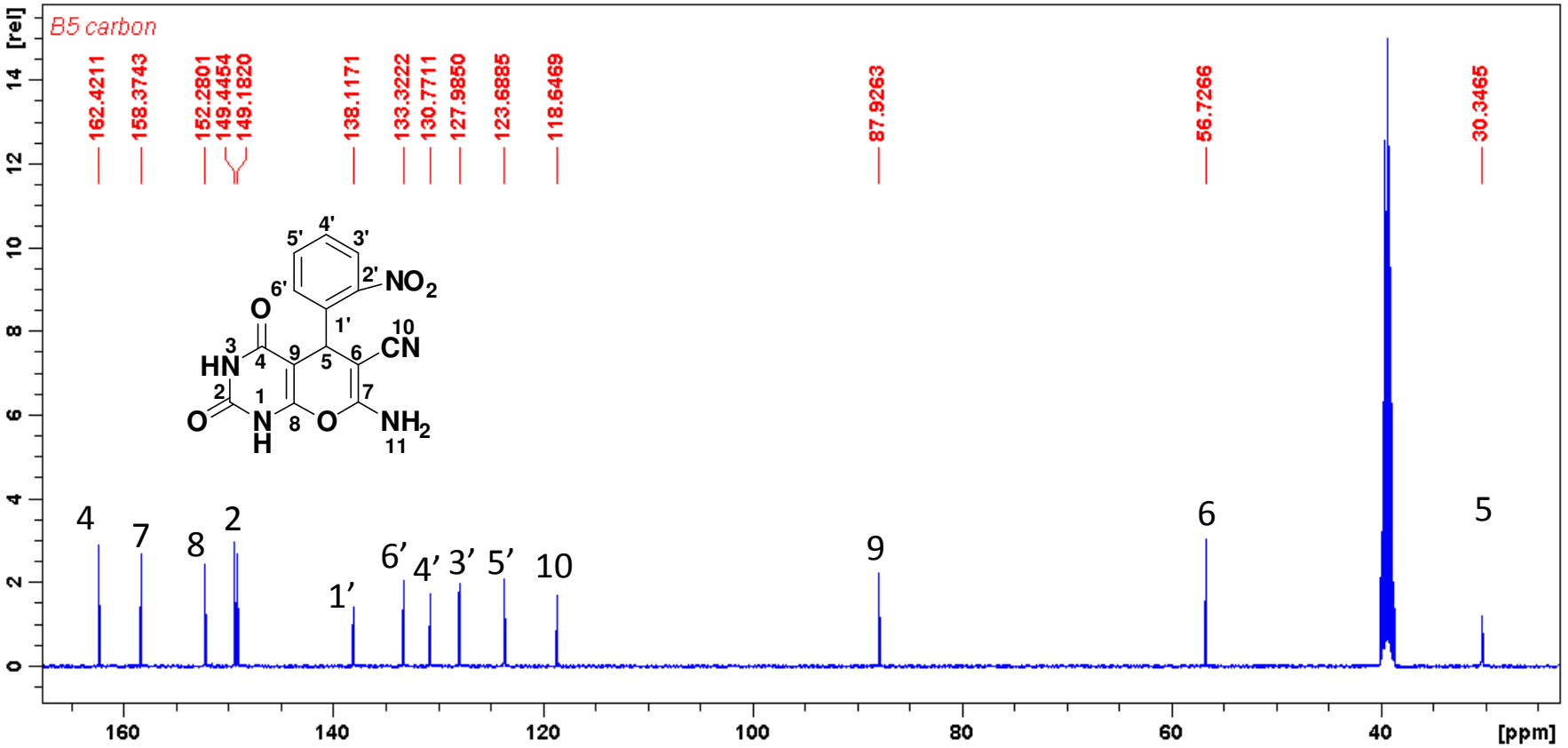
UV spectrum of 7-amino-5-(4-chlorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B4)



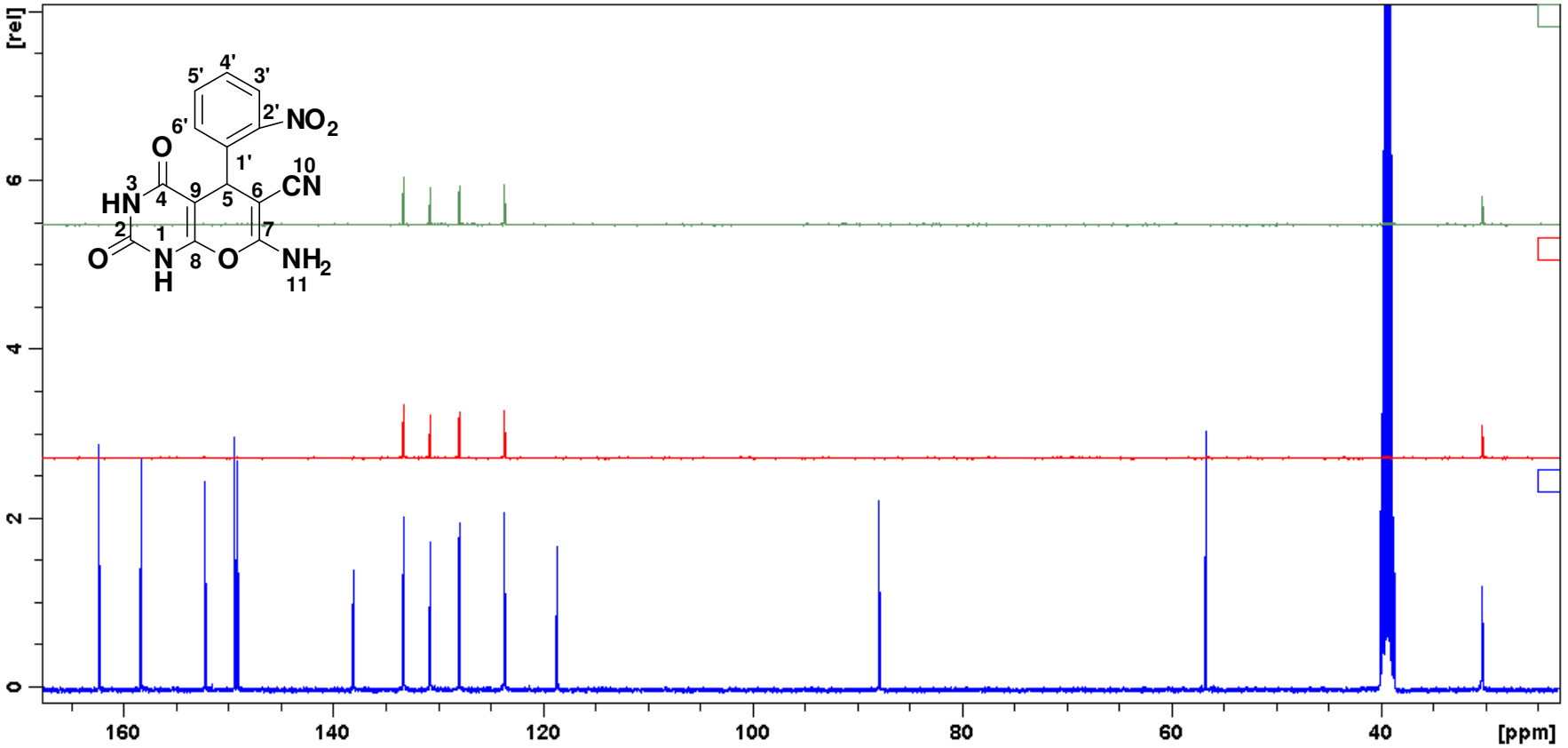
^{1}H NMR spectrum of 7-amino-5-(2-nitrophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B5)



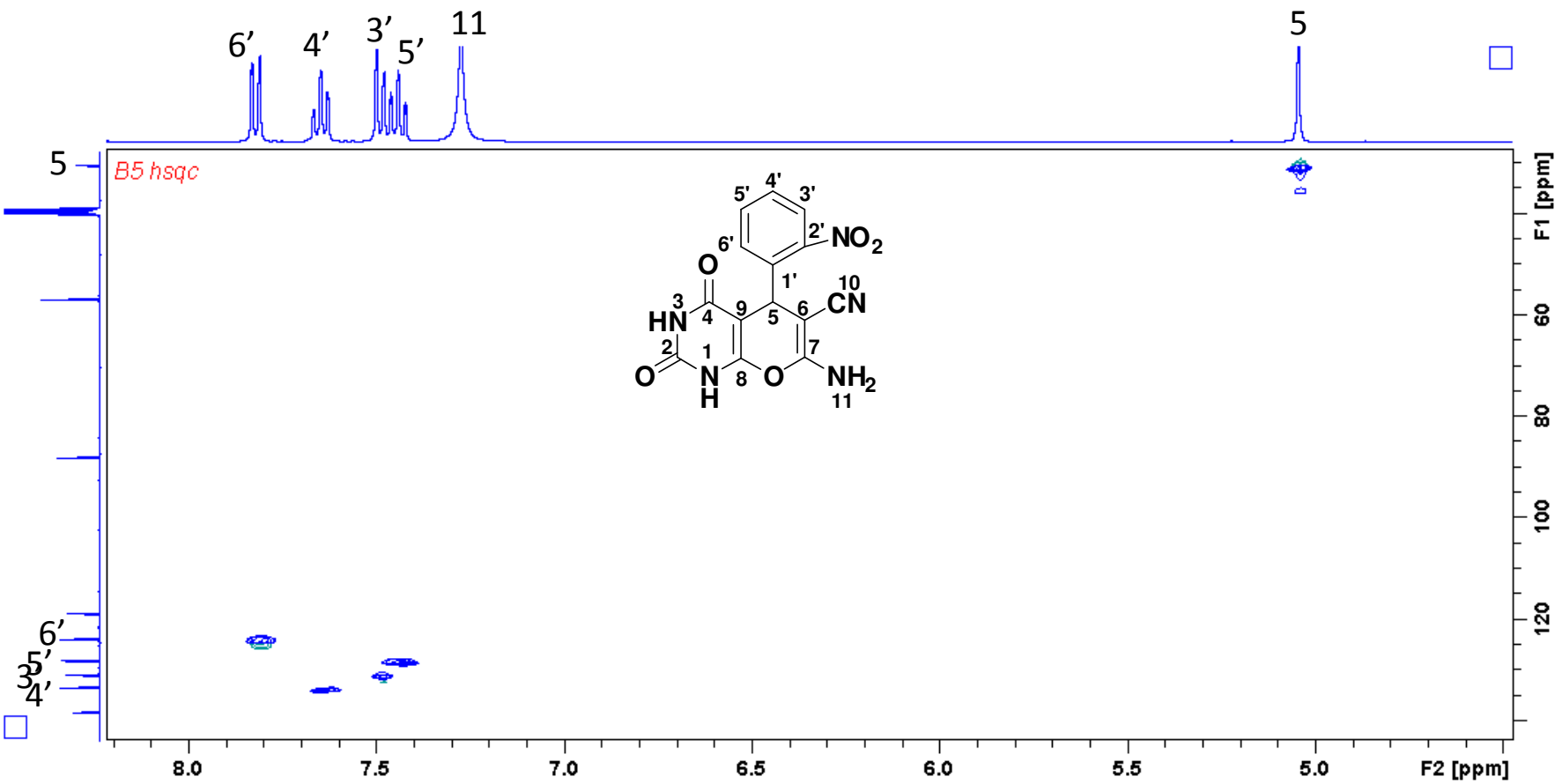
^{1}H NMR spectrum of 7-amino-5-(2-nitrophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B5) expanded



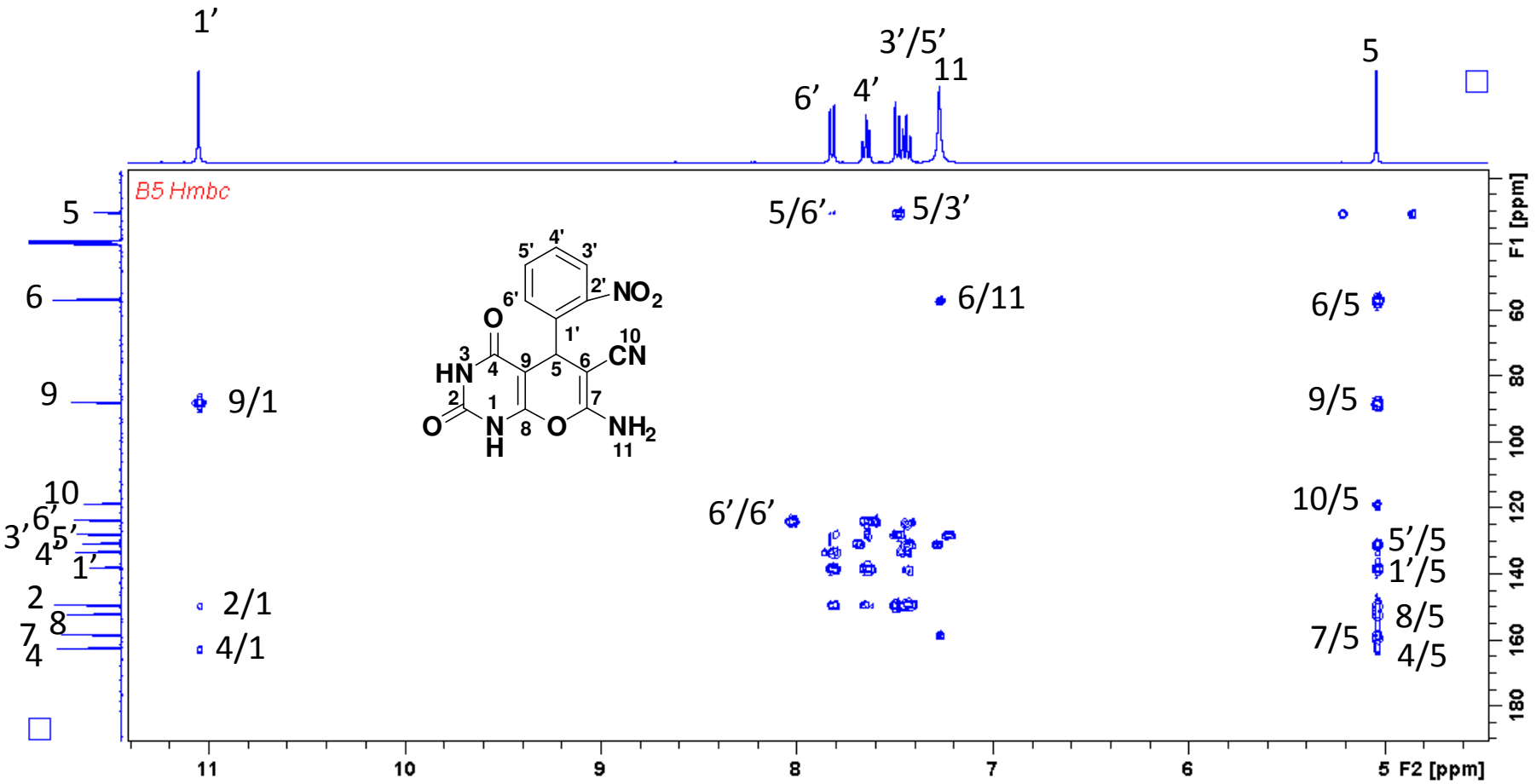
^{13}C NMR spectrum of 7-amino-5-(2-nitrophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B5)



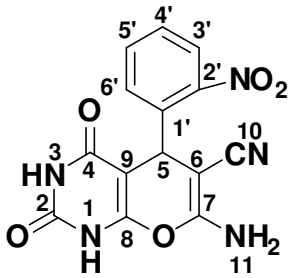
DEPT spectrum of 7-amino-5-(2-nitrophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B5)



HSQC spectrum of 7-amino-5-(2-nitrophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B5)



HMBC spectrum of 7-amino-5-(2-nitrophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B5)



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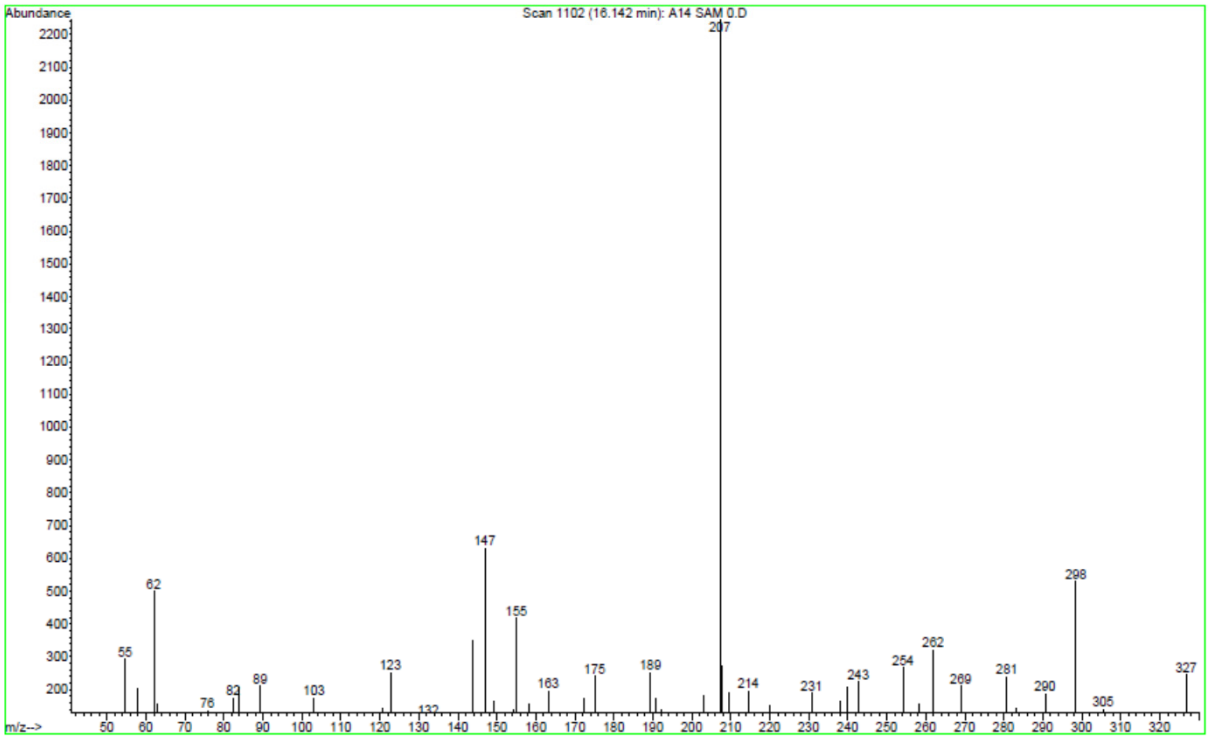
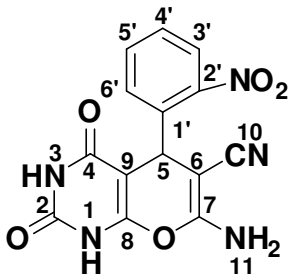
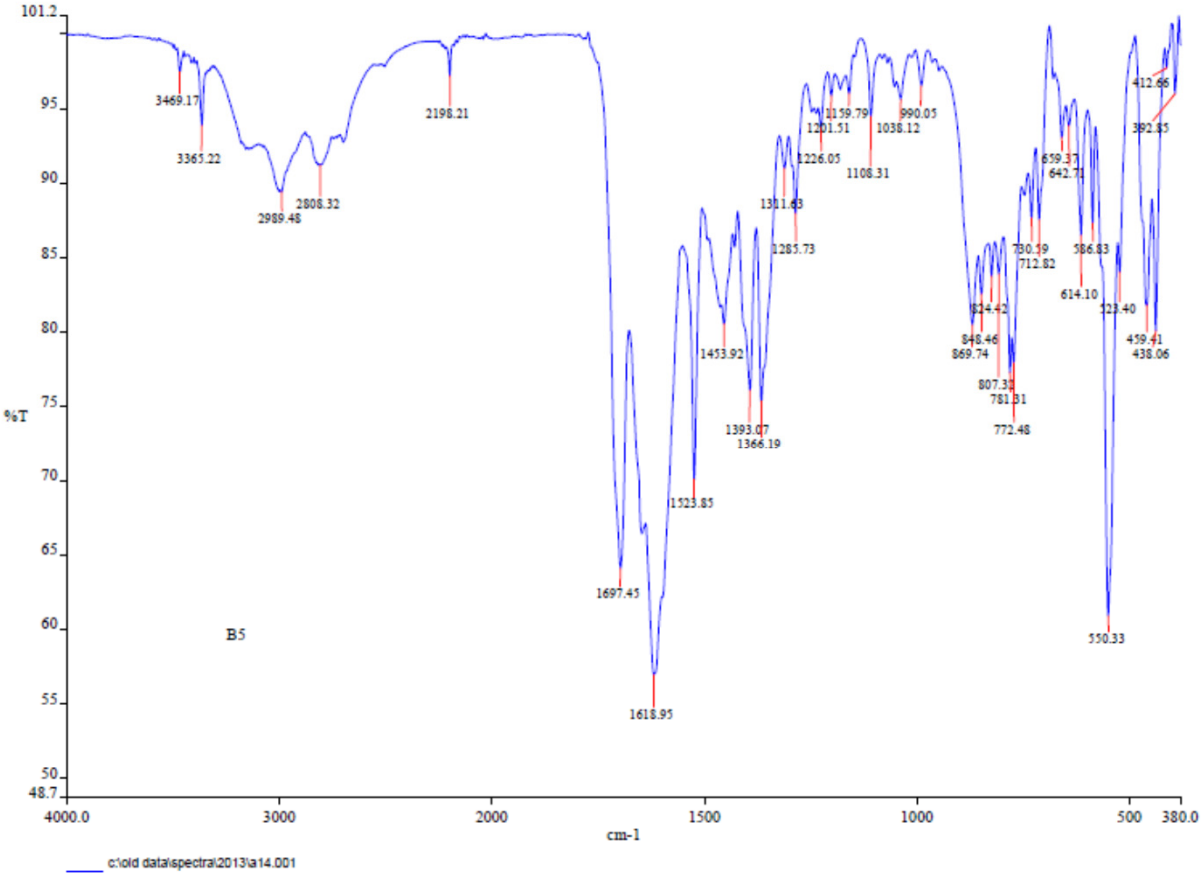
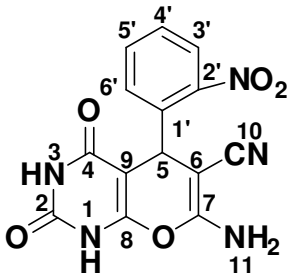


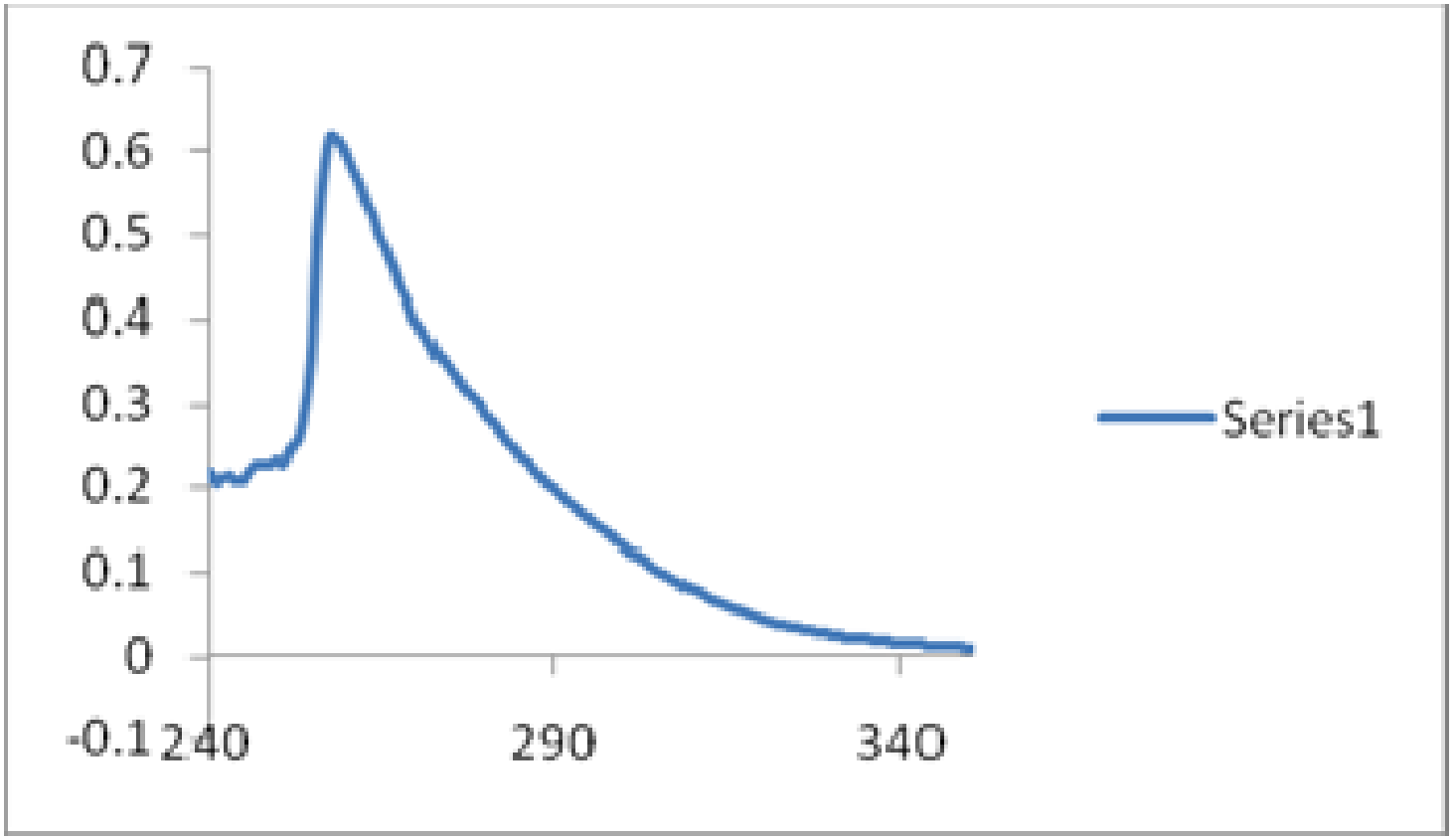
Mass spectrum of 7-amino-5-(2-nitro)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B5)



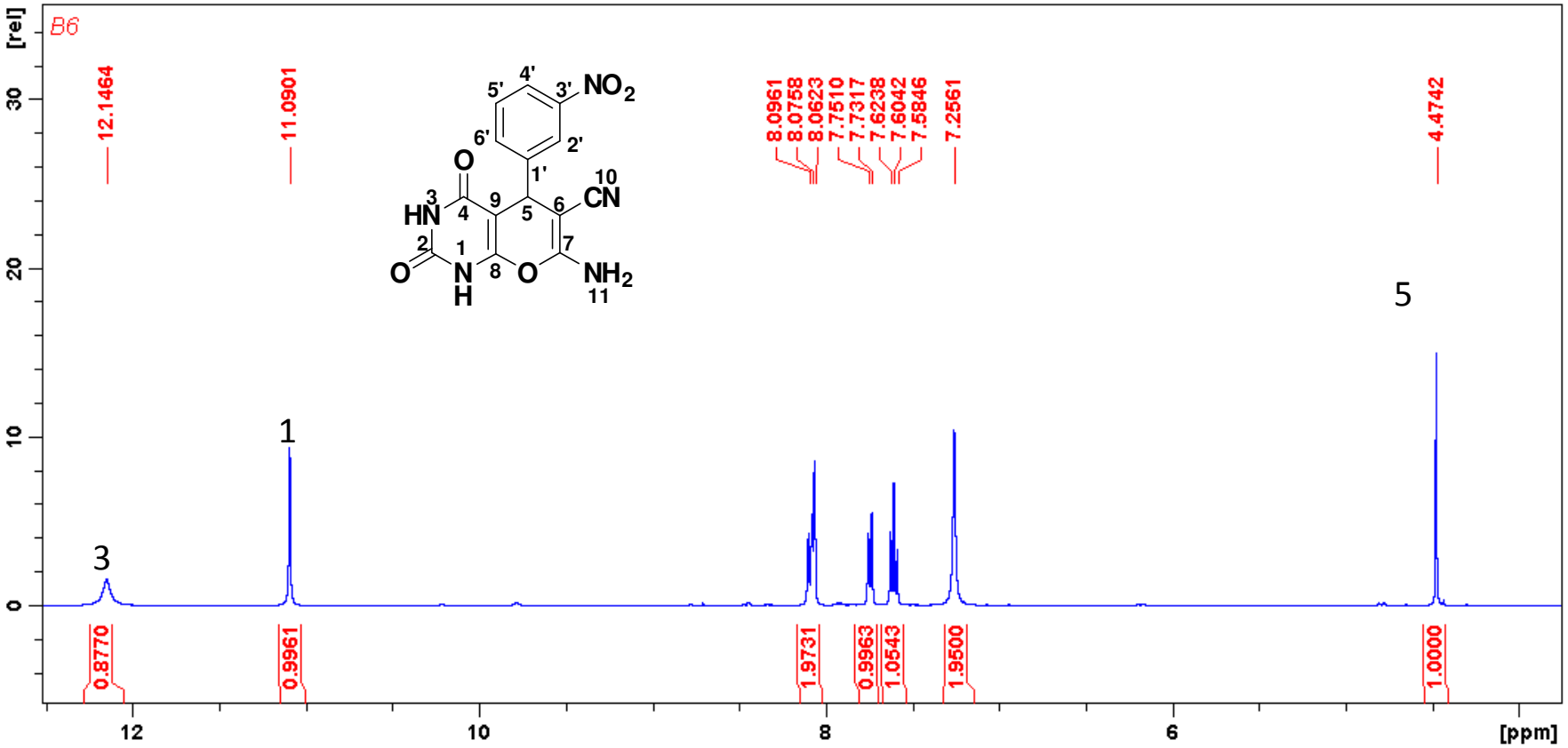


IR spectrum of 7-amino-5-(2-nitrophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B5)

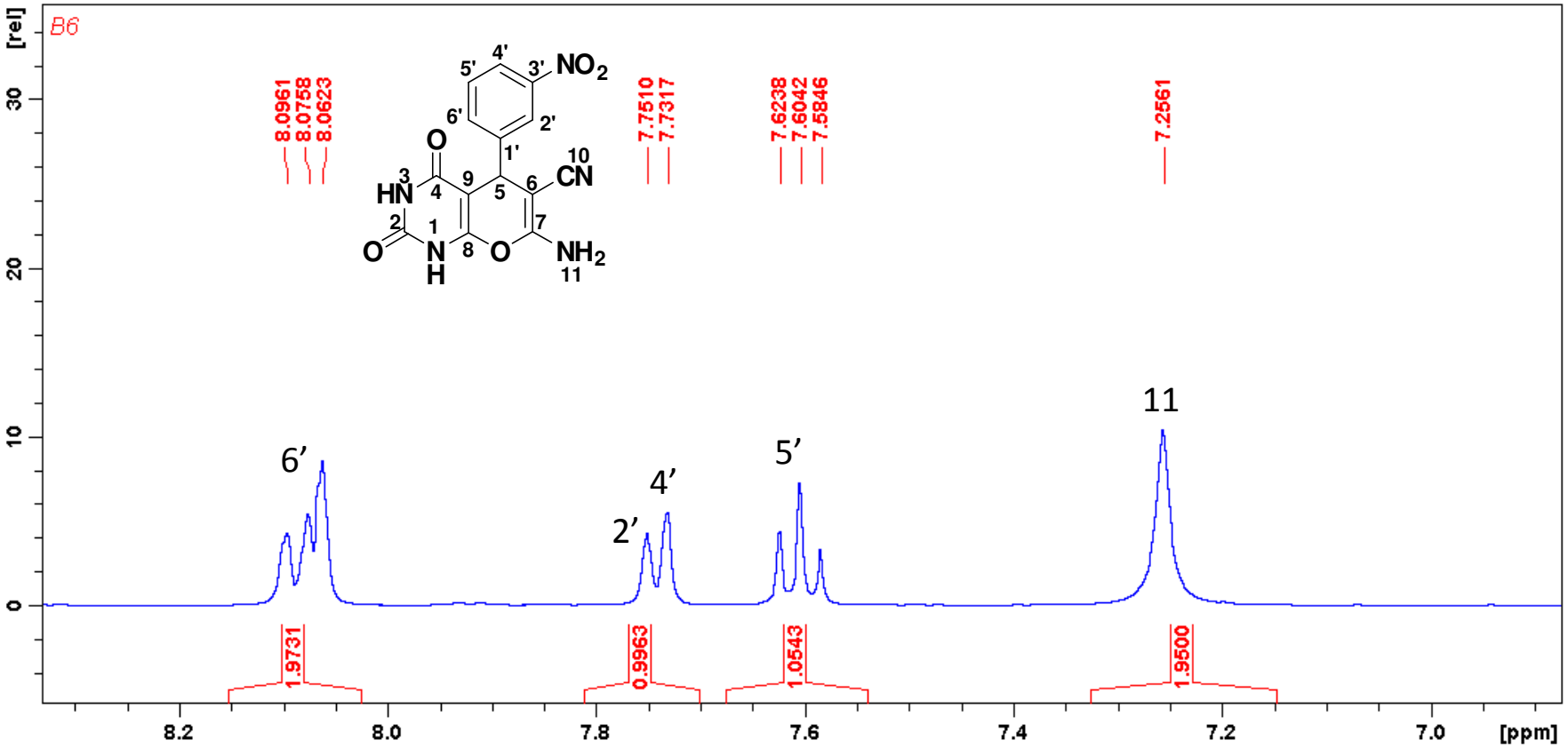




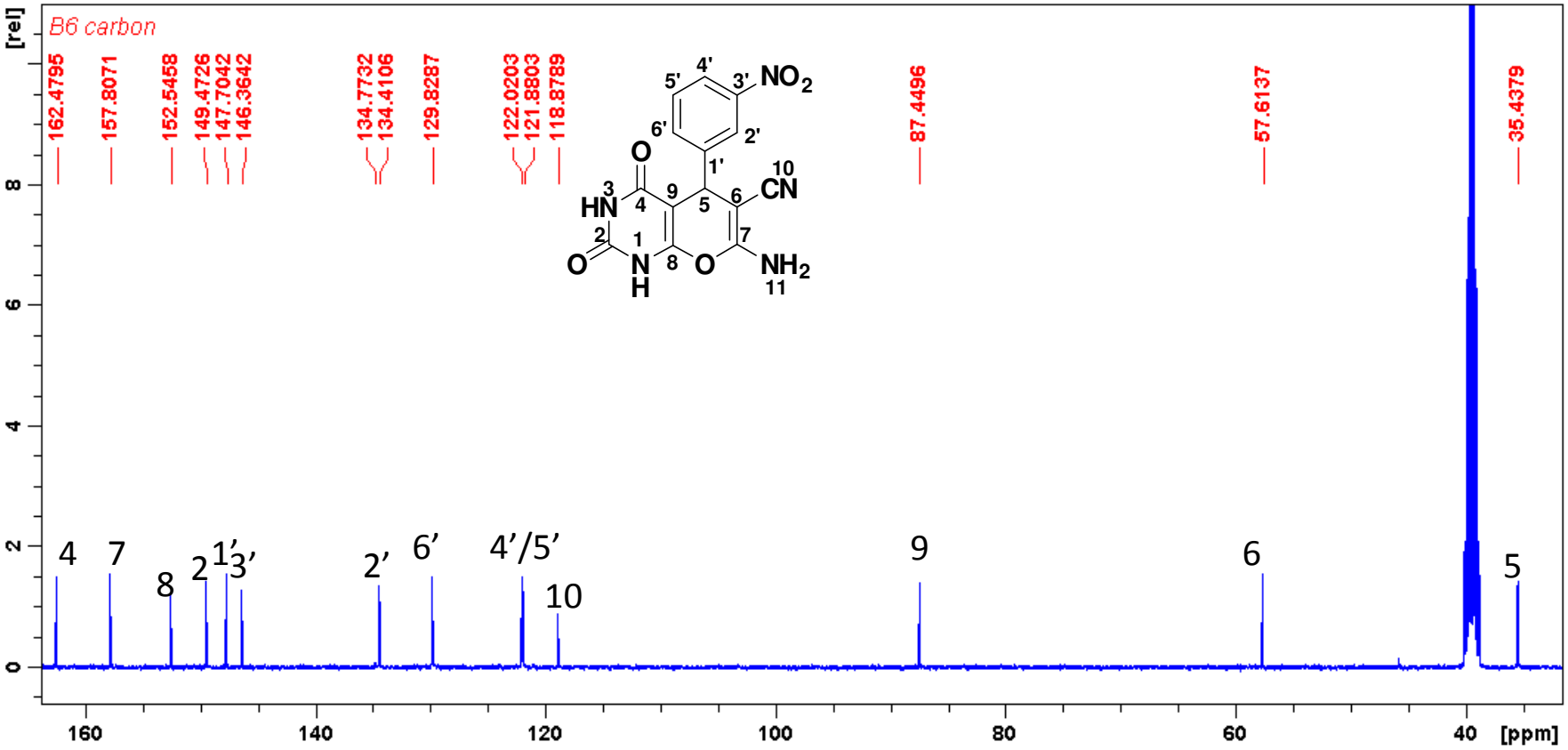
UV spectrum of 7-amino-5-(2-nitrophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B5)



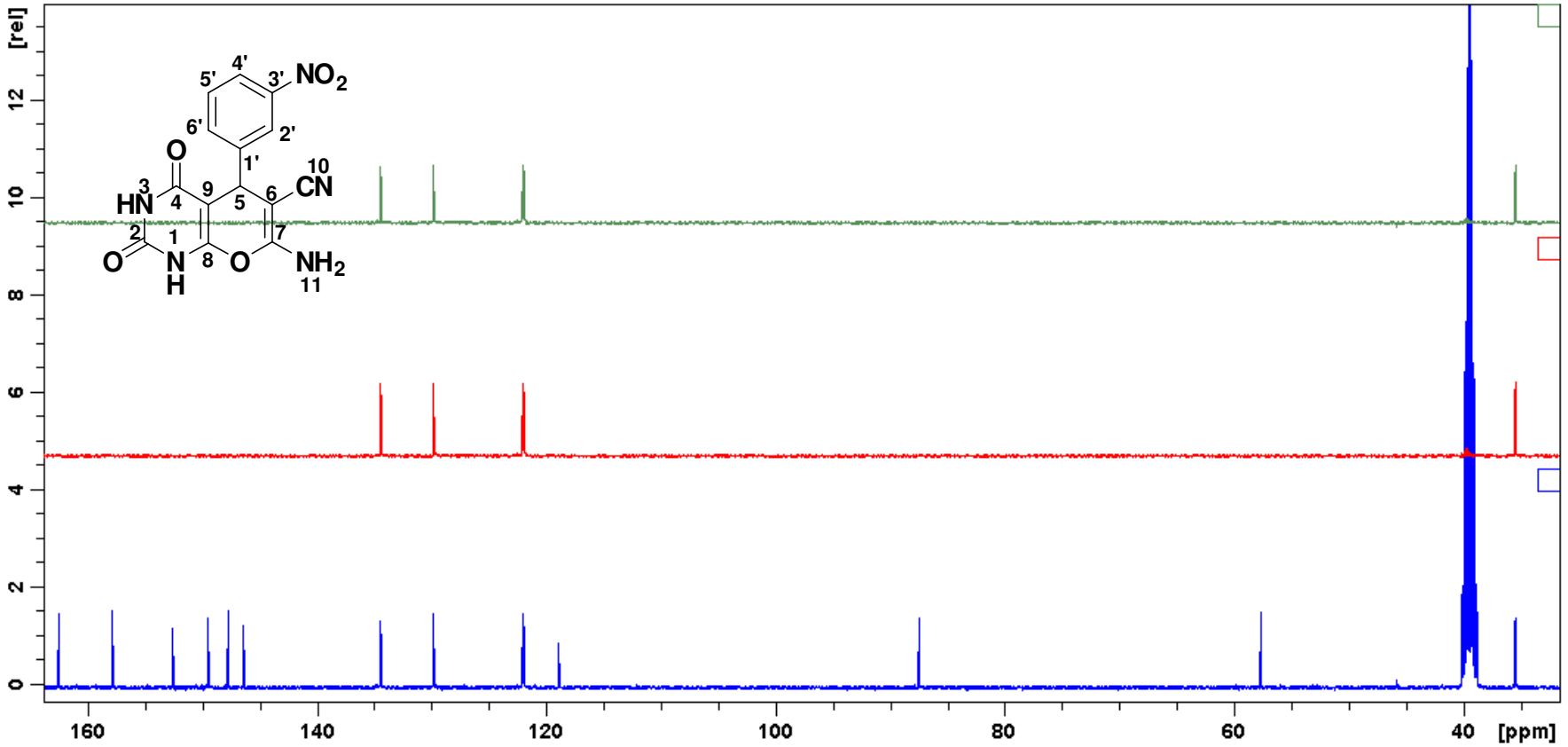
^{1}H NMR spectrum of 7-amino-5-(3-nitrophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B6)



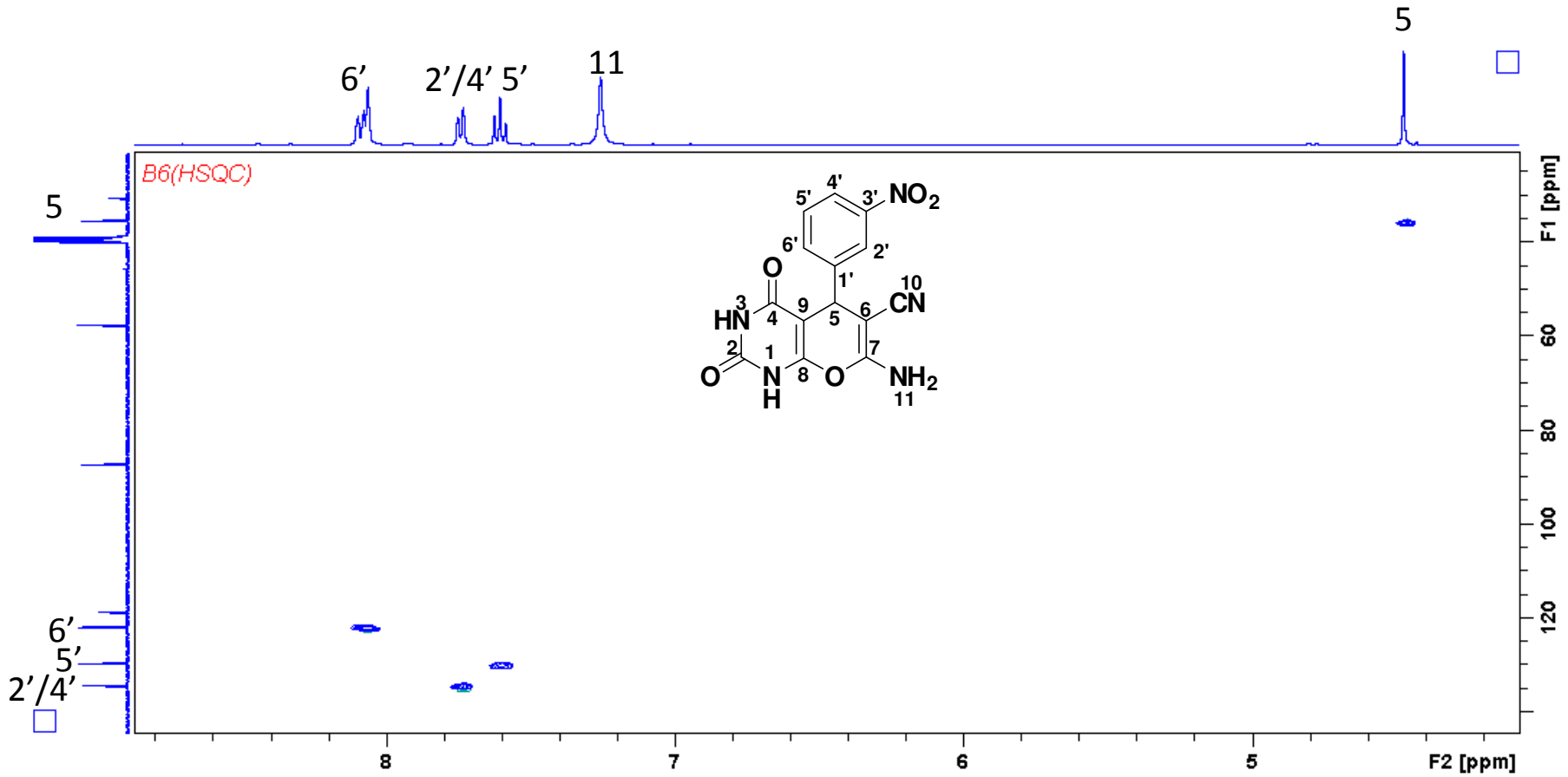
^{1}H NMR spectrum of 7-amino-5-(3-nitrophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B6) expanded



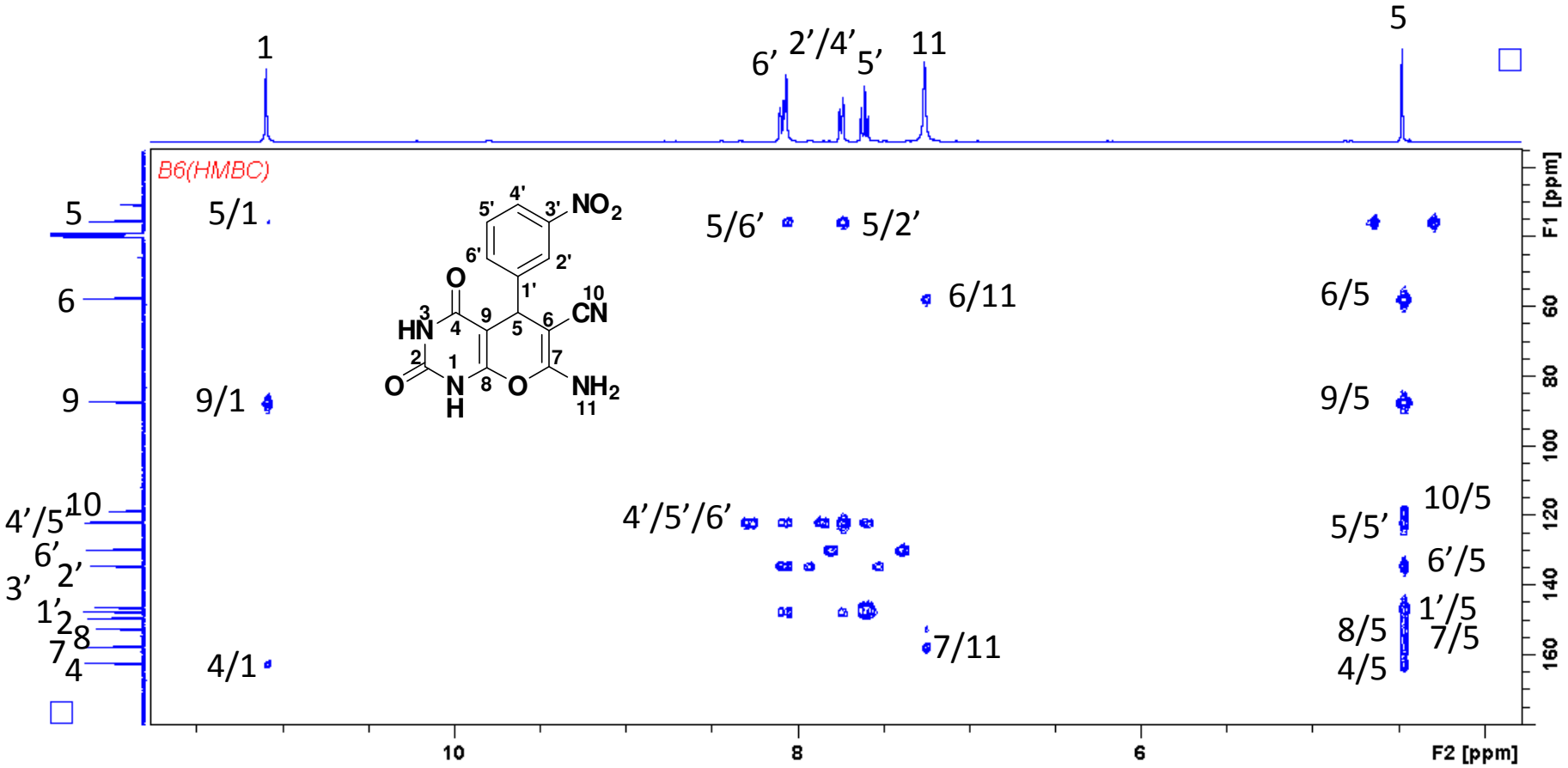
^{13}C NMR spectrum of 7-amino-5-(3-nitrophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B6)



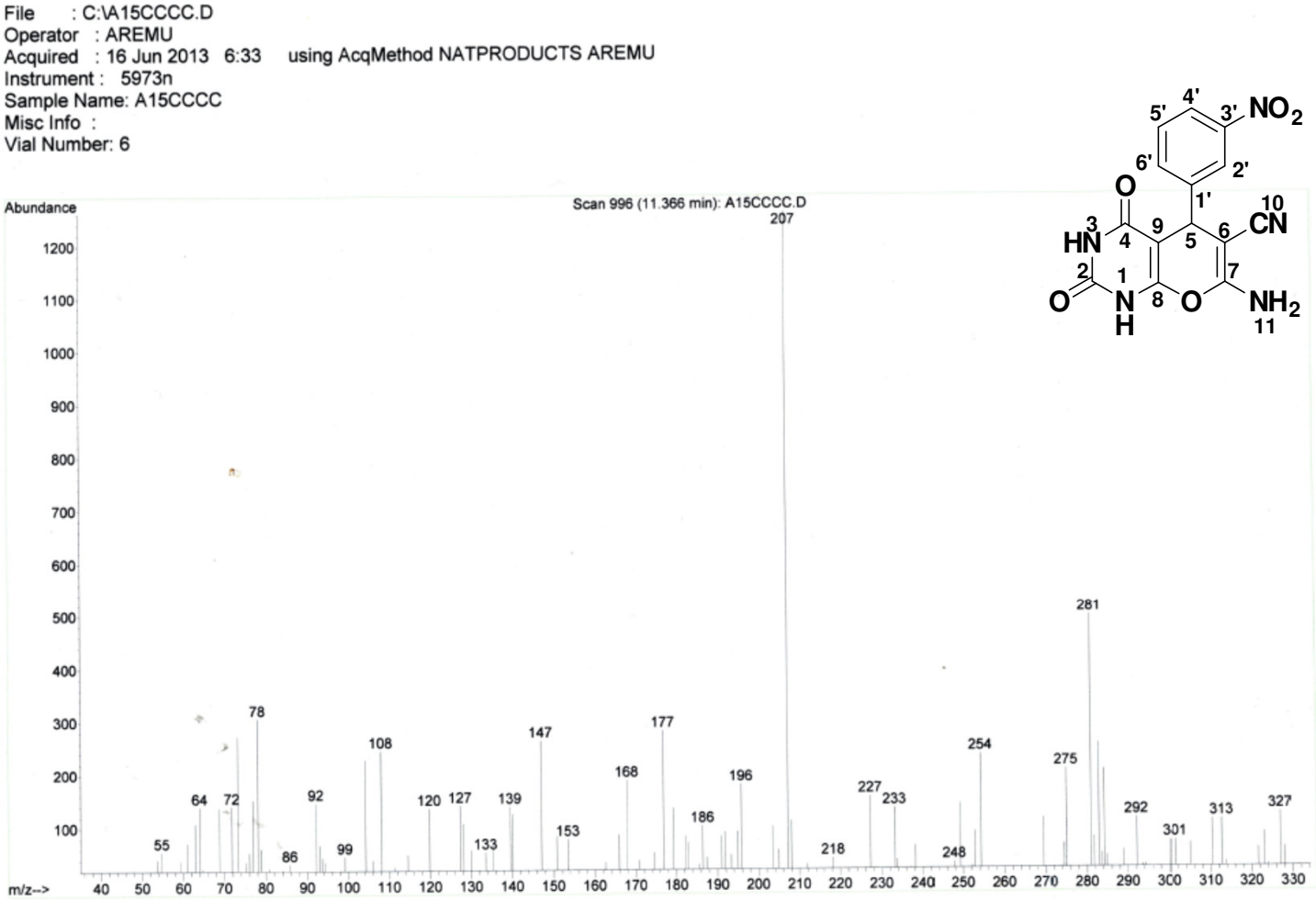
DEPT spectrum of 7-amino-5-(3-nitrophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B6)



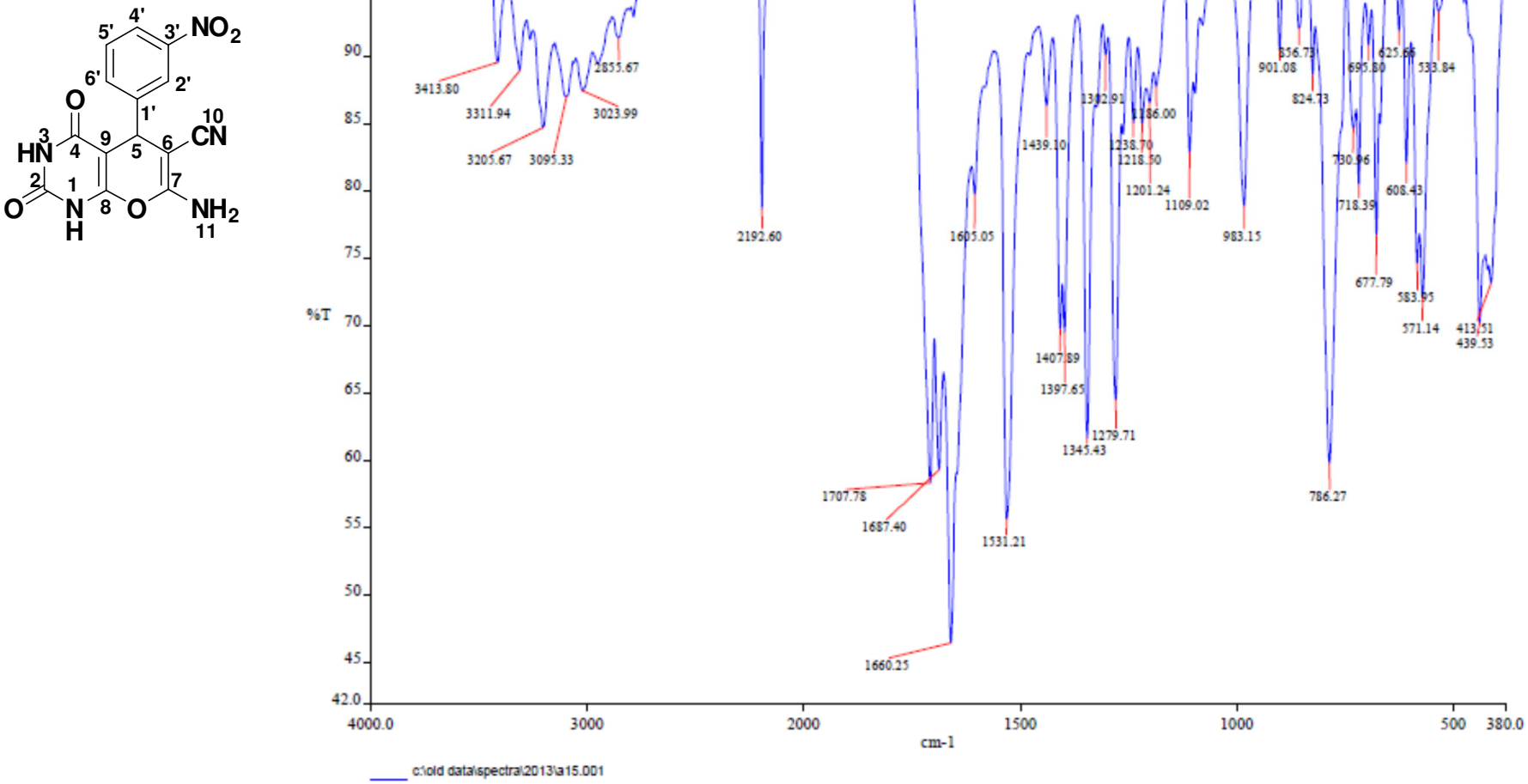
HSQC spectrum of 7-amino-5-(3-nitrophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B6)



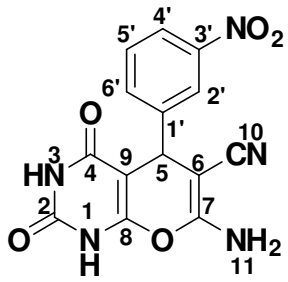
HMBC spectrum of 7-amino-5-(3-nitrophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B6)

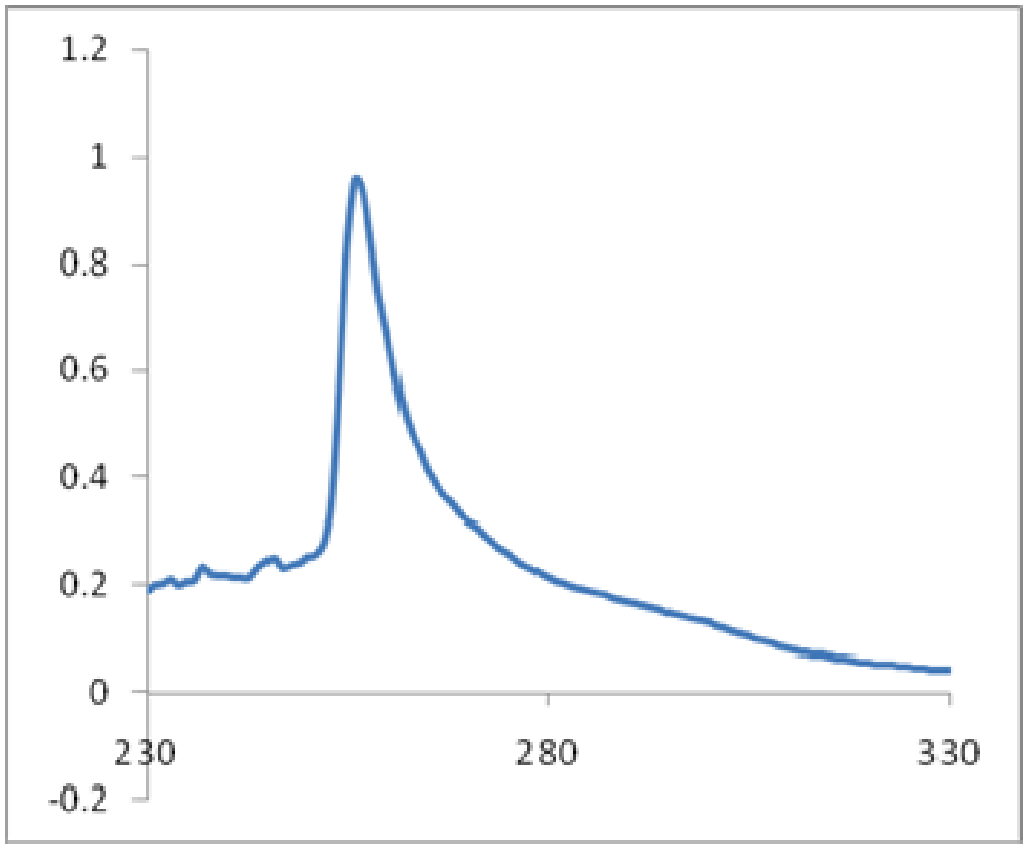


Mass spectrum of 7-amino-5-(3-nitrophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B6)

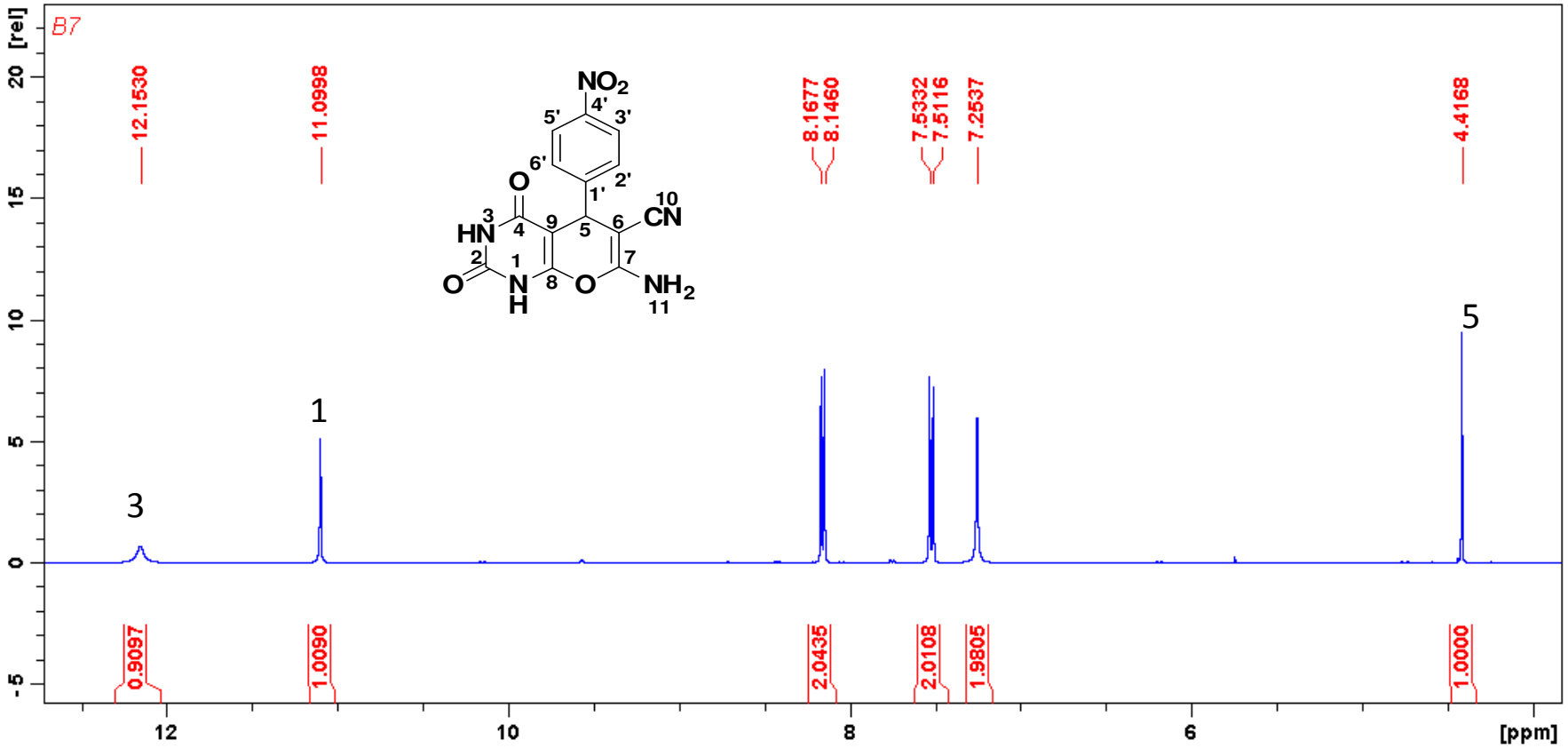


IR spectrum of 7-amino-5-(3-nitrophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B6)

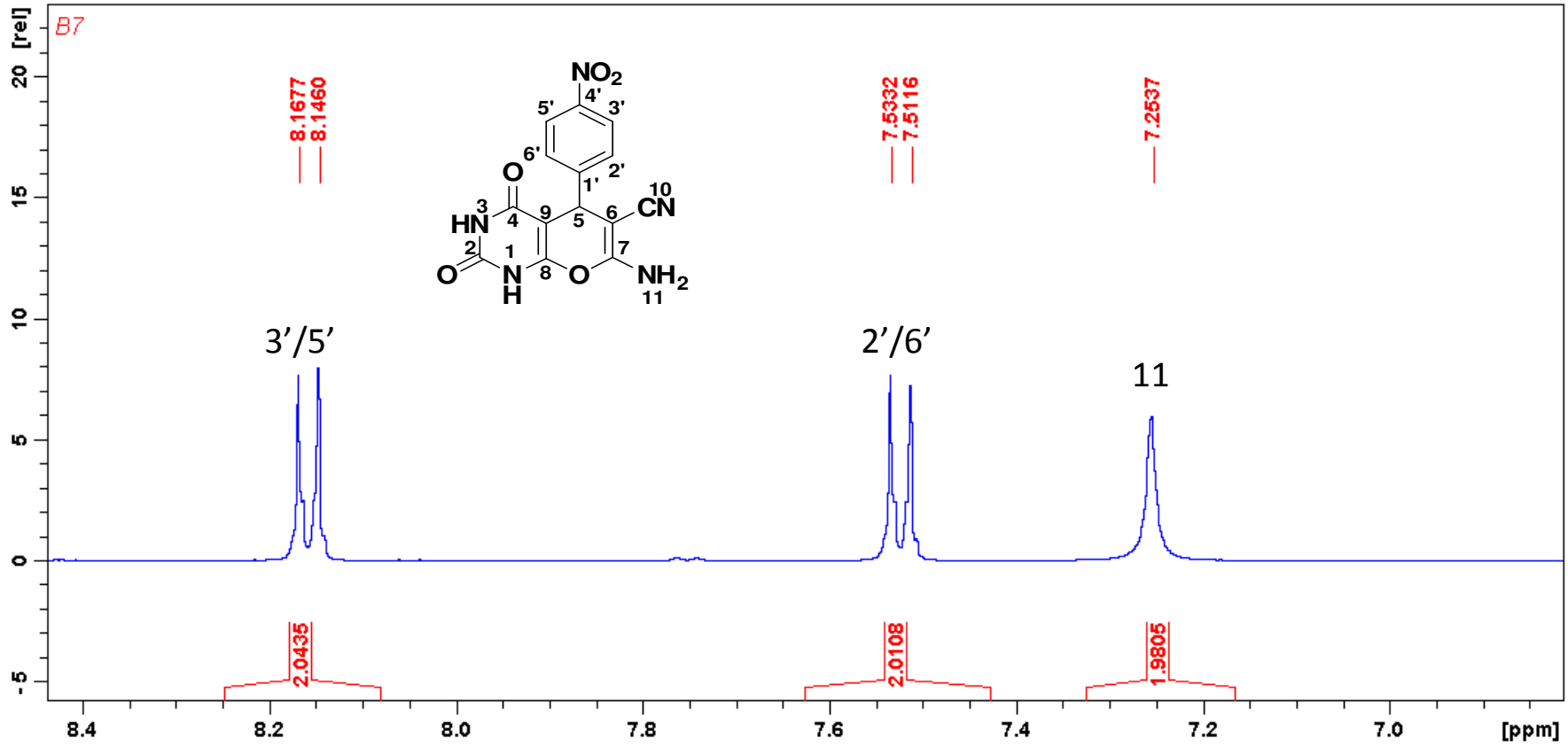




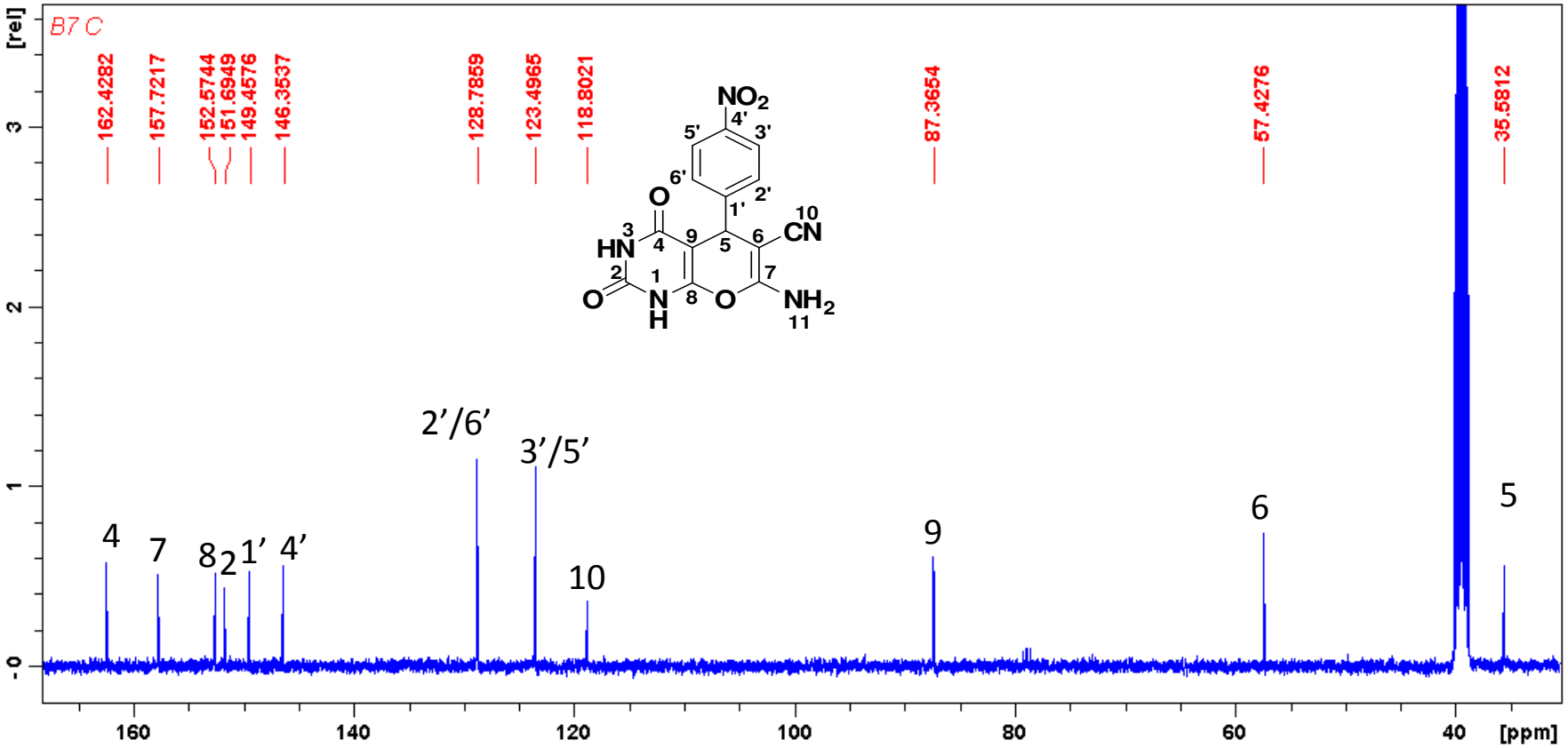
UV spectrum of 7-amino-5-(3-nitrophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B6)



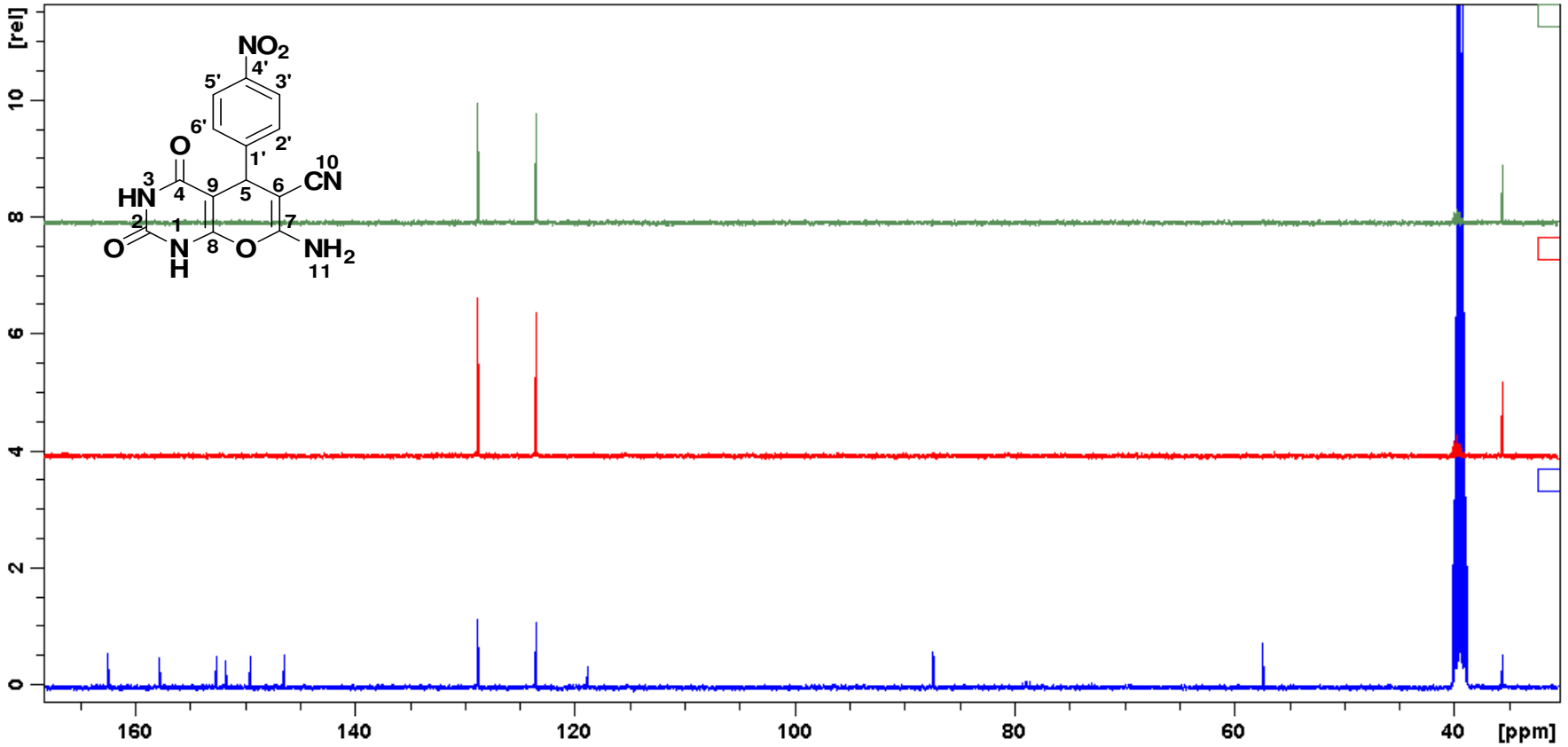
^{1}H NMR spectrum of 7-amino-5-(4-nitrophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B7)



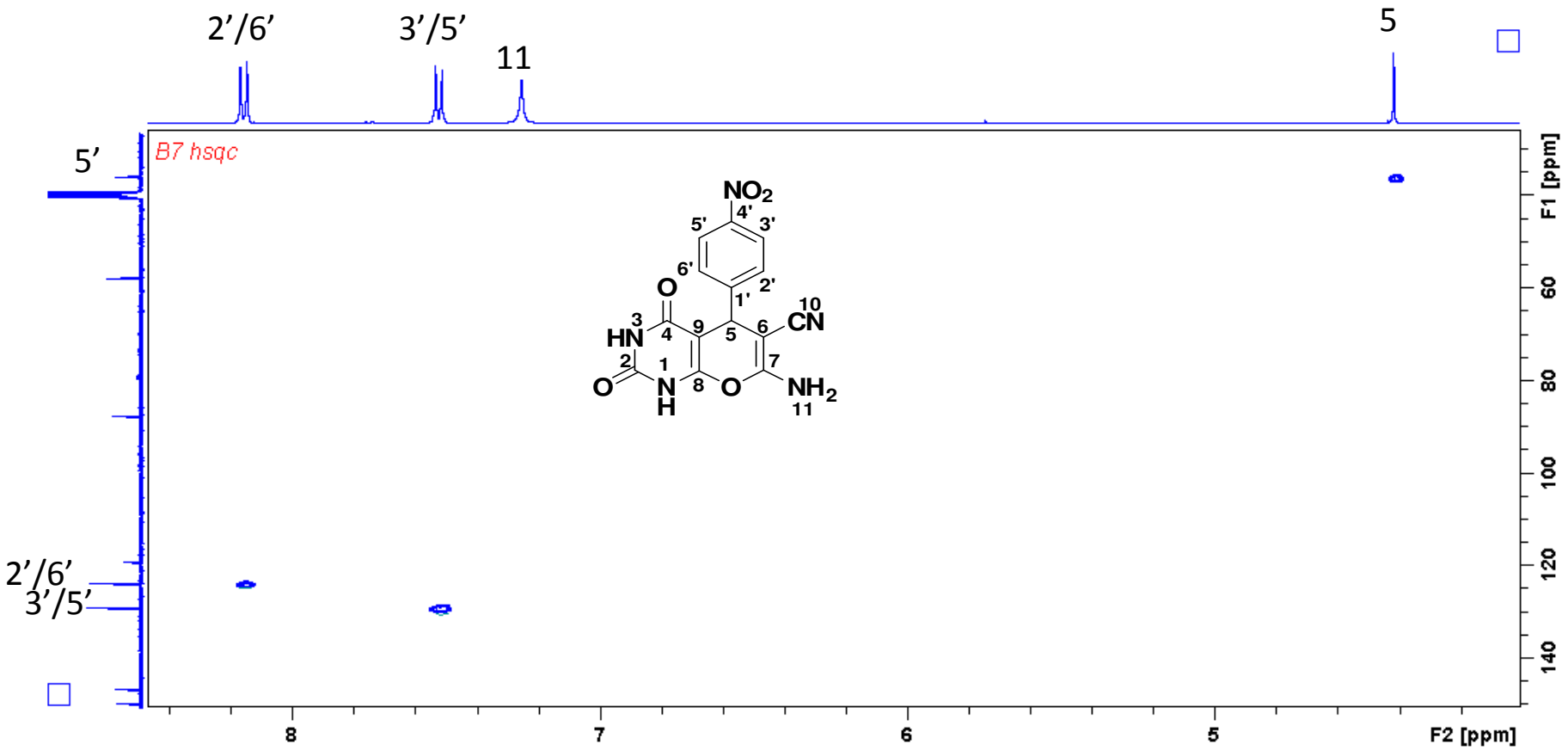
^{1}H NMR spectrum of 7-amino-5-(4-nitrophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B7) expanded



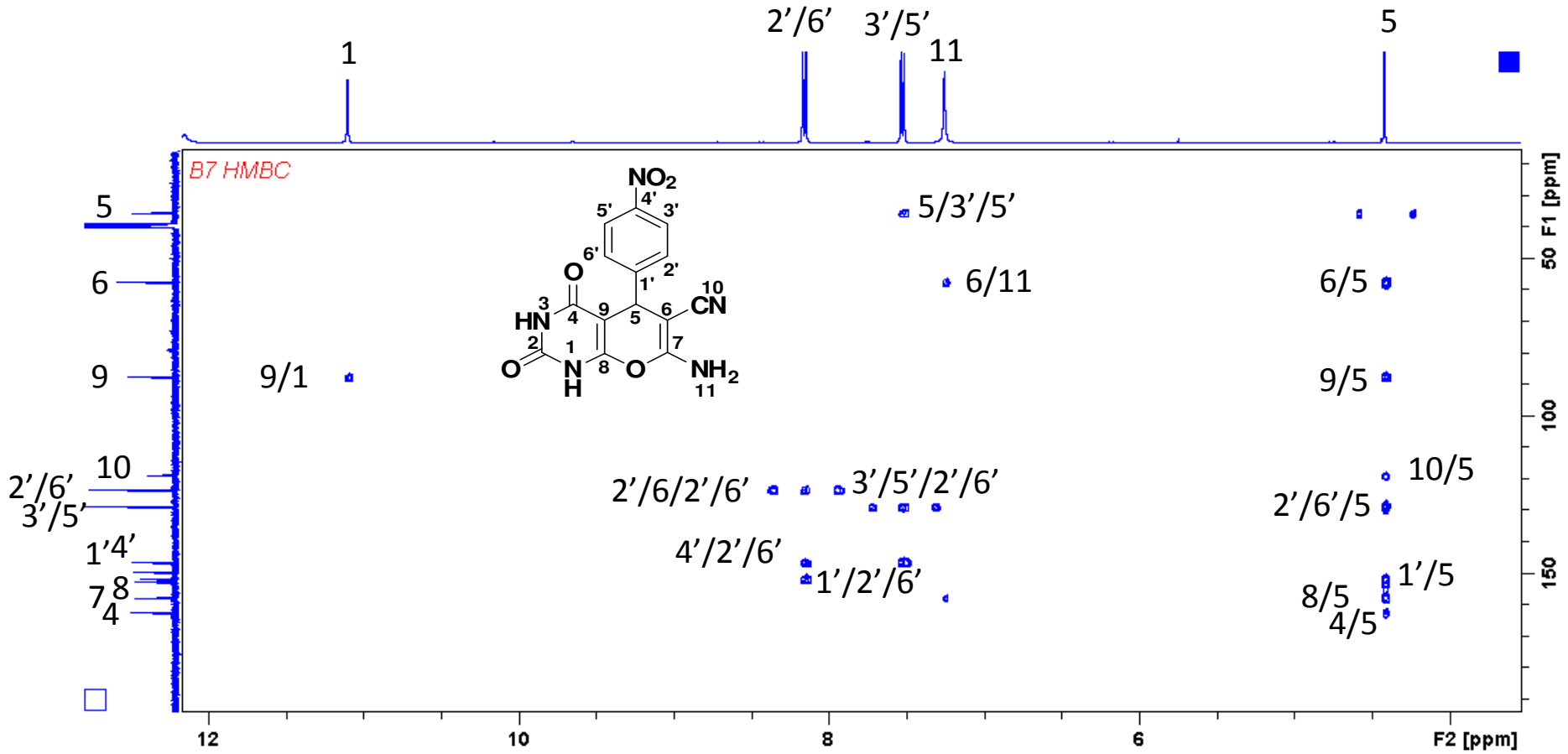
^{13}C NMR spectrum of 7-amino-5-(4-nitrophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B7)



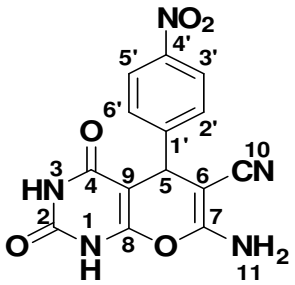
DEPT spectrum of 7-amino-5-(4-nitrophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B7)



HSQC spectrum of 7-amino-5-(4-nitrophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B7)



HMBC spectrum of 7-amino-5-(4-nitrophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B7)



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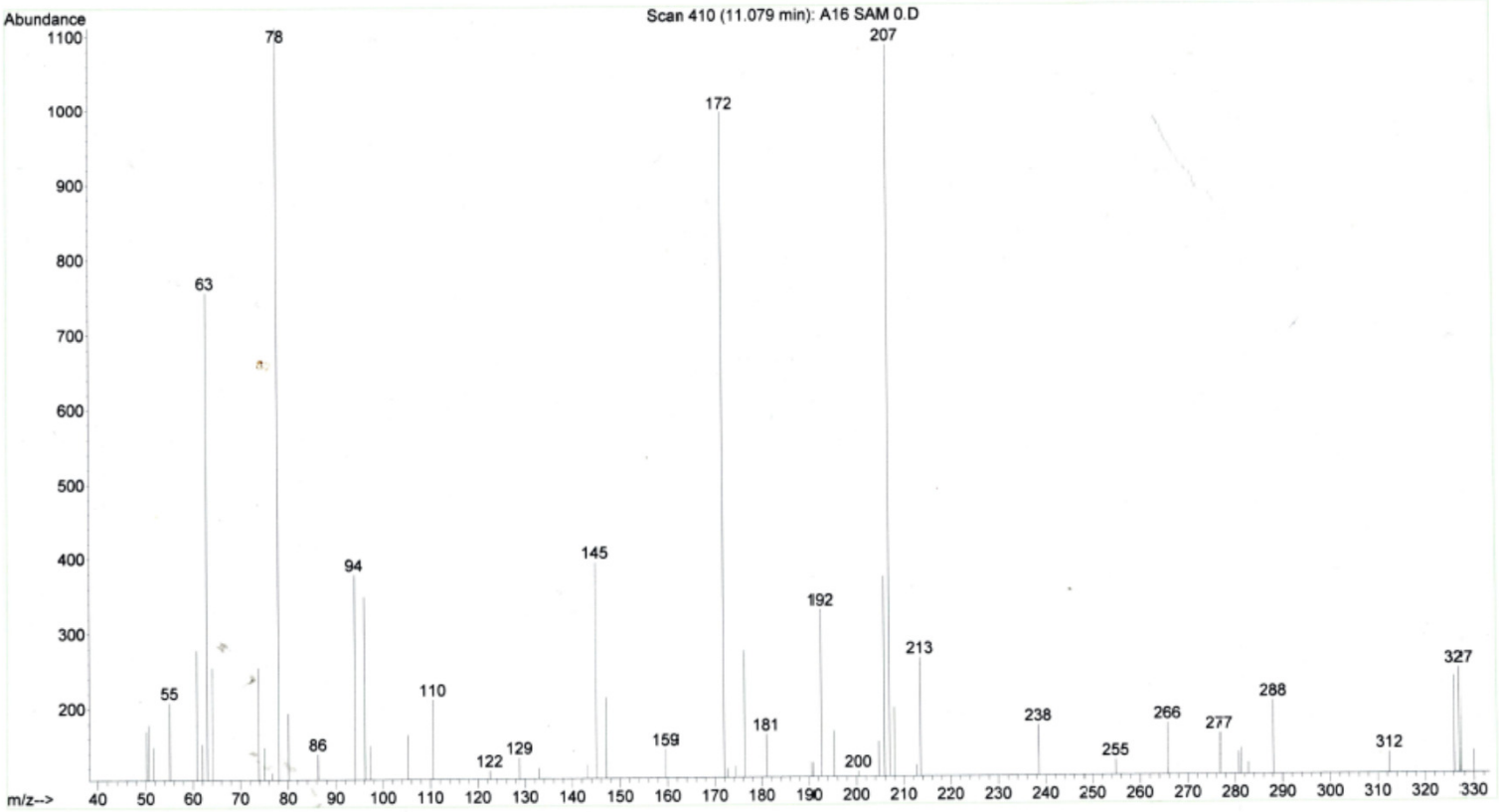
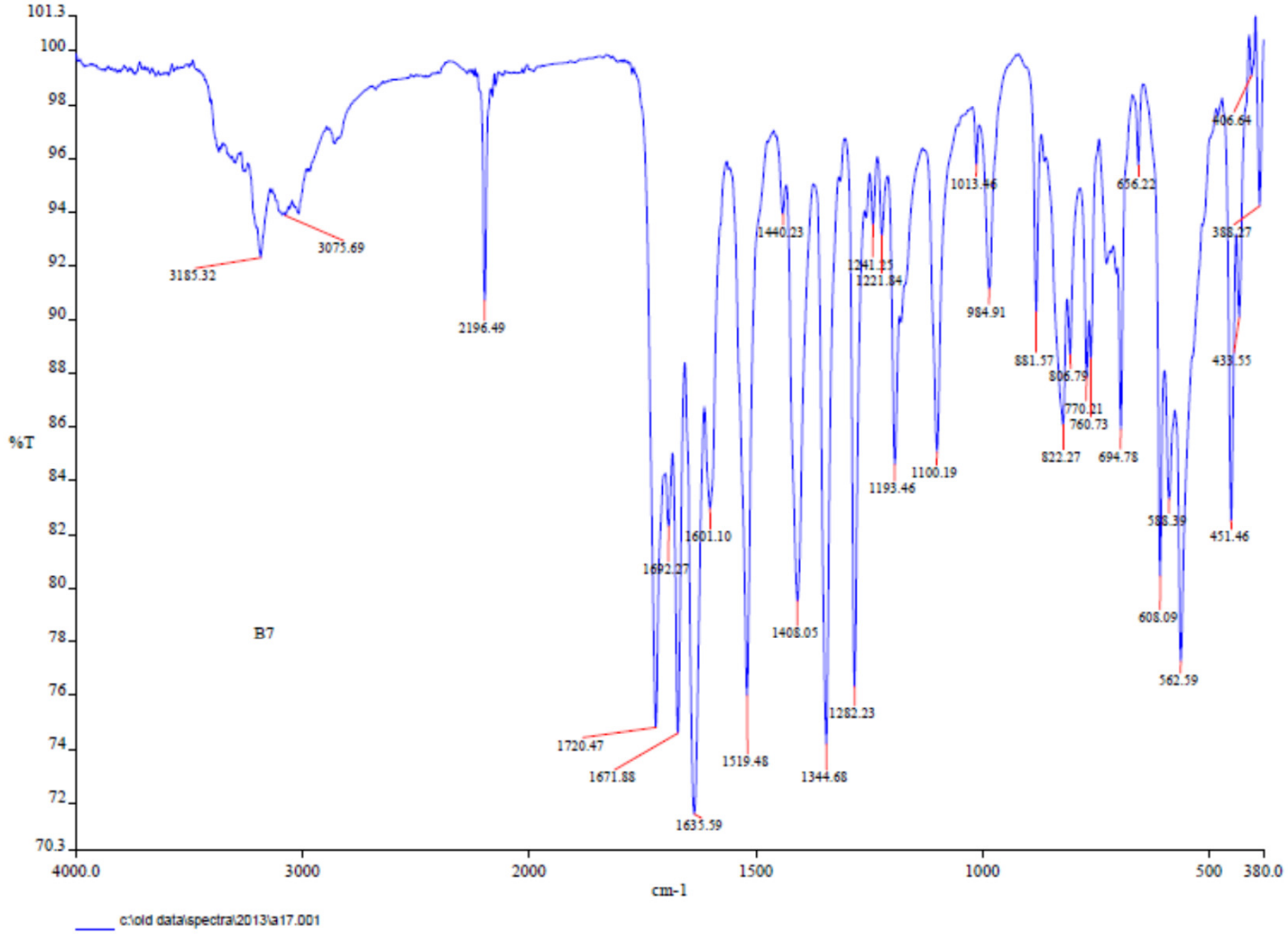
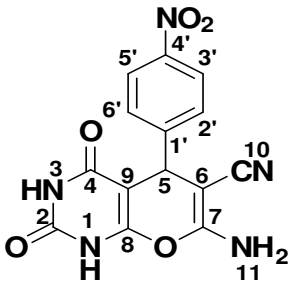
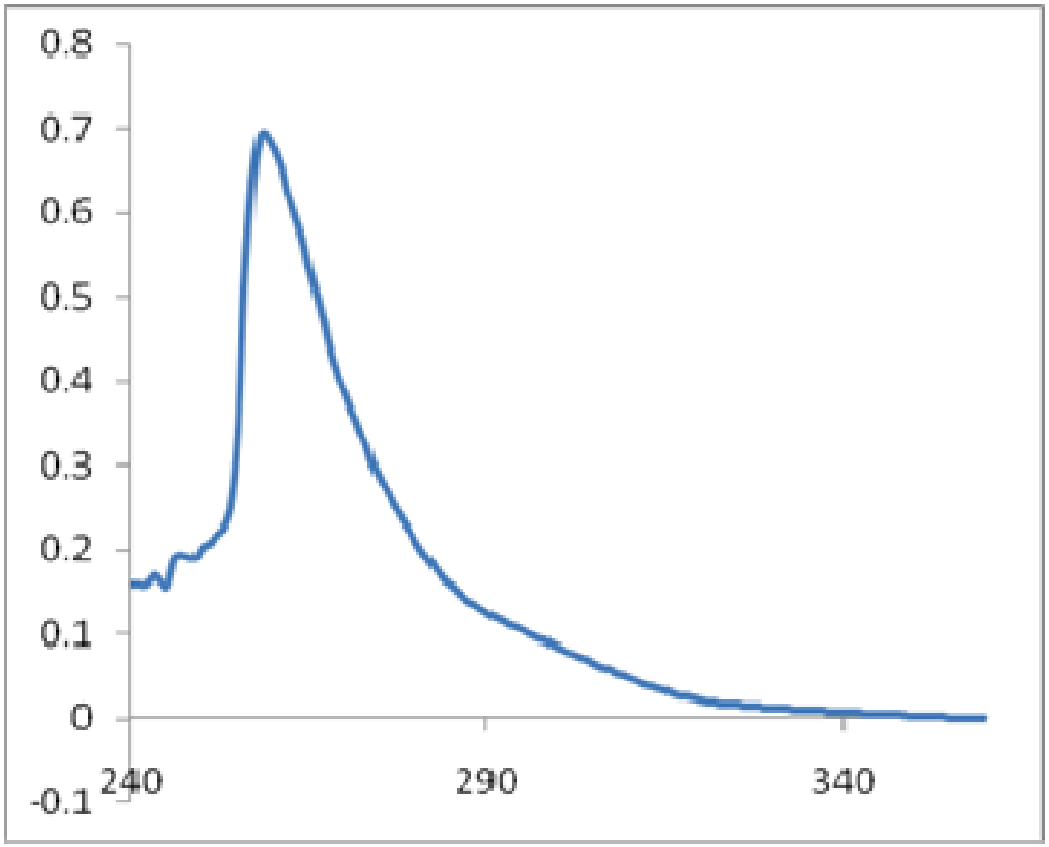


Mass spectrum of 7-amino-5-(4-nitrophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B7)

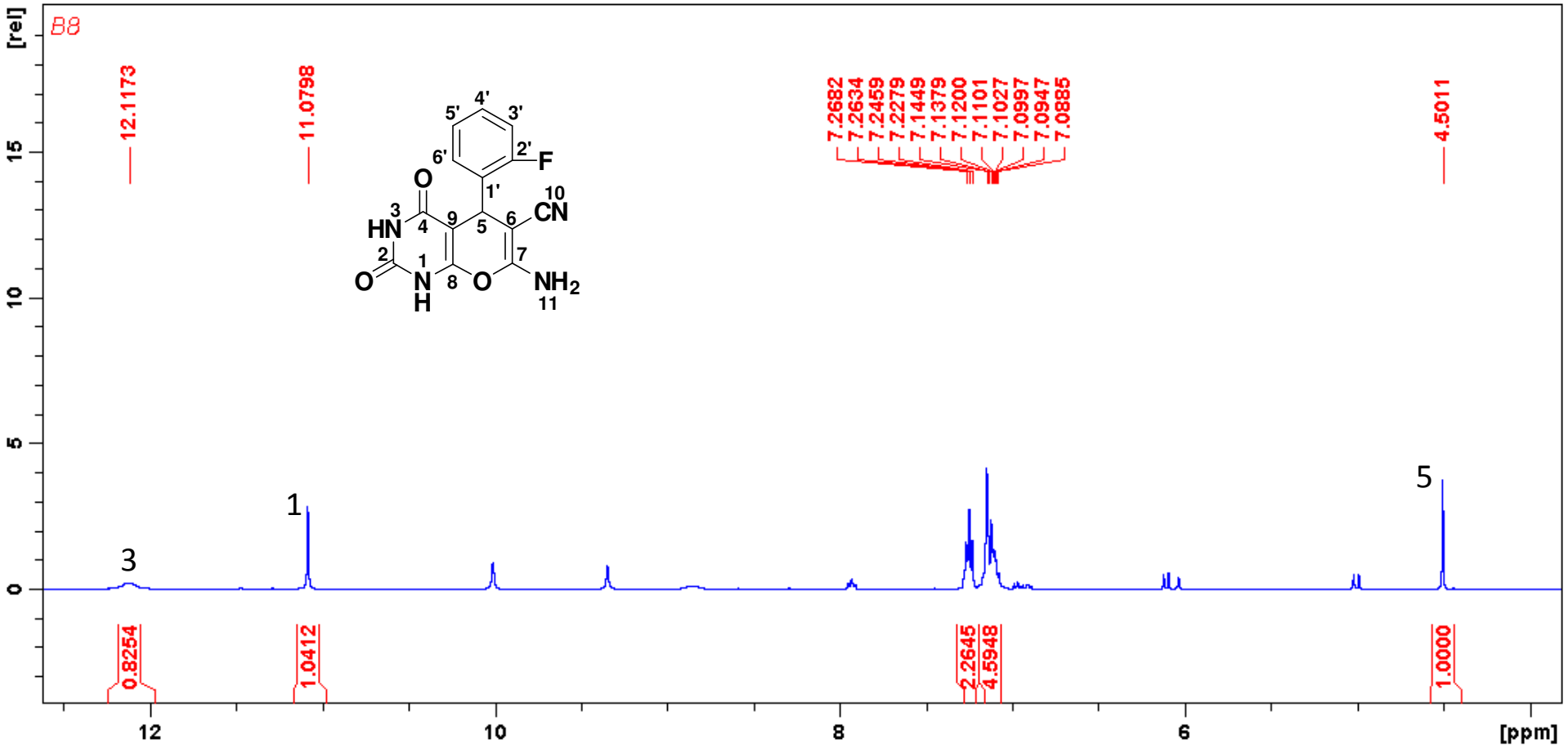


IR spectrum of 7-amino-5-(4-nitrophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B7)

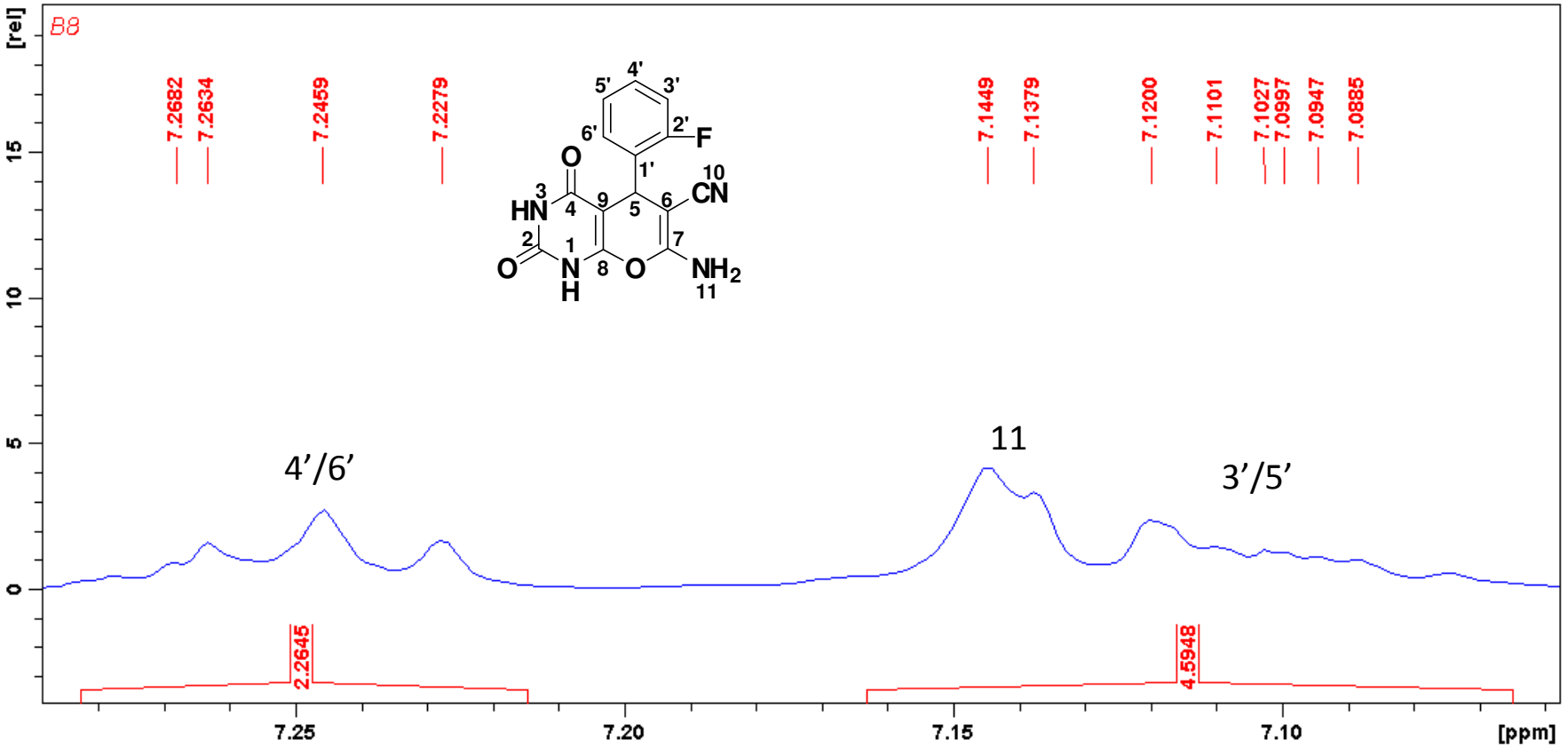




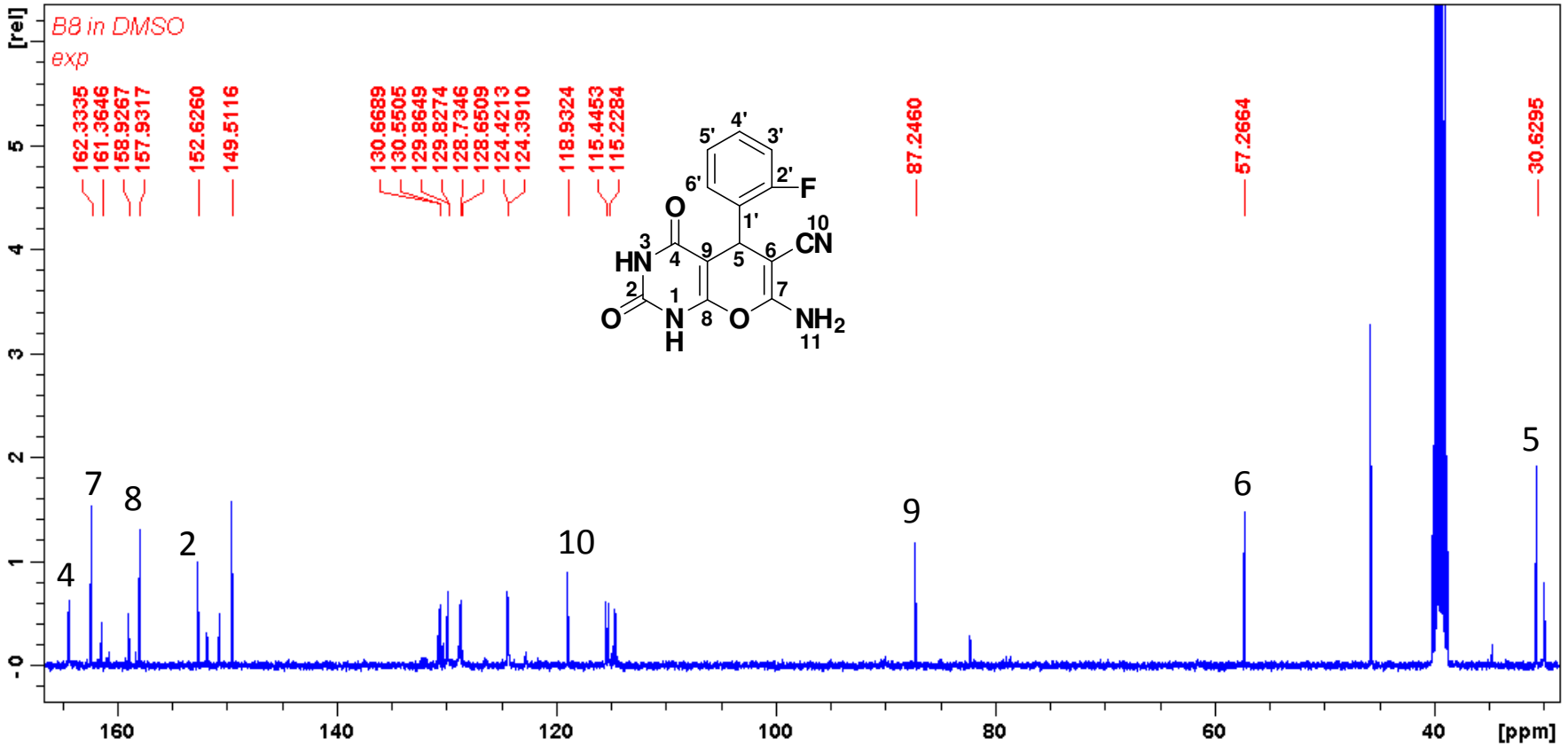
UV spectrum of 7-amino-5-(4-nitrophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B7)



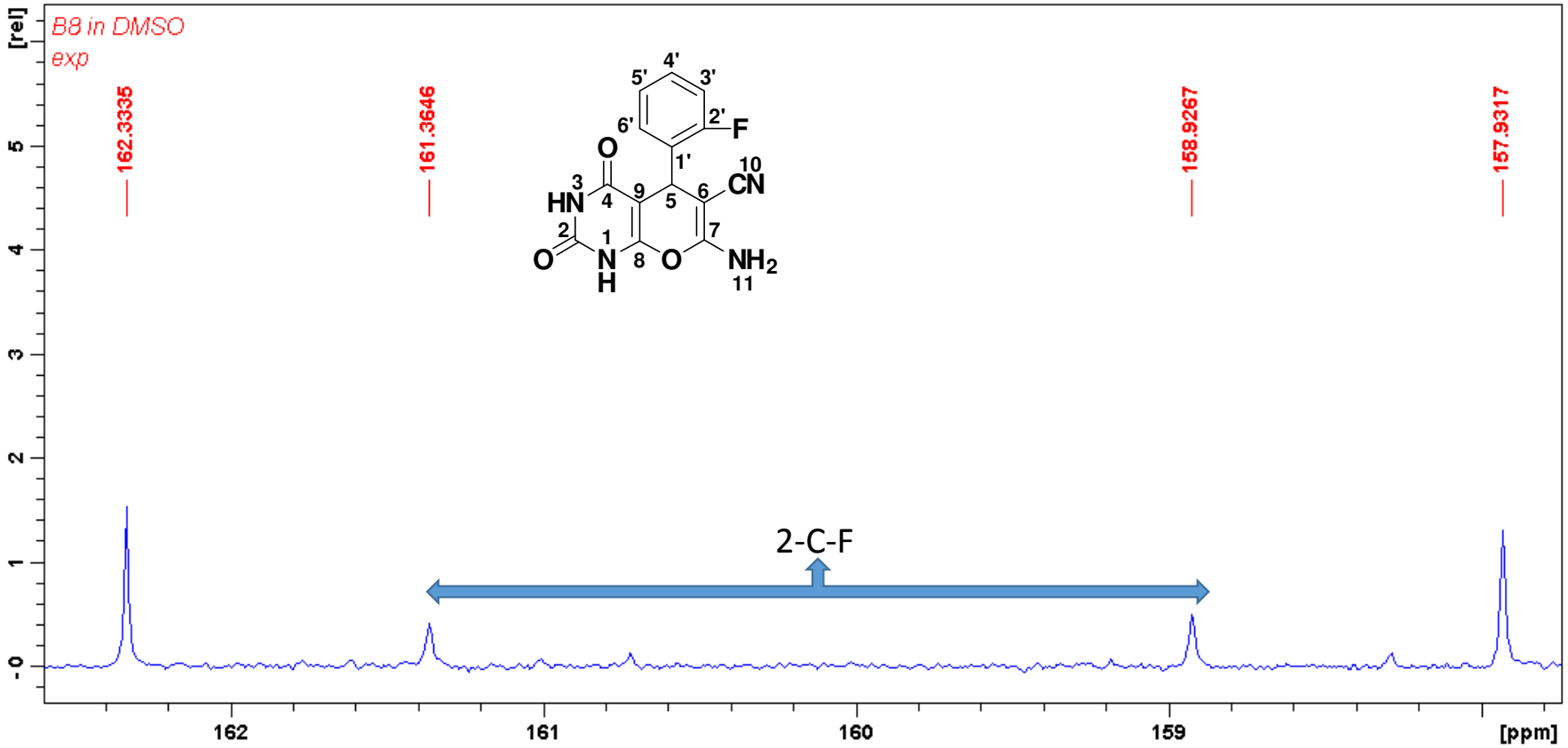
^{1}H NMR spectrum of 7-amino-5-(2-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B8)



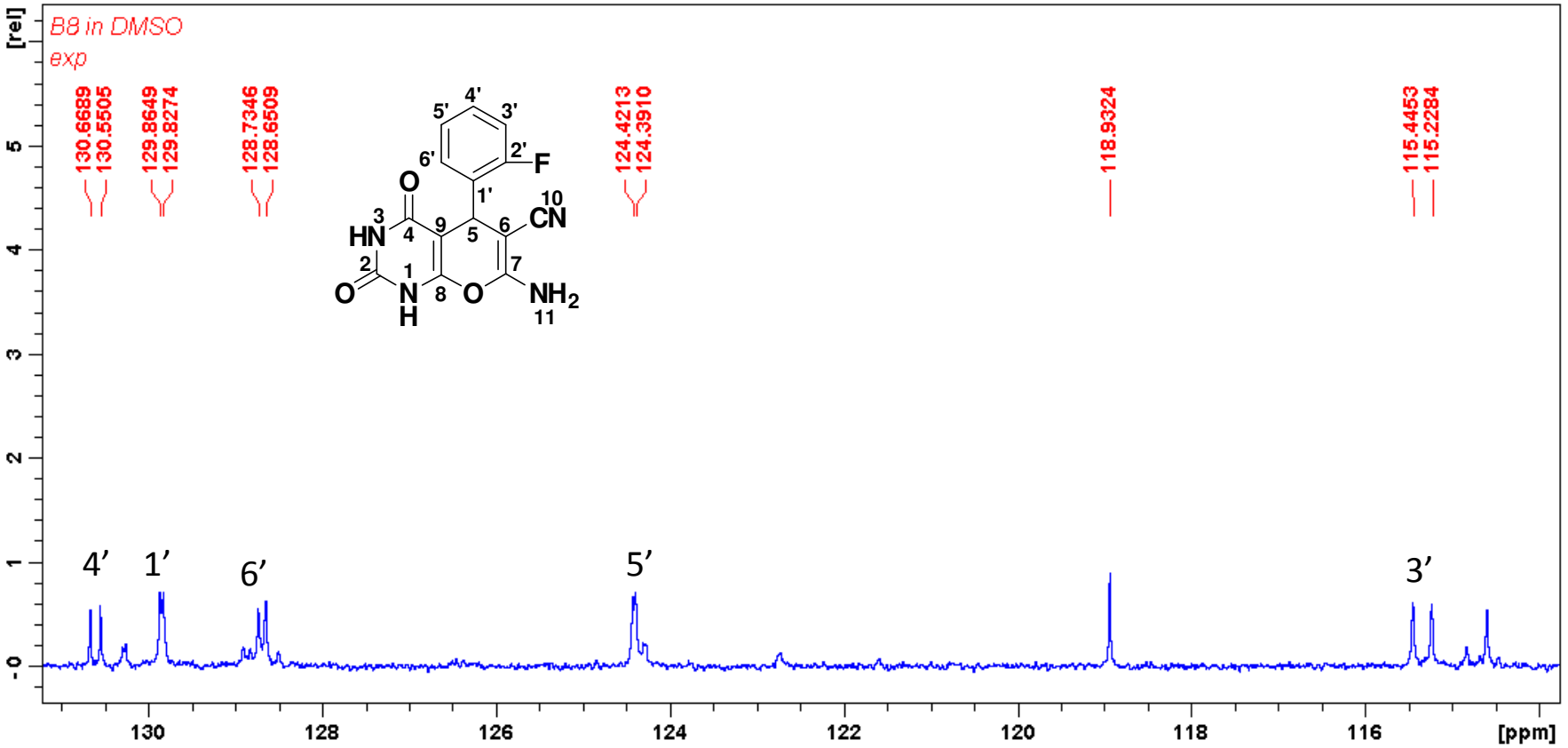
^{1}H NMR spectrum of 7-amino-5-(2-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B8) expanded



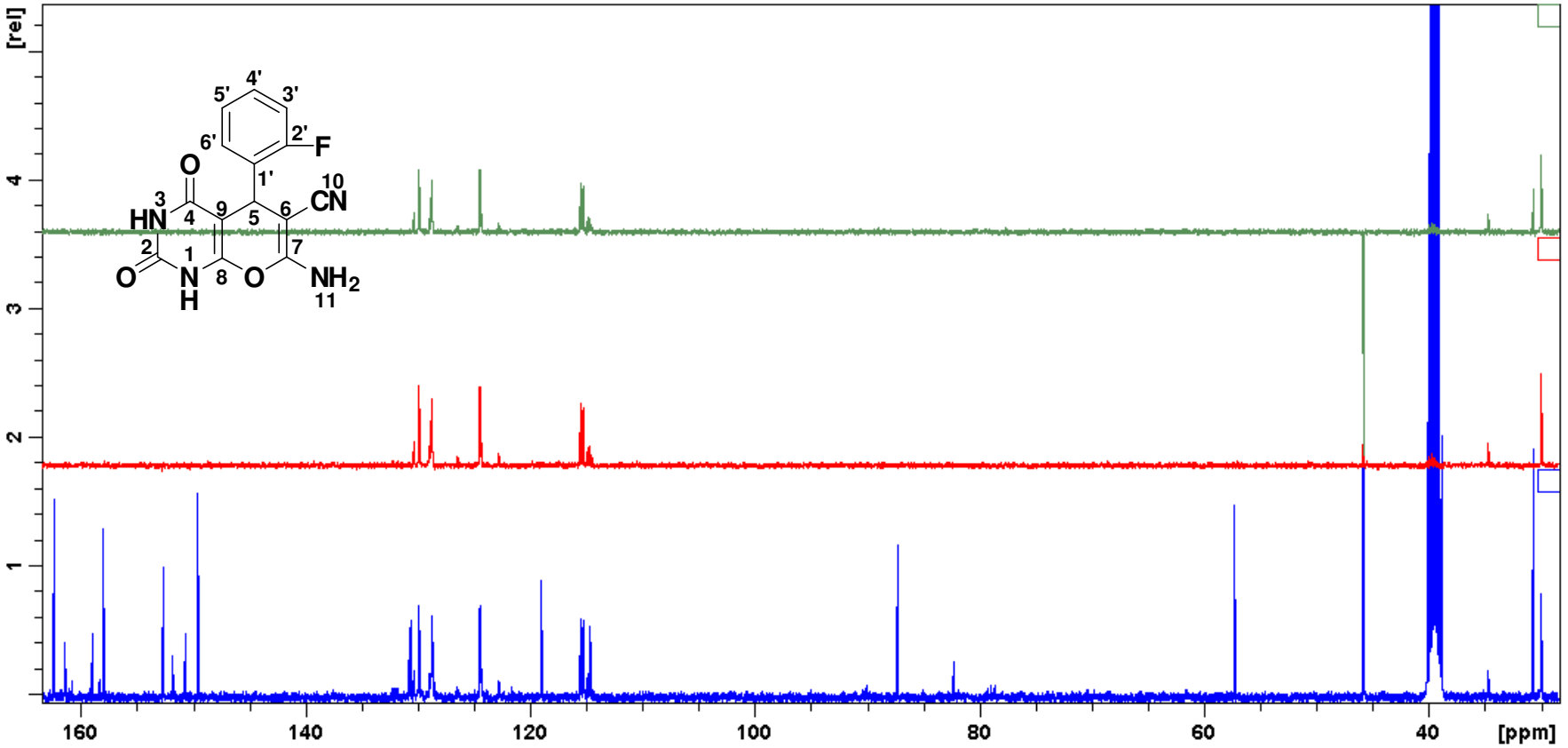
^{13}C NMR spectrum of 7-amino-5-(2-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B8)



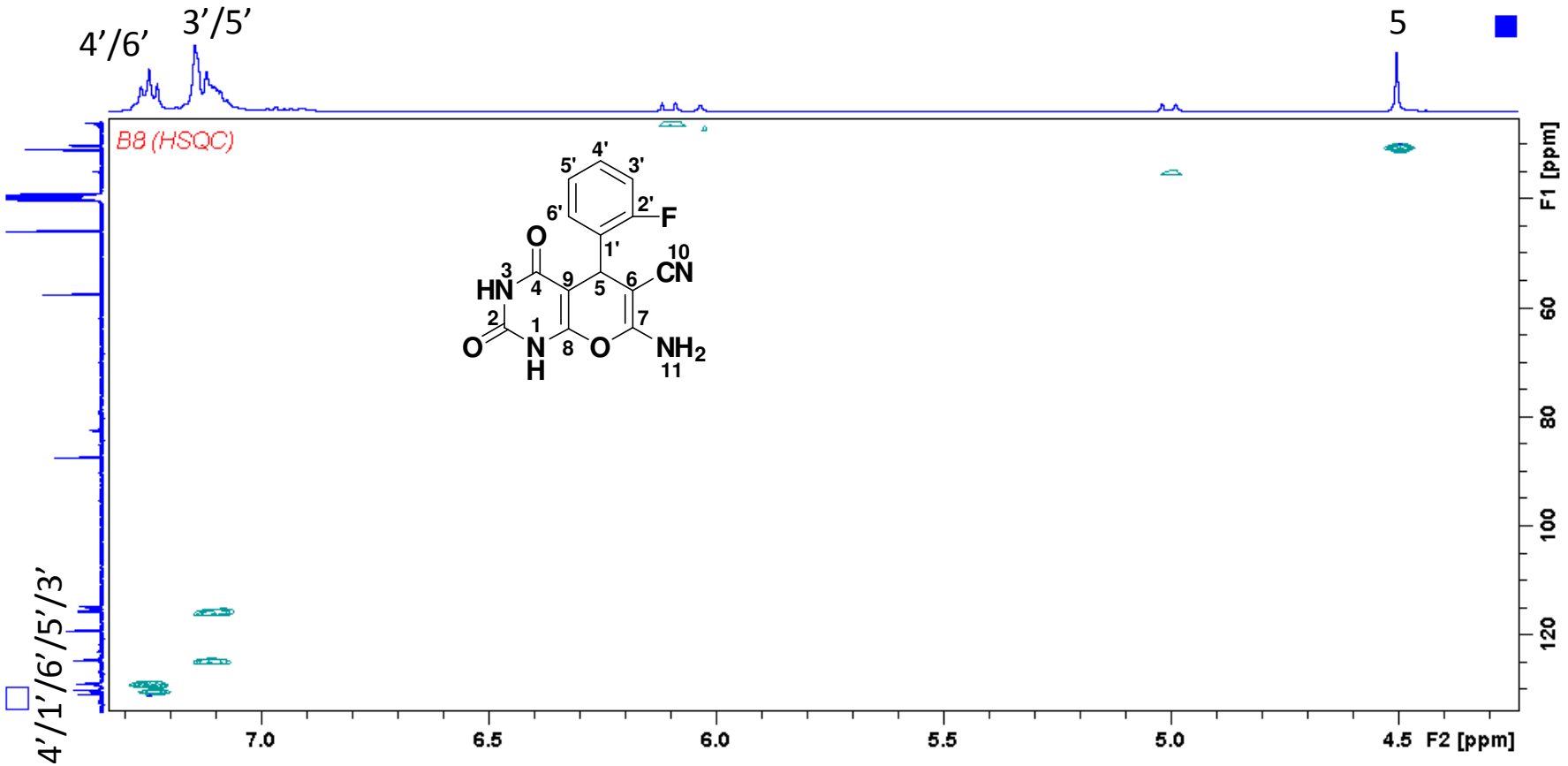
^{13}C NMR spectrum of 7-amino-5-(2-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B8) expanded



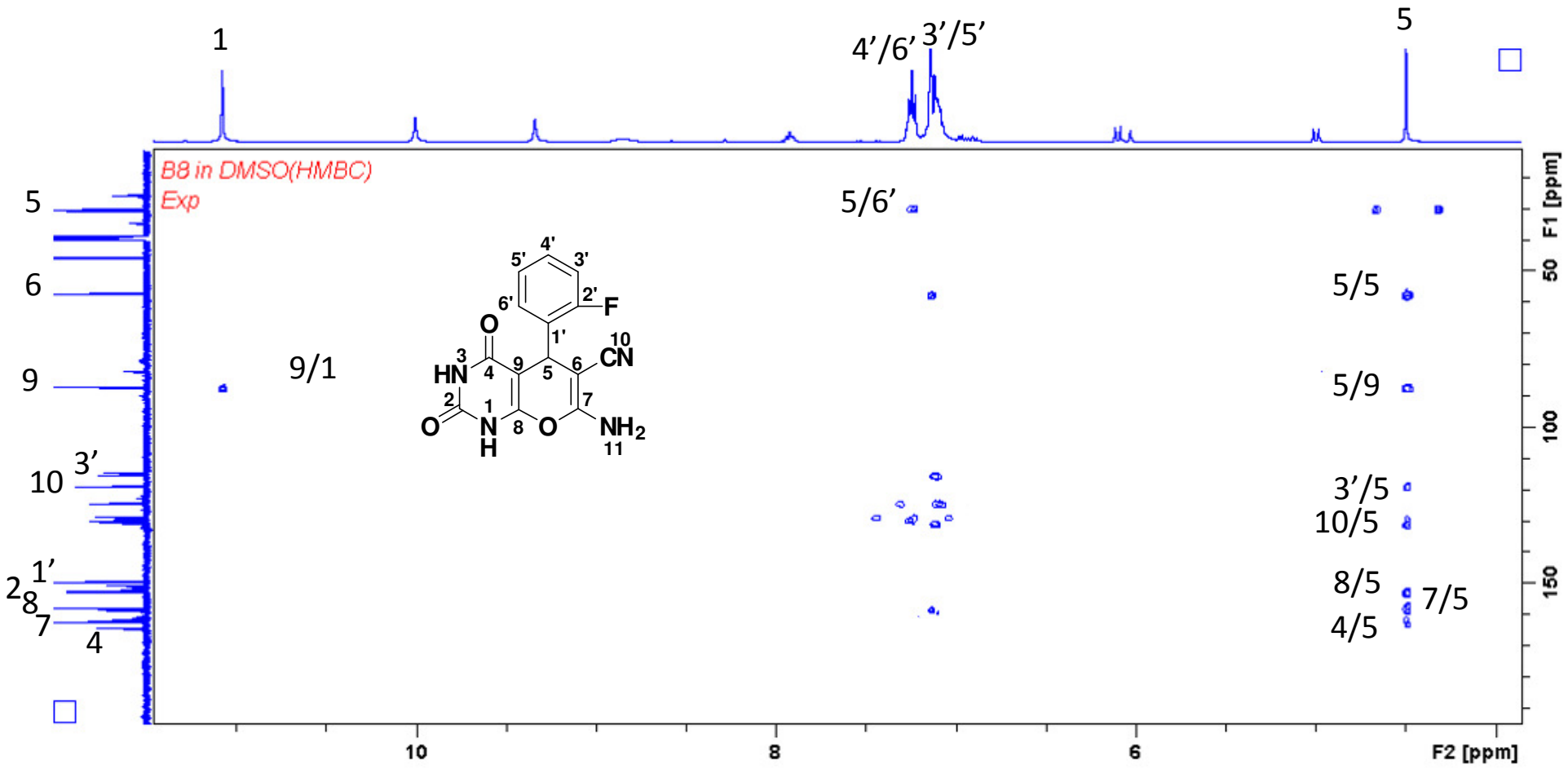
^{13}C NMR spectrum of 7-amino-5-(2-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B8) expanded



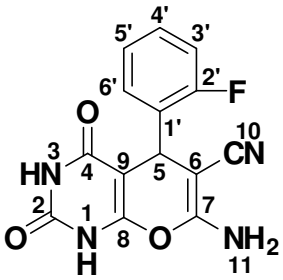
DEPT spectrum of 7-amino-5-(2-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B8)



HSQC spectrum of 7-amino-5-(2-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B8)



HMBC spectrum of 7-amino-5-(2-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B8)

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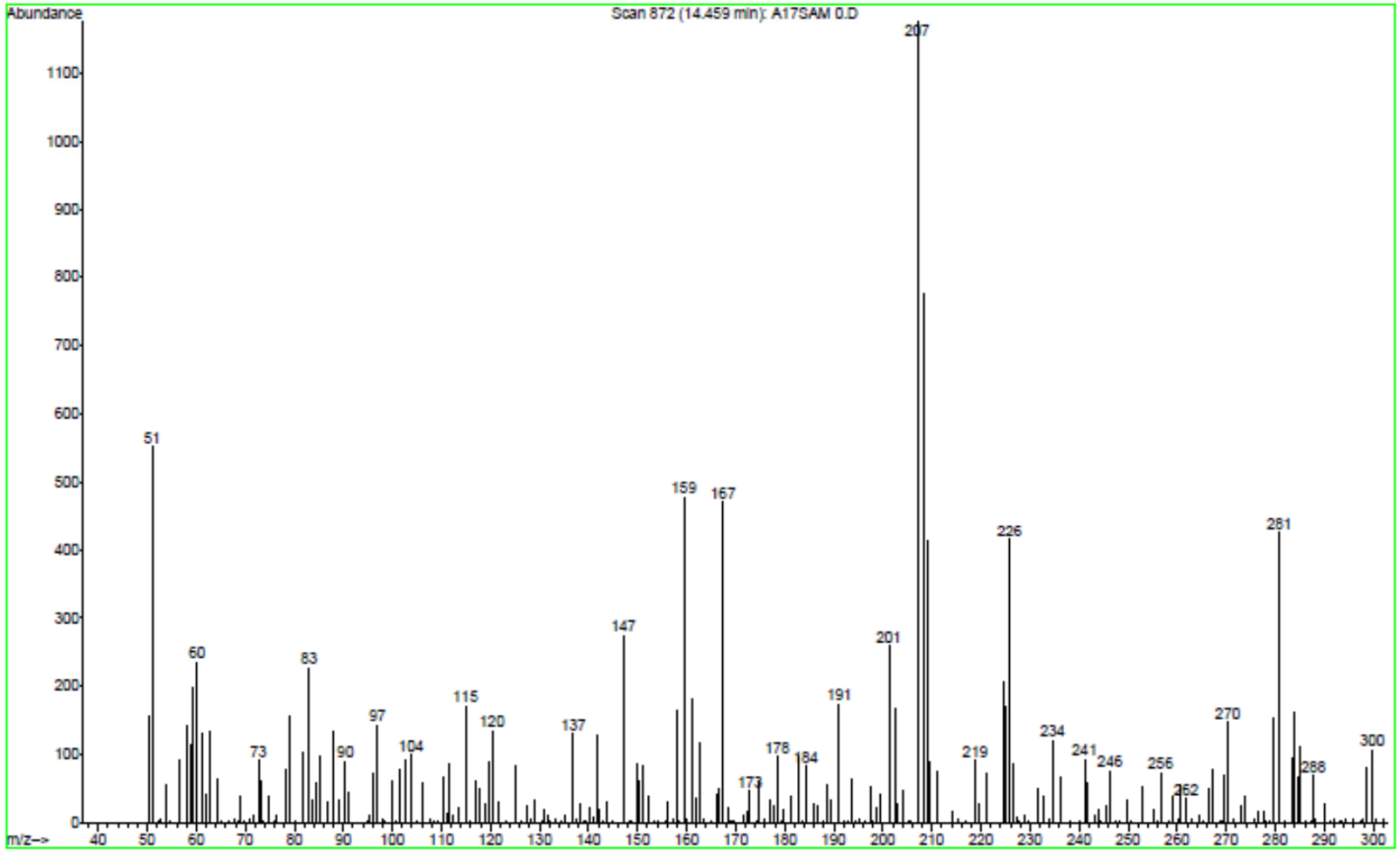
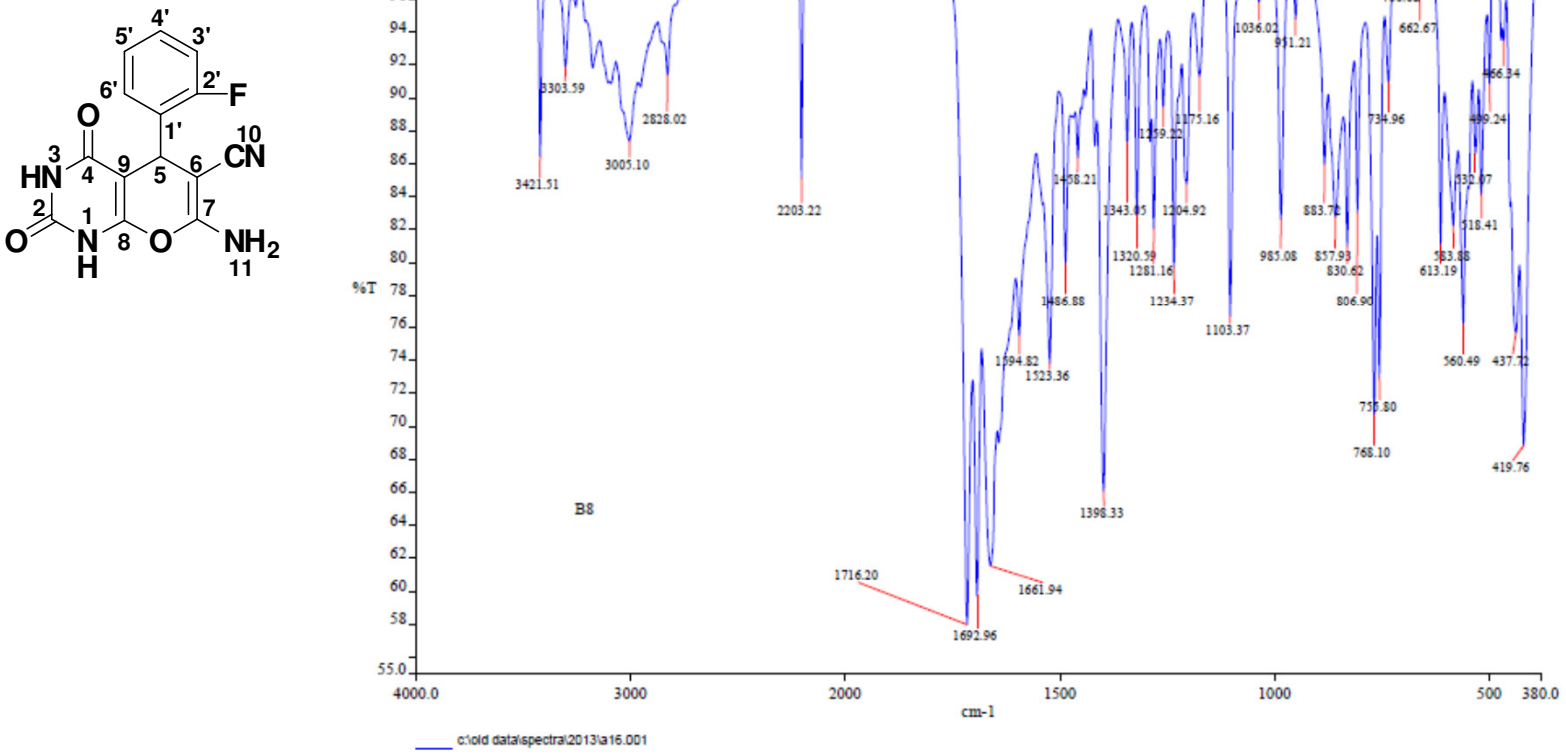
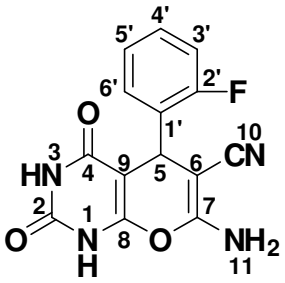
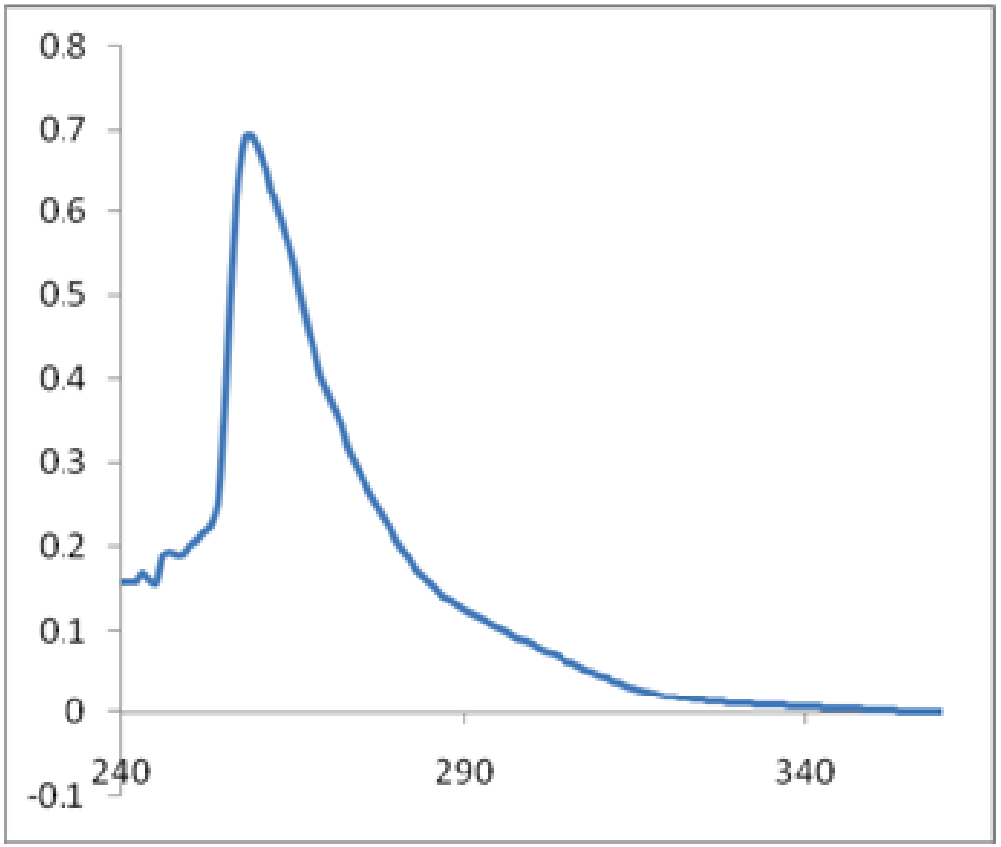


Mass spectrum of 7-amino-5-(2-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B8)

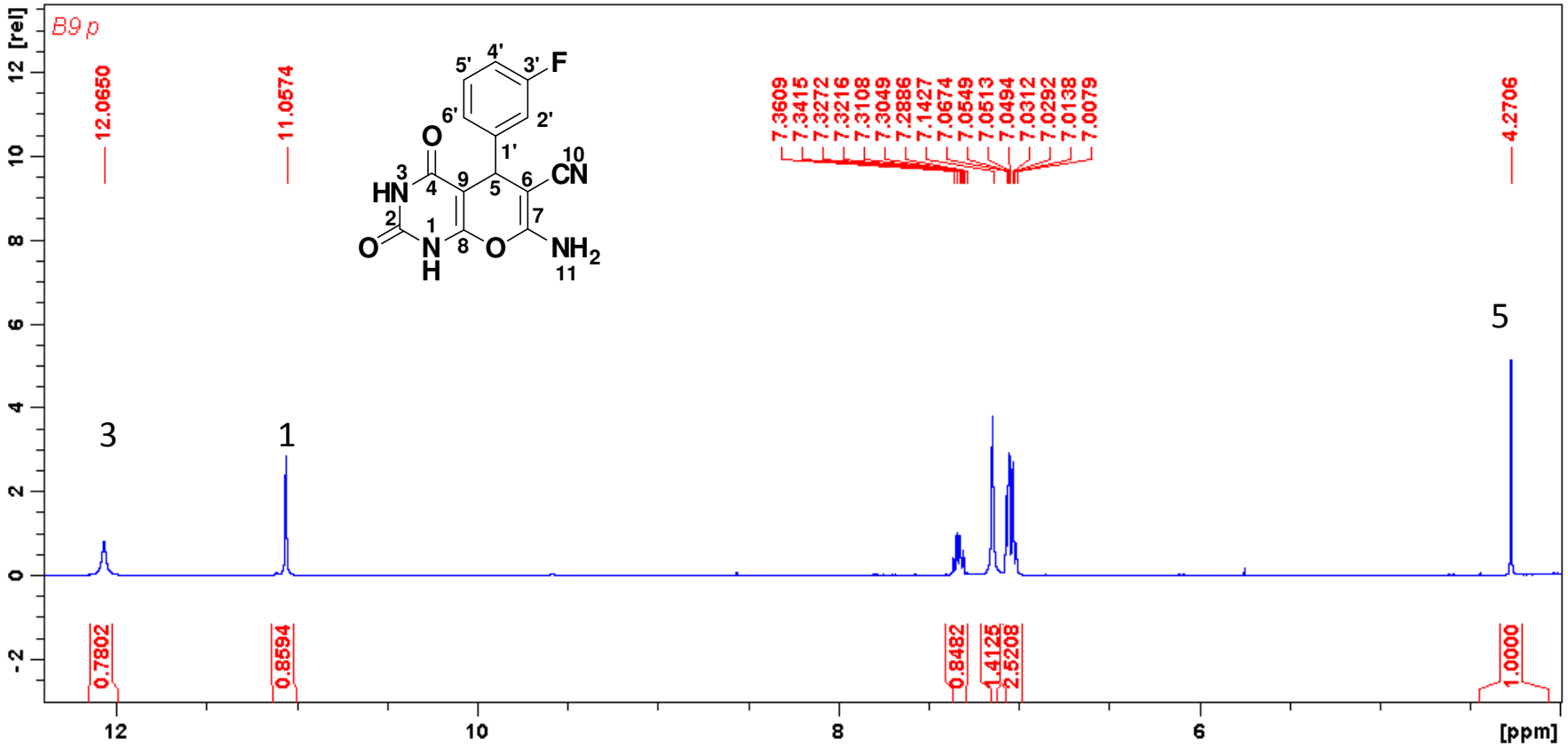


IR spectrum of 7-amino-5-(2-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B8)

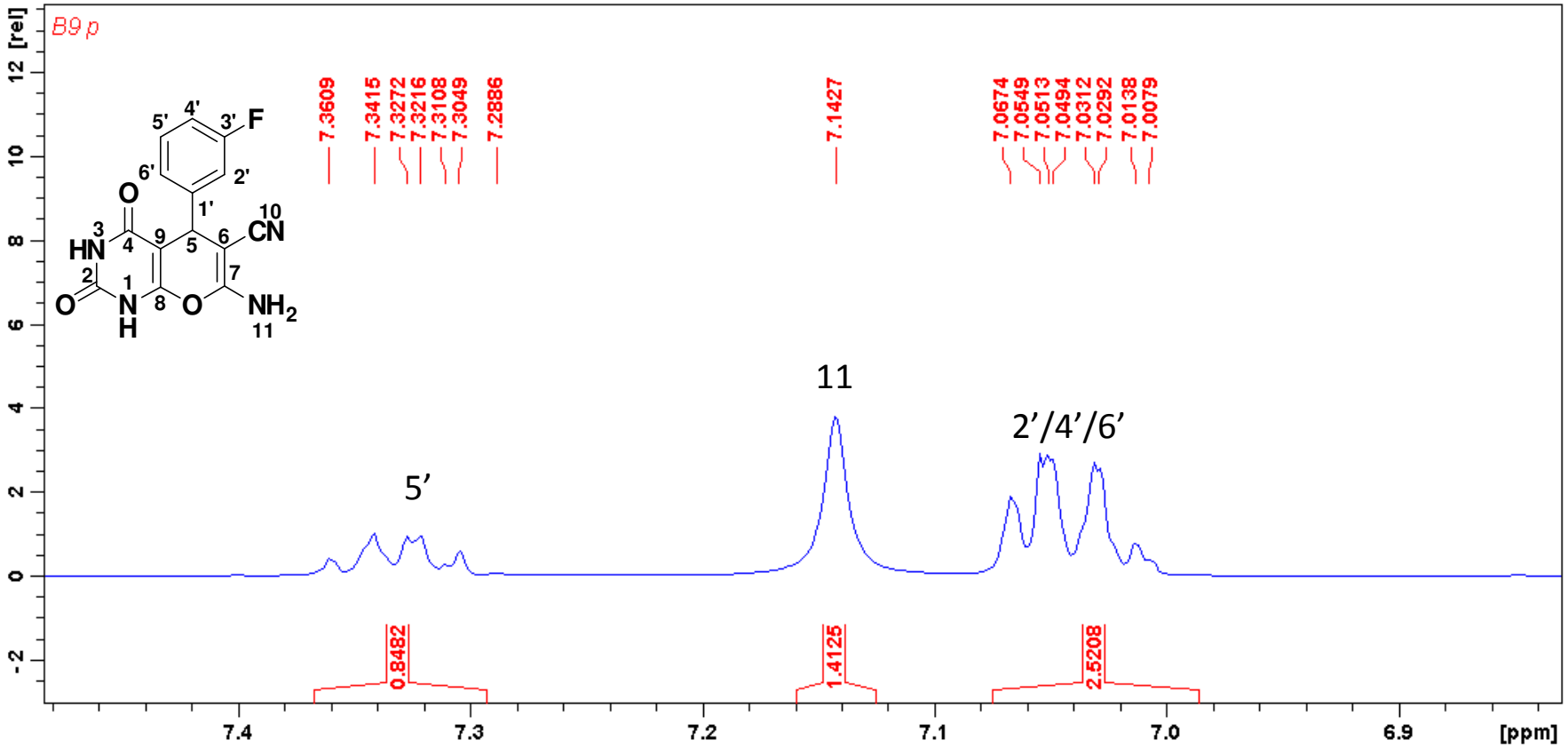




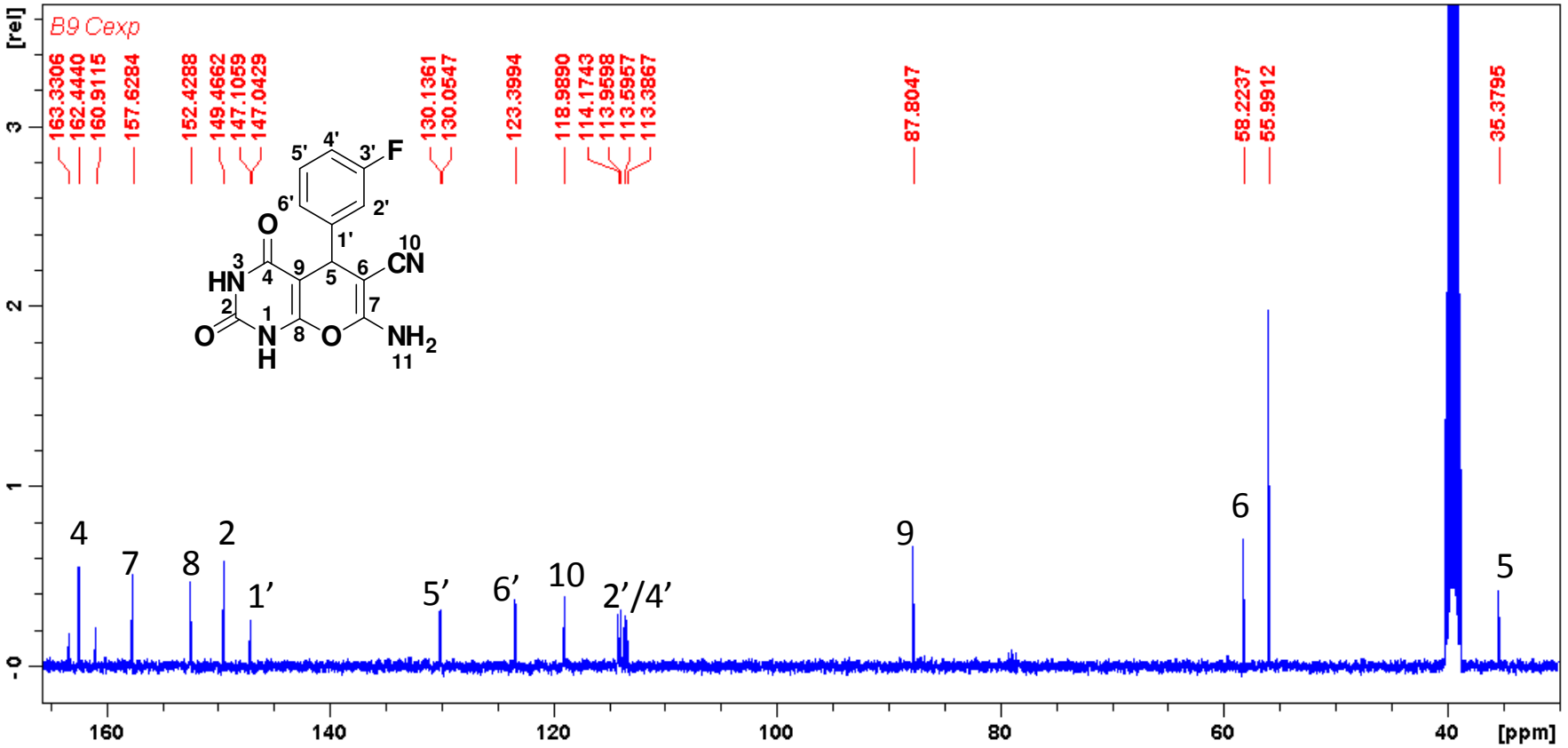
UV spectrum of 7-amino-5-(2-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B8)



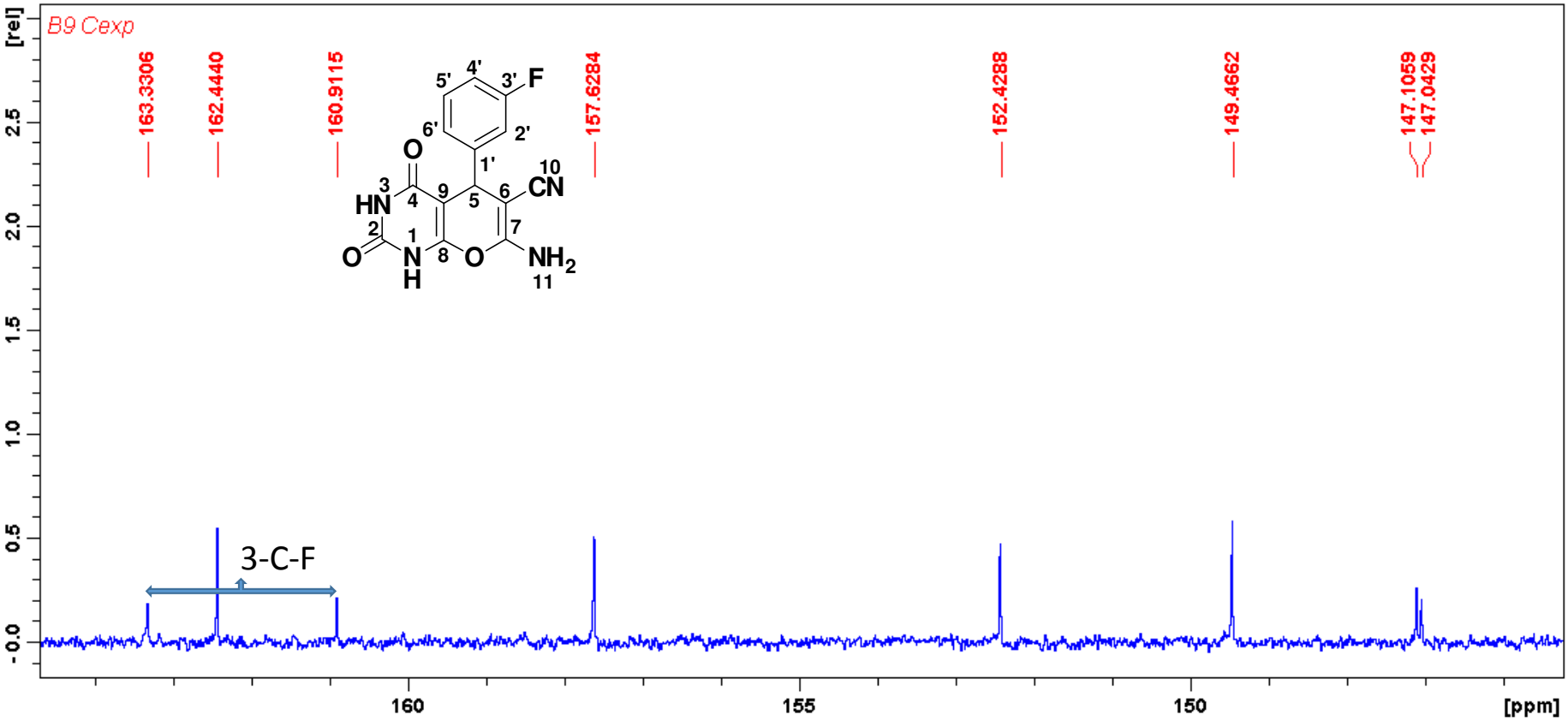
^{1}H NMR spectrum of 7-amino-5-(3-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B9)



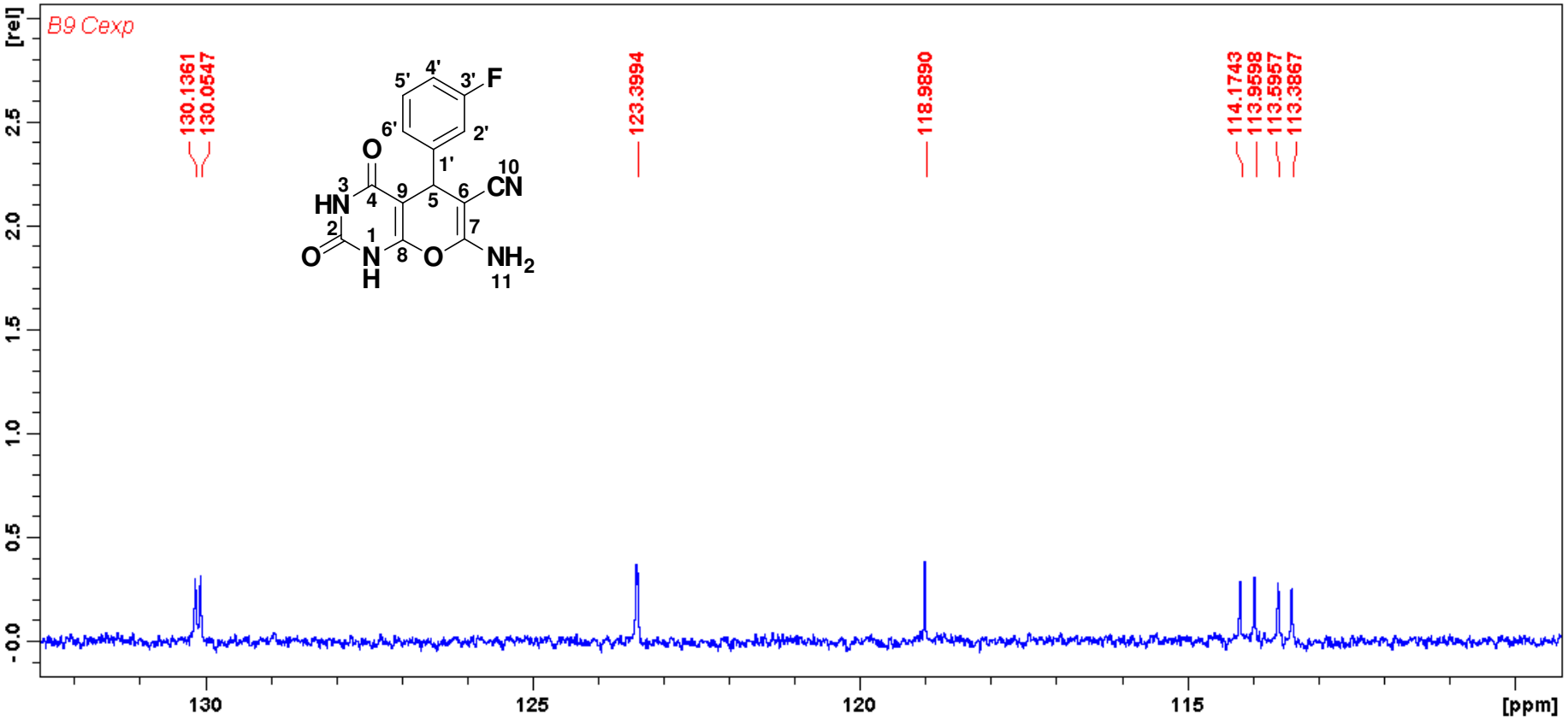
^{1}H NMR spectrum of 7-amino-5-(2-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B9) expanded



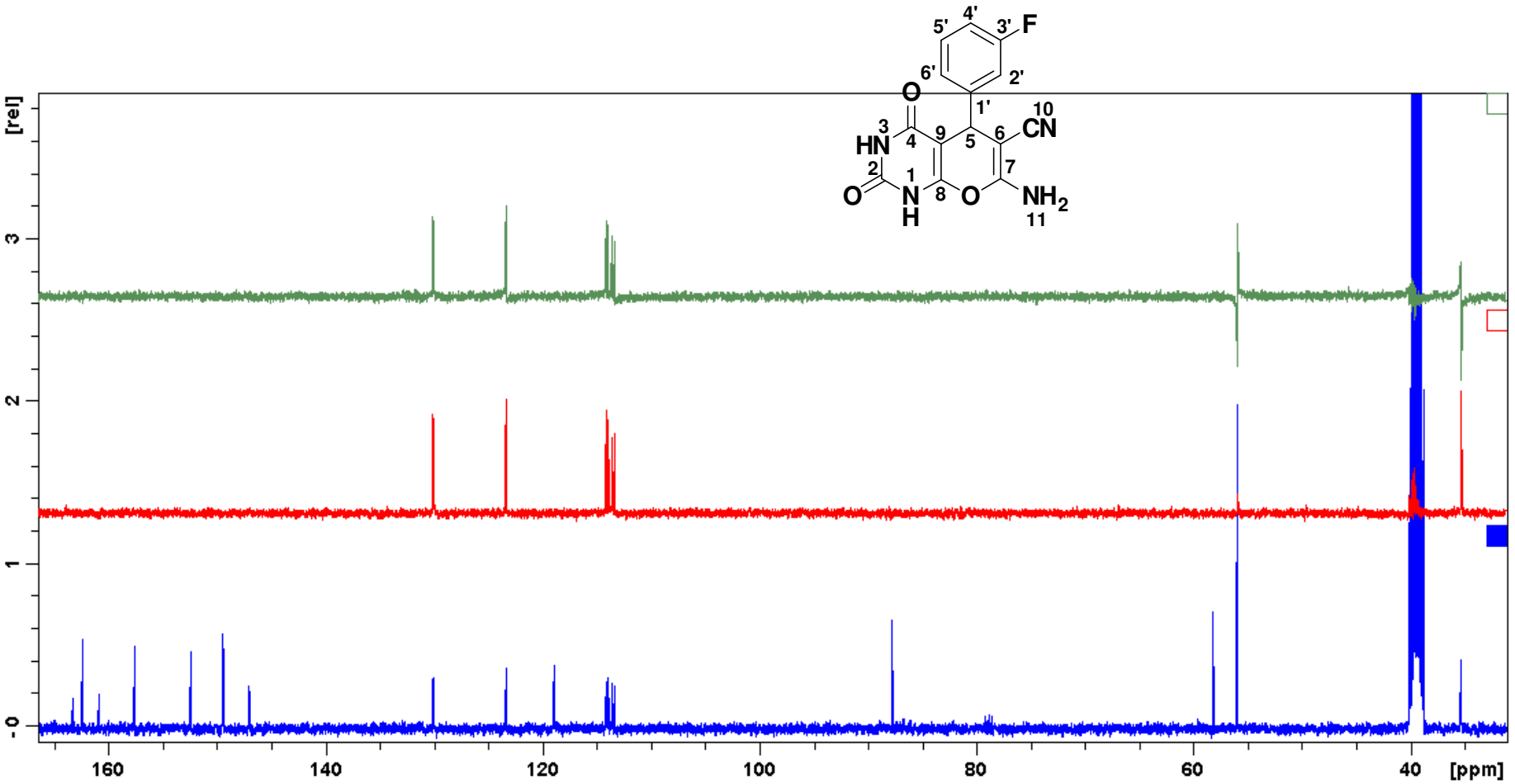
^{13}C NMR spectrum of 7-amino-5-(3-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B9)



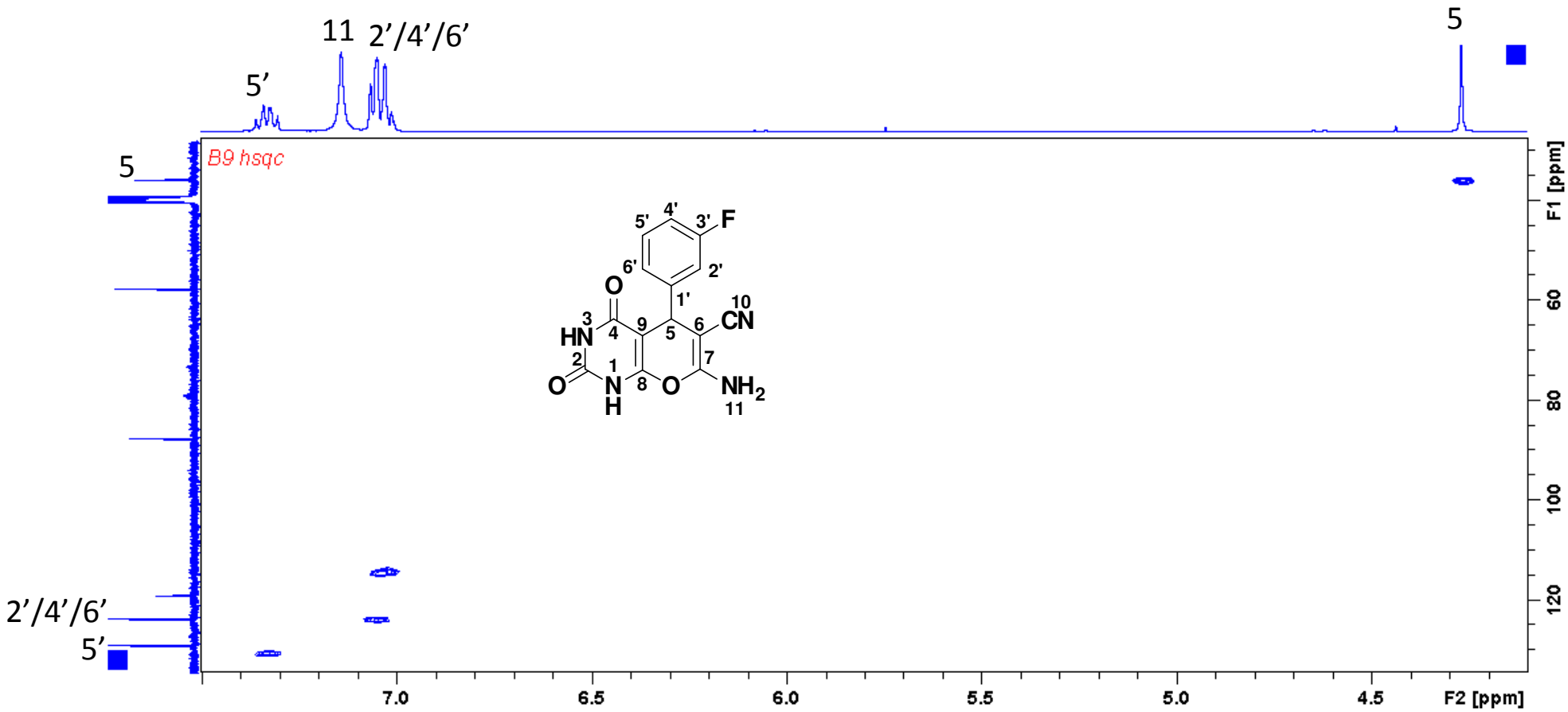
^{13}C NMR spectrum of 7-amino-5-(3-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B9) expanded



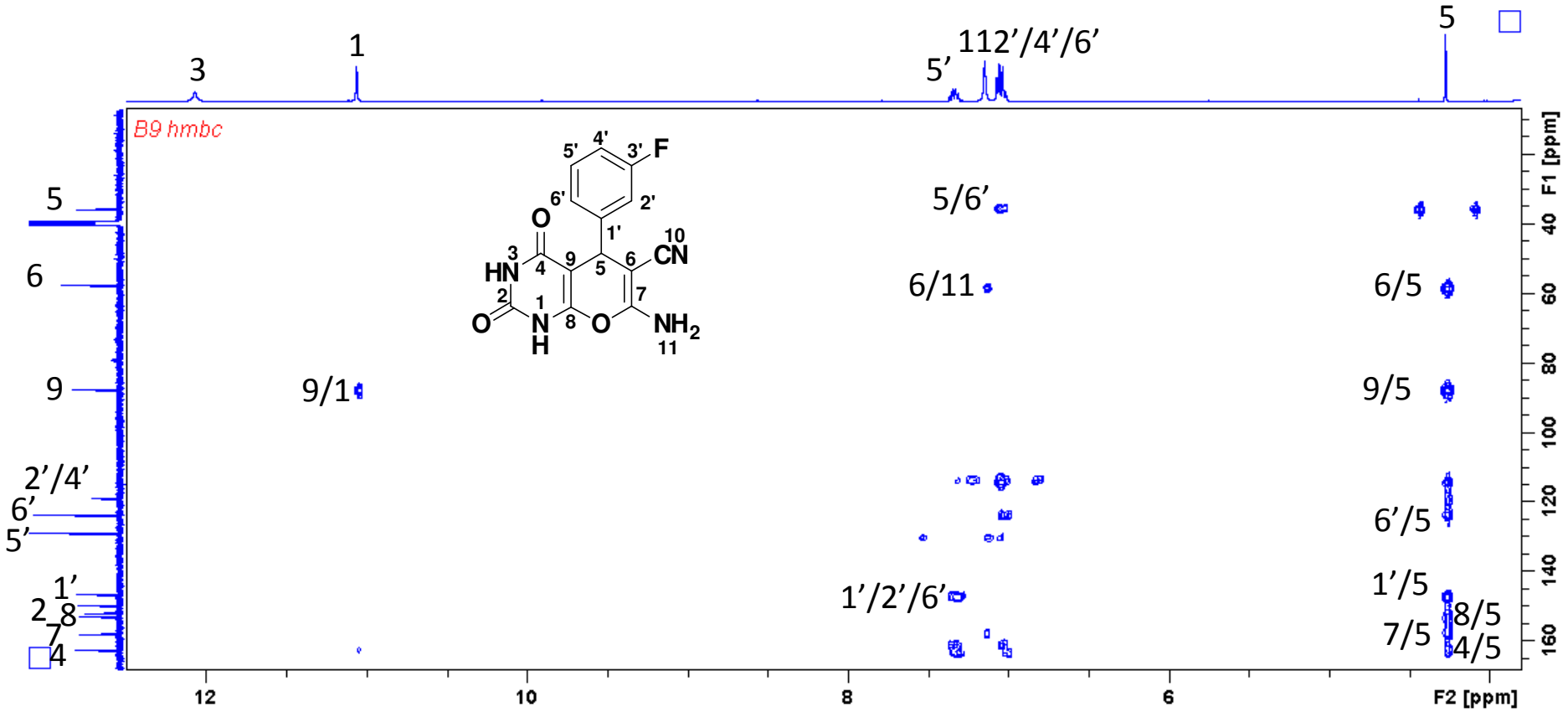
^{13}C NMR spectrum of 7-amino-5-(3-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B9) expanded



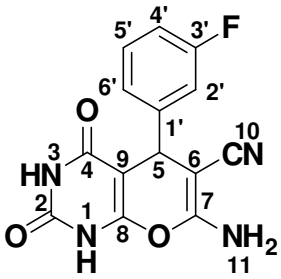
DEPT spectrum of 7-amino-5-(3-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B9)



HSQC spectrum of 7-amino-5-(3-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B9)



HMBC spectrum of 7-amino-5-(3-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B9)



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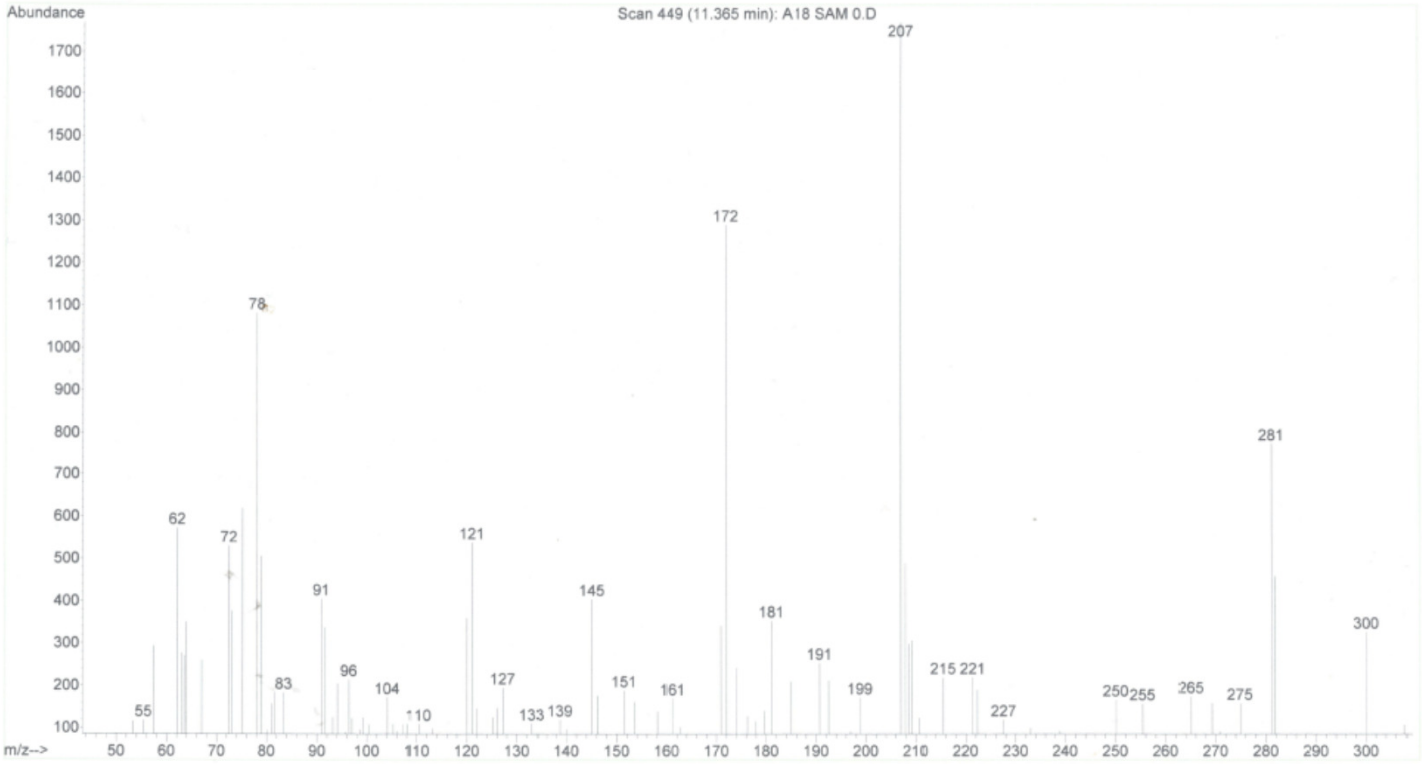
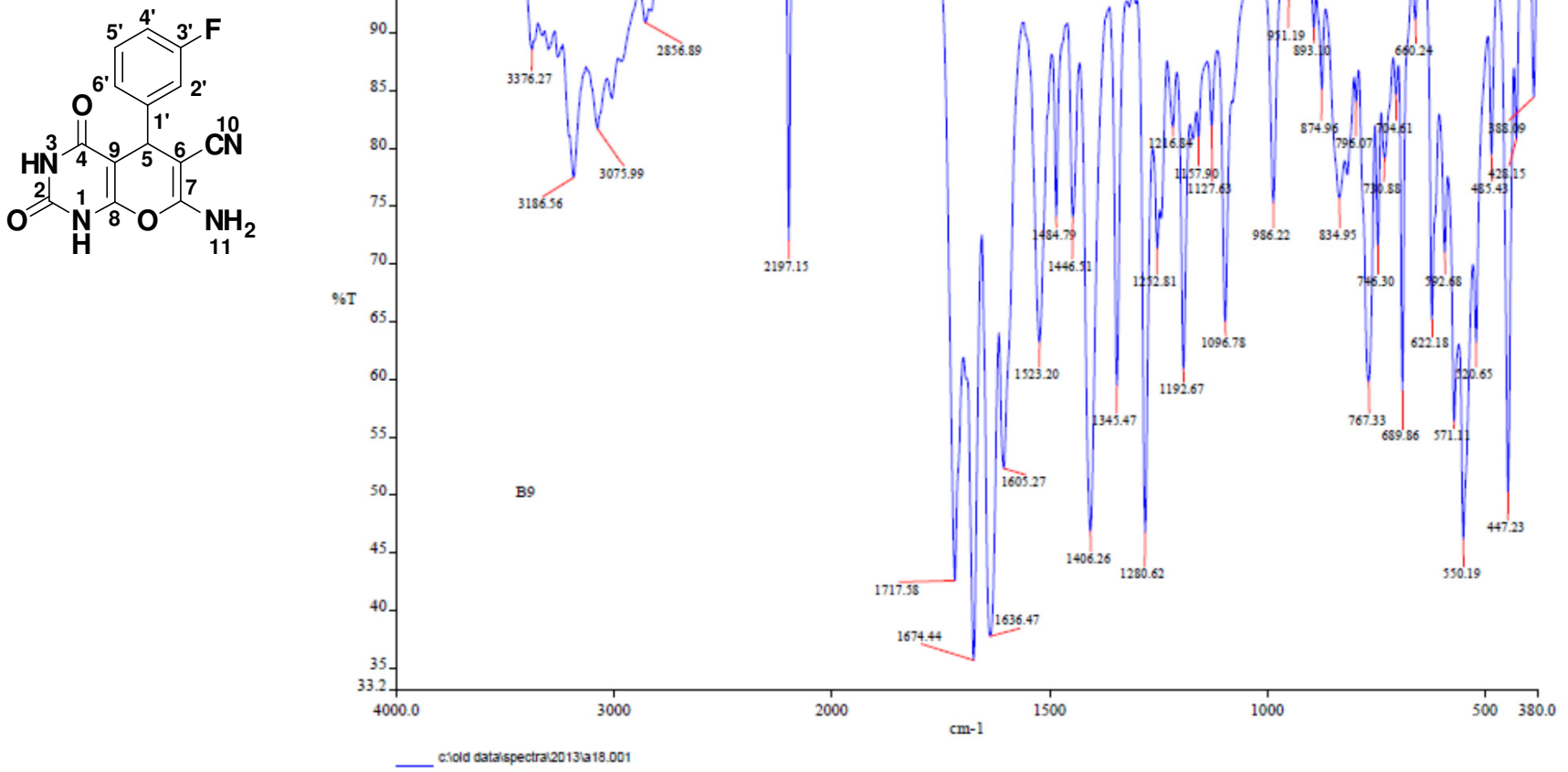
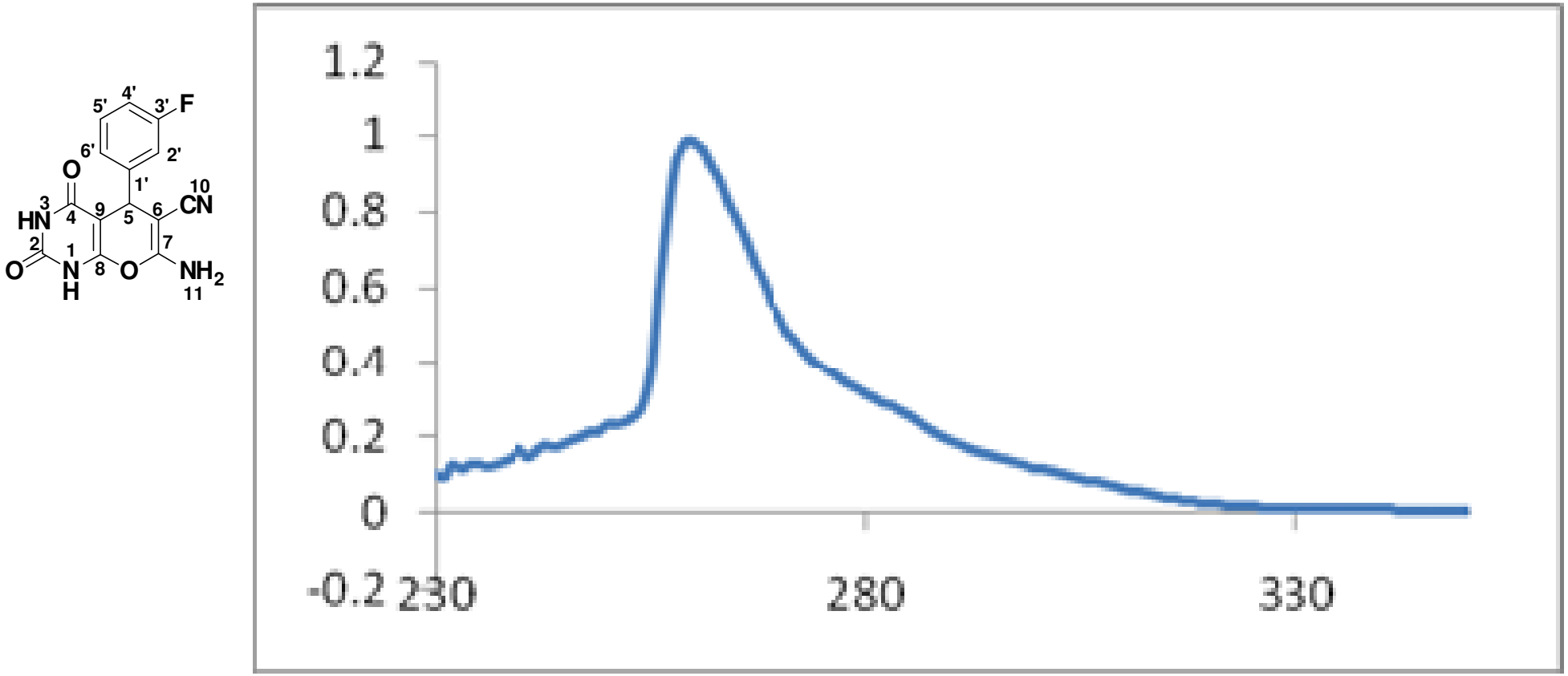


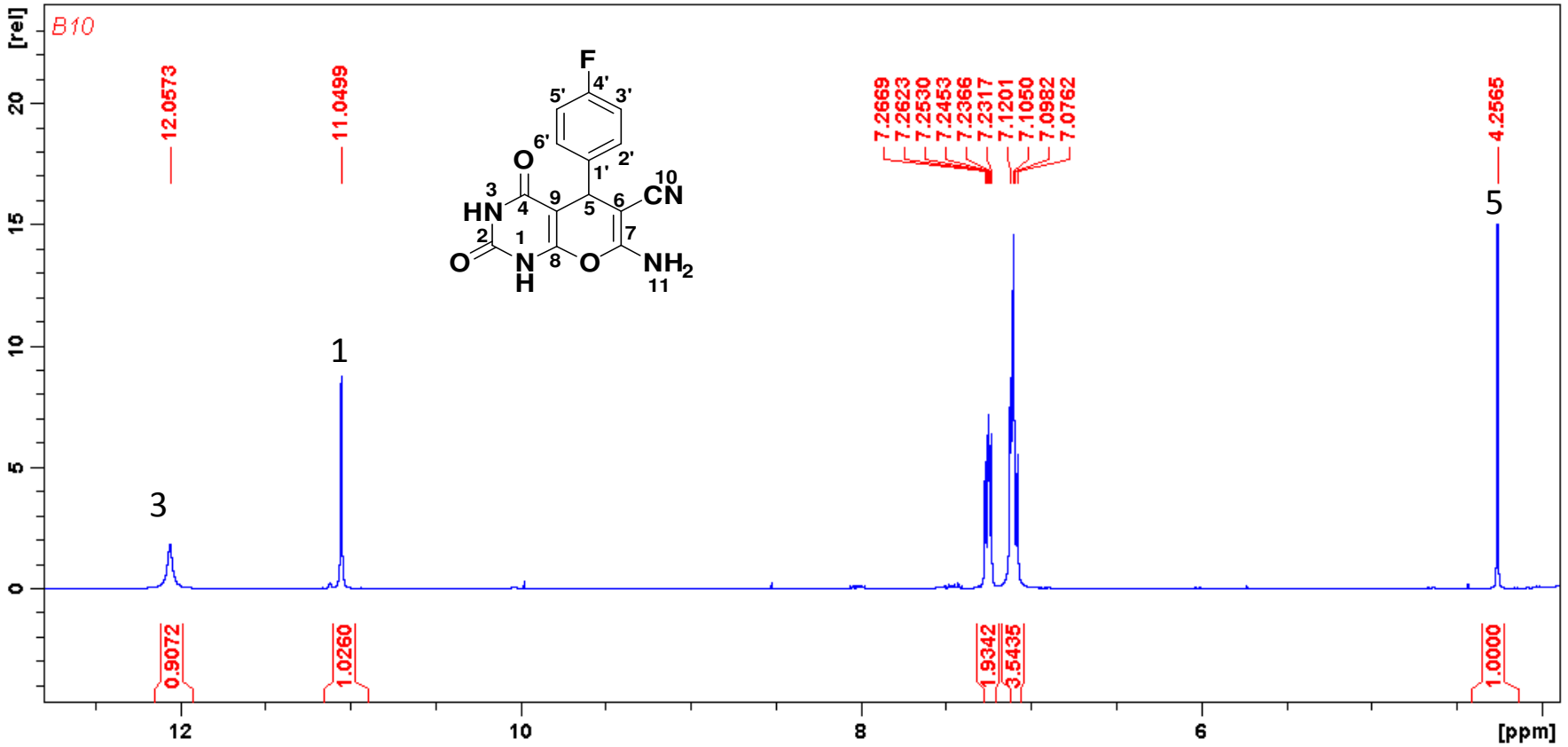
Mass spectrum of 7-amino-5-(3-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B9)



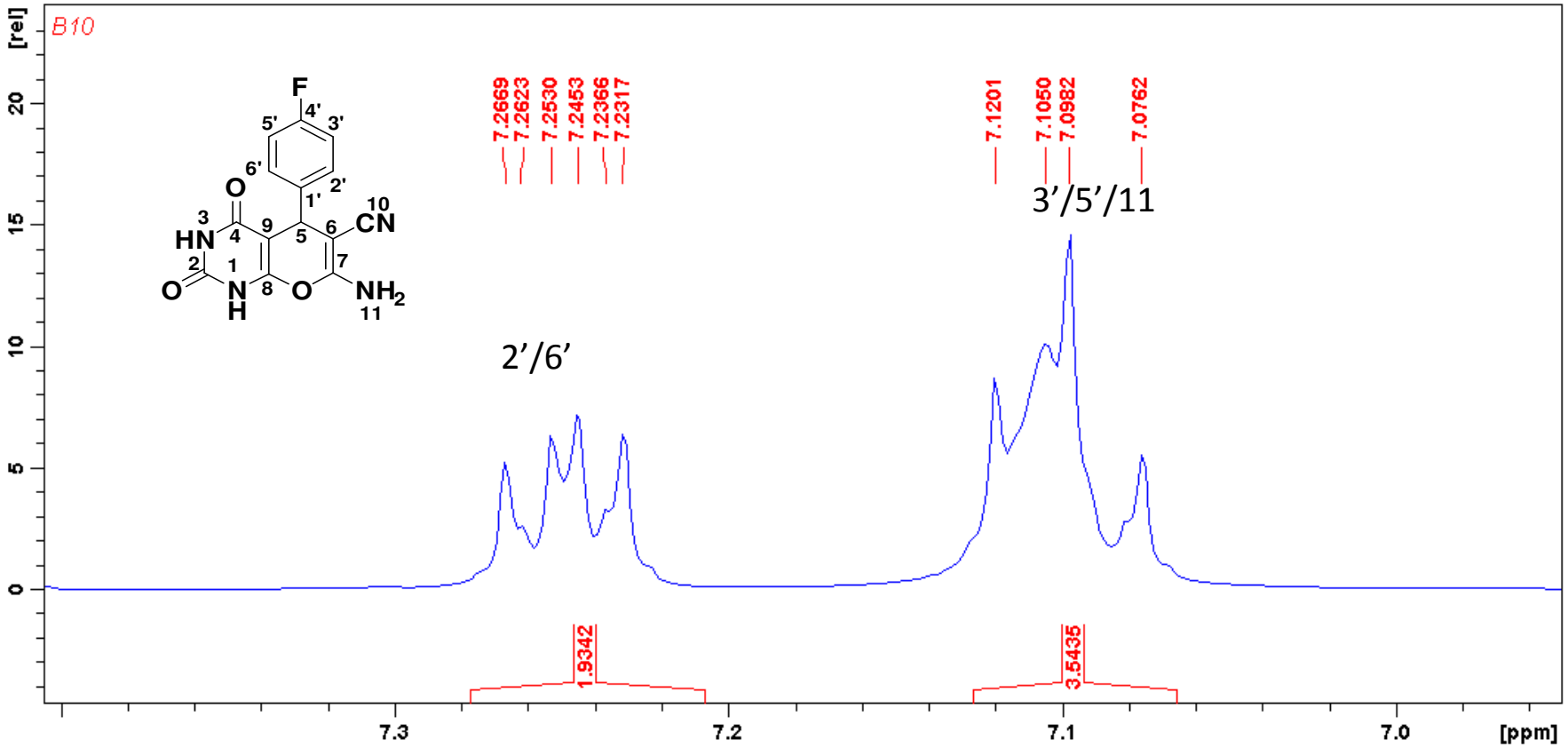
IR spectrum of 7-amino-5-(3-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B9)



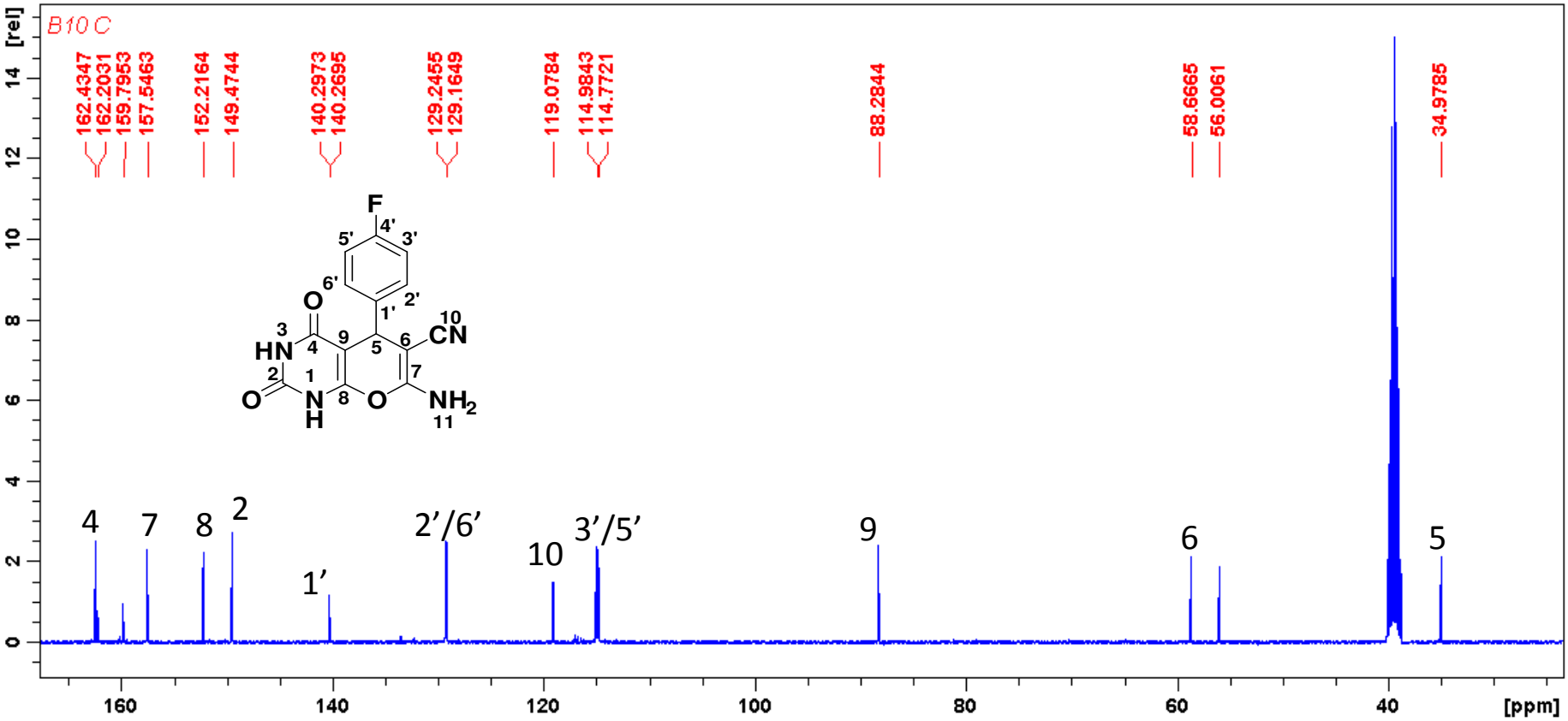
UV spectrum of 7-amino-5-(3-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-diones (B9)



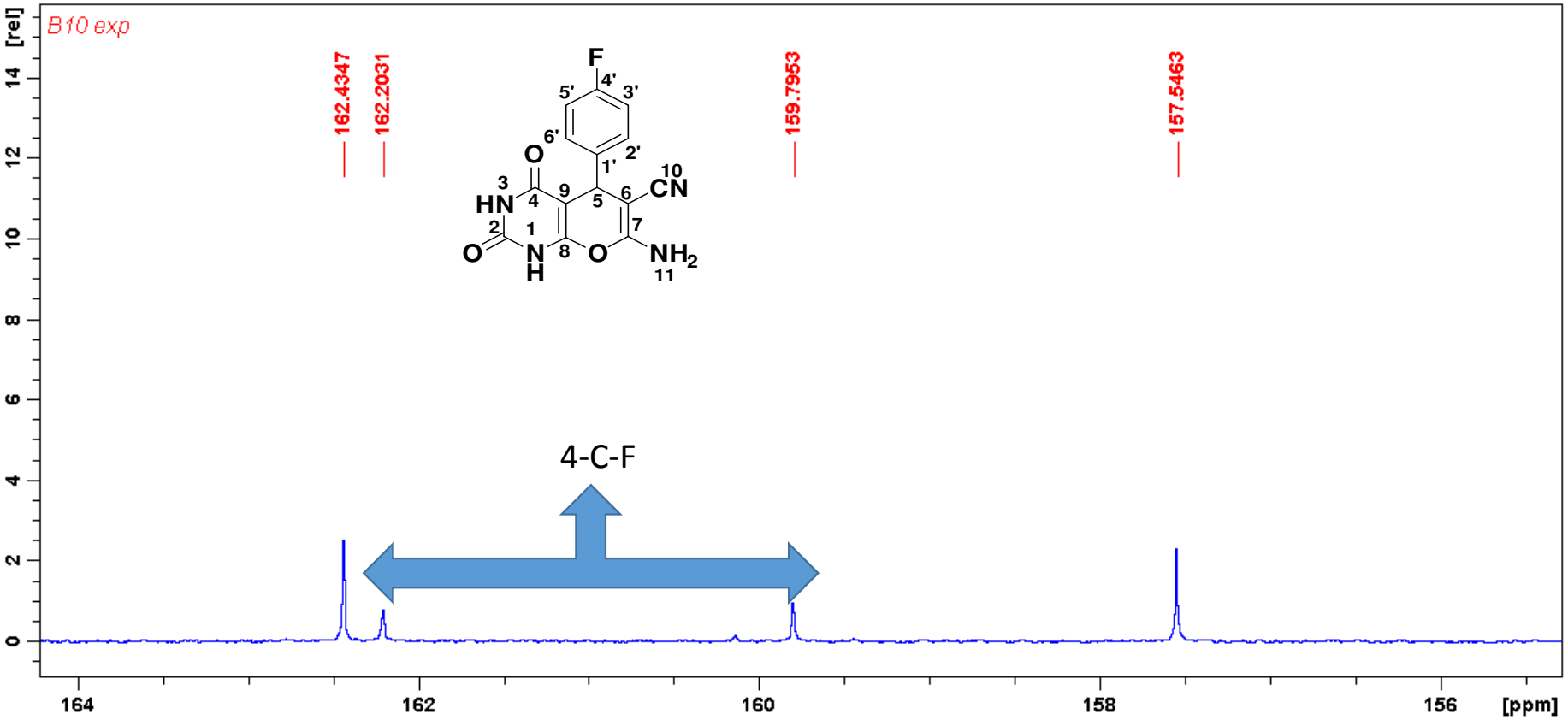
^{1}H NMR spectrum of 7-amino-5-(4-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B10)



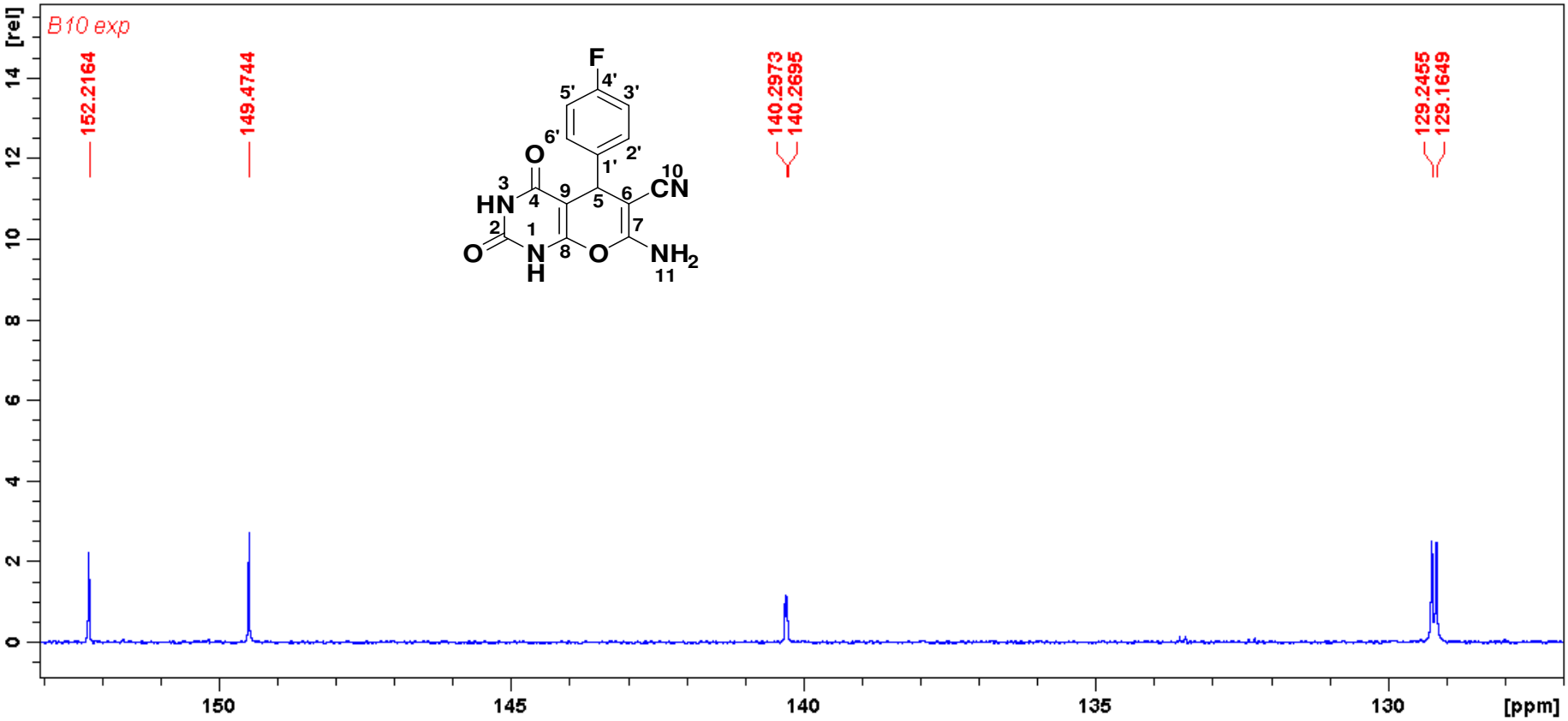
^{1}H NMR spectrum of 7-amino-5-(4-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B10) expanded



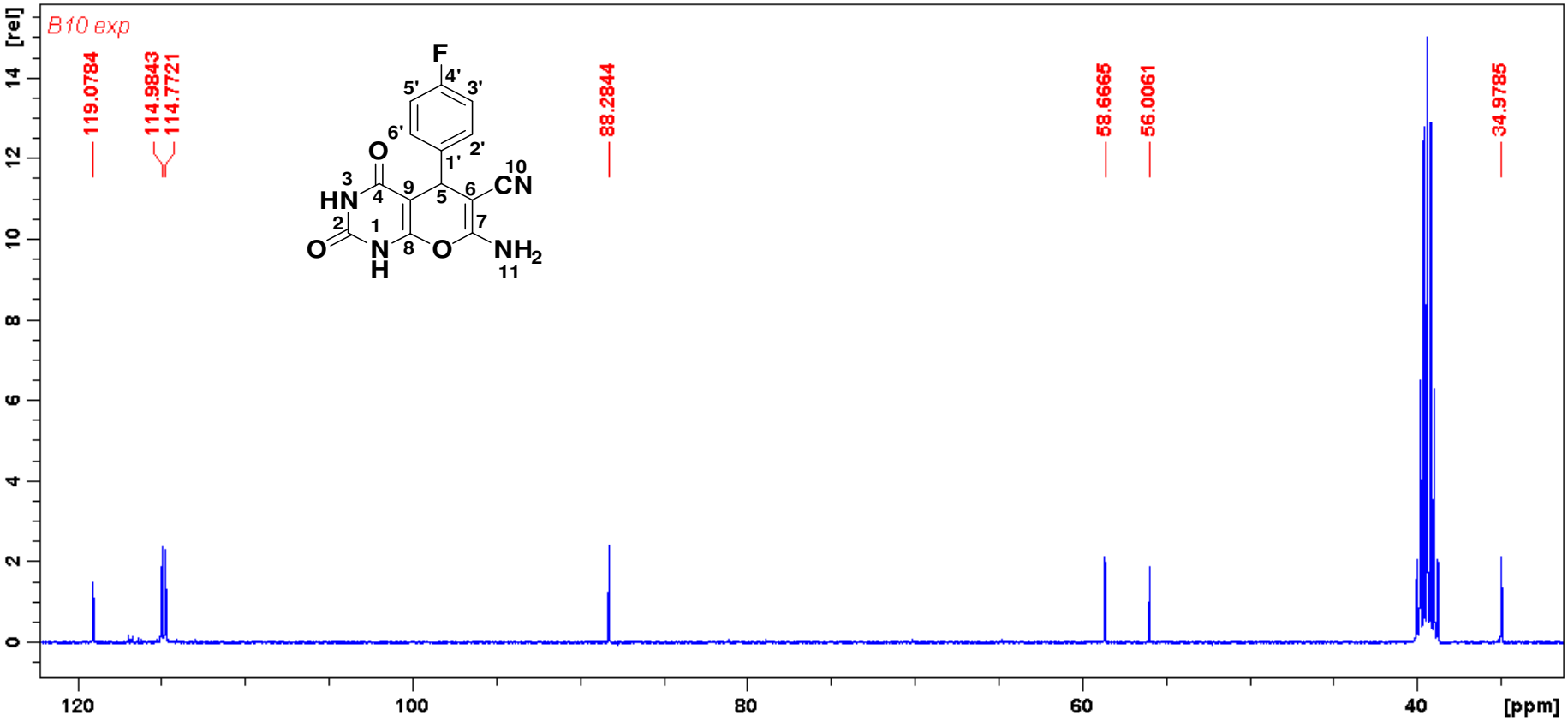
^{13}C NMR spectrum of 7-amino-5-(4-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B10)



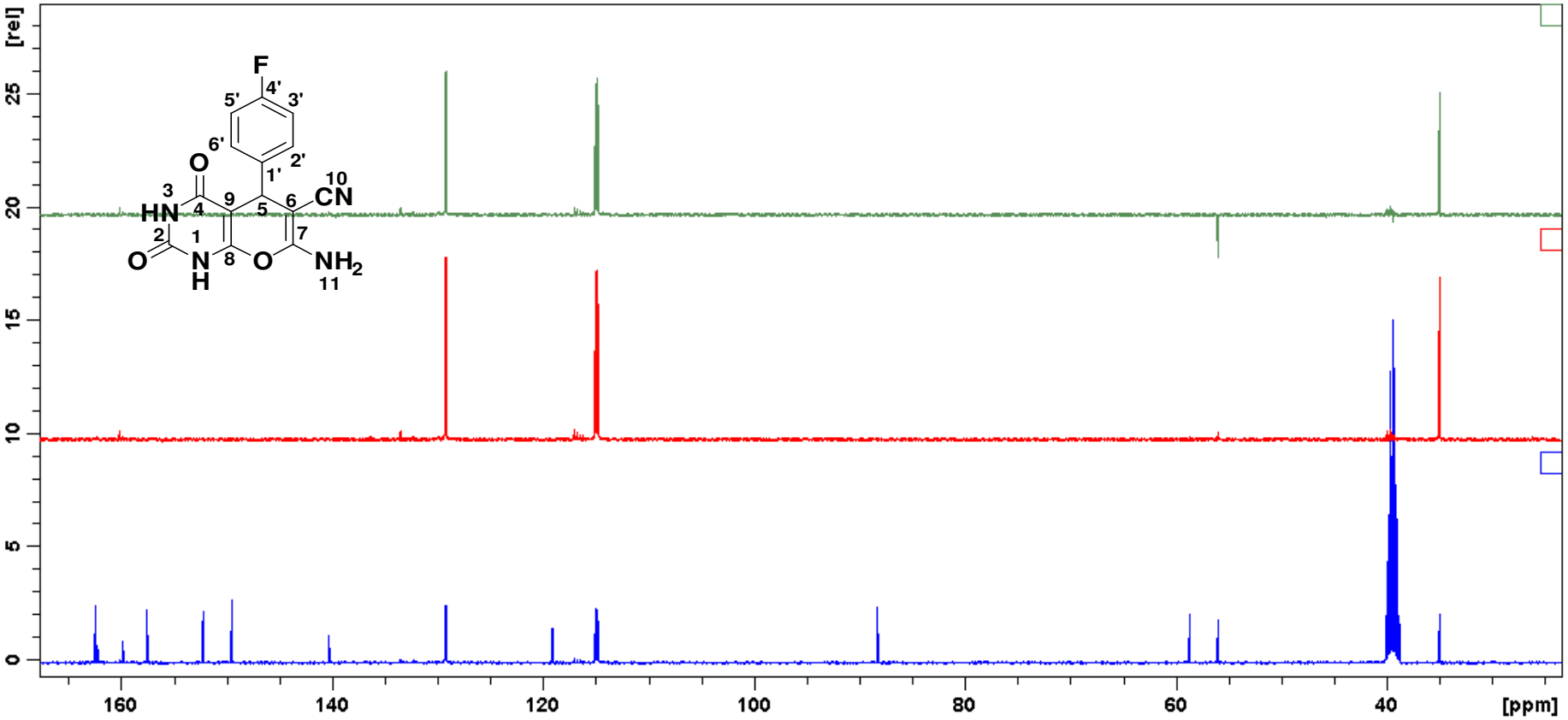
^{13}C NMR spectrum of 7-amino-5-(4-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B10) expanded



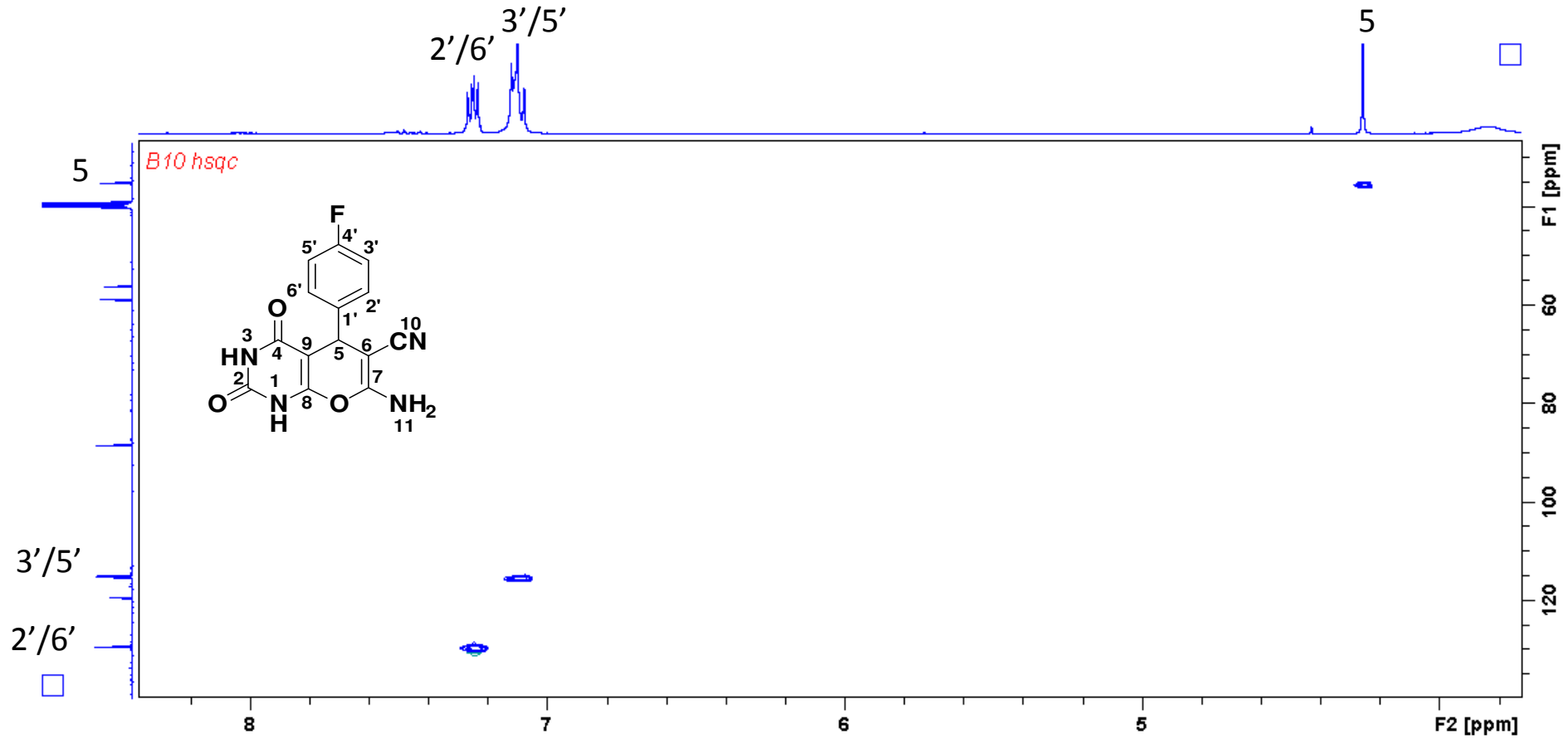
^{13}C NMR spectrum of 7-amino-5-(4-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B10) expanded



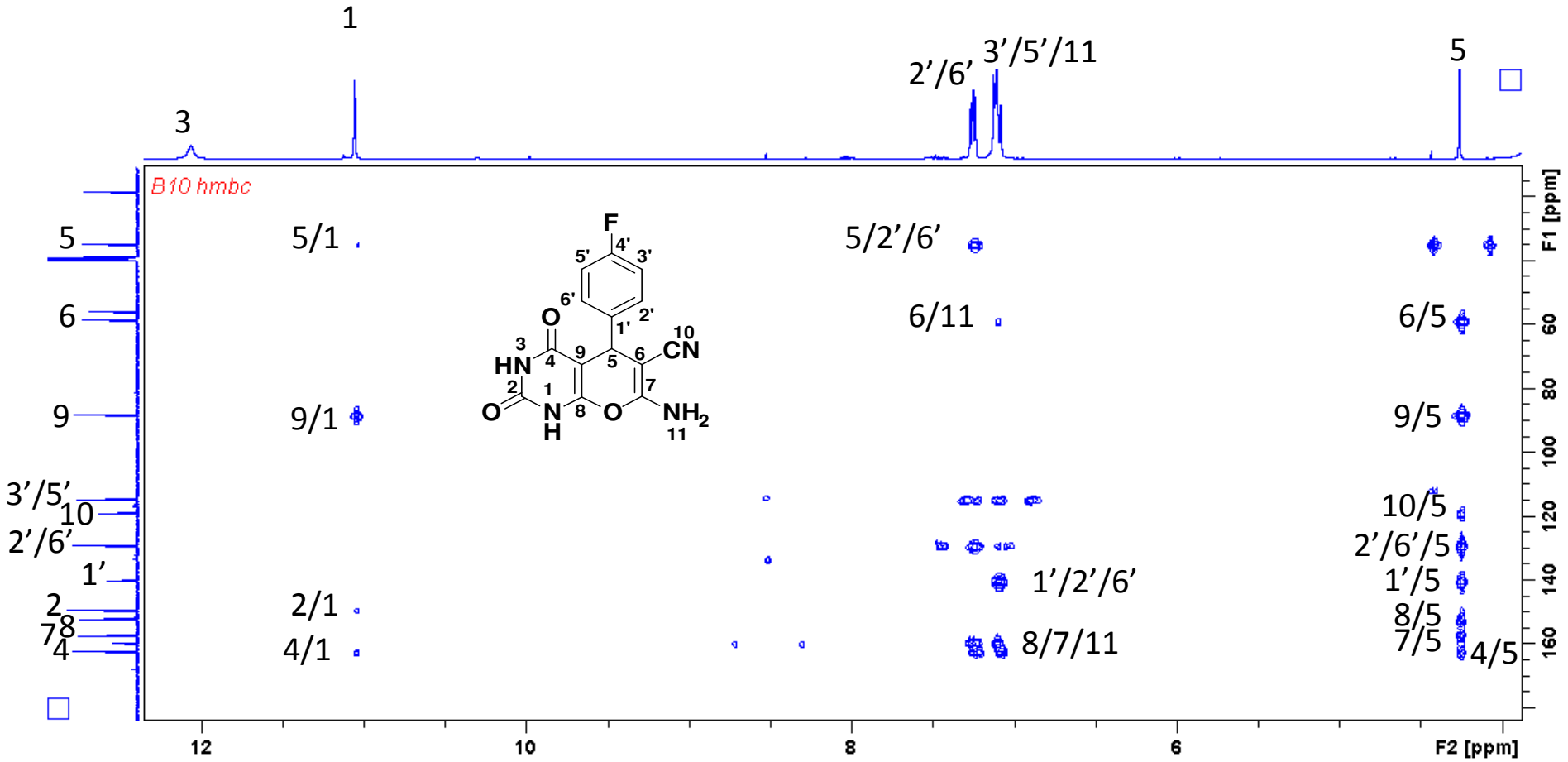
^{13}C NMR spectrum of 7-amino-5-(4-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B10) expanded



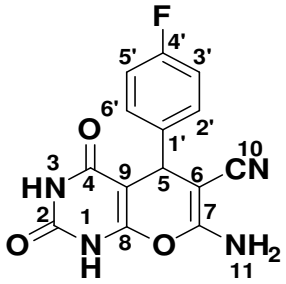
DEPT spectrum of 7-amino-5-(4-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B10)

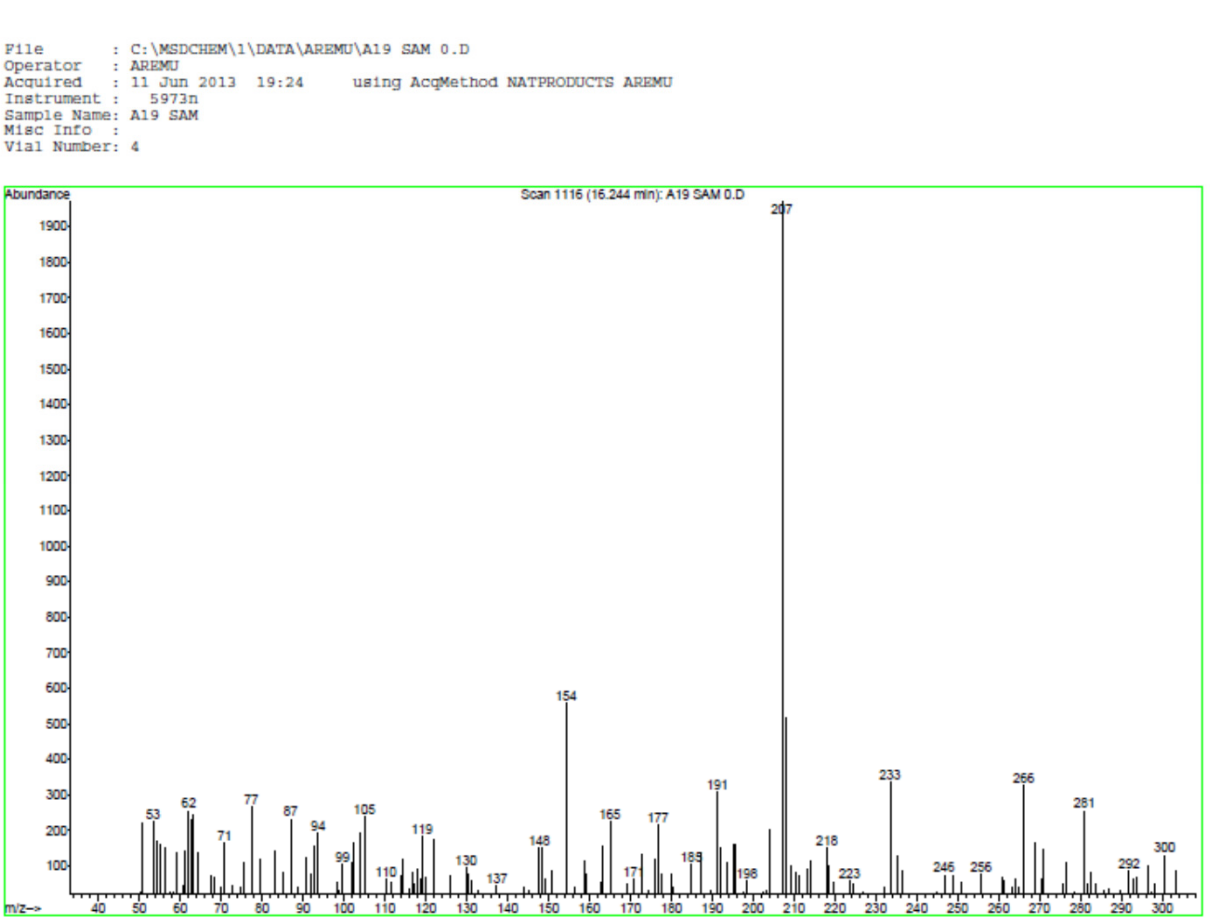


HSQC spectrum of 7-amino-5-(4-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B10)

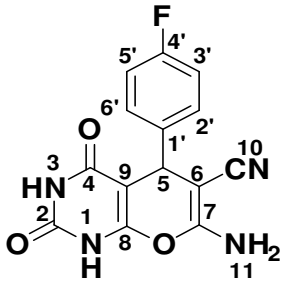


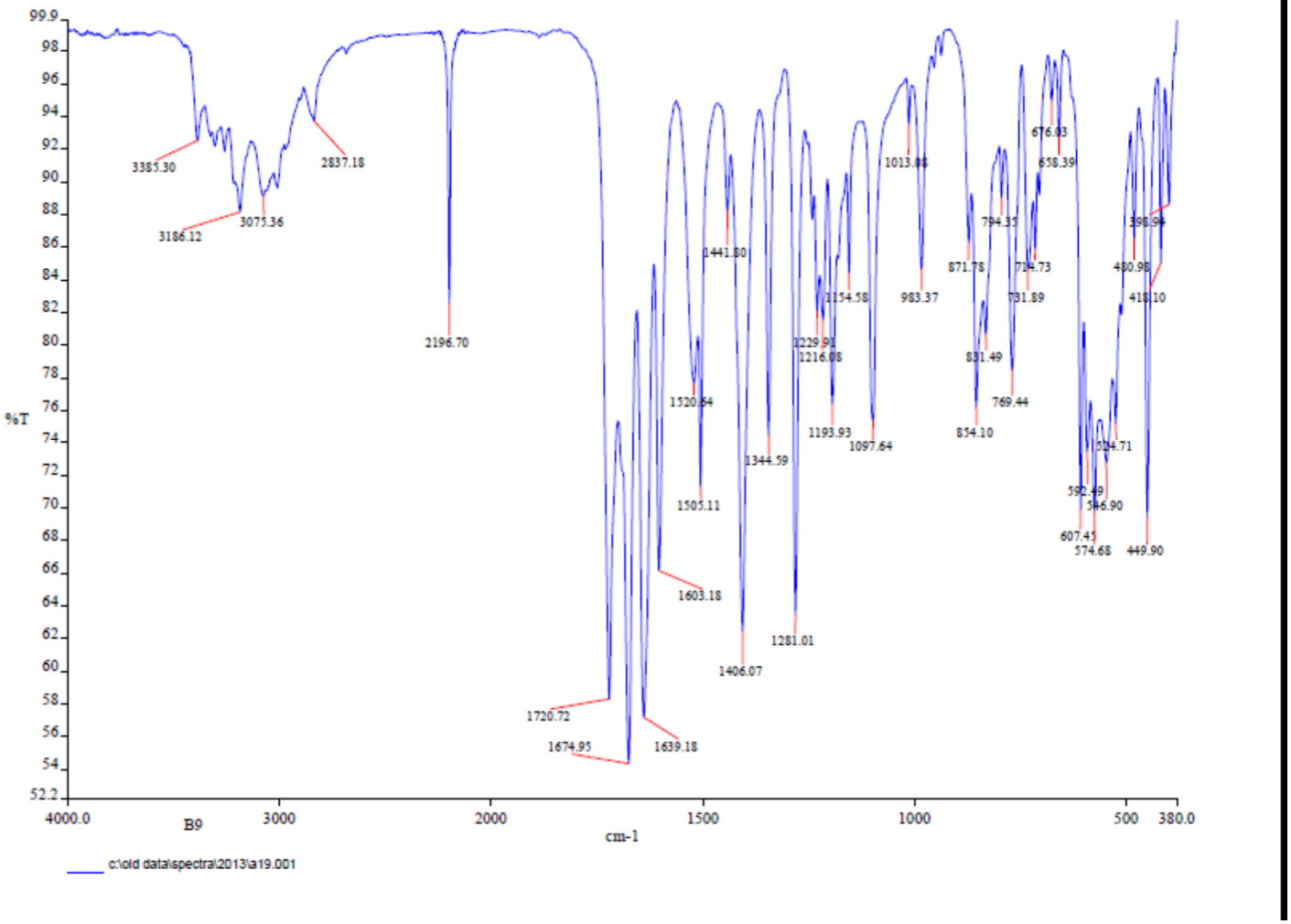
HMBC spectrum of 7-amino-5-(4-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B10)



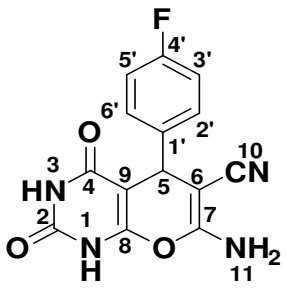


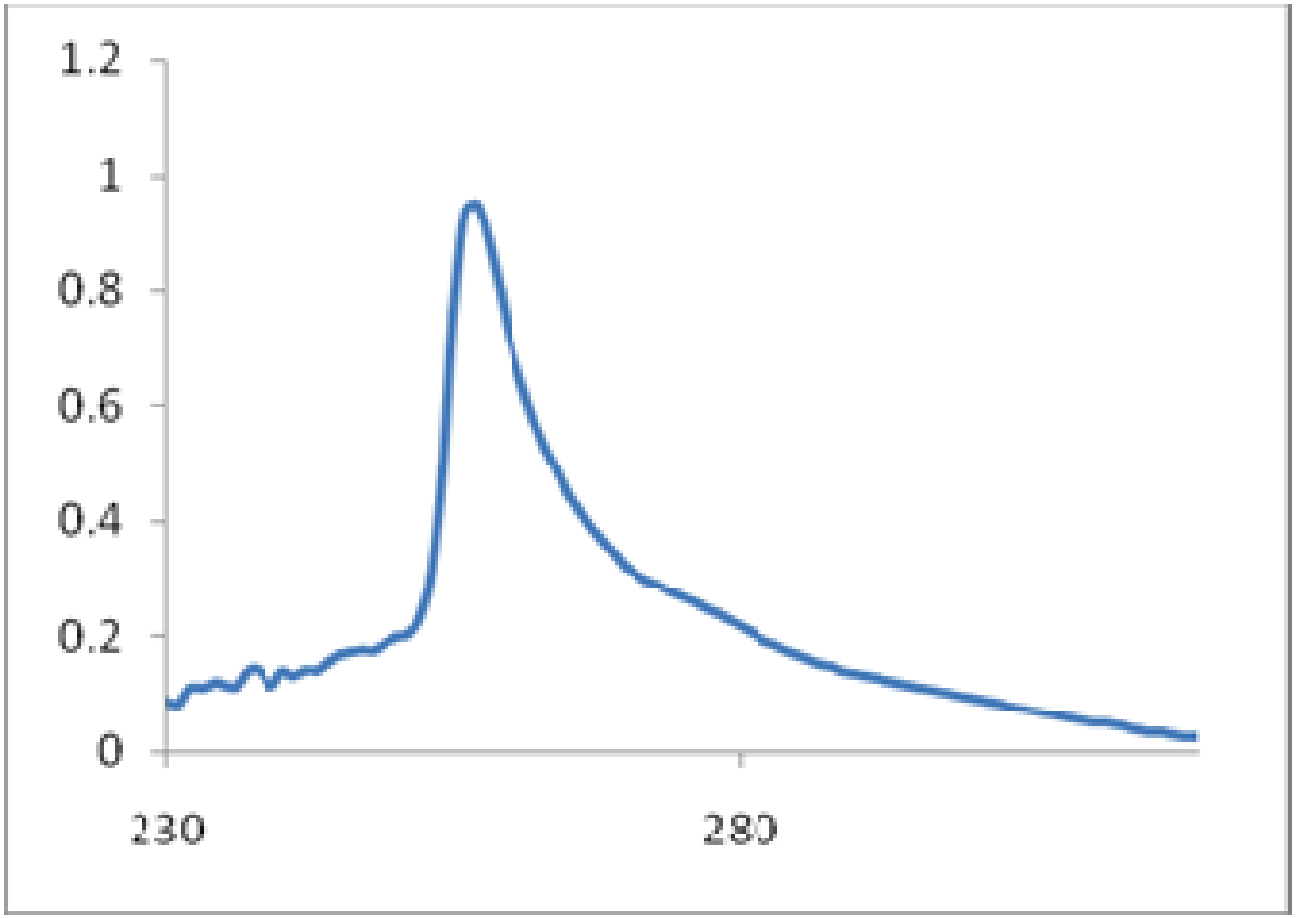
Mass spectrum of 7-amino-5-(4-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B10)



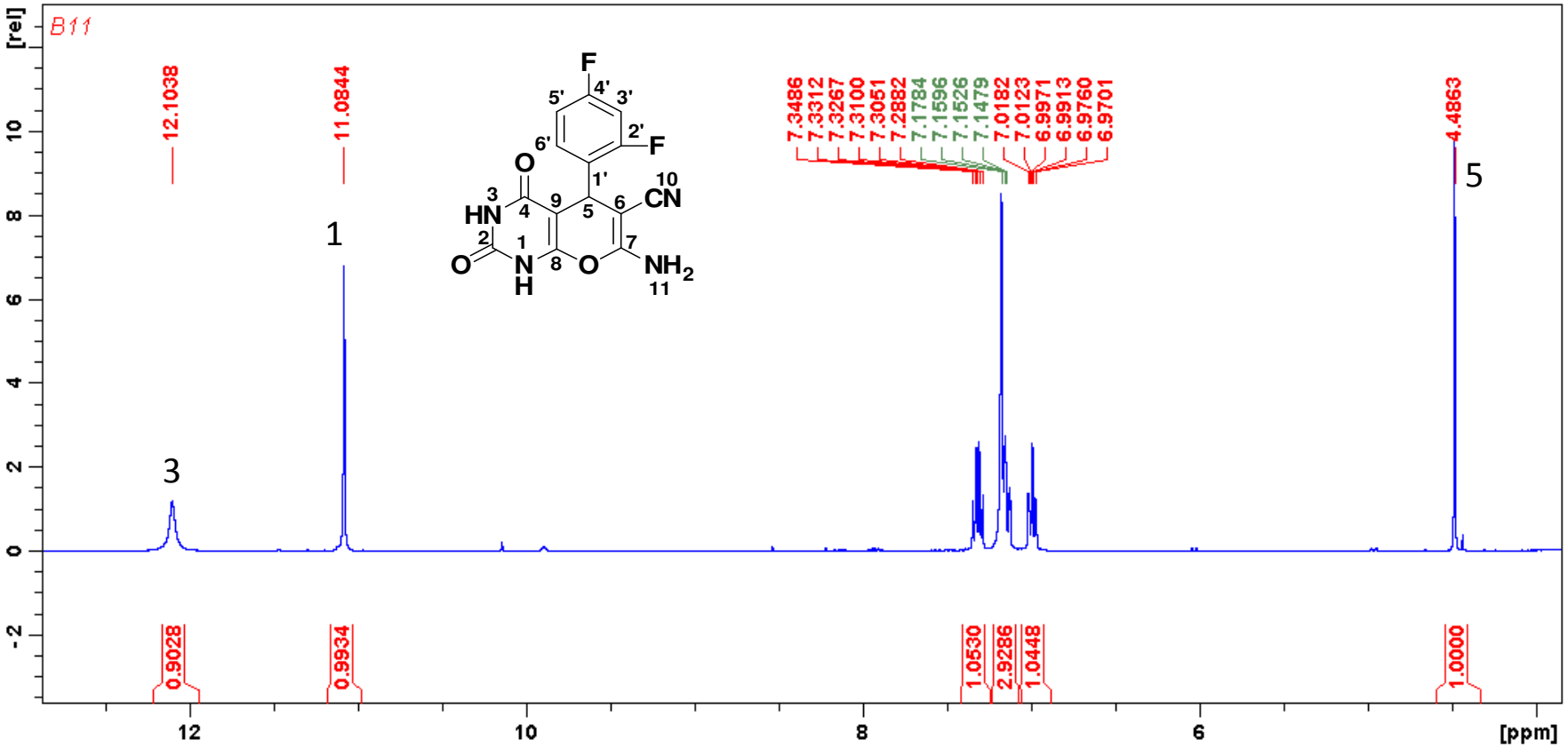


IR spectrum of 7-amino-5-(4-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B10)

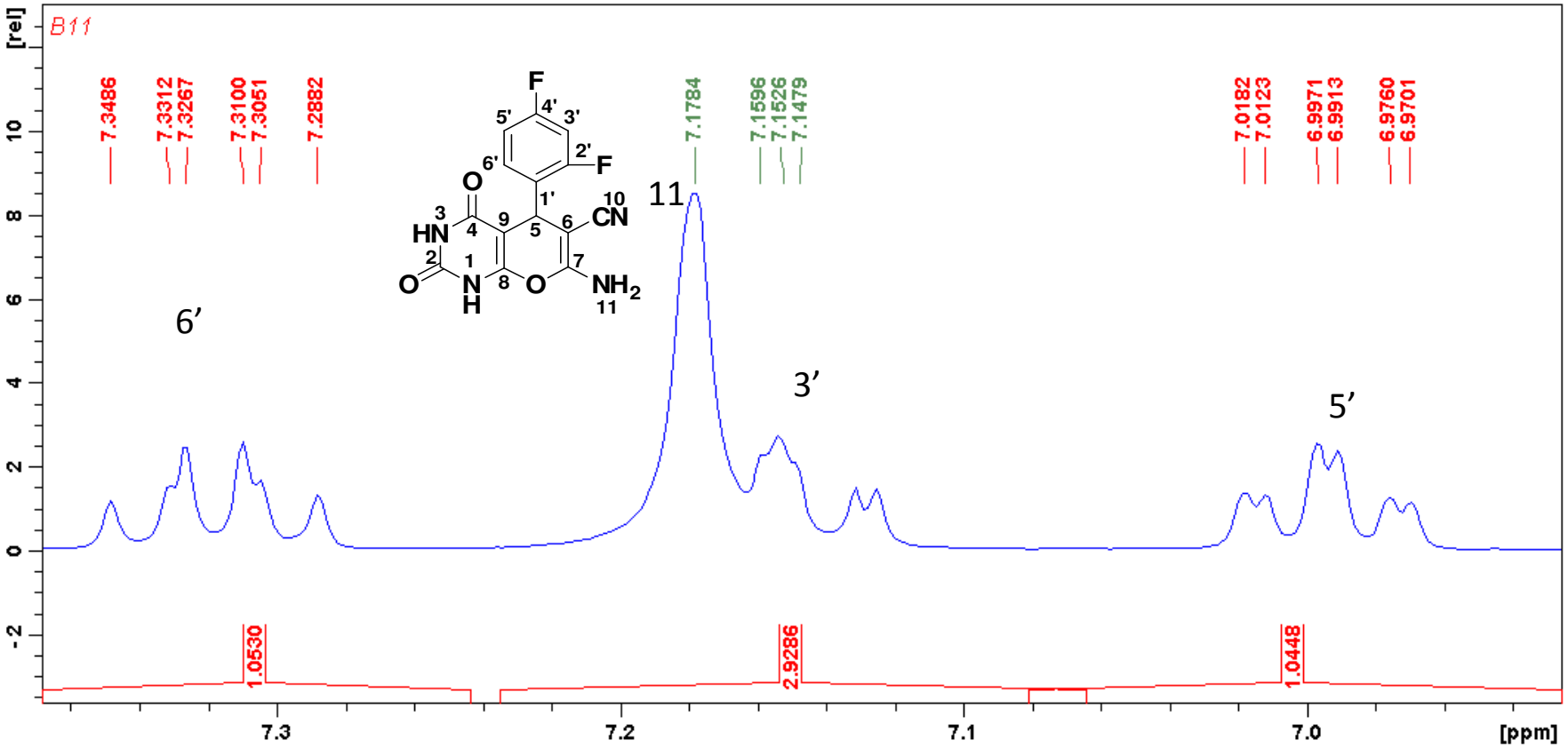




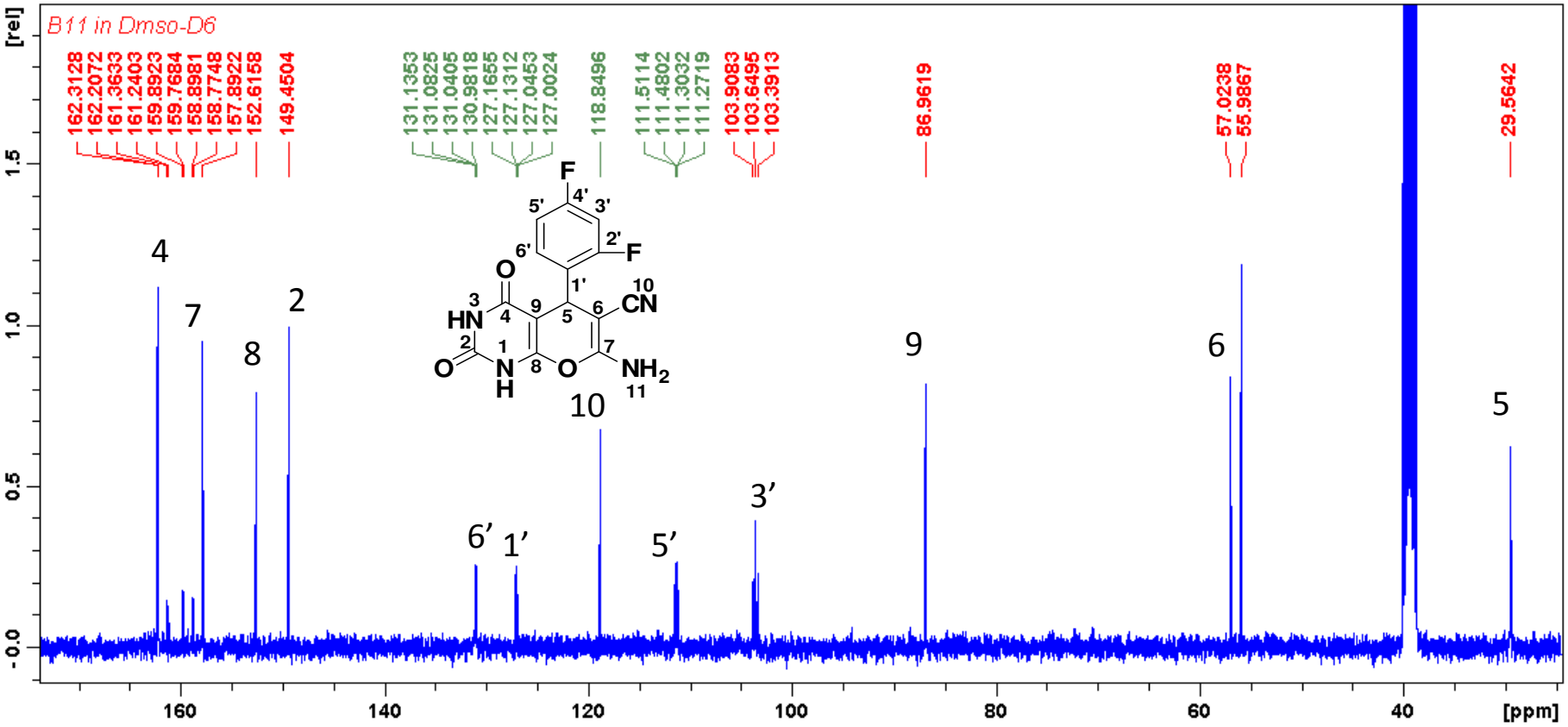
UV spectrum of 7-amino-5-(4-fluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B10)



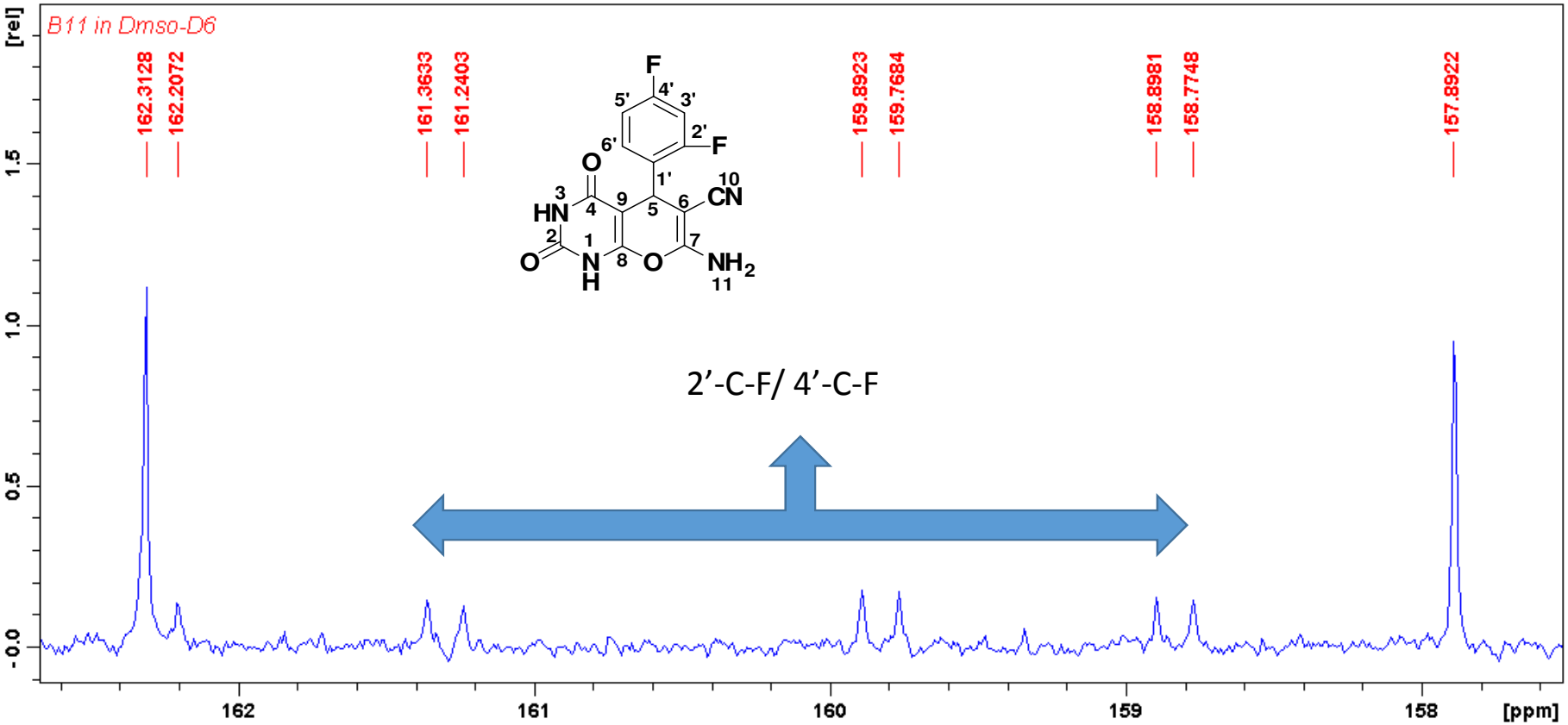
[1]H NMR spectrum of 7-amino-5-(2,4-difluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B11)



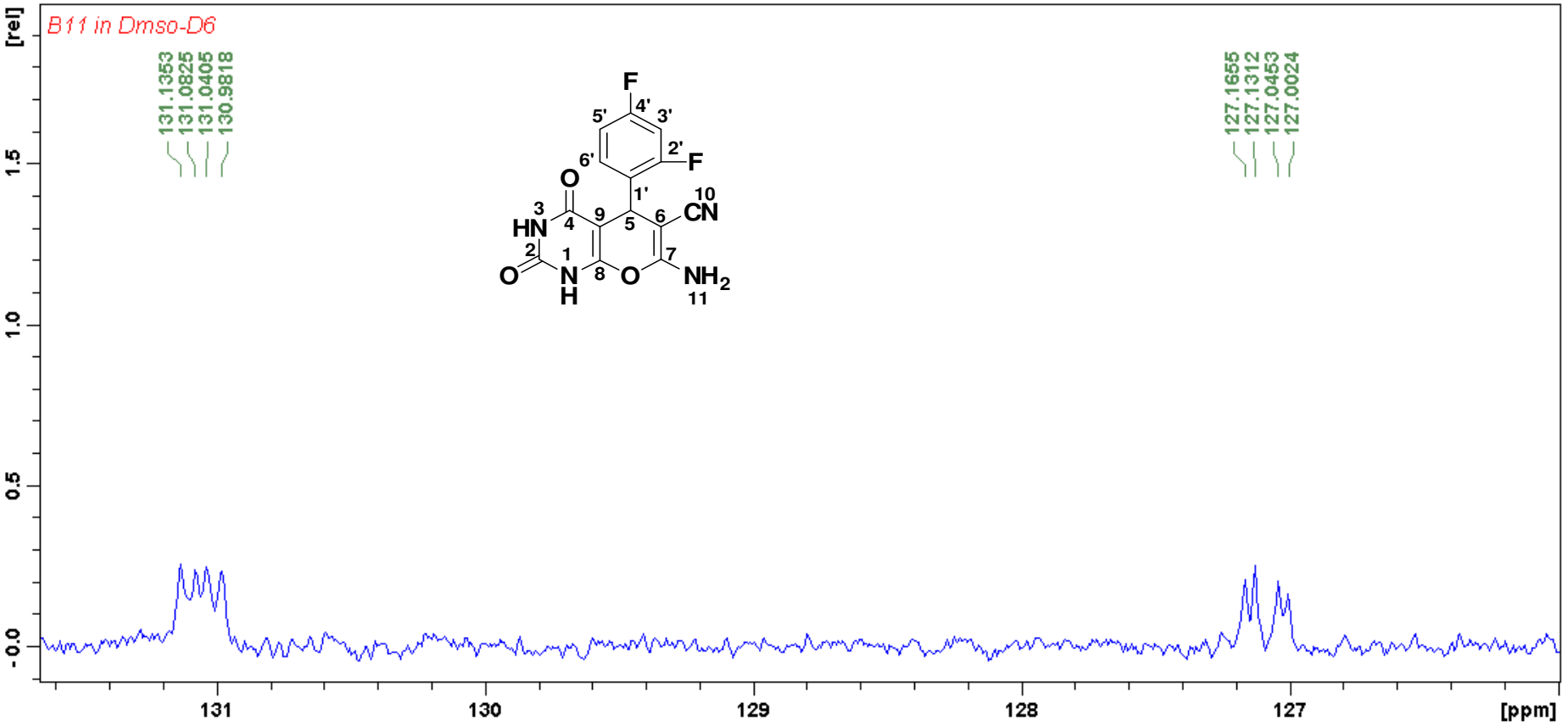
^{1}H NMR spectrum of 7-amino-5-(2,4-difluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B11) expanded



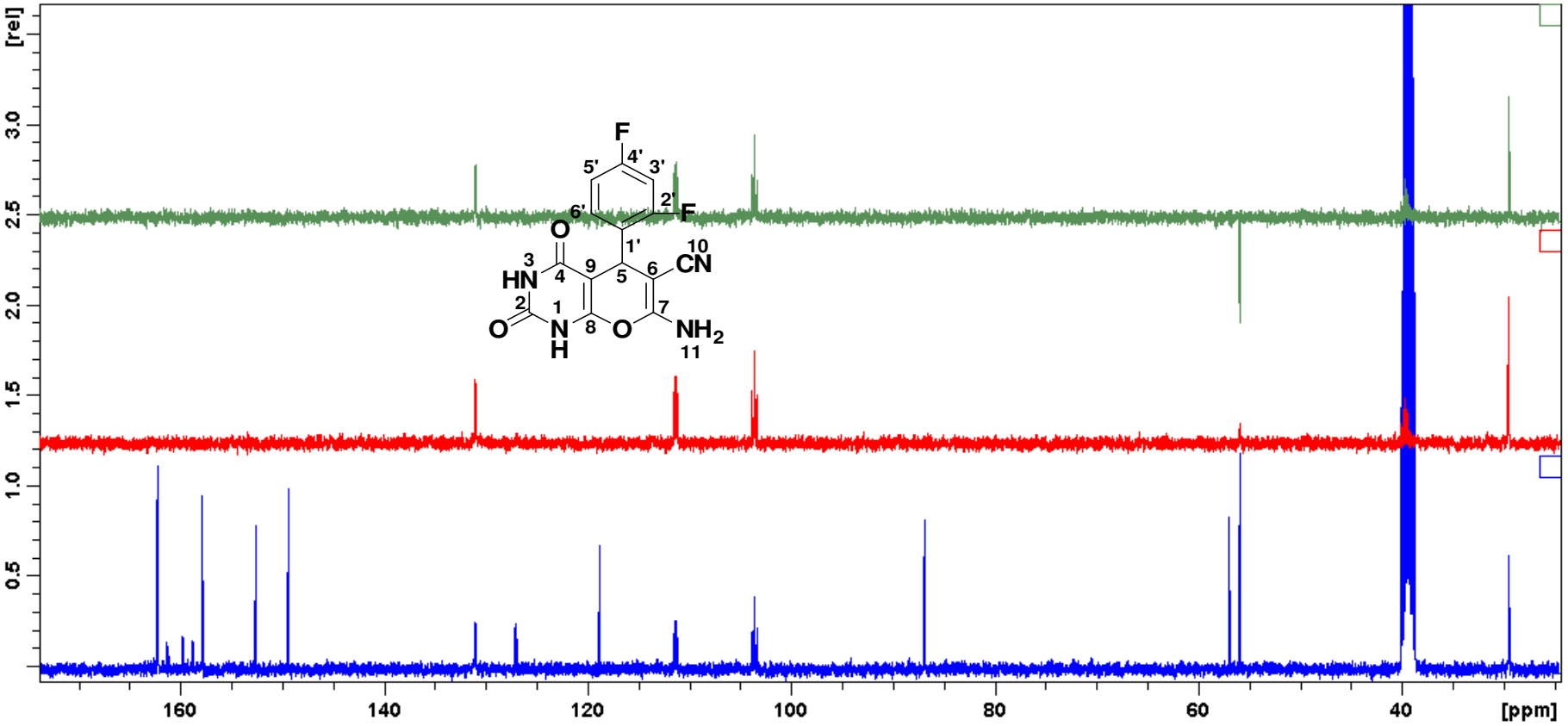
^{13}C NMR spectrum of 7-amino-5-(2,4-difluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B11)



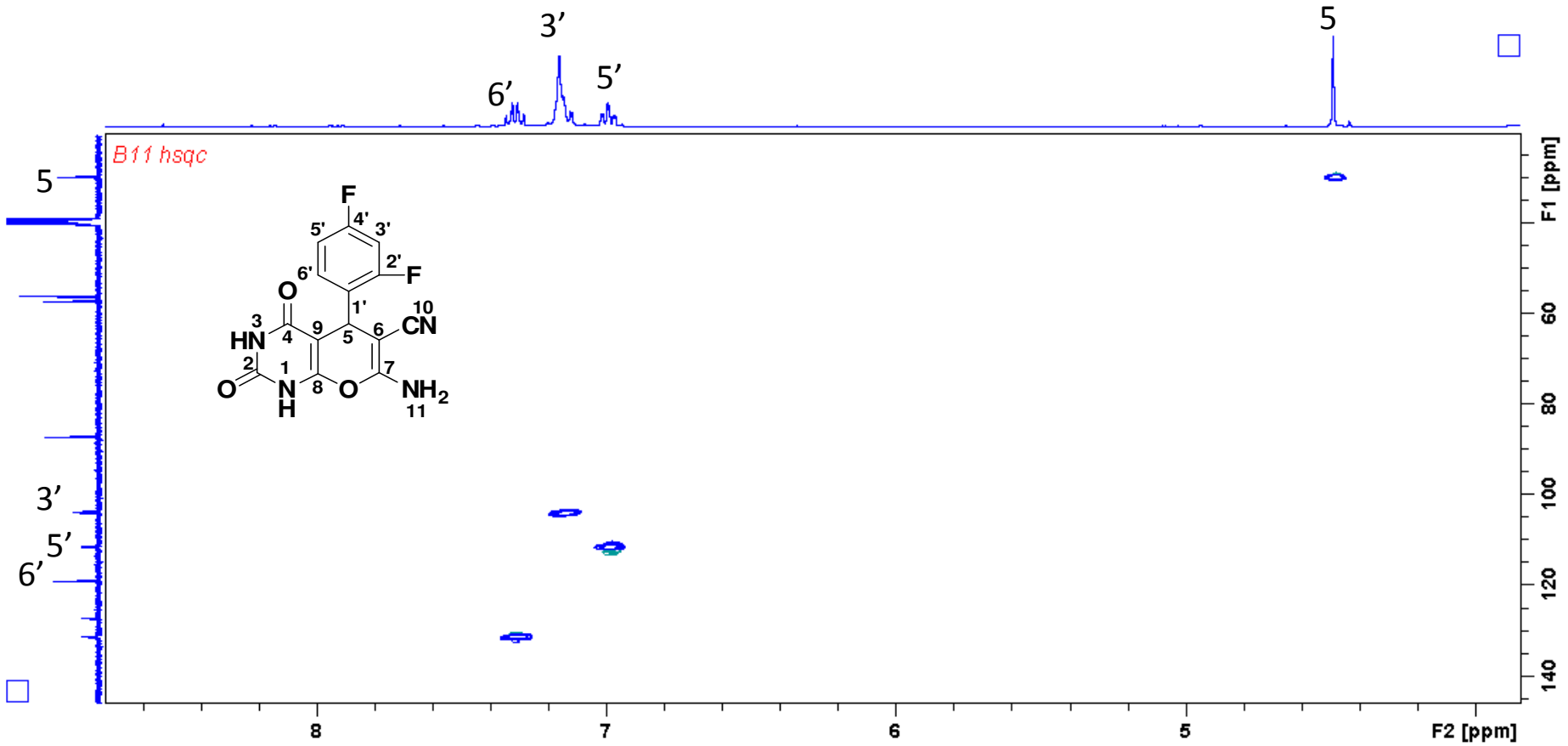
^{13}C NMR spectrum of 7-amino-5-(2,4-difluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B11) expanded



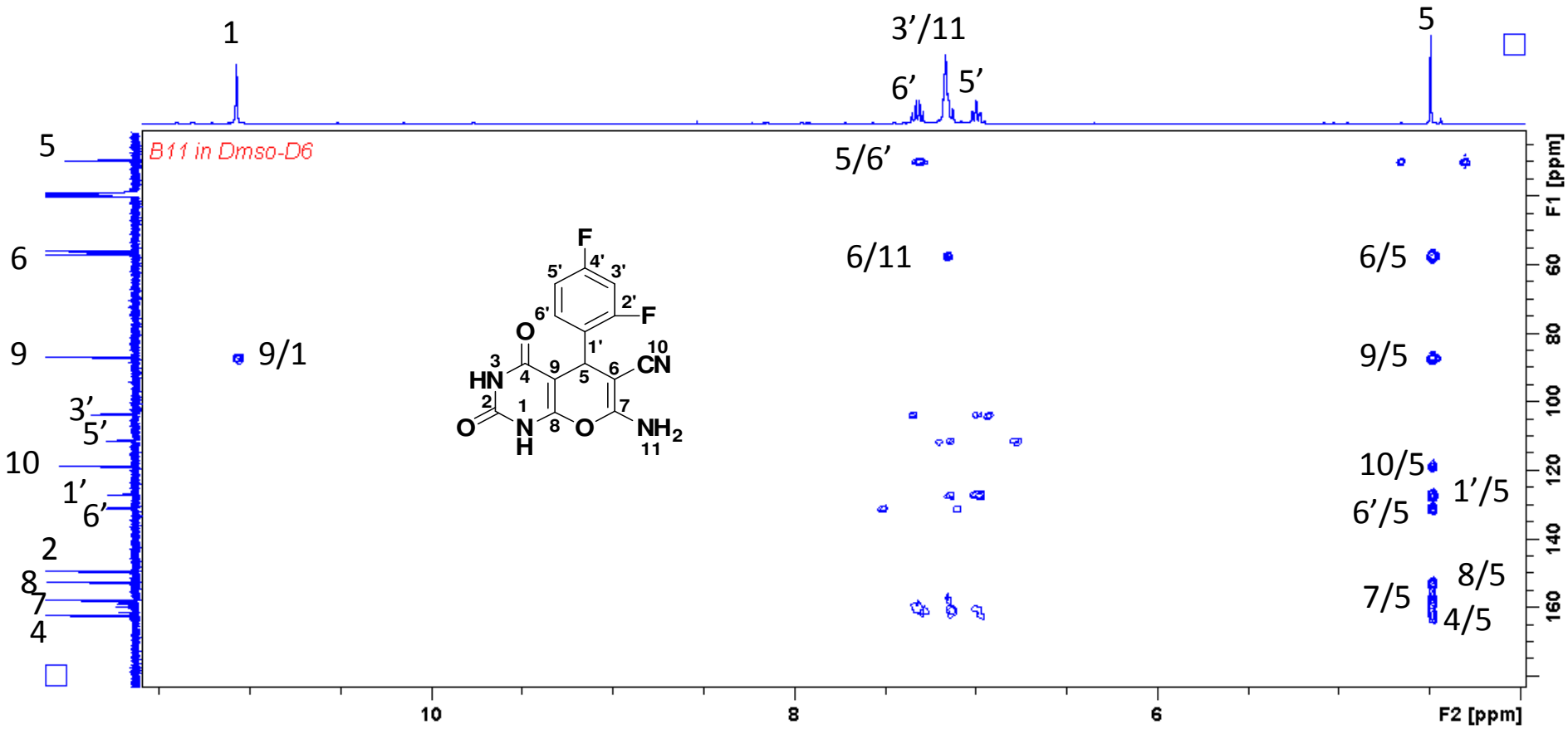
^{13}C NMR spectrum of 7-amino-5-(2,4-difluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B11) expanded



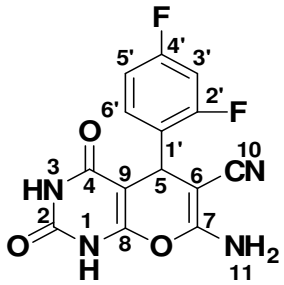
DEPT spectrum of 7-amino-5-(2,4-difluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B11)



HSQC spectrum of 7-amino-5-(2,4-difluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B11)



HMBC spectrum of 7-amino-5-(2,4-difluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B11)

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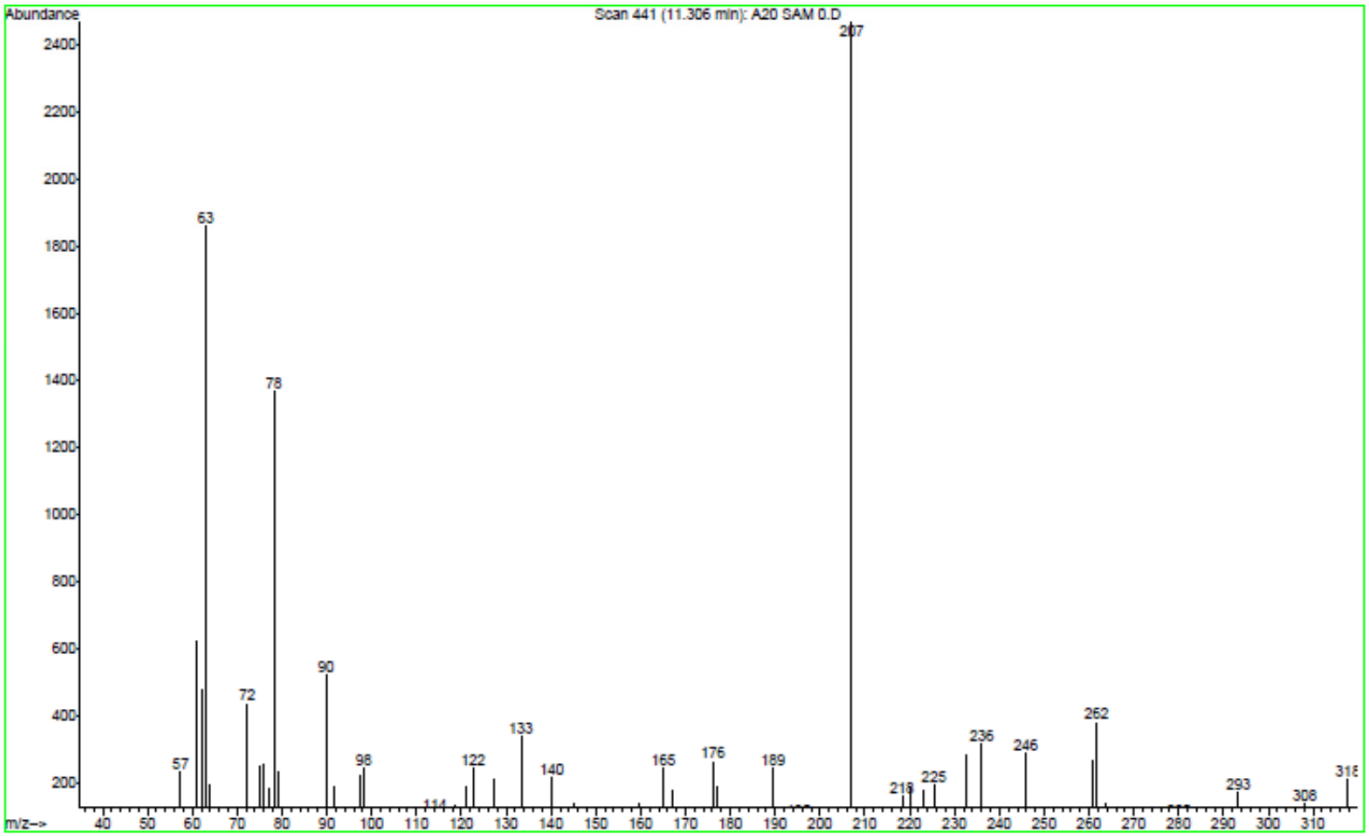
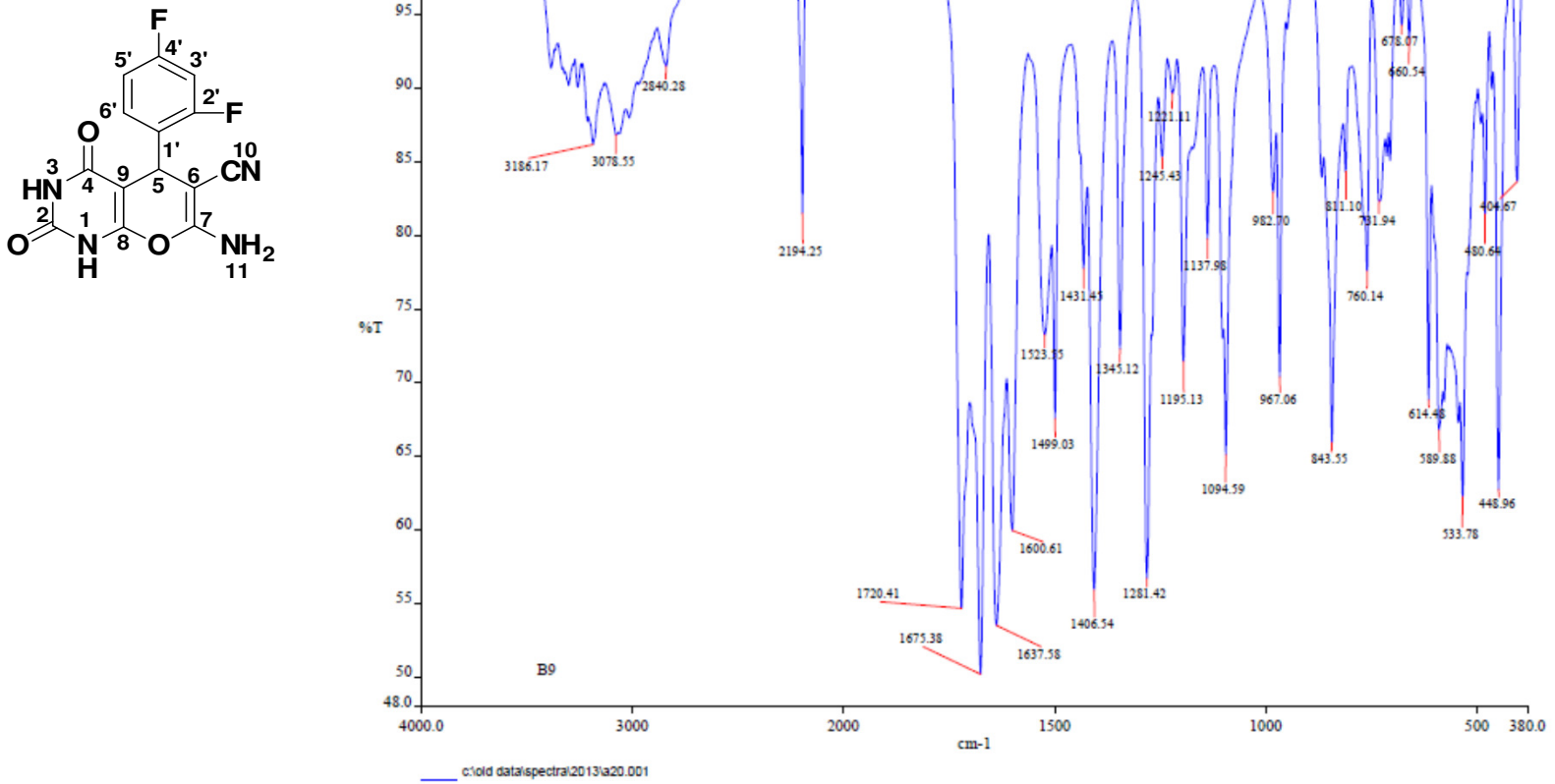
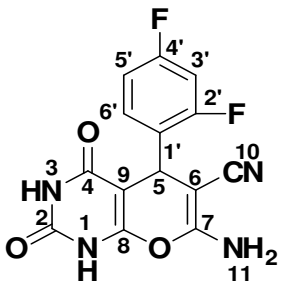
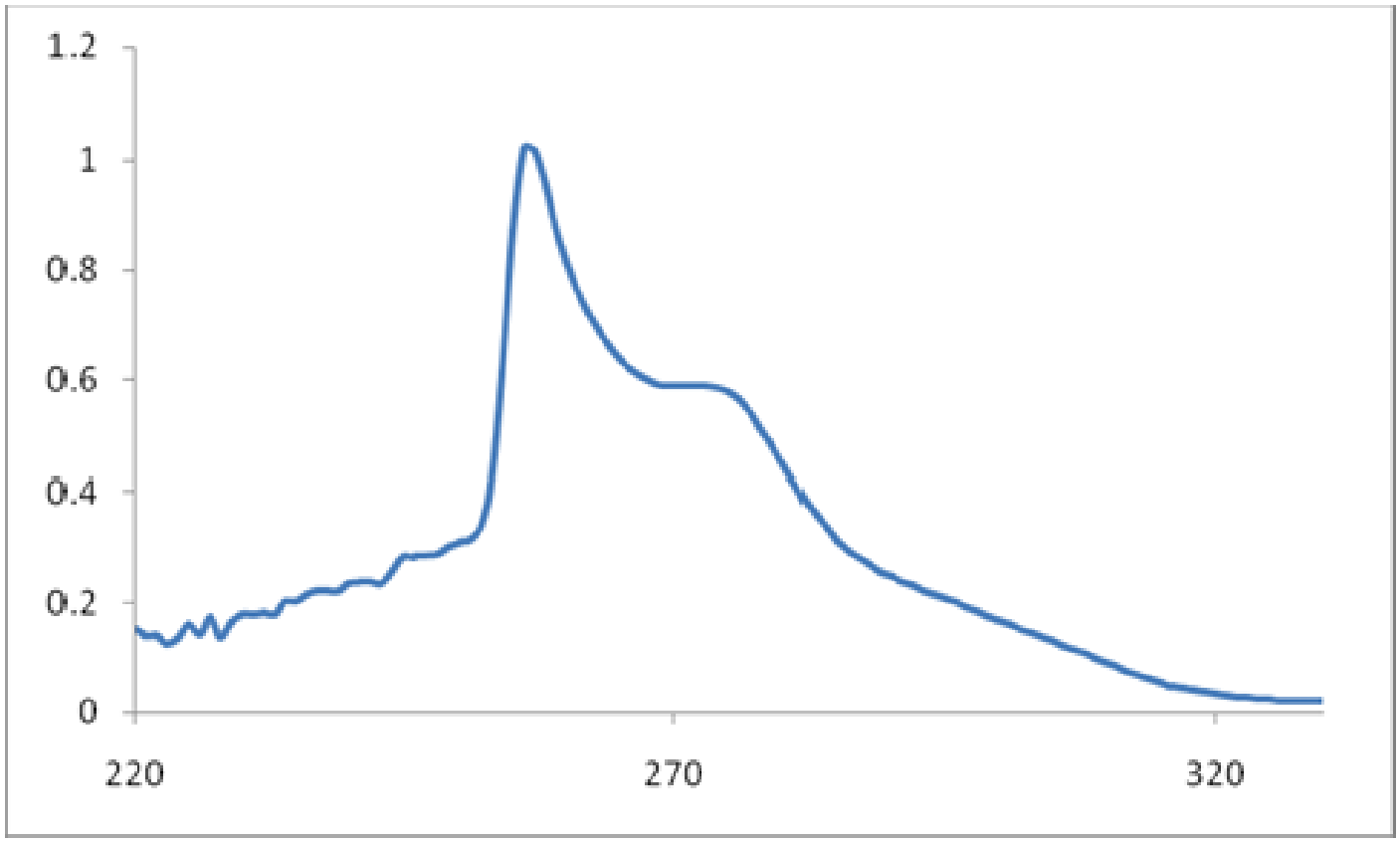


Mass spectrum of 7-amino-5-(2,4-difluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B11)

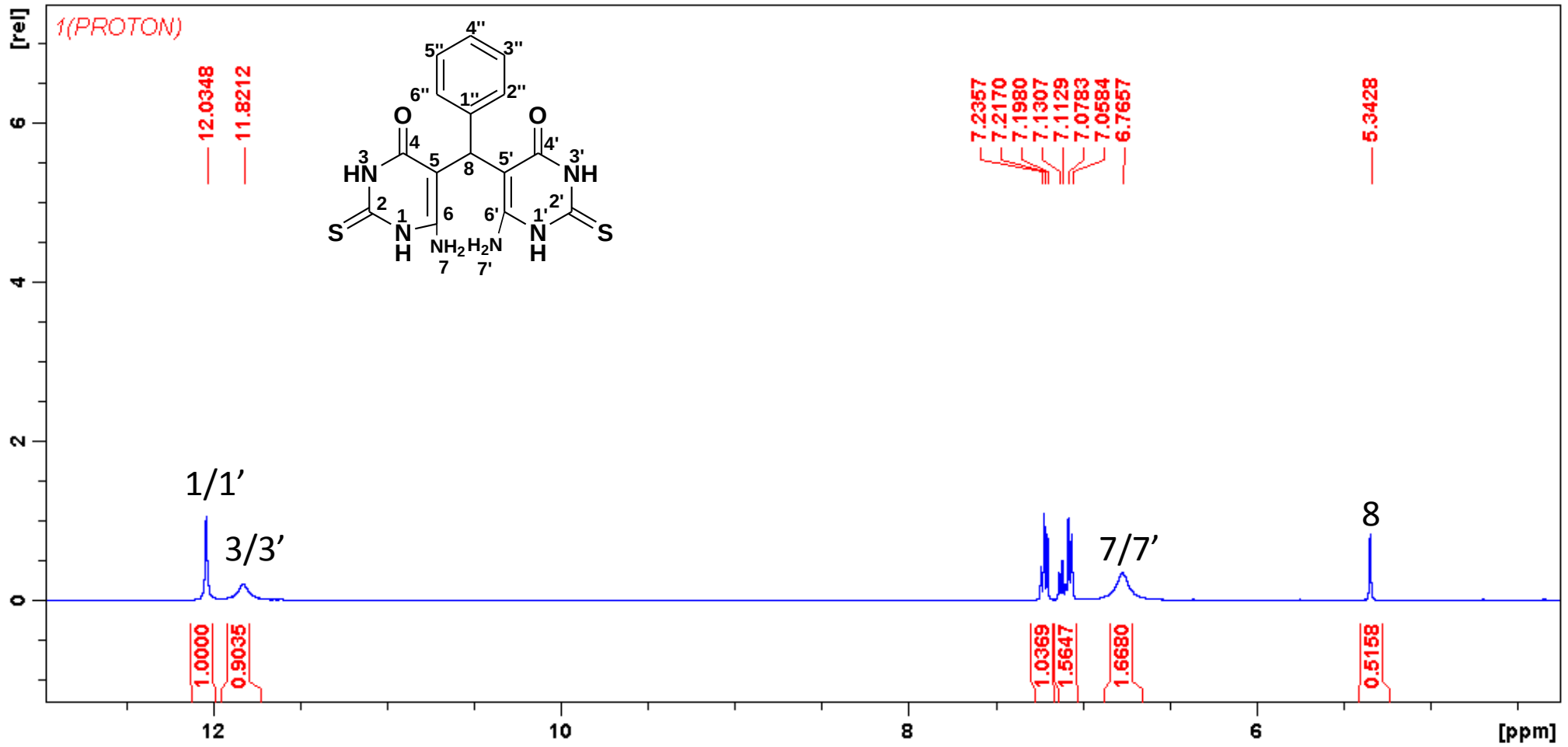


IR spectrum of 7-amino-5-(2,4-difluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B11)

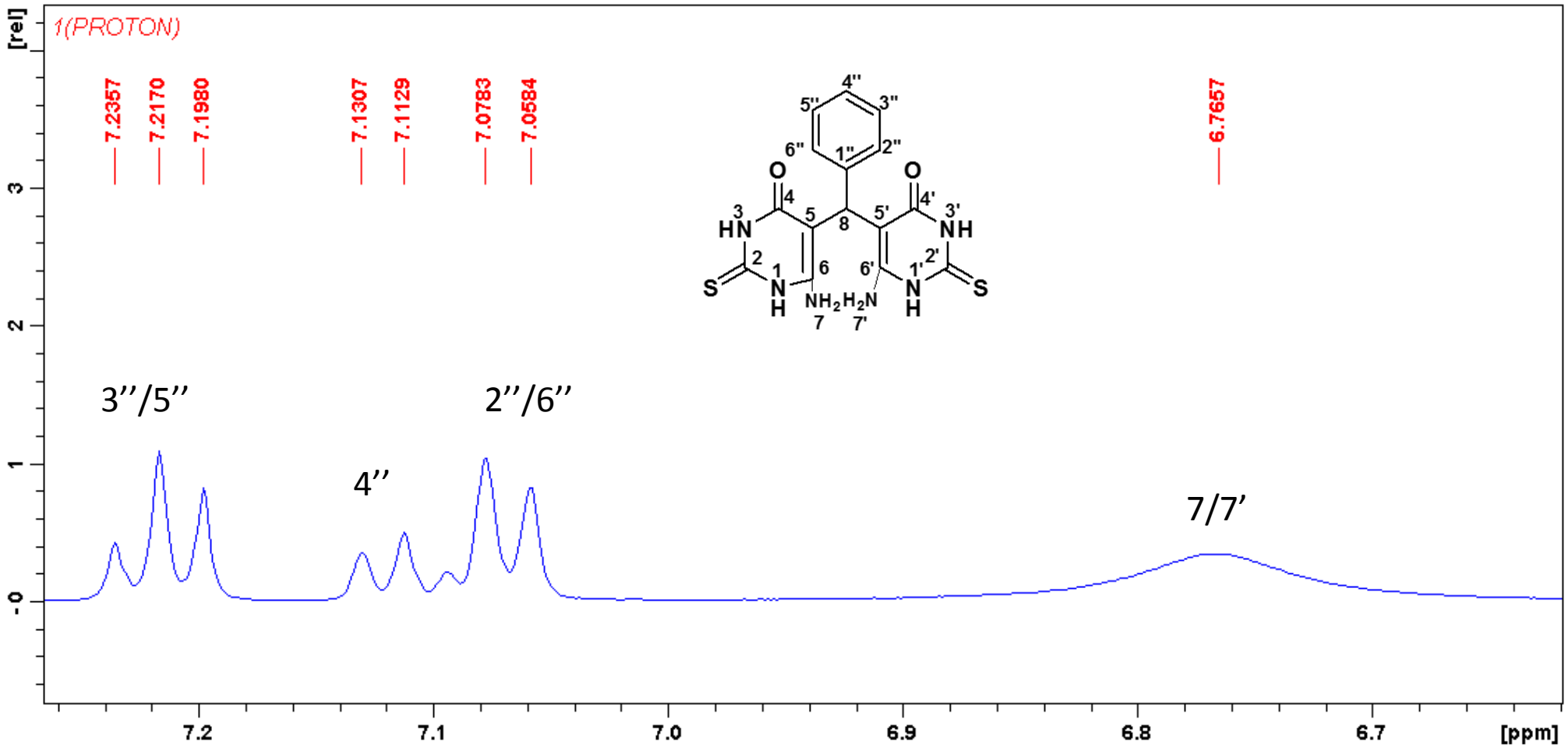




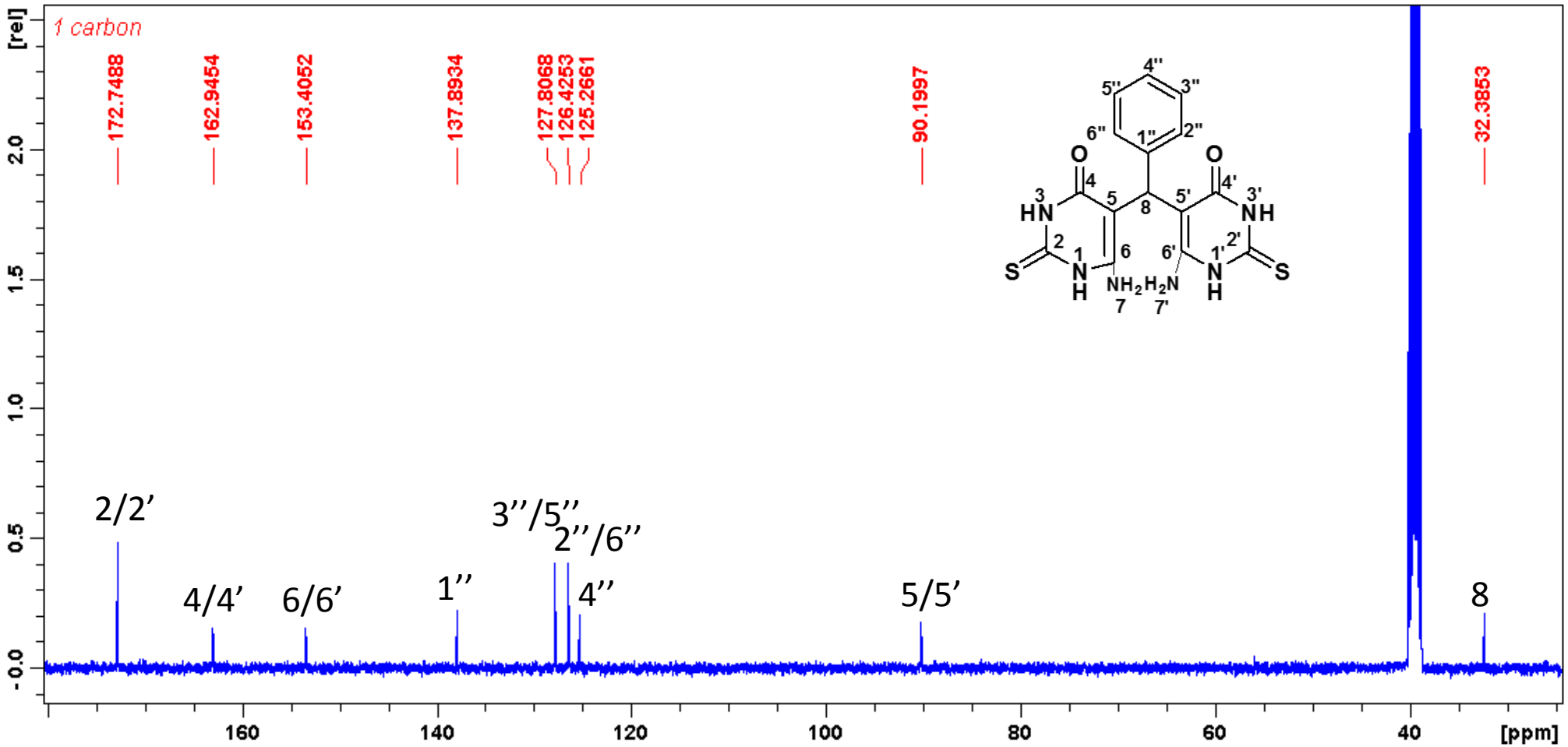
UV spectrum of 7-amino-5-(2,4-difluorophenyl)-6-cyano-5*H*-pyrano[2,3-*d*]pyrimidin-(1*H*,3*H*)-2,4-dione (B11)



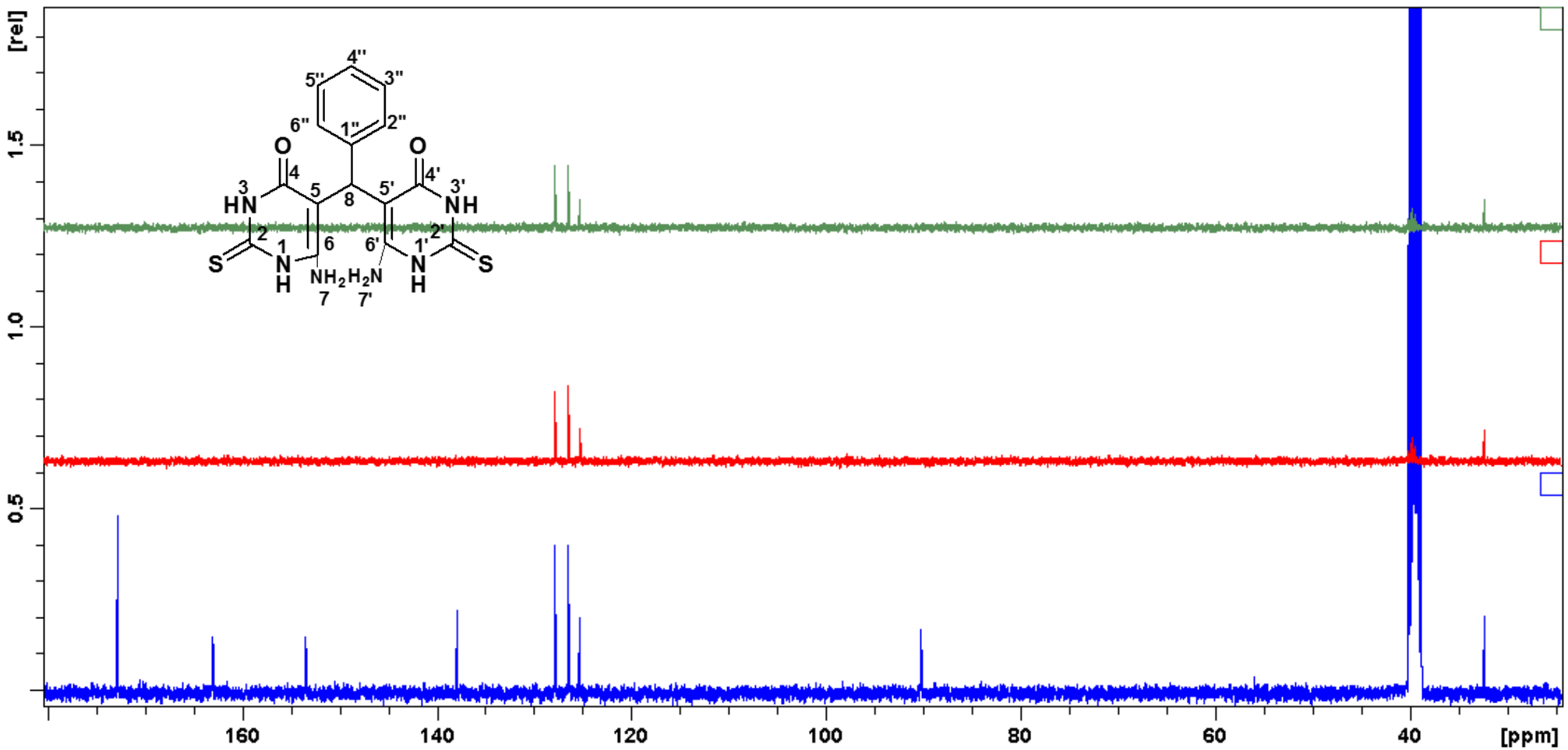
^{1}H NMR spectrum of 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) (C1)



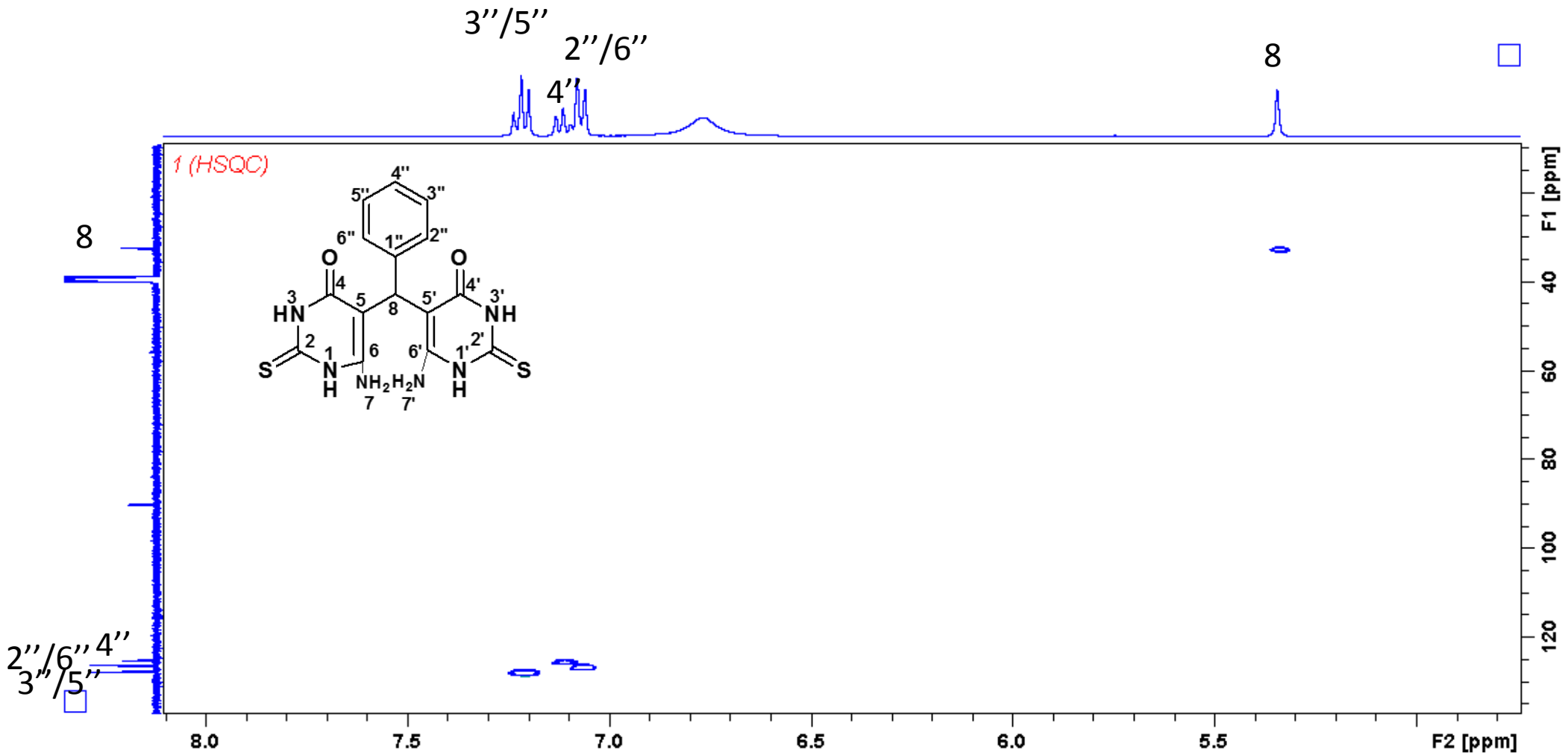
^{1}H NMR spectrum of 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) (C1)



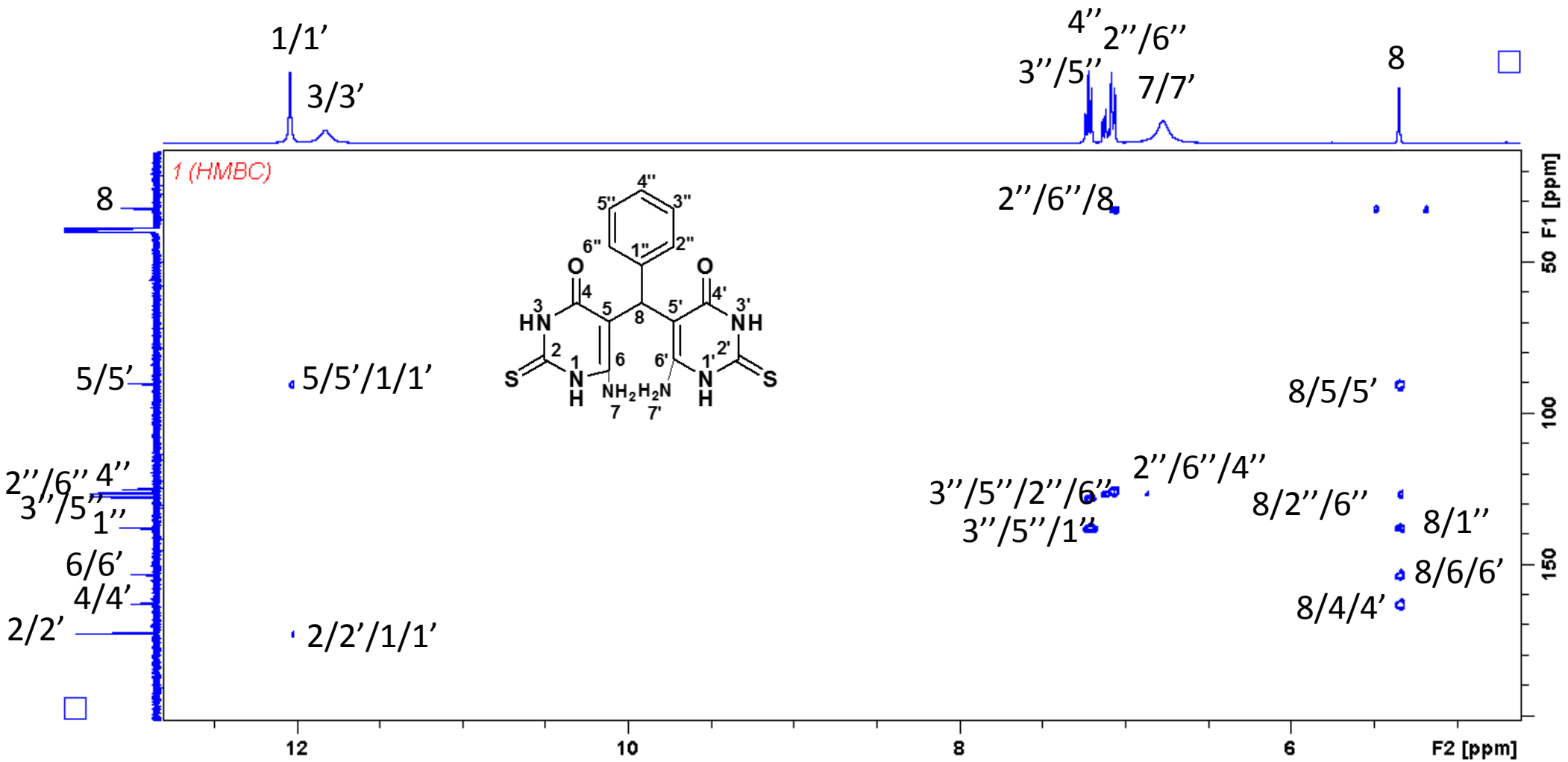
^{13}C NMR spectrum of 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) (C1)



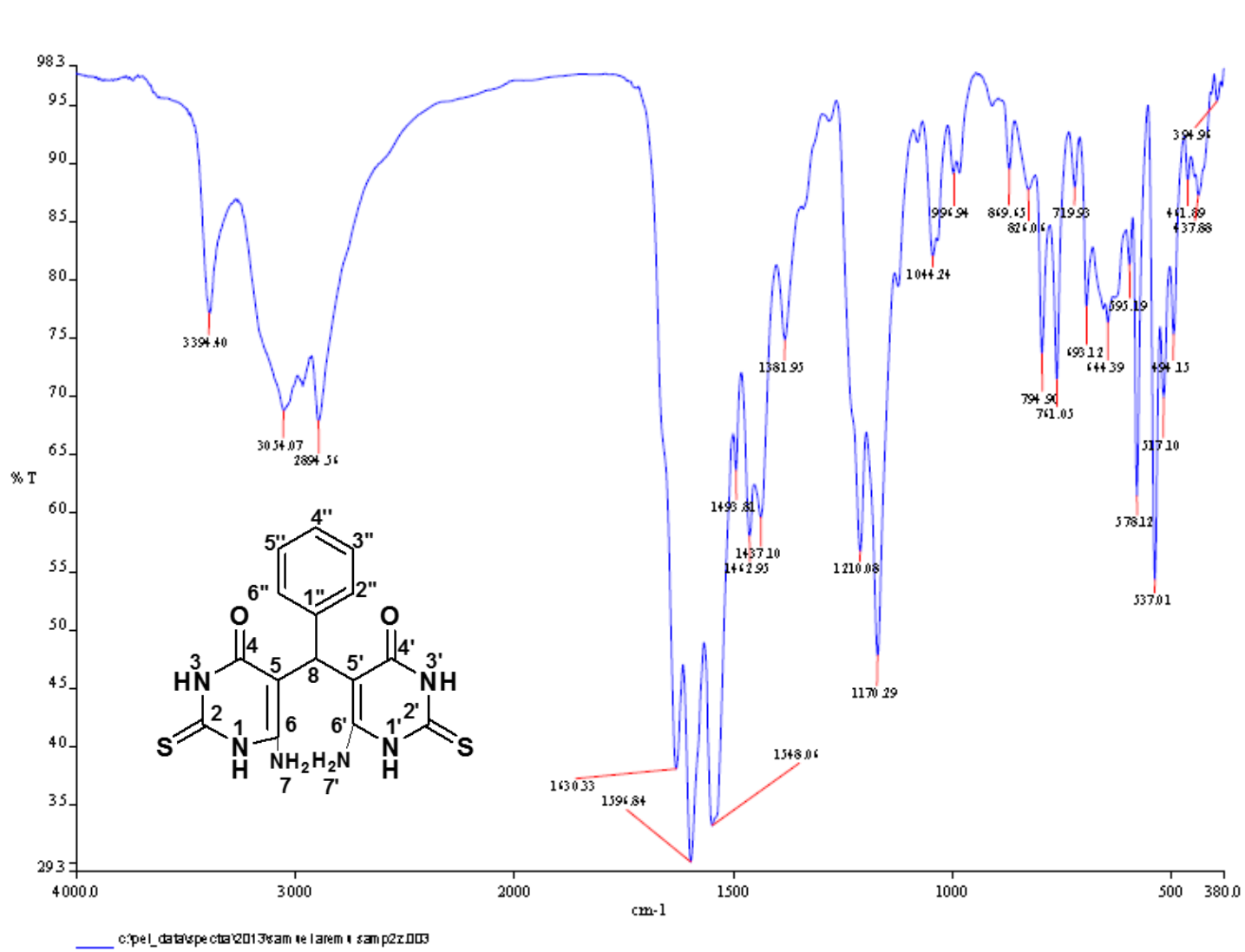
DEPT spectrum of 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) (C1)



HSQC spectrum of 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) (C1)

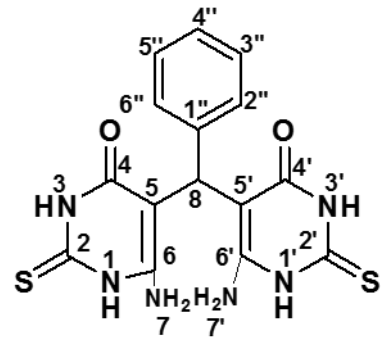


HMBC spectrum of 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) (C1)



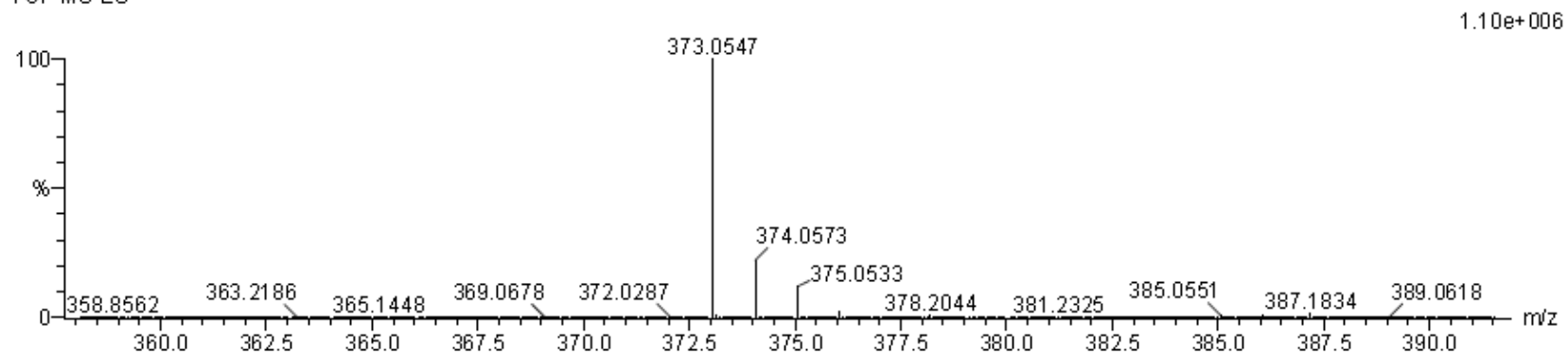
IR spectrum of 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) (C1)

Elemental Composition Report



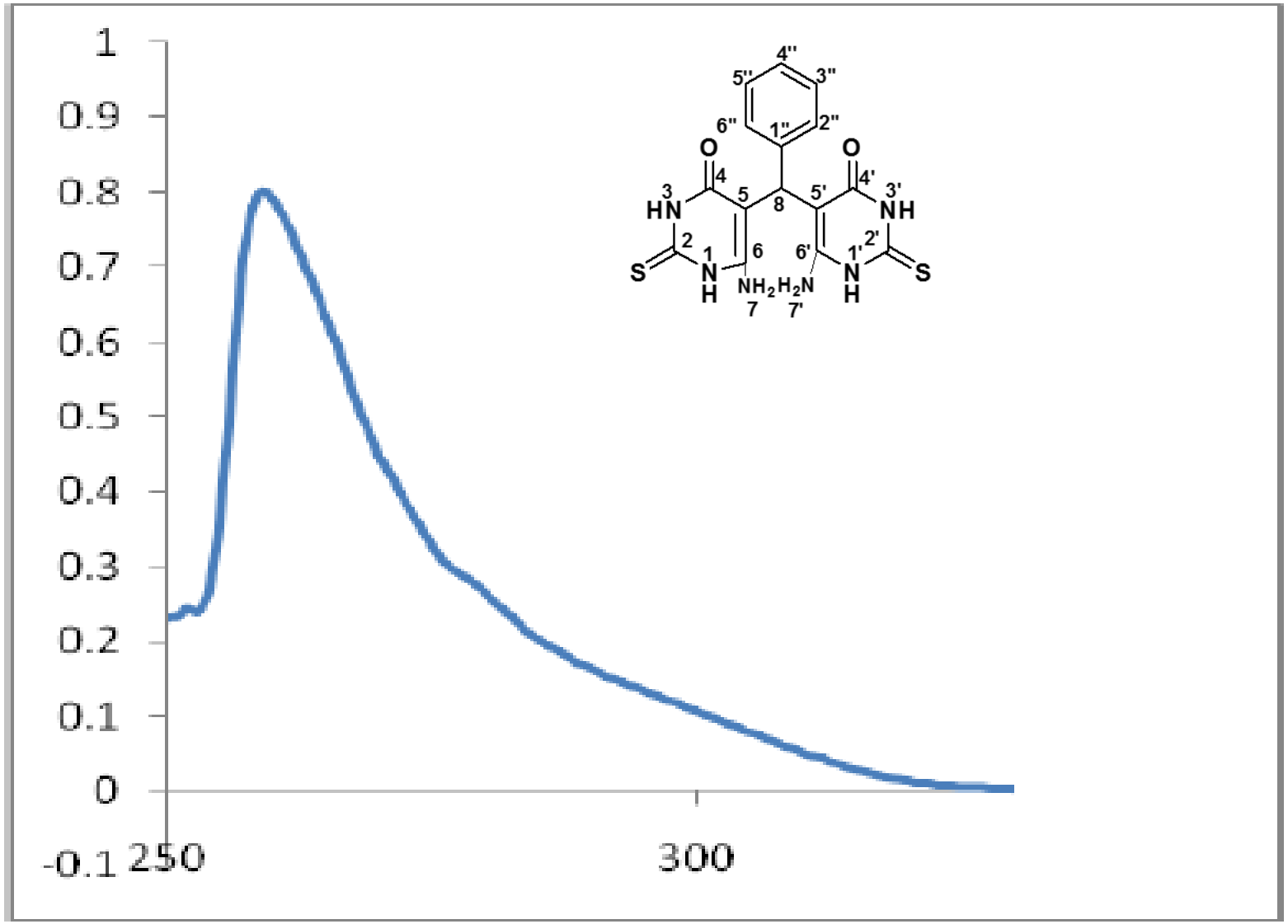
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
3489 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-30 H: 10-25 N: 5-15 O: 0-10 F: 0-10 S: 0-10
7 32 (1.047) Cm (1:61)
TOF MS ES-



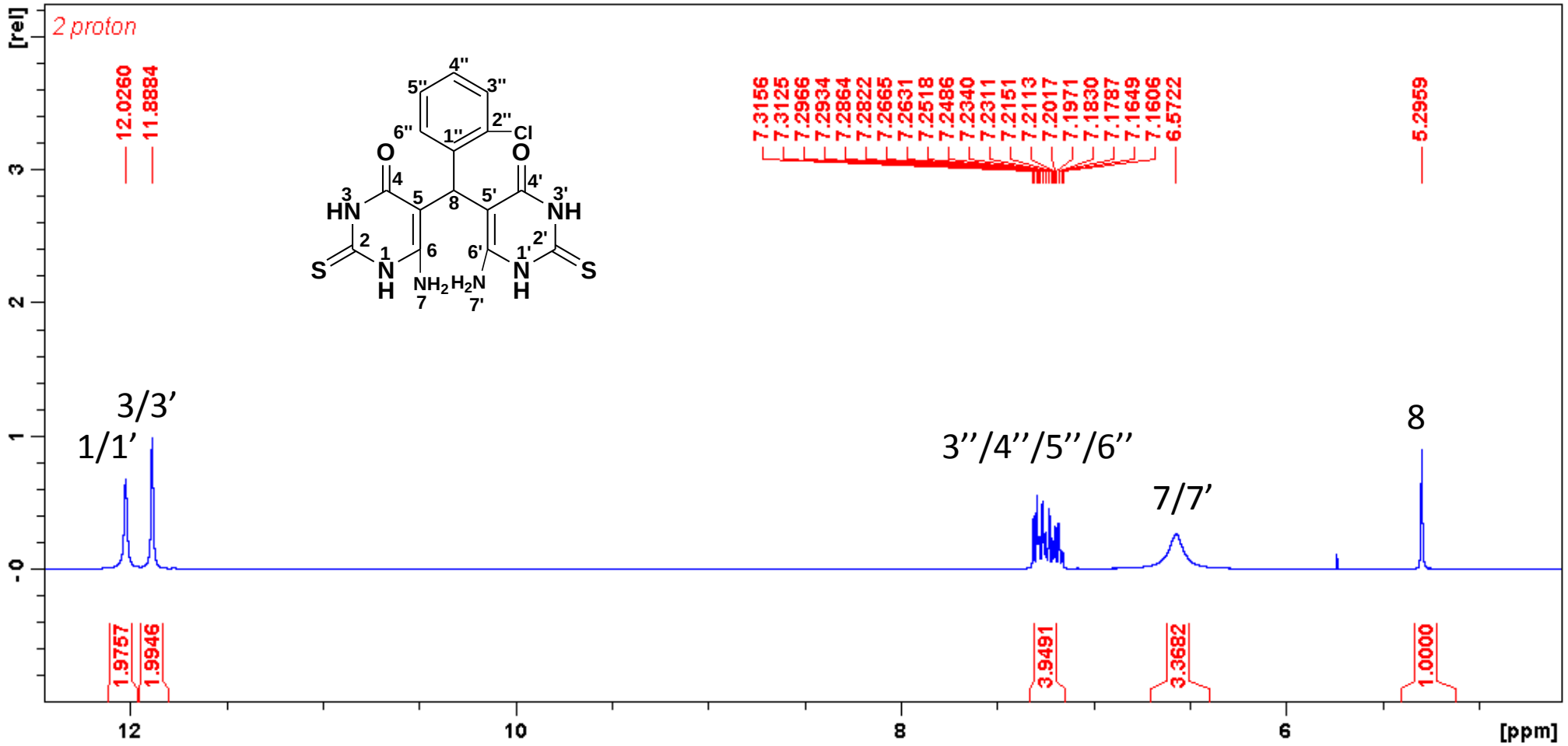
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
373.0547	373.0541	0.6	1.6	12.5	720.3	0.0	C15 H13 N6 O2 S2

HRMS of 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) (C1)

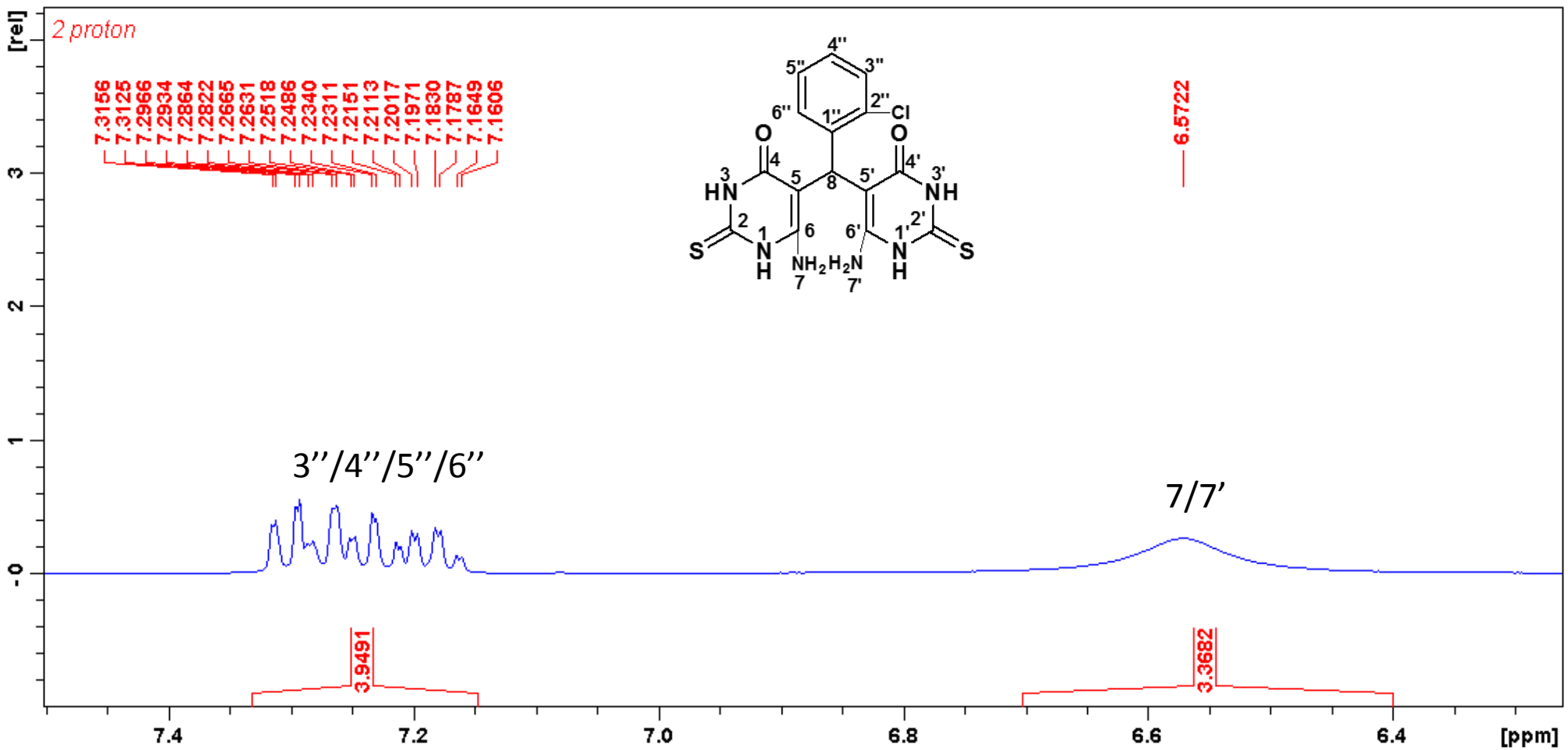


3a

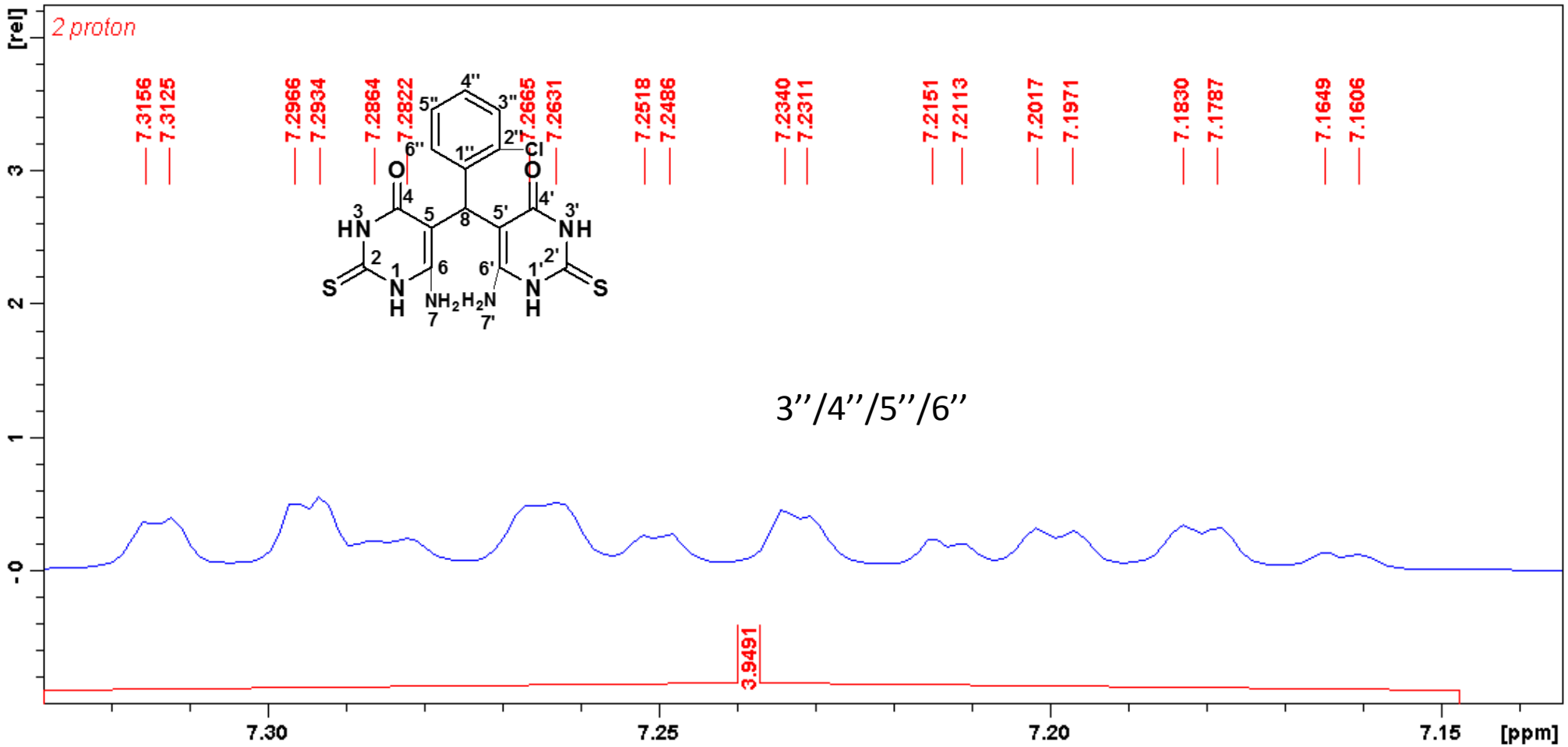
UV spectrum of 5,5'-(phenylmethylene)bis(6-amino-2-thiouracil) (C1)



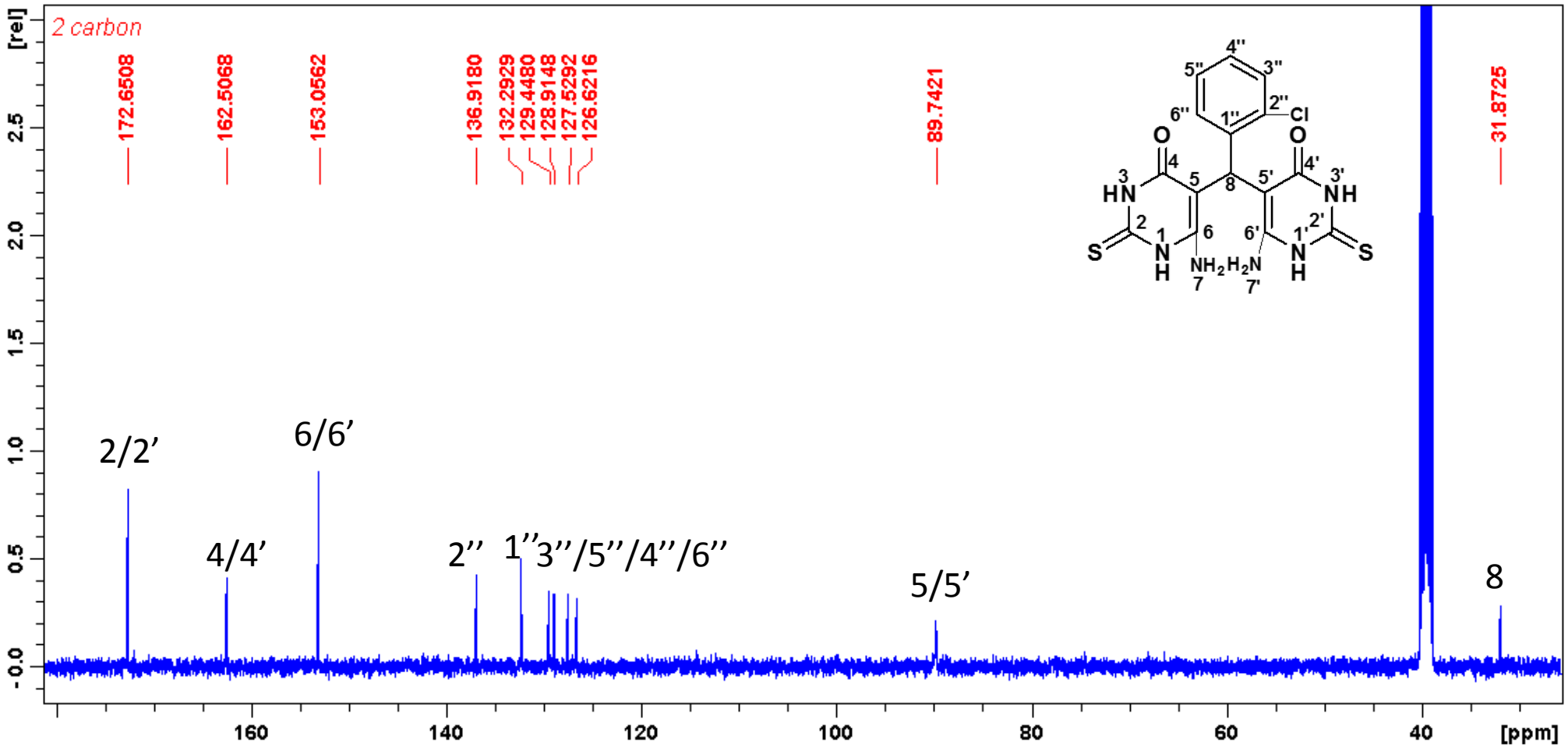
^{1}H NMR spectrum of 5,5'-((2-chlorophenyl)methylene)bis(6-amino-2-thiouracil) (C2)



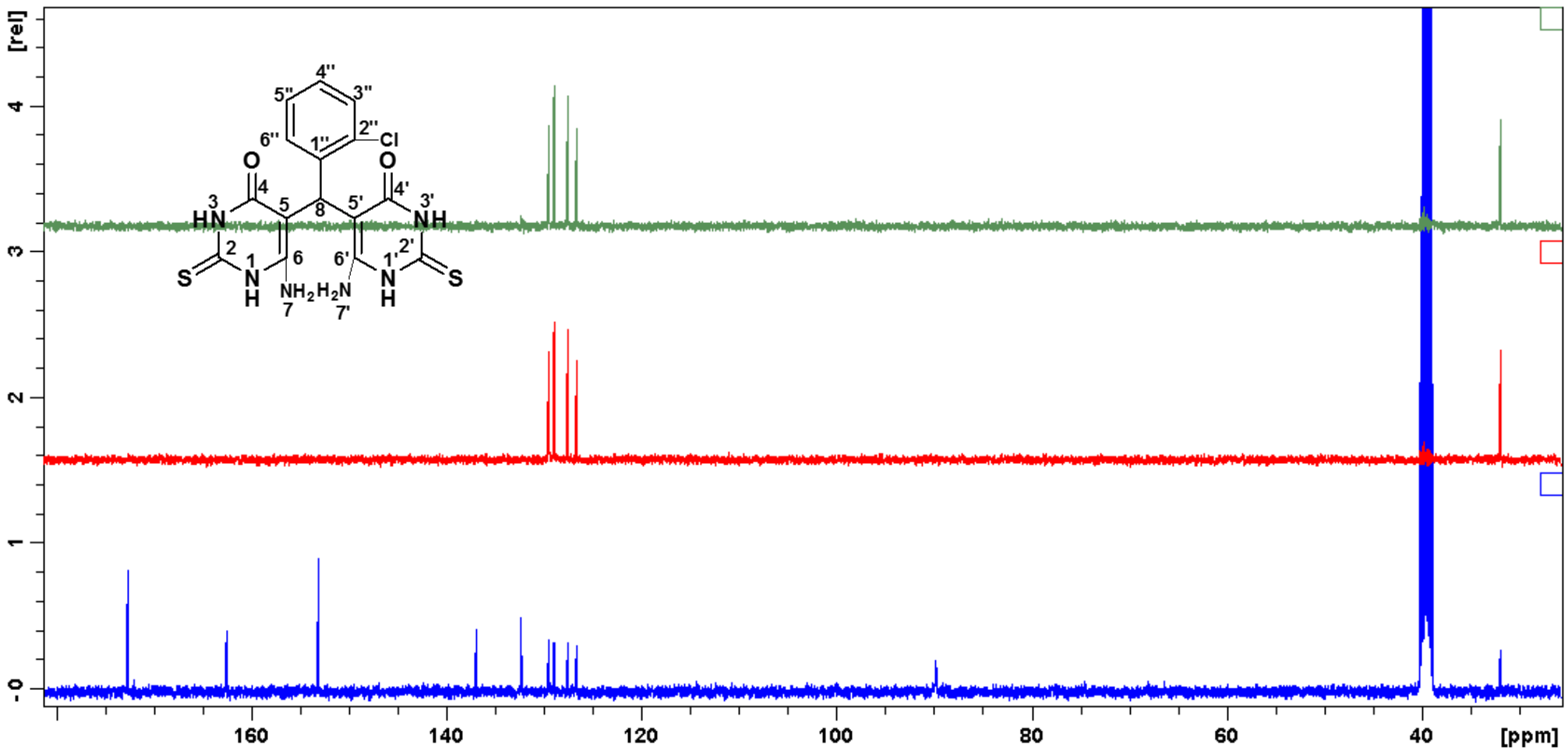
^{1}H NMR spectrum of 5,5'-((2-chlorophenyl)methylene)bis(6-amino-2-thiouracil) (C2)



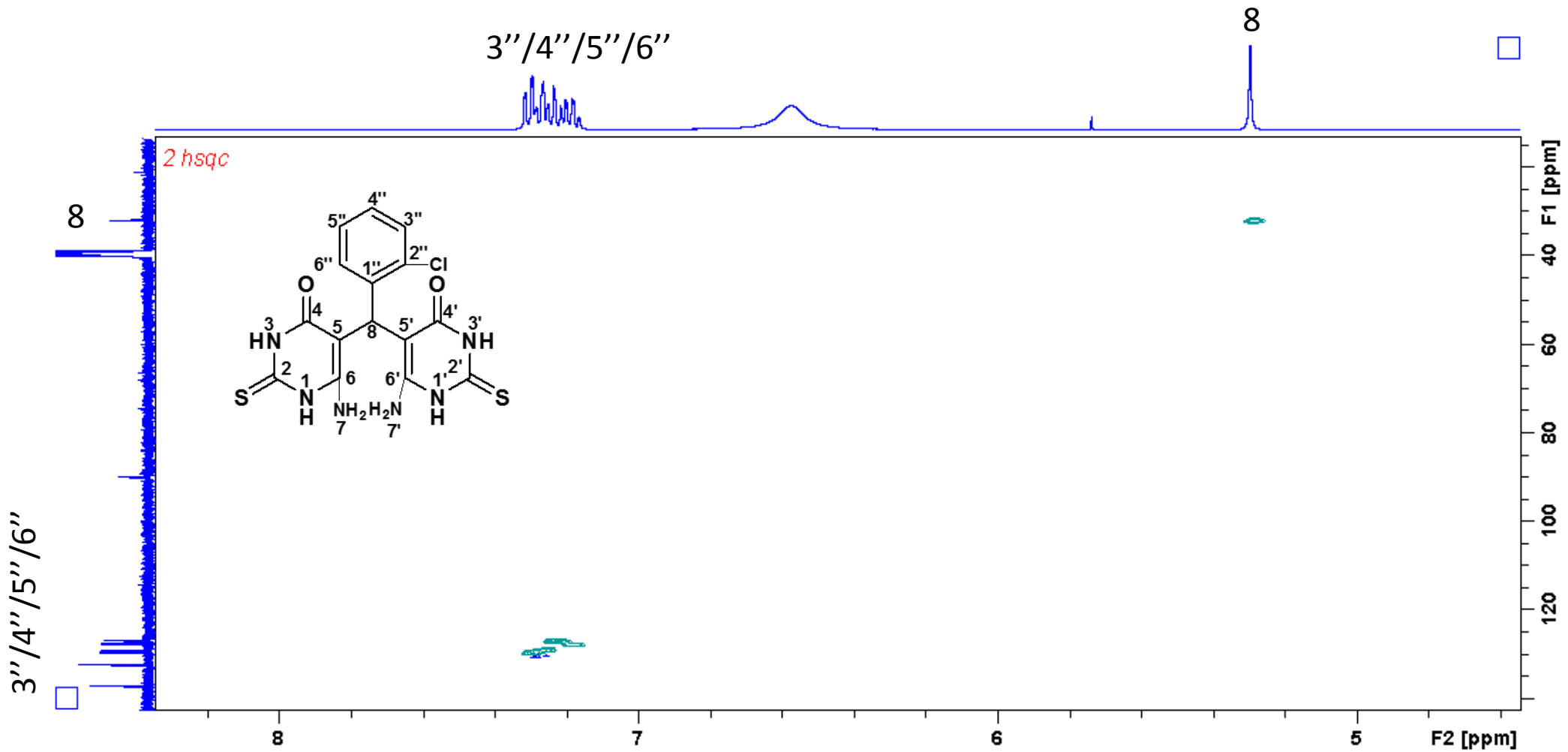
^{1}H NMR spectrum of 5,5'-((2-chlorophenyl)methylene)bis(6-amino-2-thiouracil) (C2)



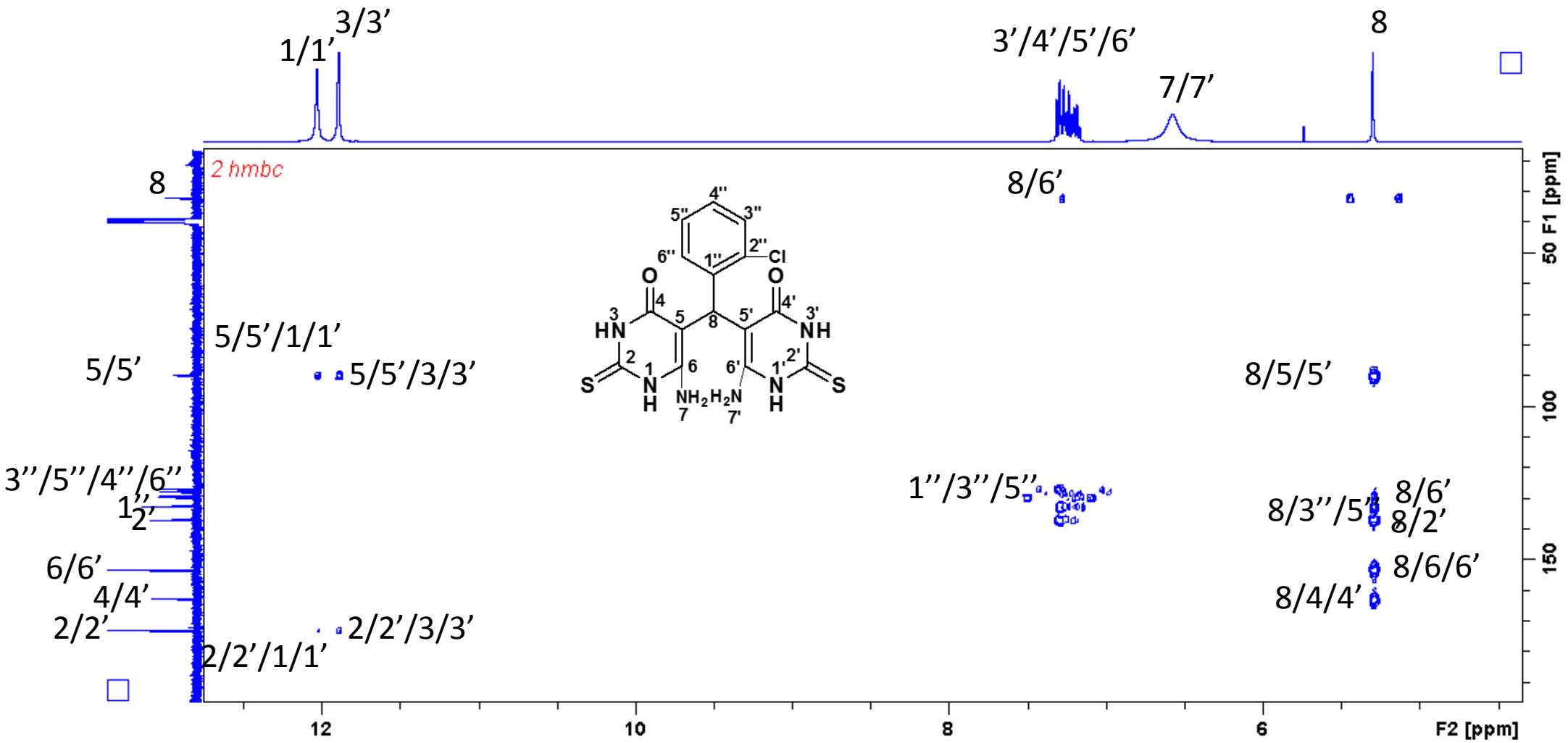
^{13}C NMR spectrum of 5,5'-((2-chlorophenyl)methylene)bis(6-amino-2-thiouracil) (C2)



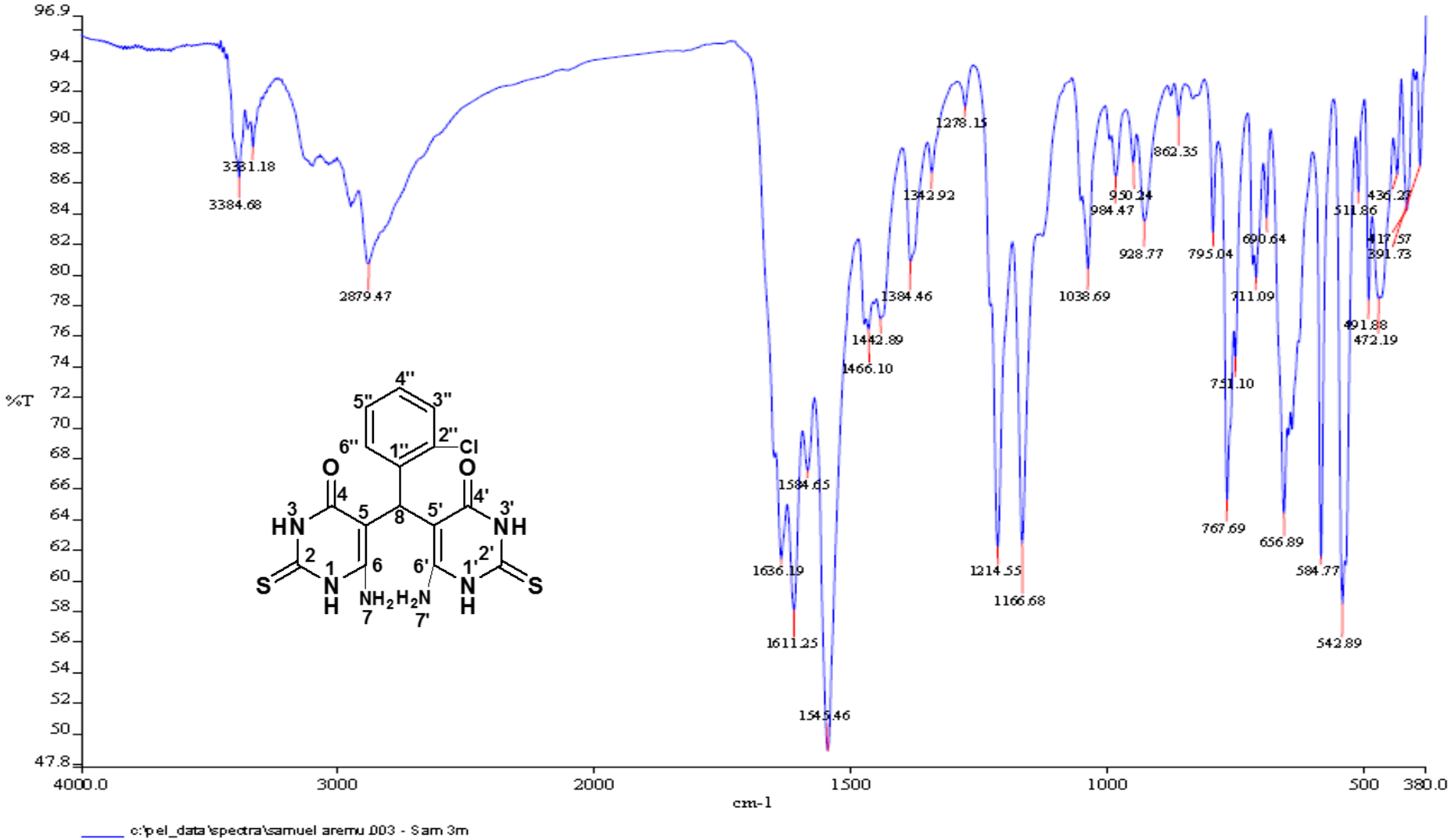
DEPT spectrum of 5,5'-((2-chlorophenyl)methylene)bis(6-amino-2-thiouracil) (C2)



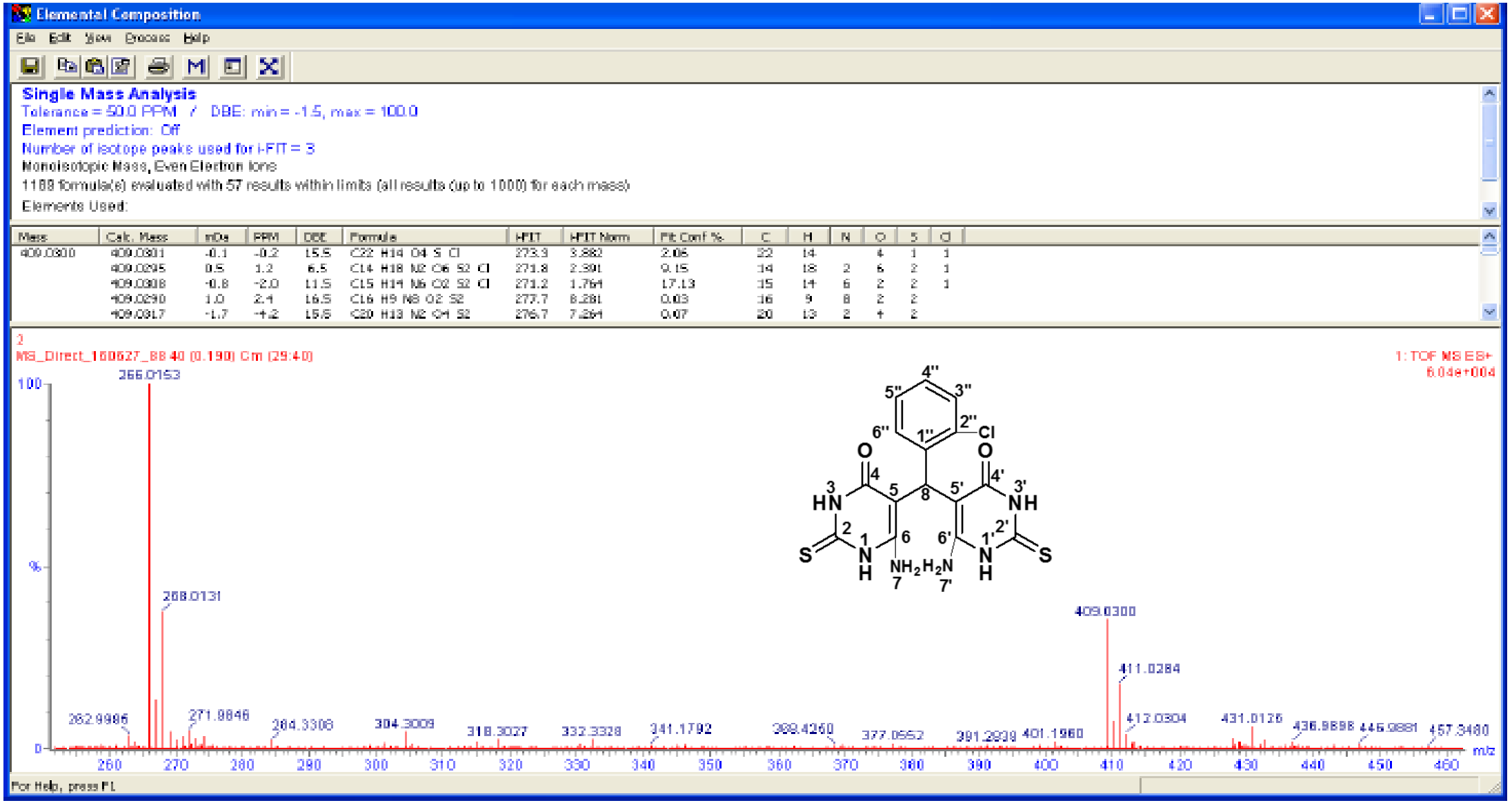
HSQC spectrum of 5,5'-((2-chlorophenyl)methylene)bis(6-amino-2-thiouracil) (C2)



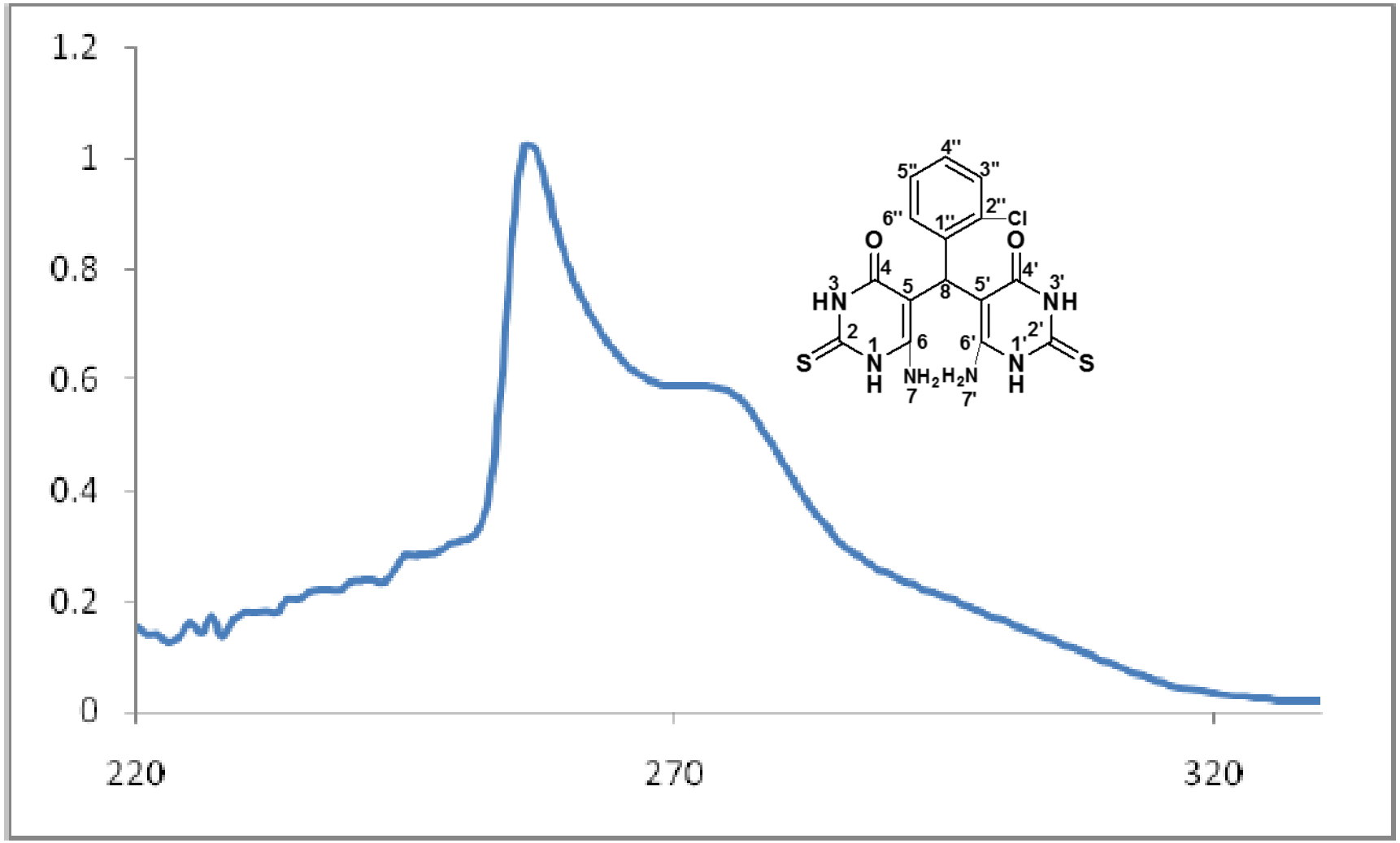
HMBC spectrum of 5,5'-((2-chlorophenyl)methylene)bis(6-amino-2-thiouracil) (C2)



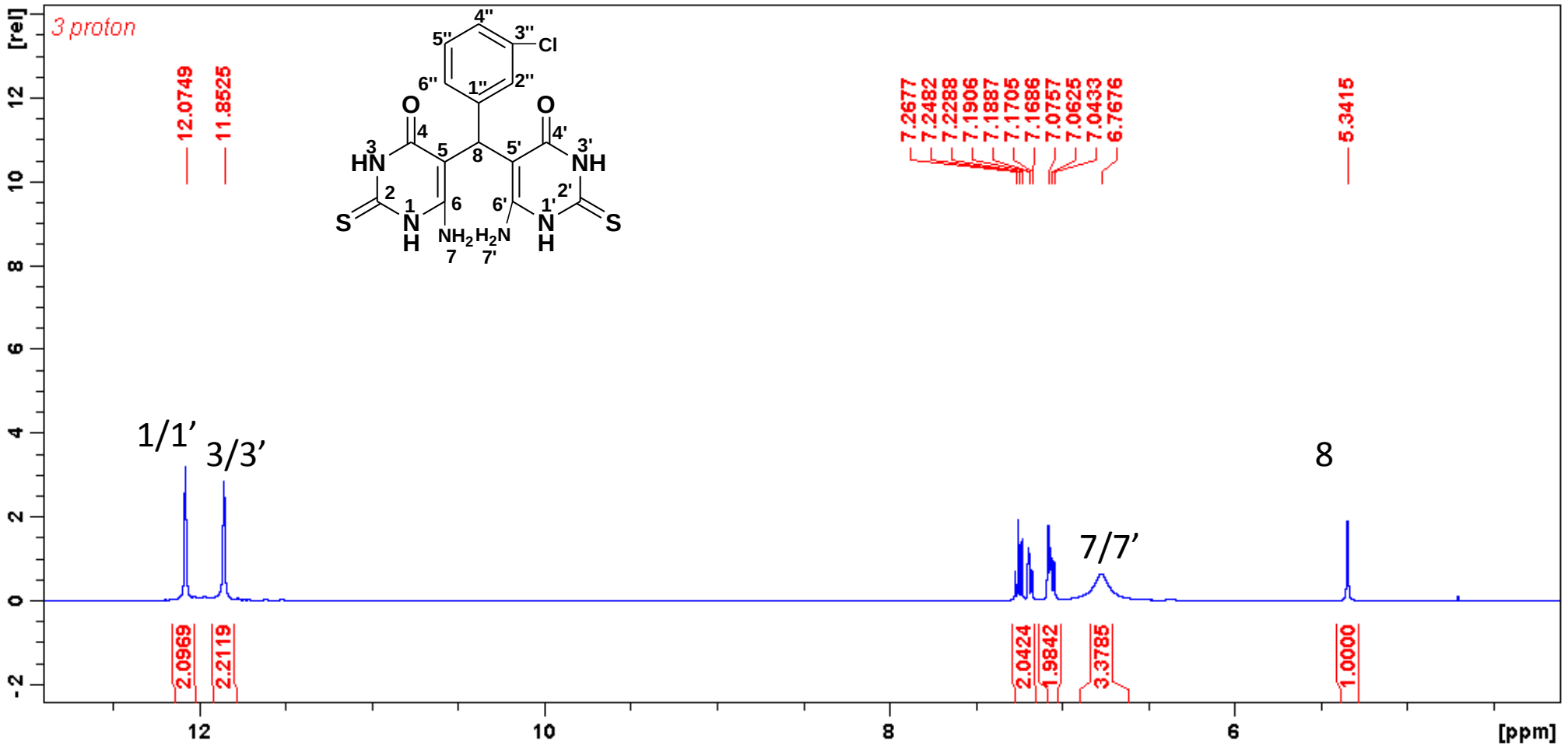
IR spectrum of 5,5'-((2-chlorophenyl)methylene)bis(6-amino-2-thiouracil) (C2)



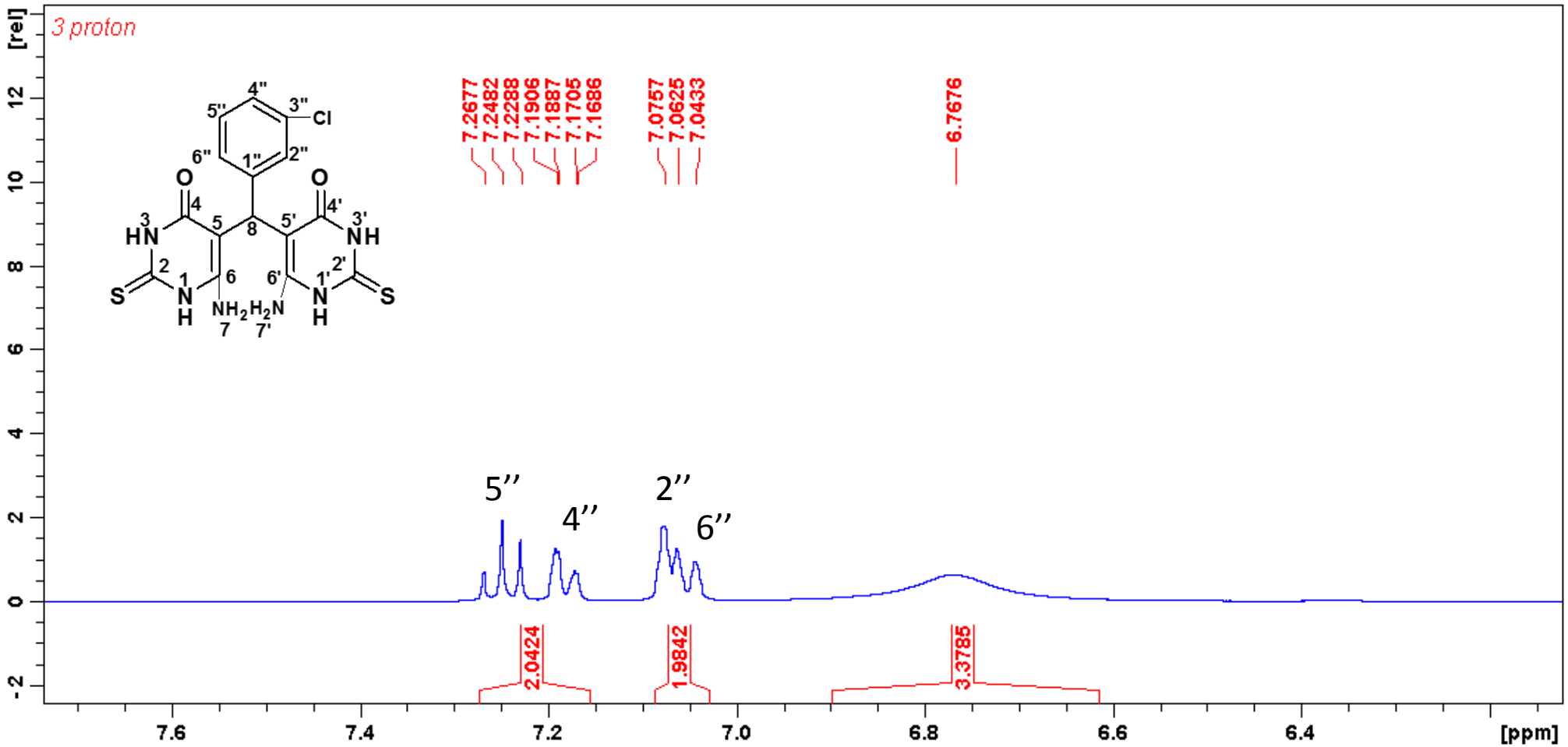
HRMS of 5,5'-((2-chlorophenyl)methylene)bis(6-amino-2-thiouracil) (C2)



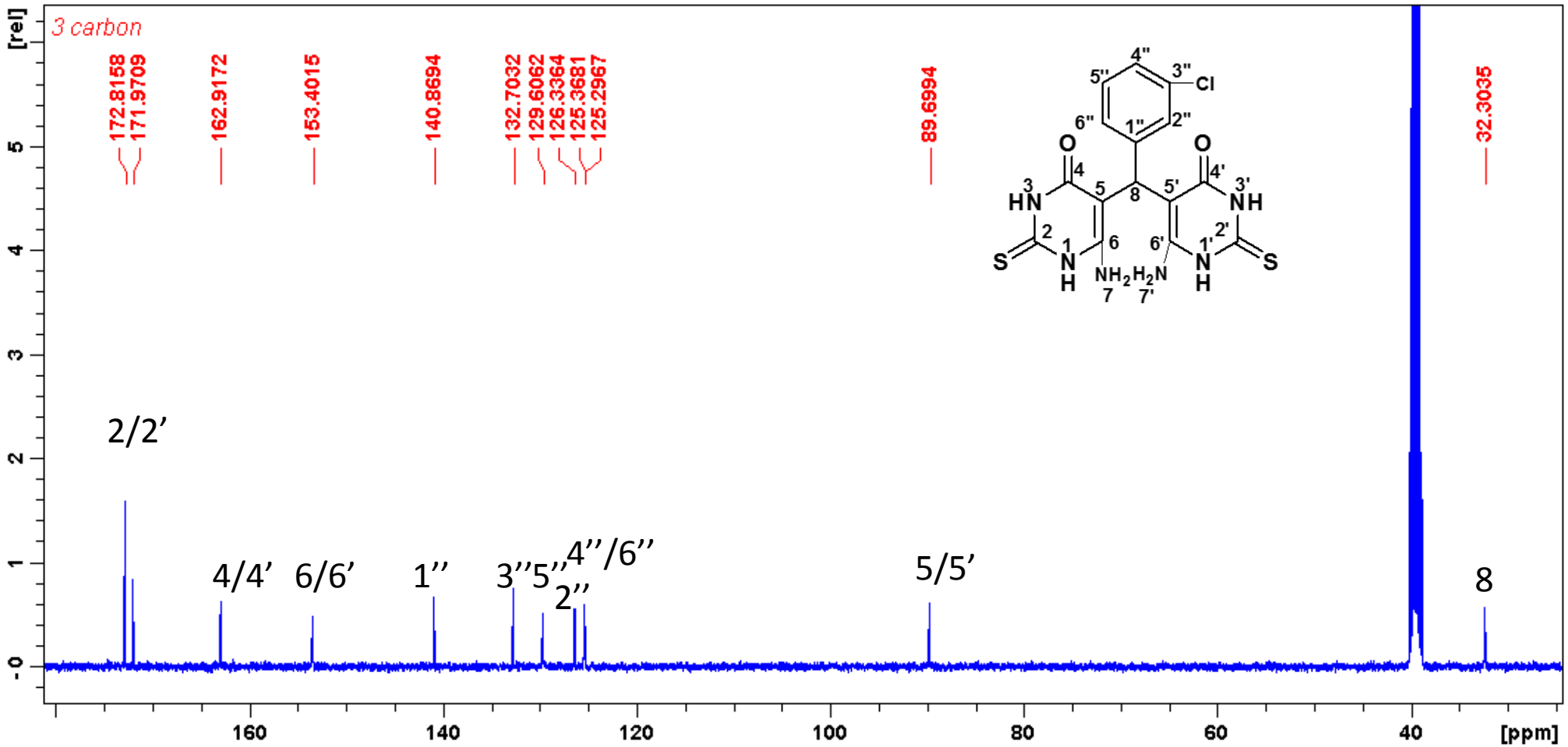
UV spectrum of 5,5'-((2-chlorophenyl)methylene)bis(6-amino-2-thiouracil) (C2)



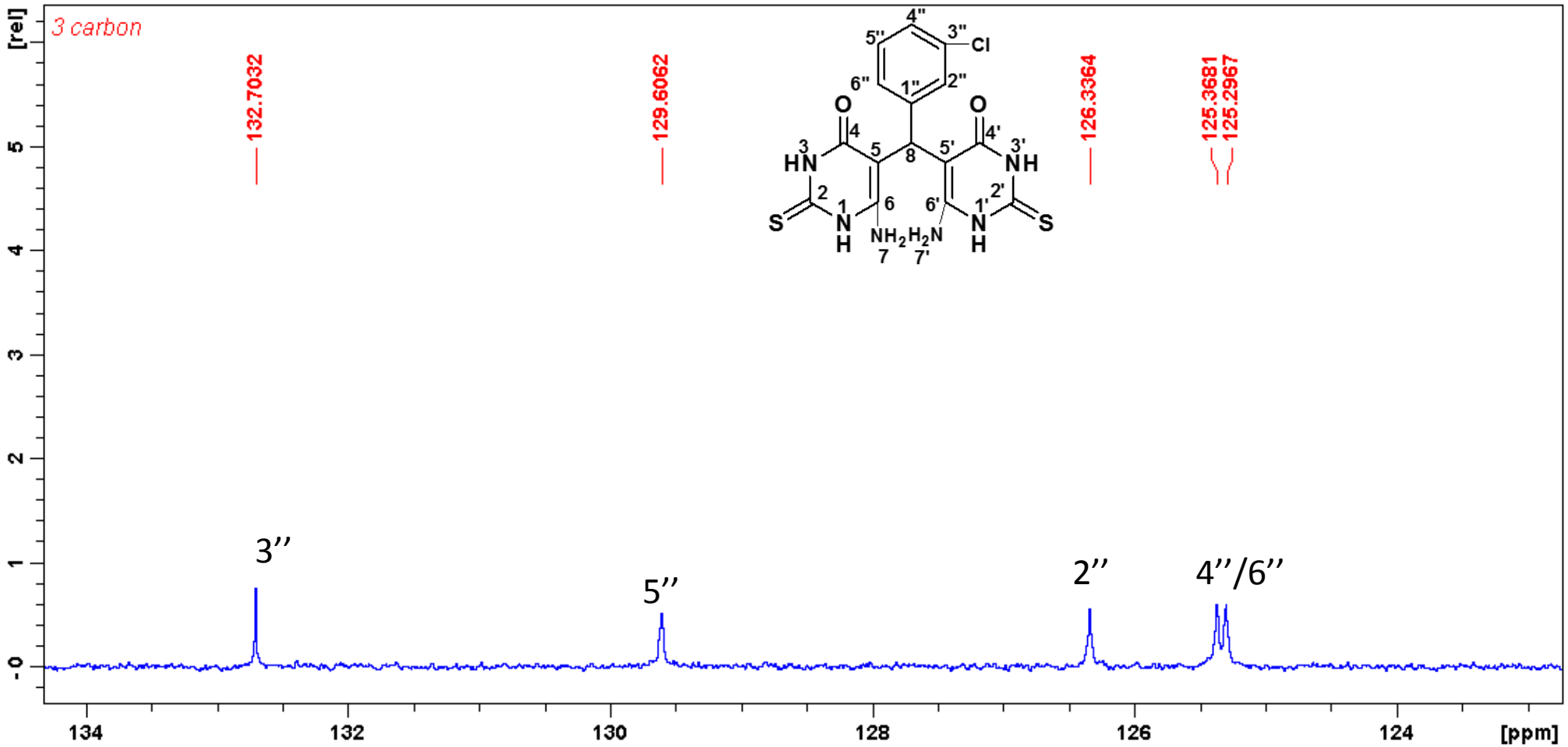
^{1}H NMR spectrum of 5,5'-((3-chlorophenyl)methylene)bis(6-amino-2-thiouracil) (C3)



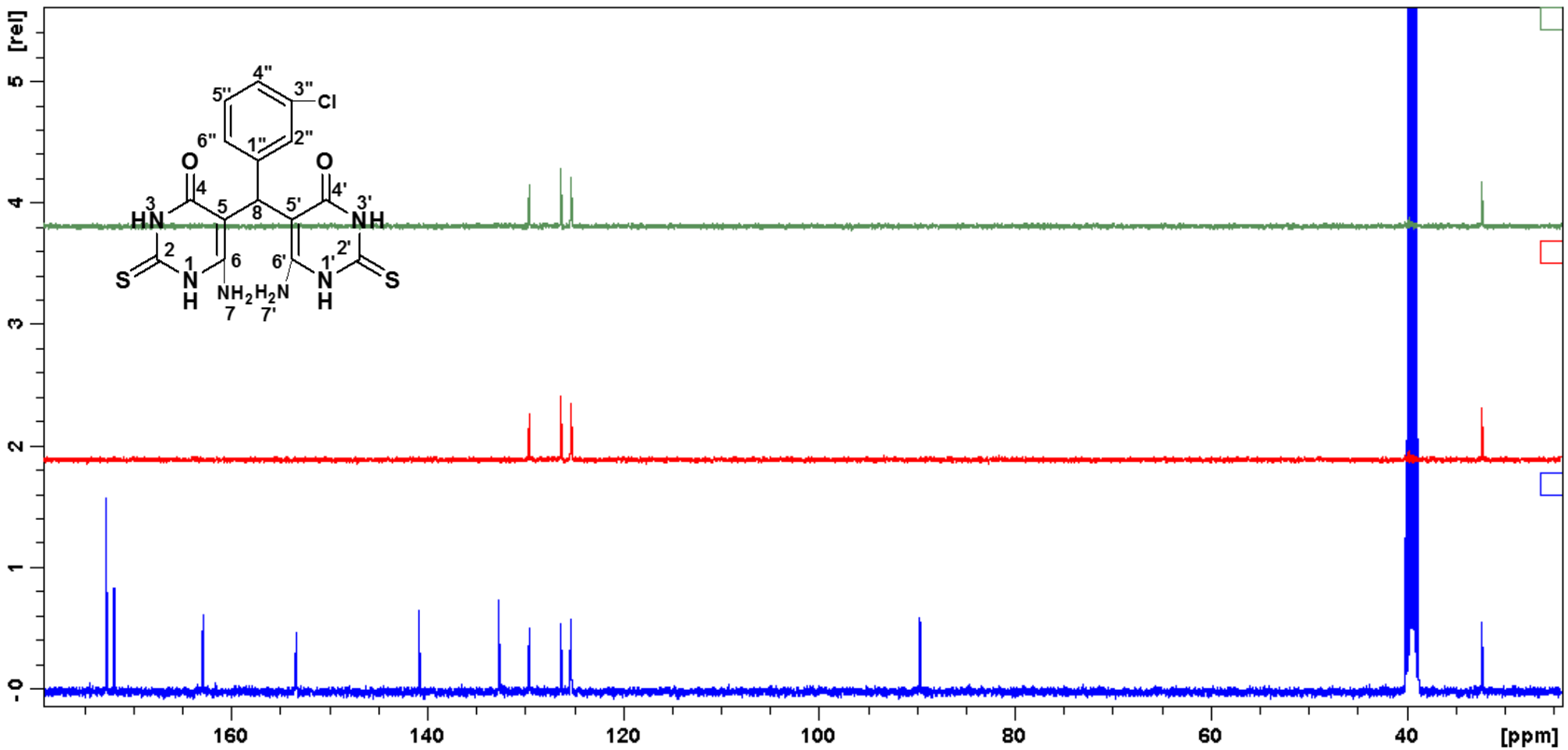
^{1}H NMR spectrum of 5,5'-((3-chlorophenyl)methylene)bis(6-amino-2-thiouracil) (C3)



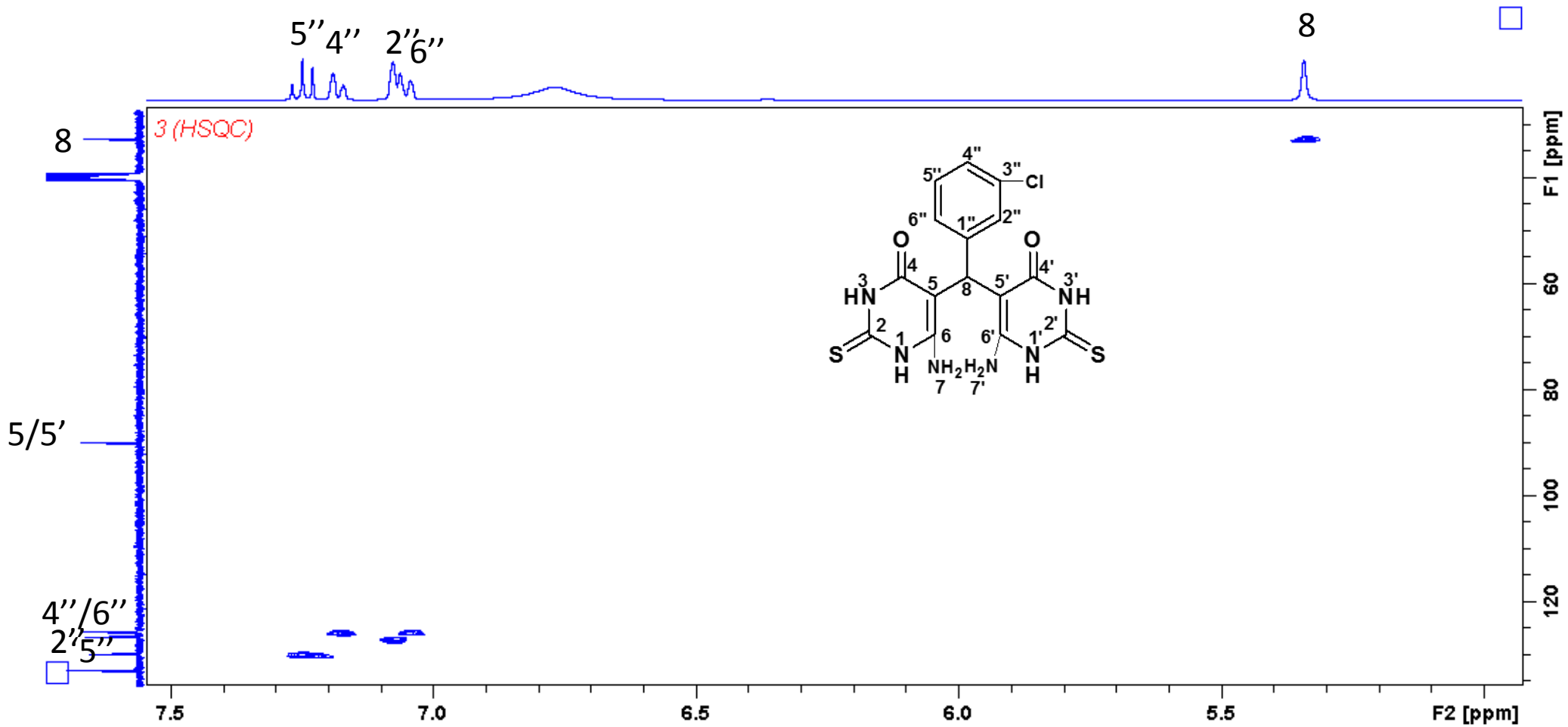
^{13}C NMR spectrum of 5,5'-((3-chlorophenyl)methylene)bis(6-amino-2-thiouracil) (C3)



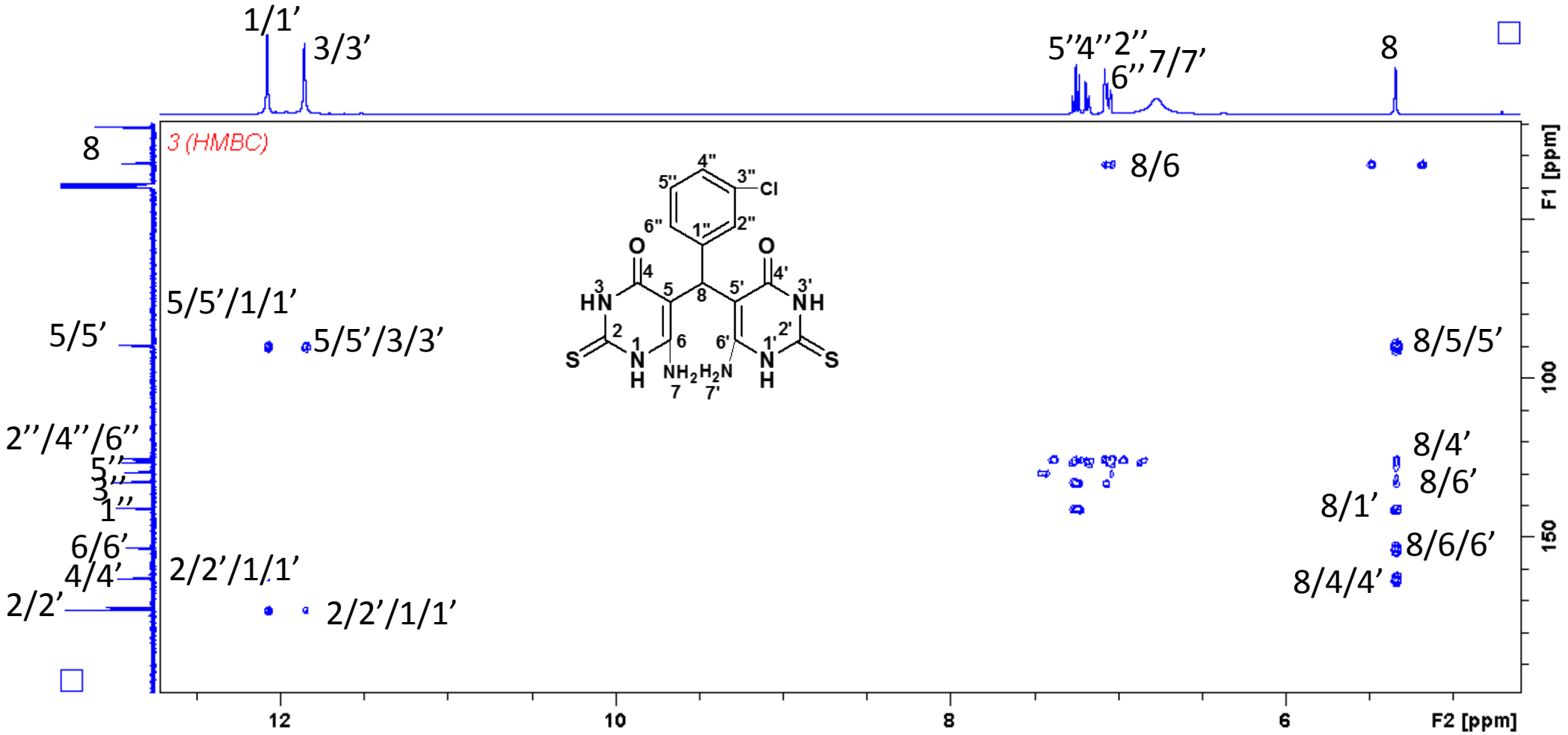
^{13}C NMR spectrum of 5,5'-((3-chlorophenyl)methylene)bis(6-amino-2-thiouracil) (C3)



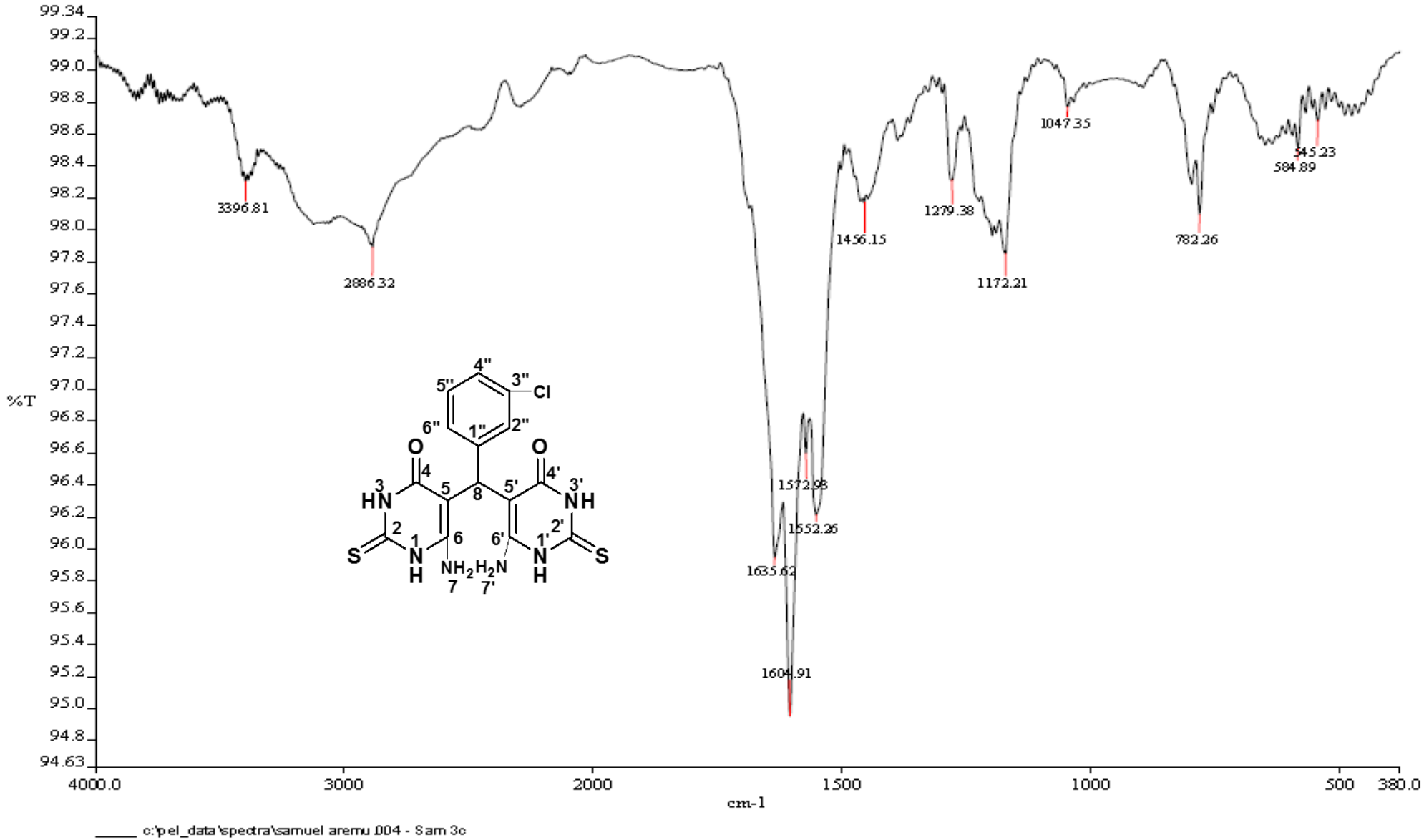
DEPT spectrum of 5,5'-((3-chlorophenyl)methylene)bis(6-amino-2-thiouracil) (C3)



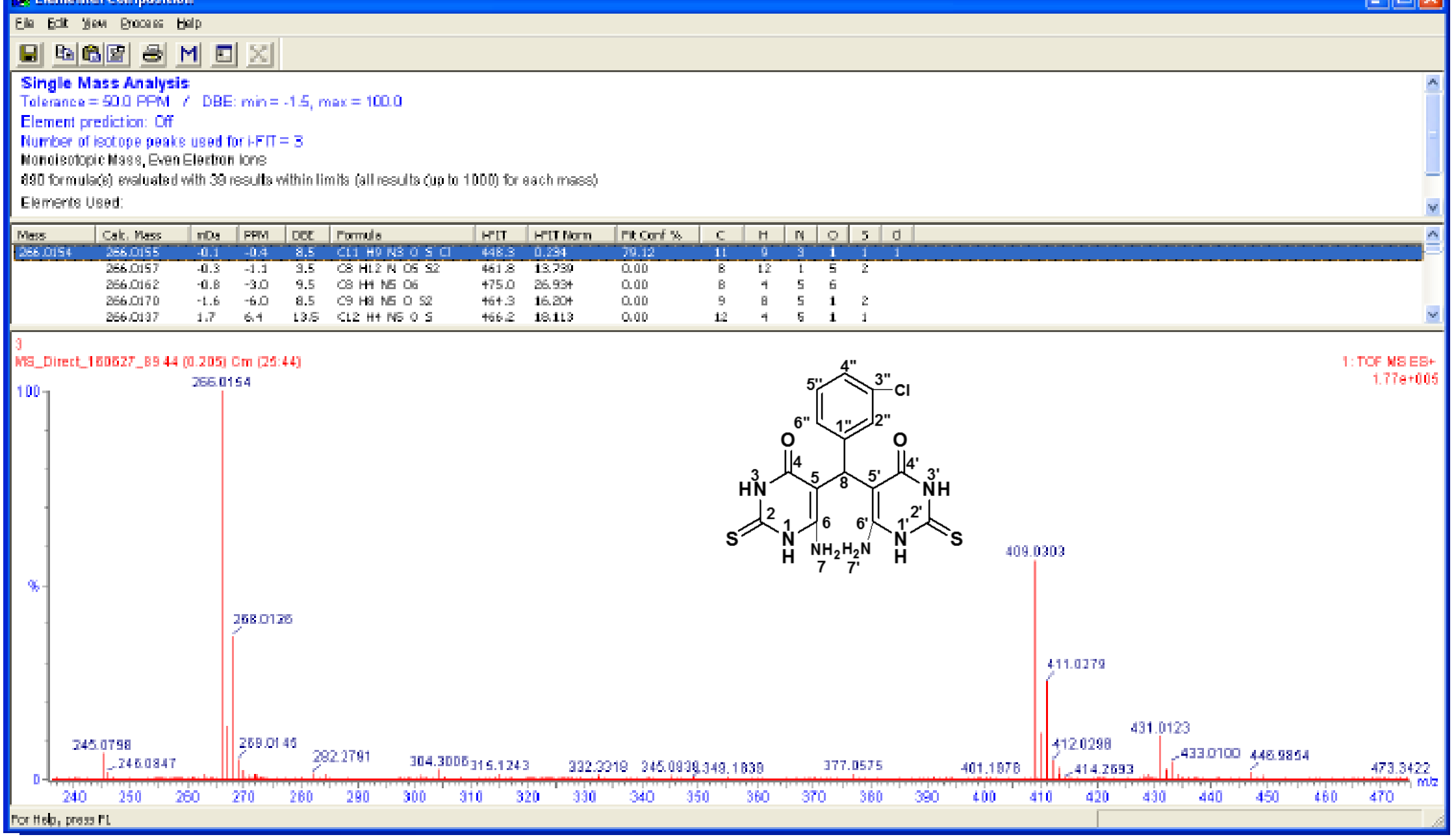
HSQC spectrum of 5,5'-((3-chlorophenyl)methylene)bis(6-amino-2-thiouracil) (C3)



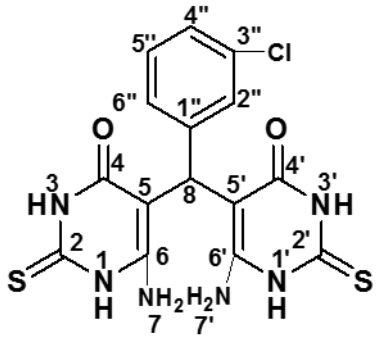
HMBC spectrum of 5,5'-((3-chlorophenyl)methylene)bis(6-amino-2-thiouracil) (C3)

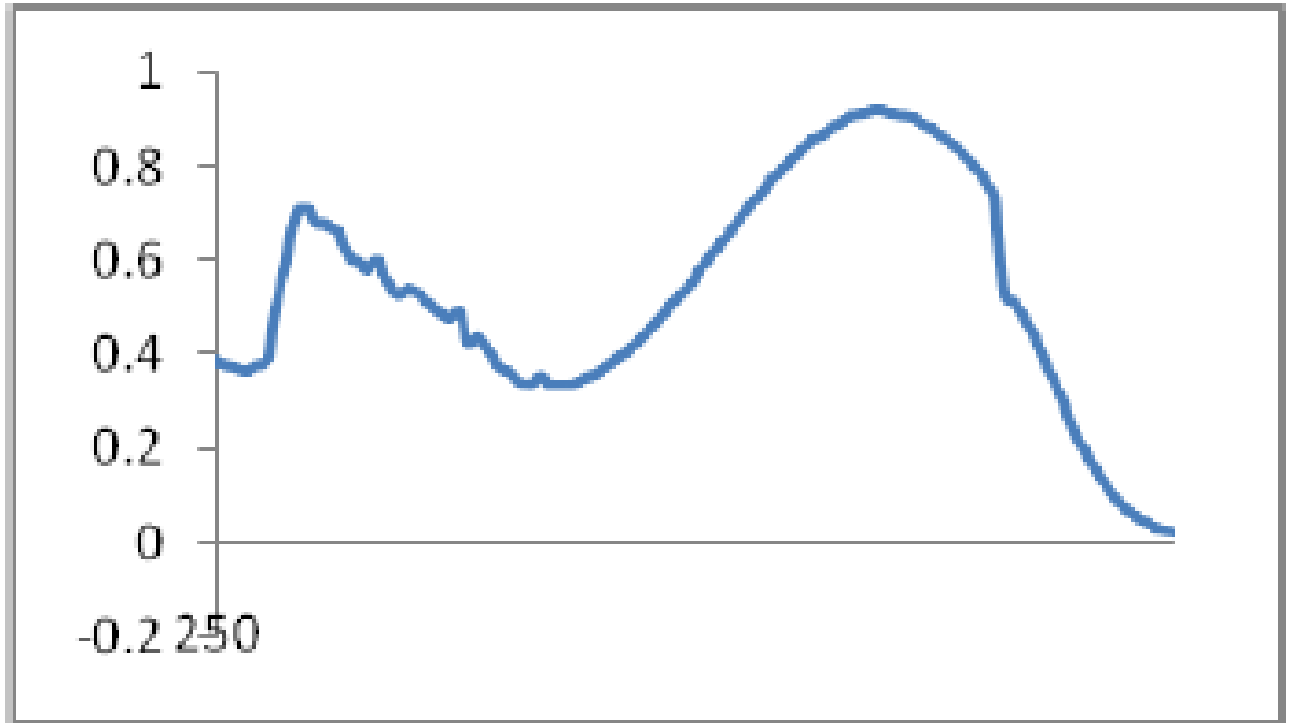


IR spectrum of 5,5'-((3-chlorophenyl)methylene)bis(6-amino-2-thiouracil) (C3)

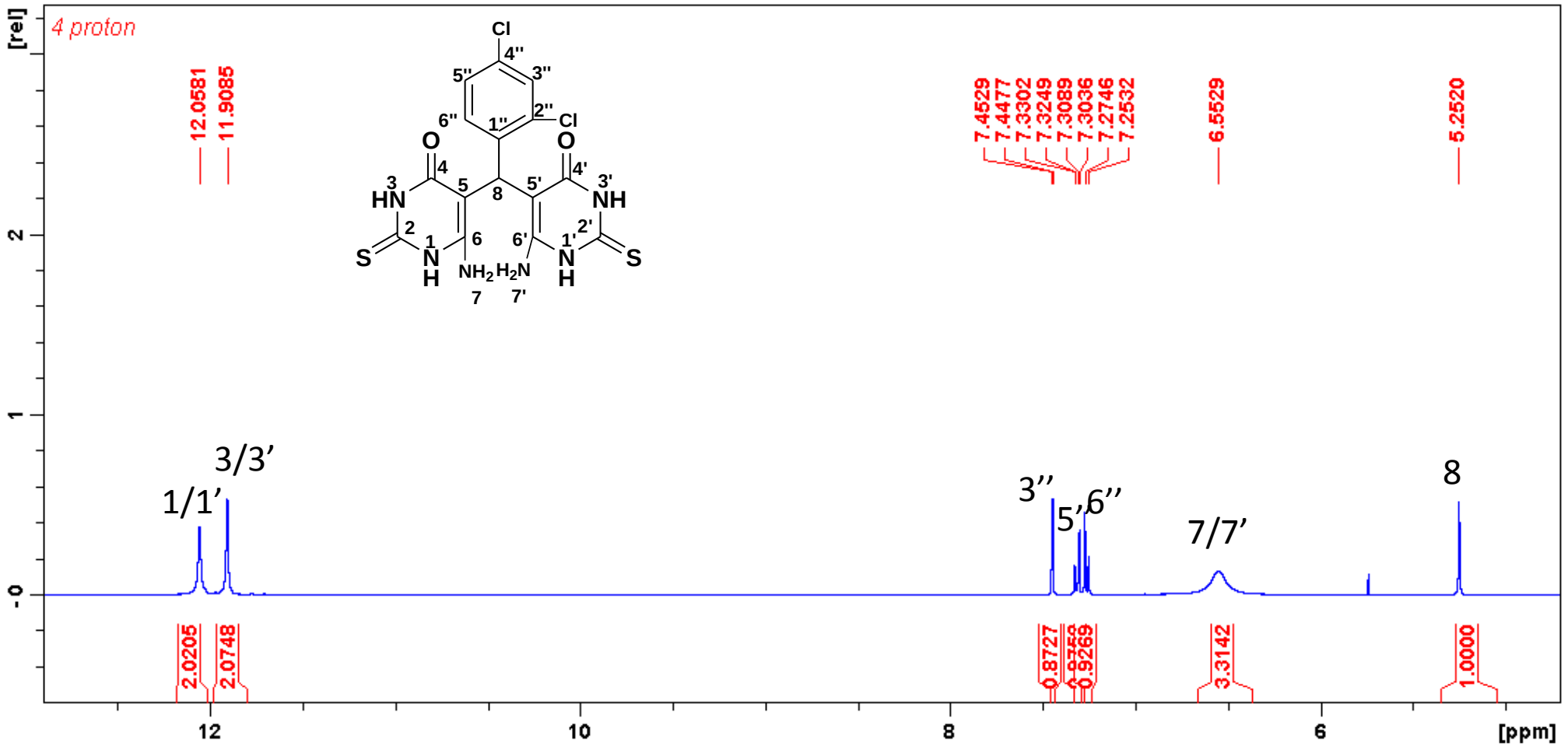


HRMS of 5,5'-((3-chlorophenyl)methylene)bis(6-amino-2-thiouracil) (C3)

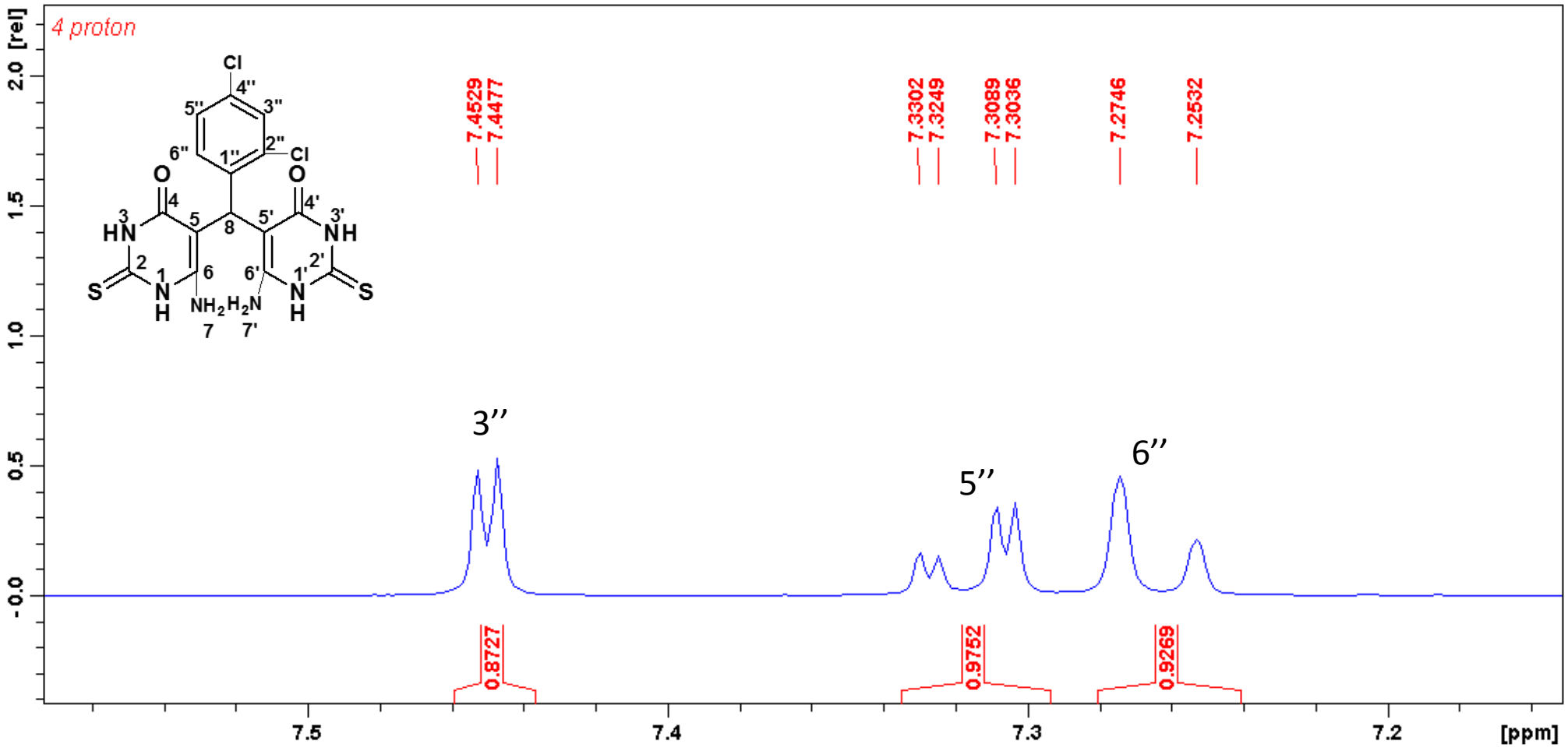




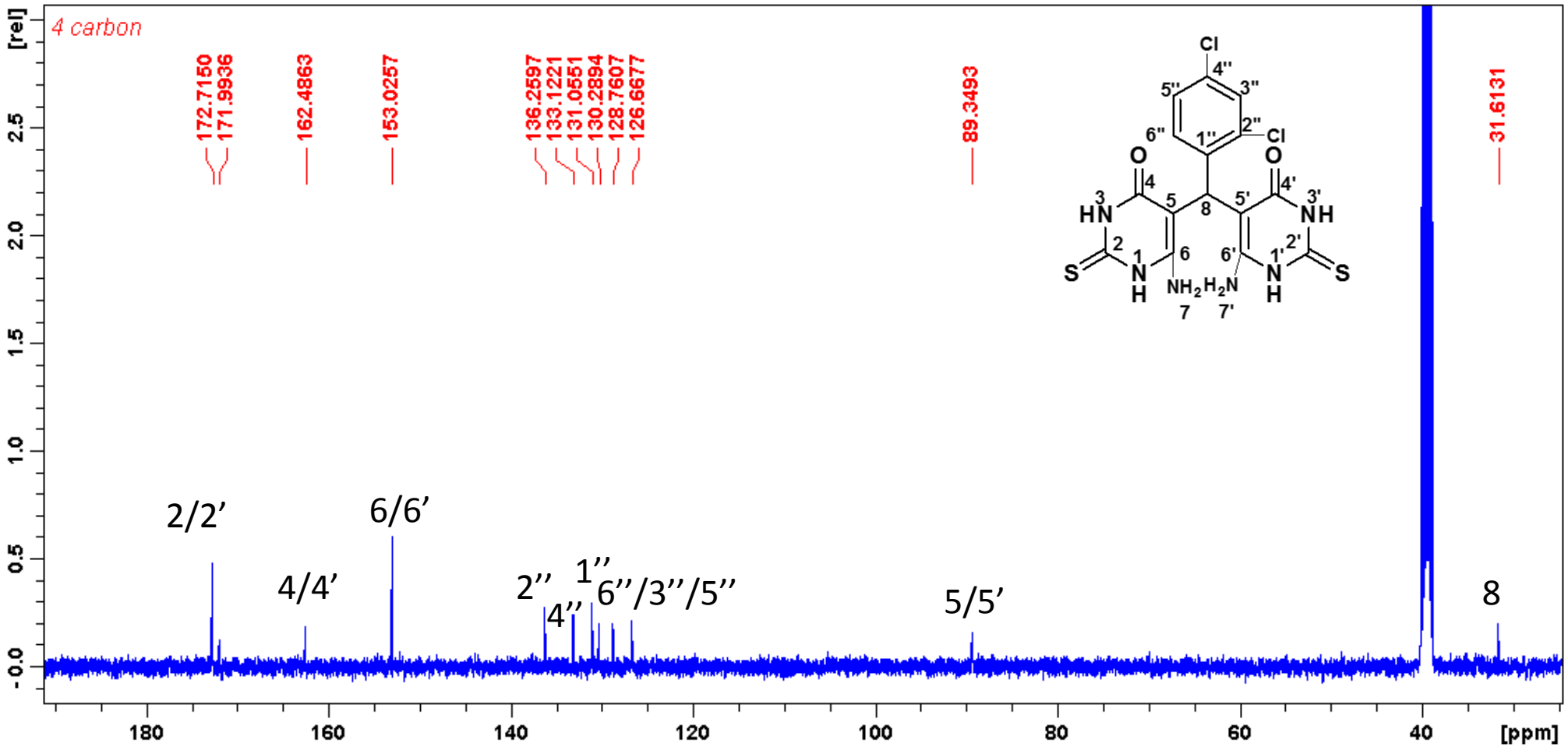
UV spectrum of 5,5'-((3-chlorophenyl)methylene)bis(6-amino-2-thiouracil) (C3)



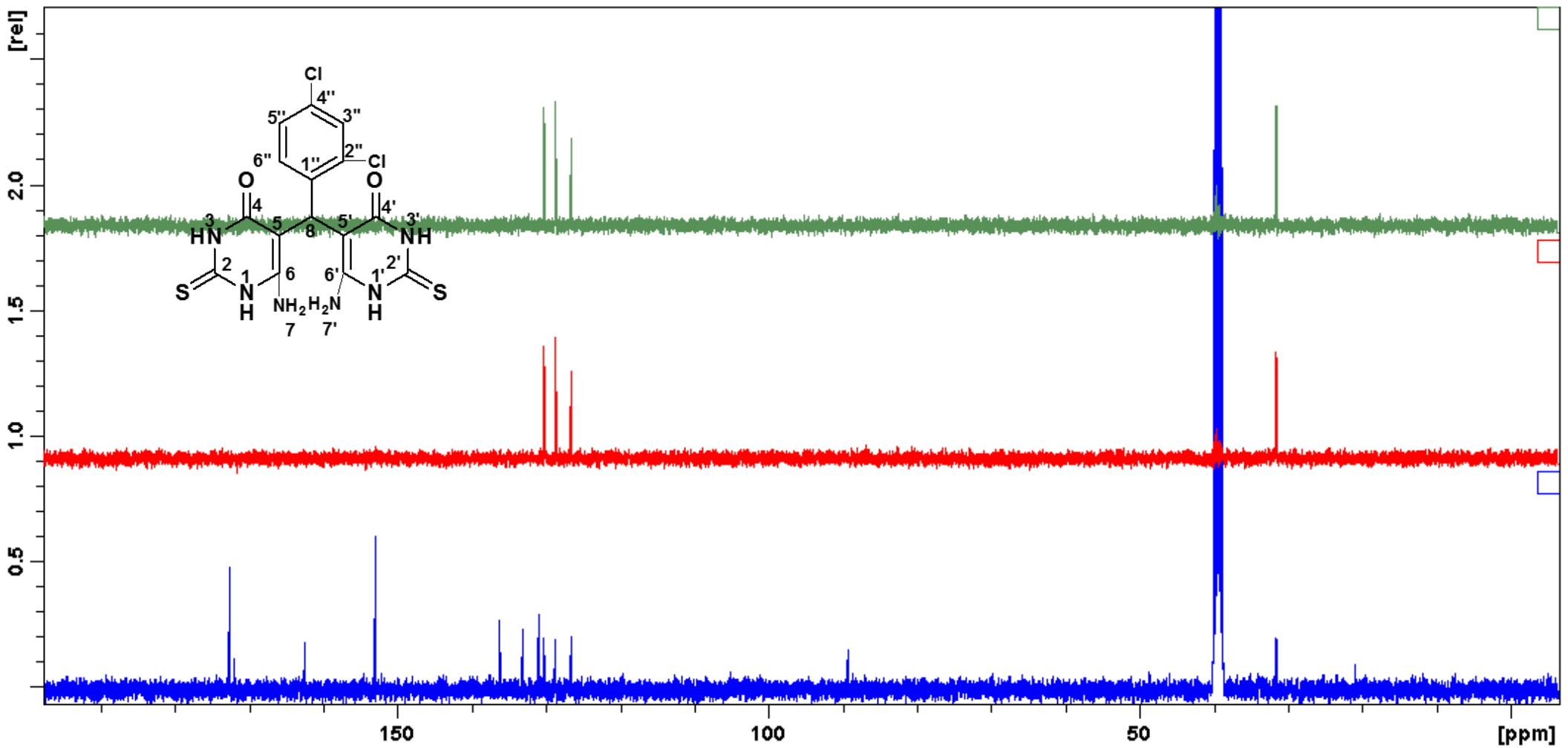
^{1}H NMR spectrum of 5,5'-((2,4-dichlorophenyl)methylene)bis(6-amino-2-thiouracil) (C4)



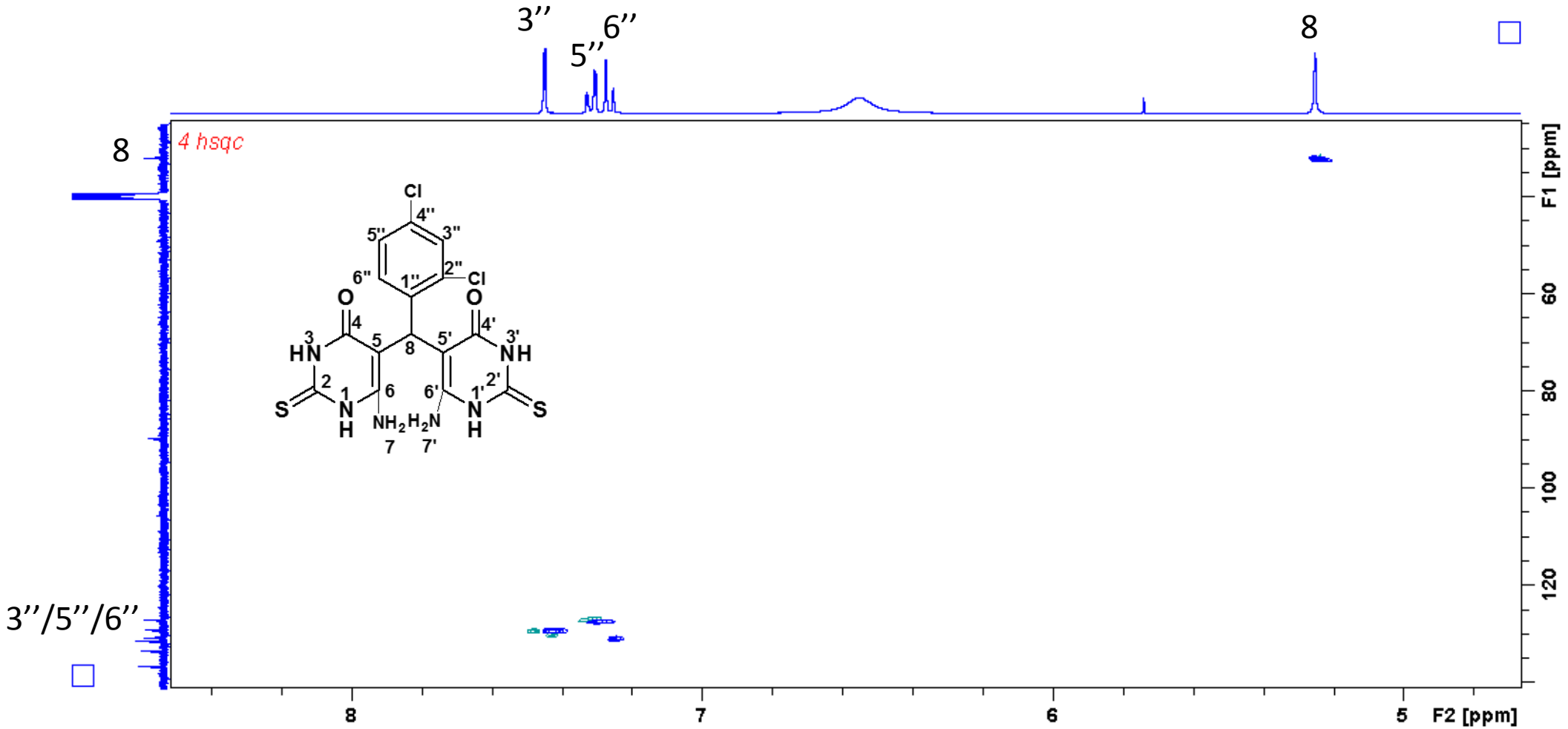
^{1}H NMR spectrum of 5,5'-((2,4-dichlorophenyl)methylene)bis(6-amino-2-thiouracil) (C4)



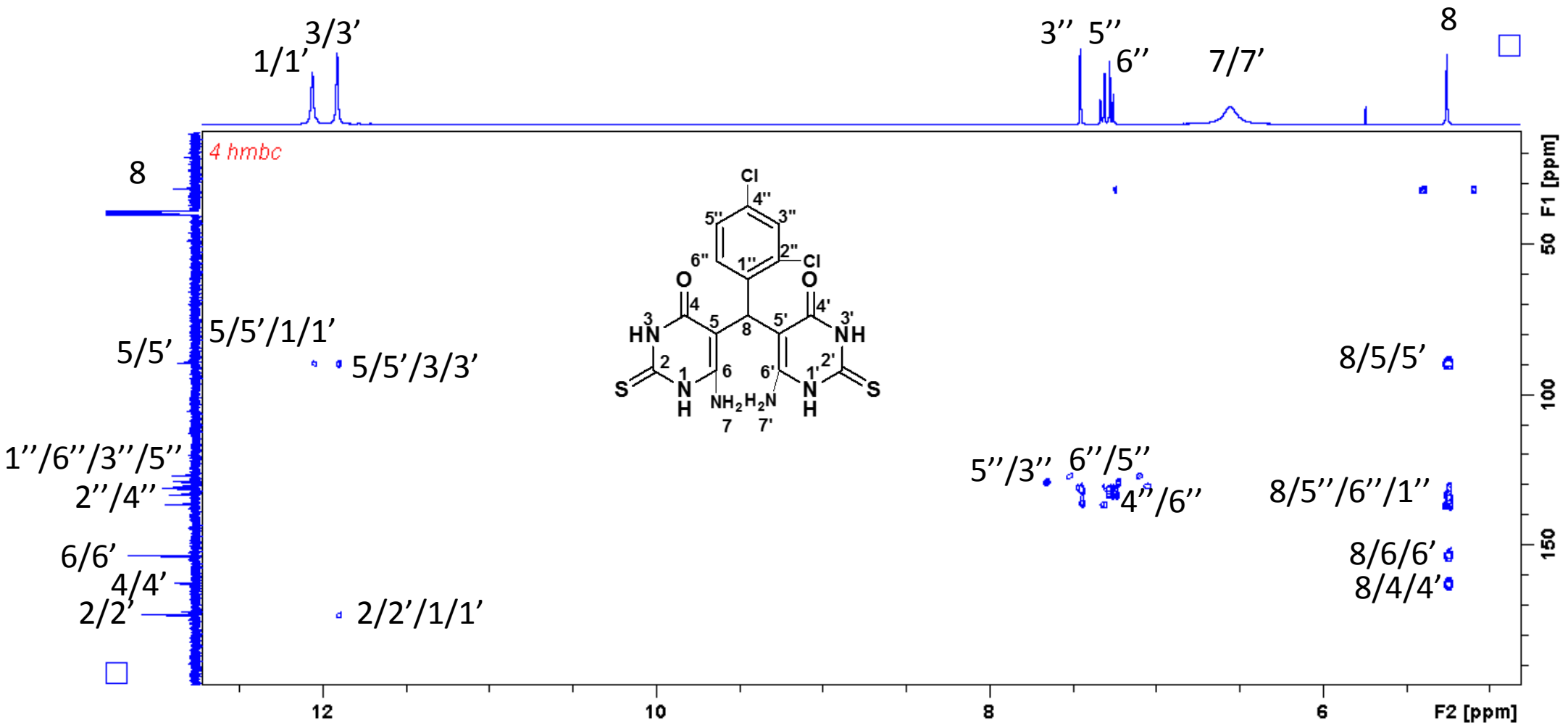
^{13}C NMR spectrum of 5,5'-((2,4-dichlorophenyl)methylene)bis(6-amino-2-thiouracil) (C4)



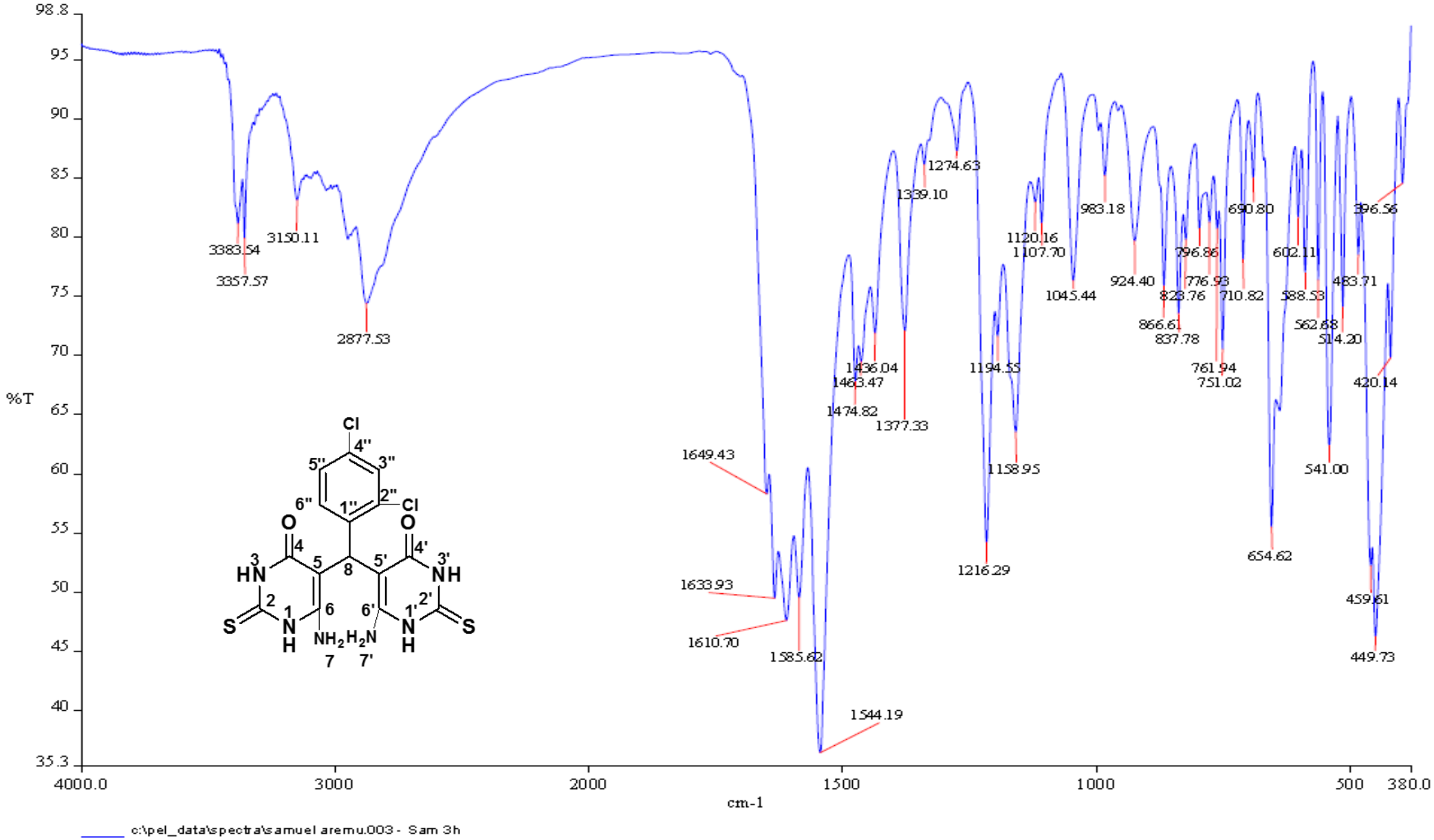
DEPT spectrum of 5,5'-((2,4-dichlorophenyl)methylene)bis(6-amino-2-thiouracil) (C4)



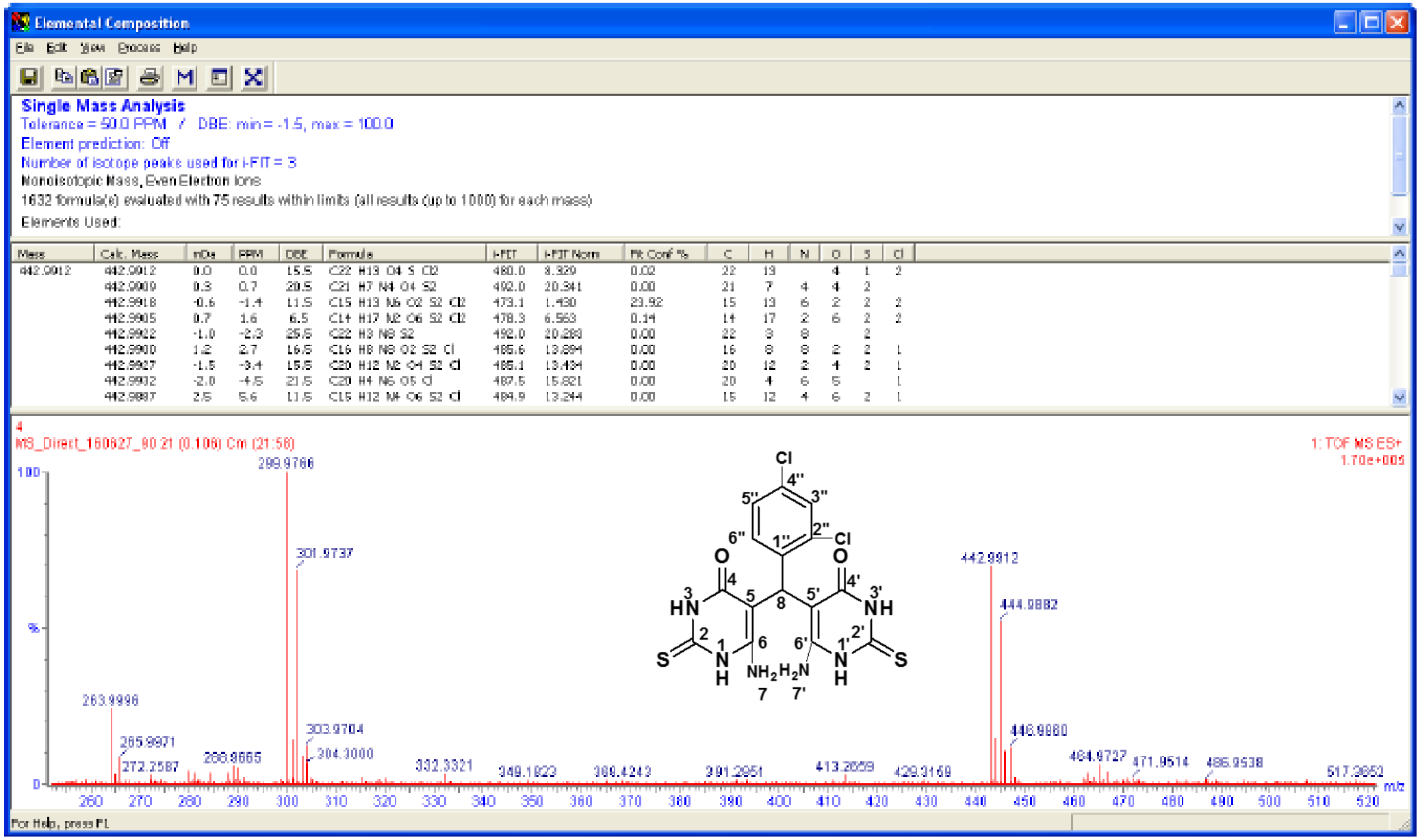
HSQC spectrum of 5,5'-((2,4-dichlorophenyl)methylene)bis(6-amino-2-thiouracil) (C4)



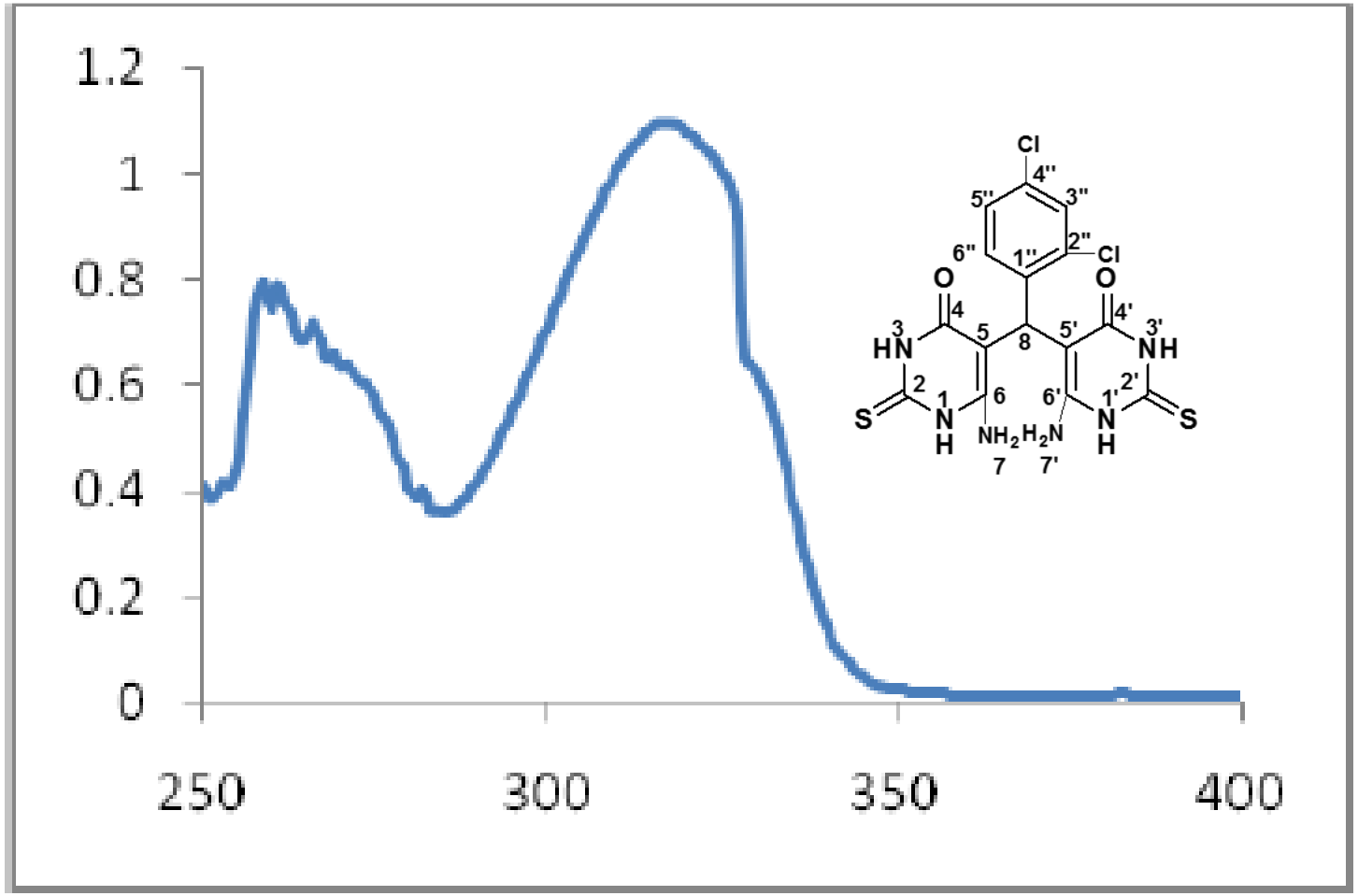
HMBC spectrum of 5,5'-((2,4-dichlorophenyl)methylene)bis(6-amino-2-thiouracil) (C4)



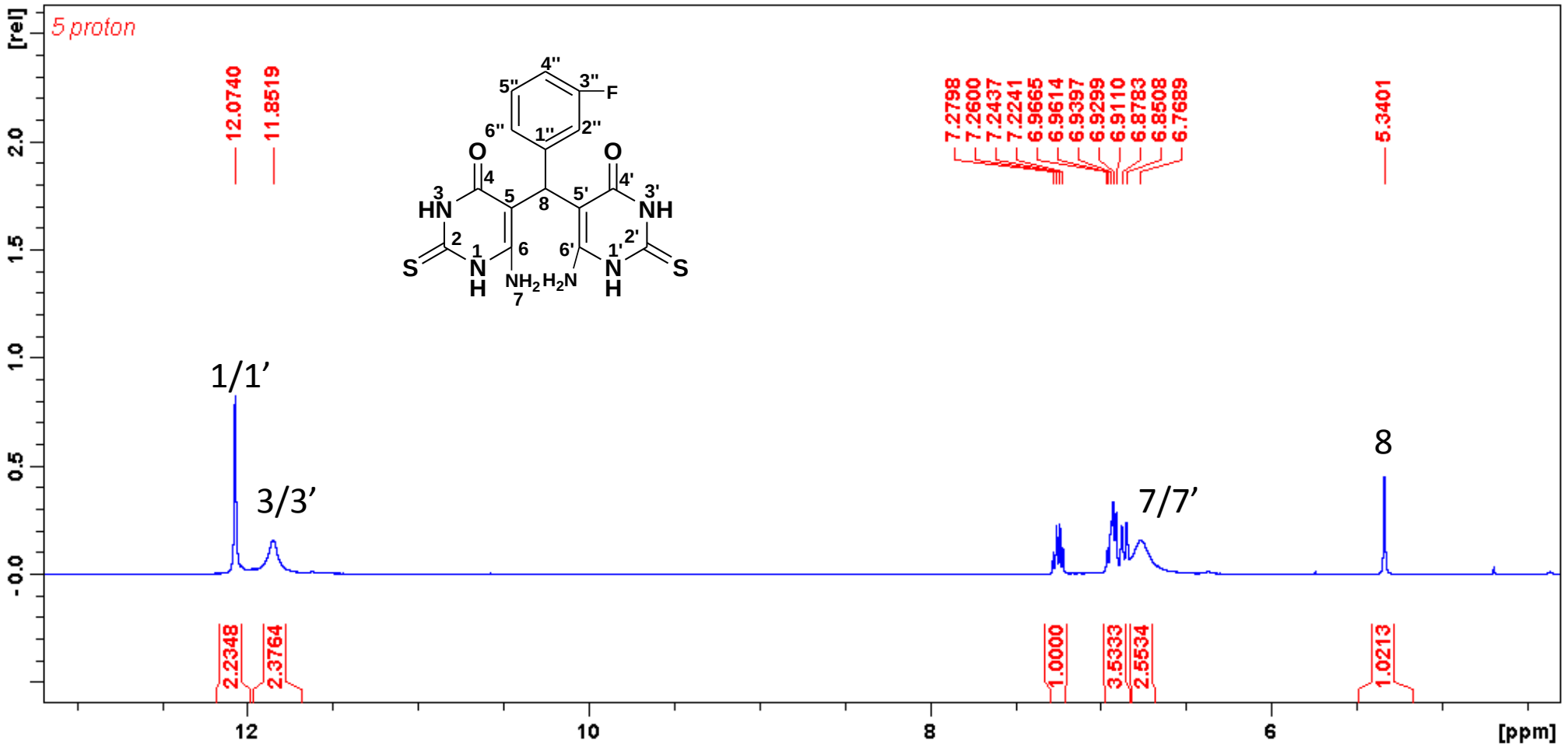
IR spectrum of 5,5'-((2,4-dichlorophenyl)methylene)bis(6-amino-2-thiouracil) (C4)



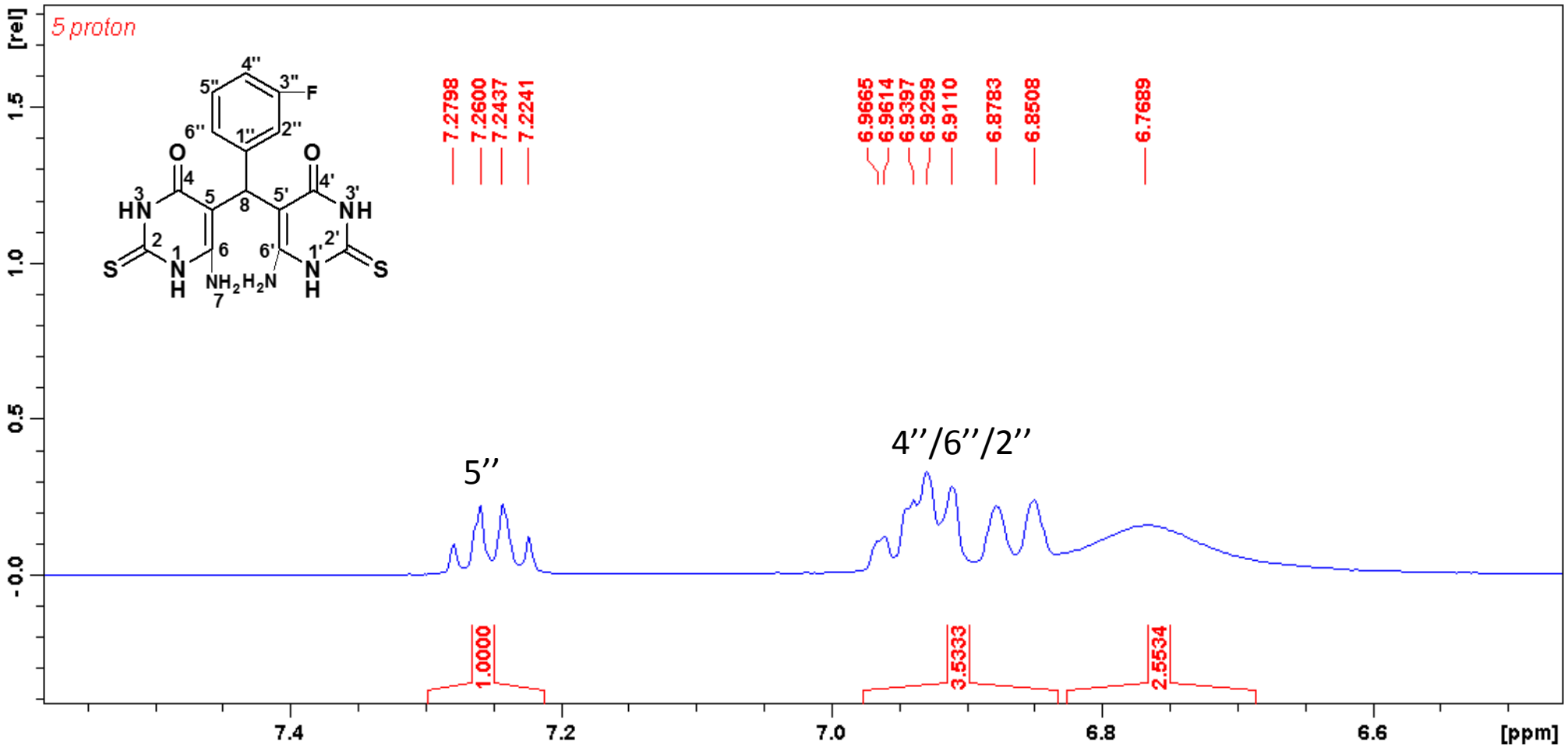
HRMS of 5,5'-((2,4-dichlorophenyl)methylene)bis(6-amino-2-thiouracil) (C4)



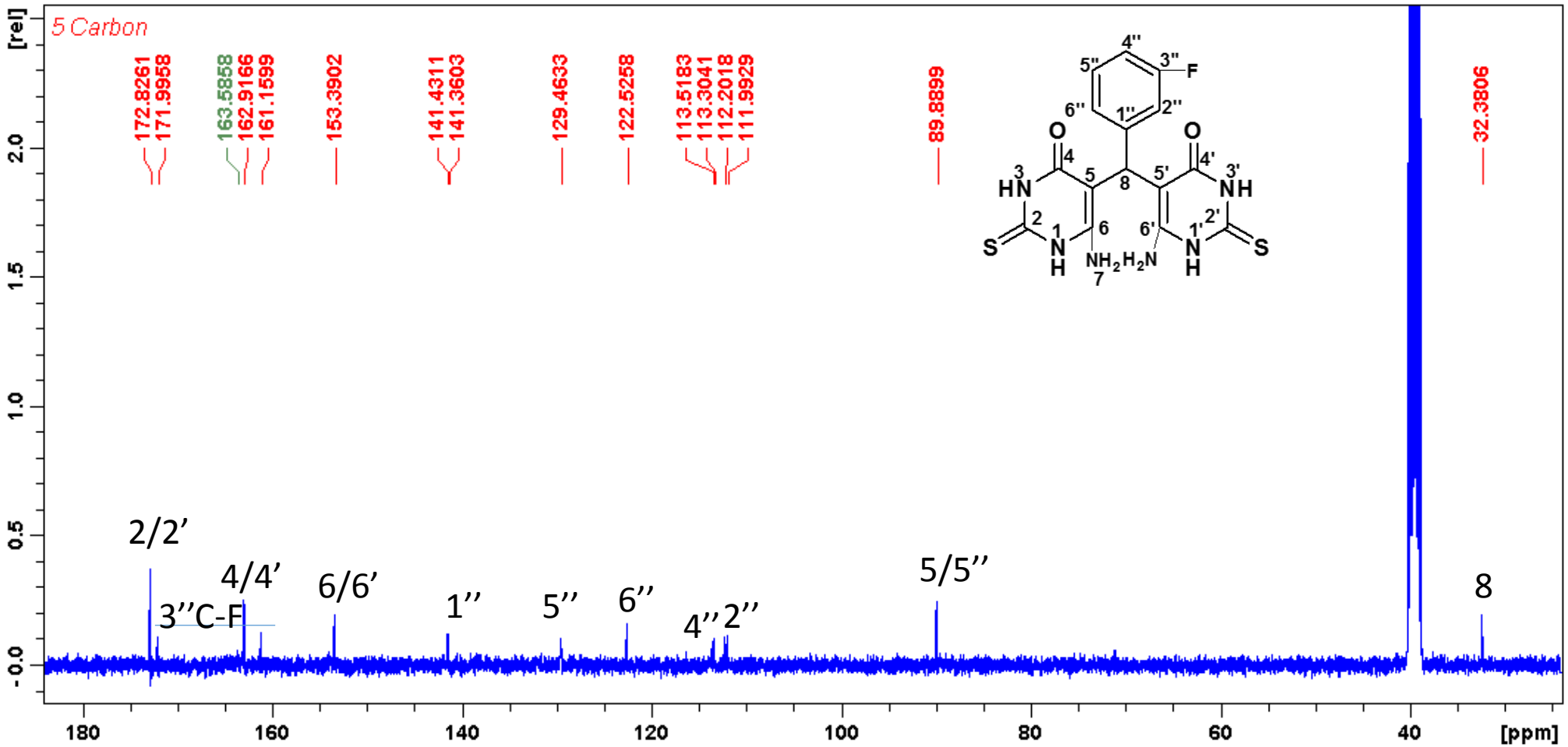
UV spectrum of 5,5'-((2,4-dichlorophenyl)methylene)bis(6-amino-2-thiouracil) (C4)



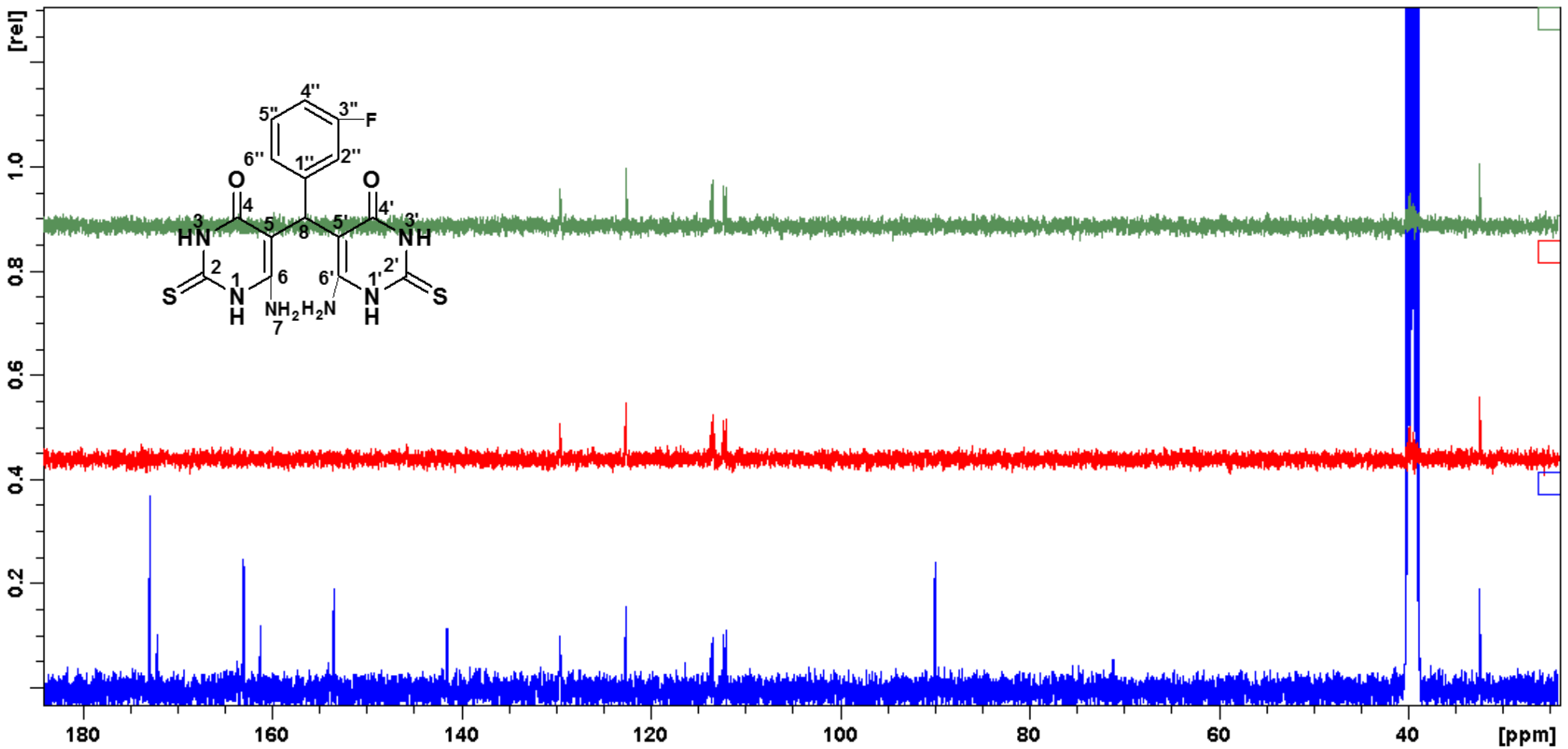
^{1}H NMR spectrum of 5,5'-((3-fluorophenyl)methylene)bis(6-amino-2-thiouracil) (C5)



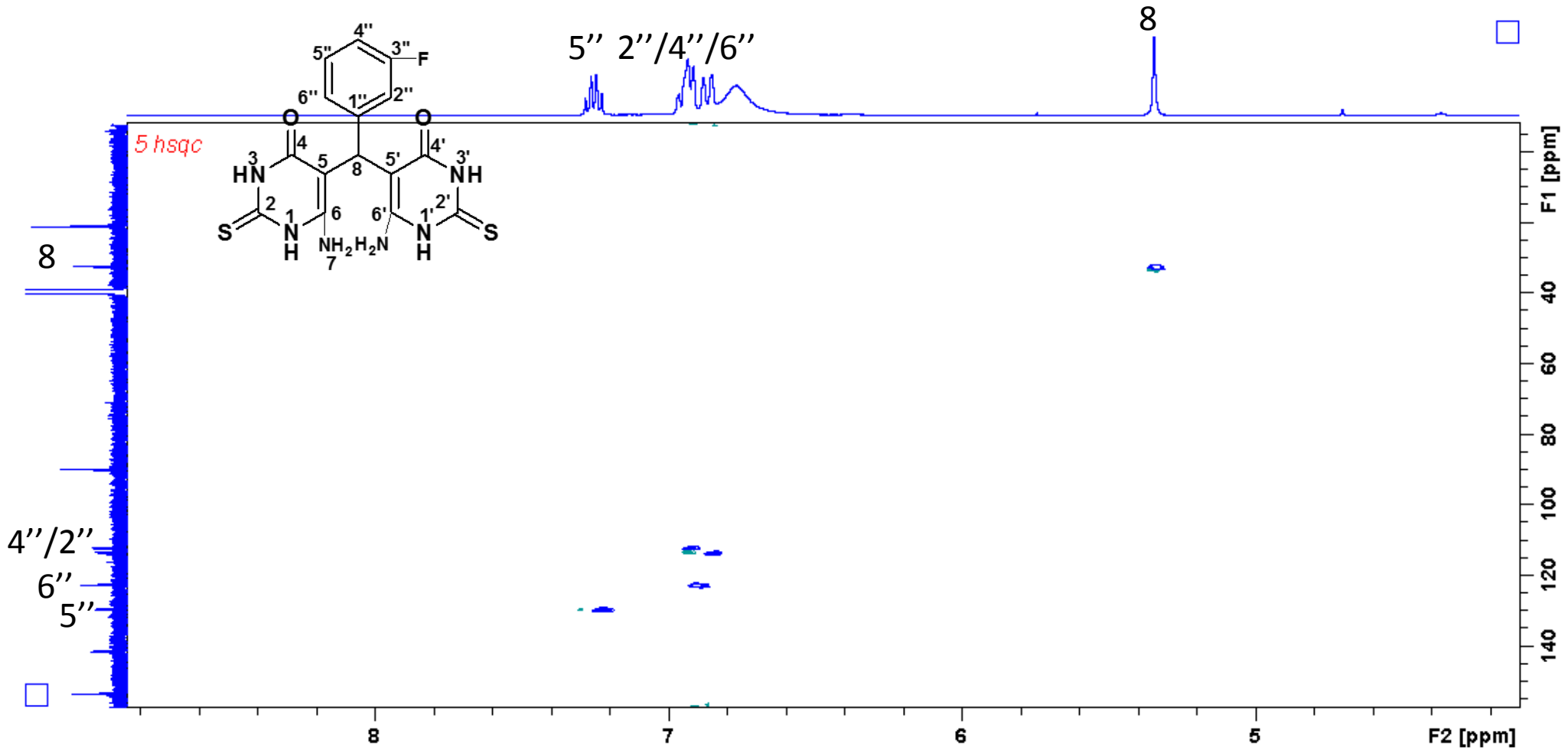
^{1}H NMR spectrum of 5,5'-((3-fluorophenyl)methylene)bis(6-amino-2-thiouracil) (C5)



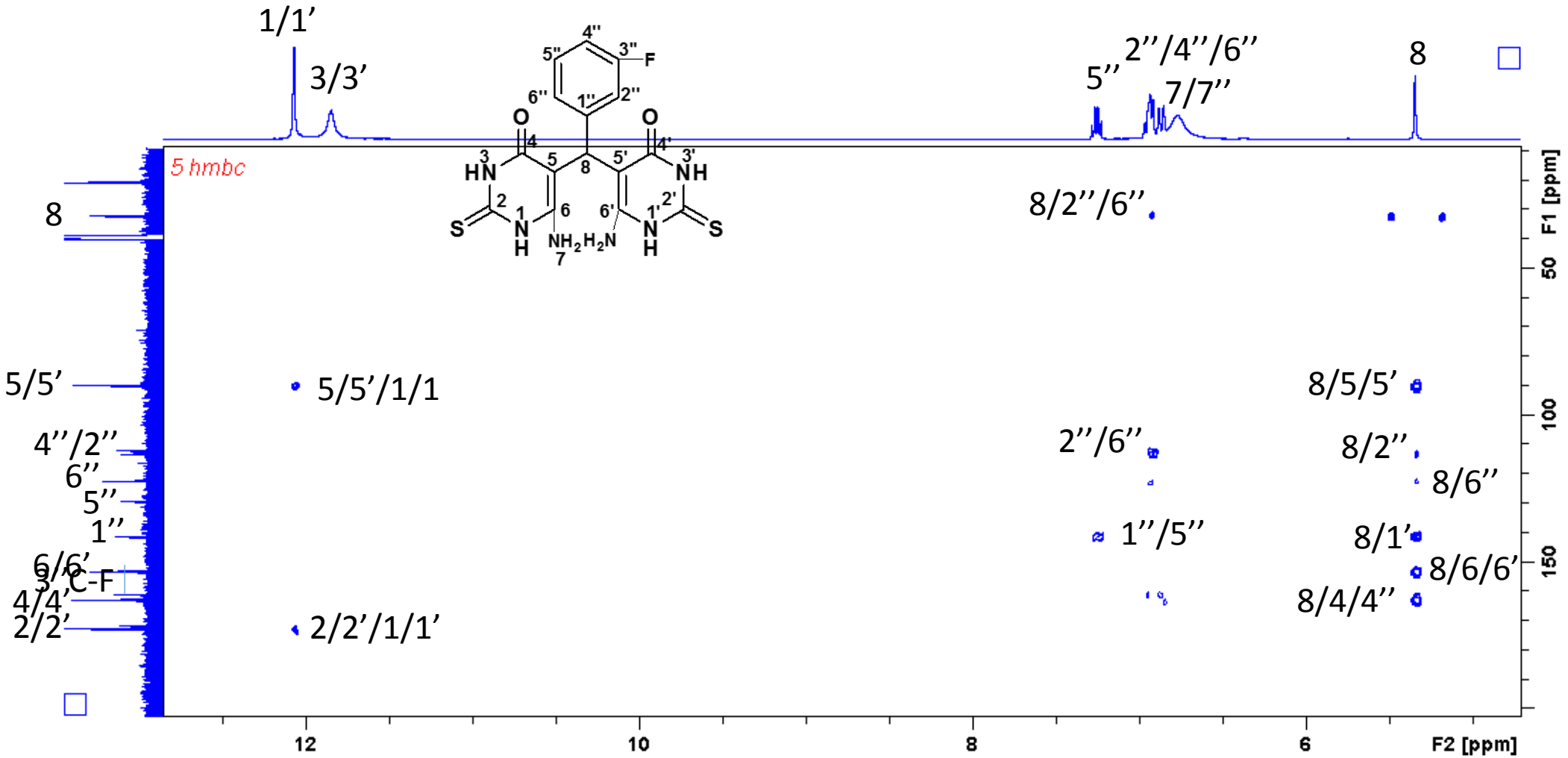
^{13}C NMR spectrum of 5,5'-((3-fluorophenyl)methylene)bis(6-amino-2-thiouracil) (C5)



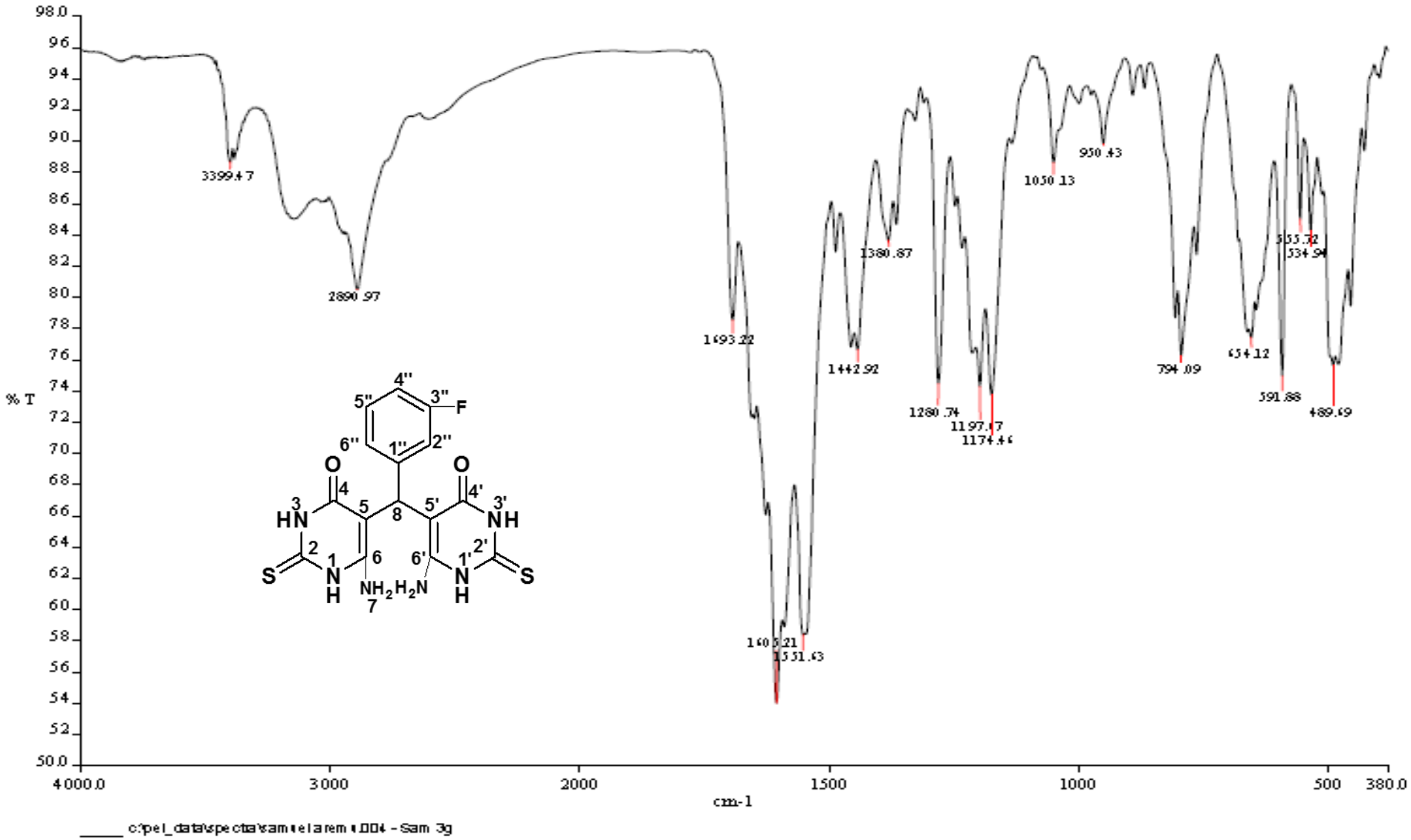
DEPT spectrum of 5,5'-((3-fluorophenyl)methylene)bis(6-amino-2-thiouracil) (C5)



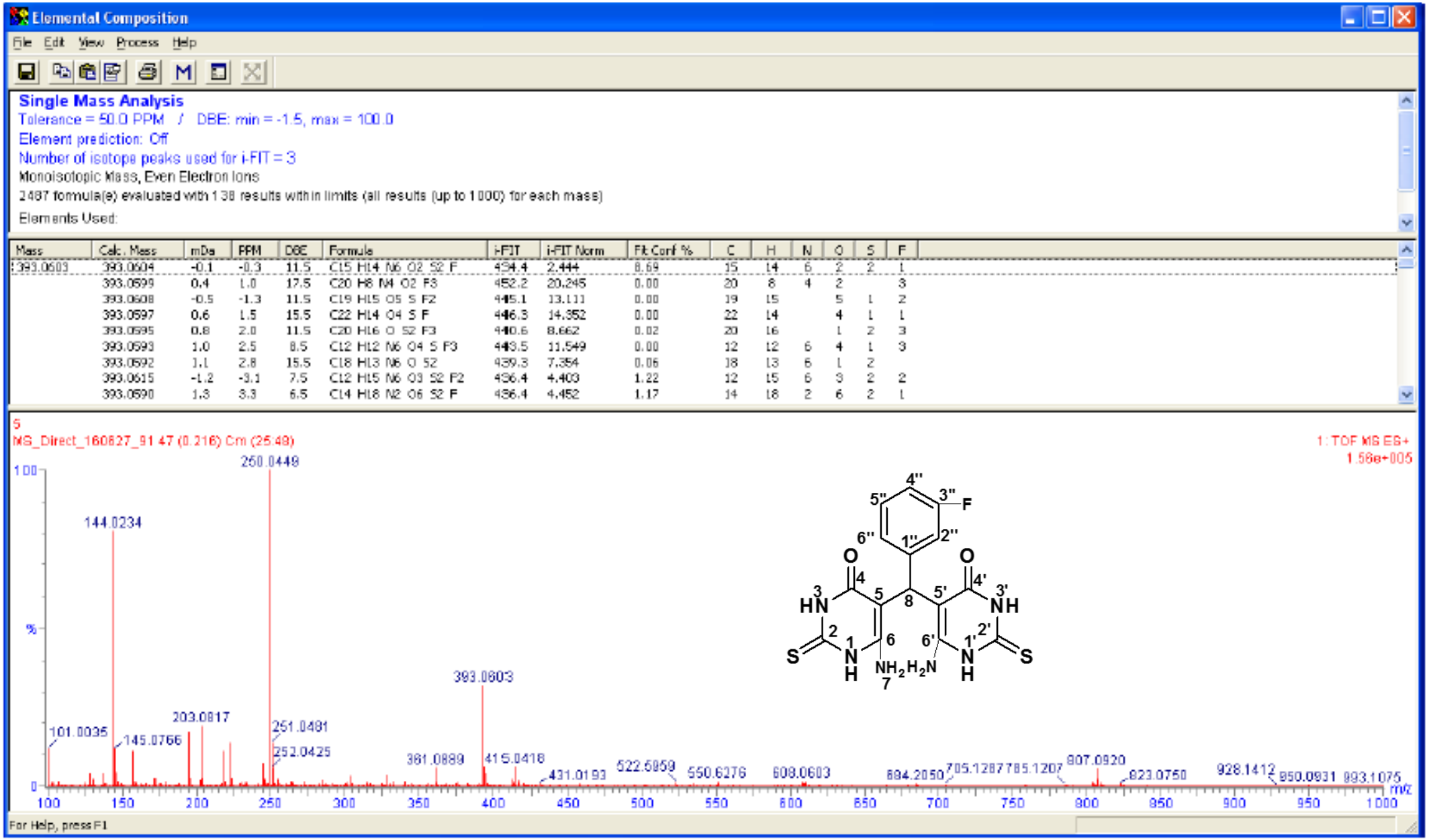
HSQC spectrum of 5,5'-((3-fluorophenyl)methylene)bis(6-amino-2-thiouracil) (C5)



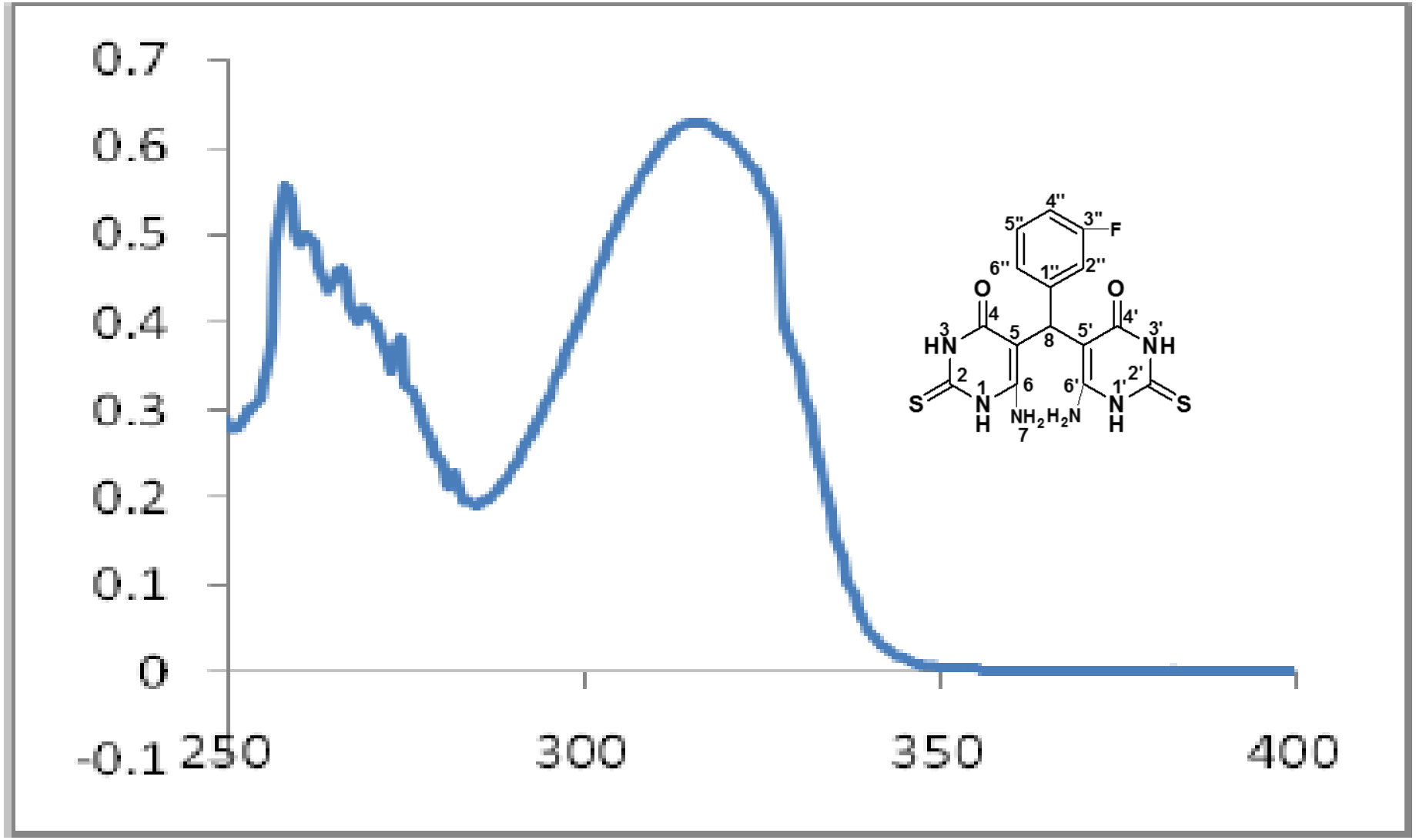
HMBC spectrum of 5,5'-((3-fluorophenyl)methylene)bis(6-amino-2-thiouracil) (C5)



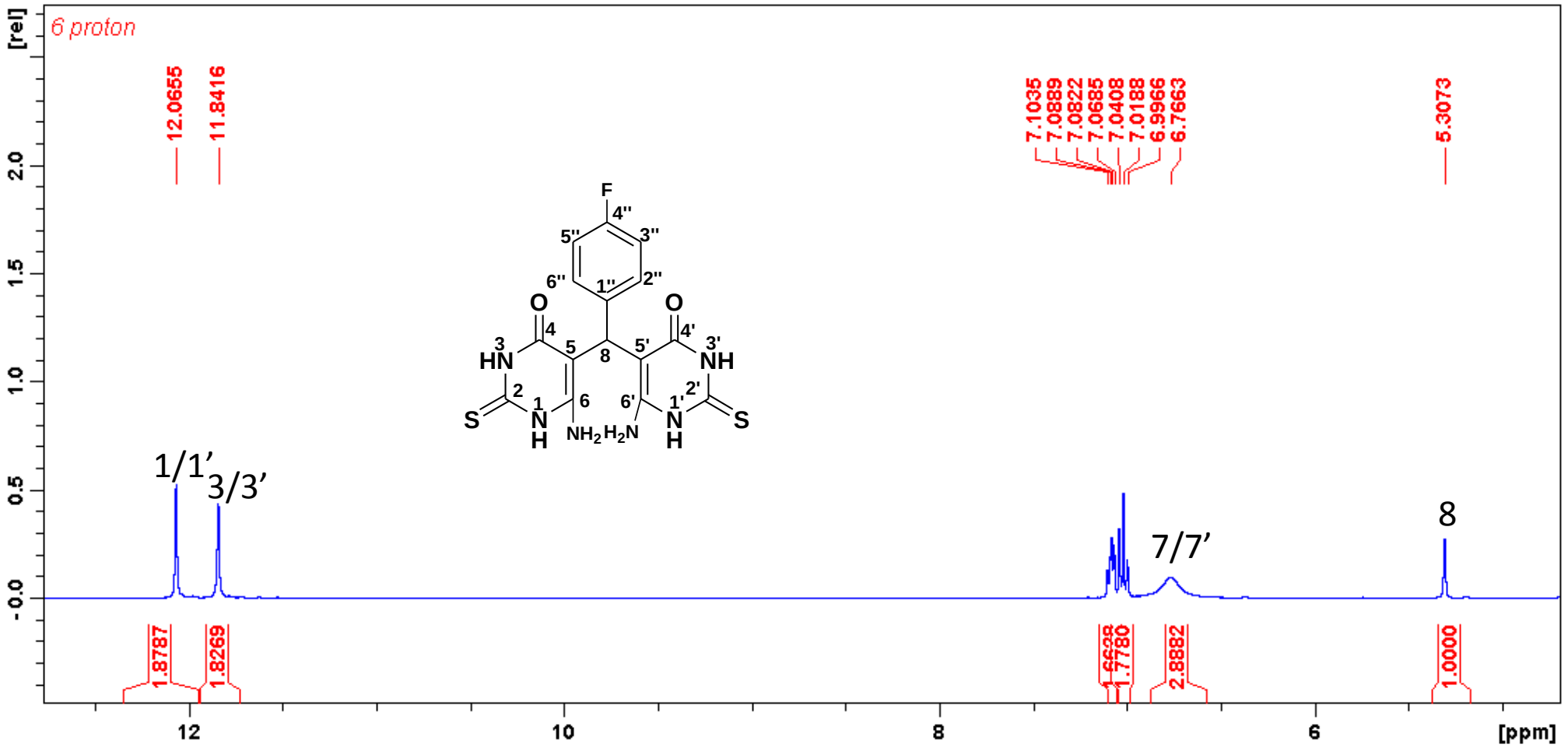
IR spectrum of 5,5'-((3-fluorophenyl)methylene)bis(6-amino-2-thiouracil) (C5)



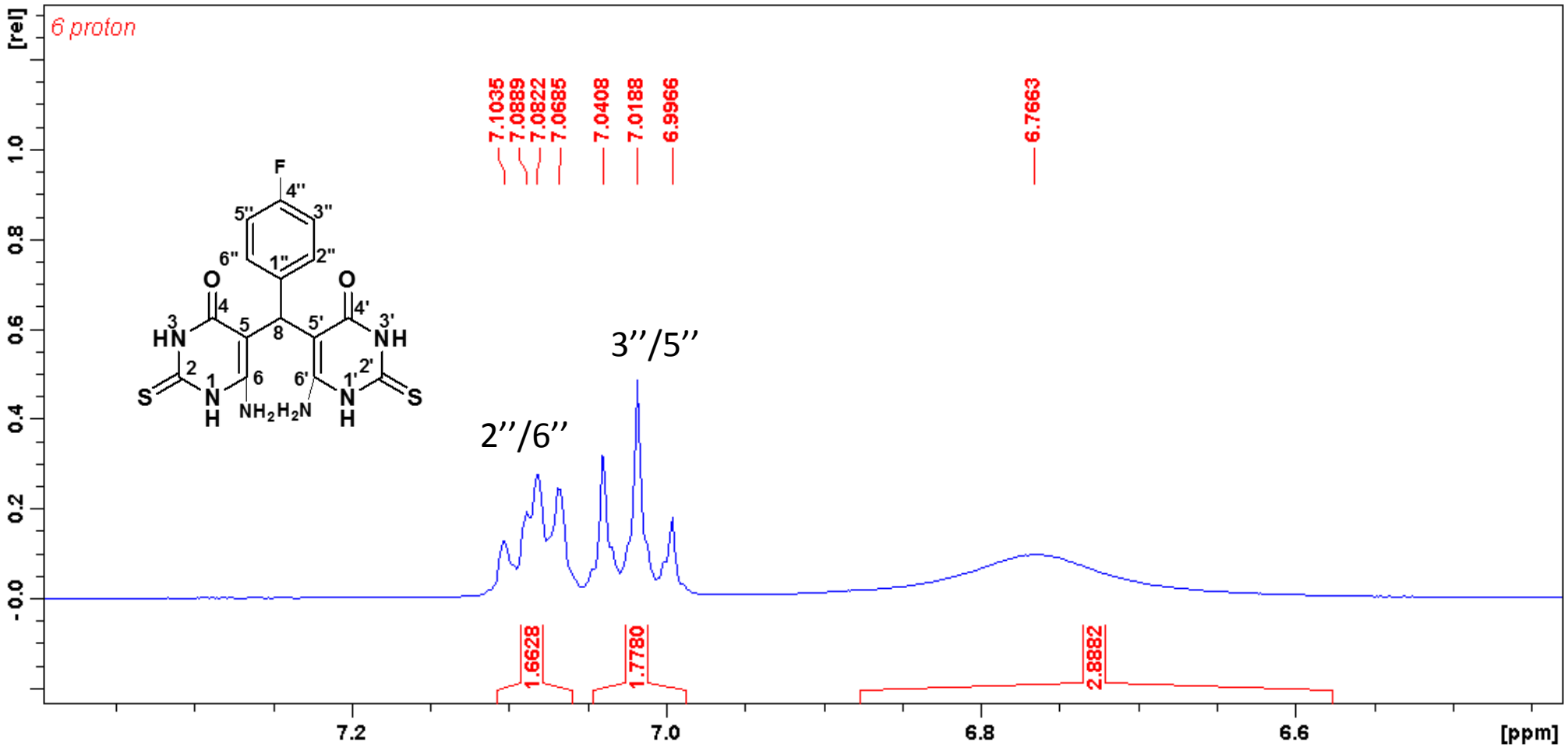
HRMS spectrum of 5,5'-((3-fluorophenyl)methylene)bis(6-amino-2-thiouracil) (C5)



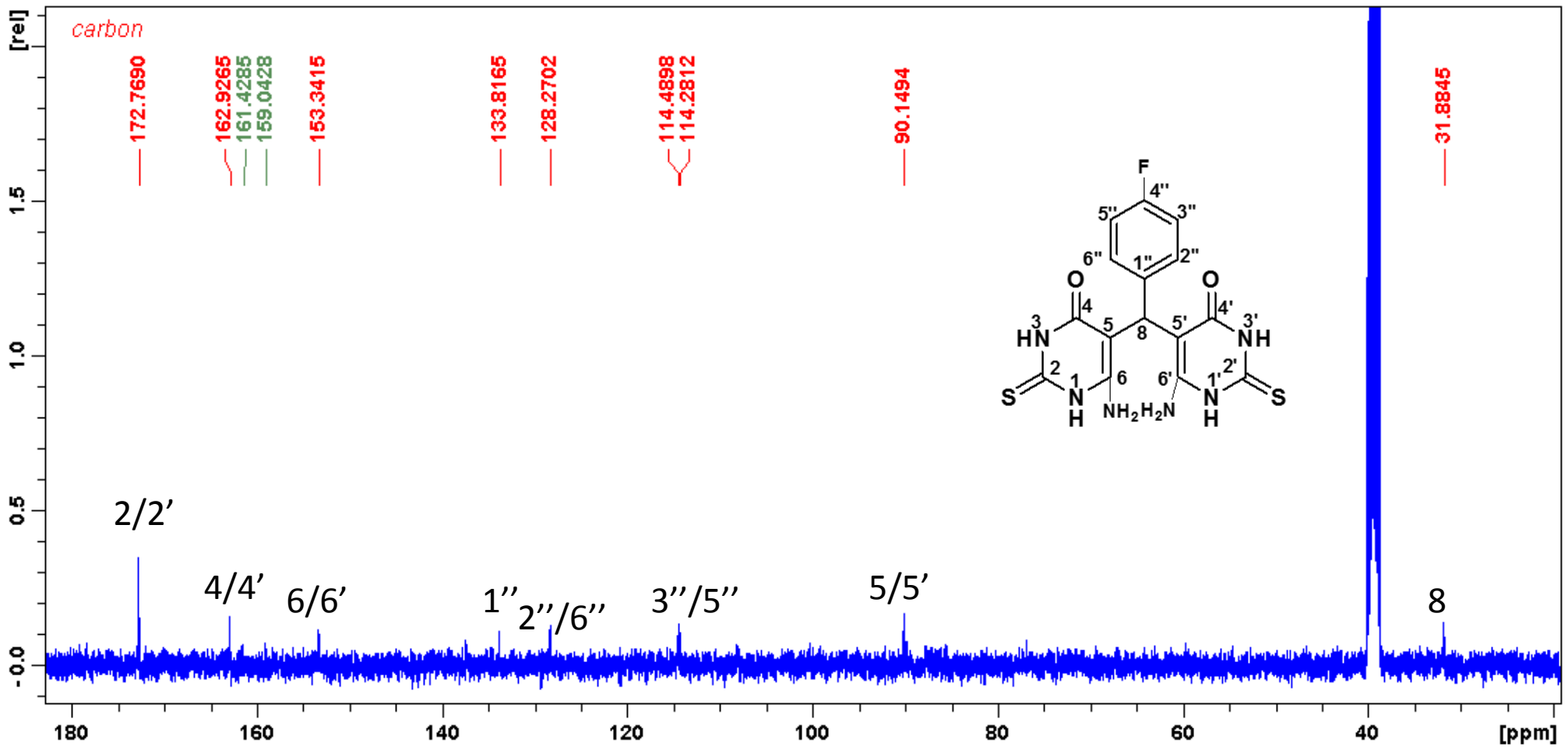
UV spectrum of 5,5'-((3-fluorophenyl)methylene)bis(6-amino-2-thiouracil) (C5)



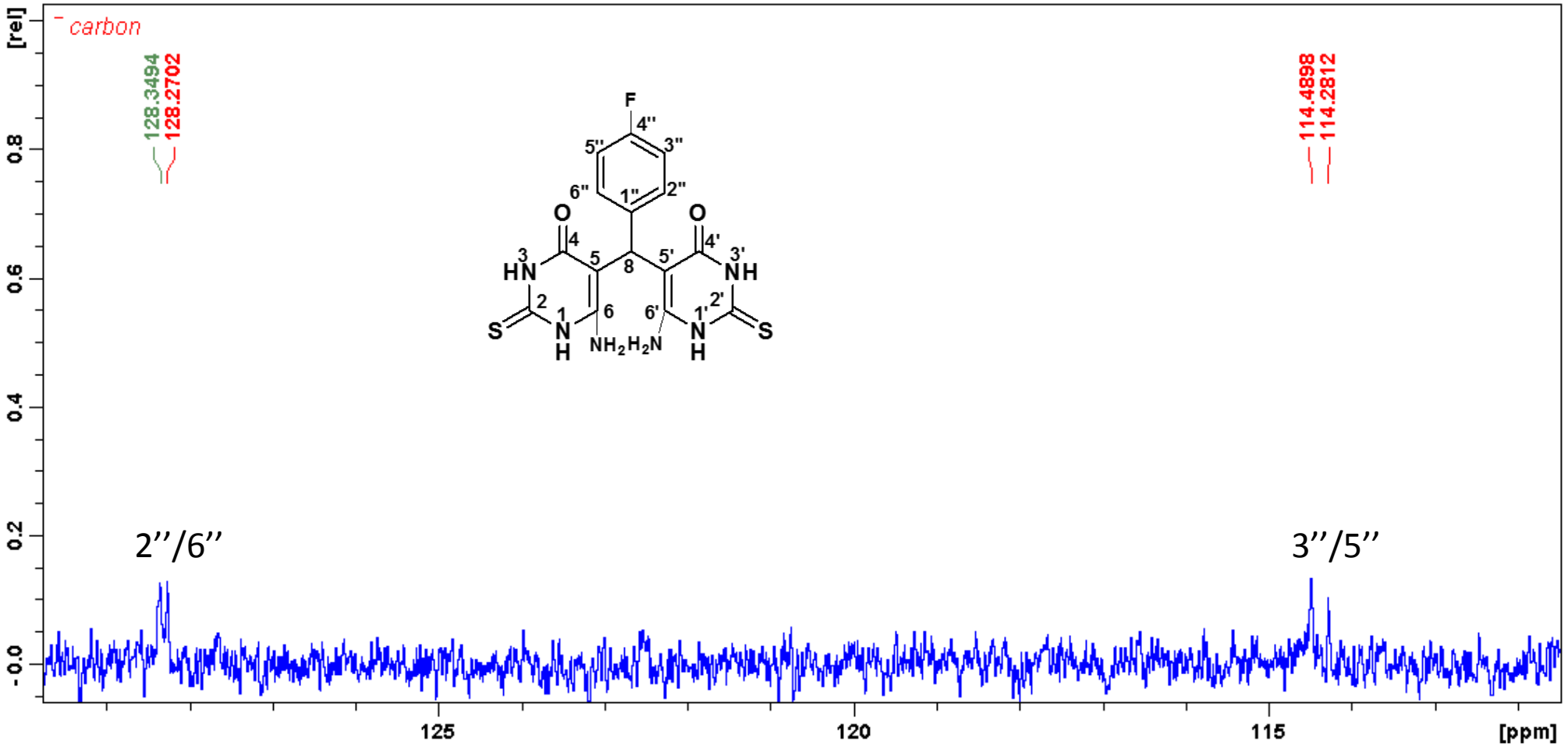
^{1}H NMR spectrum of 5,5'-((4-fluorophenyl)methylene)bis(6-amino-2-thiouracil) (C6)



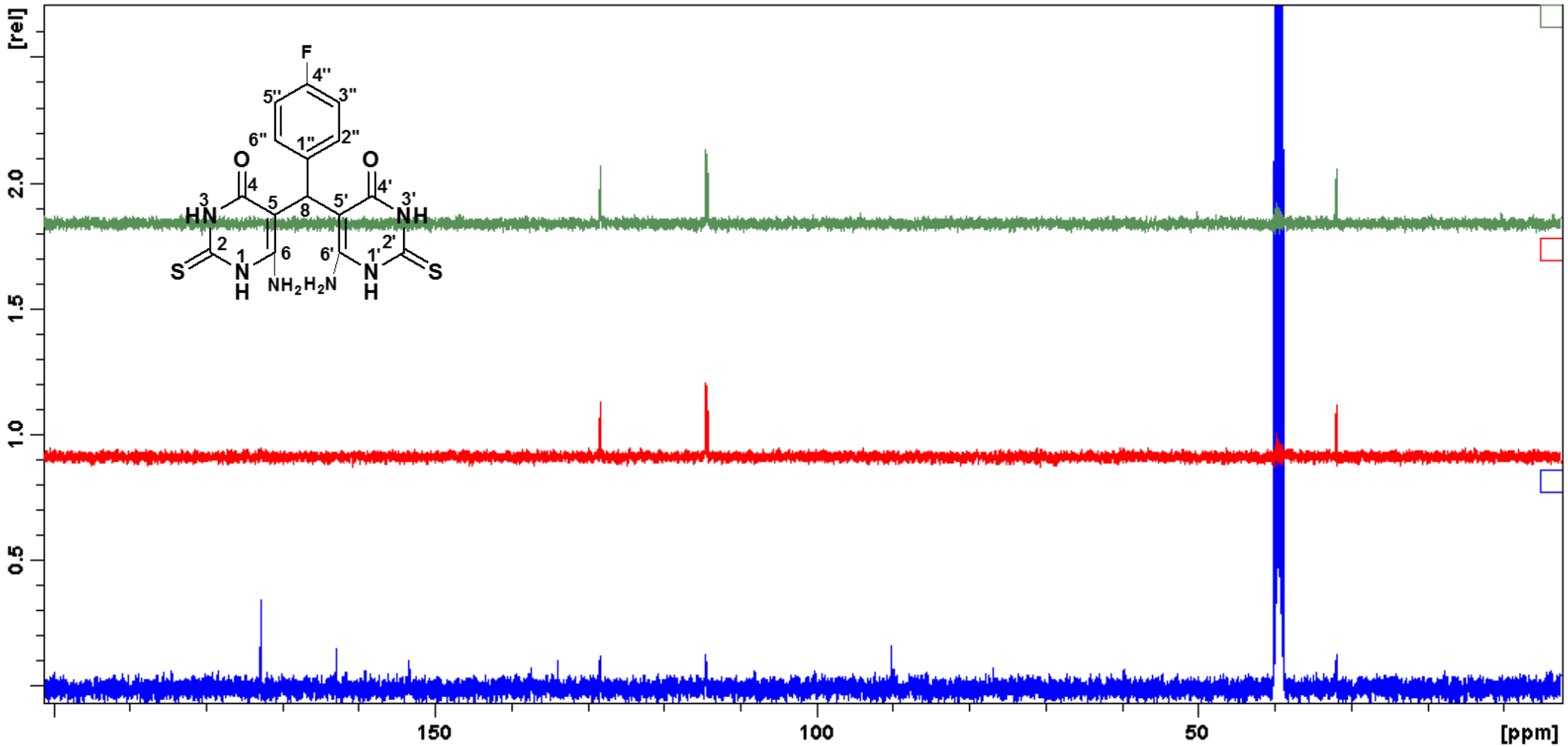
^{1}H NMR spectrum of 5,5'-((4-fluorophenyl)methylene)bis(6-amino-2-thiouracil) (C6) expanded



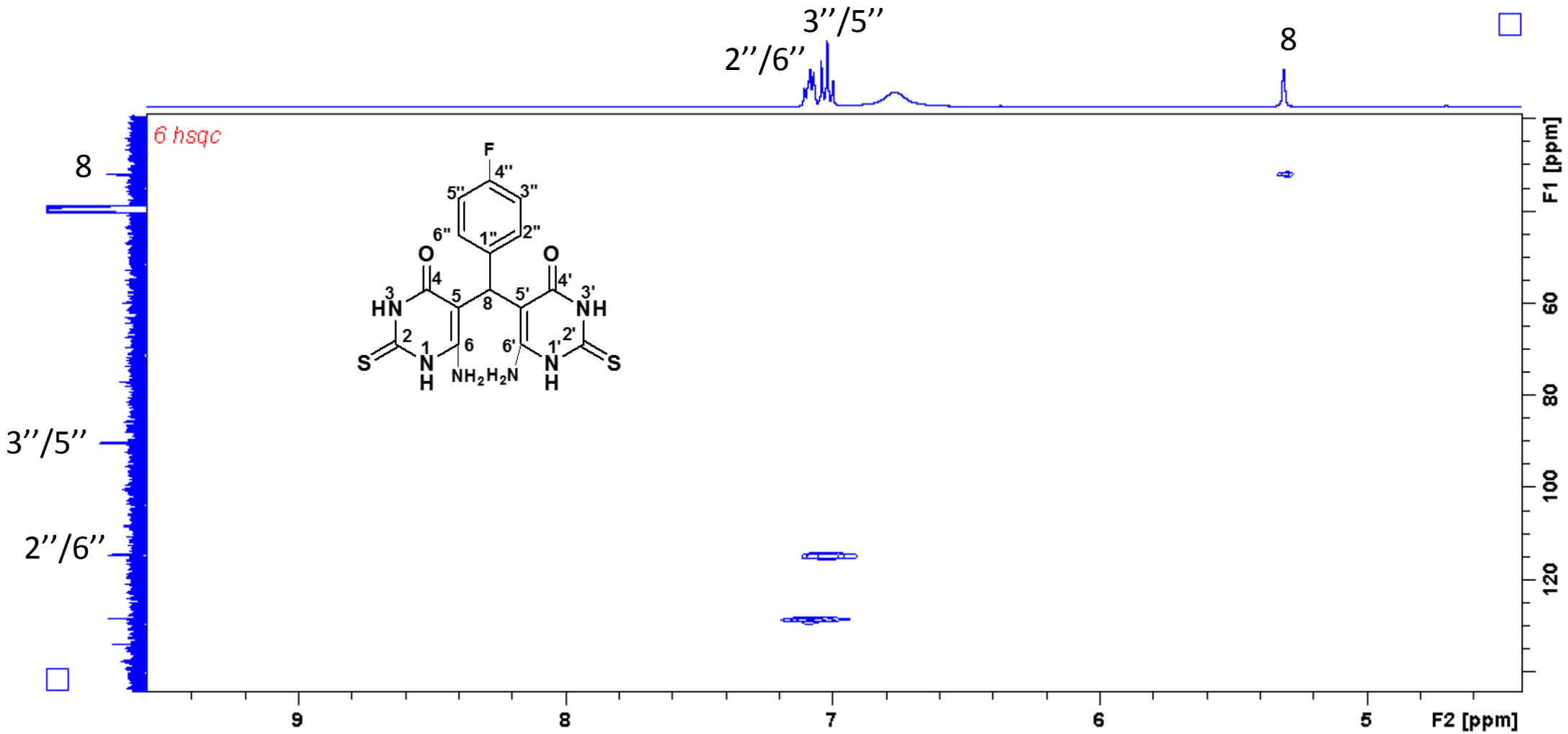
^{13}C NMR spectrum of 5,5'-((4-fluorophenyl)methylene)bis(6-amino-2-thiouracil) (C6)



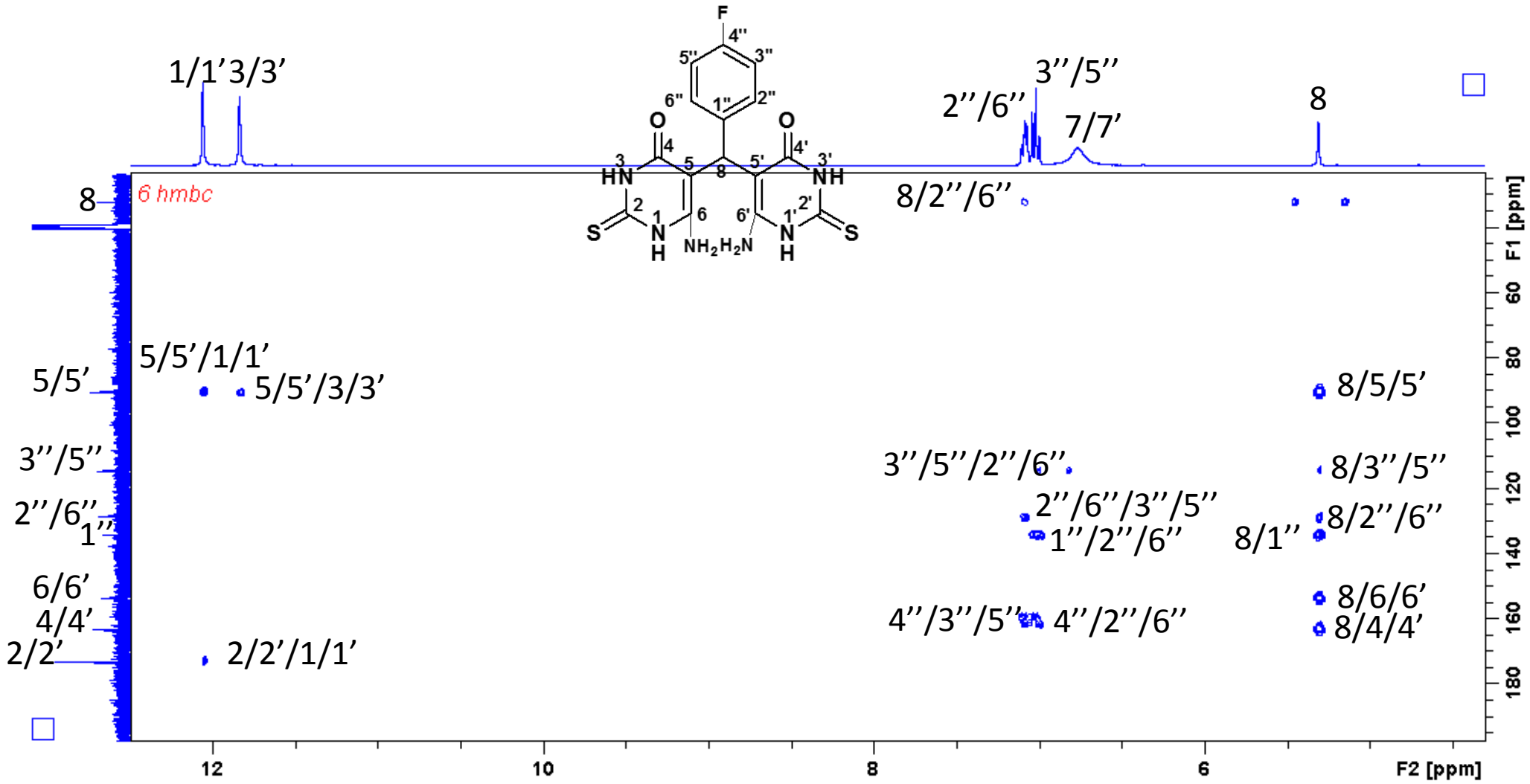
^{13}C NMR spectrum of 5,5'-((4-fluorophenyl)methylene)bis(6-amino-2-thiouracil) (C6)



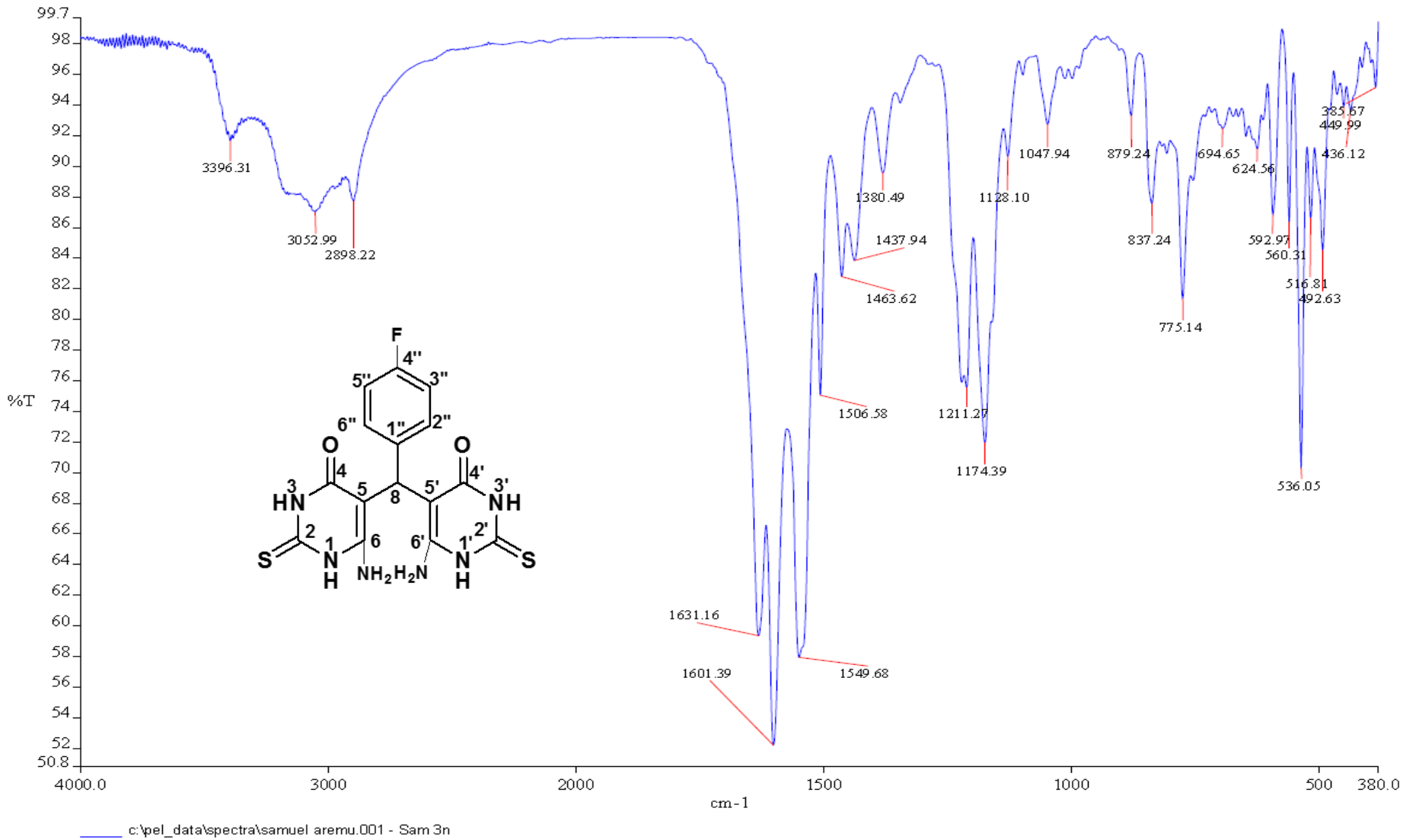
DEPT spectrum of 5,5'-((4-fluorophenyl)methylene)bis(6-amino-2-thiouracil) (C6)



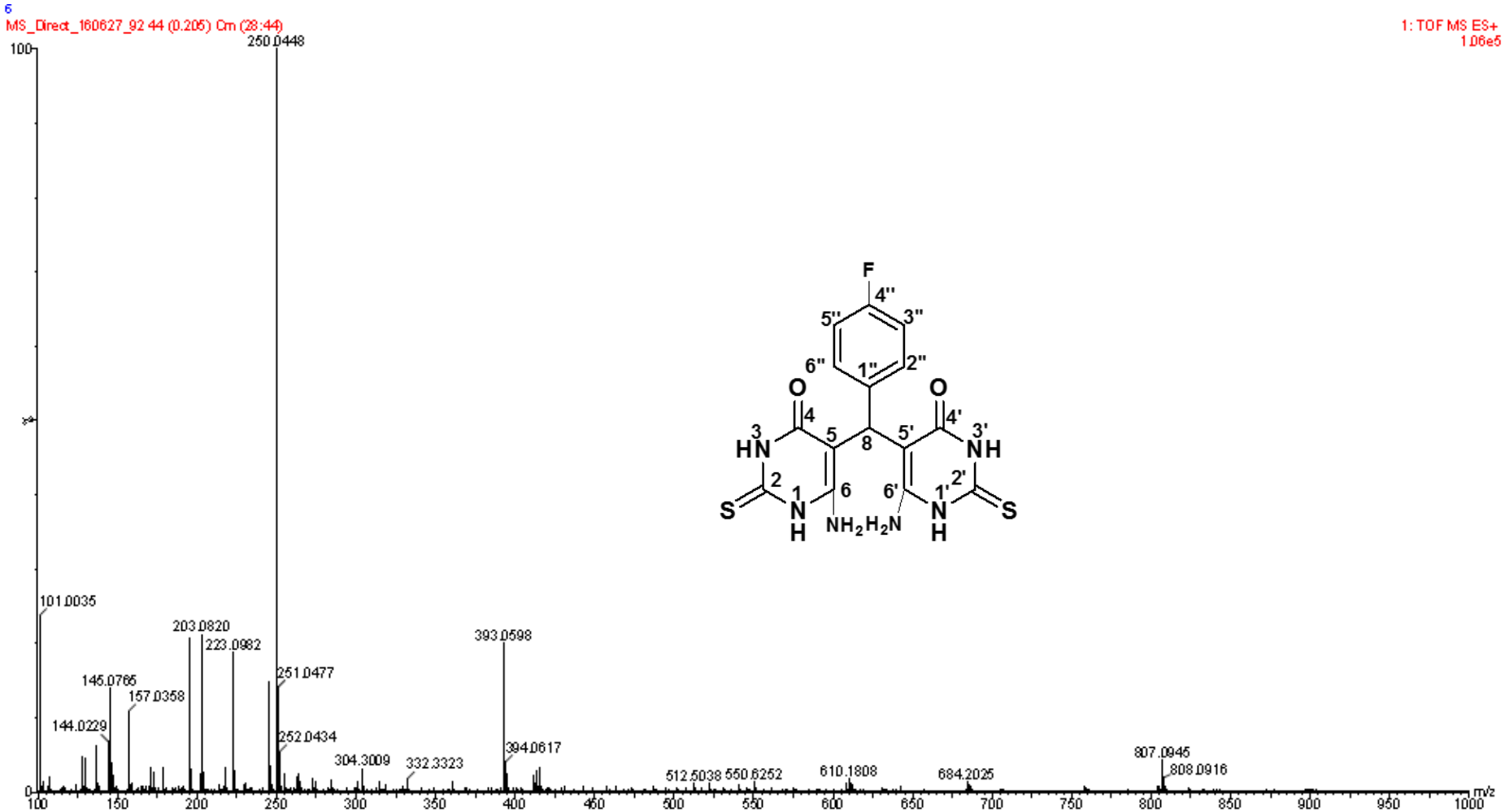
HSQC spectrum of 5,5'-((4-fluorophenyl)methylene)bis(6-amino-2-thiouracil) (C6)



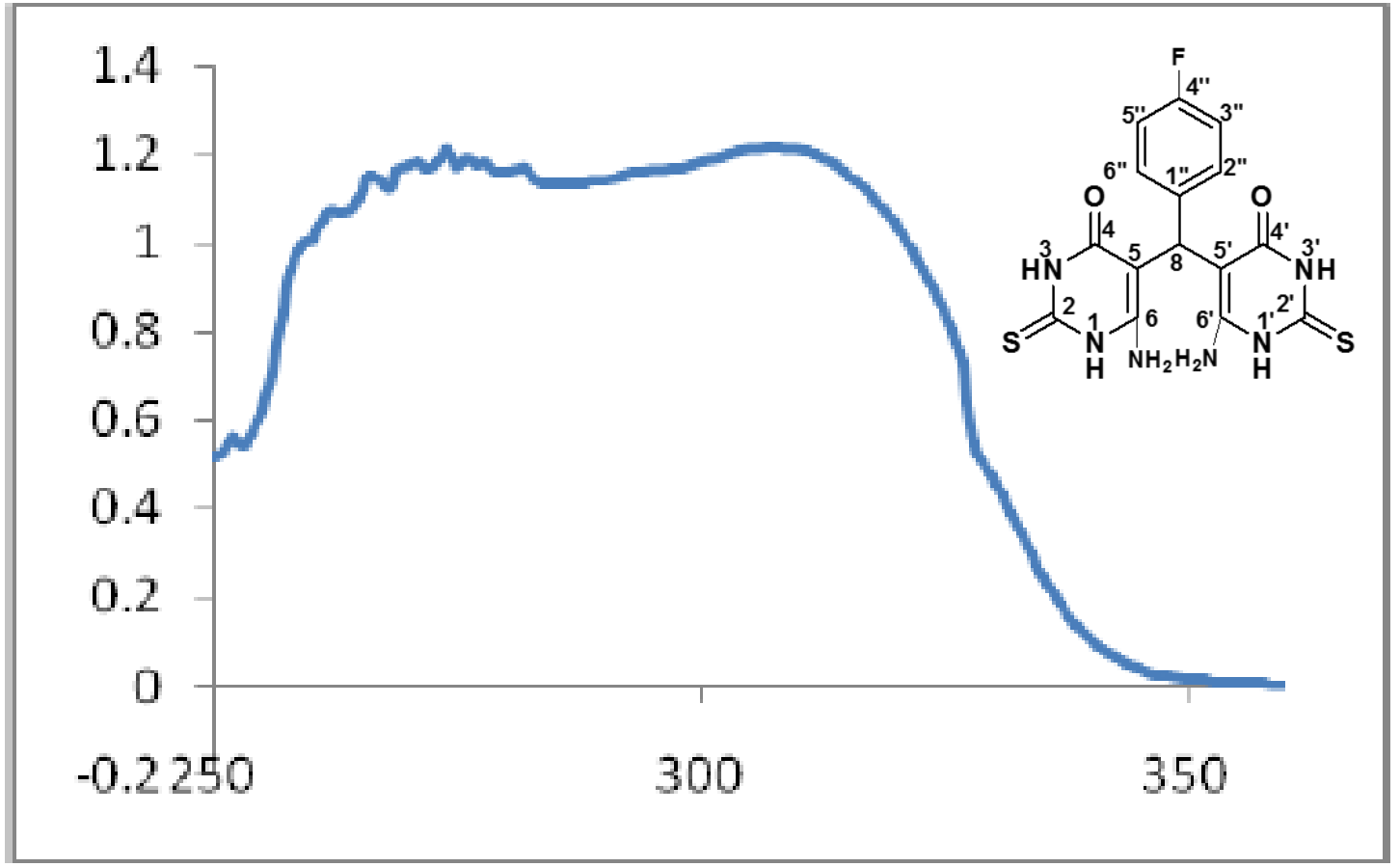
HMBC spectrum of 5,5'-((4-fluorophenyl)methylene)bis(6-amino-2-thiouracil) (C6)



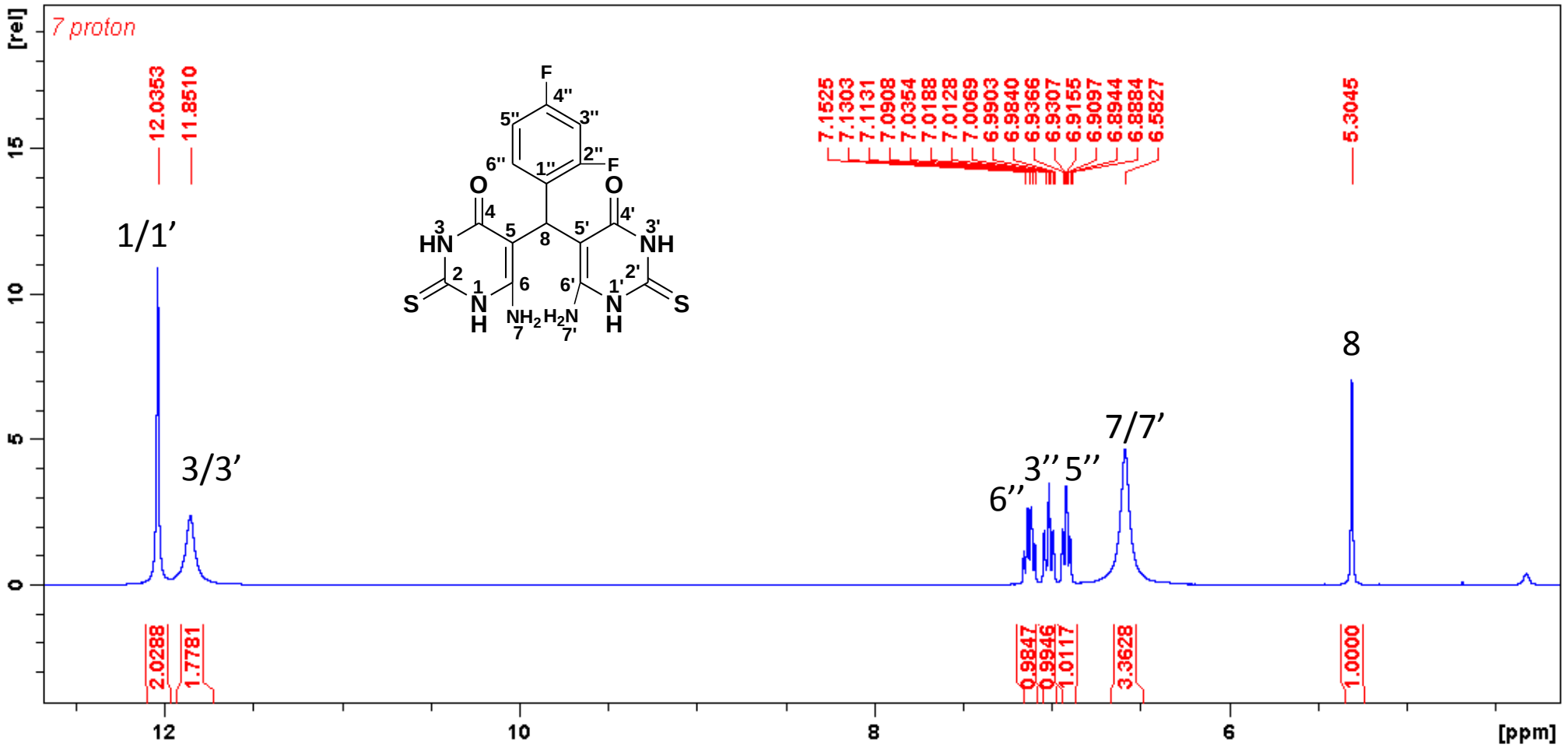
IR spectrum of 5,5'-((4-fluorophenyl)methylene)bis(6-amino-2-thiouracil) (C6)



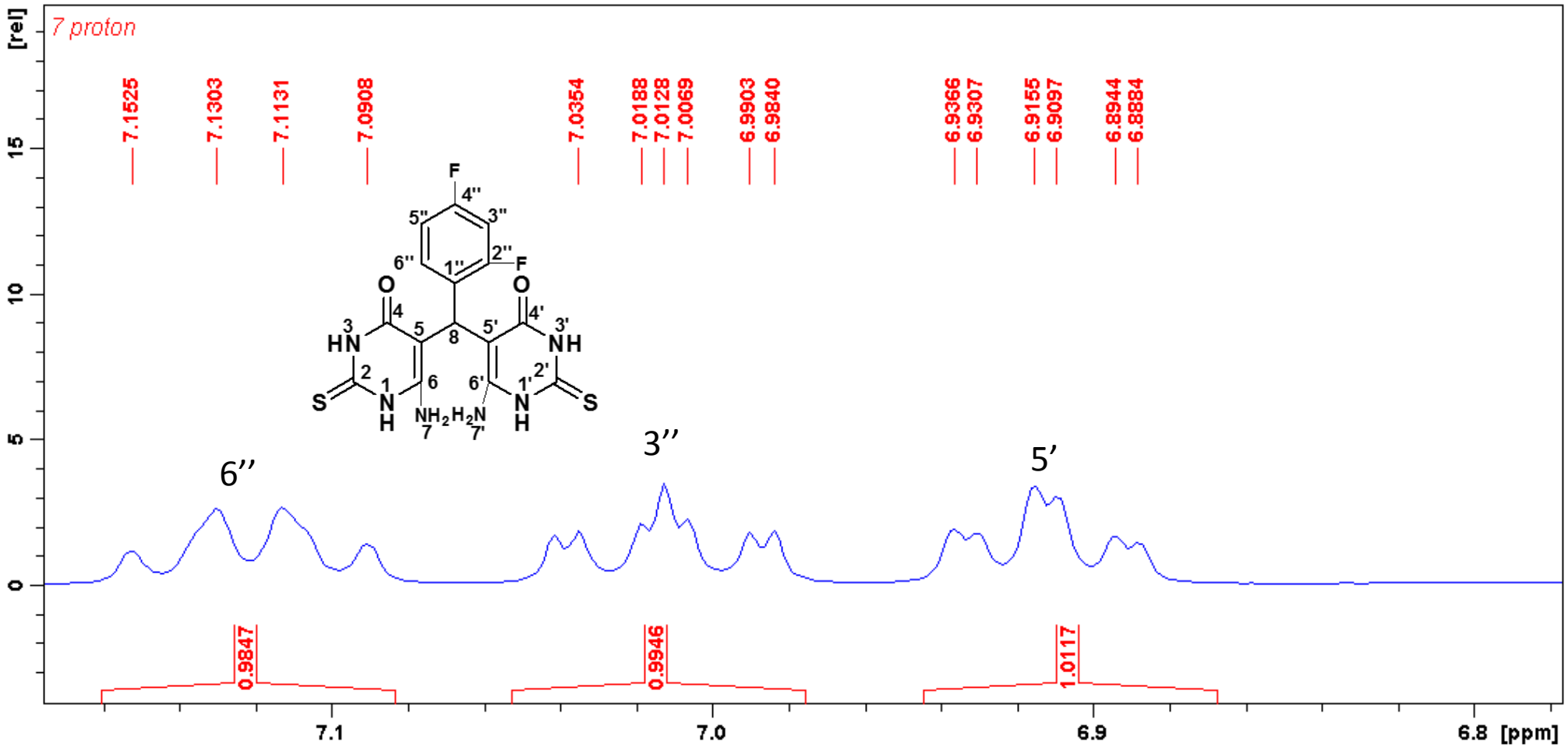
HRMS of 5,5'-((4-fluorophenyl)methylene)bis(6-amino-2-thiouracil) (C6)



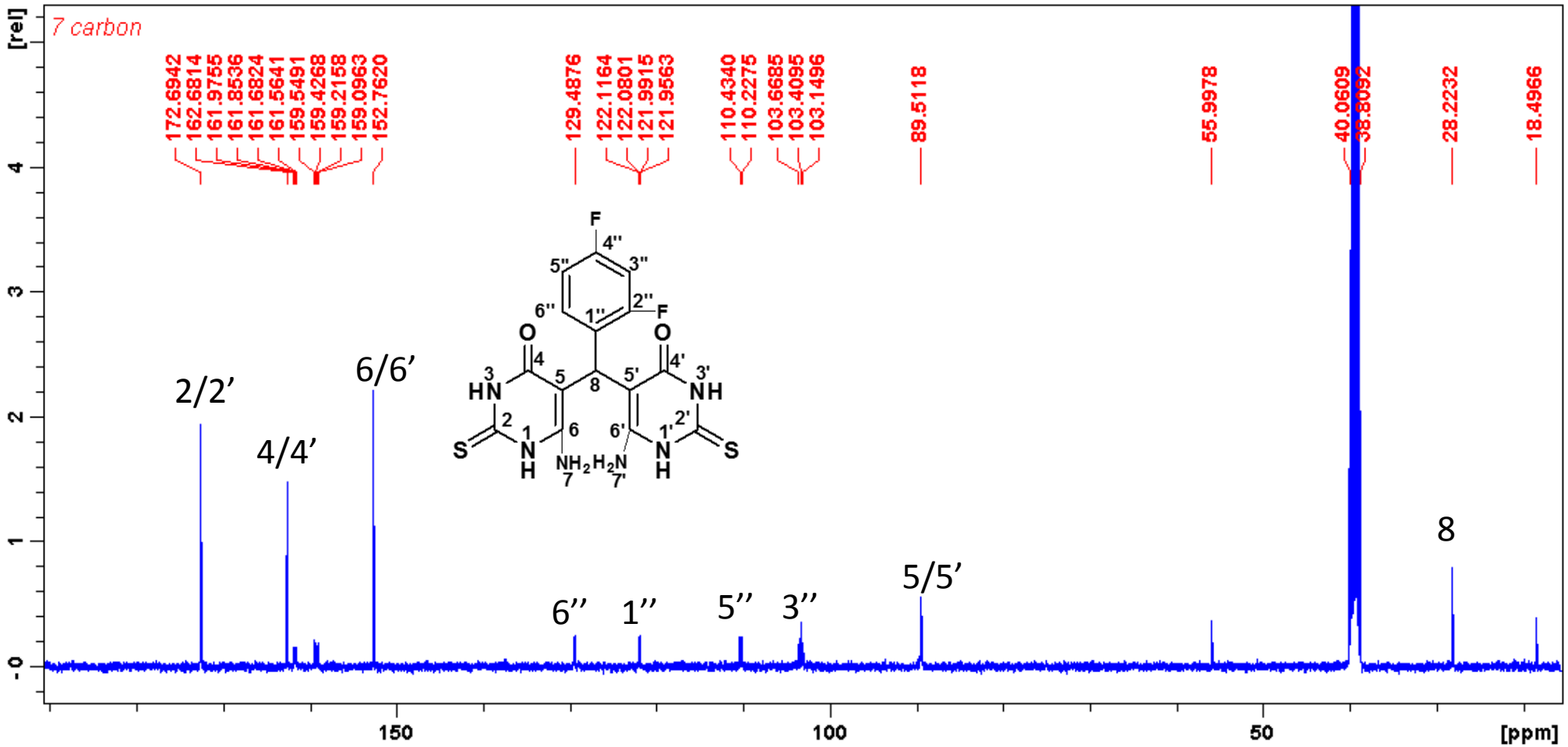
UV spectrum of 5,5'-((4-fluorophenyl)methylene)bis(6-amino-2-thiouracil) (C6)



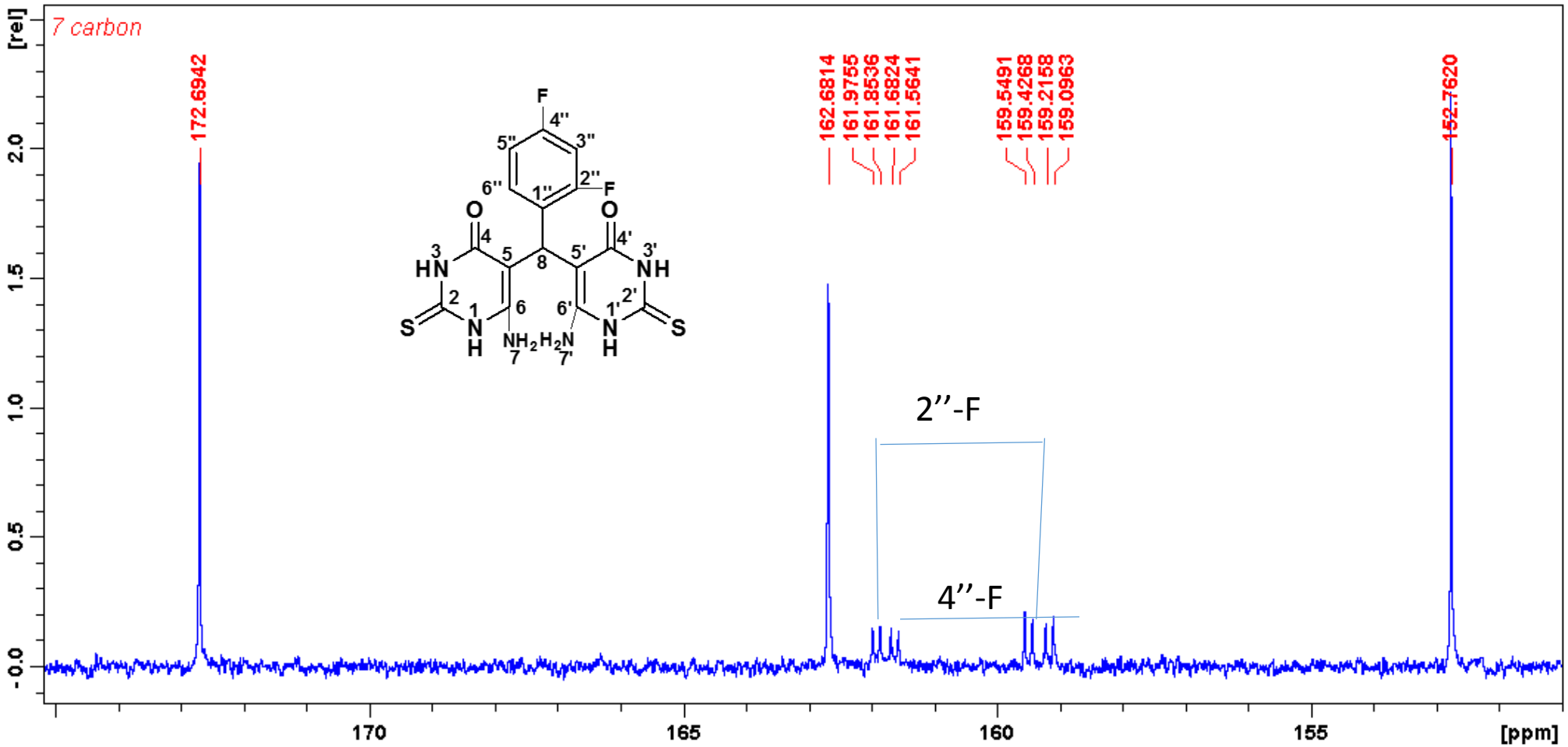
^{1}H NMR spectrum of 5,5'-((2,4-difluorophenyl)methylene)bis(6-amino-2-thiouracil) (C7)



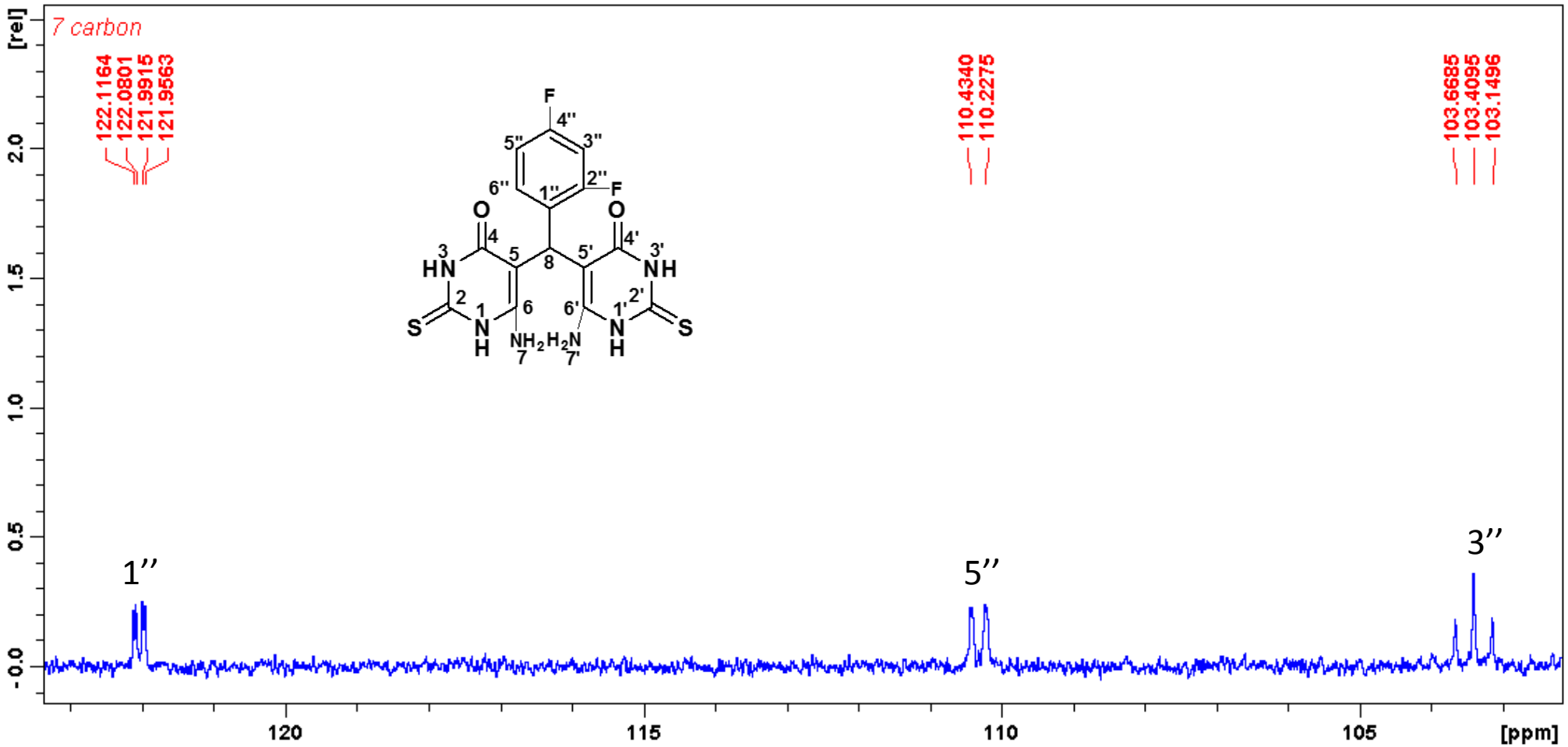
^{1}H NMR spectrum of 5,5'-((2,4-difluorophenyl)methylene)bis(6-amino-2-thiouracil) (C7)



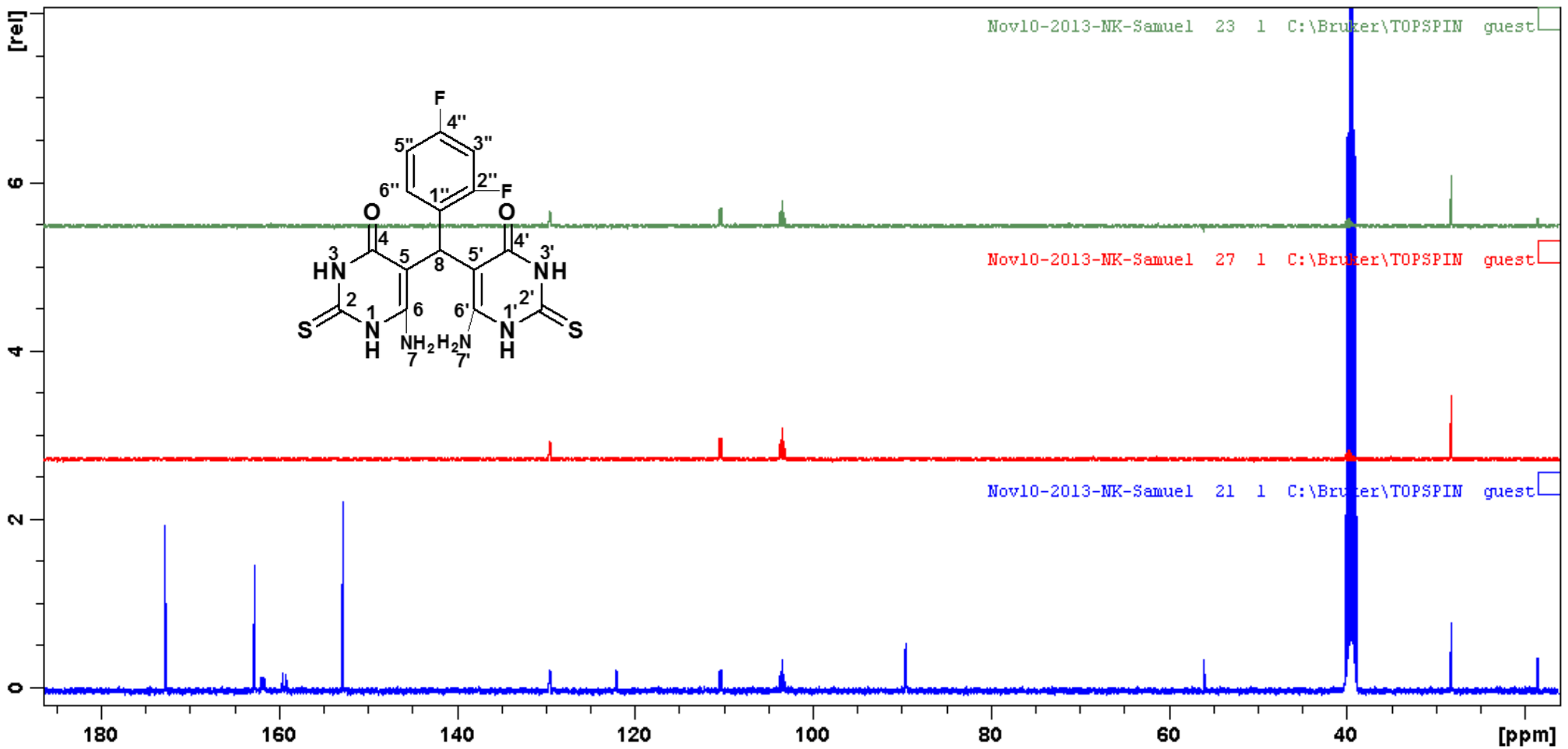
^{13}C NMR spectrum of 5,5'-((2,4-difluorophenyl)methylene)bis(6-amino-2-thiouracil) (C7)



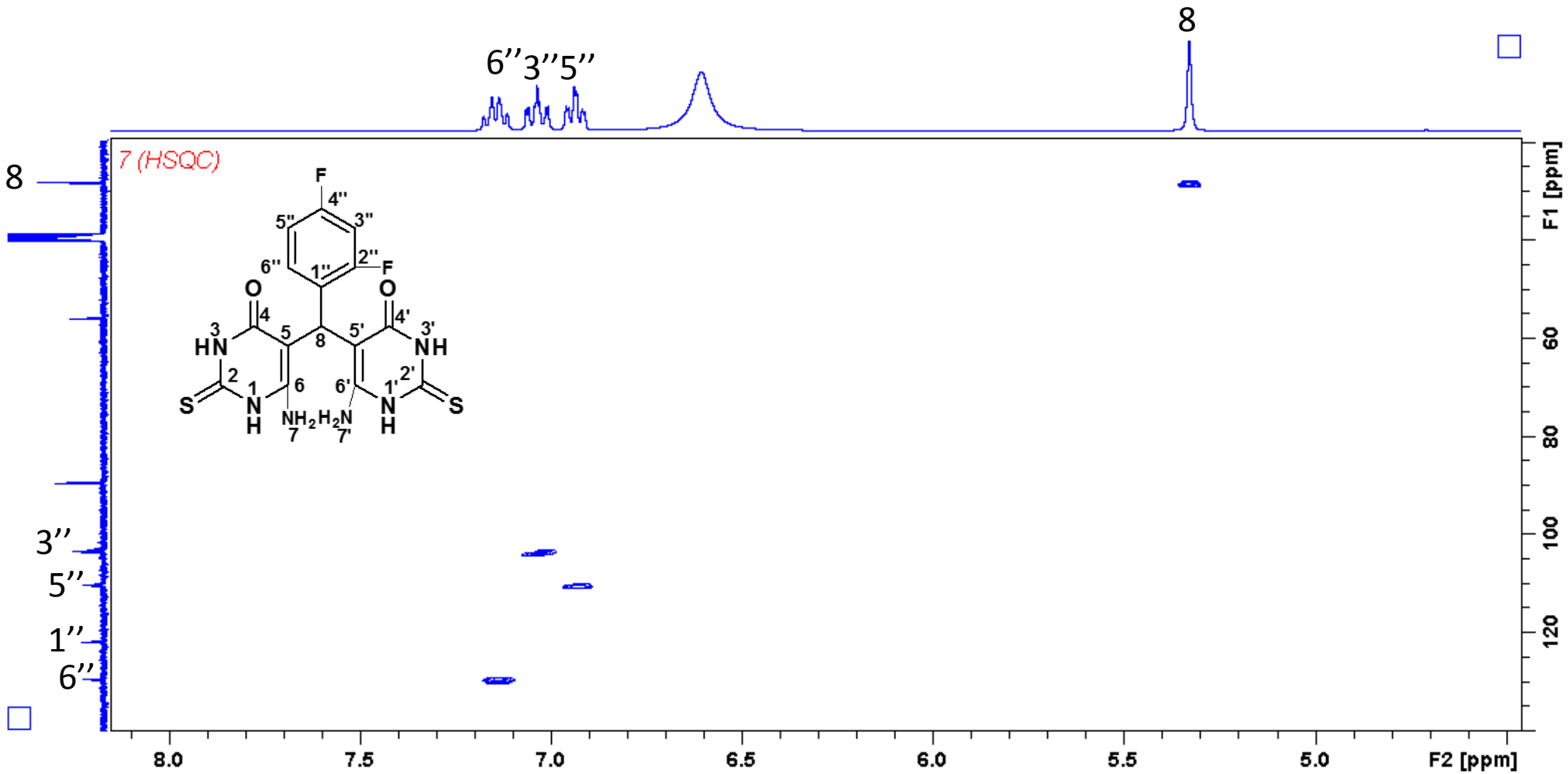
^{13}C NMR spectrum of 5,5'-((2,4-difluorophenyl)methylene)bis(6-amino-2-thiouracil) (C7)



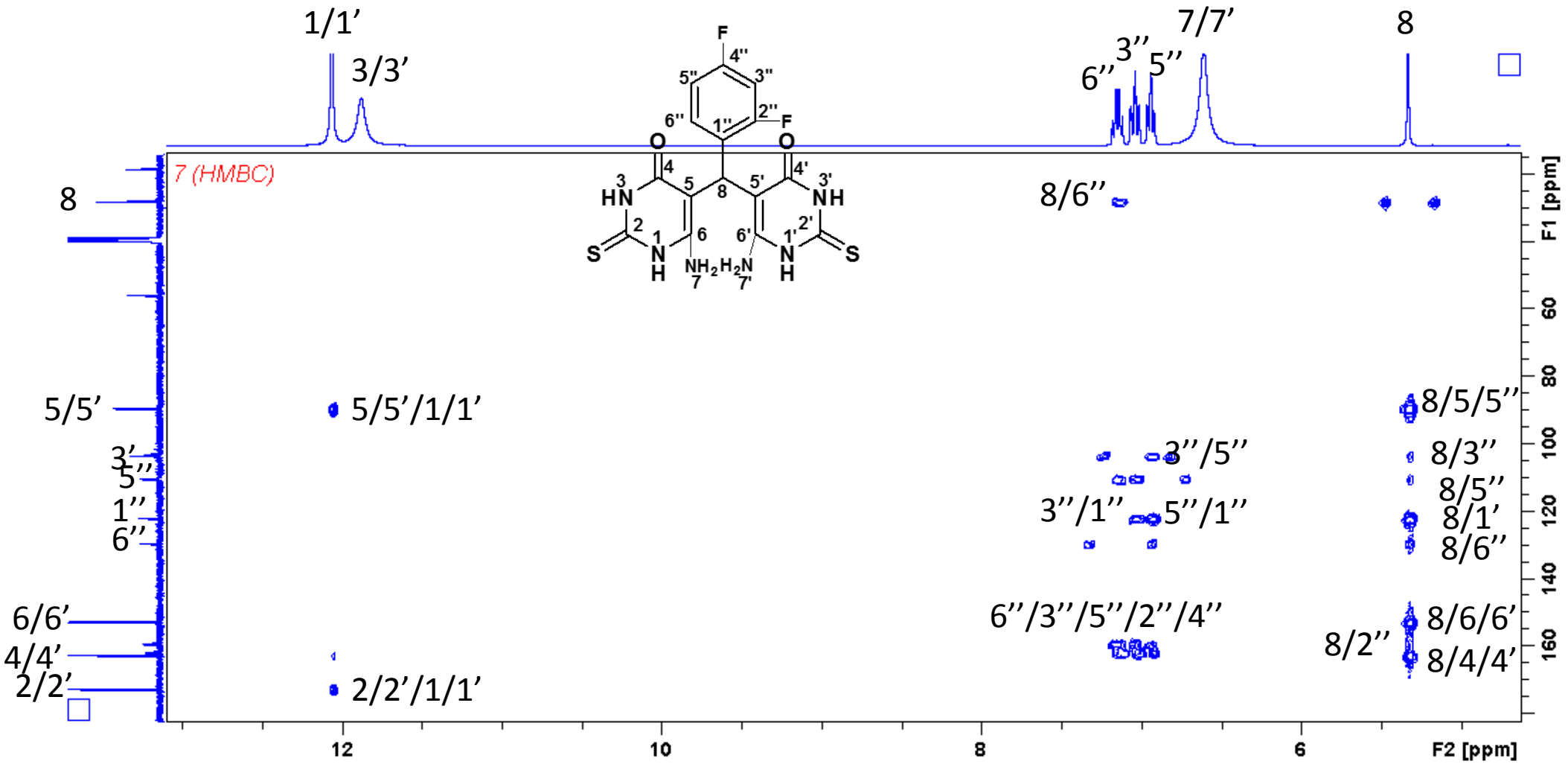
^{13}C NMR spectrum of 5,5'-((2,4-difluorophenyl)methylene)bis(6-amino-2-thiouracil) (C7) expanded



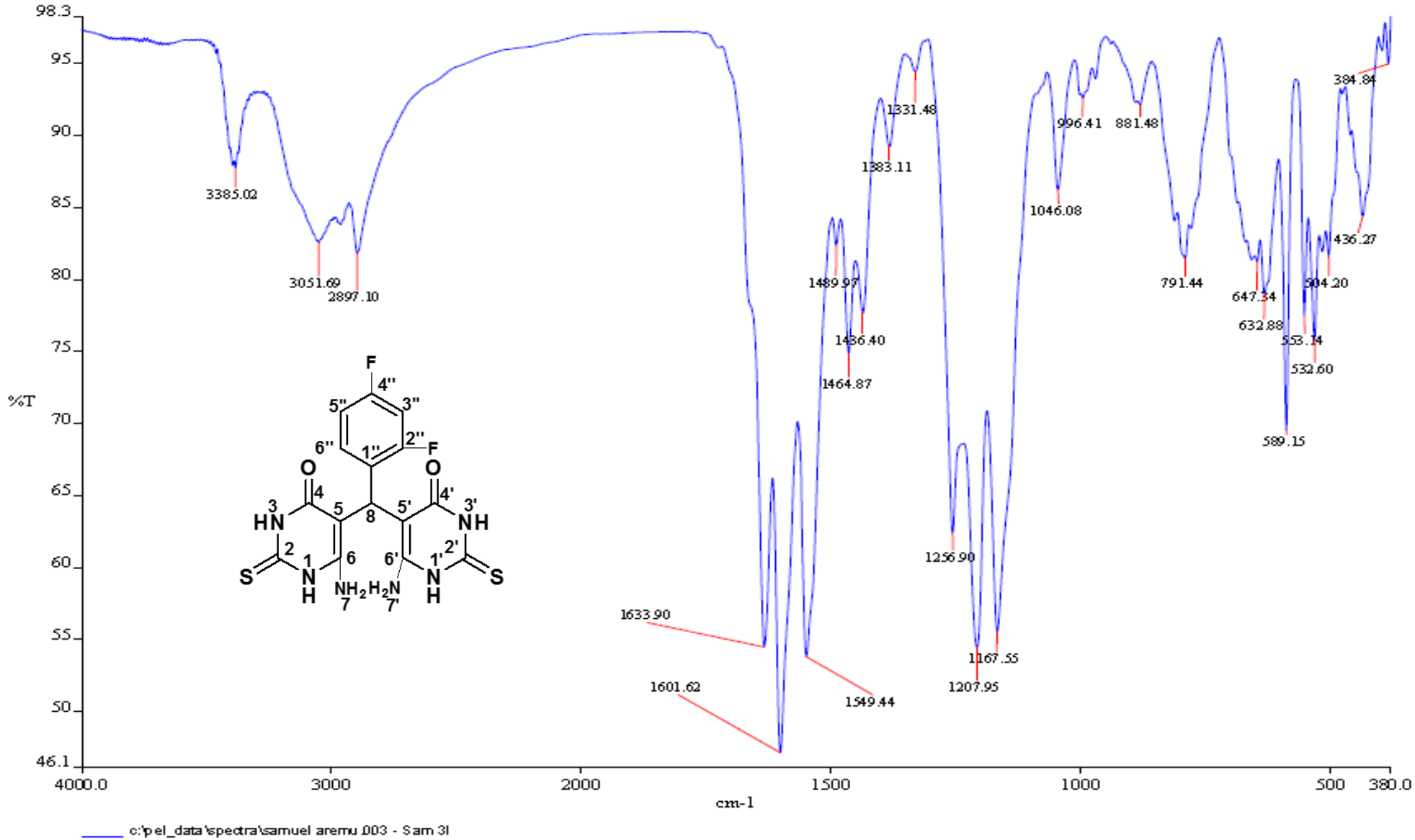
DEPT spectrum of 5,5'-((2,4-difluorophenyl)methylene)bis(6-amino-2-thiouracil) (C7)



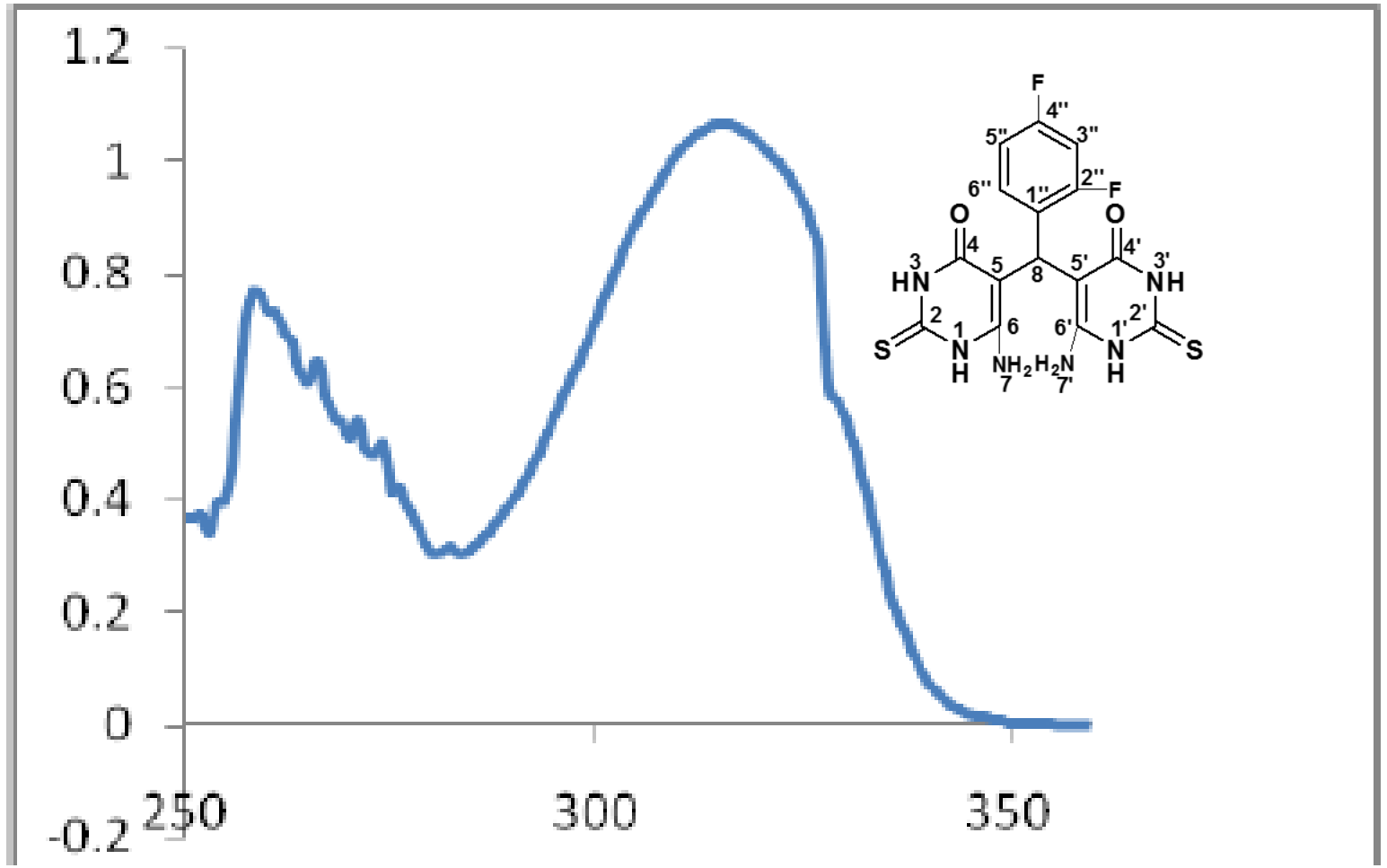
HSQC spectrum of 5,5'-((2,4-difluorophenyl)methylene)bis(6-amino-2-thiouracil) (C7)



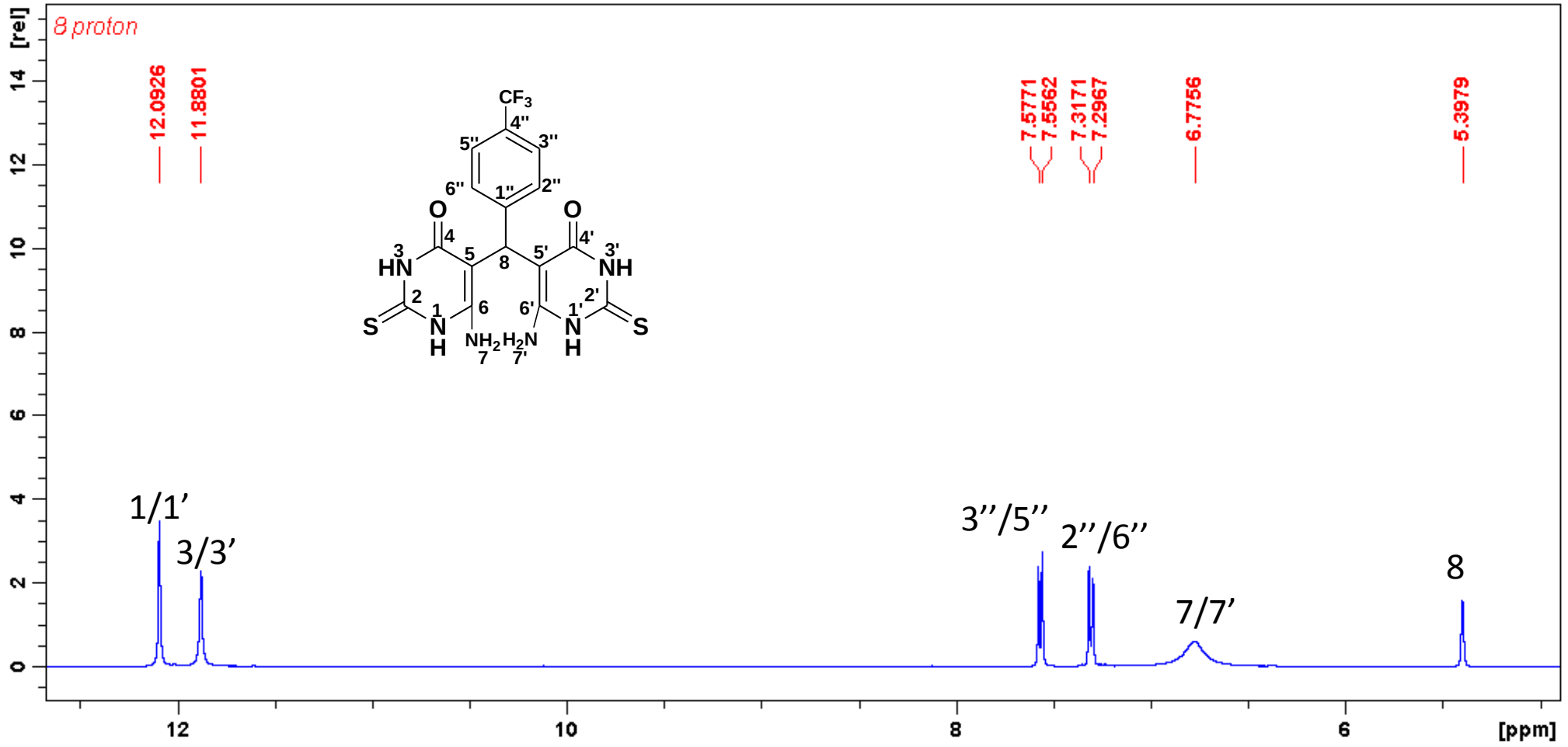
HMBC spectrum of 5,5'-((2,4-difluorophenyl)methylene)bis(6-amino-2-thiouracil) (C7)



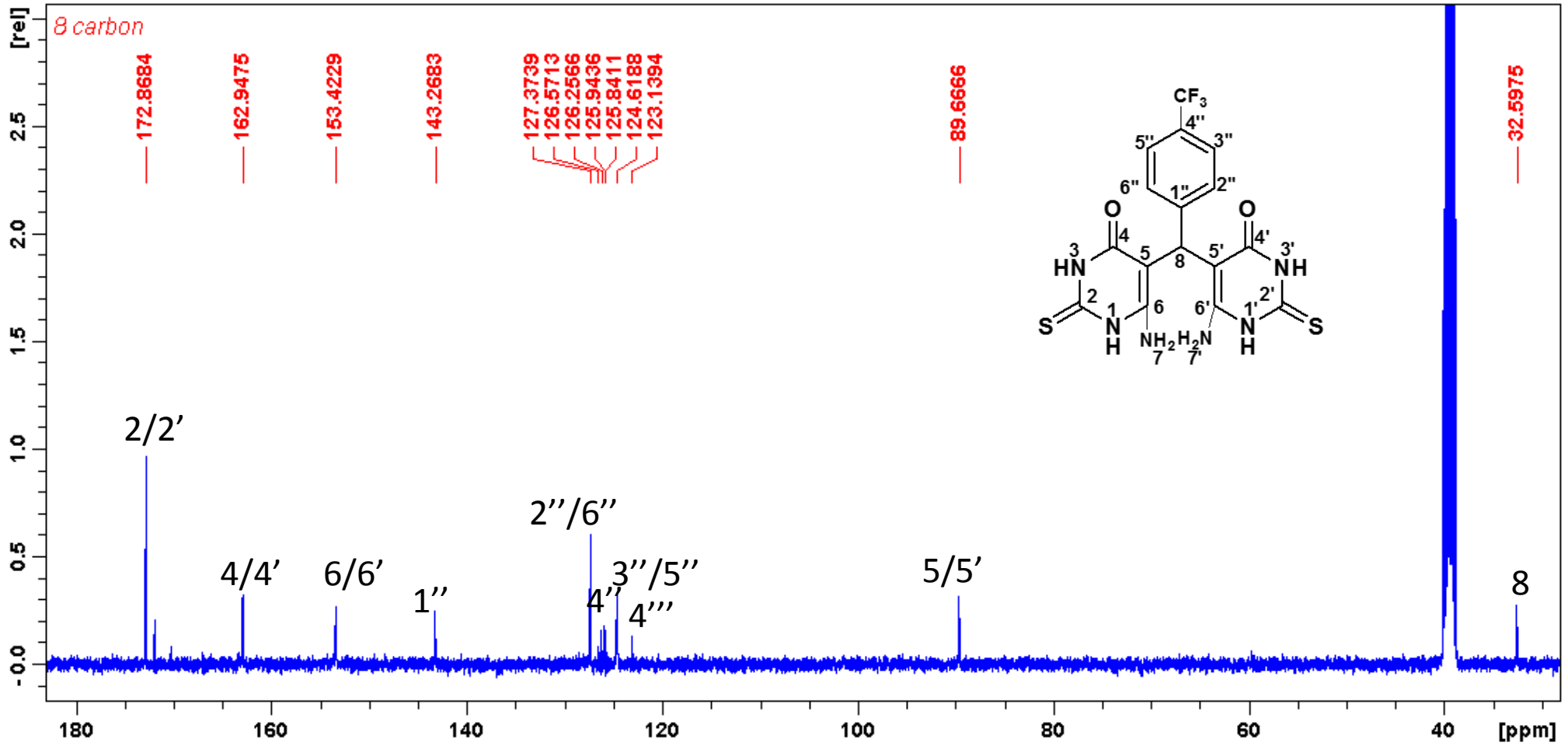
IR spectrum of 5,5'-((2,4-difluorophenyl)methylene)bis(6-amino-2-thiouracil) (C7)



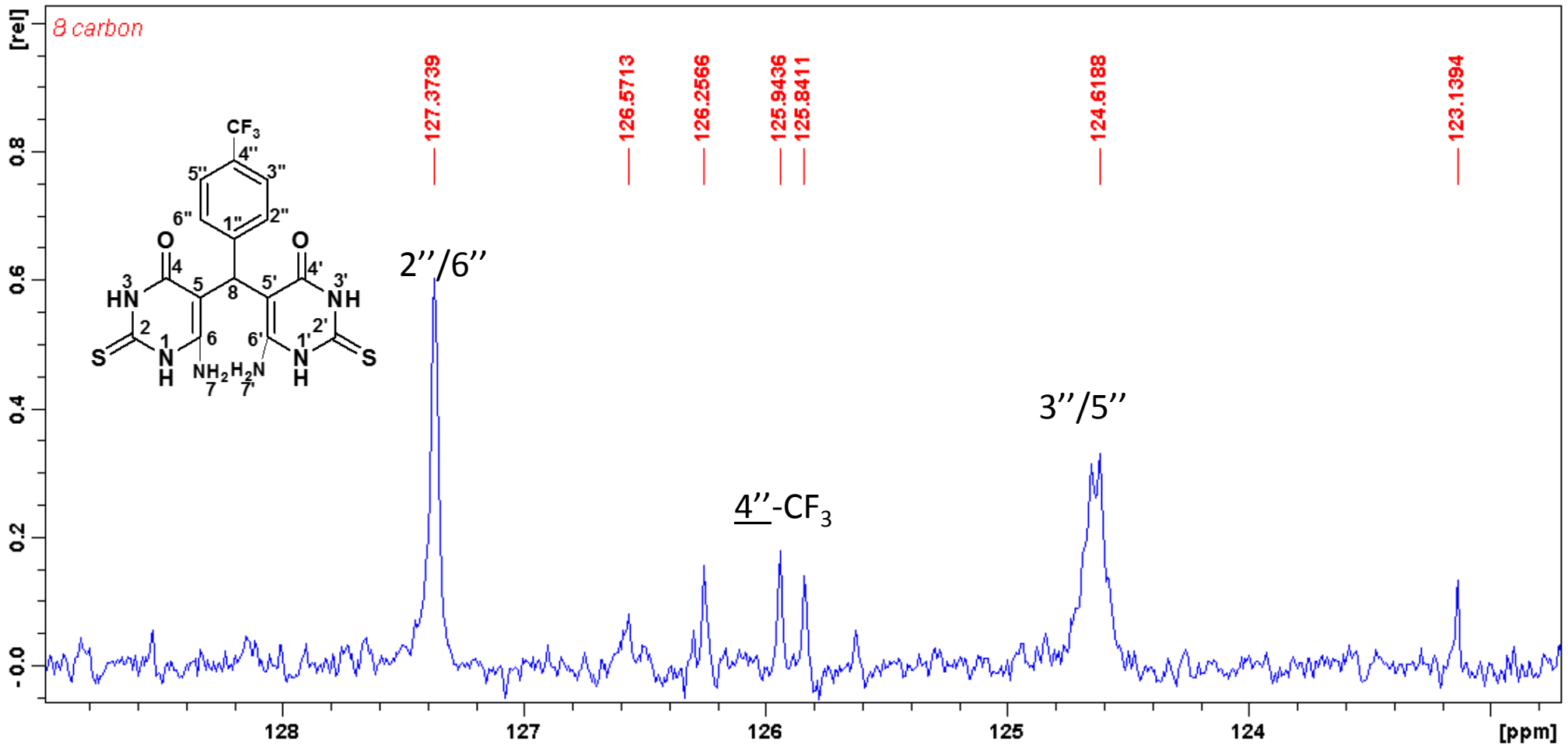
UV spectrum of 5,5'-((2,4-difluorophenyl)methylene)bis(6-amino-2-thiouracil) (C7)



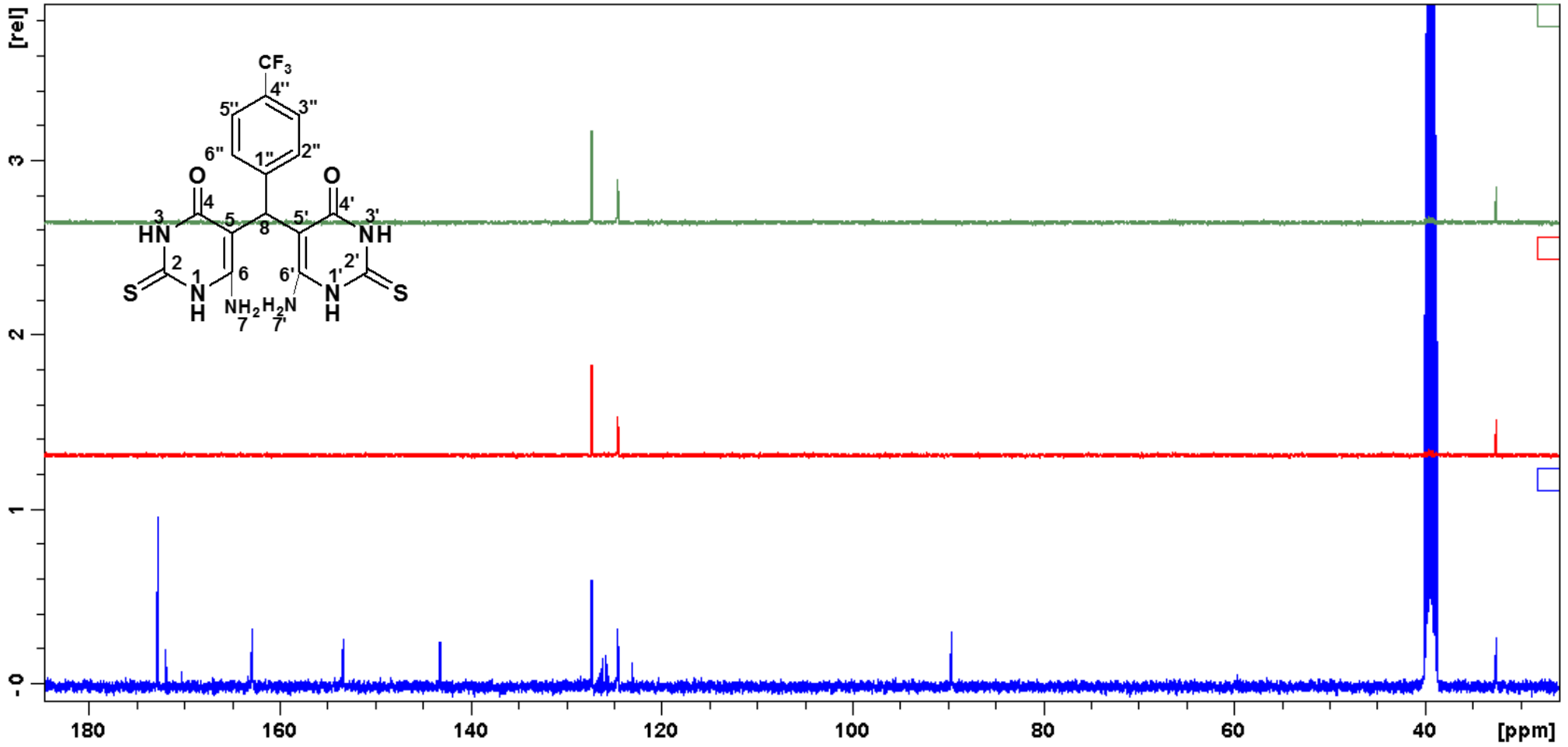
^{1}H NMR spectrum of 5,5'-((4-trifluoromethylphenyl)methylene)bis(6-amino-2-thiouracil) (C8)



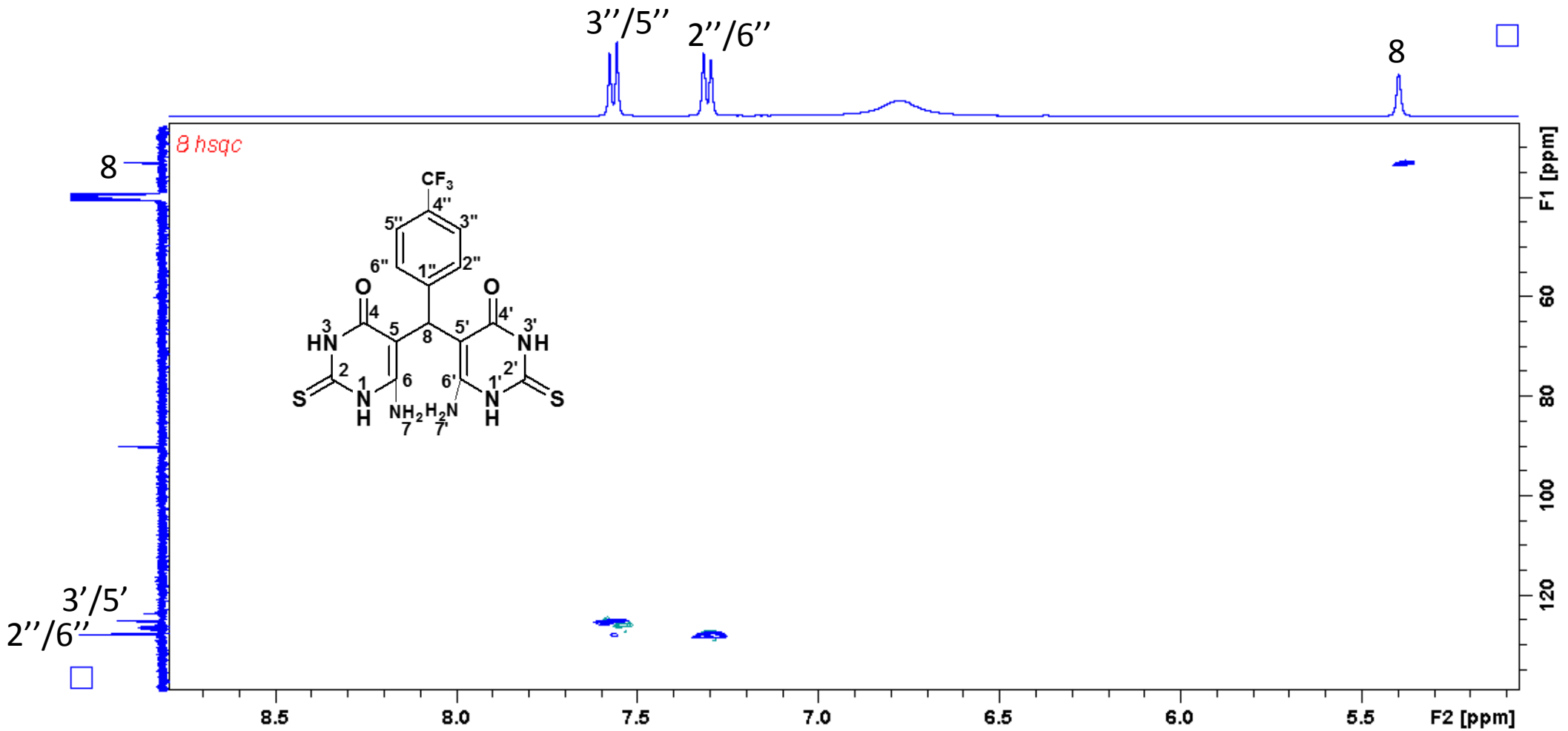
^{13}C NMR spectrum of 5,5'-((4-trifluoromethylphenyl)methylene)bis(6-amino-2-thiouracil) (C8)



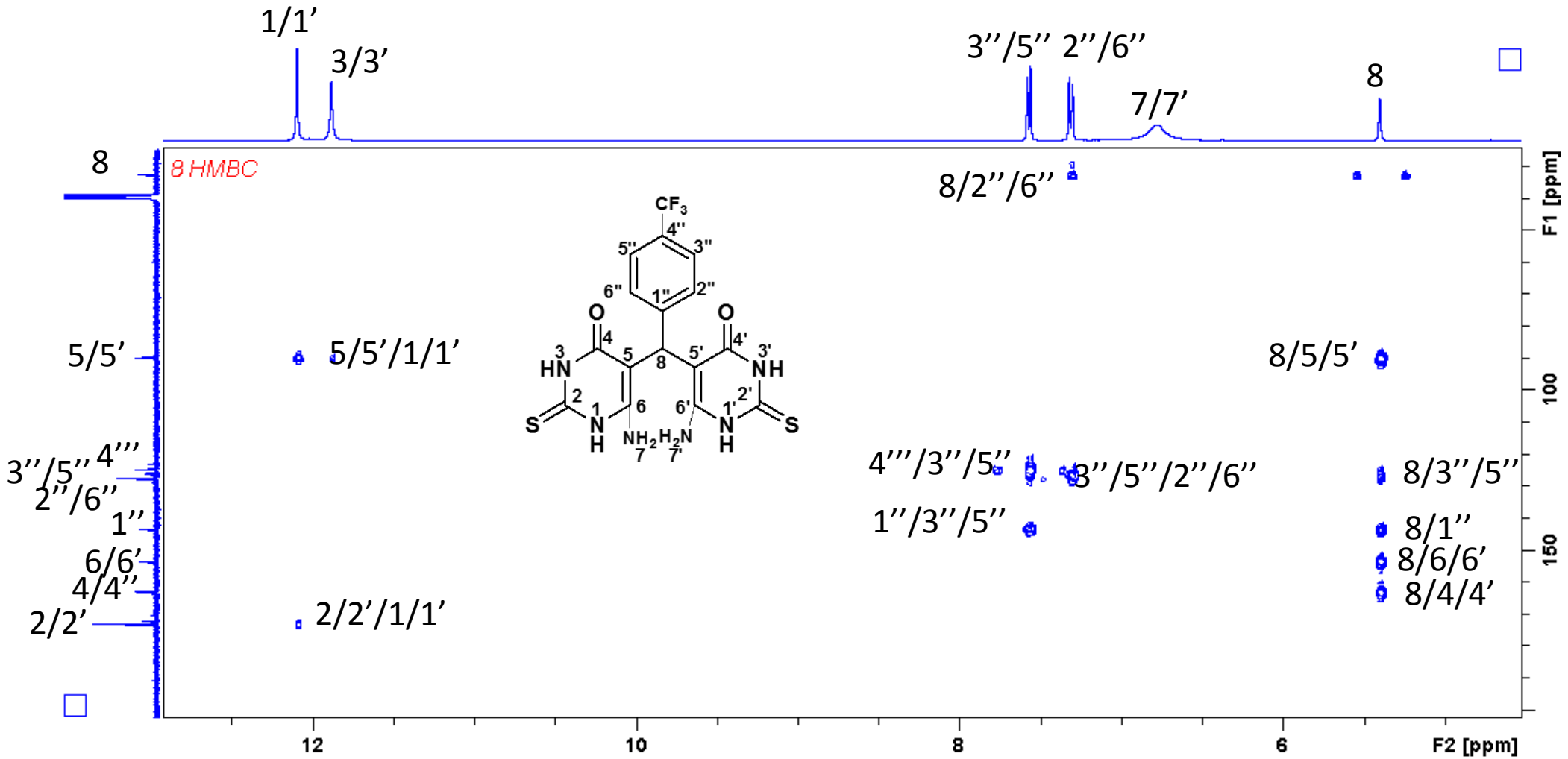
^{13}C NMR spectrum of 5,5'-((4-trifluoromethylphenyl)methylene)bis(6-amino-2-thiouracil) (C8) expanded



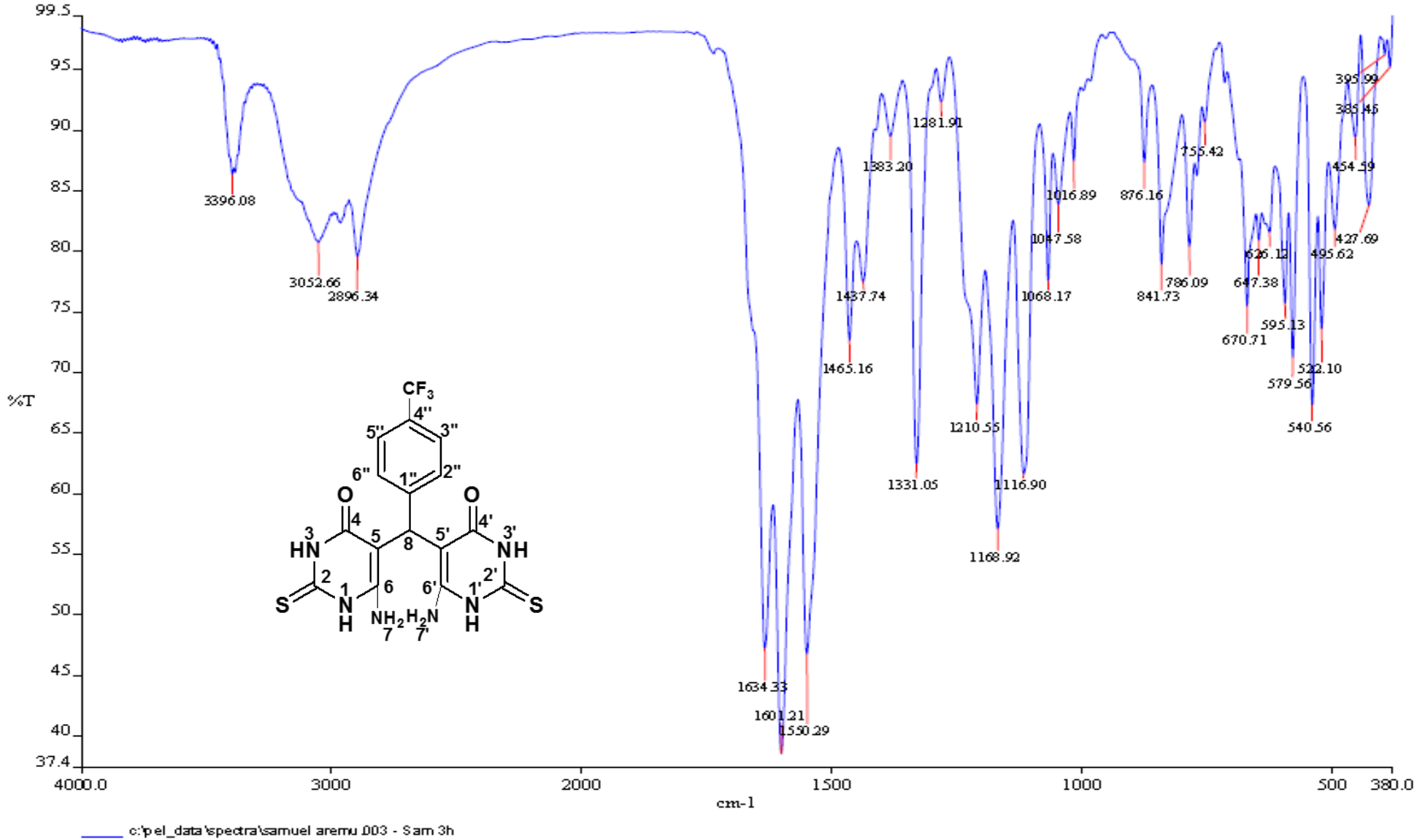
DEPT spectrum of 5,5'-((4-trifluoromethylphenyl)methylene)bis(6-amino-2-thiouracil) (C8)



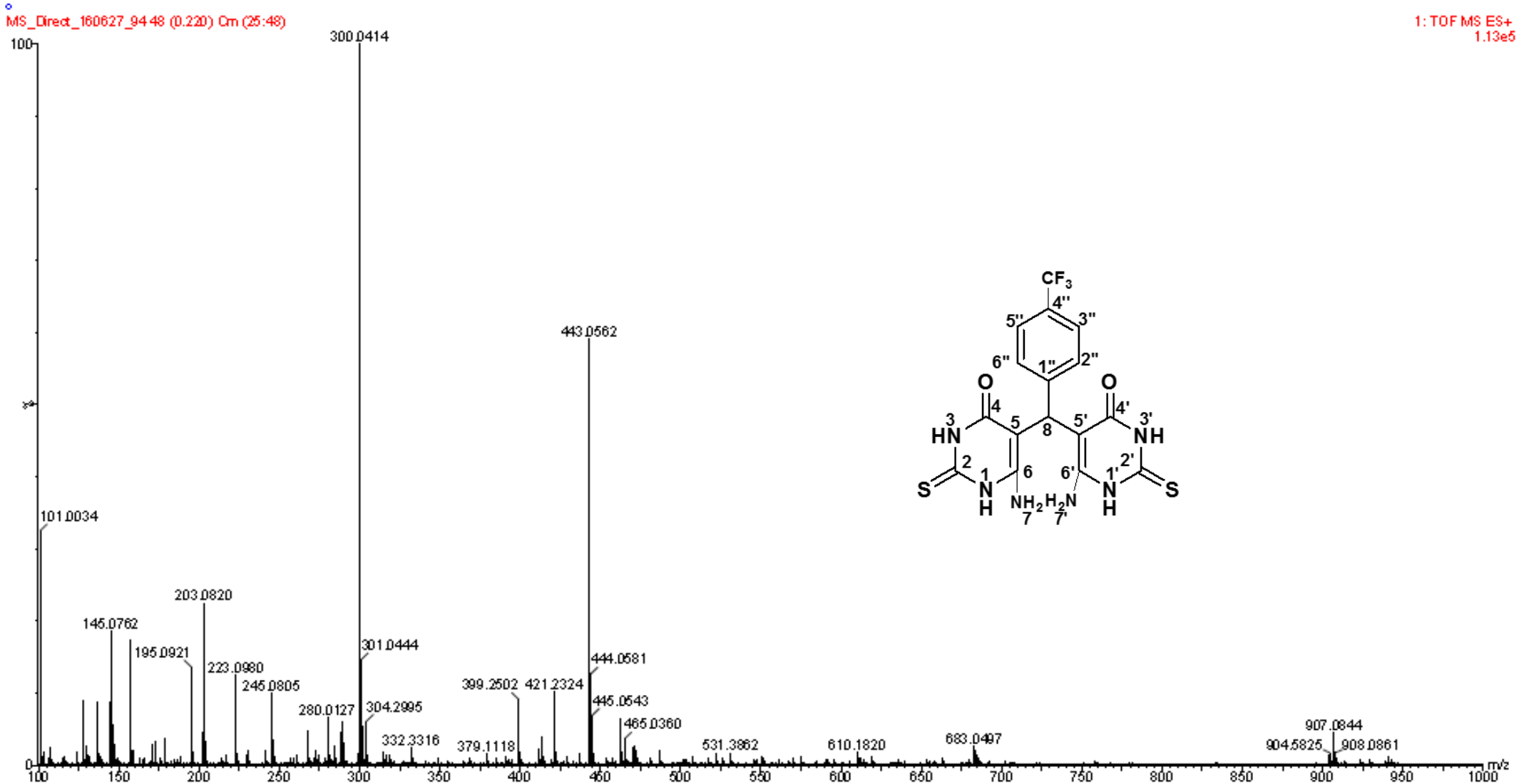
HSQC spectrum of 5,5'-((4-trifluoromethylphenyl)methylene)bis(6-amino-2-thiouracil) (C8)



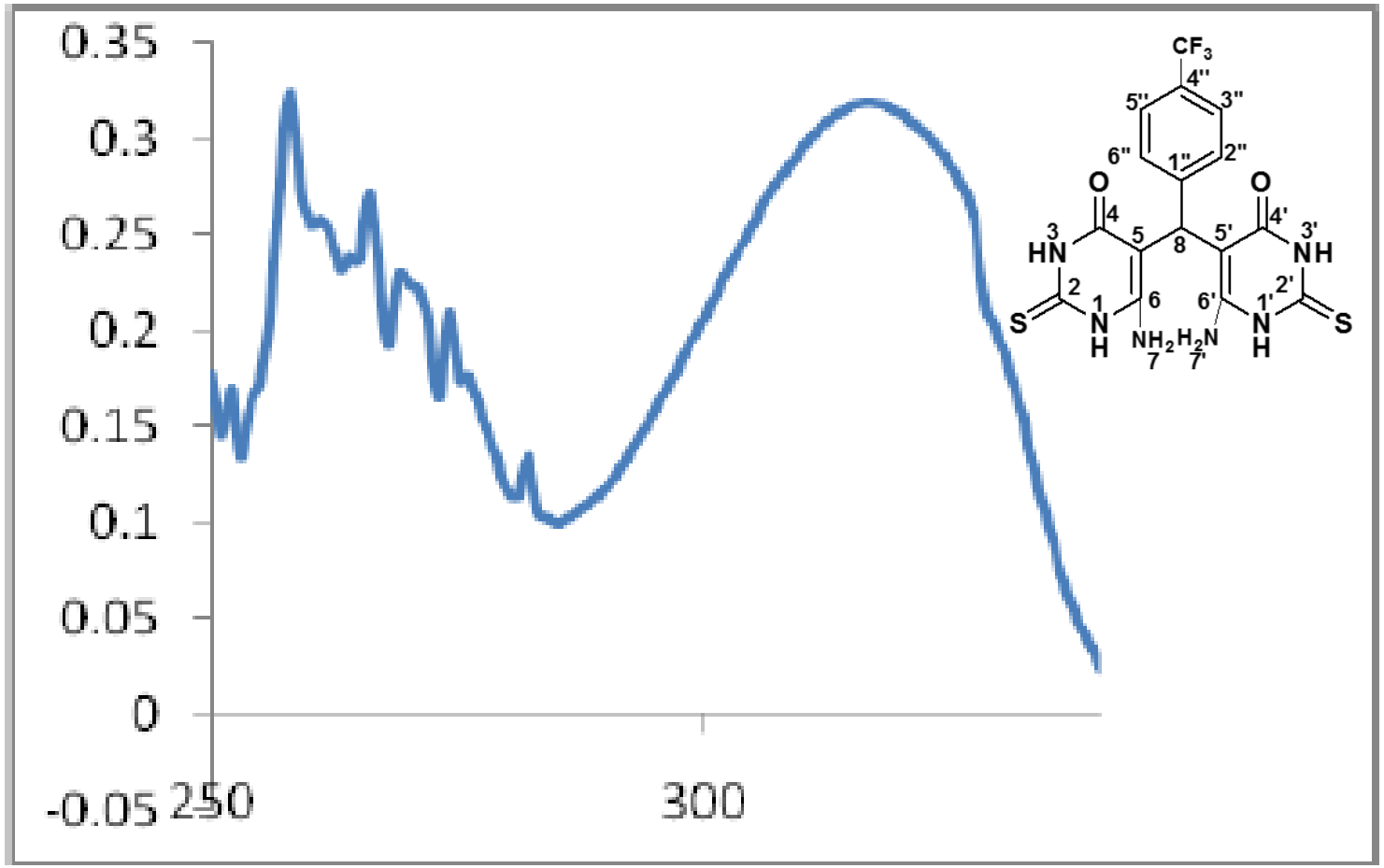
HMBC spectrum of 5,5'-((4-trifluoromethylphenyl)methylene)bis(6-amino-2-thiouracil) (C8)



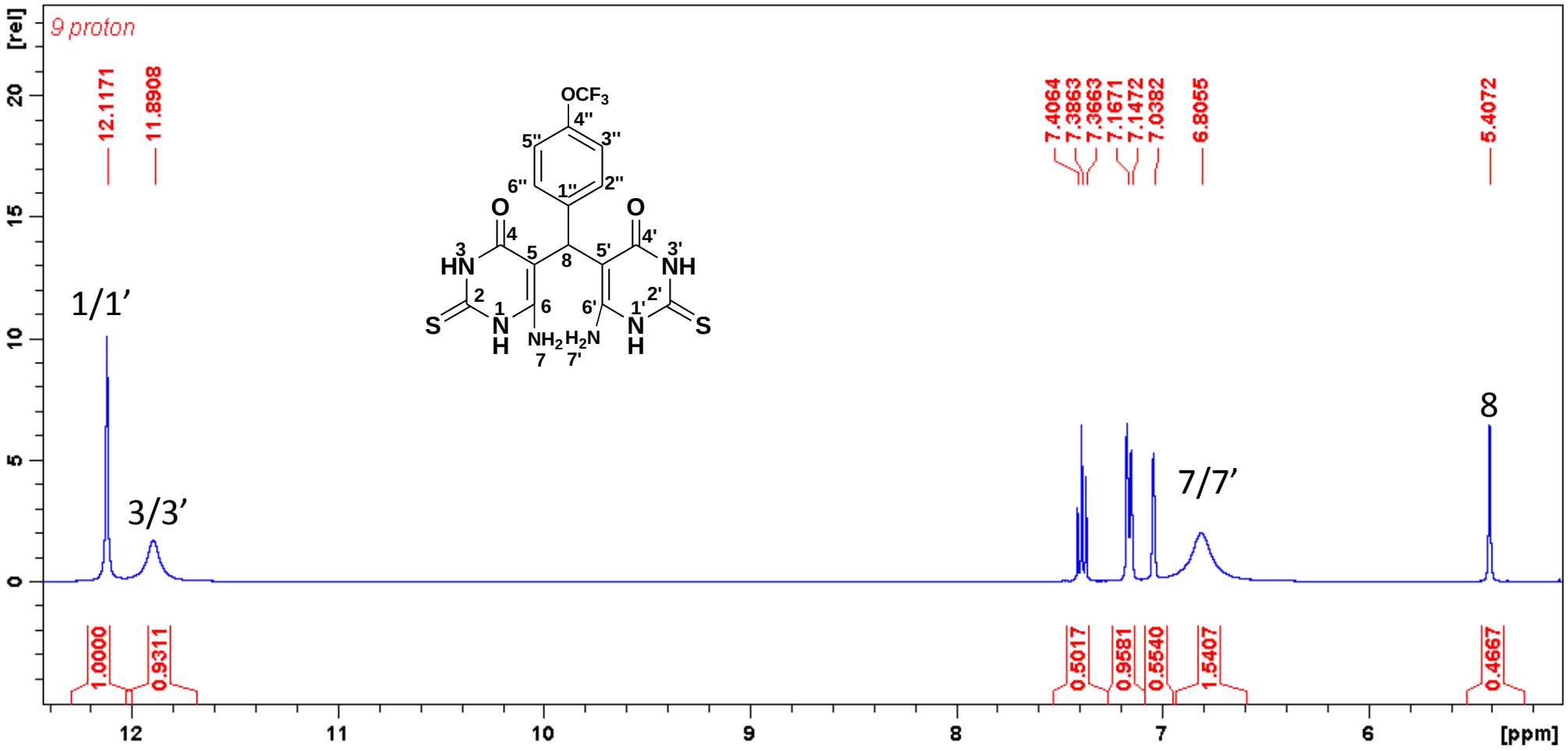
IR spectrum of 5,5'-((4-trifluoromethylphenyl)methylene)bis(6-amino-2-thiouracil) (C8)



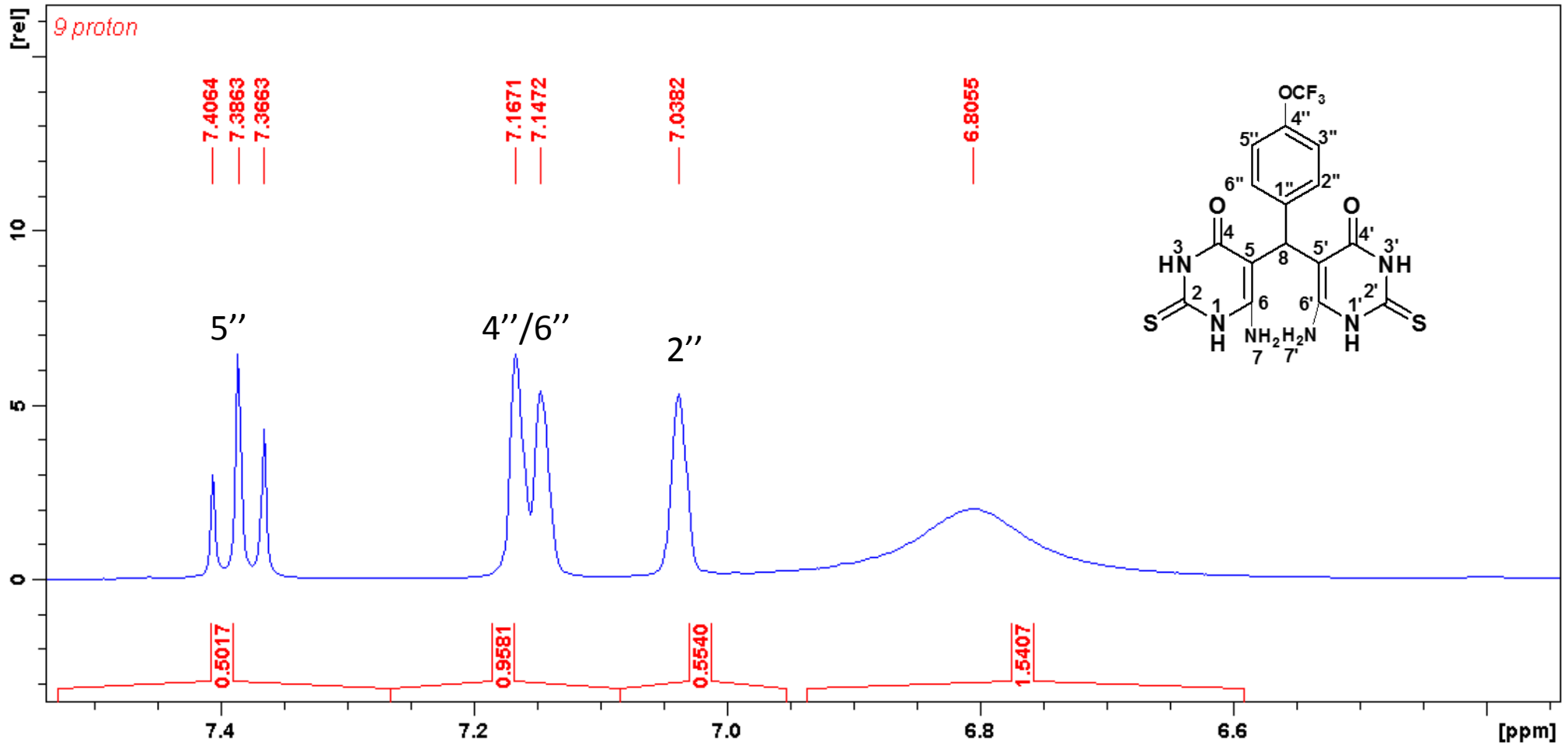
HRMS of 5,5'-((4-trifluoromethylphenyl)methylene)bis(6-amino-2-thiouracil) (C8)



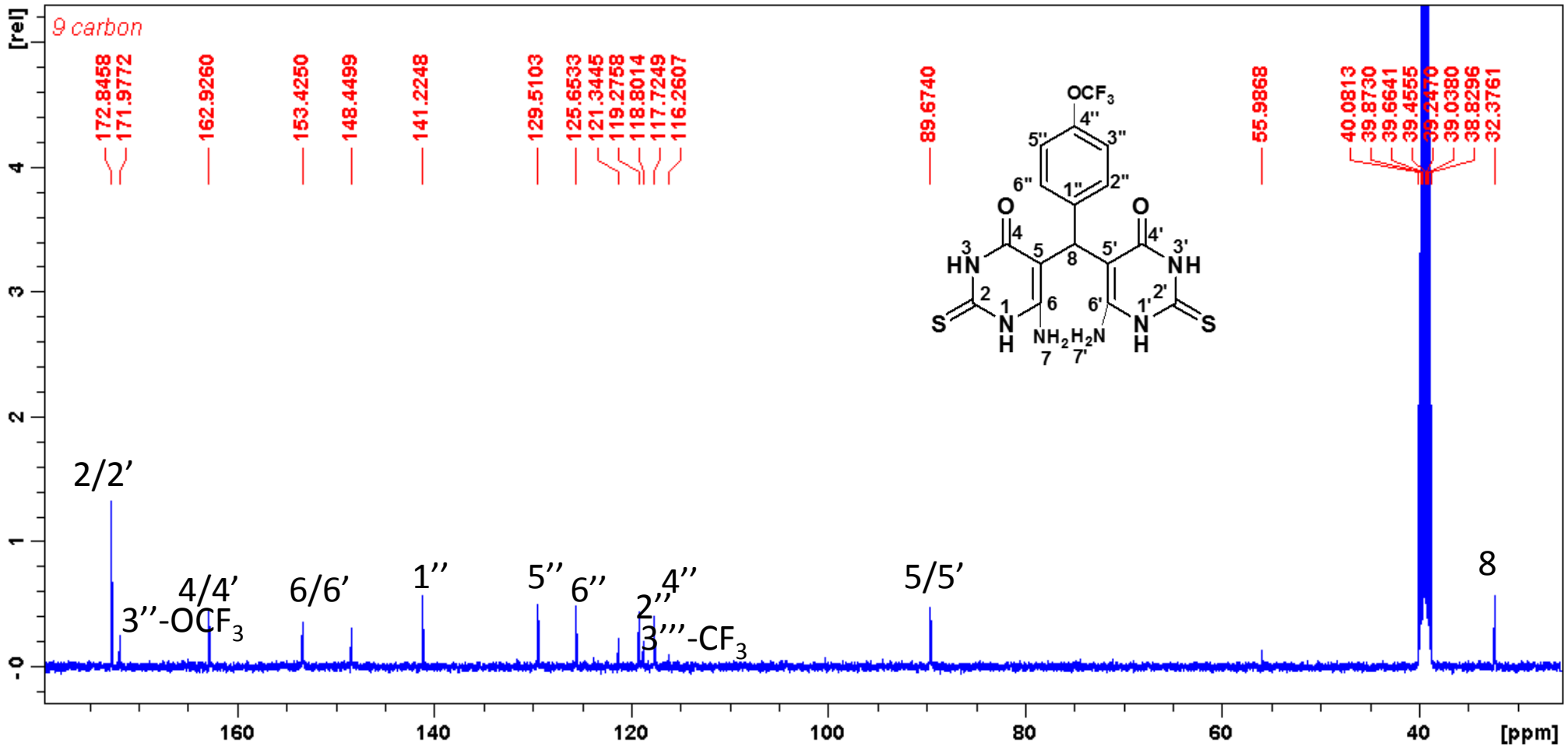
UV spectrum of 5,5'-((4-trifluoromethylphenyl)methylene)bis(6-amino-2-thiouracil) (C8)



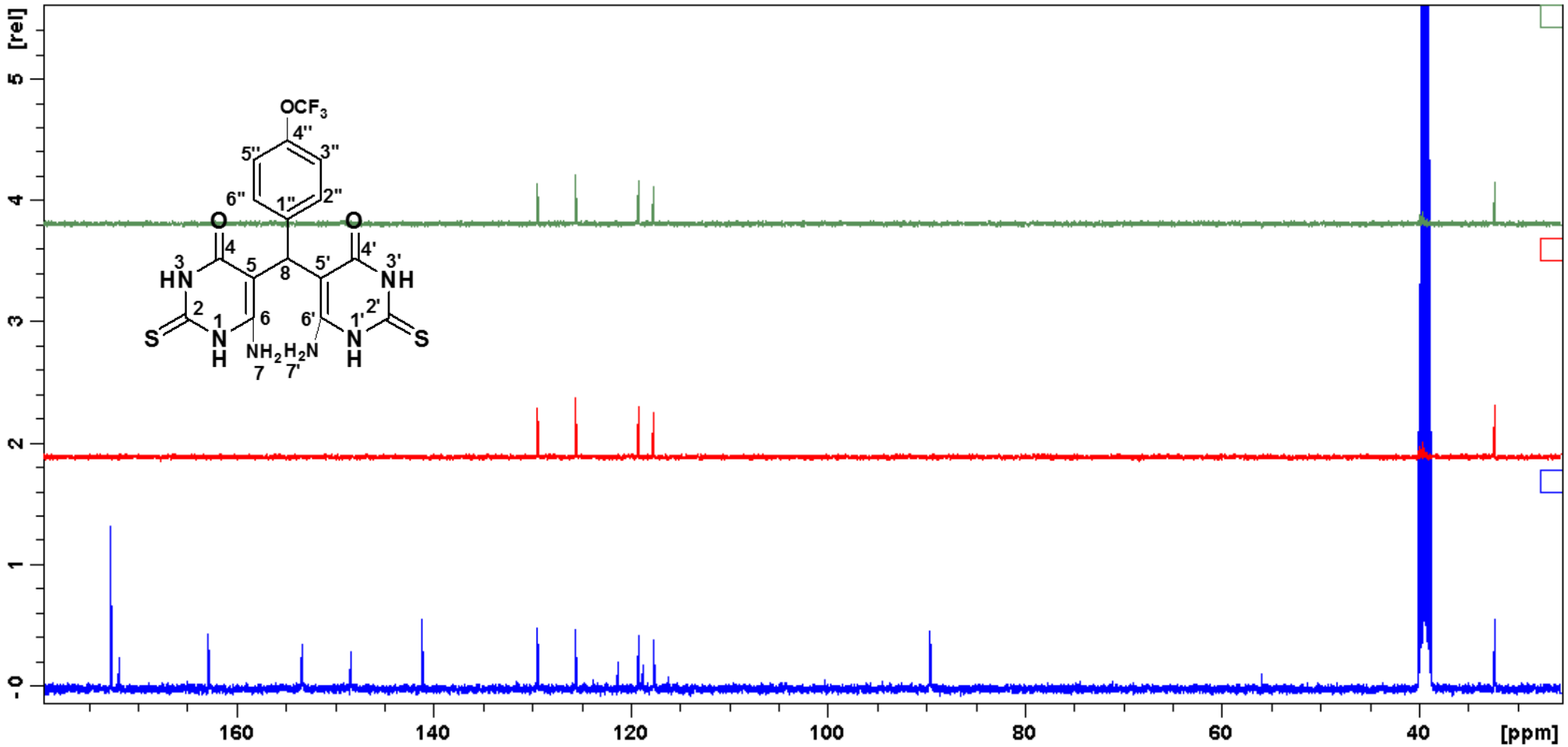
^{1}H NMR spectrum of 5,5'-((4-trifluoromethoxylphenyl)methylene)bis(6-amino-2-thiouracil) (C9)



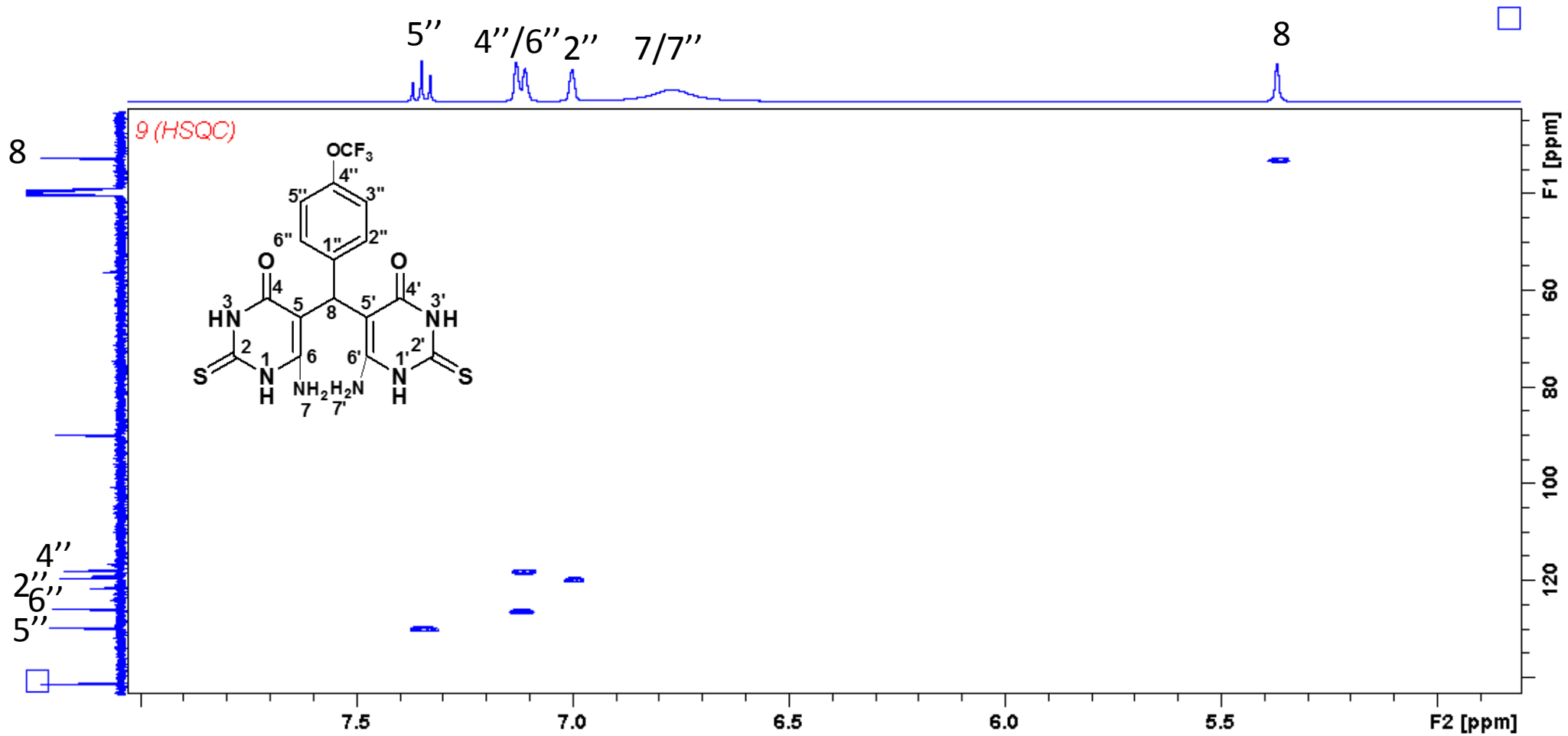
^{1}H NMR spectrum of 5,5'-((4-trifluoromethoxylphenyl)methylene)bis(6-amino-2-thiouracil) (C9) expanded



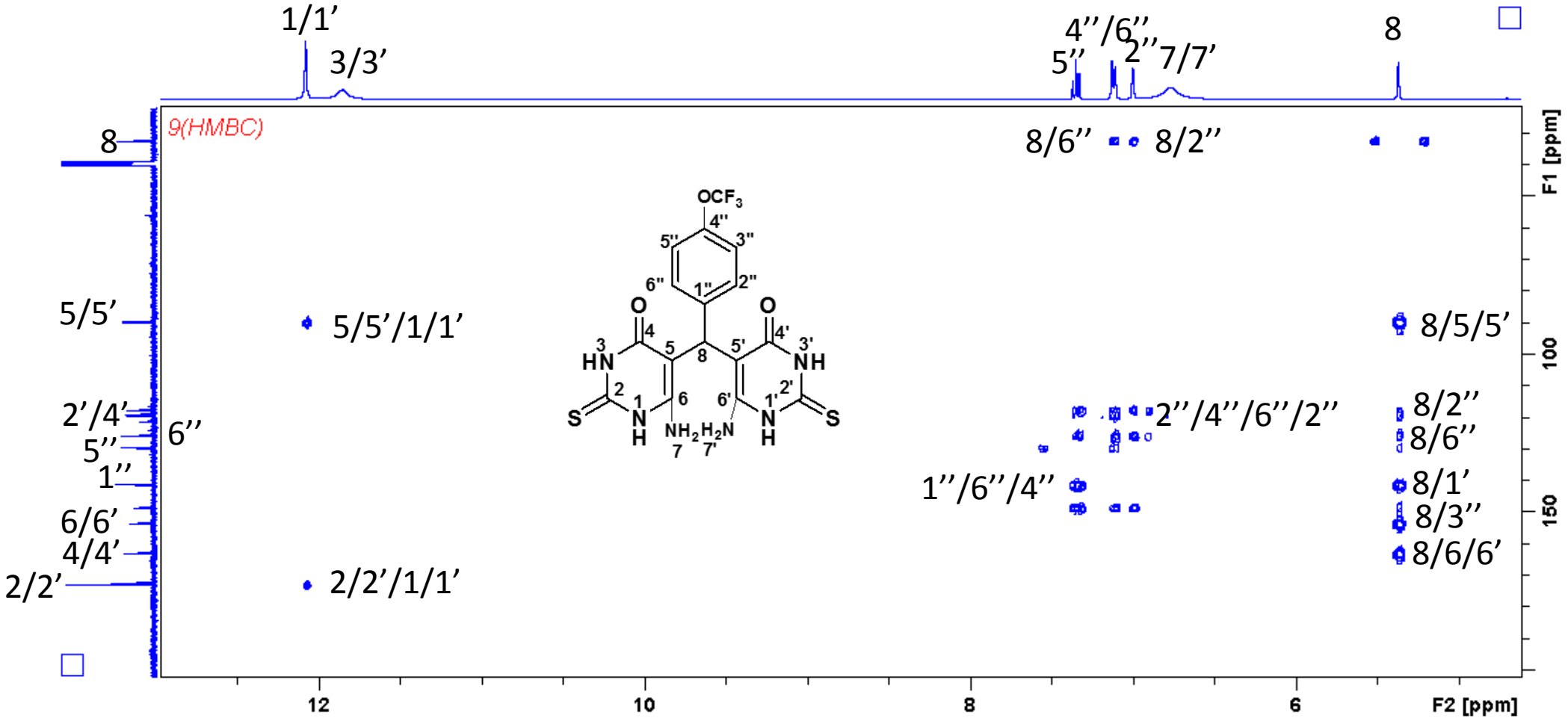
^{13}C NMR spectrum of 5,5'-((4-trifluoromethoxylphenyl)methylene)bis(6-amino-2-thiouracil) (C9)



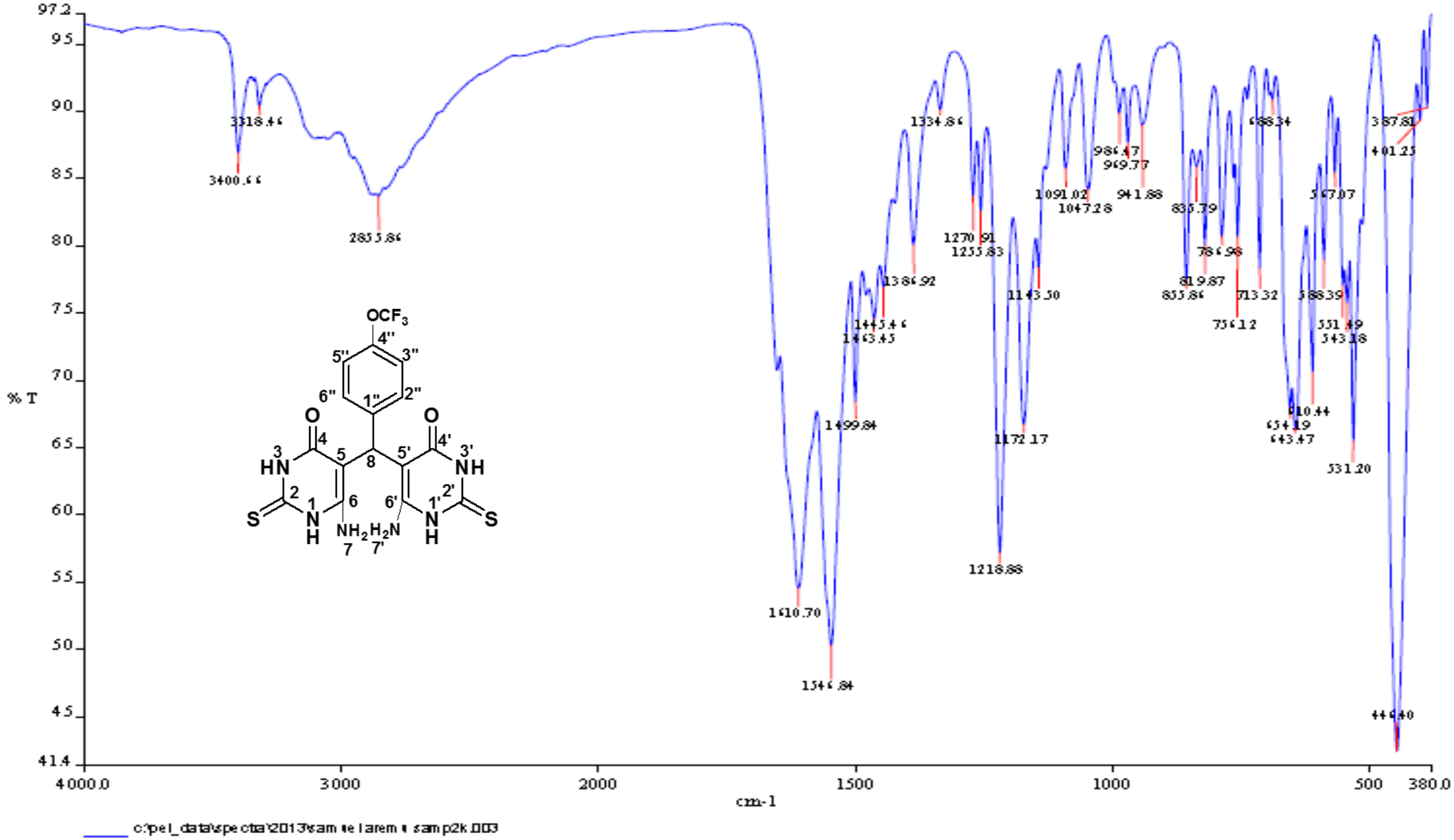
DEPT spectrum of 5,5'-((4-trifluoromethoxylphenyl)methylene)bis(6-amino-2-thiouracil) (C9)



HSQC spectrum of 5,5'-((4-trifluoromethoxylphenyl)methylene)bis(6-amino-2-thiouracil) (C9)



HMBC spectrum of 5,5'-((4-trifluoromethoxylphenyl)methylene)bis(6-amino-2-thiouracil) (C9)

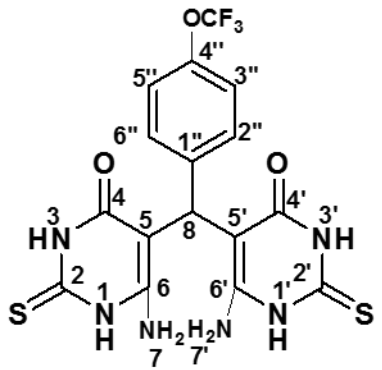


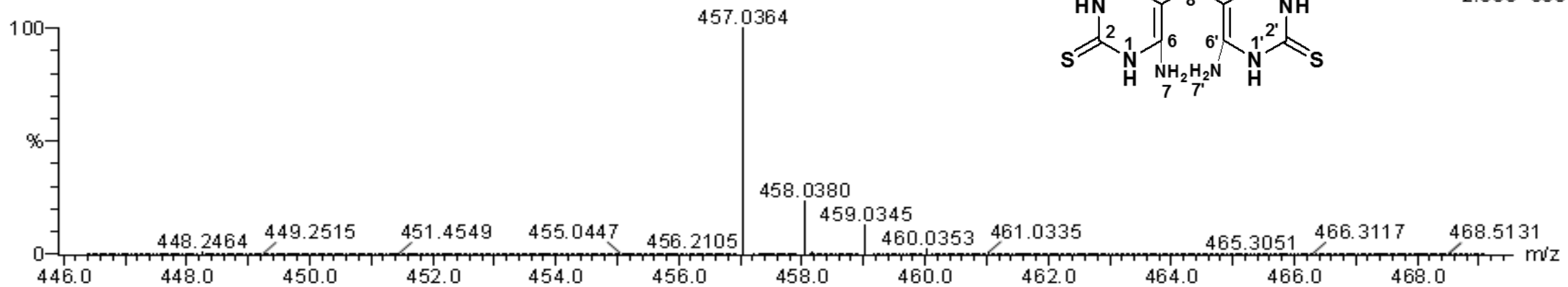
IR spectrum of 5,5'-((4-trifluoromethoxylphenyl)methylene)bis(6-amino-2-thiouracil) (C9)

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

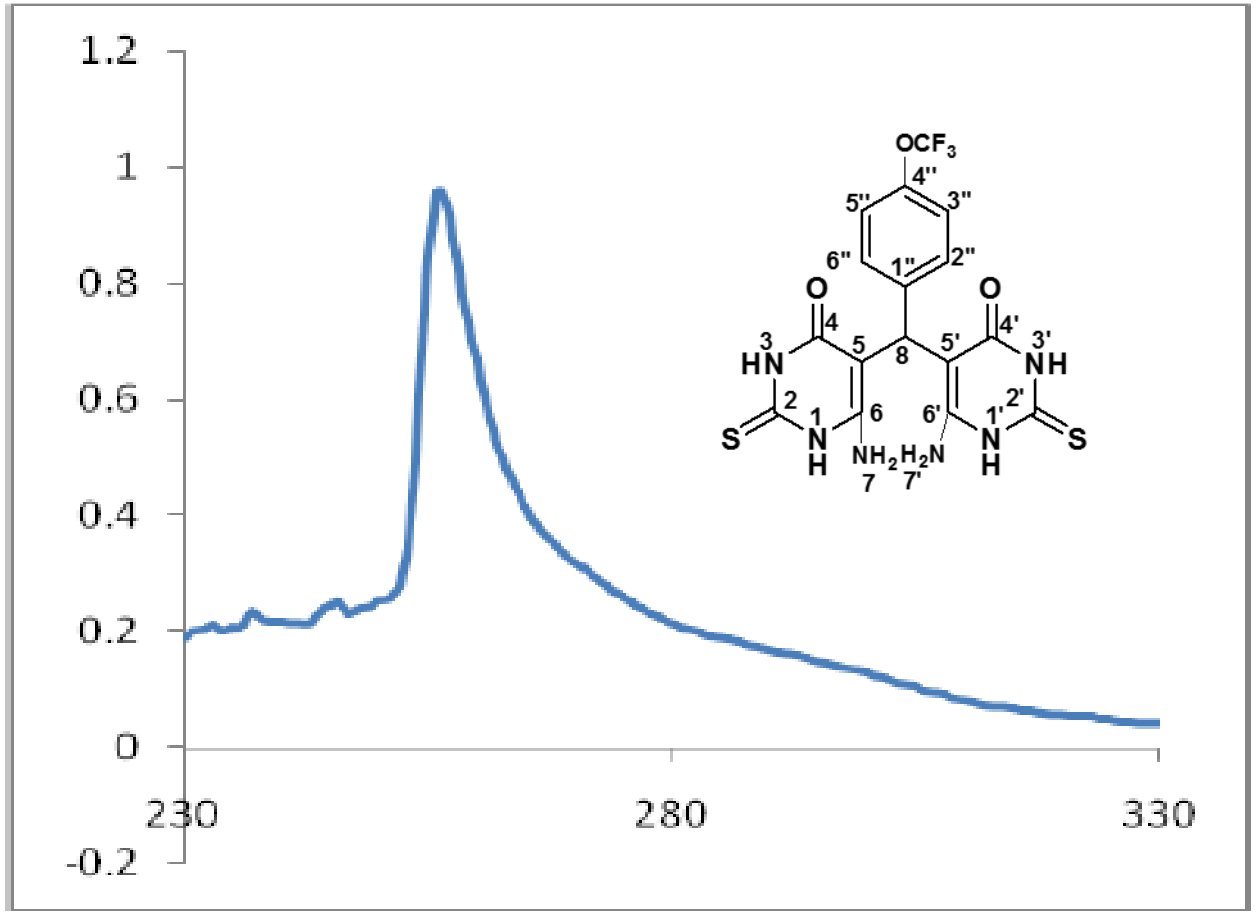
Monoisotopic Mass, Even Electron Ions
563 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-18 H: 10-15 N: 0-10 O: 0-5 F: 0-3 S: 0-2
9 24 (0.777) Cm (1:61)
TOF MS ES-



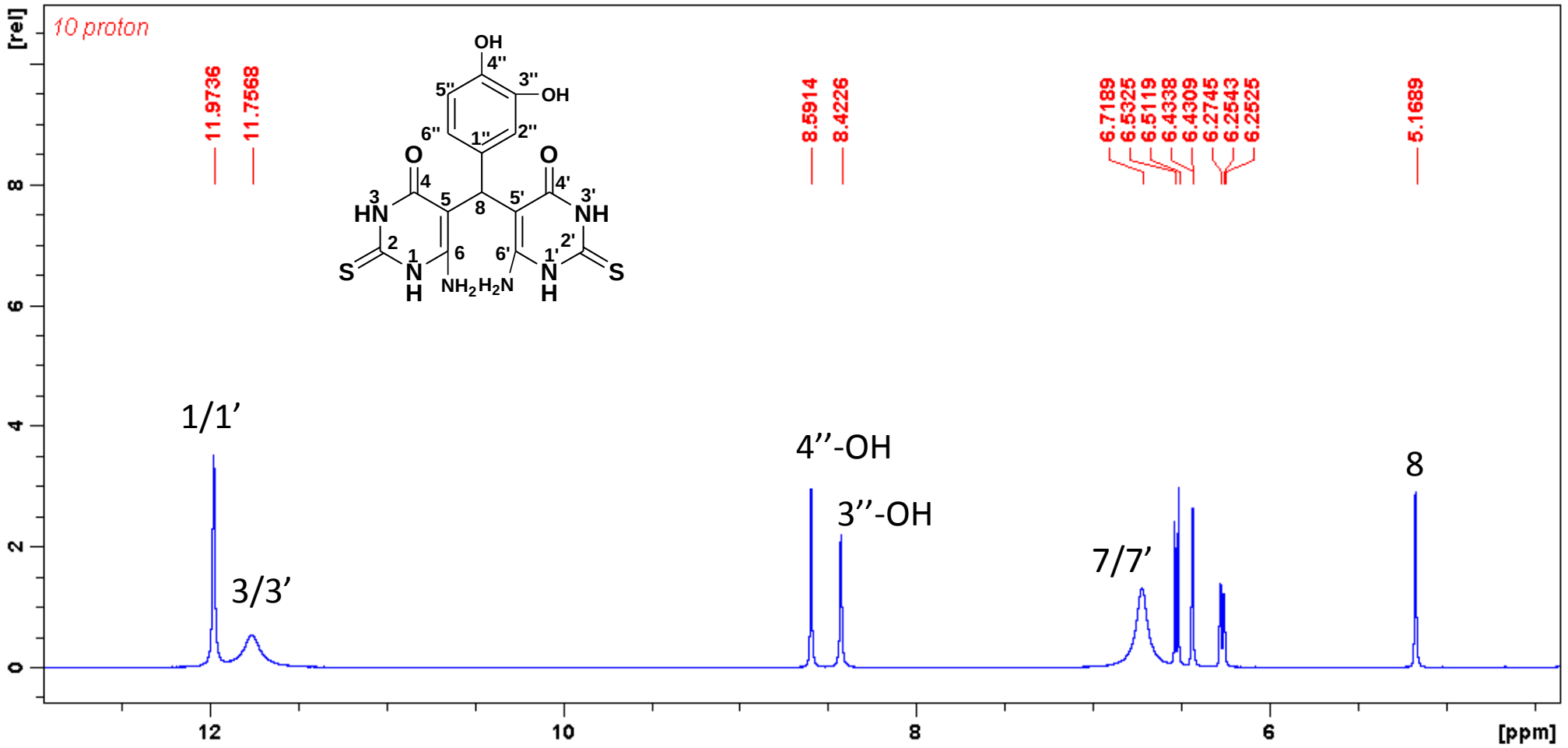


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
457.0364	457.0364	0.0	0.0	12.5	551.0	0.0	C16 H12 N6 O3 F3 S2

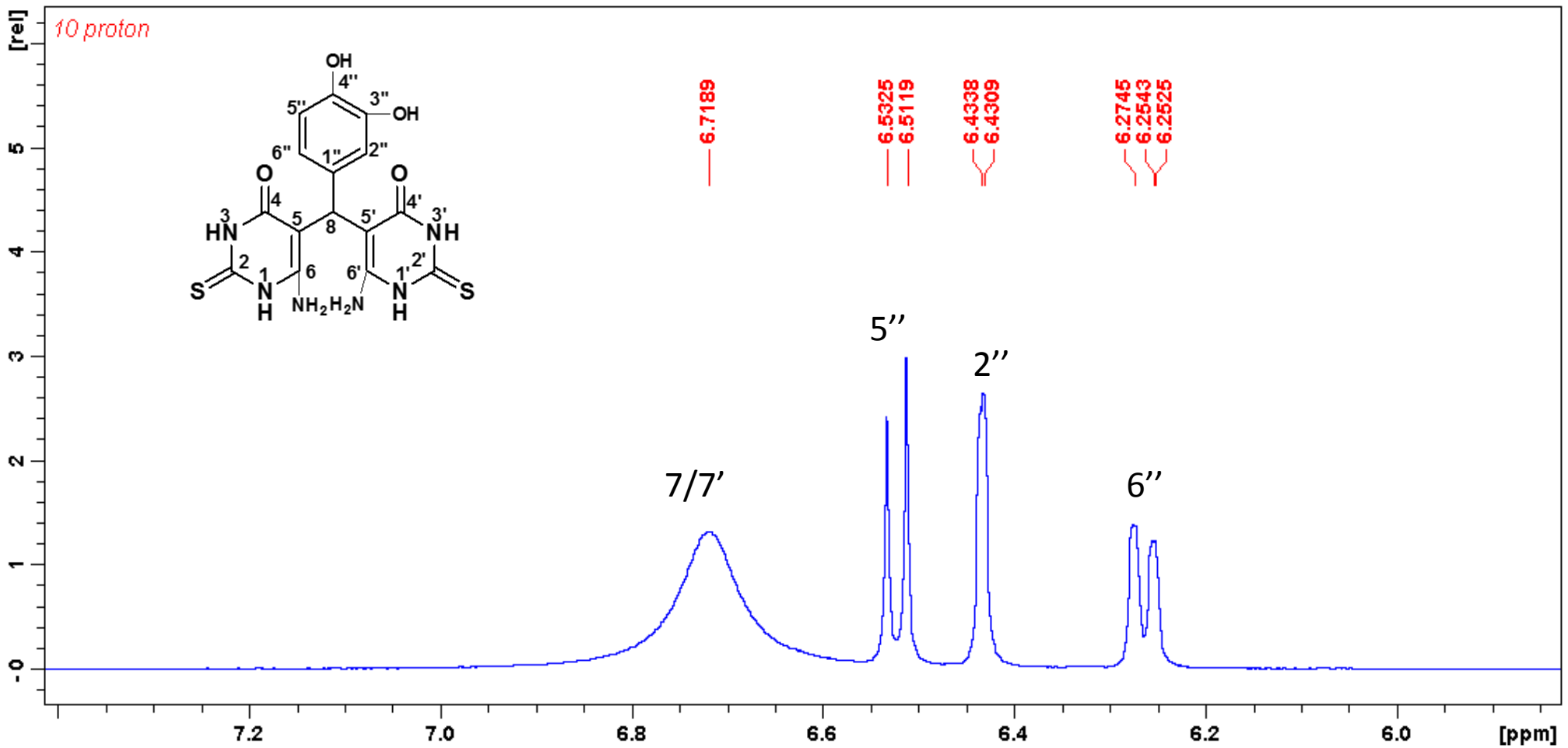
HRMS of 5,5'-((4-trifluoromethoxylphenyl)methylene)bis(6-amino-2-thiouracil) (C9)



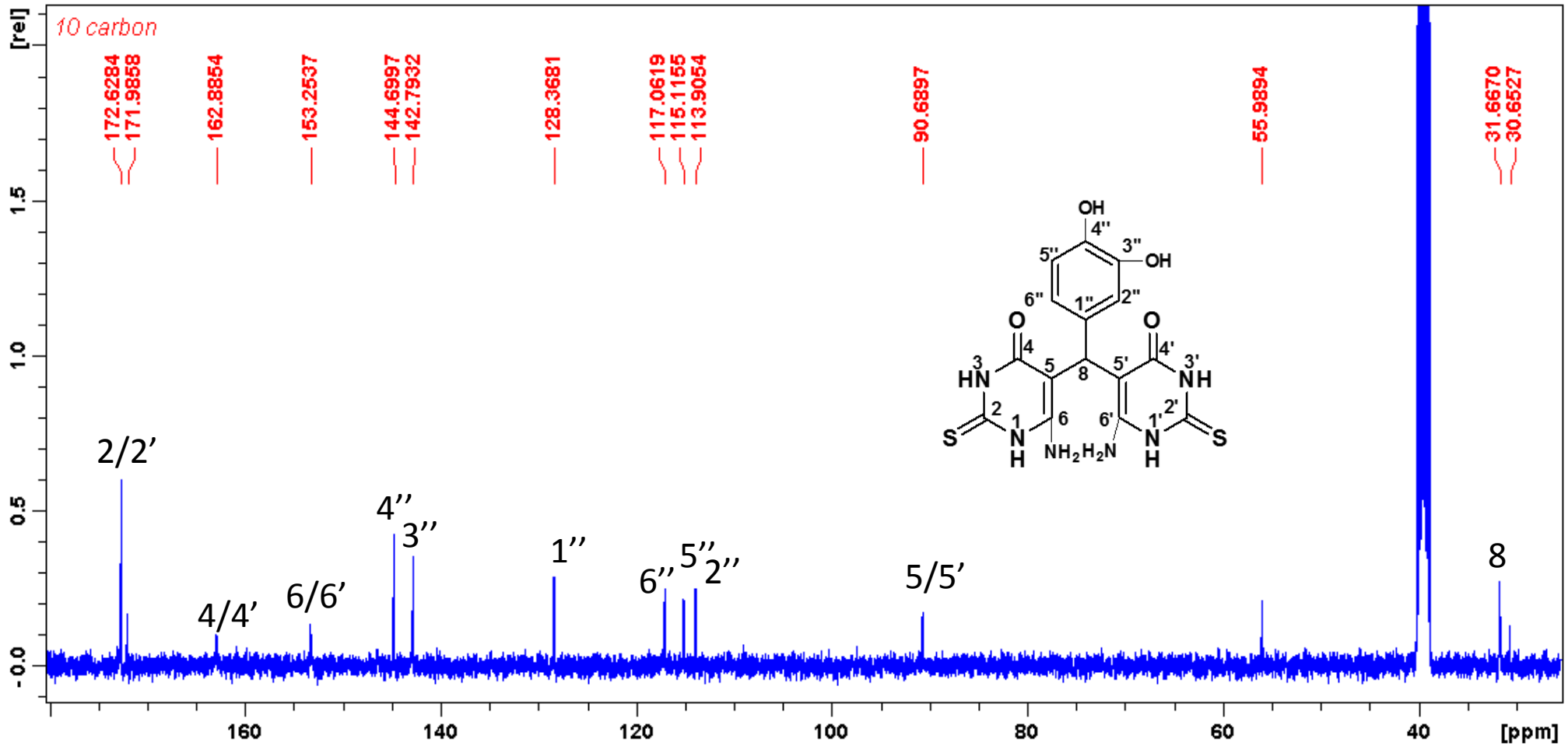
UV spectrum of 5,5'-((4-trifluoromethoxylphenyl)methylene)bis(6-amino-2-thiouracil) (C9)



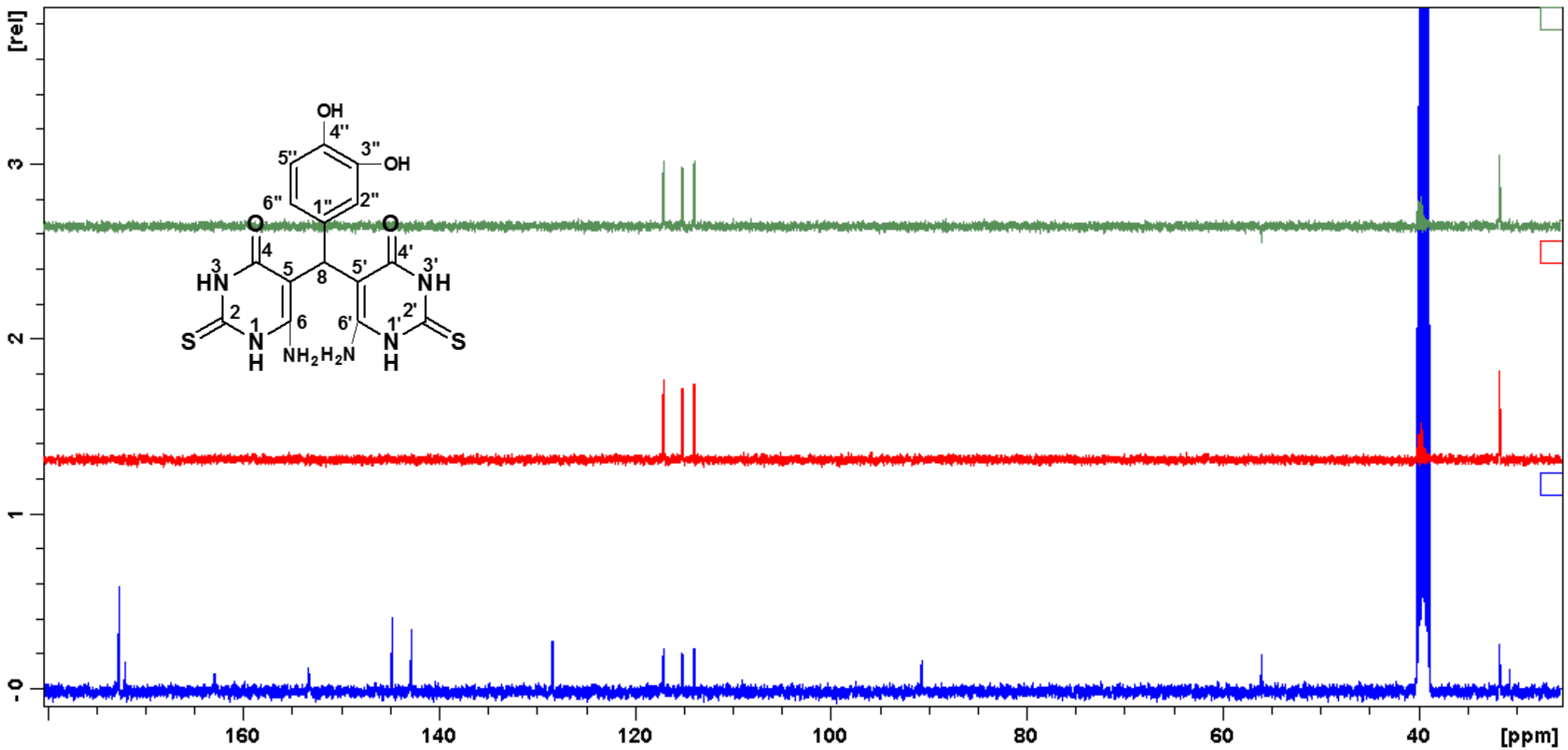
^{1}H NMR spectrum of 5,5'-((3,4-dihydroxylphenyl)methylene)bis(6-amino-2-thiouracil) (C10)



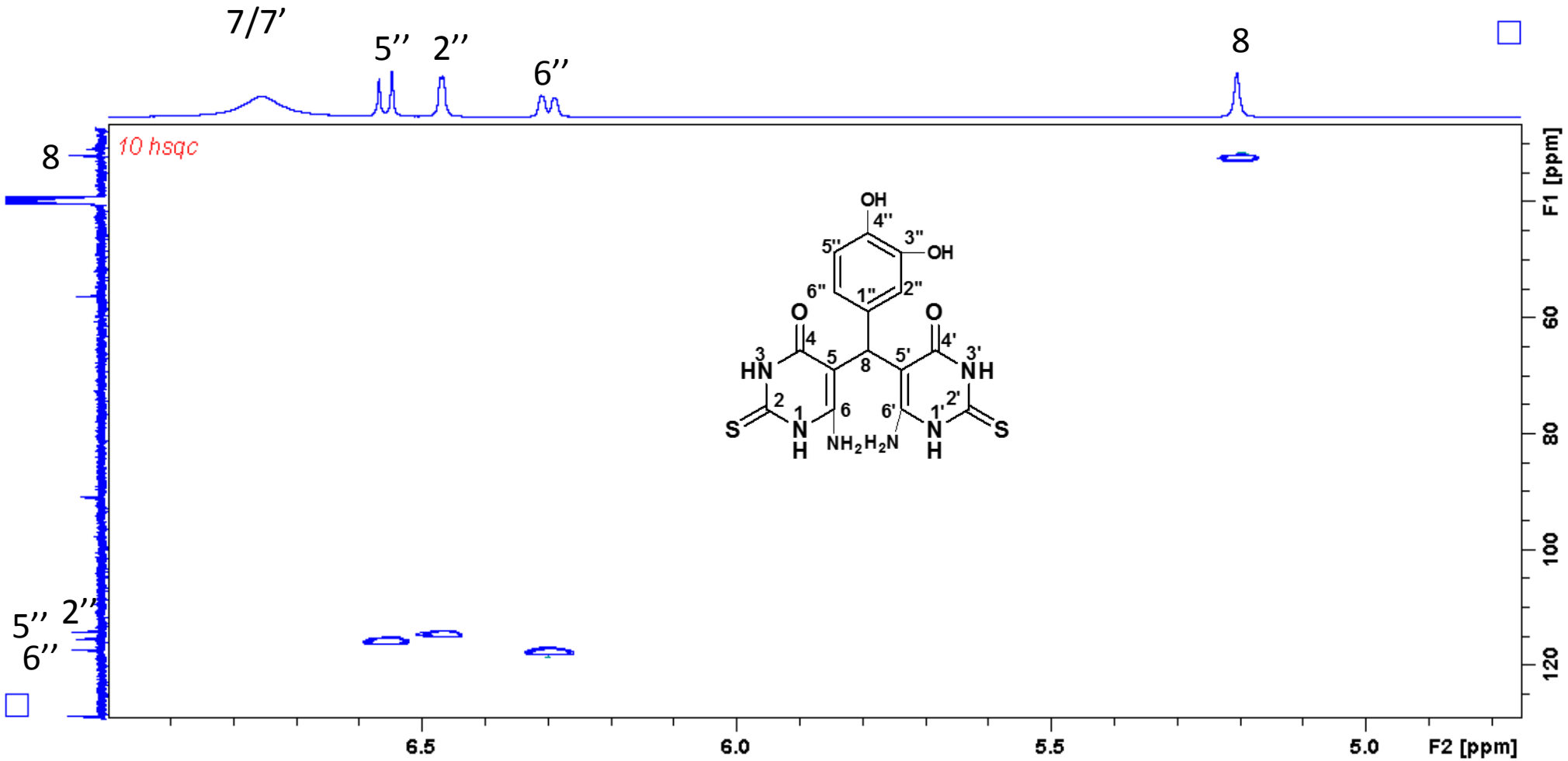
^{1}H NMR spectrum of 5,5'-(3,4-dihydroxylphenylmethylene)bis(6-amino-2-thiouracil) (C10) expanded



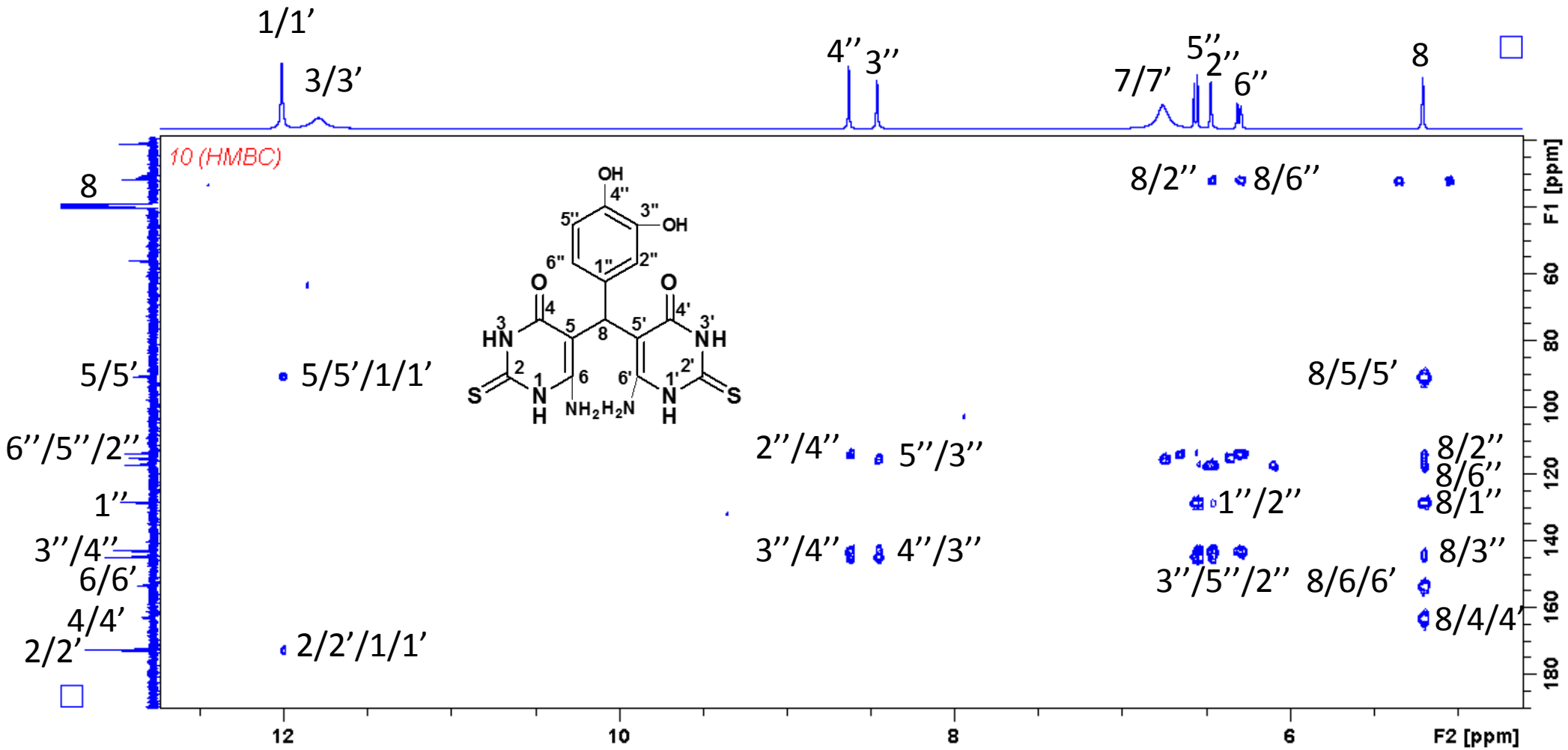
^{13}C NMR spectrum of 5,5'-((3,4-dihydroxylphenyl)methylene)bis(6-amino-2-thiouracil) (C10)



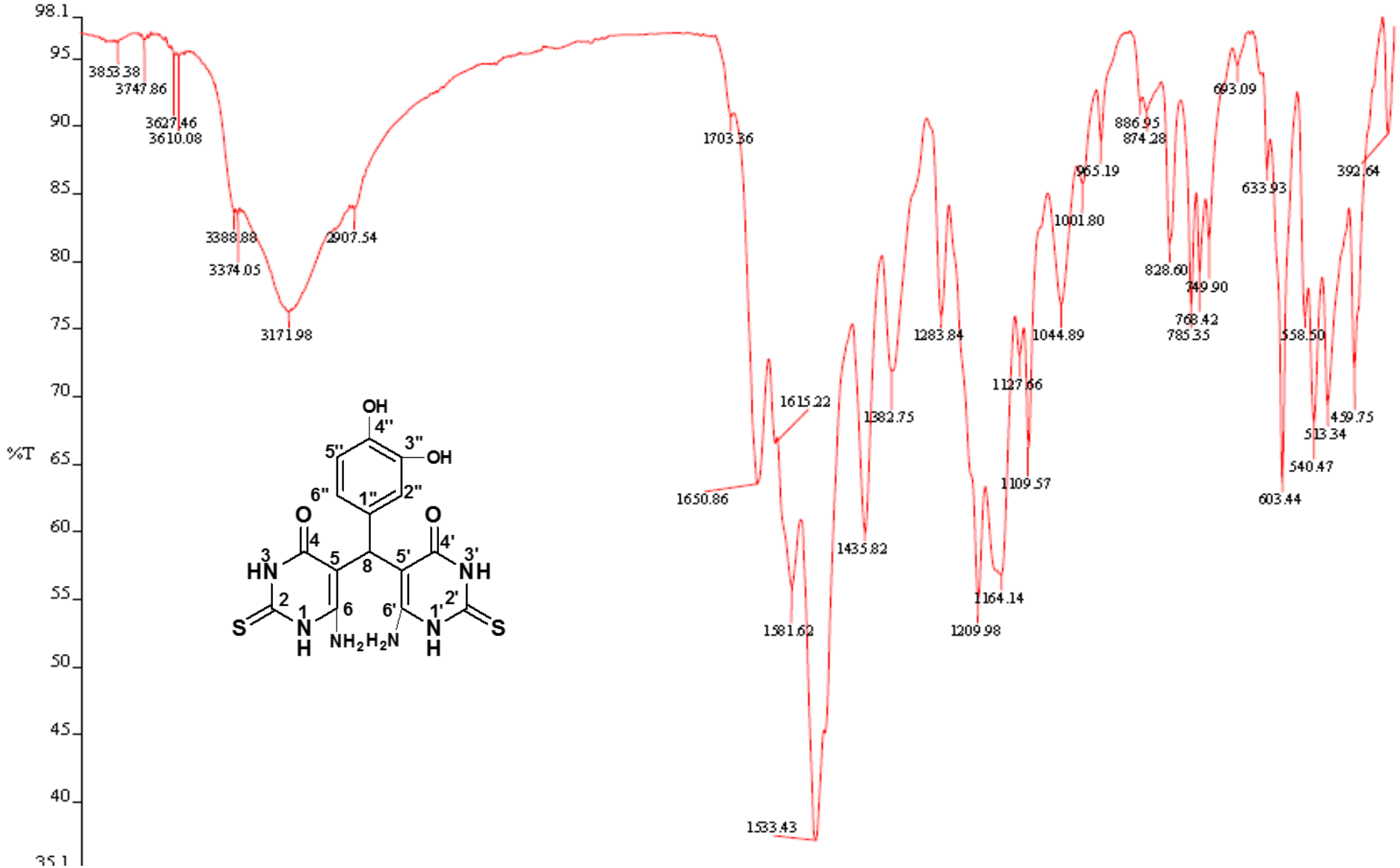
DEPT spectrum of 5,5'-((3,4-dihydroxylphenyl)methylene)bis(6-amino-2-thiouracil) (C10)



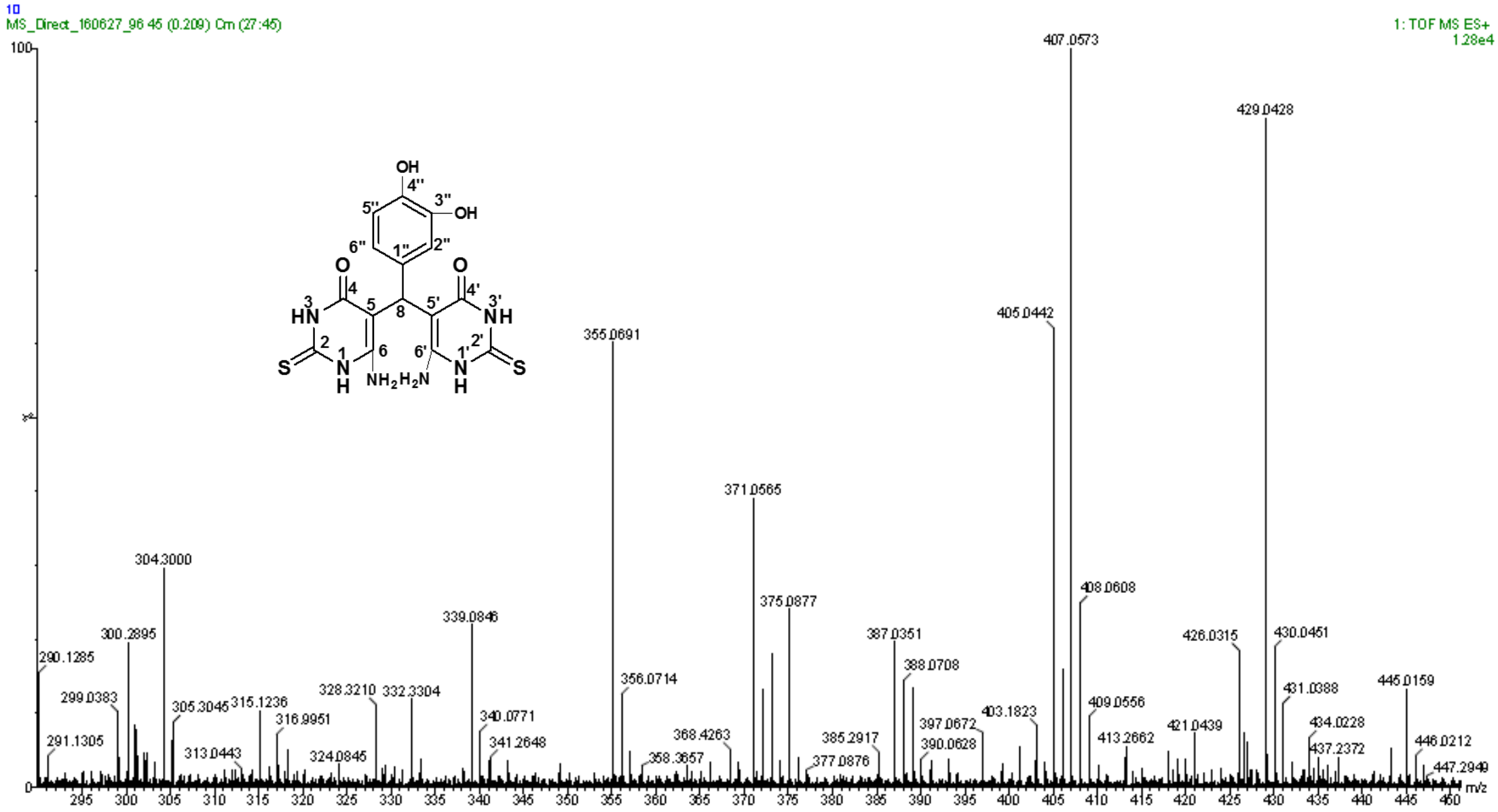
HSQC spectrum of 5,5'-((3,4-dihydroxylphenyl)methylene)bis(6-amino-2-thiouracil) (C10)



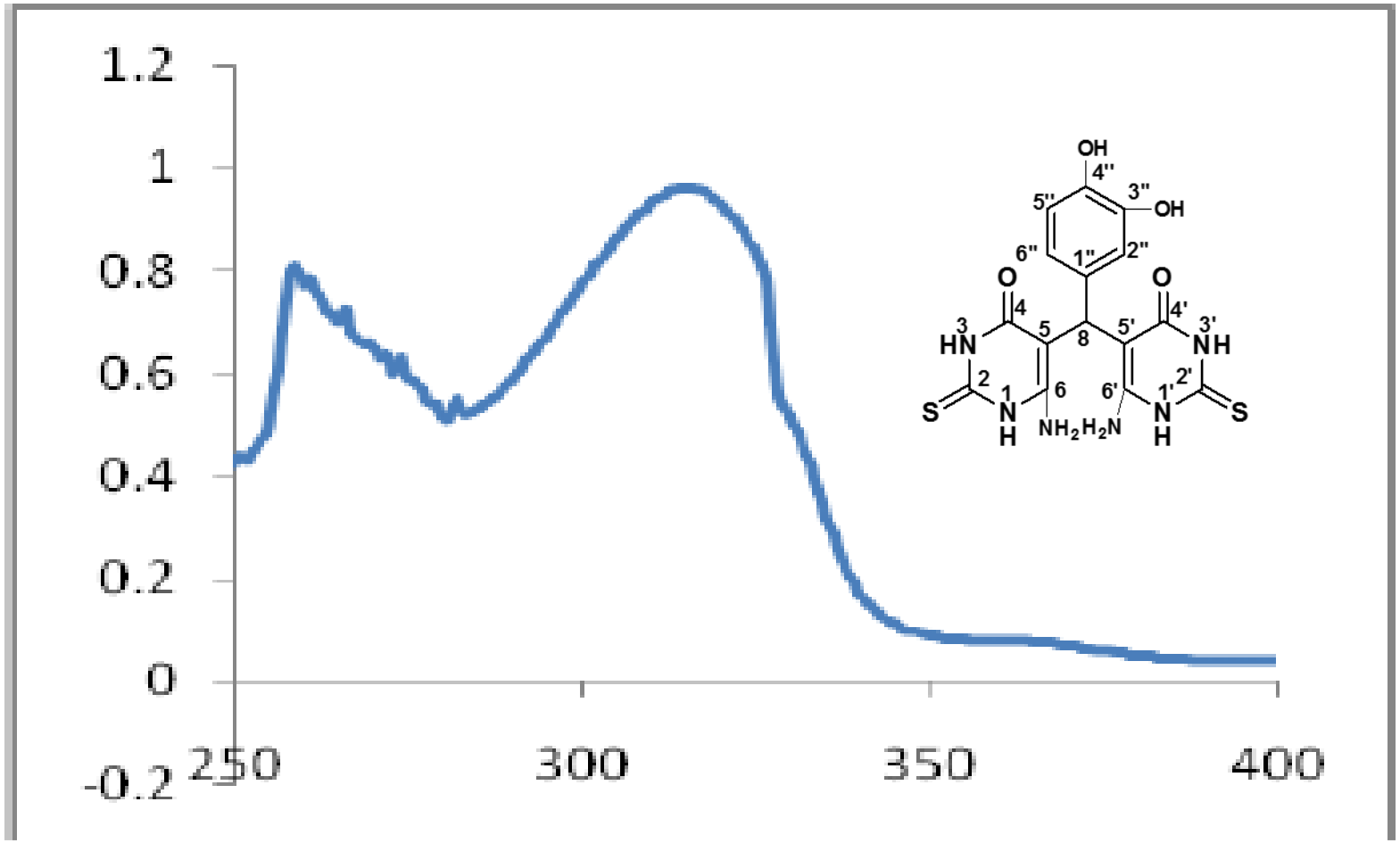
HMBC spectrum of 5,5'-((3,4-dihydroxylphenyl)methylene)bis(6-amino-2-thiouracil) (C10)



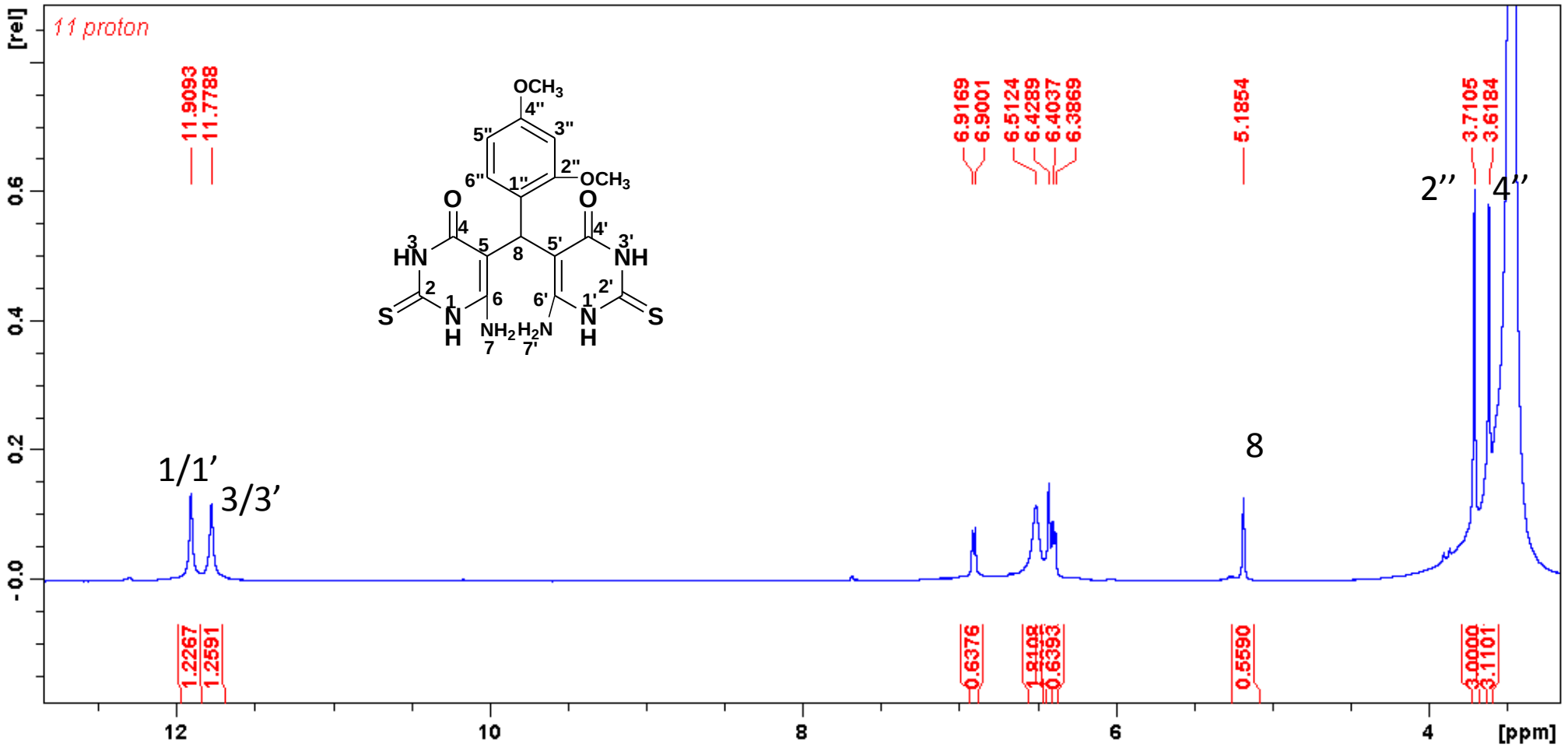
IR spectrum of 5,5'-((3,4-dihydroxylphenyl)methylene)bis(6-amino-2-thiouracil) (C10)



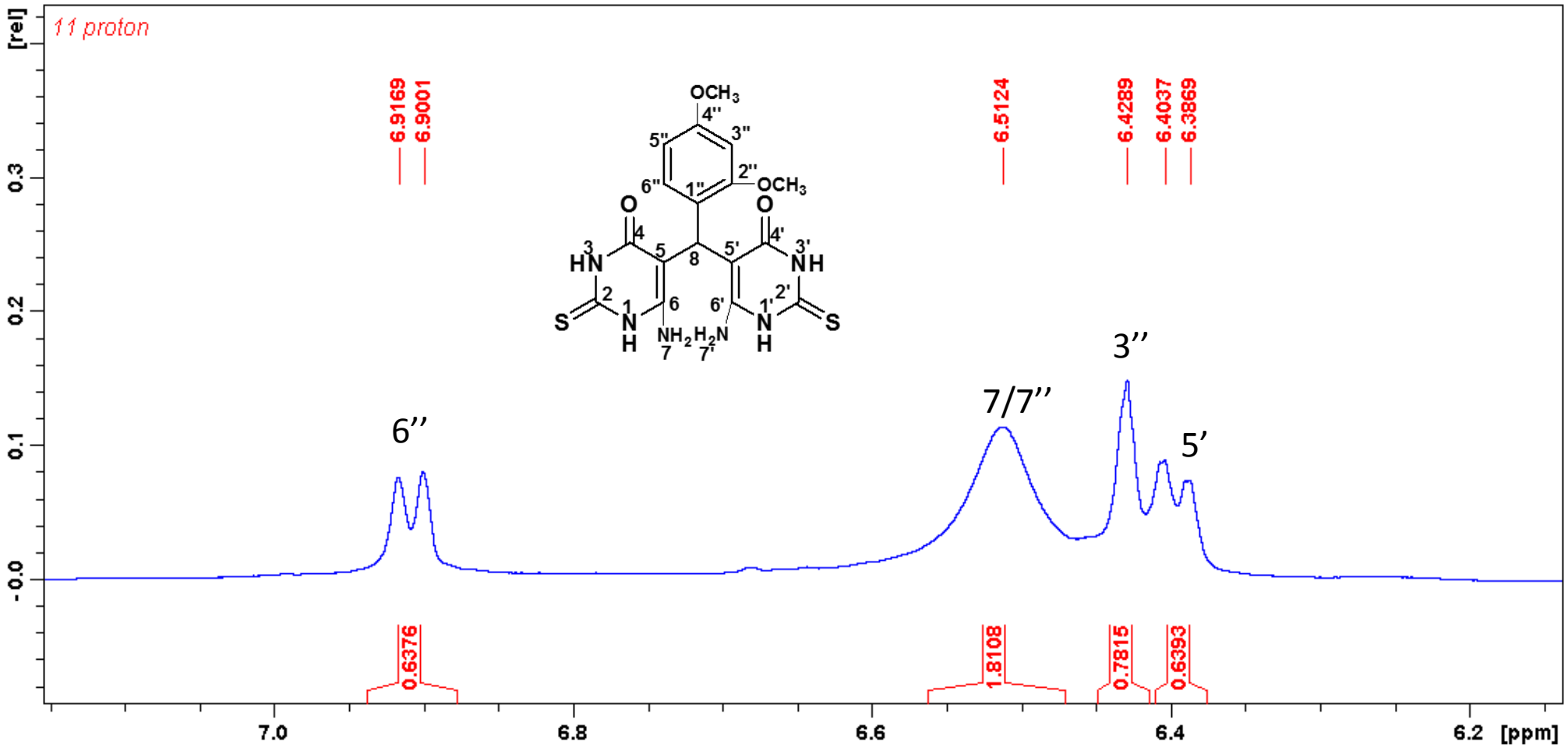
HRMS of 5,5'-((3,4-dihydroxylphenyl)methylene)bis(6-amino-2-thiouracil) (C10)



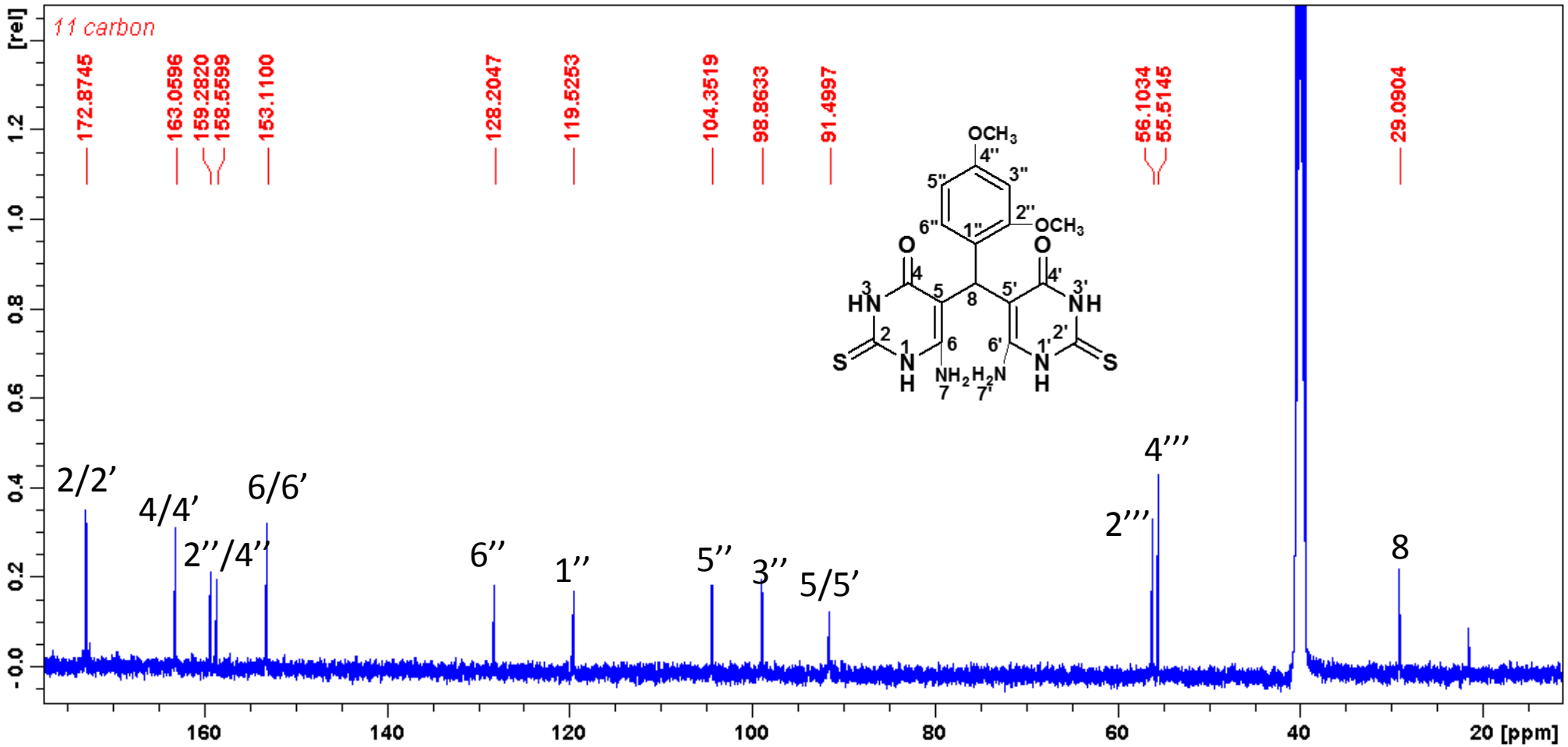
UV spectrum of 5,5'-((3,4-dihydroxylphenyl)methylene)bis(6-amino-2-thiouracil) (C10)



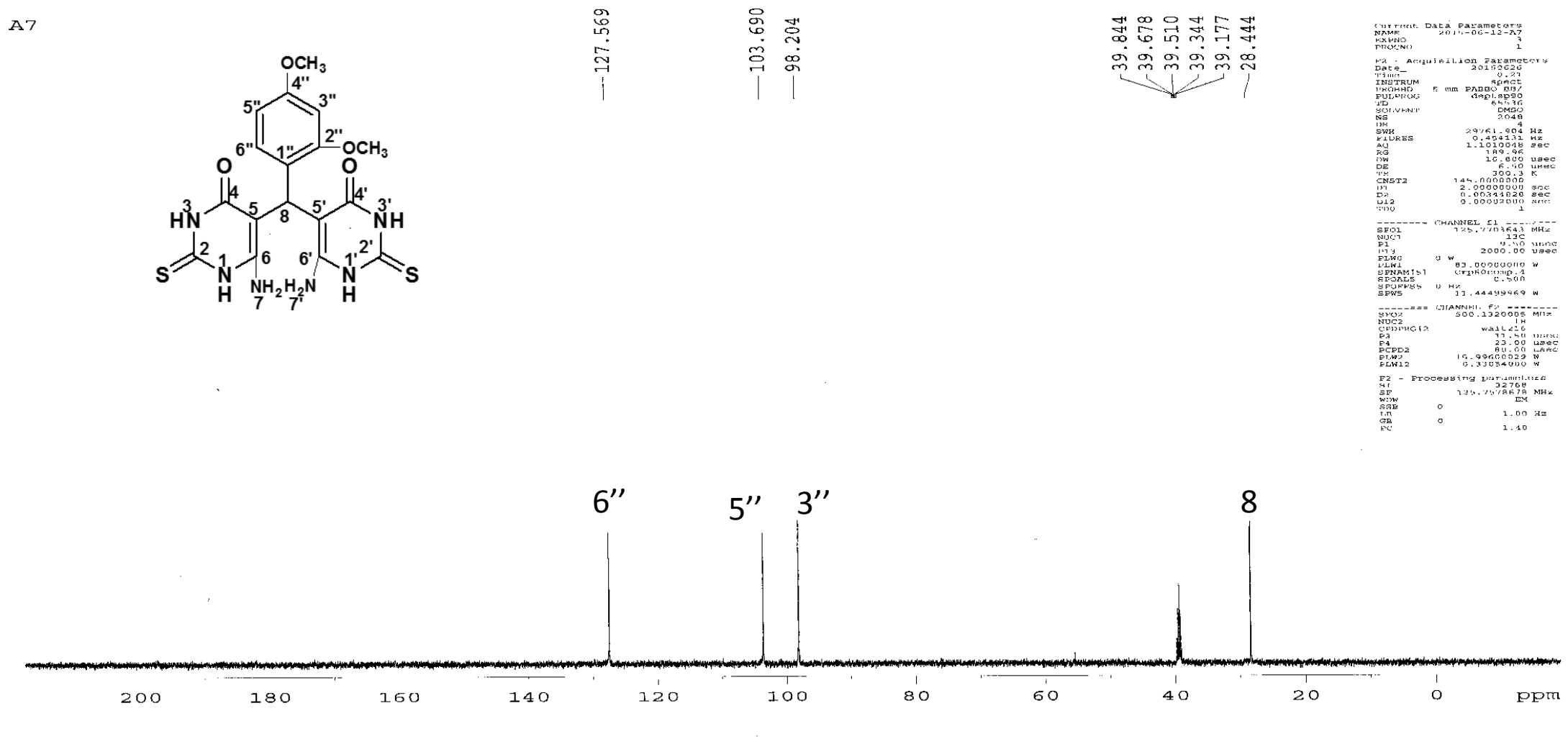
^{1}H NMR spectrum of 5,5'-((2,4-dimethoxylphenyl)methylene)bis(6-amino-2-thiouracil) (C11)



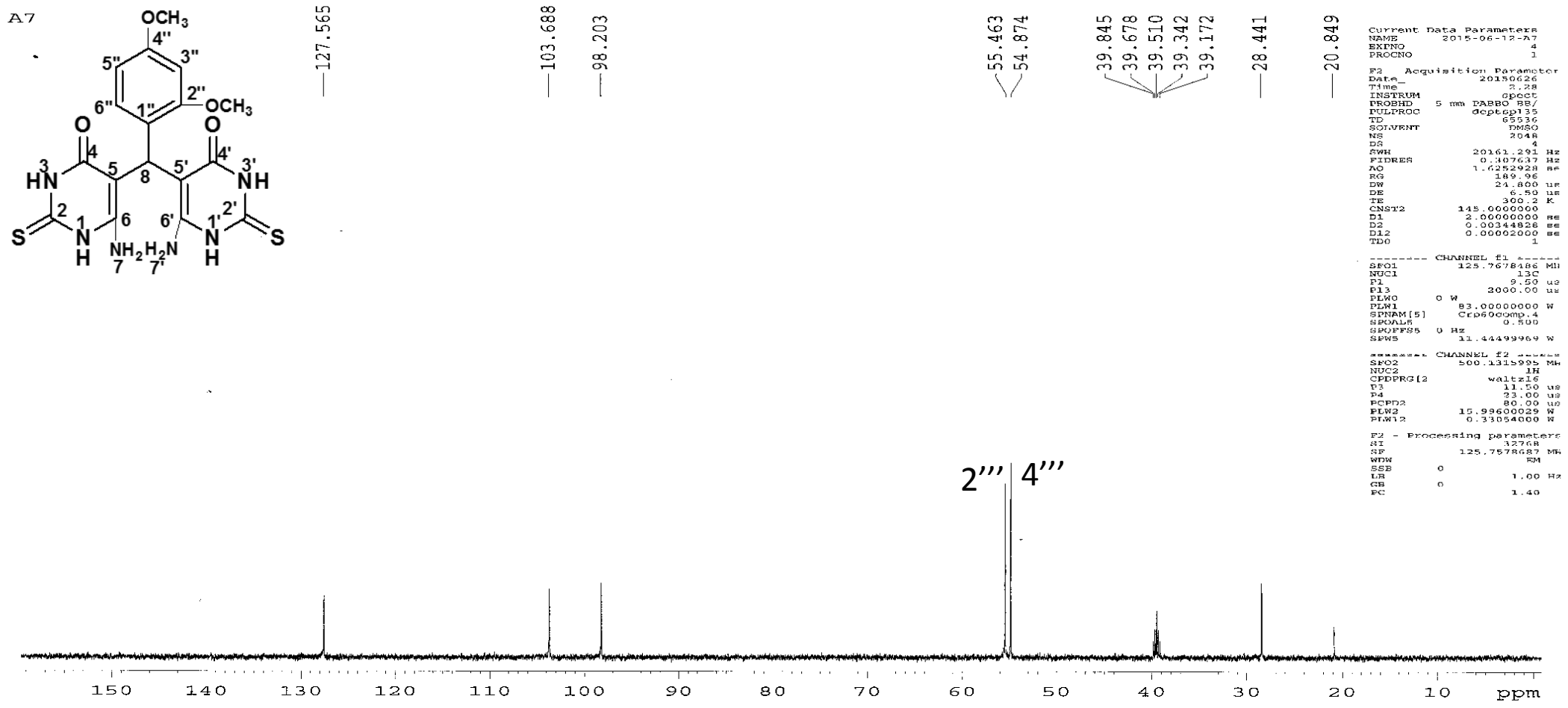
^{1}H NMR spectrum of 5,5'-((2,4-dimethoxylphenyl)methylene)bis(6-amino-2-thiouracil) (C11) expanded



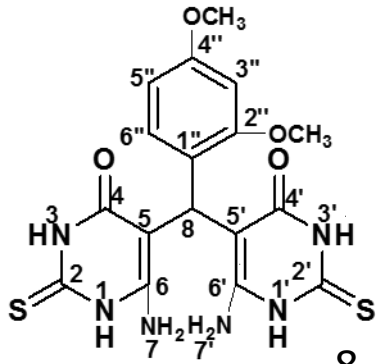
^{13}C NMR spectrum of 5,5'-((2,4-dimethoxylphenyl)methylene)bis(6-amino-2-thiouracil) (C11)

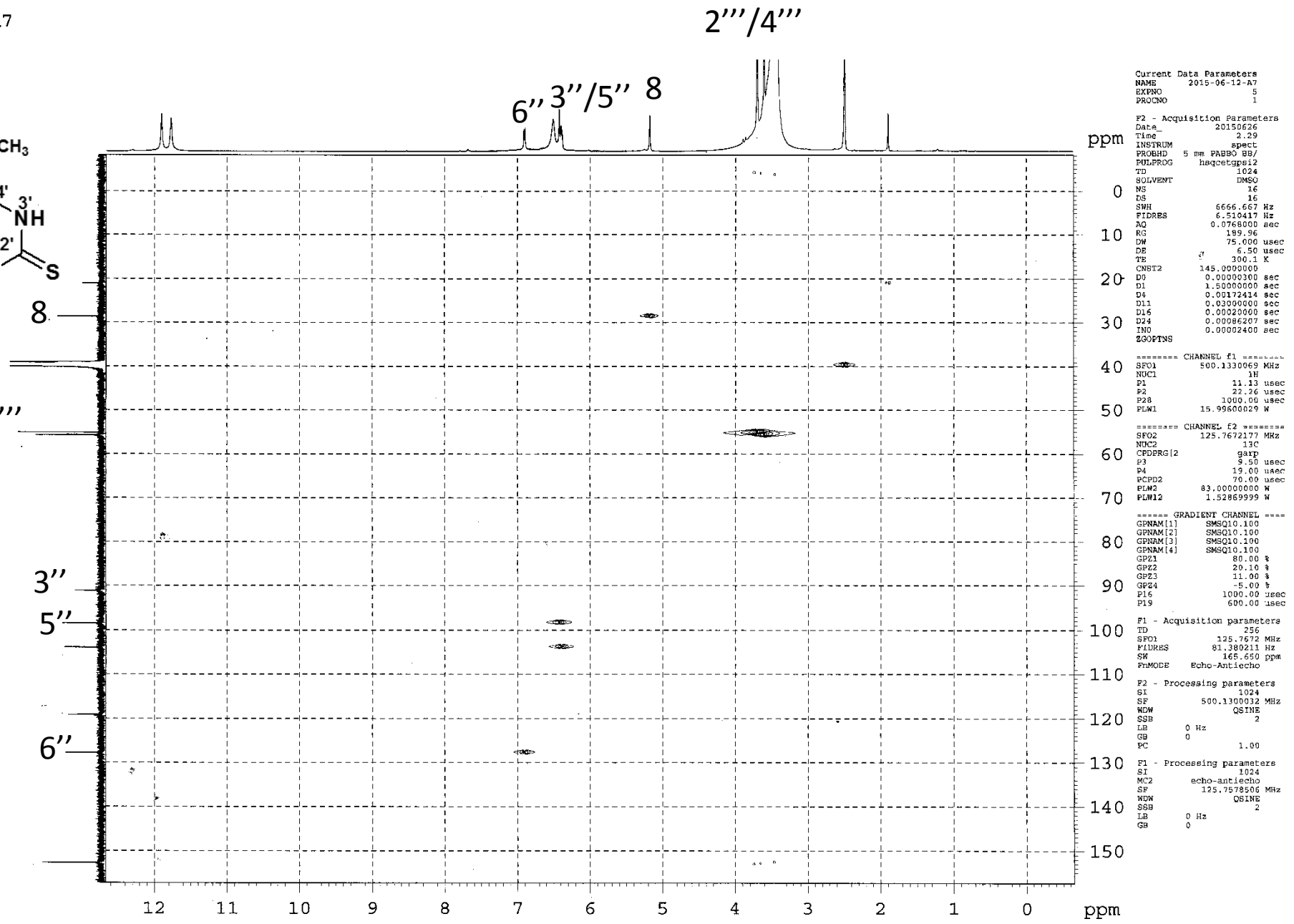


DEPT 90 spectrum of 5,5'-((2,4-dimethoxylphenyl)methylene)bis(6-amino-2-thiouracil) (C11)

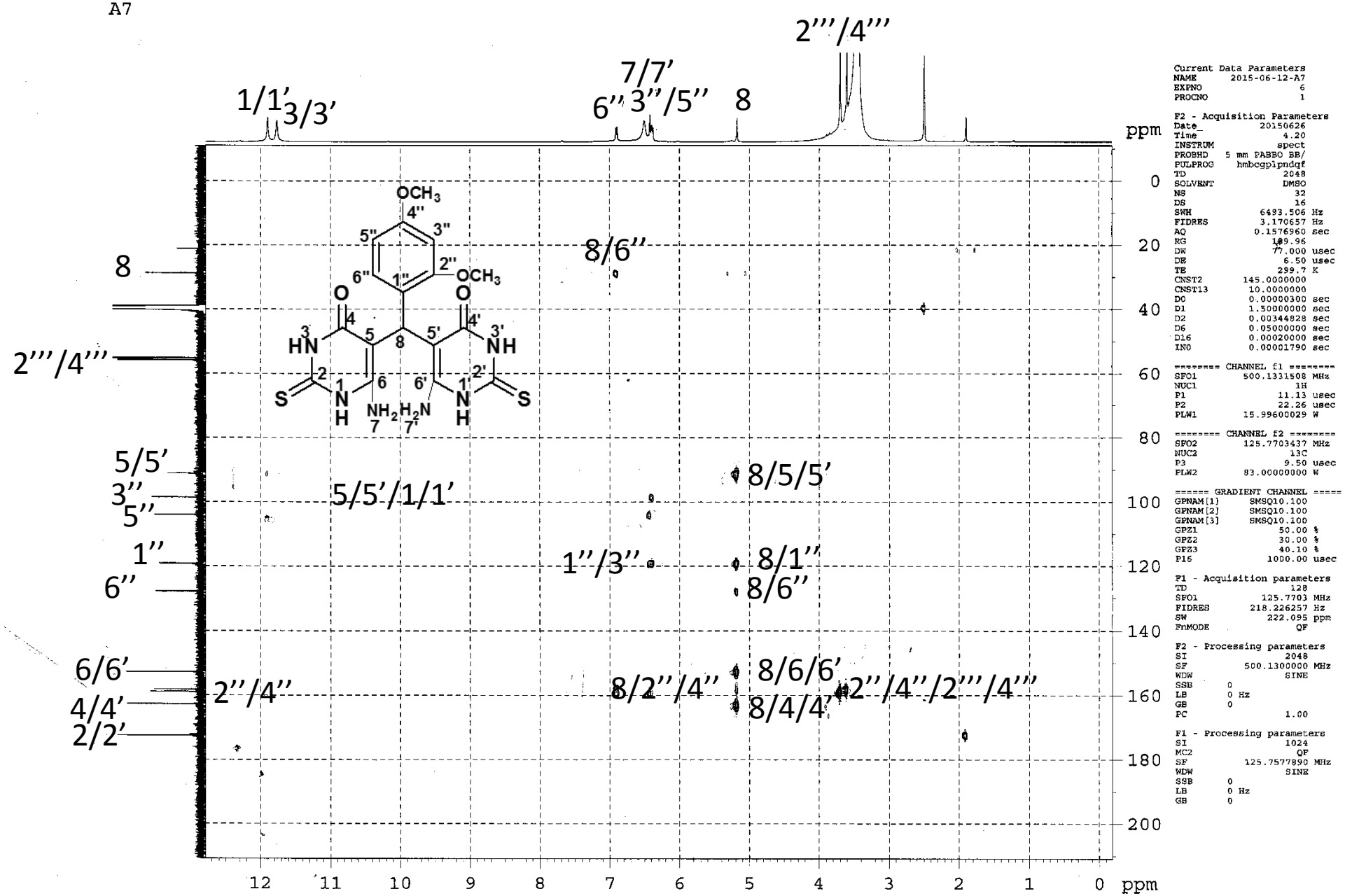


DEPT 135 spectrum of 5,5'-((2,4-dimethoxylphenyl)methylene)bis(6-amino-2-thiouracil) (C11)





HSQC spectrum of 5,5'-((2,4-dimethoxylphenyl)methylene)bis(6-amino-2-thiouracil) (C11)

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PROCNO                 1

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PULPROG    hmbcgplpndqf
TD                 2048
SOLVENT            DMSO
NS                   32
DS                   16
SWH           6493.506 Hz
FIDRES        3.170657 Hz
AQ            0.1576960 sec
RG                189.96
DW                77.000 usec
DE                 6.50 usec
TE                299.7 K
CNST2        145.0000000
CNST13        10.0000000
D0            0.00000300 sec
D1            1.50000000 sec
D2            0.00344828 sec
D6            0.05000000 sec
D16           0.00020000 sec
IN0           0.00001790 sec

======== CHANNEL f1 ========
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NUC1               1H
P1                11.13 usec
P2                22.26 usec
PLW1         15.99600029 W

======== CHANNEL f2 ========
SFO2        125.7703437 MHz
NUC2               13C
P3                 9.50 usec
PLW2         83.00000000 W

====== GRADIENT CHANNEL =====
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GPNAM[2]      SMSQ10.100
GPNAM[3]      SMSQ10.100
GPZ1              50.00 %
GPZ2              30.00 %
GPZ3              40.10 %
P16             1000.00 usec

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FIDRES      218.226257 Hz
SW          222.095 ppm
FnMODE             QF

F2 - Processing parameters
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LB         0 Hz
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GB         0
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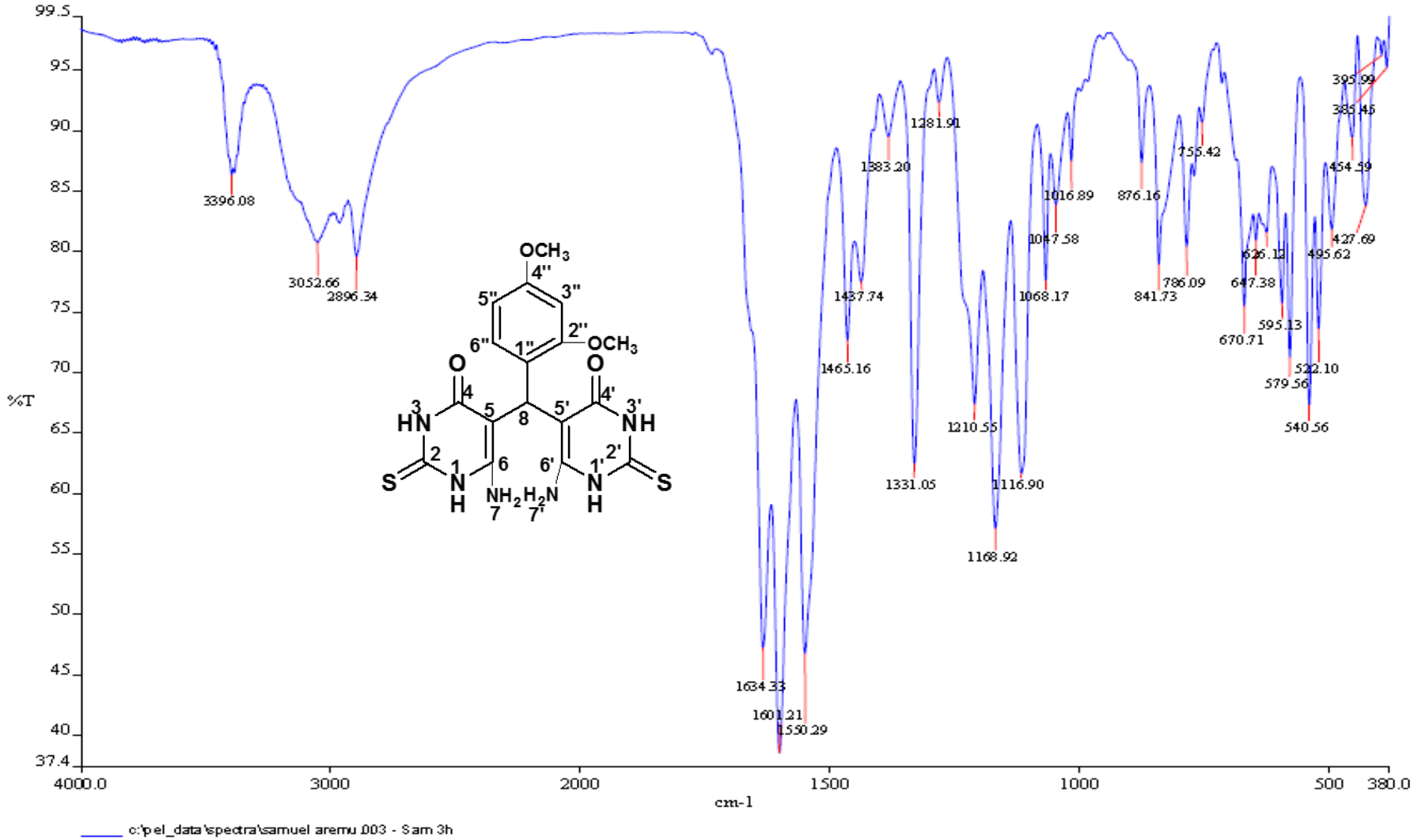
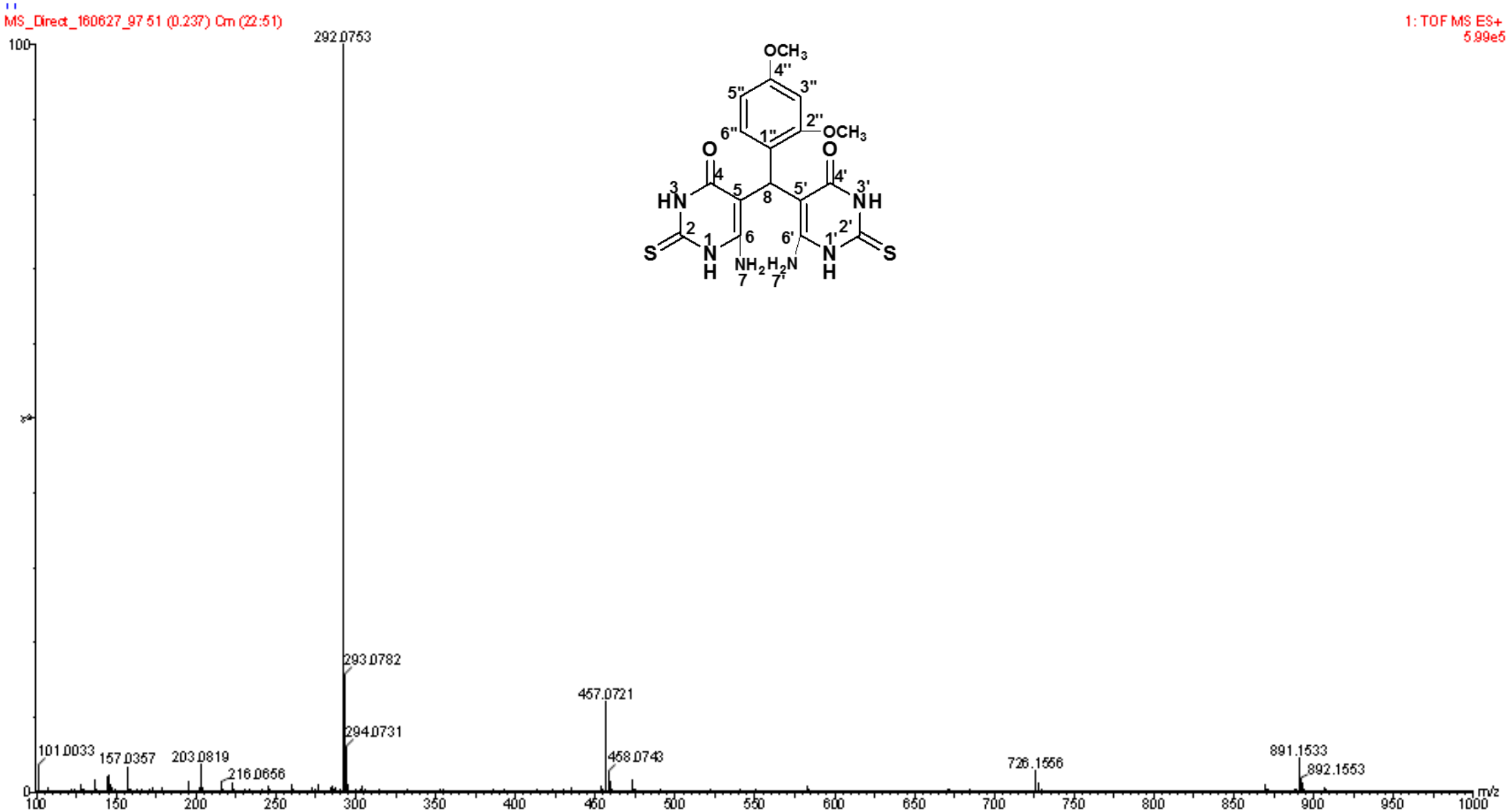
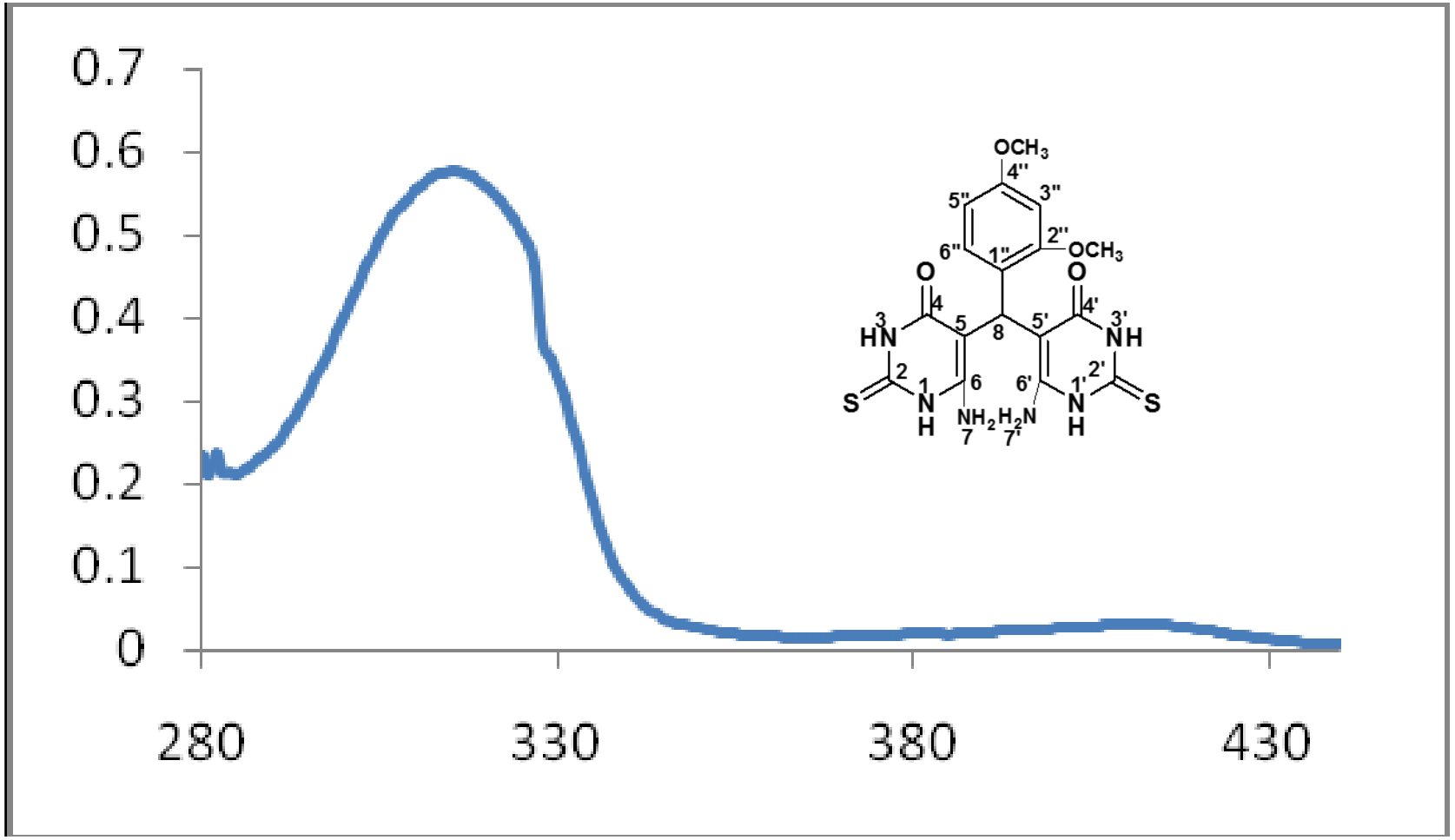
HMBC spectrum of 5,5'-((2,4-dimethoxylphenyl)methylene)bis(6-amino-2-thiouracil) (C11)



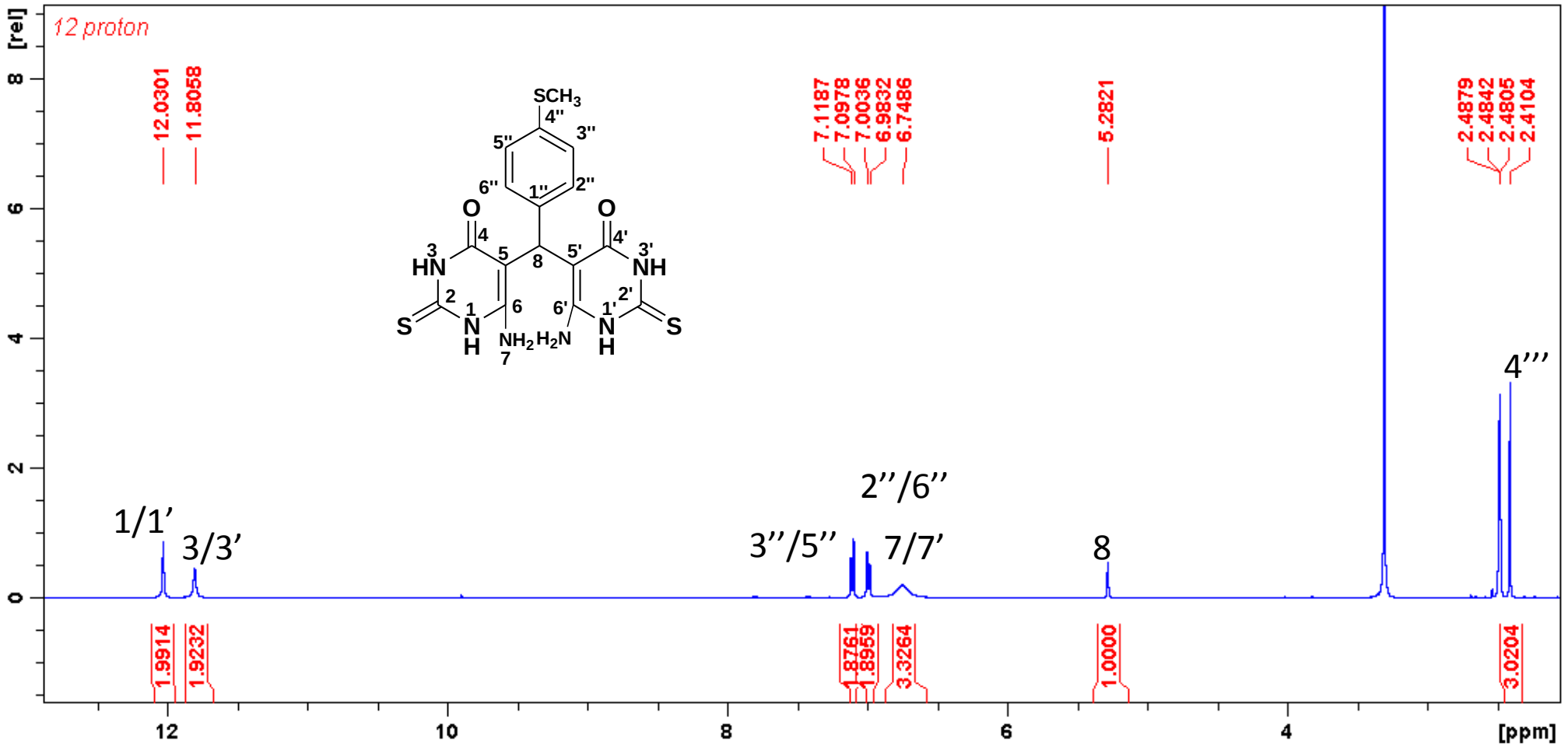
IR spectrum of 5,5'-((2,4-dimethoxylphenyl)methylene)bis(6-amino-2-thiouracil) (C11)



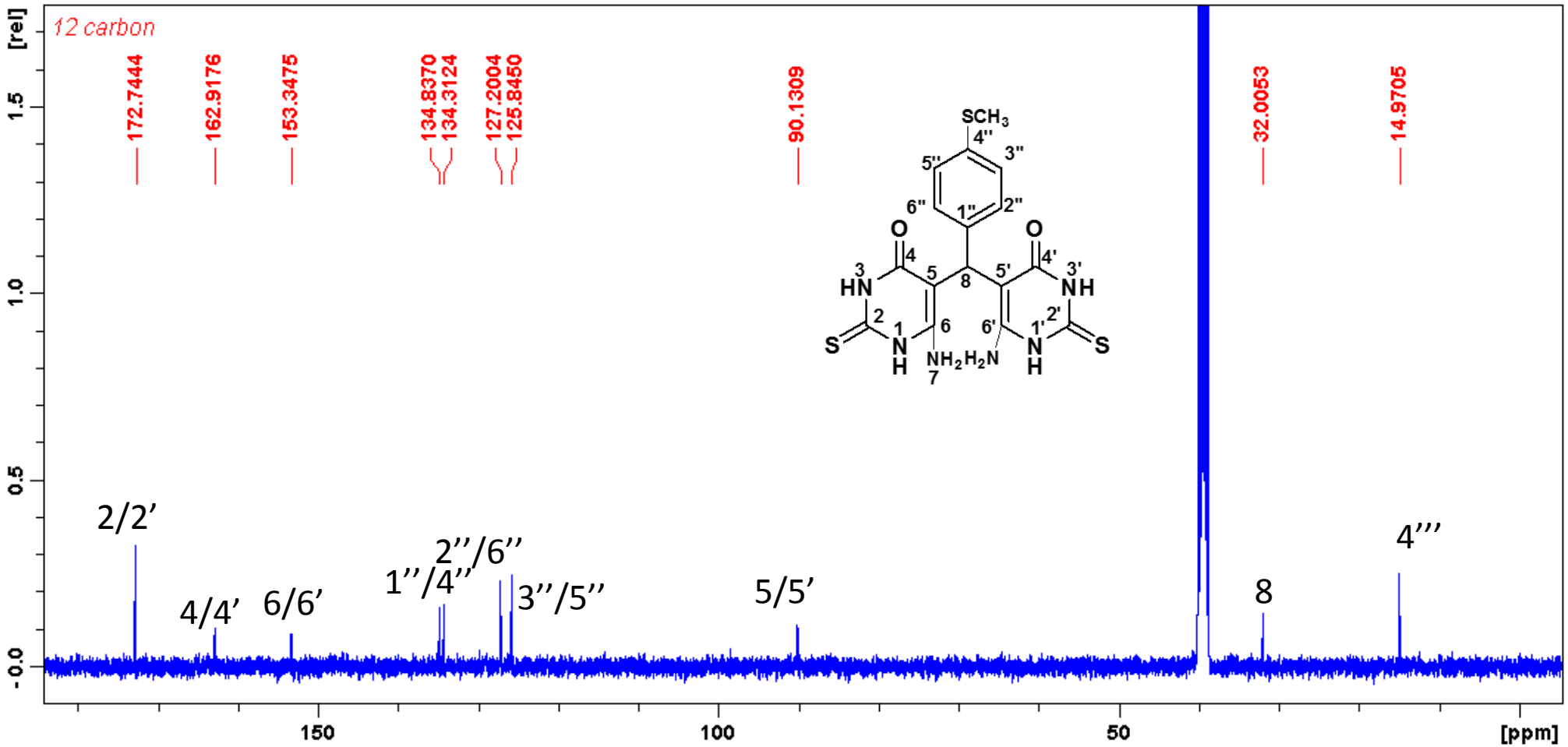
HRMS of 5,5'-((2,4-dimethoxylphenyl)methylene)bis(6-amino-2-thiouracil) (C11)



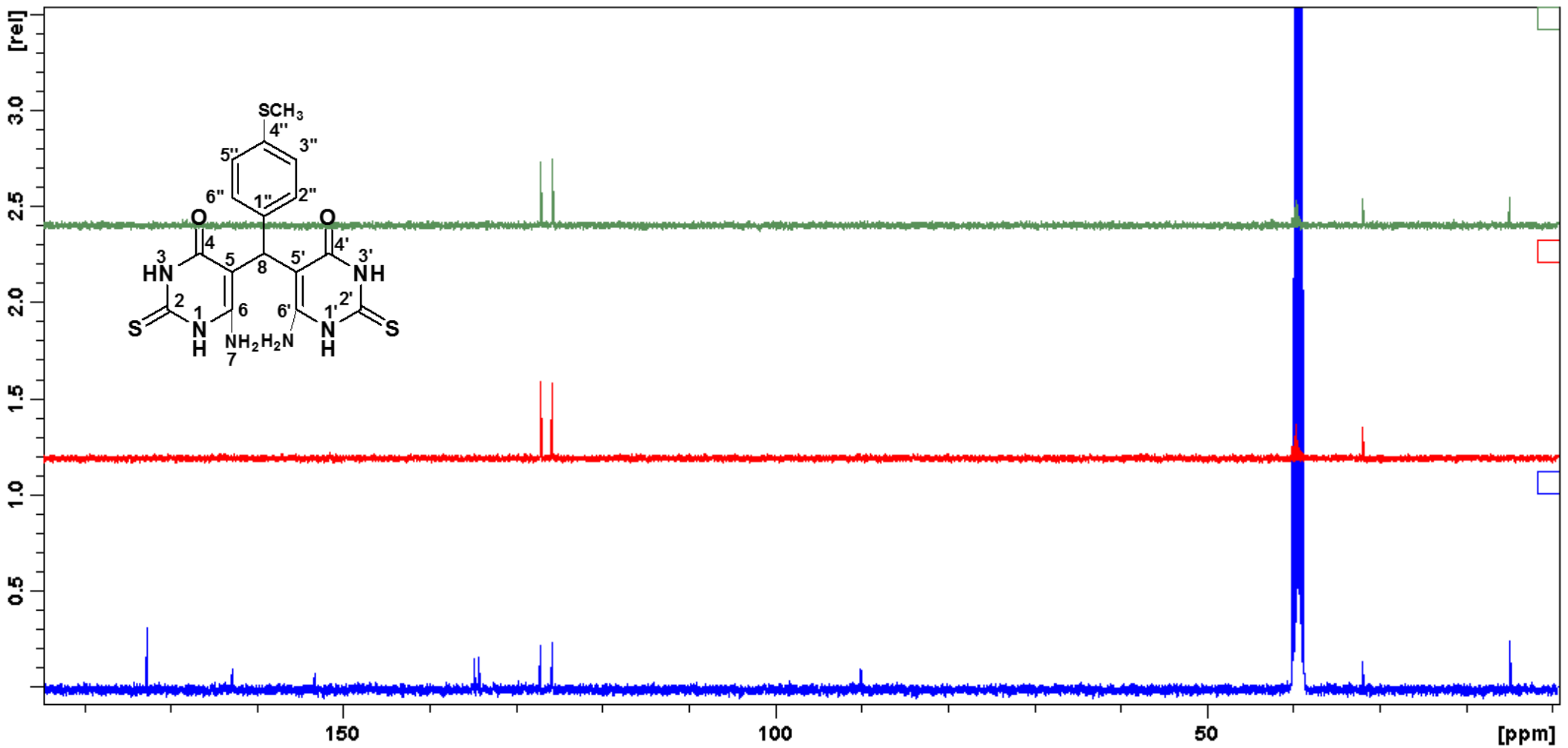
UV spectrum of 5,5'-((2,4-dimethoxylphenyl)methylene)bis(6-amino-2-thiouracil) (C11)



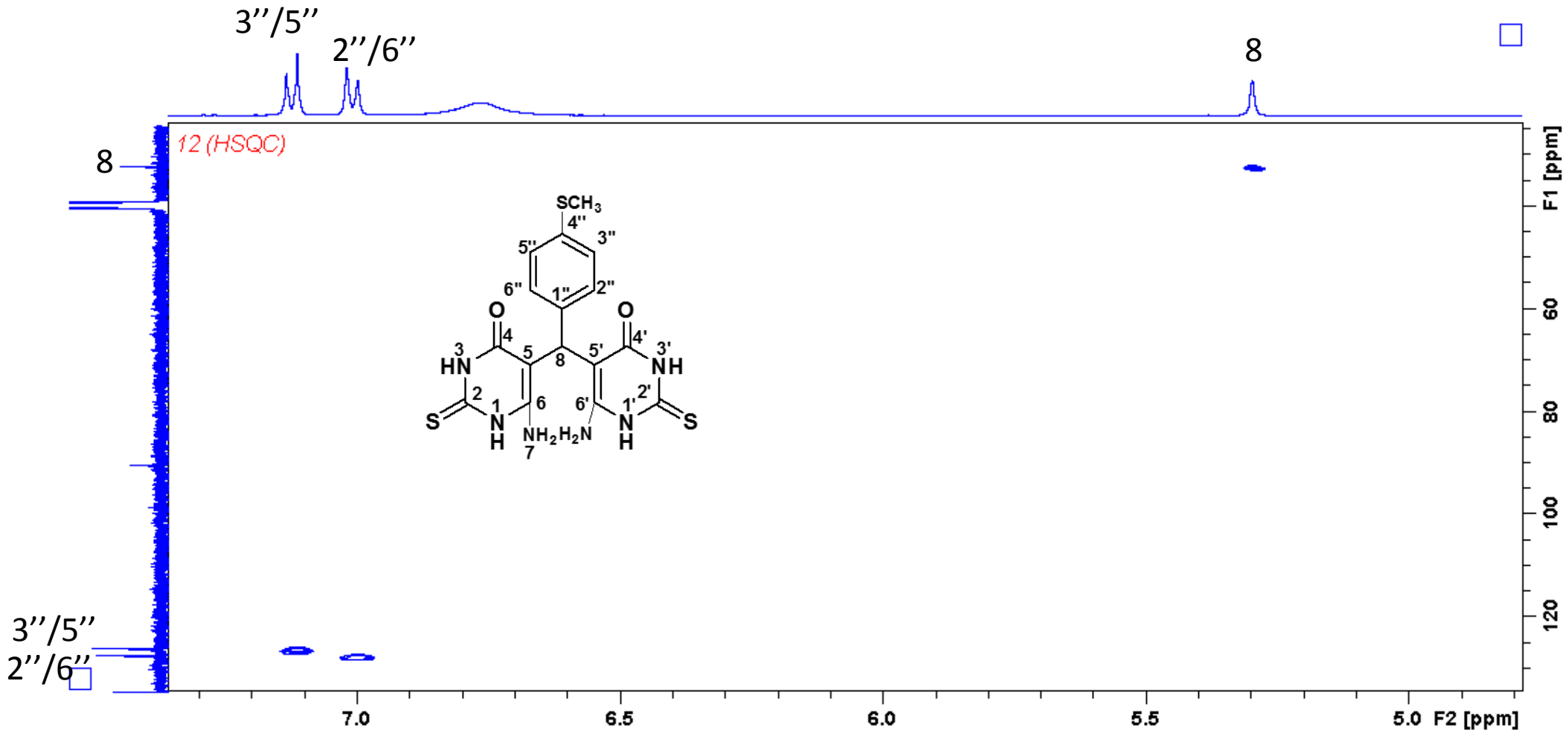
^{1}H NMR spectrum of 5,5'-((4-methylthiolphenyl)methylene)bis(6-amino-2-thiouracil) (C12)



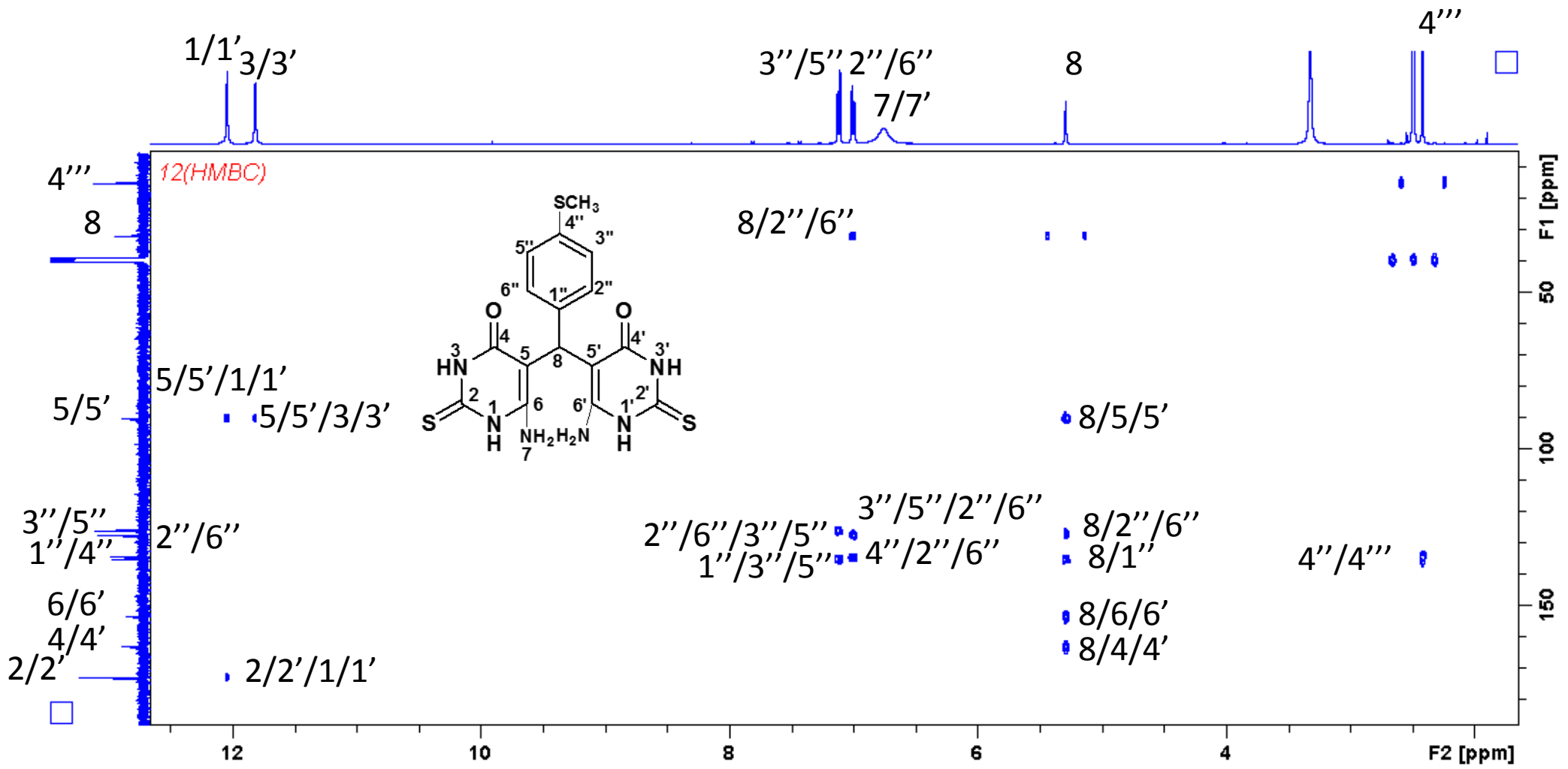
^{13}C NMR spectrum of 5,5'-((4-methylthiolphenyl)methylene)bis(6-amino-2-thiouracil) (C12)



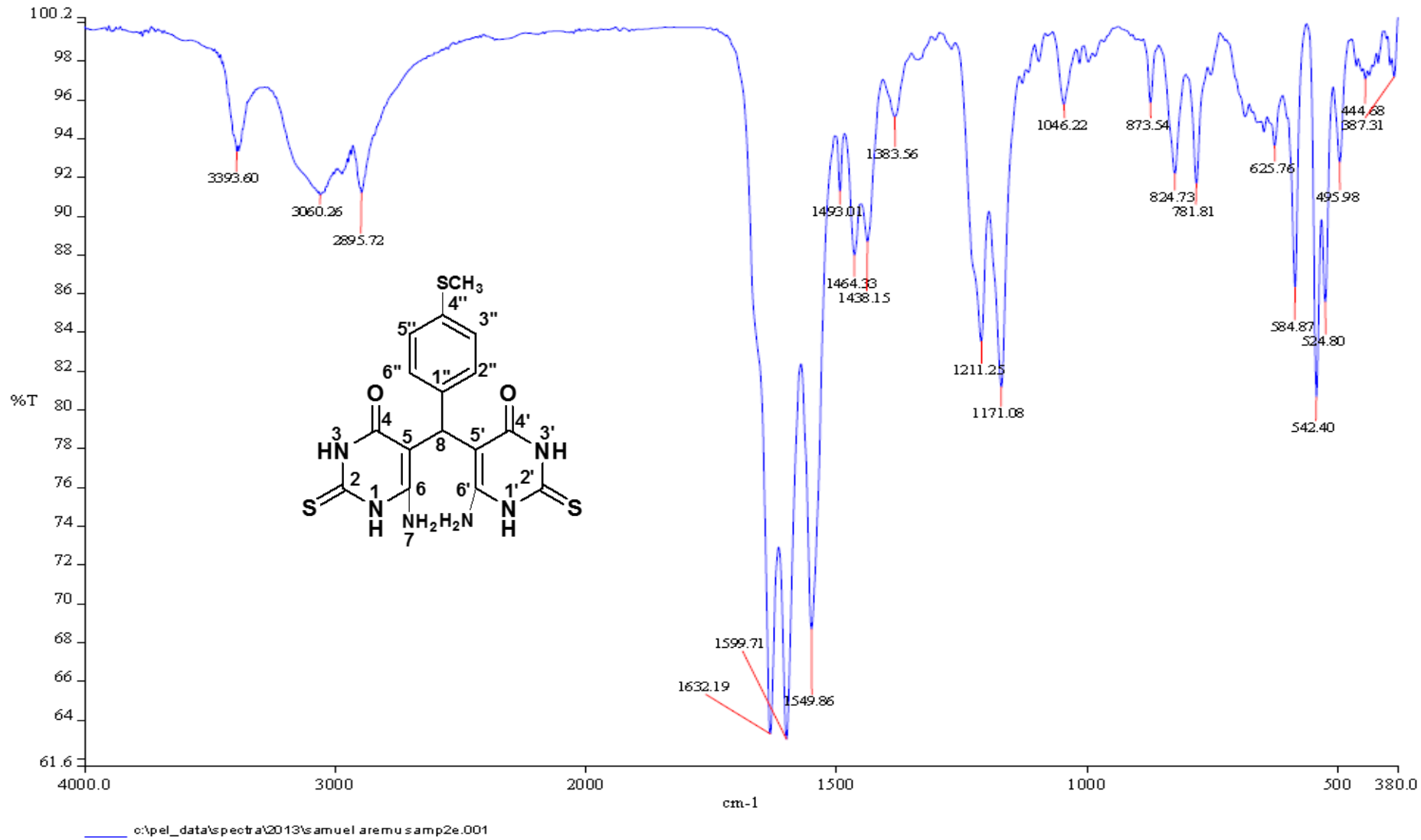
DEPT spectrum of 5,5'-((4-methylthiolphenyl)methylene)bis(6-amino-2-thiouracil) (C12)



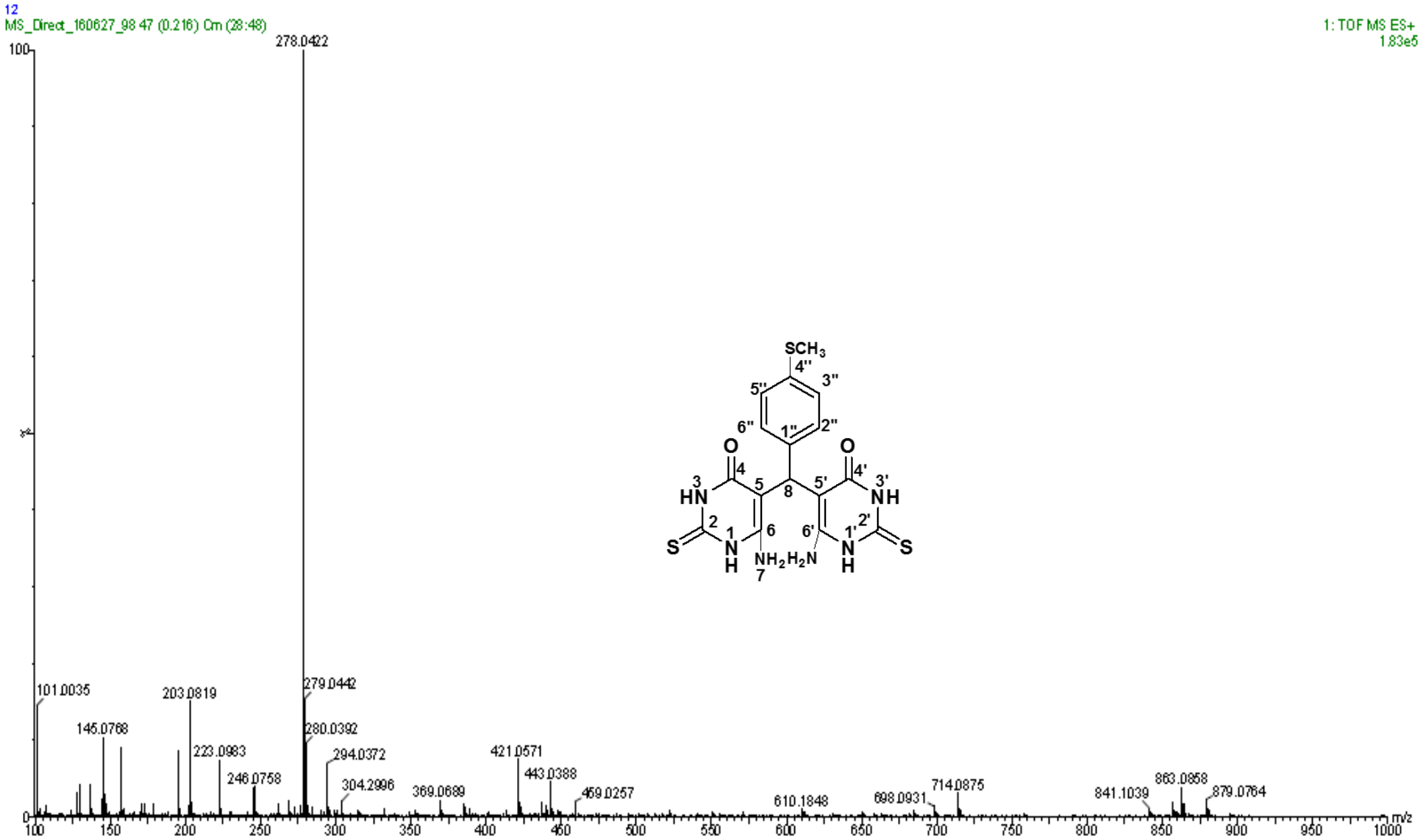
HSQC spectrum of 5,5'-((4-methylthiolphenyl)methylene)bis(6-amino-2-thiouracil) (C12)



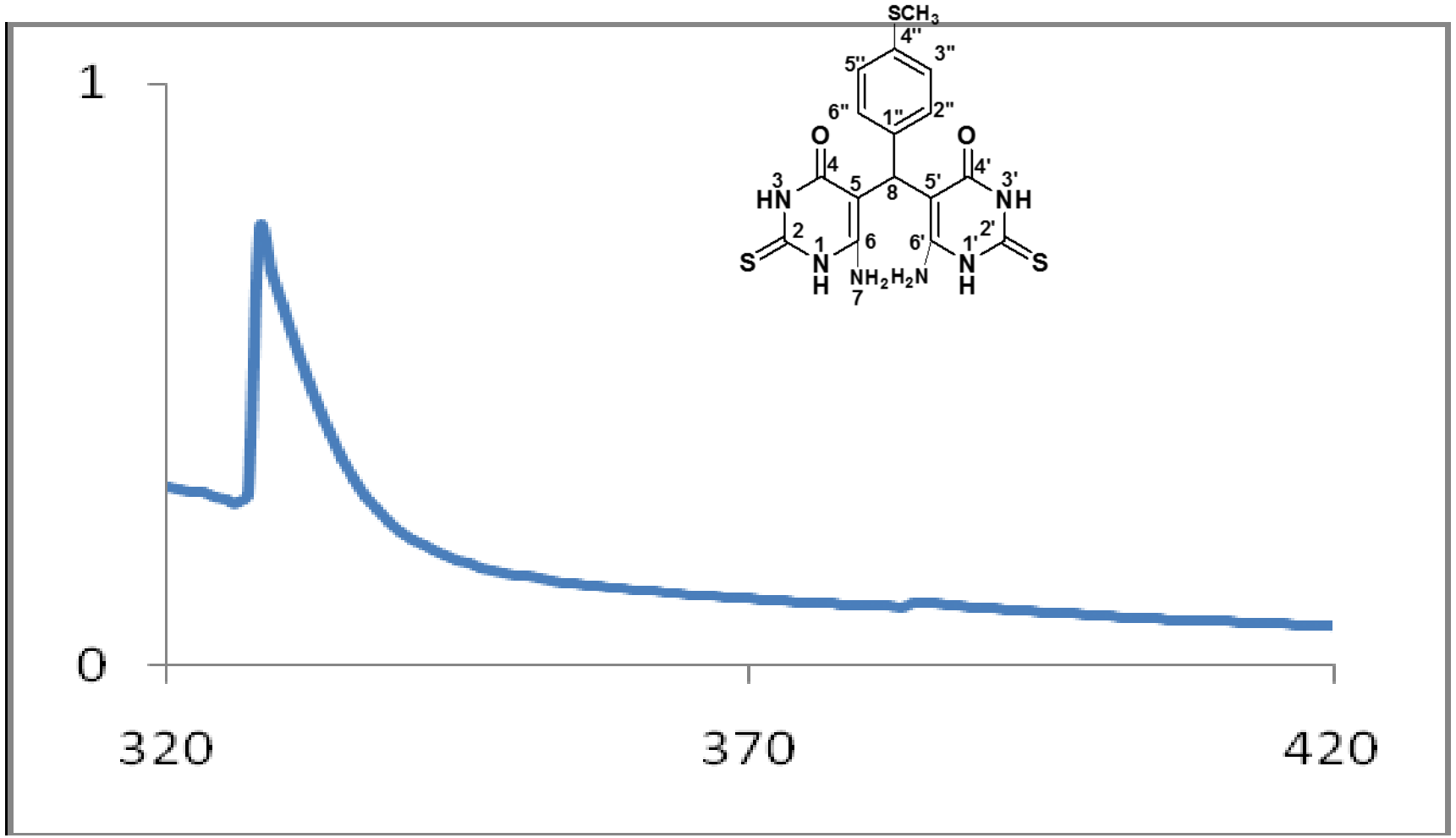
HMBC spectrum of 5,5'-((4-methylthiolphenyl)methylene)bis(6-amino-2-thiouracil) (C12)



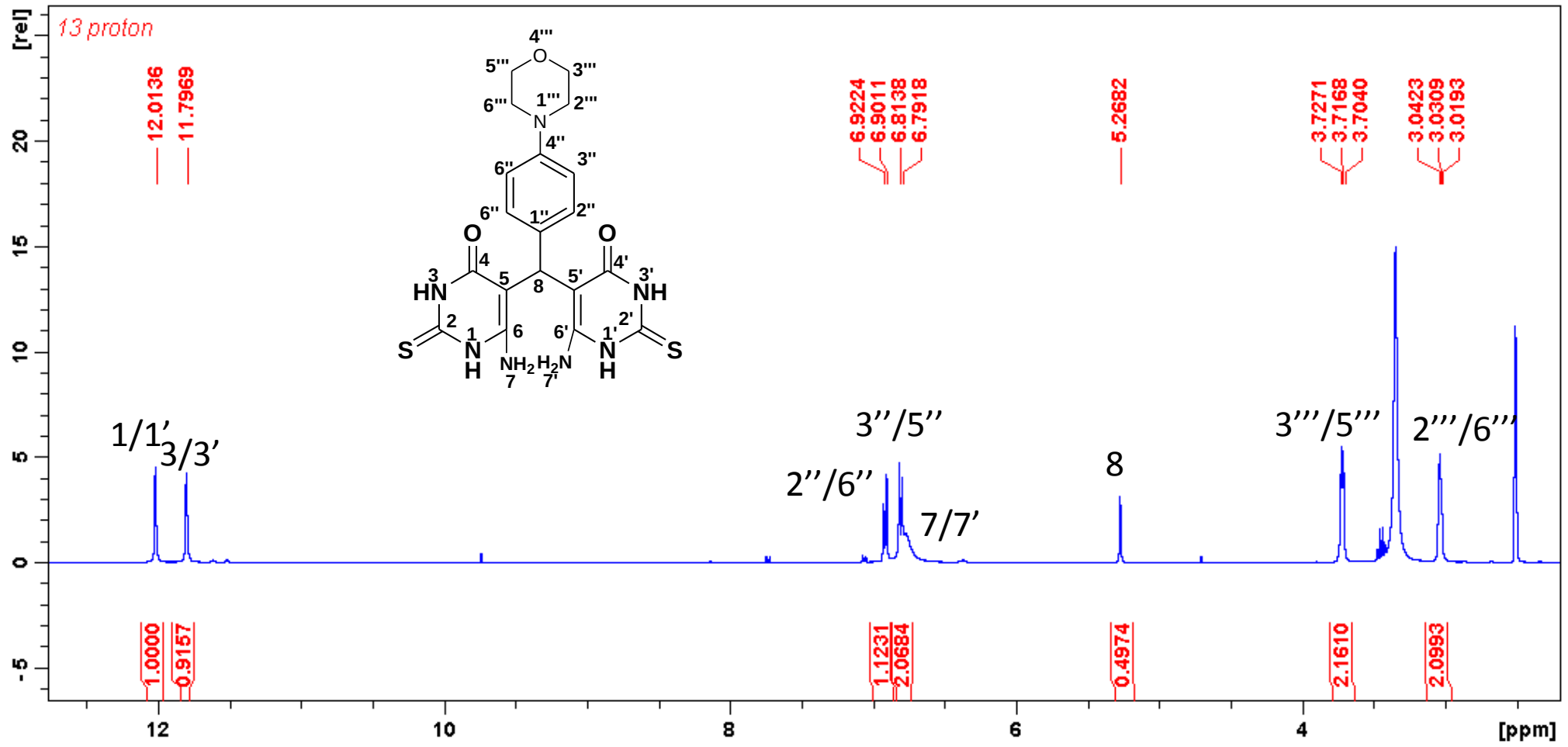
IR spectrum of 5,5'-((4-methylthiolphenyl)methylene)bis(6-amino-2-thiouracil) (C12)



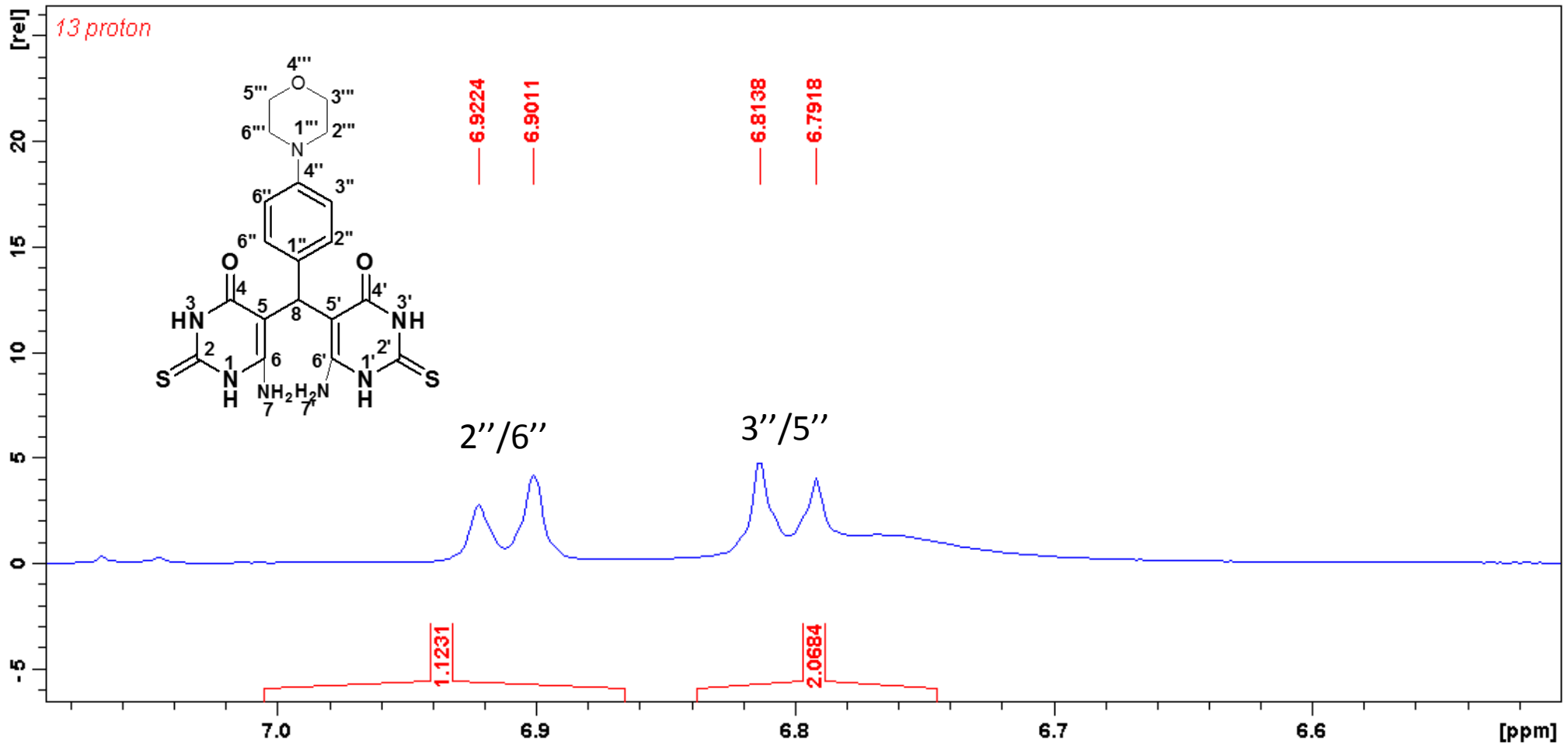
HRMS of 5,5'-((4-methylthiolphenyl)methylene)bis(6-amino-2-thiouracil) (C12)



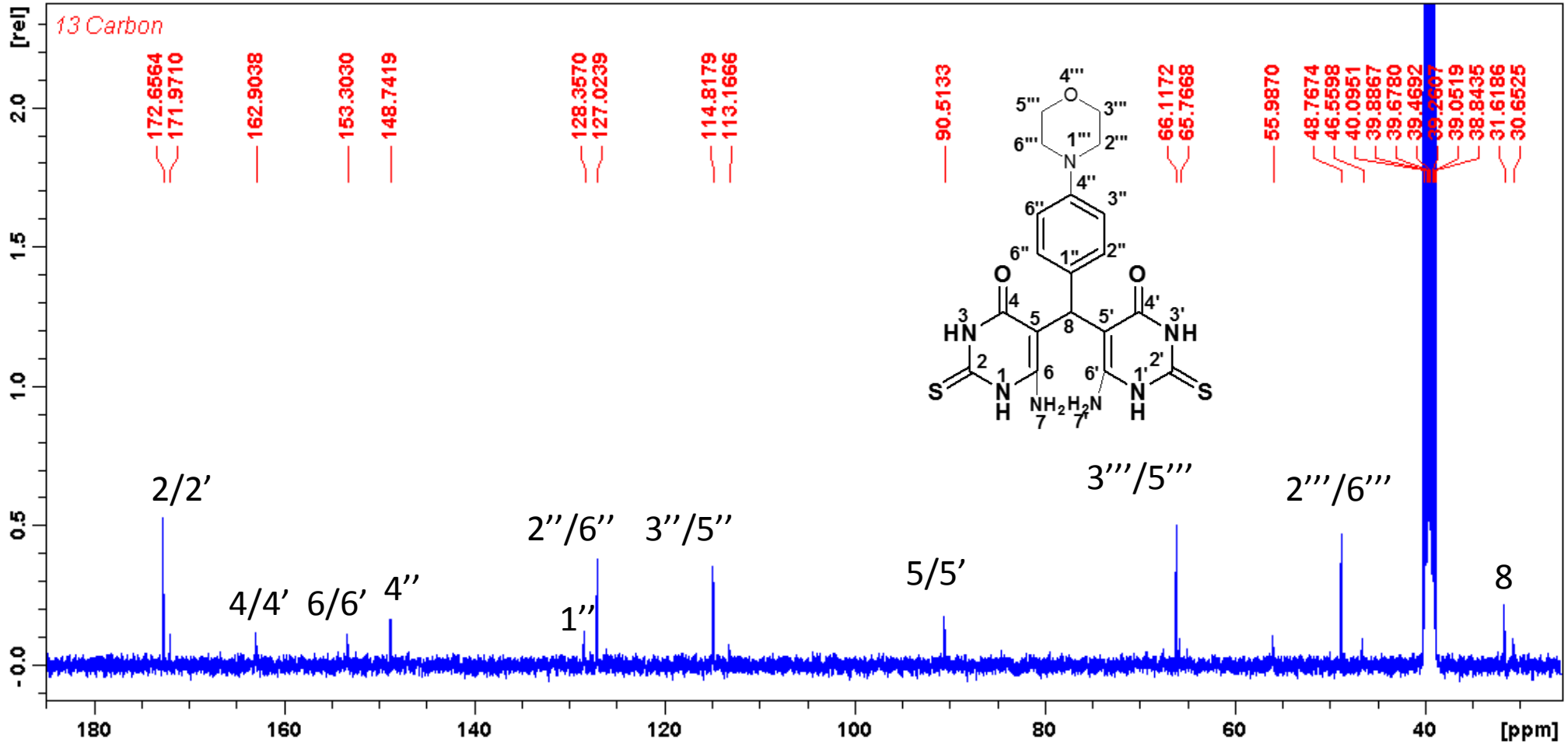
UV spectrum of 5,5'-((4-methylthiolphenyl)methylene)bis(6-amino-2-thiouracil) (C12)



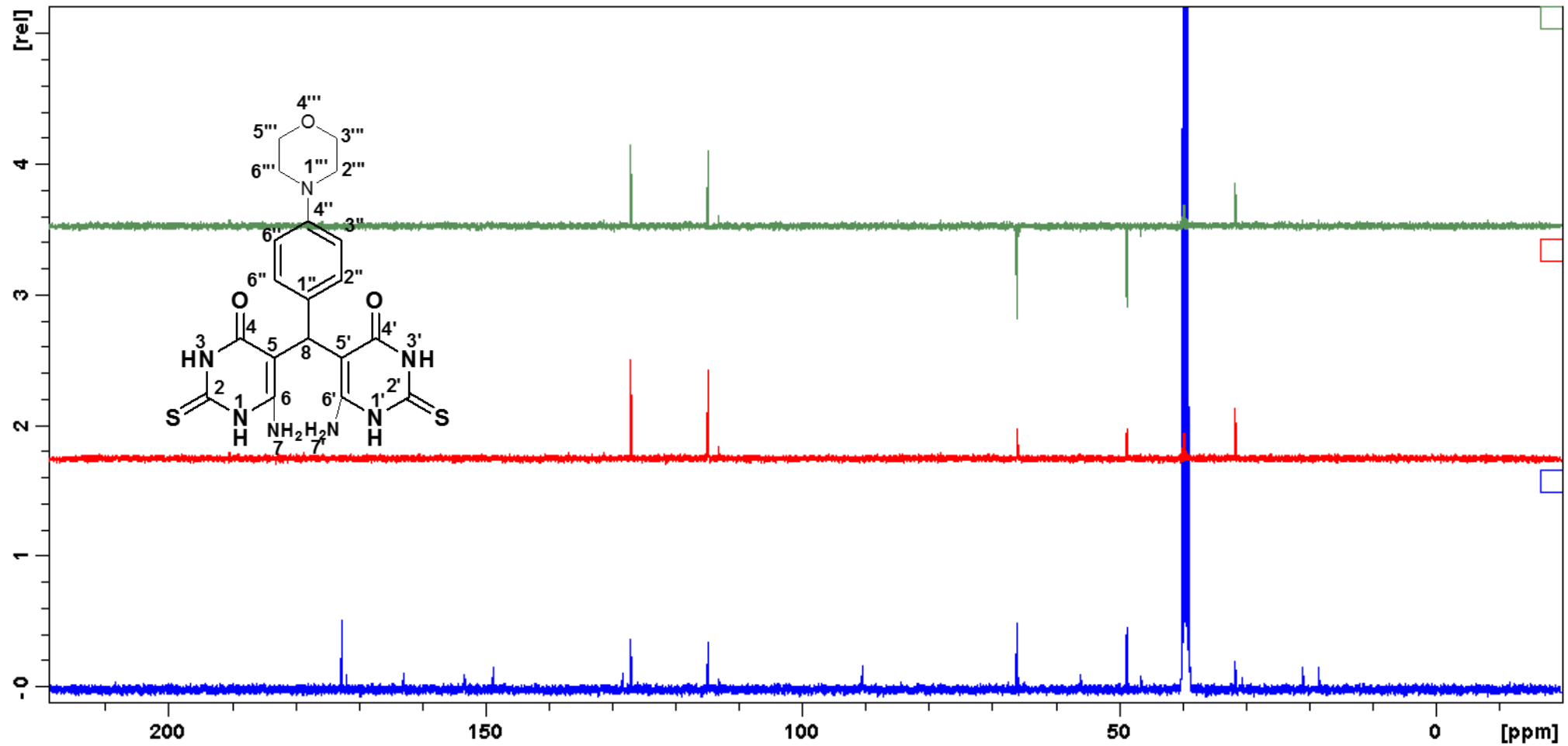
^{1}H NMR spectrum of 5,5'-((4-morpholinylphenyl)methylene)bis(6-amino-2-thiouracil) (C13)



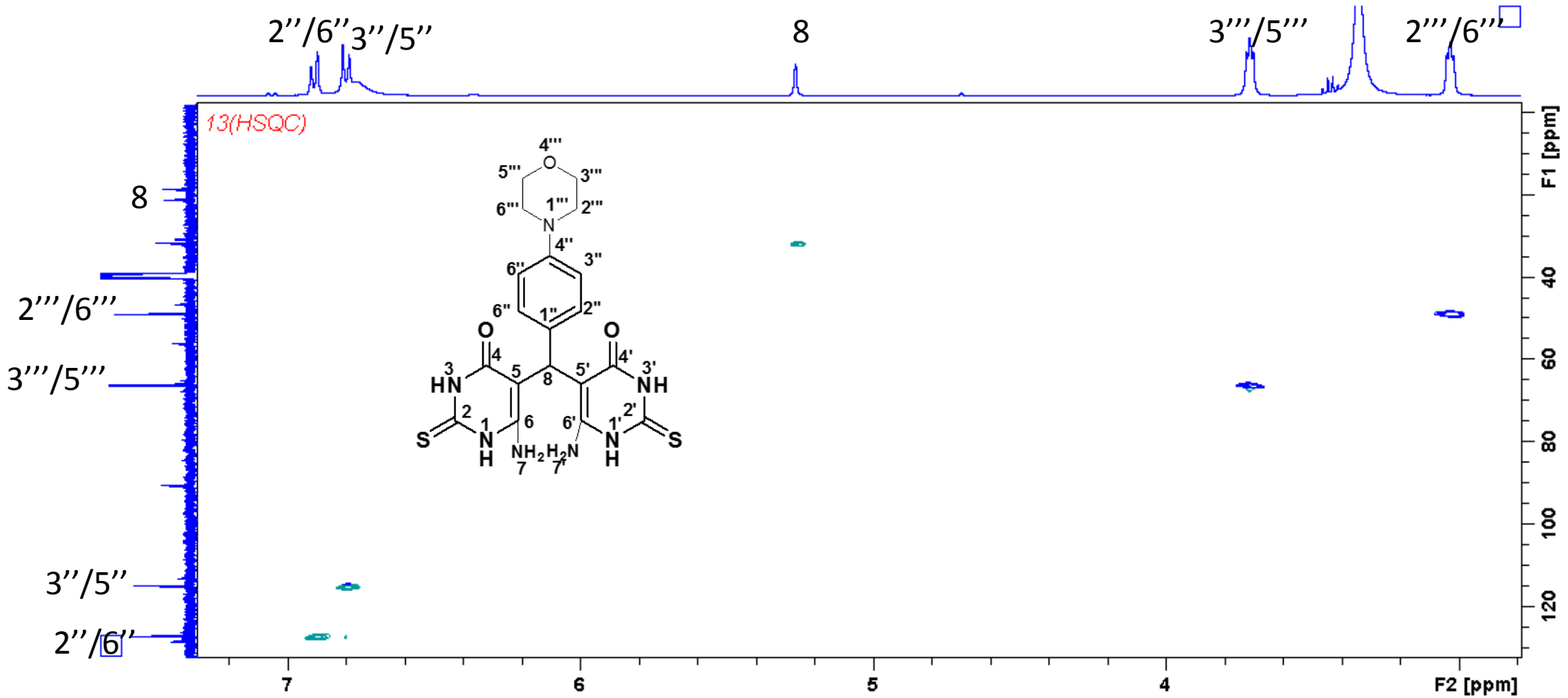
^{1}H NMR spectrum of 5,5'-((4-morpholinylphenyl)methylene)bis(6-amino-2-thiouracil) (C13) expanded



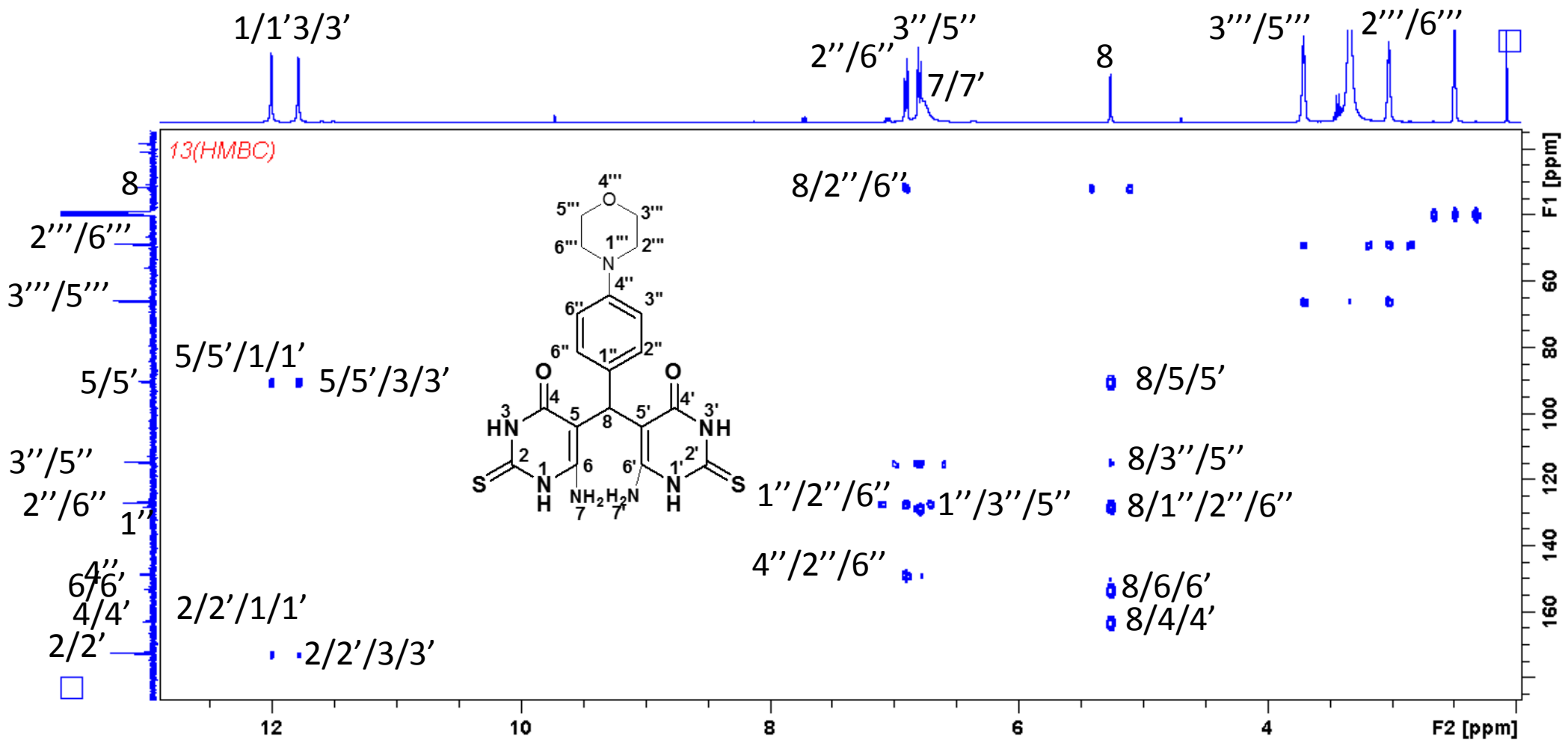
^{13}C NMR spectrum of 5,5'-((4-morpholinylphenyl)methylene)bis(6-amino-2-thiouracil) (C13)



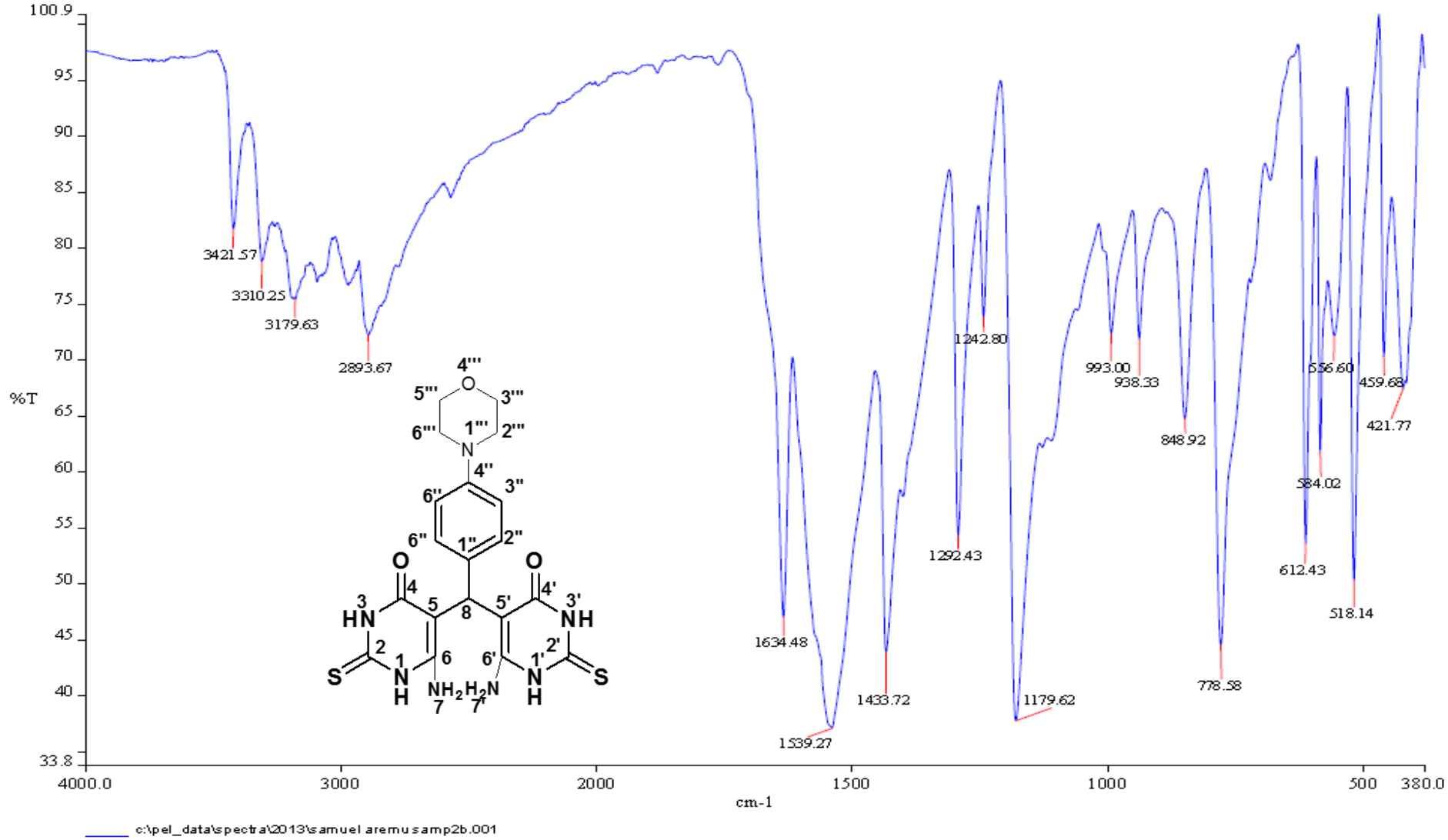
DEPT spectrum of 5,5'-((4-morpholinylphenyl)methylene)bis(6-amino-2-thiouracil) (C13)



HSQC spectrum of 5,5'-((4-morpholinylphenyl)methylene)bis(6-amino-2-thiouracil) (C13)



HMBC spectrum of 5,5'-((4-morpholinylphenyl)methylene)bis(6-amino-2-thiouracil) (C13)



IR spectrum of 5,5'-((4-morpholinylphenyl)methylene)bis(6-amino-2-thiouracil) (C13)

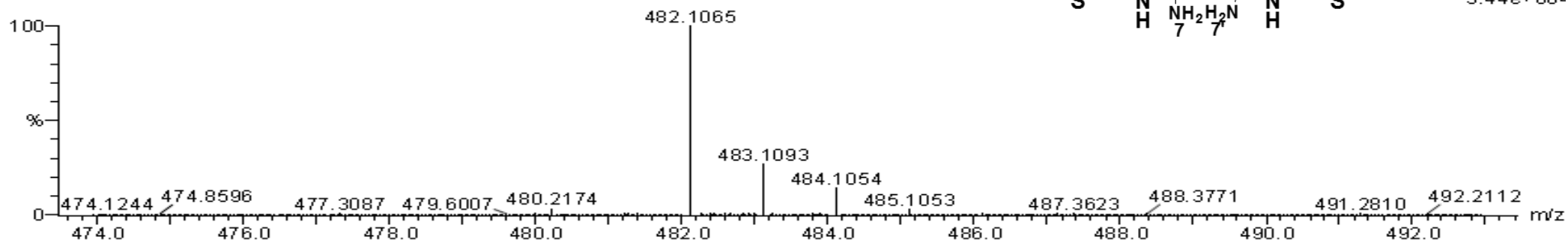
Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

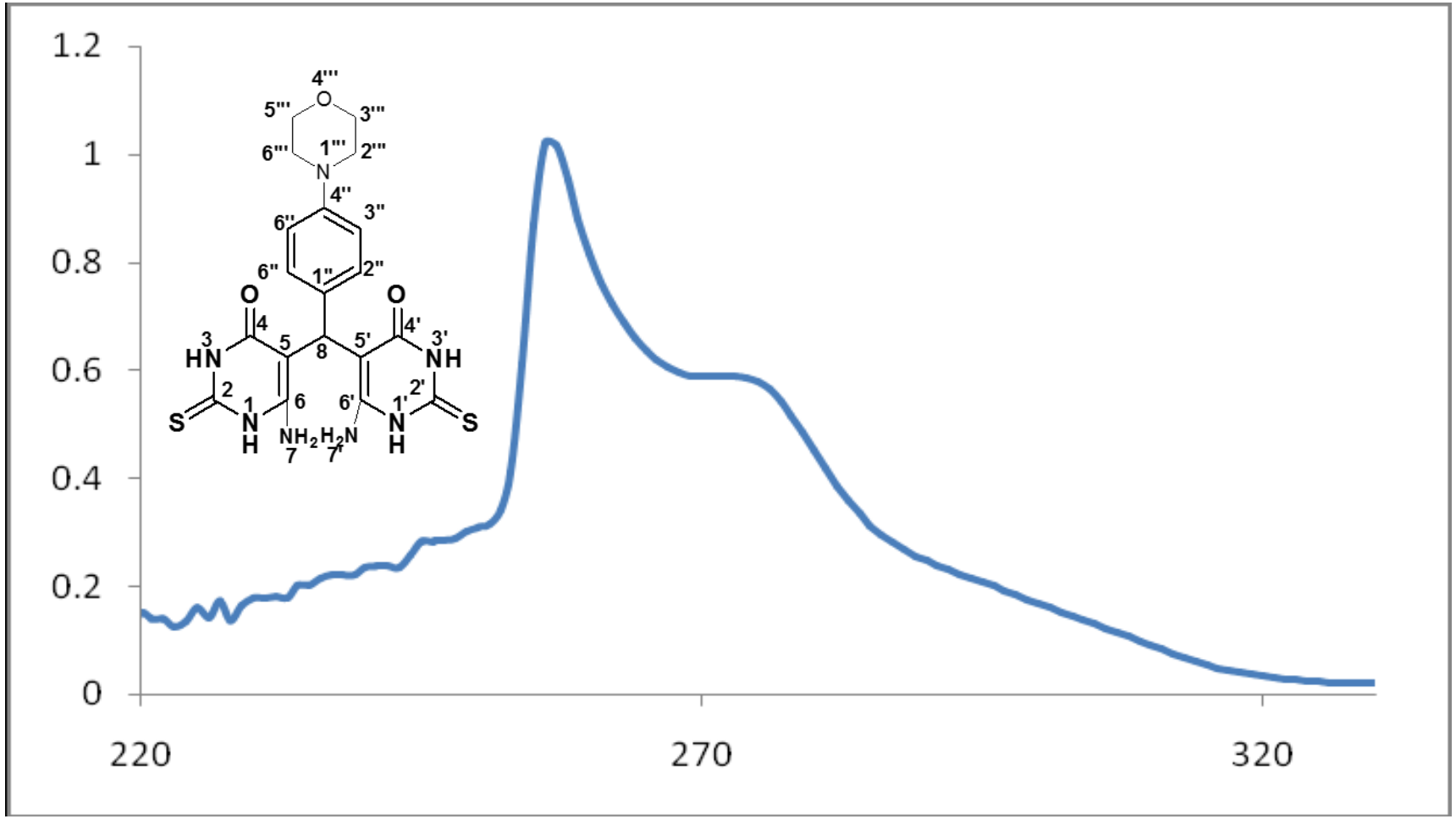
Monoisotopic Mass, Even Electron Ions
131 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-25 H: 15-25 N: 5-10 O: 0-5 S: 1-5

B-2b 61 (2.025) 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
482.1065	482.1069	-0.4	-0.8	15.5	467.1	0.0	C21 H20 N7 O3 S2

HRMS of 5,5'-((4-morpholinylphenyl)methylene)bis(6-amino-2-thiouracil) (C13)



UV spectrum of 5,5'-((4-morpholinylphenyl)methylene)bis(6-amino-2-thiouracil) (C13)