University of KwaZulu-Natal

Synthesis and Biological Studies of Novel Pyranochromene Derivatives

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Synthesis and Biological Studies of Novel Pyranochromene Derivatives

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Sunayna Sachin Pawar

2015

Supervisor: Prof. N.A. Koorbanally

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By

Sunayna Sachin Pawar

2015

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

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

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

Preface

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

Signed: _____
Sunayna Sachin Pawar
M. Sc.

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

Signed: _____

Prof. Neil A. Koorbanally
Ph.D (Natal)

Declaration 1 – Plagiarism

I, **Sunayna Sachin Pawar**, declare that:

1. The research reported in this thesis, except where otherwise indicated is my original research.
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3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
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Declaration 2 – Publications

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

Publication 1

Sunayna S. Pawar and Neil A. Koorbanally, Synthesis and structure educidation of a series of pyranochromene chalcone and flavanones using 1D and 2D NMR spectroscopy and X-ray crystallography. *Magnetic Resonance in Chemistry* **2014**, 52, 279-288.
Contributions: The candidate designed the project, synthesized and characterized all the compounds and wrote the paper. Neil Koorbanally supervised and was responsible for the work. He edited the manuscript and checked the data.

Publication 2

1-(5-Hydroxy-2,2,8,8-tetramethyl-2H,8H-pyrano[2,3-f]chromen-6-yl)-3-(4-methoxyphenyl)prop-2-en-1-one, Sunayna Pawar, Kaalin Gopaul, Thrineshan Moodley, Bernard Omondi and Neil Koorbanally. *Acta Crystallographica Section E* **2013**, E69, o484
Contributions: The candidate synthesized, characterised and grew the crystal of the compound as well as wrote the paper. Bernard Omondi helped to solve the structure. Kaalin Gopaul and Thrineshan Moodley helped to assemble the data. Neil Koorbanally supervised and was responsible for the work. He edited the manuscript and checked the data.

Publication 3

1-(5-Hydroxy-2,2,8,8-tetramethyl-2H,8H-pyrano[2,3- f]chromen-6-yl)ethanone
Sunayna Pawar, Adele Cheddie, Bernard Omondi and Neil Anthony Koorbanally. *Acta Crystallographica Section E* **2012**, E68, o3048
Contributions: The candidate synthesized, characterised and grew the crystal of the compound as well as wrote the paper. Adele Cheddie helped to write the paper. Bernard Omondi solved the structure. Neil Koorbanally supervised and was responsible for the work. He edited the manuscript and checked the data.

Publication 4

The synthesis and structural elucidation of novel pyranochromene chalcones and flavanones, Sunayna S. Pawar, Bernard. O. Omondi and Neil A. Koorbanally. Paper in preparation.
Contributions: The candidate has synthesized all the compounds and wrote the paper. Bernard Omondi solved the structures. Neil Koorbanally supervised and was responsible for the work. He edited the manuscript and checked the data.

Publication 5

Antimicrobial and antioxidant study of pyranoflavonoid analogues, Sunayna S. Pawar, Kandappa Himmakar Reddy, Patrick Govender and Neil A. Koorbanally. Paper in preparation.
Contributions: The candidate has synthesized all the compounds and wrote the paper. Kandappa Himmakar Reddy and Patrick Govender assisted with the biological activity. Neil Koorbanally supervised and was responsible for the work. He edited the manuscript and checked the data.

Publication 6

Synthesis and evaluation of pyridine, furan and fluoro-substituted phenyl pyranochromene chalcones for antibacterial and antifungal activity, Sunayna S. Pawar, Kandappa Himmakar Reddy, Patrick Govender and Neil A. Koorbanally. Paper in preparation.
Contributions: The candidate has synthesized all the compounds and wrote the paper. Kandappa Himmakar Reddy and Patrick Govender assisted with the biological activity. Neil Koorbanally supervised the work and was responsible for the project. He edited the manuscript and checked the data.

Publication 7

Synthesis, characterization, antimicrobial and antioxidant study of pyranopyrazoline derivatives, Sunayna S. Pawar, Kandappa Himmakar Reddy, Patrick Govender and Neil A. Koorbanally. Paper in preparation.
Contributions: The candidate has synthesized all the compounds and wrote the paper. Kandappa Himmakar Reddy and Patrick Govender assisted with biological activity. Neil Koorbanally supervised the work and was responsible for the project.

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~Om Shree Ganeshaya Namaha~

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I thank to my brother-in-law Sujeet, my mother-in-law Leela and father-in-law Ambadas Pawar and all my family members for their support while doing these studies.

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Last and very important person in my life, my baby Shikhar (Shree).

This thesis is dedicated to the memory of my beloved father, Mr Lachhindra S. Shelar (Anna). I miss him every day…

Sunayna Sachin Pawar

Abstract

The aim of the study was to synthesise new pyranochromene chalcones, flavanones and pyrazolines with different substituents, test them for their antimicrobial and antioxidant activity and identify the most appropriate substitution pattern for antioxidant and antibacterial activity in order to identify lead compounds to develop into antimicrobial and antioxidant drugs.

A series of 20 pryanochromene chalcones were synthesized by first adding pyran rings onto 2,4,6-trihydroxyacetophenone using 2-methyl-3-butenal forming octandrenolone (a dichromene acetophenone) with various benzaldehydes as well as pyridine 2-carbaldehyde and furan 2-carbaldehyde to form the corresponding chalcones. A selection of 13 of these chalcones was successfully converted to the flavanones using sodium acetate via an intramolecular ring closure step. In addition, 11 of the chalcones were converted to pyrazolines using hydrazine (for one set) and phenylhydrazine (for a second set). An attempt to condense octandrenolone with *p*-nitrobenzaldehyde resulted in the flavone instead of the chalcone. No further reactions were carried out on the flavone. The chalcone and flavanone with the unsubstituted B ring, **A-3a** and **A-4a** were known. In addition, the chalcone with a *p*-methoxy substituent on the B ring was known. Apart from that, 52 novel molecules including 18 chalcones, 12 flavanones, the flavone and 22 pyrazolines were all reported for the first time in this work or publications emanating from this work. The reactions were fairly easy to carry out and resulted in good yields of product.

All synthesized compounds were fully characterized using NMR and IR spectroscopy and HRMS. In addition, 9 chalcones, 2 flavanones and octandrenolone was characterized using single crystal X-Ray crystallography. This confirmed that the double bond was in the *E*-

conformation in the chalcones and that the configuration at C-2 in the flavanones was *S*. The X-Ray structures also revealed the planarity of the chromene chalcones. Comprehensive NMR tables assigning each of the ^{1}H and ^{13}C NMR resonances were constructed and will be a useful reference to assign the resonances of similar chromene chalcones, flavanones or pyrazolines.

The synthesised pyranochromene compounds were screened for their antibacterial, antifungal and antioxidant activities. There was no real trend between any of the substituents on the B ring and bioactivity; however certain compounds with particular substitution patterns showed much better activity than others. With regard to antibacterial activity, two Gram positive strains, *Staphylococcus aureus* and *Enterococcus faecalis* and three Gram negative strains, *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* were used in the study. The pyrazoline with the *meta* fluorinated B ring (**C-4e**) showed the best antibacterial activity with IC_{50} values of between 14.9-29.7 μM. The *ortho* trifluoromethyl pyrazoline derivative (**C-4i**) also showed good antibacterial activity, but only against the Gram negative bacteria with IC_{50} values of between 13.3-26.6 μM. The two most active flavanones had a much larger range than the two pyrazolines mentioned above, with **A-4a** (the unsubstituted flavanone) having MICs between 18.7-64.4 μM and the *ortho* chloroflavanone (**A4i**) having MICs between 29.6-118.2 μM. Amongst the three sets of compounds, the chalcones showed the worst antibacterial activity with the two best compounds, **A-3a** (unsubstituted B ring) and **A-3g** (4-methoxychalcone) having MICs of between 32.2-128.7 and 119.5-239.0 μM respectively. The activity of the test compounds were compared to that of neomycin, which had MIC values of between 8.1-32.5 μM.

Two other compounds, **A-5** (the *para* nitroflavone) and the 2-trifluoromethyl pyranochromene chalcone (**B-6**) showed very strain specific activity. **A-5** was active against *S. aureus* at a MIC of 2.3 μM and **B-6** against all Gram negative strains at MICs between 13.7-27.5 μM compared to neomycin with a MIC of 16.3 μM.

The synthesized compounds were also tested against four fungal strains (two strains of *Candida albicans*, *Candida krusei* and *Candida parapsilosis*). Several of the chalcones and flavanones **3a/4a** (unsubstituted B ring), **3d/4d** (4-fluorinated B ring), **4f** (3-methoxylated B ring), **4l** (3-nitrated B ring) and **3m** (B ring being a naphthalene group) showed good activity against all four strains of fungi having MIC values of 28.5-64.4 μM. The pyrazolines **4c** and **5c** (4-brominated B ring), **4f** (4-fluorinated B ring), and **4h** and **5h** (3,4-difluorinated B ring) showed good activity (IC_{50} between 28.5-104.0 μM) against all the fungal species tested against, on average approximately 20 times lower in activity than the standard, amphotericin B (1.3 μM), with the best activity being shown by **4f** (29.7 μM) and **4h** (28.5 μM) against *C. krusei*.

The antioxidant properties of the synthesized compounds were also tested using the DPPH radical scavenging assay. Several compounds in all three classes of compounds synthesized showed better antioxidant activity than the ascorbic acid standard used, which had an IC_{50} value of 220.0 μM. The chalcones that showed the best antioxidant activity were **A-3e** (2,4-difluoro) (IC_{50} of 230.4 μM), **A-3h** (2-fluoro-3-methoxy) (IC_{50} of 233.2 μM), **A-3i** (2-chloro) (IC_{50} of 228.7 μM) and **A-3l** (3-nitro) (IC_{50} of 220.3 μM). The flavanones **A-4i** (2-chloro) and **A-4l** (3-nitro) also showed comparable antioxidant activity to the chalcones with IC_{50} values of 225.8 and 234.7 μM. The pyrazolines all showed excellent antioxidant activity being up to four times better than ascorbic acid (220.0 μM) in the DPPH assay. In addition,

the H_2O_2 scavenging assay was carried out on the pyrazolines where again, several of the compounds showed 2 fold better activity than ascorbic acid.

In general, several of the compounds synthesised may be good leads for antibacterial, antifungal and antioxidant compounds. The best lead for an antibacterial compound is the pyrazoline **C-4e** (with a *meta* fluorinated B ring), while several compounds mentioned above may be good leads for antifungals and the pyrazolines in general may be good leads for antioxidant supplements.

List of Abbreviations

^{1}H NMR - Proton Nuclear Magnetic Resonance Spectroscopy

^{13}C NMR - Carbon-13 Nuclear Magnetic Resonance Spectroscopy

^{19}F NMR – Fluorine-19 Nuclear Magnetic Resonance Spectroscopy

°C - Degrees Celsius

CCDC - Cambridge Crystallographic Data Centre

$CDCl_3$ - Deuterated Chloroform

CD_3OD- Deuterated Methanol

CFU - Colony-forming unit

COSY - Correlated Nuclear Magnetic Resonance Spectroscopy

d- Doublet

dd - Double doublet

ddd - Doublet of doublet of doublets

DMSO - Dimethyl sulfoxide

DPPH - 2,2'-diphenly-1-picrylhydrazyl

dq - Doublet of quartets

dt - Doublet of triplets

EC_{50} - Half maximal effective concentration

EtOAc - Ethyl actetate

EtOH – Ethanol

FDA - Food and Drug Administration

FT-IR - Fourier Transform – Infrared Spectroscopy

GC-MS - Gas Chromatography - Mass Spectrometry

HCl - Hydrochloric acid

HIV - Human Immuno-deficiency Virus

HMBC - Heteronuclear Multiple Bond Coherence

HPLC - High Pressure Liquid Chromatography

HRMS - High Resolution Mass Spectrometry

HSQC - Heteronuclear Multiple Quantum Coherence

Hz - Hertz

IC_{50} - Half maximal inhibitory concentration

m – Multiplet

$MgSO_4$ - Magnesium sulfate

MBC- Minimum bactericidal concentration

MeOH - Methanol

MIC - Minimum inhibitory concentration

MS - Mass Spectrometry

MRSA - Methicillin-resistant *Staphylococcus aureus*

NADH - Nicotinamide adenine dinucleotide

NOESY - Nuclear Overhauser effect spectroscopy

NCCLS - National Committee for Clinical Laboratory Standards

NMR- Nuclear Magnetic Resonance Spectroscopy

R_f - Retention Factor

RPMI - Roswell Park Memorial Institute

s - Singlet

t – Triplet

TFT - Trifluorotoluene

td - Triplet of Doublets

THF - Tetrahydrofuran

TLC - Thin Layer Chromatography

TMS - Tetramethylsilane

UV - Ultraviolet Spectroscopy

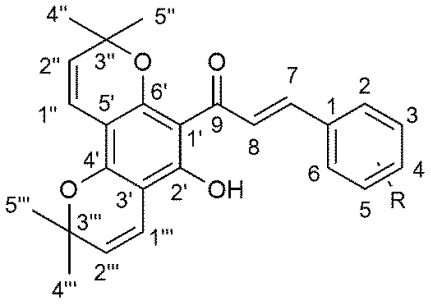
WHO – World Health Organization

Xtt - 2,3-bis-(2-methoxy-4-nitro- 5-sulfophenyl)-2*H*-tetrazolium- 5-carboxanilide

Structures and numbering of compounds contained in the thesis

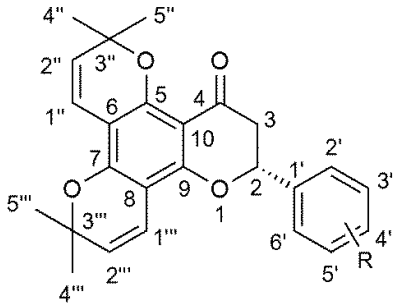
Individual chapters contain their own numbering of compounds as each is written as discrete chapters in paper format. Where reference is made to compounds within each of these chapters in the abstract and conclusion, the following numbering is used. This numbering is also used in the appendices.

Compounds contained in Chapters 2-4



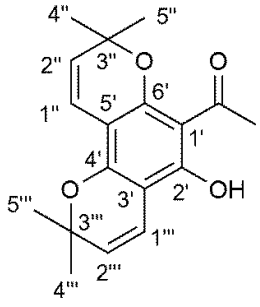
R

A-3a = H Flemiculosin
A-3b = 2-F
A-3c = 3-F
A-3d = 4-F
A-3e = 2,4-F
A-3f = 3-OMe
A-3g = 4-OMe
A-3h = 2-F,3-OMe
A-3i = 2-Cl
A-3j = 4-Cl
A-3k = 4-Br
A-3l = 3-NO_2
A-3m = Napthalene



R

A-4a = H Deoxy-MS-II
A-4b = 2-F
A-4c = 3-F
A-4d = 4-F
A-4e = 2,4-F
A-4f = 3-OMe
A-4g = 4-OMe
A-4h = 2-F,3-OMe
A-4i = 2-Cl
A-4j = 4-Cl
A-4k = 4-Br
A-4l = 3-NO_2
A-4m = Napthalene



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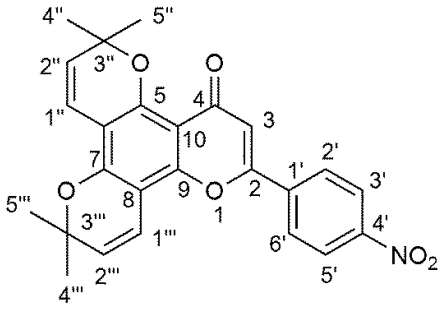
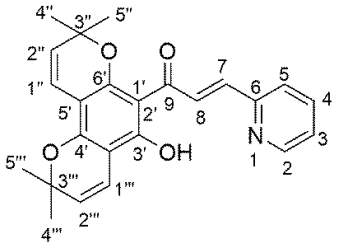
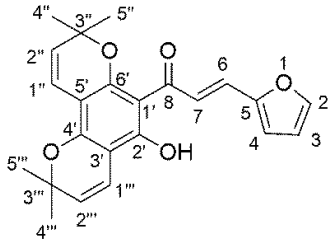


A-5

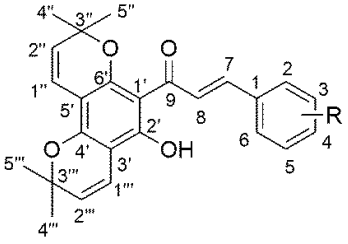
Compounds contained in Chapter 5



B-3

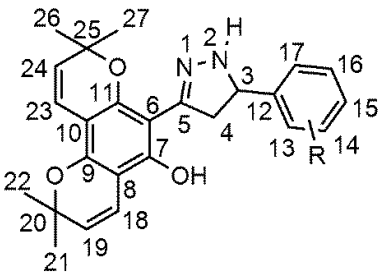


B-4

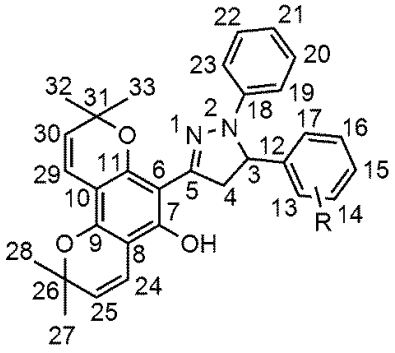


B-5 R = 2,4,6-tri-OCH_3
B-6 R = 2-CF_3
B-7 R = 3-CF_3
B-8 R = 4-CF_3
B-9 R = 3,4-diF

Compounds contained in Chapter 6



R	R
C-4a = 2-Cl	**C-4g** = 2,4-F
C-4b = 4-Cl	**C-4h** = 3,4-F
C-4c = 4-Br	**C-4i** = 2-CF_3
C-4d = 2-F	**C-4j** = 3-CF_3
C-4e = 3-F	**C-4k** = 4-CF_3
C-4f = 4-F	



R	R
C-5a = 2-Cl	**C-5g** = 2,4-F
C-5b = 4-Cl	**C-5h** = 3,4-F
C-5c = 4-Br	**C-5i** = 2-CF_3
C-5d = 2-F	**C-5j** = 3-CF_3
C-5e = 3-F	**C-5k** = 4-CF_3
C-5f = 4-F	

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Chapter 1. Introduction

Chalcones are colored compounds and occur as bright yellow and in some instances red due to their highly conjugated nature. The term chalcone was coined by Kostanecki and Tambor in 1899 [1]. They are also known as benzalacetophenones, benzylideneacetophenones, phenyl styryl ketones, *β*-phenyl acrylphenones, *γ*-oxo-*α*-*γ*-diphenyl-*α*-propylenes and *α*-phenyl-*β*-benzoethylenes. Their structure is characterized by two aromatic rings joined together by an *α*,*β*-unsaturated ketone (**Figure 1-1**). They are an important class of heterocyclic compounds, which are found abundantly in nature, have potential pharmacological use and are the precursors to the flavonoids [2-12]. This class of compounds have been found to be active in many medicinal bioassays such as anticancer [4, 7, 9, 13-20], antibacterial [5, 10, 21-26], antifungal [21, 24-26], antioxidant [7, 27, 28], anti-inflammatory [7, 15], antileishmanial [29], antidiabetic [14] and anti HIV assays [30].

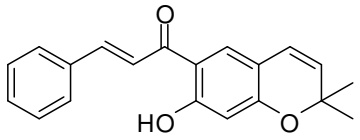


Figure 1-1 The basic structure of a linear pyranochromene chalcone

Pyrano chalcones and flavonoids arise from cyclisation of a prenyl group onto the aromatic ring, resulting in a 2,2-dimethyl-2*H*-pyrano subunit (**Figure 1-1**), which has been investigated in recent years due to their interesting pharmacological properties. They have shown interesting docking results with antibacterial enzymes [31-33] as well as antibacterial [34], antioxidant [35], antileishmanial [36, 37], protein kinase C inhibition [38], anti-inflammatory [39, 40] and anticancer [19, 20, 41, 42]. Prenyl (2-methyl-2-butenyl) groups substituted onto the chalcone core skeleton have been shown to increase the lipophilicity of

the molecule [43] which makes it easier for these compounds to enter into cells. It has also been shown that prenylation increases the biological activity of the original flavonoid [37, 44, 45]. Prenylated flavonoids are found abundantly in nature in the Leguminosae [46-49], Moraceae [47, 50-53], Euphorbiaceae [47, 54-57], Rutaceae [58] and Fabaceae [59] families.

Chalcones can easily be converted to heterocyclic compounds by reacting them with different chemical reagents (**Figure 1-2**). In this work, pyranochromenochalcones were converted to the flavanones by an additional step using sodium acetate and ethanol, and pyrazolines by the Michael addition using substituted hydrazine hydrates. Thus, three structural backbones, the pyranochromenochalcones, pyranochromenoflavanones and pyranochromenopyrazolines were synthesized with the chalcone as an intermediate. All three scaffolds were tested for their bioactivity.

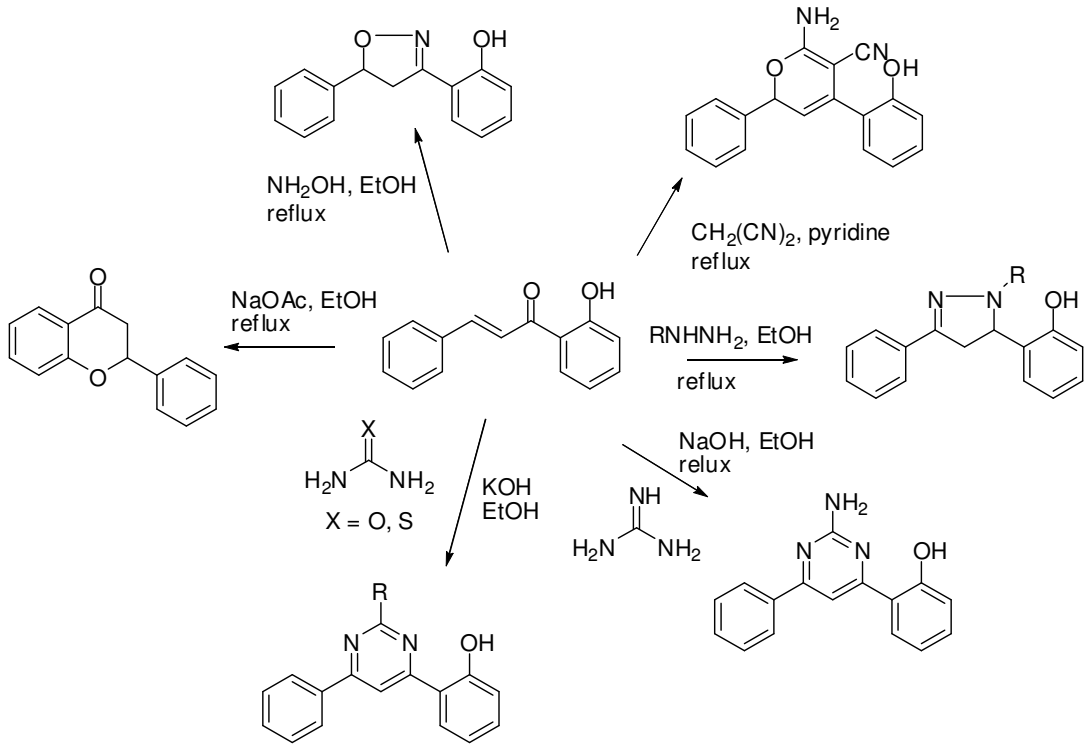


Figure 1-2 Various reactions that can be carried out with the chalcone intermediate

Synthetic routes to the pyranochalcones and pyranoflavanones

In order to synthesise pyranochalcones or pyranoflavanones, either the acetophenone or benzaldehyde moiety needs to be prenylated first. Once prenylated, these two substrates can then be reacted to produce the chalcone and then the flavanone.

Synthesis of prenylated acetophenone and aldehyde intermediates

There are a variety of methods available for the synthesis of pyranochalcones [38, 39, 46, 57, 59, 60, 61], however the most convenient method is the stepwise synthesis of pyranochromene acetophenones, which are then condensed with aldehydes in a Claisen-Schmidt condensation in the presence of alcoholic bases to form the chalcone. The pyranochromene acetophenone intermediate has been synthesized by the condensation of hydroxyacetophenones with 3-methylcrotonaldehyde (3-methyl-2-butenal) in either anhydrous pyridine [36, 37, 39], $Ca(OH)_2$ [62, 63] or ethylenediamine diacetate [46, 57, 59]. Other reagents used for pyran ring formation with acetophenones are 3-chloro-3-methylbut-1-yne in acetone [64]. Prenylation with prenyl bromide followed by cyclisation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) also resulted in pyranochromene chalcones [65]. These reactions were easy to perform producing good yields. Beside prenylating the acetophenone portion of the molecule, the aldehyde portion was also prenylated using 2-chloro-2-methyl-but-3-yne and 3,4-dihydroxybenzaldehyde [66], alkylation of 3-hydroxybenzaldehyde with 2-methyl-3-butyn-2-ol followed by cyclisation in *N,N*-dimethylaniline [67]. In this case the prenylated aldehydes were then reacted with acetophenones to produce the prenylated chalcones.

Lee *et al*. [46, 59] investigated the mechanism of the prenylation reaction of aromatic acetophenones with 3-methyl-2-butenal and ethylenediaminediacetate (EDDA). They reported a [3+3]-cycloaddition via a 6π electrocyclization (**Figure 1-3**).

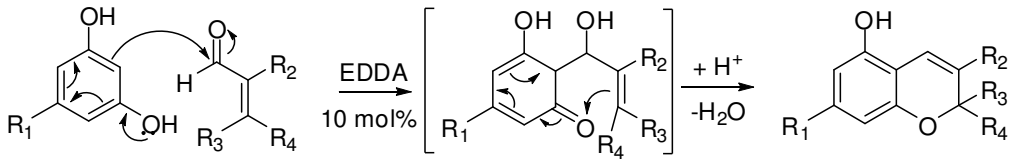


Figure 1-3 Mechanism of prenylation

Condensation reactions of acetophenone and benzaldehyde intermediates to chalcones

The most common method of preparing chalcones is the Claisen-Smith condensation of appropriate benzaldehydes and acetophenones, which is followed by dehydration. In order for this reaction to be successful, various bases such as KOH [36-37, 40, 46, 57, 60-62, 65, 68], NaOH [19, 62], LDA [59] and NaH [36] in polar solvents such as EtOH, CH_3OH and THF were used. Mali *et al.* [68] used cetyltrimethylammonium bromide (CTAB), an amine based cationic quaternary surfactant, under basic conditions to form chalcones with prenylated pyranoacetophenone and benzaldeydes that was an intermediate for (±) Maxima flavanone A. In addition to the Claisen-Smith condensation, more interesting synthetic protocols have been reported such as the Pd-mediated Suzuki coupling between cinnamoyl chlorides and phenyl boronic acids [69], and the carbonylative Heck coupling with aryl halides and styrene in the presence of carbon monoxide [70]. Chalcones were also rapidly synthesized by microwave in good yields using aqueous potassium hydroxide [71].

The mechanism (**Figure 1-4**) of this reaction involves the abstraction of a proton from the methyl group of the acetophenone forming an enolate anion, which then adds to the electrophilic carbonyl of benzaldyde forming the alcohol, which is subsequently dehydrated to the chalcone [72, 73].

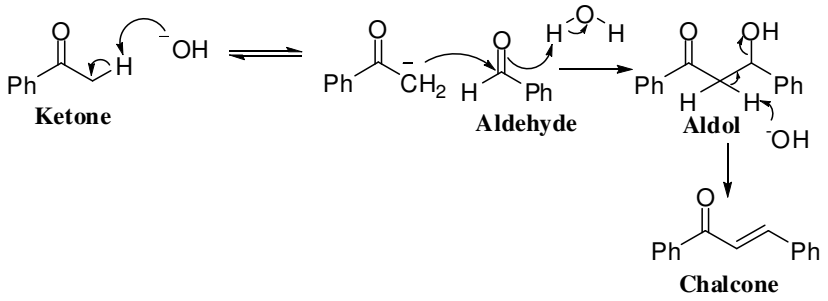


Figure 1-4 Mechanism of chalcone formation

Conversion of chalcones to flavanones

Sodium acetate [65, 66] in ethanol is most commonly used to cyclise chalcones to flavanones. The only drawback of this reaction is long reaction times of 2-4 days. Mali *et al*. [68] used KF-celite to carry out the cyclisation of chalcones to flavanones in good yields in shorter reaction times of 32-48 hours.

The mechanism (**Figure 1-5**) involves self-condensation of chalcone to produce flavanone. This reaction involves the abstraction of a proton from the ortho hydroxy group of the chalcone forming an oxyanion. This then adds to the alkene, which in turn leads to the carbanion and abstracts the proton from ethanol forming the flavanone.

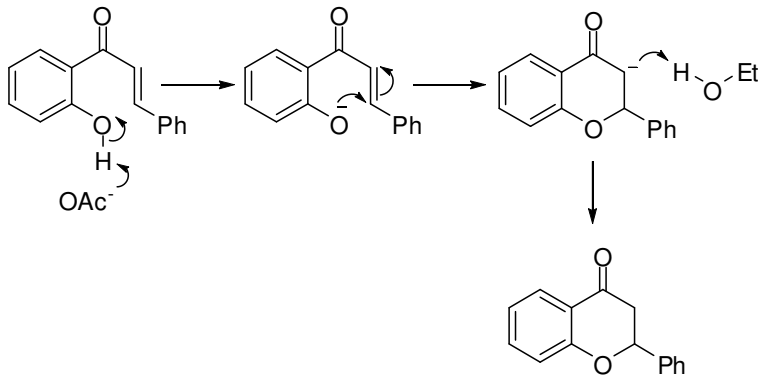


Figure 1-5 Mechanism of flavanone formation

Pyrazolines

Pyrazoline (**Figure 1-6**) is a five-membered heterocyclic ring having two adjacent nitrogen atoms within the ring. It has an endocyclic double bond, is basic in nature and has three possible tautomeric structures with the structure shown in (**Figure 1-6**) being the most stable. Pyrazolines can be considered as a cyclic hydrazine moiety. Amongst the nitrogen containing five membered heterocycles, pyrazolines have proved to be amongst the most useful frameworks for biological activity [24, 74, 75]. It is widely used as synthons in organic synthesis and the chemistry of pyrazolines was reviewed by Wiley *et al*. [76]. Pyrazolines possess a wide spectrum of biological activities. They are reported to have antiamoebic [77], antimicrobial [78-84], MAO inhibitor [85-86], antioxidant [7, 84, 87, 88], anti-inflammatory [7, 88-91], anti-analgesic [84, 90], and anticancer and antimycobacterial activities [7, 92-95].

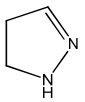


Figure 1-6 General structure of pyrazoline

Conversion of chalcones to pyrazolines

Chalcones can be converted to pyrazolines using hydrazine hydrate or phenyl hydrazine hydrate in ethanol. The reaction probably takes places through *in situ* formation of an appropriate β unsaturated hydrazone intermediate which cyclizes to produce pyrazolines.

There are two possible mechanisms for formation of pyrazolines from chalcones and hydrazine. In first mechanism (**Figure 1-7**, Mechanism **A**), hydrazine reacts with chalcone to form hydrazone which undergoes an intramolecular Michael addition to form pyrazoline. In

the second mechanism (**Figure 1-7**, Mechanism **B**), the steps are reversed, where a Michael addition of hydrazine with chalcone happens first, followed by cyclization to produce pyrazoline [81].

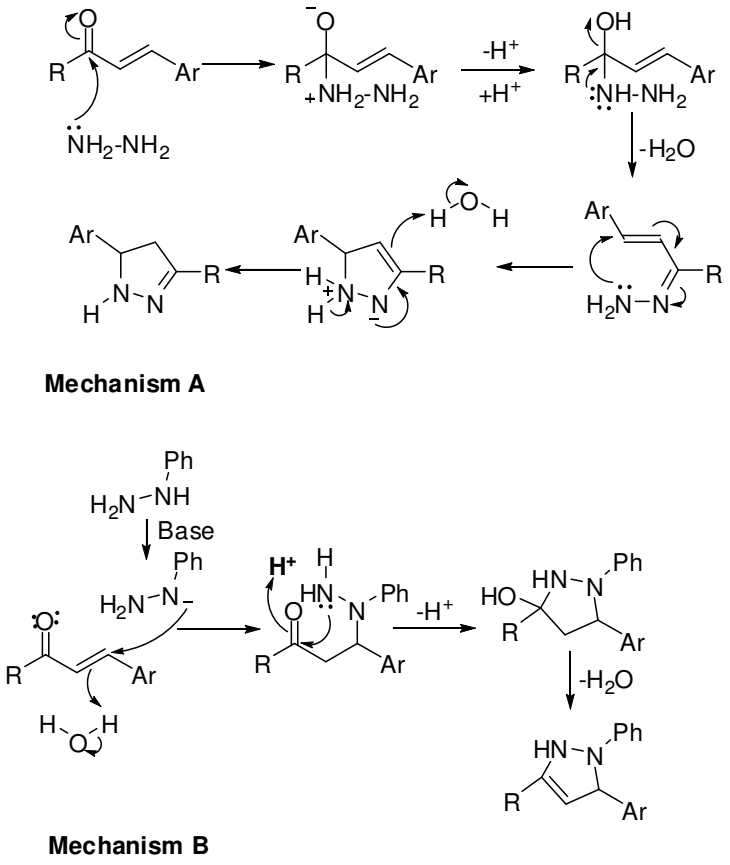


Figure 1-7 Possible mechanisms for pyrazoline formation

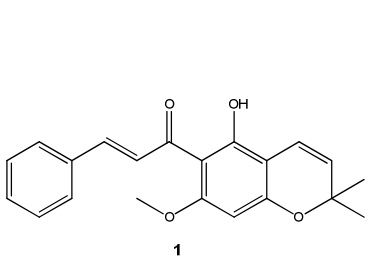
Biological importance of pyranochalcones, pyranoflavanones and pyranopyrazolines

Chalcones, flavanones and pyrazolines with a pyran group as part of the core structure have shown biological activity in a range of bioassays including antibacterial, anticancer, protein kinase inhibition, anti-inflammatory, antileishmanial, interleukin-1 inhibition, anti-HIV and NADH:ubiquinone oxido reductase inhibition.

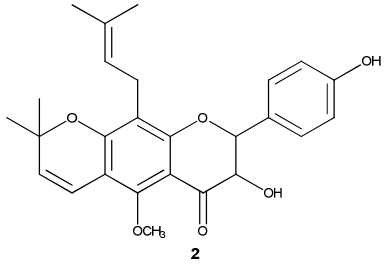
Antibacterial activity

Pongachalcone I (**1**) is a natural linear pyranochalcone which also contains a hydroxy and methoxy group. It was isolated from *Tephrosia deflexa* [96] and has been reported to have antibacterial activity against *Pseudomonas putida*. This activity was supported by molecular docking studies of pongachalcone with TtgR (multi-drug binding protein in *Pseudomonas putida* (PDB Code: 2UXI). Pongachalcone showed good binding affinity to this protein [31-33].

Prenylated dihydroflavonols from *Mundulea suberosa* also showed antibacterial activity against *Bacillus subtilis* and *Pseudomonas aeruginosa* [97]. Of these, 5-methyl lupinifolinol (**2**) exhibited MIC values of 0.01 μg mL^{-1} for both the Gram positive and negative strains.



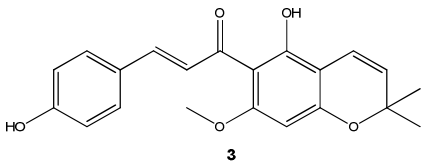
1



2

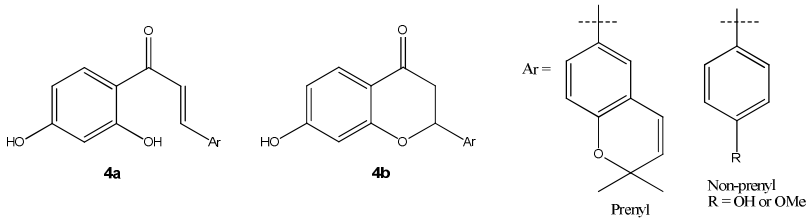
Anticancer activity

Dehydrocycloxanthohumol (**3**) isolated from hops (*Humulus lupulus*) [42] showed a dose-dependent (0.1 to 100 mM) decrease in the growth of human breast cancer (MCF-7), colon cancer (HT-29) and ovarian cancer (A-2780) cells *in vitro*, with the best activity being shown against the MCF-7 cancer cell line.

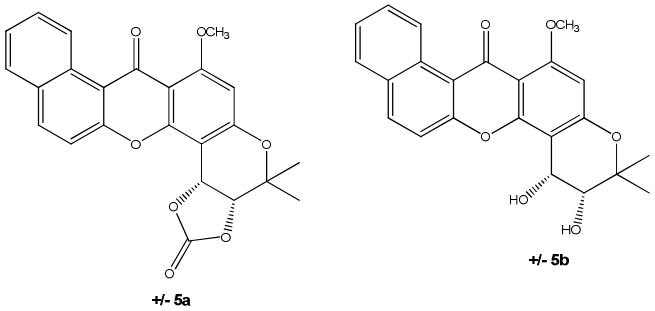


3

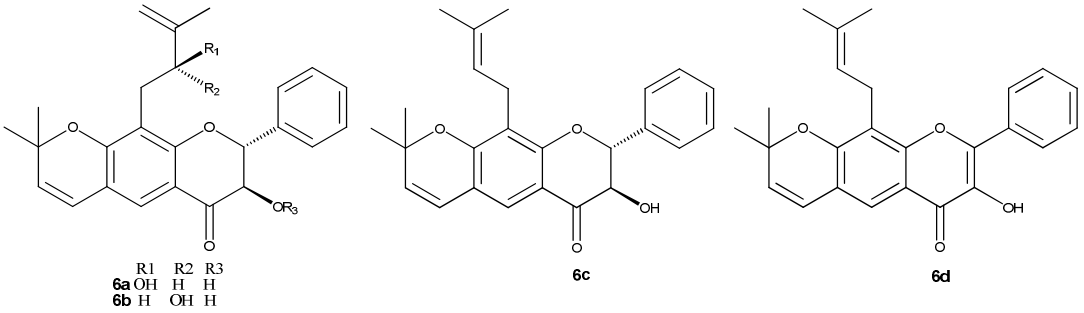
Rao *et al* [98] also observed that flavonoids containing prenyl groups showed higher inhibitory activity than their non-prenylated analogues against the MCF-7 cell line. They further observed that chalcones (**4a**) show better antioxidant activity than their respective flavanones (**4b**).



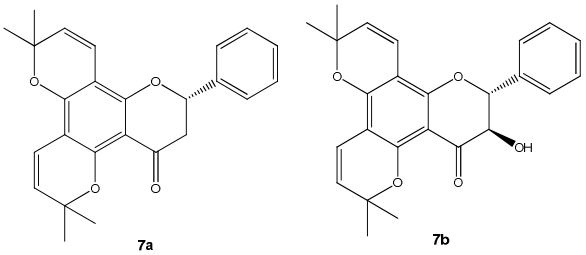
The synthetic benzopyrano xanthone carbonate (**5a**) and diol (**5b**) showed significant cytotoxicity against the murine leukemia cell line (L1210) [99].



The dihydropyranoflavanols **6a-c** isolated from *Dunbaria longeracemosa* showed weak cytotoxicity against five cancer cell lines, KB (human mouth epidermal carcinoma), HepG2 (human hepatocellular liver carcinoma), HuCCA-1 (human cholangiocarcinoma), A549 (human lung carcinoma), and S102 (human liver cancer). They were also found to inhibit aromatase with an IC_{50} value of 0.3, 0.4 and 1.2 μM respectively. The pyranoflavonol, sericetin (**6d**) also exhibited cytotoxicity against the KB and HepG2 cancer cell lines. Interestingly, sericetin **6d** did not inhibit the aromatase enzyme [100].

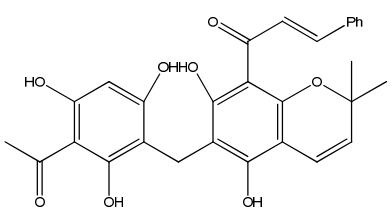


The pyranochromenoflavanones **7a** and **7b** isolated from *Mundulea chapelieri* were found to be active against the A2780 human ovarian cancer cell line with IC_{50} values of 33 and 20 μg mL^{-1} [41].

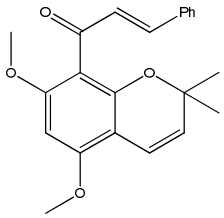


Protein kinase inhibitors

Protein kinases are enzymes that add a phosphate (PO_4) group to a protein or other organic molecules. This phosphorylation is necessary in some cancers and anti-inflammatory conditions. Inhibition of these kinases prevent phosphorylation and hence cancers and anti-inflammatory responses. Rottlierin analogues (**8a**) were shown to selectively inhibit protein kinase C delta (PKC-δ) with an IC_{50} of 3.0-6.0 μM [38]. Other analogues of rottlierin such as PK301, PK302 and PK111 (**8b**) also showed improved activity against (PKC-δ).



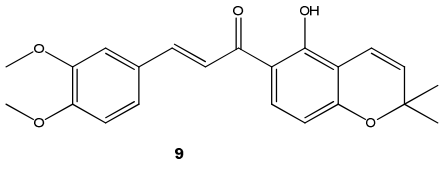
8a



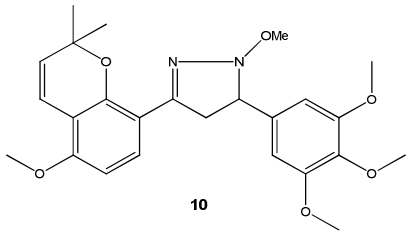
8b

Anti-inflammatory

The dimethoxypyranochalcone **9** showed remarkable *in vitro* and *in vivo* anti-inflammatory activity comparable to FDA approved indomethacin [39]. The pyrazoline derivative **10** also showed potent anti-inflammatory activity [40].



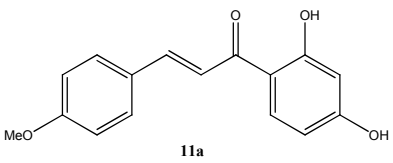
9



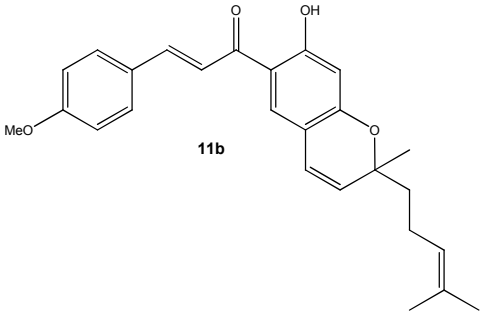
10

Antileishmanial activity

Pyranochalcones such as **11b** has shown activity against extracellular promastigotes and intracellular amastigotes of *Leishmania donavani* residing within murine macrophages in concentrations ranging from 0.25 to 50 µg mL^{-1} [37]. It was evidently clear from this study that pyran ring was a key feature to antileishmanial activity as **11b** containing the pyran group showed better activity (87 and 49% inhibition at 50 µg mL^{-1} against promastigotes and amastigotes, respectively) than **11a** (60 and 15% inhibition at 50 µg mL^{-1}), which did not contain the pyran group.

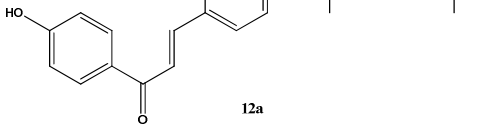


11a

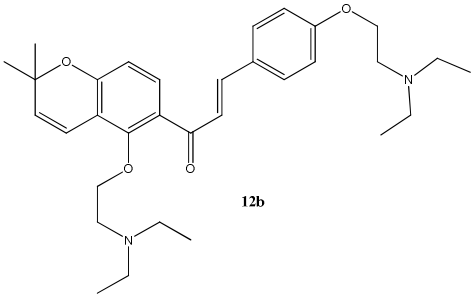


11b

The synthesized open prenylated chalcone **12a** and the pyran chalcone **12b** were also significantly more active than then standard antileishmanial drugs, Miltefosine and sodium stibogluconate (SSG) [36].



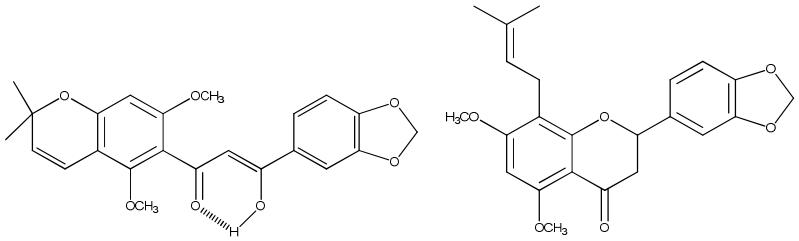
12a



12b

Interleukin-1 inhibition

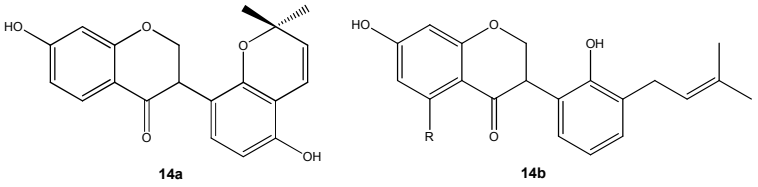
Interleukin 1 is a protein responsible for inflammatory responses. The pyran chalcone, pongapinone A **13a** and the open prenylated pongapinone B **13b** were isolated from the bark of *Pongamia pinnata.* Pongapinone A **13a** exhibited inhibitory activity upon interleukin-1 prodution with IC_{50} values of 2.5 μg mL^{-1} showing anti-inflammatory activity [101].



13a 13b

HIV reverse transcriptase inhibition

Mckee *et al*. [102] isolated six isoflavanoids from plants, marine and microbial organisms, which were tested for anti-HIV activity in the NCI's XTT-based primary screen. Two of the six compounds, 5-deoxyglyasperin F **14a** and 2′-hydroxyneobavaisoflavanone **14b** displayed moderate activity with EC_{50} values of 11.5 and 7.6 μg mL^{-1} respectively.

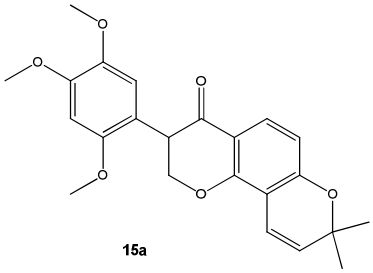


14a 14b

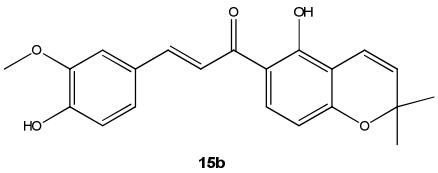
Inhibition of NADH:ubiquinone oxidoreductase

In eukaryotes, NADH:ubiquinone oxidoreductase is located in the inner mitochondrial membrane and is one of the "entry enzymes" of cellular respiration or oxidative phosphorylation in the mitochondria. It catalyzes the transfer of electrons from NADH to coenzyme Q10 (CoQ10).

Lonchocarpusone **15a** and 4-hydroxy-3-methoxylonchocarpin **15b** showed IC_{50} values of 4.4 and 4.8 μM respectively against NADH:ubiquinone oxidoreductase [103, 104].



15a

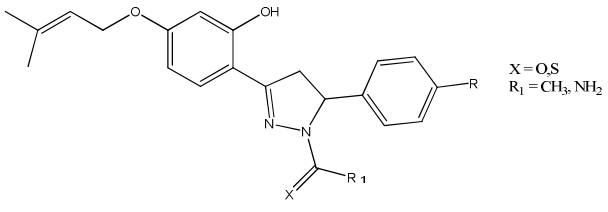


15b

Monoamine oxidase (MAO) A and B inhibitors

Monoamine oxidase inhibitors (MAO inhibitors), are chemicals which inhibit the activity of the monoamine oxidase enzyme and prevent the breakdown of monoamine oxidase neurotransmitters. Inhibition of both Type A and B monoamine oxidase treats depression and anxiety.

N-substituted-3-[(2'-hydroxy-4'-prenyloxy)-phenyl]-5-phenyl-4,5-dihydro-(1*H*)-pyrazolines **16** displayed substantial activity against human monoamine oxidase-A and B isoforms [44].



16

Hypothesis and aims

It is hypothesized that synthesizing chalcones, flavanones and pyrazolines with pyranochromene moieties would lead to bioactive molecules, since similar molecules have shown a vast array of bioactivity.

The aim of the study is to synthesise new pyranochromene chalcones, flavanones and pyrazolines and test these compounds for antimicrobial and antioxidant activity. More specifically, a variety of derivatives with different functional groups on the pyranochromene chalcone, flavanone or pyrazoline scaffold were targeted for synthesis. The main aim is to find the most appropriate substitution pattern giving the best antioxidant and antibacterial activity, which will be the best leads to develop into antimicrobial and antioxidant drugs.

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Chapter 2. Synthesis and structure elucidation of a series of pyranochromene chalcones and flavanones using 1D and 2D NMR spectroscopy and X-Ray crystallography

Abstract

A series of novel pyranochromene chalcones and corresponding flavanones were synthesized. This is the first report on the confirmation of the absolute configuration of chromene-based flavanones using X-ray crystallography. These compounds were characterized by 2D NMR spectroscopy and their assignments are reported herein. The 3D structure of the chalcone **3b** and flavanone **4g** was determined by X-ray crystallography and the structure of the flavanone confirmed to be in the *S* configuration at C-2.

Keywords

NMR; ^{1}H; ^{13}C; X-ray crystallography; pyranochromene; chalcones; flavanones

Introduction

Chalcones and flavanones form a large and important group of naturally occurring secondary metabolites [1]. Chalcones are important intermediates for the synthesis of biologically active compounds such as flavone, flavonol, flavanone, isoflavone and their derivatives [2]. Besides having a physiological role in plants, flavonoids have also been reported to have a wide variety of biological activities, including anti-inflammatory, antiviral, antiprotozoal, antioxidant, cardiovascular and anti-carcinogenic properties [2].

A five-membered prenyl moiety on a benzene ring cyclized with an oxygen atom on an adjacent position leads to a benzopyran or chromene molecule with a second such cyclisation leading to a pyranochromene such as the naturally occurring octandrenolone [3,4], *O*-methyloctandrenolone [4-5], *trans*-3'",4'"-dihydro-3'",4'"-dihydroxy-*O*-methyloctandrenolone [4], *trans*-3",4"-dihydro-3",4"-dihydroxy-*O*-methyloctandrenolone [4], flemiculosin [6], laxichalcone [7], 3-deoxy-MS-II [3,8,9] and MS-II [3,8]. The flavanone (**4a**) 3-deoxy–MS-II (**Scheme 2-1**), which incorporates pyranochromene moieties was isolated from the methanolic extracts of the bark and leaf of *Mundulea chapelieri* and exhibited activity against a human ovarian cancer cell line [8]. Shortly afterward its synthesis was reported [3]. The synthesis of six chalcones with an octandrenolone moiety was also reported [10]. Several other pyranochromene chalcones and flavonoids were isolated from *Mundela suberosa* and *Mundela sericea* and have exhibited interesting biological activities including antimicrobial and ornithine decarboxylase activity [8,11-17]. A pyranochromene chalcone, (-)-rubranine with prenyl groups on the pyran rings was also isolated from *Aniba rosaedora* [18]. Two series of prenylated chromenochalcones were also synthesized and tested for their antileishmanial and antimalarial activity where several compounds showed good activity [19-20].

In our ongoing study on synthesizing fluorinated pharmaceuticals, we have prepared several fluorinated chalcones and flavanones with the pyranochromene moiety.

Herein we report the NMR elucidation of these fluorinated pyranochromene chalcones and flavanones, which is slightly more complicated than the oxygenated or chlorinated molecules due to the fluorine atom being NMR active and coupling with both hydrogen and carbon. We used X-ray crystallography and NMR studies to provide a full structural elucidation of these novel pyranochromene chalcones and flavanones.

Experimental

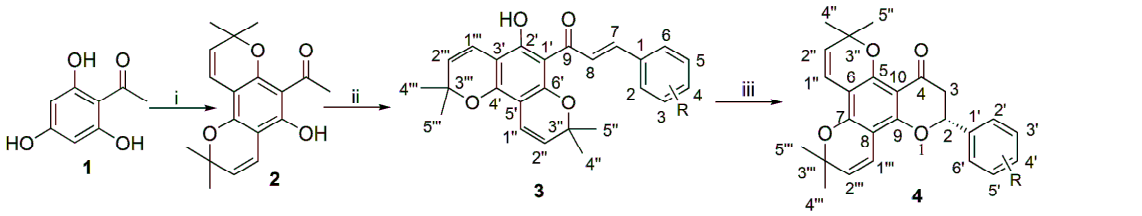
Reagents and chemicals used in this study were purchased from Sigma Aldrich via Capital Lab, South Africa and were reagent grade. All organic solvents were redistilled and dried according to standard procedures. Optical rotations were recorded using a PerkinElmer^{TM}, Model 341 Polarimeter with a 10 cm flow tube. Melting points were measured using a Stuart Scientific Melting Point Apparatus SMP3. IR spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrometer with universal ATR sampling accessory. High-resolution mass data was obtained using a Bruker microTOF-Q II ESI instrument operating at ambient temperatures, with a sample concentration of approximately 1 ppm. Thin layer chromatography (TLC) was performed using Merck Kieselgel 60 F_{254} plates. Crude compounds were purified with column chromatography using silica gel (60–120 mesh) as the stationary phase and varying combinations of solvents depending on the sample to be purified.

The ^{1}H and ^{13}C NMR spectra were recorded at 298 K with 5-10 mg samples dissolved in 0.5 mL of $CDCl_3$ in 5 mm NMR tubes using a Bruker Avance^{III} 400 MHz NMR spectrometer

(9.4 T; Bruker, Germany) (400.22 MHz for ^{1}H, 100.63 MHz for ^{13}C and 376.58 Hz for ^{19}F. The digital digitizer resolution was set at 22 for both the ^{1}H and ^{13}C NMR experiments. Chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz. The ^{1}H and ^{13}C chemical shifts of the deuterated solvent were 7.24 and 77.0 respectively, referenced to the internal standard, TMS. For the ^{19}F NMR spectra, the chemical shift of trifluorotoluene (TFT) (0.05% in $CDCl_3$) was referenced at -62.73. For the ^{1}H NMR analyses, 16 transients were acquired with a 1s relaxation delay using 32K data points. The 90° pulse duration was 10.0 μs, and the spectral width was 8223.68 Hz. The ^{13}C NMR spectra were obtained with a spectral width of 24038.46 Hz using 64K data points. The 90° pulse duration was of 8.4 μs. For the ^{19}F NMR spectra, the spectral width was 89285.71 Hz using 131K data points and the 90° pulse duration was 12.5 μs. For the two dimensional experiments including COSY, NOESY, HSQC and HMBC, all data were acquired with 4K × 128 data points ($t_2 \times t_1$). The mixing time for the NOESY experiment was 0.3 s, and the long range coupling time for the HMBC experiment was 65 ms. All data were analysed using Bruker Topspin 2.1 (2008) software.

Synthesis

The prenylated flavanones (**4a-h**) were prepared in rather moderate overall yields (30-55%) in a three step reaction (**Scheme 2-1**) where 2',4',6'-trihydroxyacetophenone was prenylated at the 2' and 4' positions with 3-methyl-2-butenal in basic conditions under reflux resulting in the dichromene acetophenone intermediate **2** [21], which then underwent a Claisen-Schmidt condensation reaction with various substituted benzaldehydes to produce chalcones **3a-h** in good yields (60-90%) before being cyclized to their corresponding flavanones **4a-h**.



Scheme 2-1 Synthetic scheme for the synthesis of pyranochromene flavanones **4a-h**. i) 3-methyl-2-butenal, pyridine, 110°C, reflux; ii) substituted benzaldehydes, KOH, EtOH/H_2O, rt; iii) NaOAc, EtOH, 80°C, reflux.

For the synthesis of octandrenolone (1-(5-Hydroxy-2,2,8,8-tetramethyl-2*H*,8*H*-pyrano[2,3-*f*]chromen-6-yl)ethanone) (**2**), the method in Pawar et al. [21] was followed and the NMR data compared with that of an authentic sample prepared earlier [21].

The pyranochromene chalcones **3a – 3h** were synthesized according to the methods reported earlier in Pawar et al. [22] and the compounds purified by flash column chromatography to produce pure brown solids. For the ^{1}H and ^{13}C NMR data, see **Table 2-1** and **Table 2-2**.

1-(5-Hydroxy-2,2,8,8-tetramethyl-2H,8H-pyrano[2,3-f]chromen-6-yl)-3-phenylpropenone (**3a**). Yield 90%; mp 99 - 100 ^{o}C. IR (neat) υ_{max} = 2970, 2928, 1631, 1582, 1546, 1132 cm^{-1}.

3-(2-Fluorophenyl)-1-(5-hydroxy-2,2,8,8-tetramethyl-2H,8H-pyrano[2,3-f]chromen-6-yl)-propenone (**3b**). Yield 64%; mp 110 - 111 ^{o}C. ^{19}F NMR (376 MHz, $CDCl_3$) δ -112.95; IR (neat) υ_{max} = 2963, 2923, 1627, 1583, 1539, 1339, 1181, 1137, 1109 cm^{-1}; HRMS *m/z* 429.1476 $[M+Na]^+$ (calcd. for $C_{25}H_{23}O_4FNa$ 429.1478).

3-(3-Fluorophenyl)-1-(5-hydroxy-2,2,8,8-tetramethyl-2H,8H-pyrano[2,3-f]chromen-6-yl)-propenone (**3c**). Yield 90%; mp 150 - 151 ^{o}C. ^{19}F NMR (376 MHz, $CDCl_3$) δ -112.63; IR (neat) υ_{max} = 2971, 2928, 1630, 1584, 1451, 1135, 1112 cm^{-1}; HRMS *m/z* 429.1472 $[M+Na]^+$ (calcd. for $C_{25}H_{23}O_4FNa$ 429.1478).

3-(4-Fluorophenyl)-1-(5-hydroxy-2,2,8,8-tetramethyl-2H,8H-pyrano[2,3-f]chromen-6-yl)-propenone (**3d**). Yield 90%; mp 143 - 144 ^{o}C. ^{19}F NMR (376 MHz, $CDCl_3$) δ -109.92; IR (neat) υ_{max} = 2971, 2928, 1631, 1585, 1468, 1135, 1113 cm^{-1}; HRMS *m/z* 429.1477 $[M+Na]^+$ (calcd. for $C_{25}H_{23}O_4FNa$ 429.1478).

3-(2,4-Difluorophenyl)-1-(5-hydroxy-2,2,8,8-tetramethyl-2H,8H-pyrano[2,3-f] chromen-6-yl)-propenone (**3e**). Yield 74%; mp 154 - 155 ^{o}C. ^{19}F NMR (376 MHz, $CDCl_3$) δ -106.57, -108.54; IR (neat) υ_{max} = 2977, 2931, 1630, 1612, 1582, 1136 cm^{-1}; HRMS *m/z* 447.1393 $[M+Na]^+$ (calcd. for $C_{25}H_{22}O_4F_2Na$ 447.1384).

1-(5-Hydroxy-2,2,8,8-tetramethyl-2H,8H-pyrano[2,3-f]chromen-6-yl)-3-(3-methoxyphenyl)-propenone (**3f**). Yield 77%; mp 95 - 96 ^{o}C. IR (neat) υ_{max} = 2975, 2924, 1640, 1625, 1586, 1534, 1465, 1153, 1131, 1112 cm^{-1}; HRMS *m/z* 441.1671 $[M+Na]^+$ (calcd. for $C_{26}H_{26}O_5Na$ 441.1678).

1-(5-Hydroxy-2,2,8,8-tetramethyl-2H,8H-pyrano[2,3-f]chromen-6-yl)-3-(4-methoxyphenyl)-propenone **(3g)**. Yield 85%; mp 125 - 126 ^{o}C. IR (neat) υ_{max} = 2977, 2968, 1602, 1578, 1532, 1509, 1145, 1131, 1110 cm^{-1}.

3-(2-Fluoro-3-methoxyphenyl)-1-(5-hydroxy-2,2,8,8-tetramethyl-2H,8H-pyrano[2,3-f]chromen-6-yl)-propenone **(3h)**. Yield 65%; mp 122 - 123 ^{o}C. ^{19}F NMR (376 MHz, $CDCl_3$) δ -136.22; IR (neat) υ_{max} = 2968, 2926, 1632, 1605, 1581, 1276, 1151, 1132, 1113 cm^{-1}; HRMS *m/z* 459.1579 $[M+Na]^+$ (calcd. for $C_{26}H_{25}O_5FNa$ 459.1584).

Table 2-1 ^{1}HNMR shifts of the pyranochromene acetophenone intermediate **2** and pyranochromene chalcones **3a** – **3h**

δ of ^{1}H (*J*, Hz)

No.	2*	3a*	3b	3c	3d	3e	3f	3g	3h
OH	14.00 (s)	14.37 (s)	14.36 (s)	14.24 (s)	14.31 (s)	14.32 (s)	14.34 (s)	14.49 (s)	14.33 (s)
OCH_3	-	-	-	-	-	-	3.84 (s)	3.85 (s)	3.91 (s)
CH_3	2.65 (s)	-	-	-	-	-	-	-	-
2	-	7.60 (s)	-	7.29 - 7.26 (m)	7.57 (dd, 8.6, 5.5)	-	7.13 (d, 1.7)	7.55 (d, 8.3)	-
3	-	7.40 (s)	7.12 (dd, 11.0, 8.8)	-	7.10 (t, 8.6)	6.95 – 6.85 (m)	-	6.93 (d, 8.3)	-
4	-	7.40 (s)	7.36 - 7.32 (m)	7.10 - 7.05 (m)	-	-	6.94 (dd, 8.08, 2.08)	-	6.98 (dt, 8.0, 1.2)
5	-	7.40 (s)	7.18 (t, 7.6)	7.38 - 7.34 (m)	7.10 (t, 8.6)	6.95 - 6.85 (m)	7.32 (t, 7.9)	6.93 (d, 8.3)	7.09 (t, 8.2)
6	-	7.60 (s)	7.57 (td,7.6, 1.4)	7.38 - 7.34 (m)	7.57 (dd, 8.6, 5.5)	7.54 (ddd, 8.5, 8.5, 6.4)	7.19 (d, 7.6)	7.55 (d, 8.3)	7.14 (td, 7.3, 1.3)
7	-	7.76 (d, 15.7)	7.83 (d, 15.9)	7.68 (d, 15.7)	7.71 (d, 15.6)	7.76 (d, 15.9)	7.73 (d, 15.6)	7.76 (d, 15.6)	7.84 (d, 15.8)
8	-	8.10 (d, 15.7)	8.23 (d, 15.9)	8.06 (d, 15.7)	8.01 (d, 15.6)	8.17 (d, 15.9)	8.08 (d, 15.6)	8.00 (d, 15.6)	8.19 (d, 15.8)
1''	6.58 (d, 10.1)	6.61 (bs)	6.60 (d, 10.0)	6.61 (d, 10.0)	6.61 (d, 10.0)	6.60 (d, 9.9)	6.61 (d, 10.0)	6.61 (d, 9.9)	6.60 (d, 9.9)
2''$^{\#}$	5.45 (d, 10.1)	5.48 (bs)	5.48 (d, 10.0)	5.49 (d, 10.0)	5.48 (d, 10.0)	5.48 (d, 9.9)	5.48 (d, 10.0)	5.47 (d, 9.9)	5.46 (d, 9.9)
4''/5''†	1.49 (s)	1.55 (s)	1.54 (s)	1.54 (s)	1.54 (s)	1.53 (s)	1.55 (s)	1.55 (s)	1.54 (s)
1'''	6.65 (d, 10.1)	6.69 (bs)	6.69 (d, 9.9)	6.68 (d, 10.0)	6.68 (d, 10.0)	6.68 (d, 9.9)	6.69 (d, 10.0)	6.69 (d, 9.9)	6.68 (d, 9.9)
2'''$^{\#}$	5.43 (d, 10.1)	5.48 (bs)	5.47 (d, 9.9)	5.47 (d, 10.0)	5.47 (d, 10.0)	5.46 (d, 9.9)	5.47 (d, 10.0)	5.47 (d, 9.9)	5.47 (d, 9.9)
4'''/5'''†	1.43 (s)	1.45 (s)	1.45 (s)	1.46 (s)	1.45 (s)	1.45 (s)	1.45 (s)	1.45 (s)	1.45 (s)

* NMR at 600 MHz; $^{\#}$ and † indicate resonances that can be interchanged.

Table 2-2 ^{13}C NMR shifts of pyranochromene chalcones **2** and **3a** – **3h**

δ of ^{13}C (*J*, Hz)

No.	2*	3a	3b	3c	3d*	3e	3f	3g	3h
1	-	135.8	123.8 (d, 11.4)	138.2 (d, 7.6)	132.1 (d, 3.2)	120.3 (dd, 11.6, 3.8)	137.2	128.6	124.5 (d, 9.2)
2	-	129.1	161.9 (d, 252.6)	114.3 (d, 21.6)	130.1 (d, 8.6)	162.1 (dd, 243.4, 12.1)	113.1	130.1	151.8 (d, 252.7)
3	-	128.4	116.5 (d, 21.9)	163.3 (d, 245.0)	116.3 (d, 21.8)	104.6 (t, 25.6)	160.1	114.5	148.5 (d, 10.6)
4	-	130.2	131.4 (d, 8.8)	117.0 (d, 20.4)	163.9 (d, 249.6)	163.8 (dd, 239.5, 12.3)	116.8	161.7	114.6 (d, 2.1)
5	-	128.4	124.7 (d, 3.6)	130.6 (d, 8.3)	116.3 (d, 21.8)	112.2 (dd, 21.6,3.6)	130.1	114.5	124.2 (d, 4.8)
6	-	129.1	130.5 (d, 8.3)	124.5 (d, 2.8)	130.1 (d, 8.6)	131.5 (dd, 9.9, 5.0)	121.2	130.1	121.0 (d, 2.1)
7	-	142.3	135.4	140.5 (d, 2.5)	140.9	134.4	142.2	142.4.	135.0 (d, 2.4)
8	-	127.8	130.5	129.1	127.6 (d, 1.7)	130.0 (dd, 8.6, 2.4)	128.0	124.9	130.6 (d, 7.7)
9	203.5	193.1	193.1	192.7	192.9	192.9	193.0	193.0	193.0
1'	105.7	106.1	106.1	106.1	106.1	106.1	106.1	106.1	106.1
2'	160.7	161.6	161.7	161.6	161.6	161.7	161.6	161.5	161.7
3'	102.5	102.8	102.8	102.8	102.8	102.8	102.8	102.8	102.8
4'	155.2	155.5	155.6	155.7	155.5	155.7	155.5	155.3	155.6
5'	102.4	102.7	102.7	102.7	102.7	102.7	102.7	102.7	102.7
6'	156.9	156.4	156.5	156.4	156.3	156.4	156.4	156.3	156.5
1''	116.6	116.8	116.6	116.7	116.8	116.6	116.4	116.8	116.6
2''[#]	125.5	125.6	125.2	125.6	125.6	125.6	125.6	125.5	125.5
3''[ǂ]	78.4	78.6	78.7	78.6	78.5	78.6	78.5	78.4	78.7
4''/5''[†]	28.5	28.6	28.6	28.6	28.6	28.6	28.6	28.6	28.6
1'''	116.3	116.4	116.4	116.3	116.4	116.4	116.3	116.5	116.4
2'''[#]	124.9	124.9	125.5	125.0	124.9	125.1	124.9	125.4	125.1
3'''[ǂ]	78.3	78.5	78.6	78.5	78.4	78.5	78.4	78.3	78.5
4'''/5'''[†]	28.2	28.3	28.0	28.3	28.3	28.1	28.3	28.3	28.1
O<u>CH$_3$/CH$_3$</u>	33.4	-	-	-	-	-	55.4	55.6	55.6

* NMR at 600 MHz; [#], [ǂ] and [†] indicate that assignments may be interchangeable.

General procedure for the synthesis of pyranochromene flavanones 4a – h

To a solution of sodium acetate (188 mg, 3.0 mmol) in ethanol (10 mL), pyranochromene chalcones **3a-h** (0.3 mmol) was added at room temperature. The reaction mixture was refluxed for 48 h at 80°C. The solvent was distilled off under reduced pressure and the

residue was dissolved in water (20 mL). After acidifying with 2 M HCl (20 mL), the mixture was extracted with ethyl acetate (3 × 30 mL), washed with water and dried over anhydrous $MgSO_4$. Removal of the solvent followed by flash column chromatography on silica gel afforded the pure pyranochromene flavanones as yellow solids. For the ^1H and ^{13}C NMR data see **Table 2-3** and **Table 2-4**.

6,6,10,10-Tetramethyl-2-phenyl-2,3-dihydro-6H,10H-dipyrano[2,3-f;2',3'-h]chromen-4-one **(4a)**. Yield 55%; mp 144 – 145 ^oC. IR (neat) υ_{max} = 2923, 2853, 1729, 1677, 1637, 1571, 1434, 1363, 1136, 1116 cm^{-1}.

2-(2-Fluorophenyl)-6,6,10,10-tetramethyl-2,3-dihydro-6H,10H-dipyrano[2,3-f;2',3'-h]chromen-4-one (**4b**) Yield 38%; mp 148 – 150 ^oC. ^{19}F NMR (376 MHz, $CDCl_3$) δ -118.38; IR (neat) υ_{max} = 2966, 2922, 1677, 1637, 1571, 1433, 1364, 1135, 1117 cm^{-1}; HRMS *m/z* 429.1476 $[M+Na]^+$ (calcd. for $C_{25}H_{23}O_4FNa$ 429.1478).

2-(3-Fluorophenyl)-6,6,10,10-tetramethyl-2,3-dihydro-6H,10H-dipyrano[2,3-f;2',3'-h]chromen-4-one (**4c**) Yield 46%; mp 92 – 93 ^oC. ^{19}F NMR (376 MHz, $CDCl_3$) δ -112.06; IR (neat) υ_{max} = 2968, 2923, 1678, 1643, 1571, 1162, 1136, 1118 cm^{-1}; HRMS *m/z* 429.1471 $[M+Na]^+$ (calcd. for $C_{25}H_{23}O_4FNa$ 429.1478).

2-(4-Fluorophenyl)-6,6,10,10-tetramethyl-2,3-dihydro-6H,10H-dipyrano[2,3-f;2',3'-h]chromen-4-one (**4d**) Yield 36%; mp 112 – 113 ^oC. ^{19}F NMR (376 MHz, $CDCl_3$) δ -113.29; IR (neat) υ_{max} = 2968, 2924, 2854, 1681, 1638, 1572, 1512, 1462, 1436, 1132, 1116 cm^{-1}; HRMS *m/z* 429.1475 $[M+Na]^+$ (calcd. for $C_{25}H_{23}O_4FNa$ 429.1478).

2-(2,4-Difluorophenyl)-6,6,10,10-tetramethyl-2,3-dihydro-6H,10H-dipyrano[2,3-f;2',3'-h]chromen-4-one (**4e**). Yield 31%; mp 158 – 159 ^{o}C. ^{19}F NMR (376 MHz, $CDCl_3$) δ -109.62, -114.22; IR (neat) υ_{max} = 2978, 2925, 1679, 1641, 1590, 1570, 1132, 1113 cm^{-1}; HRMS *m/z* 447.1384 $[M+Na]^+$ (calcd. for $C_{25}H_{22}O_4F_2Na$ 447.1384).

2-(3-Methoxyphenyl)-6,6,10,10-tetramethyl-2,3-dihydro-6H,10H-dipyrano[2,3-f;2',3'-h]chromen-4-one (**4f**) Yield 40%; mp 80 – 81 ^{o}C. IR (neat) υ_{max} = 2969, 2923, 2853, 1678, 1638, 1570, 1431, 1361, 1134, 1115 cm^{-1}; HRMS *m/z* 441.1672 $[M+Na]^+$ (calcd. for $C_{26}H_{26}O_5Na$ 441.1678).

2-(4-Methoxyphenyl)-6,6,10,10-tetramethyl-2,3-dihydro-6H,10H-dipyrano[2,3-f;2',3'-h]chromen-4-one (**4g**). Yield 46%; mp 141 – 142 ^{o} C. IR (neat) υ_{max} = 2975, 2923, 2853, 1684, 1634, 1613. 1592, 1571, 1245, 1136, 1115 cm^{-1}; HRMS *m/z* 441.1674 $[M+Na]^+$ (calcd. for $C_{26}H_{26}O_5Na$ 441.1678).

2-(2-Fluoro-3-methoxyphenyl)-6,6,10,10-tetramethyl-2,3-dihydro-6H,10H-dipyrano[2,3-f;2',3'-h]chromen-4-one (**4h**). Yield 54%; mp 100 – 101^{o} C. ^{19}F NMR (376 MHz, $CDCl_3$) δ -140.91; IR (neat) υ_{max} = 2969, 2926, 1681, 1634, 1590, 1572, 1135, 1115, 1088 cm^{-1}; HRMS *m/z* 459.1576 $[M+Na]^+$ (calcd. for $C_{26}H_{25}O_5FNa$ 459.1584).

Table 2-3 ^{1}H NMR shifts of pyranochromene flavanones **4a** – **h**

δ of ^{1}H (J, Hz)

No.	4a	4b	4c	4d	4e	4f*	4g	4h
2	5.39 (dd, 13.1, 2.8)	5.69 (dd, 12.9, 3.1)	5.39 (dd, 12.8, 3.1)	5.37 (dd, 13.0, 2.9)	5.63 (dd, 12.9, 3.0)	5.36 (dd, 13.3, 2.9)	5.33 (dd, 13.1, 2.8)	5.70 (dd, 12.9, 2.9)
3a	2.77 (dd, 16.5, 2.8)	2.81 (dd, 16.6, 3.1)	2.77 (dd, 16.5, 3.1)	2.75 (dd, 16.5, 2.9)	2.78 (dd, 16.5, 3.0)	2.77 (dd, 16.5, 2.9)	2.73 (dd, 16.5, 2.8)	2.80 (dd, 16.6, 2.9)
3b	2.96 (dd, 16.5, 13.1)	2.93 (dd, 16.6, 12.9)	2.93 (dd, 16.5, 12.8)	2.93 (dd, 16.5, 13.0)	2.91 (dd, 16.5, 12.9)	2.94 (dd, 16.5, 13.3)	2.97 (dd, 16.5, 13.1)	2.93 (dd, 16.6, 12.9)
2'	7.42 - 7.36 (m)	-	7.19 (d, 7.5)	7.42 (dd, 8.5, 5.4)	-	7.01 (s)	7.37 (d, 8.6)	-
3'	7.42 - 7.36 (m)	7.11 – 7.06 (m)	-	7.09 (t, 8.6)	6.85 (dt, 9.6, 2.5)	-	6.93 (d, 8.7)	-
4'	7.42 - 7.36 (m)	7.37 – 7.31 (m)	7.07 - 7.02 (m)	-	-	6.90 – 6.89 (m)	-	7.15 - 7.11 (m)
5'	7.42 - 7.36 (m)	7.22 (t, 7.3)	7.37 (dd, 13.9, 7.8)	7.09 (t, 8.6)	6.95 (dt, 10.2, 1.6)	7.32 (t, 8.2)	6.93 (d, 8.7)	6.99 - 6.95 (m)
6'	7.42 - 7.36 (m)	7.60 (td, 8.6, 1.5)	7.19 (d, 7.5)	7.42 (dd, 8.5, 5.4)	7.57 (ddd, 8.4, 8.4, 6.4)	7.02 (d, 7.3)	7.37 (d, 8.6)	7.15 - 7.11 (m)
1''[#]	6.60 (d, 10.0)	6.58 (d, 10.0)	6.59 (d, 10.0)	6.57 (d, 10.0)	6.56 (d, 9.9)	6.60 (d, 10.0)	6.57 (d, 10.0)	6.57 (d, 10.0)
2''	5.46 (d, 10.0)	5.46 (d, 10.0)	5.48 (d, 10.0)	5.46 (d, 10.0)	5.47 (d, 9.9)	5.46 (d, 10.0)	5.44 (d, 10.0)	5.45 (d, 10.0)
4''[†]	1.53 (s)	1.52 (s)	1.52 (s)	1.51 (s)	1.52 (s)	1.52 (s)	1.52 (s)	1.51 (s)
5''[†]	1.49 (s)	1.49 (s)	1.48 (s)	1.48 (s)	1.49 (s)	1.49 (s)	1.49 (s)	1.49 (s)
1'''[#]	6.57 (d, 10.0)	6.57 (d, 9.9)	6.57 (d, 9.9)	6.57 (d, 10.0)	6.55 (d, 10.0)	6.57 (d, 9.9)	6.56 (d, 10.0)	6.56 (d, 10.0)
2'''	5.50 (d, 10.0)	5.51 (d, 9.9)	5.51 (d, 9.9)	5.49 (d, 10.0)	5.51 (d, 10.0)	5.50 (d, 9.9)	5.49 (d, 10.0)	5.50 (d, 10.0)
4'''[†]	1.46 (s)	1.46 (s)	1.46 (s)	1.45 (s)	1.46 (s)	1.46 (s)	1.45 (s)	1.45 (s)
5'''[†]	1.44 (s)	1.44 (s)	1.44 (s)	1.43 (s)	1.44 (s)	1.44 (s)	1.43 (s)	1.43 (s)
OCH_3	-	-	-	-	-	3.83 (s)	3.82 (s)	3.90 (s)

* NMR frequency at 600 MHz; [#] and [†] indicate resonances that can be interchanged.

Table 2-4 ^{13}C NMR shifts of pyranochromene flavanones **4a- h**

δ of ^{1}H (J, Hz)

No.	4a	4b	4c	4d	4e	4f*	4g	4h
2	79.1	73.5 (d, 3.1)	78.3 (d, 1.3)	78.5	73.1 (d, 2.6)	79.0	78.9	73.4 (d, 4.28)
3	46.1	45.0	46.0	46.0	44.9	46.2	45.9	44.9
4	188.9	188.5	188.3	188.6	188.3	188.9	189.1	188.6
5	154.5	154.5	154.5	154.4	155.4	154.5	154.4	154.5
6	102.5	102.6	102.5	102.5	102.5	102.6	102.5	102.6
7	156.1	156.2	156.1	156.1	156.2	156.1	156.1	156.2
8	104.8	104.9	104.9	104.8	105.0	104.9	104.7	104.9
9	157.6	157.5	157.2	157.4	157.3	157.6	157.7	157.5
10	105.7	105.7	105.6	105.7	105.6	105.8	105.7	105.7
1'	139.3	126.7 (d, 12.9)	141.8 (d, 7.2)	135.1 (d, 3.3)	122.7 (dd, 13.1, 3.8)	140.9	131.3	127.5 (d, 10.2)
2'	126.1	159.8 (d, 246.2)	113.1 (d, 22.5)	127.9 (d, 8.2)	163.0 (dd, 236.3, 12.0)	118.4	127.7	149.6 (d, 246.4)
3'	128.9	115.8 (d, 21.1)	163.1 (d, 245.0)	115.8 (d, 21.6)	104.3 (t, 25.3)	160.1	114.2	147.9 (d, 10.2)
4'	128.6	130.1 (d, 8.2)	115.5 (d, 21.0)	162.8 (d, 245.7)	160.0 (dd, 237.0, 12.0)	113.9	159.9	124.5 (d, 4.9)
5'	128.8	124.6 (d, 3.6)	130.5 (d, 8.1)	115.8 (d, 21.6)	111.8 (dd, 21.2, 3.6)	130.0	114.2	113.4 (d, 1.6)
6'	126.1	127.4 (d, 3.7)	121.6 (d, 2.9)	127.9 (d, 8.2)	128.5 (dd, 9.7, 5.3)	111.9	127.7	118.3 (d, 2.6)
1''$^{\#}$	116.4	116.3	116.3	116.3	116.2	116.5	116.5	116.4
2''	126.4	126.9	126.9	126.5	126.6	126.5	126.3	126.5
3''	78.1	78.1	78.1	78.1	78.1	78.1	78.0	78.1
4''†	28.6	28.6	28.6	28.6	28.7	28.7	28.6	28.7
5''†	28.4	28.4	28.4	28.4	28.4	28.4	28.4	28.4
1'''$^{\#}$	116.0	116.0	116.0	116.0	115.9	116.0	116.0	116.0
2'''	126.8	126.5	126.6	126.8	126.9	126.8	126.7	126.9
3'''	77.9	78.0	78.0	77.9	78.0	77.9	77.9	77.9
4'''†	28.3	28.3	28.3	28.3	28.3	28.3	28.3	28.3
5'''†	28.1	28.1	28.0	28.1	28.1	28.1	28.0	28.1
OCH_3	-	-	-	-	-	55.4	55.5	56.5

* NMR frequency at 600 MHz; $^{\#}$ and † indicate resonances that can be interchanged.

Single crystal X-ray diffraction analysis

A cube-shaped single crystal was selected and glued onto the tip of a glass fibre and mounted in a stream of cold nitrogen at 100(1) K and centred in the X-ray beam using a video camera. The crystal evaluation and data collection were performed on a Bruker Smart APEXII

diffractometer with Mo Kα radiation (λ = 0.71073 Å). The diffractometer to crystal distance was set at 4.00 cm. The initial cell matrix was obtained from three series of scans at different starting angles. Each series consisted of 12 frames collected at intervals of 0.5º in a 6º range with the exposure time of 10 seconds per frame. The reflections were successfully indexed by an automated indexing routine built in the APEXII program suite. The final cell constants were calculated from a set of 6460 strong reflections from the actual data collection. Data collection method involved ω scans of width 0.5°. Data reduction was carried out using the program *SAINT+*. The structure was solved by direct methods using SHELXS and refined. Non-H atoms were first refined isotropically and then by anisotropic refinement with full-matrix least-squares calculations based on F^2 using SHELXS. All H atoms were positioned geometrically and allowed to ride on their respective parent atoms. All H atoms were refined isotropically. The absorption correction was based on fitting a function to the empirical transmission surface as sampled by multiple equivalent measurements. The final least-squares refinement of 286 parameters against 5126 data resulted in residuals *R* (based on *F*2 for $I \geq 2\sigma$) and *wR* (based on F^2 for all data) of 0.0404 and 0.1035, respectively. The final difference Fourier map was featureless. The programs Olex-2 and Ortep-3 were used within the WinGX software package to prepare artwork representation. The molecular diagram is drawn with 50% probability ellipsoids.

Results and Discussion

The full characterisation (^{1}H and ^{13}C NMR assignments) of the pyranochromene chalcones **3a-h** and flavanones **4a-h** are presented in **Table 2-1** to **Table 2-4** and the ^{1}H spectra of a representative fluorinated chalcone and fluorinated flavanone is presented in **Figure 2-1** and **Figure 2-2**. The spectra of these compounds were well resolved. With the aid of HSQC, HMBC, COSY and NOESY data as well as multiplicity patterns, the unambiguous chemical shift assignments were made.

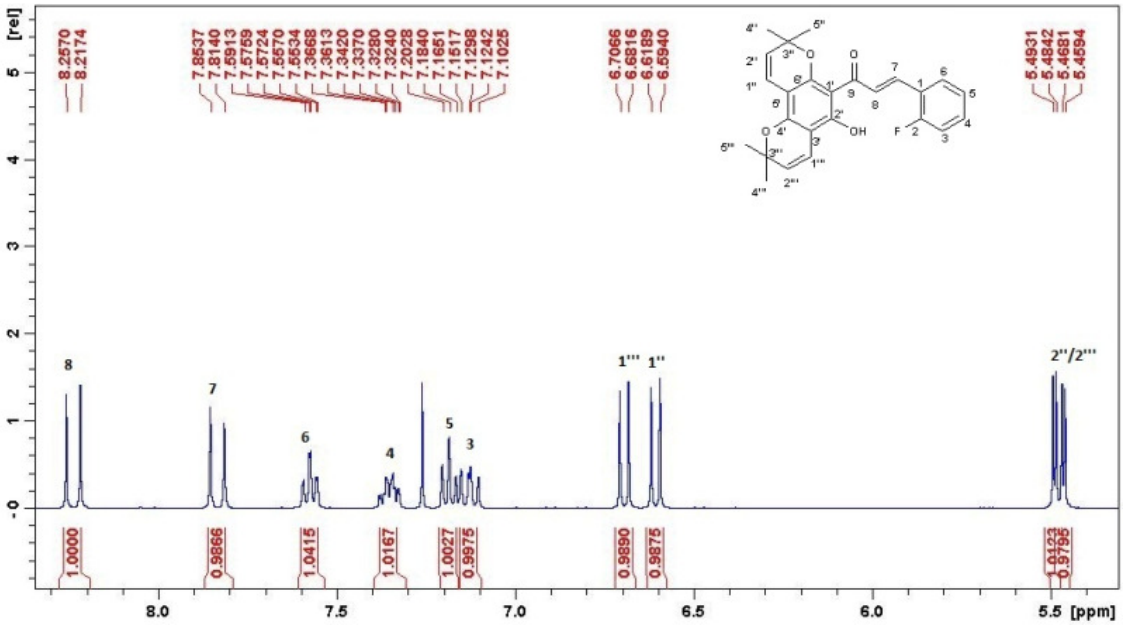


Figure 2-1 ^{1}H NMR spectrum of 2'-fluoropyranochromene chalcone (**3b**)

For the pyranochromene chalcones, typical ^{1}H and ^{13}C NMR resonances can be observed for the pyran rings and the α,β-unsaturated ketone moiety. Taking the 2-fluoro derivative (**3b**) as an example, the double bond of the α,β-unsaturated ketone moiety occurs as a distinct pair of doublets at δ 8.23 (H-7) and 7.83 (H-8) with a coupling constant of 15.9 Hz, typical of *trans* olefinic protons (**Figure 2-1**). The carbonyl resonance (C-9) is distinctly separated from the other carbon resonances at δ 193.1 and shows HMBC correlations to both H-7 and H-8 (**Figure 2-3**), confirming the assignments of the α,β-unsaturated ketone moiety. Due to conjugation between the double bond and the carbonyl group, C-8 (δ_C 130.5) is more shielded than C-7 (δ_C 135.4) and is therefore assigned further upfield than C-7. This is however not the case for H-7 and H-8 and HSQC correlations show that H-8 appears more deshielded than H-7. A ^{3}J correlation in the HMBC spectrum can also be observed between H-7 and C-2 and C-6, verifying the assignment of H-7. The H-8 resonance is more

deshielded due to the through space deshielding effect of the oxygen on the nearby pyran ring.

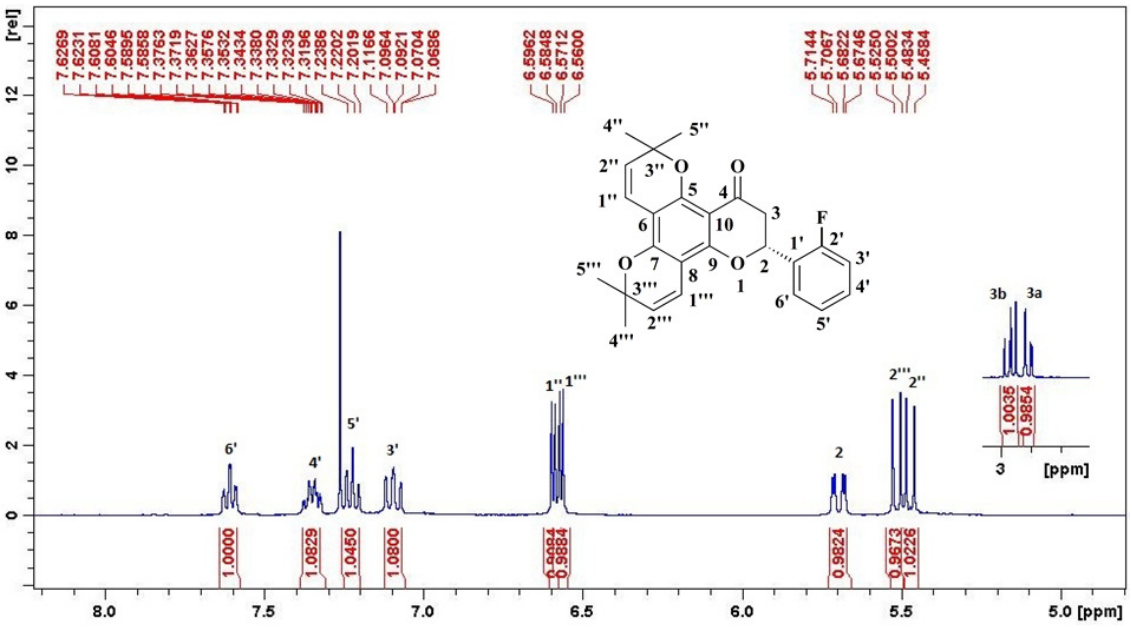
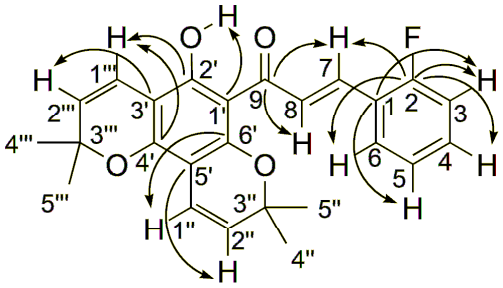


Figure 2-2 ^{1}H NMR spectrum of 2'-fluoropyranochromene flavanone (**4b**)



3b

Figure 2-3 HMBC correlations of compound **3b**, the 2-fluoropyranochromene chalcone

There are four resonances between 7.10 and 7.60. These are attributed to the aromatic proton resonances of the fluorinated aromatic ring B. C-7 shows a HMBC correlation to the doublet of triplets (dt) at δ_H 7.57 (H-6). This doublet of triplets arises from the coalescing of the

doublet of doublet of doublets (ddd) brought about by coupling with the fluorine atom and H-5 ($J_{H6,F} = J_{H6,H5} = 7.6$ Hz) and the meta coupling with H-4 ($J_{H4,H6} = 1.4$ Hz). The H-6 proton resonance shows a COSY correlation to another triplet also arising because of coalescing of the doublet doublet that arises by coupling with both H-6 and H-4 with $J_{H6,H5} = J_{H5,H4} = 7.5$ Hz. No *meta* coupling between H-5 and H-3 is observed. The other two aromatic resonances at δ_H 7.32-7.36 (m) and δ_H 7.12 (dd, J = 11.0, 8.8 Hz) were assigned to H-4 and H-3 respectively and could be distinguished by HMBC correlations between C-1 (d, J = 11.4 Hz) and H-3. C-1 also showed HMBC correlations to H-5. $J_{H3,F}$ (11.0 Hz) was larger than $J_{H3,H4}$ (8.8 Hz). The C-3 carbon resonance was identified by an HSQC correlation with H-3 and was seen overlapping with C-1''' and C-1'' as a doublet at δ_C 116.5 (J = 21.9 Hz). C-4, C-5 and C-6 were all identified similarly at δ_C 131.4 (d, J = 8.8 Hz, *meta* C-F coupling), 124.7 (d, J = 3.6 Hz, *para* C-F coupling) and 130.5 (d, J = 8.3 Hz, *meta* C-F coupling) respectively. The fluorine bonded C-2 showed HMBC correlations with H-7, H-6, H-4 and H-3 and was identified as a large doublet at δ_C 162.0 (J = 252.5 Hz).

For the two pyran rings on the A ring (closer to the ketone), the H-2''/2''' resonances overlap as a pair of doublets, with four peaks for the two doublet resonances, the first and the third peak being assigned to H-2'' and the second and fourth peak being assigned to H-2'''. These doublets occur at δ_H 5.48 and δ_H 5.47 and have coupling constants of 10.0 Hz, characteristic of *cis* olefinic protons and can be interchanged. The H-1'' and H-1''' proton resonances, although occurring close to each other do not overlap and can be distinguished as two separate resonances at δ_H 6.60 and δ_H 6.69 (J = 10.0 Hz). The H-1'' and H-2'', and H-1''' and H-2''' resonances show clear coupling in the COSY spectrum. Both H-2''' and H-2'' show HMBC correlations to C-3' and C-5' respectively at δ_C 102.80 and δ_C 102.76. However, both these sets of resonances can be interchanged as they occur so close to each other that it is

difficult to tell them apart. The H-1''' resonance was distinguished from the H-1'' resonance by a HMBC correlation between H-1''' and C-2' at δ_C 161.8. The identification of H-1''' and H-1'' allowed the unambiguous assignments of C-4' (through an HMBC with H-1''') and C-6' (HMBC with H-1''). The C-1' resonance at δ_C 106.17 was detected by a HMBC correlation to the 2'-hydroxy resonance at δ_H 14.36.

The corresponding carbon resonances of H-1''' and H-1'' was found at δ_C 116.4 (C-1''') and δ_C 116.7 (C-1'') in the HSQC spectrum. There are two methyl proton resonances for the four methyl groups since the methyl groups on each of the pyran rings are equivalent due to the planar nature of the molecule. Thus, H-4''/5'' occurs at δ 1.54 and H-4'''/5''' occurs at δ 1.46. These methyl resonances show HMBC correlations to C-2''/C-2''' and C-3'' and C-3''', the latter two carbon resonances occuring at δ_C 78.7 and 78.6, which can be interchanged. The C-4''/5'' and C-4'''/5''' resonances occur at δ_C 28.6 and δ_C 28.1. The HMBC correlations of **3b** are reflected in **Figure 2-3**.

A crystal structure of **3b** (**Figure 2-4**) was solved in the monoclinic space group P21/c, with one molecule in the asymmetric unit. The bond length and angles are in agreement with the expected average values for related compounds [21, 22]. The *trans* double bond is clearly evident for C-7 and C-8 and the 2'-hydroxy group is on the same side of the carbonyl group with weak intramolecular hydrogen bonds being experienced by the 2'-hydroxy and C-9 carbonyl group. Each of the methyl groups on a particular pyran ring is in a similar chemical environment being on different sides of the planar molecule. The fluorine atom is seen pointing toward the angular pyran ring. Both pyranochromene rings exist in a half chair conformation with Φ = 0.3779 (11) Å, θ = 112.14 (18)°, Ψ = 219.32 (19)°and Φ = 0.3460

(11) Å, θ = 113.85 (18)° and Ψ = 146.4 (2)° respectively. This is similar to our previous reports on similar structures [21, 22].

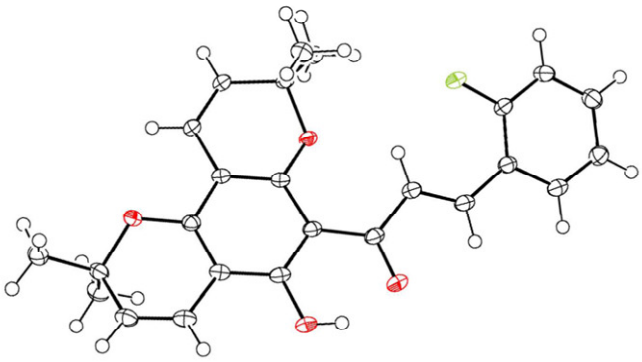


Figure 2-4 ORTEP diagram of **3b**

The cyclisation of the chalcones to the flavanones is carried out with sodium acetate under reflux at 80 ºC in ethanol. The spectra of the flavanones using **4b** as an example (**Figure 2-2**), is typified by a doublet doublet at δ_H 5.69 (H-2) deshielded by the oxygen and two double doublets at δ_H 2.93 (H-3a) and 2.81(H-3b) respectively. The two protons at C-3 and the one proton at C-2 all couple with each other with $J_{H\text{-}3a,H\text{-}3b}$ = 16.6 Hz, $J_{H\text{-}3a,H\text{-}2}$ = 3.2 Hz and $J_{H\text{-}3b,H\text{-}2}$ = 12.9 Hz. These coupling constants indicate that the H-2/H-3a dihedral angle is closer to 90º and the H-2/H-3b dihedral angle closer to 180º. These occurrences take place with the concomitant disappearance of the distinctive H-7 and H-8 pair of doublets discussed above.

Interestingly enough, the change in structure from the pyranochromene chalcone to the flavanone led to differences in the NMR resonances of the pyran rings. In particular, whereas H-2" and H-2"' overlapped in the chalcones and H-1" and H-1"' could clearly be distinguished, the opposite now occurs with the flavanones. As with the H-2"/H-2"'

resonances above the H-1''/H-1''' resonances appear as a series of four peaks, the first and the third being the doublet of H-1'' and the second and the fourth being the doublet of H-1'''. These occur at δ_H 6.58 and δ_H 6.57 respectively with $J_{H\text{-}1'',2''}$ = 10.0 Hz and $J_{H\text{-}1''',2'''}$ = 9.9 Hz. These coupling constants were used to distinguish the olefinic methine resonances on each of the rings.

Unlike in the chalcones where there was a clear correlation between H-1''' and C-2' which allowed us to assign each of the pyran ring resonances unambiguously, there is no correlation between H-1''' and C-9 in the flavanones to make this distinction. Therefore each of the pyran ring resonances as well as C-5 and C-7, and C-6 and C-8 may be interchangeable. Nevertheless, H-1'' shows a HMBC correlation to C-6 and H-1''' a HMBC correlation to C-8. Since the planarity of the molecule changes in forming the flavanone, each of the methyl resonances in both the ^{1}H and ^{13}C NMR spectra appear as separate resonances at δ_H 1.52, 1.50, 1.46 and 1.44, and δ_C 28.7, 28.4, 28.3 and 28.1. However, it is impossible to assign any one of these resonances to a specific methyl group and these resonances are interchangeable. The chemical shifts and splitting patterns of the fluorinated aromatic ring of **4b** were very similar to that of the chalcone **3b**.

The crystal structure of a related flavanone **4g** (**Figure 2-5**) with a *para* methoxy group on the phenyl ring shows the absolute stereochemistry to be in the *S* configuration at C-2. The dihedral angle between the axial H-2 and the axial H-3b is 137.8 (4)º, whereas the dihedral angle between the axial H-2 and the equatorial H-3a is 94.8 (3)º which explains the coupling constants above. Both the pyran rings are now in an angular position with the phenyl ring being out of the plane of the benzopyranone ring with the dihedral angle between the least-squares plane of the pyranochromene ring system and the phenyl ring being 40.65 (19)°.

The flavanone core ring exists in the half chair conformation (Φ = 0.453 (5) Å, θ = 53.8 (6)° and Ψ = 284.5 (7)°.

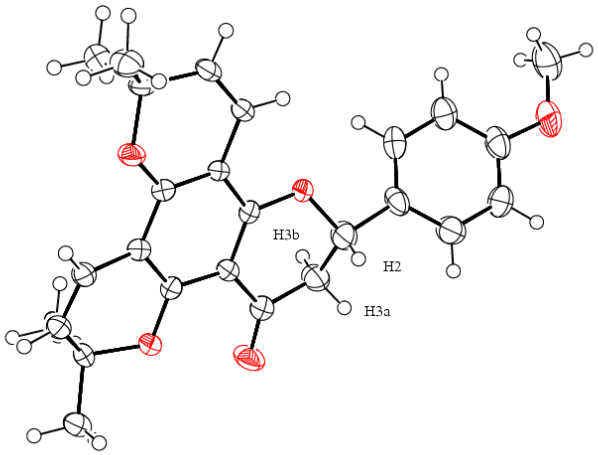


Figure 2-5 ORTEP diagram of **4g**

Conclusion

A series of pyranochromene chalcones and flavanones were synthesized and characterized by 1D and 2D NMR spectroscopy and X-ray crystallography. The stereochemistry of C-2 for **4g** was shown to be in the *S* configuration for the flavanones and since the NMR spectra of all the other compounds prepared with the same method are similar, the series of compounds is deduced to have the *S*-configuration for C-2. The ^{1}H and ^{13}C NMR assignments of the pyranochromene chalcones and flavanones were made with the aid of HSQC and HMBC data and will provide a reference point for other pyranochromene chalcones synthesized or isolated.

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Chapter 3. The synthesis and structural elucidation of novel pyranochromene chalcones and flavanones

Abstract

Chloro, bromo, nitro and naphthyl pyranochromene chalcones and their corresponding flavanones were synthesized and characterized by NMR spectroscopy and X-ray crystallography. The *E* configuration of chalcone **3a** and the *S* configuration of C-2 in **4c** were determined by single crystal X-Ray diffraction. The ^{1}H and ^{13}C NMR resonances of the synthesized compounds were unambiguously assigned.

Keywords

^{1}H NMR; ^{13}C NMR; X-ray crystallography; pyranochromene chalcone; pyranochromene flavanone

Introduction

Pyranoflavonoids, a rare class of flavonoids characterised by the presence of a 2,2-dimethyl-*2H*-pyrano moiety, have been widely investigated in recent years due to their interesting pharmacological properties [1-8]. These pyran groups on the flavonoid core skeleton increase the lipophilicity of these molecules, a desirable property for pharmaceutical compounds as lipophilic compounds have strong affinity to biological membranes [9]. A wide variety of chalcones and flavanones have been isolated from natural sources, however the prenylated chalcones and flavonoids are not as common as their hydroxy and methoxy counterparts.

The synthesis of pyranoflavonoids has been reported with several methods [10-18]. An attractive and simple method is a three step route in which the hydroxylated benzaldehydes or acetophenone precursors are first reacted with 3-methyl-2-butenal to form the pyrano benzaldehydes or acetophenones and then condensed to form the 2'-hydroxychalcones and finally cyclised to the flavanones [10, 14, 18]. It is essential that the *ortho* hydroxy group of the acetophenone is left unreacted as this is used to form the flavanones. Pyran rings can be inserted into hydroxylated acetophenones using prenyl bromide to first prenylate the acetophenone followed by cyclisation with DDQ [10, 12, 14], or in one step using 3-chloro-3-methylbut-1-yne, CuI and acetone [15], or β-methyl crotonaldehyde (3-methyl-2-butenal) and ethylene diamine diacetate (EDDA) [13] or β-methyl crotonaldehyde and pyridine [11].

Chalcone synthesis involves the Claisen-Schmidt condensation between acetophenones and benzaldehydes in aqueous alkaline bases [19]. Subsequent cyclization of 2'-hydroxychalcone intermediates lead to pyranoflavanones. This can be done with either I_2 and DMSO to produce flavones [10] or with sodium acetate and ethanol to produce flavanones [14]. We have recently reported the synthesis and structural elucidation of a series of fluorinated and

methoxylated pyranochalcones and flavanones [18]. We have used similar chemistry to provide novel chloro, bromo, nitro and naphthalene derivatives of pyranochromene chalcones and flavonoids. Herein we report the synthesis and characterization of these molecules by NMR and X-ray crystallography.

Experimental

General Experimental Procedures

Reagents and chemicals used in this study were purchased from Sigma Aldrich via Capital Lab, South Africa and were reagent grade. All organic solvents were redistilled and dried according to standard procedures. Optical rotations were recorded using a PerkinElmer^{TM}, Model 341 Polarimeter with a 10 cm flow tube. Melting points were measured using a Stuart Scientific Melting Point Apparatus SMP3. IR spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrometer with universal ATR sampling accessory. High-resolution mass data was obtained using a Bruker microTOF-Q II ESI instrument operating at ambient temperatures, with a sample concentration of approximately 1 ppm. Thin layer chromatography (TLC) was performed using Merck Kieselgel 60 F_{254} plates. Crude compounds were purified with column chromatography using silica gel (60–120 mesh) as the stationary phase and varying combinations of solvents depending on the sample to be purified.

^{1}H and ^{13}C NMR spectra were recorded at 298 K with 5-10 mg samples dissolved in 0.5 mL of $CDCl_3$ in 5 mm NMR tubes using a Bruker Avance^{III} 400 and 600 MHz NMR spectrometer (9.4 T; Bruker, Germany) (400.22 and 600.00 MHz for ^{1}H, 100.63 and 150.00 MHz for ^{13}C and 376.58 Hz for ^{19}F). Chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz. The ^{1}H and ^{13}C NMR chemical shifts of the deuterated solvent were 7.24

and 77.0 respectively, referenced to the internal standard, TMS. For the ^{19}F NMR spectra, the chemical shift of trifluorotoluene (TFT) (0.05% in $CDCl_3$) was referenced at -62.73.

General procedure for the synthesis of the pyranochromene intermediate, octandrenolone (2)

Using our previous method [11], 2,4,6-trihydroxyacetophenone (4.0 g, 0.0238 mol) was dissolved in pyridine (2.70 g) in a round bottom flask and 3-methyl-2-butenal (8.0 g, 0.0952 mol) then added before heating the reaction to 110° C for 24 h. The reaction was monitored by TLC using EtOAc:hexane (5:95, *R*f = 0.6). On completion, HCl (30 ml) was used to neutralize the reaction and the organic portion extracted with ethyl acetate (4 × 40 ml). The combined organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure. The residue was purified by column chromatography on silica gel using 100% hexane to afford octandrenolone (1-(5-hydroxy-2,2,8,8-tetramethyl-2*H*,8*H*-pyrano[2,3-*f*]chromen-6-yl)ethanone) (**2**) as a yellow crystalline solid (70.6% yield) with a melting point of 89–90 °C.

^1H NMR (400 MHz, $CDCl_3$): δ 13.99 (1H, s, OH), 6.65 (1H, d, *J* = 10.1 Hz), 6.58 (1H, d, *J* = 10.1 Hz), 5.45 (1H, d, *J* = 10.1 Hz), 5.43 (1H, d, *J* = 10.1 Hz), 2.65 (3H, s), 1.49 (6H, s), 1.43 (6H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 203.2, 160.5, 156.6, 154.9, 125.3, 124.6, 116.4, 116.1, 105.7, 102.2, 102.1, 78.2, 78.1, 33.1, 28.5, 28.2.

General procedure for the synthesis of pyranochromene chalcones 3a – 3e

The chalcones were prepared according to our previously developed method [17] as outlined in **Scheme 3-1**. To a solution of octandrenolone (**2**) (150 mg, 0.500 mmol) in ethanol (10 mL) and water (2 mL), the substituted benzaldehydes (84.3 mg, 0.700 mmol) were added at room temperature with KOH (112 mg, 2.00 mmol). The reaction mixture was stirred for 24 –

48 h at room temperature and monitored by TLC using EtOAc:hexane (10:90, R_f = 0.4). The solvent was distilled off under reduced pressure and the residue was dissolved in water (20 mL) and made neutral with 2 M HCl (20 mL). The organic portion was extracted with ethyl acetate (3 × 30 mL), washed with water and dried over anhydrous $MgSO_4$. Removal of the solvent followed by flash column chromatography on silica gel gave the pure compounds **3a-e** as brown solids. ^1H and ^{13}C NMR data for **3a-e** are reported in **Table 3-1** and **Table 3-2**.

3-(2-Chlorophenyl)-1-(5-hydroxy-2,2,8,8-tetramethyl-2H,8H-pyrano[2,3-f]chromen-6-yl)-propenone (**3a**) brown solid; Yield 90%; mp 114-115 ^oC; R_f = 0.5 (EtOAc:hexane, 10:90); IR υ_{max} 2972, 1625, 1583, 1467 cm^{-1}; HRESIMS *m/z* = 445.1185 $[M+Na]^+$ (calcd. for $C_{25}H_{23}O_4ClNa$ 445.1183).

3-(4-Chlorophenyl)-1-(5-hydroxy-2,2,8,8-tetramethyl-2H,8H-pyrano[2,3-f]chromen-6-yl)-propenone (**3b**) brown solid; Yield 90%; mp 138-139 ^oC; R_f = 0.5 (EtOAc:hexane, 10:90); IR υ_{max} 2974, 2935, 1629, 1603, 1583, 1543, 1461 cm^{-1}; HRESIMS *m/z* = 445.1182 $[M+Na]^+$ (calcd. for $C_{25}H_{23}O_4ClNa$ 445.1183).

3-(4-Bromophenyl)-1-(5-hydroxy-2,2,8,8-tetramethyl-2H,8H-pyrano[2,3-f]chromen-6-yl)-propenone (**3c**) brown solid; Yield 85%; mp 140-141 ^oC; R_f = 0.5 (EtOAc:hexane, 10:90); IR υ_{max} 2973, 2962, 2934, 1629, 1603, 1583, 1544, 1418 cm^{-1}; HRESIMS *m/z* = 489.0677 $[M+Na]^+$ (calcd. for $C_{25}H_{23}O_4BrNa$ 489.0677).

3-naphthalen-2-yl-1-(5-hydroxy-2,2,8,8-tetramethyl-2H,8H-pyrano[2,3-f]chromen-6-yl)-propenone (**3d**) brown solid; Yield 68%; mp 134-135 ^oC; R_f = 0.5 (EtOAc:hexane, 10:80); IR

υ_{max} 2956, 1626, 1578, 1543 cm^{-1}; HRESIMS *m/z* = 461.1729 $[M+Na]^+$ (calcd. for $C_{29}H_{26}O_4Na$ 461.1729).

3-(3-nitrophenyl)-1-(5-hydroxy-2,2,8,8-tetramethyl-2H,8H-pyrano[2,3-f]chromen-6-yl)-propenone (**3e**) brown solid; Yield 90%; mp 193-194 ^oC; R_f = 0.6 (EtOAc:hexane, 20:80); IR υ_{max} 2971, 2925, 1634, 1580, 1530, 1457 cm^{-1}; HRESIMS *m/z* = 456.1424 $[M+Na]^+$ (calcd. for $C_{25}H_{23}NO_6Na$ 456.1423).

General procedure for the synthesis of pyranochromene flavanones 4a-4e

Pyranochromene chalcones **3a-e** (0.3 mmol) were added to a solution of sodium acetate (188 mg, 3.0 mmol) in ethanol (10 mL) at room temperature and refluxed for 48 h at 80 °C. Upon completion, the solvent was removed under reduced pressure and the residue dissolved in water (20 mL), acidifed with 2 M HCl (20 mL) and the organic portion extracted with ethyl acetate (3 × 30 mL), washed with water and dried over anhydrous $MgSO_4$. Removal of the solvent followed by flash column chromatography on silica gel afforded the pure pyranochromene flavanones **4a-e** as yellow solids. The ^1H and ^{13}C NMR data of **4a-e** are reported in **Table 3-3** and **Table 3-4**.

2-(2-Chlorophenyl)-6,6,10,10-tetramethyl-2,3-dihydro-6H,10H-dipyrano[2,3-f;2',3'-h]chromen-4-one (**4a**) yellow solid; Yield 36%; mp 130-131 ^oC; R_f = 0.5 (EtOAc:hexane, 20:80); IR υ_{max} cm^{-1} 2970, 2924, 1678, 1636, 1591, 1571, 1431 cm^{-1}; HRESIMS *m/z* = 445.1179 $[M+Na]^+$ (calcd. for $C_{25}H_{23}O_4ClNa$ 445.1183).

2-(4-Chlorophenyl)-6,6,10,10-tetramethyl-2,3-dihydro-6H,10H-dipyrano[2,3-f;2',3'-h]chromen-4-one (**4b**) yellow solid; Yield 45%; mp 135-136 ^oC; R_f = 0.5 (EtOAc:hexane,

20:80); IR υ_{max} 2975, 2925, 1682, 1636, 1571, 1438 cm^{-1}; HRESIMS *m/z* = 445.1178 $[M+Na]^+$ (calcd. for $C_{25}H_{23}O_4ClNa$ 445.1183).

2-(4-Bromophenyl)-6,6,10,10-tetramethyl-2,3-dihydro-6H,10H-dipyrano[2,3-f;2',3'-h]chromen-4-one (**4c**) yellow solid; Yield 50%; mp 171-172 ^oC; R_f = 0.5 (EtOAc:hexane, 20:80); IR υ_{max} 2977, 2960, 1683, 1666, 1638, 1591, 1572, 1412 cm^{-1}; HRESIMS *m/z* = 489.0684 $[M+Na]^+$ (calcd. for $C_{25}H_{23}O_4BrNa$ 489.0677).

2-(Naphthalen-2-yl)-6,6,10,10-tetramethyl-2,3-dihydro-6H,10H-dipyrano[2,3-f;2',3'-h]chromen-4-one (**4d**) yellow solid; Yield 43%; mp 161-162 ^oC; R_f = 0.5 (EtOAc:hexane, 20:80); IR υ_{max} 2925, 1671, 1634, 1574, 1432 cm^{-1}; HRESIMS *m/z* = 461.1729 $[M+Na]^+$ (calcd. for $C_{29}H_{26}O_4Na$ 461.1729).

2-(3-Nitrophenyl)-6,6,10,10-tetramethyl-2,3-dihydro-6H,10H-dipyrano[2,3-f;2',3'-h]chromen-4-one (**4e**) yellow solid; Yield 38%; mp 107-108 ^oC; R_f = 0.5 (EtOAc:hexane, 10:80); IR υ_{max} 2969, 2923, 2853, 1684, 1639, 1591, 1570, 1526, 1429 cm^{-1}; HRESIMS *m/z* = 456.1418 $[M+Na]^+$ (calcd. for $C_{25}H_{23}NO_6Na$ 456.1423).

Synthesis of the 4-nitrophenyl pyranochromene flavone (5)

4-Nitrobenzaldehyde (105 mg, 0.7 mmol) and potassium hydroxide (112 mg, 2.0 mmol) were added to a solution of octandrenolone (150 mg, 0.5 mmol) in ethanol (10 mL) and water (2 mL) and stirred for 2 h at room temperature. The product precipitated out of the reaction mixture. It was then filtered, washed with cold ethanol and dried under vaccum, yielding the pure compound 2-(4-nitrophenyl)-6,6,10,10-tetramethyl-6*H*,10*H*-dipyrano[2,3-f;2',3'-h]chromen-4-one **(5)** (149 mg, 70% yield) as a yellow solid with a melting point of 270-271

°C; IR υ_{max} 2967, 2926, 1700, 1669, 1633, 1613, 1582, 1506 cm^{-1}; HRESIMS *m/z* = 454.1269 $[M+Na]^+$ (calcd. for $C_{25}H_{21}NO_6Na$ 454.1267). ^1H and ^{13}C NMR data of **5** are reported in **Table 3-3** and **Table 3-4**.

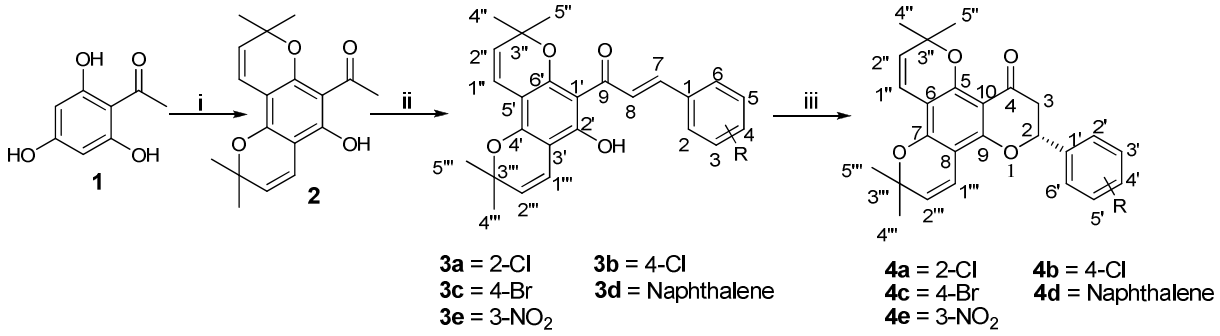
Single crystal X-ray diffraction analysis

Cube-shaped single crystals of compounds **3a** and **4c** were selected and glued onto glass fibre tips and mounted in a stream of cold nitrogen at 173(1) K and centred in the X-ray beam using a video camera. Crystal evaluation and data collection were performed on a Bruker Smart APEXII diffractometer with Mo Kα radiation (λ = 0.71073 Å). The diffractometer to crystal distance was set at 4.00 cm. The initial cell matrix was obtained from three series of scans at different starting angles. Each series consisted of 12 frames collected at intervals of 0.5º in a 6º range with the exposure time of 10 seconds per frame. The reflections were successfully indexed by an automated indexing routine built in the APEXII program suite. The final cell constants were calculated from a set of 6460 strong reflections from the actual data collection. Data collection method involved ω scans of width 0.5°. Data reduction was carried out using the program *SAINT+*. The structure was solved by direct methods using SHELXS and refined. Non-H atoms were first refined isotropically and then by anisotropic refinement with full-matrix least-squares calculations based on F^2 using SHELXS. All H atoms were positioned geometrically and allowed to ride on their respective parent atoms. All H atoms were refined isotropically. The absorption correction was based on fitting a function to the empirical transmission surface as sampled by multiple equivalent measurements. The program *ORTEP*3 was used to prepare artwork representation. The molecular diagrams are drawn with 50% probability ellipsoids. Crystallographic data (excluding structure factors) for the structure in this paper has been deposited with the Cambridge Crystallographic Data Centre, CCDC, 12 Union Road, Cambridge CB21EZ, UK.

Copies of the data can be obtained free of charge on quoting the depository numbers CCDC-1031442 (for **3a**) and CCDC-1031443 (for **4c**) (Fax: +44-1223-336-033; E-Mail: deposit@ccdc.cam.ac.uk, http://www.ccdc.cam.ac.uk).

Results and Discussion

The synthesis of pyranochromene flavanones involved three steps (**Scheme 3-1**), formation of the pyran rings with 2',4',6'-trihydroxyacetophenone, condensation with aldehydes to produce chalcones and cyclisation of the chalcones to flavanones. 3-Methyl-2-butenal was used as the prenylating agent, which forms pyran rings under basic conditions at high temperatures (110 °C) at the 2'- and 4'- positions of 2',4',6'-trihydroxyacetophenone. Various substituted benzaldehydes were used to produce several chalcones and flavanones. We had previously reported on the synthesis of fluorinated and methoxylated chalcones and flavanones of this type and herein report the chloro, bromo, nitro and naphthyl derivatives.



Scheme 3-1 Synthetic scheme for the synthesis of pyranochromene flavanones **4a-e**. i) 3-methyl-2-butenal, pyridine, 110°C, reflux; ii) substituted benzaldehydes, KOH, EtOH/H_2O, rt; iii) NaOAc, EtOH, 80°C, reflux.

The chalcone intermediates **3a-e**, were synthesized in good yields of between 60-90%. The overall yields obtained for the flavanones were moderate at between 35-50%. These yields were consistent with that reported for the fluoro and methoxy derivatives [18]. Interestingly,

the 4-nitrobenzaldehyde did not form the chalcone on condensation with octandrenolone (**2**), but rather formed the flavone (**5**) in one single step. We were however able to form the chalcone intermediate **3e** and the flavanone **4e** with 3-nitrobenzaldehyde. The structures of the compounds were determined with IR, ^{1}H NMR, ^{13}C NMR, and mass spectral data. In addition, X-ray crystallography was carried out on **3a** and **4c** to see how the packing of the crystal with the larger chlorine atom compares to the corresponding fluoro chalcones and how the *para* bromine atom compares to the corresponding methoxy flavanone reported in Pawar et al. [18]. We also used the crystal structure to determine the configuration at C-2 in the flavanones and see whether or not this is consistent with the *2S* configuration for the methoxy flavanone [18].

Formation of the pyranochromene chalcones were shown typically by the H-7 and H-8 ^{1}H NMR resonances of the α,β-unsaturated ketone moiety, which occurred at δ 8.15 (H-8) and 8.06 (H-7) with $J_{H7,H8}$ = 15.6 Hz for **3a** (**Table 3-1**). The 2'-hydroxy group occurred as a singlet at approximately δ 14.26 for all the chalcones **3a-e**, with the exception of **3d** (naphthyl chalcone), where it appeared at δ 14.43. The two pyran rings were evident from the two pairs of doublets that were present at δ 6.61 and δ 5.48 ($J_{H1'',H2''}$ = 10.0 Hz) and δ 6.69 and δ 5.46 ($J_{H1''',2'''}$ = 10.0 Hz). The carbonyl resonance appeared at δ 192-193 in the ^{13}C NMR spectrum and all carbon resonances for the dipyrano aromatic ring and α,β-conjugated system are consistent with that reported earlier [18]. These are tabulated in **Table 3-2**.

Table 3-1 ^{1}H NMR shifts of pyranochromene chalcones **3a – 3e** ($CDCl_3$, *J* in Hz)

No.	3a[$]	3b*	3c*	3d*	3e*
OH	14.26 (s)	14.28 (s)	14.26 (s)	14.43 (s)	14.17 (s)
1	-	-	-	8.00 (s)	-
2	-	7.51 (d, 8.3)	7.54 (d, 8.3)	-	8.52(s),
3	7.45 - 7.43 (m)	7.38 (d, 8.3)	7.45 (d, 8.3)	7.75 (dd, 8.2, 0.9)	-
4	7.31 - 7.29 (m)	-	-	7.87 (d, 8.6)	8.22 (d, 7.9)
5	7.31 - 7.29 (m)	7.38 (d, 8.3)	7.45 (d, 8.3)	7.87 (d, 8.6)	7.59 (t, 7.9)
6	7.71 - 7.68 (m)	7.51 (d, 8.3)	7.54 (d, 8.3)	7.53 - 7.50 (m)	7.83 (d, 7.6)
7	8.15 (d, 15.6)	7.68 (d, 15.7)	7.67 (d, 15.8)	7.53 - 7.50 (m)	7.73 (d, 15.6)
8	8.06 (d, 15.6)	8.04 (d, 15.7)	8.06 (d, 15.8)	7.85 - 7.83(m)	8.23 (d, 15.6),
11	-	-	-	7.93 (d, 15.7)	-
12	-	-	-	8.21 (d, 15.7)	-
1''	6.61 (d, 10.0)	6.61 (d, 10.2)	6.61 (d, 10.1)	6.63 (d, 9.9)	6.61 (d, 9.8)
2''[#]	5.48 (d, 10.0)	5.48 (d, 10.2)	5.48 (d, 10.1)	5.50 (d, 9.9)	5.50 (d, 9.8)
4''/5''[†]	1.52 (s)	1.53 (s)	1.53 (s)	1.59 (s)	1.58 (s)
1'''	6.69 (d, 10.0)	6.68 (d, 10.0)	6.68 (d, 9.8)	6.71 (d, 9.9)	6.68 (d, 10.1)
2'''[#]	5.46 (d, 10.0)	5.47 (d, 10.0)	5.47 (d, 9.8)	5.49 (d, 9.9)	5.49 (d, 10.1)
4'''/5'''[†]	1.46 (s)	1.46 (s)	1.46 (s)	1.47 (s)	1.46 (s)

[$] 400 MHz; * 600 MHz; [#] and [†] indicate resonances that can be interchanged.

Table 3-2 ^{13}C NMR shifts of pyranochromene chalcones **3a** – **3e** ($CDCl_3$)

No.	3a[$]	3b*	3c*	3d*	3e*
1	134.1	134.4	124.4	130.5	137.7
2	135.7	129.5	132.4	134.4	121.8
3	130.6	129.4	129.7	123.8	148.9
4	127.2	136.1	134.8	128.9	124.3
5	130.9	129.4	129.7	128.8	130.2
6	127.5	129.5	132.4	127.3	134.6
7	137.8	140.6	140.7	126.9	138.7
8	130.2	128.4	128.5	128.0	130.7
9	192.8	192.8	192.8	133.4	192.2
10	-	-	-	133.7	-
11	-	-	-	142.3	-
12	-	-	-	128.1	-
13	-	-	-	193.0	-
1'	106.2	106.1	106.1	106.2	106.1
2'	161.7	161.3	161.6	161.7	161.7
3'	102.9	102.8	102.8	102.9	102.9
4'	155.7	155.7	155.7	155.5	156.0
5'	102.7	102.8	102.8	102.8	102.8
6'	156.4	156.4	156.4	156.4	156.5
1''	116.8	116.8	116.8	116.8	116.7
2''	125.6	125.7	125.7	125.6	125.8
3''	78.6	78.6	78.6	78.6	78.9
4''/5''	28.6	28.6	28.6	28.6	28.7
1'''	116.4	116.4	116.4	116.5	116.3
2'''	124.9	125.0	125.0	125.0	125.1
3'''	78.5	78.5	78.5	78.5	78.8
4'''/5'''	28.3	28.3	28.3	28.3	28.3

[$] 400 MHz; * NMR at 600 MHz

The chalcones **3a-e** were cyclized to the flavanones **4a-e** with sodium acetate and were confirmed to have occurred due to the disappearance of the Δ^7 *trans* double bond and the appearance of an ABX system for the H-2 and two methylene H-3 resonances. For instance in **4b**, H-2, H-3a and H-3b occurred as double doublets at δ 5.37, δ 2.75 and δ 2.91 with $J_{H2,H3a}$ = 2.9 Hz, $J_{H2,H3b}$ = 13.0 Hz and $J_{H3a,H3b}$ = 16.5 Hz, consistent with our previous set of

flavanones [18]. An exception was **4a**, the 2-chloro derivative, where H-2/H-3b coupling was not observed. In this instance H-2 appeared as a doublet at δ 5.75 (J = 13.1 Hz) and H-3a and H-3b appeared at δ 2.77 (dd, J = 16.6, 13.1 Hz) and δ 2.91 (d, J = 16.6 Hz) respectively. This peculiarity was not observed in the fluoro and methoxy derivatives and is specific to the 2-chloro derivative [18]. The NMR data for **4a-e** are tabulated in **Table 3-3** and **Table 3-4**.

The position of the substituents on the phenyl ring of flavanones affect the H-2 resonance. With *ortho* positioned substituents such as 2-chloropyranochromene flavanone **4a**, H-2 is the most deshielded occurring at δ 5.75 and becoming more shielded as the substituent moves to the 3-position (δ 5.48 in the 3-nitro derivative **4e**) and the 4-position (δ 5.37 and δ 5.35 in **4b** and **4c**, respectively). With the naphthyl group, H-2 appears at δ 5.56.

In the pyran rings, all the resonances of the flavanones **4a-e** are similar to that of the chalcones **3a-e**, with the exception that the H-4", H-5", H-4"' and H-5"' resonances now appear as separate resonances. For example δ 1.53 (H-4"), δ 1.50 (H-5"), δ 1.46 (H-4"') and δ 1.44 (H-5"') in **4a** appear as separate resonances, since the flavanone backbone is not as planar as the chalcone backbone and hence the methyl groups on either side are not equivalent.

Table 3-3 ^{1}H NMR shifts of pyranochromene flavanones **4a** – **e** and flavone **5** ($CDCl_3$, *J* in Hz)

No.	4a[2]	4b[2]	4c[1]	4d[1]	4e[1]	5[1]
2	5.75 (d, 13.1)	5.37 (dd, 13.0, 2.7)	5.35 (dd, 13.0, 2.9)	5.56 (dd, 13.1, 2.8)	5.48 (dd, 12.7, 3.1)[4]	-
3a	2.77 (dd, 16.6, 13.1)[5]	2.75 (dd, 16.5, 2.7)	2.75 (dd, 16.6, 2.9)	2.86 (dd, 16.5, 2.8)	2.75 (dd, 16.4, 3.1)	6.68 (s)
3b	2.91 (d, 16.6)	2.91 (dd, 16.5, 13.0)	2.90 (dd, 16.6, 13.0)	3.07 (dd, 16.5, 13.1)	2.93 (dd, 16.4, 12.7)	-
1'	**-**	**-**	**-**	7.91 (s)	**-**	**-**
2'	-	7.39 (s)	7.54 (d, 8.1)	-	8.36 (s),	8.26 (d, 8.8)
3'	7.40 - 7.35 (m)	7.39 (s)	7.33 (d, 8.1)	7.57 (dd, 8.5, 1.7)	-	7.95 (d, 8.8)
4'	7.40 - 7.35 (m)	-	-	7.90 (d, 8.5)	8.22 (d, 7.9)	-
5'	7.31 - 7.28 (m)	7.39 (s)	7.33 (d, 8.1)	7.88 – 7.85 (m)	7.61 (t, 7.9)	7.95 (d, 8.8)
6'	7.70 (d, 7.6)	7.39 (s)	7.54 (d, 8.1)	7.53 - 7.50 (m)	7.76 (d, 7.7)	8.26 (d, 8.8)
7'	**-**	**-**	**-**	7.53 - 7.50 (m)	**-**	**-**
8'	**-**	**-**	**-**	7.88 – 7.85 (m)	**-**	**-**
1''	6.58 (d, 10.0)[6]	6.57 (d, 10.0)	6.56 (d, 10.1)[7]	6.58 (d, 10.2)	6.58 (d, 10.0)	6.56 (d, 10.1)
2''	5.47 (d, 10.0)	5.48 (d, 10.0)	5.48 (d, 10.1)	5.52 (d, 10.2)	5.51 (d, 10.0)[4]	5.66 (d, 10.0)
4''[3]	1.53 (s)	1.52 (s)	1.52 (s)	1.54 (s)	1.52 (s)	1.54 (s)
5''[3]	1.50 (s)	1.48 (s)	1.48 (s)	1.50 (s)	1.49 (s)	1.54 (s)
1'''	6.58 (d, 10.0)[6]	6.56 (d, 10.0)	6.56 (d, 10.1)[7]	6.63 (d, 9.9)	6.57 (d, 9.9)	6.62 (d, 10.0)
2'''	5.51 (d, 10.0)	5.51 (d, 10.0)	5.51 (d, 10.1)	5.48 (d, 9.9)	5.52 (d, 9.9)	5.53 (d, 10.0)
4'''[3]	1.46 (s)	1.46 (s)	1.46 (s)	1.47 (s)	1.47 (s)	1.52 (s)
5'''[3]	1.44 (s)	1.44 (s)	1.44 (s)	1.45 (s)	1.46 (s)	1.52 (s)

[1] 400 MHz; [2] NMR at 600 MHz; [3] indicate resonances that can be interchanged; [4] signals overlap; [5] due to coalescing of resonances, this appears as a triplet with J = 14.9 Hz; [6] 1" and 1" coalesce and appear as a triplet; [7] 1" and 1'" overlap.

Table 3-4 ^{13}C NMR shifts of pyranochromene flavanones **4a**-**e** and flavone **5** ($CDCl_3$)

No.	4a*	4b*	4c$	4d*	4e$	5$
2	76.3	78.5	78.5	78.0	78.0	139.6
3	44.7	46.0	46.0	46.1	46.0	106.9
4	188.6	188.5	188.3	188.8	187.6	179.8
5	154.5	154.5	154.5	154.5	154.7	154.0
6	102.6	102.5	102.5	102.6	102.6	99.2
7	156.2	156.2	156.2	156.2	156.2	156.9
8	105.0	105.0	105.0	104.9	105.2	105.7
9	157.6	157.3	157.3	157.7	156.8	161.5
10	105.7	105.7	105.7	105.8	105.6	106.9
1'	137.2	134.5	122.6	125.3	141.5	147.3
2'	132.0	129.1	132.1	136.7	121.2	131.4
3'	129.9	126.6	127.8	128.8	148.8	124.1
4'	127.5	137.8	138.4	124.0	123.6	150.1
5'	129.6	126.6	127.8	128.0	130.0	124.1
6'	127.2	129.1	132.1	126.8	132.0	131.4
7'	-	-	-	126.7	-	-
8'	-	-	-	128.3	-	-
9'	-	-	-	133.4	-	-
10'	-	-	-	133.5	-	-
1''	116.4	116.3	116.3	116.5	116.1	115.7
2''	126.6	127.5	126.6	126.6	127.1	127.9
3''	78.2	78.2	78.1	79.4	78.3	79.4
4''	28.7	28.7	28.7	28.7	28.7	28.7
5''	28.4	28.4	28.4	28.4	28.4	28.7
1'''	116.0	116.0	116.0	116.1	115.9	114.5
2'''	126.9	126.9	126.9	126.5	127.0	127.2
3'''	78.0	78.0	78.0	78.1	78.2	79.3
4'''	28.3	28.3	28.3	28.3	28.3	28.6
5'''	28.1	28.1	28.1	28.1	28.1	28.6

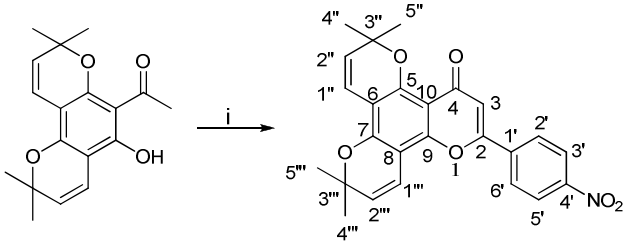
$ 400 MHz; * NMR at 600 MHz

As expected, the major differences in the ^1H and ^{13}C NMR spectra compared to the fluoro and methoxy derivatives reported in Pawar et al. [18] were in the phenyl ring. For the 2-chloro dipyranochalcone derivative, the H-3 and H-6 resonances occurred separately as multiplets at δ 7.43-7.45 and δ 7.68-7.71, with H-4 and H-5 overlapping as a multiplet at δ

7.29-7.31. On conversion to the flavanone, the resonances occurred at approximately the same chemical shift, but now H-6 was a distinct doublet at δ 7.70 (J = 7.6 Hz) and the H-3 and H-4 resonances overlapped at δ 7.35-7.40, while H-5 appeared separately at δ 7.28-7.31 as a multiplet. As expected, a pair of doublets were seen for the 4-chloro chalcones derivative at δ 7.51 (H-2/6) and δ 7.38 (H-3/5) with J = 8.3 Hz. The H-3/5 resonance was more shielded than H-2/6 due to the electron donating chloro substituent. Interestingly, the H-7 resonance, which appeared at δ 8.15, now resonated at δ 7.68. The more deshielded resonance in the 2-chloro derivative must be due to an interaction between H-7 and the 2-Cl substituent, which deshields H-7. More interesting was the fact that upon cyclisation to the flavanone, the H-2/6 and H-3/5 resonances, both coalesced into one four-proton singlet resonance at δ 7.39. This indicates that the $-CH_2\underline{C}HO$ group (C-2) in the pyranone ring attached to C-1' has the same effect on H-2'/6' that the chloro group at C-4' has on C-3'/5'. The pair of doublets H-2/6 and H-3/5 for the 4-bromo chalcone derivatives were similar to that of the 4-chloro derivative appearing at δ 7.54 and δ 7.45 respectively with J = 8.3 Hz. In the corresponding flavanone, the resonances did not coalesce into one as in the 4-chloro derivative, but H-3/5 was observed to be more shielded than in the chalcones.

For the 3-nitro derivative, a typical 3-substituted pattern was seen for both the chalcone and flavanone derivatives with H-2 being present at δ 8.51 (s), H-4 at δ 8.22 (d, J = 7.9 Hz), H-5 at δ 7.59 (t, J = 7.9 Hz) and H-6 at δ 7.83 (d, J = 7.6 Hz). For the napthyl derivate of the chalcones, H-1 was a singlet at δ 8.00, H-3 at δ 7.75 (dd, J = 8.2, 0.9 Hz), H-4 and H-5 as an overlapping doublet at δ 7.87 (J = 8.6 Hz), H-6 and H-7 at δ 7.50-7.53 (occurring together as a multiplet) and H-8 as a multiplet at δ 7.83-7.85. These resonances were very similar when the chalcone was cyclized to the flavanone, with the exception that the H-1 and H-3 resonances were now more shielded at δ 7.91 (s) and δ 7.57 (dd, J = 8.5, 1.7 Hz) respectively.

The condensation of 4-nitrobenzaldehyde with octandrenolone resulted in the formation of the flavone (**Scheme 3-2**), without proceeding through the chalcone and flavanone intermediates, even on repeating the experiment. This was quite surprising, but was confirmed by the ^{1}H and ^{13}C NMR spectra with the presence of the olefinic H-3 at δ 6.68 as a singlet and the olefininc C-2 and C-3 at δ 139.6 and δ 106.9 and the carbonyl C-4, which was more shielded than that of the flavanones at δ 179.8. The 4-nitrophenyl ring was evident with a pair of doublets at δ 8.26 and δ 7.95 with an *ortho* coupled *J* value of 8.8 Hz. The planarity of this flavone ring to the flavanone ring was also demonstrated by the fact that the two pairs of methyl groups, one on each of the two pyran rings were equivalent and hence two six-proton methyl resonances, similar to the that present in the chalcones were seen at δ 1.54 (H-4"/5") and δ 1.52 (H-4'"/5'").



2 **5**

Scheme 3-2 Reaction for the synthesis of the 4-nitropyranodichromene flavone **5**. i) 4-nitrobenzaldehyde, KOH, EtOH/H_2O, rt.

Compound **3a** (**Figure 3-1**) crystallizes in the monoclinic $P2_1/c$ space group with one molecule in the asymmetric unit. The molecular structure displays three fused rings composed of a phenol ring and two dimethyl substituted pyran rings. These are linked to a chlorobenzene ring through a C(=O)—C=C moiety whose C=C double bond is *trans* (torsion angle for H7-C7-C8-H8 = 177.5(2)°). The plane of the phenol ring fused to the pyrans rings

and that of the chlorobenzene ring are coplanar with a small deviation of 3.8(3)°. The C(=O)—C=C moiety, however, deviates from the two planes by an angle of 10.8(4) to the phenol ring and 11.7(4)° to the chlorobenzene ring. Both pyranochromene rings exist in a half chair conformation with Φ = 0.2456 (15) Å, θ = 63.7 (4)°, Ψ = 30.2 (4)°and Φ = 0.2966 (15) Å, θ = 63.2 (3)° and Ψ = 26.3 (3)°, respectively. Each of the methyl groups on their respective pyran rings are equivalent due to the planarity of the molecule. All bond lengths and angles are in agreement with the expected average values for related compounds [11, 17]. In addition to the O—H…O intramolecular hydrogen bond, the 2'-hydroxy and the carbonyl group experiences other weak intramolecular hydrogen bonds and π…π intermolecular interactions that stabilise the crystal lattice which compares well with our previous reports on similar structures [11, 17]. However, unlike the 2-fluoro derivative [18], where the fluorine and carbonyl groups were on different sides, the larger chlorine atoms in ring B is now on the same side of the carbonyl group.

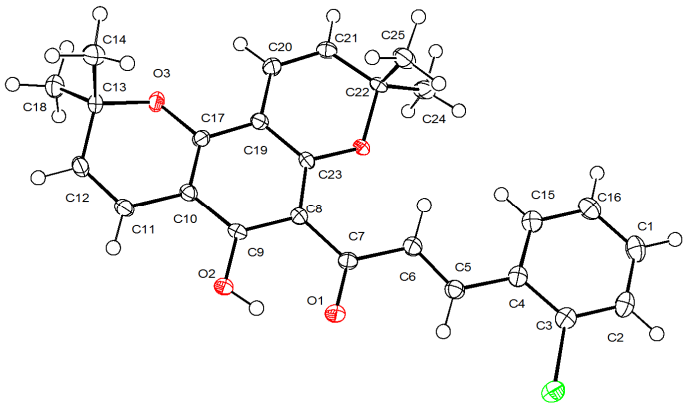


Figure 3-1 ORTEP diagram of **3a**

Compound **4c** (**Figure 3-2**) crystallizes with two symmetrically unrelated molecules of 4-bromo-dipryanoflavanone in the asymmetric unit. Both molecules display a central essentially planar backbone composed of a phenyl ring that is fused to two pyran rings and a

pyranone ring attached to a bromobenzene ring through a chiral carbon. The structure of **4c** shows the absolute stereochemistry to be in the *S* configuration at this carbon (see C-2 of **4c** in **Scheme 3-1**). The planar backbone of the two molecules in the asymmetric unit are oriented parallel to each other (dihedral angle between the two phenyl groups = 7.6(2)°), with the benzophenyl moieties oriented differently in the two 38.8(10) in one and 85.7 (10)° in the second. The dihedral angle between H-2 and the axial H-3b is 136.9 (3)°, whereas the dihedral angle between the H-2 and the equatorial H-3a is 90.2 (2)°. These dihedral angles were similar to earlier results [18]. The flavanone core ring exists in the half chair conformation (Φ = 0.380 (4) Å, θ = 124.9 (6)° and Ψ = 249.0 (6)°.

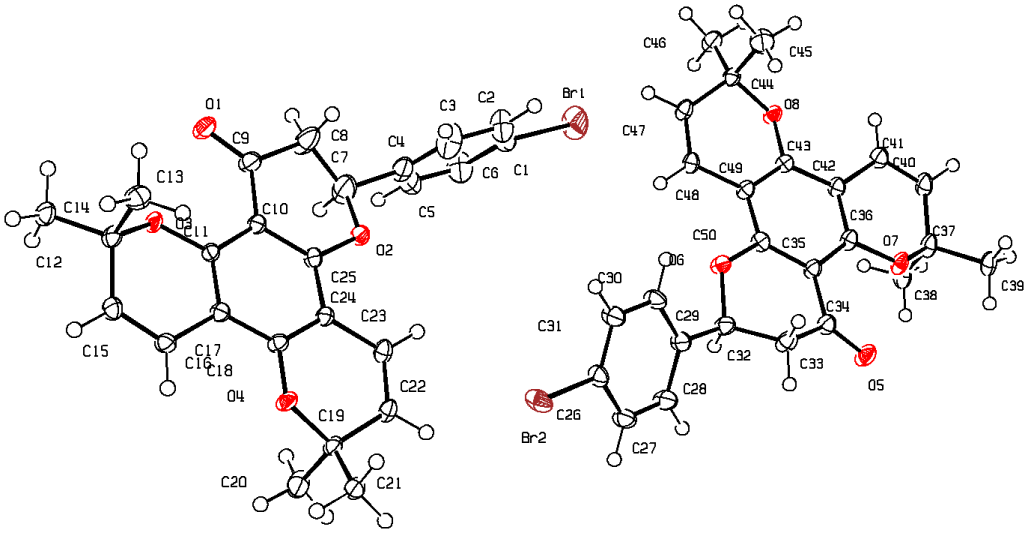


Figure 3-2 ORTEP diagram of **4c**

Conclusion

A series of novel pyranochromene chalcones and flavanones were synthesized and characterized by 2D NMR spectroscopy and X-ray crystallography. The chalcones were converted to the flavanones with the *2S* configuration. Some interesting observations were

made. The 4-nitrobenzaldehyde was converted straight to the flavone on condensation with the dipryanated acetophenone. The 4-chloro flavanone derivative contained a single singlet resonance instead of the two pairs of doublets that is normally observed for a *para*-substituted aromatic ring.

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Chapter 4. Antimicrobial and antioxidant activity of pyranoflavonoid analogues

Abstract

A series of pyranochromene chalcones **3a-m**, flavanones **4a-m** and flavone **5** with F, Cl, Br, OCH_3, NO_2 and naphthalene substituents on the B ring and two pyran rings fused to the A ring were tested for their antibacterial, antifungal and antioxidant activity. Chalcones **3a**, **3d**, **3i** (unsubstituted, 4-F and 2-Cl derivatives) and flavanones **4a** and **4i** (unsubstituted and 2-Cl) displayed potent antibacterial activity, whereas chalcones **3a**, **3b**, **3d**, **3l** and **3m** (unsubstituted, 2-F, 4-F, 3-NO_2 and naphthalene derivatives) and flavanone **4b**, **4d**, **4f** and **4l** (2-F, 4-F, 3-OCH_3 and 3-NO_2 derivatives) exhibited moderate to significant antifungal activity. In general, the pyranochromenes showed good antioxidant activity.

Keywords: Pyranochromene chalcones, pyranochromene flavanones, antibacterial activity, antifungal and antioxidant activity.

Introduction

Flavonoids are a group of common and naturally occurring polyphenolic compounds that are widely found in the plant kingdom. Prenylated chalcones and flavanones are a unique class of naturally occurring flavonoids characterized by the presence of a prenylated side chain in the flavonoid skeleton. Cyclisation of prenyl groups onto the aromatic ring result in chromene and pyranochromene compounds. Prenyl and pyran groups increase the lipophilicity and consequently enhance their interaction with cellular membranes [1]. Favorable properties and enhanced biological activities of halogens and hydroxyl functional groups were attributed to its dipole character, hydrogen bonding capability, rigidity and stability under *in vivo* conditions.

Prenylated chalcones and flavanones are rapidly gaining importance in medicinal and synthetic chemistry due to their diverse biological properties. These include anticancer activity [2-4], tyrosine protein kinase inhibition [2], antileishmanial activity [5], protein kinase C inhibition for the treatment of neurological or inflammatory responses [6], anti-inflammatory activity [7] and as trypanosomal glyceraldehyde-3-phosphate dehydrogenase inhibitors [8]. Paul and Choudhury [9-10] reported molecular docking studies of Pongachalcone I, which exhibited potent inhibitory activity against the multidrug resistant bacterium *Pseudomonas putida.*

This work forms part of an ongoing study on the synthesis of biologically active pyranochromene compounds and presents the antimicrobial and antioxidant evaluations of synthesized pyranochromene chalcones and flavanones. There is always a need for new antibiotics due to the growing problem of antibiotic resistance [11]. This paper explores the potential of pyranochromene chalcones and flavanones to have antimicrobial properties.

Experimental

Test compounds

The pyranochromene chalcones (**3a-m**) and flavanones (**4a-m**) **(Figure 4-1)** were previously prepared in our laboratory [12]. These molecules contained a pyranochromene skeleton on ring A and either an unsubstituted or F, Cl, Br, OCH_3 and NO_2 group at various positions on the phenyl ring or a naphthalene group instead of the phenyl ring. Attempts to make the chalcones and flavanones with the 4-NO_2 benzaldehyde did not yield the target molecules but the flavone (**5**) (**Figure 3-1**) instead. This was also used for the bioassays.

Antimicrobial assay

Antifungal/antibacterial agents: Stock solutions with a concentration of 2mM of the test compounds were prepared in DMSO and diluted before assays. Amphotericin-B and neomycin purchased from Sigma Aldrich were used as reference drugs for the antifungal and antibacterial assays, respectively. The final concentration of the synthesized compounds ranged from 0.0012 to 200 µg mL^{-1}, amphotericin-B ranged from 0.0015 to 100 µg mL^{-1} and neomycin from 0.0076 to 500 µg mL^{-1}. All drug dilutions were carried out in 96-well flat bottom microtitre plates. The final concentration of DMSO in each well was 2 nM or 0.001%.

Antifungal susceptibility test: Evaluation of the susceptibility of *Candida albicans* and non-*Candida albicans* species were performed using the broth micro dilution method according to M27-A2 for yeast guidelines. Yeast strains were grown aerobically overnight at 35 °C on Sabouraud dextrose agar plates. Yeasts were harvested and suspended in 1% sterile saline and the turbidity of the supernatants measured using a spectrophotometer at 625 nm with an absorbance of 0.08-0.1 equivalents to a 0.5 McFarland standard following the

NCCLS M27-A2 guidelines. The working suspension was diluted to 1:20 in a mixture containing RPMI 1640 medium and 0.165 M morpholinepropanesulfonic acid buffered to pH 7.0. The working suspension was further diluted with the medium (1:50) to obtain the final test inoculums ($1\text{-}5\times10^3$ CFU mL^{-1}). The microtitre plates containing different concentrations of test compounds were allowed to thaw and equilibrate to room temperature under aseptic conditions. Aliquots of working inoculum suspensions were dispensed into each well and the plates incubated in an aerobic environment at 35 °C for 24 h. After incubation, 20 µL of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium salt (MTS, Promega Corporation, Madison, USA) was added to each well, incubated at 37 ºC for 4 h and the absorbance recorded at 490 nm on a 96-well plate reader (Biotek, Powerwave XS2). All analyses were performed in triplicate and the data was found to be reproducible. The minimum inhibitory concentration (MIC) is the lowest concentration at which growth of the fungi was inhibited.

Antibacterial susceptibility test: Bacterial susceptibility tests were carried out using the micro broth dilution method. Overnight cultures after 16-18 h of incubation at 37 ºC were adjusted to the turbidity of a 0.5 McFarland standard. Inocula were adjusted to an absorbance of 0.08 - 0.10 to yield a stock suspension of $0.4\text{-}5\times10^8$ CFU mL^{-1}, which was diluted one hundred fold to obtain a working suspension of 10^6 CFU mL^{-1} at 625 nm. Microtitre plates were placed in a laminar flow unit to equilibrate to room temperature under aseptic conditions. Aliquots of 100 µL of bacterial inoculate were added to the microtiter plates containing different concentrations of test compounds. Plates were incubated aerobically for 16-18 h at 37 °C. Following incubation, 40 µL of freshly prepared iodonitrotetrazolium chloride [2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2*H* tetrazolium chloride] (INT) solution (200 µg mL^{-1}) was added to each well and the plate further incubated for 45 minutes

at 37 ºC in the dark. If the colorless INT is reduced to red after incubation, persistent growth of bacteria is indicated. No color change denotes the lack of bacterial growth. Neomycin was used as a control drug in this study. All analyses were performed in triplicate and the data was found to be reproducible. The minimum inhibitory concentration (MIC) is the lowest concentration at which growth of the bacteria was inhibited.

In vitro antioxidant studies

All the newly synthesized compounds were screened for radical scavenging (antioxidant properties) on 2,2'-diphenly-1-picrylhydrazyl (DPPH) following the method by Burits and Bucar [13]. Various concentrations of the compounds were dissolved in methanol and 1 mL was added to 4 mL of a 0.004% solution of DPPH in methanol. After a 30 min incubation period at room temperature, the absorbance was read against a blank (containing MeOH only) at 517 nm. The percentage scavenging activity of free radicals by DPPH was calculated using the following equation:

% scavenging activity = [(A control – A sample) / A blank] x 100.

Where 'A control' is the absorbance of the control reaction (containing all reagents except the test compound) and 'A sample' is the absorbance of the reagents with a particular test compound. The IC_{50} value was obtained by plotting a graph of concentration (in $\mu g\ mL^{-1}$) against % scavenging activity using Microsoft Excel.

Results and Discussion

Chemical structures

The chemical structures of the compounds used in the structure activity relationship (SAR), have either a chalcone (**3a**-**m**) or flavanone (**4a-m**) backbone (**Figure 4-1**). The chalcones

had a pyranochromene moiety, which was acetophenone derived. The same moiety transformed into the flavanones resulting in three pyran rings surrounding the acetophenone derived benzene ring. The aldehyde derived phenyl ring was either unsubstituted (**3a/4a**), monosubstituted, containing F at the *ortho*, *meta* or *para* positions (**3b-d/4b-d**), OCH_3 at the *meta* or *para* positions (**3f-g/4f-g**), Cl at the *ortho* or *para* positions (**3i-j/4i-j**), Br at the *para* position (**3k/4k**) and NO_2 at the *meta* position (**3l/4l**). In addition, the disubstituted compounds, 2,4-difluoro (**3e/4e**) and 2-fluoro-3-methoxy (**3h/4h**) and the naphthalene derivative were also used. The flavone (**5**) had the same backbone as the flavanones, but had a double bond at Δ^2 and a *para* NO_2 group on the phenyl ring.

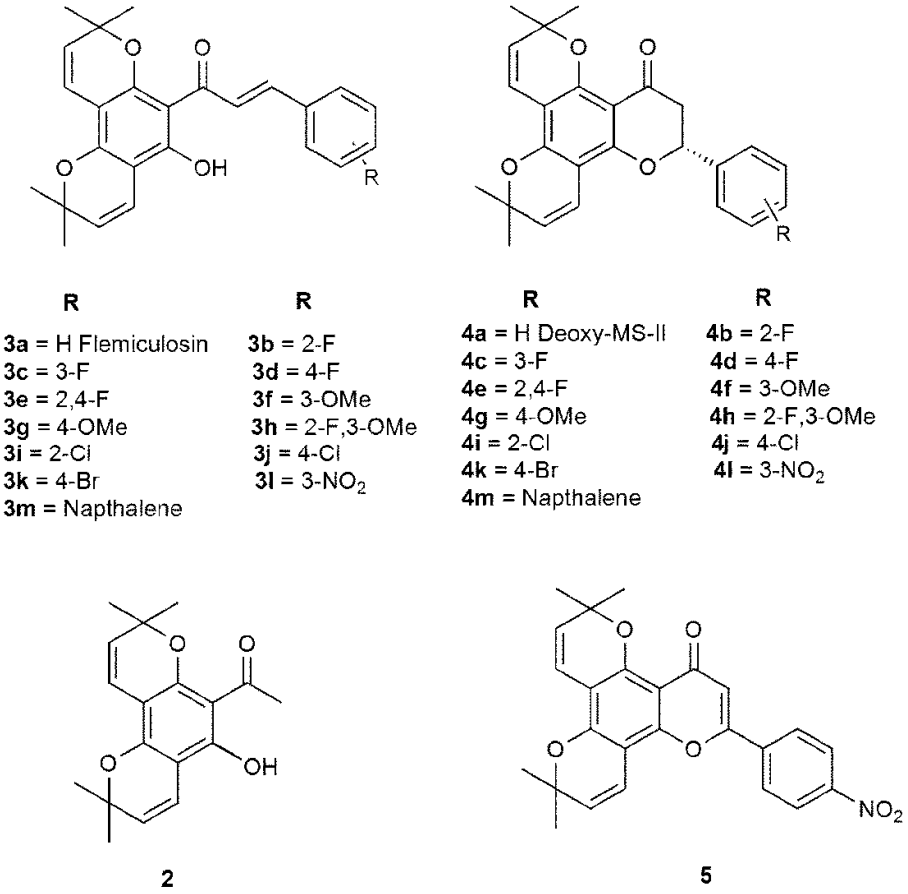

Figure 4-1 Compounds used in the antibacterial and antioxidant assays

Antibacterial activity

The compounds were evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis, Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. **Table 4-1** summarizes the MIC results obtained for the active compounds against the five different bacterial species. Four compounds showed a broad spectrum of activity, having antimicrobial activity against all the bacterial strains used in the assay. These were the unsubstituted chalcone **3a**, with MICs between 32.2 and128.7 μM, the unsubstituted flavanone **4a** (MIC between 18.7 and 64.4 μM), the 4-OCH_3 chalcone **3g** (MICs between 119.5 and 239.0 μM) and the 2-Cl flavanone **4i** (MICs between 29.6 and 118.2 μM). The best activity was shown by **4a**, better than the acetophenone precursor (**2**), indicating that not only were the pyran rings on the flavanone nucleus important, but also the flavanone core skeleton.

Table 4-1 MIC (μM) of test compounds on Gram positive and Gram negative bacterial strains

MIC (μM)					
	Gram positive		**Gram negative**		
Compounds*	***S. aureus***	***E. faecalis***	***E. coli***	***K. pneumonia***	***P. aeruginosa***
2	_	_	83.2	166.5	166.5
3a	32.2	32.2	32.2	128.7	128.7
4a	32.2	18.7	32.2	64.4	64.4
3b	_	_	123.0	_	_
4b	_	_	246.0	_	_
3d	61.5	_	30.7	61.5	30.7
3e	_	_	117.8	_	_
3g	239.0	119.5	239.0	119.5	119.5
3i	29.6	_	29.6	29.6	29.6
4i	59.1	29.6	59.1	59.1	118.2
4j	_	236.5	_	_	_
5	2.3	_	463.6	46.3.6	463.6
Neomycin	16.3	32.5	8.1	16.3	8.1

* The following compounds had no activity against all the bacterial species and were omitted from the table: **3c**, **4c**, **4d**, **4e**, **3f**, **4f**, **4g**, **3h**, **4h**, **3j**, **3k**, **4k**, **3l**, **4l, 3m** and **4m**; "-" indicates no activity

In general, the flavanone **4a** had better activity than its chalcone precursor **3a**, indicating that the pyranone nucleus in **4a** contributed to better activity than the α,β-unsaturated moiety in **3a**. The activity of the 2-Cl derivative **4i** was not as good as the unsubstituted derivatives, being 2-fold less active than **3a** and **4a** in some of the bacterial strains. The 4-OCH_3 derivative **3g** in some cases showed a 8 fold decrease in activity compared to **3a** and **4a**. Compared to the neomycin control, **3a** and **4a** had a 2-fold decrease in activity with regard to *S. aureus* and **4a** was 2-fold better than neomycin against *E. faecalis*. The chalcone **3a** had the same activity to that of neomycin against *E. faecalis*. The 2-Cl chalcone **3i** showed the best activity against the Gram negative strains, being active at 29.6 μM for all Gram negative strains and *S. aureus*. Likewise, the 4-F chalcone **3d** showed good activity, being active at 30.7 μm for *E.coli* and *P. aeruginosa* and 61.5 μM against *K. pneumonia* and *S. aureus*. Both **3d** and **3i** were inactive against *E. faecalis*.

The *para* NO_2 flavone **5** showed excellent activity against *S. areus* at 2.3 μM, but did not show any activity against *E. faecalis* and very high MIC values against the Gram negative strains. This was synthesized as a product of one of the reactions, where the target molecule was unable to be synthesized and hence a series of these flavones were not tested for comparison. However, this could be an excellent lead for an antibiotic against *S. aureus*.

Antifungal activity

The same set of compounds were evaluated for their *in vitro* antifungal activity against four *Candida* species comprising *C. albicans ATCC 90028*, *C. albicans ATCC 10231*, *C. krusei ATCC 6258,* and *C. parapsilosis ATCC 22019.* The results are reported in **Table 4-2**. Compounds **3a/4a**, **3d/4d**, **4f**, **4l** and **3m** showed good activity against all four strains of fungi having MIC values of 28.5 to 64.4 μM. Of these, only **3a** had an MIC of 128.7 against *C.*

albicans ATCC 90028. These compounds were either unsubstituted on the phenyl ring (**3a/4a**) or contained a 4-F (**3d/4d**), 3-OCH_3 (**4f**), 3-NO_2 (**4l**) or naphthalene group (**3m**) on the skeleton of the chalcone or flavanone. In addition, **4b** (2-F), showed good activity against both *C. albicans* species (30.8 μM), but not in *C. krusei* and *C. parapsilosis*. These results indicate that the pyran groups on the chalcone or flavanone skeleton is a good scaffold for antifungal activity and that this activity can be increased by fluoro, methoxy and nitro groups being substituted on the phenyl ring or by substituting the phenyl ring with a naphthalene group. However, these compounds were not as active as the standard drug amphotericin-B (Amp-B), whose MIC was at least 1 order of magnitude lower against all the fungal species tested against.

Table 4-2 Minimum Inhibitory Concentration (MIC, μM) of test compounds on *Candida* species

Compound	*C. albicans ATCC 90028*	*C. albicans ATCC 10231*	*C. krusei ATCC 6258*	*C. parapsilosis ATCC 22019*
2	332.9	332.9	332.9	332.9
3a	128.7	64.4	32.2	32.2
4a	64.4	64.4	64.4	64.4
3b	30.8	61.5	246.0	246.0
4b	30.8	30.8	61.5	246.0
3d	30.8	61.5	61.5	61.5
4d	30.8	61.5	30.8	30.8
3e	117.8	117.8	117.8	117.8
4e	-	-	-	-
3f	59.7	59.7	119.5	239.0
4f	29.9	29.9	59.7	29.9
3h	-	-	-	229.1
4h	57.3	57.3	114.6	28.6
3k	53.5	53.5	107.0	107.0
4k	214.0	214.0	214.0	427.9
3l	57.7	57.7	28.8	115.3
4l	28.8	57.7	57.7	57.7
3m	57.0	57.0	28.5	28.5
4m	228.1	228.1	228.1	114.0
Amp-B	1.3	1.3	5.4	1.3

Amp- B indicates Amphotericin-B; "—'' indicates no activity

Antioxidant Activity:

The antioxidant activity of most of the chalcones was comparable to that of the control, ascorbic acid. The chalcones that showed the best activity were **3e** (2,4-difluoro), **3h** (2-F, 3-OCH_3), **3i** (2-Cl) and **3l** (3-NO_2). The flavanones **4i** (2-Cl) and **4l** (3-NO_2) (**Table 4-3**) also showed good antioxidant activity. This indicates that a halogen substituted at position 2 or a withdrawing group such as nitro substituted at position 3 imparts good antioxidant activity on the prenylated chalcones and flavanones.

Table 4-3 Antioxidant activity of the synthesized compounds by the DPPH assay (IC_{50}, μM)

Compound	IC_{50}	Compound	IC_{50}
2	322.16	**4a**	427.25
3a	281.70	**4b**	349.86
3b	418.97	**4c**	339.35
3c	250.26	**4d**	340.29
3d	273.24	**4e**	381.02
3e	230.42	**4f**	291.27
3f	330.51	**4g**	423.92
3g	433.52	**4h**	258.41
3h	233.21	**4i**	225.80
3i	228.73	**4j**	464.55
3j	292.93	**4k**	260.81
3k	269.61	**4l**	234.68
3l	220.30	**4m**	328.68
3m	282.71	**5**	254.96
Ascorbic acid	220.02		

Conclusion

In summary, these findings show that the presence of phenolic hydroxyl and lipophilic pyranochromene rings contribute to the antimicrobial activity of chalcones and flavanones. The chalcones showed better activity than the flavanones in the antibacterial assay and the opposite was shown in the antifungal assays. These pyranochromene containing chalcones

and flavanones could be good lead compounds for antimicrobial agents. The antioxidant assay also indicate that these compounds could be good antioxidant leads.

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Chapter 5. Synthesis and evaluation of pyridine, furan and fluoro-substituted phenyl pyranochromene chalcones for antibacterial and antifungal activity

Abstract

A series of novel pyridine, furan and fluorosubstituted pyranochromene chalcones with different substituents on the phenyl ring were synthesized and tested for their antibacterial activity. The synthesized compounds displayed moderate to significant *in vitro* antibacterial activity with the highest inhibitory activity being shown by the 2-trifluoromethyl derivative (**6**) (MIC of 13.7-27.5 μM) against all Gram negative strains. The X-ray structure of **6** is also reported here.

Keywords: fluorosubstituted, pyranochromene, chalcones, antibacterial, antifungal

Introduction

Pyranochalcones are synthetic or naturally occurring heterocyclic molecules and the precursors to flavonoids, both synthetically and naturally. They consist of two aromatic rings joined by a three-carbon α,β-unsaturated carbonyl system with the pyran rings attached to the aromatic ring derived from the acetophenone portion of the molecule. These pyranochalcones have been associated with a wide variety of biological activities, including antibacterial [1-2] and anticancer [3-4] activity. They have also been reported to be used for neurological problems and inflammation [5-6]. This wide range of biological properties has stimulated interest in the synthesis of naturally occurring pyranochalcones [7-10]. Some of the naturally occurring pyranochalcones were isolated from *Lonchocarpus subglaucescens* [11], *Lonchocarpus utilus* and *Lonchocarpus urucu* [12], *Tephrosia tunicate* [13], *Tephrosia deflexa* [14], *Glycosmis citrifolia* [15] and *Mallotus philippensis* [16-17]. The simple structure of these chalcones and easy method of preparation makes this class of compounds a desirable target for drug scaffolds with the aim of having important therapeutic potential.

The bioactivities of these compounds have prompted us to investigate and develop a convenient and efficient method for synthesizing molecules with a pyranochromene moiety. To establish more advanced structure activity relationships with chromanochalcones, the chroman ring A was fixed, and more diverse substituents (both electron-donating and electron-withdrawing groups) such as methoxy, trifluoromethyl and fluorine groups were introduced at different positions on ring B of the chalcones (**Scheme 5-1**). Further to this, pyridine and furan groups were also incorporated into the chromanochalcone skeleton. The initial aim was to explore the influence that the different substituents and different heterocyclic groups had on biological activity. To the best of our knowledge this is the first report on the synthesis of pyridine, furan and trifluoromethylphenyl chromenochalcones.

Experimental Procedures

General

Reagent grade chemicals used in this study were purchased from Sigma Aldrich through Capital Laboratories, South Africa. Organic solvents were distilled prior to being used and dried according to standard drying procedures. Melting points were measured using a Stuart Scientific Melting Point Apparatus SMP3. IR spectra were recorded on a Perkin Elmer spectrum 100 FT-IR instrument with a universal ATR attachment. All ^{1}H and ^{13}C NMR spectra were recorded on Bruker AVANCE III 400 or 600 MHz instruments in $CDCl_3$ referenced to the internal standard TMS at δ 7.24 and 77.0 for ^{1}H and ^{13}C NMR respectively. For the ^{19}F NMR spectra, the chemical shift of trifluorotoluene (0.05% in $CDCl_3$) was referenced at -62.73 relative to TMS. Chemical shifts are reported in ppm and coupling constants (*J*) in Hz. High-resolution mass data was obtained using a Bruker microTOF-Q II ESI instrument operating at ambient temperatures, with a sample concentration of approximately 1 ppm. Thin layer chromatography (TLC) was performed using Merck Kieselgel 60 F_{254} plates. Crude compounds were purified with column chromatography using silica gel (60–120 mesh).

Synthesis of octandrenolone

Octandrenolone (**2**), was synthesized according to our previous method [18]. Briefly, 2,4,6-trihydroxyacetophenone (2.00 g, 0.0119 mol), 2,3-dimethylbutenal (4.00 g, 0.0476 mol) and pyridine (1.35 g) were stirred for 24 h at 110°C. The reaction was monitored by TLC using EtOAc:hexane (5:95, R_f = 0.6). Upon completion, hydrochloric acid (30 mL) was added to neutralize the reaction mixture, which was then extracted with ethyl acetate (4 × 40 mL). The organic portions were combined, dried over $MgSO_4$ and the solvent evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using

100% hexane as the eluent to afford the pyranochromene as a yellow crystalline solid (2.58 g, yield 72.8%) with a melting point of 89–90 °C;

*1-(5-Hydroxy-2,2,8,8-tetramethyl-2H,8H-pyrano[2,3-f]chromen-6-yl)ethanone (**2**)*

^{1}H NMR (600 MHz, $CDCl_3$): δ 13.99 (1H, s, OH), 6.65 (1H, d, J = 10.1 Hz, H-1'''), 6.58 (1H, d, J = 10.1 Hz, H-1''), 5.45 (1H, d, J = 10.1 Hz, H-2''), 5.43 (1H, d, J = 10.1 Hz, H-2'''), 2.65 (3H, s, CH_3), 1.49 (6H, s, H-4''/5''), 1.43 (6H, s, H-4'''/H-5'''); ^{13}C NMR (150 MHz, $CDCl_3$): 203.5 (C=O), 160.7 (C-2'), 156.9 (C-6'), 155.2 (C-4'), 125.5 (C-2''), 124.9 (C-2'''), 116.6 (C-1''), 116.3 (C-1'''), 105.7 (C-1'), 102.5 (C-3'), 102.4 (C-5'), 78.4 (C-3''), 78.3 (C-3'''), 33.4 (CH_3), 28.5 (C-4''/5''), 28.2 (C-4'''/5'''); IR υ_{max} cm^{-1} (neat): 2972, 2932, 1638, 1592, 1466.

General procedure for the synthesis of the pyranochromene chalcones 3-9

Octandrenolone **2** (150 mg, 0.500 mmol) was dissolved in ethanol (10 mL) and water (2 mL), after which, potassium hydroxide (112 mg, 2.00 mmol) and substituted aldehydes (0.7 mmol) were added at room temperature. The reaction mixture was stirred for 24-48 h at room temperature and monitored by TLC using EtOAc:hexane (10:90, R_f = 0.4). On completion, the solvent was removed under reduced pressure. The residue was dissolved in water (20 mL), acidified with 2 M HCl (20 mL), extracted with ethyl acetate (3 × 30 mL), washed with water and dried over anhydrous $MgSO_4$. Removal of the solvent followed by flash column chromatography on silica gel gave the pure compound as brown solids.

1-(5-Hydroxy-2,2,8,8-tetramethyl-2H,8H-pyrano[2,3-f]chromen-6-yl)-3-pyridin-2-yl-propenone (**3**), brown solid, 70% yield; mp. 65-66 ^{o}C; R_f = 0.6 (EtOAc:Hexane, 10:90); IR υ_{max} cm^{-1}: 2966, 2922, 2852, 1638, 1583; ^{1}H NMR (600 MHz, $CDCl_3$): δ 14.18 (1H, s, OH), 8.66 (1H, d, J = 4.1 Hz, H-2), 8.67 (1H, d, J = 15.4 Hz, H-8), 7.70 (1H, td, J = 8.1, 1.6 Hz, H-

4), 7.64 (1H, d, *J* = 15.4 Hz, H-7), 7.44 (1H, d, *J* = 7.9 Hz, H-5), 7.22-7.24 (1H, m, H-3), 6.68 (1H, d, *J* = 10.0 Hz, H-1'''), 6.59 (1H, d, *J* = 10.2 Hz, H-1''), 5.48 (1H, d, *J* = 10.2 Hz, H-2''), 5.47 (1H, d, *J* = 10.0 Hz, H-2'''), 1.56 (6H, s, H4''/5''), 1.45 (6H, s, H-4'''/5'''); ^{13}C NMR (150 MHz, $CDCl_3$): 193.6 (C-9), 161.5 (C-2'), 156.7 (C-6'), 155.7 (C-4'), 154.3 (C-6), 150.4 (C-2), 140.0 (C-7), 136.7 (C-4), 132.3 (C-8), 125.6 (C-2''), 125.3 (C-2'''), 124.5 (C-5), 123.9 (C-3), 116.5 (C-1''), 116.4 (C-1'''), 106.3 (C-1'), 102.8 (C-3'), 102.7 (C-5'), 78.7 (C-3''), 78.6 (C-3'''), 28.6 (C-4'''/5'''), 28.0 (C-4''/5''); HRMS *m/z* 388.1553 (Calcd. for $C_{24}H_{22}NO_4$, 388.1549).

3-Furan-2-yl-1-(5-hydroxy-2,2,8,8-tetramethyl-2H,8H-pyrano[2,3-f]chromen-6-yl)-propenone (**4**), brown solid; 67% yield; mp. 77-78 ^{o}C; R_f = 0.6 (EtOAc:Hexane, 10:80); IR υ_{max} cm^{-1} (neat): 2972, 2923, 2853, 1631, 1583, 1534; ^{1}H NMR (600 MHz, $CDCl_3$): δ 14.43 (1H, s, OH), 8.04 (1H, d, *J* = 15.4 Hz, H-7), 7.54 (1H, d, *J* = 15.4 Hz, H-6), 7.51 (1H, s, H-2), 6.68 (1H, d, *J* = 10.0 Hz, H-1'''), 6.66 (1H, d, *J* = 3.3 Hz, H-4), 6.59 (1H, d, *J* = 9.8 Hz, H-1''), 6.49-6.50 (1H, m, H-3), 5.47 (1H, d, *J* = 9.8 Hz, H-2''), 5.47 (1H, d, *J* = 10.0 Hz, H-2'''), 1.56 (6H, s, H4''/5''), 1.45 (6H, s, H-4'''/5'''); ^{13}C NMR (150 MHz, $CDCl_3$): 192.6 (C8), 161.7 (C-2'), 156.4 (C-6'), 155.5 (C-4'), 152.6 (C-5), 144.9 (C-2), 128.8 (C-6), 125.5 (C-7), 125.1 (C-2''/2'''), 116.6 (C-1''), 116.4 (C-1'''), 115.4 (C-4), 112.7 (C-3), 106.1 (C-1'), 102.76 (C-3'), 102.75 (C-5'), 78.6 (C-3''), 78.5 (C-3'''), 28.6 (C-4'''/5'''), 28.0 (C-4''/5''); HRMS *m/z* 377.1389 (Calcd. for $C_{23}H_{21}O_5$, 377.1389).

1-(5-Hydroxy-2,2,8,8-tetramethyl-2H,8H-pyrano[2,3-f]chromen-6-yl)-3-(2,4,6-trimethoxyphenyl)-propenone (**5**), brown solid; 72% yield; mp. 89-90 ^{o}C; R_f = 0.6 (EtOAc:Hexane, 10:90); IR υ_{max} cm^{-1}: 2928, 1637, 1603, 1583, 1523; ^{1}H NMR (600 MHz, $CDCl_3$): δ 14.74 (1H, s, OH), 8.26* (1H, d, *J* = 15.8 Hz, H-8), 8.23* (1H, d, *J* = 15.8 Hz, H-

7), 6.69 (1H, d, *J* = 9.9 Hz, H-1'''), 6.60 (1H, d, *J* = 9.8 Hz, H-1''), 6.14 (2H, s, H-3/5), 5.45 (1H, d, *J* = 9.8 Hz, H-2''), 5.44 (1H, d, *J* = 9.9 Hz, H-2'''), 3.88 (6H, s, H-10/12), 3.85 (3H, s, H-11), 1.50 (6H, s, H-4''/5''), 1.44 (6H, s, H-4'''/5'''); ^{13}C NMR (150 MHz, $CDCl_3$): 194.8 (C-9), 163.0 (C-4), 161.8 (C-2/6), 161.6 (C-2'), 156.3 (C-6'), 154.7 (C-4'), 134.1 (C-7), 127.7 (C-8), 125.3 (C-2''), 125.0 (C-2'''), 116.8 (C-1''), 116.7 (C-1'''), 107.4 (C-1), 106.5 (C-1'), 102.7 (C-3'), 102.5 (C-5'), 90.8 (C-3/5), 78.1 (C-3''), 78.0 (C-3'''), 56.1 (C10/12), 55.6 (C-11), 28.5 (C-4'''/5'''), 27.8 (C-4''/5''); HRMS *m/z* 477.1922 (calcd. for $C_{28}H_{29}O_7$, 477.1913).

* resonances coalesce

1-(5-Hydroxy-2,2,8,8-tetramethyl-2H,8H-pyrano[2,3-f]chromen-6-yl)-3-(2-trifluoromethylphenyl)-propenone (**6**), brown solid; 90% yield; mp. 112-113 ^{o}C; R_f = 0.6 (EtOAc:Hexane, 10:90); IR υ_{max} cm^{-1}: 2975, 2928, 1634, 1586, 1549; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.16 (1H, s, OH), 8.08 (1H, dq, *J* = 15.4, 2.0 Hz, H-7), 8.01 (1H, d, *J* = 15.4 Hz, H-8), 7.79 (1H, d, *J* = 7.8 Hz, H-6), 7.72 (1H, d, *J* = 7.8 Hz, H-3), 7.59 (1H, t, *J* = 7. 6 Hz, H4), 7.47 (1H, t, *J* = 7.6 Hz, H-5), 6.69 (1H, d, *J* = 10.0 Hz, H-1'''), 6.60 (1H, d, *J* = 9.9 Hz, H-1''), 5.48 (1H, d, *J* = 9.9 Hz, H-2''), 5.46 (1H, d, *J* = 10.0 Hz, H-2'''), 1.50 (6H, s, H-4''/5''), 1.45 (6H, s, H-4'''/5'''); ^{13}C NMR (100 MHz, $CDCl_3$): 192.5 (C-9), 161.6 (C-2'), 156.4 (C-6'), 155.8 (C-4'), 136.9 (q, *J* = 2.0 Hz, C-7), 134.9 (q, *J* = 1.3 Hz, C-2), 132.1 (C-4), 131.9 (C-8), 129.4 (C-5), 127.7 (C-6), 126.5 (q, *J* = 5.7 Hz, C-3), 125.6 (C-2''), 125.0 (C-2'''), 122.9 (C-1), 116.8 (C-1''), 116.4 (C-1'''), 106.1 (C-1'), 102.9 (C-3'), 102.8 (C-5'), 78.6 (C-3''), 78.6 (C-3'''), 28.6 (C-4''/5''), 28.3 (C-4'''/5'''); ^{19}F NMR ($CDCl_3$, 376.5 MHz) δ -58.84; HRMS *m/z* 455.1470 (calcd. for $C_{26}H_{22}O_4F_3$, 455.1470).

1-(5-Hydroxy-2,2,8,8-tetramethyl-2H,8H-pyrano[2,3-f]chromen-6-yl)-3-(3-trifluoromethylphenyl)-propenone (**7**), brown solid; 87% yield; mp. 74-75 ^{o}C; R_f = 0.6

(EtOAc:Hexane, 10:90); IR υ_{max} cm^{-1}: 2977, 2930, 1632, 1582, 1539; 1454; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.23 (1H, s, OH), 8.16 (1H, d, *J* = 15.7 Hz, H-8), 7.90 (1H, s, H-2), 7.73 (1H, d, *J* = 15.7 Hz, H-7), 7.71 (1H, d, *J* = 7.8 Hz, H-6), 7.62 (1H, d, *J* = 7.8 Hz, H-4), 7.54 (1H, t, *J* = 7.6 Hz, H-5), 6.69 (1H, d, *J* = 10.0 Hz, H-1'''), 6.61 (1H, d, *J* = 9.9 Hz, H-1''), 5.48 (2H, d, *J* = 10.0 Hz, H-2''/2'''), 1.55 (6H, s, H-4''/5''), 1.46 (6H, s, H-4'''/5'''); ^{13}C NMR (100 MHz, $CDCl_3$): 192.6 (C-9), 161.7 (C-2'), 156.5 (C-6'), 155.8 (C-4'), 140.0 (C-7), 136.7 (C-1), 132.0 (C-6), 131.6 (q, *J* = 32.3, C-3), 129.8 (C-5), 129.7 (C-8), 126.4 (q, *J* = 3.7 Hz, C-4), 125.7 (C-2''), 125.1 (C-2'''), 124.2 (q, *J* = 3.8, C-2), 116.8 (C-1''), 116.3 (C-1'''), 106.0 (C-1'), 102.9 (C-3'), 102.8 (C-5'), 78.7 (C-3''), 78.5 (C-3'''), 28.7 (C-4''/5''), 28.2 (C-4'''/5'''); ^{19}F NMR ($CDCl_3$, 376.5 MHz) δ -63.01; HRMS *m/z* 455.1470 (Calcd. for $C_{26}H_{22}O_4F_3$, 455.1470).

1-(5-Hydroxy-2,2,8,8-tetramethyl-2H,8H-pyrano[2,3-f]chromen-6-yl)-3-(4-trifluoromethylphenyl)-propenone (**8**), brown solid, 91% yield; mp. 125-126 ^{o}C; R_f = 0.6 (EtOAc:Hexane, 10:90); IR υ_{max} cm^{-1}: 2979, 2934, 1629, 1581, 1545; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.18 (1H, s, OH), 8.12 (1H, d, *J* = 15.6 Hz, H-8), 7.72 (1H, d, *J* = 15.6 Hz, H-7), 7.67 (4H, br s, H-2/3/5/6), 6.68 (1H, d, *J* = 10.0 Hz, H-1'''), 6.61 (1H, d, *J* = 9.9 Hz, H-1''), 5.49 (1H, d, *J* = 9.9 Hz, H-2''), 5.47 (1H, d, *J* = 9.9 Hz, H-2'''), 1.54 (6H, s, H-4''/5''), 1.46 (6H, s, H-4'''/5'''); ^{13}C NMR (100 MHz, $CDCl_3$): 192.7 (C-9), 161.6 (C-2'), 156.4 (C-6'), 155.9 (C-4'), 139.9 (C-7), 139.3 (C-1), 131.5 (q, *J* = 32.5 Hz, C-4), 130.3 (C-8), 128.4 (C-2/6), 126.1 (q, *J* = 3.8 Hz, C-3/5), 125.7 (C-2''), 125.0 (C-2'''), 116.8 (C-1''), 116.3 (C-1'''), 106.1 (C-1'), 102.9 (C-3'), 102.8 (C-5'), 78.7 (C-3''), 78.6 (C-3'''), 28.7 (C-4''/5''), 28.3 (C-4'''/5'''); ^{19}F NMR ($CDCl_3$, 376.5 MHz) δ -62.79; HRMS *m/z* 455.1472 (Calcd. for $C_{26}H_{22}O_4F_3$, 455.1470).

3-(3,4-Difluorophenyl)-1-(5-hydroxy-2,2,8,8-tetramethyl-2H,8H-pyrano[2,3-f]chromen-6-yl)-propenone (**9**), brown solid, 90% yield, mp. 149-150 ^{o}C; R_f = 0.6 (EtOAc:Hexane, 10:90); IR υ_{max} cm^{-1}: 2972, 2929, 1633, 1587, 1510; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.00 (1H, s, OH), 7.98 (1H, d, *J* = 15.6 Hz, H-8), 7.62 (1H, d, *J* = 15.6 Hz, H-7), 7.39 (1H, ddd, *J* = 10.0, 7.6, 1.8, H-2), 7.29-7.32 (1H, m, H-6), 7.19 (1H, dd, *J* = 17.9, 8.3 Hz, H-5), 6.68 (1H, d, *J* = 10.0 Hz, H-1'''), 6.61 (1H, d, *J* = 9.9 Hz, H-1''), 5.49 (2H, d, 9.9 Hz, H-2''/2'''), 1.54 (6H, s, H-4''/5''), 1.46 (6H, s, H-4'''/5'''); ^{13}C NMR (100 MHz, $CDCl_3$): 192.6 (C-9), 161.6 (C-2'), 156.4 (C-6'), 155.8 (C-4'), 151.5 (dd, *J* = 251.5, 12.9 Hz, C-3), 150.8 (dd, *J* = 247.7, 12.9 Hz, C-4), 139.6 (C-7), 133.2 (dd, *J* = 5.6, 4.4 Hz, C-1), 128.8 (d, *J* = 2.3 Hz, C-8), 125.7 (C-2''), 125.1 (dd, *J* = 6.5, 3.5 Hz, C-6), 124.9 (C-2'''), 118.1 (d, *J* = 17.5 Hz, C-5), 116.8 (C-1''), 116.34 (C-1'''), 116.33 (d, *J* = 17.5 Hz, C-2), 106.1 (C-1'), 102.9 (C-3'), 102.8 (C-5'), 78.7 (C-3''), 78.6 (C-3'''), 28.6 (C-4'''/5'''), 28.3 (C-4''/5''); ^{19}F NMR ($CDCl_3$, 376.5 MHz) δ -134.63 (d, *J* = 20.9 Hz), -136.68 (d, *J* = 20.9 Hz); HRMS *m/z* 423.1409 (Calcd. for $C_{25}H_{21}O_4F_2$, 423.1408).

Single crystal X-Ray diffraction analysis

Cube-shaped single crystals of compound **6** was selected and glued onto a glass fibre tip, mounted in a stream of cold nitrogen at 173(1) K and centred in the X-ray beam using a video camera. Crystal evaluation and data collection were performed on a Bruker Smart APEXII diffractometer with Mo Kα radiation (λ = 0.71073 Å). The diffractometer to crystal distance was set at 4.00 cm. The initial cell matrix was obtained from three series of scans at different starting angles. Each series consisted of 12 frames collected at intervals of 0.5º in a 6º range with the exposure time of 10 seconds per frame. The reflections were successfully indexed by an automated indexing routine built in the APEXII program suite. The final cell constants were calculated from a set of 6460 strong reflections from the actual data collection. Data collection method involved ω scans of width 0.5°. Data reduction was carried out using the

program *SAINT+*. The structure was solved by direct methods using SHELXS and refined. Non-H atoms were first refined isotropically and then by anisotropic refinement with full-matrix least-squares calculations based on F^2 using SHELXS. All H atoms were positioned geometrically and allowed to ride on their respective parent atoms. All H atoms were refined isotropically. The absorption correction was based on fitting a function to the empirical transmission surface as sampled by multiple equivalent measurements. The program *ORTEP*3 was used to prepare artwork representation. The molecular diagrams are drawn with 50% probability ellipsoids. Crystallographic data (excluding structure factors) for the structure in this paper has been deposited with the Cambridge Crystallographic Data Centre, CCDC, 12 Union Road, Cambridge CB21EZ, UK. Copies of the data can be obtained free of charge on quoting the depository numbers CCDC- 1047148 (Fax: +44-1223-336-033; E-Mail: deposit@ccdc.cam.ac.uk, http://www.ccdc.cam.ac.uk).

Antimicrobial activity

Antimicrobial agents: Stock solutions of 2mM of the test compounds were prepared in DMSO and diluted before assays. Neomycin purchased from Sigma Aldrich was used as a reference drug for the antibacterial assays and amphotericin-B was used as a reference for the antifungal assays. The final concentration of antimicrobial agents ranged from 2.5 nM to 530.3 µM, neomycin was between 12.3 nM to 813.5 µM and amphotericin-B from 8.2 nM to 541.4 µM. All drug dilutions were carried out in 96-well flat bottom microtitre plates. The final concentration of DMSO in each well was 2 nM or 0.001%.

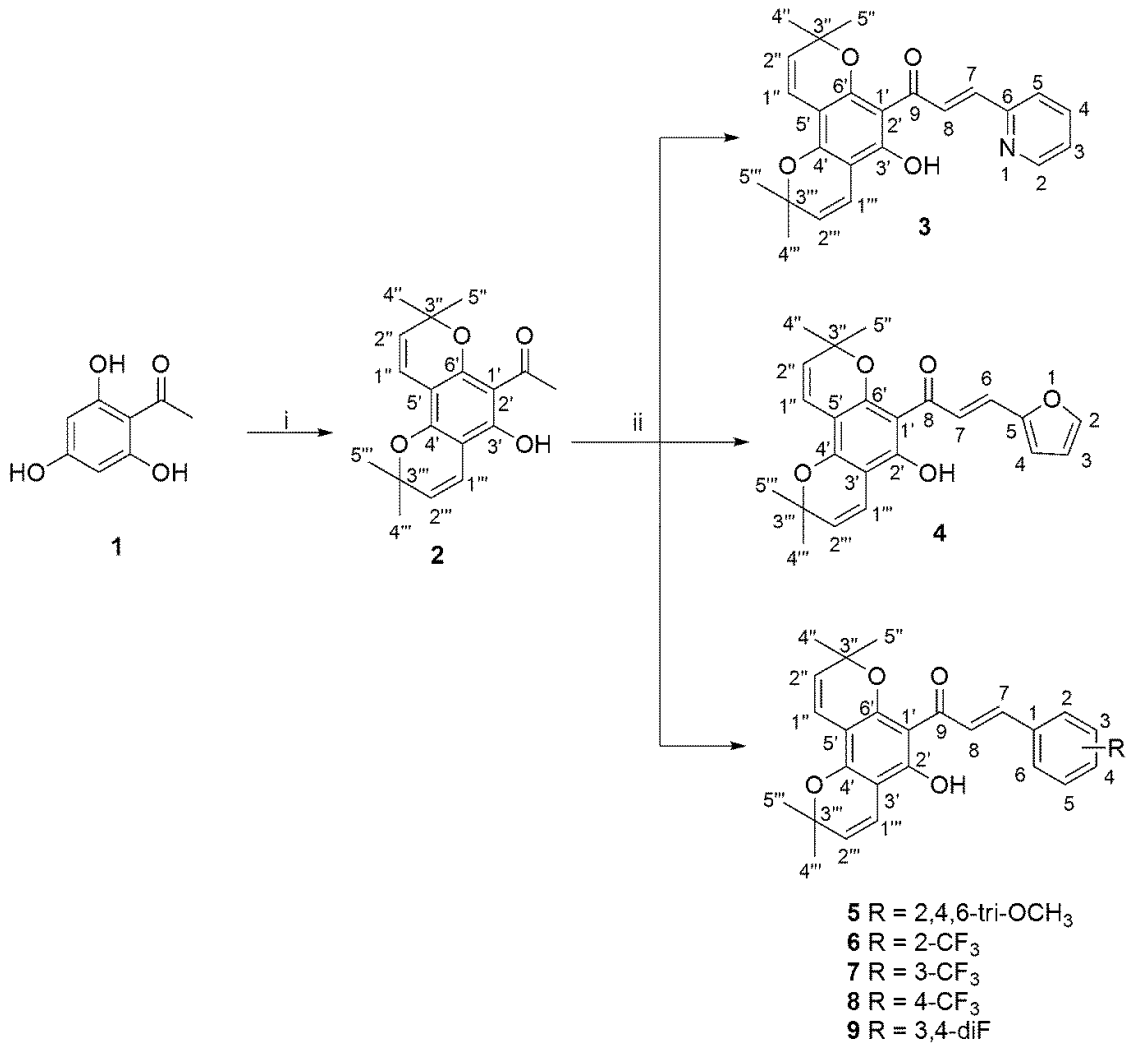
Antimicrobial susceptibility tests: Microbial susceptibility tests were carried out using the micro broth dilution method. Overnight cultures (after 16-18 h of incubation at 37 ºC) were adjusted to the turbidity of a 0.5 McFarland standard. Inocula were adjusted to 0.08 - 0.1 to yield a stock suspension of $0.4\text{-}5\times10^8$ CFU mL^{-1}, which was diluted one hundred fold to

obtain a working suspension of 10^6 CFU mL^{-1} at 625 nm. Microtitre plates were placed in a laminar flow unit to equilibrate to room temperature under aseptic conditions. Aliquots of 100 µL of bacterial/fungal inoculate were added to the microtiter plates containing different concentrations of test compounds. Plates were incubated aerobically for 16-18 h at 37 °C. Following incubation, 40 µL of freshly prepared iodonitrotetrazolium chloride [2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2*H* tetrazolium chloride] (INT) solution (200 µg mL^{-1}) was added to each well and the plate further incubated for 45 minutes at 37 ºC in the dark. Persistent growth of bacteria/fungi is indicated if the colourless INT is reduced to red after incubation. No color change denotes the lack of bacterial growth. All analyses were performed in triplicate.

Results and Discussion

Chemistry

A series of pyranochromene chalcones **3-9** (**Scheme 5-1**) were synthesized in quantitative yields from commercially available 2,4,6-trihydroxy acetophenone (**1**) in two steps, forming the pyranochromene acetophenone, octandrenolone (**2**) in the first step with 3-methyl-2-butenal [18], followed by a Claisen-Schmidt condensation with various benzaldehydes, picolinaldehyde and furan-2-carbaldehyde.



Scheme 5-1 Synthetic route to the pyranochromene chalcones. Reagents: i) 3-methy 2-butenal, pyridine, 110°C, reflux; ii) substituted benzaldehydes, KOH, EtOH/H_2O, rt.

The compounds were characterized by 1D and 2D NMR spectroscopy. In the ^{1}H NMR spectrum of **3** (pyridine derivative), the *trans* α,β-unsaturated double bond occurred at the typical resonances of δ 7.64 (H-7) and δ 8.67 (H-8) with J = 15.4 Hz. The hydroxyl proton occurred as a singlet at δ 14.18. The pyran rings were indicated by the olefinic resonances at δ 6.68 and δ 5.47 (H-1''' and H-2''' respectively) with J = 10.0 Hz and δ 6.59 and δ 5.48 (H-1'' and H-2'' respectively) with J = 10.2 Hz. In addition, the equivalent methyl groups of the

pyran rings could be seen as singlets at δ 1.56 (H-4"/H-5") and δ 1.45 (H-4'"/5'"). The pyridine proton resonances occurred as doublets at δ 8.66 (*J* = 4.1 Hz, H-2) and δ 7.44 (*J* = 7.9 Hz, H-5), a triplet of doublets at δ 7.70 (*J* = 8.1, 1.6 Hz, H-4) and a multiplet at δ 7.22-7.24 (H-3). For the furan derivative **4**, the furan protons were present at δ 7.51 (s, H-2), δ 6.66 (d, *J* = 3.3 Hz, H-4) and δ 6.49-6.50 (m, H-3).

For the 2,4,6-trimethoxy derivative **5**, a singlet was seen for the equivalent H-3 and H-5 protons at δ 6.14. For the 2-trifluoromethylphenyl (2-CF_3) derivative **6**, H-3 and H-6 occurred as doublets at δ 7.72 and δ 7.79 respectively with *J* = 7.8 Hz each and H-4 and H-5 appeared as triplets at δ 7.59 and δ 7.47 respectively with *J* = 7.6 Hz each. In compound **7**, the 3-CF_3 derivative, H-2 occurs as a singlet at δ 7.90, both H-4 and H-6 occur as doublets at δ 7.62 and 7.71 with *J* = 7.8 Hz each and H-5 is present as a triplet at δ 7.54 (*J* = 7.6 Hz). In the 4-CF_3 derivative **8**, the equivalent H-2/6 and H-3/5 resonances co-incided and appeared as a broad singlet at δ 7.67. For the difluorinated phenyl ring in compound **9**, H-F coupling was also evident, which resulted in H-2 being present as a ddd at δ 7.39 with *J* = 10.0, 7.6 and 1.8 Hz, H-5 occurring as a dd at δ 7.19 (*J* = 17.9 and 8.3 Hz) and H-6 occurring as a multiplet at δ 7.29-7.32. C-F coupling was also evident in the ^{13}C NMR spectrum with the two carbon resonances where the F was present occurring as a dd at δ 151.5 (*J* = 251.5, 12.9 Hz, C-3) and δ 150.8 (*J* = 247.7, 12.9 Hz, C-4), the carbon atoms *ortho* to the F occurring as doublets at δ 116.3 (*J* = 17.5 Hz, C-2) and δ 118.1 (*J* = 17.5 Hz, C-5) and C-6 being present as a dd at δ 125.1 (*J* = 6.5, 3.5 Hz).

Crystal structure

The crystal structure of **6** showed the planarity of these molecules with the methyl groups of the pyran rings going into and out of the plane respectively. The *trans* double bond was also

indicated by the torsion angle of C17-C18-C19-C20, which was found to be -177.20º (9) in the crystal. The crystal structure was solved in the monoclinic space group P21/c, with one molecule in the asymmetric unit (**Table 5-1**). The bond length and angles are in agreement with the expected average values for related compounds [18-19]. The carbonyl group is in a *cis* configuration with respect to the olefinic double bond. The crystal structure is stabilized by C=O—O-H intramolecular hydrogen bonding (**Figure 5-1**). Both pyranochromene rings exist in a half chair conformation with Q = 0.3400(10) Å, θ = 113.84(18)°, Ψ = 218.20 (19)° and Q = 0.3382(10) Å, θ = 64.76 (17)° and Ψ = 33.2(2)° respectively. This is similar to our previous reported structures, which also resemble this conformation [18]. The dihedral angle between the least-squares plane of the pyranochromene ring system and its nearest phenyl ring is 15.04 (10)°.

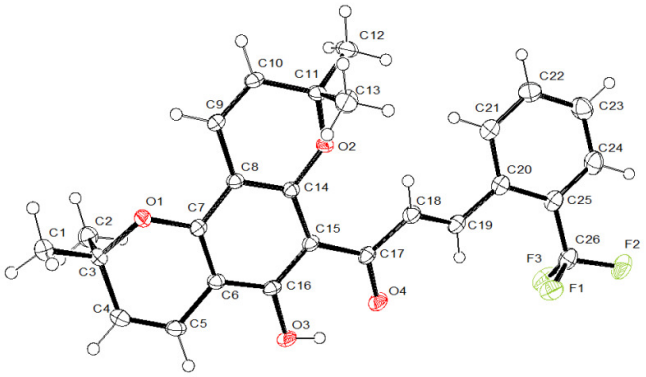


Figure 5-1 Crystal structure of 1-(5-hydroxy-2,2,8,8-tetramethyl-2*H*,8*H*-pyrano[2,3-f]chromen-6-yl)-3-(2-trifluoromethyl-phenyl)-propenone **(6)** (010) plane

Table 5-1 Crystal data and structure refinement for 1-(5-hydroxy-2,2,8,8-tetramethyl-2*H*,8*H*-pyrano[2,3-f]chromen-6-yl)-3-(2-trifluoromethyl-phenyl)-propenone (**6**)

Empirical formula	$C_{26}\ H_{23}\ F_3\ O_4$
Formula weight	456.44
Temperature	173(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	P1
Unit cell dimensions	a = 9.5858(5) Å ; α = 81.443(3)°
	b = 10.0120(5) Å ; β = 80.132(3)°
	c = 12.0479(7) Å ; γ = 74.075(2)°
Volume	1089.11(10) $Å^3$
Z	2
Density (calculated)	1.392 Mg/m^3
Absorption coefficient	0.110 mm^{-1}
F(000)	476
Crystal size	0.32 x 0.17 x 0.16 mm^3
Theta range for data collection	1.73 to 28.31°
Reflections collected	28617
Independent reflections	5420 [R(int) = 0.0195]
Completeness to theta = 28.31°	99.7 %
Goodness-of-fit on F^2	1.048
Final R indices [I>2sigma(I)]	R1 = 0.0354, wR2 = 0.09
R indices (all data)	R1 = 0.0409, wR2 = 0.10

Antimicrobial activity

All synthesized compounds were evaluated for their antimicrobial activity on a panel of five bacterial strains and four fungal strains. For each compound, the minimum inhibitory concentration (MIC) was calculated (**Table 5-2**).

Most of the compounds showed good activity against the Gram negative strains i.e. *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (MICs between 13.7 – 132.6 μM) in comparison to neomycin (16.3 μM). All synthesized compounds did not exhibit any activity against the Gram positive strains (*S. aureus* and *E. faecalis*). The best

activity was shown by compound **6** (2-CF_3 derivative), with MICs of 13.7 μM for *E. coli* and *K. pneumonia* and 27.5 μM for *P. aeruginosa*. The activity against *E. coli* and *K. pneumonia* was similar to the control, neomycin, which had a MIC of 16.3 μM in the same assay. The other compounds showed activity in the order of **7** (3-CF_3 derivative) > **9** (difluoro derivative) > **3** (pyridine derivative) > **5** (trimethoxy derivative) > **4** (furan derivative). Surprisingly, compound **8** (4-CF_3 derivative) showed no activity against these Gram negative strains. In general, the fluorinated derivatives showed better activity than the heterocyclic and trimethoxyphenyl derivatives.

Table 5-2 Antimicrobial activity of pyranochromene chalcones **3-9** (MIC, μM)

	Antibacterial activity			**Antifungal activity**			
Compound	***E. coli ATCC 35218***	***K. pneumonia ATCC 700603***	***P. aeruginosa ATCC 27853***	***C. albicans ATCC 90028***	***C. albicans ATCC 10231***	***C. krusei ATCC 6258***	***C. parapsilosis ATCC 22019***
3	32.2	32.2	32.2	257.6	257.6	128.8	128.8
4	66.3	132.6	132.6	-	-	-	530.3
5	52.4	52.4	52.4	104.8	104.8	209.6	26.2
6	13.7	13.7	27.5	-	-	-	-
7	27.5	27.5	27.5	-	-	-	-
8	-	-	-	27.5	27.5	54.9	54.9
9	29.5	29.5	29.5	-	-	472.7	-
Control	16.3	16.3	16.3	6.8	6.8	3.4	3.4

"-'' indicates no activity. Data reported as the mean ±standard error of the mean ≤5; * Neomycin served as the control for the antibacterial assay and Amphotericin-B as the control for the antifungal assay; All compounds had no activity against *S. aureus* (ATCC 43300) and *E. faecalis* (ATCC 5129)

Four strains of *Candida* were employed in evaluating the antifungal potency of the compounds. Here, only compound **8** (the inactive antibacterial compound), showed appreciable activity against the antifungal strains tested with MICs of between 27.5-54.9 μM (**Table 5-2**). This was 1 to 2 orders of magnitude higher than amphotericin-B, the control, which had MICs ranging from 3.4 – 6.8 μM. All the other compounds showed either high or

no activity. The only exception was compound **5** (trimethoxy derivative), which showed a MIC of 26.2 μM for *C. parapsilosis* only.

Conclusions

Seven novel pryanochalcones bearing pyridine, furan, trimethoxyphenyl, trifluoromethylphenyl and difluorophenyl moieties were synthesized in good yields. Compound **6** (2-CF_3 derivative) showed excellent antibacterial activity against *E. coli*, *K. pneumoniae* and *P. aeruginosa*, comparable to neomycin, with the compounds **7** (3-CF_3 derivative), **9** (difluoro derivative) and **3** (pyridine derivative) also showing comparable activity to neomycin. Only compound **8** (4-CF_3 derivative) showed appreciable activity against the fungal species used in this assay. The data indicates that the CF_3 moiety is a good substituent for antimicrobial activity, better than other heterocyclic rings and the fluoro substituent. The best position for this group with regard to antibacterial activity is C-2 on the B ring of the chalcone and C-4 with regard to antifungal activity.

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Chapter 6. Synthesis, characterization, antimicrobial and antioxidant study of pyranopyrazoline derivatives

Abstract

Novel halogenated pyranochromene pyrazolines **4a – 4k** and *N*-pyrazolines **5a - 5k** were synthesized. The compounds were screened for *in vitro* antimicrobial and antioxidant activity. Compounds **4e** and **4i** showed significant antibacterial activity against a –ve strain of bacteria (*Pseudomonas aeruginosa*). Some of these derivatives also exhibited moderate antifungal activity. These compounds showed moderate to potent antioxidant properties in comparison to ascorbic acid.

Keywords: pryanochromene, pyrazolines, antibacterial, antifungal, antioxidant

Introduction

The overuse of antibiotics has led to microbes becoming resistant to them and some strains are resistant to almost all available antibiotics [1]. In a recent World Health Organisation Report on antimicrobial resistance [2], strains of *E. Coli* and *K. pneumonia* were reportedly resistant to cephalosporins in many regions and in some regions, resistance to carbapenems were also reported. These are antibiotics used as a last resort to these infections. In addition, second line drugs are now being used for MRSA infections as first line drugs are no longer effective [2]. These second line drugs are more expensive and have more side effects. *Streptococcus pneumonia* was also reported to be resistant to penicillin in all WHO areas and diarrhea causing non-typhoidal *Salmonella* and *Shigella* species were reported in some instances to be resistant to the fluoroquinolones [2]. There is thus an urgent need to find new and safe antibiotics, which can be used in the fight against antimicrobial resistance.

Pyrazolines can easily be prepared from the reaction of hydrazine or hydrazine derivatives with chalcone precursors, reacting at the α,β-unsaturated ketone moiety of the molecule. In the last ten years, pyrazolines and their *N*-substituted analogues prepared in this manner have shown promising antimicrobial activity [3-10]. There has been a recent interest in the antibacterial activity of these compounds with numerous reports on the synthesis and antibacterial activity of pyrazolines [11-20]. In addition they have also displayed several other medicinal applications such as antiamoebics [21], monoamine oxidase (MAO) inhibitors [22-23], antioxidants [10, 24-26], anti-inflammatory [25-27] and anticancer agents [26, 28-30]. In search of more potent antibiotics, we herein report an efficient and simple methodology for the synthesis of halogenated pyranopyrazolines from 2,4,6-trihydroxy acetophenone. The synthesized compounds were screened for their *in vitro* antibacterial, antifungal and antioxidant activities.

Experimental

Reagent grade chemicals used in this study were purchased from Sigma Aldrich through Capital Laboratories, South Africa. Organic solvents were distilled prior to being used and dried according to standard drying procedures. Melting points were measured using a Stuart Scientific Melting Point Apparatus SMP3. IR spectra were recorded on a Perkin Elmer spectrum 100 FT-IR instrument with a universal ATR attachment. All ^{1}H and ^{13}C NMR spectra were recorded on Bruker AVANCE III 400 or 600 MHz instruments in $CDCl_3$ referenced to the internal standard TMS at δ 7.24 and 77.0 for ^{1}H and ^{13}C NMR respectively. For the ^{19}F NMR spectra, the chemical shift of trifluorotoluene (0.05% in $CDCl_3$) was referenced at -62.73 relative to TMS. Chemical shifts are reported in ppm and coupling constants (*J*) in Hz. High-resolution mass data was obtained using a Bruker microTOF-Q II ESI instrument operating at ambient temperatures, with a sample concentration of approximately 1 ppm. Thin layer chromatography (TLC) was performed using Merck Kieselgel 60 F_{254} plates. Crude compounds were purified with column chromatography using silica gel (60–120 mesh).

Synthesis of *1-(5-hydroxy-2,2,8,8-tetramethyl-2H,8H-pyrano[2,3-f]chromen-6-yl) ethanone* (**2**)

For the synthesis of octandrenolone (*1-(5-hydroxy-2,2,8,8-tetramethyl-2H,8H-pyrano[2,3-f]chromen-6-yl)ethanone*) (**2**), the method in Pawar et al. [31] was followed and the NMR data compared with those of an authentic sample prepared earlier.

General procedure for the synthesis of pyranochromene-chalcones (3a-k)

The chalcones **3a-k** were prepared according to our previously described methods in chapters 4 and 5 (**Scheme 6-1**). Briefly, substituted benzaldehydes (0.6 mmol) were added at room

temperature along with KOH (112 mg, 2 mmol) to a solution of octandrenolone (**2**) (150 mg, 0.5 mmol) in ethanol (10 mL) and water (2 mL). The reaction mixture was stirred for 24 – 48 h at room temperature and monitored by TLC using EtOAc:hexane (10:90, R_f = 0.4). Thereafter, the solvent was evaporated, dissolved in water (20 mL), neutralised with 2 M HCl (20 mL) and extracted with ethyl acetate (3 × 30 mL), washed with water and dried over anhydrous $MgSO_4$. Removal of the solvent followed by flash column chromatography on silica gel gave the pure compounds **3a – 3k** as brown solids. These compounds are fully characterized in chapters 4 and 5.

Synthesis of pyranochromene-pyrazolines (4a – 4k)

Hydrazine hydrate (80 µL, 1.44 mmol) was added under a nitrogen atmosphere to a solution of substituted chalcone (0.5 mmol) in ethanol (7 mL). The reaction mixture was heated under reflux at 80 ^oC for 2-4 h and the progress monitored by TLC using EtOAc:hexane (10:90, R_f = 0.4). After completion, the resulting solution was cooled and poured onto crushed ice. The solution was filtered and recrystallized from EtOH to yield the solid pyrazolines (**4a-k**), each with Rf values of approximately 0.6 in EtOAc:Hexane (10:90). The ^1H and ^{13}C NMR data of **4a-k** are reported in **Table 6-1** and **Table 6-2**.

6-(5-(2-Chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2,2,8,8-tetramethyl-2,8-dihydropyrano[2,3-f]chromen-5-ol (**4a**) yellow solid; Yield 69%; mp 78-79 ^oC; IR υ_{max} 2973, 2926, 1638, 1593, 1437, 1361 cm^{-1}; HRMS *m/z* 435.1465 $[M-H]^+$ (calcd. for $C_{25}H_{24}N_2O_3Cl$ 435.1475).

6-(5-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2,2,8,8-tetramethyl-2,8-dihydropyrano[2,3-f]chromen-5-ol (**4b**) white solid; Yield 78%; mp 113-114 ^oC; IR υ_{max} 3352, 2969, 2926, 1642, 1592, 1490 1357; HRMS *m/z* 435.1473 $[M-H]^+$ (calcd. for $C_{25}H_{24}N_2O_3Cl$ 435.1475)

6-(5-(4-Bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2,2,8,8-tetramethyl-2,8-dihydropyrano[2,3-f]chromen-5-ol (**4c**) yellow solid; Yield 73%; mp 96-97 ^{o} C; IR υ_{max} 3277, 2972, 2922, 1640, 1592, 1429, 1358 cm^{-1}; HRMS *m/z* 479.0968 $[M-H]^+$ (calcd. for $C_{25}H_{24}N_2O_3Br$ 479.0970).

6-(5-(2-Fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2,2,8,8-tetramethyl-2,8-dihydropyrano[2,3-f]chromen-5-ol (**4d**) yellow solid; Yield 76%; mp 79-80 ^{o}C; ^{19}F NMR (376 MHz, $CDCl_3$) δ -114.60; IR υ_{max} 2974, 2926, 1637, 1591, 1455, 1361 cm^{-1}; HRMS *m/z* 419.1783 $[M-H]^+$ (calcd. for $C_{25}H_{24}N_2O_3F$ 419.1771).

6-(5-(3-Fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2,2,8,8-tetramethyl-2,8-dihydropyrano[2,3-f]chromen-5-ol (**4e**) yellow solid; Yield 69%; mp 89-90 ^{o}C; ^{19}F NMR (376 MHz, $CDCl_3$) δ -112.26; IR υ_{max} 3319, 3282, 2973, 2926, 1634, 1590, 1433, 1356 cm^{-1}; HRMS *m/z* 419.1776 $[M-H]^+$ (calcd. for $C_{25}H_{24}N_2O_3F$ 419.1771).

6-(5-(4-Fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2,2,8,8-tetramethyl-2,8-dihydropyrano[2,3-f]chromen-5-ol (**4f**) brown solid; Yield 70%; mp 91-92 ^{o}C; ^{19}F NMR (376 MHz, $CDCl_3$) δ -114.61; IR υ_{max} 3290, 2971, 2923, 1633, 1592, 1509, 1358 cm^{-1}; HRMS *m/z* 419.1783 $[M-H]^+$ (calcd. for $C_{25}H_{24}N_2O_3F$ 419.1771).

6-(5-(2,4-Difluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2,2,8,8-tetramethyl-2,8-dihydropyrano[2,3-f]chromen-5-ol (**4g**) yellow solid; Yield 80%; mp 55-56 ^{o}C; ^{19}F NMR (376 MHz, $CDCl_3$) δ -111.16 (d, *J* = 7.3 Hz), -114.70 (d, *J* = 7.3 Hz); IR υ_{max} 2973, 2930, 1635, 1594, 1503, 1362 cm^{-1}; HRMS *m/z* 437.1687 $[M-H]^+$ (calcd. for $C_{25}H_{23}N_2O_3F_2$ 437.1677).

6-(5-(3,4-Difluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2,2,8,8-tetramethyl-2,8-dihydropyrano[2,3-f]chromen-5-ol (**4h**) brown solid; Yield 65%; mp 82-83 ^{o}C; ^{19}F NMR (376 MHz, $CDCl_3$) δ -136.75 (d, *J* = 21.2 Hz), -139.12 (d, *J* = 21.2 Hz); IR υ_{max} 3327, 2978, 2926, 1640, 1631, 1613, 1591, 1518, 1366 cm^{-1}; HRMS *m/z* 437.1687 $[M-H]^+$ (calcd. for $C_{25}H_{23}N_2O_3F_2$ 437.1677).

2,2,8,8-Tetramethyl-6-(5-(2-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2,8-dihydropyrano[2,3-f]chromen-5-ol (**4i**) yellow solid; Yield 97%; mp 69-70 ^{o}C; ^{19}F NMR (376 MHz, $CDCl_3$) δ -58.63; IR υ_{max} 3333, 2975, 2932, 1640, 1593, 1455, 1431, 1362, 1311 cm^{-1}; HRMS *m/z* 469.1747 $[M-H]^+$ (calcd. for $C_{26}H_{24}N_2O_3F_3$ 469.1739).

2,2,8,8-Tetramethyl-6-(5-(3-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2,8-dihydropyrano[2,3-f]chromen-5-ol (**4j**) yellow solid; Yield 55%; mp 51-52 ^{o}C; ^{19}F NMR (376 MHz, $CDCl_3$) δ -62.58; IR υ_{max} 2975, 2928, 1639, 1593, 1325 cm^{-1}; HRMS *m/z* 469.1747 $[M-H]^+$ (calcd. for $C_{26}H_{24}N_2O_3F_3$ 469.1739).

2,2,8,8-Tetramethyl-6-(5-(4-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2,8-dihydropyrano[2,3-f]chromen-5-ol (**4k**) white solid; Yield 77%; mp 124-125 ^{o}C; ^{19}F NMR (376 MHz, $CDCl_3$) δ -62.56; IR υ_{max} 3299, 3237, 2981, 2922, 1643, 1614, 1591, 1328 cm^{-1}; HRMS *m/z* 469.1752 $[M-H]^+$ (calcd. for $C_{26}H_{24}N_2O_3F_3$ 469.1739).

General procedure for the synthesis of pyranochromene-*N*-pyrazolines (5a – 5k)

Phenyl hydrazine hydrate (56 µl, 0.52 mmol) was added under nitrogen atmosphere using a micropippette to a solution of substituted chalcone (0.5 mmol) in ethanol (7 mL). The reaction mixture was heated under reflux at 80 ^{o}C for 2 – 4 h and the progress of the reaction

monitored by TLC using EtOAc:hexane (10:90, R_f = 0.4). After completion of the reaction, the resulting solution was cooled and poured onto crushed ice. The solid pyrazoline was filtered and recrystallized from EtOH to produce the pyranochromene-*N*-pyrazolines (**5a-k**). For the ^{1}H and ^{13}C NMR data, see **Table 6-3** and **Table 6-4**.

6-(5-(2-Chlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-2,2,8,8-tetramethyl-2,8-dihydropyrano[2,3-f]chromen-5-ol (**5a**) brown solid; Yield 71%; mp 92-93 ^{o}C; IR υ_{max} 2975, 2927, 1633, 1592, 1500, 1367 cm^{-1}; HRMS *m/z* 511.1778 $[M-H]^+$ (calcd. for $C_{31}H_{28}N_2O_3Cl$ 511.1788).

6-(5-(4-Chlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-2,2,8,8-tetramethyl-2,8-dihydropyrano[2,3-f]chromen-5-ol (**5b**) yellow solid; Yield 79%; mp 177-178 ^{o}C; IR υ_{max} 3042, 2968, 2920, 1646, 1634, 1616, 1592, 1492, 1358 cm^{-1}; HRMS *m/z* 511.1798 $[M-H]^+$ (calcd. for $C_{31}H_{28}N_2O_3Cl$ 511.1788).

6-(5-(4-Bromophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-2,2,8,8-tetramethyl-2,8-dihydropyrano[2,3-f]chromen-5-ol (**5c**) yellow solid; Yield 79%; mp 176-177 ^{o}C; IR υ_{max} 3041, 2963, 2916, 1633, 1616, 1592, 1488, 1357 cm^{-1}; HRMS *m/z* 555.1287 $[M-H]^+$ (calcd. for $C_{31}H_{28}N_2O_3Br$ 555.1283).

6-(5-(2-Fluorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-2,2,8,8-tetramethyl-2,8-dihydropyrano[2,3-f]chromen-5-ol (**5d**) brown solid; Yield 70%; mp 119-120 ^{o}C; ^{19}F NMR (376 MHz, $CDCl_3$) δ -119.08; IR υ_{max} 3057, 2978, 2929, 1644, 1631, 1614, 1590, 1500, 1486, 1366 cm^{-1}; HRMS *m/z* 495.2098 $[M-H]^+$ (calcd. for $C_{31}H_{28}N_2O_3F$ 495.2084).

6-(5-(3-Fluorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-2,2,8,8-tetramethyl-2,8-dihydropyrano[2,3-f]chromen-5-ol (**5e**) brown solid; Yield 66%; mp 166-167 ^{o}C; ^{19}F NMR

(376 MHz, $CDCl_3$) δ -111.78; IR υ_{max} 2974, 2924, 1641, 1610, 1588, 1499, 1363 cm^{-1}; HRMS *m/z* 495.2092 $[M-H]^+$ (calcd. for $C_{31}H_{28}N_2O_3F$ 495.2084)

6-(5-(4-Fluorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-2,2,8,8-tetramethyl-2,8-dihydropyrano[2,3-f]chromen-5-ol (**5f**) brown solid; Yield 60%; mp 110 - 111 ^{o}C; ^{19}F NMR (376 MHz, $CDCl_3$) δ -111.78; IR υ_{max} 3041, 2975, 2923, 1633, 1593, 1497, 1358 cm^{-1}; HRMS *m/z* 495.2092 $[M-H]^+$ (calcd. for $C_{31}H_{28}N_2O_3F$ 495.2084).

6-(5-(2,4-Difluorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-2,2,8,8-tetramethyl-2,8-dihydropyrano[2,3-f]chromen-5-ol (**5g**) yellow solid; Yield 70%; mp 171-172 ^{o}C; ^{19}F NMR (376 MHz, $CDCl_3$) δ -110.95 (d, *J* = 6.77 Hz), -115.02 (d, *J* = 7.14 Hz); IR υ_{max} 3057, 2973, 2923, 1641, 1592, 1501, 1367 cm^{-1}; HRMS *m/z* 513.2006 $[M-H]^+$ (calcd. for $C_{31}H_{27}N_2O_3F_2$ 513.1990).

6-(5-(3,4-Difluorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-2,2,8,8-tetramethyl-2,8-dihydropyrano[2,3-f]chromen-5-ol (**5h**) brown solid; Yield 85%; mp 138-139 ^{o}C; ^{19}F NMR (376 MHz, $CDCl_3$) δ -136.75 (d, *J* = 21.4 Hz), -139.12 (d, *J* = 21.4 Hz); IR υ_{max} 3055, 2968, 2921, 2856, 1643, 1592, 1501, 1360 cm^{-1}; HRMS *m/z* 513.2006 $[M-H]^+$ (calcd. for $C_{31}H_{27}N_2O_3F_2$ 513.1990).

2,2,8,8-Tetramethyl-6-(1-phenyl-5-(2-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2,8-dihydropyrano[2,3-f]chromen-5-ol (**5i**) yellow solid; Yield 80%; mp 130-131 ^{o}C; ^{19}F NMR (376 MHz, $CDCl_3$) δ -59.01; IR υ_{max} 2974, 2926, 2854, 1643, 1629, 1589, 1499, 1363, 1310 cm^{-1}; HRMS *m/z* 545.2070 $[M-H]^+$ (calcd. for $C_{32}H_{28}N_2O_3F_3$ 545.2052).

2,2,8,8-Tetramethyl-6-(1-phenyl-5-(3-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2,8-dihydropyrano[2,3-f]chromen-5-ol (**5j**) yellow solid; Yield 88%; mp 62-63 ^{o}C; ^{19}F NMR (376 MHz, $CDCl_3$) δ -62.58; IR υ_{max} 2975, 2928, 1641, 1593, 1499, 1325 cm^{-1}; HRMS *m/z* 545.2064 $[M-H]^+$ (calcd. for $C_{32}H_{28}N_2O_3F_3$ 545.2052).

2,2,8,8-Tetramethyl-6-(1-phenyl-5-(4-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2,8-dihydropyrano[2,3-f]chromen-5-ol (**5k**) yellow solid; Yield 71%; mp 168-169 ^{o}C; ^{19}F NMR (376 MHz, $CDCl_3$) δ -62.51; IR υ_{max} 2975, 2928, 1645, 1631, 1591, 1499, 1360, 1323 cm^{-1}; HRMS *m/z* 545.2070 $[M-H]^+$ (calcd. for $C_{32}H_{28}N_2O_3F_3$ 545.2052).

Pharmacological evaluation

***In vitro* antioxidant studies**

All synthesized compounds were screened for their antioxidant properties using the DPPH radical scavenging and the H_2O_2 scavenging assay. These assays were chosen since they were "in house" assays that provide a good indication of antioxidant activity.

DPPH radical scavenging activity

Free radical scavenging activity was determined by using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) method described by Burits and Bucar [32]. The synthesized compounds were dissolved in methanol and made to various concentrations of which 1 mL was added to 4 mL of a 0.004% solution of DPPH made up in methanol. After a 30 min incubation period at room temperature, the absorbance was read against a methanol blank at 517 nm. The percentage scavenging activity of free radicals by DPPH was calculated by using the following equation:

% scavenging activity = [(A control – A sample) / A blank] x 100.

Where A control is the absorbance of the control reaction (containing all reagents except the test compound), and A sample is the absorbance of the test compound.

H_2O_2 scavenging activity

The H_2O_2 scavenging activity of pyrazolines was determined according to the method of Ruch et al. [33]. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations of compounds made up in 3.4 mL phosphate buffer were added to the H_2O_2 solution (0.6 mL). The absorbance value of the reaction mixture was recorded at 230 nm. The % scavenging activity was calculated as follows:

% scavenging activity = [(A control – A sample) / A blank] x 100,

Where "A control" is the absorbance of the control reaction (containing all reagents except the test compound), and "A sample" is the absorbance of the test compound.

Antimicrobial assay

Antifungal/antibacterial agents: All the synthesized compounds were dissolved in DMSO and diluted accordingly before being assayed. Amphotericin-B and neomycin were purchased from Sigma Aldrich and used as reference drugs for antifungal and antibacterial assays respectively. The final concentration of the test compounds ranged from 0.0012 to 200 µg/mL. Amphotericin-B ranged from 0.0015 to 100 µg/mL and neomycin from 0.0076 to 500 µg/mL. All drug dilutions were carried out in 96-well flat bottom microtitre plates. The final concentration of DMSO in each well was 2 nM or 0.001%.

Antifungal susceptibility test: Evaluation of the susceptibility of *Candida albicans* and non-*Candida albicans* species to the synthesized compounds were performed using the broth microdilution method according to M27-A2 for yeast guidelines. Yeast strains were grown aerobically overnight at 35 °C on Sabouraud dextrose agar plates. Yeasts were harvested and

suspended in 1% sterile saline solution and the turbidity of the supernatants measured spectrophotometrically at 625 nm with an absorbance of 0.08 - 0.1 equivalents to a 0.5 McFarland standard following the NCCLS M27-A2 guidelines. The working suspension was diluted 1:20 in a mixture containing RPMI 1640 medium and 0.165 M morpholinepropanesulfonic acid buffered to pH 7.0. The working suspension was further diluted with the medium (1:50) to obtain the final test inoculums (1-5x10^3 CFU mL). The microtitre plates were allowed to thaw and equilibrate to room temperature under aseptic conditions and different concentrations of test compounds were added to each well. Aliquots of working inoculum suspensions were dispensed into each well and the plates incubated in an aerobic environment at 35 °C for 24 h. After incubation, 20 µL of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium salt (MTS, Promega Corporation, Madison, USA) was added directly to each well, incubated at 37 ºC for 4 h and the absorbance recorded at 490 nm on a 96-well plate reader (Biotek, Powerwave XS2). All analyses were performed in triplicate and data are reported as the mean ± standard error of the mean of ≤ 5.

Antibacterial susceptibility test

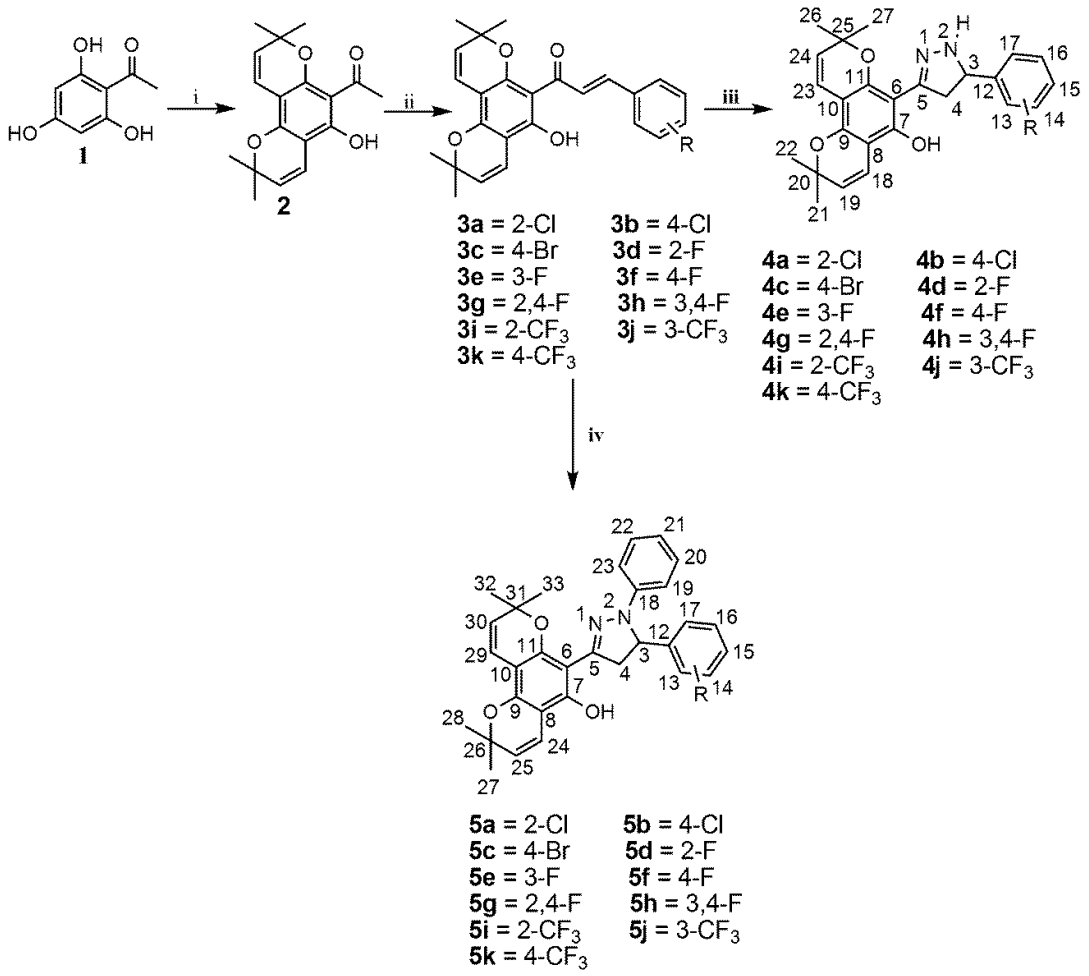
Bacterial susceptibility test was carried out using the broth microdilution method. Cultures were incubated overnight for 16 - 18 h at 37 ºC and adjusted to a turbidity equivalent to that of a 0.5 McFarland standard. The inoculum was adjusted to 0.08 - 0.1 to yield a stock suspension of 0.4-5x10^8 CFU/mL, which was diluted one hundred fold to obtain a working suspension of 10^6 CFU/mL at 625 nm. Microtitre plates were placed in a laminar flow unit to equilibrate to room temperature under aseptic conditions. Aliquots of 100 µL of bacterial inoculate were added to the microtiter plates containing different concentrations of test compounds. Plates were incubated aerobically for 16 - 18 h at 37 °C. Following incubation, 40 µL of freshly prepared iodonitrotetrazolium chloride (INT) (2-(4-iodophenyl)-3-(4-

nitrophenyl)-5-phenyl-2H tetrazolium chloride) solution (200 μg mL^{-1}) was added to each well and the plate further incubated for 45 minutes at 37 ºC in the dark. If the colorless INT is reduced to red after incubation, persistent growth of bacteria is indicated. No color change denotes the lack of bacterial growth. Neomycin was used as a control drug in this study. All analyses were performed in triplicate and data are reported as the mean ± standard error of the mean of ≤ 5.

Results and discussion

Chemistry

The synthesis of the pyrazolines was a three step reaction starting with the prenylation of 2',4',6'-trihydroxyacetophenone using β-methyl crotonaldehyde. This formed two dimethyl pyran rings at the 2- and 4- positions resulting in the prenylated acetophenone intermediate **2**, which was condensed with various benzaldehydes by a Claisen-Schmidt condensation to form the chalcones (**3a-k**) at room temperature under basic conditions with potassium hydroxide and ethanol. The chalcones were then converted to the pyrazolines with either hydrazine or phenylhydrazine to produce the corresponding pyrazoline analogues as racemic mixtures (**4a-k** and **5a-k**, respectively) as illustrated in **Scheme 6-1**. This reaction probably takes places through *in situ* formation of an appropriate β unsaturated hydrazone intermediate which immediately cyclizes to form pyrazolines. The series of pyranochromene pyrazolines synthesized contained a single fluorine atom or trifluoromethyl group at the *ortho, meta* and *para* positions of the phenyl ring as well fluorine at the 2',4'- and 3',4'- positions on the phenyl ring. These substitution patterns were chosen to observe the effect of fluorine on biological activity as well as to determine the best position for these groups. For comparison, 2-Cl, 4-Cl and 4-Br analogues were also synthesized.



Scheme 6-1 General synthesis of chalcones and pyrazoline compounds. Reagents and conditions: (i) 3-methy 2-butenal, pyridine, 110 °C reflux, 48 hrs; (ii) substituted benzaldehyde, KOH, EtOH/H_2O, 5 – 16 hrs; (iii) NH_2NH_2, EtOH, 70 °C, 2 - 4 hrs; (iv) $PhNHNH_2$, EtOH, 70 °C, 2 - 4 hrs.

Structural elucidation

The structures of the pyrazoline derivatives were established on the basis of IR and NMR spectroscopy (^1H, ^{13}C and 2D). The full characterization (^1H and ^{13}C NMR assignments) of the pyranochromene pyrazolines **4a-k** and **5a-k** are presented in **Table 6-1** -**Table 6-4** respectively. The spectra of these compounds were well resolved and with the aid of HSQC, HMBC, COSY and NOESY data as well as multiplicity patterns, unambiguous chemical shift assignments were made.

For the pyranochromene pyrazoline, typical ^{1}H resonances can be observed for the pyran rings and the pyrazoline moiety. Taking the 3-fluoro derivative (**4e**) as an example (**Figure 6-1**), the presence of the pyrazoline ring was indicated by the typical ABX system of H-4a (δ 3.30, *J* = 17.6, 8.9 Hz), H-4b (δ 3.80, *J* = 17.6, 10.5 Hz) and H-3, which appeared as a triplet due to second order coupling at δ 4.76 (*J* = 9.7 Hz). Coupling between H-3, H-4a and H-4b was clearly seen in the COSY spectrum. C-3 showed HMBC correlations to H-13 (δ 7.10, d, *J* = 9.8 Hz) and H-17 (δ 7.15, d, *J* = 7.7 Hz), allowing these two aromatic protons to be distinguished from H-15 at δ 7.00 (td, *J* = 8.3, 2.0 Hz) and H-16 at δ 7.32 (dd, *J* = 13.8, 7.8 Hz), which in turn could be distinguished by their coupling constants. HMBC correlations between H-4a and H-4b with C-12 and C-5 at δ 145.5 (d, *J* = 6.6 Hz) and δ 154.4 allowed these two carbon resonances to be assigned.

The olefinic protons on the pyran rings appeared as two pairs of doublets with *J* = 10.0 Hz at δ 6.71 (H-18) and δ 5.47 (H-19) and δ 6.61 (H-23) and δ 5.40 (H-24), each pair being coupled in the COSY spectrum. HMBC correlations between these olefinic protons and the carbon resonances overlapping with the solvent peak at δ 77.3 allowed C-20 and C-25 to be assigned to this resonance. The methyl groups, H-21/H-22 at δ 1.43 and 1.42, and H-26/H-27 at δ 1.41 and 1.37 were assigned based on HMBC correlations with C-19 and C-24 respectively. The NH proton of the pyrazoline unit and the hydroxyl proton on the phenyl ring A appeared as two broad singlets at δ 5.73 and δ 12.36 respectively.

The carbon resonance of C-8 (δ 103.2) and C-10 (δ 102.8) were assigned due to HMBC correlations with H-19 and H-24 respectively and C-6, the other non-oxygenated aromatic proton at δ 100.0 was then assigned to C-6. The C-9 and C-11 oxygenated carbon resonances on the A ring at δ 150.8 and δ 152.7 could be assigned since C-9 showed HMBC correlations

to both H-18 and H-23 while C-11 showed HMBC correlations to H-23 only. The C-7 oxygenated carbon resonance at δ 155.5 was assigned as such, since it showed HMBC correlations to H-18. The six carbon resonances on the fluorinated B ring each appeared as doublets with C-14, directly bonded to fluorine having the largest *J* value of 244.9 Hz, followed by the two carbon resonances *ortho* to the fluorine (C-13 at δ 113.7, *J* = 21.7 Hz and C-15 at δ 114.9, *J* = 21.0 Hz), the two *meta* carbon resonances (C-12 at δ 145.5, *J* = 6.6 Hz; C-16 at δ 130.6, *J* = 8.2 Hz) and the *para* carbon resonance at δ 122.3 (d, *J* = 2.8 Hz).

The pyrazolines **5a-k** had similar NMR resonances, with the exception of extra aromatic proton resonances between δ 6.81 to 7.23, for example H-19/23 occurring as a doublet at δ 6.86 (*J* = 8.1 Hz), H-20/22 occuring as a multiplet at δ 7.16-7.23 and H-21 occurring as a triplet at δ 6.81 (*J* = 7.6 Hz).

Table 6-1 ^{1}H NMR data of pyranochromene pyrazolines **4a** – **4k** (400 MHz, $CDCl_3$, *J* in Hz)

No.	4a	4b	4c	4d	4e	4f	4g	4h*	4i	4j	4k
OH	12.39 (bs)	12.36 (bs)	12.35 (bs)	12.39 (bs)	12.36 (bs)	12.39 (bs)	12.39 (bs)	12.30 (bs)	12.35 (bs)	12.38 (bs)	-
NH	5.72-5.80 (bs)	5.70 (bs)	5.71 (bs)	-	5.73 (bs)	5.70 (bs)	-	5.72 (bs)	5.76 (bs)	-	-
3	5.18 (t, 9.8)	4.74 (t, 9.7)	4.73 (t, 9.7)	5.05 (t, 9.7)	4.76 (t, 9.7)	4.75 (t, 9.7)	5.03 (t, 9.8)	4.73 (t, 9.9)	5.19 (t, 9.9)	4.83 (t, 10.2)	4.85 (t, 9.7)
4a	3.22 (dd, 17.6, 9.8)	3.27 (dd, 17.6, 9.7)	3.27 (dd, 17.6, 9.7)	3.28 (dd, 17.5, 9.0)	3.30 (dd, 17.6, 9.0)	3.29 (dd, 17.6, 8.8)	3.24 (dd, 17.4, 9.2)	3.24 (dd, 17.6, 9.4)	3.25 (dd, 17.8, 9.1)	3.27 (dd, 17.6, 9.7)	3.31 (dd, 17.6, 9.1)
4b	3.95 (dd, 17.6, 9.8)	3.79 (dd, 17.6, 9.7)	3.79 (dd, 17.6, 9.7)	3.81 (dd, 17.5, 10.5)	3.81 (dd, 17.6, 10.5)	3.79 (dd, 17.6, 10.5)	3.84 (dd, 17.5, 10.6)	3.80 (dd, 17.6, 10.4)	3.90 (dd, 17.8, 10.6)	3.86 (dd, 17.6, 10.5)	3.86 (dd, 17.6, 10.5)
13	-	7.32 (bs)	7.26 (d, 8.3)	-	7.10 (d, 9.8)	7.35 (dd, 8.0, 5.6)	-	7.22-7.25 (m)	-	7.66 (s)	7.51 (d, 8.0)
14	7.39 (dd, 7.6, 1.1)	7.32 (bs)	7.47 (d, 8.4)	7.24-7.27 (m)	-	7.03 (t, 8.5)	6.89 - 6.80 (m)	-	7.66 (d, 7.9)	-	7.62 (d, 8.0)
15	7.27-7.29 (m)	-	-	7.09-7.03 (m)	7.00 (td, 8.3, 2.0)	-	-	-	7.39 (t, 7.6)	7.56 (d, 7.8)	-
16	7.20-7.23 (m)	7.32 (bs)	7.47 (d, 8.4)	7.01-7.06 (m)	7.32 (dd, 13.8, 7.8)	7.03 (t, 8.5)	6.89 - 6.80 (m)	7.11 - 7.16 (m)	7.56 (t, 7.6)	7.48 (t, 7.7)	7.62 (d, 8.0)
17	7.59 (dd, 7.6, 1.5)	7.32 (bs)	7.26 (d, 8.3)	7.43-7.47 (m)	7.15 (d, 7.7)	7.35 (dd, 8.0, 5.6,8.0)	7.47 (dd, 14.8, 8.0)	7.07 - 7.11 (m)	7.83 (d, 7.9)	7.60 (d, 7.7)	7.51 (d, 8.0)
18	6.71 (d, 10.0)	6.71 (d, 9.9)	6.71 (d, 9.9)	6.68 (d, 9.8)	6.71 (d, 10.0)	6.71 (d, 9.9)	6.70 (d, 10.0)	6.71 (d, 9.9)	6.71 (d, 10.0)	6.71 (d, 9.9)	6.71 (d, 9.9)
19	5.46 (d, 10.0)	5.47 (d, 9.9)	5.47 (d, 9.9)	5.43 (d, 9.9)	5.47 (d, 10.0)	5.47 (d, 9.9)	5.47 (d, 10.0)	5.47 (d, 9.9)	5.47 (d, 10.0)	5.47 (d, 9.9)	5.47 (d, 9.9)
21	1.43 (s)	1.43 (s)	1.43 (s)	1.42 (s)	1.43 (s)	1.43 (s)	1.43 (s)	1.43 (s)	1.43 (s)	1.44 (s)	1.43 (s)
22	1.42 (s)	1.42 (s)	1.42 (s)	1.40 (s)	1.42 (s)	1.42 (s)	1.43 (s)	1.42 (s)	1.42 (s)	1.43 (s)	1.42 (s)
23	6.60 (d, 9.8)	6.60 (d, 9.8)	6.60 (d, 9.8)	6.57 (d, 9.9)	6.61 (d, 10.0)	6.60 (d, 10.0)	6.60 (d, 9.9)	6.60 (d, 9.8)	6.60 (d, 10.0)	6.60 (d, 9.9)	6.60 (d, 9.9)
24	5.40 (d, 9.8)	5.40 (d, 9.8)	5.40 (d, 9.8)	5.37 (d, 9.9)	5.40 (d, 10.0)	5.40 (d, 10.0)	5.40 (d, 9.9)	5.40 (d, 9.8)	5.39 (d, 10.0)	5.40 (d, 9.9)	5.40 (d, 9.9)
26	1.42 (s)	1.40 (s)	1.40 (s)	1.39 (s)	1.40 (s)	1.40 (s)	1.42 (s)	1.41 (s)	1.39 (s)	1.41 (s)	1.40 (s)
27	1.37 (s)	1.36 (s)	1.36 (s)	1.35 (s)	1.37 (s)	1.37 (s)	1.38 (s)	1.37 (s)	1.34 (s)	1.36 (s)	1.36 (s)

Table 6-2 ^{13}C NMR data of pyranochromene pyrazolines **4a – 4k** (100 MHz, $CDCl_3$, *J* in Hz)

No.	4a	4b	4c	4d†	4e	4f	4g*	4h*	4i	4j	4k
3	59.2	62.1	62.1		62.2 (d, 1.4)	62.0	55.3	61.9	57.8	62.4	62.3
4	44.0	45.9	45.9		45.8	45.9	44.3	46.0	46.4	46.0	46.0
5	154.5	154.4	154.4		154.4	154.4	154.7	154.3	154.0	154.4	154.4
6	100.1	100.0	100.0		100.0	100.0	100.0	99.9	99.9	99.9	99.9
7	155.5	155.5	155.5		155.5	155.5	155.5	155.5	155.5	155.5	155.5
8	103.2	103.2	103.2		103.2	103.2	103.2	103.2	103.2	103.2	103.2
9	150.7	150.8	150.8		150.8	150.7	150.8	150.9	150.8	150.8	150.9
10	102.8	102.8	102.8		102.8	102.8	102.8	102.8	102.8	102.8	102.8
11	152.7	152.6	152.6		152.7	152.6	152.7	152.7	152.7	152.7	152.7
12	140.0	141.4	141.9		145.5 (d, 6.6)	138.6 (d, 3.1)	-	139.8 (t, 2.92)	141.8	143.8	146.8
13	133.1	129.1	128.5		113.7 (d, 21.7)	128.4 (d, 8.0)	162.5 (dd, 247.3, 12.1)	115.8 (d, 17.5)	123.2#	123.8 (q, 3.8)	127.2
14	125.2	128.1	132.1		163.3 (d, 244.9)	116.0 (d, 21.3)	104.1 (t, 25.4)	150.1 (dd, 247.6, 12.3)	126.1 (q, 5.8)	131.3 (q, 31.7)	126.0 (q, 3.8)
15	117.0	133.7	121.8		114.9 (d, 21.0)	162.6 (d, 244.5)	160.6 (dd, 247.1, 11.3)	150.7 (dd, 244.1, 8.9)	127.7	124.8 (q, 3.8)	##
16	117.2	128.1	132.1		130.6 (d, 8.2)	116.0 (d, 21.3)	111.8 (dd, 21.0, 3.2)	117.8 (d, 17.2)	132.9	129.6	126.0 (q, 3.8)
17	125.9	129.1	128.5		122.3 (d, 2.8)	128.4 (d, 8.0)	128.6 (dd, 9.2, 5.7)	122.7 (dd, 5.7, 3.4)	127.8	130.2	127.2
18	127.5	117.2	117.2		117.2	117.2	117.2	117.2	117.2	117.2	117.2
19	129.9	125.9	125.9		125.9	125.9	125.9	125.9	125.9	125.9	126.0
20	77.3	77.3	77.3		77.3	77.3	77.4	77.4	77.3	77.4	77.4
21	28.3	28.3	28.3		28.3	28.3	28.30	28.29	28.3	28.3	28.3
22	28.2	28.2	28.2		28.2	28.2	28.26	28.26	28.2	28.2	28.2
23	127.4	117.0	117.0		117.1	117.1	117.1	117.1	117.0	117.0	117.0
24	128.9	125.2	125.2		125.2	125.2	125.2	125.2	125.3	125.2	125.2
25	77.2	77.3	77.3		77.3	77.3	77.4	77.3	77.3	77.4	77.4
26	28.0	28.0	28.0		28.0	28.0	28.02	28.02	28.0	28.01	28.0
27	27.9	27.8	28.0		28.0	28.0	27.98	28.00	27.8	27.97	27.9

†^{13}C NMR data could not be acquired as the sample is unstable and decomposes on standing in solution; * NMR at 600 MHz; # quartet could not be observed; ## could not be observed.

Table 6-3 ^{1}H NMR data of pyranochromene *N*-pyrazolines **5a** – **5k** (400 MHz, $CDCl_3$, *J* in Hz)

No.	5a	5b	5c	5d	5e	5f	5g	5h	5i	5j	5k
OH	12.21 (s)	12.20 (s)	12.23 (s)	-	12.20 (s)	12.15 (s)	12.15 (s)	12.06 (s)	-	-	12.16 (s)
3	5.45 (dd, 12.4, 7.3)	5.04 (dd, 12.1, 7.8)	5.06 (dd, 8.0, 11.5)	5.42 – 5.38 (m)	5.05 (dd, 12.0, 7.8)	4.98 (dd, 12.0, 7.7)	5.35 (dd, 12.1,6.9)	4.94 (dd, 12.0, 7.7)	5.60 - 5.50 (m)	5.53 - 5.50 (m)	5.13 (dd, 12.1, 7.8)
4a	3.36 (dd, 18.4, 7.0)	3.37 (dd, 18.4, 7.8)	3.41 (dd, 18.2, 7.7)	3.42 (dd, 18.3, 7.0)	3.40 (dd, 18.4, 7.8)	3.31 (dd, 18.3, 7.7)	3.39 (dd, 18.3, 6.9)	3.30 (dd, 18.3, 7.6)	3.38 (dd, 18.6, 6.9)	3.38 (dd, 18.6, 6.8)	3.39 (dd, 18.4, 7.8)
4b	4.30 (dd, 18.5, 12.4)	4.15 (dd, 18.3, 12.1)	4.19 (dd, 18.2, 12.3)	4.20 (dd, 18.3, 12.2)	4.16 (dd, 18.4, 12.2)	4.08 (dd, 18.4, 12.2)	4.18 (dd, 18.3, 12.1)	4.07 (dd, 18.3, 12.1)	4.23 (dd, 18.6, 12.4)	4.23 (dd, 18.6, 12.4)	4.20 (dd, 18.4, 12.2)
13	-	7.28 (d, 8.6)	7.27 (d, 7.8)	-	7.14 (d, 7.7)	7.25 (dd, 8.7, 5.4)	-	7.23 - 7.21 (m)	-	7.75 (d, 7.8)	7.47 (d, 8.1)
14	7.45 (d, 7.4)	7.33 (d, 8.6)	7.51 (d, 7.8)	7.14-7.09 (m)	-	7.11 (t, 7.9)	6.80 (t, 7.3)	-	7.75 (d, 7.8)	-	7.62 (d, 8.2)
15	7.23 – 7.16 (m)	-	-	7.09 - 7.04 (m)	6.97 (dt, 8.4, 2.1)	-	-	-	7.47 – 7.44 (m)	7.47 – 7.44 (m)	-
16	7.45 (d, 7.4)	7.33 (d, 8.6)	7.51 (d, 7.8)	7.09 - 7.04 (m)	7.32 (ddd, 12.5, 6.2, 1.5)	7.11 (t, 7.9)	6.89 (d, 8.2)	7.20 - 7.19 (m)	7.39-7.37 (m)	7.40 - 7.37 (m)	7.62 (d, 8.2)
17	7.23 – 7.16 (m)	7.28 (d, 8.6)	7.27 (d, 7.8)	7.25 - 7.19 (m)	7.06 (d, 9.1)	7.28 – 7.22 (m)	7.21 (t, 7.8)	7.14 - 7.07 (m)	7.47 - 7.44 (m)	7.47 - 7.44 (m)	7.47 (d, 8.1)
19/23	6.86 (d, 8.1)	6.88 (d, 8.1)	6.91 (d, 8.1)	6.91 (d, 8.1)	6.90 (d, 8.0)	6.82 (d, 8.0)	6.89 (d, 8.2)	6.80 (d, 8.1)	6.85 (d, 8.2)	6.85 (d, 8.1)	6.87 (d, 8.0)
20/22	7.16 – 7.23 (m)	7.18 (t, 8.4)	7.22 (t, 8.1)	7.25 - 7.19 (m)	7.19 (t, 7.6)	6.95 (t, 8.6)	7.21 (t, 7.8)	7.06 - 7.01 (m)	7.19 (t, 7.9)	7.19 (t, 7.9)	7.19 (dd, 7.6, 8.4)
21	6.81 (t, 7.6)	6.81 (t, 7.4)	6.85 (t, 7.0)	6.81 (t, 7.3)	6.82 (t, 7.3)	6.74 (t, 7.4)	6.84 (t, 7.8)	6.75 (t, 7.4)	6.81 (t, 7.4)	6.81 (t, 7.4)	6.83 (t, 7.3)
24	6.76 (d, 9.9)	6.75 (d, 9.9)	6.79 (d, 9.8)	6.75 (d, 9.9)	6.76 (d, 10.0)	6.69 (d, 9.9)	6.75 (d, 10.0)	6.67 (d, 9.9)	6.76 (d, 10.0)	6.76 (d, 10.0)	6.75 (d, 10.0)
25	5.51 (d, 10.0)	5.51 (d, 10.0)	5.55 (d, 10.0)	5.50 (d, 10.0)	5.51 (d, 10.0)	5.44 (d, 10.0)	5.50 (d, 10.0)	5.43 (d, 9.9)	5.60 – 5.50 (m)	5.53 - 5.50 (m)	5.51 (d, 10.0)
27	1.45 (s)	1.44 (s)	1.48 (s)	1.45 (s)	1.45 (s)	1.38 (s)	1.45 (s)	1.37 – 1.36 (m)	1.45 (s)	1.45 (s)	1.45 (s)
28	1.45 (s)	1.43 (s)	1.48 (s)	1.43 (s)	1.44 (s)	1.36 (s)	1.43 (s)	1.35 (s)	1.43 (s)	1.43 (s)	1.44 (s)
29	6.60 (d, 9.9)	6.60 (d, 9.9)	6.64 (d, 9.8)	6.60 (d, 9.9)	6.60 (d, 9.8)	6.54 (d, 9.9)	6.60 (d, 9.8)	6.59 (d, 9.8)	6.59 (d, 9.8)	6.59 (d, 9.8)	6.60 (d, 9.9)
30	5.40 (d, 9.8)	5.40 (d, 9.9)	5.44 (d, 9.8)	5.42 - 5.38 (m)	5.40 (d, 9.8)	5.33 (d, 9.8)	5.41 (d, 9.9)	5.33 (d, 9.8)	5.40 (d, 9.9)	5.39 (d, 9.9)	5.40 (d, 9.9)
32	1.43 (s)	1.40 (s)	1.44 (s)	1.42 (s)	1.41 (s)	1.33 (s)	1.42 (s)	1.33 (s)	1.39 (s)	1.39 (s)	1.41 (s)
33	1.43 (s)	1.35 (s)	1.39 (s)	1.37 (s)	1.36 (s)	1.28 (s)	1.38 (s)	1.28 (s)	1.33 (s)	1.33 (s)	1.35 (s)

Table 6-4 ^{13}C NMR shifts of pyranochromene *N*-pyrazolines **5a** – **5k** (100 MHz, $CDCl_3$, *J* in Hz)

No.	5a	5b	5c	5d	5e	5f	5g	5h	5i	5j	5k
3	60.2	63.0	63.0	56.5 (d, 2.7)	63.1	62.8	56.2 (d, 2.0)	62.6	59.1	63.3	63.1
4	46.6	48.2	48.1	46.8	48.1	48.3	46.8	48.1	48.3	48.2	48.1
5	150.6	150.1	150.1	150.6	150.2	150.1	150.6	150.1	150.0	150.2	150.2
6	100.0	99.9	99.9	100.0	99.9	99.9	99.9	99.8	99.9	99.8	99.8
7	154.8	154.8	154.8	154.8	154.9	154.8	154.6	154.8	154.8	154.9	154.8
8	103.2	103.3	103.1	103.2	103.3	103.3	103.3	103.3	103.2	103.3	103.3
9	150.8	150.9	151.0	150.9	150.9	150.8	150.9	151.0	150.9	151.0	151.0
10	103.1	103.1	103.0	103.1	103.1	103.0	103.1	103.1	103.1	103.1	103.1
11	152.8	152.8	152.8	152.8	152.8	152.7	152.6	152.7	152.8	152.8	152.8
12	139.6	141.4	141.9	#	145.5 (d, 6.5)	138.6 (d, 3.2)	#	139.8 (t, 3.9)	141.1	144.0	146.9
13	132.0	127.7	128.1	160.0 (d, 243.9)	113.3 (d, 23.7)	127.9 (d, 8.0)	162.3 (dd, 243.9, 11.3)	115.3 (d, 17.8)	103.1 (q, 14.7)	123.2 (q, 3.7)	126.7
14	130.1	129.6	132.5	115.8 (d, 21.1)	163.5 (d, 245.2)	116.3 (d, 21.3)	104.4 (t, 25.4)	151.0 (dd, 246.3, 14.3)	126.6 (d, 5.7)	131.6 (q, 15.4)	126.5 (q, 3.8)
15	127.6	133.6	121.7	129.2 (d, 15.7)	114.8 (d, 21.1)	162.4 (d, 244.6)	159.7 (dd, 241.6, 12.8)	150.0 (dd, 231.0, 20.8)	133.3	124.8 (q, 3.8)	-
16	130.1	129.6	132.5	125.0 (d, 3.2)	131.0 (d, 8.1)	116.3 (d, 21.3)	112.2 (dd, 21.2, 3.7)	118.2 (d, 17.1)	127.8	130.0	126.5 (d, 3.8)
17	127.8	127.7	128.1	127.8 (d, 3.9)	121.8 (d, 2.8)	127.9 (d, 8.0)	128.8 (dd, 9.7, 5.5)	122.2 (dd, 5.9, 3.6)	127.4	129.7	126.7
18	144.4	144.6	144.6	144.5	144.7	144.7	144.4	144.5	144.0	144.7	144.6
19/23	113.1	113.3	113.4	113.1	113.4	113.4	113.2	113.4	113.0	113.5	113.4
20/22	129.4	129.3	129.3	129.3	129.3	129.3	129.4	129.4	129.4	129.4	129.4
21	119.7	119.9	119.9	119.7	119.9	119.8	119.9	120.1	119.6	120.1	120.0
24	117.1	117.1	117.1	117.1	117.1	117.1	117.0	117.03	117.0	117.04	117.04
25	126.2	126.2	126.2	126.2	126.2	126.2	126.2	126.3	126.2	126.2	126.3
26	77.5	77.5	77.5	77.5	77.4	77.4	77.4	77.4	77.5	77.4	77.4
27	28.3	28.3	28.3	28.3	28.3	28.3	28.3	28.3	28.3	28.3	28.3
28	28.2	28.2	28.2	28.2	28.2	28.2	28.2	28.2	28.2	28.2	28.2
29	116.9	117.0	117.0	116.9	117.0	117.0	116.9	116.98	116.9	116.96	116.98
30	125.3	125.3	125.3	125.3	125.3	125.3	125.4	125.3	125.4	125.3	125.3
31	77.3	77.3	77.3	77.3	77.3	77.3	77.4	77.4	77.3	77.3	77.4
32	28.1	28.0	28.0	28.1	28.1	28.1	28.1	28.1	28.0	28.0	28.08
33	28.0	28.0	28.0	28.0	28.0	28.0	28.0	28.0	27.8	28.0	28.04

could not be detected.

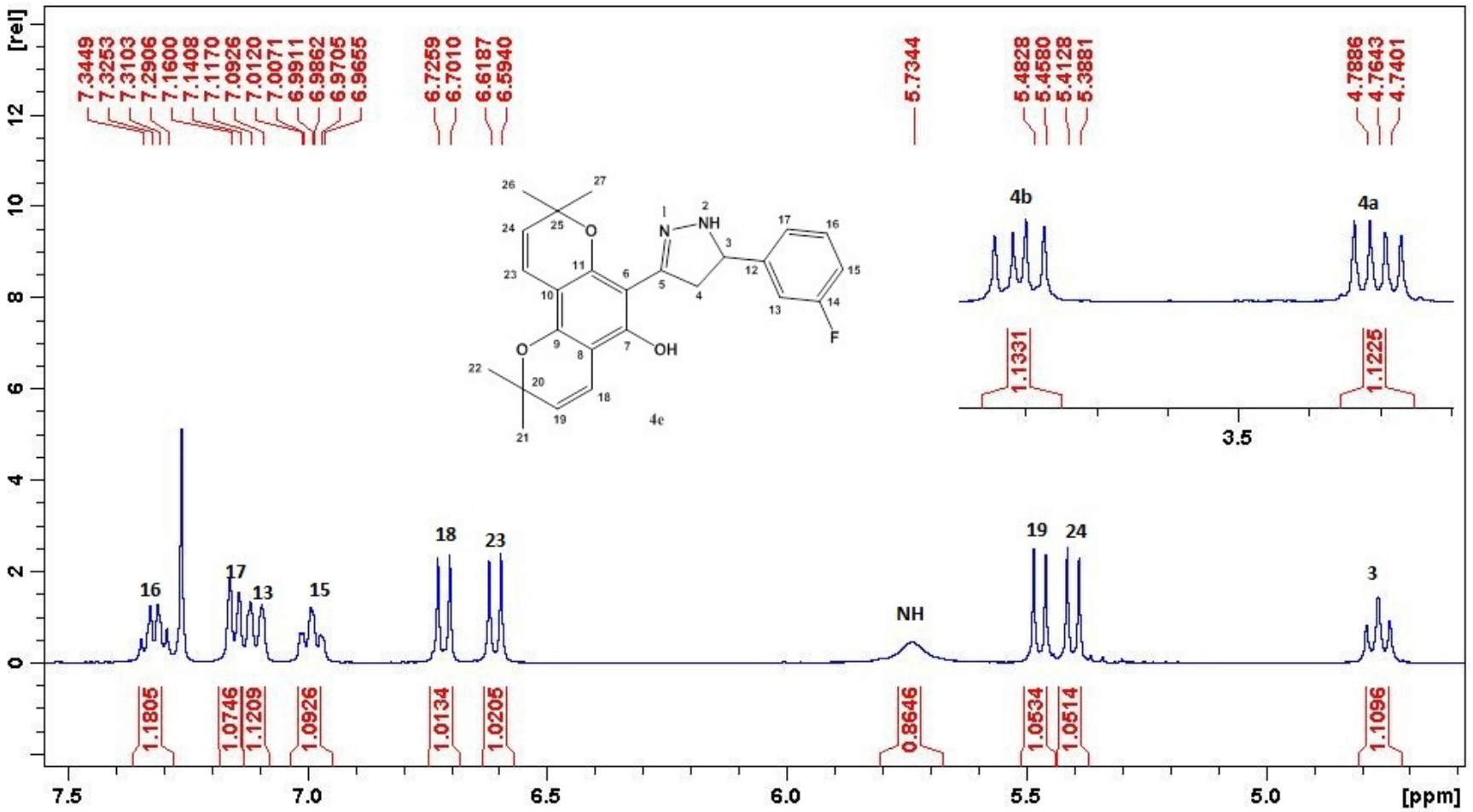


Figure 6-1 [1]H NMR of 6-(5-(3-fluorophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)-2,2,8,8-tetramethyl-2,8-dihydropyrano[2,3-f]chromen-5-ol (**4e**)

Antimicrobial activity

All of the synthesized pyrazoline analogues were screened for their *in vitro* anti-bacterial activity against Gram +ve (*Staphylococcus aureus* (43300) and *Enterococcus faecalis* (5129)) and Gram -ve (*Escherichia coli* (35218), *Klebsiella pneumoniae* (700603) and *Pseudomonas aeruginosa* (27853)) strains of bacteria. The observed anti-bacterial activities are shown in (**Table 6-5**). Pyrazoline and *N*-pyrazoline analogues **4b**, **5b** (*p*-Cl) and **4e**, **5e** (*m*-F) displayed good activity against both Gram +ve bacteria with MIC values between 14.9 to 57.2 μM. Of these compounds, the best activity was shown by **4e** with an MIC of 14.9 μM against *P. aeruginosa*, similar to the standard neomycin, which had an MIC of 16.3 μM against the same bacterial species. Compounds **4i** and **5i** (*o*-CF_3) and **4j** (*m*-CF_3) showed excellent activity against the Gram –ve bacterial species, with **4i** having similar activity (MIC of 13.3 μM) to neomycin (16.3 μM) against *P. aeruginosa*.

Compounds **4c** and **5c** (4-Br), **4f** (4-F), and **4h** and **5h** (3,4-F) showed good activity against all the fungal species tested against, on average 8-10 times higher than the standard, amphotericin B, with the best activity being shown by **4f** (29.7 μM) and **4h** (28.5 μM) against *C. krusei*.

Antioxidant activity

Antioxidant properties of the synthesized compounds were tested by both the DPPH radical scavenging activity (testing the ability of the compounds to scavenge free radicals) and H_2O_2 scavenging activity (testing the ability to scavenge hydroxyl radicals). These results are presented in **Table 6-6**. With the exception of the 3,4-difluoro (**4h** and **5h**) and the trifluoromethyl compounds (**4i**, **5i**, **4j**, **5j**, **4k** and **5k**), all other compounds showed very good antioxidant activity, comparable to or better than ascorbic acid, which was used as a control.

The pyrazolines **4c** and **5c** (4-Br), and the monofluorinated compounds **5d** (2-F), **4e** and **5e** (3-F), and **4f** and **5f** (4-F) exhibited excellent antioxidant activity with IC_{50} values of between 48.4-63.0 μM for the DPPH assay and 79.7-95.6 μM for the H_2O_2 assay.

Table 6-5 Antimicrobial activity of the synthesized compounds

No.	Minimum inhibitory concentrations (μM)								
	Antibacterial					Antifungal			
	Gram-positive		Gram-negative						
	S. aureues	***E. faecalis***	***E. coli***	***P. aeruginosa***	***K. pneumoniae***	***C. albicans 90028***	***C. albicans 1023***	***C. krusei***	***C. parapsilosis***
4a	57.2	57.2	-	-	-	-	-	-	-
5a	48.7	97.5	-	-	-	-	-	-	-
4b	28.6	57.2	57.2	57.2	57.2	228.9	228.9	228.9	457.7
5b	24.4	48.7	48.7	48.7	48.7	97.5	97.5	97.5	121.8
4c	-	-	104.1	104.1	208.2	52.0	52.0	104.1	52.0
5c	-	-	89.7	89.7	89.7	44.8	44.8	-	112.1
4d	-	-	-	-	-	-	-	-	-
5d	-	402.8	-	-	-	201.4	201.4	402.8	201.4
4e	29.7	29.7	29.7	14.9	29.7	-	-	-	-
5e	25.2	50.4	50.4	50.4	50.4	-	-	-	-
4f	148.6	148.6	237.8	237.8	237.8	59.5	59.5	29.7	59.5
5f	125.9	125.9	201.4	201.4	201.4	-	-	-	-
4g	57.0	57.0	-	-	-	456.1	456.1	-	456.1
5g	48.6	48.6	-	-	-	194.3	194.3	-	-
4h	228.1	456.1	-	-	-	57.0	57.0	28.5	57.0
5h	194.3	388.7	-	-	-	48.6	48.6	48.6	48.6
4i	212.6	53.1	26.6	13.3	26.6	-	-	-	-
5i	183.0	45.7	22.9	22.9	22.9	-	-	-	-
4j	106.3	106.3	26.6	26.6	26.6	132.8	212.6	425.1	425.1
5j	91.5	91.5	45.7	91.5	91.5	114.4	183.0	365.9	365.9
4k	106.3	212.6	53.1	53.1	53.1	-	-	-	-
5k	183.0	183.0	91.5	114.4	91.5	-	-	-	-
C1*	16.3	32.5	16.3	16.3	16.3	-	-	-	-
C2**	-	-	-	-	-	6.8	6.8	3.4	3.4

* C1 = neomycin; **C2 = amphotericin B

Table 6-6 Antioxidant activity of the synthesized compounds

No.	DPPH IC_{50}(μM)	H_2O_2 IC_{50}(μM)	No.	DPPH IC_{50}(μM)	H_2O_2 IC_{50}(μM)
4a	126.2	103.4	**4g**	131.25	126.35
5a	117.3	84.5	**5g**	89.40	85.35
4b	145.1	115.0	**4h**	175.45	167.63
5b	103.4	95.3	**5h**	>388.7	184.58
4c	55.2	89.24	**4i**	>365.9	>183.0
5c	48.4	95.57	**5i**	>425.1	>212.6
4d	114.3	134.96	**4j**	-	>457.4
5d	59.9	85.14	**5j**	-	>531.4
4e	57.98	90.80	**4k**	>183.0	128.1
5e	50.99	79.69	**5k**	>212.6	127.9
4f	62.95	90.90	**Ascorbic acid**	220.02	187.71
5f	52.44	87.36			

Conclusion

A series of novel halogen substituted pyranopyrazolines and *N*-pyrazoline analogues (**4a** - **4k** and **5a** – **5k**) were easily synthesized and evaluated for their antimicrobial and antioxidant activities. Amongst the synthesized compounds, **4e** (3-F) and **4i** (2-CF_3) were found to have excellent activity against the Gram -ve *P. aeruginosa* (MICs of 14.9 and 13.3 μM, respectively). Compound **4e** showed the best broad spectrum activity with MICs of 29.7 μM for all the other bacterial strains tested against. Compounds **4i**, **5i** (2-CF_3) and **4j** (3-CF_3) showed excellent activity against the Gram –ve strains only, with MICs between 13.3 and 26.6 μM. The best antifungal activity was shown by **4f** (29.7 μM) and **4h** (28.5 μM) against *C. krusei*. The monofluorinated compounds **5d**, **4e**, **5e**, **4f** and **5f** and the bromo compounds **4c** and **5c** showed excellent antioxidant activity, much better than that of ascorbic acid with IC_{50} values of between 48.4-63.0 μM for the DPPH assay and 79.7-95.6 μM for the H_2O_2 assay. The compounds synthesized here could be lead compounds for antibacterial activity and possible antioxidant supplements. The data indicate that the both the F and CF_3 groups result in good activity of the pyranopyrazolines with the *ortho* and *meta* positions on the

phenyl ring showing better activity than the *para* position.

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Chapter 7. Conclusion

The aim of the study was to synthesise novel pyranochromene chalcones, which were to be tested for their antimicrobial and antioxidant activity as it was hypothesized that the chromene moiety would make a difference to the bioactivity of the molecules. The chromene moiety was chosen to be studied since molecules isolated or synthesized previously containing a chromene moiety had shown good bioactivity [1, 2].

In total, 20 pyranochromene chalcones were synthesized, of which 18 were novel. These pyranochromene chalcones were synthesized by adding the pyran moiety onto 2,4,6-trihydroxyacetophone using 3-methyl-2-butenal in the presence of pyridine forming the dipyrano acetophenone, before condensing it with various substituted benzaldehydes as well as naphthalene-2-carbaldehyde, furan-2-carbaldehyde and pyridine-2-carbaldehylde (picolinaldehyde). The varying substituents on benzaldehyde included halogens, methoxy, trifluoromethyl and nitro groups.

Two series of pyranochromene chalcones (those with halogens, methoxy and nitro phenyl moieties and the naphthyl moiety) were cyclized with NaOAc to the flavanones. A total of 13 flavanones were synthesized, of which 12 were novel. In addition, a flavone was also synthesized. The halogenated and trifluoromethylated chalcones were also converted to pyrazolines using either hydrazine or phenylhydrazine to prepare pyrazoline derivatives of the pyranochalcones. A total of 22 pyrazoline derivatives were synthesized, of which all were novel.

In general, the syntheses were fairly easy and the compounds were prepared in good yields. The α,β-unsaturated ketone moiety of the chalcones were reactive and this reactivity was

taken advantage of to make two other series of molecules, the flavanones and the pyrazolines, which could also be tested for bioactivity.

A full structural elucidation of each set of compounds was carried out using 2D NMR and X-ray crystallography. Unequivocal NMR assignments for each set of compounds were carried out and will provide a nice basis for identification and assignment of resonances for compounds with similar structures. In addition, the crystal structures for several compounds were obtained, which established the *trans* configuration of the double bond and the planarity of the synthesized chalcones. In addition, X-Ray crystallography was also used to determine the absolute configuration at C-2 of the flavanones as *S*.

The synthesized compounds were tested for their antibacterial, antifungal and antioxidant activity. The unsubstituted flavanone **A-4a** (MIC between 18.7-64.4 μM) showed the best antibacterial activity, followed by the 2-chloroflavanone **A-4i** (MICs between 29.6-118.2 μM), the unsubstituted chalcone **A-3a** (MICs between 32.2-128.7 μM), and lastly the 4-methoxychalcone **A-3g** (MICs between 119.5-239.0 μM). In some cases, the synthesized compounds had similar activity to the standard, neomycin (8.1-32.5 μM). The *para* NO_2 flavone **A-5** showed excellent activity against *S. aureus* at 2.3 μM, far better than neomycin at 16.3 μM. This flavone could be an excellent lead for an anti-Staphylococcal antibiotic. The 2-trifluoromethyl pyranochromene chalcone **B-6** showed excellent antibacterial activity against all Gram negative strains (13.7-27.5 μM), comparable to the control neomycin (16.3 μM).

The chalcones **A-3a** (unsubstituted), **A-3d** (4-fluoro) and **A-3m** (naphthyl) and the flavanones **A-4a** (unsubstituted), **A-4d** (4-fluoro), **A-4f** (3-methoxy) and **A-4l** (3-nitro) all showed good

activity against all four strains of fungi having MIC values of 28.5 to 64.4 μM. The standard amphotericin B used for comparison showed lower activity with MIC values of between 1.3-5.4 μM. However, the activity of the chalcones and flavanones is still good and these compounds may have fewer side effects than drugs currently on the market.

With the exception of a few compounds, the antioxidant activity of most chalcones was comparable to that of the control, ascorbic acid (IC_{50} of 220.0 μM). The chalcones that showed the best activity were **A-3e** (2,4-difluoro) (IC_{50} of 230.4 μM), **A-3h** (2-fluoro-3-methoxy) (IC_{50} of 233.2 μM), **A-3i** (2-chloro) (IC_{50} of 228.7 μM) and **A-3l** (3-nitro) (IC_{50} of 220.3 μM). The flavanones **A-4i** (2-chloro) (IC_{50} of 225.8 μM) and **A-4l** (3-nitro) (IC_{50} of 234.7 μM) also showed good antioxidant activity. These results indicated that halogenation at C-2 of the phenyl ring or nitration or methoxylation at C-3 results in good antioxidant activity of the prenylated chalcones and flavanones.

The pyrazoline **C-4e** (3-fluoro derivative) showed good antibacterial activity against both Gram positive and Gram negative strains with IC_{50} values of between 14.9-29.7 μM. The 2-trifluoromethyl derivative **C-4i** showed good antibacterial activity against the Gram negative strains only with IC_{50} values of between 13.3-26.6 μM in comparison to neomycin, which showed activity in the same assay at 16.3-32.5 μM for the Gram positive strains and 16.3 μM for the Gram negative strains. Several pyrazolines showed better antioxidant activity than the standard ascorbic acid, with IC_{50} values up to four times lower in both the DPPH and the H_2O_2 assay.

With regard to structure activity relationship, these results indicate that pyran groups on the chalcone or flavanone skeleton is a good scaffold for antifungal activity and that this activity

can be increased by fluoro, methoxy and nitro groups being substituted on the phenyl ring or by substituting the phenyl ring with a naphthalene group. It was further found that the CF_3 moiety is a good substituent for antimicrobial activity, better than other heterocyclic rings and the fluoro substituent. The best position for this group with regard to antibacterial activity is C-2 on the B ring of the chalcone and C-4 on the same ring with regard to antifungal activity. For the pyranopyrazolines, the F and CF_3 groups result in good activity with the *ortho* and *meta* positions on the phenyl ring showing better activity than the *para* position.

In conclusion, several of the synthesized compounds can be considered leads in finding drugs with antibacterial, antifungal and antioxidant activities. The most promising leads for broad spectrum antibiotics are the unsubstituted pyranochromene flavanone (**A-4a**) and the 3-fluoro pyrazoline derivative (**C-4e**), whilst the 2-trifluoromethyl pyranochromene chalcone (**B-6**) and its pyrazoline derivative (**C-4i**) are good leads for Gram negative antibiotics and the *p*-nitropyranochromene flavone derivative, an excellent lead for a *S. aureus* strain specific antibiotic. Several of the pyranochromene chalcones mentioned above could be good antifungal and antioxidant leads, whilst several pyrazolines can also be considered good leads for antioxidant activity. The mechanism of action of their antimicrobial and antioxidant activity could be due to their ability to complex with metals [3] or their ability to interact with cell membranes [4].

Future work can be carried out on these lead molecules to see if they can be developed further into marketable pharmaceuticals.

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Appendix

Spectral Data of Synthesised Compounds

Synthesis and Biological Studies of Novel Pyranochromene Derivatives

2015

Sunayna Sachin Pawar

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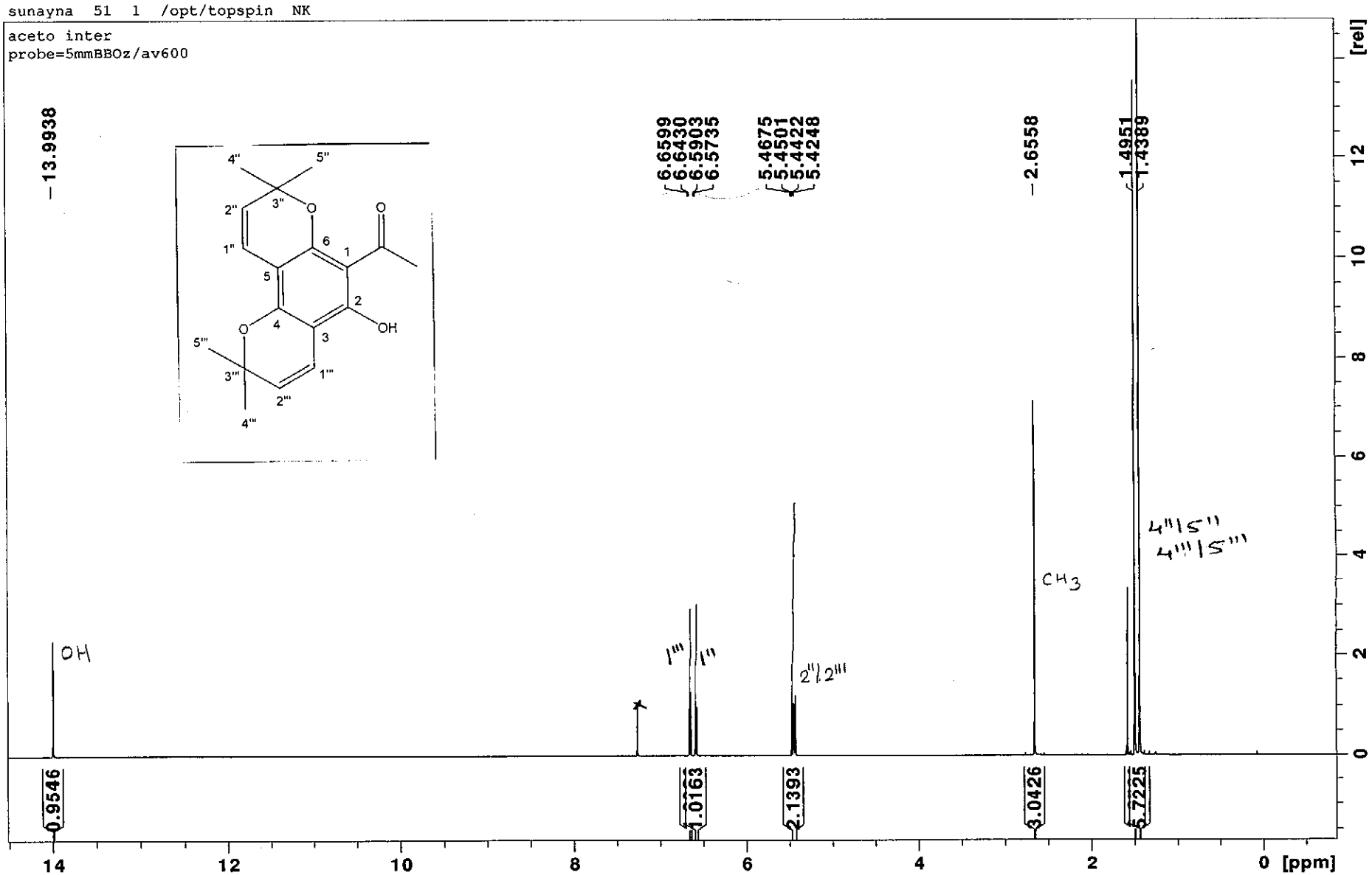
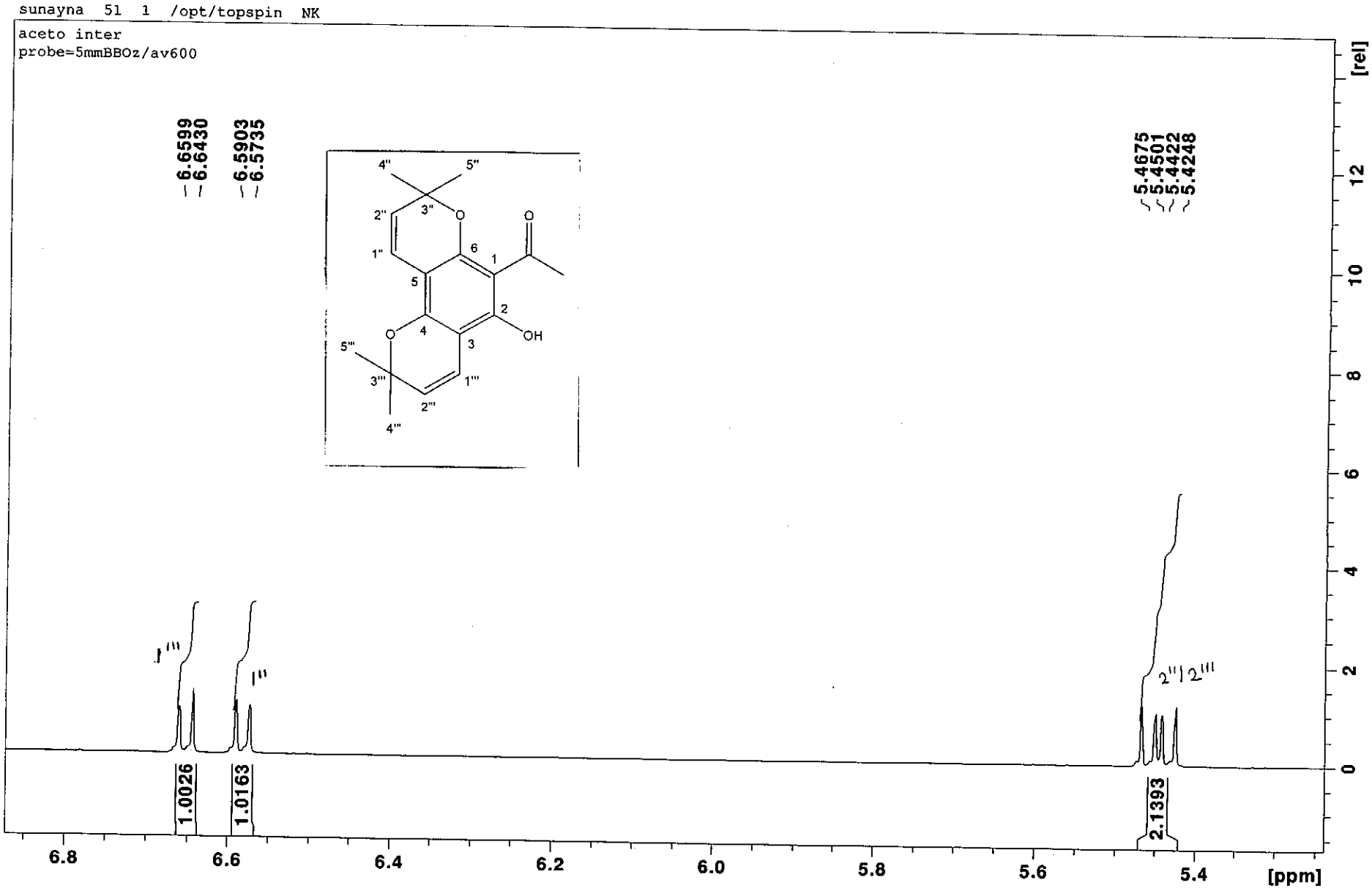


^{1}H NMR spectrum of 2 – pyranochromene intermediate (A-2)



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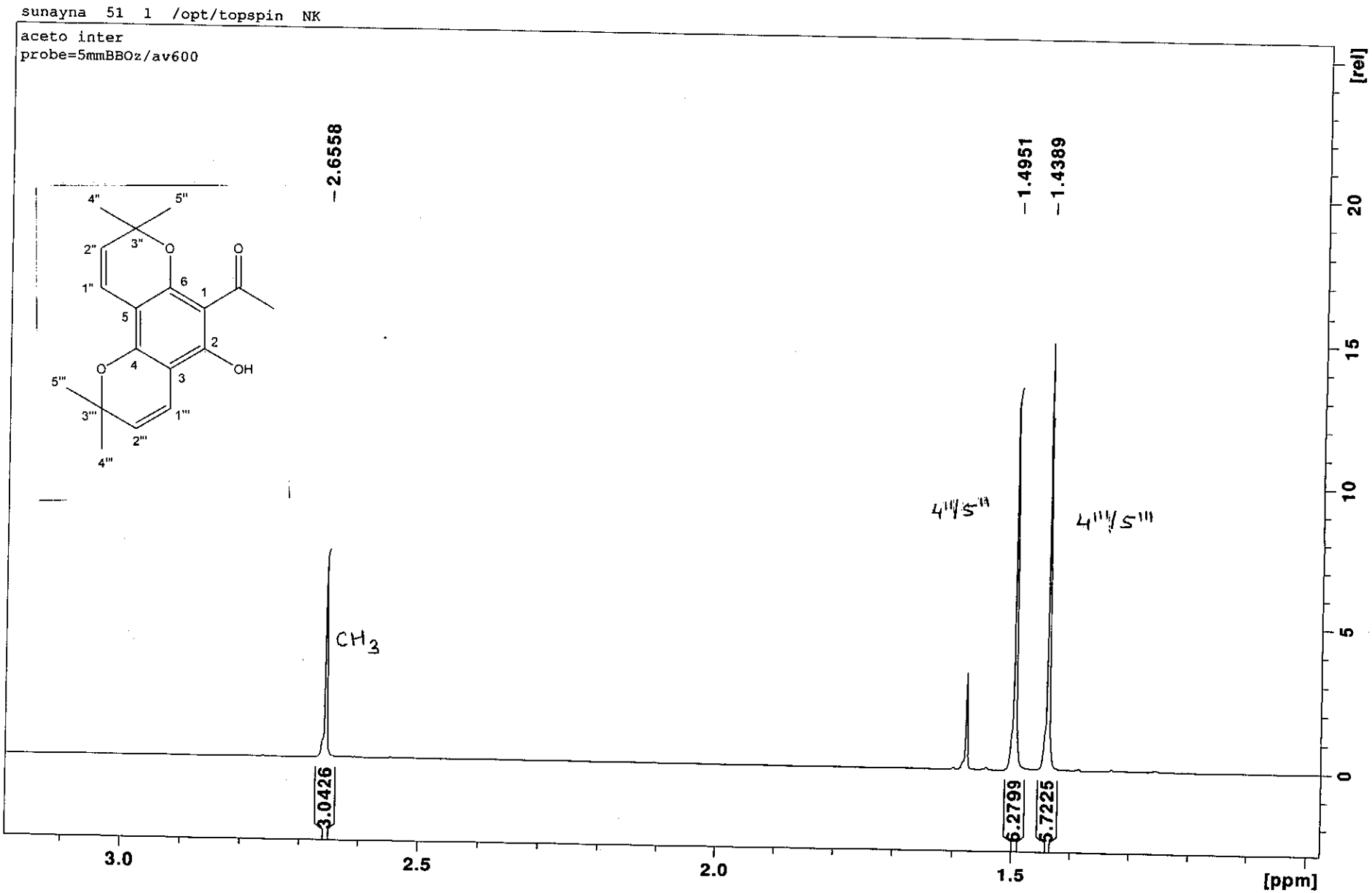

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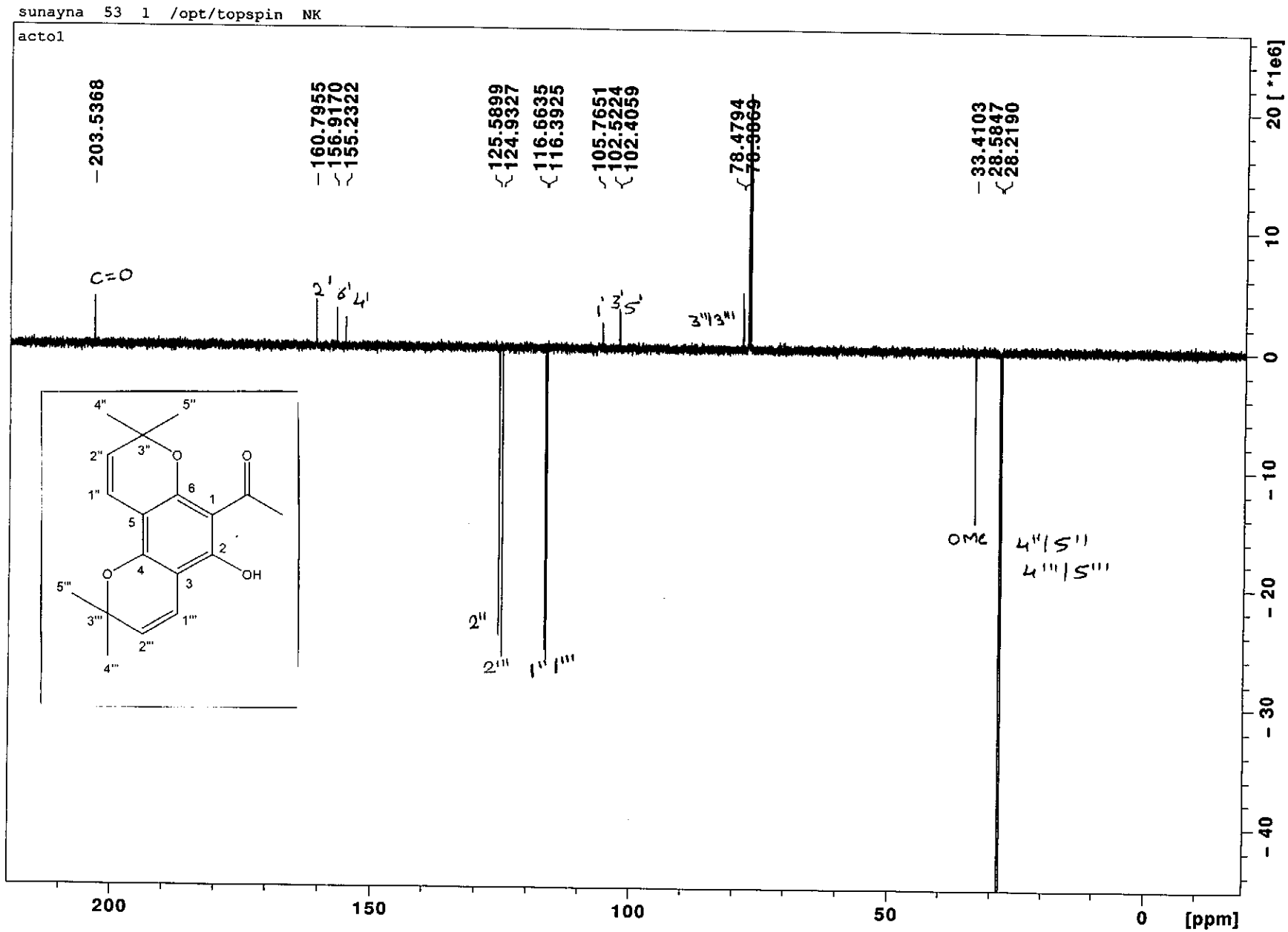

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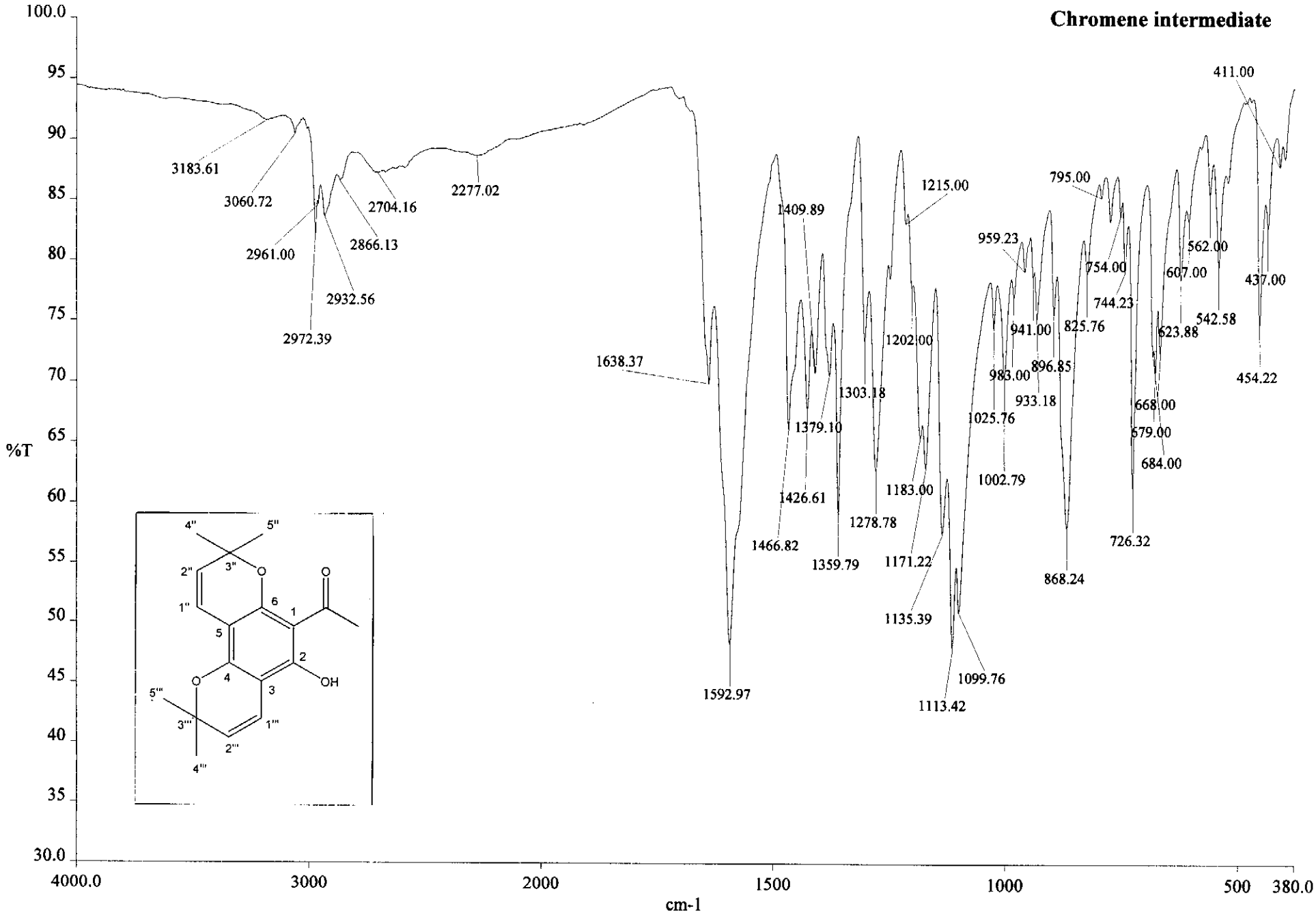

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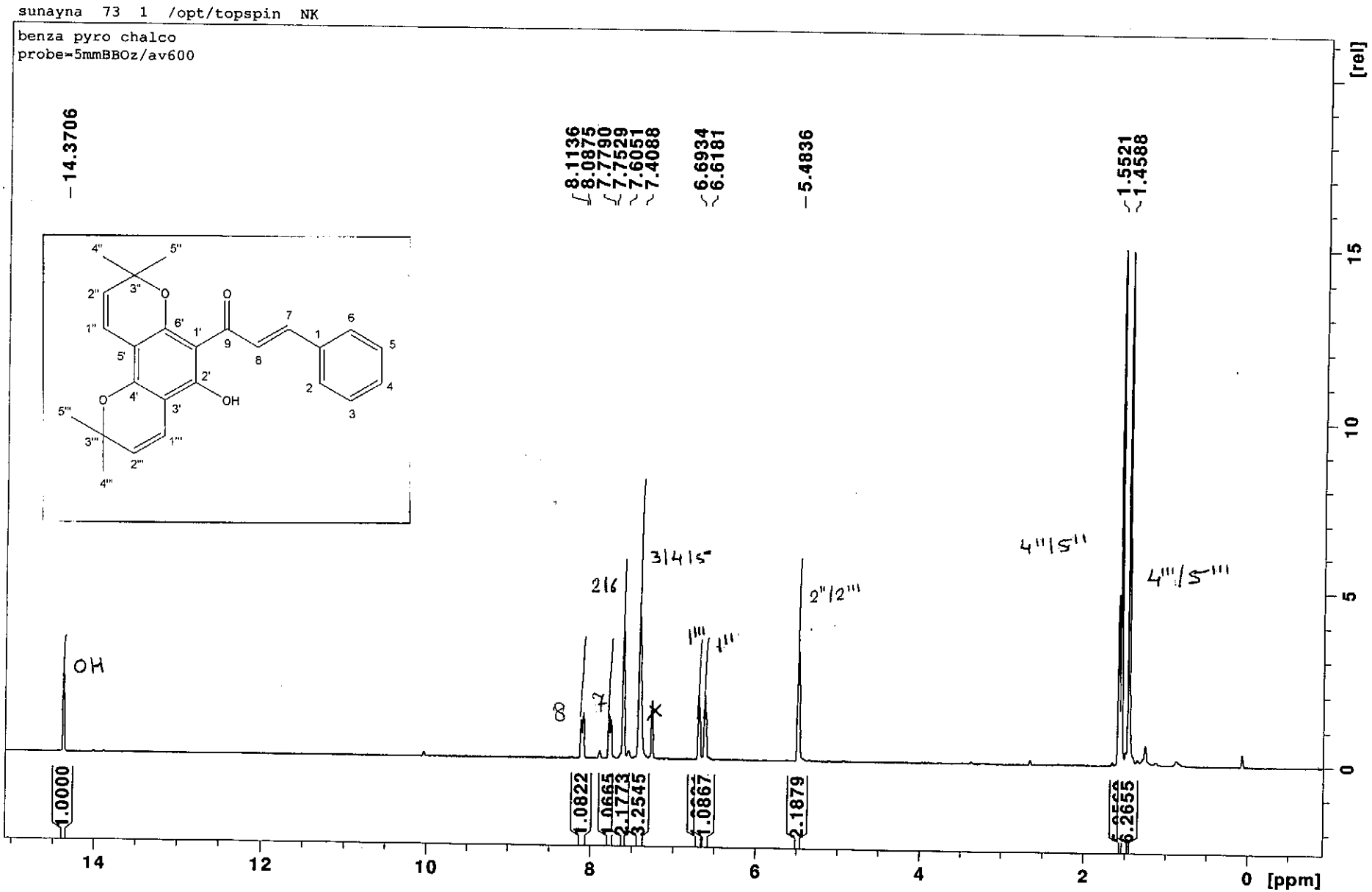

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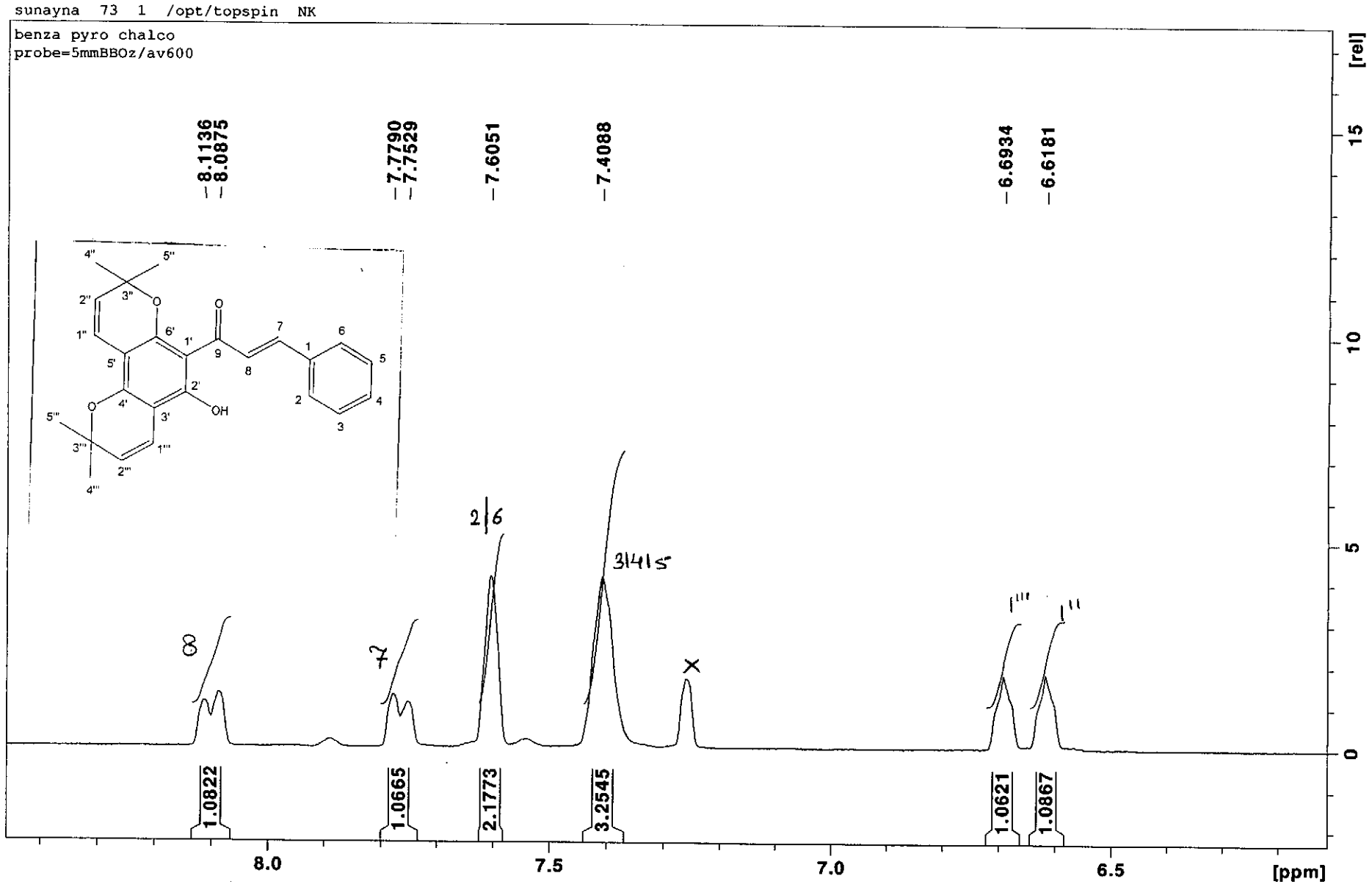

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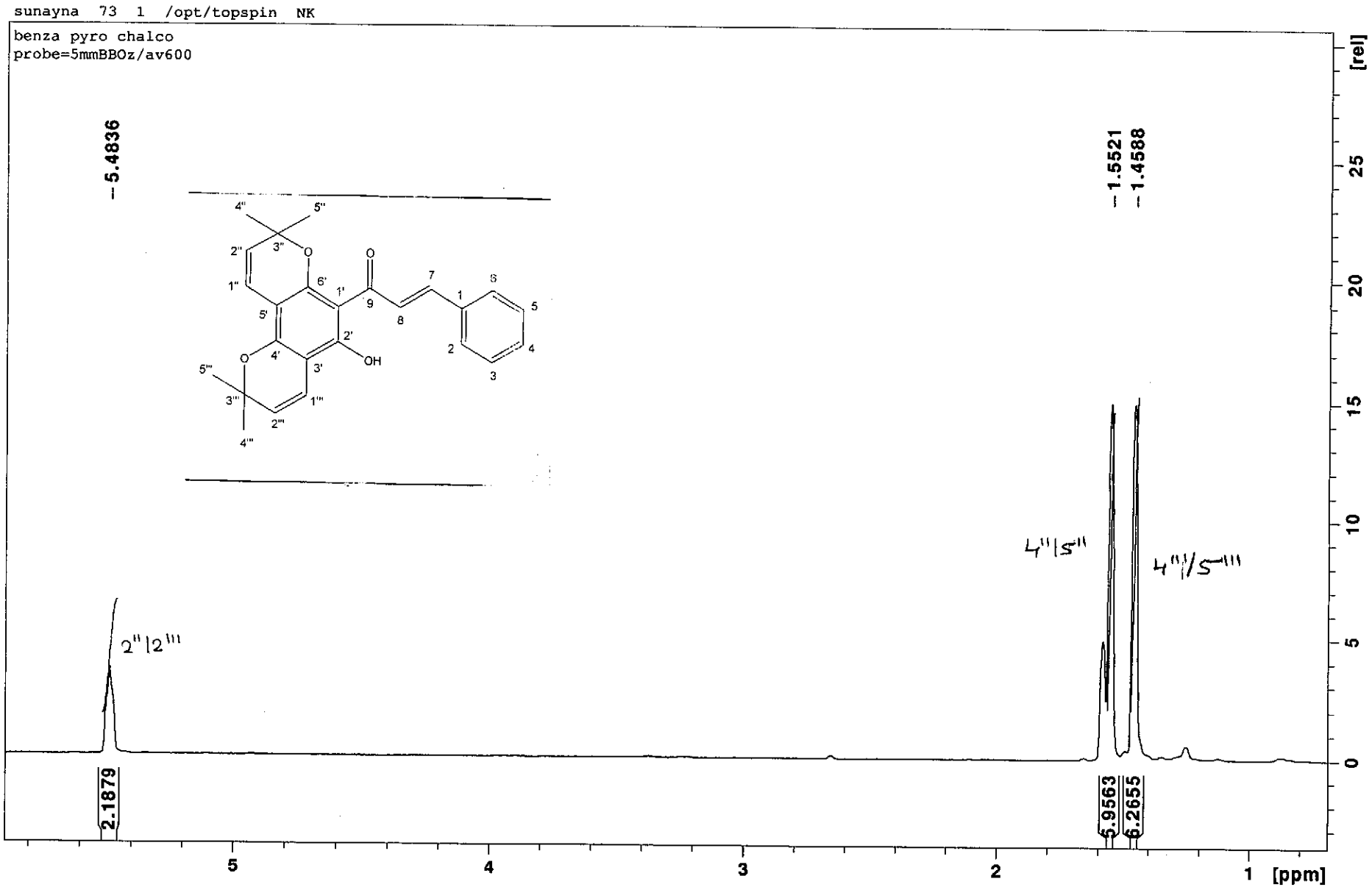

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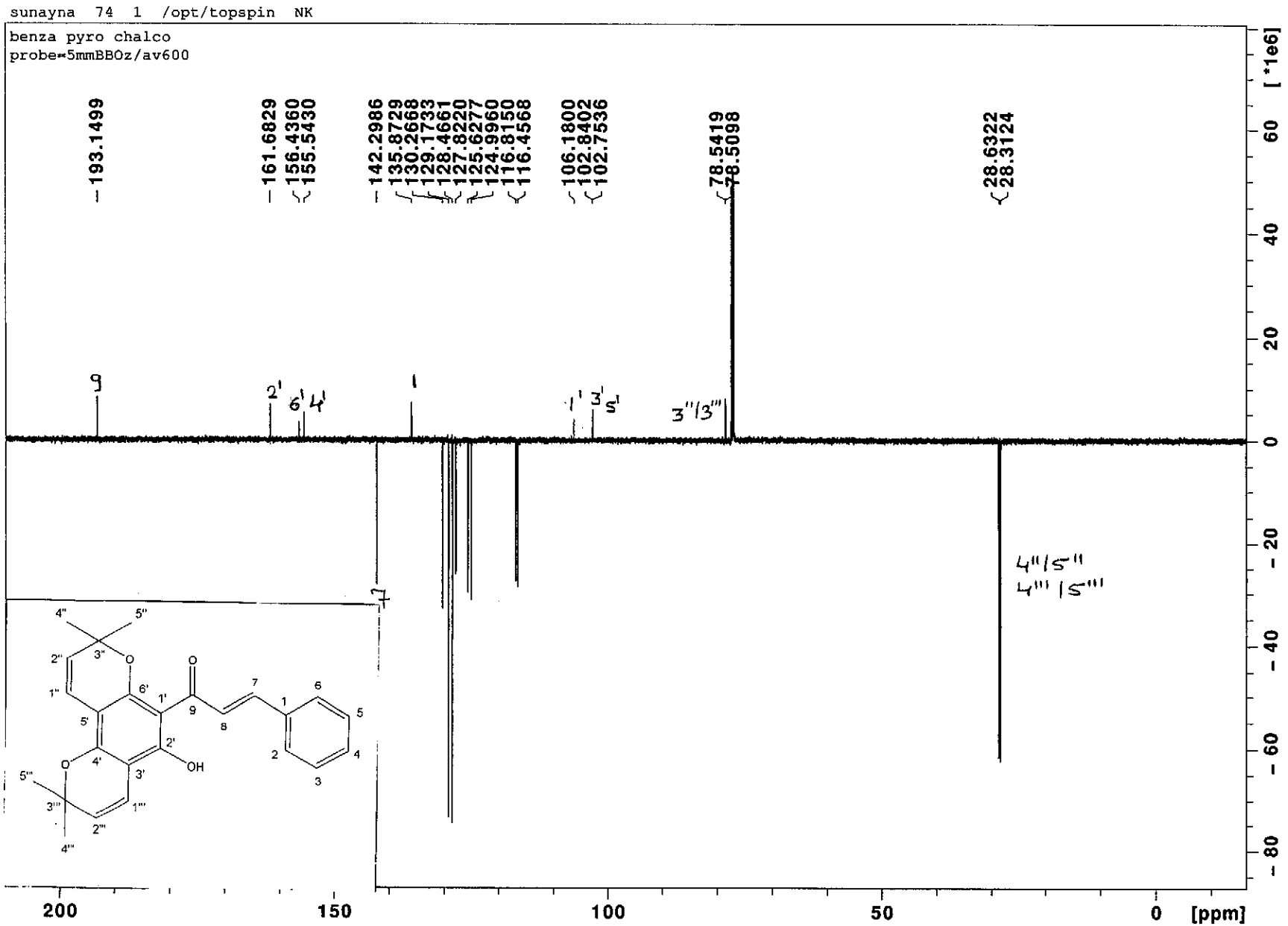

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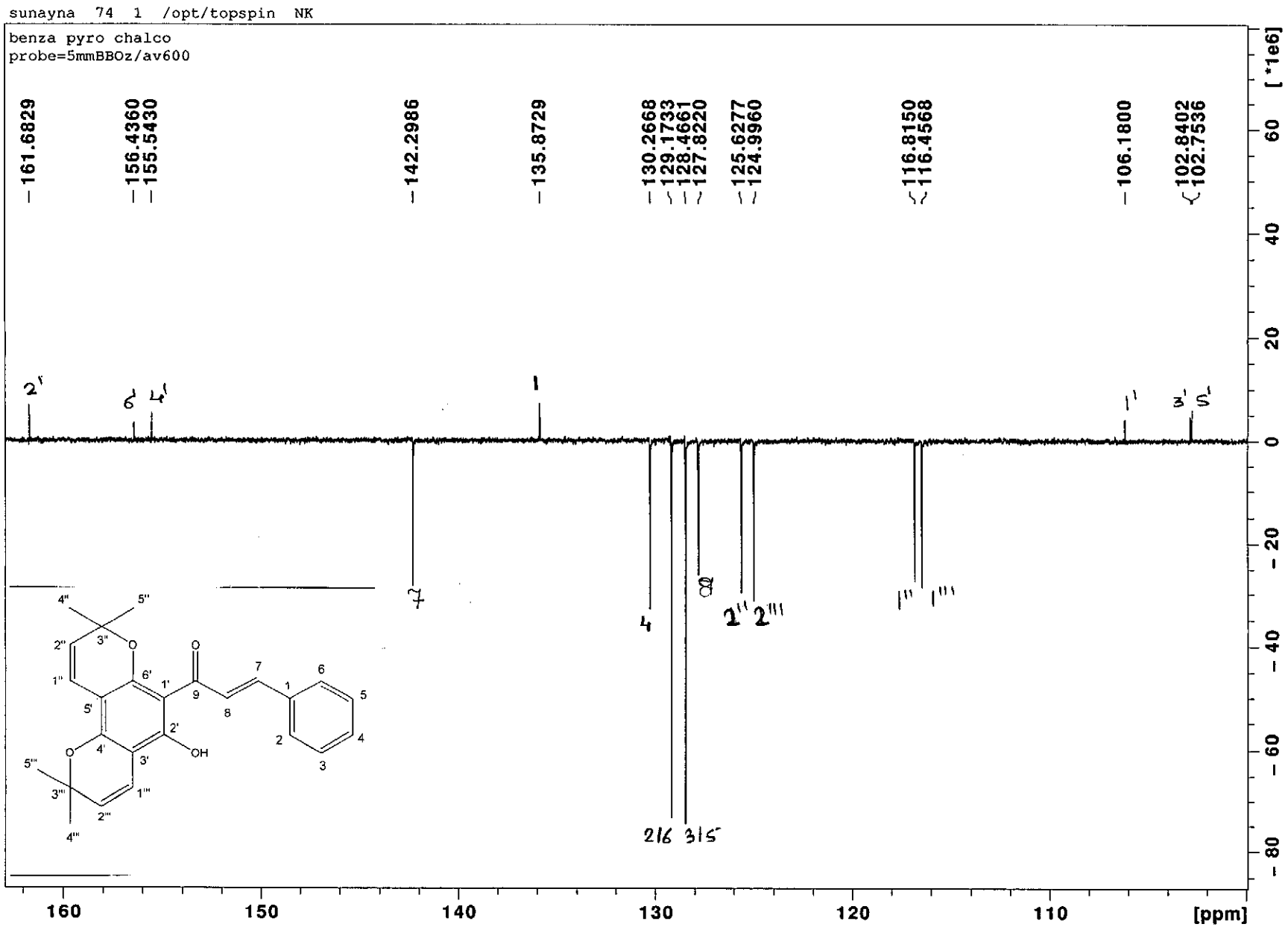

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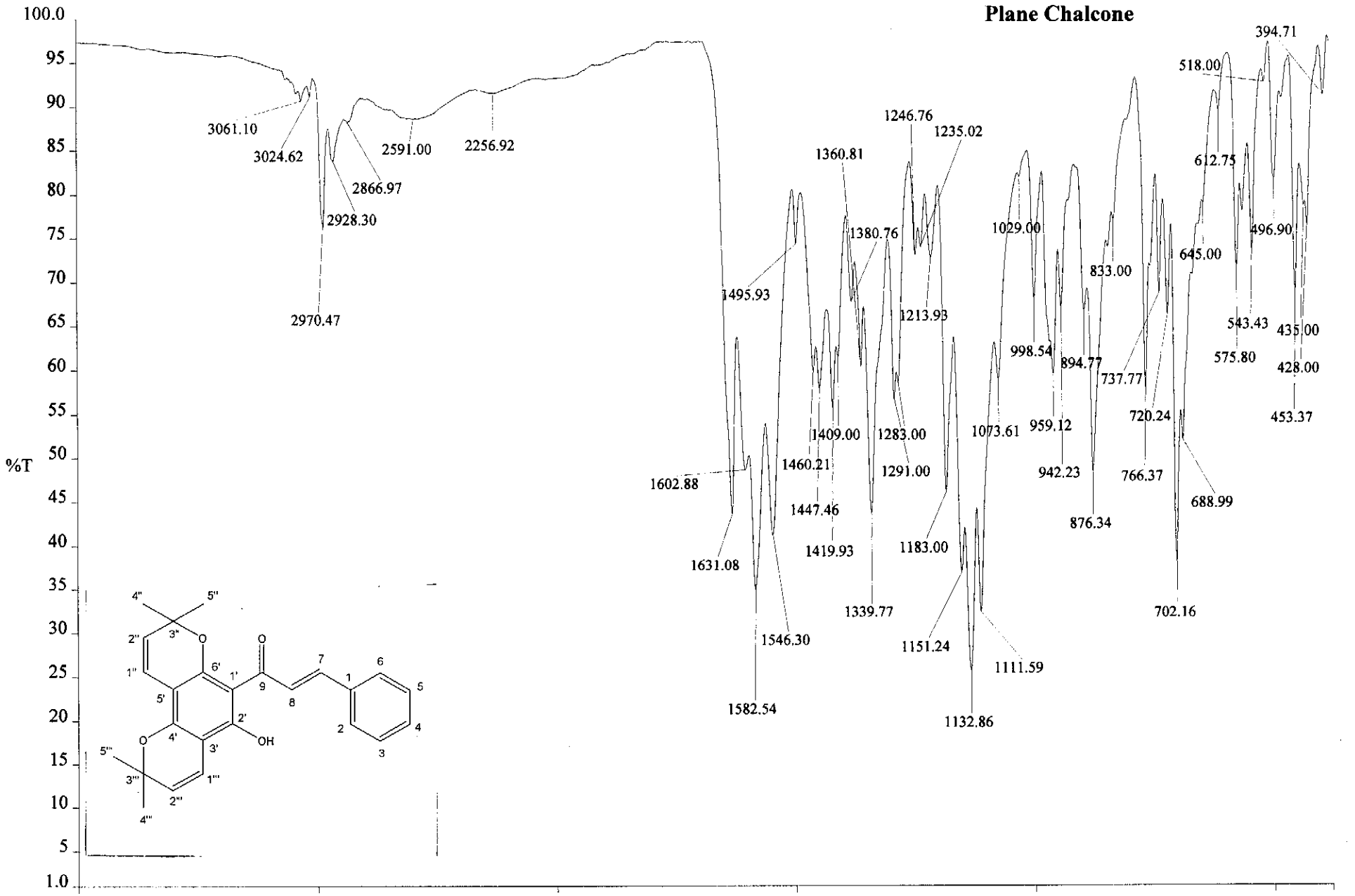

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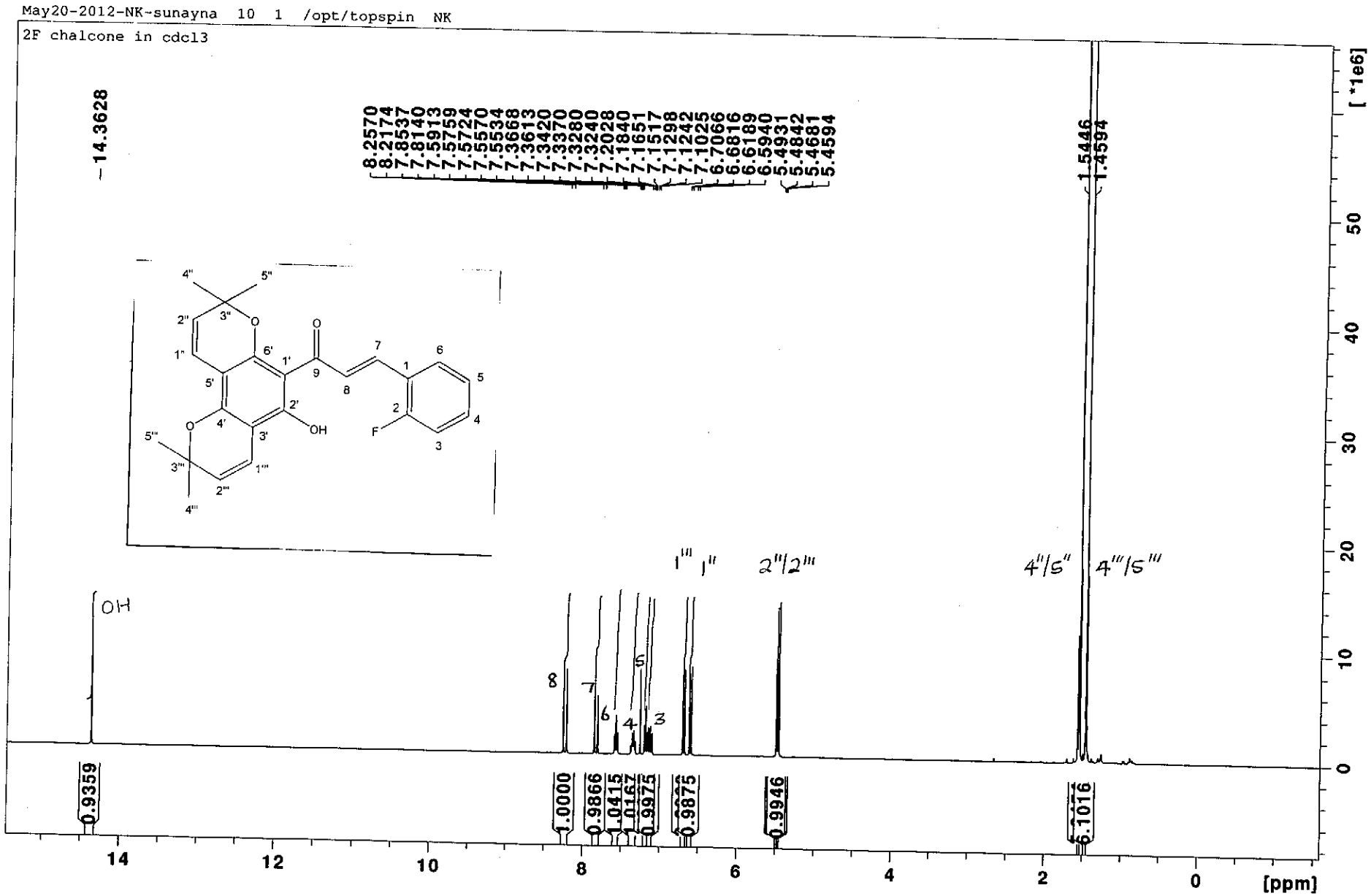

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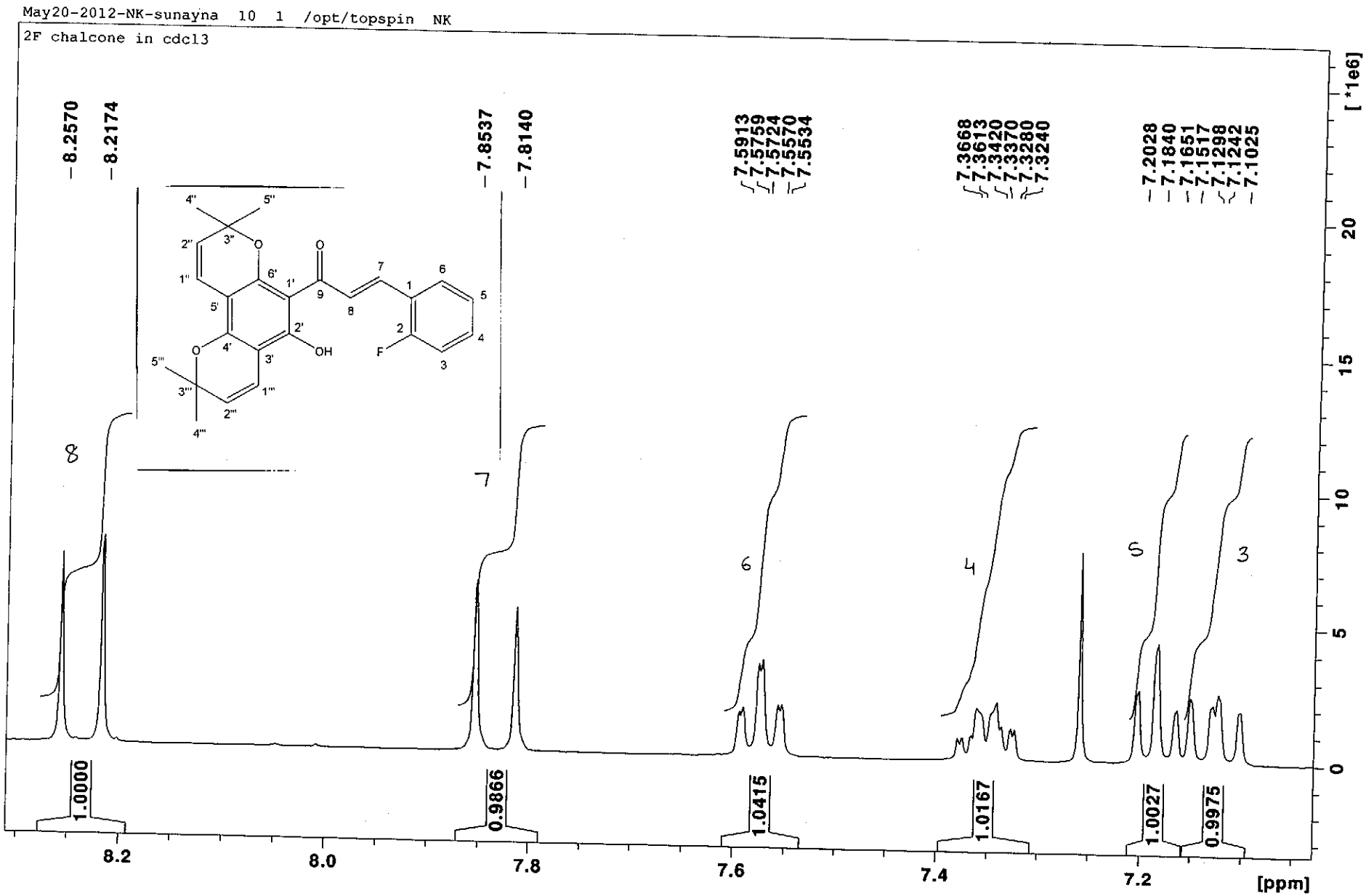

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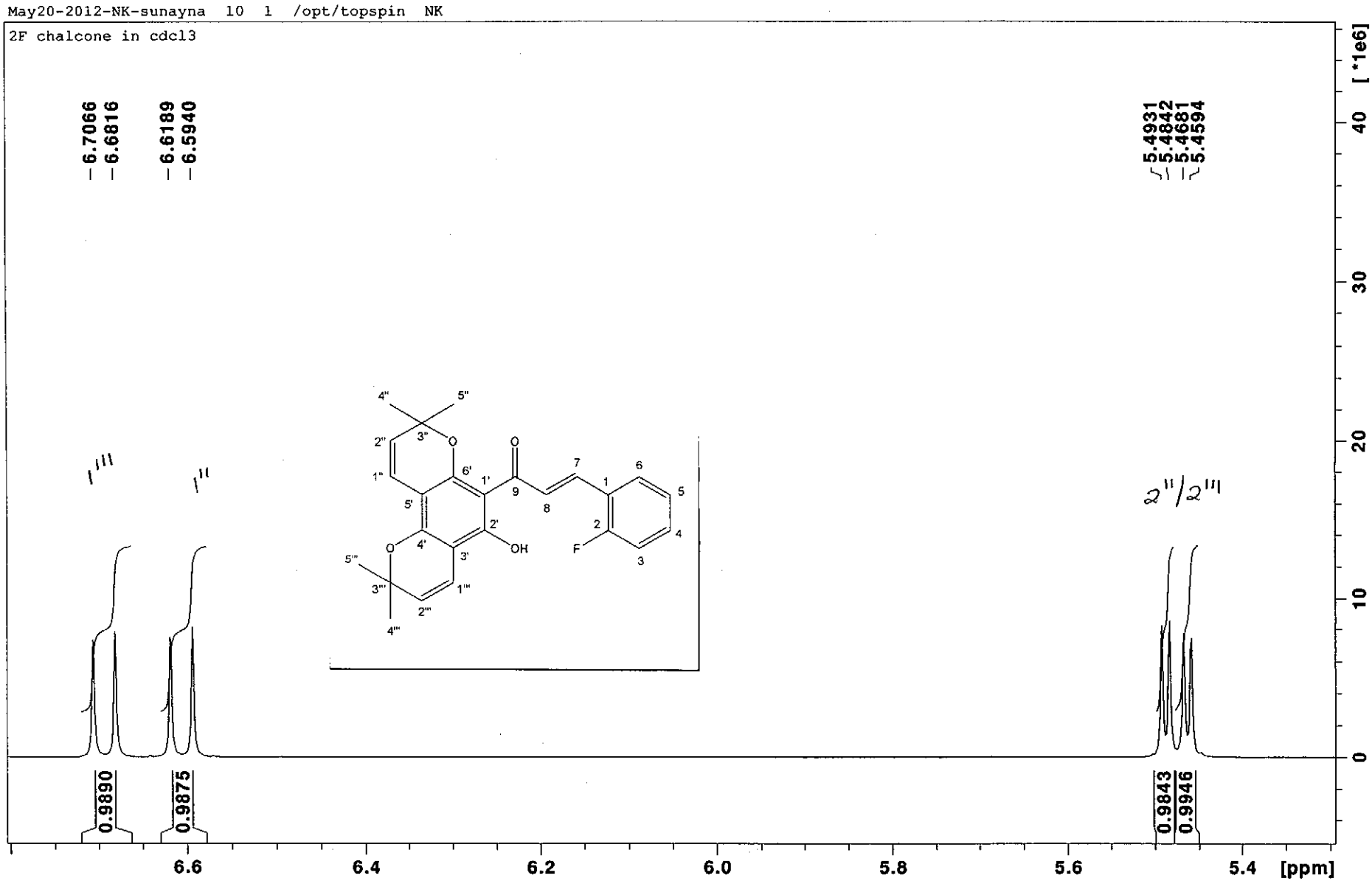

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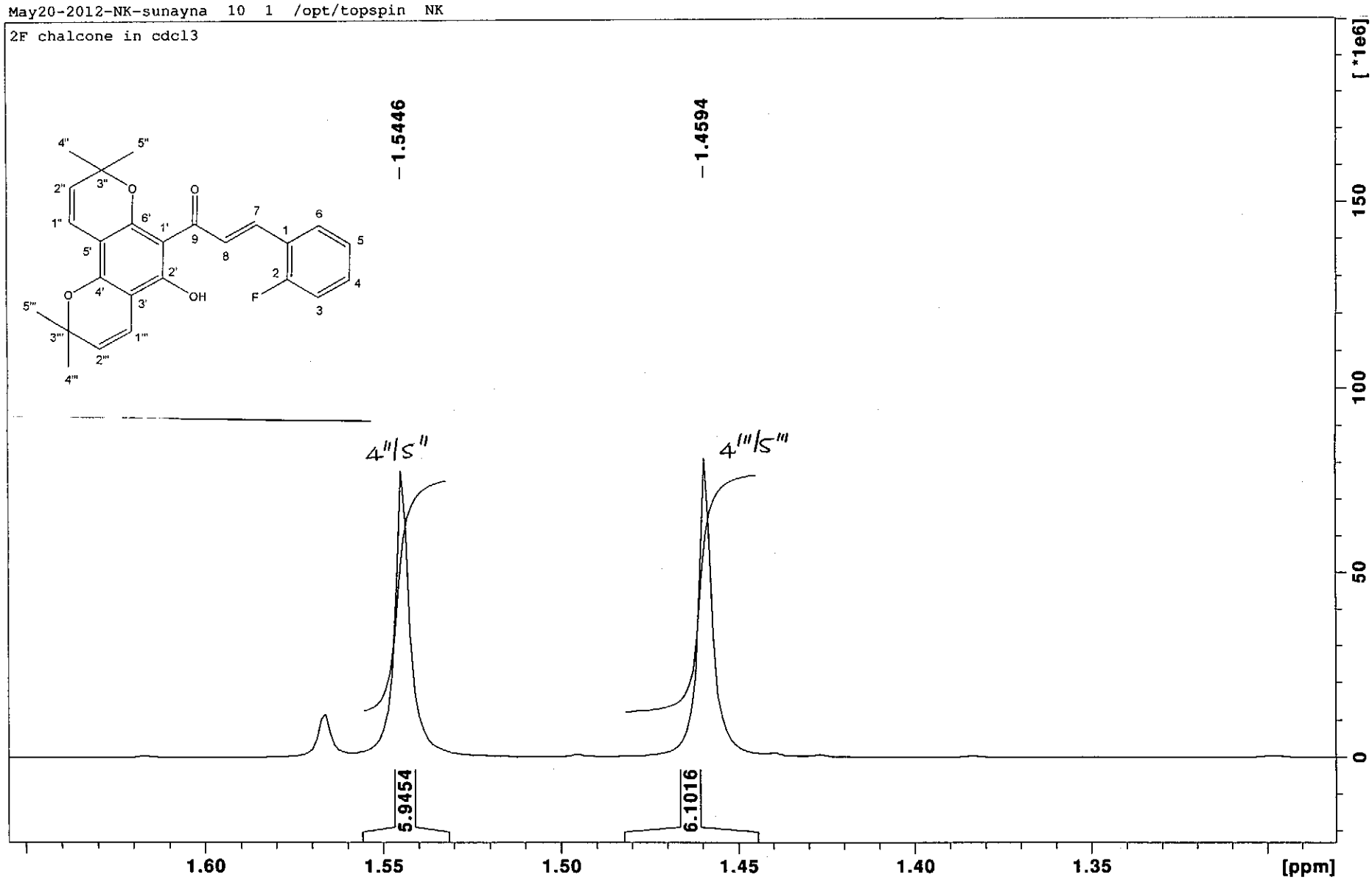

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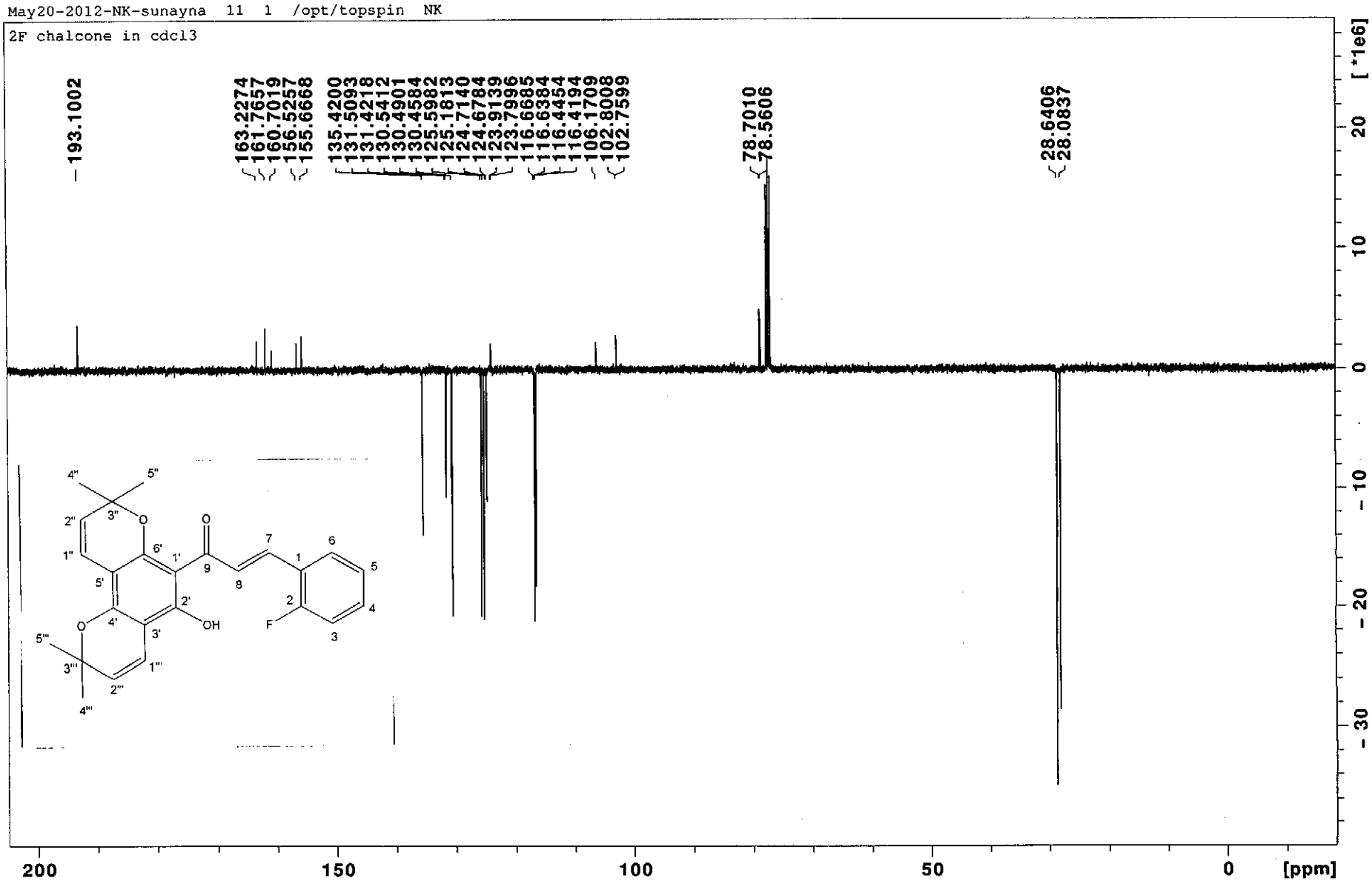

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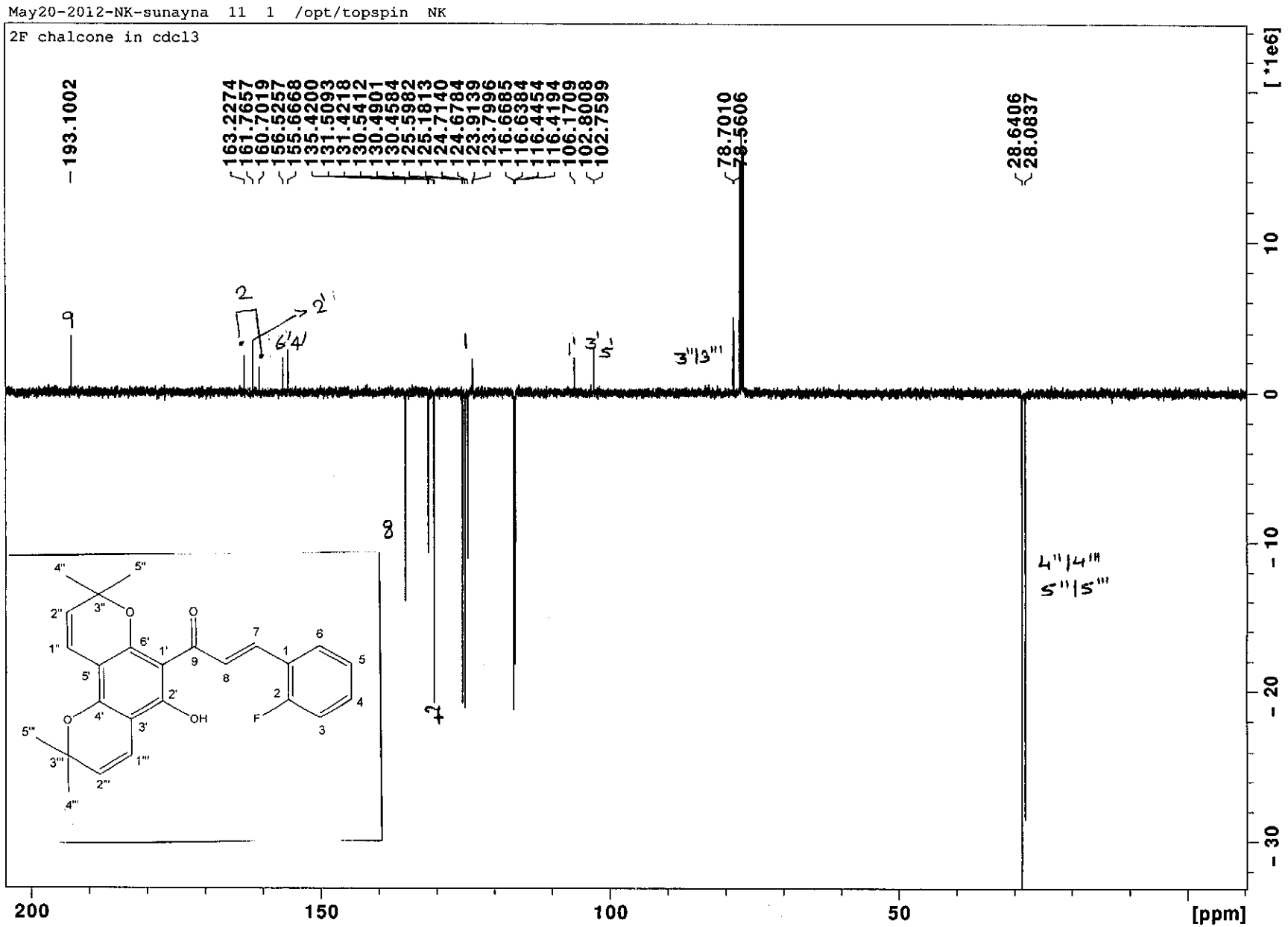

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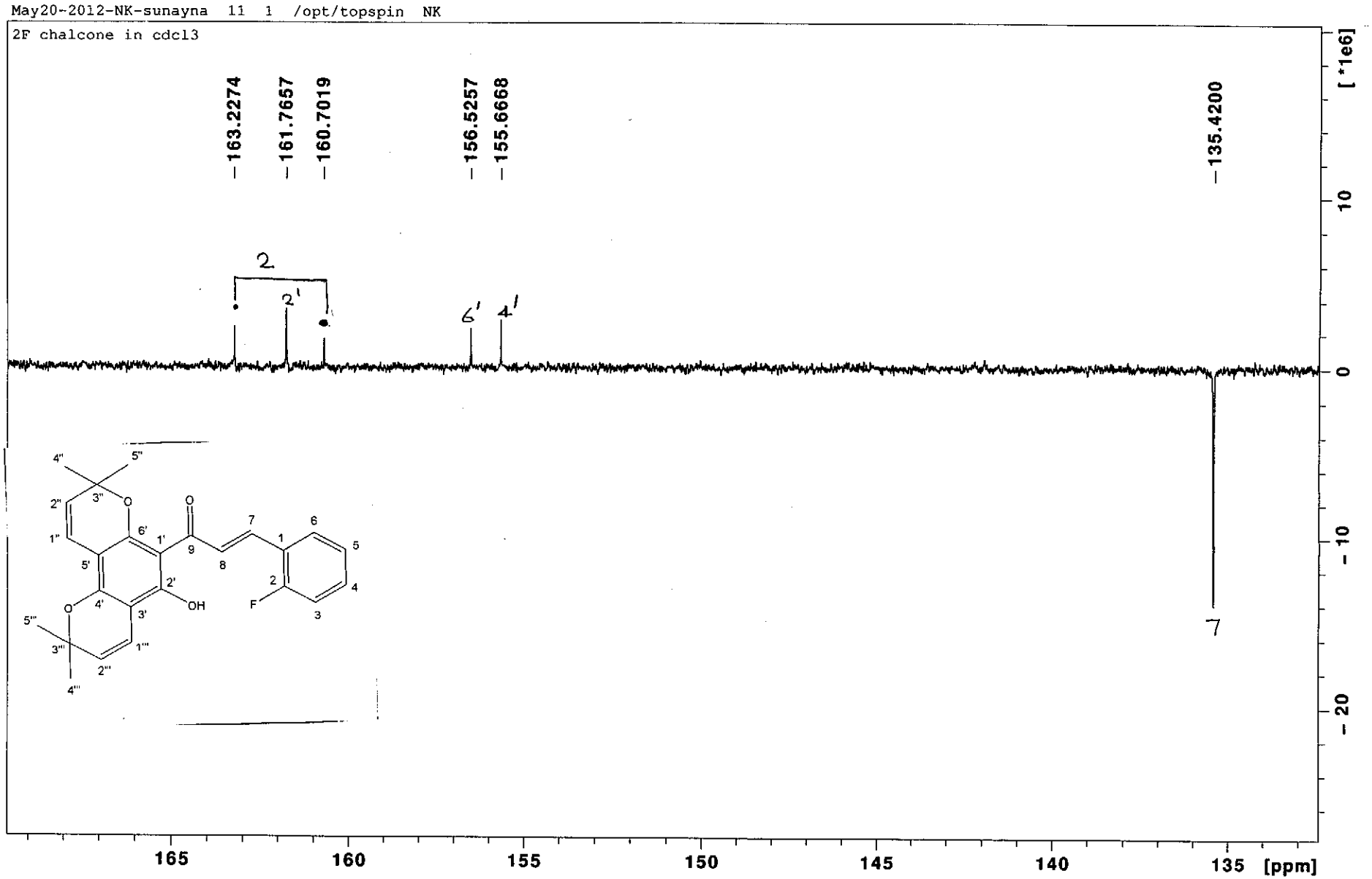

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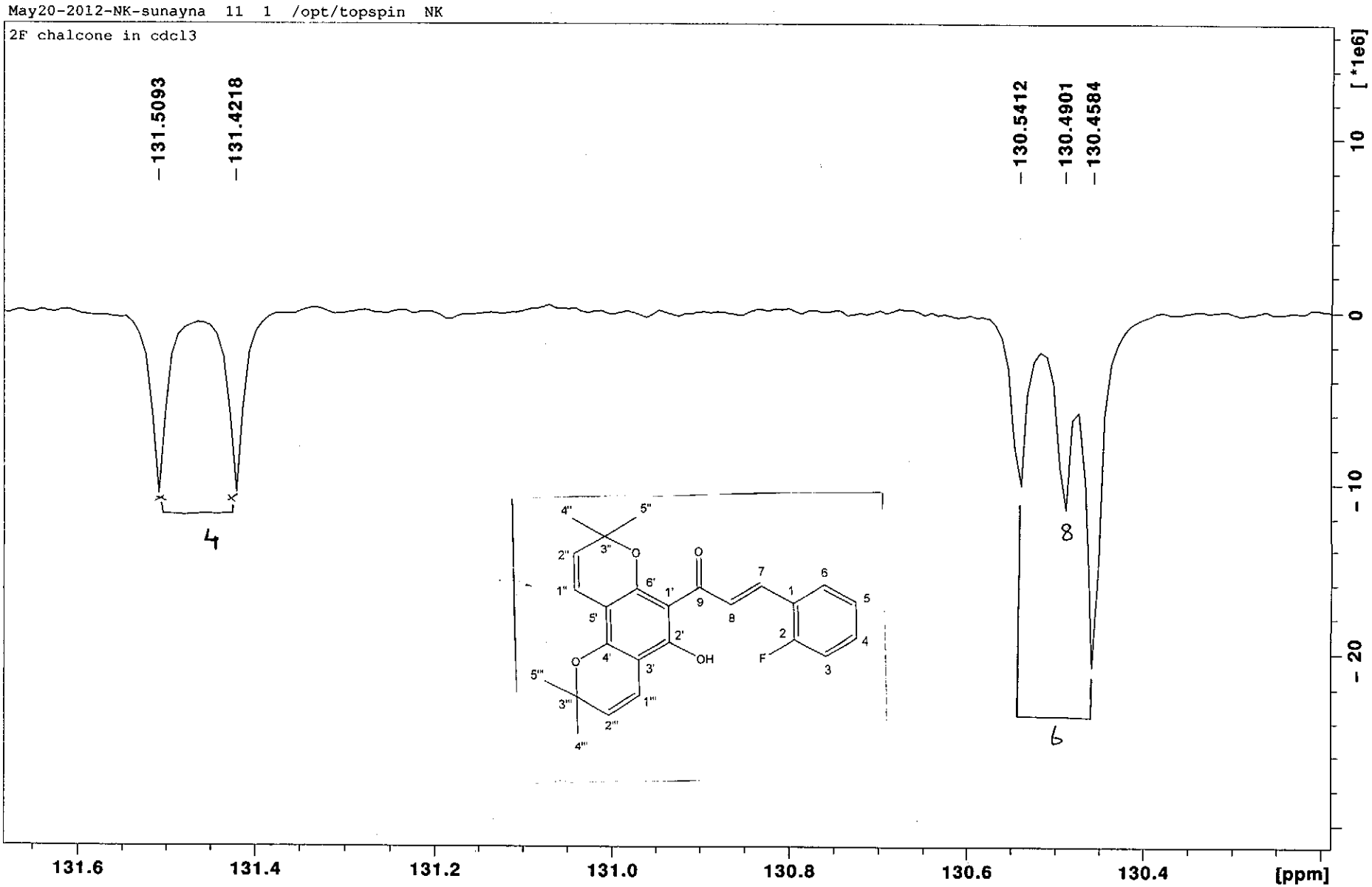

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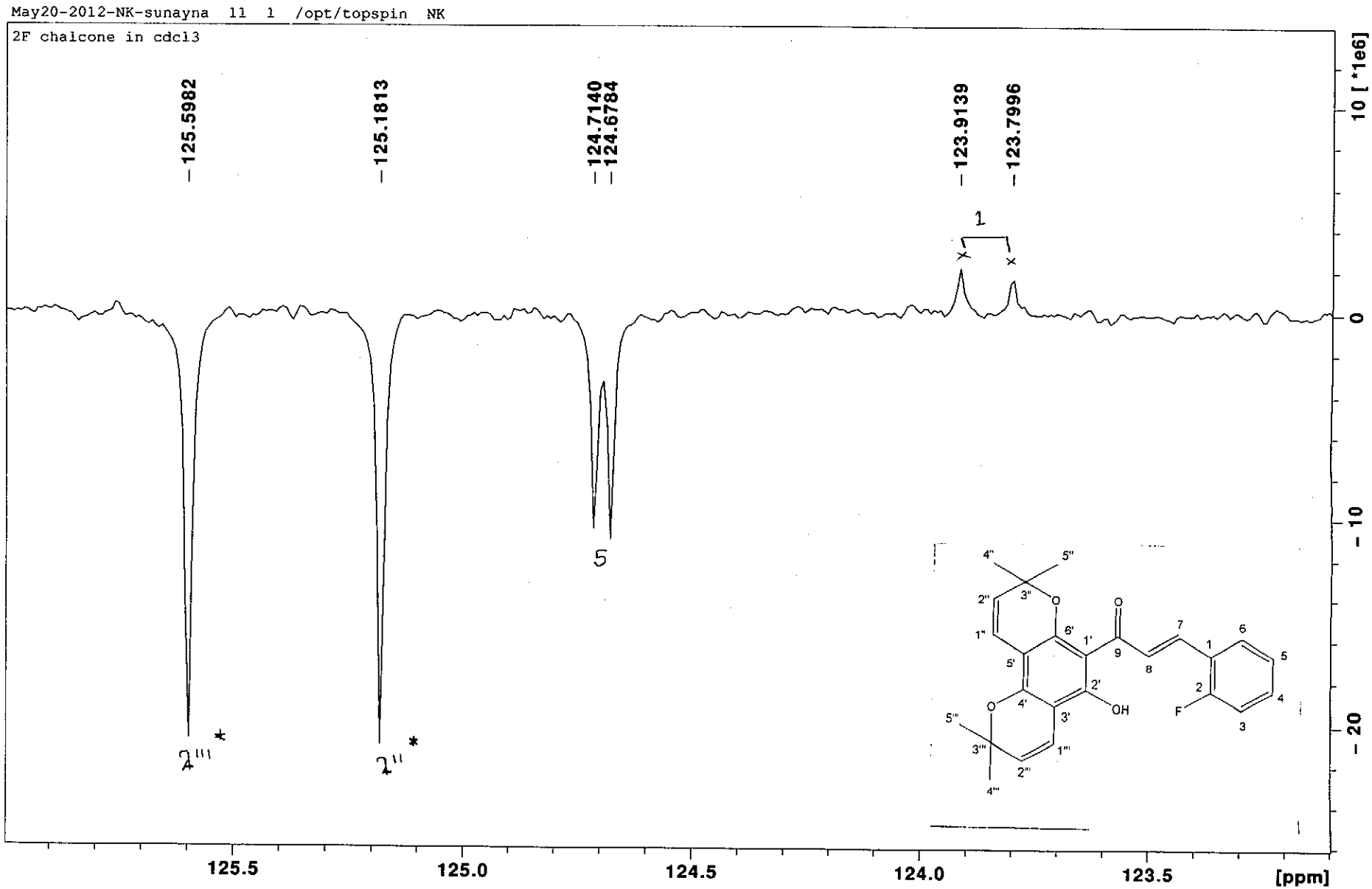

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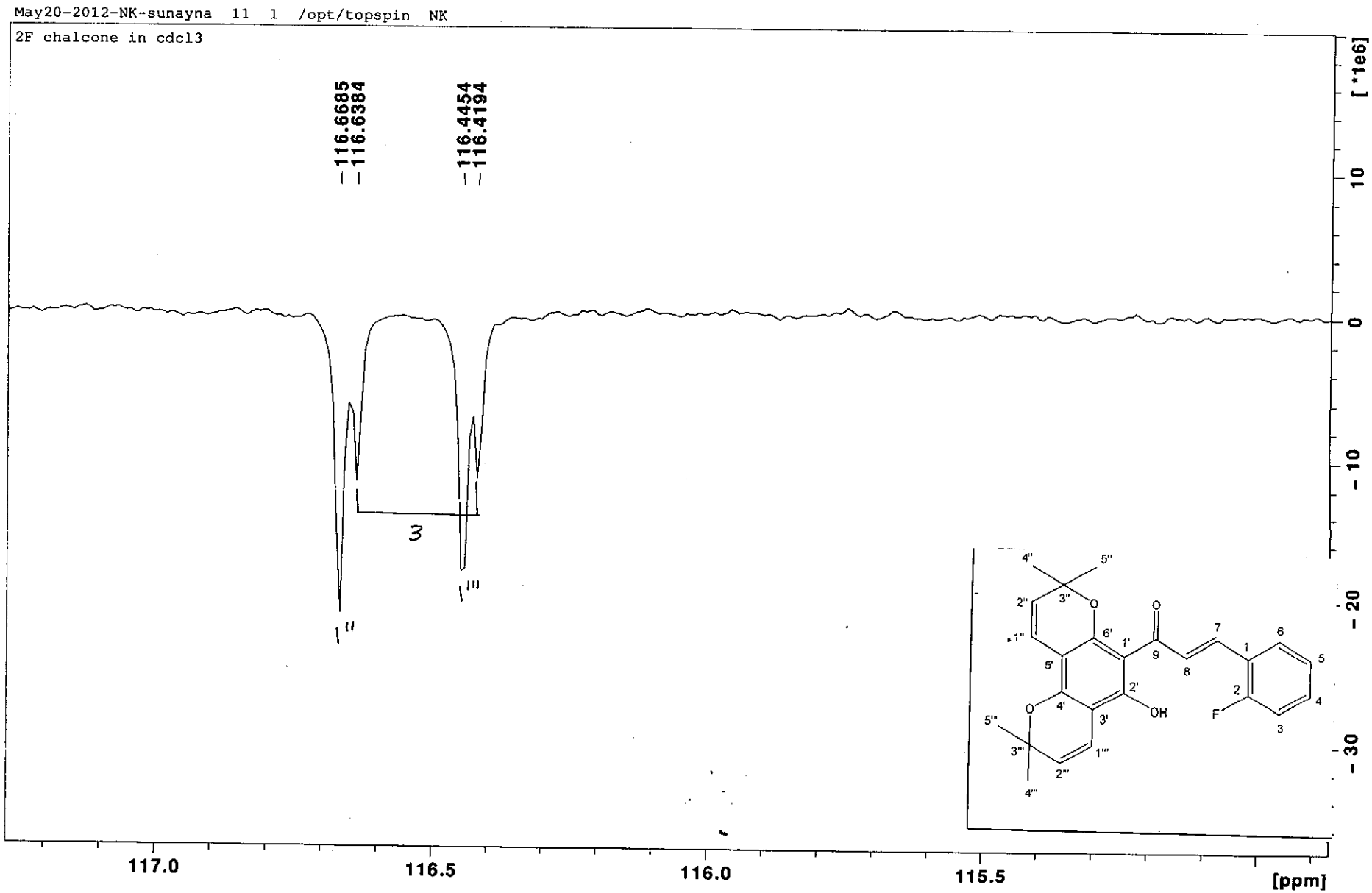

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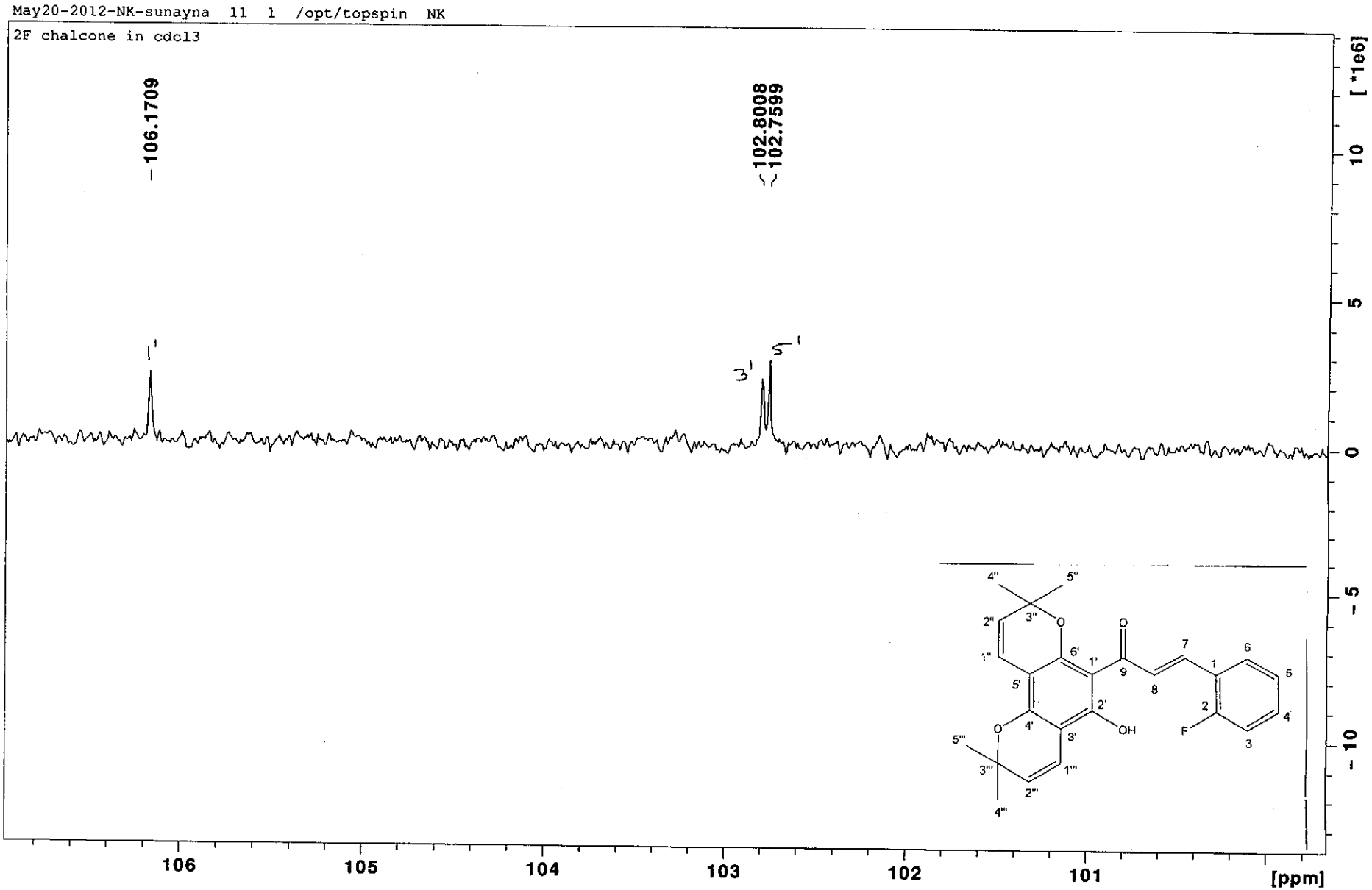

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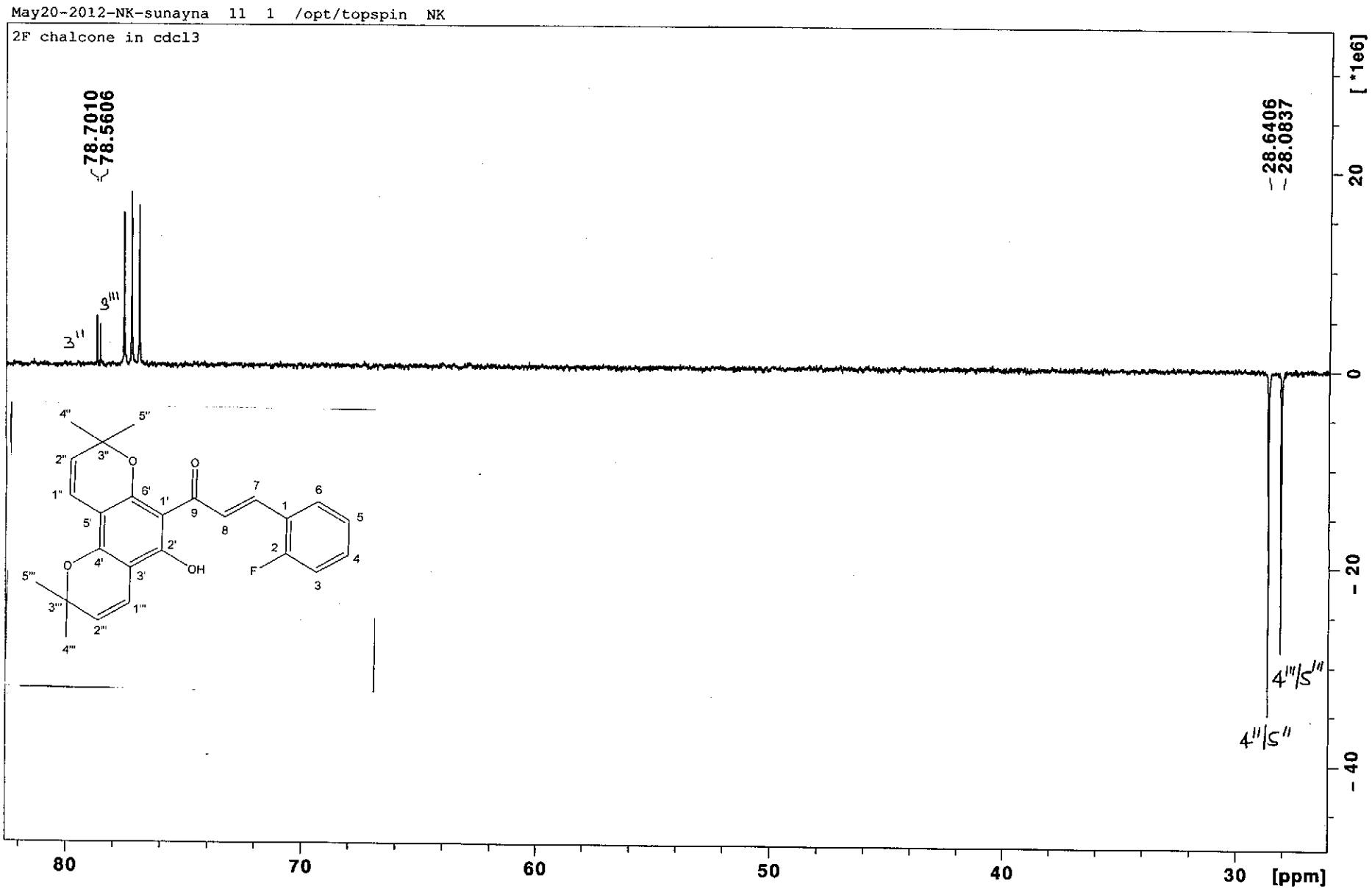

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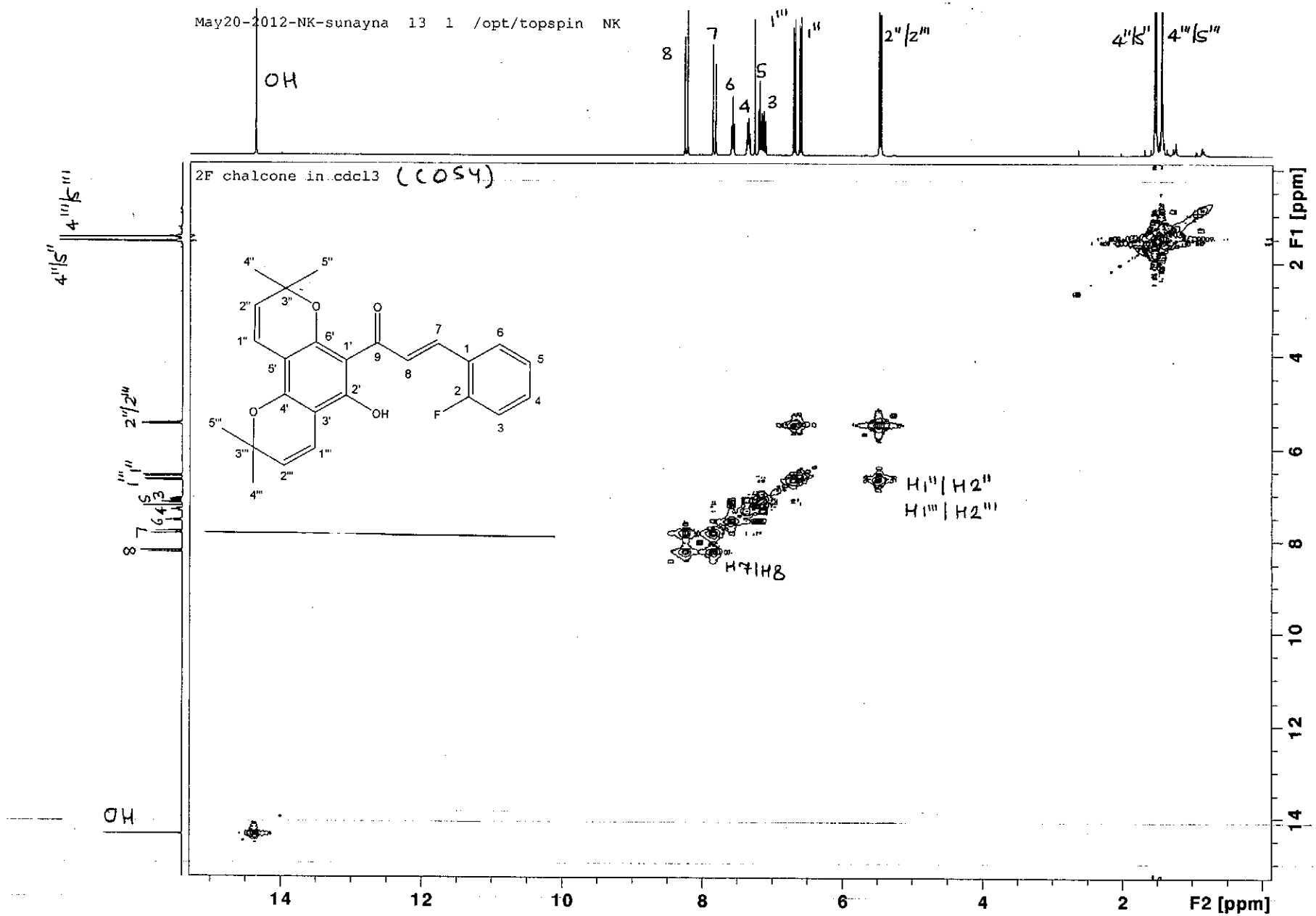

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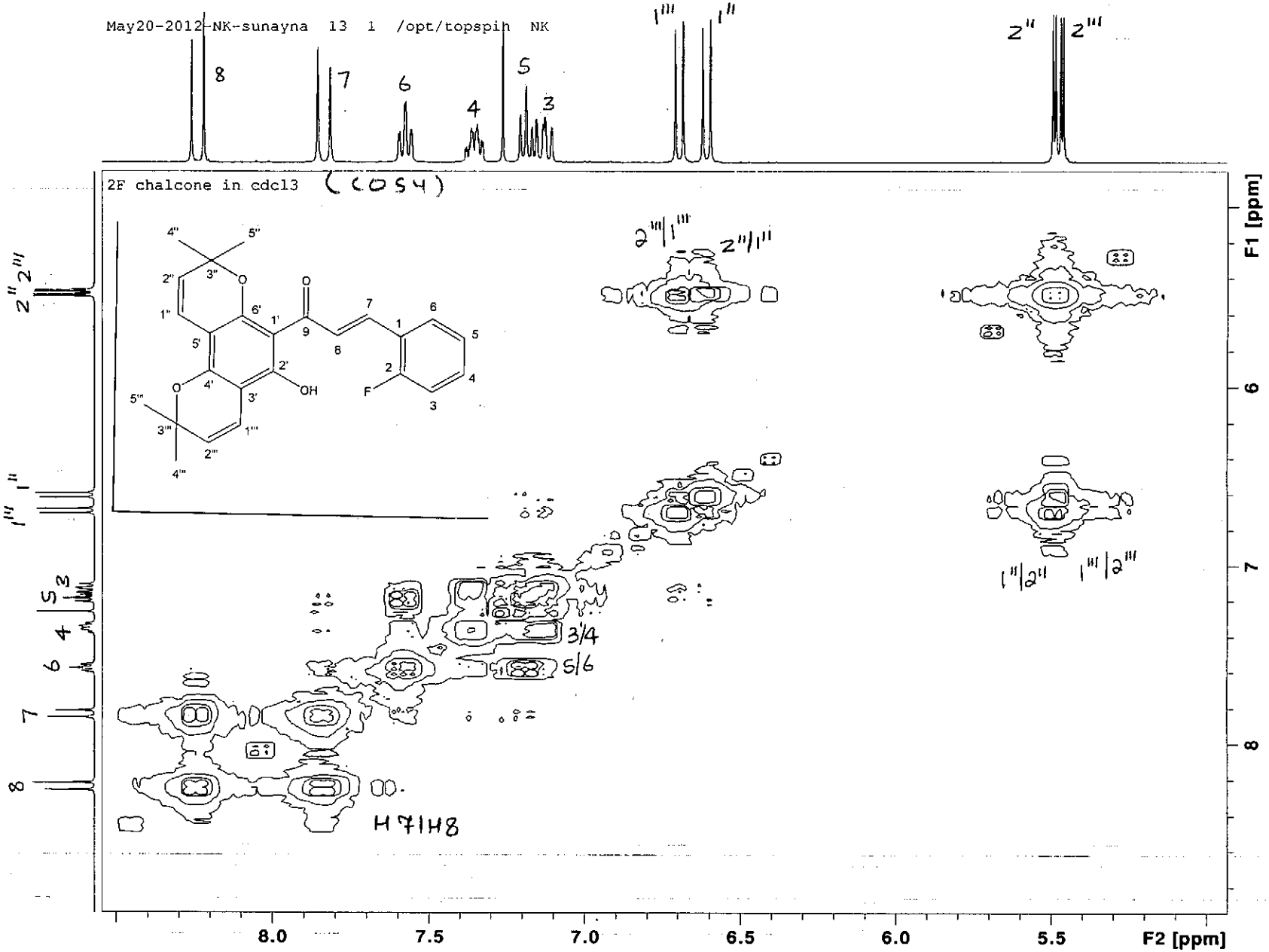

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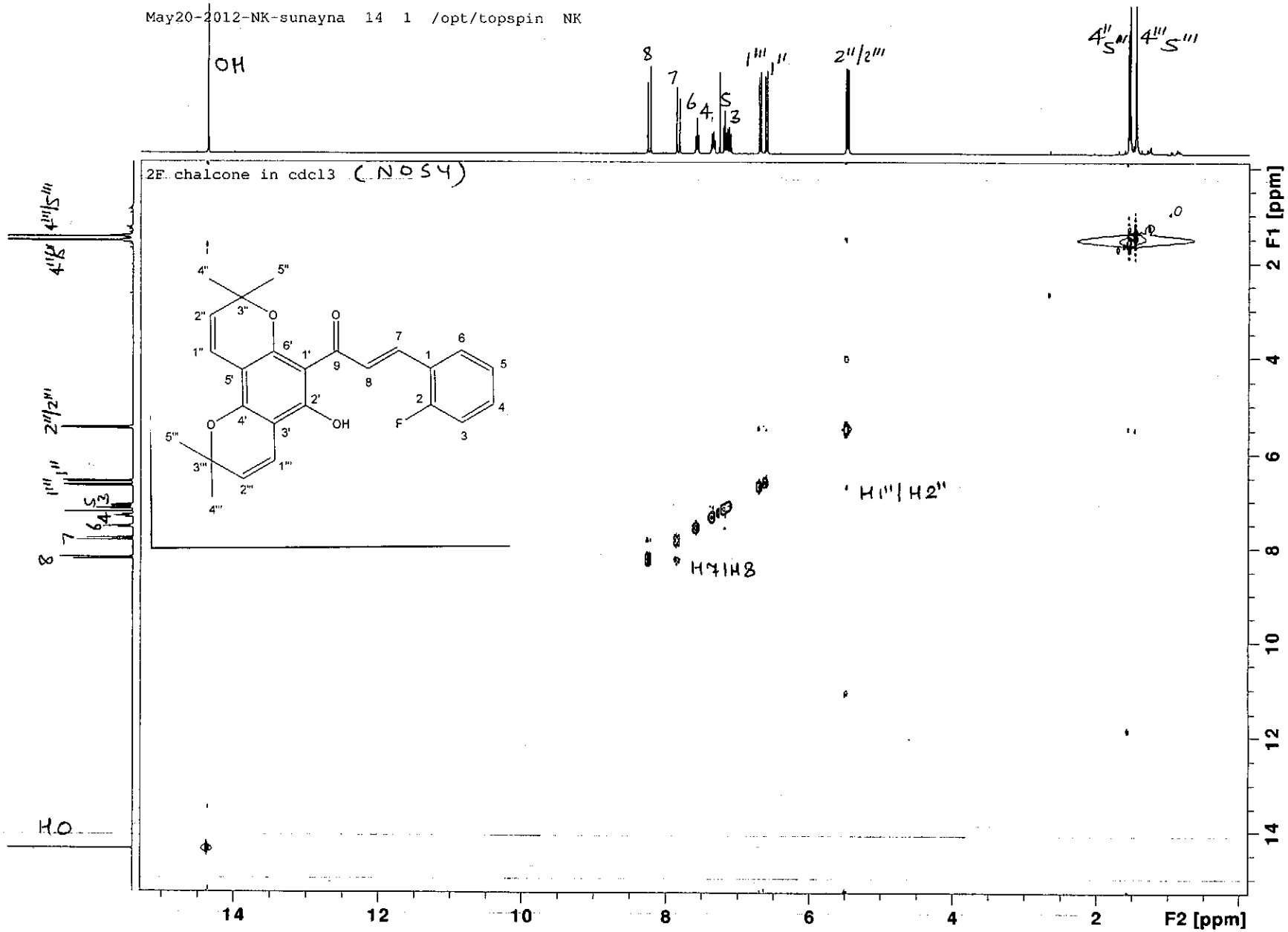

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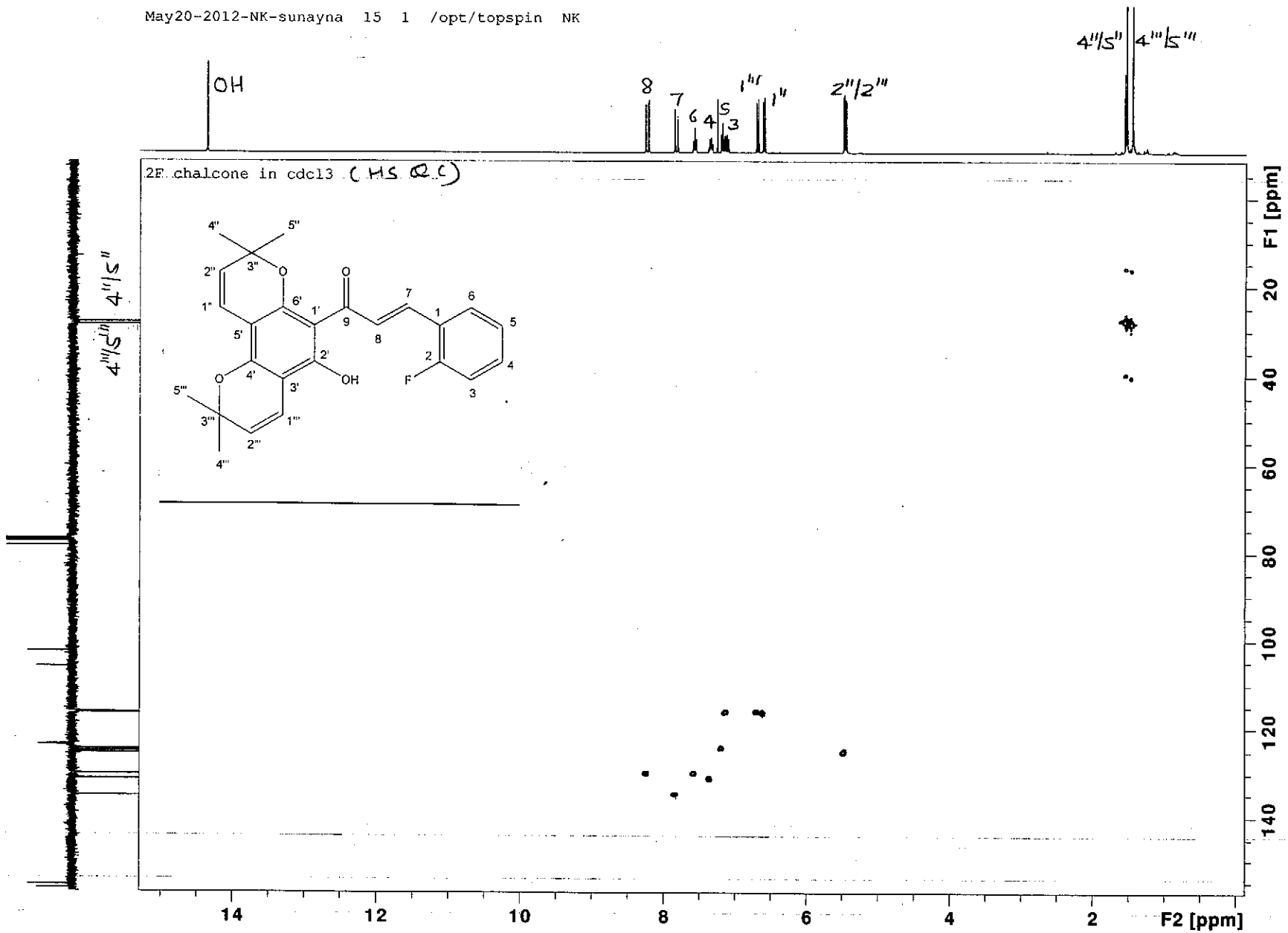

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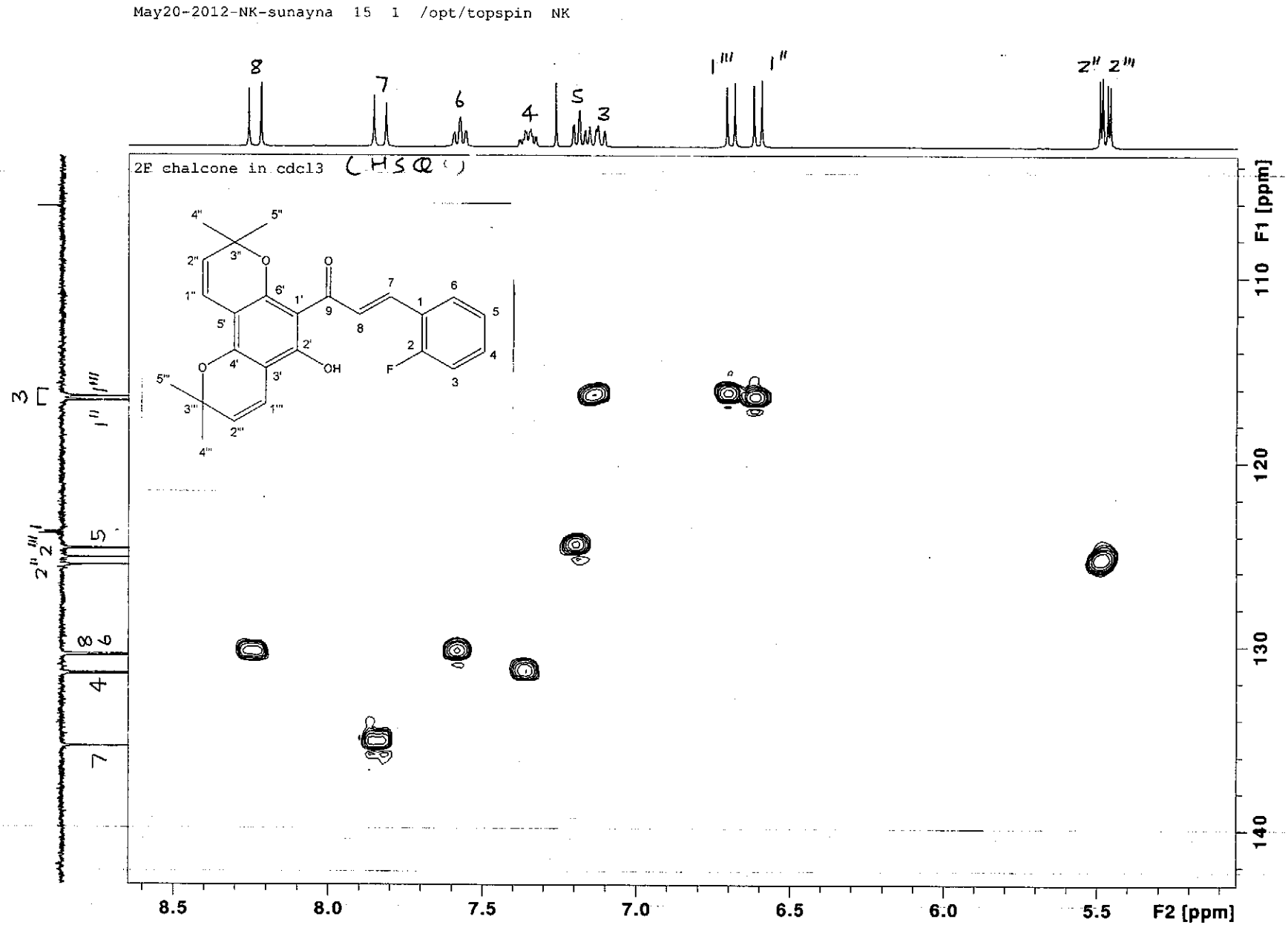

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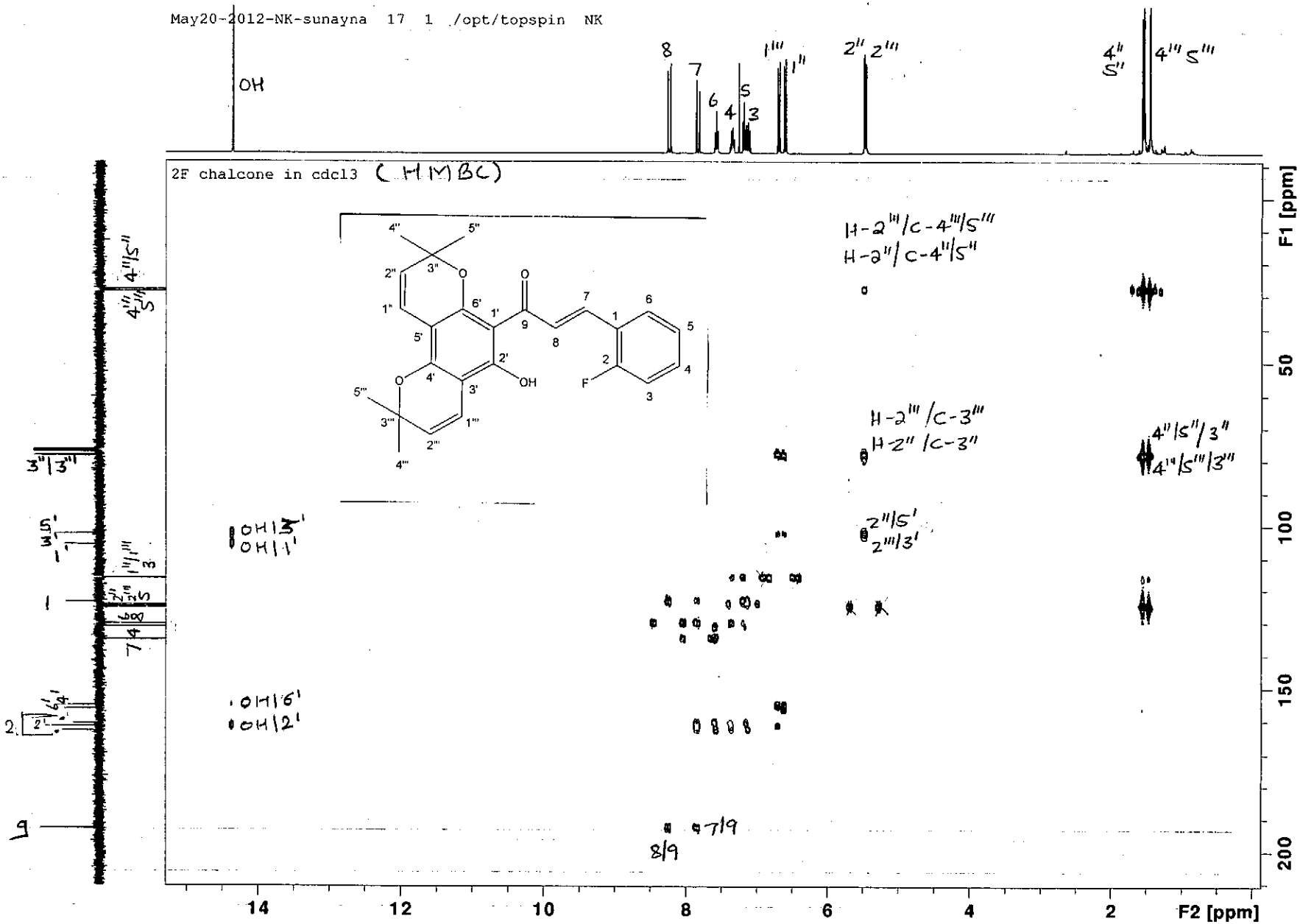

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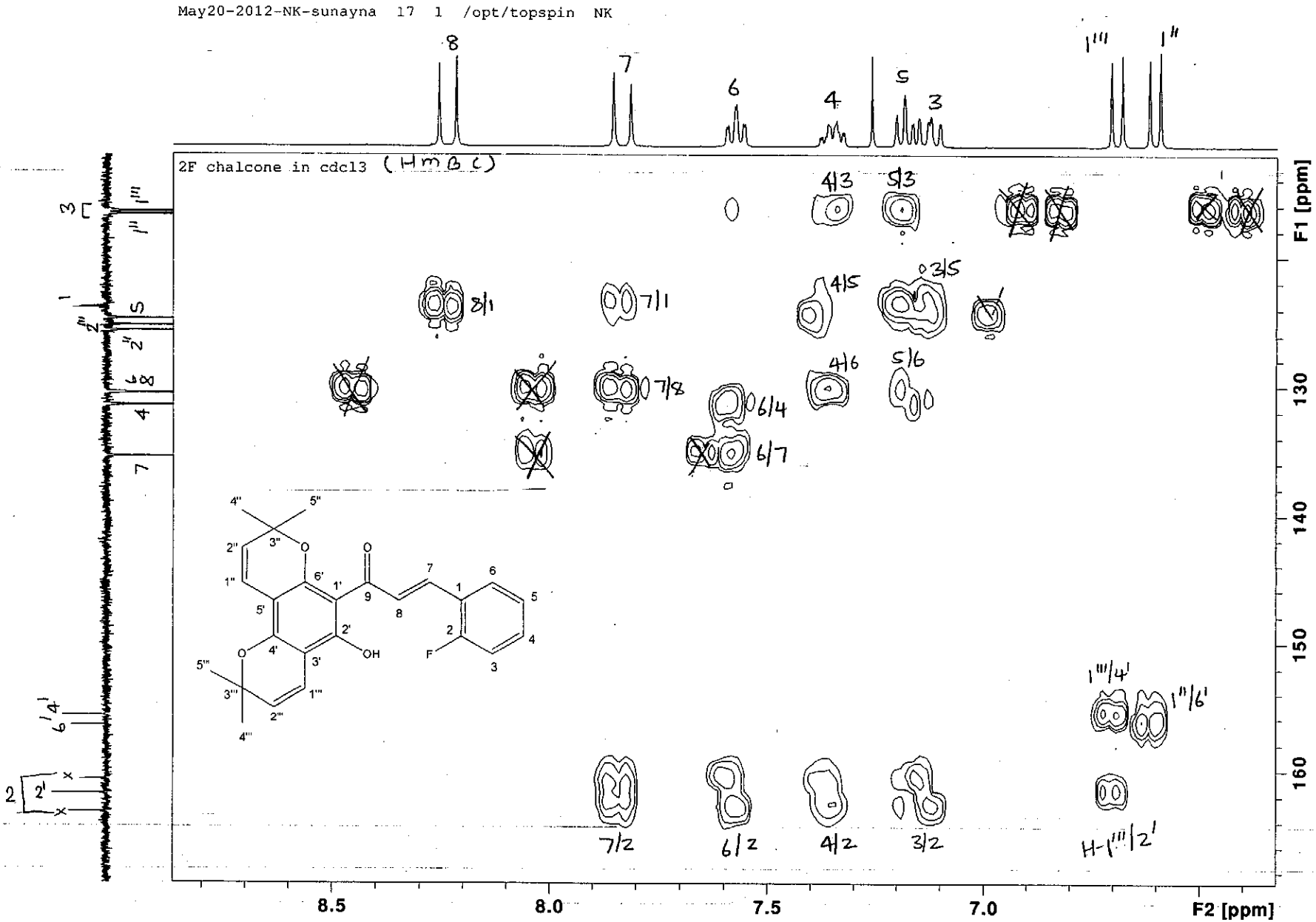

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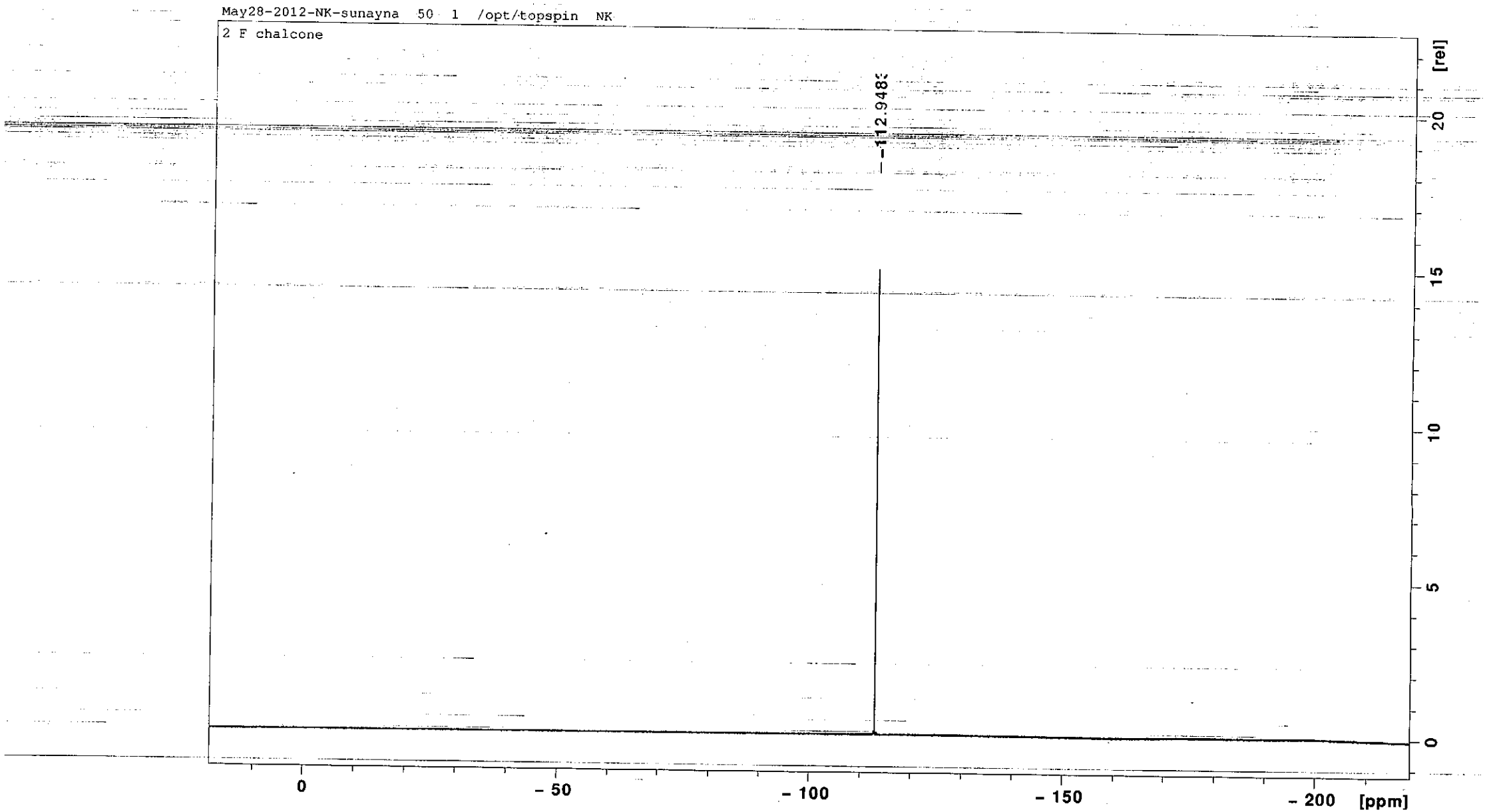

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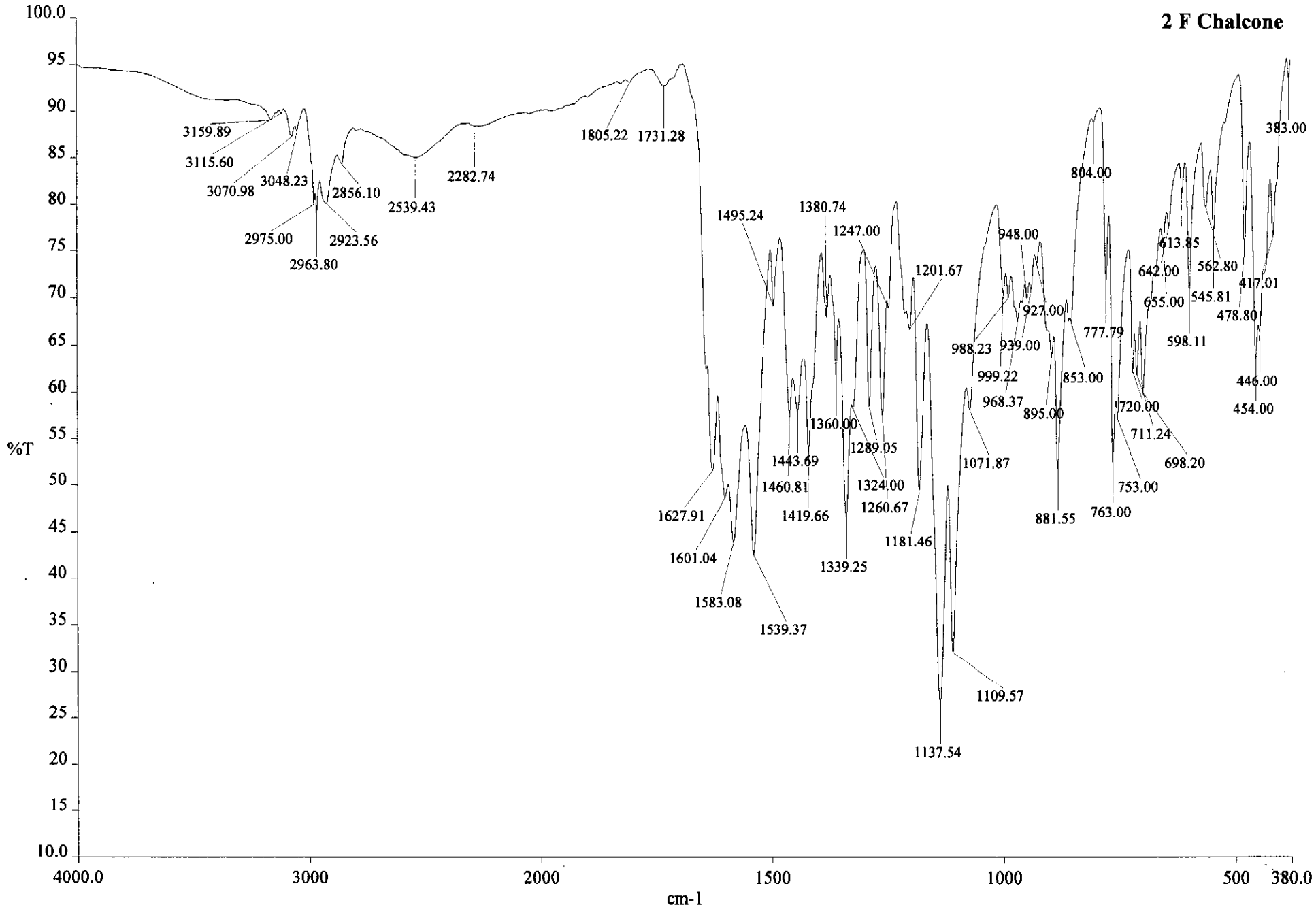

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IR spectrum of 3b - 2-F pyranochromene chalcone (A-3b)

Single Mass Analysis

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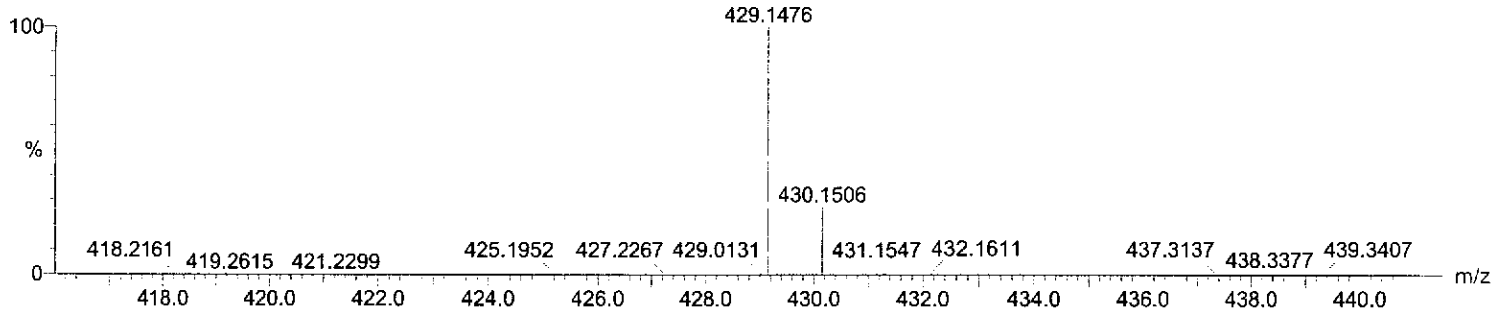
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
6 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-30 H: 20-25 O: 0-5 F: 0-1 Na: 0-1

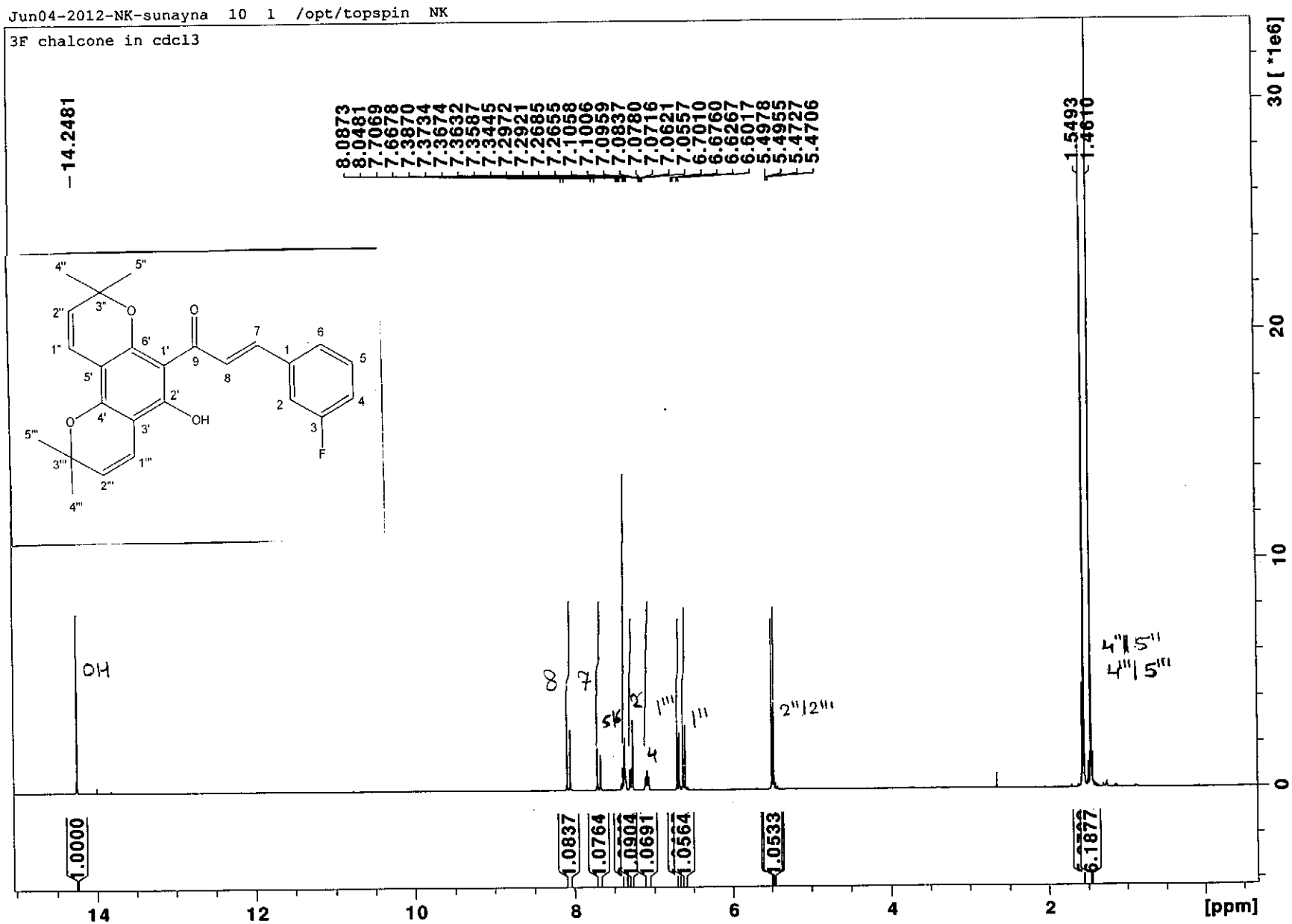
Dr. Nizam
10 2 (0.017) Cm (1:30)

TOF MS ES+
2.40e+004



Minimum:				-1.5			
Maximum:		5.0	1.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
429.1476	429.1478	-0.2	-0.5	13.5	402.8	0.0	C25 H23 O4 F Na

HRMS of 3b – 2-F pyranochromene chalcone (A-3b)



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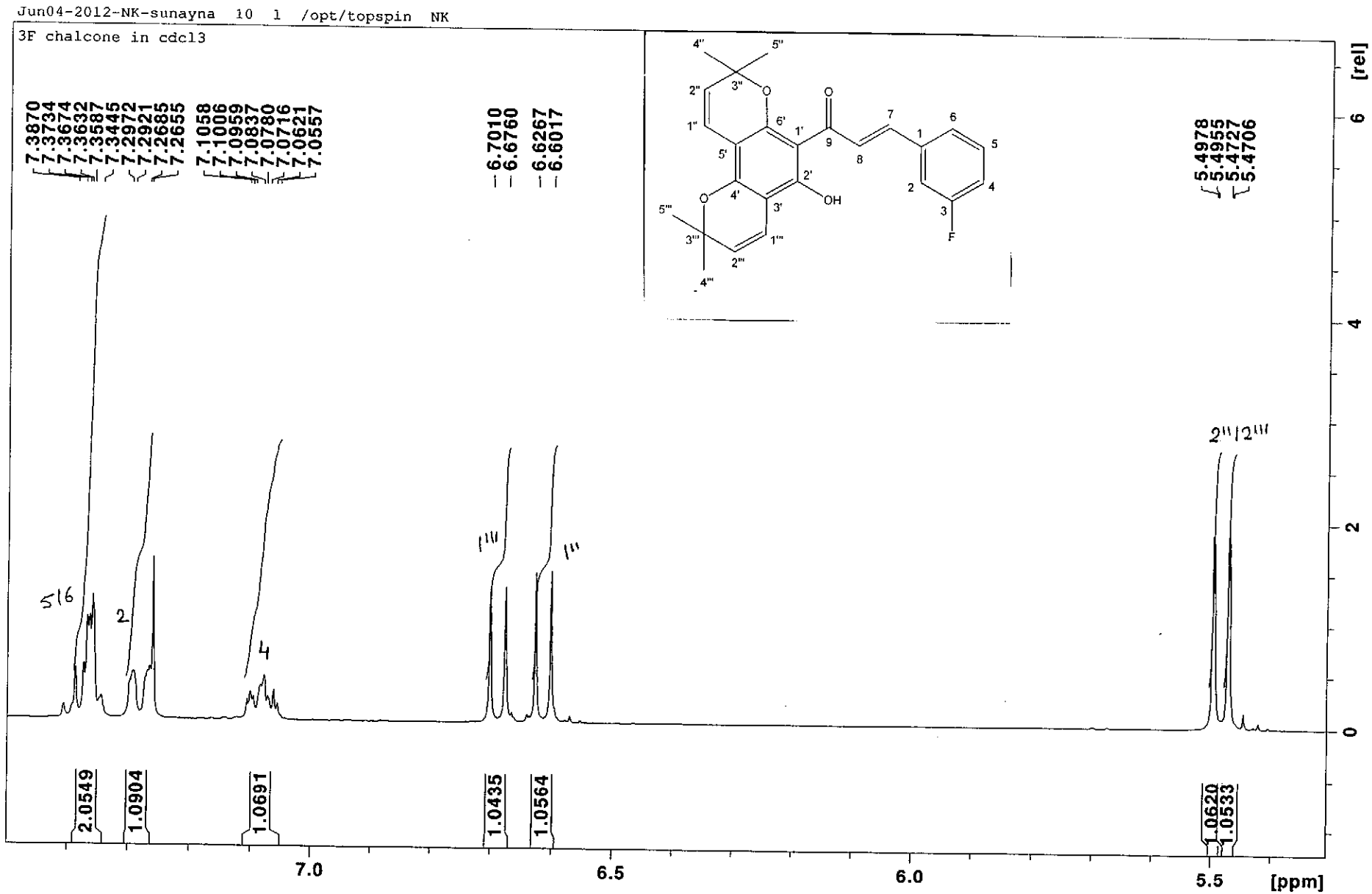

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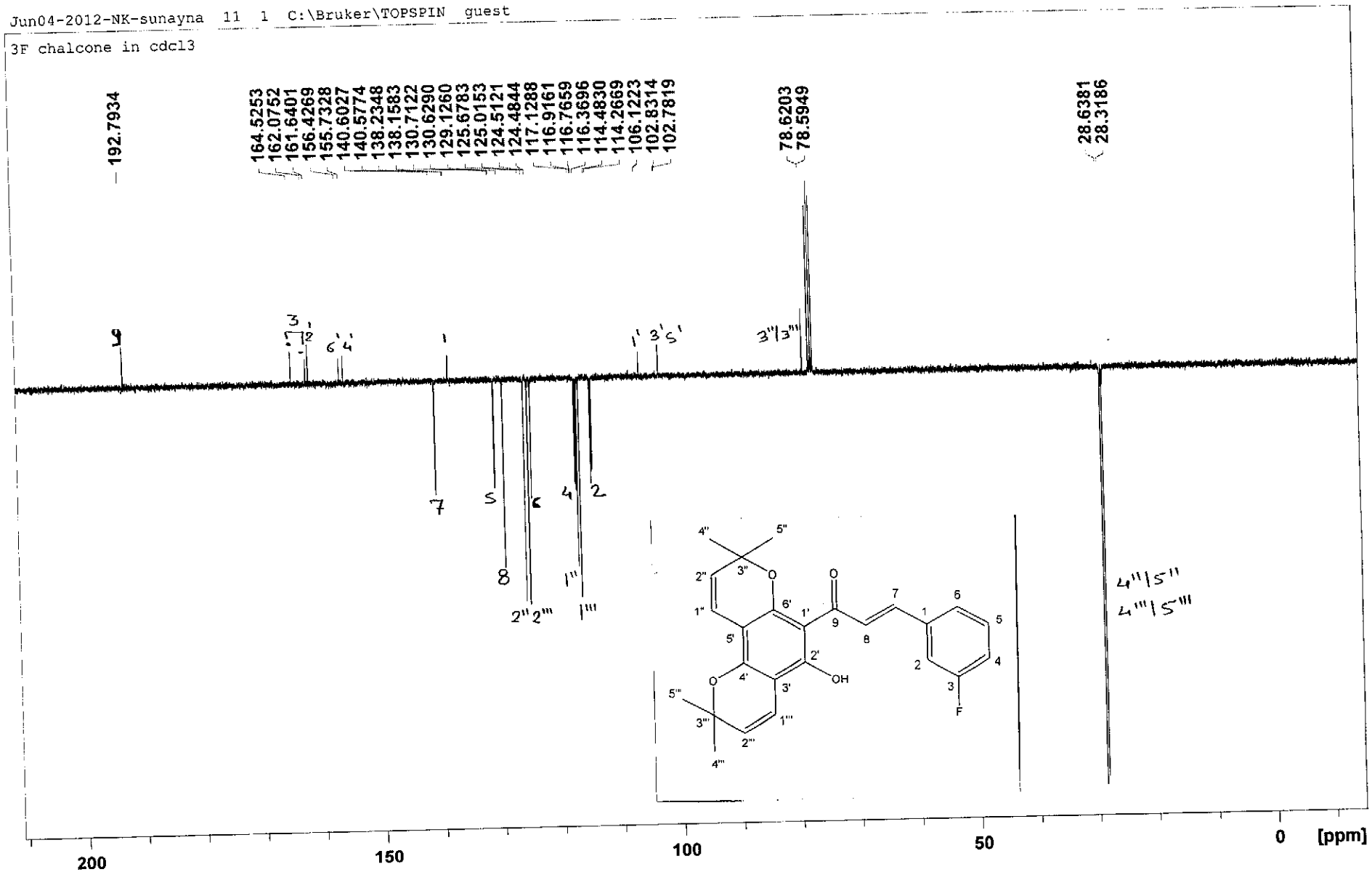

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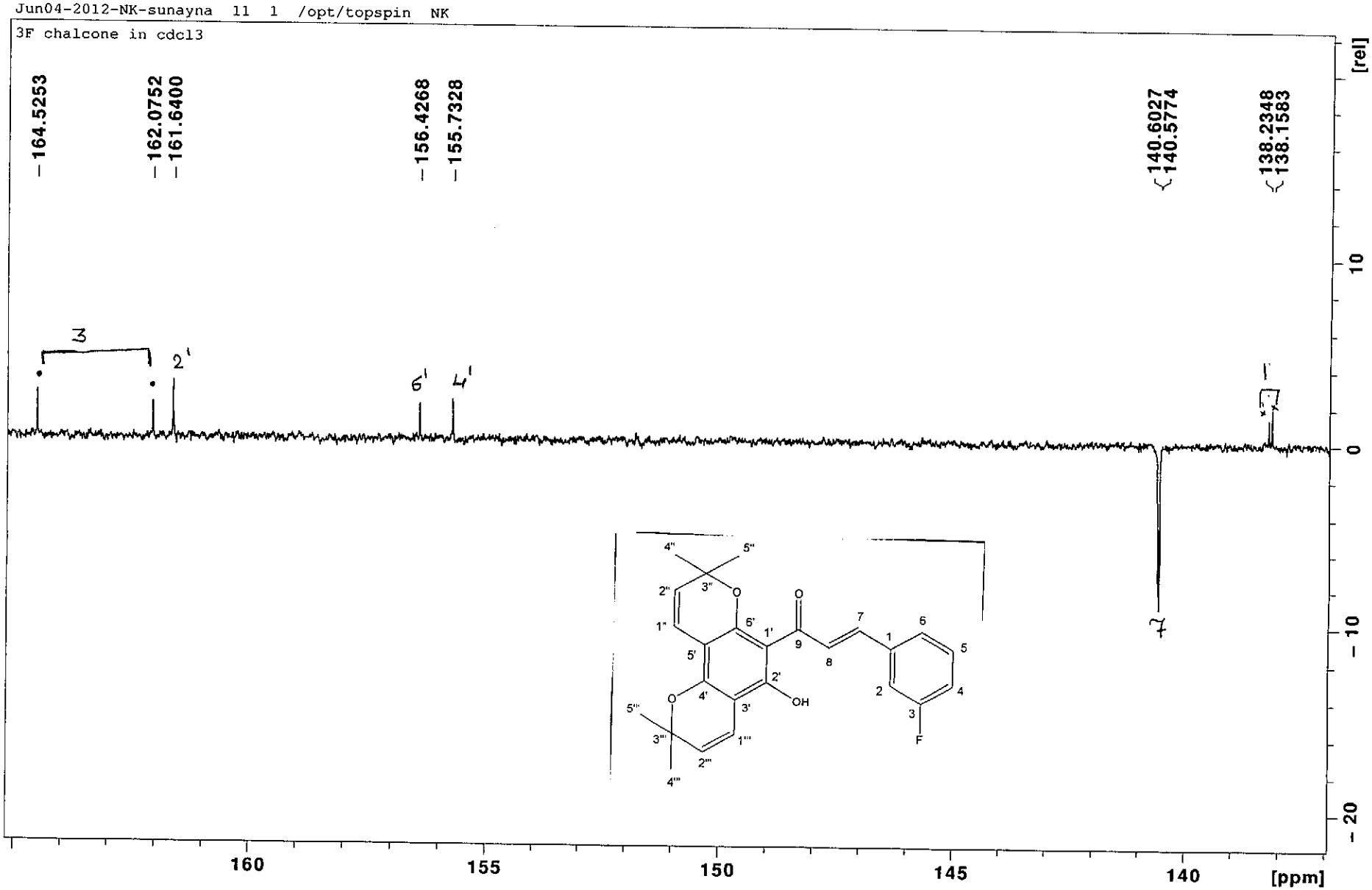

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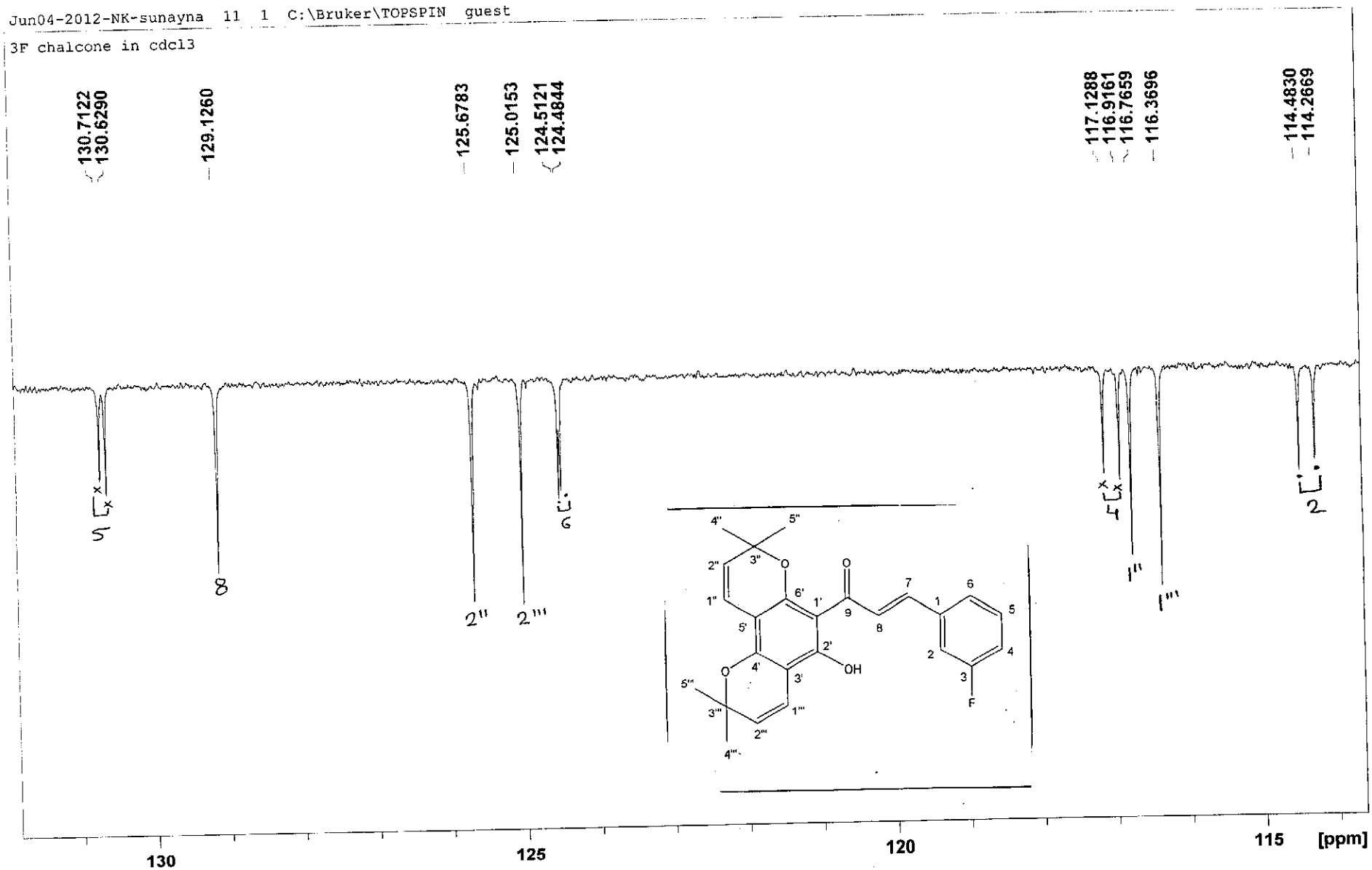

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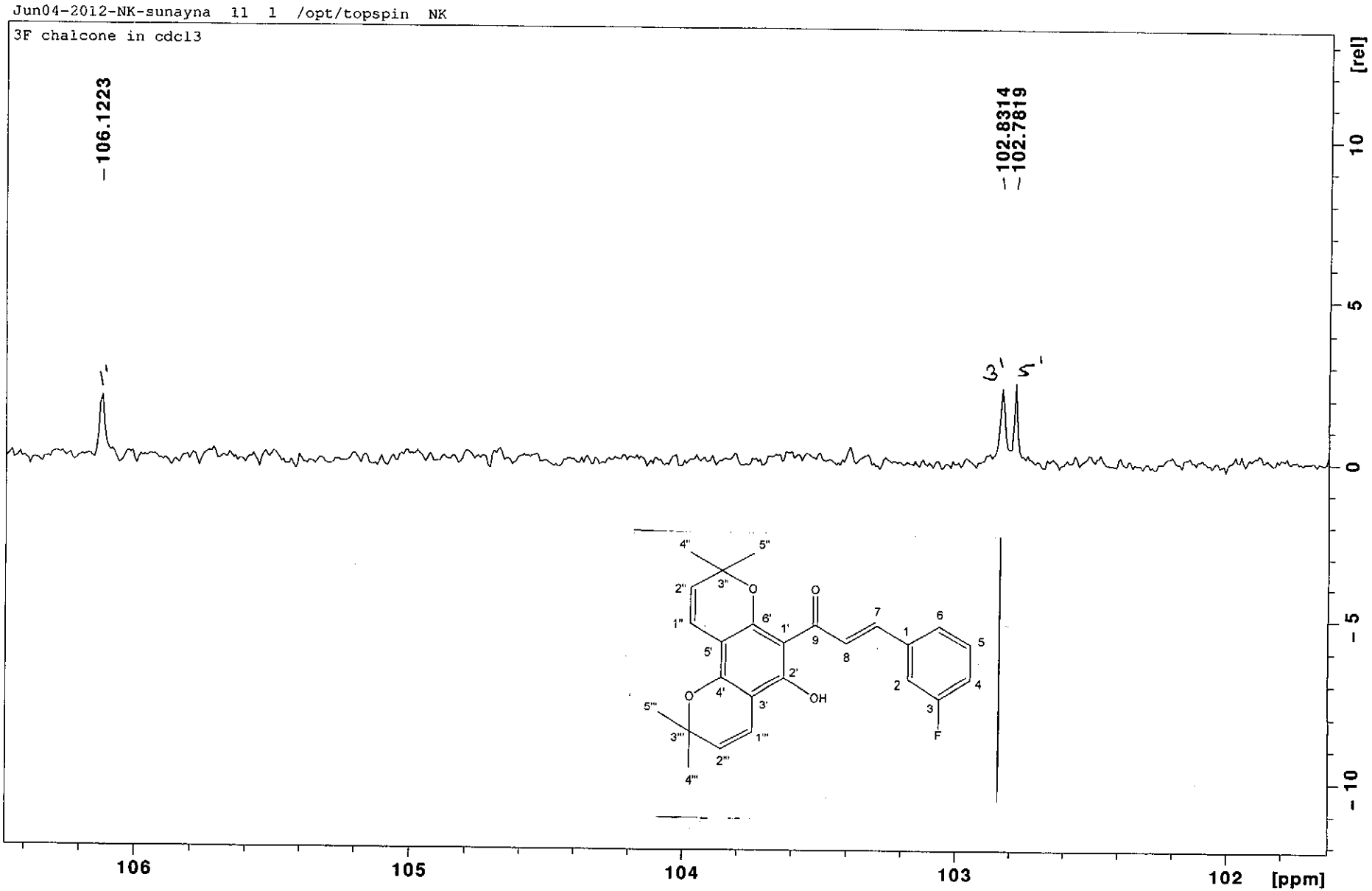
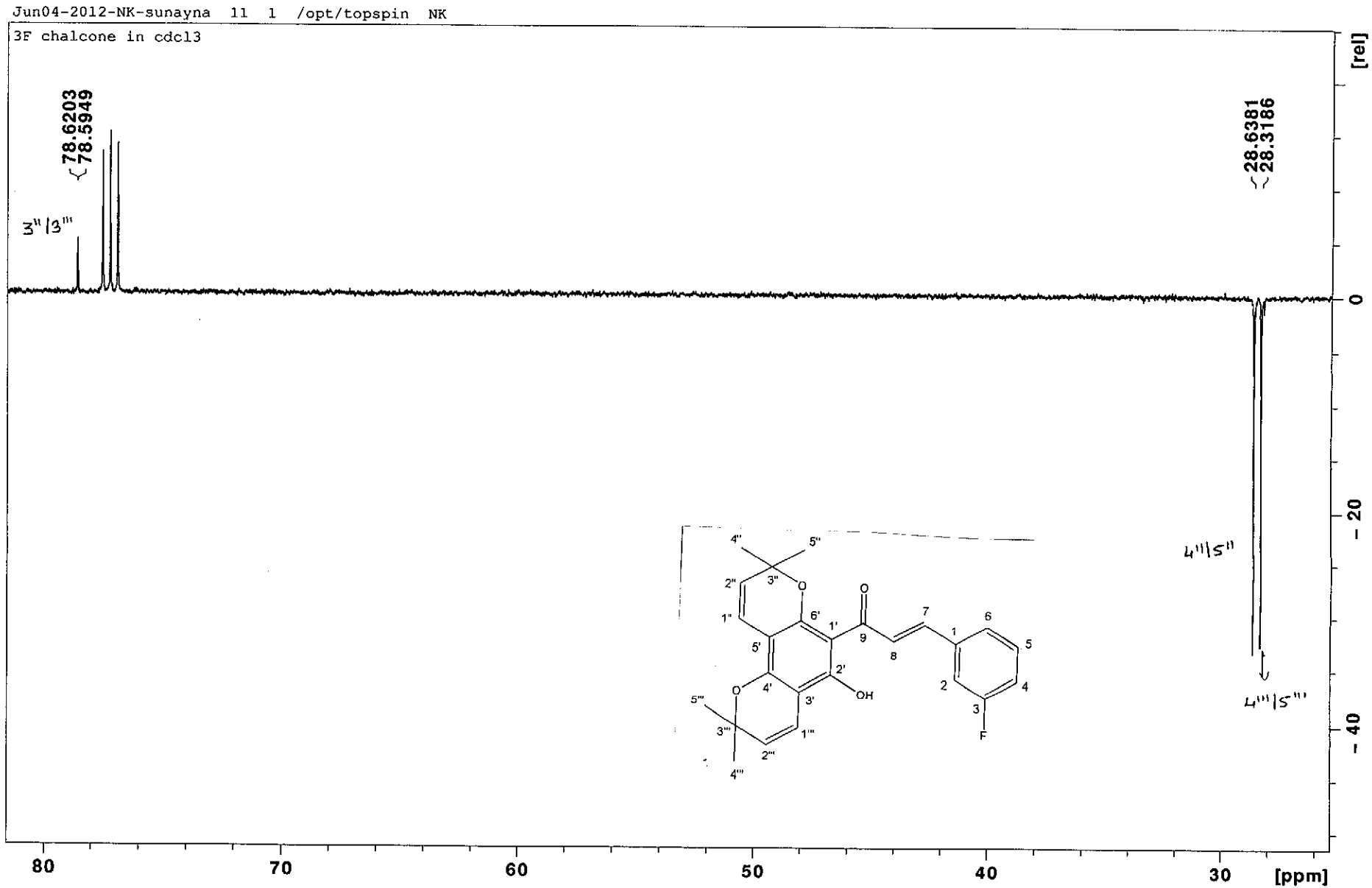
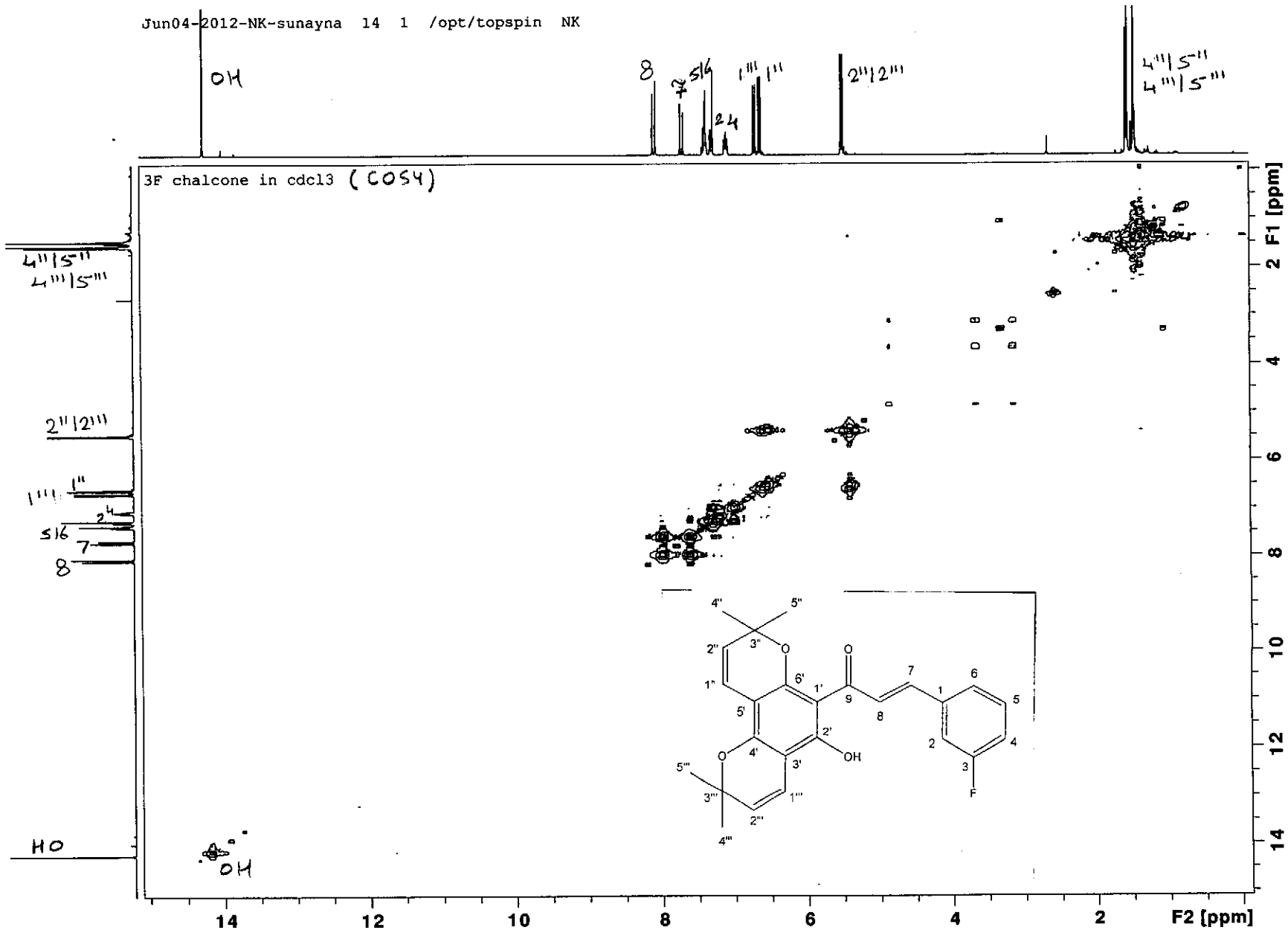


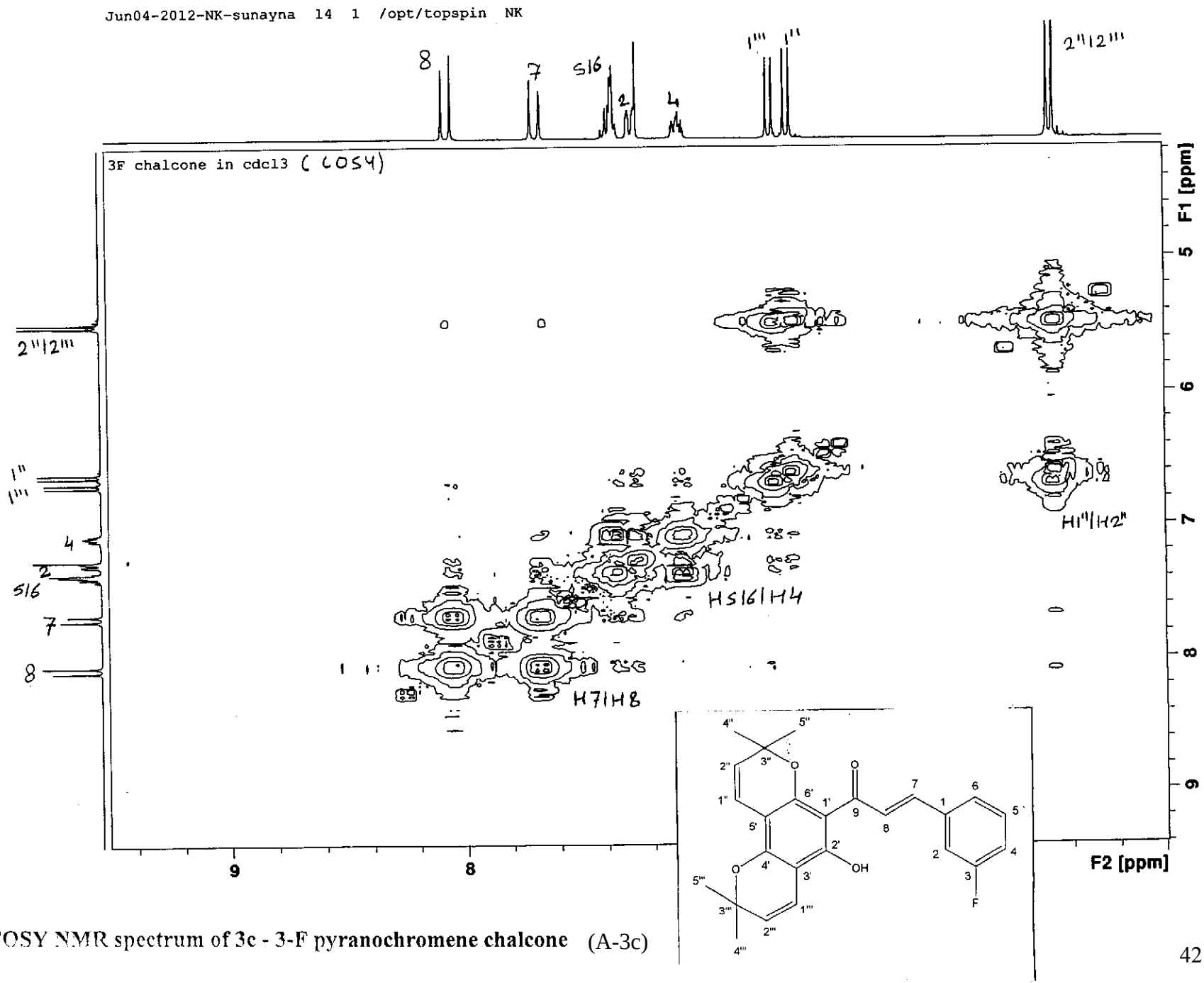
^{13}C NMR spectrum of 3c - 3-F pyranochromene chalcone (A-3c)



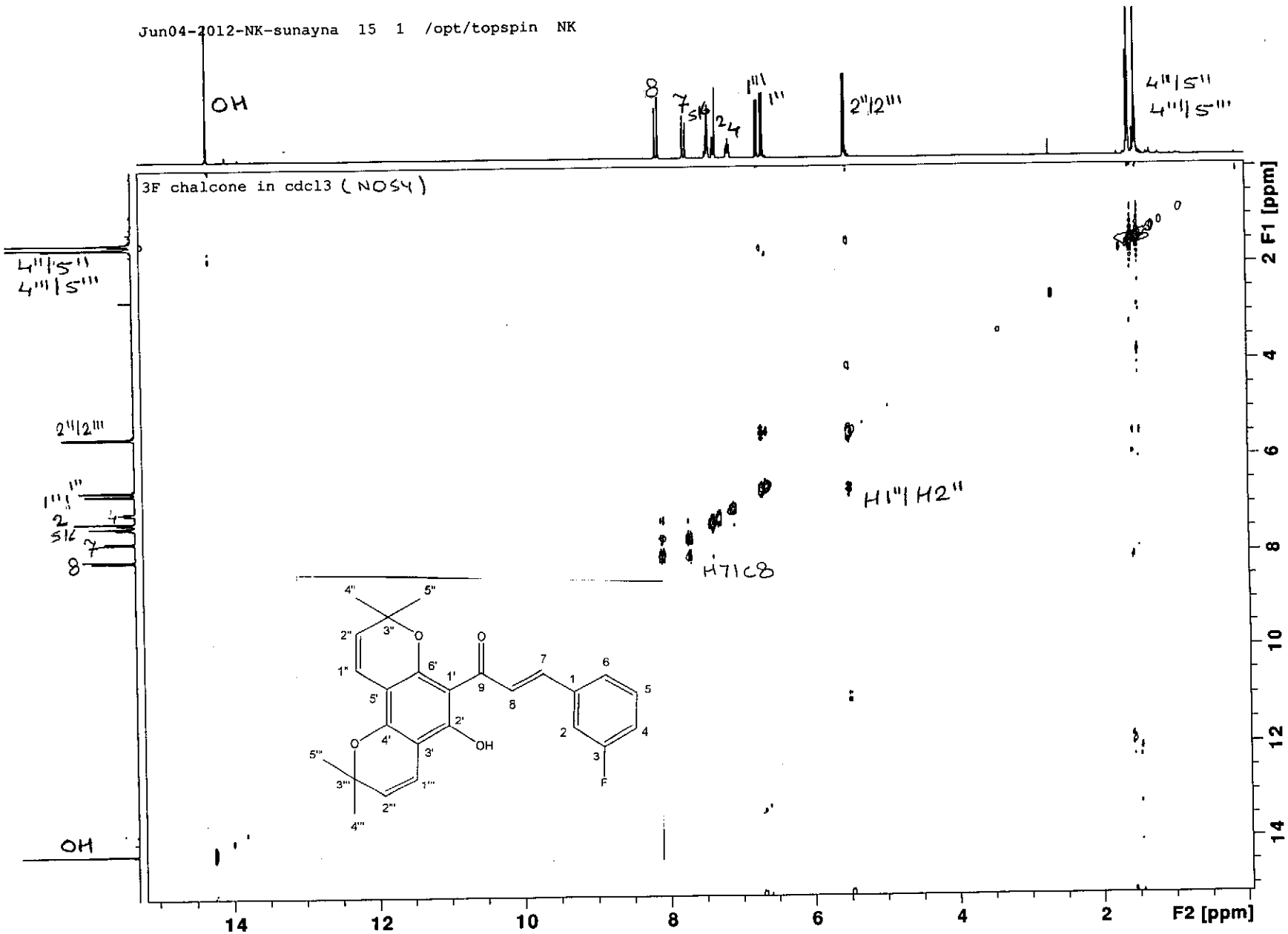
^{13}C NMR spectrum of 3c - 3-F pyranochromene chalcone (A-3c)



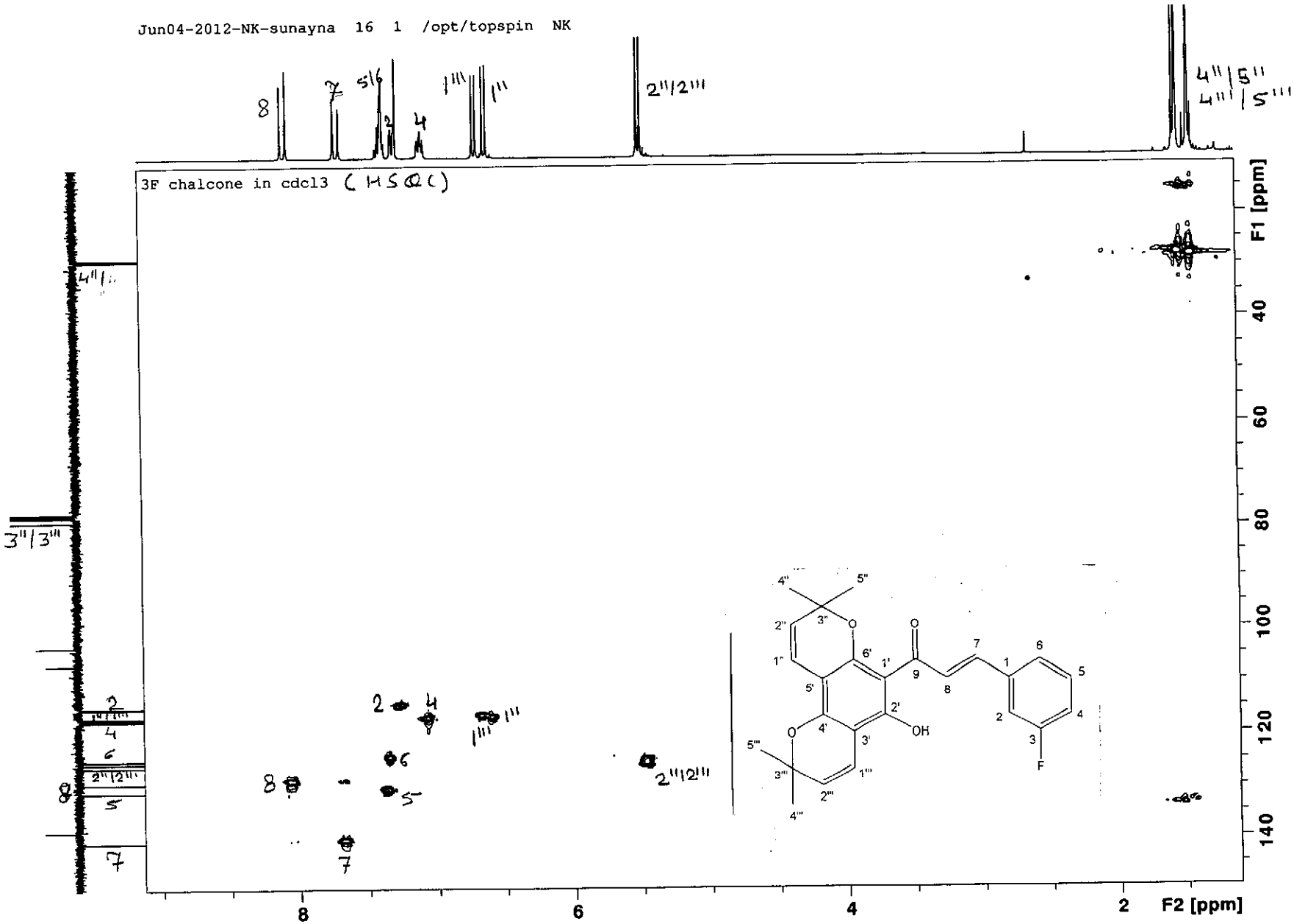
COSY NMR spectrum of 3c - 3-F pyranochromene chalcone (A-3c)



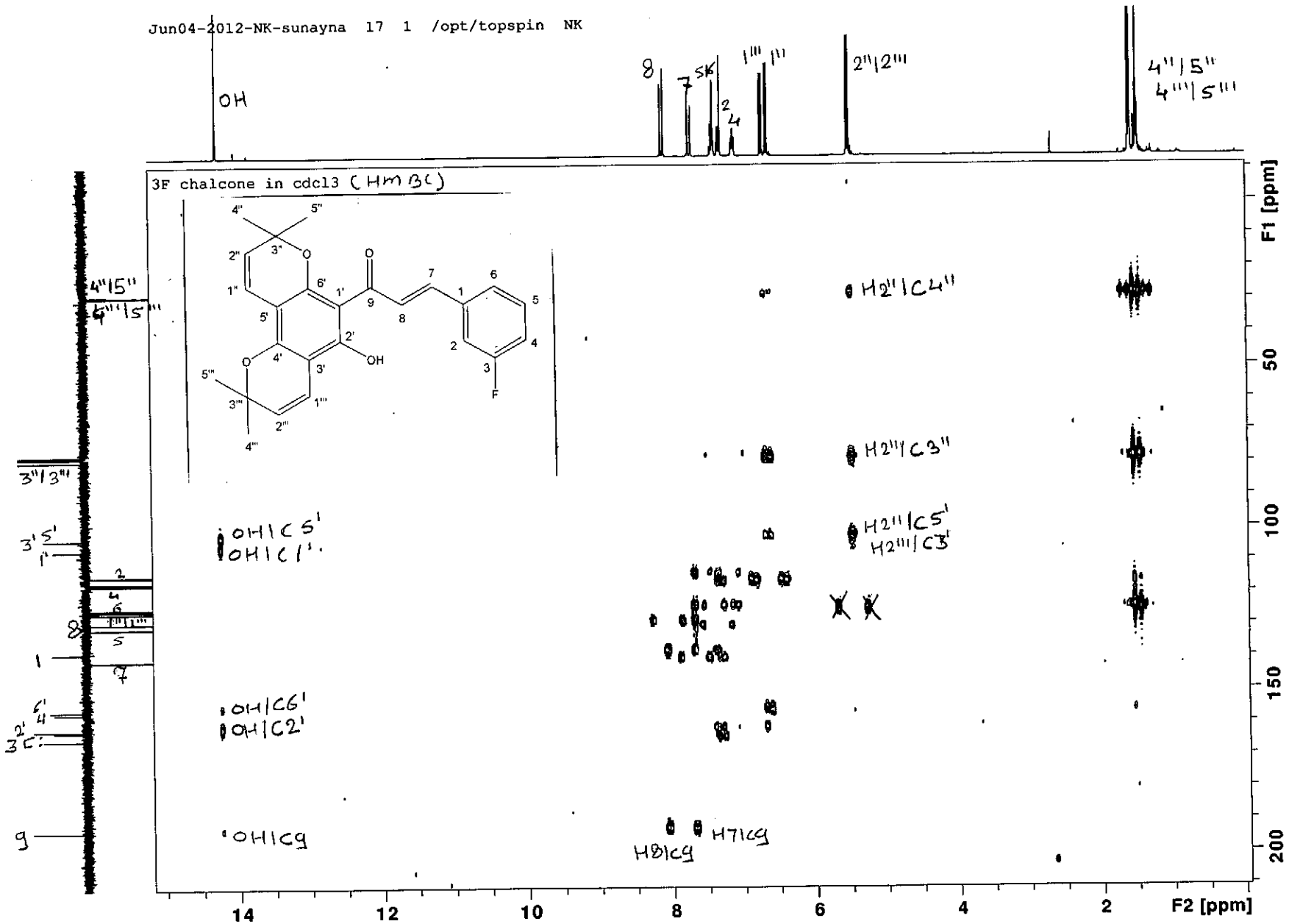
COSY NMR spectrum of **3c - 3-F pyranochromene chalcone** (A-3c)



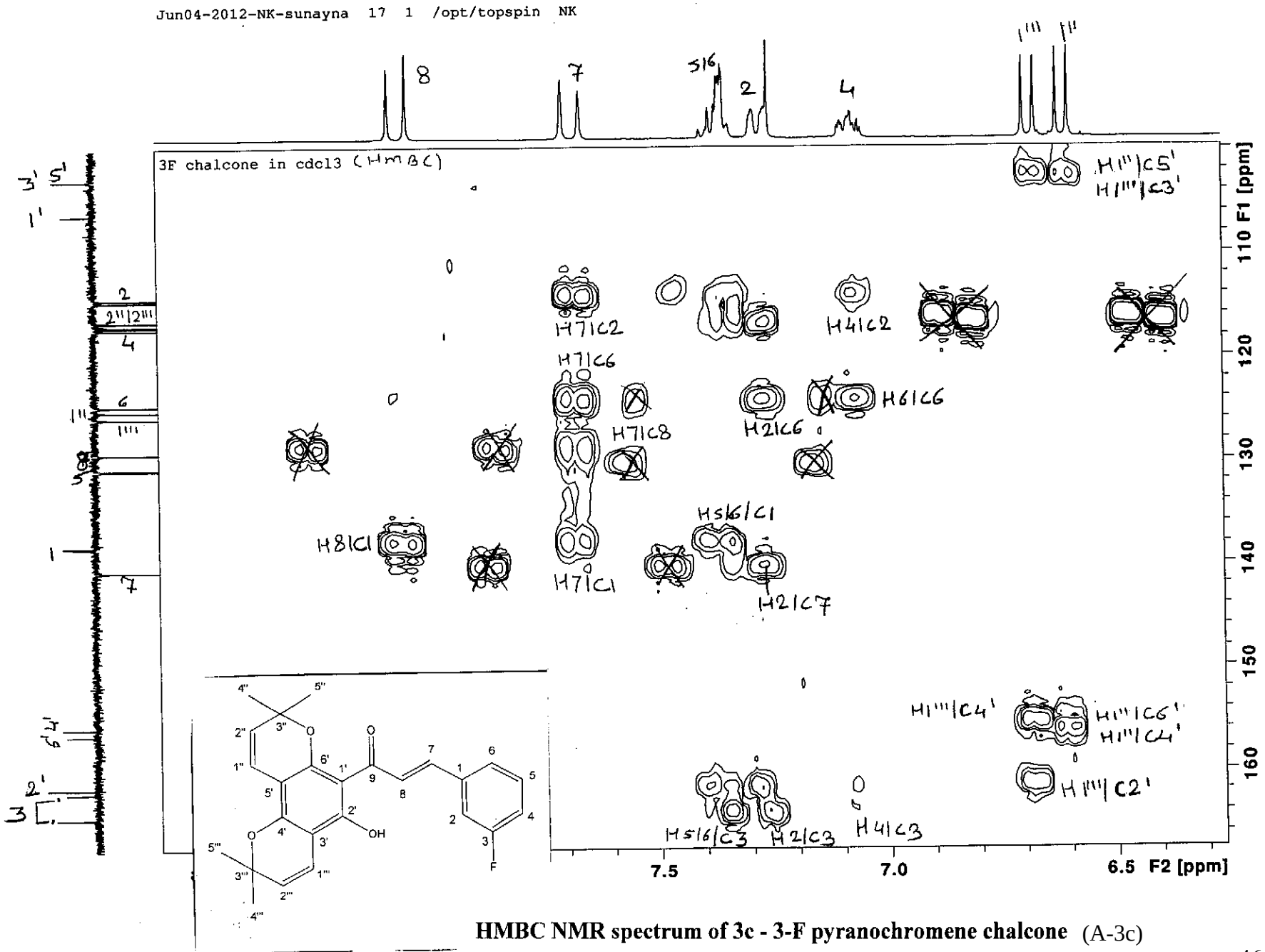
NOSY NMR spectrum of 3c - 3-F pyranochromene chalcone (A-3c)



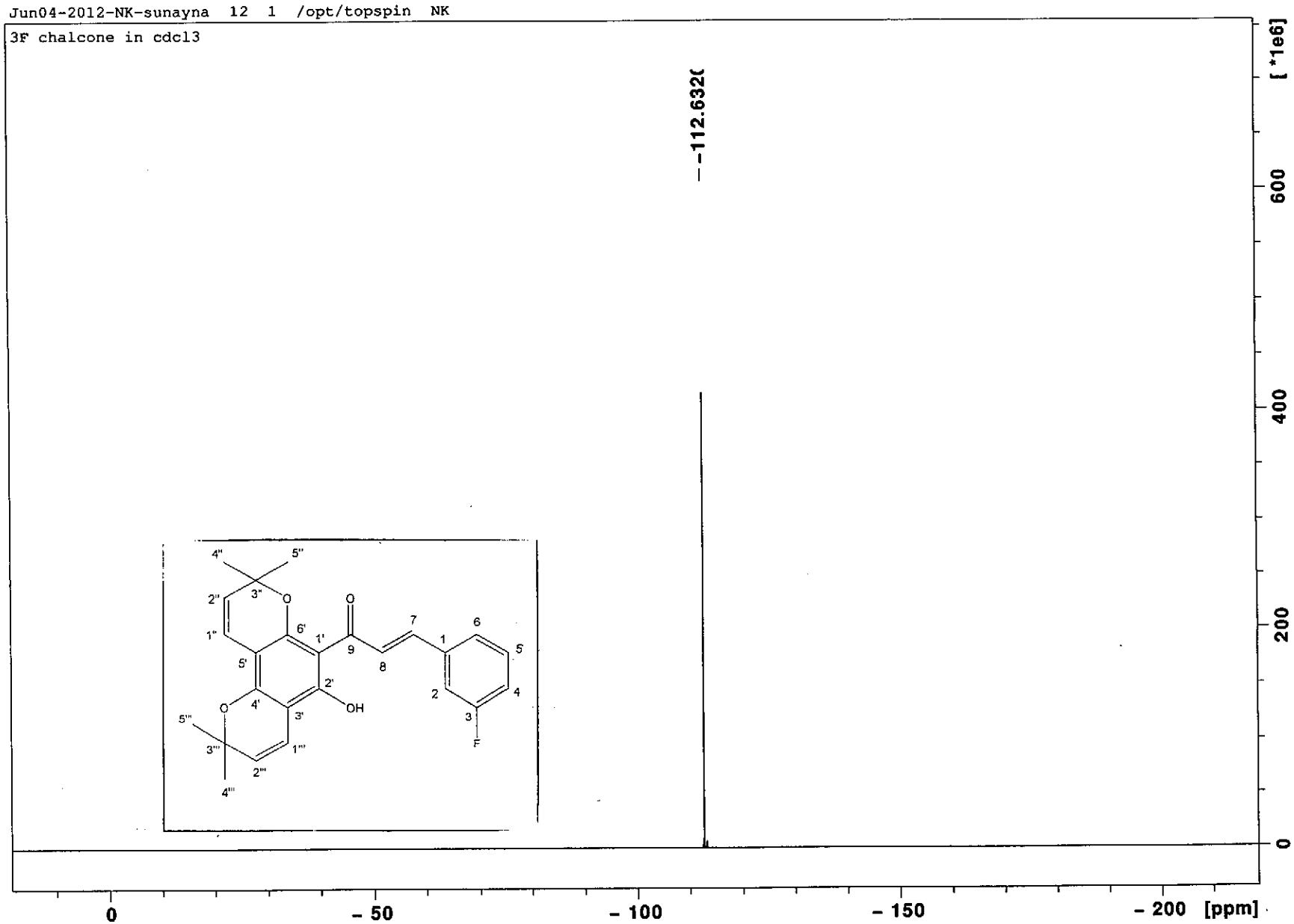
HSQC NMR spectrum of 3c - 3-F pyranochromene chalcone (A-3c)



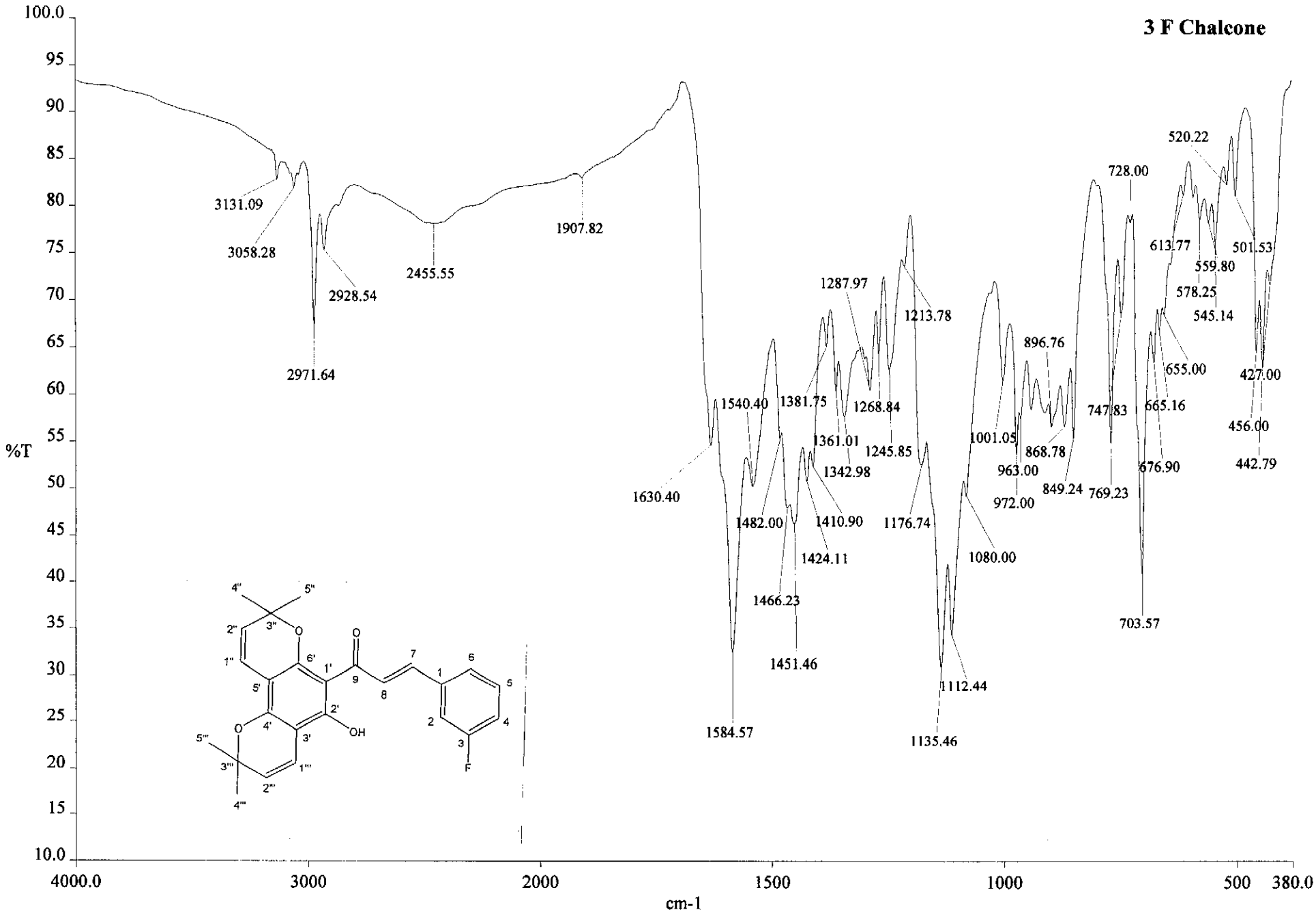
HMBC NMR spectrum of 3c - 3-F pyranochromene chalcone (A-3c)



HMBC NMR spectrum of 3c - 3-F pyranochromene chalcone (A-3c)

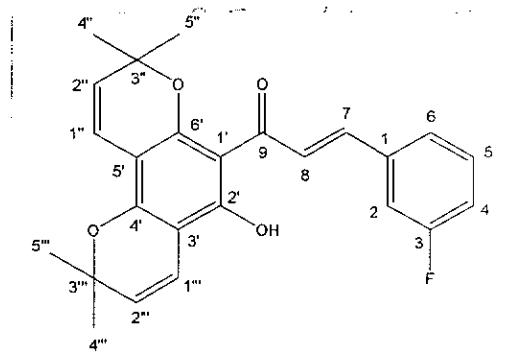


^{19}F NMR spectrum of 3c - 3-F pyranochromene chalcone (A-3c)



IR spectrum of 3c - 3-F pyranochromene chalcone (A-3c)

Elemental Composition Report



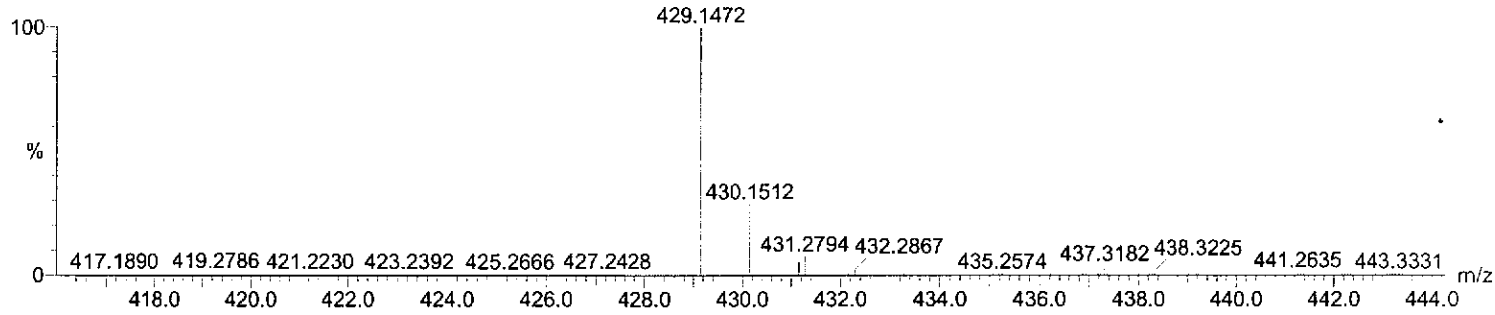
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
3 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-30 H: 20-25 O: 0-5 F: 1-1 Na: 0-1

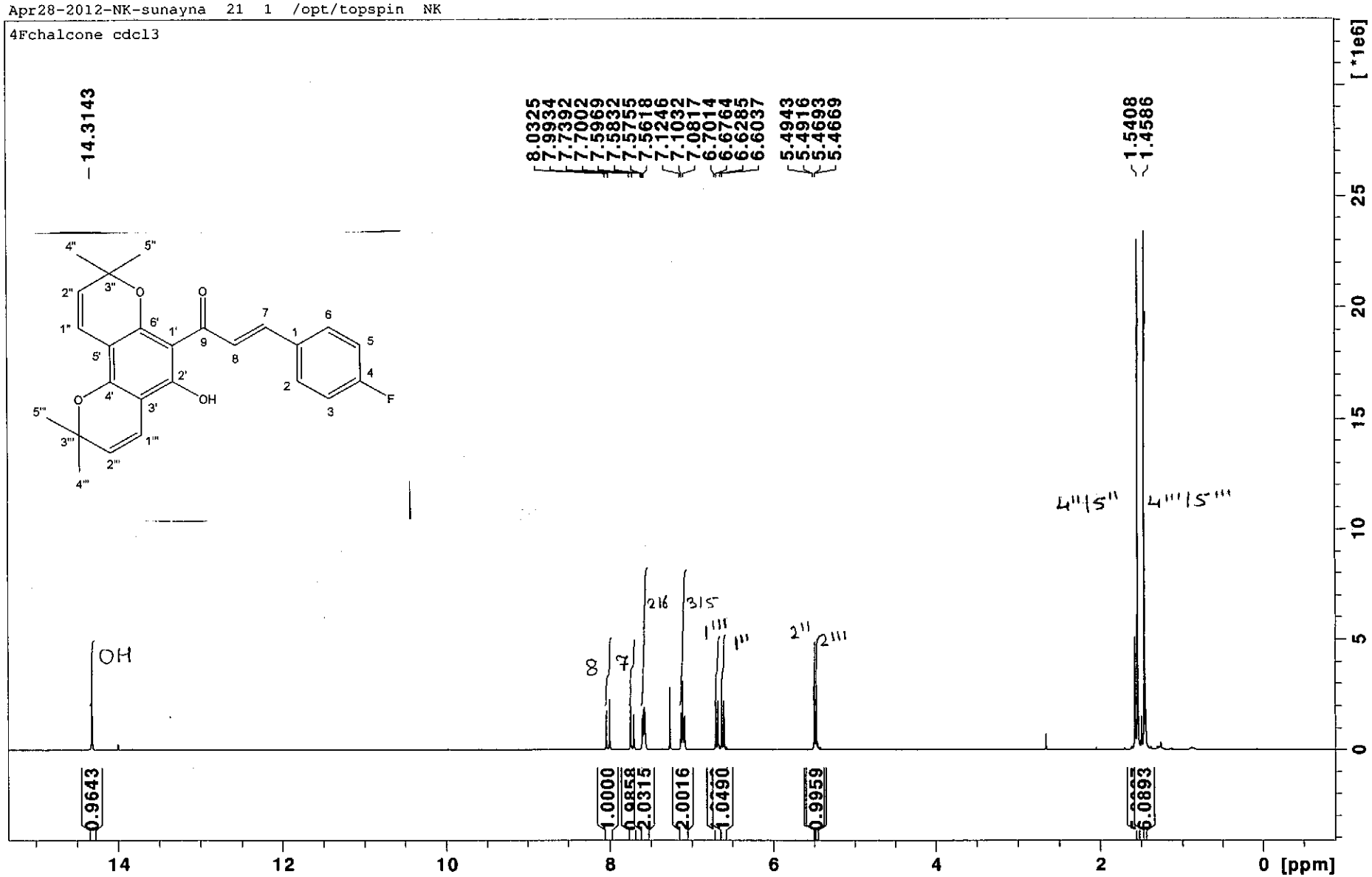
Dr. Nizam
11 19 (0.307) Cm (1:31)

TOF MS ES+
1.01e+004

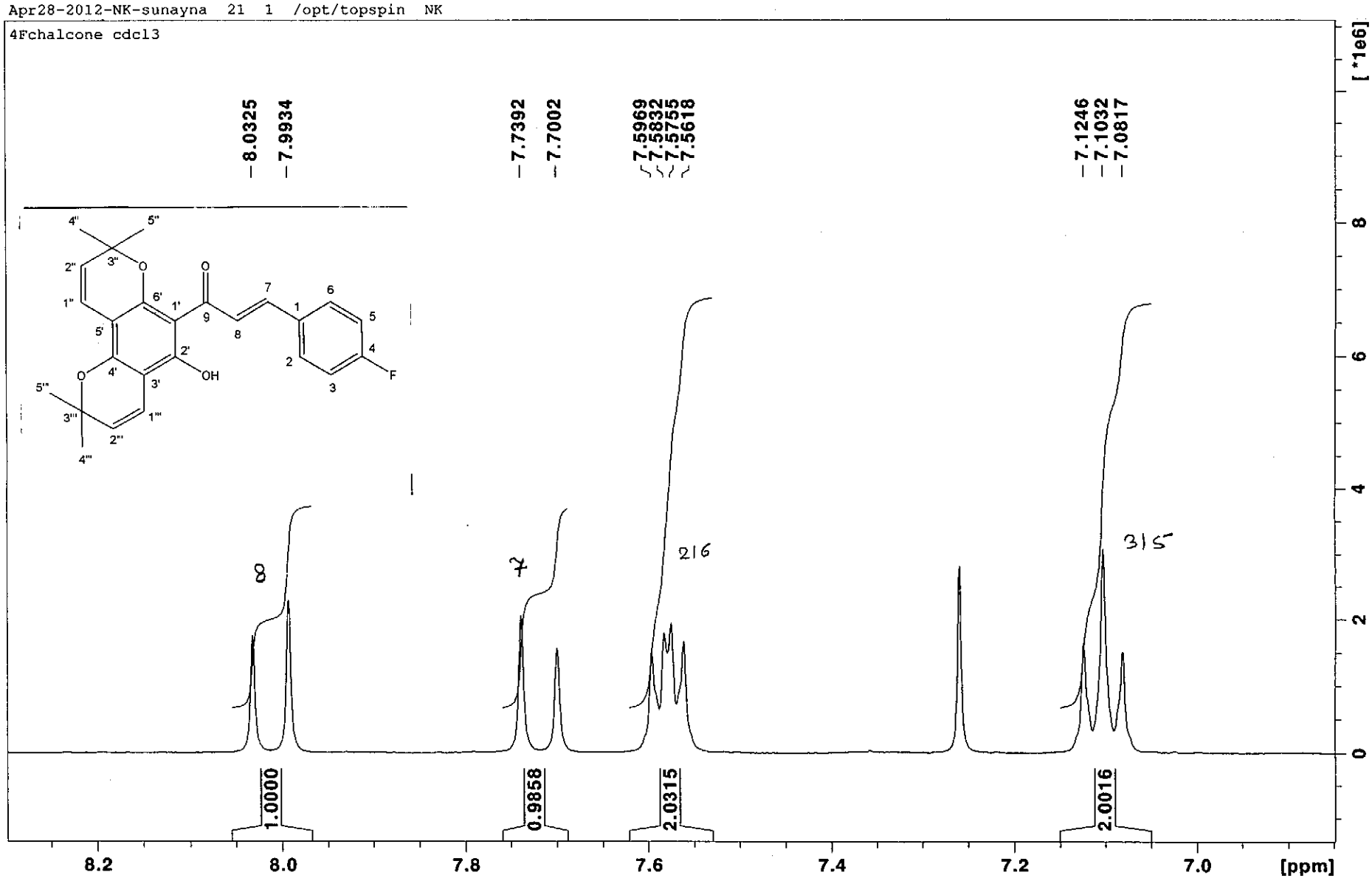


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
429.1472	429.1478	-0.6	-1.4	13.5	344.4	0.0	C25 H23 O4 F Na

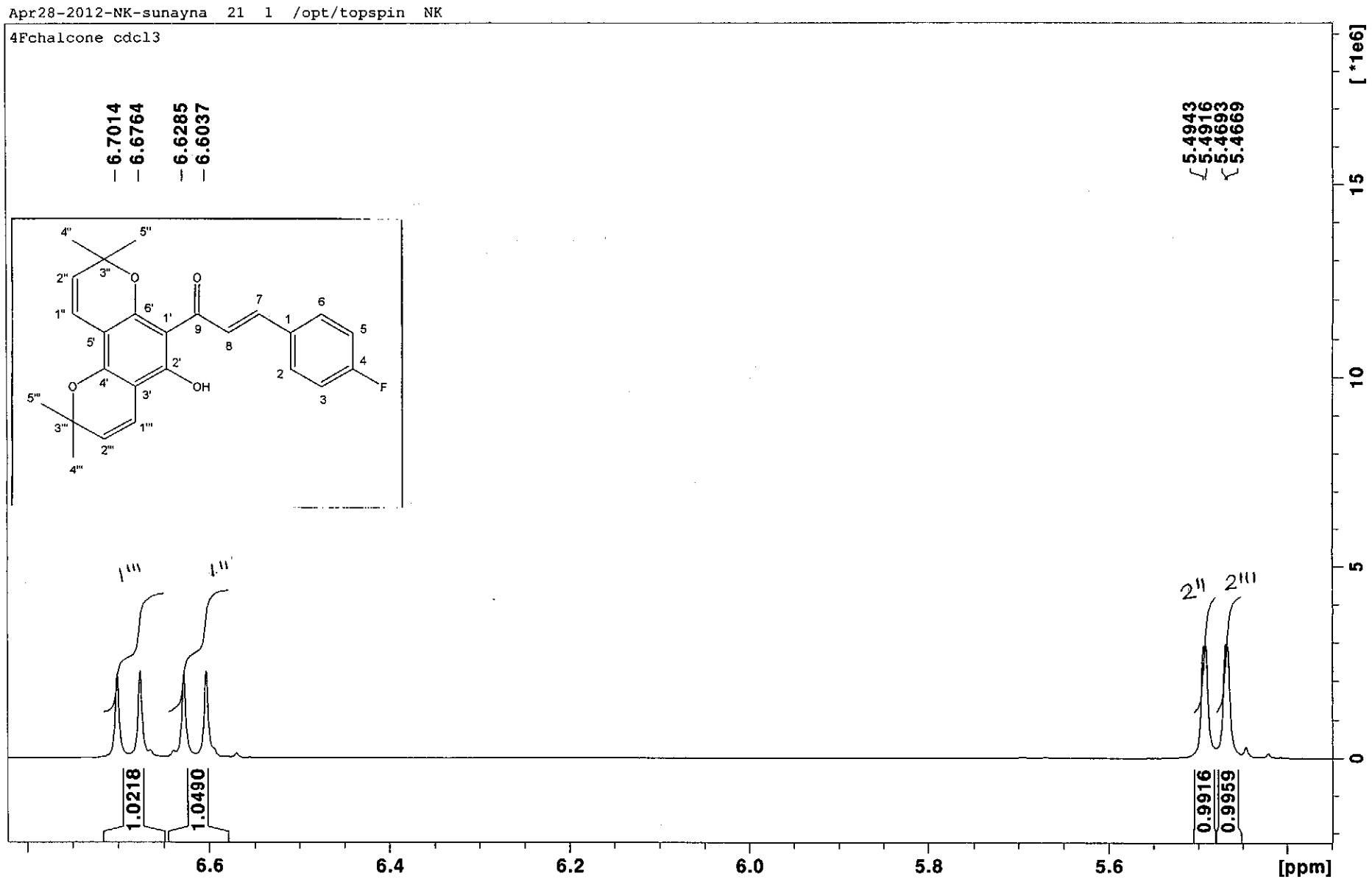
HRMS of 3c – 3-F pyranochromene chalcone (A-3c)



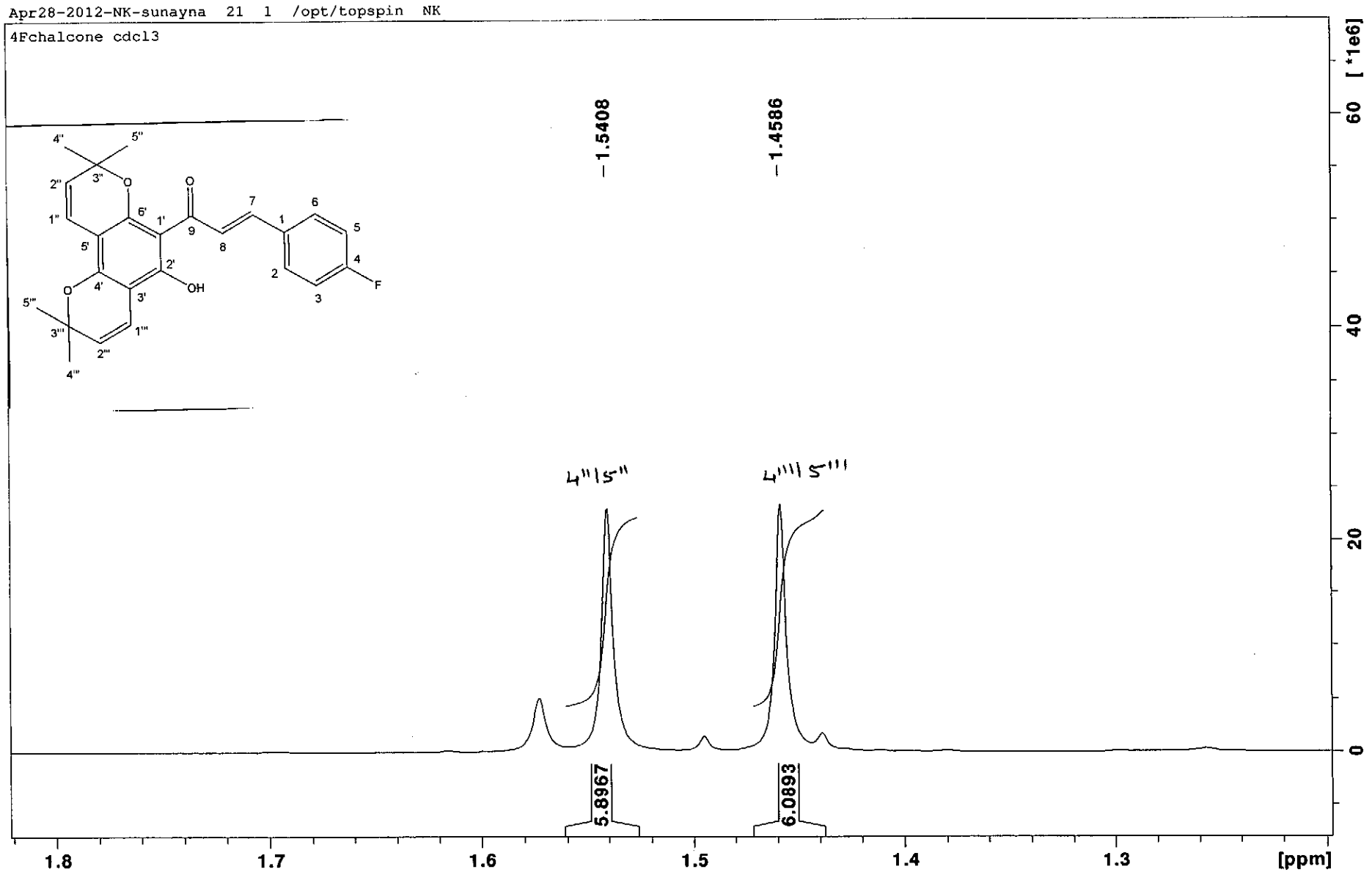
^{1}H NMR spectrum of 3d - 4-F pyranochromene chalcone (A-3d)



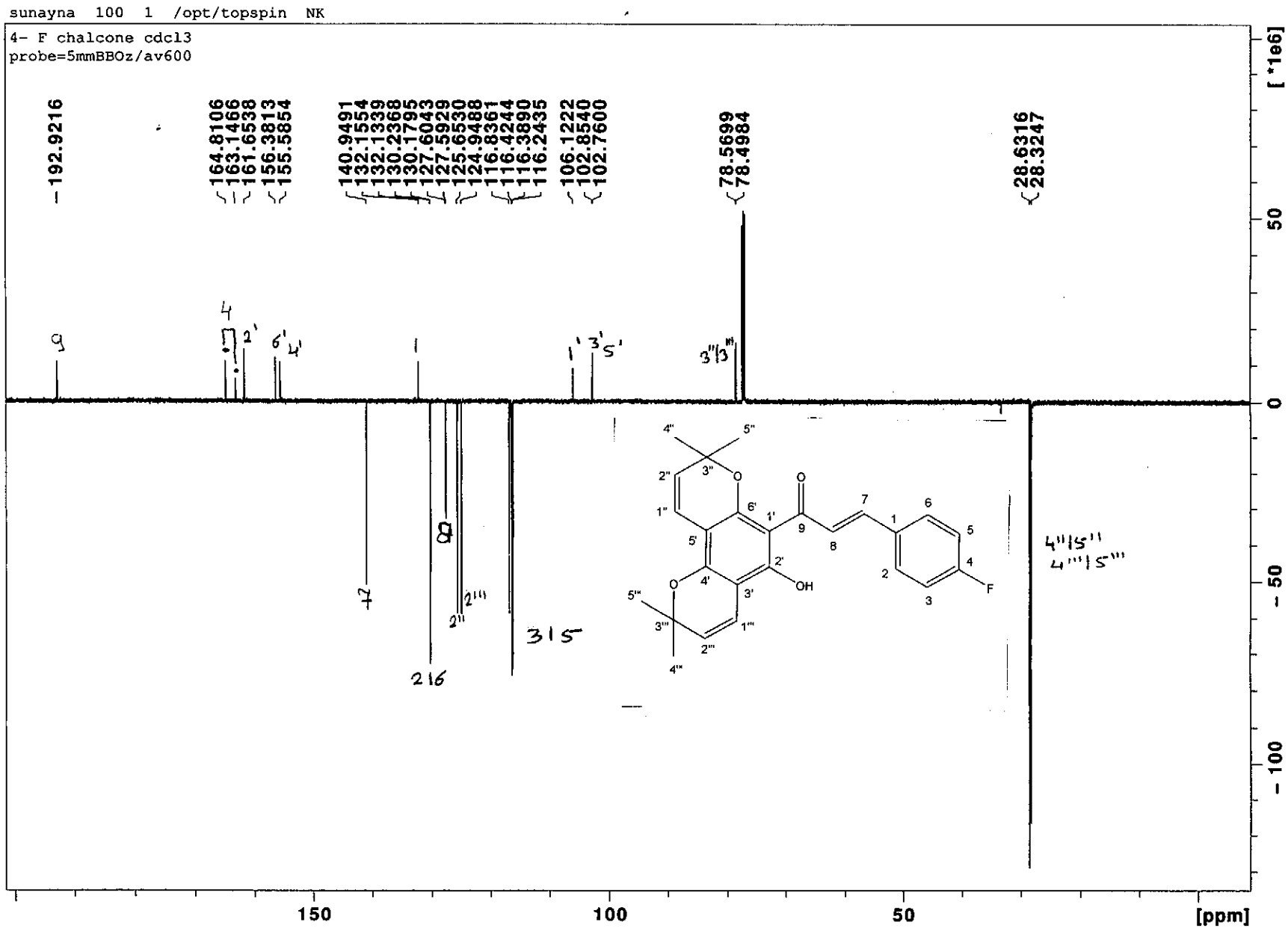
^{1}H NMR spectrum of 3d - 4-F pyranochromene chalcone (A-3d)



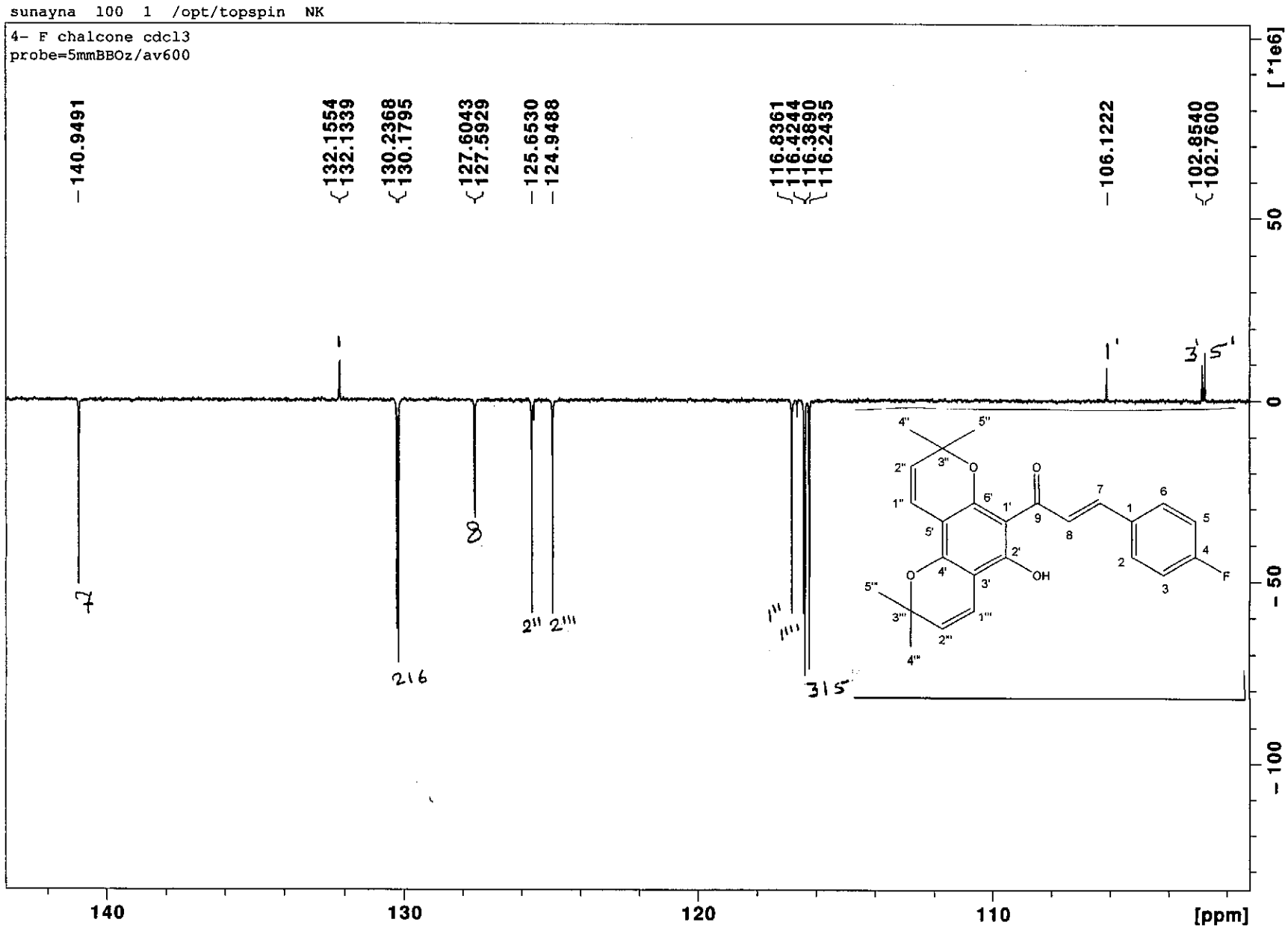
^{1}H NMR spectrum of 3d - 4-F pyranochromene chalcone (A-3d)



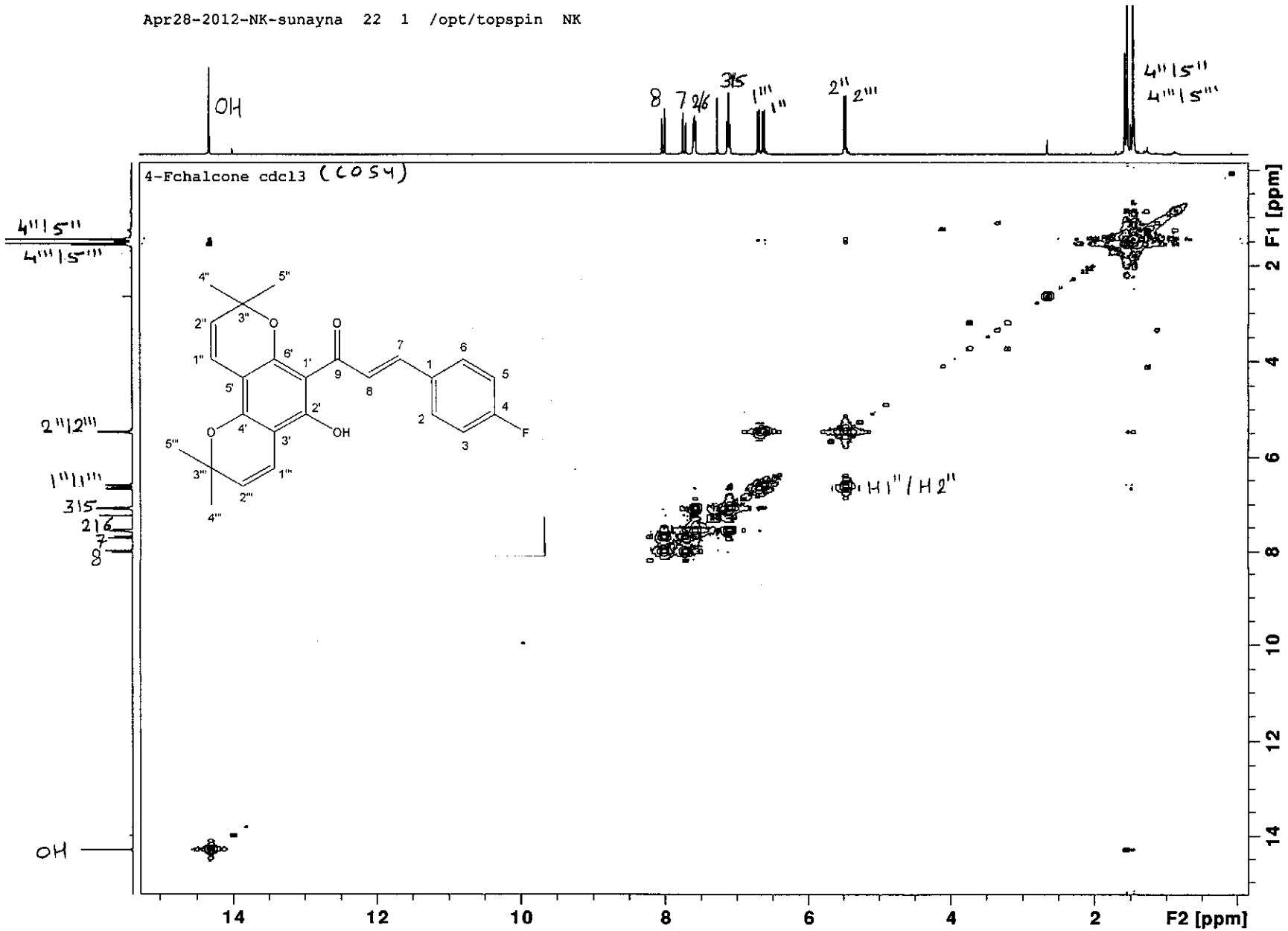
^{1}H NMR spectrum of 3d - 4-F pyranochromene chalcone (A-3d)



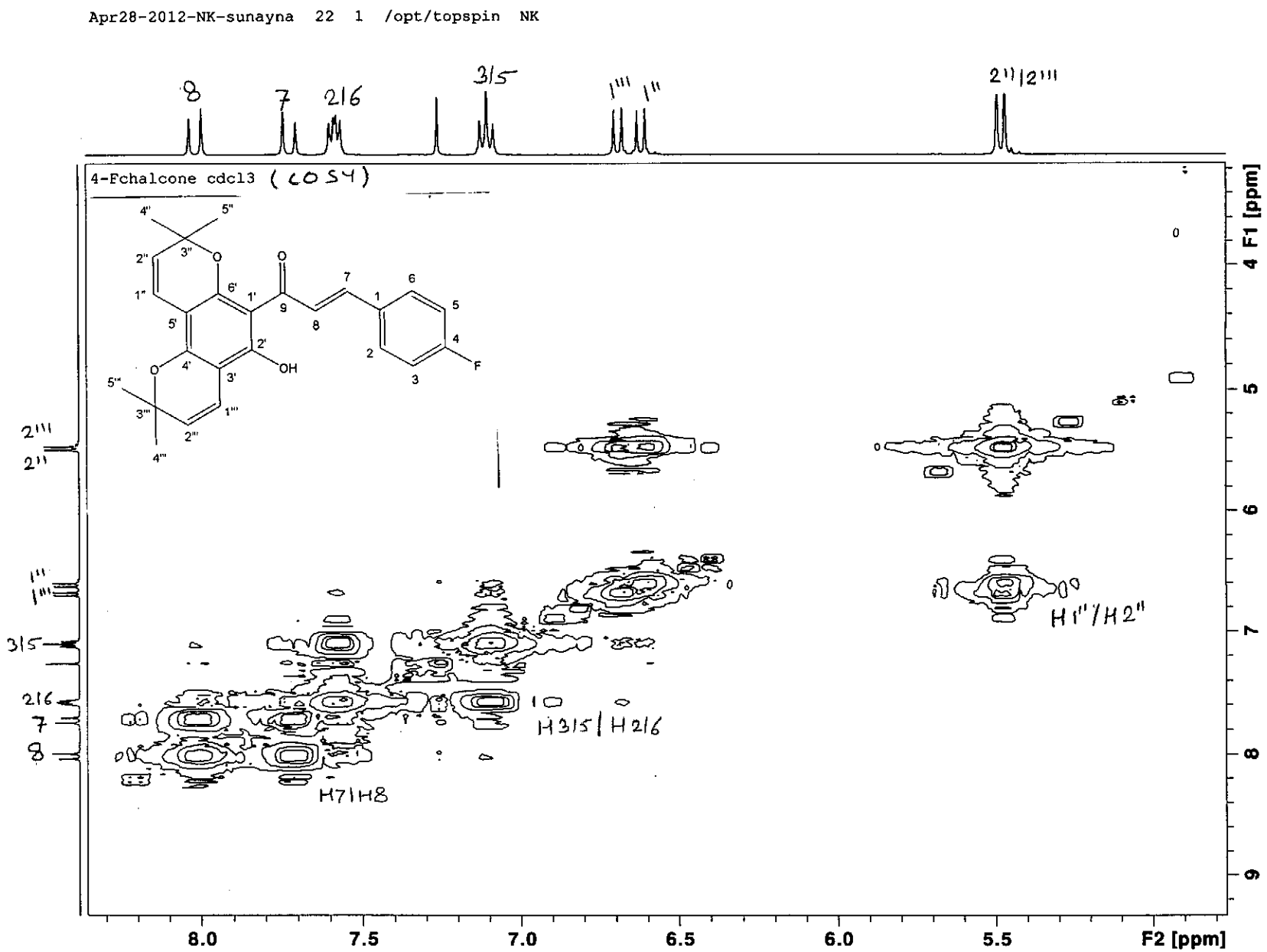
^{13}C NMR spectrum of 3d - 4-F pyranochromene chalcone (A-3d)



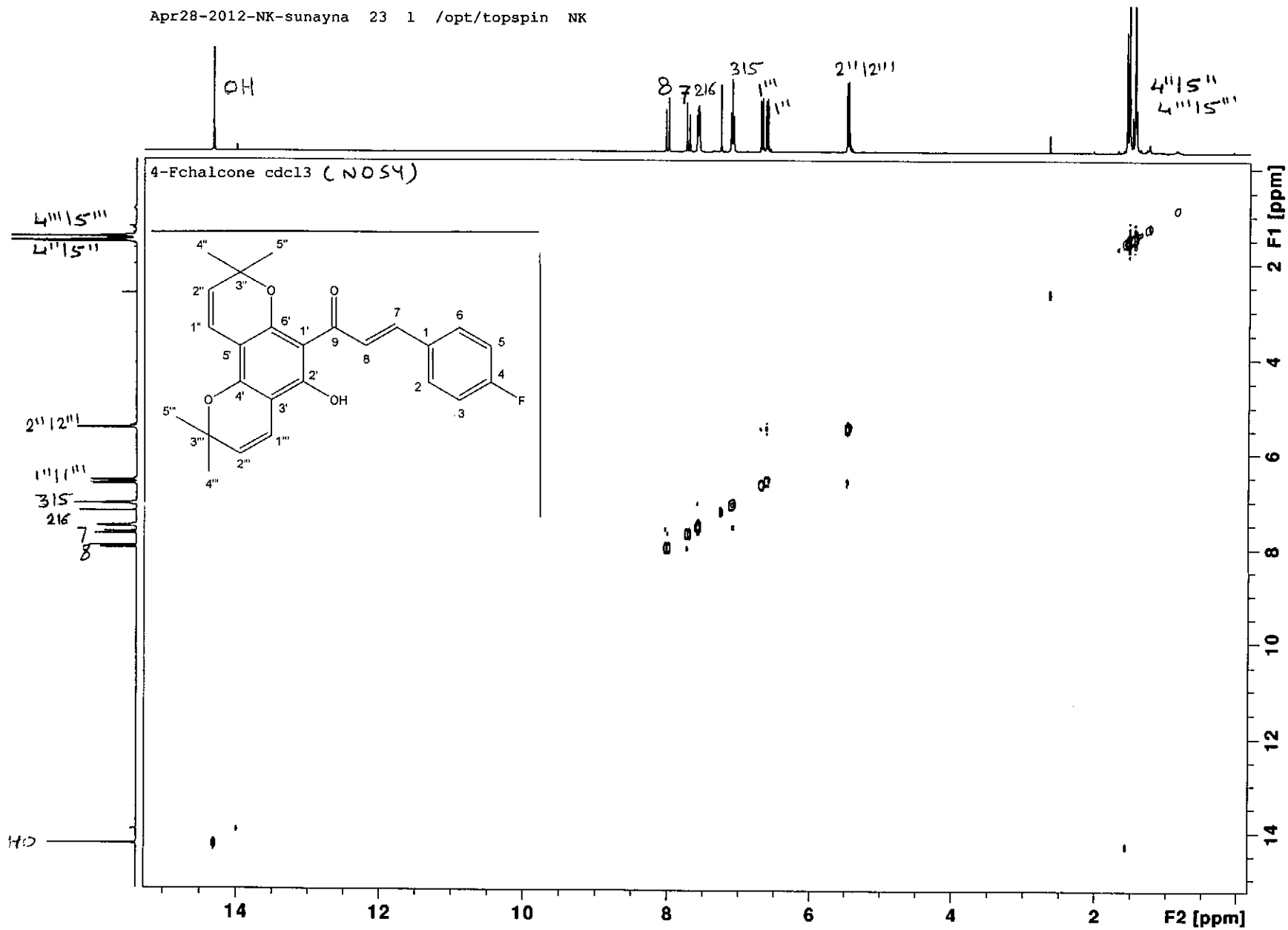
^{13}C NMR spectrum of 3d - 4-F pyranochromene chalcone (A-3d)



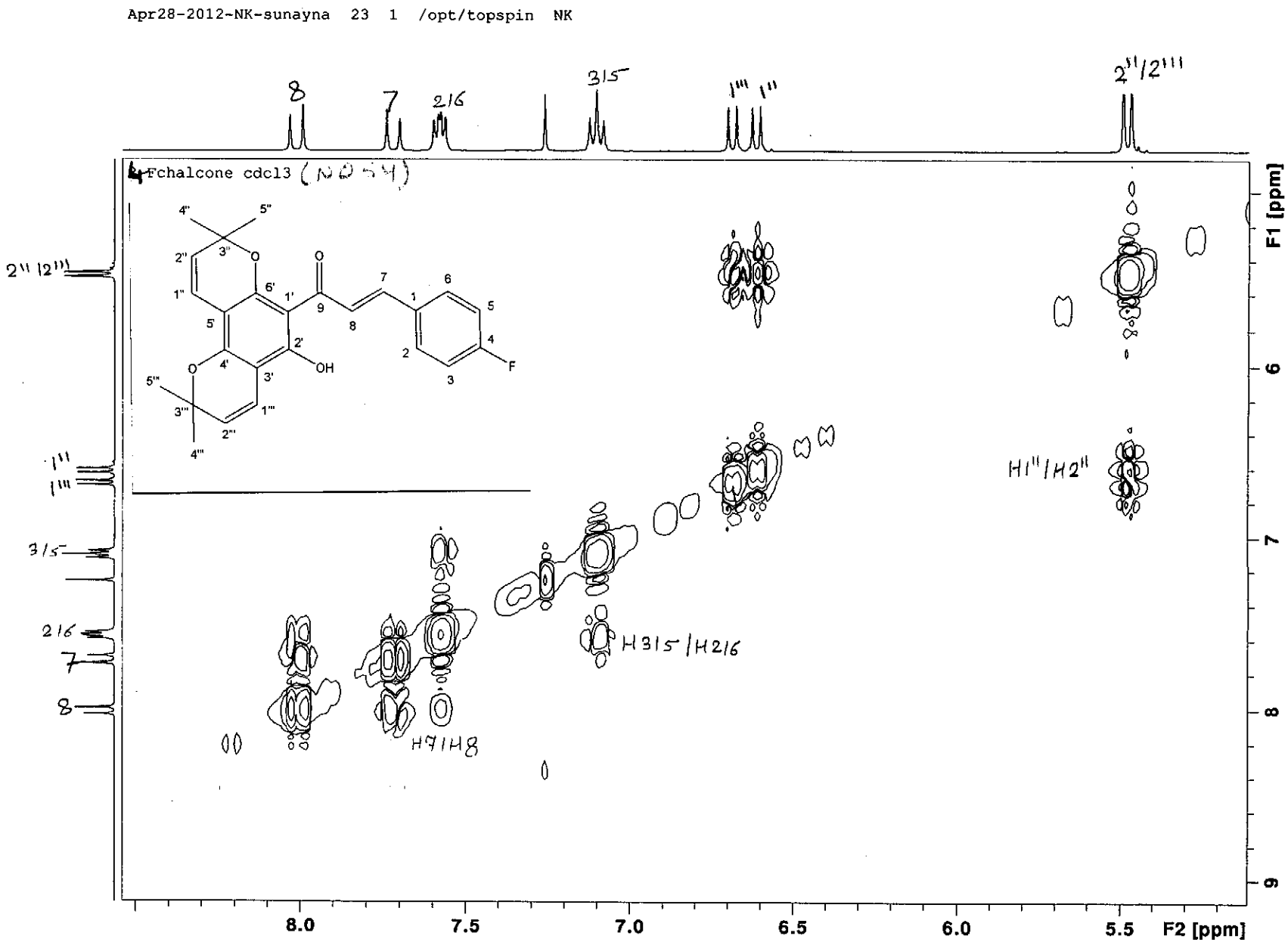
COSY NMR spectrum of 3d - 4-F pyranochromene chalcone (A-3d)



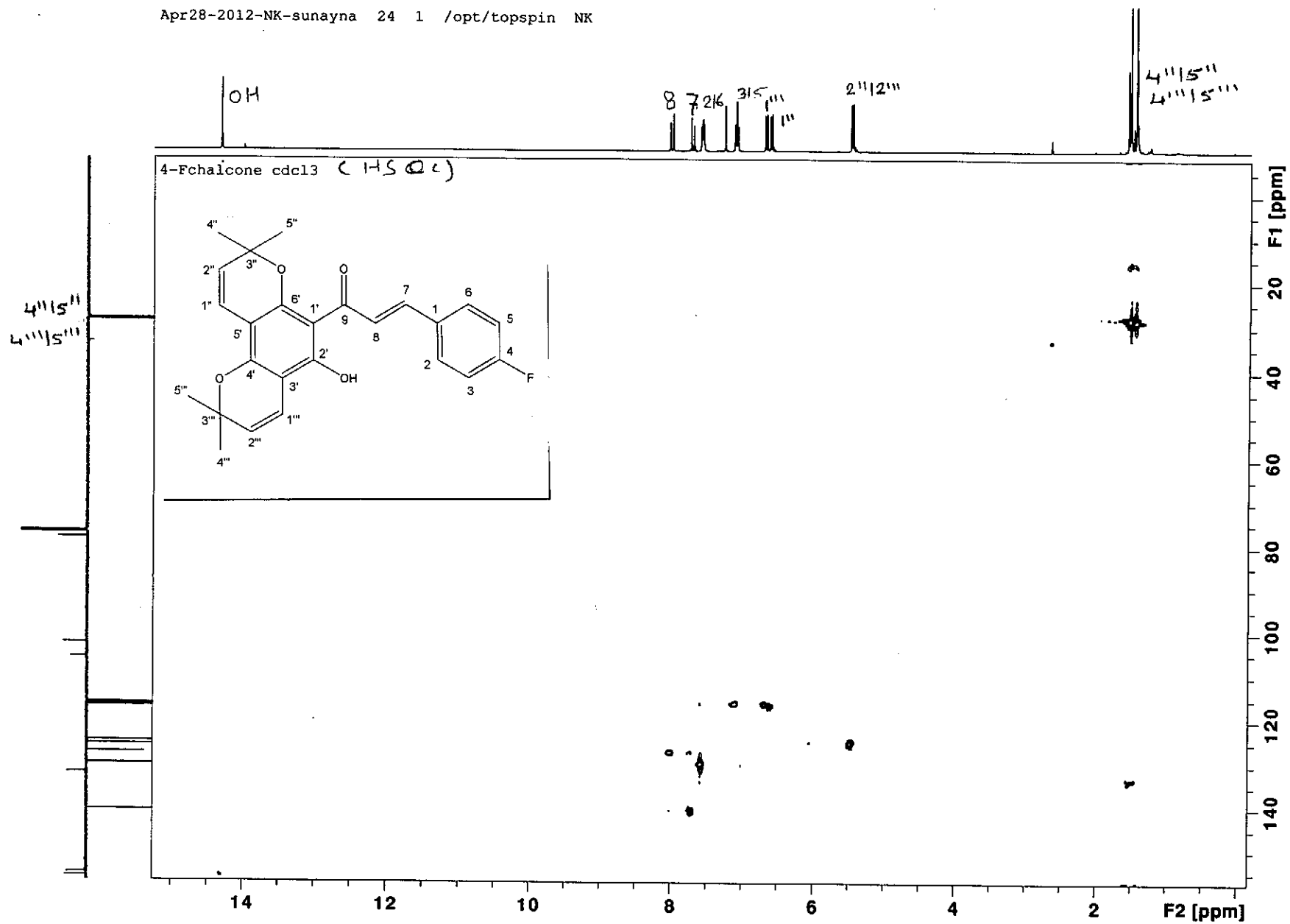
COSY NMR spectrum of 3d - 4-F pyranochromene chalcone (A-3d)



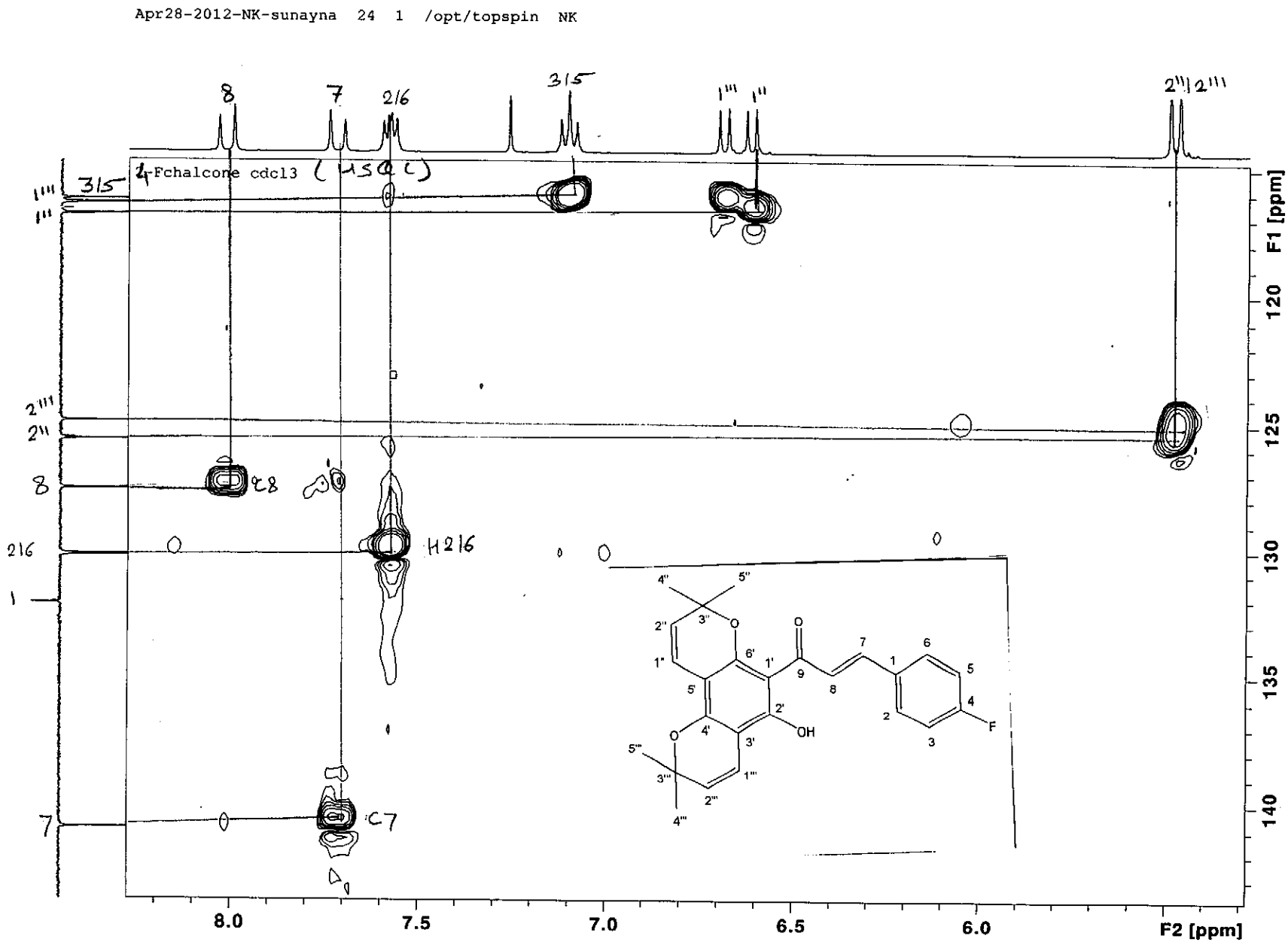
NOSY NMR spectrum of 3d - 4-F pyranochromene chalcone (A-3d)



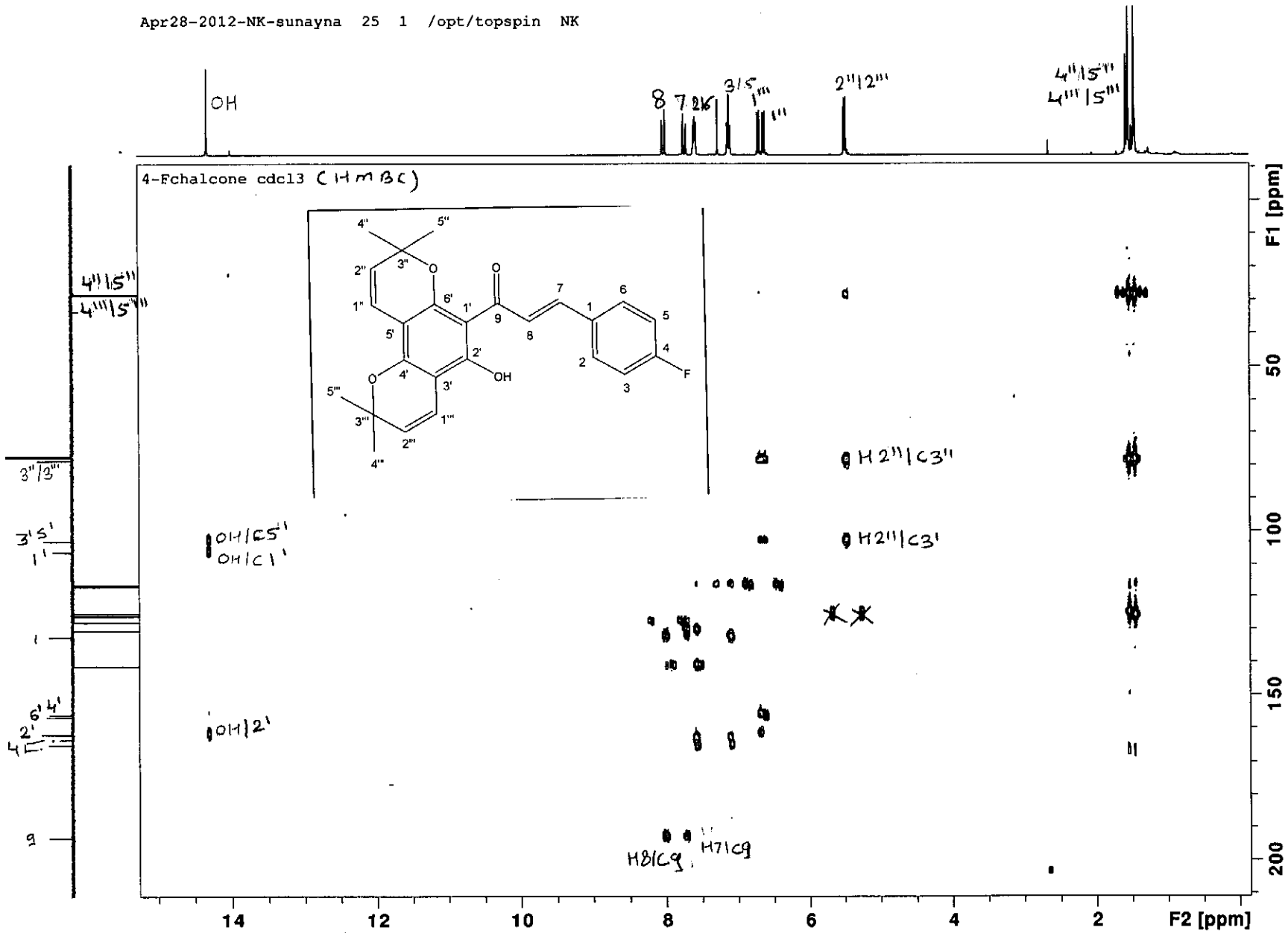
NOSY NMR spectrum of 3d - 4-F pyranochromene chalcone (A-3d)



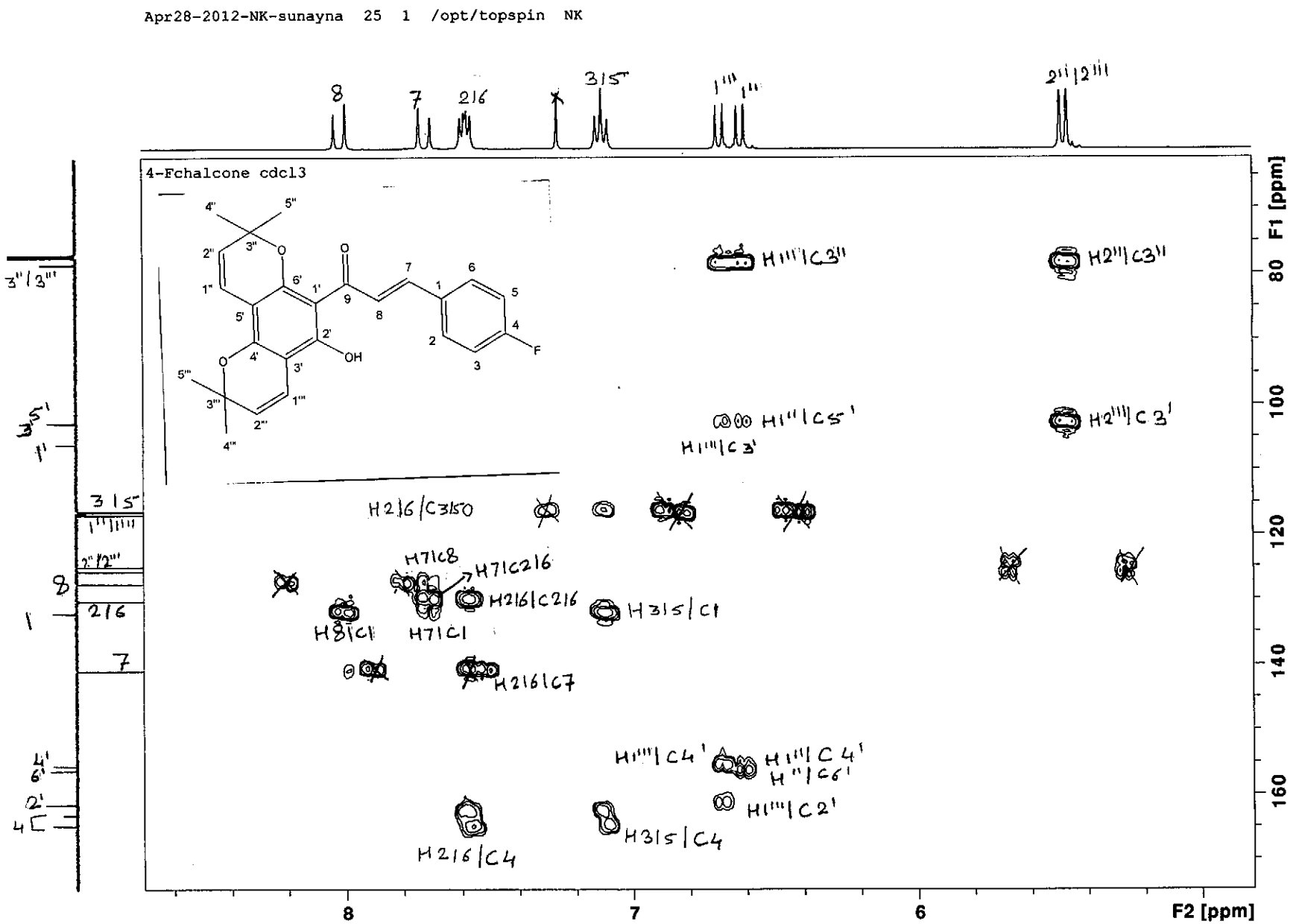
HSQC NMR spectrum of 3d - 4-F pyranochromene chalcone (A-3d)



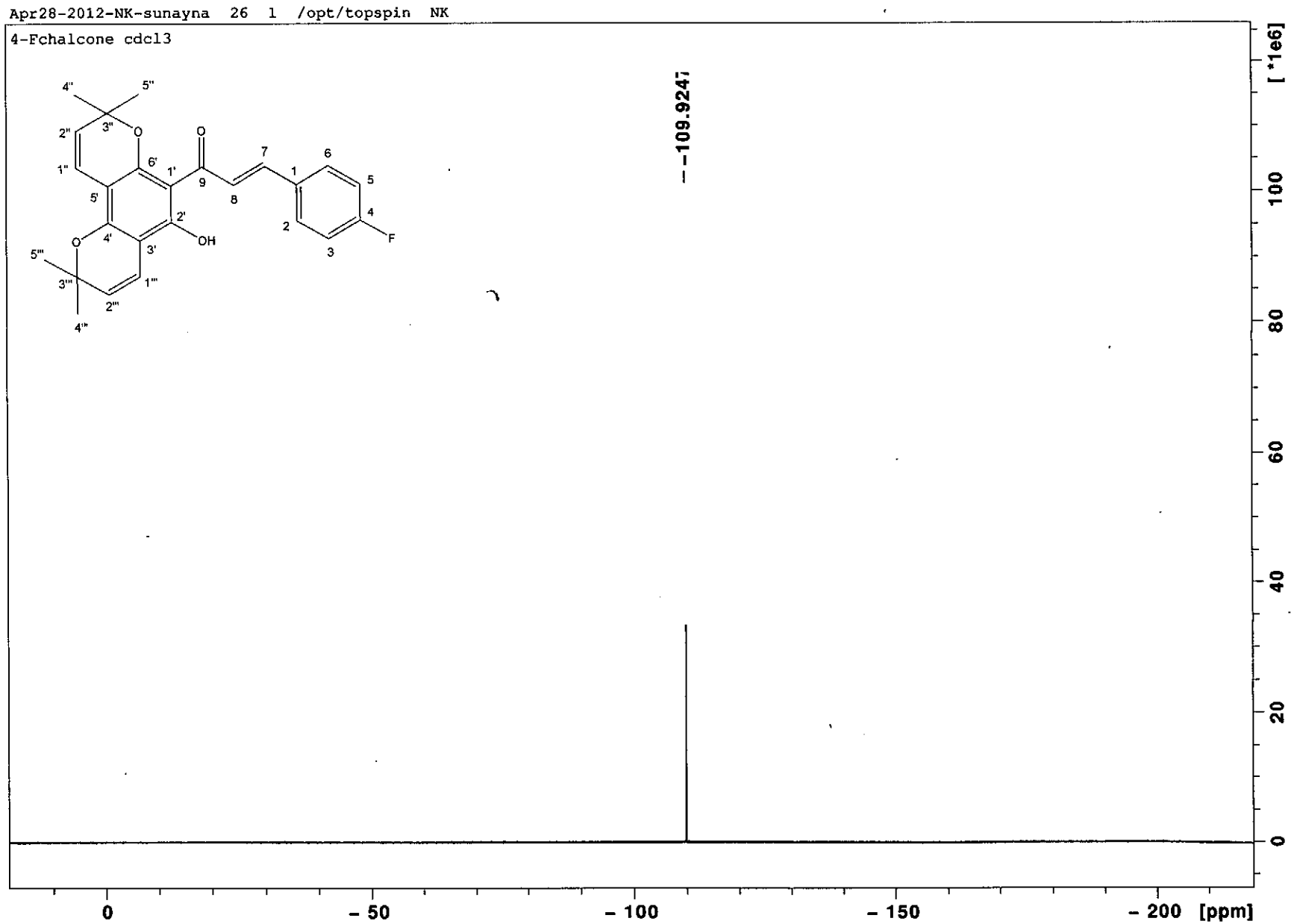
HSQC NMR spectrum of 3d - 4-F pyranochromene chalcone (A-3d)



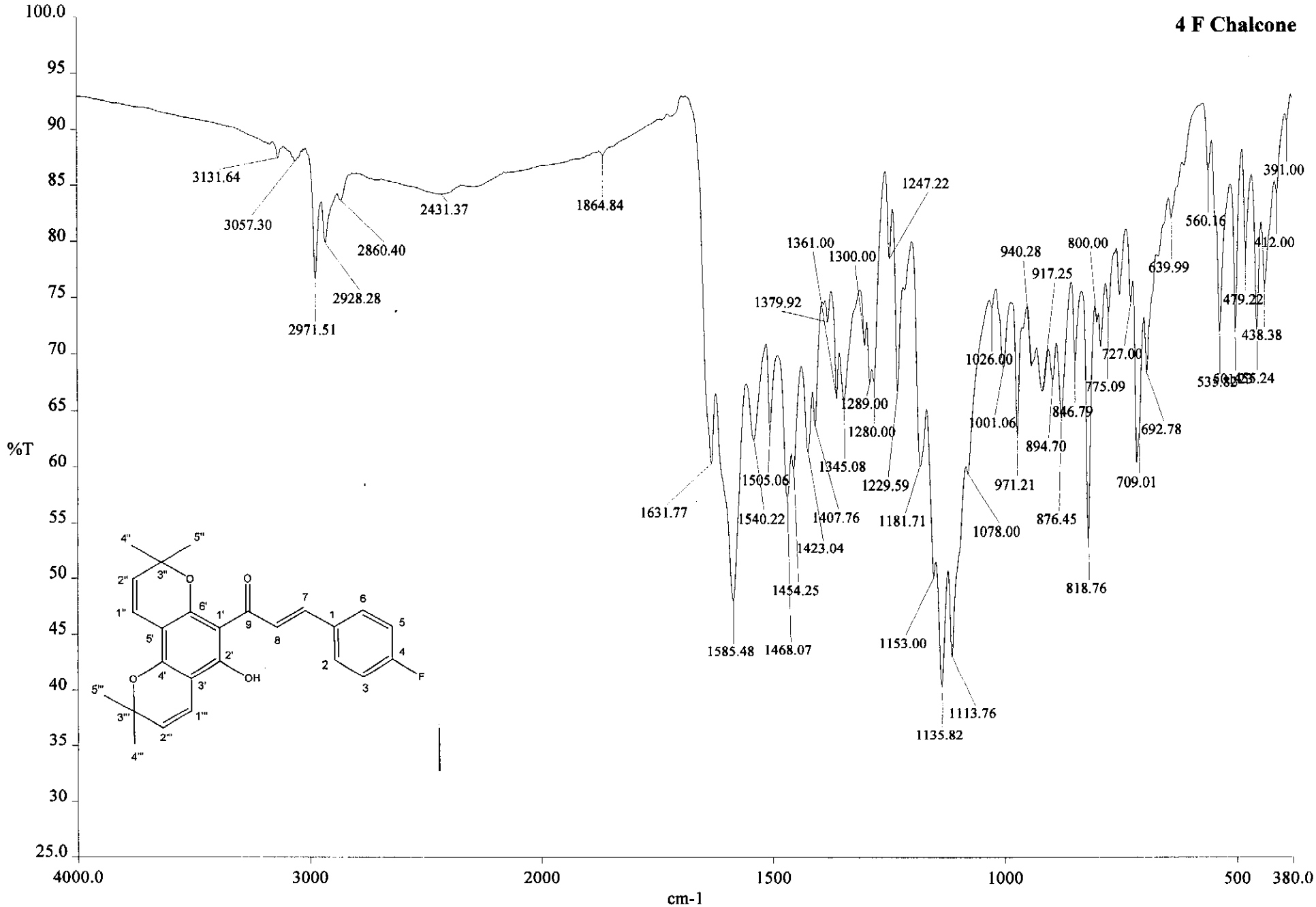
HMBC NMR spectrum of 3d - 4-F pyranochromene chalcone (A-3d)



HMBC NMR spectrum of 3d - 4-F pyranochromene chalcone (A-3d)

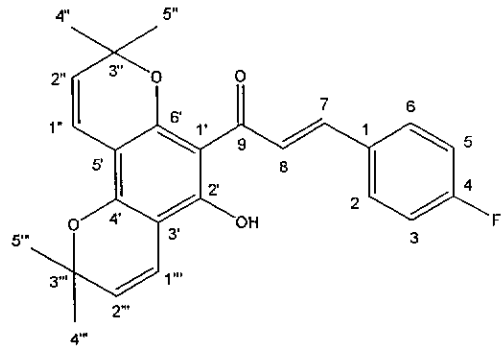


^{19}F NMR spectrum of 3d - 4-F pyranochromene chalcone (A-3d)



IR spectrum of 3d - 4-F pyranochromene chalcone (A-3d)

Elemental Composition Report



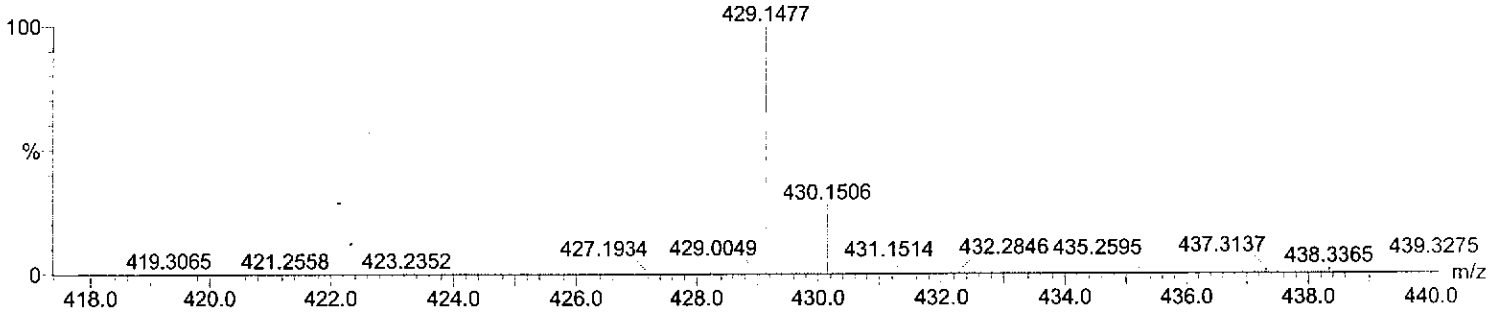
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
3 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-30 H: 20-25 O: 0-5 F: 1-1 Na: 0-1

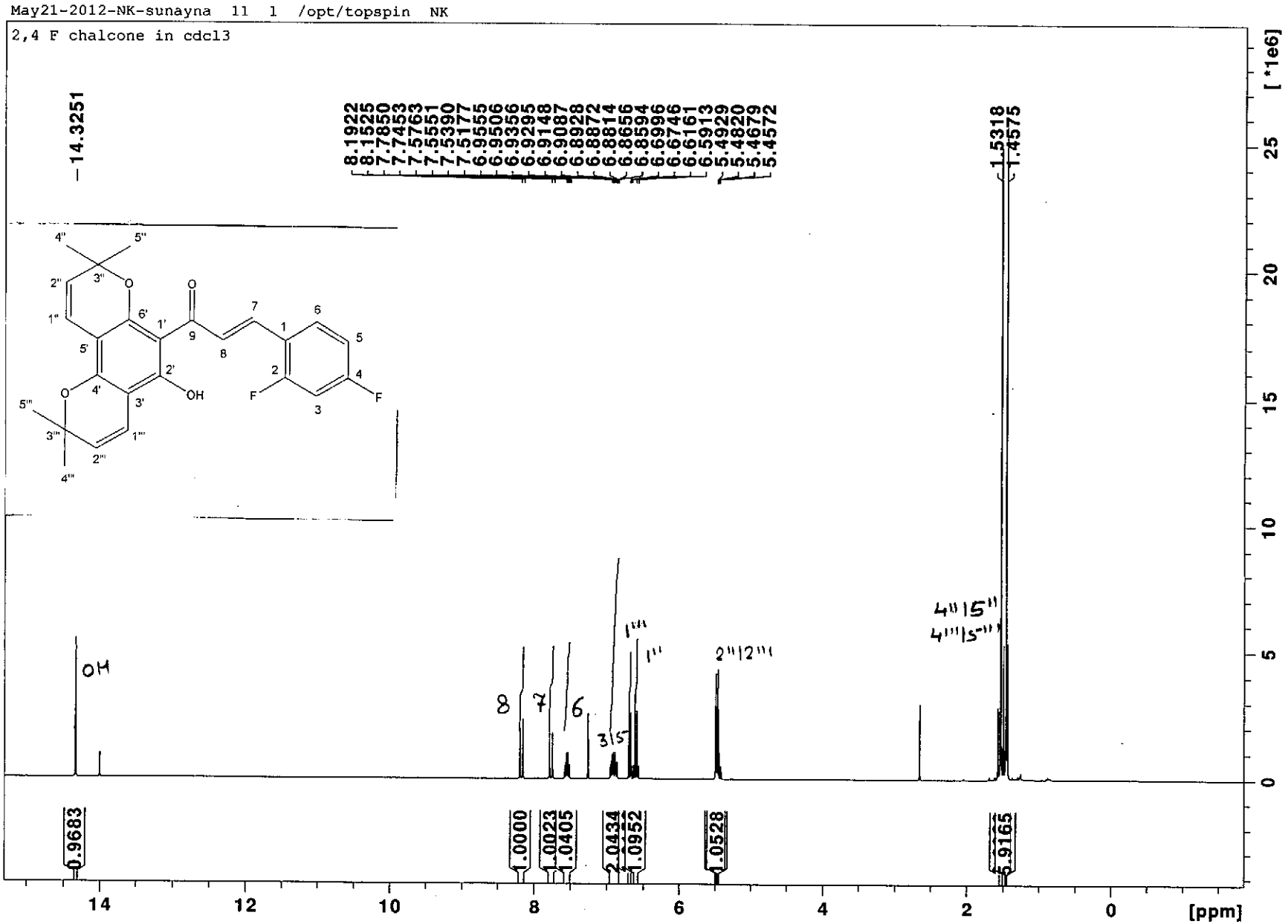
Dr. Nizam
13 11 (0.171) Cm (1:31)

TOF MS ES+
1.10e+004

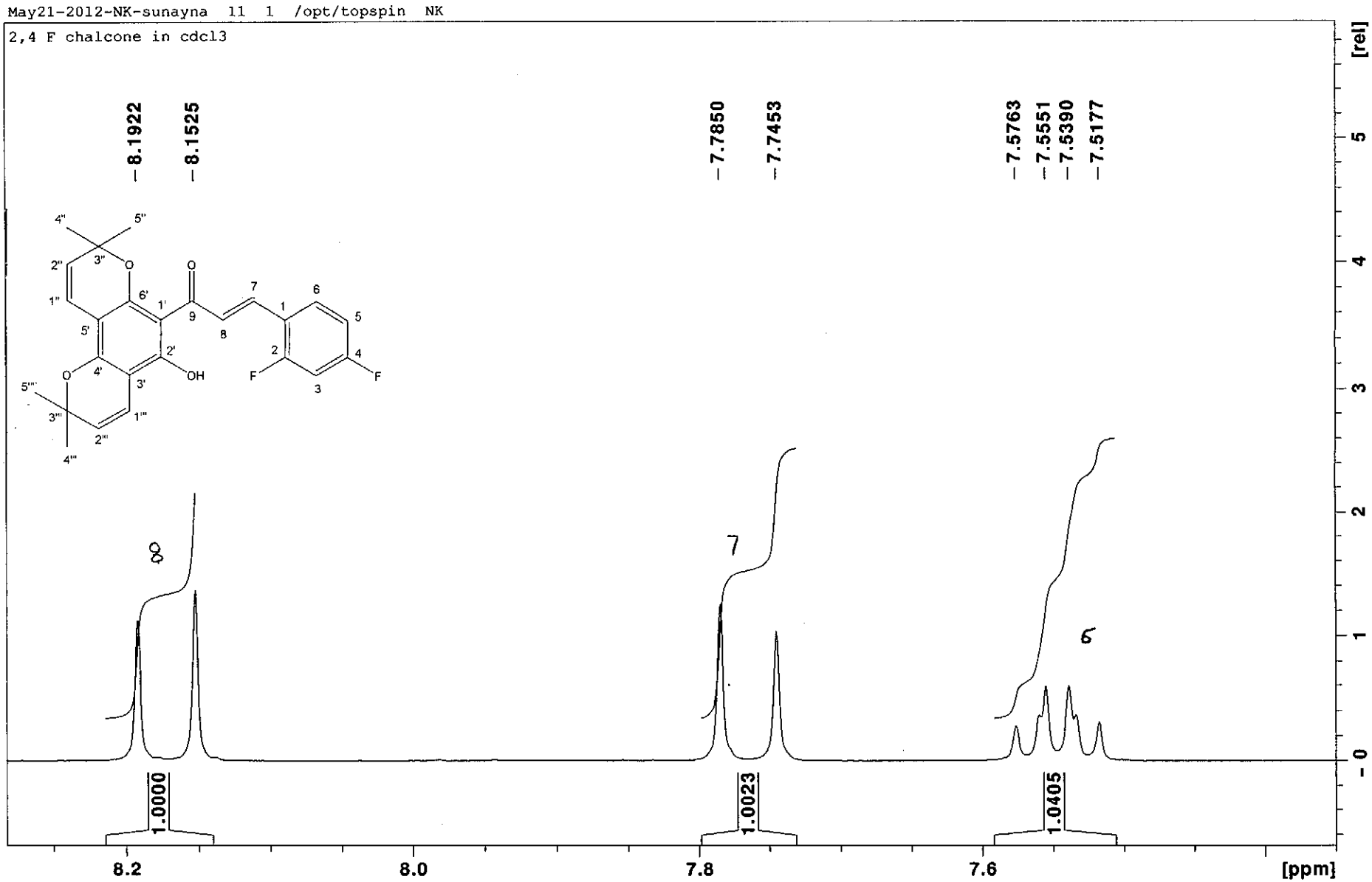


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
429.1477	429.1478	-0.1	-0.2	13.5	350.9	0.0	C25 H23 O4 F Na

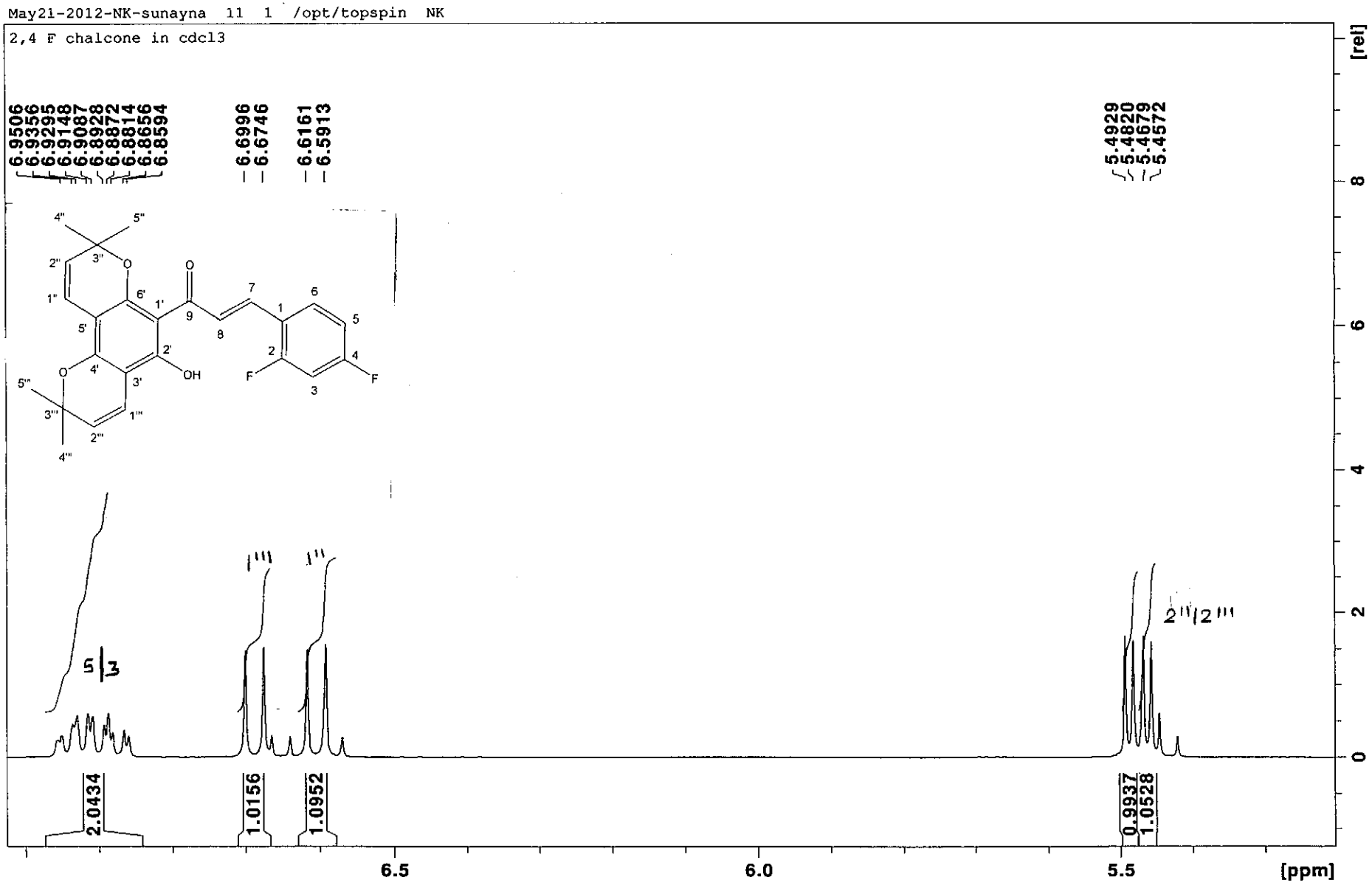
HRMS of 3d – 4-F pyranochromene chalcone (A-3d)



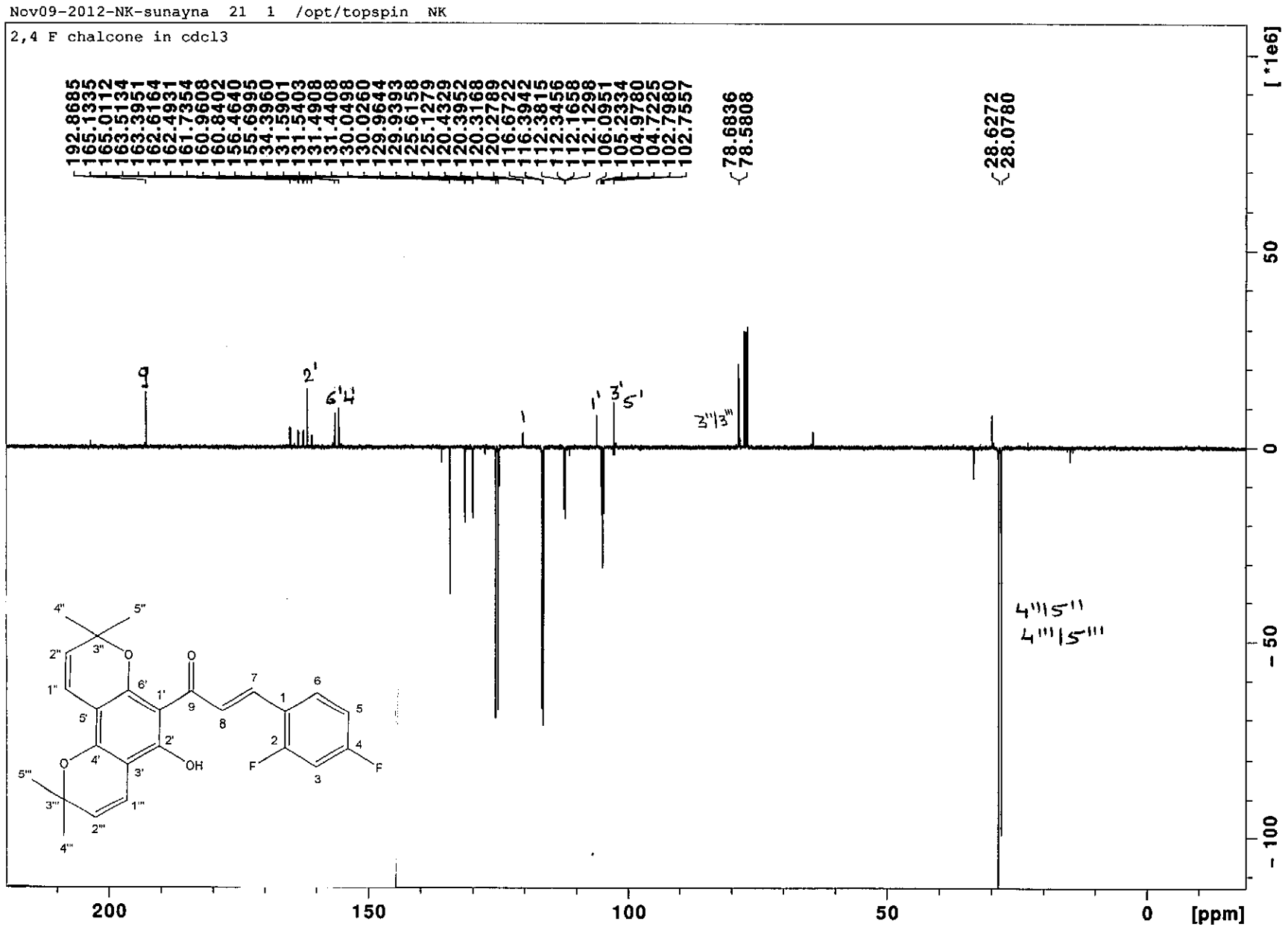
^{1}H NMR spectrum of 3e – 2,4-F pyranochromene chalcone (A-3e)



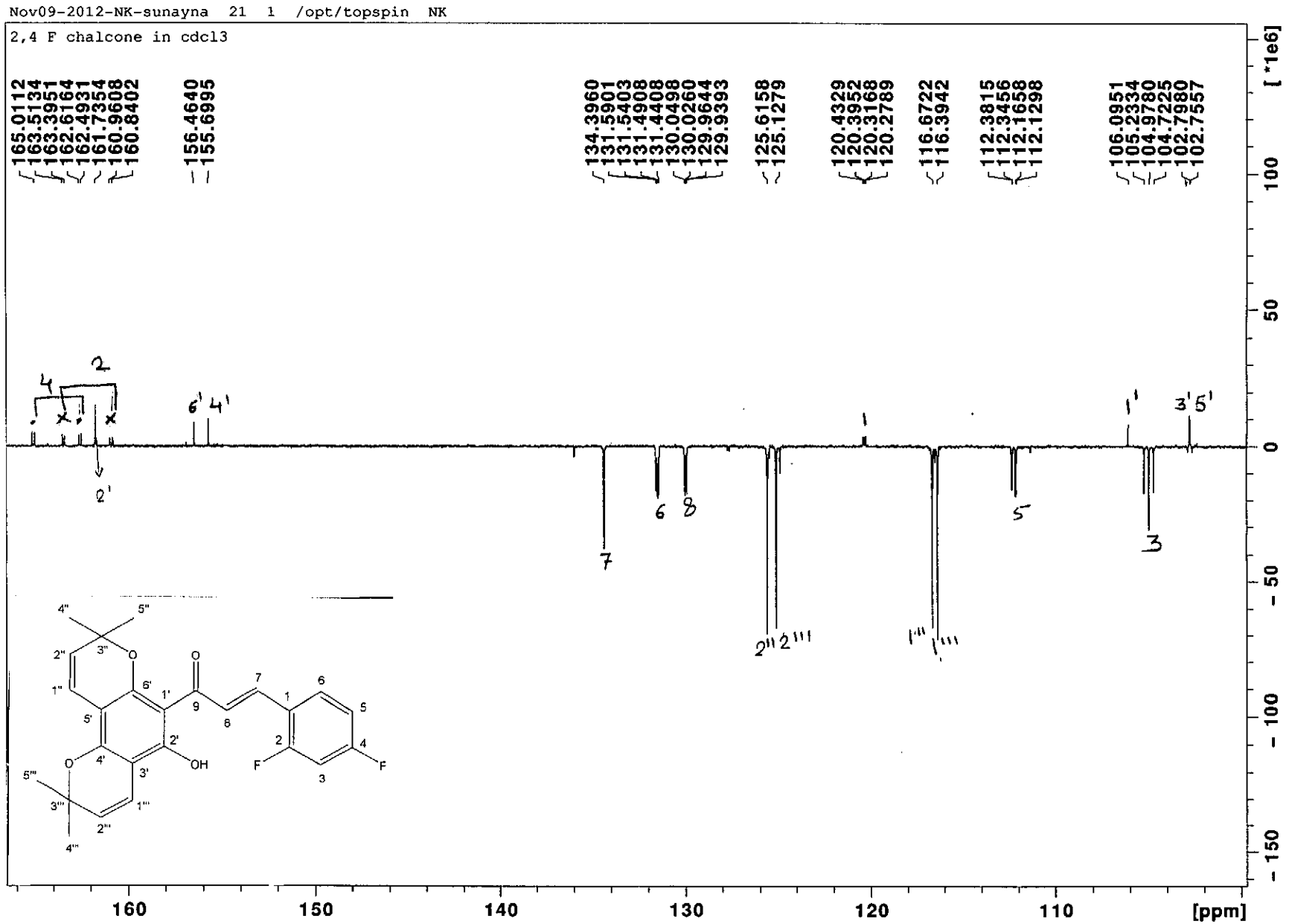
^{1}H NMR spectrum of 3e – 2,4-F pyranochromene chalcone (A-3e)



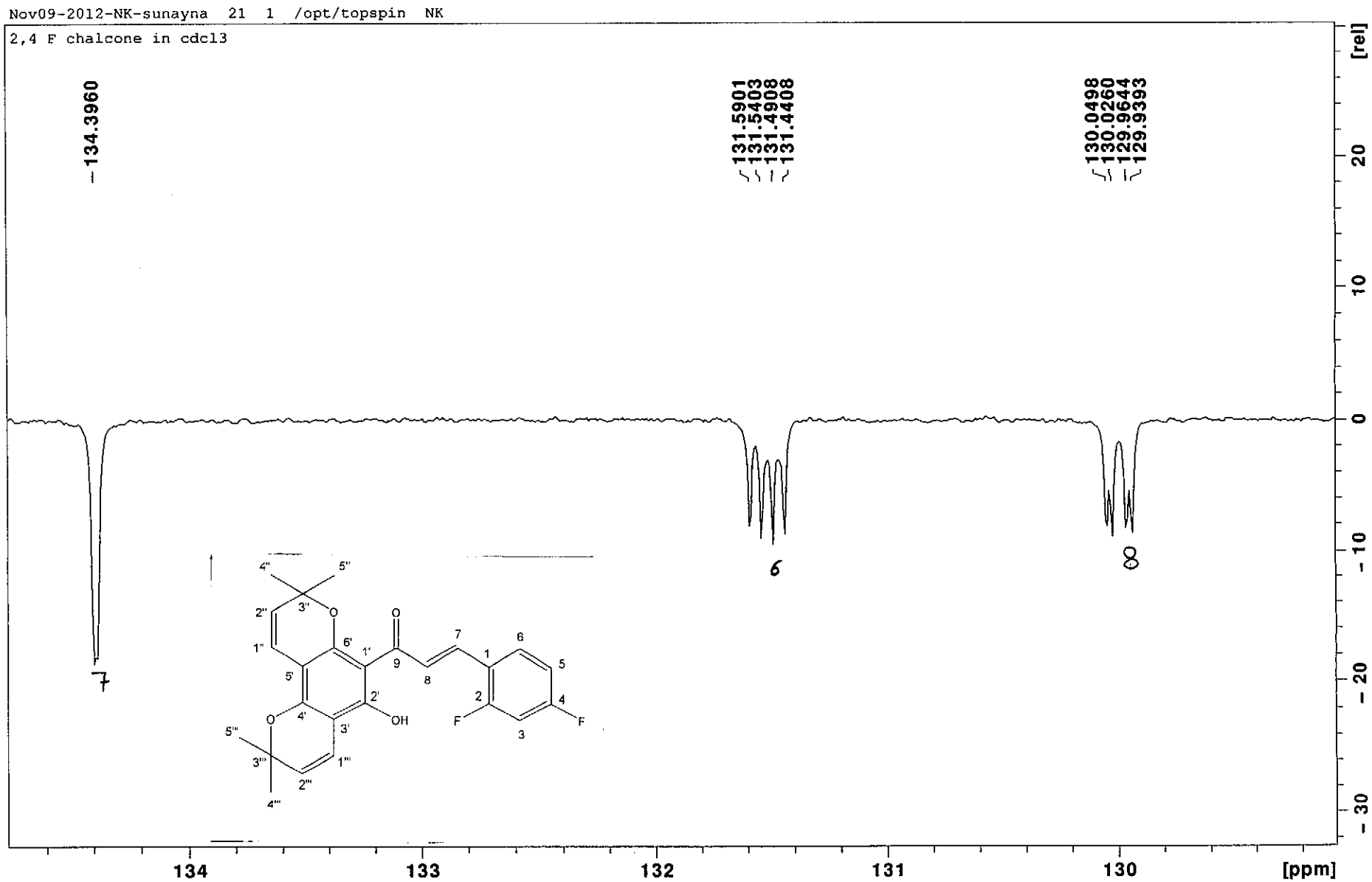
^{1}H NMR spectrum of 3e – 2,4-F pyranochromene chalcone (A-3e)



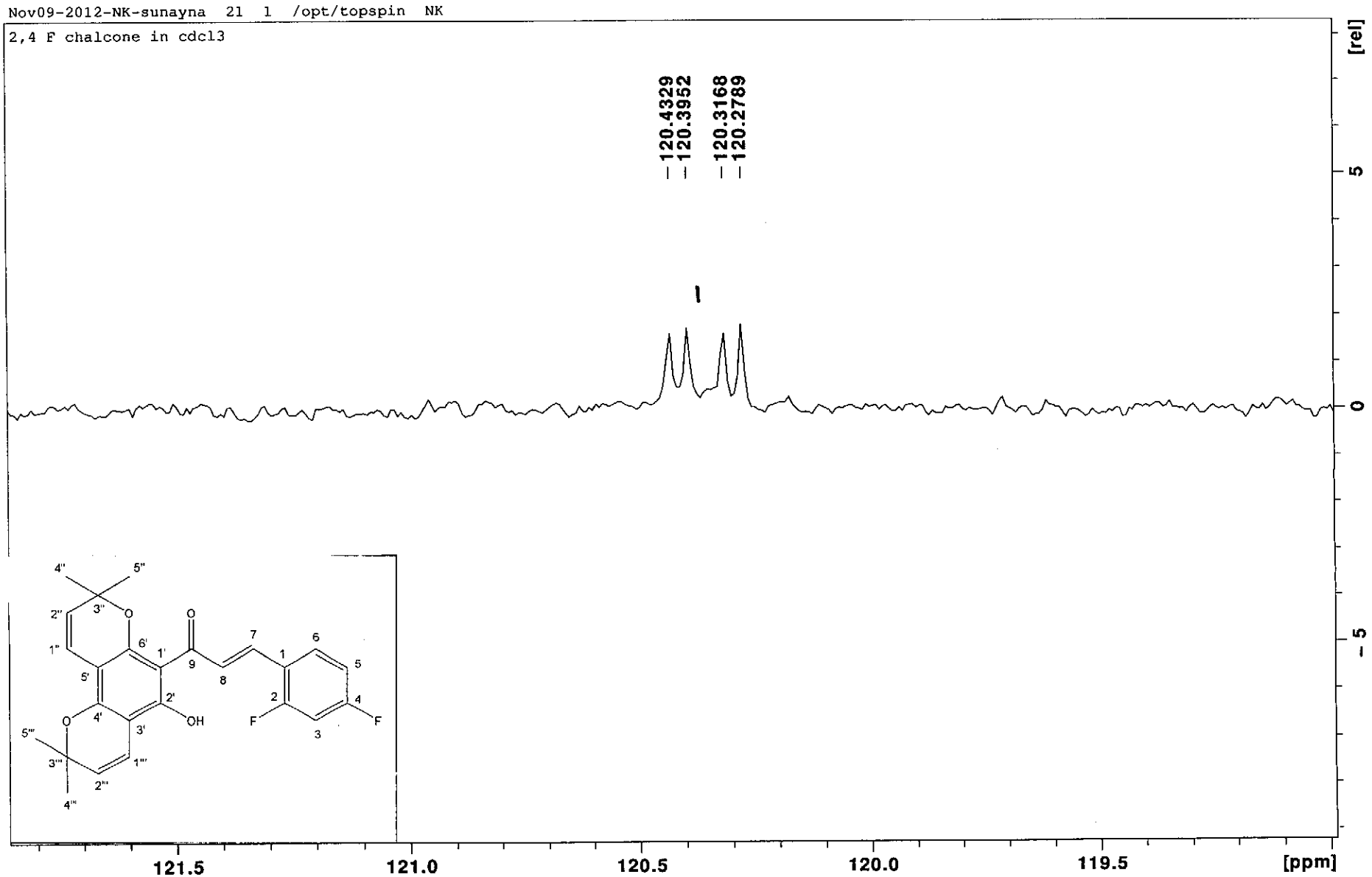
^{13}C NMR spectrum of 3e – 2,4-F pyranochromene chalcone (A-3e)



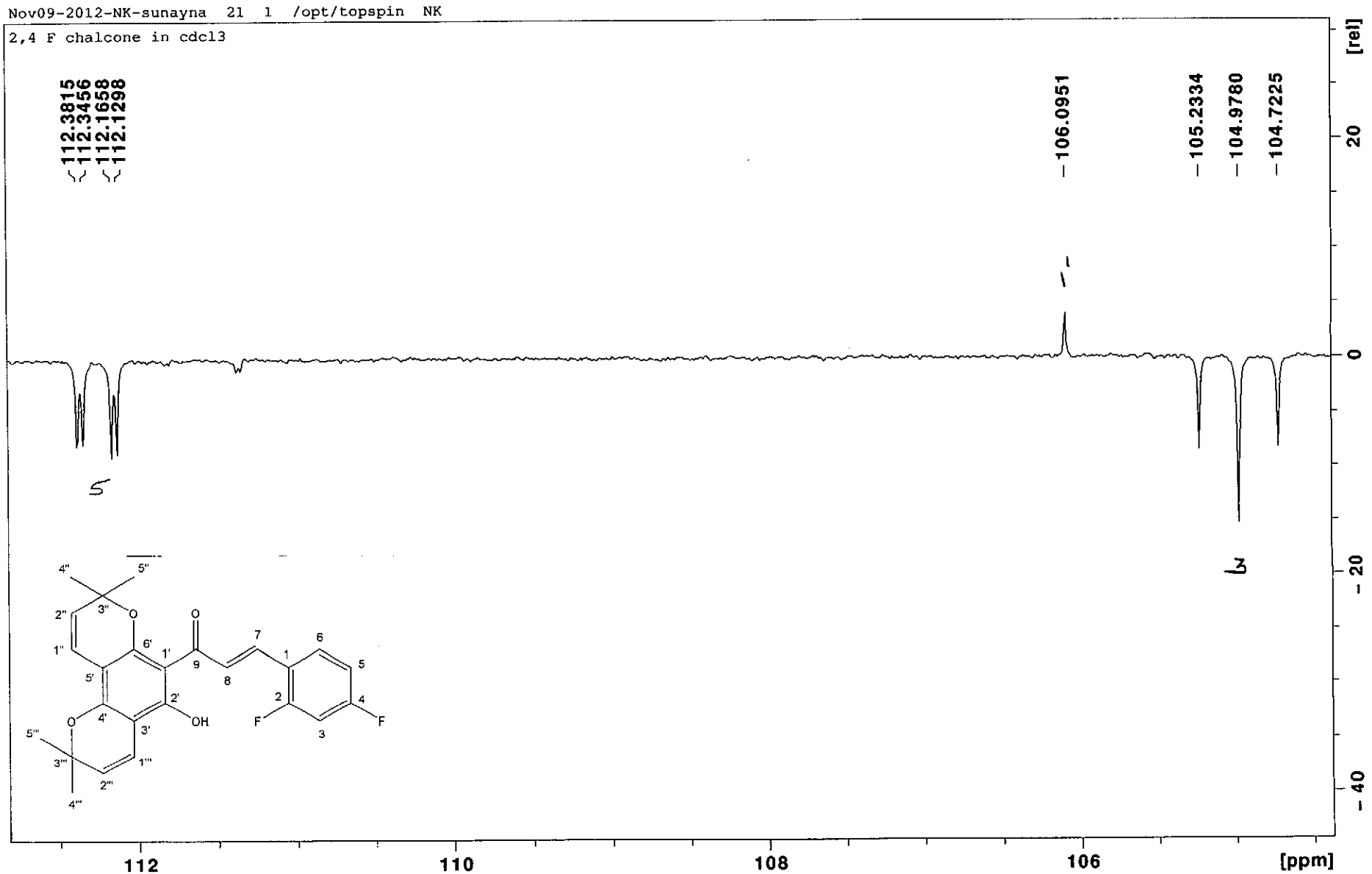
^{13}C NMR spectrum of 3e – 2,4-F pyranochromene chalcone (A-3e)



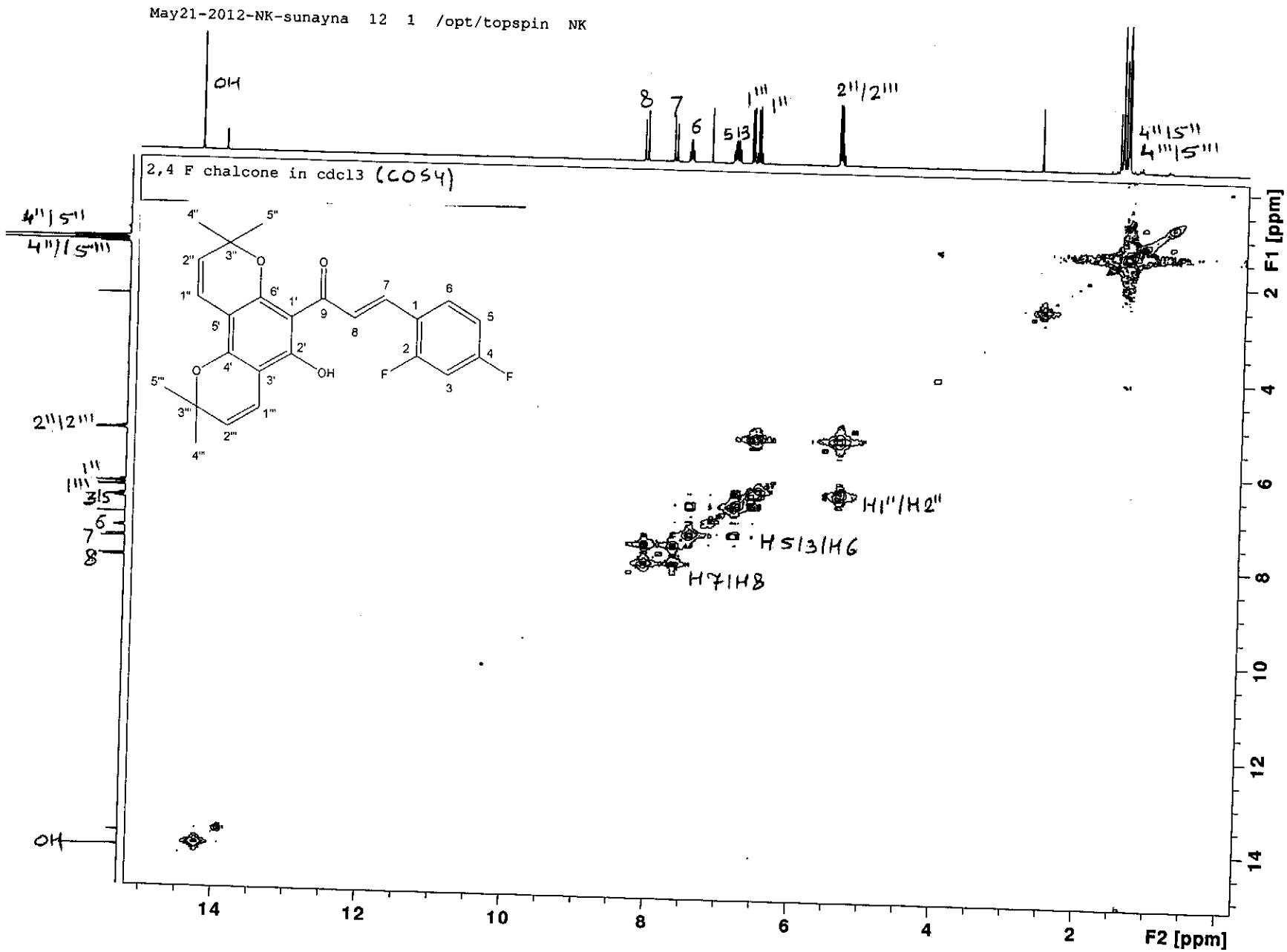
^{13}C NMR spectrum of 3e – 2,4-F pyranochromene chalcone (A-3e)



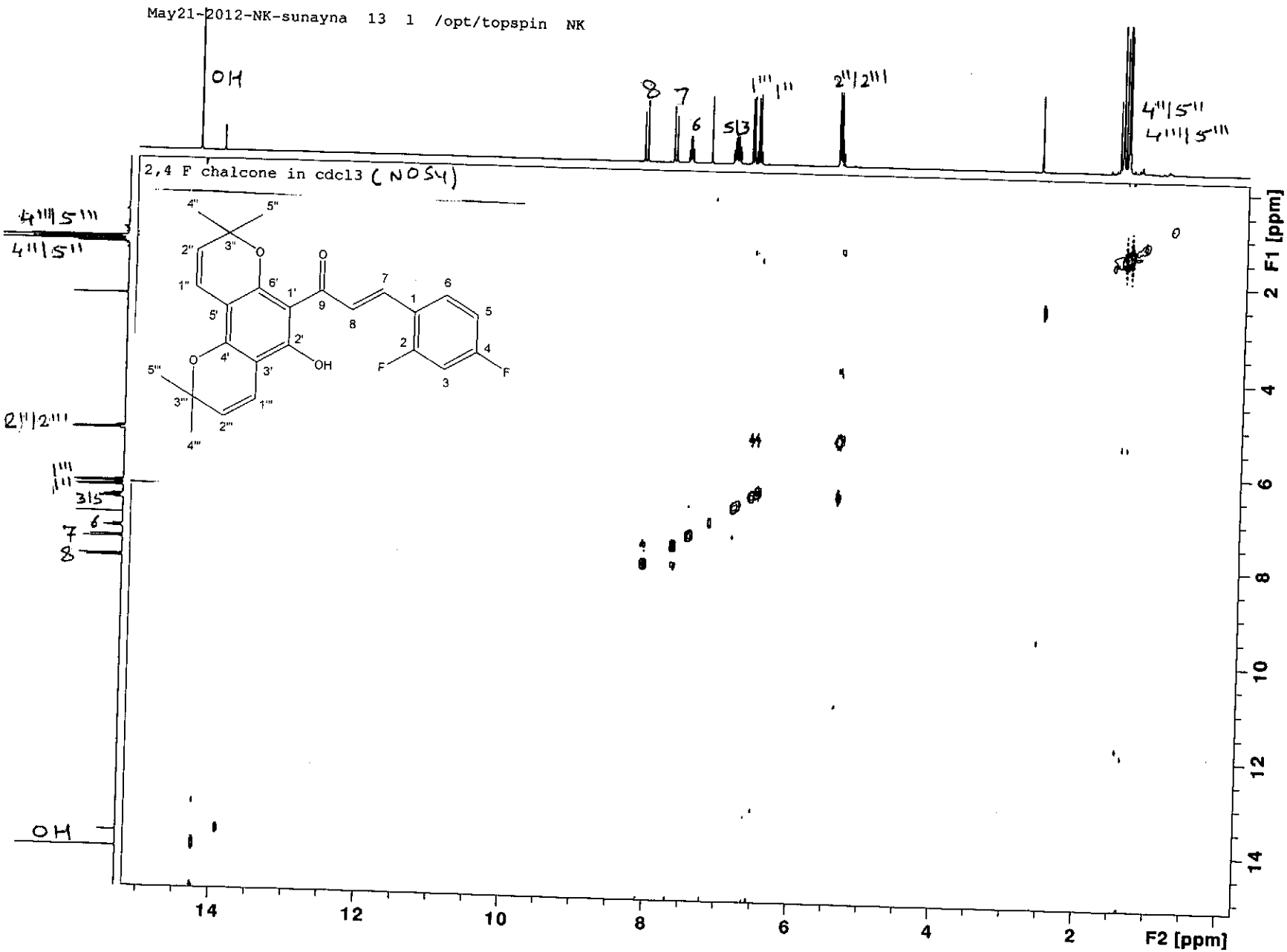
^{13}C NMR spectrum of 3e – 2,4-F pyranochromene chalcone (A-3e)



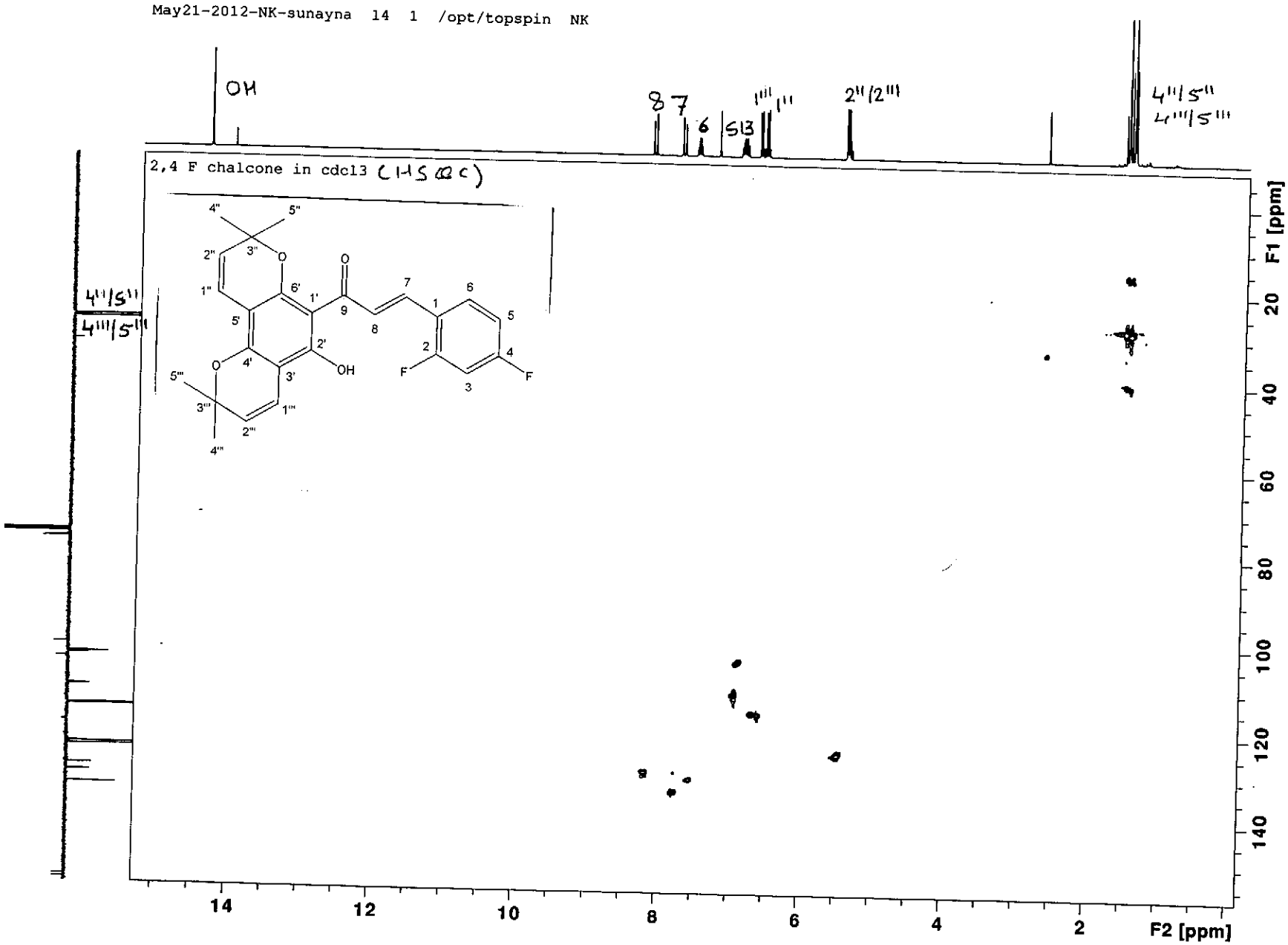
^{13}C NMR spectrum of 3e – 2,4-F pyranochromene chalcone (A-3e)



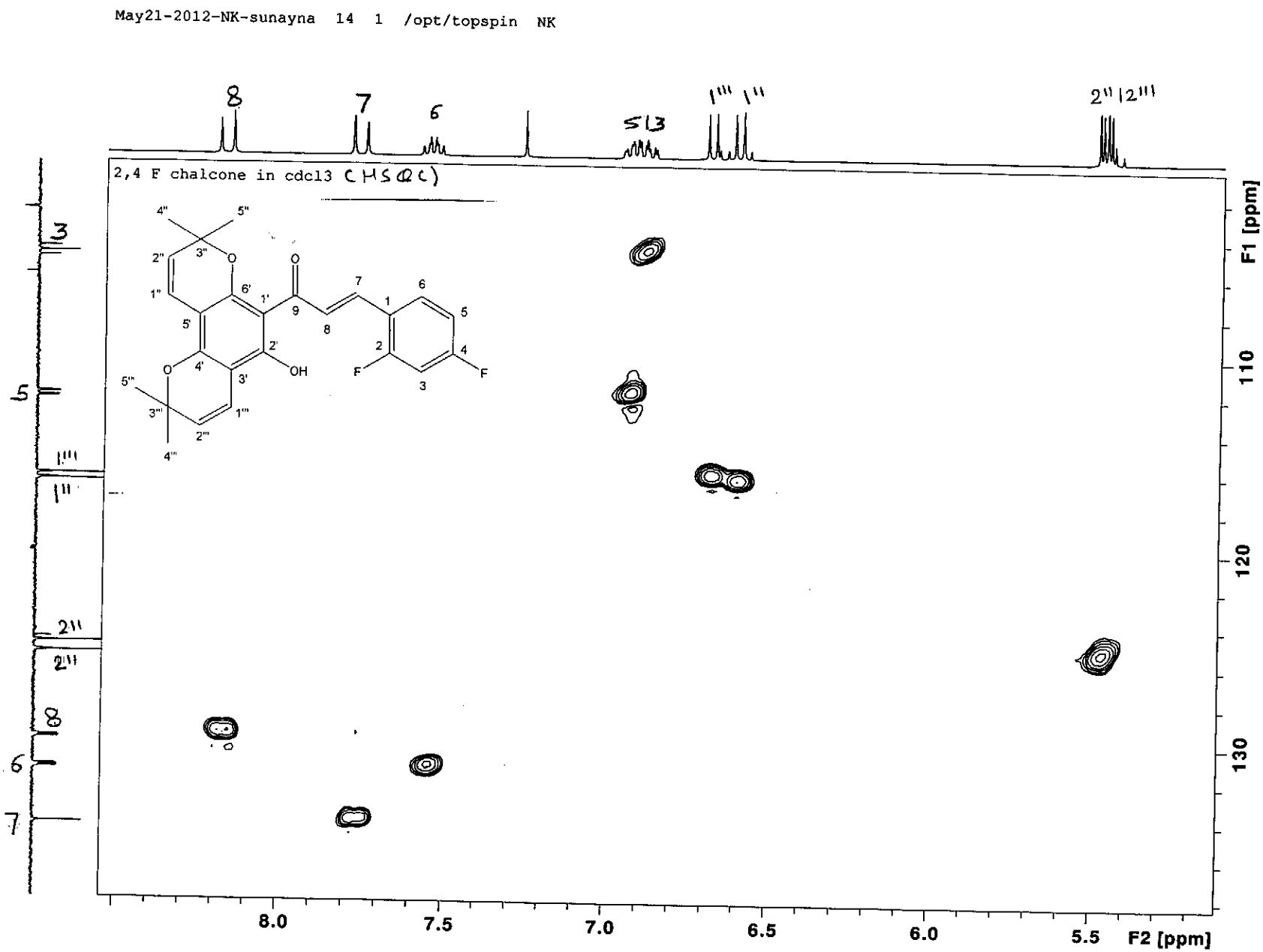
COSY NMR spectrum of 3e – 2,4-F pyranochromene chalcone (A-3e)



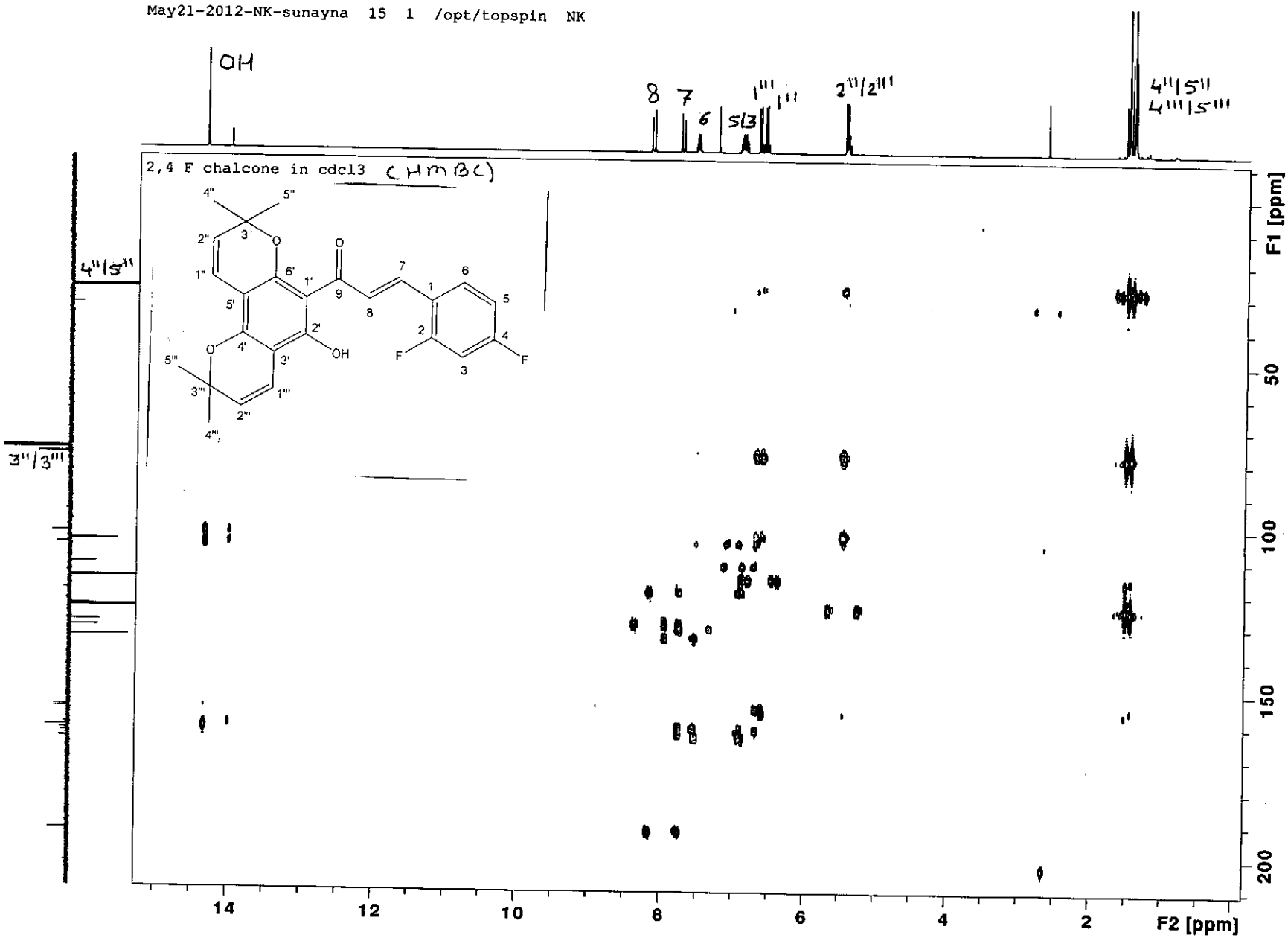
NOSY NMR spectrum of 3e – 2,4-F pyranochromene chalcone (A-3e)



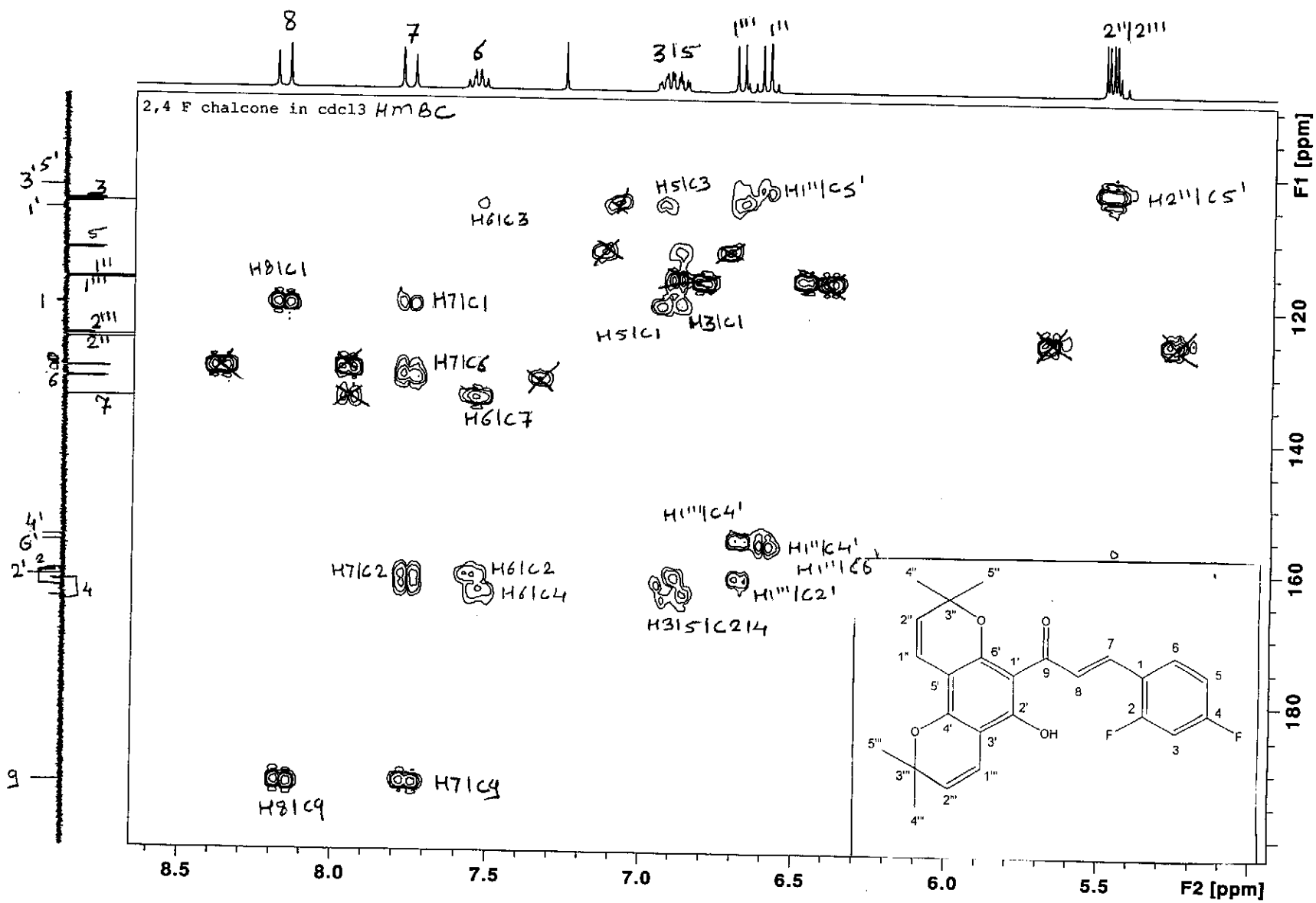
HSQC NMR spectrum of 3e – 2,4-F pyranochromene chalcone (A-3e)



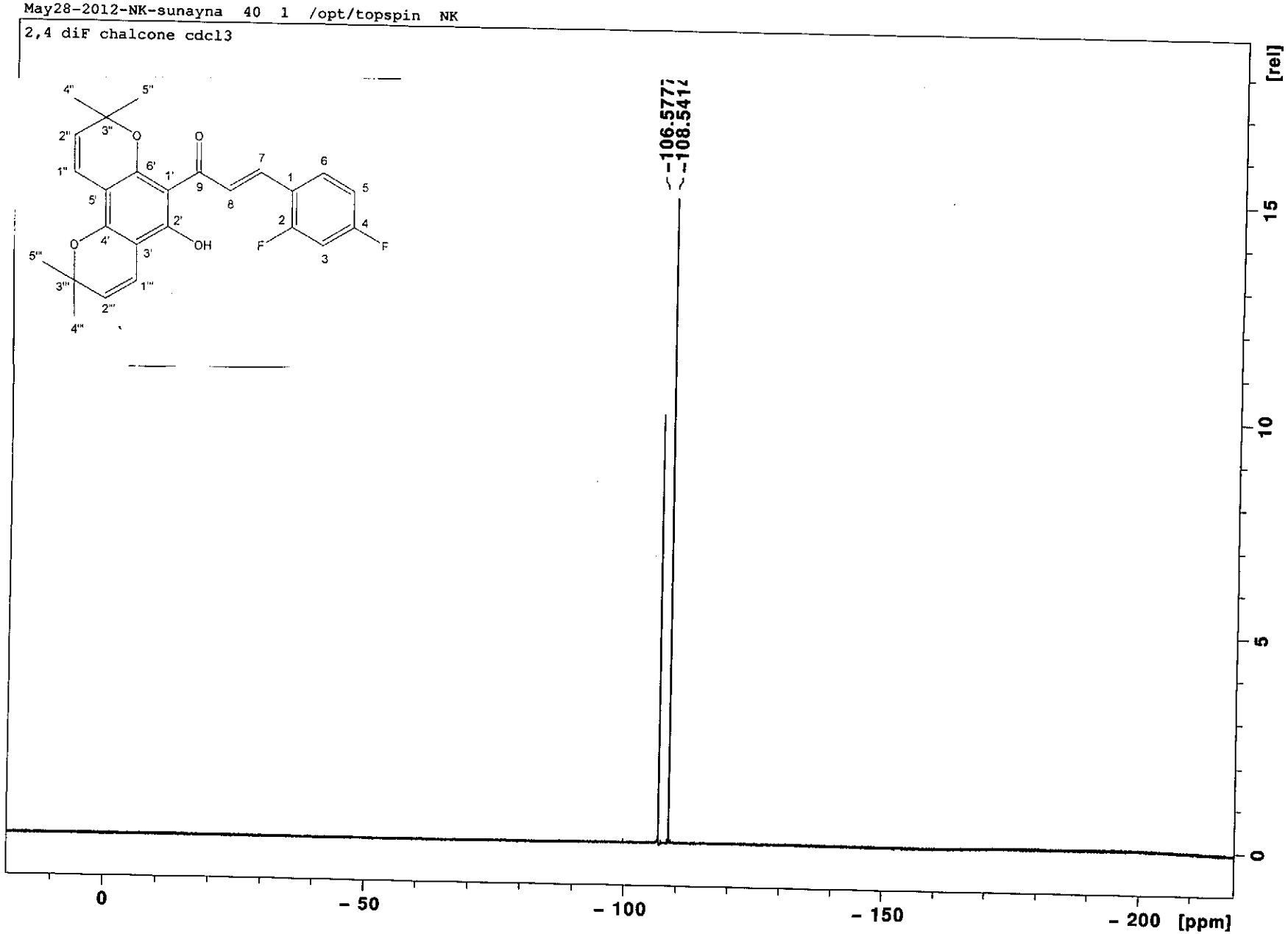
HSQC NMR spectrum of 3e – 2,4-F pyranochromene chalcone (A-3e)



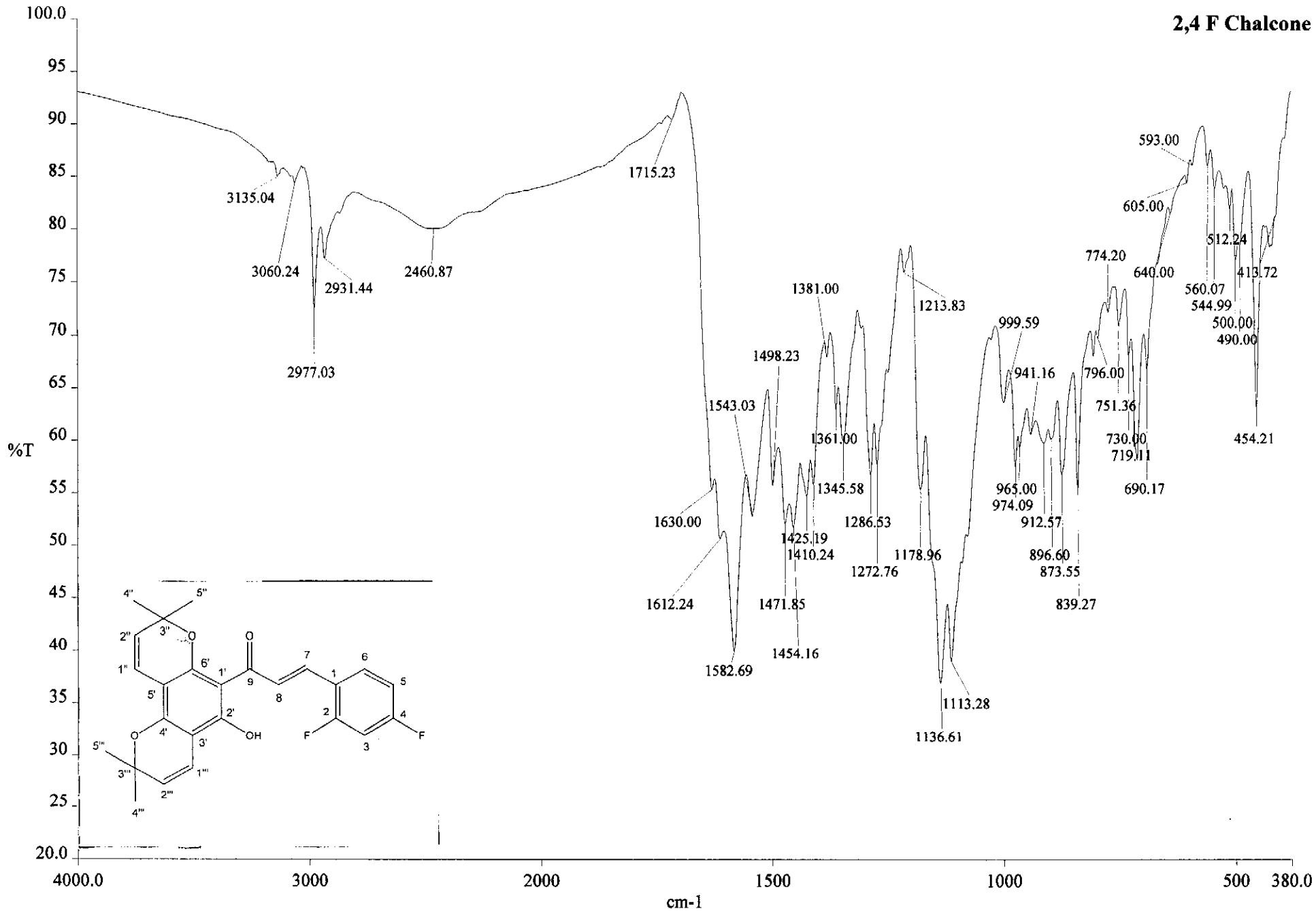
HMBC NMR spectrum of 3e – 2,4-F pyranochromene chalcone (A-3e)



HMBC NMR spectrum of 3e – 2,4-F pyranochromene chalcone (A-3e)

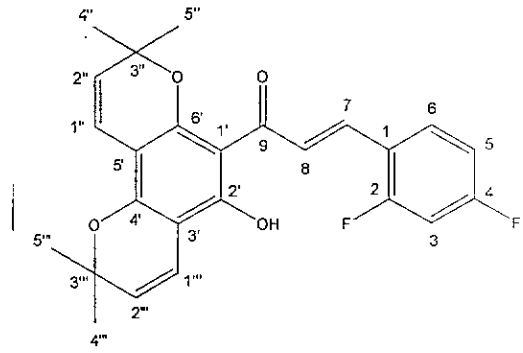


^{19}F NMR spectrum of 3e – 2,4-F pyranochromene chalcone (A-3e)



IR spectrum of 3e – 2,4-F pyranochromene chalcone (A-3e)

Elemental Composition Report



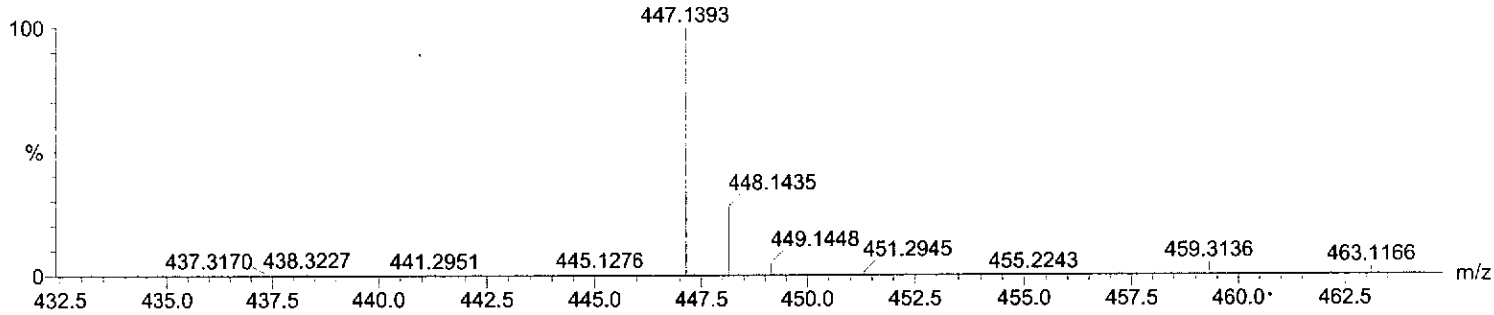
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
15 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-26 H: 20-25 O: 0-5 F: 0-2 Na: 0-1

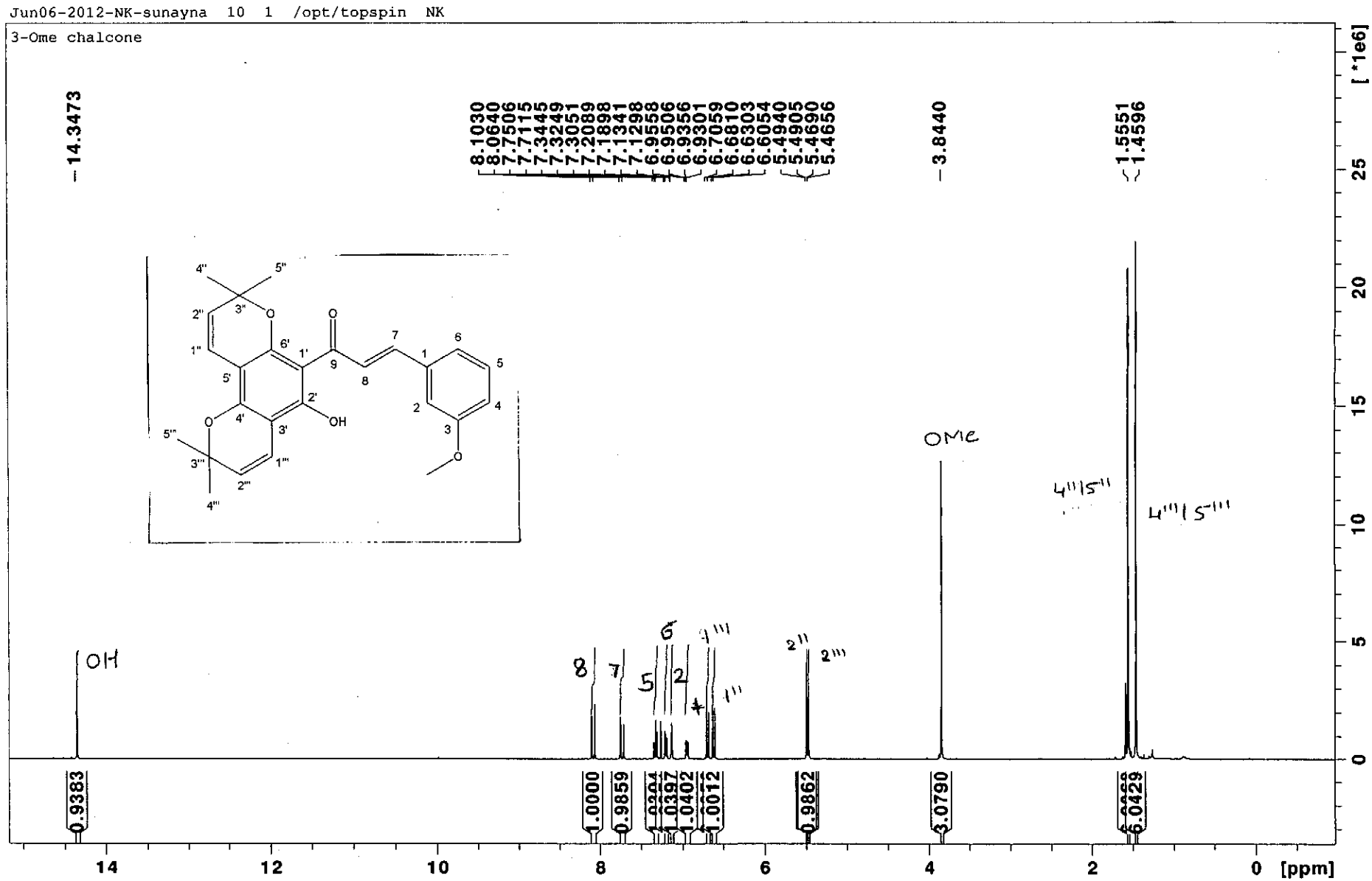
Dr. Nizam
15 10 (0.154) Cm (1:30)

TOF MS ES+
1.03e+004

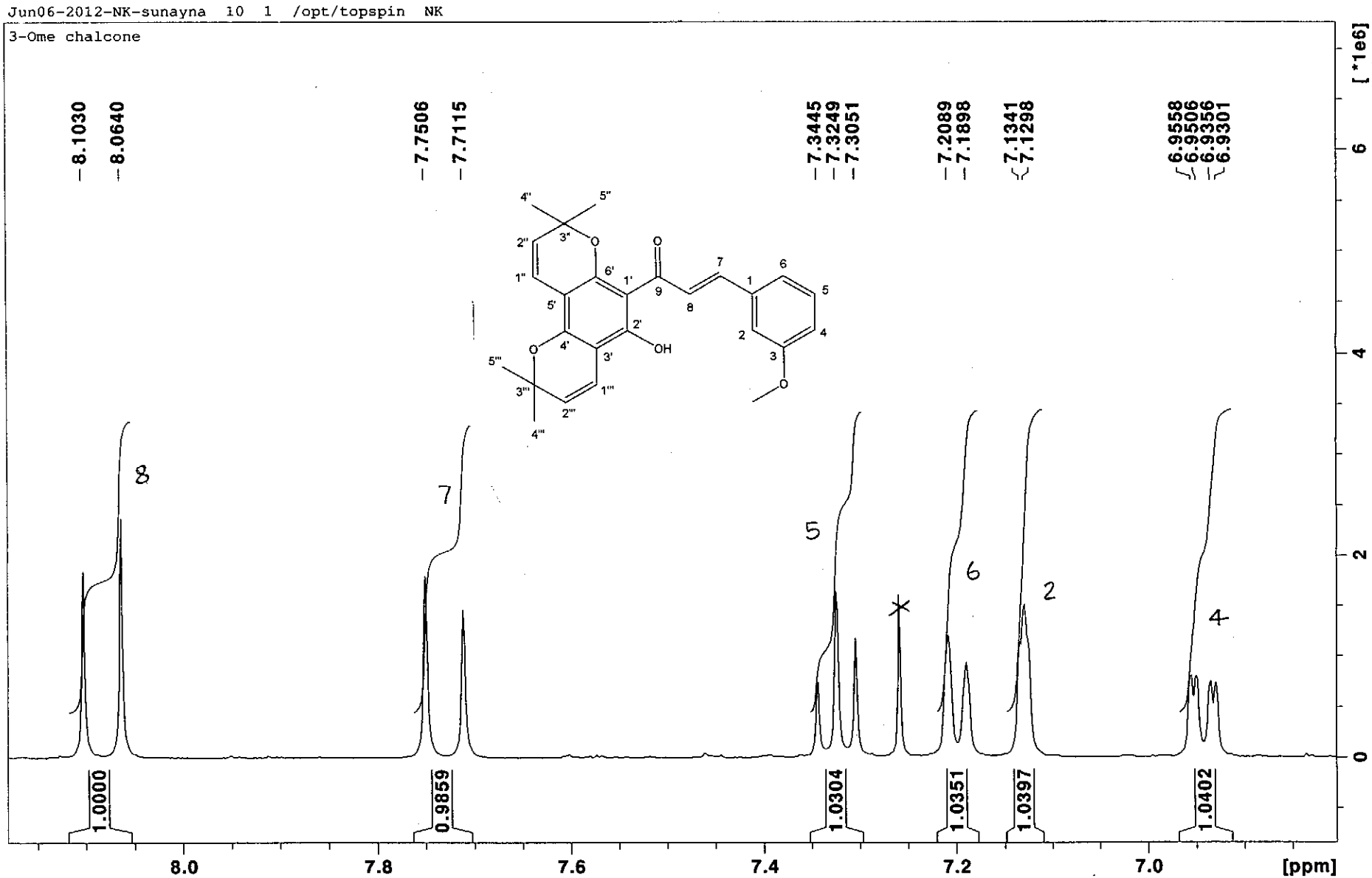


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
447.1393	447.1384	0.9	2.0	13.5	337.2	0.0	C25 H22 O4 F2 Na

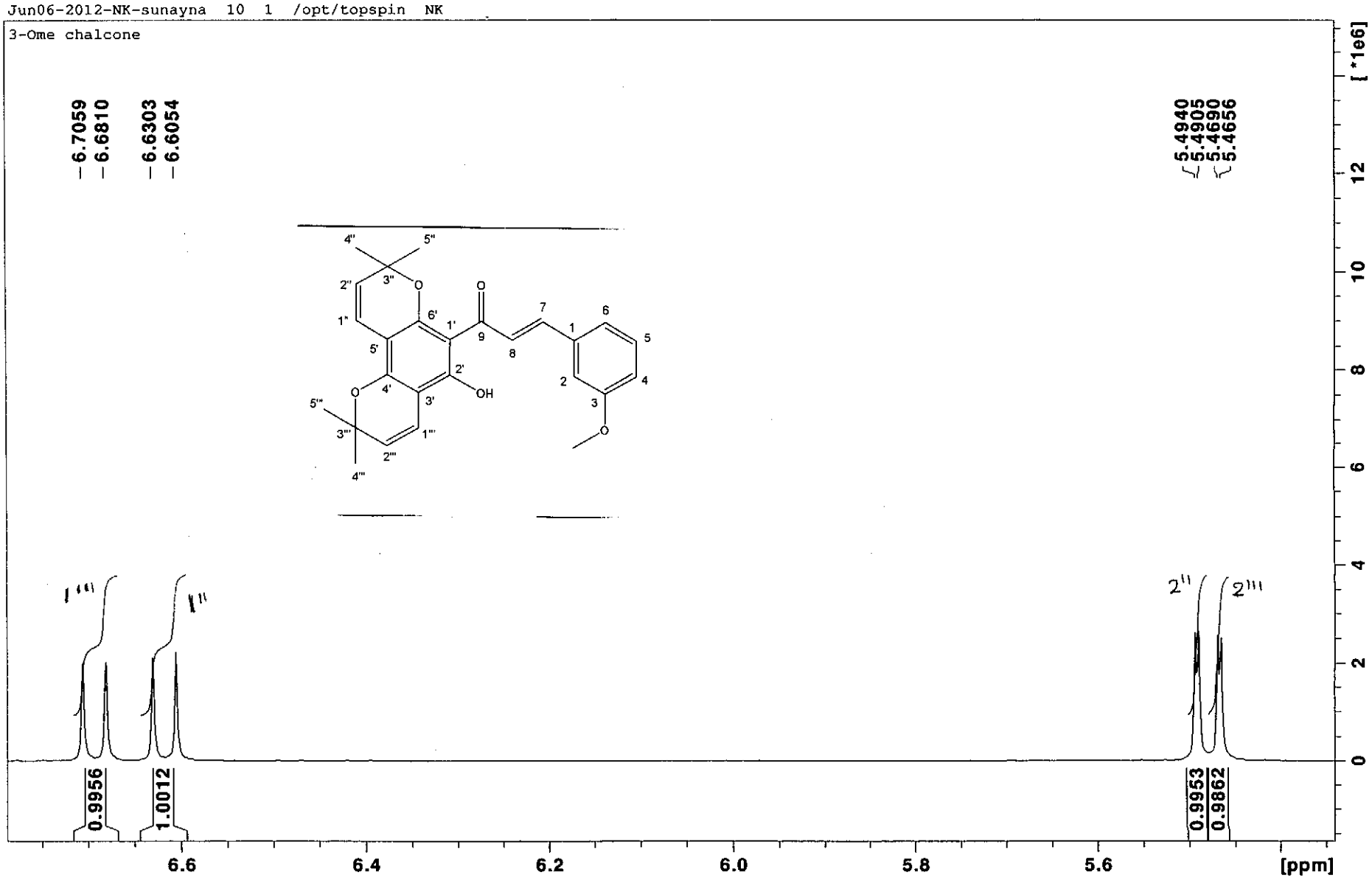
HRMS of 3e – 2,4-F pyranochromene chalcone (A-3e)



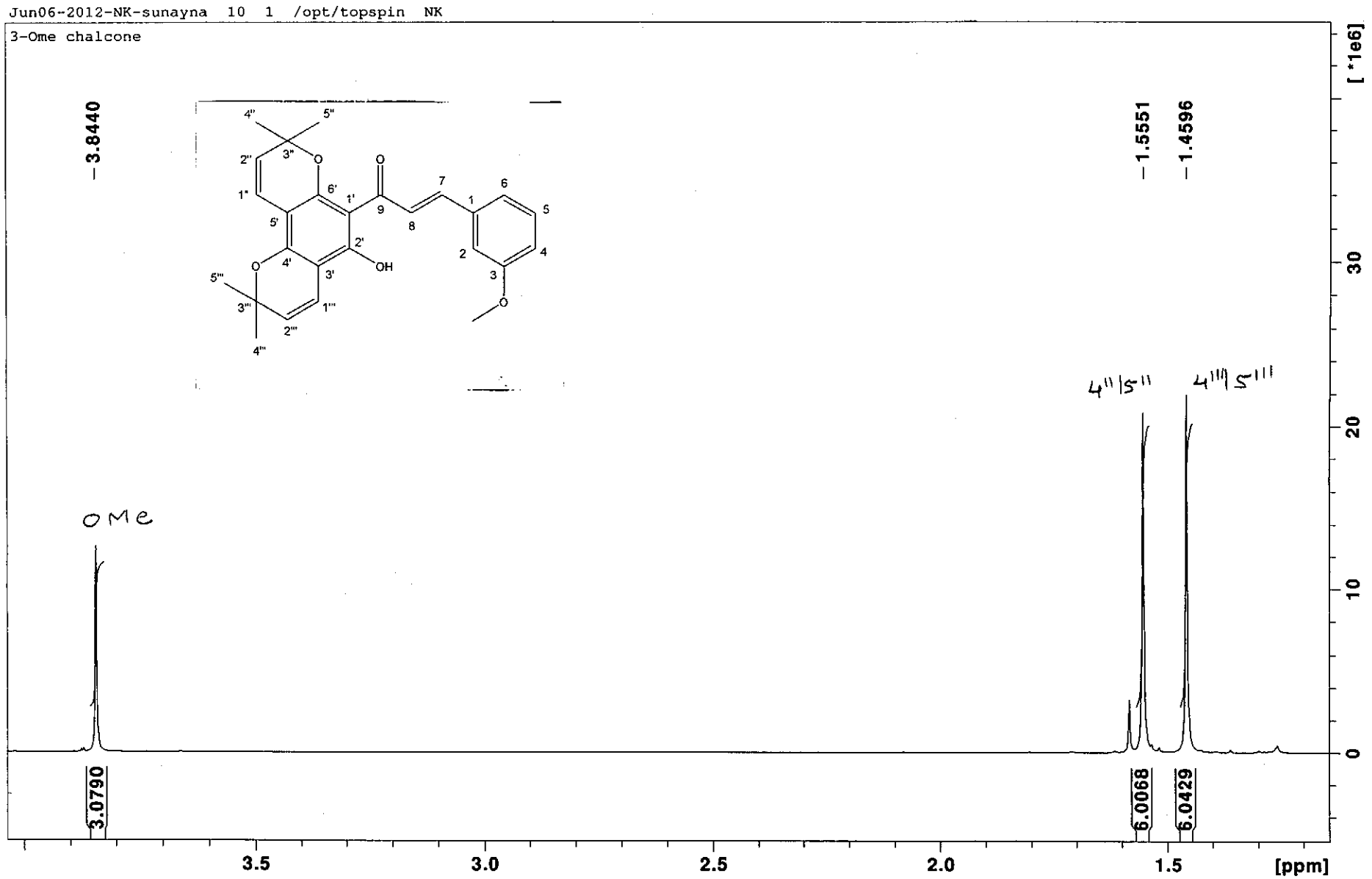
^{1}H NMR spectrum of 3f - 3-OMe pyranochromene chalcone (A-3f)



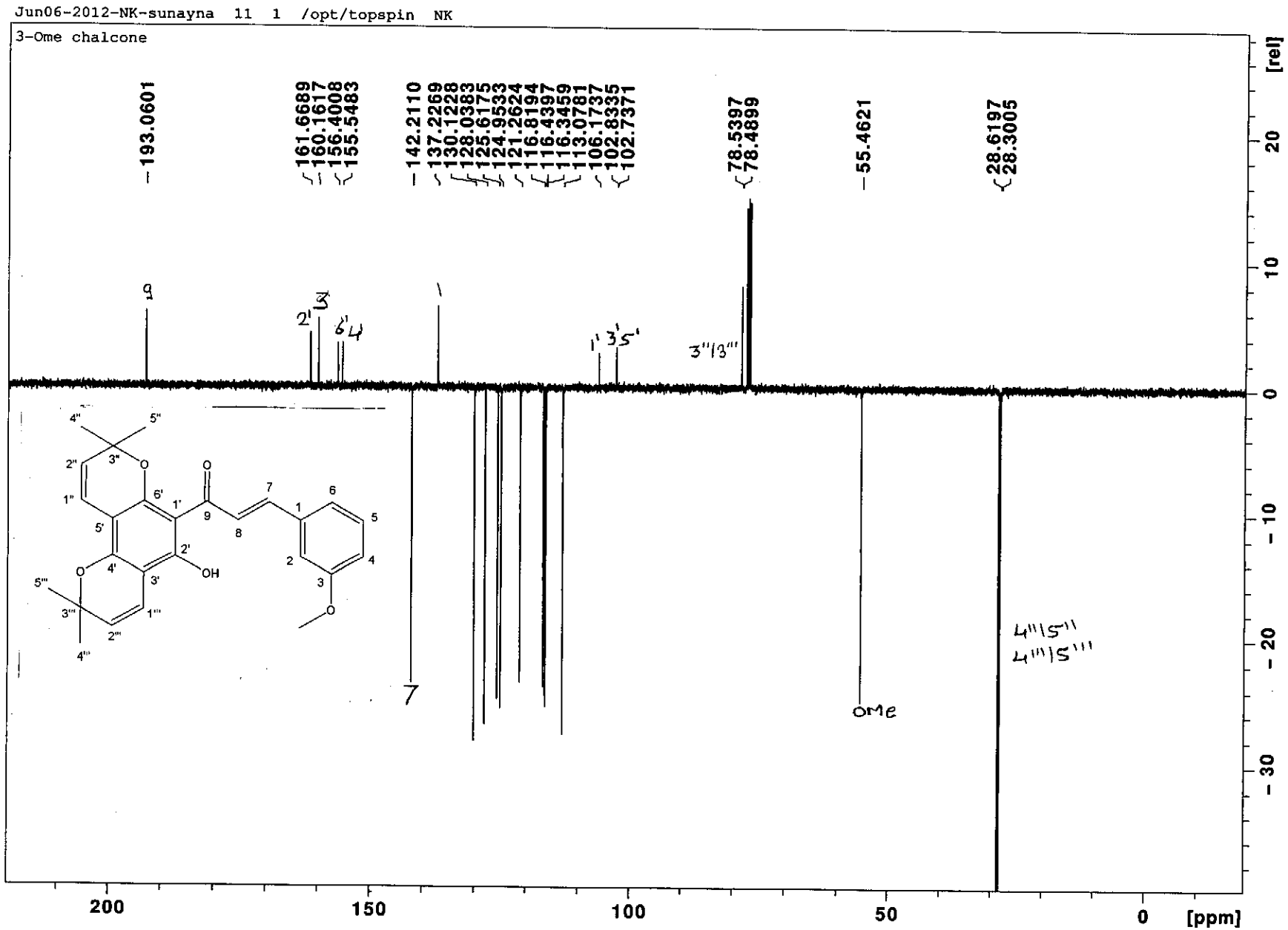
^{1}H NMR spectrum of 3f - 3-OMe pyranochromene chalcone (A-3f)



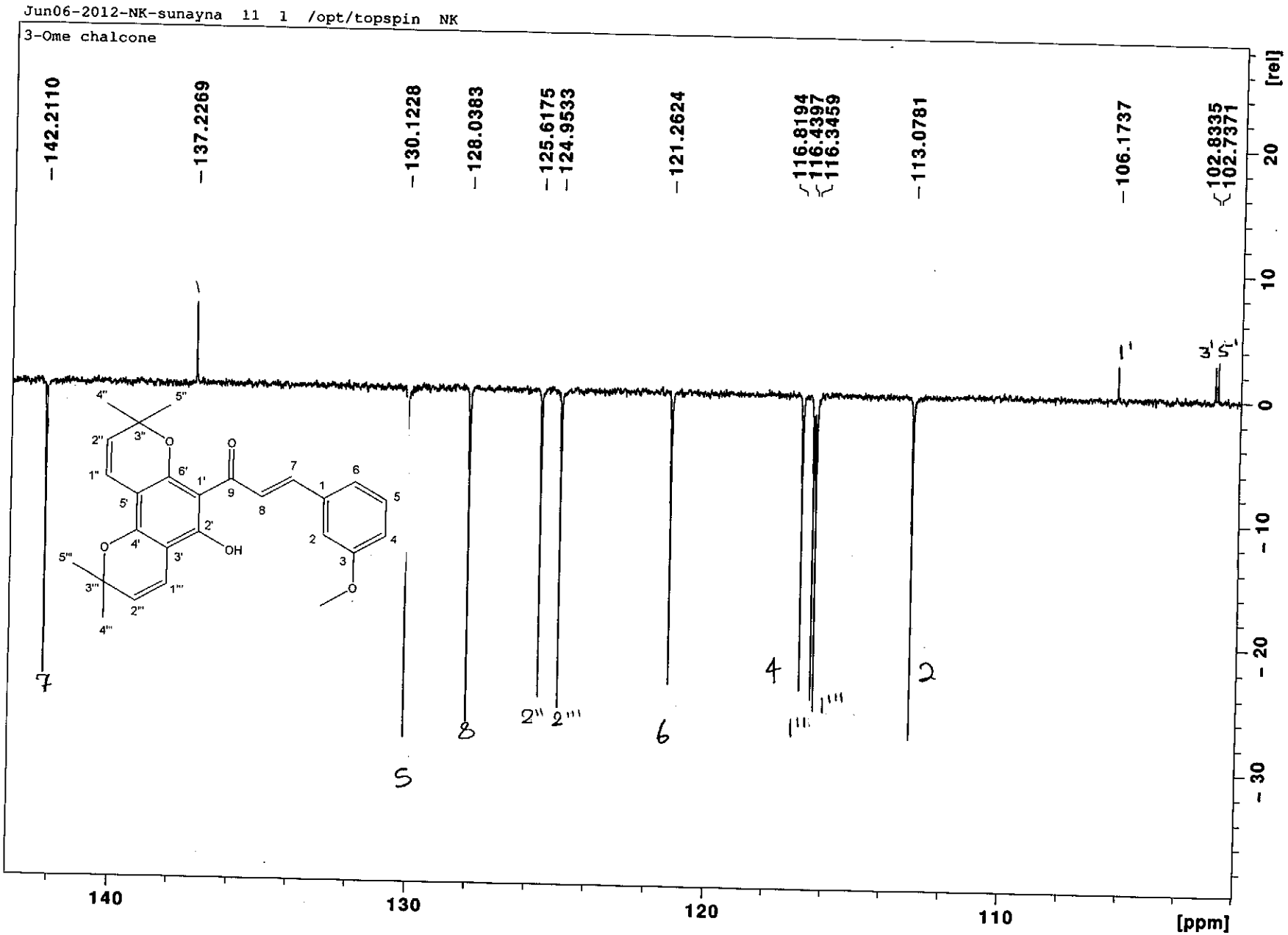
^{1}H NMR spectrum of 3f - 3-OMe pyranochromene chalcone (A-3f)



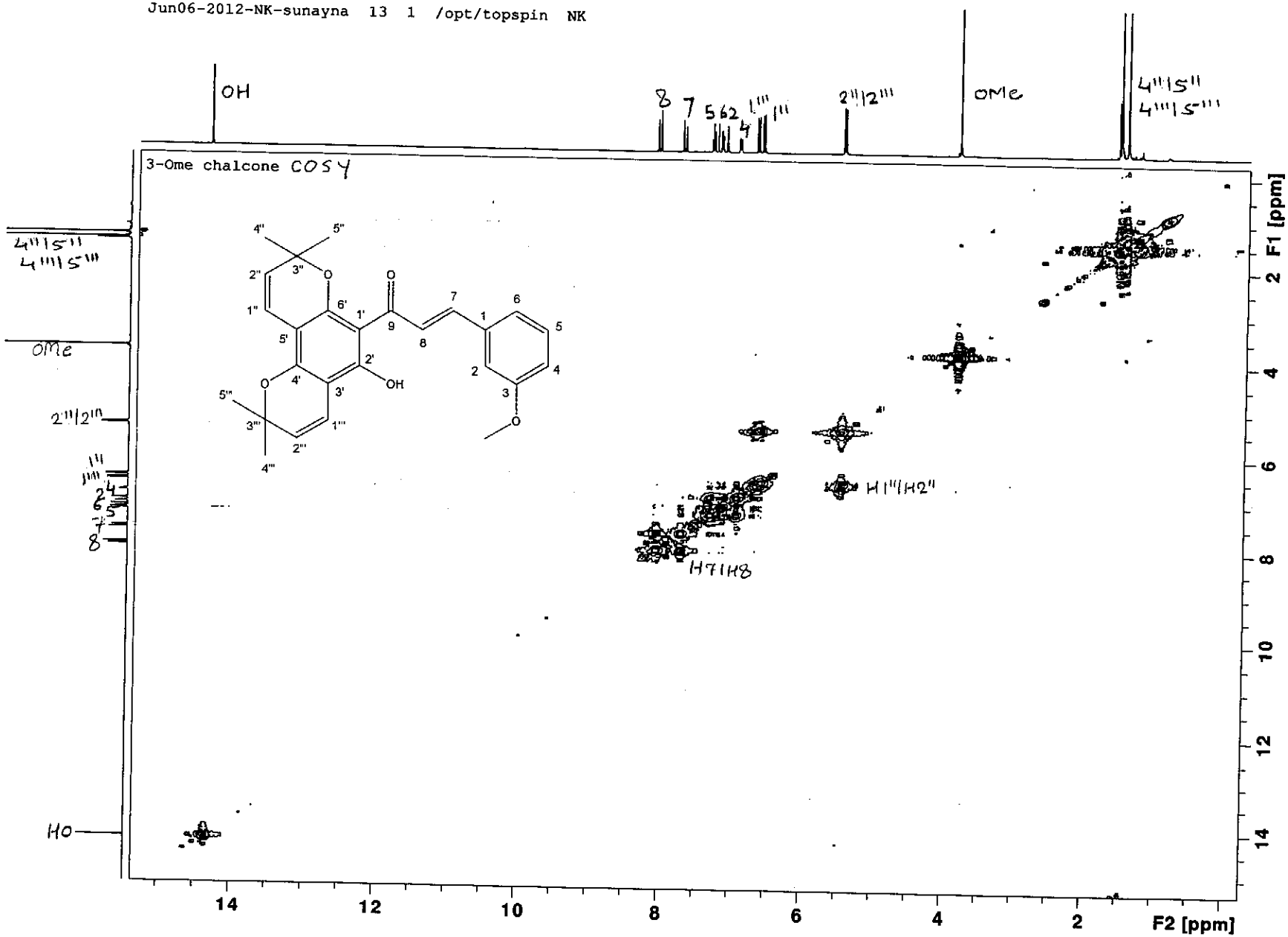
^{1}H NMR spectrum of 3f - 3-OMe pyranochromene chalcone (A-3f)



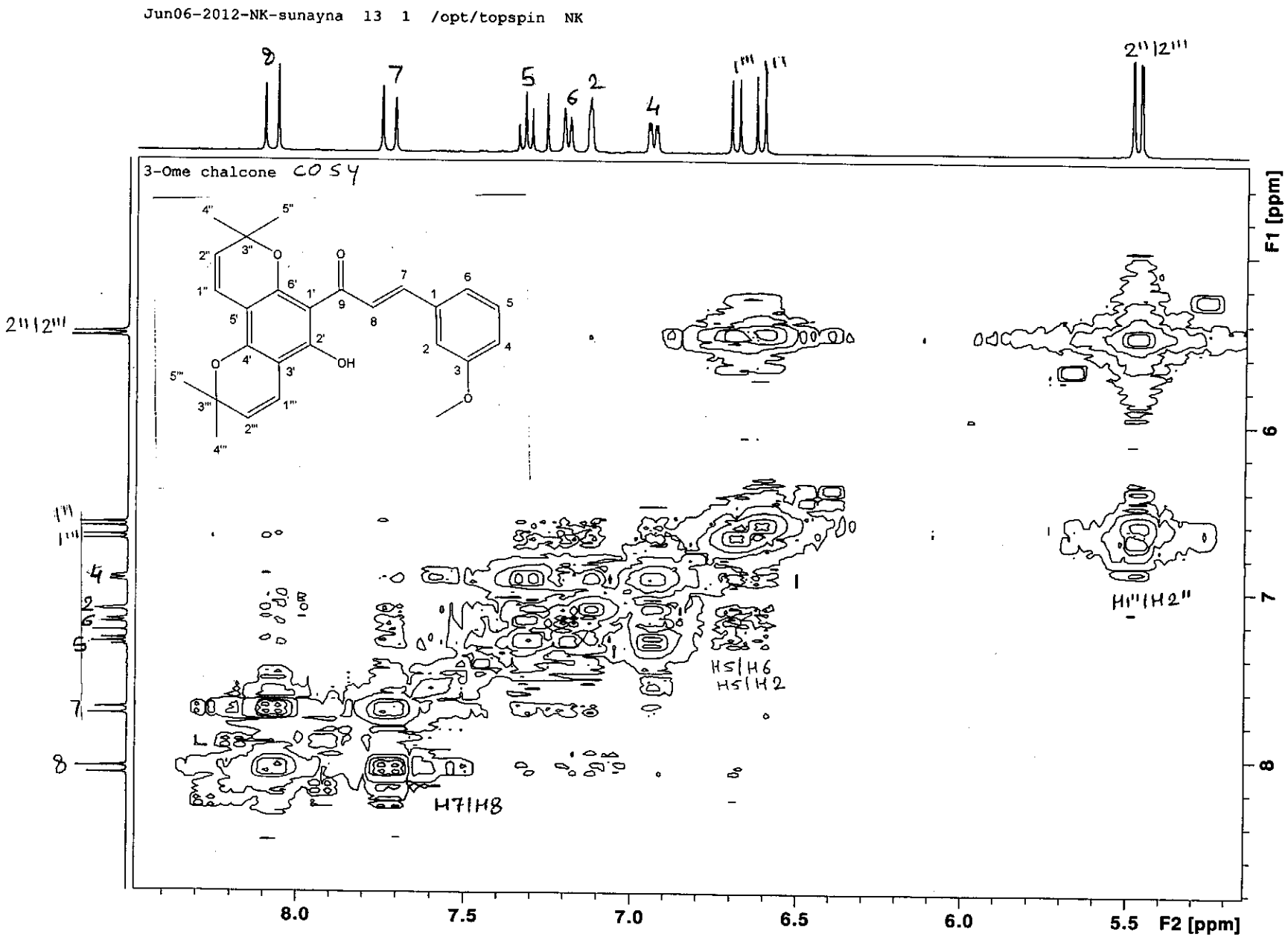
^{13}C NMR spectrum of 3f - 3-OMe pyranochromene chalcone (A-3f)



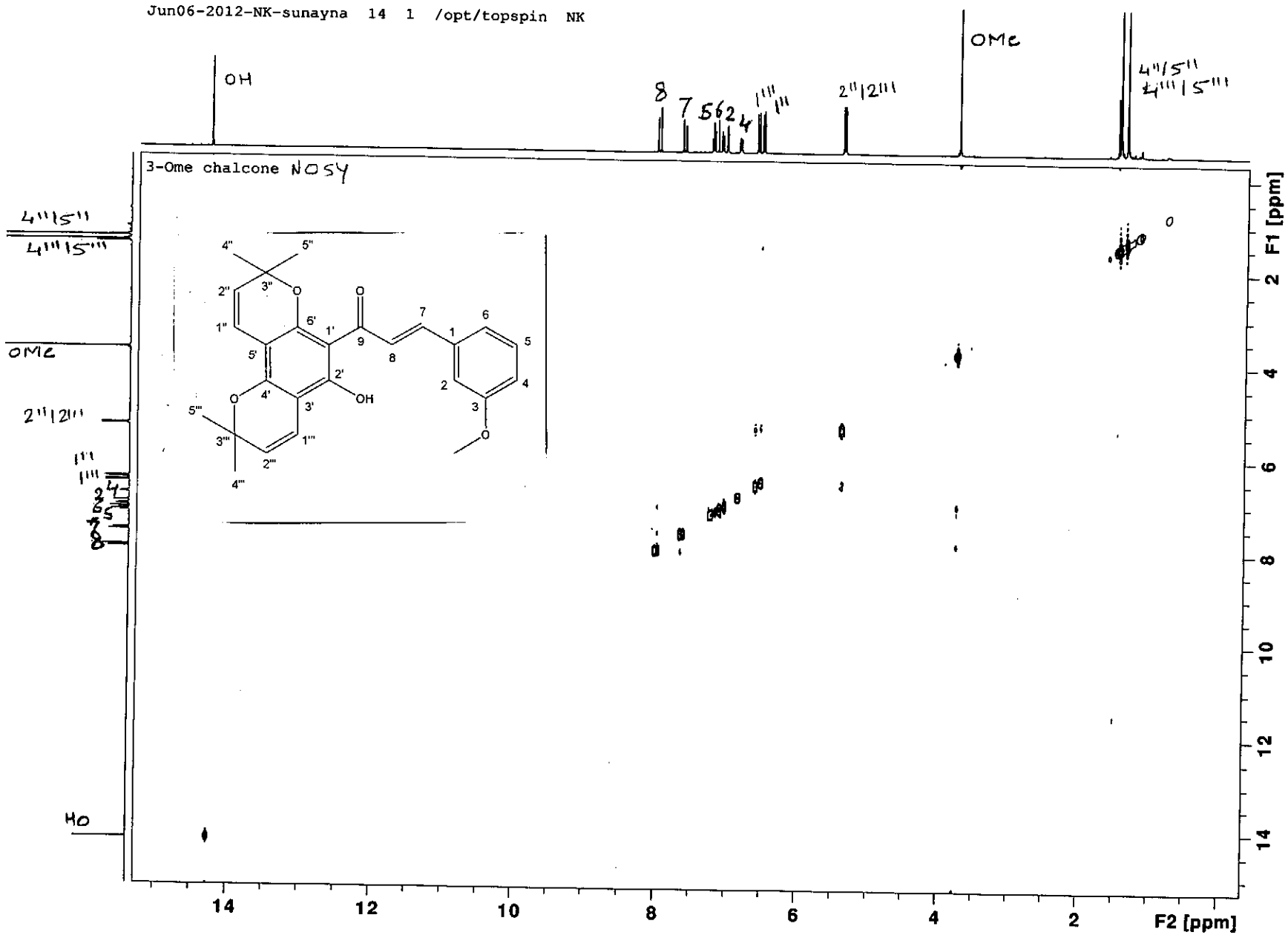
^{13}C NMR spectrum of 3f - 3-OMe pyranochromene chalcone (A-3f)



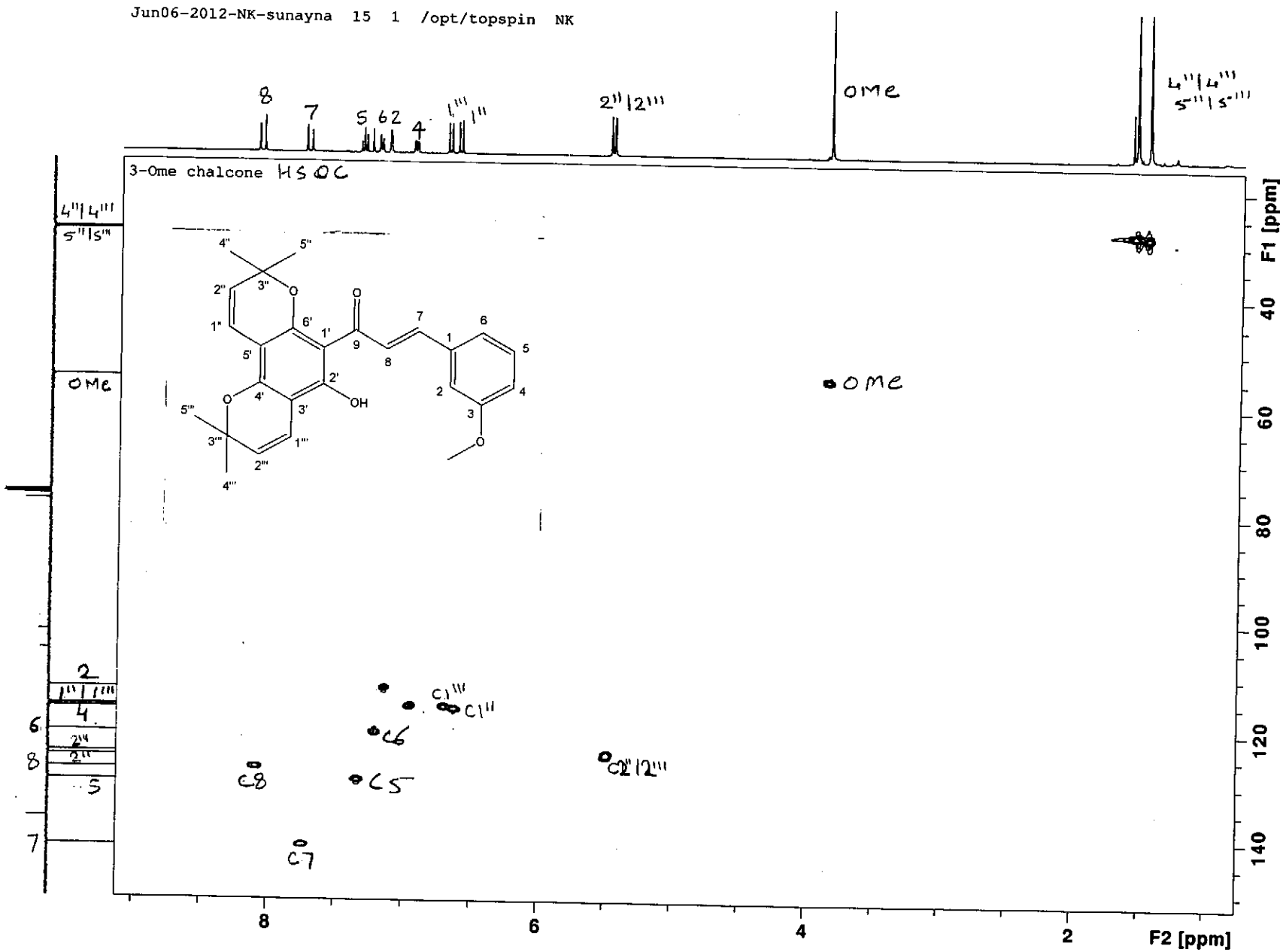
COSY NMR spectrum of 3f - 3-OMe pyranochromene chalcone (A-3f)



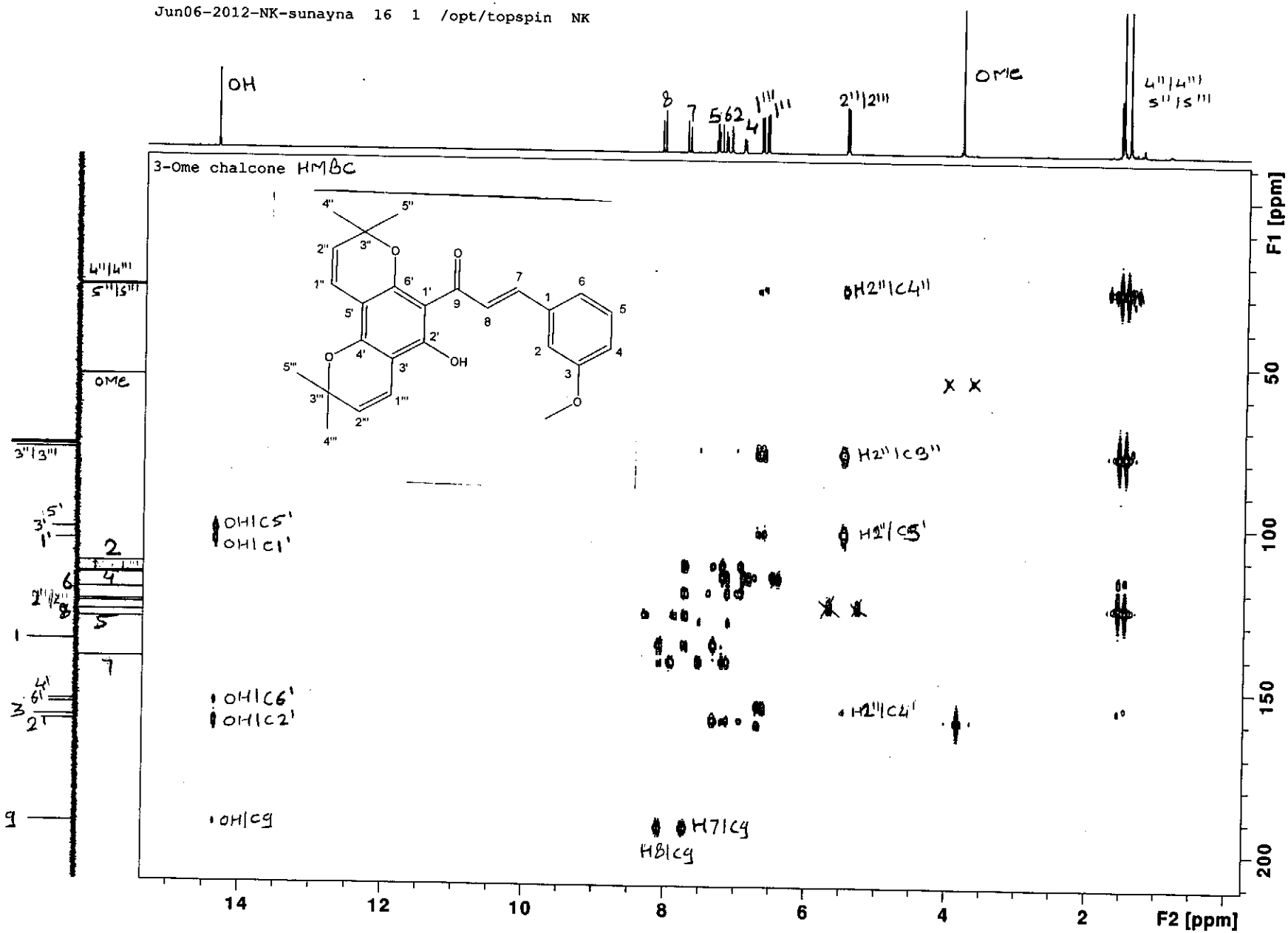
COSY NMR spectrum of 3f - 3-OMe pyranochromene chalcone (A-3f)



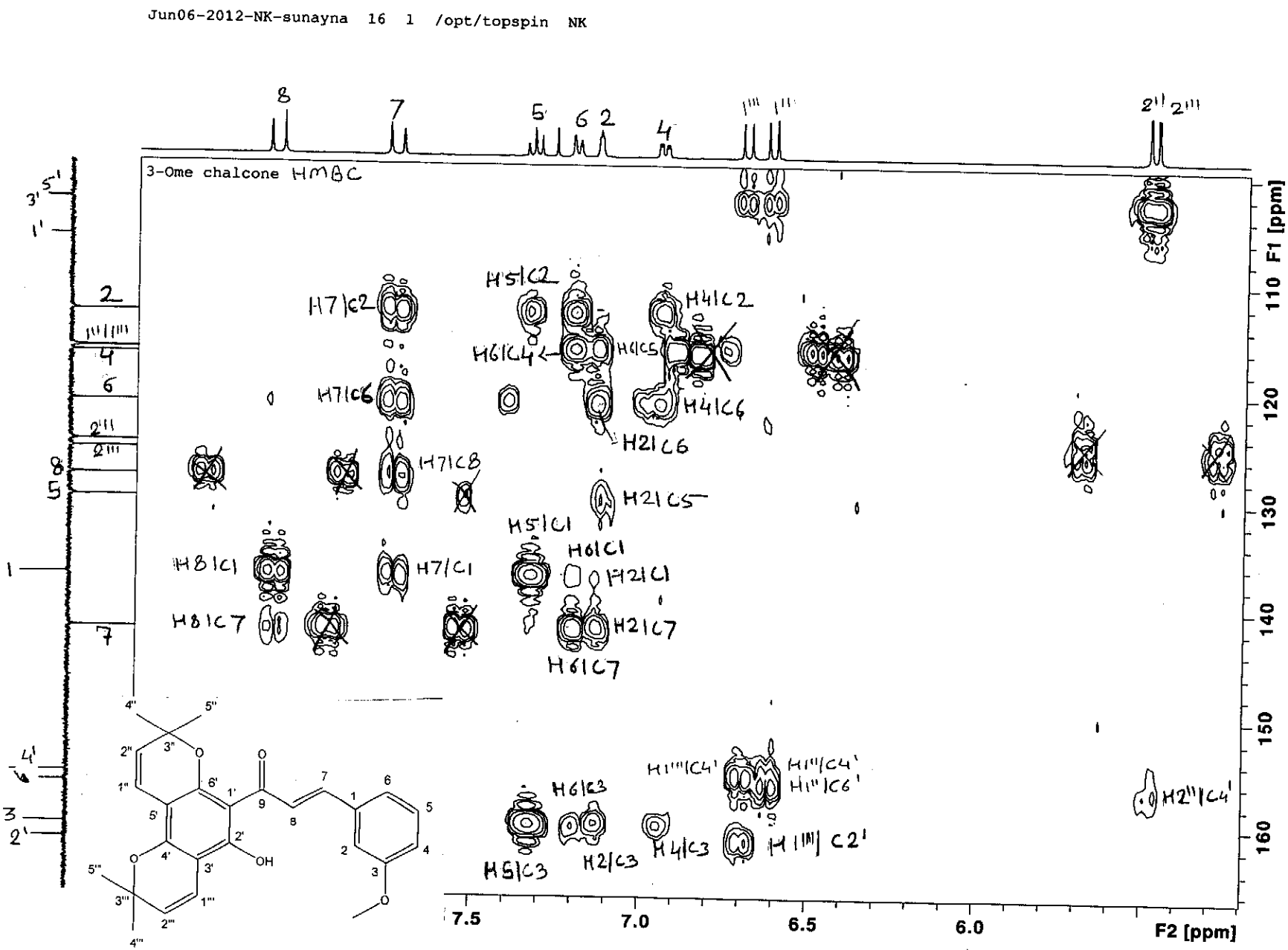
NOSY NMR spectrum of 3f - 3-OMe pyranochromene chalcone (A-3f)



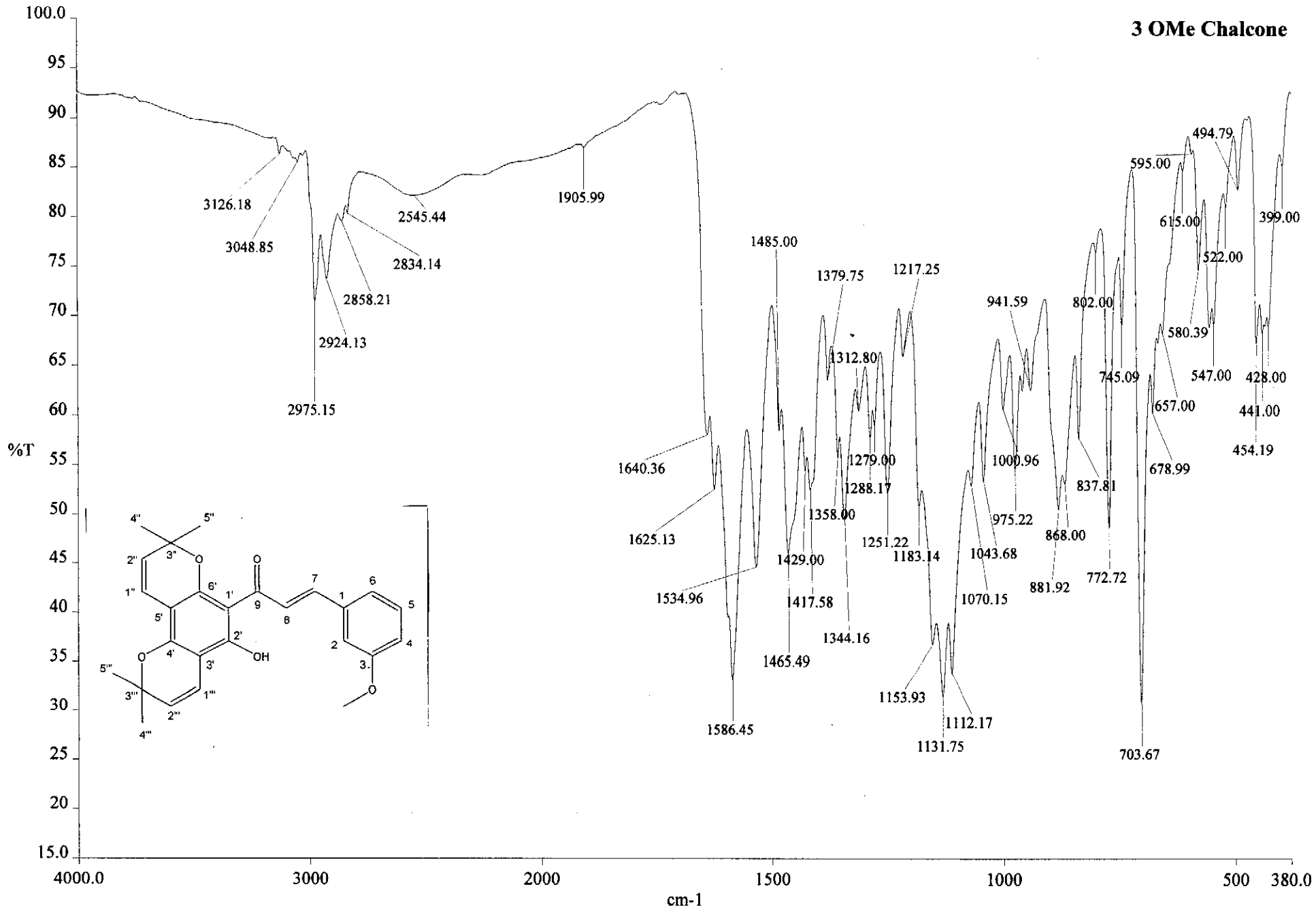
HSQC NMR spectrum of 3f - 3-OMe pyranochromene chalcone (A-3f)



HBMC NMR spectrum of 3f - 3-OMe pyranochromene chalcone (A-3f)

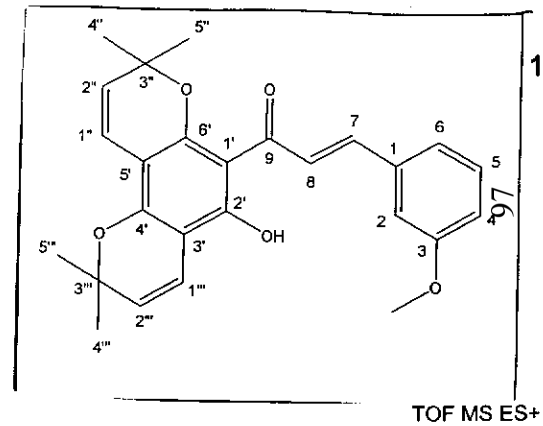


HMBC NMR spectrum of 3f - 3-OMe pyranochromene chalcone (A-3f)



IR spectrum of 3f - 3-OMe pyranochromene chalcone (A-3f)

Elemental Composition Report

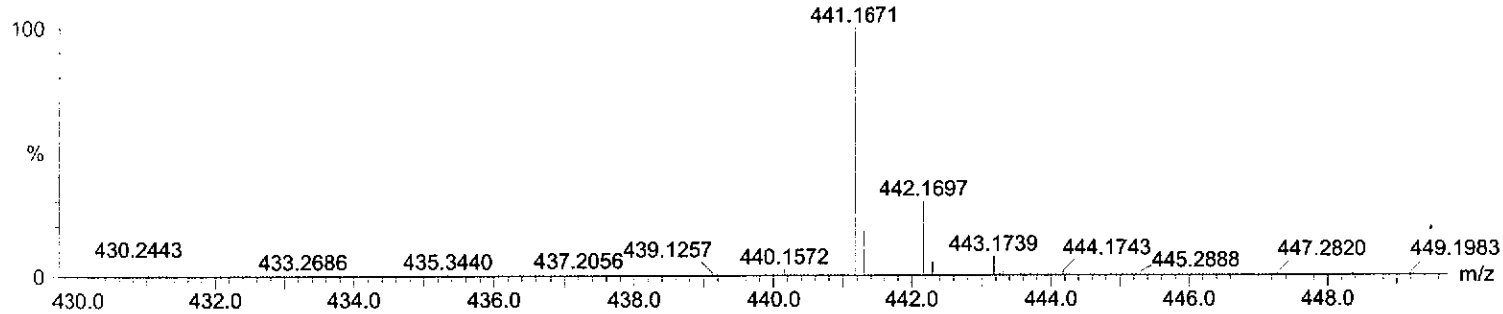


Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

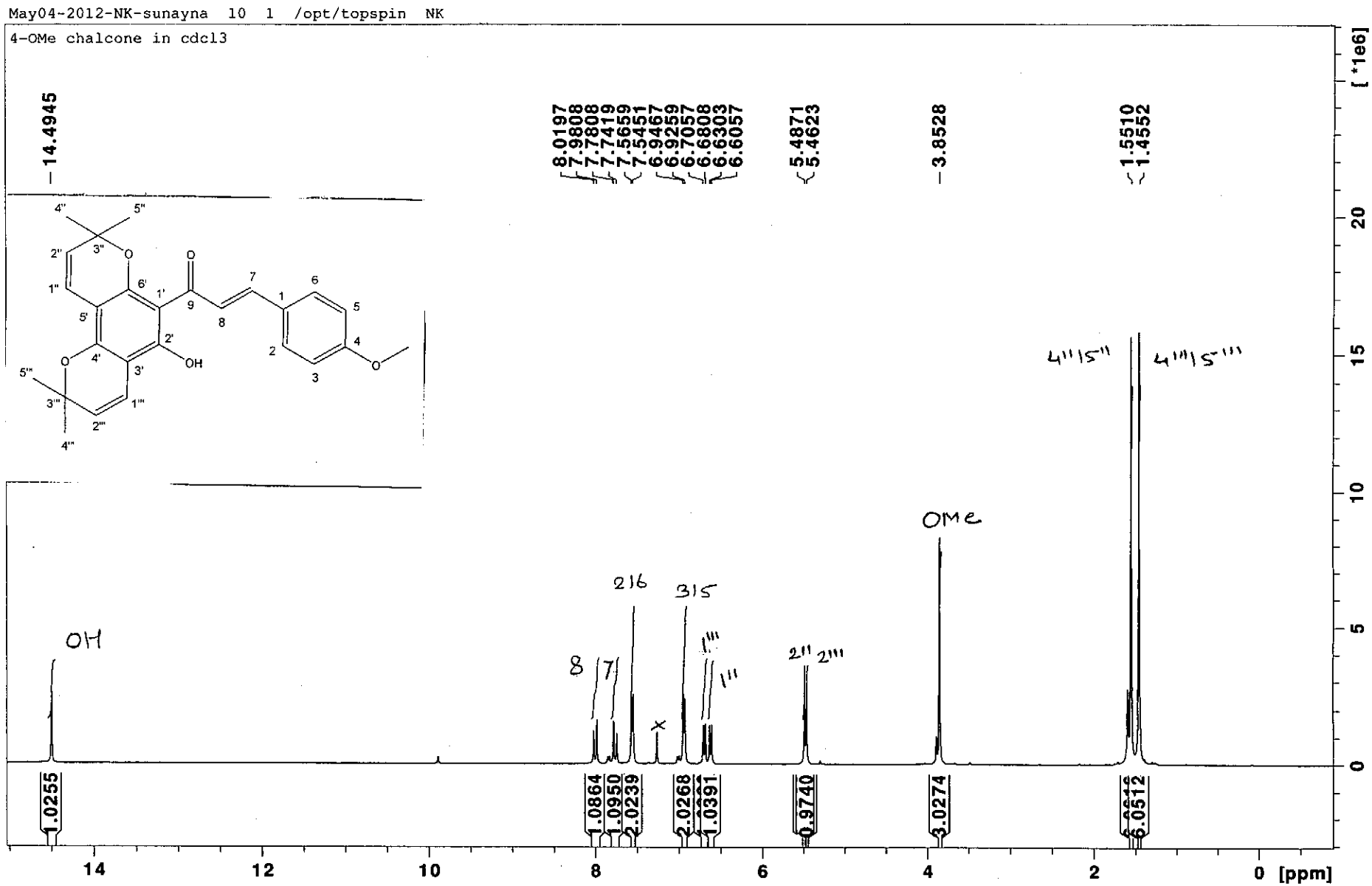
Monoisotopic Mass, Even Electron Ions
5 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-30 H: 25-30 O: 0-5 Na: 0-1

Dr. Nizam
5 14 (0.222) Cm (1:31)

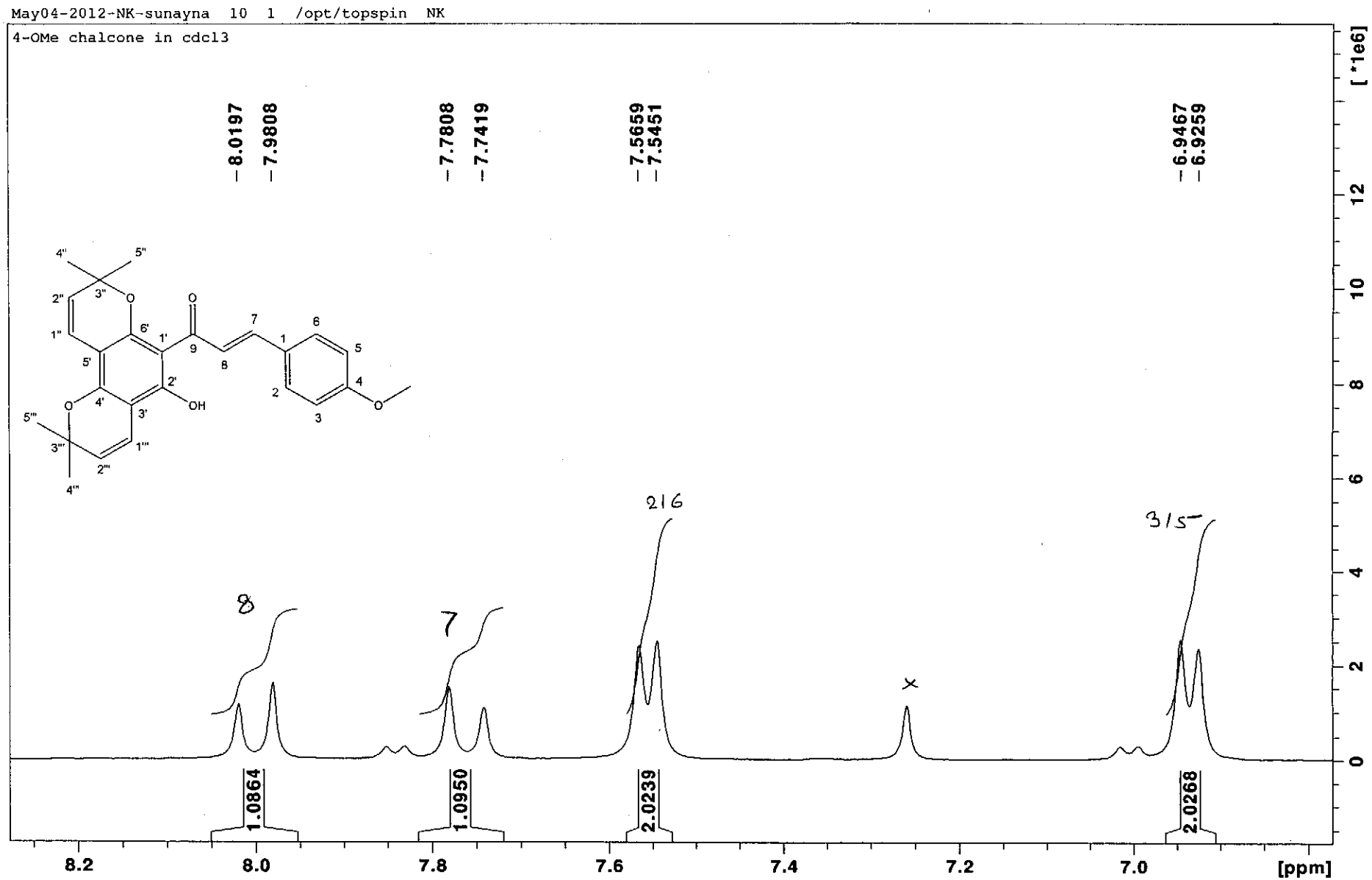


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
441.1671	441.1678	-0.7	-1.6	13.5	345.3	0.0	C26 H26 O5 Na

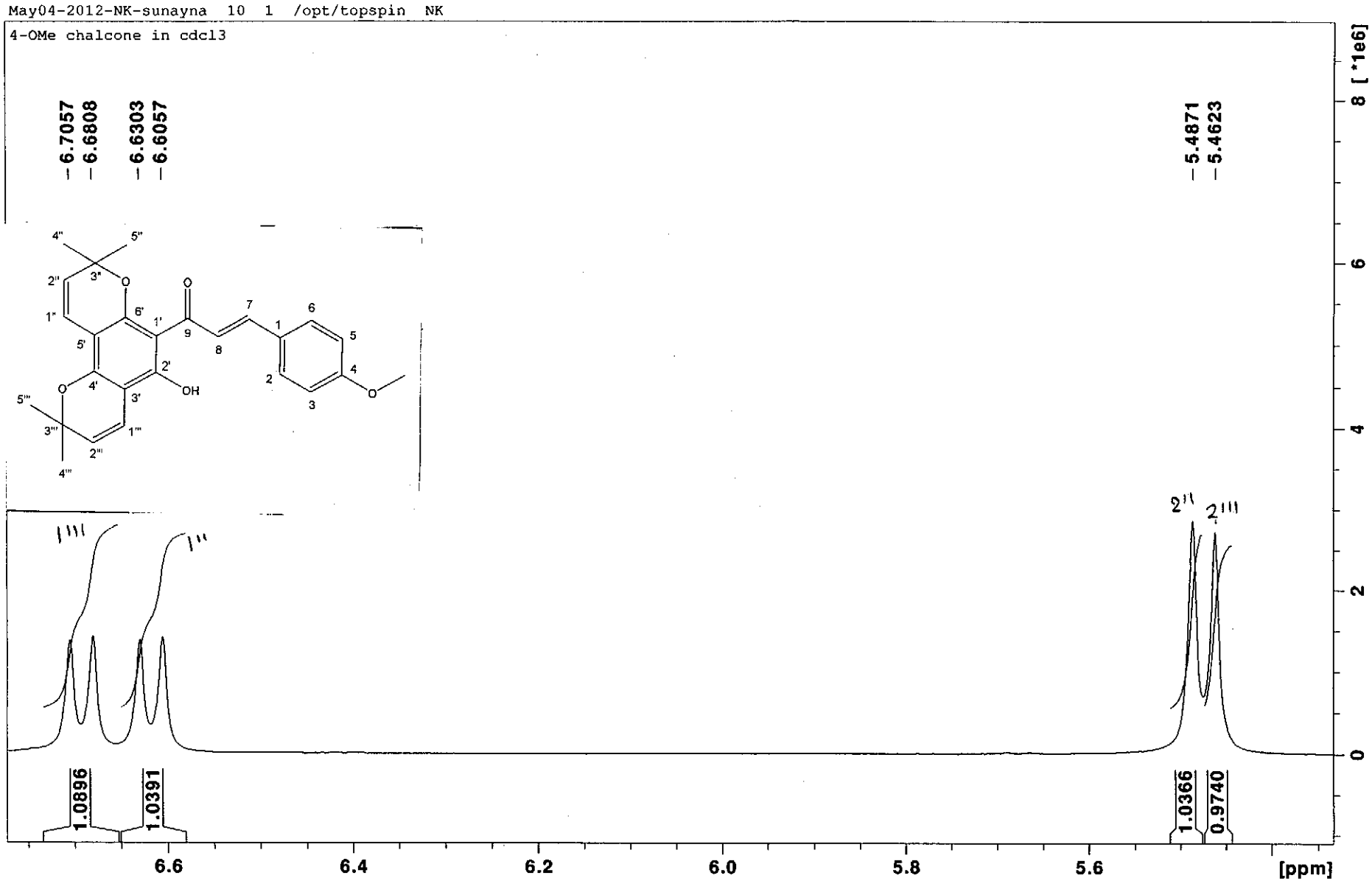
HRMS of 3f – 3-OMe pyranochromene chalcone (A-3f)



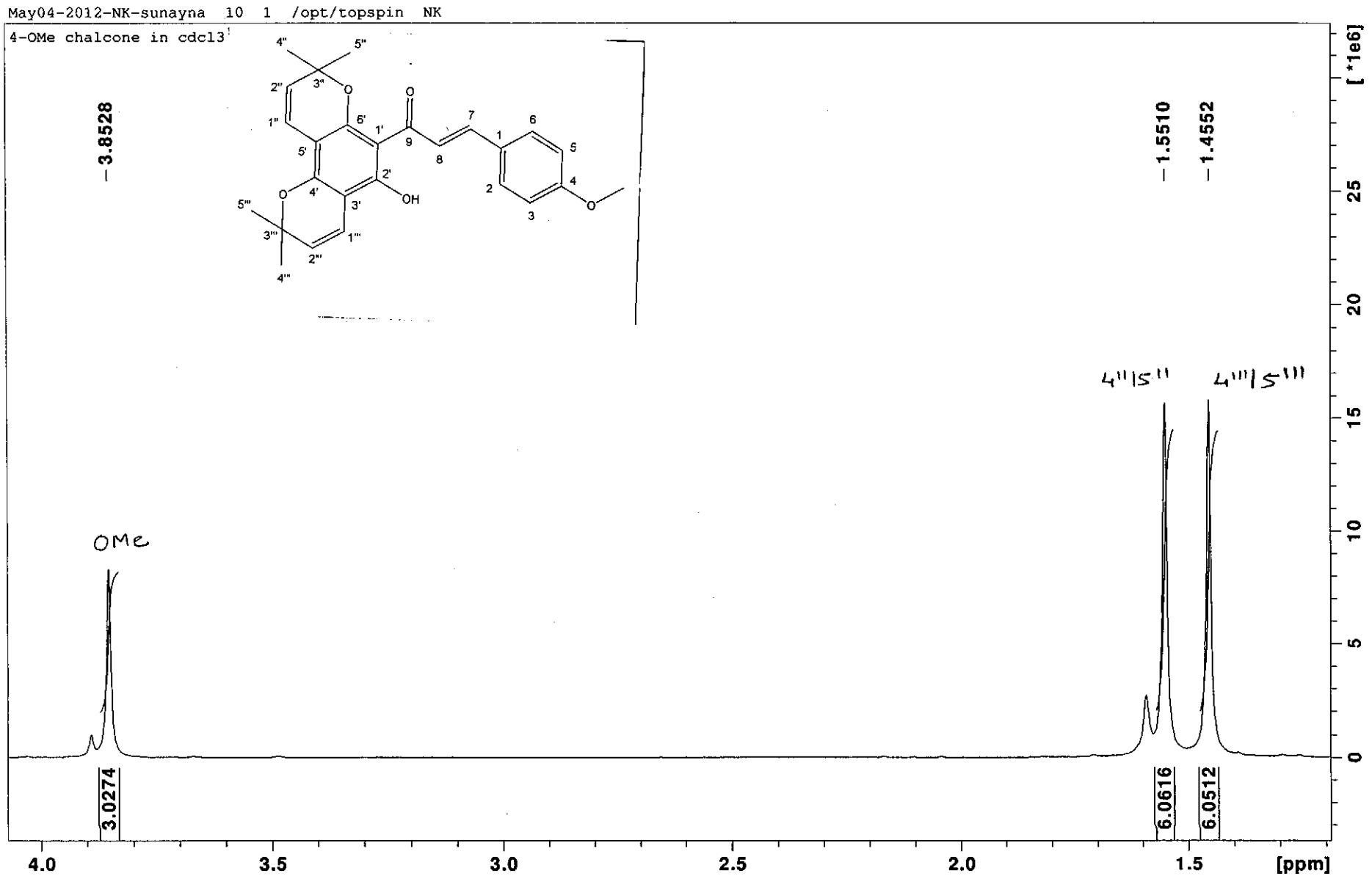
^{1}H NMR spectrum of 3g - 4-OMe pyranochromene chalcone (A-3g)



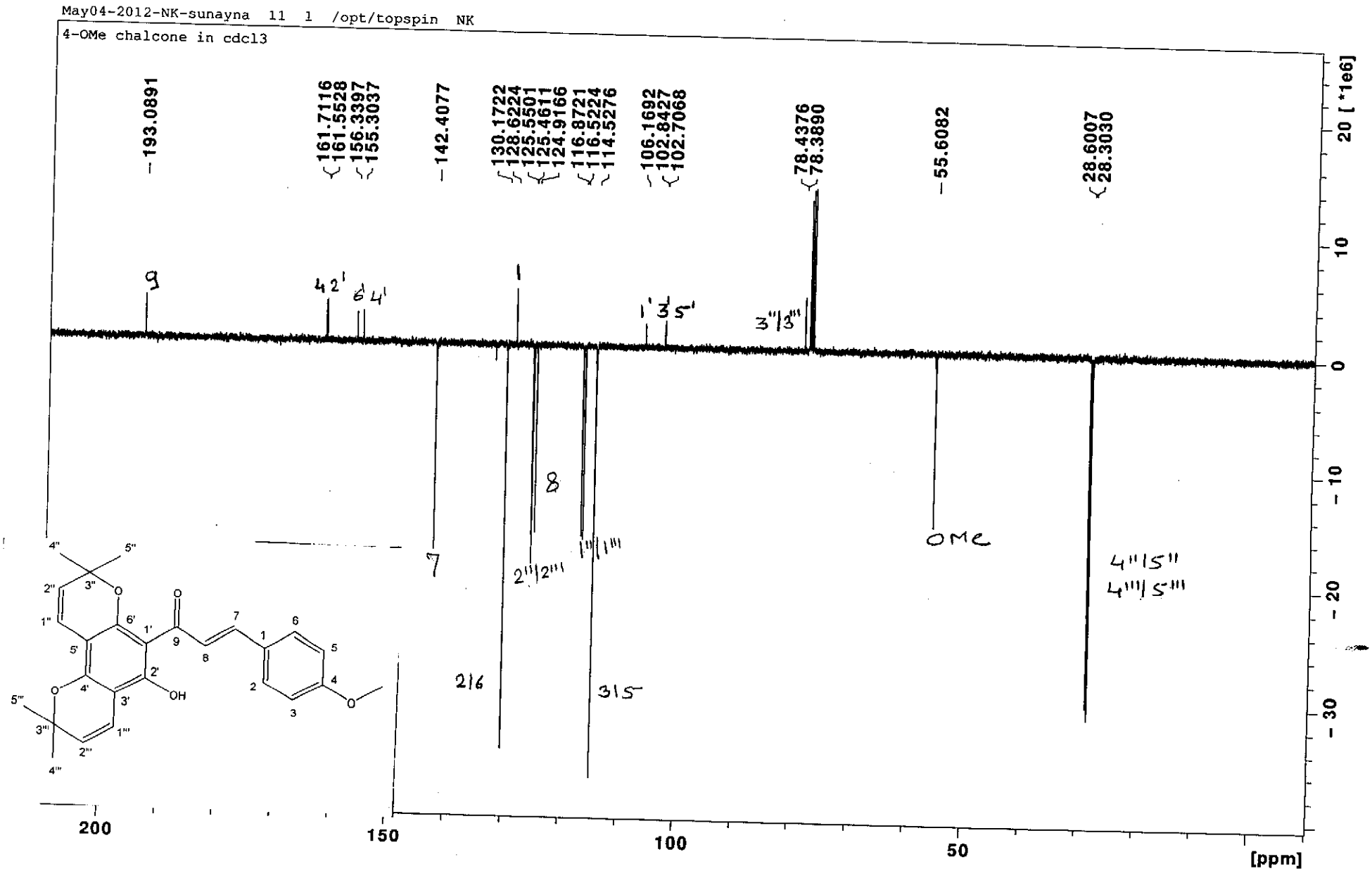
^{1}H NMR spectrum of 3g - 4-OMe pyranochromene chalcone (A-3g)



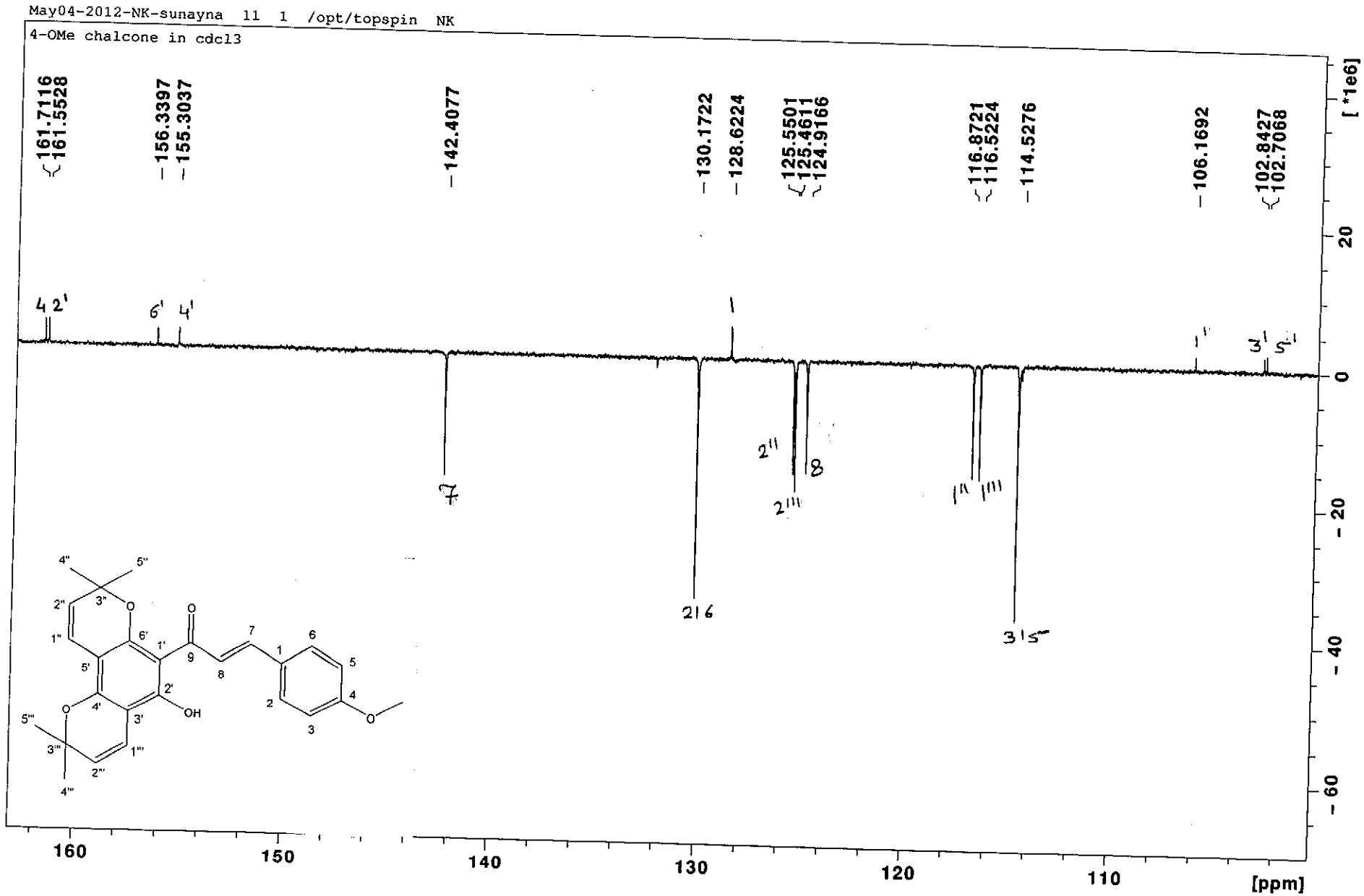
^{1}H NMR spectrum of 3g - 4-OMe pyranochromene chalcone (A-3g)



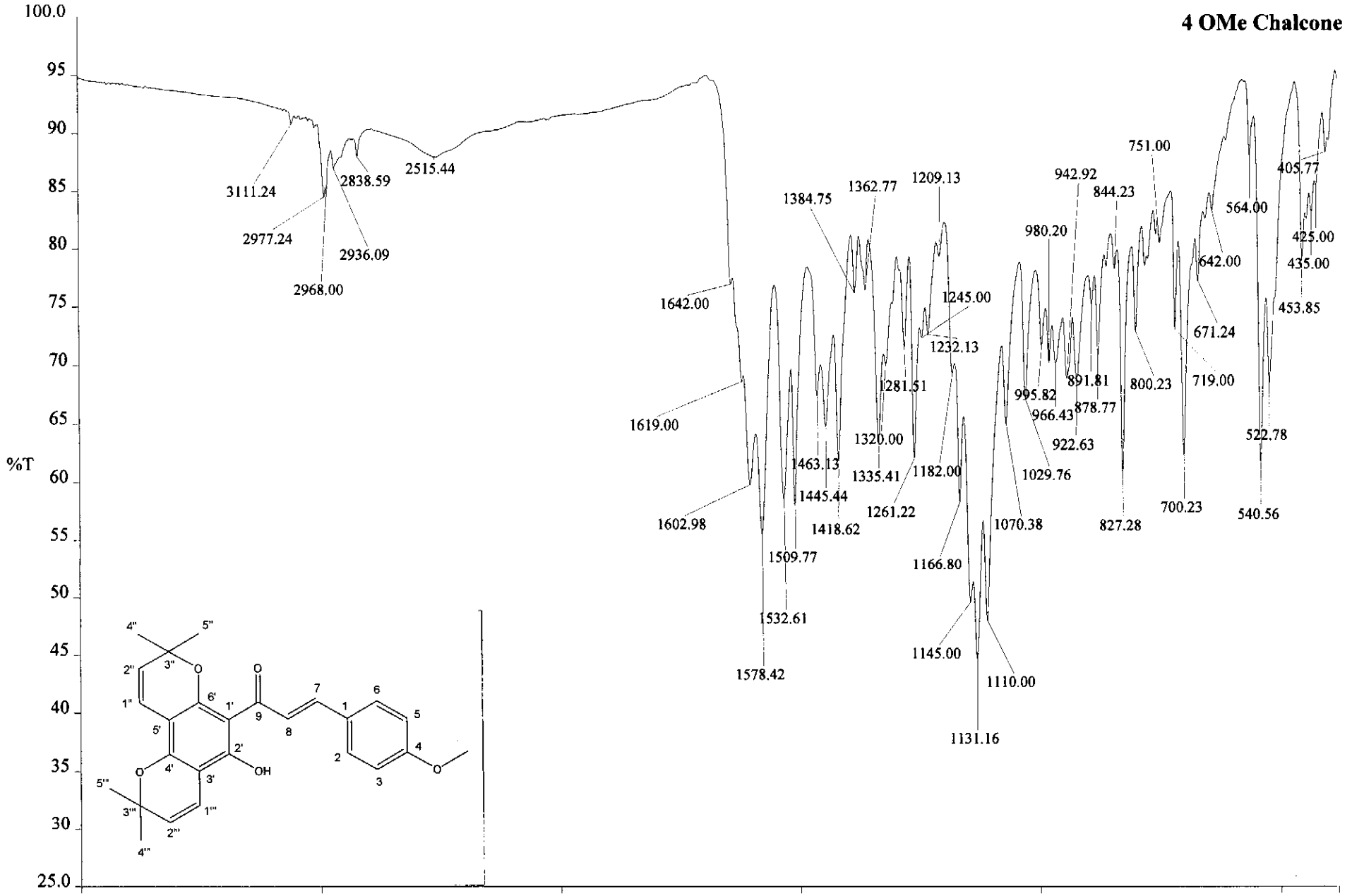
^{1}H NMR spectrum of 3g - 4-OMe pyranochromene chalcone (A-3g)



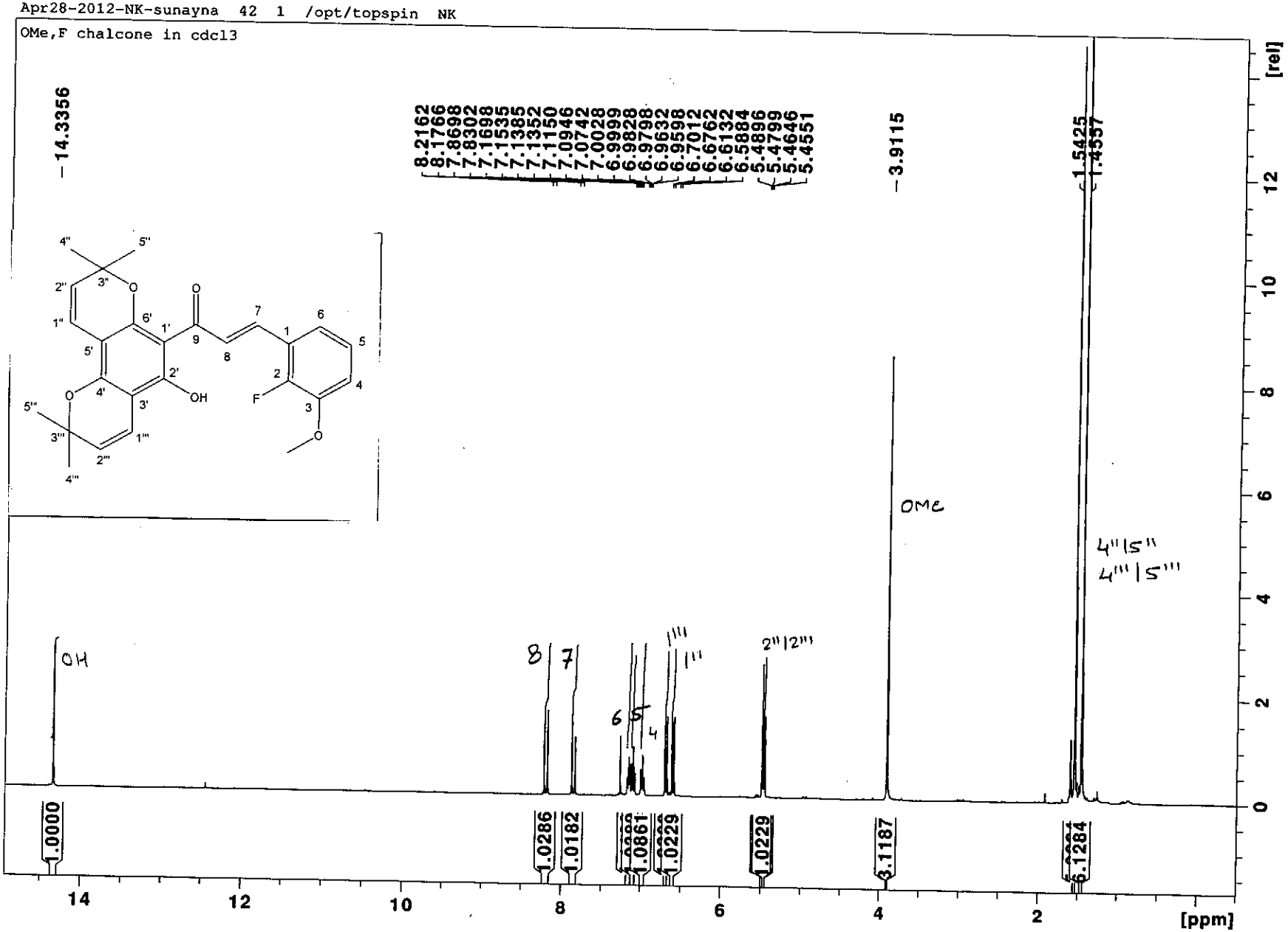
^{13}C NMR spectrum of 3g - 4-OMe pyranochromene chalcone (A-3g)



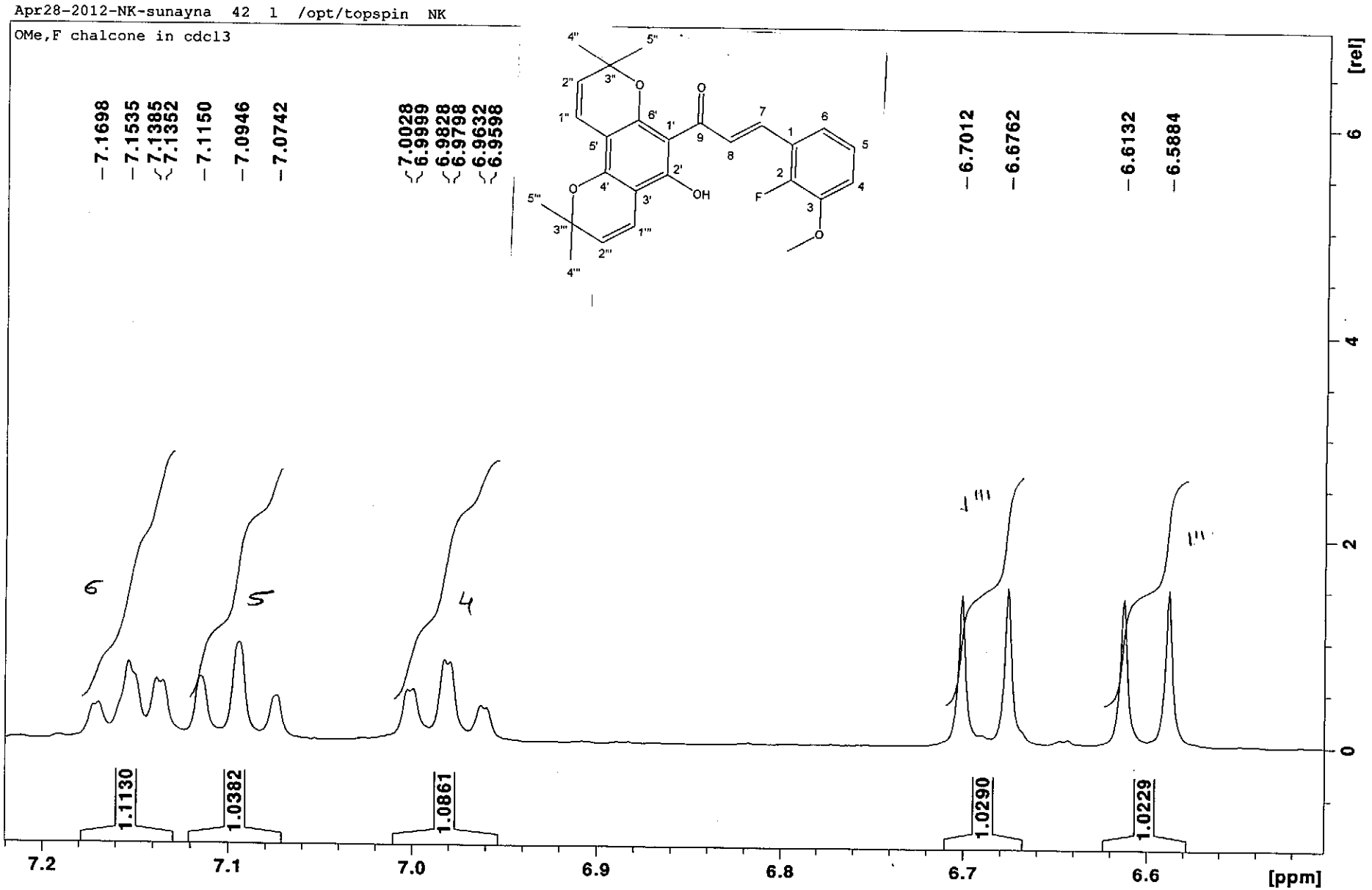
^{13}C NMR spectrum of 3g - 4-OMe pyranochromene chalcone (A-3g)



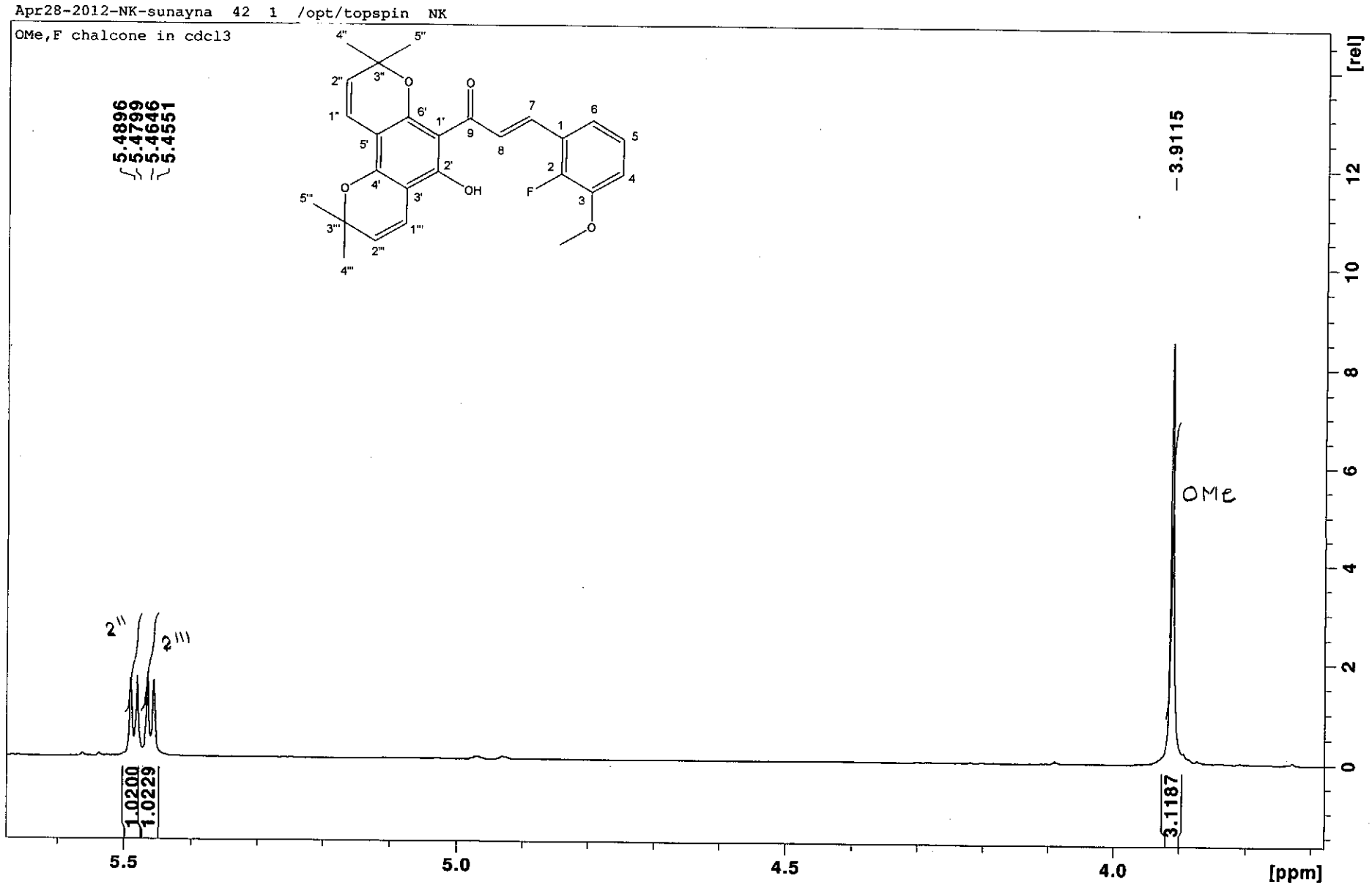
IR spectrum of 3g - 4-OMe pyranochromene chalcone (A-3g)



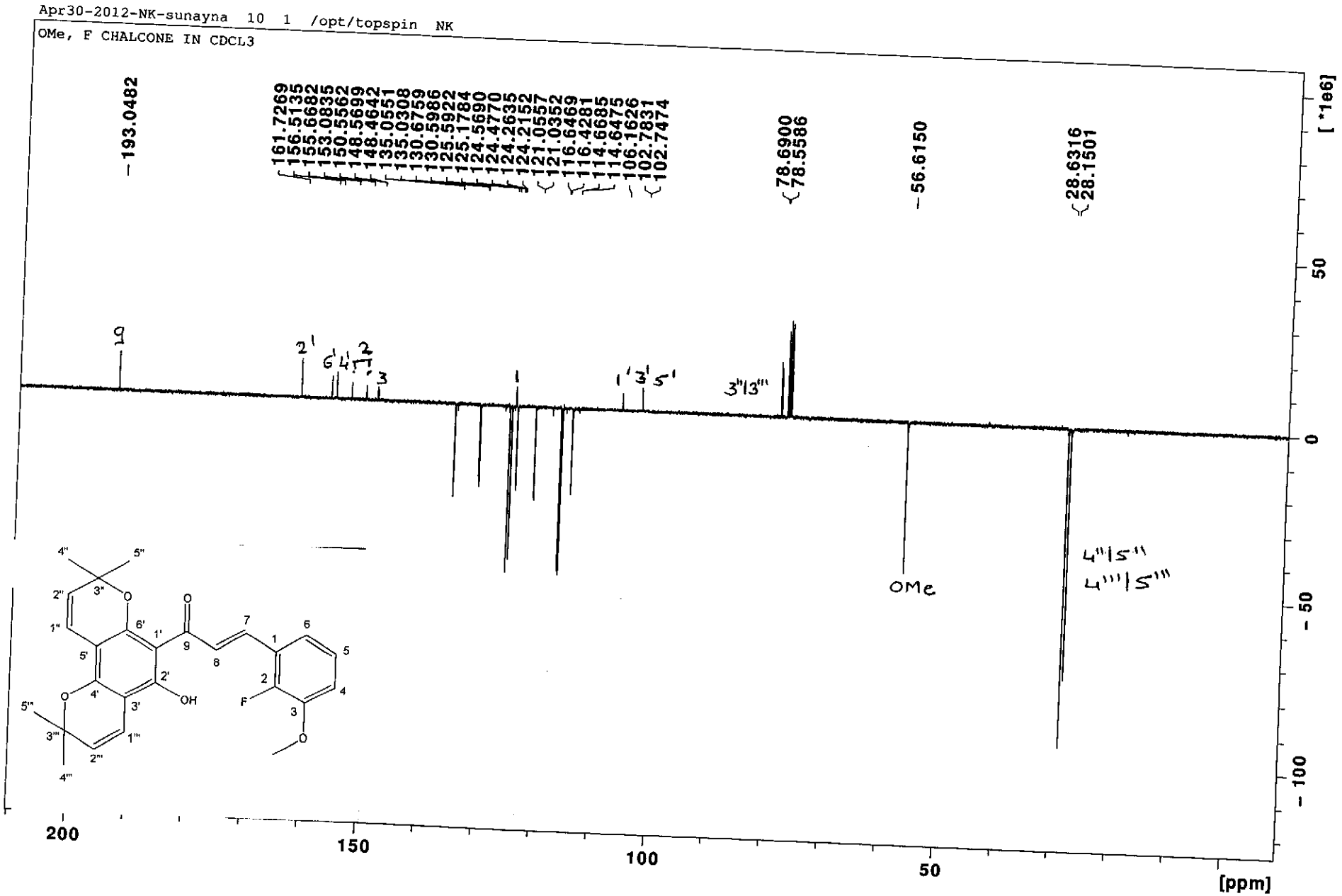
[1]H NMR spectrum of 3h- 2-F, 3-OMe pyranochromene chalcone (A-3h)



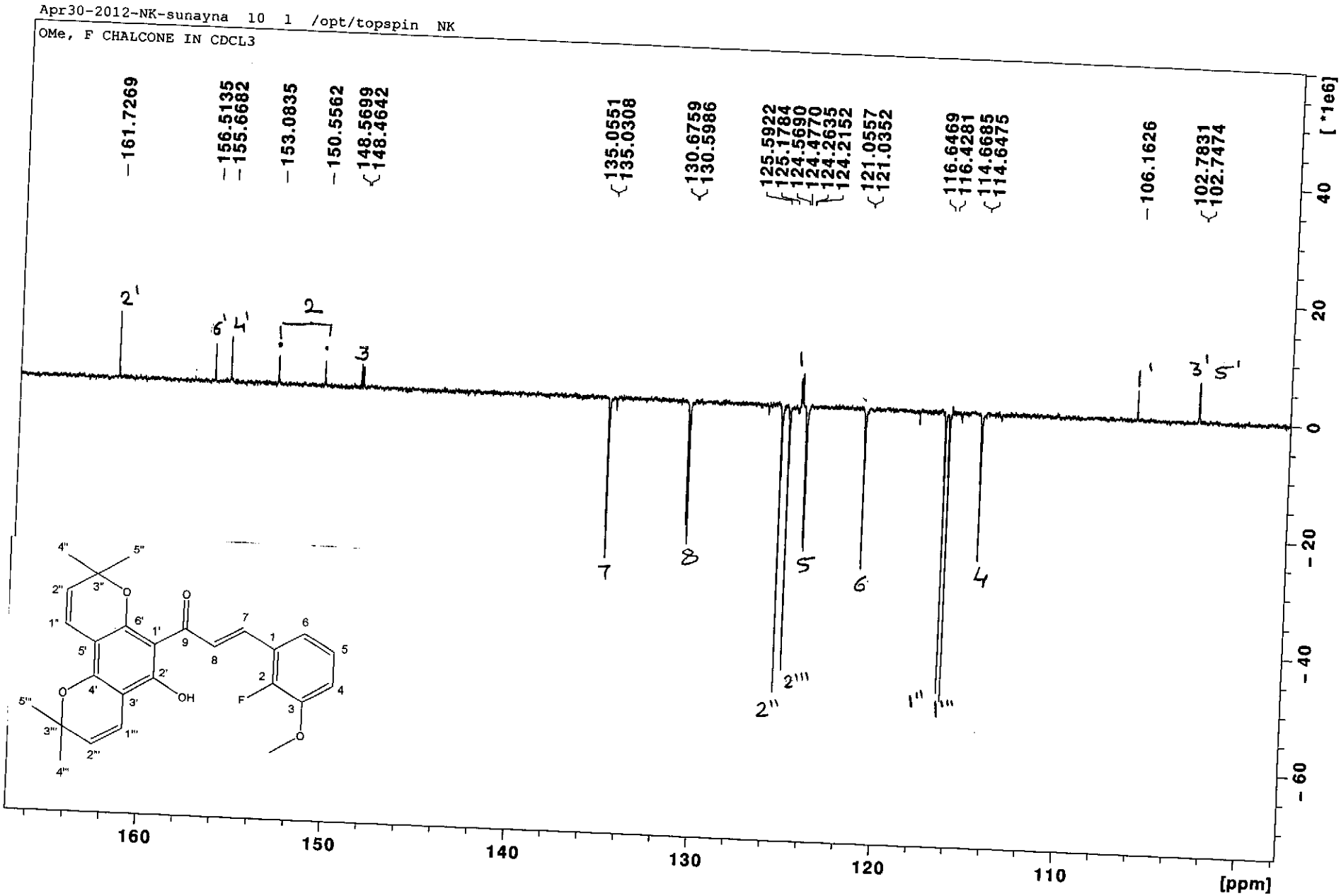
^{1}H NMR spectrum of 3h- 2-F, 3-OMe pyranochromene chalcone (A-3h)



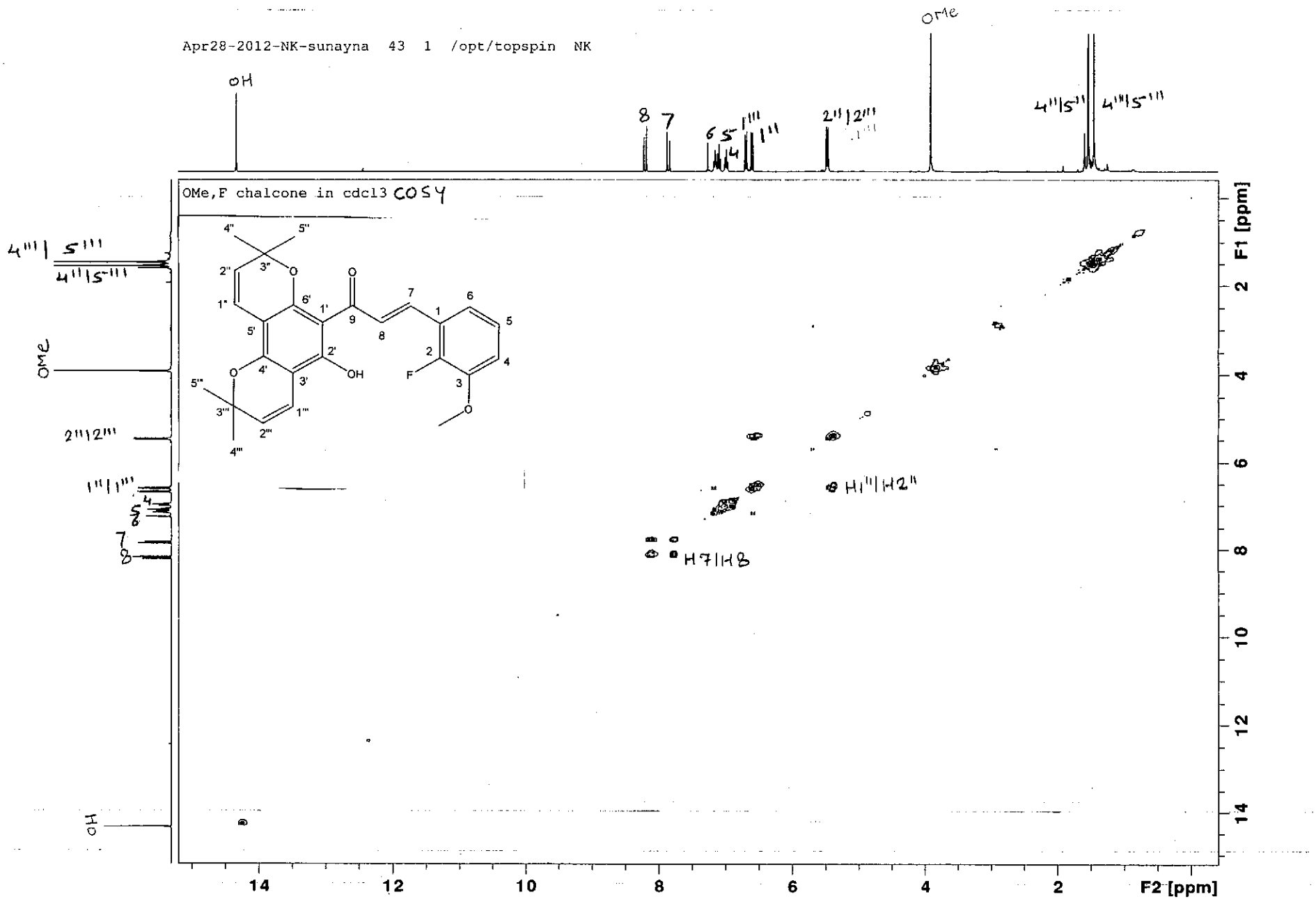
^{1}H NMR spectrum of 3h- 2-F, 3-OMe pyranochromene chalcone (A-3h)



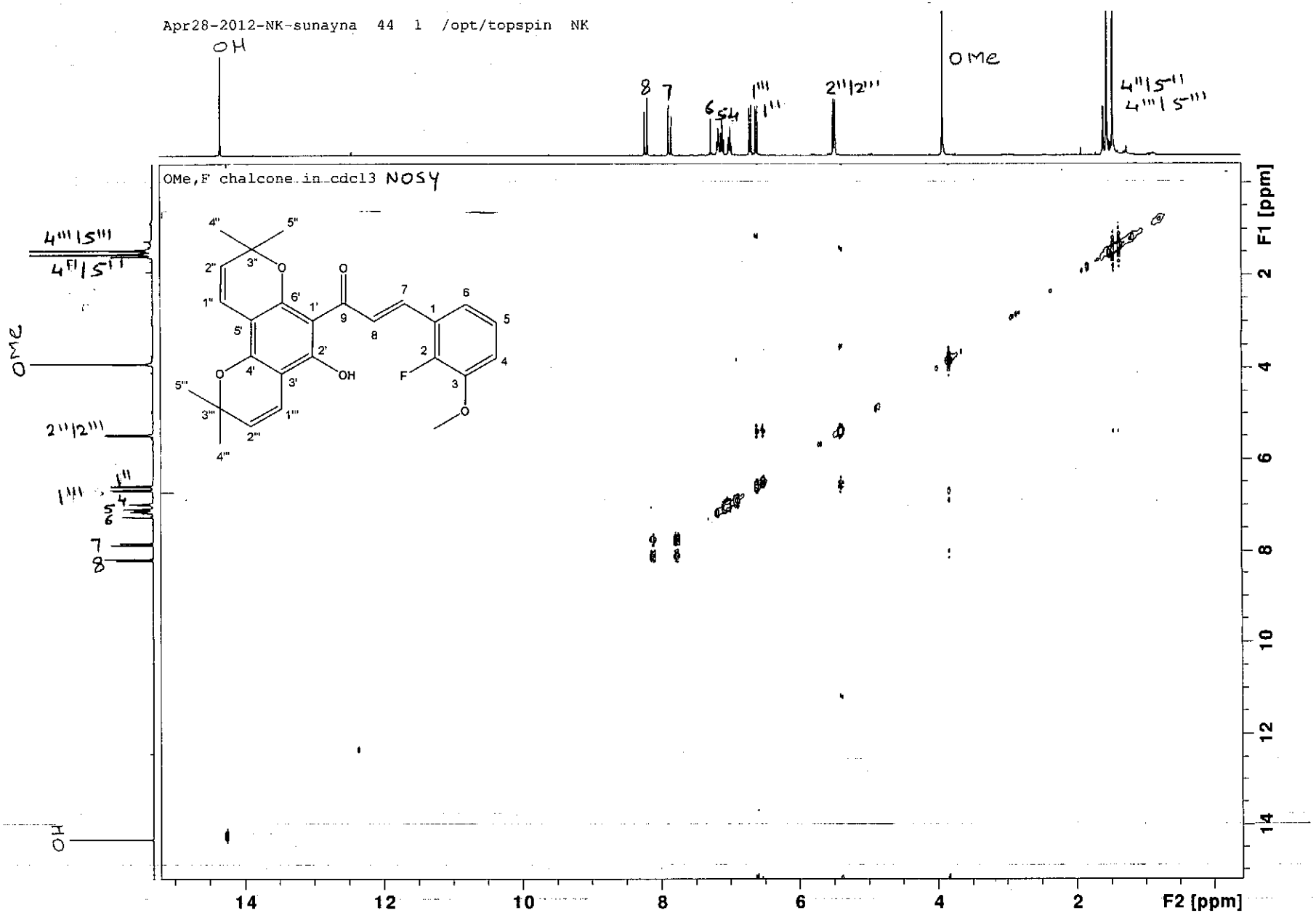
^{13}C NMR spectrum of 3h- 2-F, 3-OMe pyranochromene chalcone (A-3h)



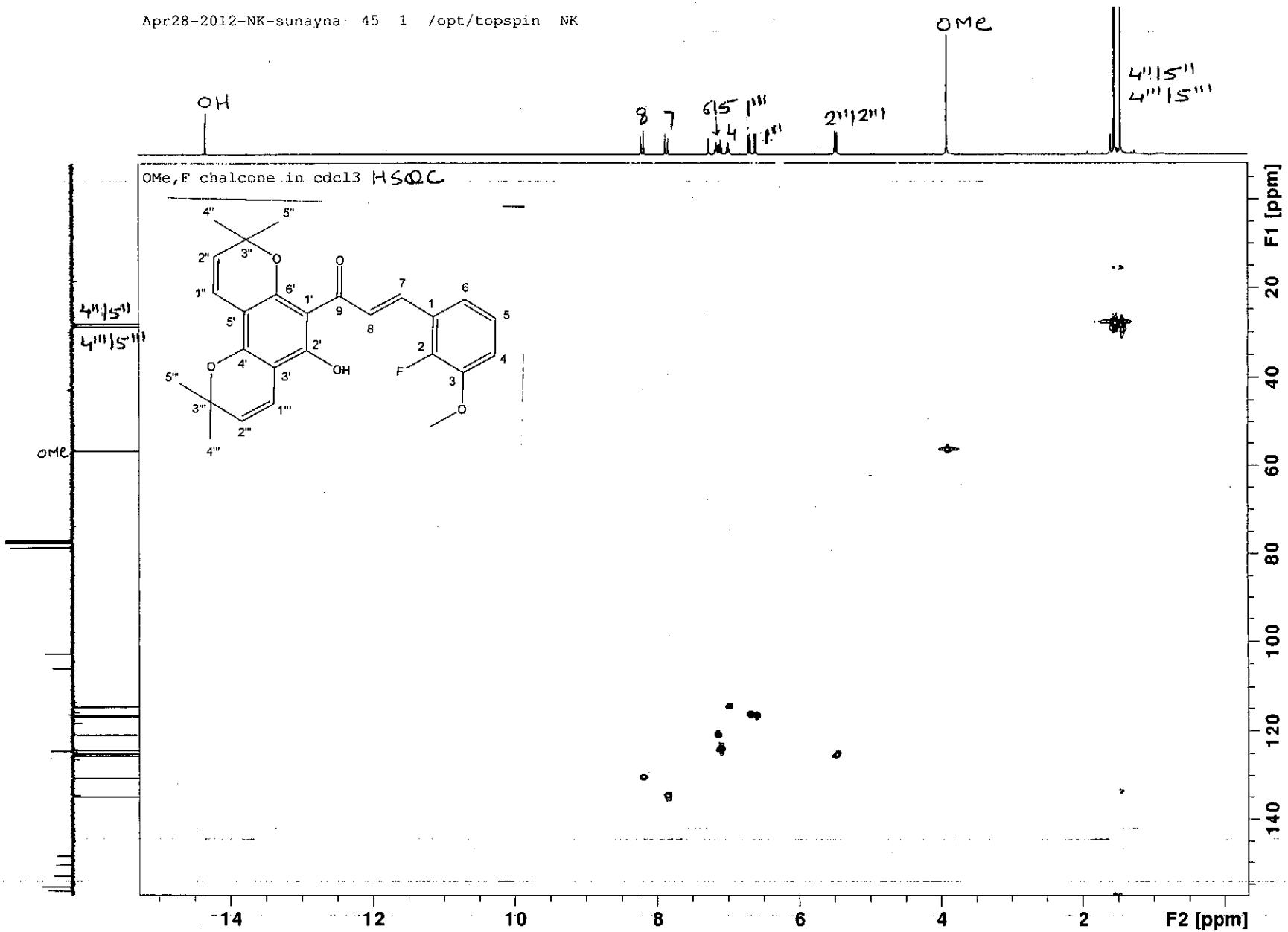
^{13}C NMR spectrum of 3h- 2-F, 3-OMe pyranochromene chalcone (A-3h)



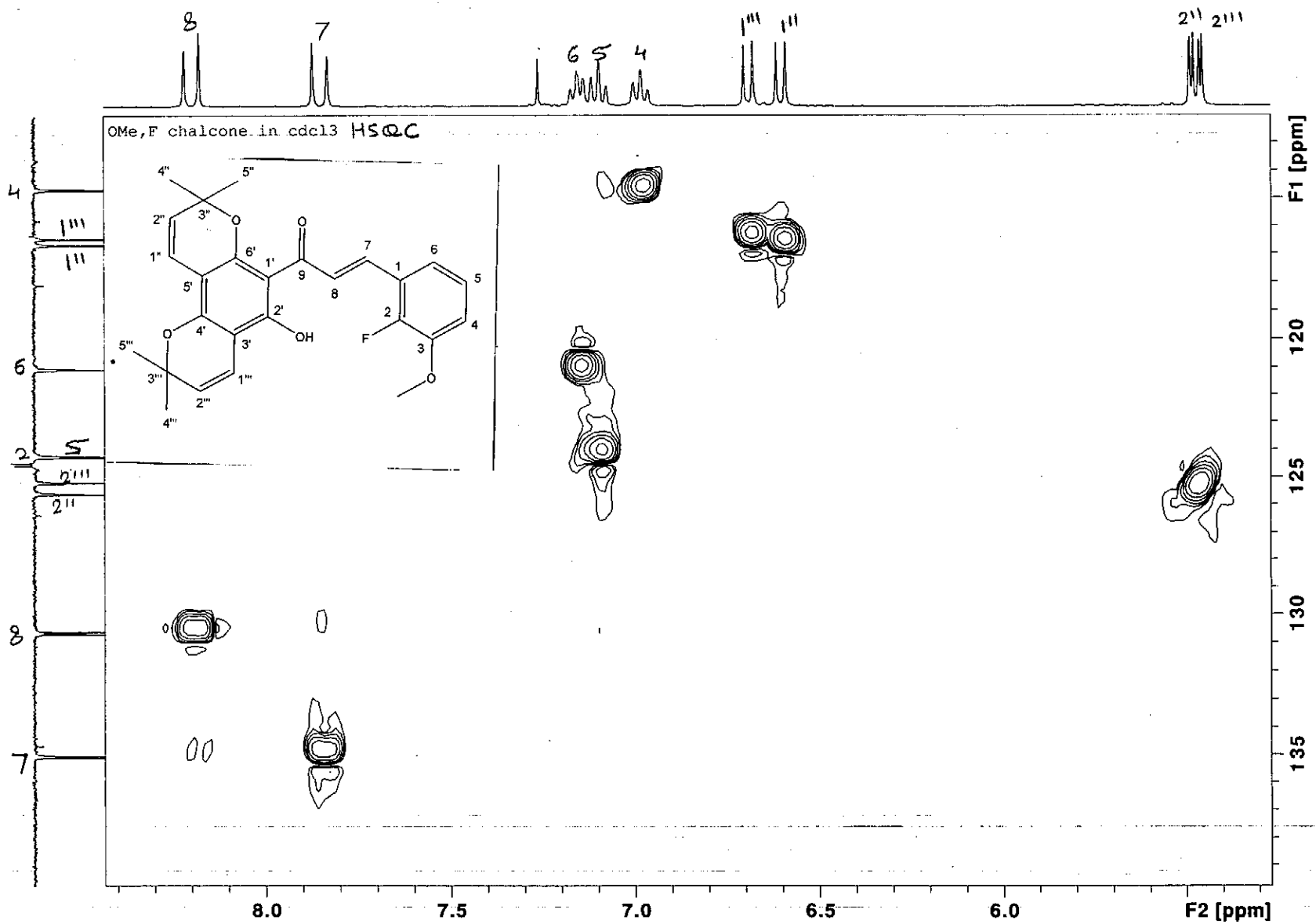
COSY NMR spectrum of 3h- 2-F, 3-OMe pyranochromene chalcone (A-3h)



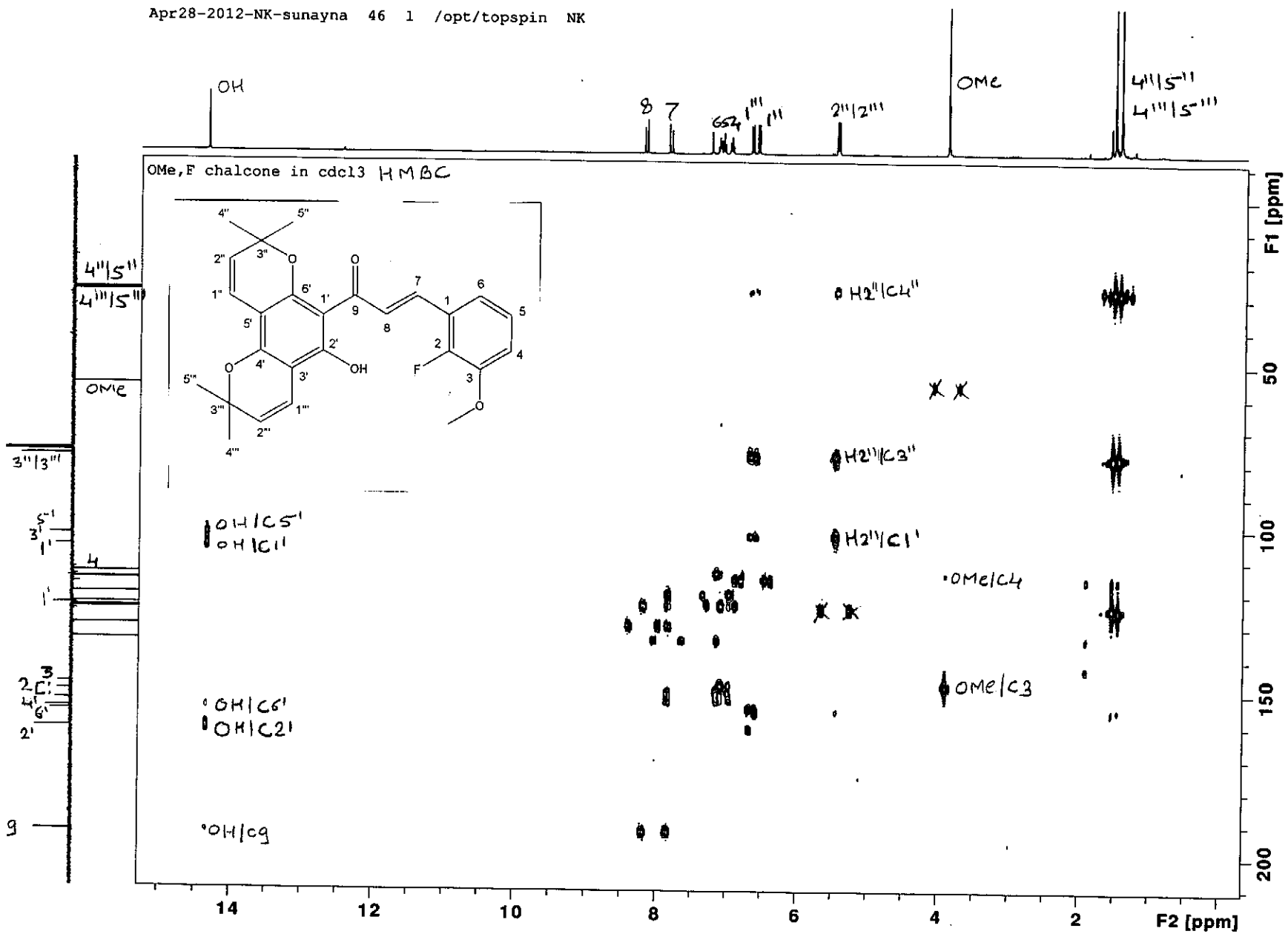
NOSY NMR spectrum of 3h- 2-F, 3-OMe pyranochromene chalcone (A-3h)



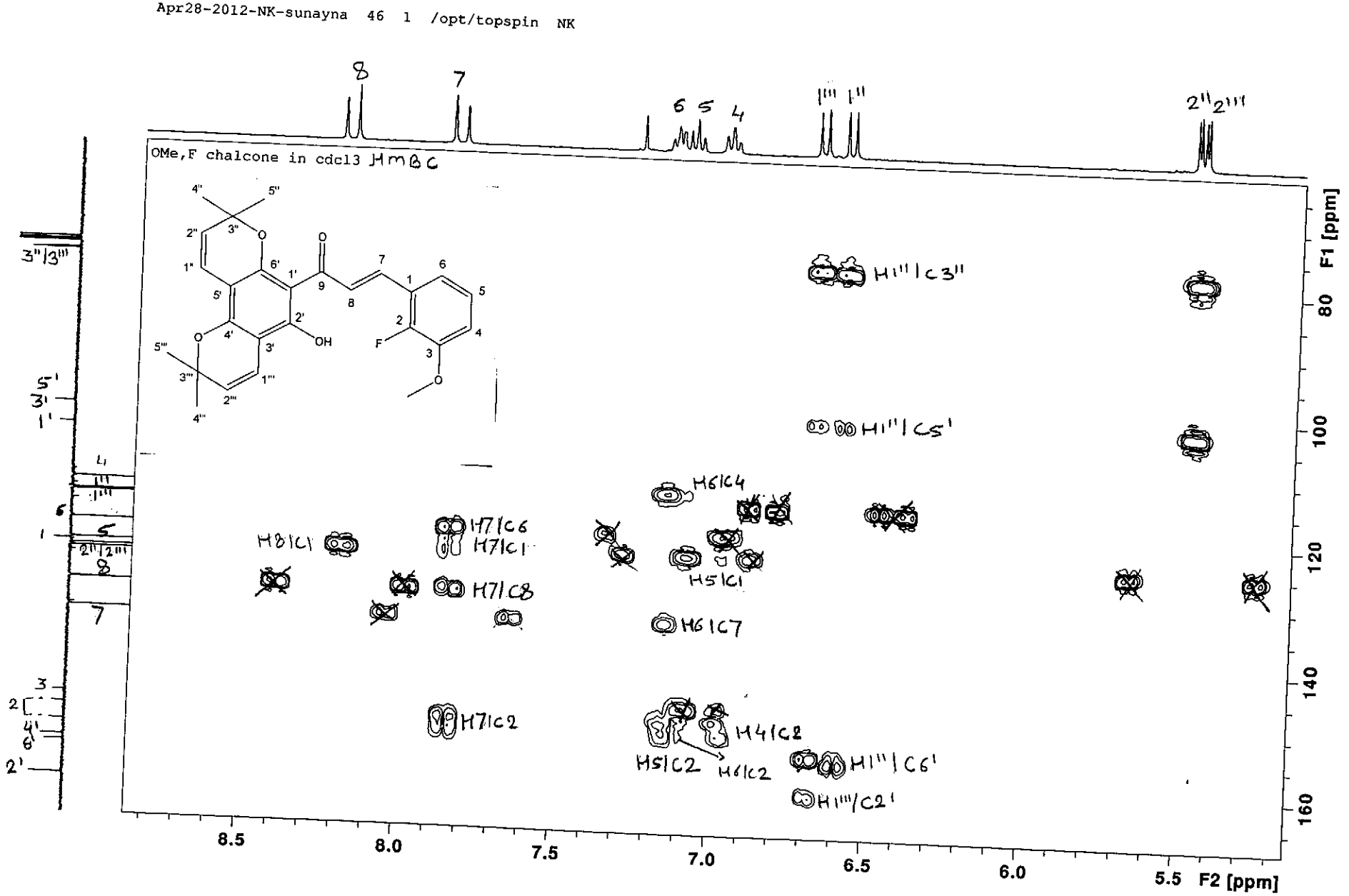
HSQC NMR spectrum of 3h- 2-F, 3-OMe pyranochromene chalcone (A-3h)



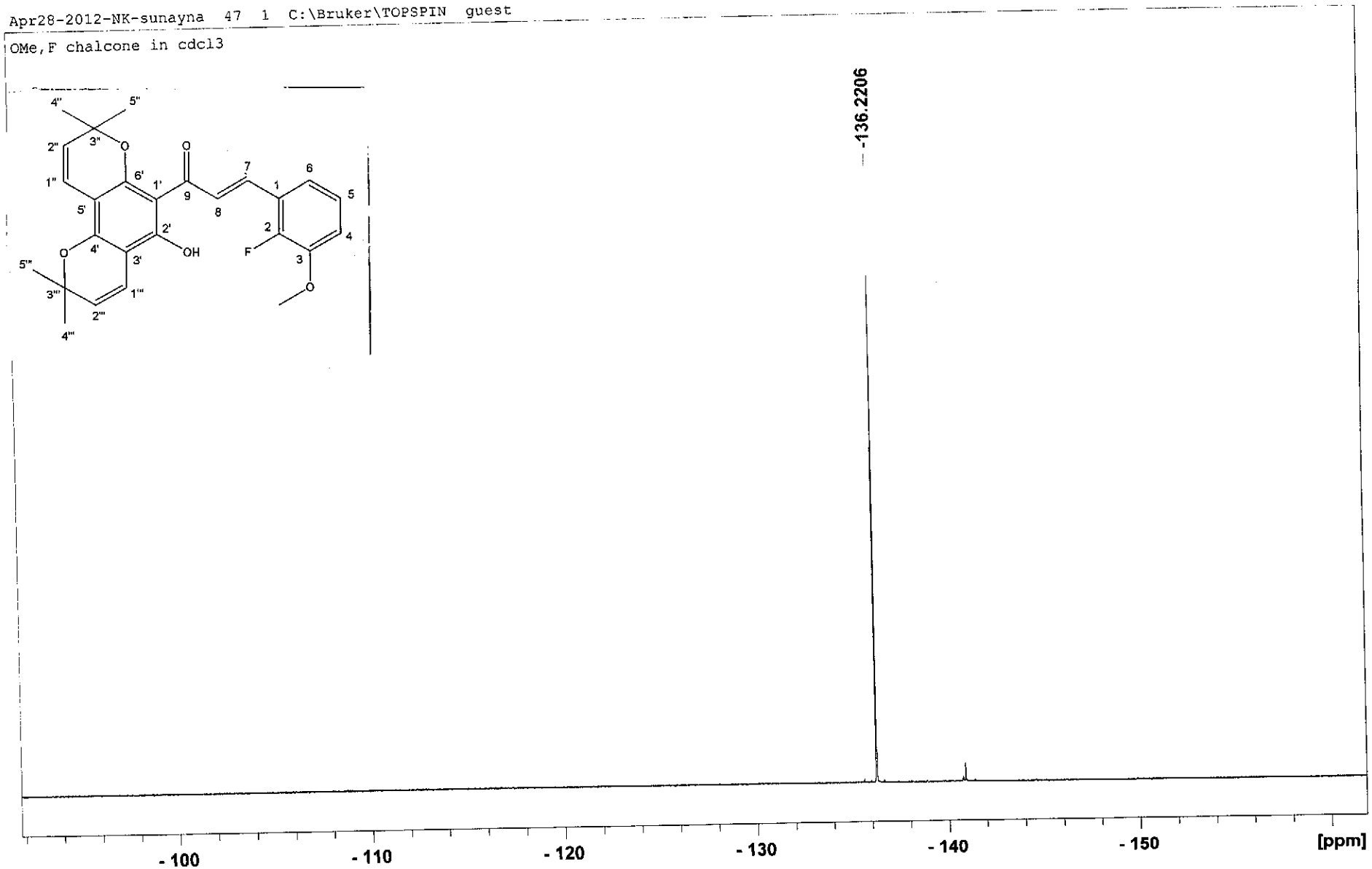
HSQC NMR spectrum of 3h- 2-F, 3-OMe pyranochromene chalcone (A-3h)



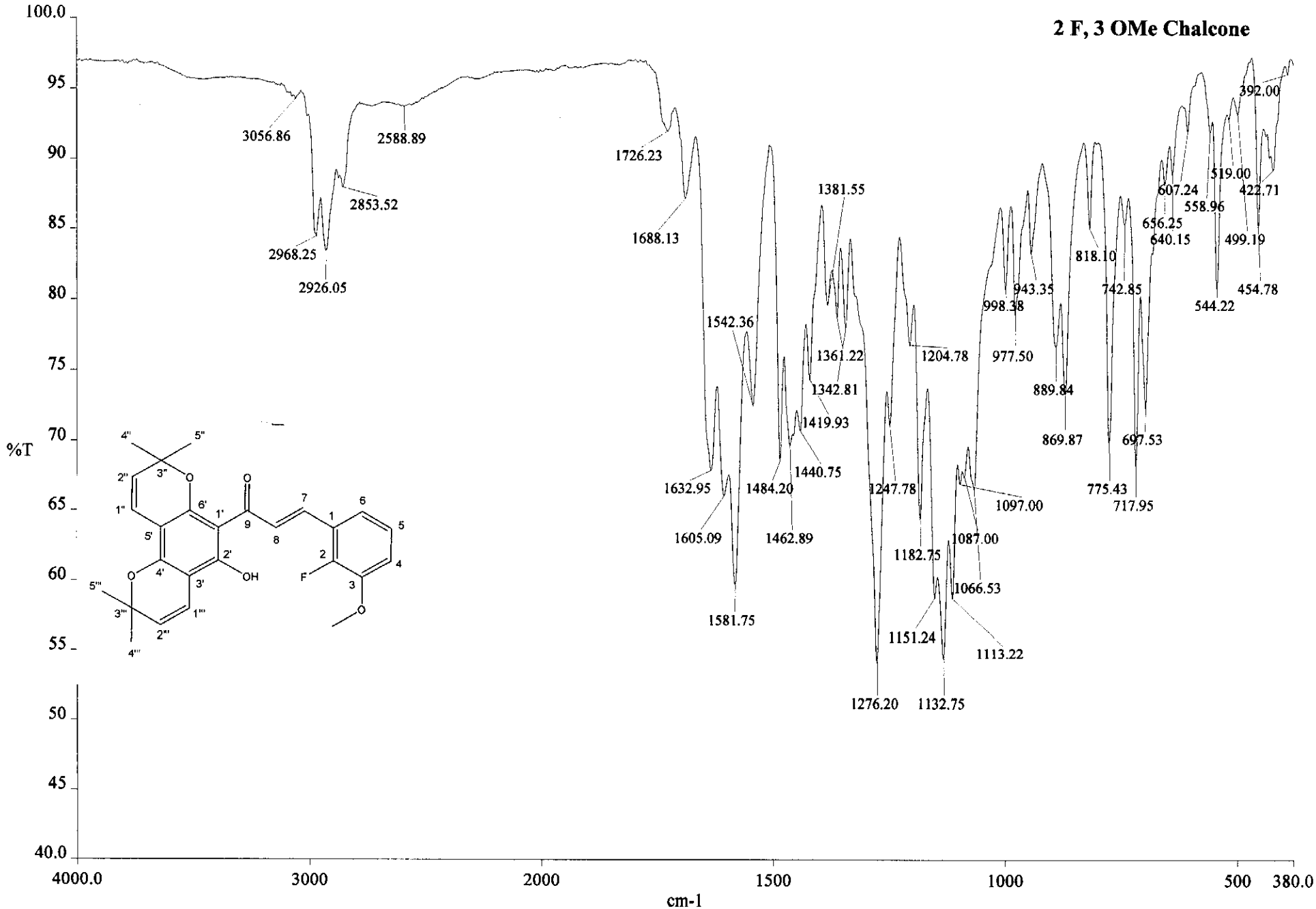
HMBC NMR spectrum of 3h- 2-F, 3-OMe pyranochromene chalcone (A-3h)



HMBC NMR spectrum of 3h- 2-F, 3-OMe pyranochromene chalcone (A-3h)

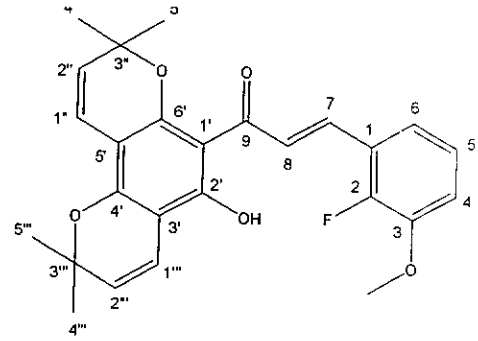


^{19}F NMR spectrum of 3h- 2-F, 3-OMe pyranochromene chalcone (A-3h)



IR spectrum of 3h- 2-F, 3-OMe pyranochromene chalcone (A-3h)

Elemental Composition Report



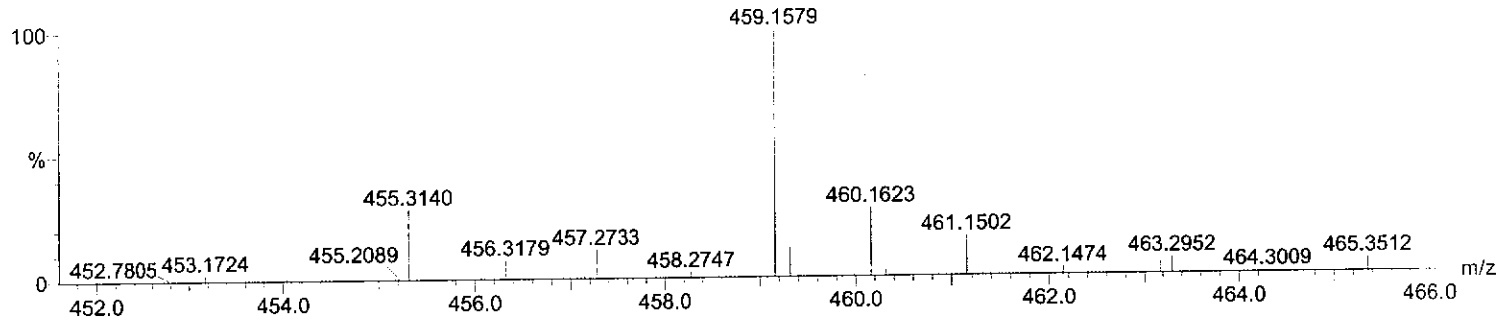
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
6 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-30 H: 25-30 O: 0-5 F: 0-1 Na: 0-1

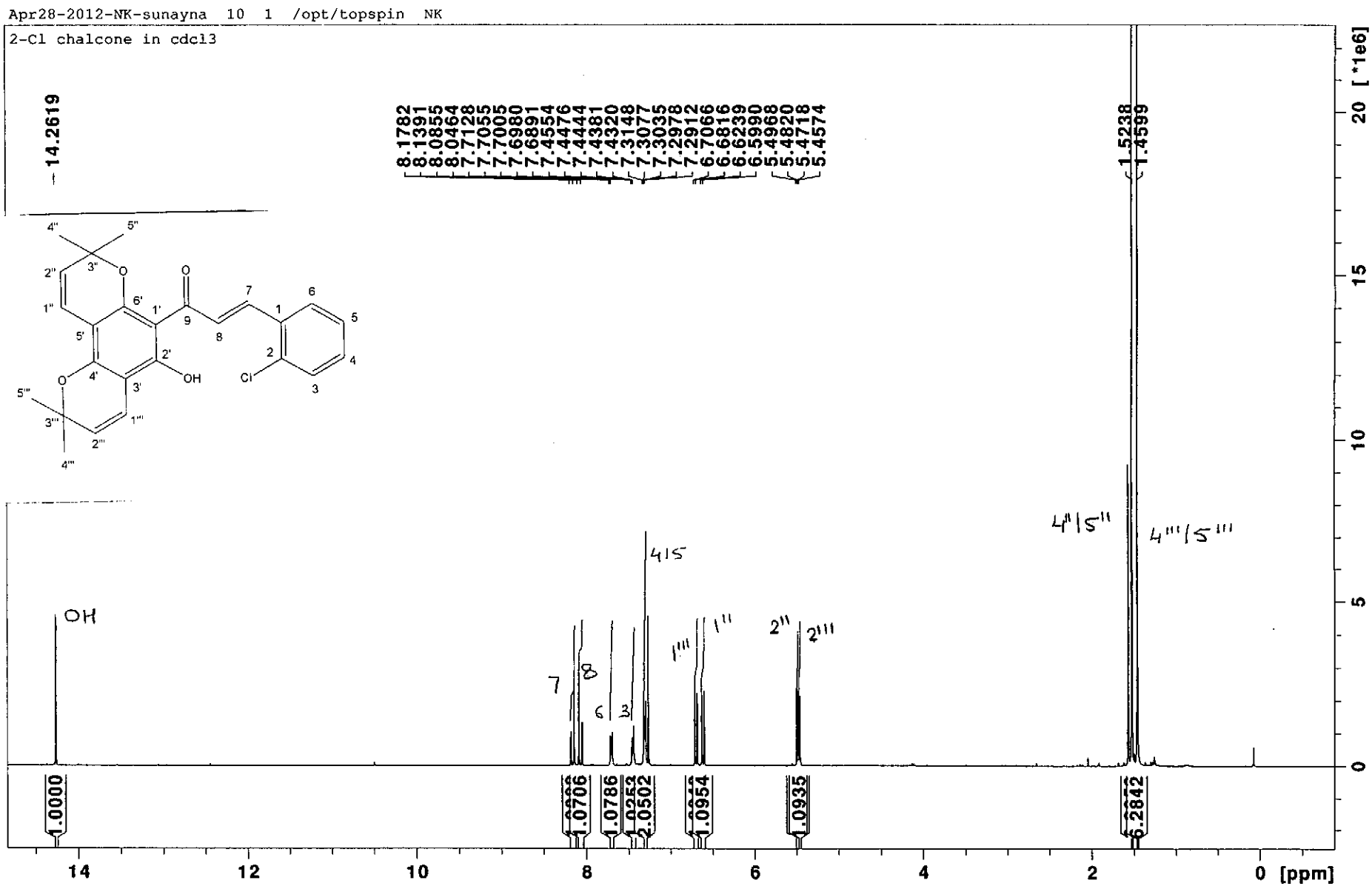
Dr. Nizam
7 7 (0.102) Cm (1:31)

TOF MS ES+
7.60e+004

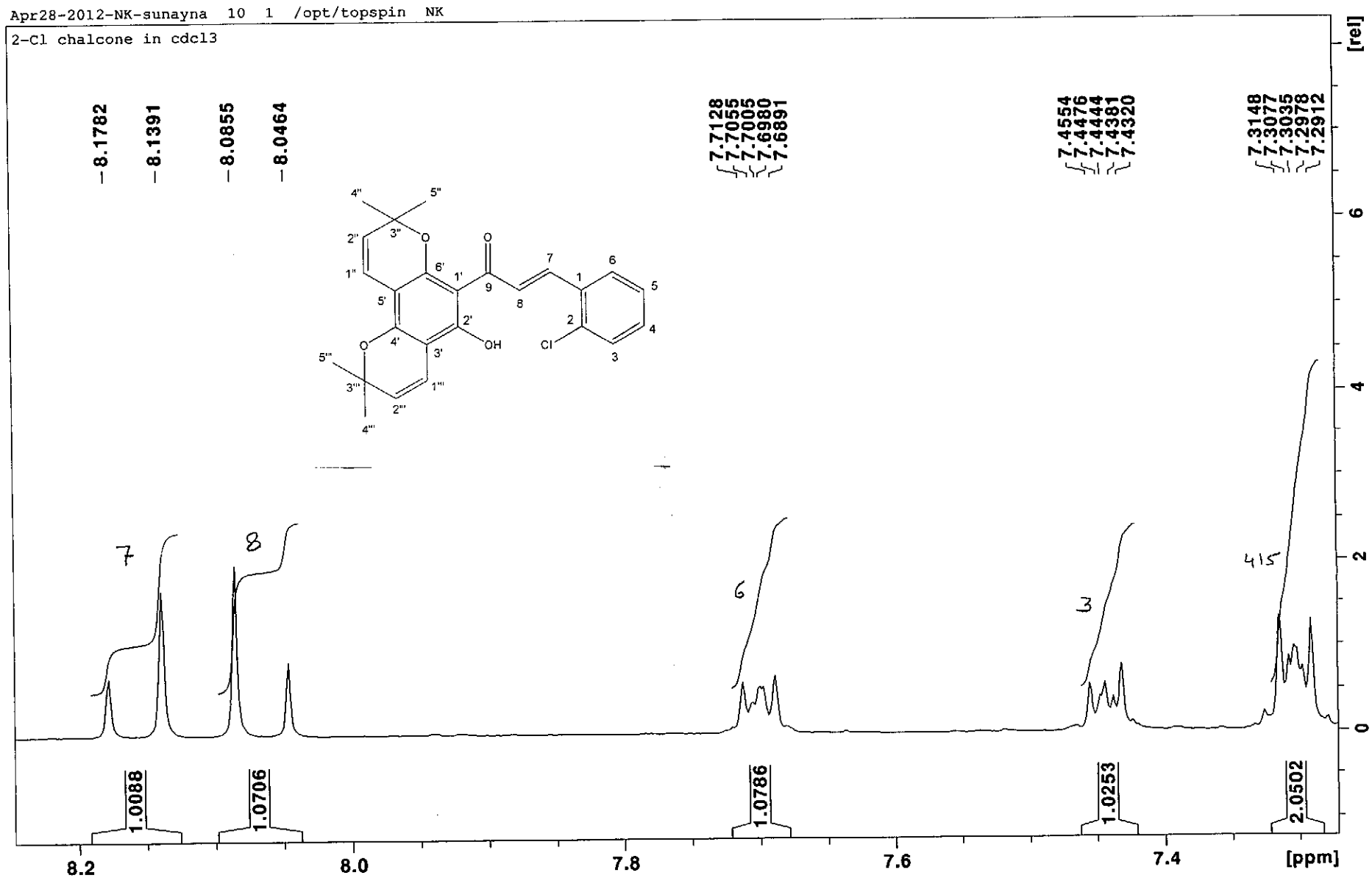


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
459.1579	459.1584	-0.5	-1.1	13.5	483.7	0.0	C26 H25 O5 F Na

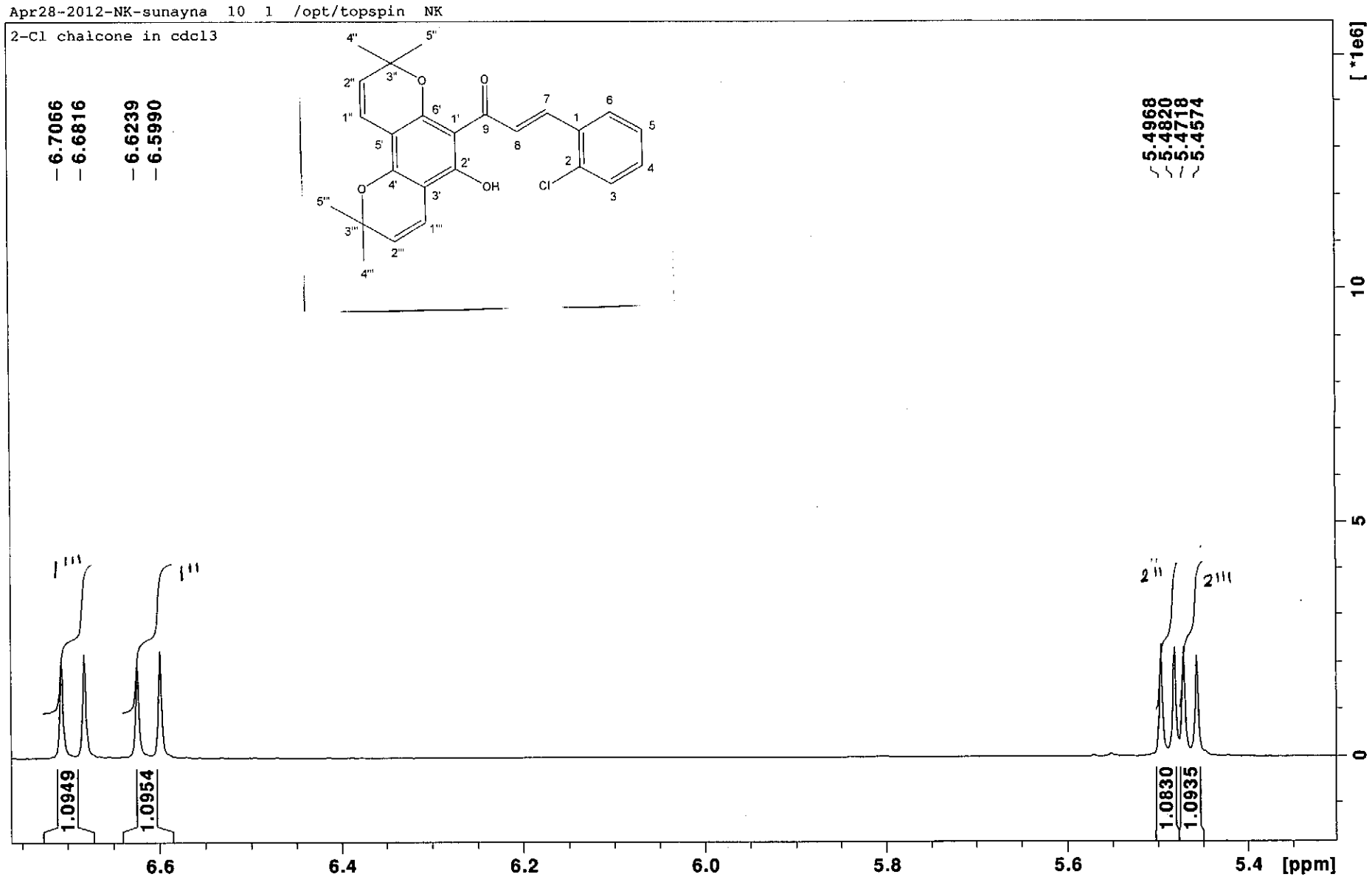
HRMS of 3h – 2-F, 3-OMe pyranochromene chalcone (A-3h)



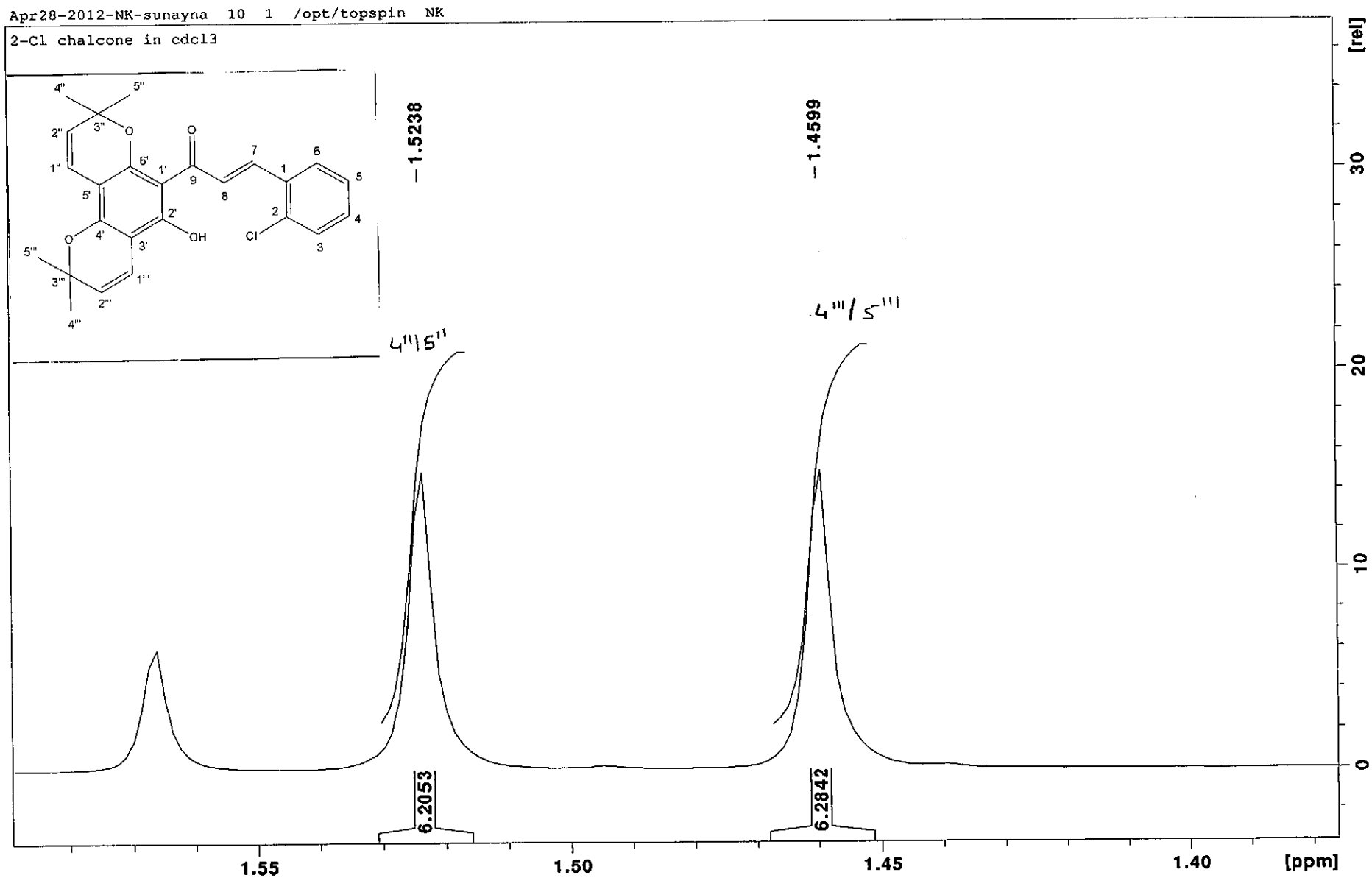
1H NMR spectrum of 2-chloropyranochromene chalcone (A-3i)



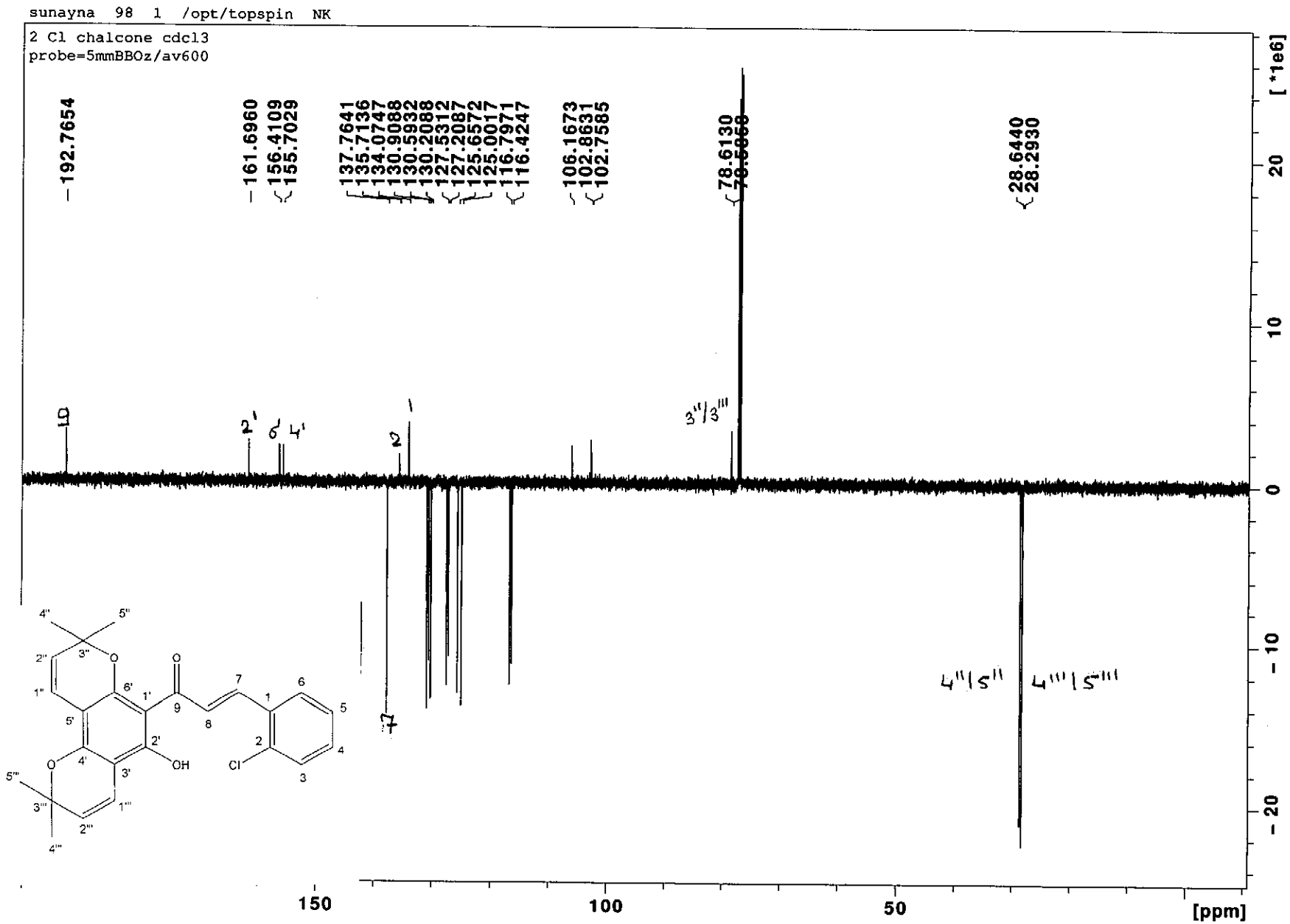
Expanded 1H NMR spectrum of 2-chloropyranochromene chalcone (A-3i)



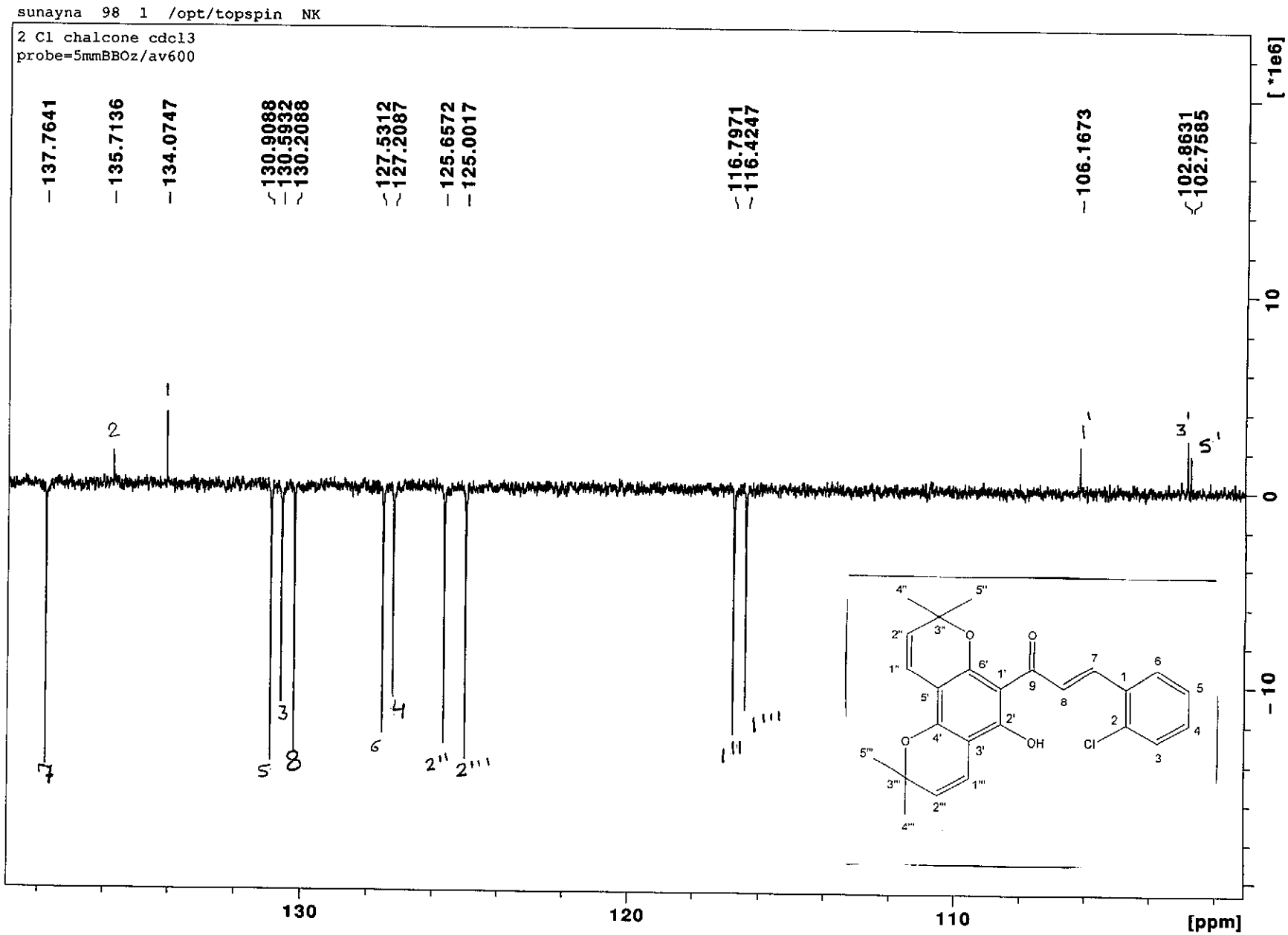
Expanded 1H NMR spectrum of 2-chloropyranochromene chalcone (A-3i)



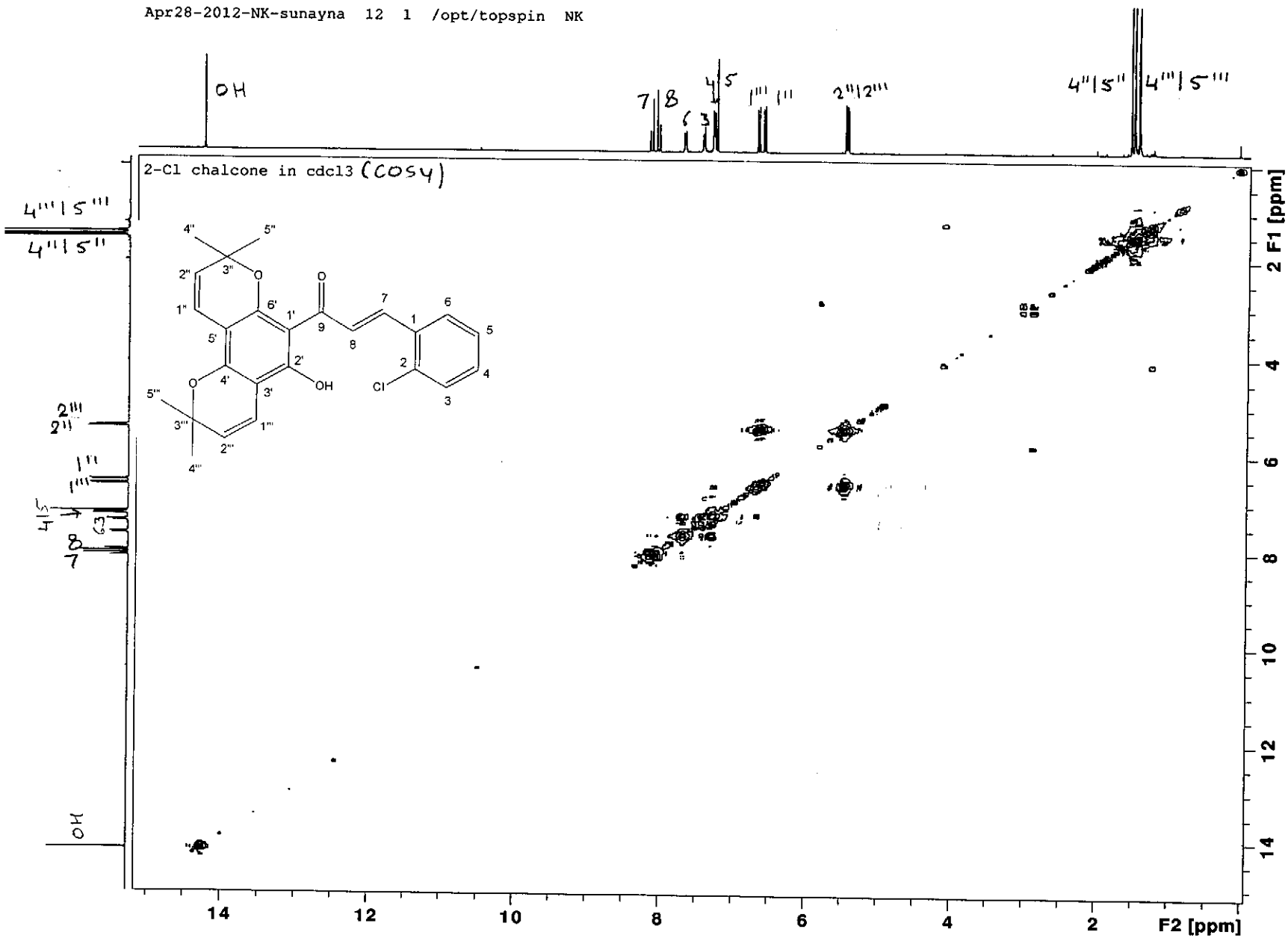
Expanded 1H NMR spectrum of 2-chloropyranochromene chalcone (A-3i)



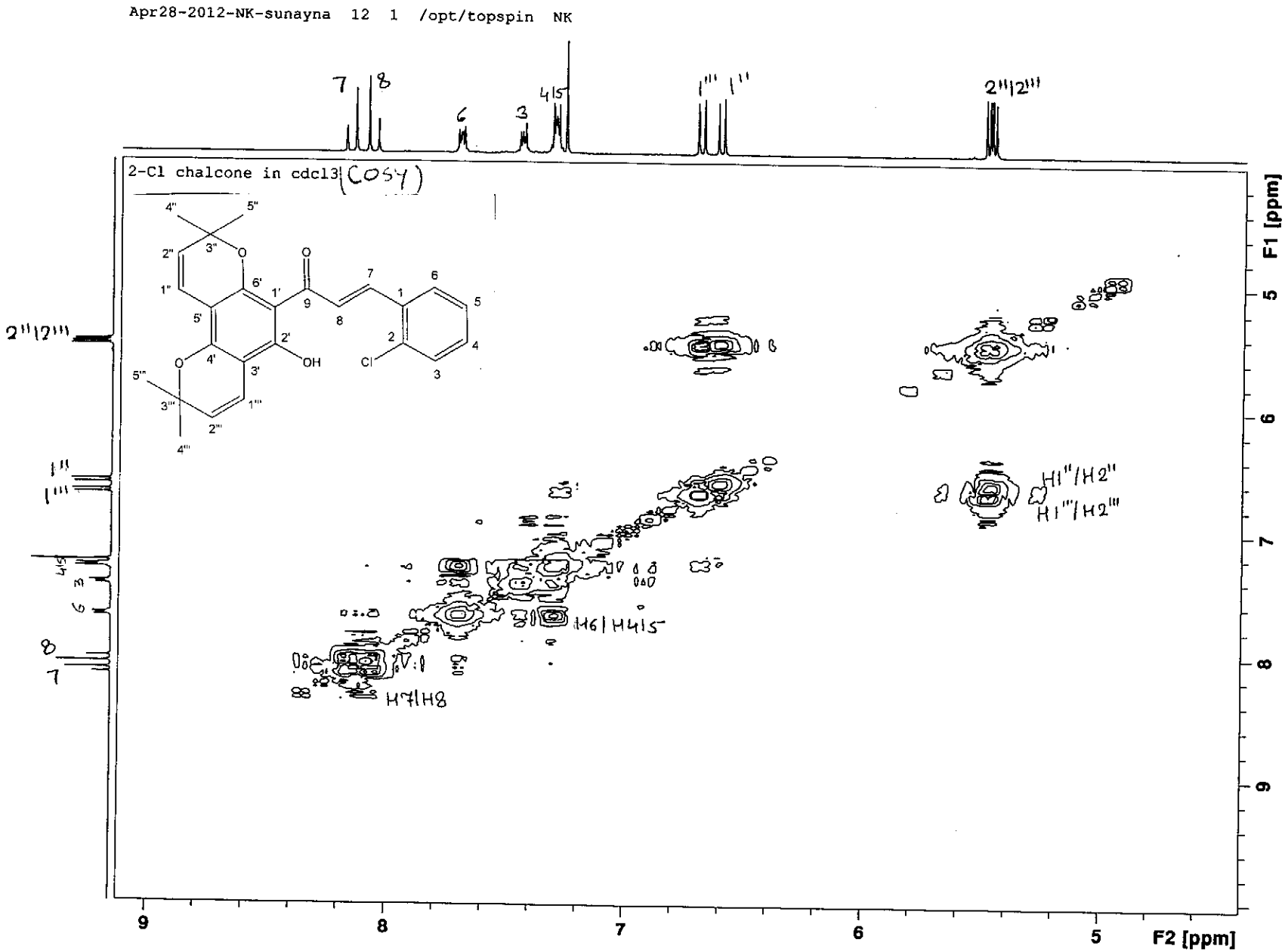
13C NMR spectrum of 2-chloropyranochromene chalcone (A-3i)



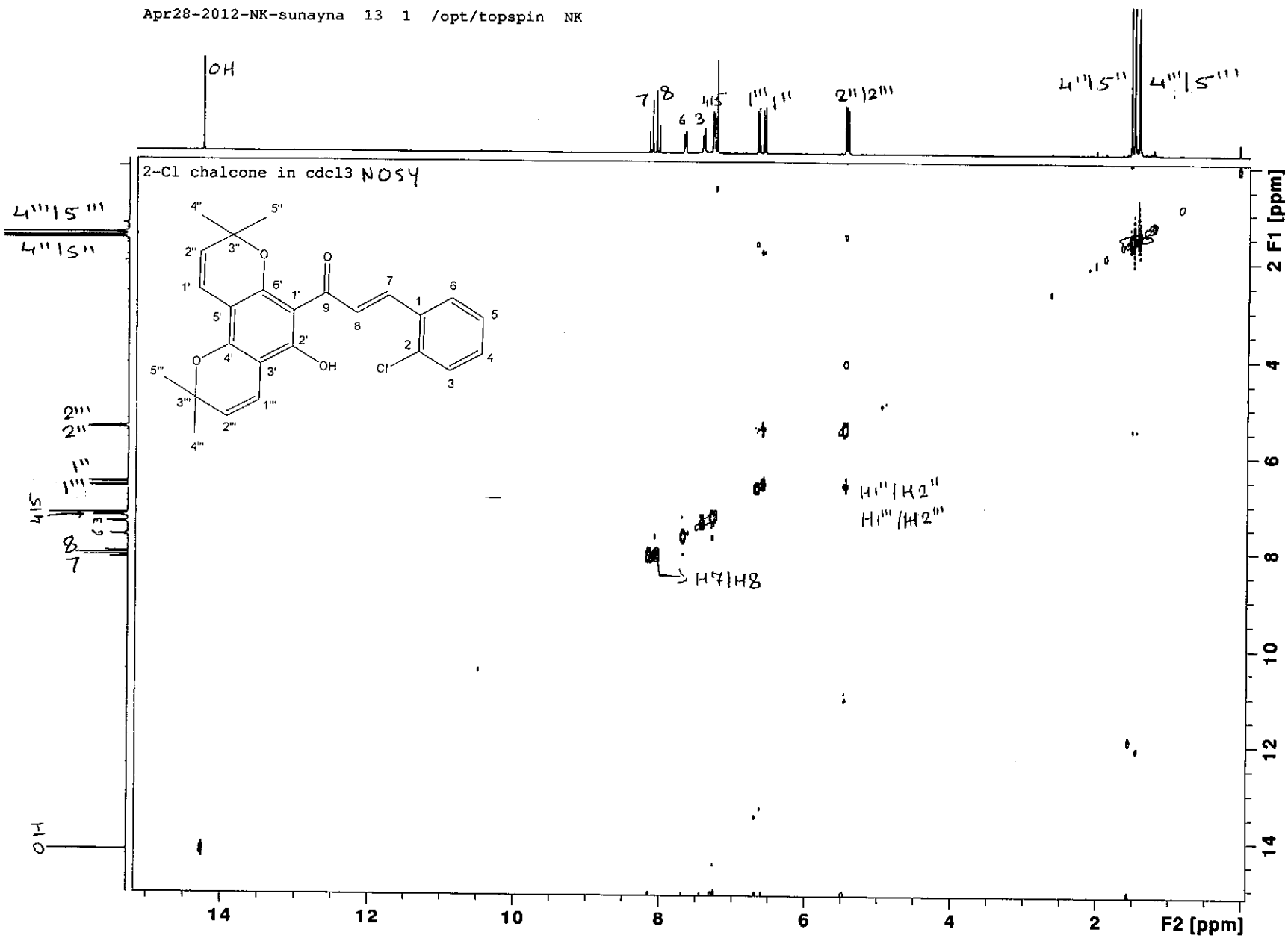
Expanded 13C NMR spectrum of 2-chloropyranochromene chalcone (A-3i)



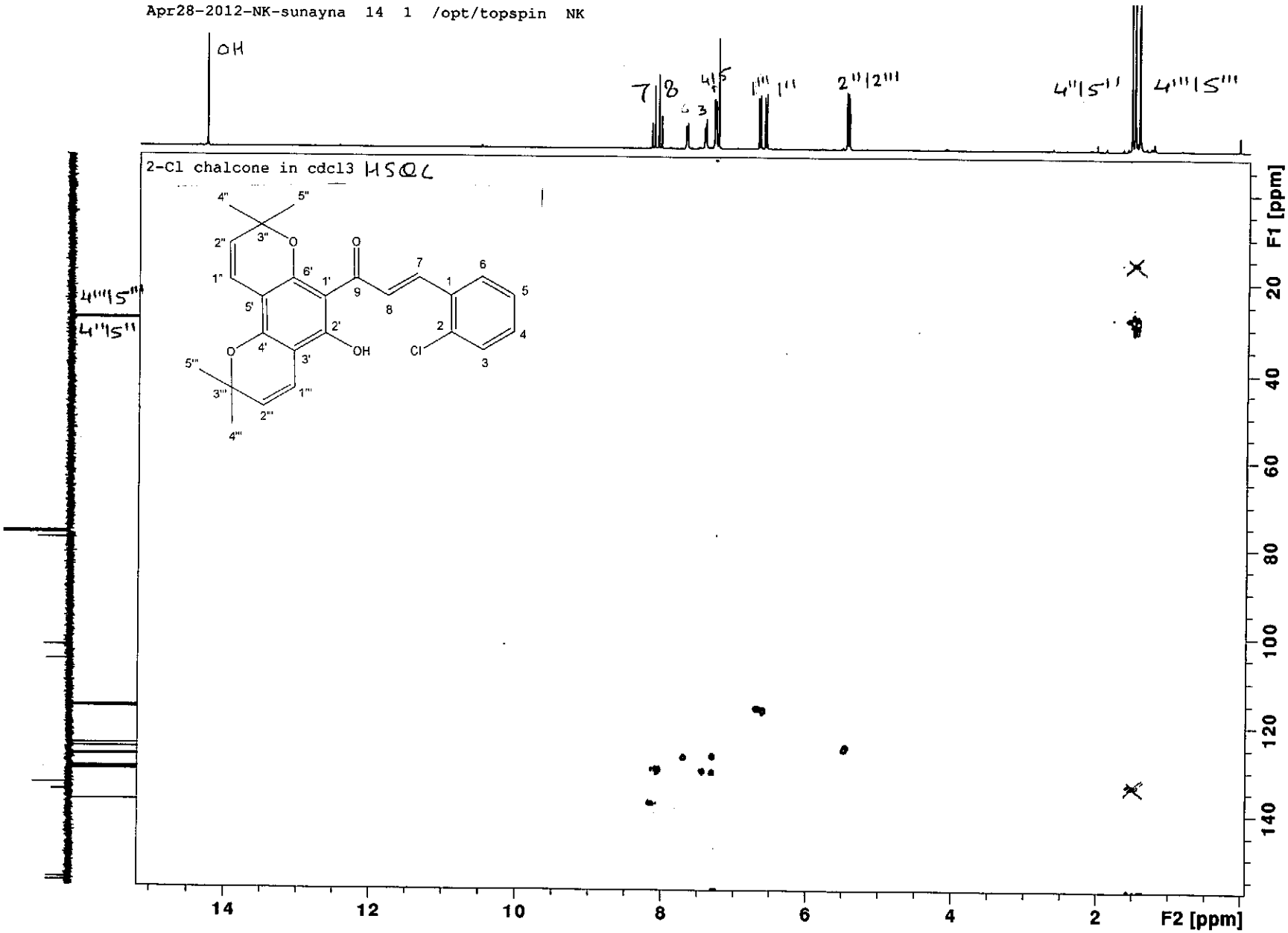
COSY spectrum of 2-chloropyranochromene chalcone (A-3i)



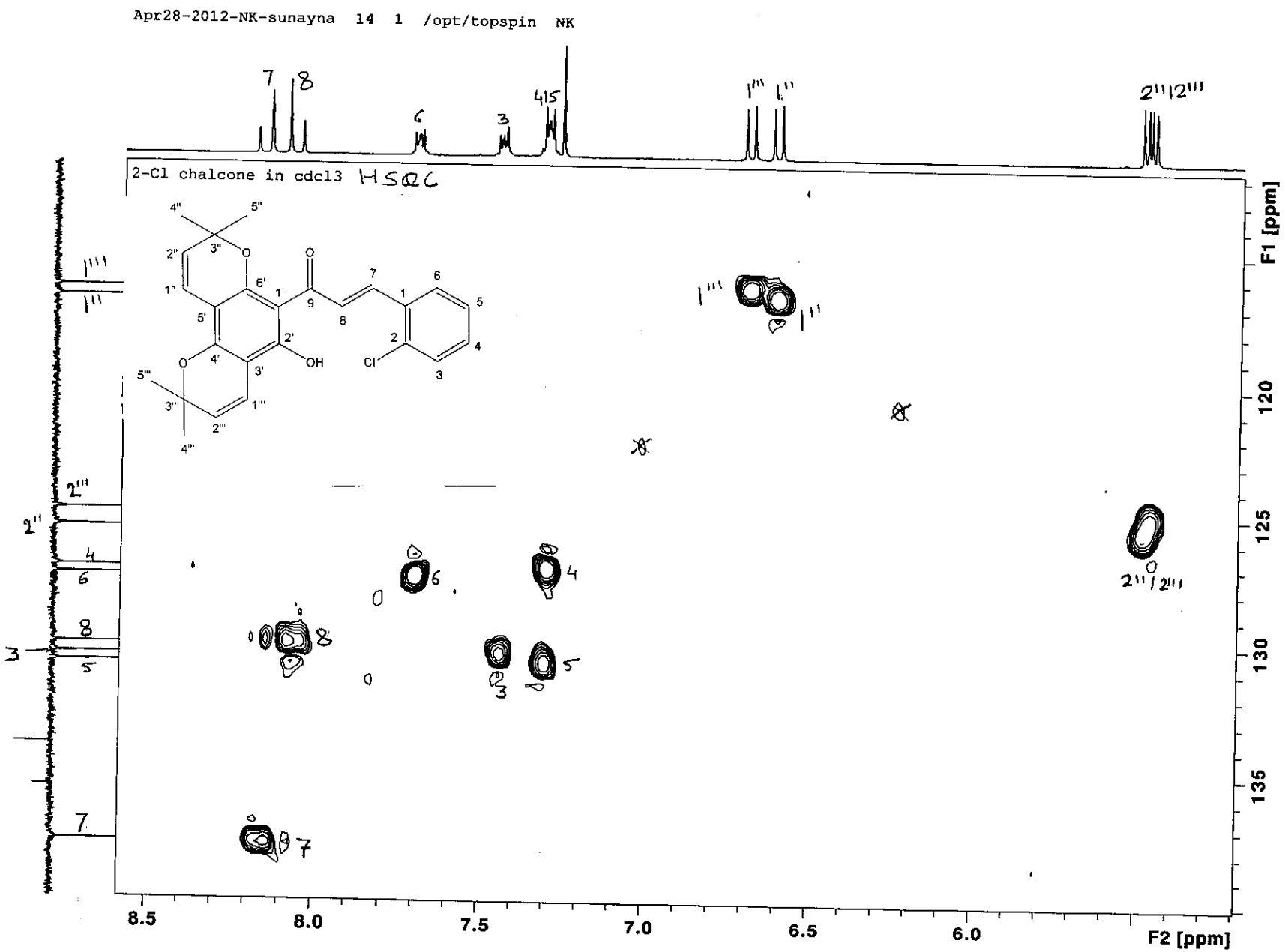
Expanded COSY spectrum of 2-chloropyranochromene chalcone (A-3i)



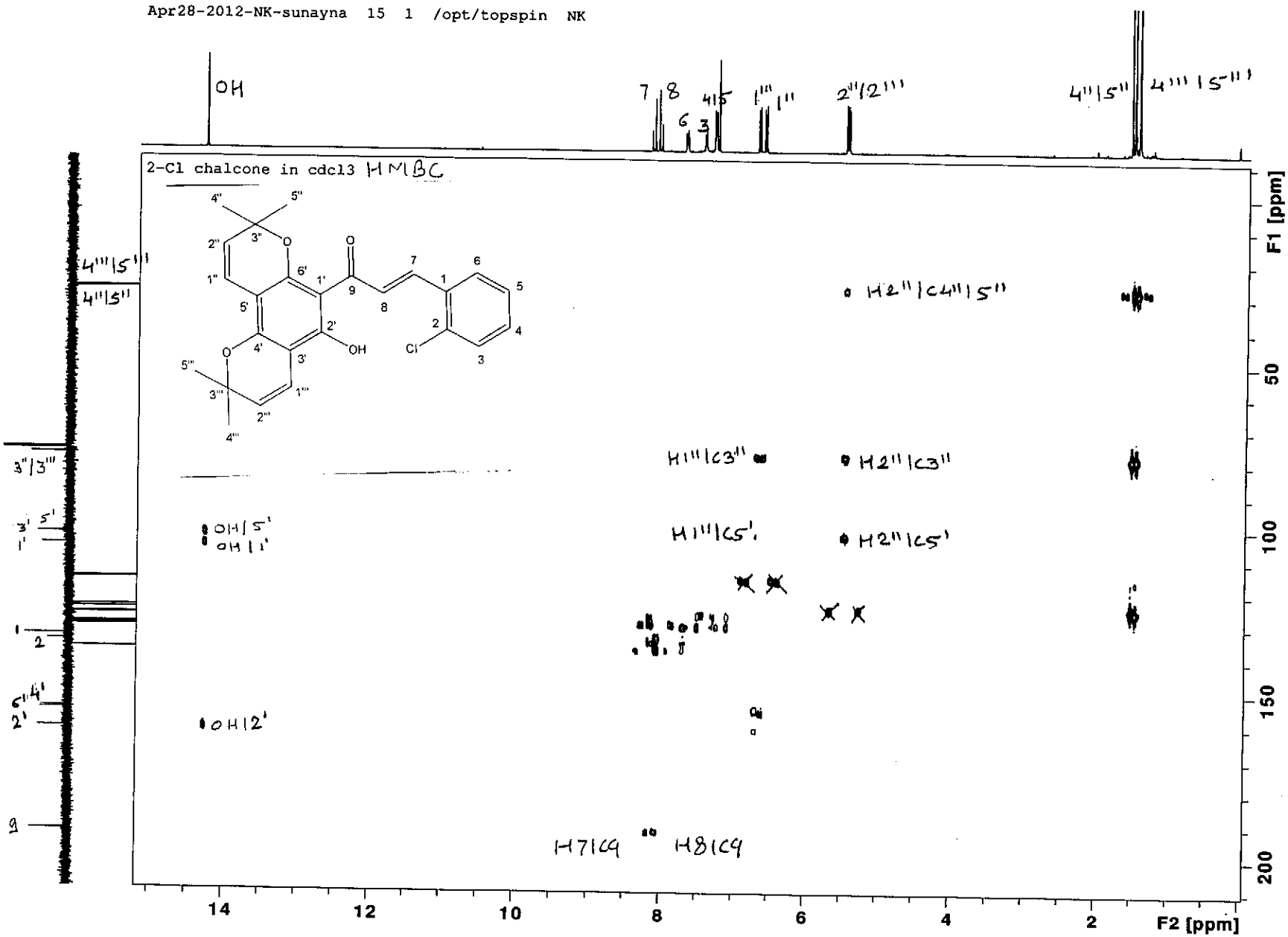
NOESY spectrum of 2-chloropyranochromene chalcone (A-3i)



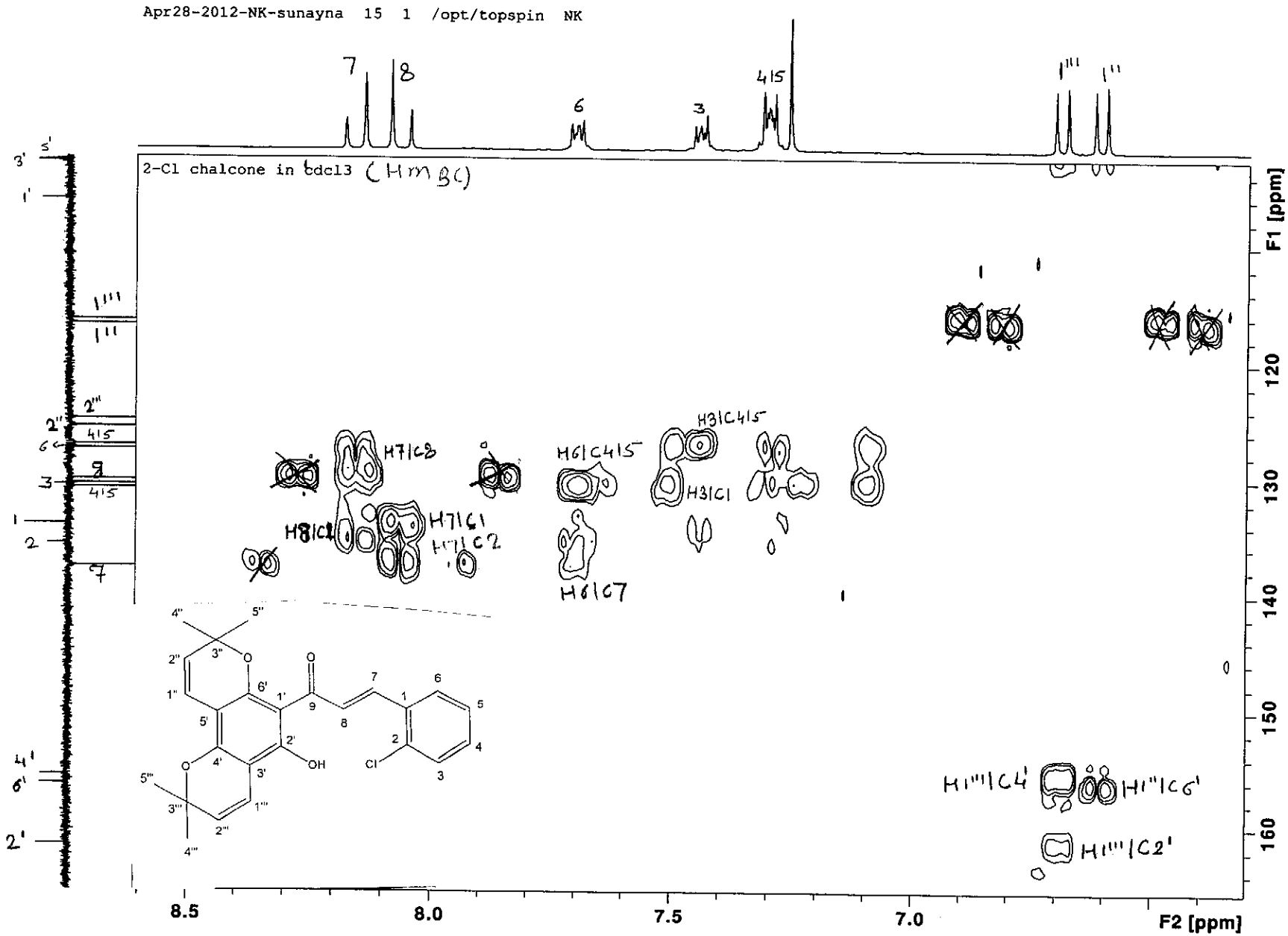
HSQC spectrum of 2-chloropyranochromene chalcone (A-3i)



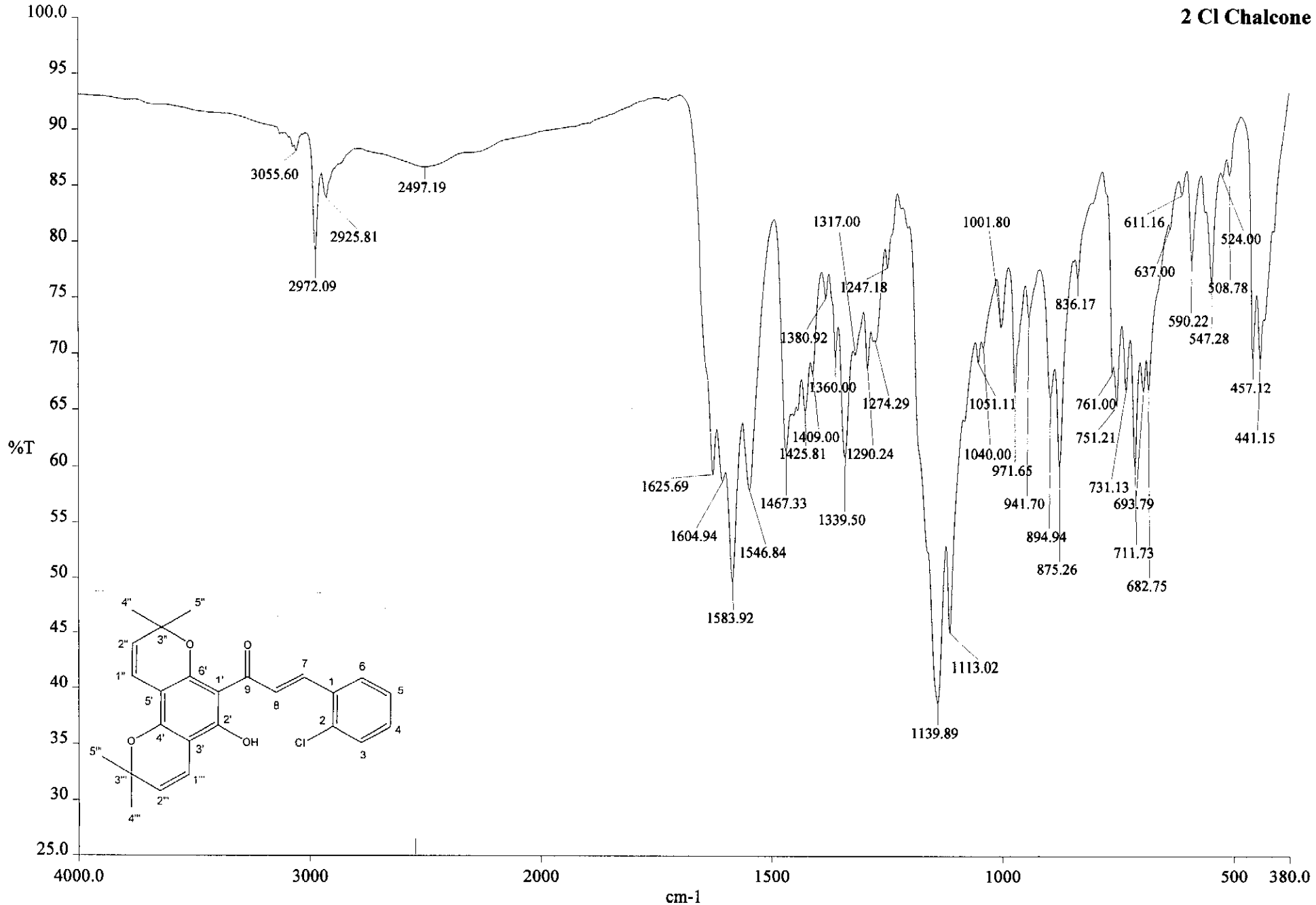
HSQC spectrum of 2-chloropyranochromene chalcone (A-3i)



HMBC spectrum of 2-chloropyranochromene chalcone (A-3i)

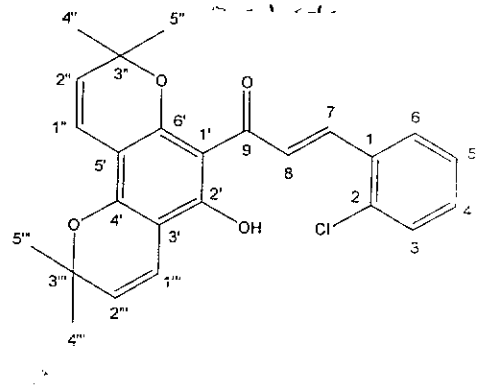


Expanded HMBC spectrum of 2-chloropyranochromene chalcone (A-3i)



Infrared spectrum of 2-chloropyranochromene chalcone (A-3i)

Elemental Composition Report

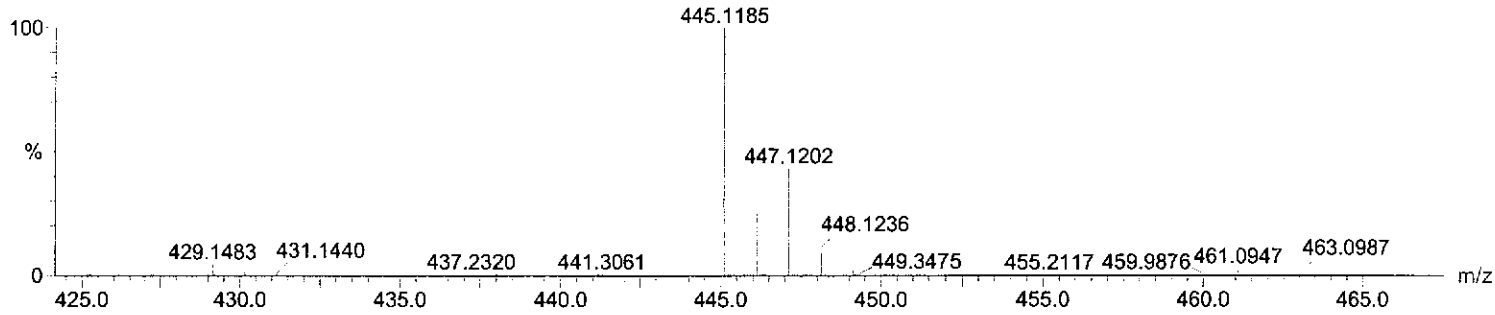


Single Mass Analysis
Tolerance = 50000.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
9 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-26 H: 20-25 O: 0-5 Na: 0-1 Cl: 0-1

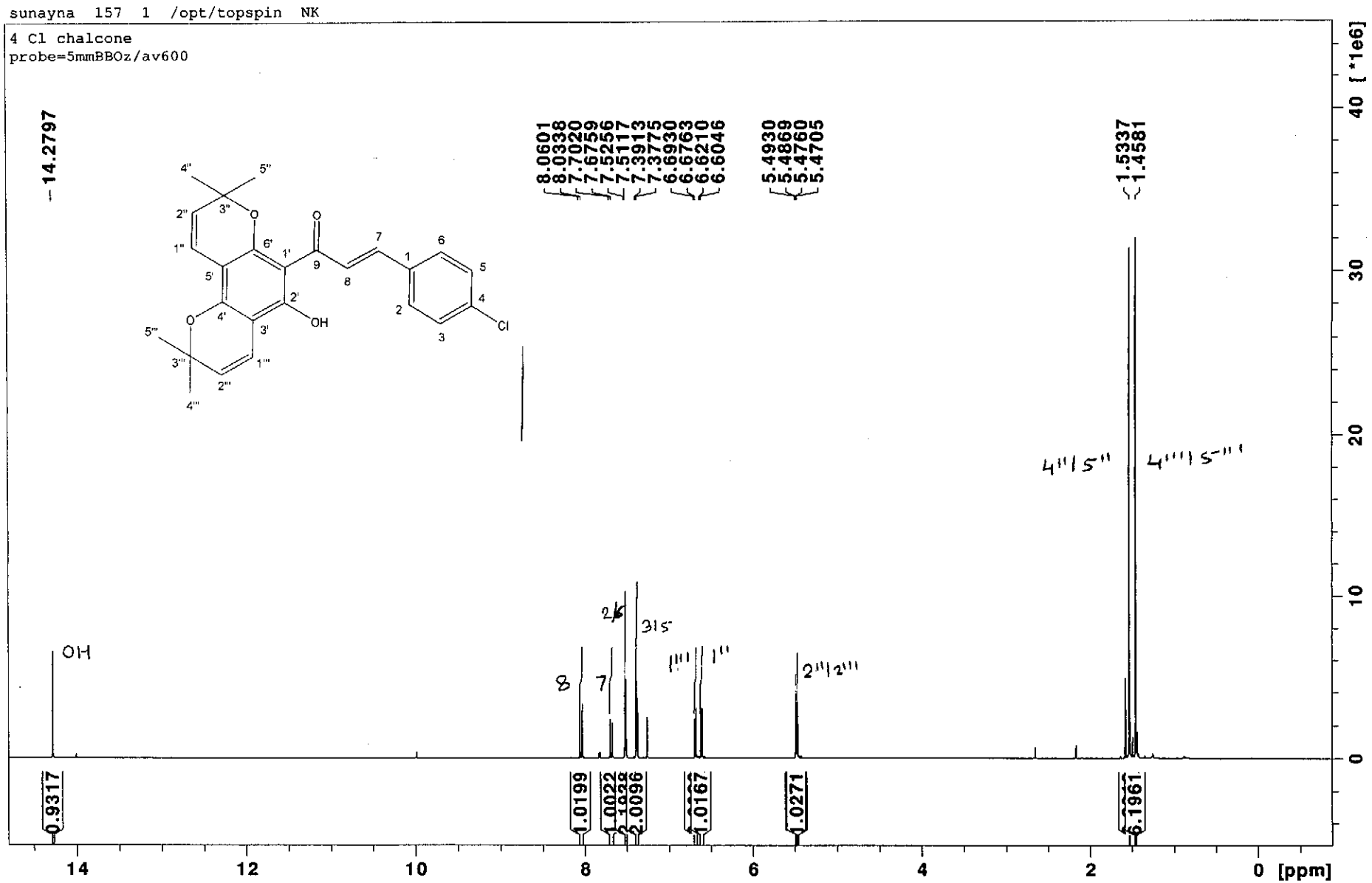
Dr. Nizam
17 8 (0.119) Cm (1:31)

TOF MS ES+
1.54e+004

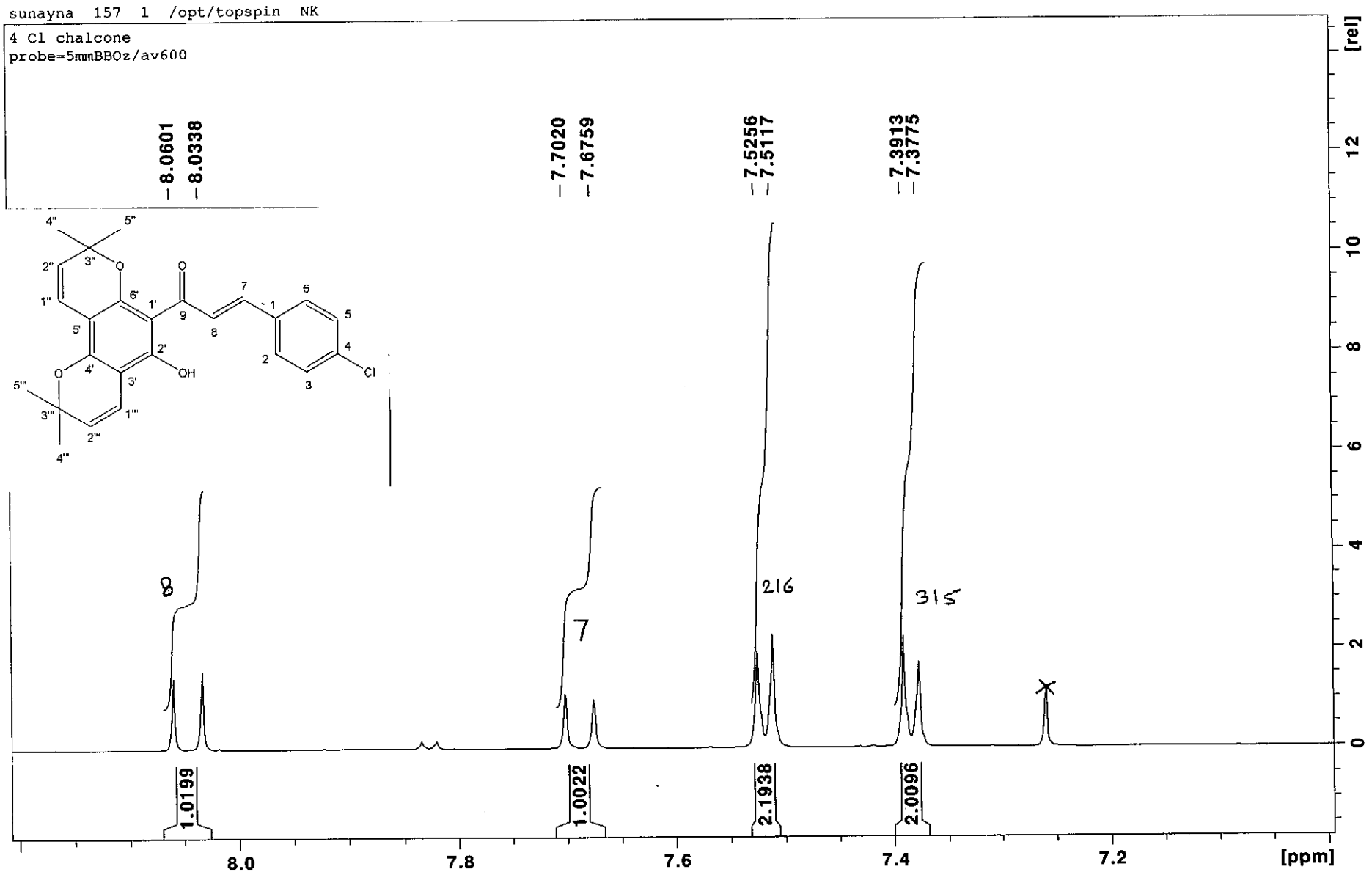


Minimum:				-1.5						
Maximum:		5.0	50000.0	50.0						
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula			
445.1185	445.1183	0.2	0.4	13.5	368.9	0.0	C25	H23	O4	Na Cl

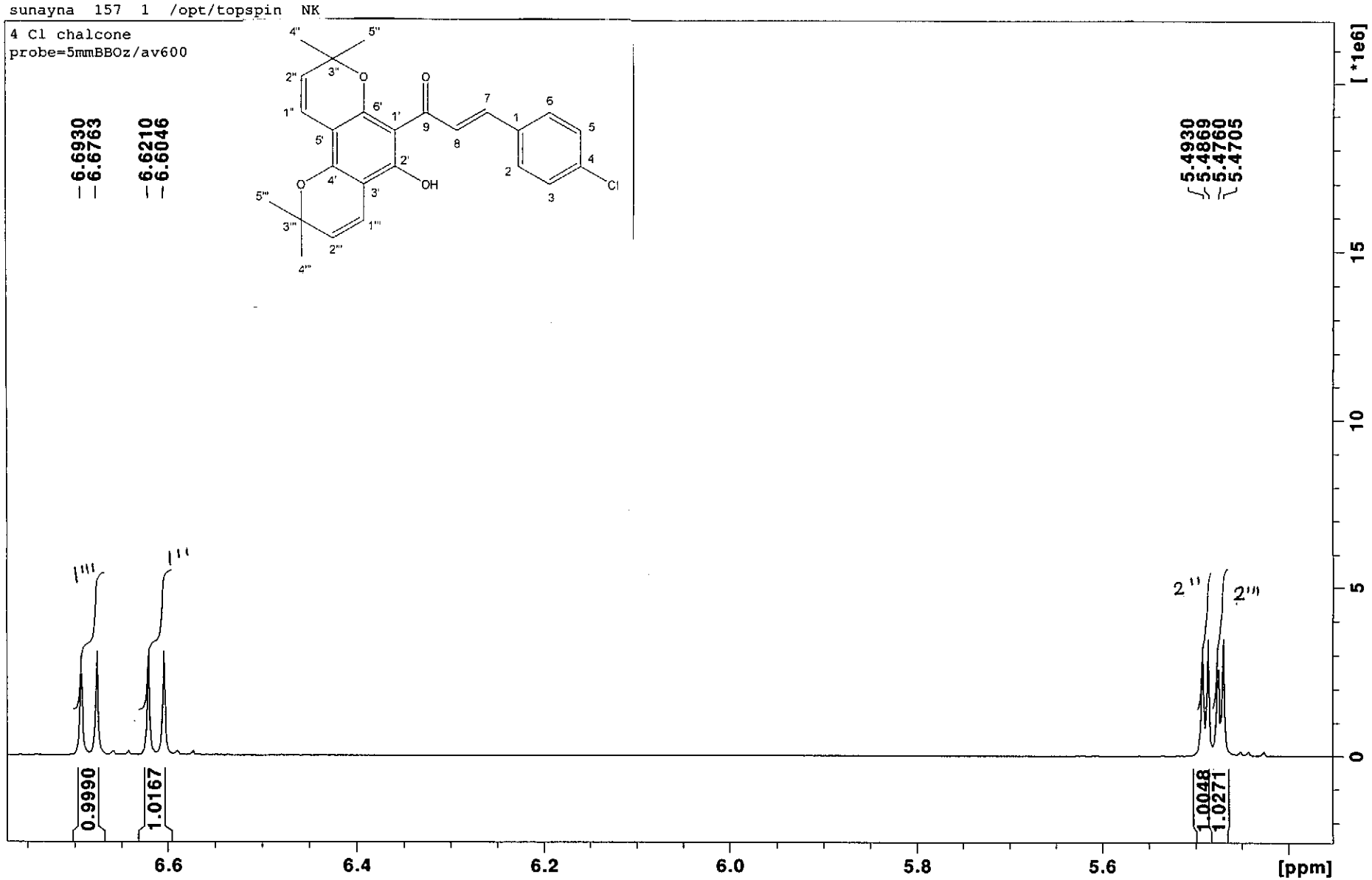
HRMS spectrum of 2-chloropyranochromene chalcone (A-3i)



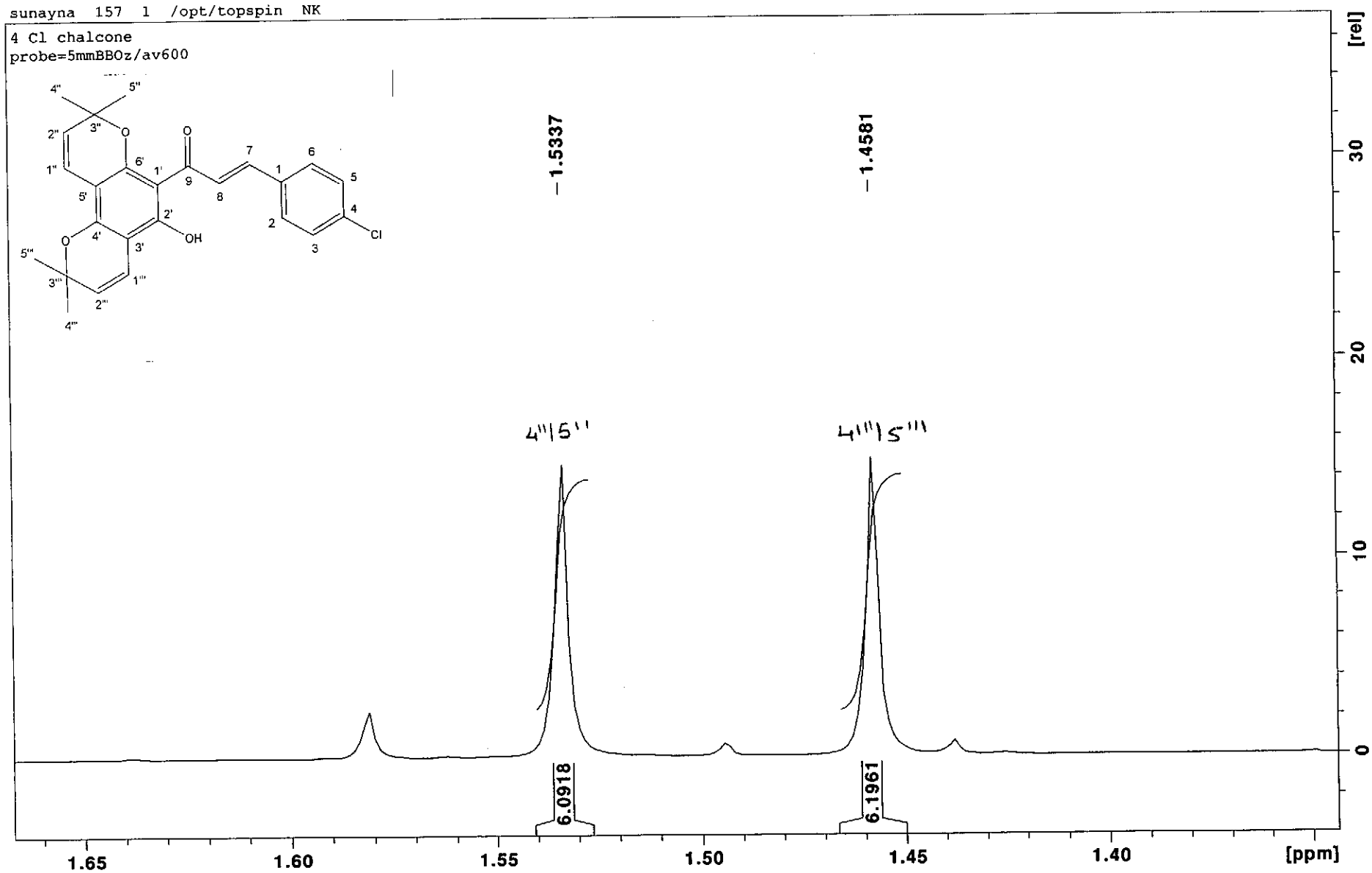
1H NMR spectrum of 4-chloropyranochromene chalcone (A-3j)



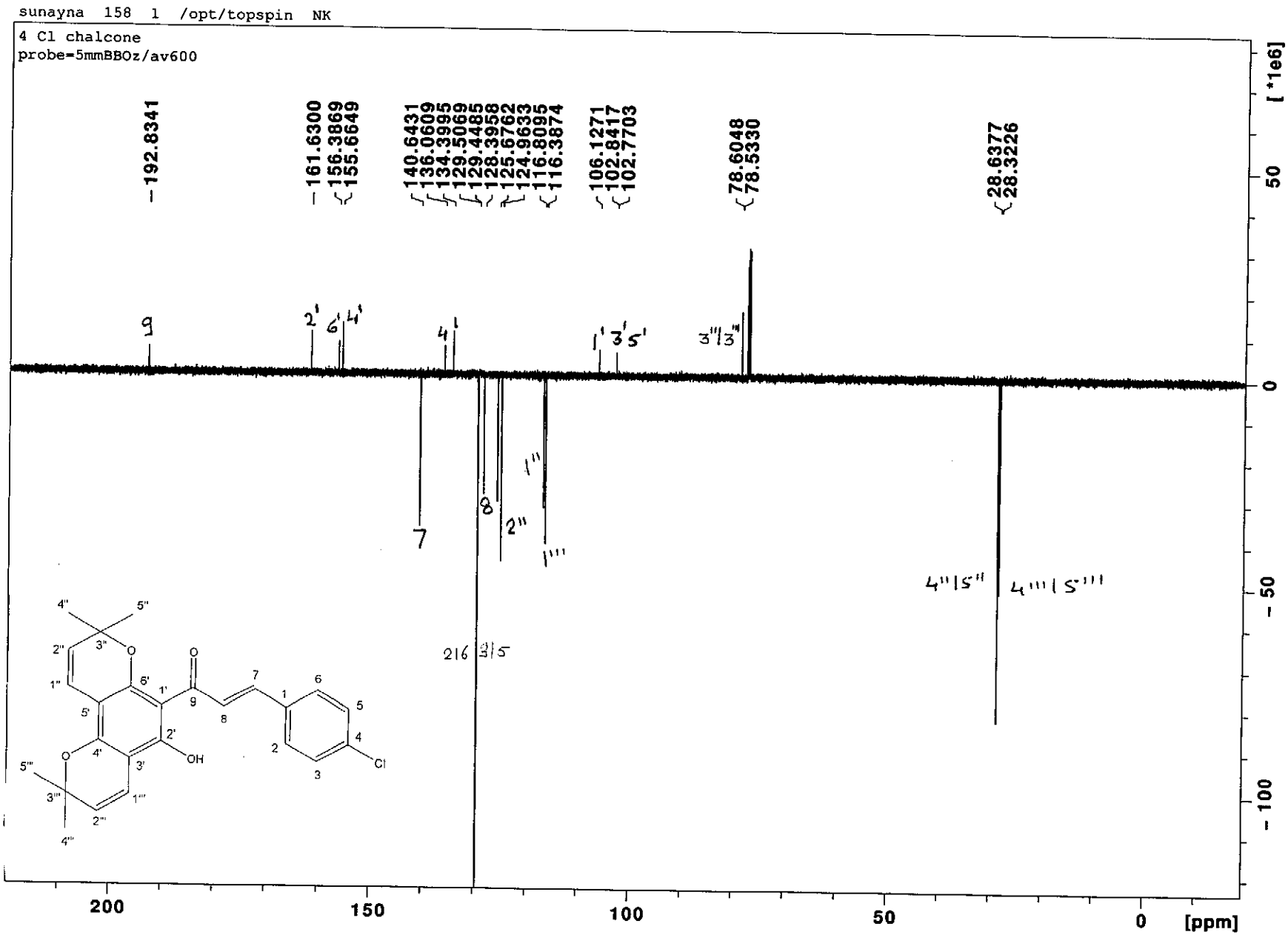
Expanded 1H NMR spectrum of 4-chloropyranochromene chalcone (A-3j)



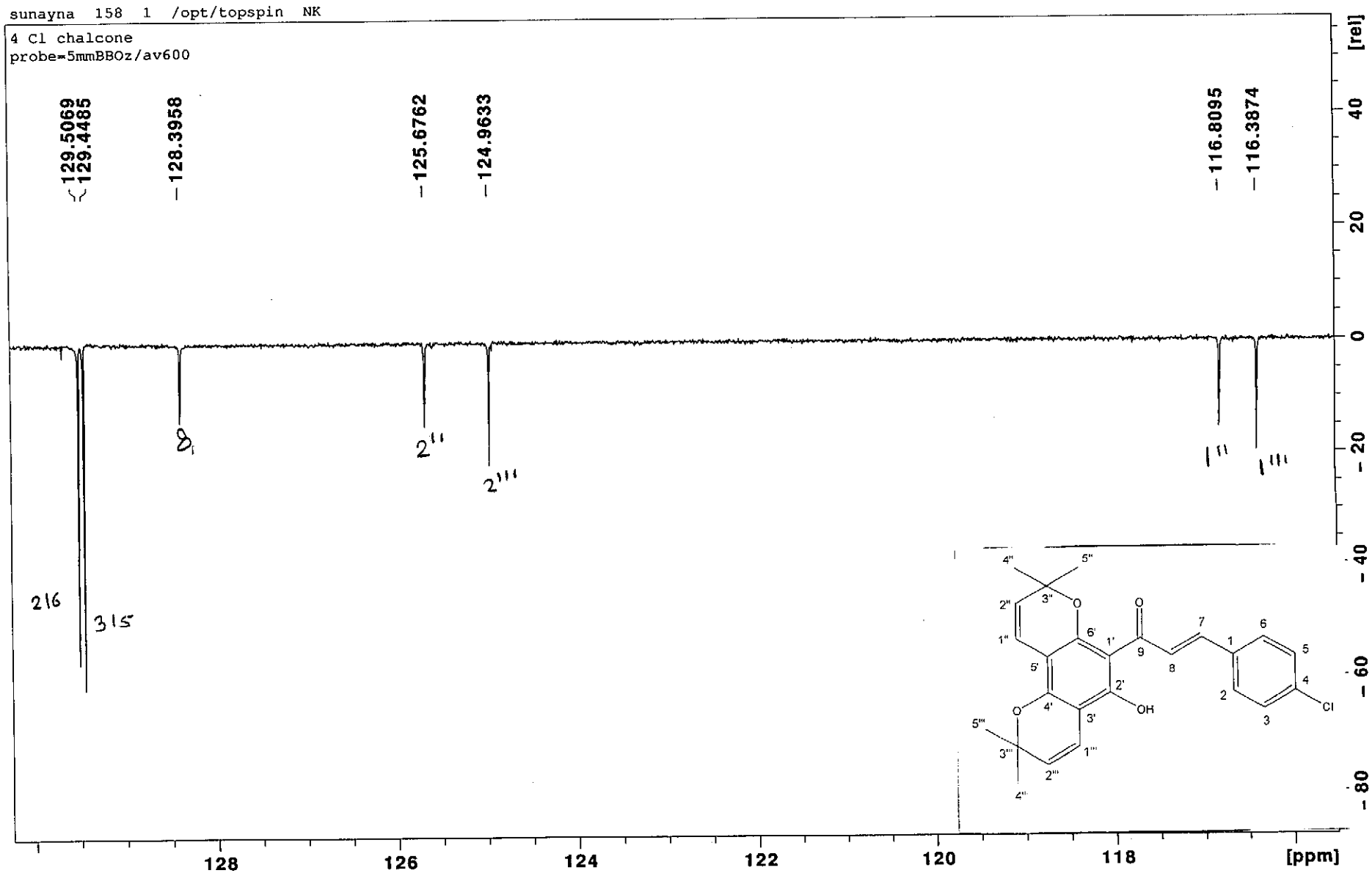
Expanded 1H NMR spectrum of 4-chloropyranochromene chalcone (A-3j)



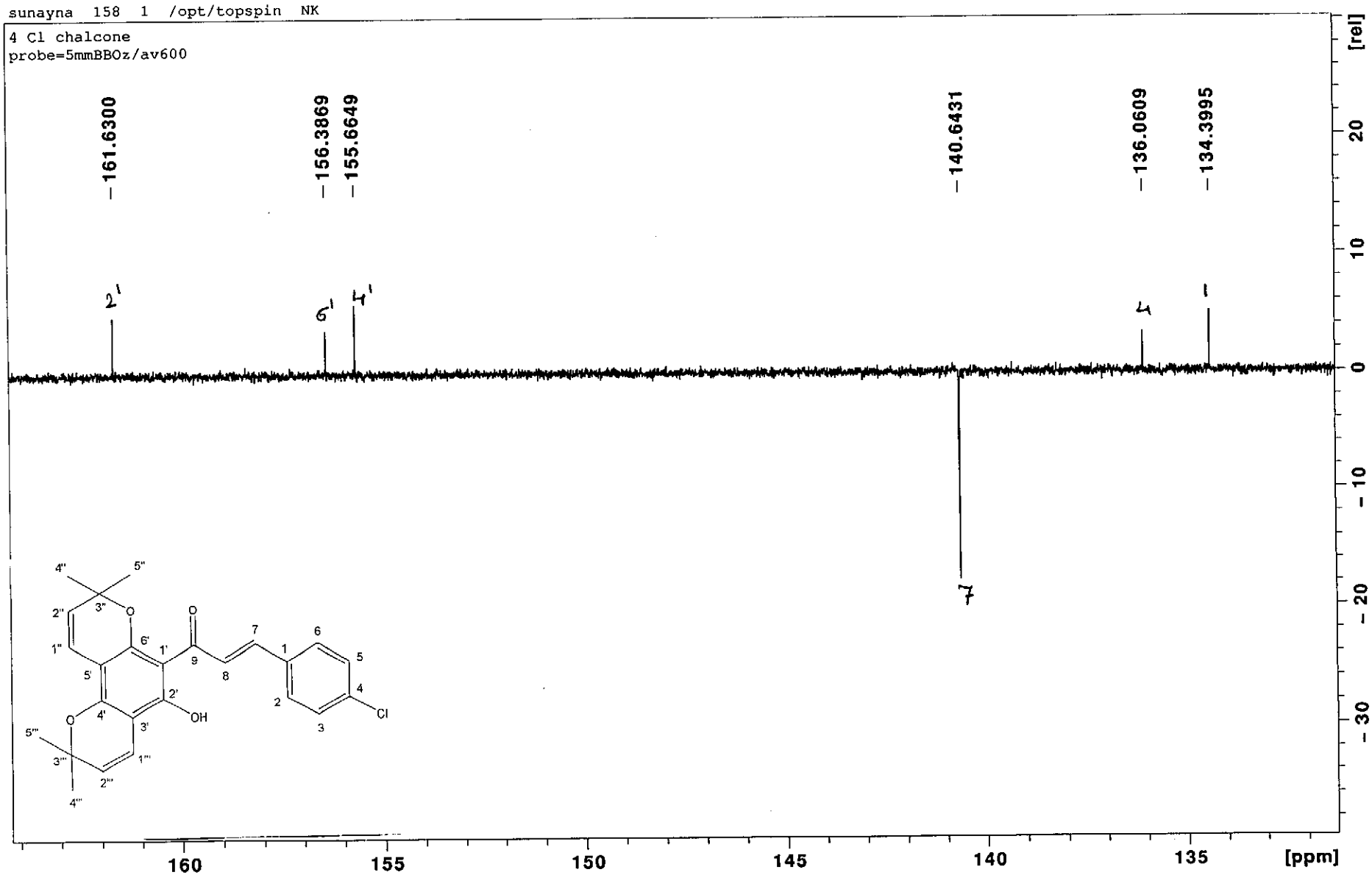
Expanded 1H NMR spectrum of 4-chloropyranochromene chalcone (A-3j)



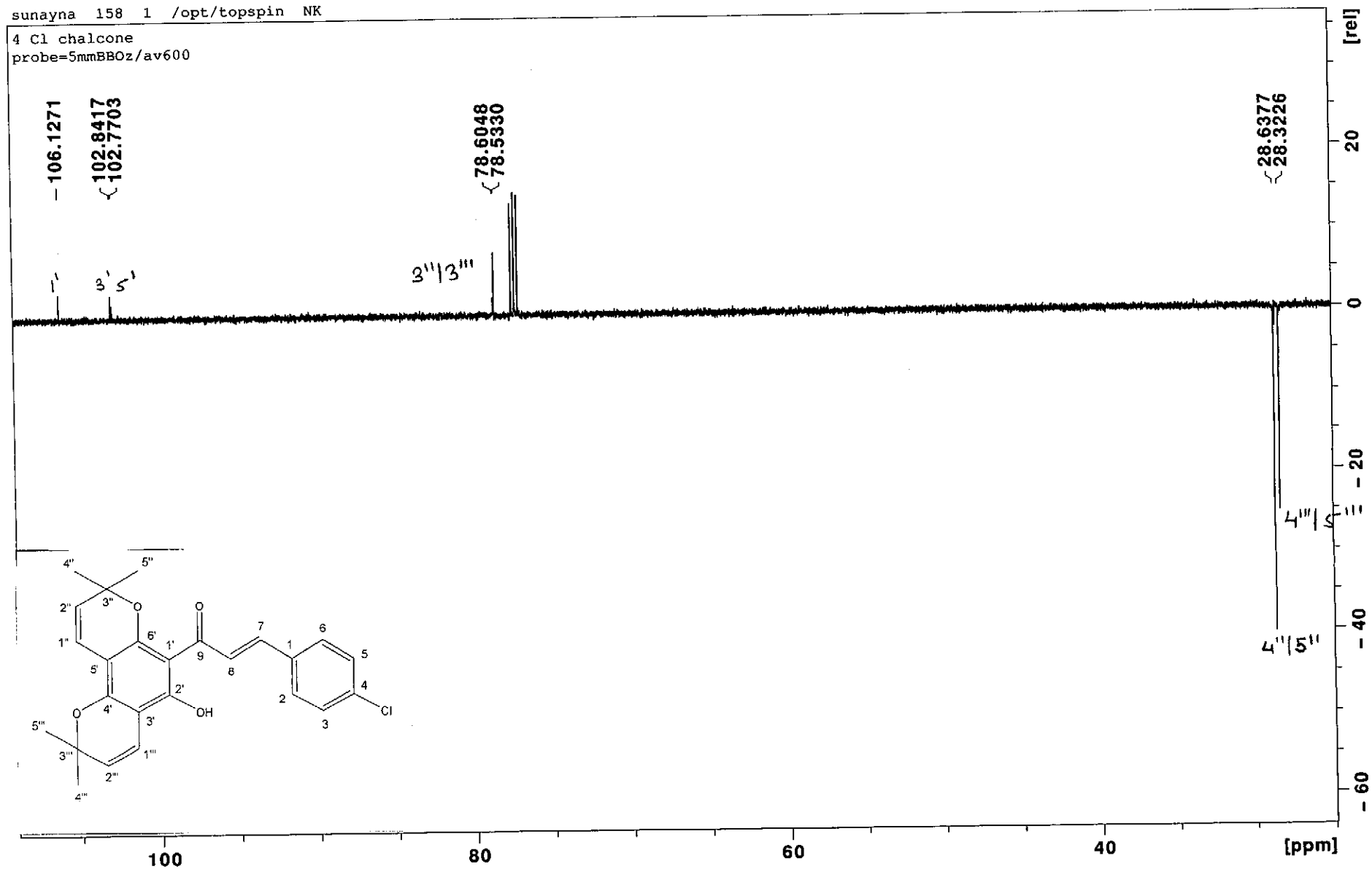
13C NMR spectrum of 4-chloropyranochromene chalcone (A-3j)



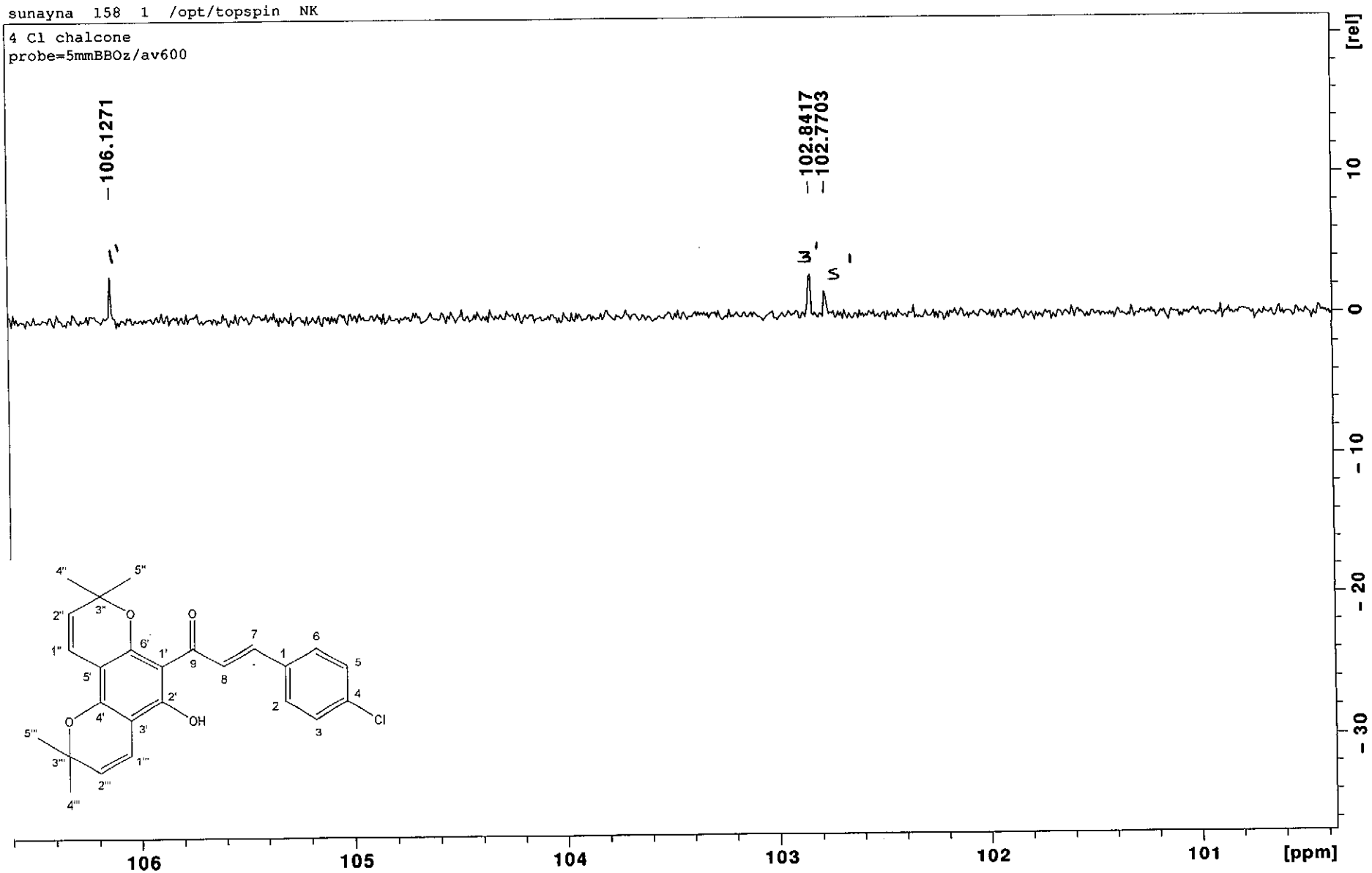
Expanded 13C NMR spectrum of 4-chloropyranochromene chalcone (A-3j)



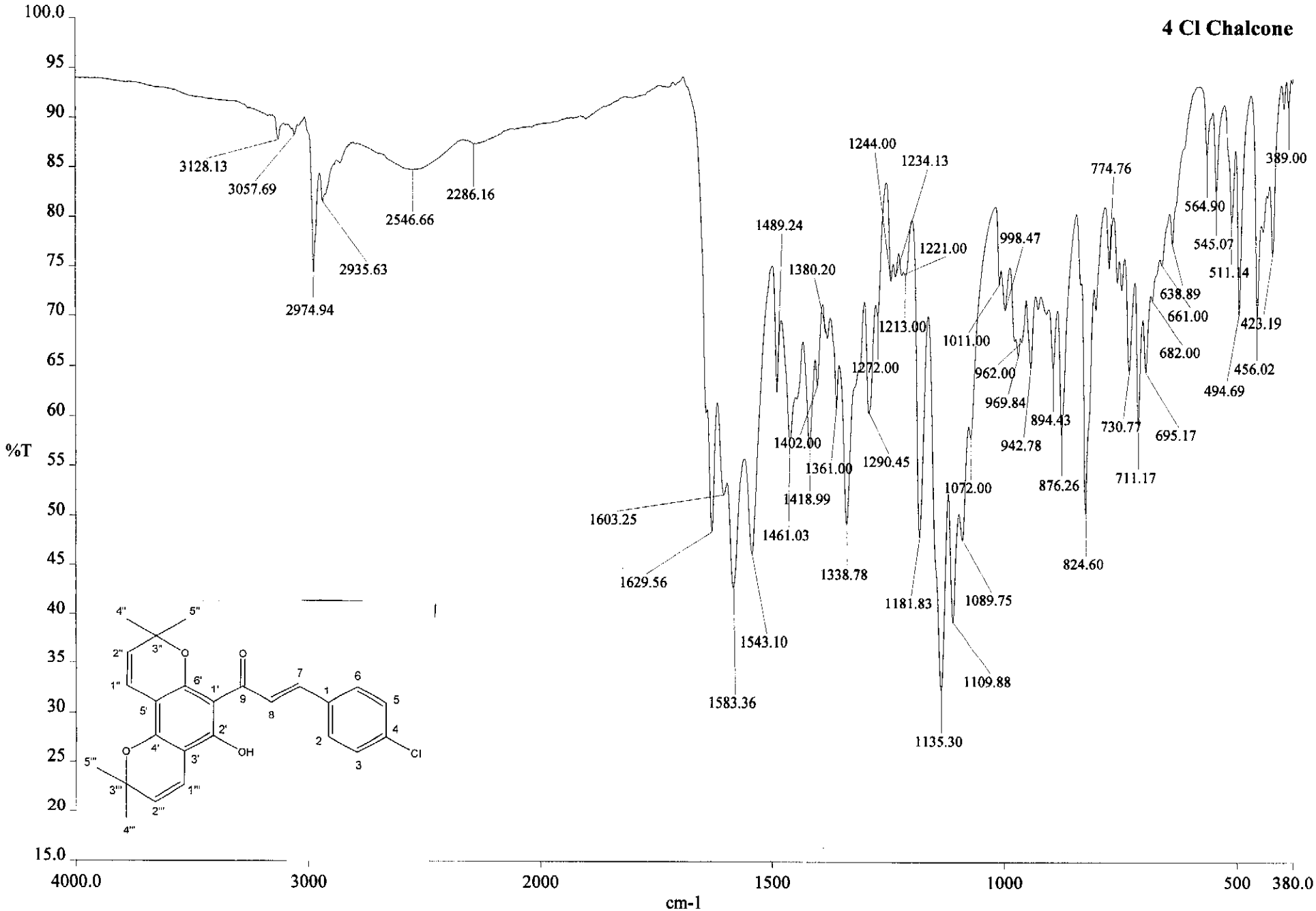
Expanded 13C NMR spectrum of 4-chloropyranochromene chalcone (A-3j)



Expanded 13C NMR spectrum of 4-chloropyranochromene chalcone (A-3j)

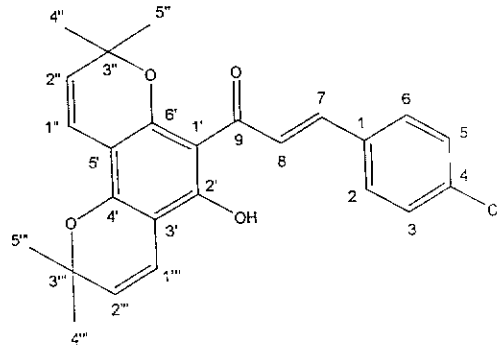


Expanded 13C NMR spectrum of 4-chloropyranochromene chalcone (A-3j)



Infrared spectrum of 4-chloropyranochromene chalcone (A-3j)

Elemental Composition Report



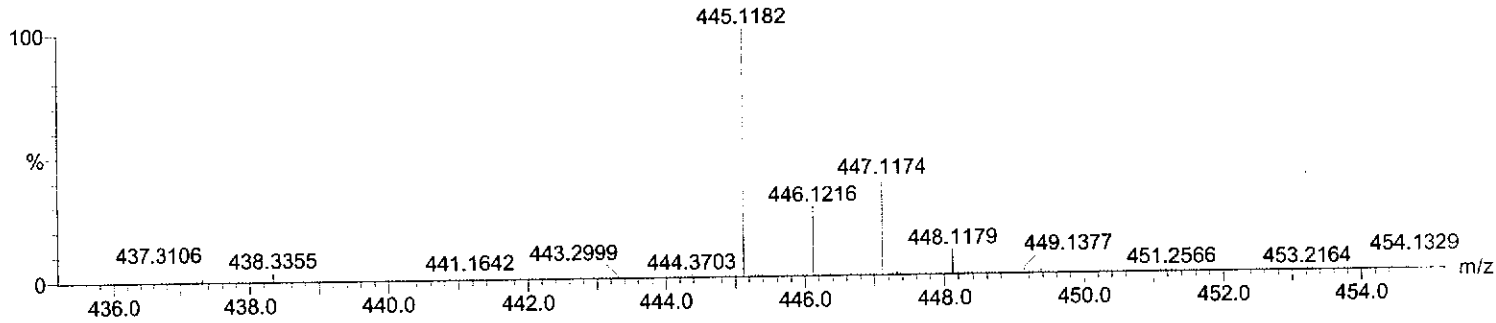
Single Mass Analysis

Tolerance = 50000.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
9 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-26 H: 20-25 O: 0-5 Na: 0-1 Cl: 0-1

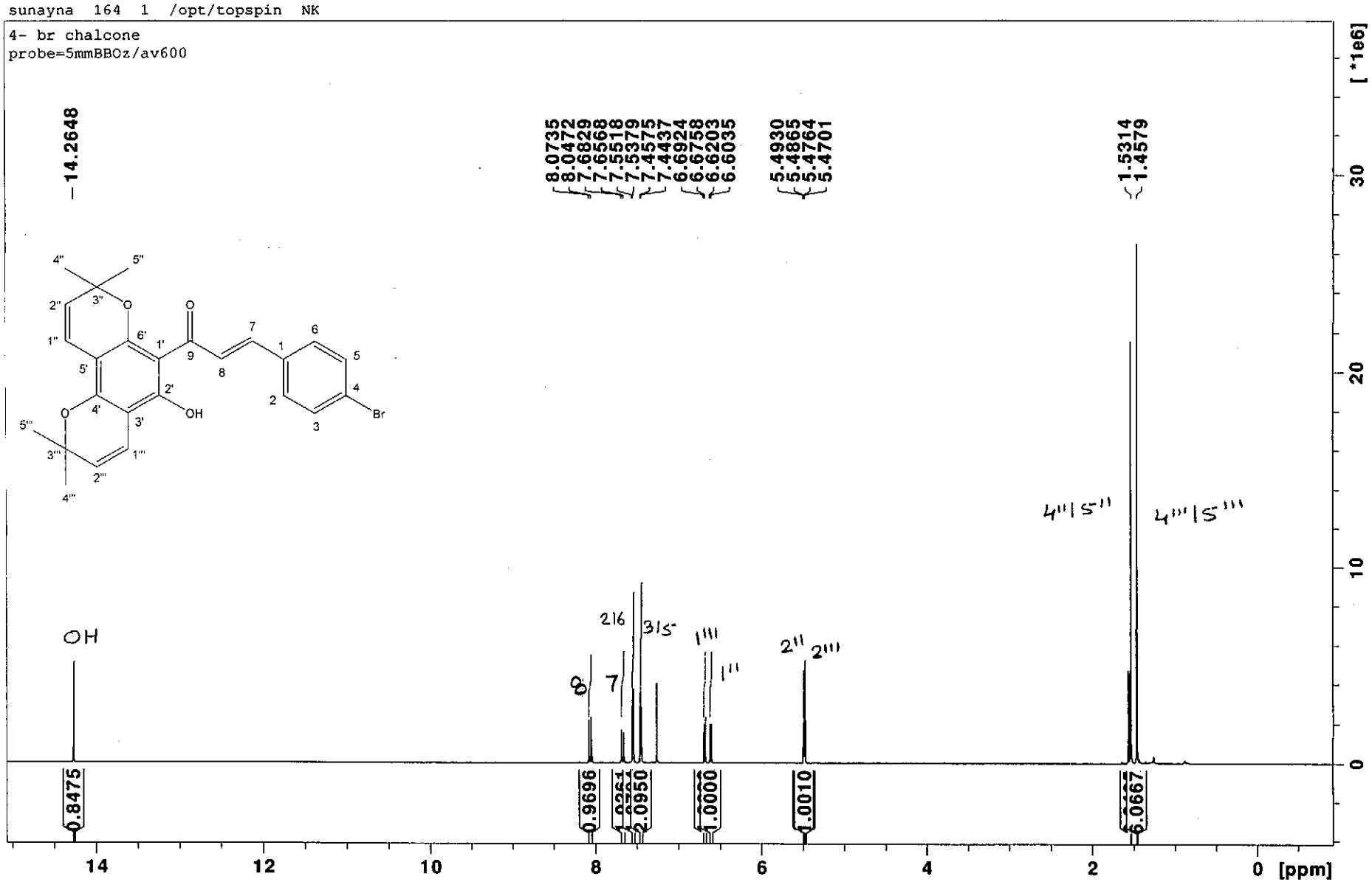
Dr. Nizam
19 4 (0.051) Cm (1:31)

TOF MS ES+
8.73e+003

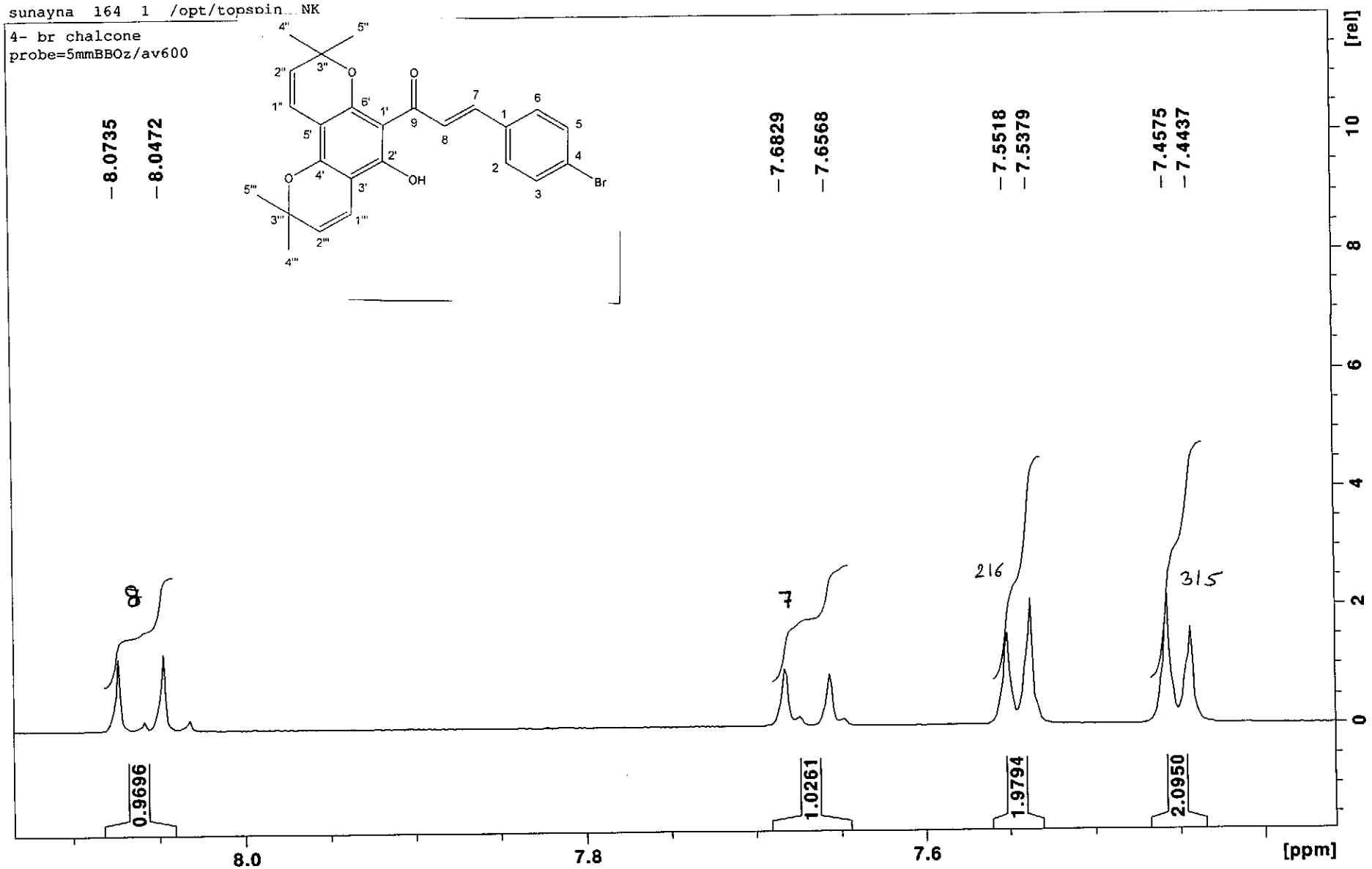


Minimum:				-1.5			
Maximum:		5.0	50000.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
445.1182	445.1183	-0.1	-0.2	13.5	342.2	0.0	C25 H23 O4 Na Cl

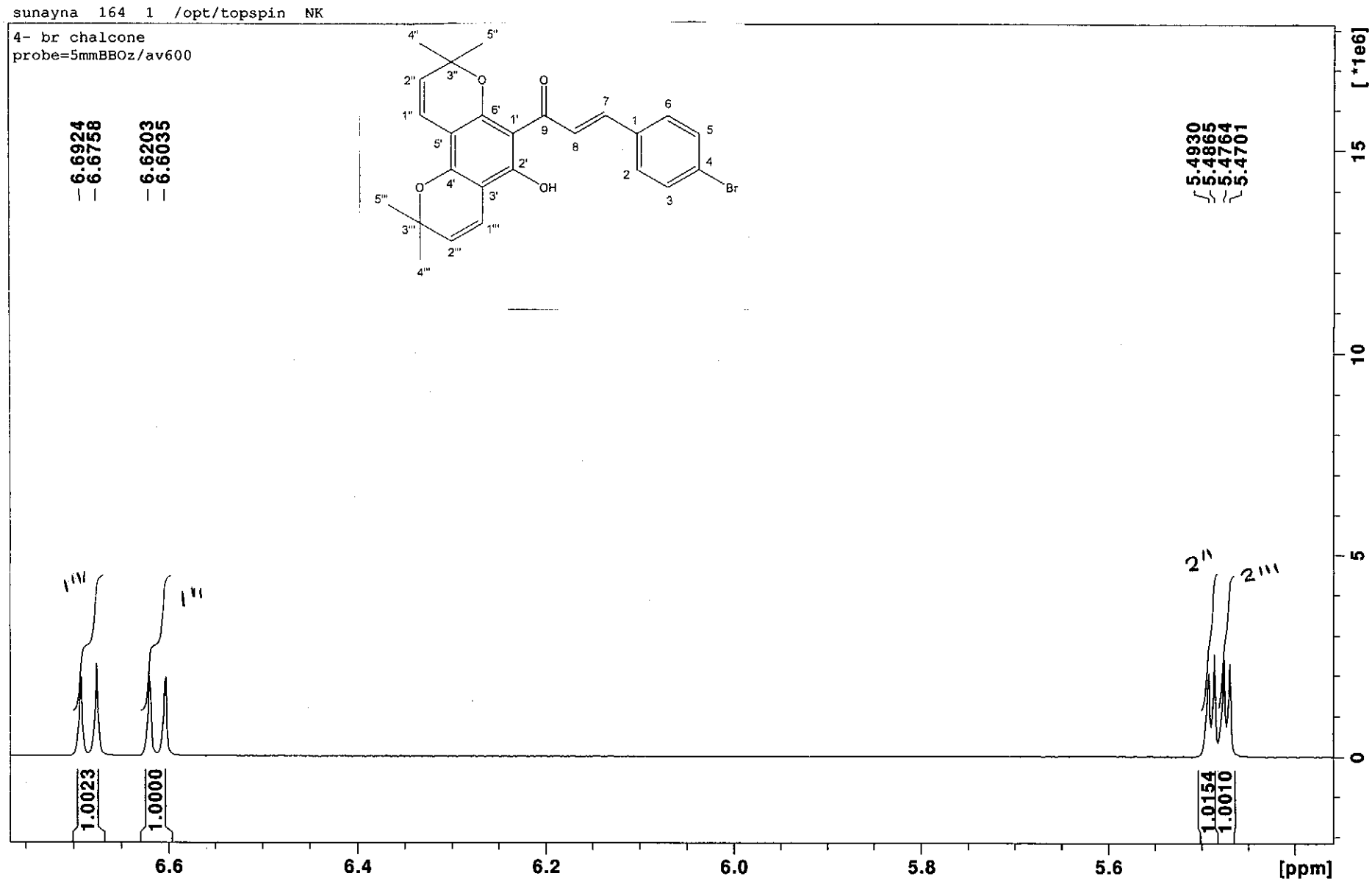
HRMS spectrum of 4-chloropyranochromene chalcone (A-3j)



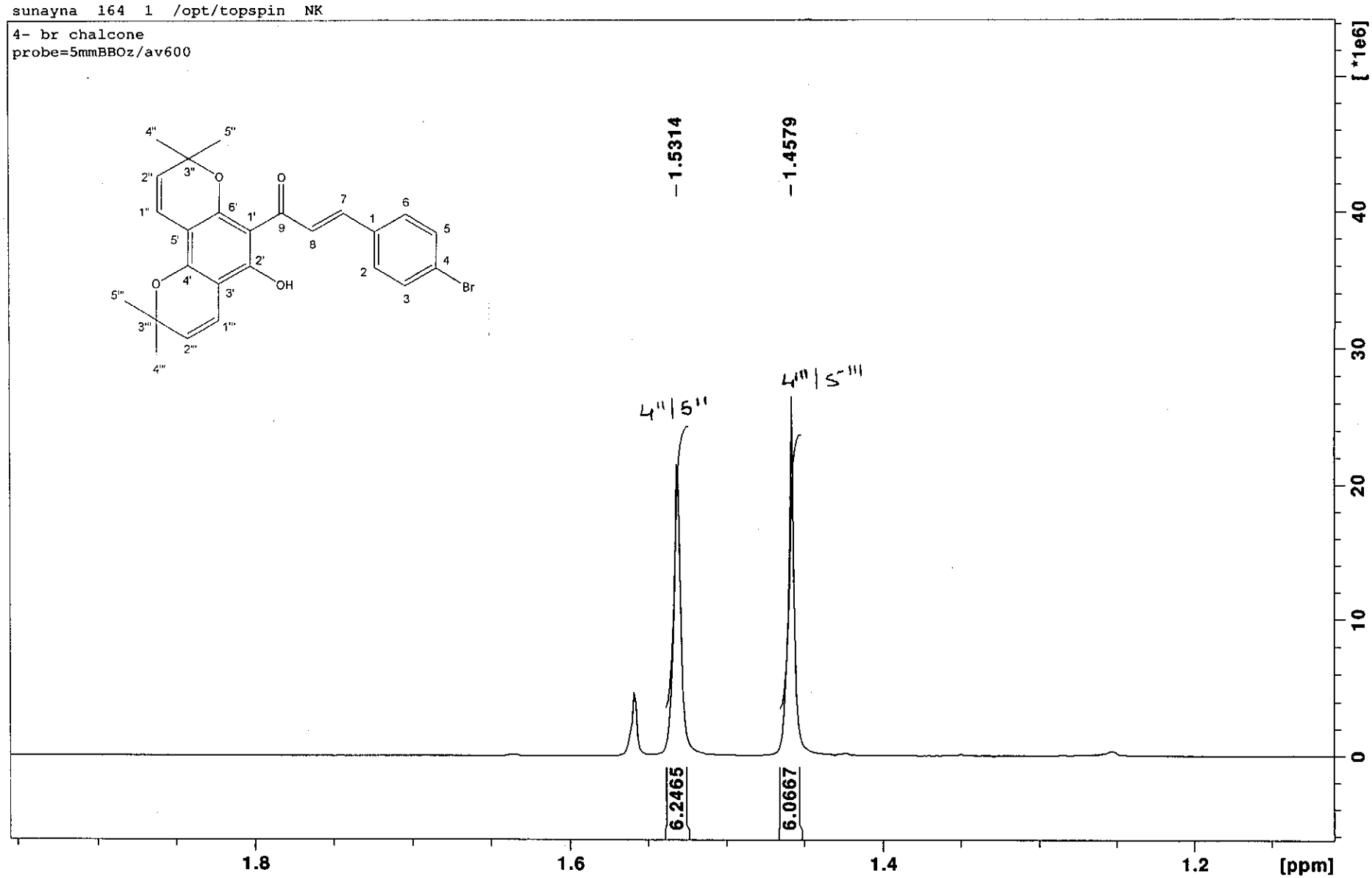
1H NMR spectrum of 4-bromopyranochromene chalcone (A-3k)



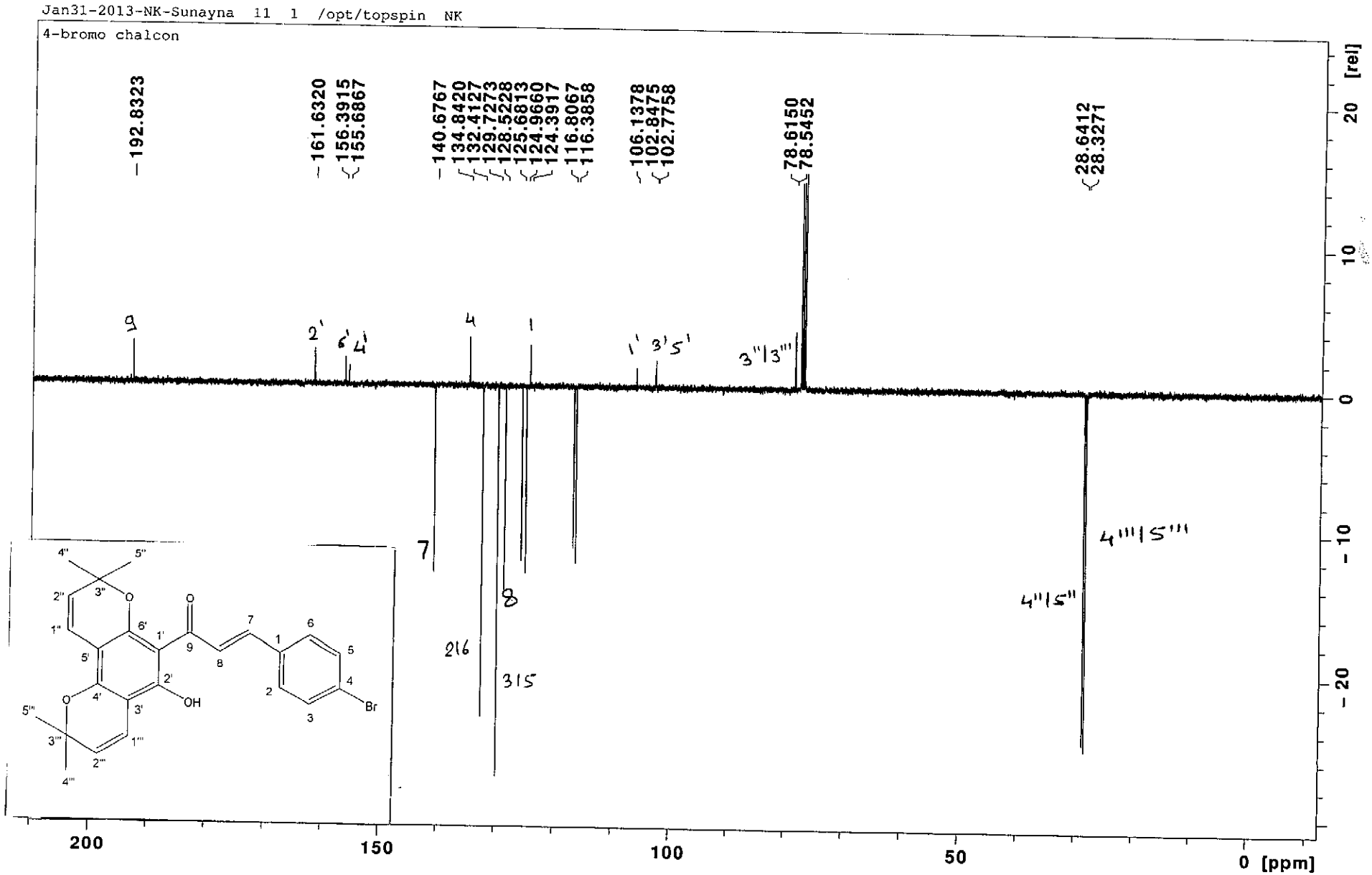
Expanded 1H NMR spectrum of 4-bromopyranochromene chalcone (A-3k)



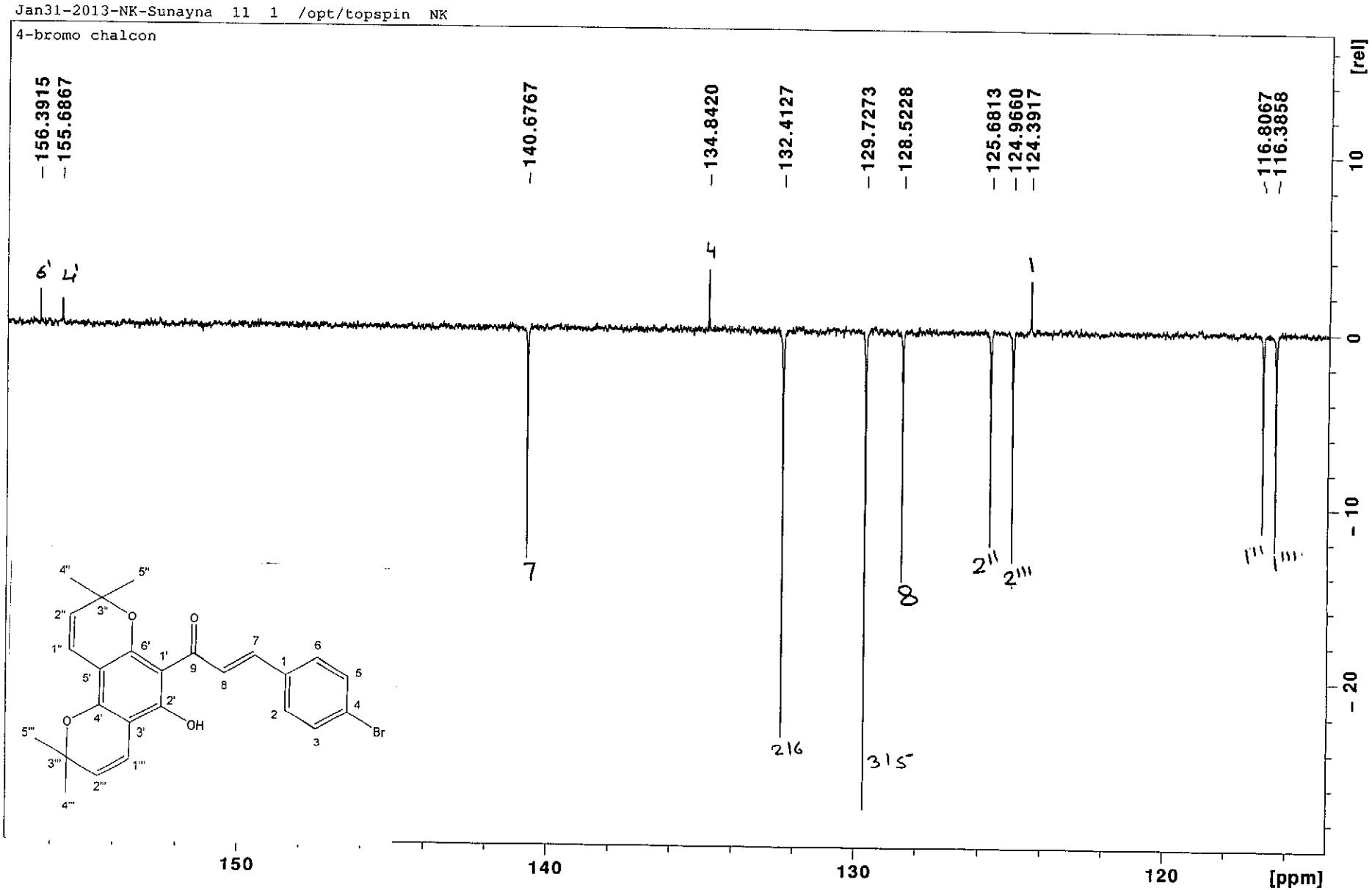
Expanded 1H NMR spectrum of 4-bromopyranochromene chalcone (A-3k)



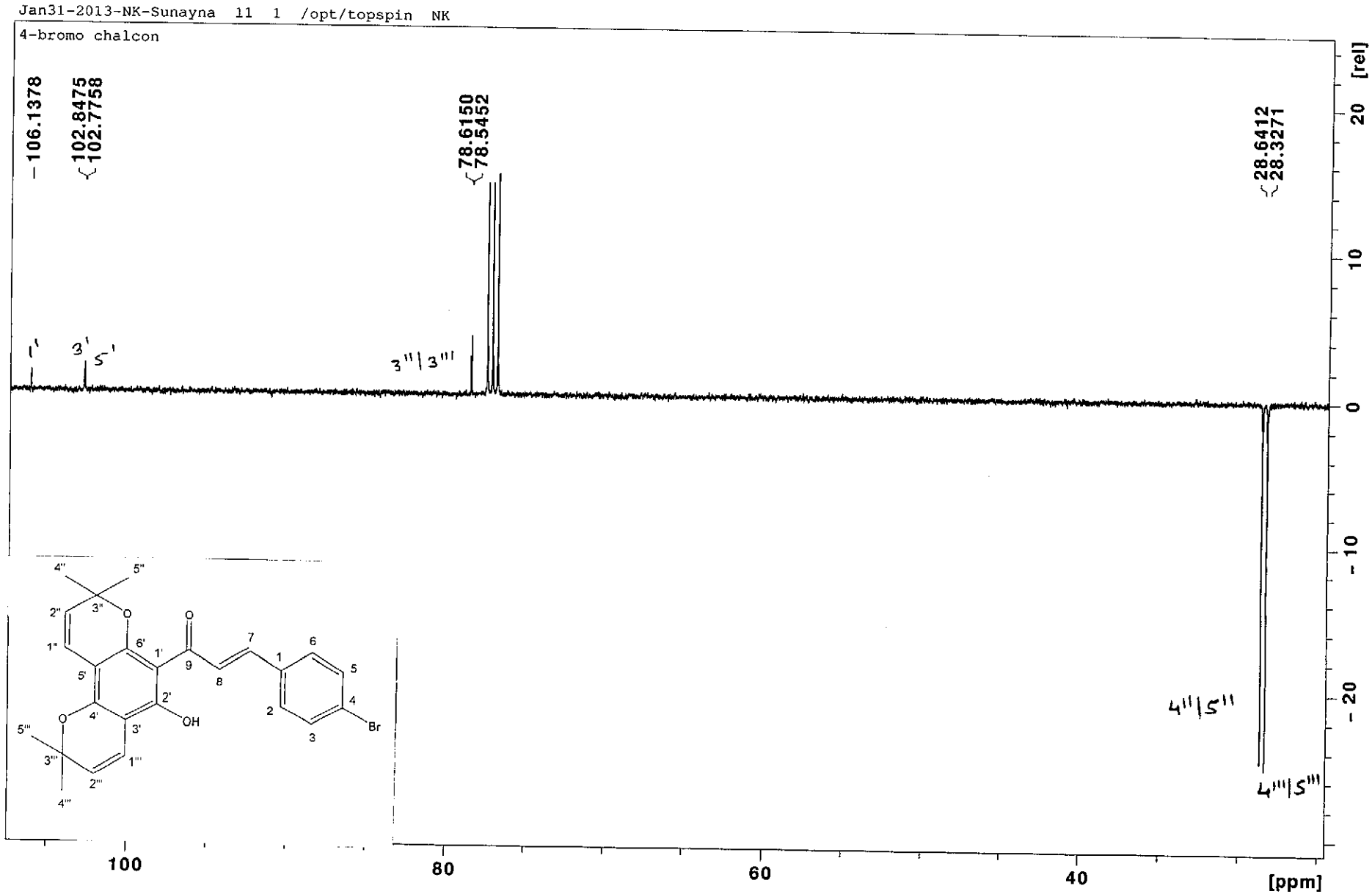
Expanded 1H NMR spectrum of 4-bromopyranochromene chalcone (A-3k)



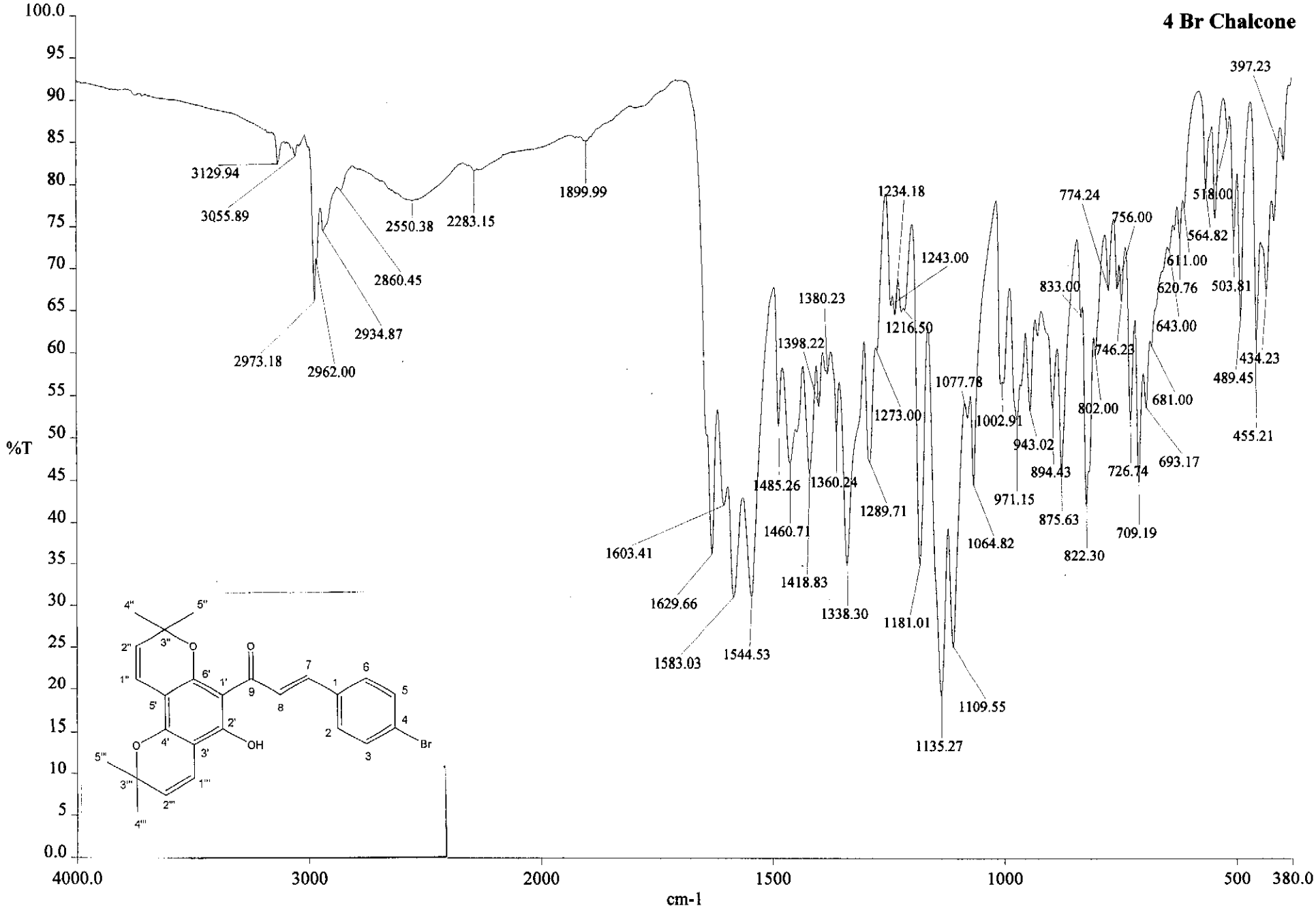
Expanded 13C NMR spectrum of 4-bromopyranochromene chalcone (A-3k)



Expanded 13C NMR spectrum of 4-bromopyranochromene chalcone (A-3k)



Expanded 13C NMR spectrum of 4-bromopyranochromene chalcone (A-3k)



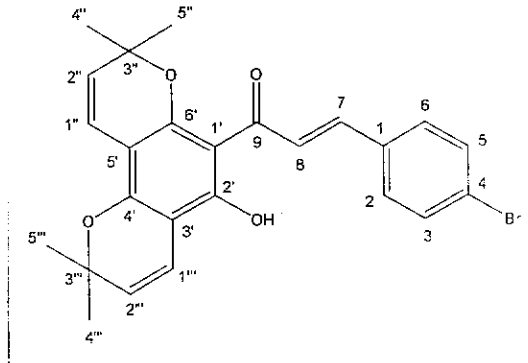
Infrared spectrum of 4-bromopyranochromene chalcone (A-3k)

Elemental Composition Report

Single Mass Analysis

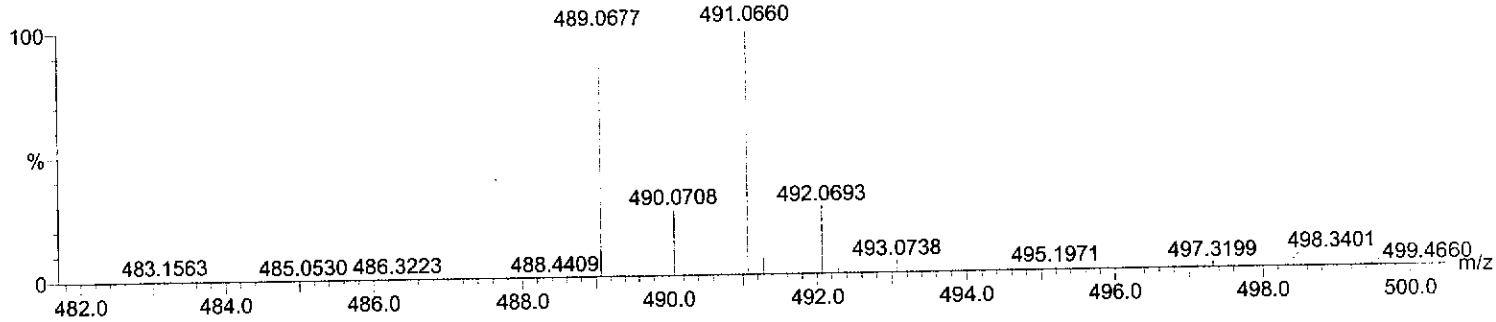
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
14 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-26 H: 20-25 O: 0-5 Na: 0-1 Br: 0-1



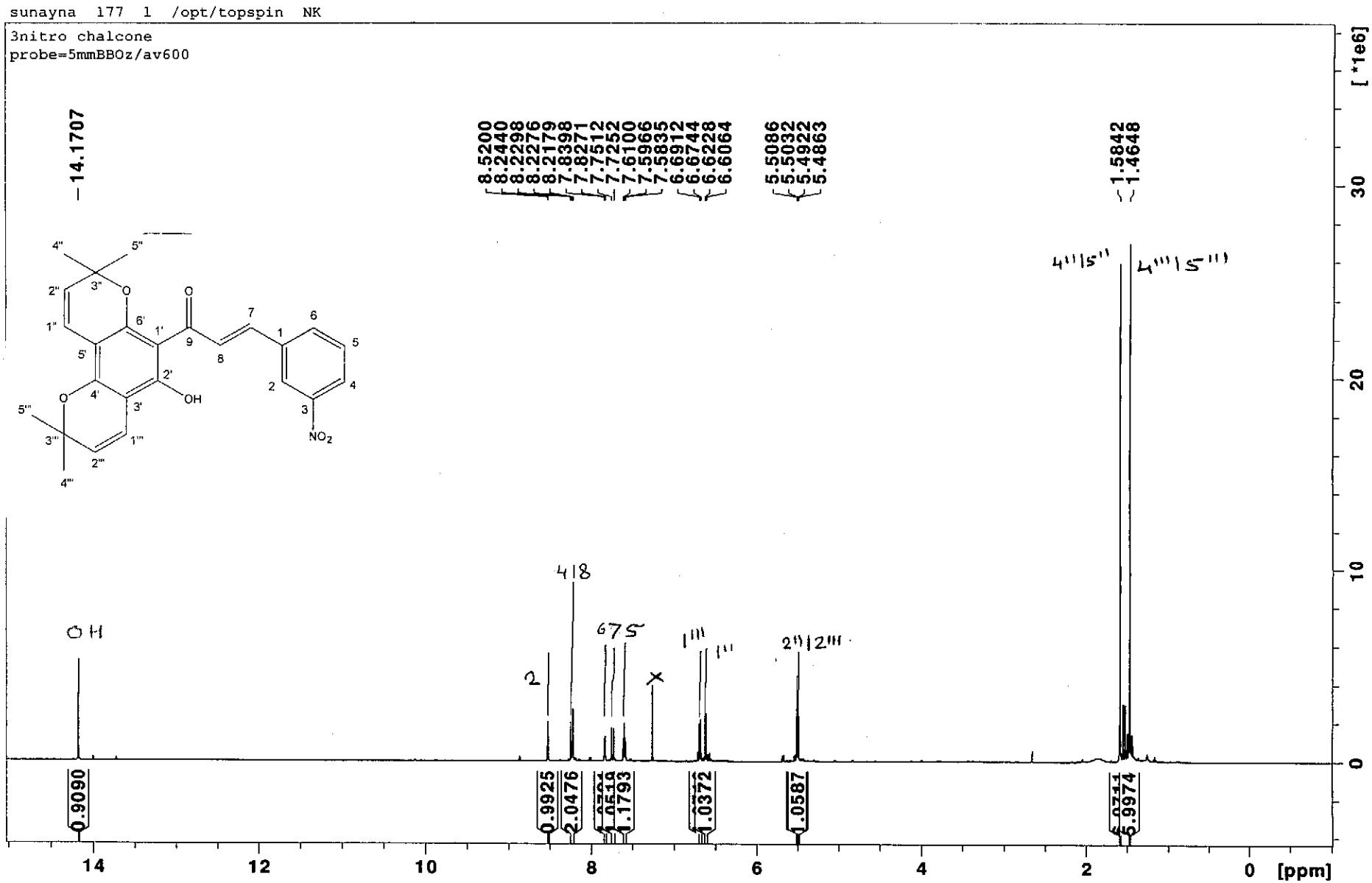
Dr. Nizam
21 9 (0.136) Cm (1:30)

TOF MS ES+
1.16e+005

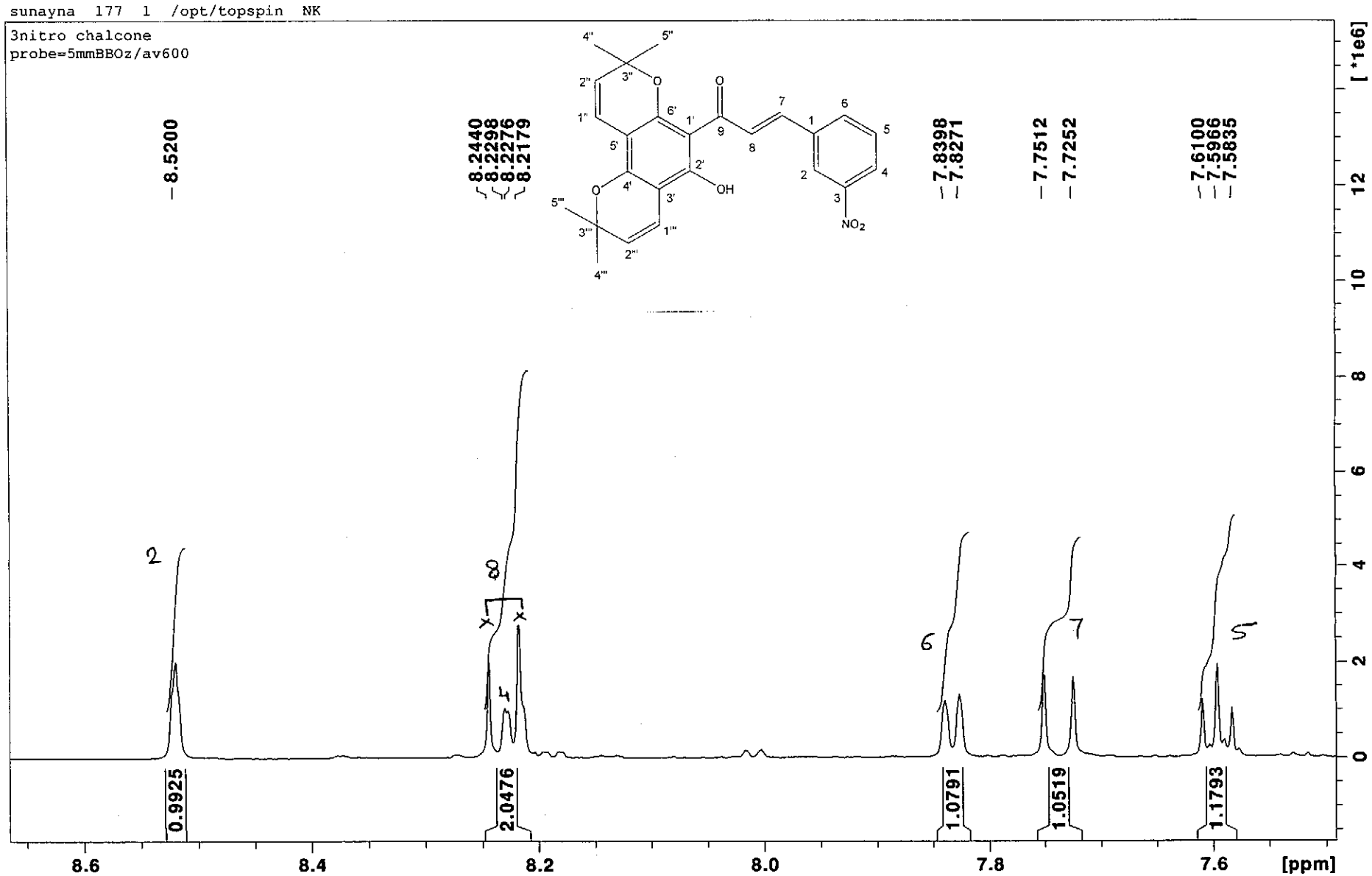


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
489.0677	489.0677	0.0	0.0	13.5	445.5	0.0	C25 H23 O4 Na Br

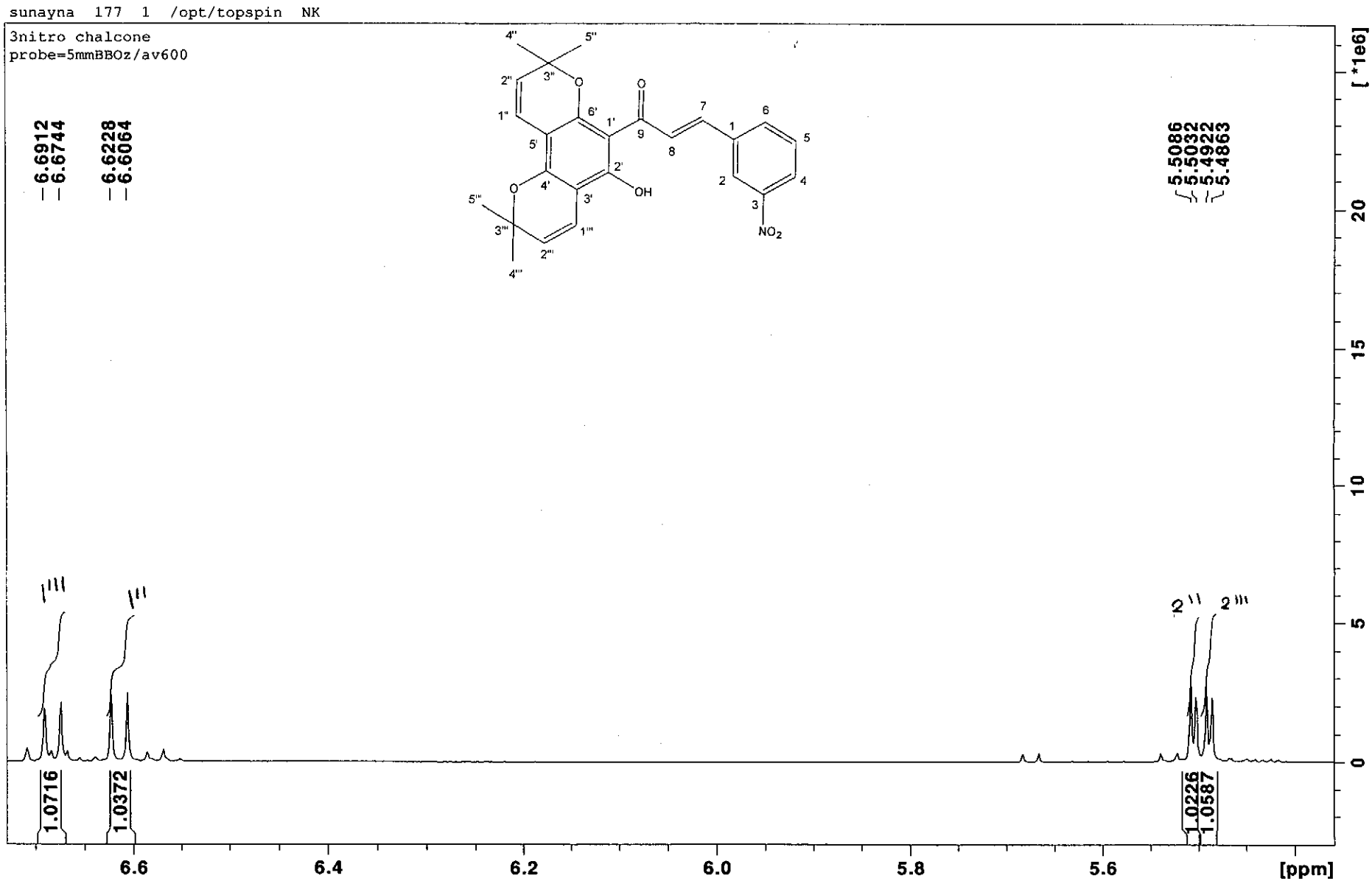
HRMS spectrum of 4-bromopyranochromene chalcone (A-3k)



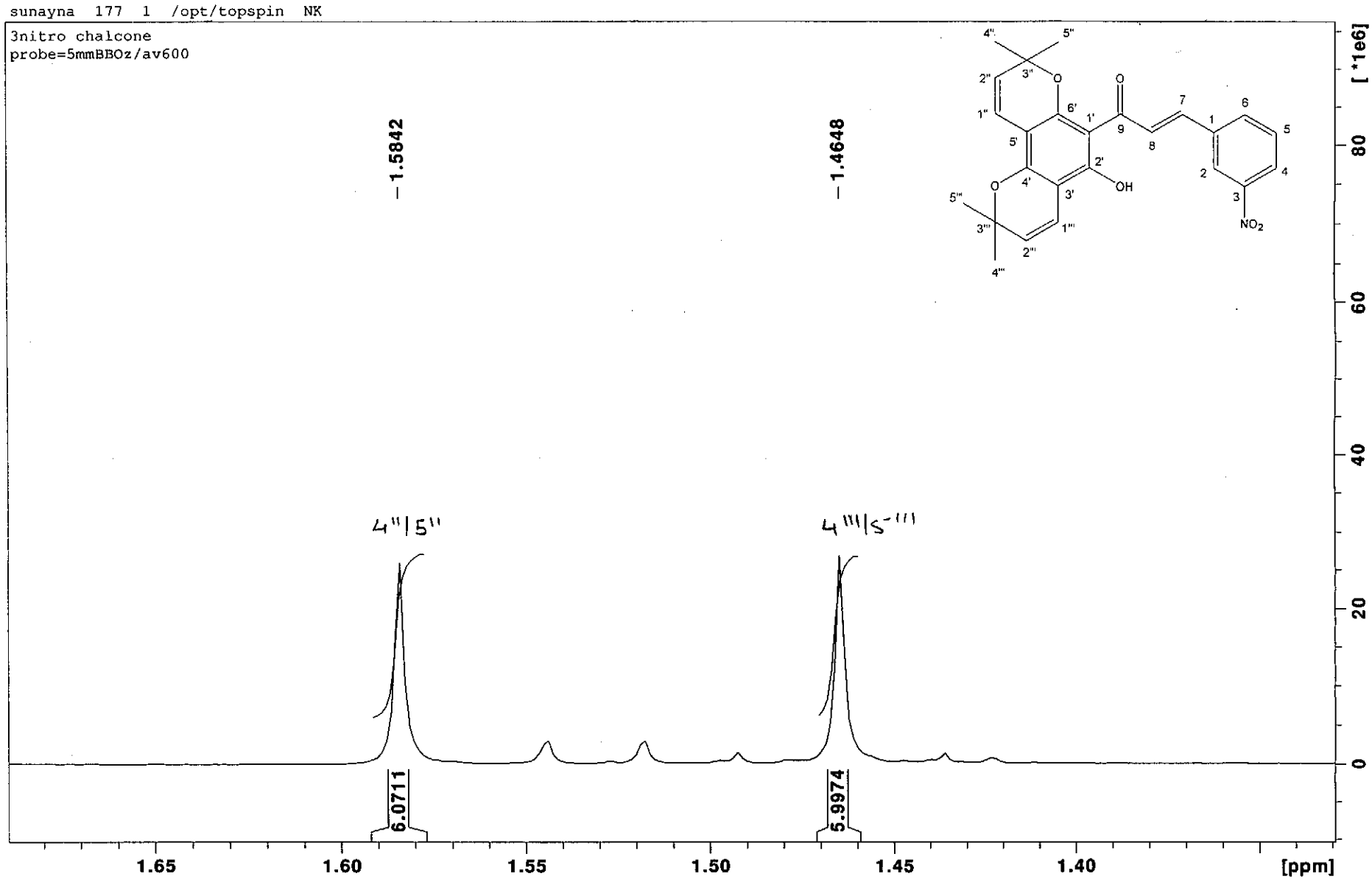
1H NMR spectrum of 3-nitropyranochromene chalcone (A-3l)



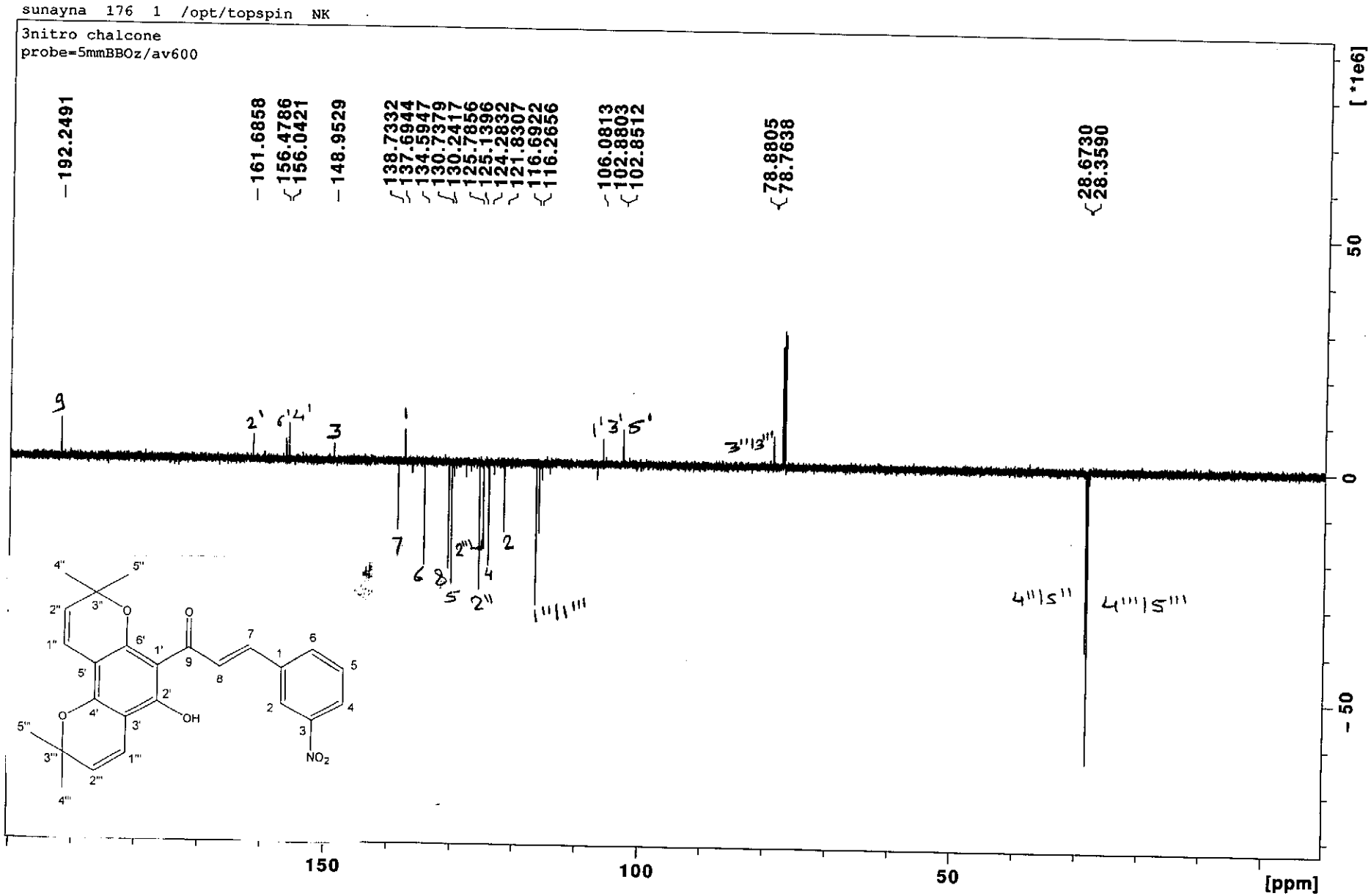
Expanded 1H NMR spectrum of 3-nitropyranochromene chalcone (A-3l)



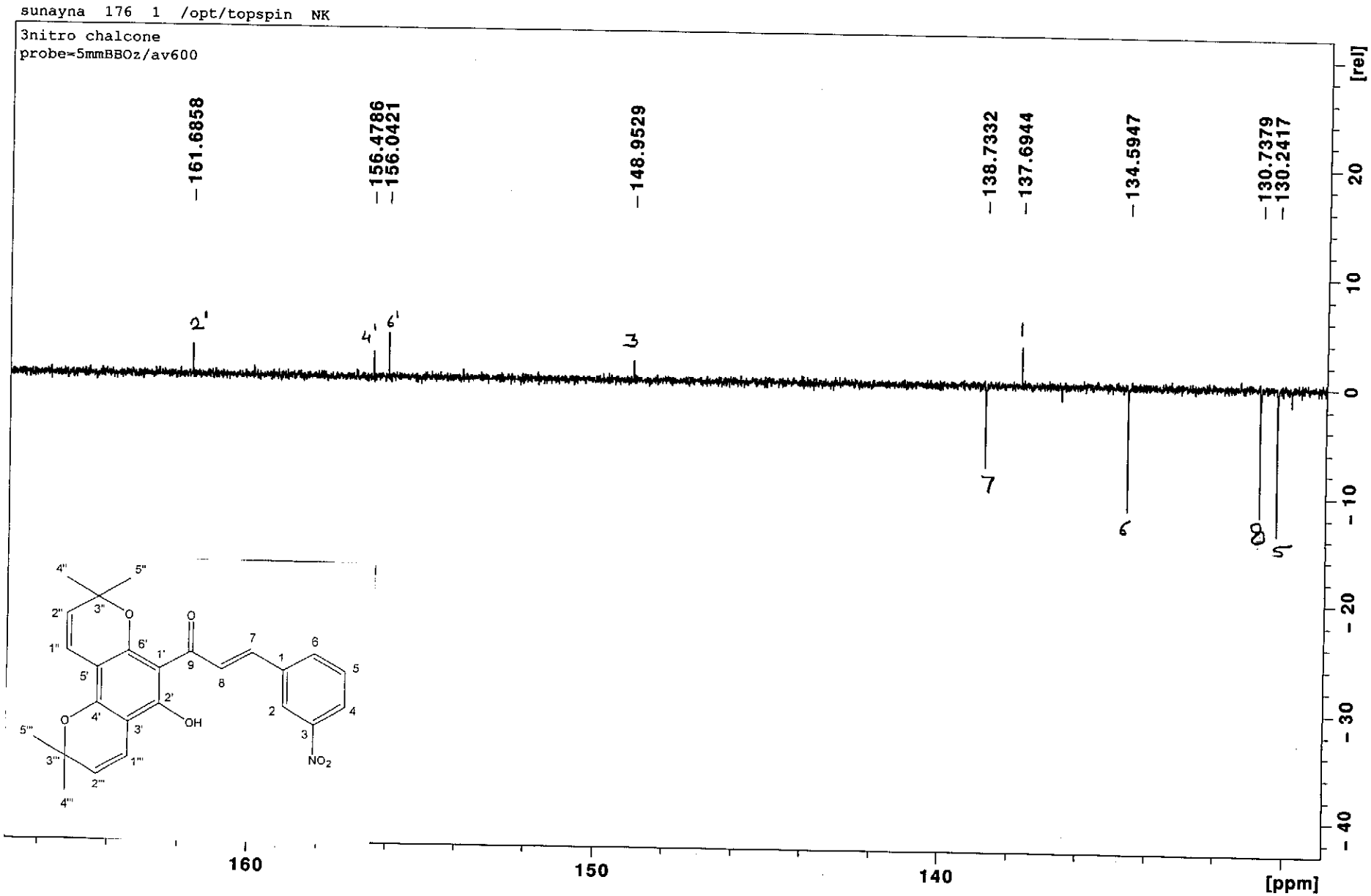
Expanded 1H NMR spectrum of 3-nitropyranochromene chalcone (A-3l)



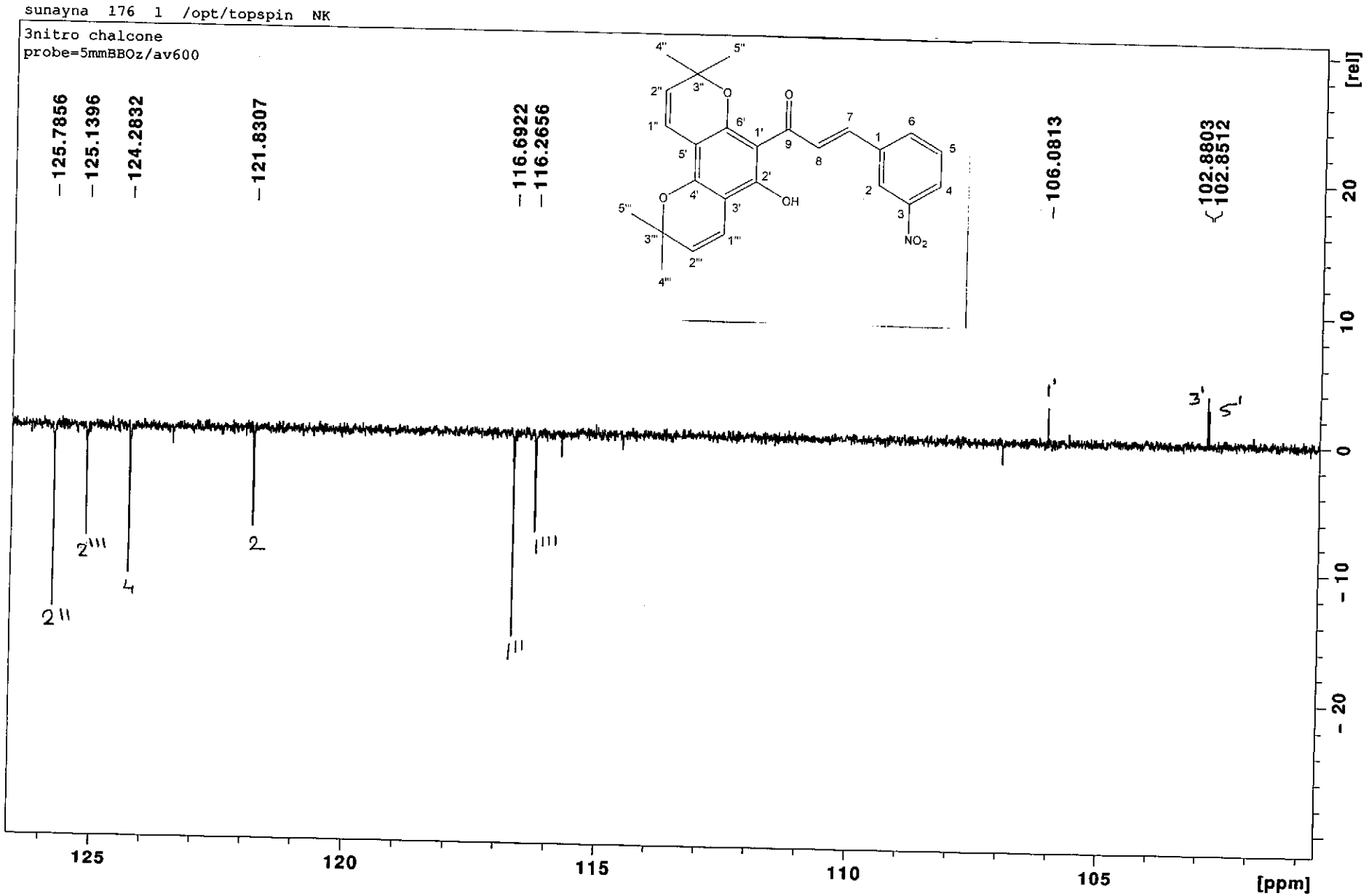
Expanded 1H NMR spectrum of 3-nitropyranochromene chalcone (A-3l)



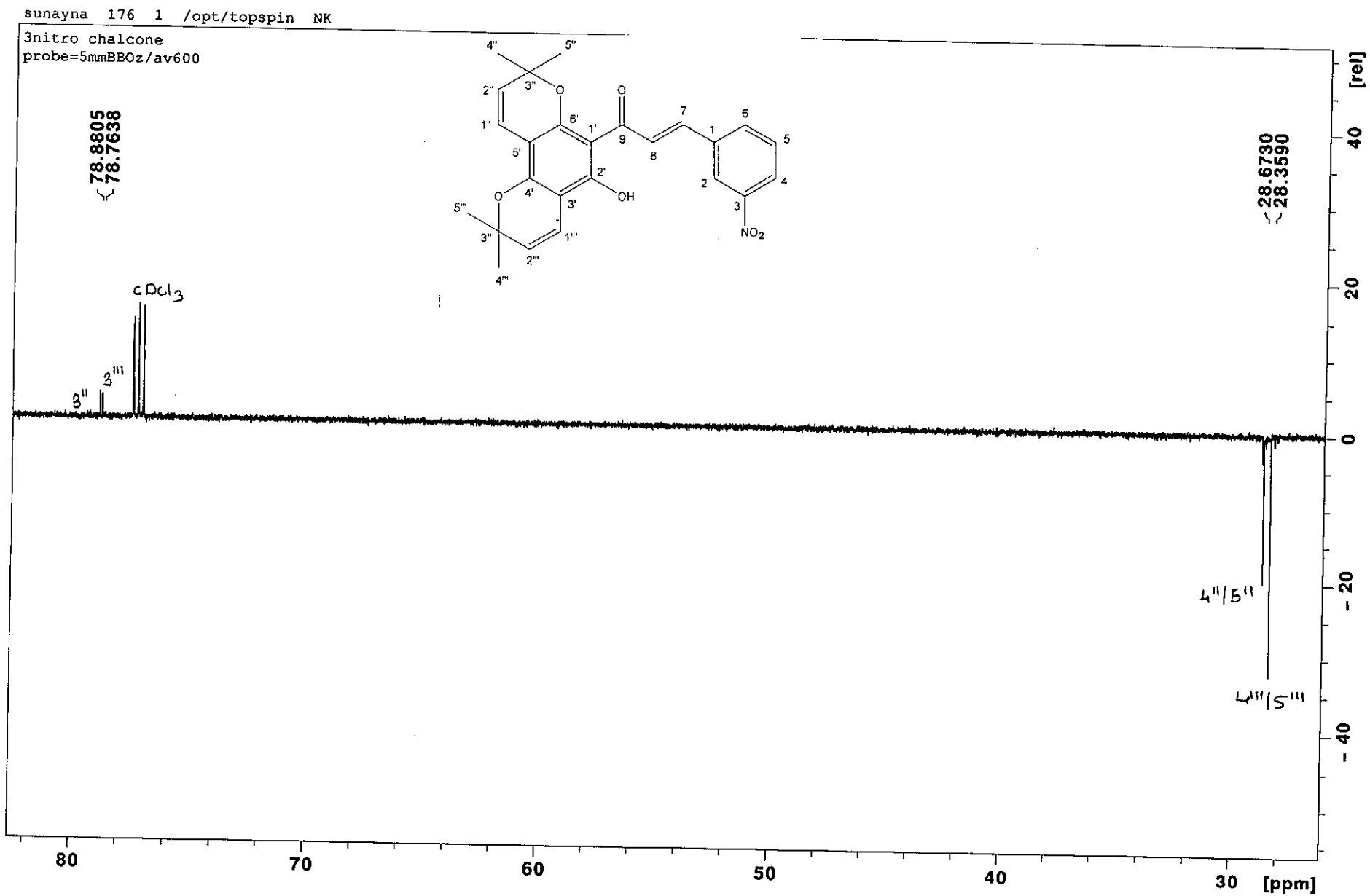
13C NMR spectrum of 3-nitropyranochromene chalcone (A-3l)



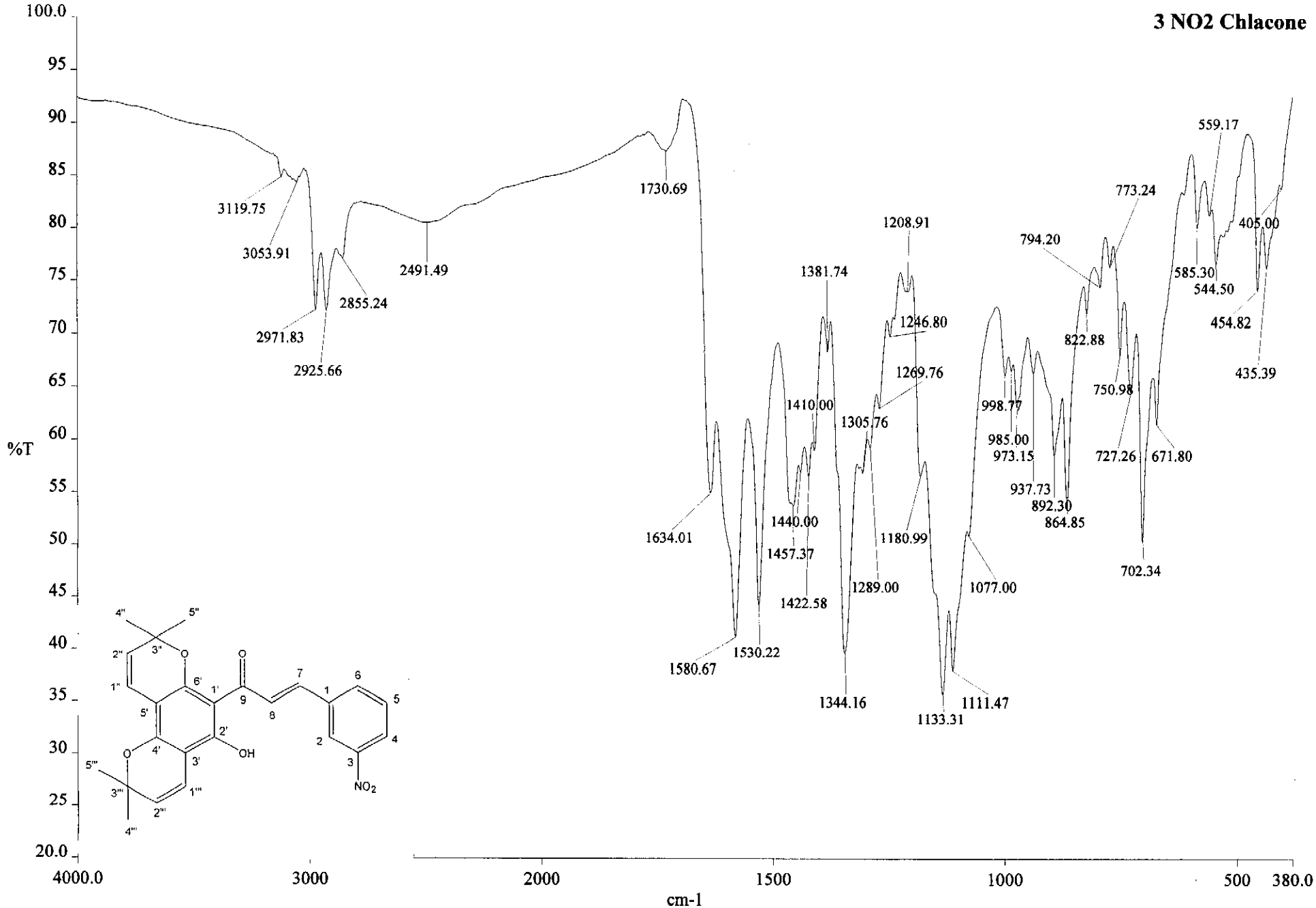
Expanded 13C NMR spectrum of 3-nitropyranochromene chalcone (A-3l)



Expanded 13C NMR spectrum of 3-nitropyranochromene chalcone (A-3l)

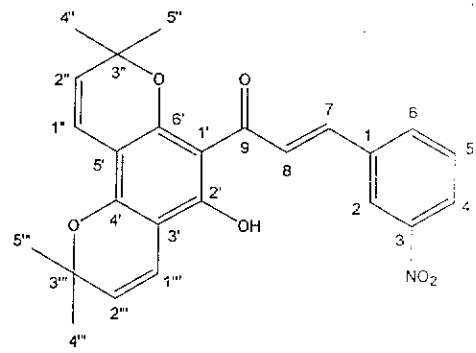


Expanded 13C NMR spectrum of 3-nitropyranochromene chalcone (A-3l)



Infrared spectrum of 3-nitropyranochromene chalcone (A-3l)

Elemental Composition Report



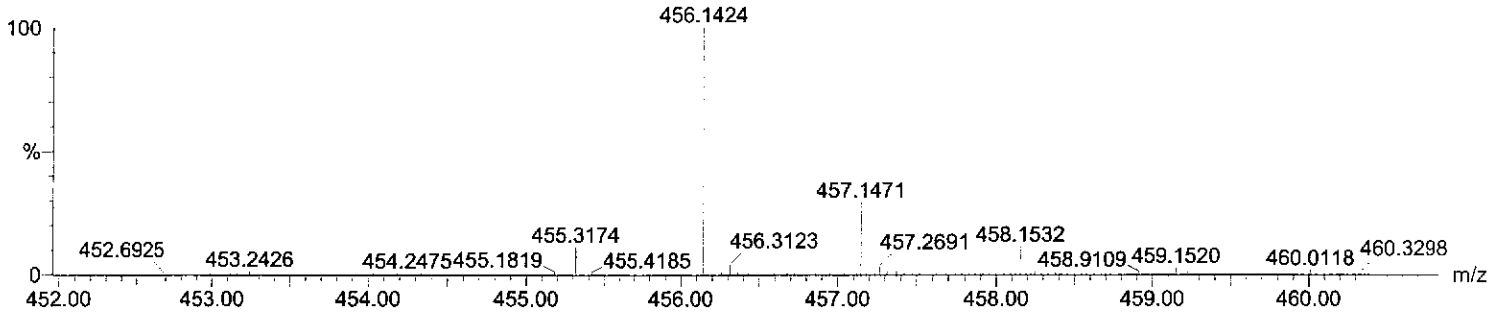
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
67 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-30 H: 20-25 N: 0-5 O: 0-10 Na: 0-1

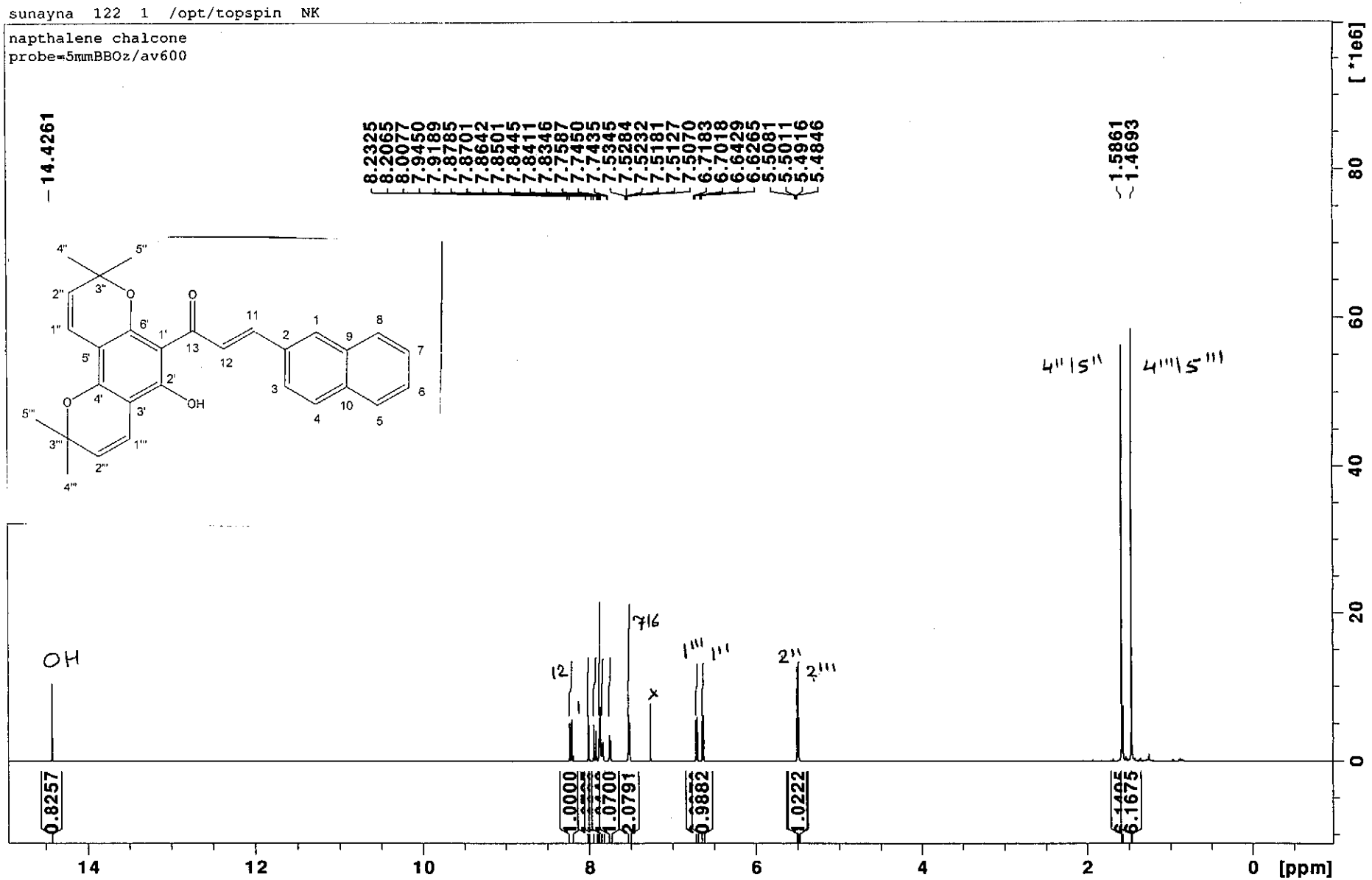
Dr. Nizam
25 2 (0.017) Cm (1:30)

TOF MS ES+
3.61e+003

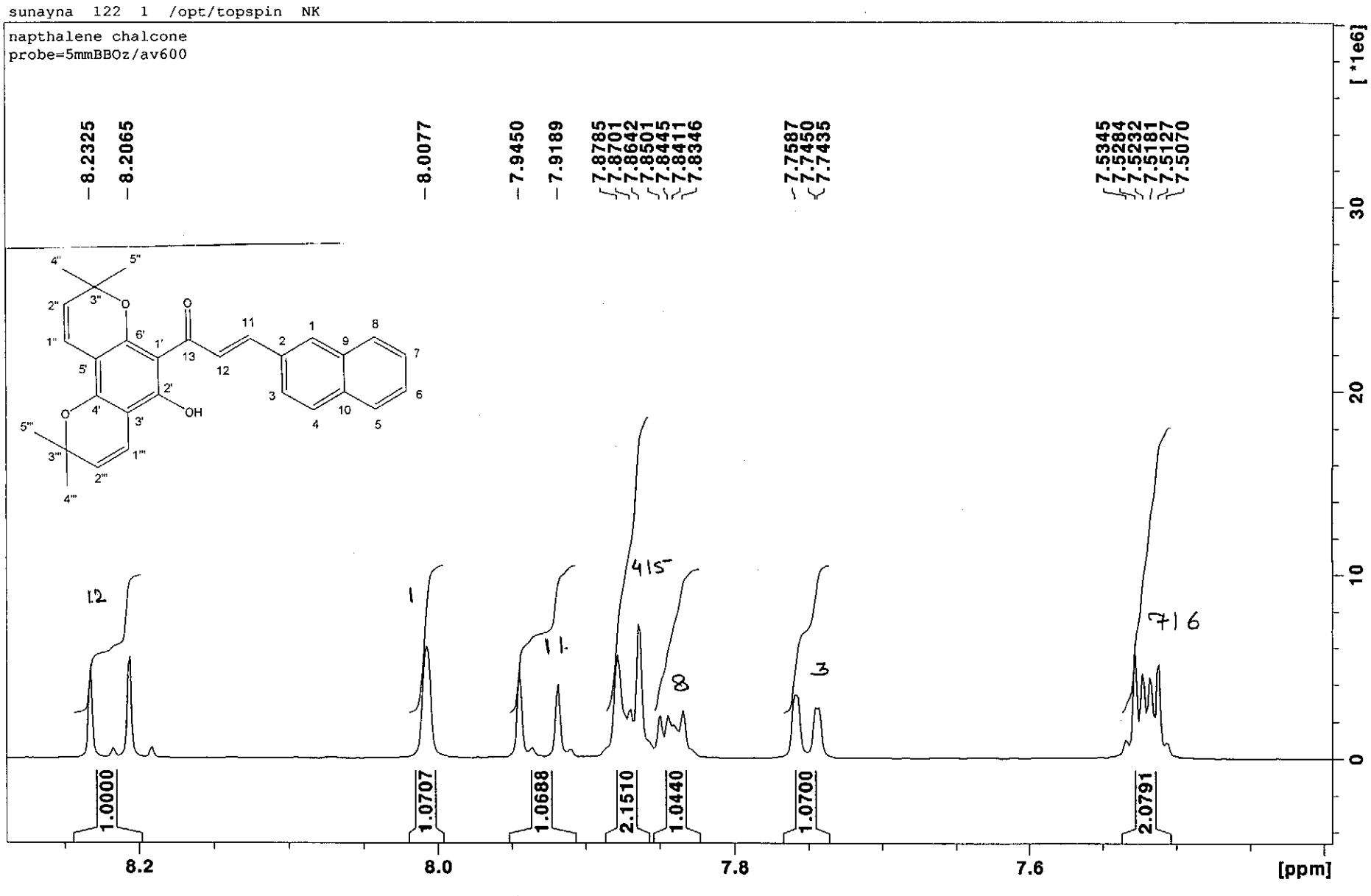


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
456.1424	456.1423	0.1	0.2	14.5	321.3	0.0	C25 H23 N O6 Na

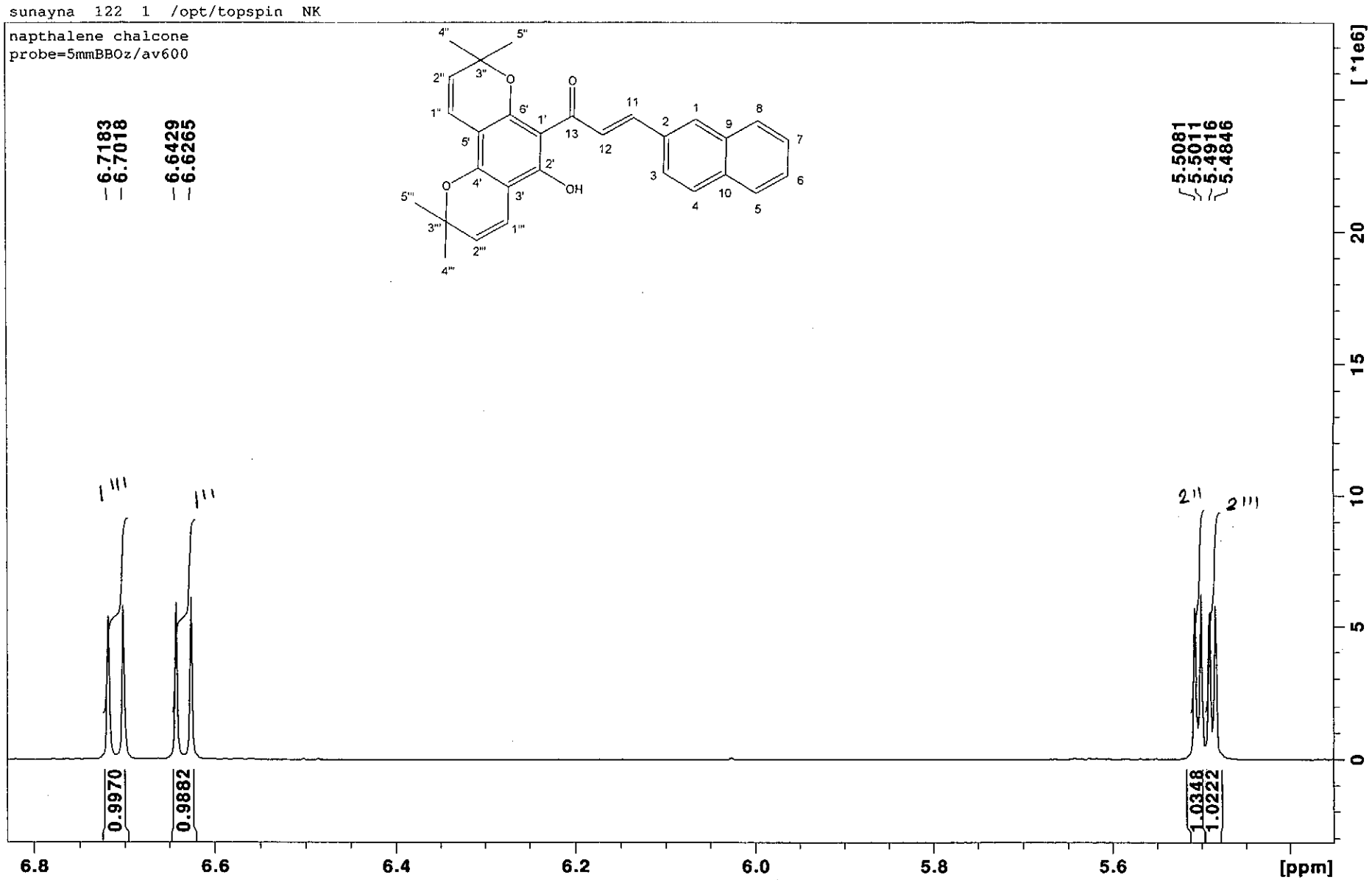
HRMS of 3-nitropyranochromene chalcone (A-3l)



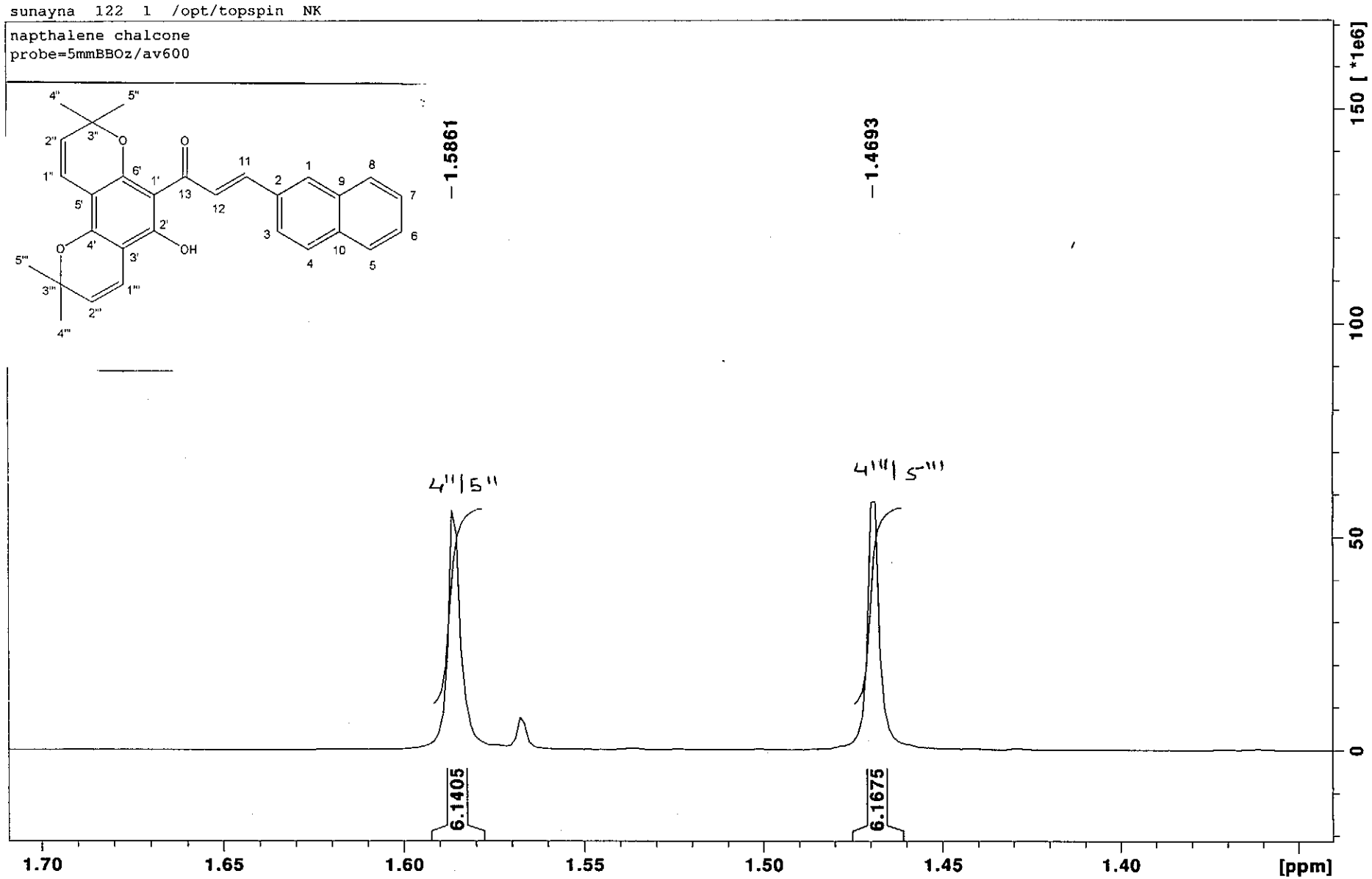
1H NMR spectrum of naphthylpyranochromene chalcone (A-3m)



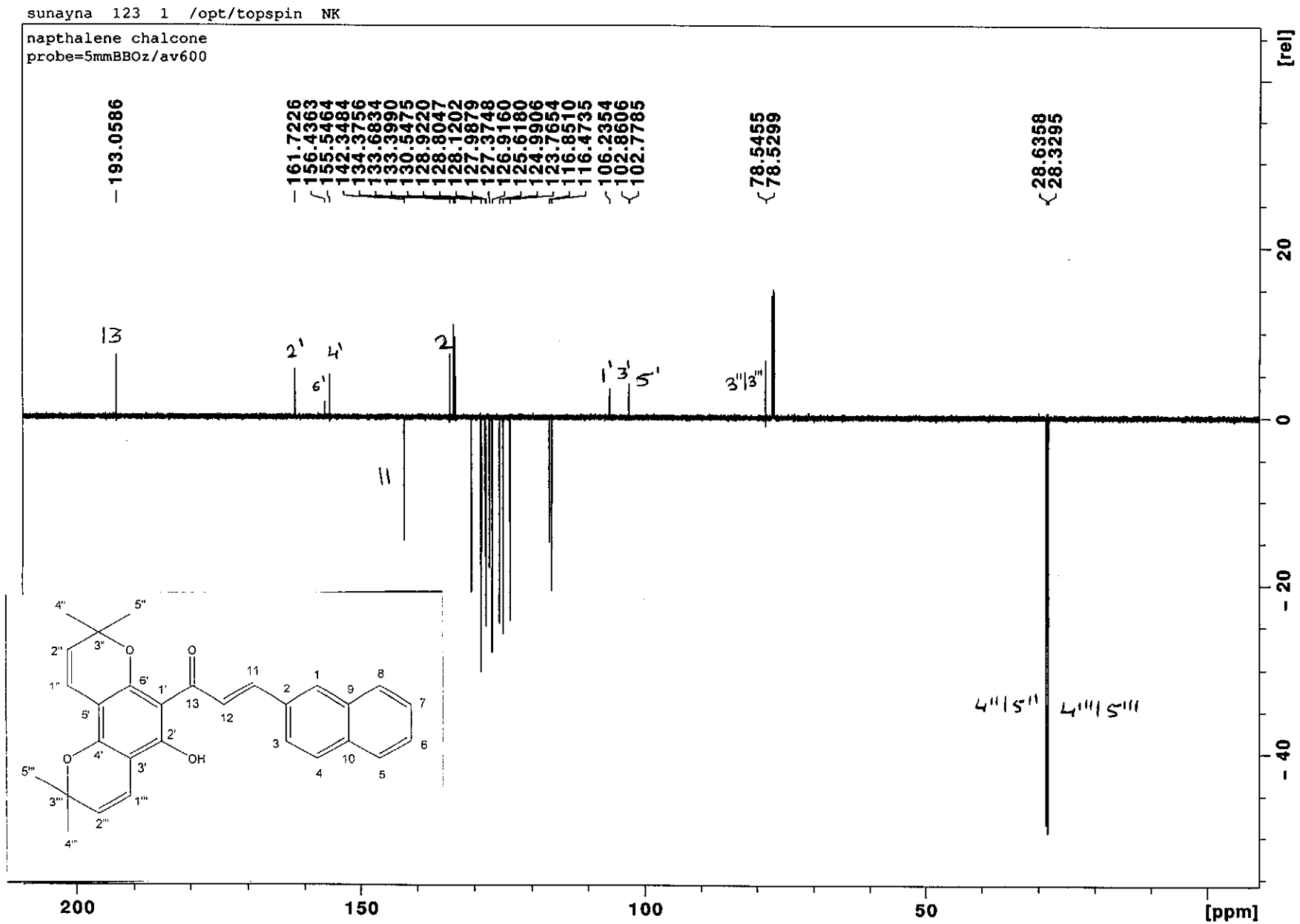
Expanded 1H NMR spectrum of naphthylpyranochromene chalcone (A-3m)



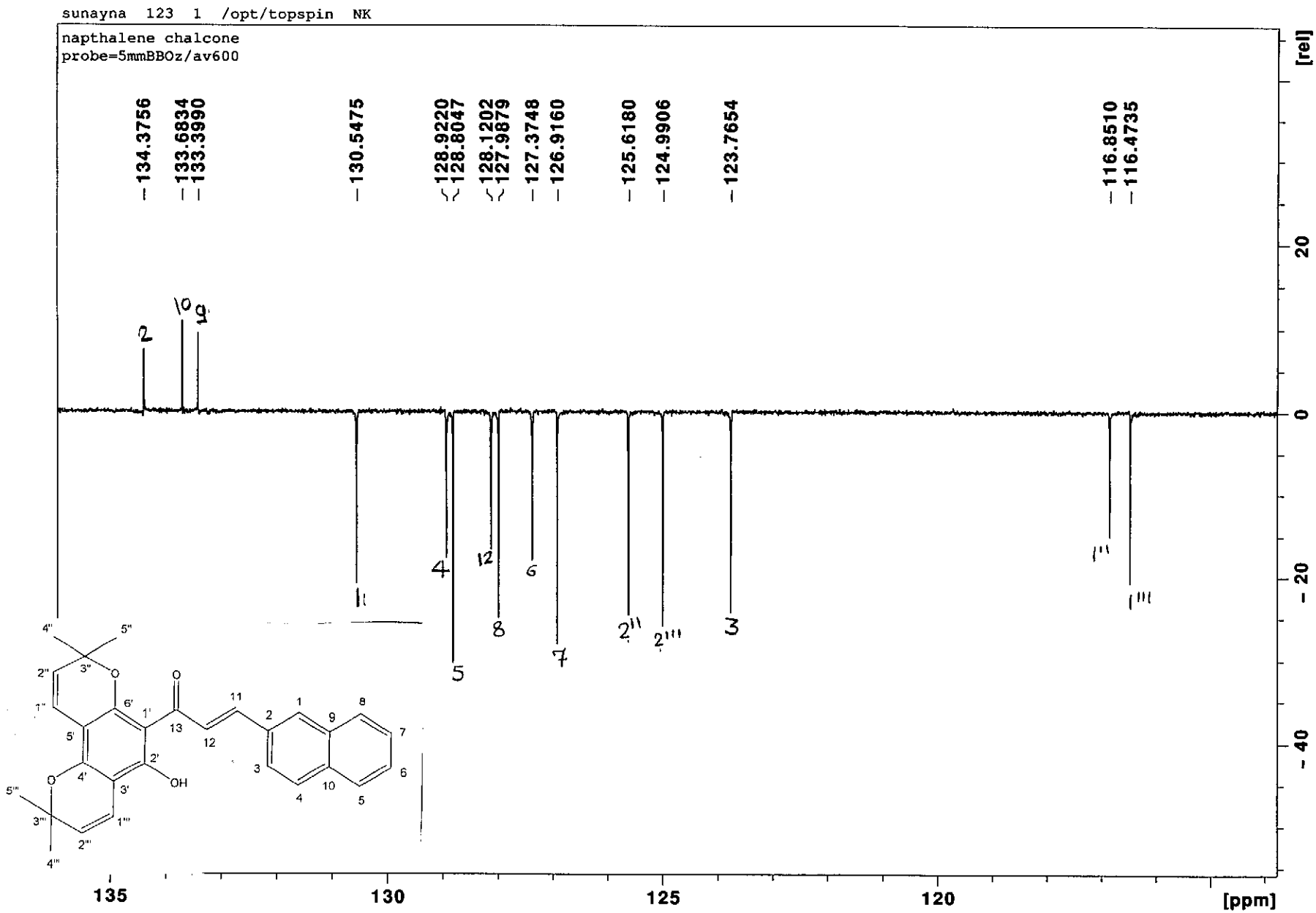
Expanded 1H NMR spectrum of naphthylpyranochromene chalcone (A-3m)



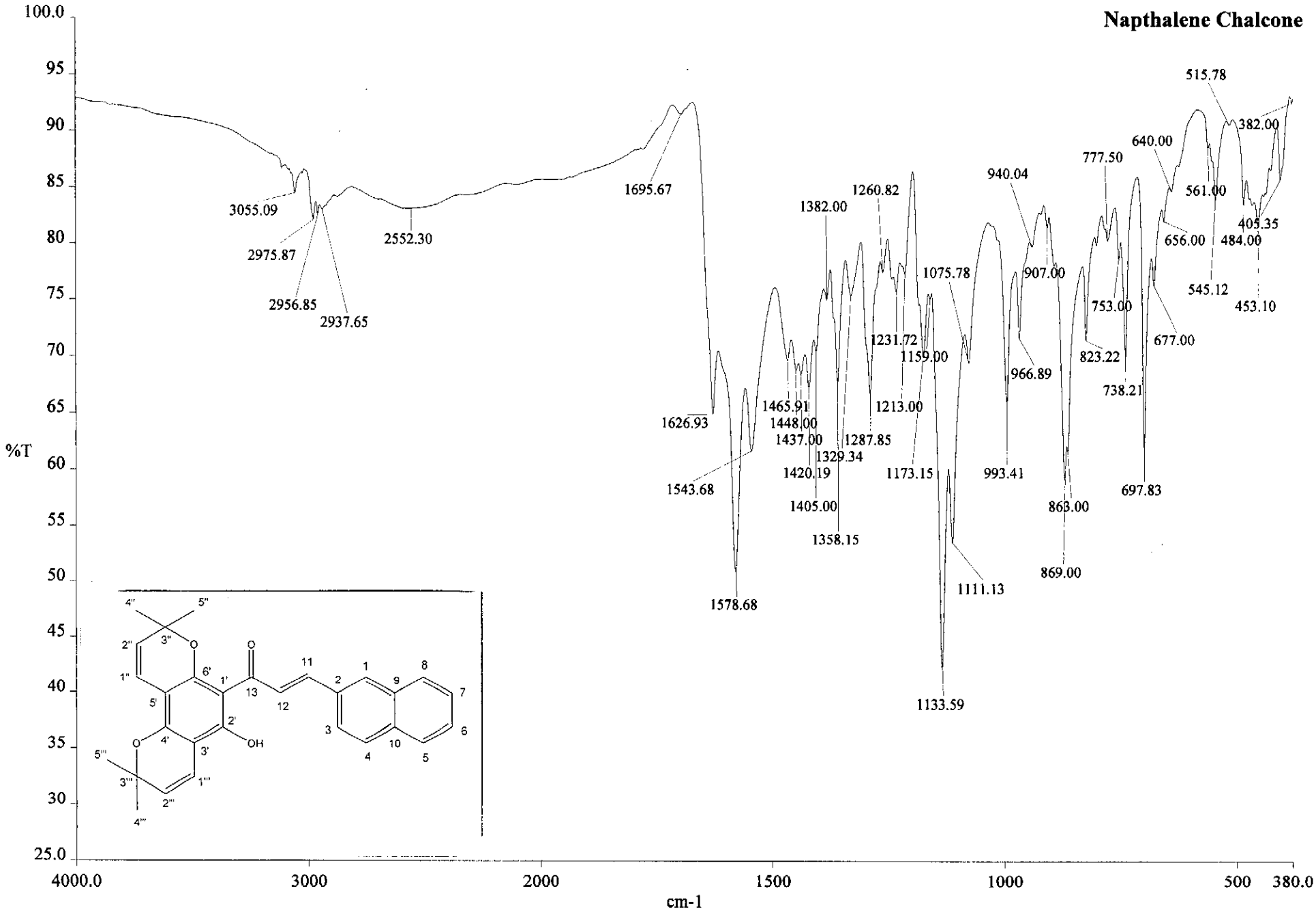
Expanded 1H NMR spectrum of naphthylpyranochromene chalcone (A-3m)



13C NMR spectrum of naphthylpyranochromene chalcone (A-3m)



Expanded 13C NMR spectrum of naphthylpyranochromene chalcone (A-3m)

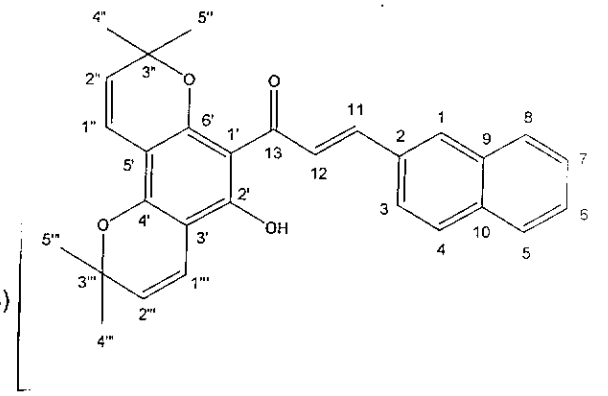


Infrared spectrum of naphthylpyranochromene chalcone (A-3m)

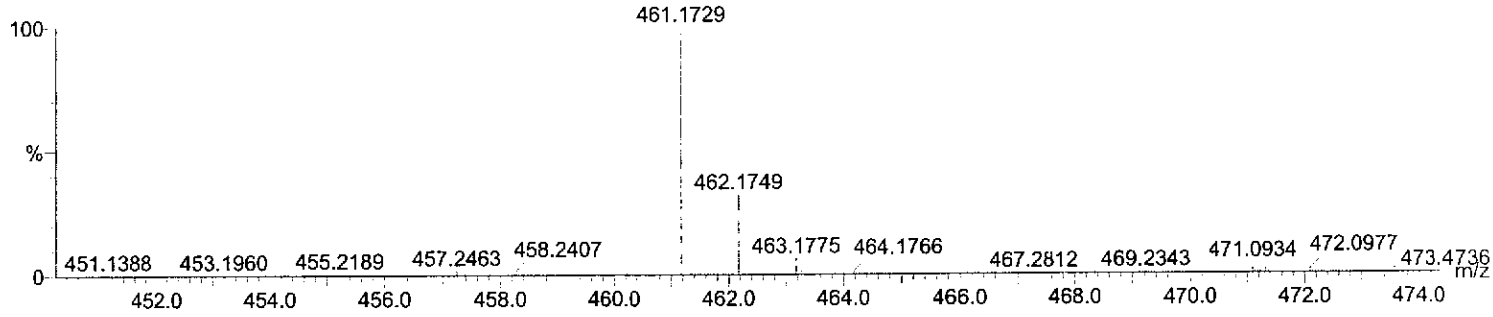
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
3 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-30 H: 25-30 O: 0-5 Na: 0-1
Dr. Nizam
24 11 (0.170) Cm (1:29)

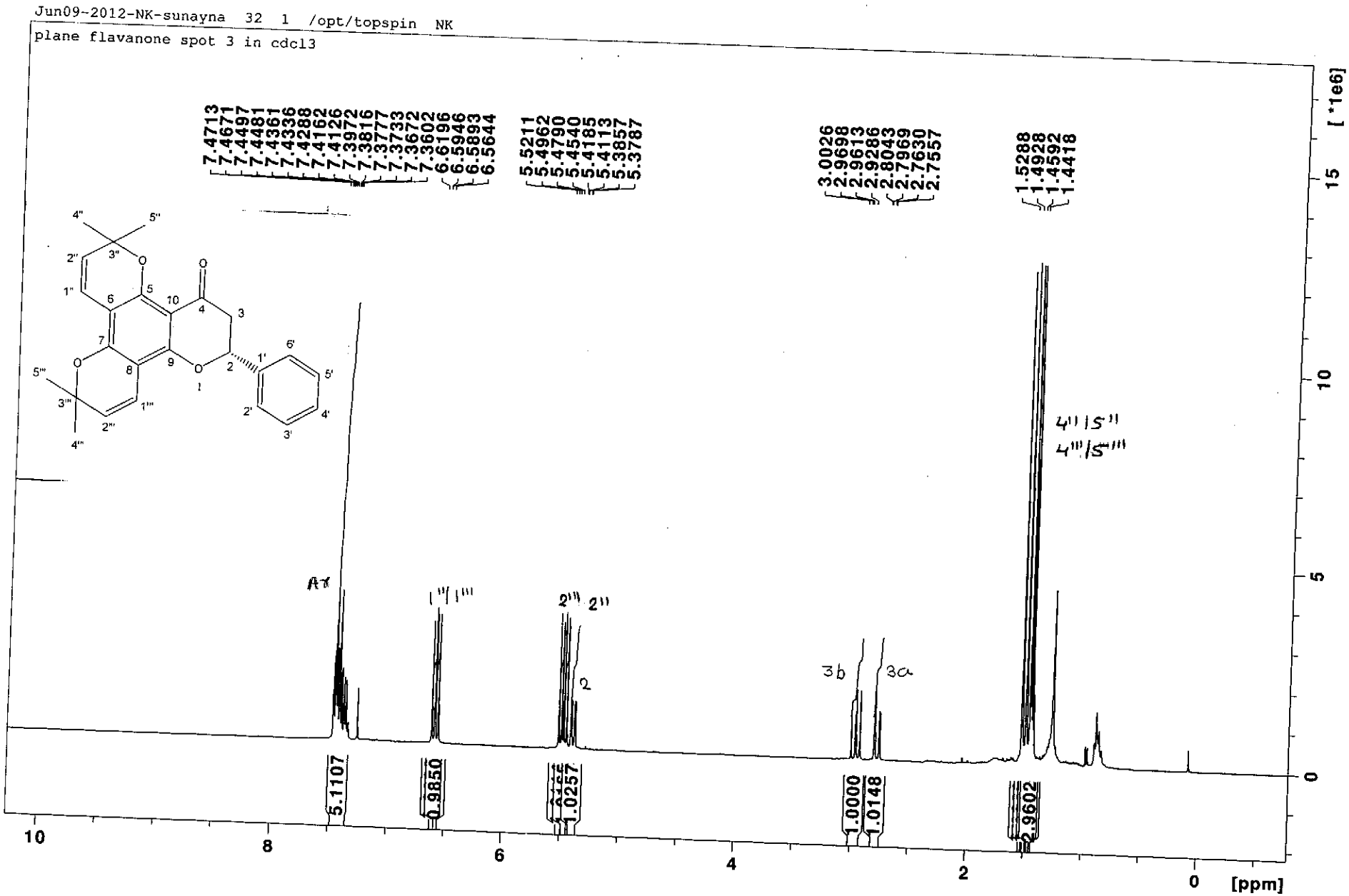


TOF MS ES+
8.08e+003

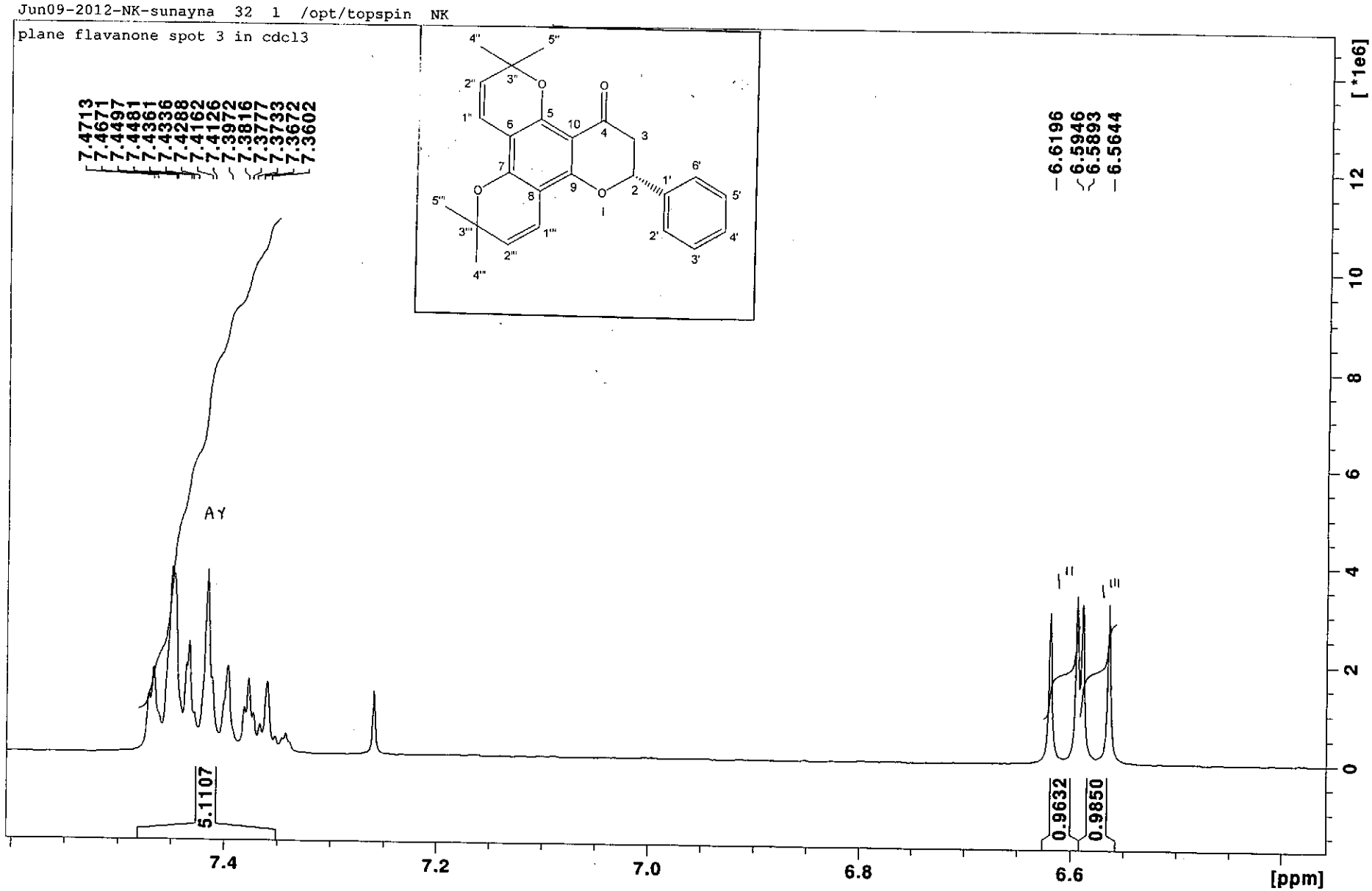


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
461.1729	461.1729	0.0	0.0	16.5	322.1	0.0	C29 H26 O4 Na

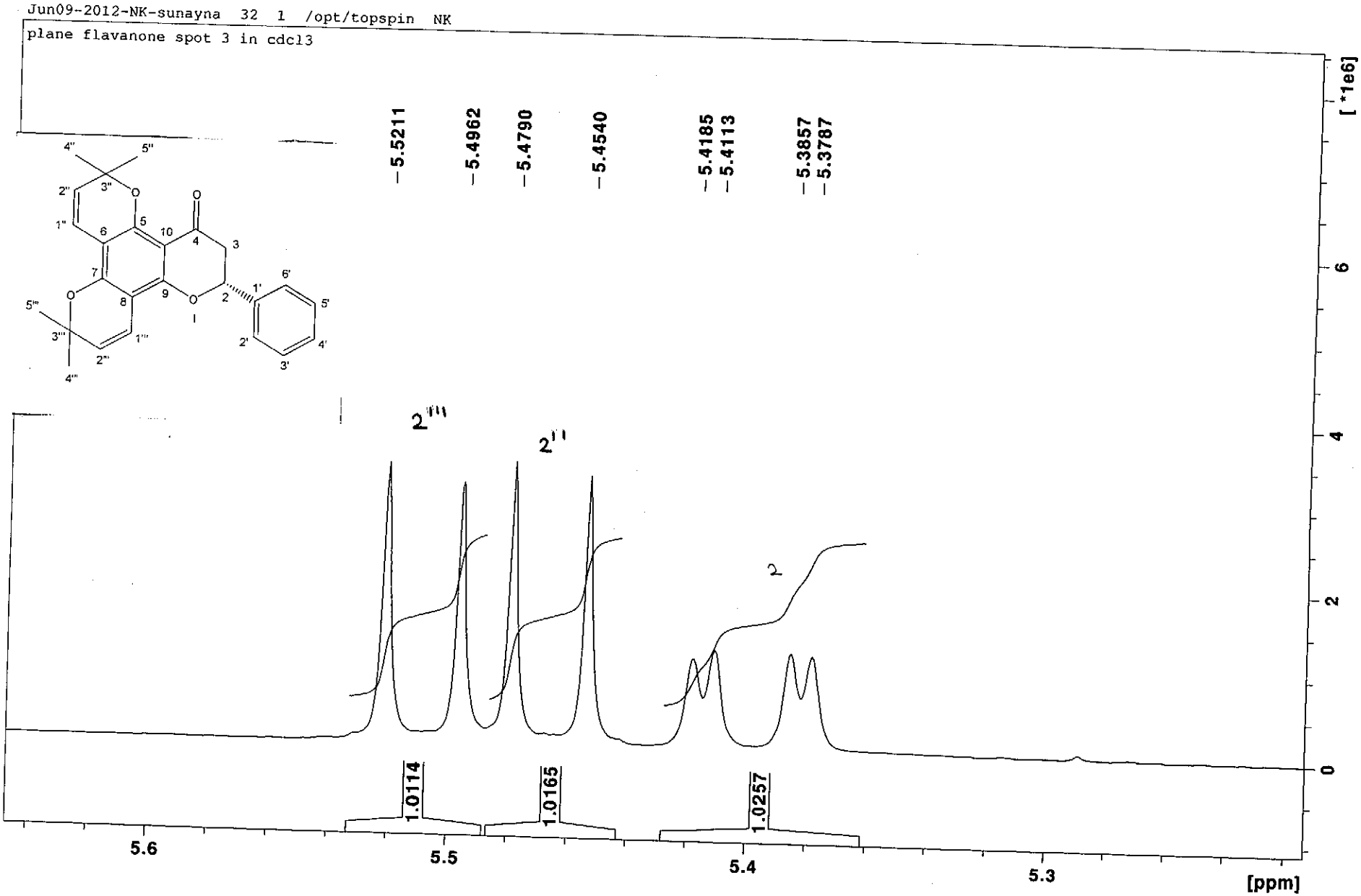
HRMS spectrum of naphthylpyranochromene chalcone (A-3m)



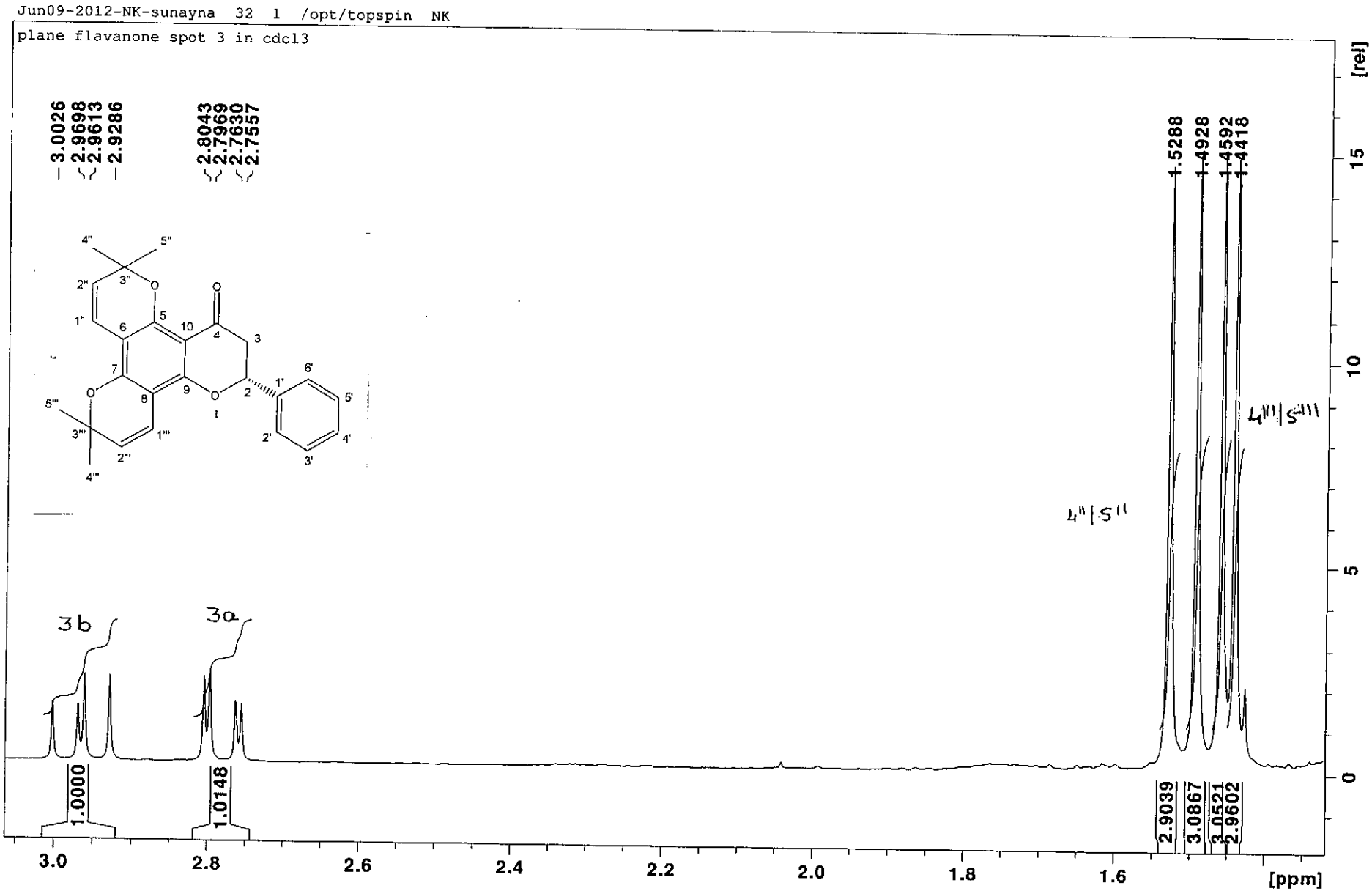
^{1}H NMR spectrum of 4a – Deoxy-MS-II (A-4a)



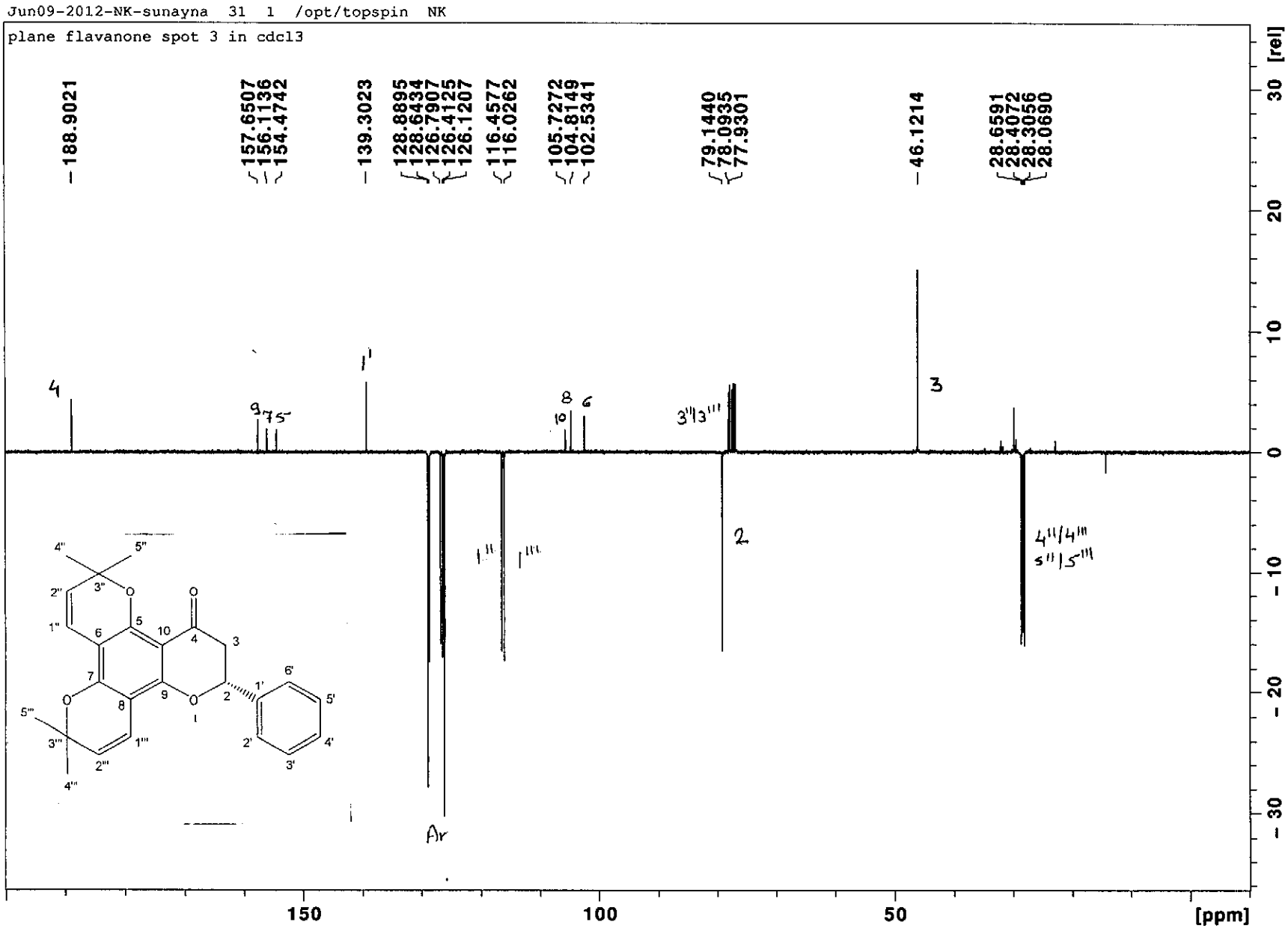
^{1}H NMR spectrum of 4a – Deoxy-MS-II (A-4a)



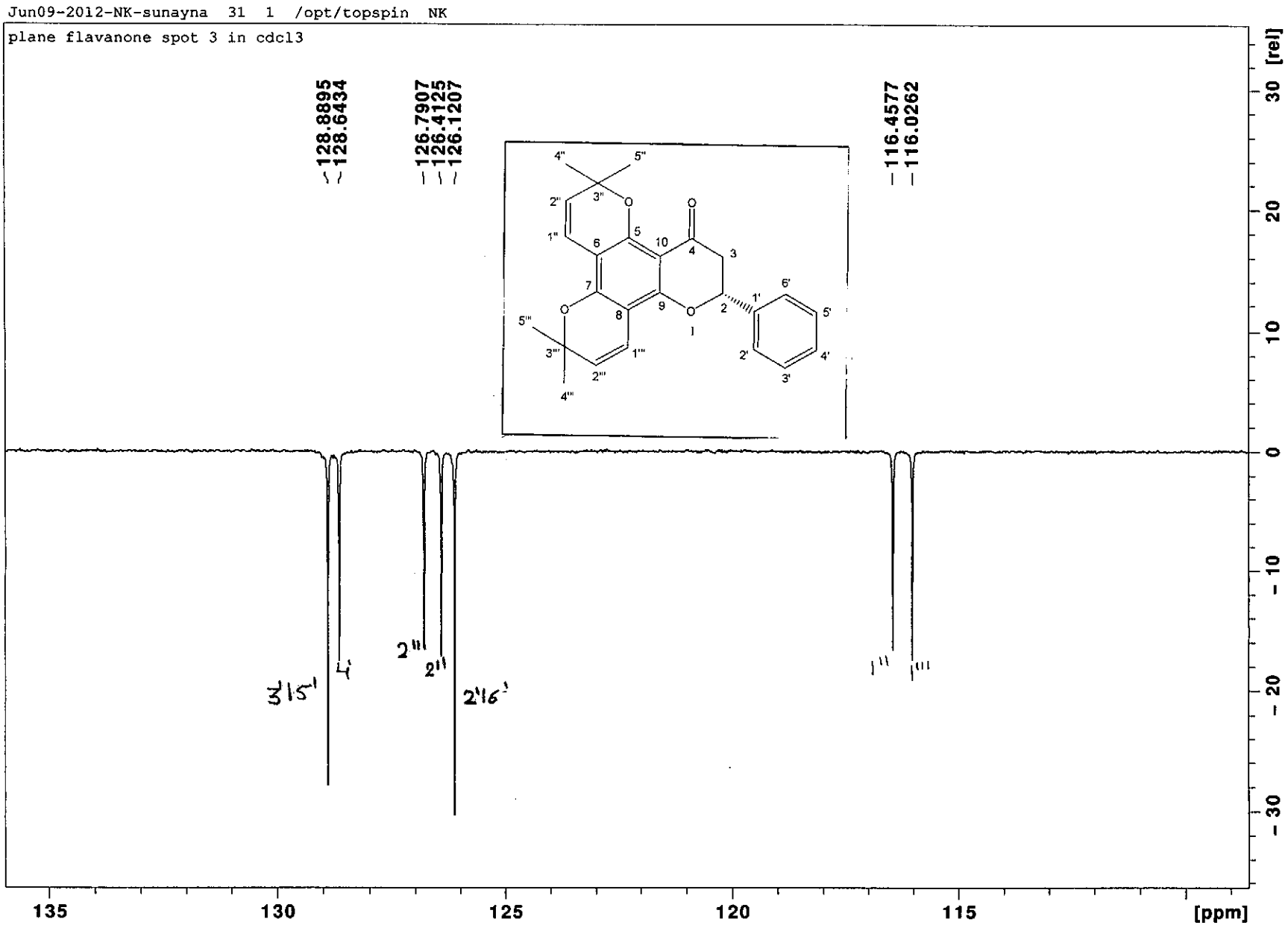
^{1}H NMR spectrum of 4a – Deoxy-MS-II (A-4a)



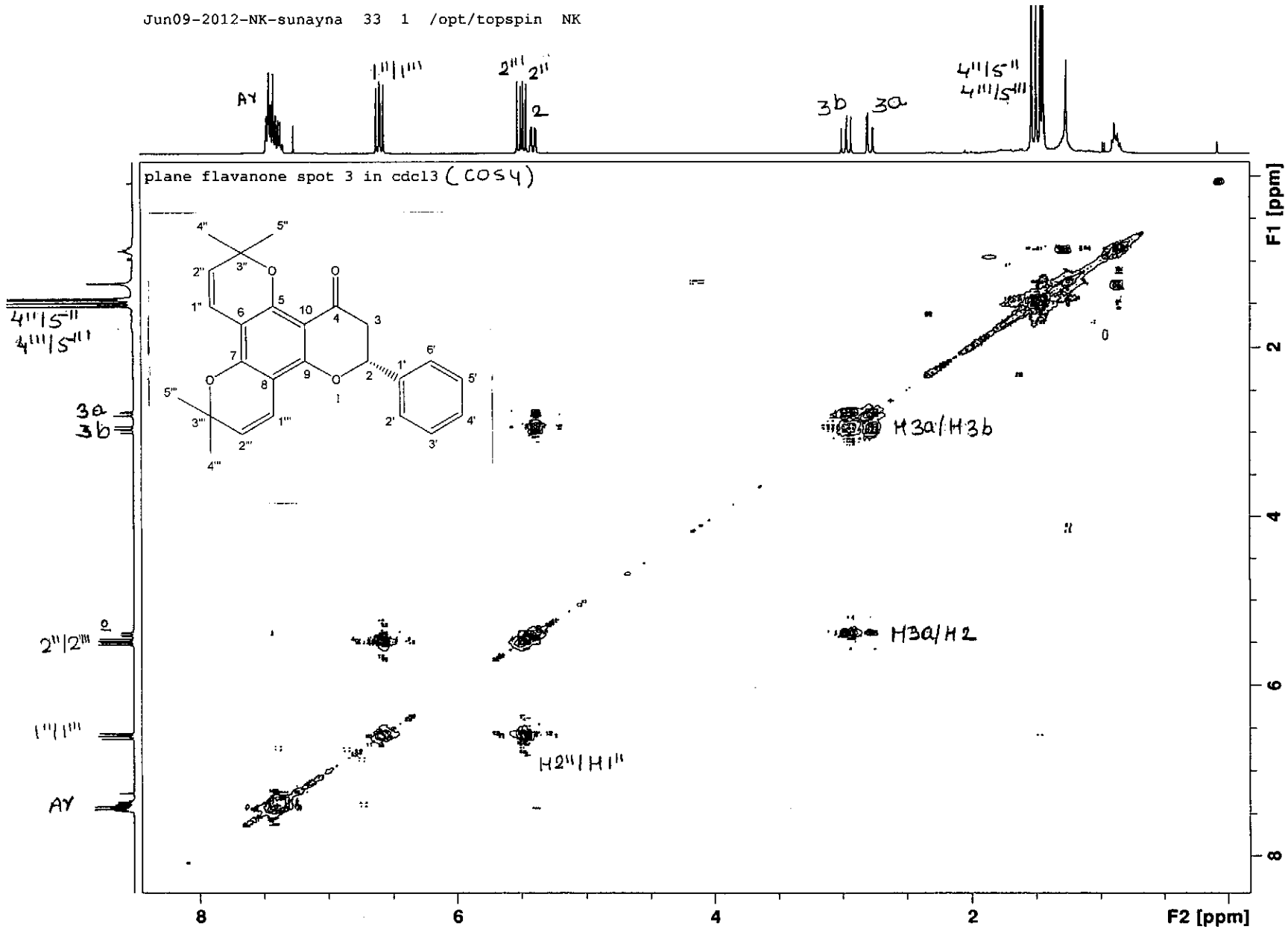
^{1}H NMR spectrum of 4a – Deoxy-MS-II (A-4a)



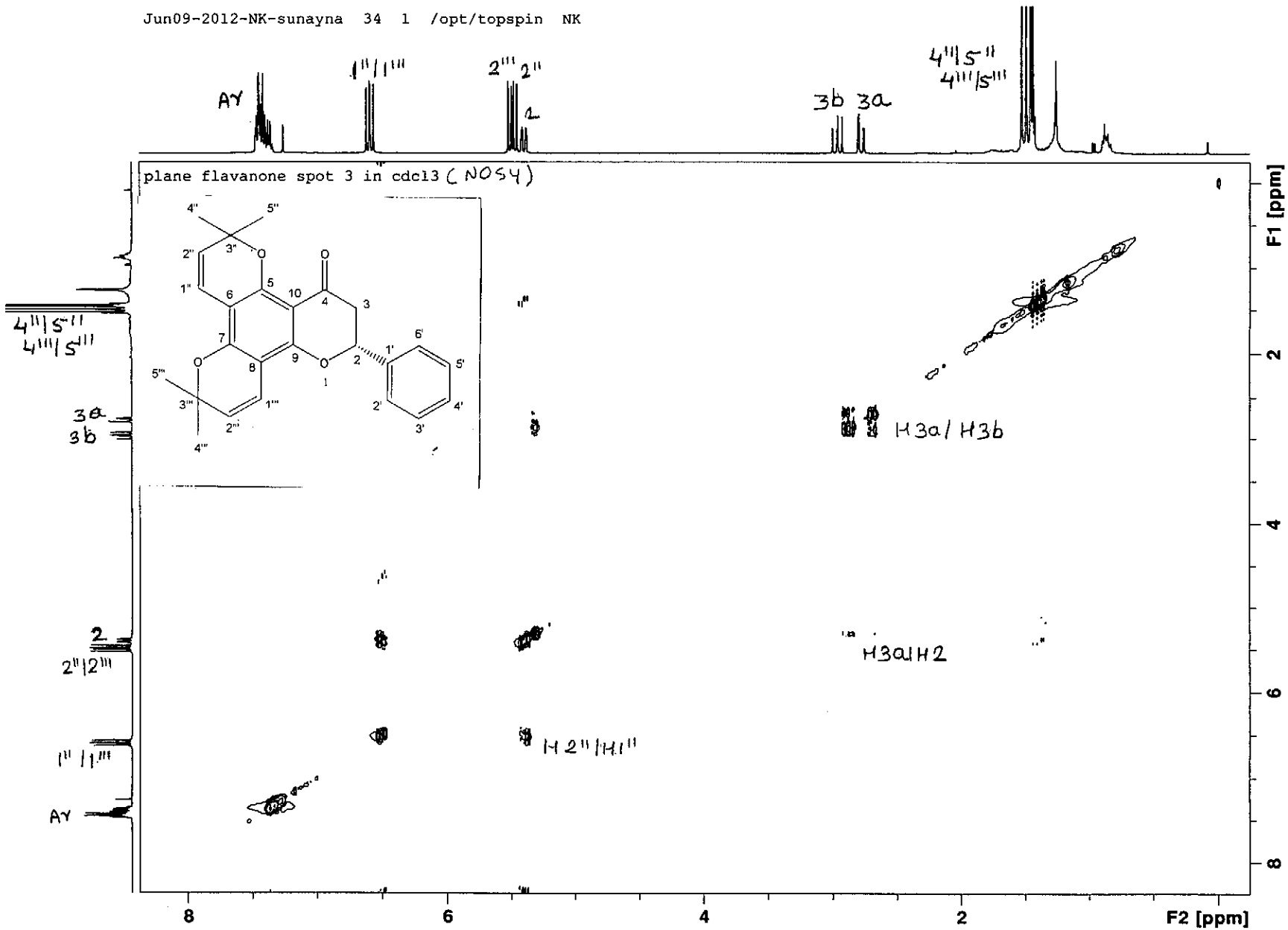
^{13}C NMR spectrum of 4a – Deoxy-MS-II (A-4a)



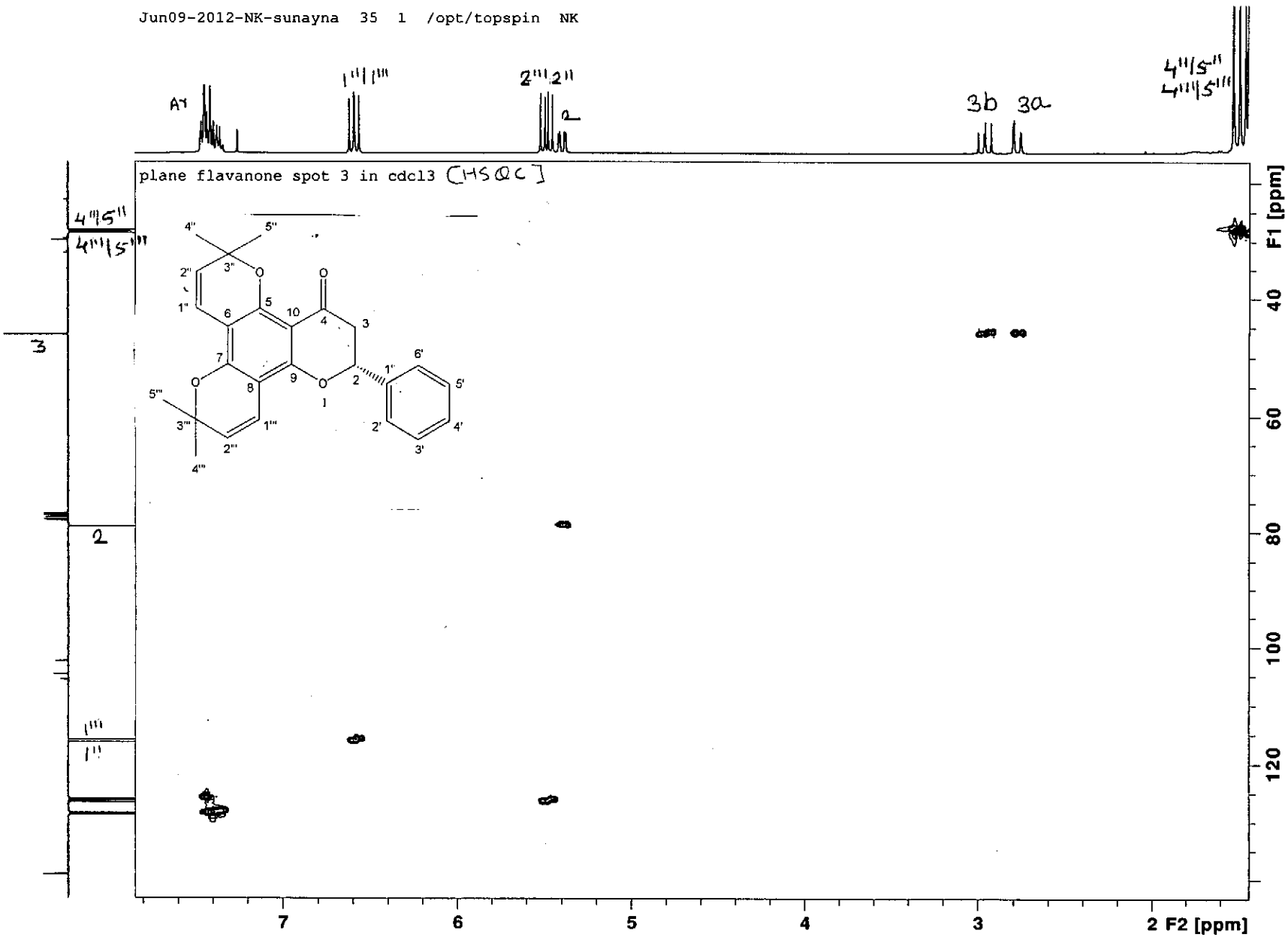
^{13}C NMR spectrum of 4a – Deoxy-MS-II (A-4a)



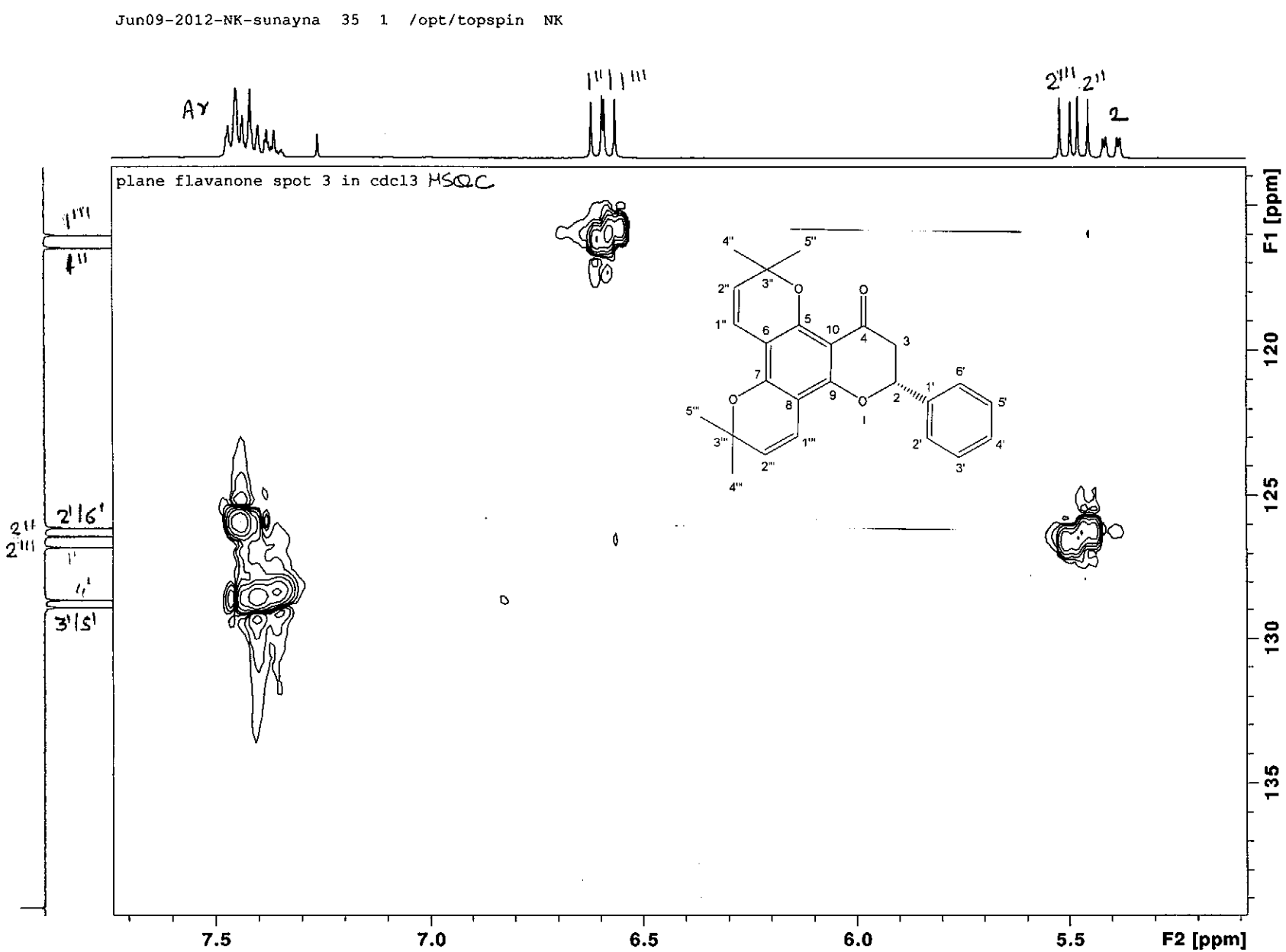
COSY NMR spectrum of 4a – Deoxy-MS-II (A-4a)



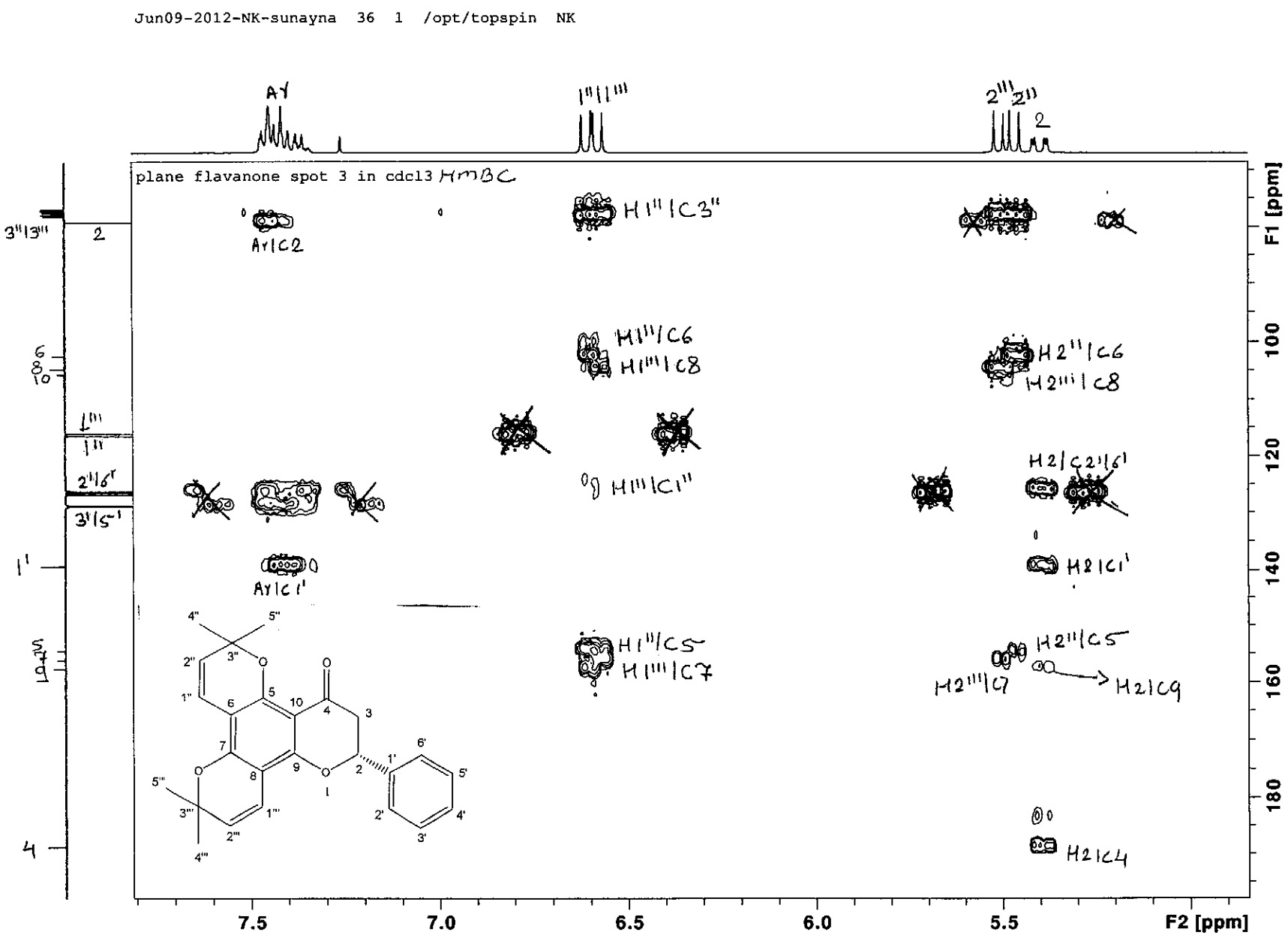
NOSY NMR spectrum of 4a – Deoxy-MS-II (A-4a)



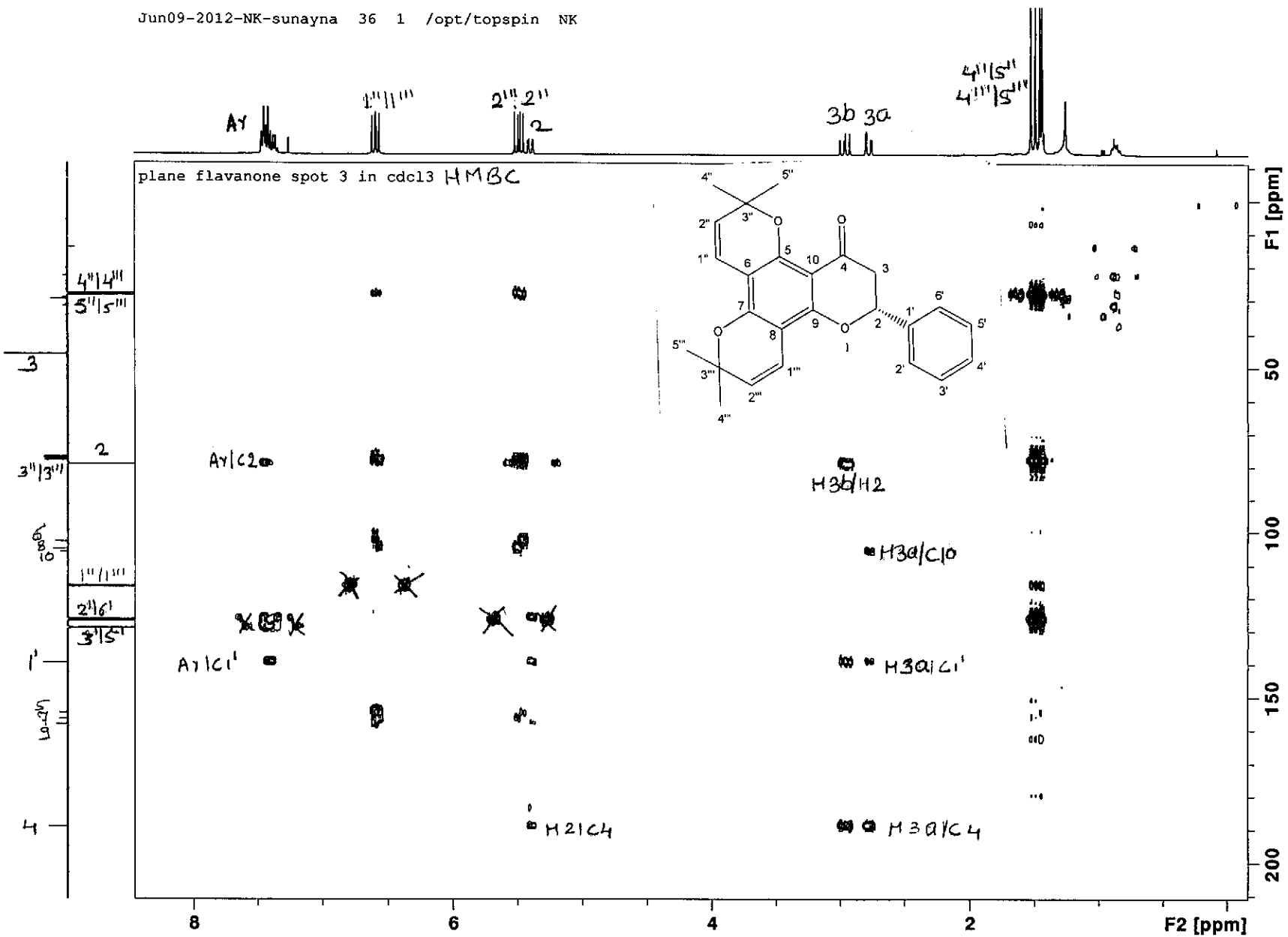
HSQC NMR spectrum of 4a – Deoxy-MS-II (A-4a)



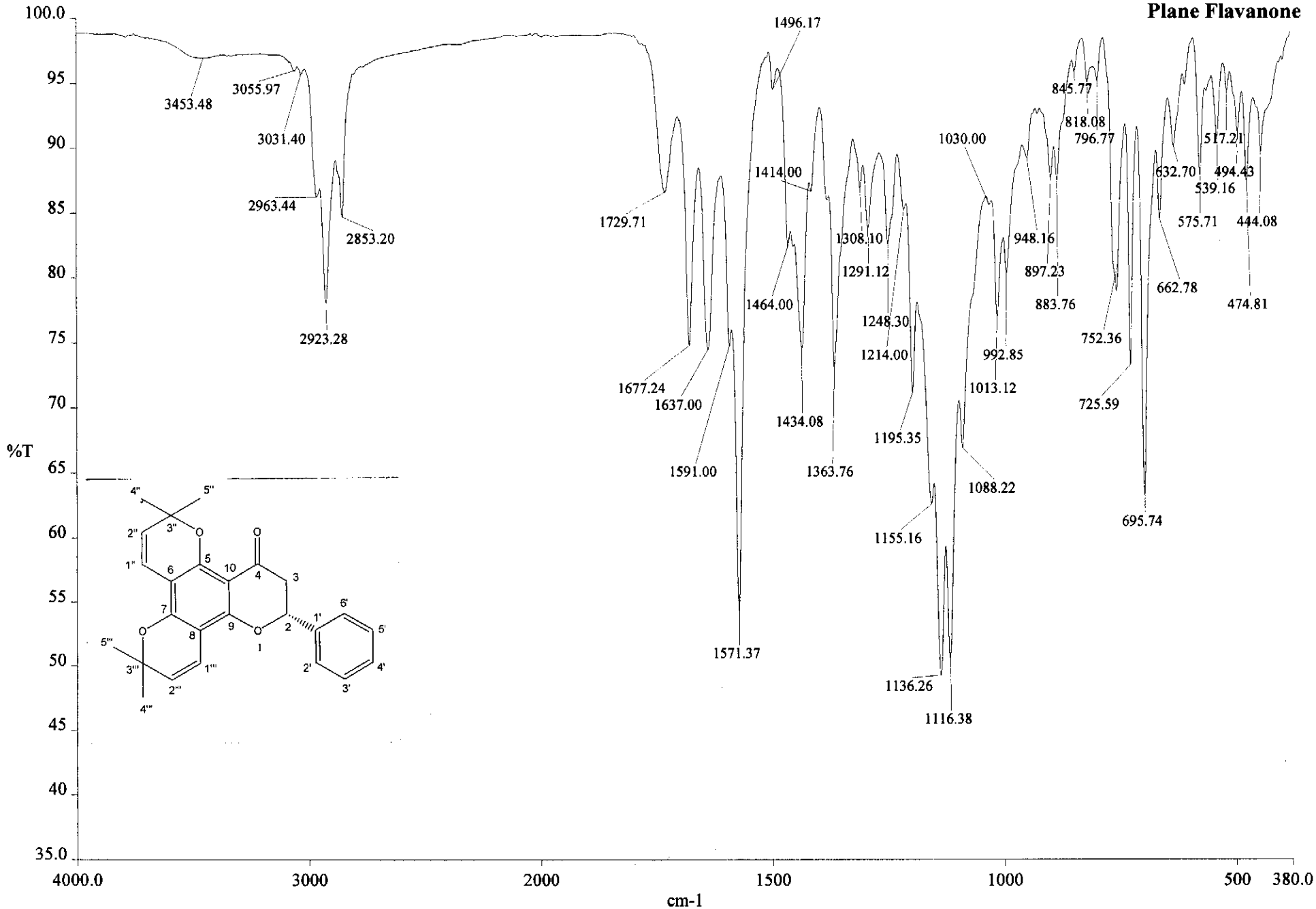
HSQC NMR spectrum of 4a – Deoxy-MS-II (A-4a)



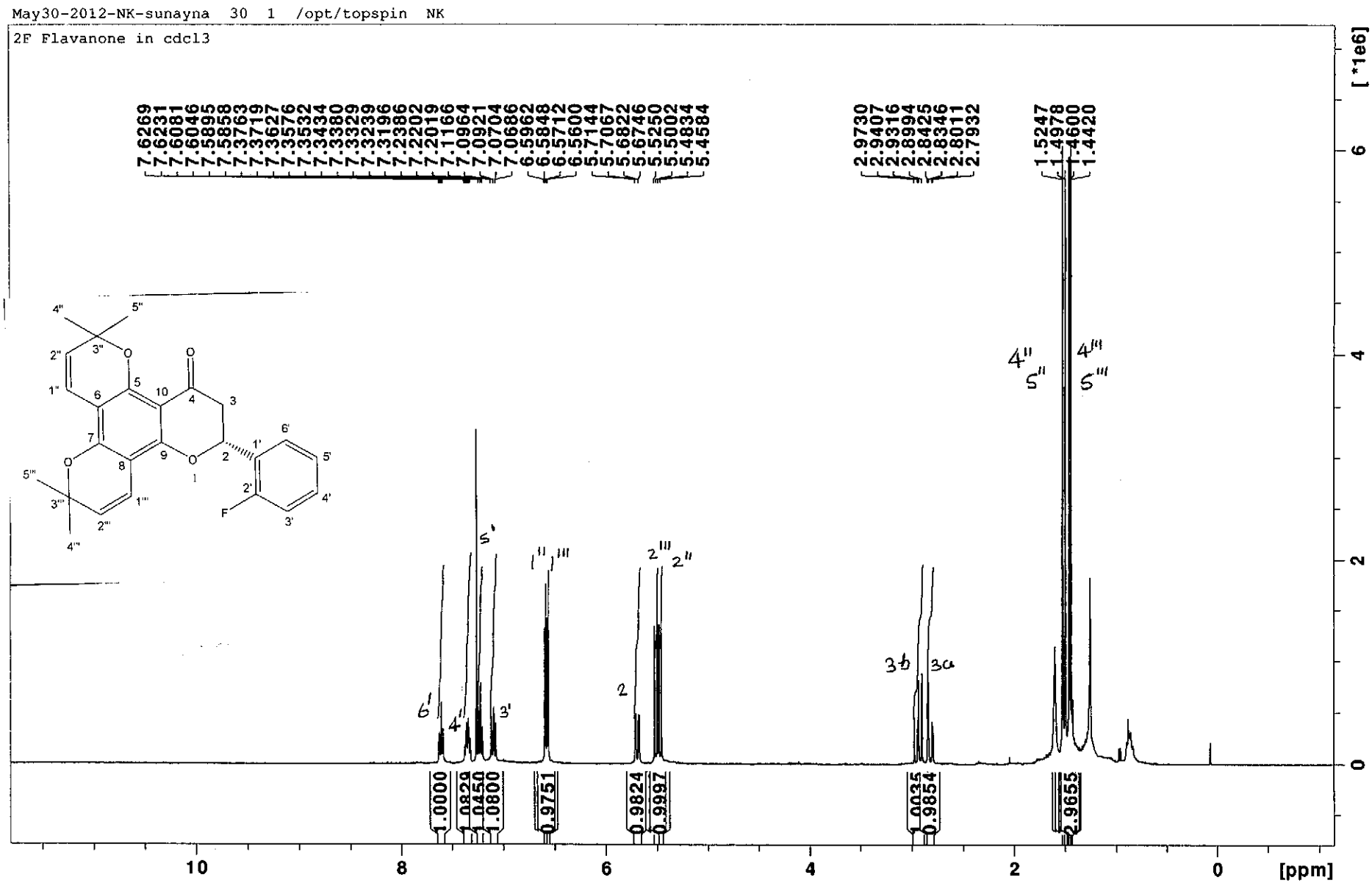
HMBC NMR spectrum of 4a – Deoxy-MS-II (A-4a)



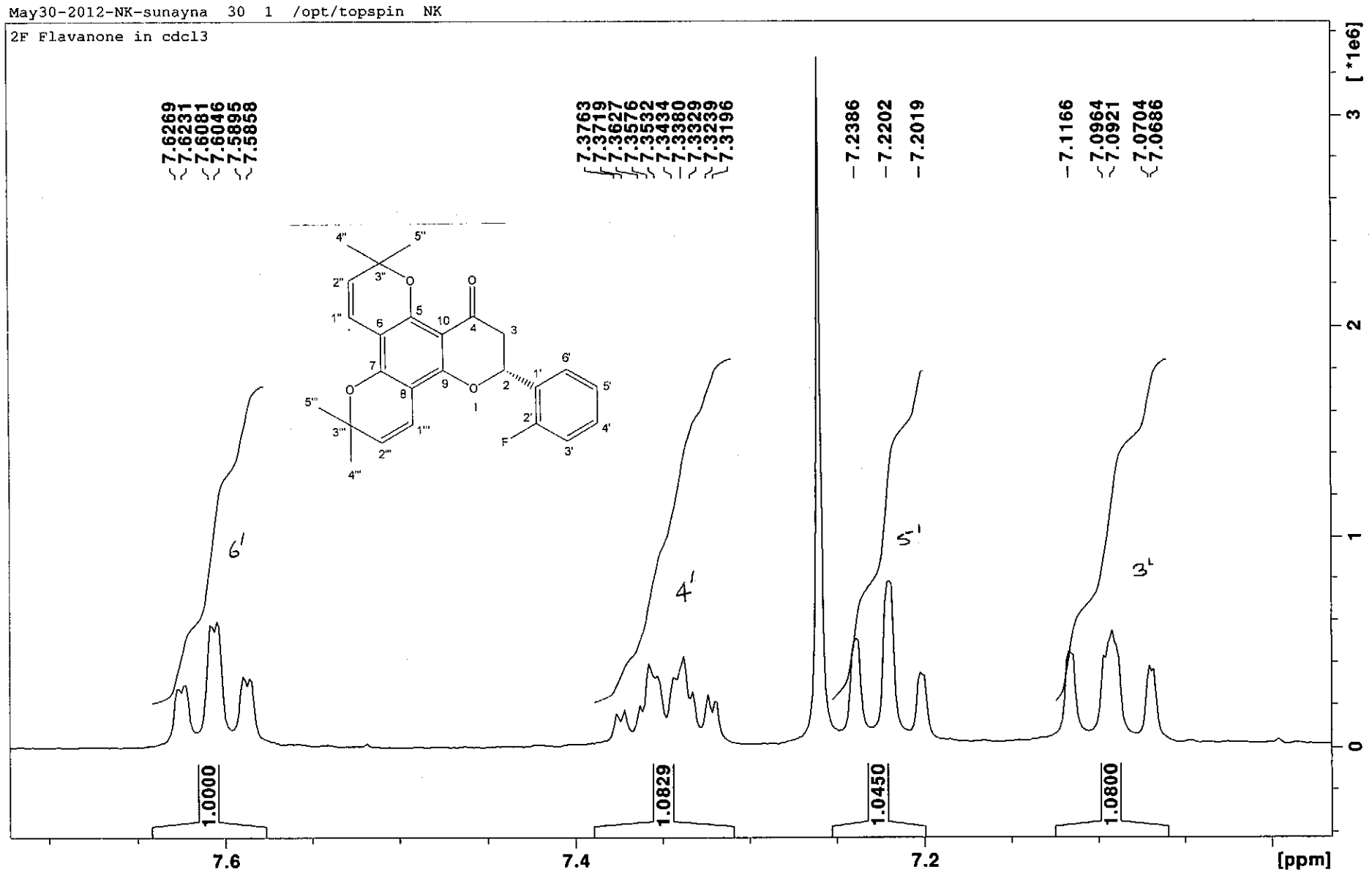
HMBC NMR spectrum of 4a – Deoxy-MS-II (A-4a)



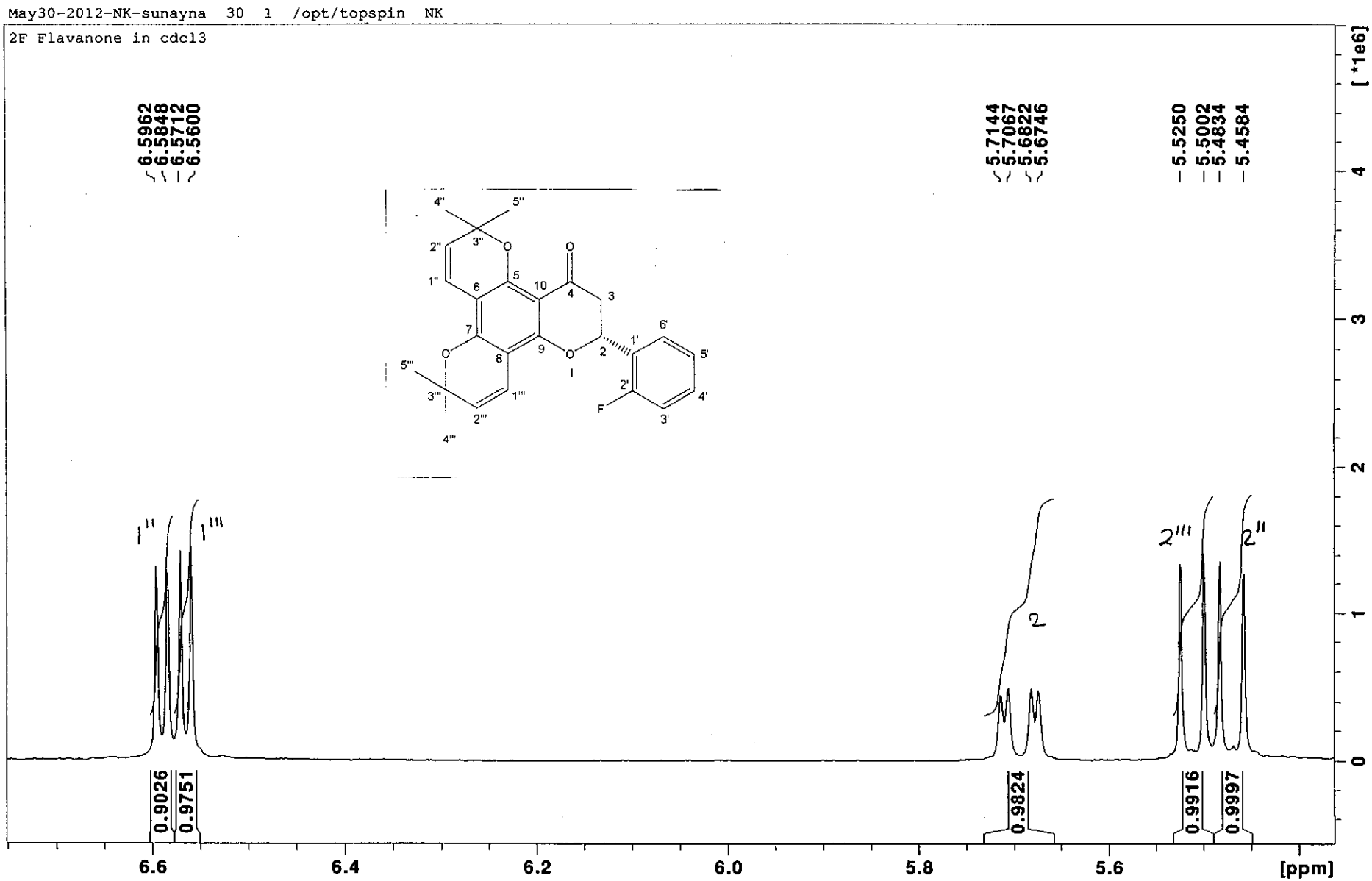
IR spectrum of 4a – Deoxy-MS-II (A-4a)



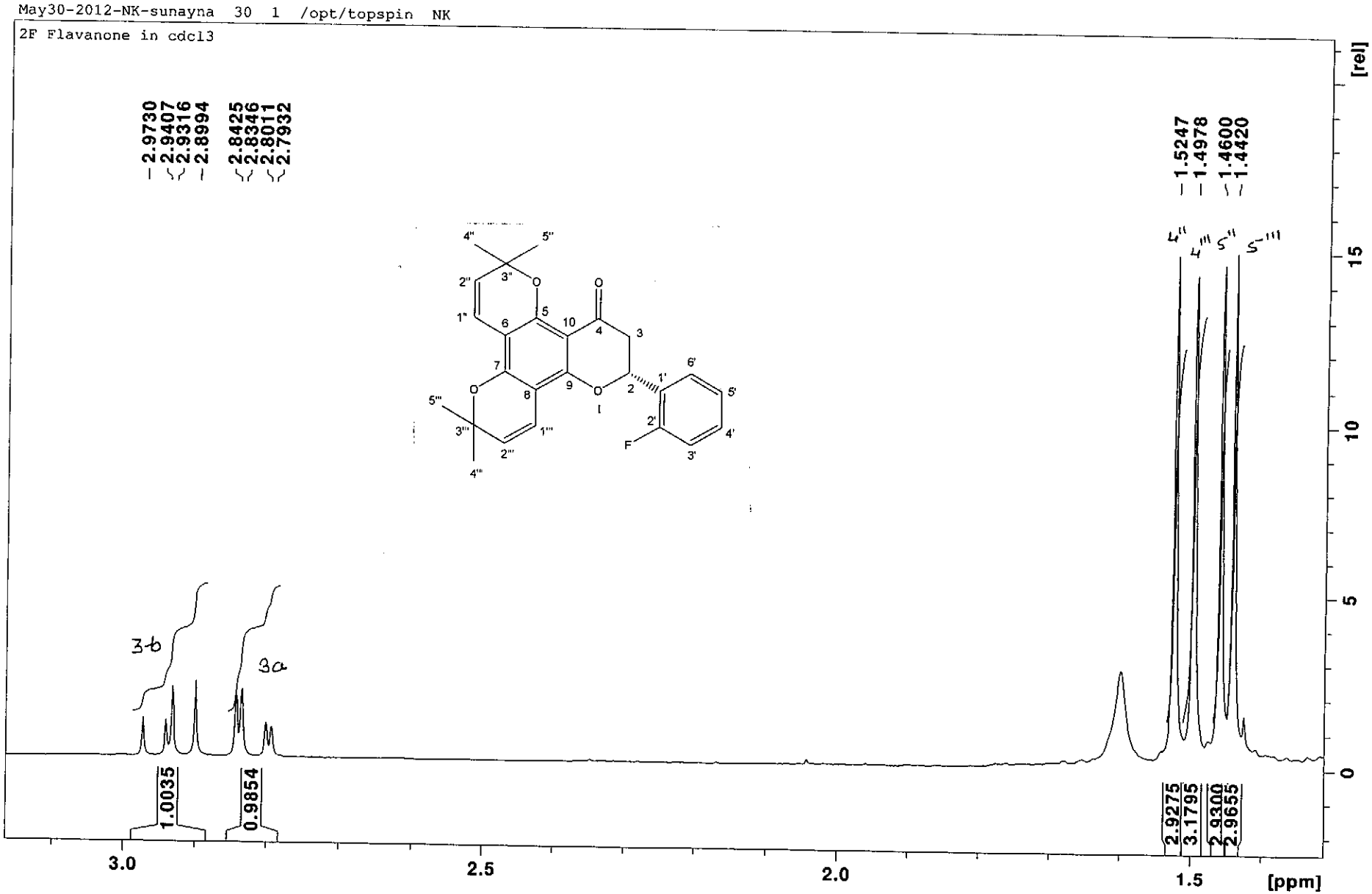
^{1}H NMR spectrum of 4b - 2'-F pyranochromene flavanone (A-4b)



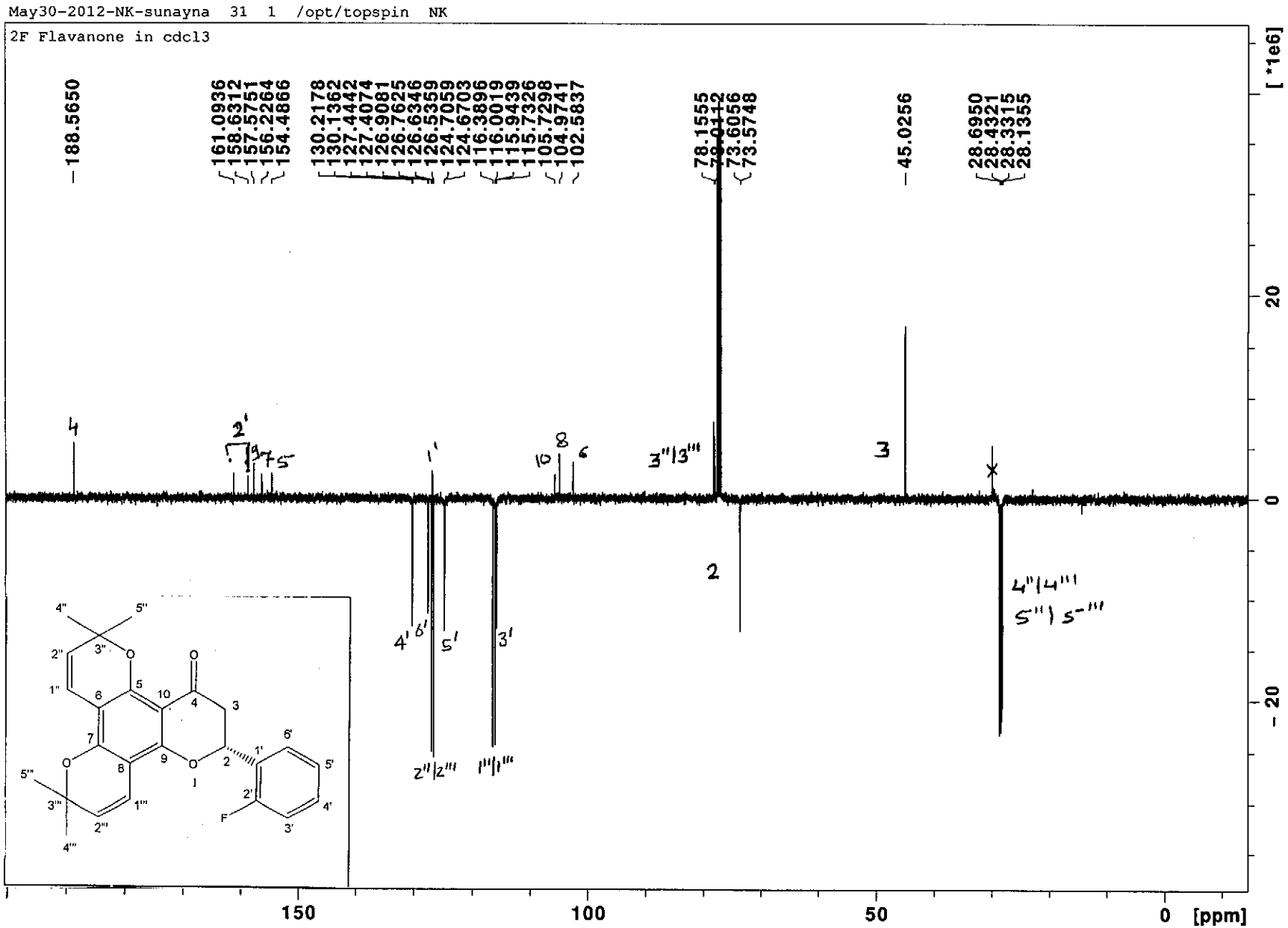
^{1}H NMR spectrum of 4b - 2'-F pyranochromene flavanone (A-4b)



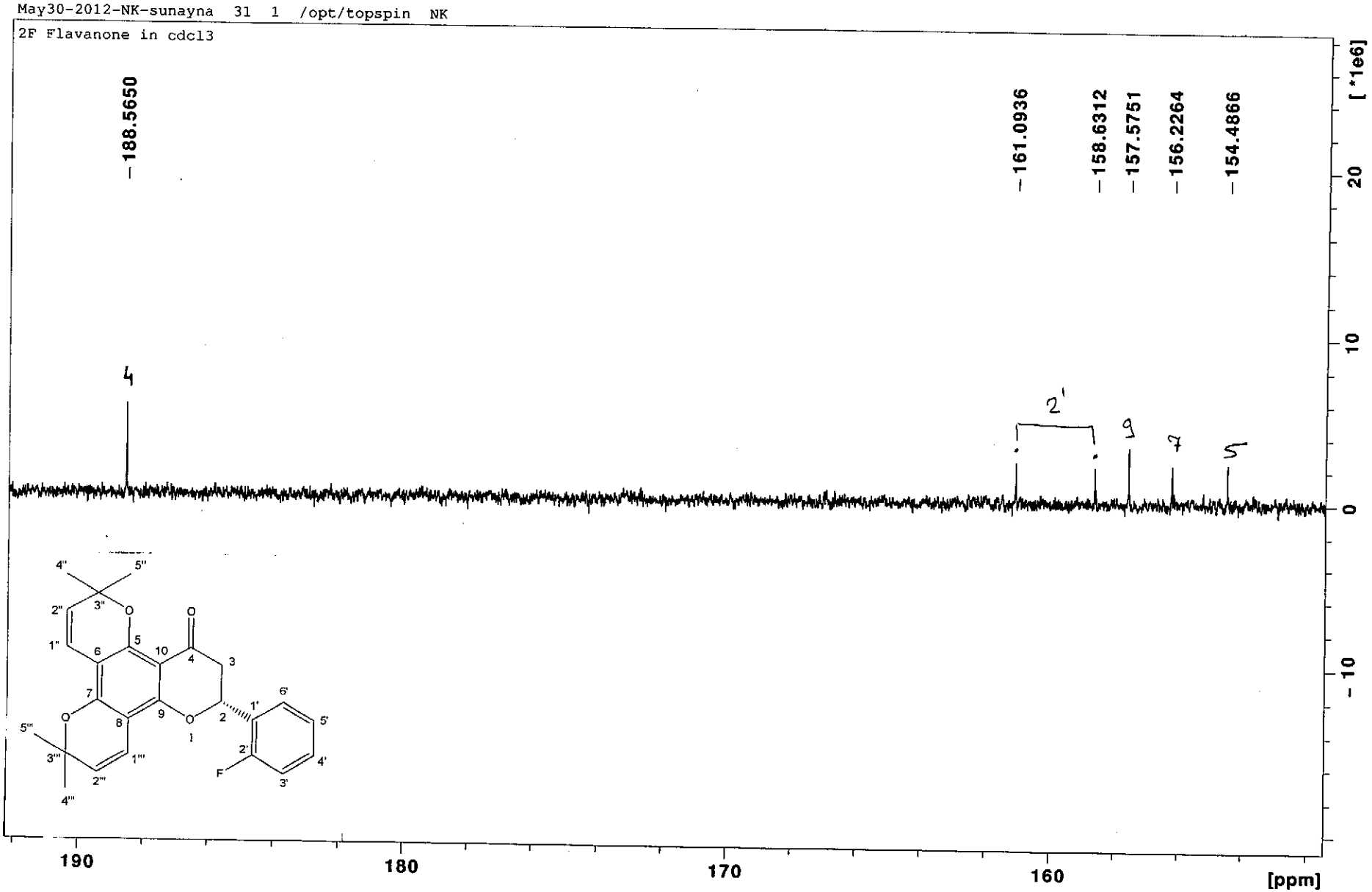
^{1}H NMR spectrum of 4b - 2'-F pyranochromene flavanone (A-4b)



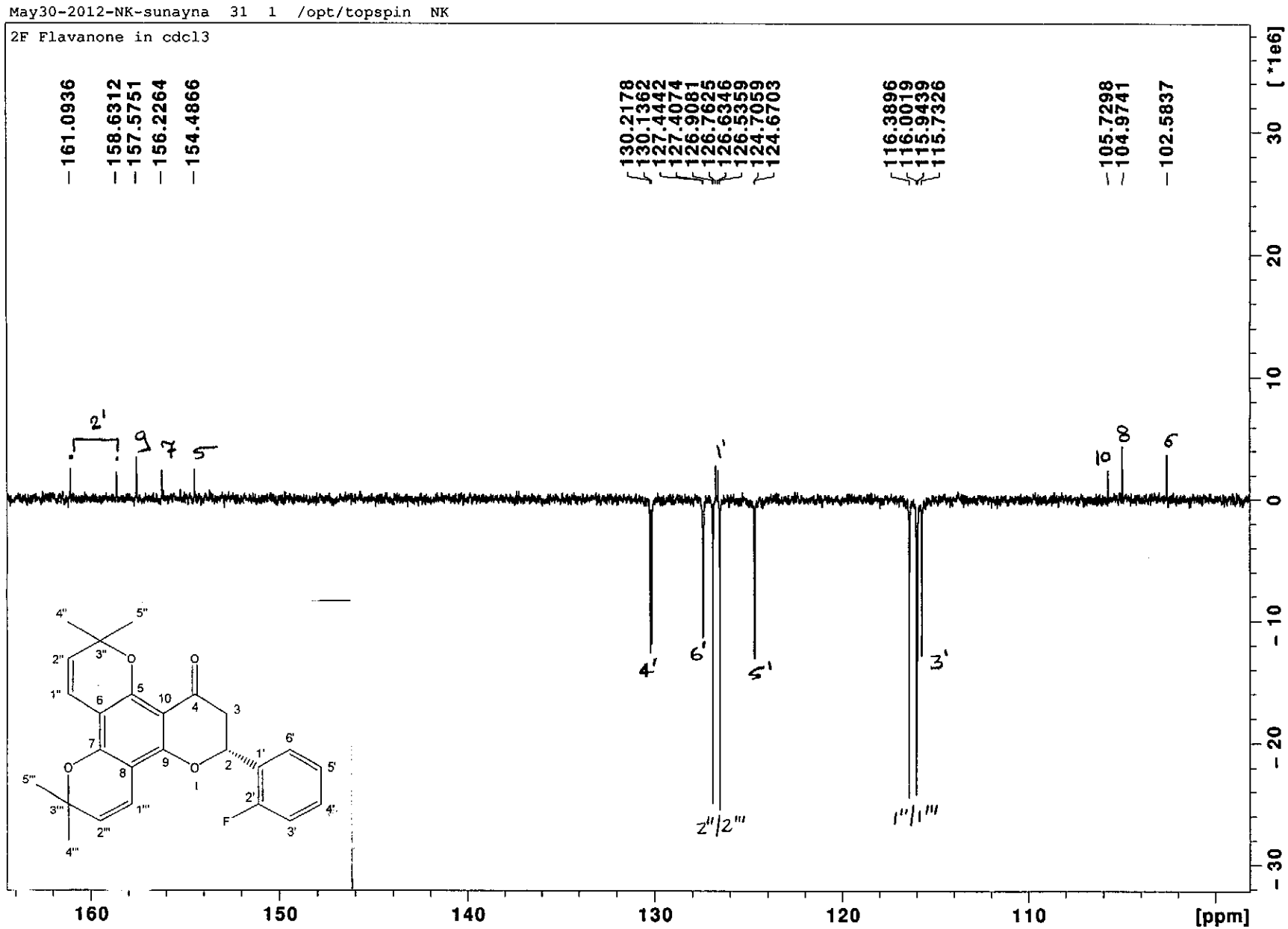
^{1}H NMR spectrum of 4b - 2'-F pyranochromene flavanone (A-4b)



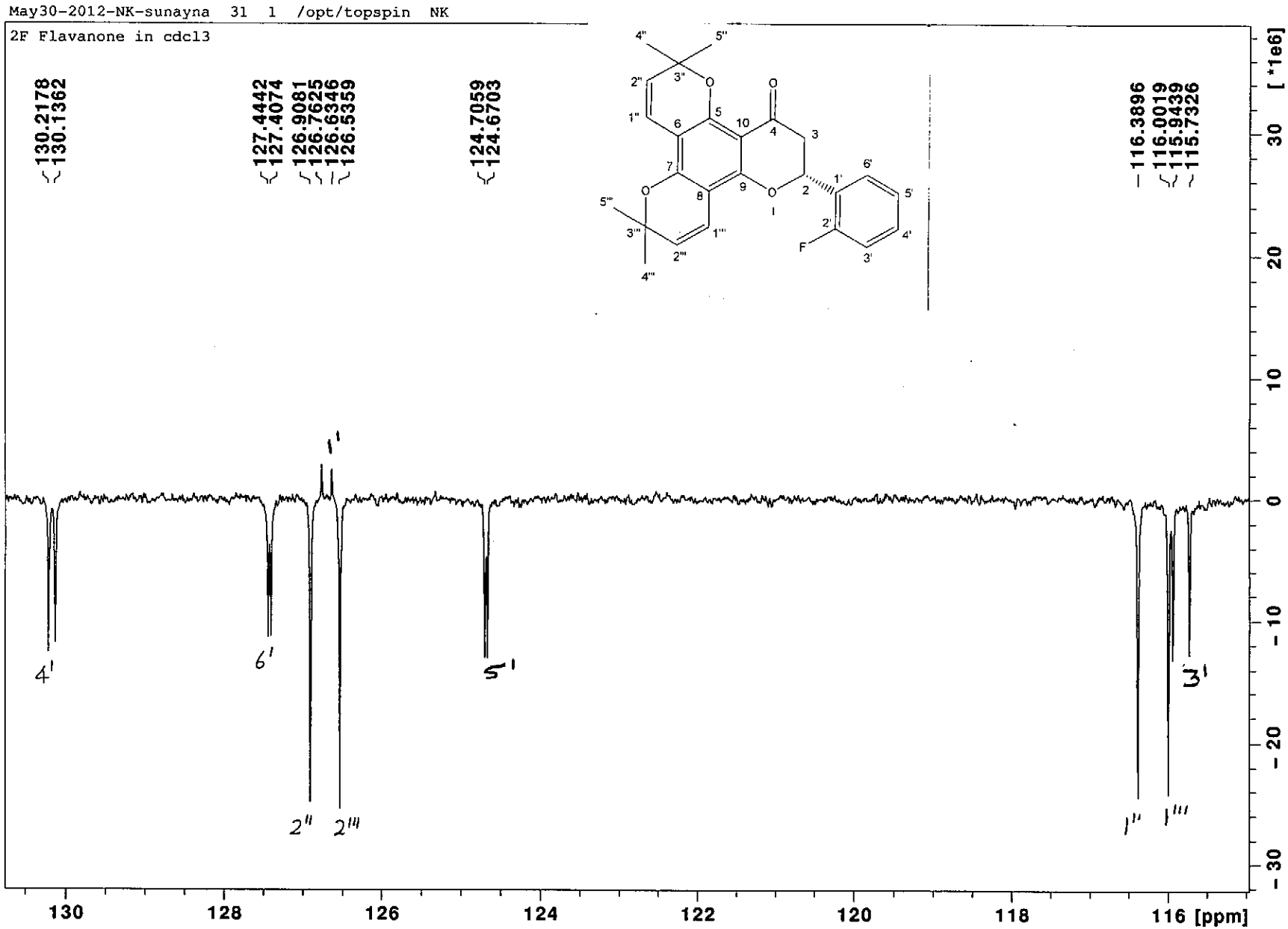
^{13}C NMR spectrum of 4b - 2'-F pyranochromene flavanone (A-4b)



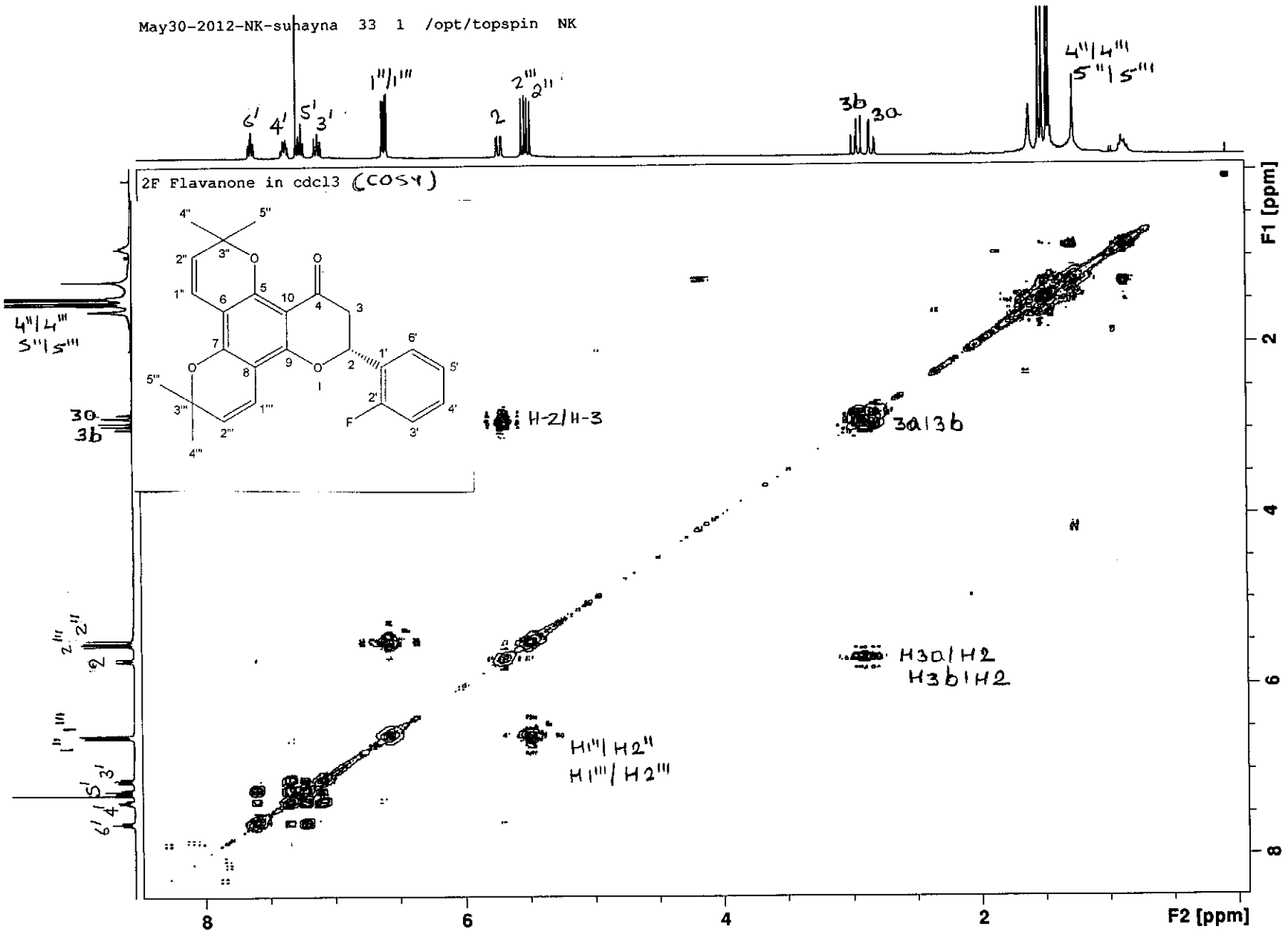
^{13}C NMR spectrum of 4b - 2'-F pyranochromene flavanone (A-4b)



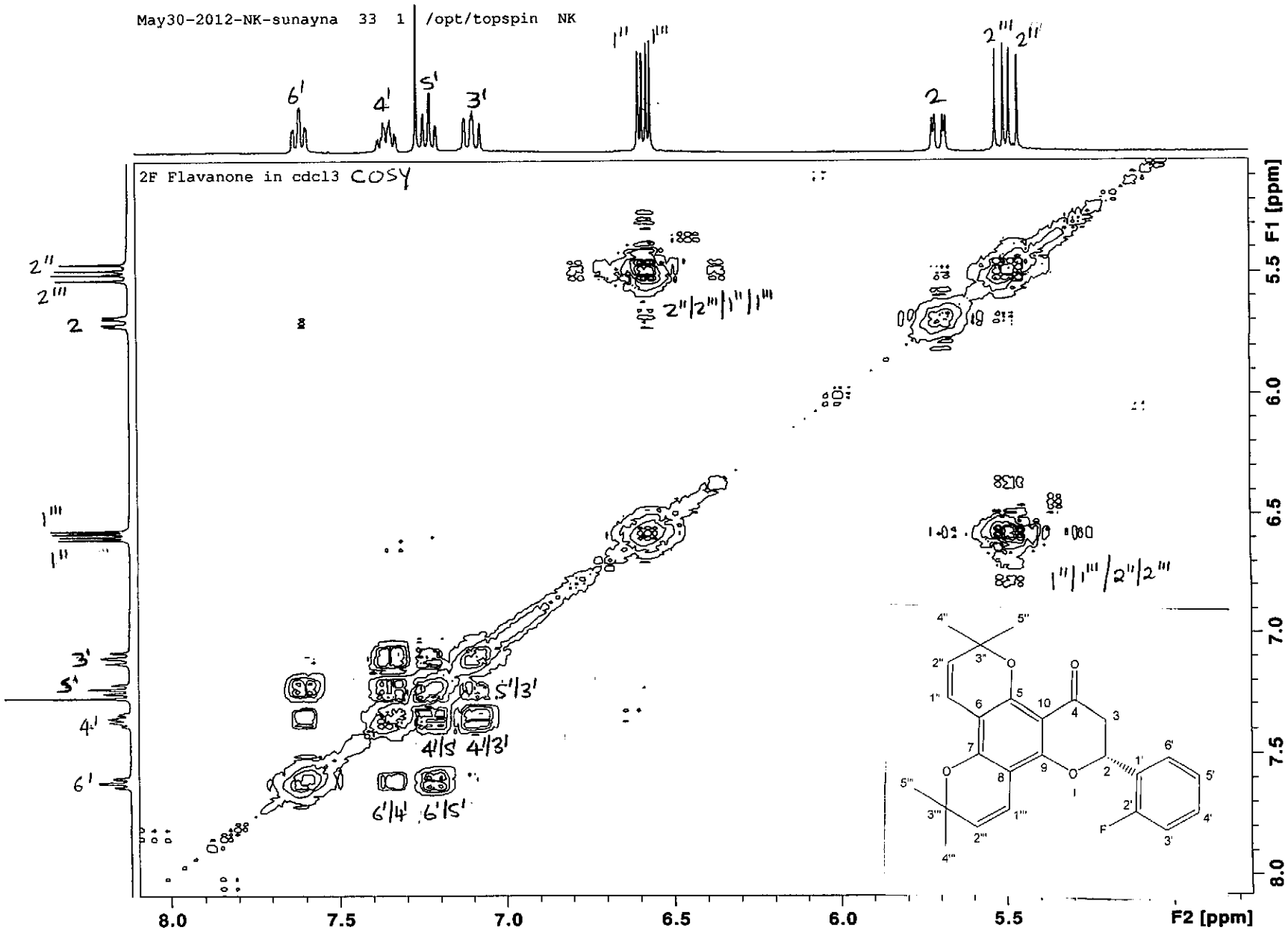
^{13}C NMR spectrum of 4b - 2'-F pyranochromene flavanone (A-4b)



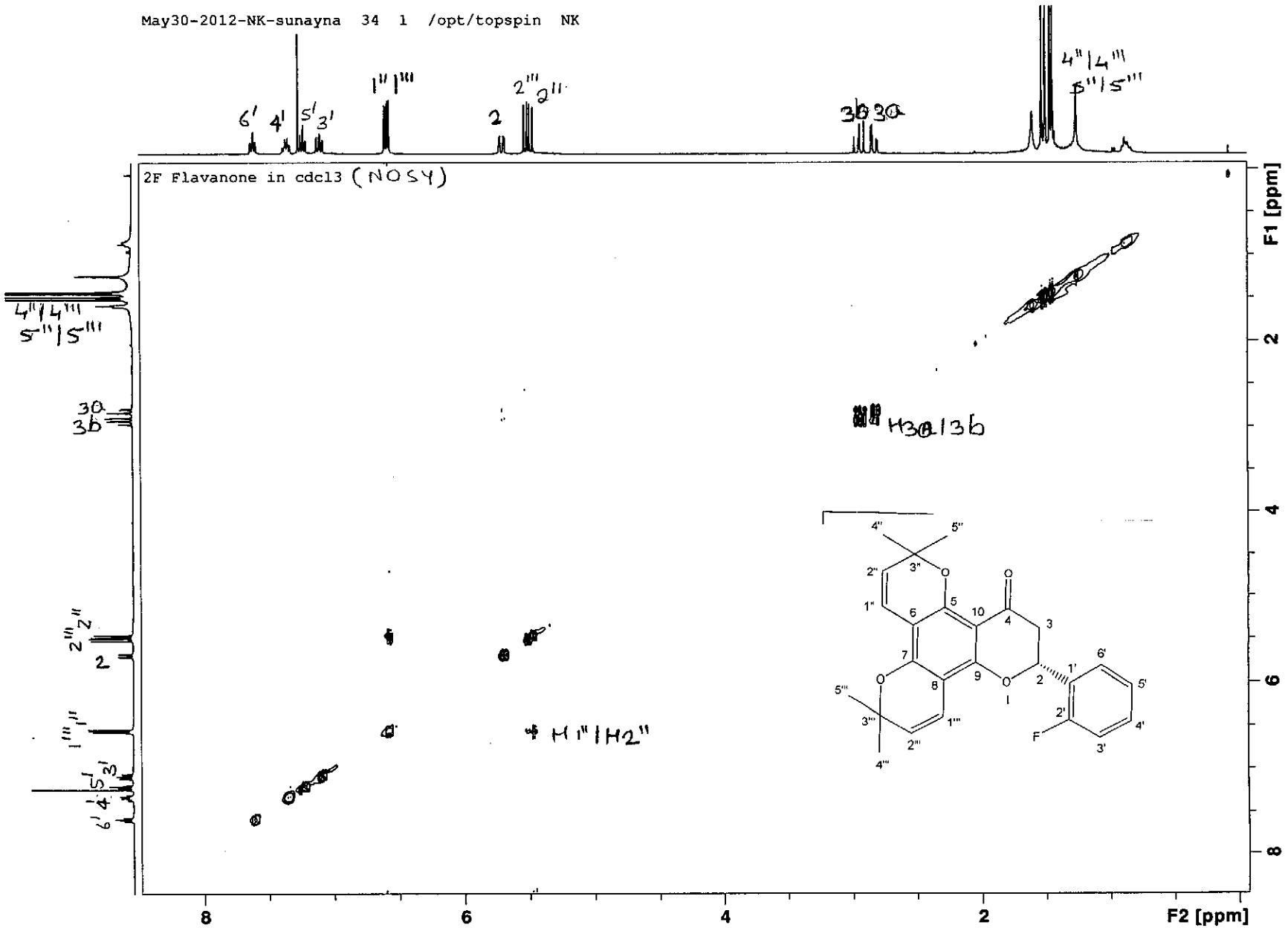
^{13}C NMR spectrum of 4b - 2'-F pyranochromene flavanone (A-4b)



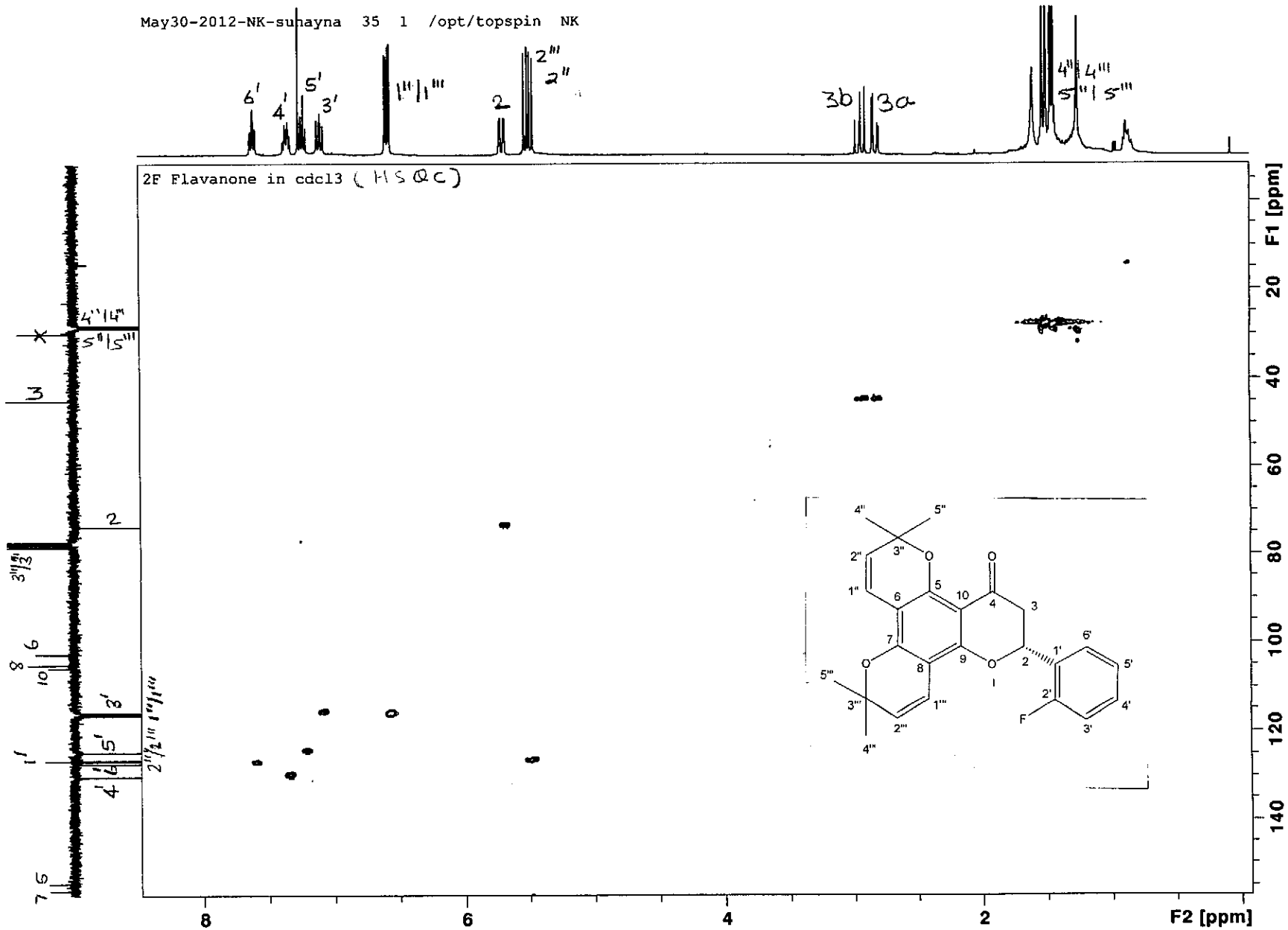
COSY NMR spectrum of 4b - 2'-F pyranochromene flavanone (A-4b)



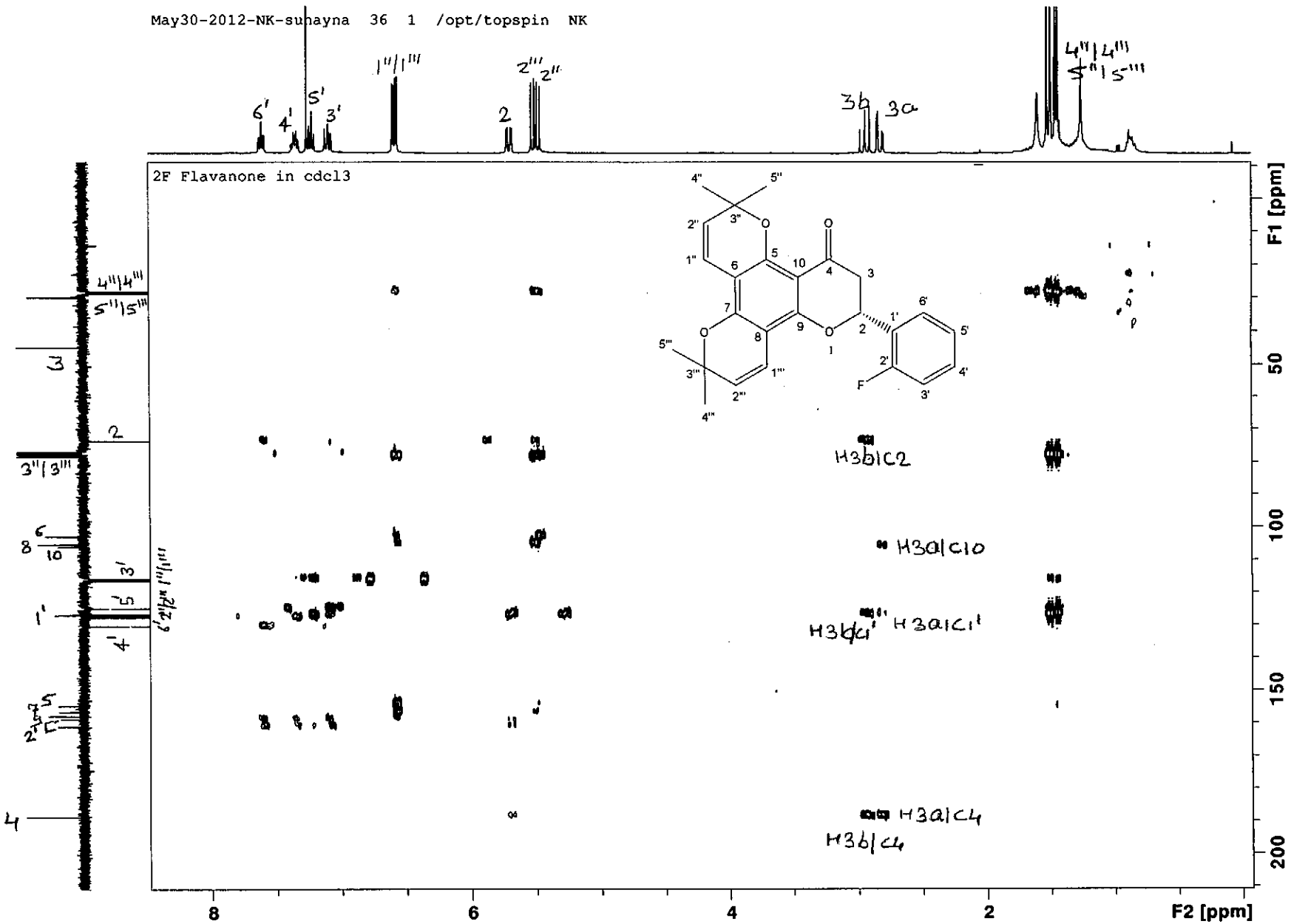
COSY NMR spectrum of 4b - 2'-F pyranochromene flavanone (A-4b)



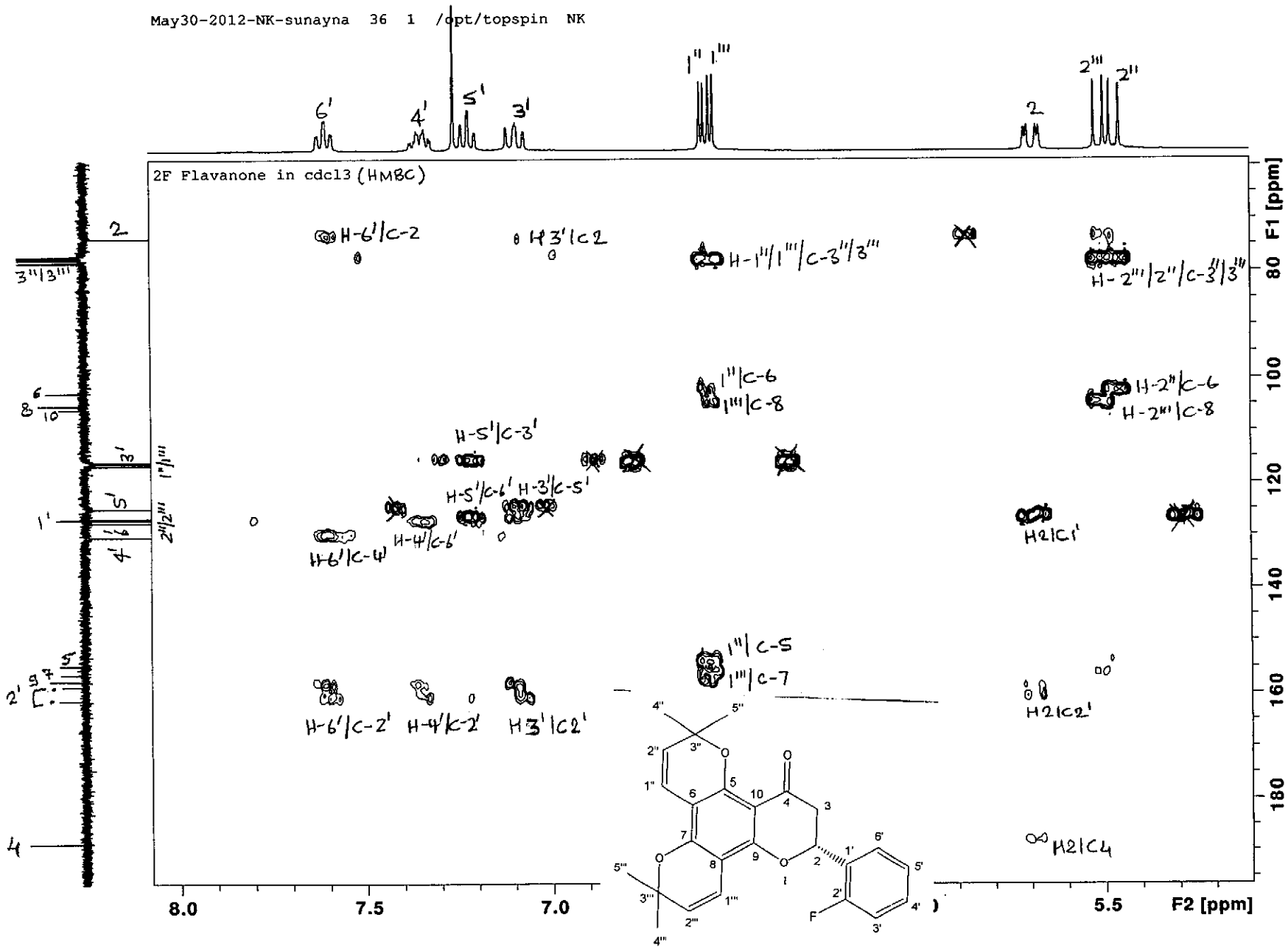
NOSY NMR spectrum of 4b - 2'-F pyranochromene flavanone (A-4b)



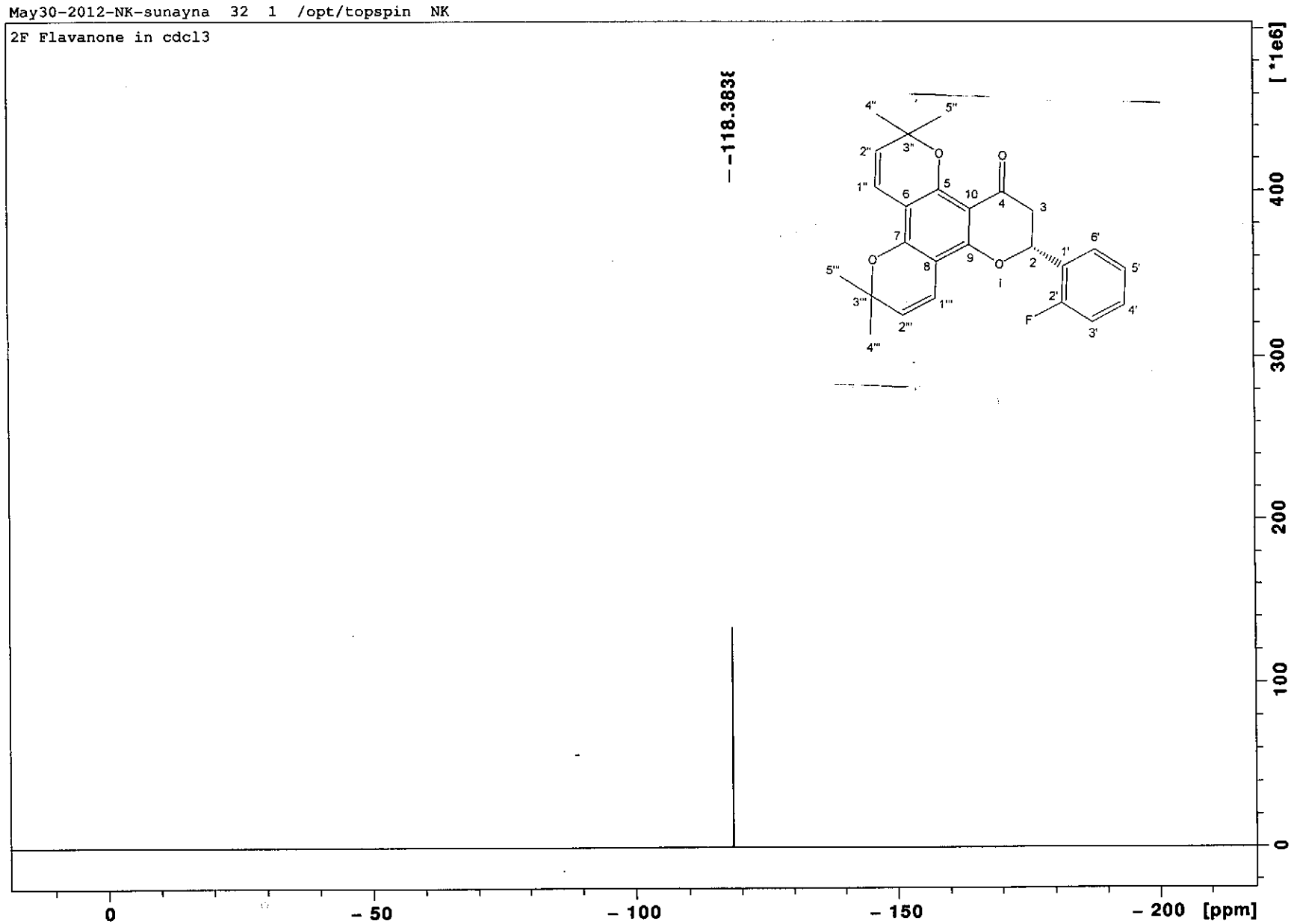
HSQC NMR spectrum of 4b - 2'-F pyranochromene flavanone (A-4b)



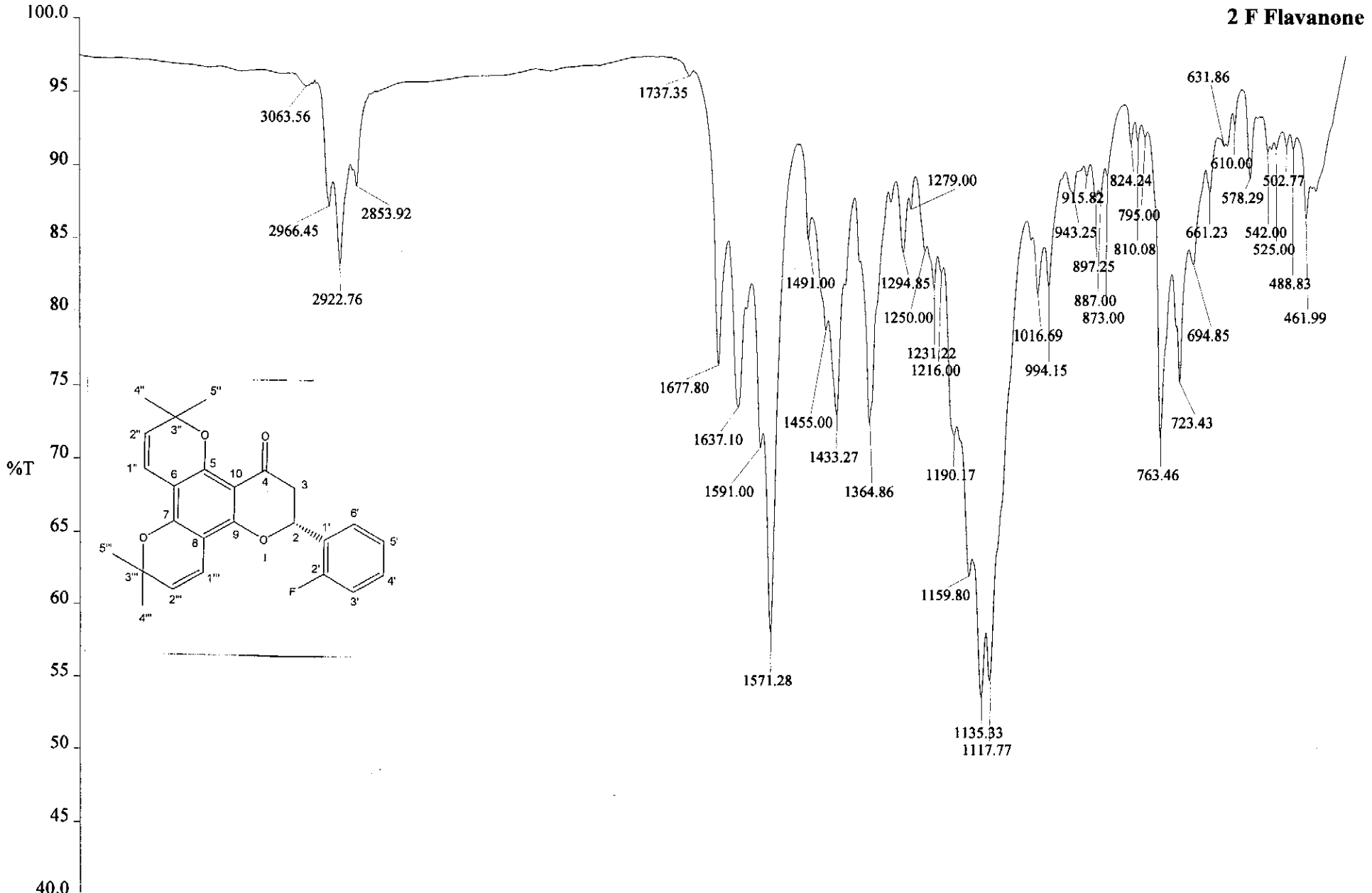
HMBC NMR spectrum of 4b - 2'-F pyranochromene flavanone (A-4b)



HMBC NMR spectrum of 4b - 2'-F pyranochromene flavanone (A-4b)

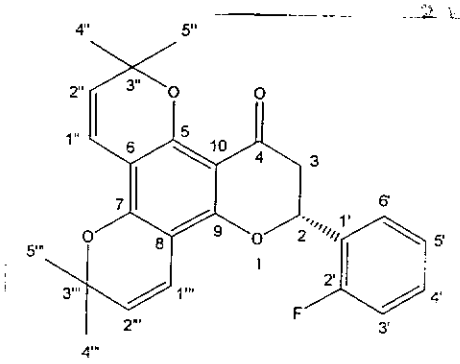


^{19}F NMR spectrum of 4b - 2'-F pyranochromene flavanone (A-4b)



IR spectrum of 4b - 2'-F pyranochromene flavanone (A-4b)

Elemental Composition Report



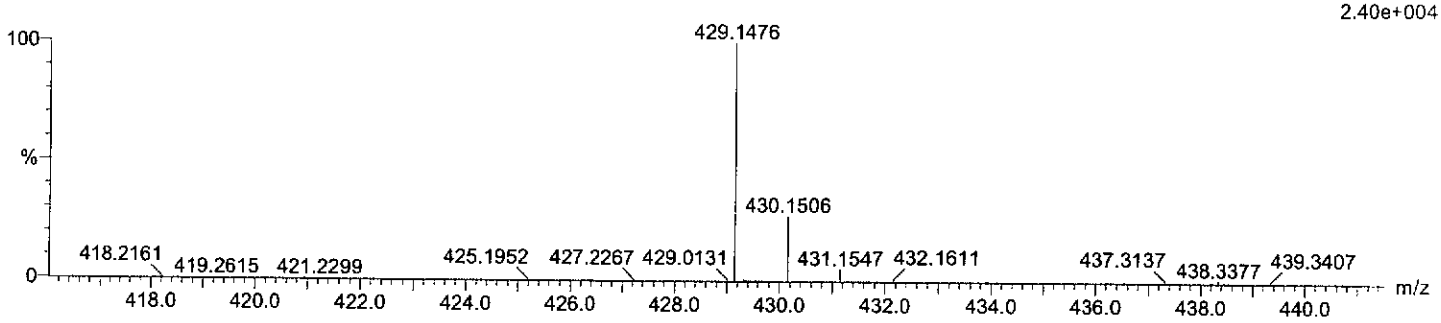
Single Mass Analysis

Tolerance = 1.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
6 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-30 H: 20-25 O: 0-5 F: 0-1 Na: 0-1

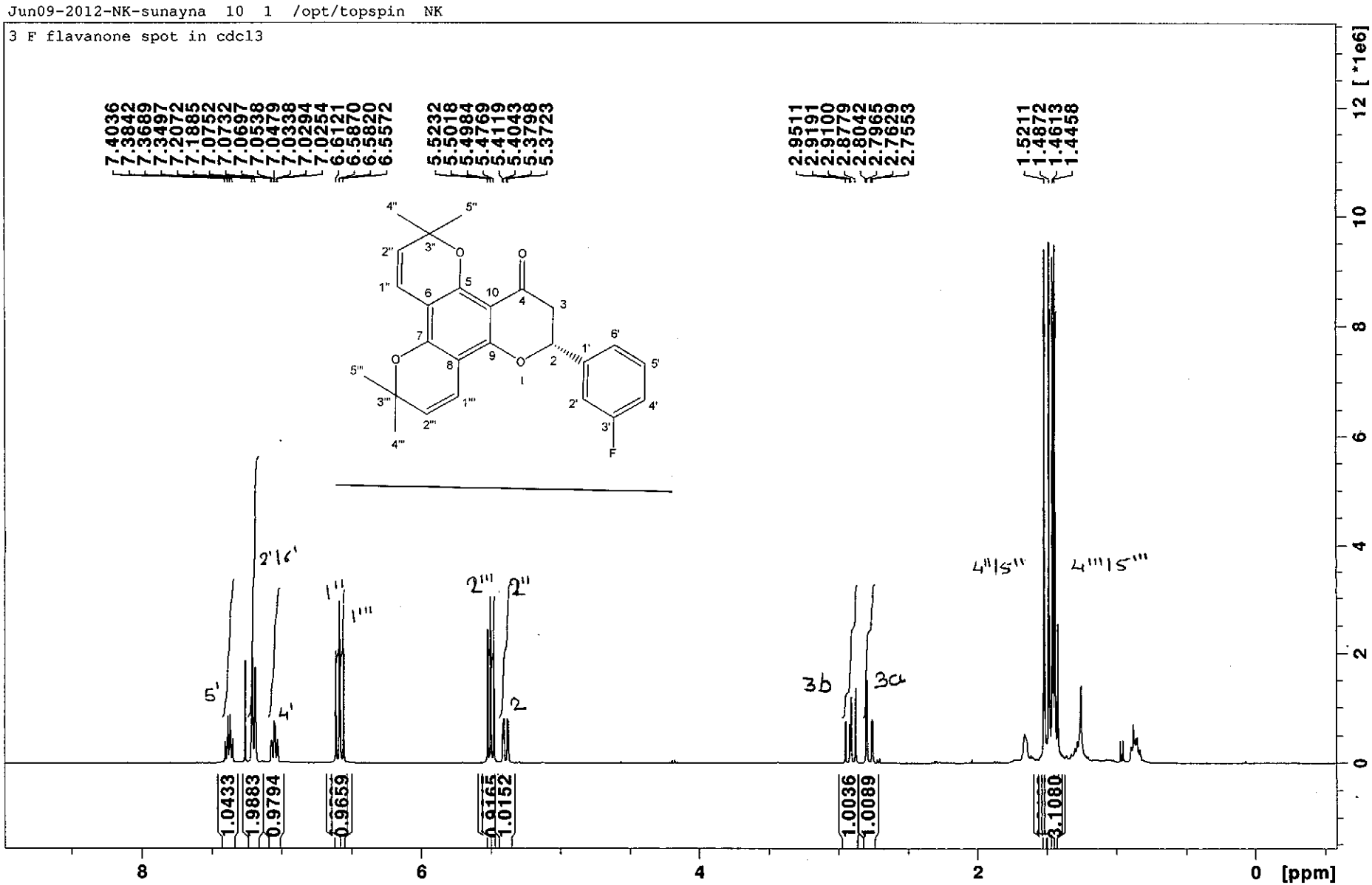
Dr. Nizam
10 2 (0.017) Cm (1:30)

TOF MS ES+
2.40e+004

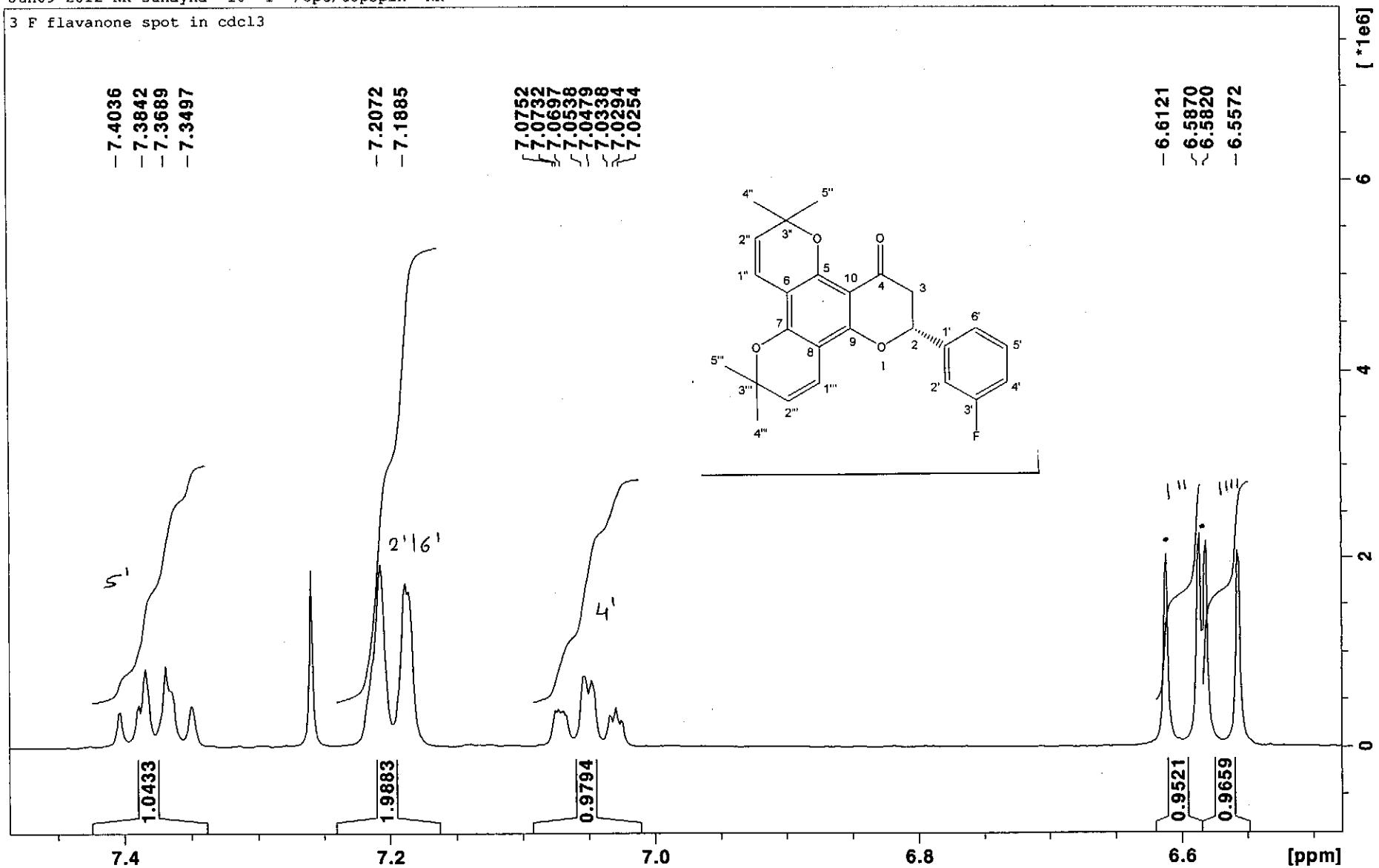


Minimum:				-1.5			
Maximum:		5.0	1.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
429.1476	429.1478	-0.2	-0.5	13.5	402.8	0.0	C25 H23 O4 F Na

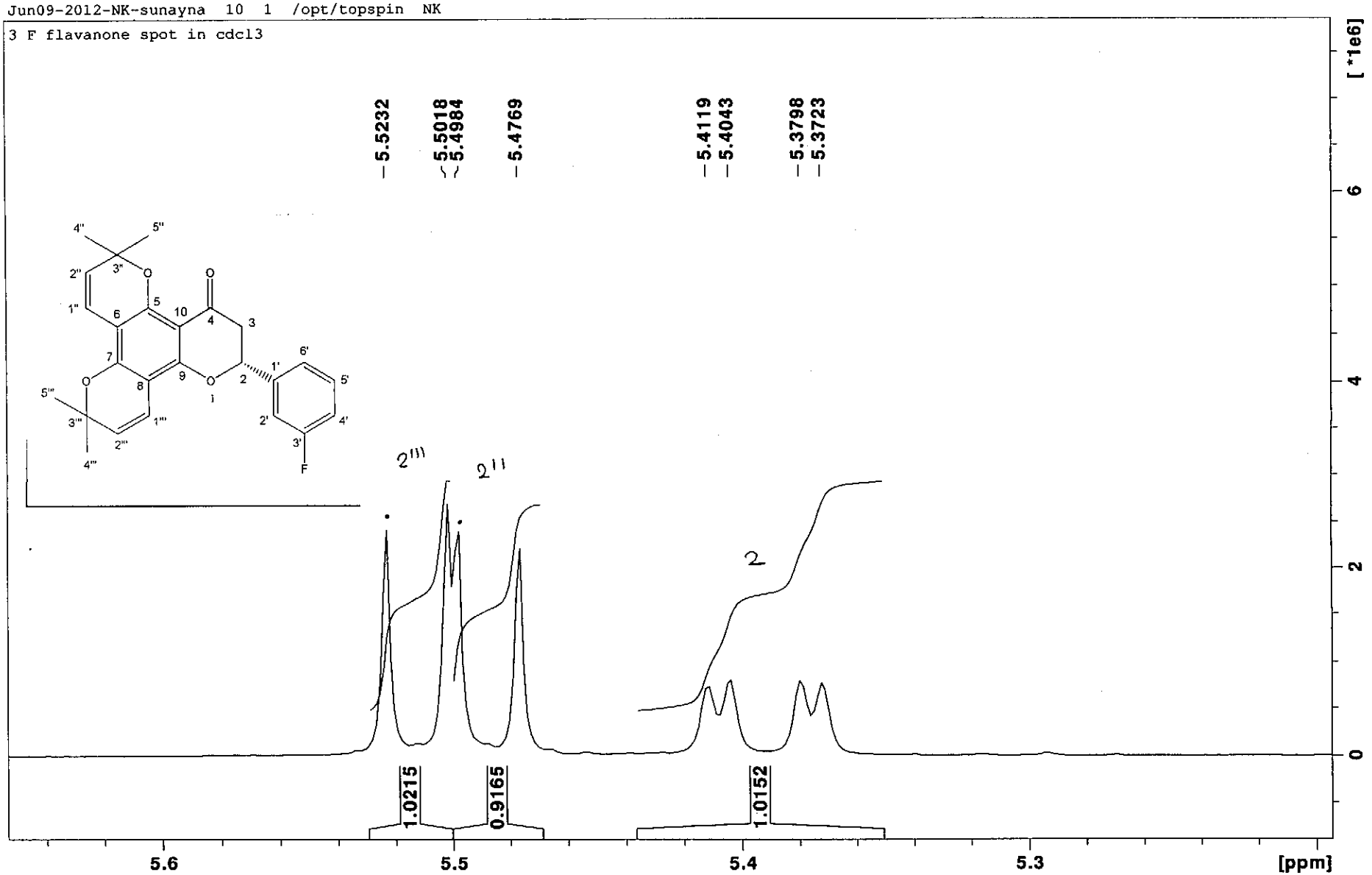
HRMS of 4b – 2'-F pyranochromene flavanone (A-4b)



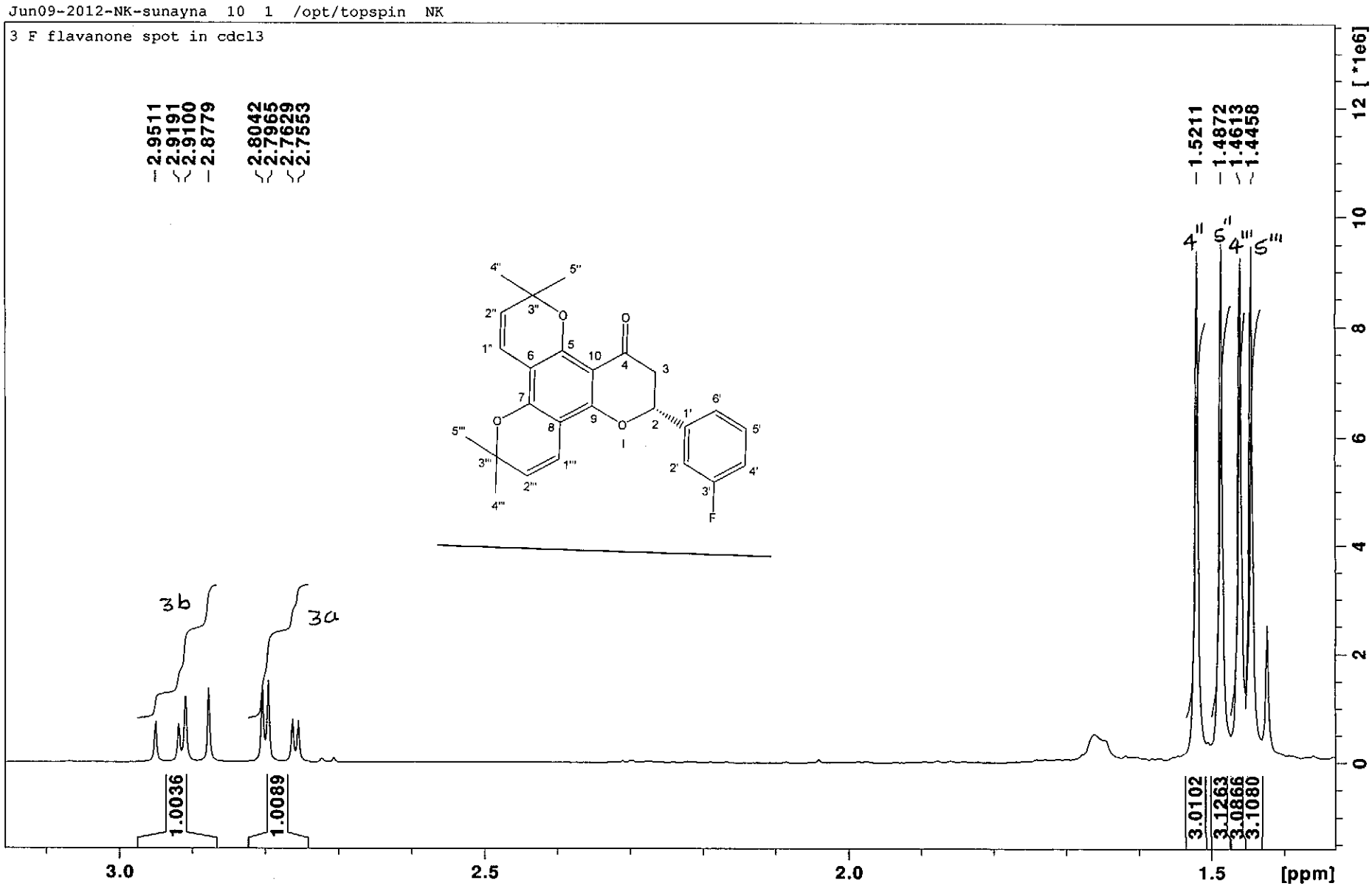
^{1}H NMR spectrum of 4c - 3'-F pyranochromene flavanone (A-4c)



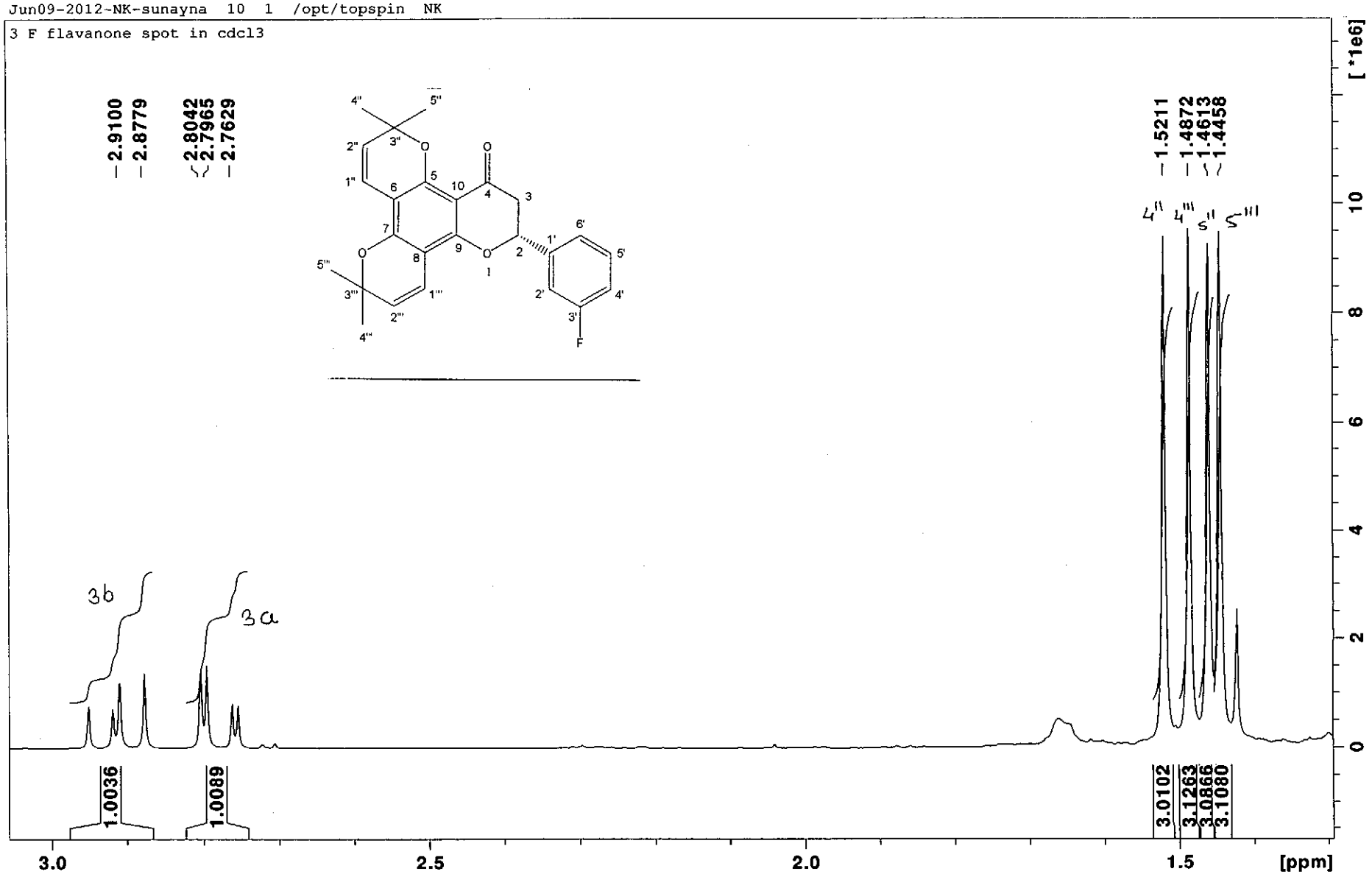
^{1}H NMR spectrum of 4c - 3'-F pyranochromene flavanone (A-4c)



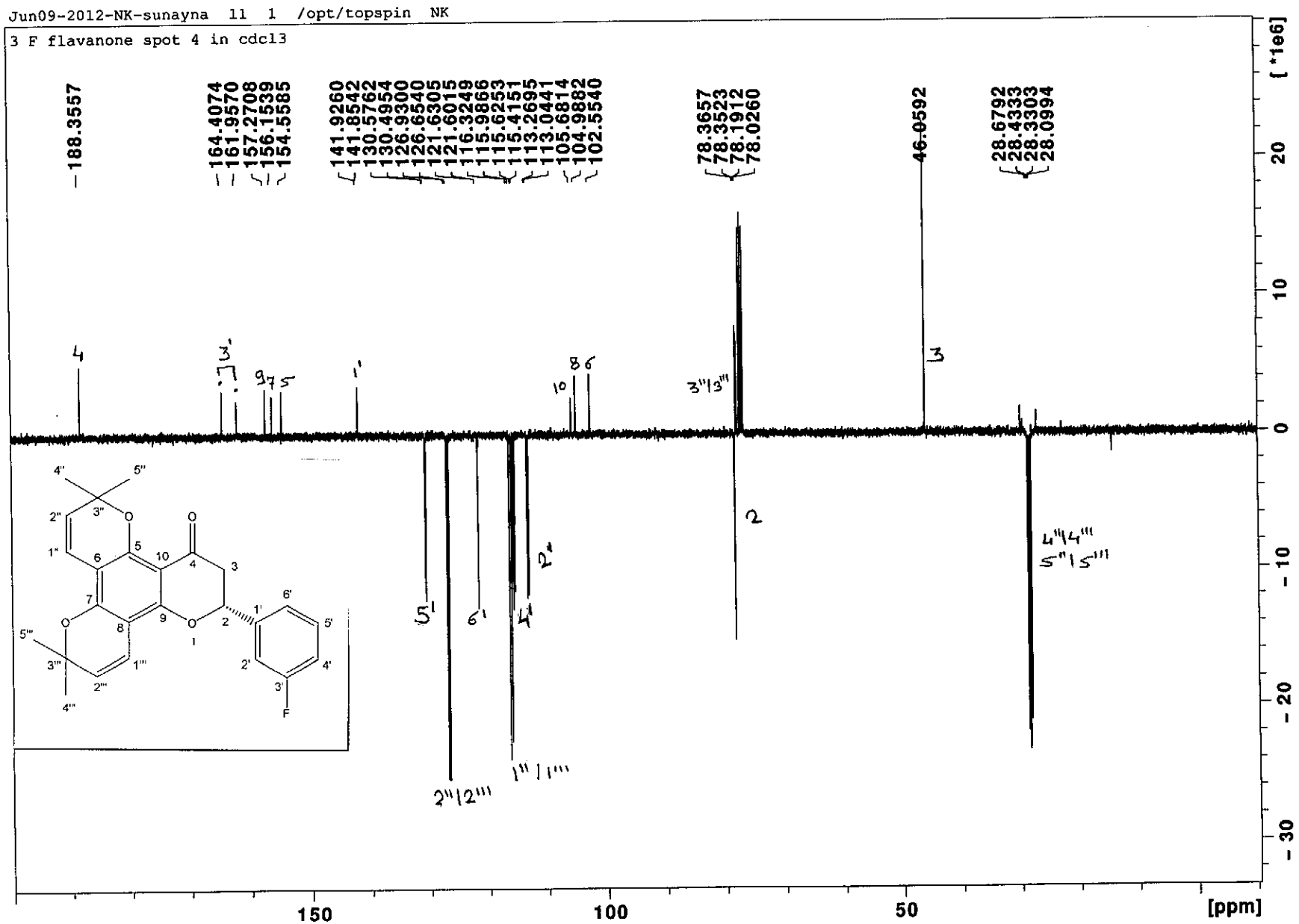
^{1}H NMR spectrum of 4c - 3'-F pyranochromene flavanone (A-4c)



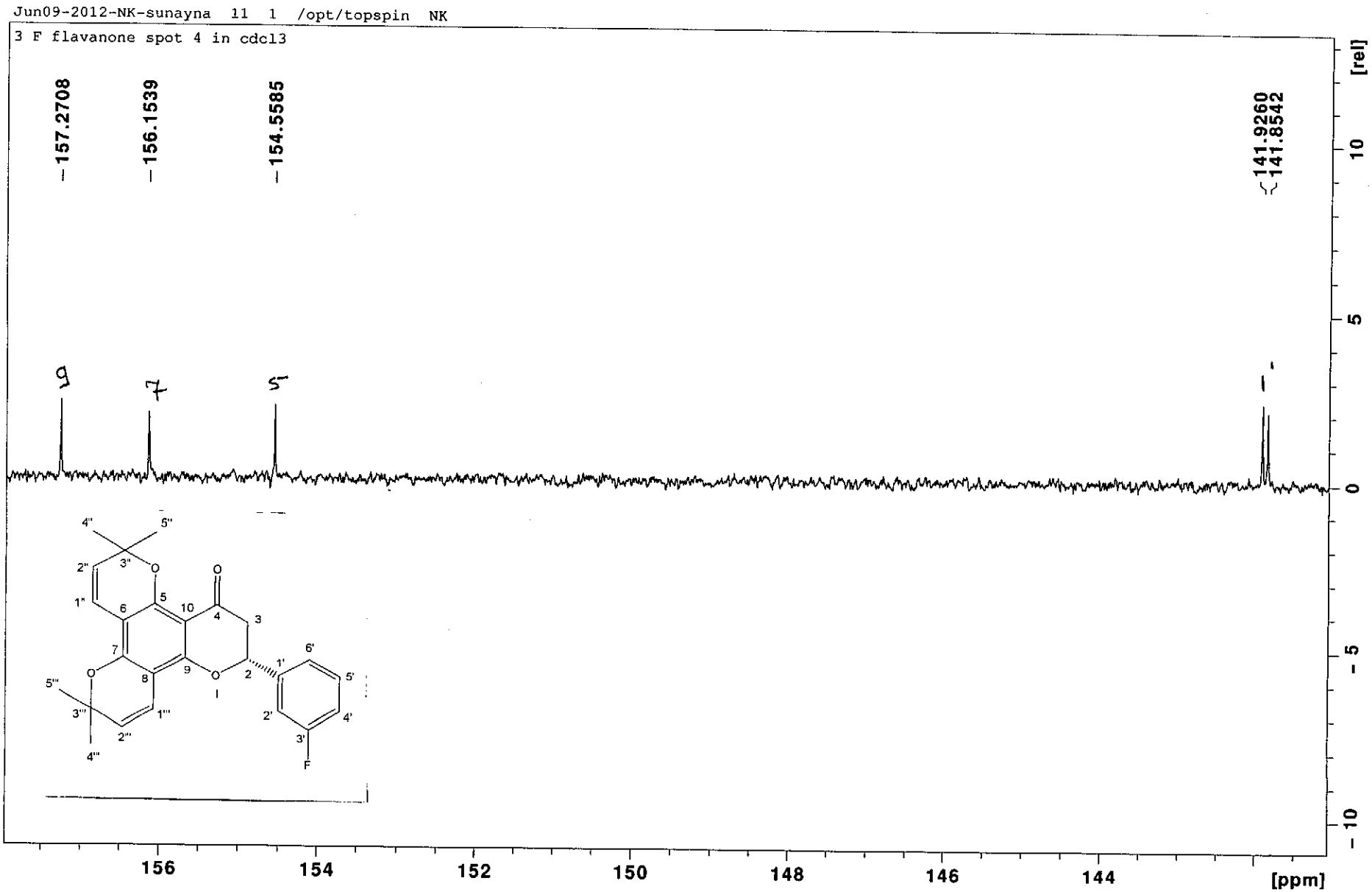
^{1}H NMR spectrum of 4c - 3'-F pyranochromene flavanone (A-4c)



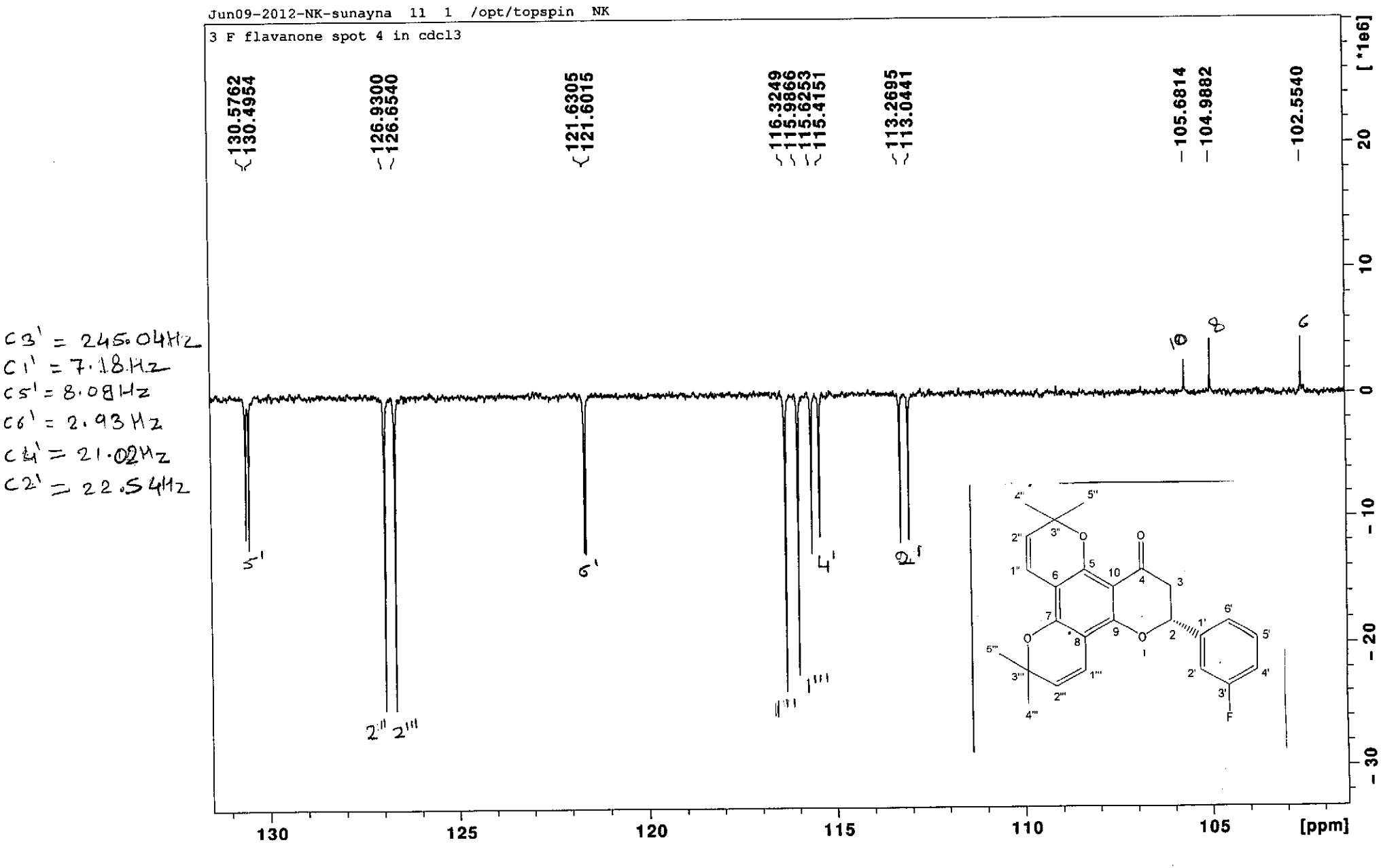
^{1}H NMR spectrum of 4c - 3'-F pyranochromene flavanone (A-4c)



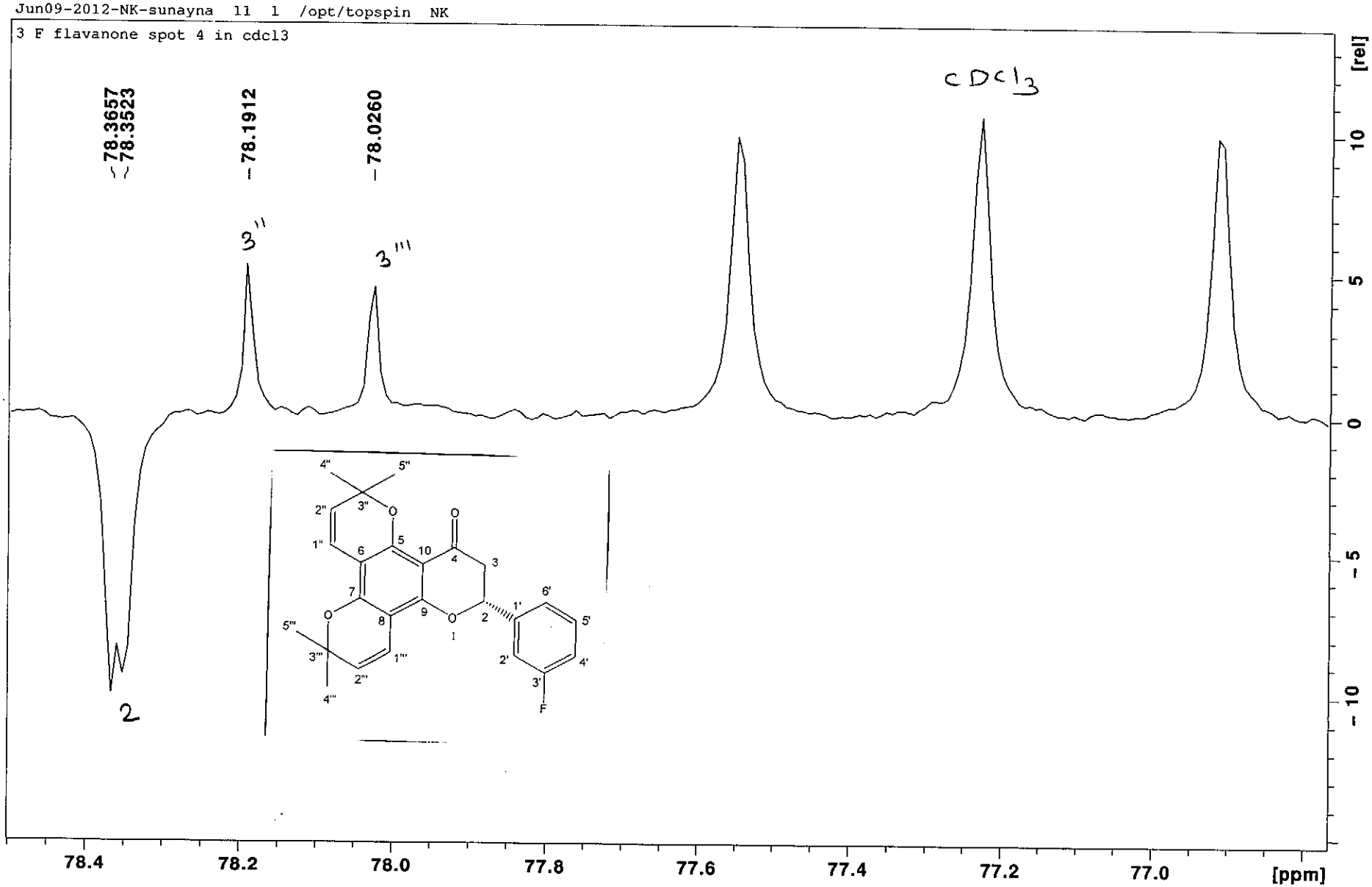
^{13}C NMR spectrum of 4c - 3'-F pyranochromene flavanone (A-4c)



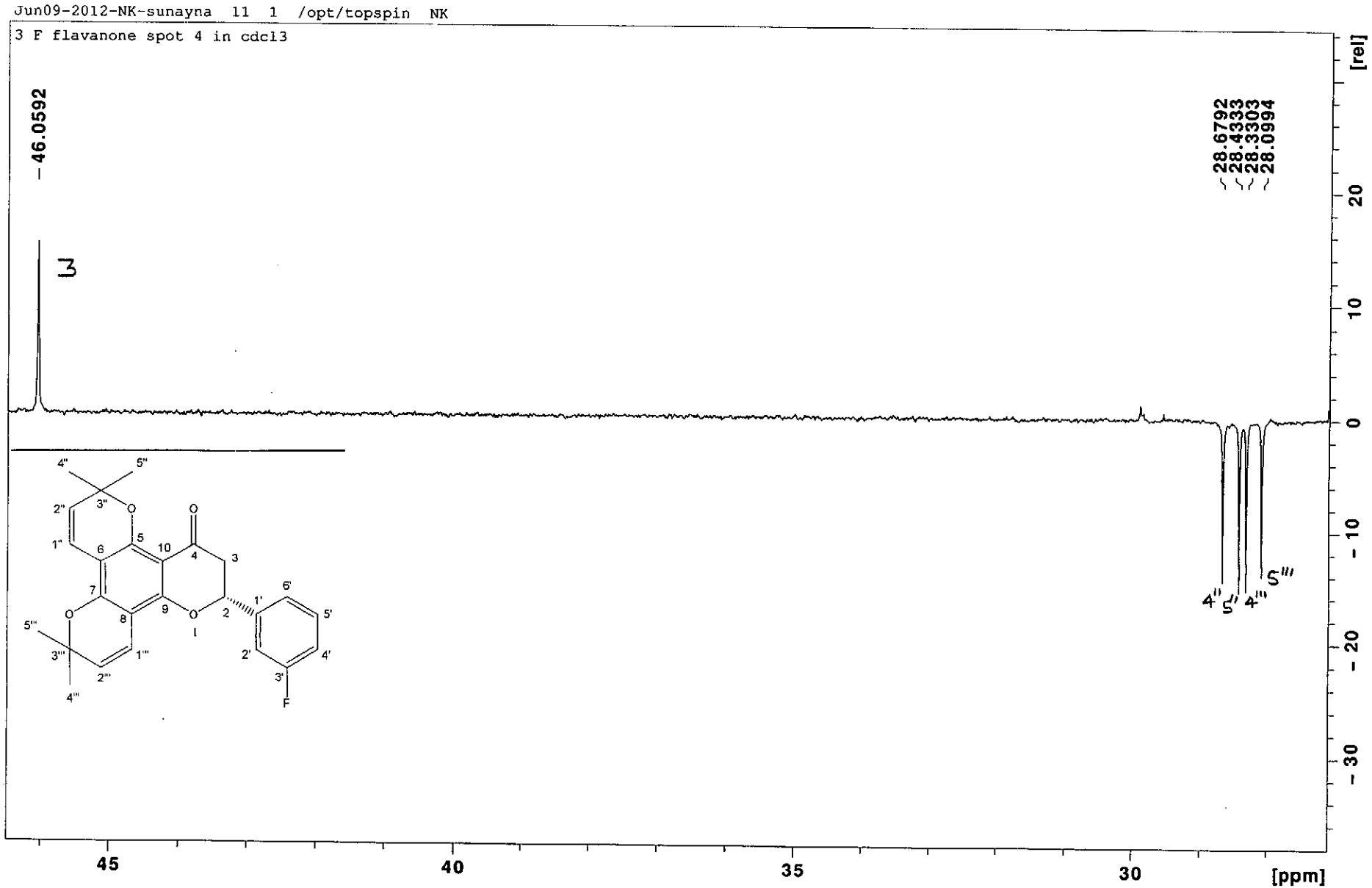
^{13}C NMR spectrum of 4c - 3'-F pyranochromene flavanone (A-4c)



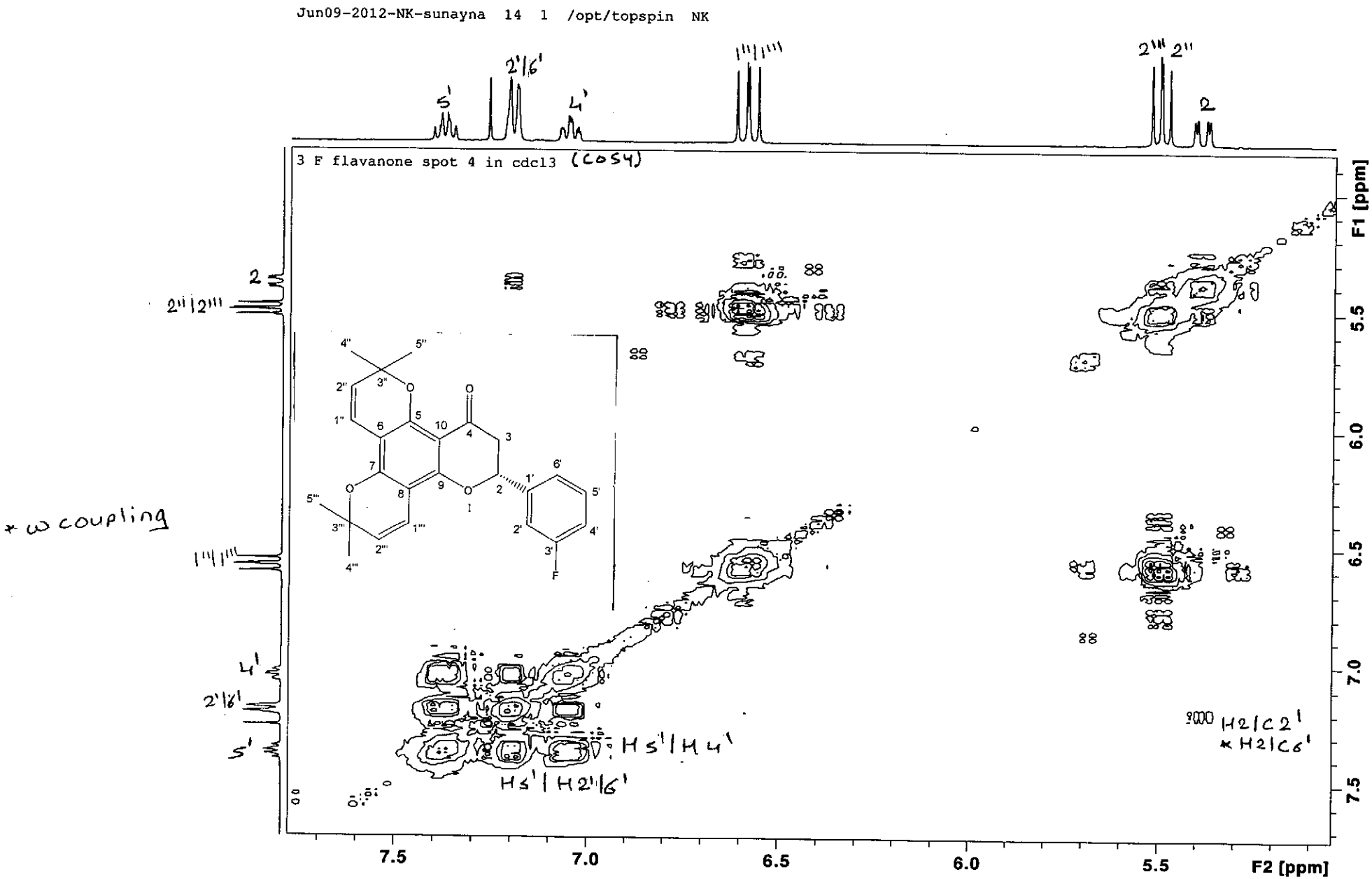
^{13}C NMR spectrum of 4c - 3'-F pyranochromene flavanone (A-4c)



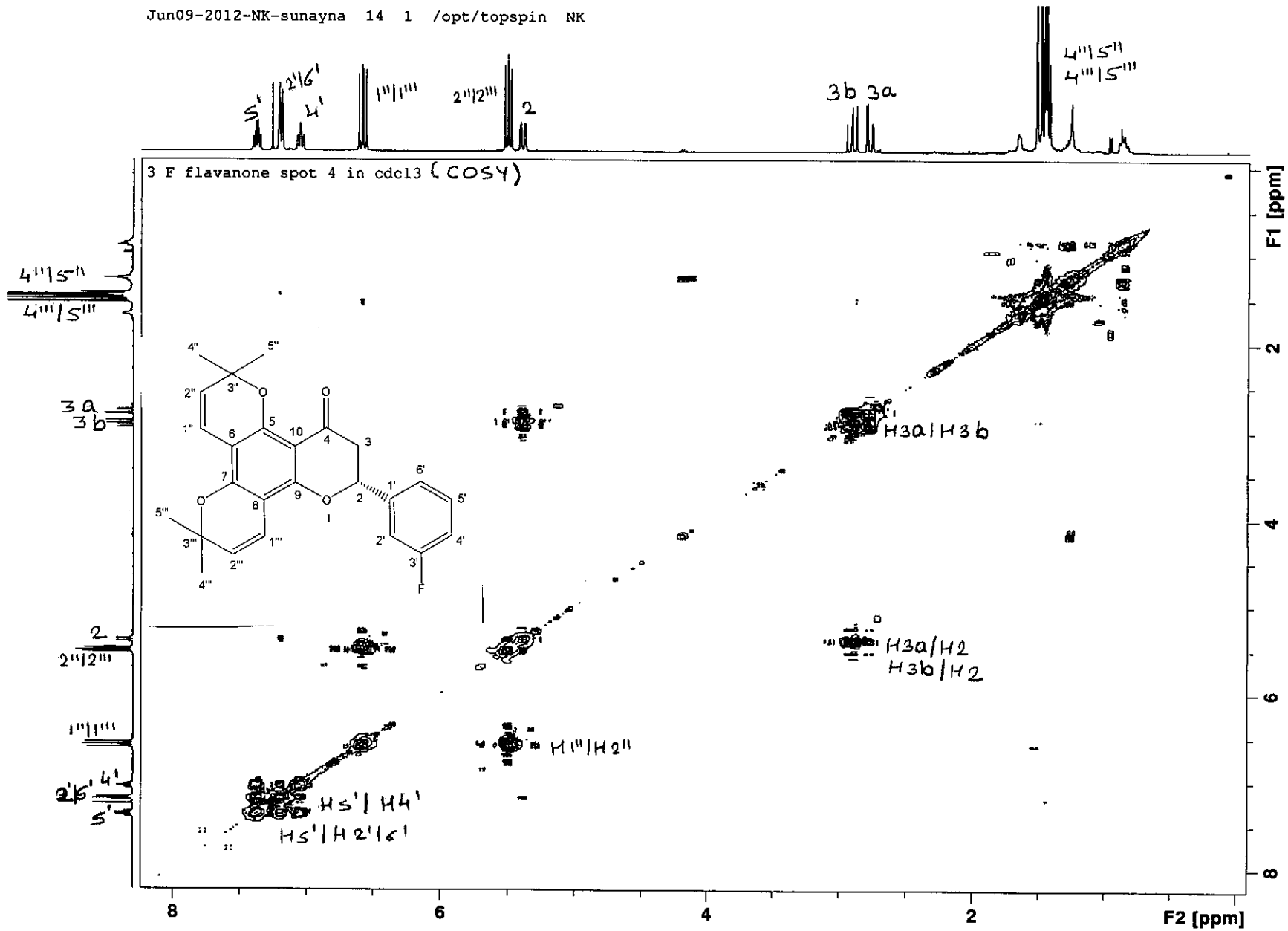
^{13}C NMR spectrum of 4c - 3'-F pyranochromene flavanone (A-4c)



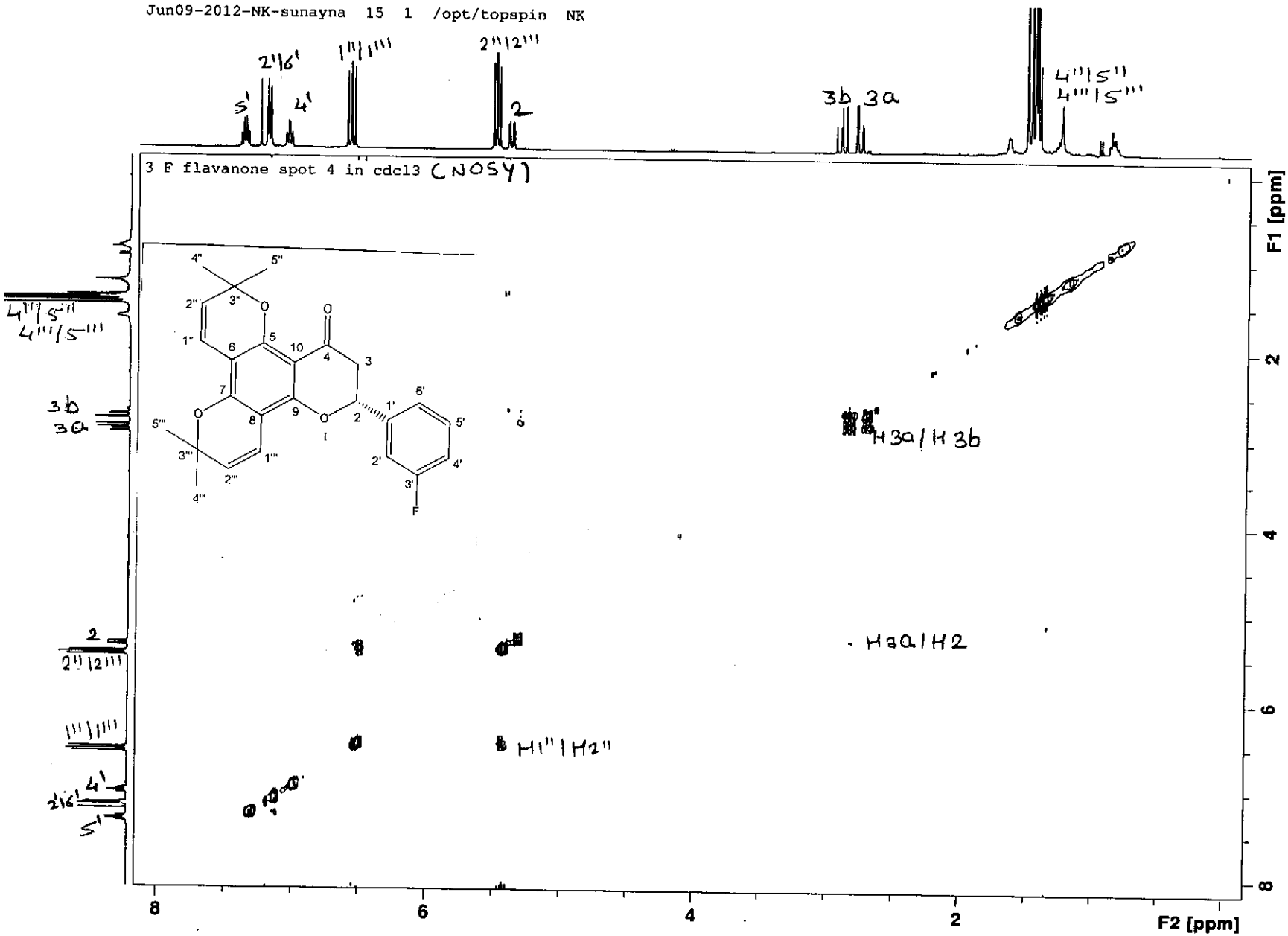
^{13}C NMR spectrum of 4c - 3'-F pyranochromene flavanone (A-4c)



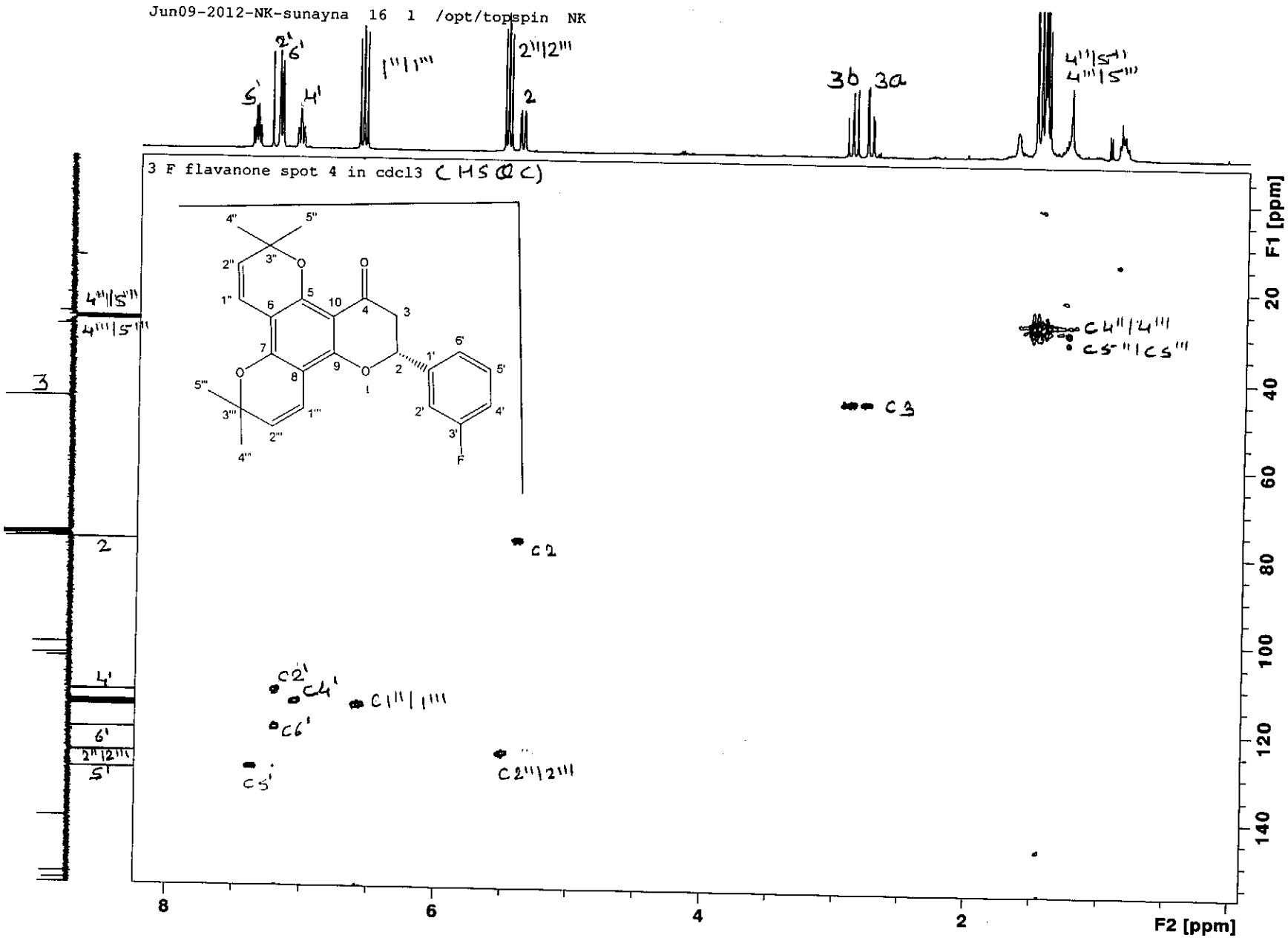
COSY NMR spectrum of 4c - 3'-F pyranochromene flavanone (A-4c)



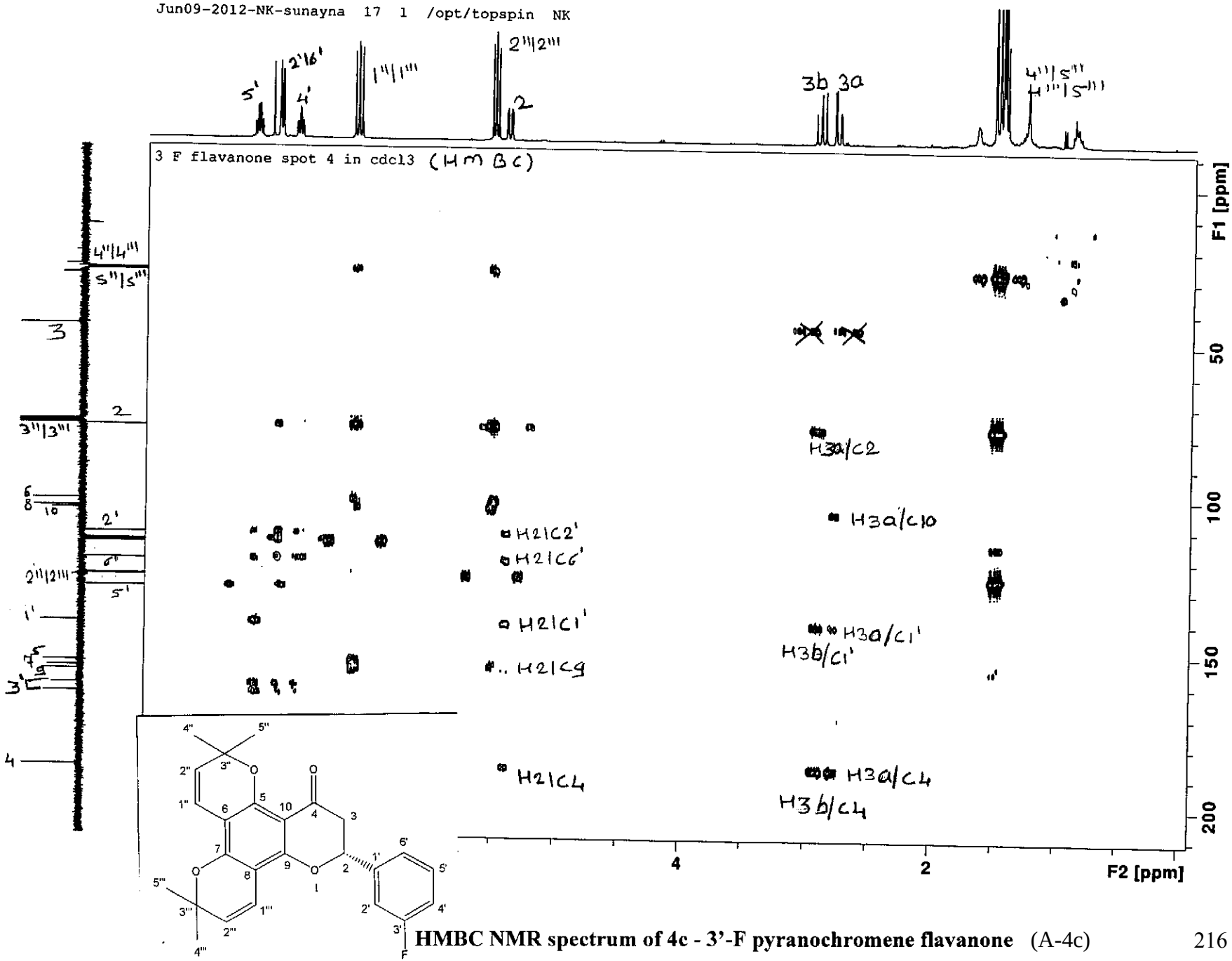
COSY NMR spectrum of 4c - 3'-F pyranochromene flavanone (A-4c)



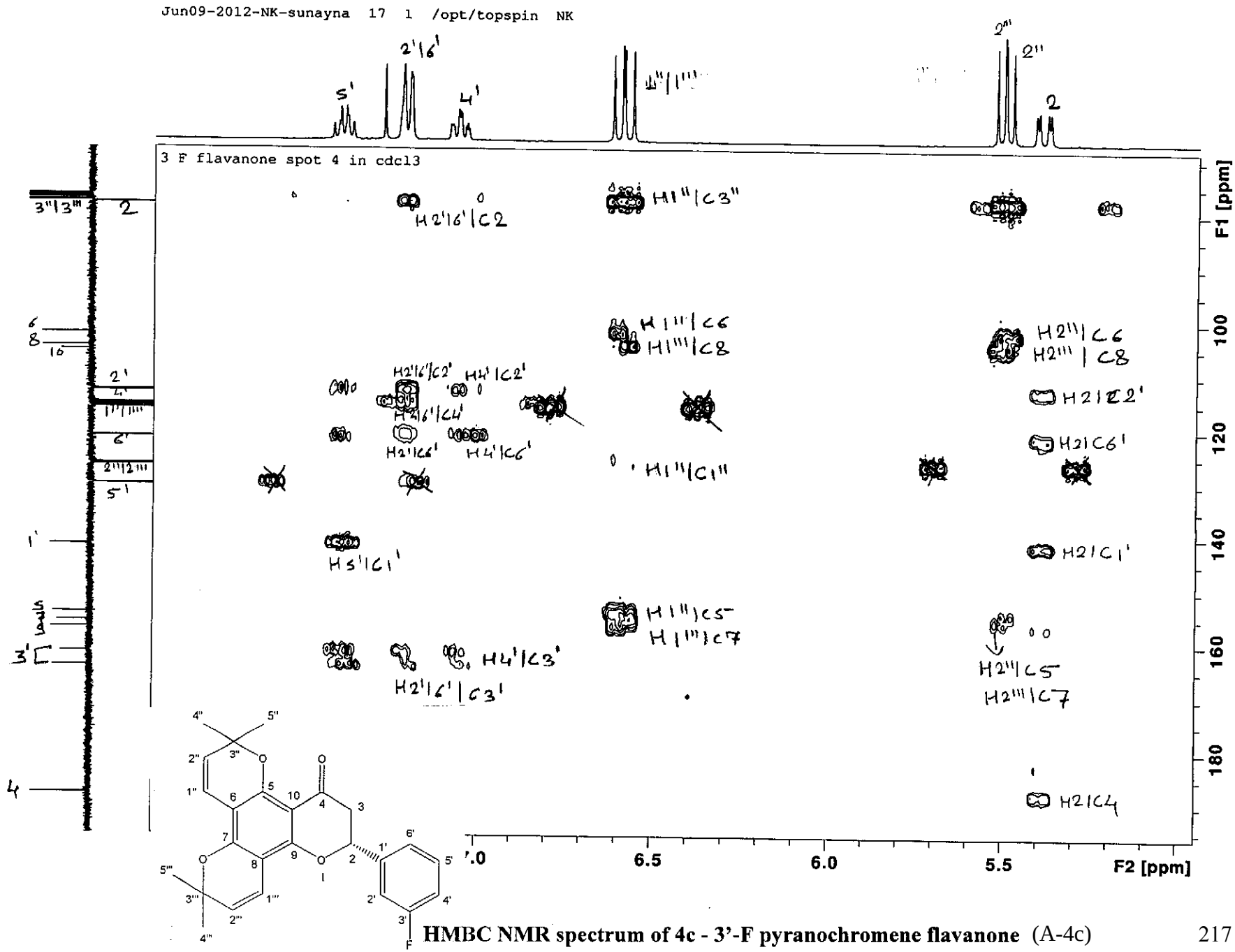
NOSY NMR spectrum of 4c - 3'-F pyranochromene flavanone (A-4c)



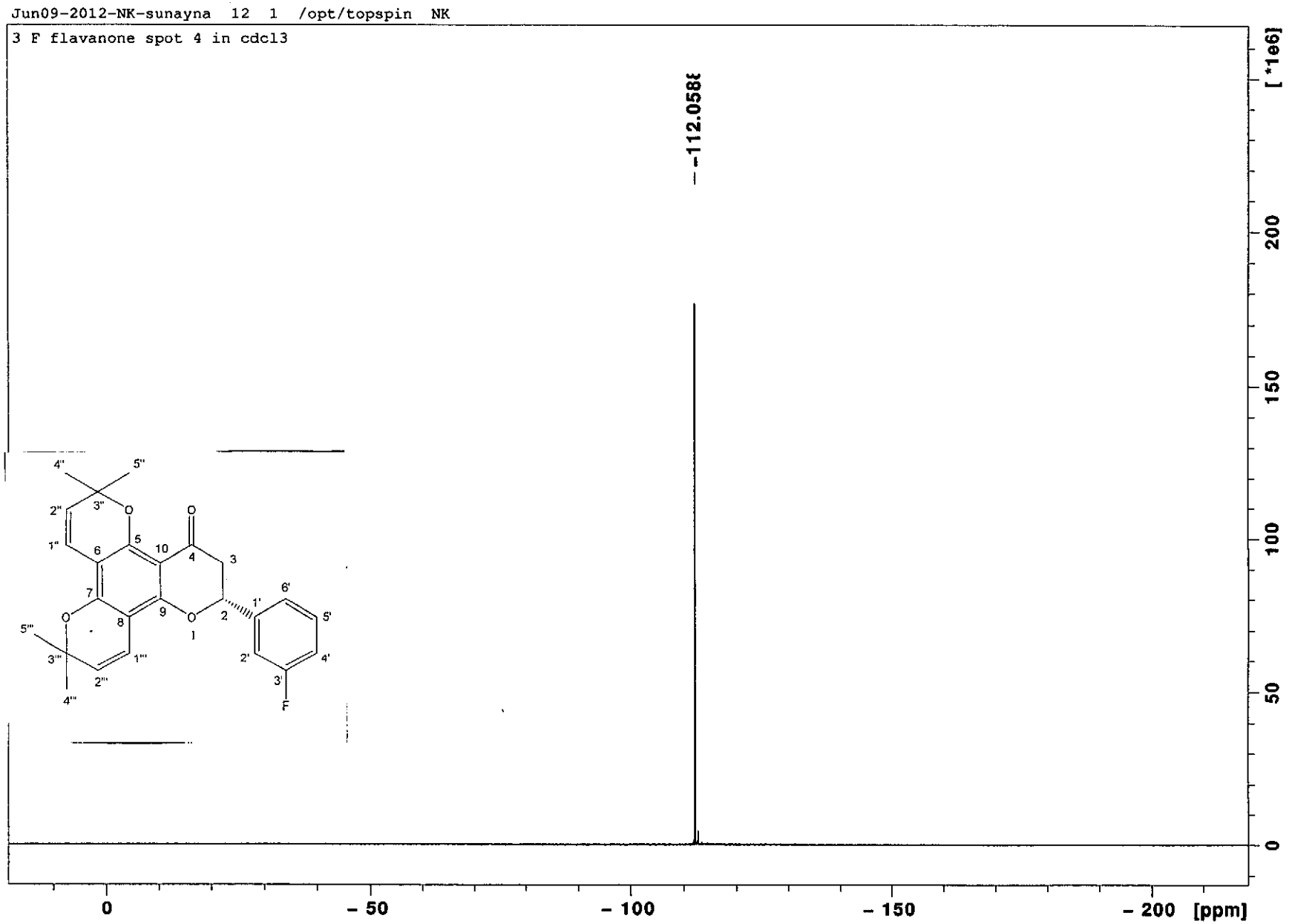
HSQC NMR spectrum of 4c - 3'-F pyranochromene flavanone (A-4c)



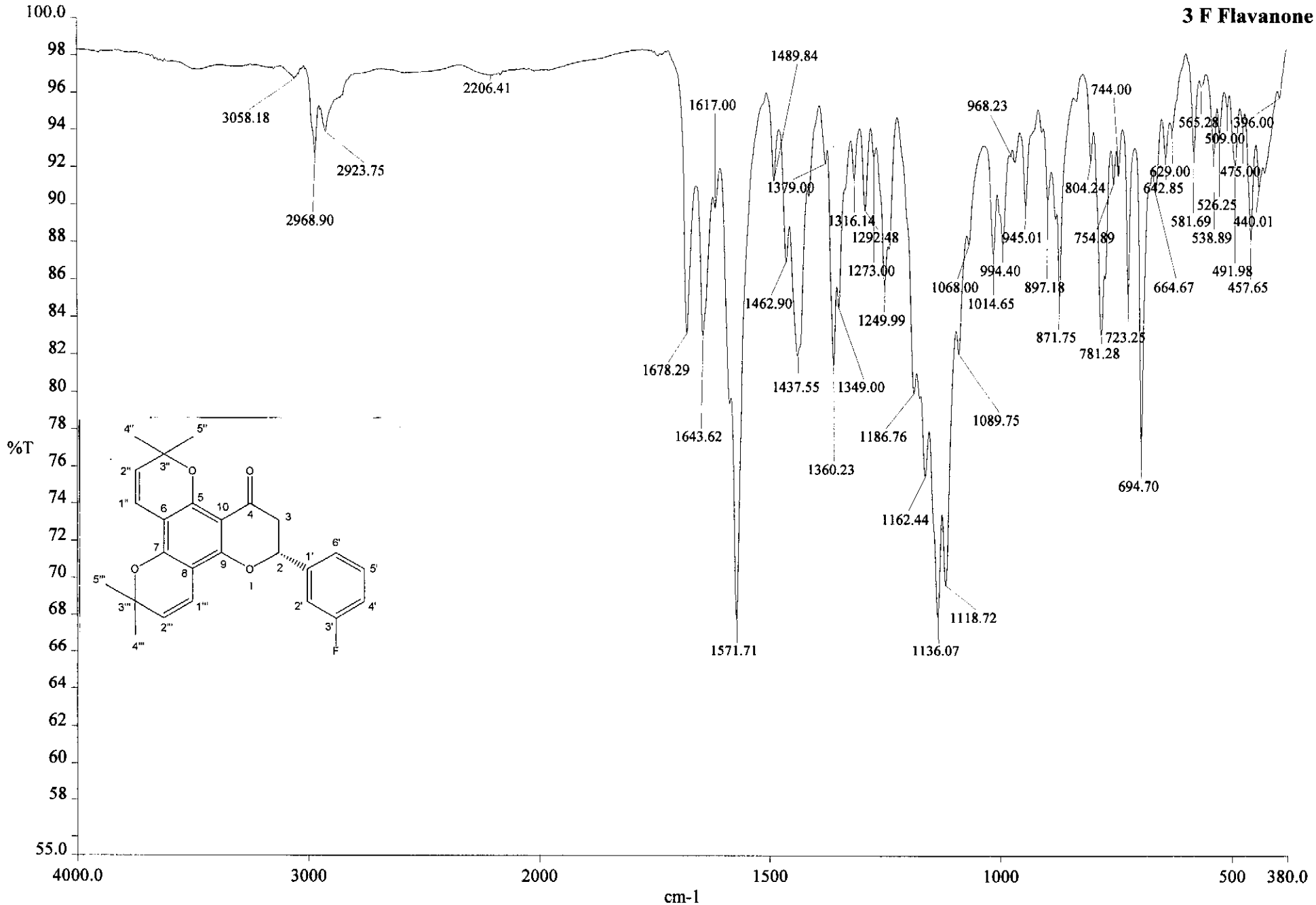
HMBC NMR spectrum of 4c - 3'-F pyranochromene flavanone (A-4c)



HMBC NMR spectrum of 4c - 3'-F pyranochromene flavanone (A-4c)

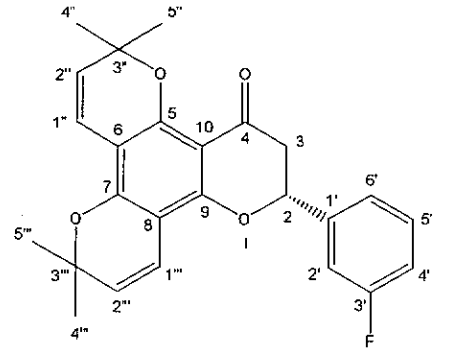


^{19}F NMR spectrum of 4c - 3'-F pyranochromene flavanone (A-4c)



IR spectrum of 4c - 3'-F pyranochromene flavanone (A-4c)

Elemental Composition Report



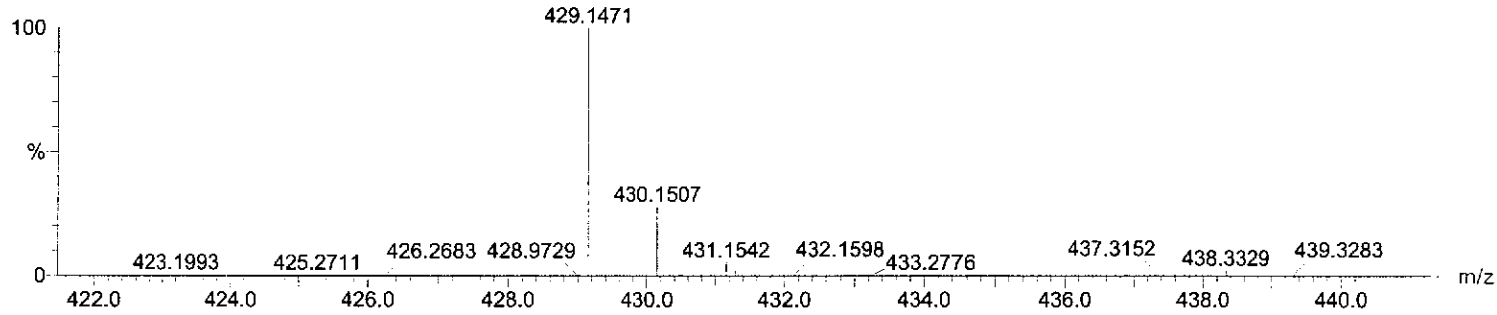
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
3 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-30 H: 20-25 O: 0-5 F: 1-1 Na: 0-1

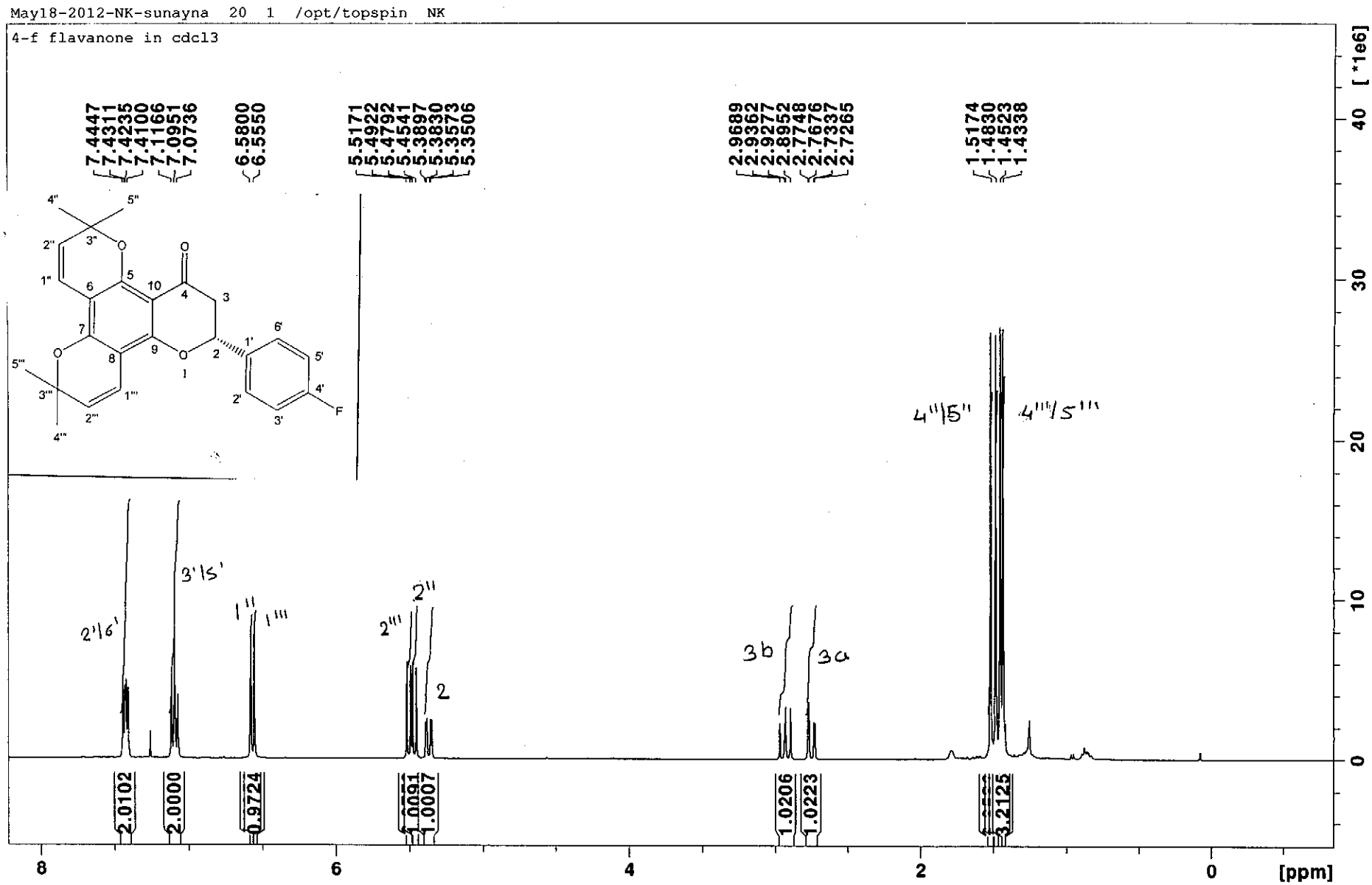
Dr. Nizam
12 27 (0.443) Cm (1:30)

TOF MS ES+
2.19e+004

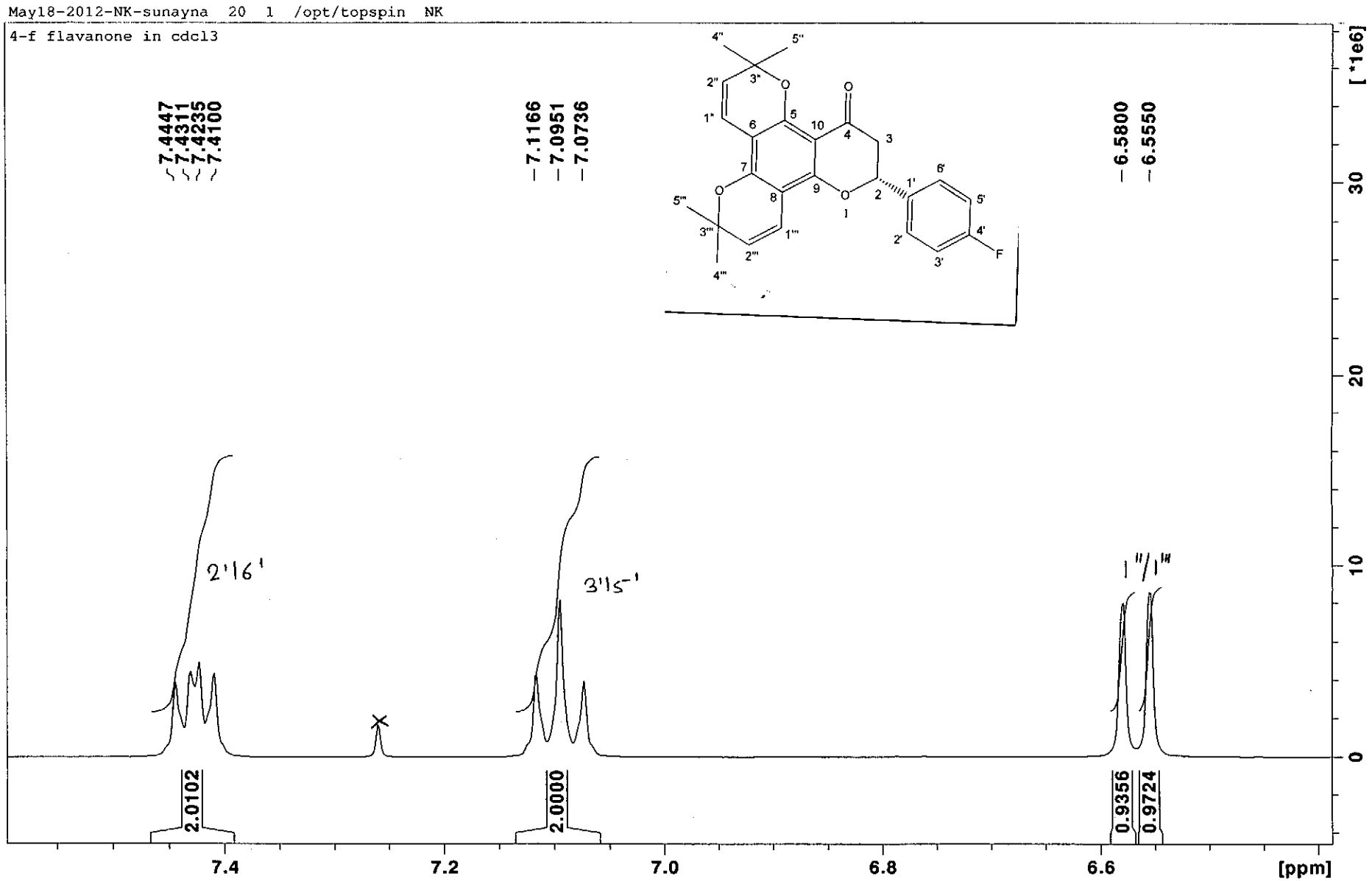


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
429.1471	429.1478	-0.7	-1.6	13.5	384.3	0.0	C25 H23 O4 F Na

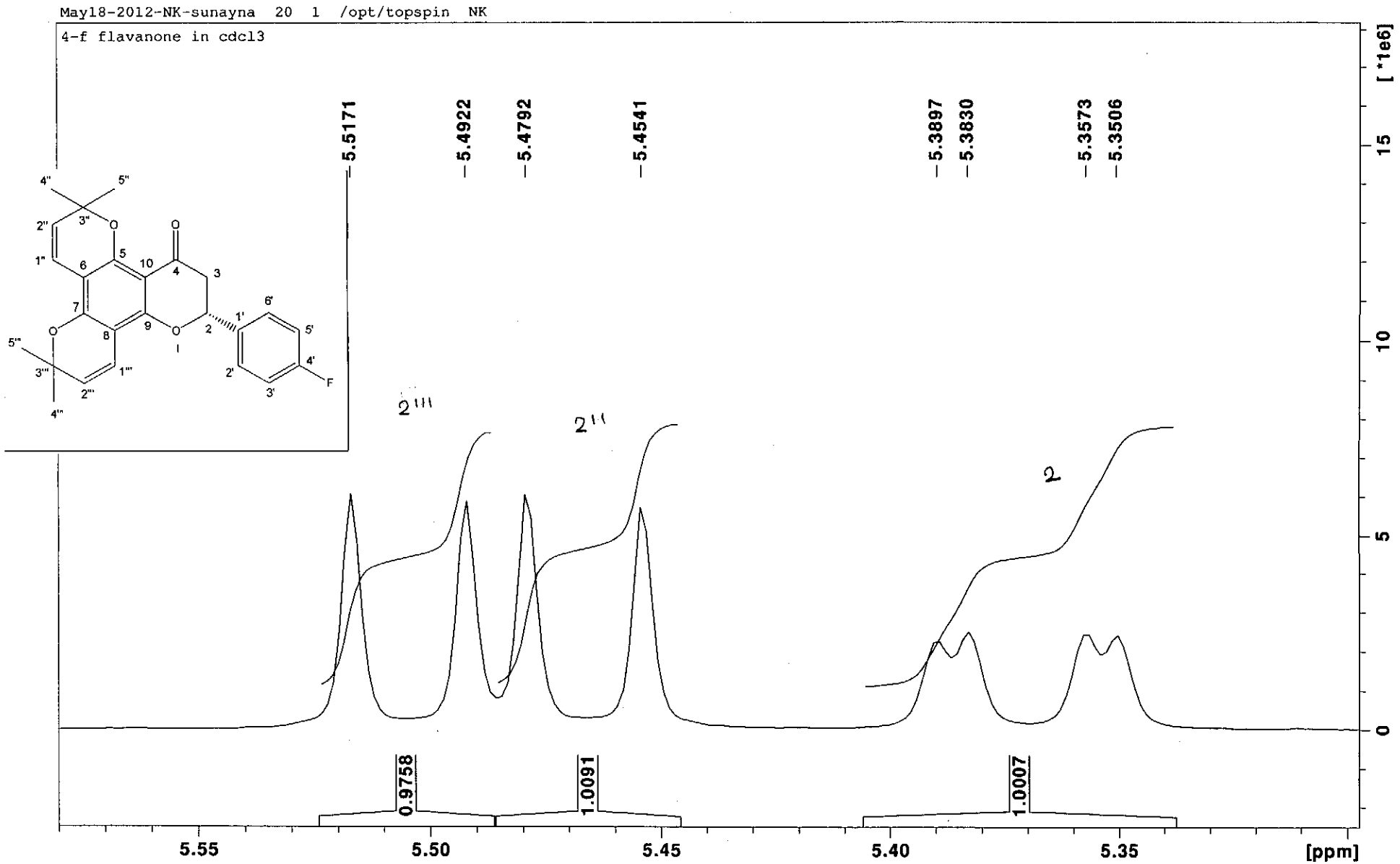
HRMS of 4c – 3'-F pyranochromene flavanone (A-4c)



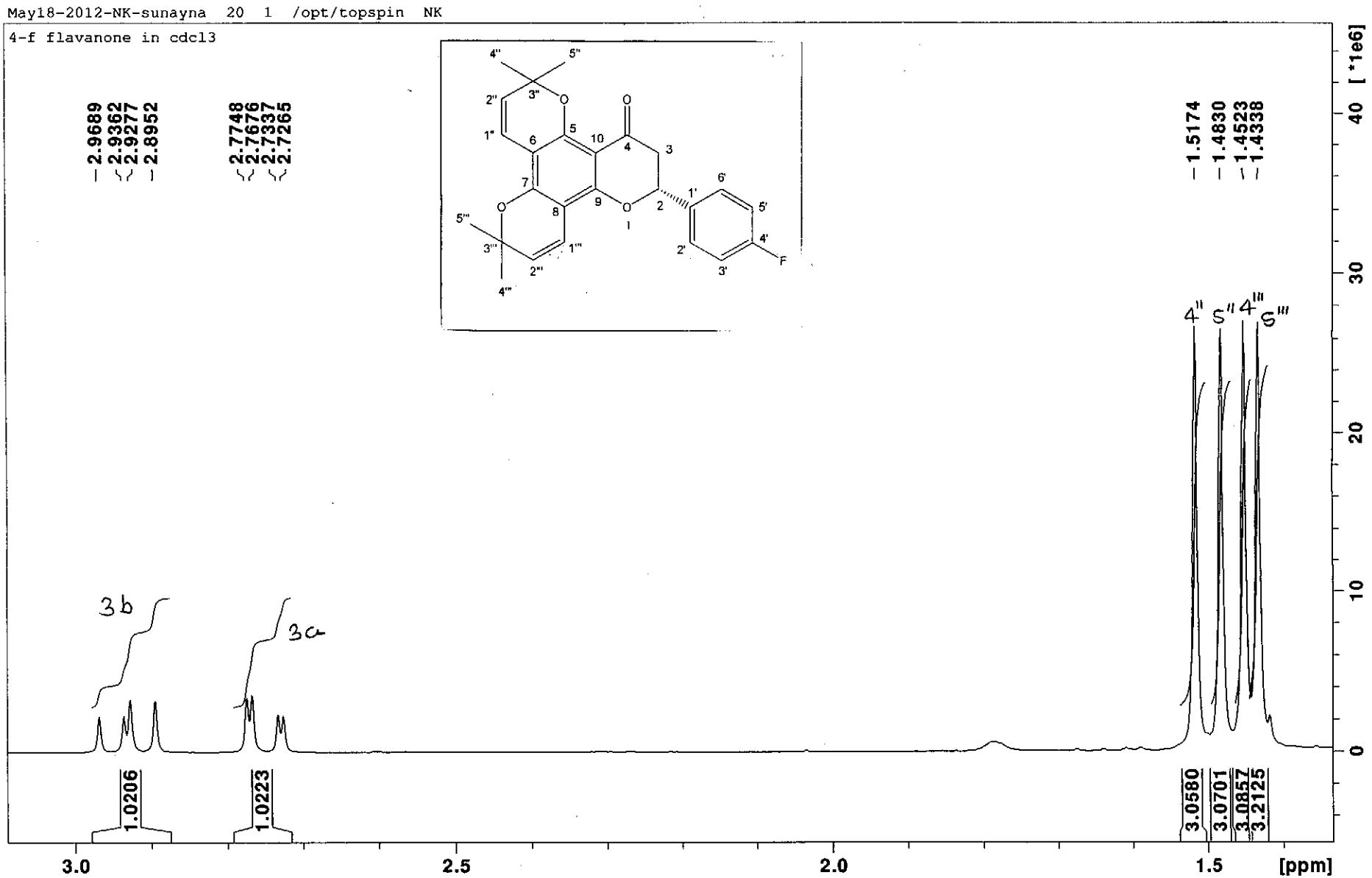
[1]H NMR spectrum of 4d - 4'-F pyranochromene flavanone (A-4d)



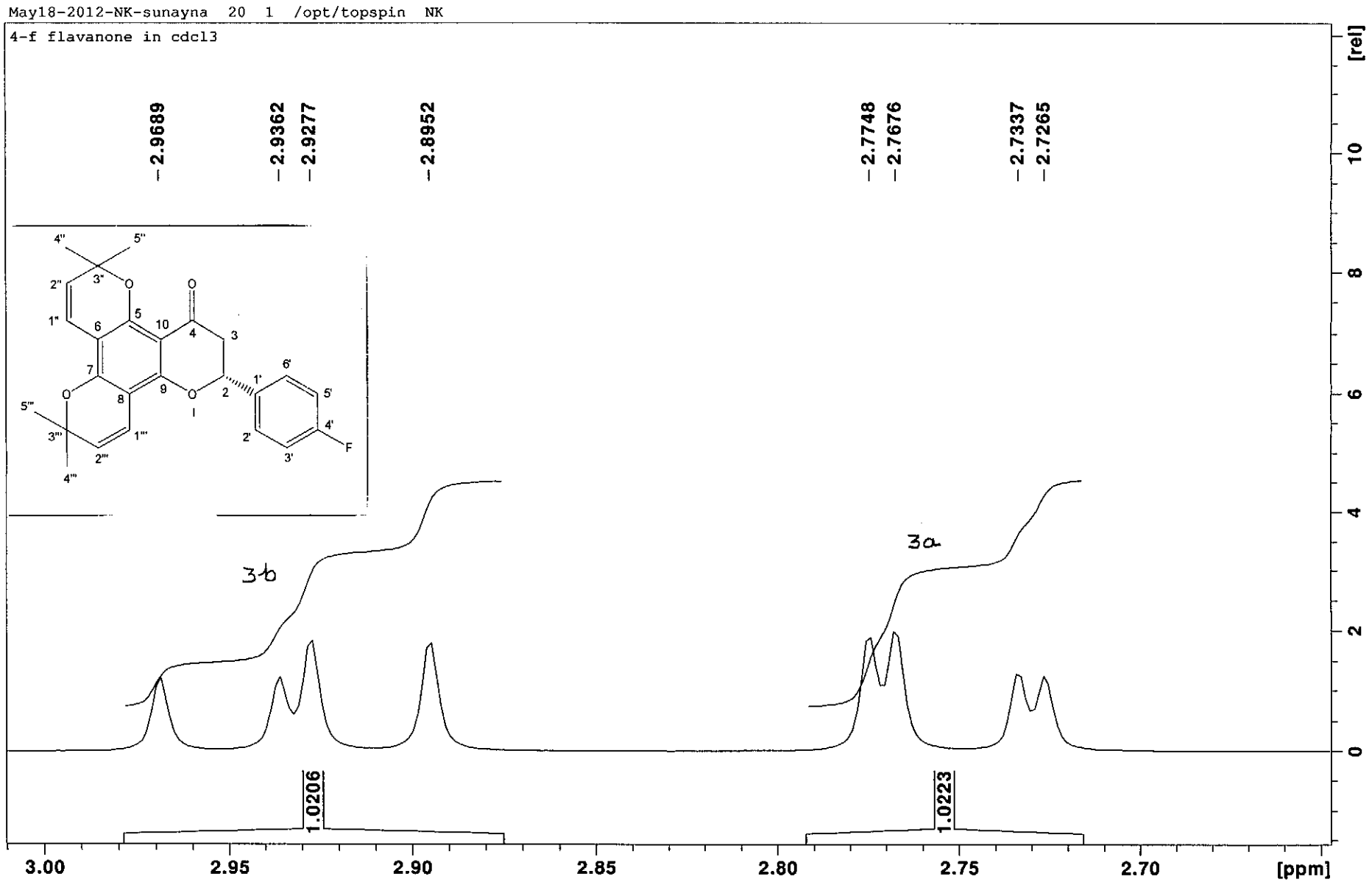
^{1}H NMR spectrum of 4d - 4'-F pyranochromene flavanone (A-4d)



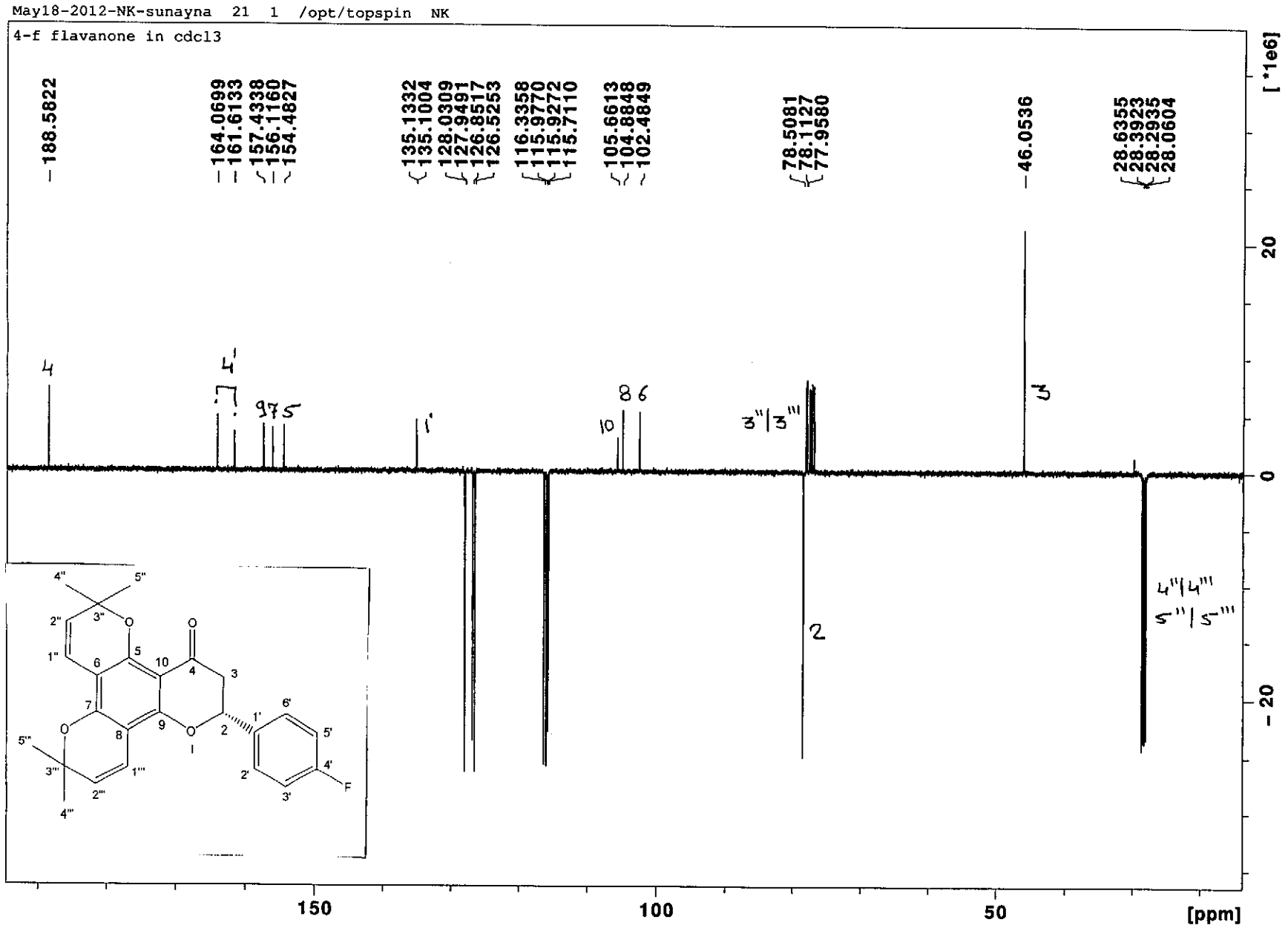
^{1}H NMR spectrum of 4d - 4'-F pyranochromene flavanone (A-4d)



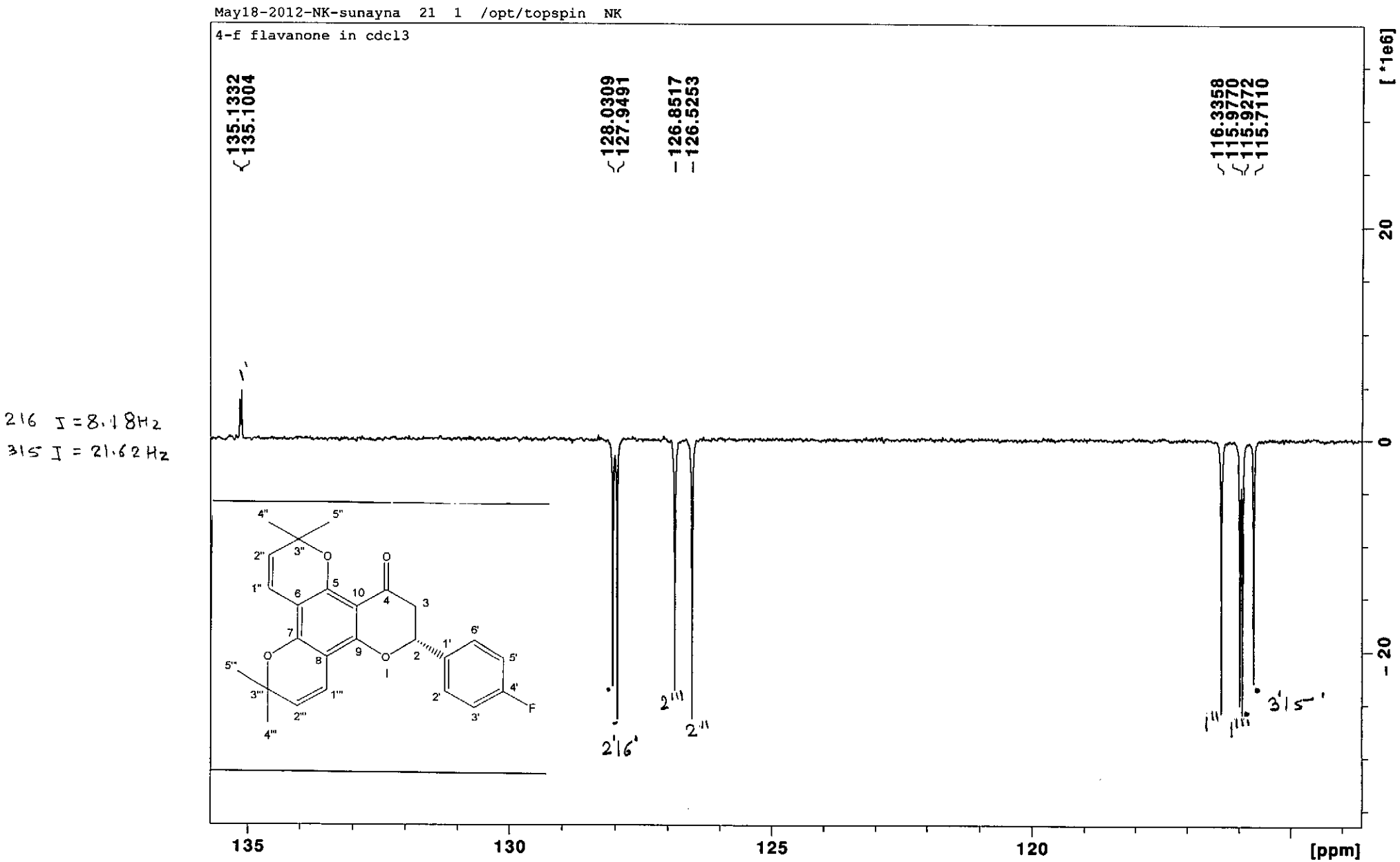
^{1}H NMR spectrum of 4d - 4'-F pyranochromene flavanone (A-4d)



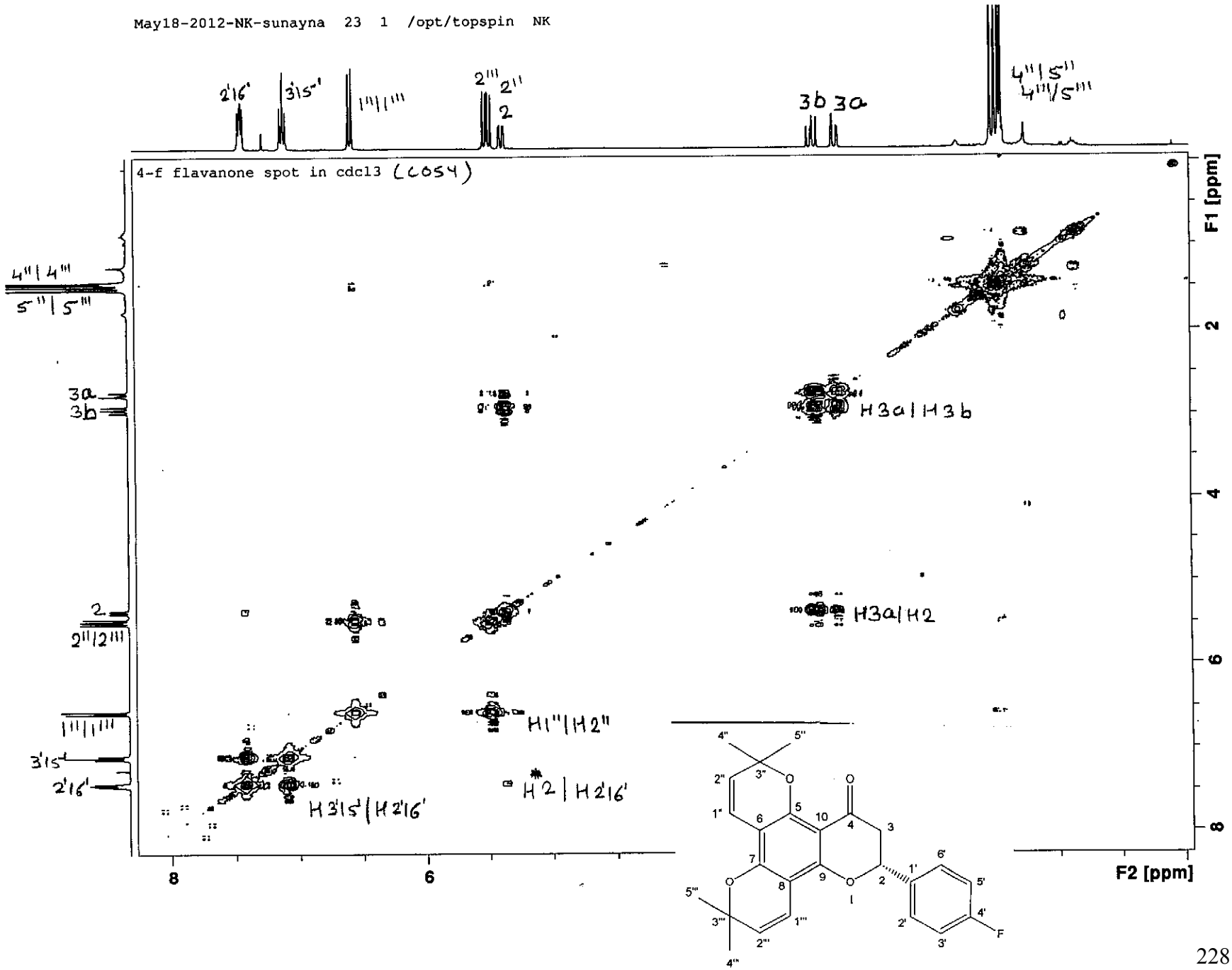
^{1}H NMR spectrum of 4d - 4'-F pyranochromene flavanone (A-4d)



^{13}C NMR spectrum of 4d - 4'-F pyranochromene flavanone (A-4d)

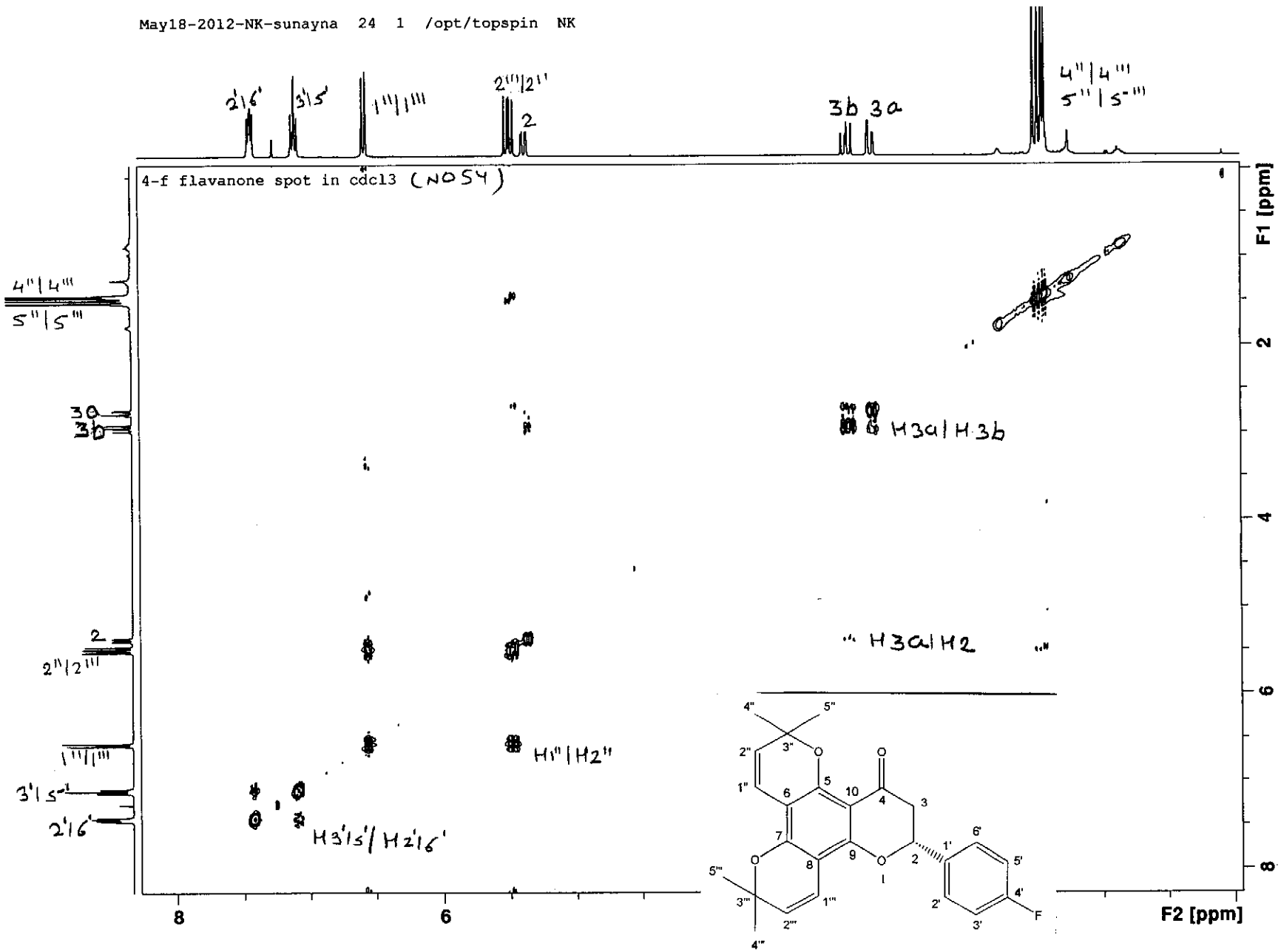


^{13}C NMR spectrum of 4d - 4'-F pyranochromene flavanone (A-4d)

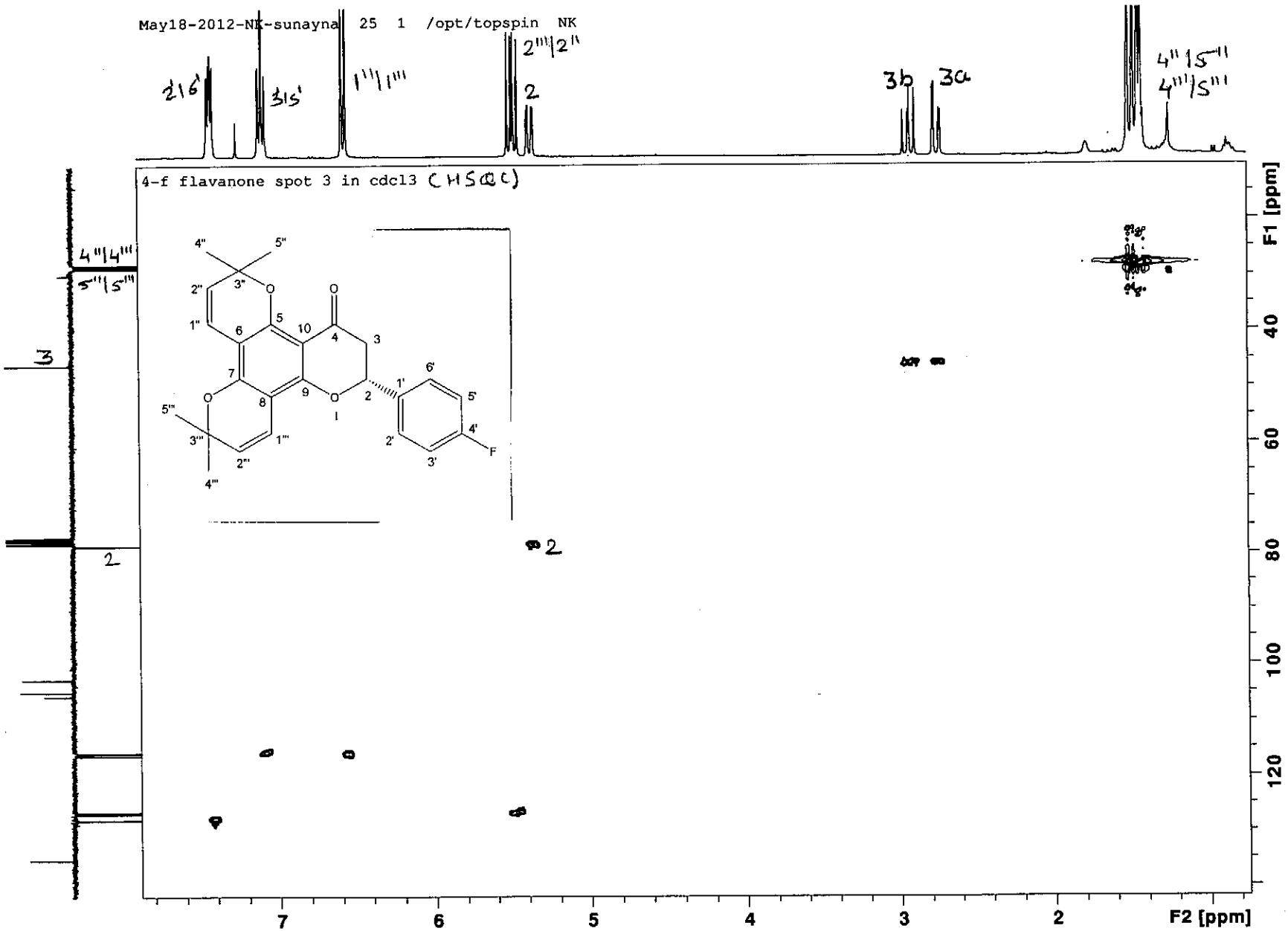


*'ω Coupling

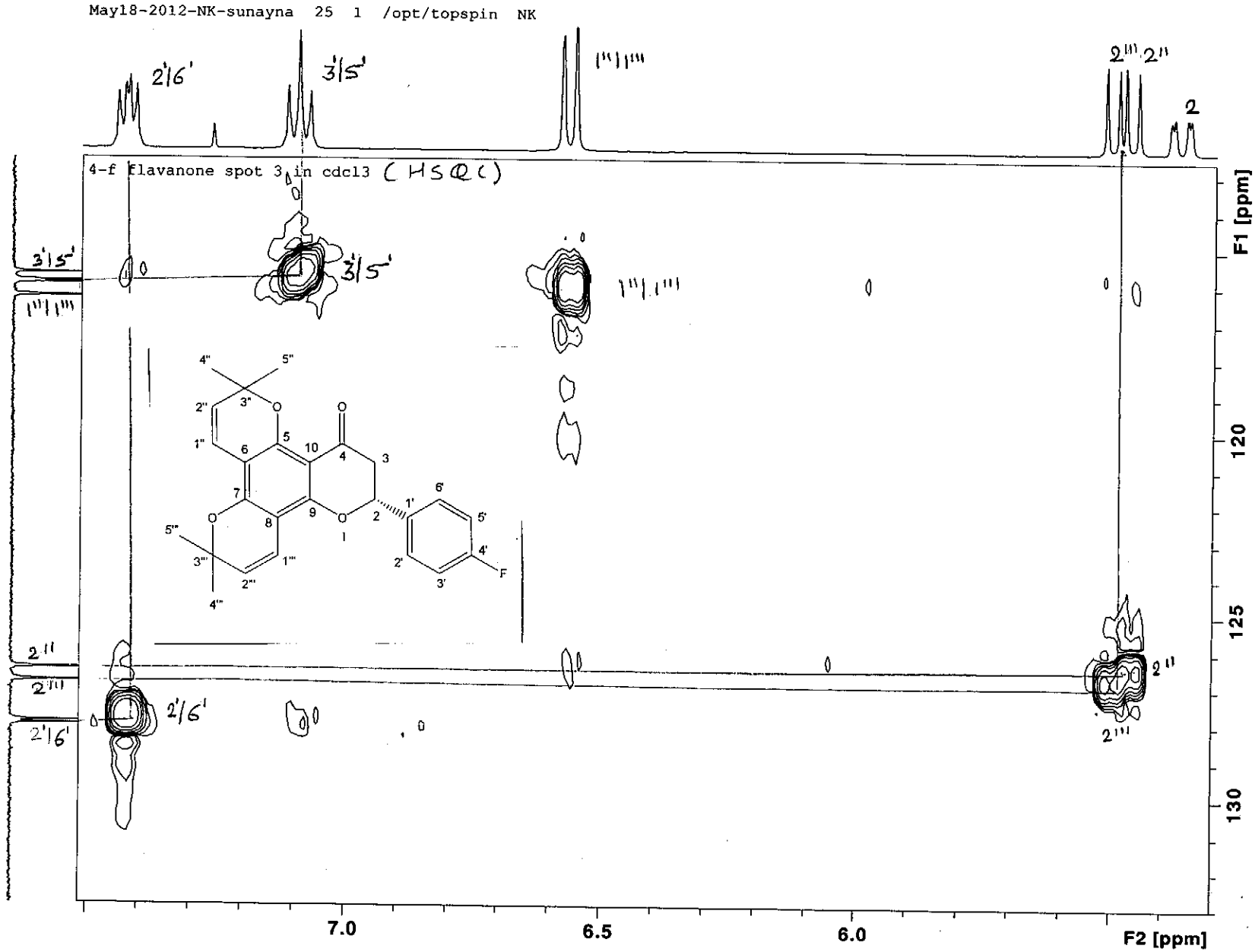
COSY NMR spectrum of 4d - 4'-F pyranochromene flavanone (A-4d)



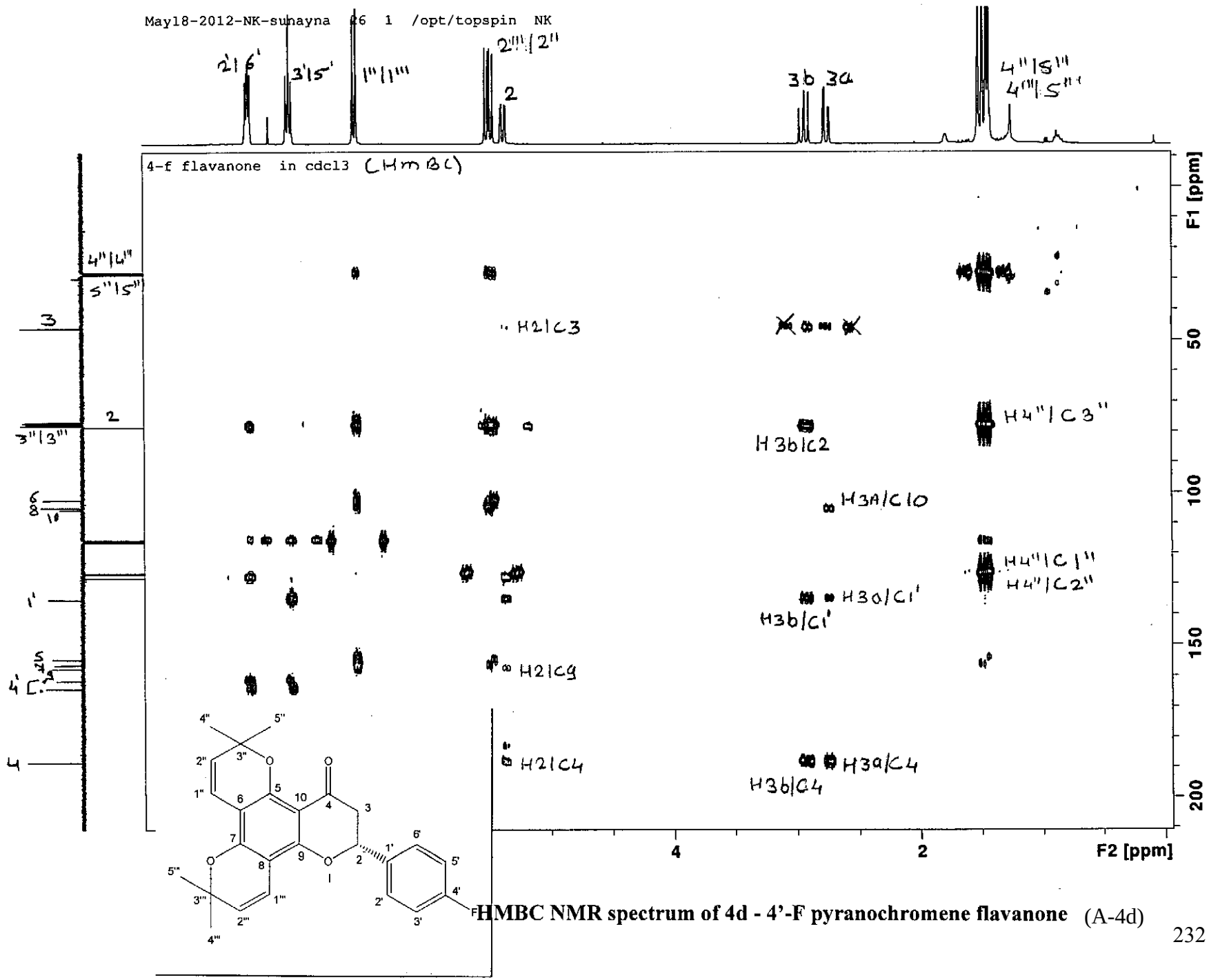
NOSY NMR spectrum of 4d - 4'-F pyranochromene flavanone (A-4d)



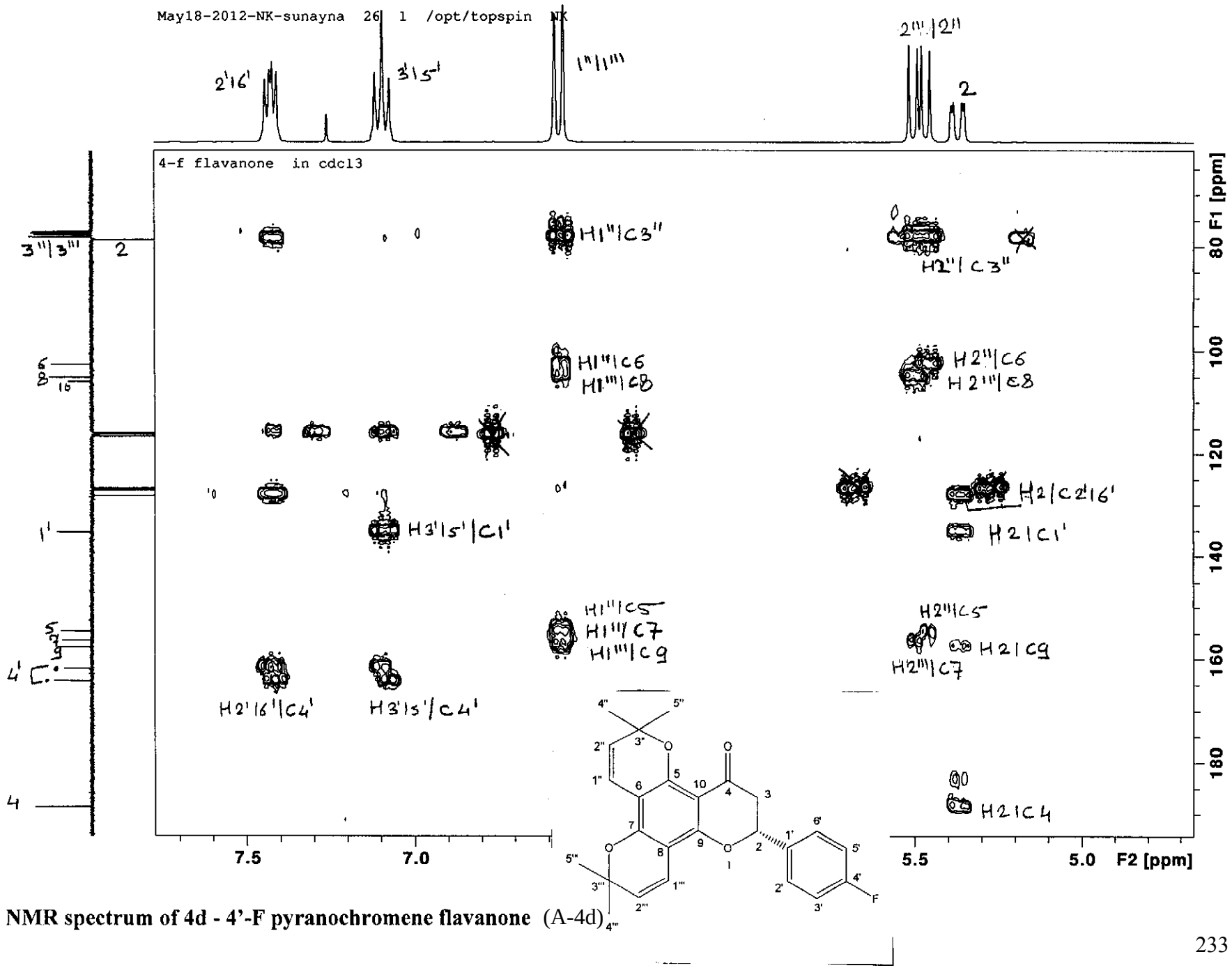
HSQC NMR spectrum of 4d - 4'-F pyranochromene flavanone (A-4d)



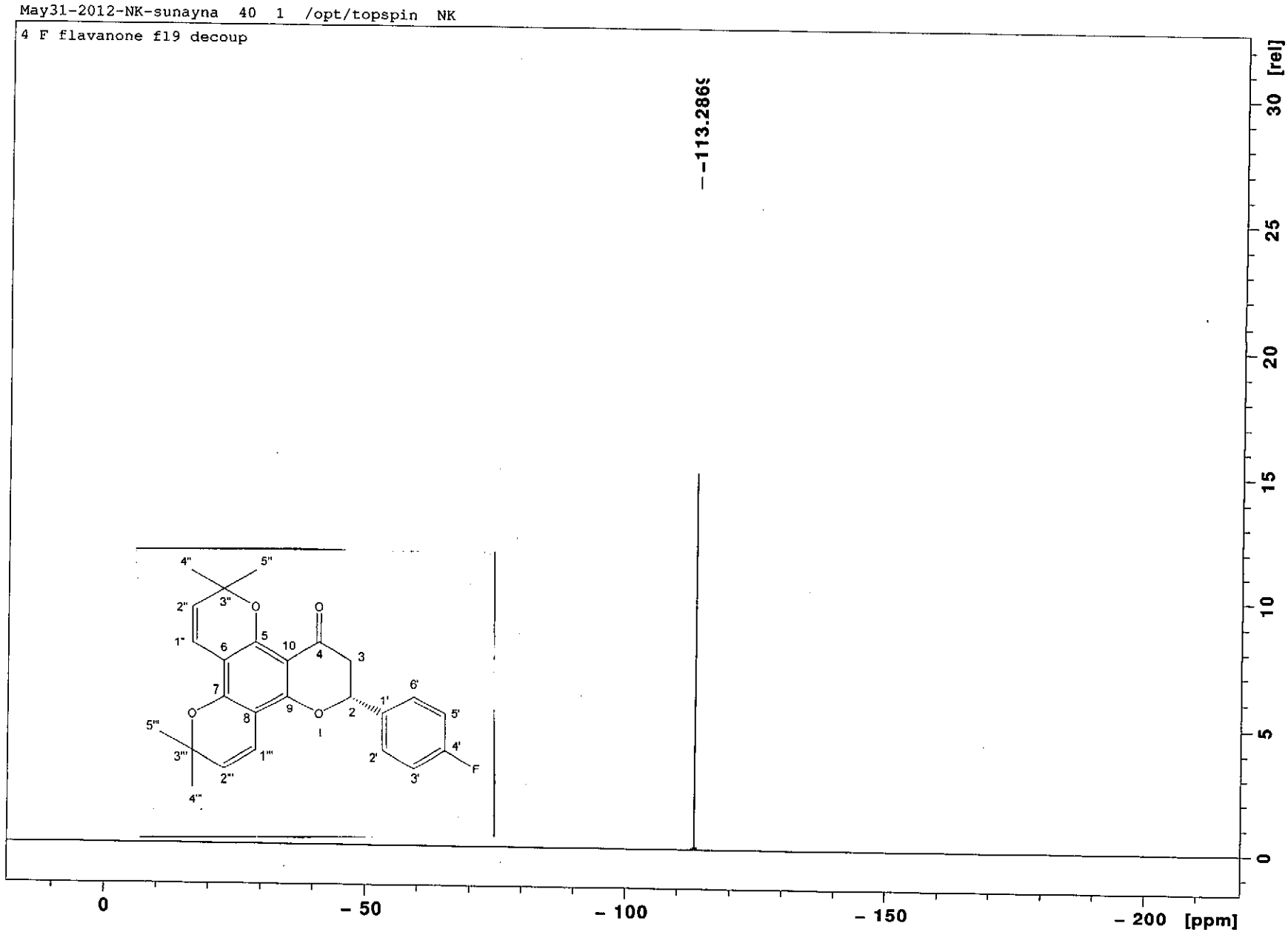
HSQC NMR spectrum of 4d - 4'-F pyranochromene flavanone (A-4d)



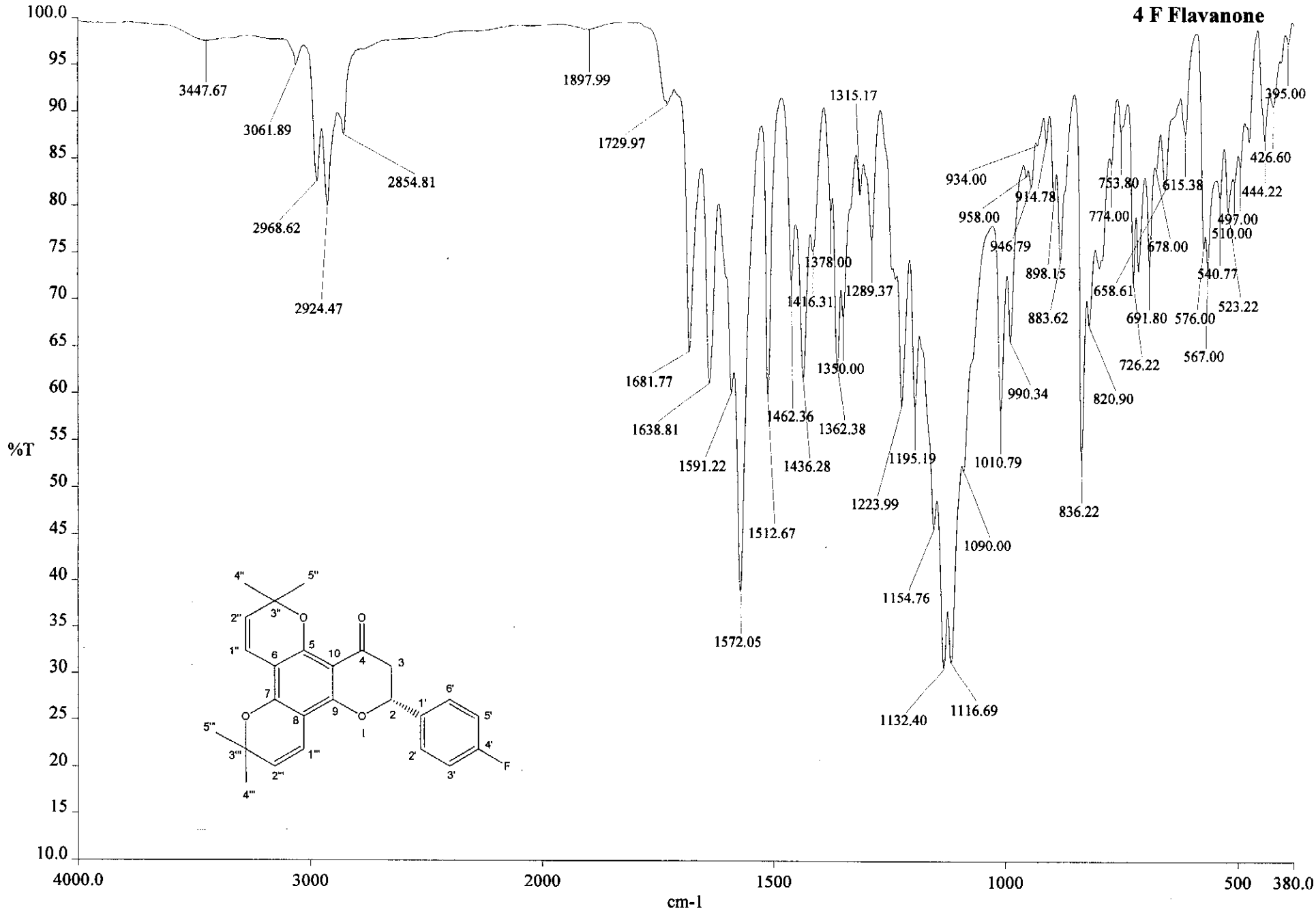
HMBC NMR spectrum of 4d - 4'-F pyranochromene flavanone (A-4d)



HMBC NMR spectrum of 4d - 4'-F pyranochromene flavanone (A-4d)



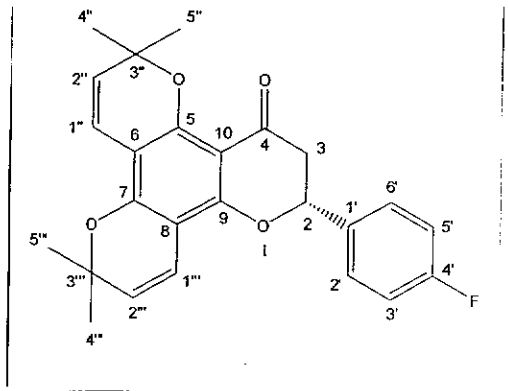
^{19}F NMR spectrum of 4d - 4'-F pyranochromene flavanone (A-4d)



c:\pel_data\spectra\sunayna cha\4 f fla.002

IR spectrum of 4d - 4'-F pyranochromene flavanone (A-4d)

Elemental Composition Report



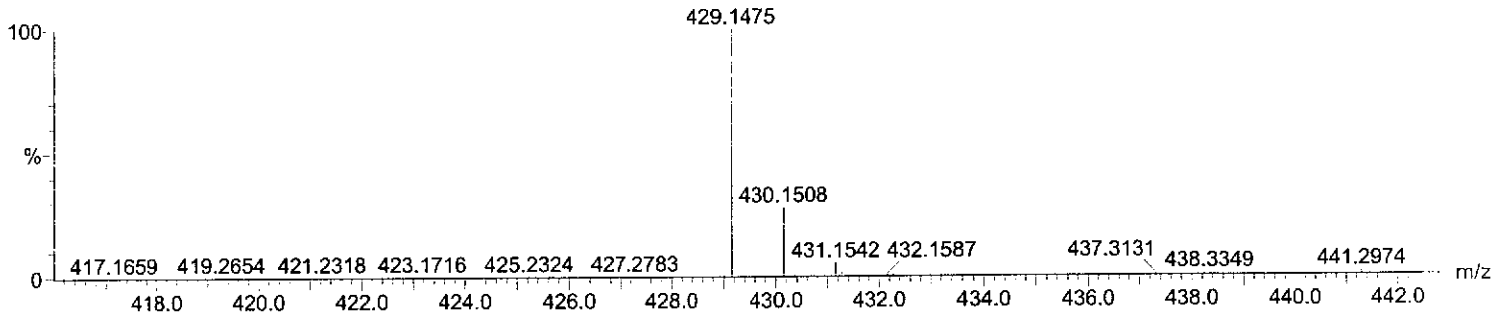
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
3 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-30 H: 20-25 O: 0-5 F: 1-1 Na: 0-1

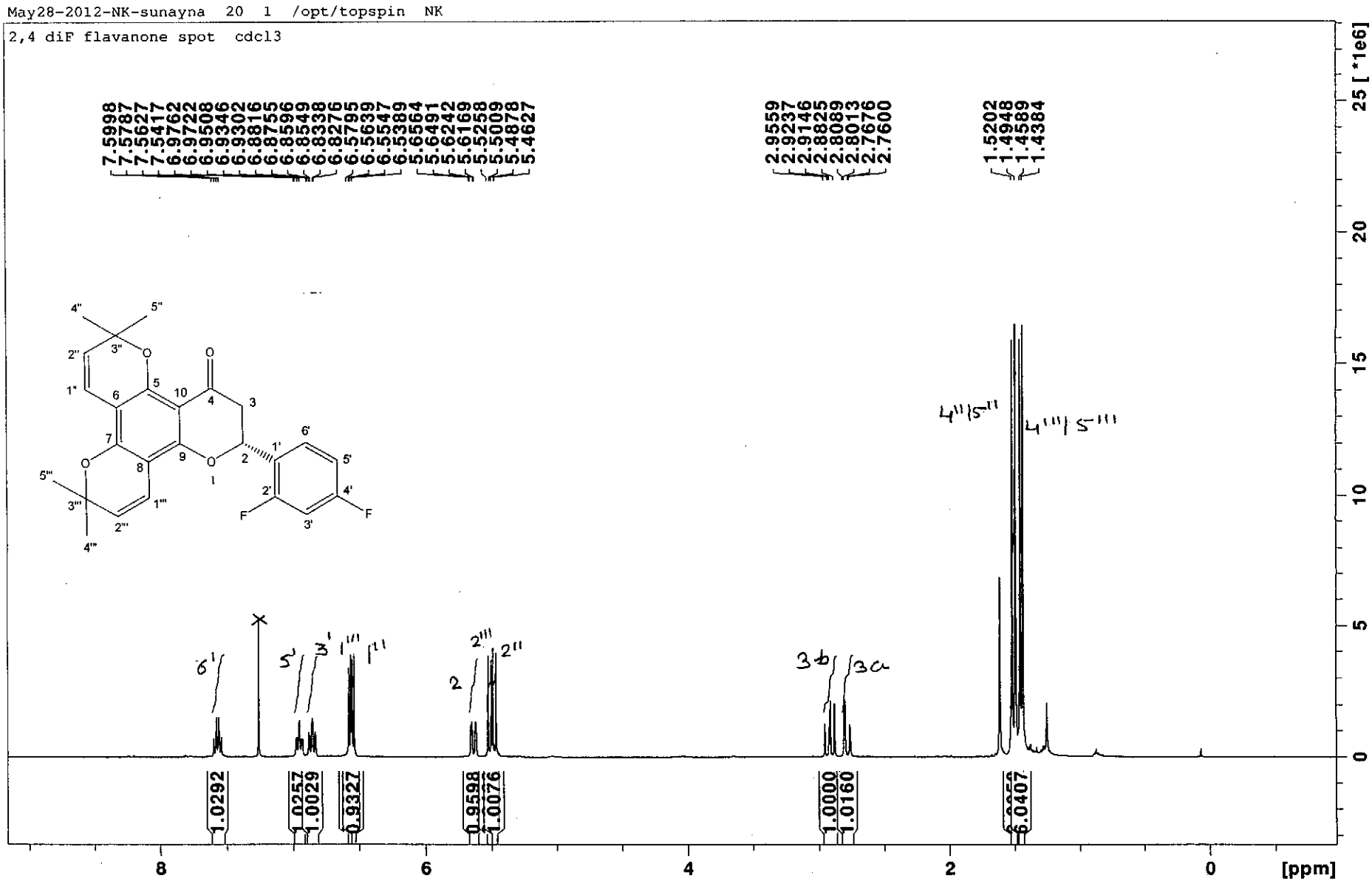
Dr. Nizam
14 2 (0.017) Cm (1:30)

TOF MS ES+
2.87e+004

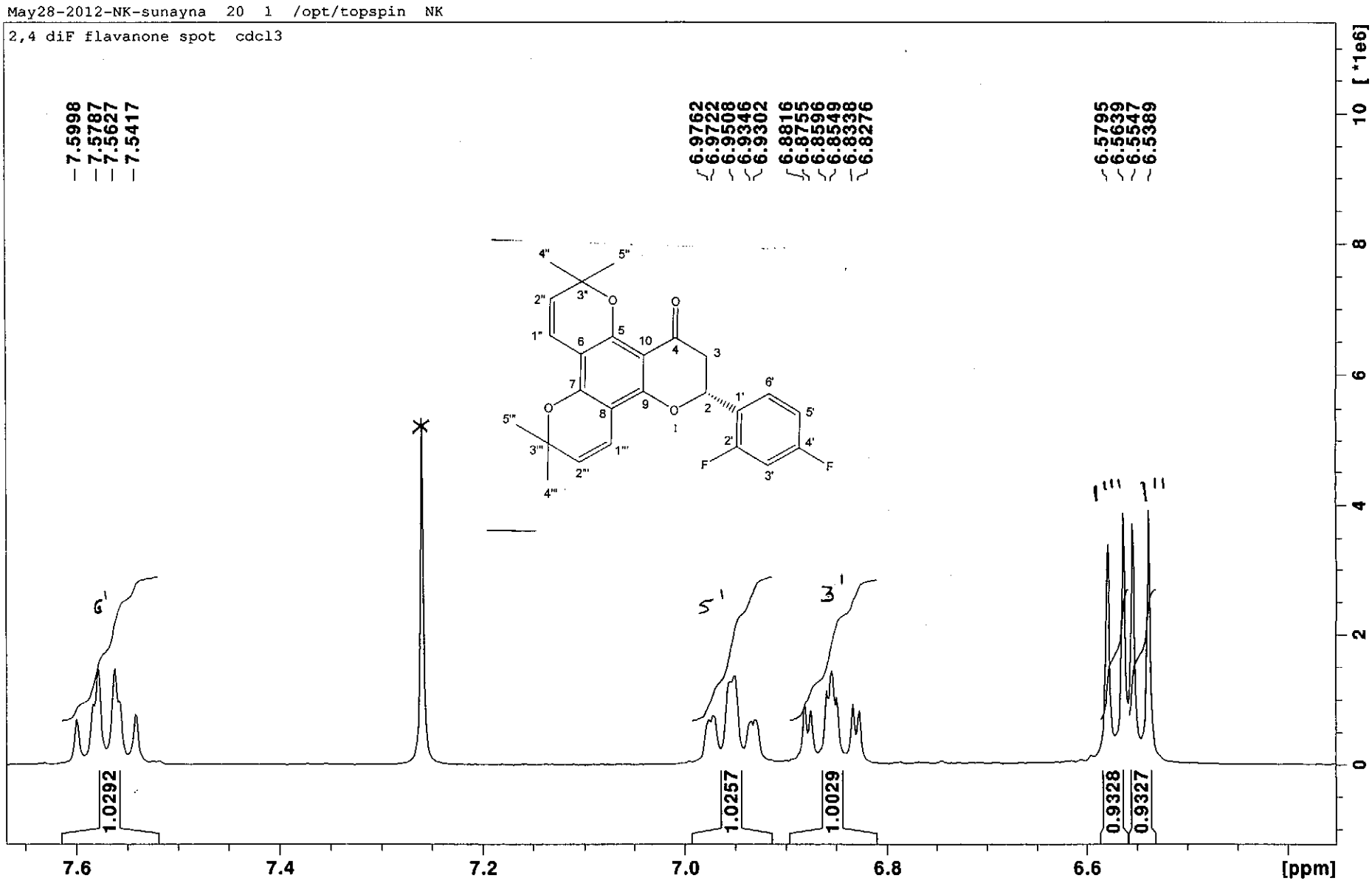


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
429.1475	429.1478	-0.3	-0.7	13.5	394.5	0.0	C25 H23 O4 F Na

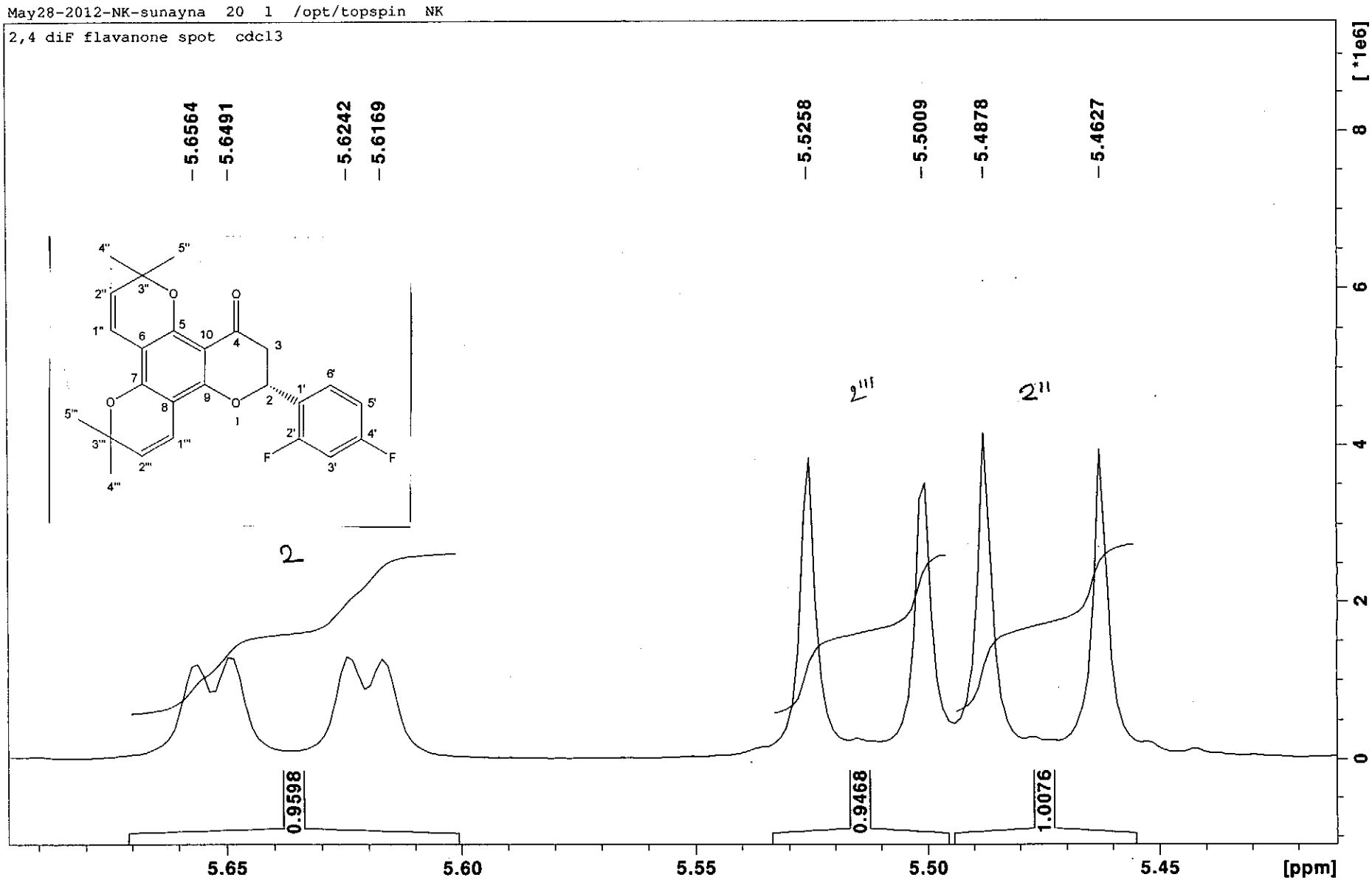
HRMS of 4d – 4'-F pyranochromene flavanone (A-4d)



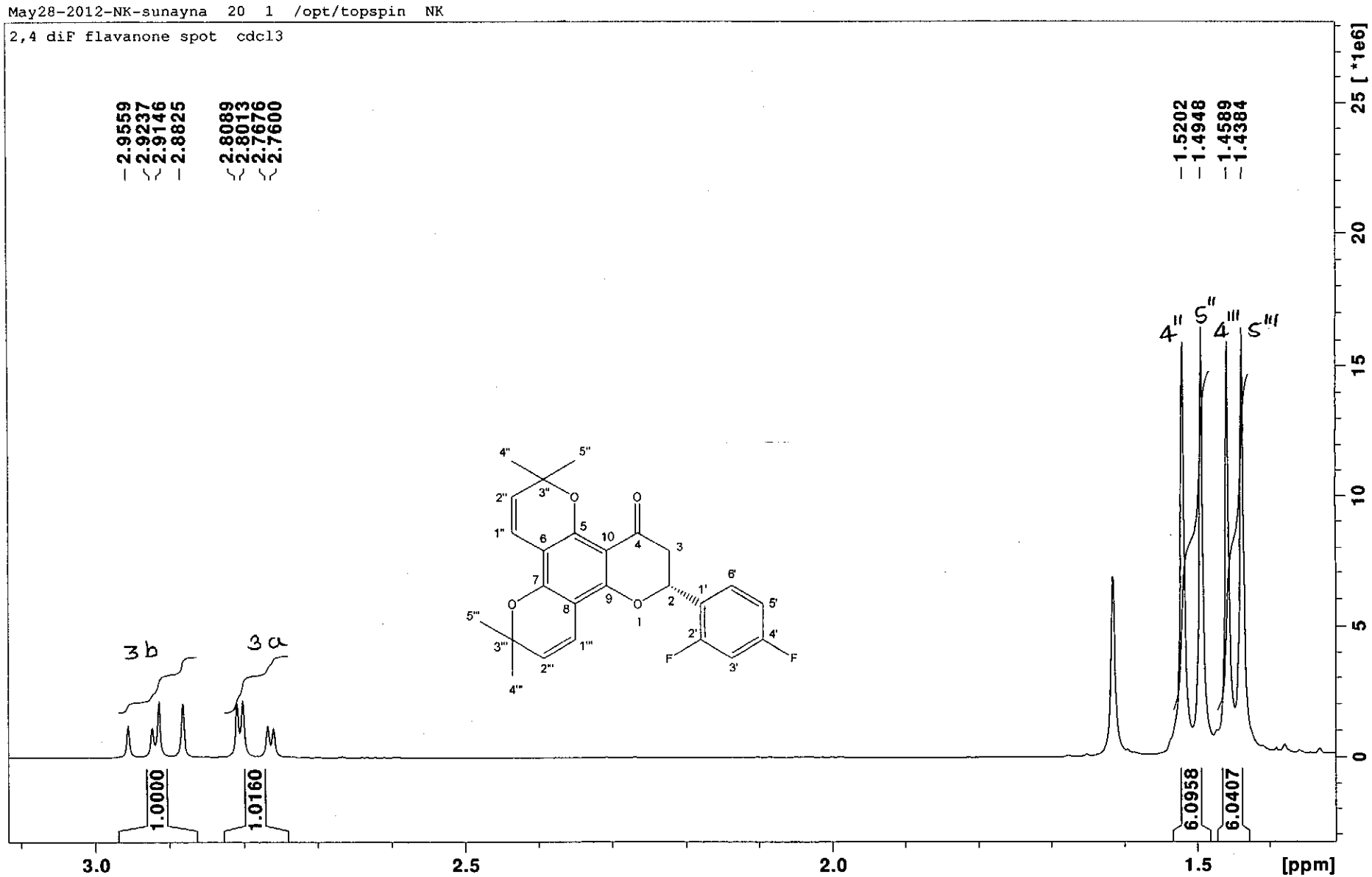
^{1}H NMR spectrum of 4e - 2',4'-F pyranochromene flavanone (A-4e)



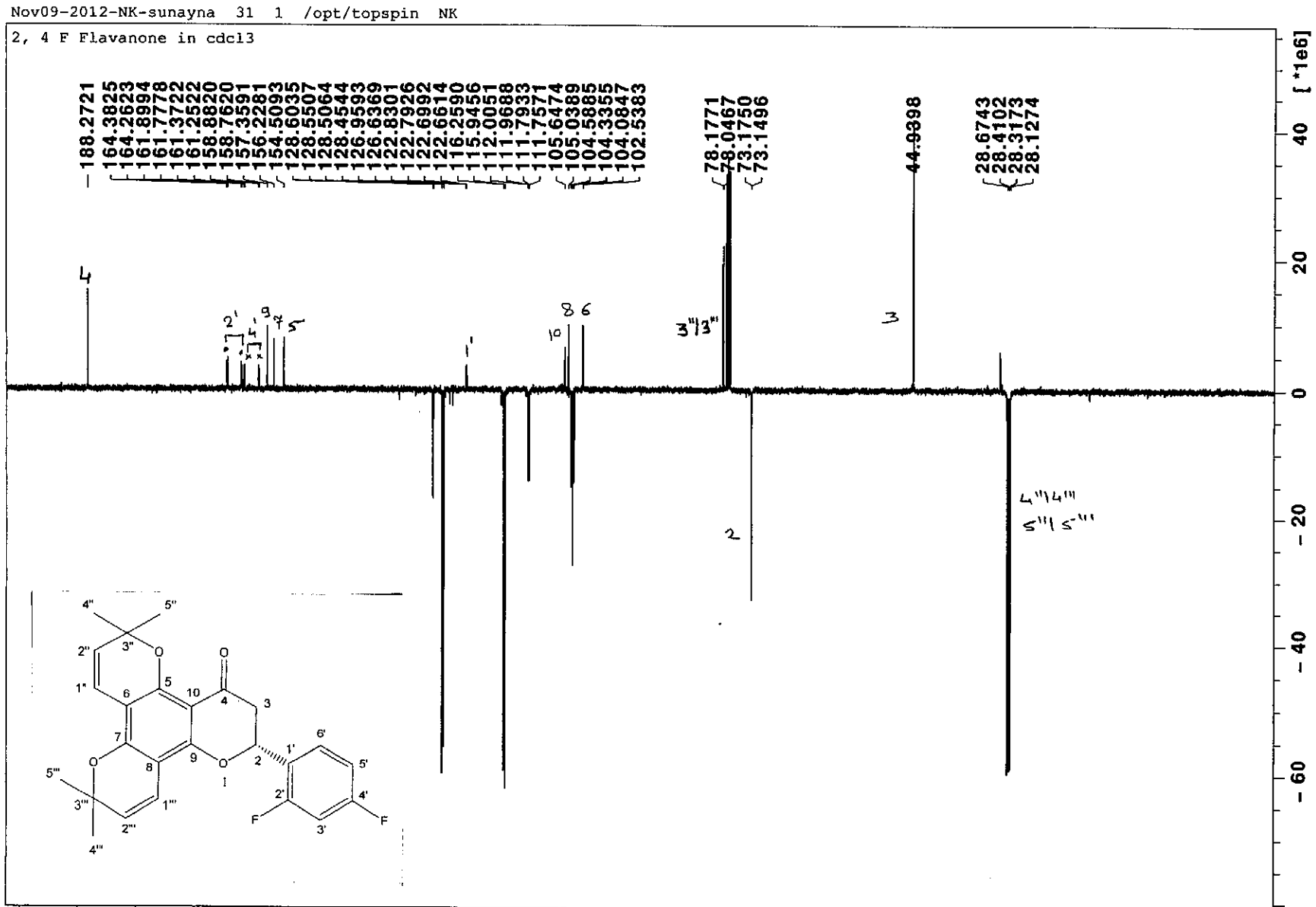
^{1}H NMR spectrum of 4e - 2',4'-F pyranochromene flavanone (A-4e)



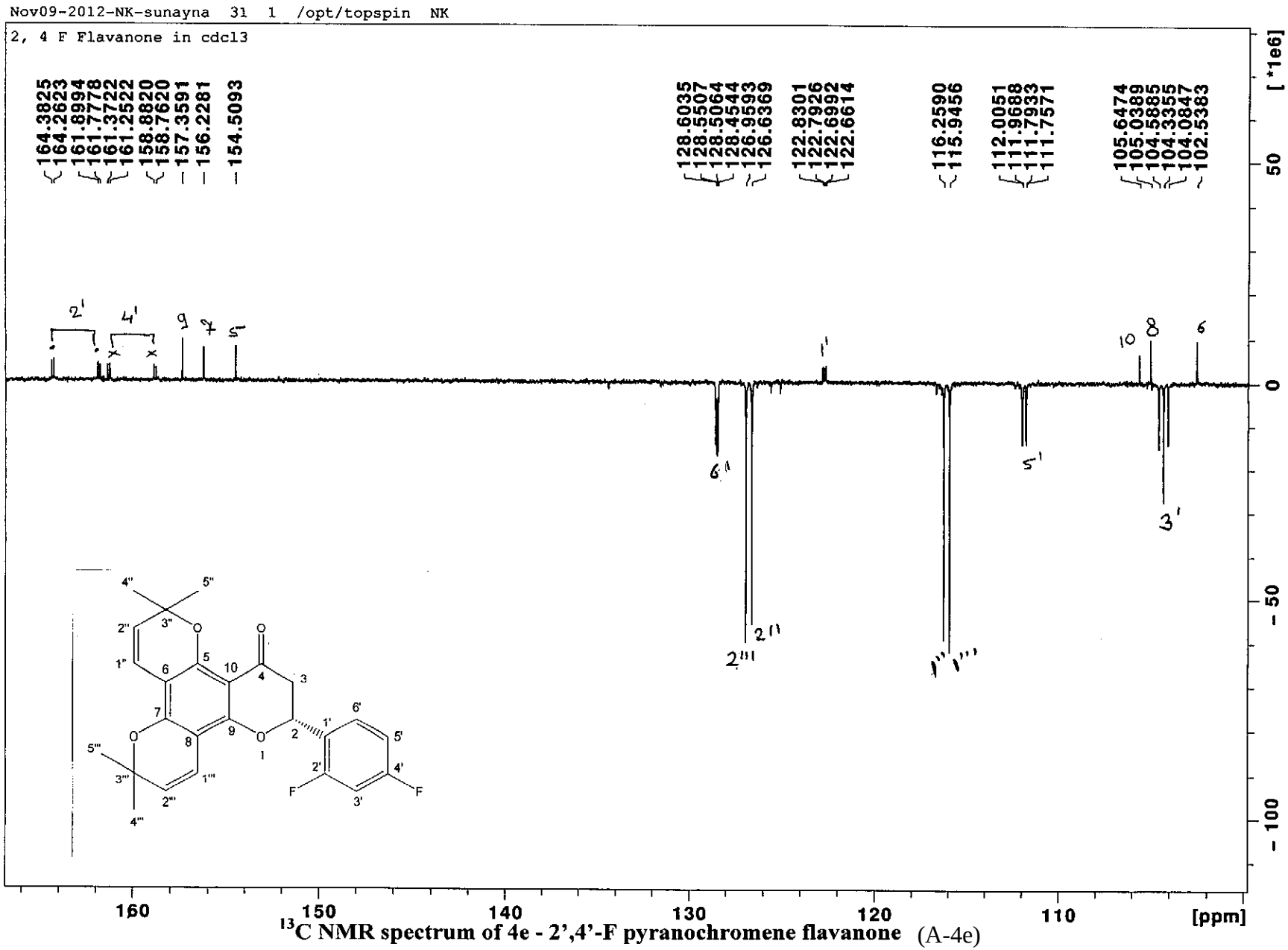
^{1}H NMR spectrum of 4e - 2',4'-F pyranochromene flavanone (A-4e)



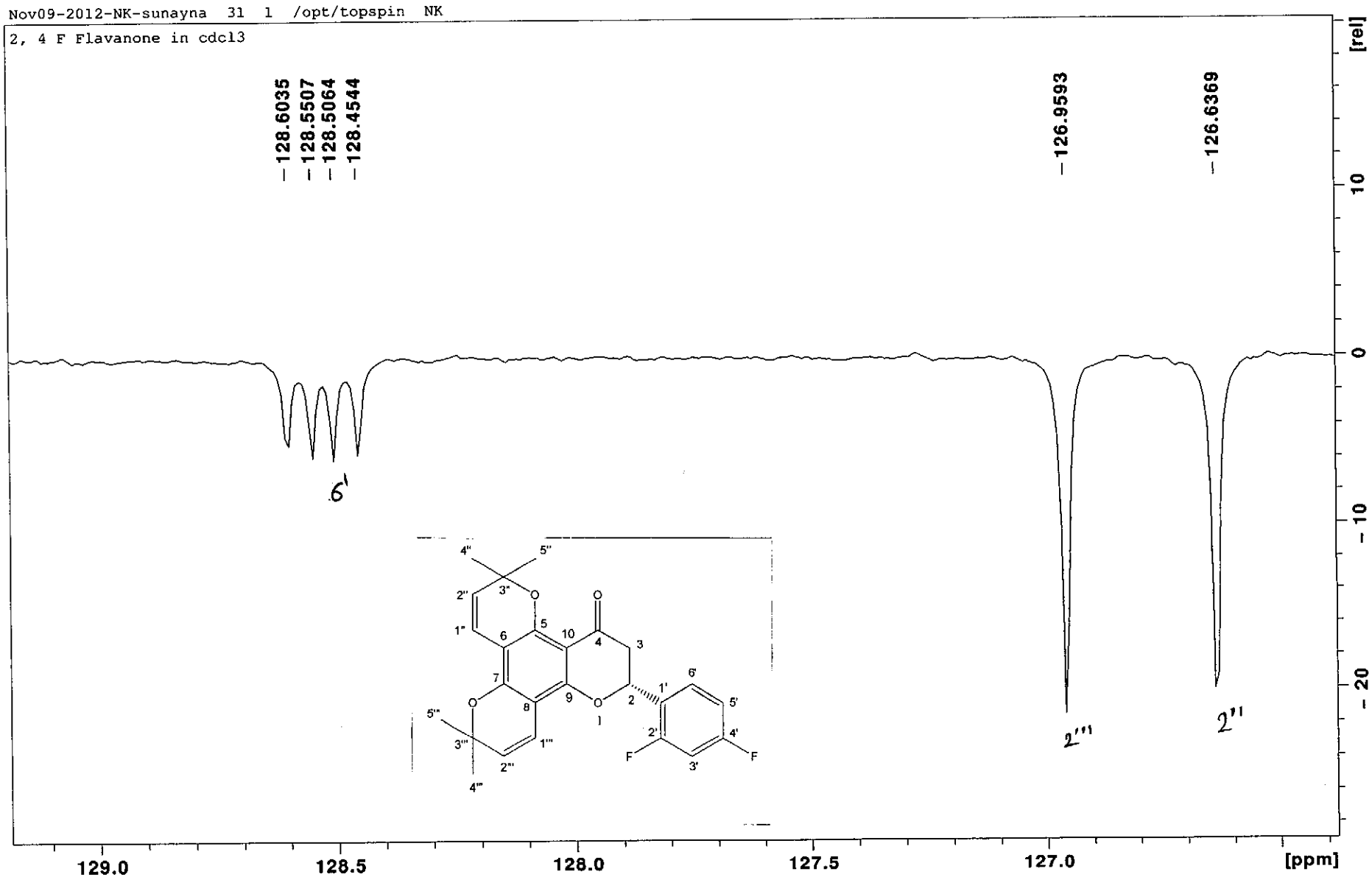
^{1}H NMR spectrum of 4e - 2',4'-F pyranochromene flavanone (A-4e)



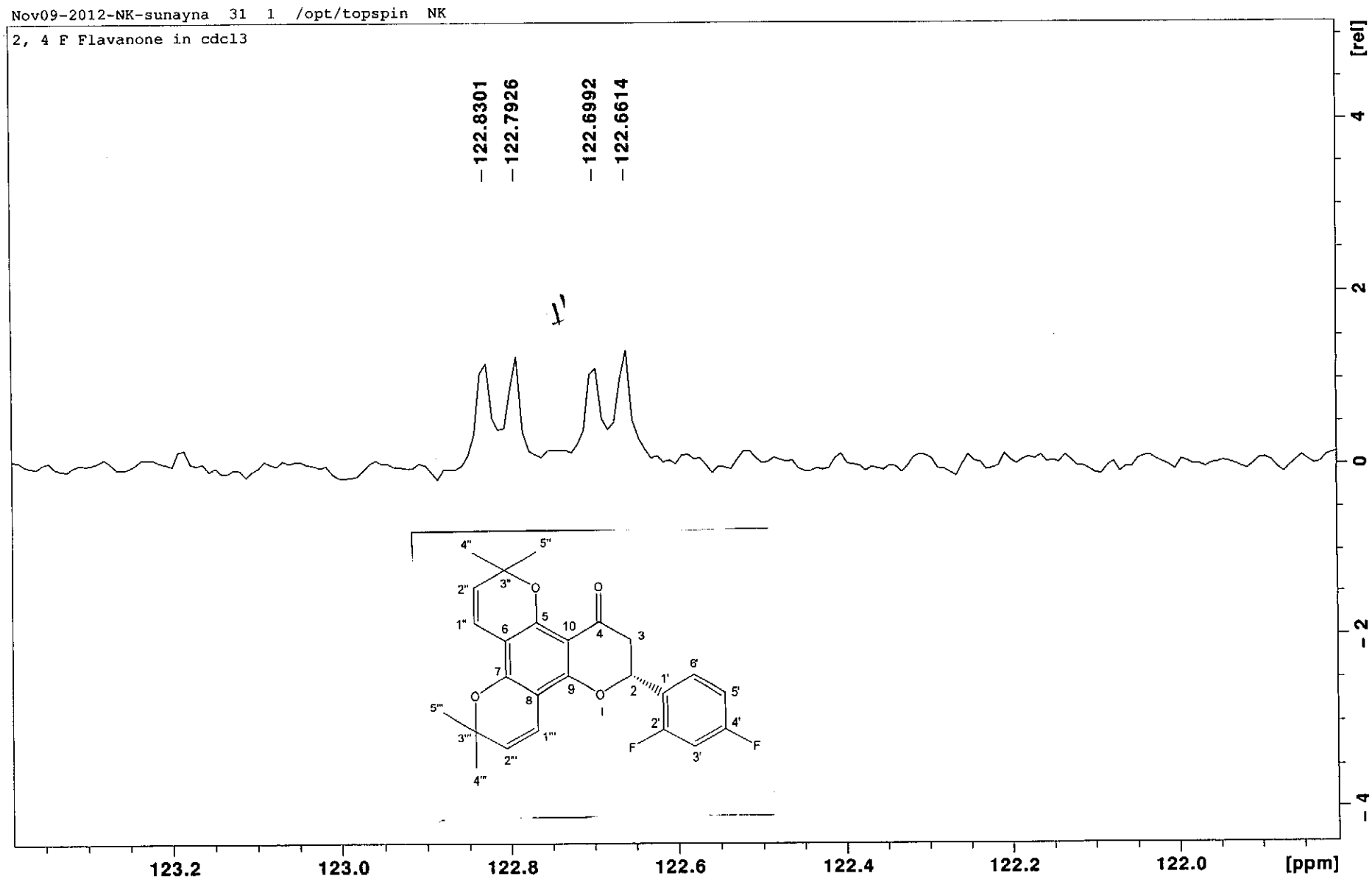
^{13}C NMR spectrum of 4e - 2',4'-F pyranochromene flavanone (A-4e)



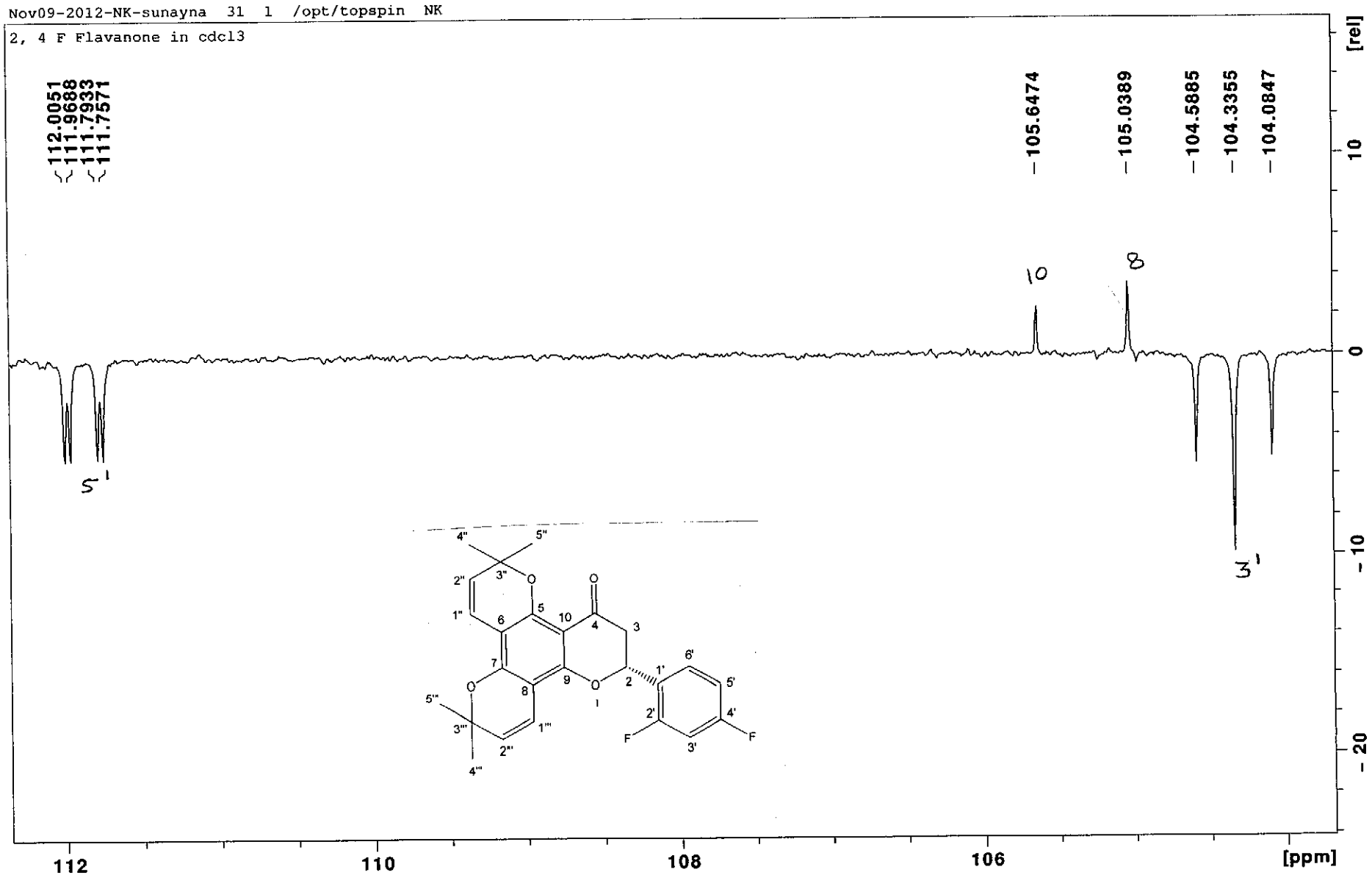
^{13}C NMR spectrum of 4e - 2',4'-F pyranochromene flavanone (A-4e)



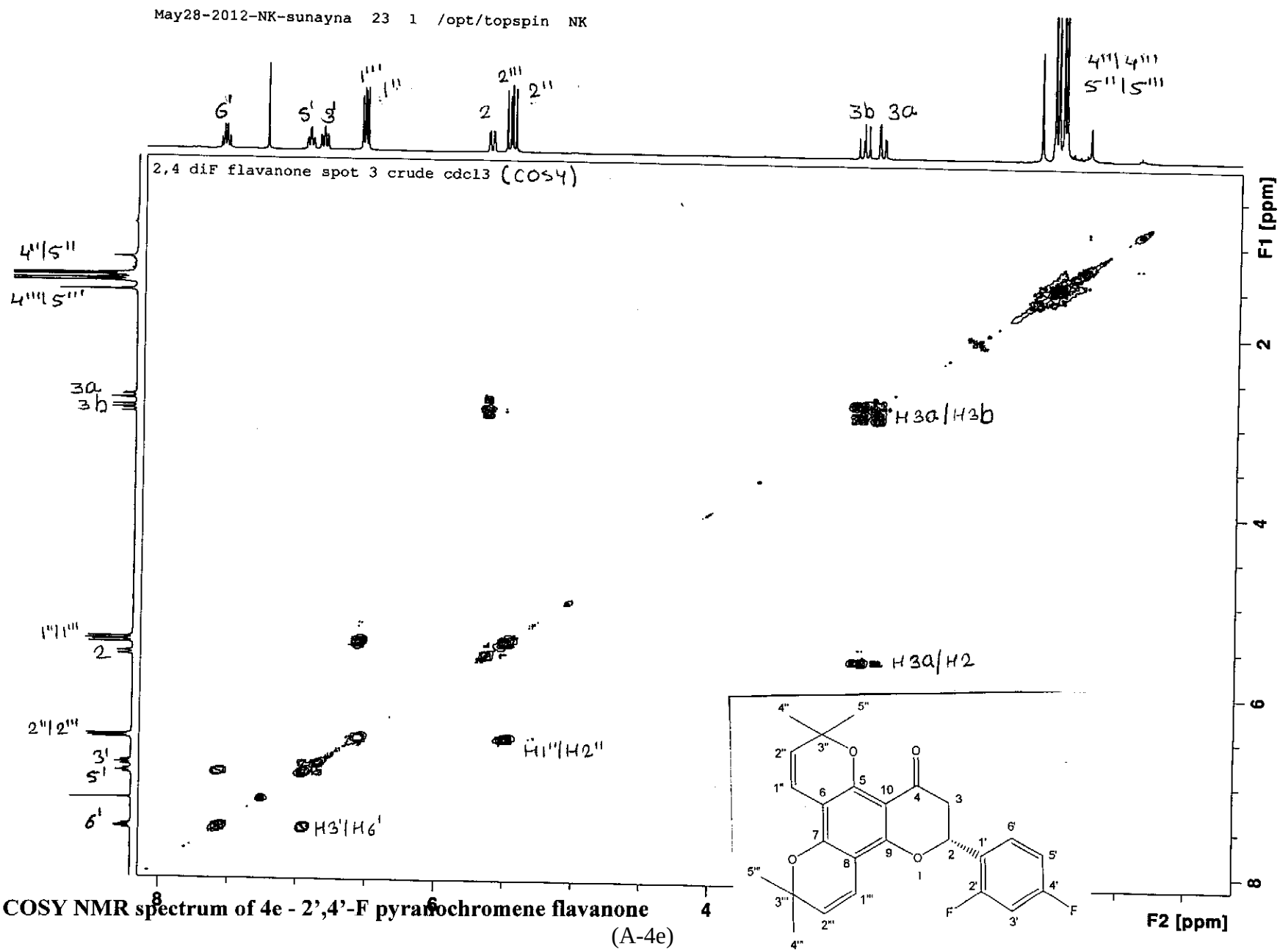
^{13}C NMR spectrum of 4e - 2',4'-F pyranochromene flavanone (A-4e)



^{13}C NMR spectrum of 4e - 2',4'-F pyranochromene flavanone (A-4e)

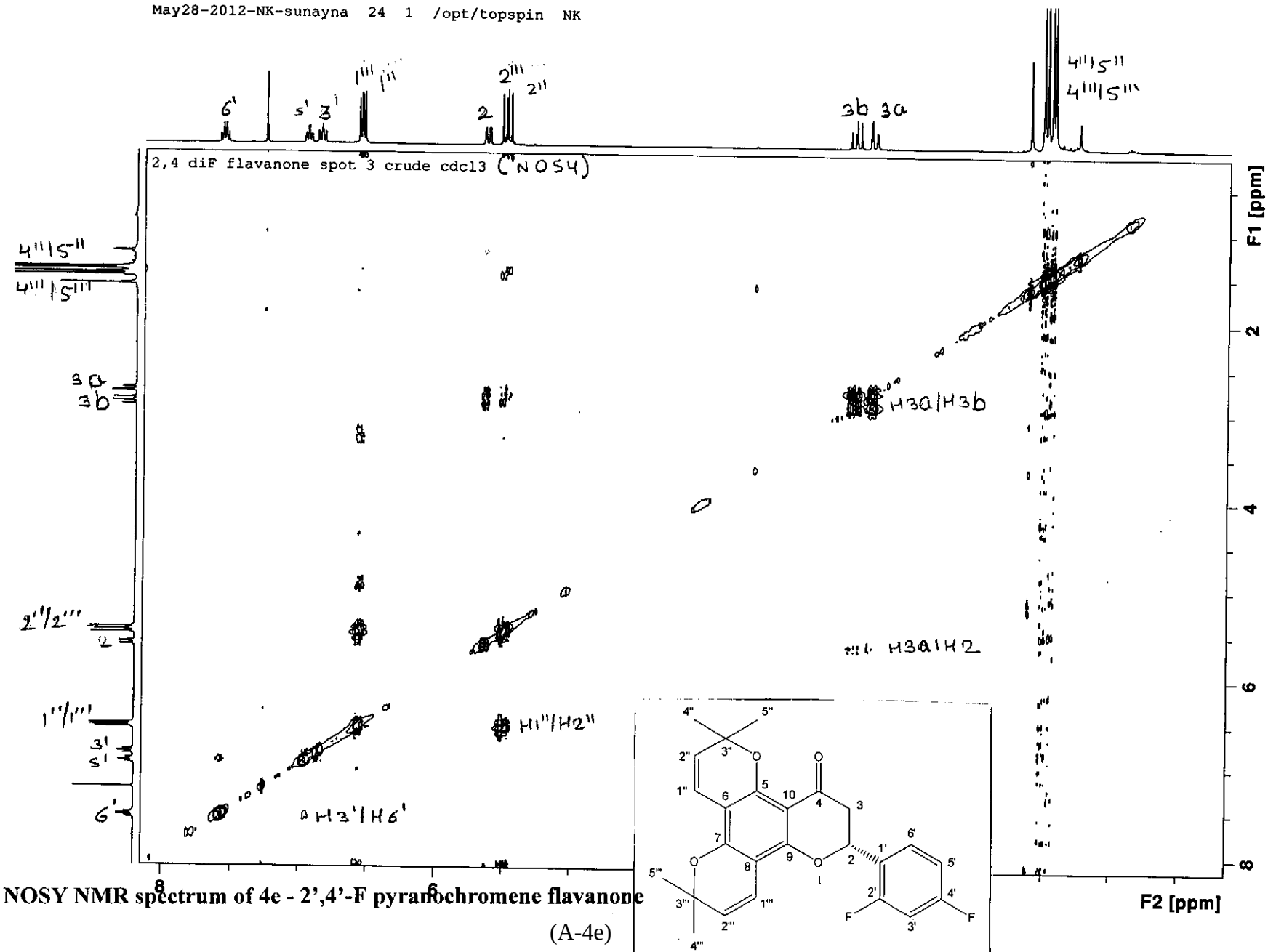


^{13}C NMR spectrum of 4e - 2',4'-F pyranochromene flavanone (A-4e)



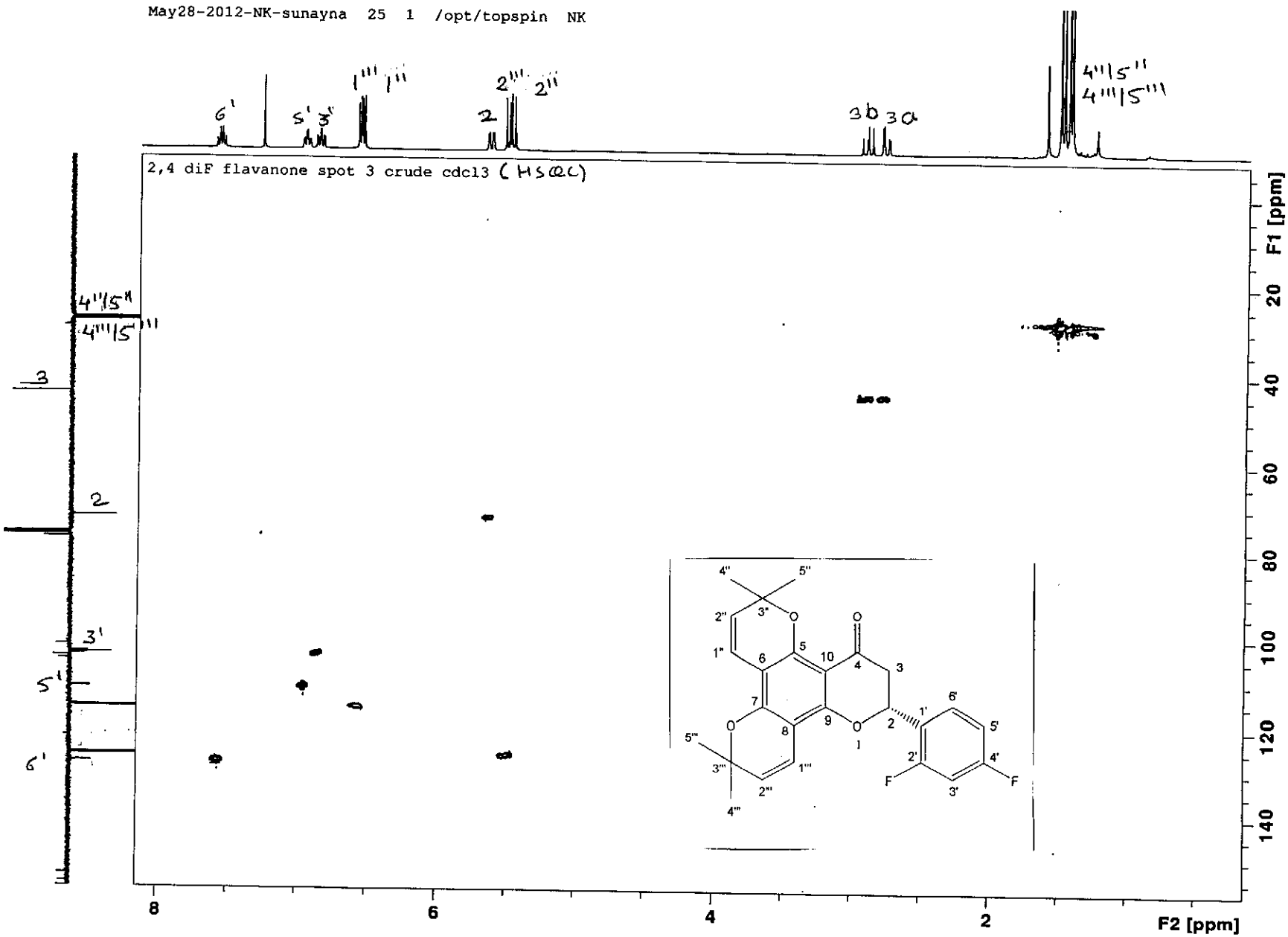
COSY NMR spectrum of 4e - 2',4'-F pyranochromene flavanone

(A-4e)

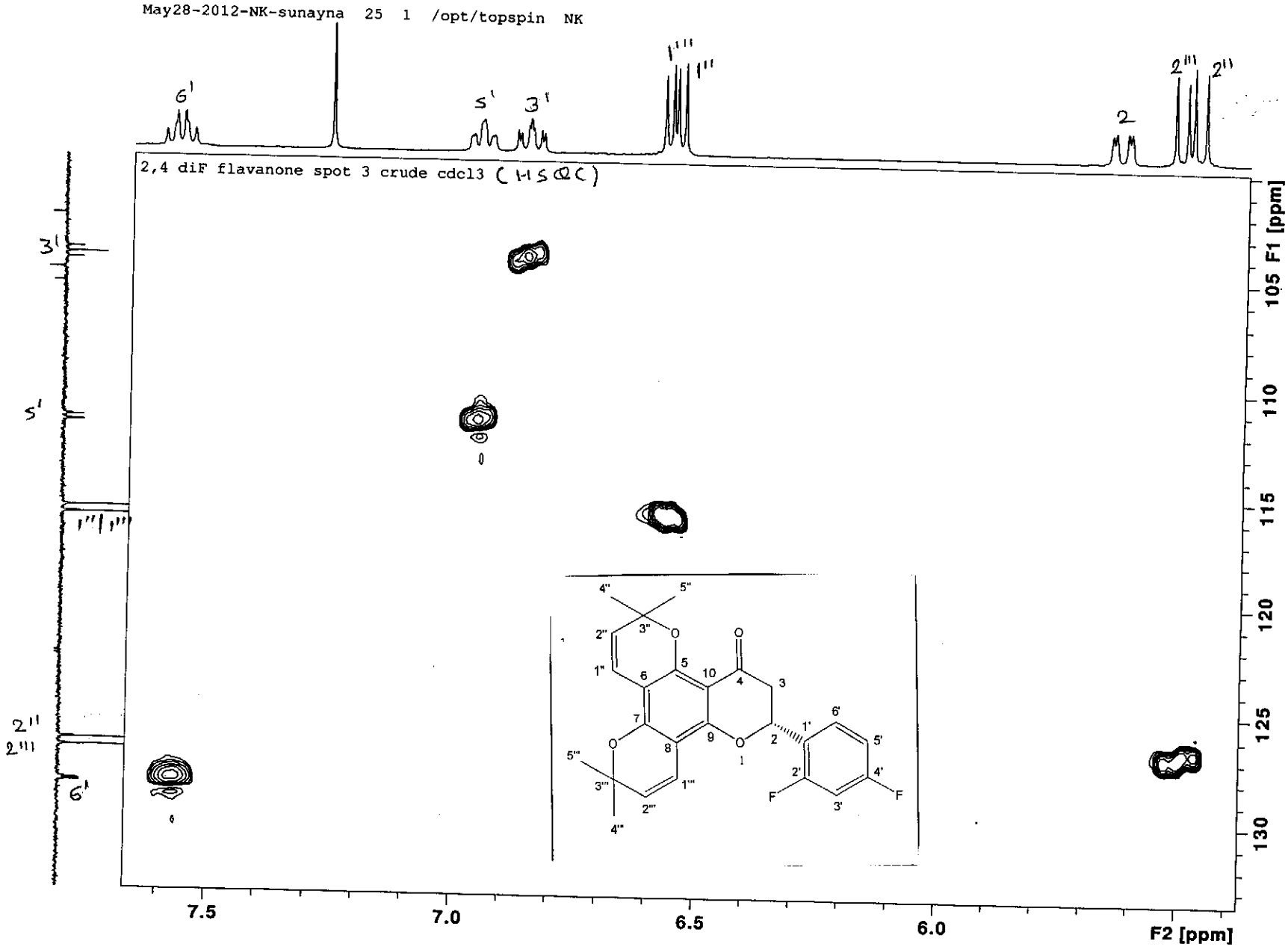


NOSY NMR spectrum of 4e - 2',4'-F pyranochromene flavanone

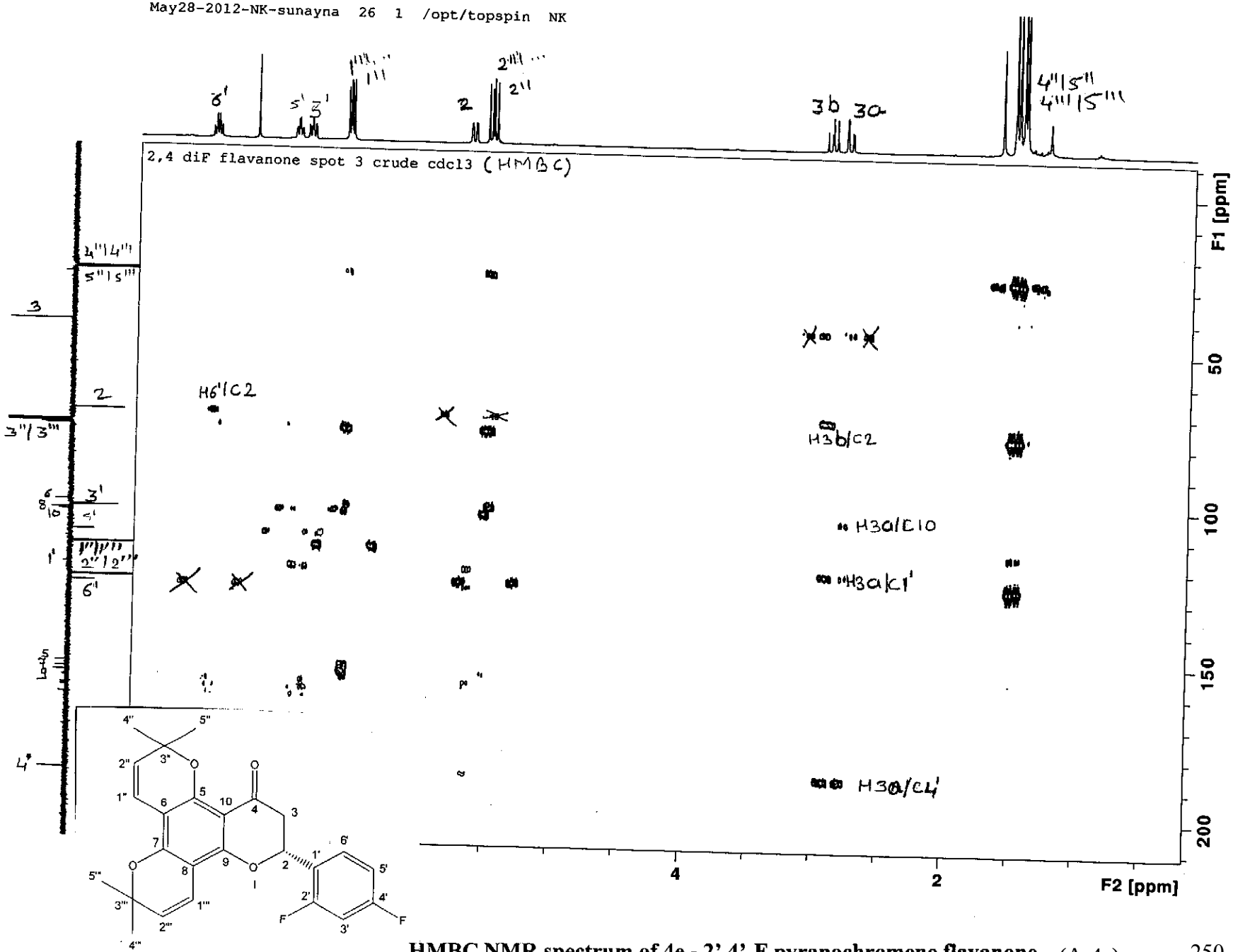
(A-4e)



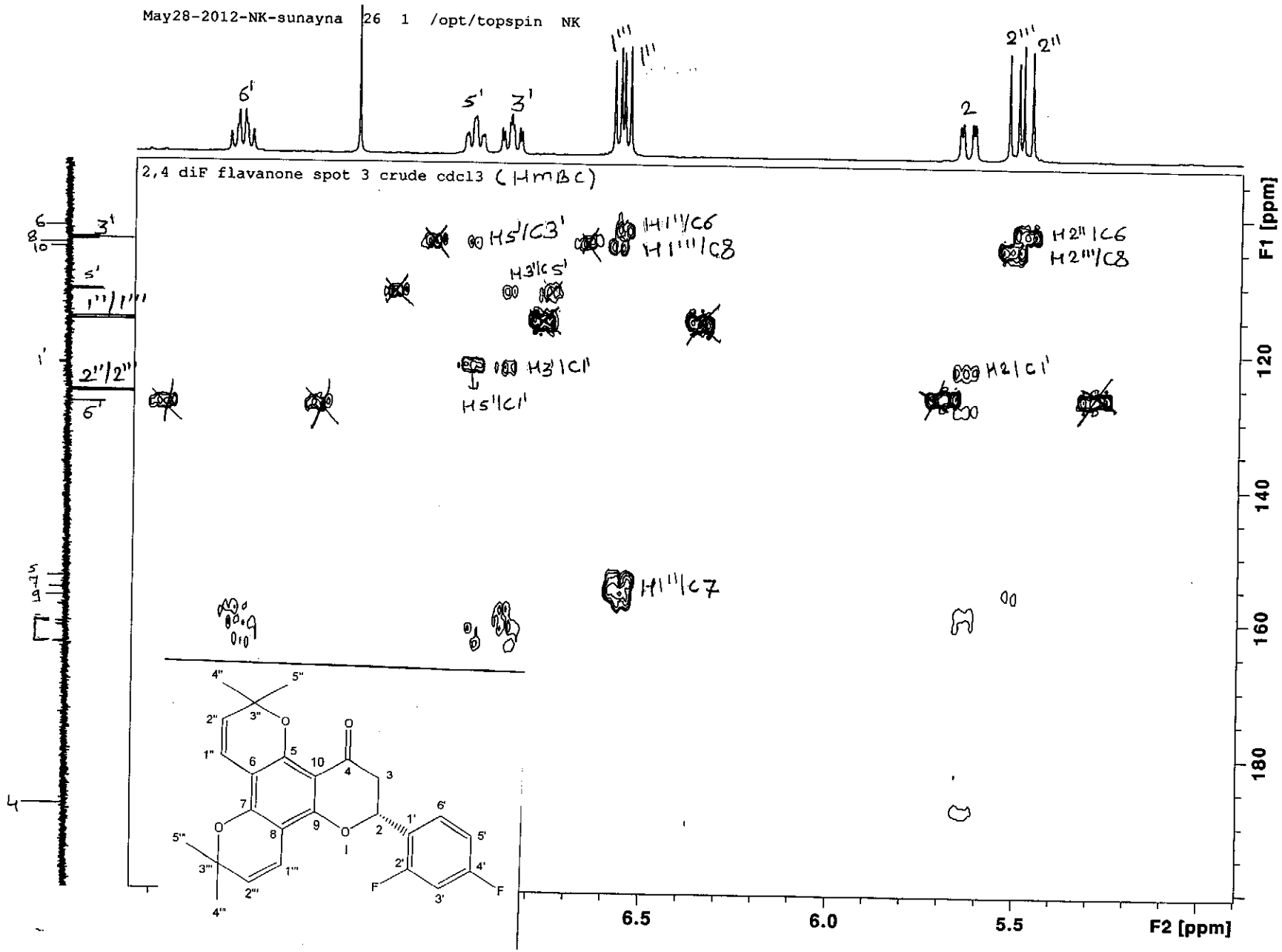
HSQC NMR spectrum of 4e - 2',4'-F pyranochromene flavanone (A-4e)



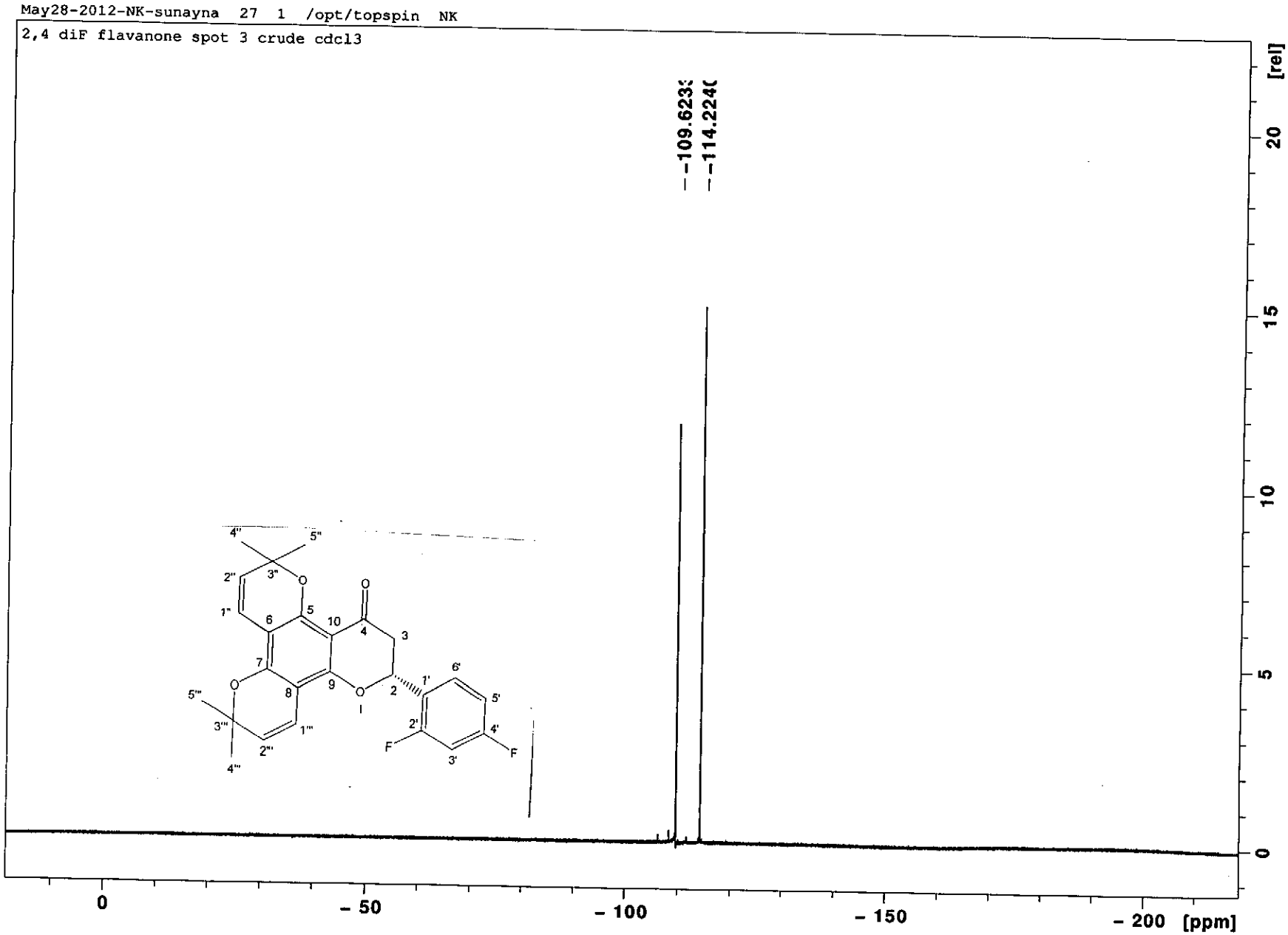
HSQC NMR spectrum of 4e - 2',4'-F pyranochromene flavanone (A-4e)



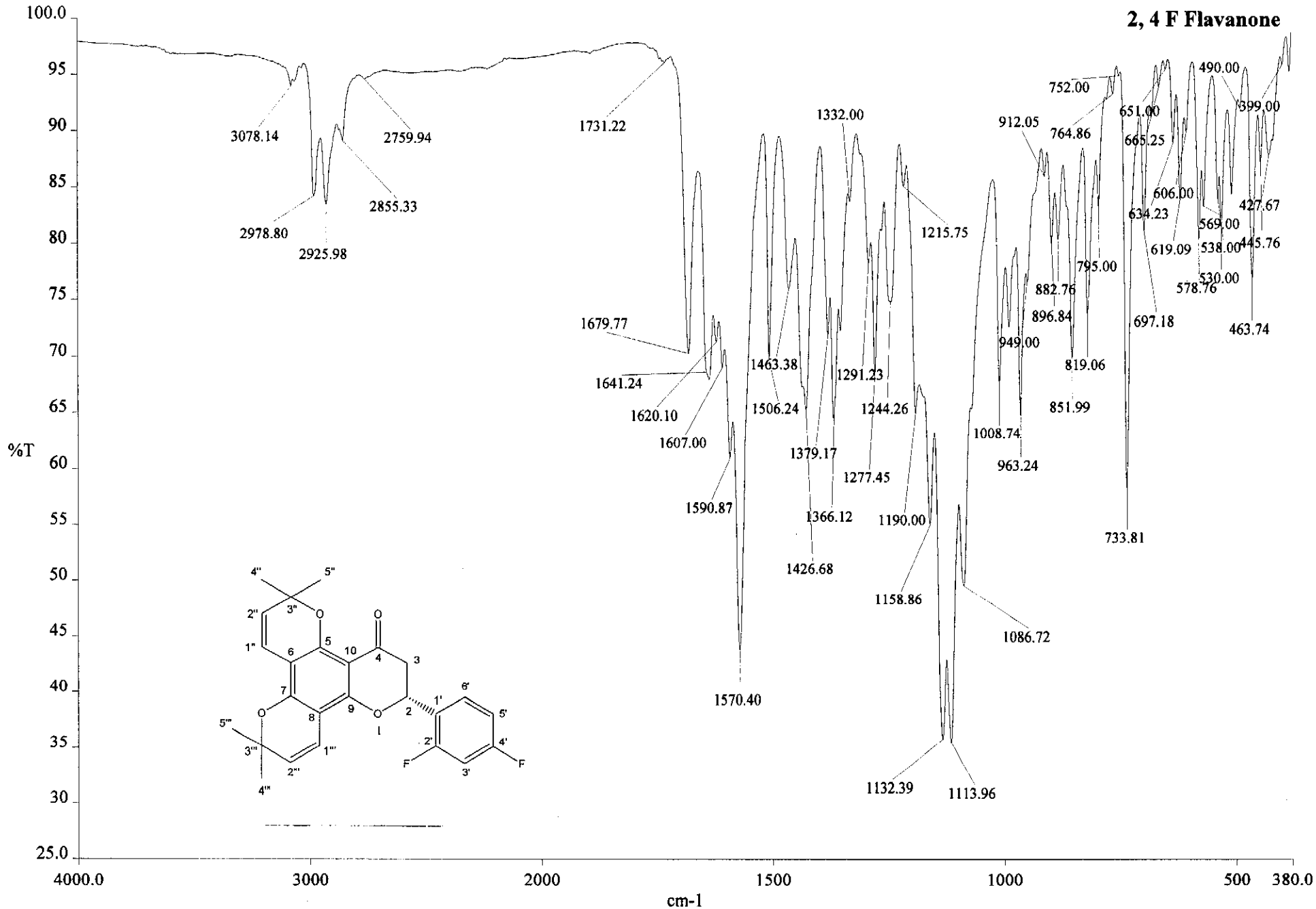
HMBC NMR spectrum of 4e - 2',4'-F pyranochromene flavanone (A-4e)



HMBC NMR spectrum of 4e - 2',4'-F pyranochromene flavanone (A-4e)



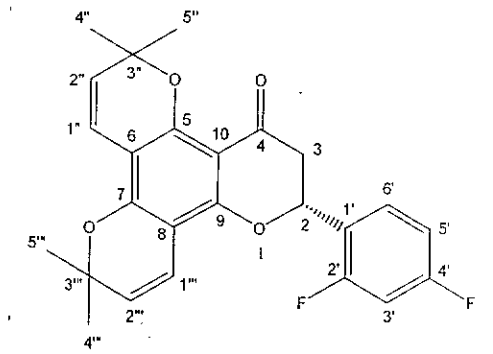
^{19}F NMR spectrum of 4e - 2',4'-F pyranochromene flavanone (A-4e)



c:\pel_data\spectra\sunayna cha\2, 4 f fla.0

IR spectrum of 4e - 2',4'-F pyranochromene flavanone (A-4e)

Elemental Composition Report



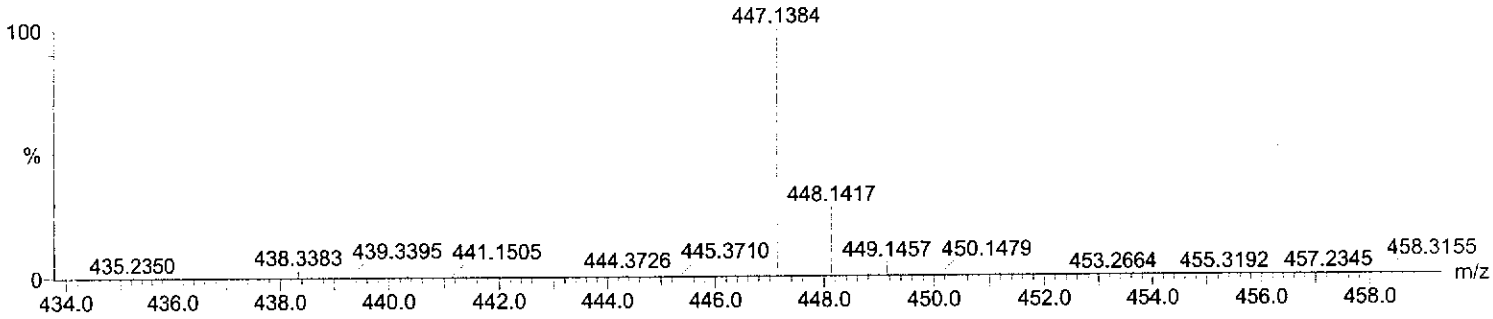
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
15 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-26 H: 20-25 O: 0-5 F: 0-2 Na: 0-1

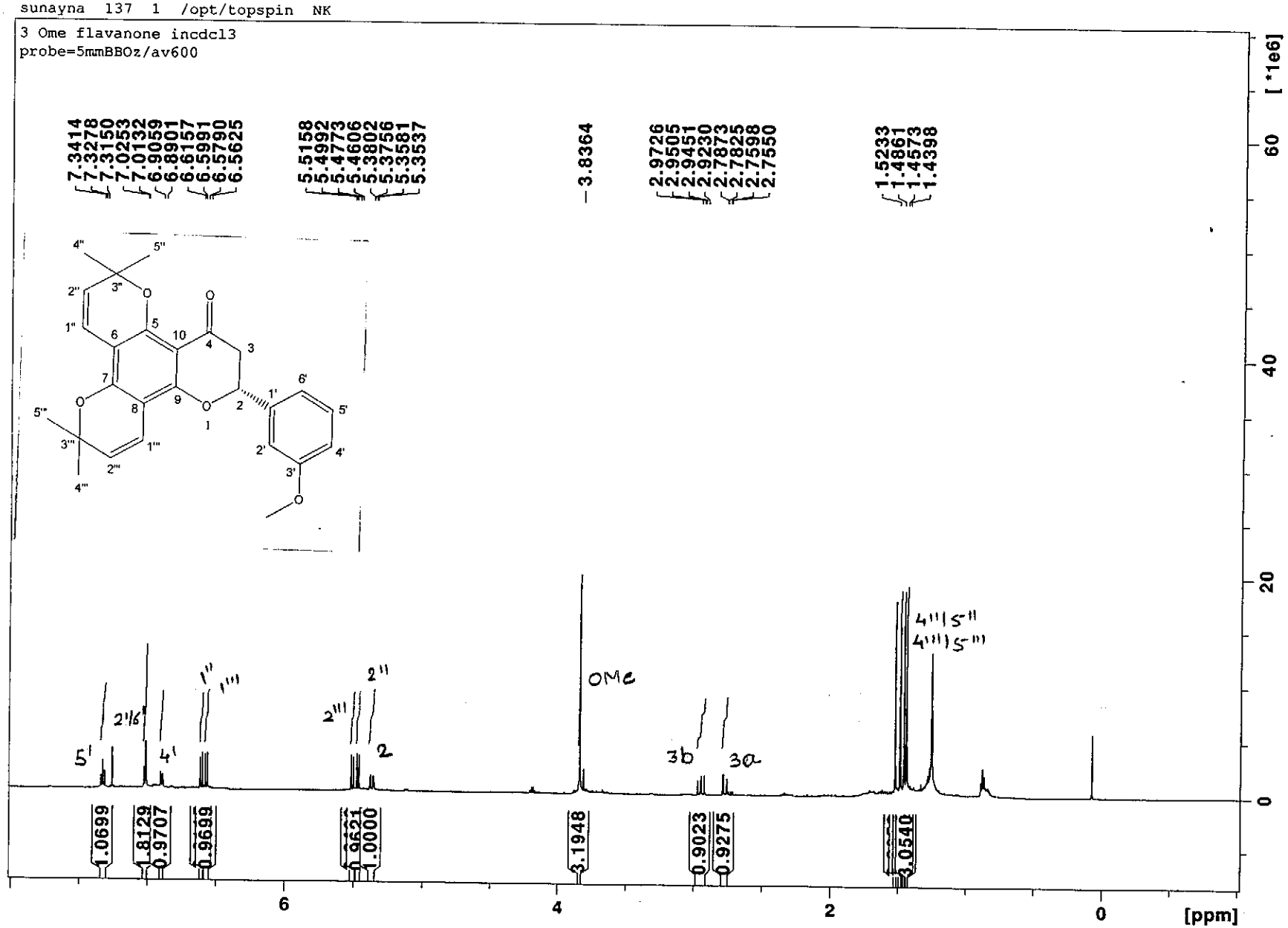
Dr. Nizam
16 29 (0.478) Cm (1:30)

TOF MS ES+
2.79e+004

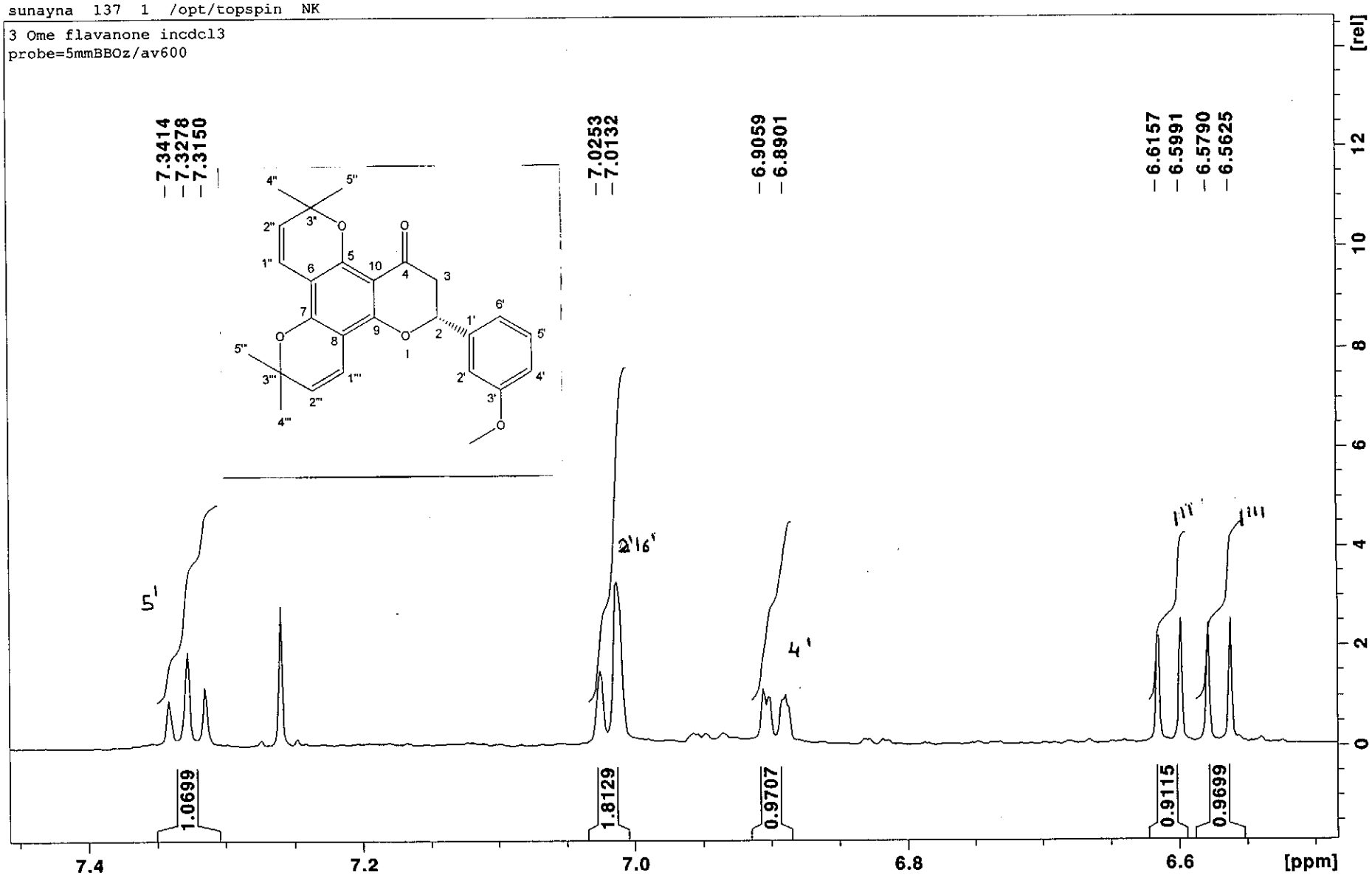


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
447.1384	447.1384	0.0	0.0	13.5	392.0	0.0	C25 H22 O4 F2 Na

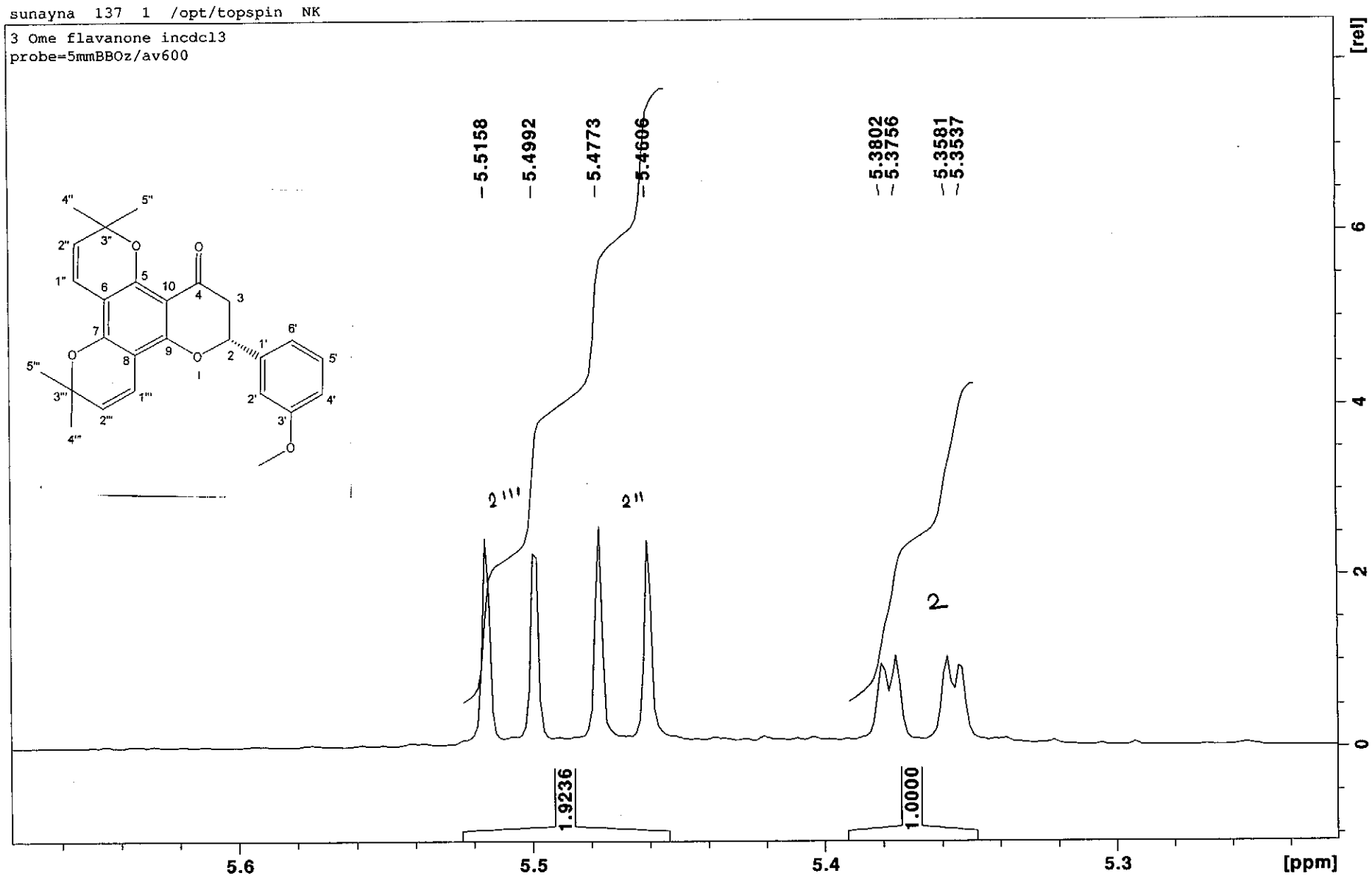
HRMS of 4e – 2',4'-F pyranochromene flavanone (A-4e)



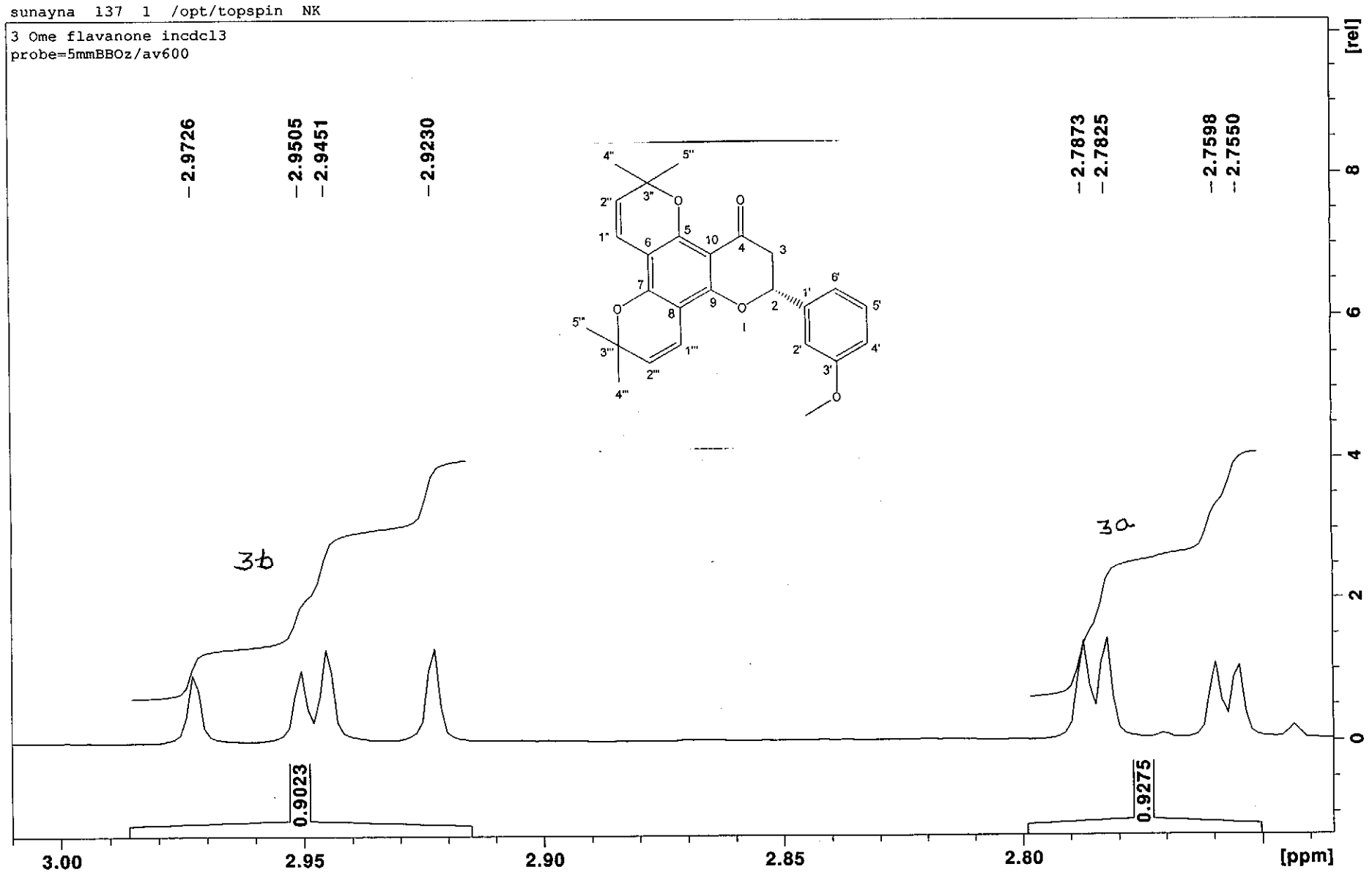
^{1}H NMR spectrum of 4f - 3'-OMe pyranochromene flavanone (A-4f)



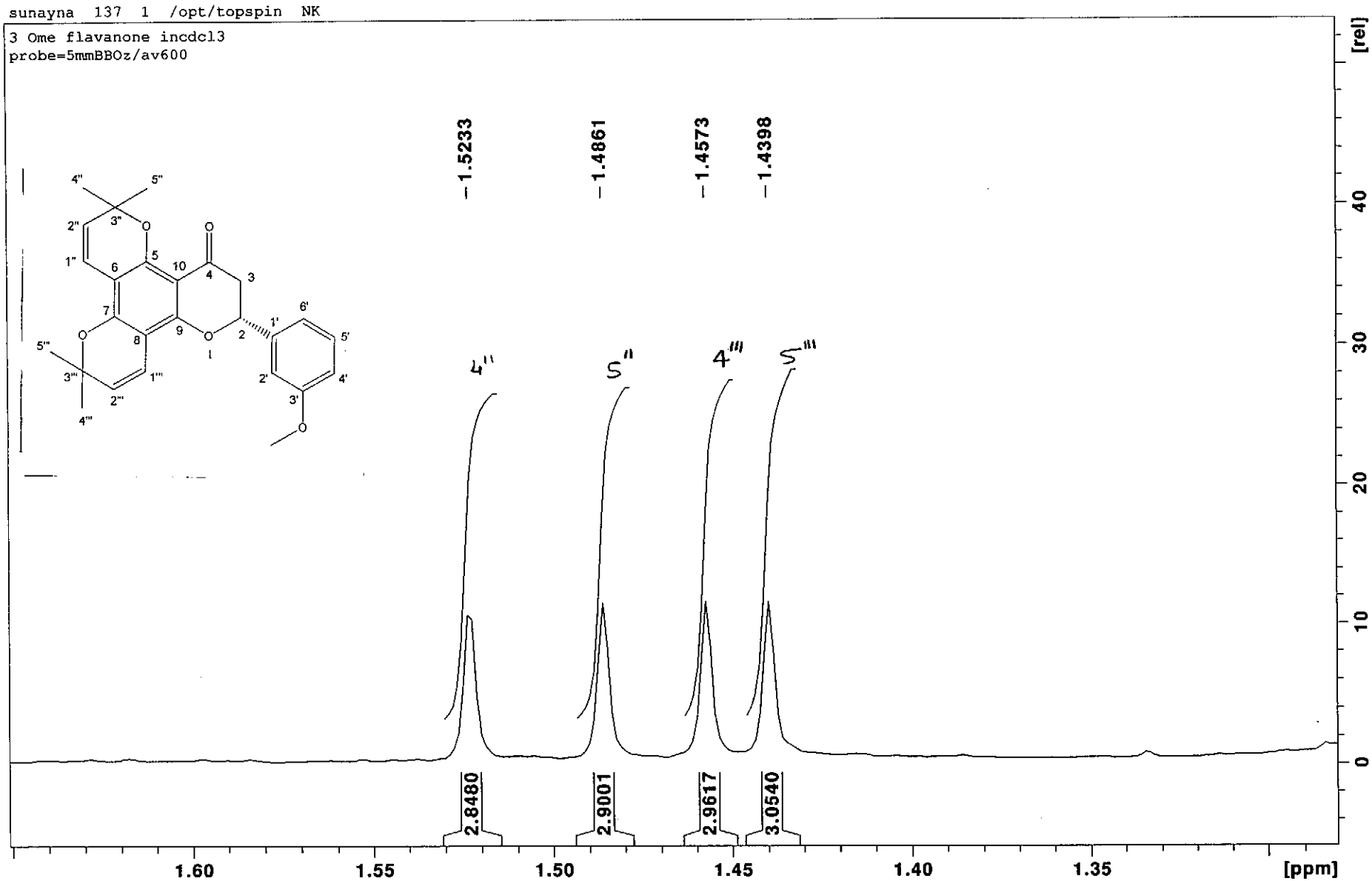
^{1}H NMR spectrum of 4f - 3'-OMe pyranochromene flavanone (A-4f)



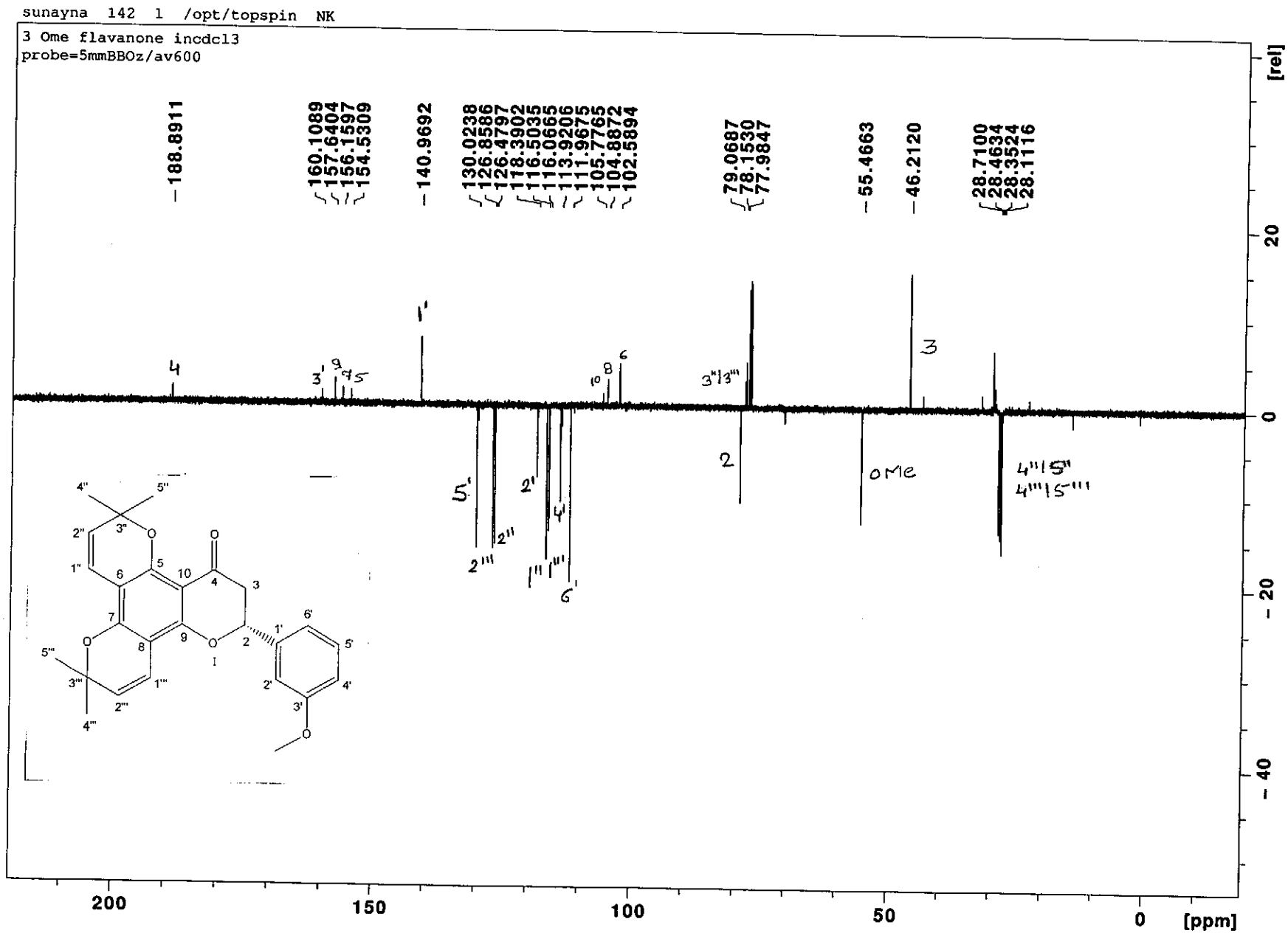
^{1}H NMR spectrum of 4f - 3'-OMe pyranochromene flavanone (A-4f)



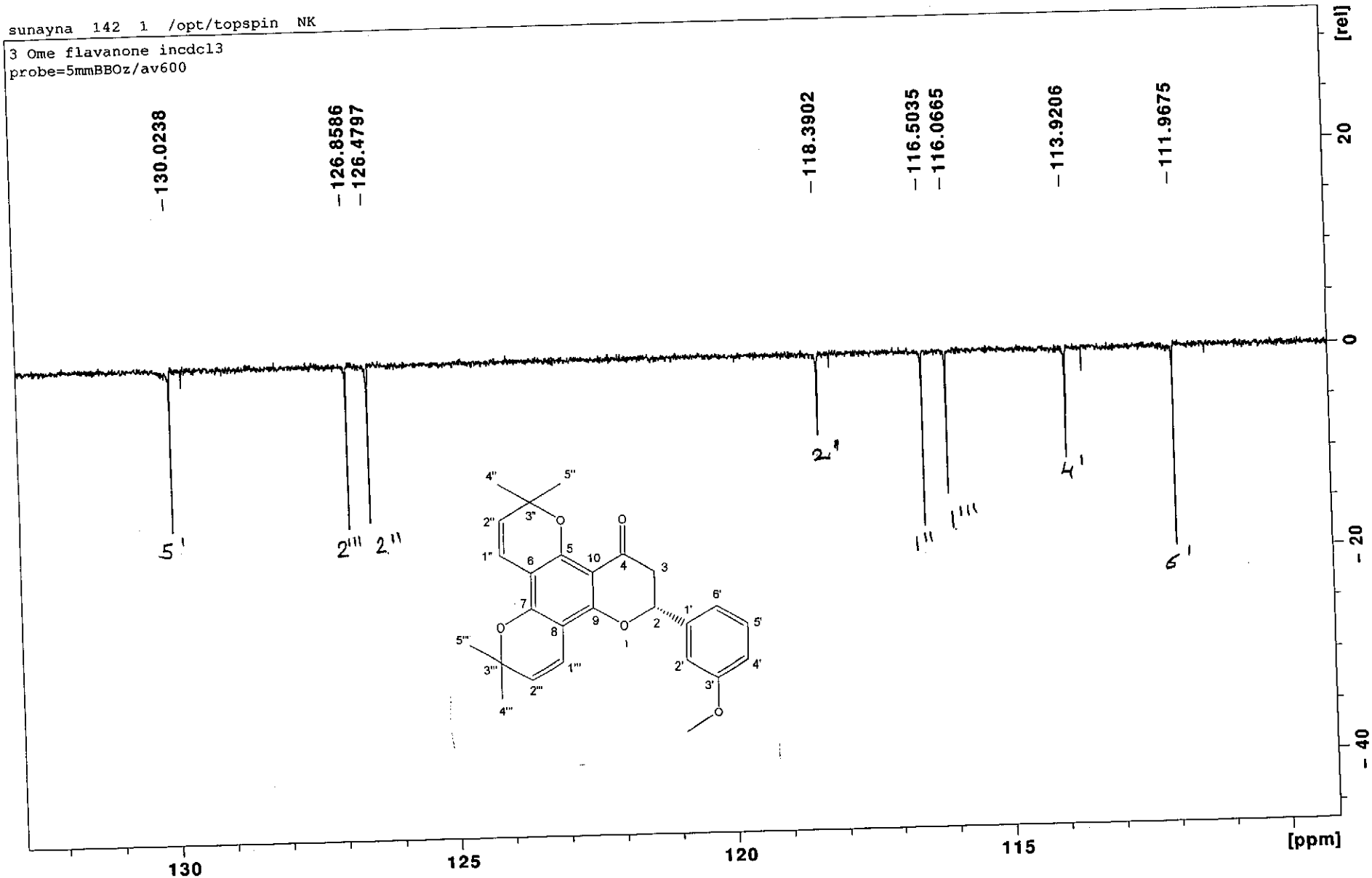
^{1}H NMR spectrum of 4f - 3'-OMe pyranochromene flavanone (A-4f)



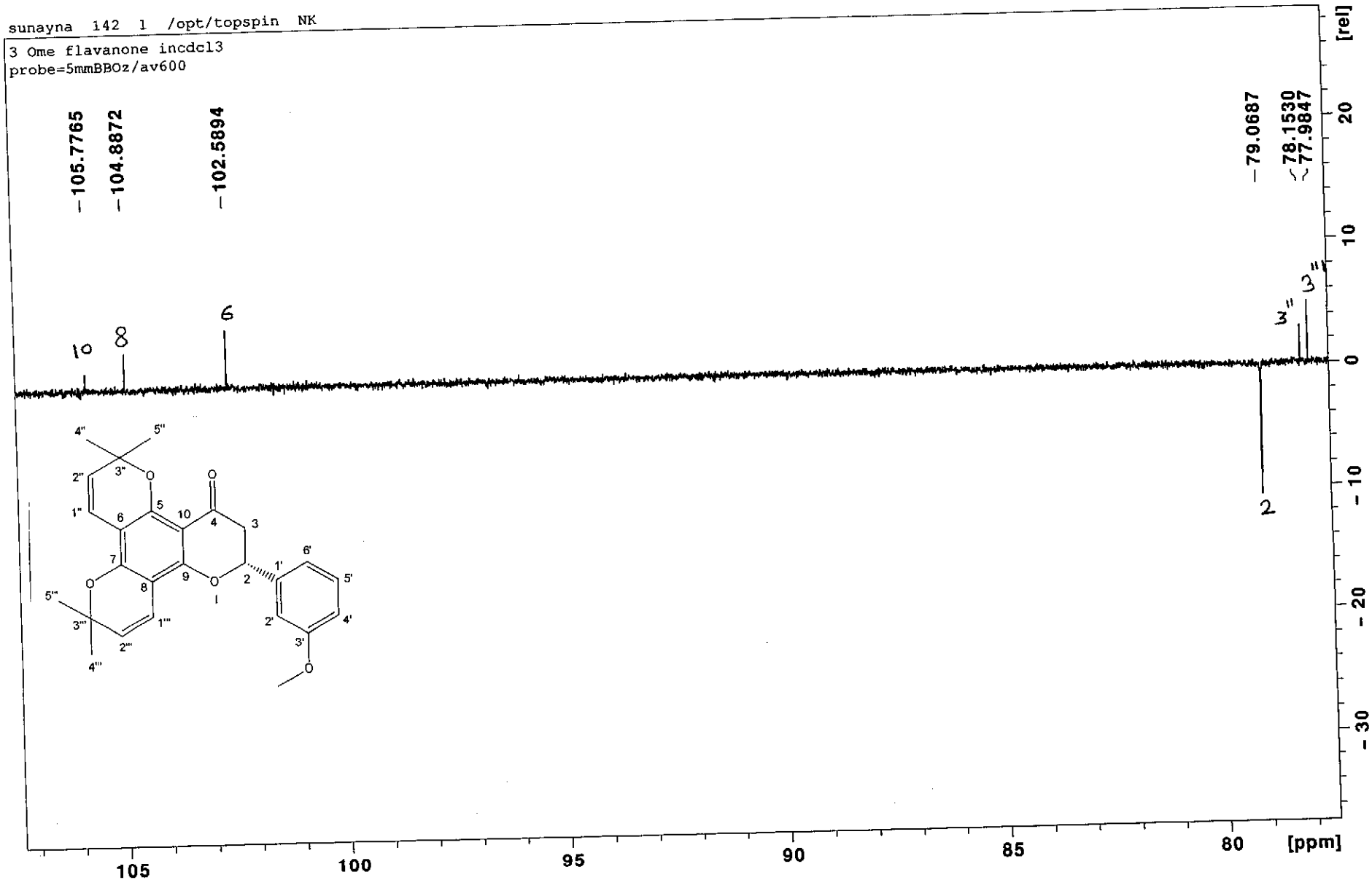
^{1}H NMR spectrum of 4f - 3'-OMe pyranochromene flavanone (A-4f)



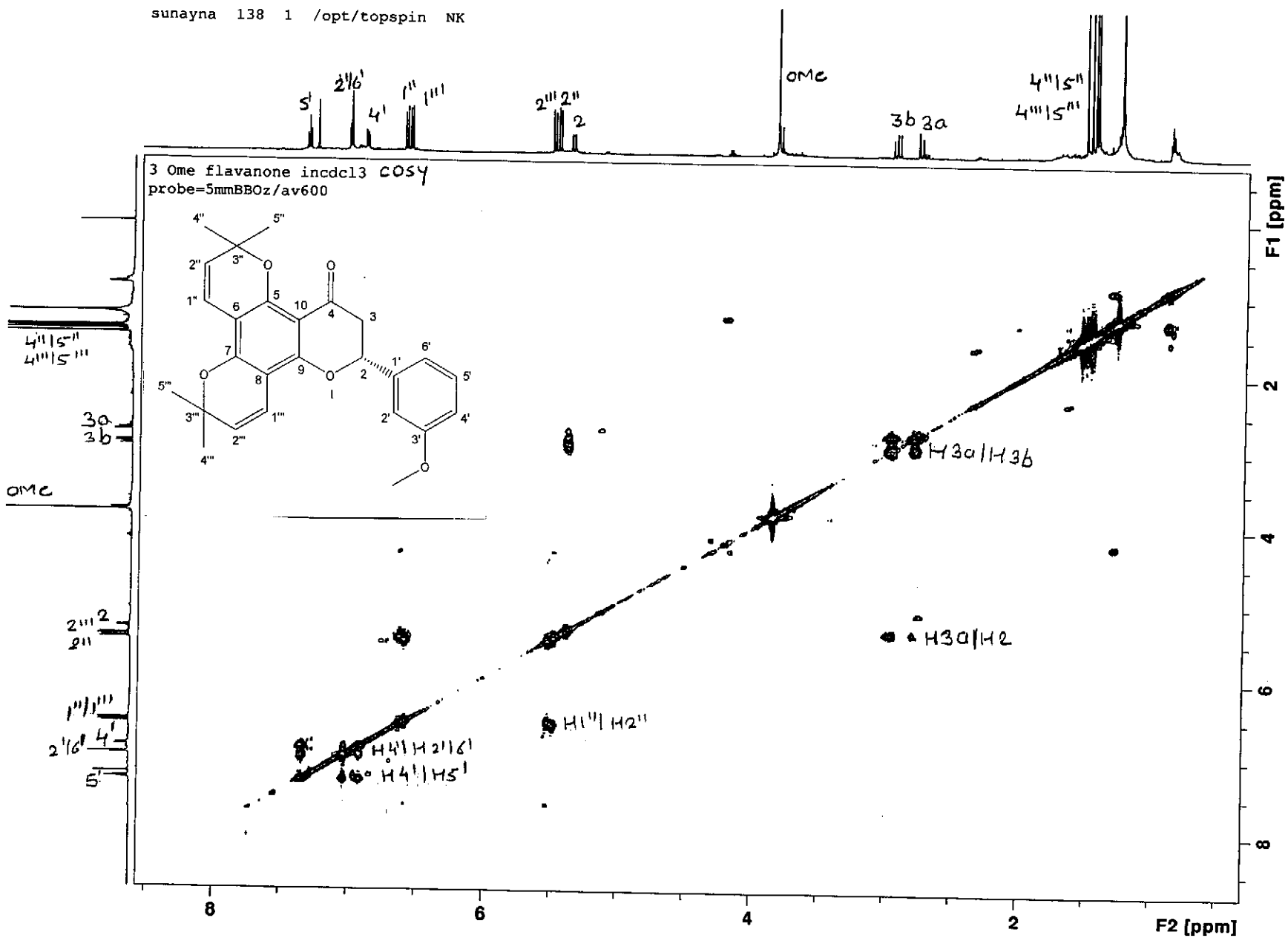
^{13}C NMR spectrum of 4f - 3'-OMe pyranochromene flavanone (A-4f)



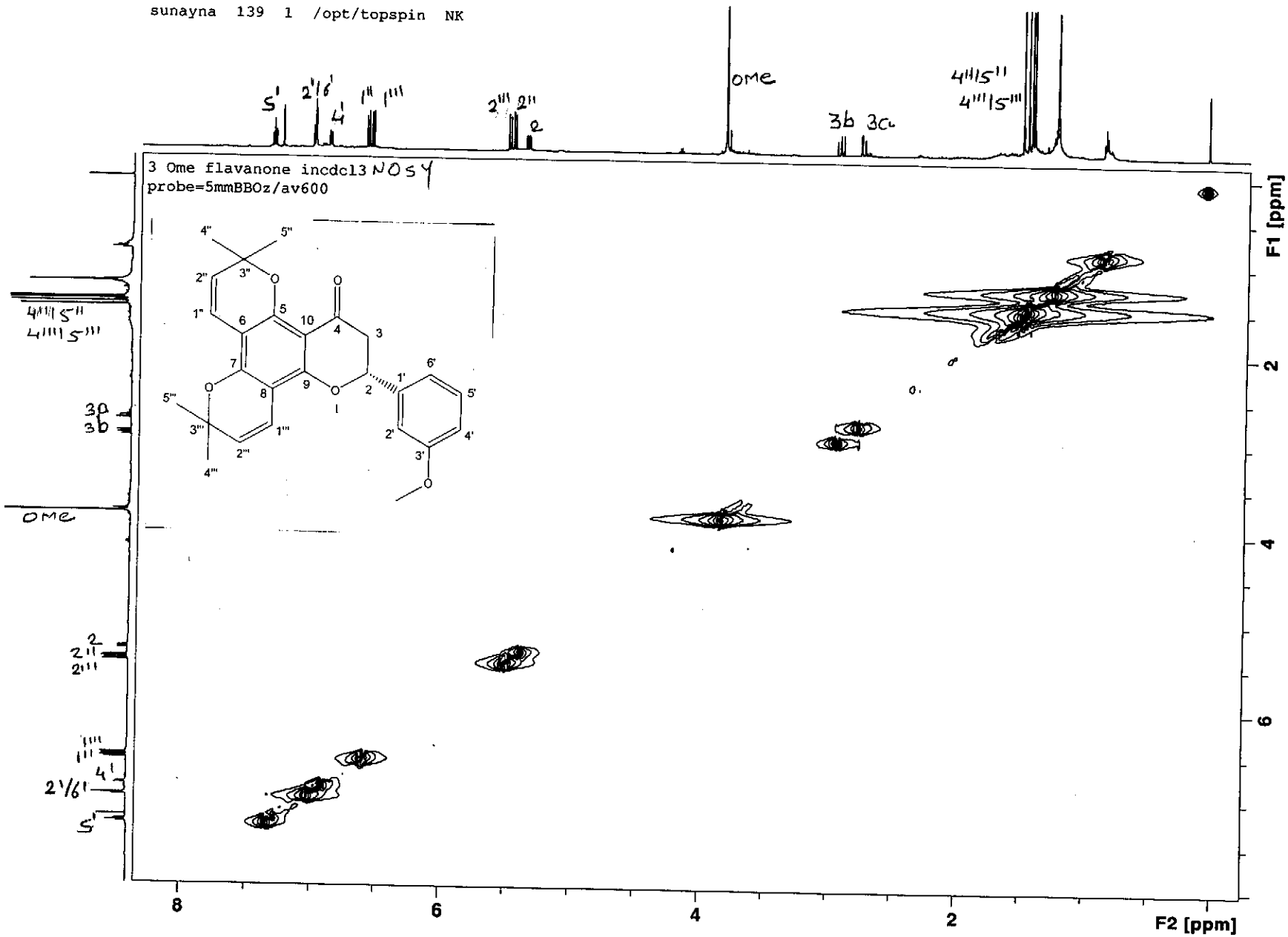
^{13}C NMR spectrum of 4f - 3'-OMe pyranochromene flavanone (A-4f)



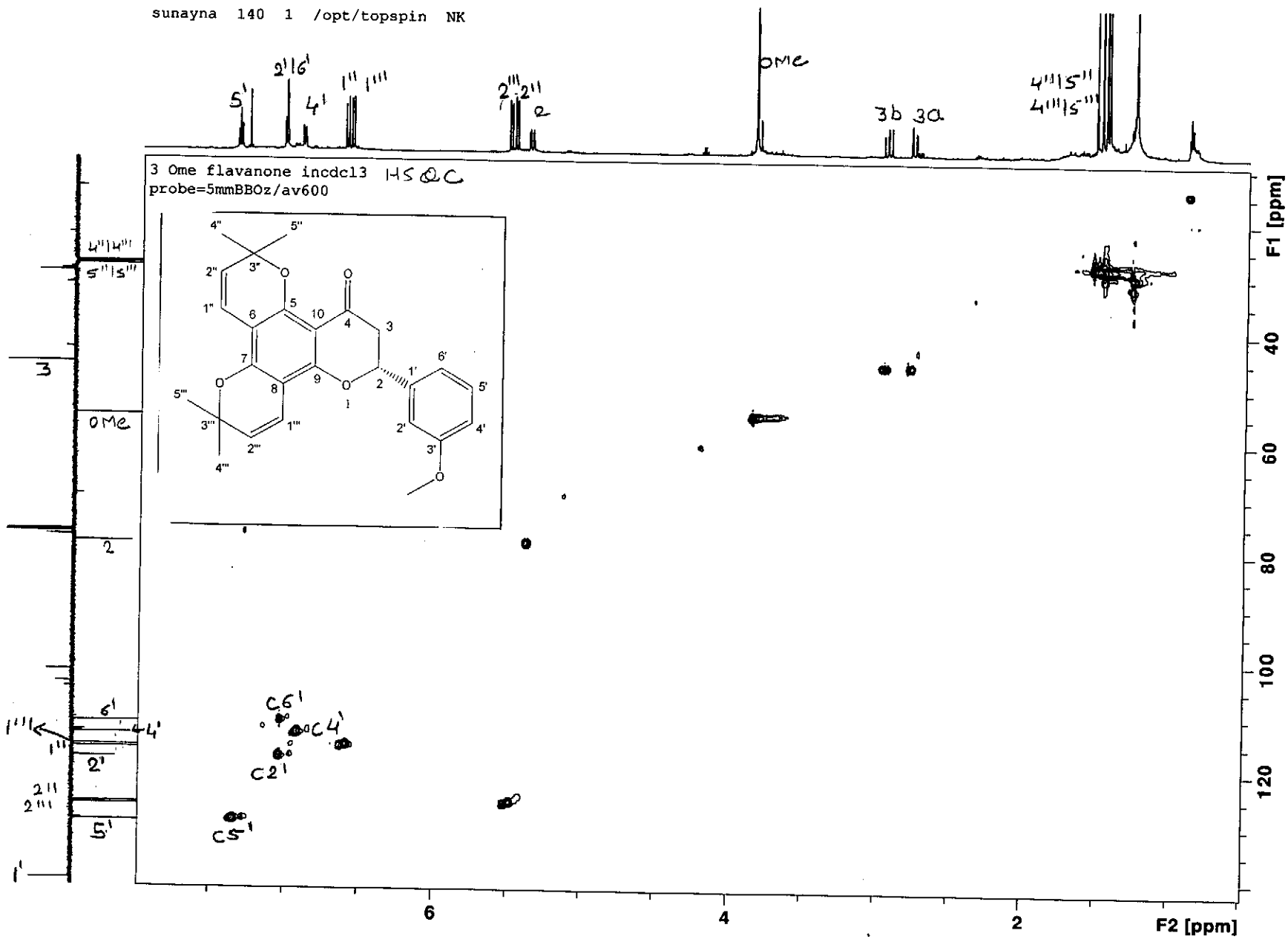
^{13}C NMR spectrum of 4f - 3'-OMe pyranochromene flavanone (A-4f)



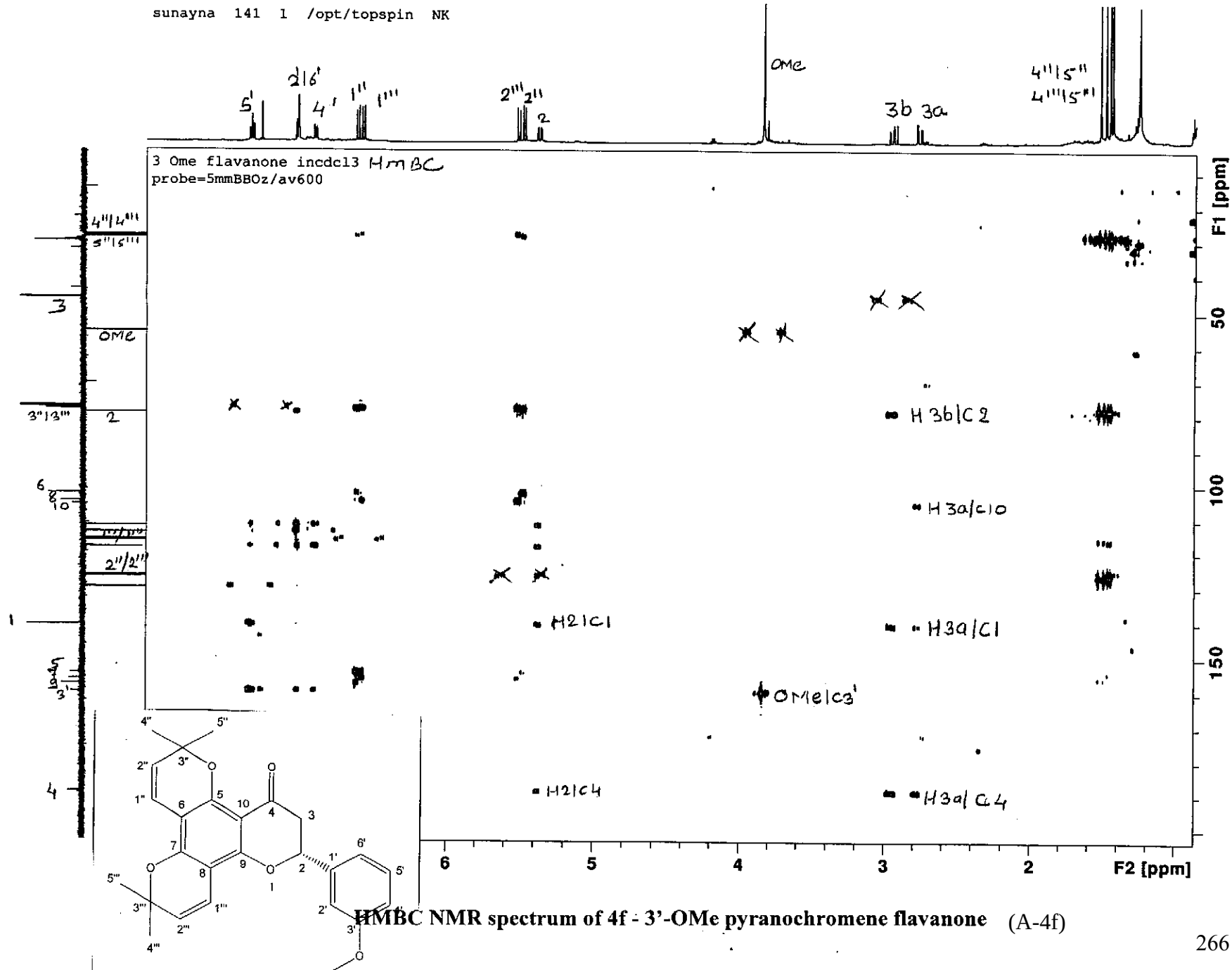
COSY NMR spectrum of 4f - 3'-OMe pyranochromene flavanone (A-4f)



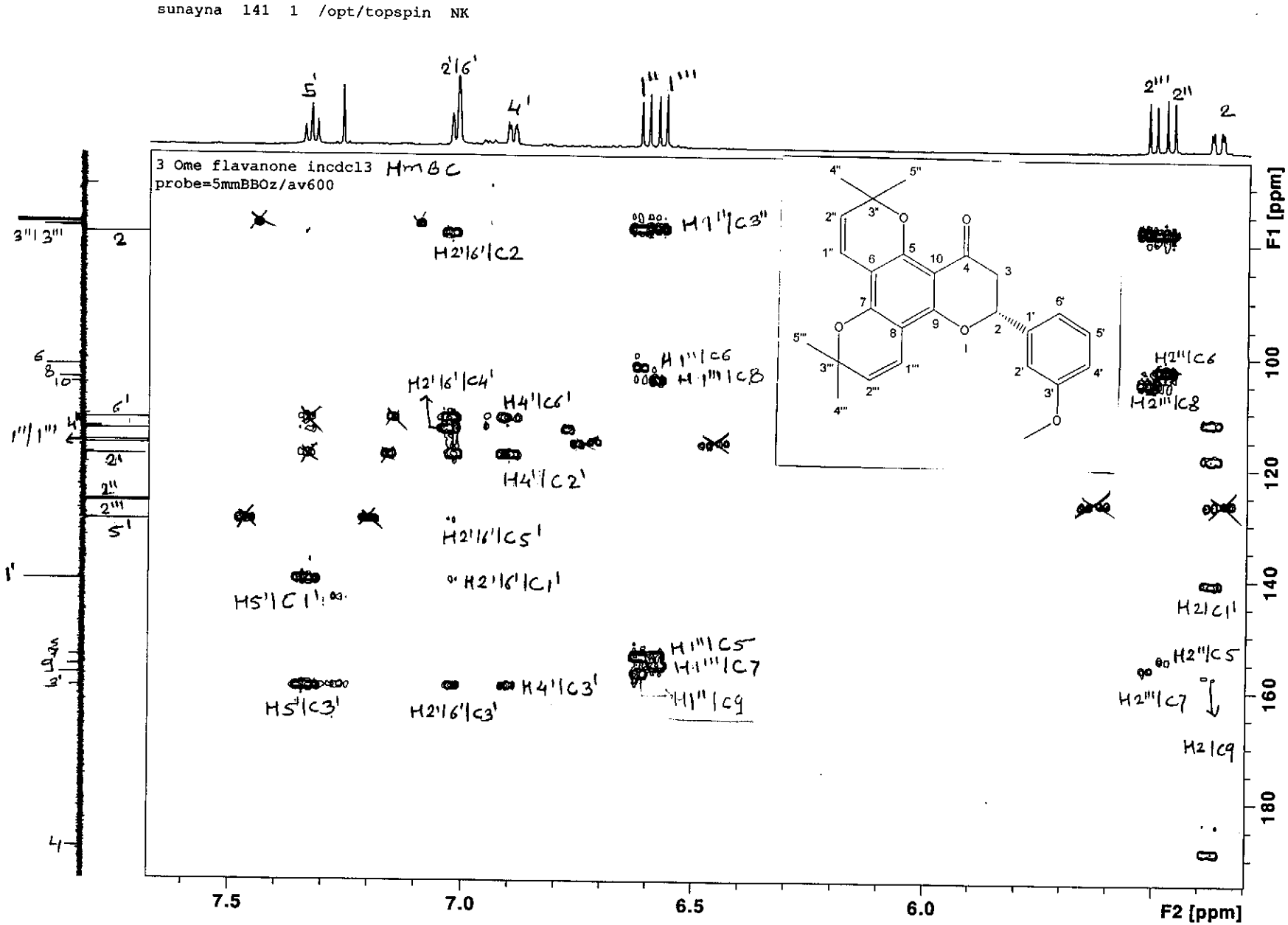
NOSY NMR spectrum of 4f - 3'-OMe pyranochromene flavanone (A-4f)



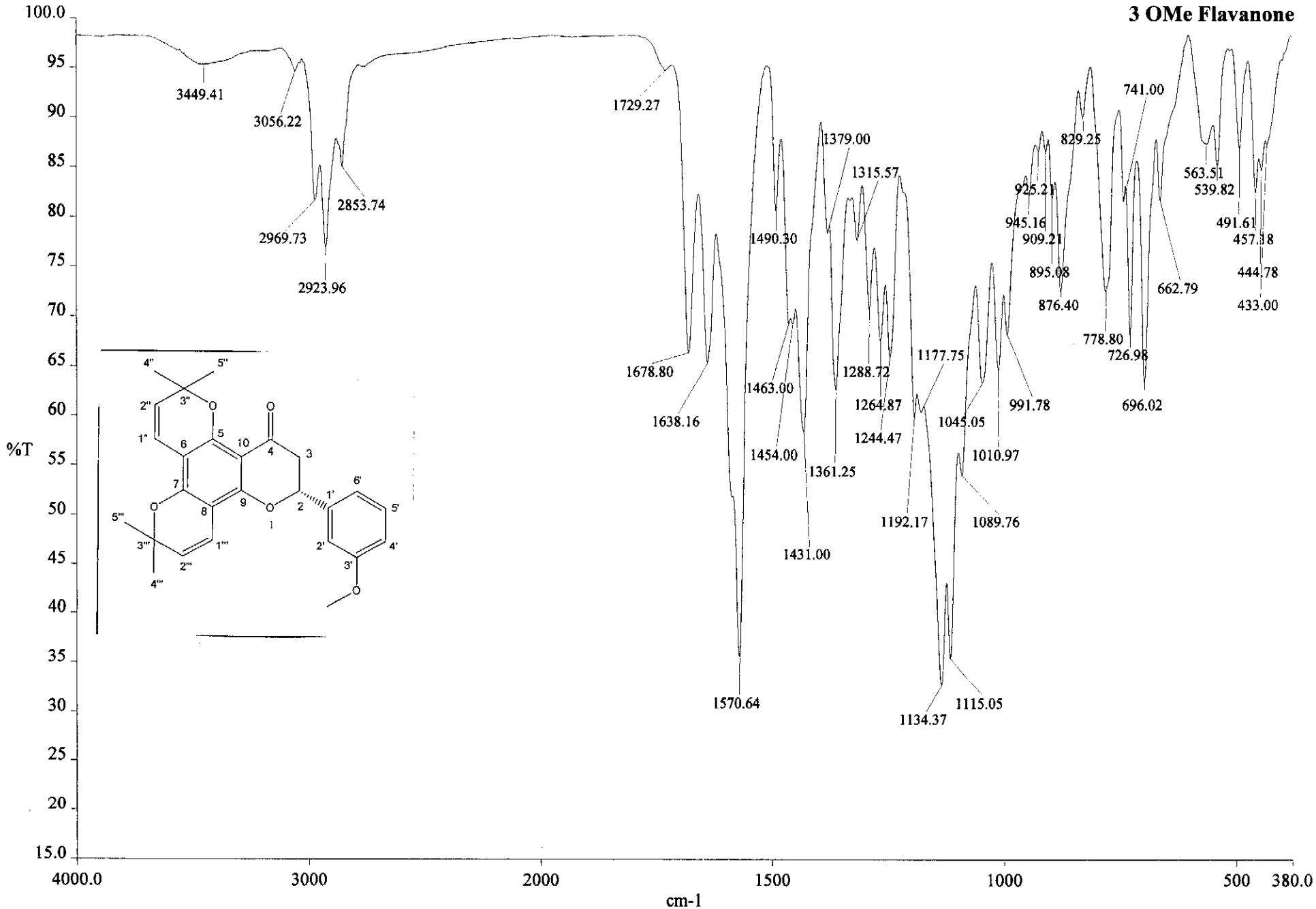
HSQC NMR spectrum of 4f - 3'-OMe pyranochromene flavanone (A-4f)



HMBC NMR spectrum of 4f - 3'-OMe pyranochromene flavanone (A-4f)

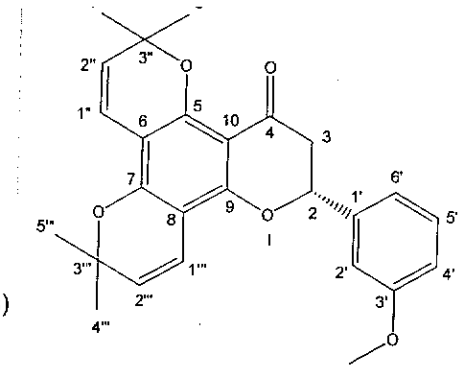


HMBC NMR spectrum of 4f - 3'-OMe pyranochromene flavanone (A-4f)



IR spectrum of 4f - 3'-OMe pyranochromene flavanone (A-4f)

Elemental Composition Report



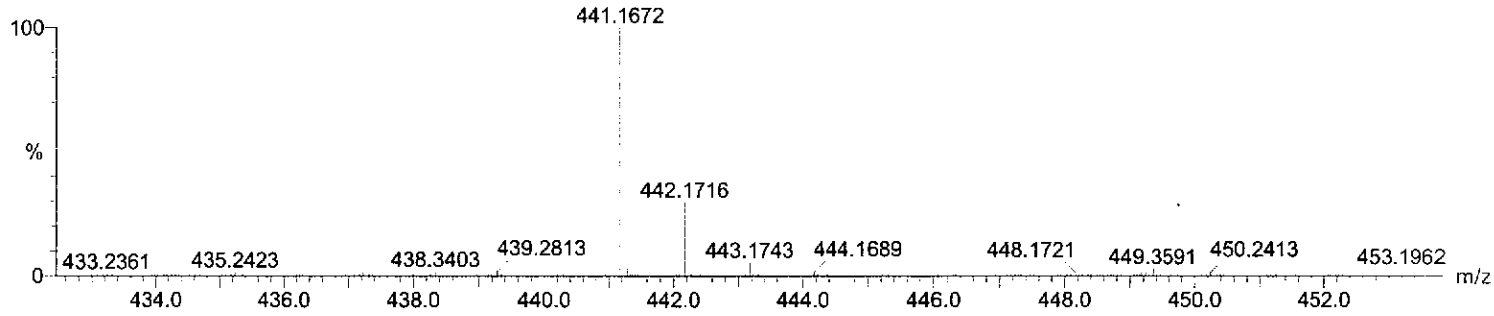
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
5 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-30 H: 25-30 O: 0-5 Na: 0-1

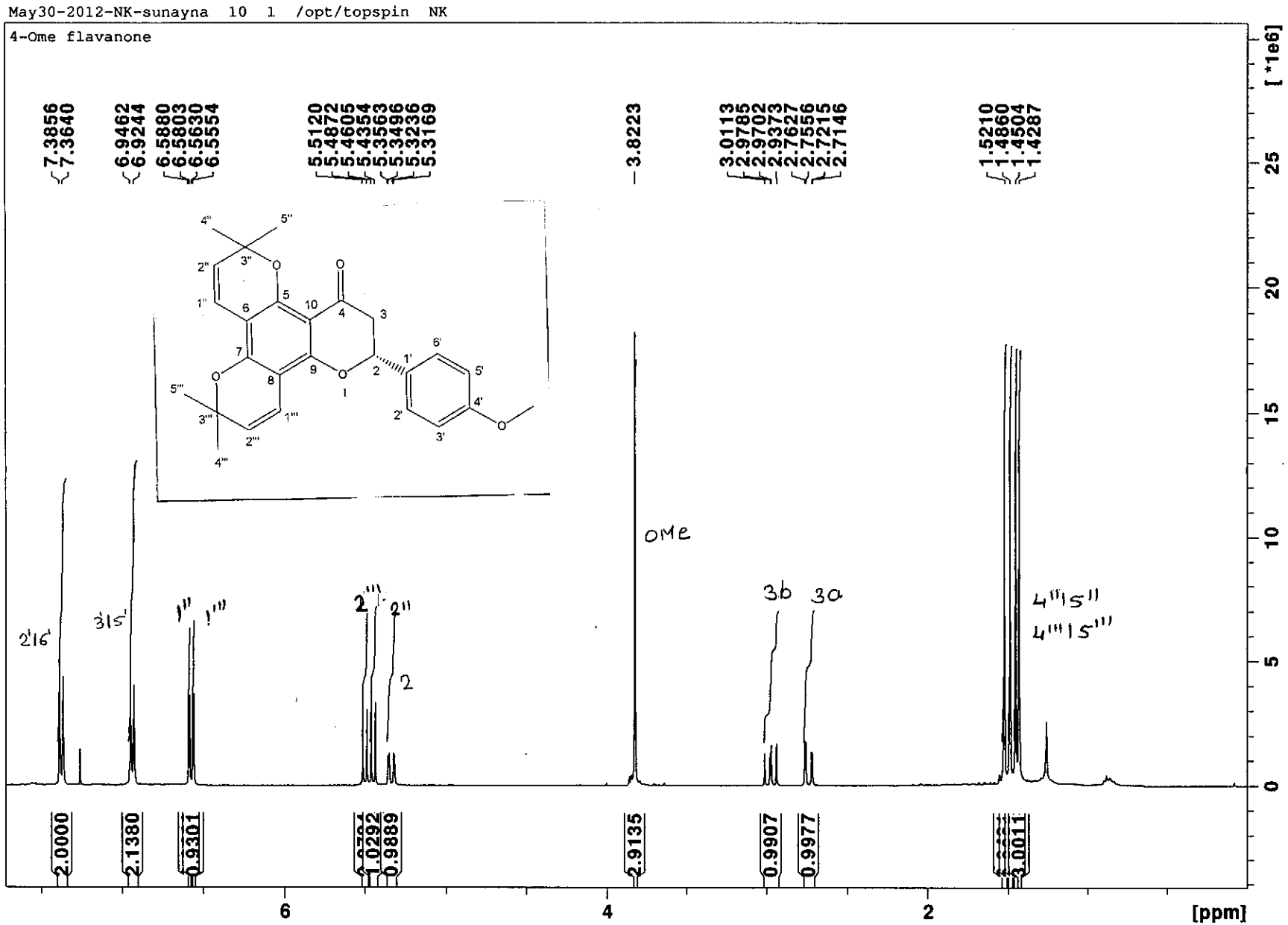
Dr. Nizam
6 2 (0.017) Cm (1:30)

TOF MS ES+
6.80e+003

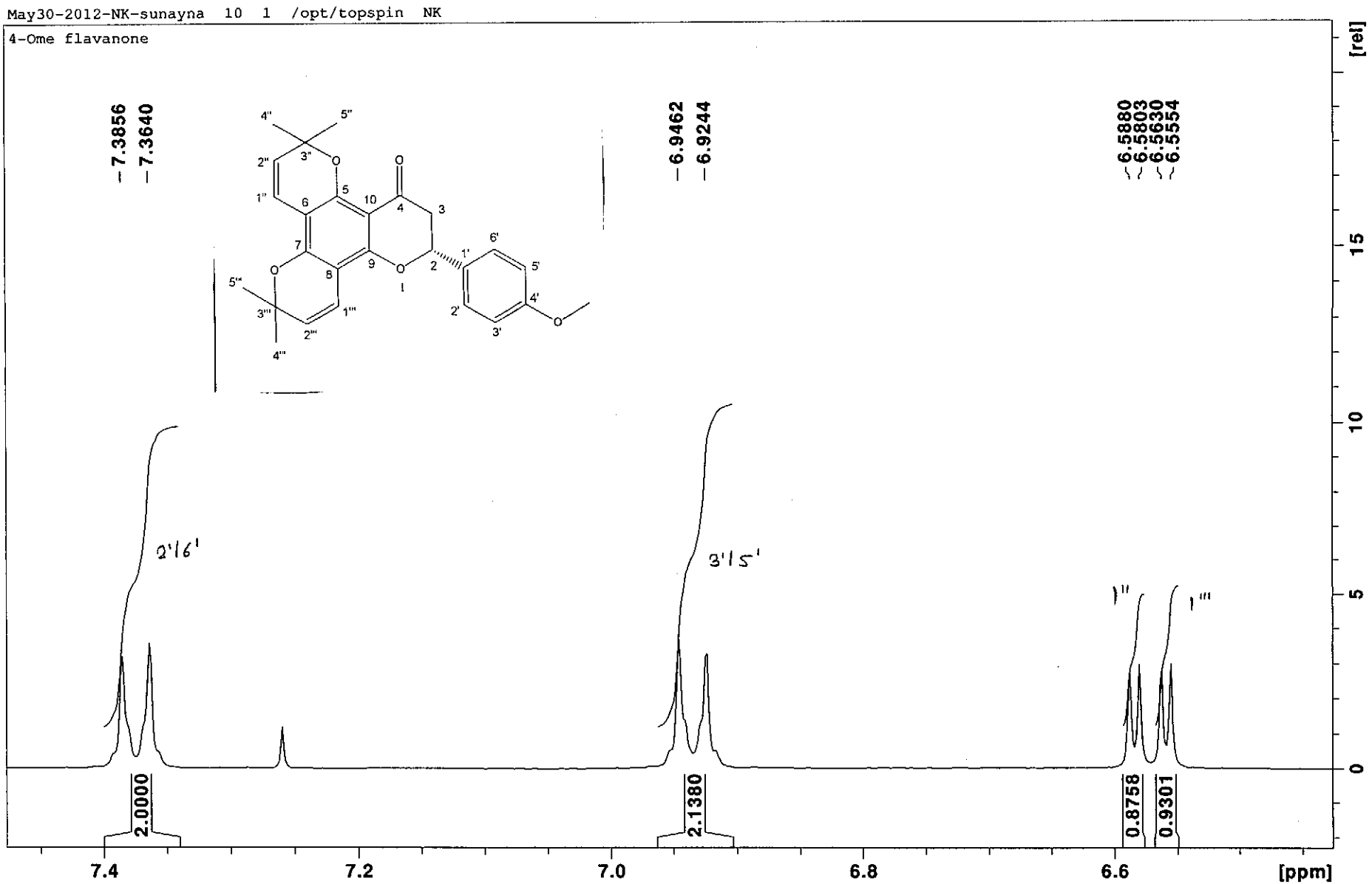


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
441.1672	441.1678	-0.6	-1.4	13.5	322.6	0.0	C26 H26 O5 Na

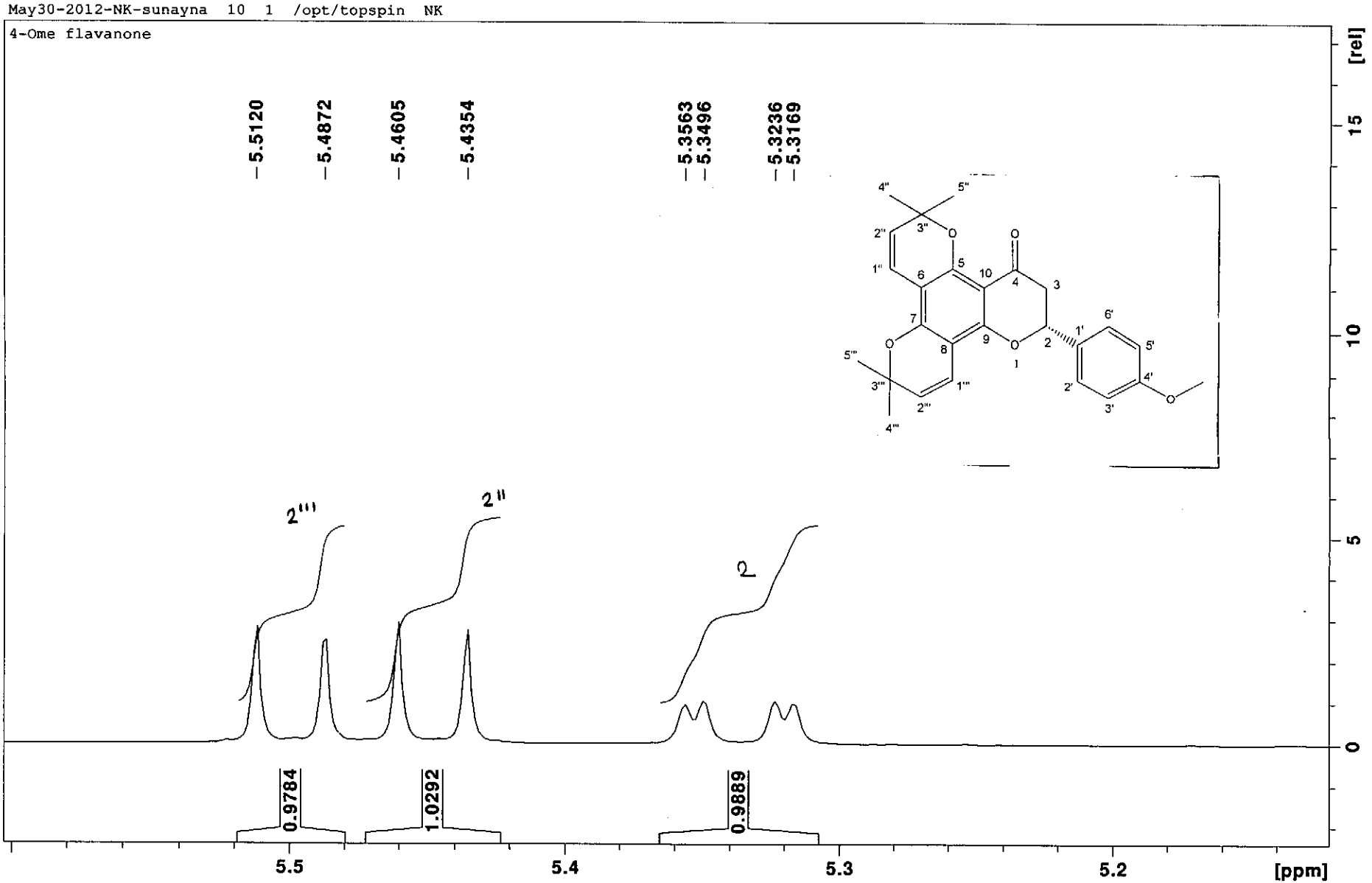
HRMS of 4f – 3'-OMe pyranochromene flavanone (A-4f)



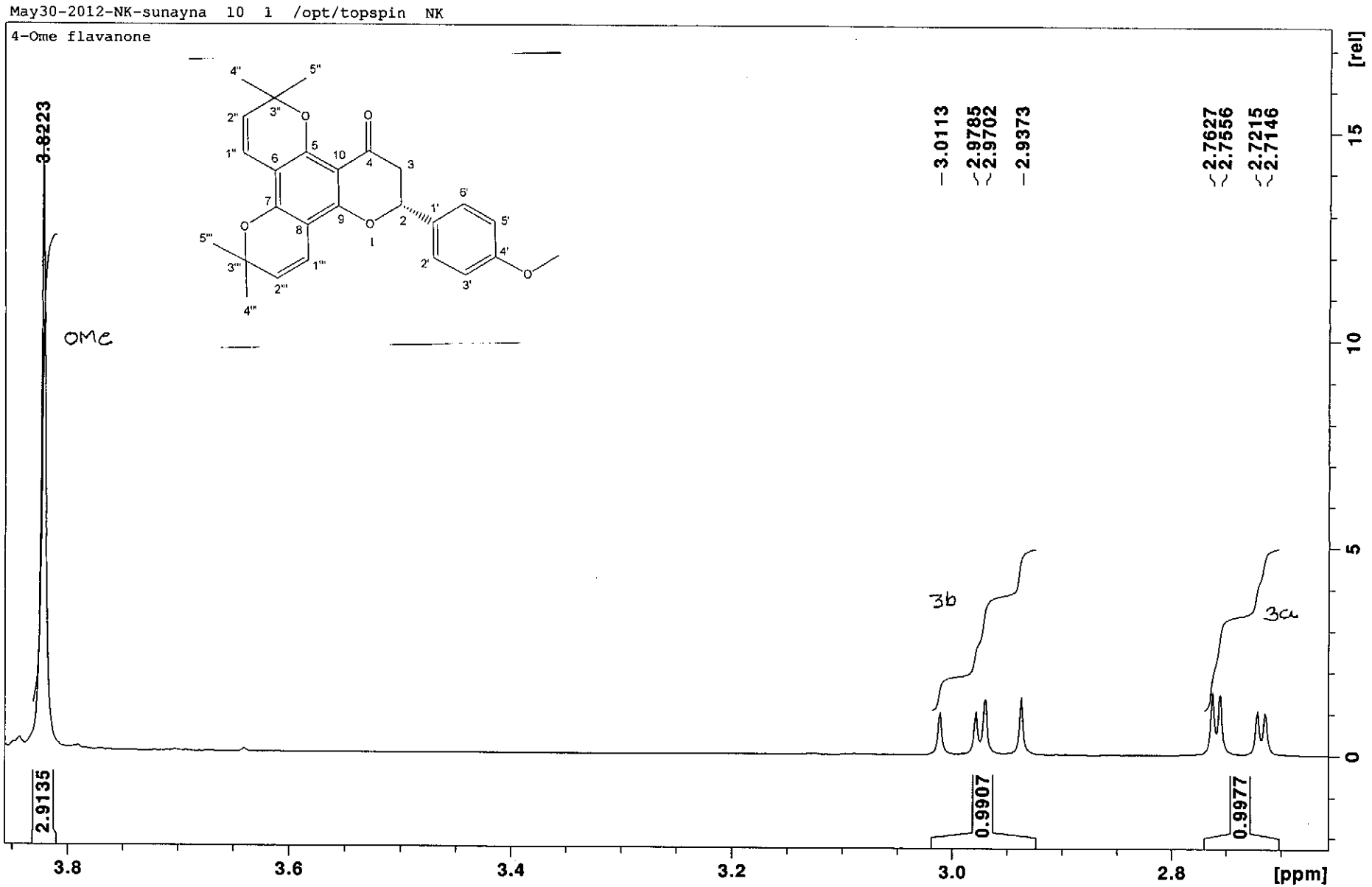
^{1}H spectrum of 4g - 4'-OMe pyranochromene flavanone (A-4g)



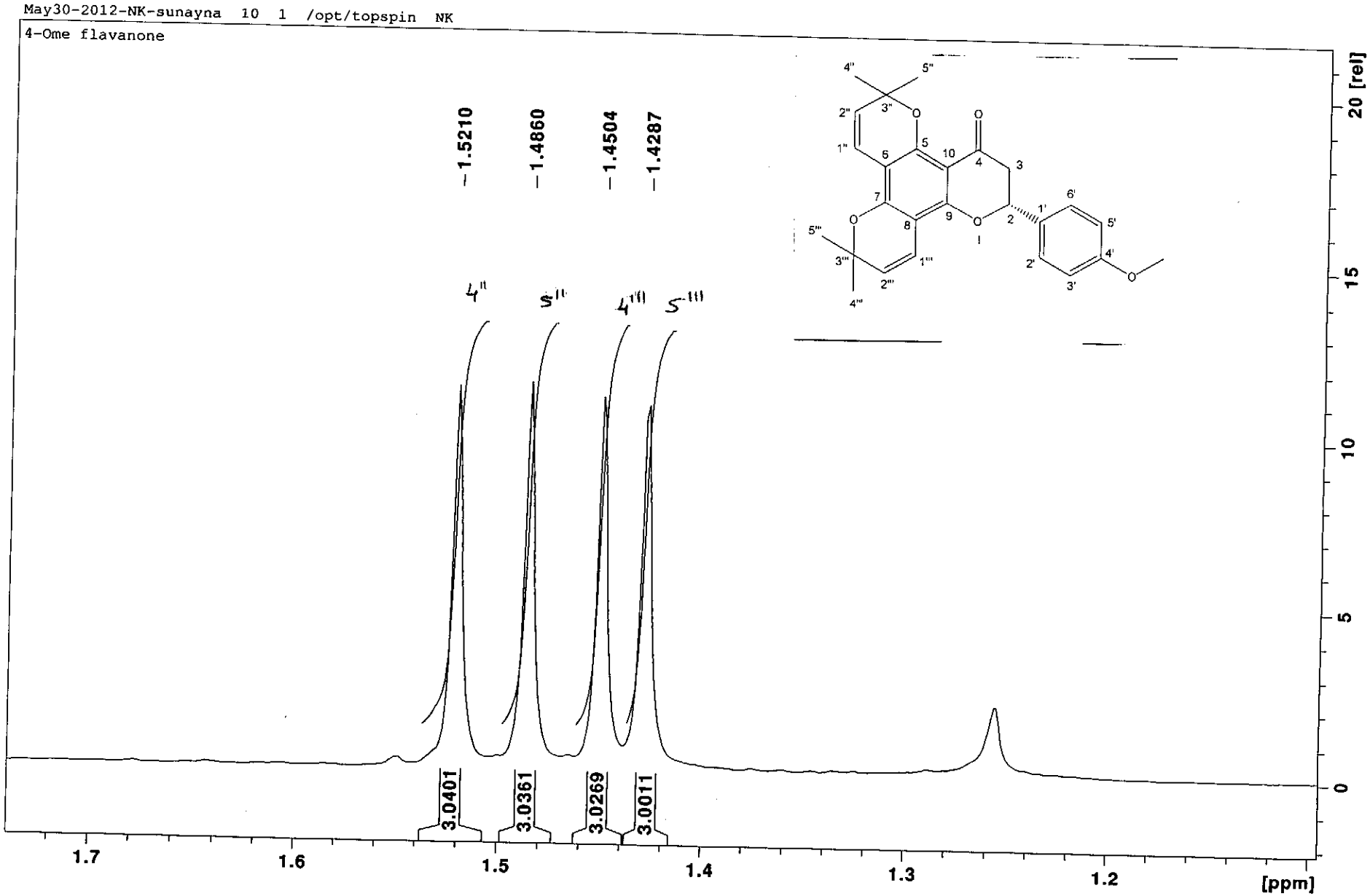
^{1}H spectrum of 4g - 4'-OMe pyranochromene flavanone (A-4g)



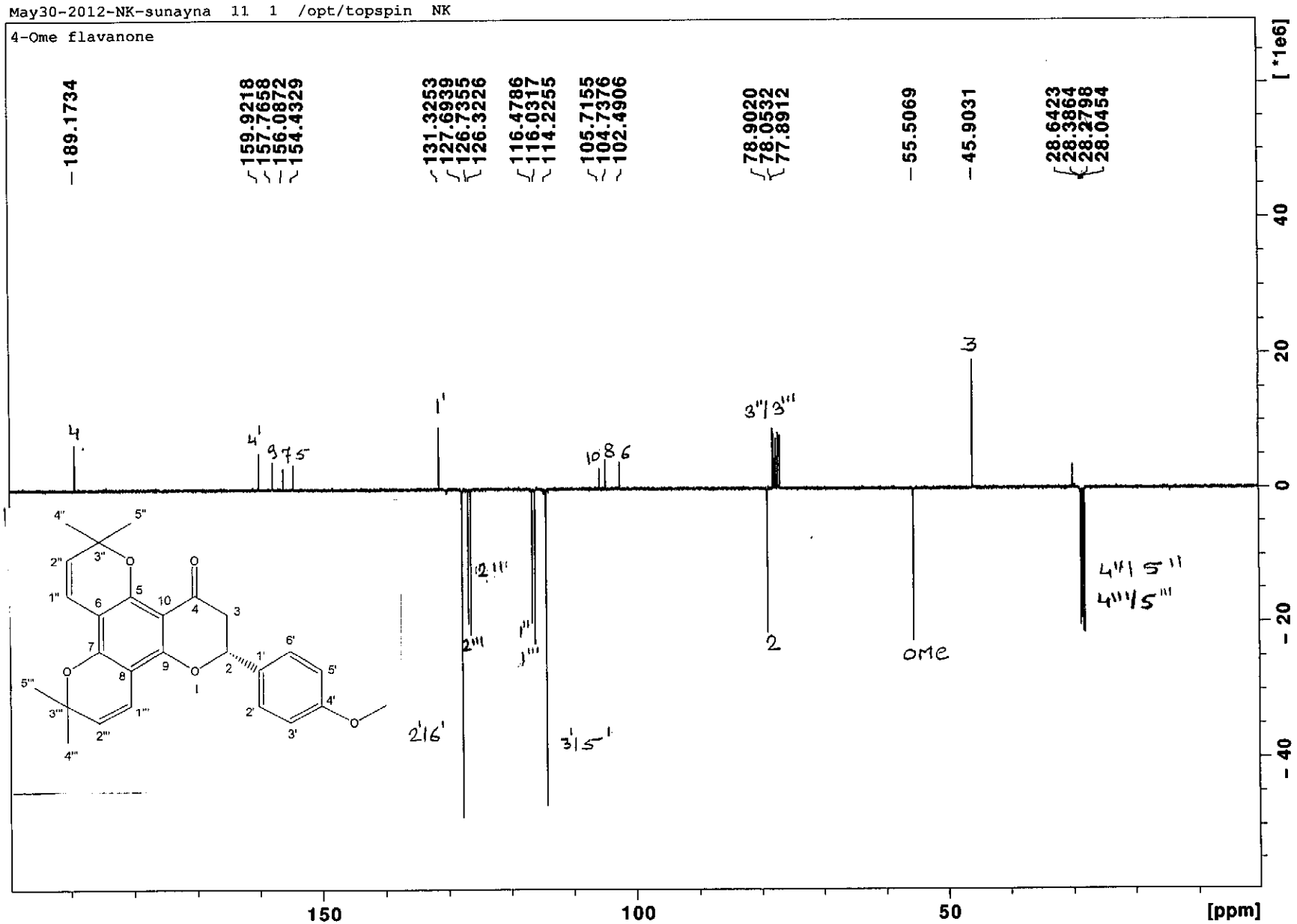
^{1}H spectrum of 4g - 4'-OMe pyranochromene flavanone (A-4g)



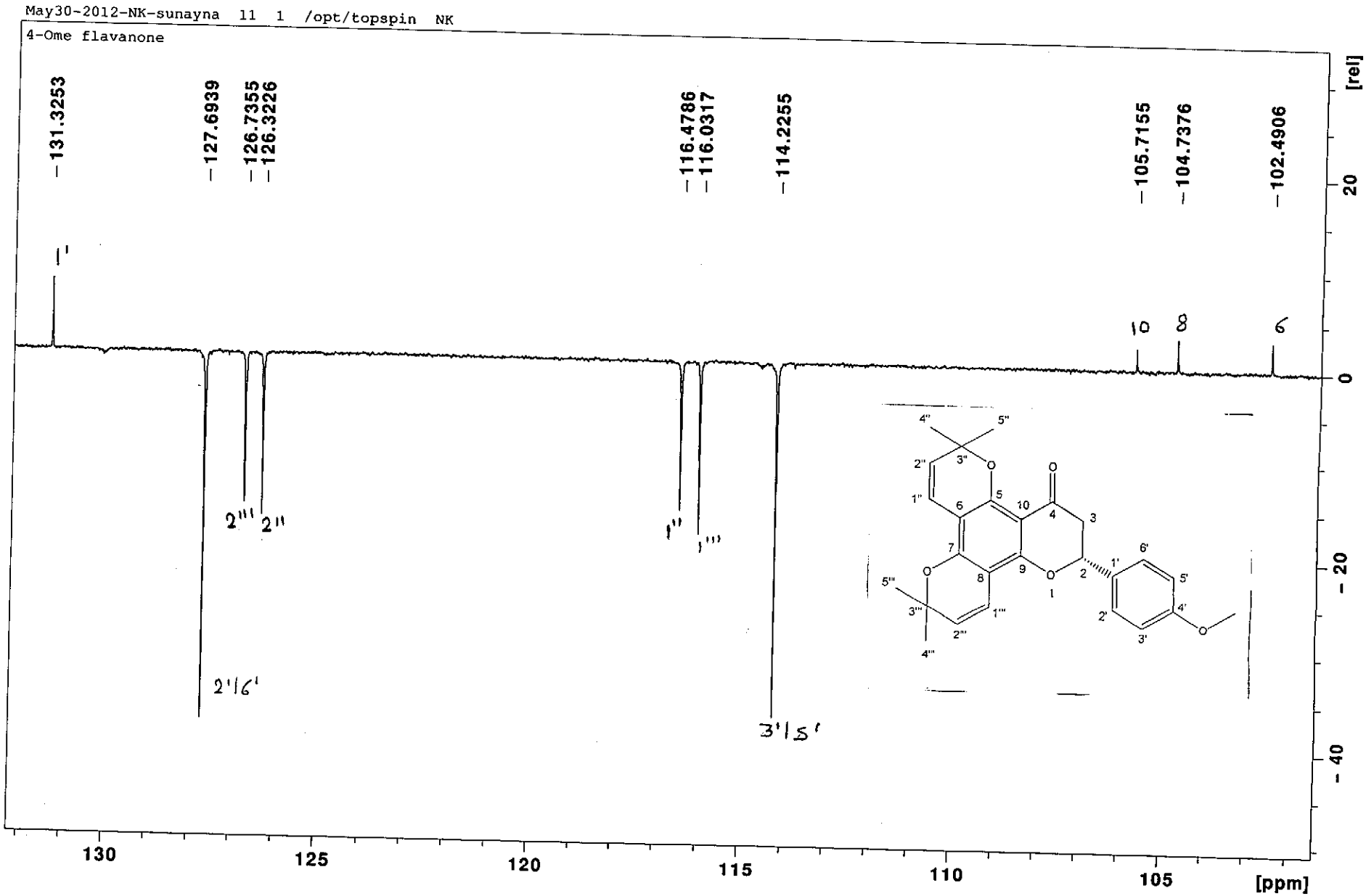
^{1}H spectrum of 4g - 4'-OMe pyranochromene flavanone (A-4g)



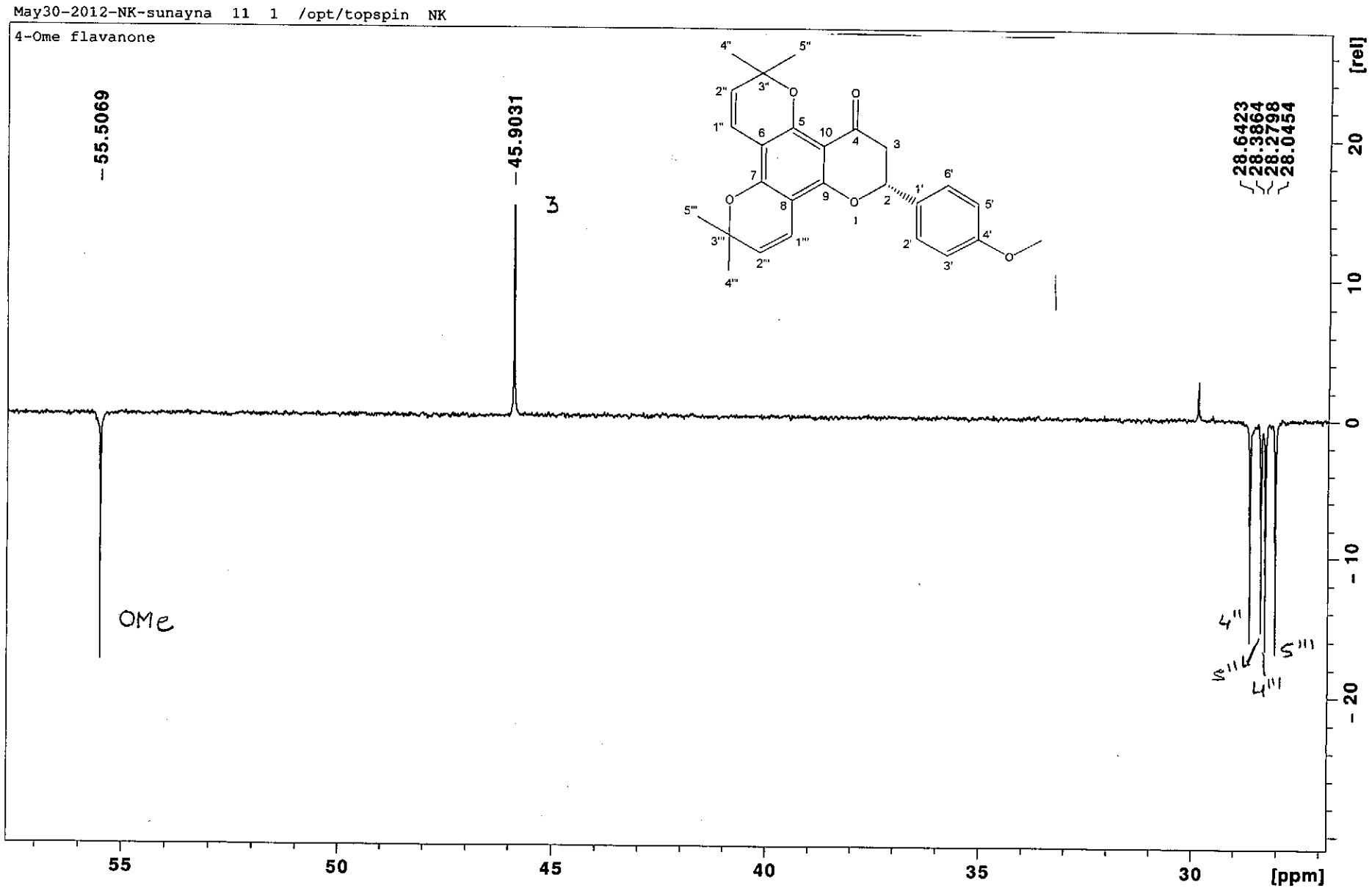
^{1}H spectrum of 4g - 4'-OMe pyranochromene flavanone (A-4g)



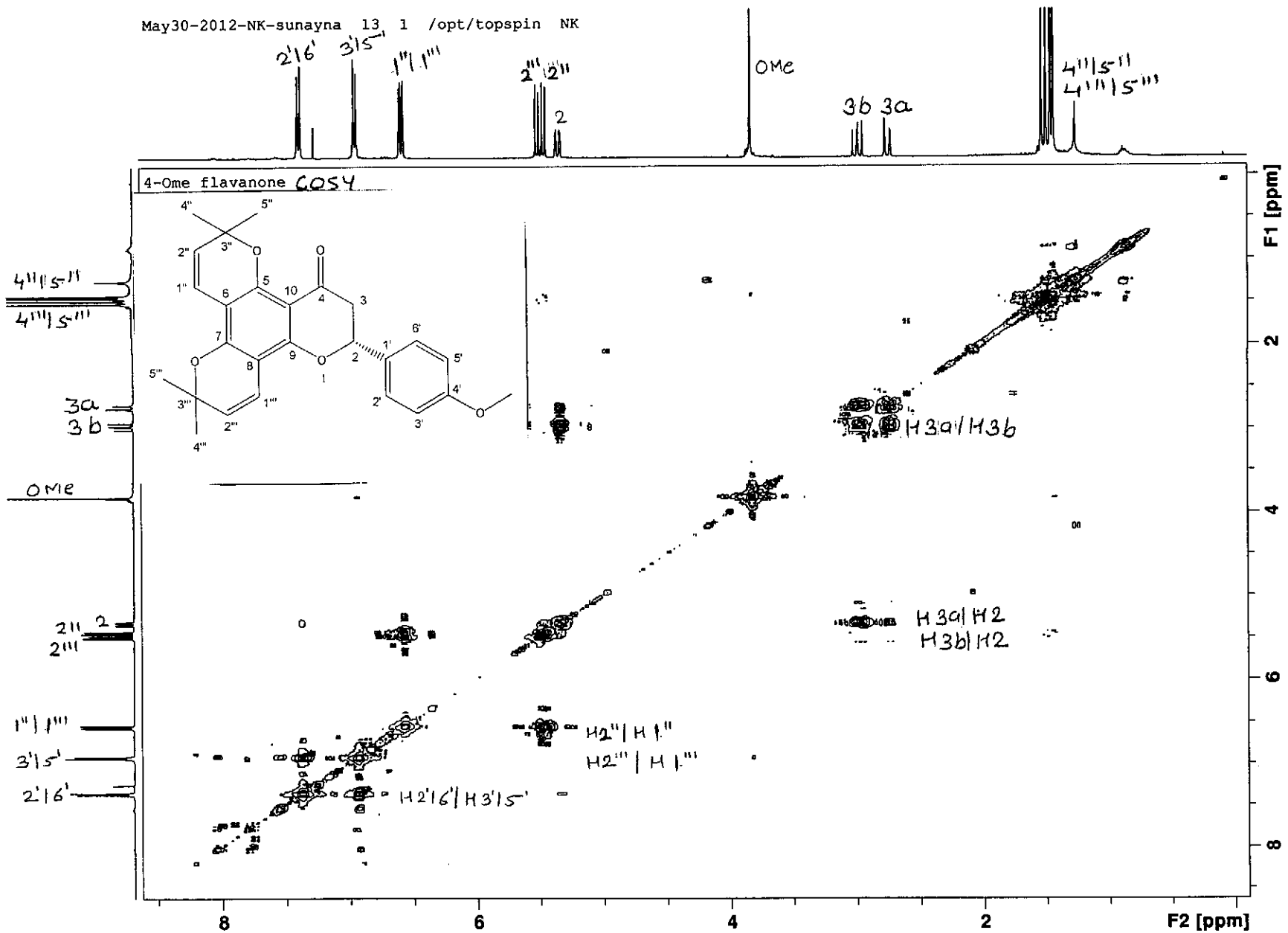
^{13}C spectrum of 4g - 4'-OMe pyranochromene flavanone (A-4g)



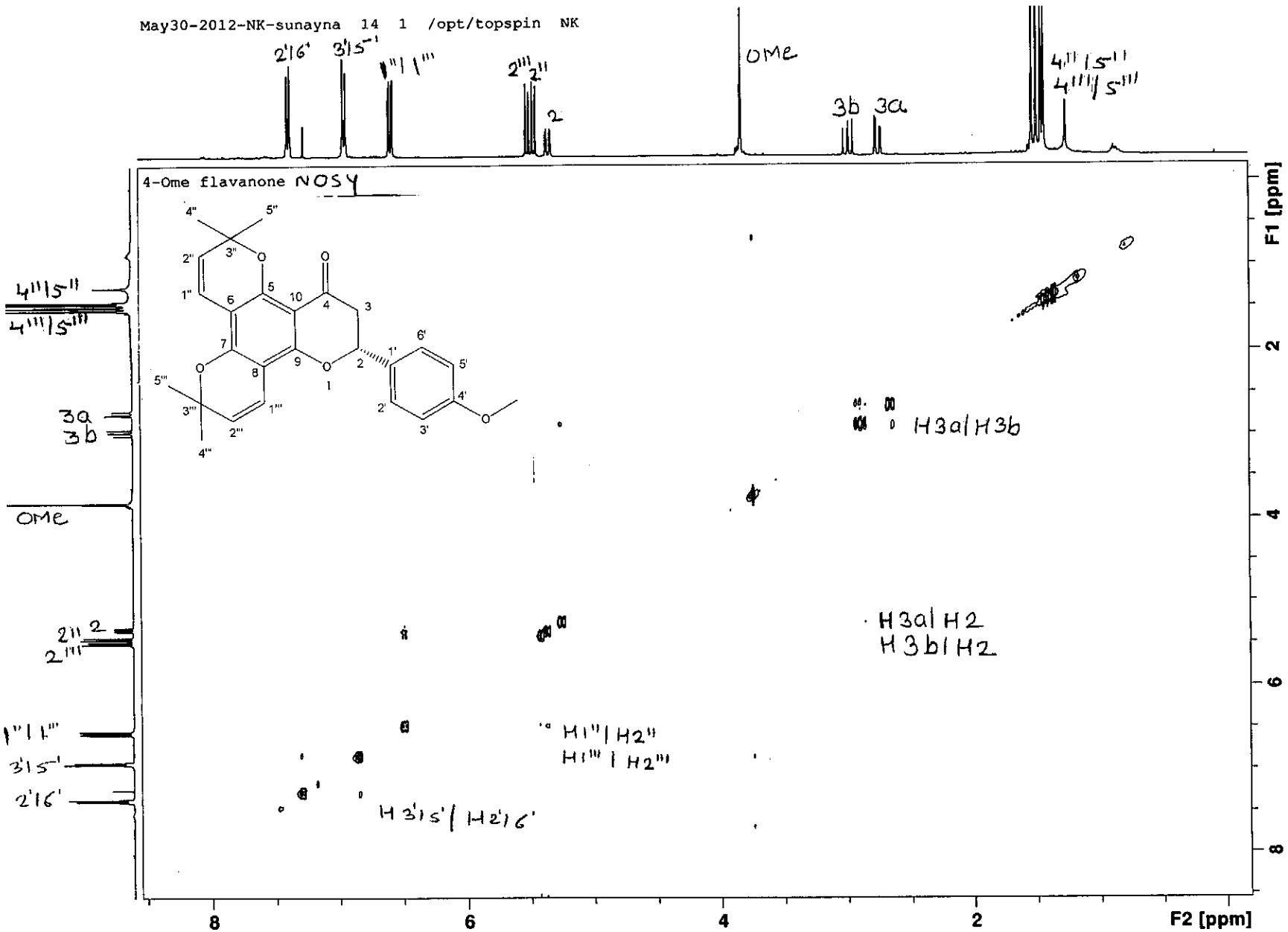
^{13}C spectrum of 4g - 4'-OMe pyranochromene flavanone (A-4g)



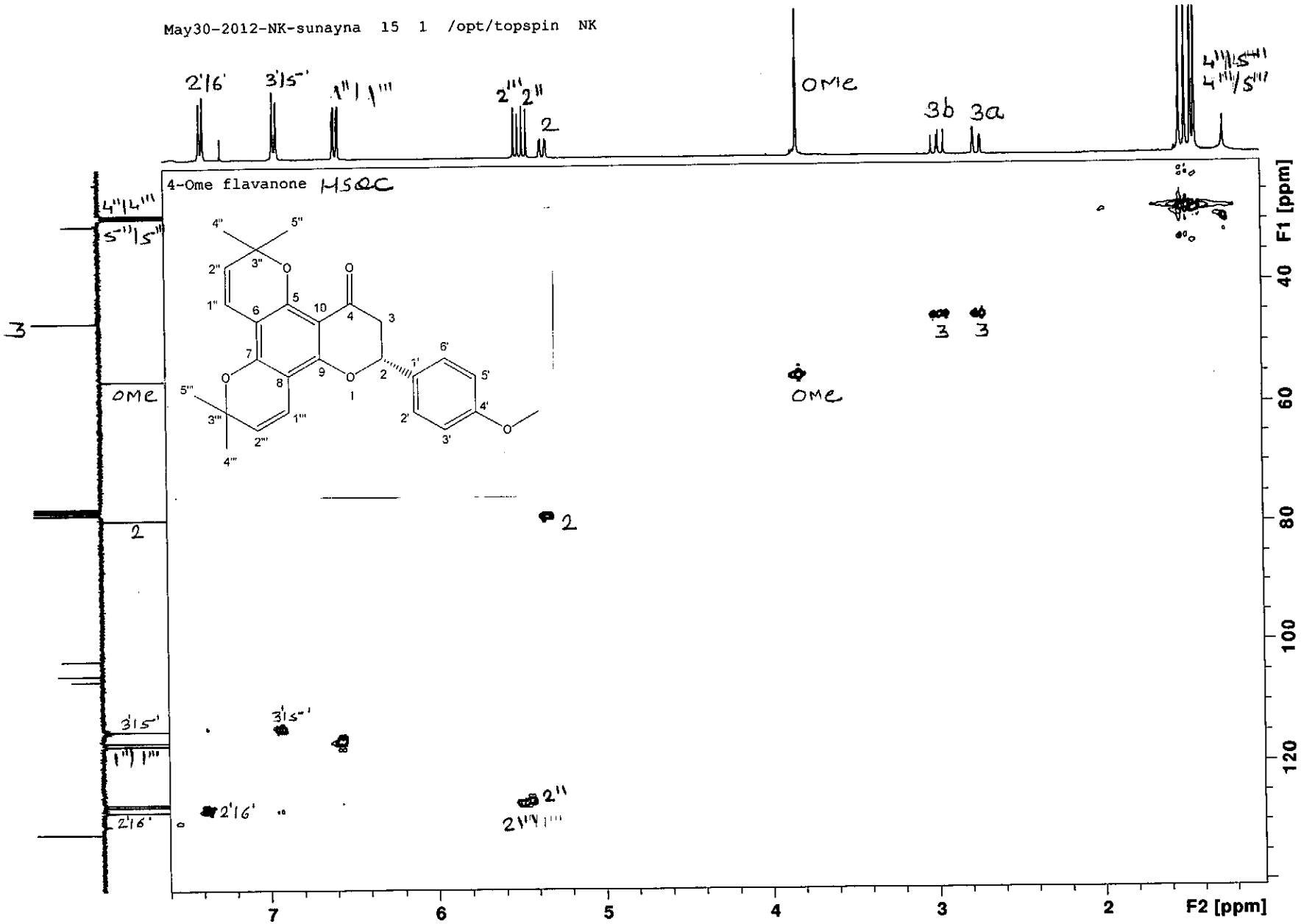
^{13}C spectrum of 4g - 4'-OMe pyranochromene flavanone (A-4g)



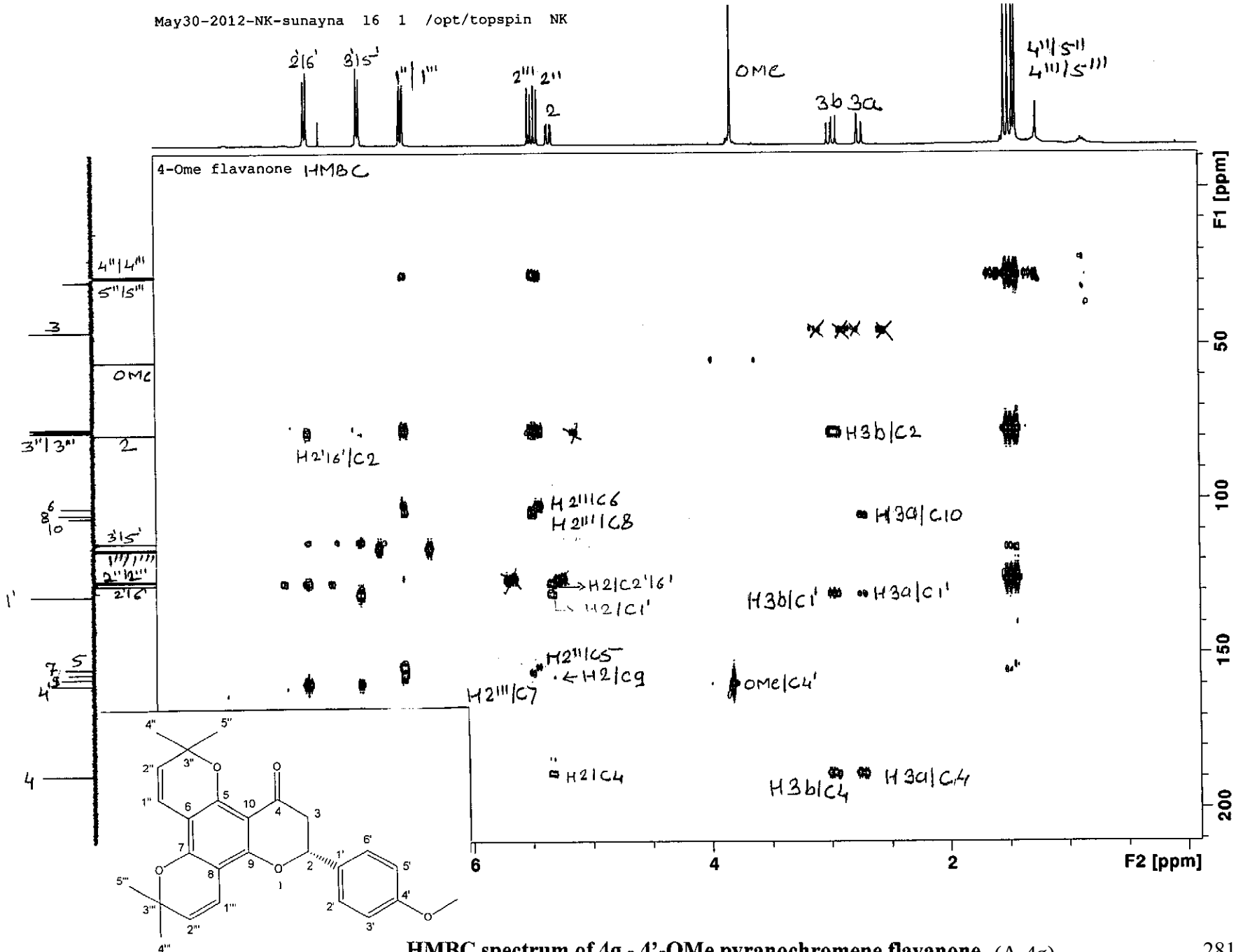
COSY spectrum of 4g - 4'-OMe pyranochromene flavanone (A-4g)



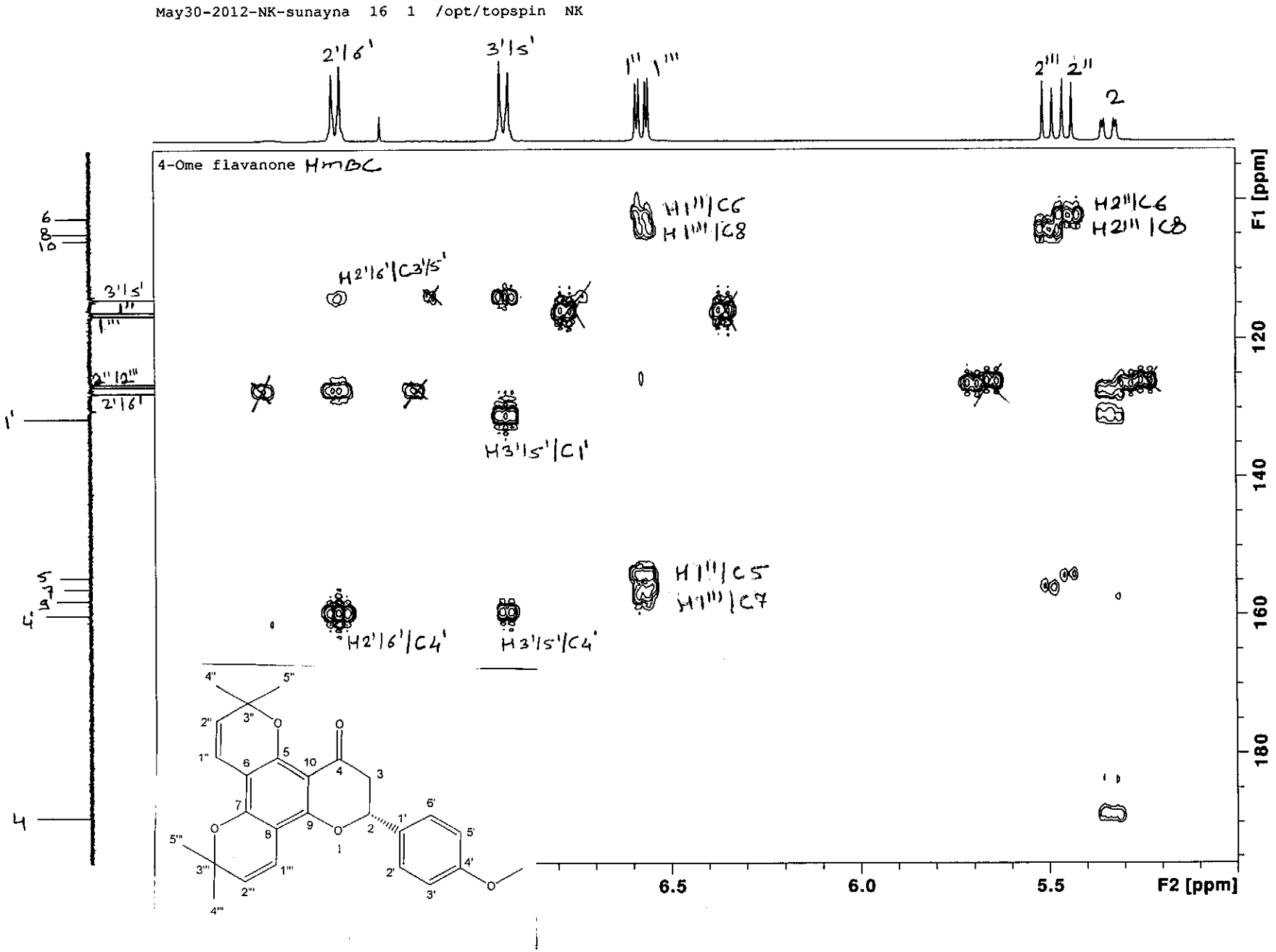
NOSY spectrum of 4g - 4'-OMe pyranochromene flavanone (A-4g)



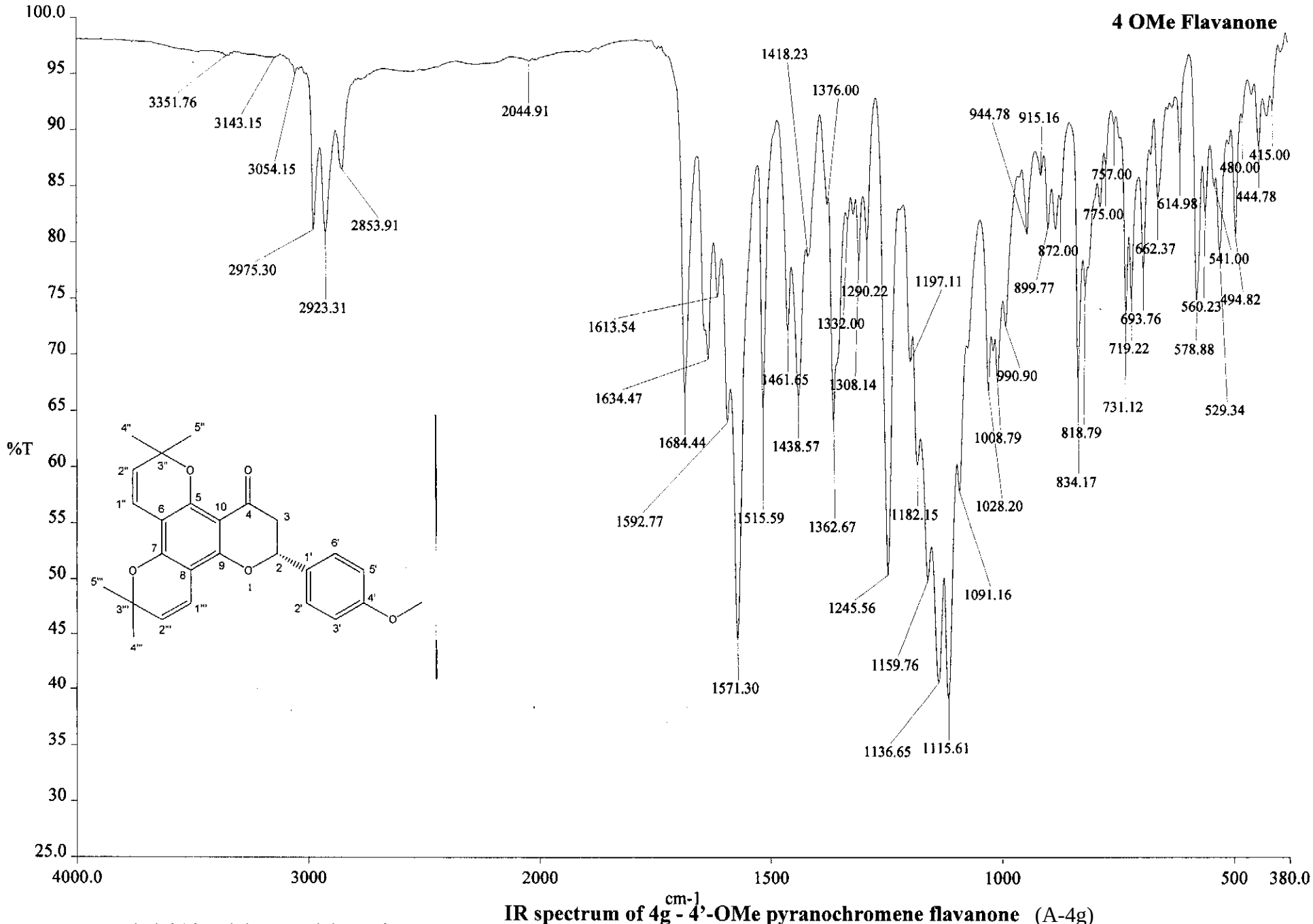
HSQC spectrum of 4g - 4'-OMe pyranochromene flavanone (A-4g)



HMBC spectrum of 4g - 4'-OMe pyranochromene flavanone (A-4g)

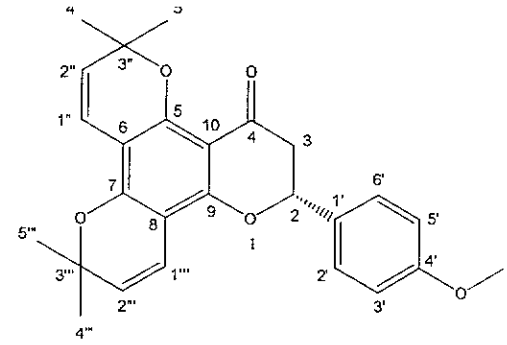


HMBC spectrum of 4g - 4'-OMe pyranochromene flavanone (A-4g)



IR spectrum of 4g - 4'-OMe pyranochromene flavanone (A-4g)

Elemental Composition Report



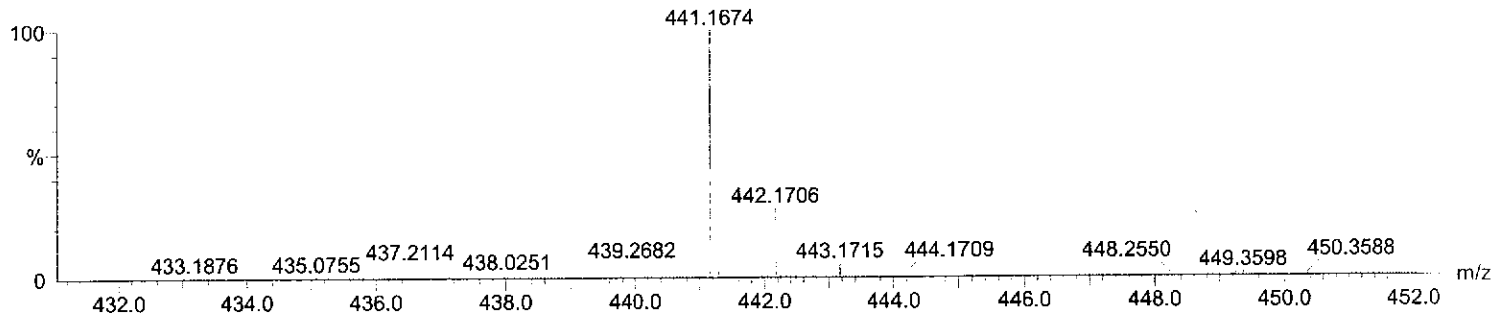
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
5 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-30 H: 25-30 O: 0-5 Na: 0-1

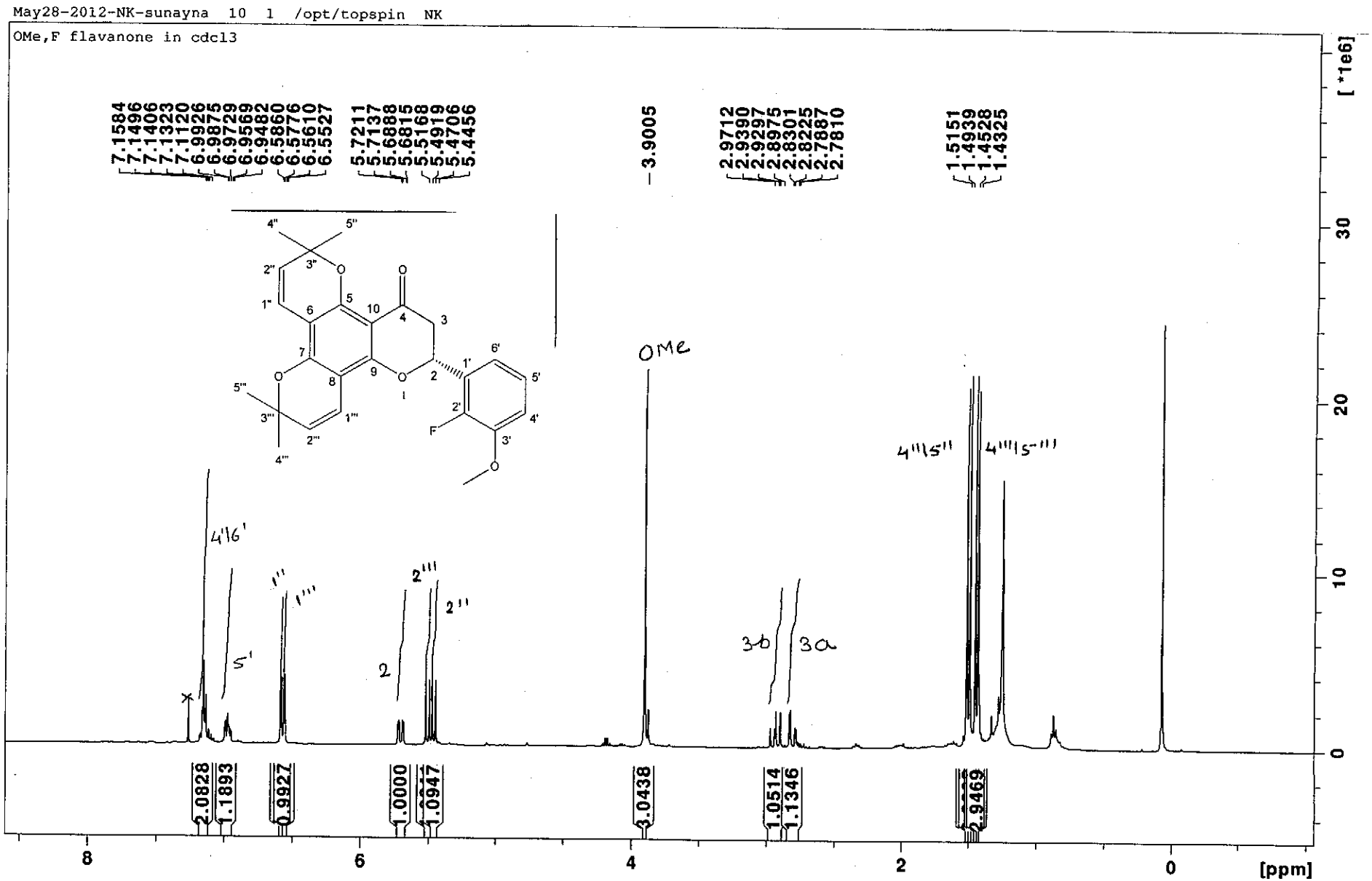
Dr. Nizam
4 4 (0.051) Cm (1:31)

TOF MS ES+
3.66e+003

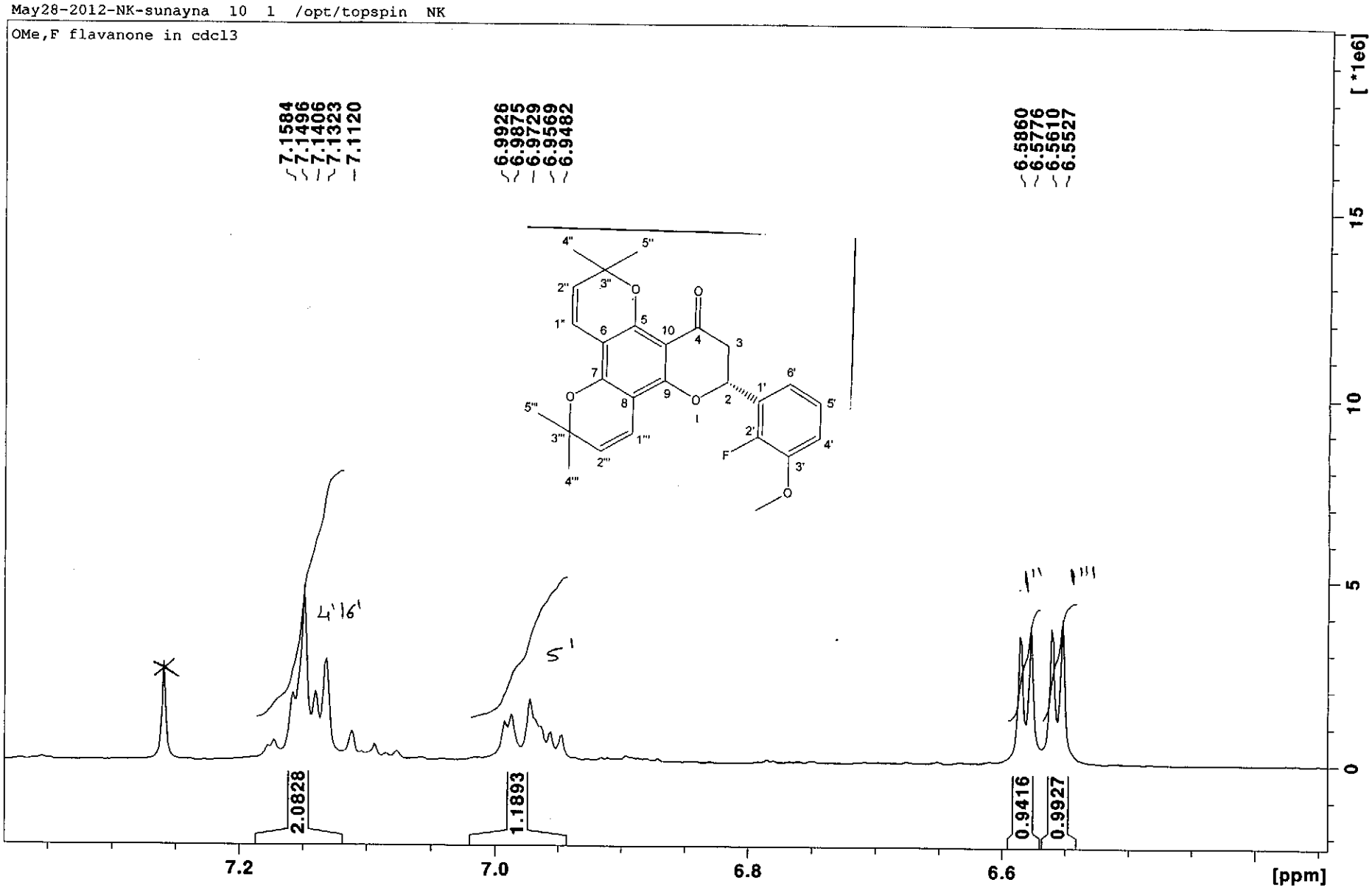


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
441.1674	441.1678	-0.4	-0.9	13.5	261.4	0.0	C26 H26 O5 Na

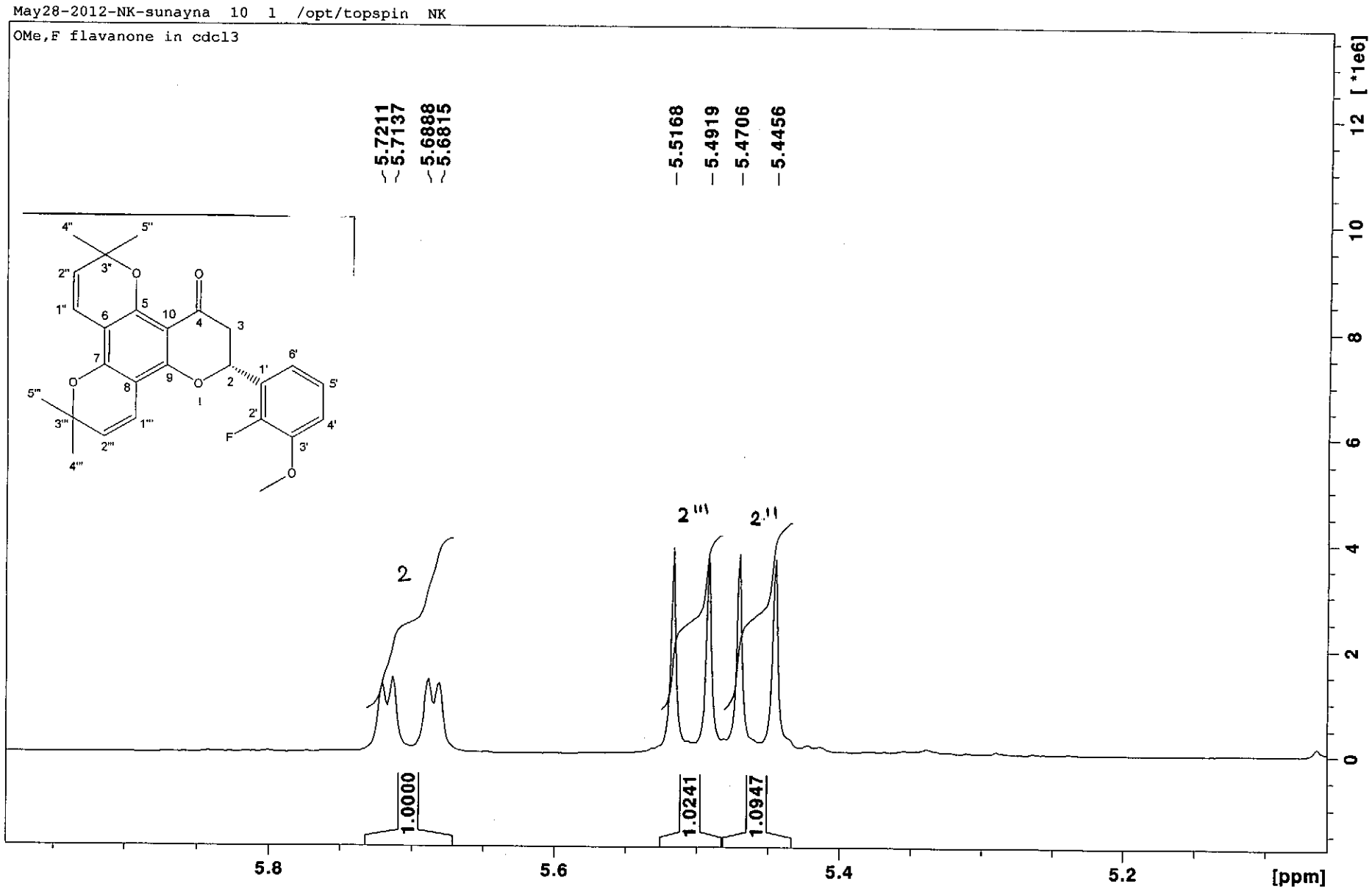
HRMS of 4g – 4'-OMe pyranochromene flavanone (A-4g)



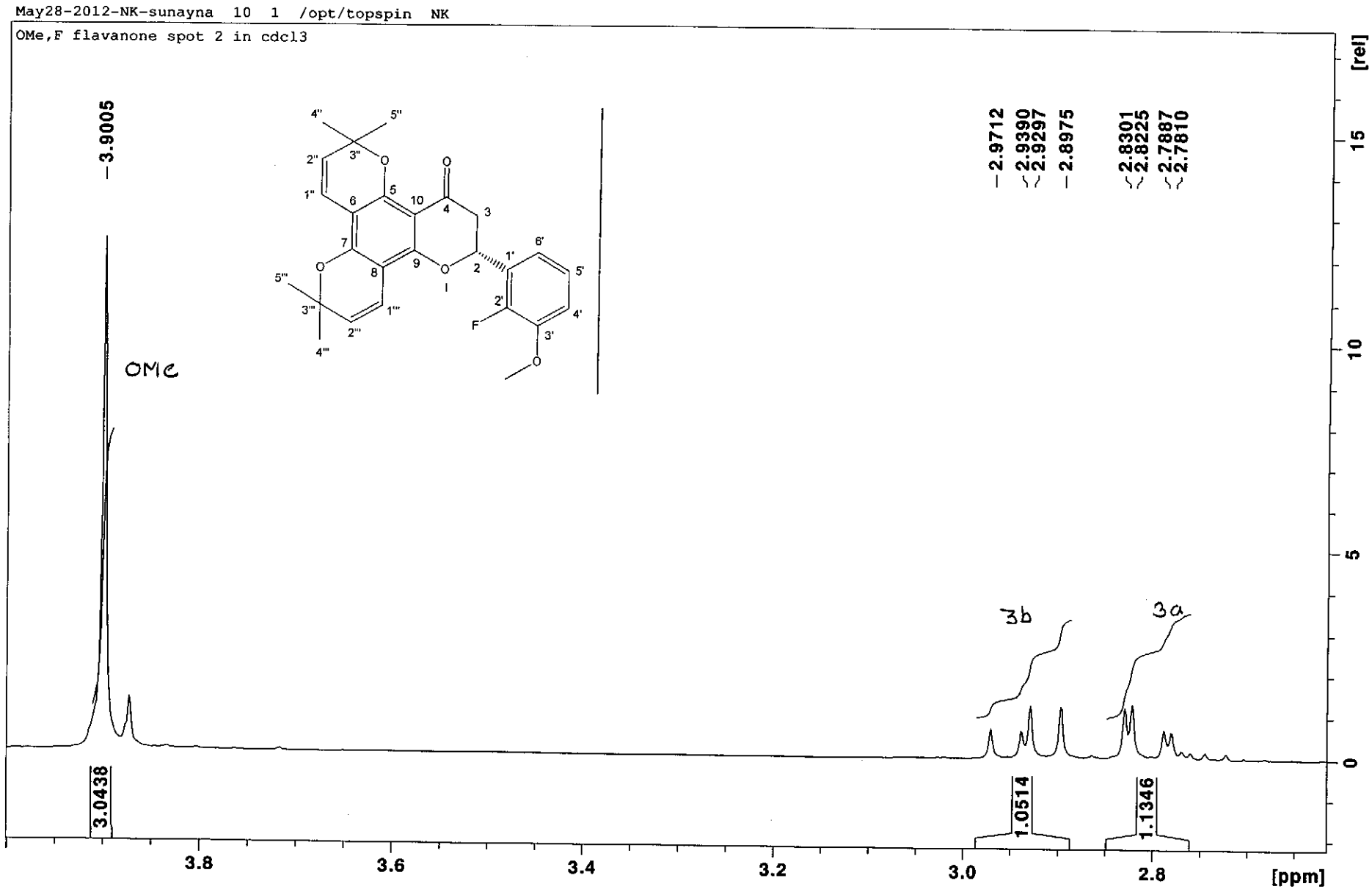
[1]H NMR spectrum of 4h - 2'-F, 3'-OMe pyranochromene flavanone (A-4h)



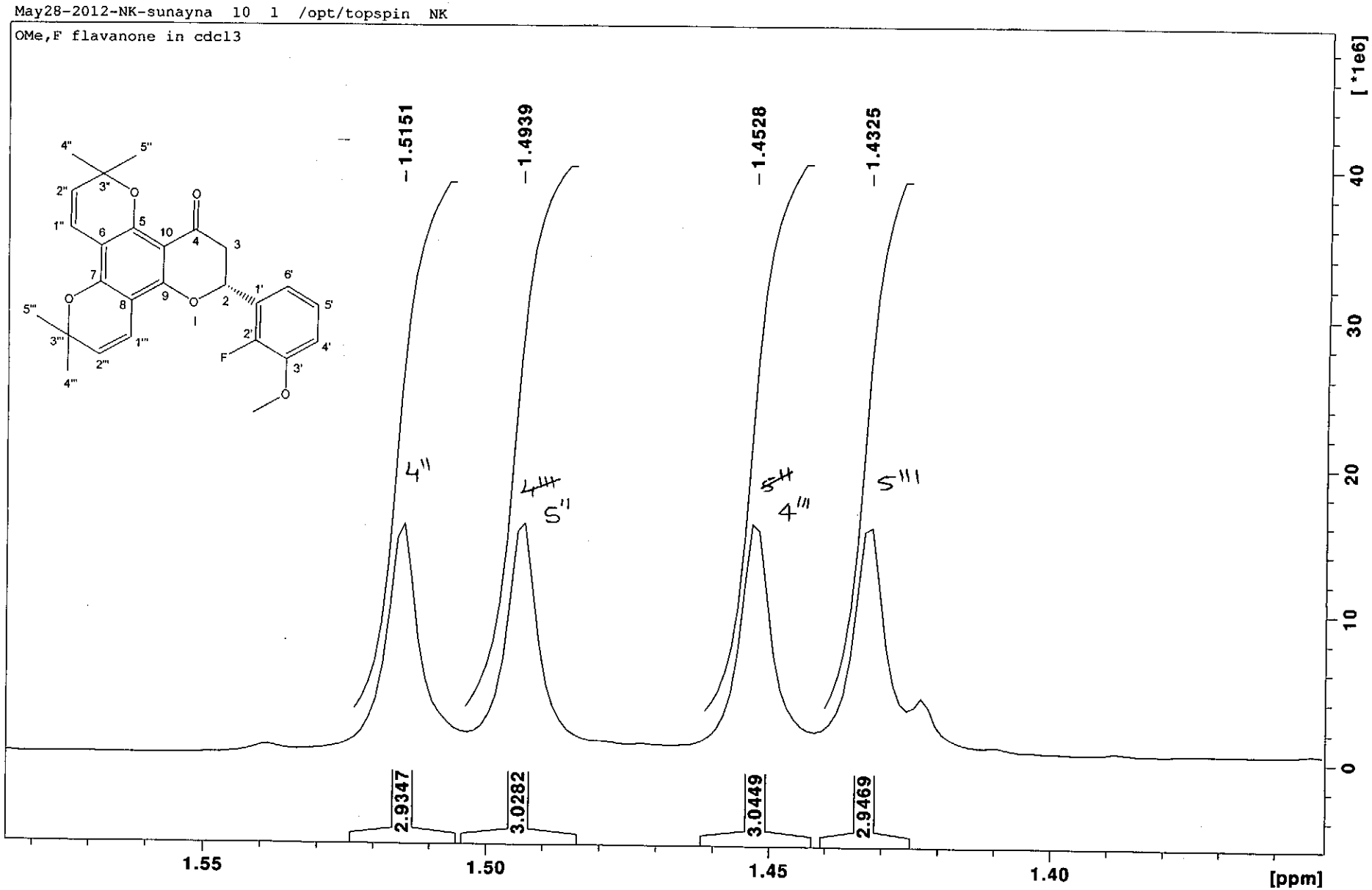
^{1}H NMR spectrum of 4h - 2'-F, 3'-OMe pyranochromene flavanone (A-4h)



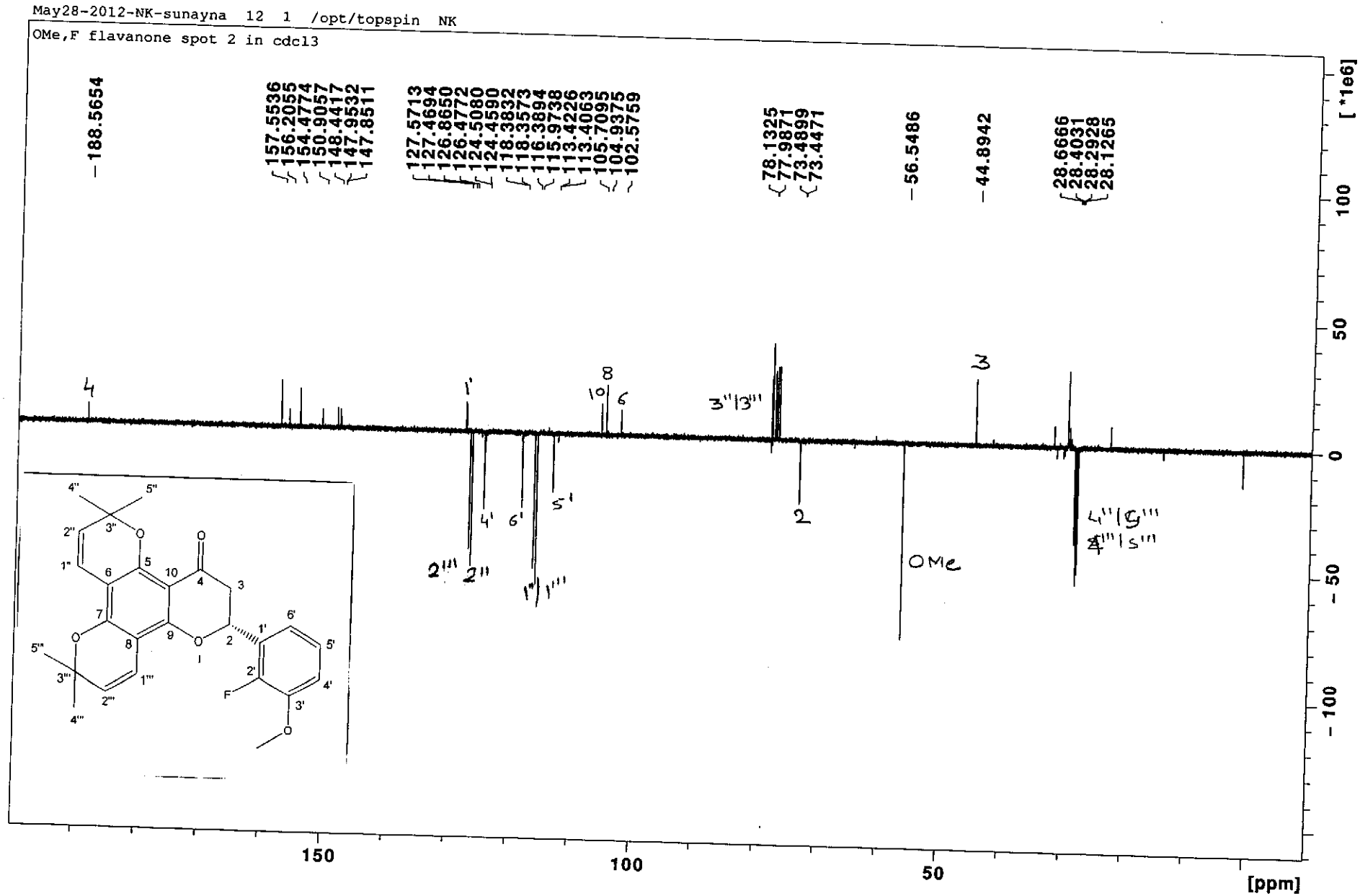
^{1}H NMR spectrum of 4h - 2'-F, 3'-OMe pyranochromene flavanone (A-4h)



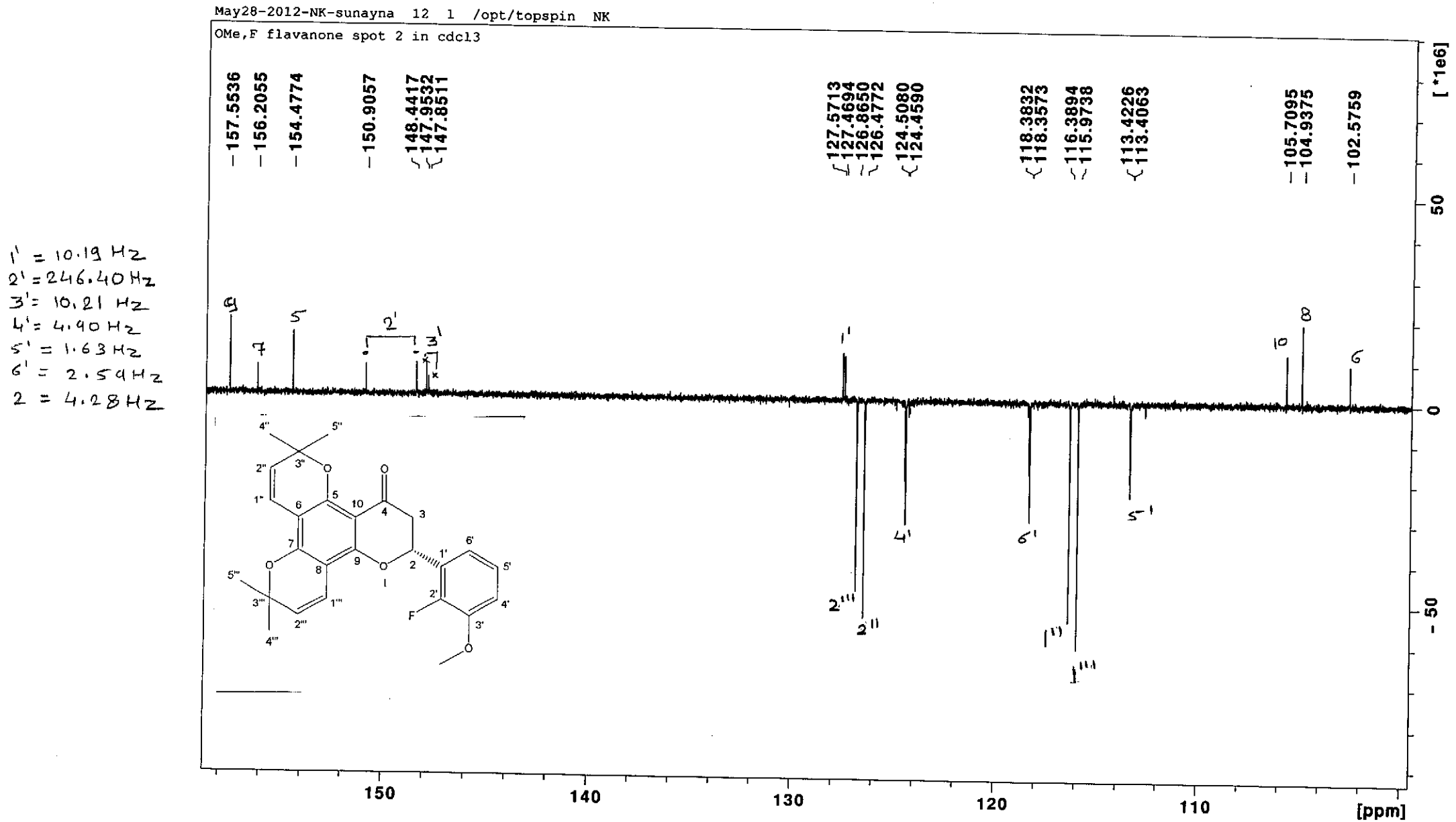
^{1}H NMR spectrum of 4h - 2'-F, 3'-OMe pyranochromene flavanone (A-4h)



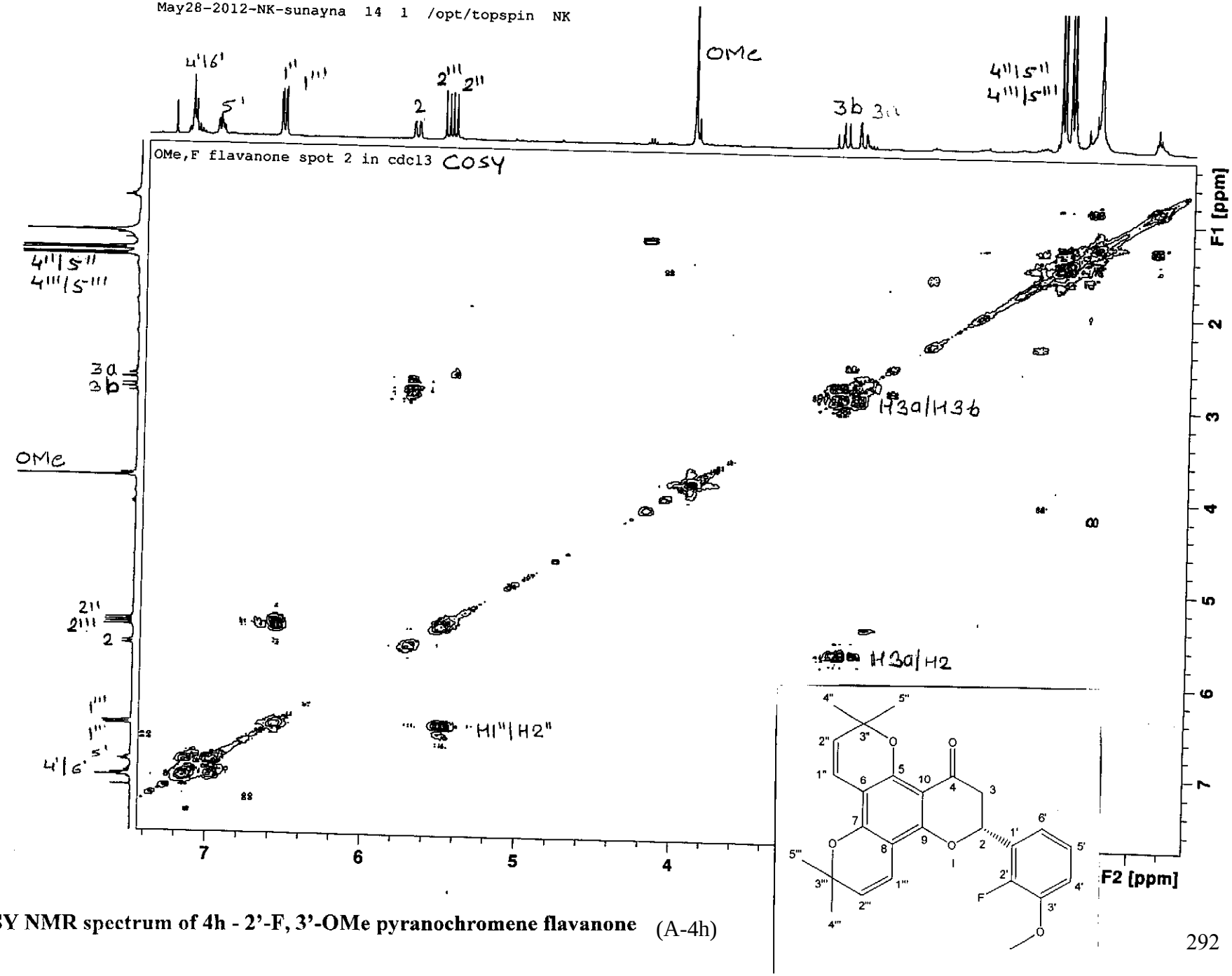
^{1}H NMR spectrum of 4h - 2'-F, 3'-OMe pyranochromene flavanone (A-4h)



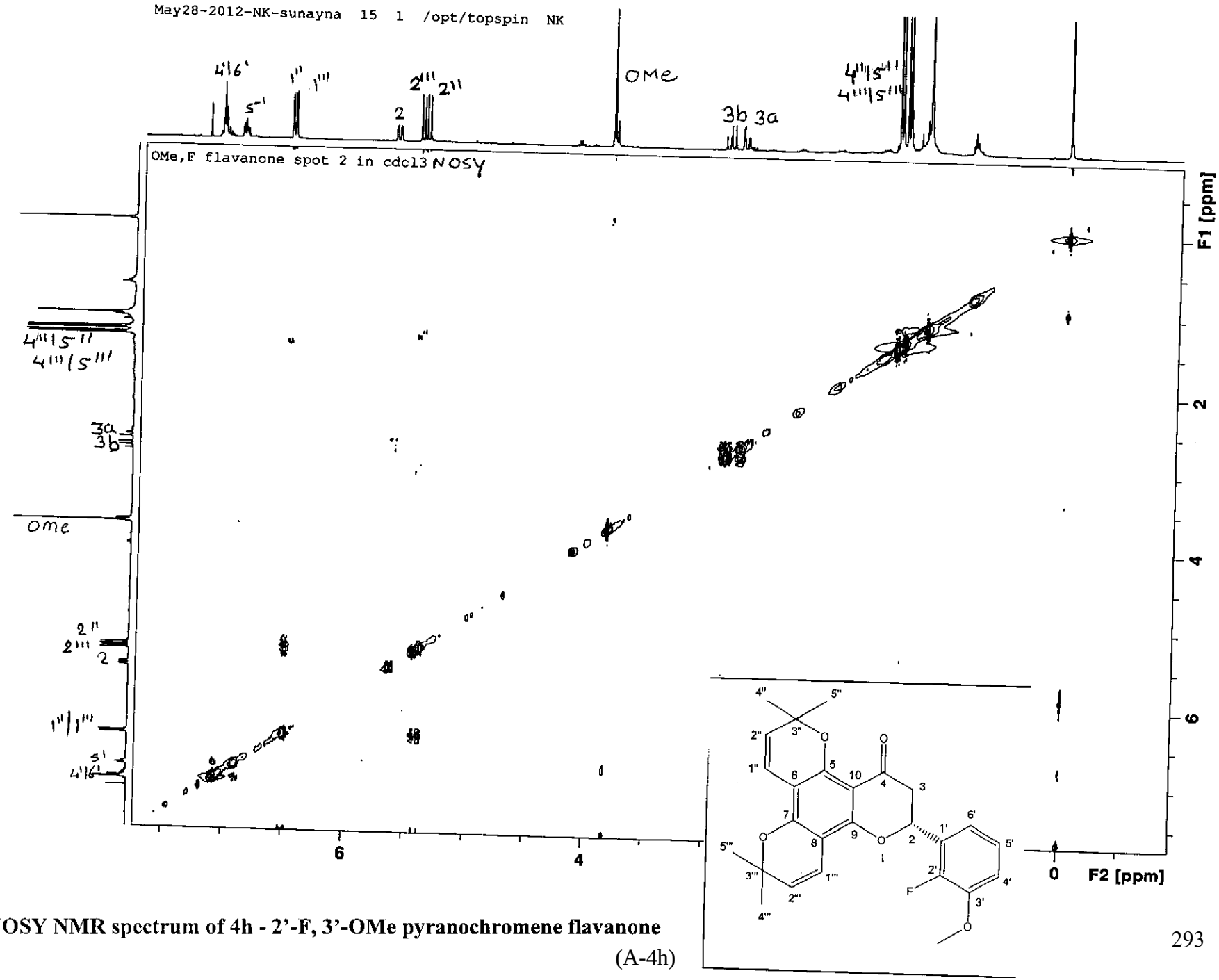
^{13}C NMR spectrum of 4h - 2'-F, 3'-OMe pyranochromene flavanone (A-4h)



^{13}C NMR spectrum of 4h - 2'-F, 3'-OMe pyranochromene flavanone (A-4h)

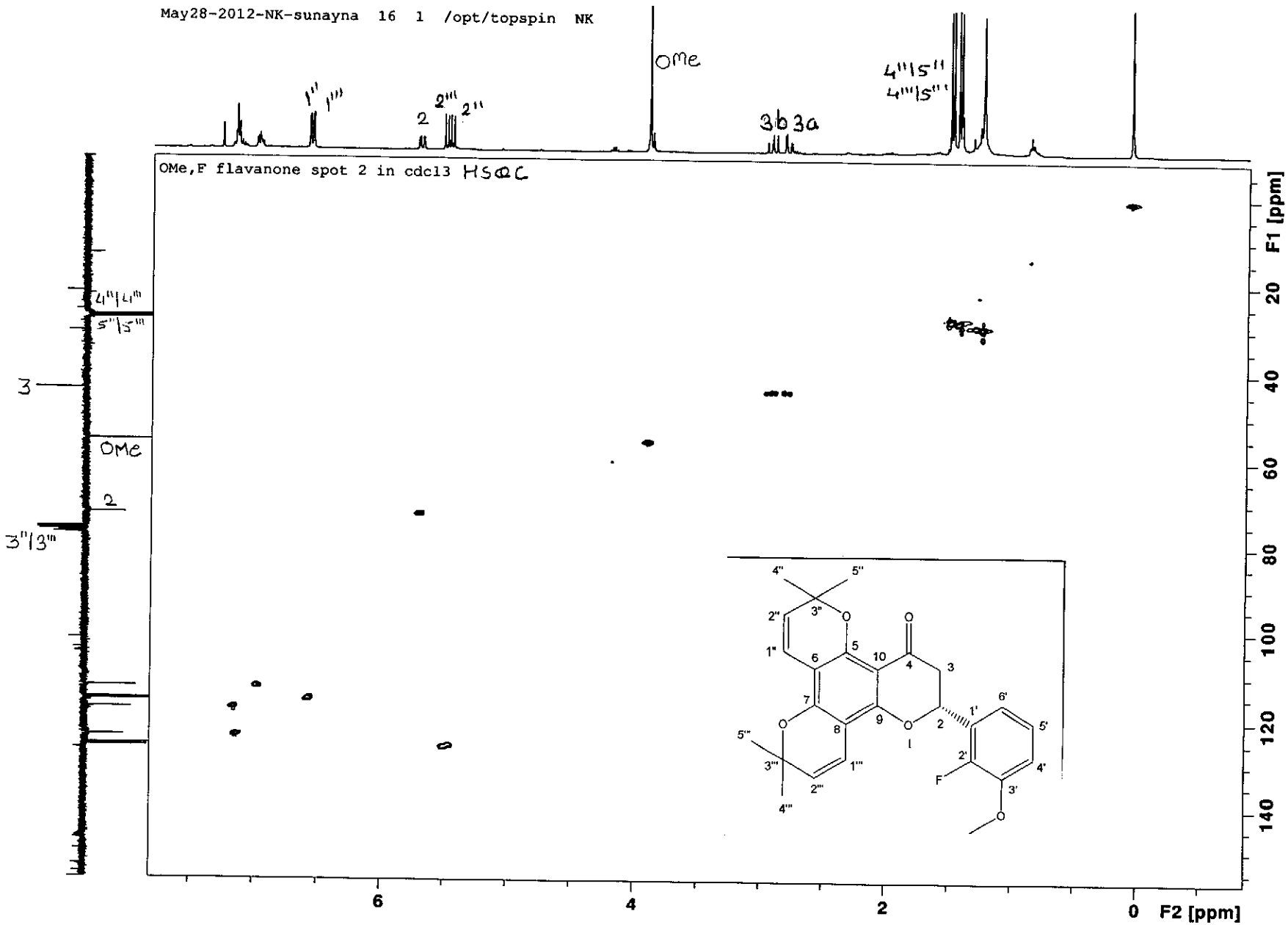


COSY NMR spectrum of 4h - 2'-F, 3'-OMe pyranochromene flavanone (A-4h)

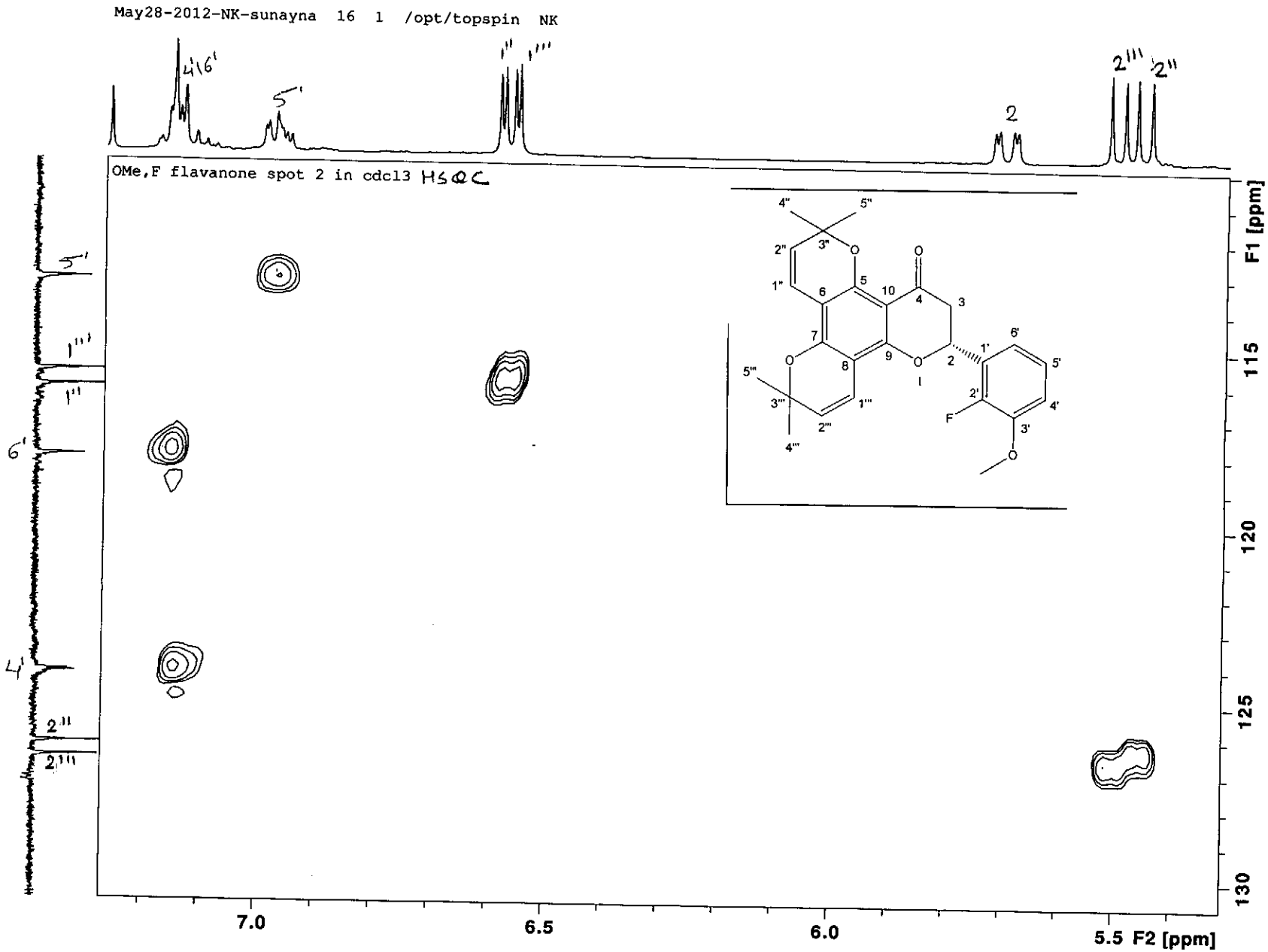


NOSY NMR spectrum of 4h - 2'-F, 3'-OMe pyranochromene flavanone

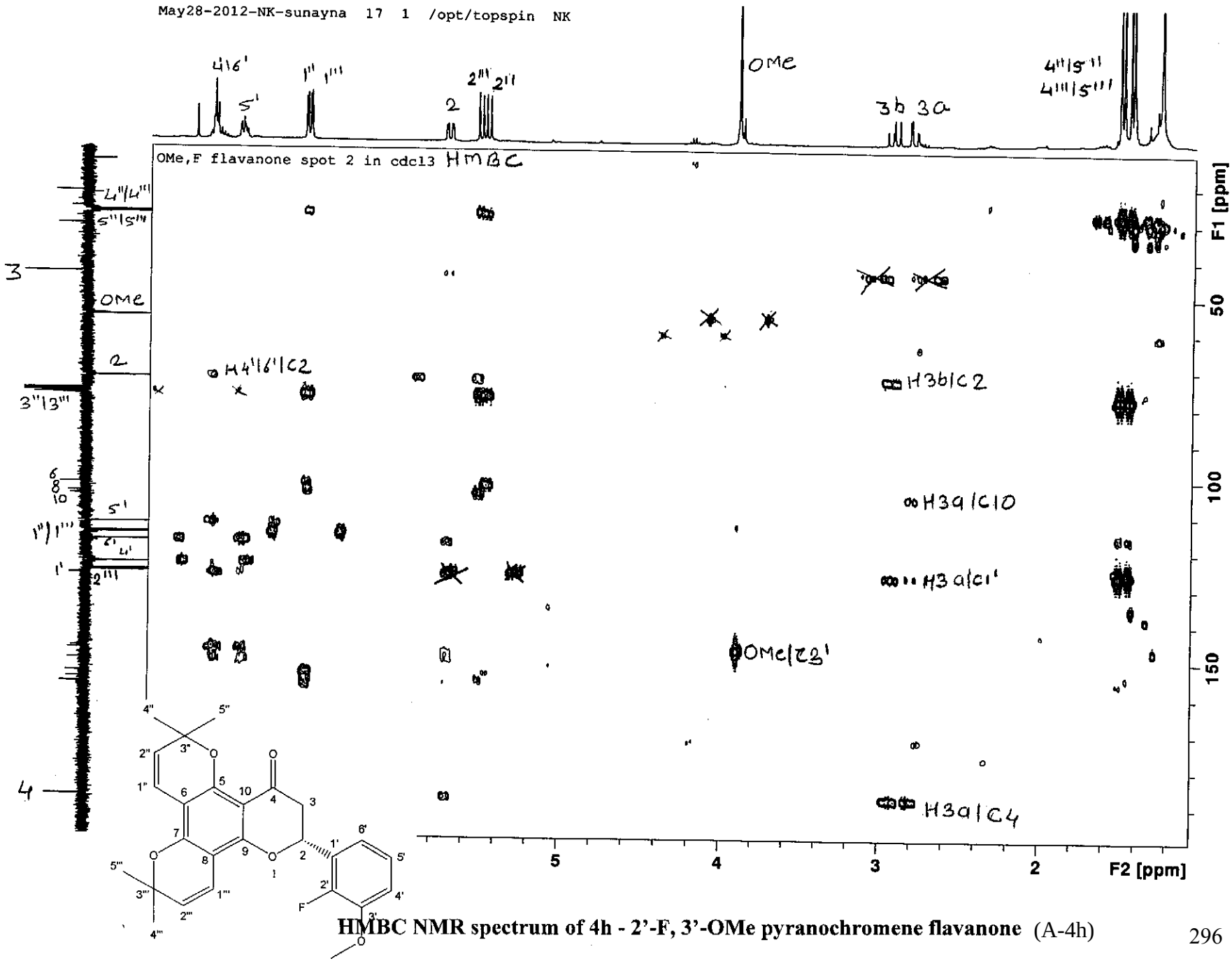
(A-4h)



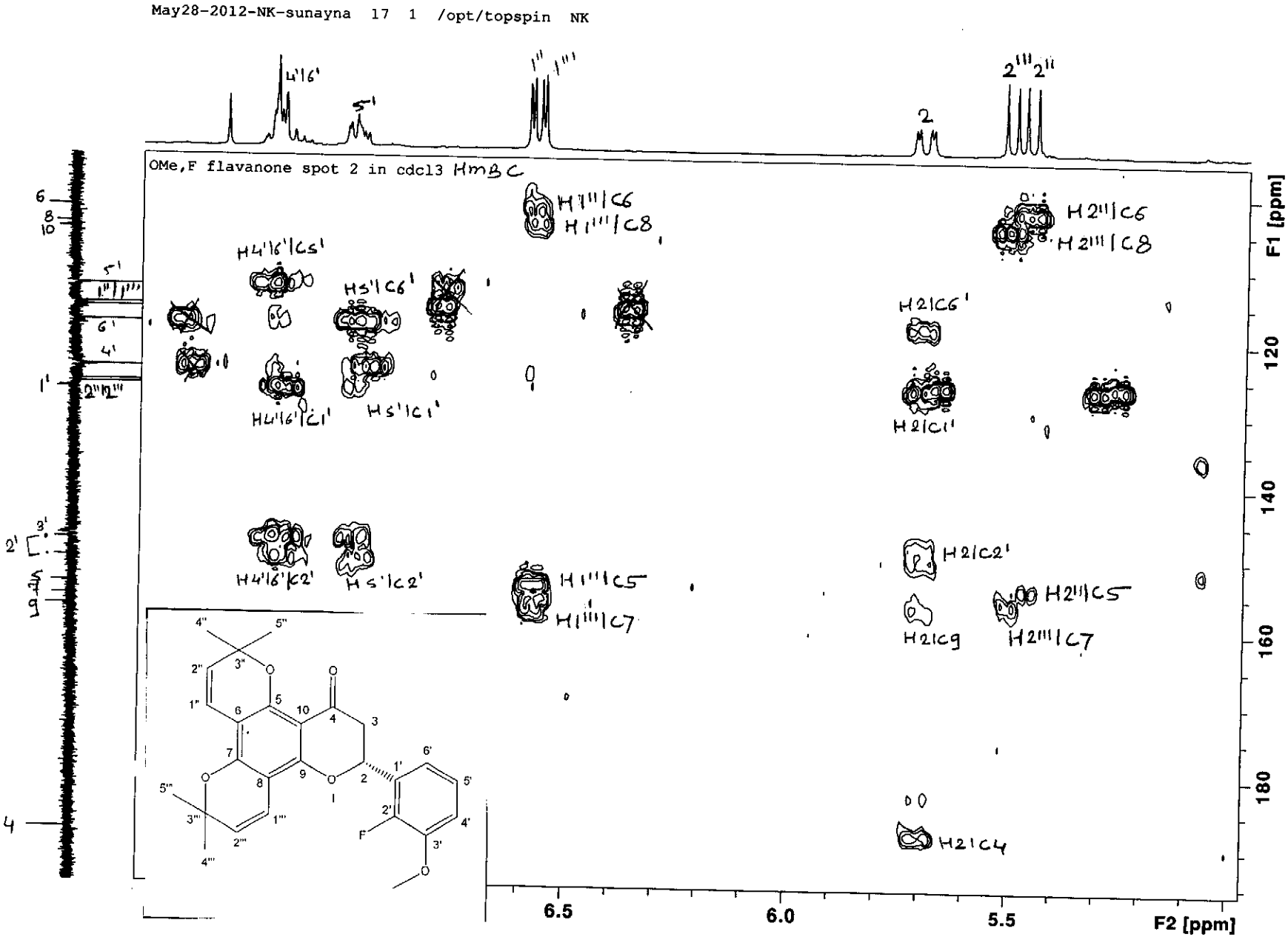
HSQC NMR spectrum of **4h - 2'-F, 3'-OMe pyranochromene flavanone** (A-4h)



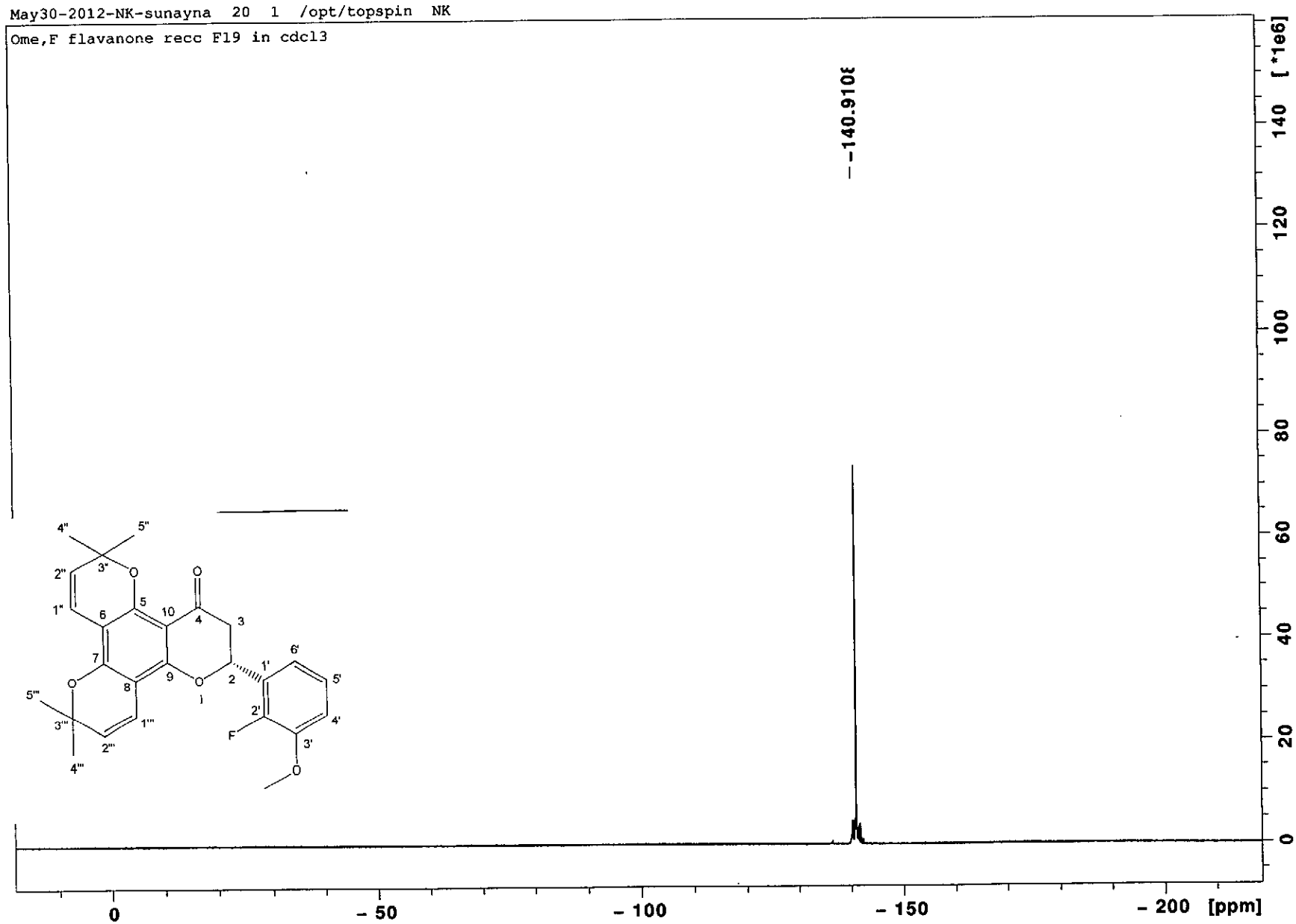
HSQC NMR spectrum of **4h - 2'-F, 3'-OMe pyranochromene flavanone** (A-4h)



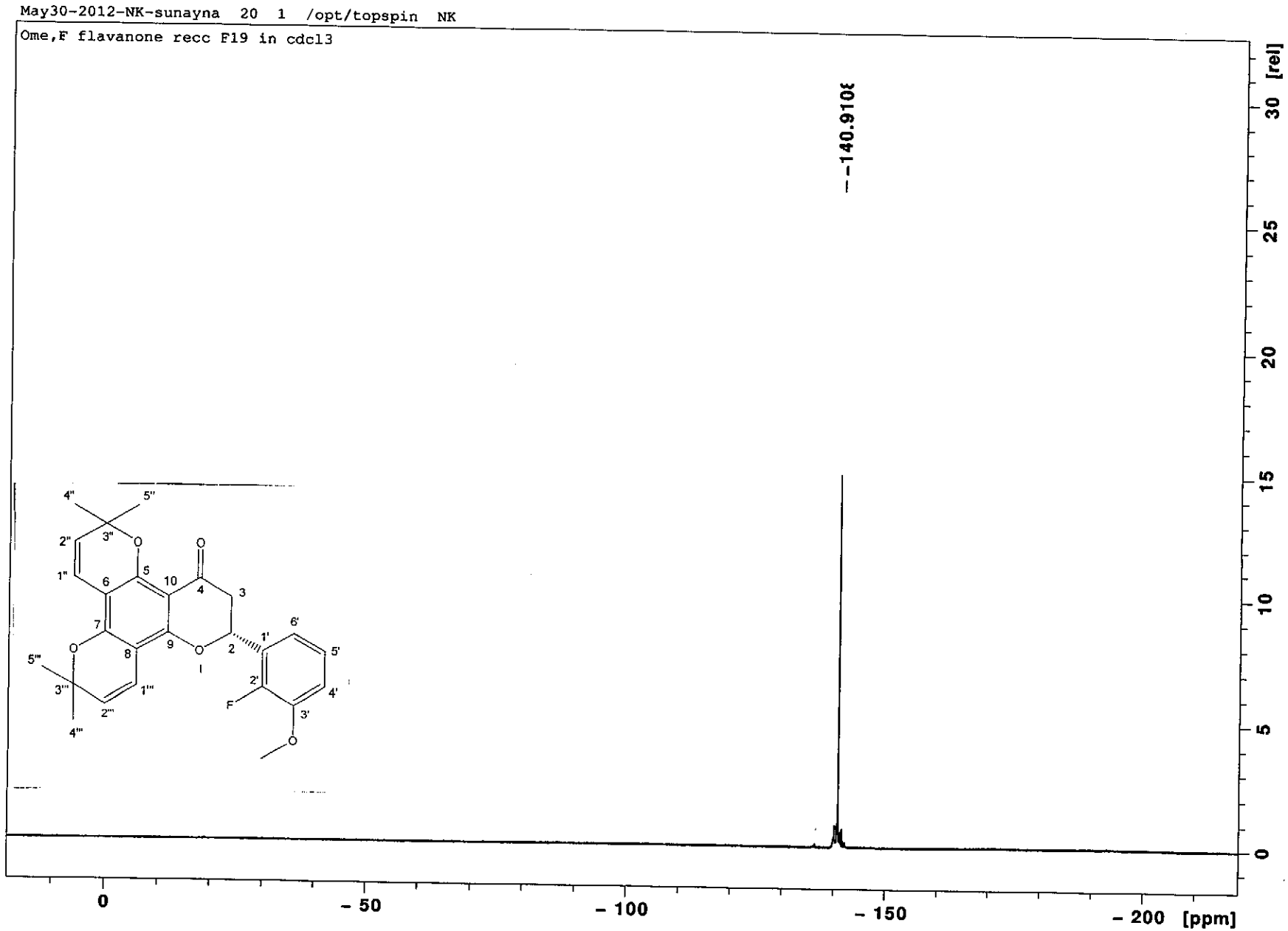
HMBC NMR spectrum of 4h - 2'-F, 3'-OMe pyranochromene flavanone (A-4h)



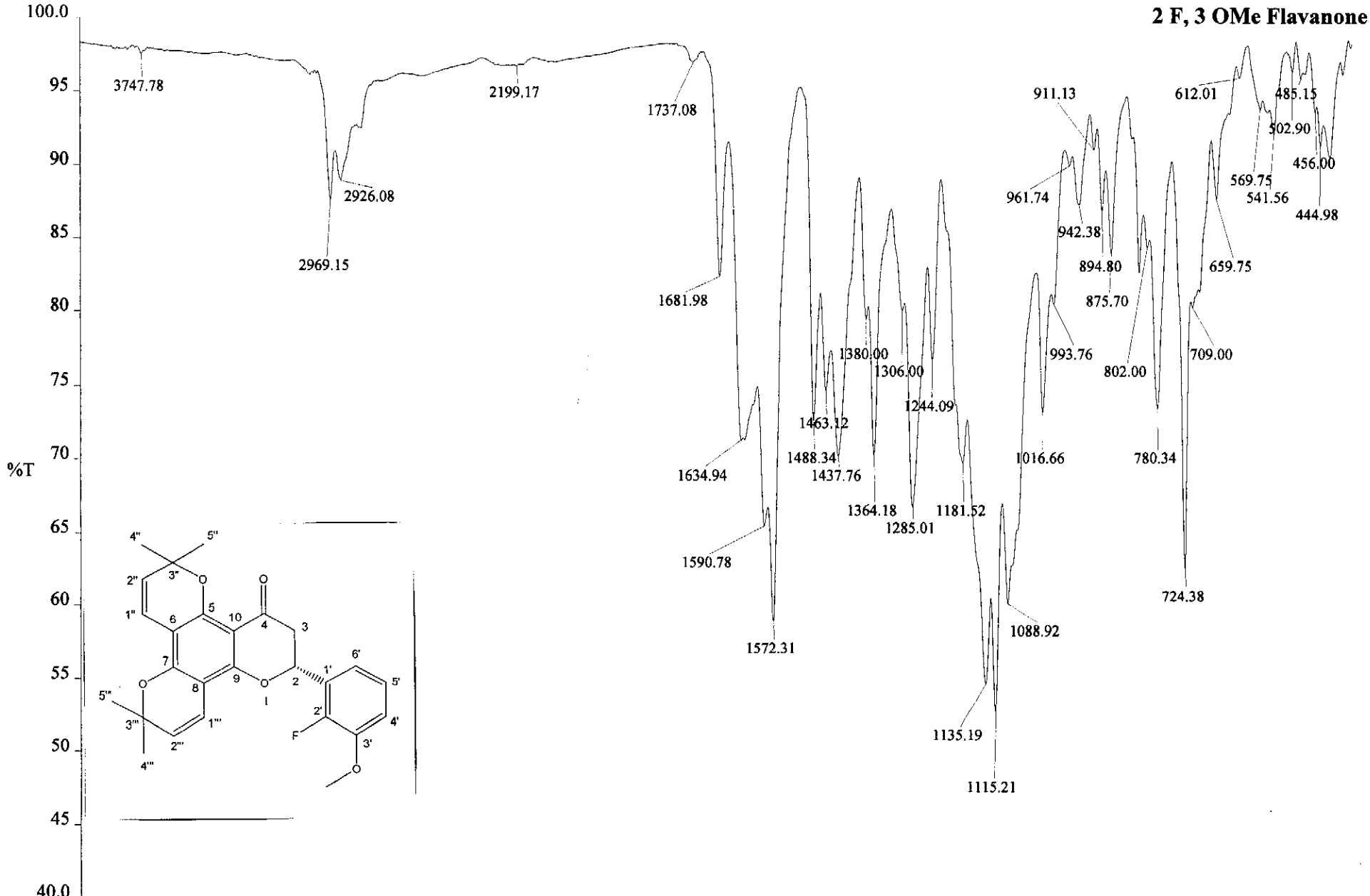
HMBC NMR spectrum of 4h - 2'-F, 3'-OMe pyranochromene flavanone (A-4h)



^{19}F NMR spectrum of 4h - 2'-F, 3'-OMe pyranochromene flavanone (A-4h)



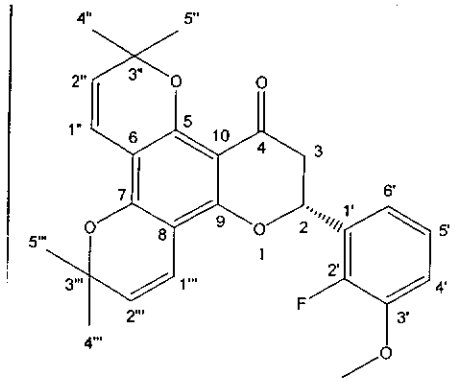
^{19}F NMR spectrum of 4h - 2'-F, 3'-OMe pyranochromene flavanone (A-4h)



IR spectrum of 4h - 2'-F, 3'-OMe pyranochromene flavanone (A-4h)

c:\pel_data\spectra\sunayna cha\2 f, 3 ome fla.002

Elemental Composition Report



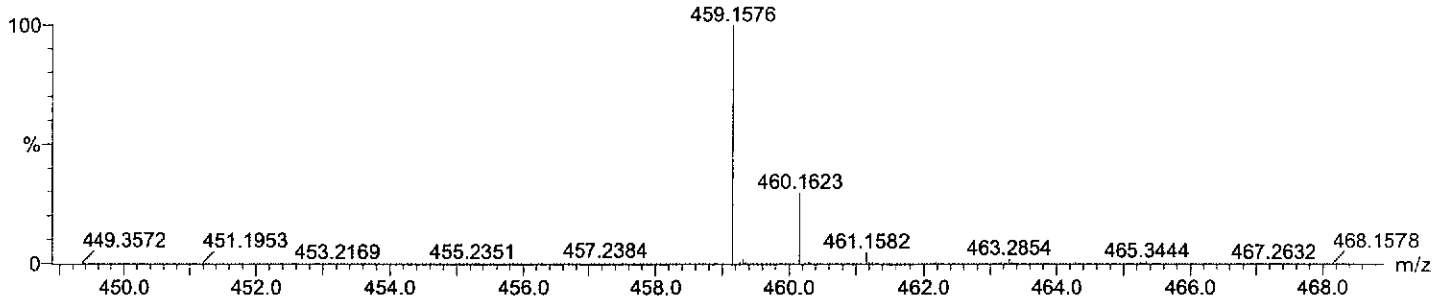
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
6 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-30 H: 25-30 O: 0-5 F: 0-1 Na: 0-1

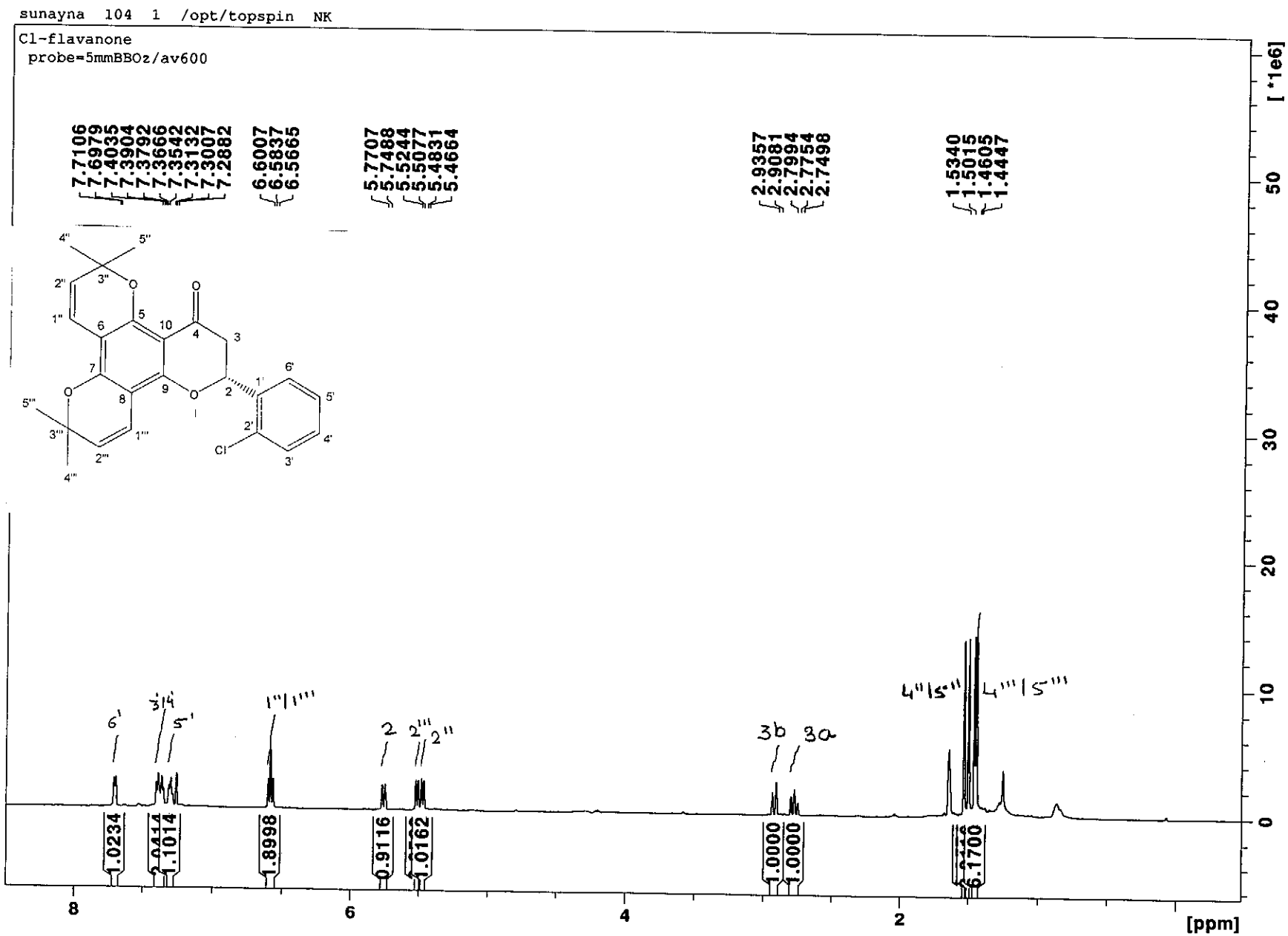
Dr. Nizam
8 10 (0.154) Cm (1:31)

TOF MS ES+
1.12e+004

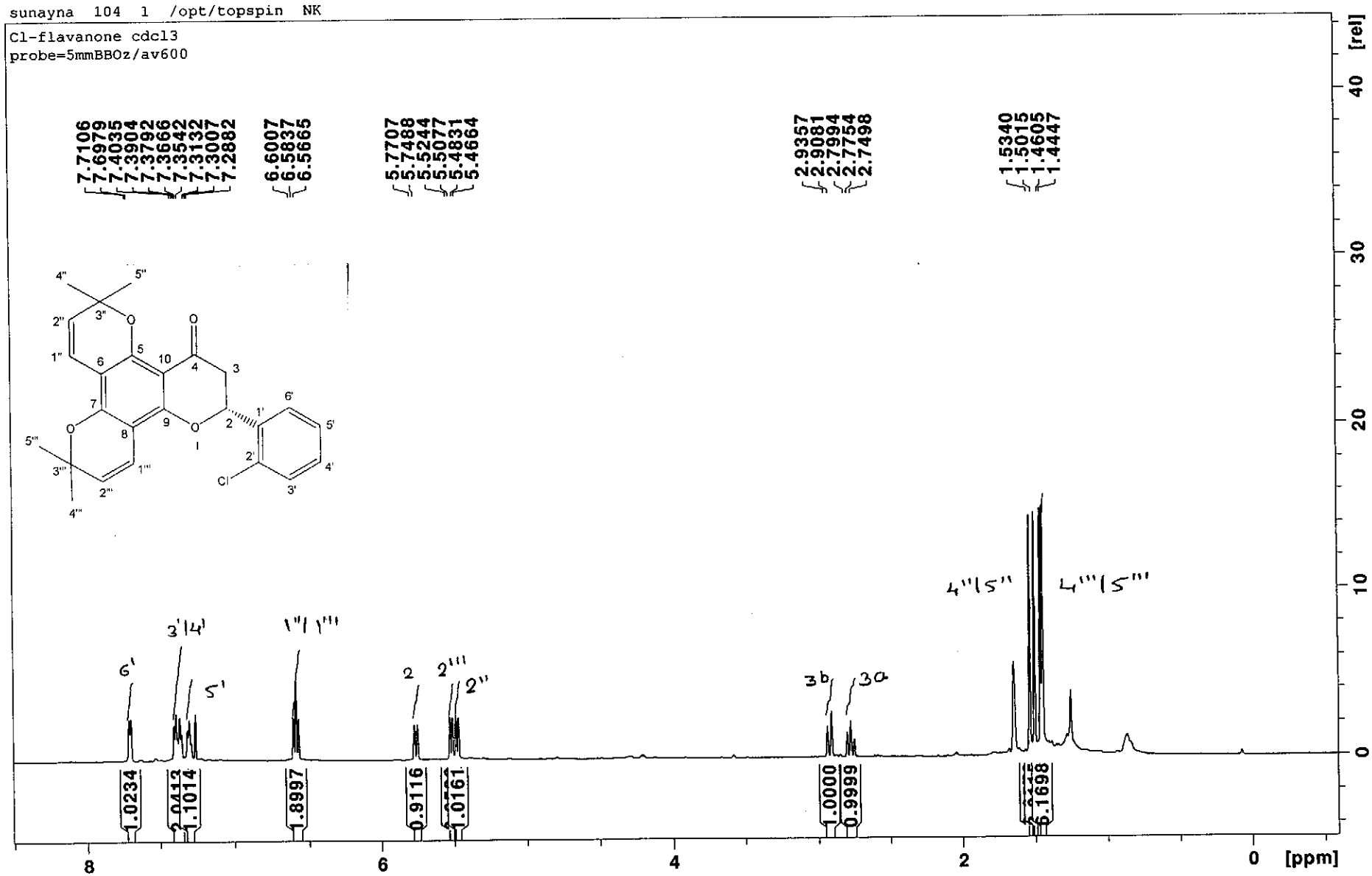


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
459.1576	459.1584	-0.8	-1.7	13.5	350.9	0.0	C26 H25 O5 F Na

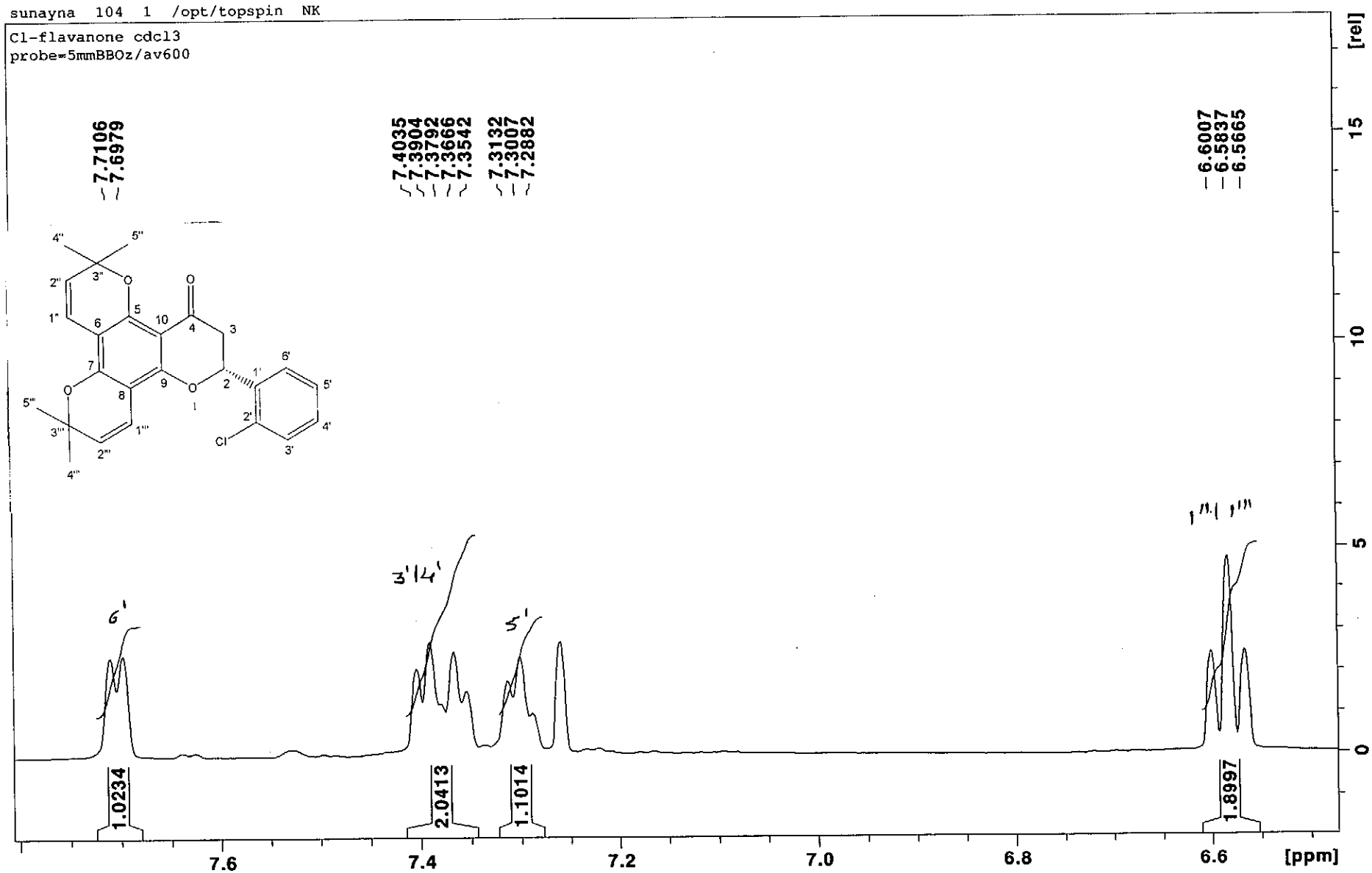
HRMS of 4h – 2'-F, 3'-OMe pyranochromene flavanone (A-4h)



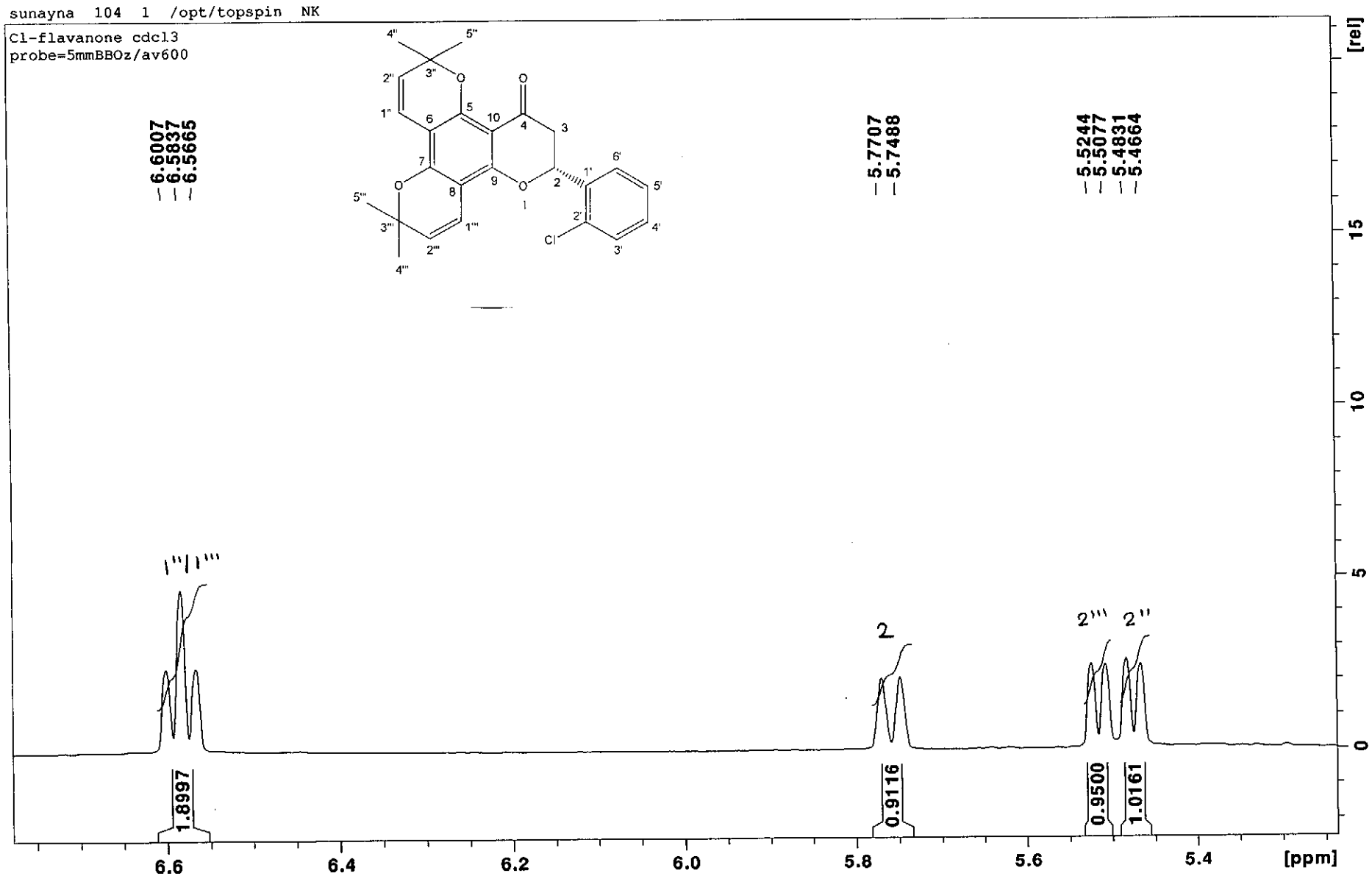
1H NMR spectrum of 2'-chloropyranochromene flavanone (A-4i)



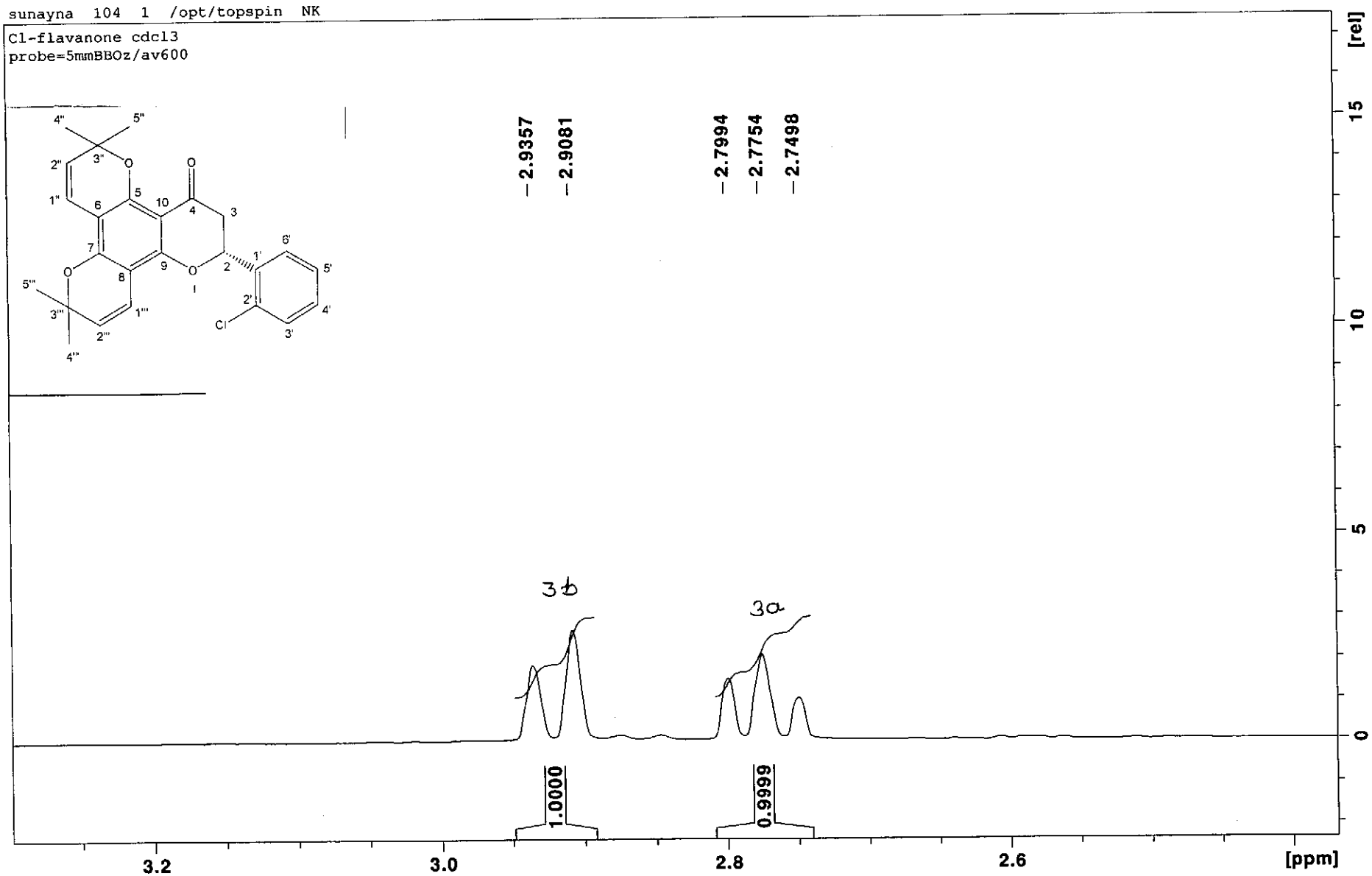
1H NMR spectrum of 2'-chloropyranochromene flavanone (A-4i)



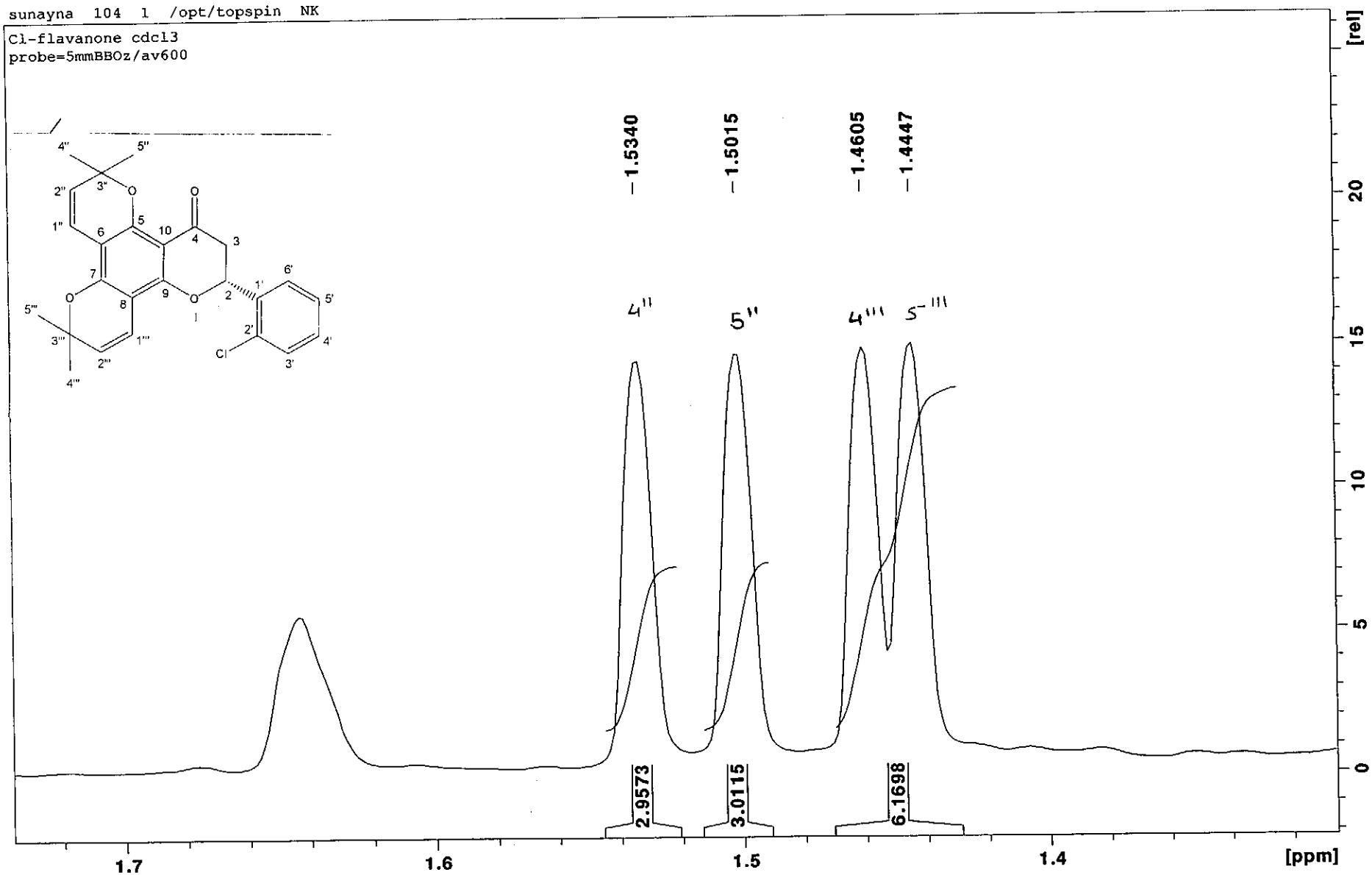
Expanded 1H NMR spectrum of 2'-chloropyranochromene flavanone (A-4i)



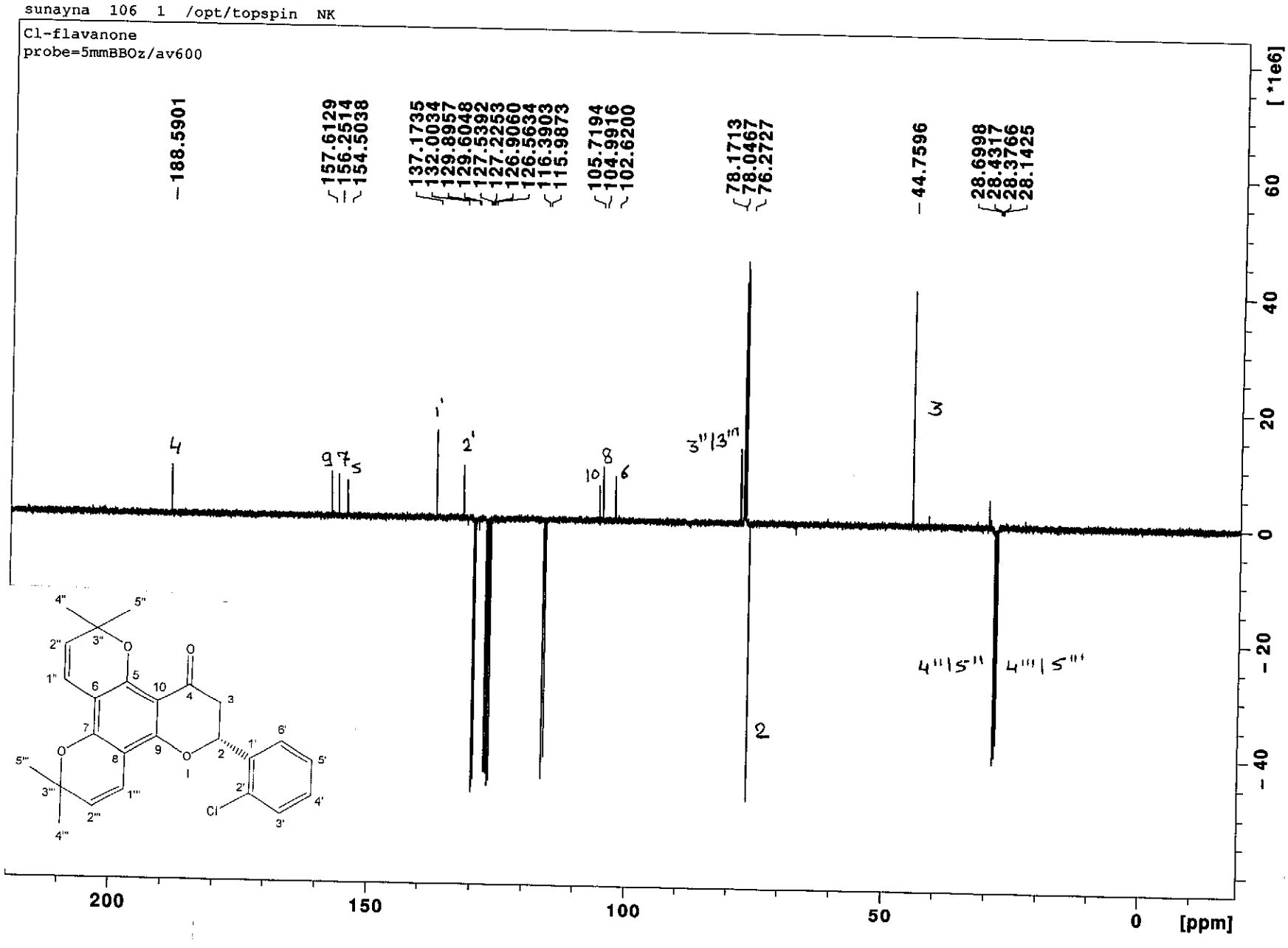
Expanded 1H NMR spectrum of 2'-chloropyranochromene flavanone (A-4i)



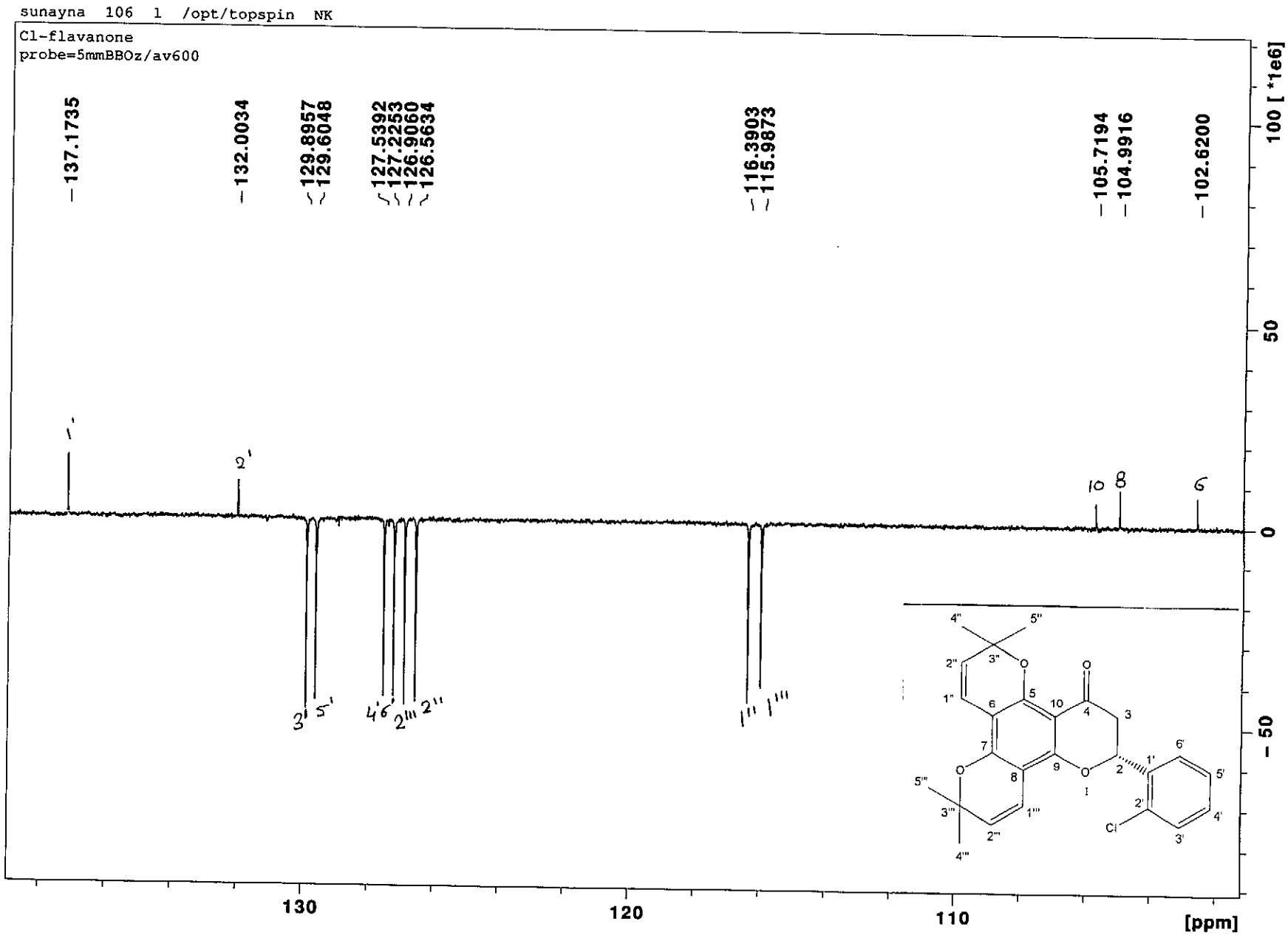
Expanded 1H NMR spectrum of 2'-chloropyranochromene flavanone (A-4i)



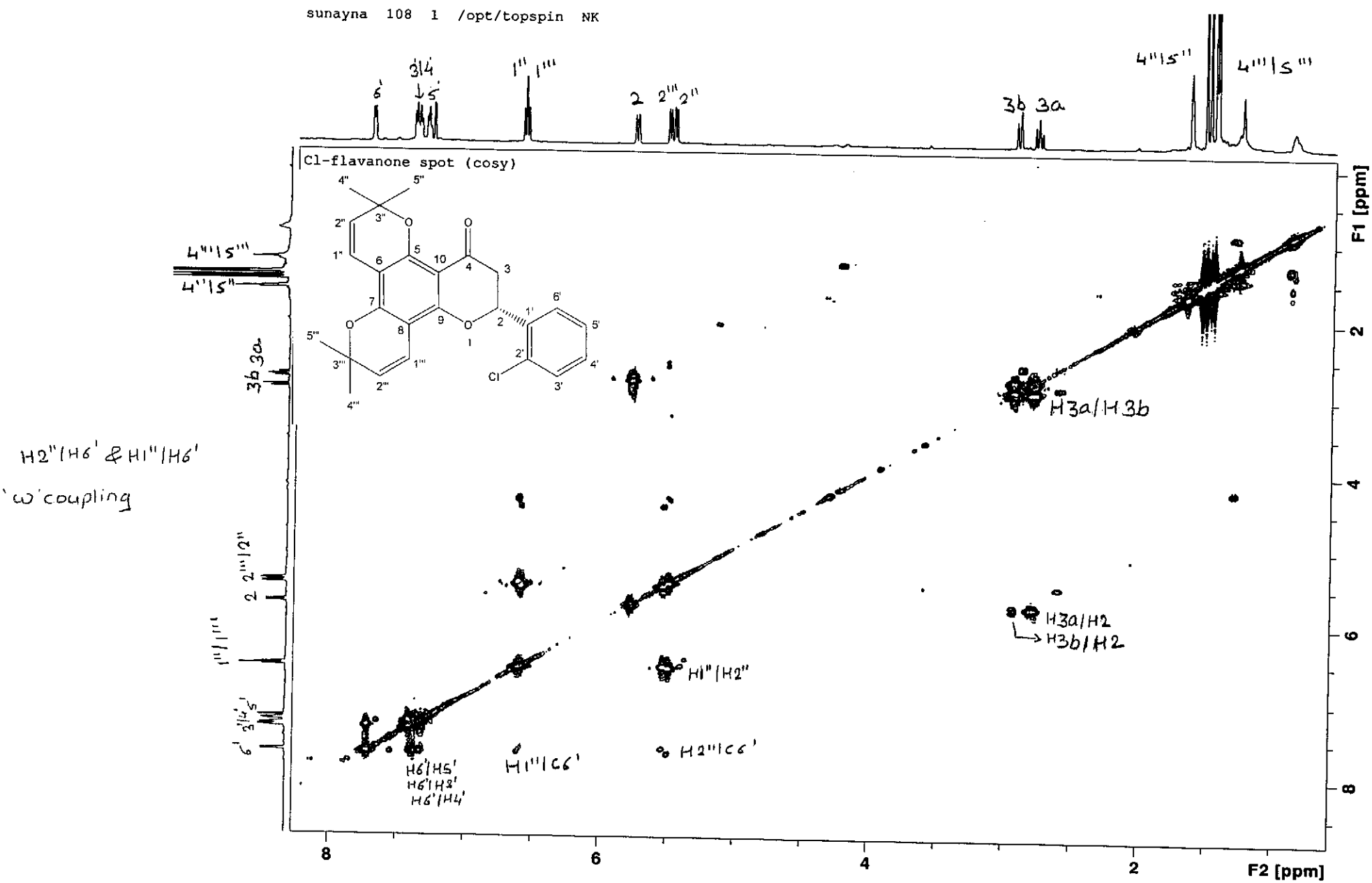
Expanded 1H NMR spectrum of 2'-chloropyranochromene flavanone (A-4i)



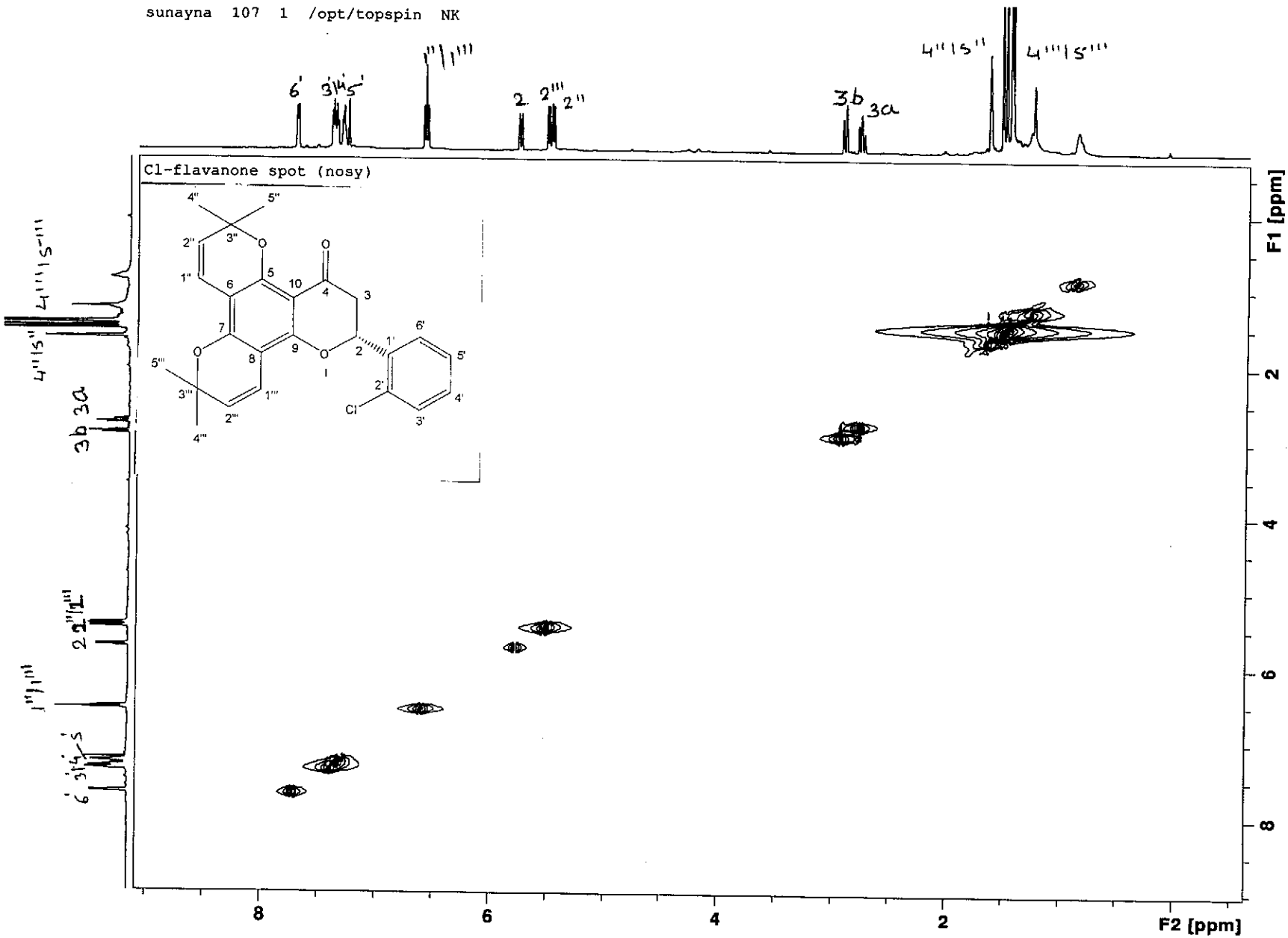
13C NMR spectrum of 2'-chloropyranochromene flavanone (A-4i)



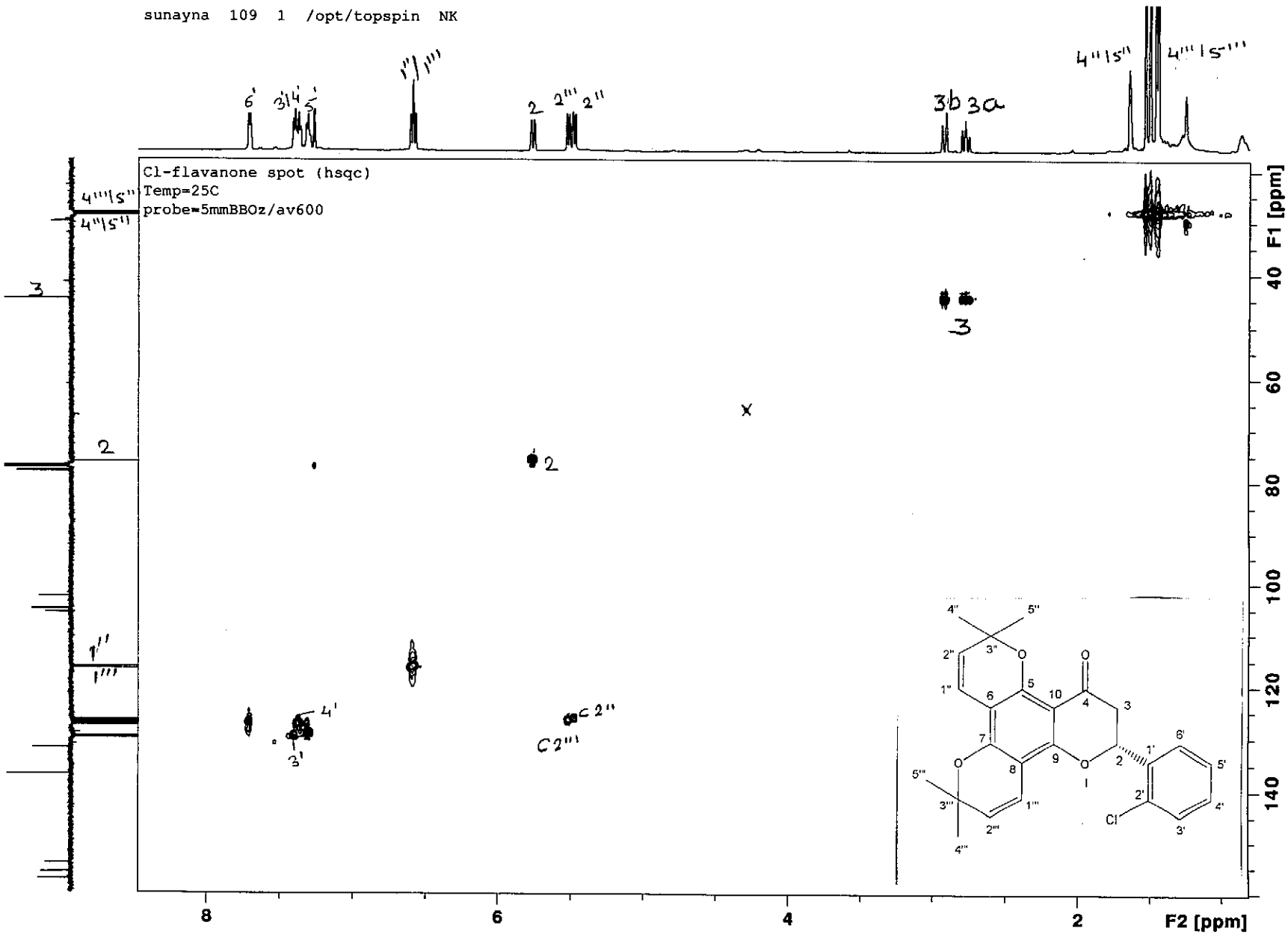
Expanded 13C NMR spectrum of 2'-chloropyranochromene flavanone (A-4i)



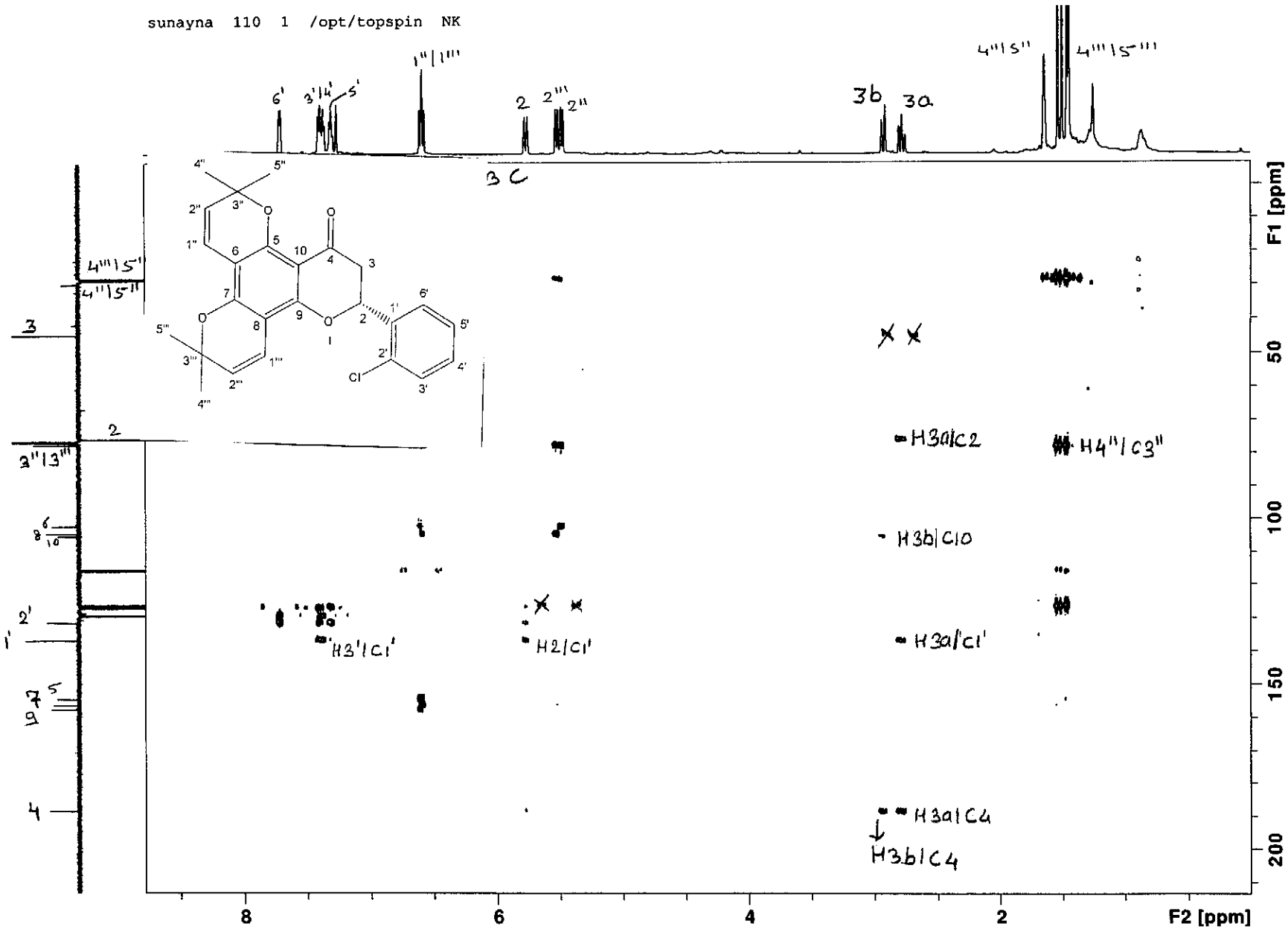
COSY spectrum of 2'-chloropyranochromene flavanone (A-4i)



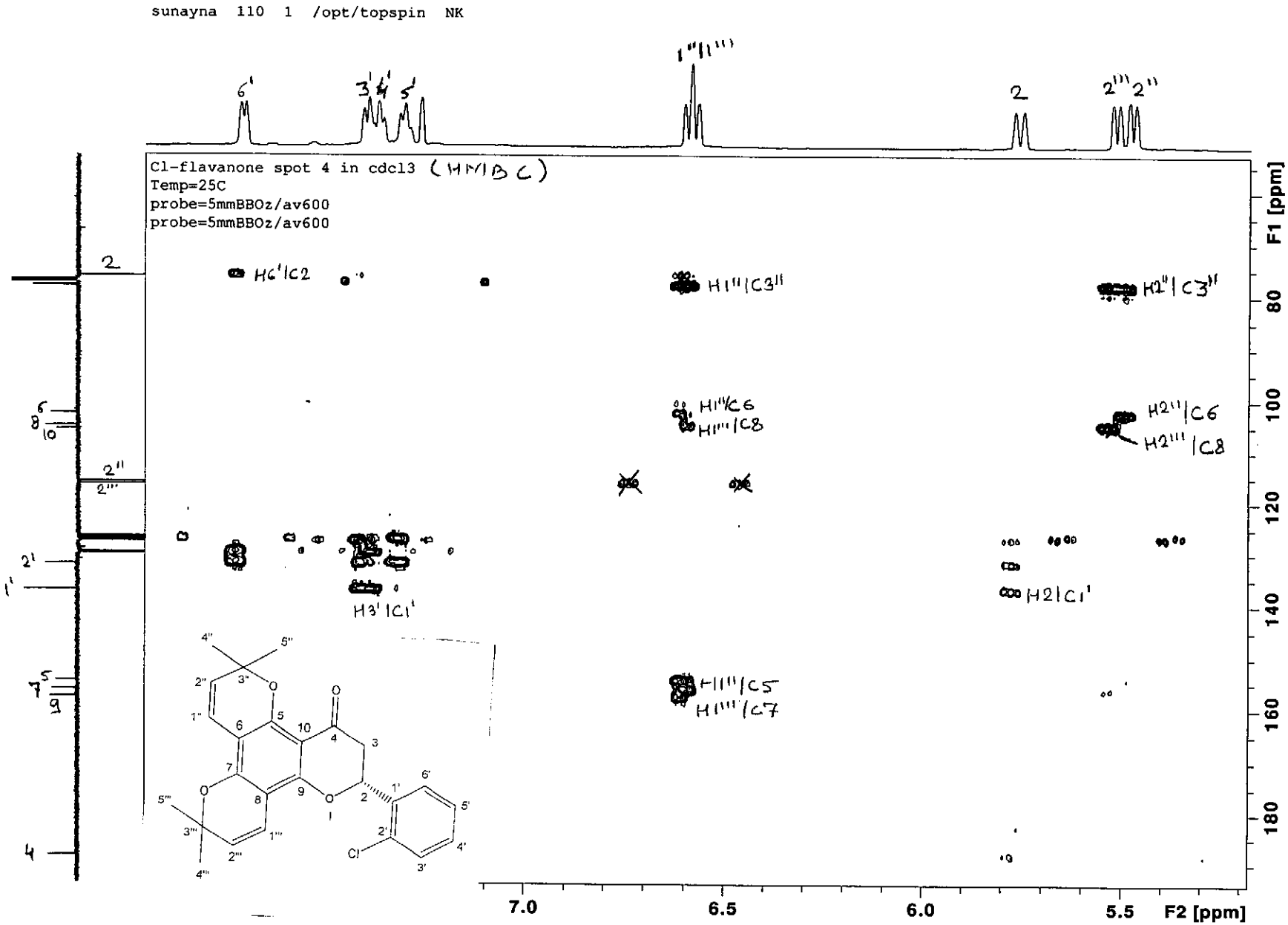
NOESY spectrum of 2'-chloropyranochromene flavanone (A-4i)



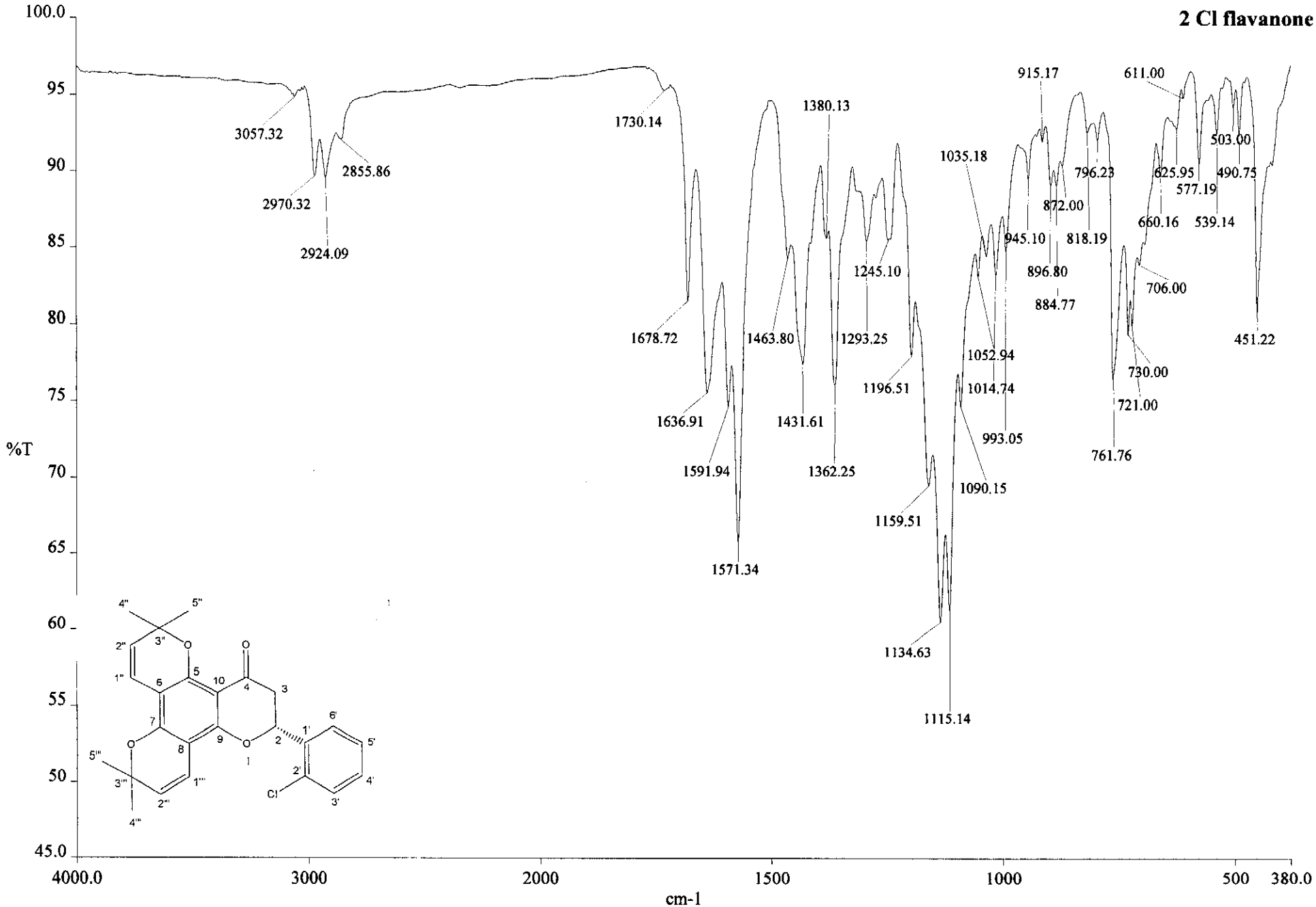
HSQC spectrum of 2'-chloropyranochromene flavanone (A-4i)



HMBC spectrum of 2'-chloropyranochromene flavanone (A-4i)

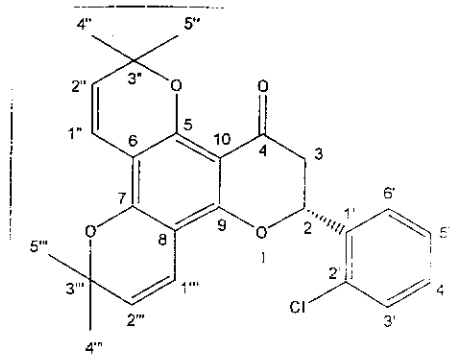


Expanded HMBC spectrum of 2'chloropyranochromene flavanone (A-4i)



Infrared spectrum of 2'chloropyranochromene flavanone (A-4i)

Elemental Composition Report

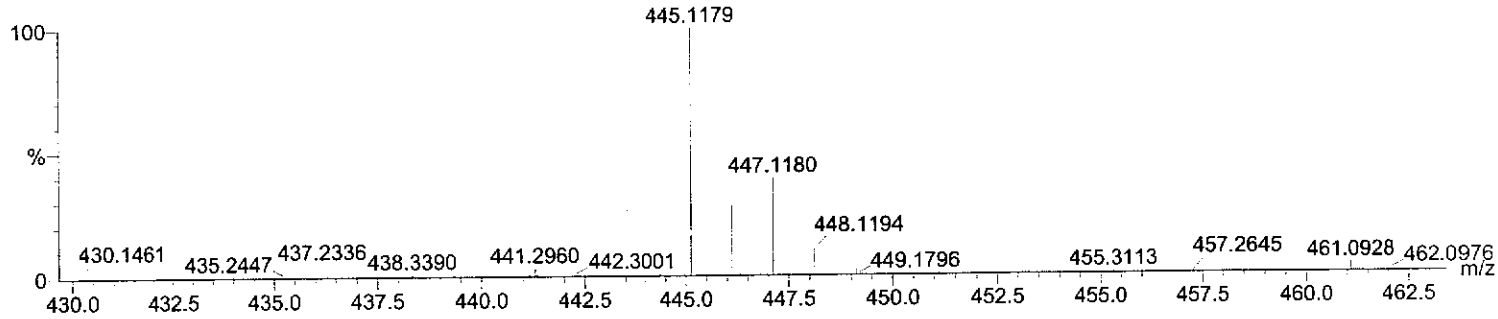


Single Mass Analysis
Tolerance = 50000.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
9 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-26 H: 20-25 O: 0-5 Na: 0-1 Cl: 0-1

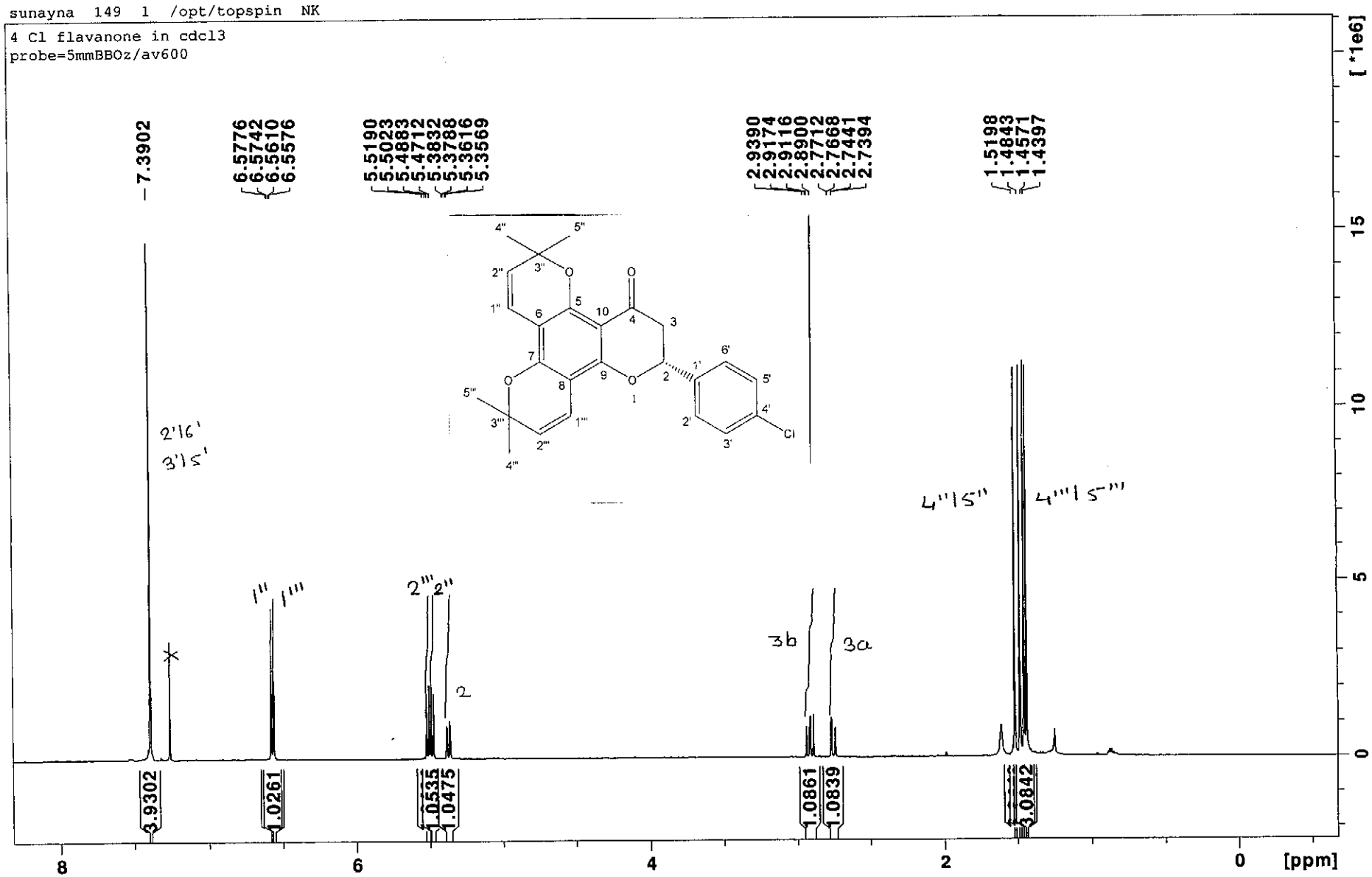
Dr. Nizam
18 24 (0.409) Cm (1:30)

TOF MS ES+
1.83e+004

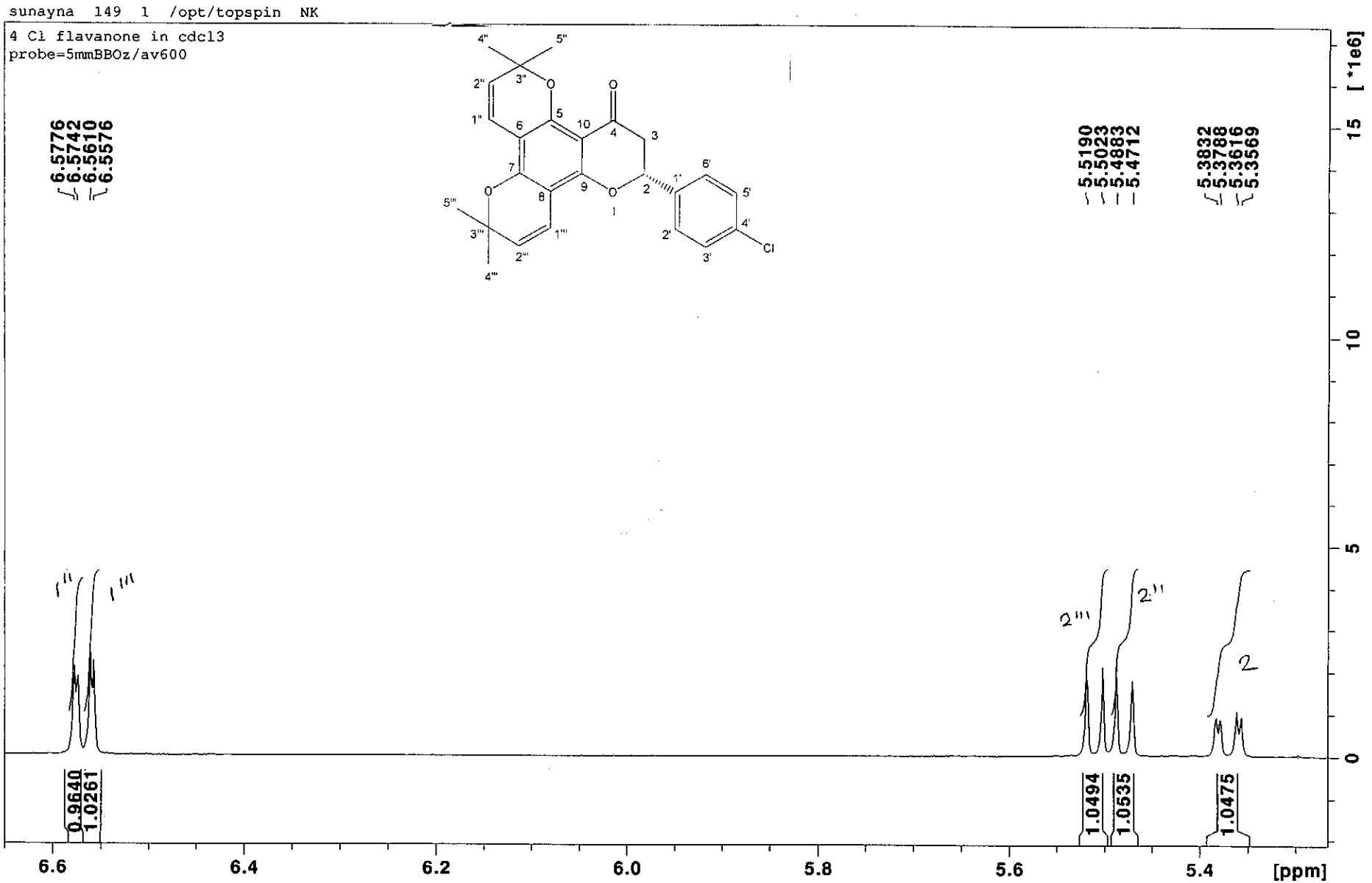


Minimum:				-1.5			
Maximum:		5.0	50000.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
445.1179	445.1183	-0.4	-0.9	13.5	379.4	0.0	C25 H23 O4 Na Cl

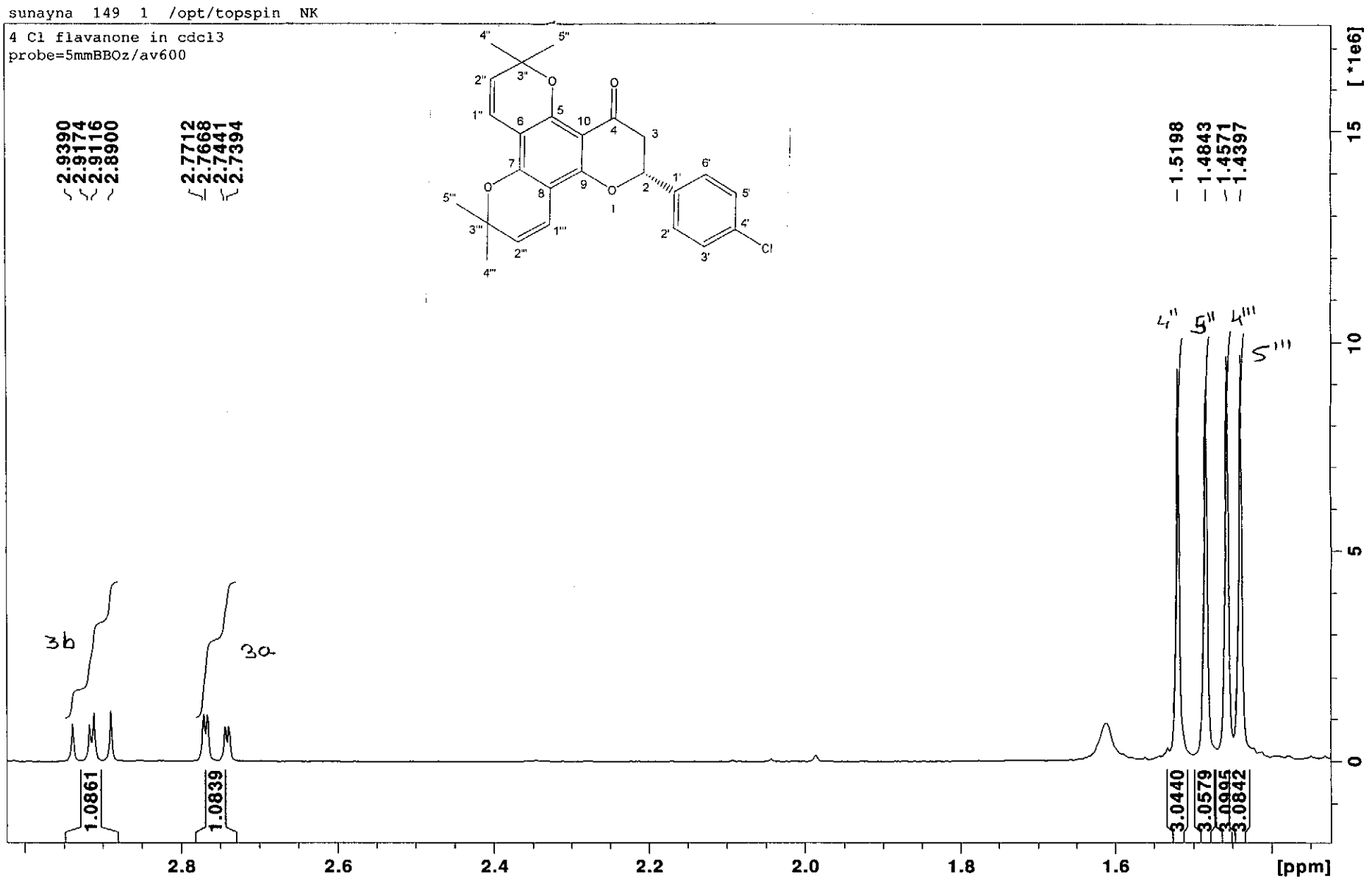
HRMS spectrum of 2'-chloropyranochromene flavanone (A-4i)



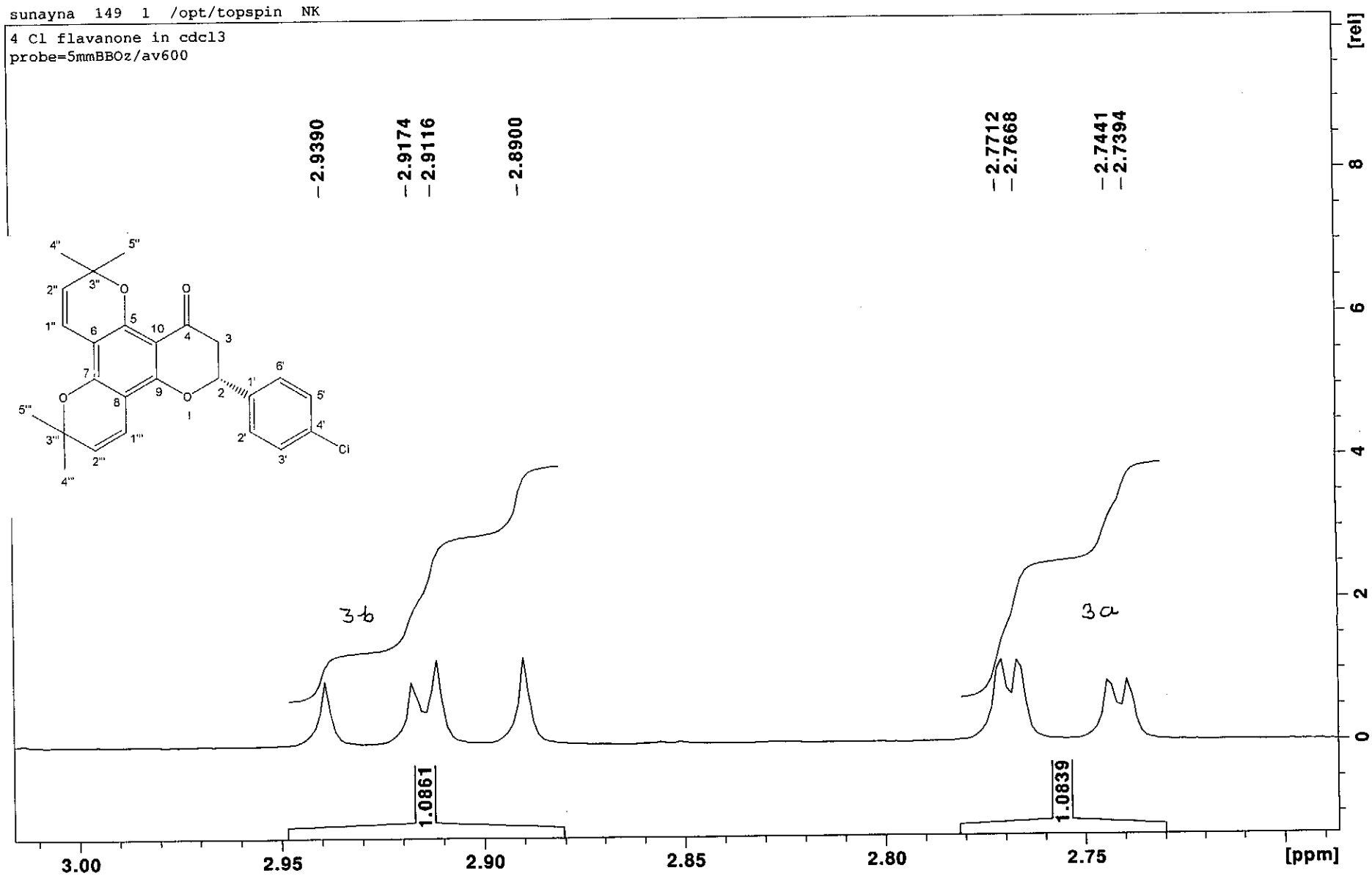
1H NMR spectrum of 4'-chloropyranochromene flavanone (A-4j)



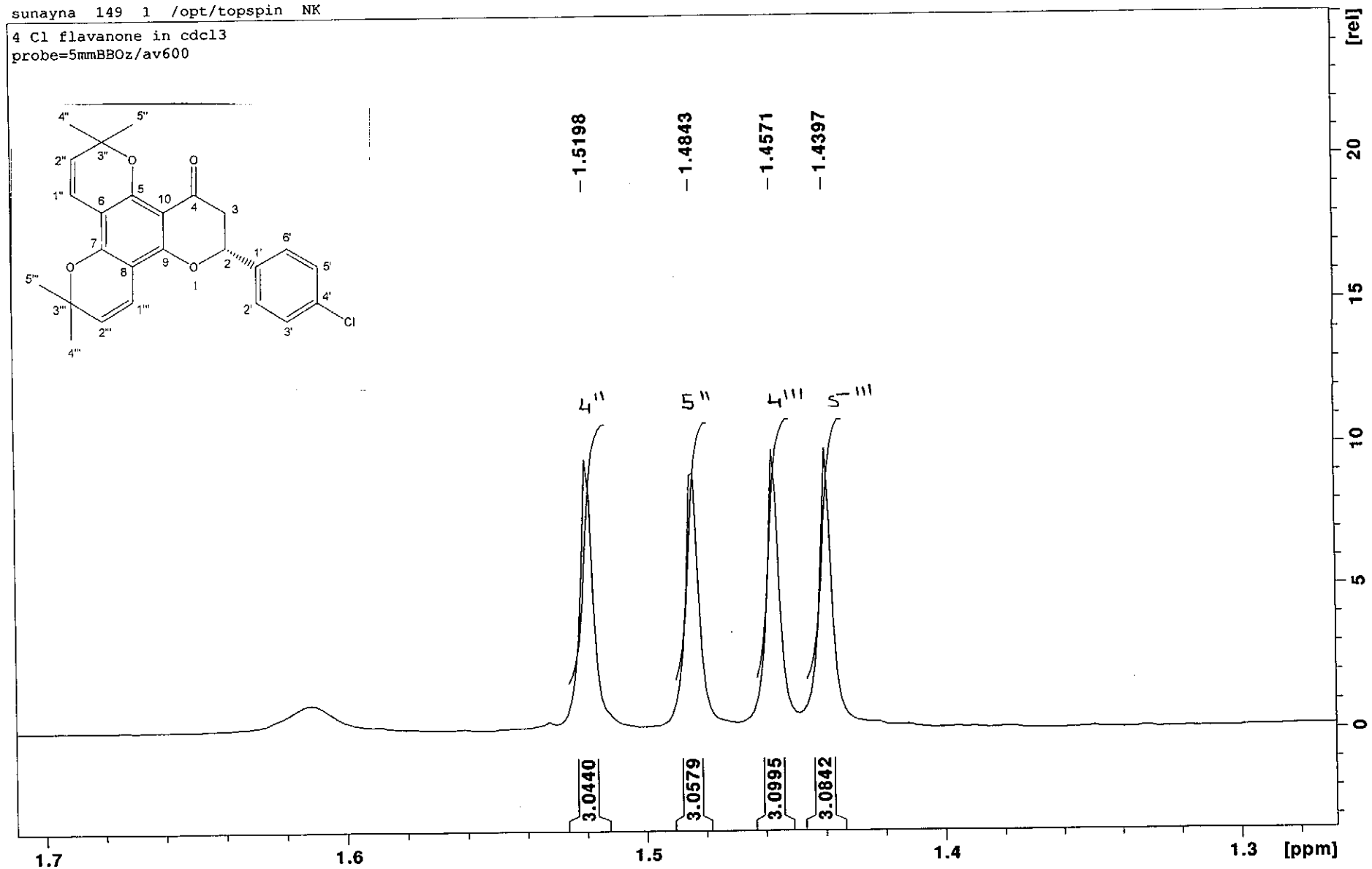
Expanded 1H NMR spectrum of 4'-chloropyranochromene flavanone (A-4i)



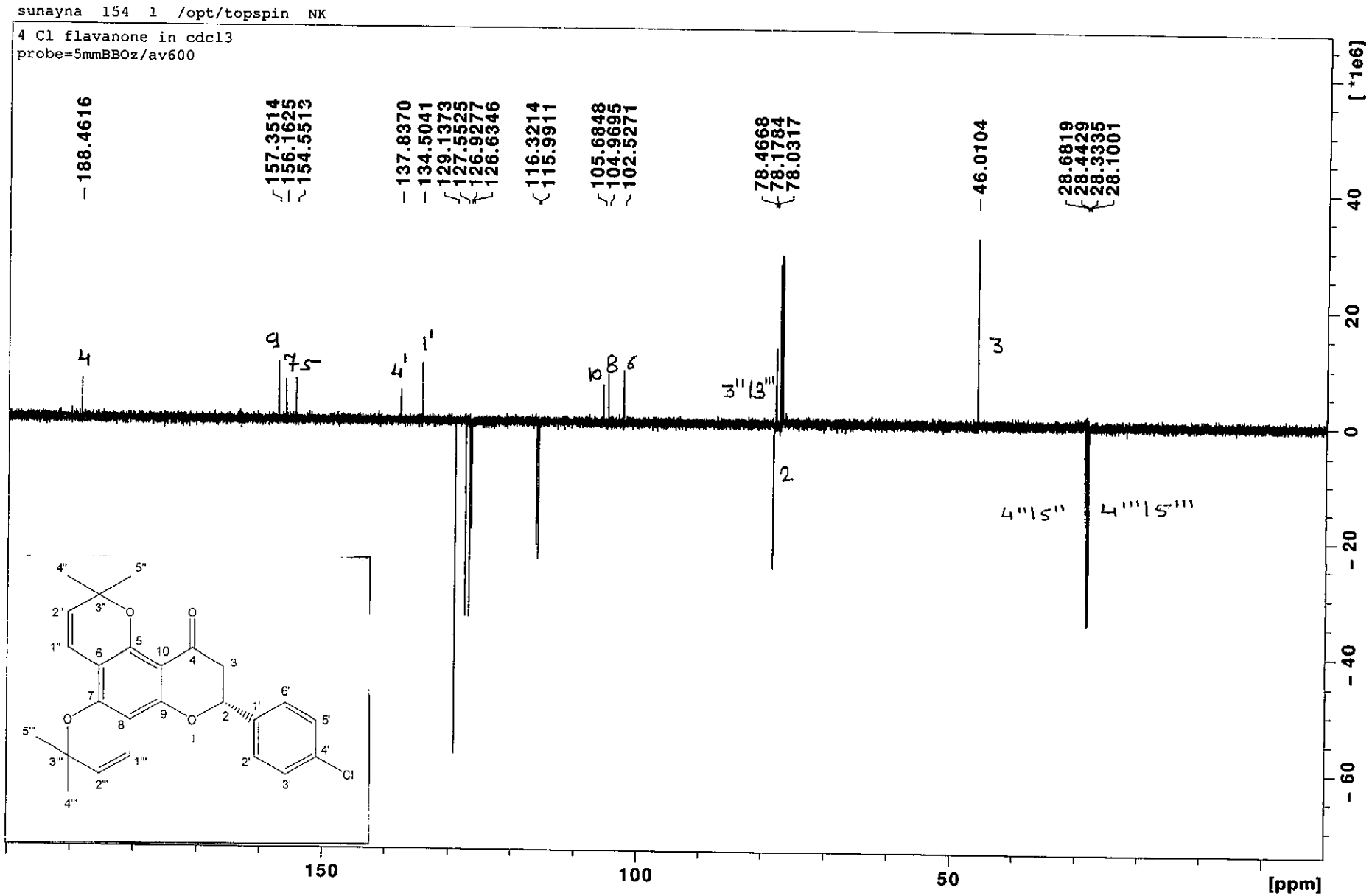
Expanded 1H NMR spectrum of 4'-chloropyranochromene flavanone (A-4j)



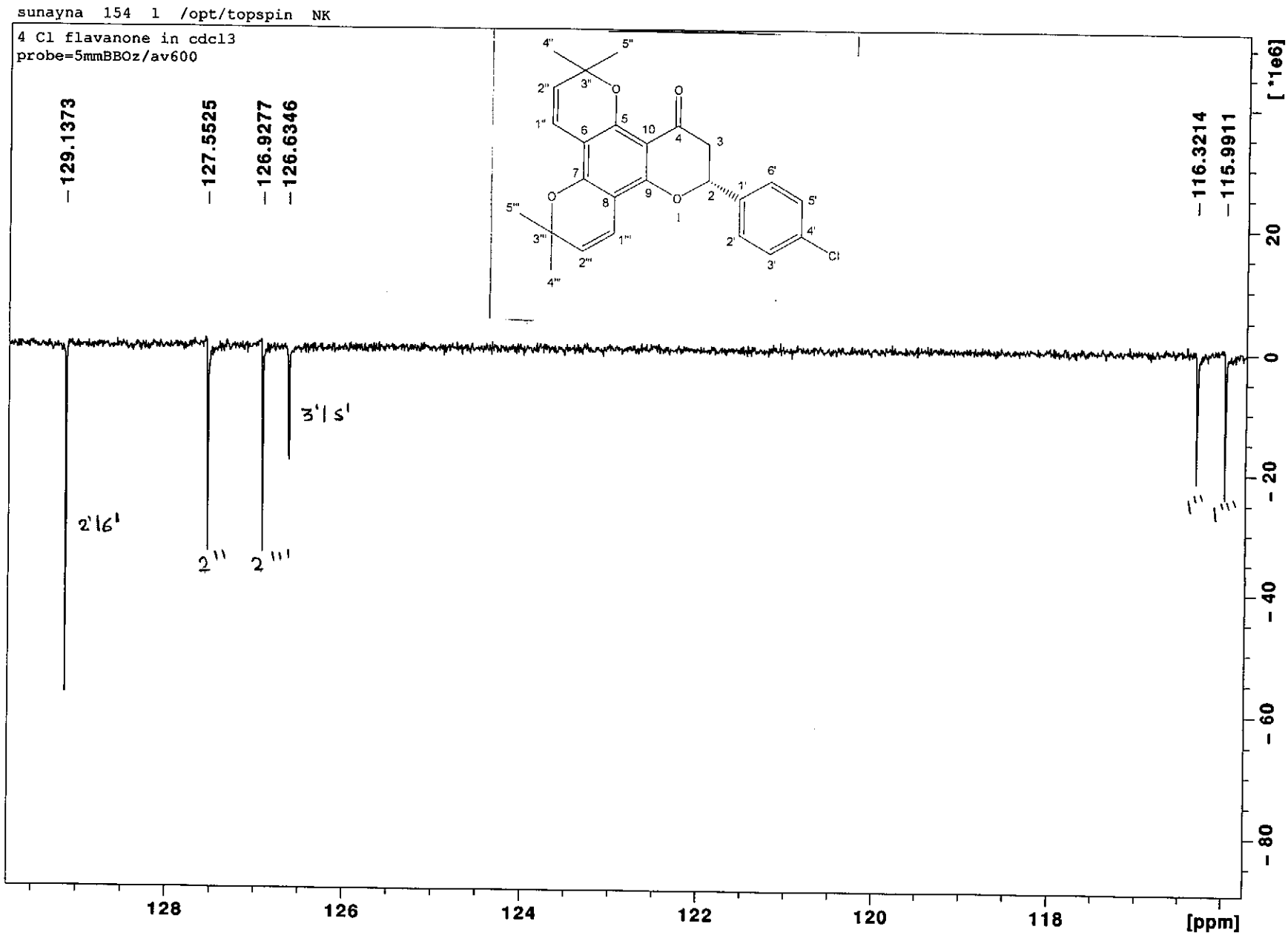
Expanded 1H NMR spectrum of 4'-chloropyranochromene flavanone (A-4j)



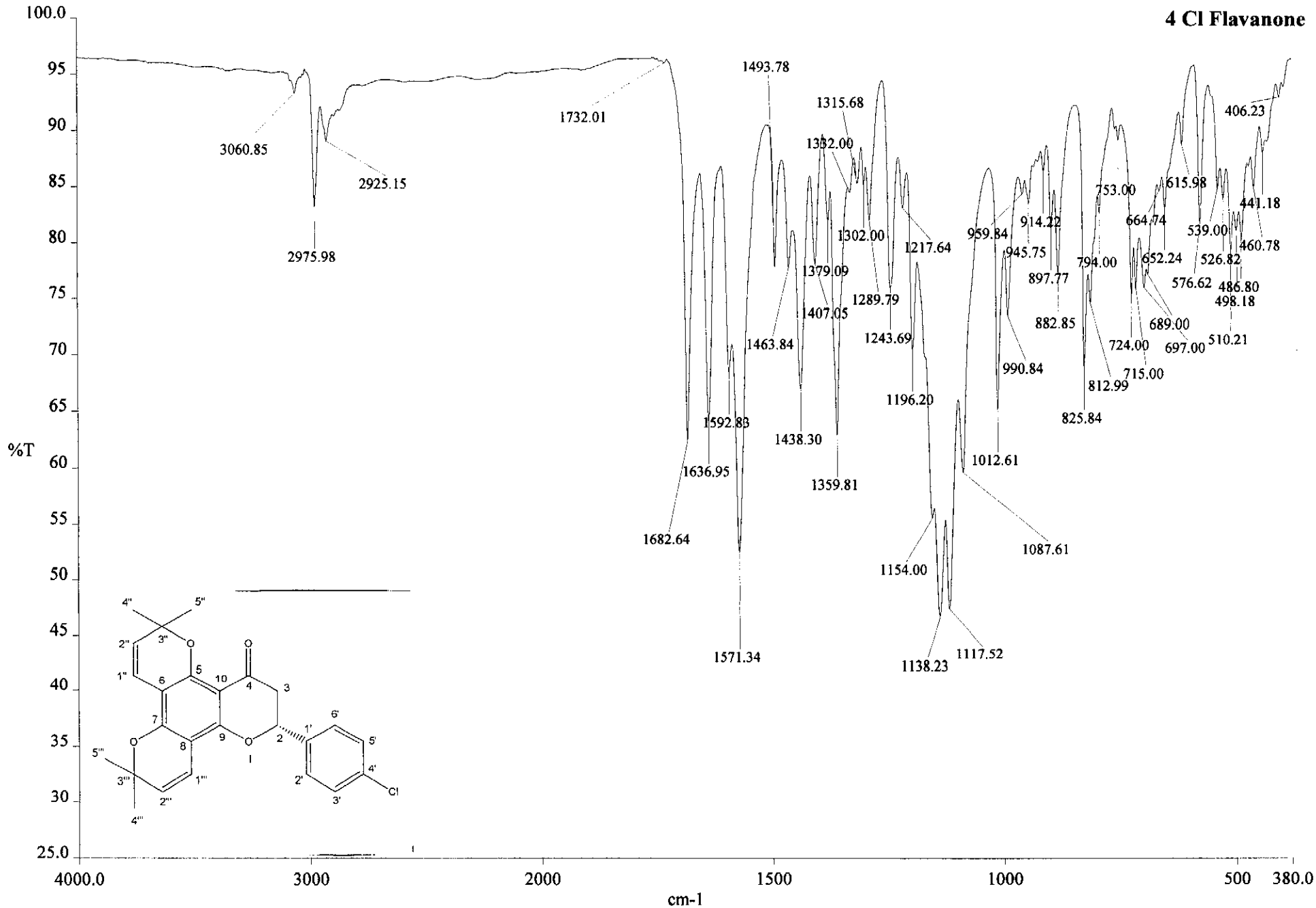
Expanded 1H NMR spectrum of 4'-chloropyranochromene flavanone (A-4j)



13C NMR spectrum of 4'-chloropyranochromene flavanone (A-4j)



13C NMR spectrum of 4'-chloropyranochromene flavanone (A-4j)



Infrared spectrum of 4'-chloropyranochromene flavanone (A-4j)

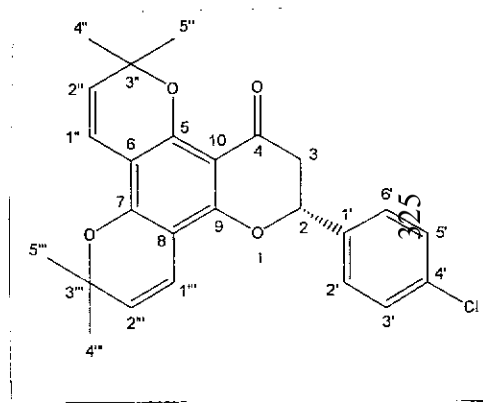
Elemental Composition Report

Single Mass Analysis

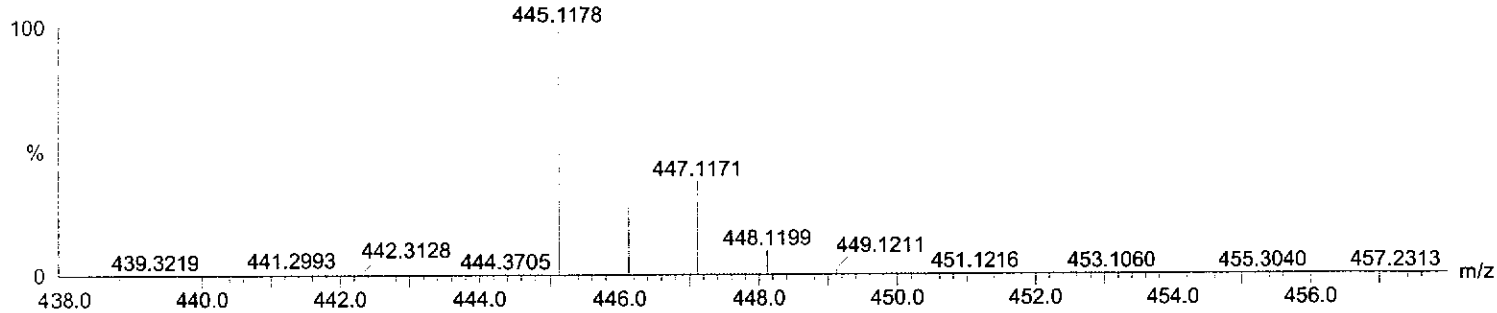
Tolerance = 50000.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
9 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-26 H: 20-25 O: 0-5 Na: 0-1 Cl: 0-1

Dr. Nizam
20 7 (0.103) Cm (1:31)

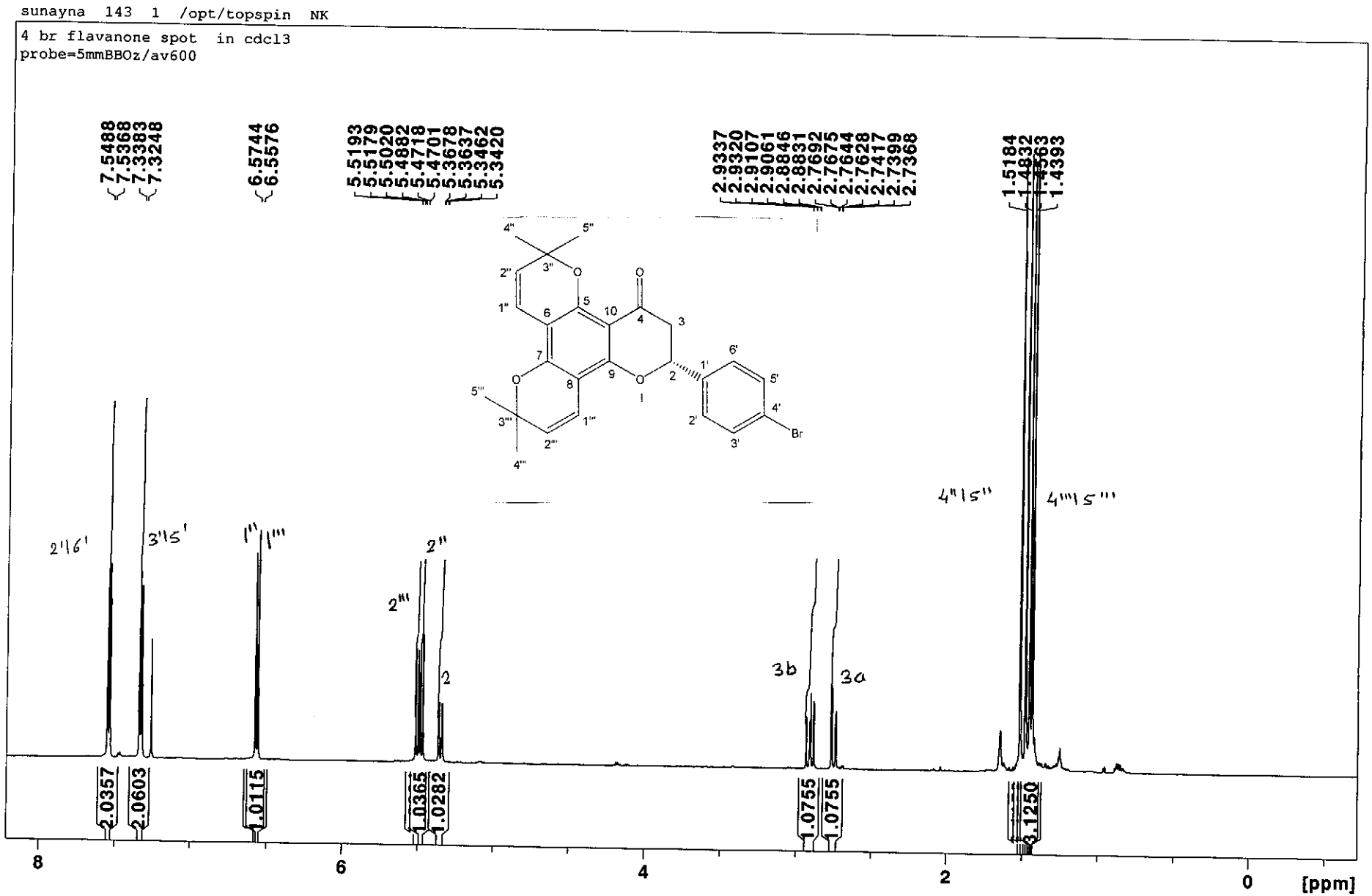


TOF MS ES+
3.00e+004

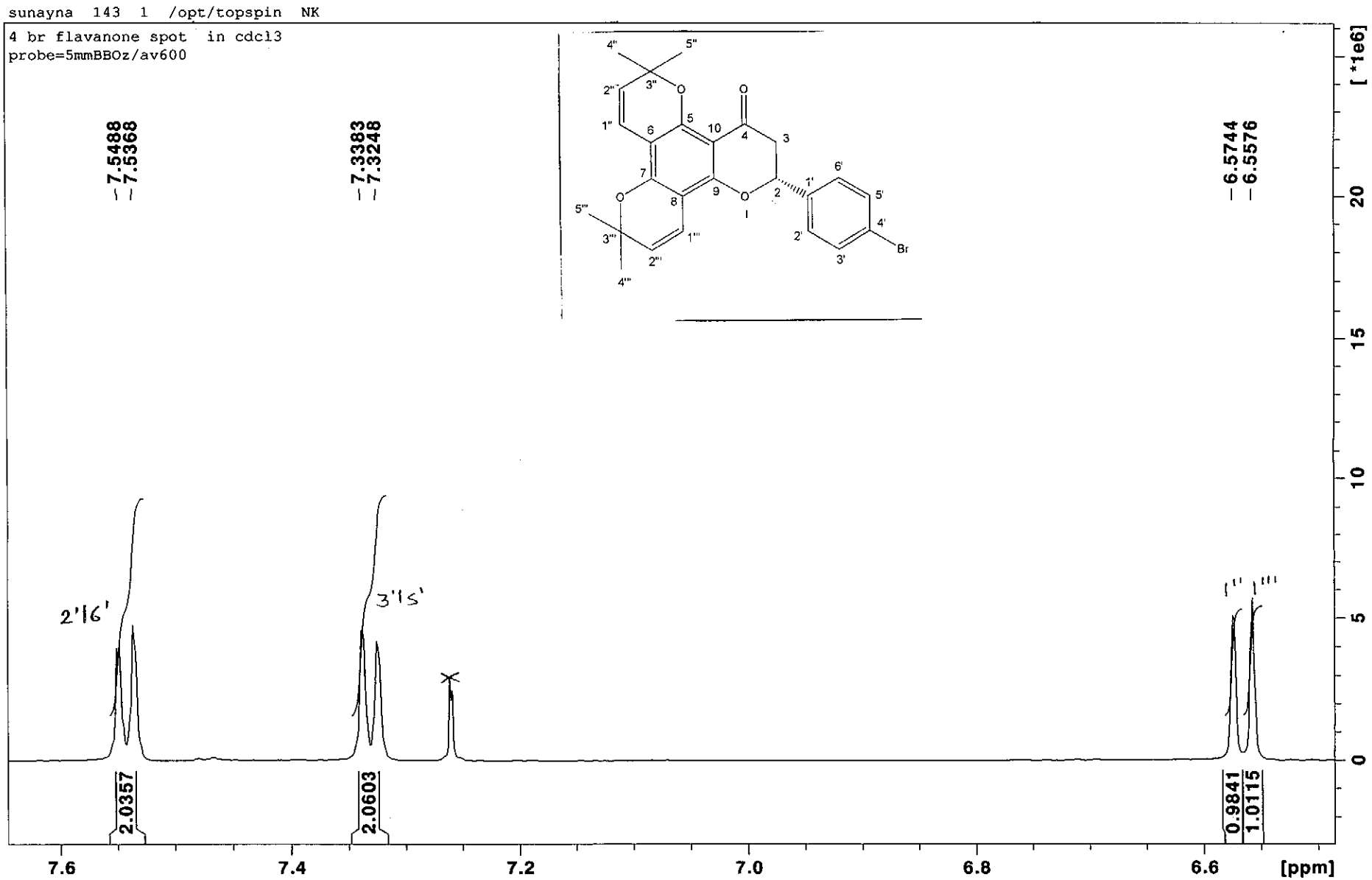


Minimum:				-1.5			
Maximum:		5.0	50000.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
445.1178	445.1183	-0.5	-1.1	13.5	394.3	0.0	C25 H23 O4 Na Cl

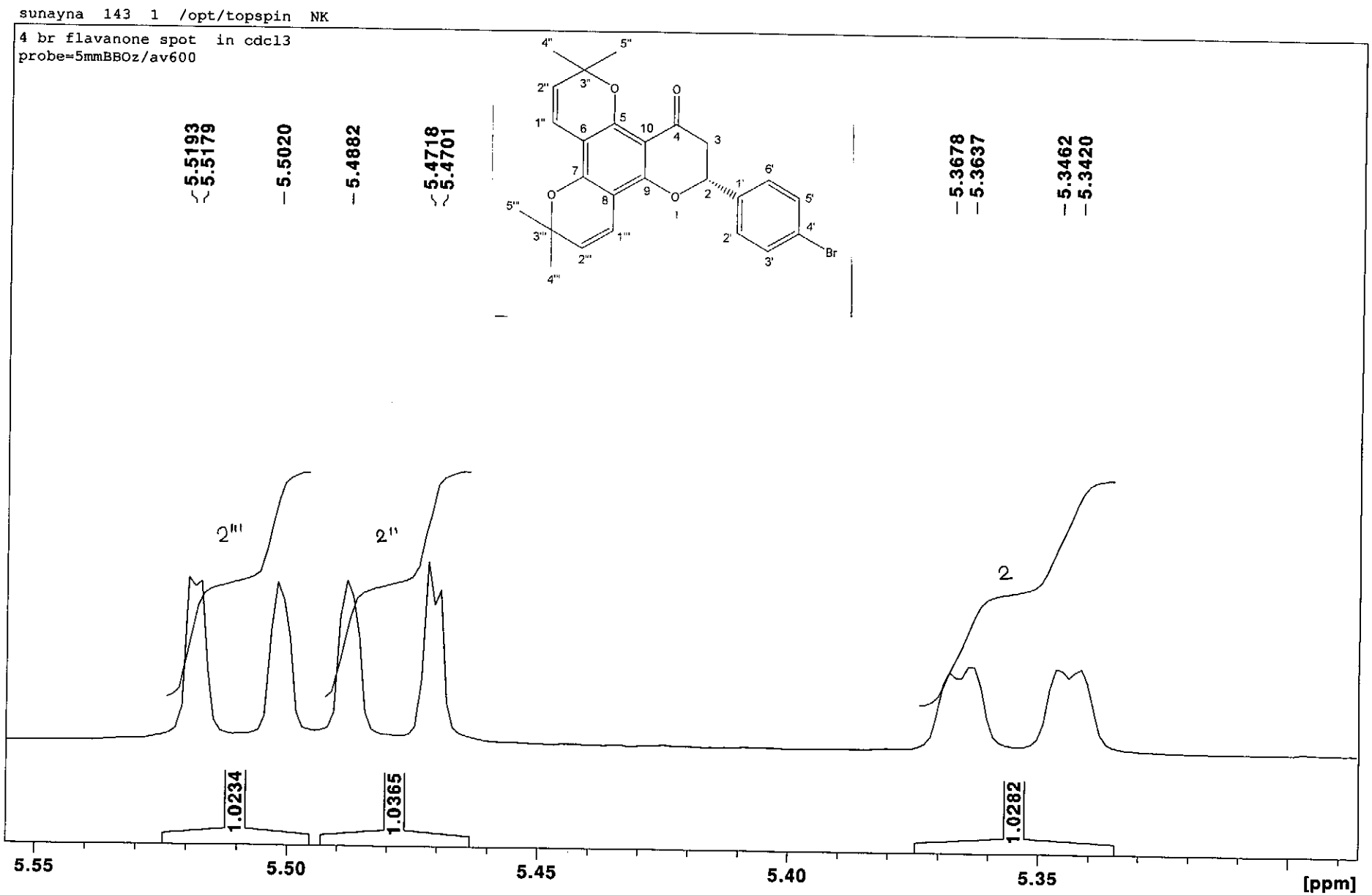
HRMS spectrum of 4'-chloropyranochromene flavanone (A-4j)



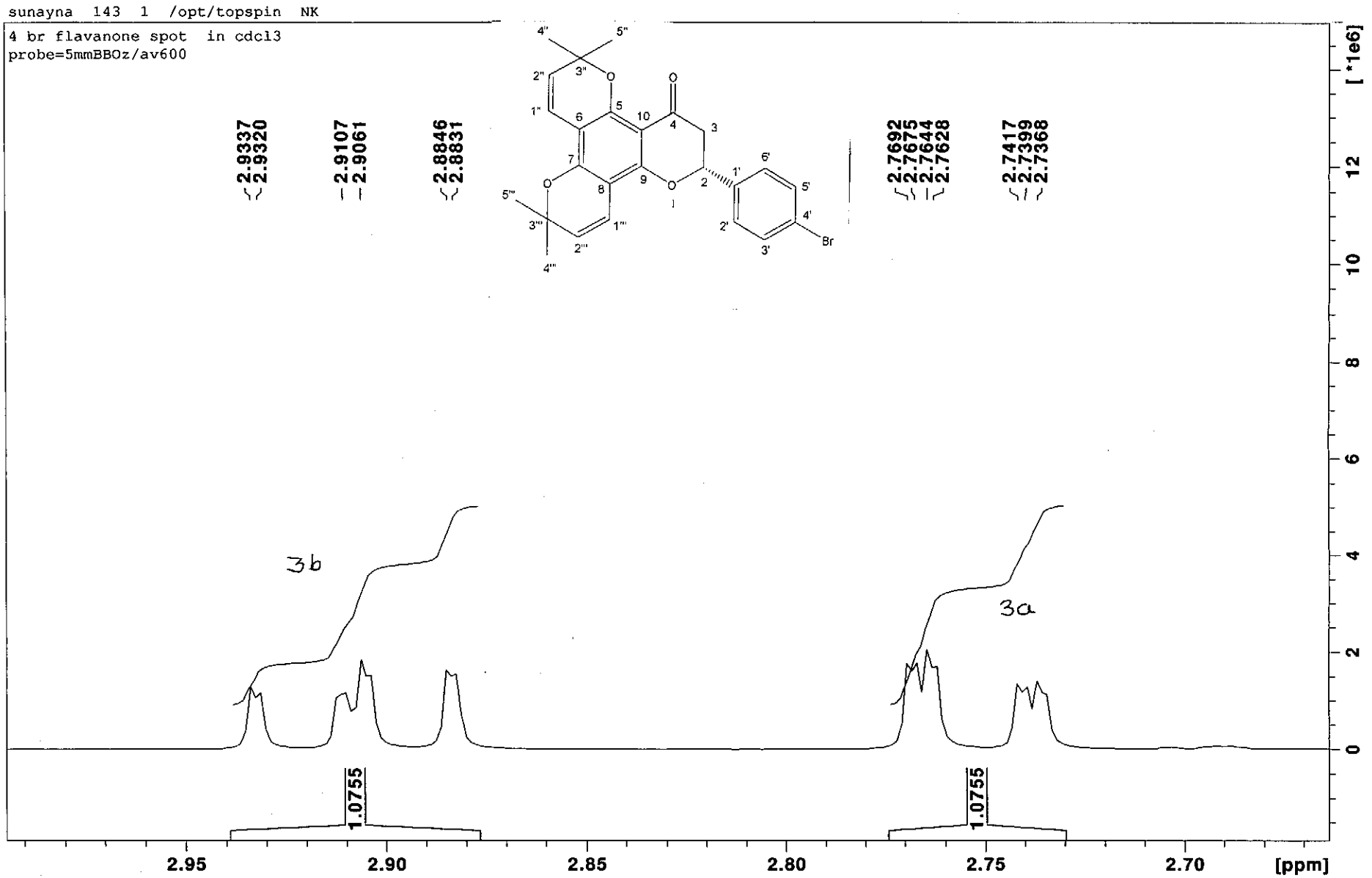
1H NMR spectrum of 4'-bromopyranochromene flavanone (A-4k)



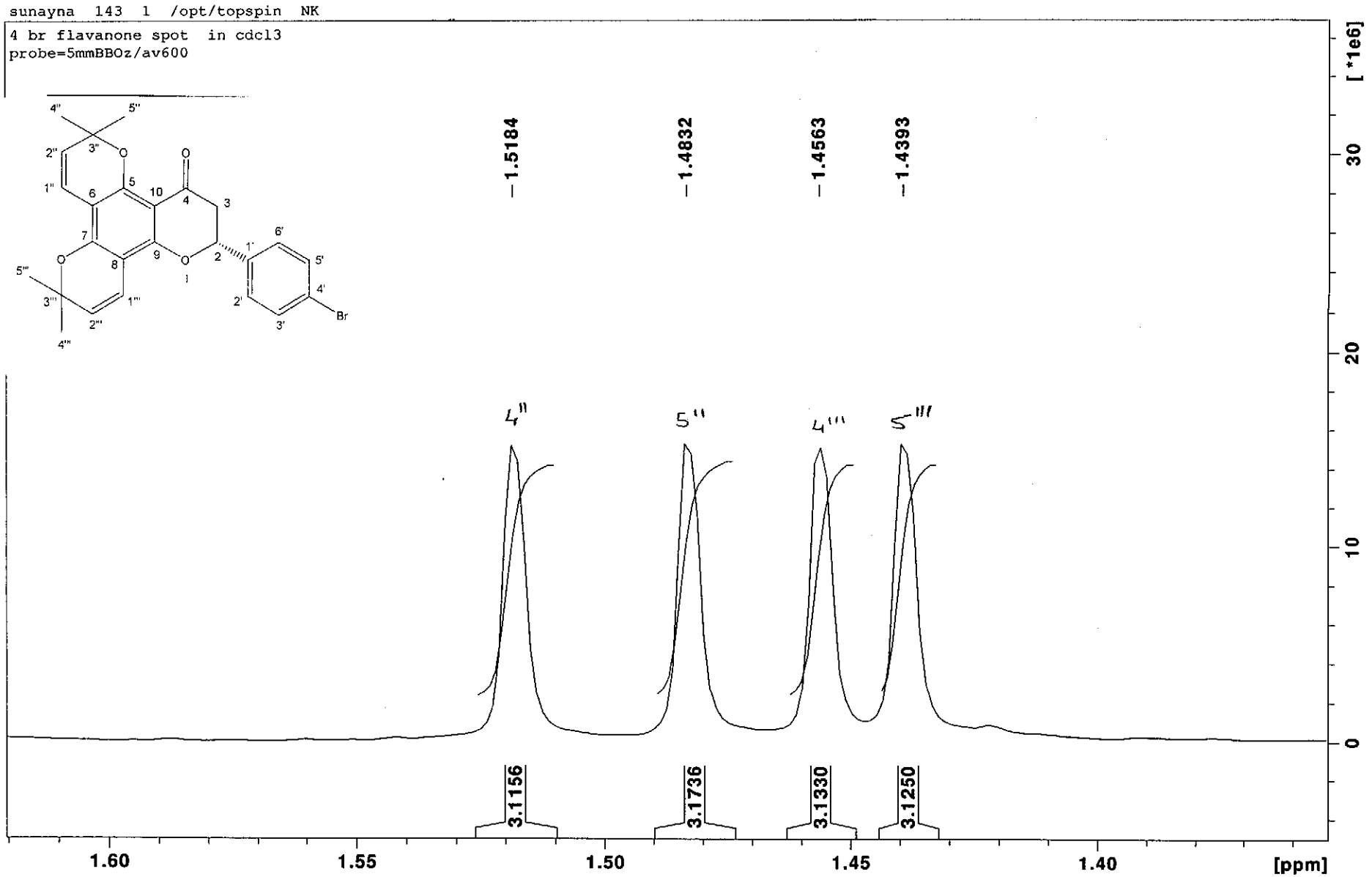
Expanded 1H NMR spectrum of 4'-bromopyranochromene flavanone (A-4k)



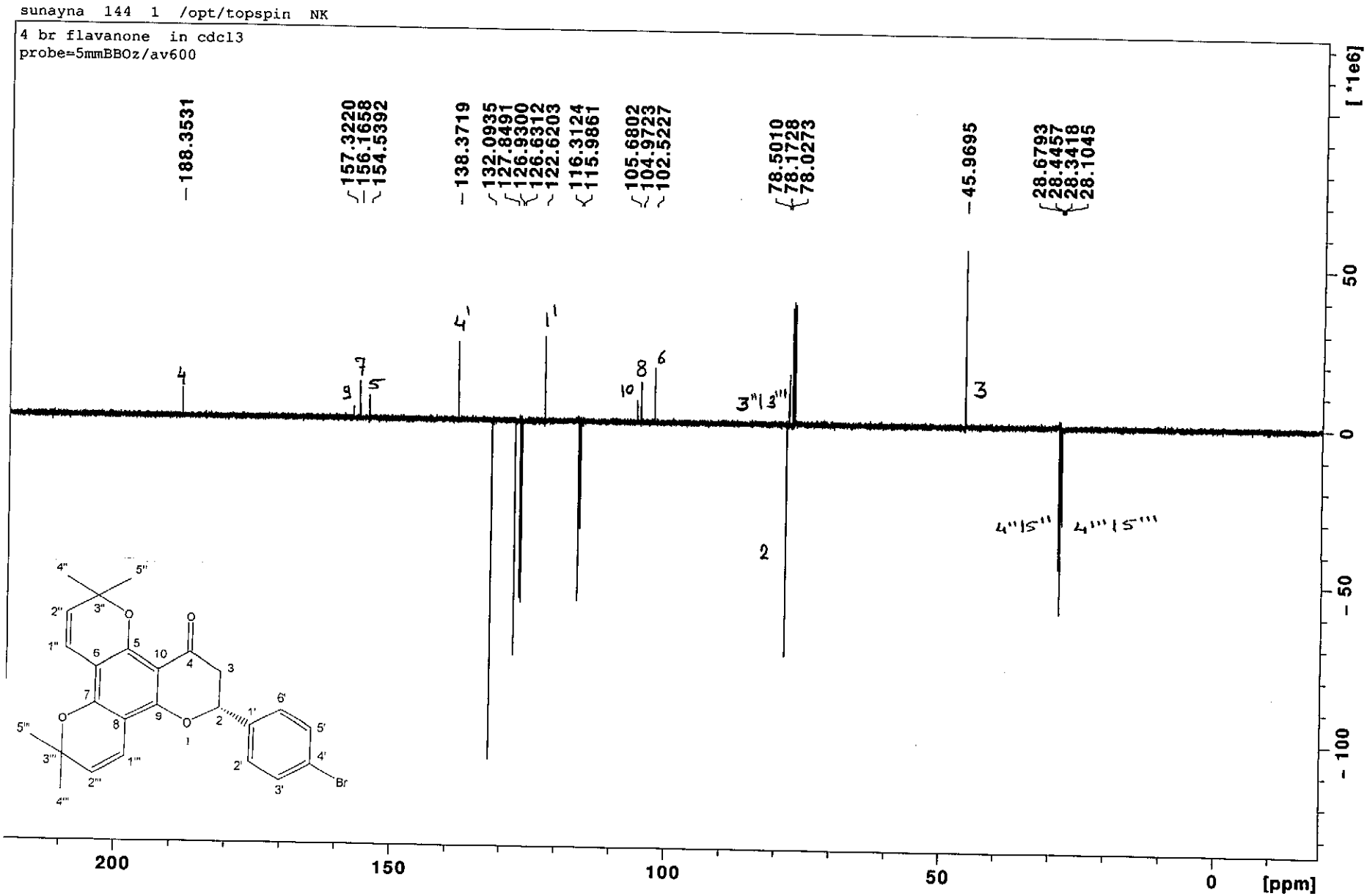
Expanded 1H NMR spectrum of 4'-bromopyranochromene flavanone (A-4k)



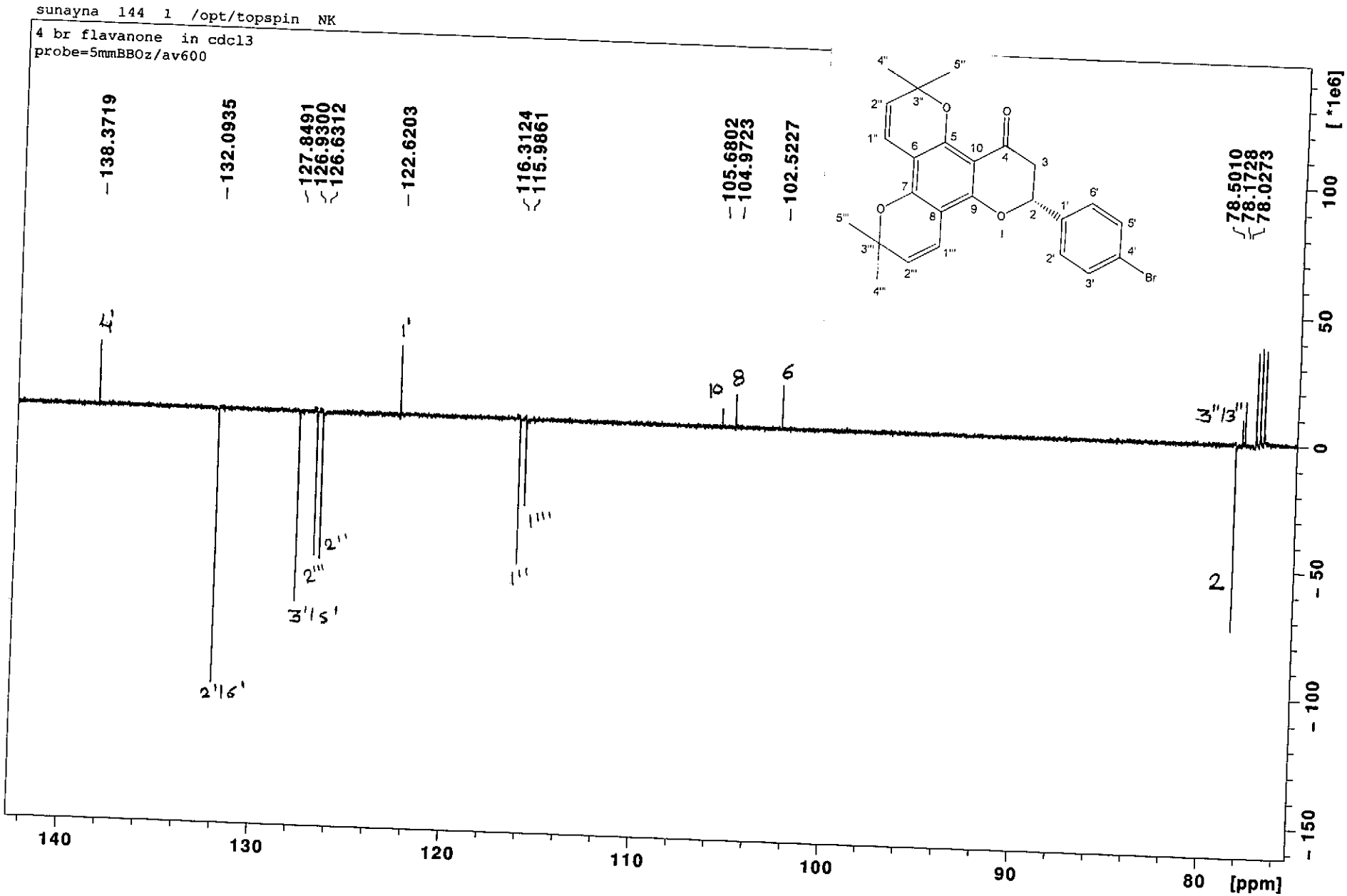
Expanded 1H NMR spectrum of 4'-bromopyranochromene flavanone (A-4k)



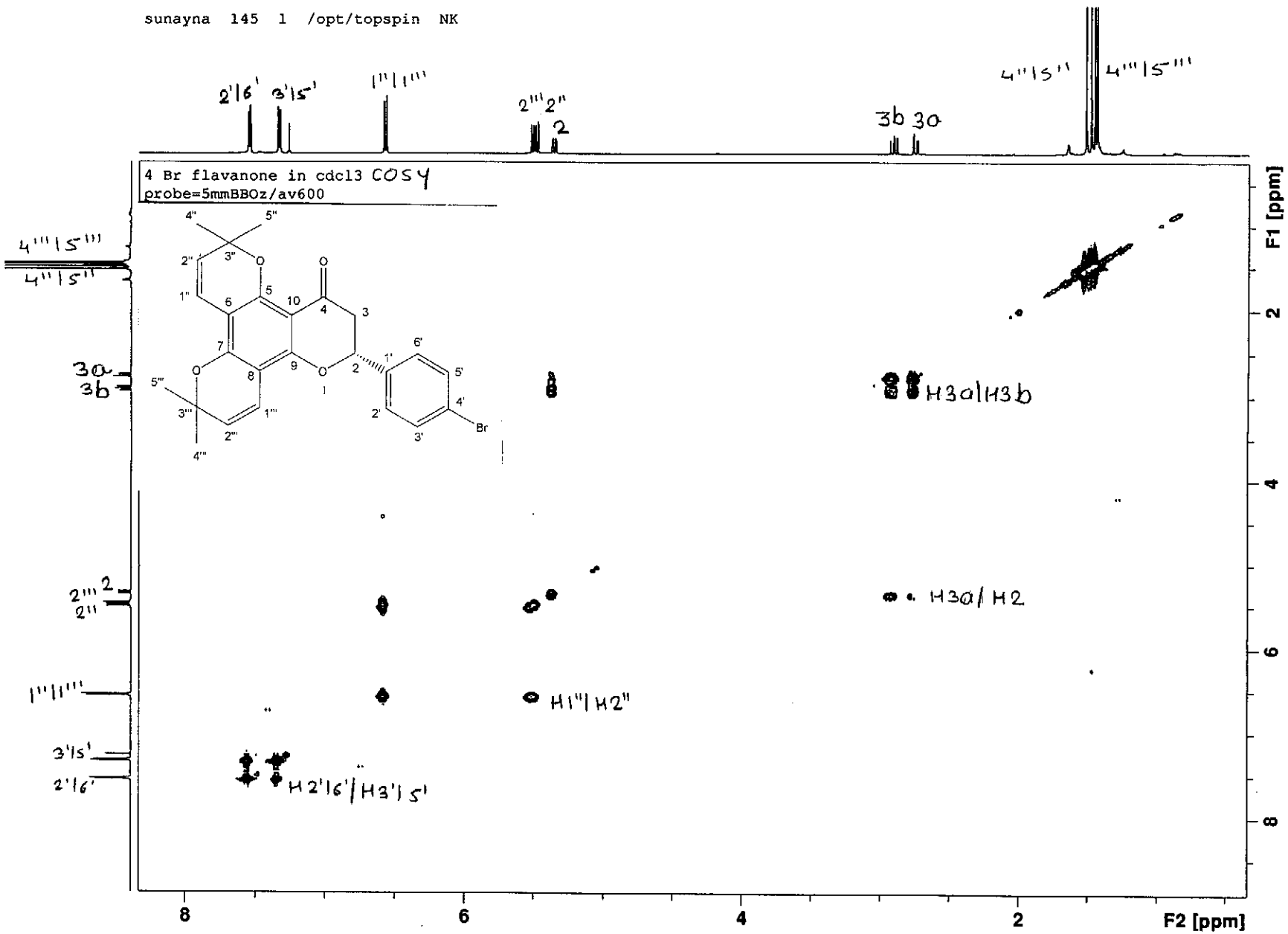
Expanded 1H NMR spectrum of 4'-bromopyranochromene flavanone (A-4k)



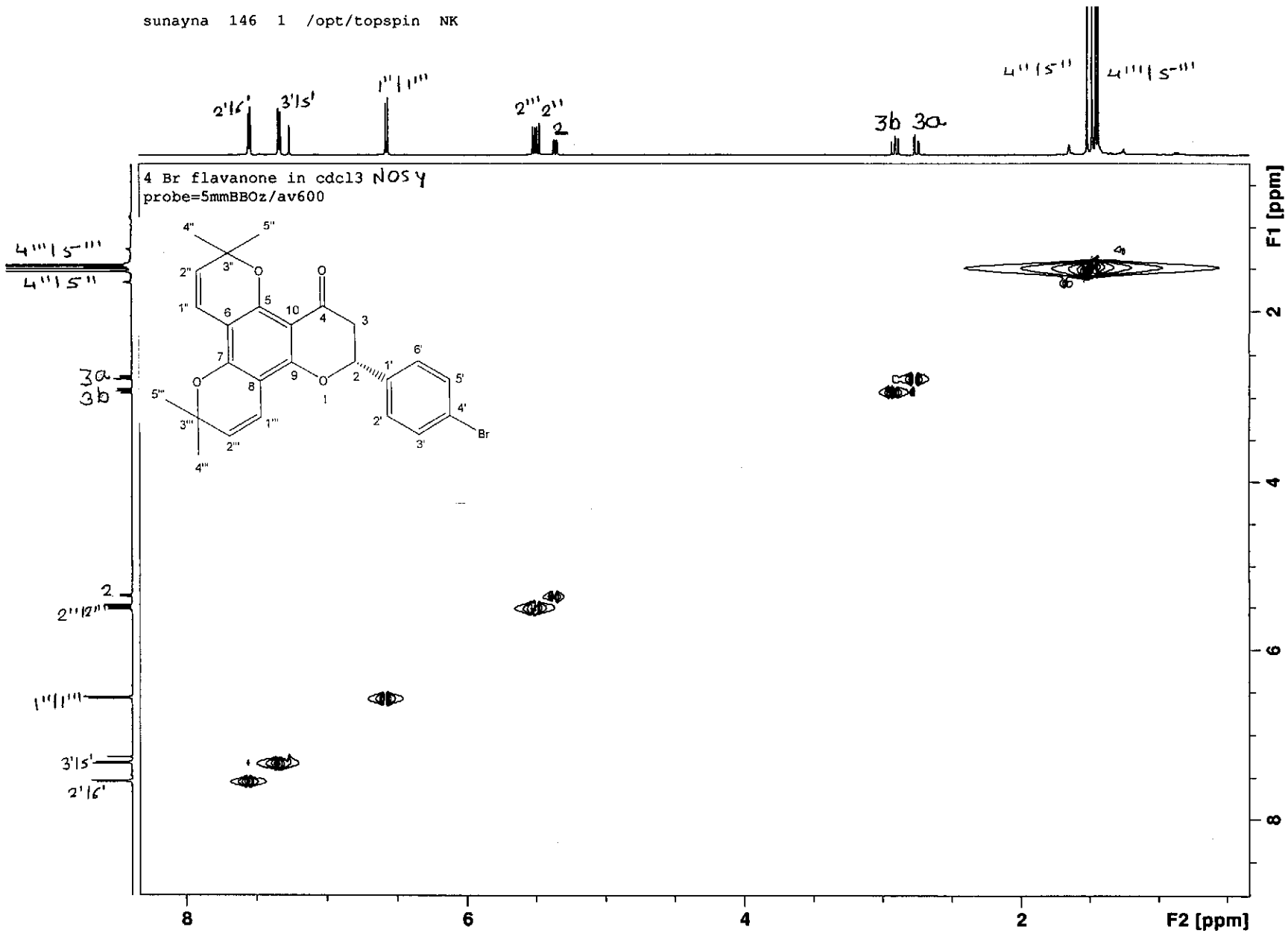
13C NMR spectrum of 4'-bromopyranochromene flavanone (A-4k)



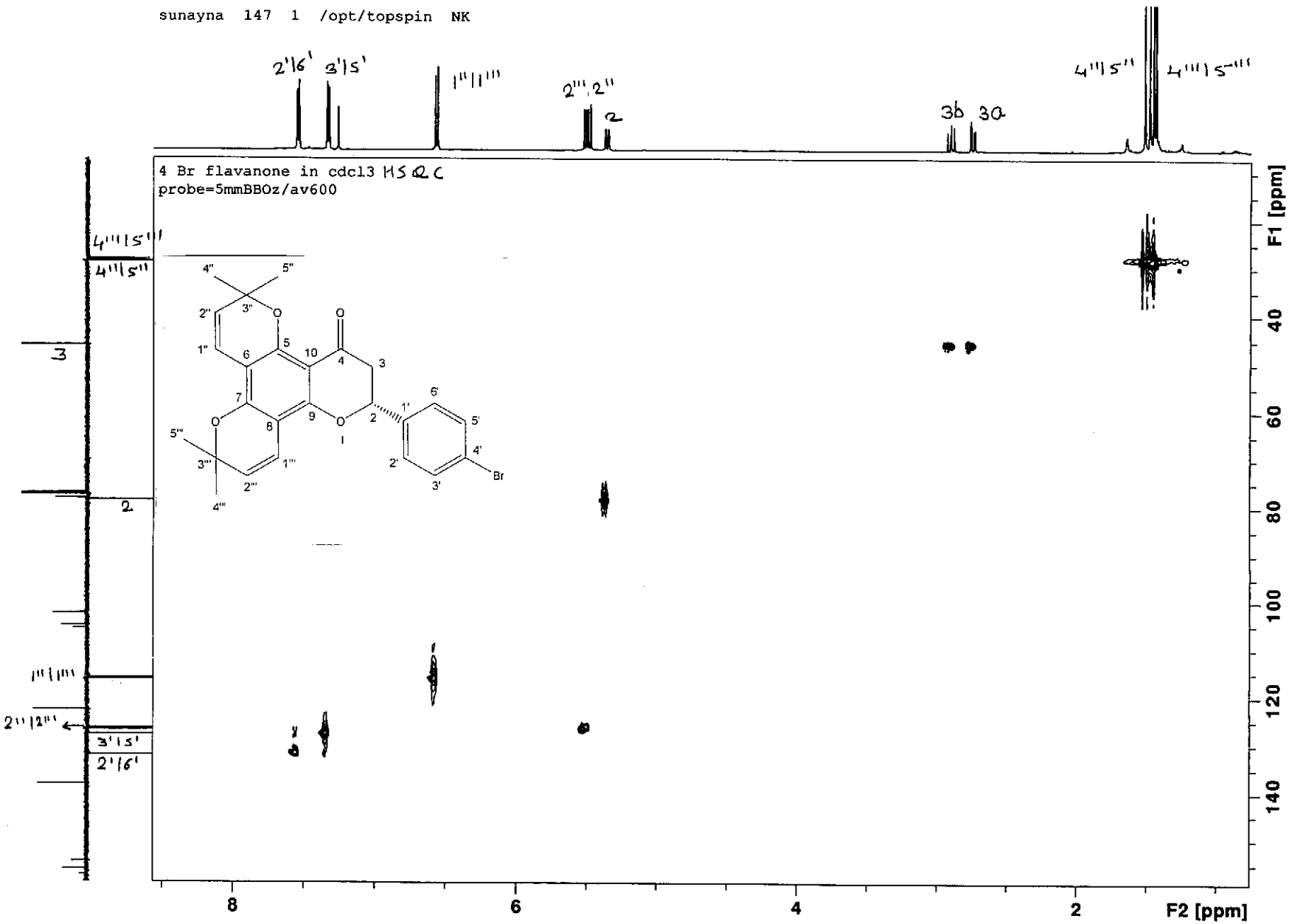
Expanded 13C NMR spectrum of 4'-bromopyranochromene flavanone (A-4k)



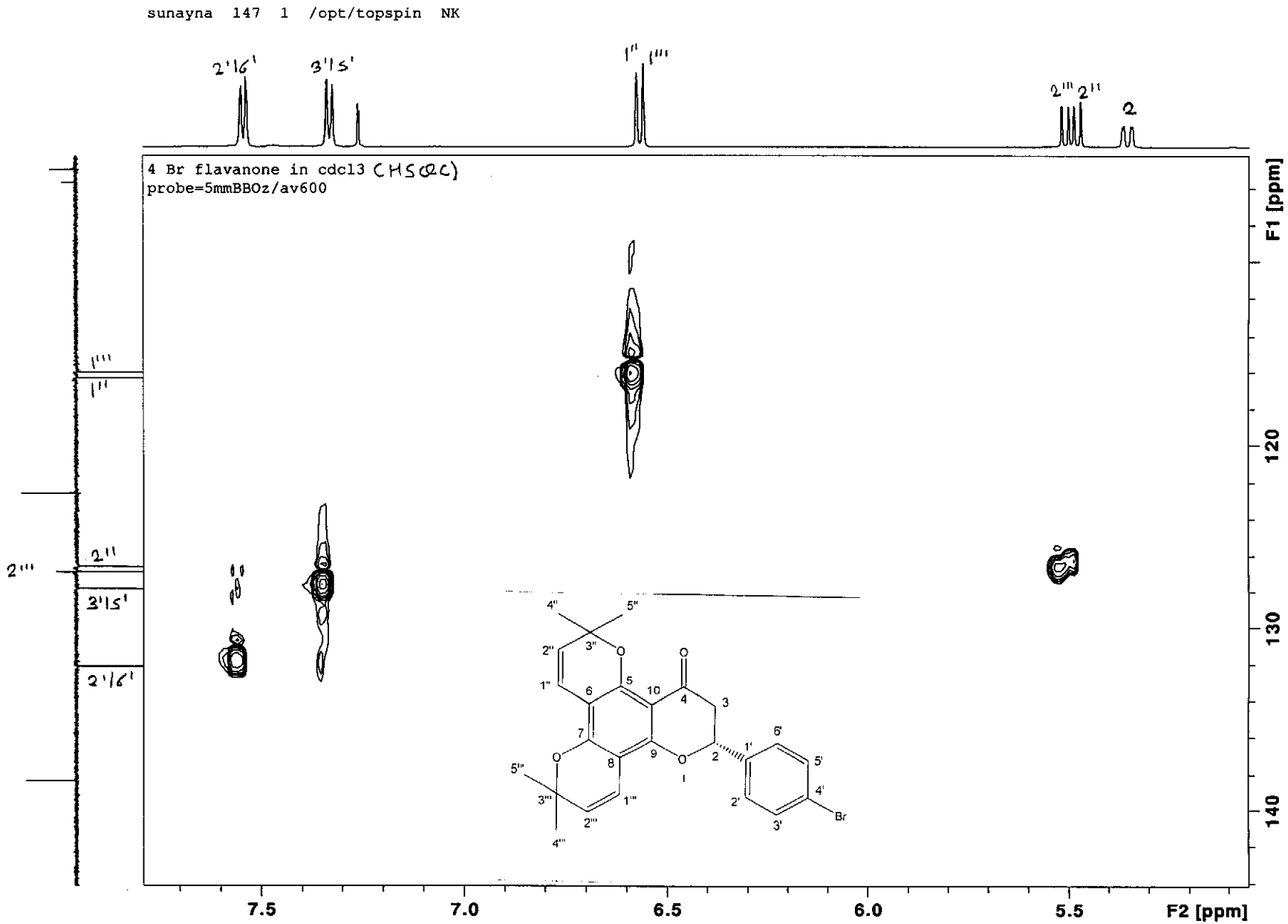
COSY spectrum of 4'-bromopyranochromene flavanone (A-4k)



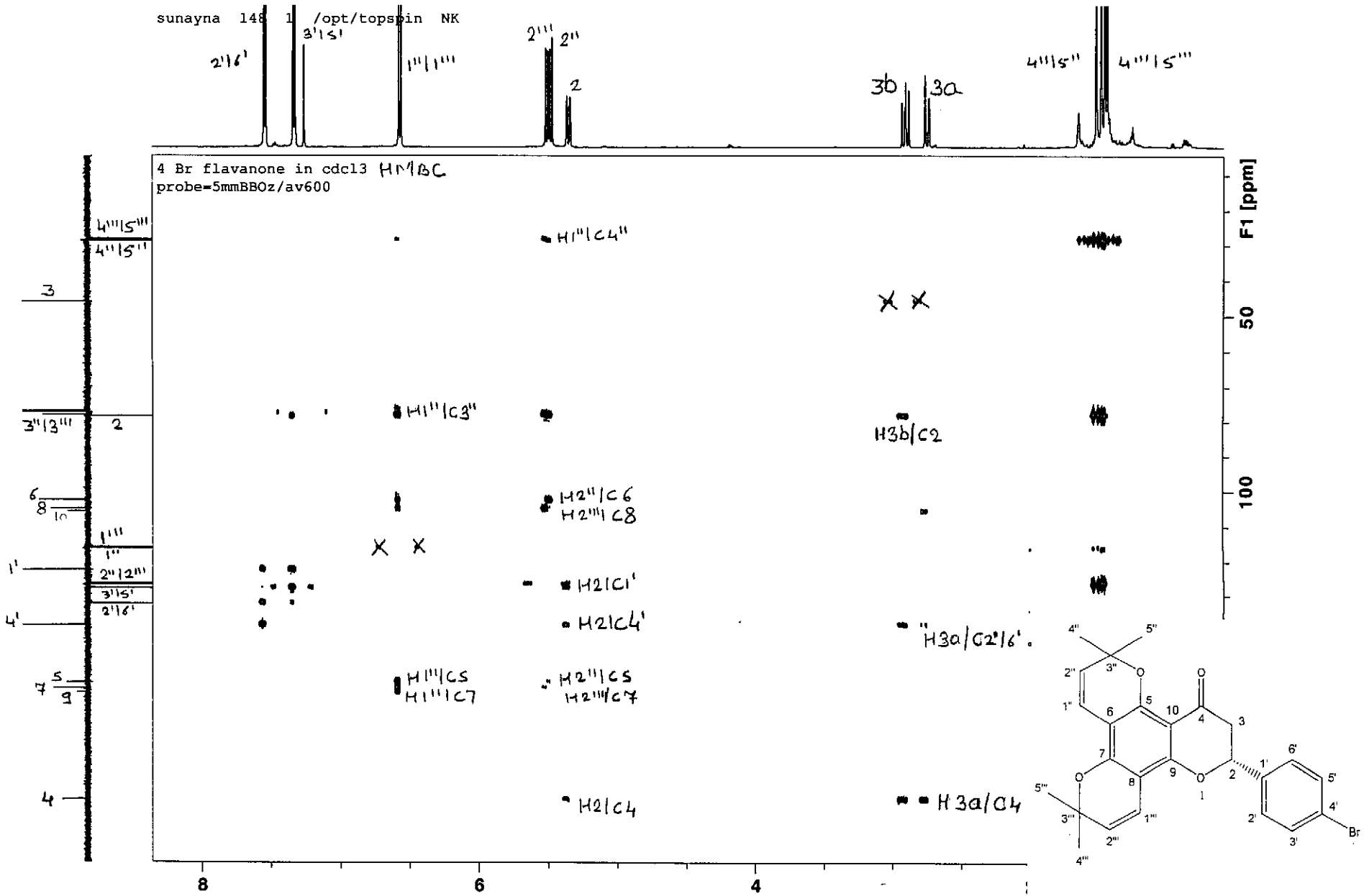
NOESY spectrum of 4'-bromopyranochromene flavanone (A-4k)



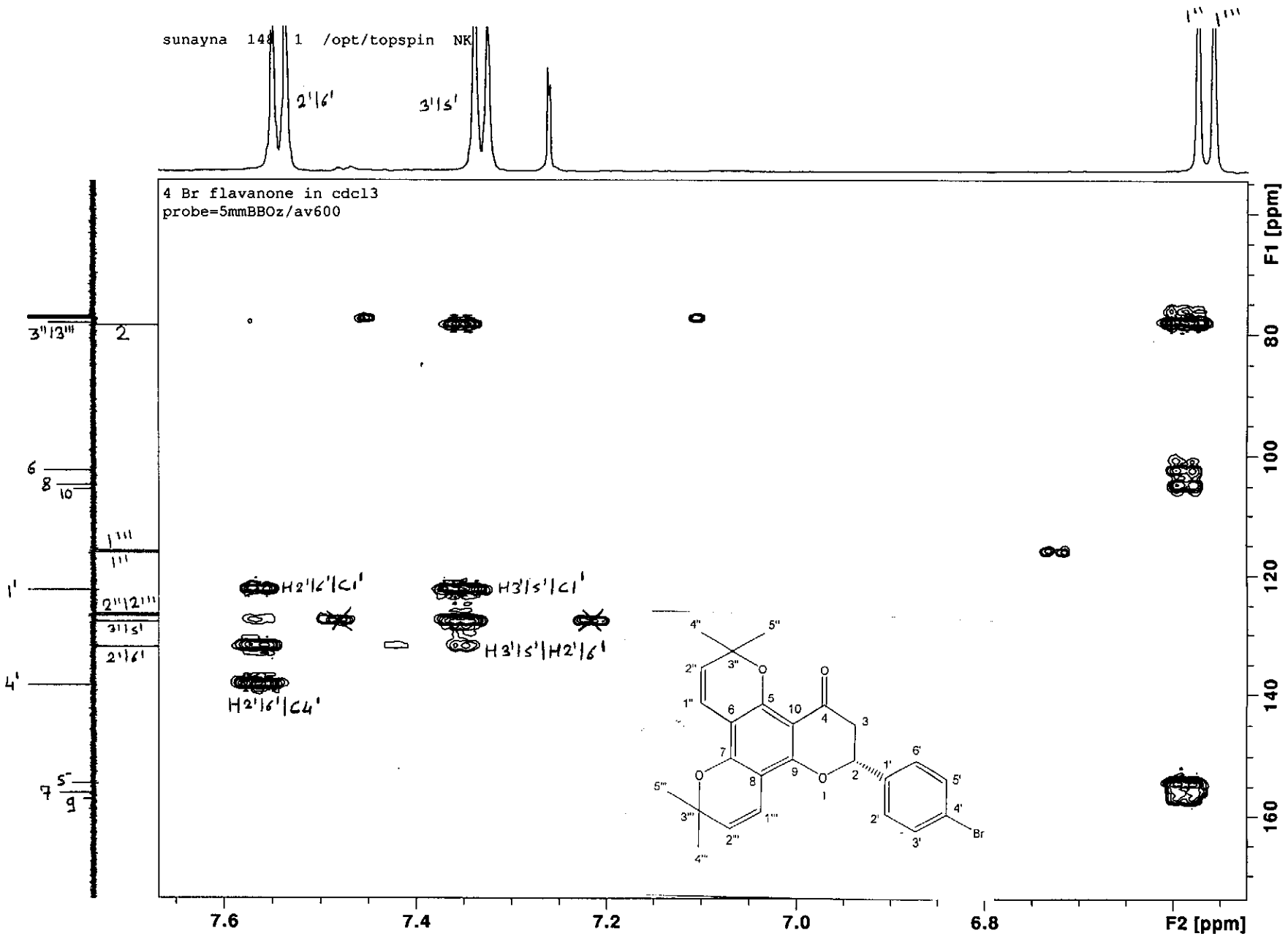
HSQC spectrum of 4'-bromopyranochromene flavanone (A-4k)



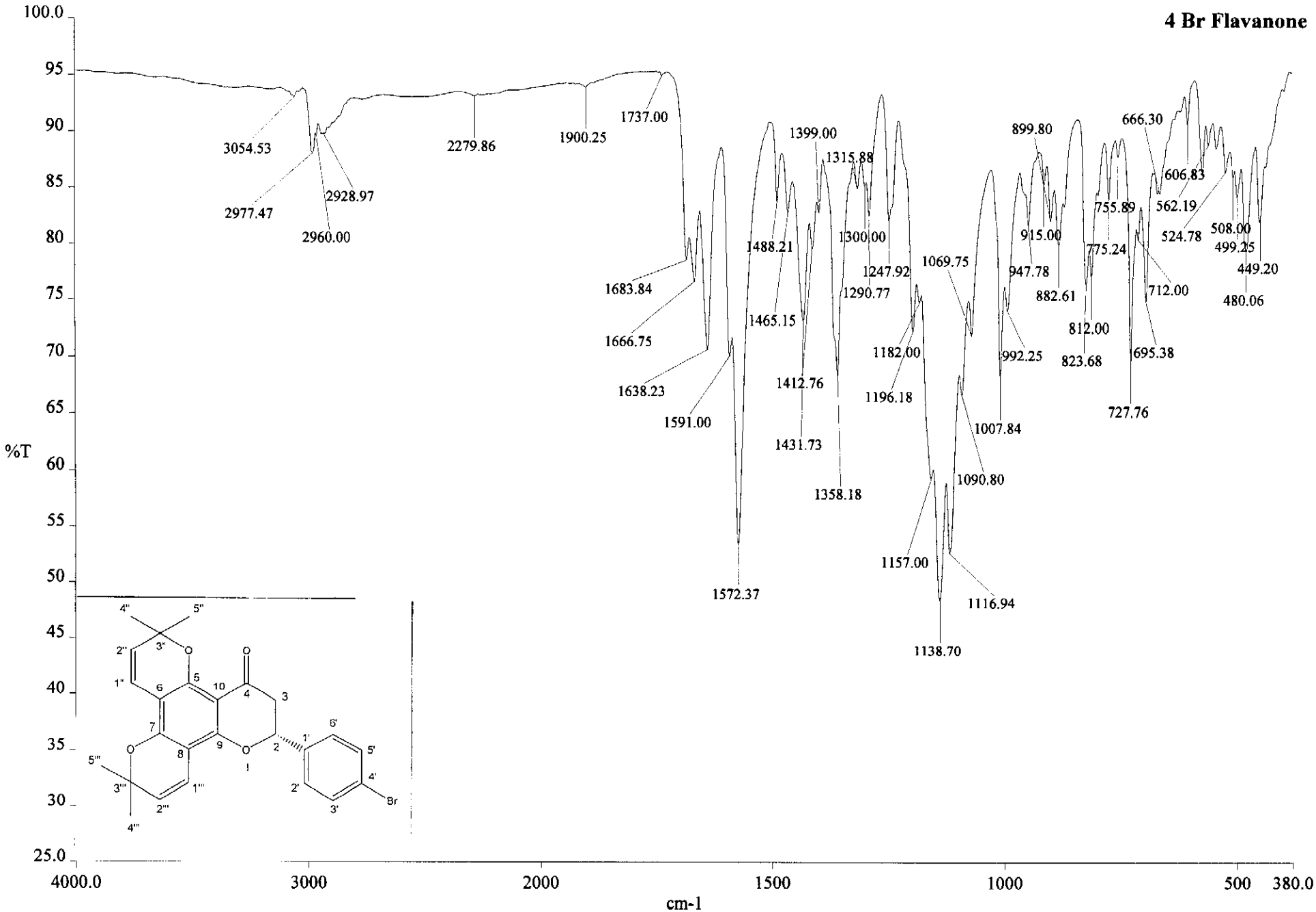
Expanded HSQC spectrum of 4'-bromopyranochromene flavanone (A-4k)



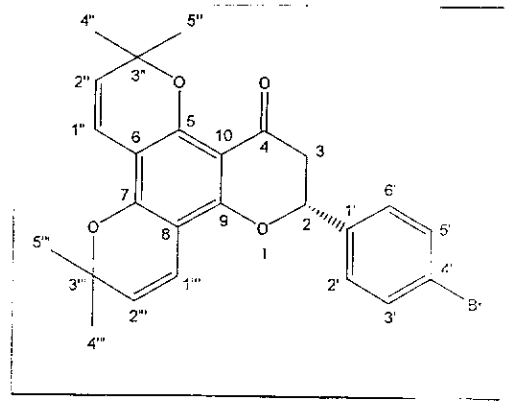
HMBC spectrum of 4'-bromopyranochromene flavanone (A-4k)



Expanded HMBC spectrum of 4'-bromopyranochromene flavanone (A-4k)



Infrared spectrum of 4'-bromopyranochromene flavanone (A-4k)



Elemental Composition Report

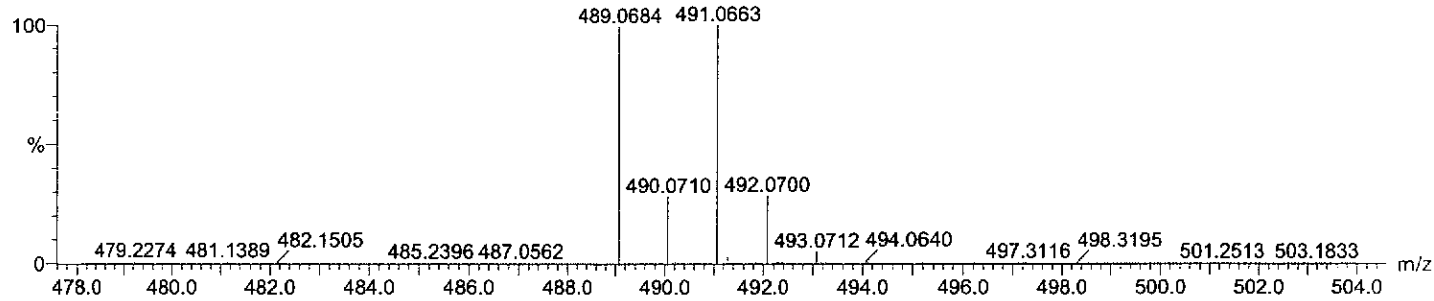
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
14 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-26 H: 20-25 O: 0-5 Na: 0-1 Br: 0-1

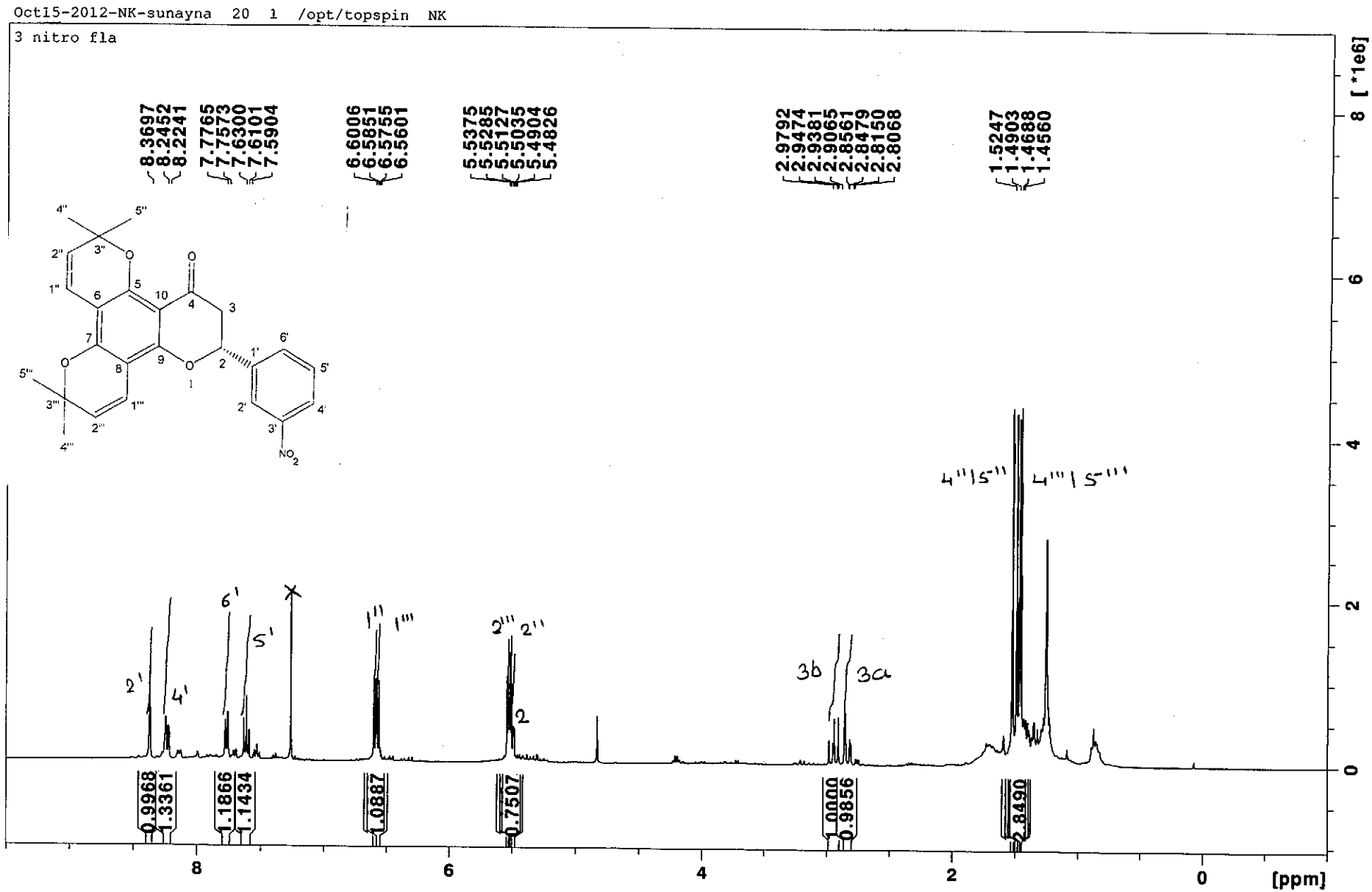
Dr. Nizam
22 2 (0.017) Cm (1:31)

TOF MS ES+
1.75e+004

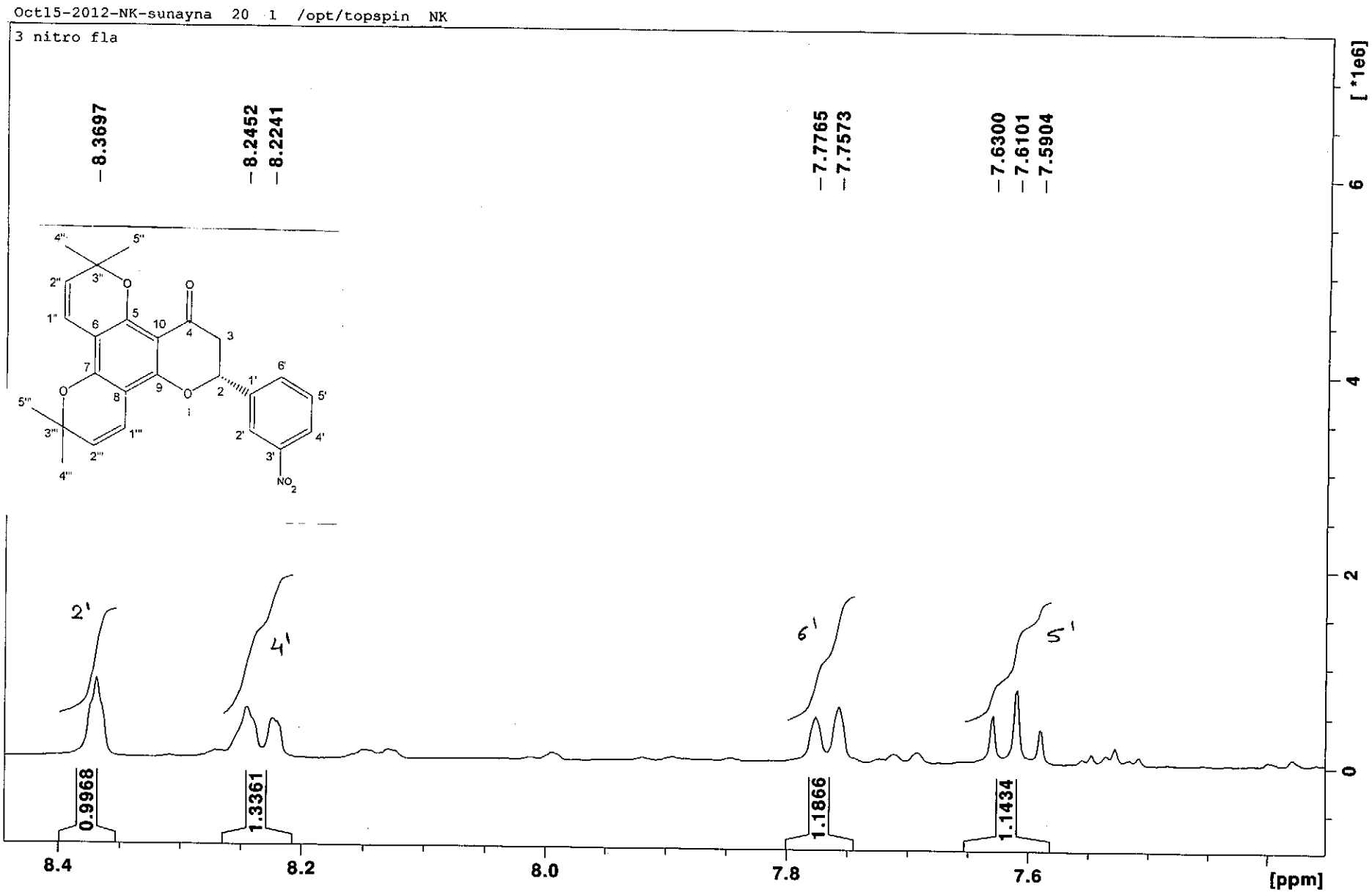


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
489.0684	489.0677	0.7	1.4	13.5	359.5	0.0	C25 H23 O4 Na Br

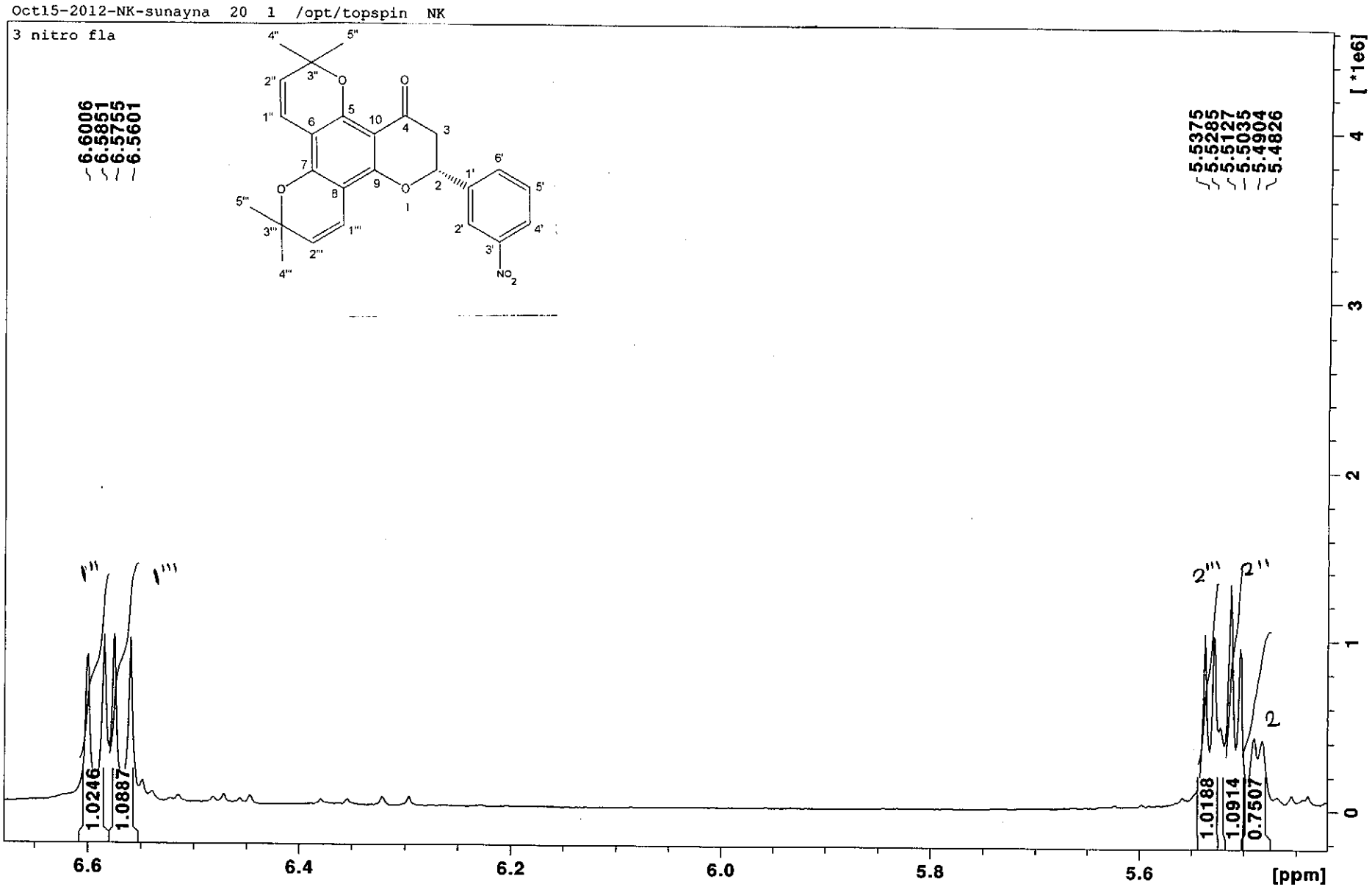
HRMS spectrum of 4'-bromopyranochromene flavanone (A-4k)



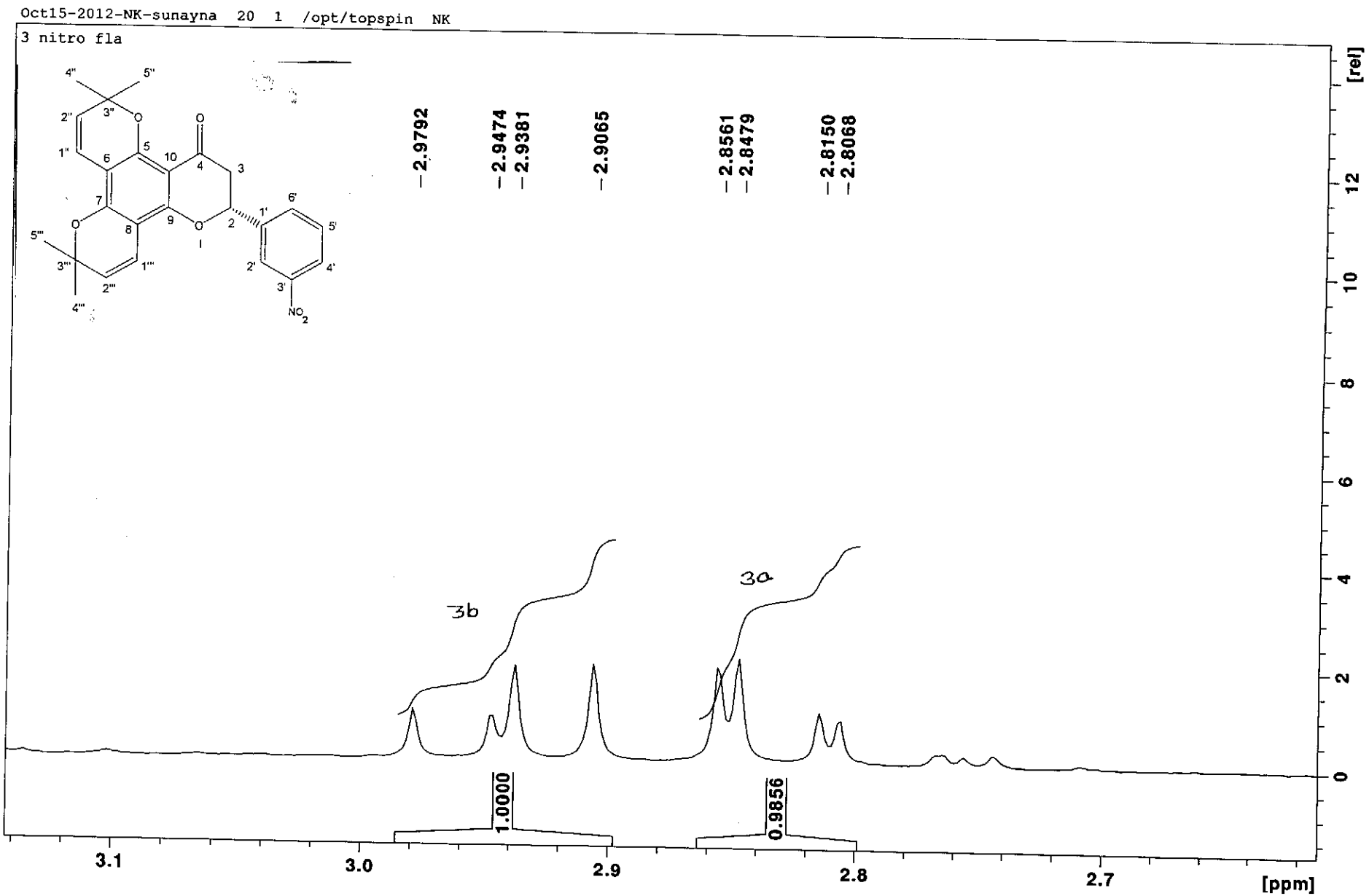
1H NMR spectrum of 3'-nitropyranochromene flavanone (A-4l)



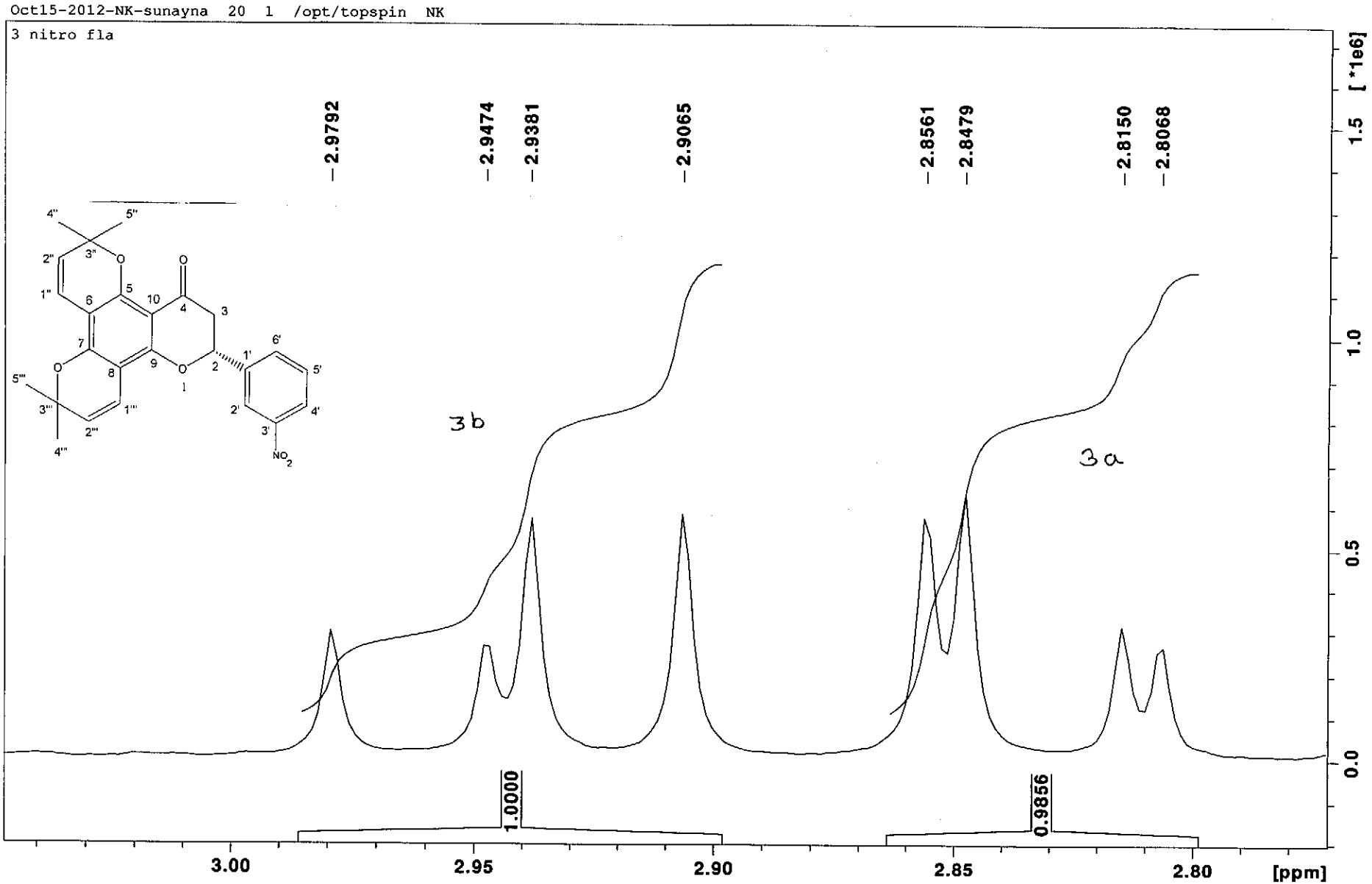
Expanded 1H NMR spectrum of 3'-nitropyranochromene flavanone (A-4l)



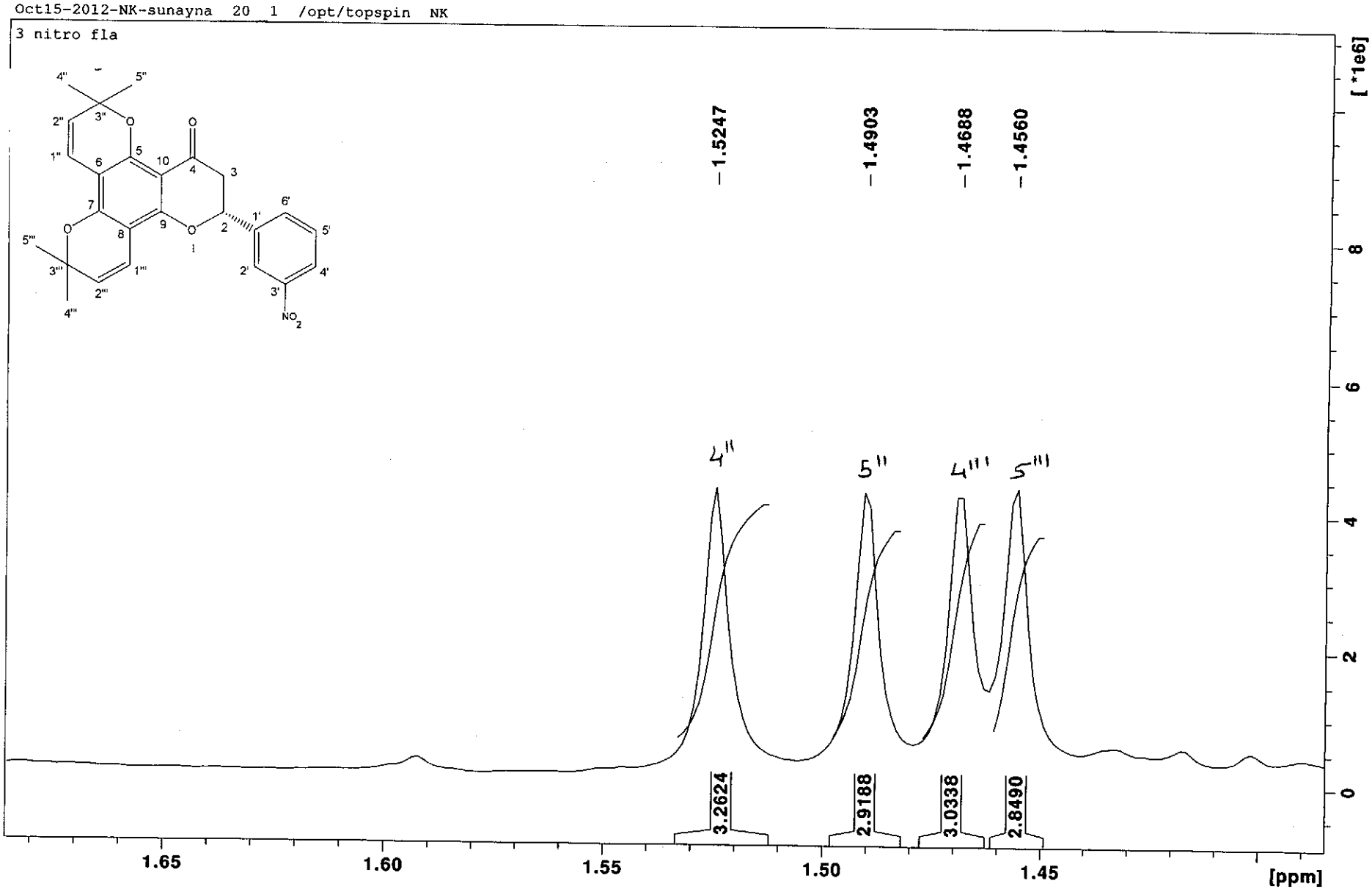
Expanded 1H NMR spectrum of 3'-nitropyranochromene flavanone (A-4l)



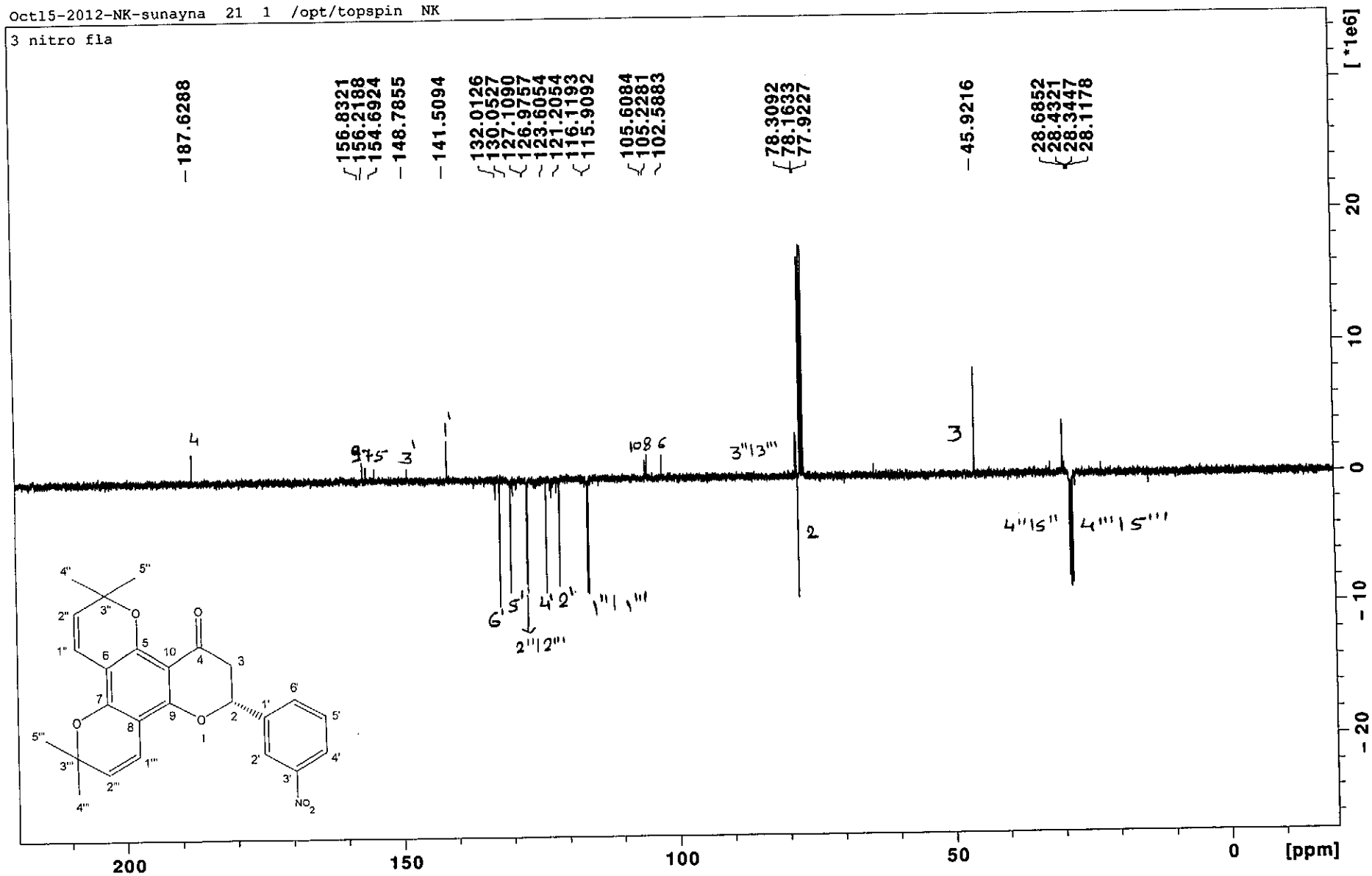
Expanded 1H NMR spectrum of 3'-nitropyranochromene flavanone (A-4l)



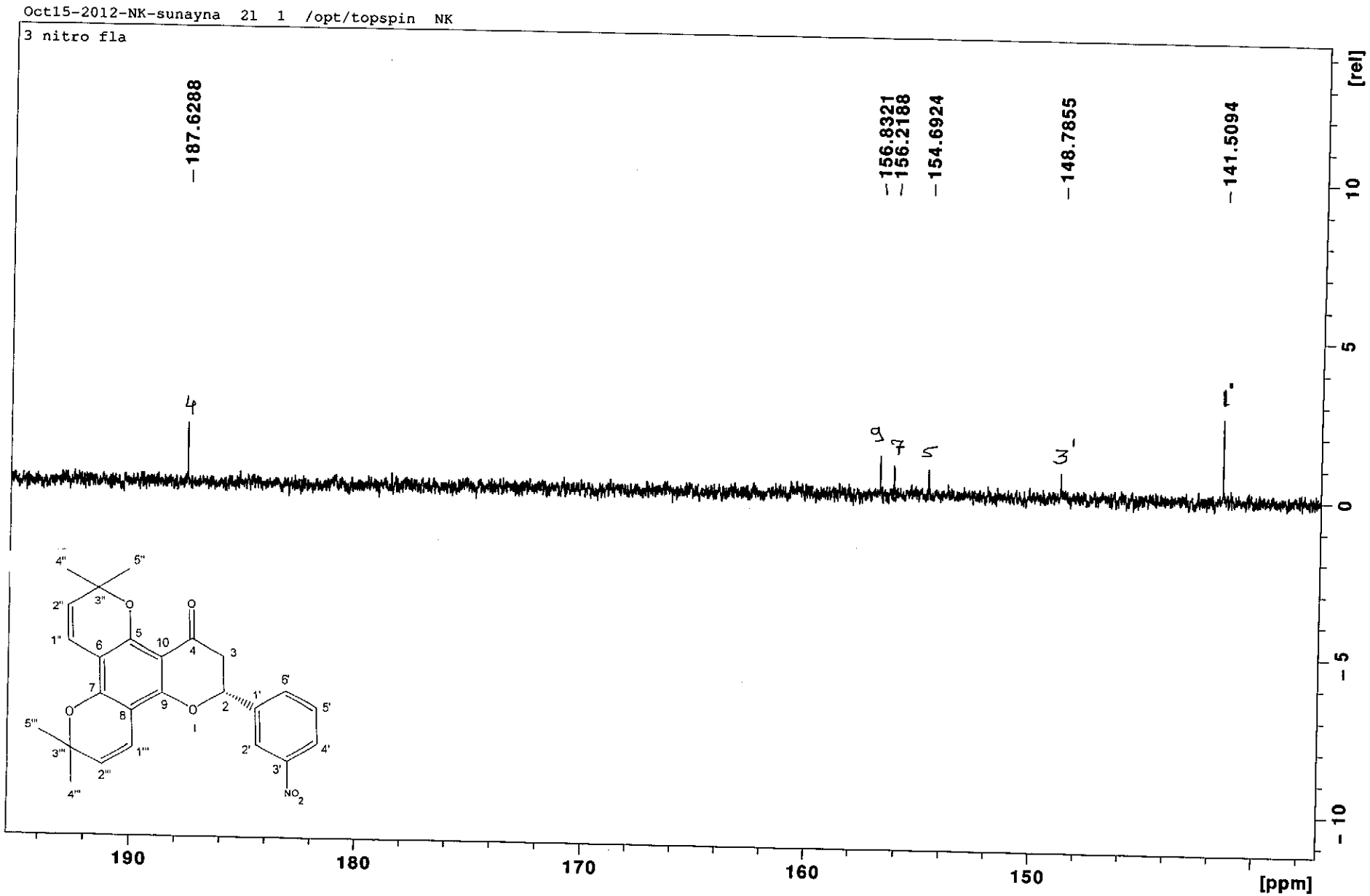
Expanded 1H NMR spectrum of 3'-nitropyranochromene flavanone (A-4l)



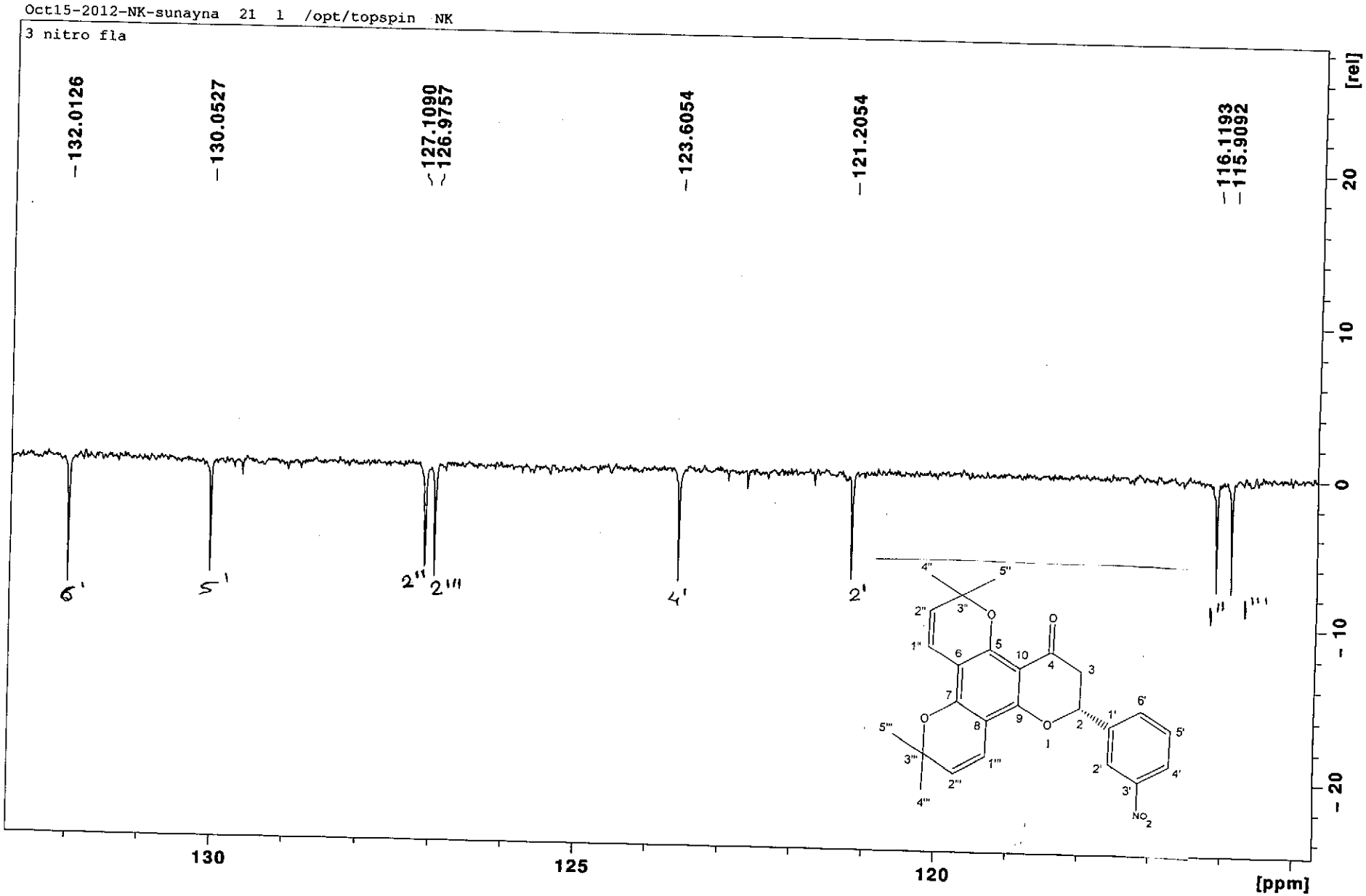
Expanded 1H NMR spectrum of 3'-nitropyranochromene flavanone (A-4l)



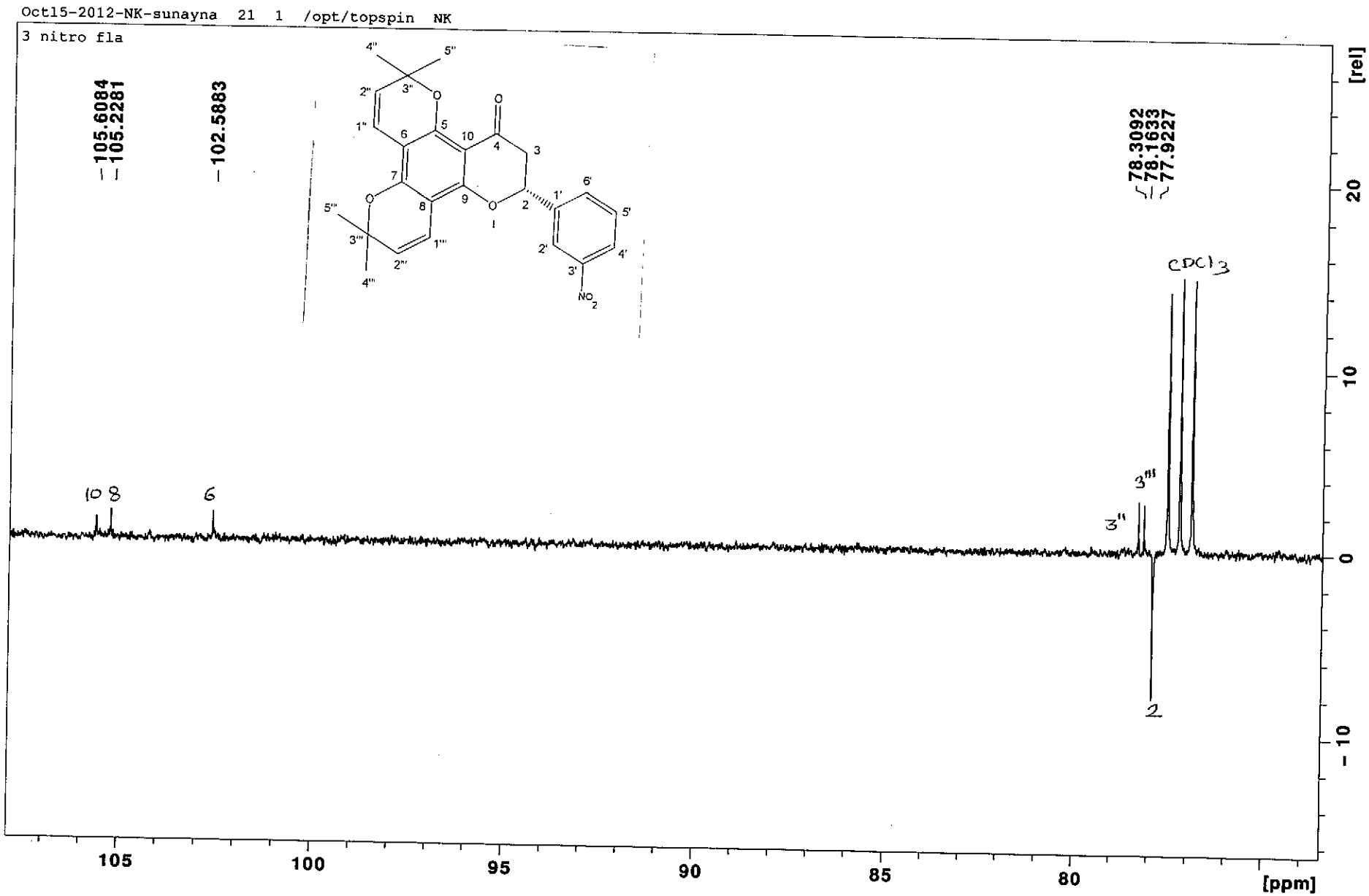
13C NMR spectrum of 3'-nitropyranochromene flavanone (A-4l)



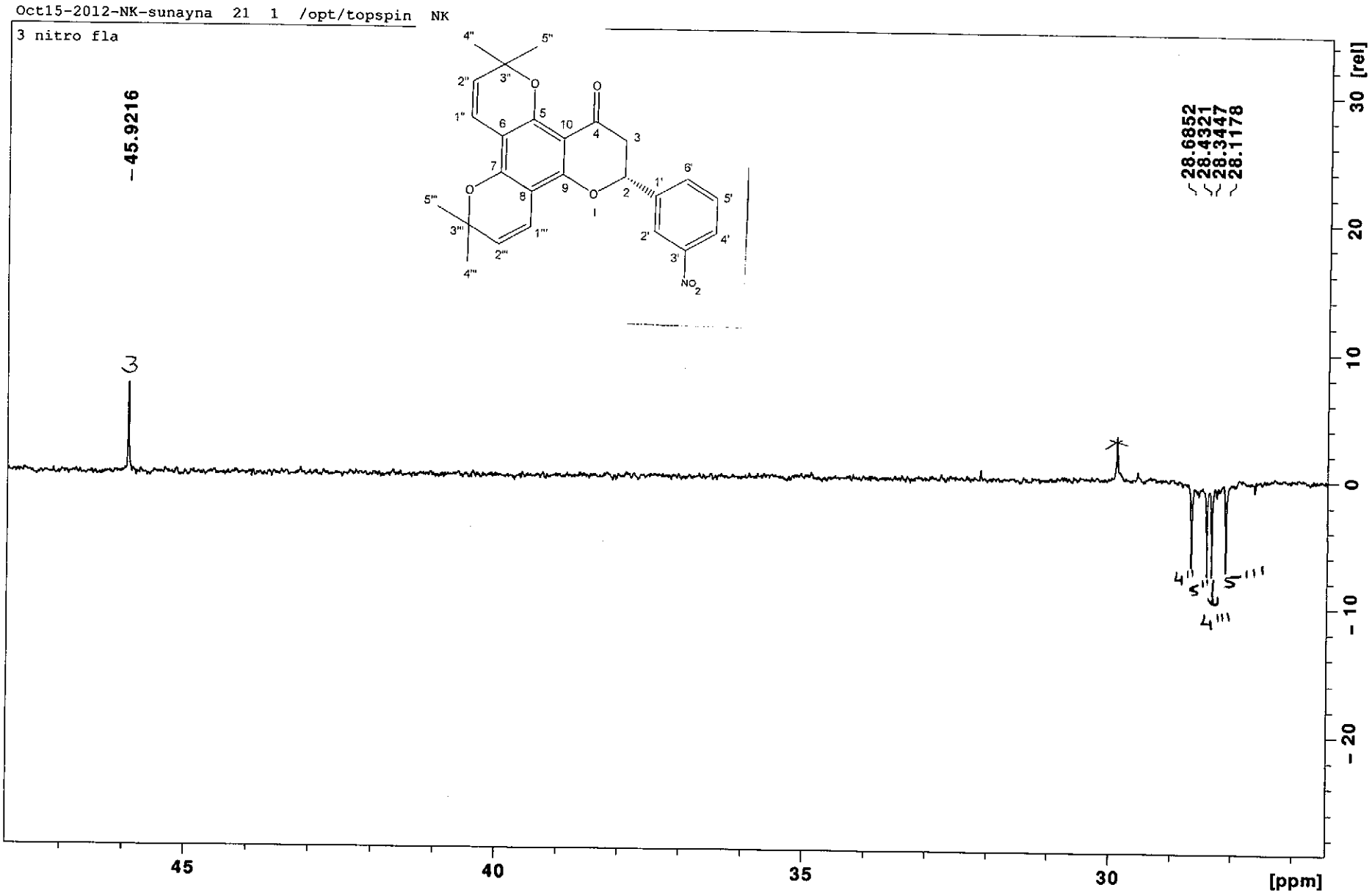
Expanded 13C NMR spectrum of 3-nitropyranochromene flavanone (A-4l)



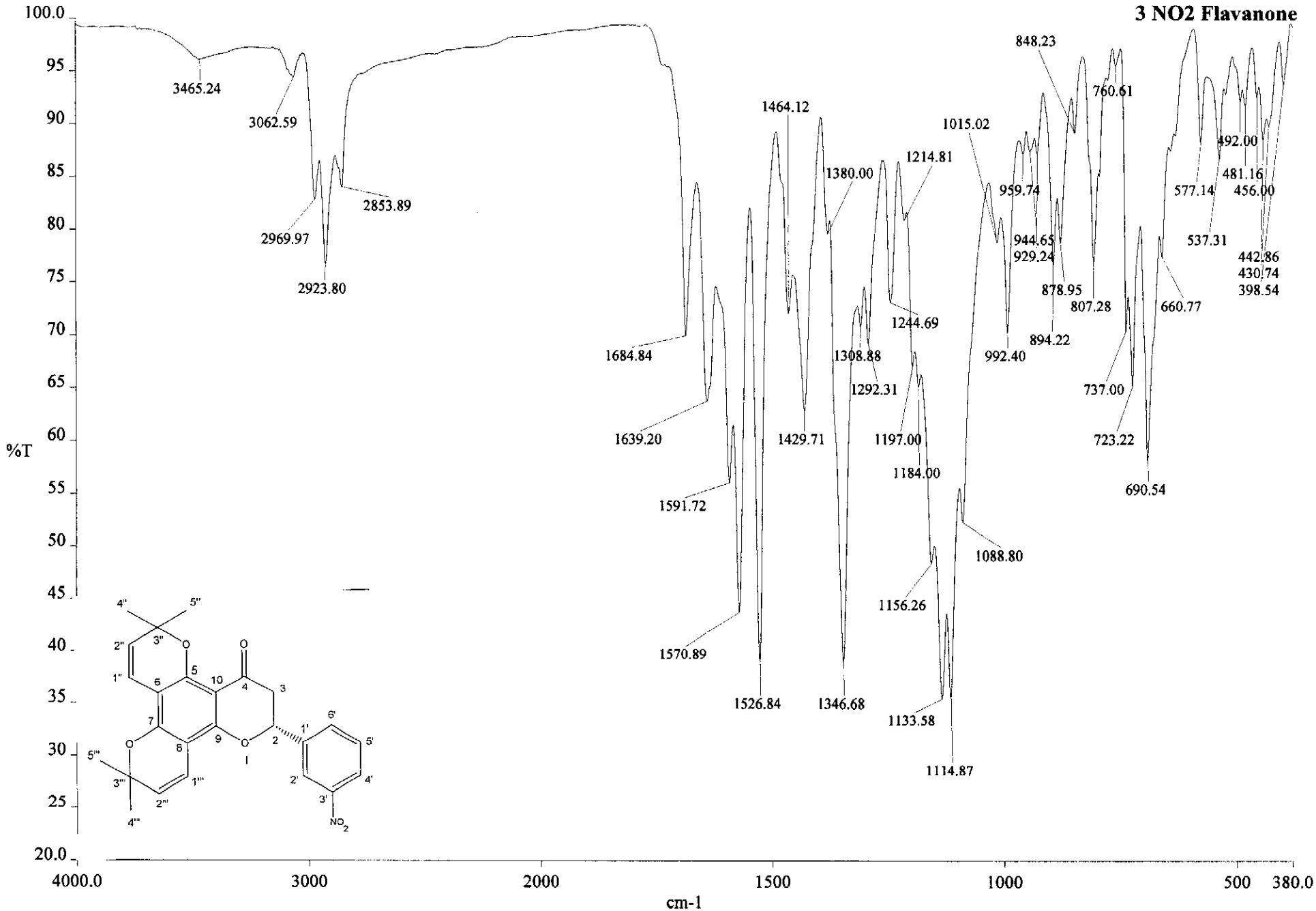
Expanded 13C NMR spectrum of 3'-nitropyranochromene flavanone (A-4l)



Expanded 13C NMR spectrum of 3'-nitropyranochromene flavanone (A-4l)

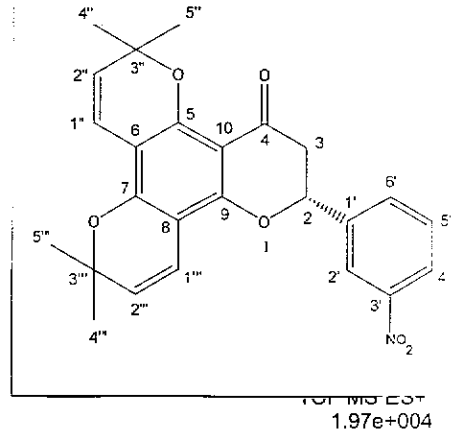


Expanded 13C NMR spectrum of 3'-nitropyranochromene flavanone (A-4l)



Infrared spectrum of 3'-nitropyranochromene flavanone (A-4l)

Elemental Composition Report

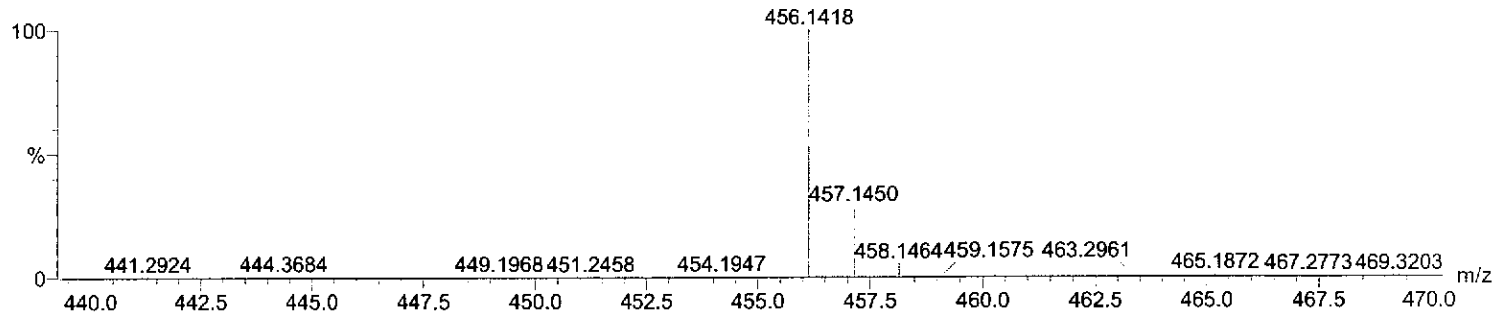


Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

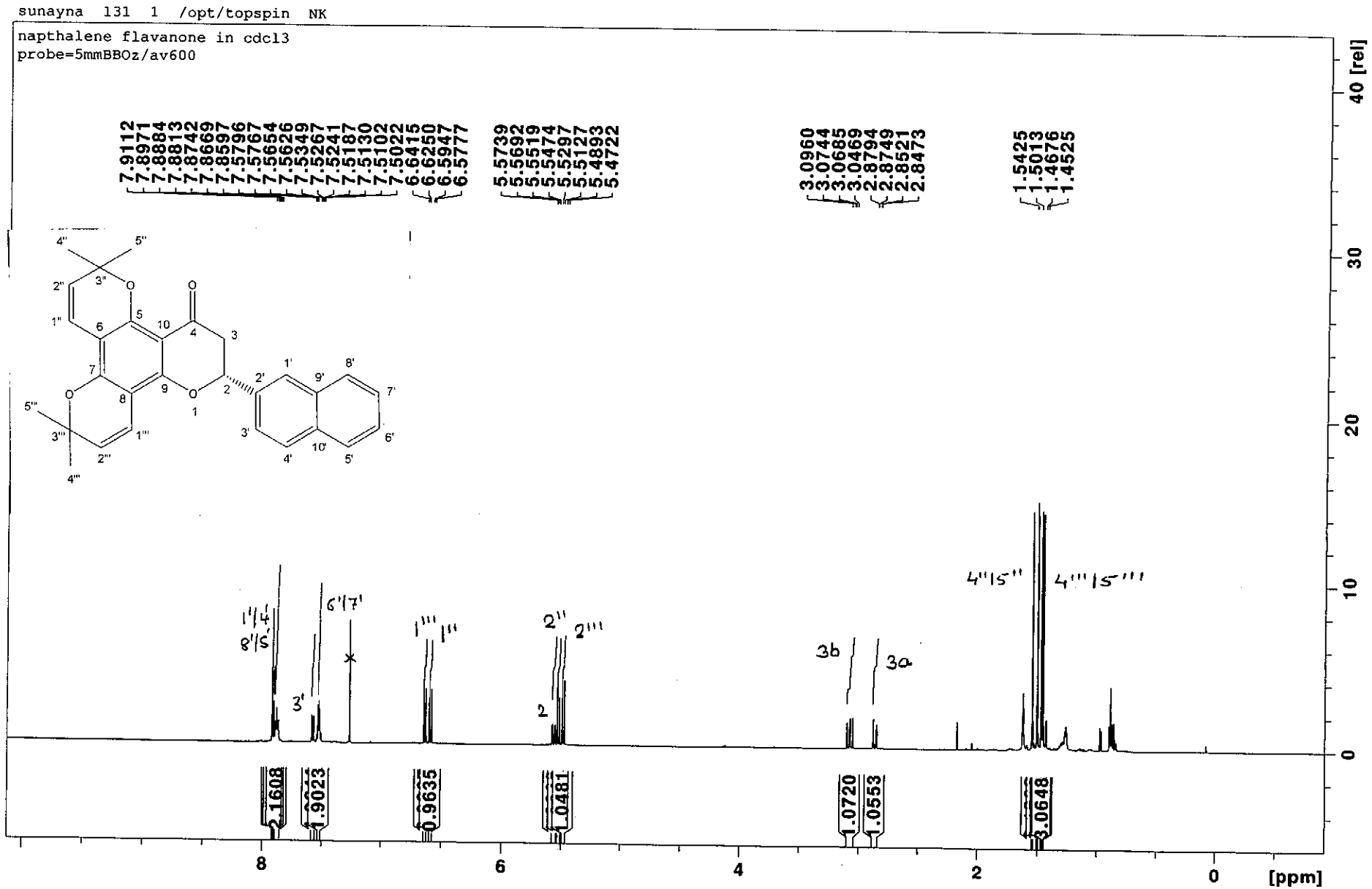
Monoisotopic Mass, Even Electron Ions
67 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-30 H: 20-25 N: 0-5 O: 0-10 Na: 0-1

Dr. Nizam
26 4 (0.052) Cm (1:31)

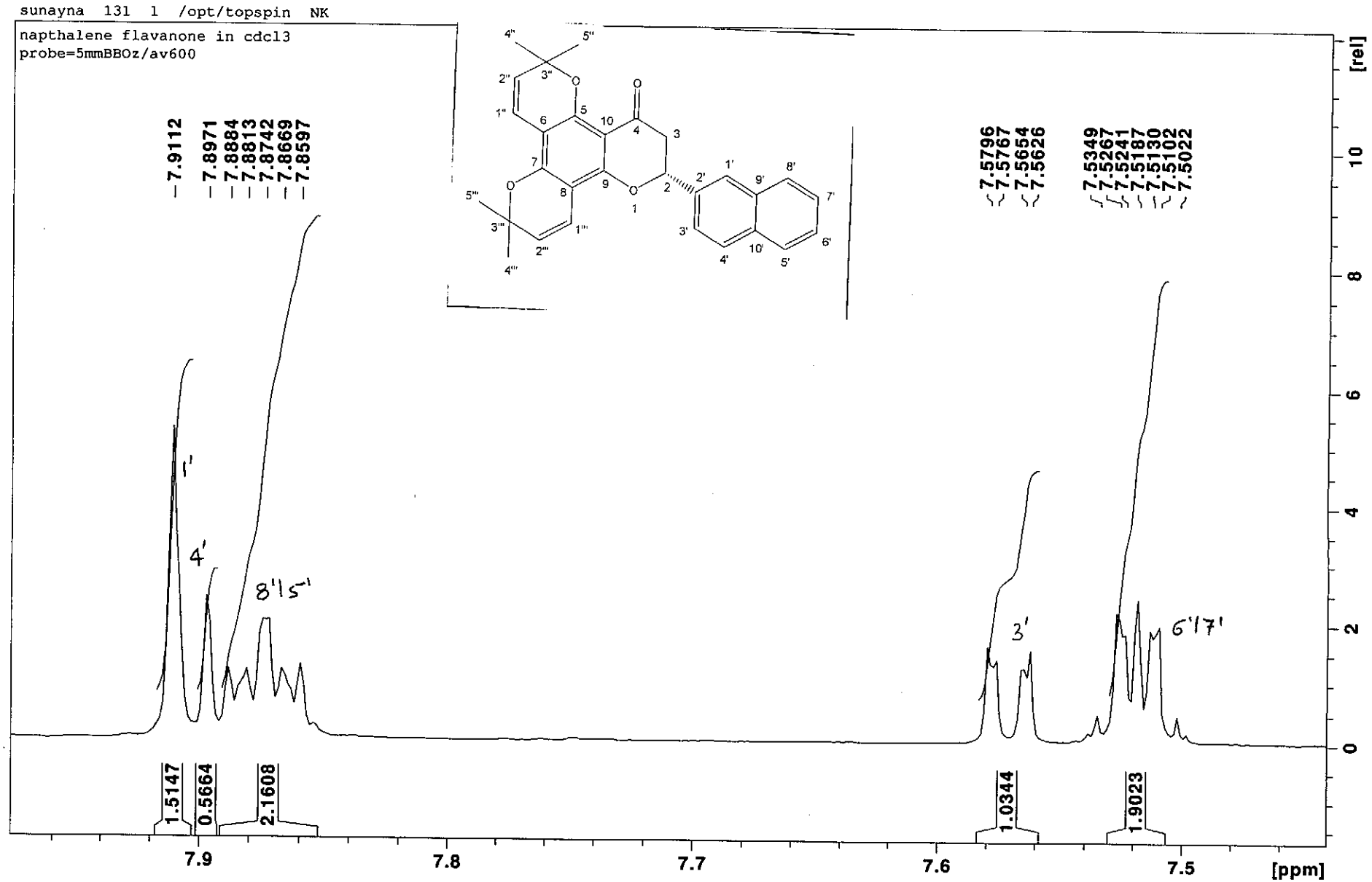


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
456.1418	456.1423	-0.5	-1.1	14.5	365.2	0.0	C25 H23 N O6 Na

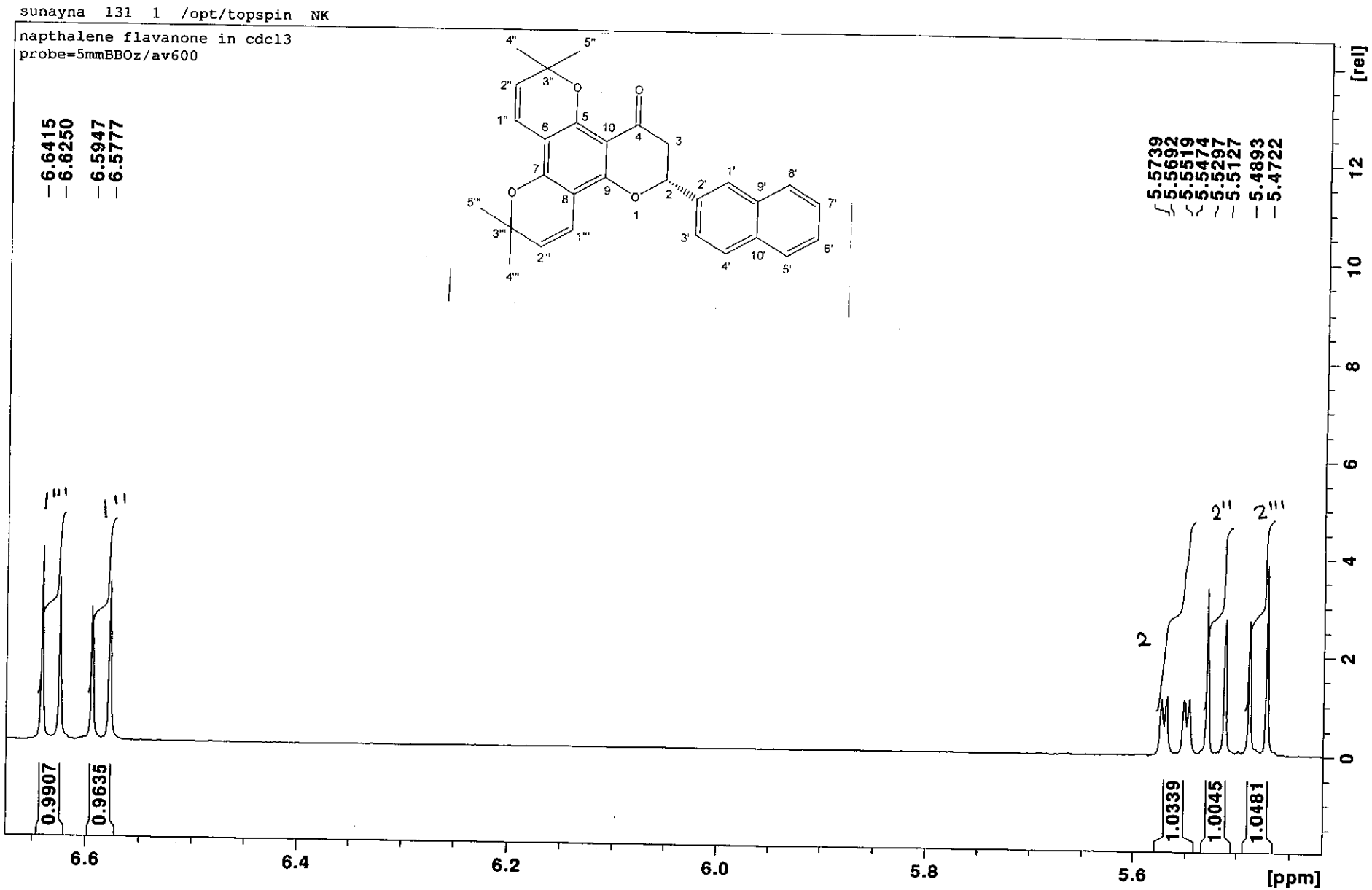
HRMS spectrum of 3'-nitropyranochromene flavanone (A-4l)



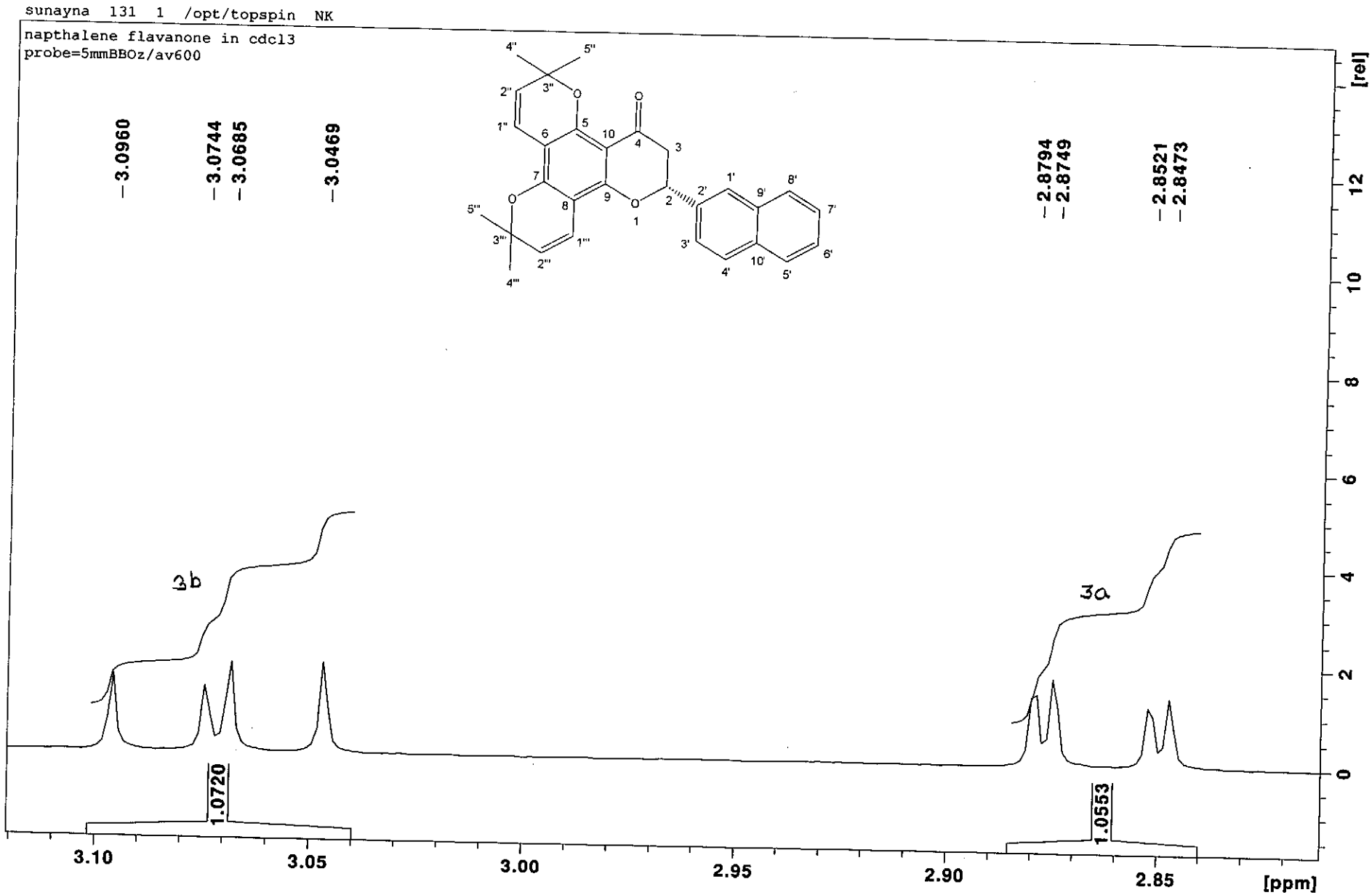
1H NMR spectrum of naphthalene pyranochromene flavanone (A-4m)



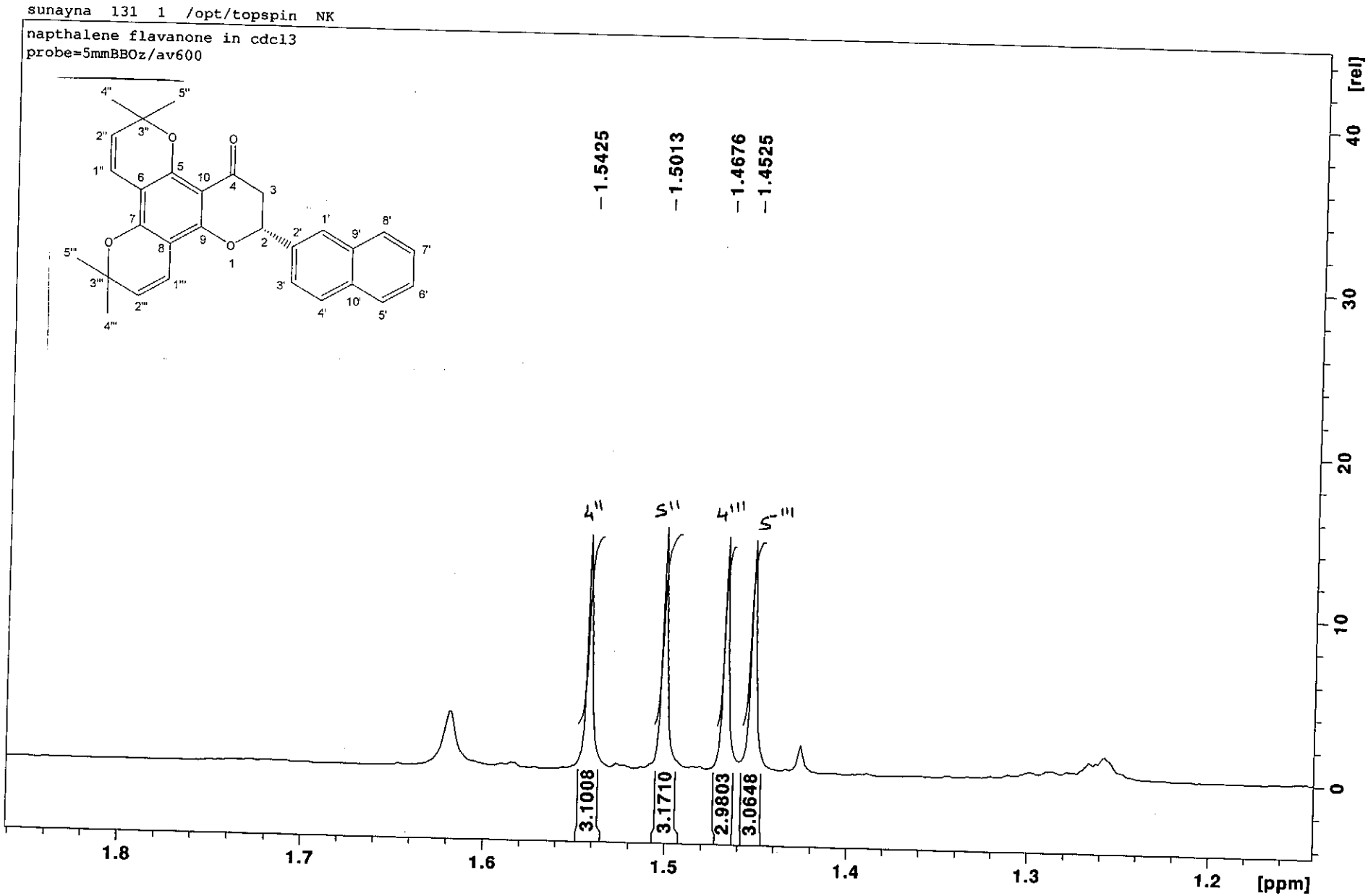
Expanded 1H NMR spectrum of naphthalene pyranochromene flavanone (A-4m)



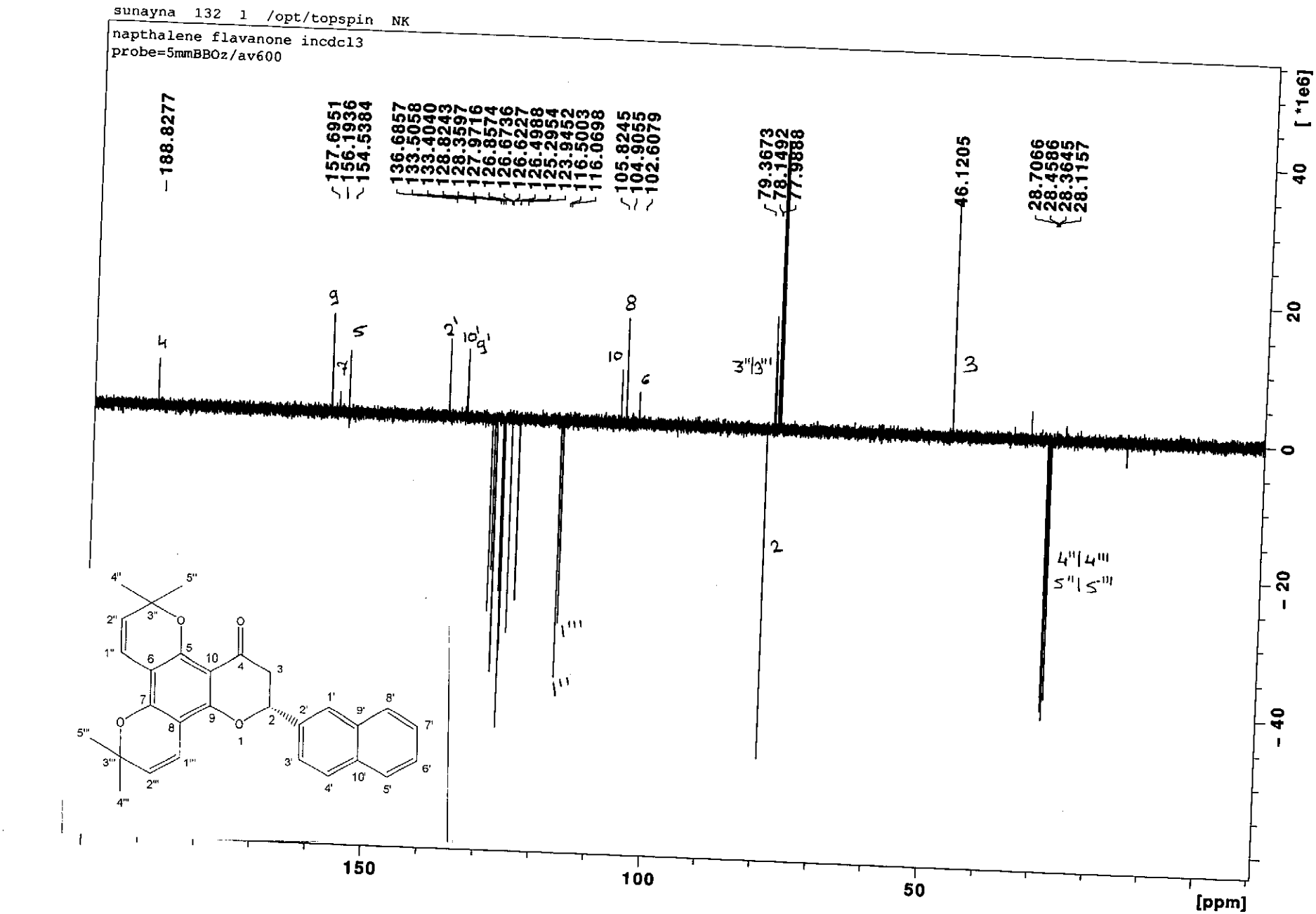
Expanded 1H NMR spectrum of naphthalene pyranochromene flavanone (A-4m)



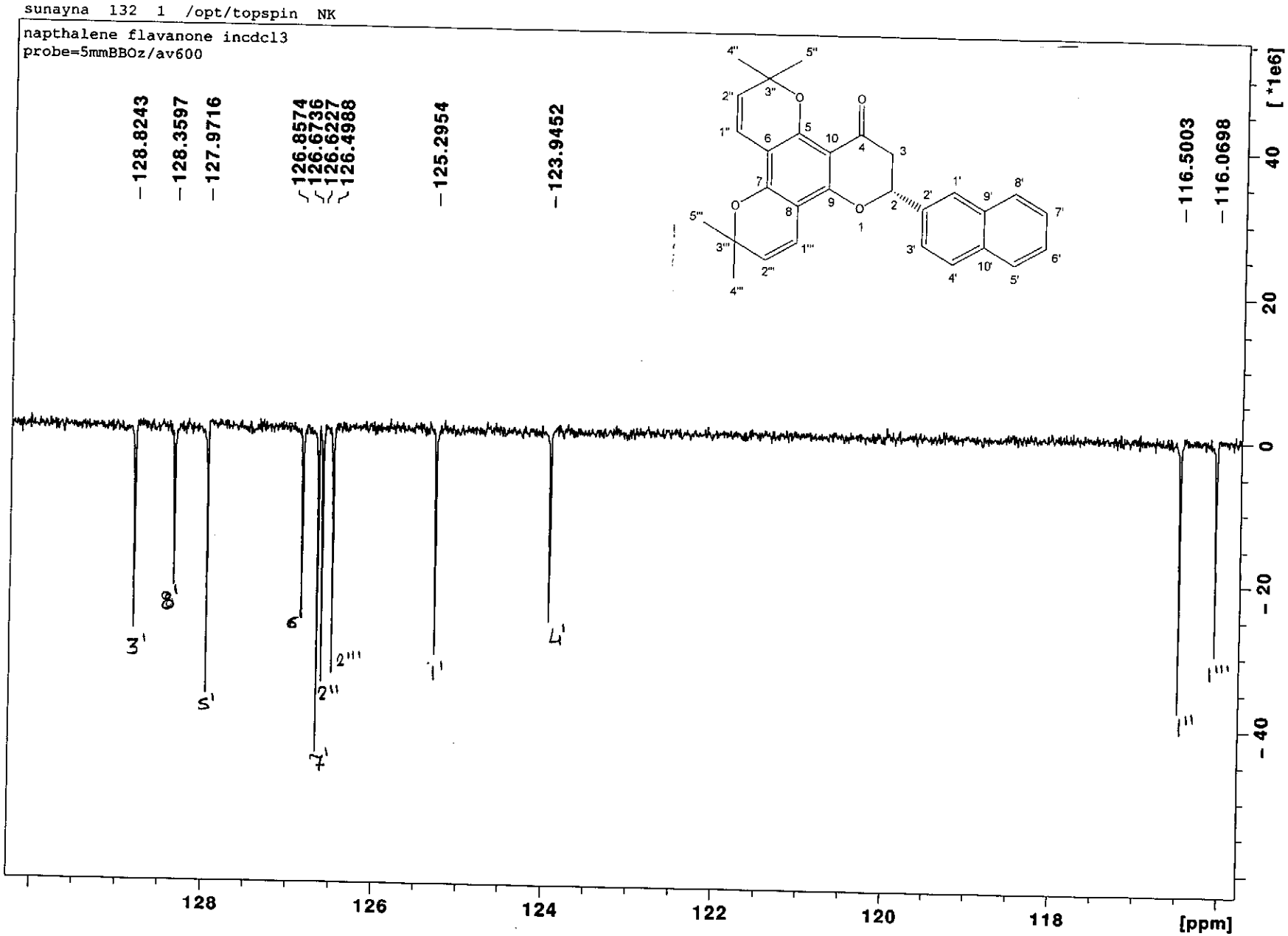
Expanded 1H NMR spectrum of naphthalene pyranochromene flavanone (A-4m)



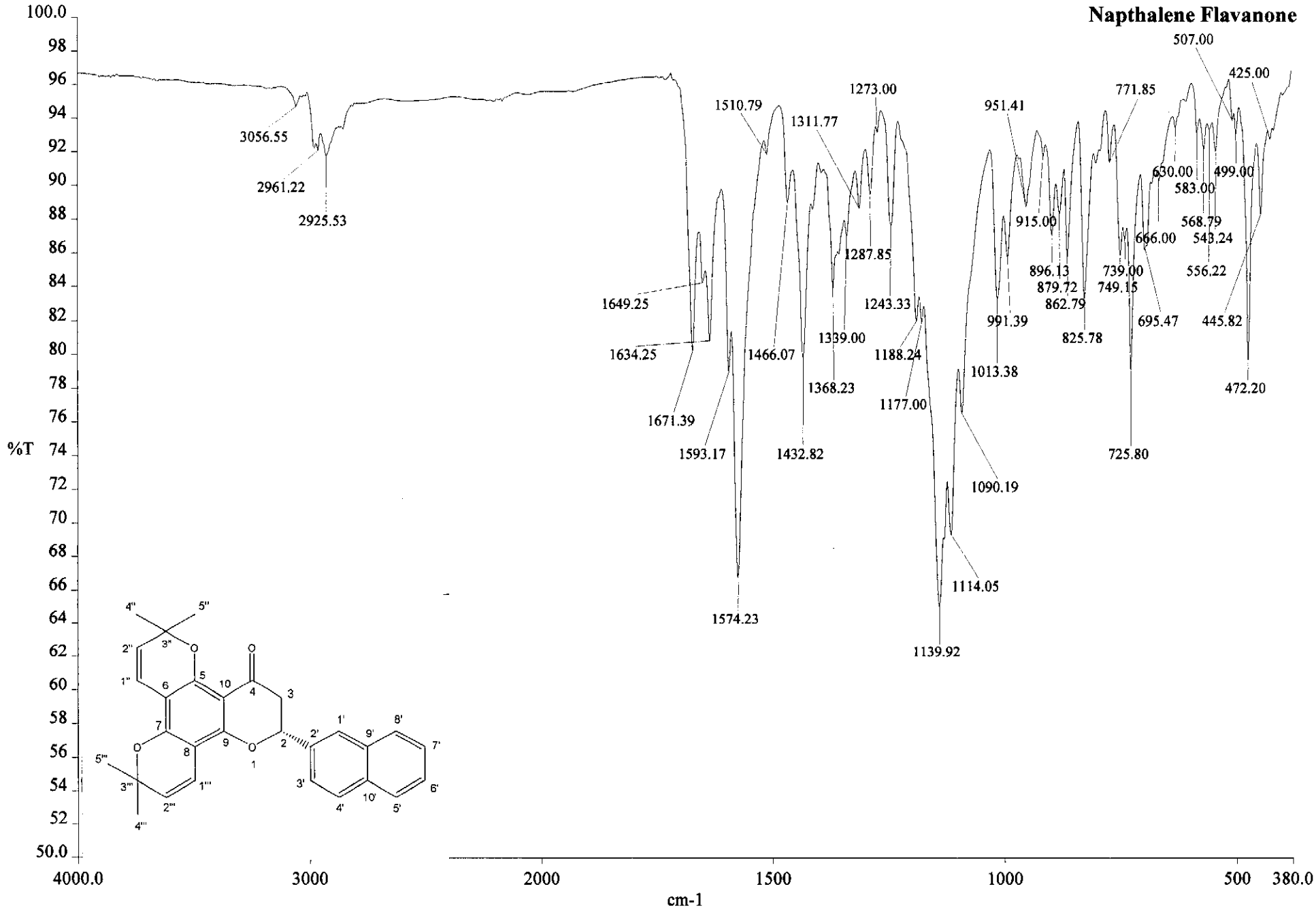
Expanded 1H NMR spectrum of naphthalene pyranochromene flavanone (A-4m)



13C NMR spectrum of naphthalene pyranochromene flavanone (A-4m)



Expanded 13C NMR spectrum of naphthalene pyranochromene flavanone (A-4m)



Infrared spectrum of naphthalene pyranochromene flavanone (A-4m)

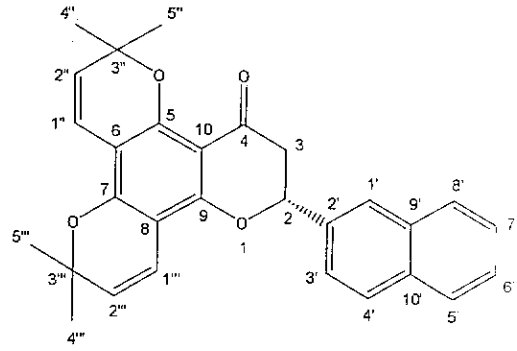
Elemental Composition Report

Single Mass Analysis

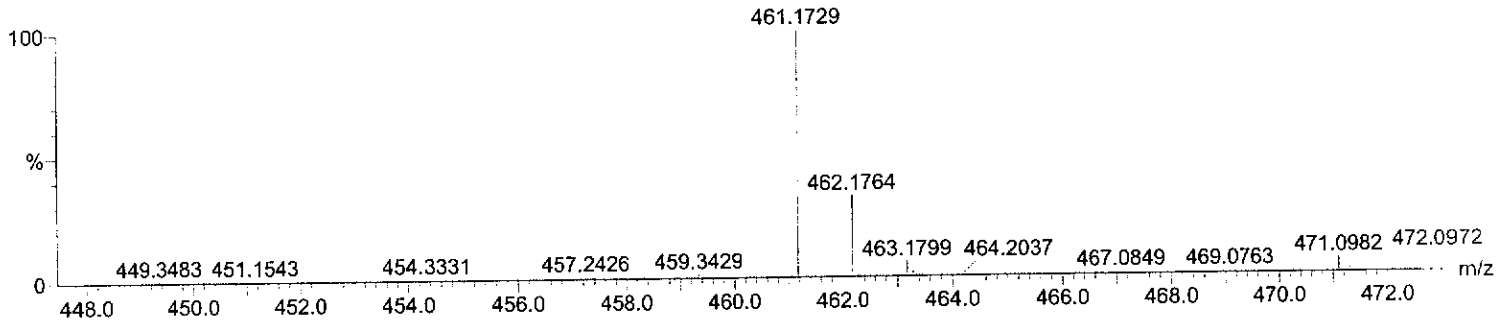
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
3 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-30 H: 25-30 O: 0-5 Na: 0-1

Dr. Nizam
23 4 (0.051) Cm (1:31)

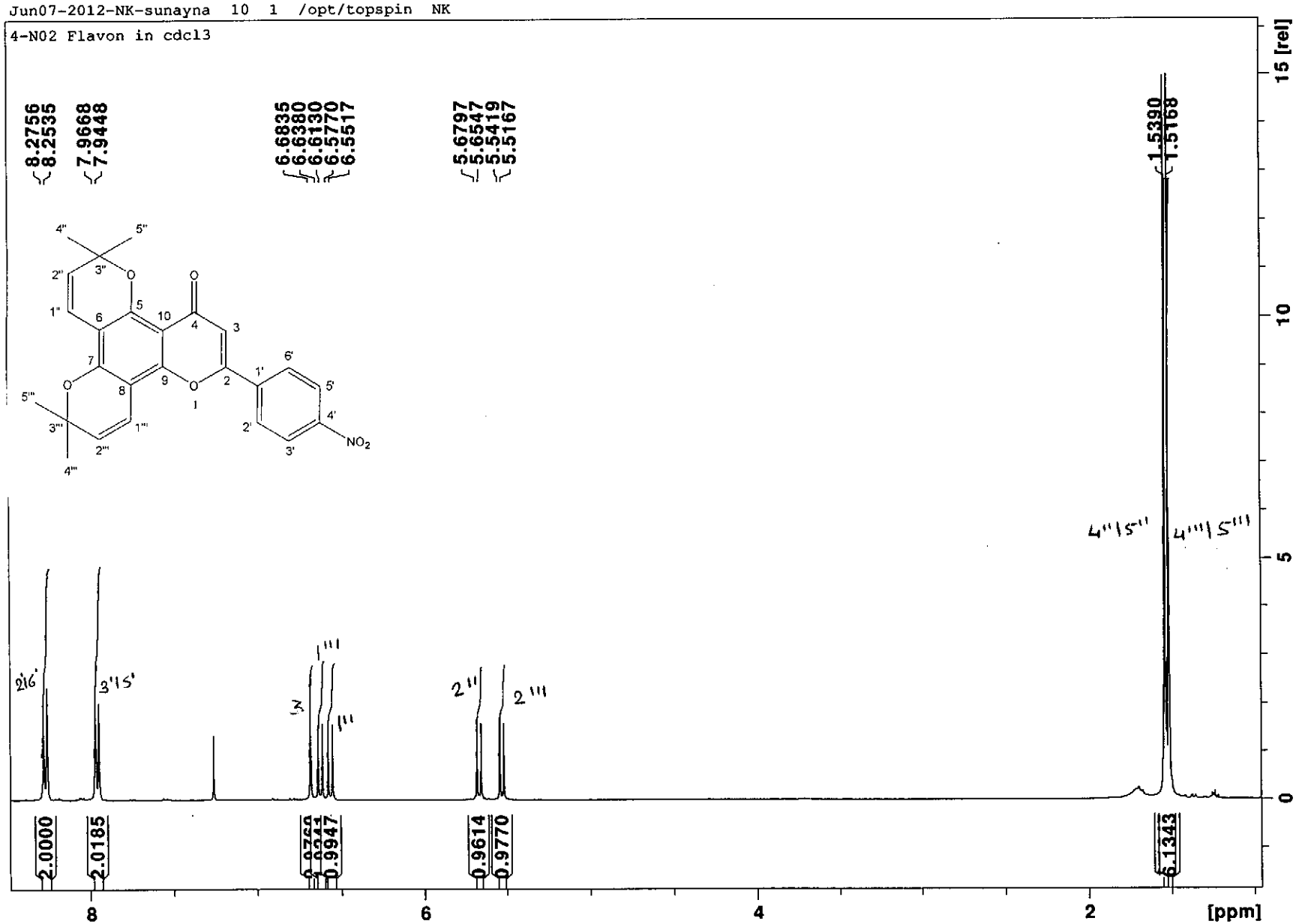


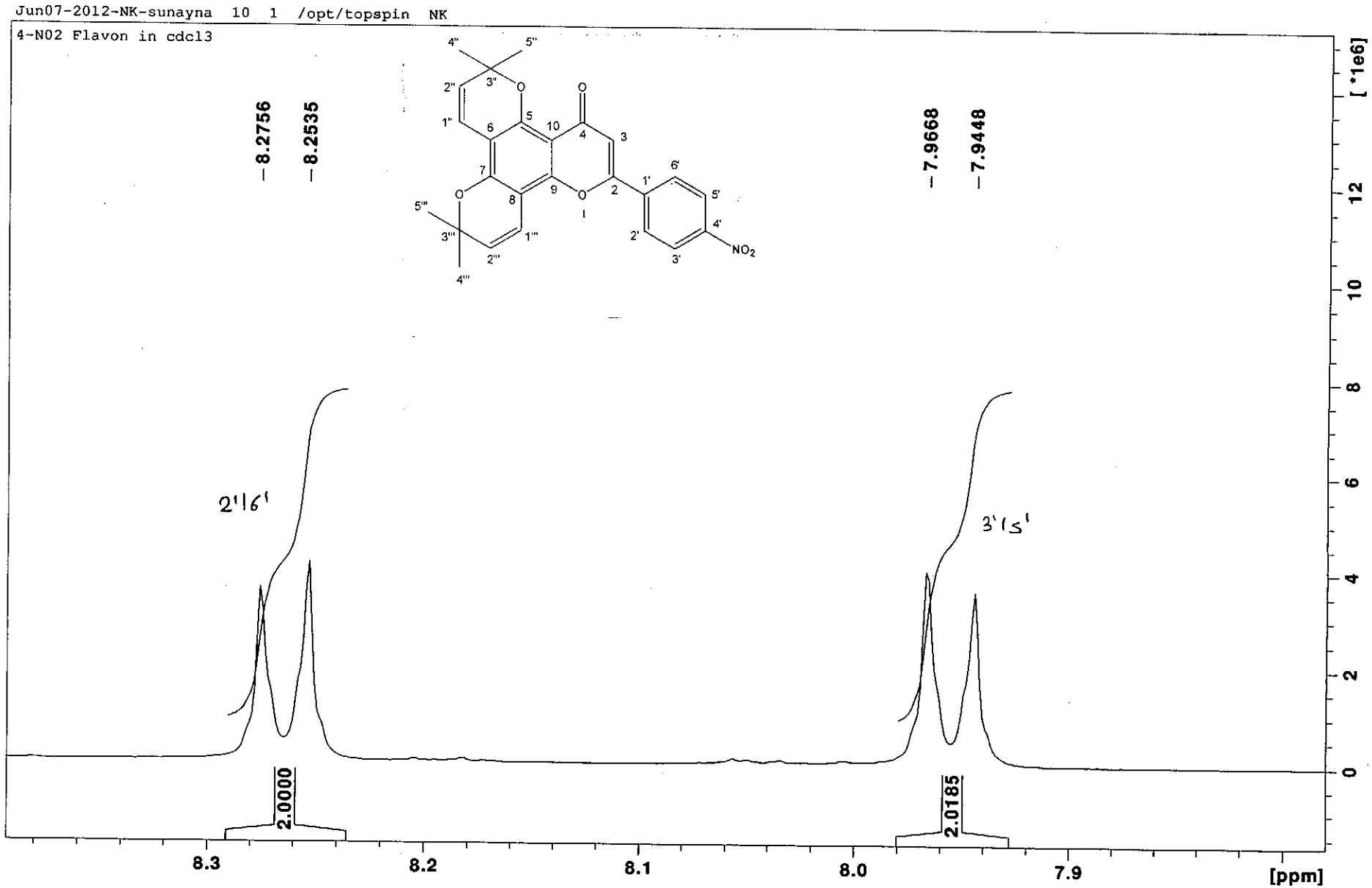
TOF MS ES+
8.13e+003

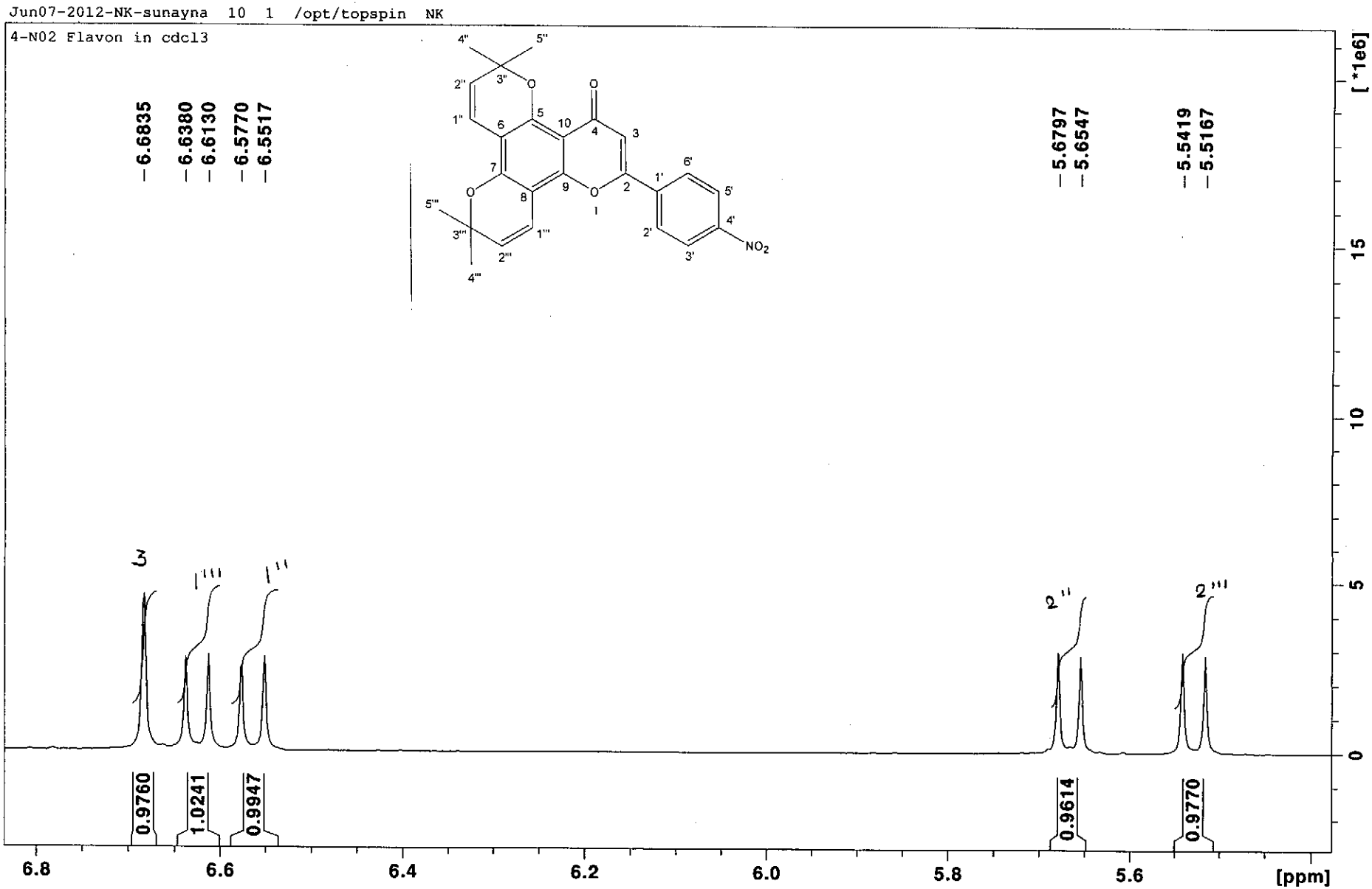


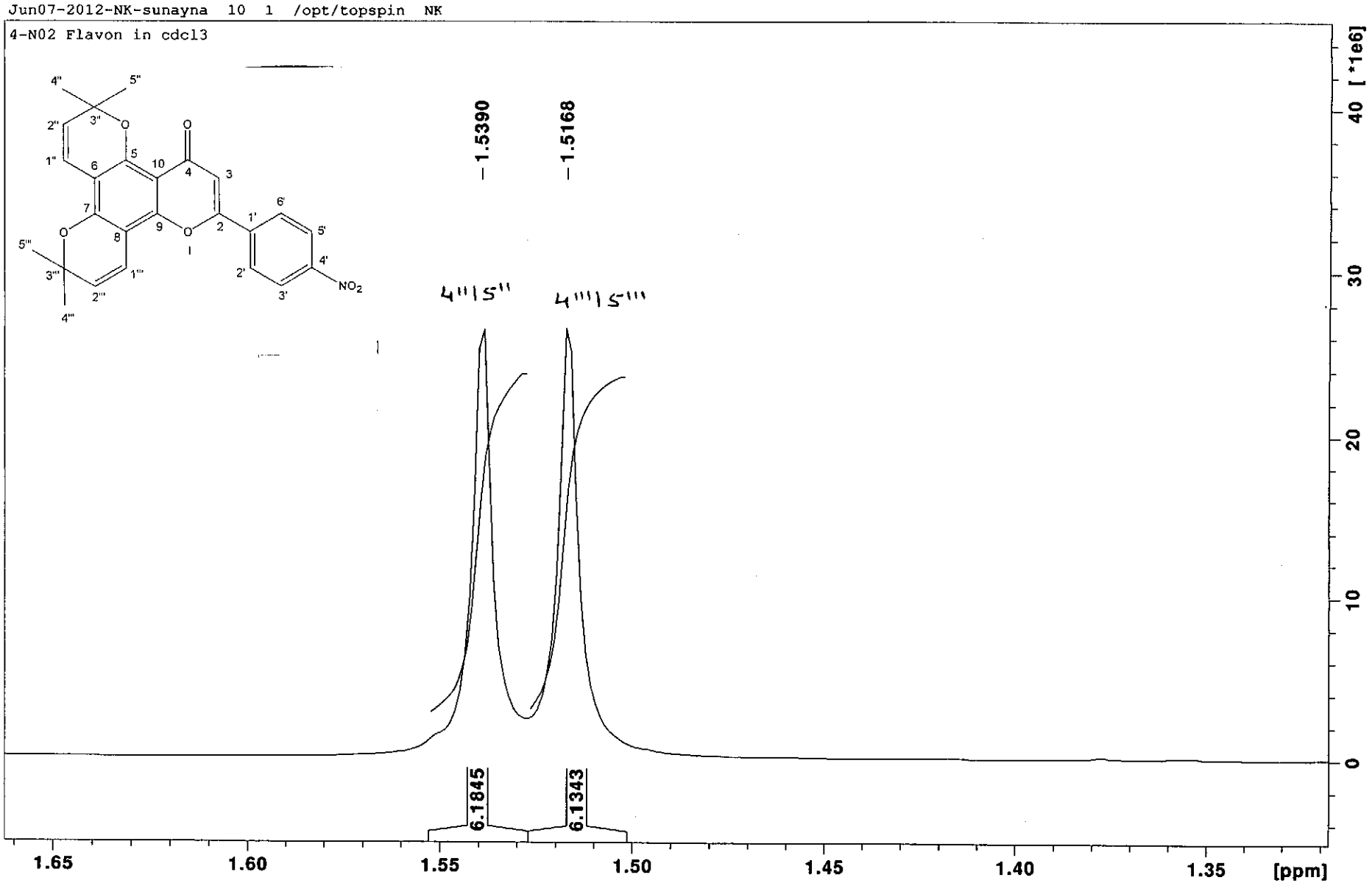
Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
461.1729	461.1729	0.0	0.0	16.5	355.1	0.0	C29 H26 O4 Na

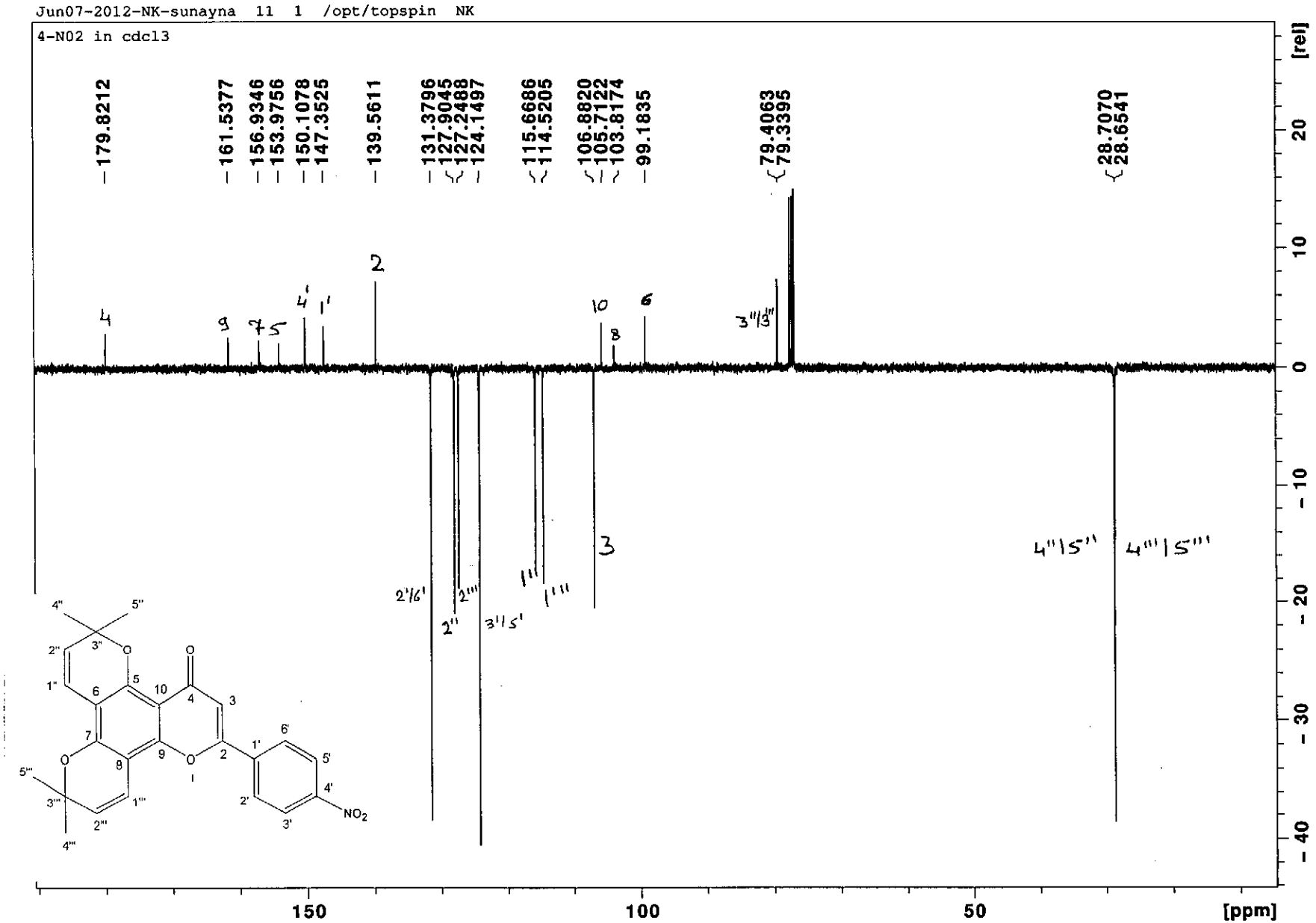
HRMS spectrum of naphthalene pyranochromene flavanone (A-4m)


Jun07-2012-NK-sunayna 10 1 /opt/topspin NK
4-N02 Flavon in cdcl3
8.2756
8.2535
7.9668
7.9448
6.6835
6.6380
6.6130
6.5770
6.5517
5.6797
5.6547
5.5419
5.5167
1.5390
1.5168
4''|5''
4'''|5'''
2'|6'
3'|5'
3
1'''
2''
2'''
2.0000
2.0185
0.9614
0.9770
6.1343
8
6
4
2
[ppm]
15 [rel]
10
5
0


Jun07-2012-NK-sunayna 10 1 /opt/topspin NK
4-N02 Flavon in cdc13
8.2756
8.2535
7.9668
7.9448
2'|6'
3'|5'
2.0000
2.0185
8.3
8.2
8.1
8.0
7.9
[ppm]
[*1e6]
NO2


Jun07-2012-NK-sunayna 10 1 /opt/topspin NK
4-N02 Flavon in cdcl3
6.6835
6.6380
6.6130
6.5770
6.5517
5.6797
5.6547
5.5419
5.5167
NO2
0.9760
1.0241
0.9947
0.9614
0.9770
6.8
6.6
6.4
6.2
6.0
5.8
5.6
[ppm]
[*1e6]


Jun07-2012-NK-sunayna 10 1 /opt/topspin NK
4-N02 Flavon in cdcl3
1.5390
1.5168
4''|5''
4'''|5'''
6.1845
6.1343
1.65
1.60
1.55
1.50
1.45
1.40
1.35
[ppm]
0
10
20
30
40
[*1e6]


Jun07-2012-NK-sunayna 11 1 /opt/topspin NK
4-N02 in cdcl3
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156.9346
153.9756
150.1078
147.3525
139.5611
131.3796
127.9045
127.2488
124.1497
115.6686
114.5205
106.8820
105.7122
103.8174
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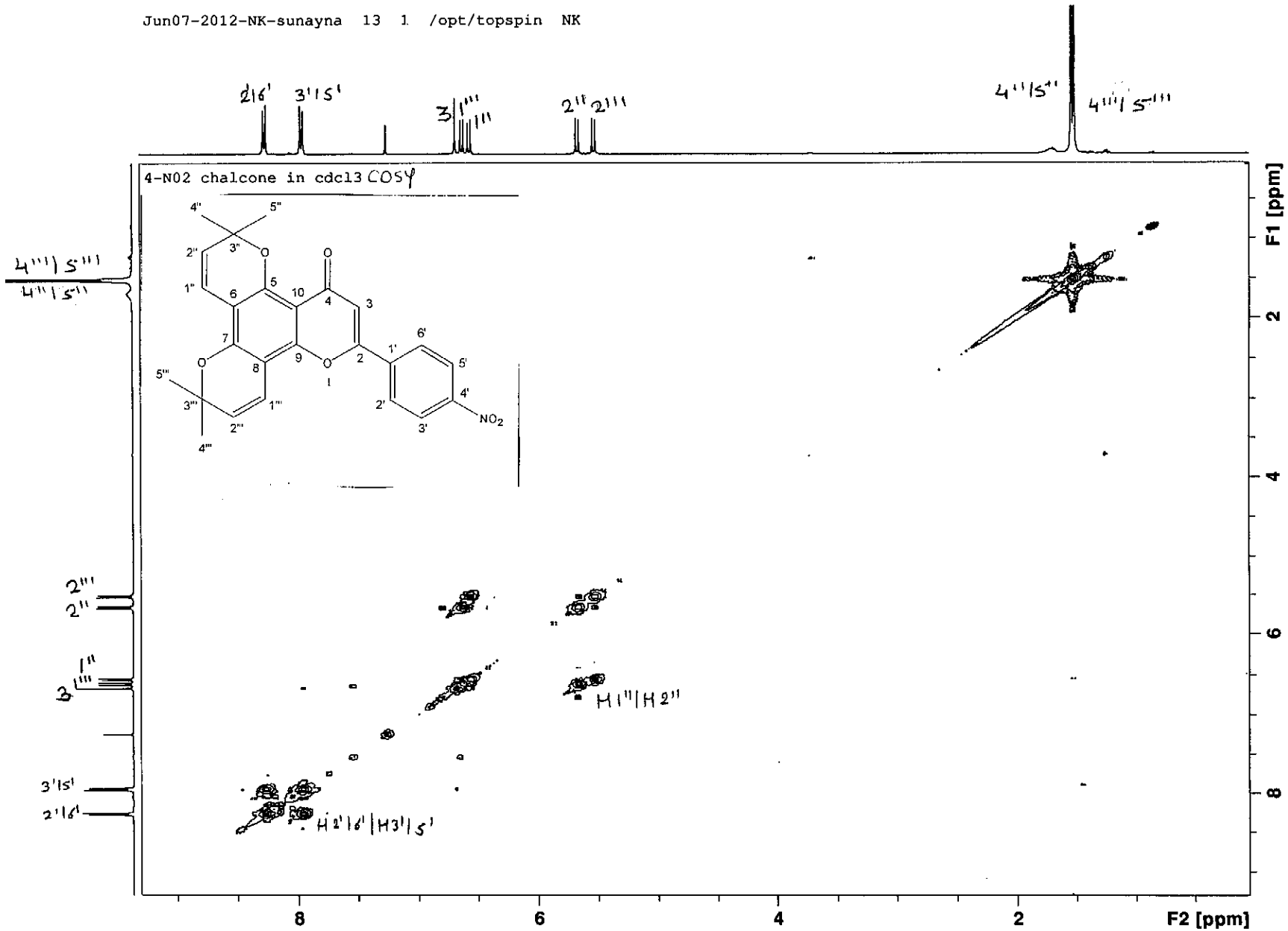
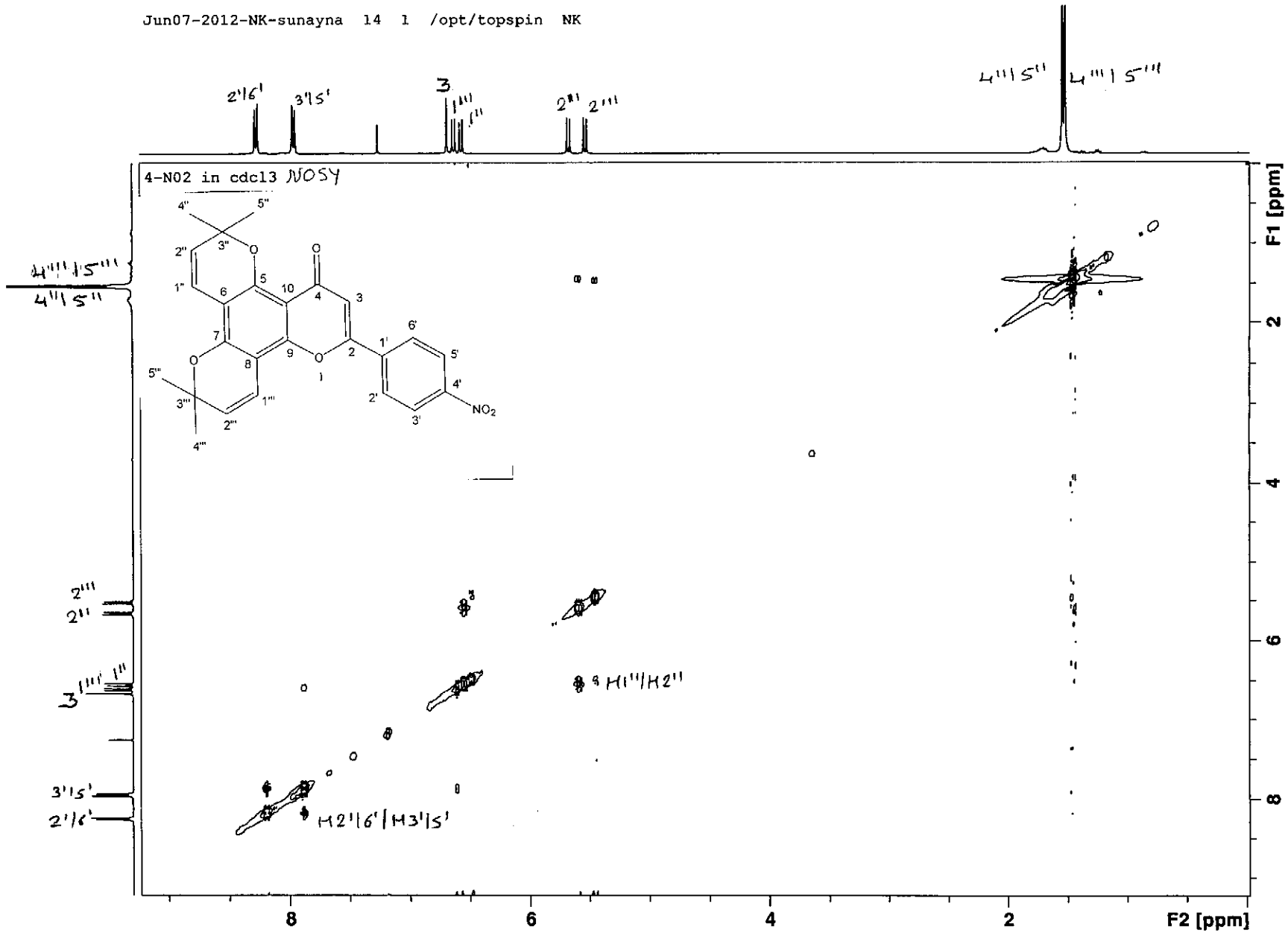
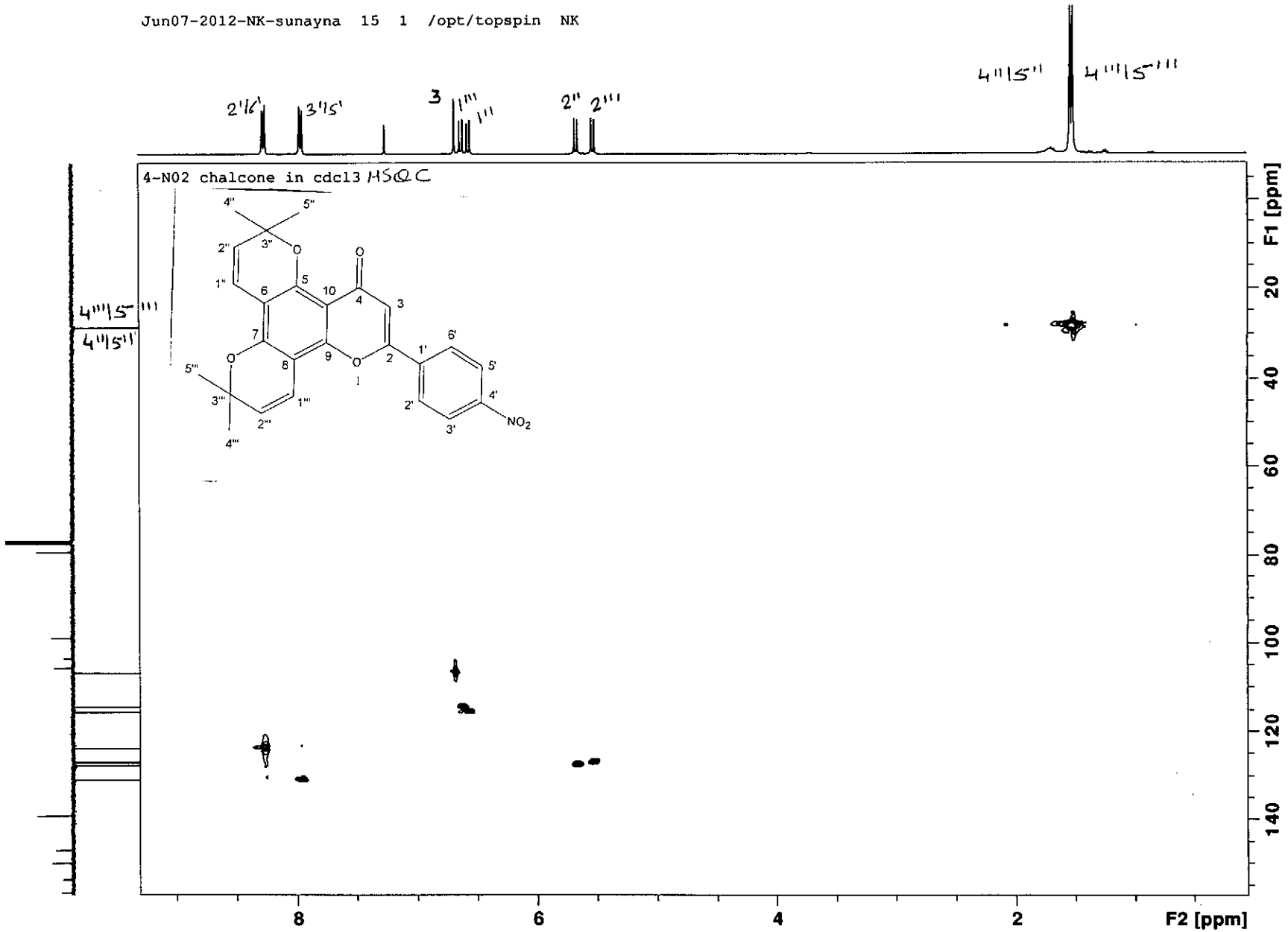
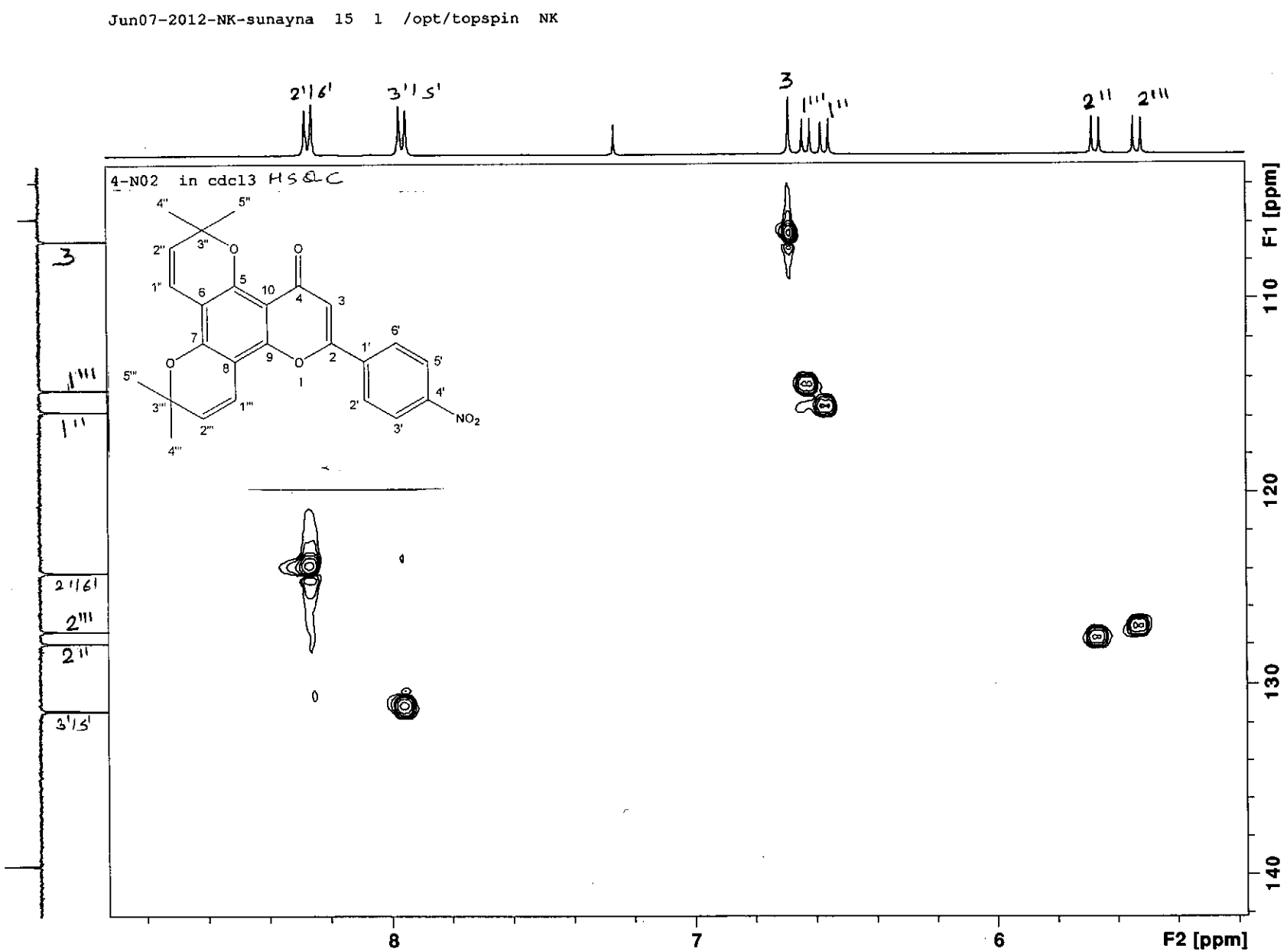
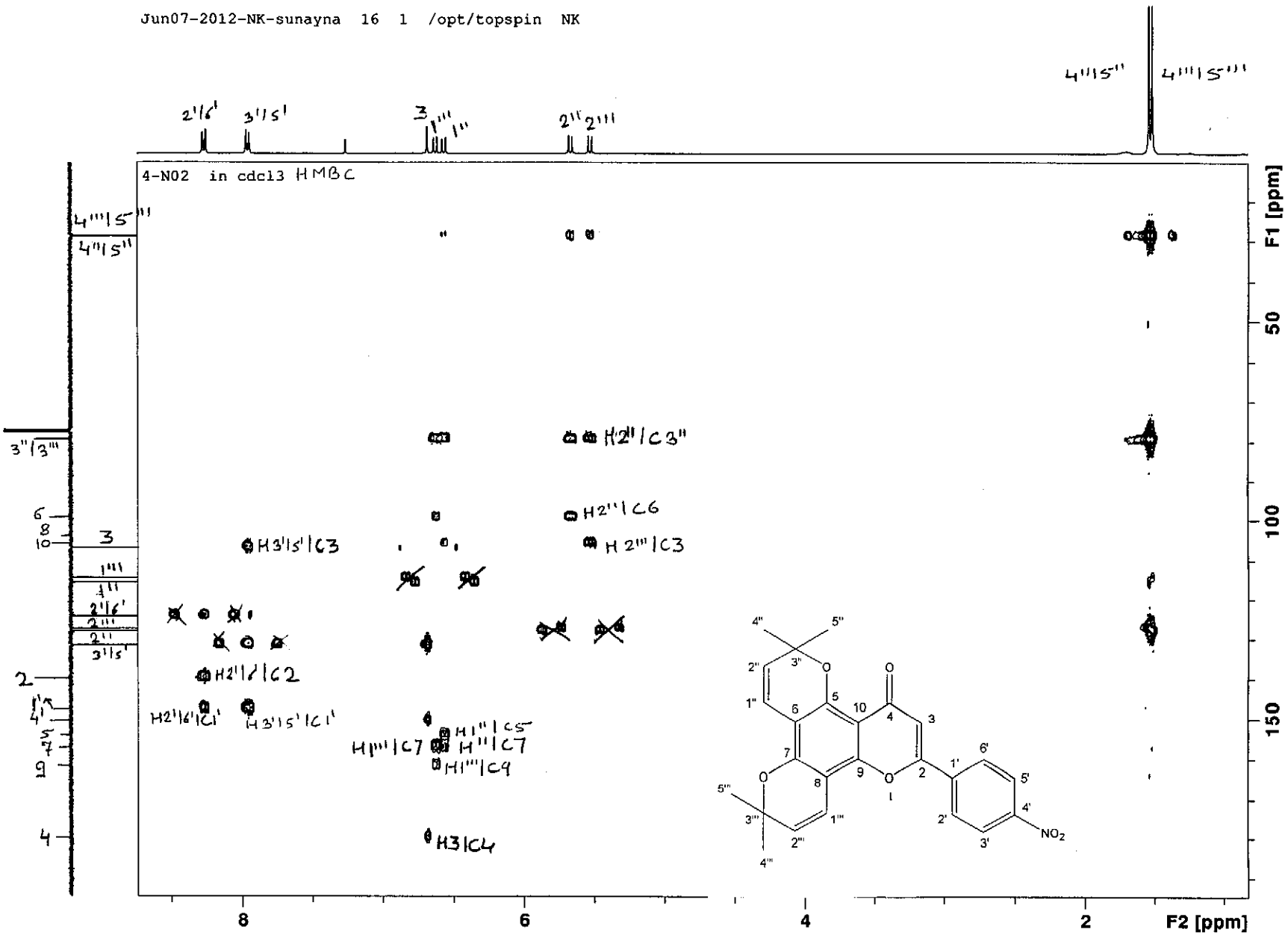
150
100
50
NO2

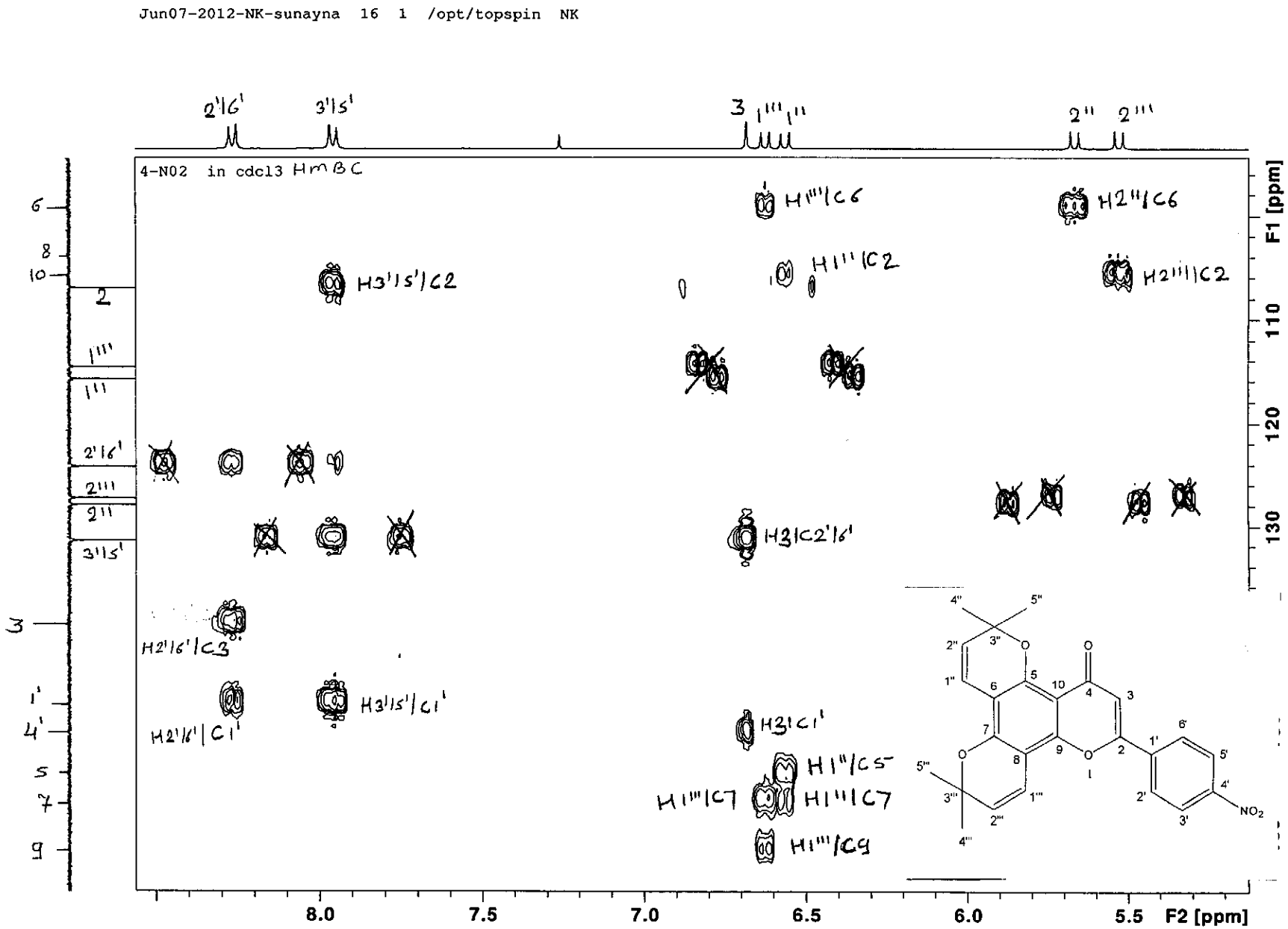

Jun07-2012-NK-sunayna 13 1 /opt/topspin NK
4-N02 chalcone in cdcl3 COSY
F1 [ppm]
F2 [ppm]
H1''|H2''
H2'|6'|H3'|5'

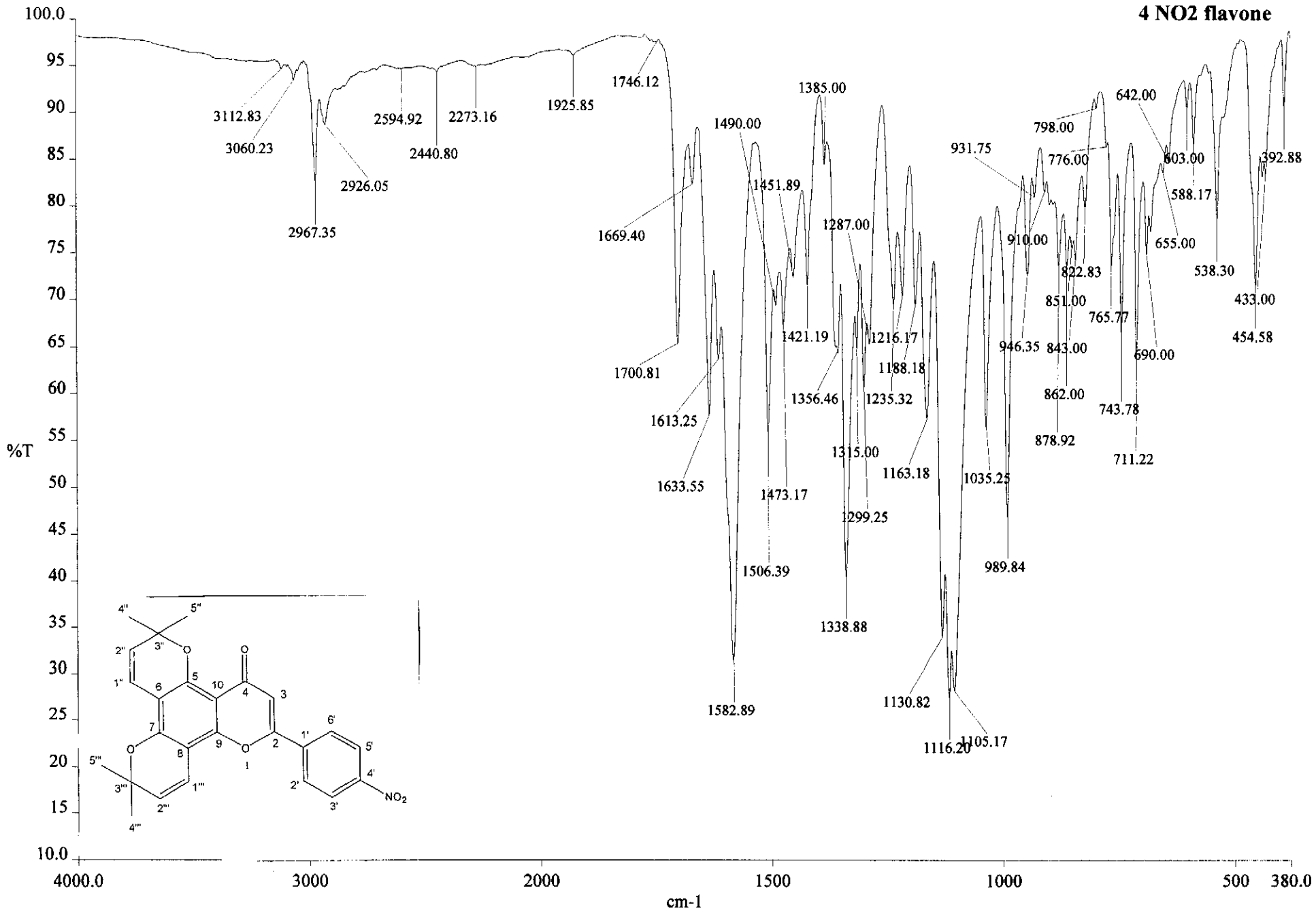

Jun07-2012-NK-sunayna 14 1 /opt/topspin NK
4-N02 in cdcl3 NOSY
F1 [ppm]
F2 [ppm]
H1'''/H2''
H2'/6'/H3'/5'


Jun07-2012-NK-sunayna 15 1 /opt/topspin NK
2'16'
3'15'
3
1''' 1'''
2'' 2'''
4''15''
4'''15'''
4-N02 chalcone in cdcl3 HSQC
4'''15'''
4''15''
F1 [ppm]
F2 [ppm]
8
6
4
2
20
40
60
80
100
120
140


Jun07-2012-NK-sunayna 15 1 /opt/topspin NK
2'/6'
3'/5'
3
1'''
1''
2'''
2''
4-N02 in cdcl3 HSQC
F1 [ppm]
110
120
130
140
8
7
6
F2 [ppm]


Jun07-2012-NK-sunayna 16 1 /opt/topspin NK
4-N02 in cdcl3 HMBC
H2''/C3''
H2'''/C6
H 2'''/C3
H3'5'/C3
H2'/C2
H2'6'/C1'
H3'5'/C1'
H1'''/C5
H1'''/C7
H1''/C7
H1'''/C9
H3/C4
F1 [ppm]
F2 [ppm]
NO2


Jun07-2012-NK-sunayna 16 1 /opt/topspin NK
4-N02 in cdcl3 HmBC
H3'1s'/C2
H1'''/C6
H2'''/C6
H1'''/C2
H2'''/C2
H3/C2'16'
H2'16'/C3
H2'16'/C1'
H3'1s'/C1'
H3/C1'
H1'''/C5
H1'''/C7
H1'''/C7
H1'''/C9
F1 [ppm]
F2 [ppm]
8.0
7.5
7.0
6.5
6.0
5.5


4 NO2 flavone
100.0
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
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15
10.0
%T
4000.0
3000
2000
1500
1000
500
380.0
cm-1
3112.83
3060.23
2967.35
2926.05
2594.92
2440.80
2273.16
1925.85
1746.12
1669.40
1700.81
1613.25
1633.55
1582.89
1490.00
1451.89
1421.19
1473.17
1506.39
1385.00
1287.00
1356.46
1315.00
1299.25
1338.88
1216.17
1188.18
1235.32
1163.18
1130.82
1116.20
1105.17
1035.25
989.84
931.75
910.00
946.35
798.00
776.00
822.83
851.00
843.00
862.00
878.92
642.00
603.00
588.17
655.00
765.77
743.78
711.22
690.00
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392.88
NO2
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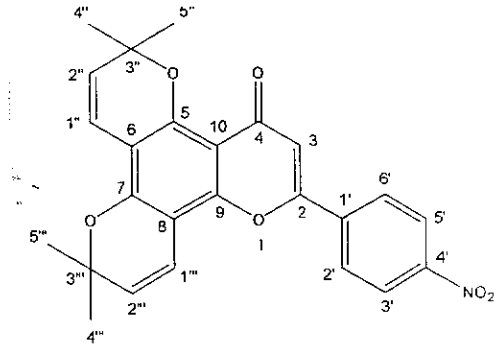
Elemental Composition Report



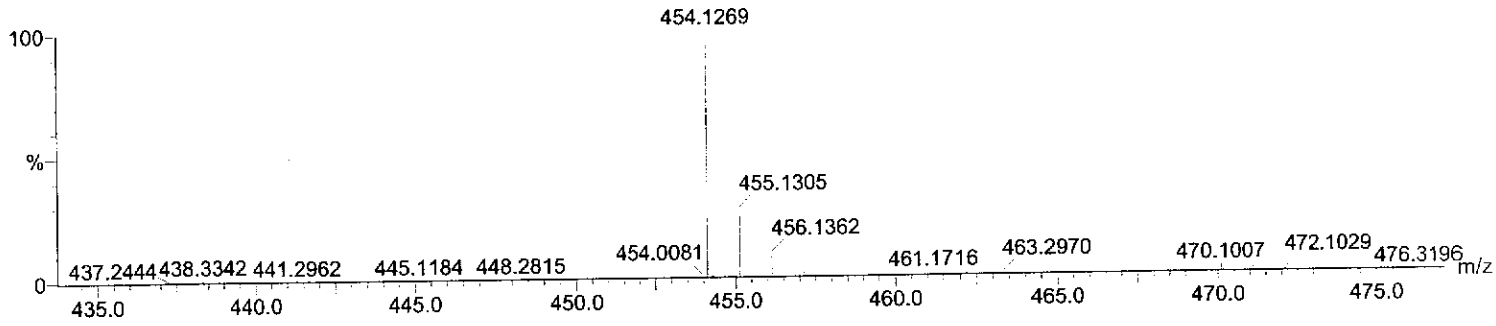
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

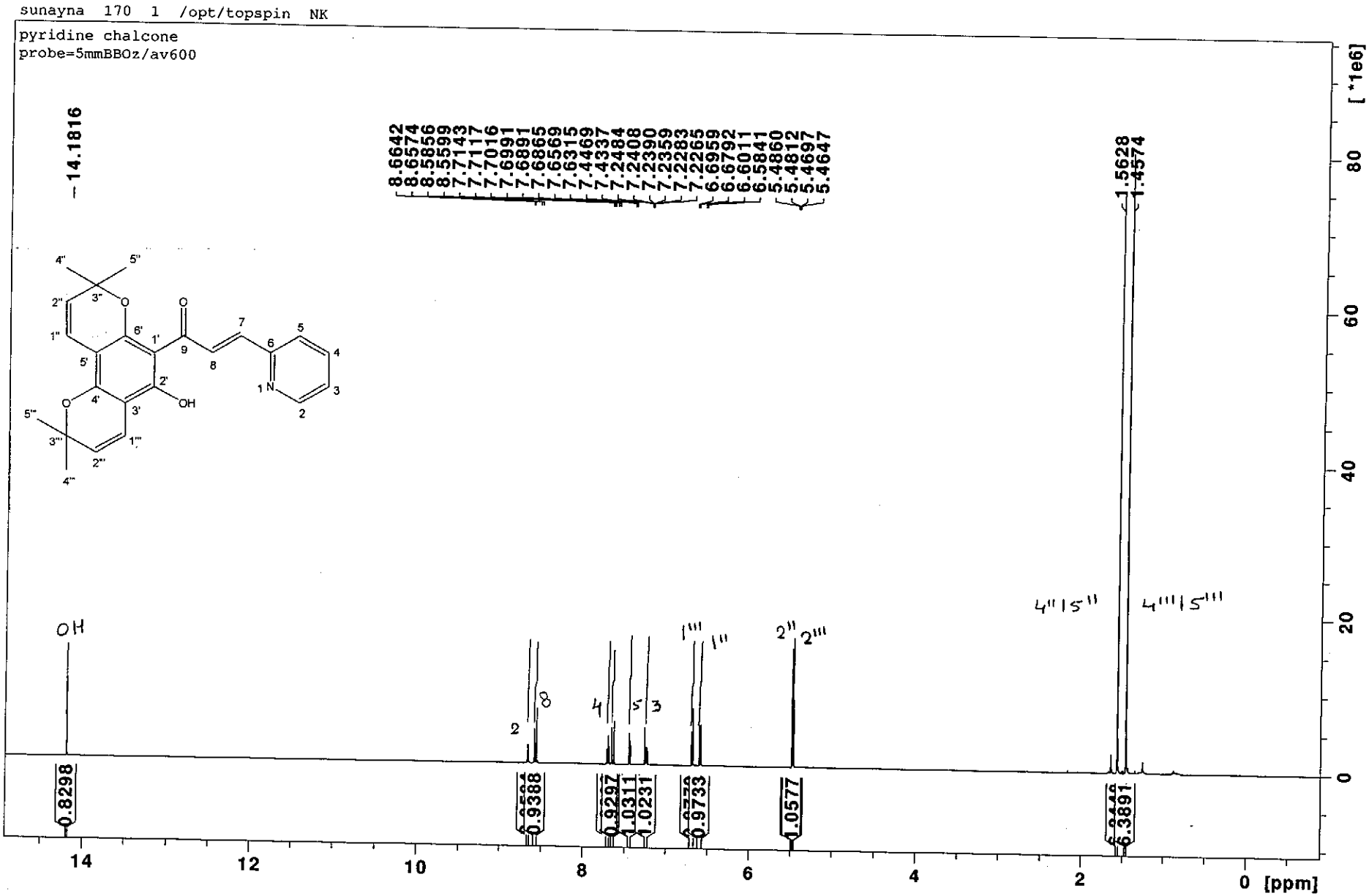
Monoisotopic Mass, Even Electron Ions
69 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-26 H: 20-25 N: 0-5 O: 0-10 Na: 0-1

Dr. Nizam
27 18 (0.291) Cm (1:31)

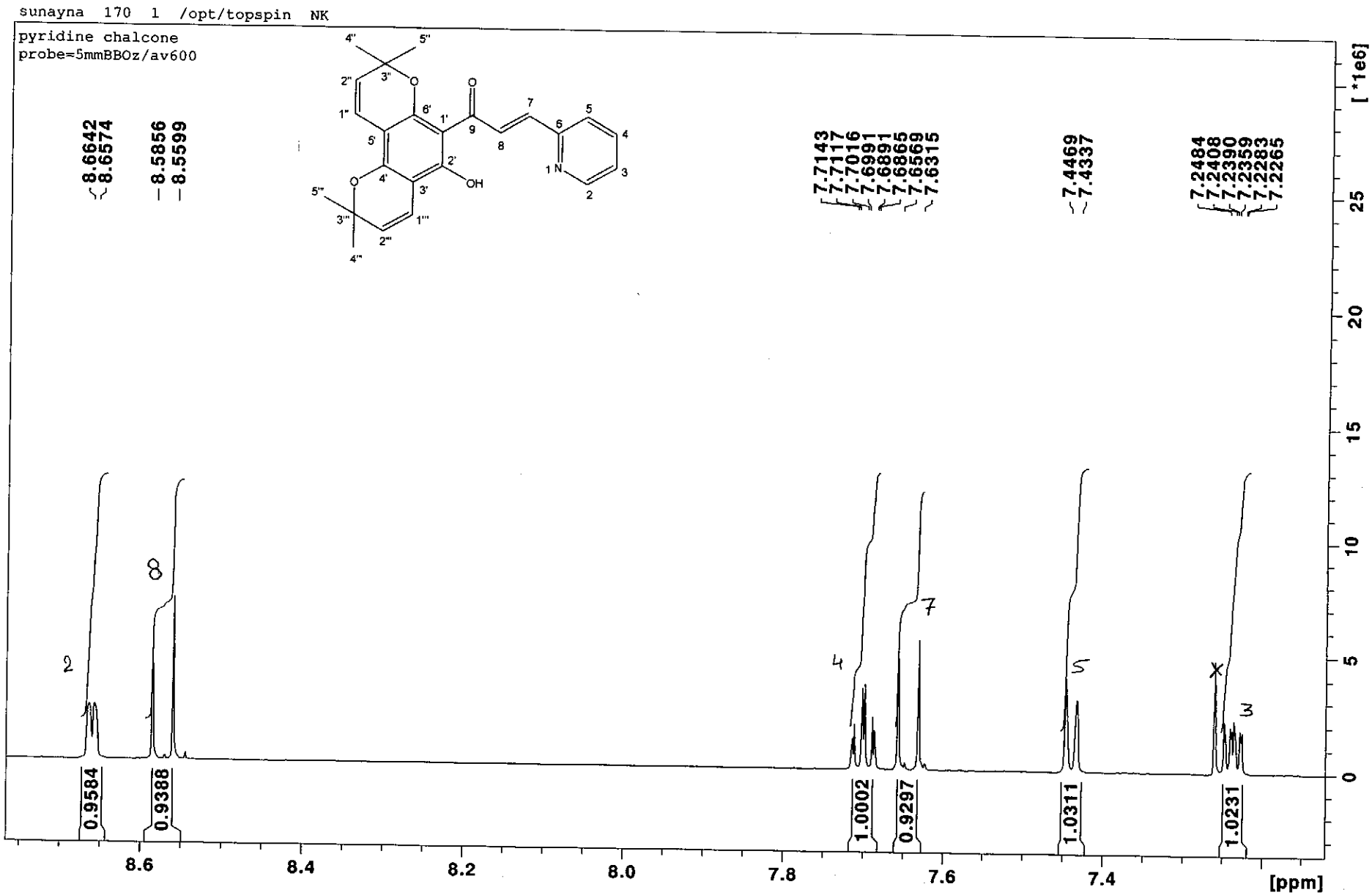
TOF MS ES+
2.11e+004



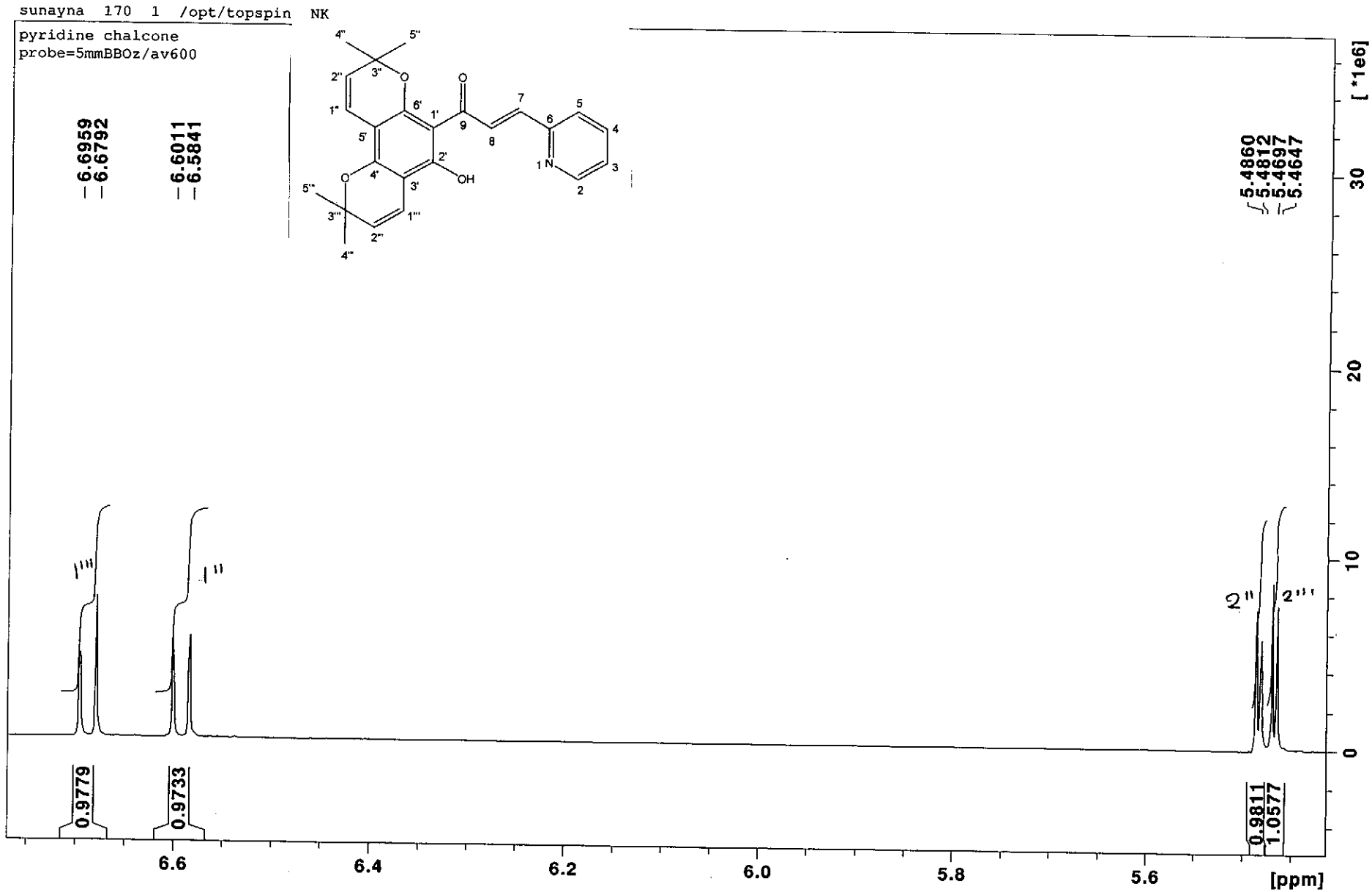
Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
454.1269	454.1267	0.2	0.4	15.5	369.6	0.0	C25 H21 N O6 Na



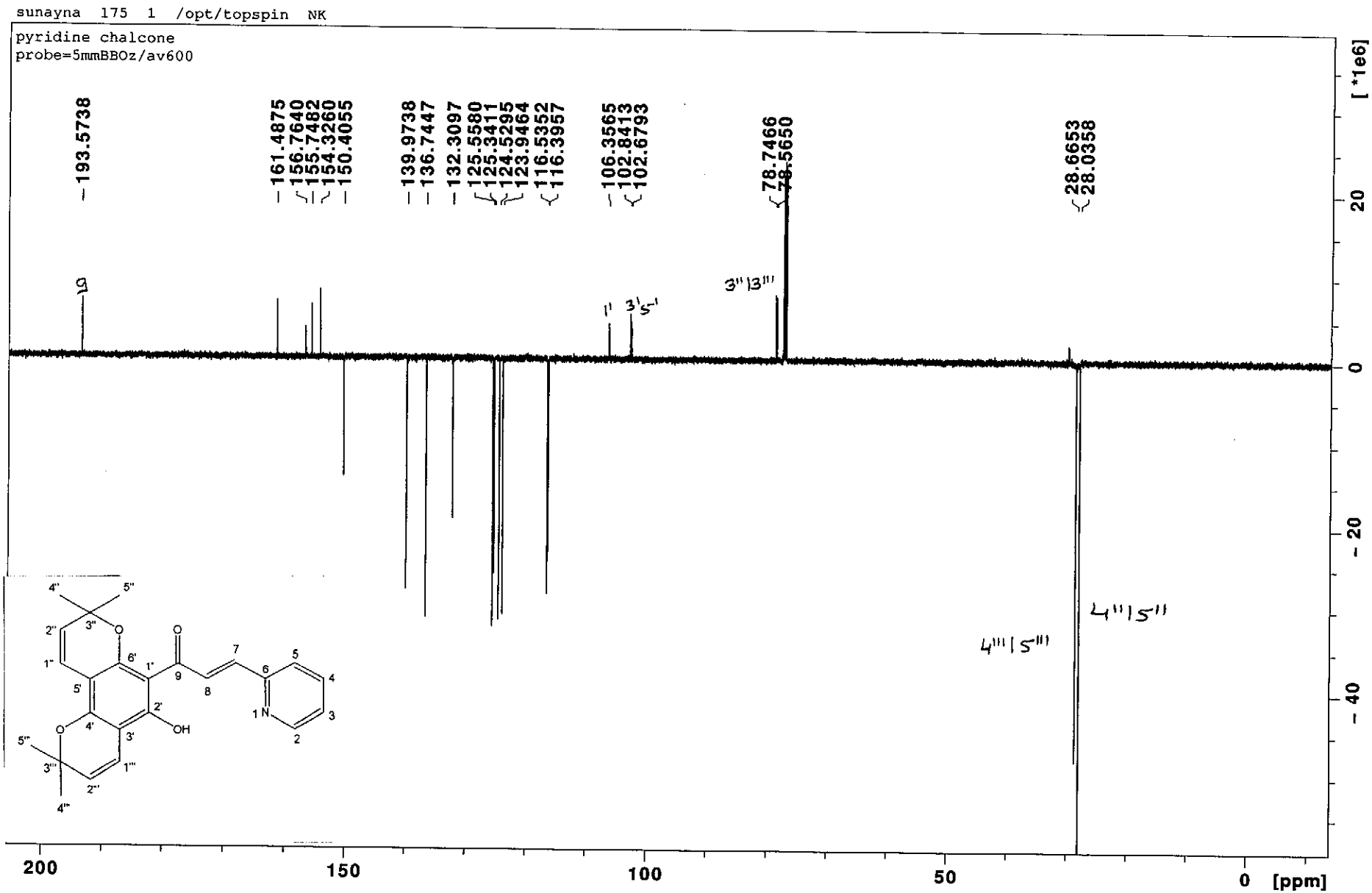
1H NMR spectrum of pyridine pyranochromene chalcone (B-3)



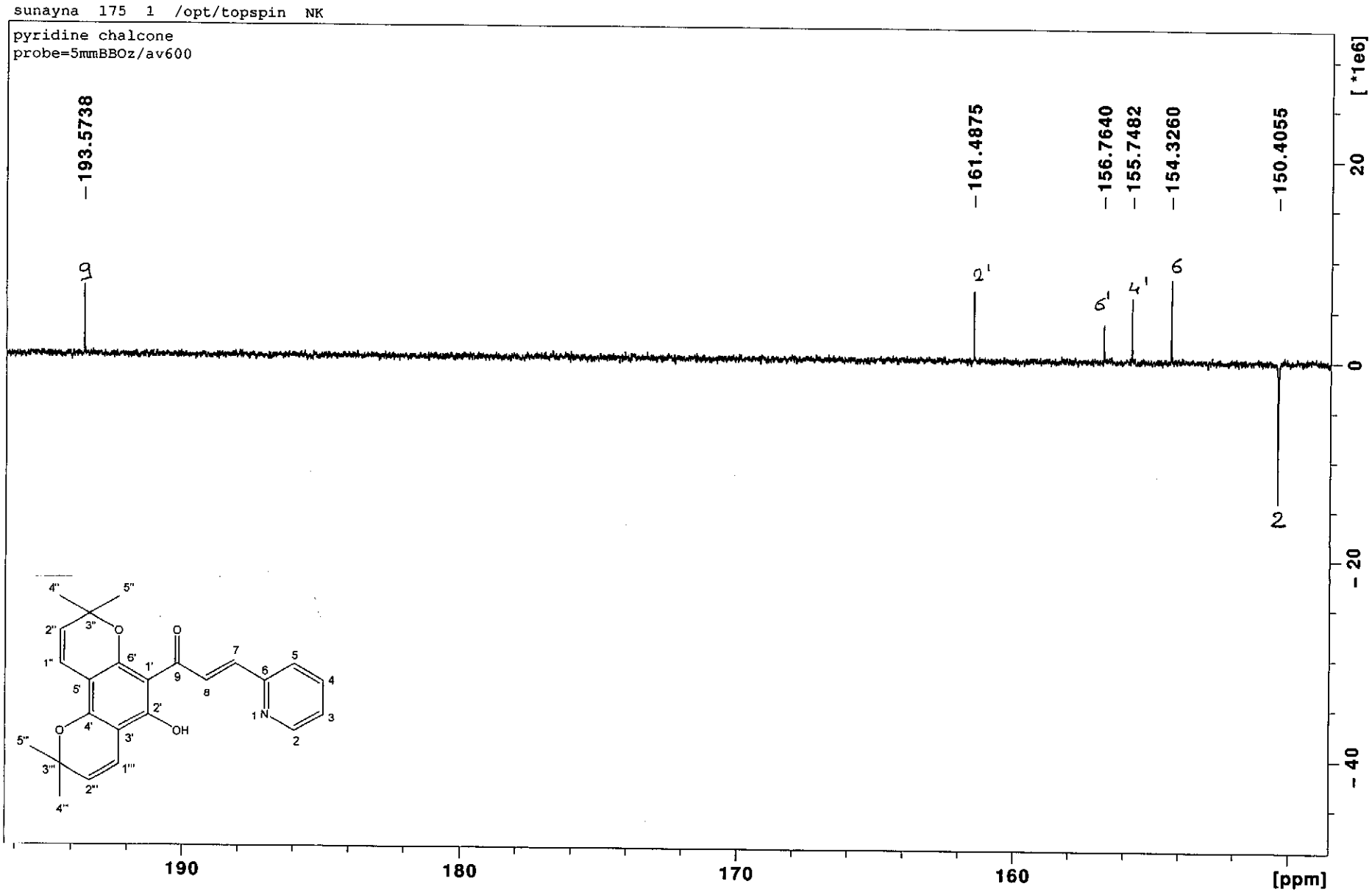
Expanded 1H NMR spectrum of pyridine pyranochromene chalcone (B-3)



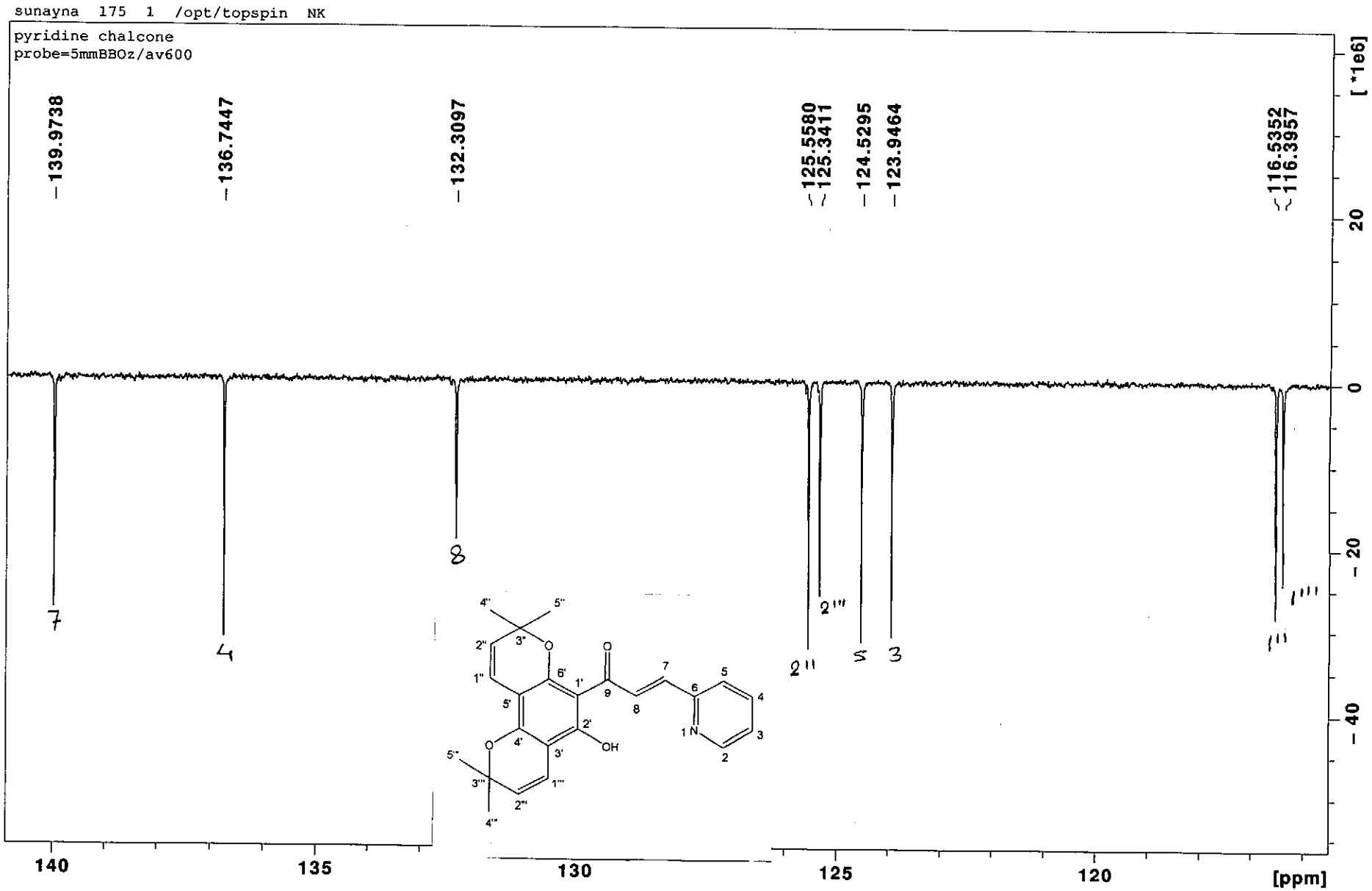
Expanded 1H NMR spectrum of pyridine pyranochromene chalcone (B-3)



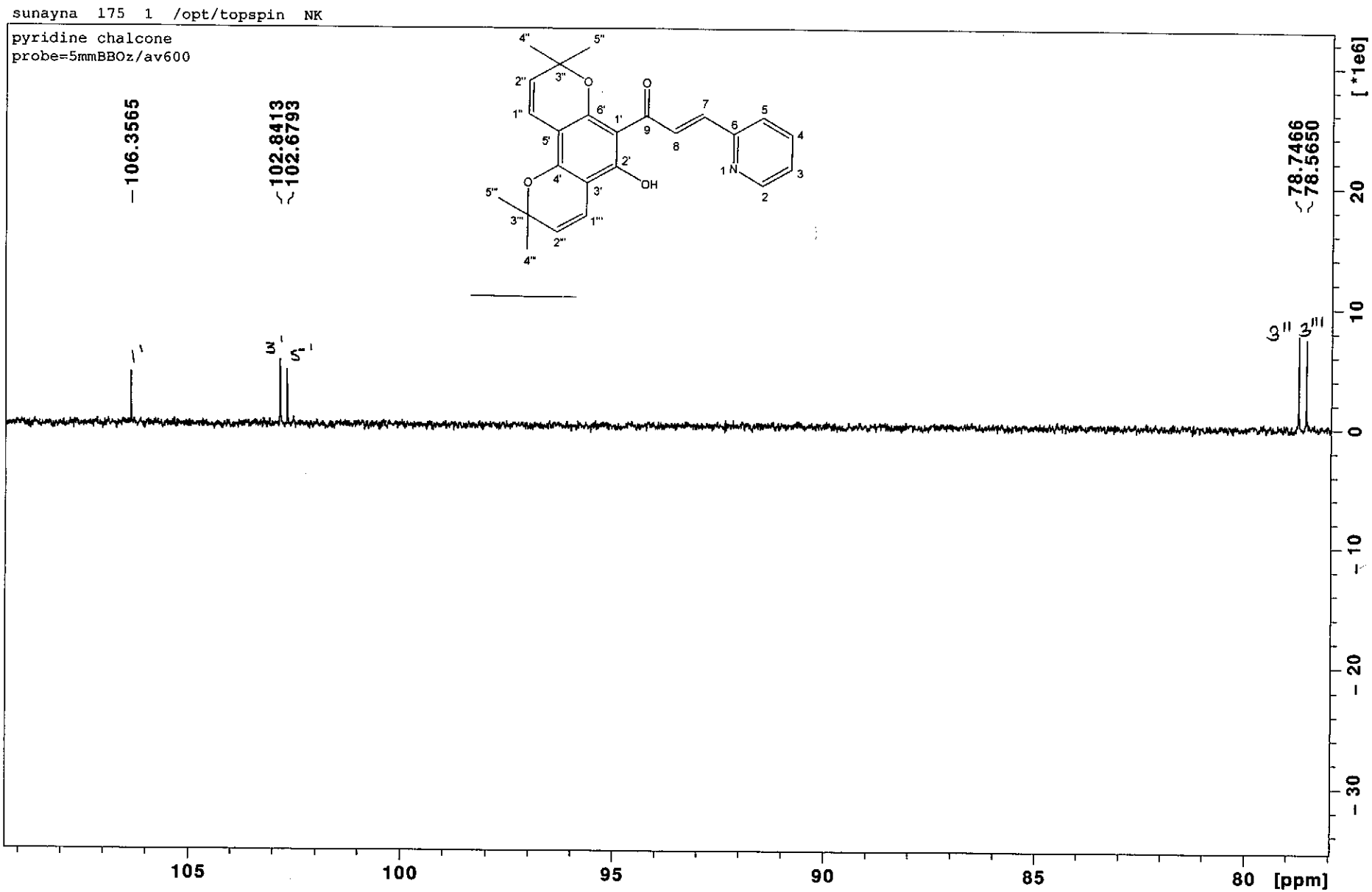
13C NMR spectrum of pyridine pyranochromene chalcone (B-3)



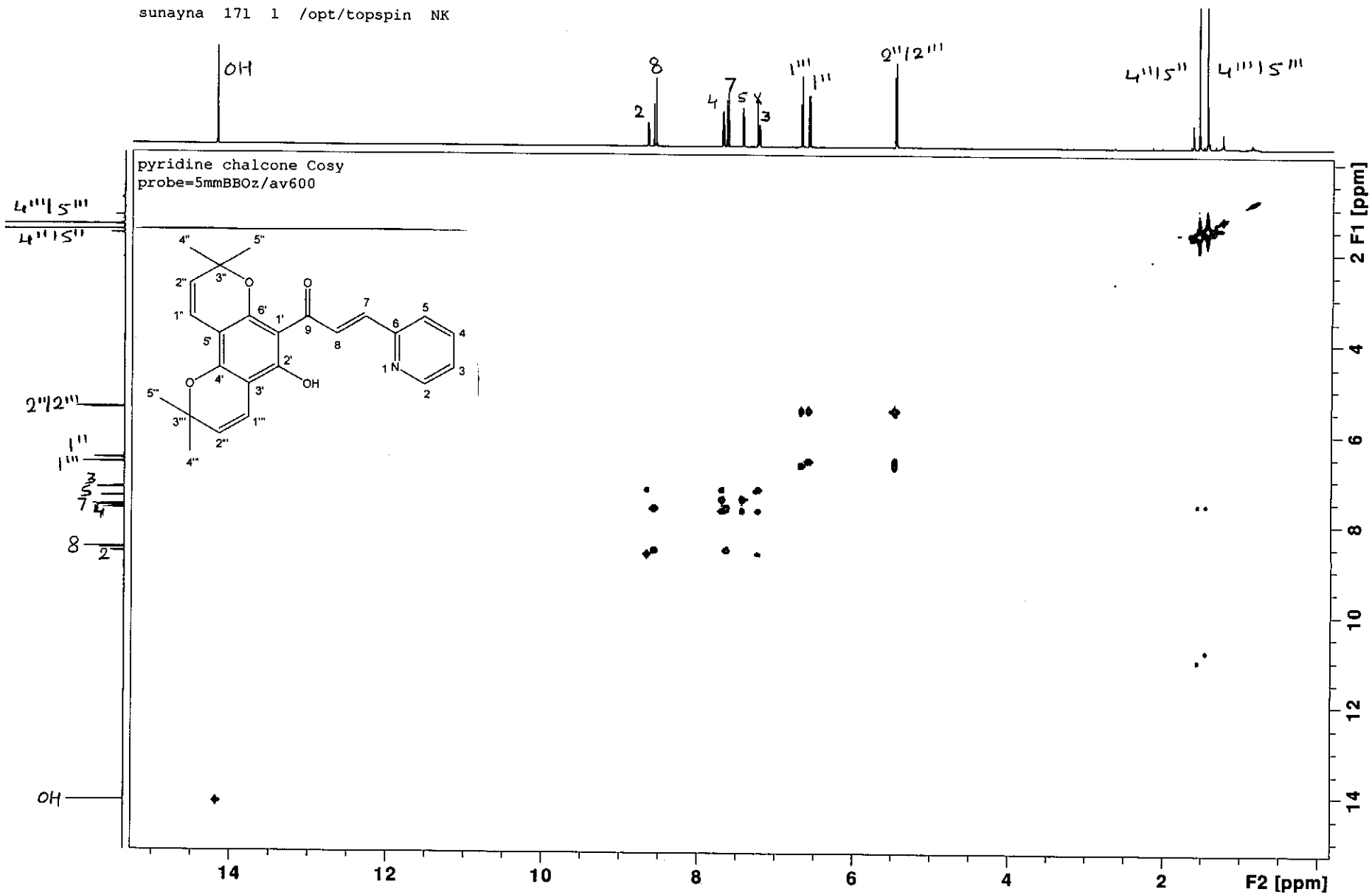
Expanded 13C NMR spectrum of pyridine pyranochromene chalcone (B-3)



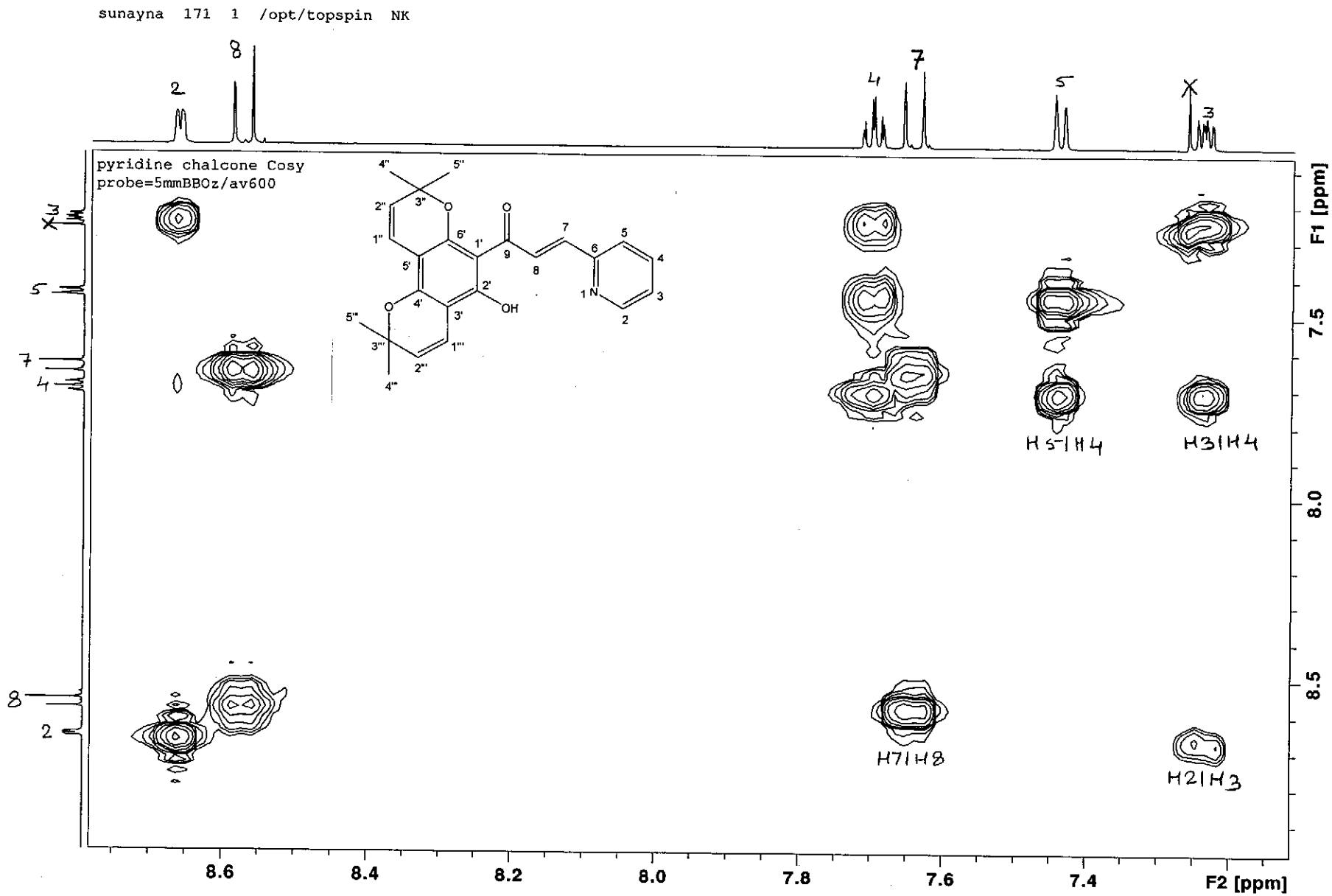
Expanded 13C NMR spectrum of pyridine pyranochromene chalcone (B-3)



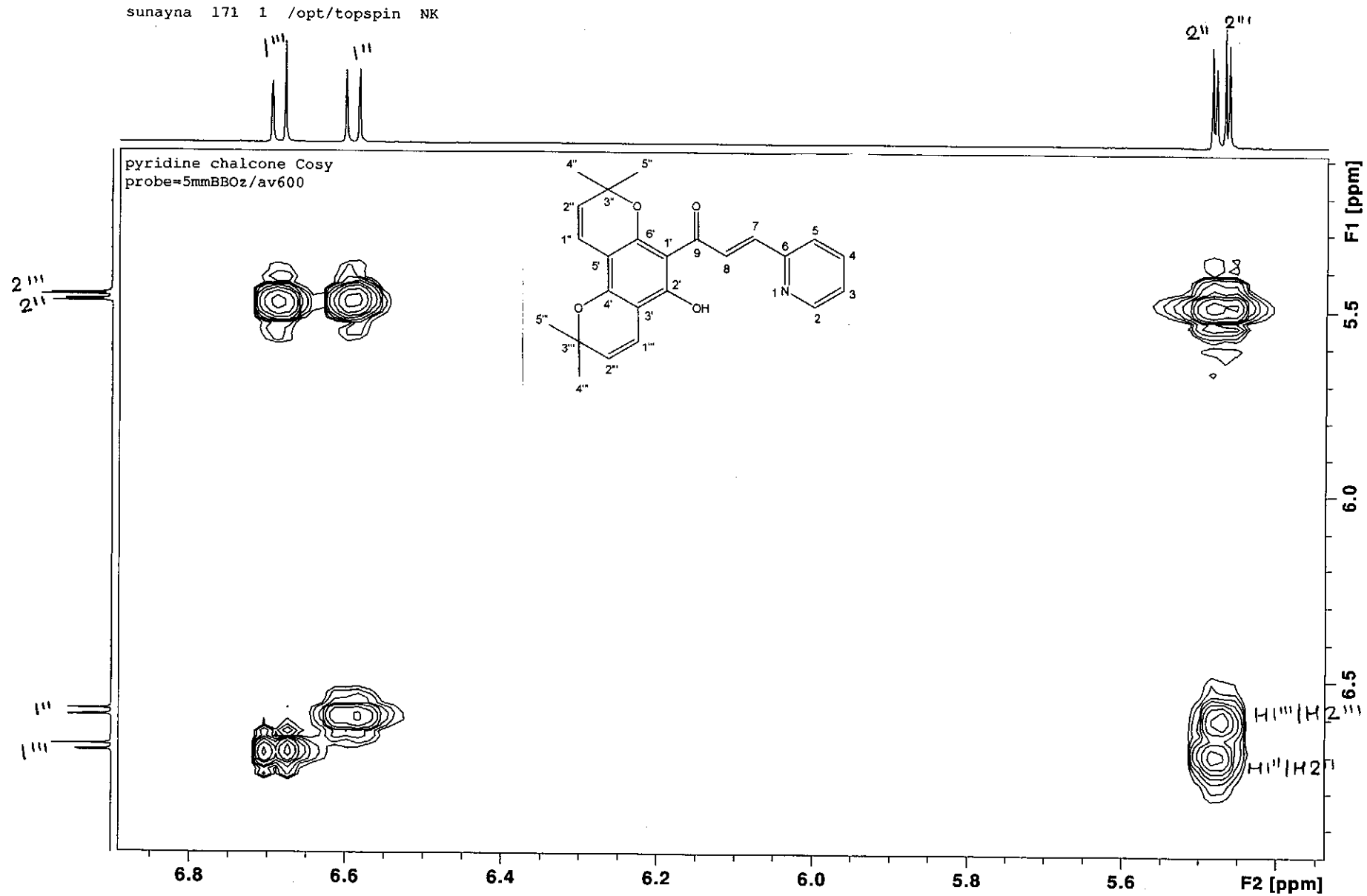
Expanded 13C NMR spectrum of pyridine pyranochromene chalcone (B-3)



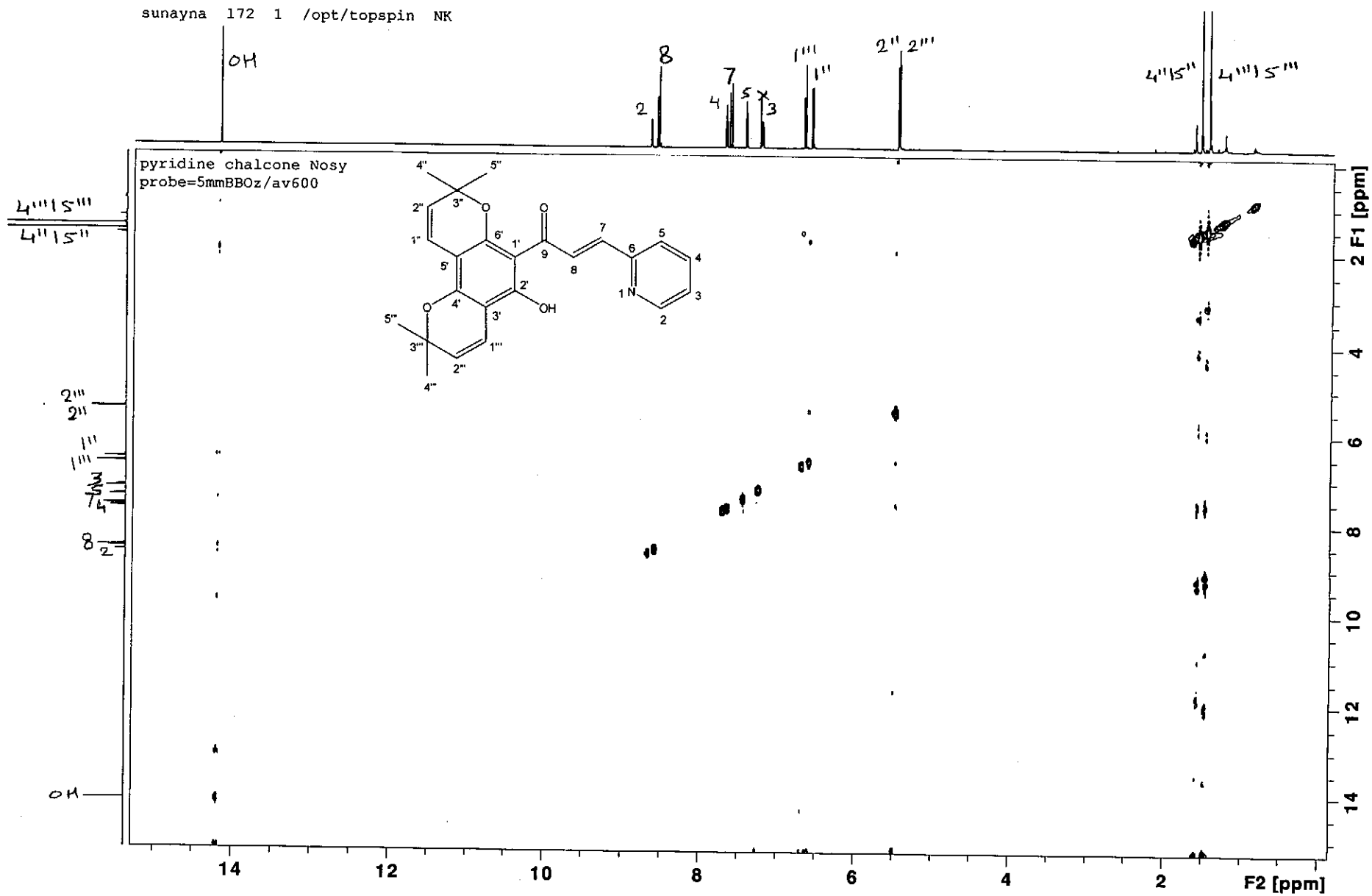
COSY NMR spectrum of pyridine pyranochromene chalcone (B-3)



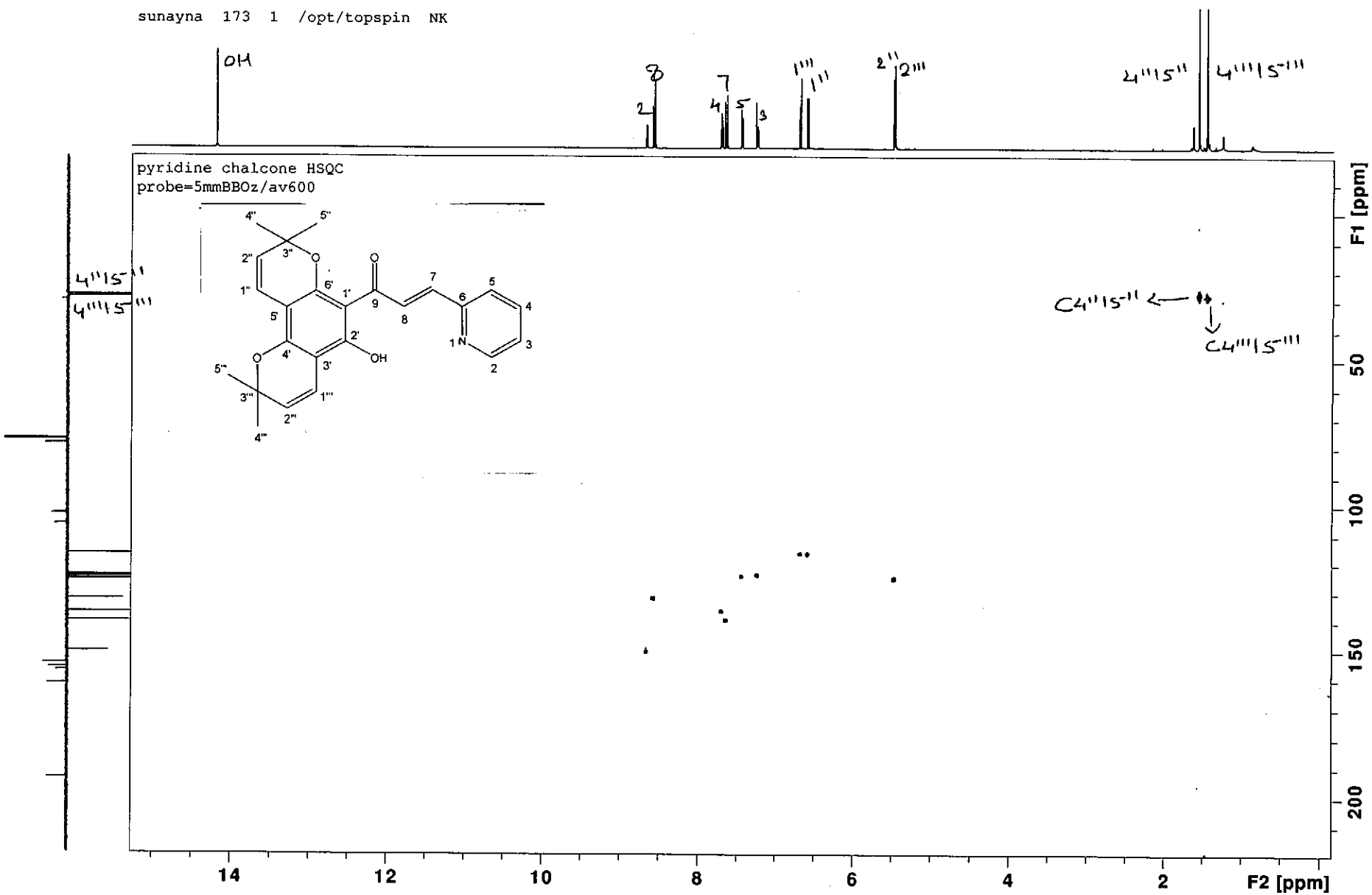
Expanded COSY NMR spectrum of pyridine pyranochromene chalcone (B-3)



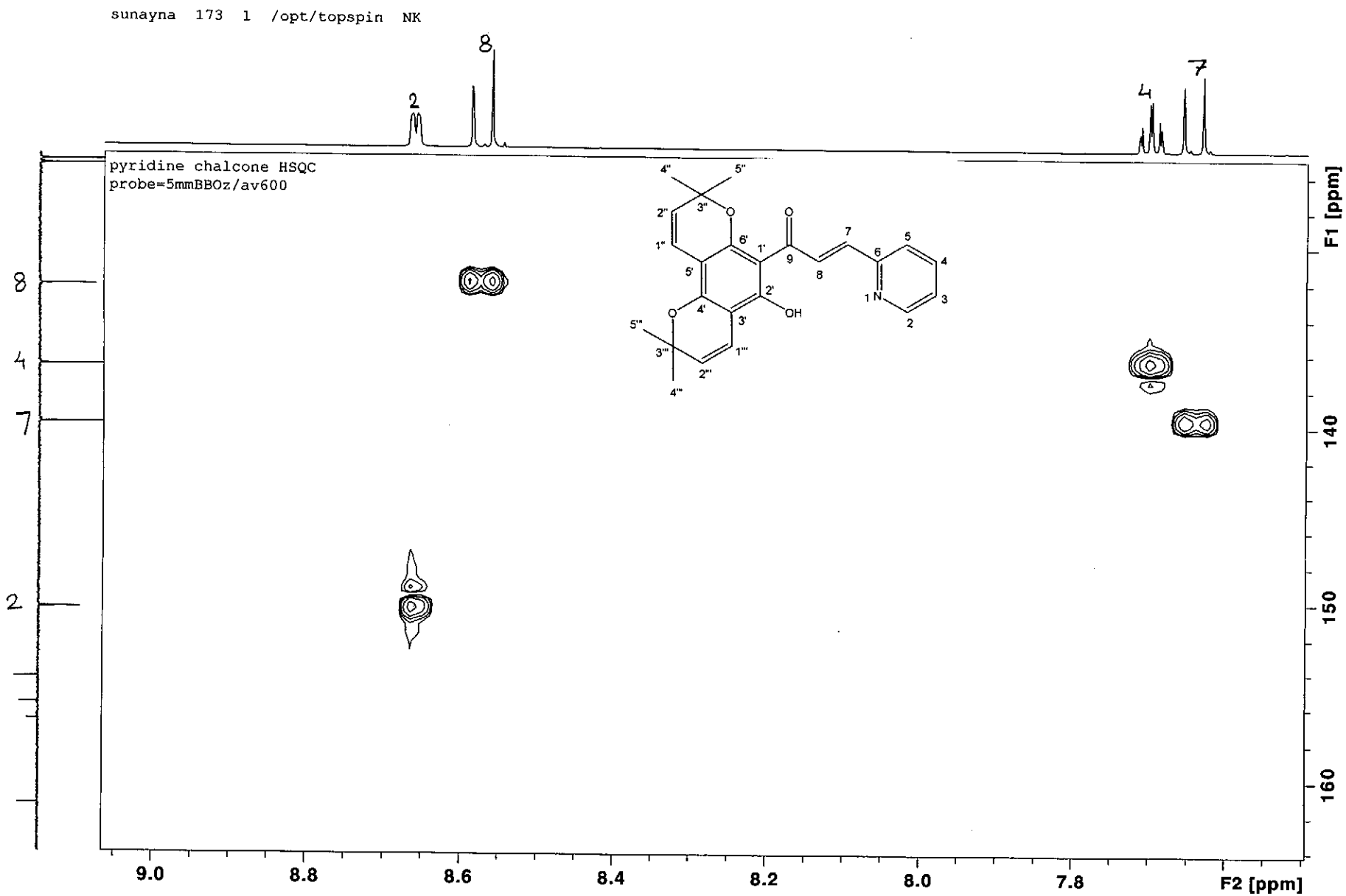
Expanded COSY NMR spectrum of pyridine pyranochromene chalcone (B-3)



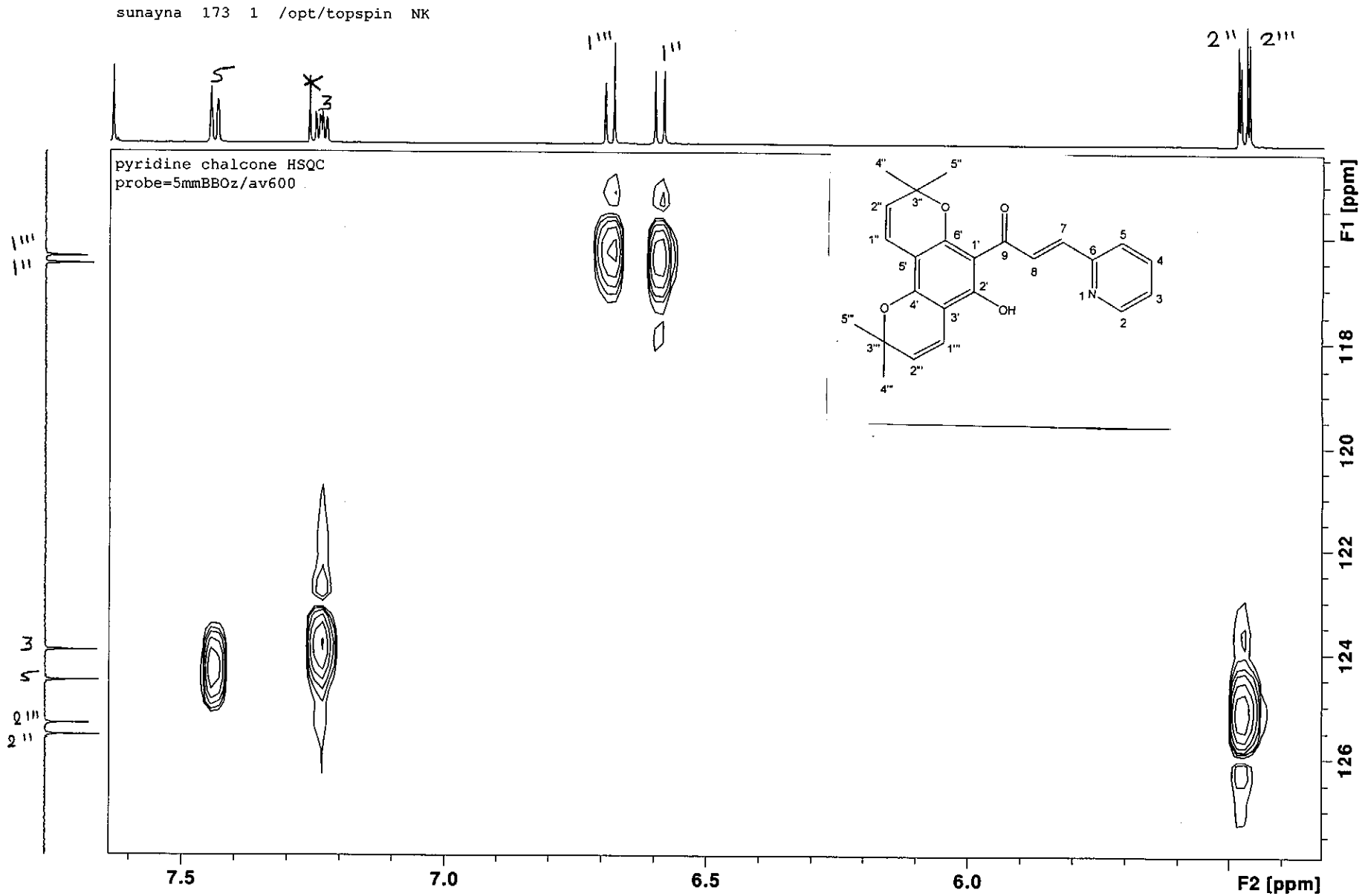
NOESY NMR spectrum of pyridine pyranochromene chalcone (B-3)



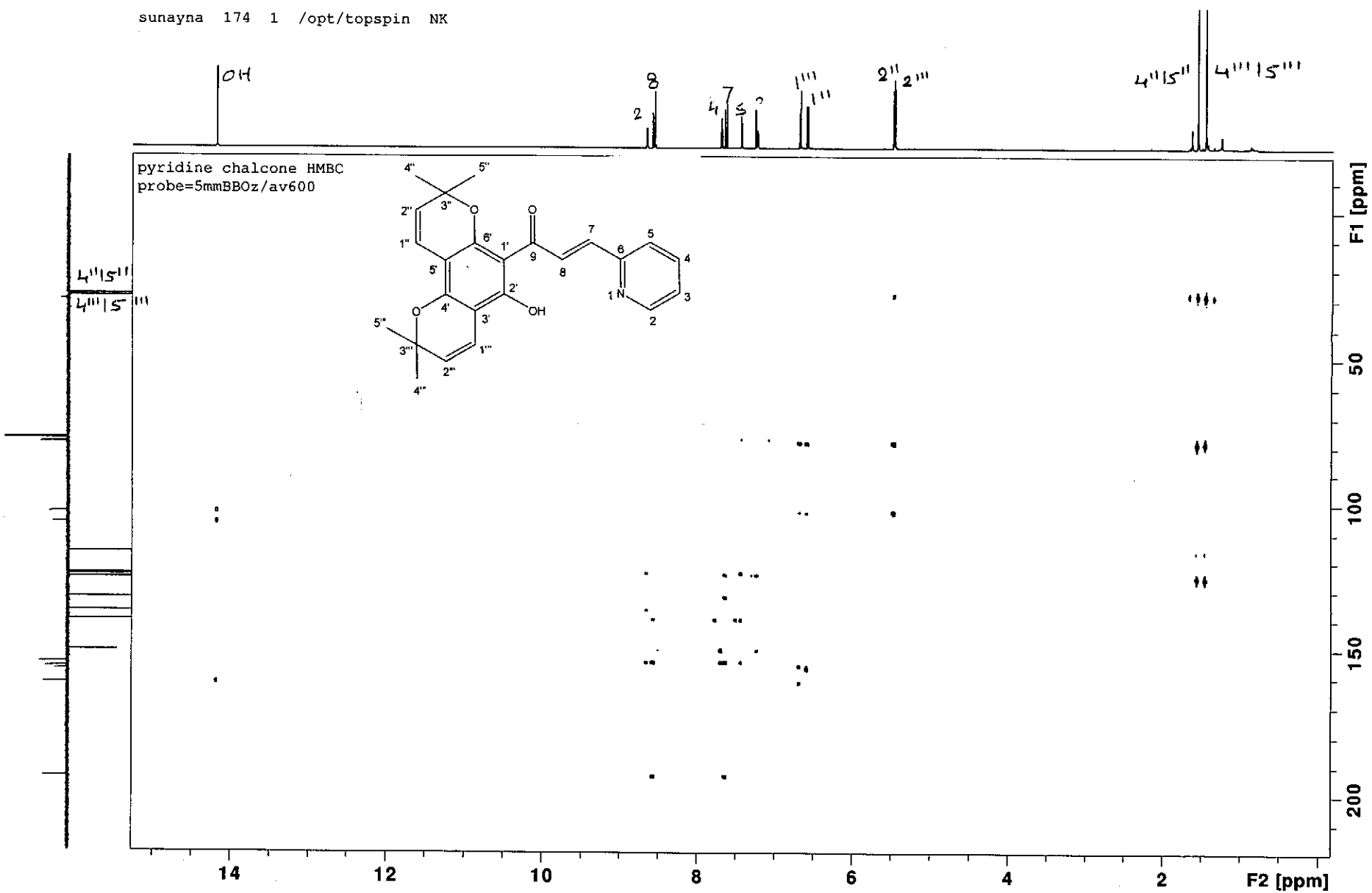
HSQC spectrum of pyridine pyranochromene chalcone (B-3)



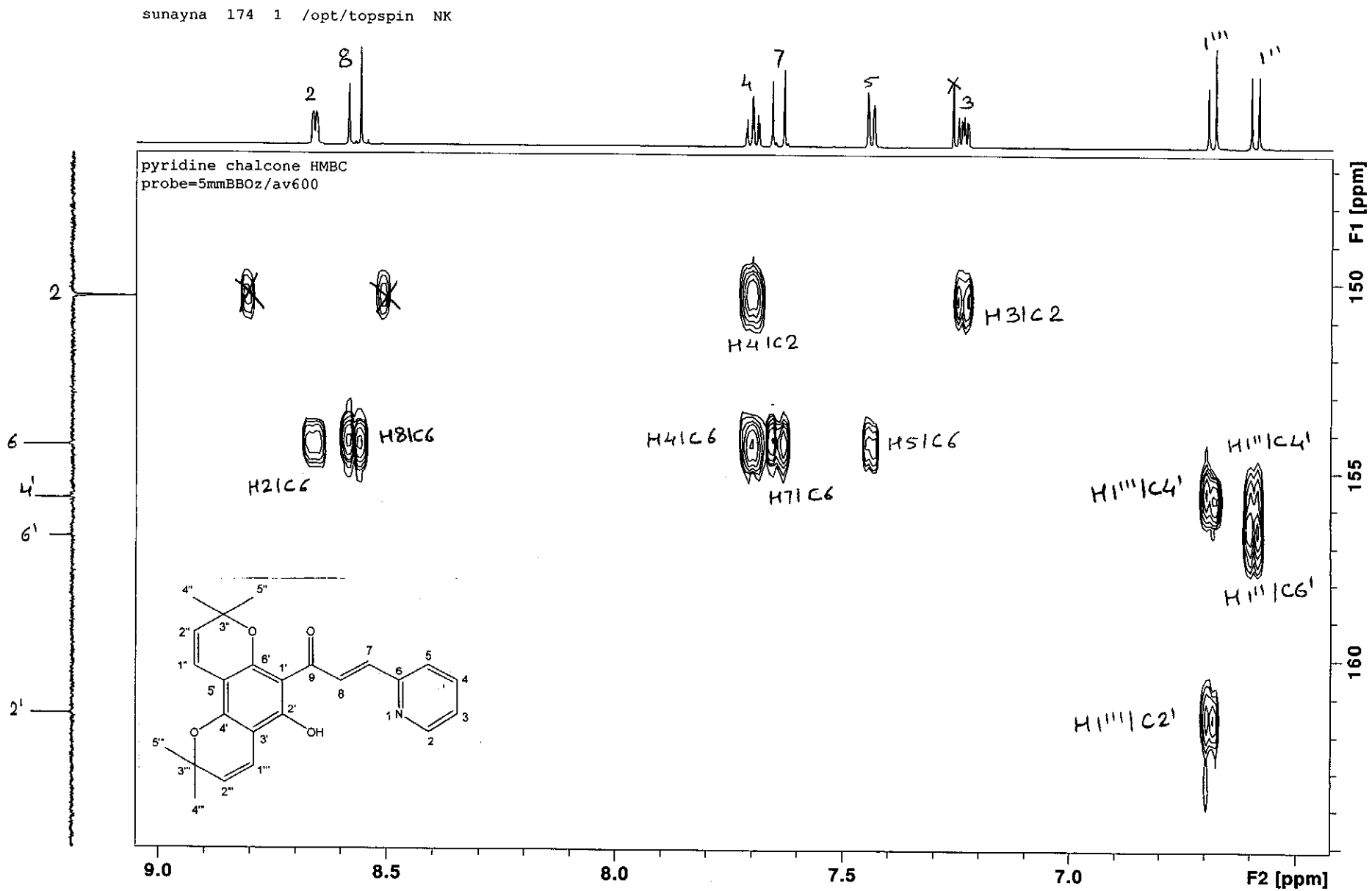
HSQC spectrum of pyridine pyranochromene chalcone (B-3)



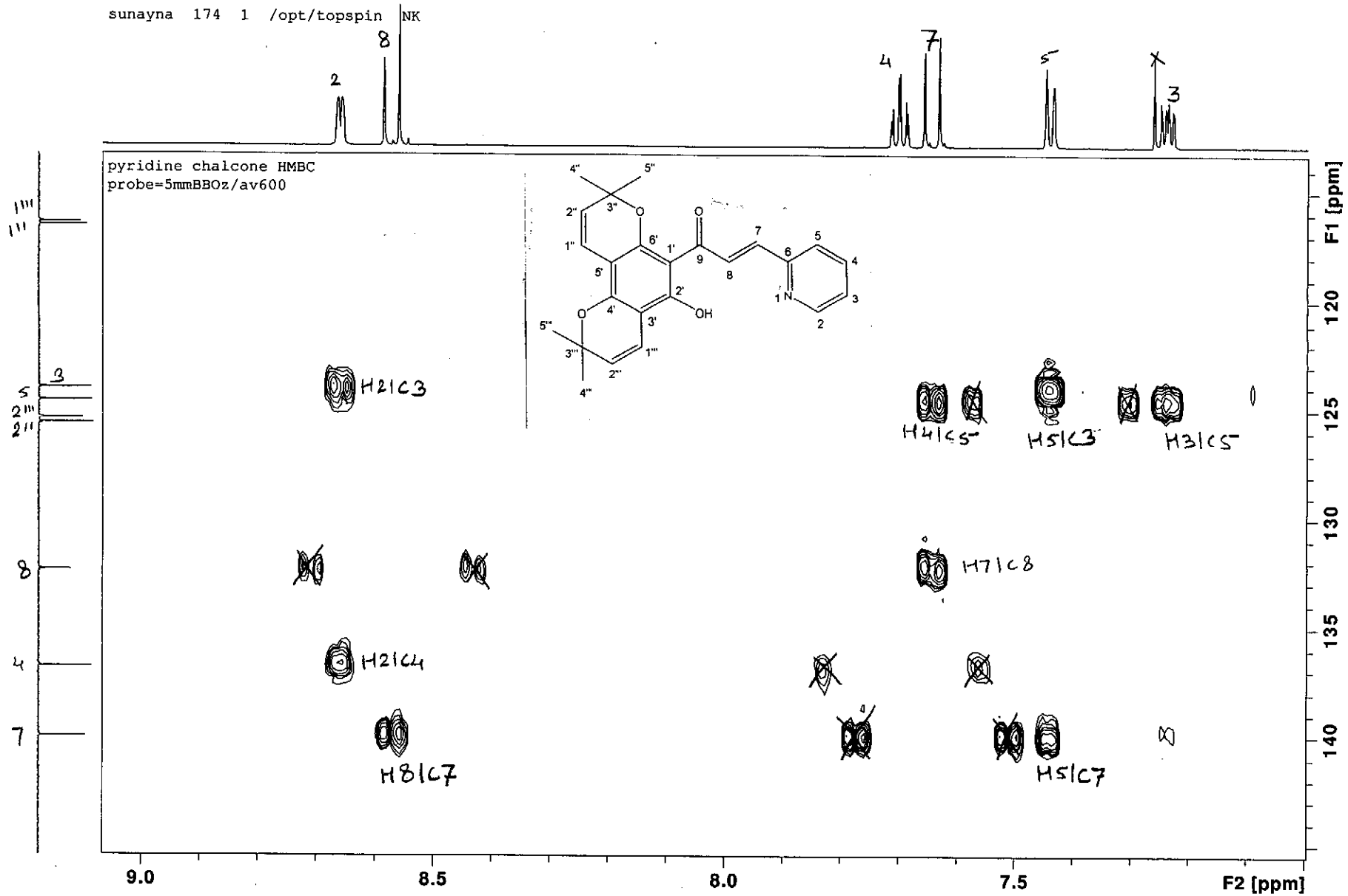
HSQC spectrum of pyridine pyranochromene chalcone (B-3)



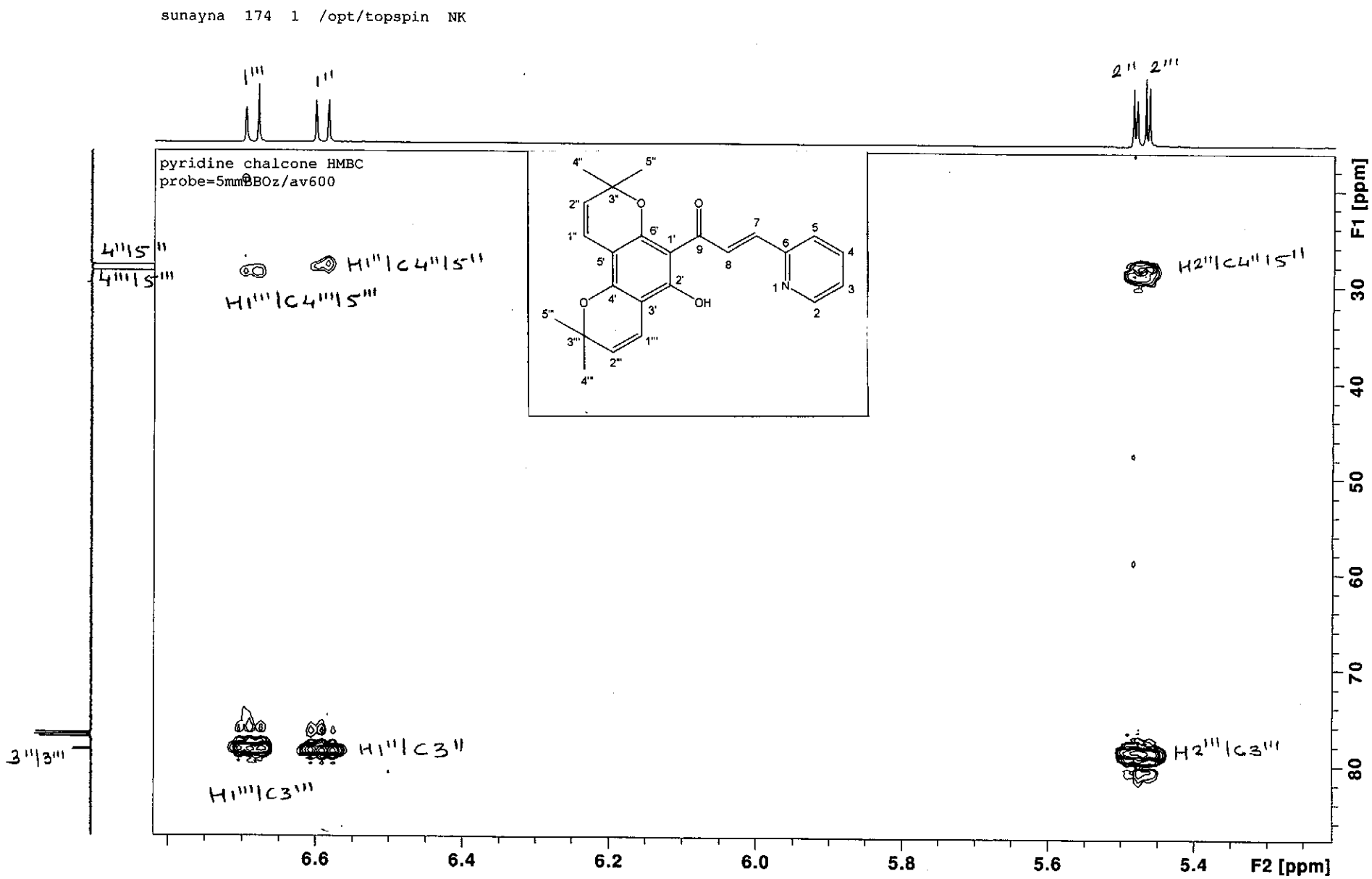
HMBC spectrum of pyridine pyranochromene chalcone (B-3)



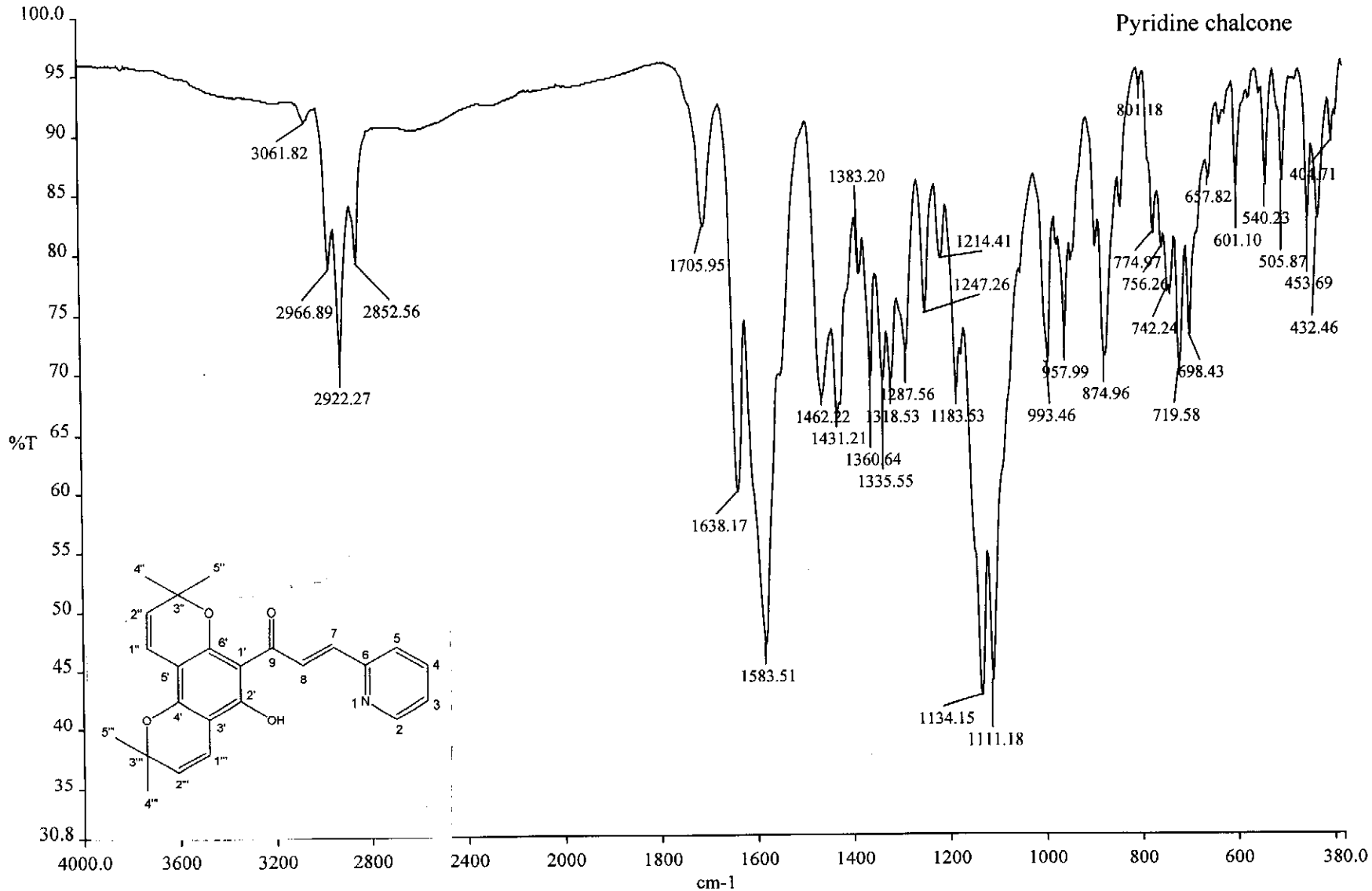
Expanded HMBC spectrum of pyridine pyranochromene chalcone (B-3)



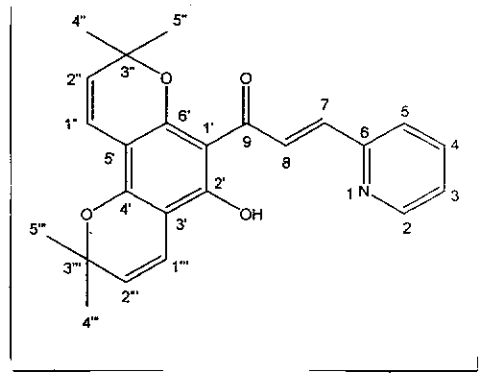
Expanded HMBC spectrum of pyridine pyranochromene chalcone (B-3)



Expanded HMBC spectrum of pyridine pyranochromene chalcone (B-3)



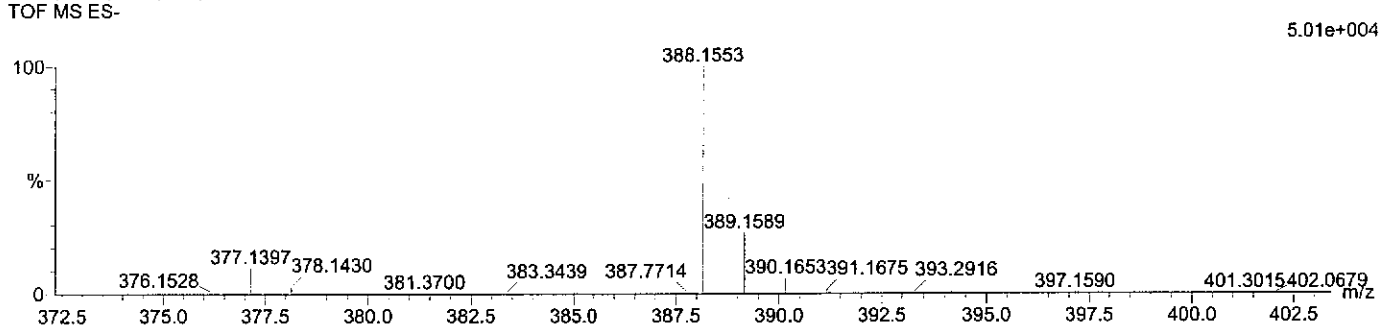
Infrared spectrum of pyridine pyranochromene chalcone (B-3)



Elemental Composition Report

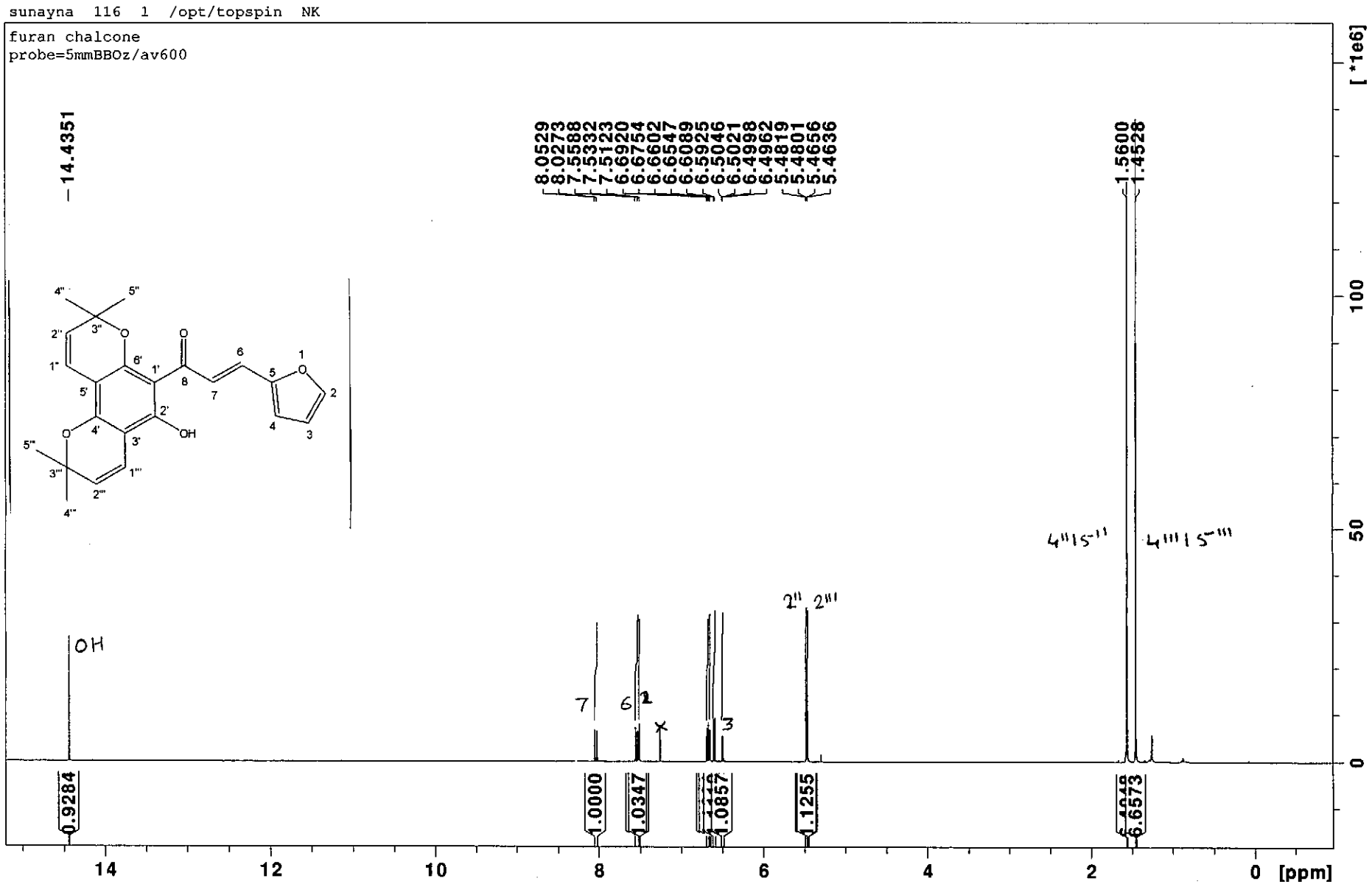
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
29 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-35 H: 20-30 N: 0-4 O: 0-5
28 2 (0.017) Cm (1:31)
TOF MS ES-

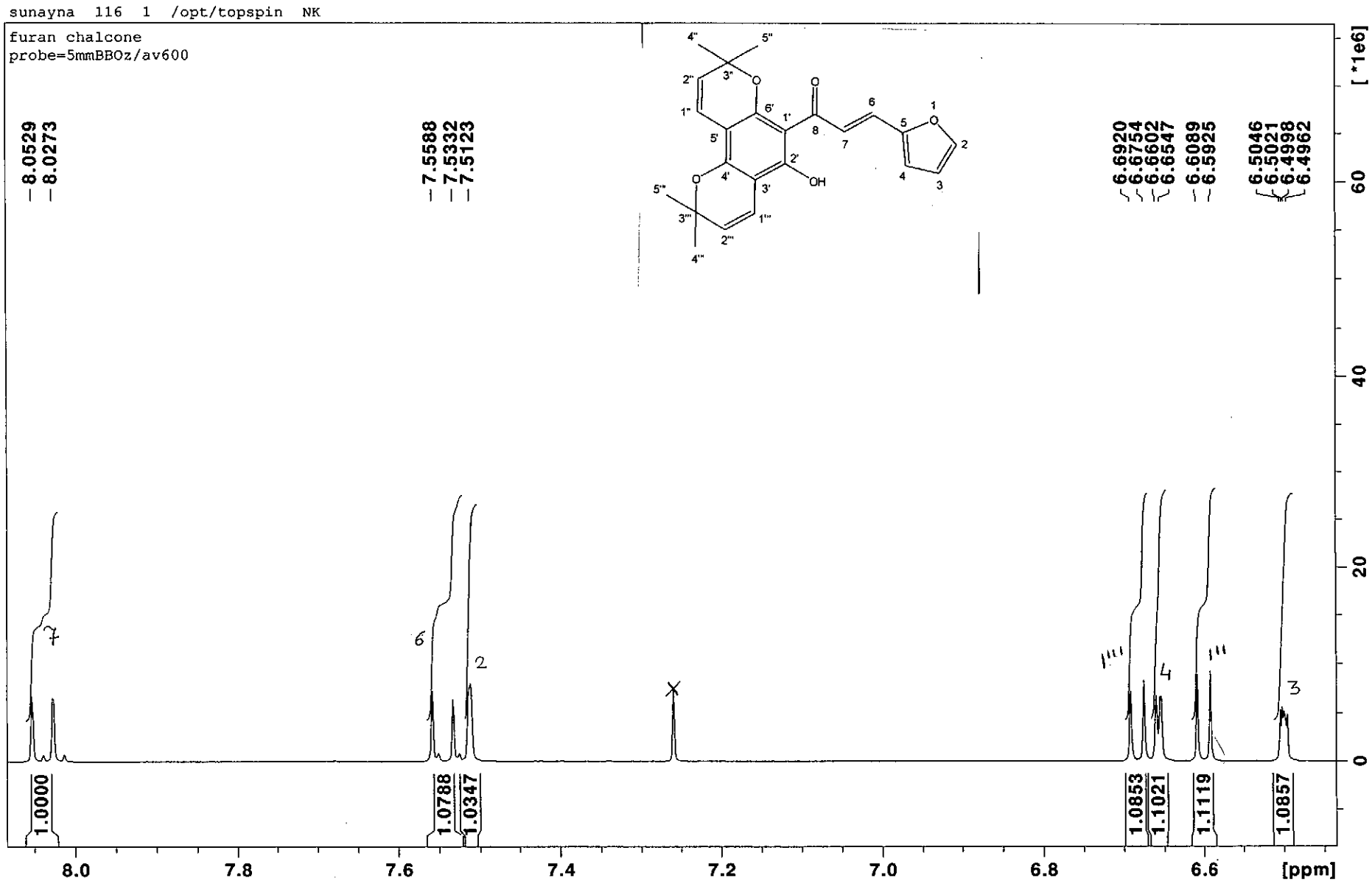


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
388.1553	388.1549	0.4	1.0	14.5	472.8	0.0	C24 H22 N O4

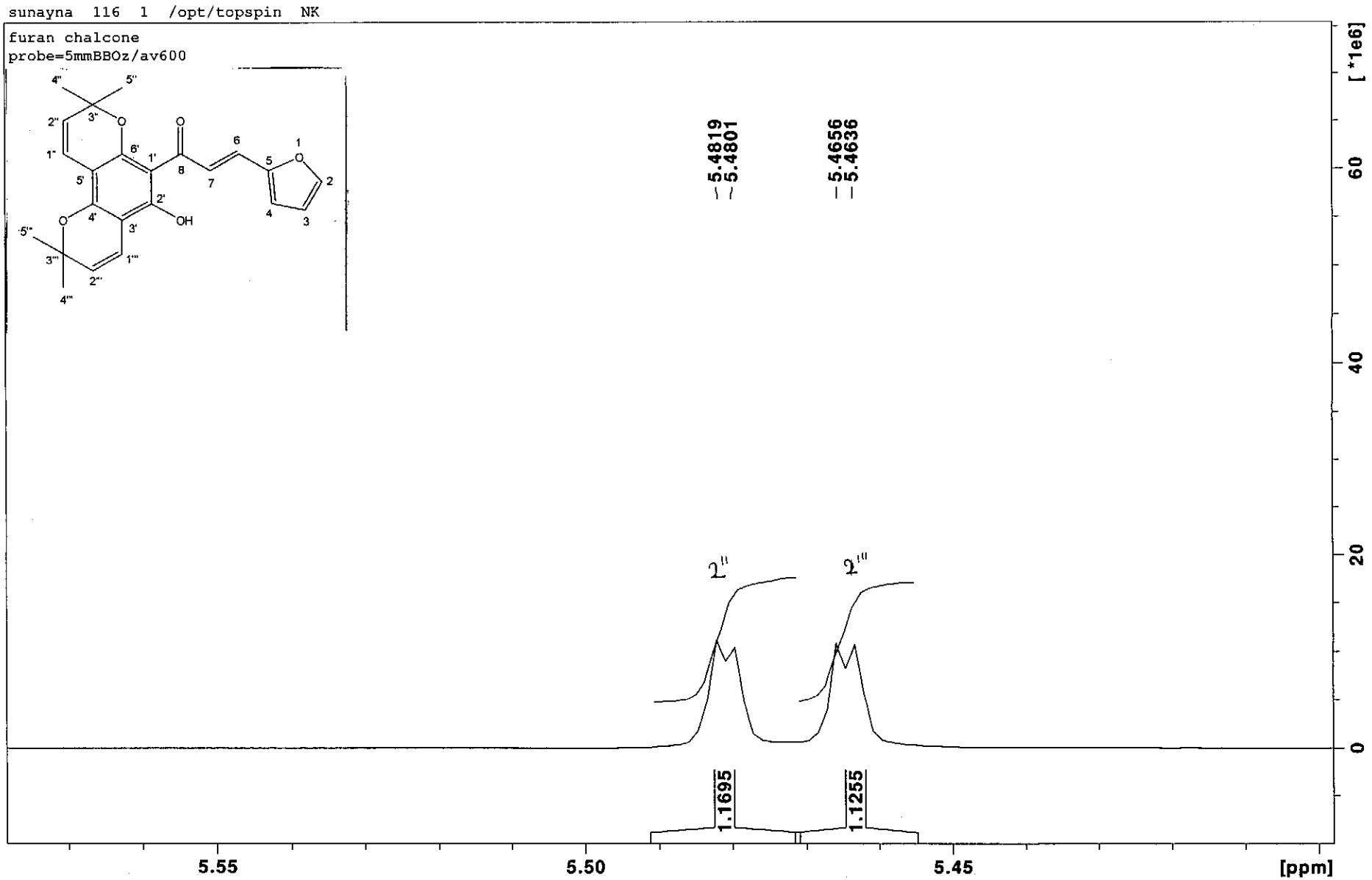
HRMS spectrum of pyridine pyranochromene chalcone (B-3)



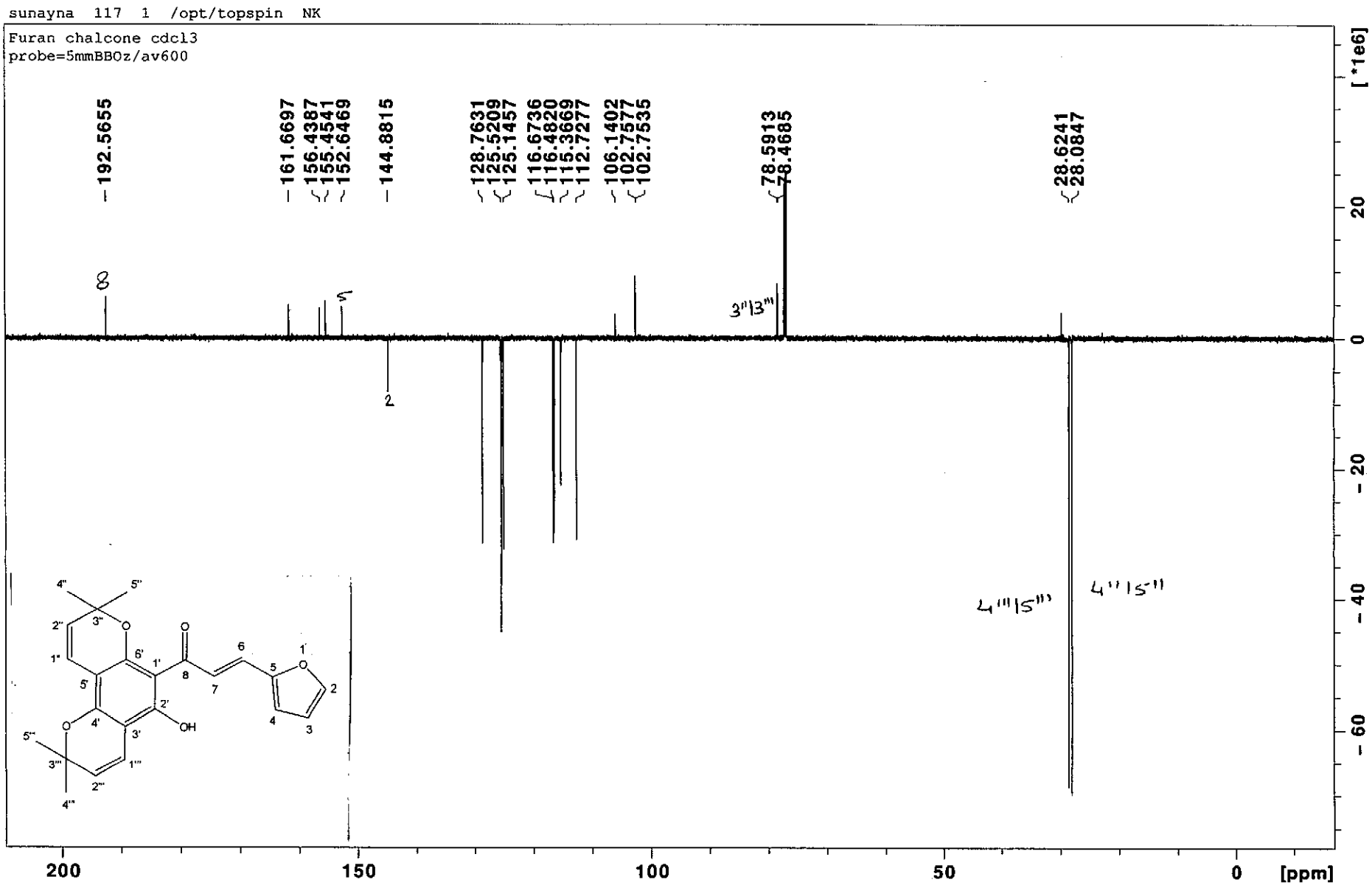
1H NMR spectrum of furan pyranochromene chalcone (B-4)



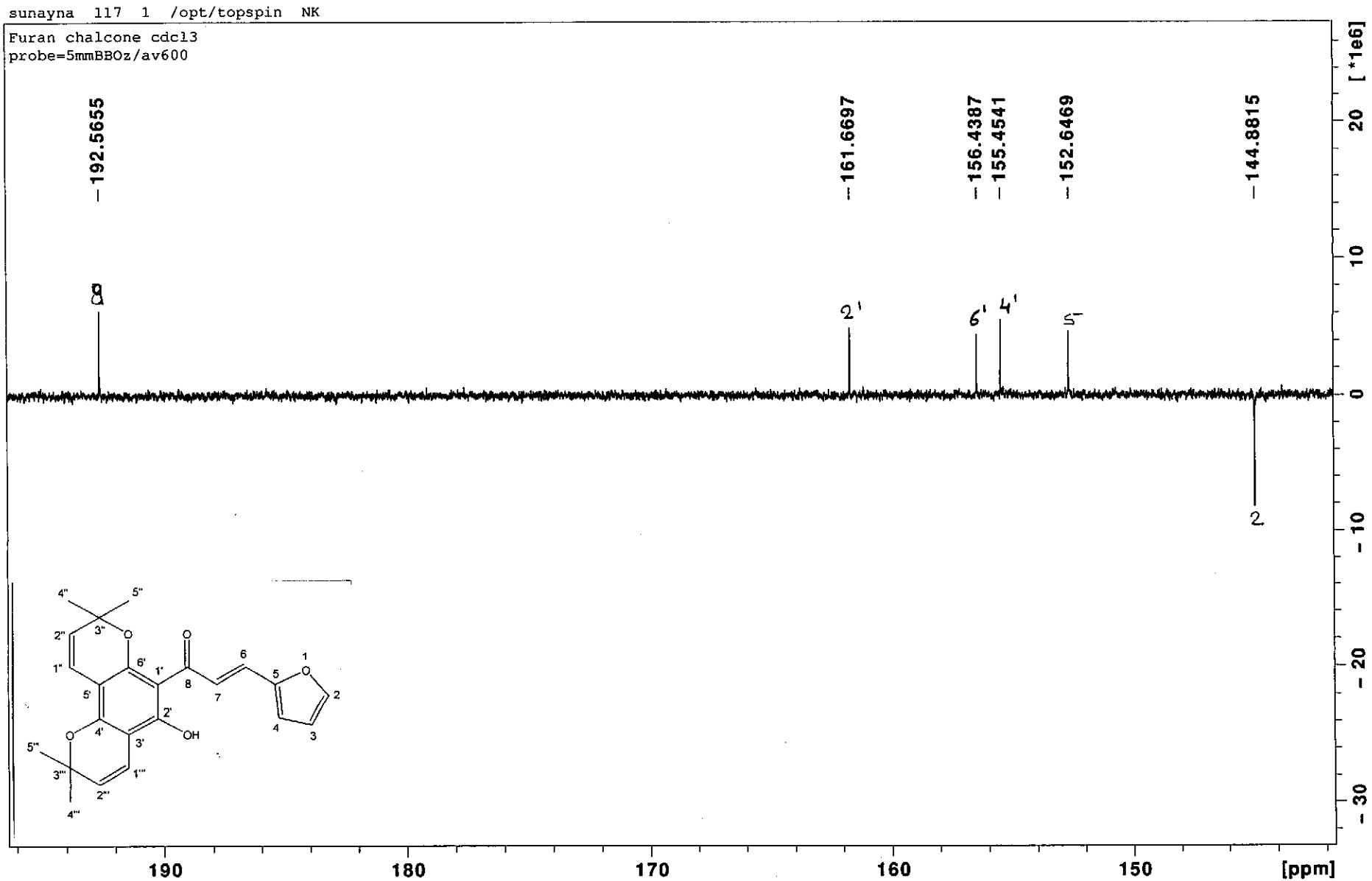
Expanded 1H NMR spectrum of furan pyranochromene chalcone (B-4)



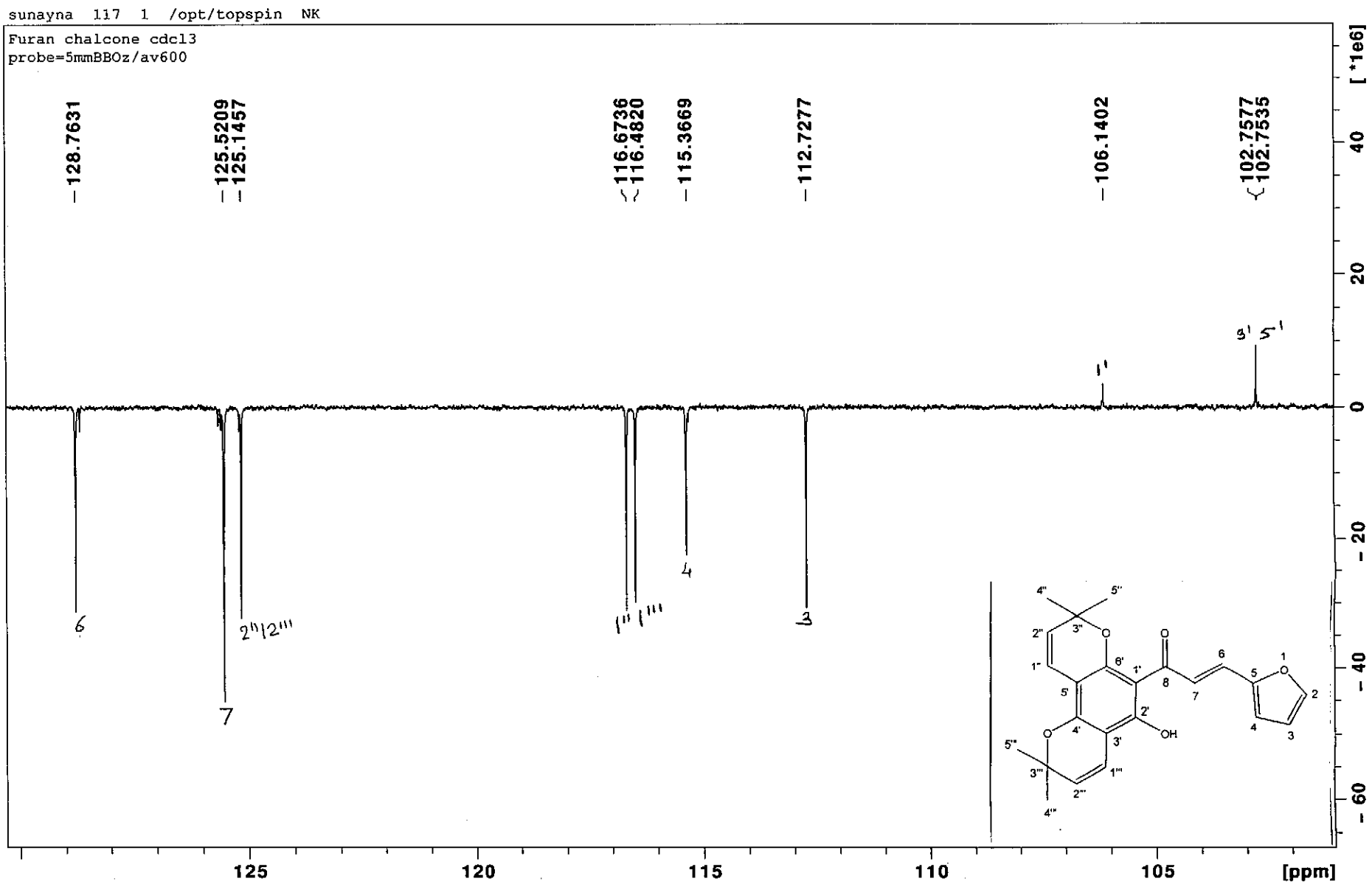
Expanded 1H NMR spectrum of furan pyranochromene chalcone (B-4)



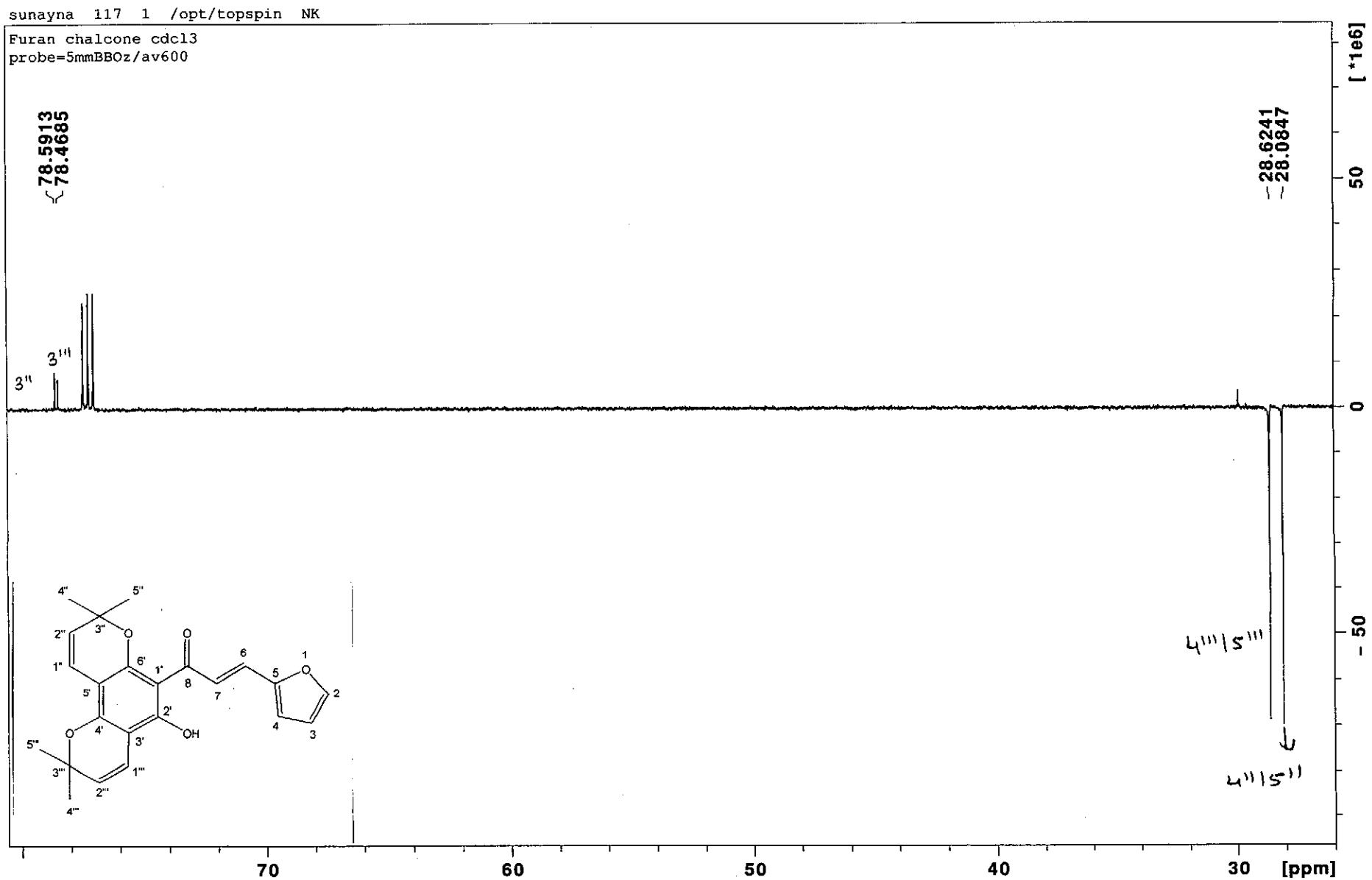
13C NMR spectrum of furan pyranochromene chalcone



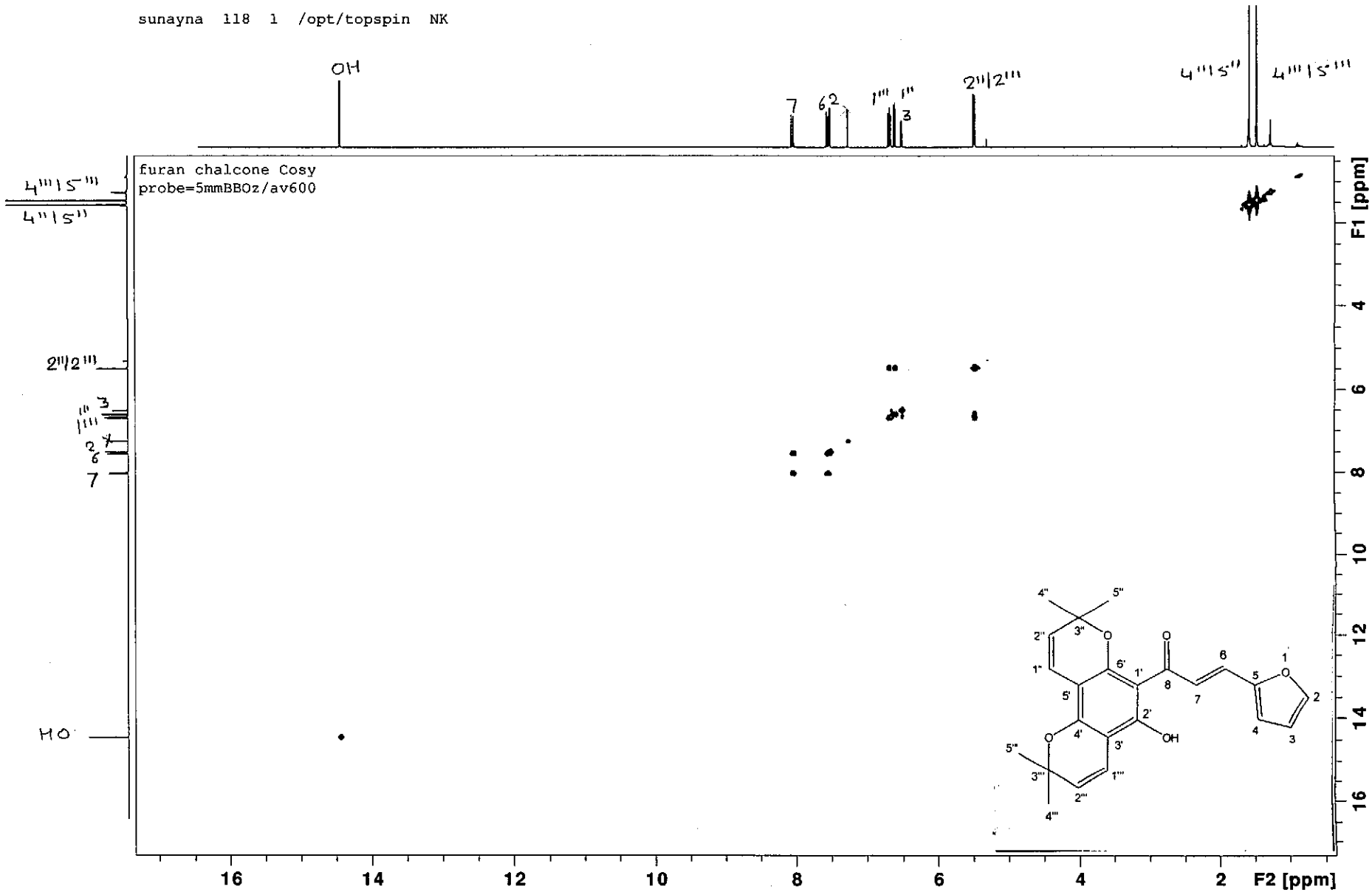
Expanded 13C NMR spectrum of furan pyranochromene chalcone (B-4)



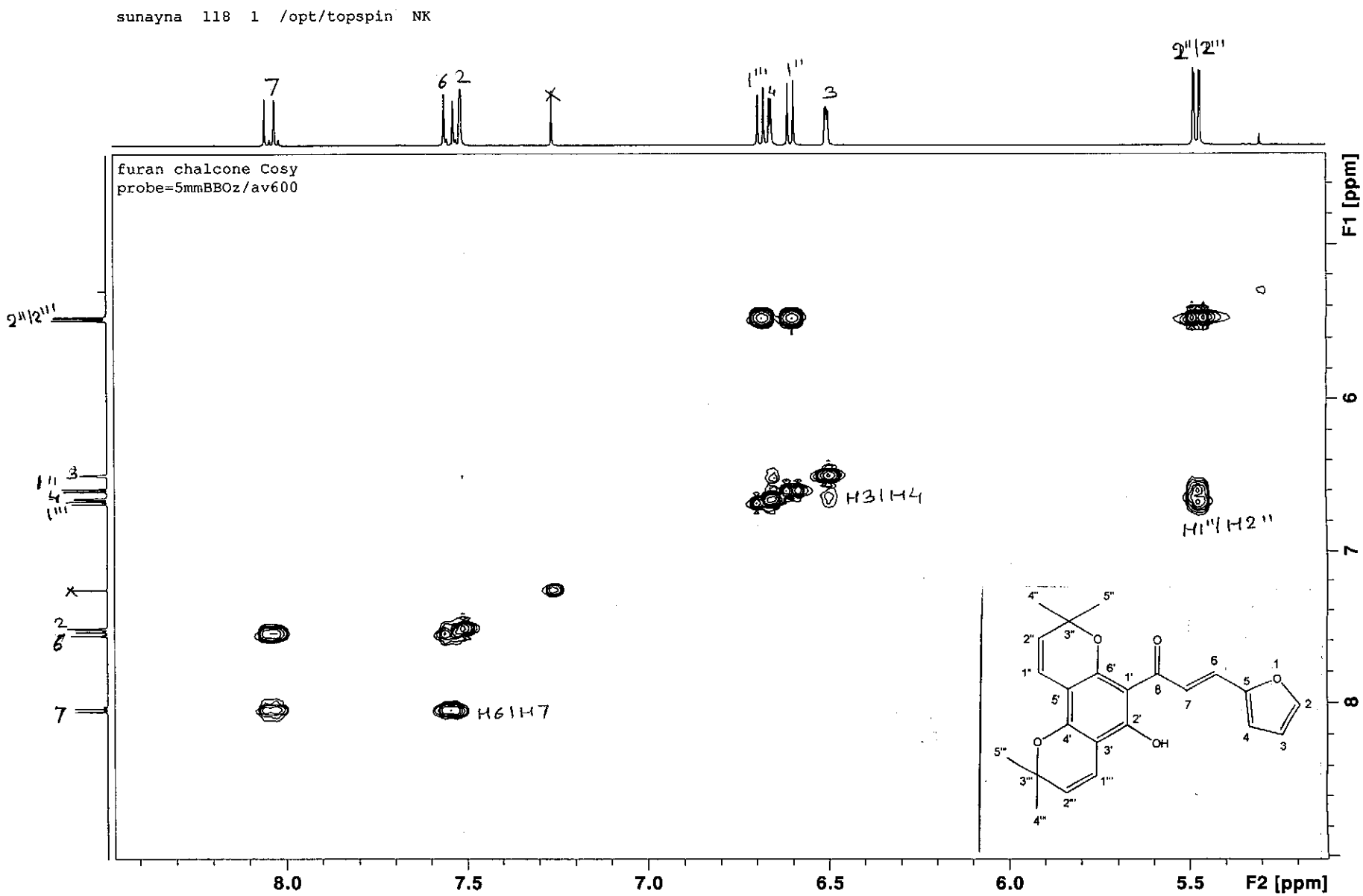
Expanded 13C NMR spectrum of furan pyranochromene chalcone (B-4)



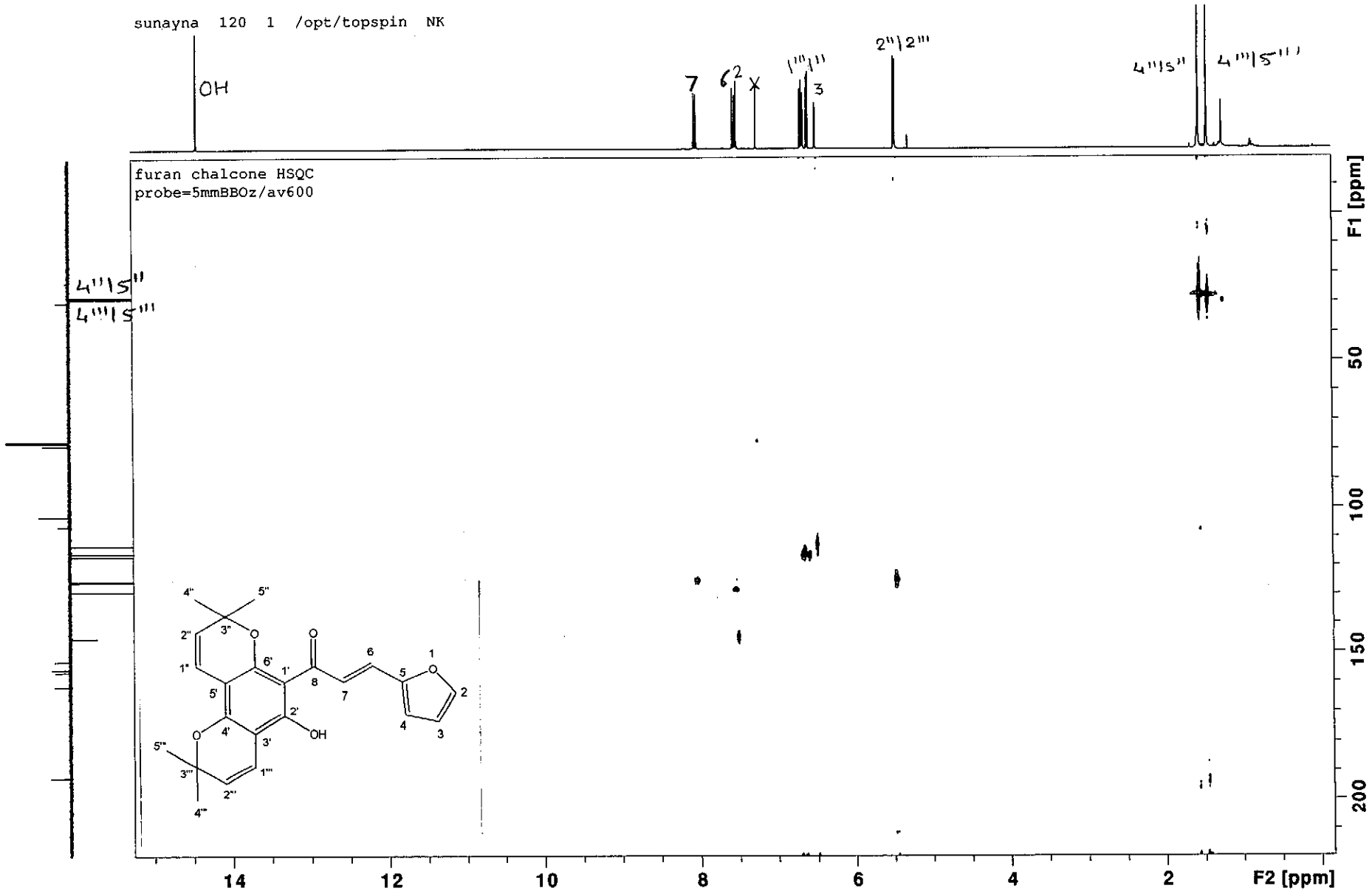
Expanded 13C NMR spectrum of furan pyranochromene chalcone (B-4)



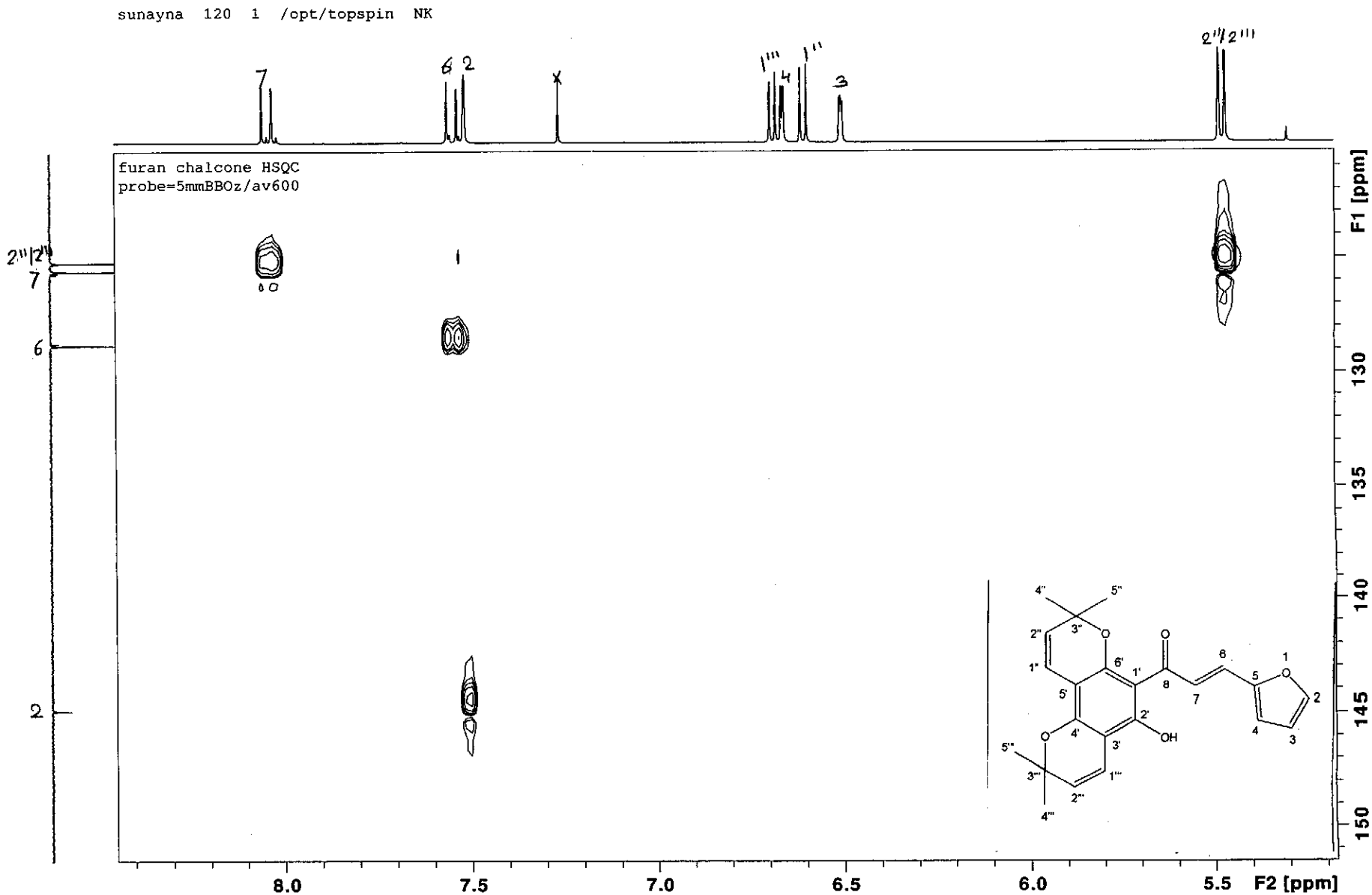
COSY NMR spectrum of furan pyranochromene chalcone (B-4)



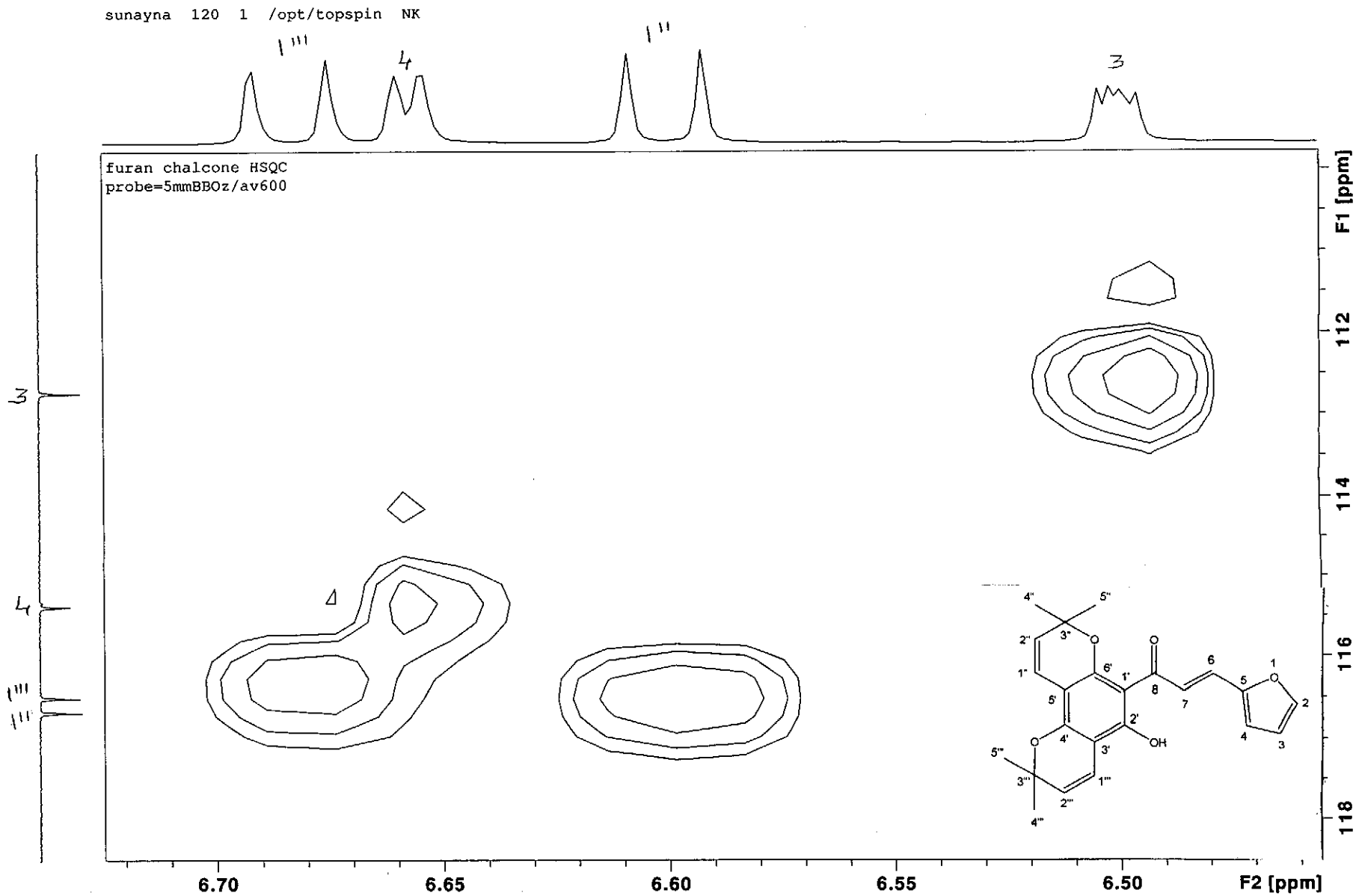
Expanded COSY NMR spectrum of furan pyranochromene chalcone (B-4)



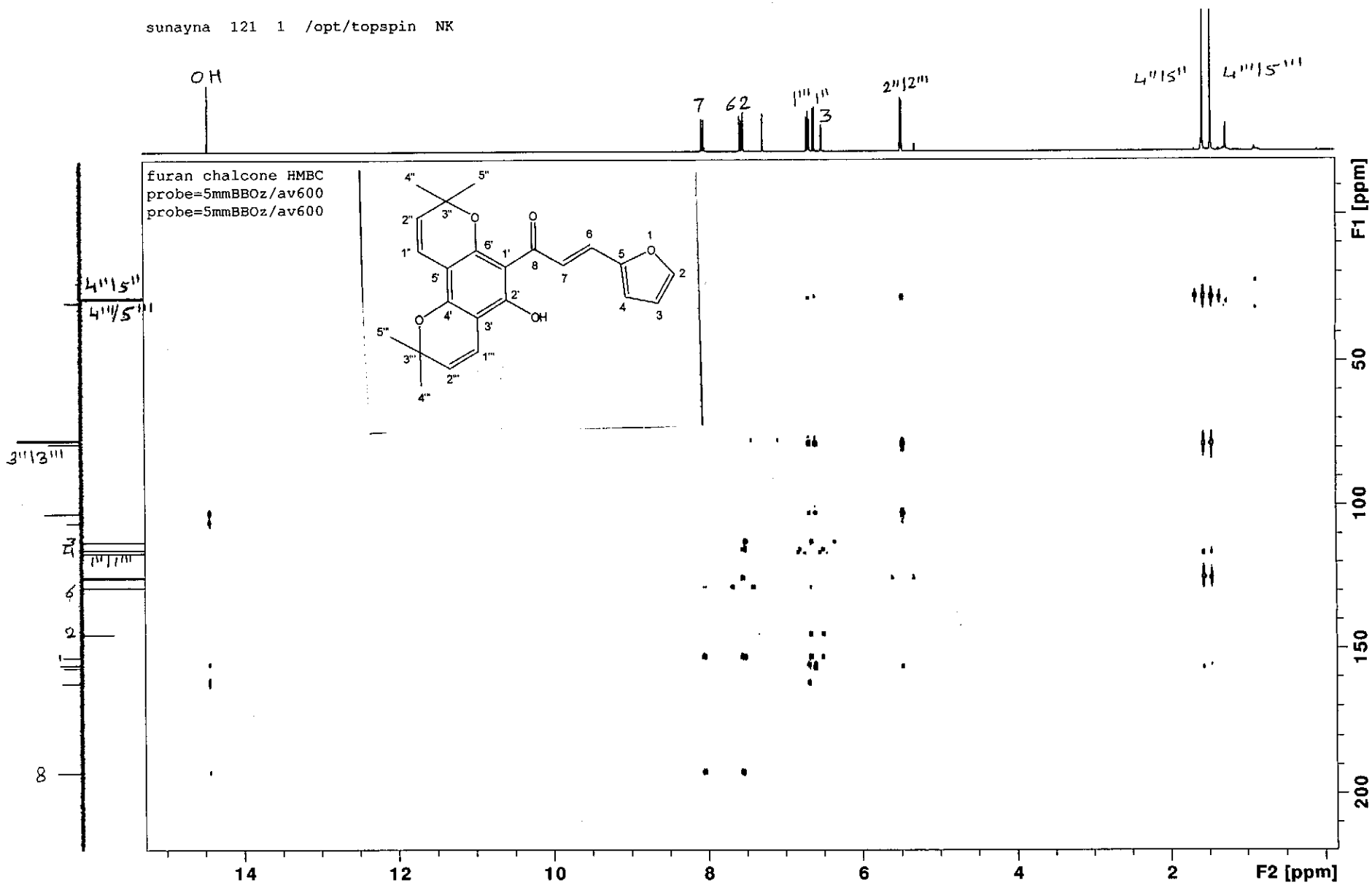
HSQC NMR spectrum of furan pyranochromene chalcone (B-4)



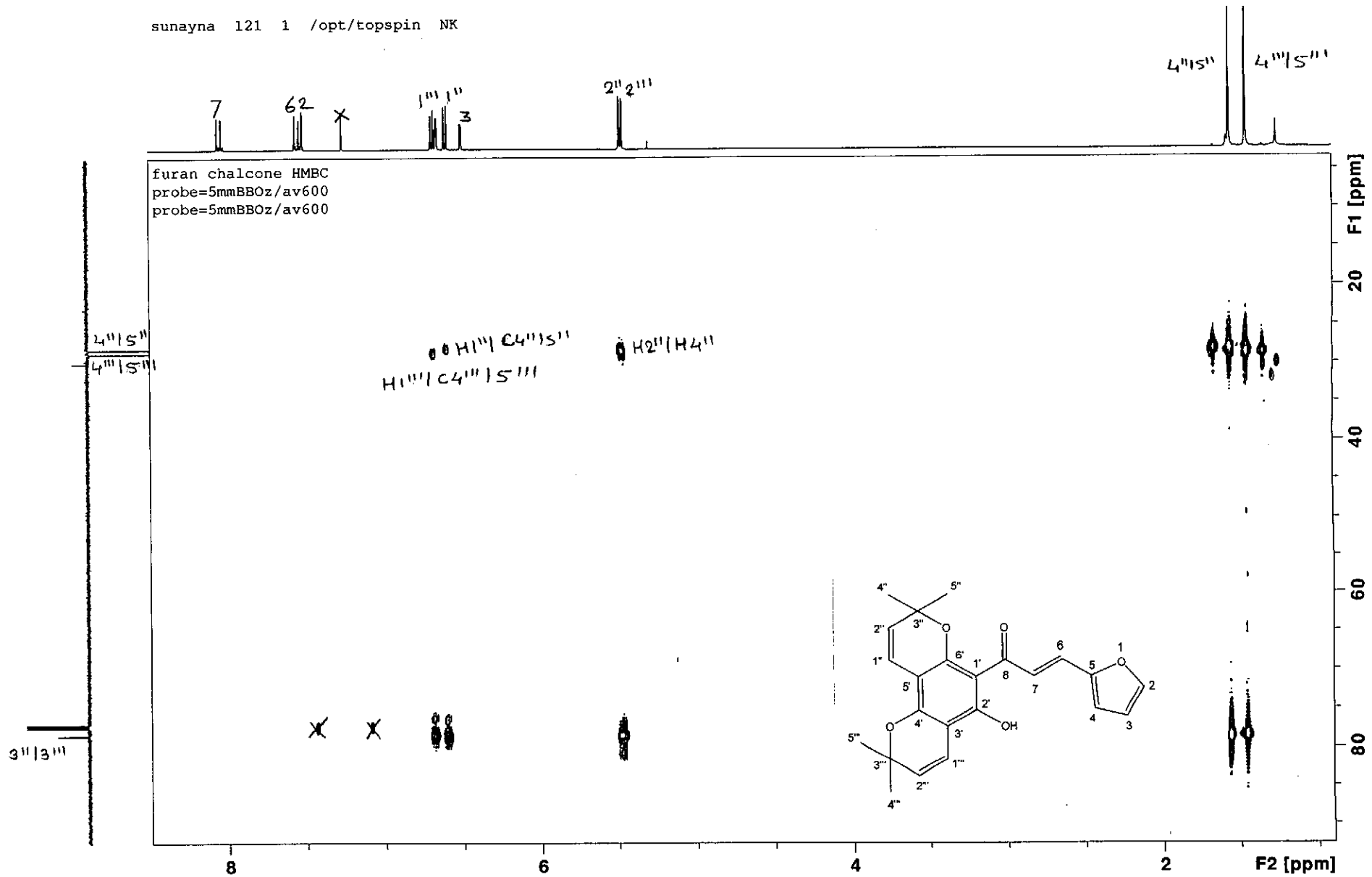
Expanded HSQC spectrum of furan pyranochromene chalcone (B-4)



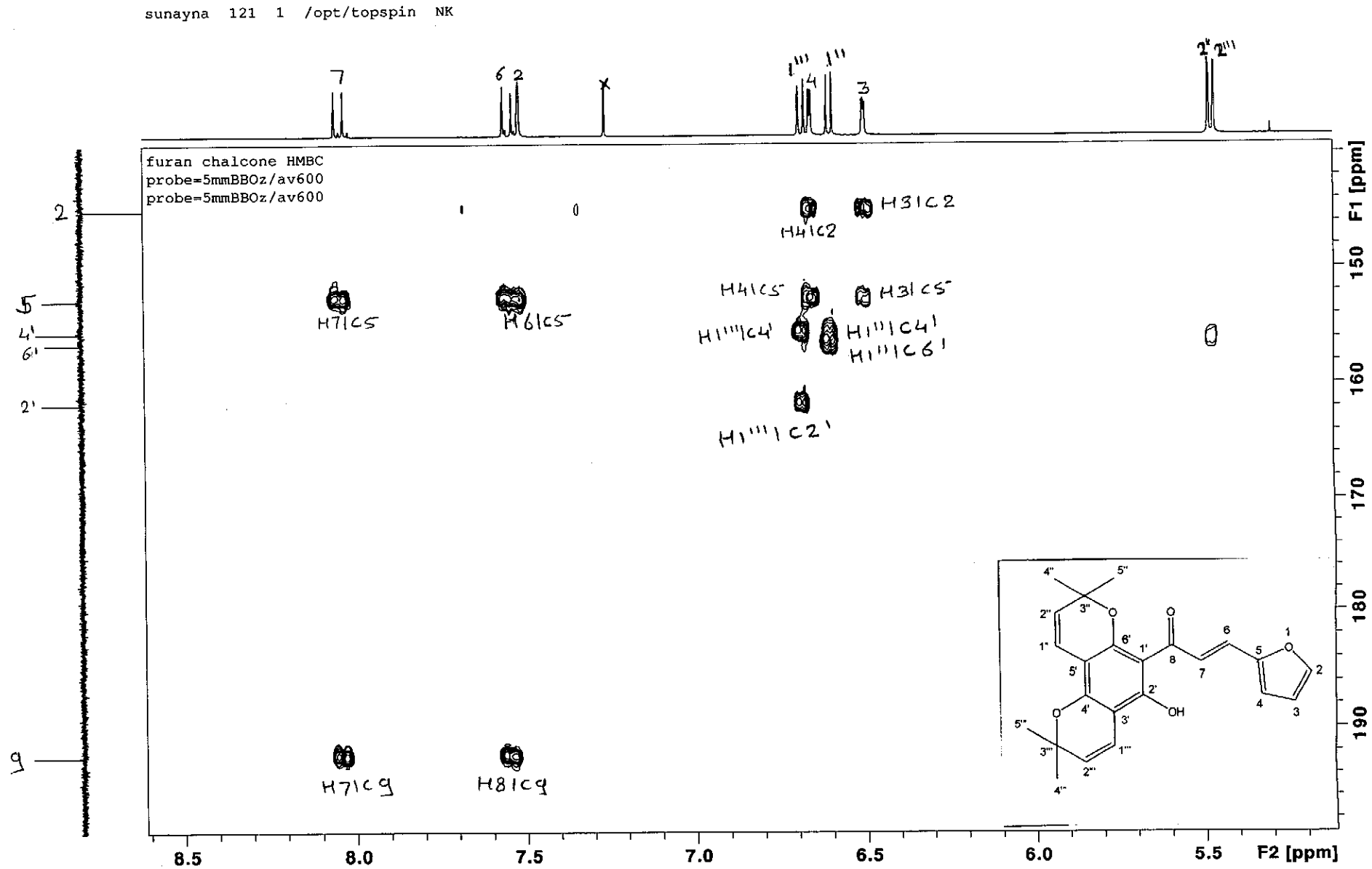
Expanded HSQC spectrum of furan pyranochromene chalcone (B-4)



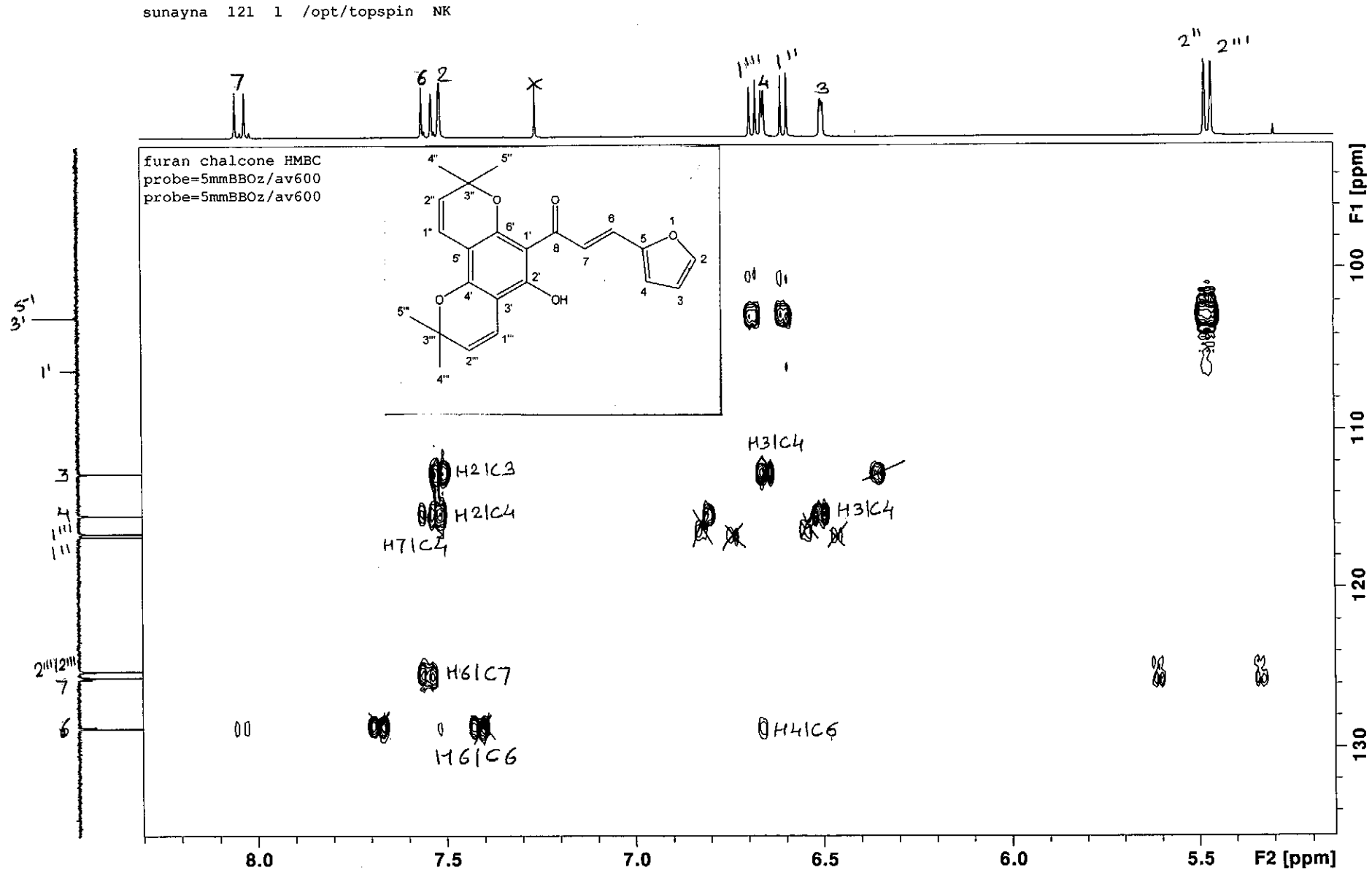
HMBC spectrum of furan pyranochromene chalcone (B-4)



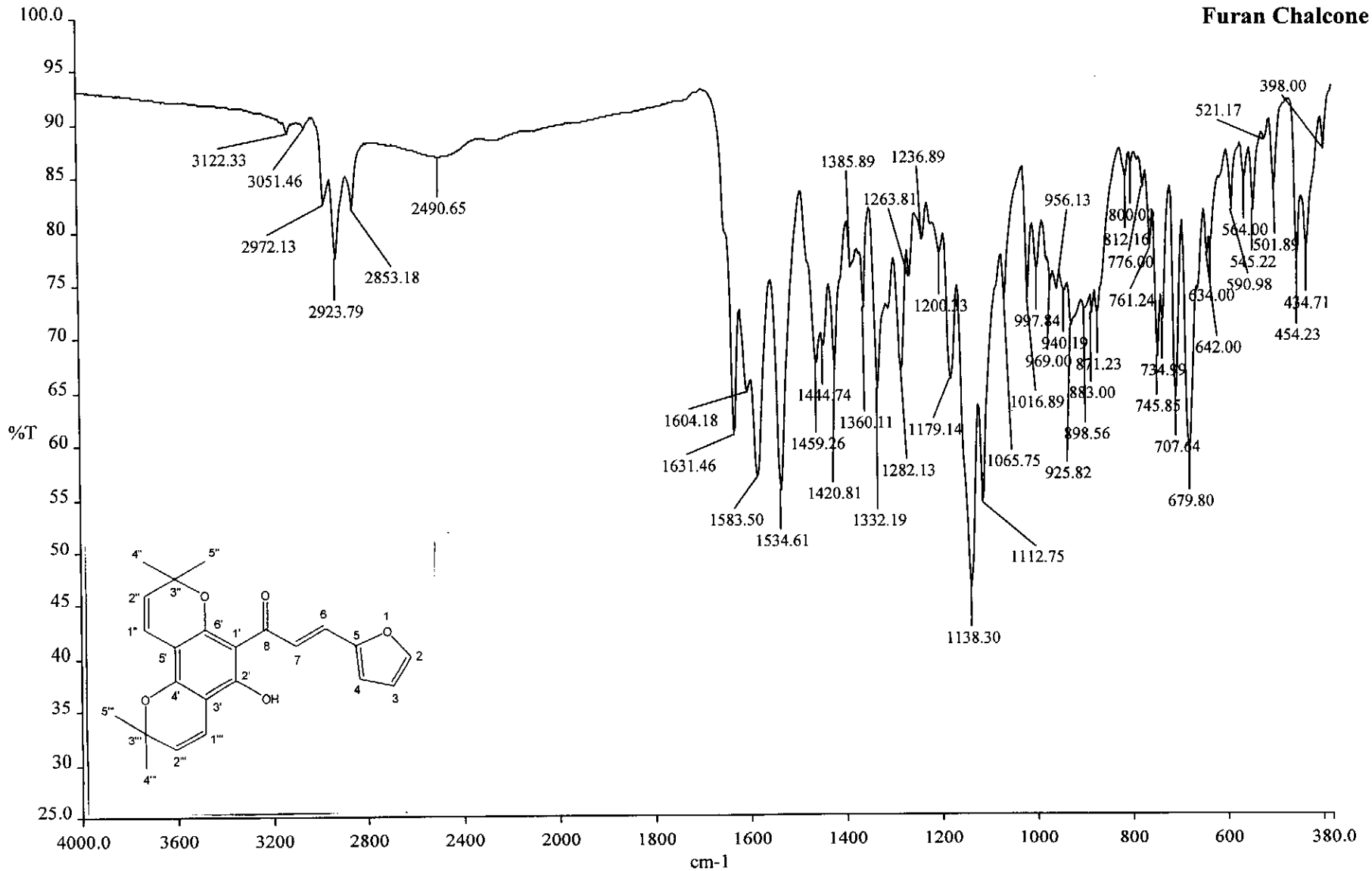
Expanded HMBC spectrum of furan pyranochromene chalcone (B-4)



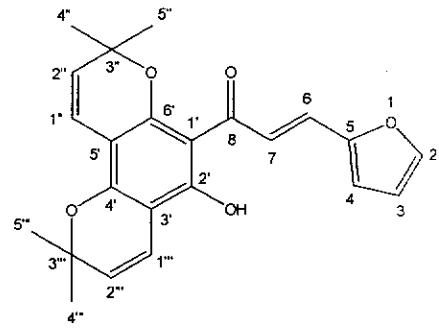
Expanded HMBC spectrum of furan pyranochromene chalcone (B-4)



Expanded HMBC spectrum of furan pyranochromene chalcone (B-4)



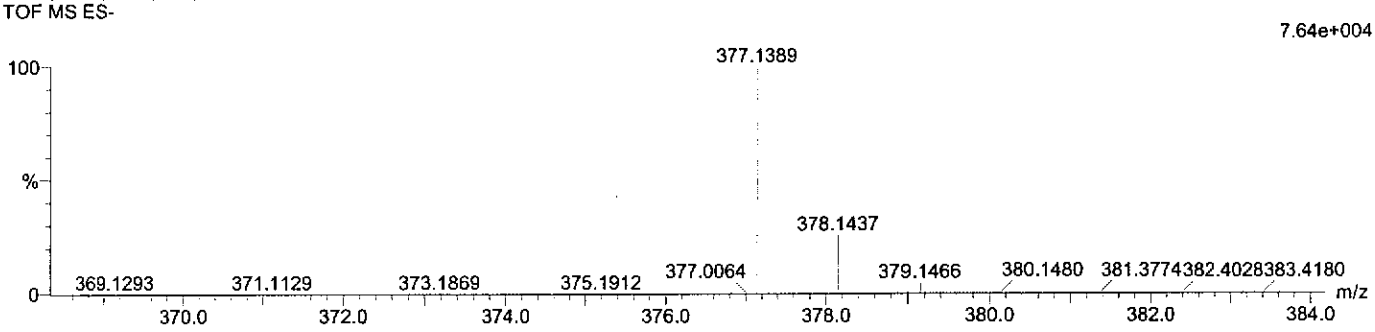
Infrared spectrum of furan pyranochromene chalcone (B-4)



Elemental Composition Report

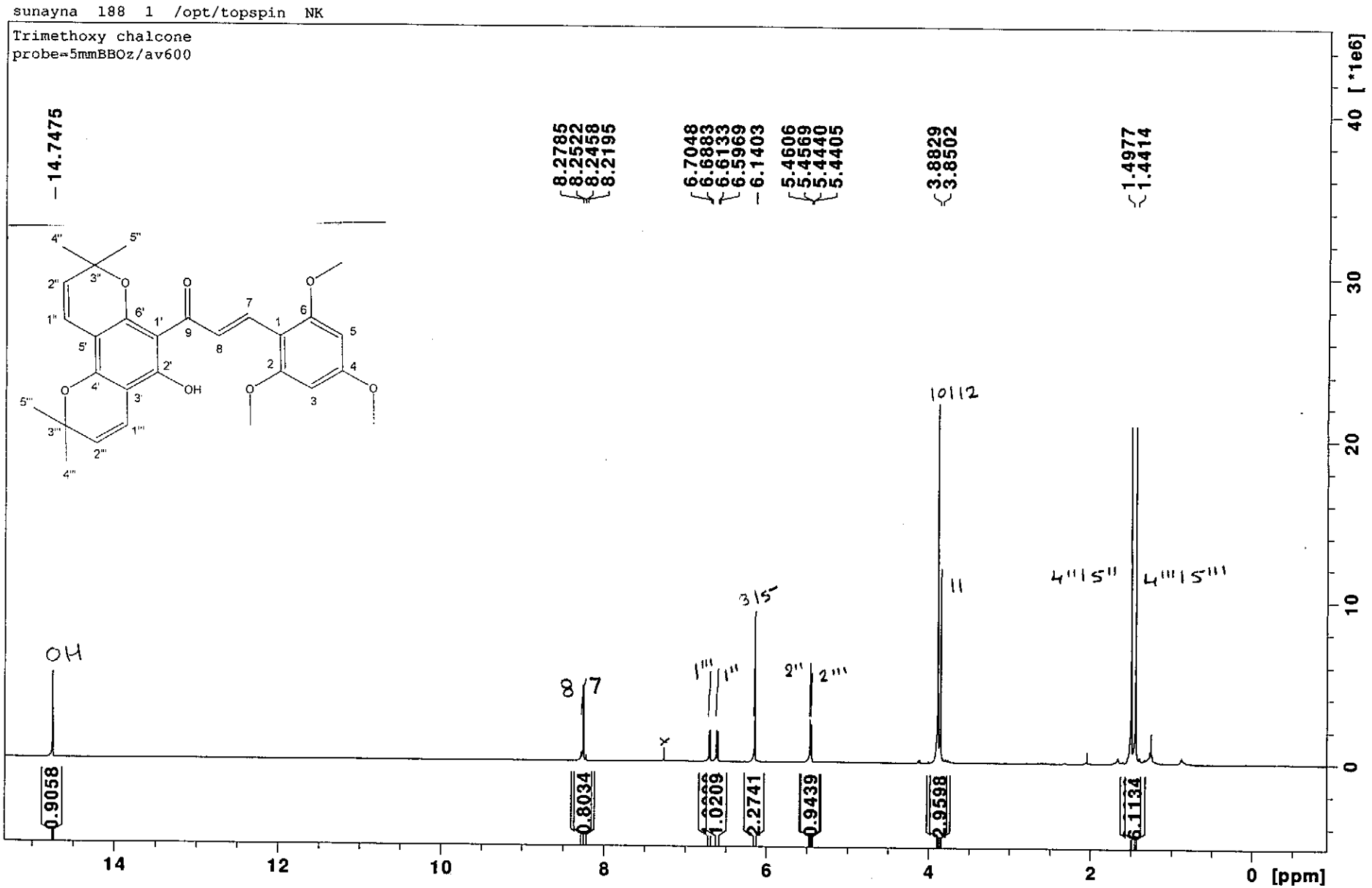
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
6 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-35 H: 20-30 O: 0-5
27 2 (0.017) Cm (1:31)
TOF MS ES-

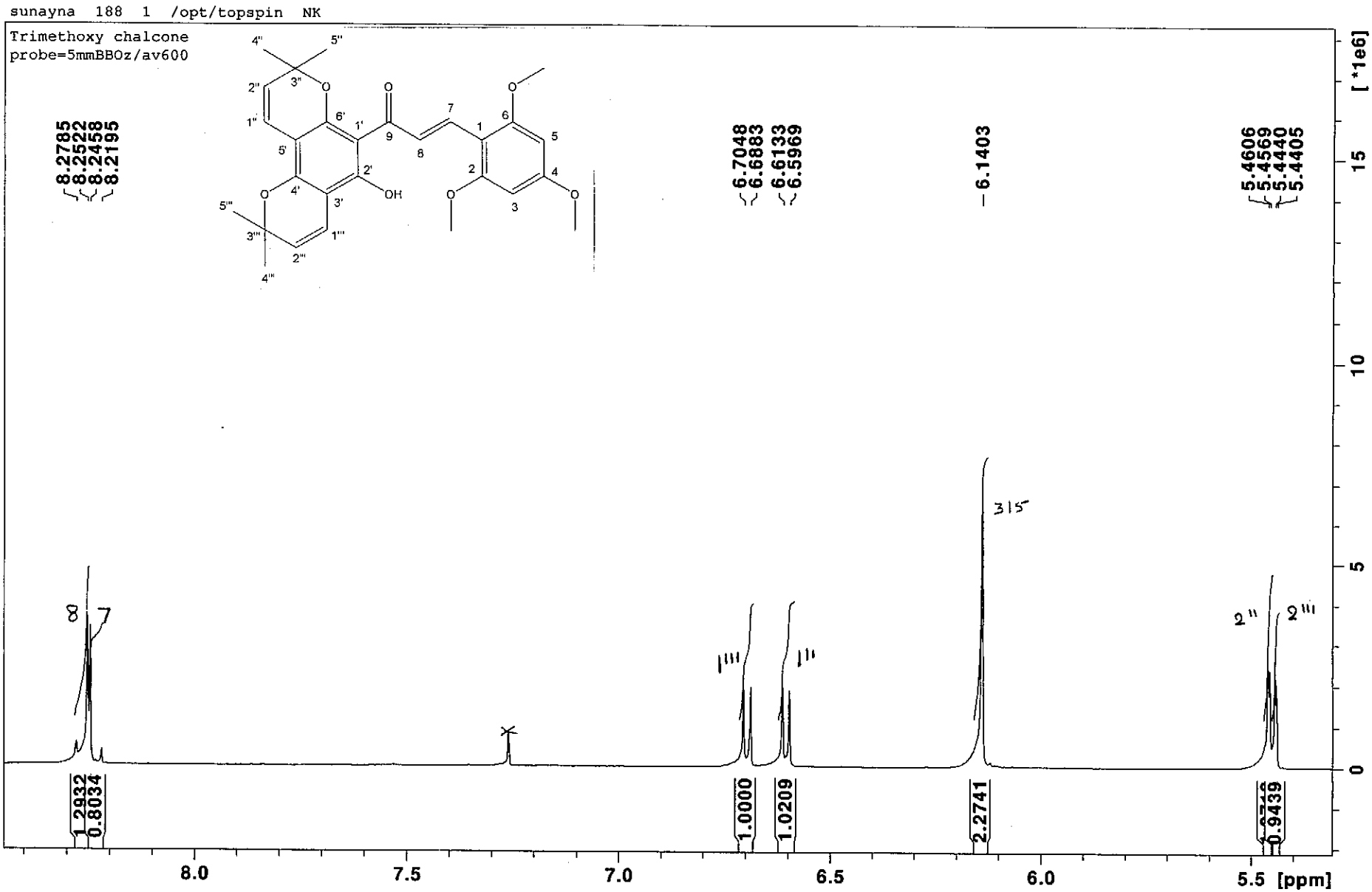


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
377.1389	377.1389	0.0	0.0	13.5	512.4	0.0	C23 H21 O5

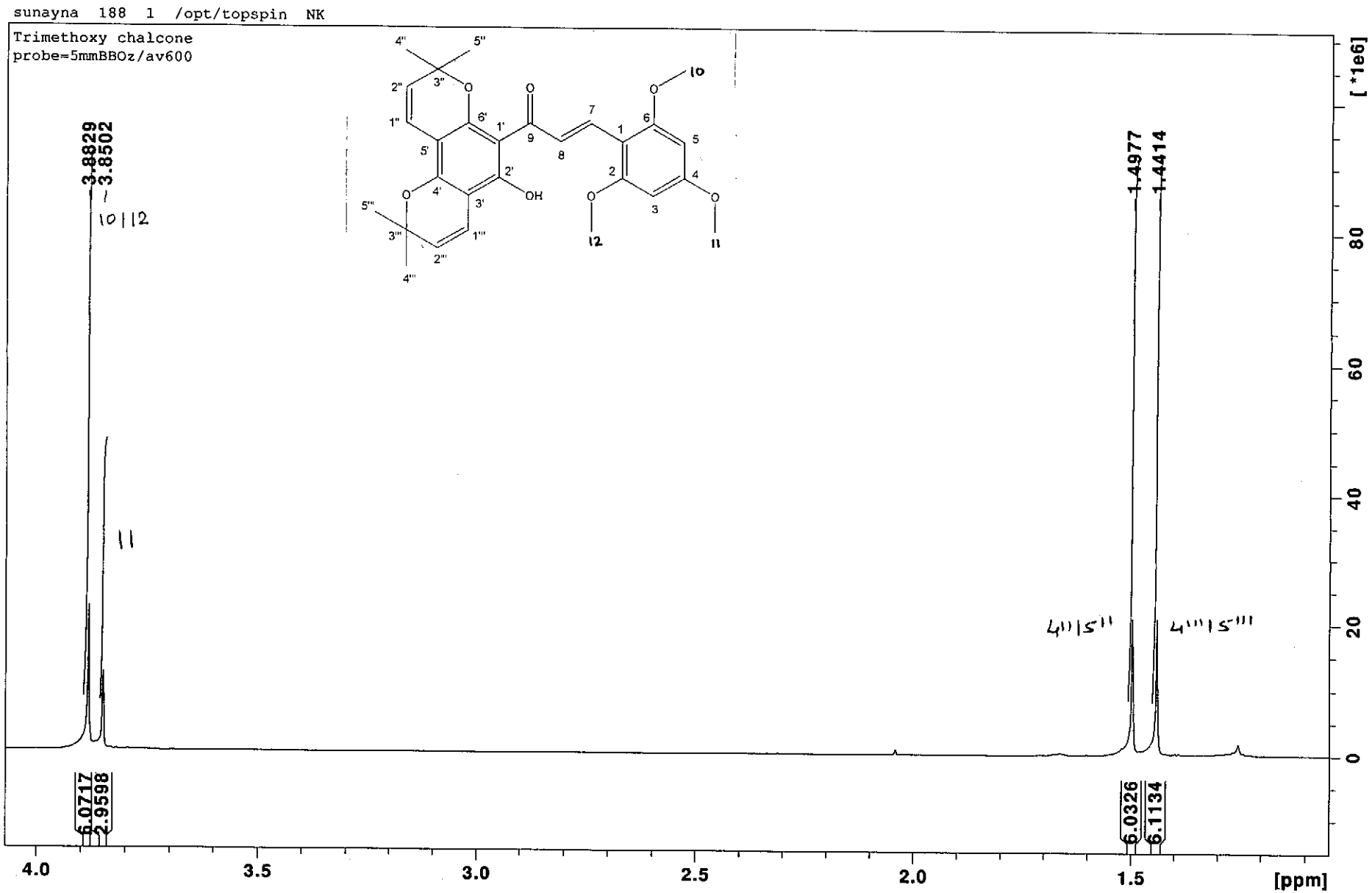
HRMS spectrum of furan pyranochromene chalcone (B-4)



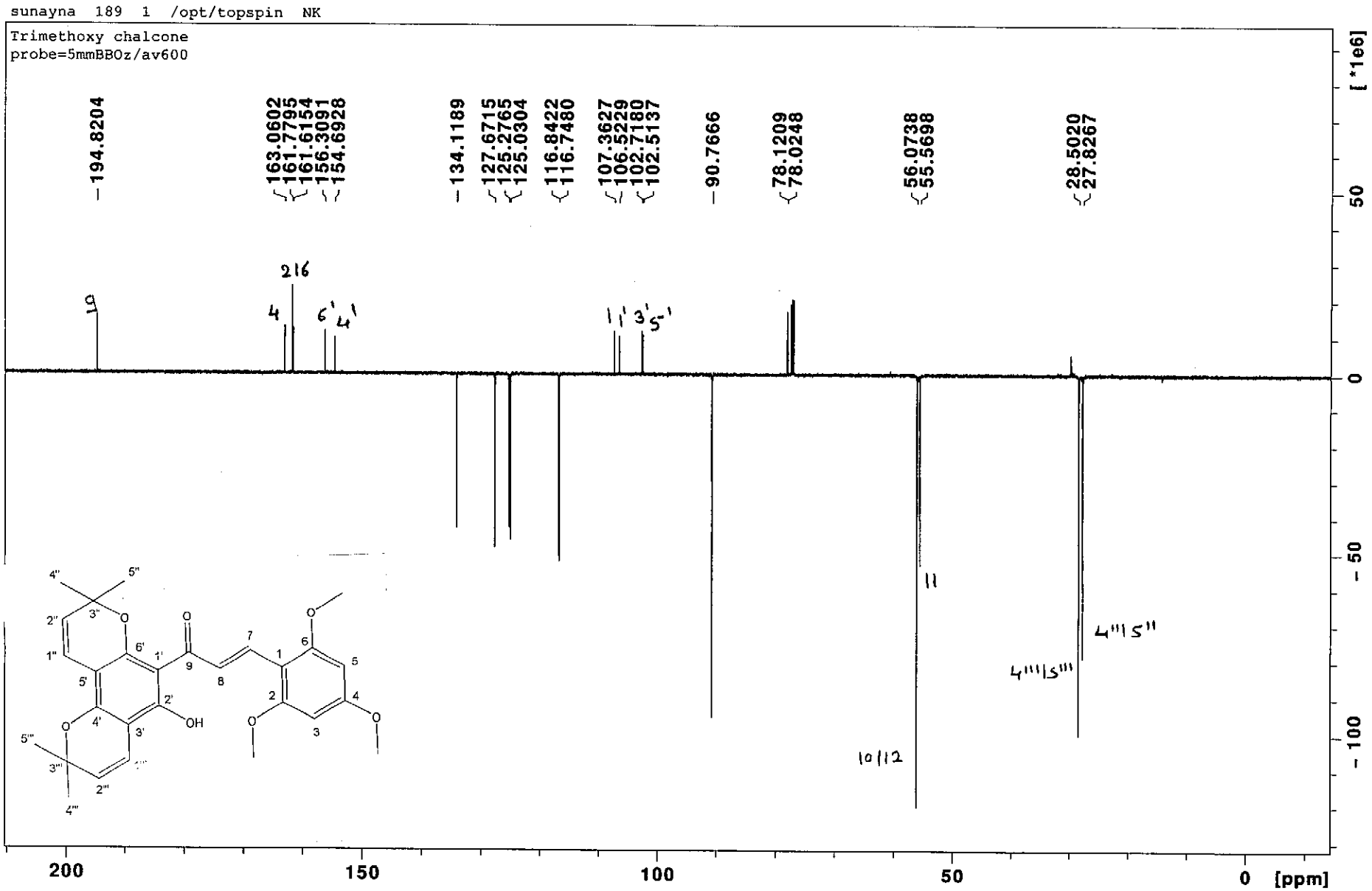
1H NMR spectrum of 2,4,6-trimethoxypyranochromene chalcone (B-5)



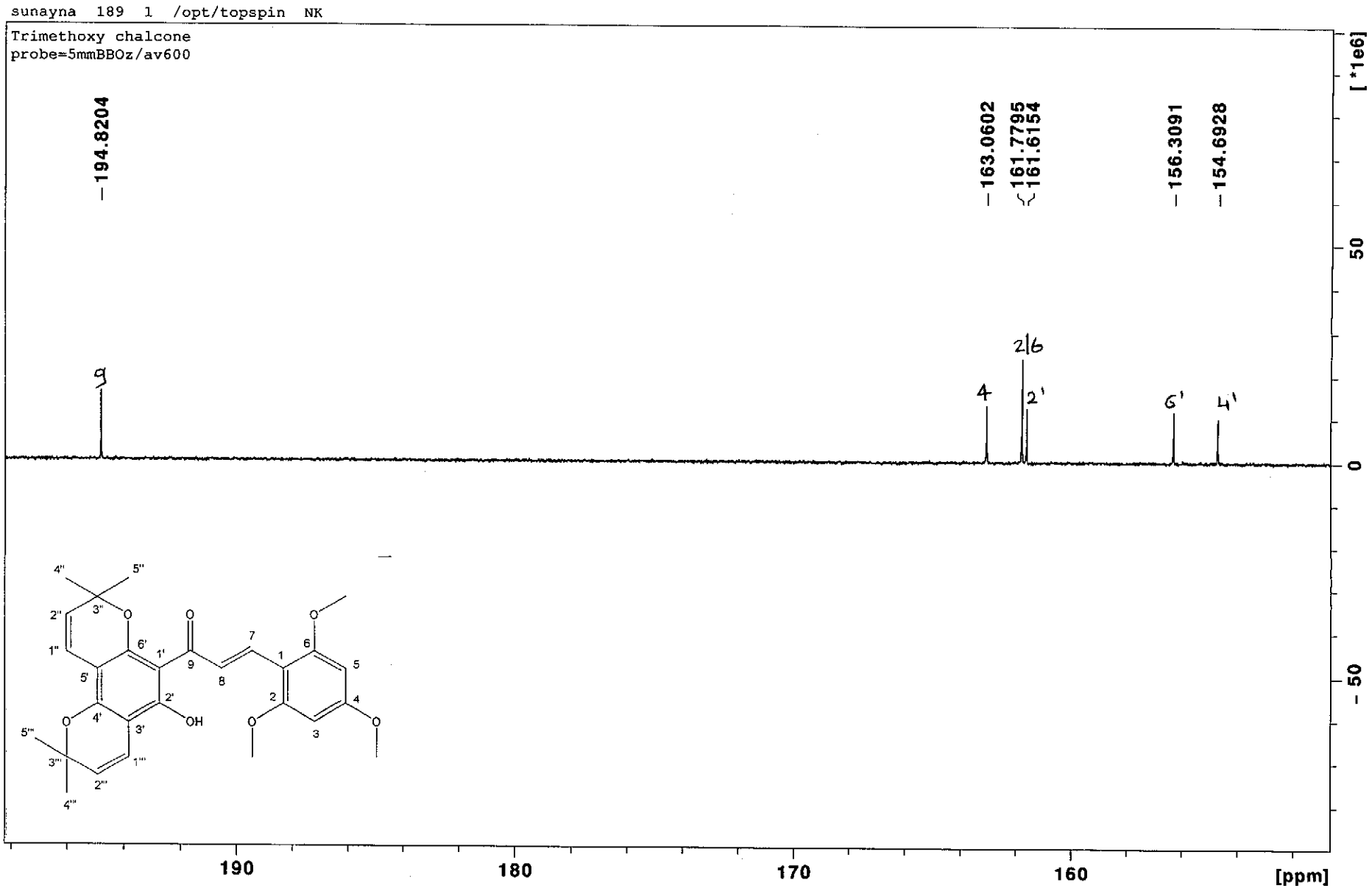
Expanded 1H NMR spectrum of 2,4,6-trimethoxypyranochromene chalcone (B-5)



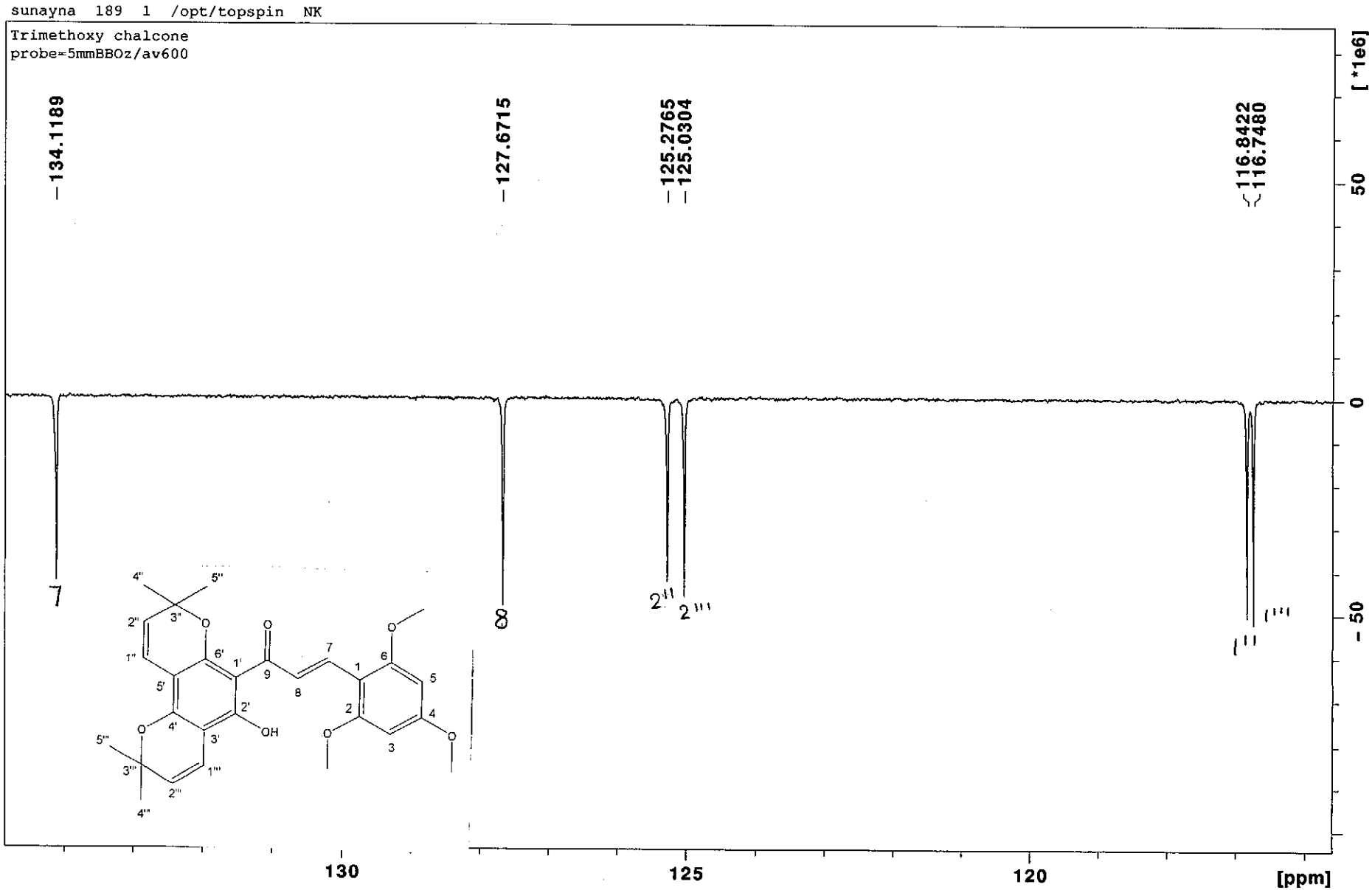
Expanded 1H NMR spectrum of 2,4,6-trimethoxypyranochromene chalcone (B-5)



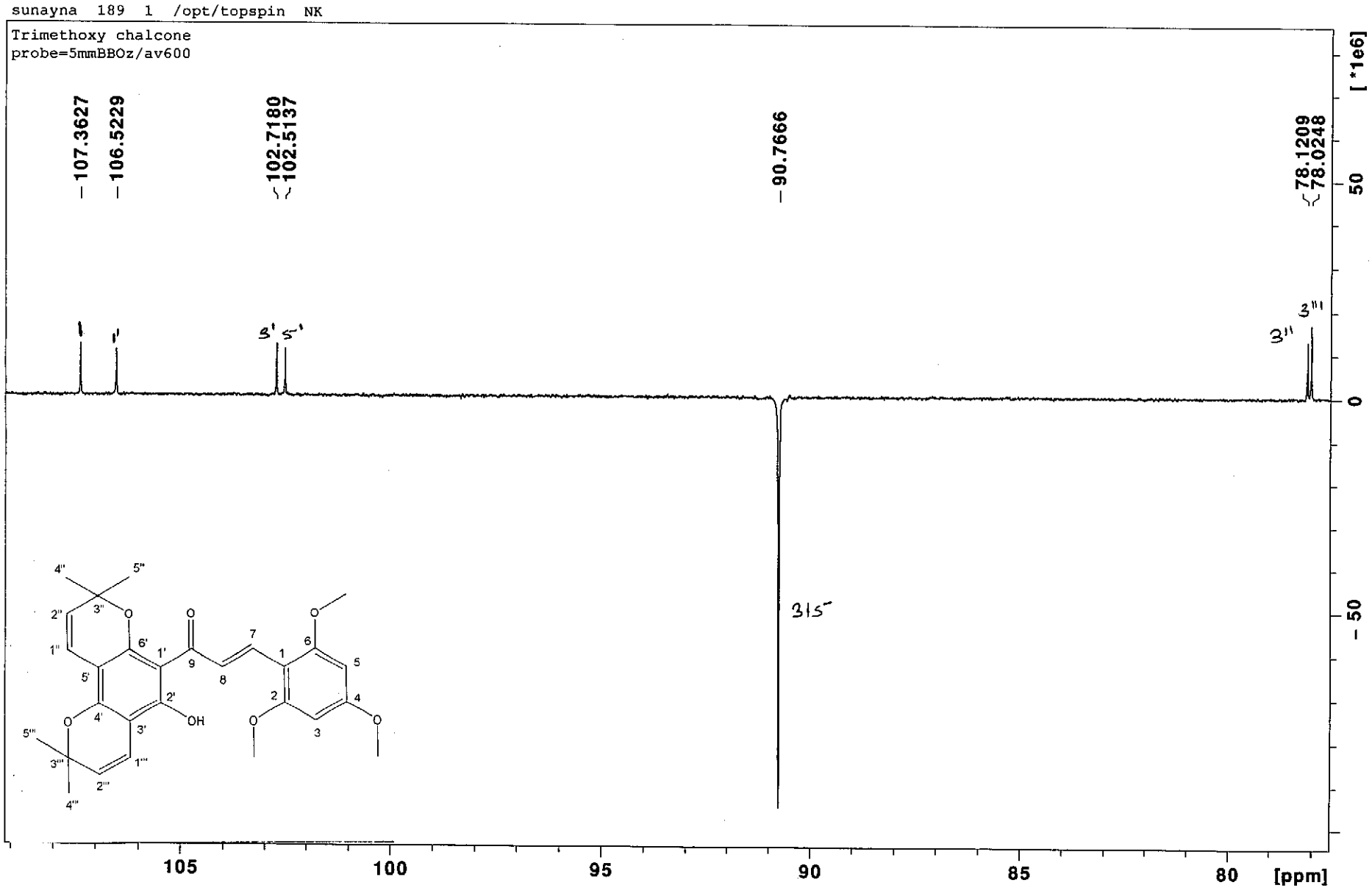
13C NMR spectrum of 2,4,6-trimethoxypyranochromene chalcone (B-5)



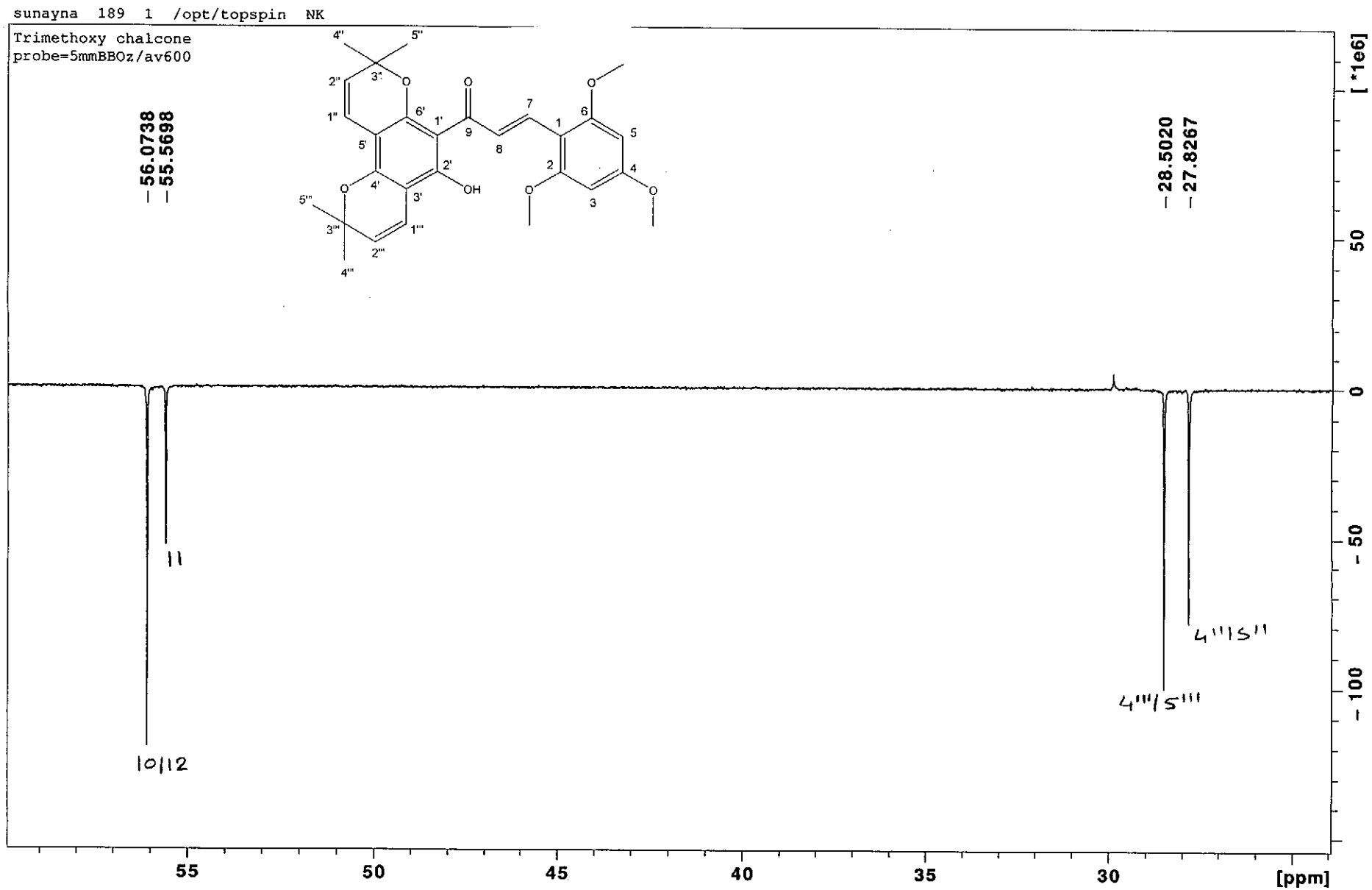
Expanded 13C NMR spectrum of 2,4,6-trimethoxypyranochromene chalcone (B-5)



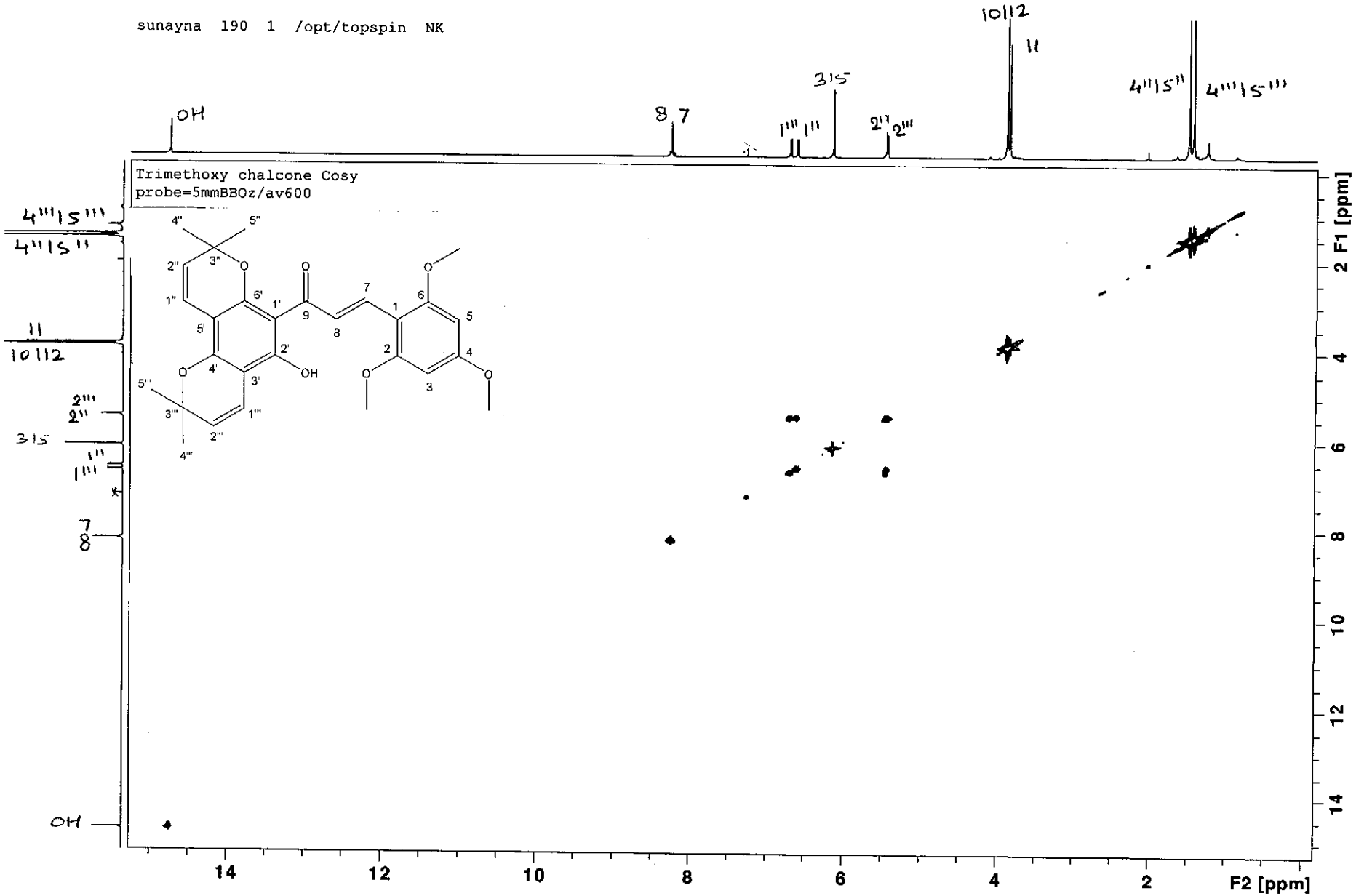
Expanded 13C NMR spectrum of 2,4,6-trimethoxypyranochromene chalcone (B-5)



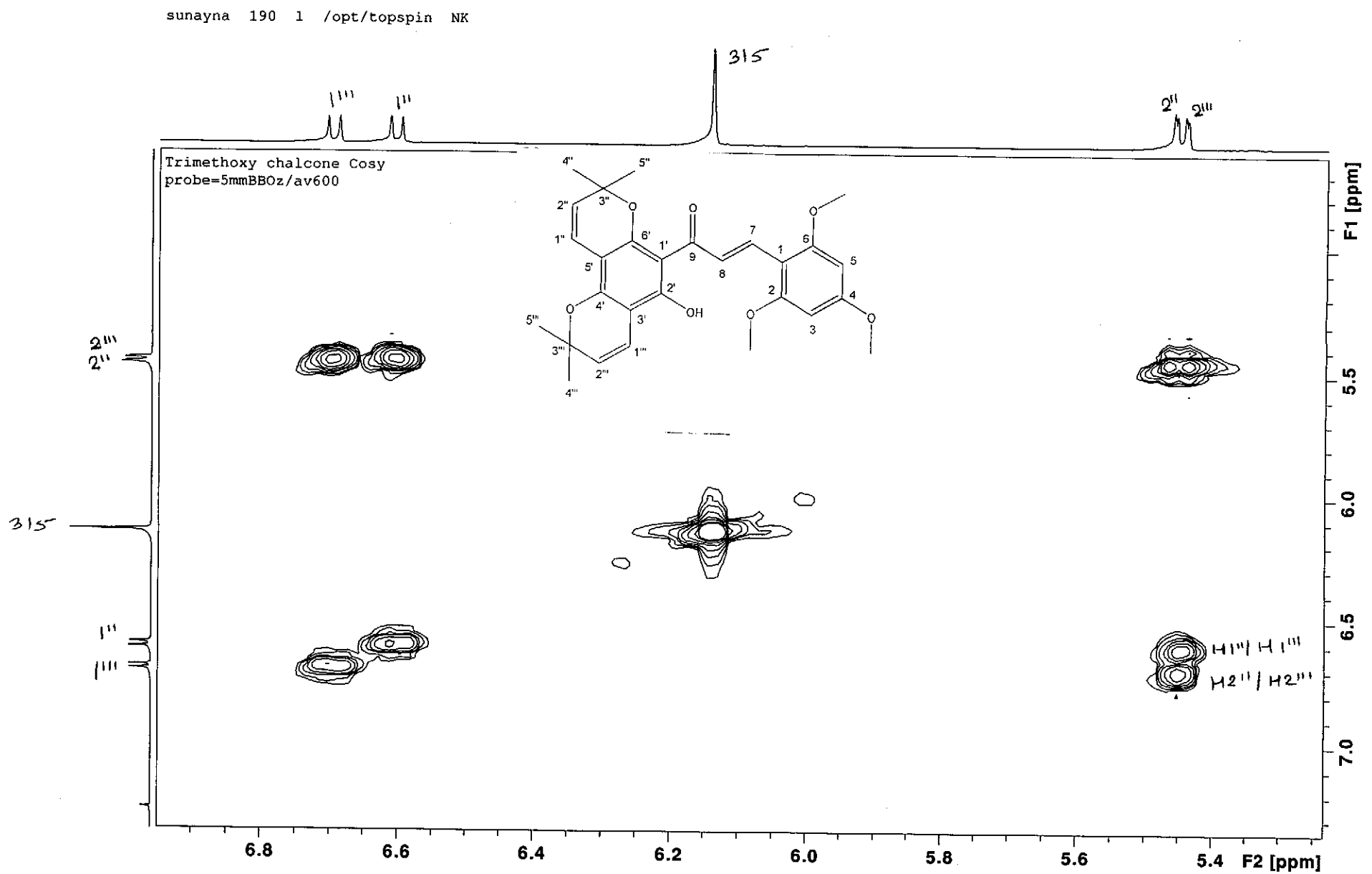
Expanded 13C NMR spectrum of 2,4,6-trimethoxypyranochromene chalcone (B-5)



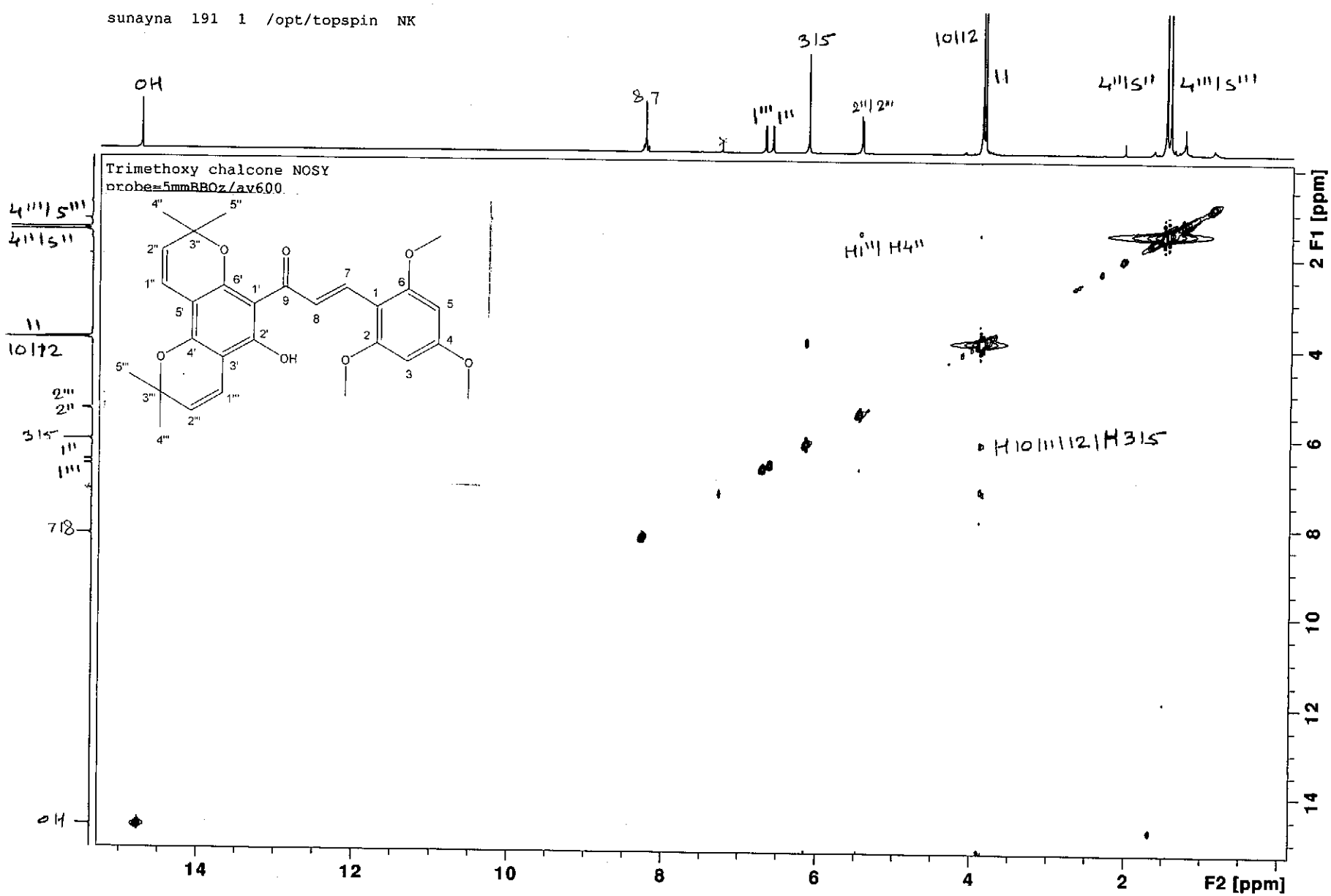
Expanded 13C NMR spectrum of 2,4,6-trimethoxypyranochromene chalcone (B-5)



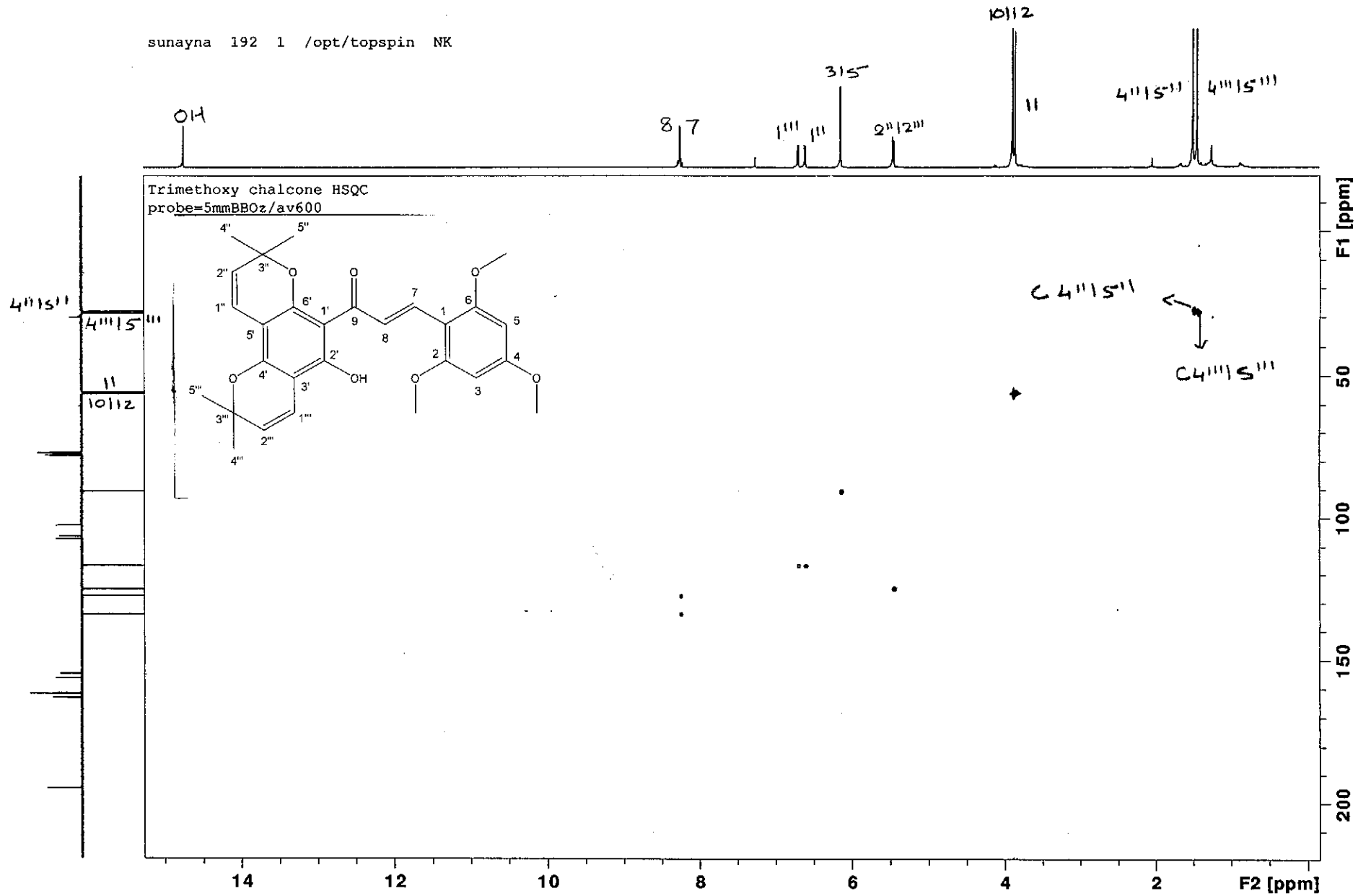
COSY NMR spectrum of 2,4,6-trimethoxypyranochromene chalcone (B-5)



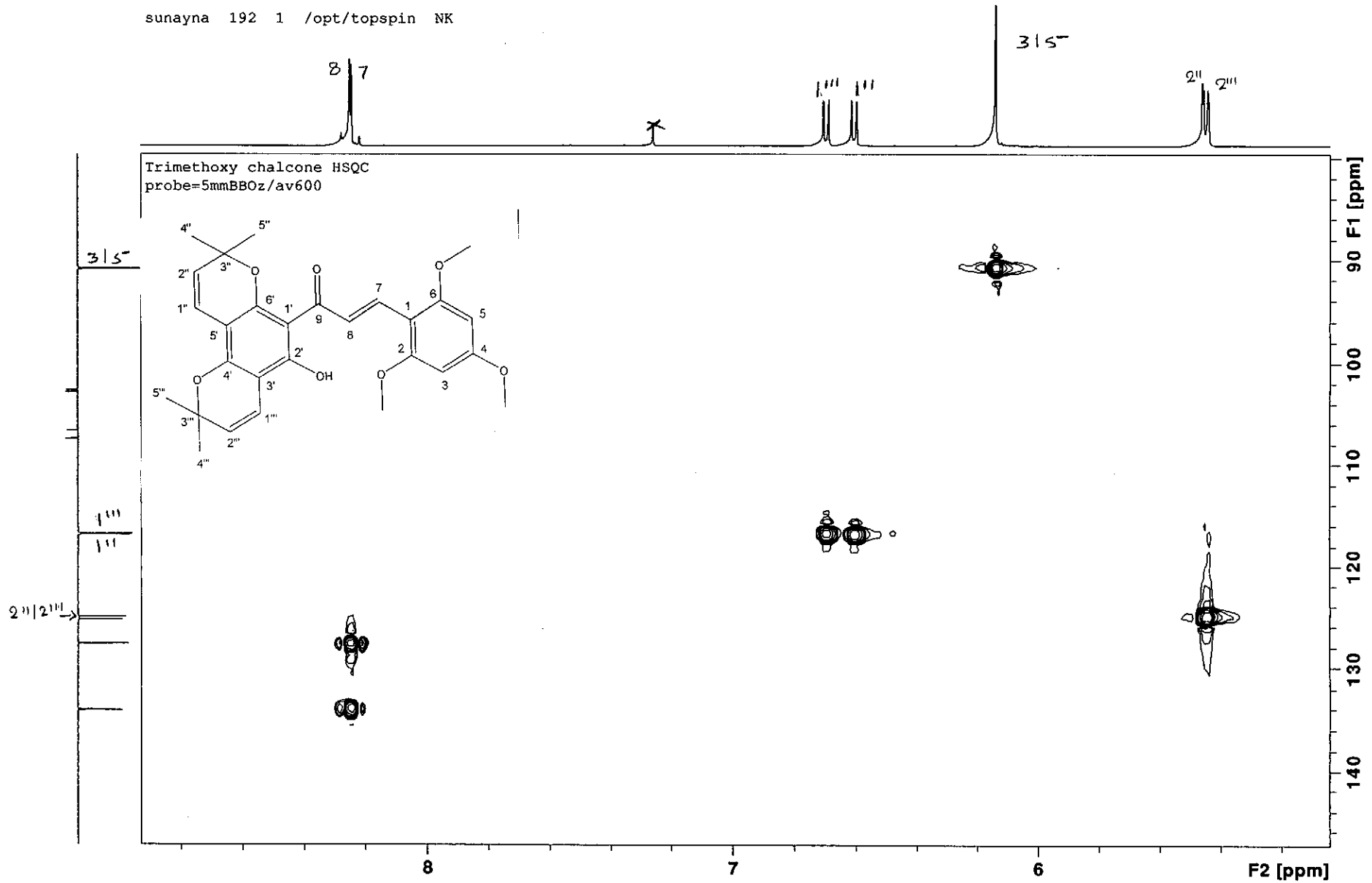
Expanded COSY NMR spectrum of 2,4,6-trimethoxypyranochromene chalcone (B-5)



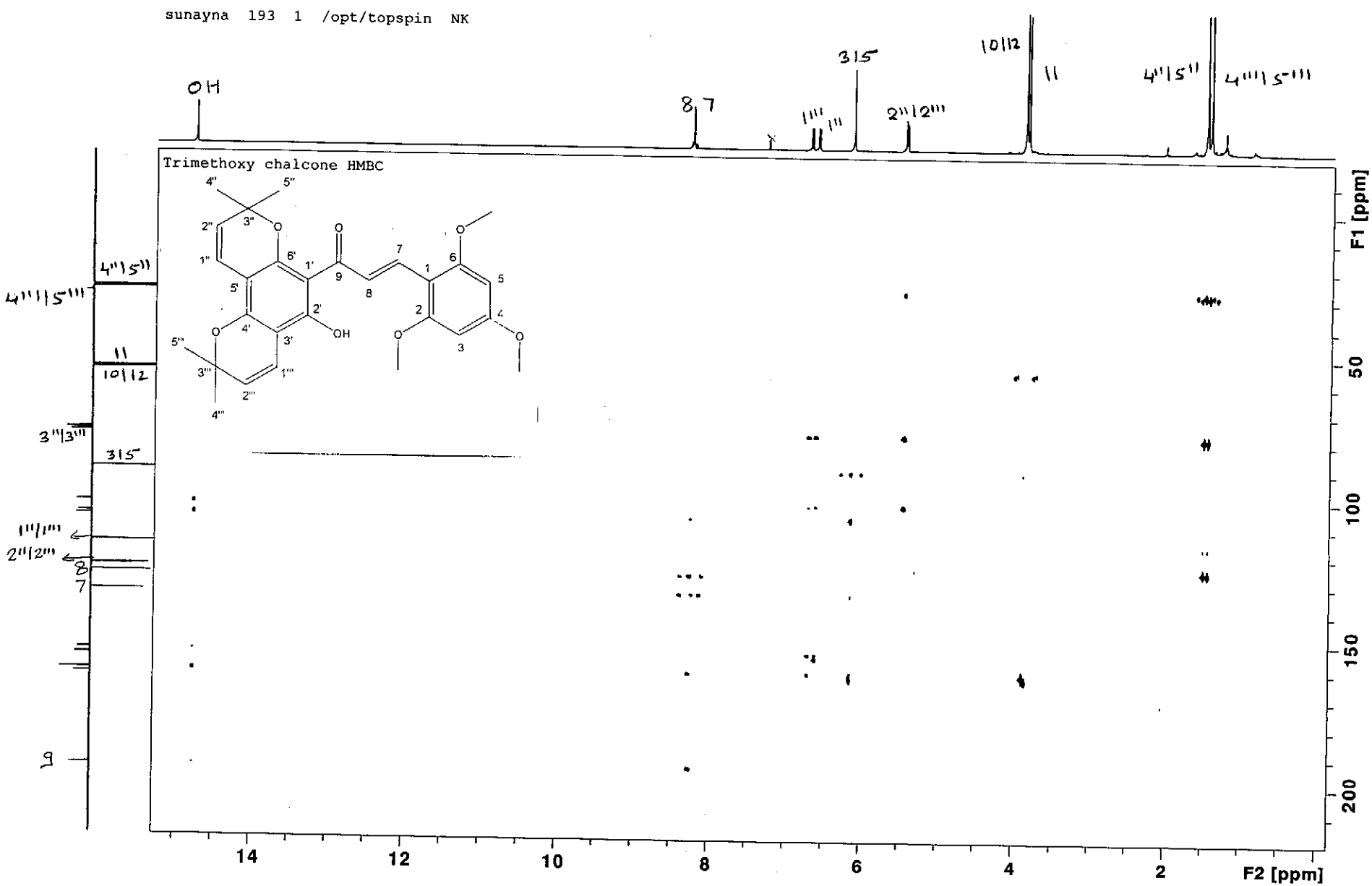
NOESY NMR spectrum of 2,4,6-trimethoxypyranochromene chalcone (B-5)



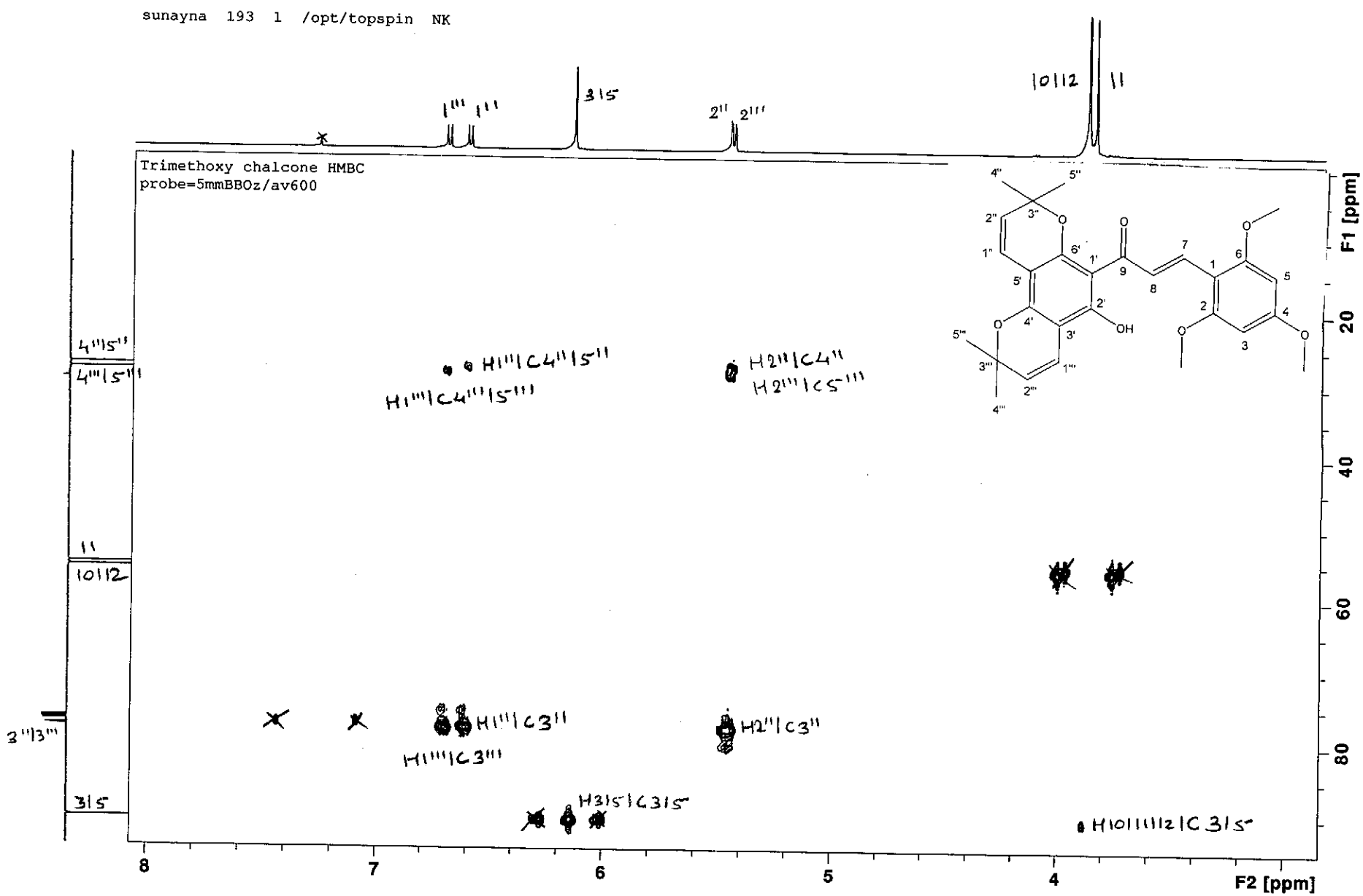
HSQC spectrum of 2,4,6-trimethoxypyranochromene chalcone (B-5)



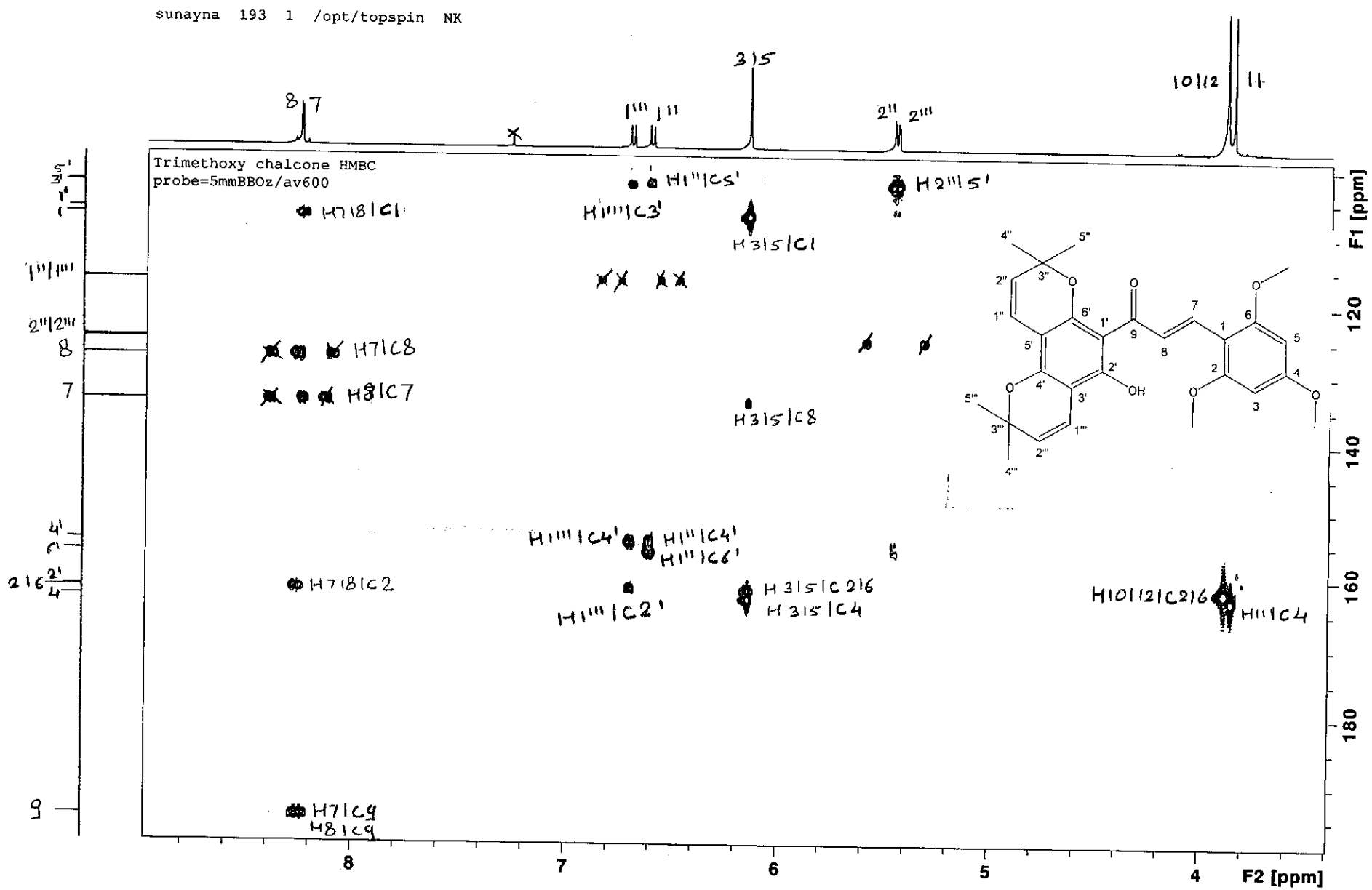
Expanded HSQC spectrum of 2,4,6-trimethoxypyranochromene chalcone (B-5)



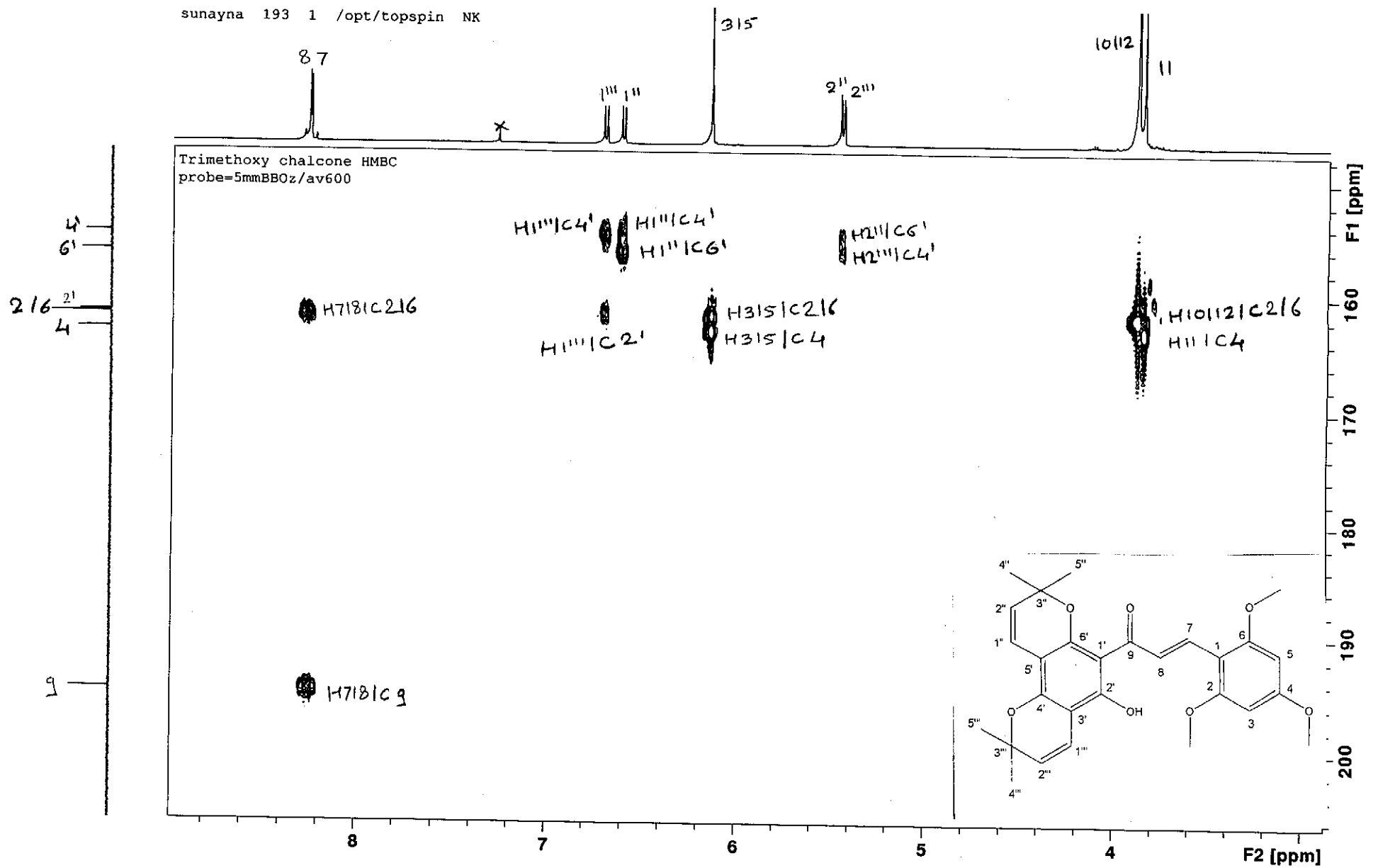
HMBC spectrum of 2,4,6-trimethoxypyranochromene chalcone (B-5)



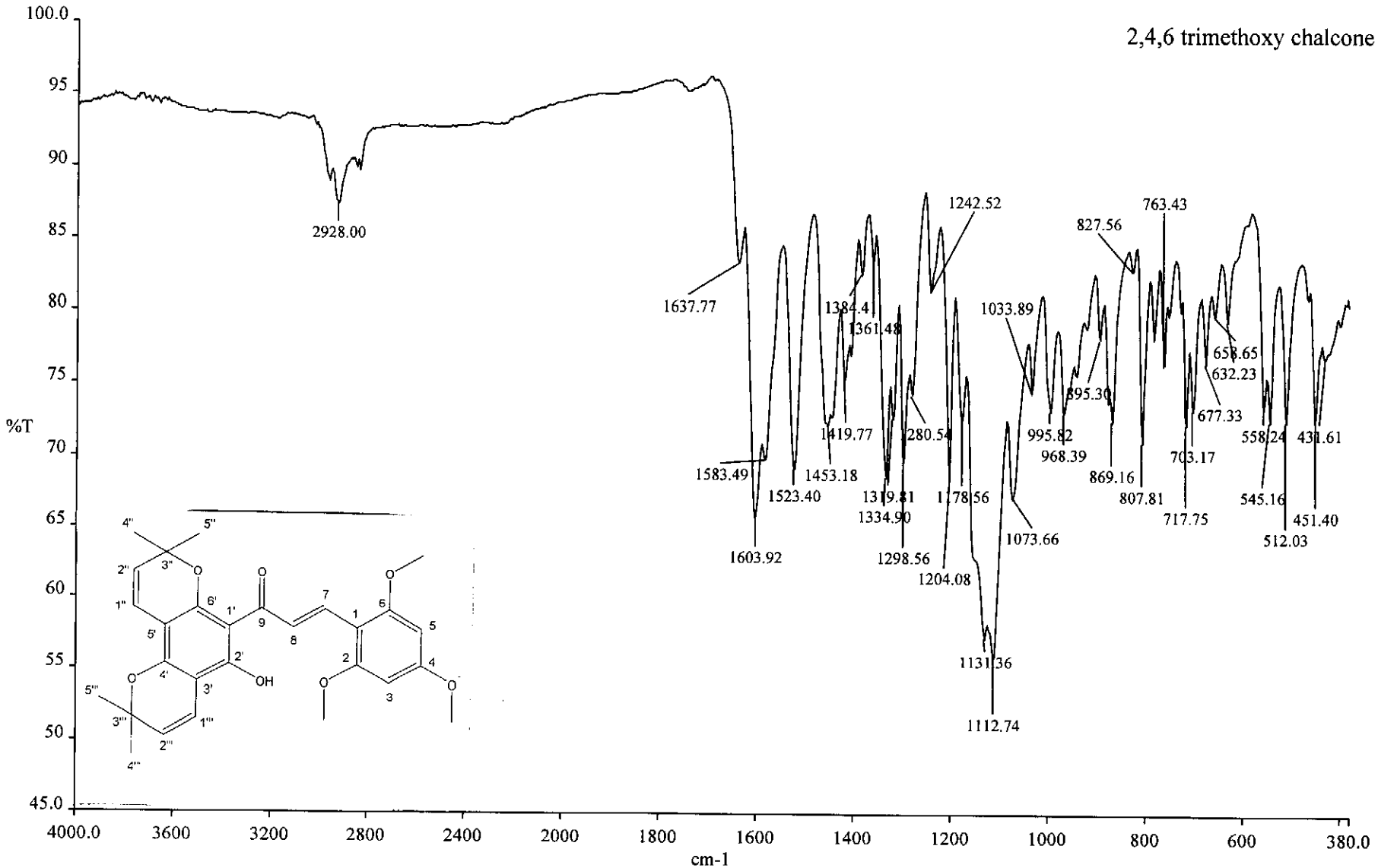
Expanded HMBC spectrum of 2,4,6-trimethoxypyranochromene chalcone (B-5)



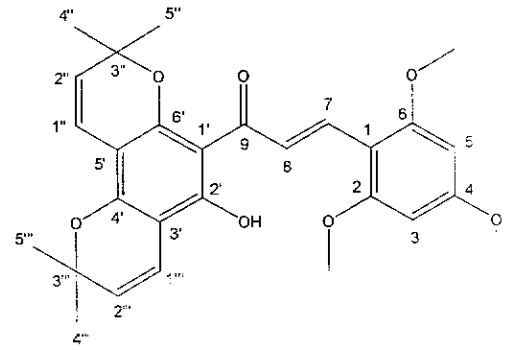
Expanded HMBC spectrum of 2,4,6-trimethoxypyranochromene chalcone (B-5)



Expanded HMBC spectrum of 2,4,6-trimethoxypyranochromene chalcone (B-5)



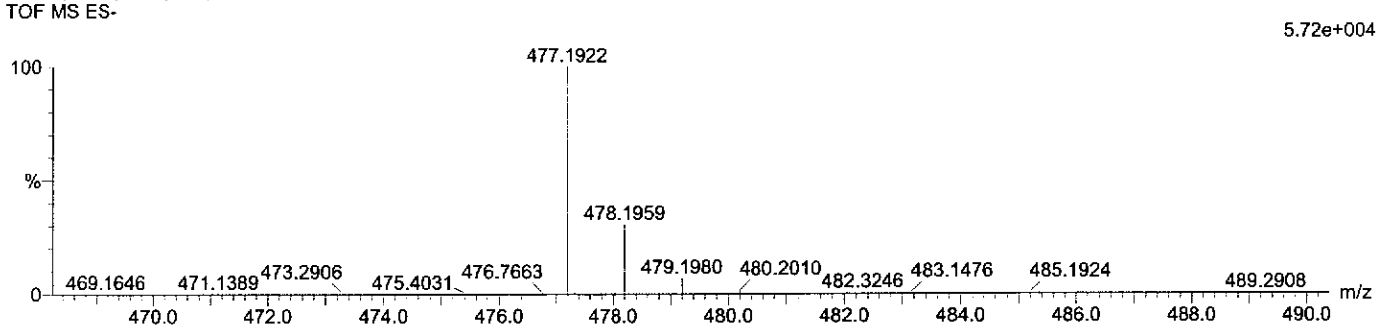
Infrared spectrum of 2,4,6-trimethoxypyranochromene chalcone (B-5)



Elemental Composition Report

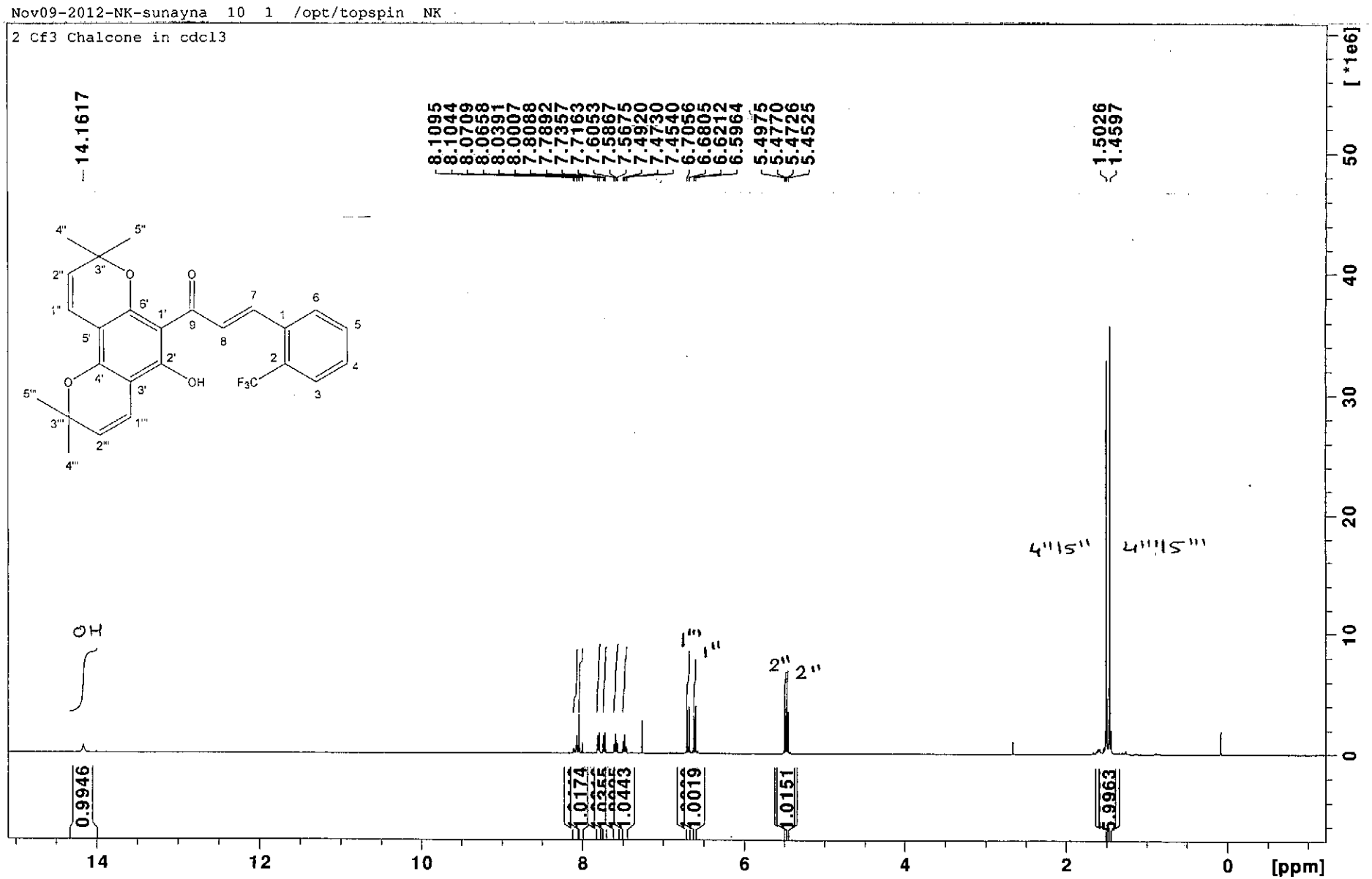
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
9 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-35 H: 20-30 O: 0-10
29 12 (0.188) Cm (1:31)
TOF MS ES-

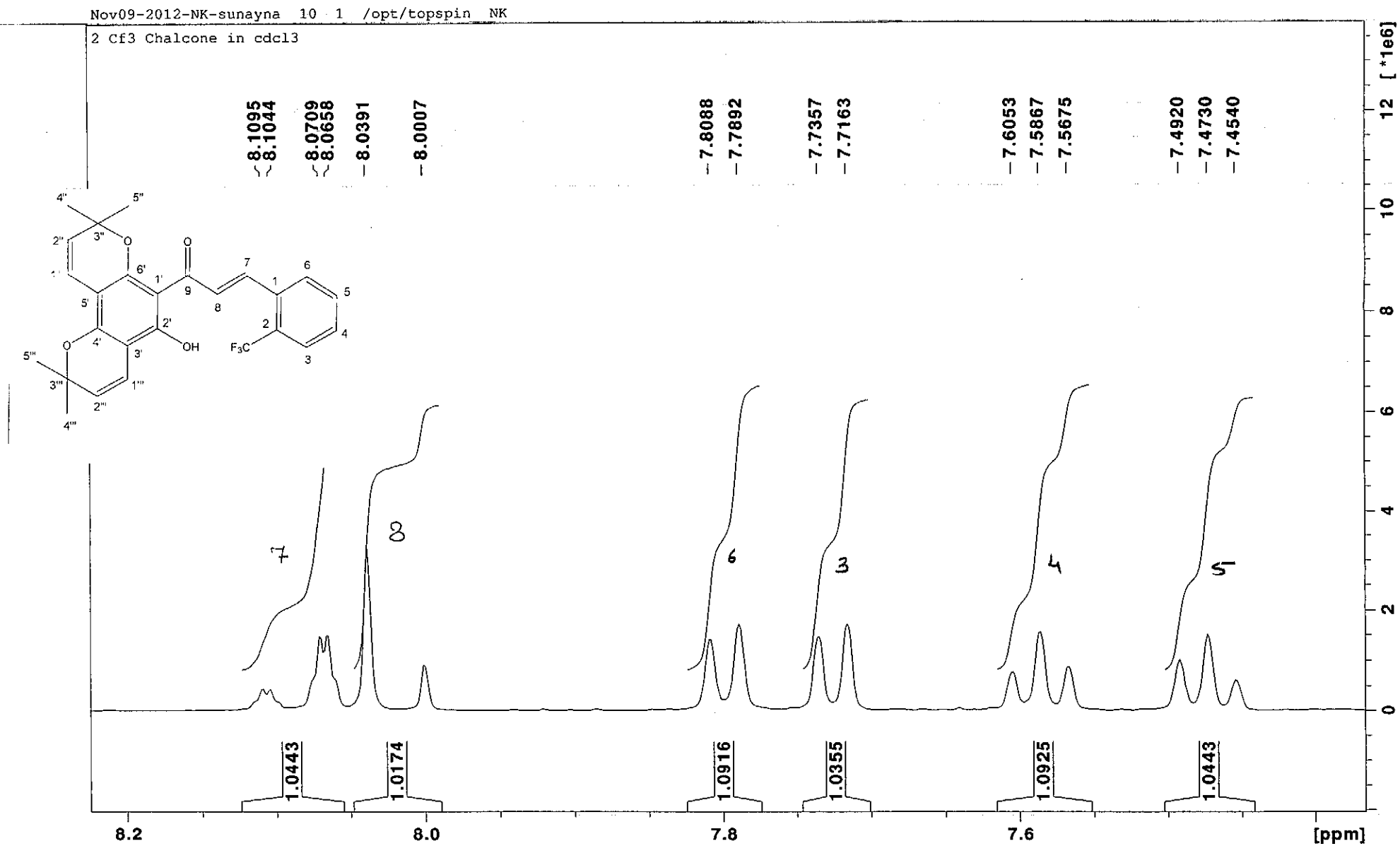


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
477.1922	477.1913	0.9	1.9	14.5	441.9	0.0	C28 H29 O7

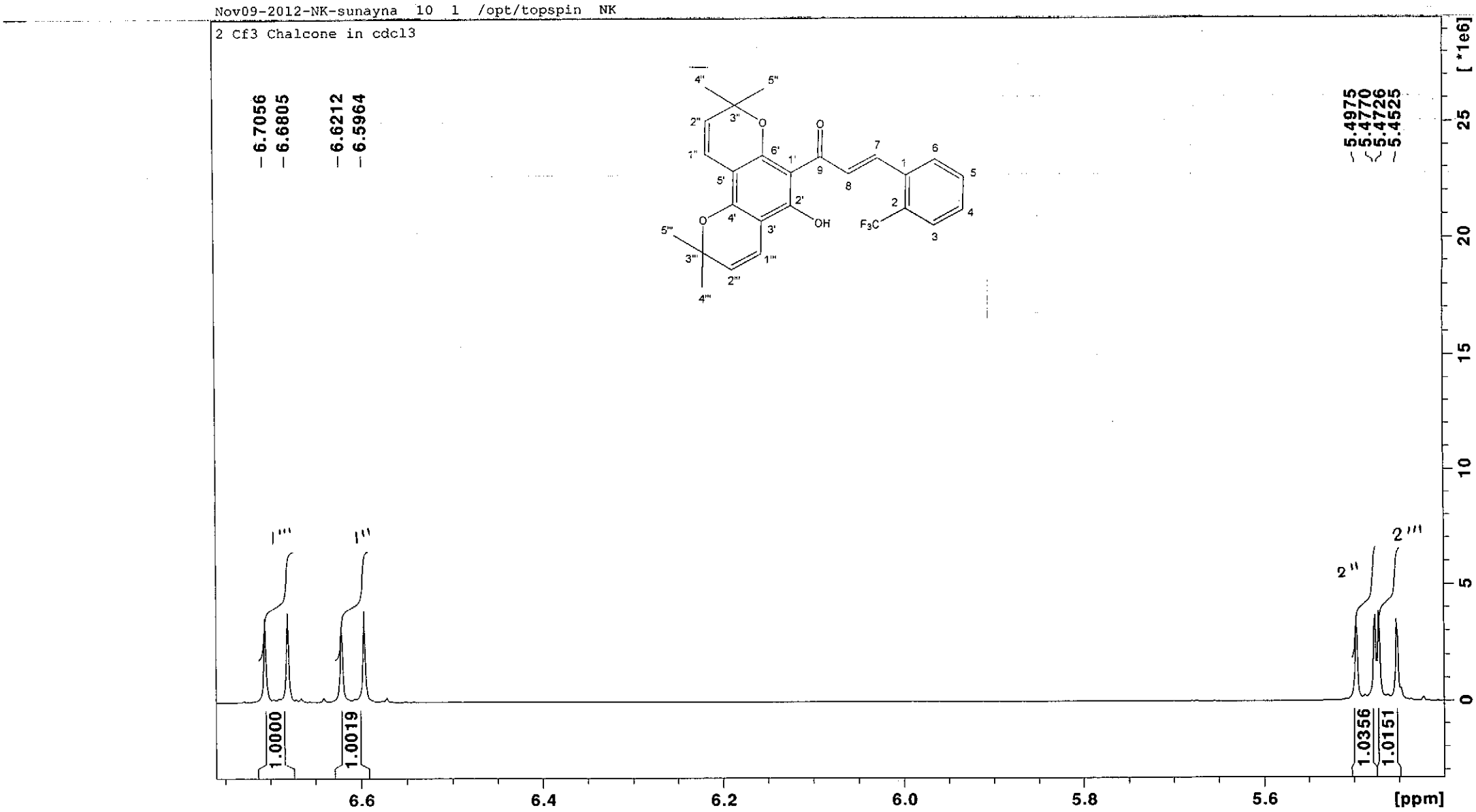
HRMS spectrum of 2,4,6-trimethoxypyranochromene chalcone (B-5)



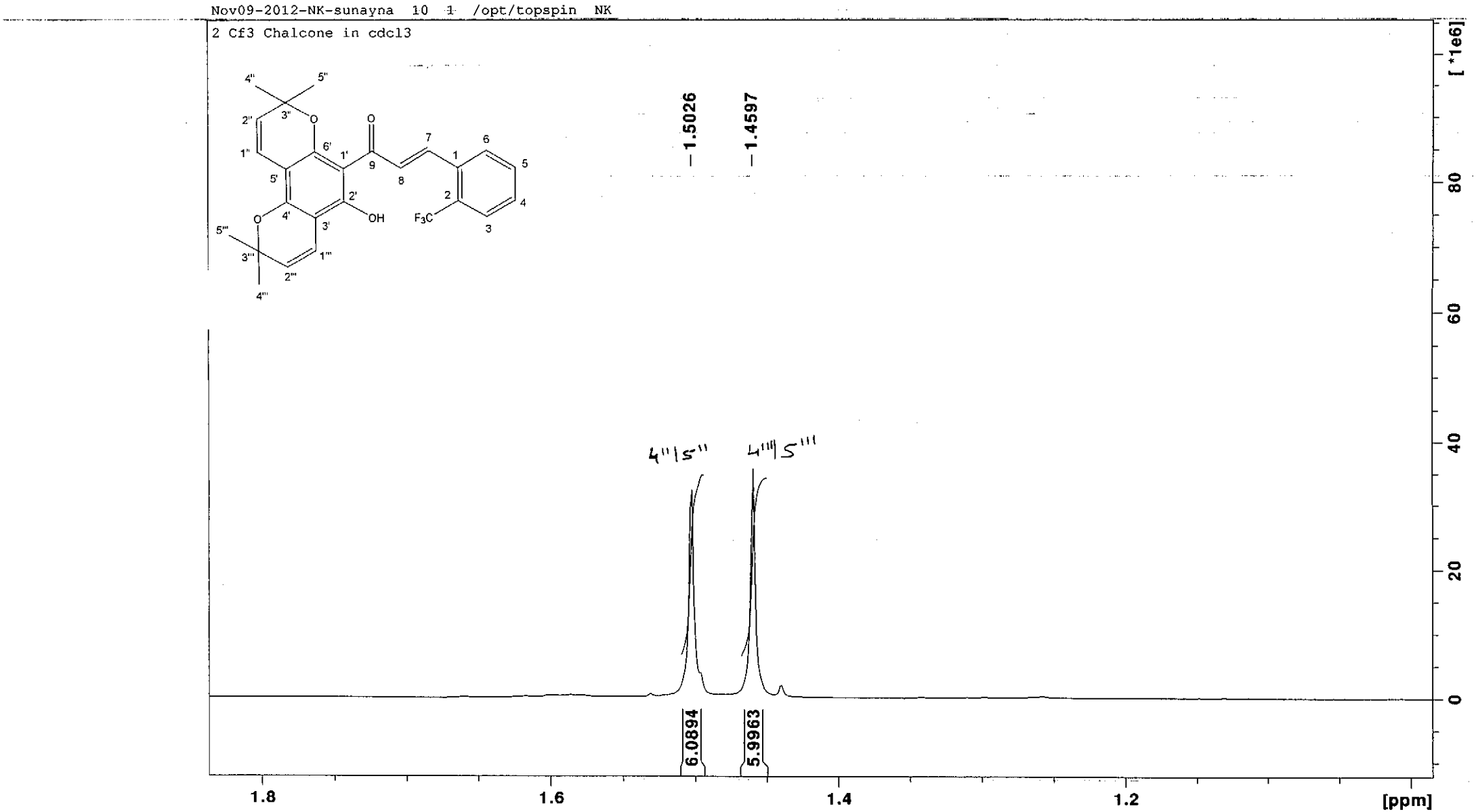
1H NMR spectrum of 2-trifluoromethylpyranochromene chalcone (B-6)



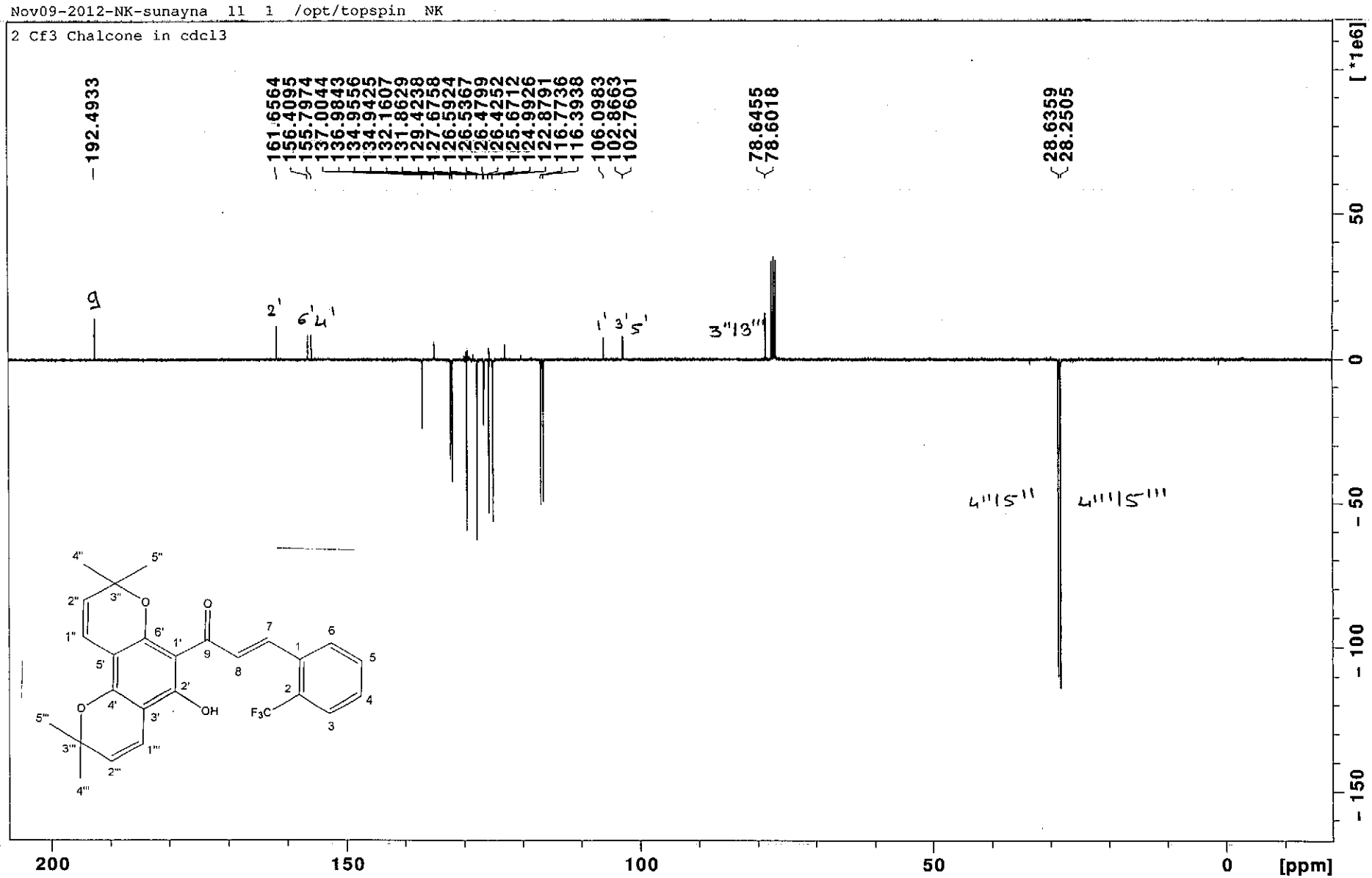
Expanded 1H NMR spectrum of 2-trifluoromethylpyranochromene chalcone (B-6)



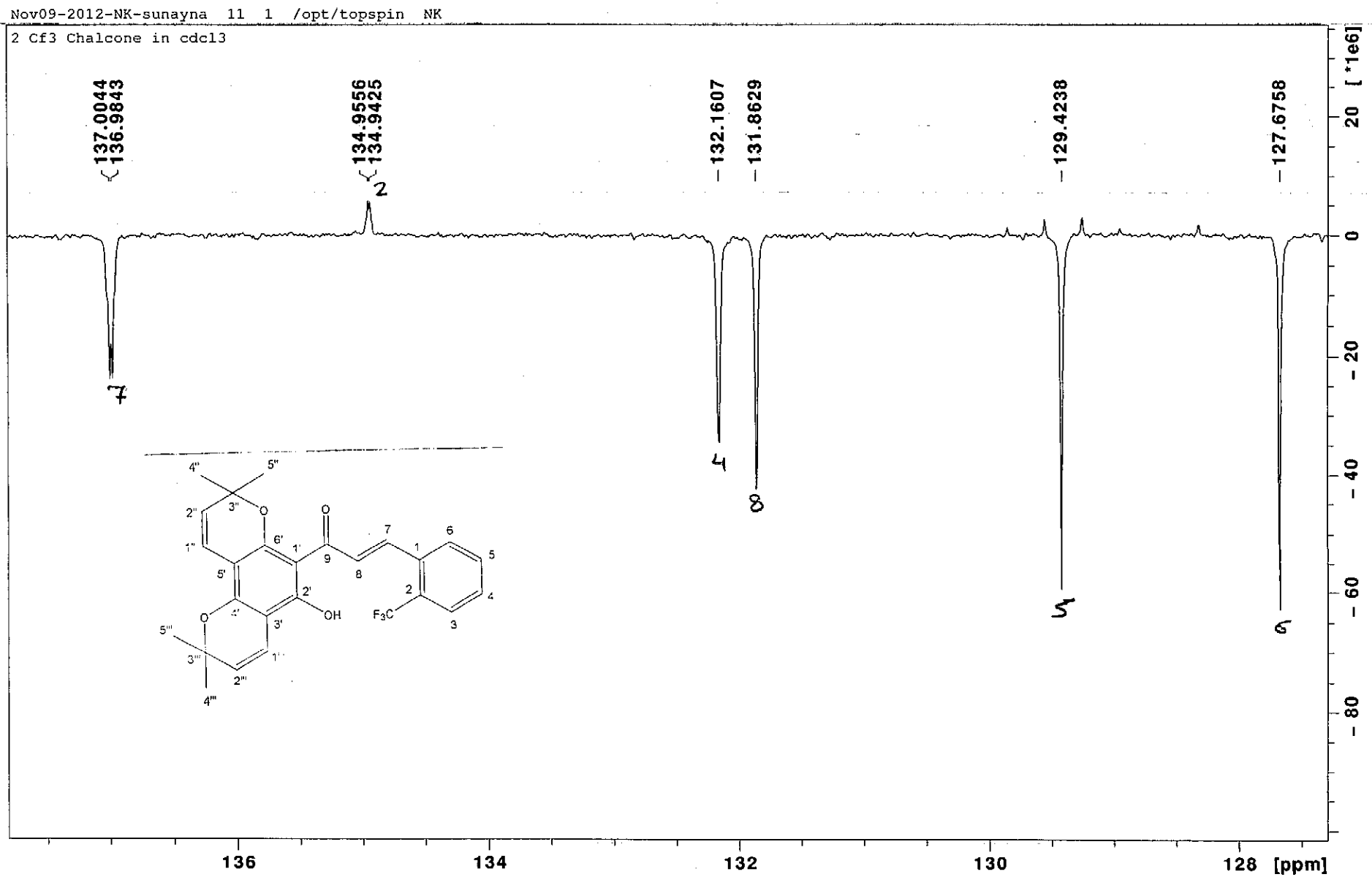
Expanded 1H NMR spectrum of 2-trifluoromethylpyranochromene chalcone (B-6)



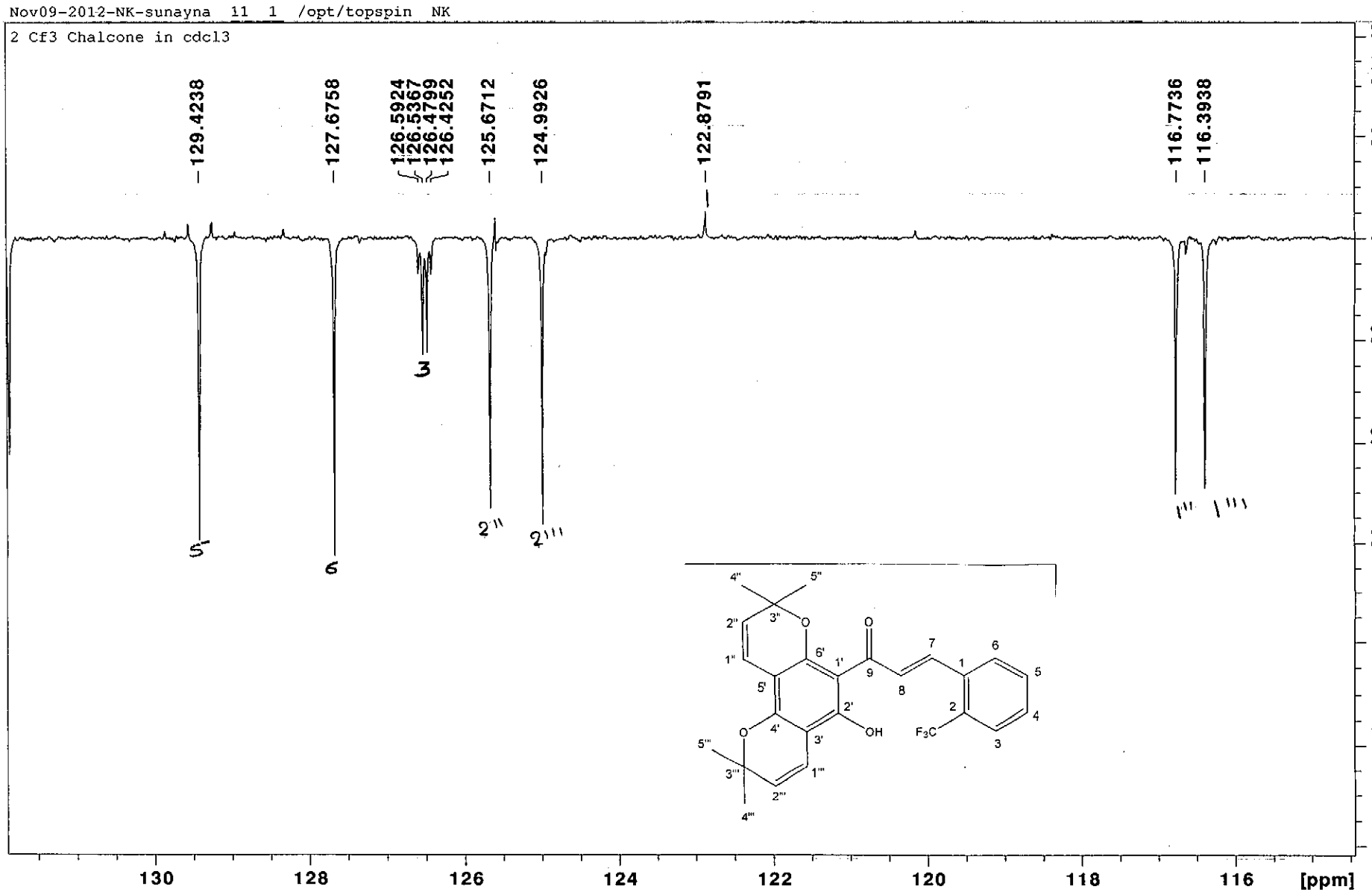
Expanded 1H NMR spectrum of 2-trifluoromethylpyranochromene chalcone (B-6)



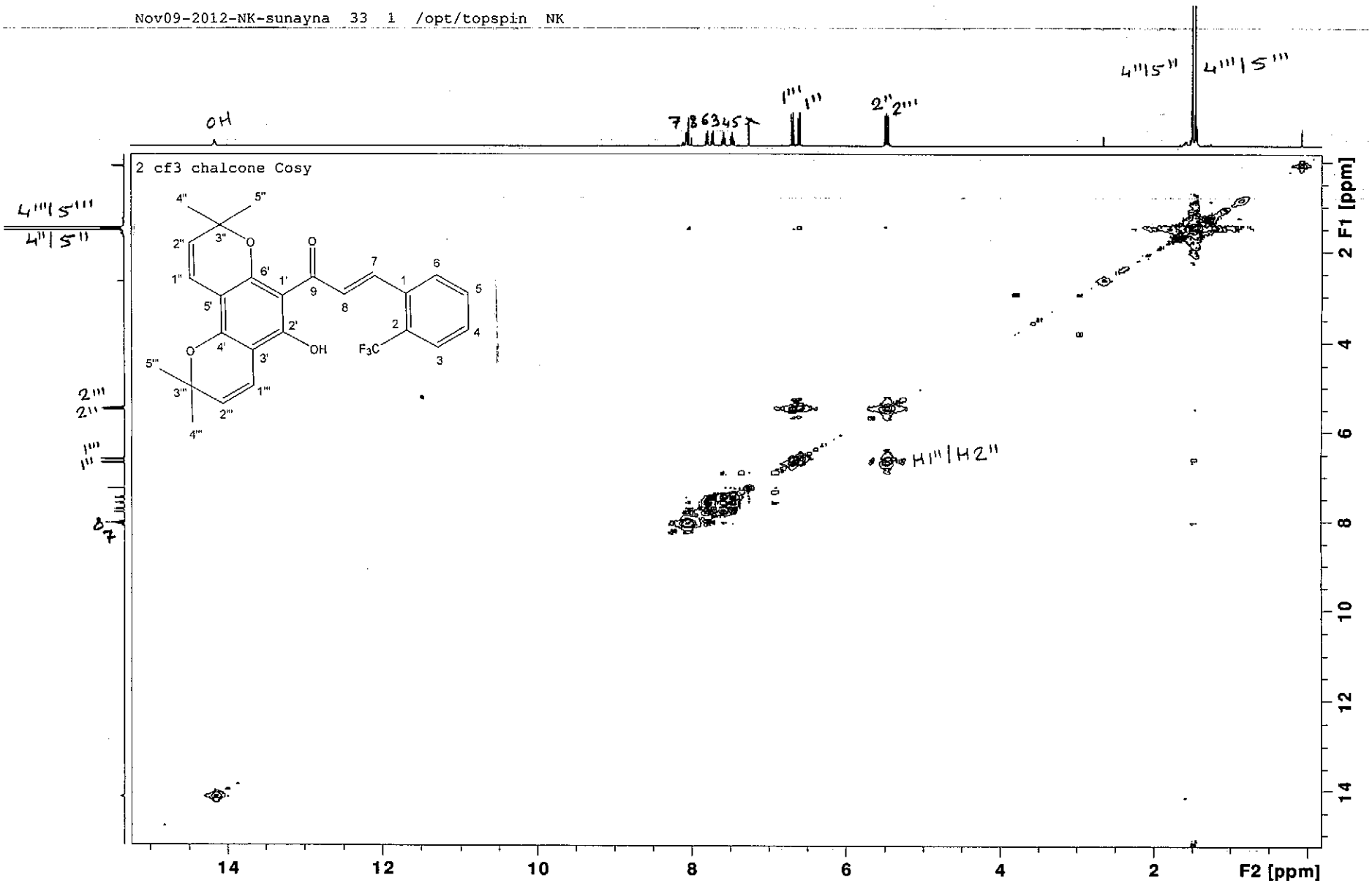
13C NMR spectrum of 2-trifluoromethylpyranochromene chalcone (B-6)



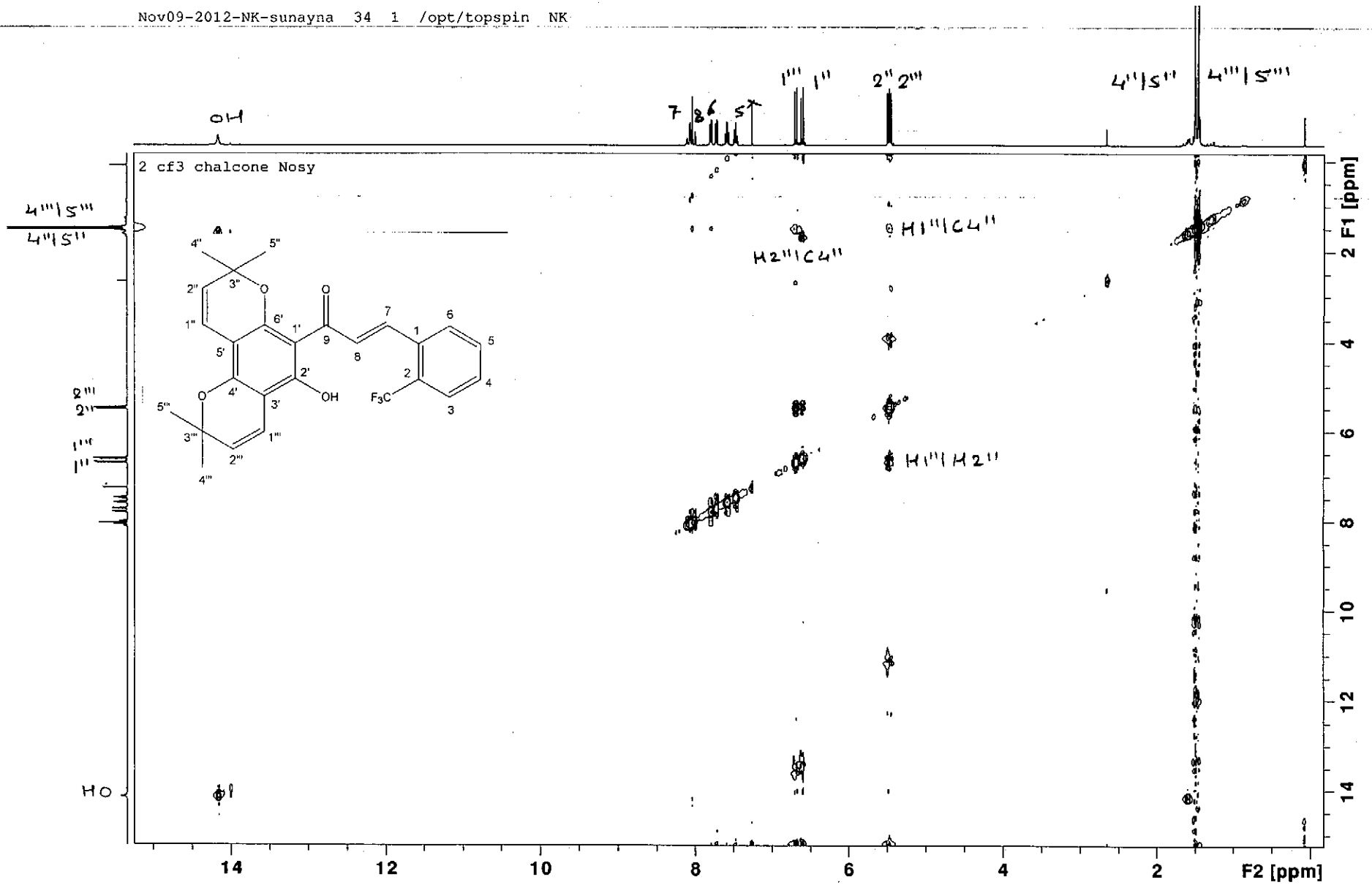
Expanded 13C NMR spectrum of 2-trifluoromethylpyranochromene chalcone (B-6)



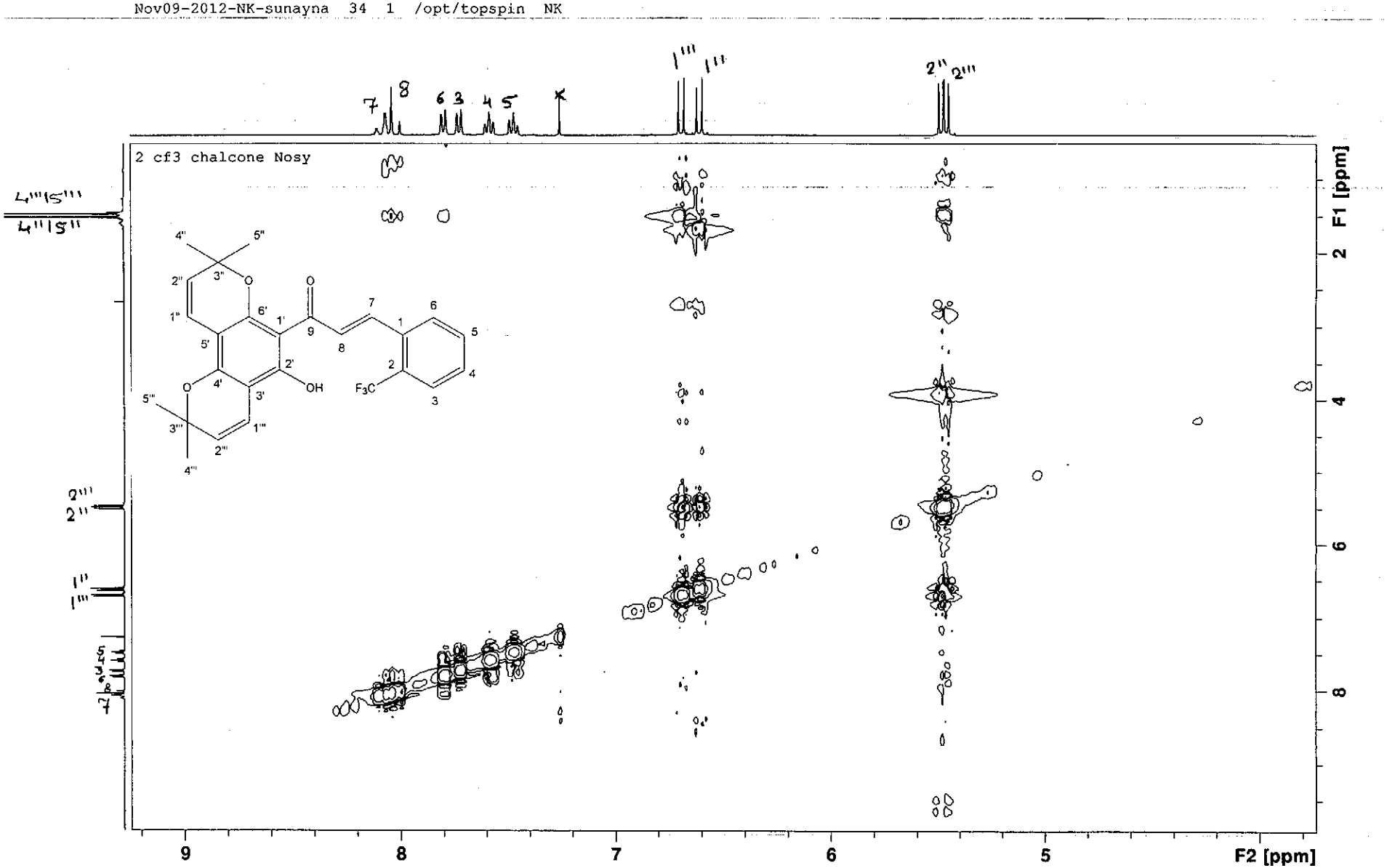
Expanded 13C NMR spectrum of 2-trifluoromethylpyranochromene chalcone (B-6)



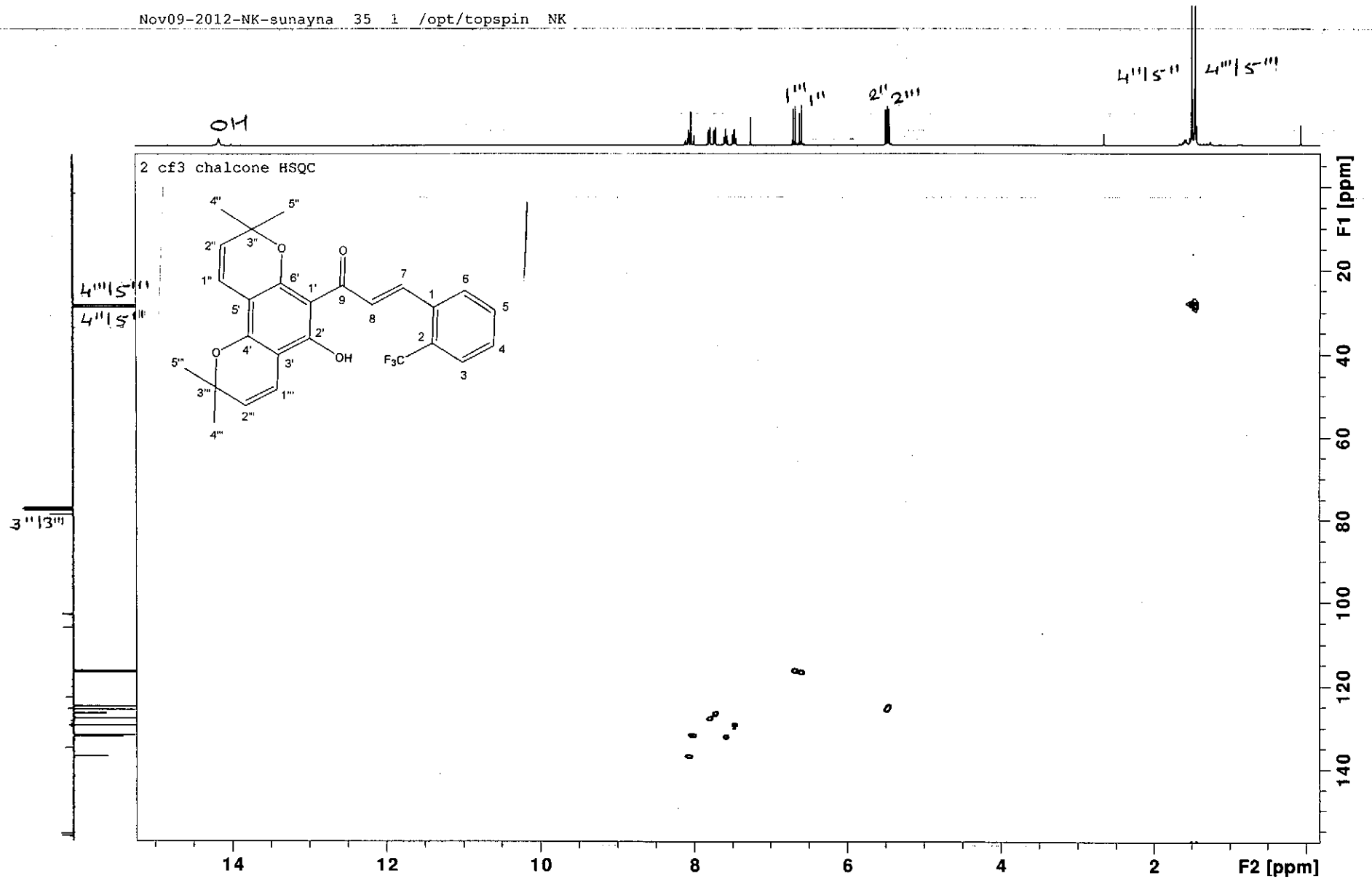
COSY spectrum of 2-trifluoromethylpyranochromene chalcone (B-6)



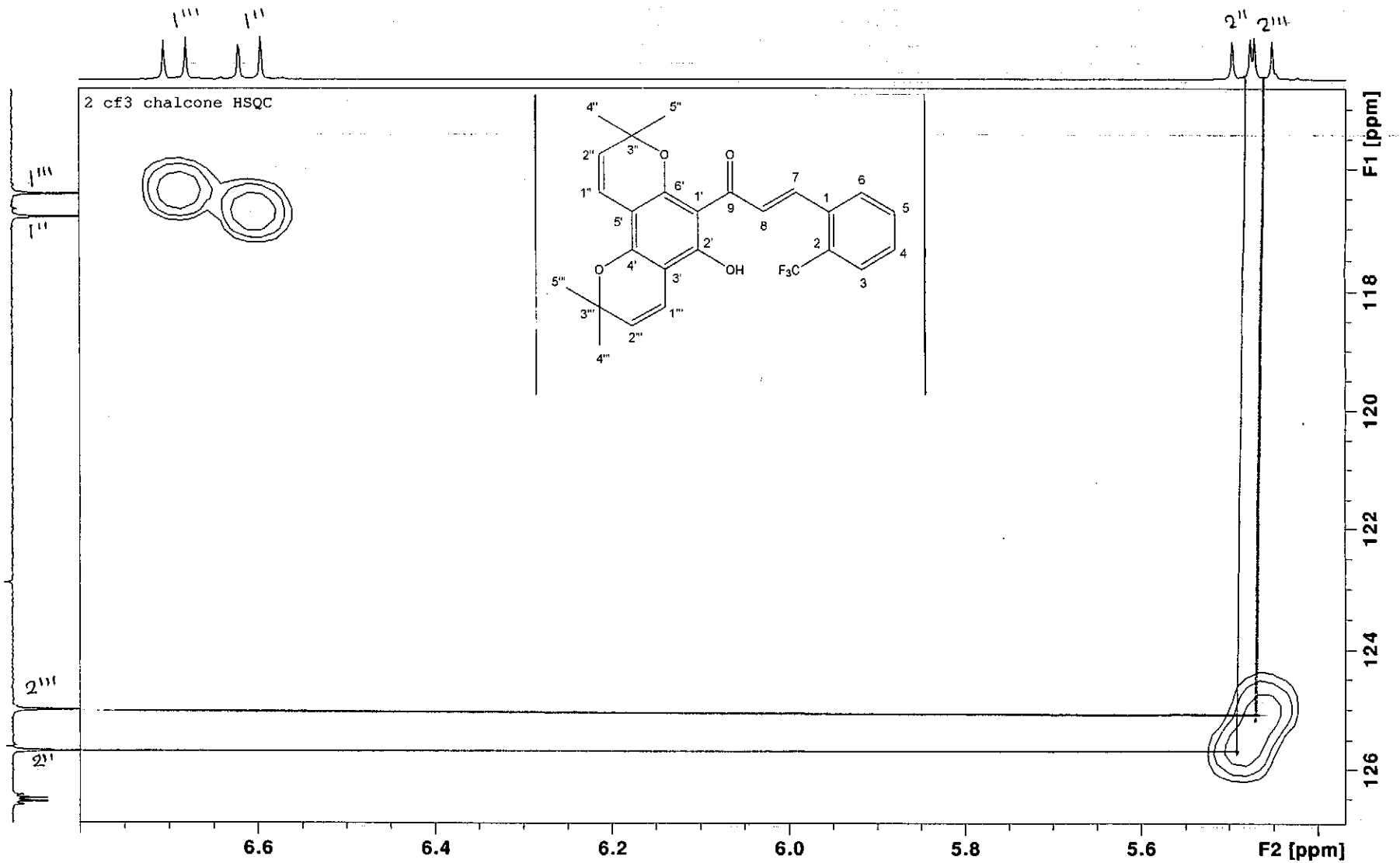
NOESY spectrum of 2-trifluoromethylpyranochromene chalcone (B-6)



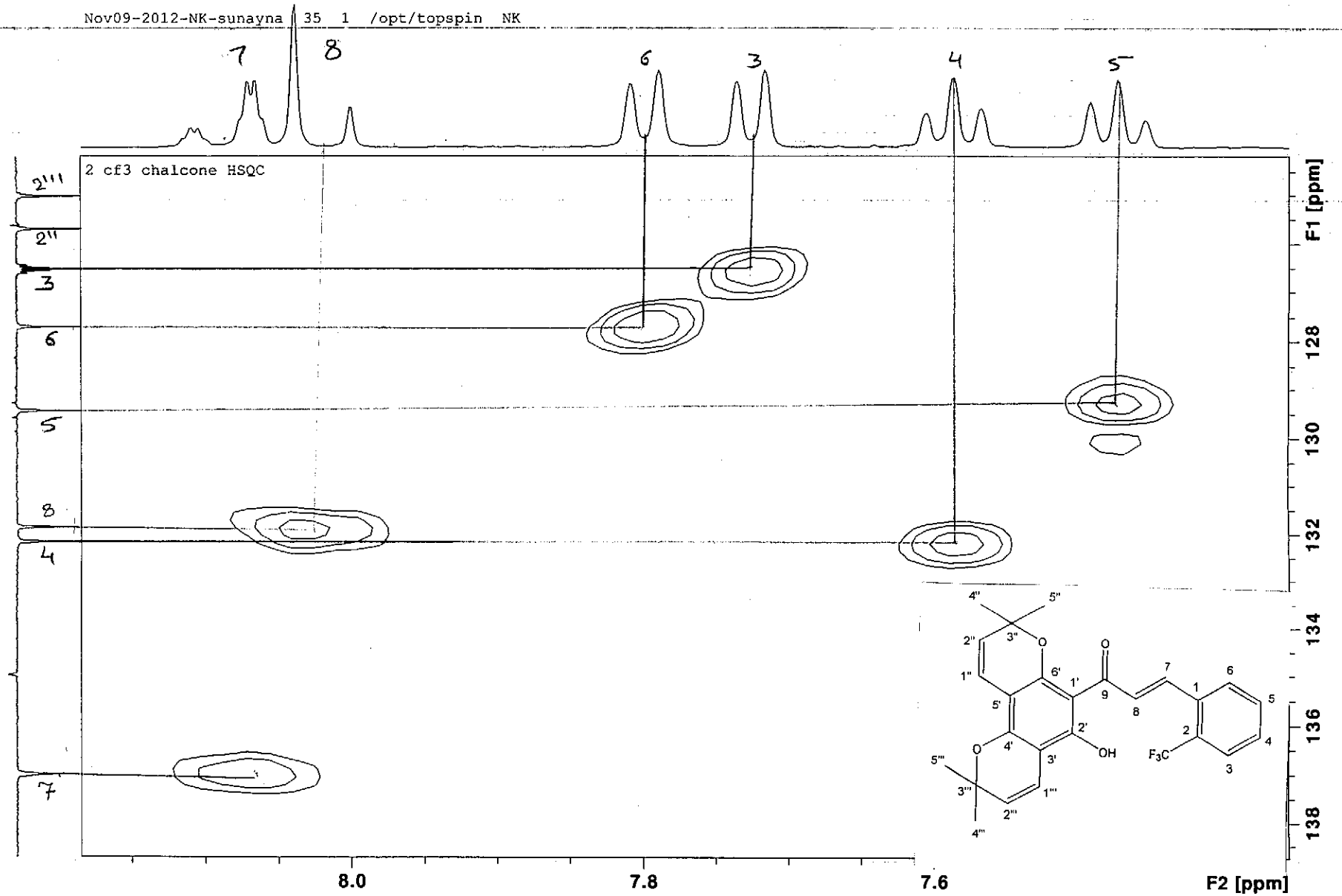
Expanded NOESY spectrum of 2-trifluoromethylpyranochromene chalcone (B-6)



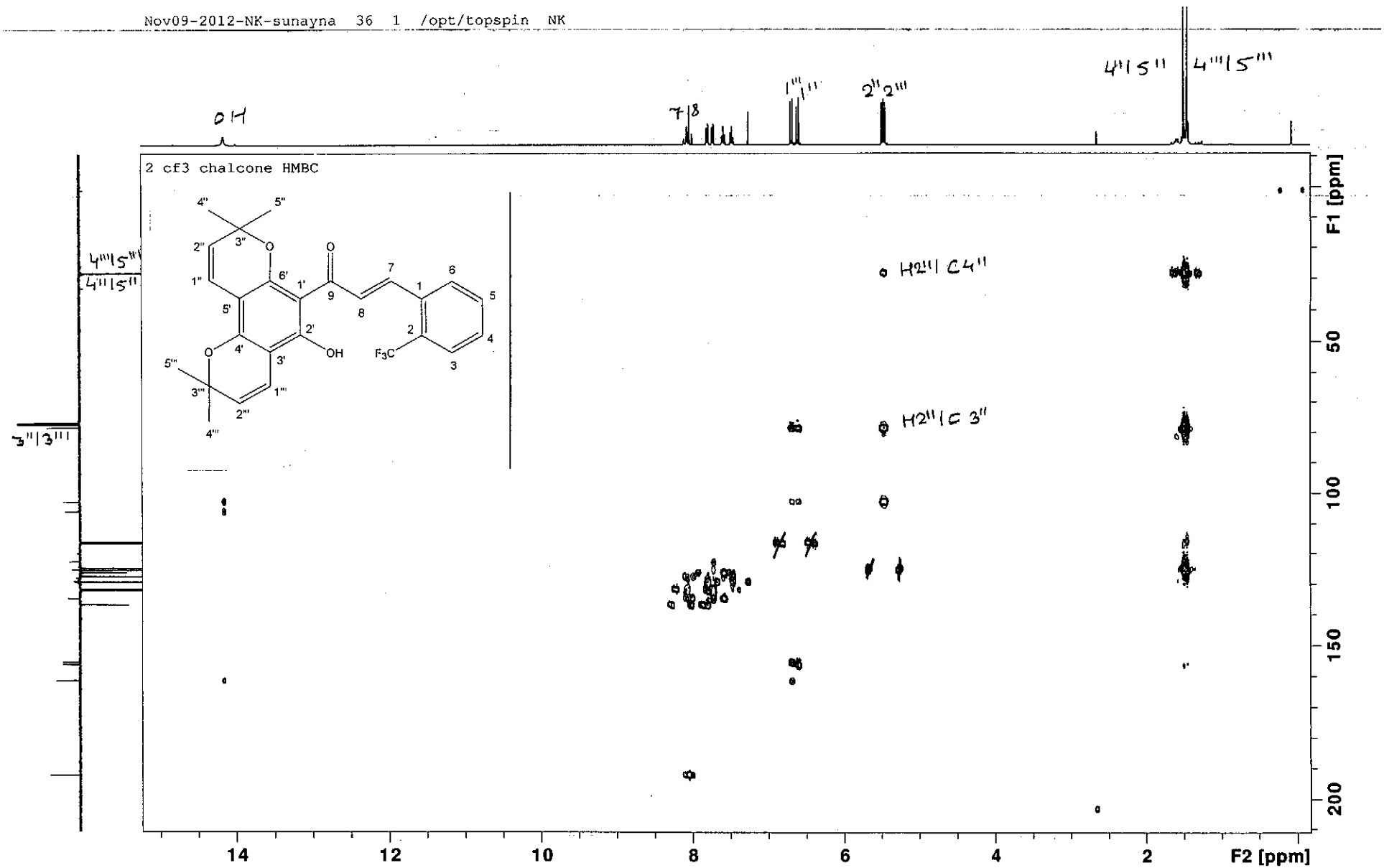
HSQC spectrum of 2-trifluoromethylpyranochromene chalcone (B-6)



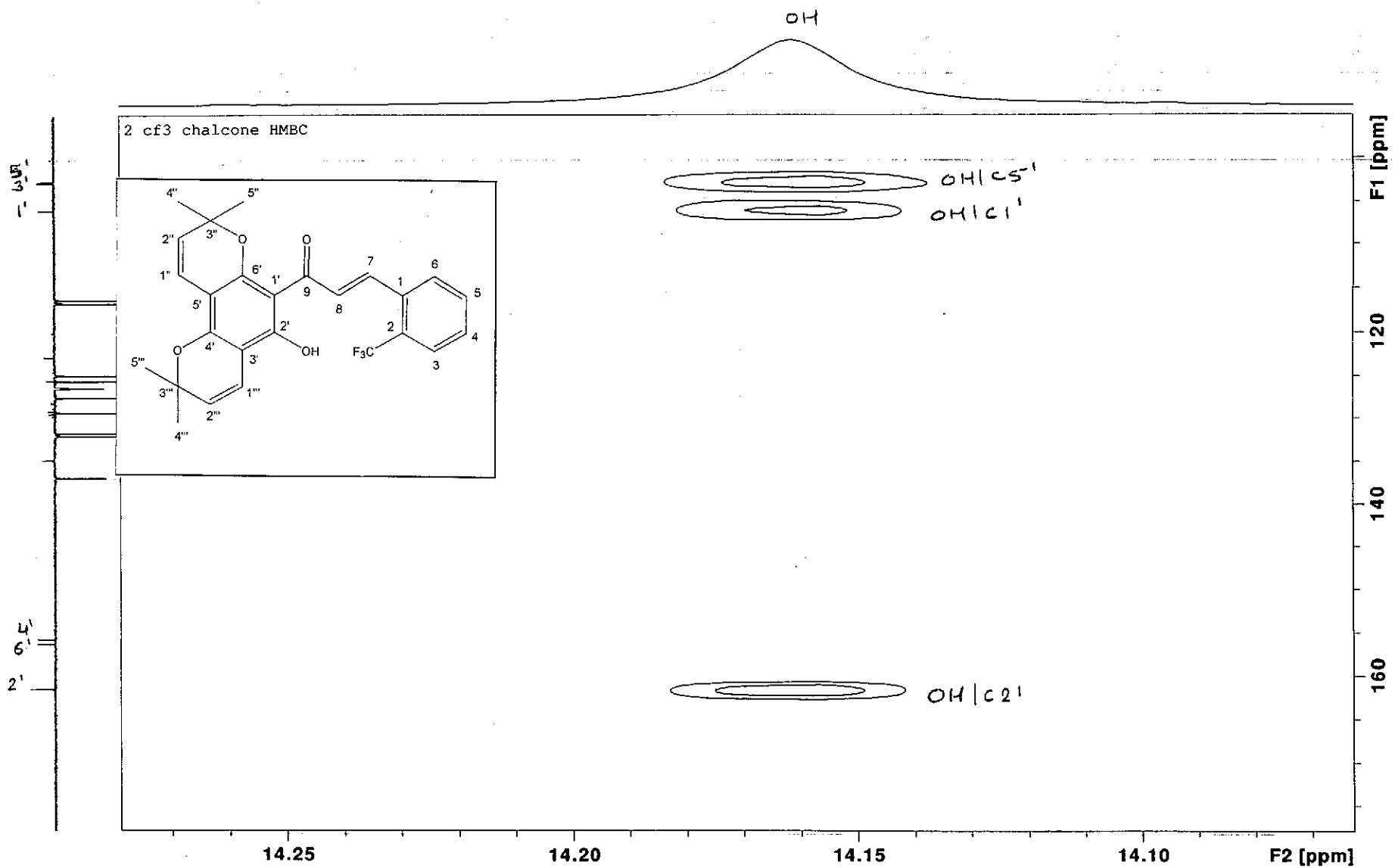
Expanded HSQC spectrum of 2-trifluoromethylpyranochromene chalcone (B-6)



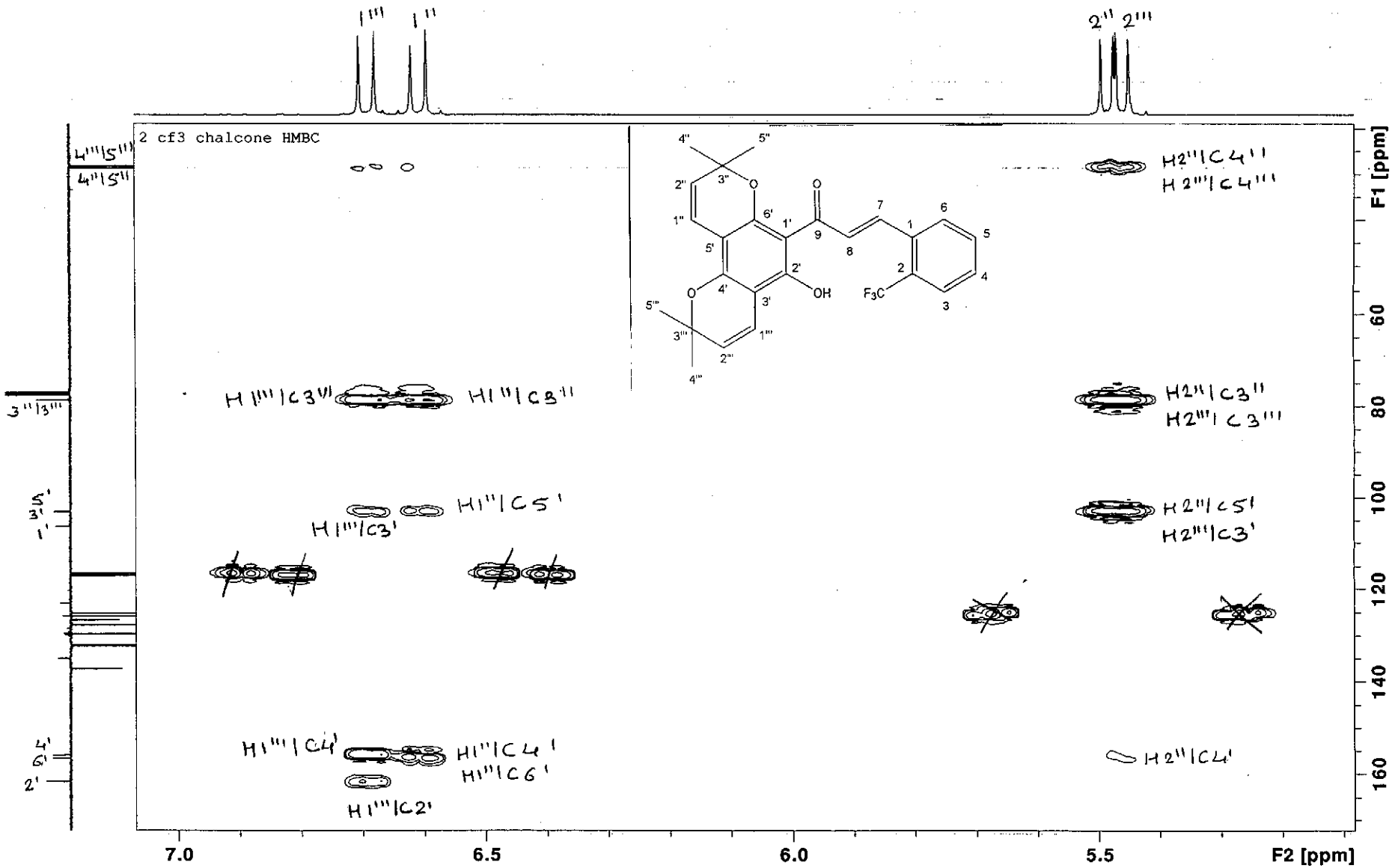
Expanded HSQC spectrum of 2-trifluoromethylpyranochromene chalcone (B-6)



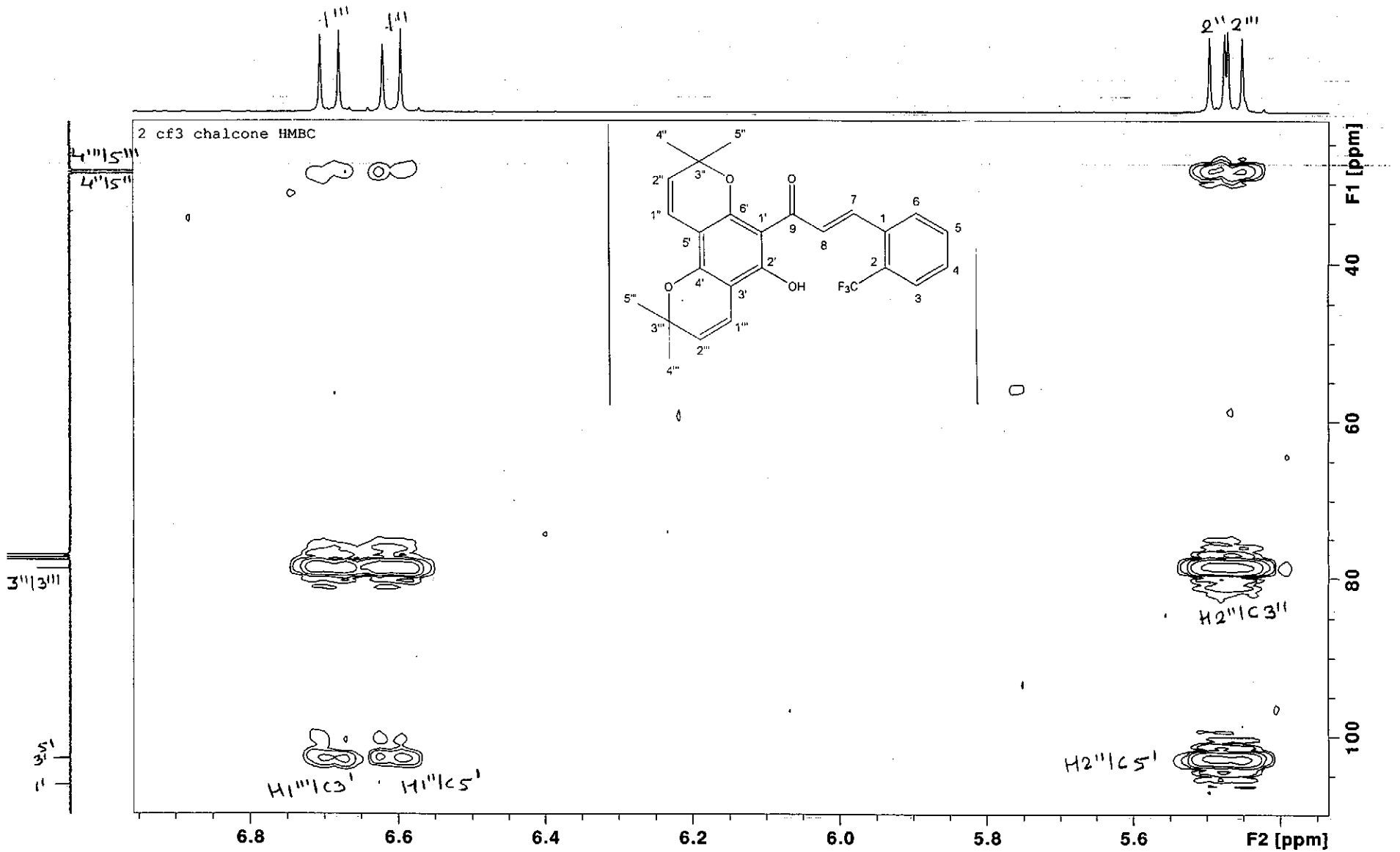
HMBC spectrum of 2-trifluoromethylpyranochromene chalcone (B-6)



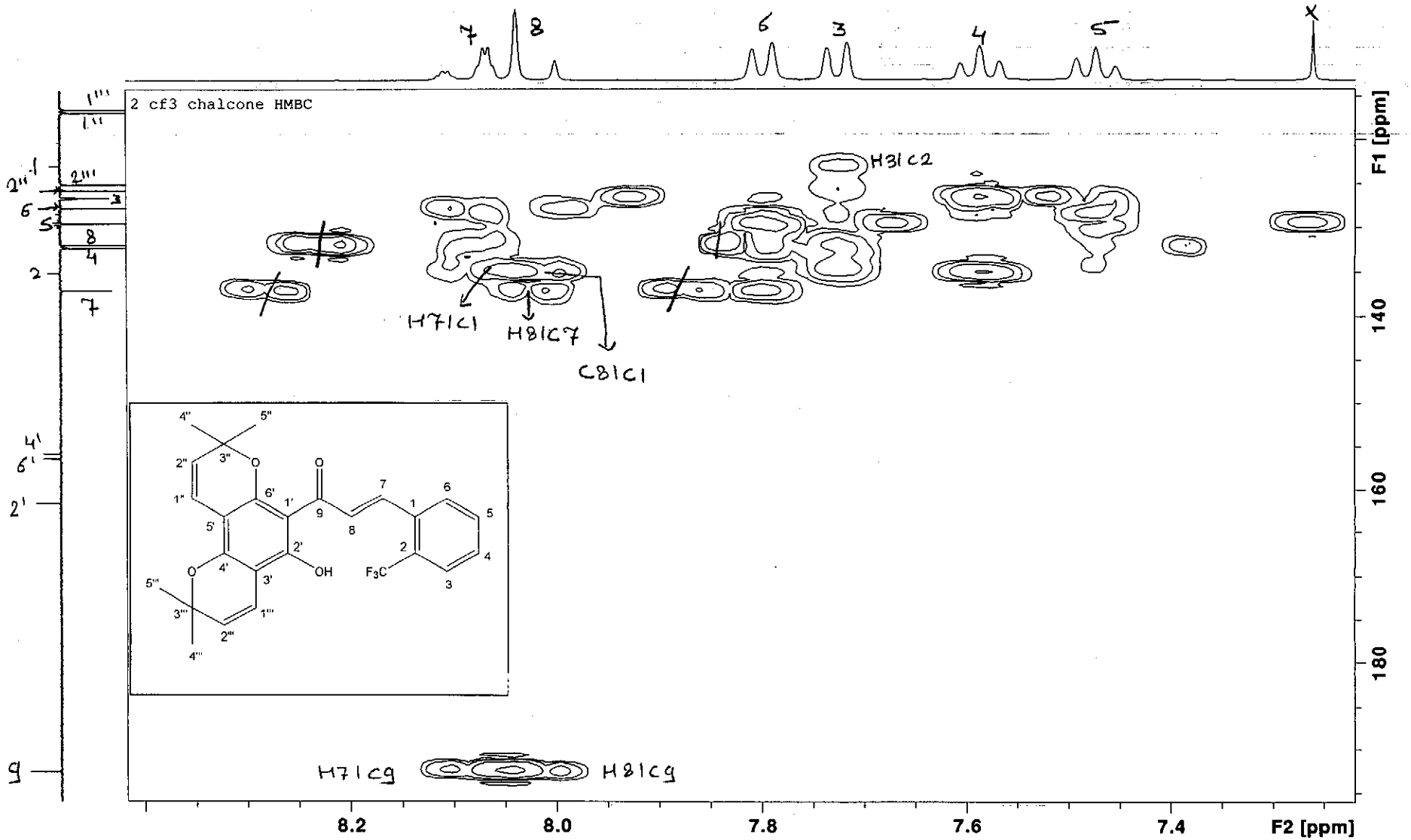
Expanded HMBC spectrum of 2-trifluoromethylpyranochromene chalcone (B-6)



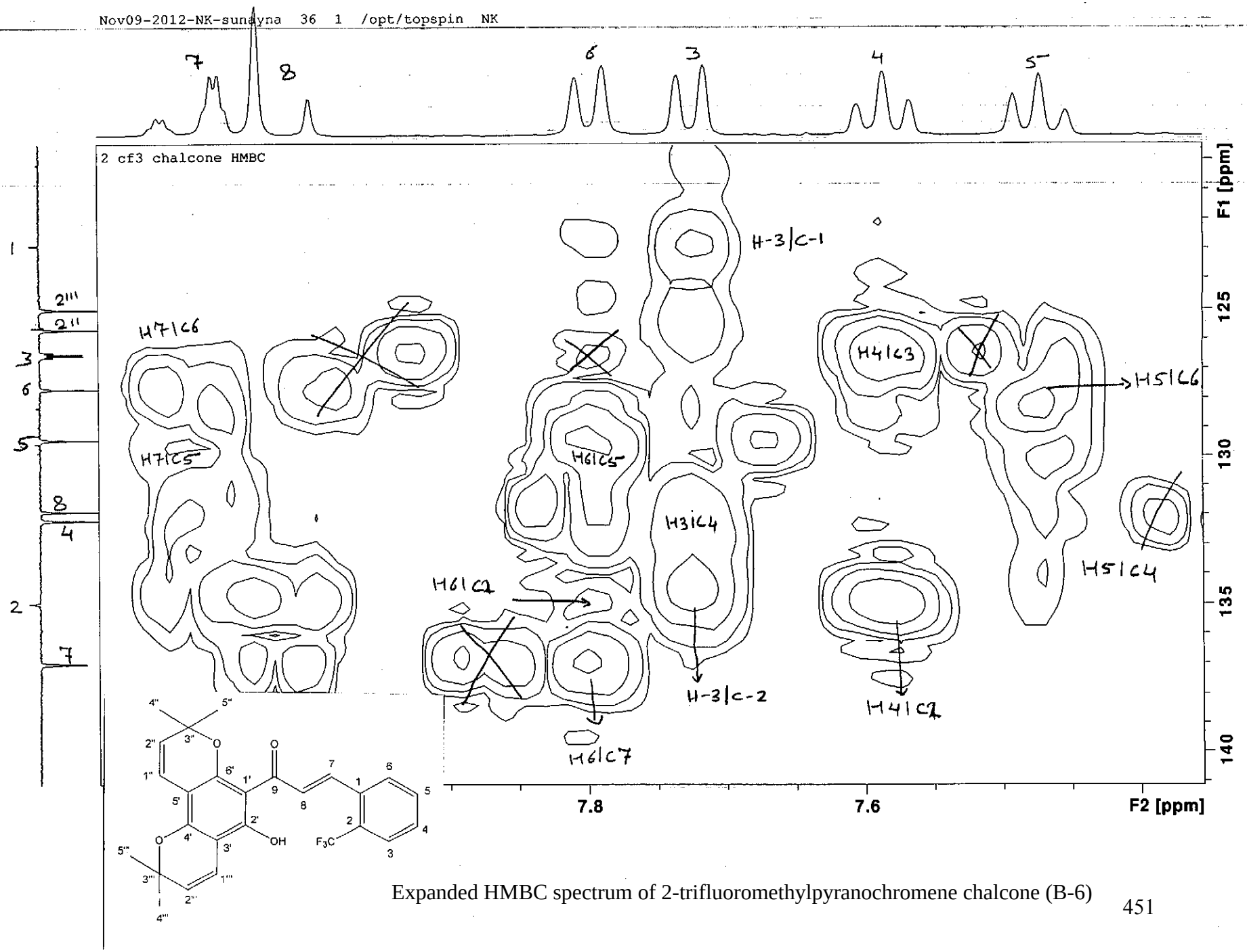
Expanded HMBC spectrum of 2-trifluoromethylpyranochromene chalcone (B-6)



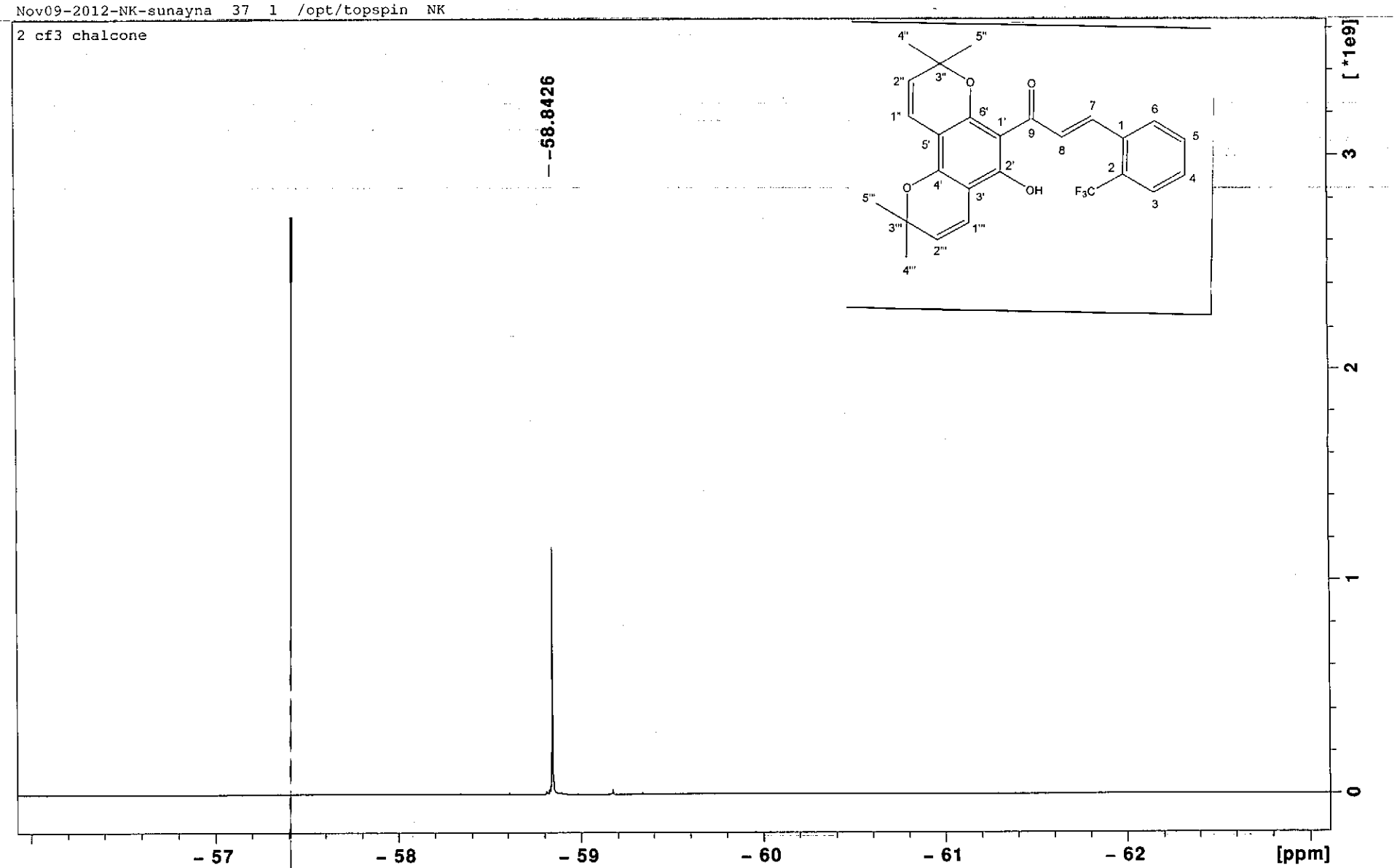
Expanded HMBC spectrum of 2-trifluoromethylpyranochromene chalcone (B-6)



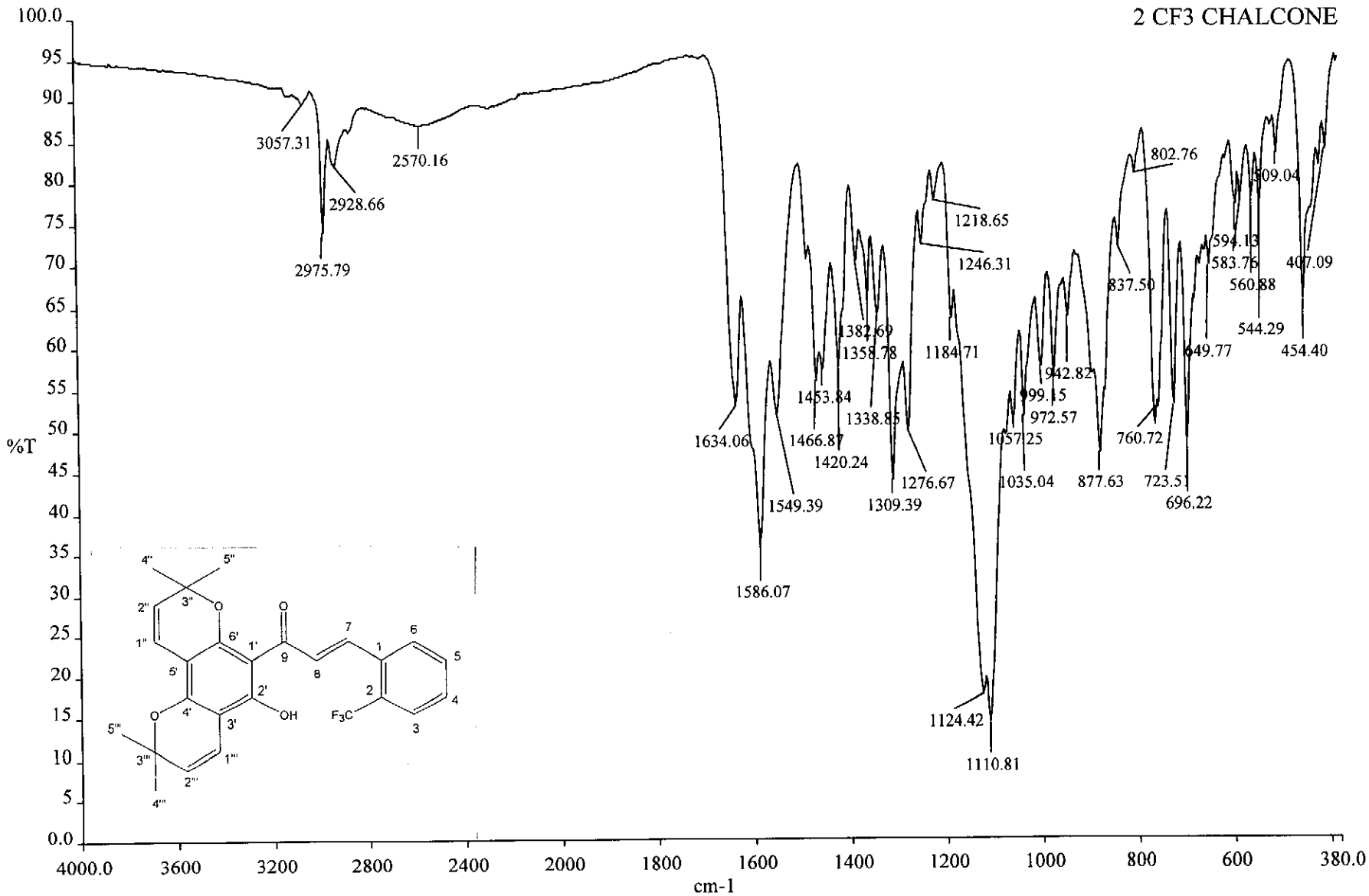
Expanded HMBC spectrum of 2-trifluoromethylpyranochromene chalcone (B-6)



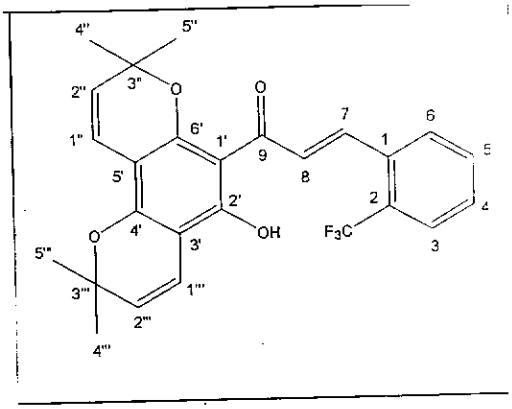
Expanded HMBC spectrum of 2-trifluoromethylpyranochromene chalcone (B-6)



19F NMR spectrum of 2-trifluoromethylpyranochromene chalcone (B-6)



Infrared spectrum of 2-trifluoromethylpyranochromene chalcone (B-6)



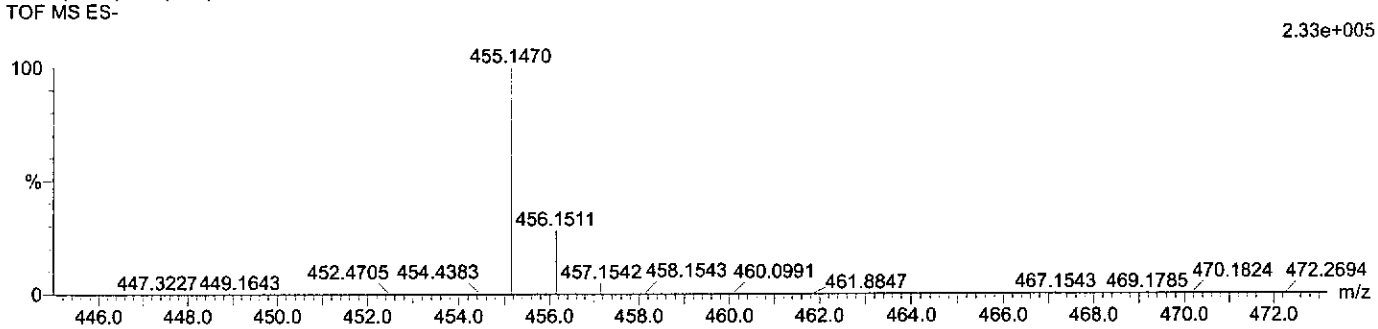
Elemental Composition Report

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

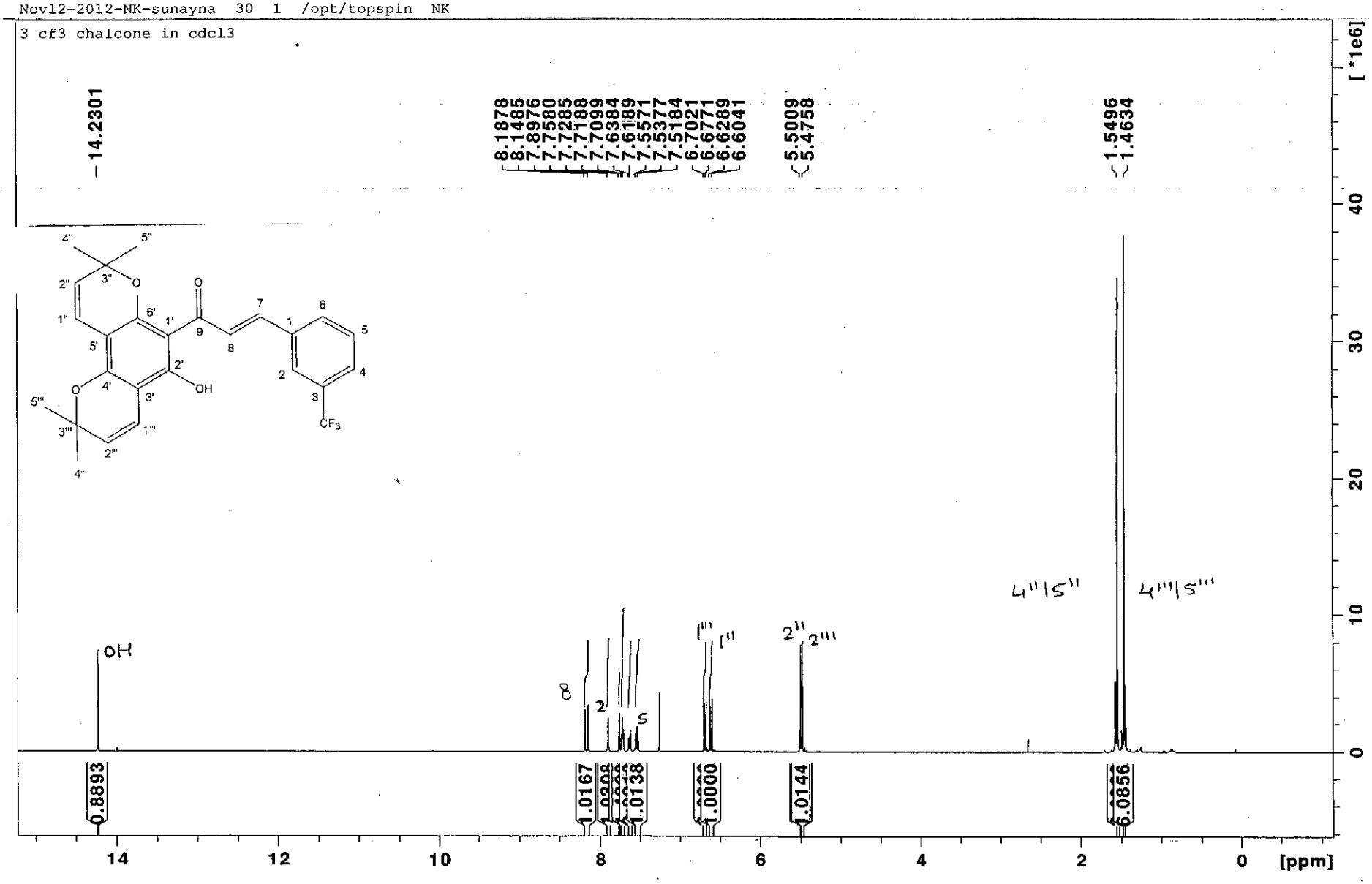
Monoisotopic Mass, Even Electron Ions
25 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-35 H: 20-30 N: 0-4 O: 0-4 F: 3-3

24 19 (0.308) Cm (1:30)
TOF MS ES-

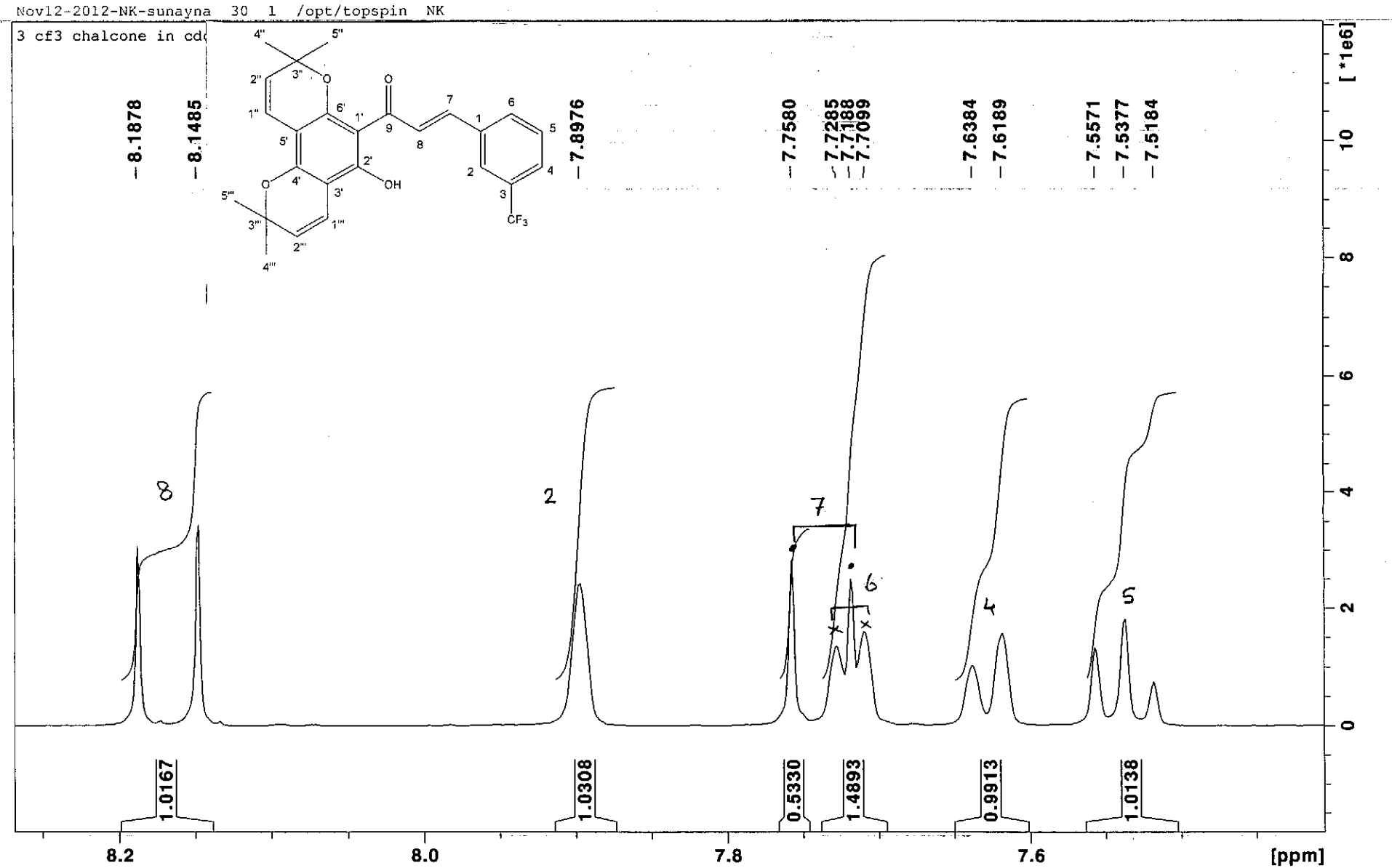


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
455.1470	455.1470	0.0	0.0	14.5	488.0	0.0	C26 H22 O4 F3

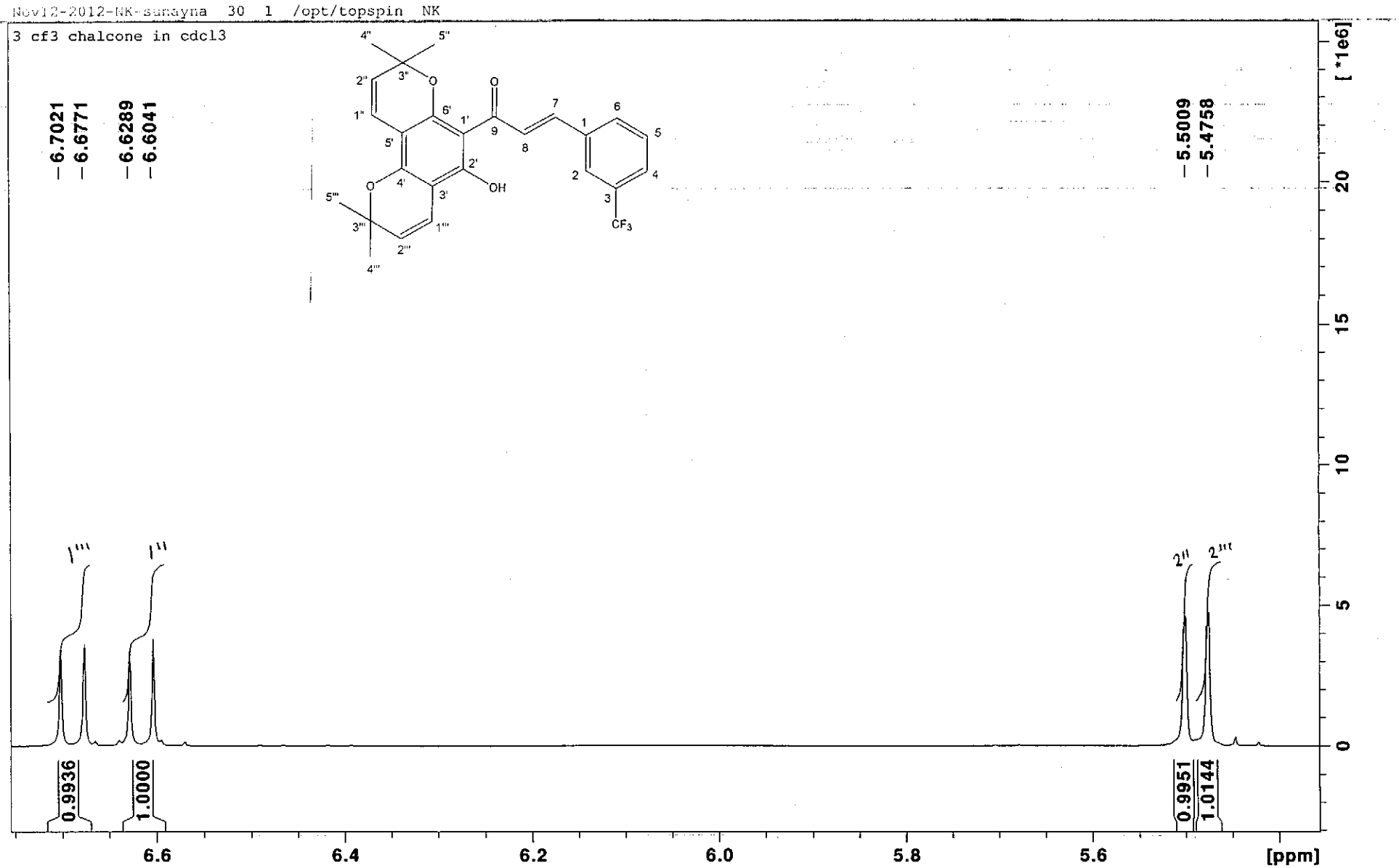
HRMS spectrum of 2-trifluoromethylpyranochromene chalcone (B-6)



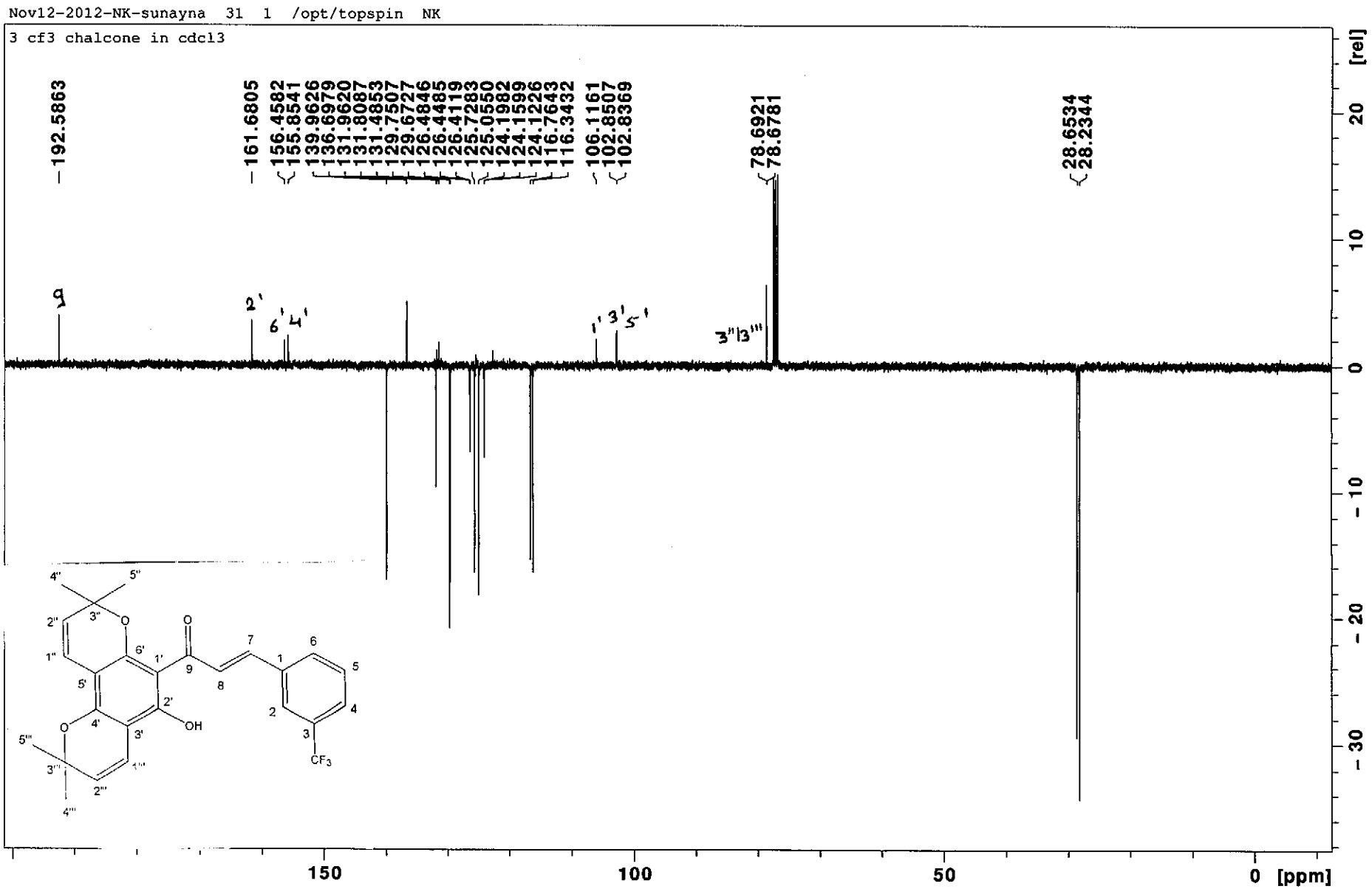
1H NMR spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



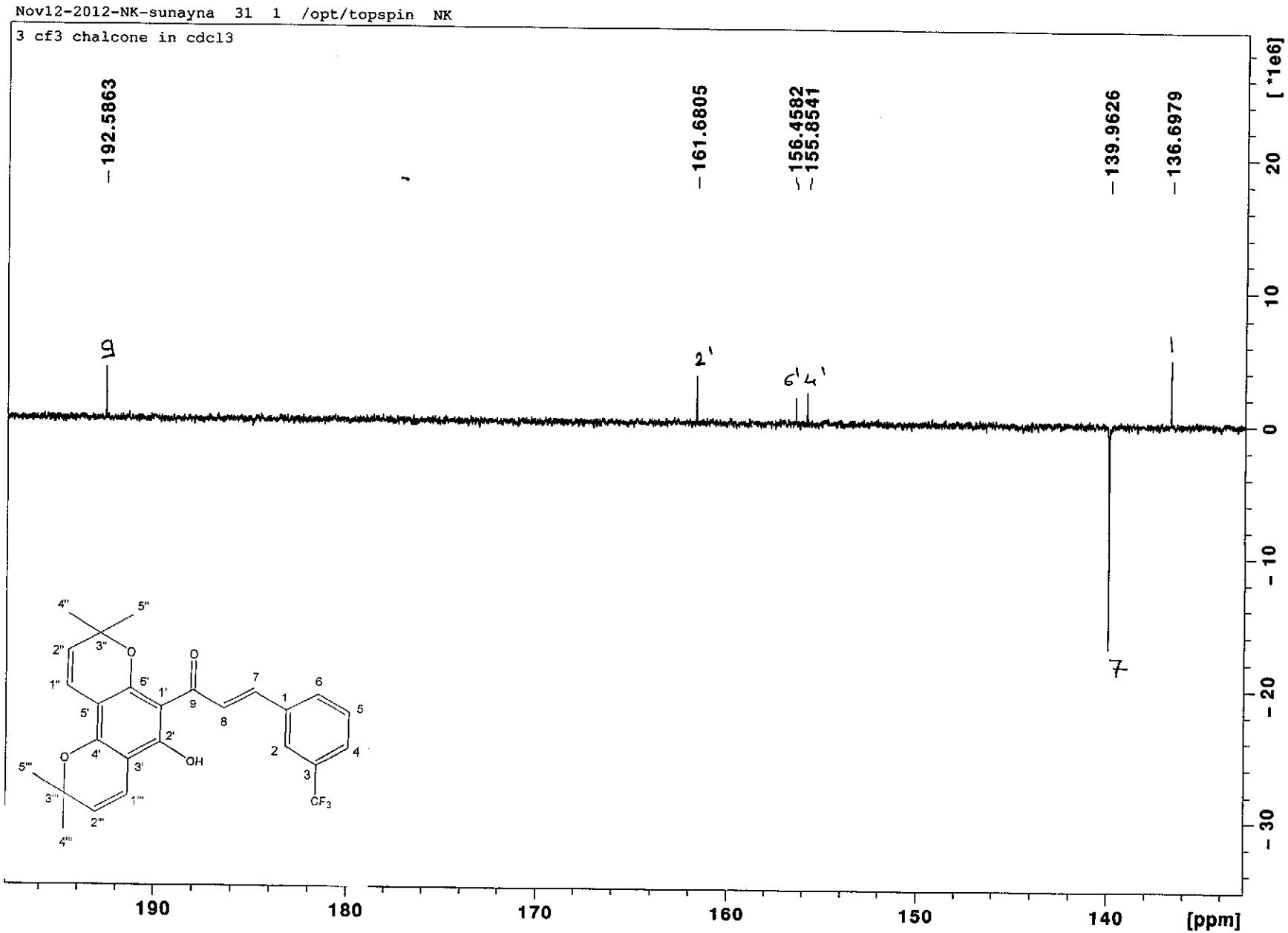
Expanded 1H NMR spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



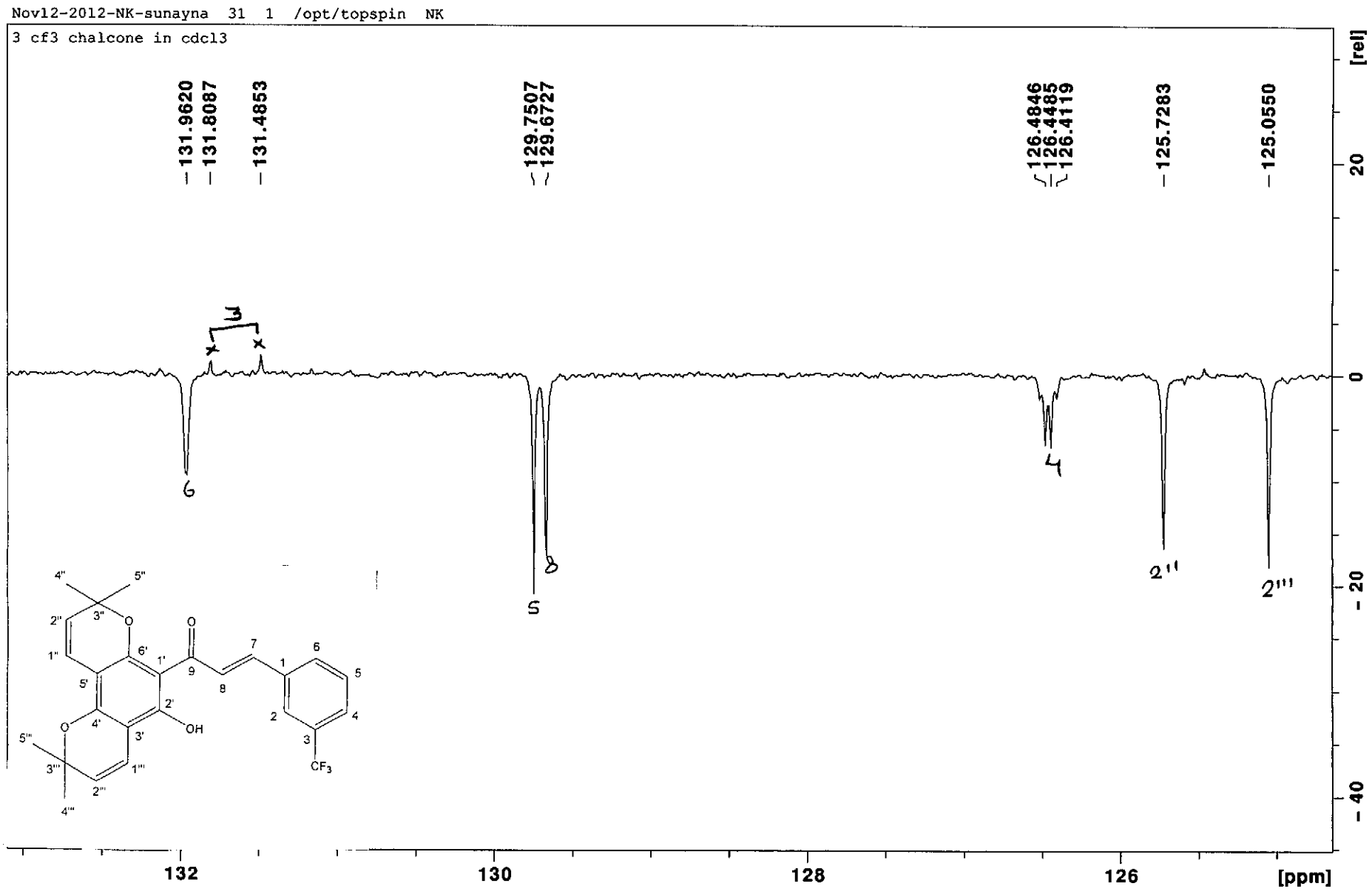
Expanded 1H NMR spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



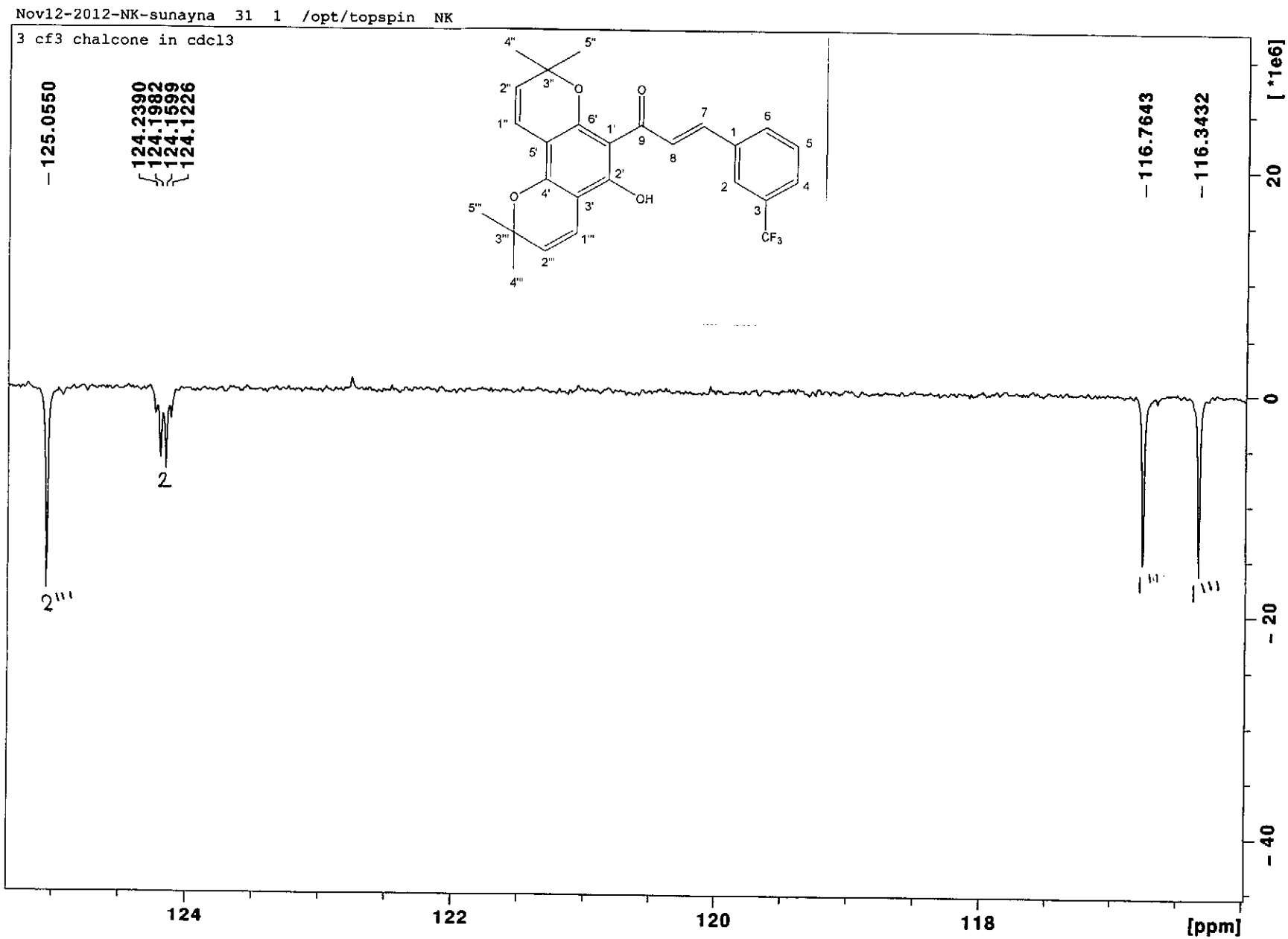
13C NMR spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



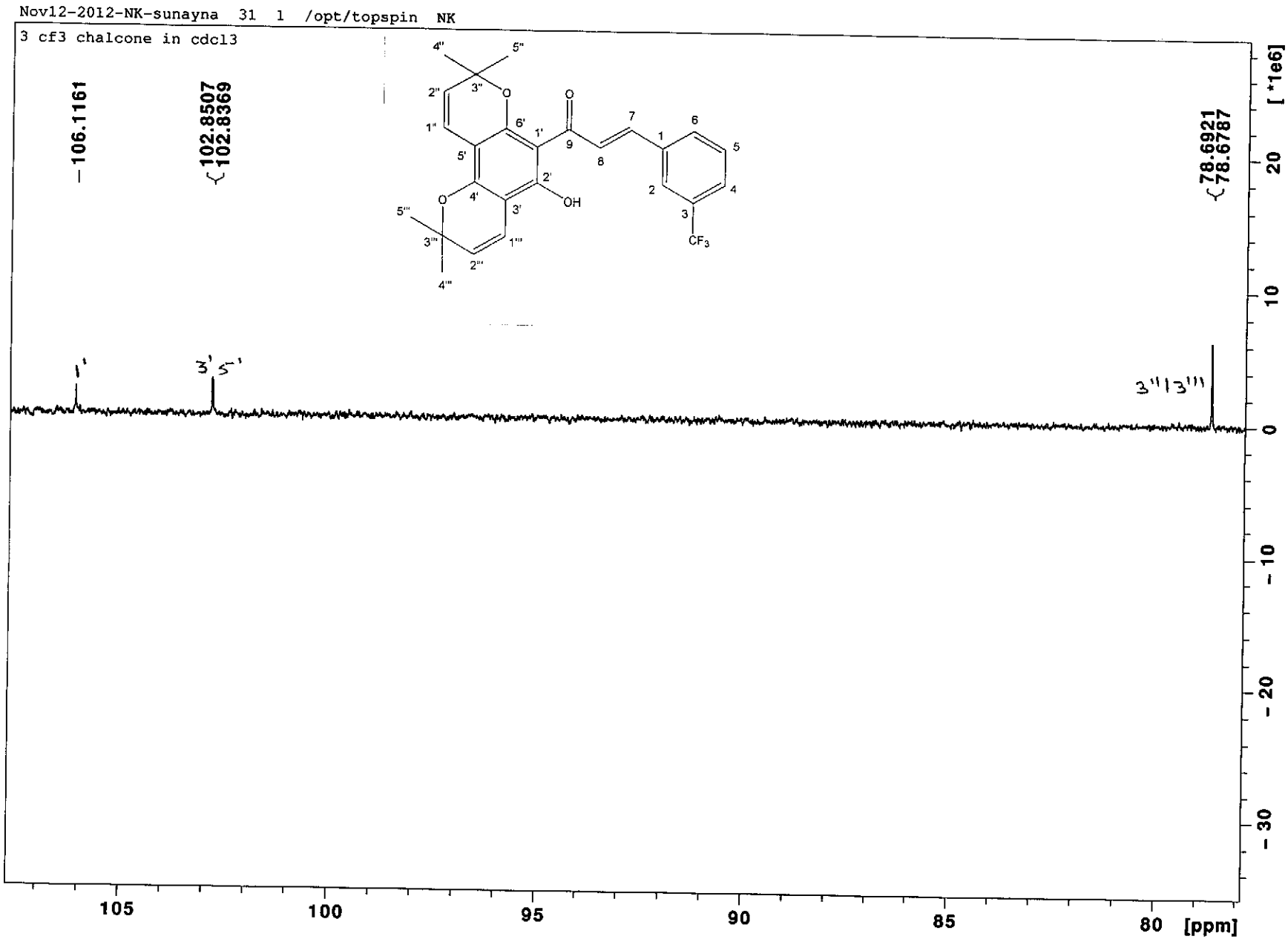
Expanded 13C NMR spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



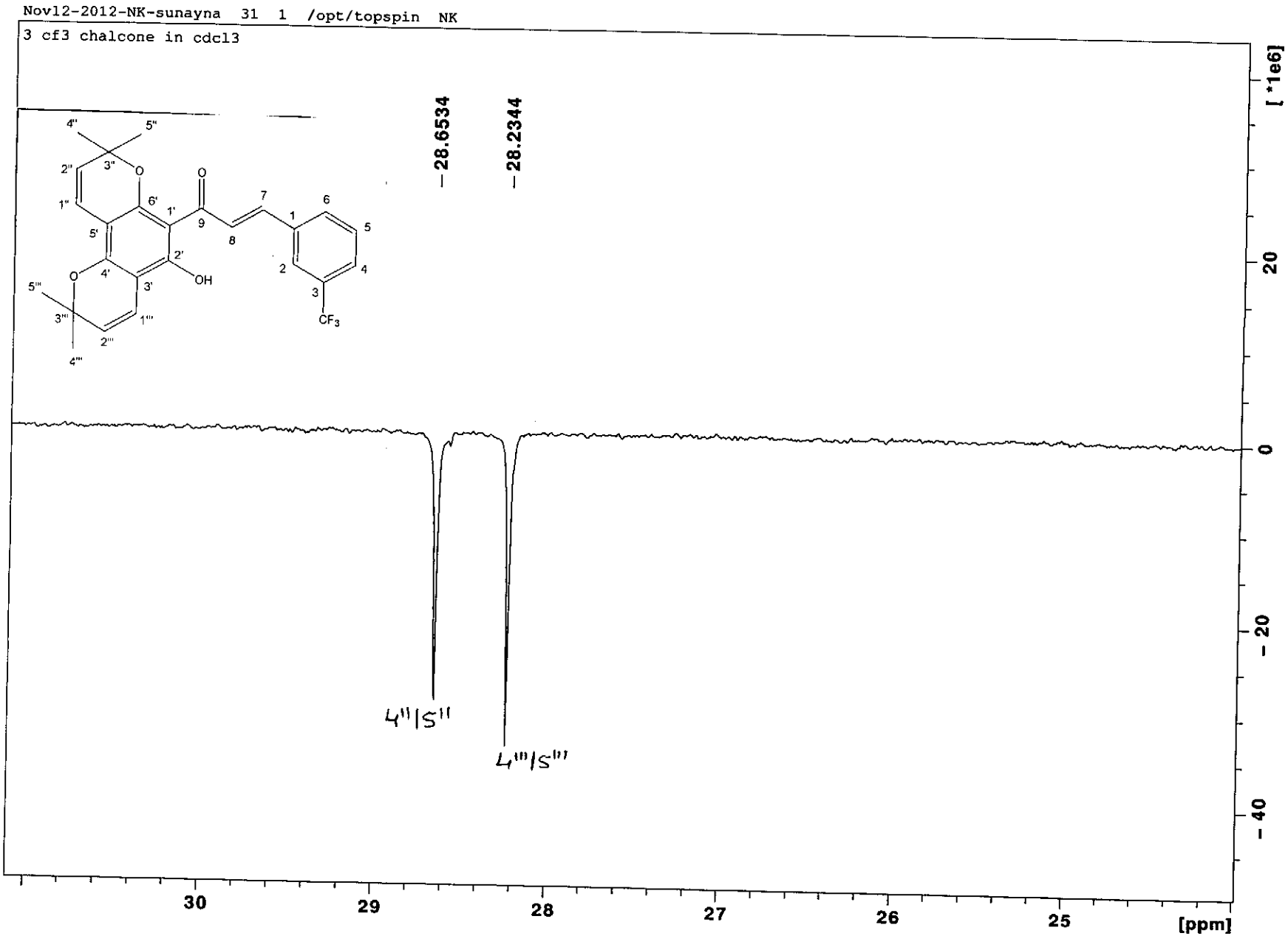
Expanded 13C NMR spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



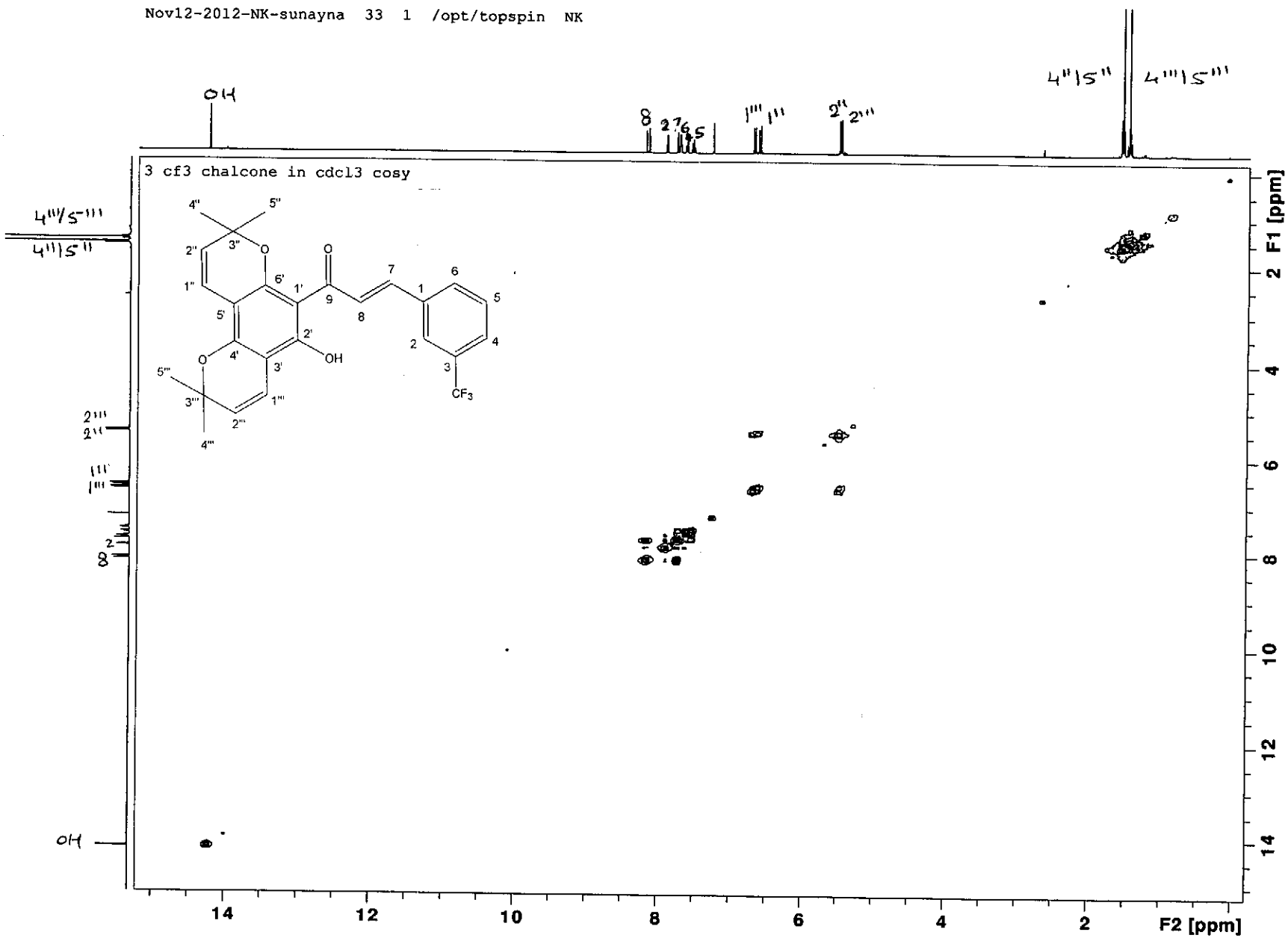
Expanded 13C NMR spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



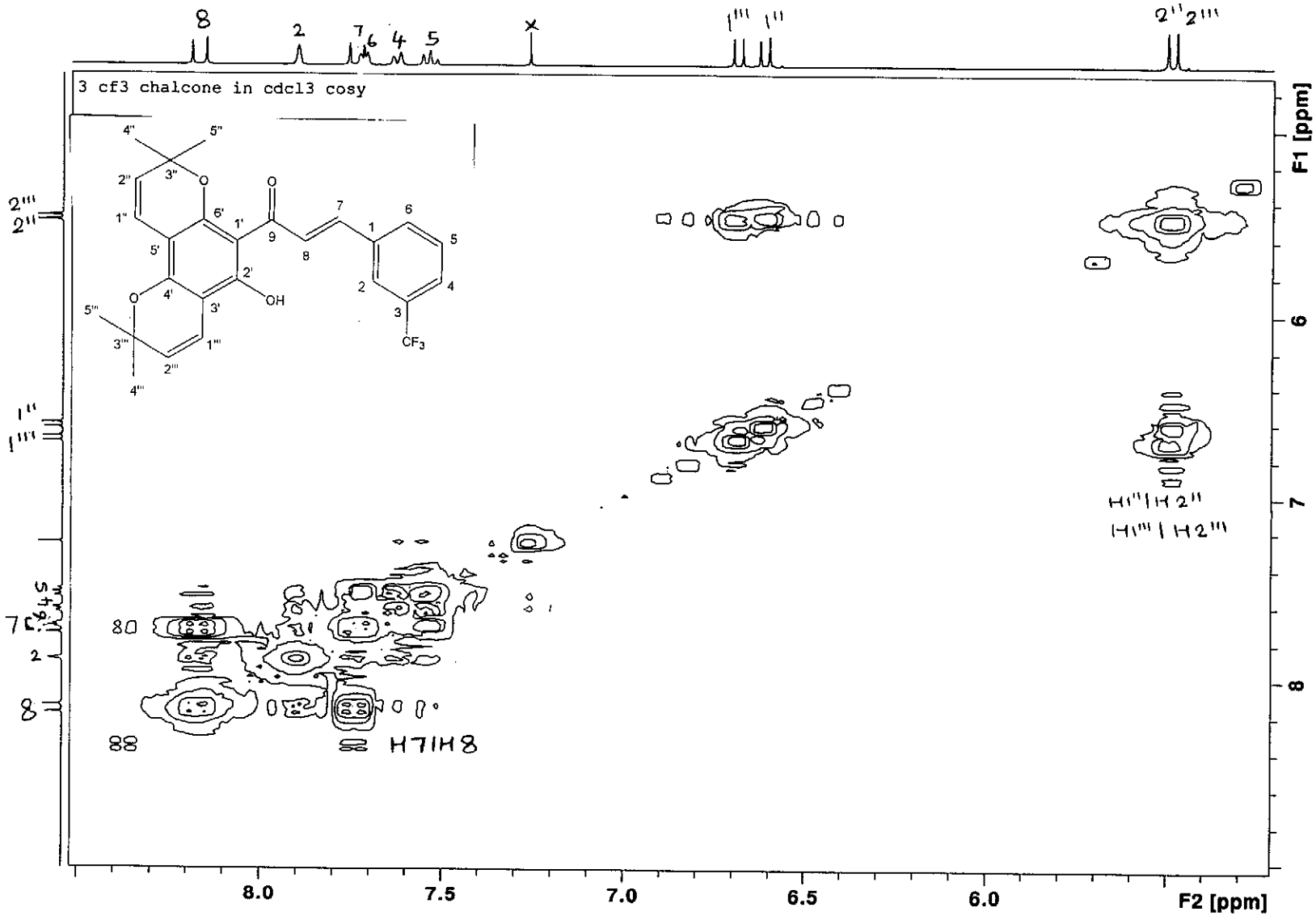
Expanded 13C NMR spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



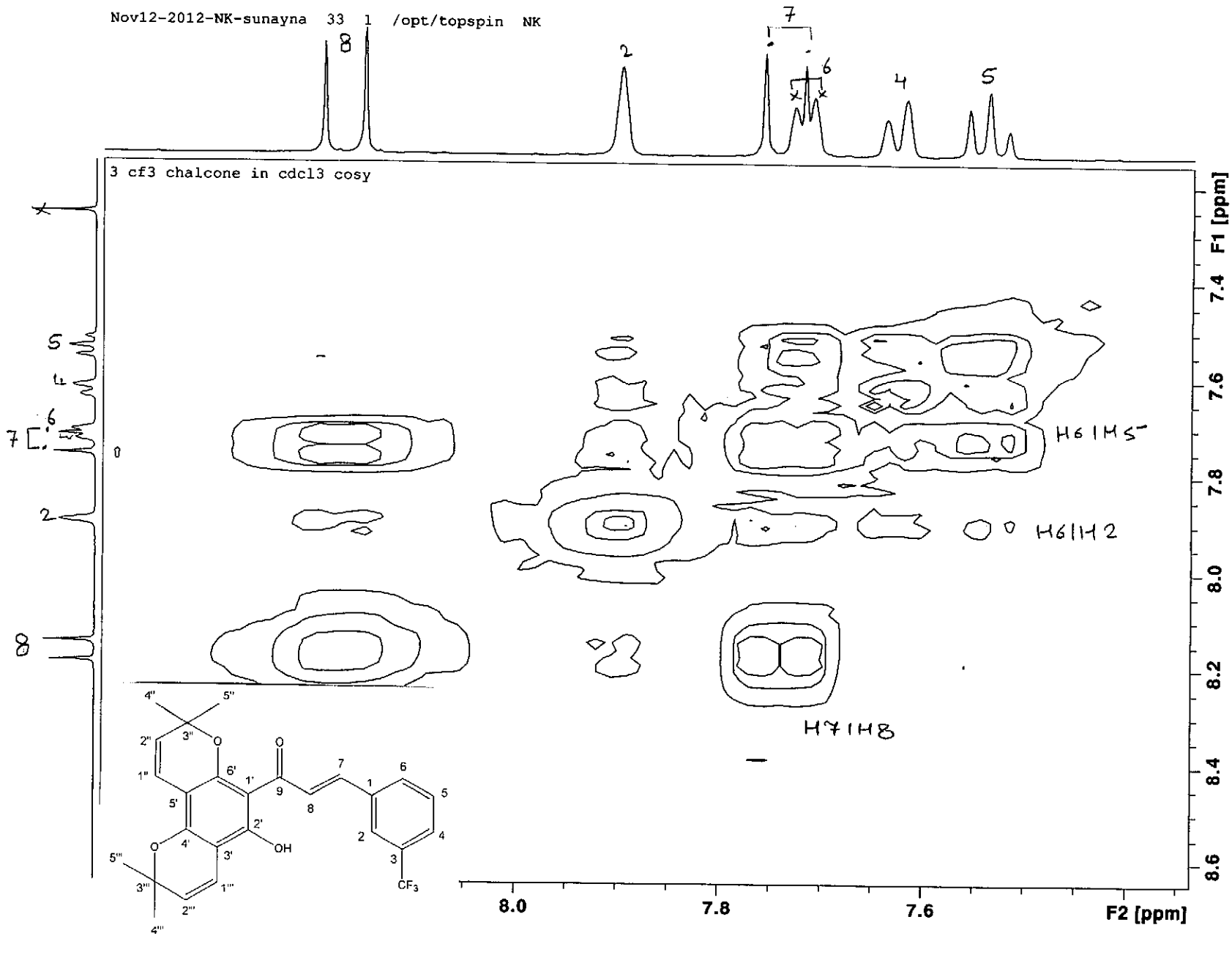
Expanded 13C NMR spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



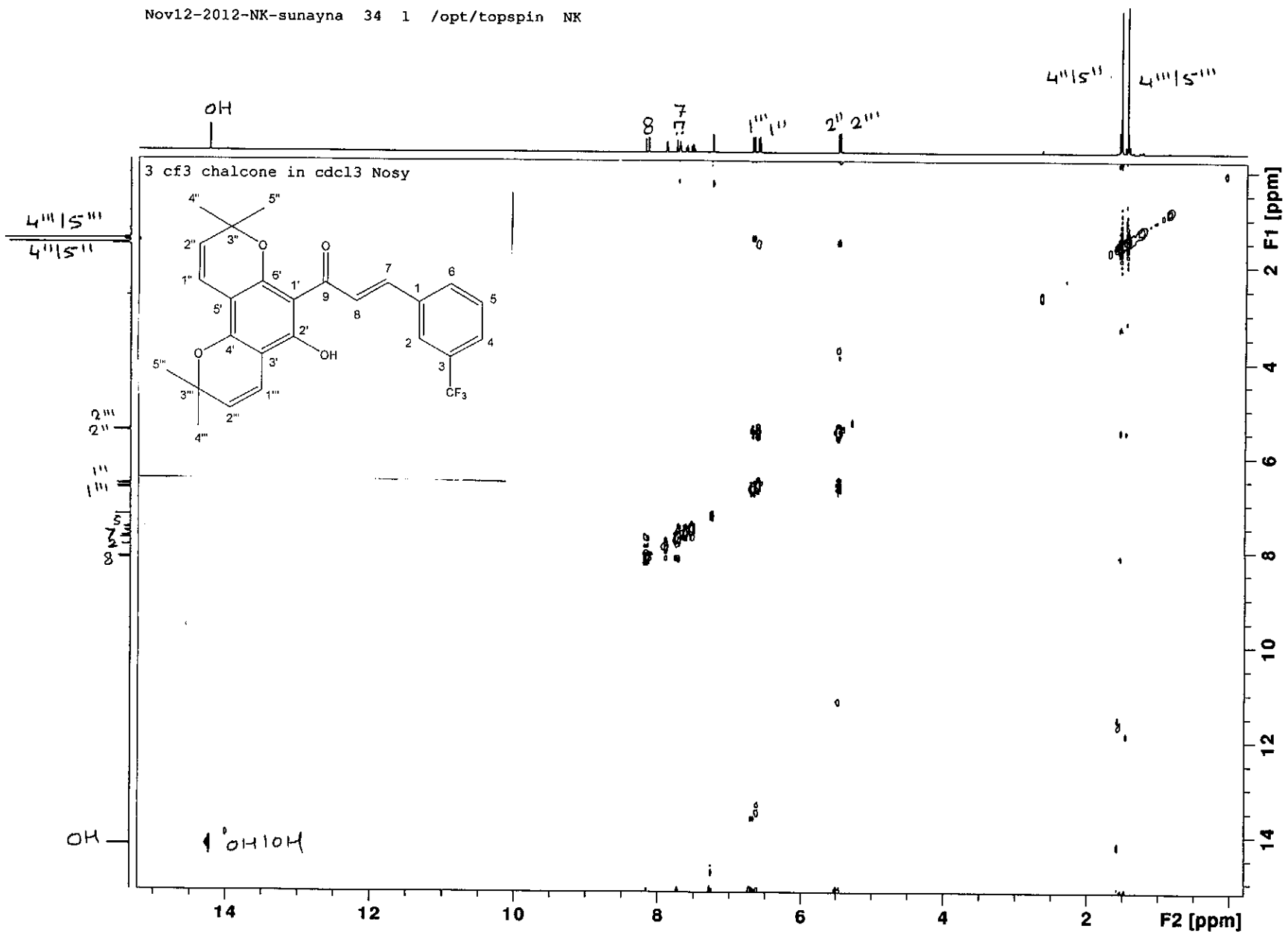
COSY NMR spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



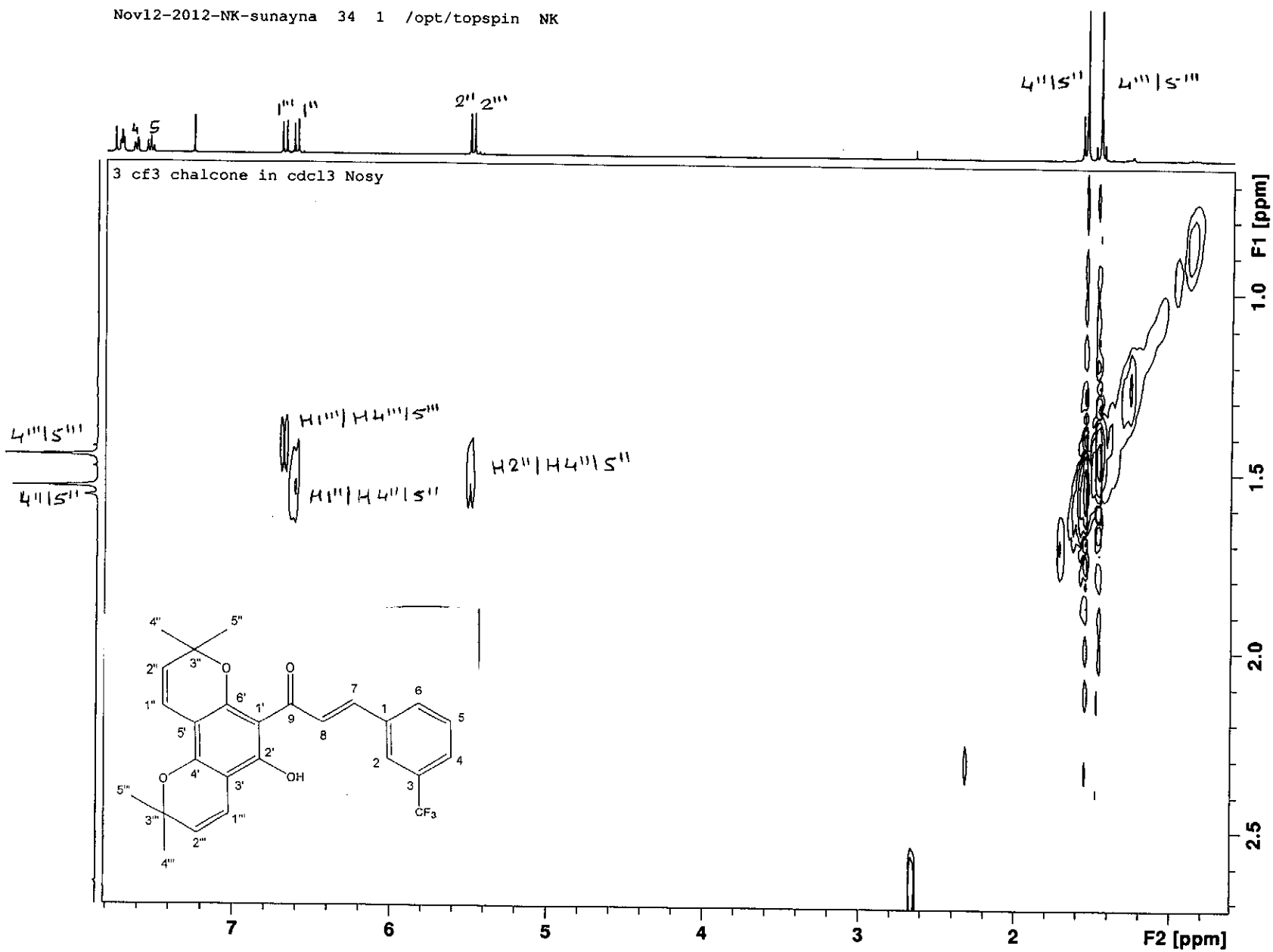
Expanded COSY NMR spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



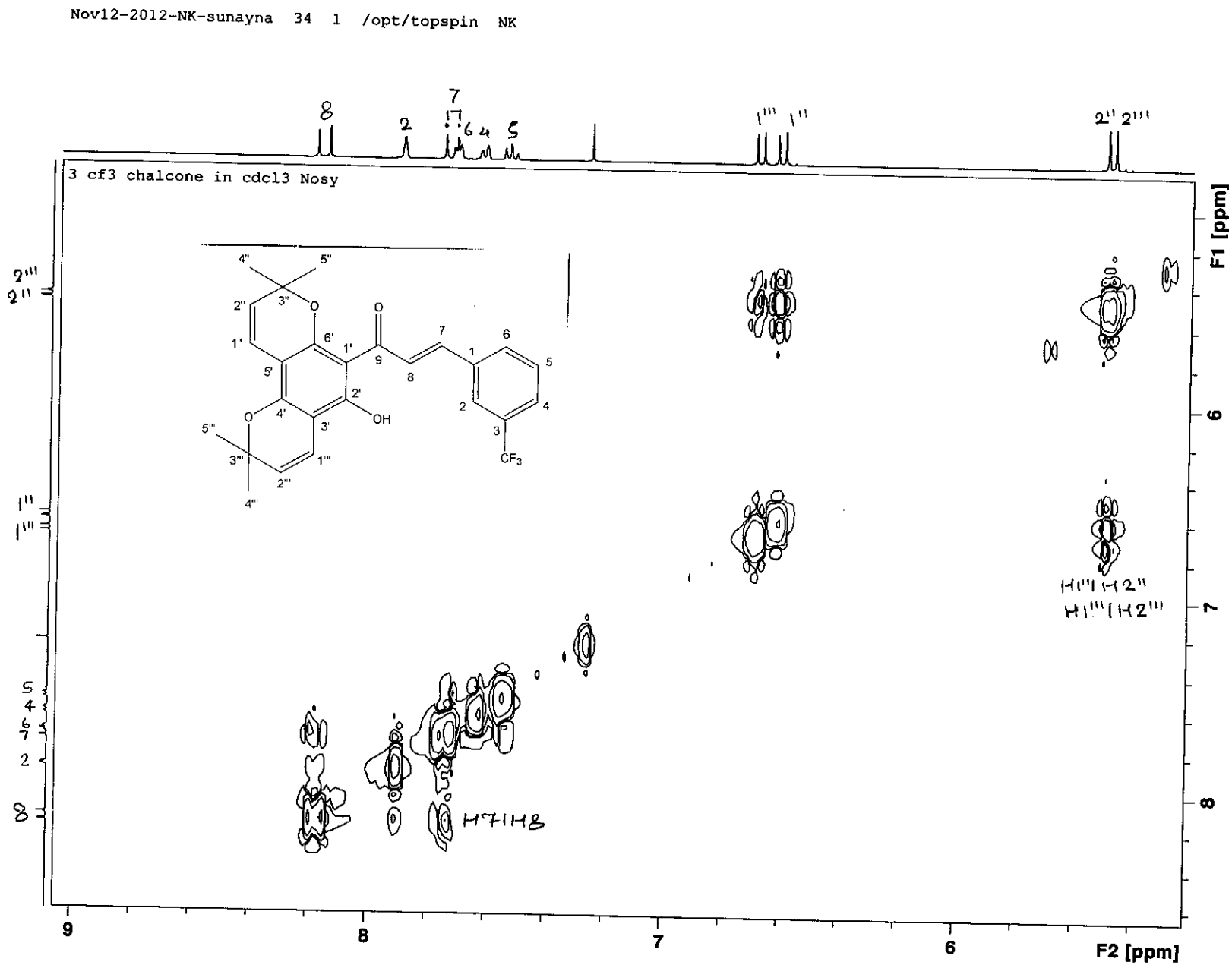
Expanded COSY NMR spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



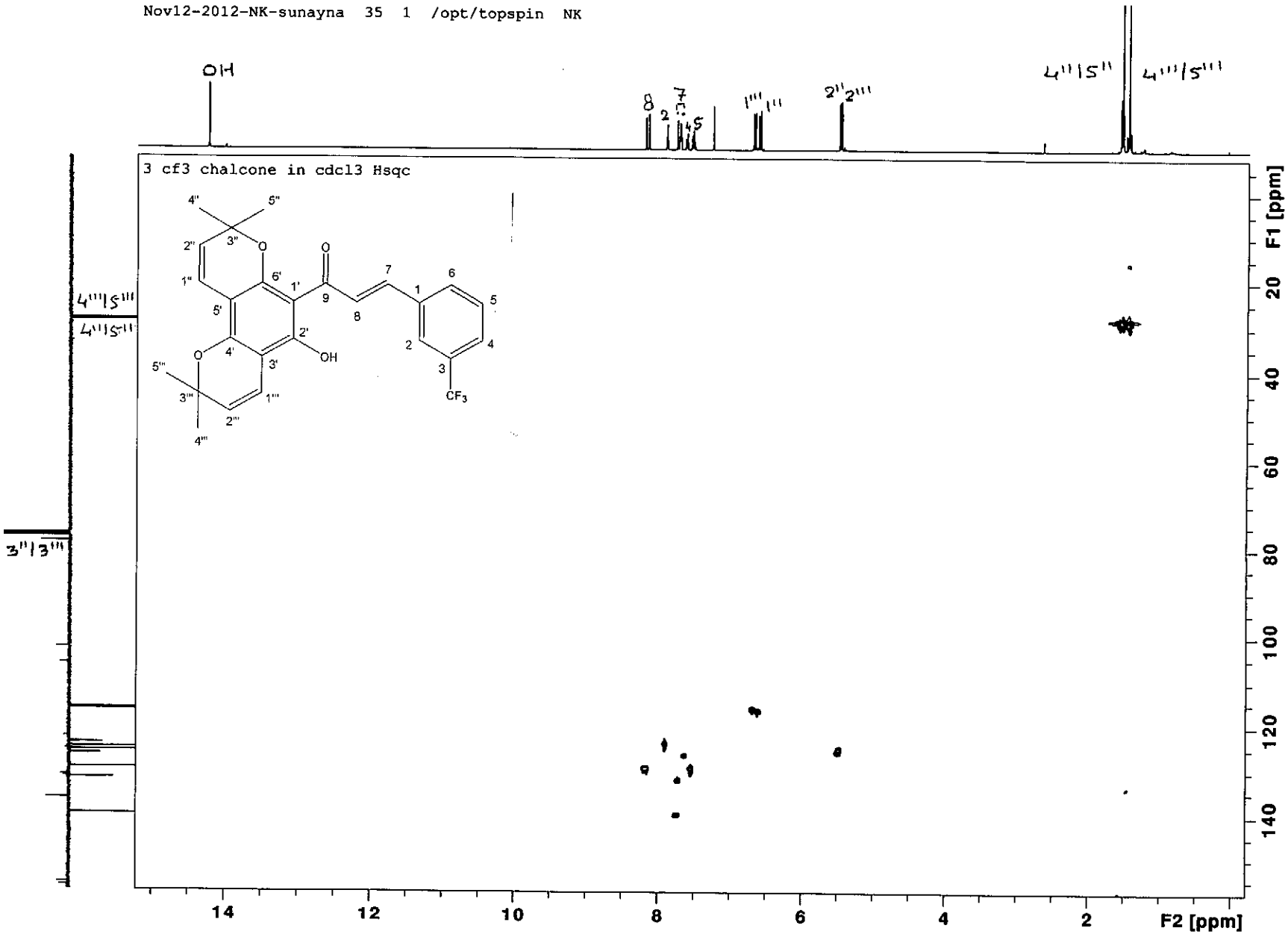
NOESY spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



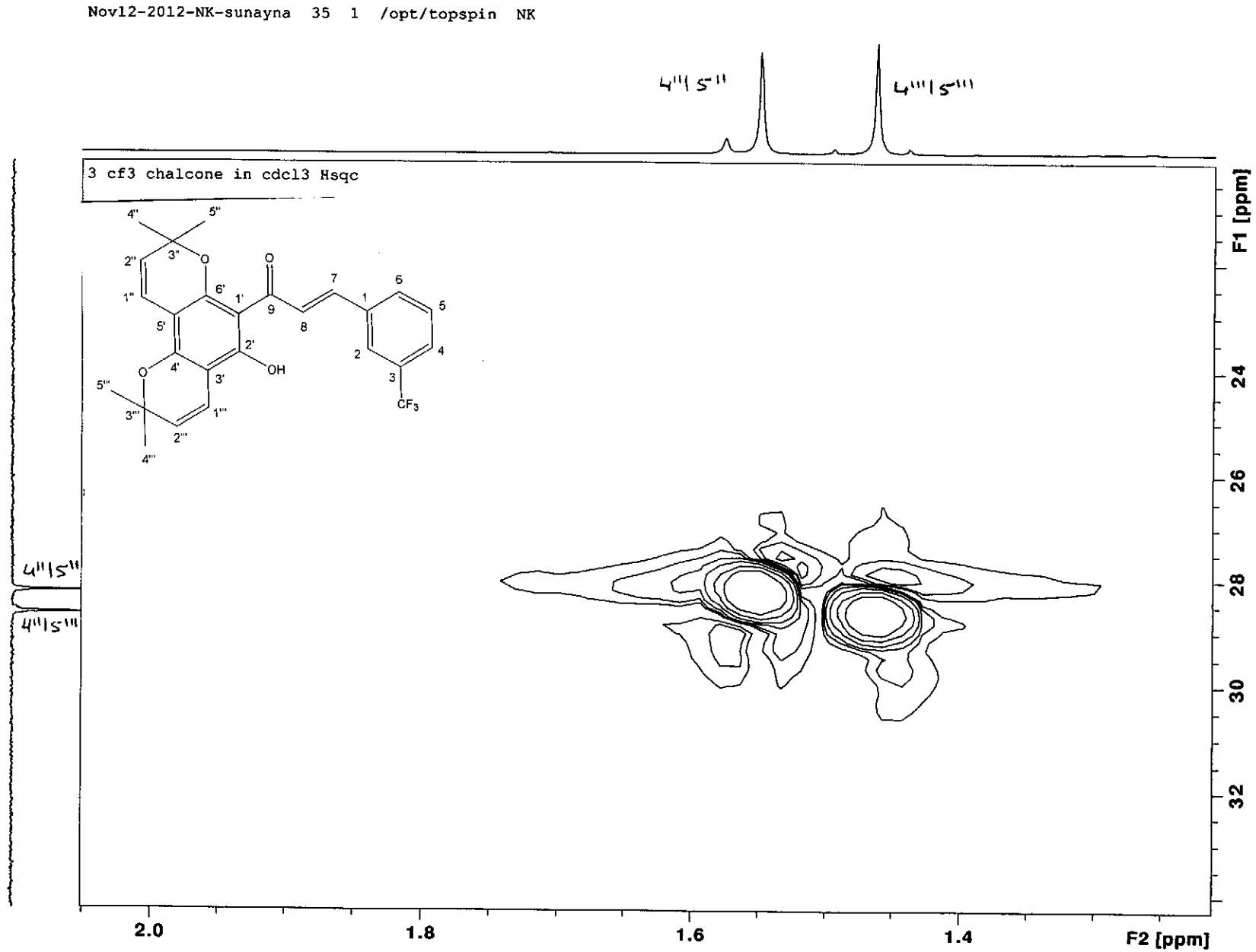
Expanded NOESY spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



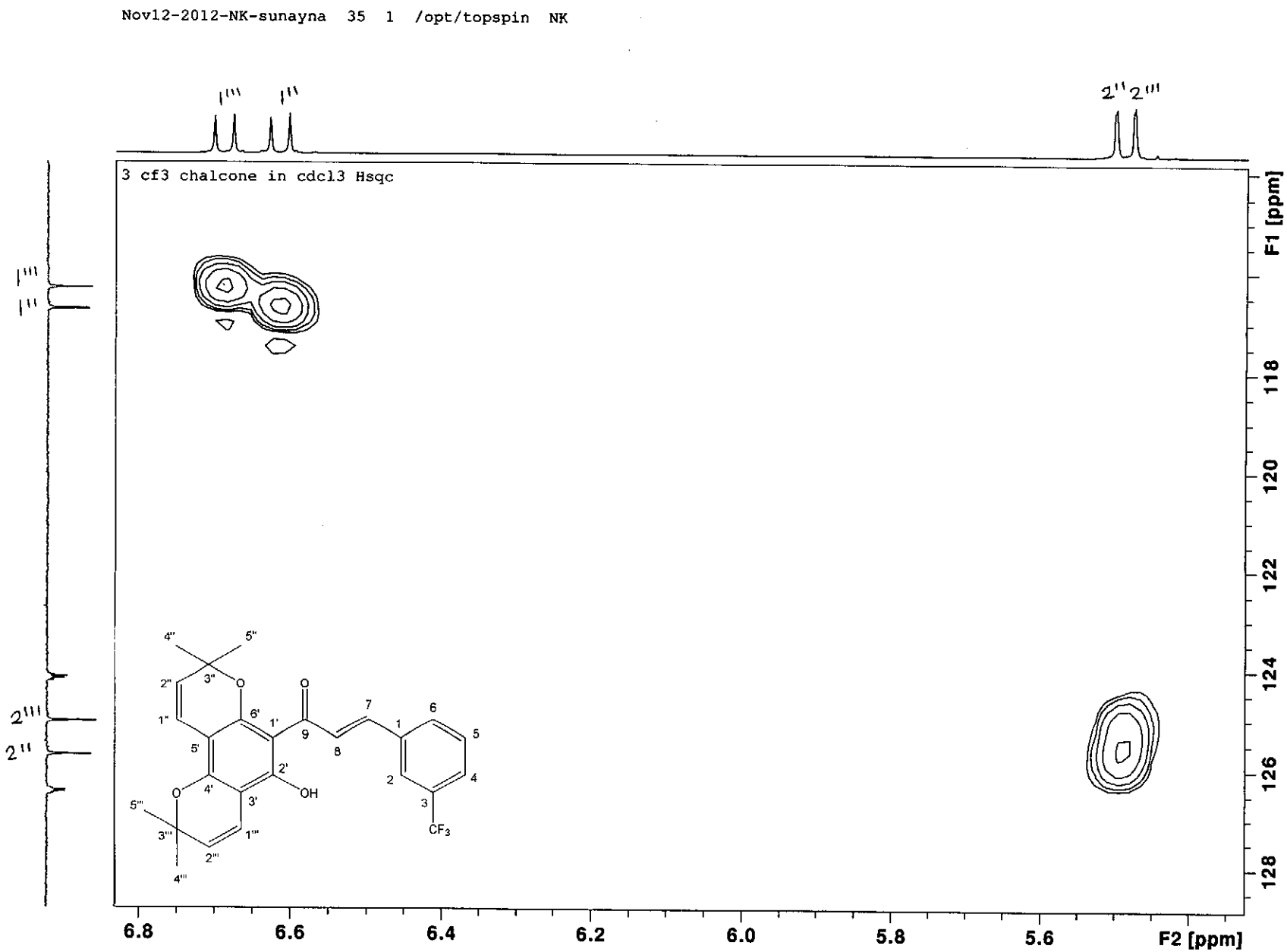
Expanded NOESY spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



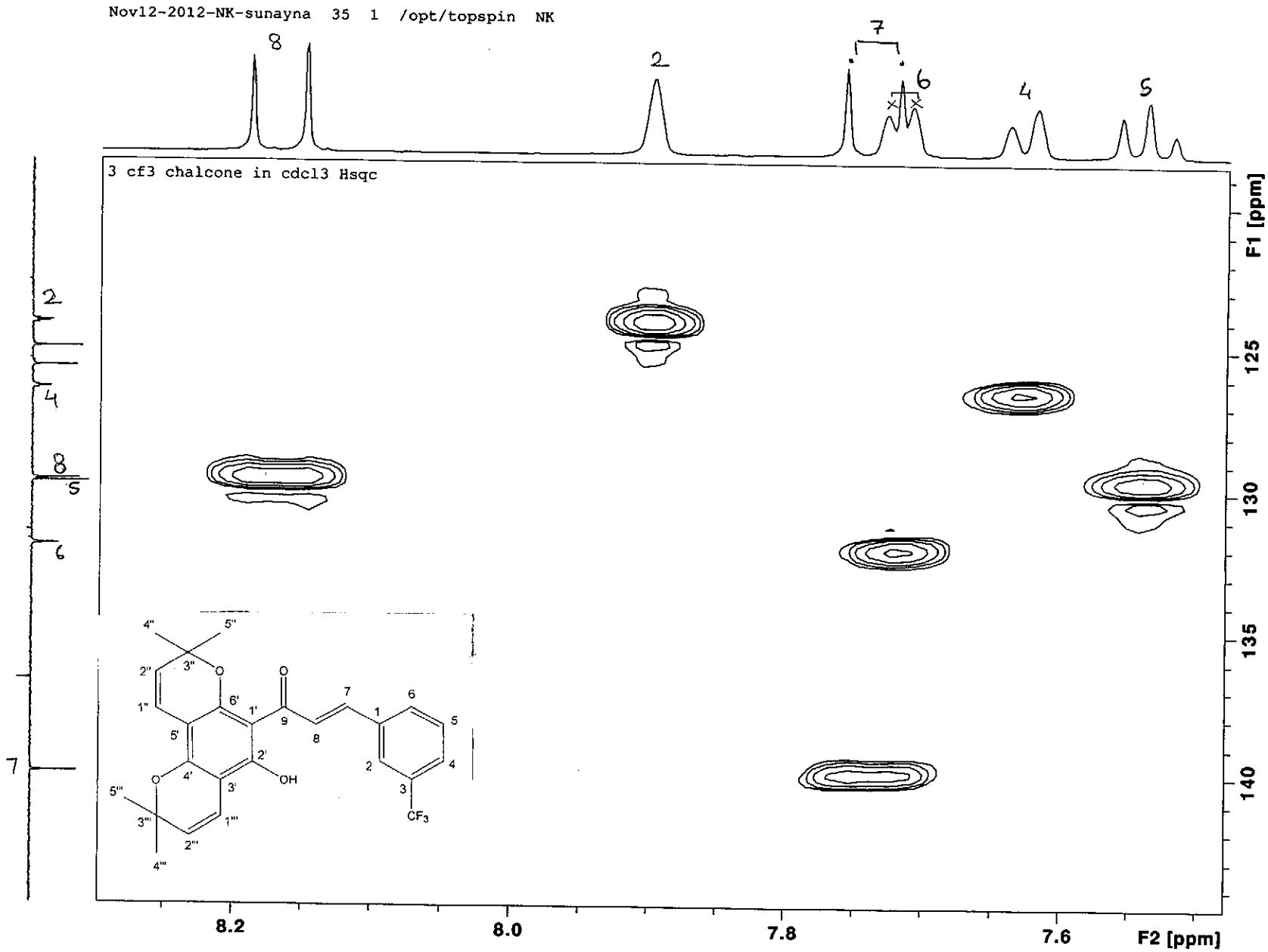
HSQC spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



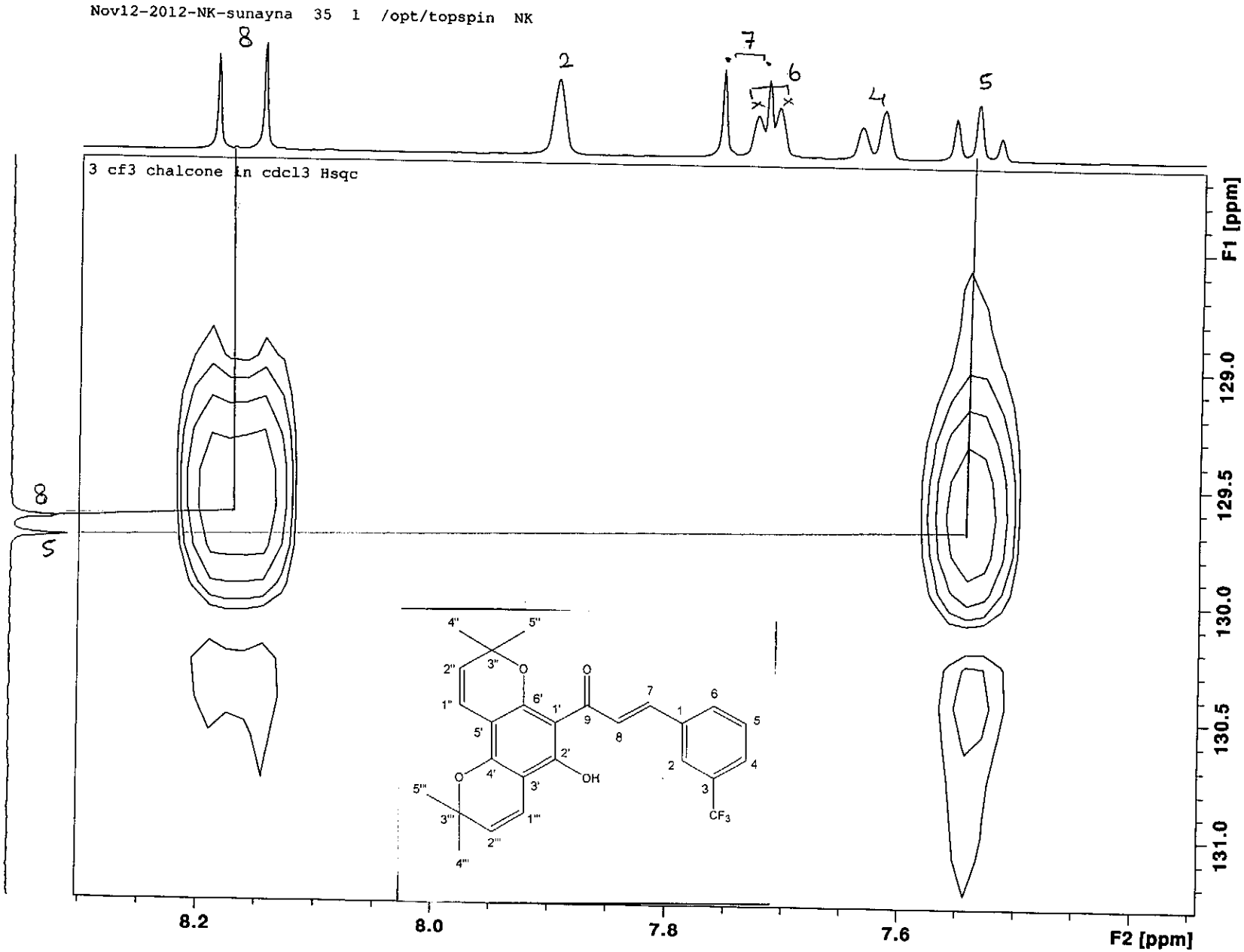
Expanded HSQC spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



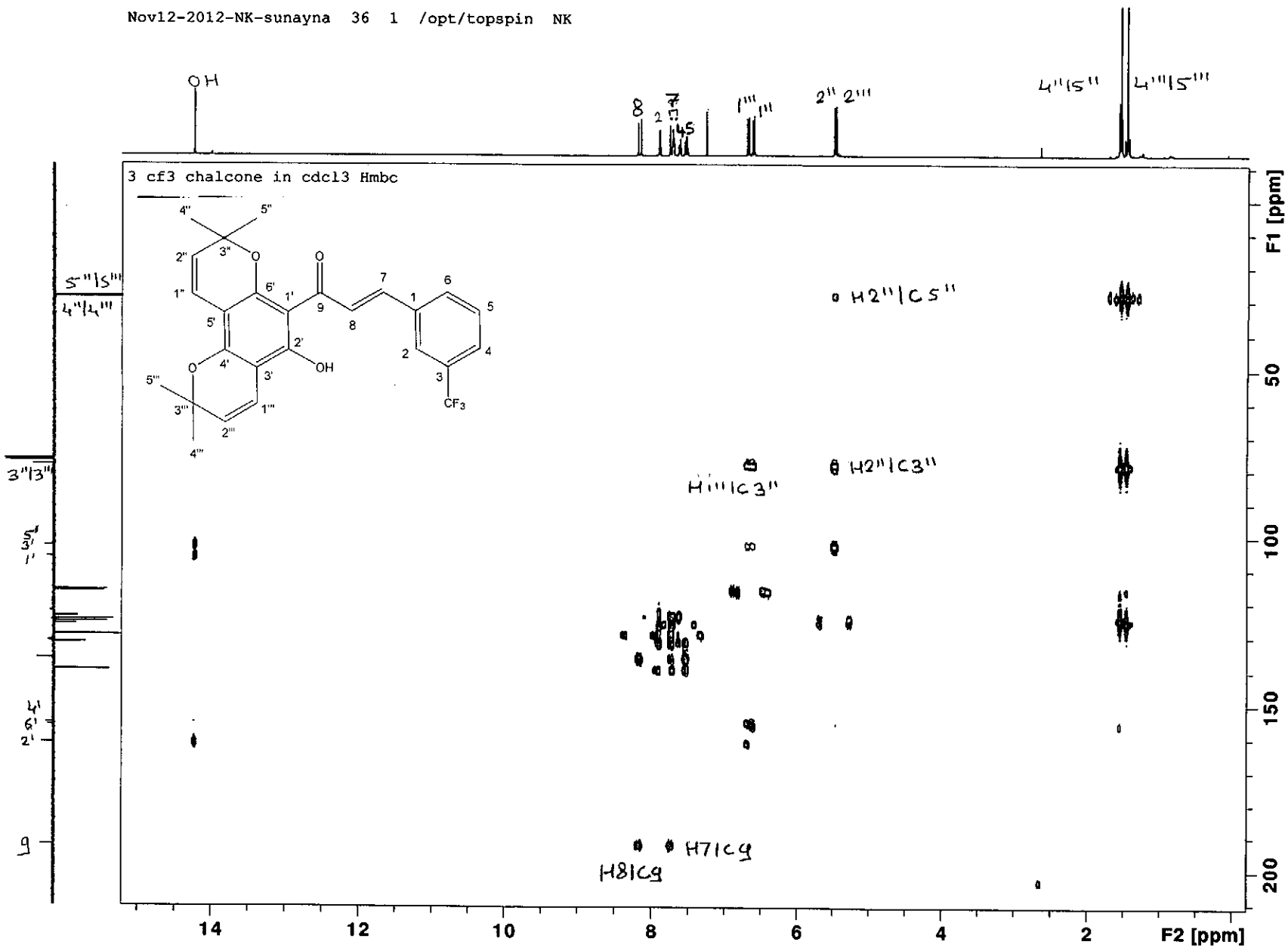
Expanded HSQC spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



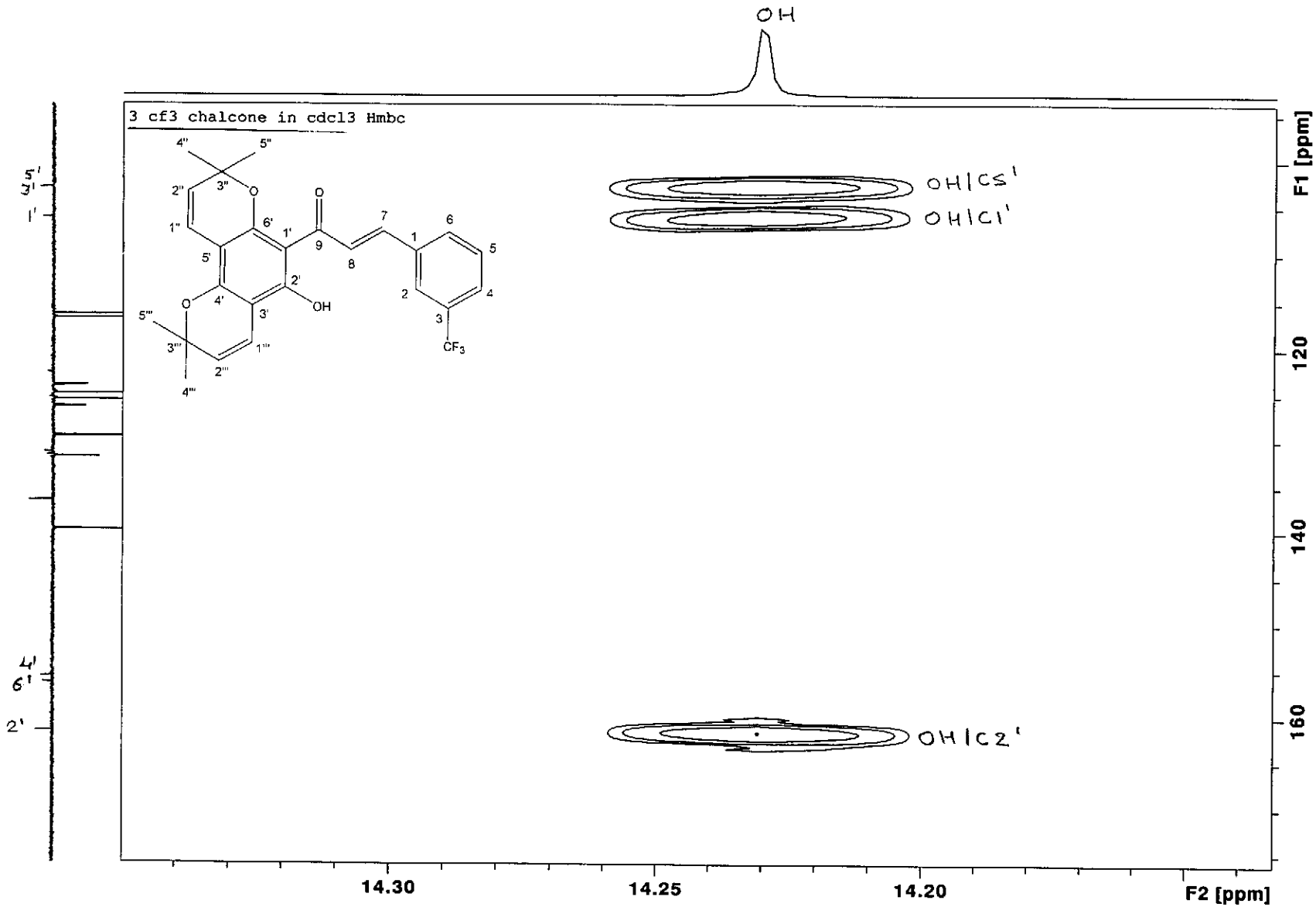
Expanded HSQC spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



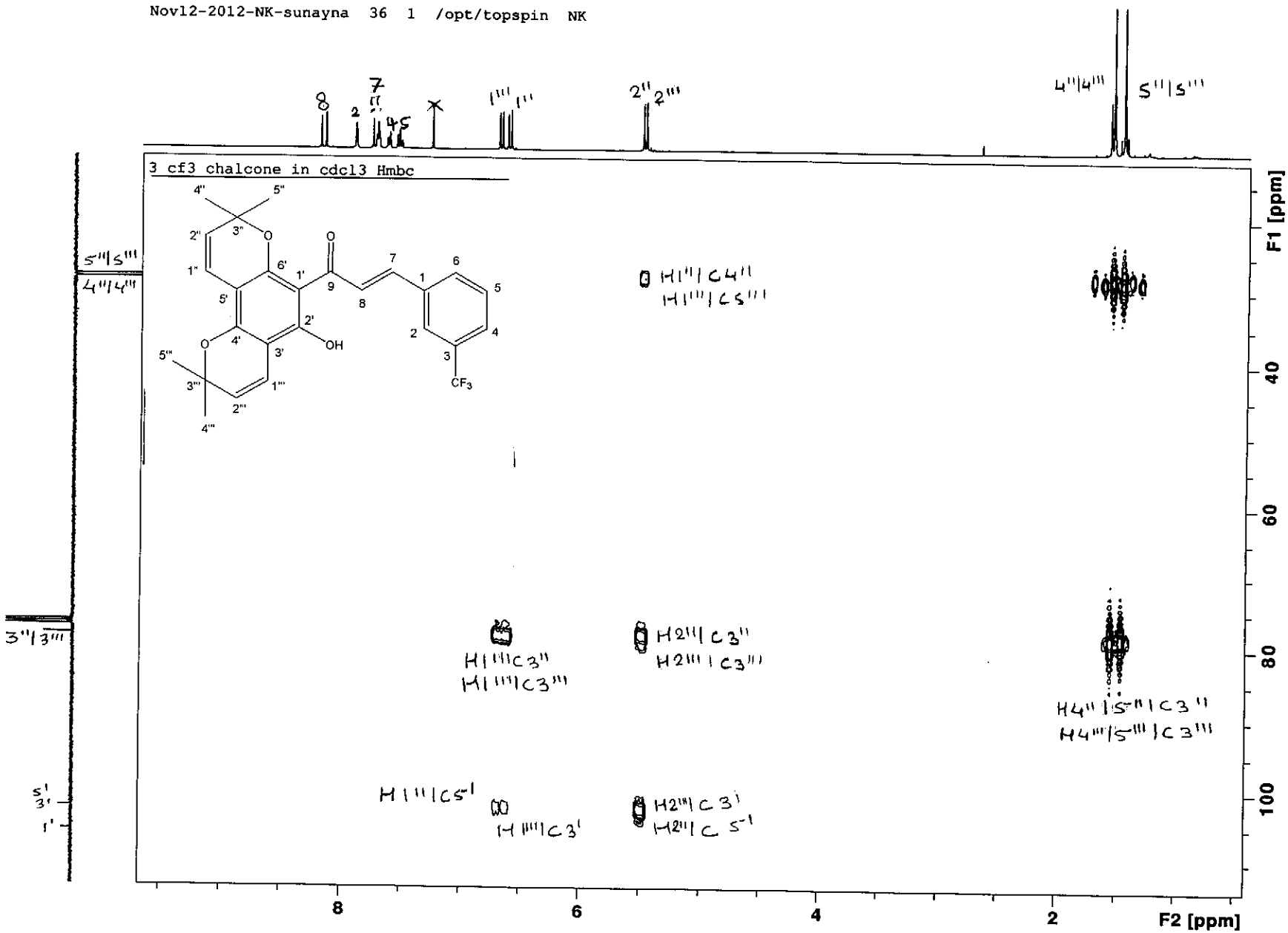
Expanded HSQC spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



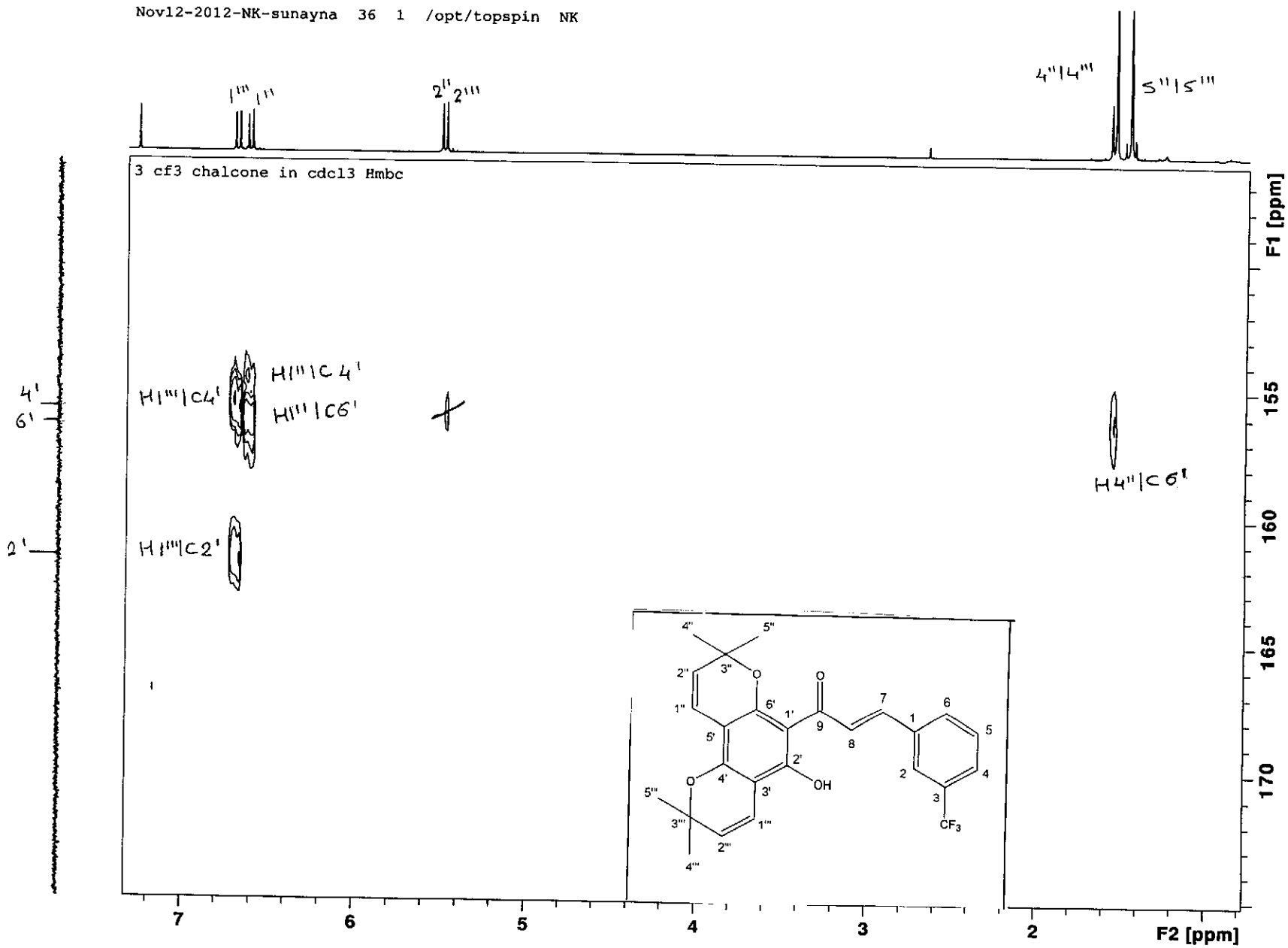
HMBC spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



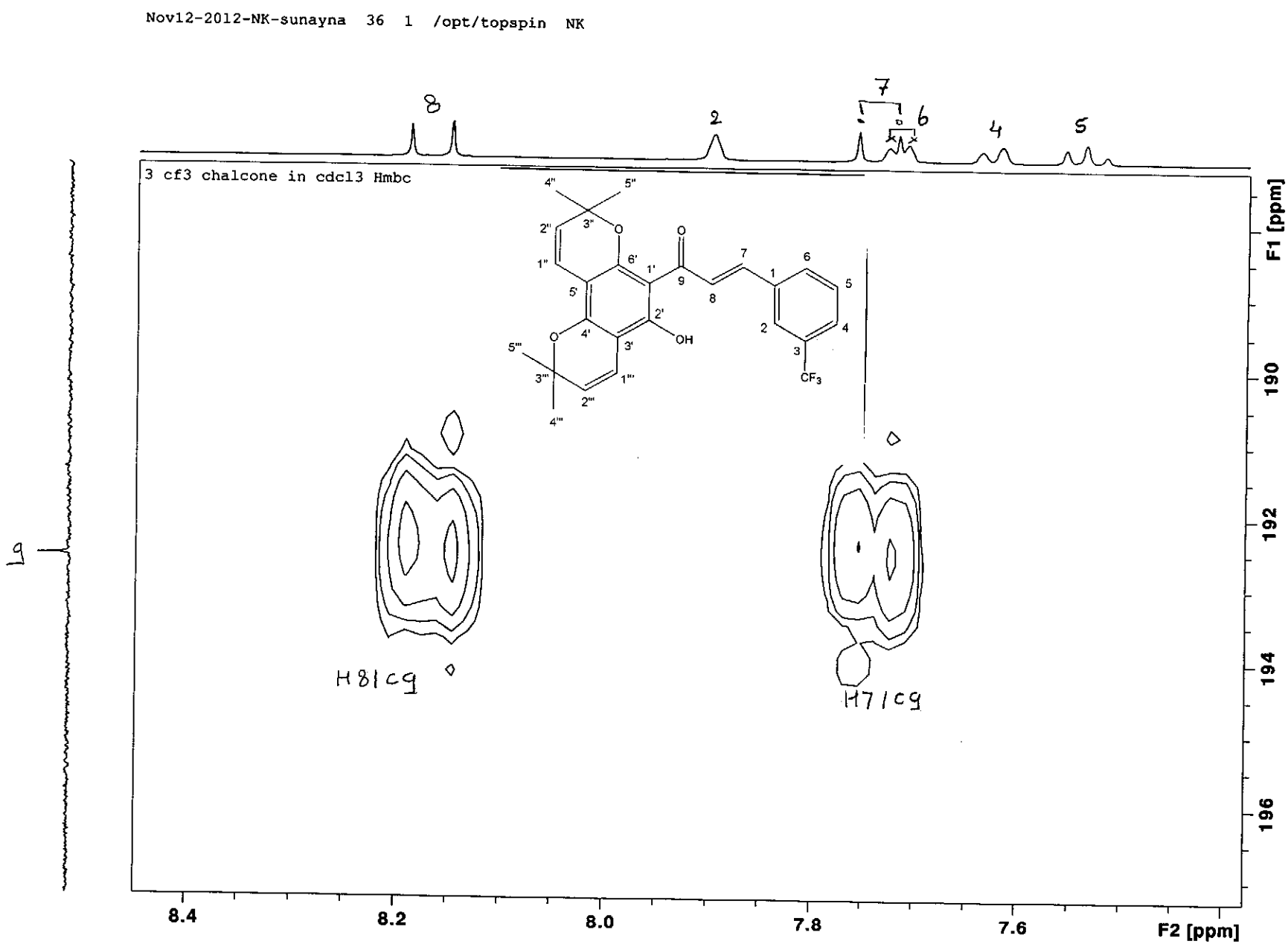
Expanded HMBC spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



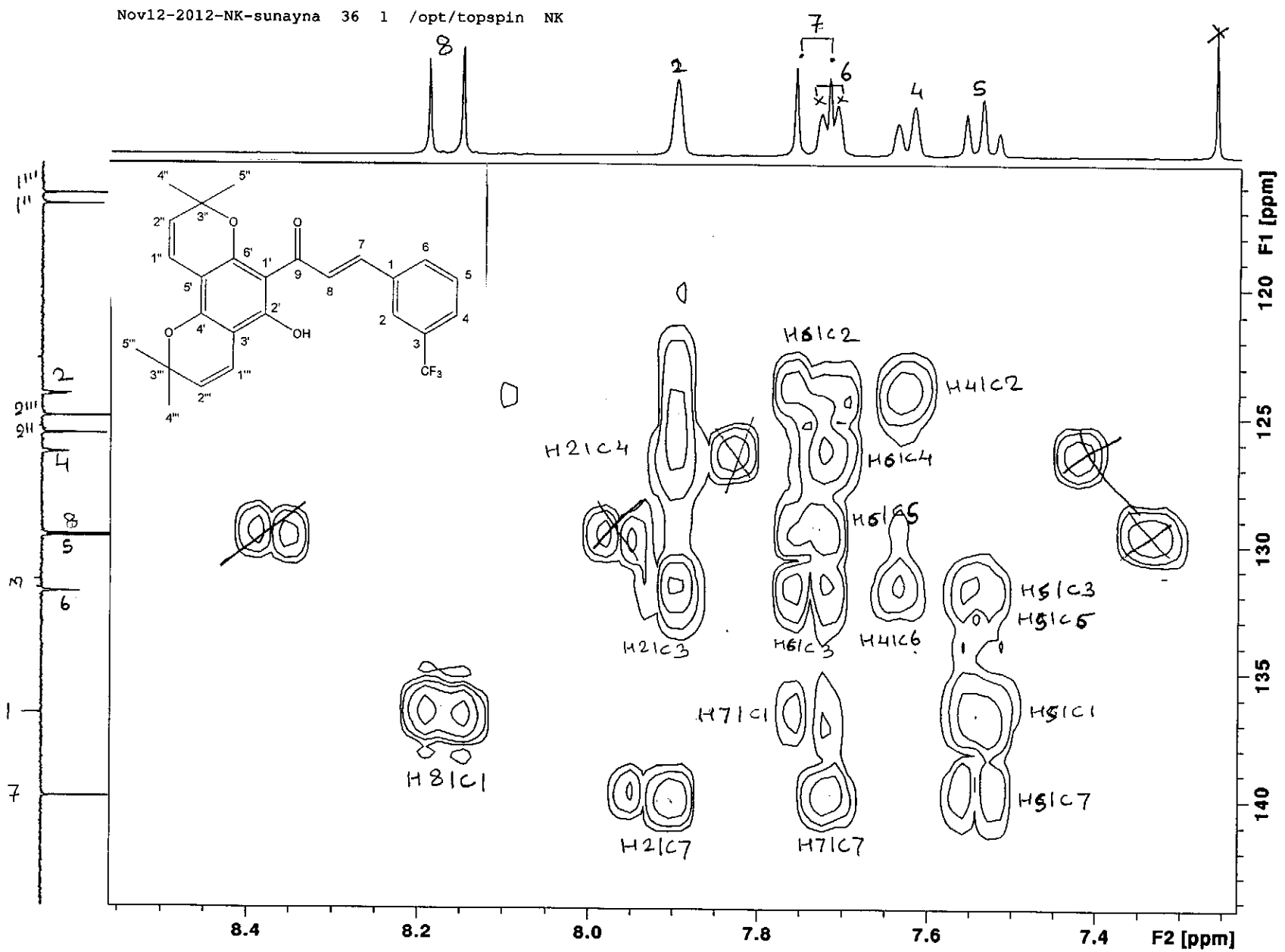
Expanded HMBC spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



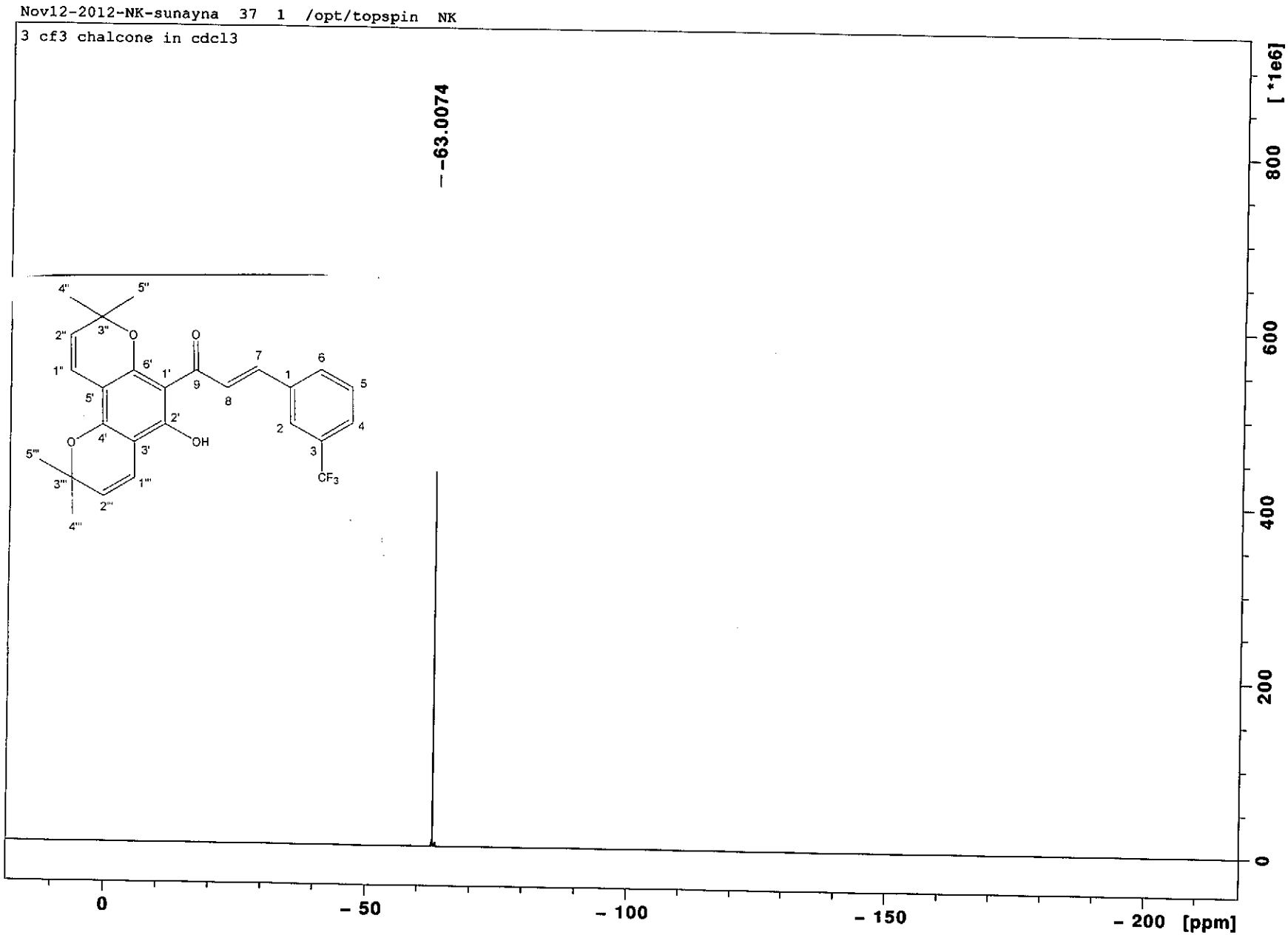
Expanded HMBC spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



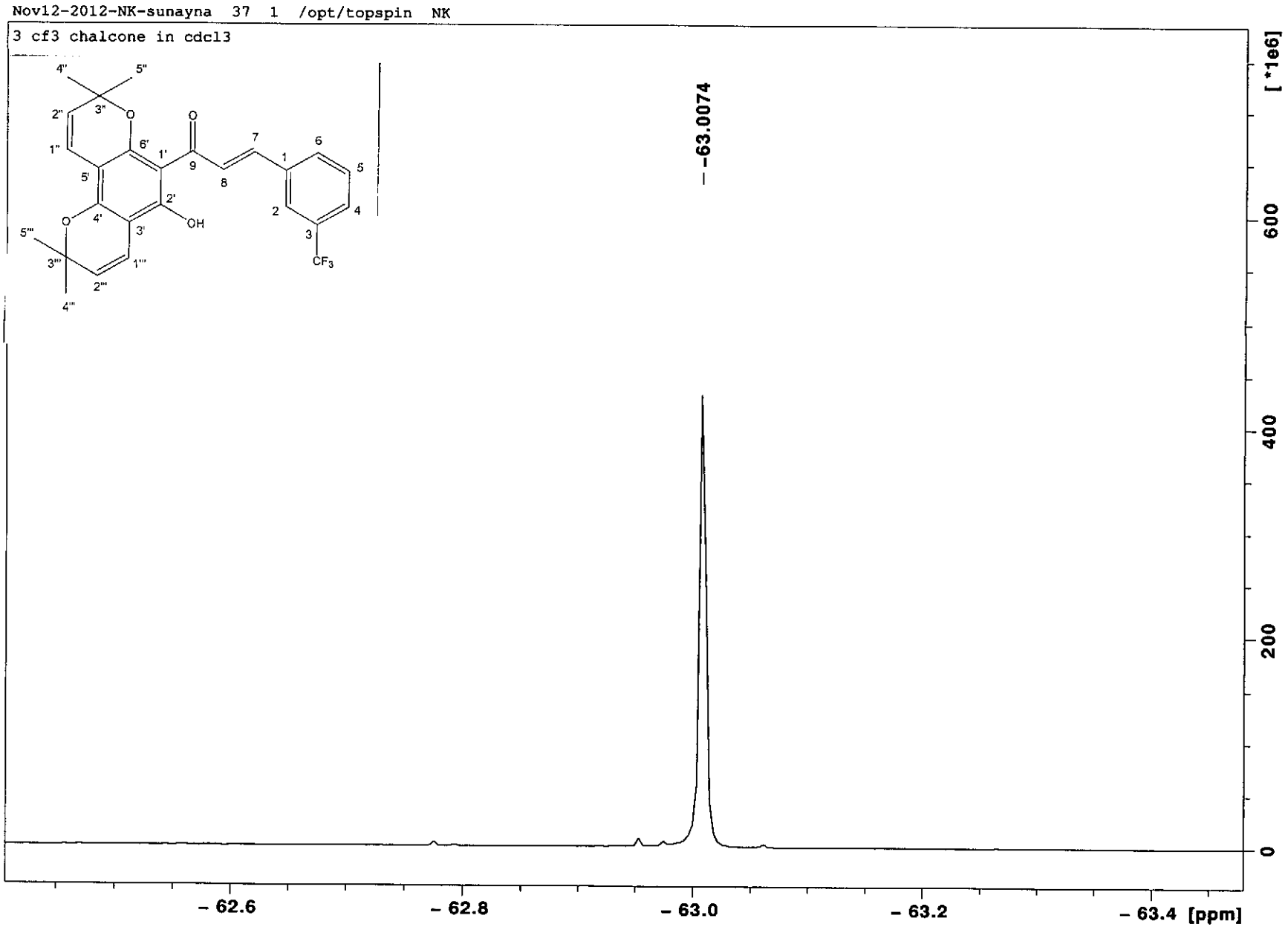
Expanded HMBC spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



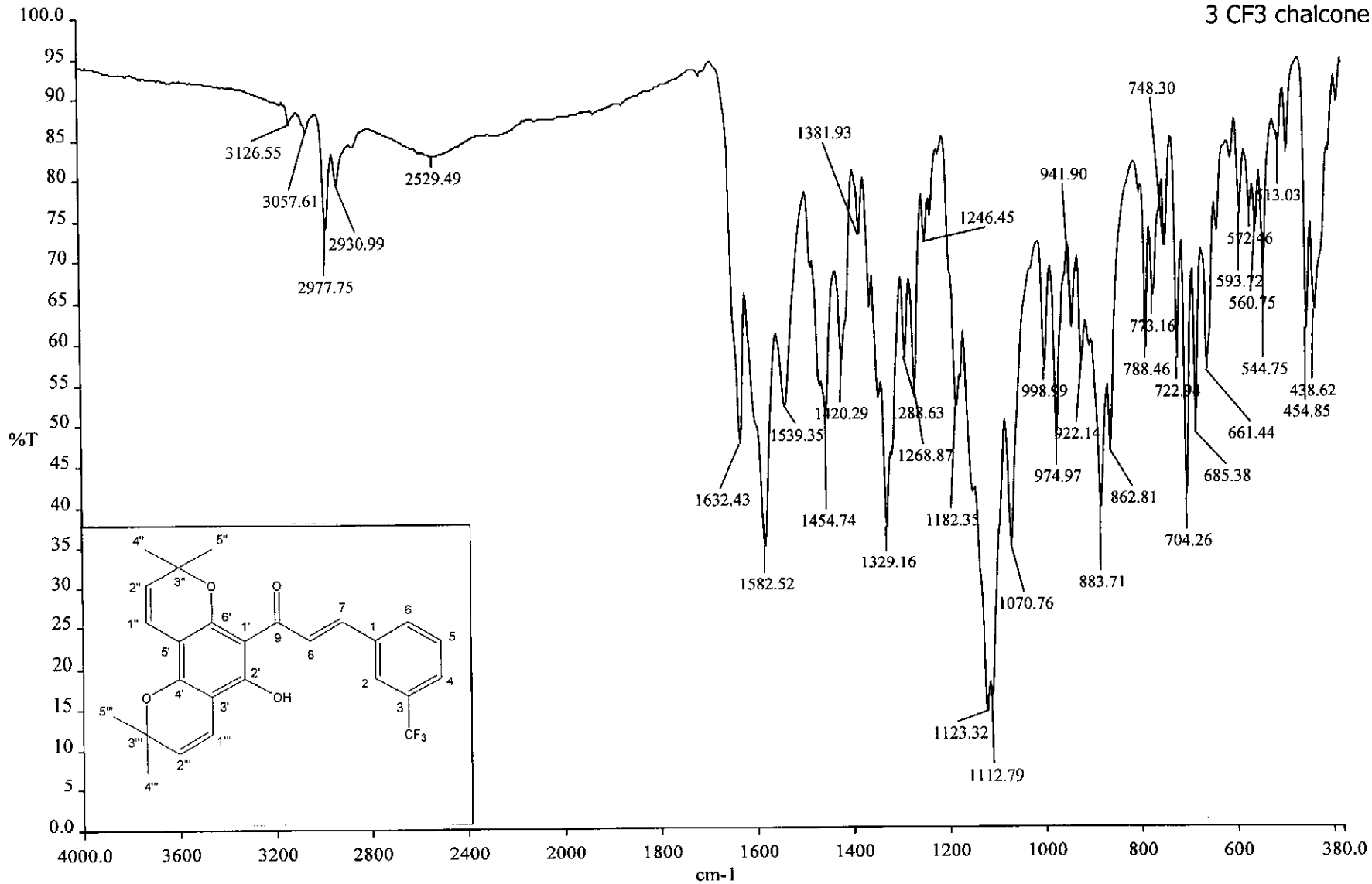
Expanded HMBC spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



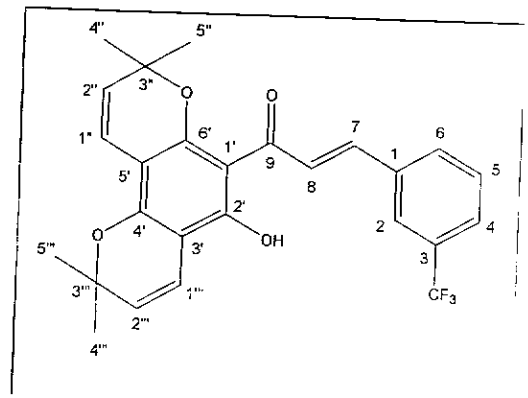
19F NMR spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



19F NMR spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



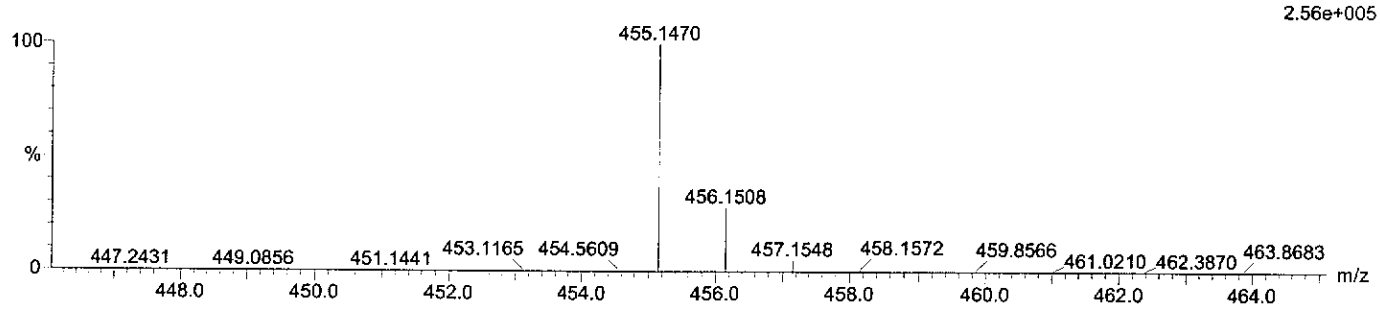
Infrared spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



Elemental Composition Report

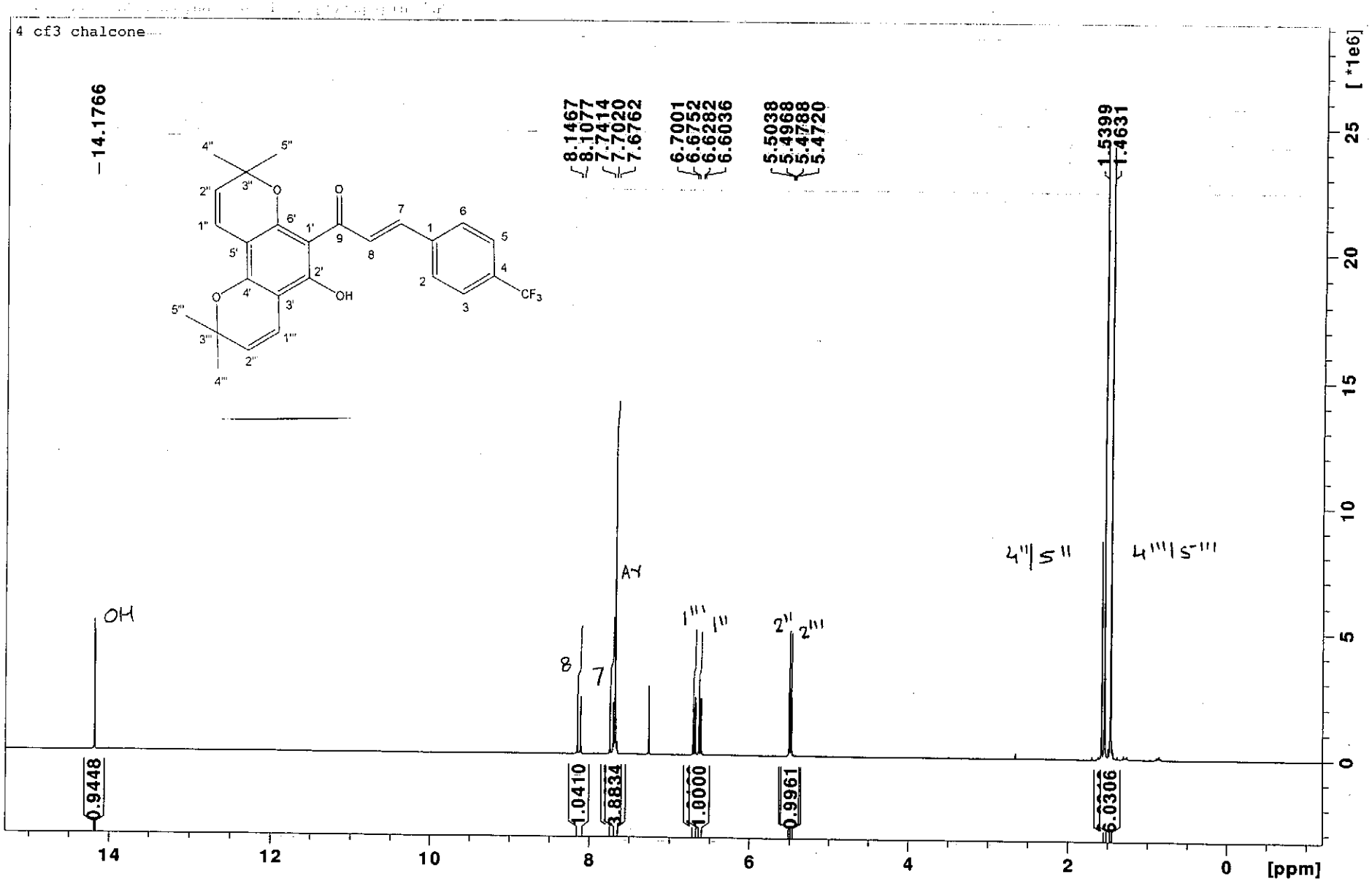
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
25 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-35 H: 20-30 N: 0-4 O: 0-4 F: 3-3
25 6 (0.085) Cm (1:30)
TOF MS ES-

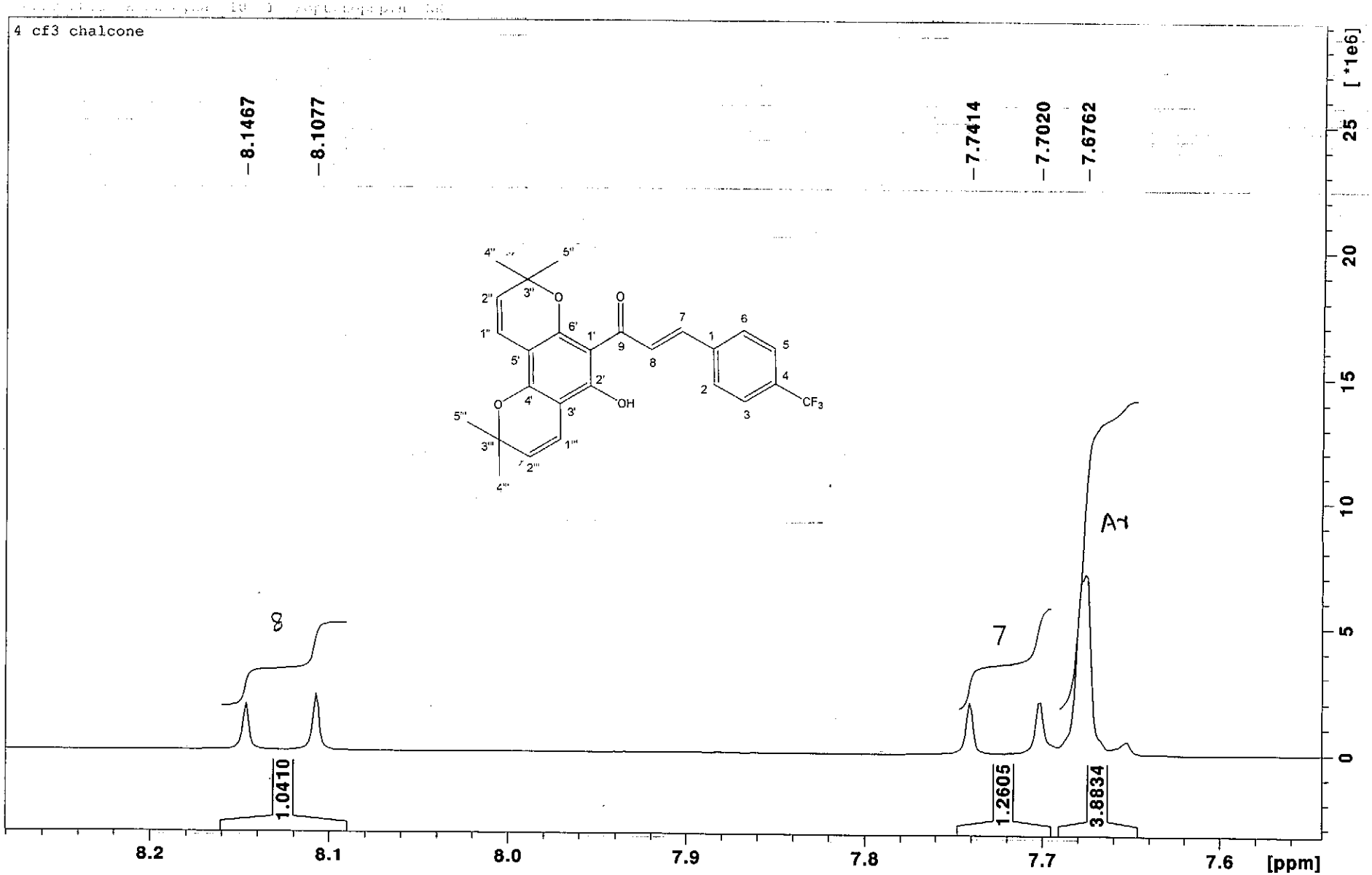


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
455.1470	455.1470	0.0	0.0	14.5	526.8	0.0	C26 H22 O4 F3

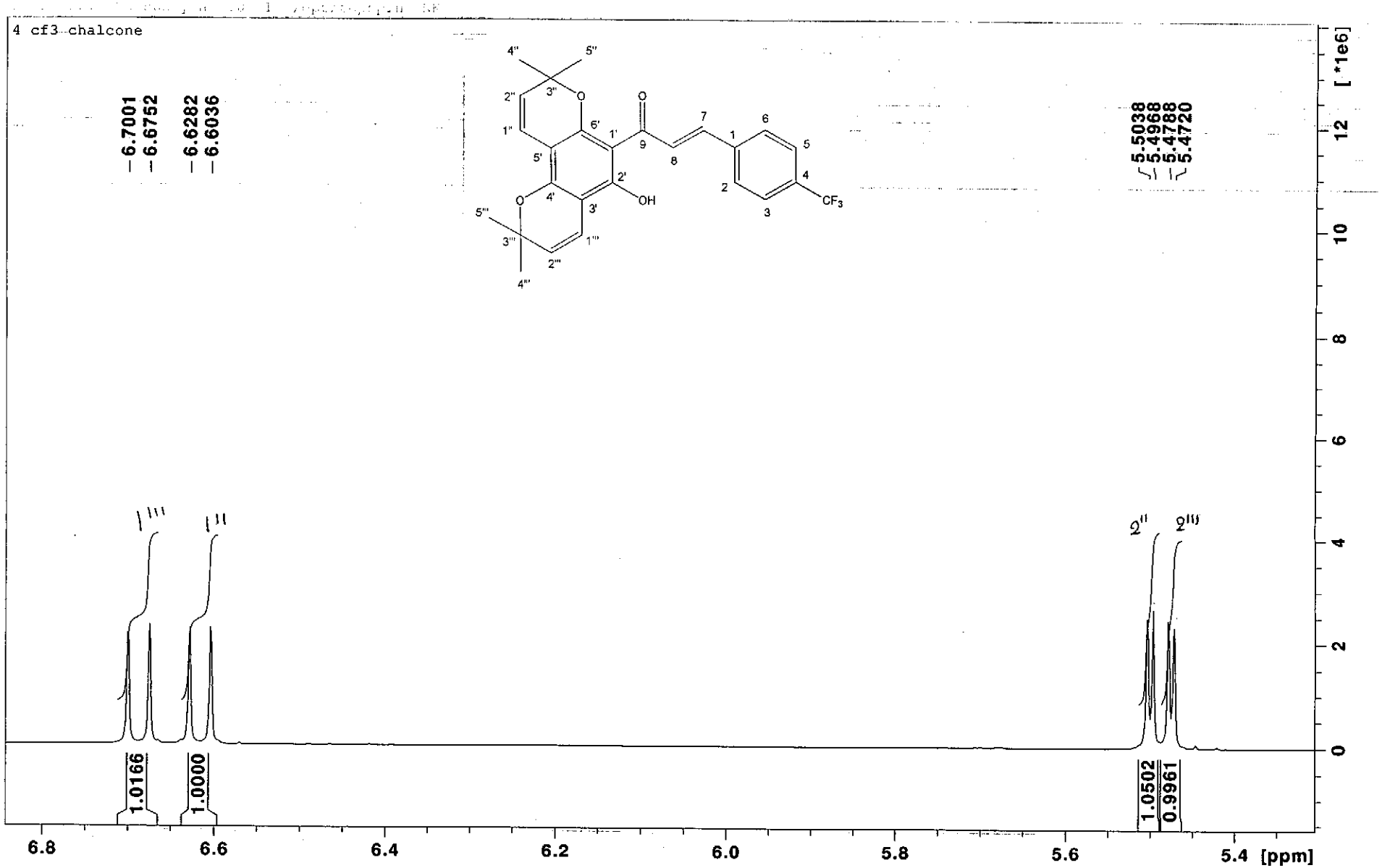
HRMS spectrum of 3-trifluoromethylpyranochromene chalcone (B-7)



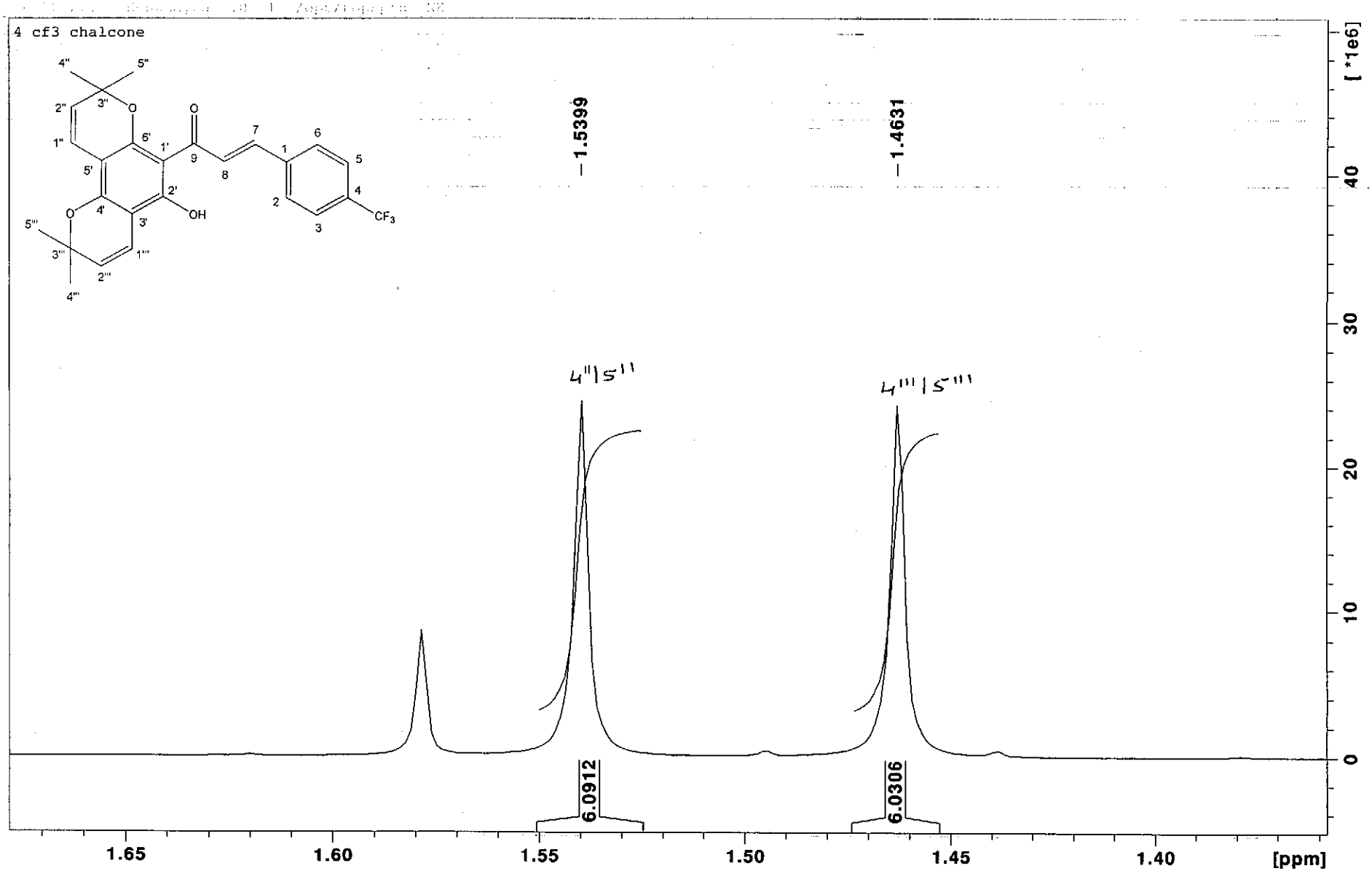
1H NMR spectrum of 4-trifluoromethylpyranochromene chalcone (B-8)



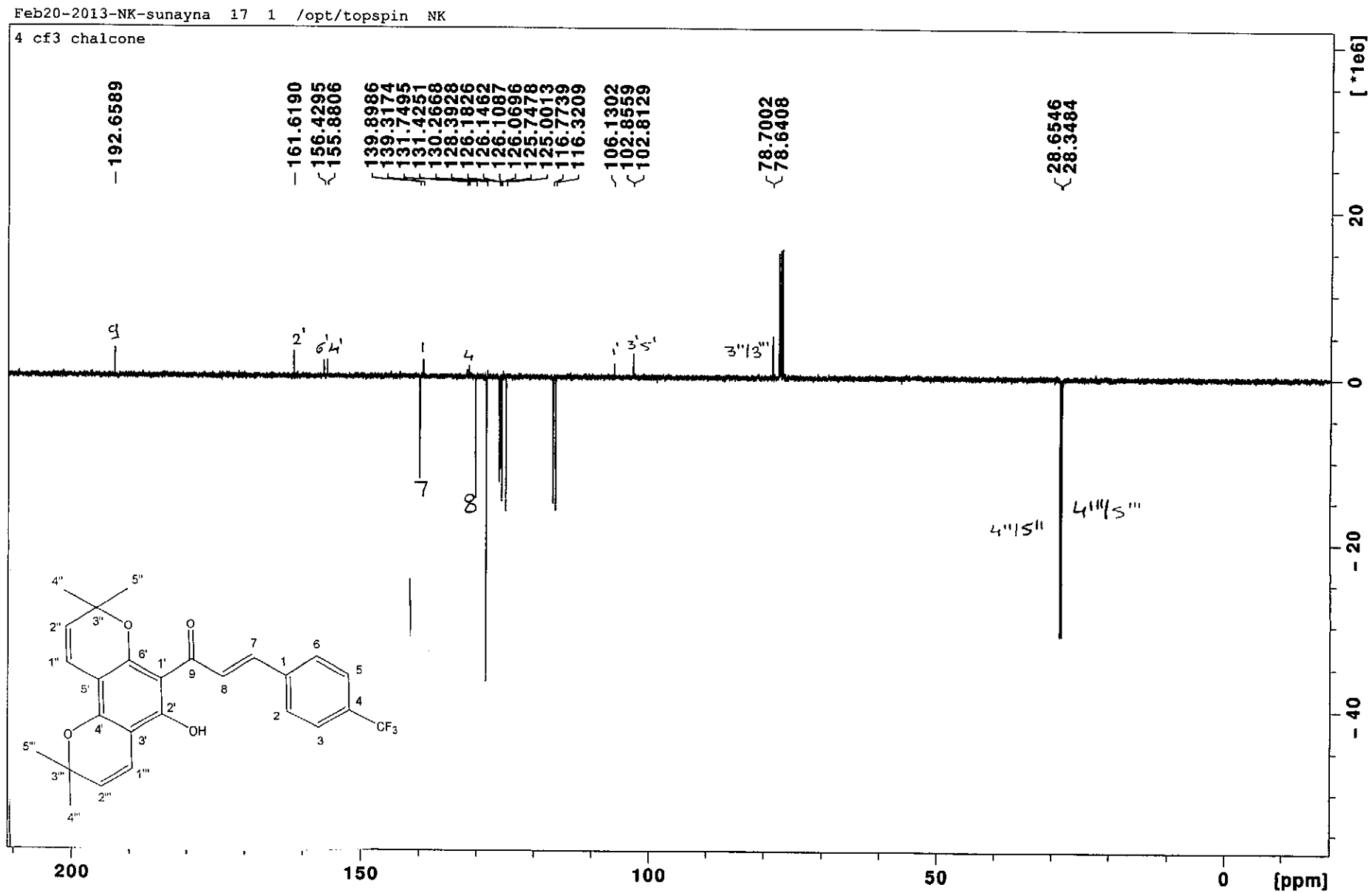
Expanded 1H NMR spectrum of 4-trifluoromethylpyranochromene chalcone (B-8)



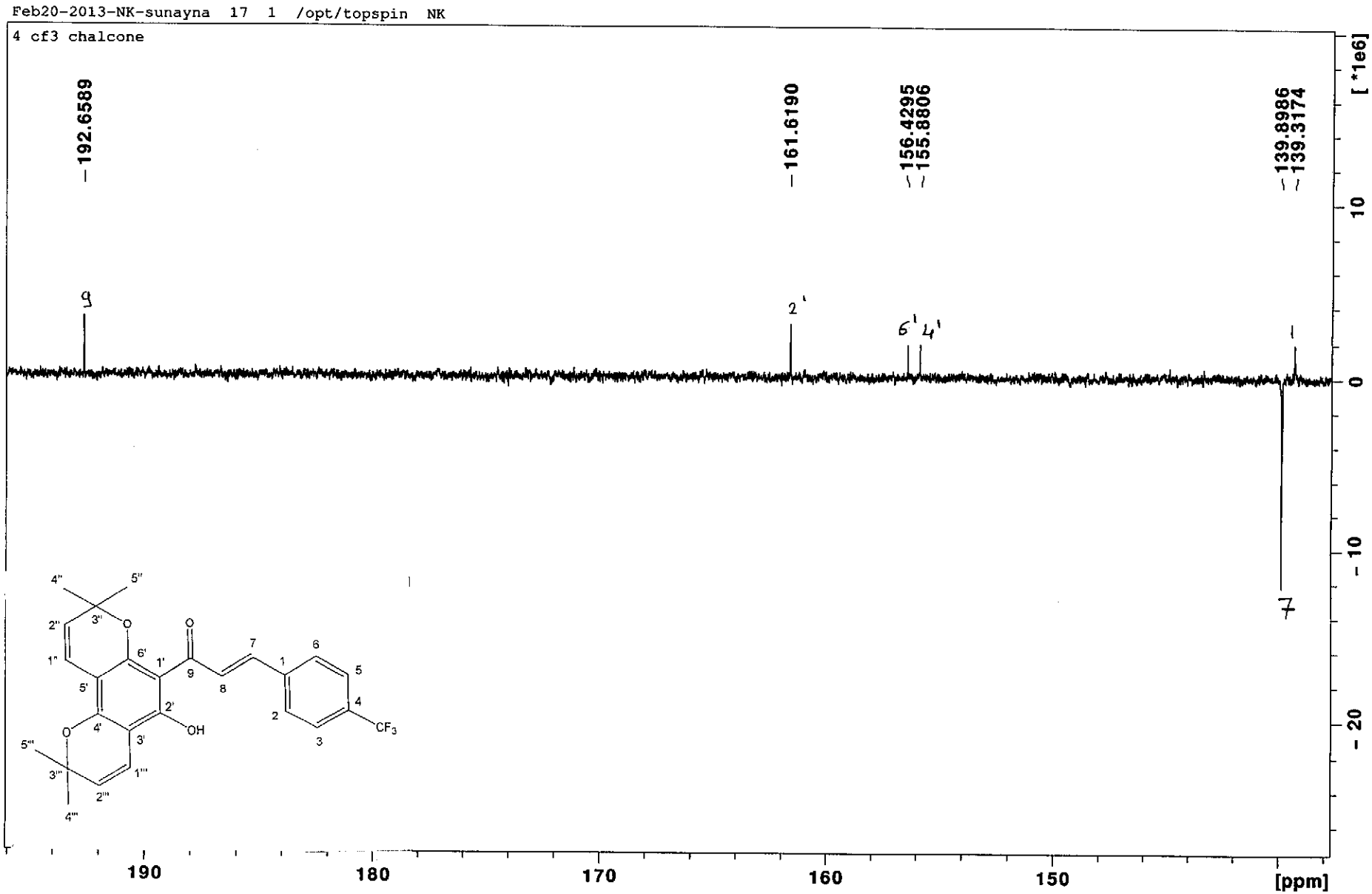
Expanded 1H NMR spectrum of 4-trifluoromethylpyranochromene chalcone (B-8)



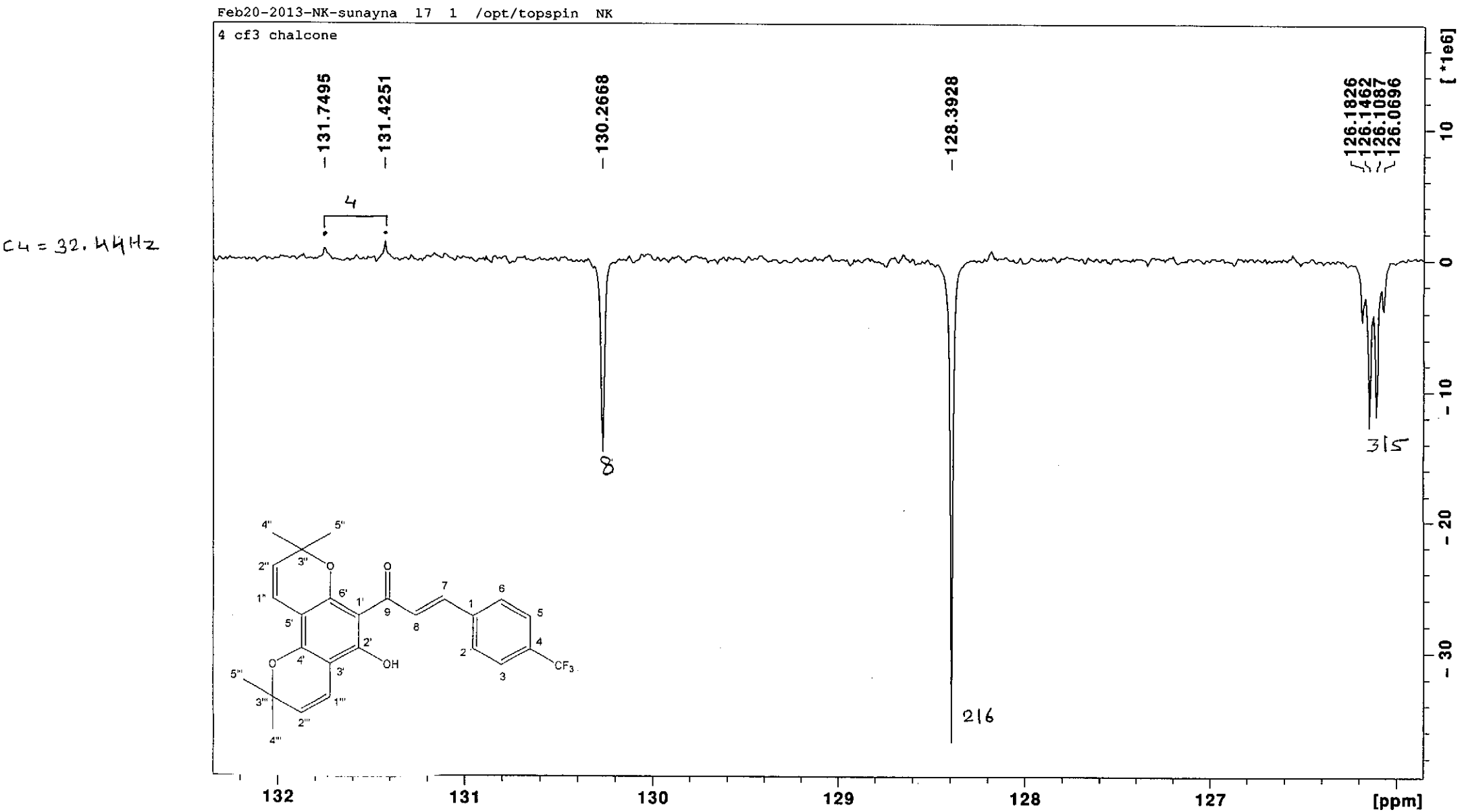
Expanded 1H NMR spectrum of 4-trifluoromethylpyranochromene chalcone (B-8)



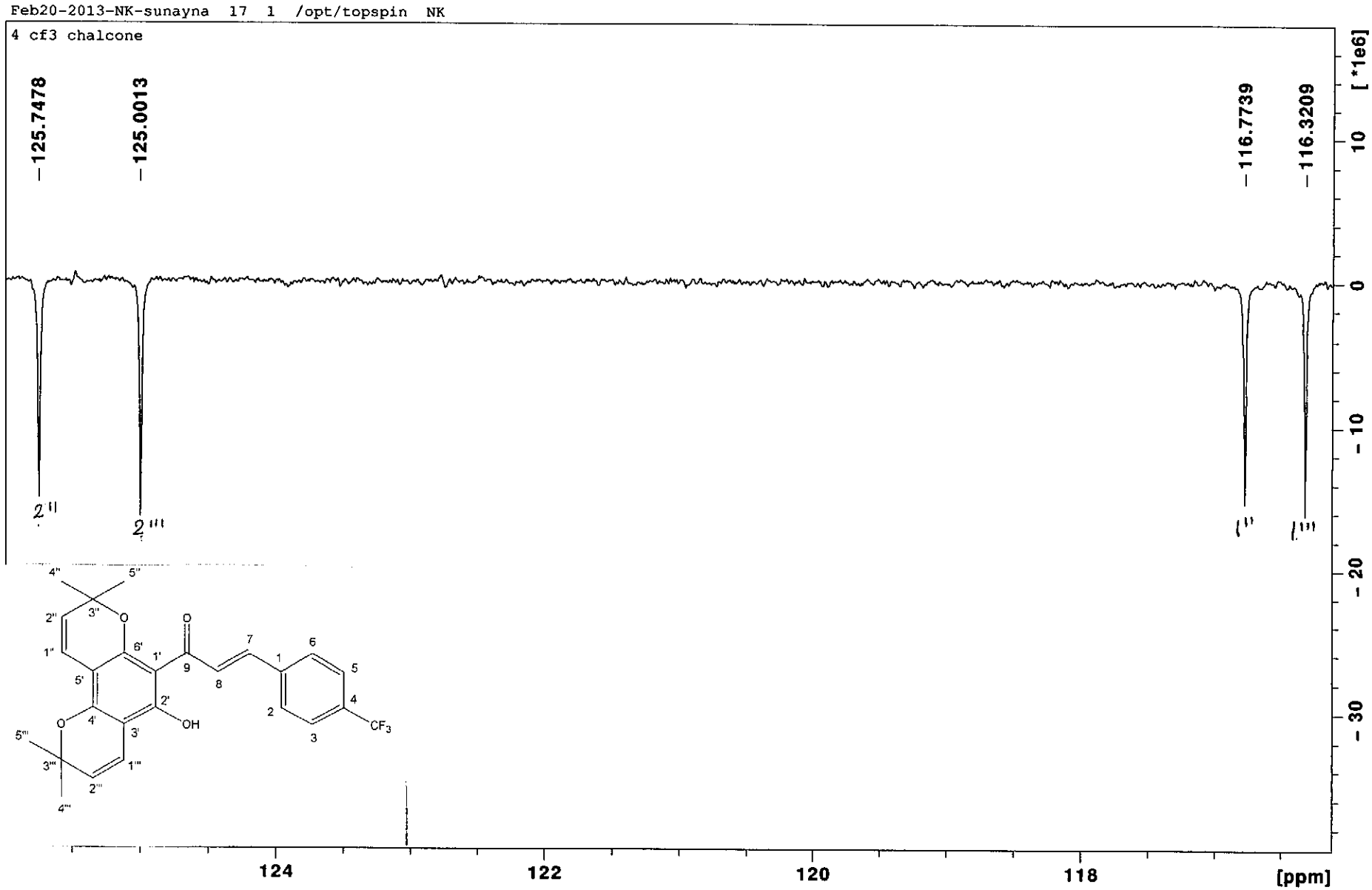
13C NMR spectrum of 4-trifluoromethylpyranochromene chalcone (B-8)



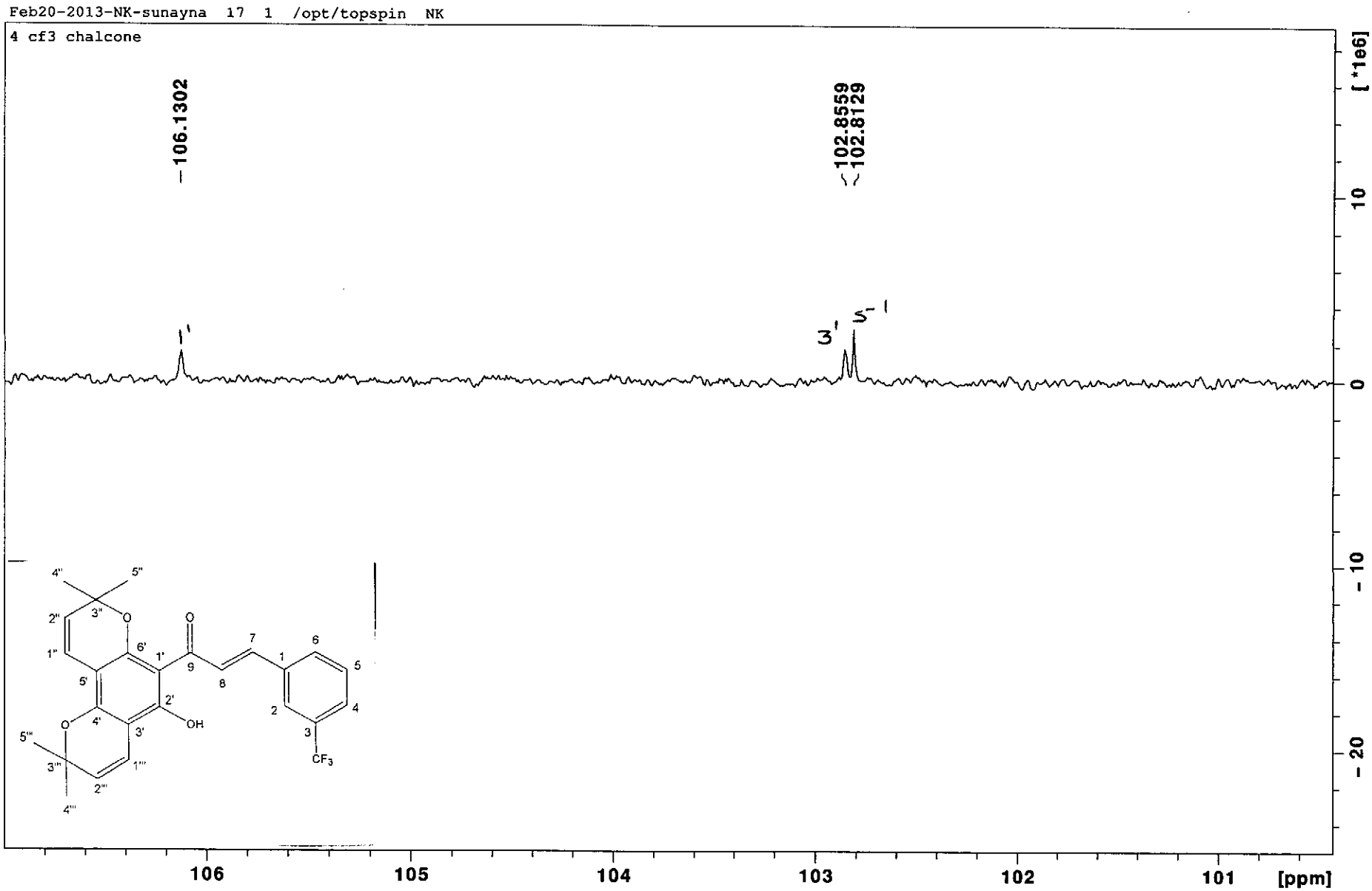
Expanded 13C NMR spectrum of 4-trifluoromethylpyranochromene chalcone (B-8)



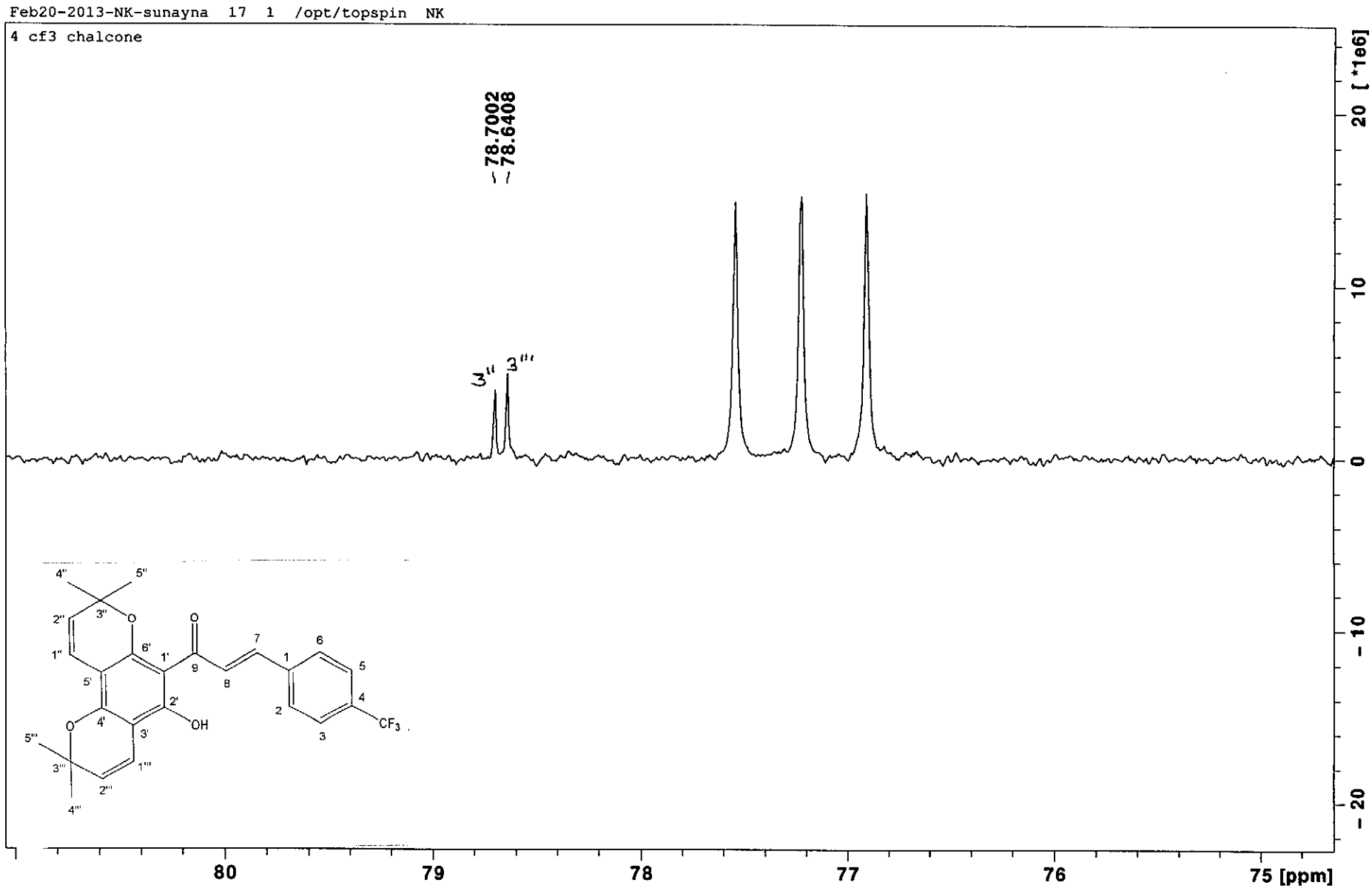
Expanded 13C NMR spectrum of 4-trifluoromethylpyranochromene chalcone (B-8)



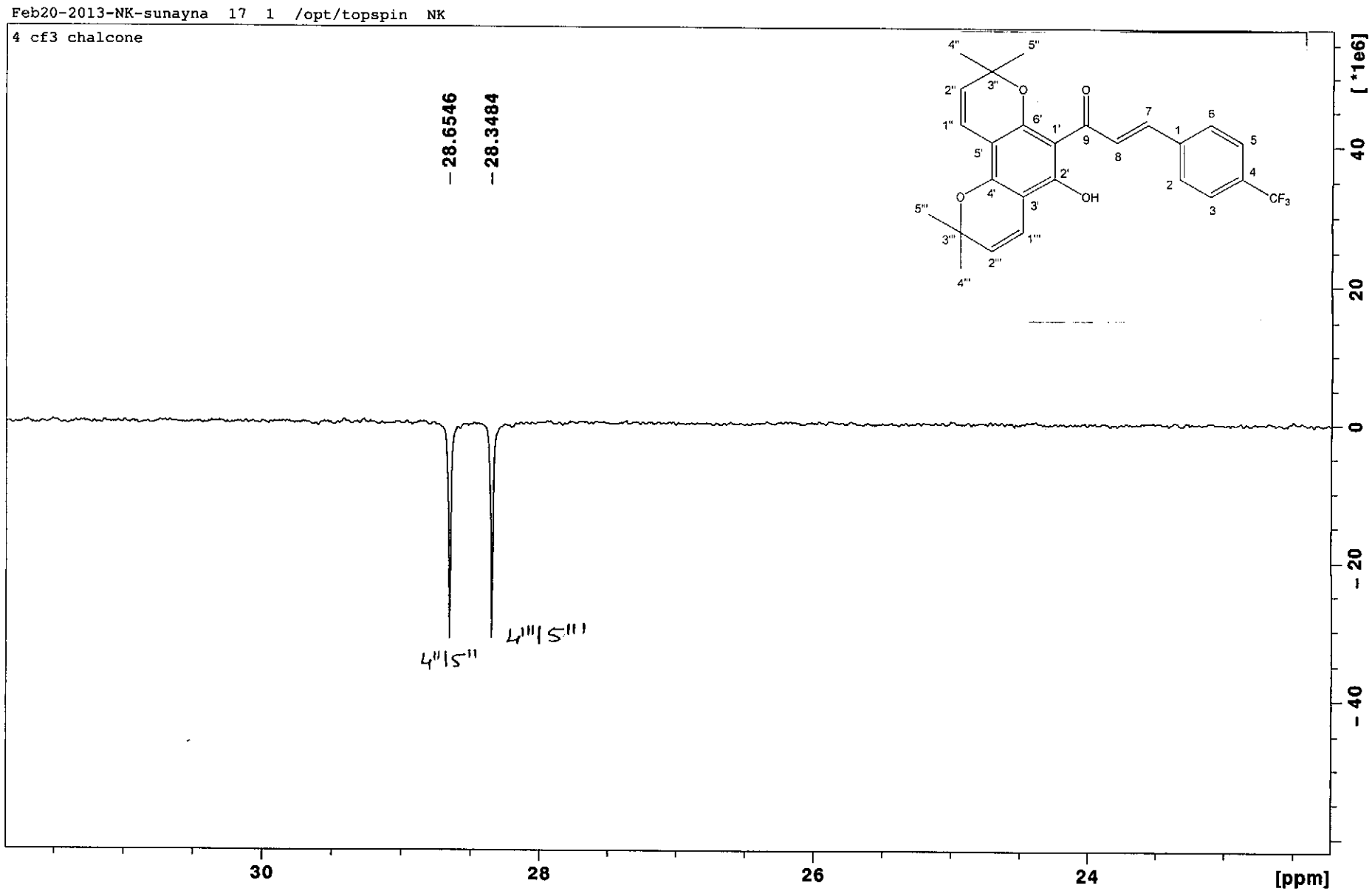
Expanded 13C NMR spectrum of 4-trifluoromethylpyranochromene chalcone (B-8)



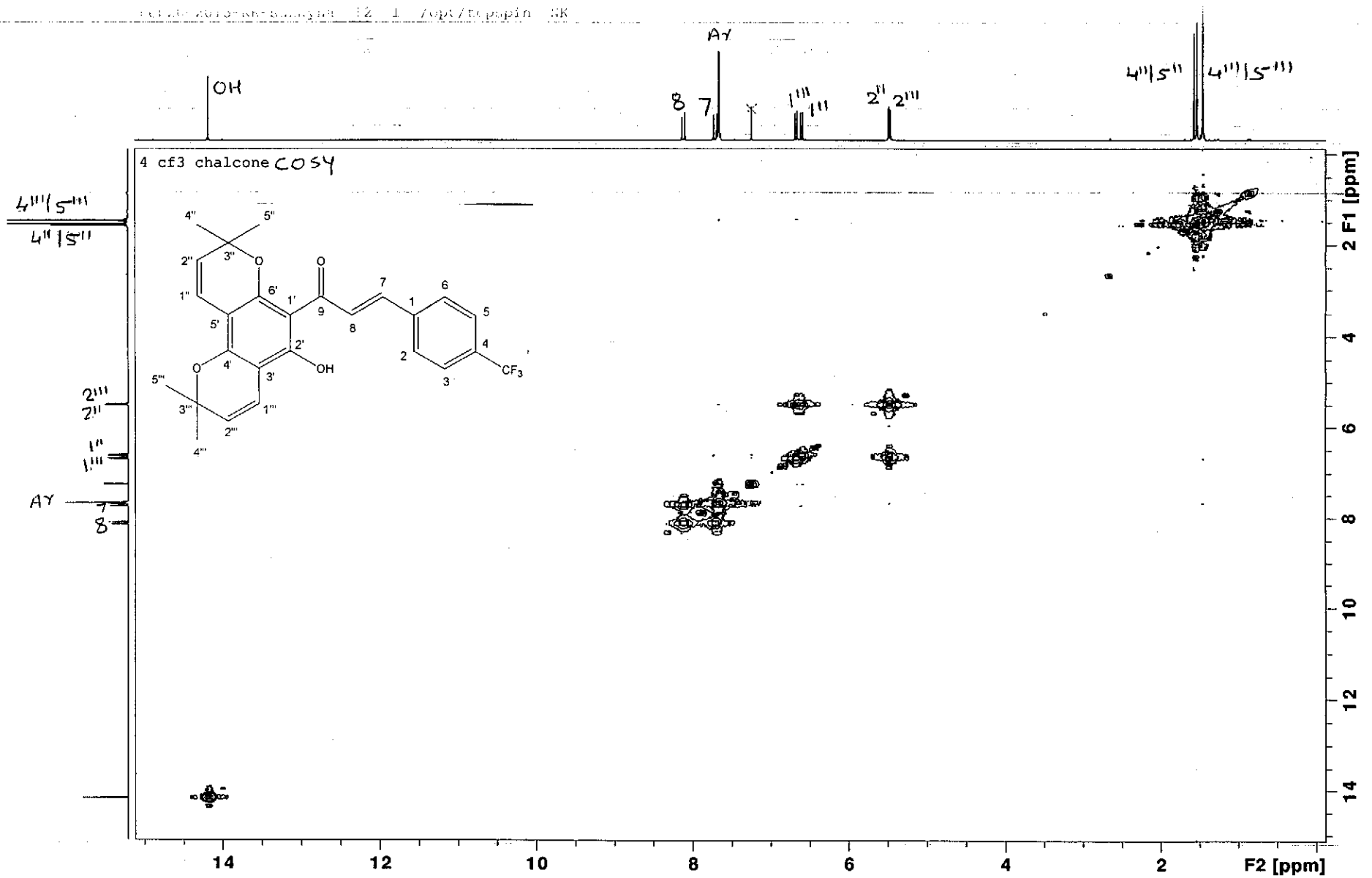
Expanded 13C NMR spectrum of 4-trifluoromethylpyranochromene chalcone (B-8)



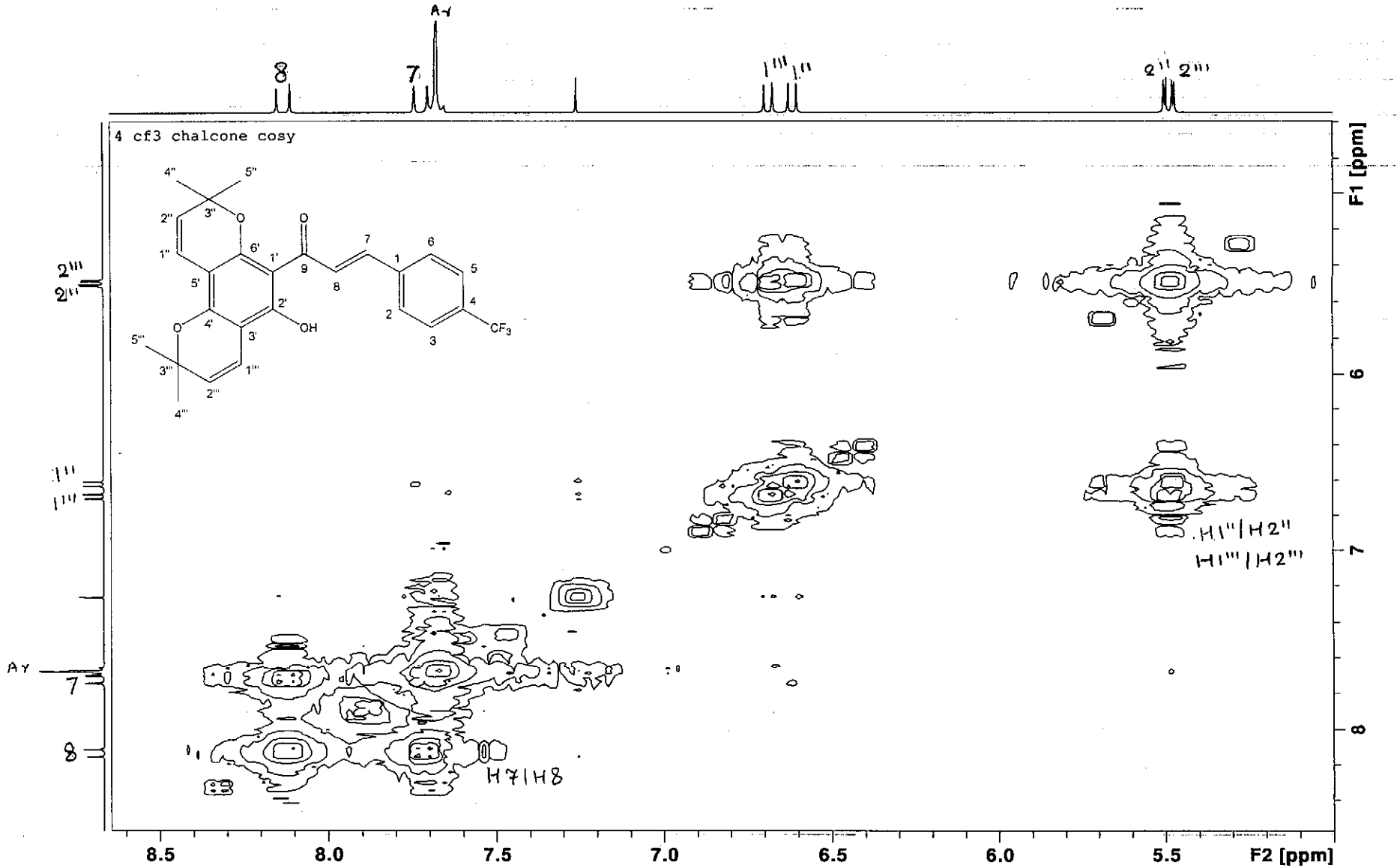
Expanded 13C NMR spectrum of 4-trifluoromethylpyranochromene chalcone (B-8)



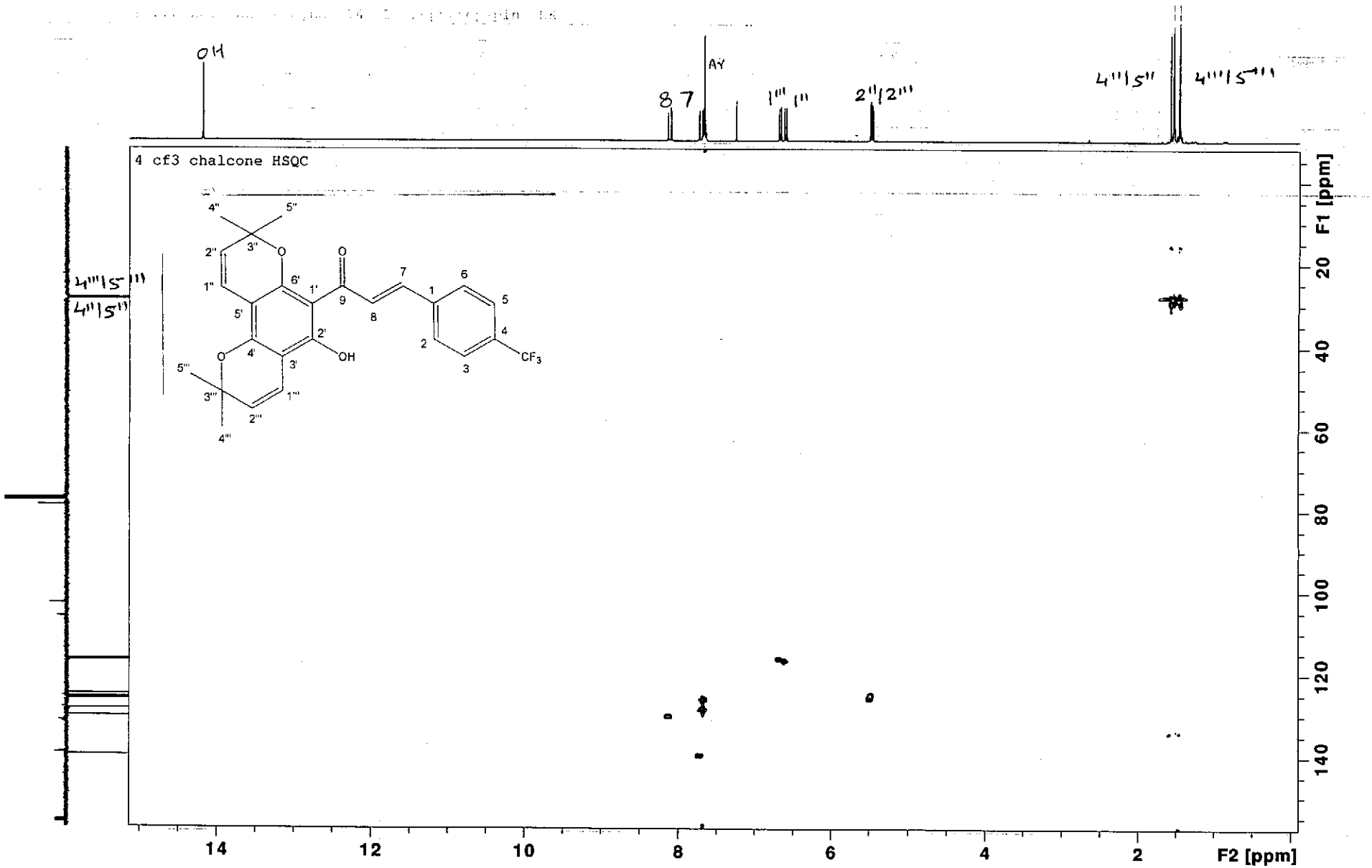
Expanded 13C NMR spectrum of 4-trifluoromethylpyranochromene chalcone (B-8)



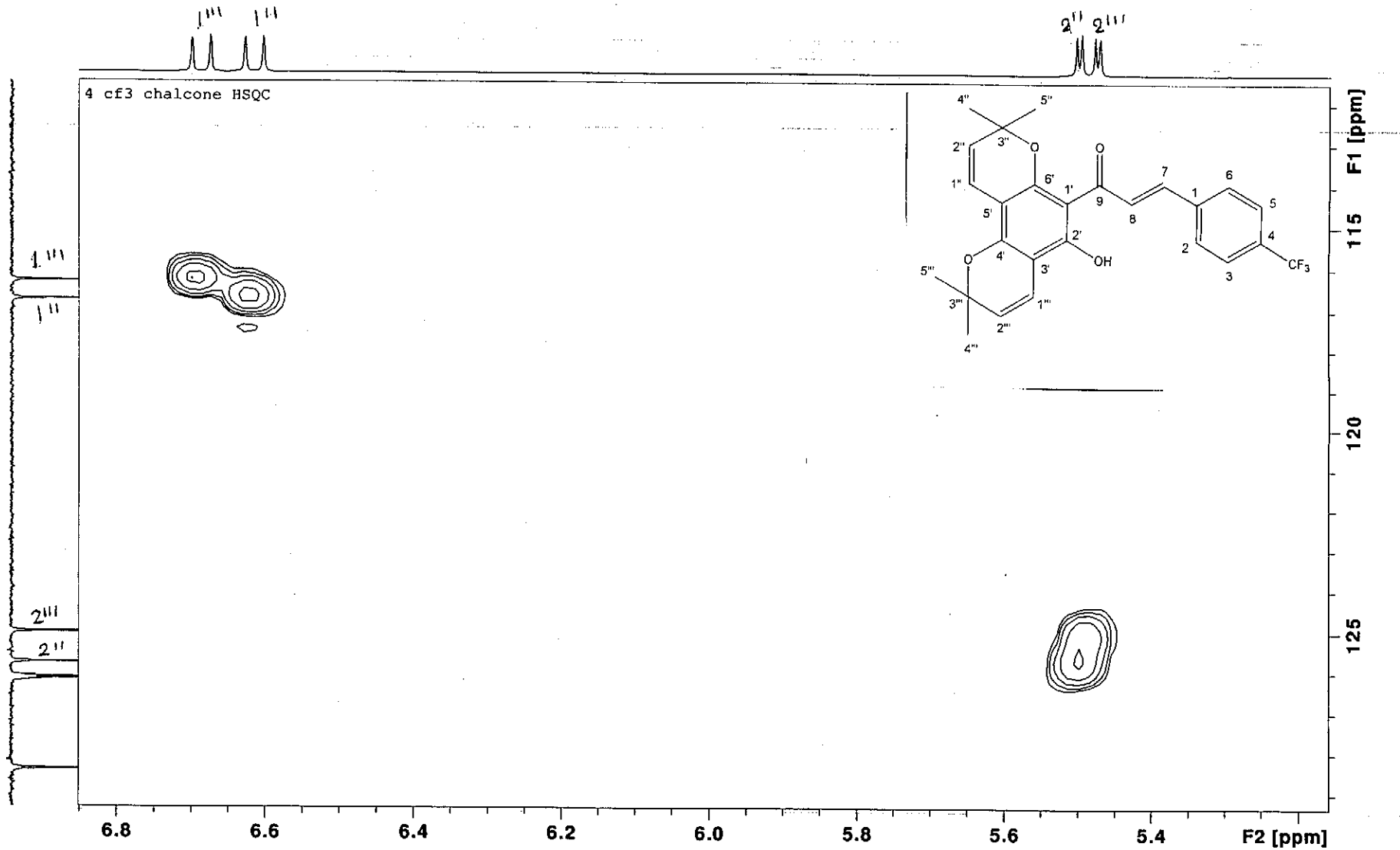
COSY NMR spectrum of 4-trifluoromethylpyranochromene chalcone (B-8)



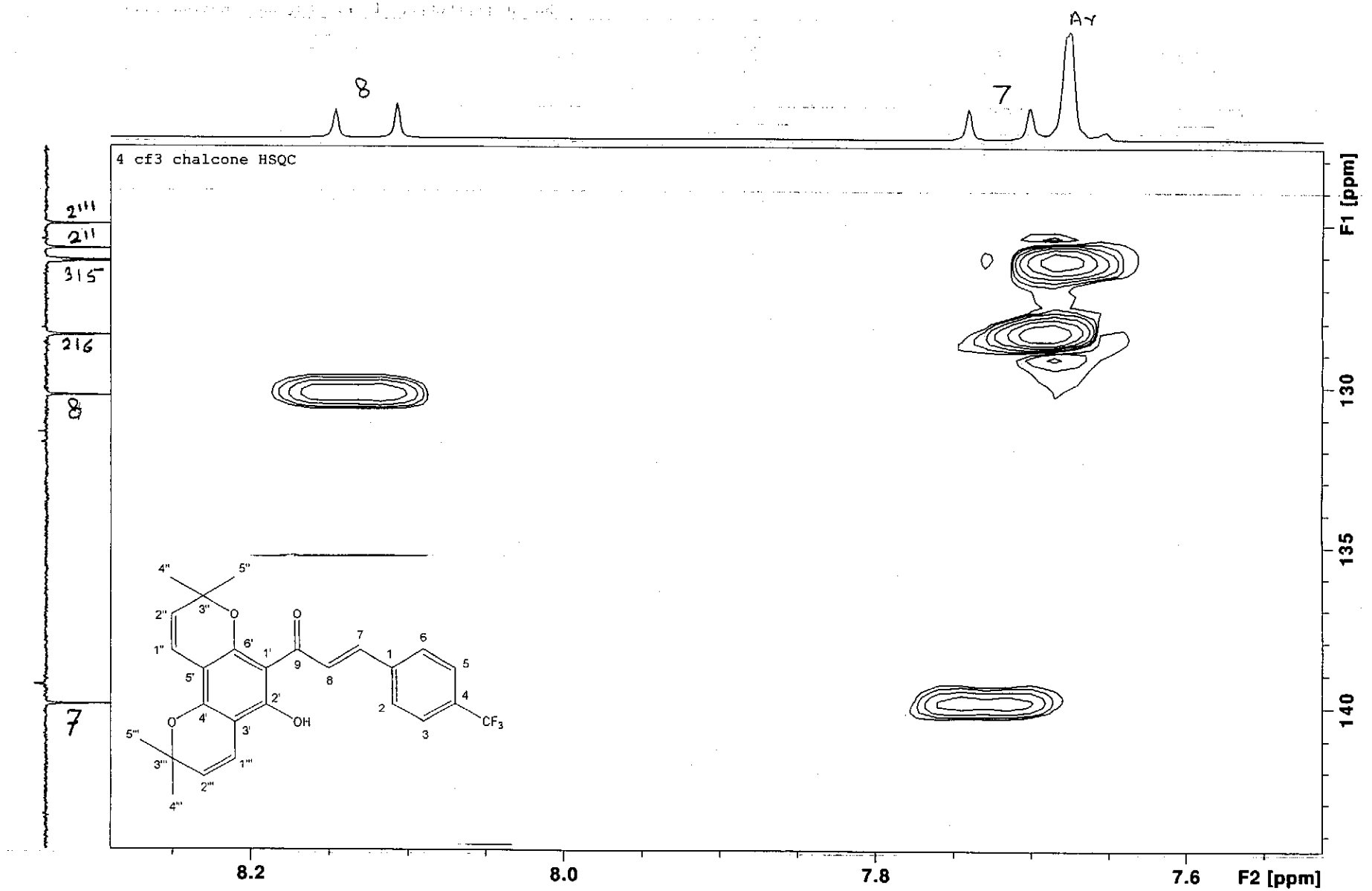
Expanded COSY NMR spectrum of 4-trifluoromethylpyranochromene chalcone (B-8)



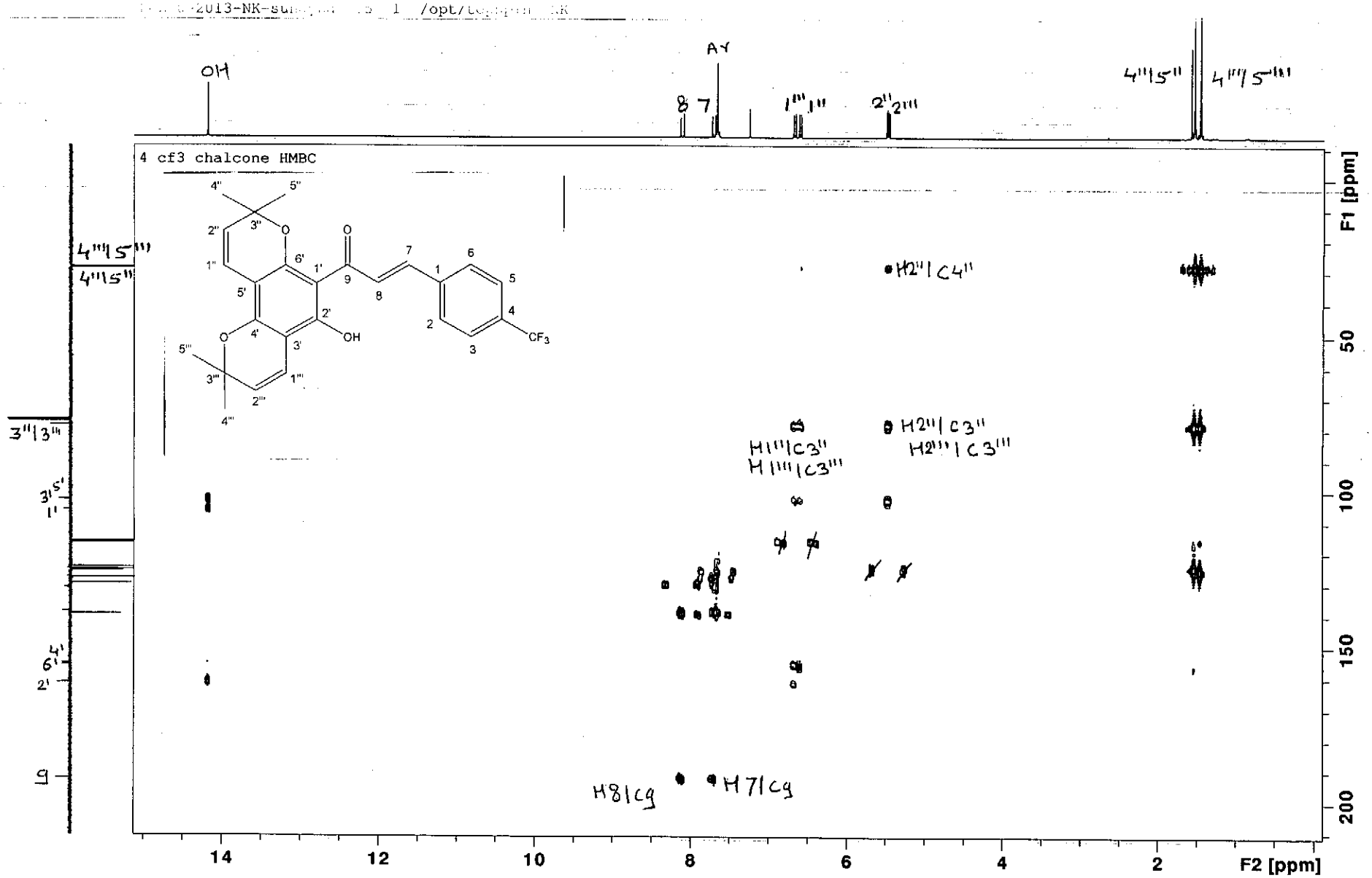
HSQC spectrum of 4-trifluoromethylpyranochromene chalcone (B-8)



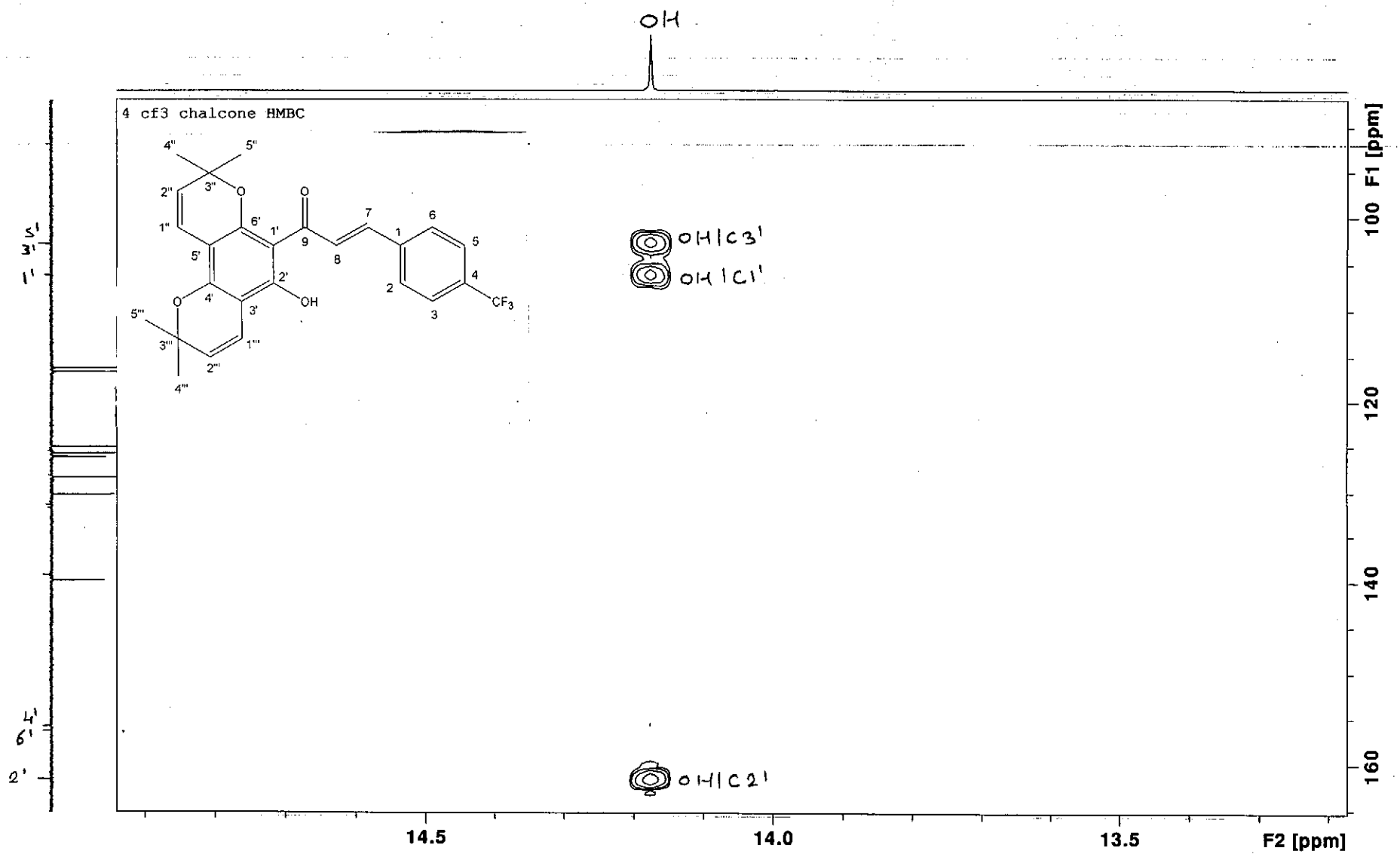
Expanded HSQC spectrum of 4-trifluoromethylpyranochromene chalcone (B-8)



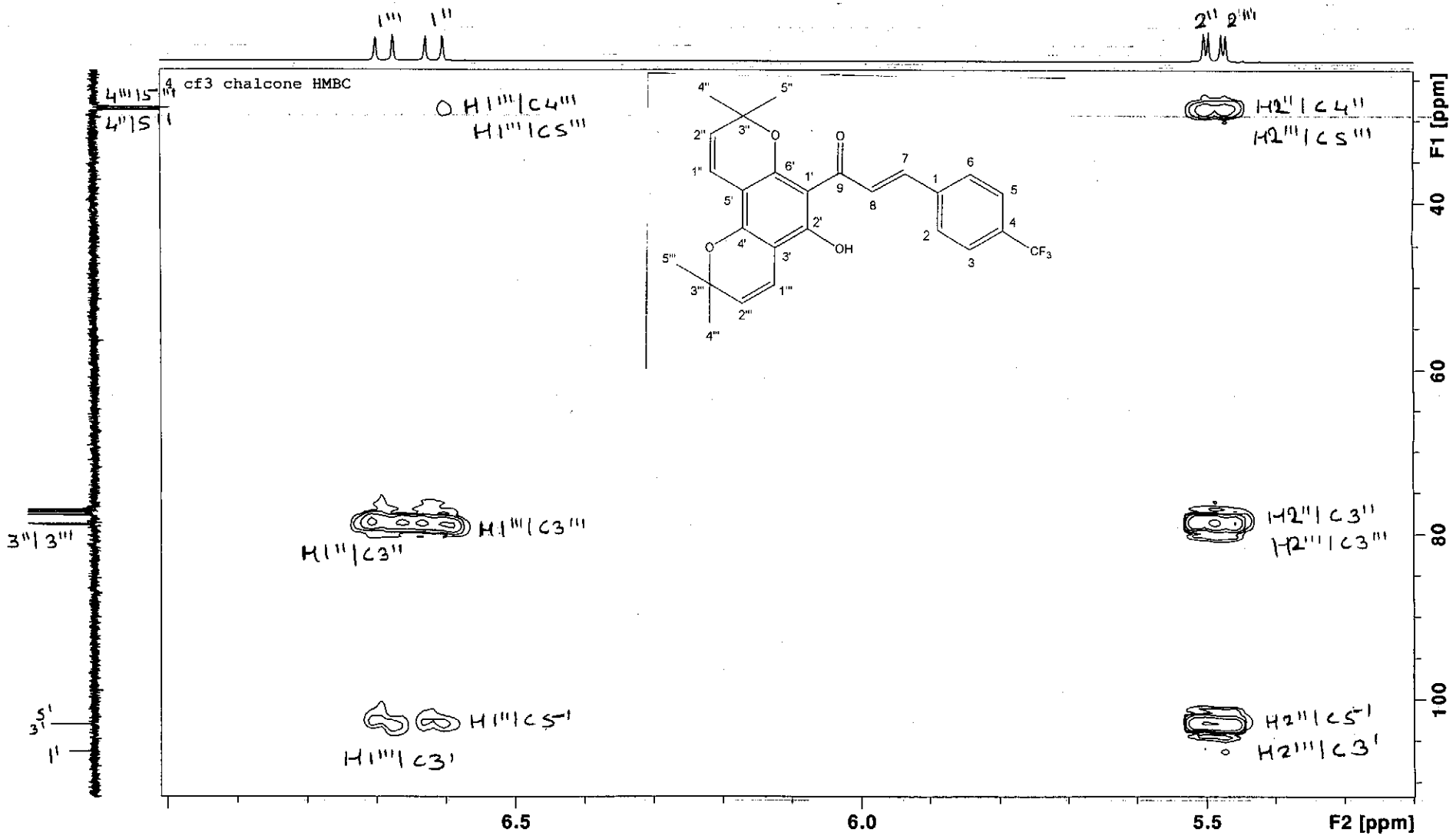
Expanded HSQC spectrum of 4-trifluoromethylpyranochromene chalcone (B-8)



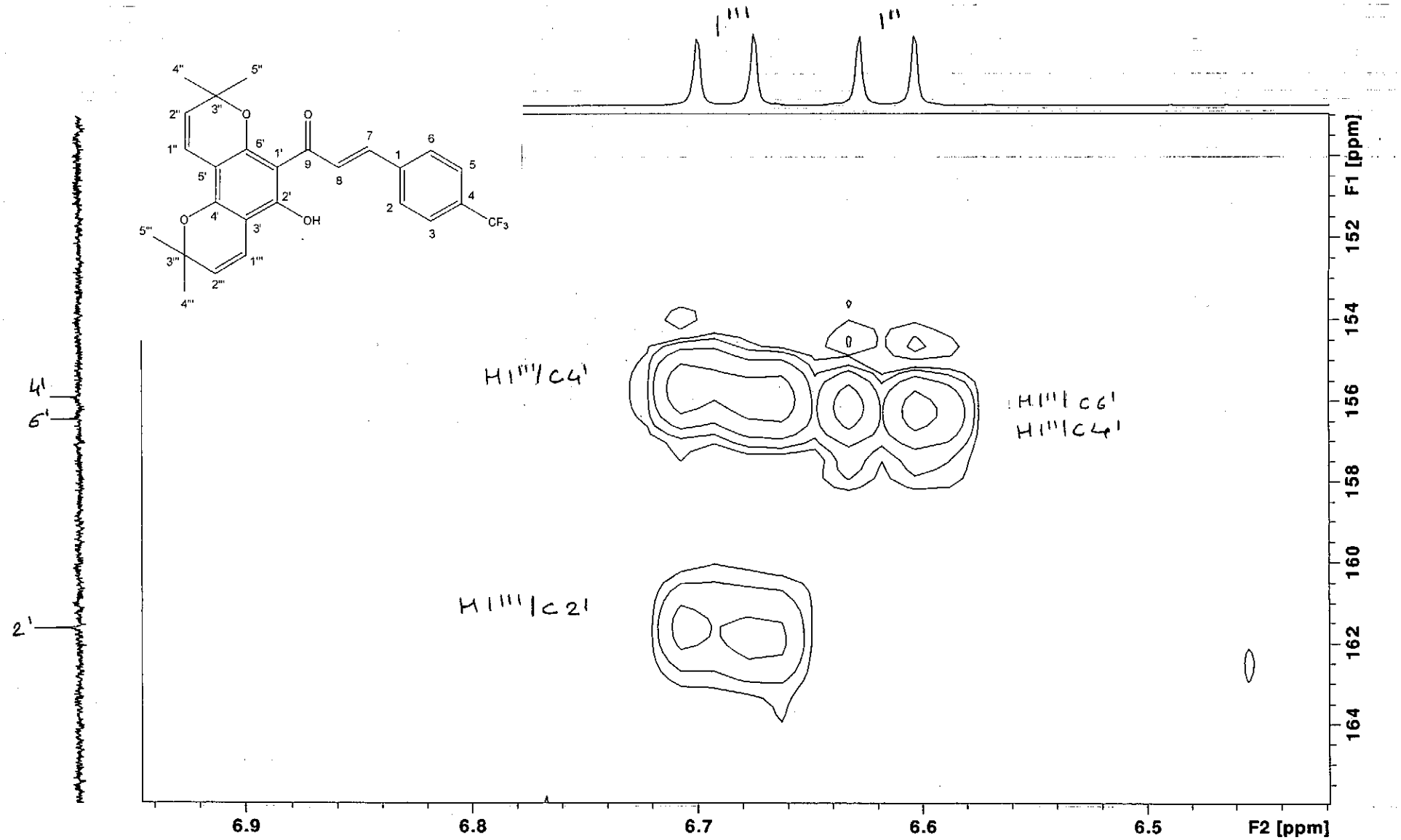
HMBC spectrum of 4-trifluoromethylpyranochromene chalcone (B-8)



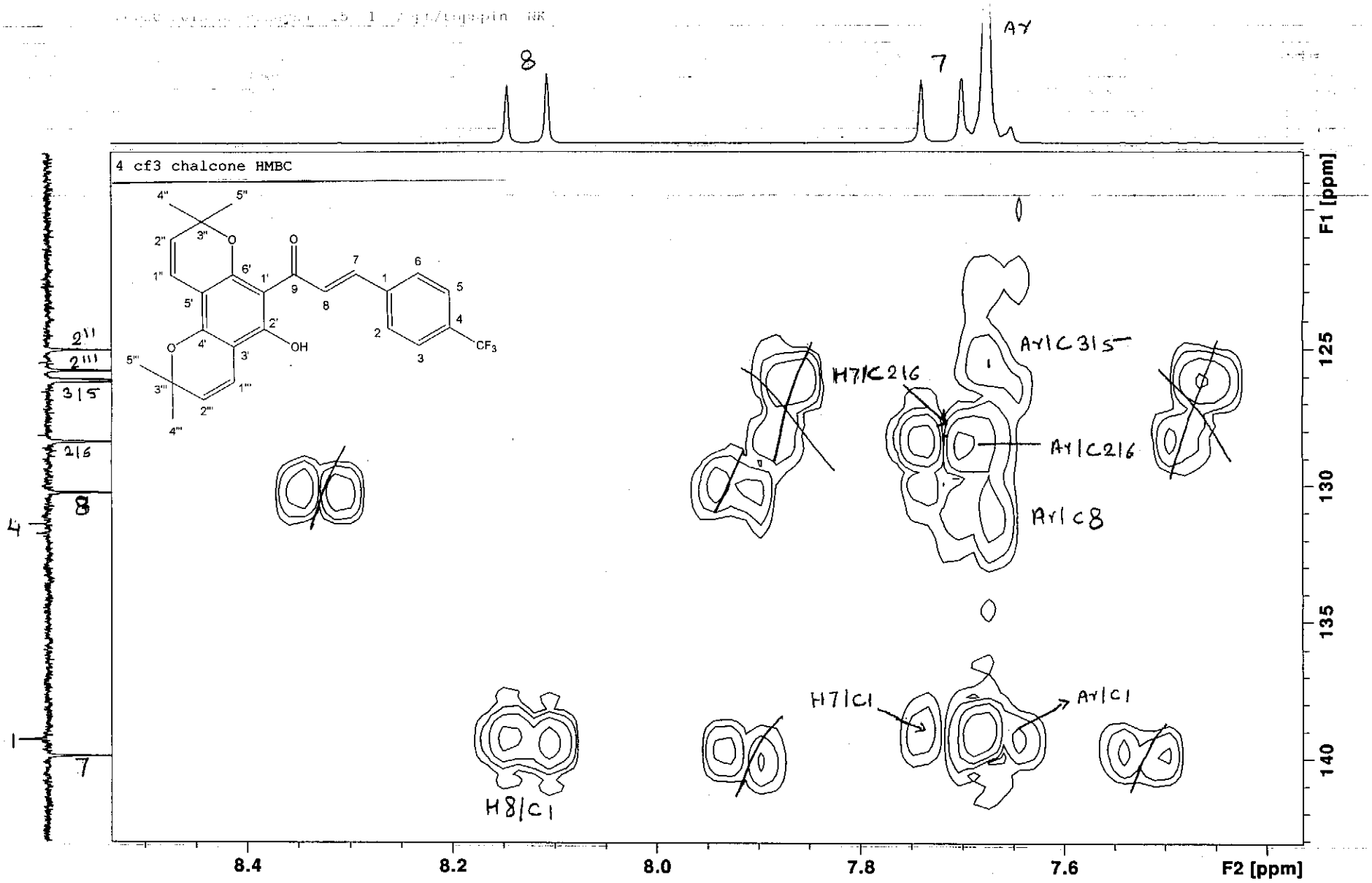
Expanded HMBC spectrum of 4-trifluoromethylpyranochromene chalcone (B-8)



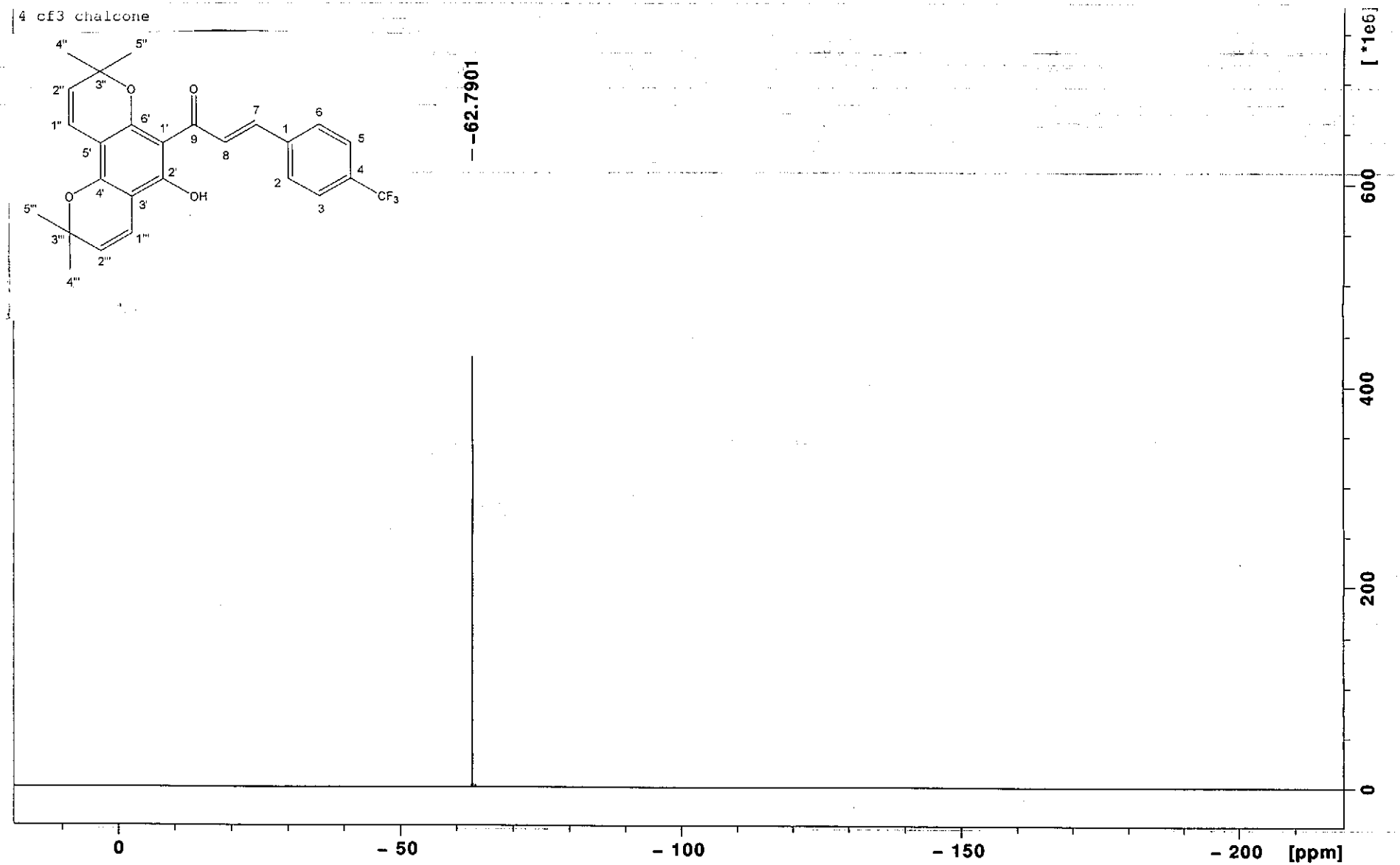
Expanded HMBC spectrum of 4-trifluoromethylpyranochromene chalcone (B-8)



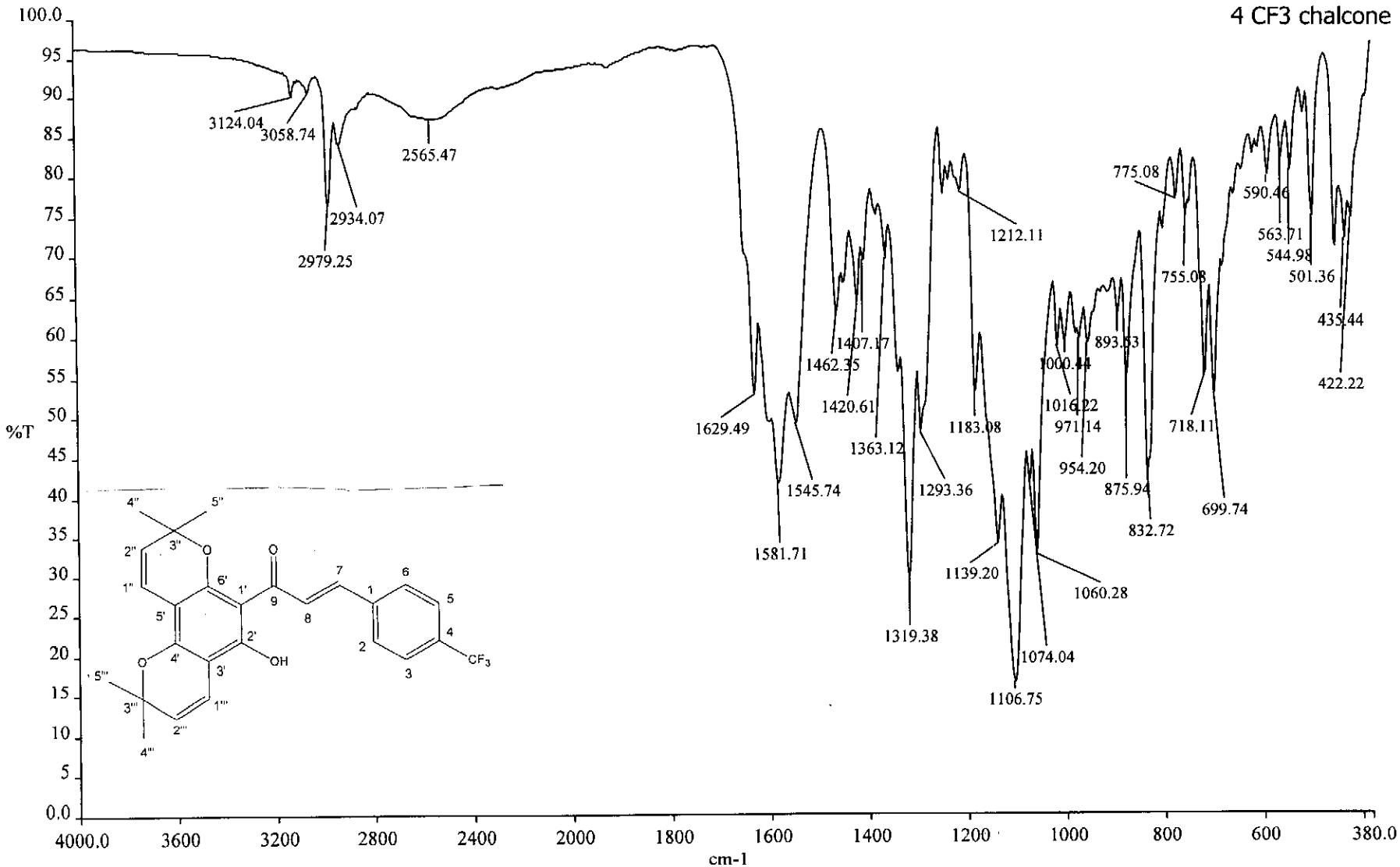
Expanded HMBC spectrum of 4-trifluoromethylpyranochromene chalcone (B-8)



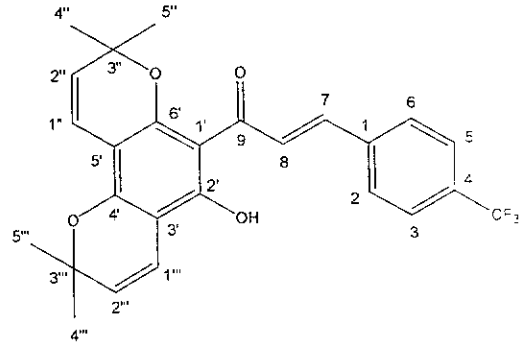
Expanded HMBC spectrum of 4-trifluoromethylpyranochromene chalcone (B-8)



19F NMR spectrum of 4-trifluoromethylpyranochromene chalcone (B-8)



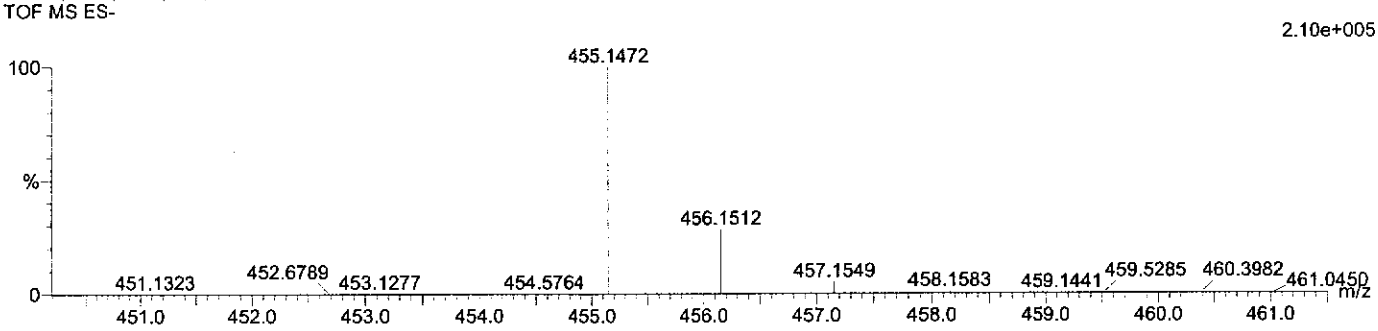
Infrared spectrum of 4-trifluoromethylpyranochromene chalcone (B-8)



Elemental Composition Report

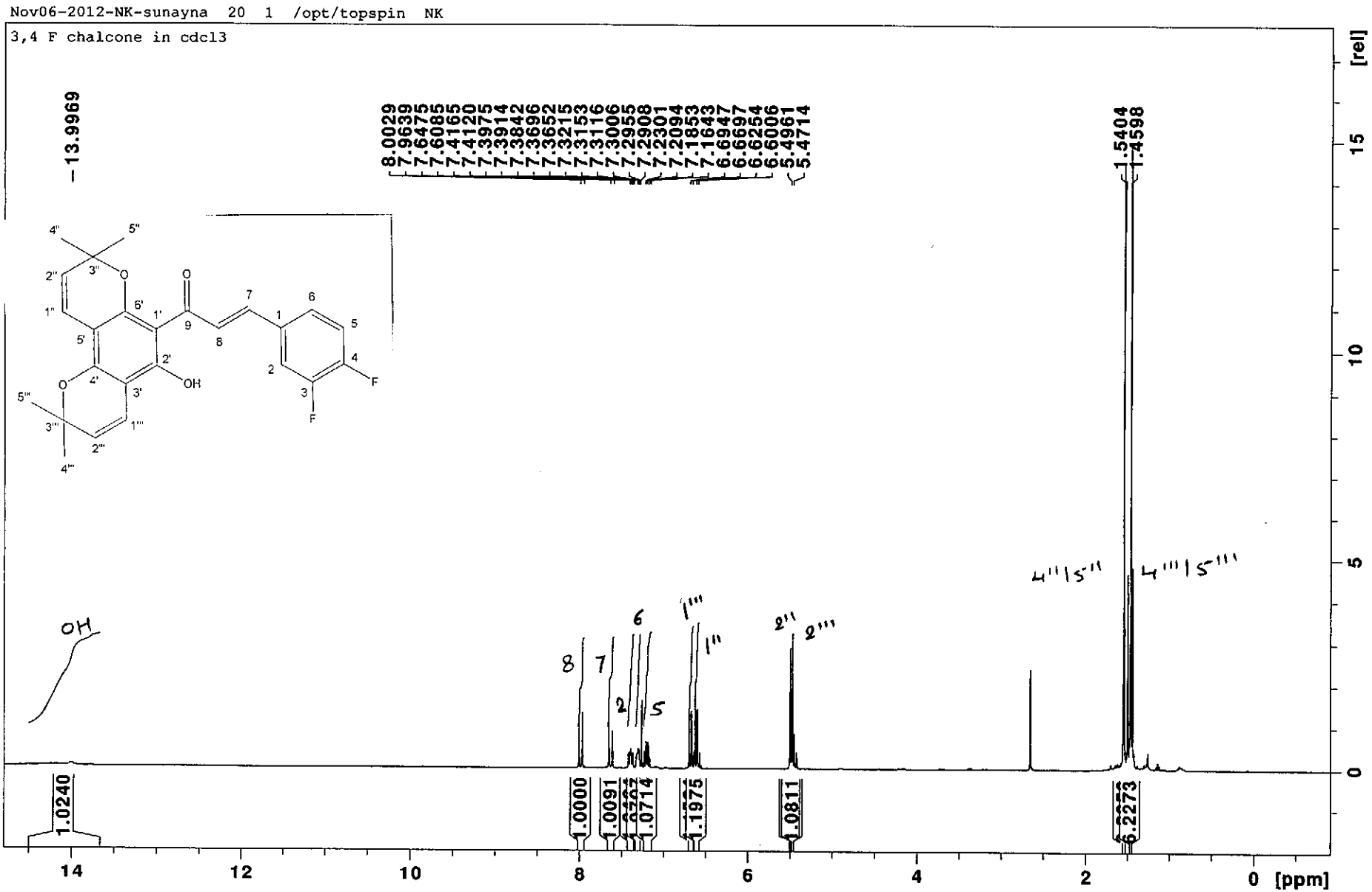
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
25 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-35 H: 20-30 N: 0-4 O: 0-4 F: 3-3
26 10 (0.153) Cm (1:31)
TOF MS ES-

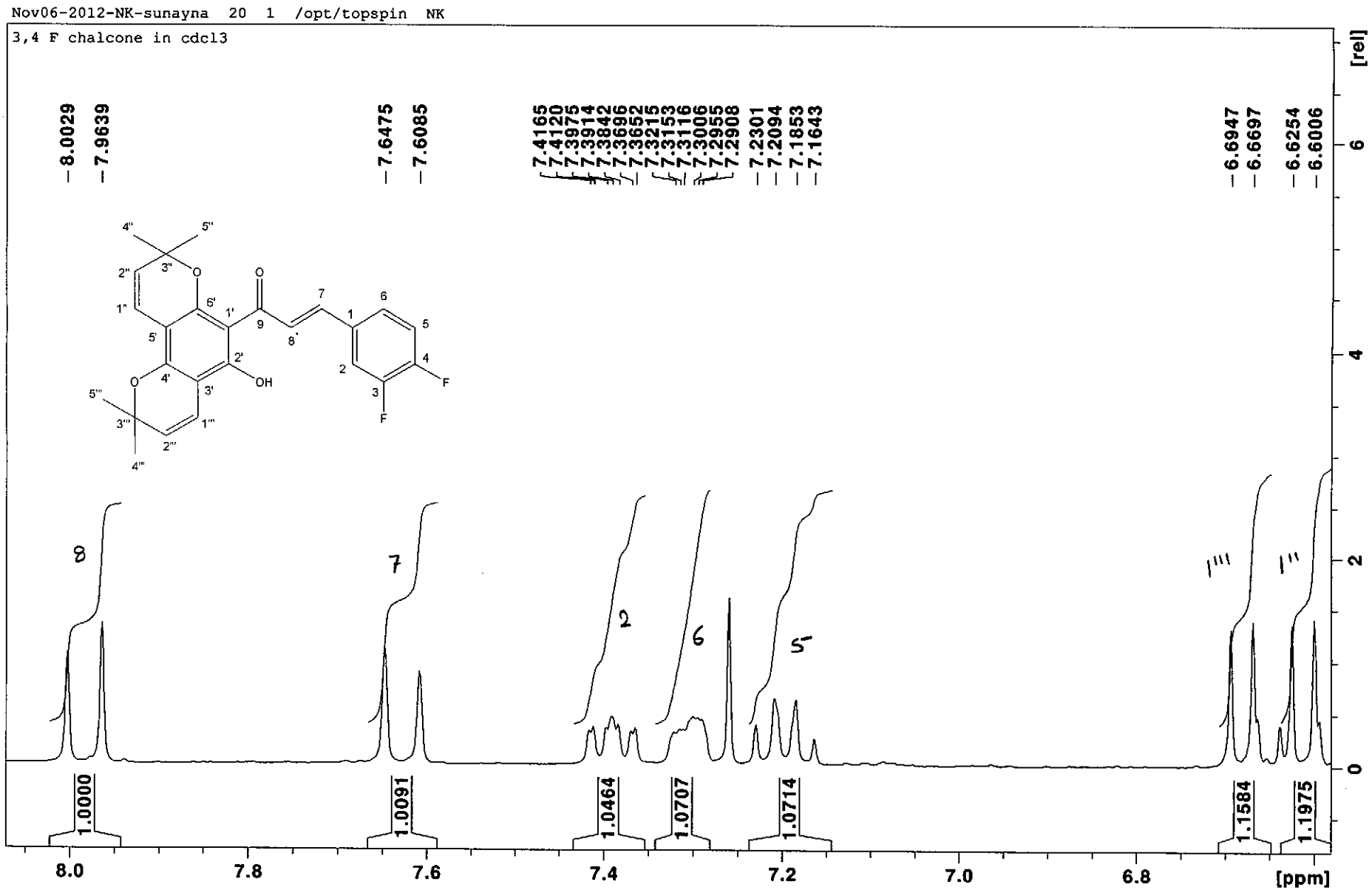


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
455.1472	455.1470	0.2	0.4	14.5	508.3	0.0	C26 H22 O4 F3

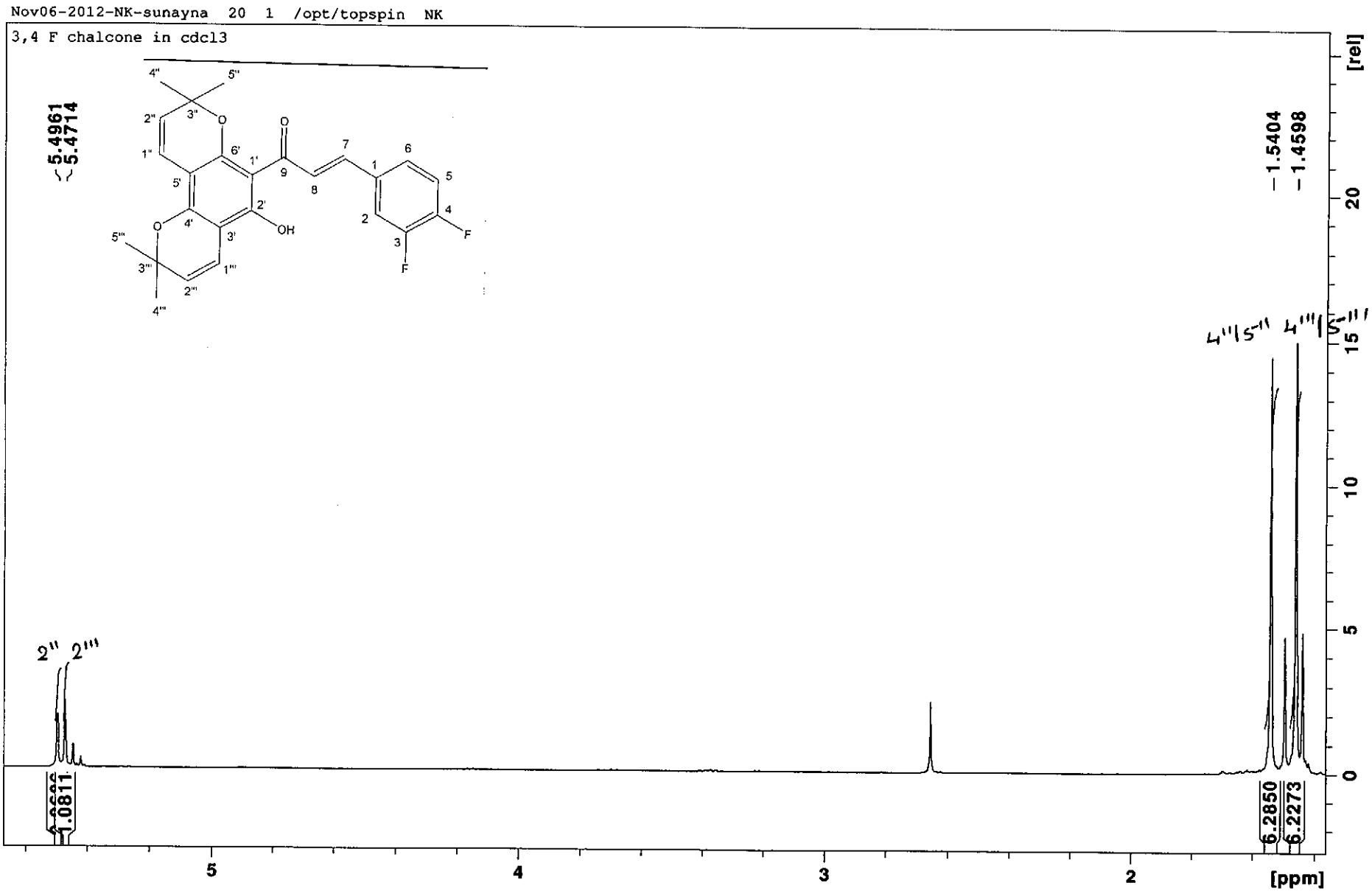
HRMS spectrum of 4-trifluoromethylpyranochromene chalcone (B-8)



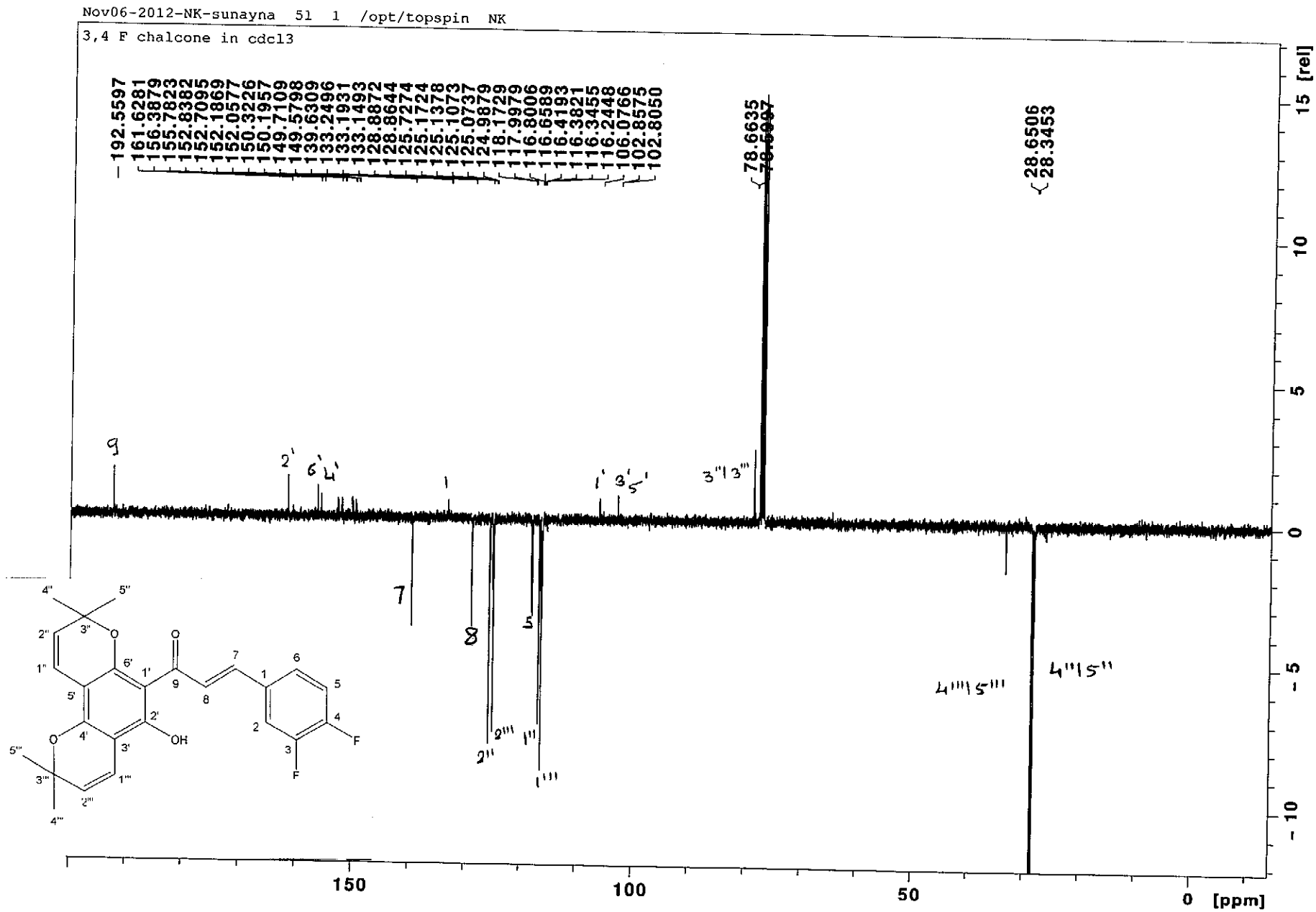
1H NMR spectrum of 3,4-difluoropyranochromene chalcone (B-9)



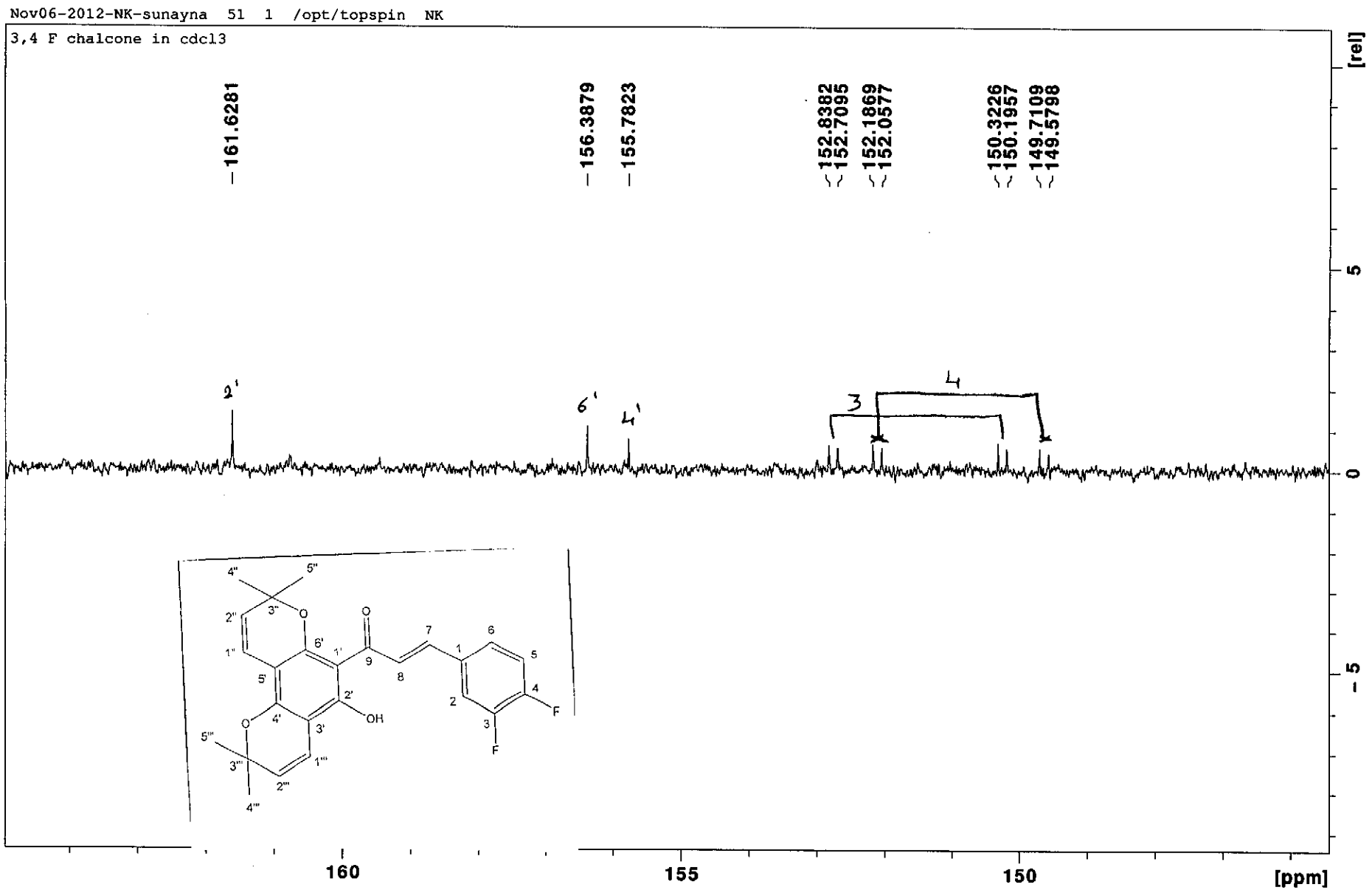
Expanded 1H NMR spectrum of 3,4-difluoropyranochromene chalcone (B-9)



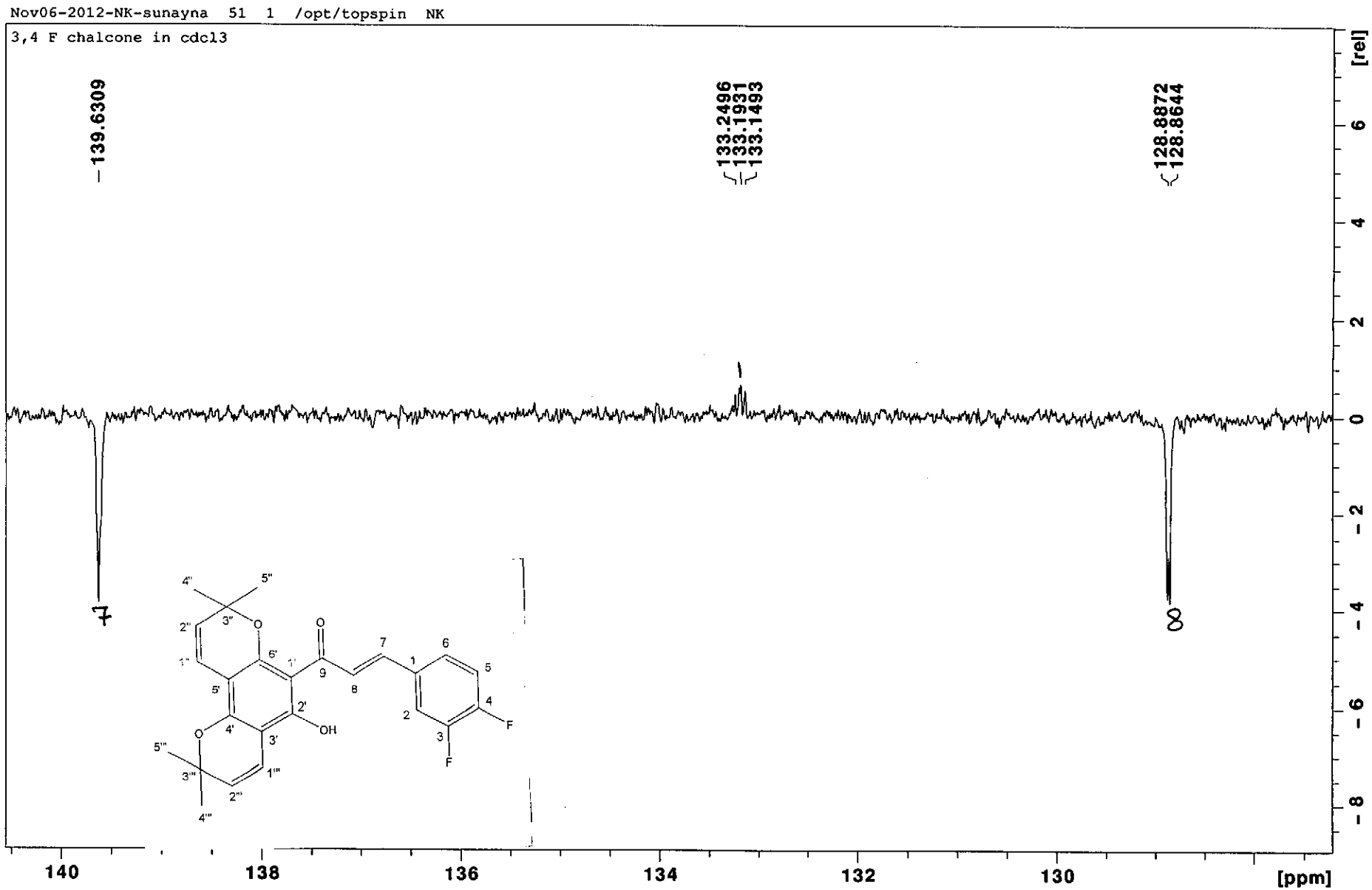
Expanded 1H NMR spectrum of 3,4-difluoropyranochromene chalcone (B-9)



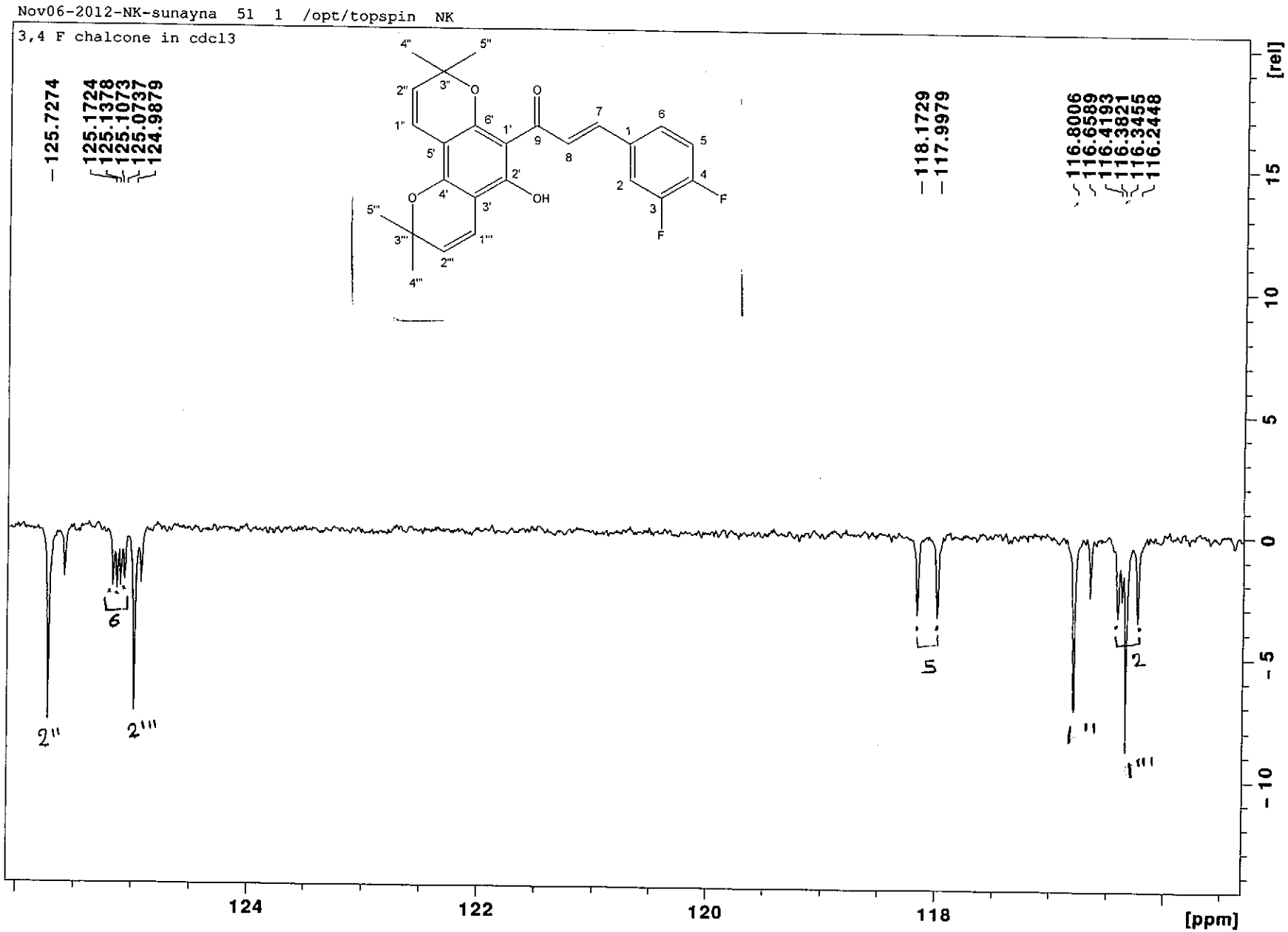
13C NMR spectrum of 3,4-difluoropyranochromene chalcone (B-9)



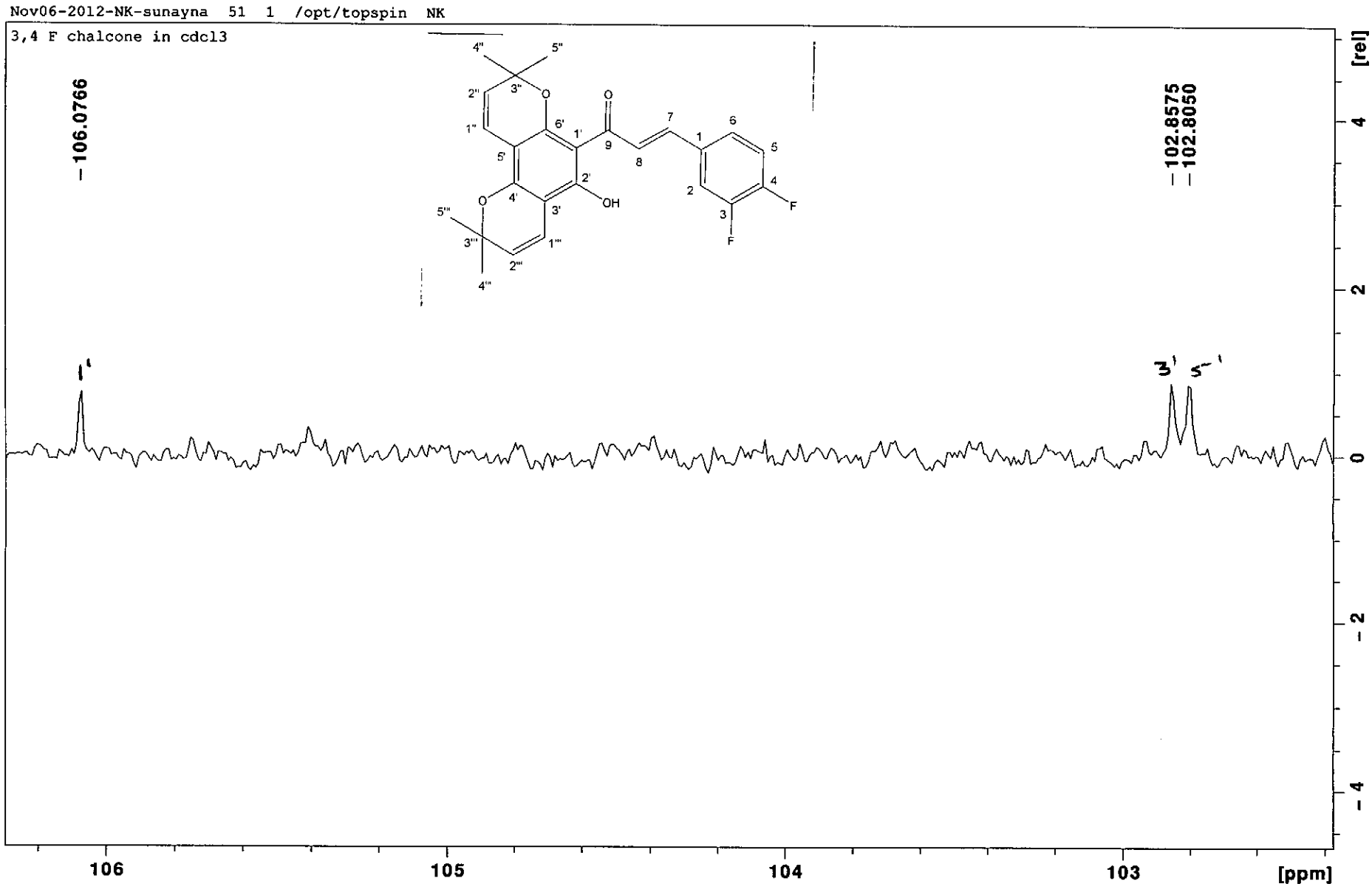
Expanded 13C NMR spectrum of 3,4-difluoropyranochromene chalcone (B-9)



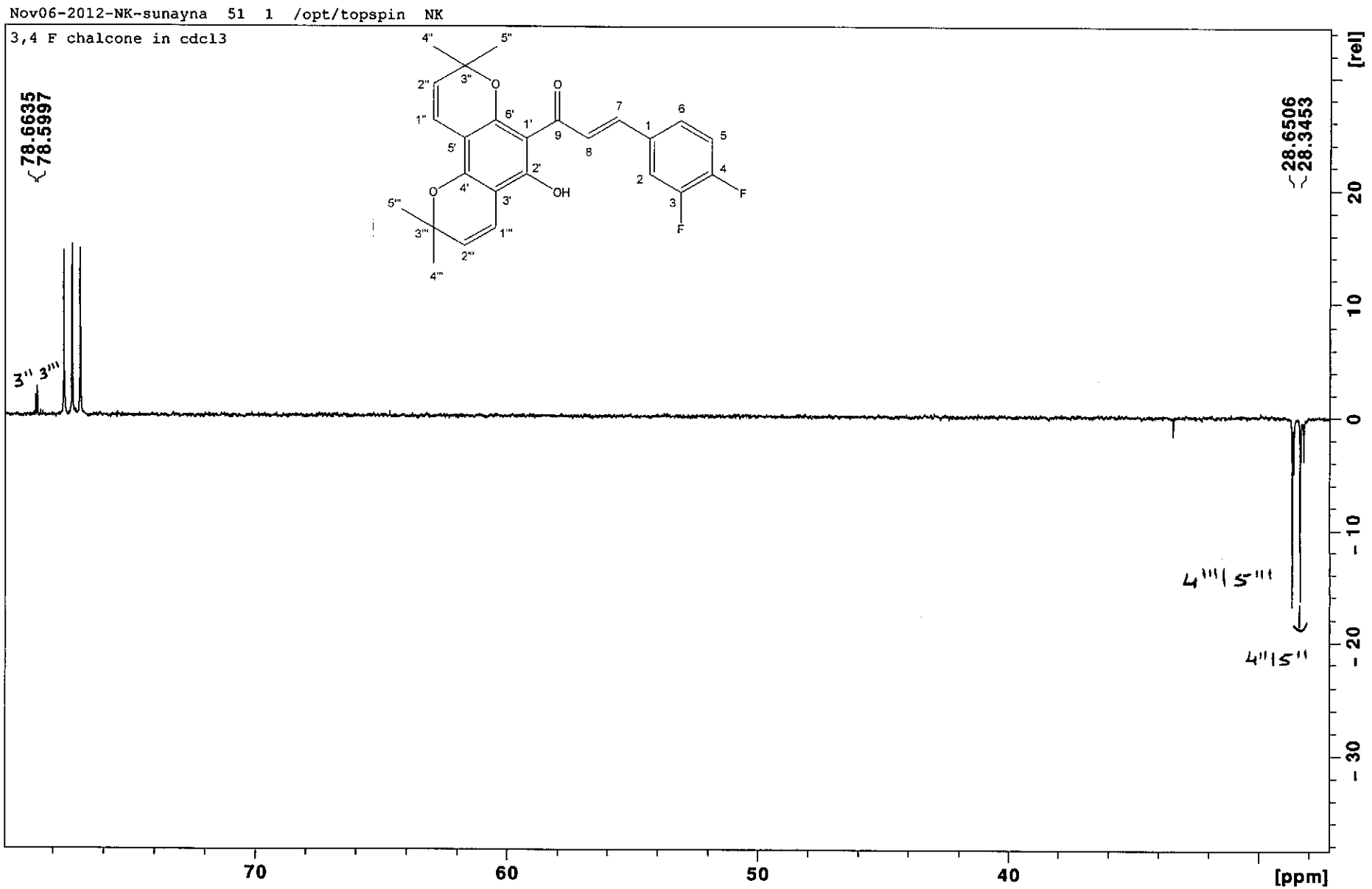
Expanded 13C NMR spectrum of 3,4-difluoropyranochromene chalcone (B-9)



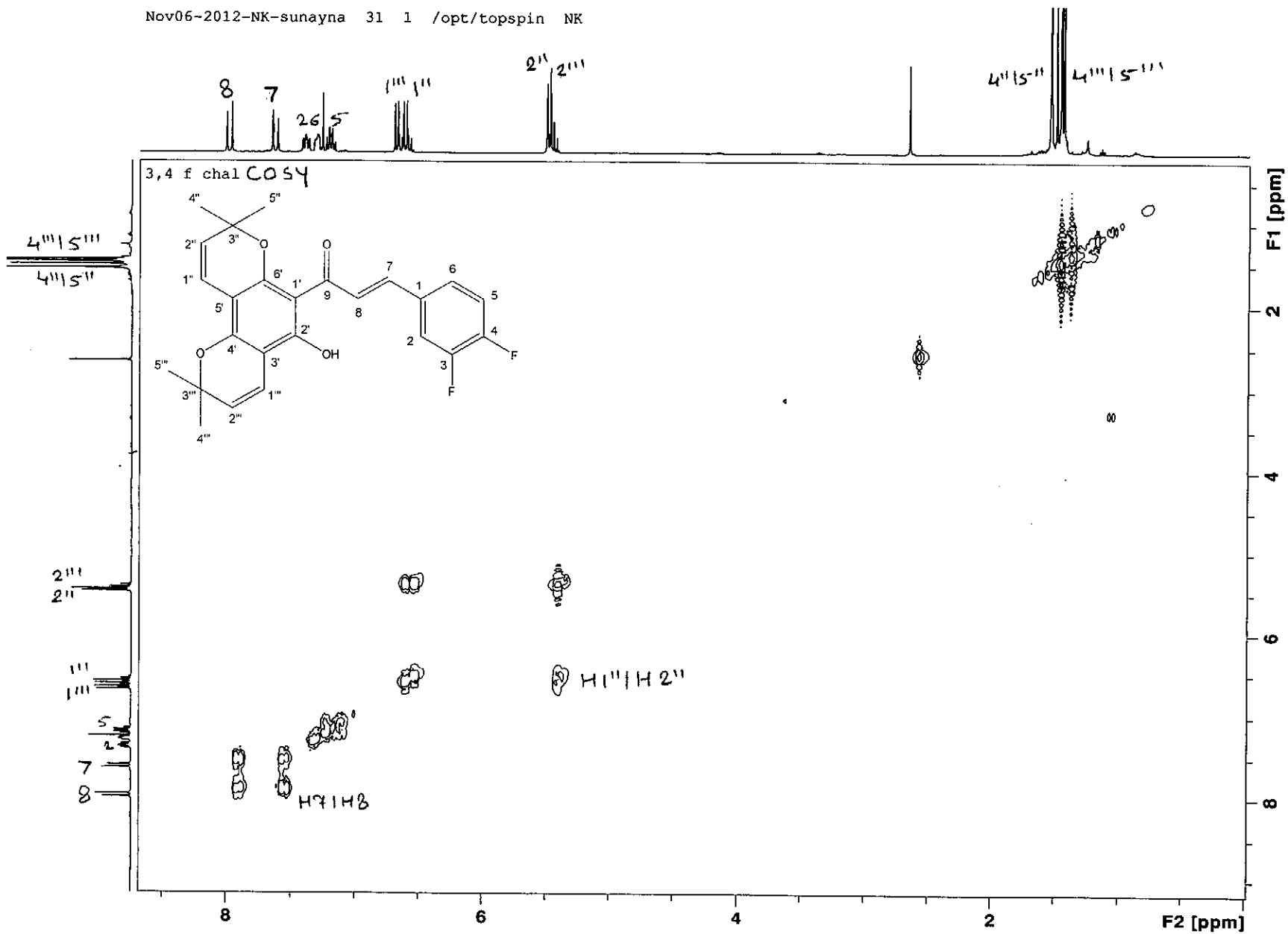
Expanded 13C NMR spectrum of 3,4-difluoropyranochromene chalcone (B-9)



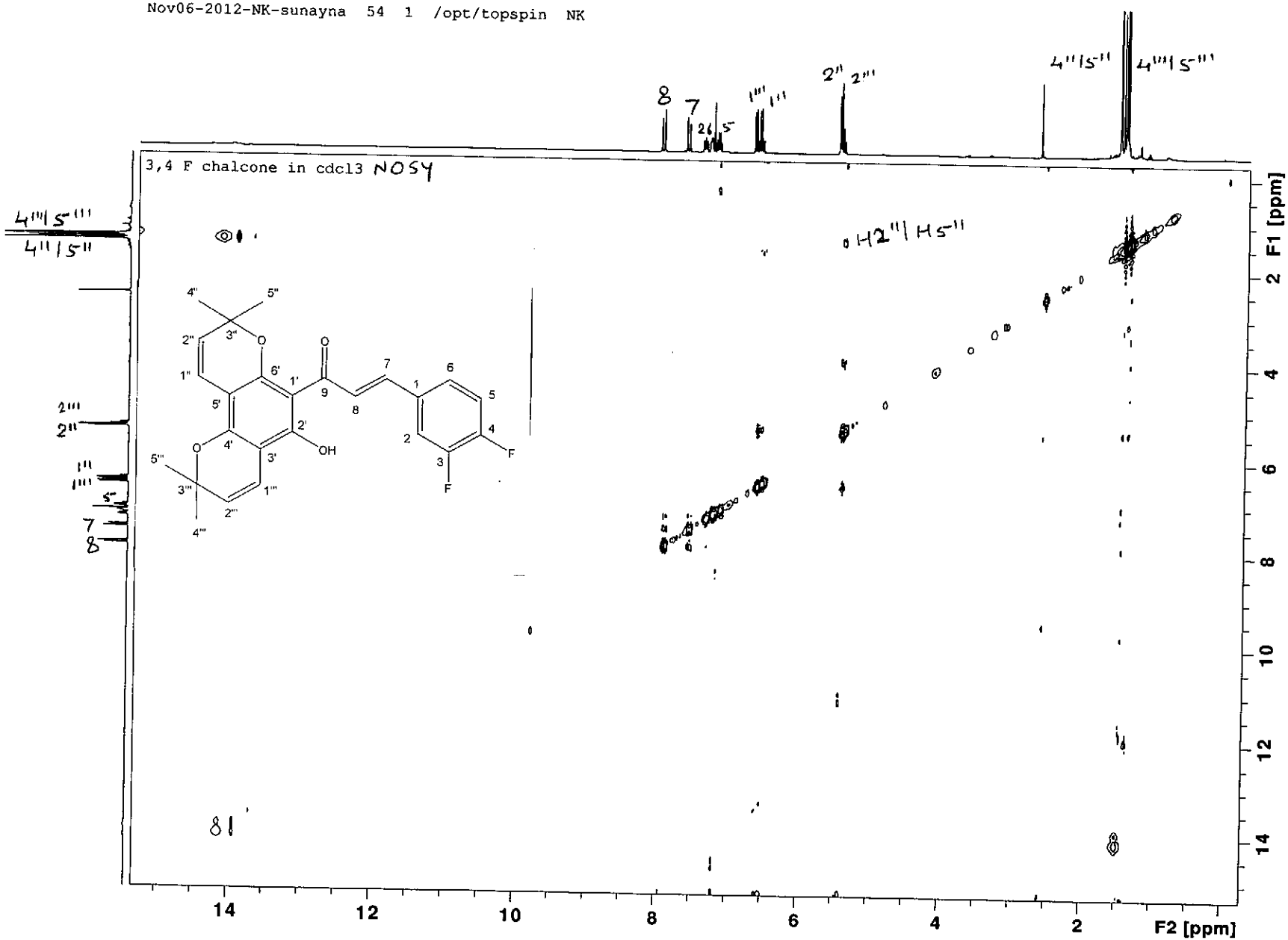
Expanded 13C NMR spectrum of 3,4-difluoropyranochromene chalcone (B-9)



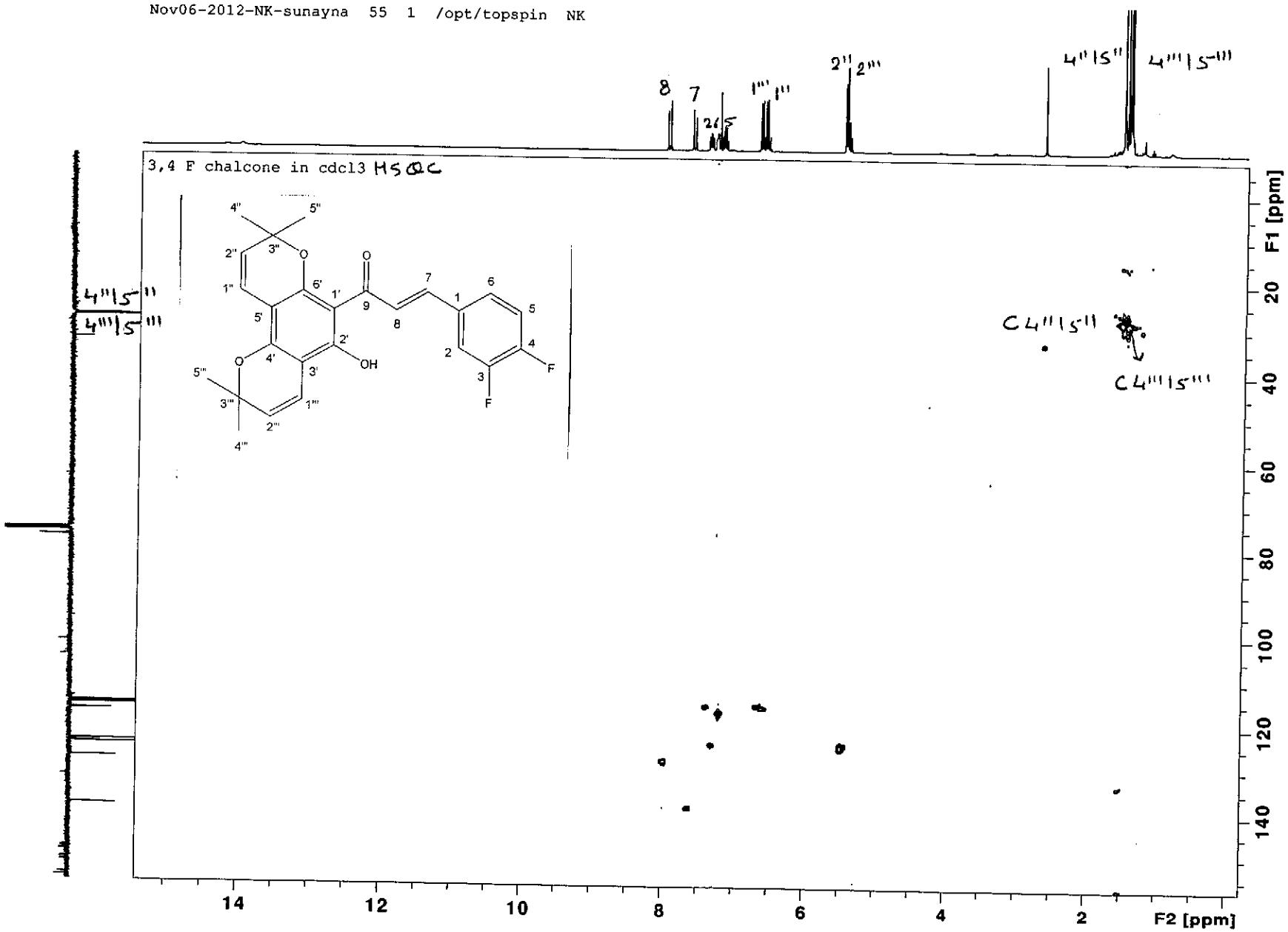
Expanded 13C NMR spectrum of 3,4-difluoropyranochromene chalcone (B-9)



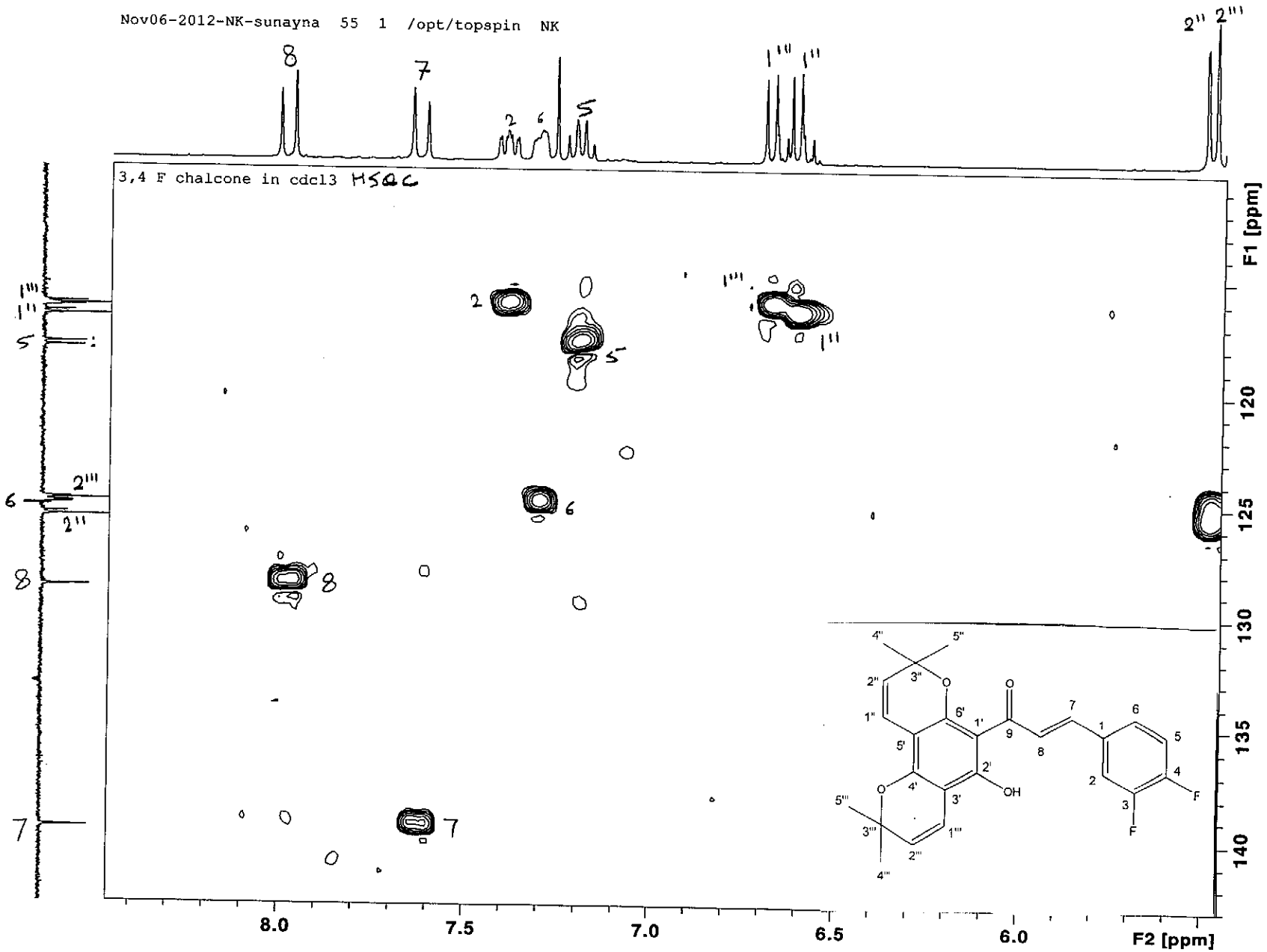
COSY NMR spectrum of 3,4-difluoropyranochromene chalcone (B-9)



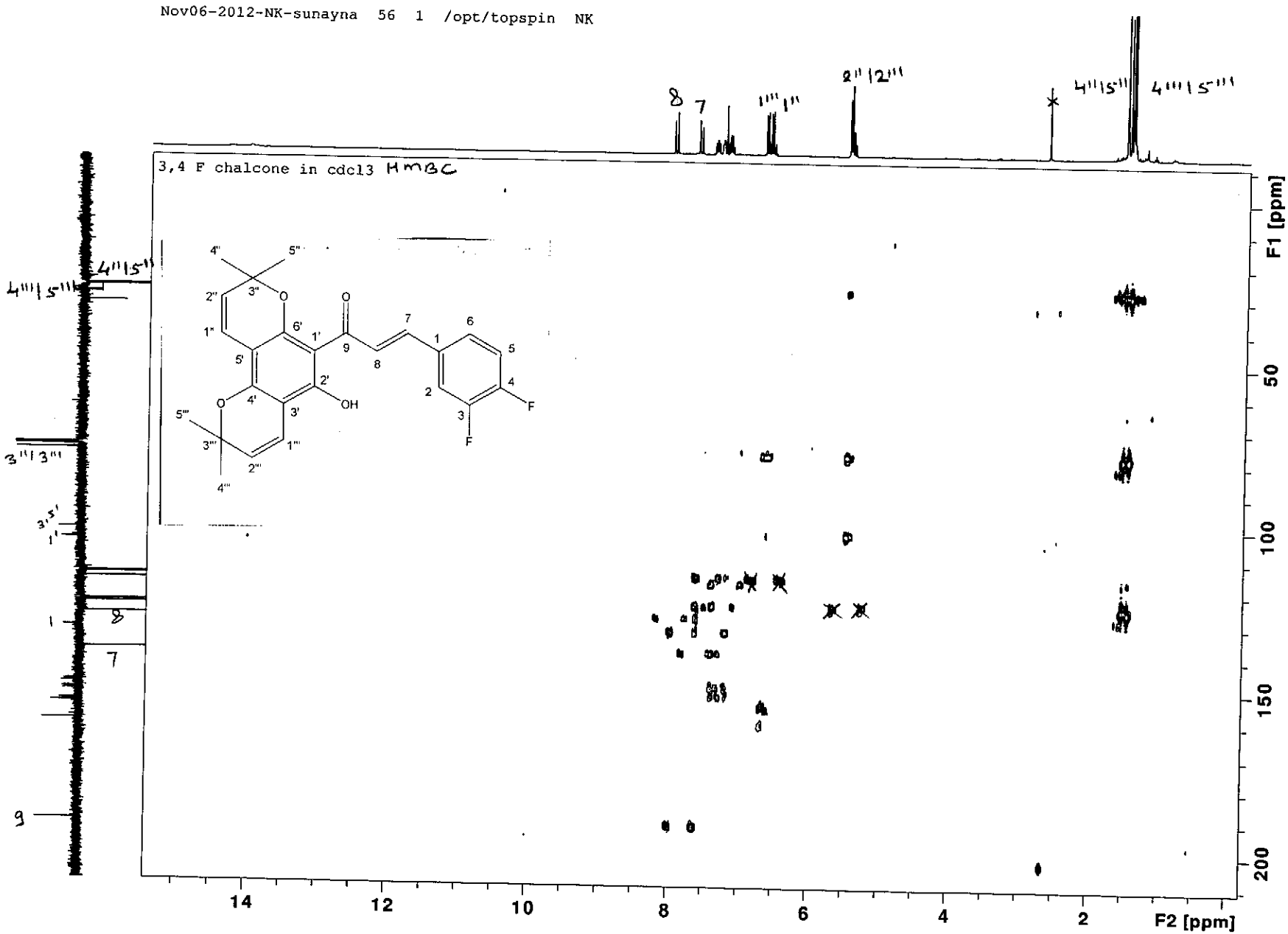
NOESY NMR spectrum of 3,4-difluoropyranochromene chalcone (B-9)



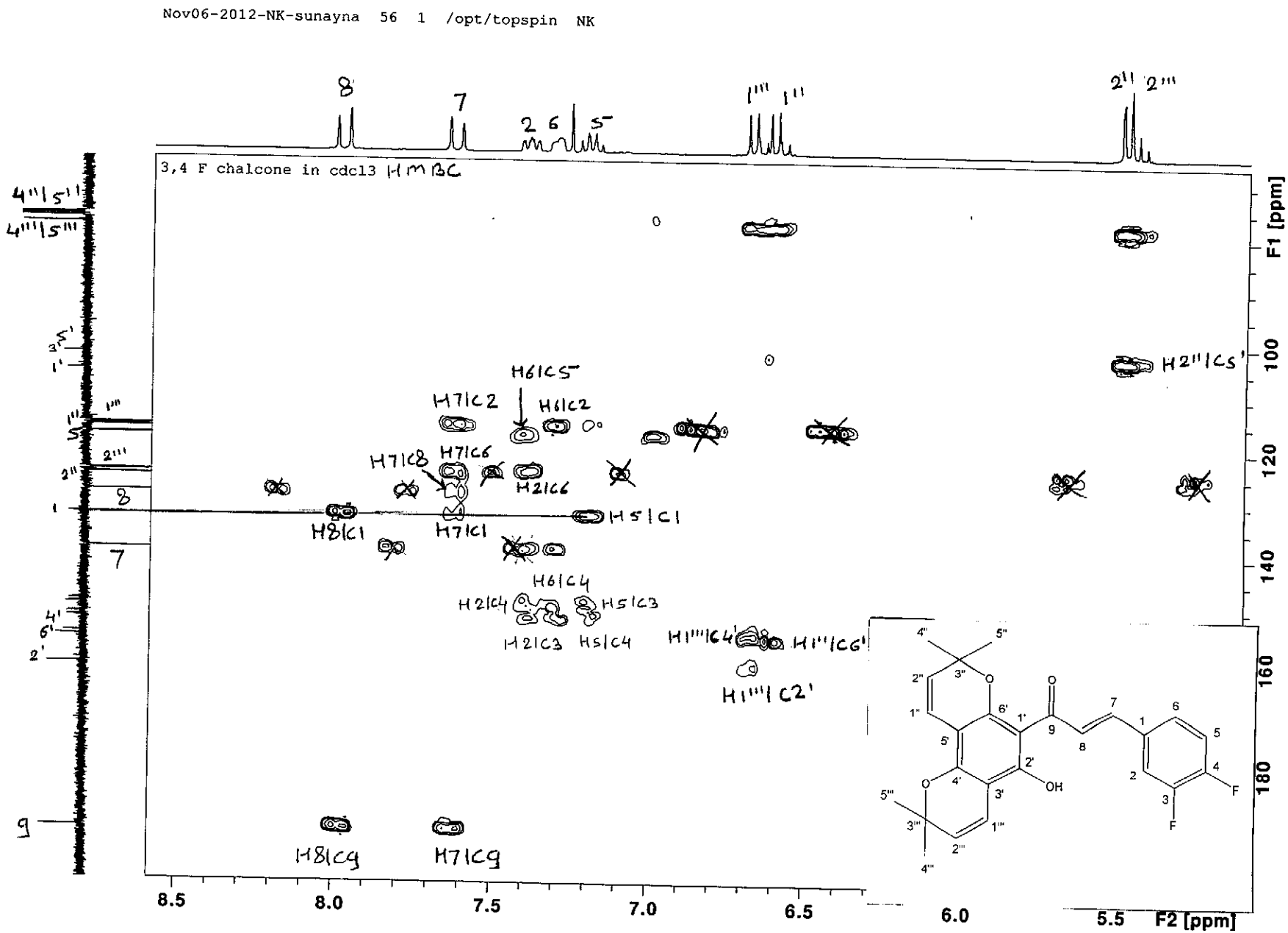
HSQC spectrum of 3,4-difluoropyranochromene chalcone (B-9)



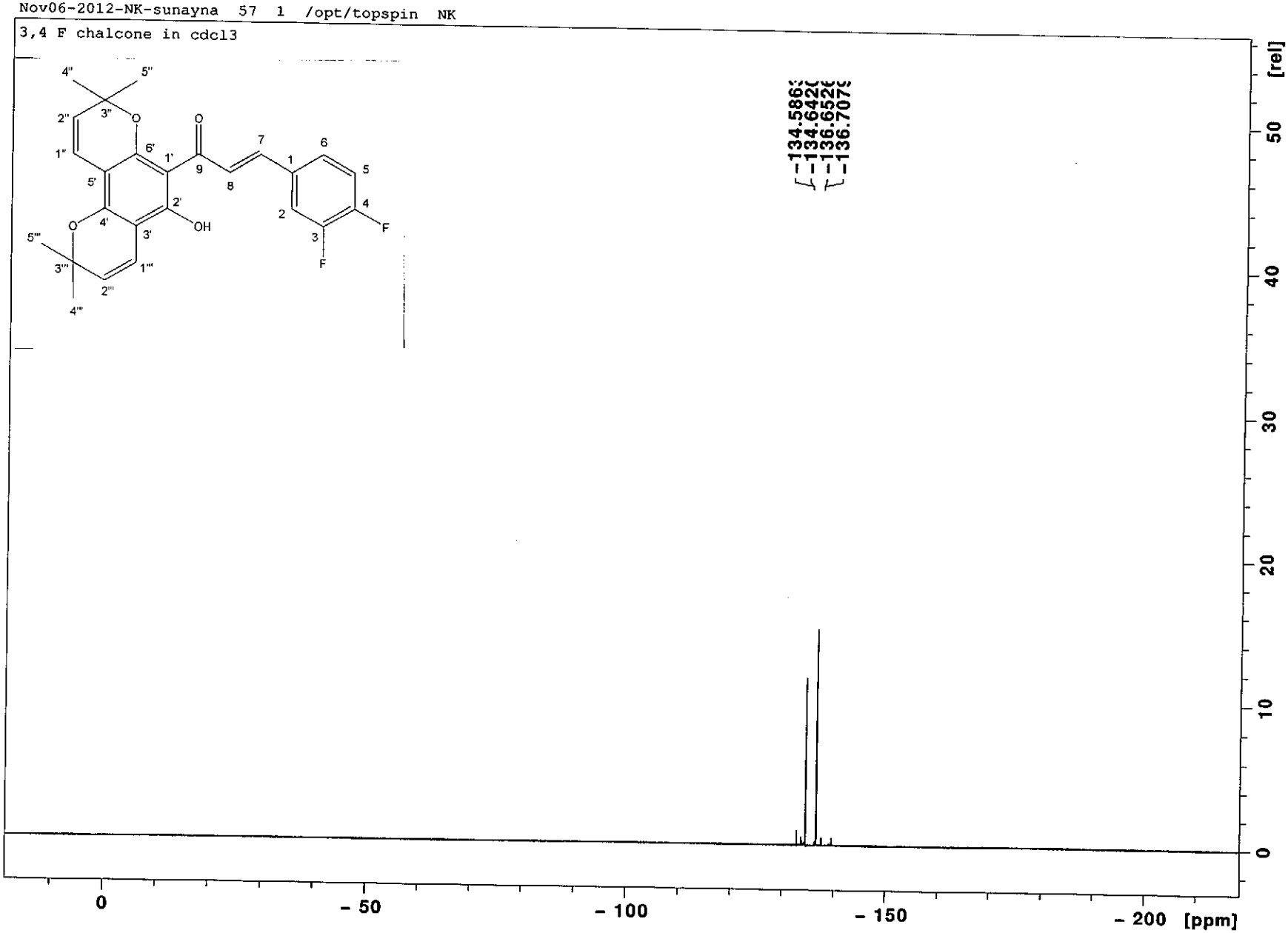
Expanded HSQC spectrum of 3,4-difluoropyranochromene chalcone (B-9)



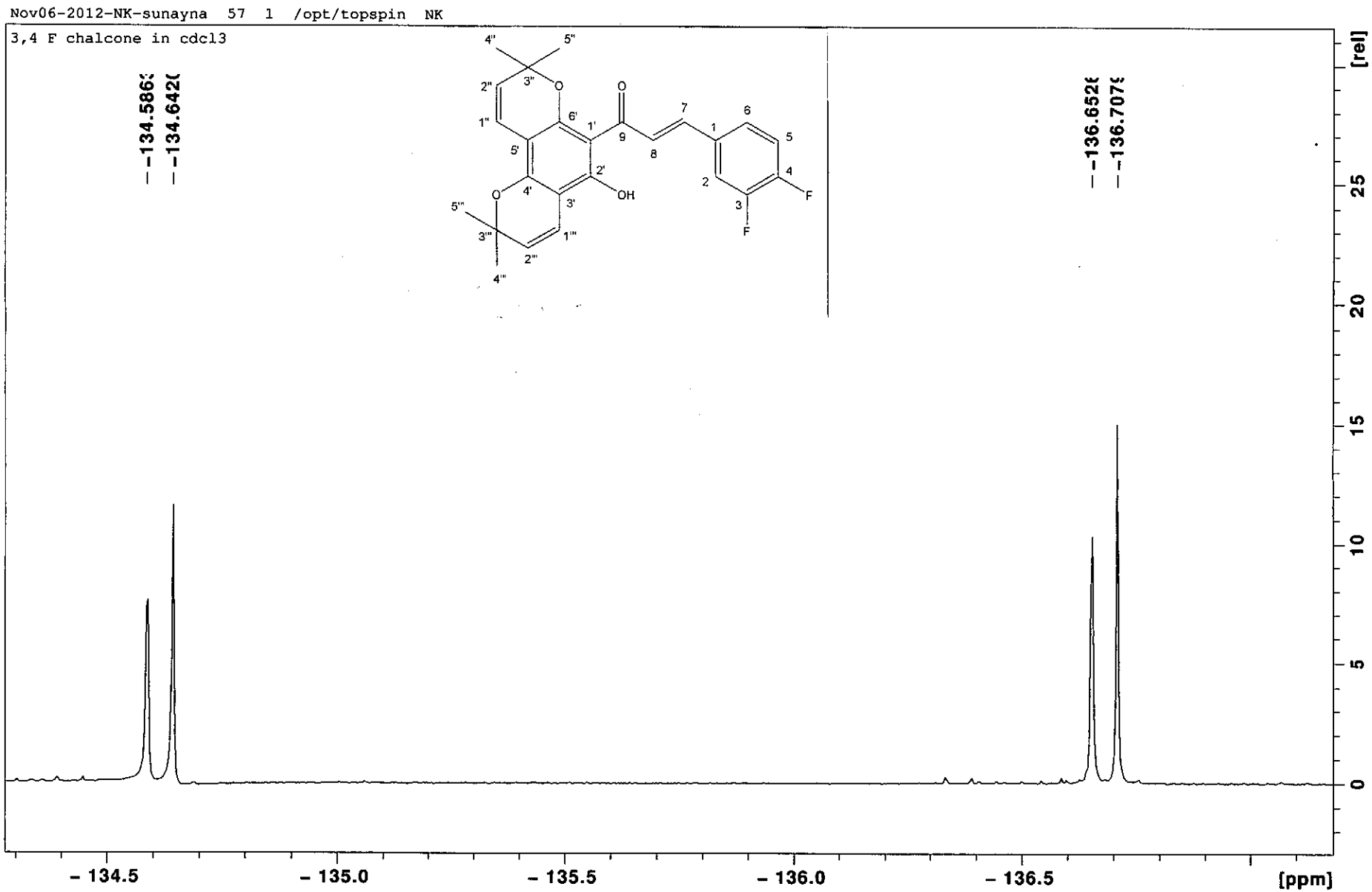
HMBC spectrum of 3,4-difluoropyranochromene chalcone (B-9)



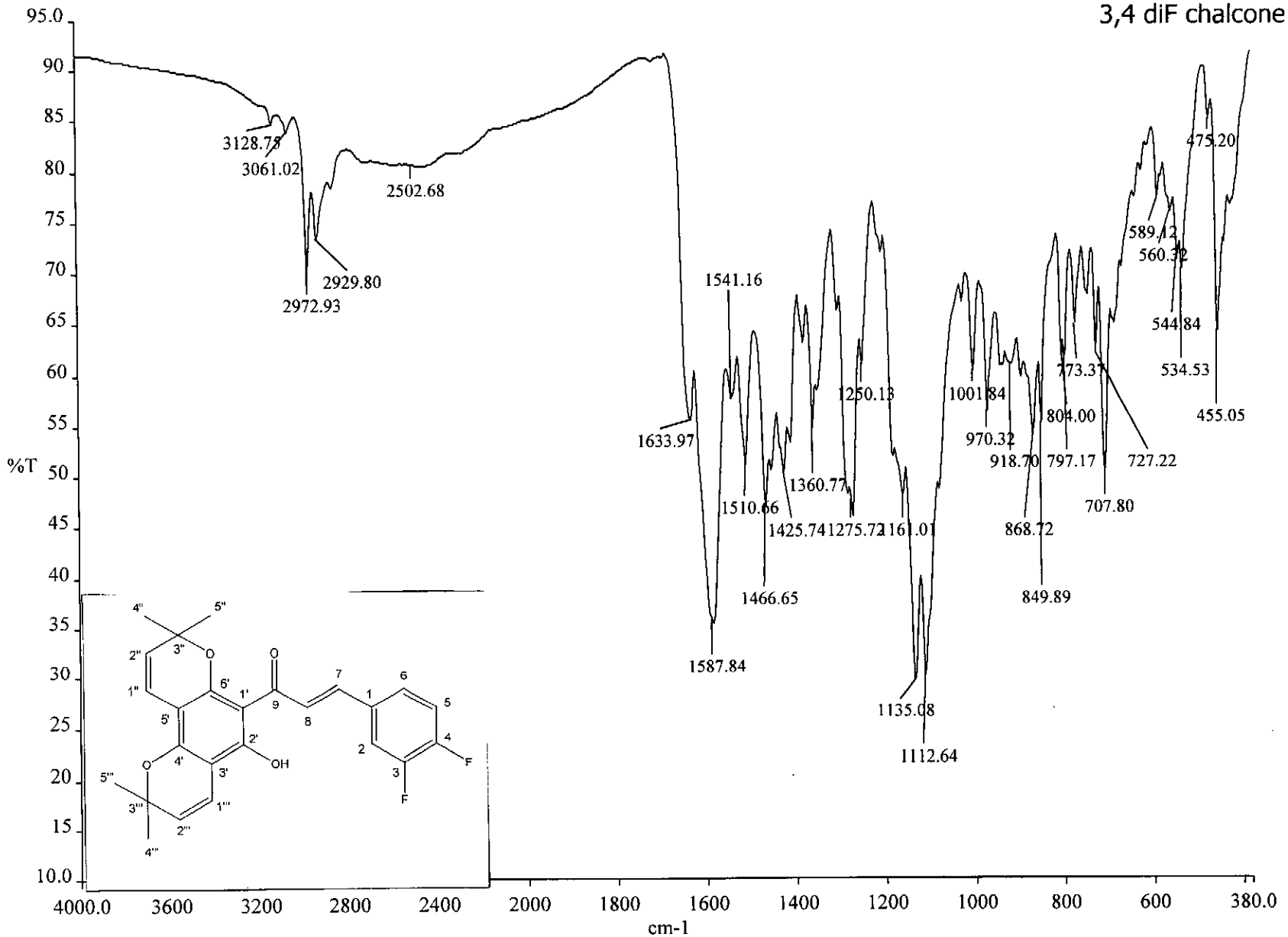
Expanded HMBC spectrum of 3,4-difluoropyranochromene chalcone (B-9)



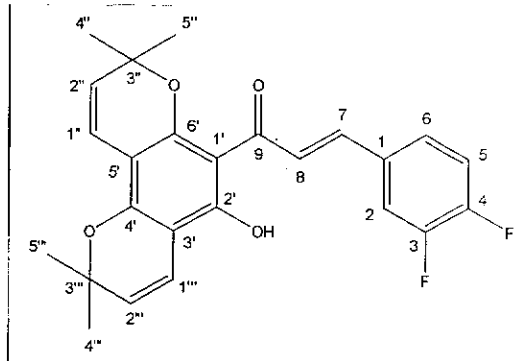
19F NMR spectrum of 3,4-difluoropyranochromene chalcone (B-9)



Expanded 19F NMR spectrum of 3,4-difluoropyranochromene chalcone (B-9)



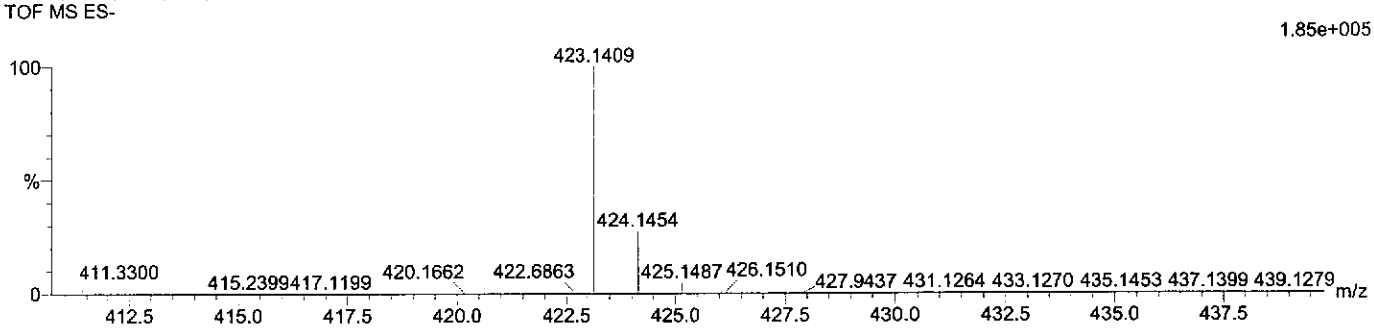
Infrared spectrum of 3,4-difluoropyranochromene chalcone (B-9)



Elemental Composition Report

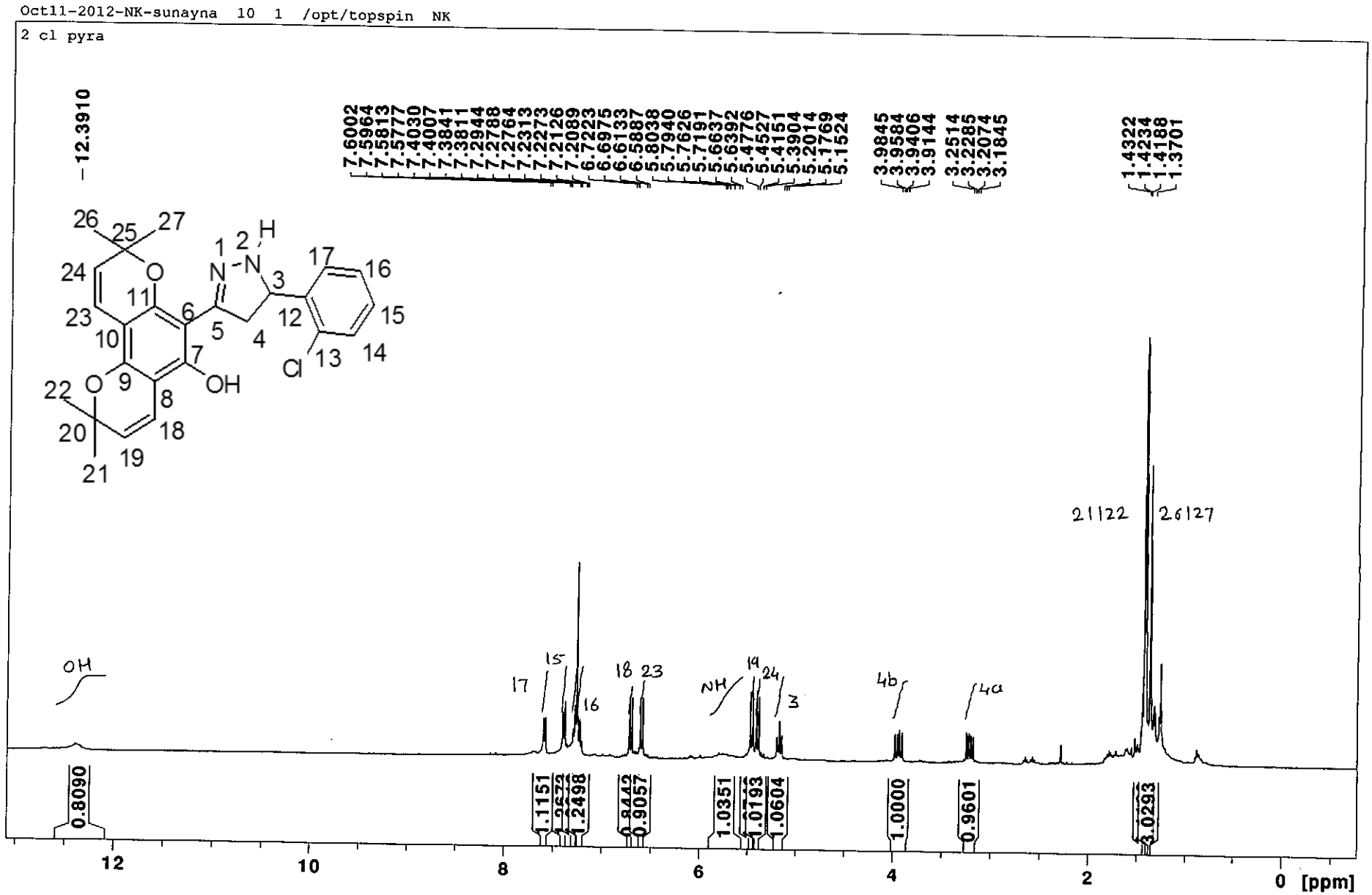
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
45 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-35 H: 20-30 N: 0-4 O: 0-4 F: 2-3
23 30 (0.495) Cm (1:31)
TOF MS ES-

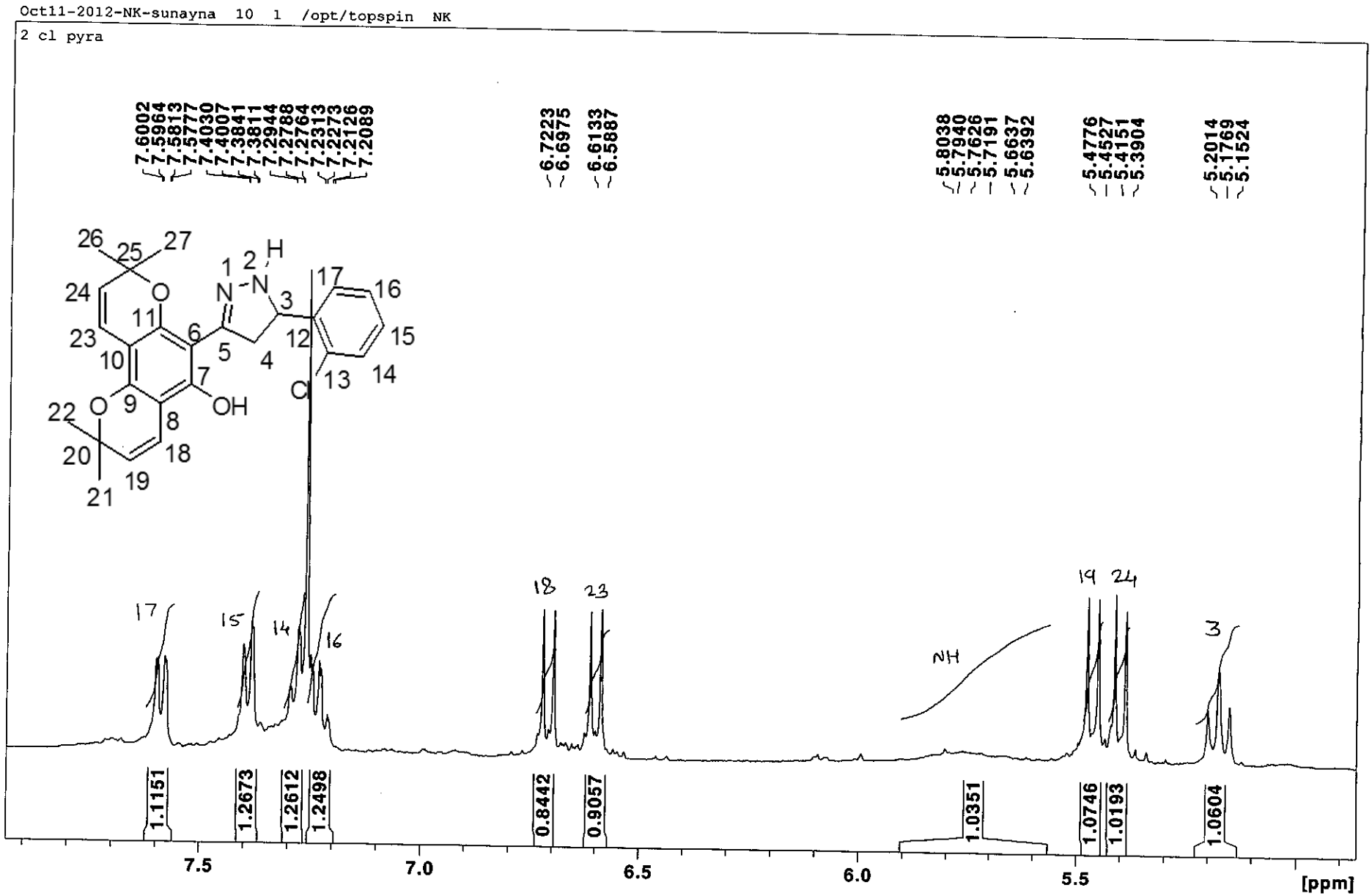


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
423.1409	423.1408	0.1	0.2	14.5	511.8	0.0	C25 H21 O4 F2

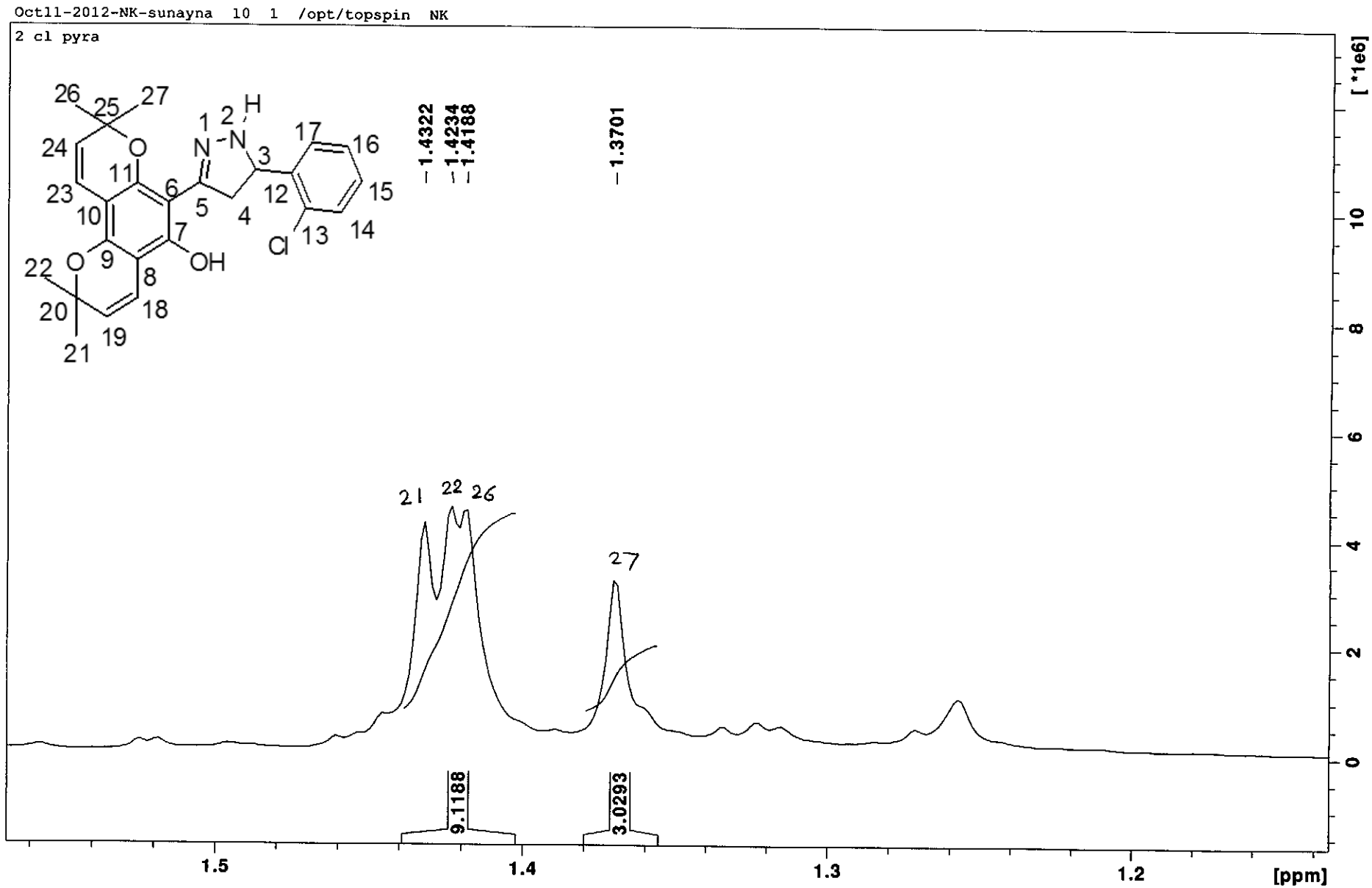
HRMS spectrum of 3,4-difluoropyranochromene chalcone (B-9)



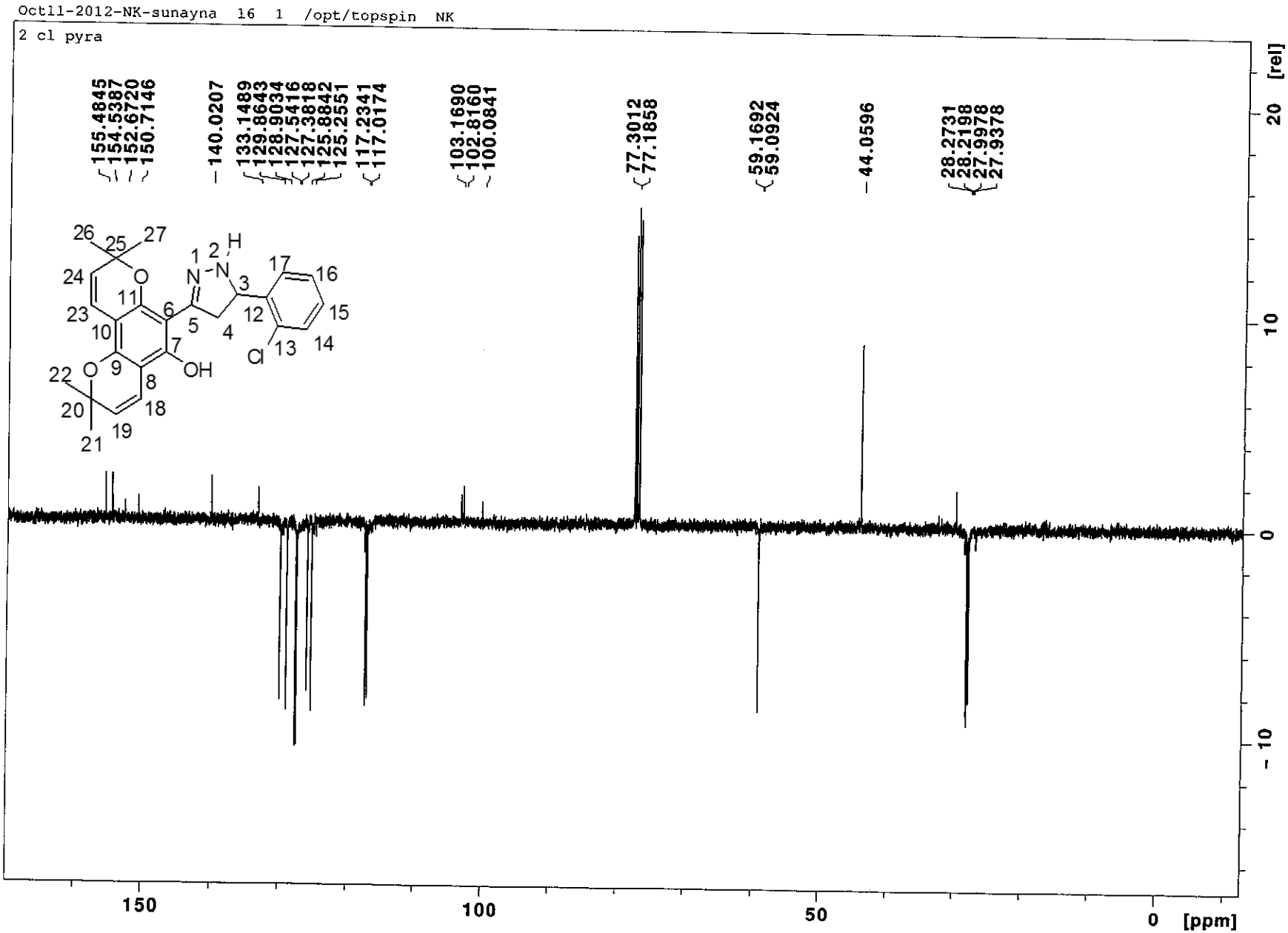
1H NMR spectrum of 3-(2-chlorophenyl)-5-pyranochromene pyrazoline (C-4a)



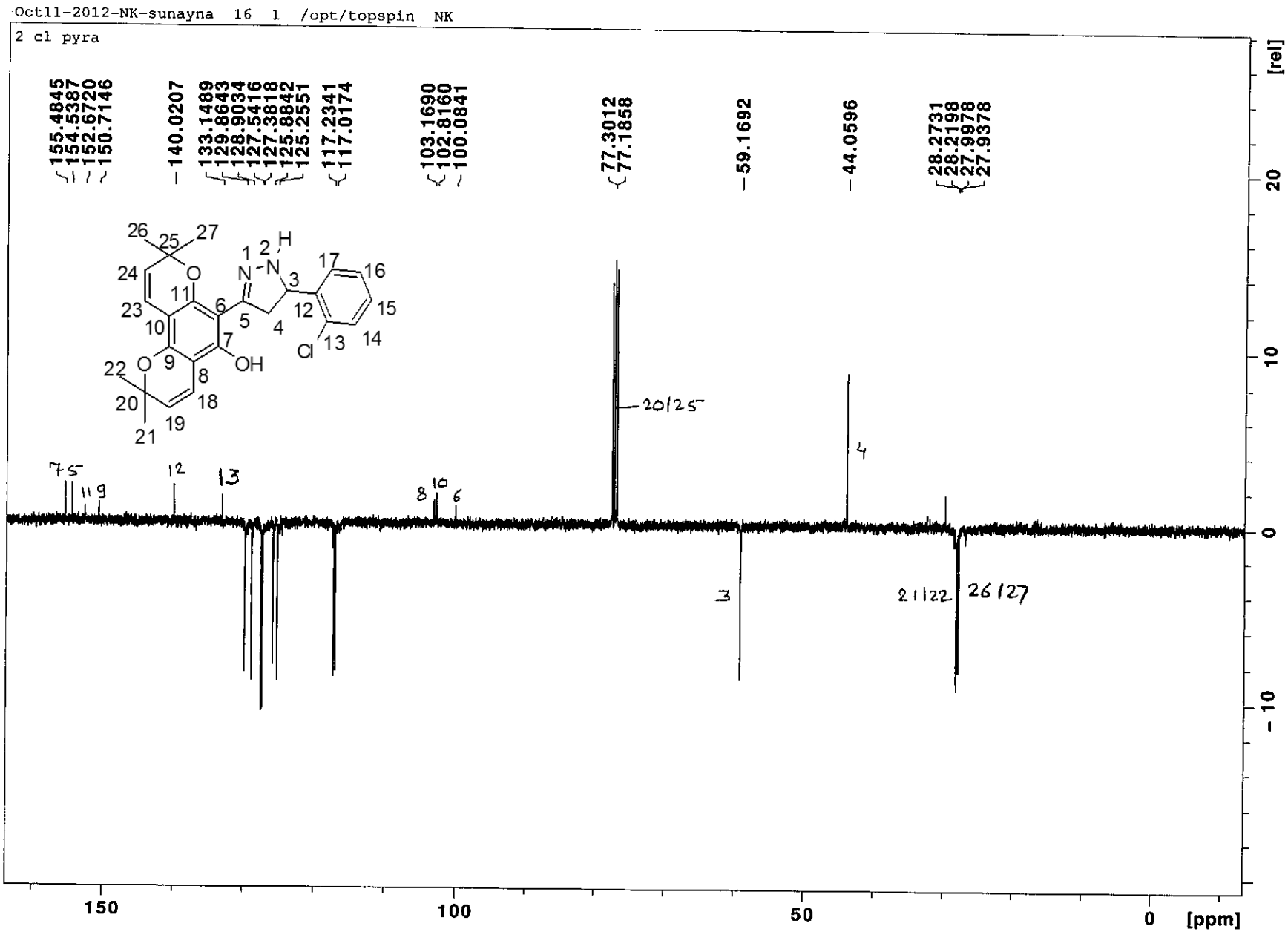
Expanded 1H NMR spectrum of 3-(2-chlorophenyl)-5-pyranochromene pyrazoline (C-4a)



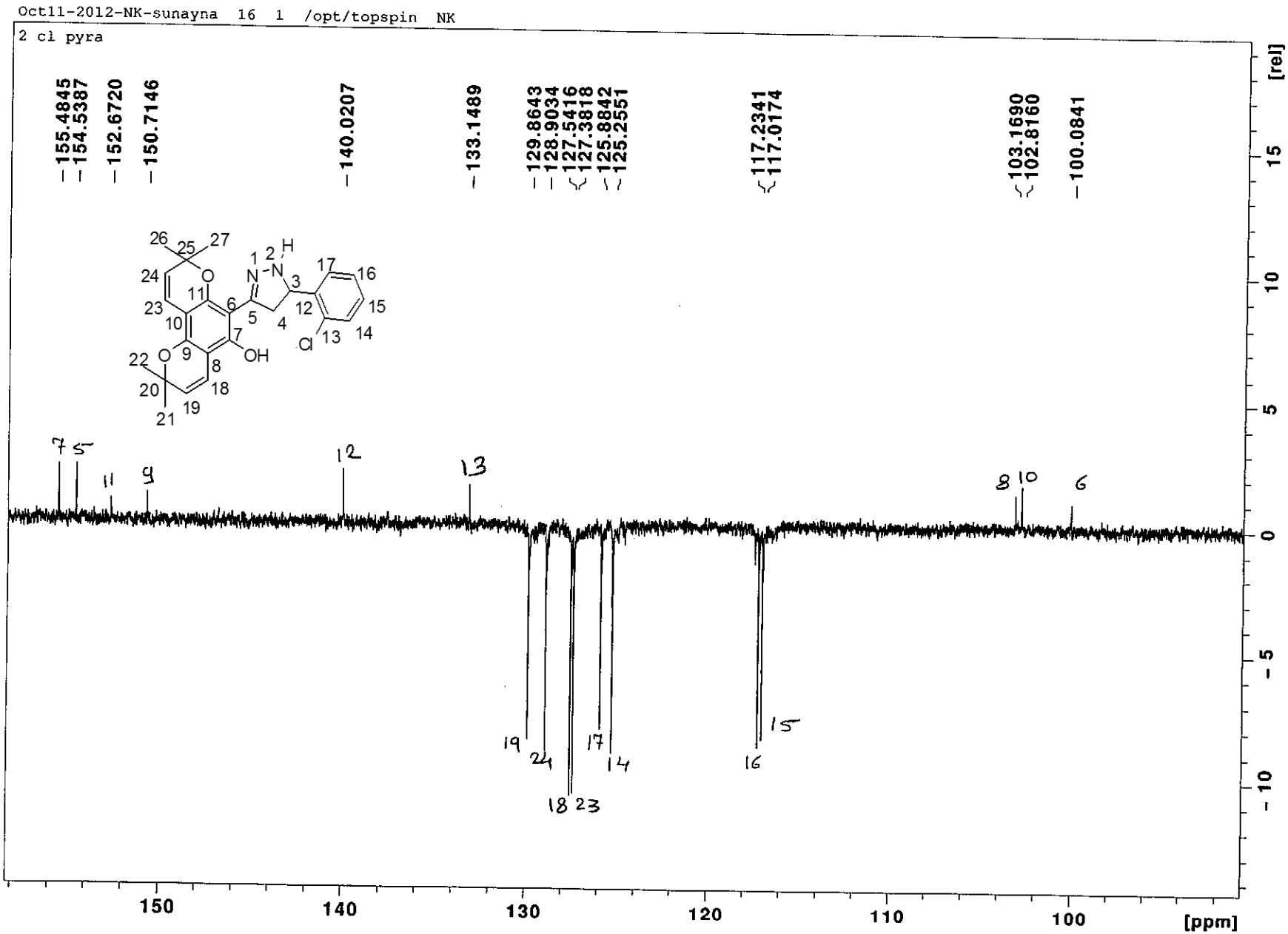
Expanded 1H NMR spectrum of 3-(2-chlorophenyl)-5-pyranochromene pyrazoline (C-4a)



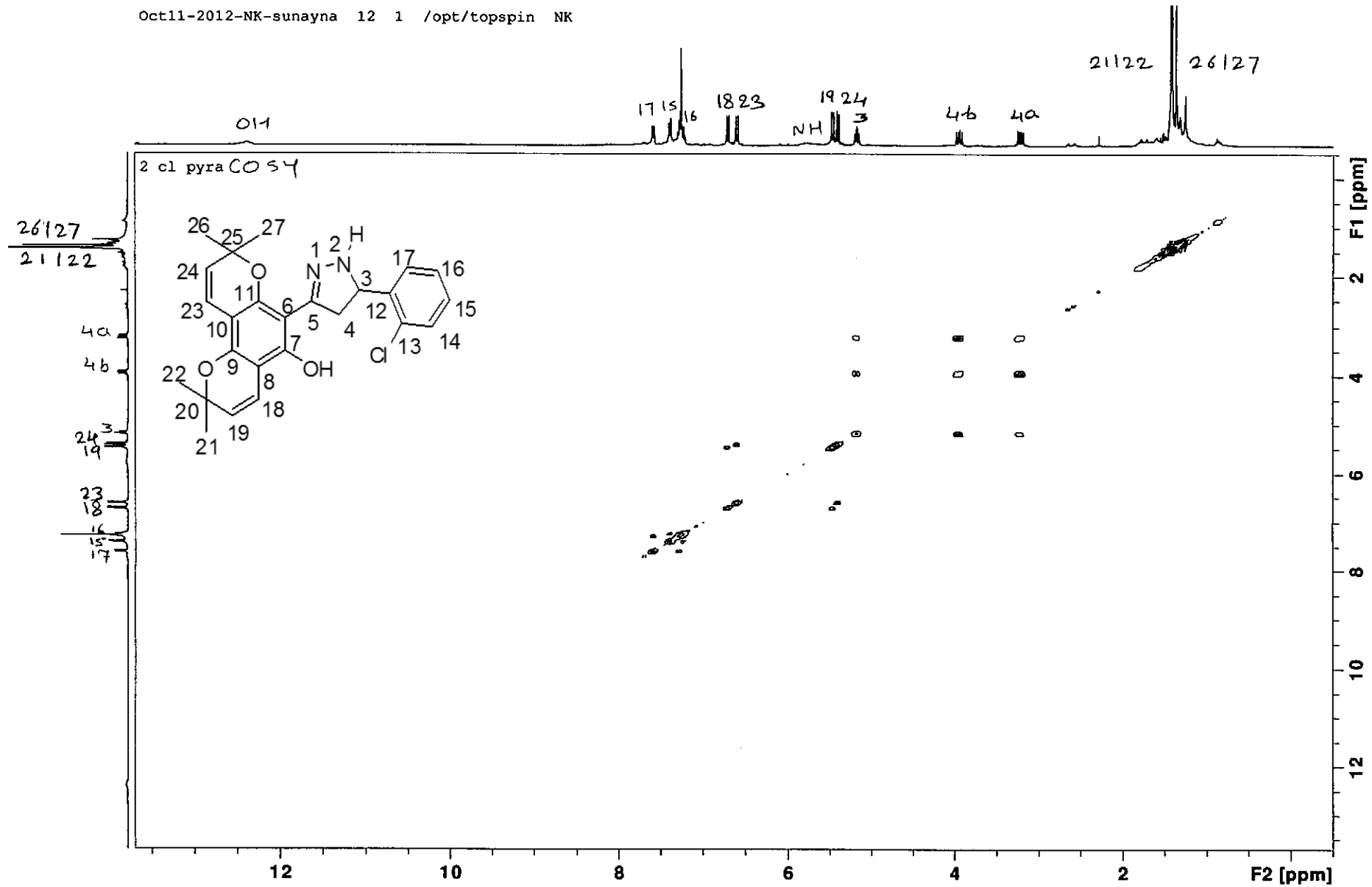
13C NMR spectrum of 3-(2-chlorophenyl)-5-pyranochromene pyrazoline (C-4a)



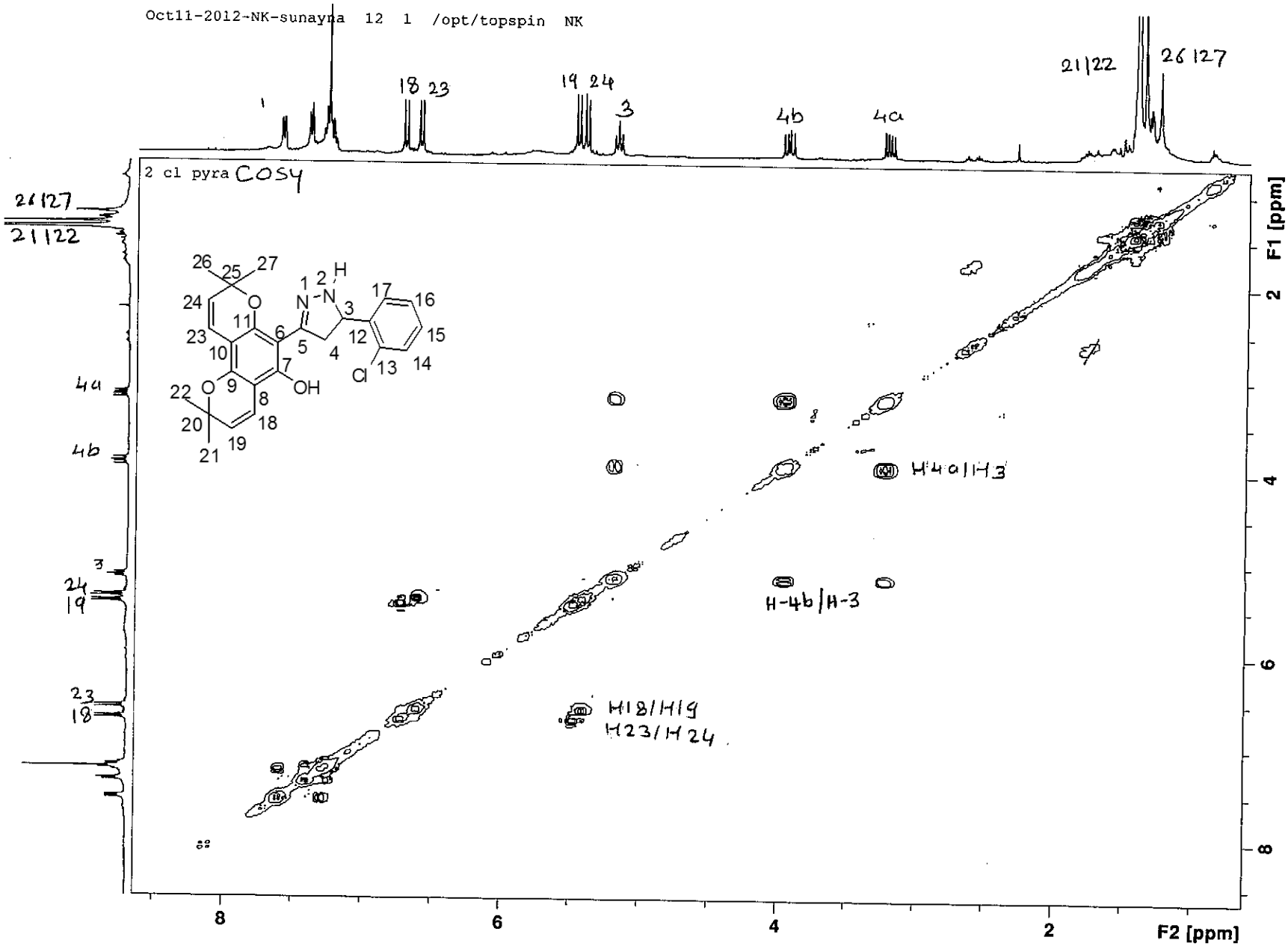
Expanded 13C NMR spectrum of 3-(2-chlorophenyl)-5-pyranochromene pyrazoline (C-4a)



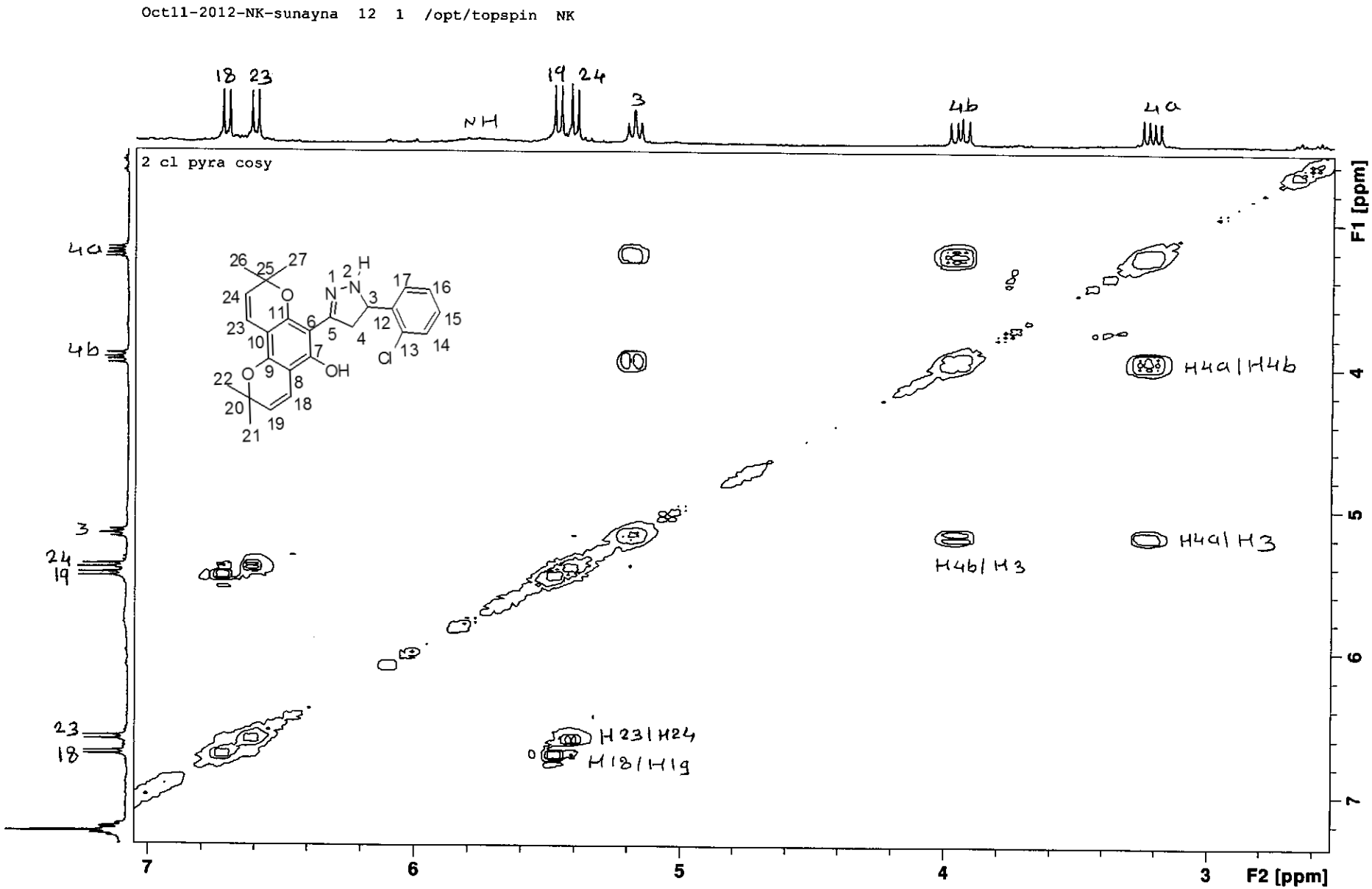
Expanded 13C NMR spectrum of 3-(2-chlorophenyl)-5-pyranochromene pyrazoline (C-4a)



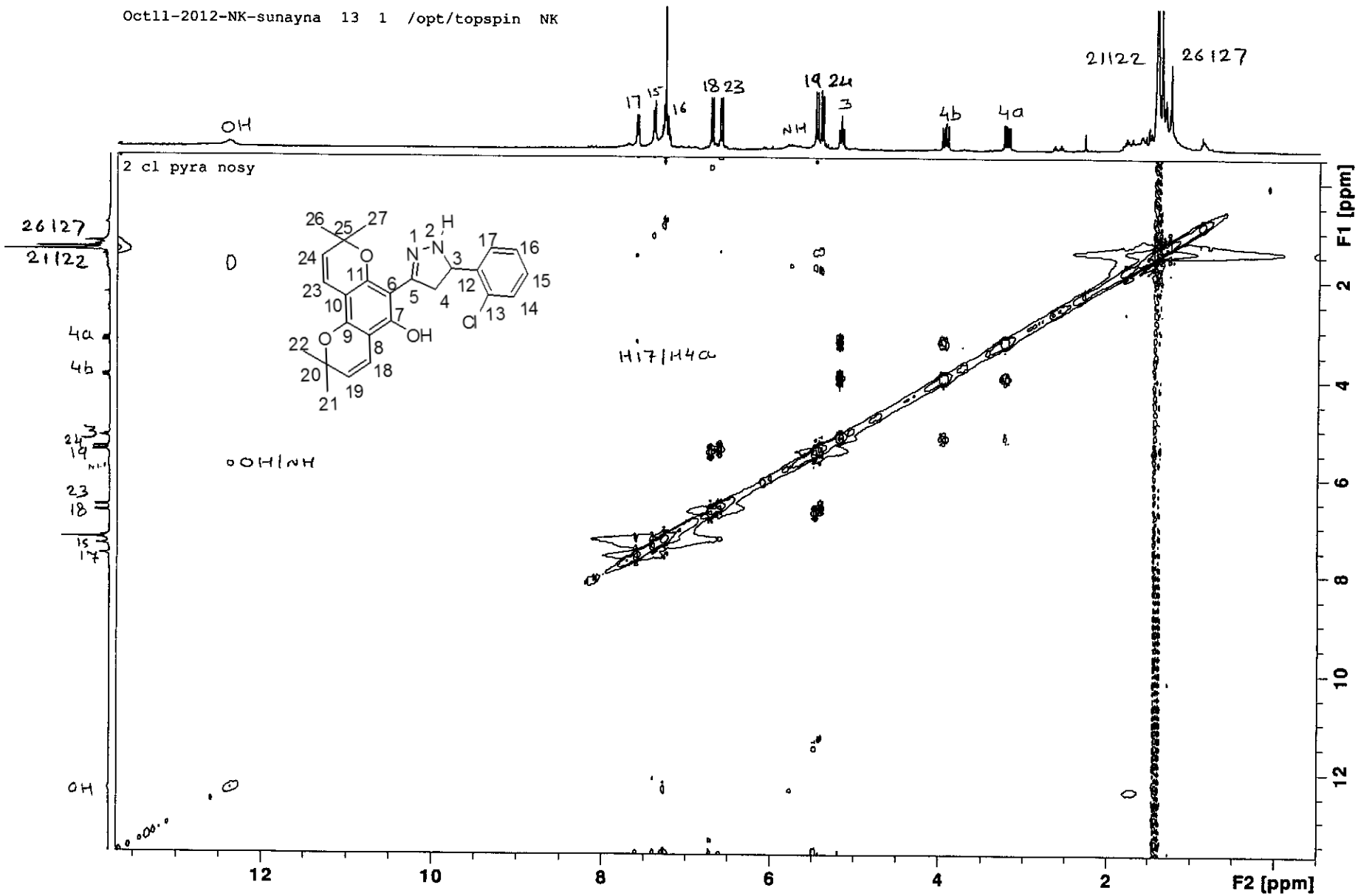
COSY NMR spectrum of 3-(2-chlorophenyl)-5-pyranochromene pyrazoline (C-4a)



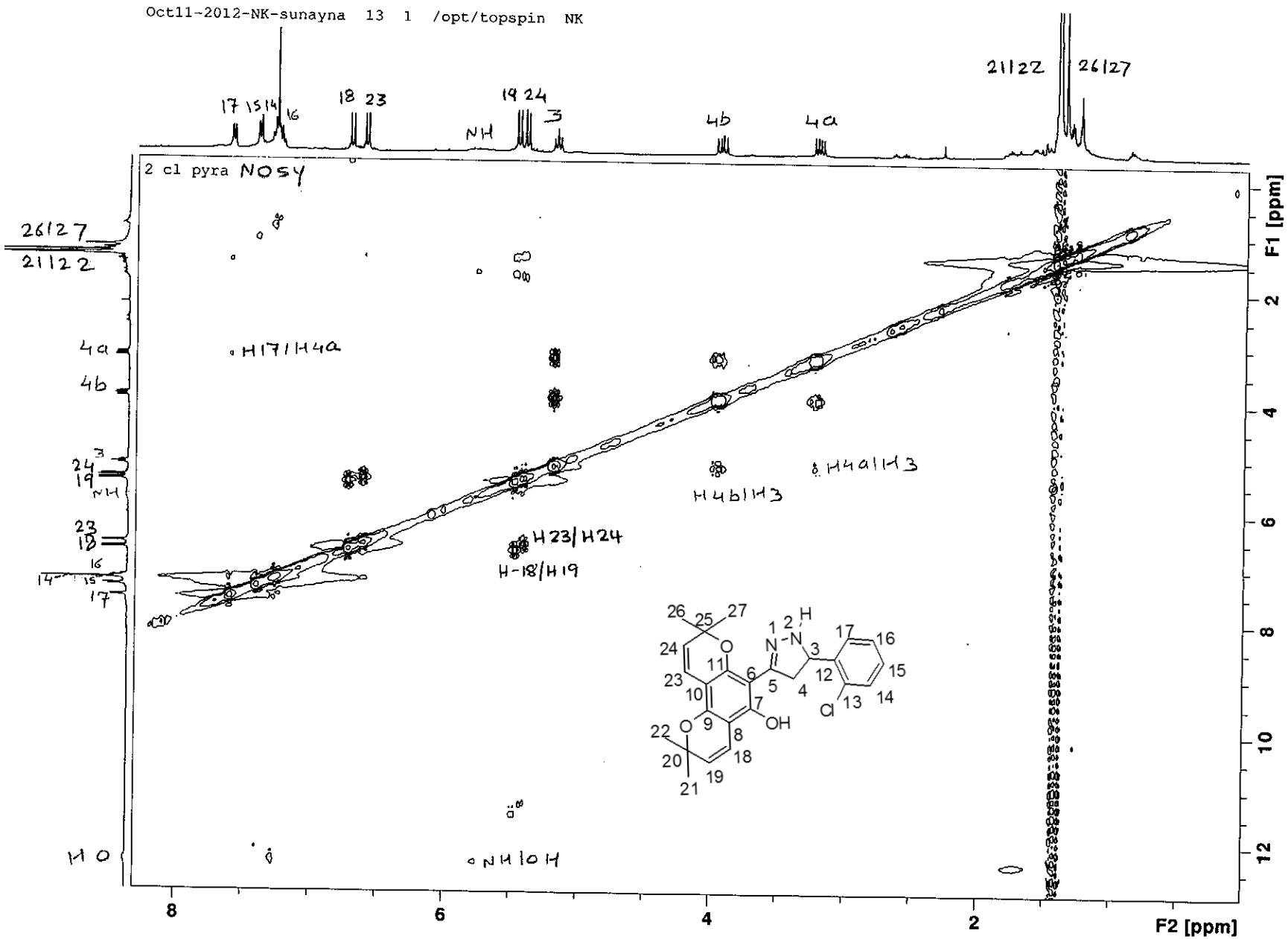
Expanded COSY NMR spectrum of 3-(2-chlorophenyl)-5-pyranochromene pyrazoline (C-4a)



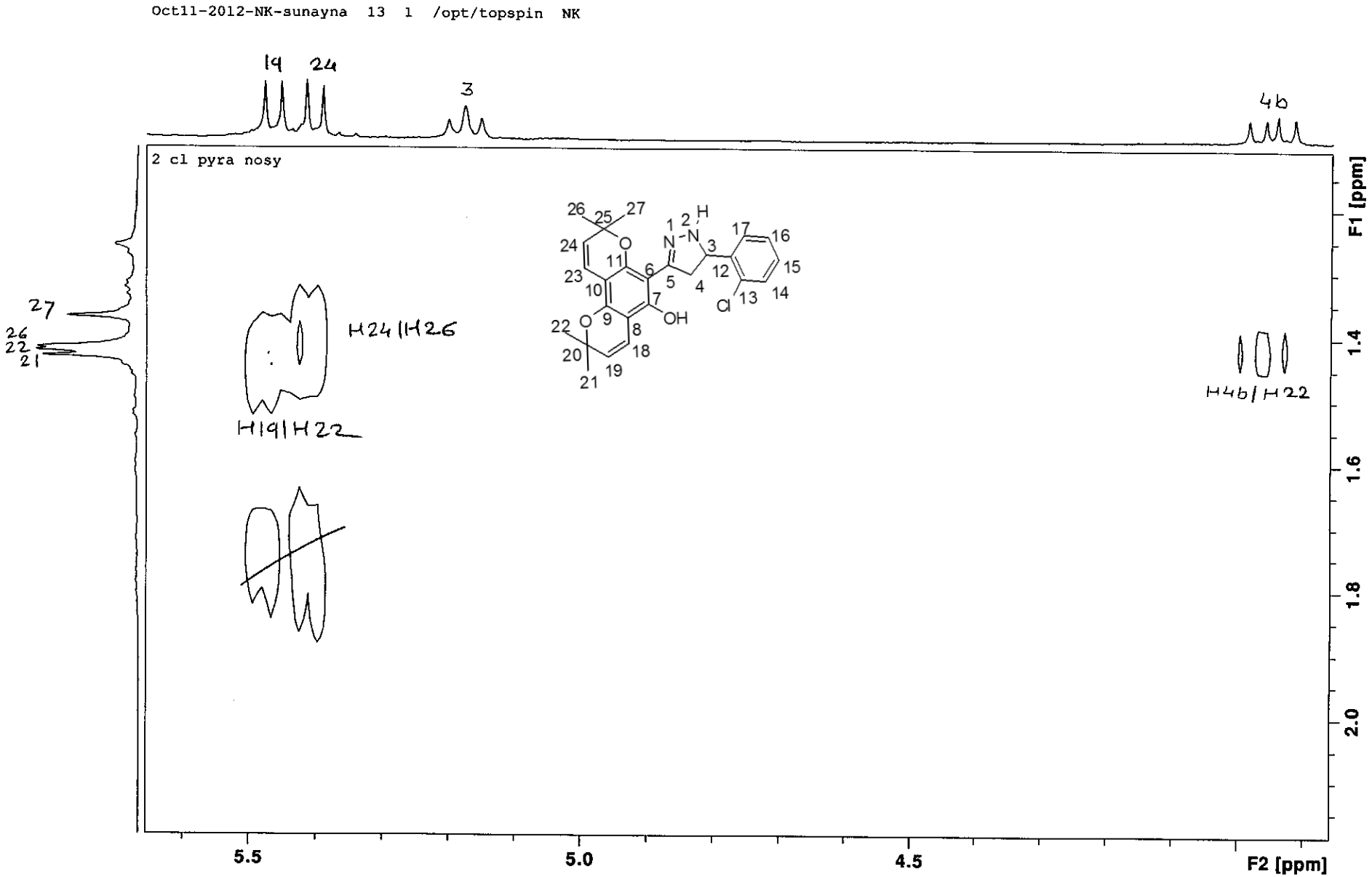
Expanded COSY NMR spectrum of 3-(2-chlorophenyl)-5-pyranochromene pyrazoline (C-4a)



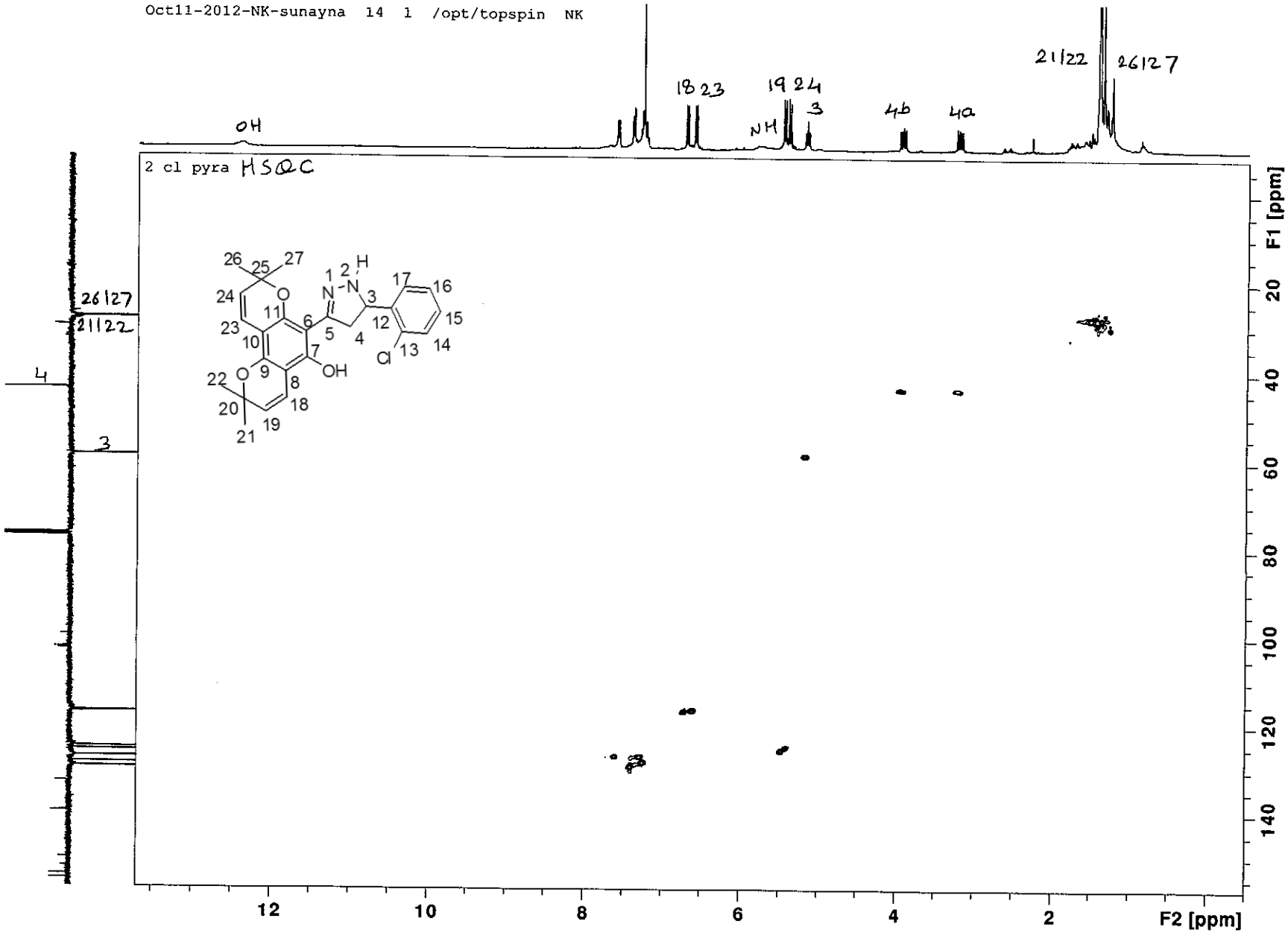
NOESY NMR spectrum of 3-(2-chlorophenyl)-5-pyranochromene pyrazoline (C-4a)



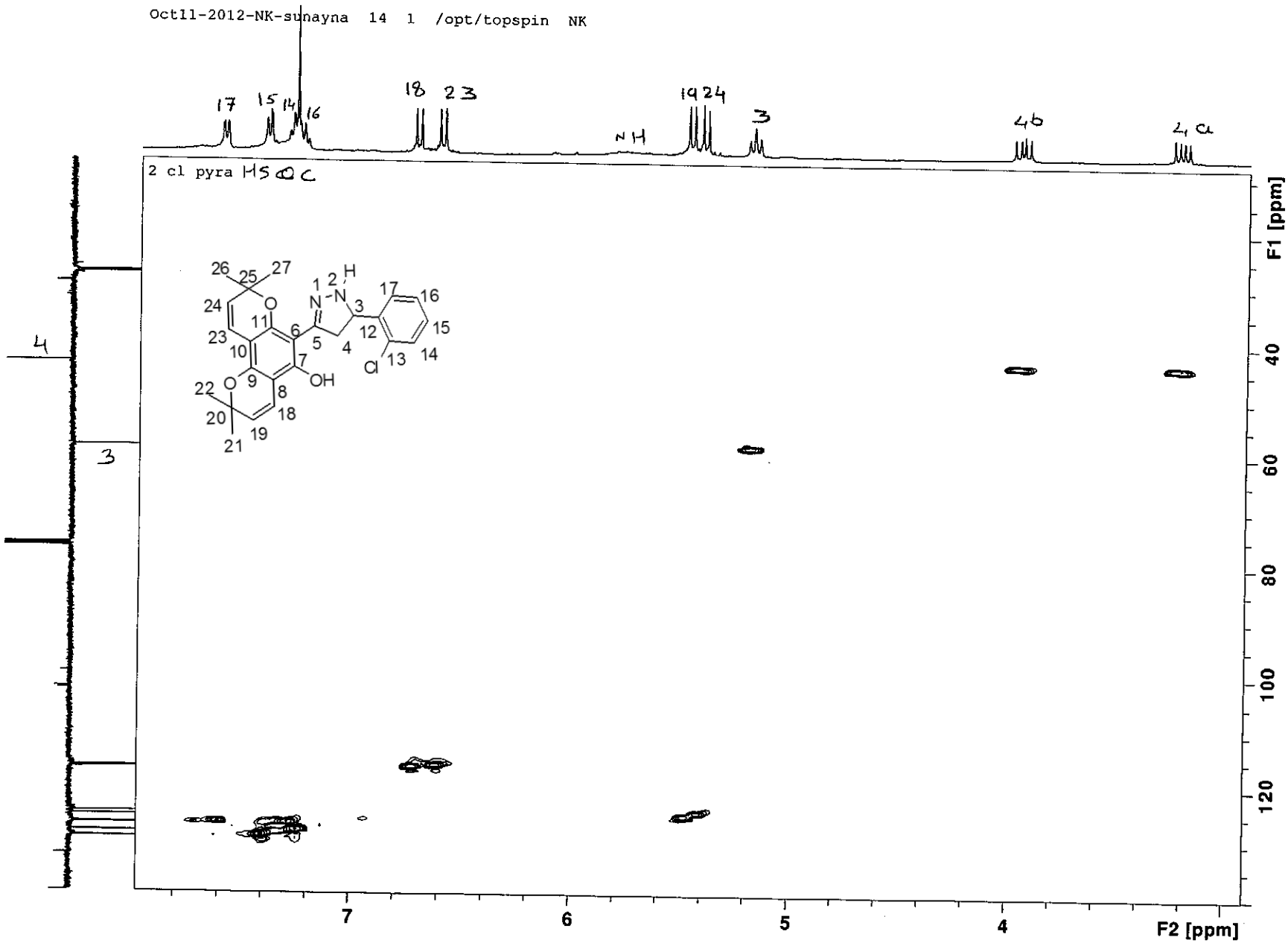
Expanded NOESY NMR spectrum of 3-(2-chlorophenyl)-5-pyranochromene pyrazoline (C-4a)



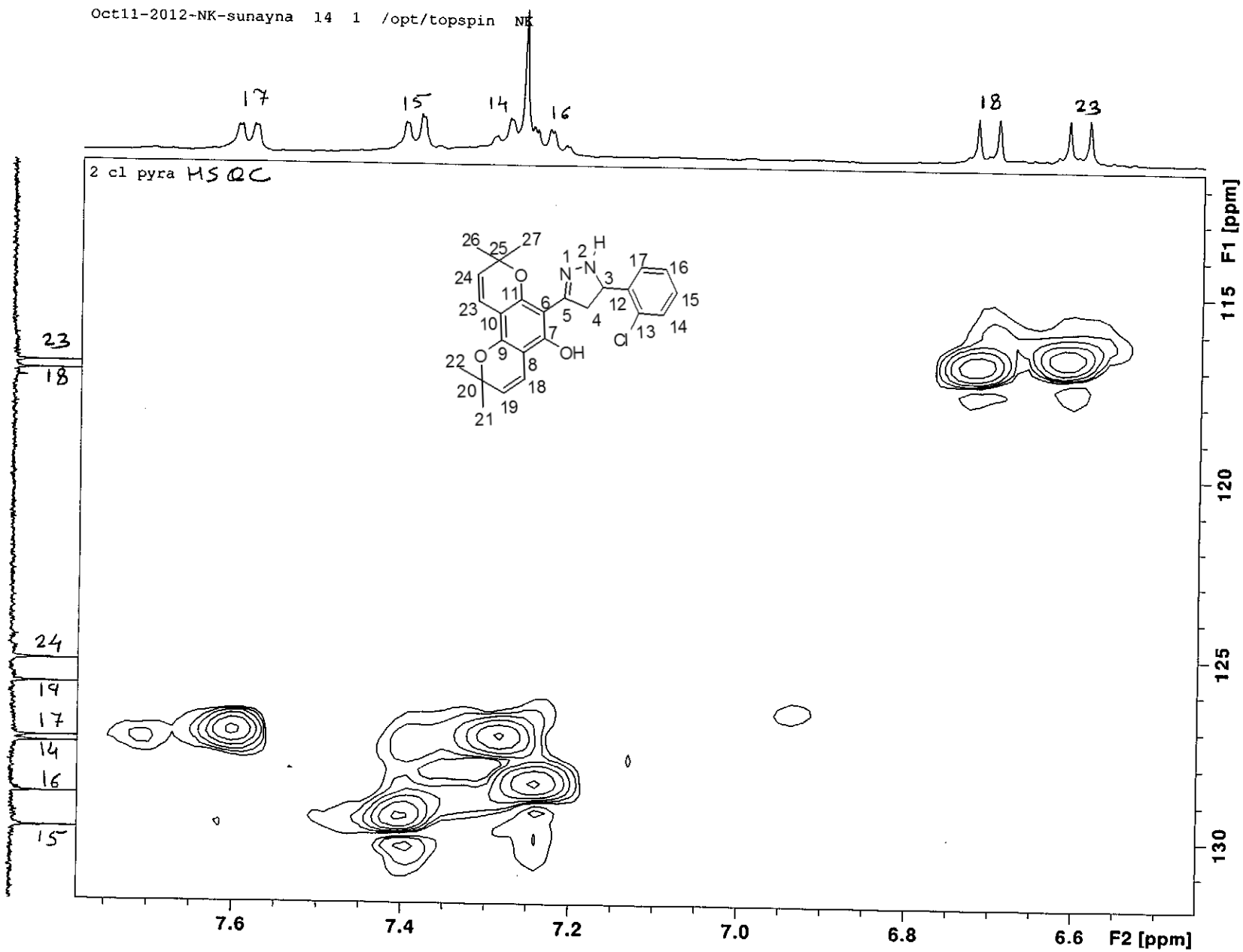
Expanded NOESY NMR spectrum of 3-(2-chlorophenyl)-5-pyranochromene pyrazoline (C-4a)



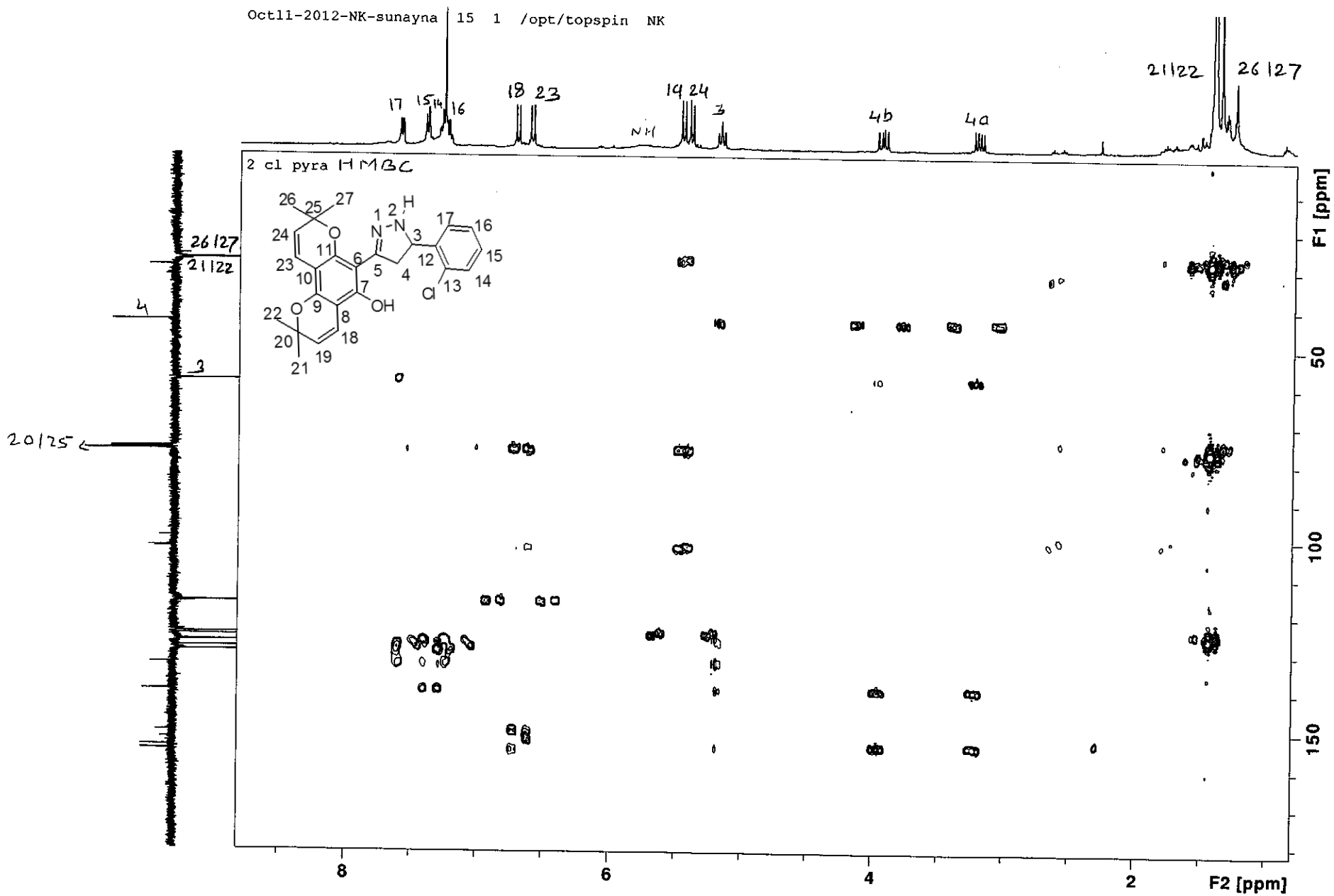
HSQC spectrum of 3-(2-chlorophenyl)-5-pyranochromene pyrazoline (C-4a)



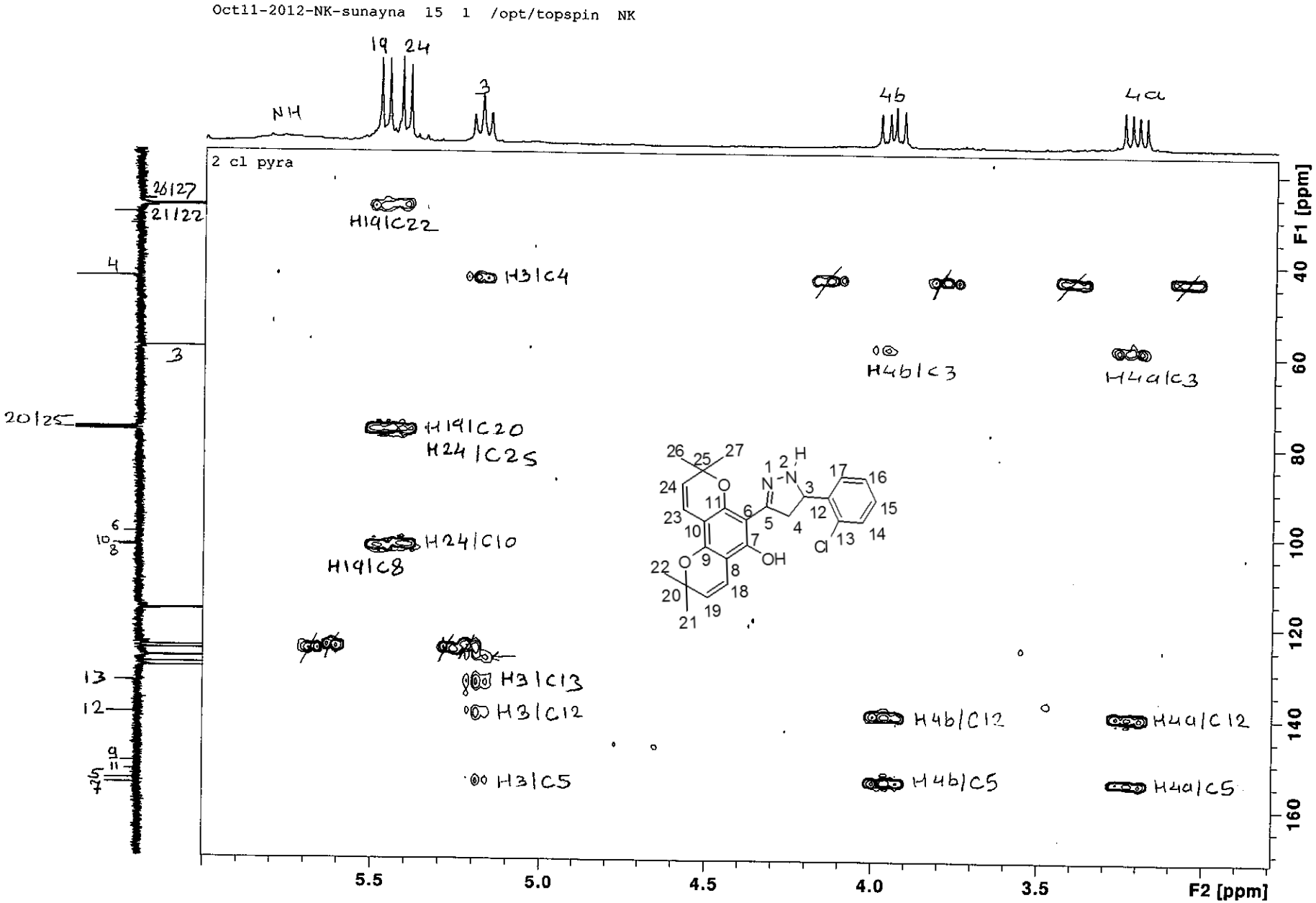
Expanded HSQC spectrum of 3-(2-chlorophenyl)-5-pyranochromene pyrazoline (C-4a)



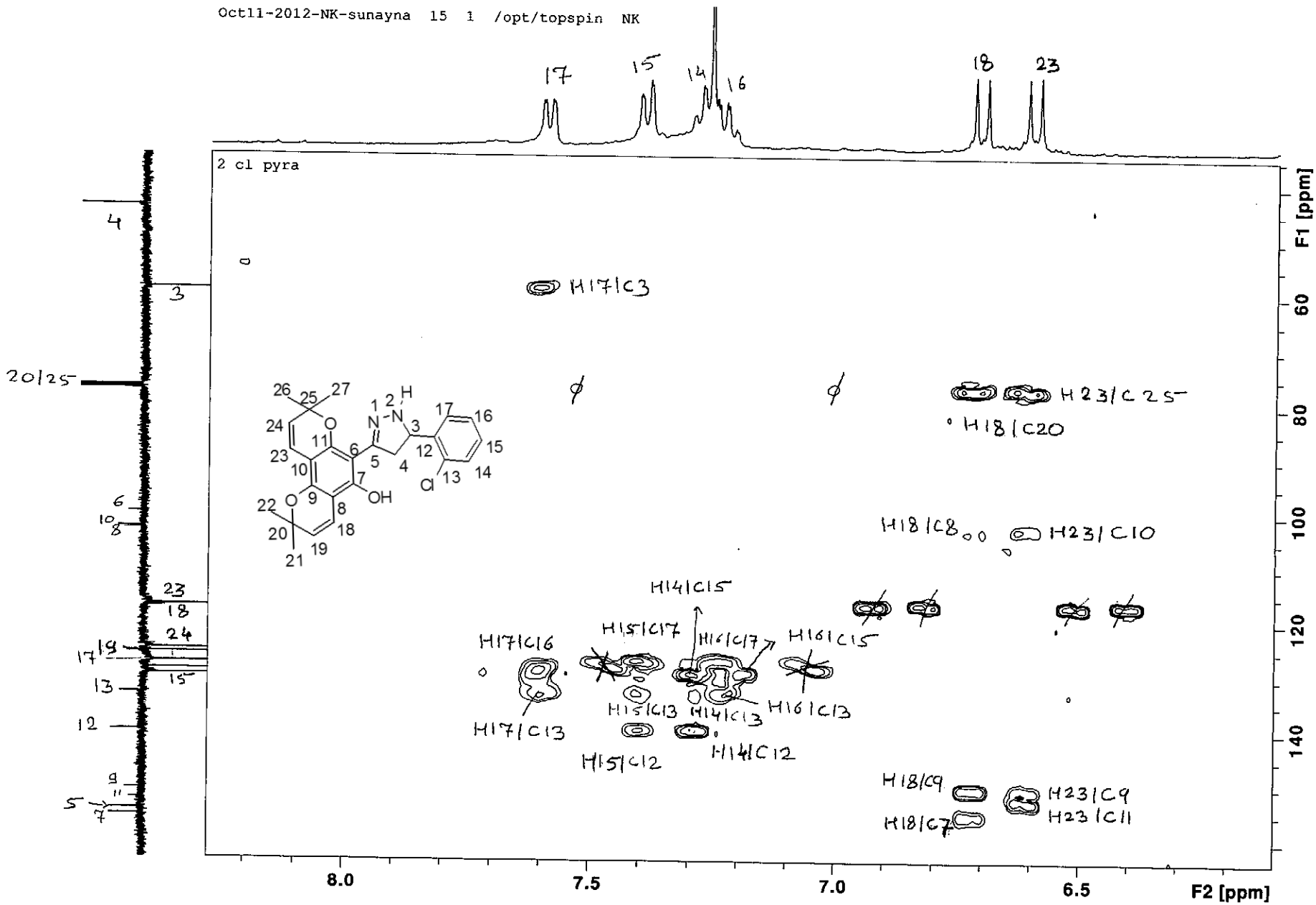
Expanded HSQC spectrum of 3-(2-chlorophenyl)-5-pyranochromene pyrazoline (C-4a)



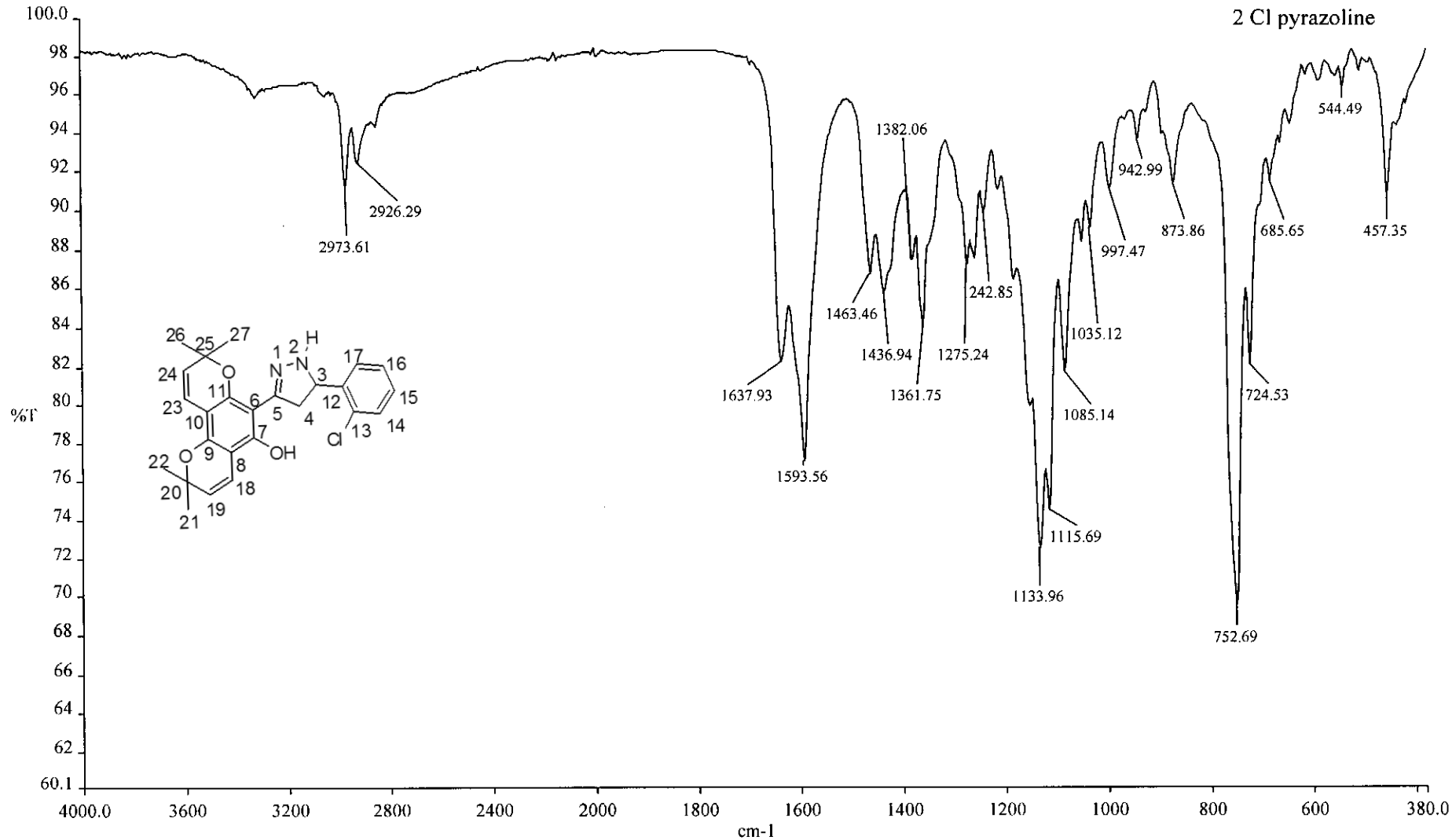
HMBC spectrum of 3-(2-chlorophenyl)-5-pyranochromene pyrazoline (C-4a)



Expanded HMBC spectrum of 3-(2-chlorophenyl)-5-pyranochromene pyrazoline (C-4a)



Expanded HMBC spectrum of 3-(2-chlorophenyl)-5-pyranochromene pyrazoline (C-4a)

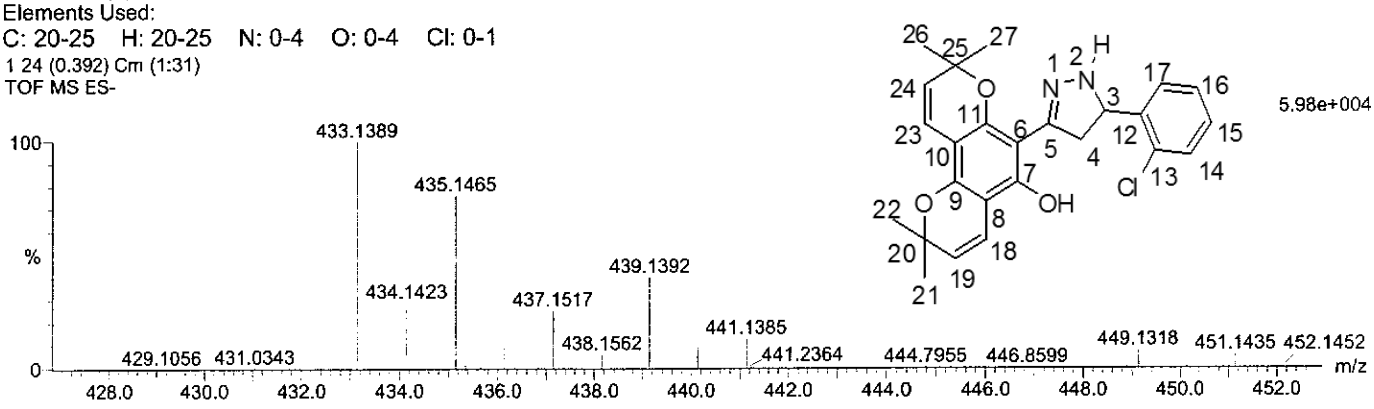


Infrared spectrum of 3-(2-chlorophenyl)-5-pyranochromene pyrazoline (C-4a)

Elemental Composition Report **Page 1**

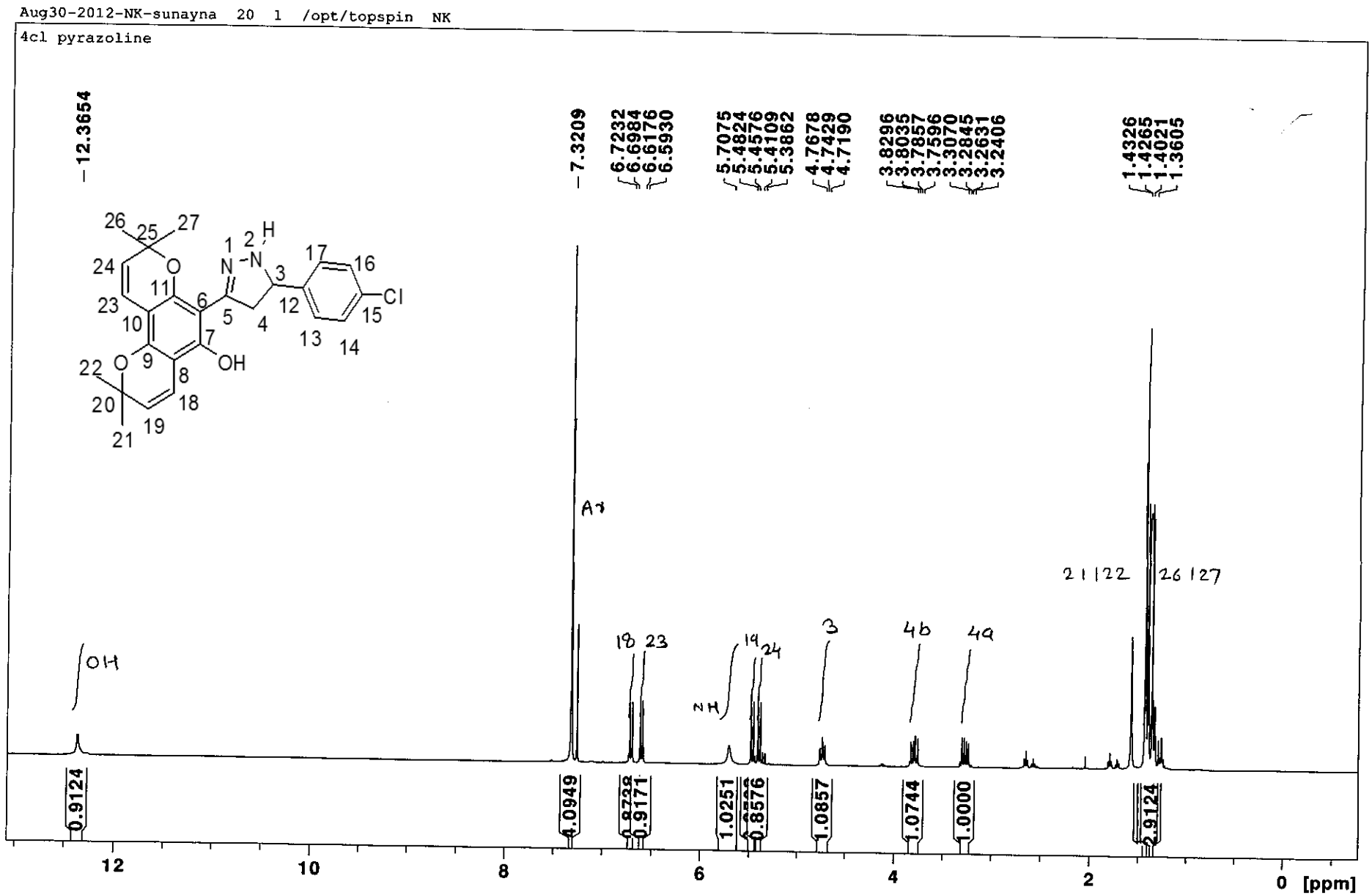
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
20 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-25 H: 20-25 N: 0-4 O: 0-4 Cl: 0-1
1 24 (0.392) Cm (1:31)
TOF MS ES-

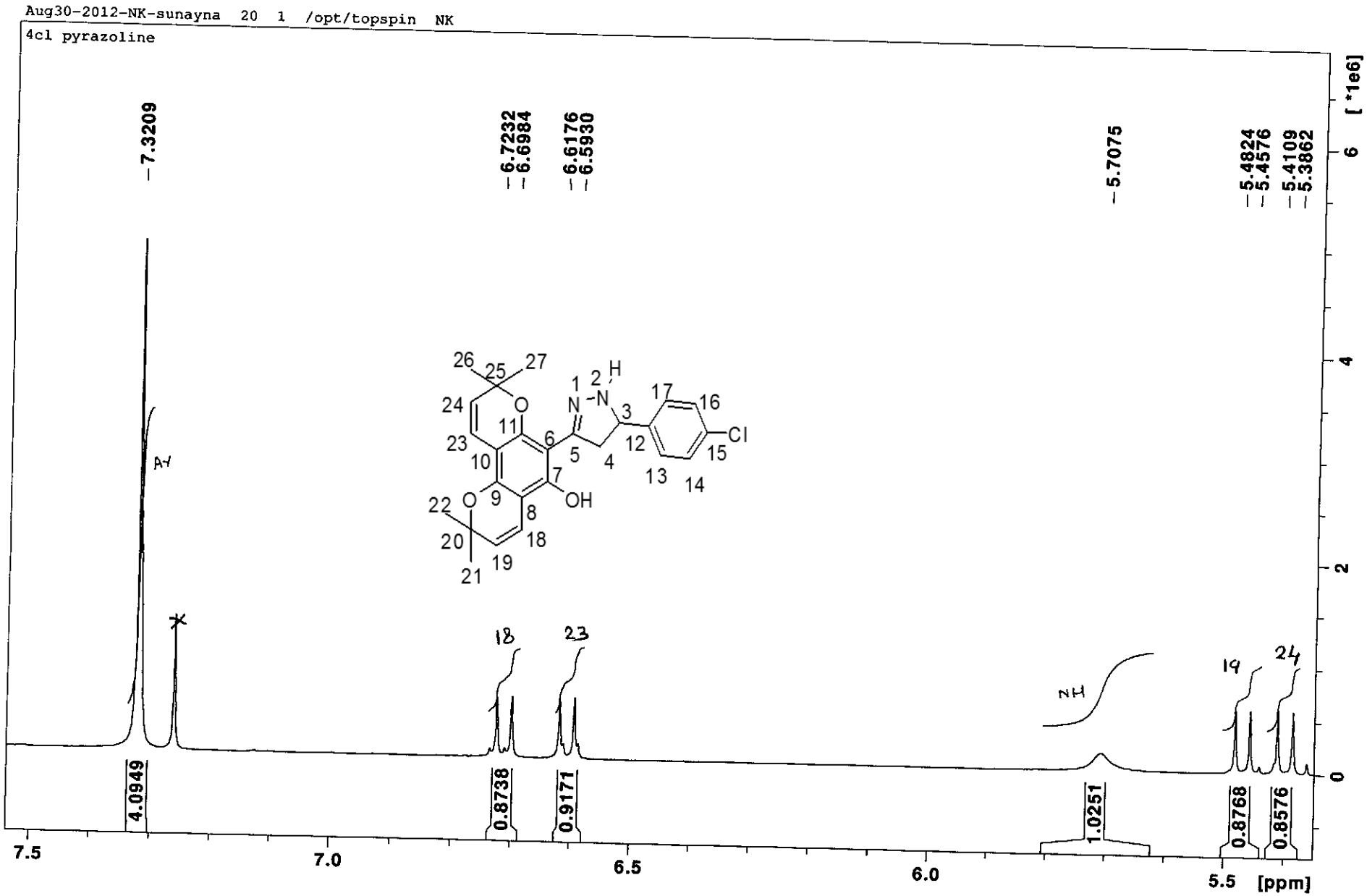


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
435.1465	435.1475	-1.0	-2.3	14.5	458.6	0.0	C25 H24 N2 O3 Cl

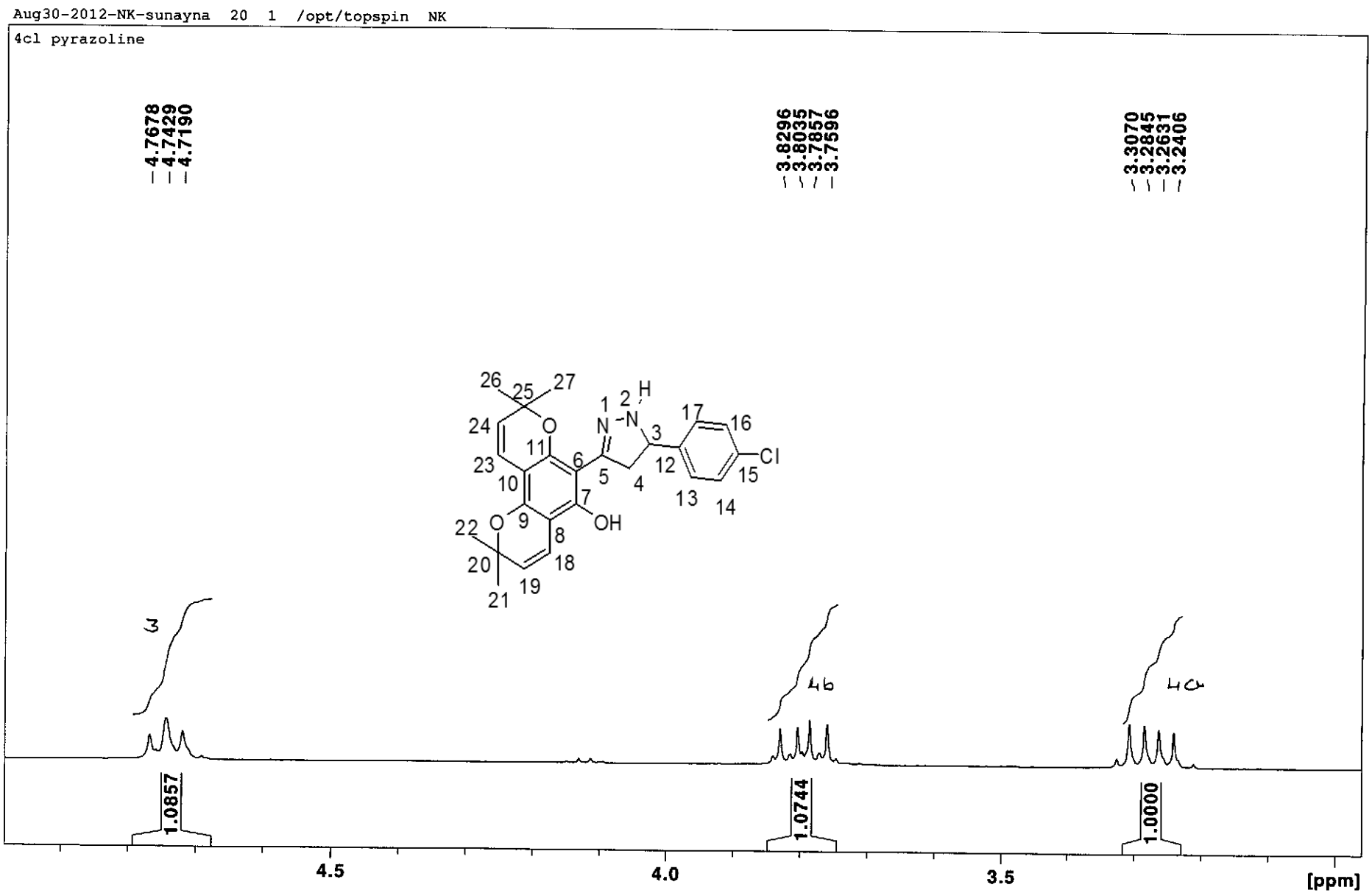
HRMS spectrum of 3-(2-chlorophenyl)-5-pyranochromene pyrazoline (C-4a)



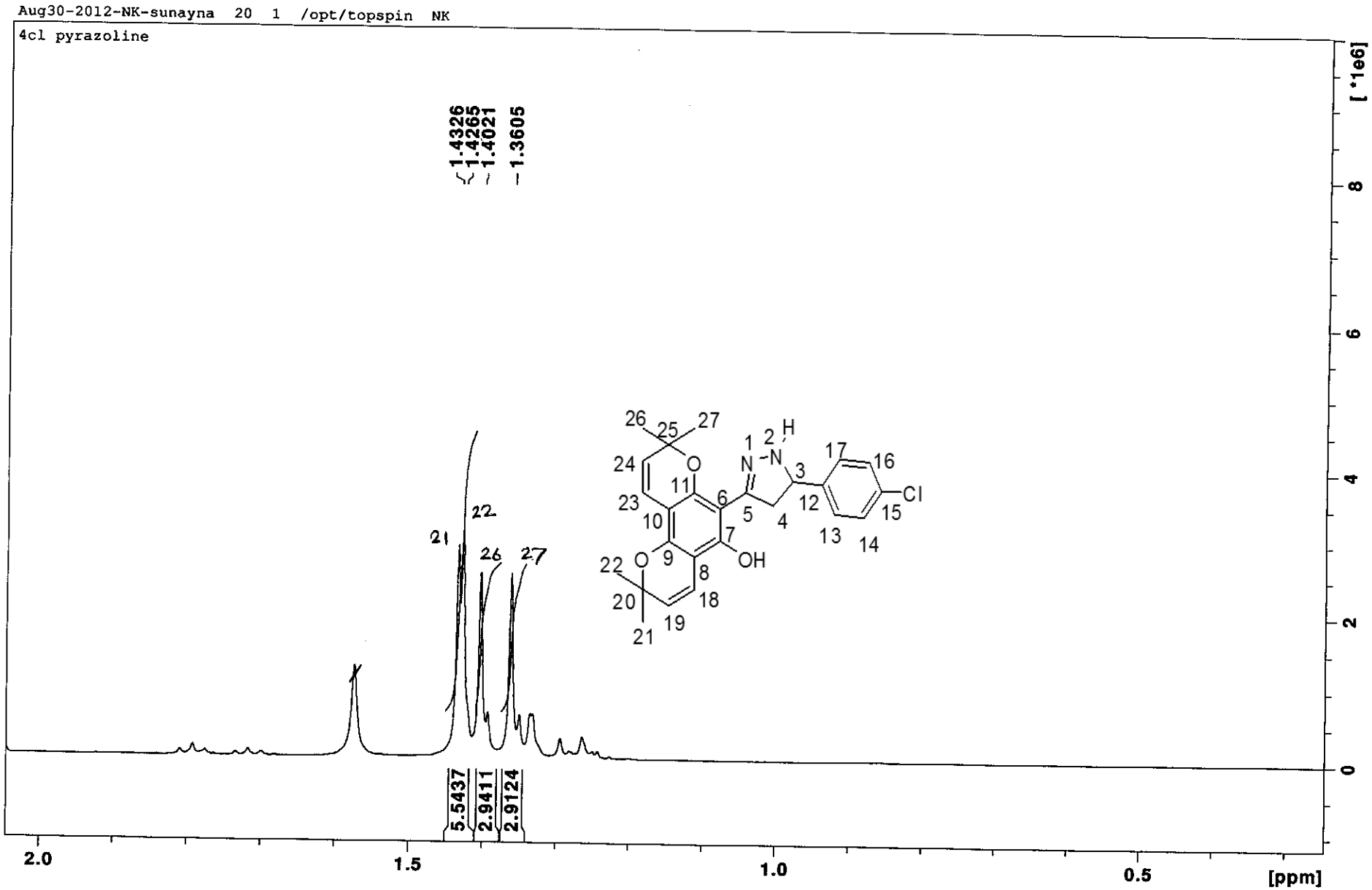
1H NMR spectrum of 3-(4-chlorophenyl)-5-pyranochromene pyrazoline (C-4b)



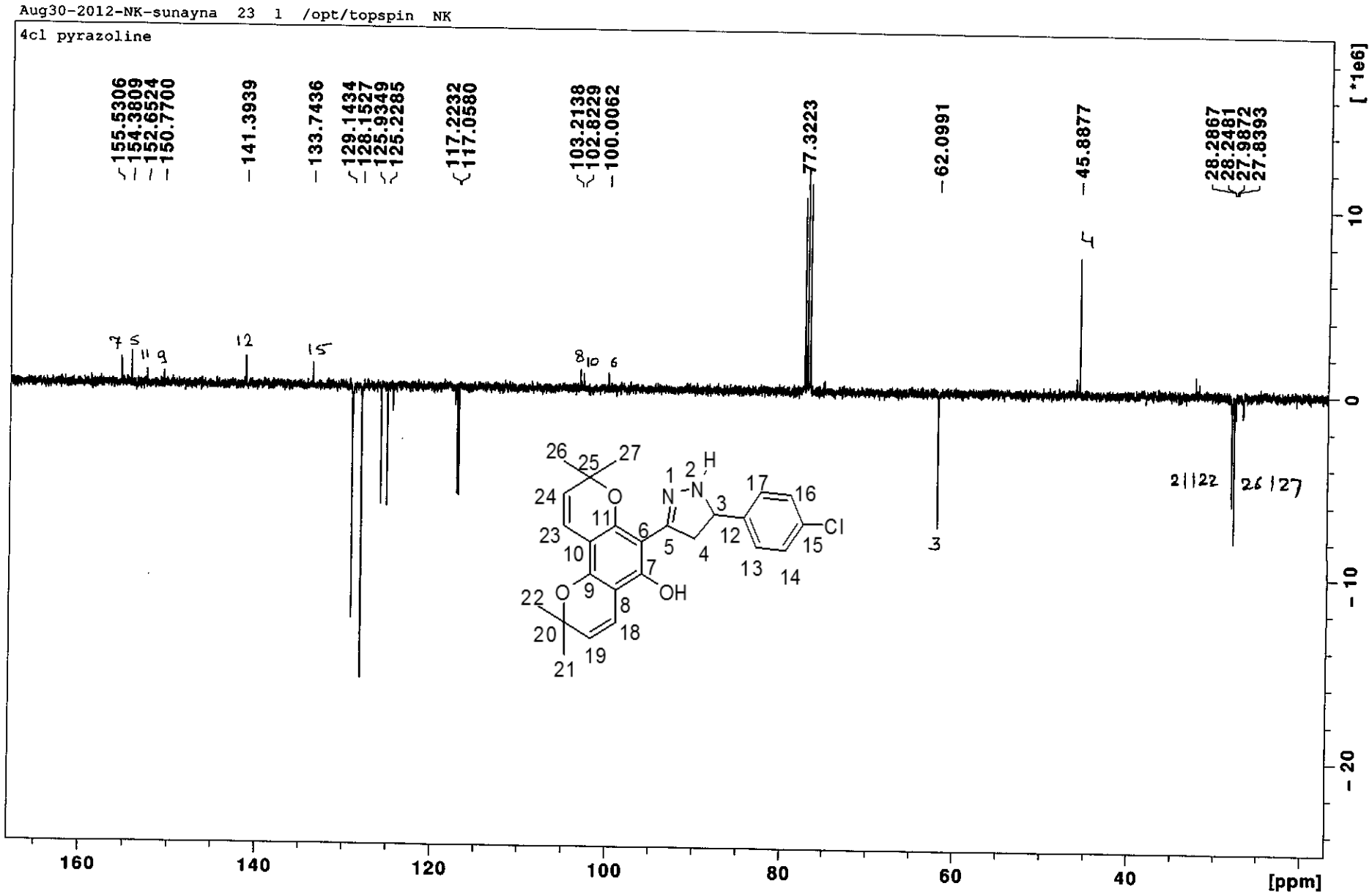
Expanded 1H NMR spectrum of 3-(4-chlorophenyl)-5-pyranochromene pyrazoline (C-4b)



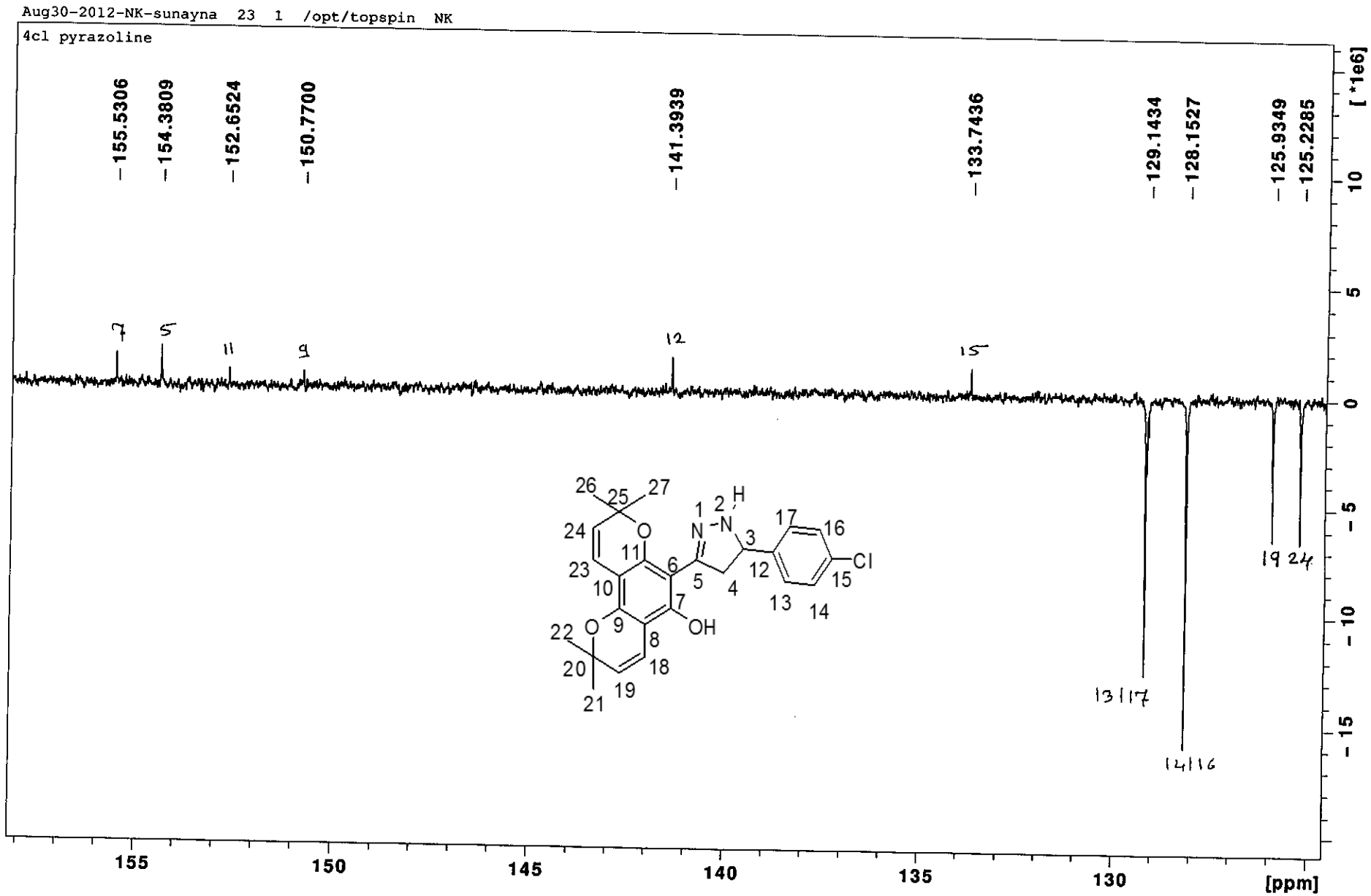
Expanded 1H NMR spectrum of 3-(4-chlorophenyl)-5-pyranochromene pyrazoline (C-4b)



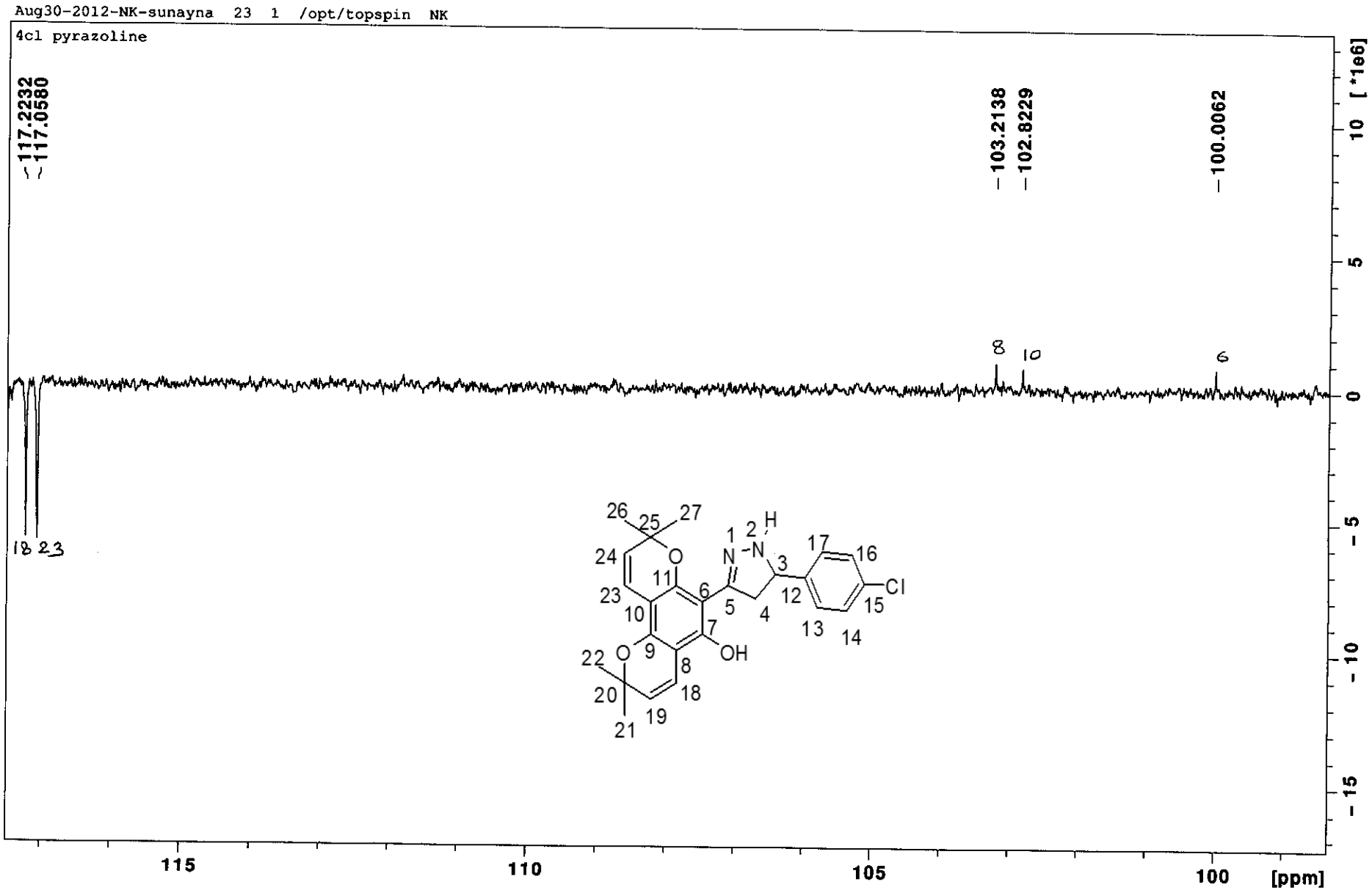
Expanded 1H NMR spectrum of 3-(4-chlorophenyl)-5-pyranochromene pyrazoline (C-4b)



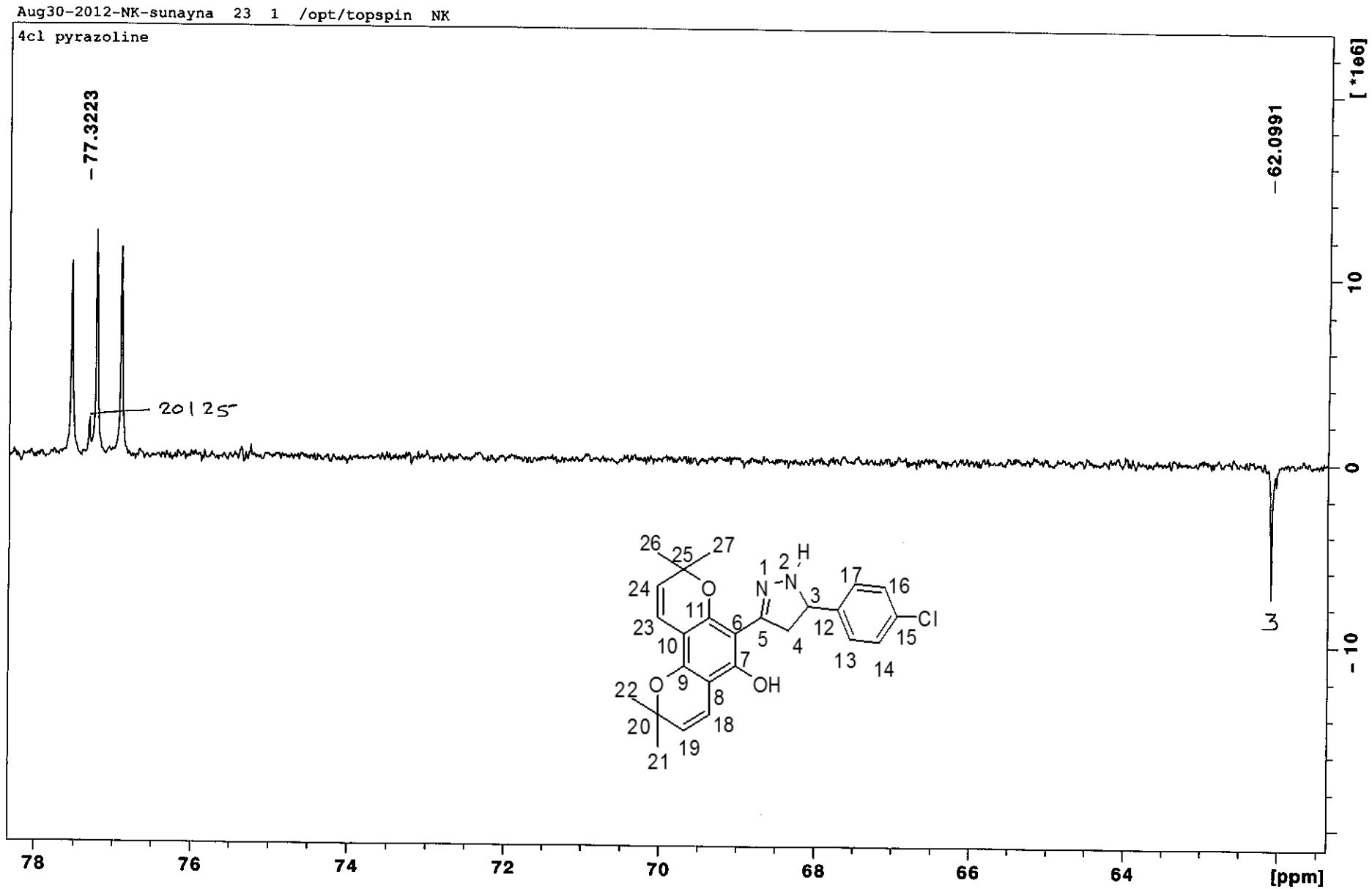
13C NMR spectrum of 3-(4-chlorophenyl)-5-pyranochromene pyrazoline (C-4b)



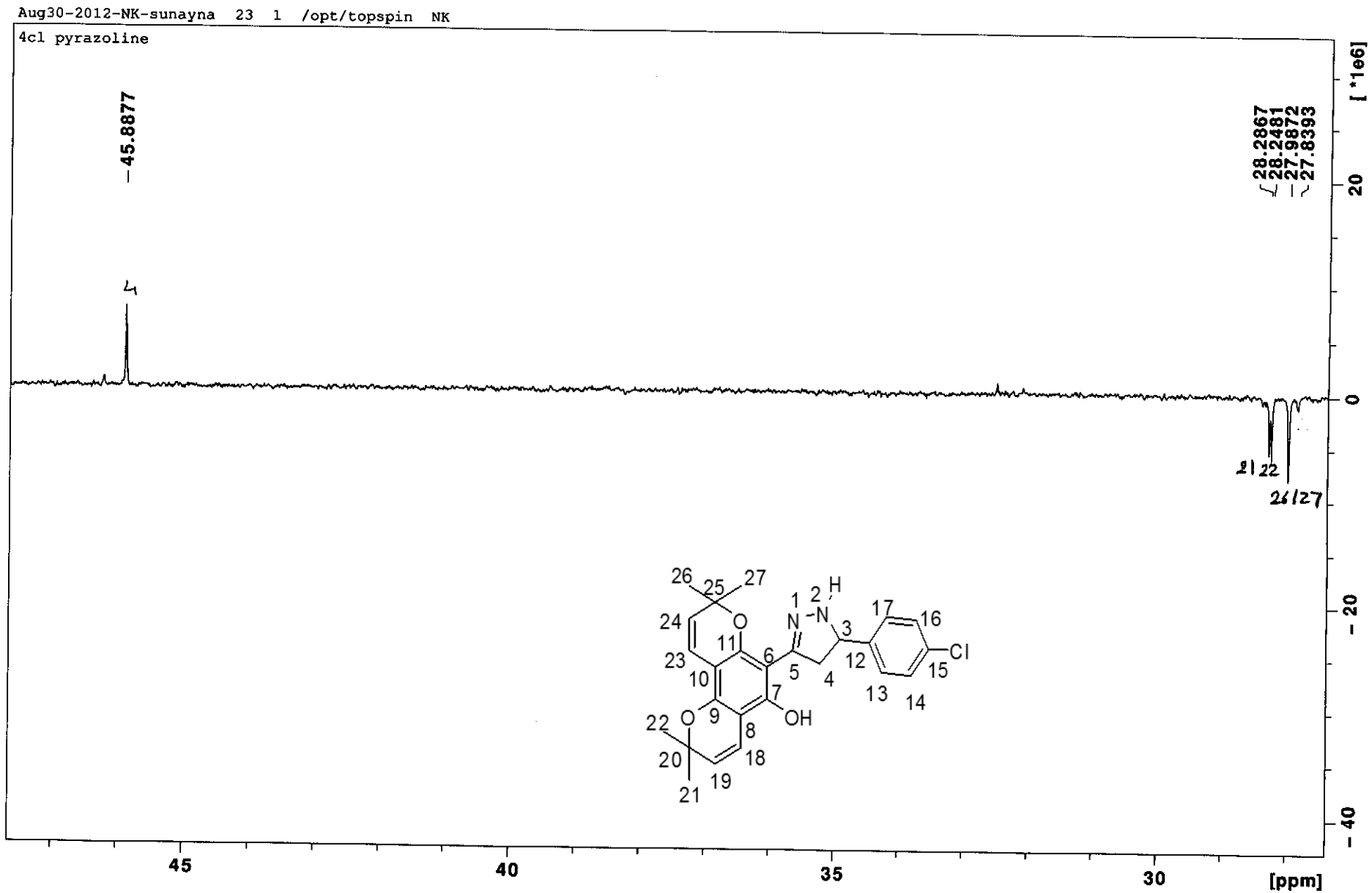
Expanded 13C NMR spectrum of 3-(4-chlorophenyl)-5-pyranochromene pyrazoline (C-4b)



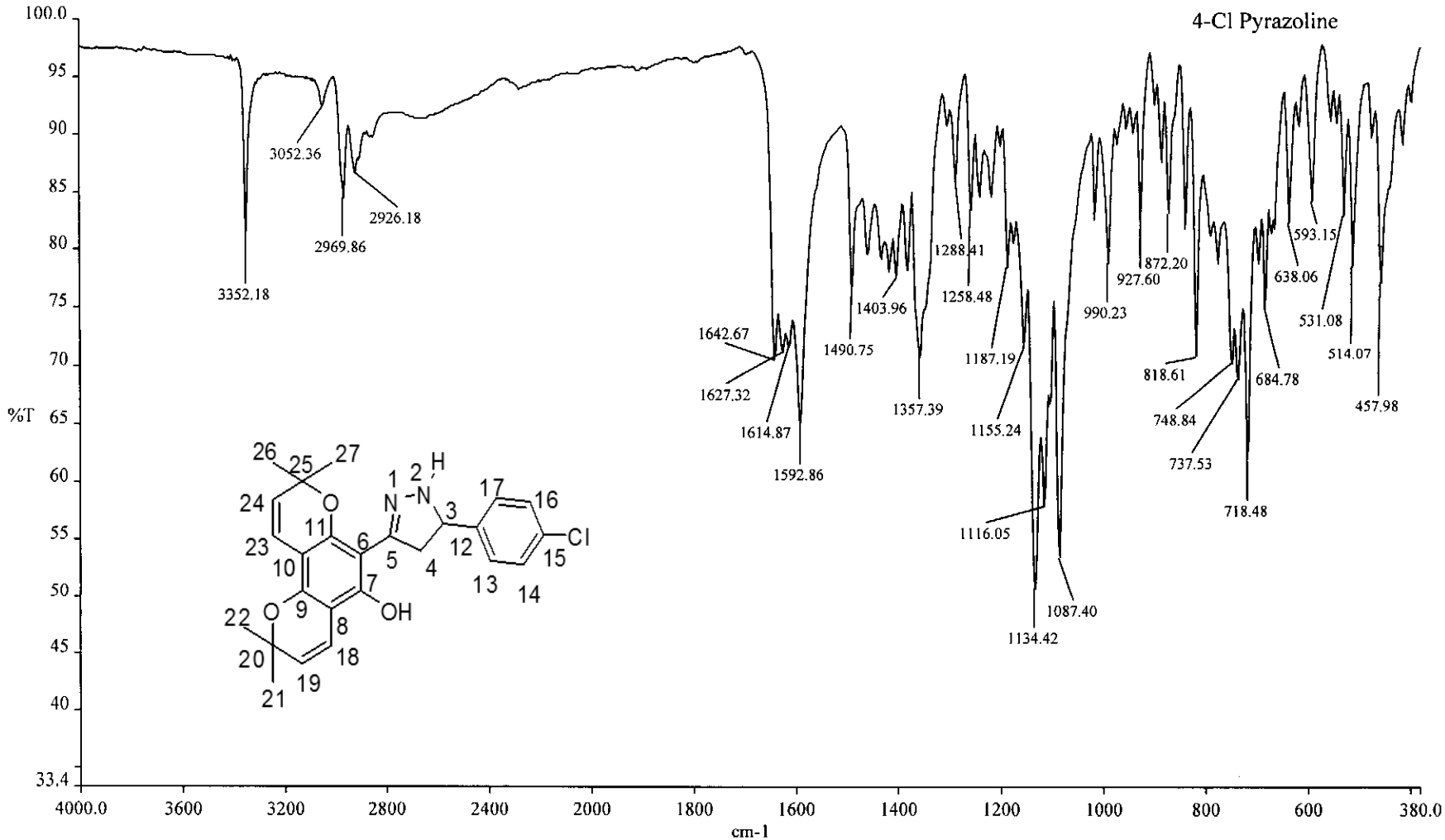
Expanded 13C NMR spectrum of 3-(4-chlorophenyl)-5-pyranochromene pyrazoline (C-4b)



Expanded 13C NMR spectrum of 3-(4-chlorophenyl)-5-pyranochromene pyrazoline (C-4b)



Expanded 13C NMR spectrum of 3-(4-chlorophenyl)-5-pyranochromene pyrazoline (C-4b)

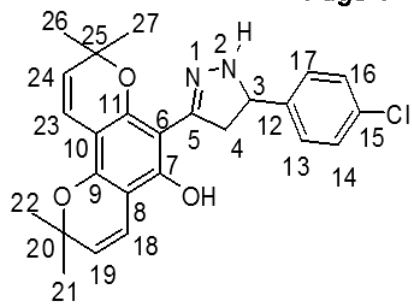


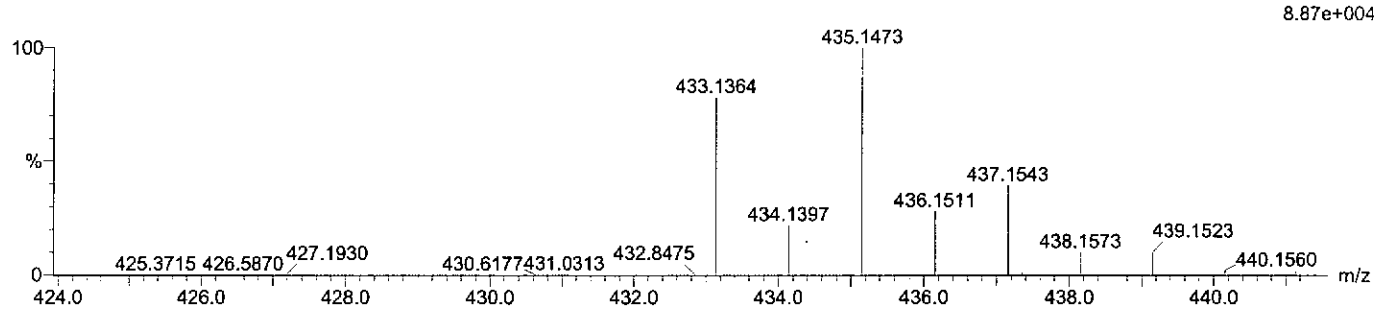
Infrared spectrum of 3-(4-chlorophenyl)-5-pyranochromene pyrazoline (C-4b)

Elemental Composition Report **Page 1**

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

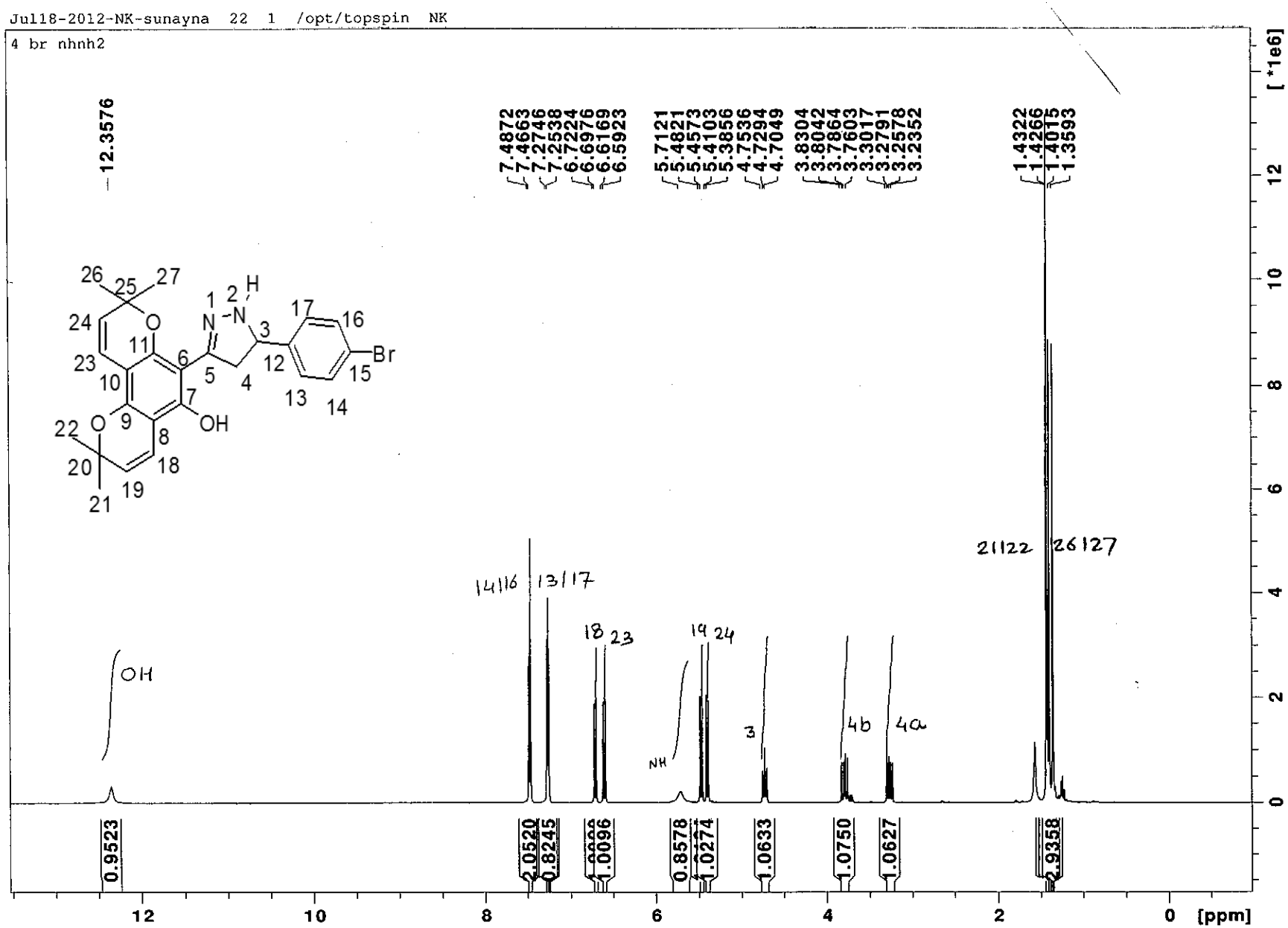
Monoisotopic Mass, Even Electron Ions
25 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-35 H: 20-30 N: 0-4 O: 0-4 Cl: 1-1
3 6 (0.086) Cm (1:31)
TOF MS ES-



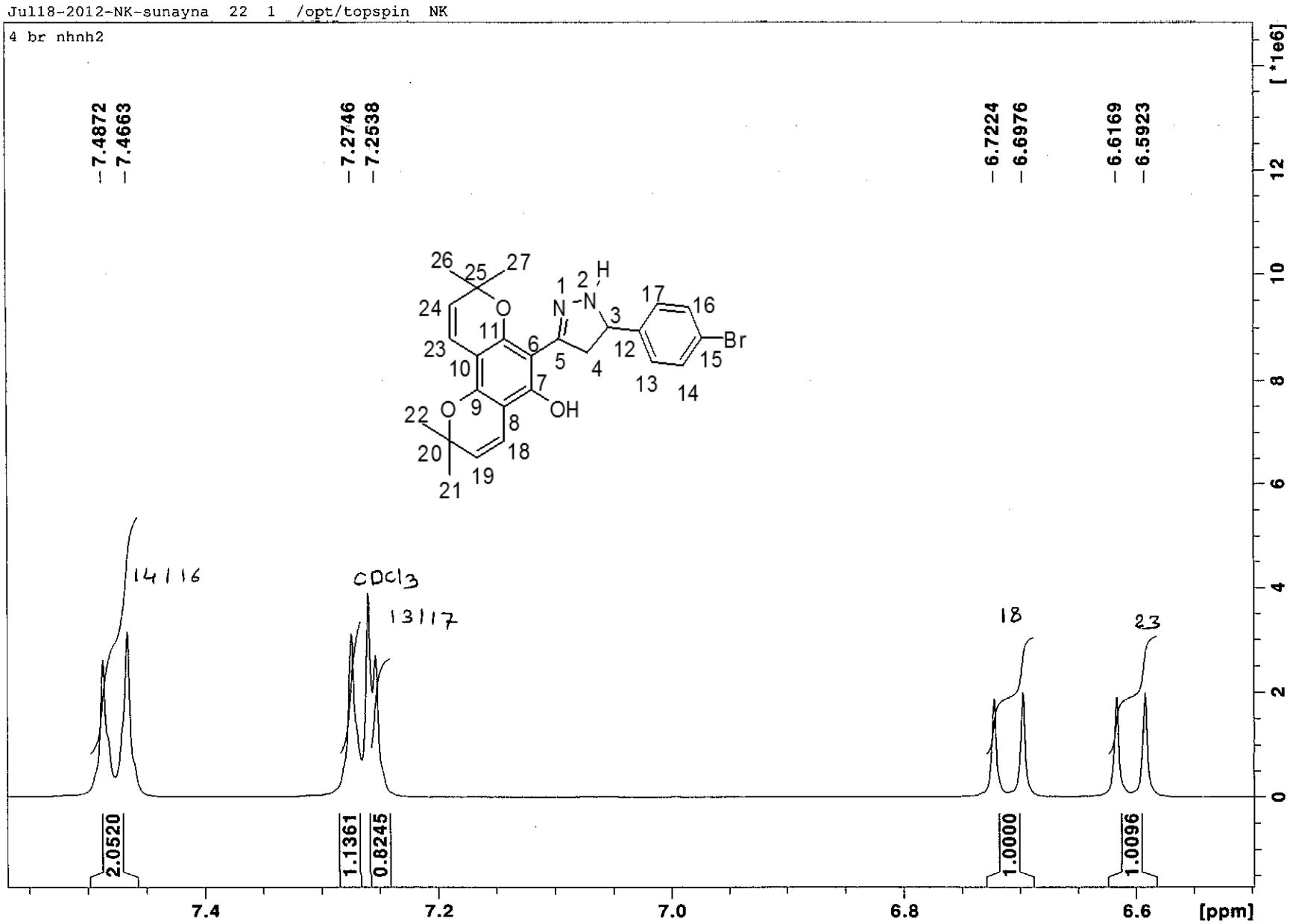


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
435.1473	435.1475	-0.2	-0.5	14.5	511.3	0.0	C25 H24 N2 O3 Cl

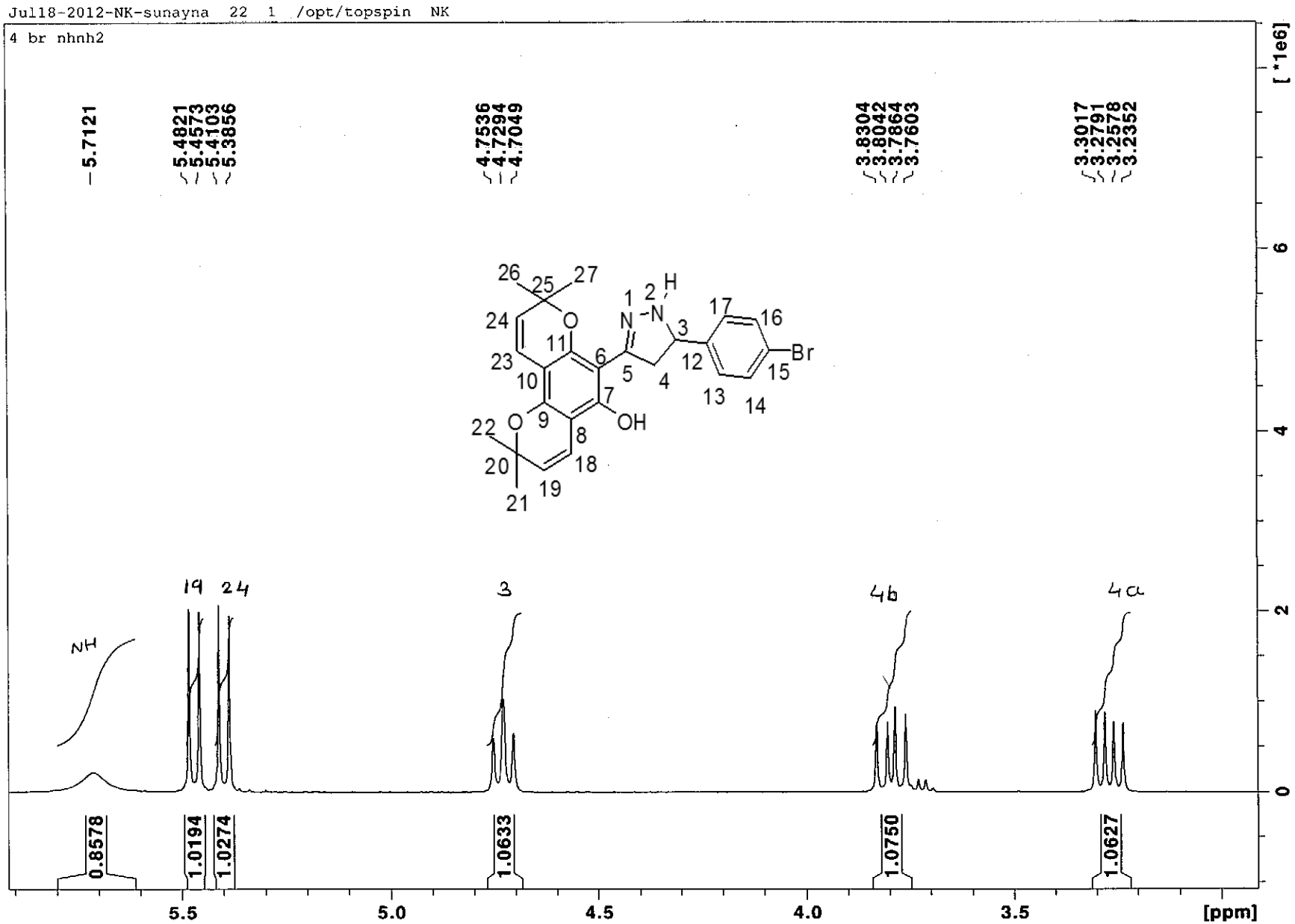
HRMS spectrum of 3-(4-chlorophenyl)-5-pyranochromene pyrazoline (C-4b)



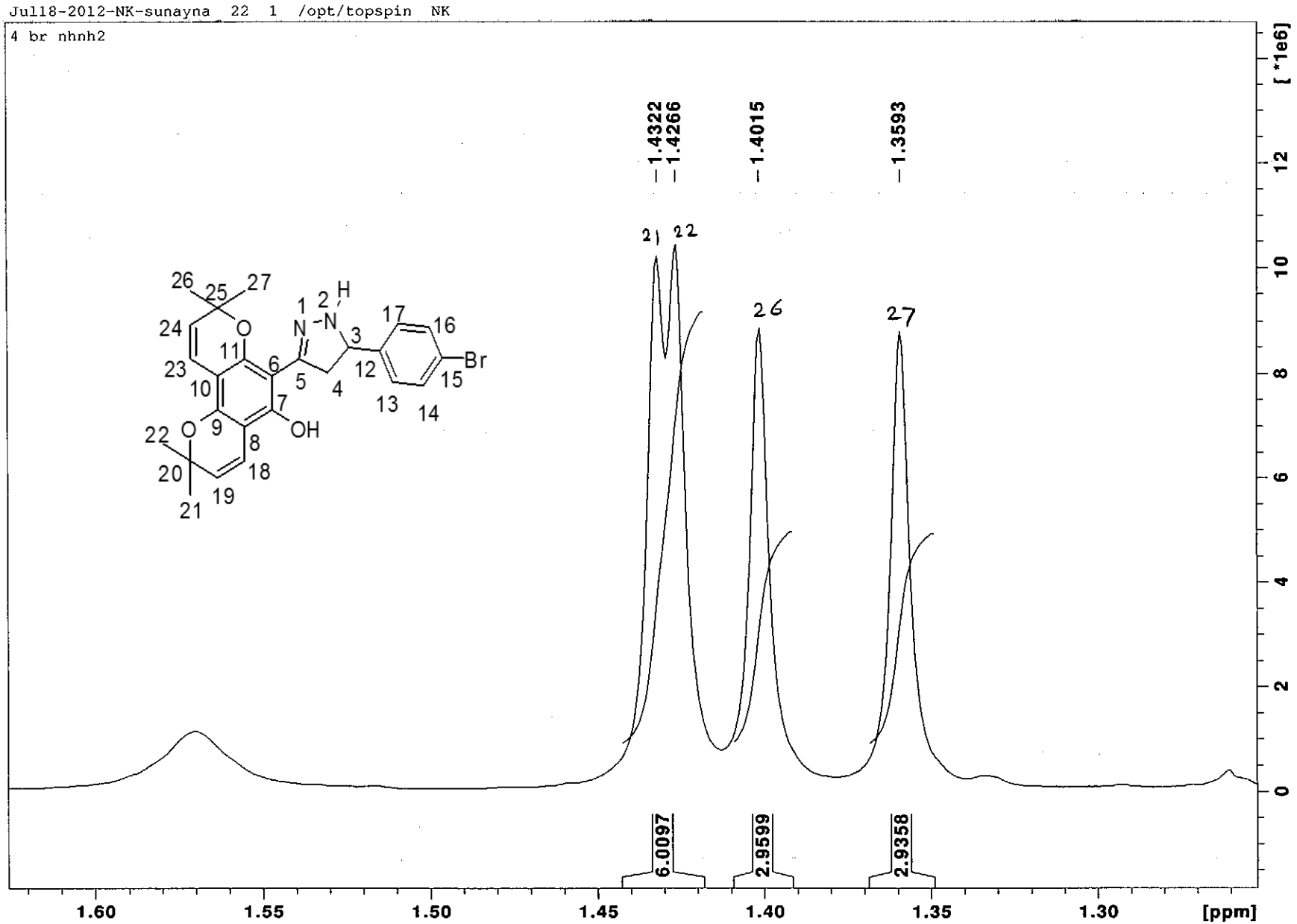
1H NMR spectrum of 3-(4-bromophenyl)-5-pyranochromene pyrazoline (C-4c)



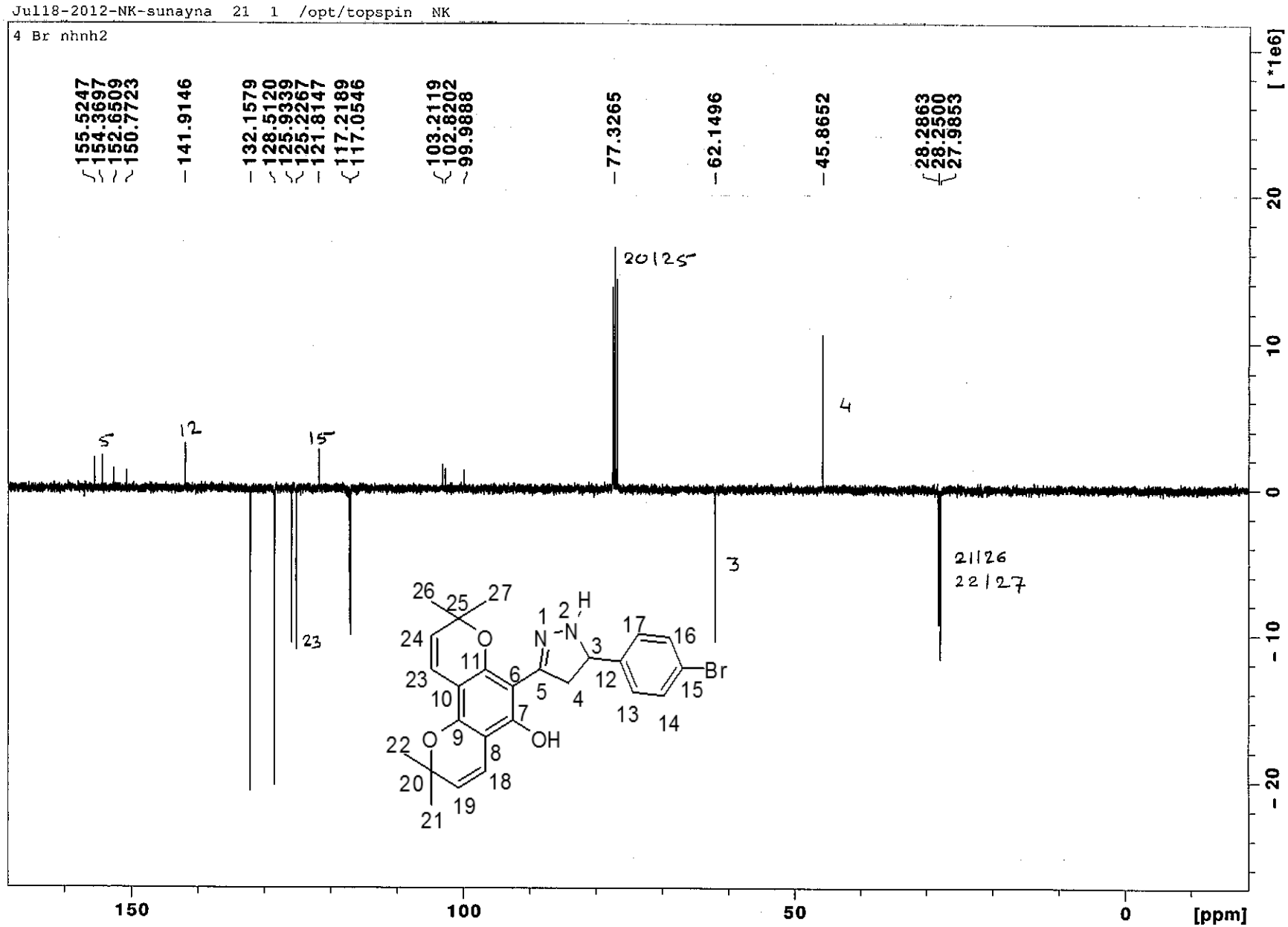
Expanded 1H NMR spectrum of 3-(4-bromophenyl)-5-pyranochromene pyrazoline (C-4c)



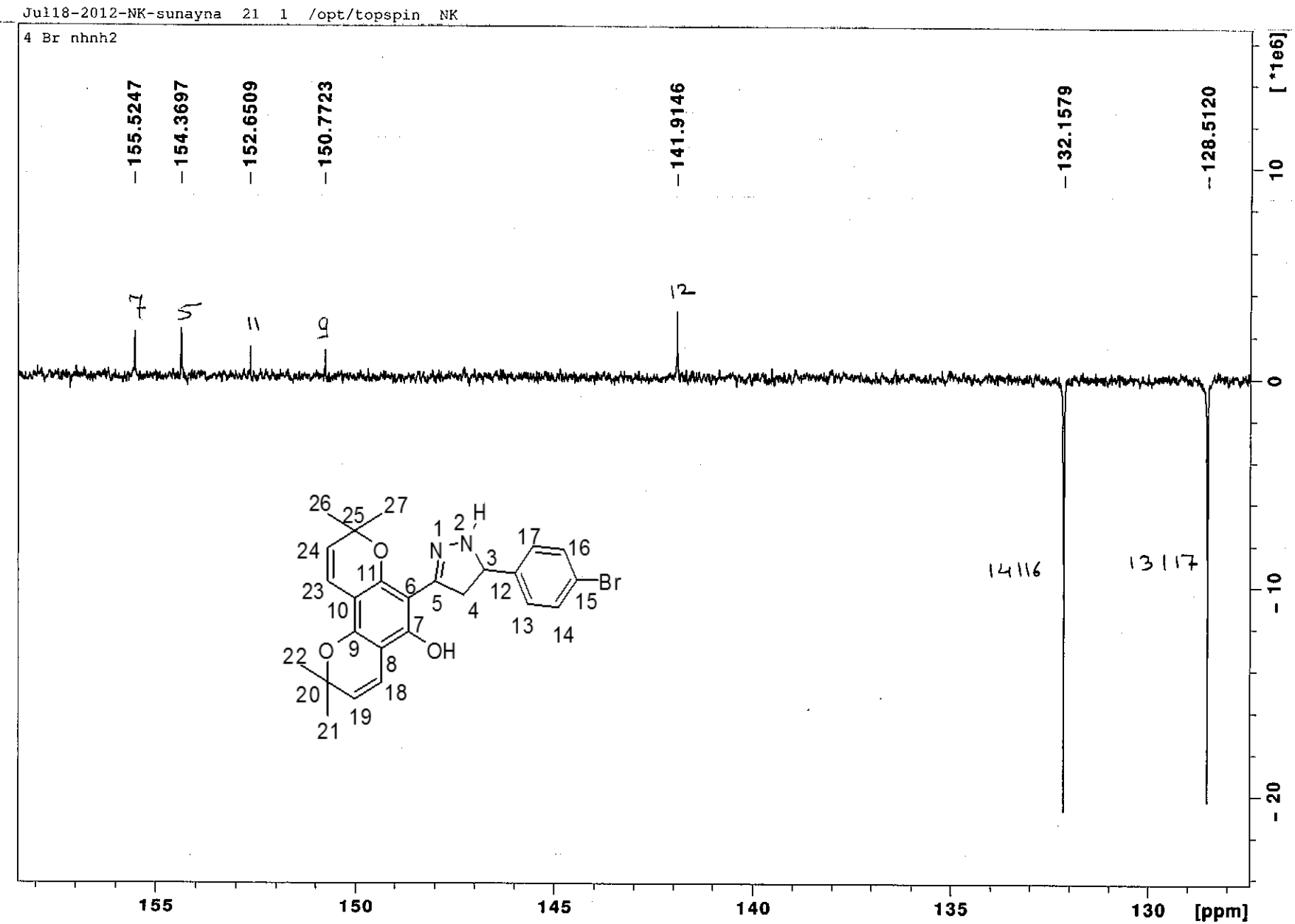
Expanded 1H NMR spectrum of 3-(4-bromophenyl)-5-pyranochromene pyrazoline (C-4c)



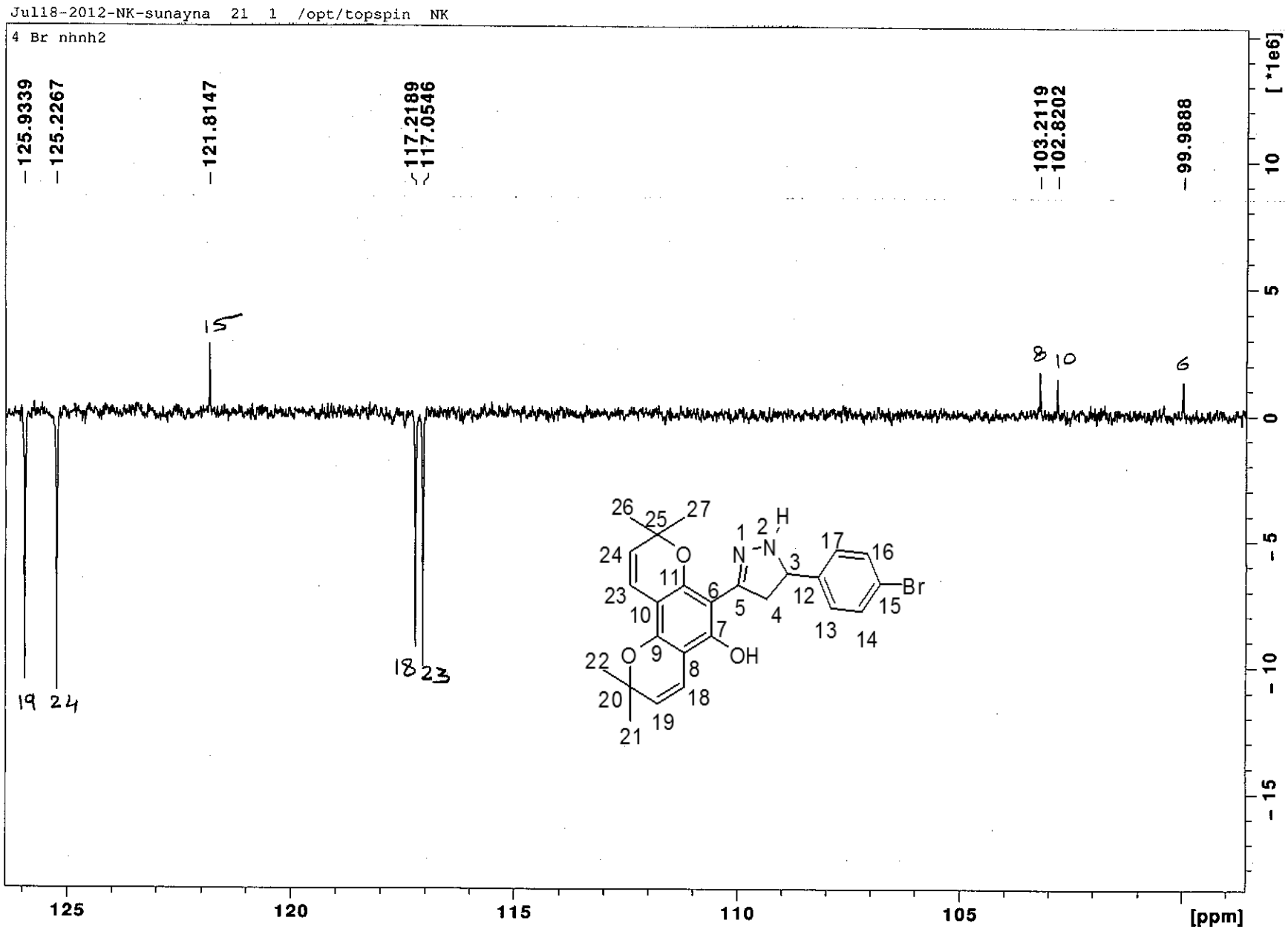
Expanded 1H NMR spectrum of 3-(4-bromophenyl)-5-pyranochromene pyrazoline (C-4c)



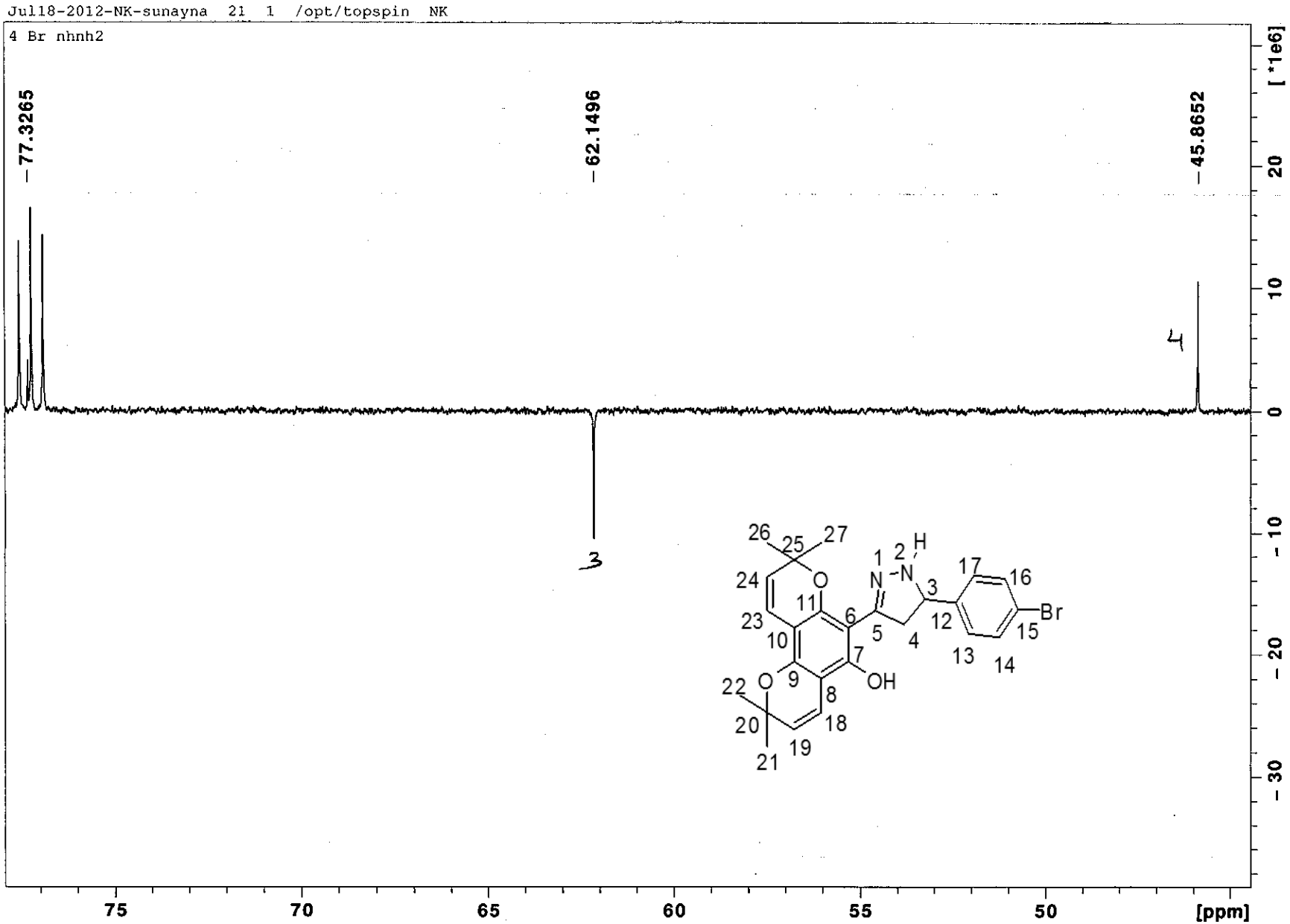
13C NMR spectrum of 3-(4-bromophenyl)-5-pyranochromene pyrazoline (C-4c)



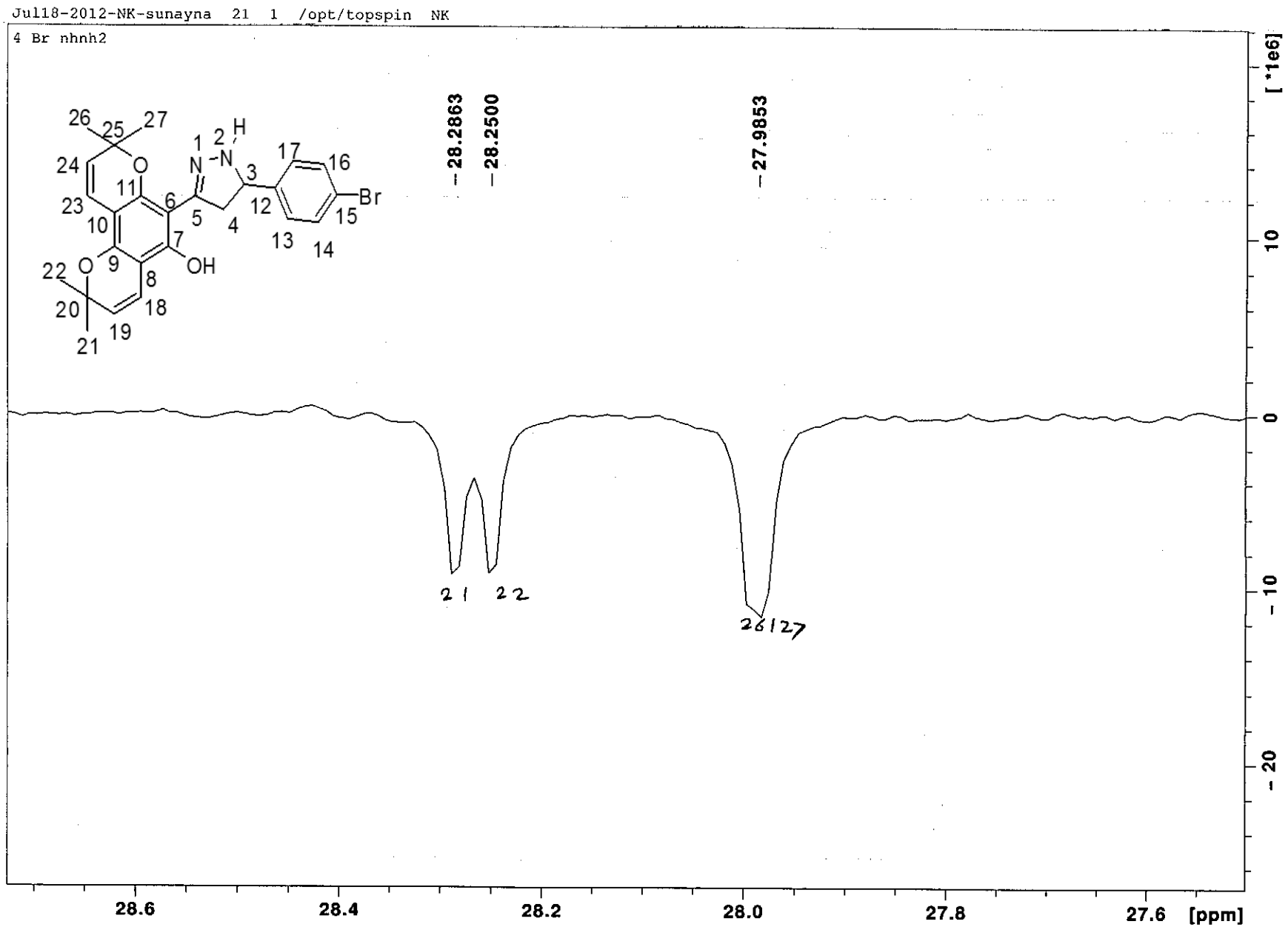
Expanded 13C NMR spectrum of 3-(4-bromophenyl)-5-pyranochromene pyrazoline (C-4c)



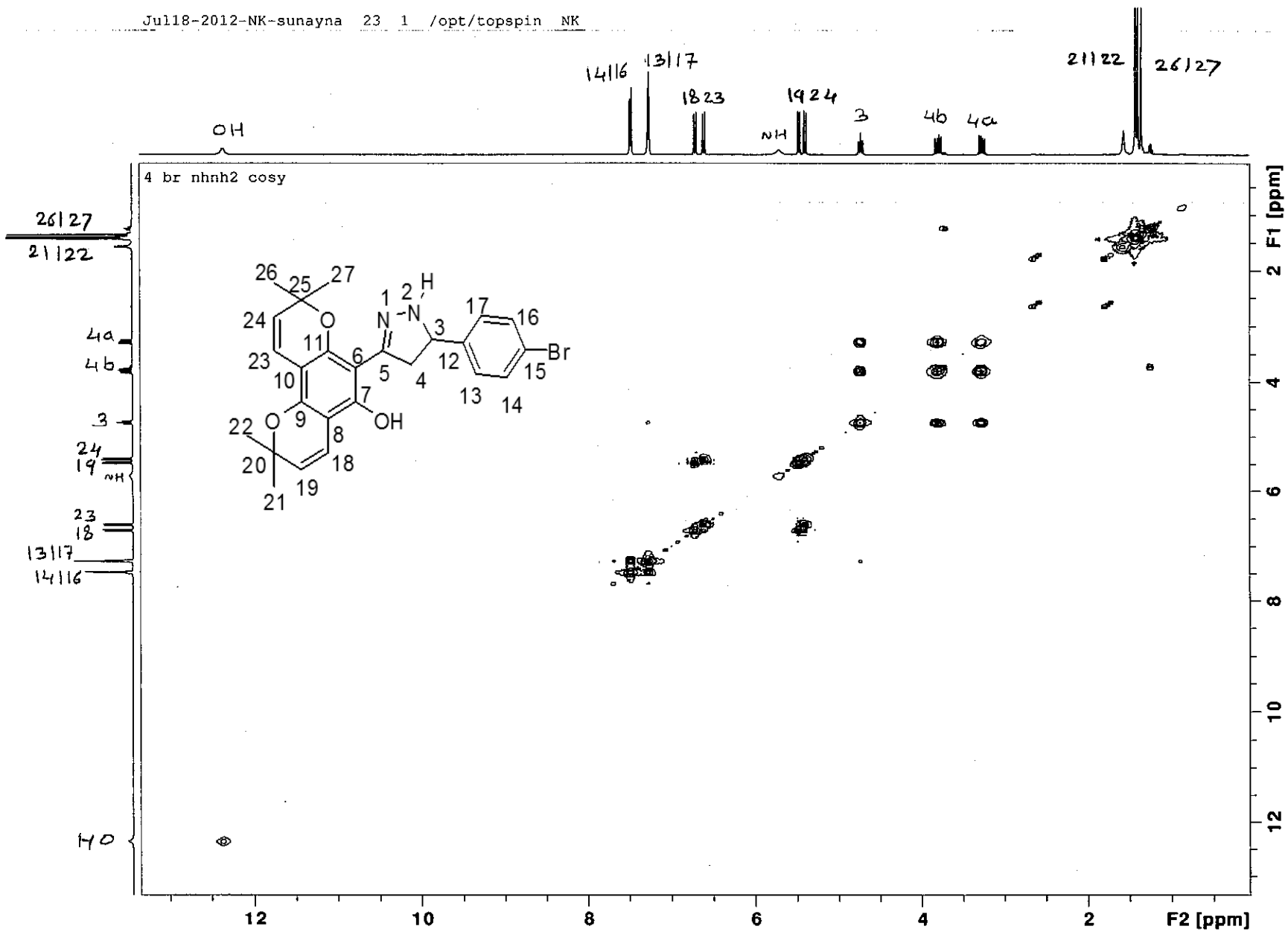
Expanded 13C NMR spectrum of 3-(4-bromophenyl)-5-pyranochromene pyrazoline (C-4c)



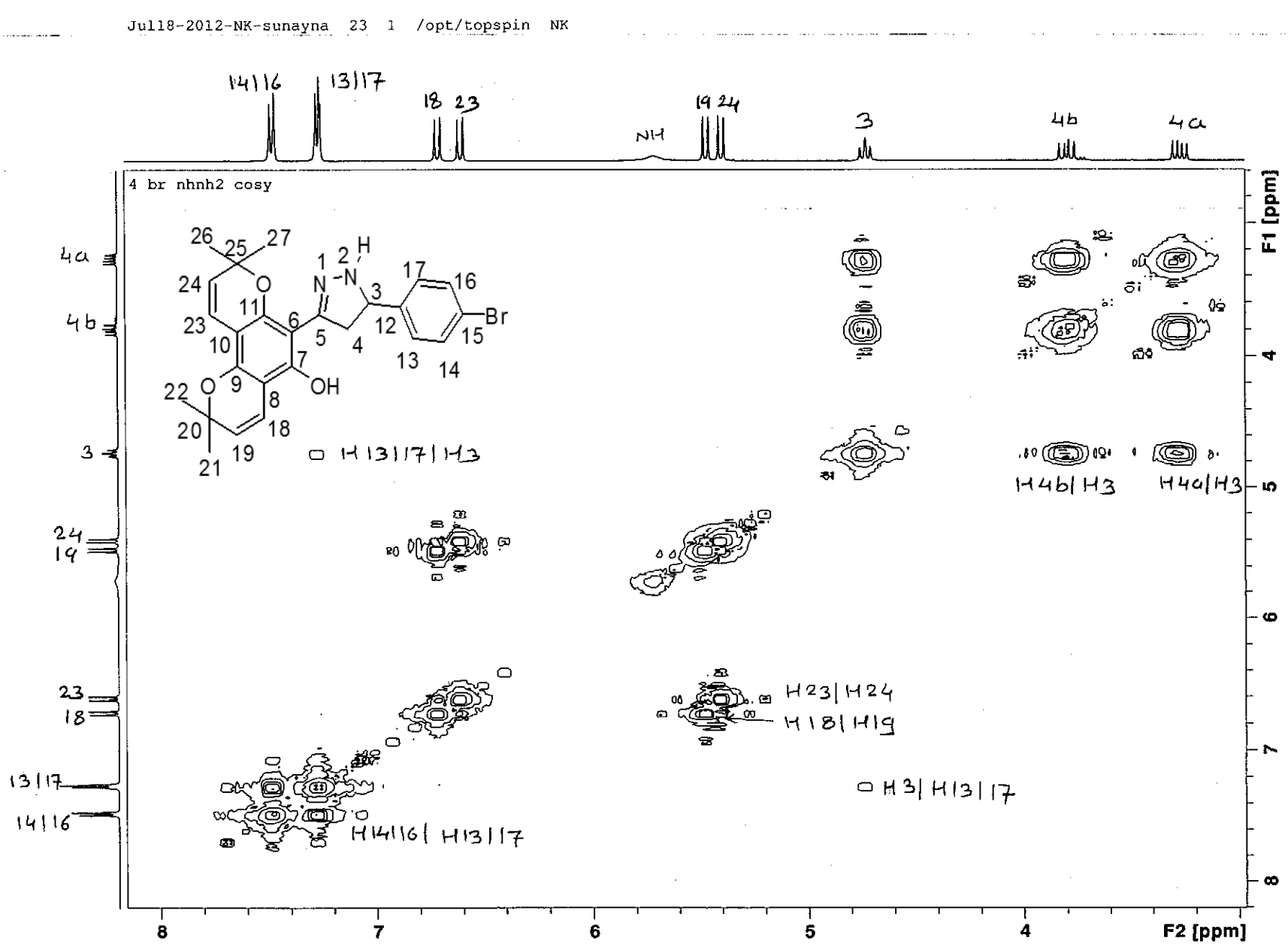
Expanded 13C NMR spectrum of 3-(4-bromophenyl)-5-pyranochromene pyrazoline (C-4c)



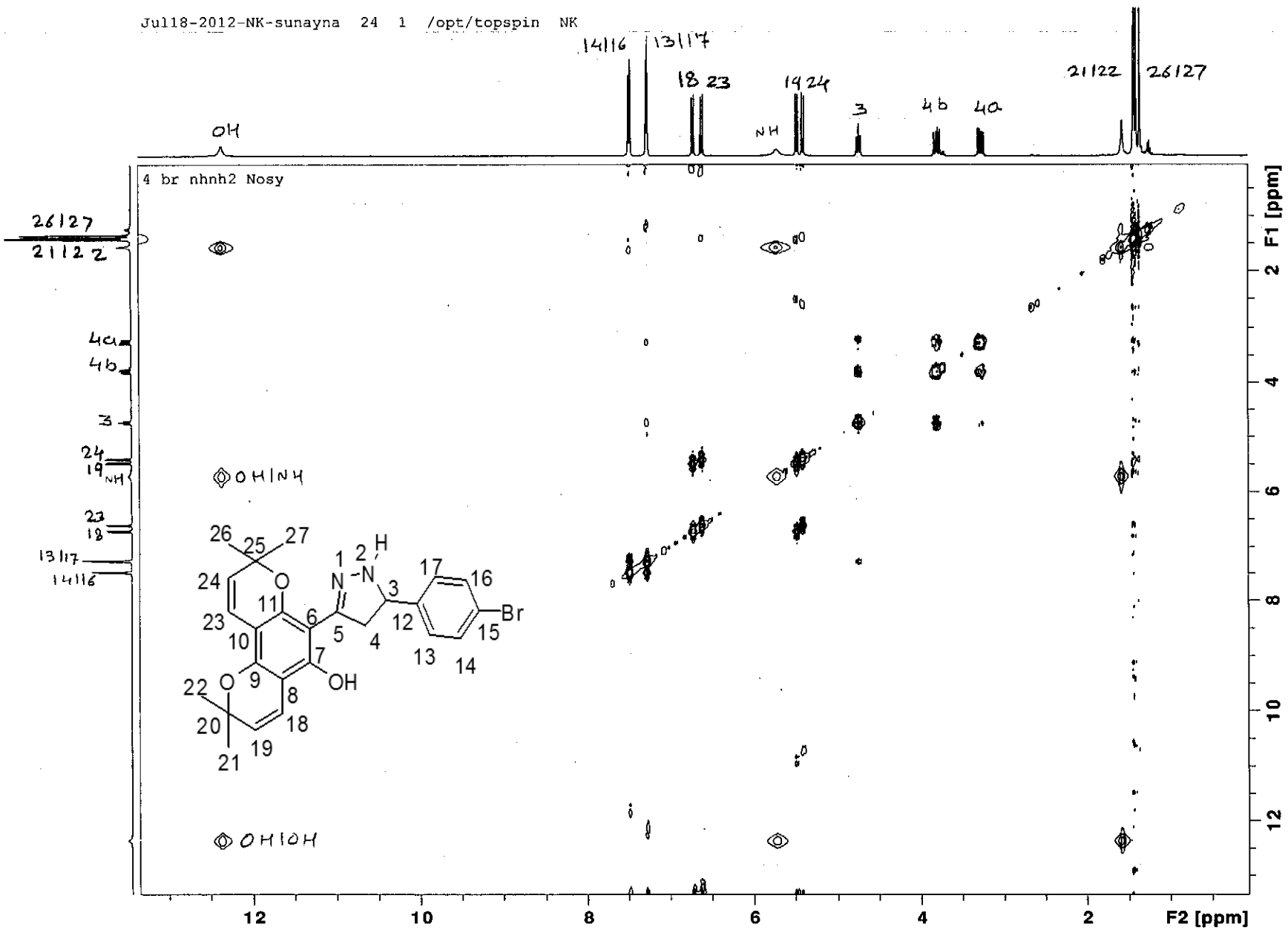
Expanded 13C NMR spectrum of 3-(4-bromophenyl)-5-pyranochromene pyrazoline (C-4c)



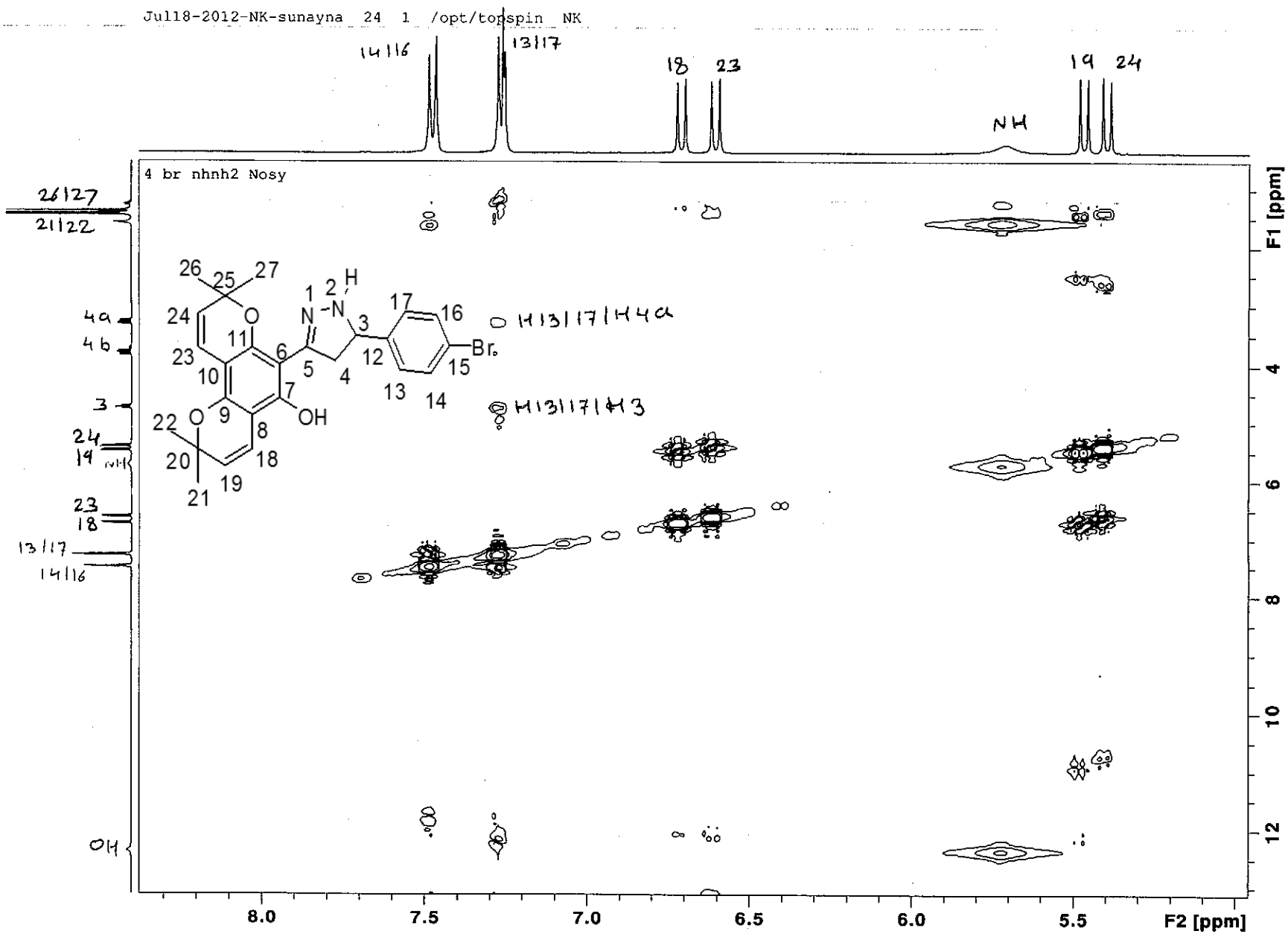
COSY NMR spectrum of 3-(4-bromophenyl)-5-pyranochromene pyrazoline (C-4c)



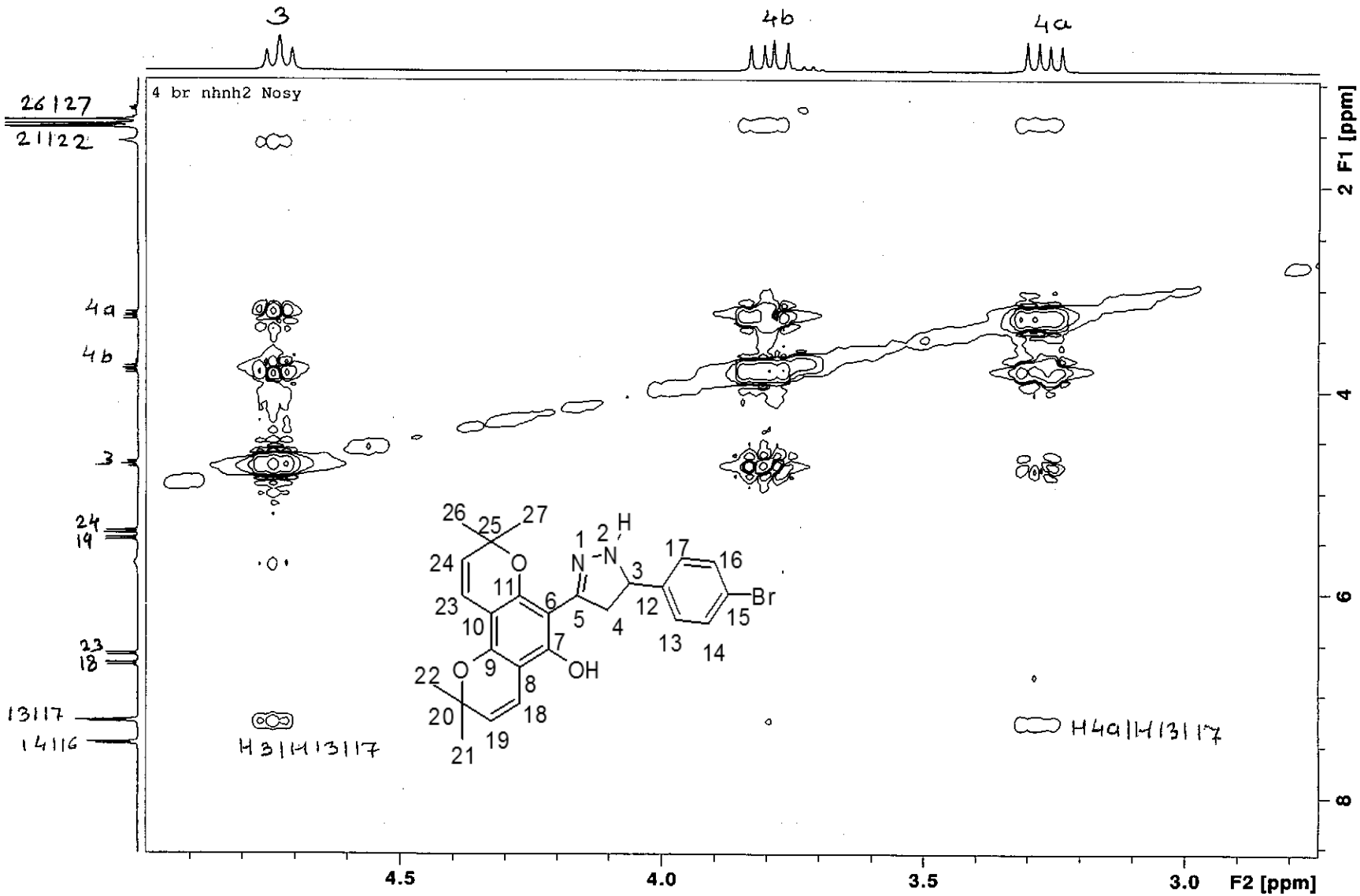
Expanded COSY NMR spectrum of 3-(4-bromophenyl)-5-pyranochromene pyrazoline (C-4c)



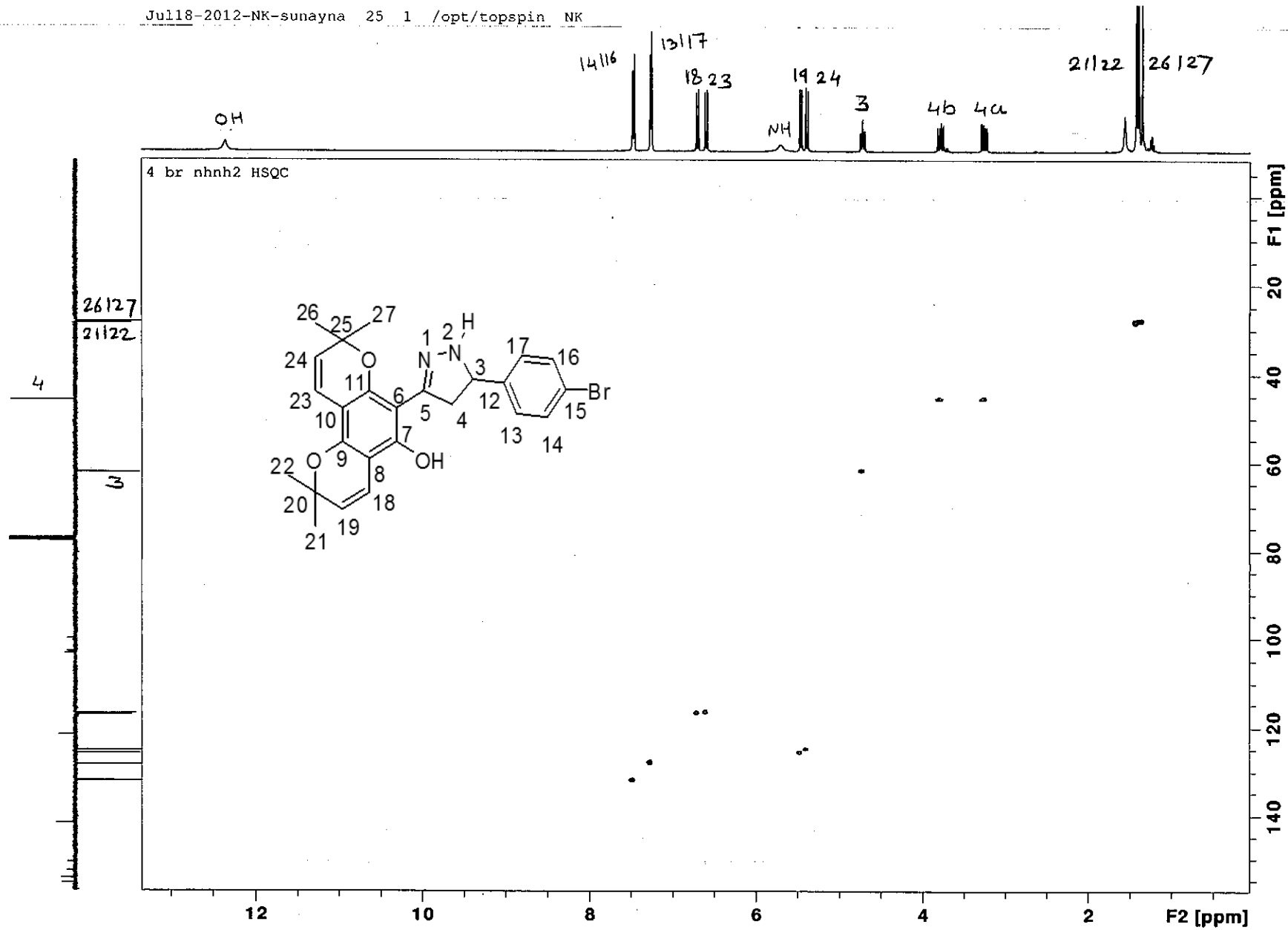
NOESY NMR spectrum of 3-(4-bromophenyl)-5-pyranochromene pyrazoline (C-4c)



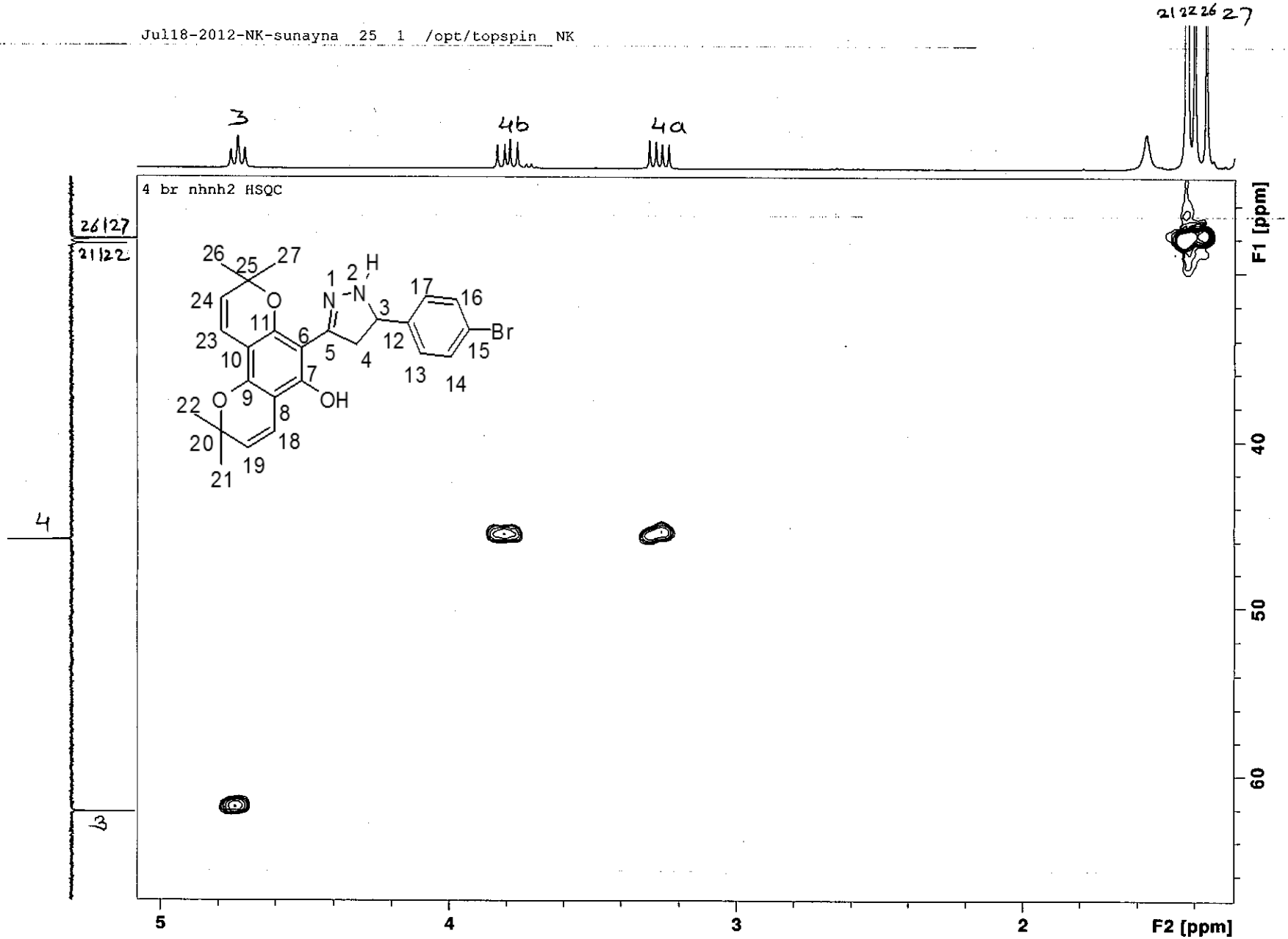
Expanded NOESY spectrum of 3-(4-bromophenyl)-5-pyranochromene pyrazoline (C-4c)



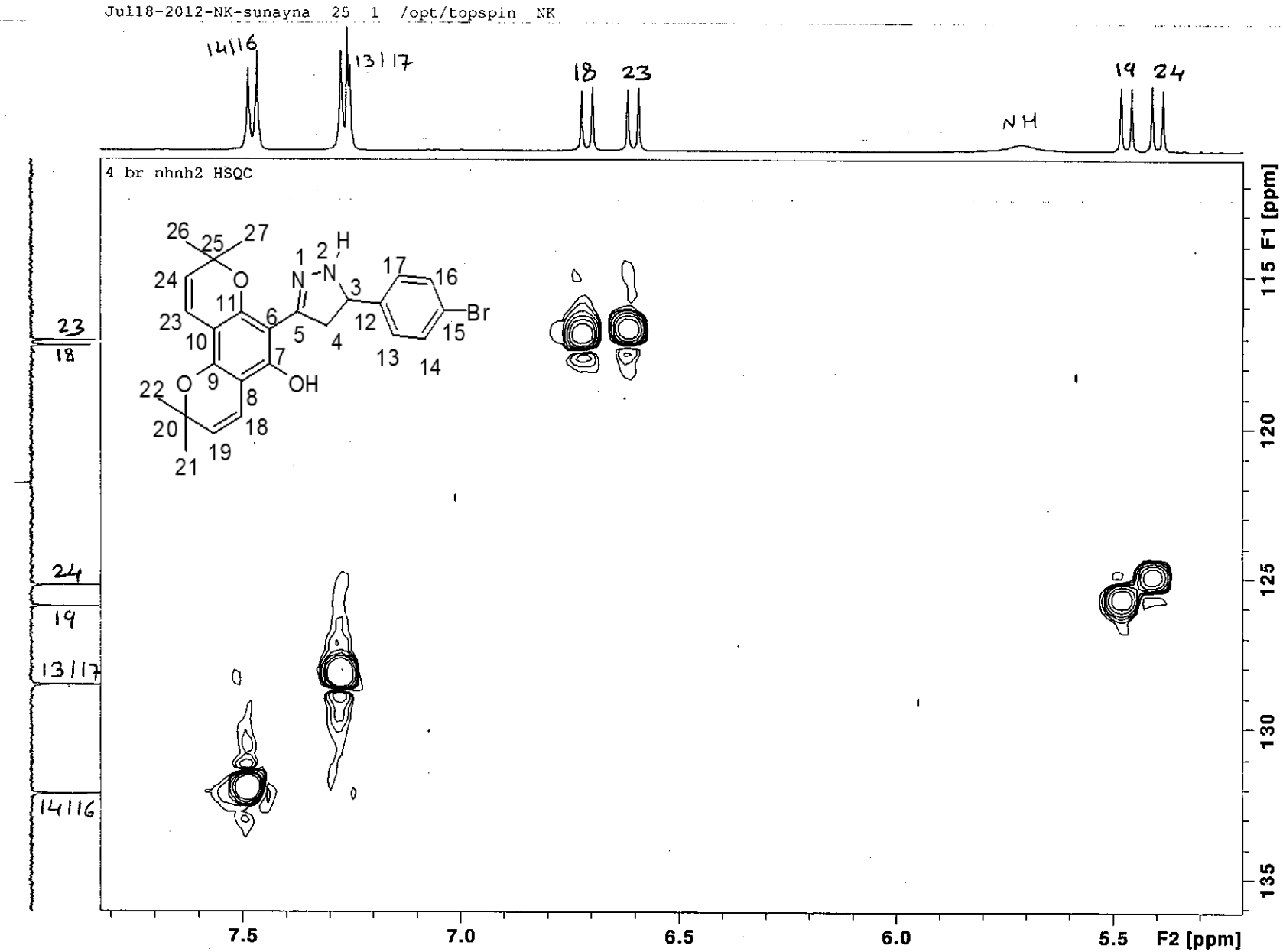
Expanded NOESY spectrum of 3-(4-bromophenyl)-5-pyranochromene pyrazoline (C-4c)



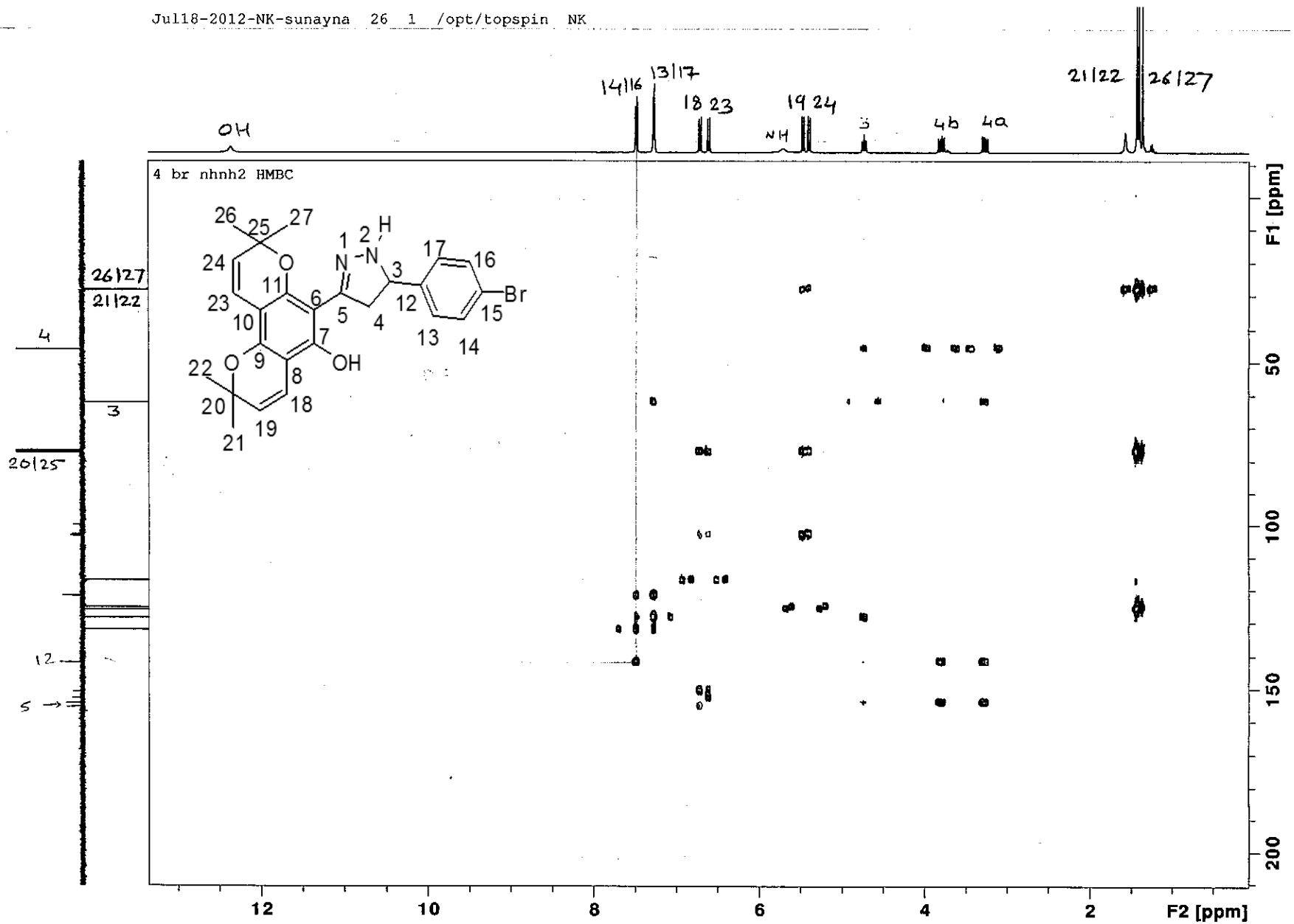
HSQC spectrum of 3-(4-bromophenyl)-5-pyranochromene pyrazoline (C-4c)



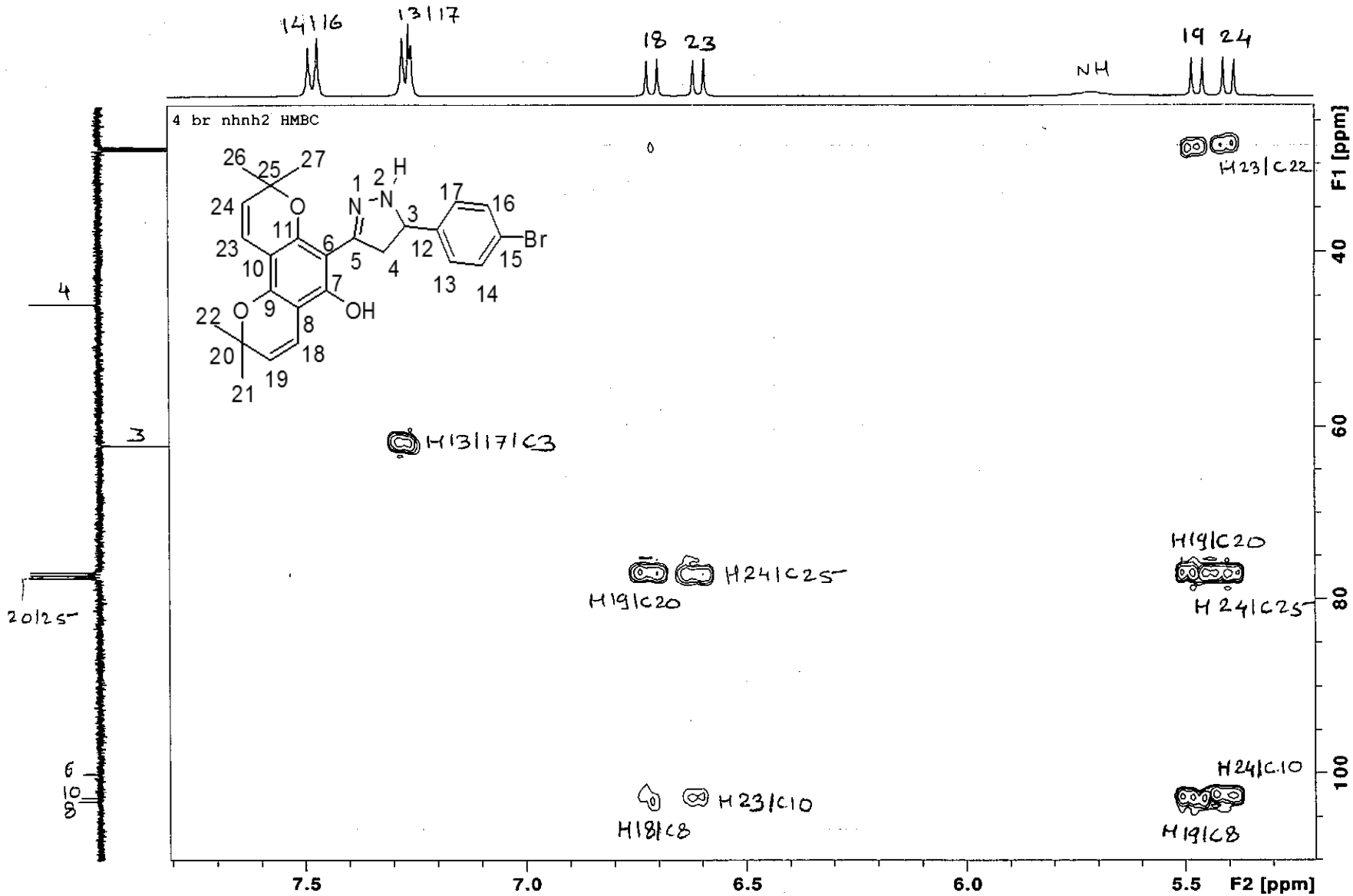
HSQC spectrum of 3-(4-bromophenyl)-5-pyranochromene pyrazoline (C-4c)



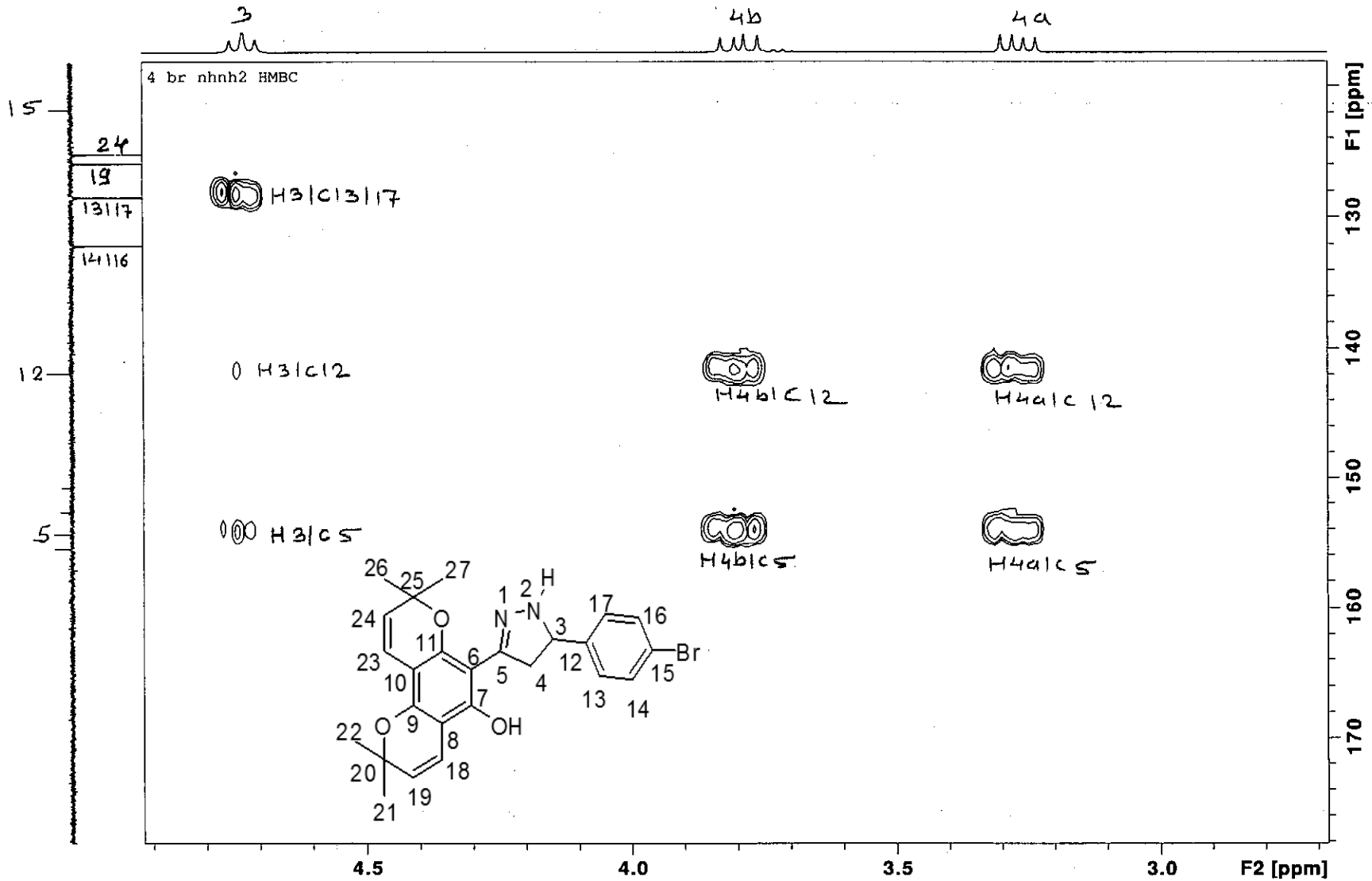
HSQC spectrum of 3-(4-bromophenyl)-5-pyranochromene pyrazoline (C-4c)



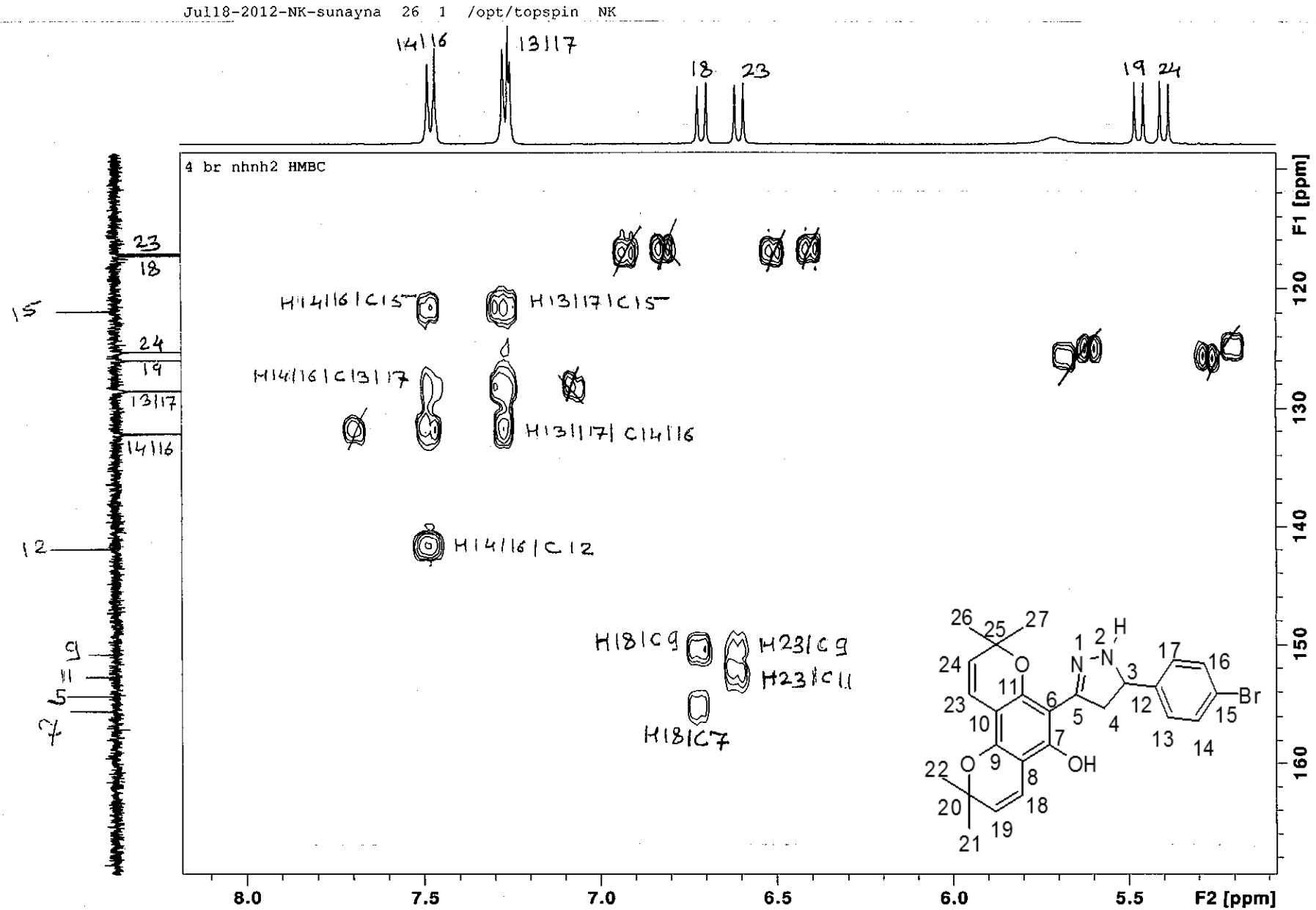
HMBC spectrum of 3-(4-bromophenyl)-5-pyranochromene pyrazoline (C-4c)



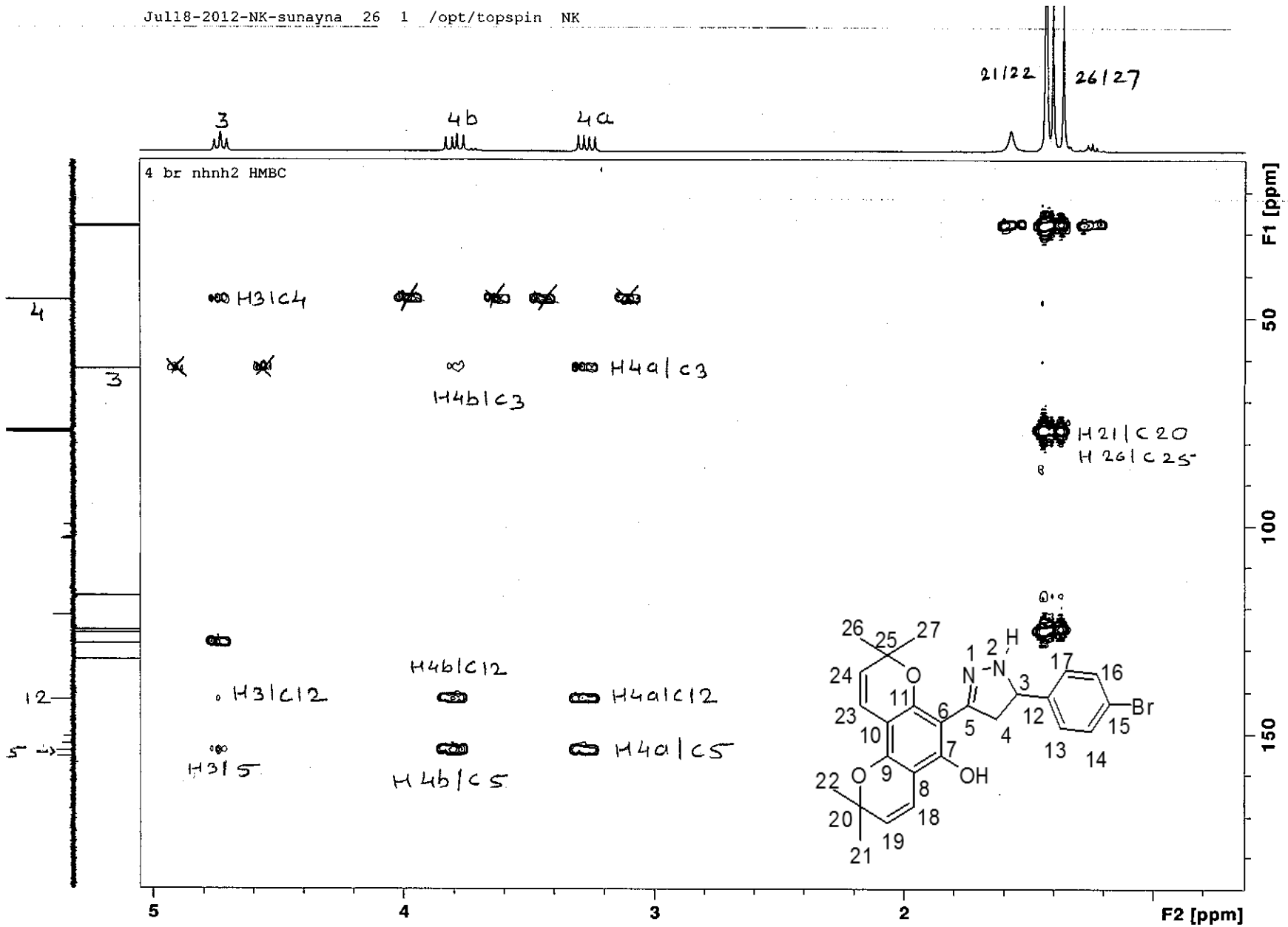
Expanded HMBC spectrum of 3-(4-bromophenyl)-5-pyranochromene pyrazoline (C-4c)



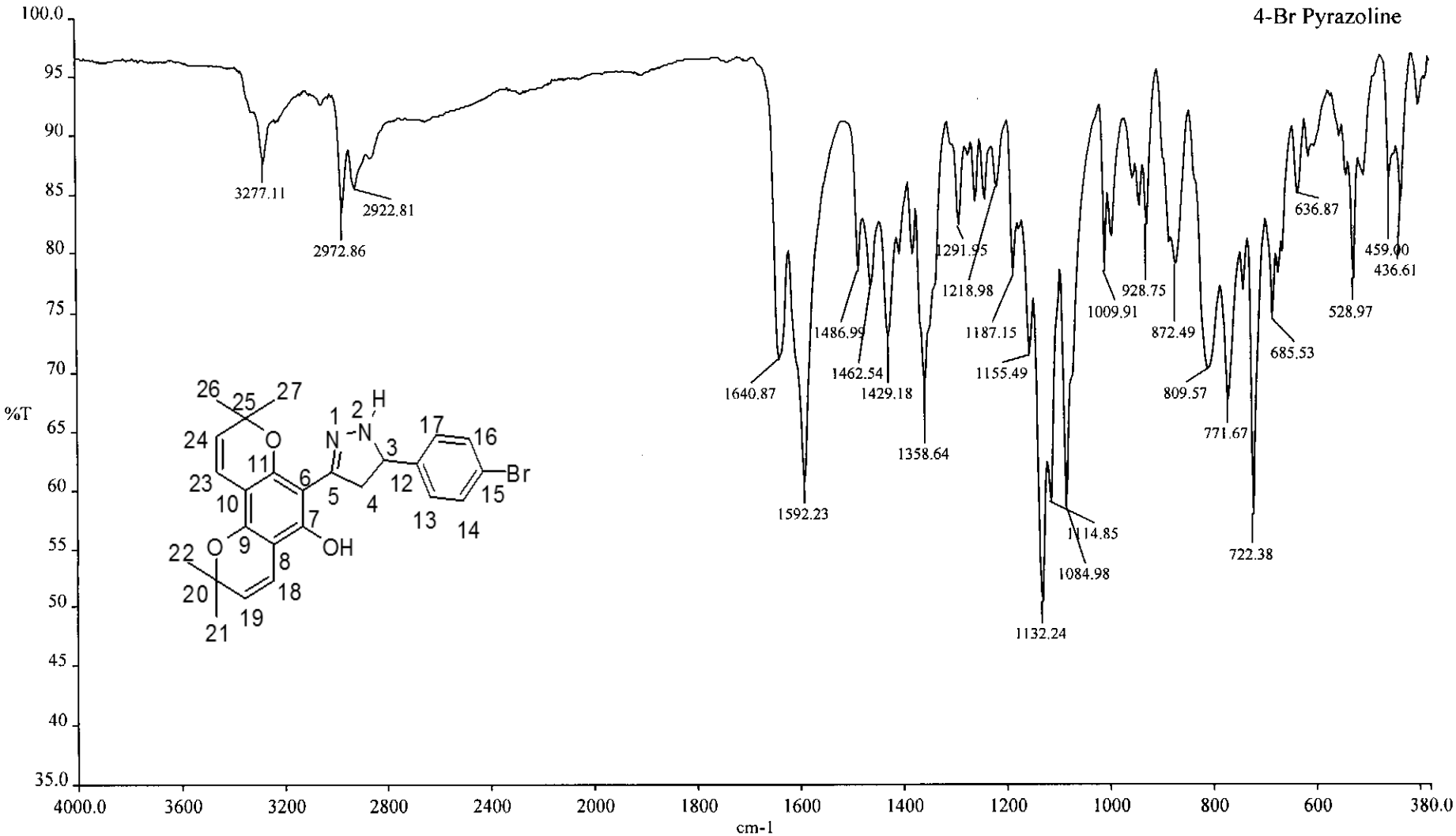
Expanded HMBC spectrum of 3-(4-bromophenyl)-5-pyranochromene pyrazoline (C-4c)



Expanded HMBC spectrum of 3-(4-bromophenyl)-5-pyranochromene pyrazoline (C-4c)



Expanded HMBC spectrum of 3-(4-bromophenyl)-5-pyranochromene pyrazoline (C-4c)

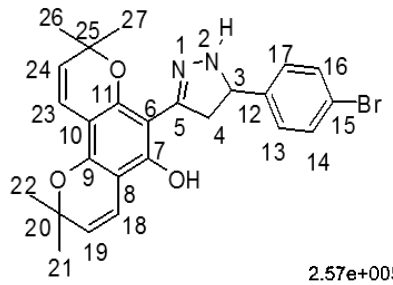


Infrared spectrum of 3-(4-bromophenyl)-5-pyranochromene pyrazoline (C-4c)

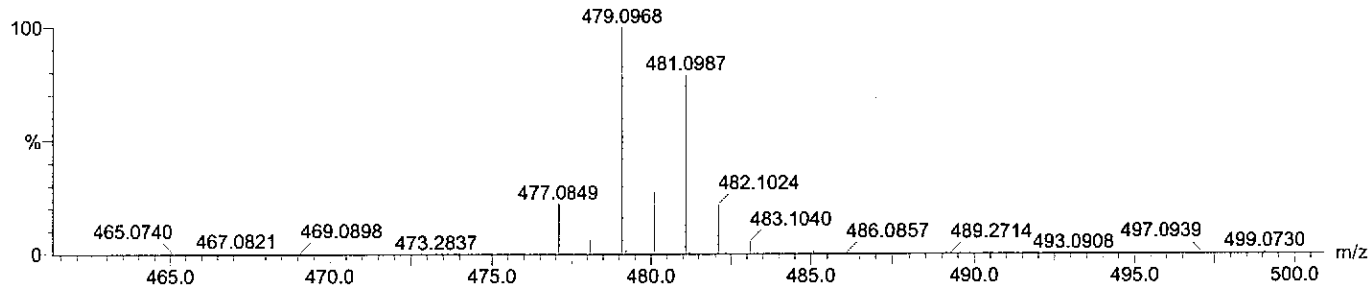
Elemental Composition Report **Page 1**

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

Monoisotopic Mass, Even Electron Ions
25 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-35 H: 20-30 N: 0-4 O: 0-4 Br: 1-1

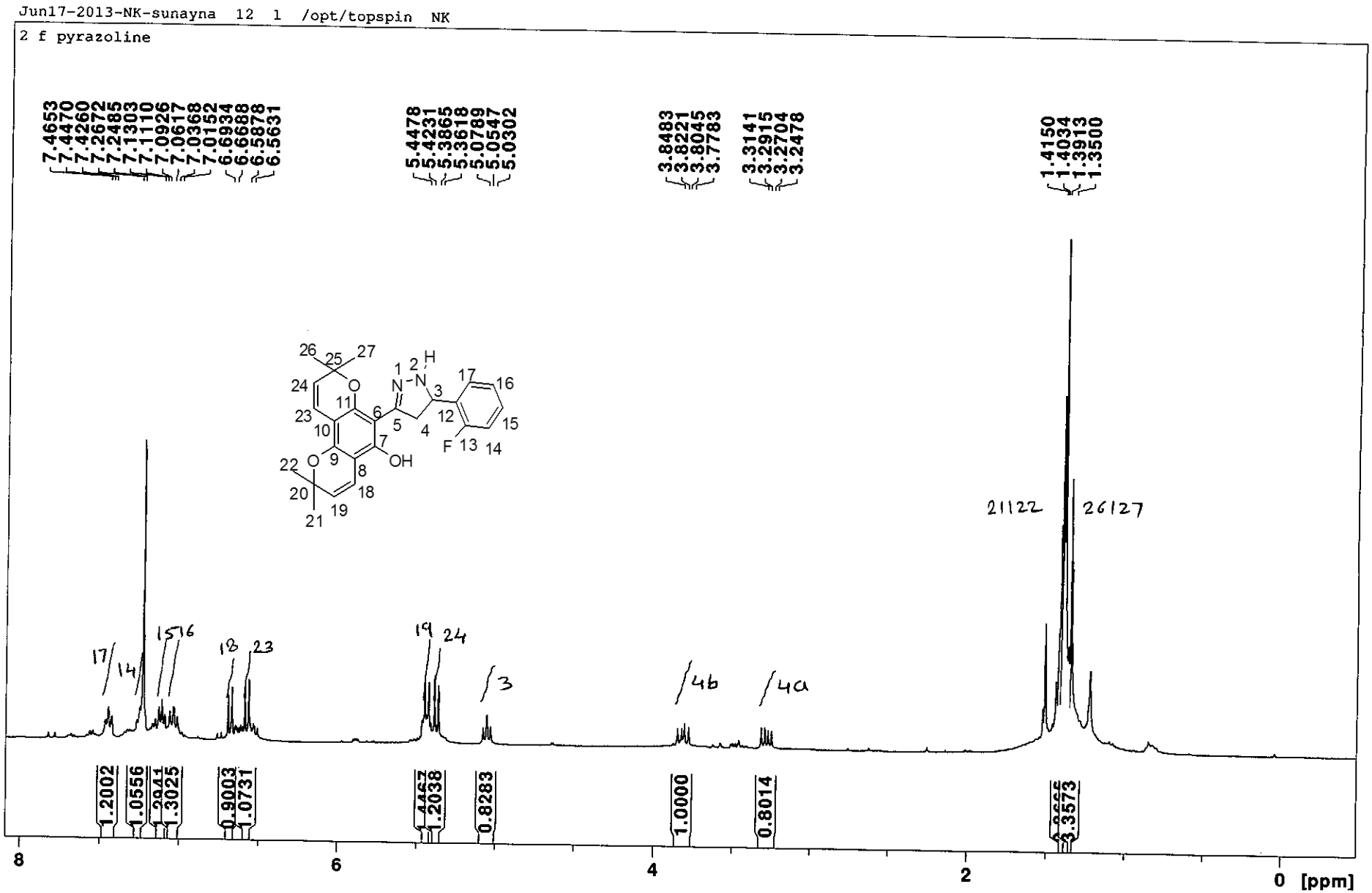


5 23 (0.375) Cm (1:31)
TOF MS ES-
2.57e+005

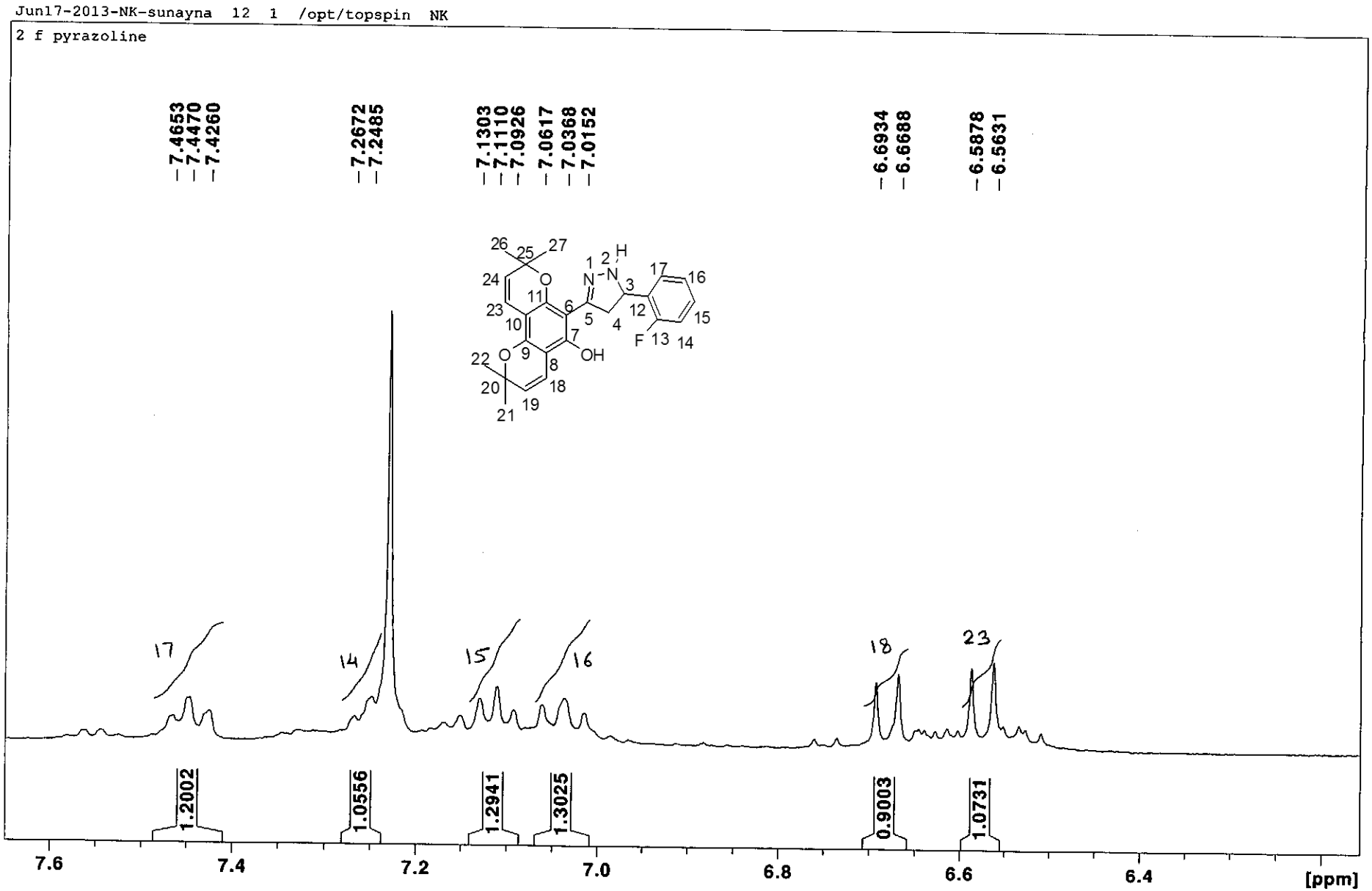


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
479.0968	479.0970	-0.2	-0.4	14.5	532.2	0.0	C25 H24 N2 O3 Br

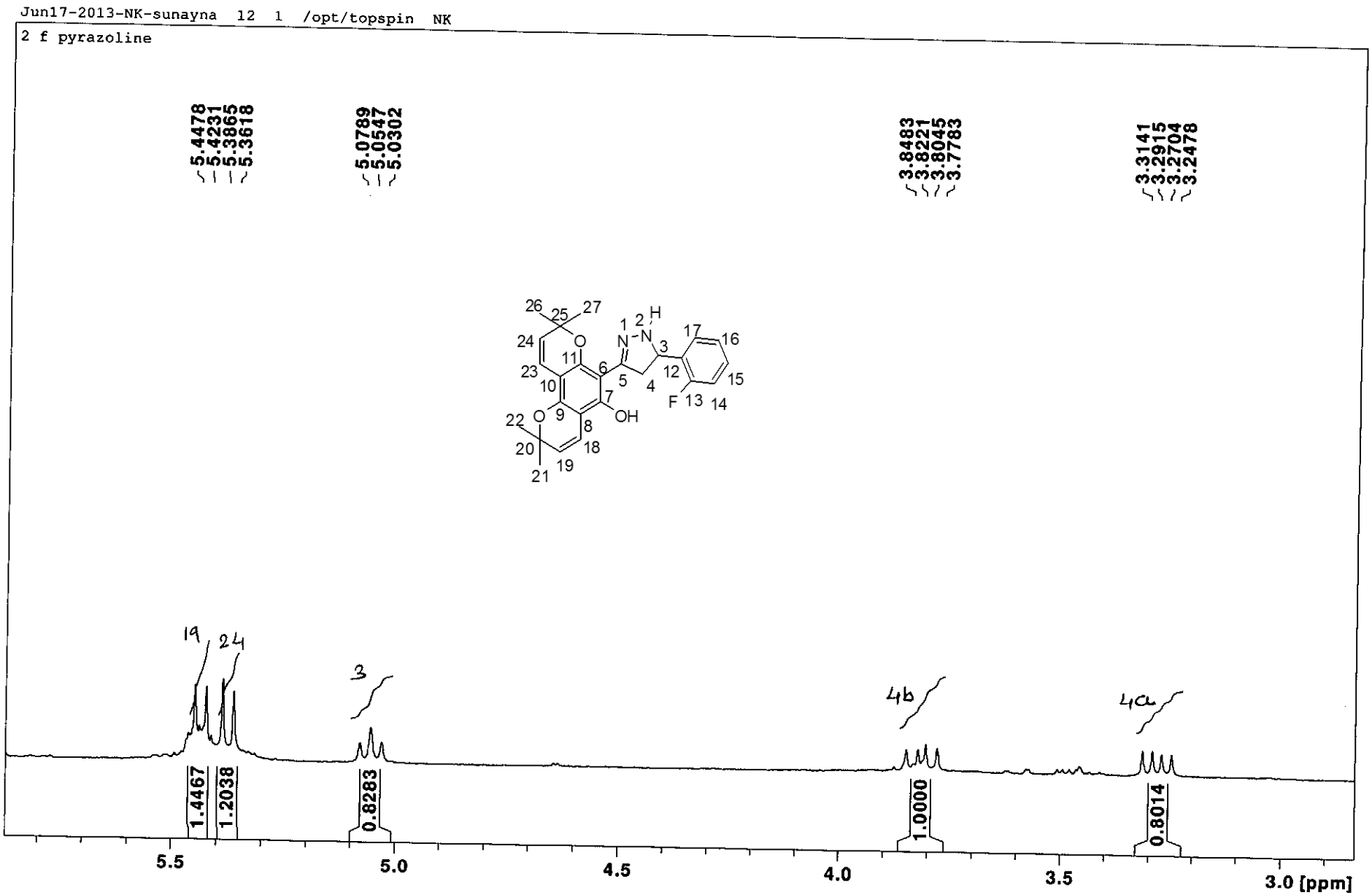
HRMS spectrum of 3-(4-bromophenyl)-5-pyranochromene pyrazoline (C-4c)



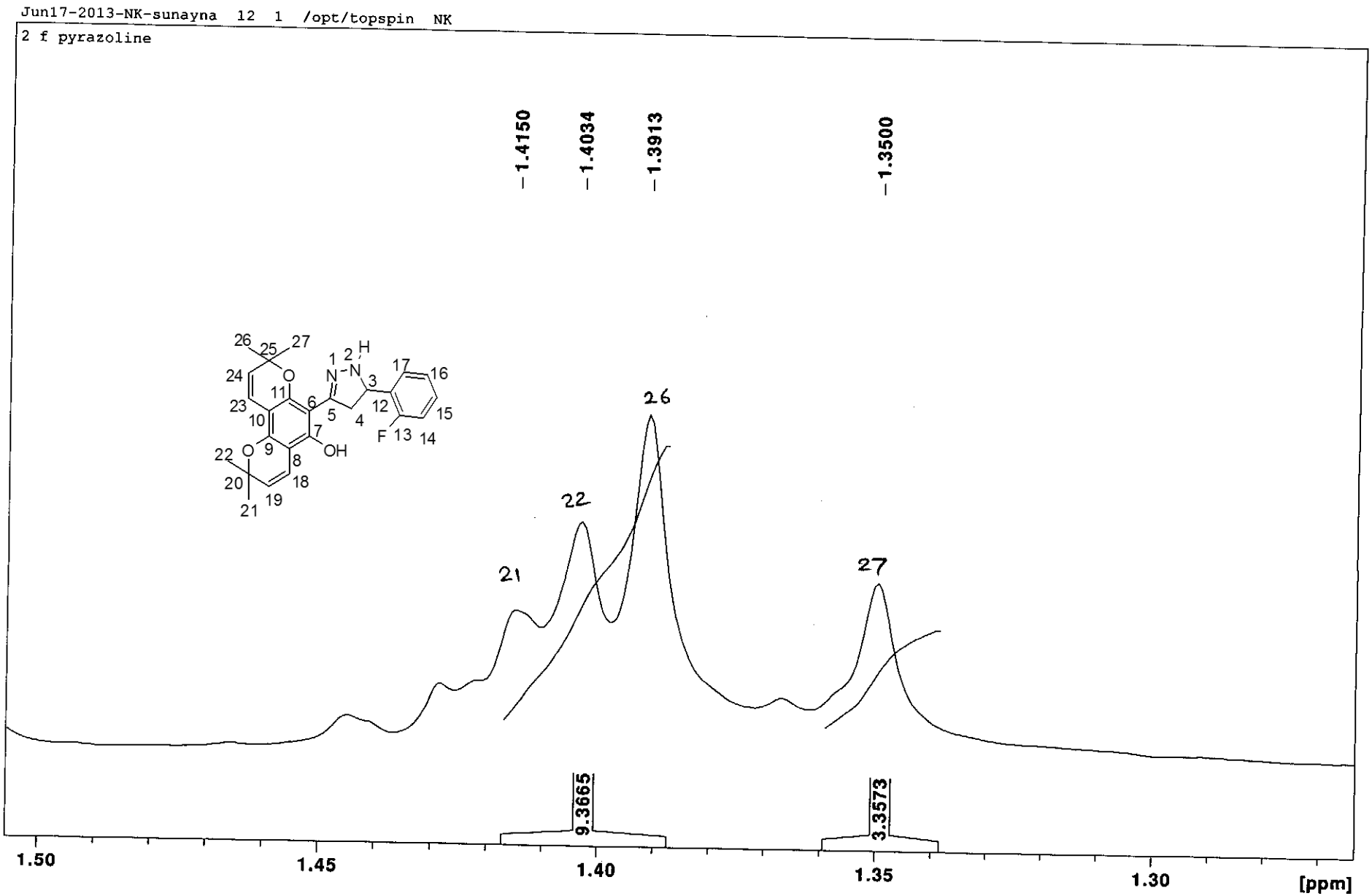
1H NMR spectrum of 3-(2-fluorophenyl)-5-pyranochromene pyrazoline (C-4d)



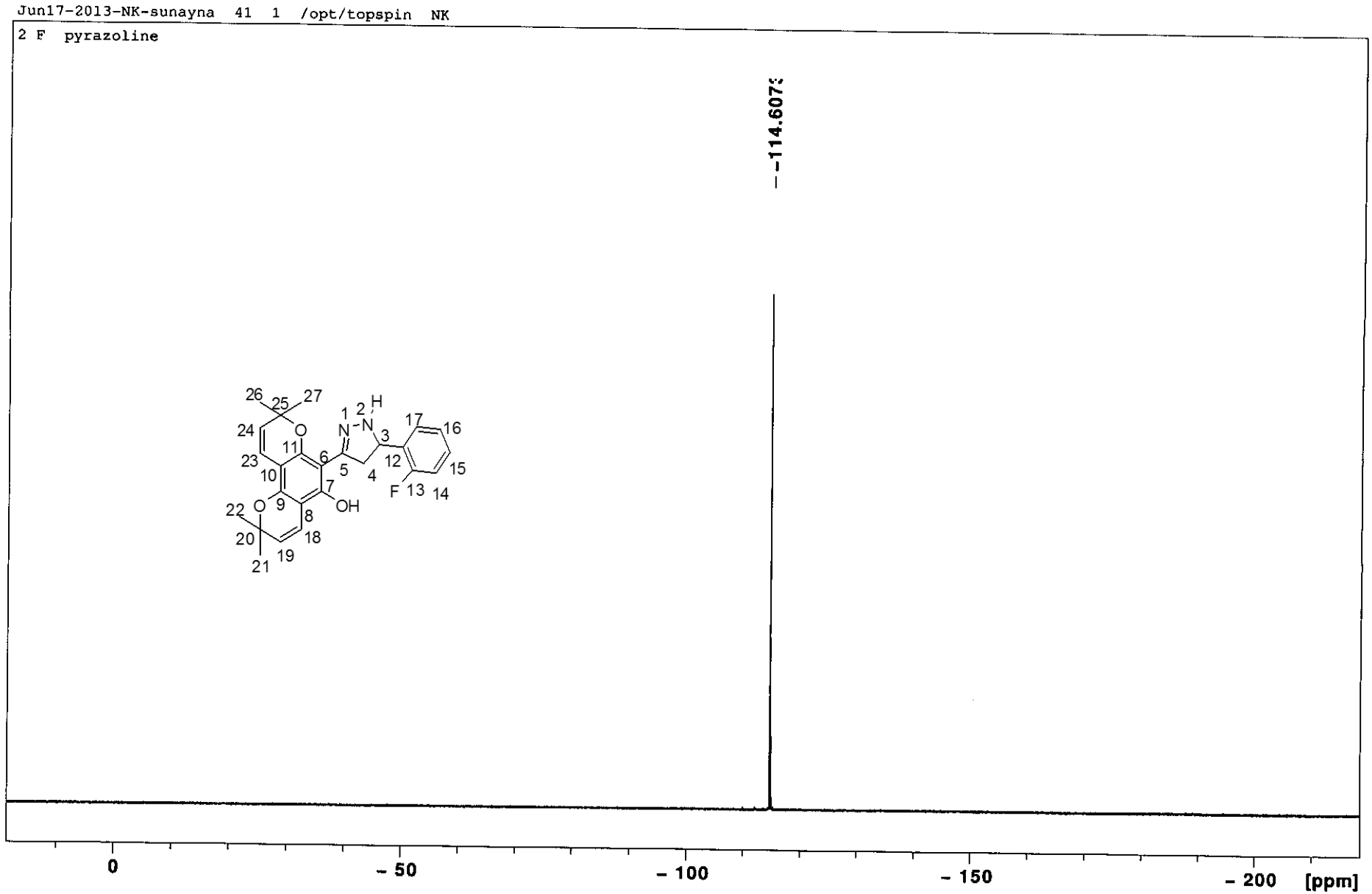
Expanded 1H NMR spectrum of 3-(2-fluorophenyl)-5-pyranochromene pyrazoline (C-4d)



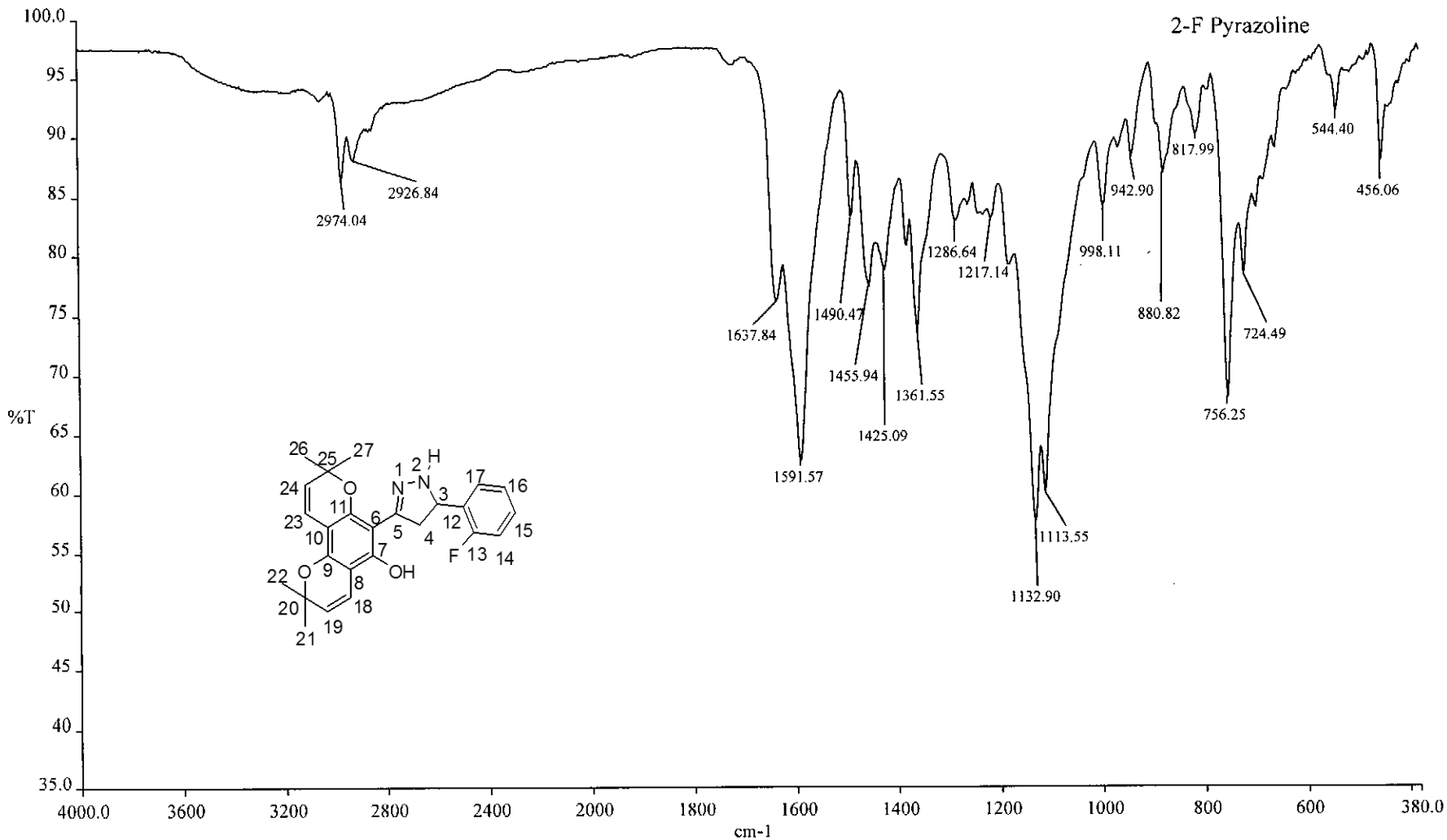
Expanded 1H NMR spectrum of 3-(2-fluorophenyl)-5-pyranochromene pyrazoline (C-4d)



Expanded 1H NMR spectrum of 3-(2-fluorophenyl)-5-pyranochromene pyrazoline (C-4d)



19F NMR spectrum of 3-(2-fluorophenyl)-5-pyranochromene pyrazoline (C-4d)

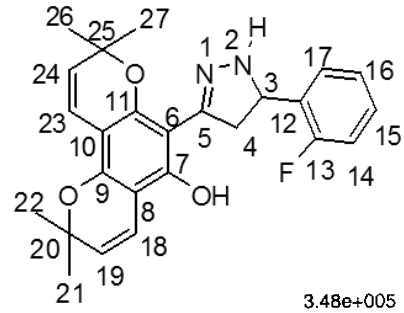


Infrared spectrum of 3-(2-fluorophenyl)-5-pyranochromene pyrazoline (C-4d)

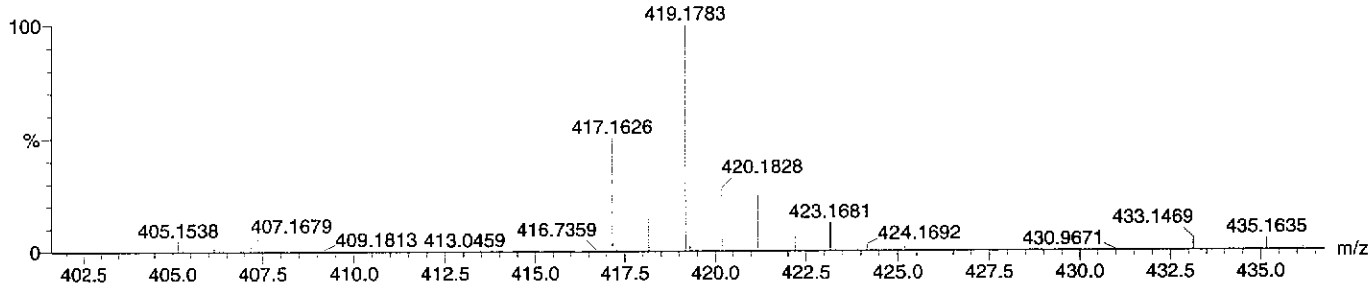
Elemental Composition Report **Page 1**

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

Monoisotopic Mass, Even Electron Ions
25 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-35 H: 20-30 N: 0-4 O: 0-4 F: 1-1
11 27 (0.444) Cm (1:30)
TOF MS ES-

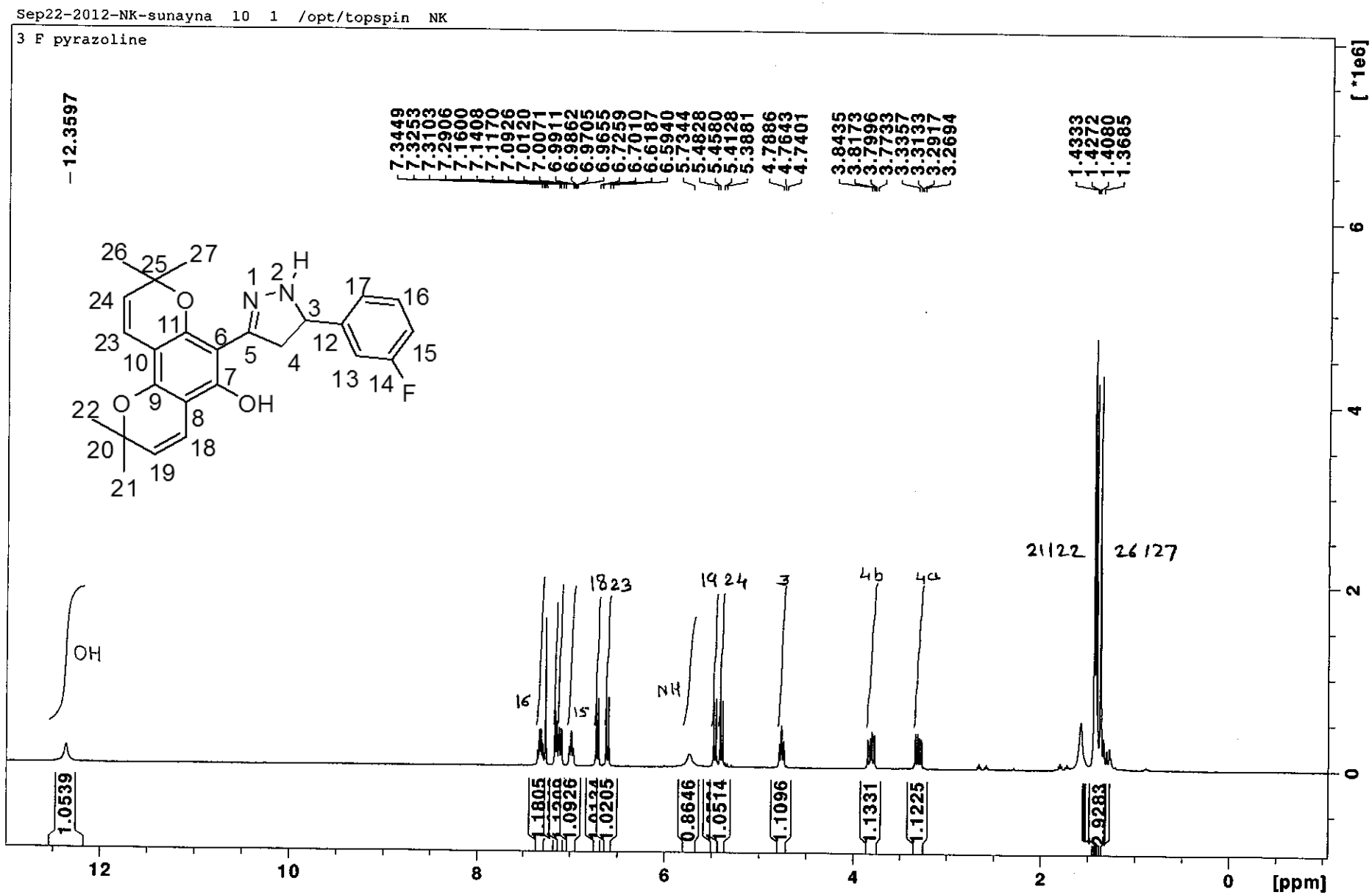


3.48e+005

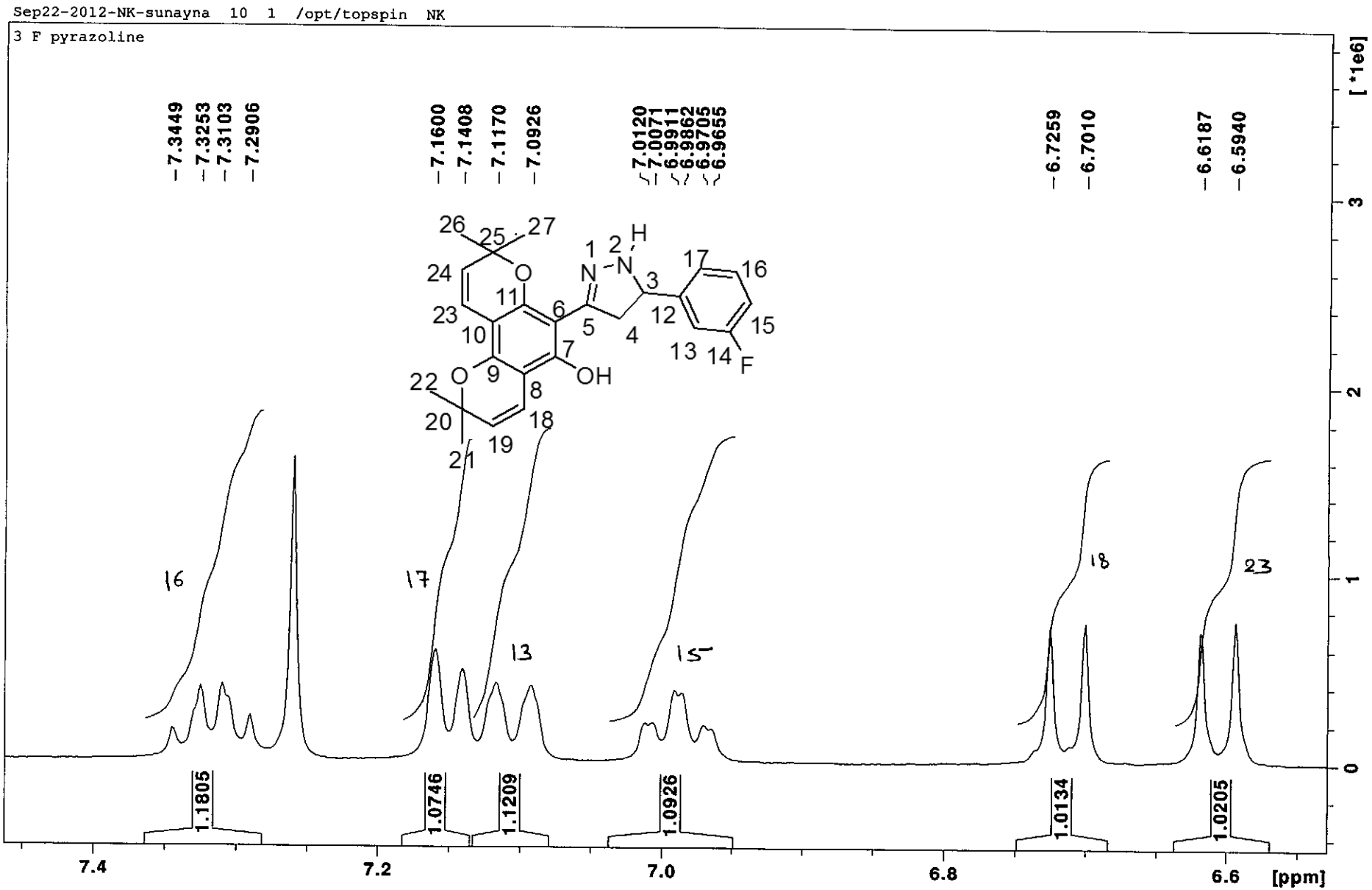


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
419.1783	419.1771	1.2	2.9	14.5	593.8	0.0	C25 H24 N2 O3 F

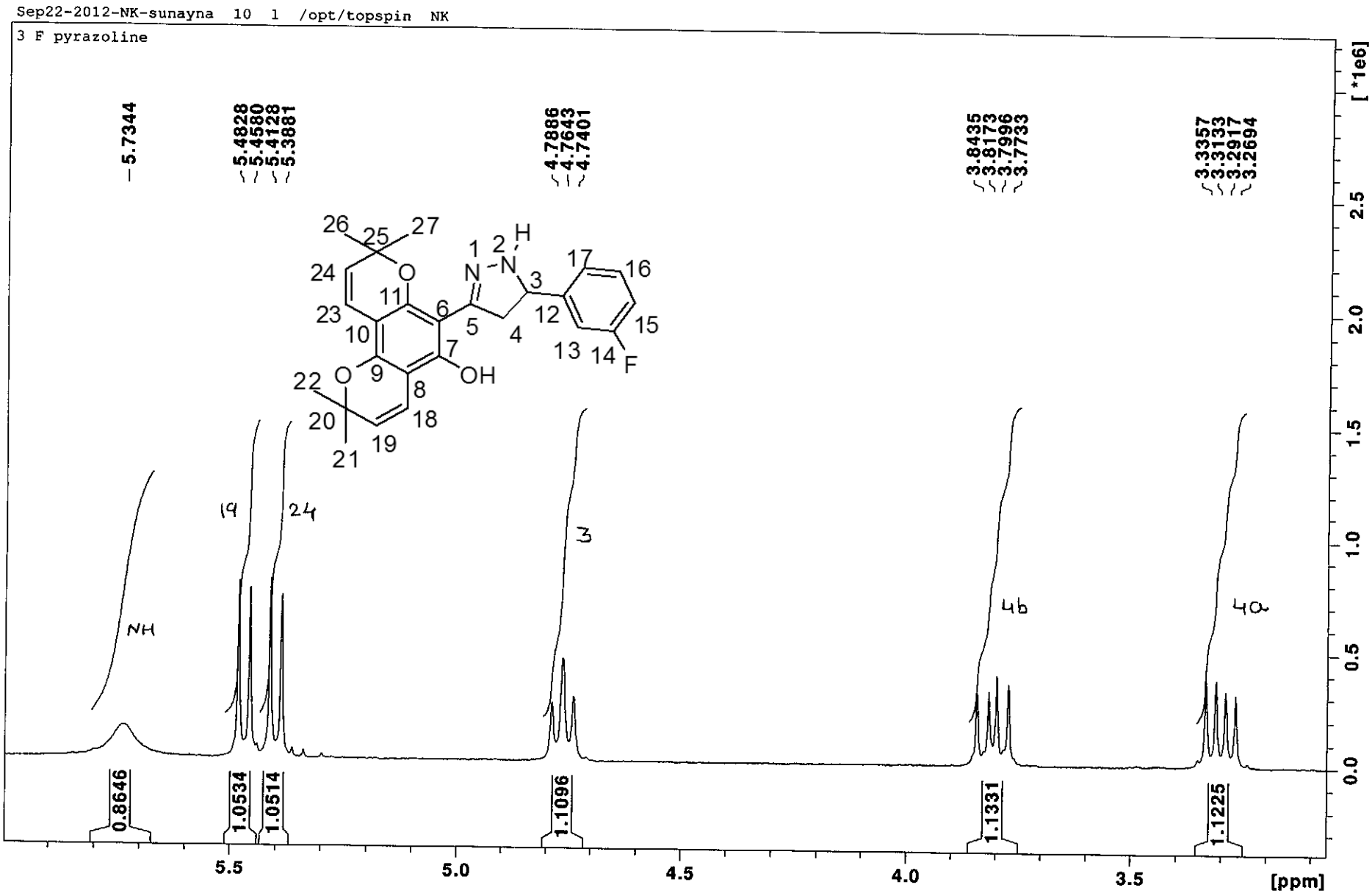
HRMS spectrum of 3-(2-fluorophenyl)-5-pyranochromene pyrazoline (C-4d)



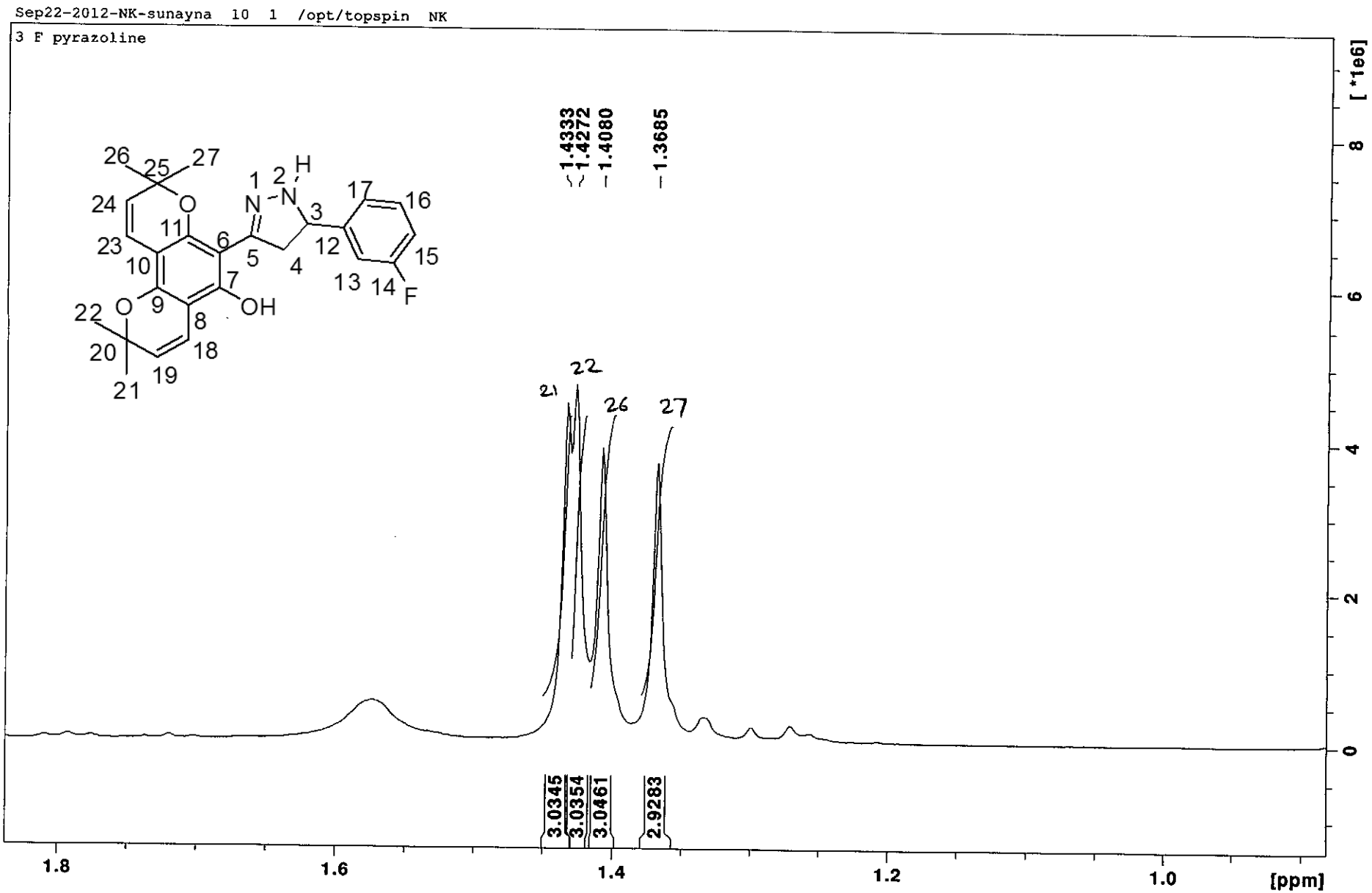
1H NMR spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



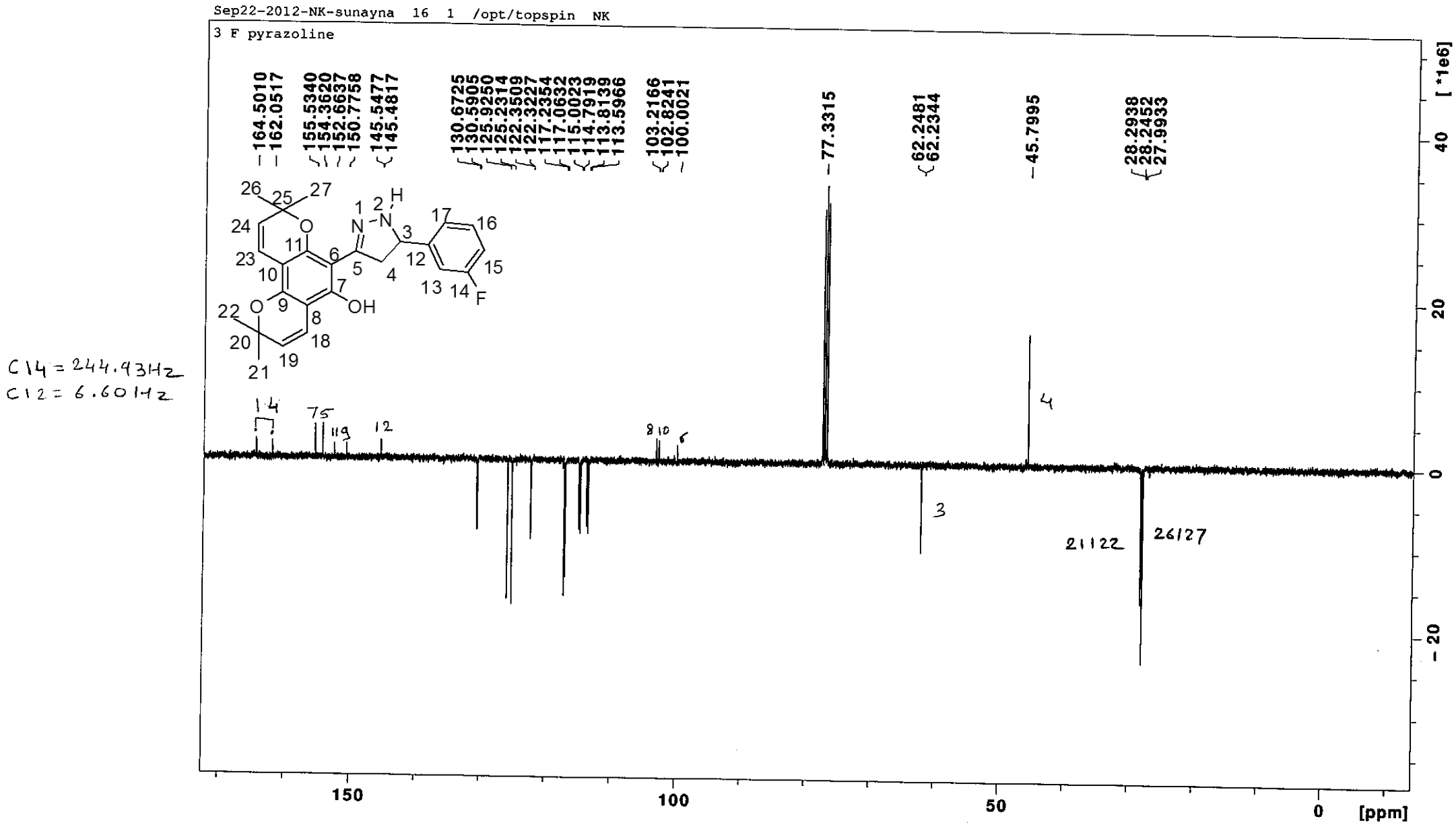
Expanded 1H NMR spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



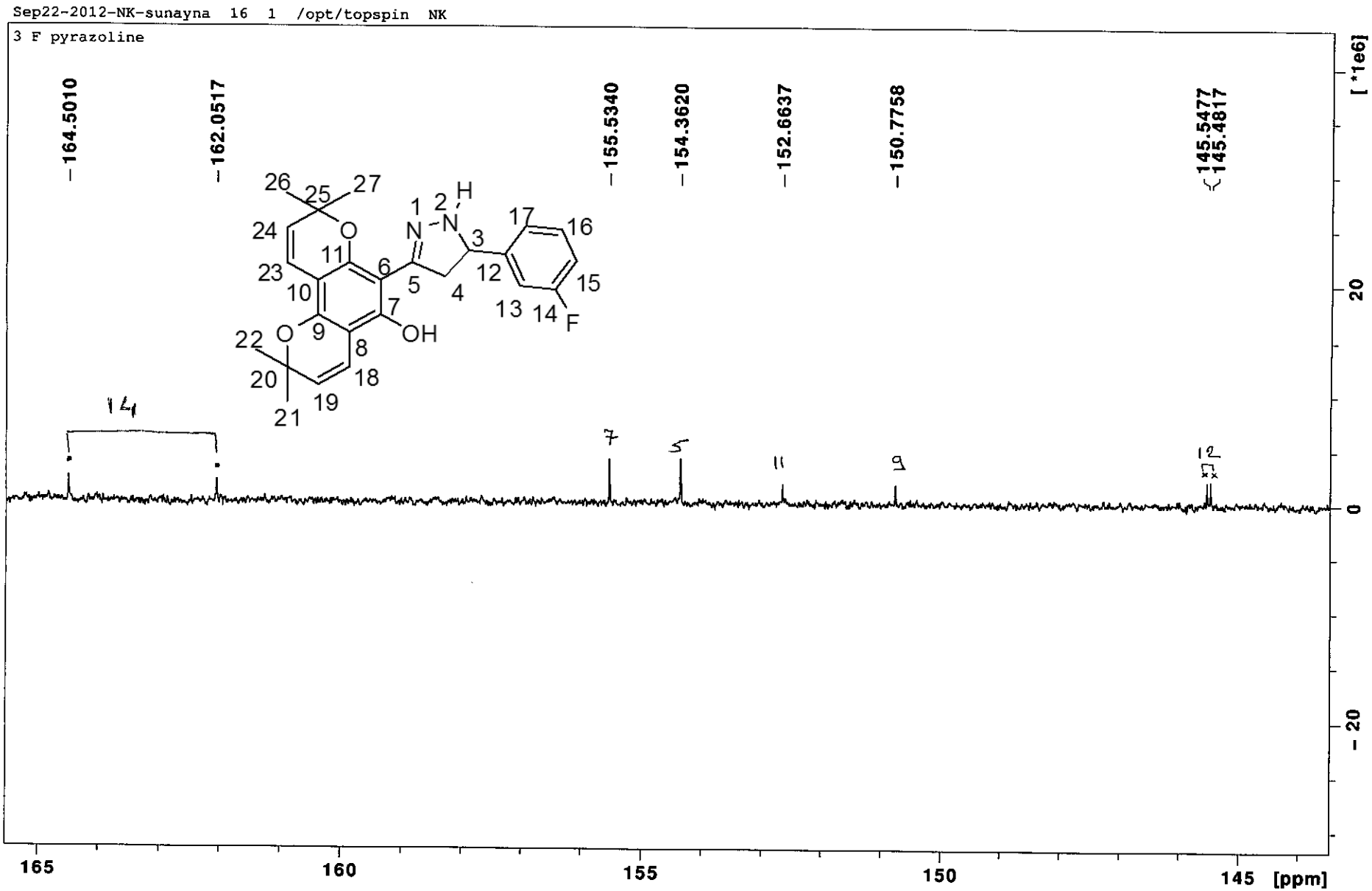
Expanded 1H NMR spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



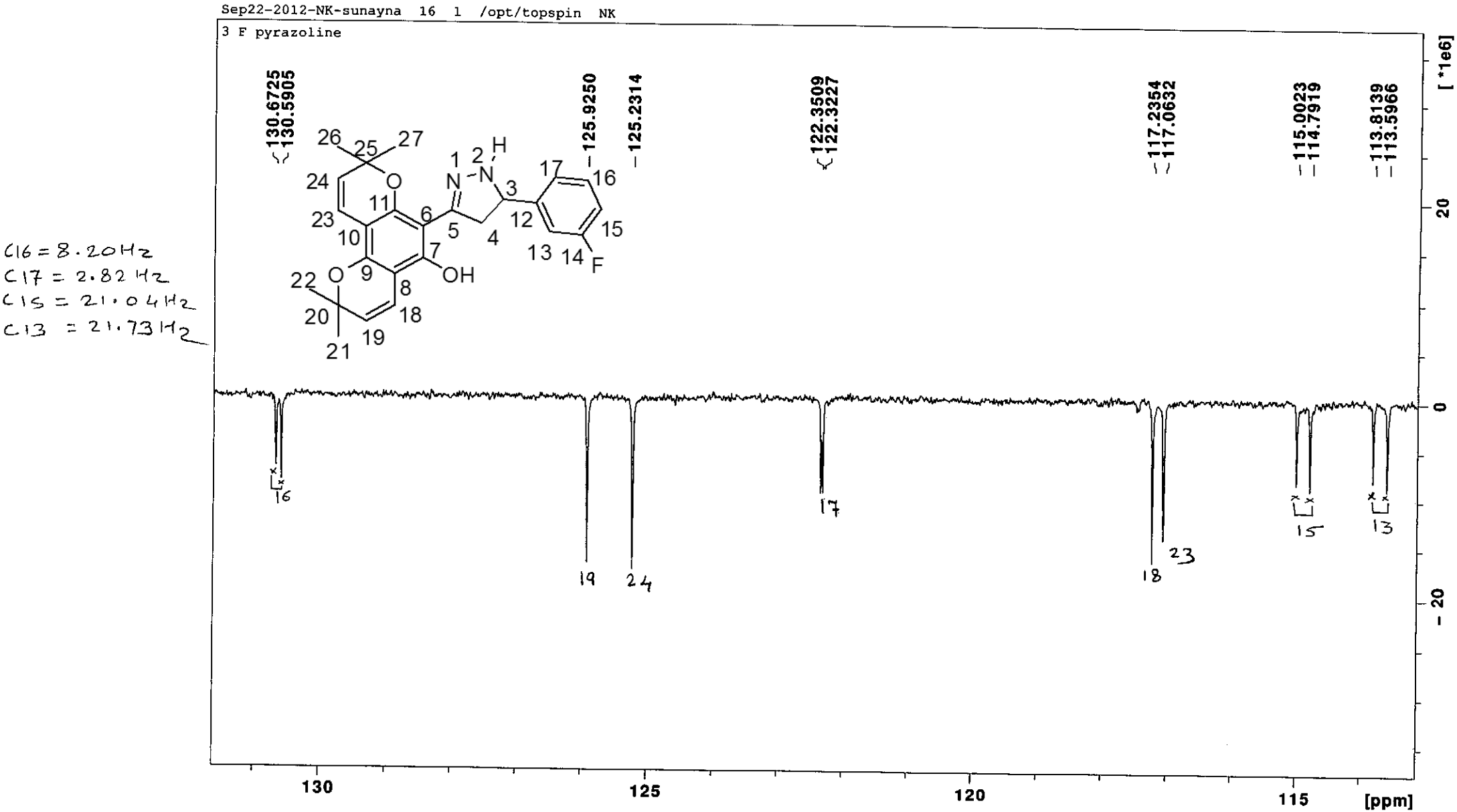
Expanded 1H NMR spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



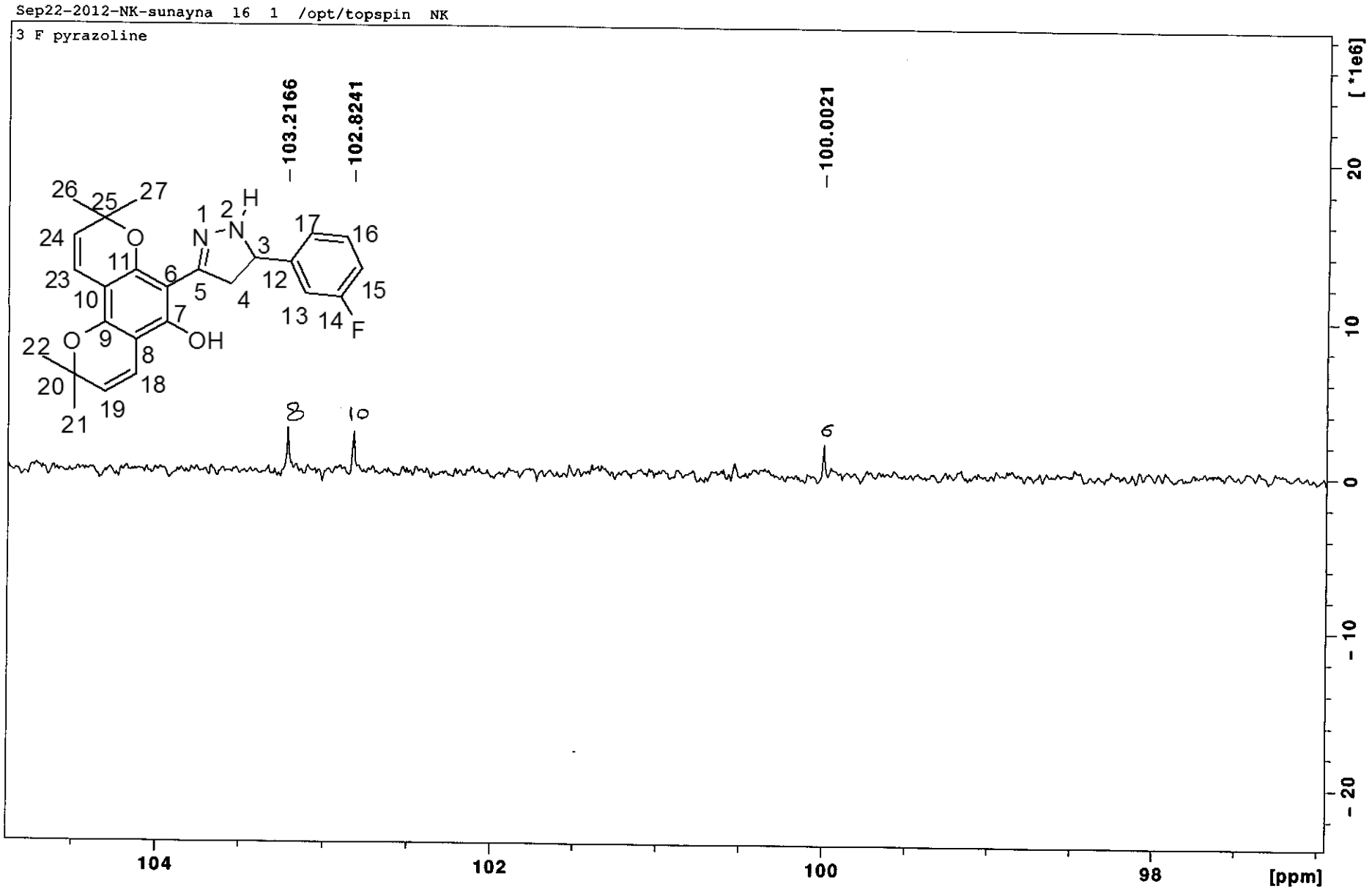
13C NMR spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



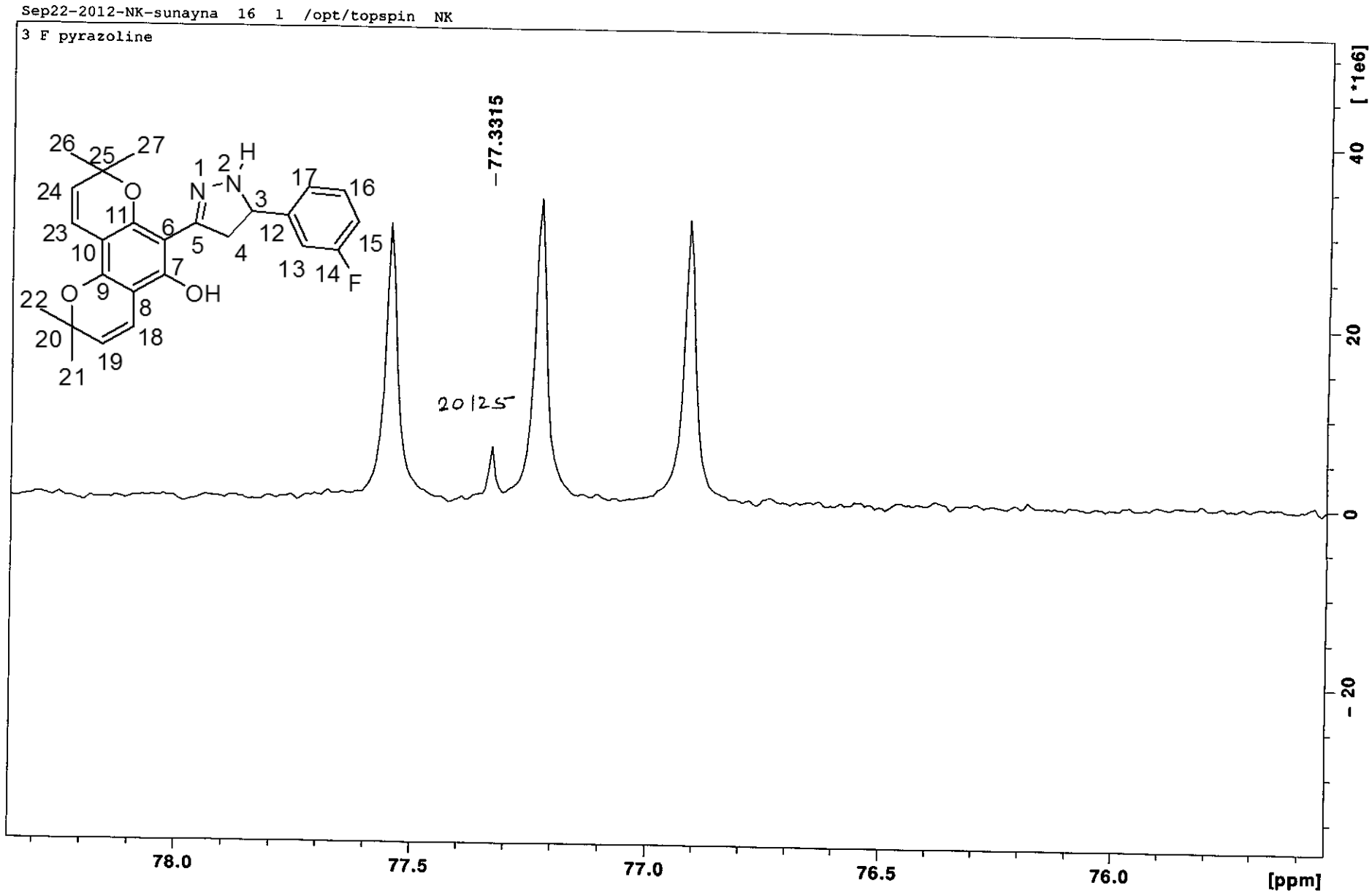
Expanded 13C NMR spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



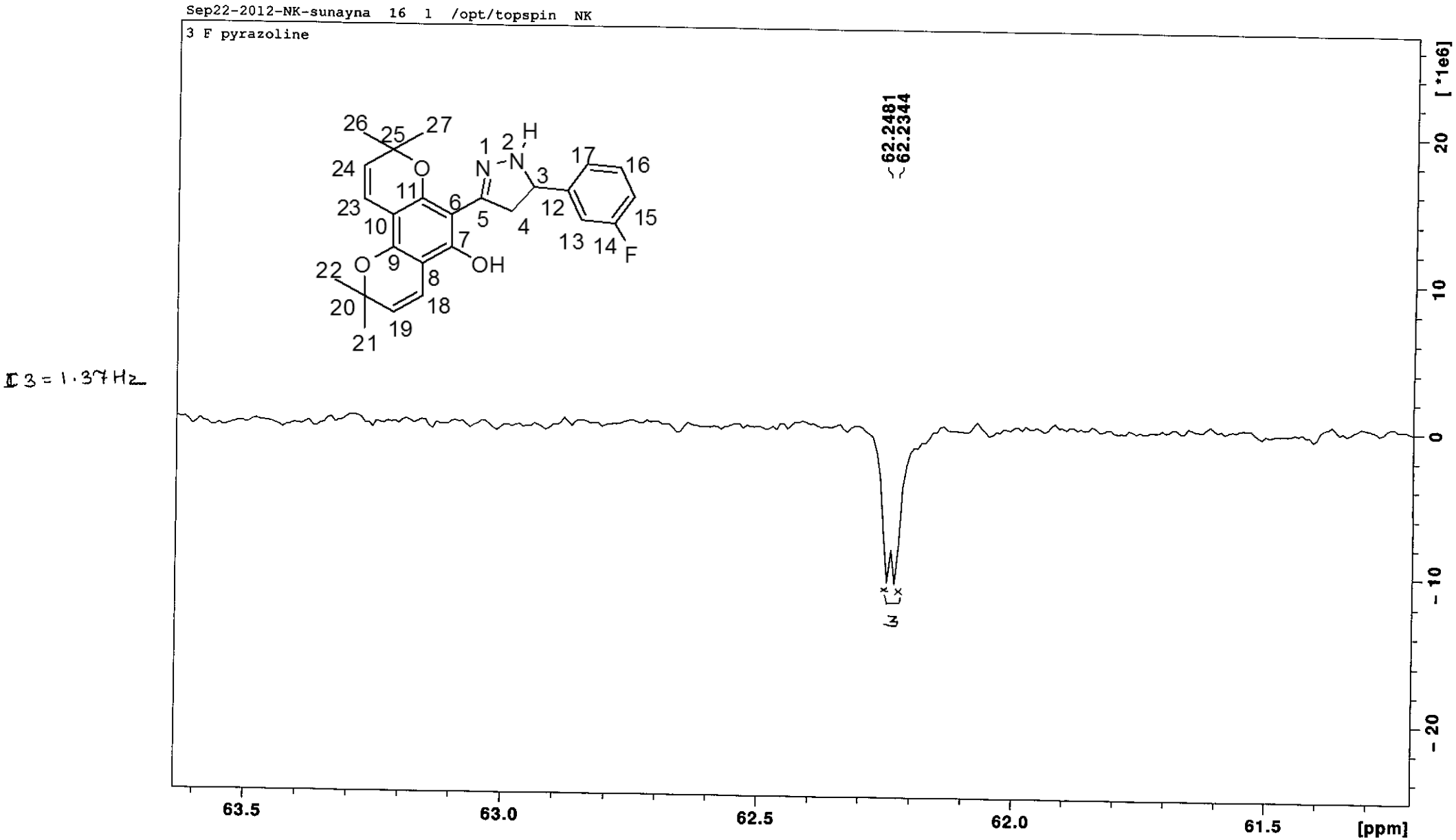
Expanded 13C NMR spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



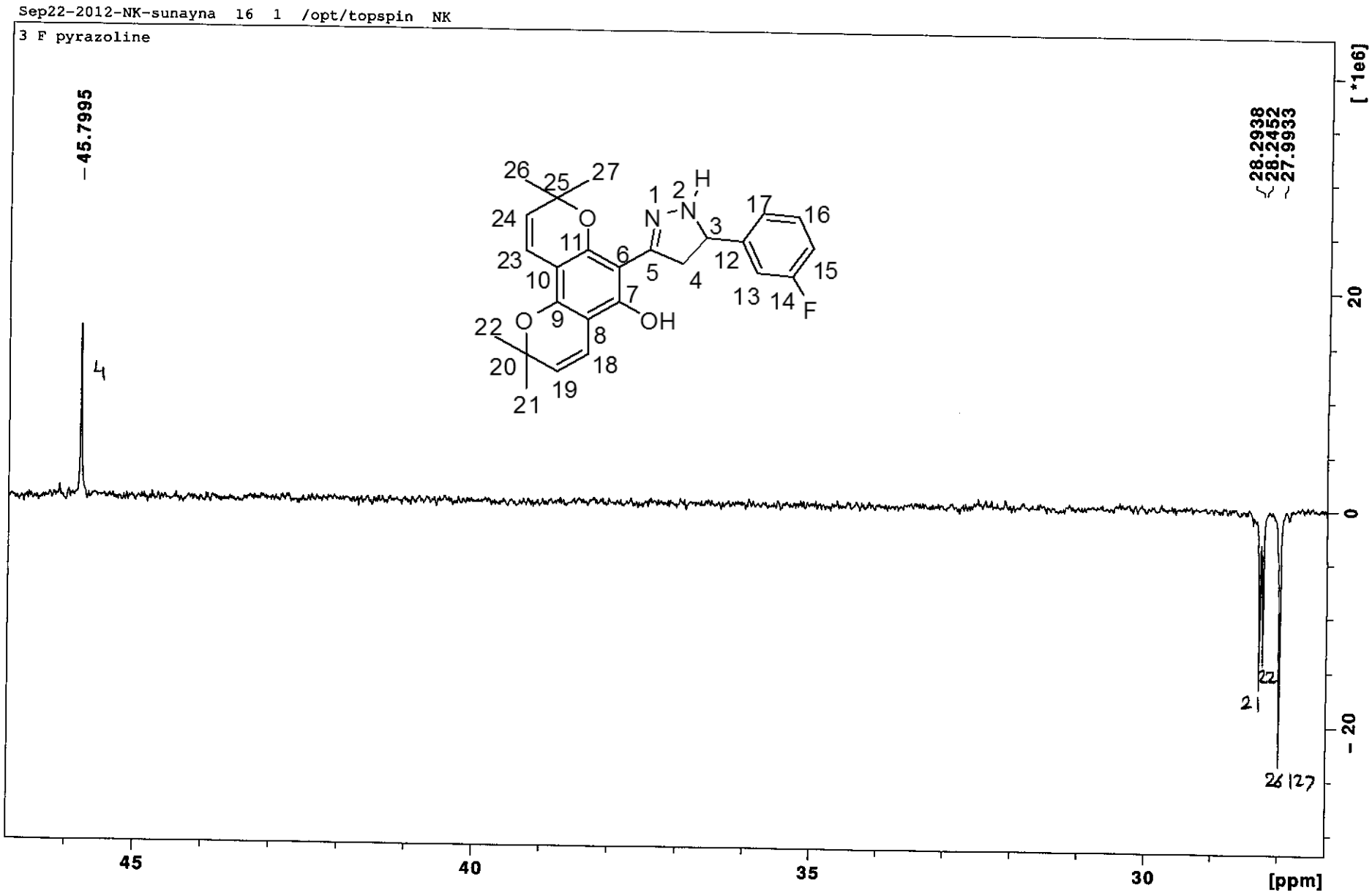
Expanded 13C NMR spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



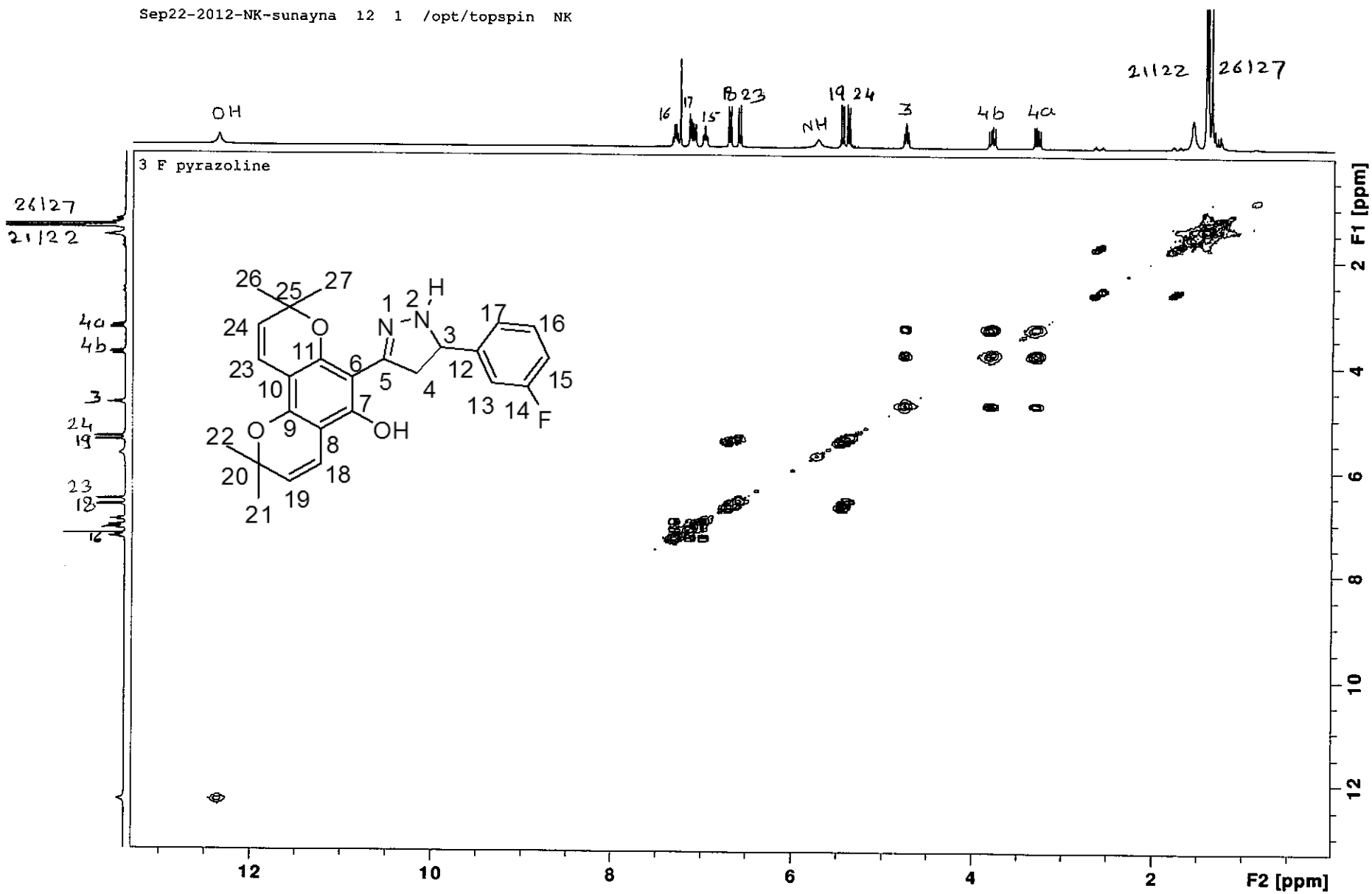
Expanded 13C NMR spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



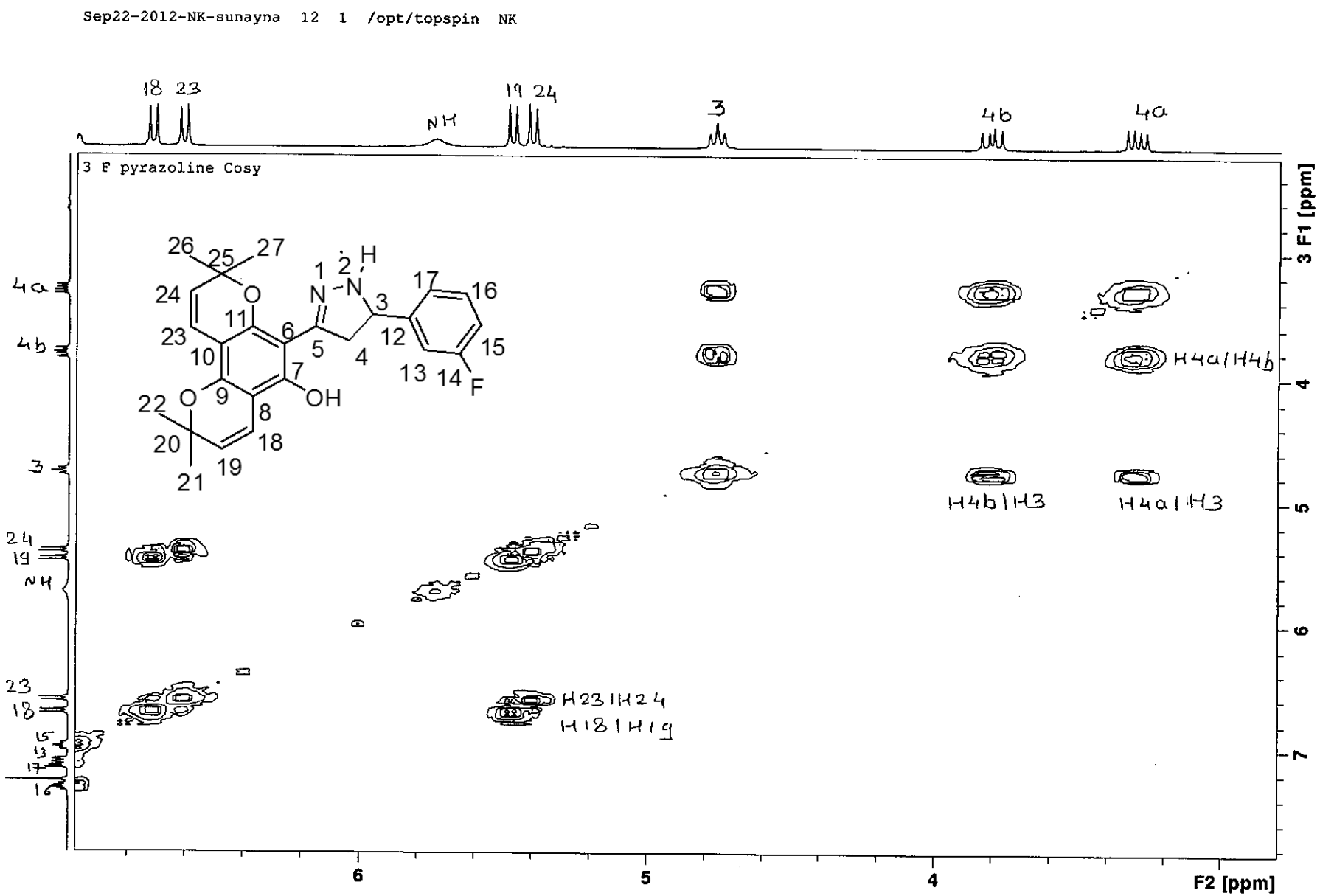
Expanded 13C NMR spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



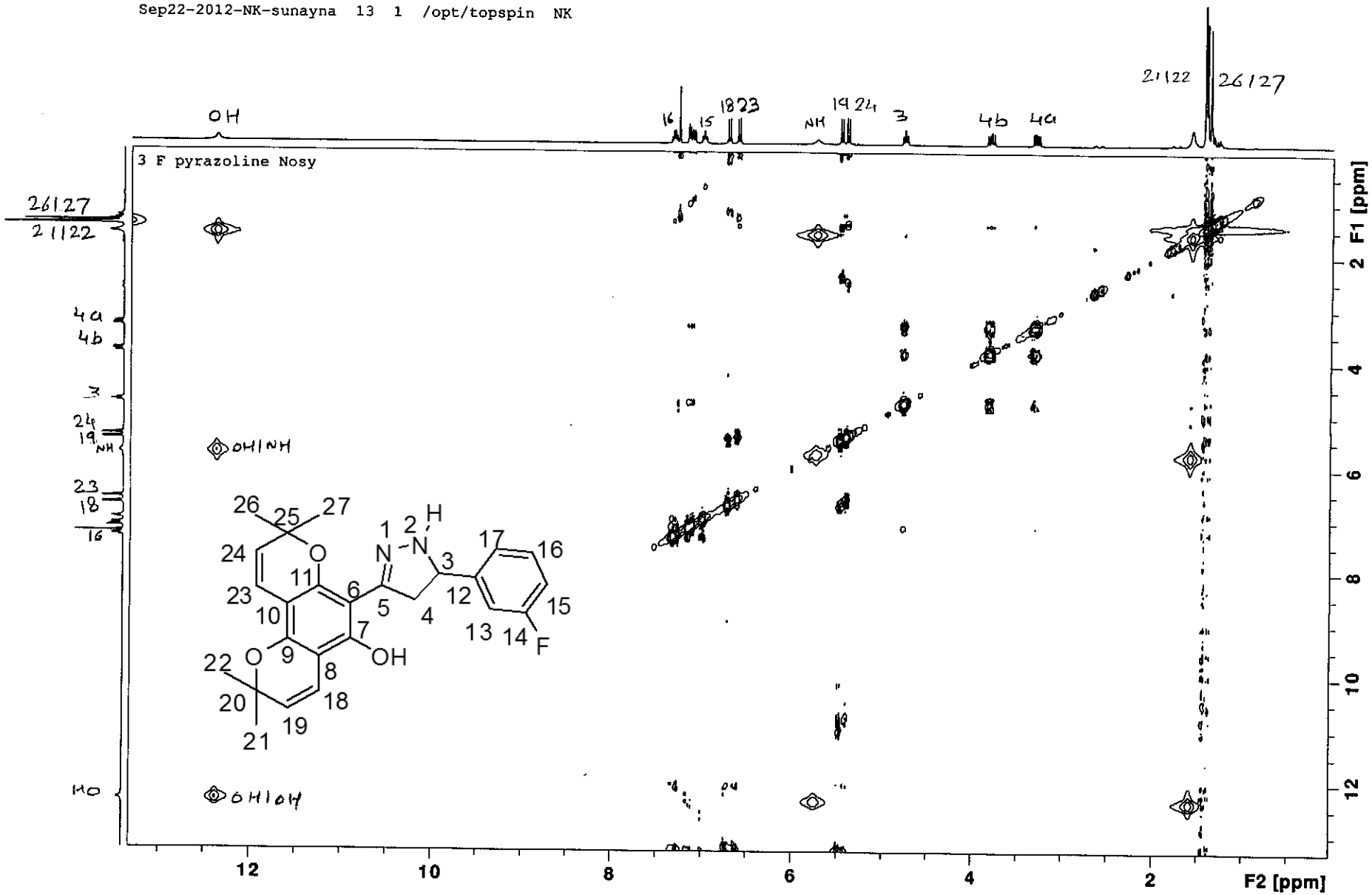
Expanded 13C NMR spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



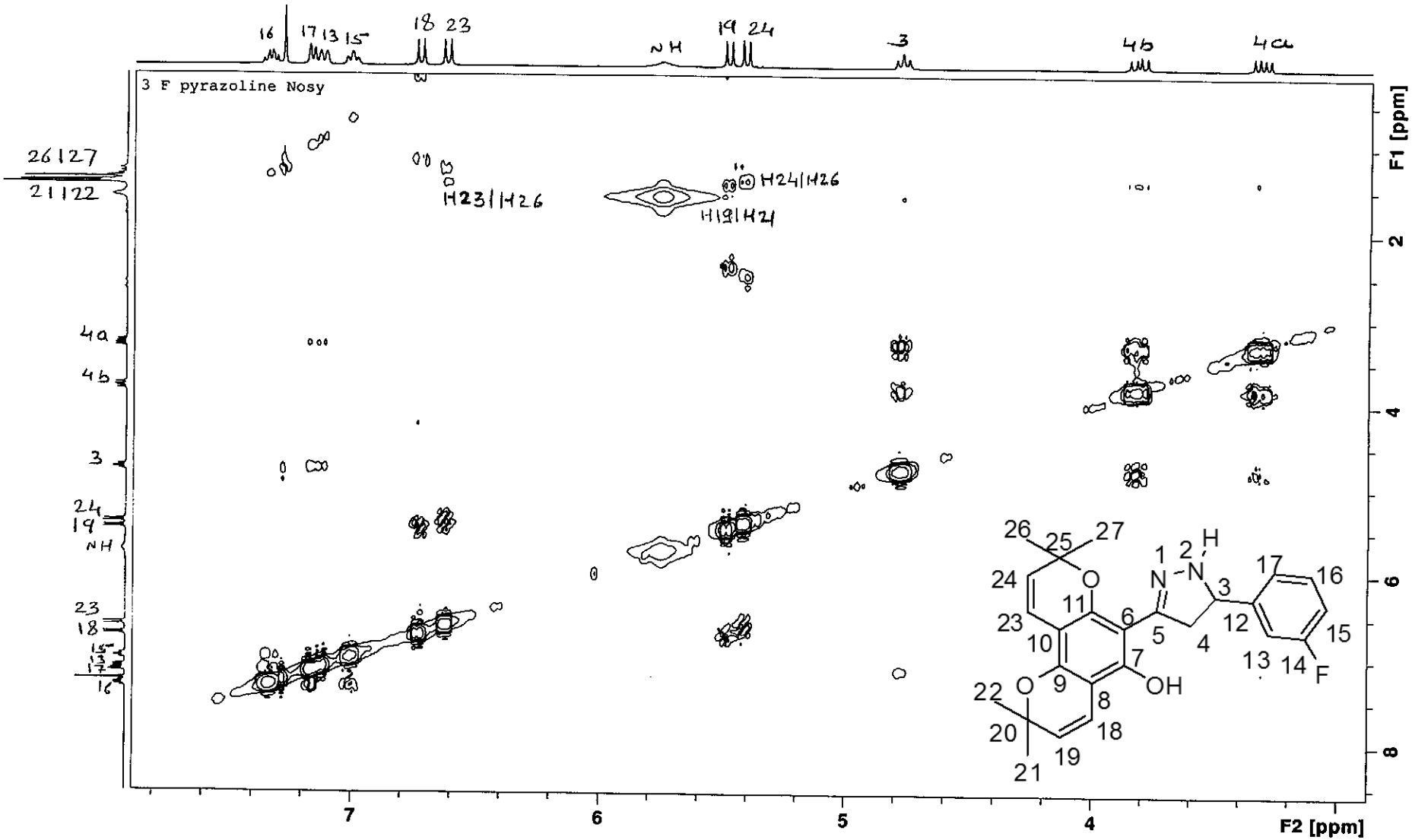
COSY NMR spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



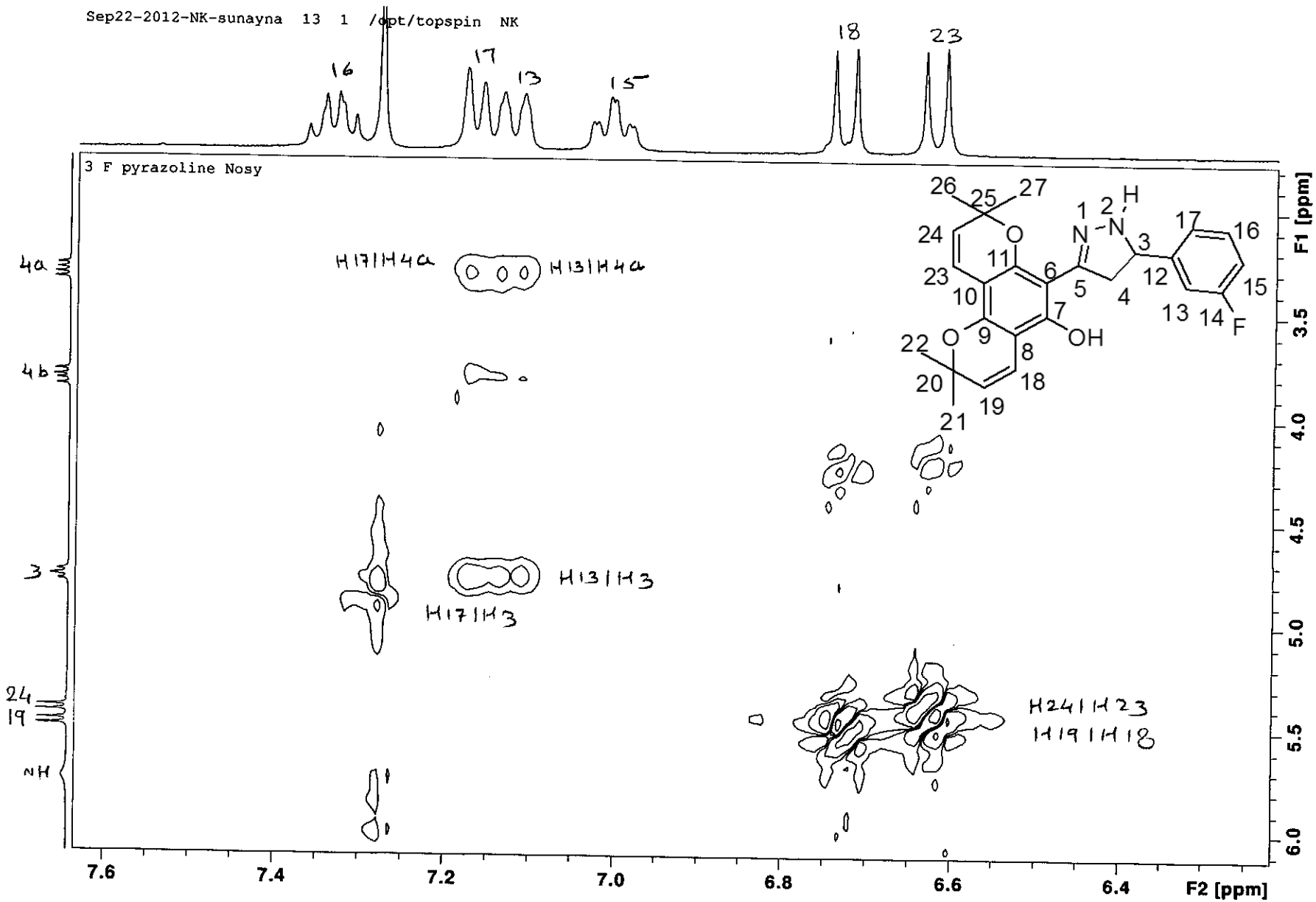
Expanded COSY NMR spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



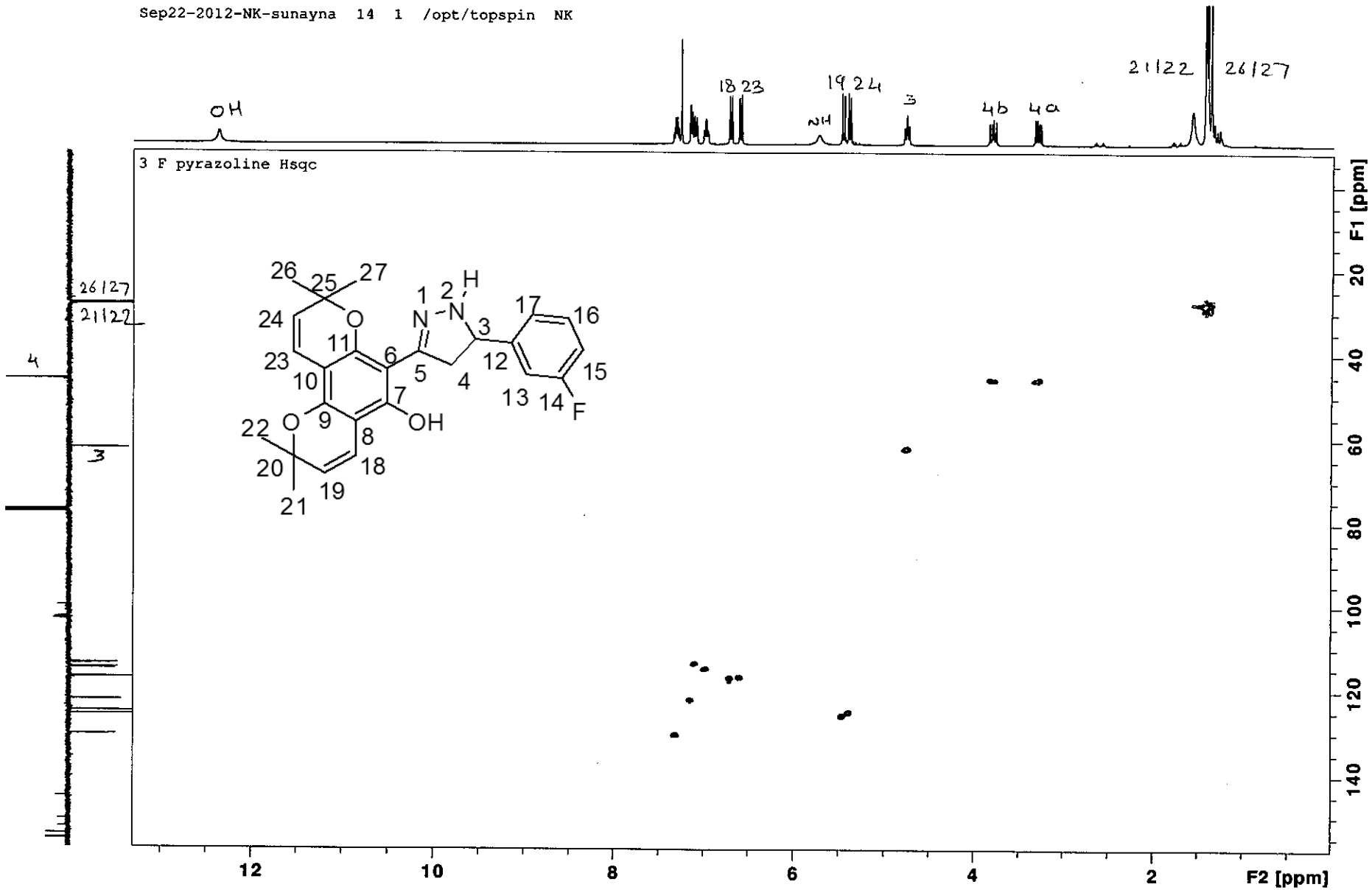
NOESY spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



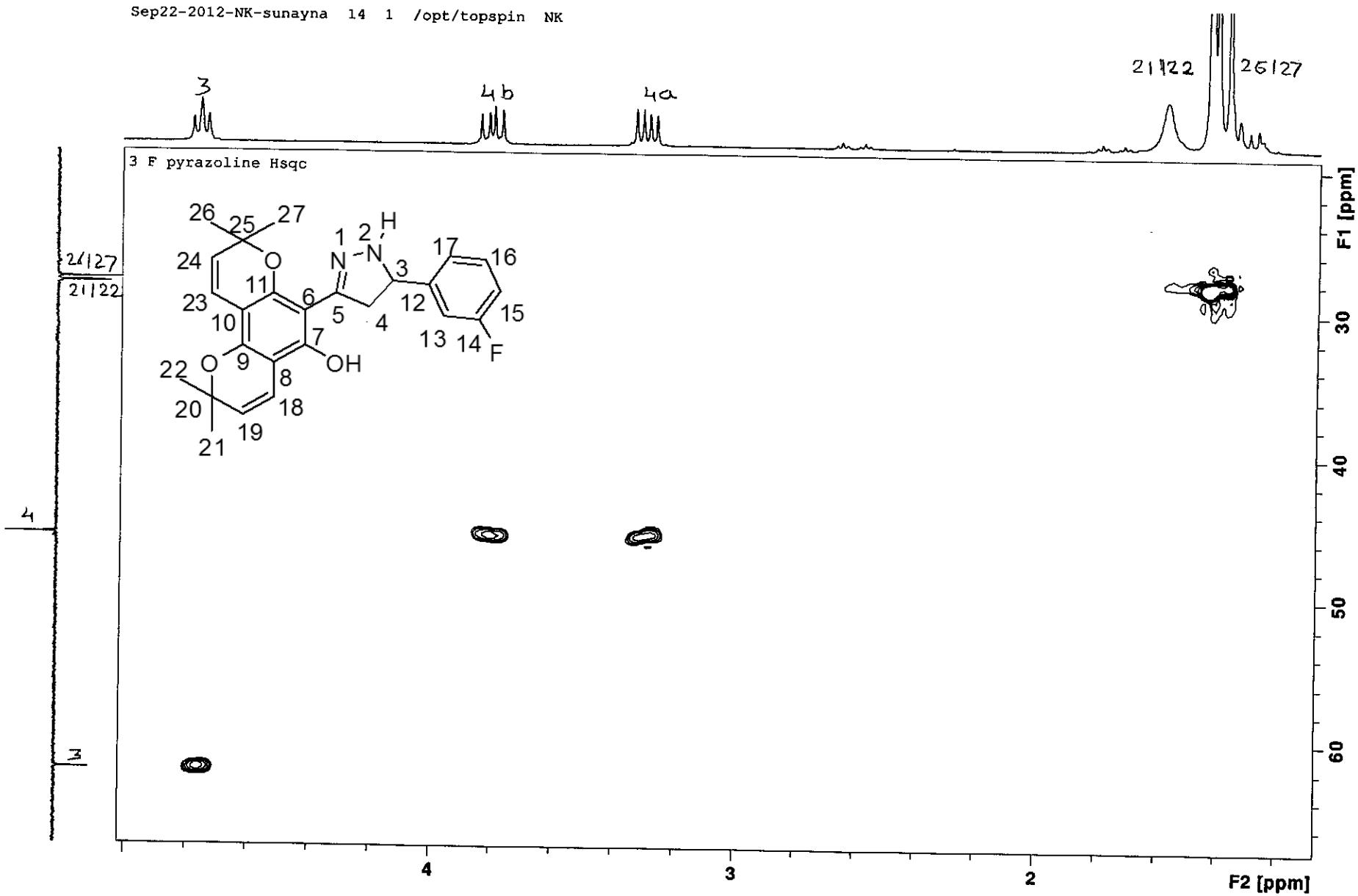
Expanded NOESY spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



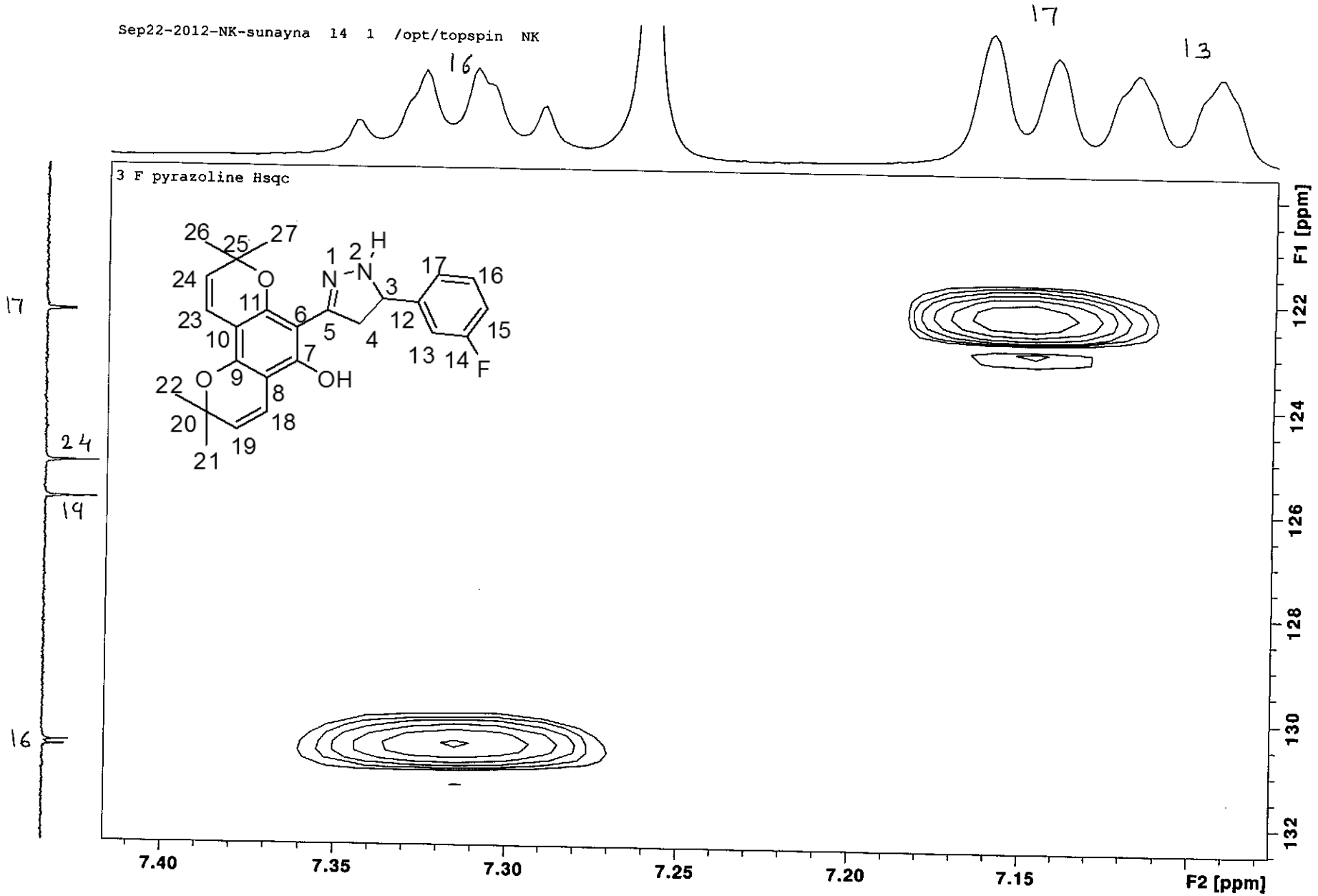
Expanded NOESY spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



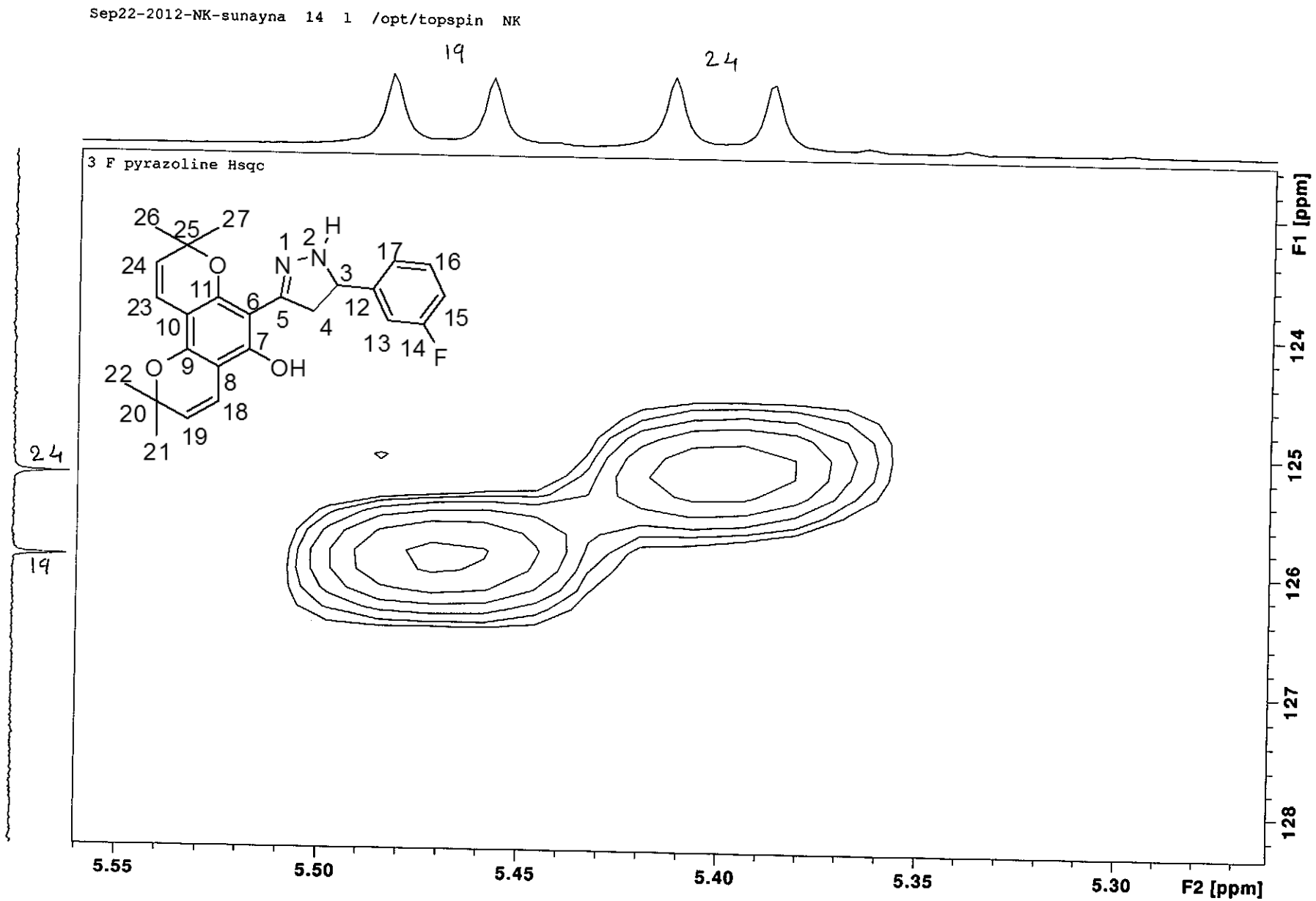
HSQC spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



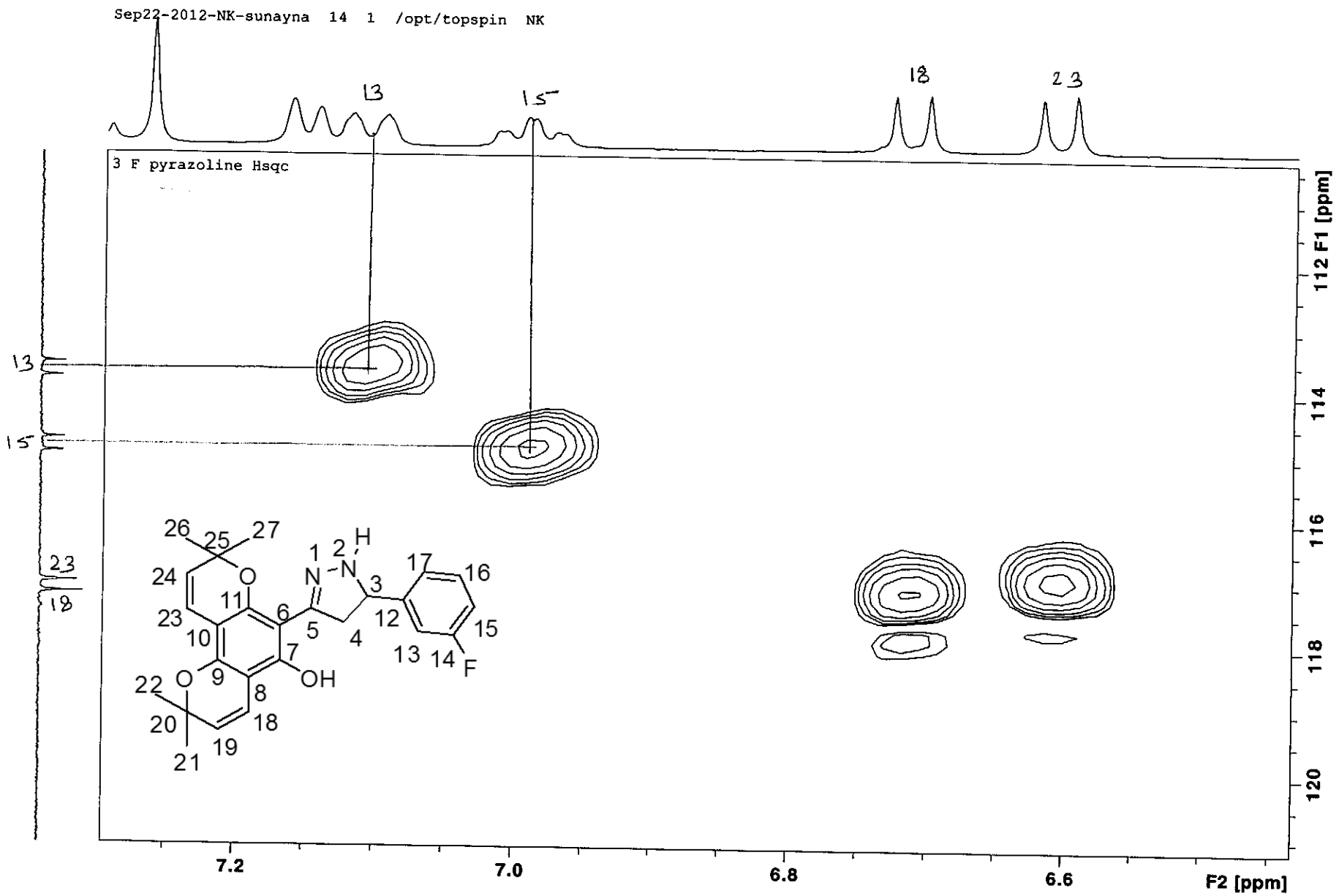
Expanded HSQC spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



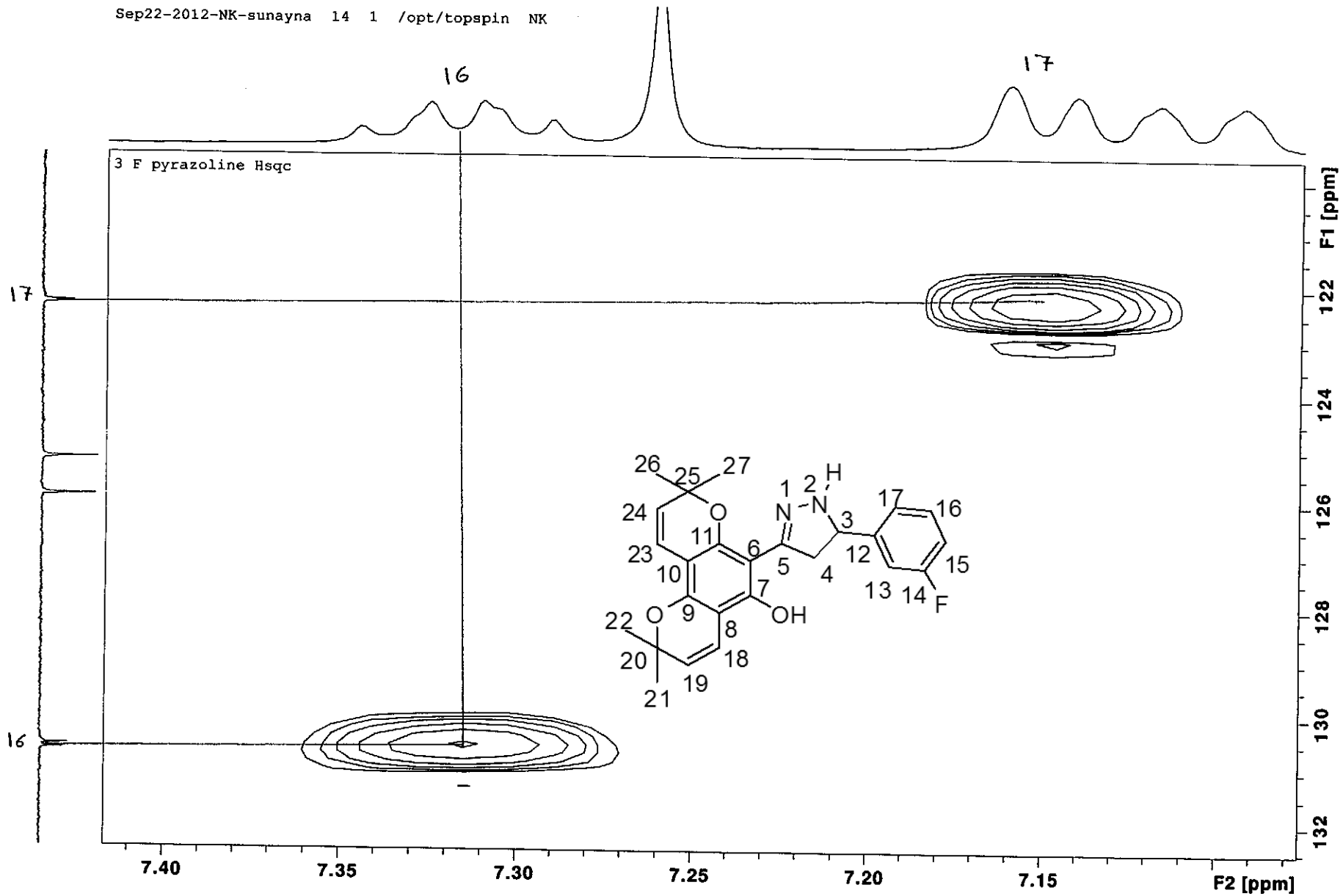
Expanded HSQC spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



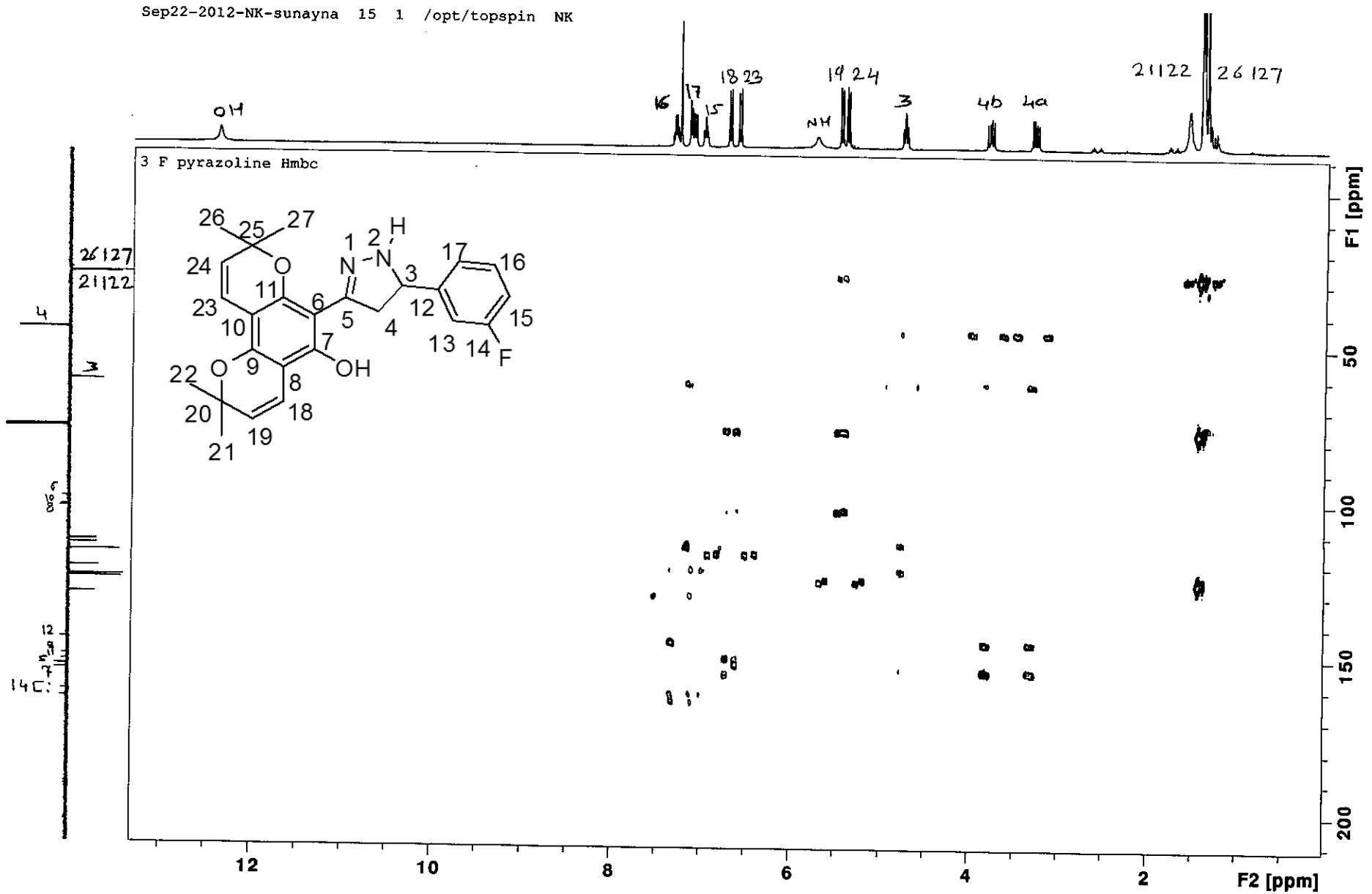
Expanded HSQC spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



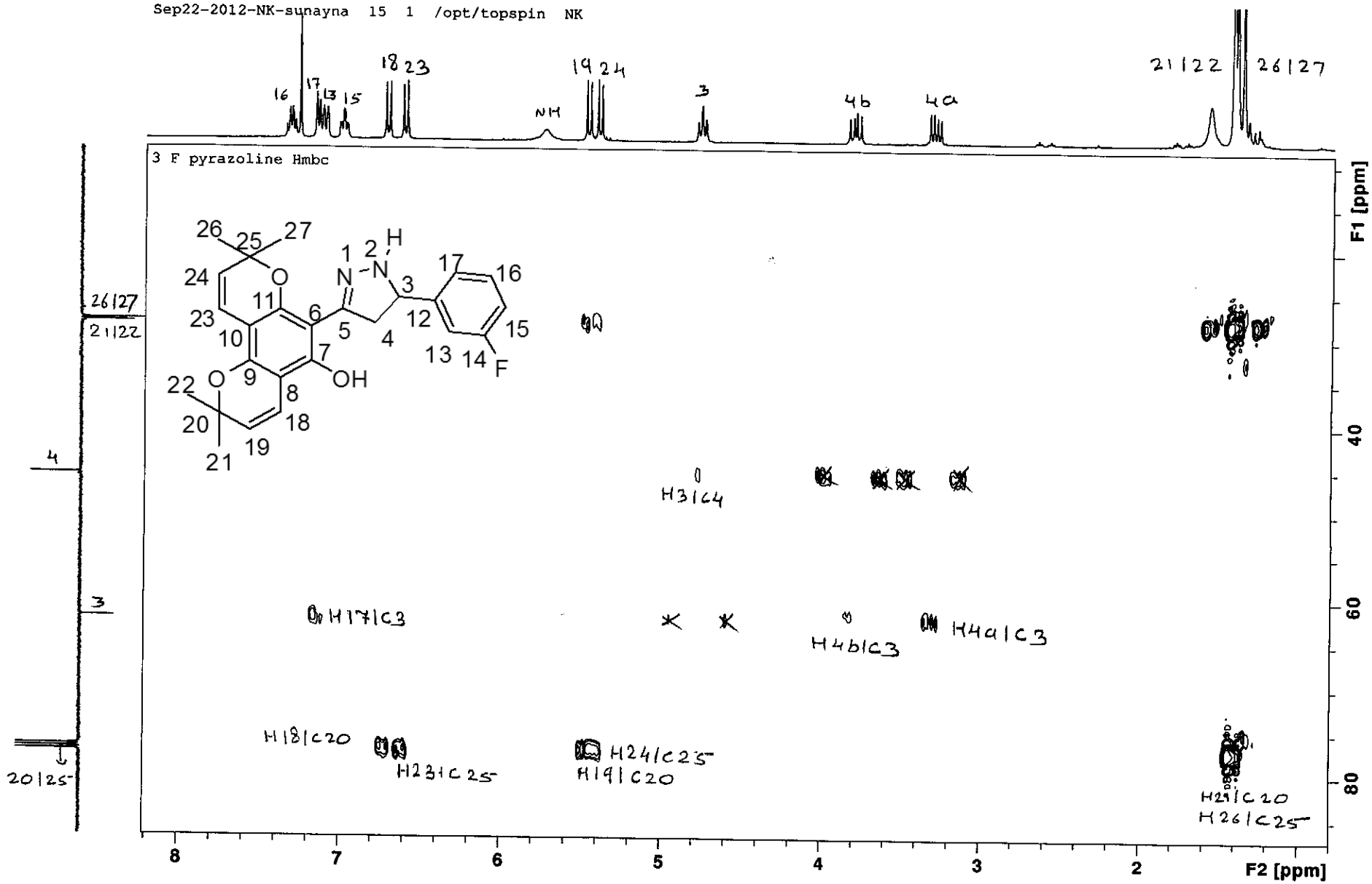
Expanded HSQC spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



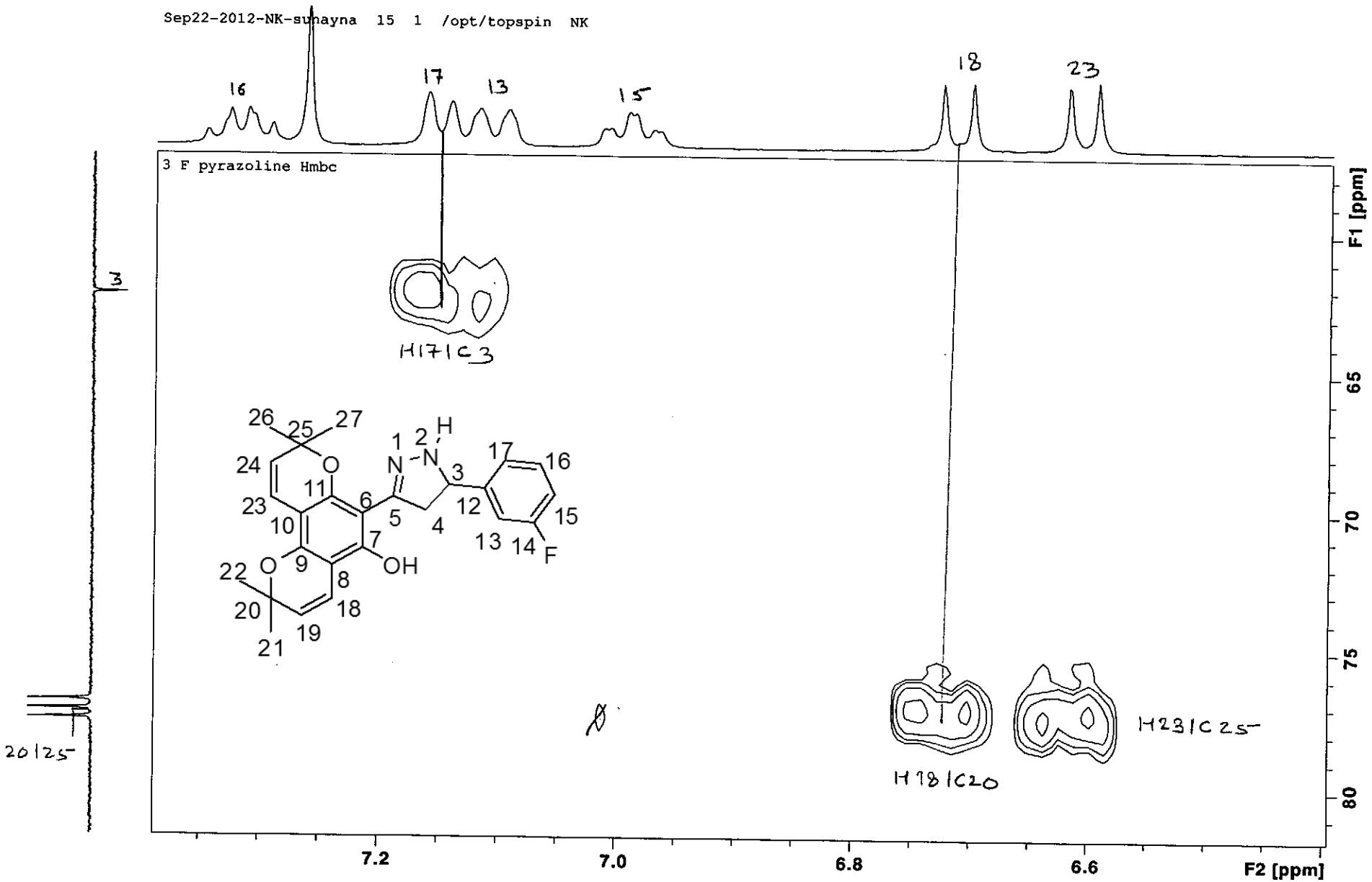
Expanded HSQC spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



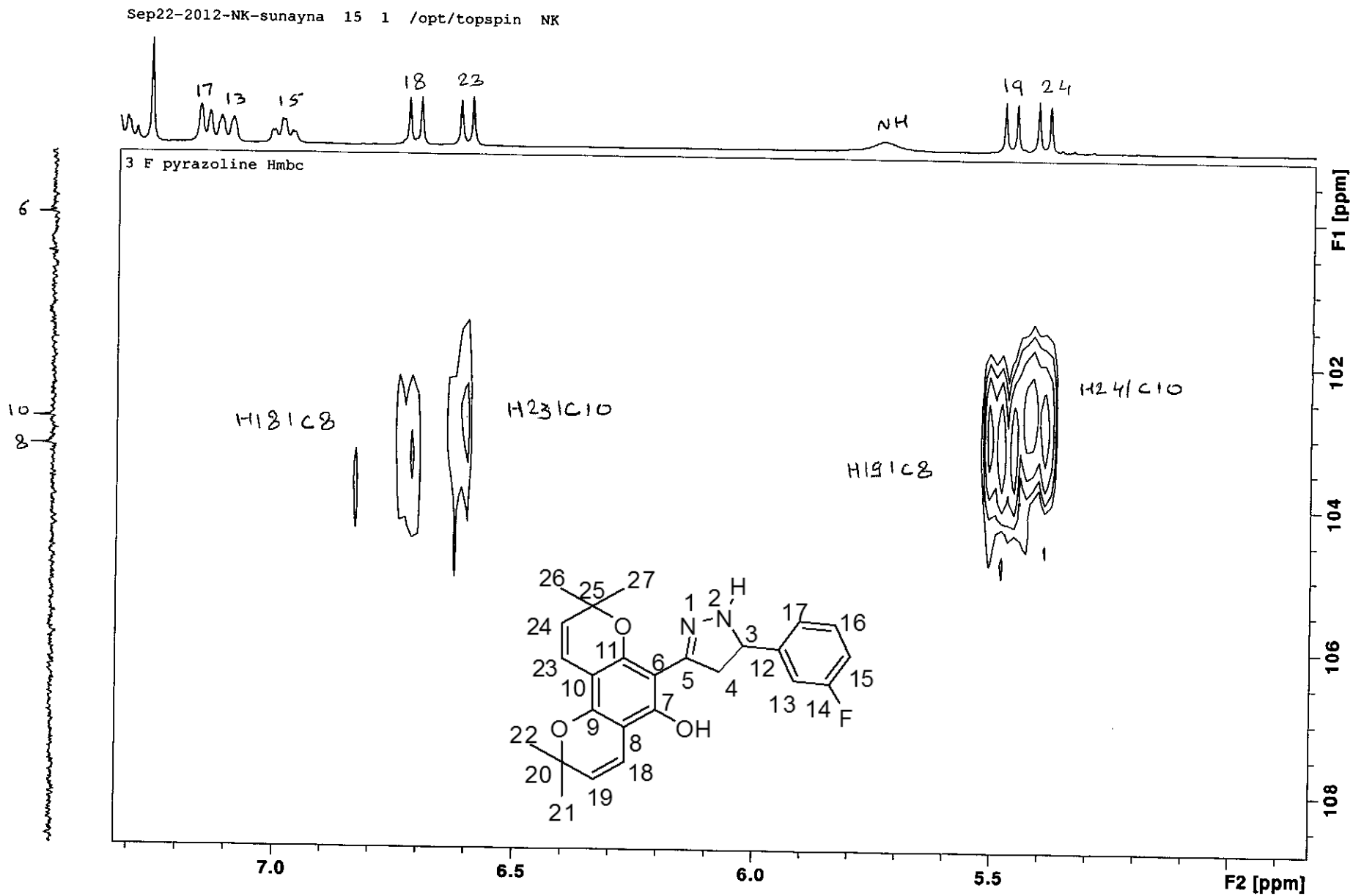
HMBC spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



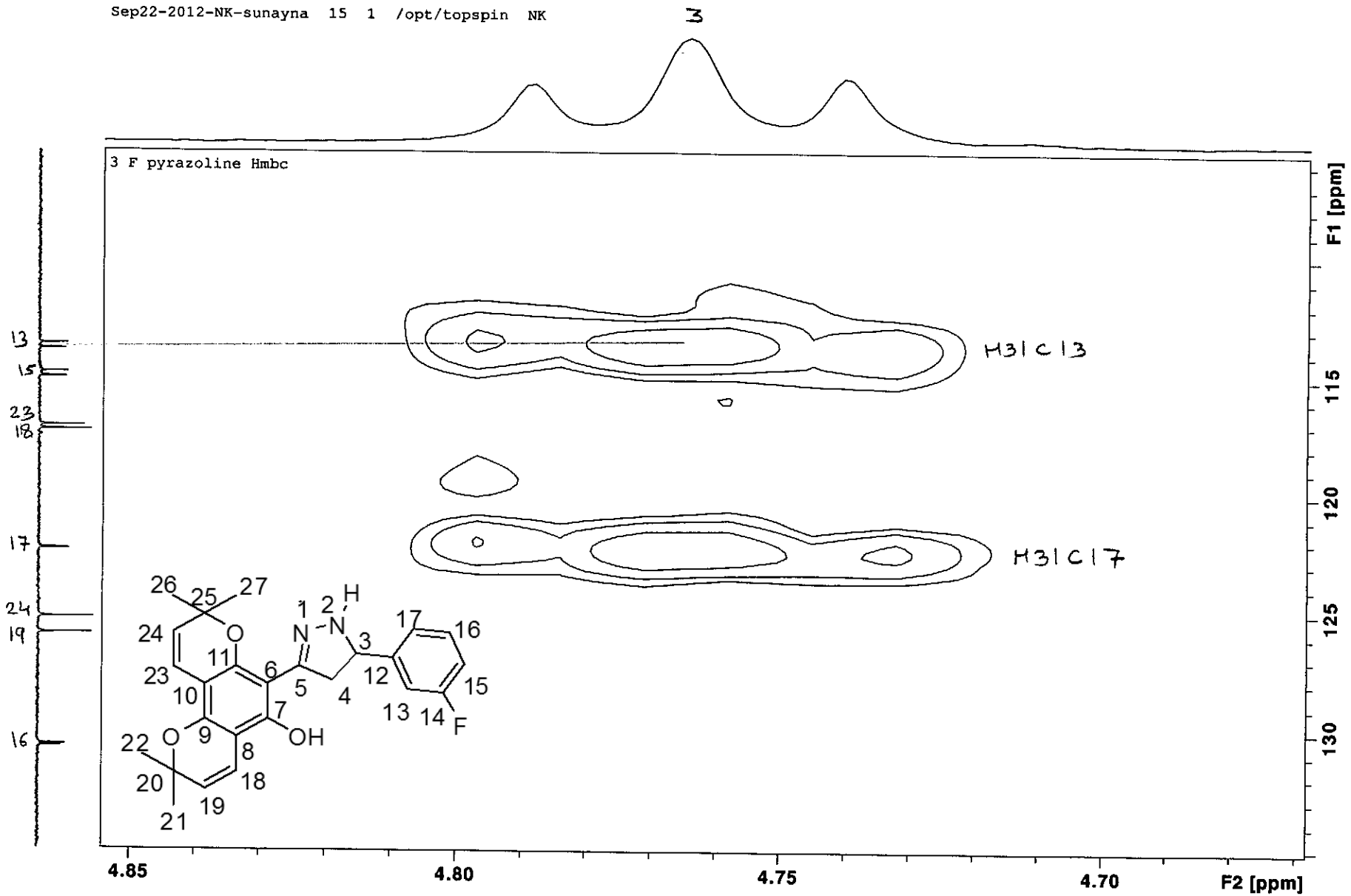
Expanded HMBC spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



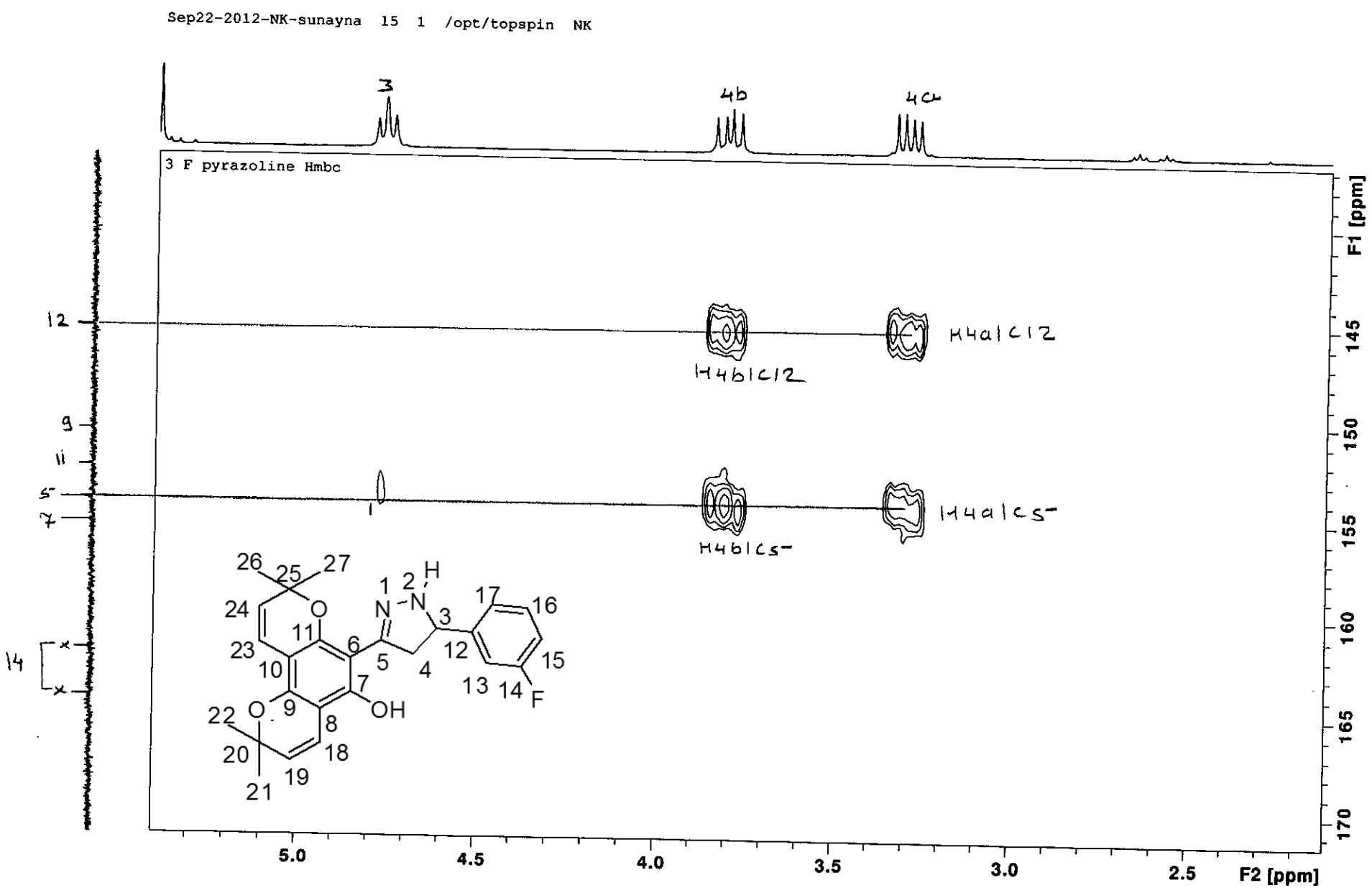
Expanded HMBC spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



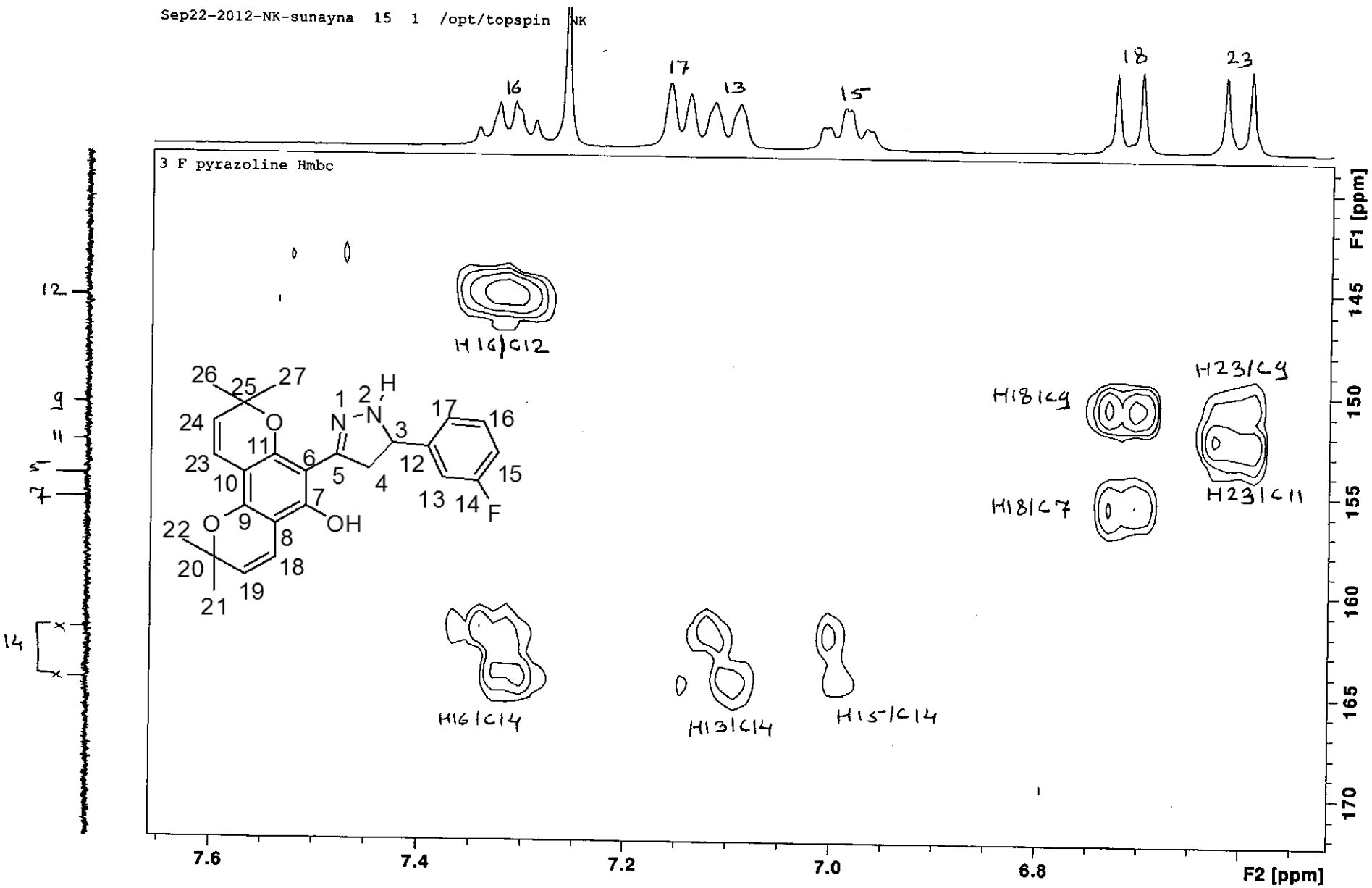
Expanded HMBC spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



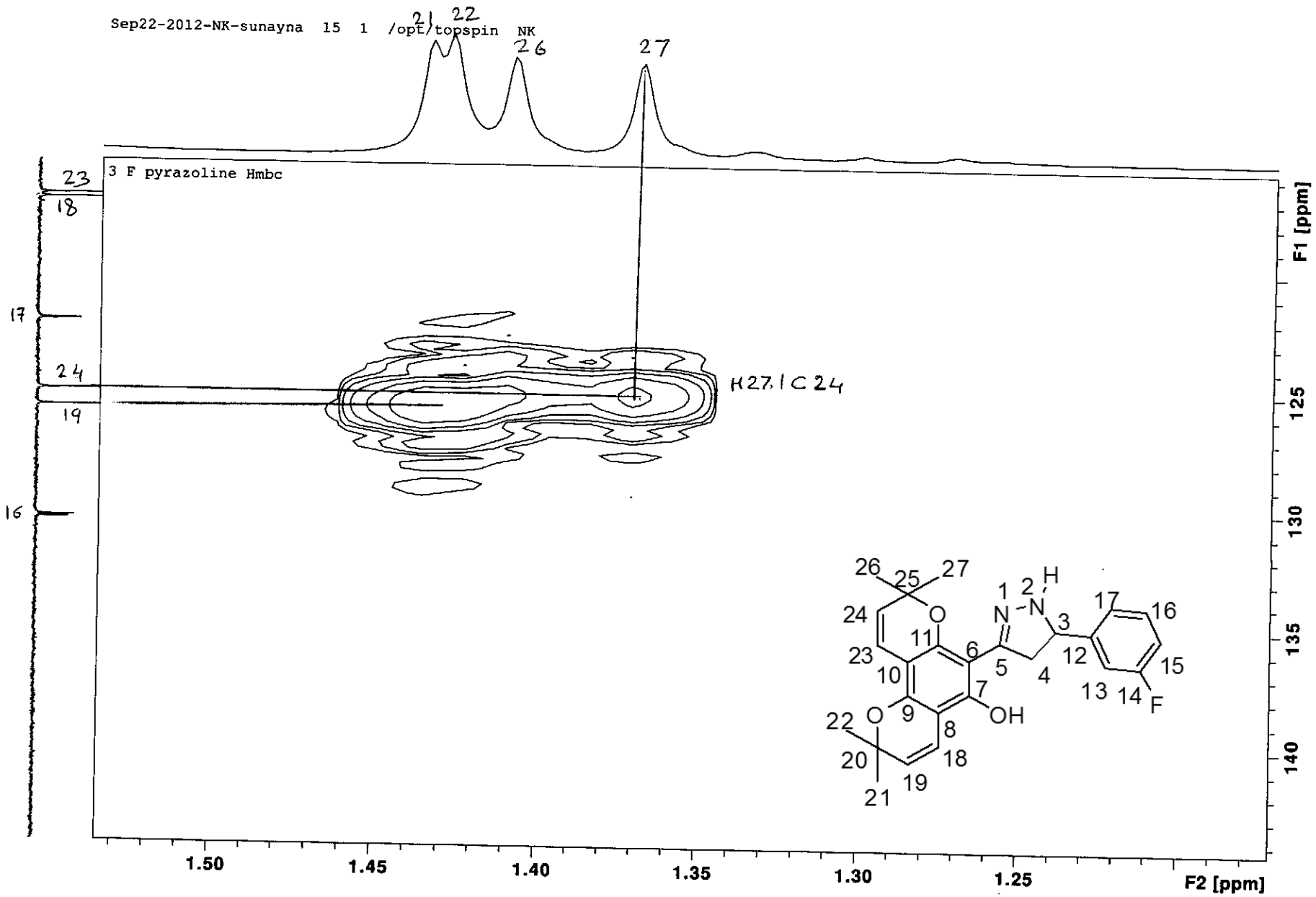
HMBC spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



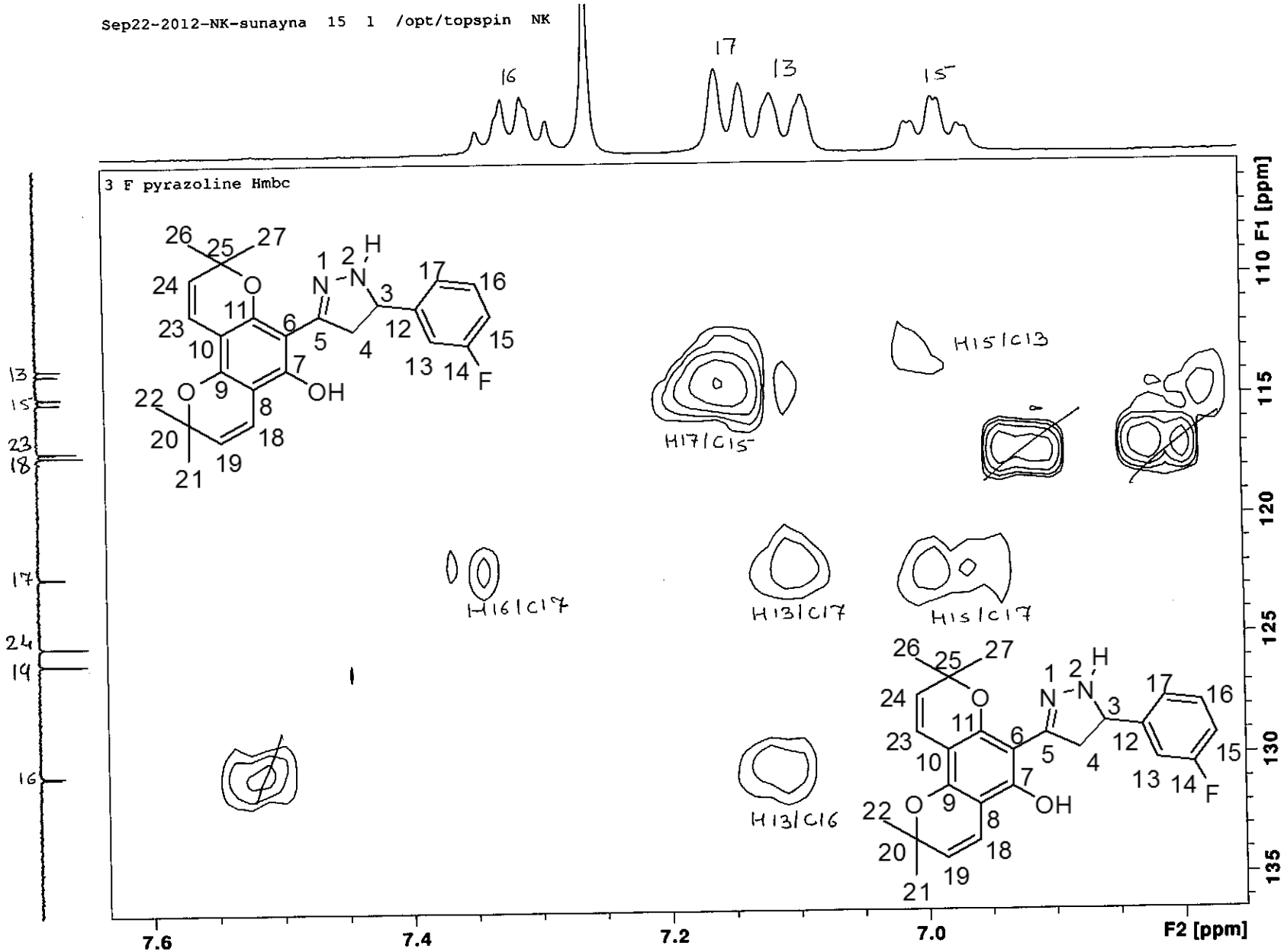
Expanded HMBC spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



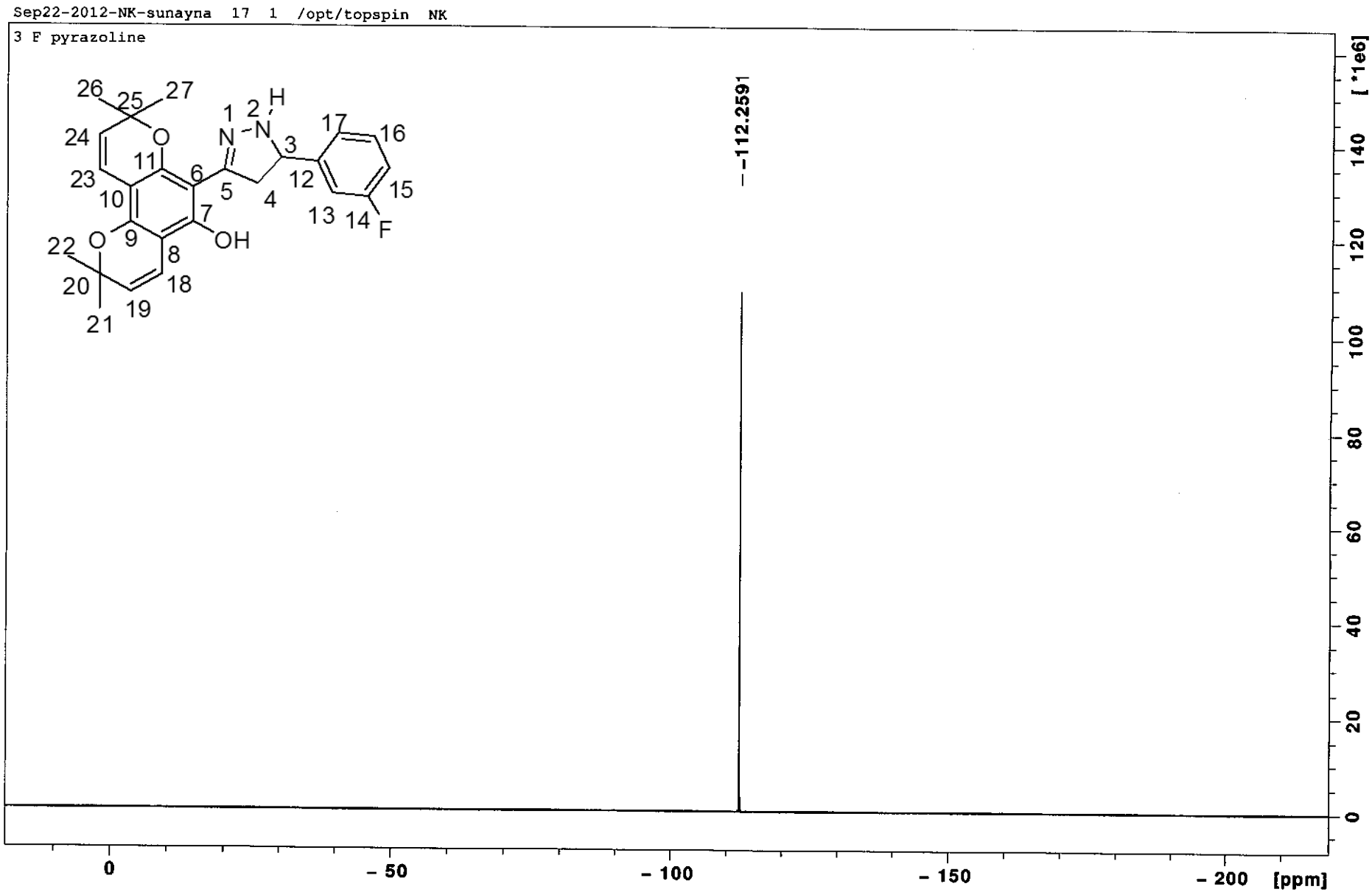
Expanded HMBC spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



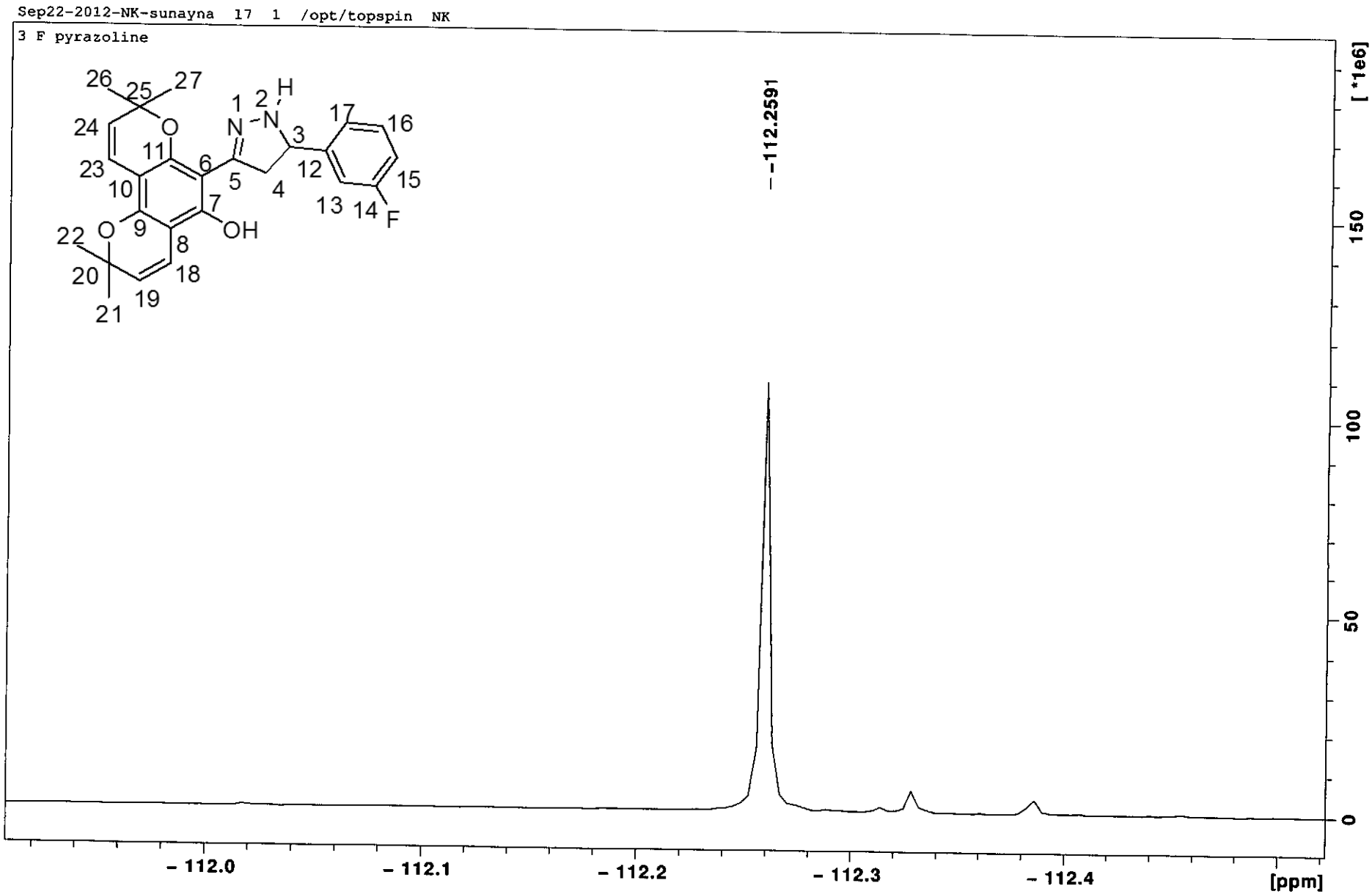
Expanded HMBC spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



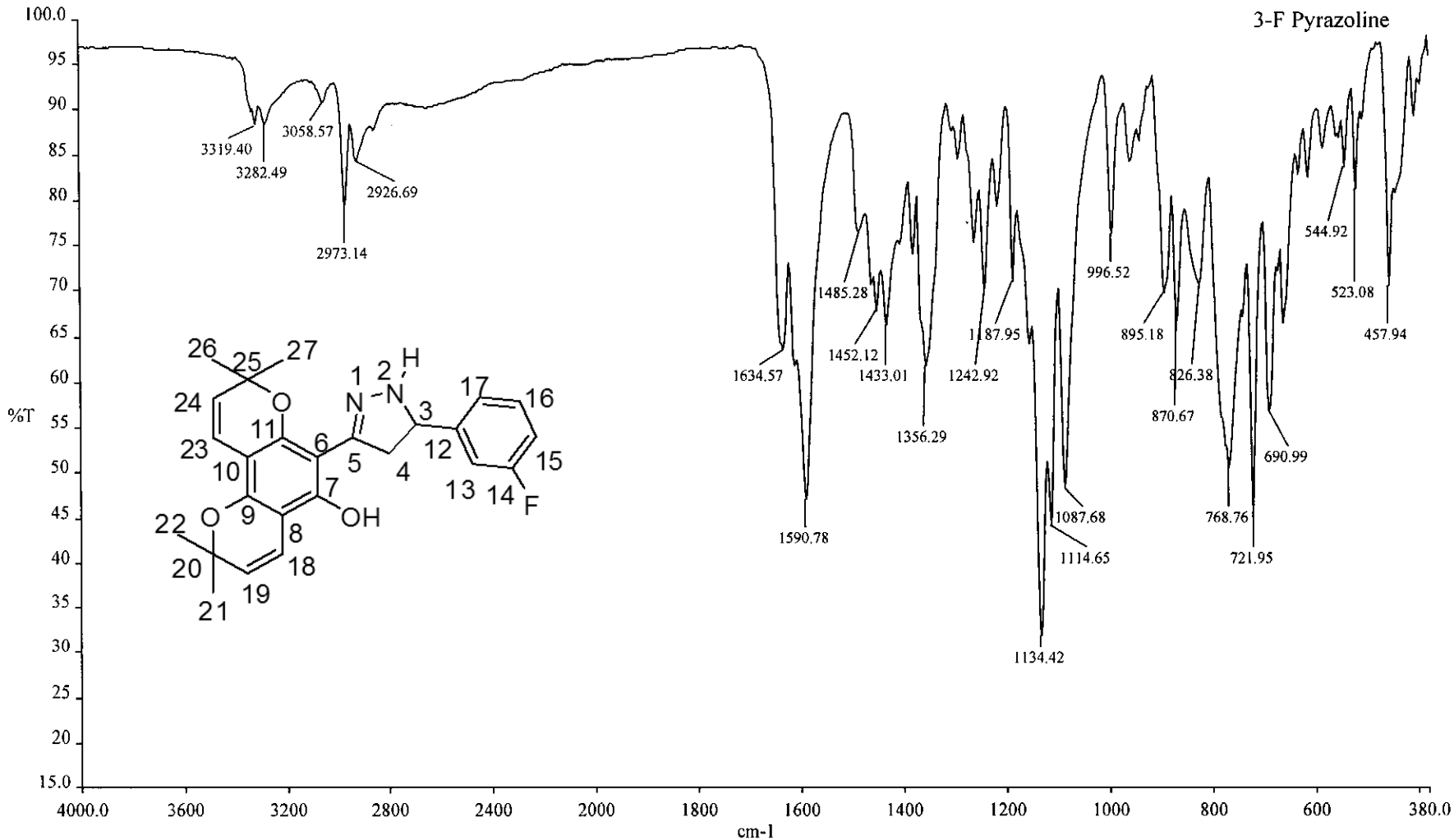
Expanded HMBC spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e) 620



19F NMR spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



Expanded 19F NMR spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)

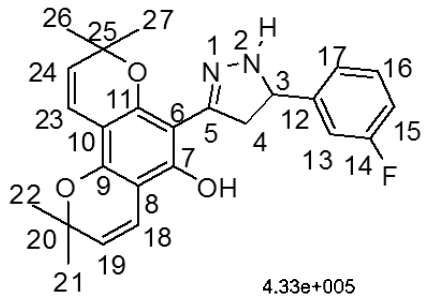


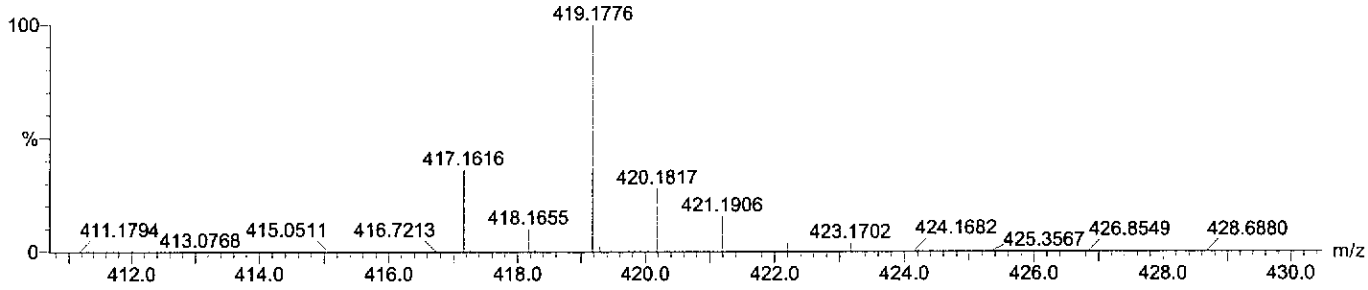
Infrared spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)

Elemental Composition Report **Page 1**

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

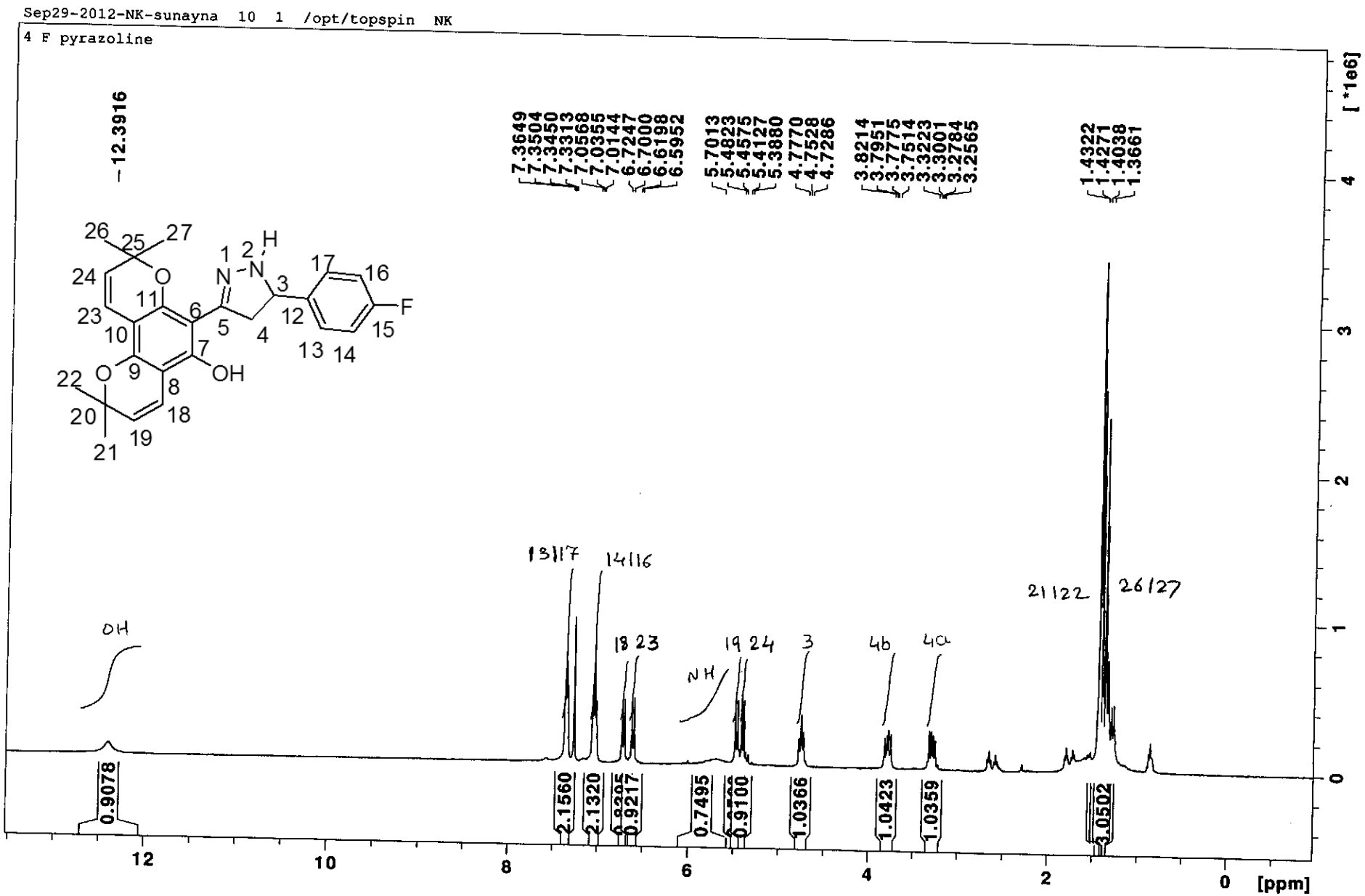
Monoisotopic Mass, Even Electron Ions
25 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-35 H: 20-30 N: 0-4 O: 0-4 F: 1-1
9 27 (0.444) Cm (1:31)
TOF MS ES-



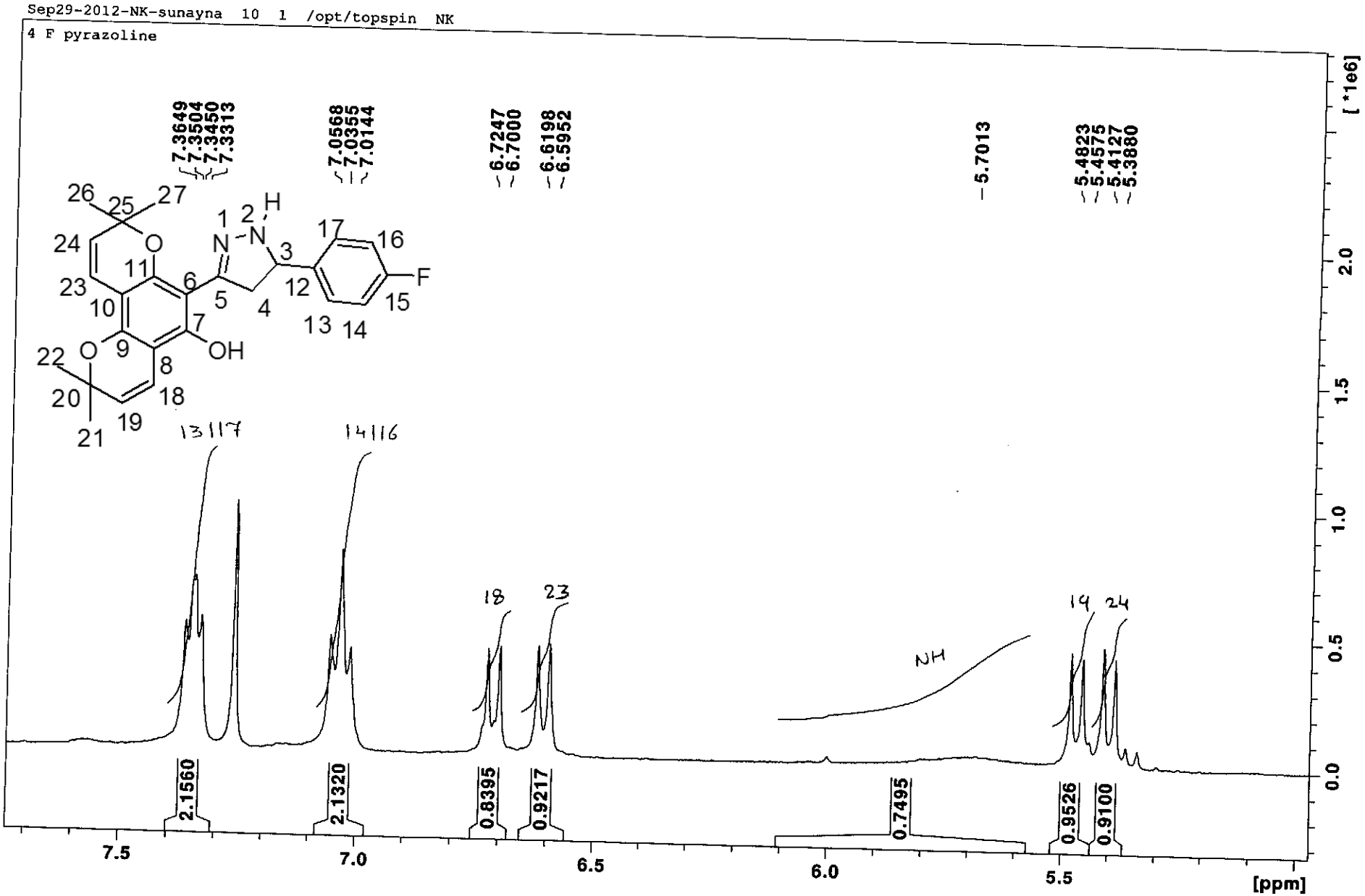


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
419.1776	419.1771	0.5	1.2	14.5	596.5	0.0	C25 H24 N2 O3 F

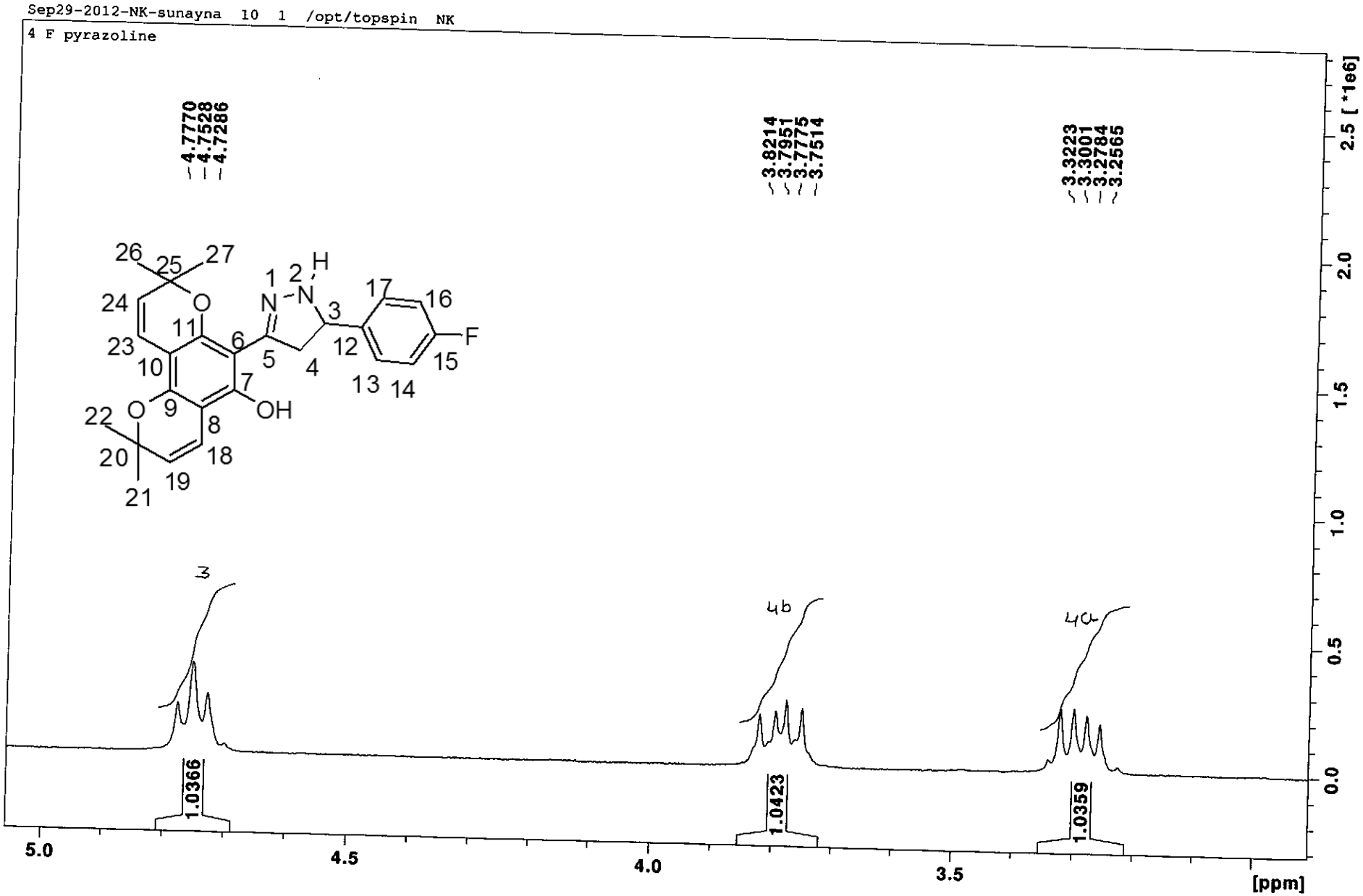
HRMS spectrum of 3-(3-fluorophenyl)-5-pyranochromene pyrazoline (C-4e)



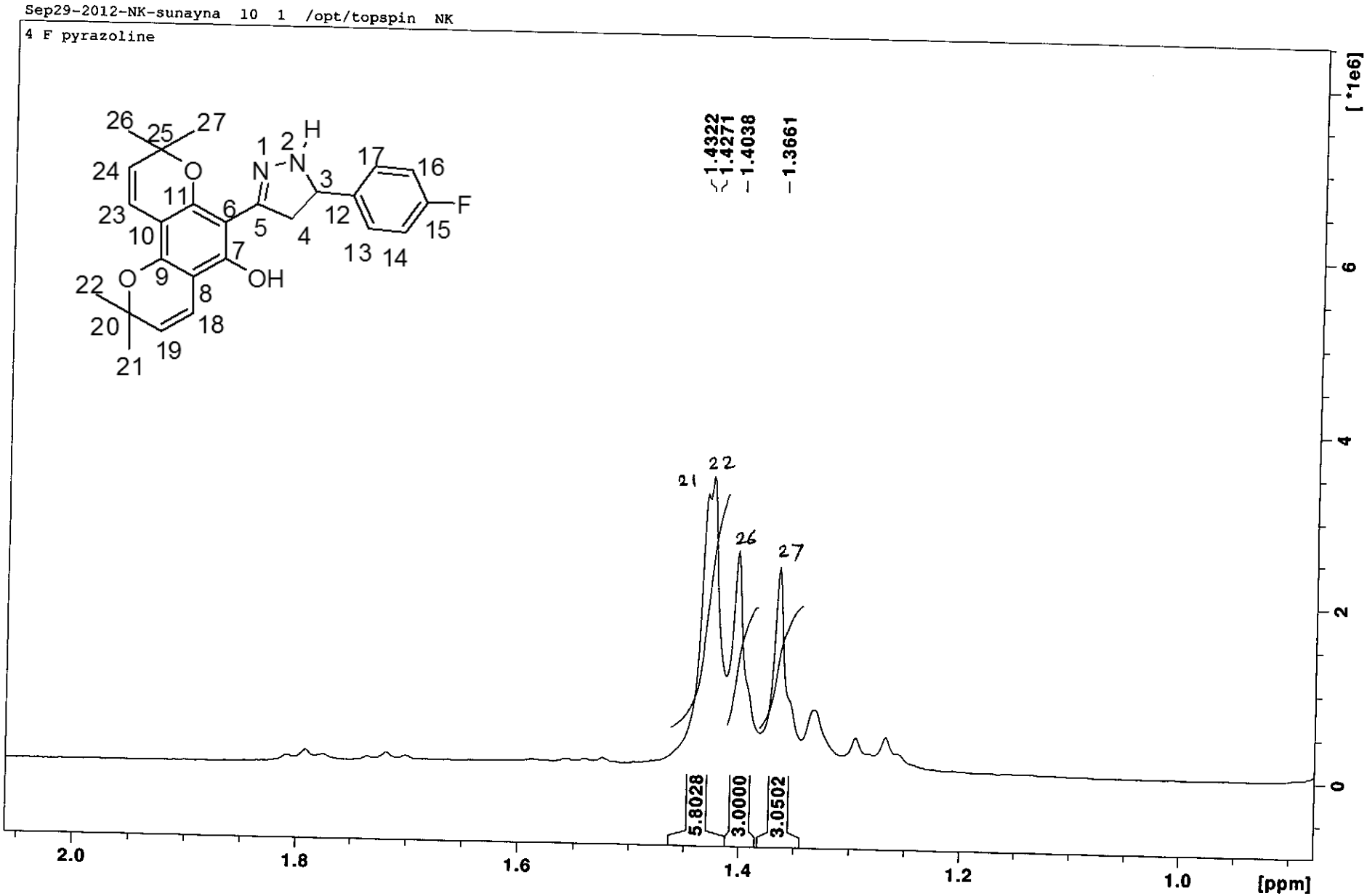
1H NMR spectrum of 3-(4-fluorophenyl)-5-pyranochromene pyrazoline (C-4f)



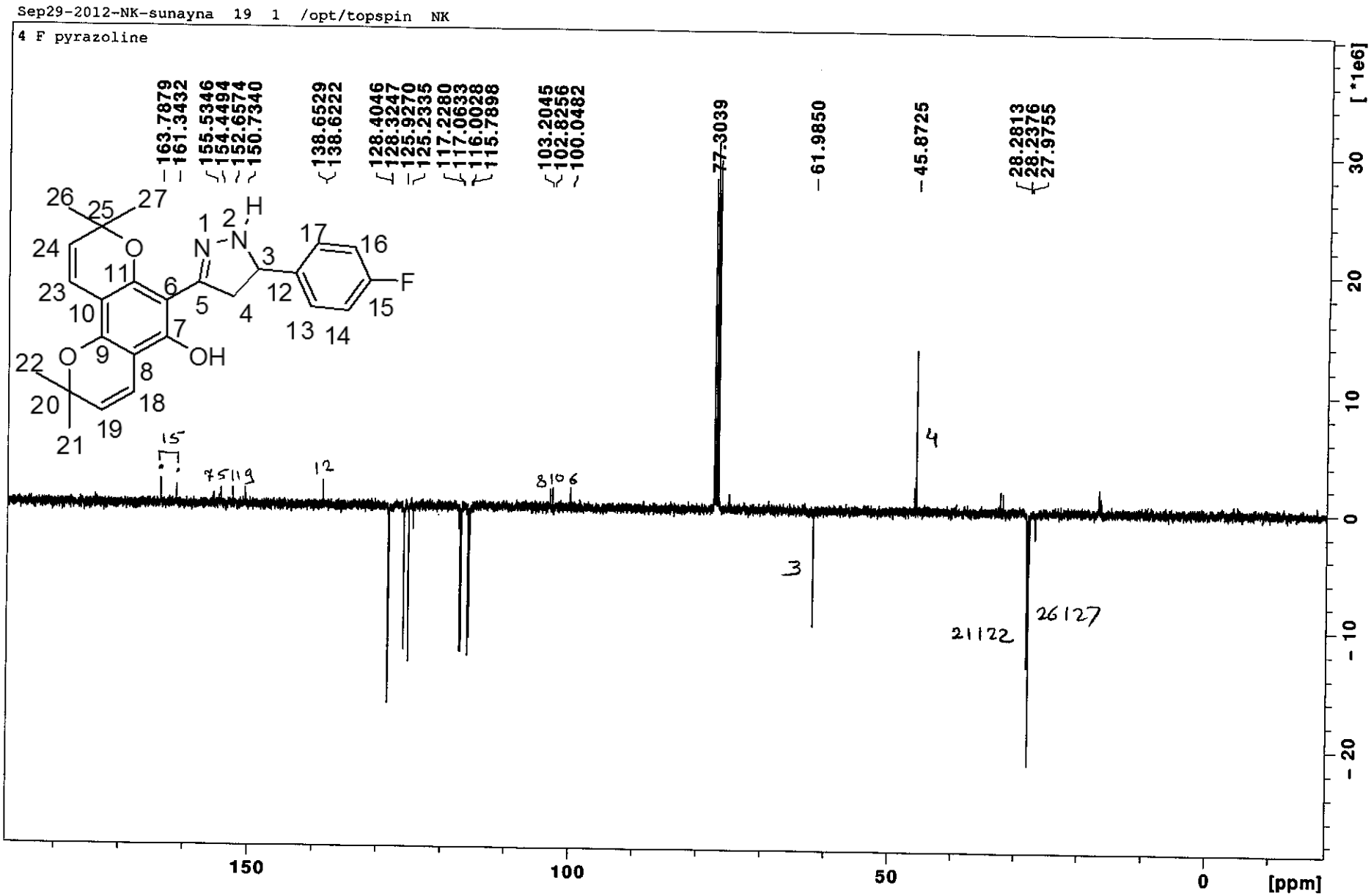
Expanded 1H NMR spectrum of 3-(4-fluorophenyl)-5-pyranochromene pyrazoline (C-4f)



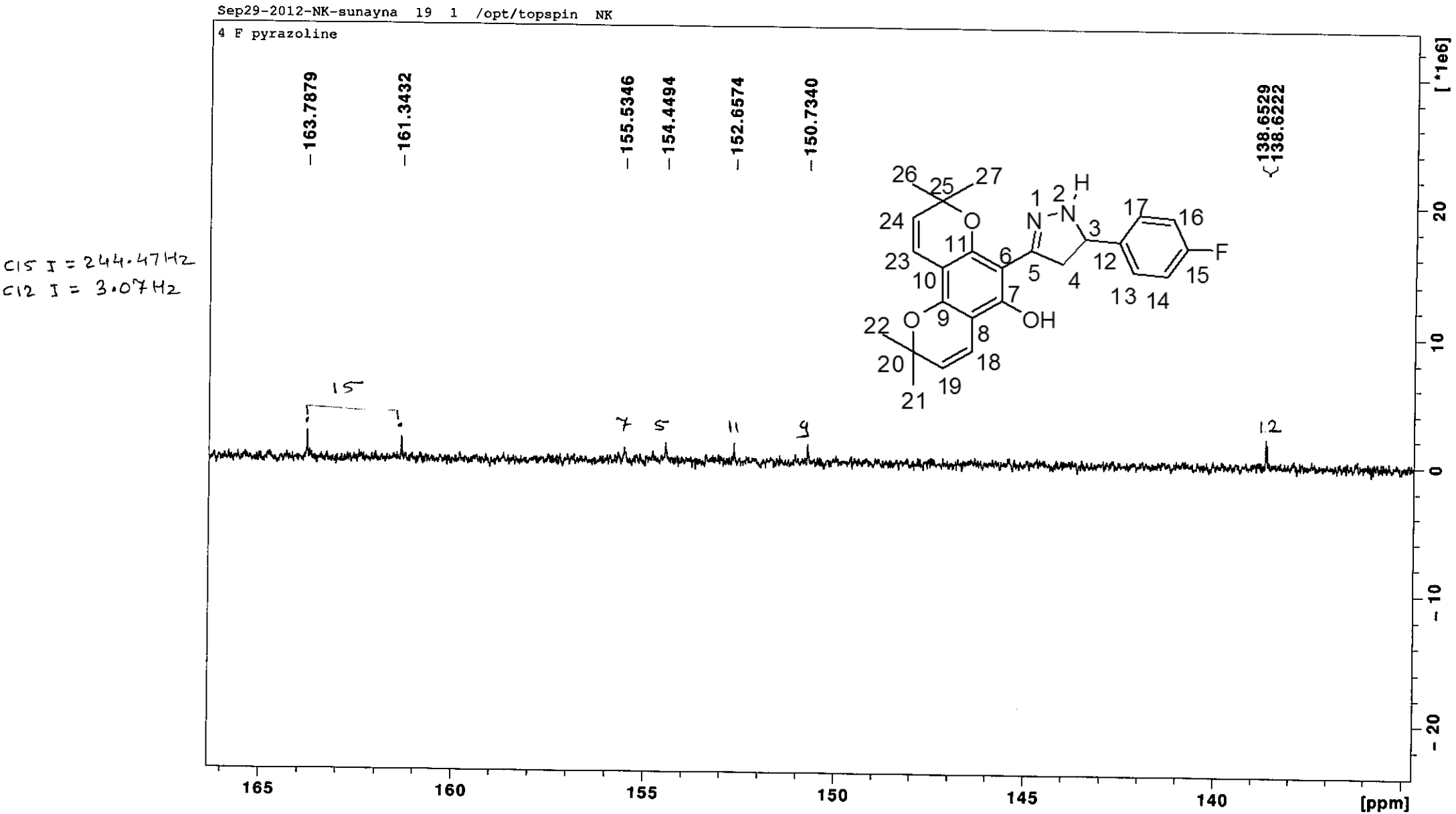
Expanded 1H NMR spectrum of 3-(4-fluorophenyl)-5-pyranochromene pyrazoline (C-4f)



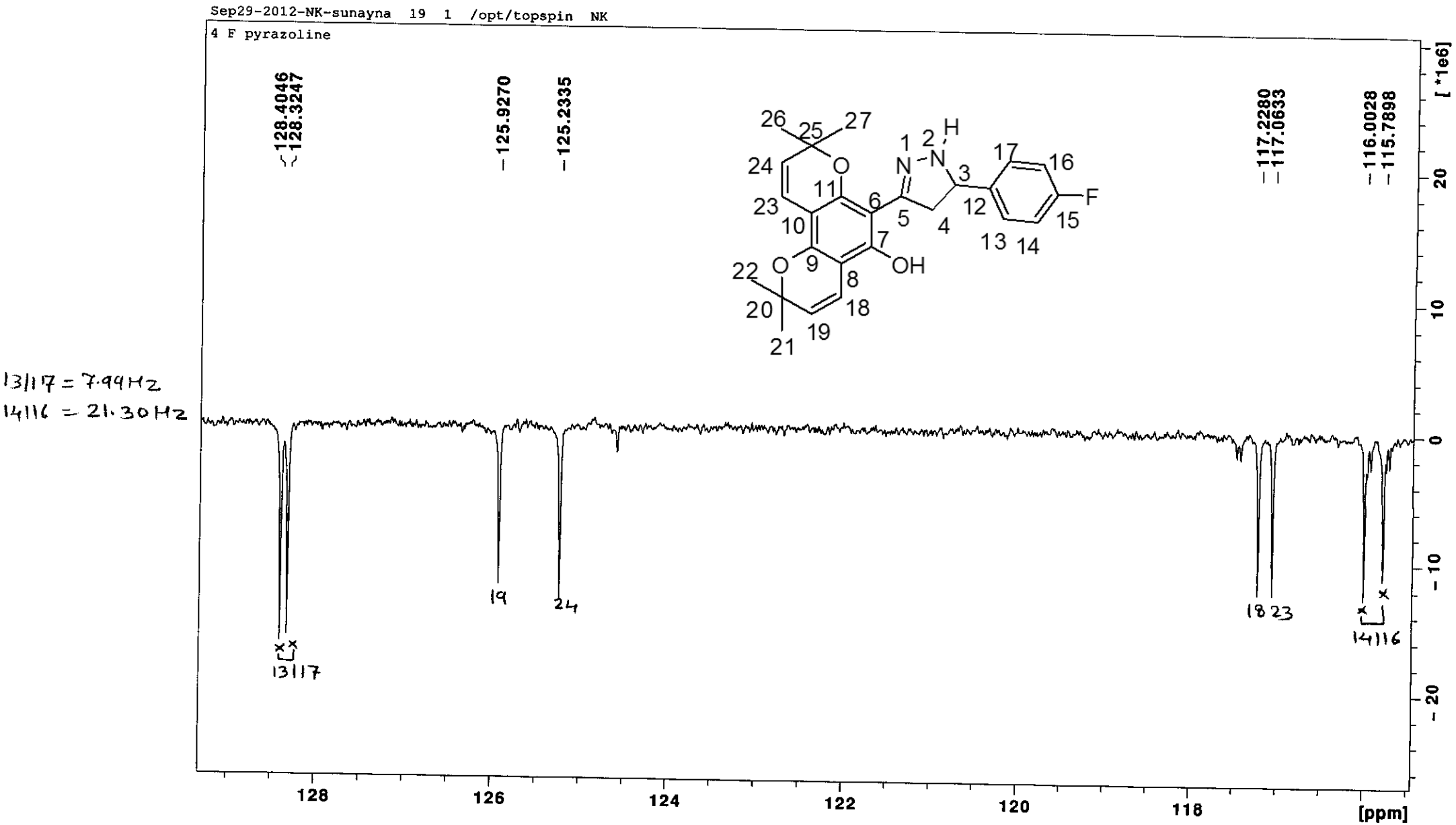
Expanded 1H spectrum of 3-(4-fluorophenyl)-5-pyranochromene pyrazoline (C-4f)



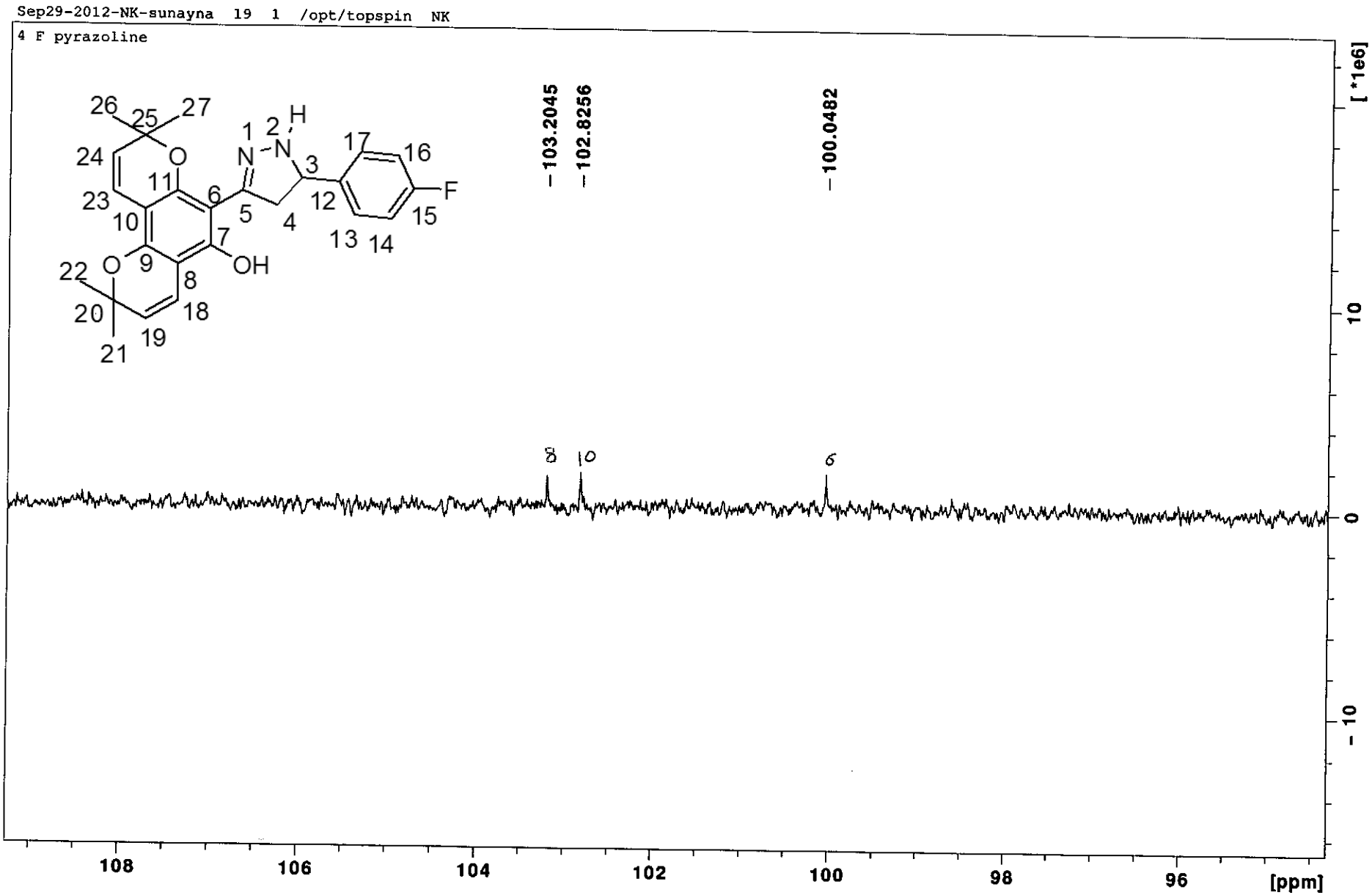
13C NMR spectrum of 3-(4-fluorophenyl)-5-pyranochromene pyrazoline (C-4f)



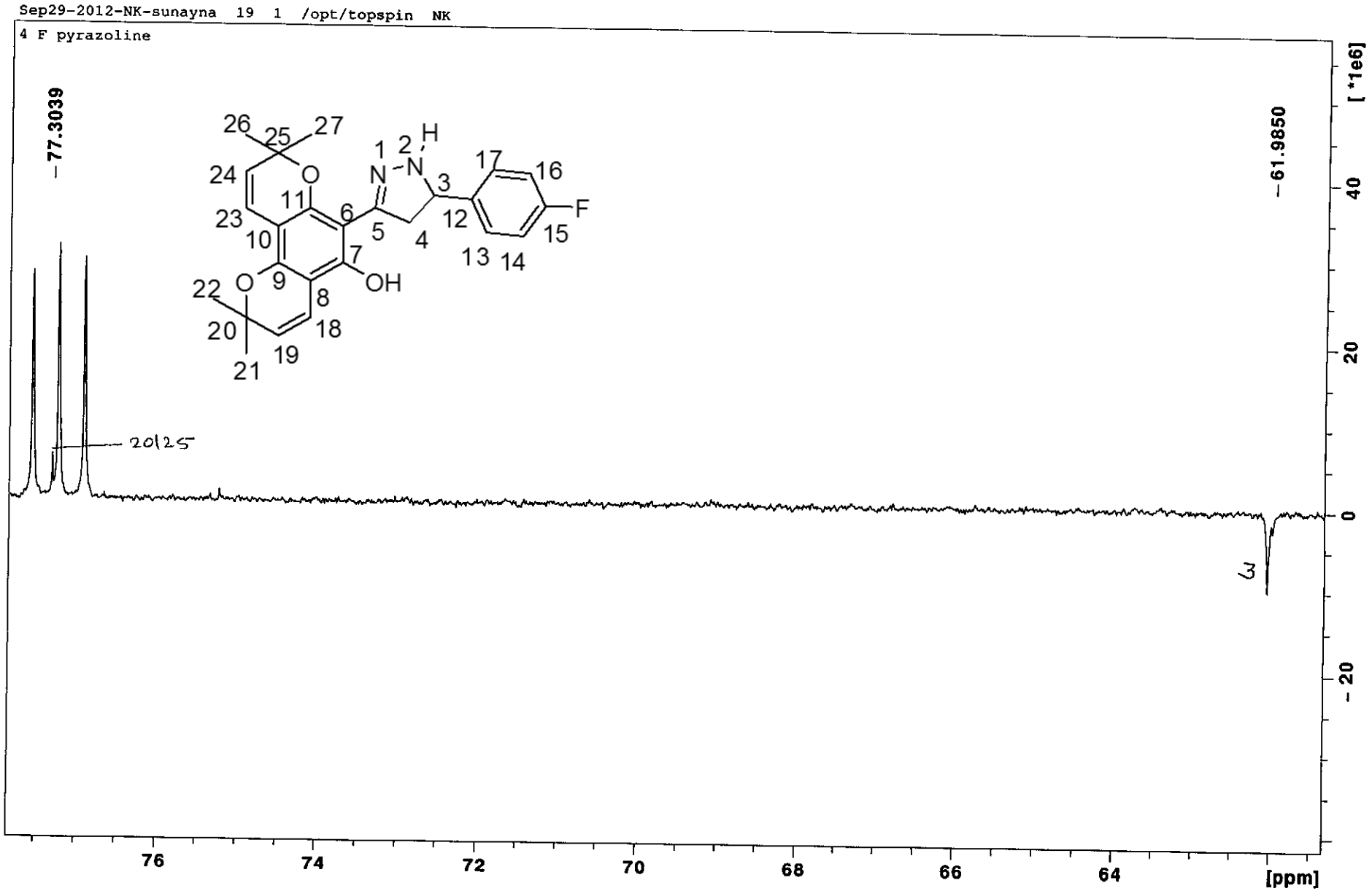
Expanded 13C NMR spectrum of 3-(4-fluorophenyl)-5-pyranochromene pyrazoline (C-4f)



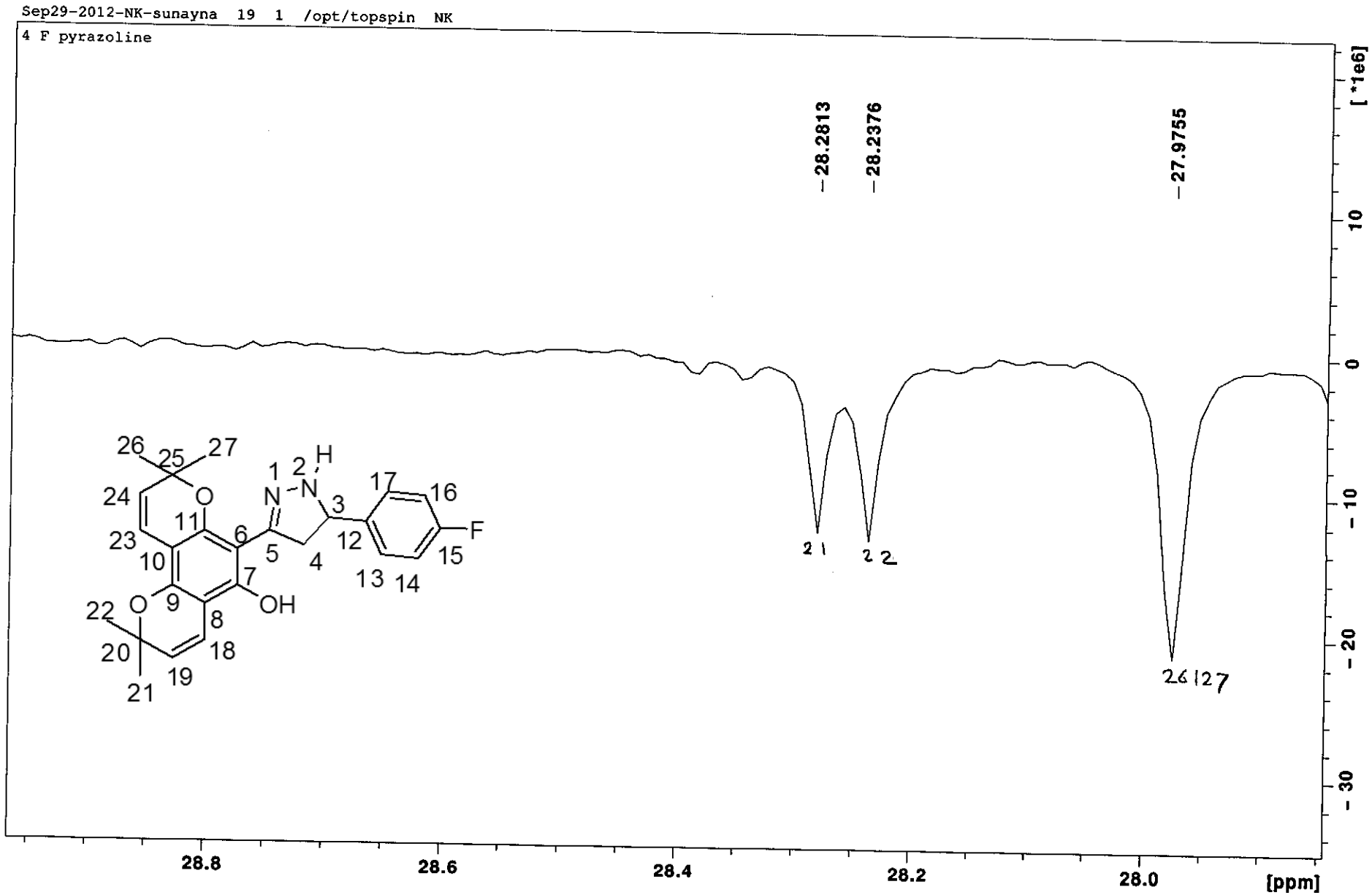
Expanded 13C NMR spectrum of 3-(4-fluorophenyl)-5-pyranochromene pyrazoline (C-4f)



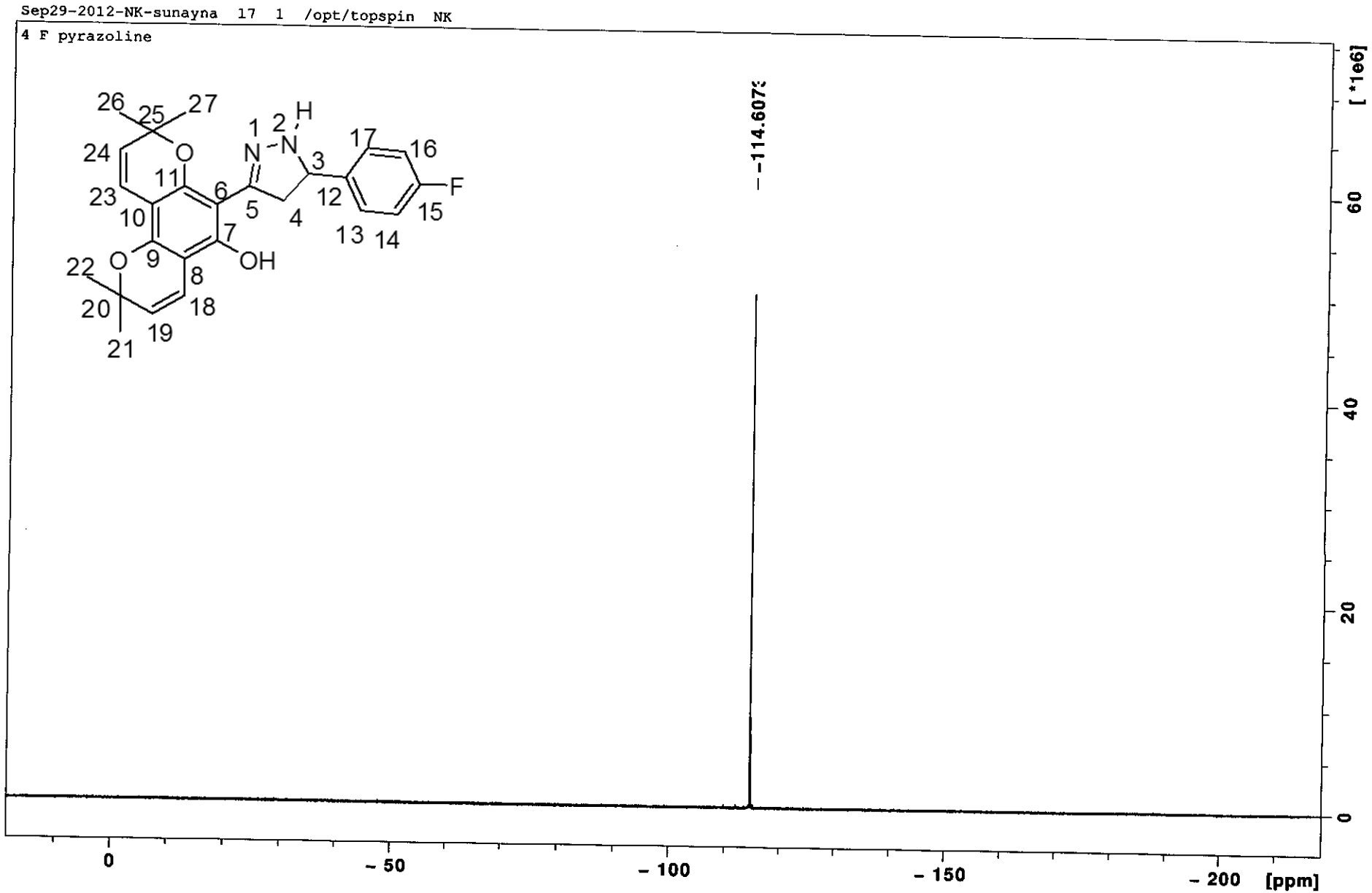
Expanded 13C NMR spectrum of 3-(4-fluorophenyl)-5-pyranochromene pyrazoline (C-4f)



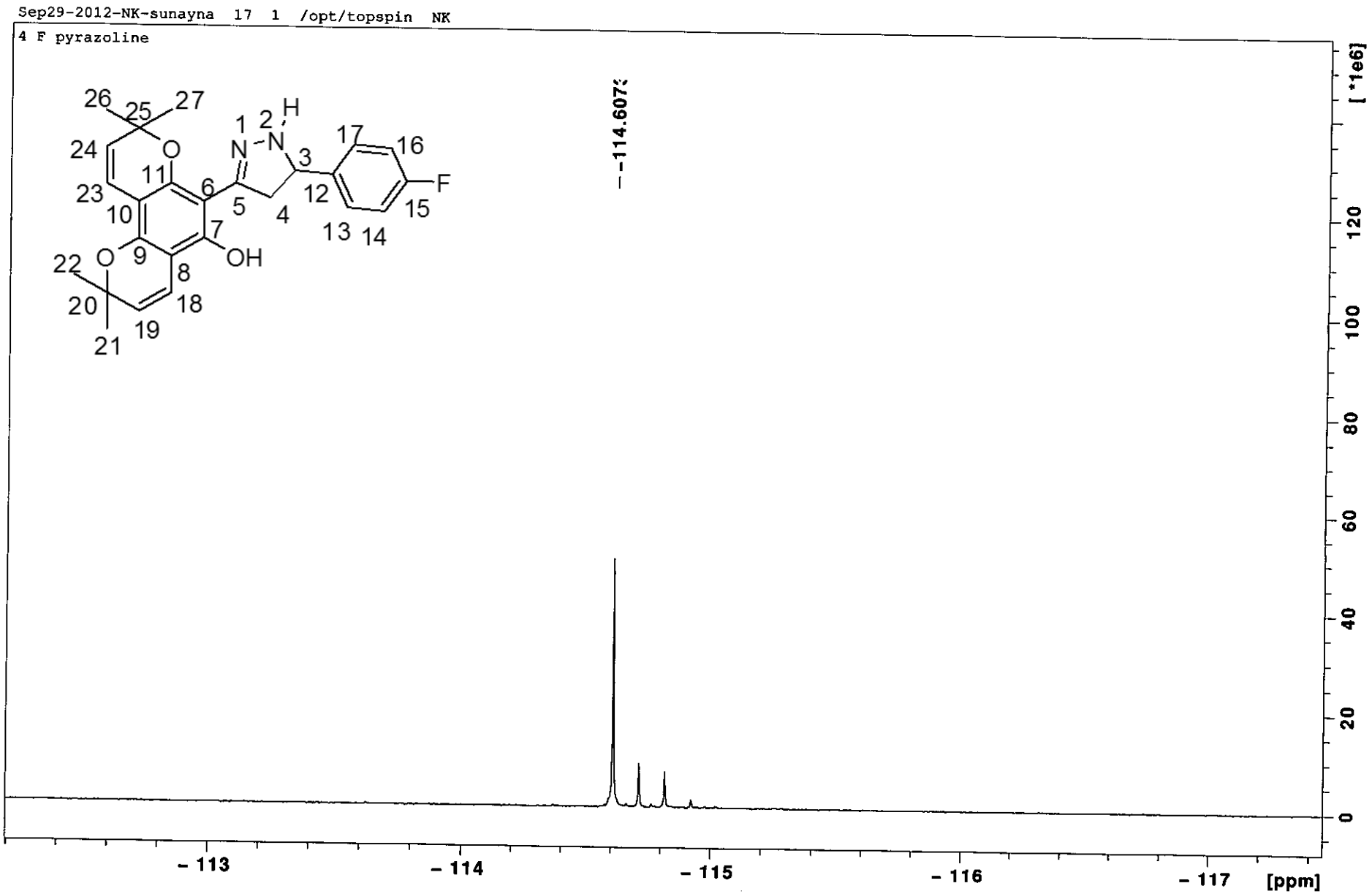
Expanded 13C NMR spectrum of 3-(4-fluorophenyl)-5-pyranochromene pyrazoline (C-4f)



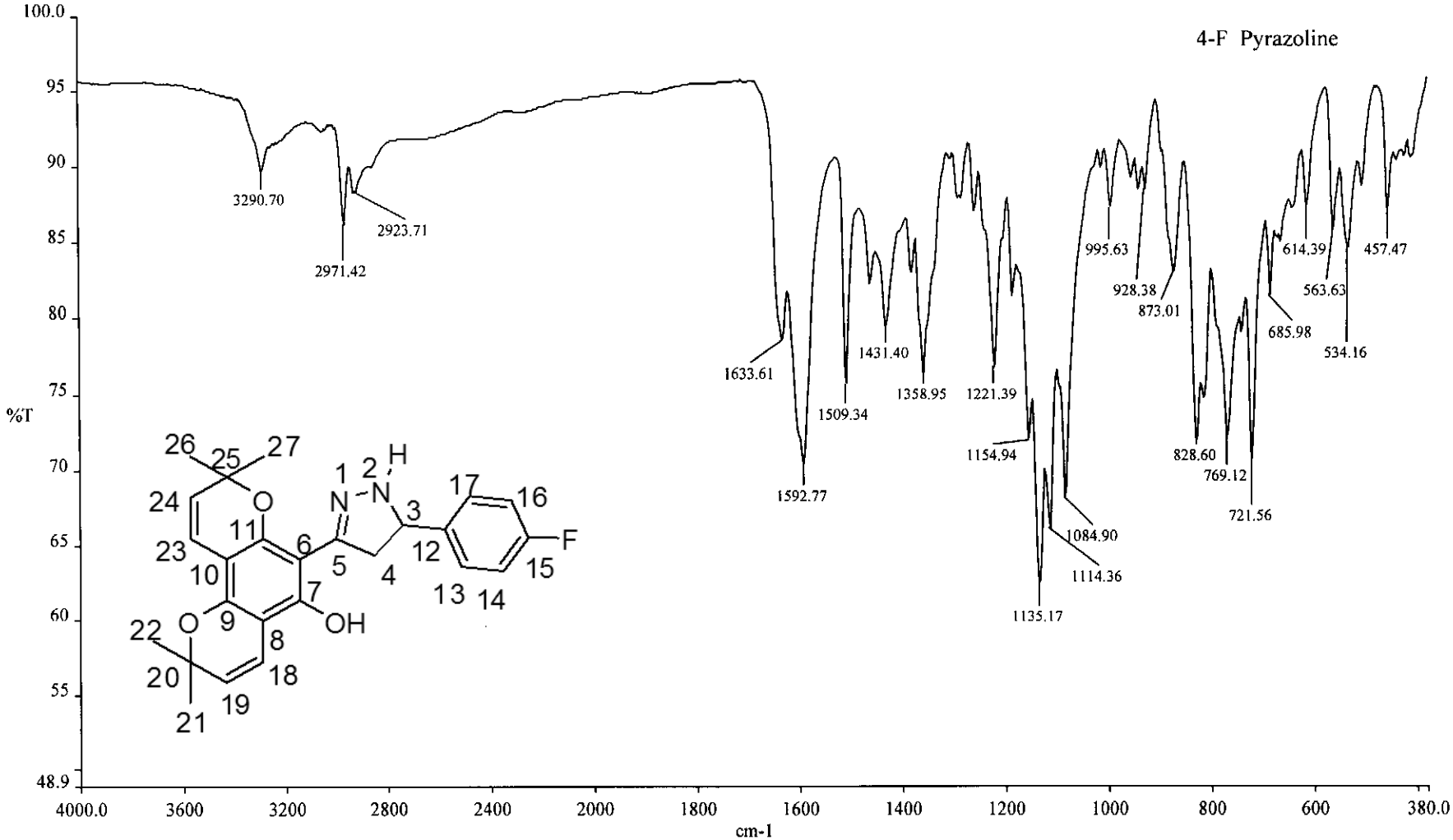
Expanded 13C NMR spectrum of 3-(4-fluorophenyl)-5-pyranochromene pyrazoline (C-4f)



19F NMR spectrum of 3-(4-fluorophenyl)-5-pyranochromene pyrazoline (C-4f)



Expanded 19F NMR spectrum of 3-(4-fluorophenyl)-5-pyranochromene pyrazoline (C-4f)

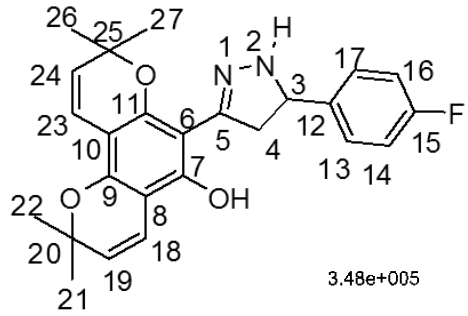


Infrared spectrum of 3-(4-fluorophenyl)-5-pyranochromene pyrazoline (C-4f)

Elemental Composition Report **Page 1**

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

Monoisotopic Mass, Even Electron Ions
25 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-35 H: 20-30 N: 0-4 O: 0-4 F: 1-1
11 27 (0.444) Cm (1:30)
TOF MS ES-



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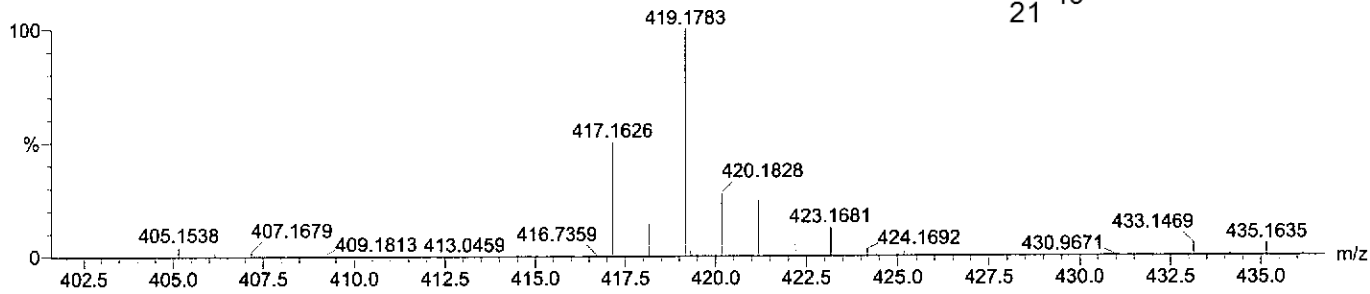
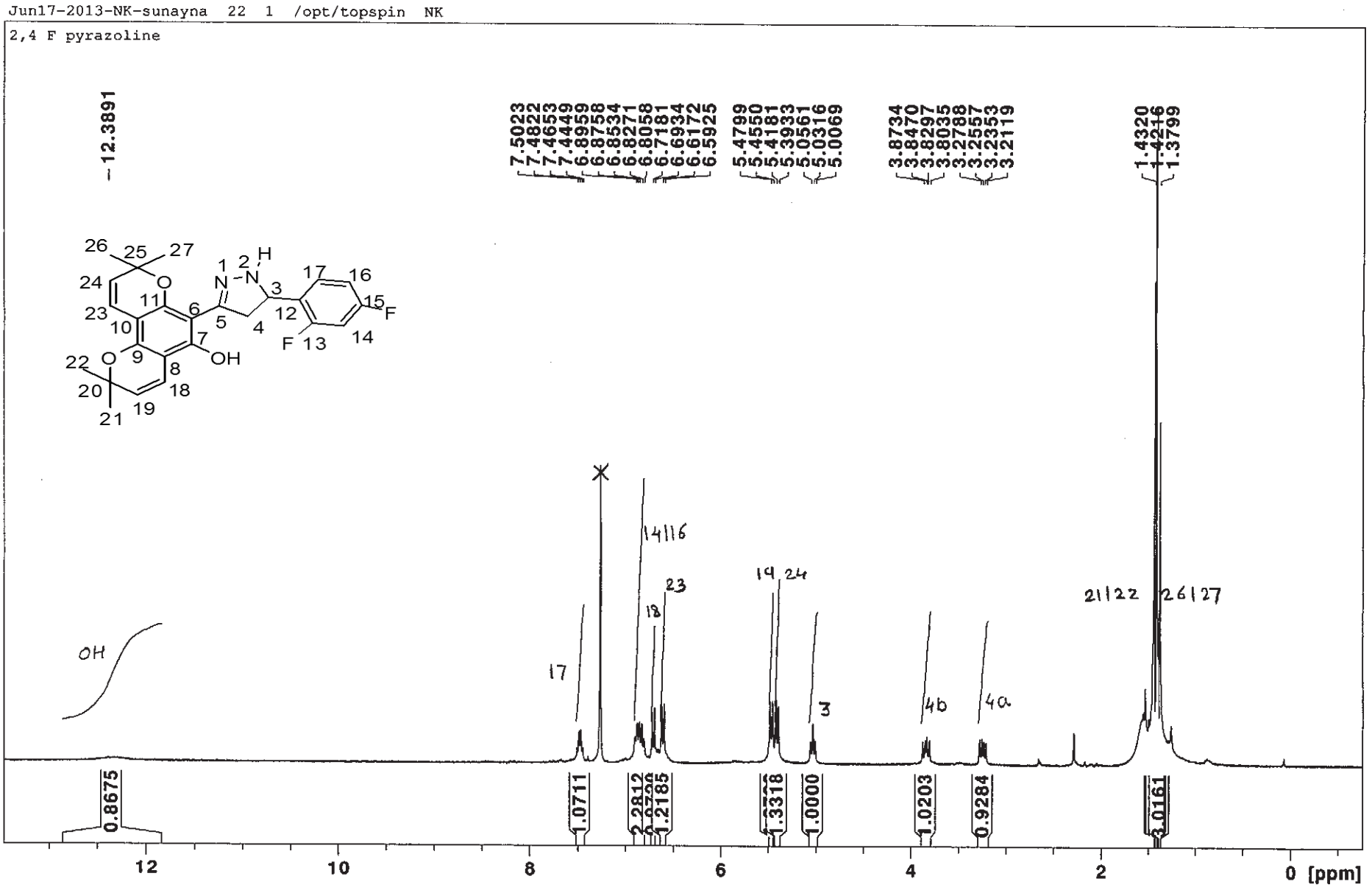
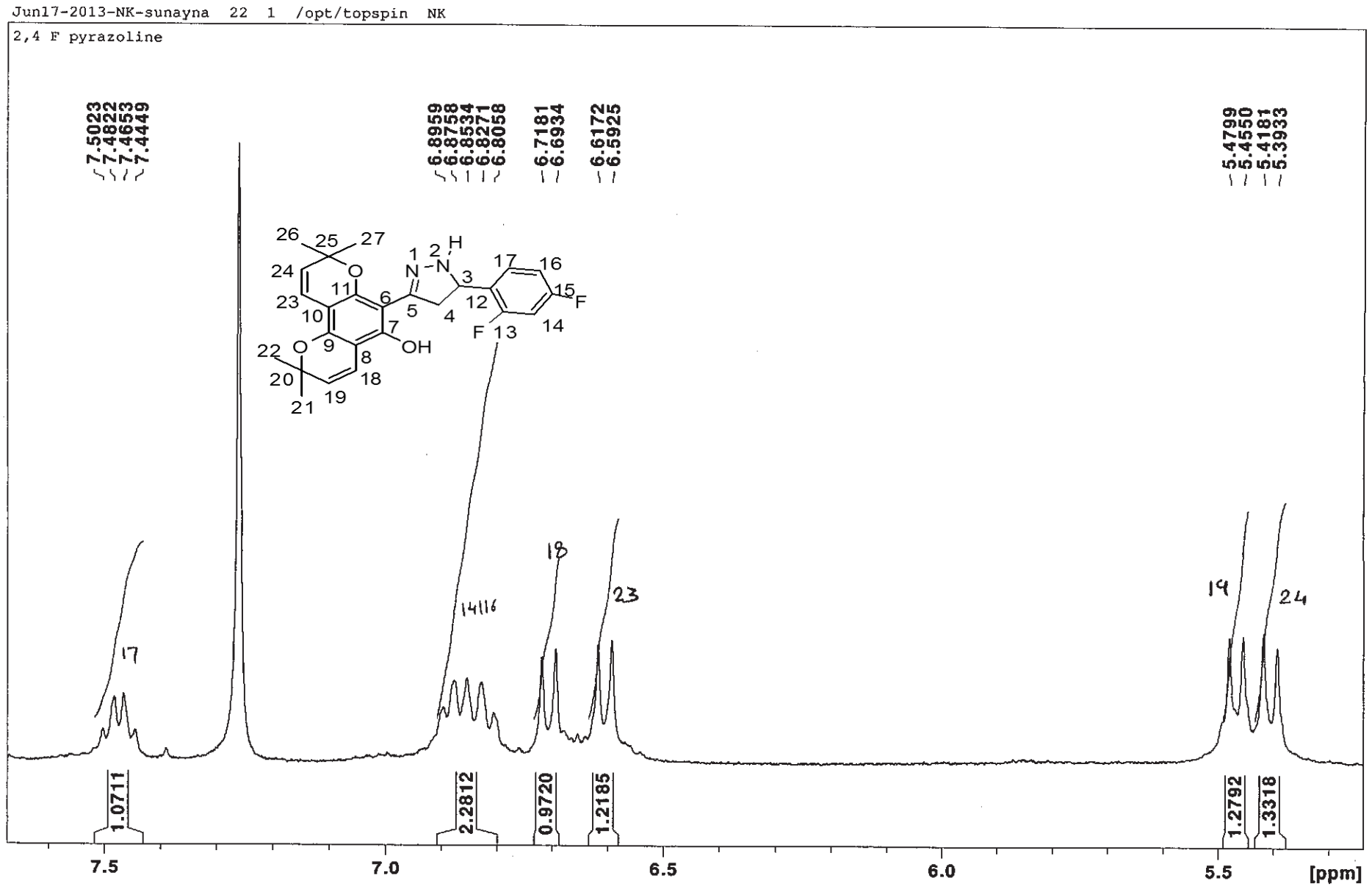


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
419.1783	419.1771	1.2	2.9	14.5	593.8	0.0	C25 H24 N2 O3 F

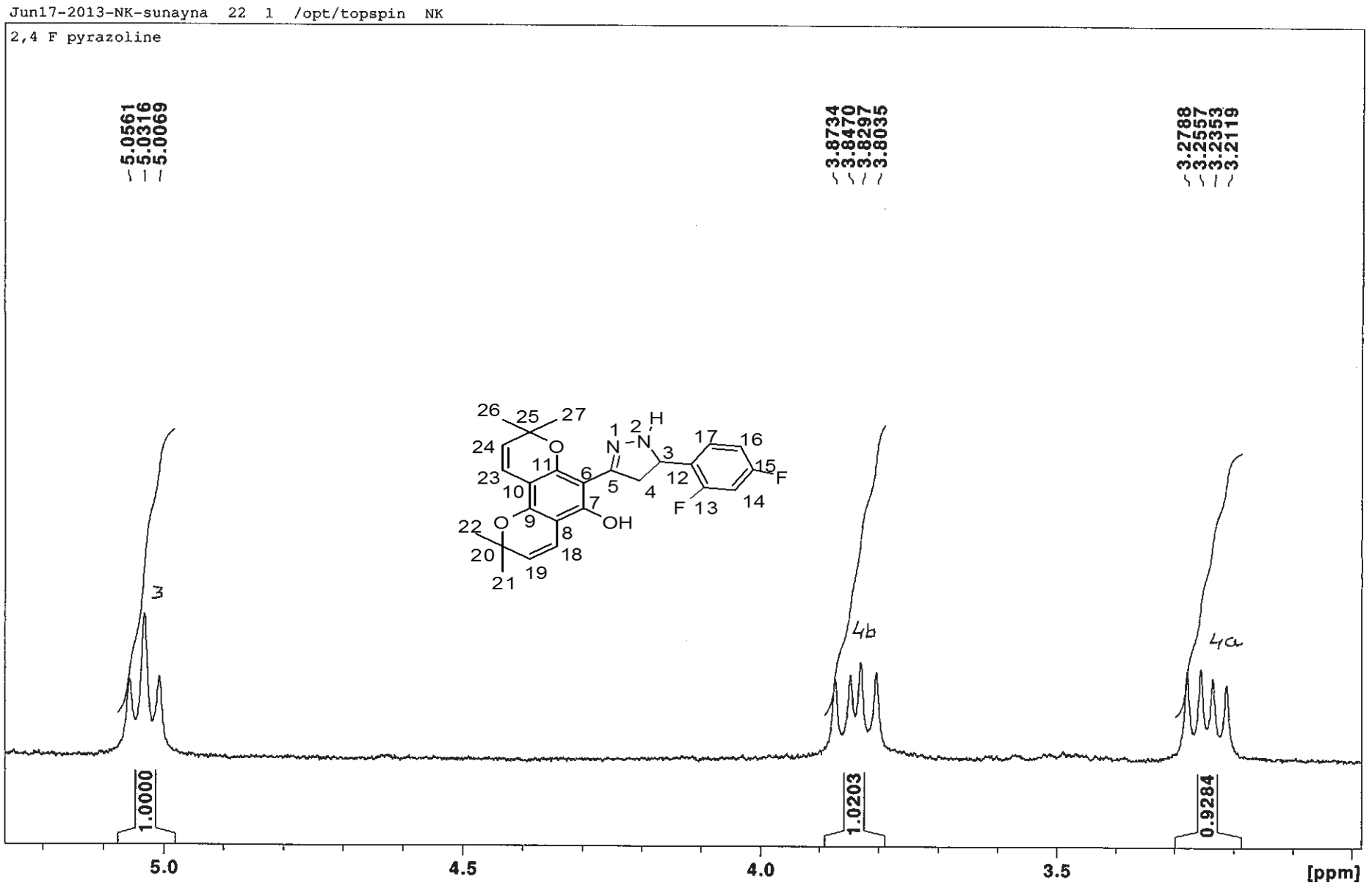
HRMS spectrum of 3-(4-fluorophenyl)-5-pyranochromene pyrazoline (C-4f)



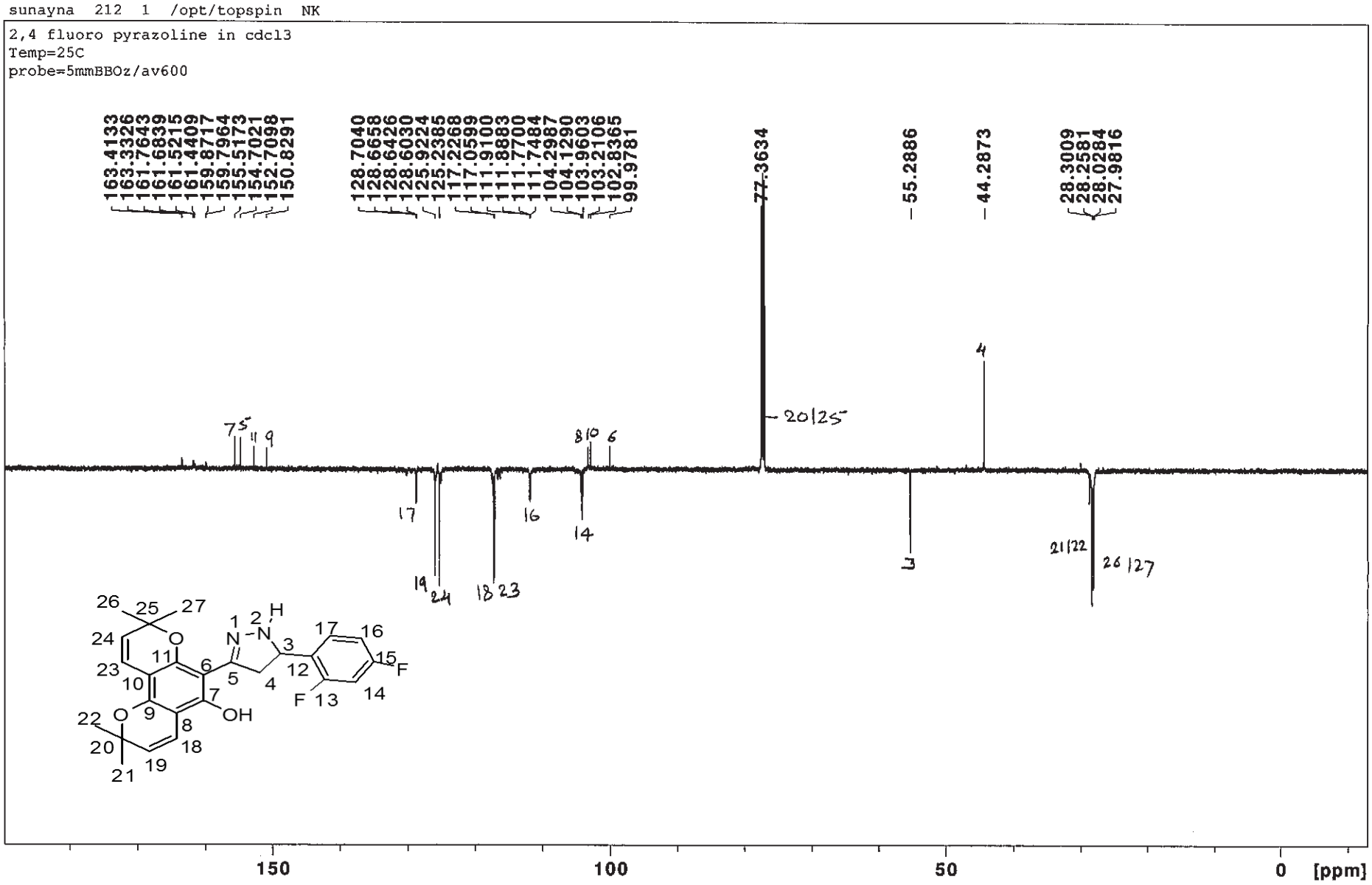
1H NMR spectrum of 3-(2,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4g)



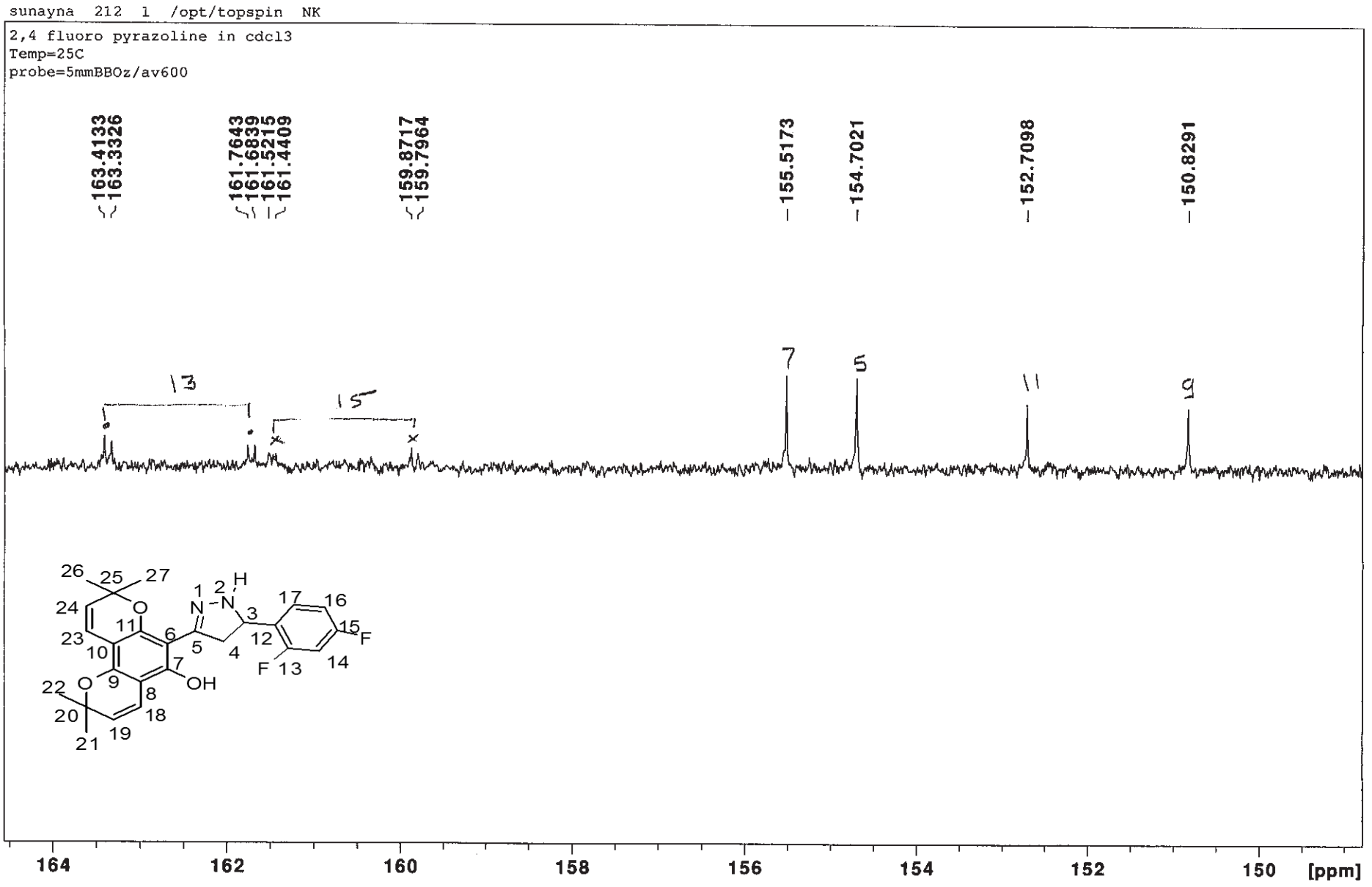
Expanded 1H NMR spectrum of 3-(2,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4g)



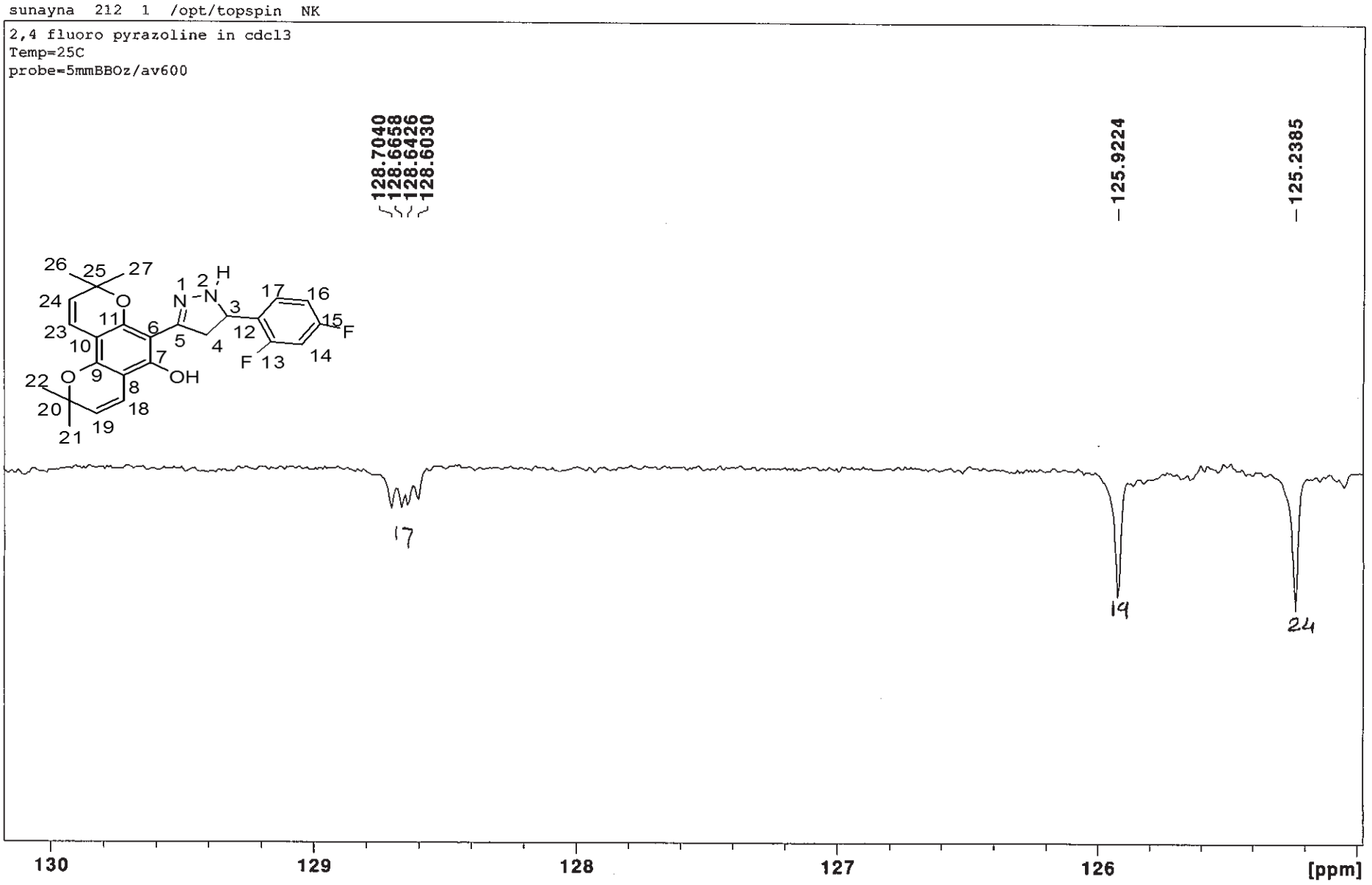
Expanded 1H NMR spectrum of 3-(2,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4g)



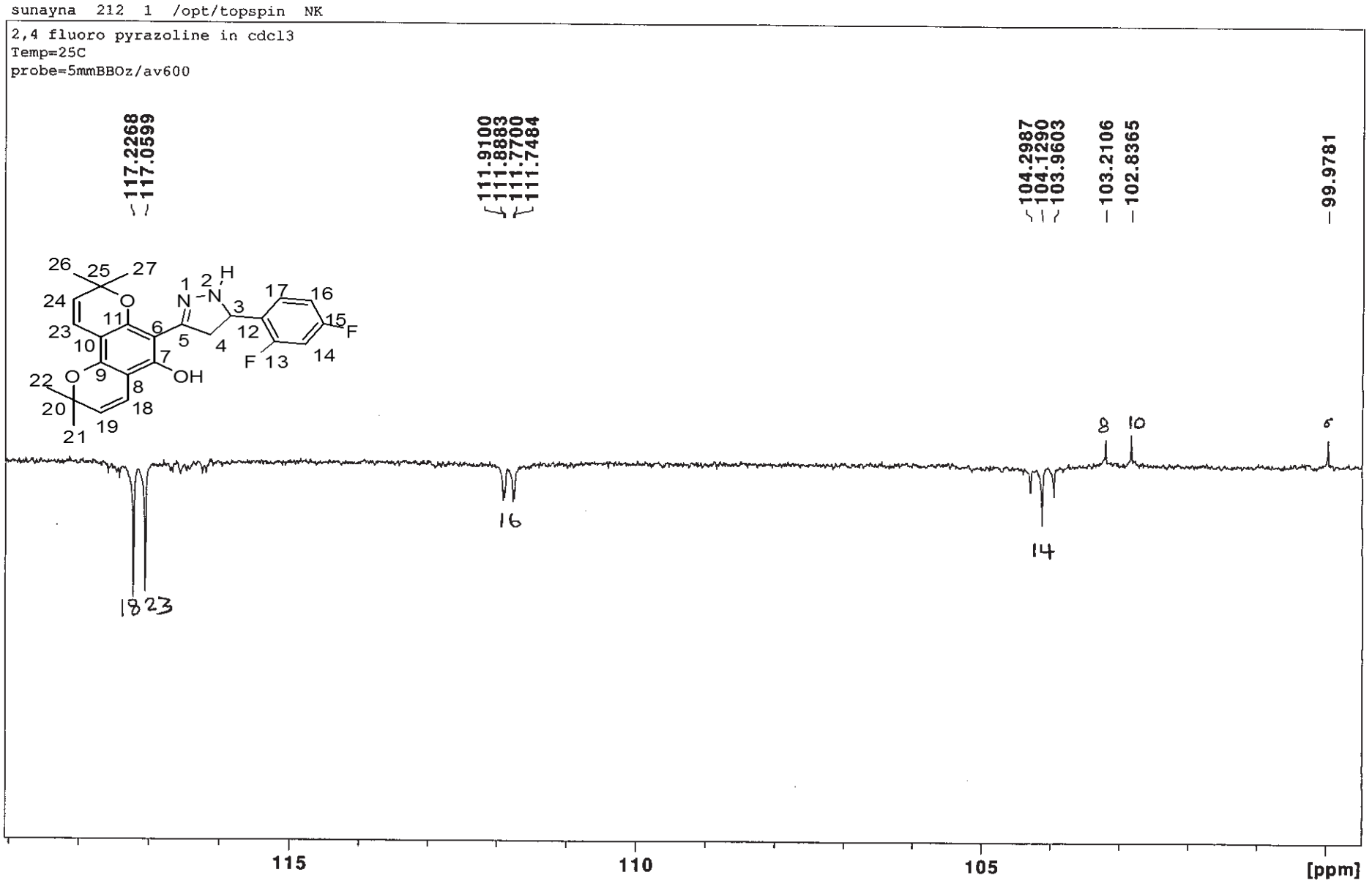
13C NMR spectrum of 3-(2,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4g)



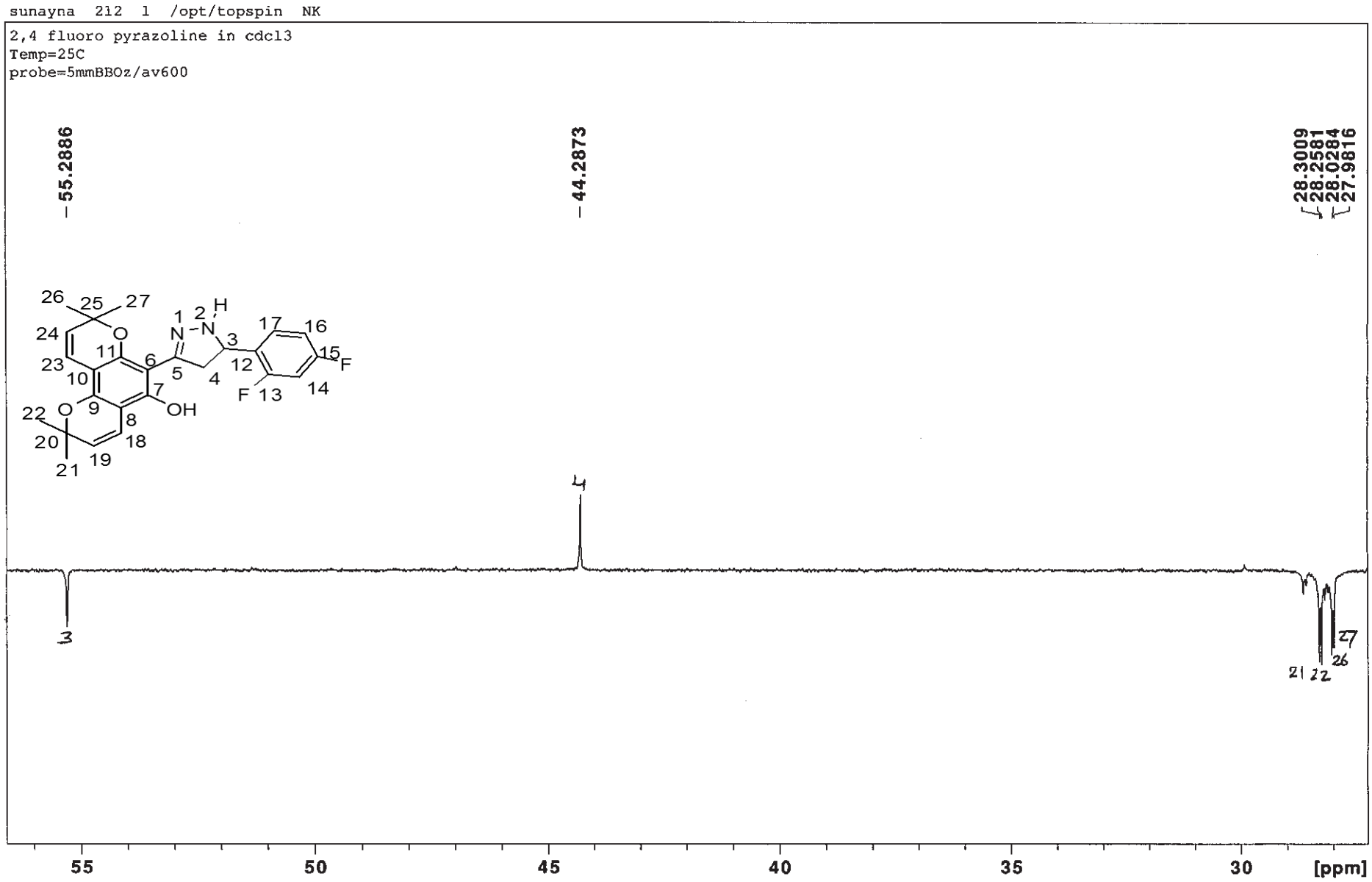
Expanded 13C NMR spectrum of 3-(2,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4g)



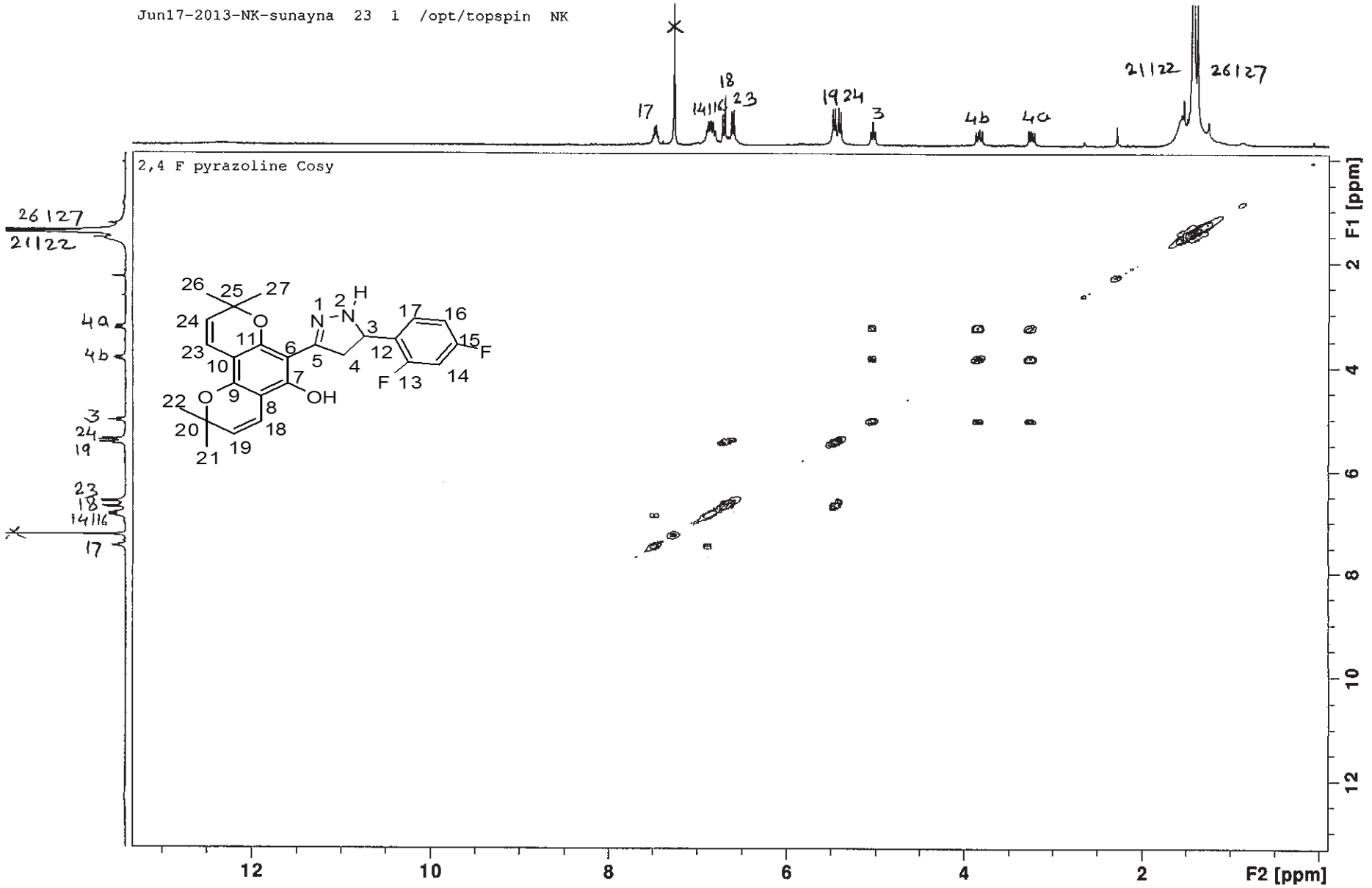
Expanded 13C NMR spectrum of 3-(2,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4g)



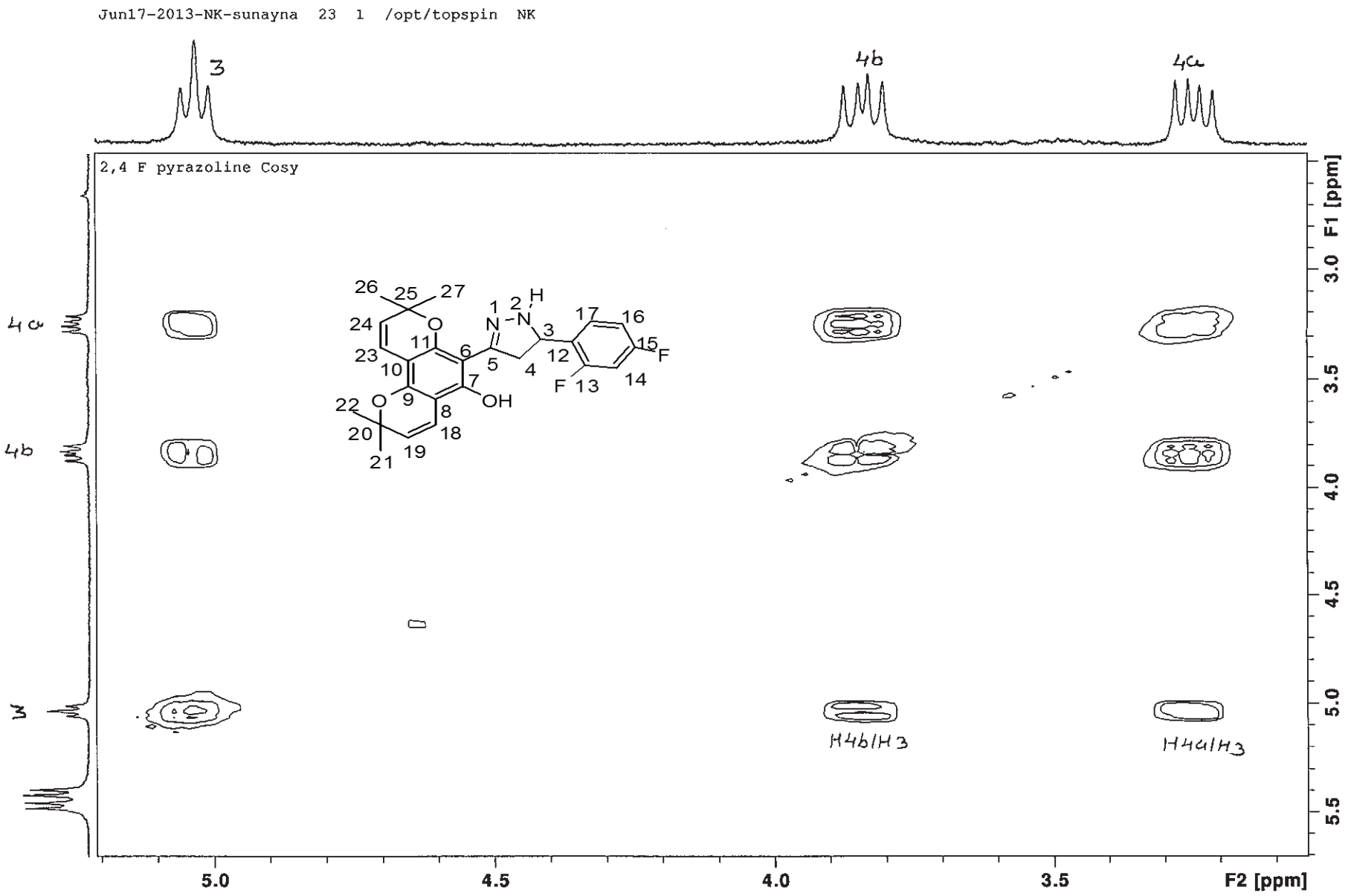
Expanded 13C NMR spectrum of 3-(2,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4g)



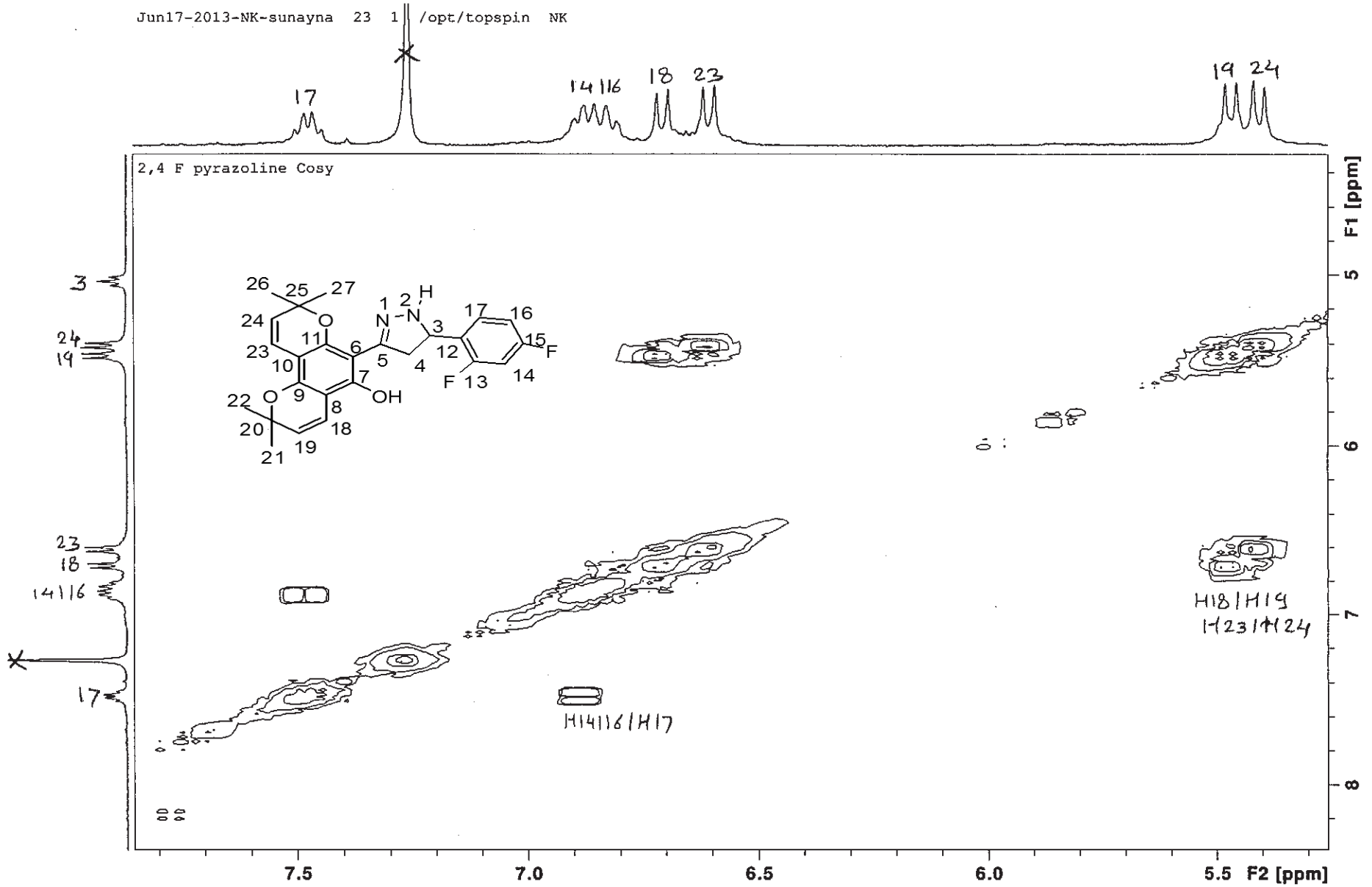
Expanded 13C NMR spectrum of 3-(2,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4g)



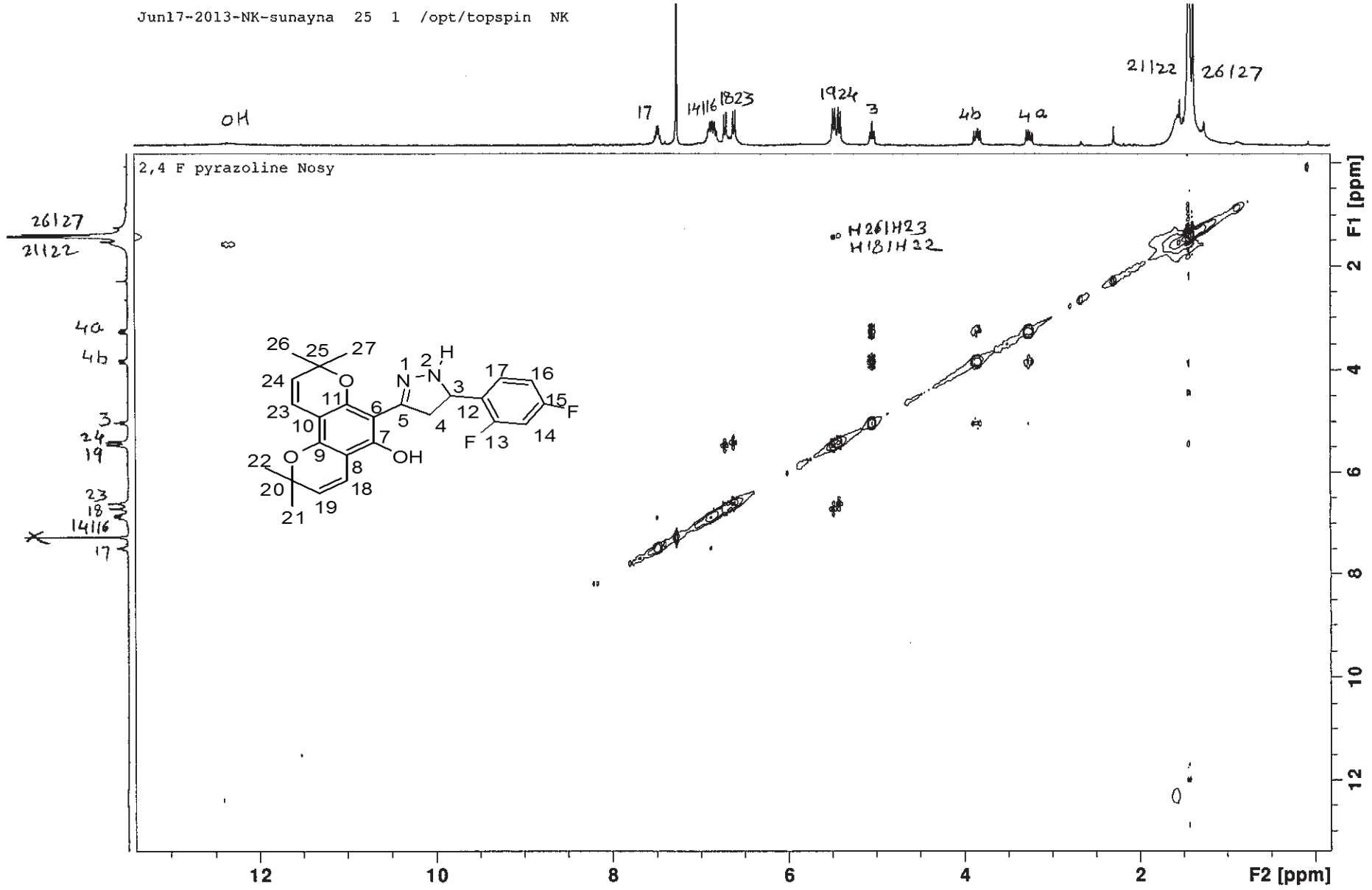
Expanded 13C NMR spectrum of 3-(2,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4g)



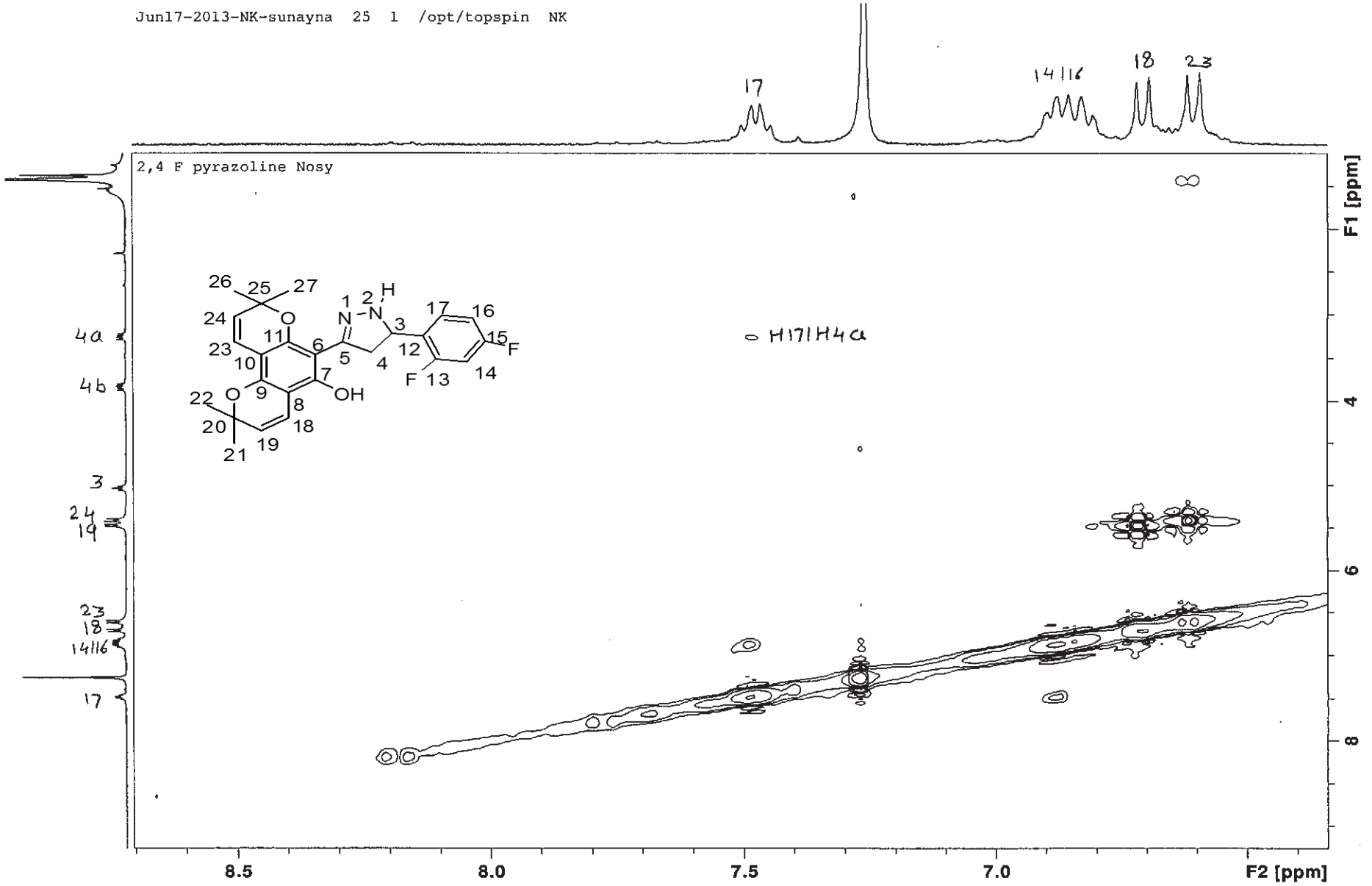
COSY NMR spectrum of 3-(2,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4g)



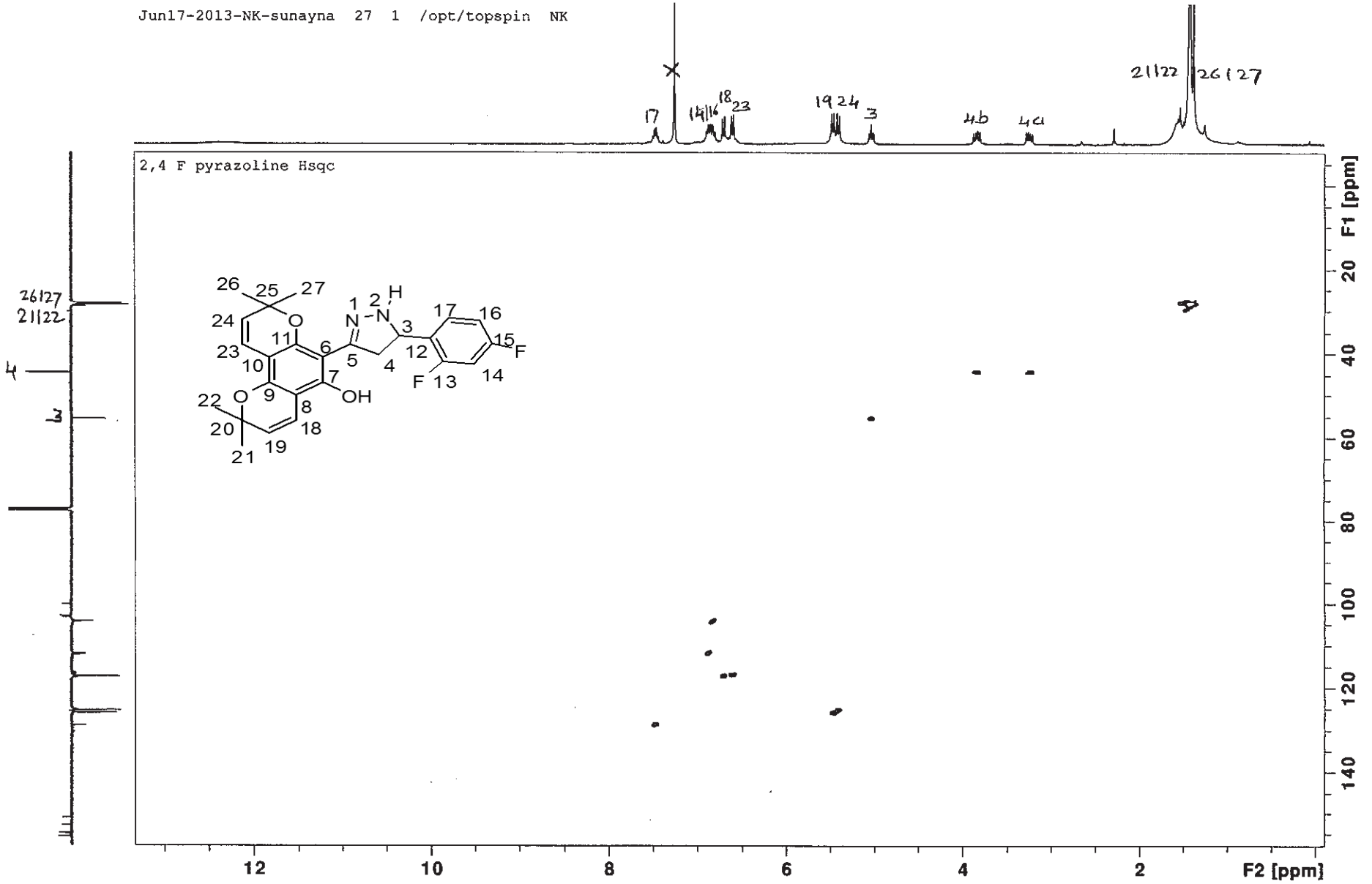
Expanded COSY NMR spectrum of 3-(2,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4g)



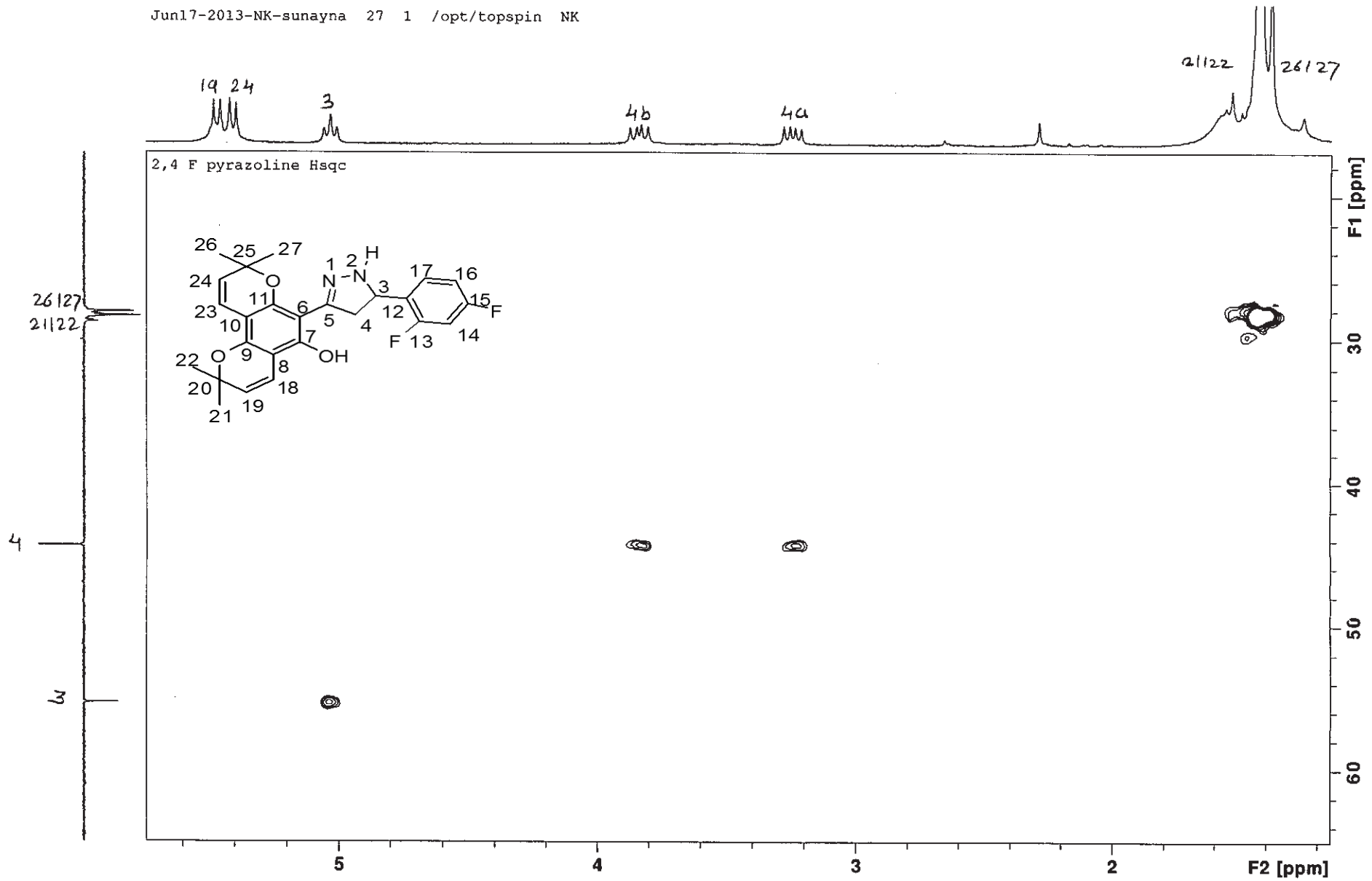
NOESY NMR spectrum of 3-(2,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4g)



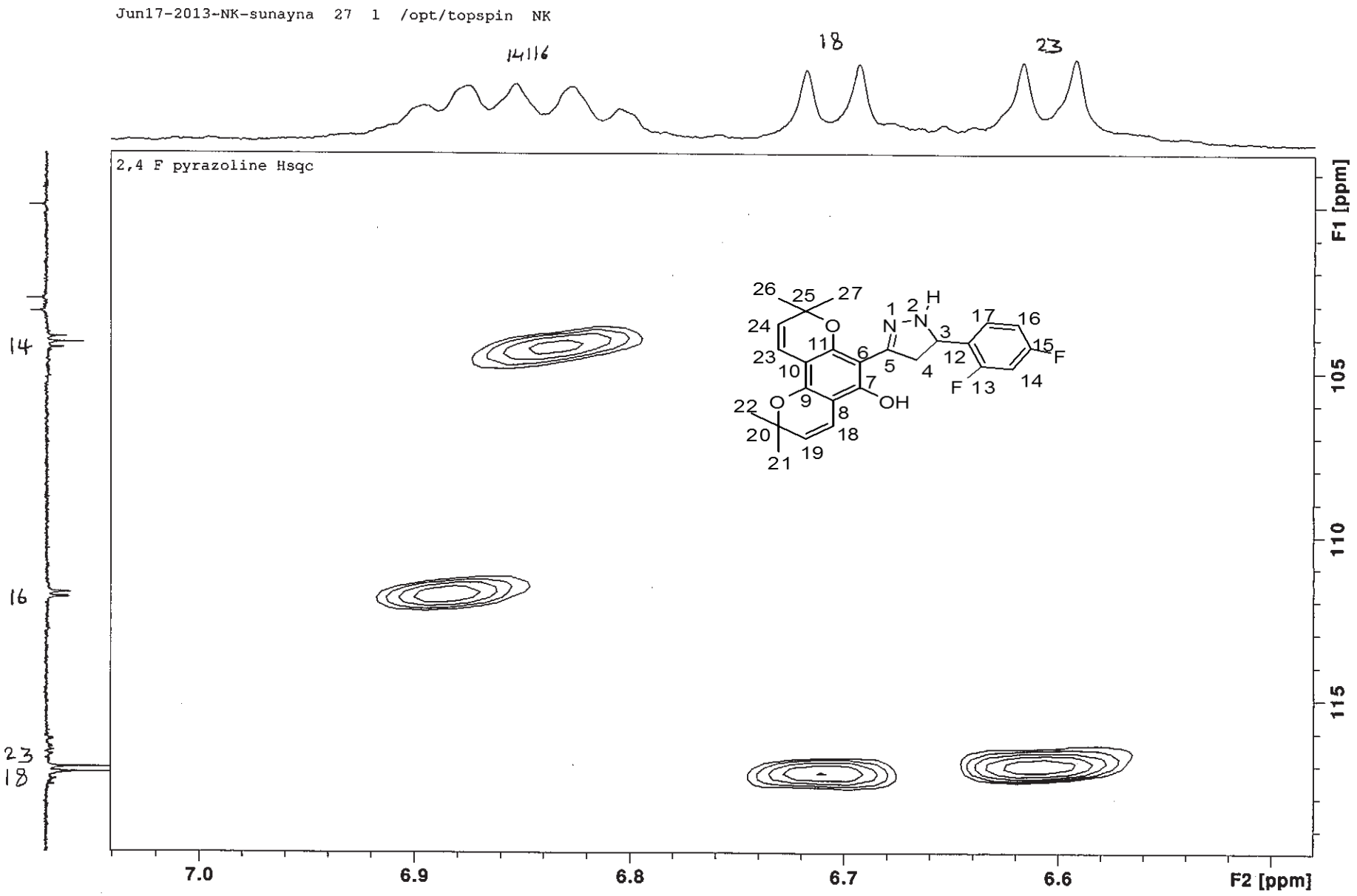
NOESY NMR spectrum of 3-(2,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4g)



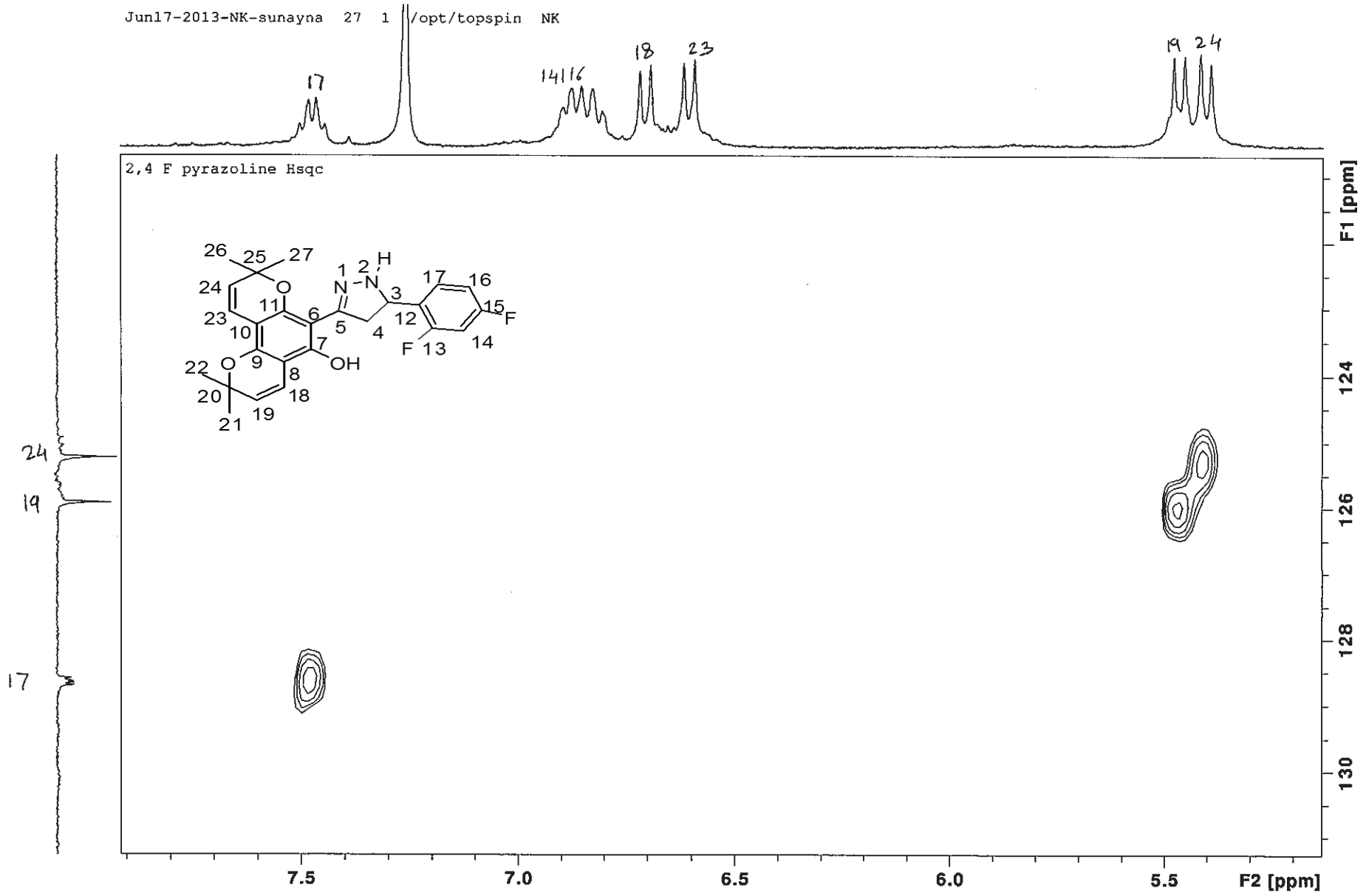
HSQC spectrum of 3-(2,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4g)



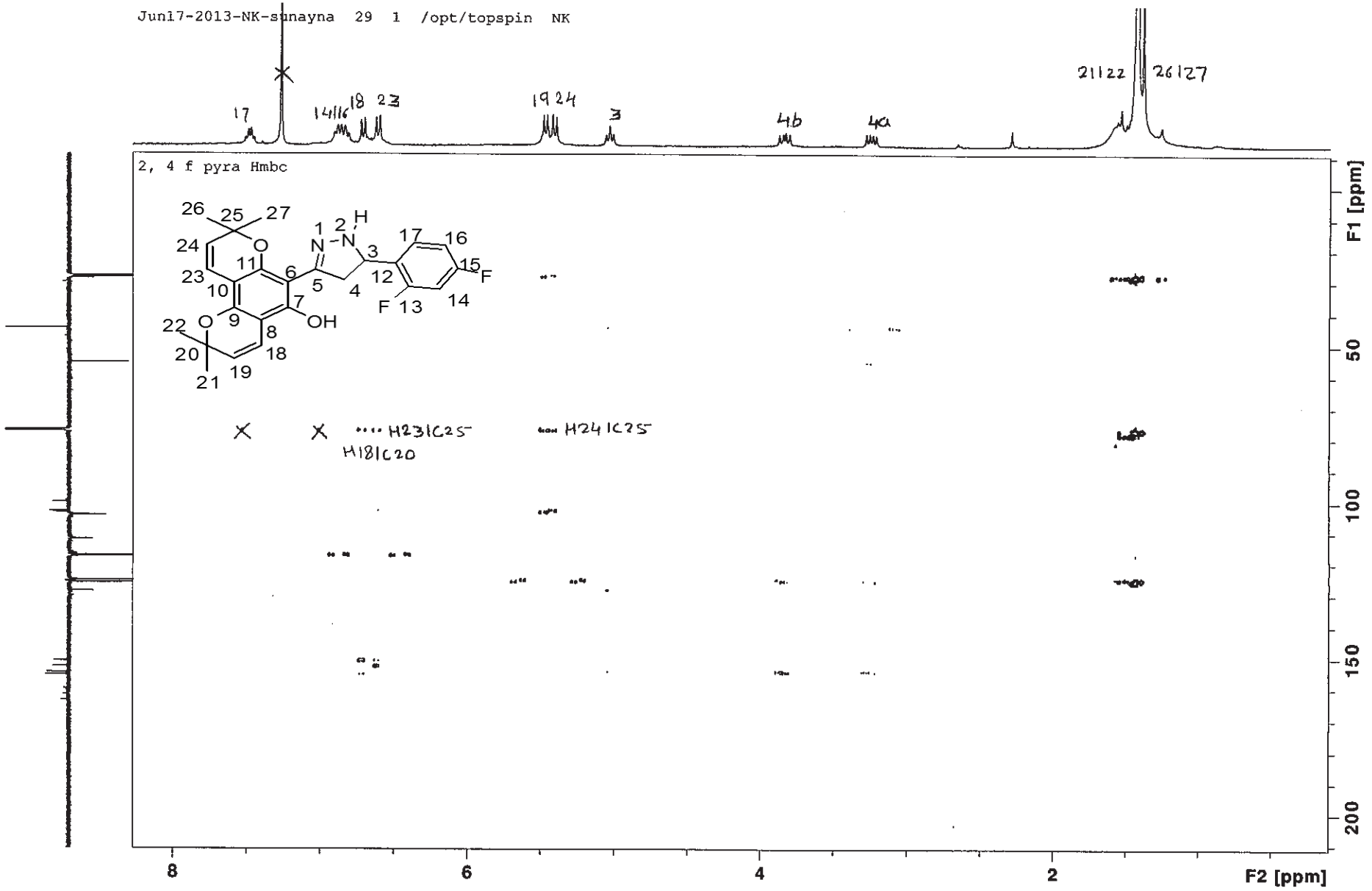
HSQC spectrum of 3-(2,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4g)



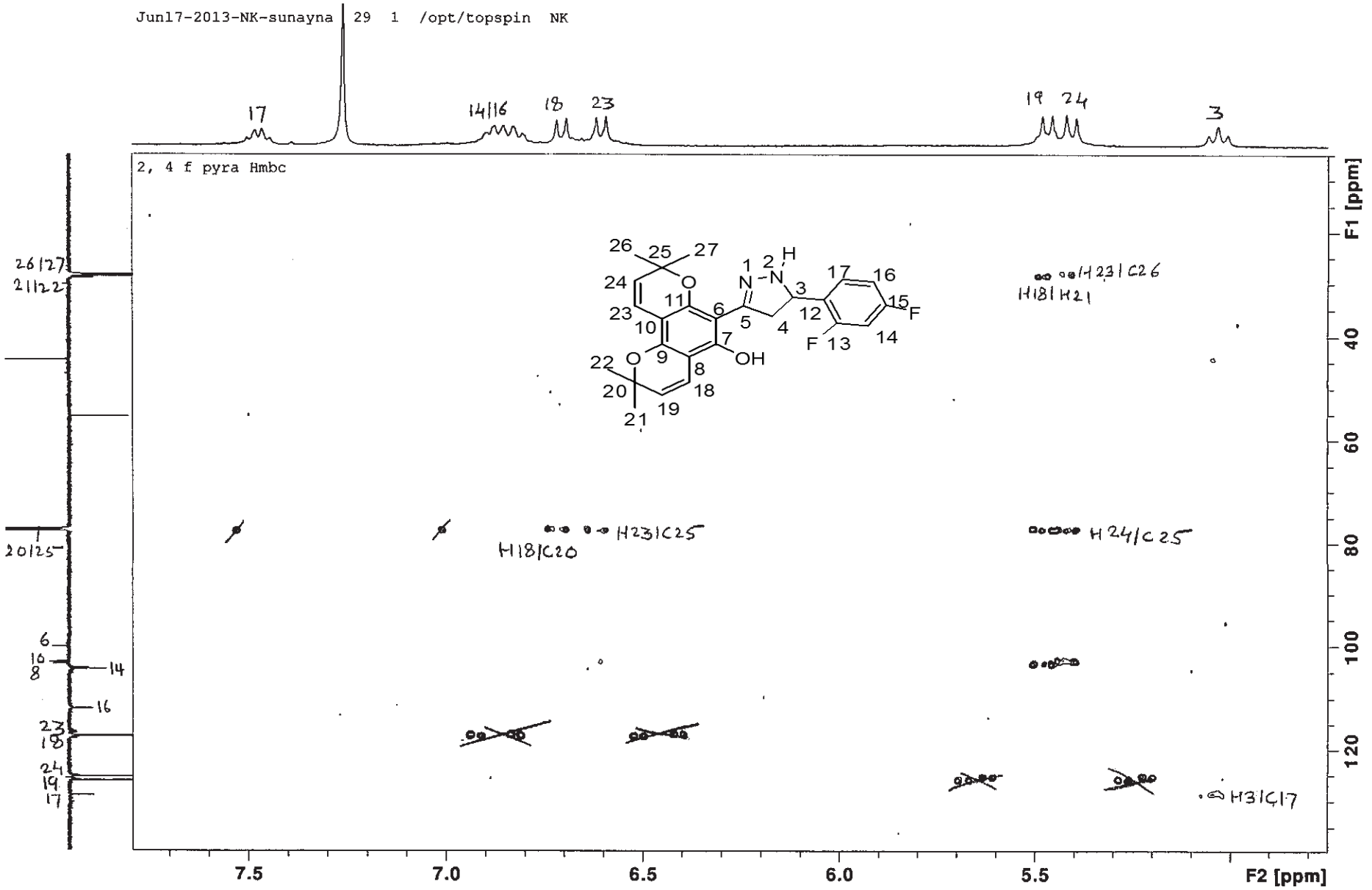
HSQC spectrum of 3-(2,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4g)



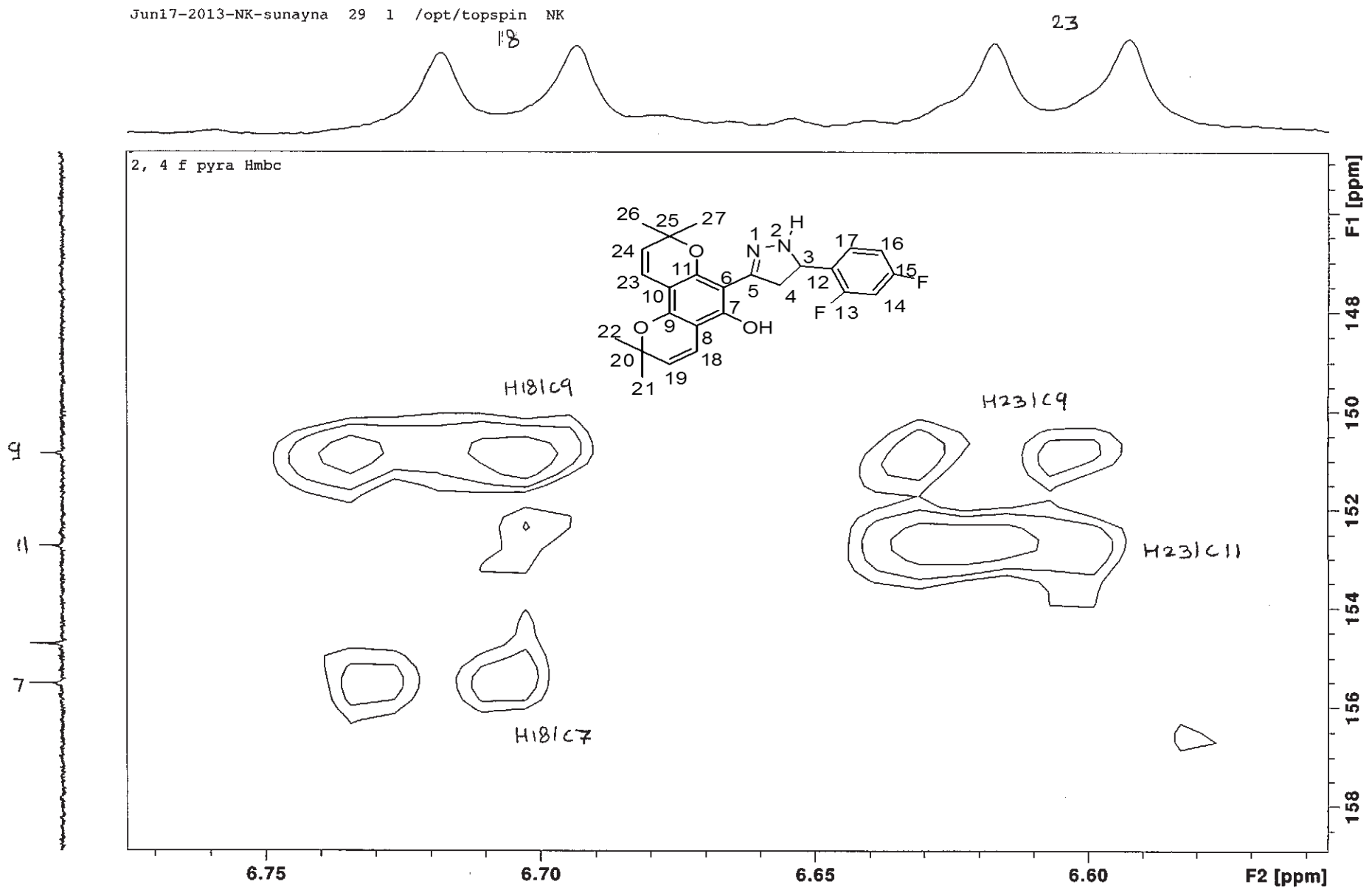
HSQC spectrum of 3-(2,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4g)



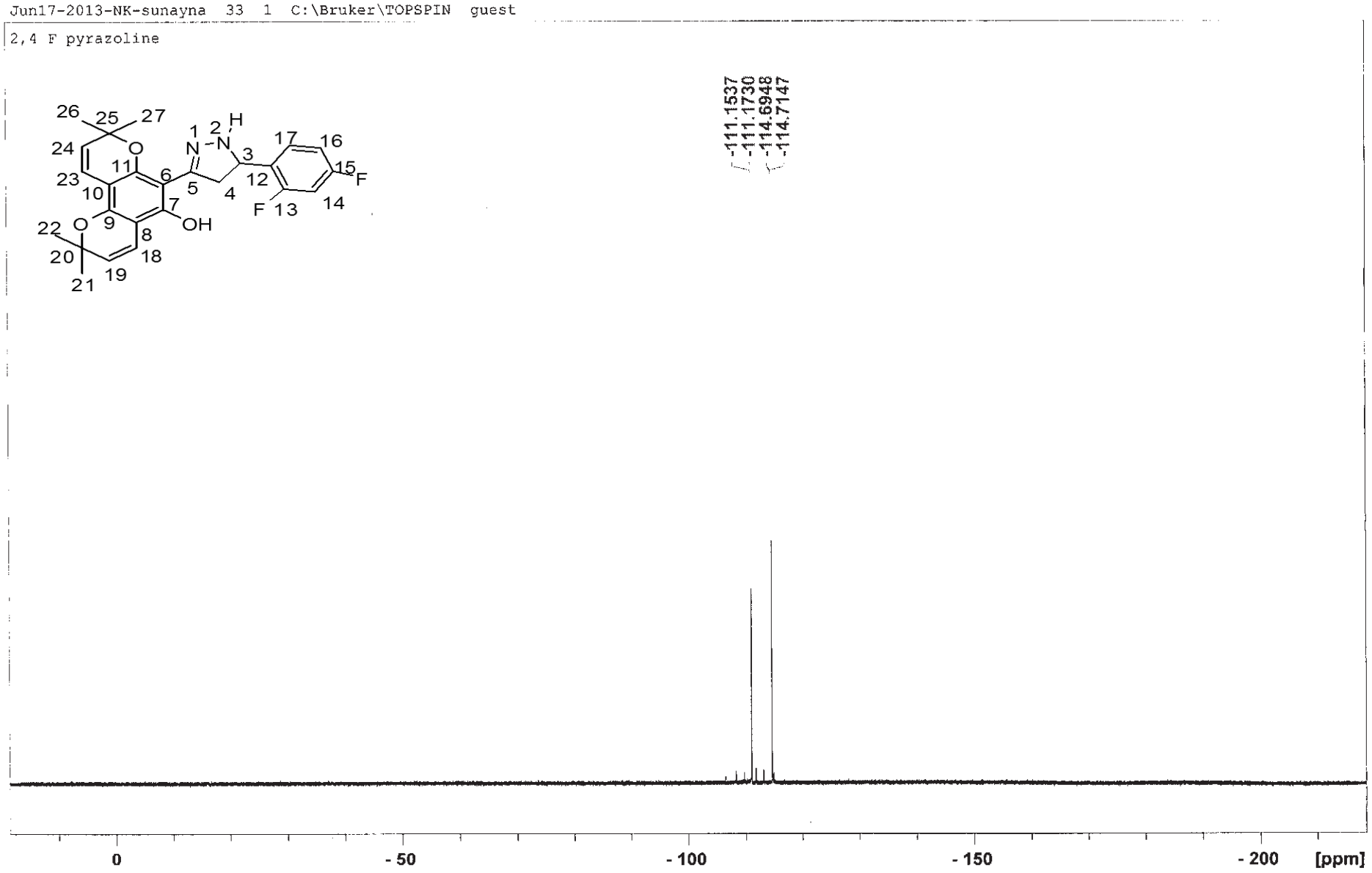
HMBC spectrum of 3-(2,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4g)



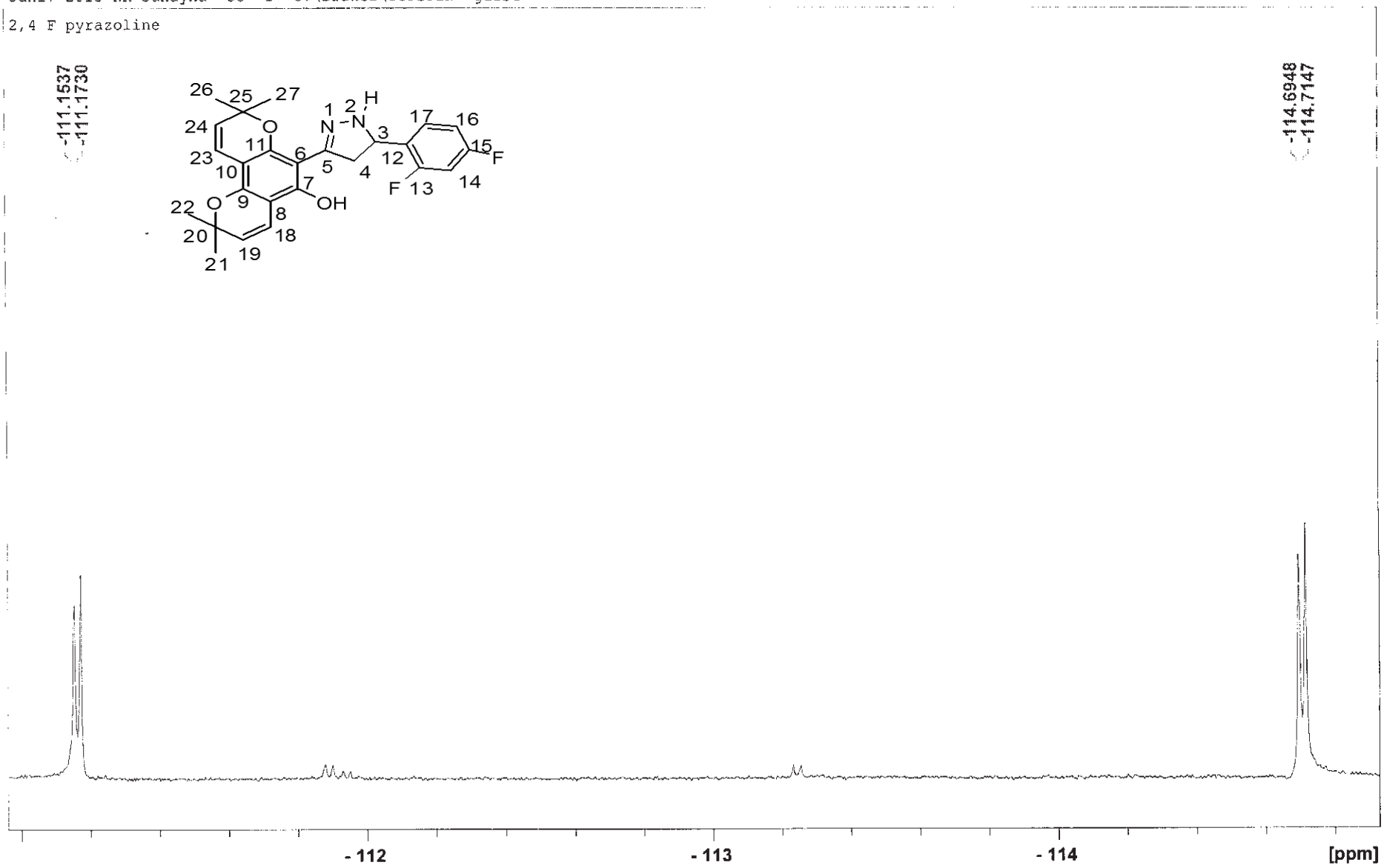
Expanded HMBC spectrum of 3-(2,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4g)



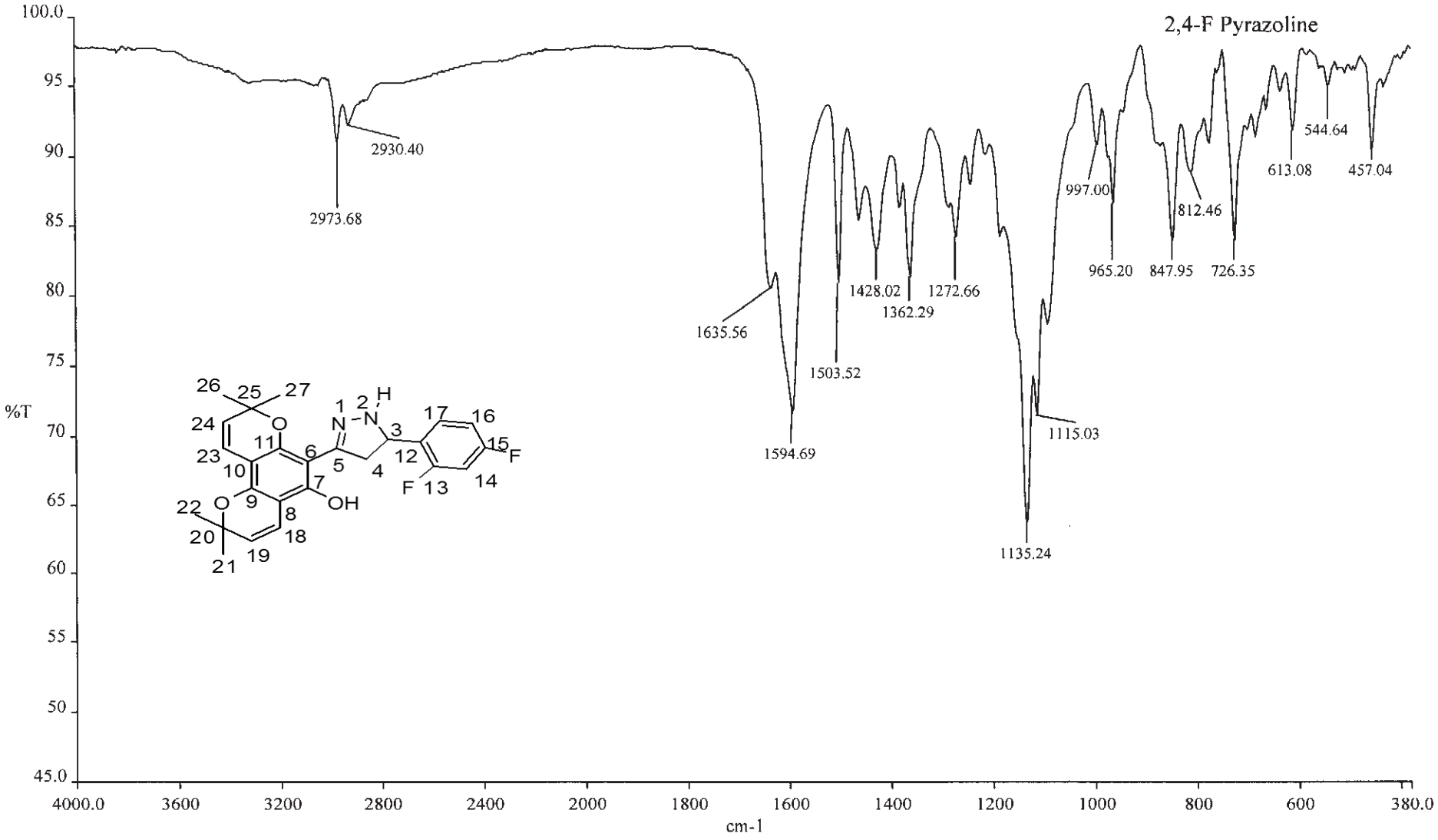
Expanded HMBC spectrum of 3-(2,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4g)



19F NMR spectrum of 3-(2,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4g)



Expanded 19F NMR spectrum of 3-(2,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4g)

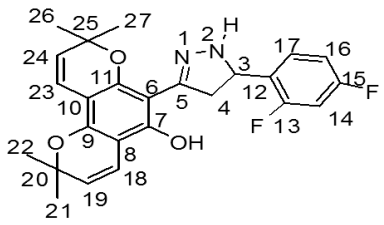


Infrared spectrum of 3-(2,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4g)

2,4 F

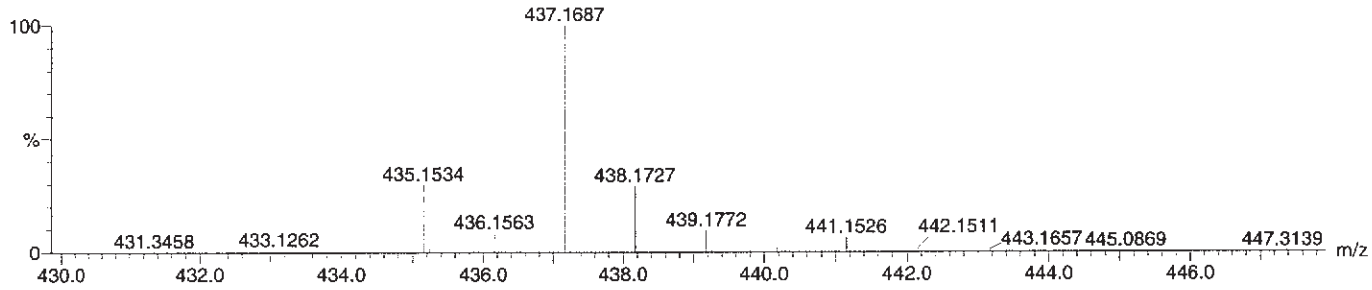
Elemental Composition Report **Page 1**

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



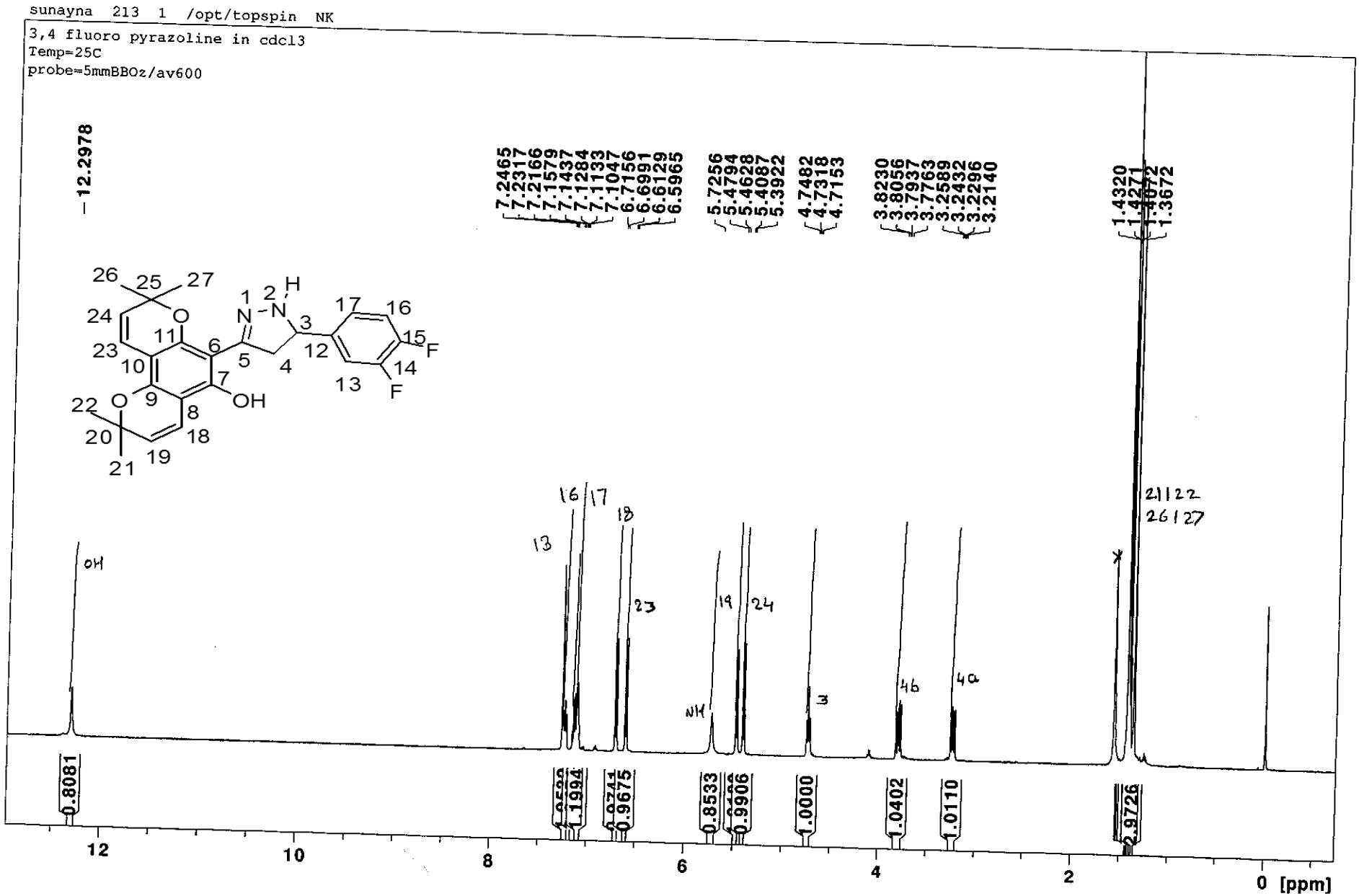
Monoisotopic Mass, Even Electron Ions
19 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-30 H: 20-30 N: 0-4 O: 0-4 F: 2-2
15 19 (0.307) Cm (1:30)
TOF MS ES-

4.24e+004

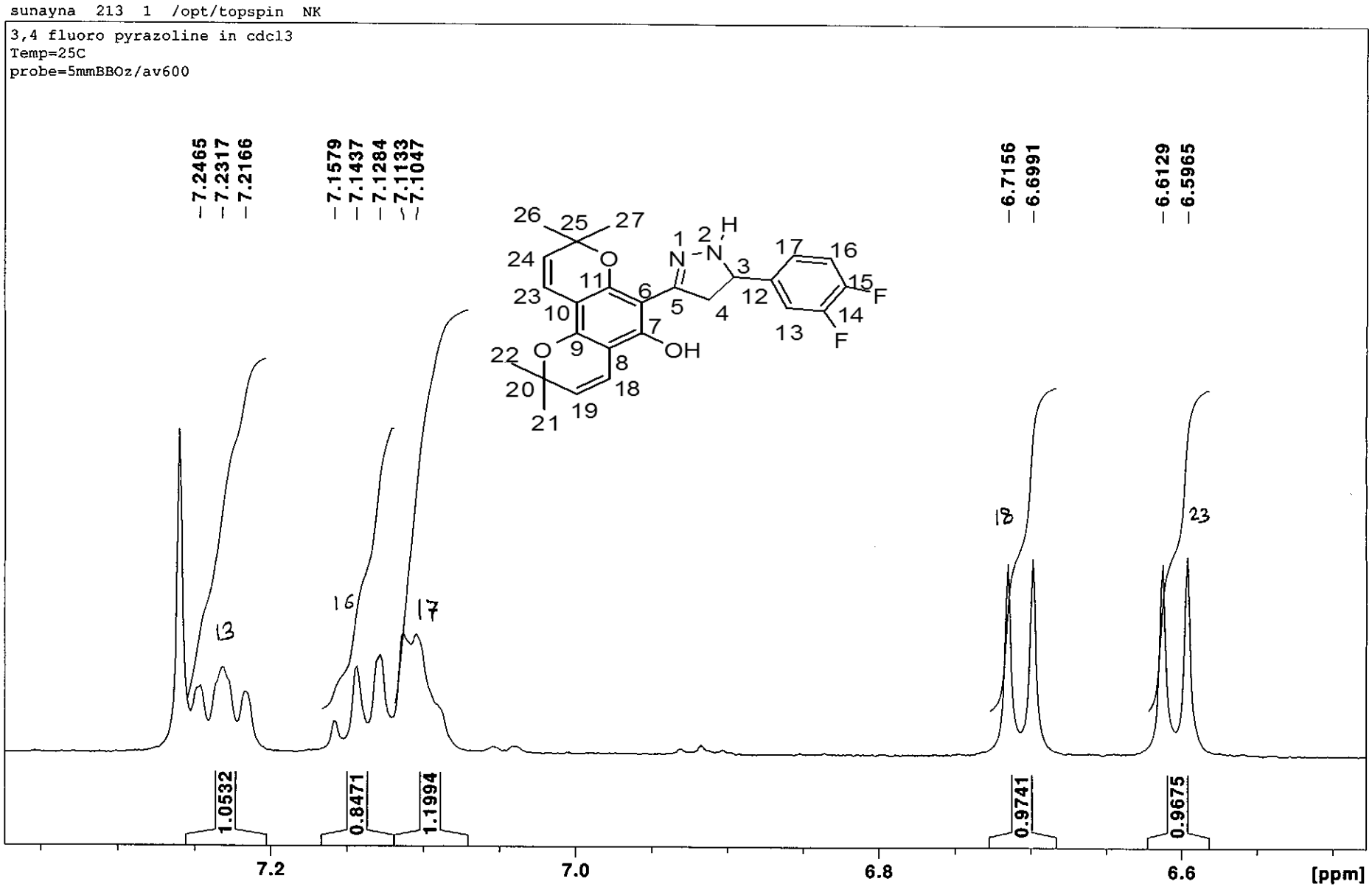


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
437.1687	437.1677	1.0	2.3	14.5	456.0	0.0	C25 H23 N2 O3 F2

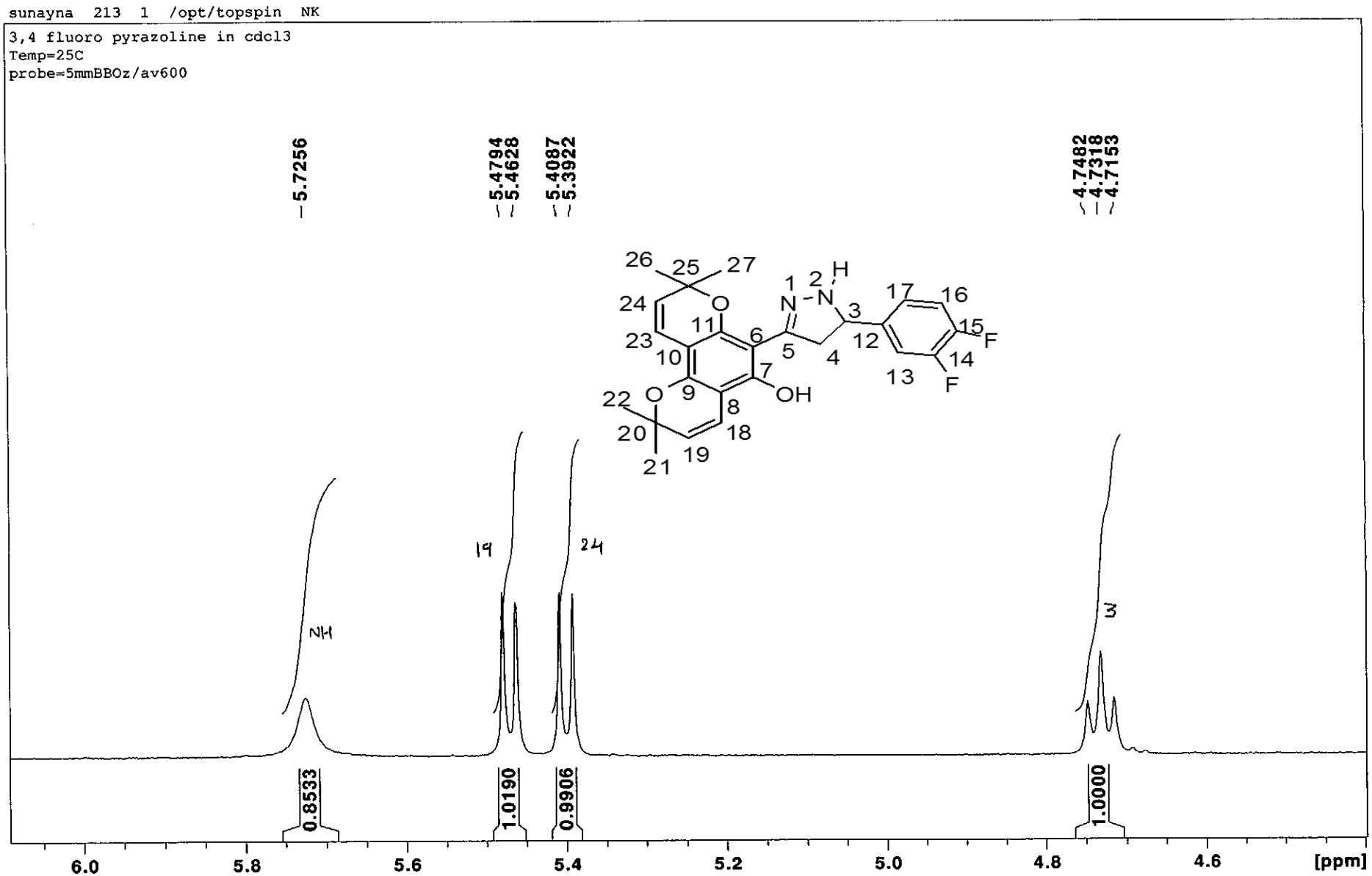
HRMS spectrum of 3-(2,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4g)



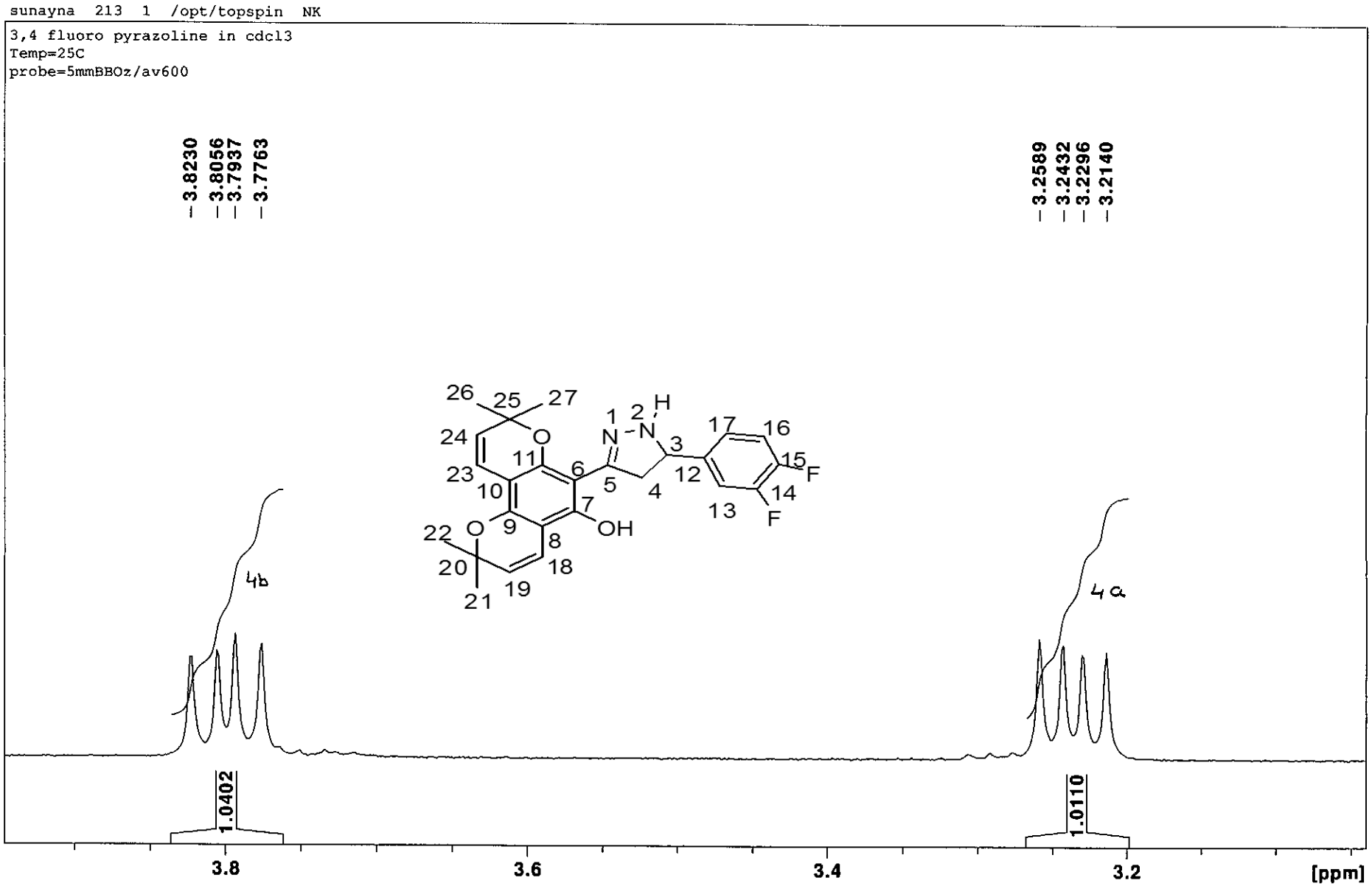
1H NMR spectrum of 3-(3,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4h)



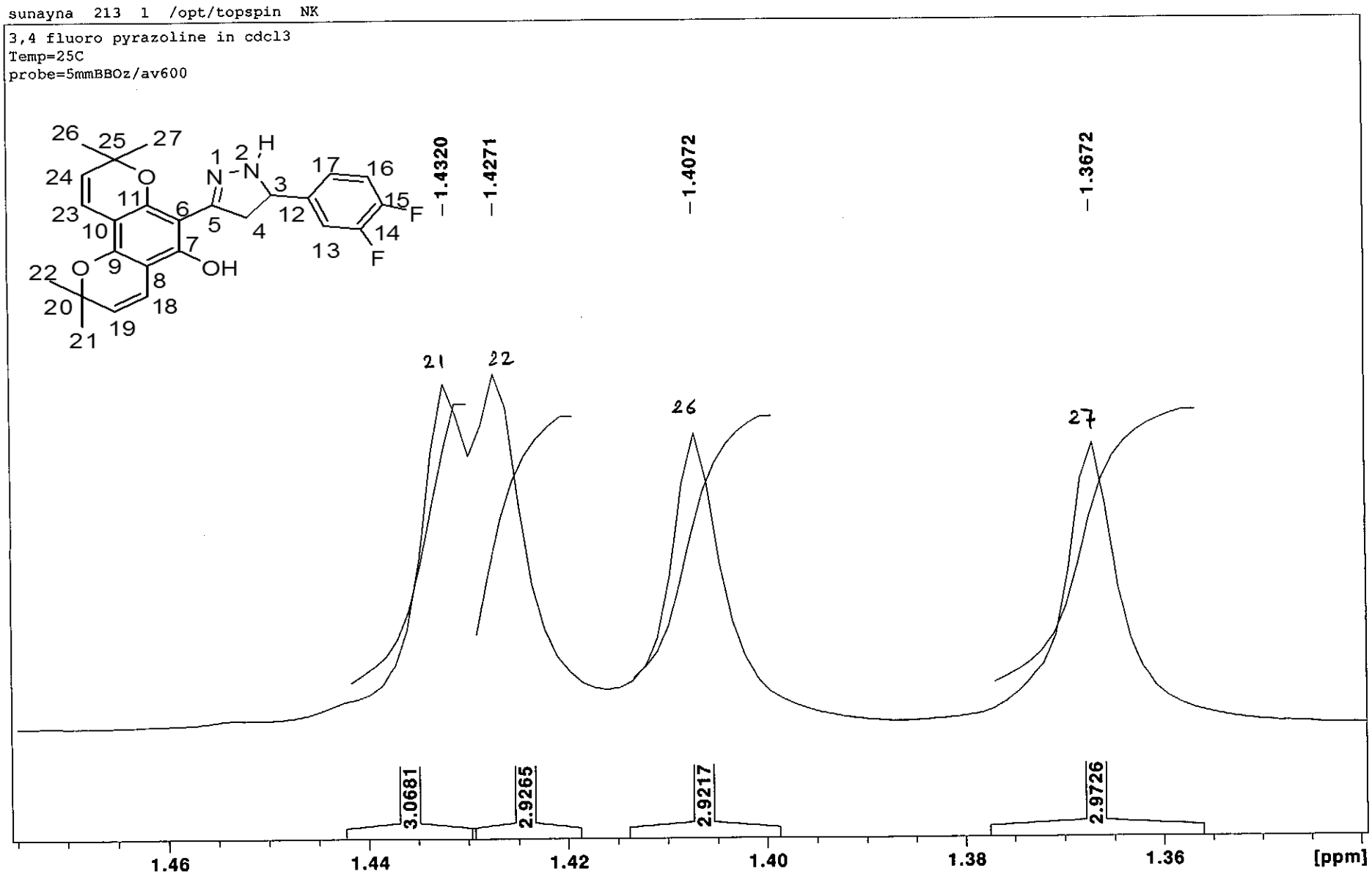
Expanded 1H NMR spectrum of 3-(3,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4h)



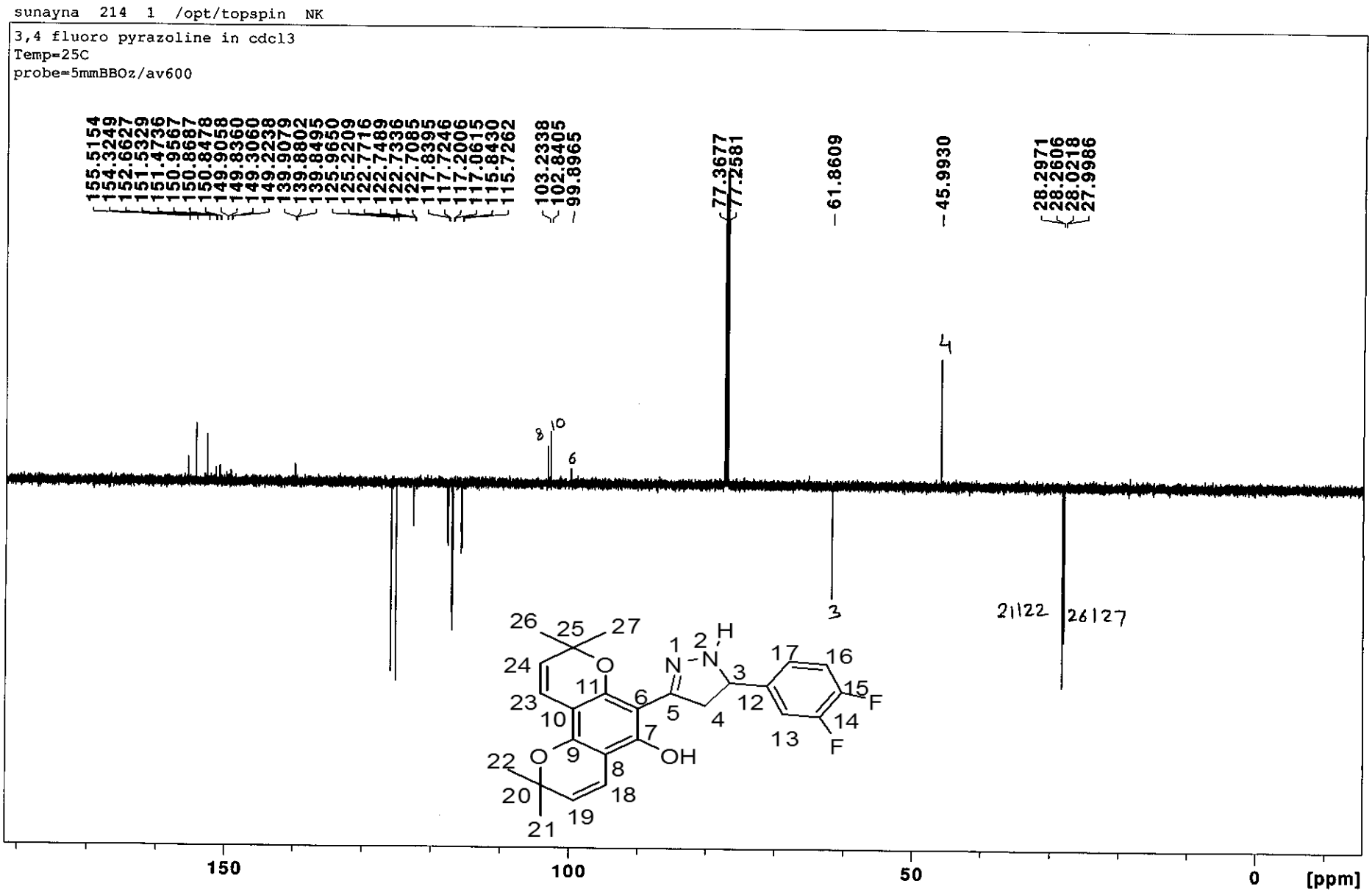
Expanded 1H NMR spectrum of 3-(3,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4h)



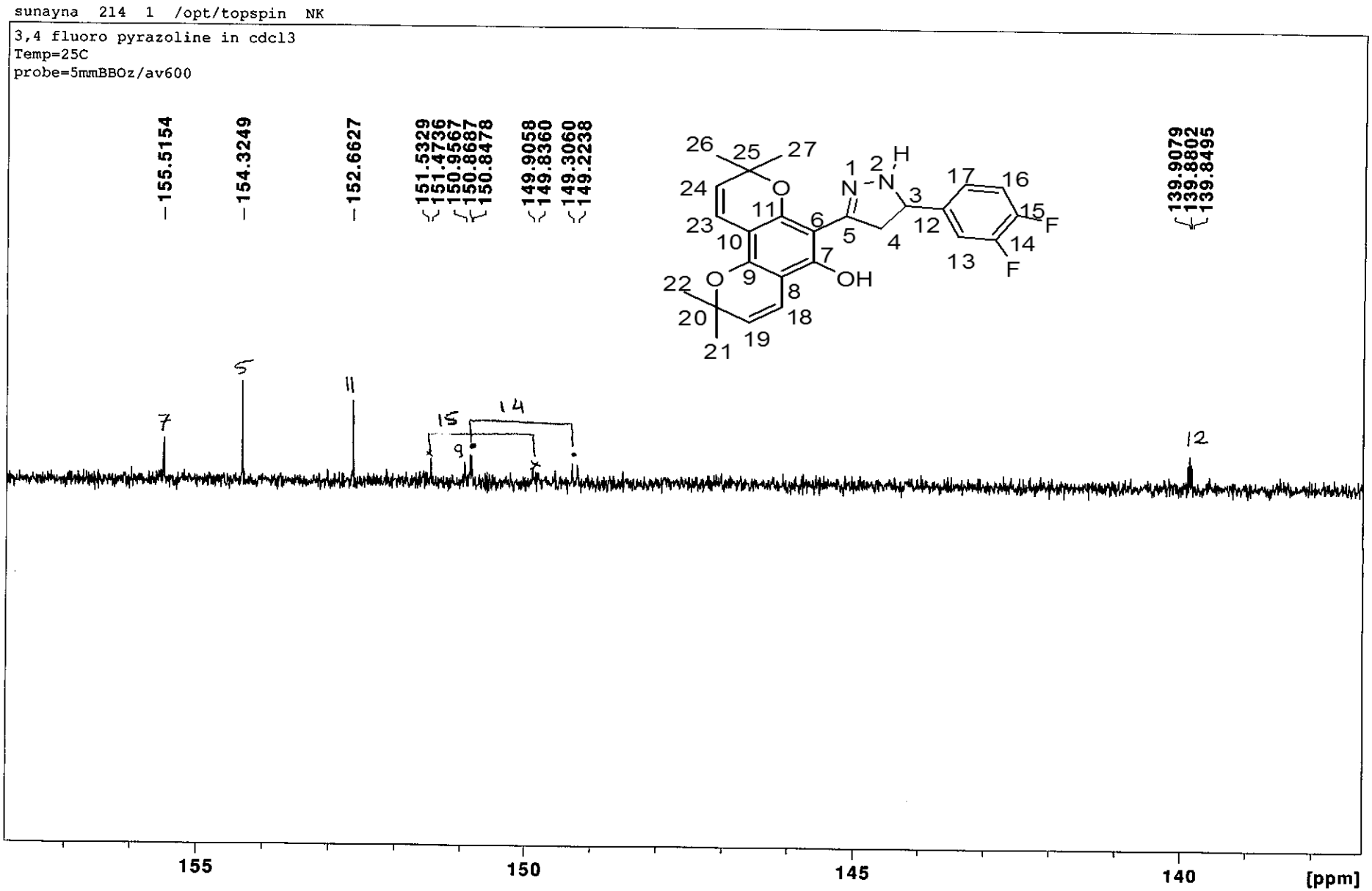
Expanded 1H NMR spectrum of 3-(3,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4h)



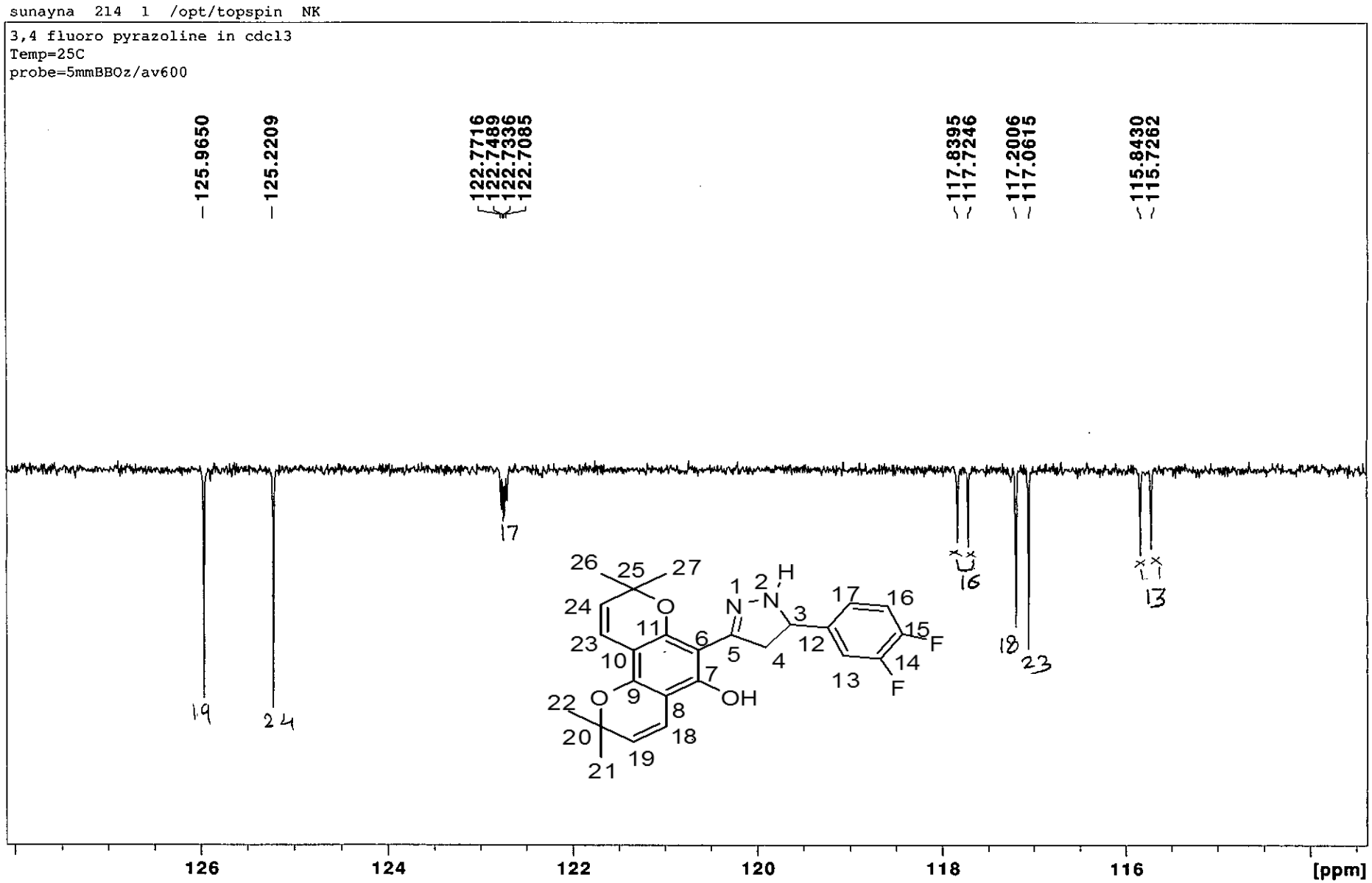
Expanded 1H NMR spectrum of 3-(3,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4h)



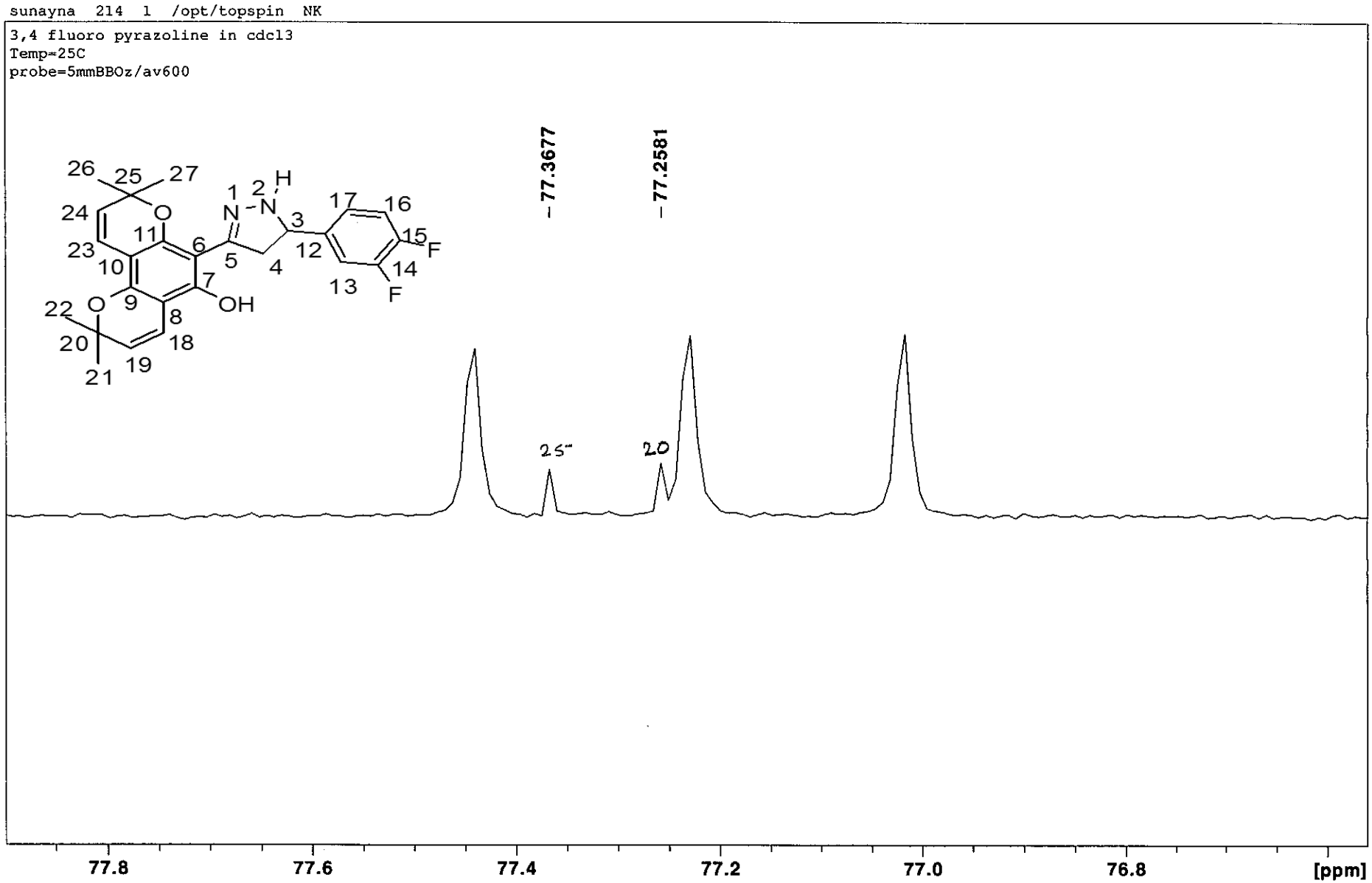
13C NMR spectrum of 3-(3,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4h)



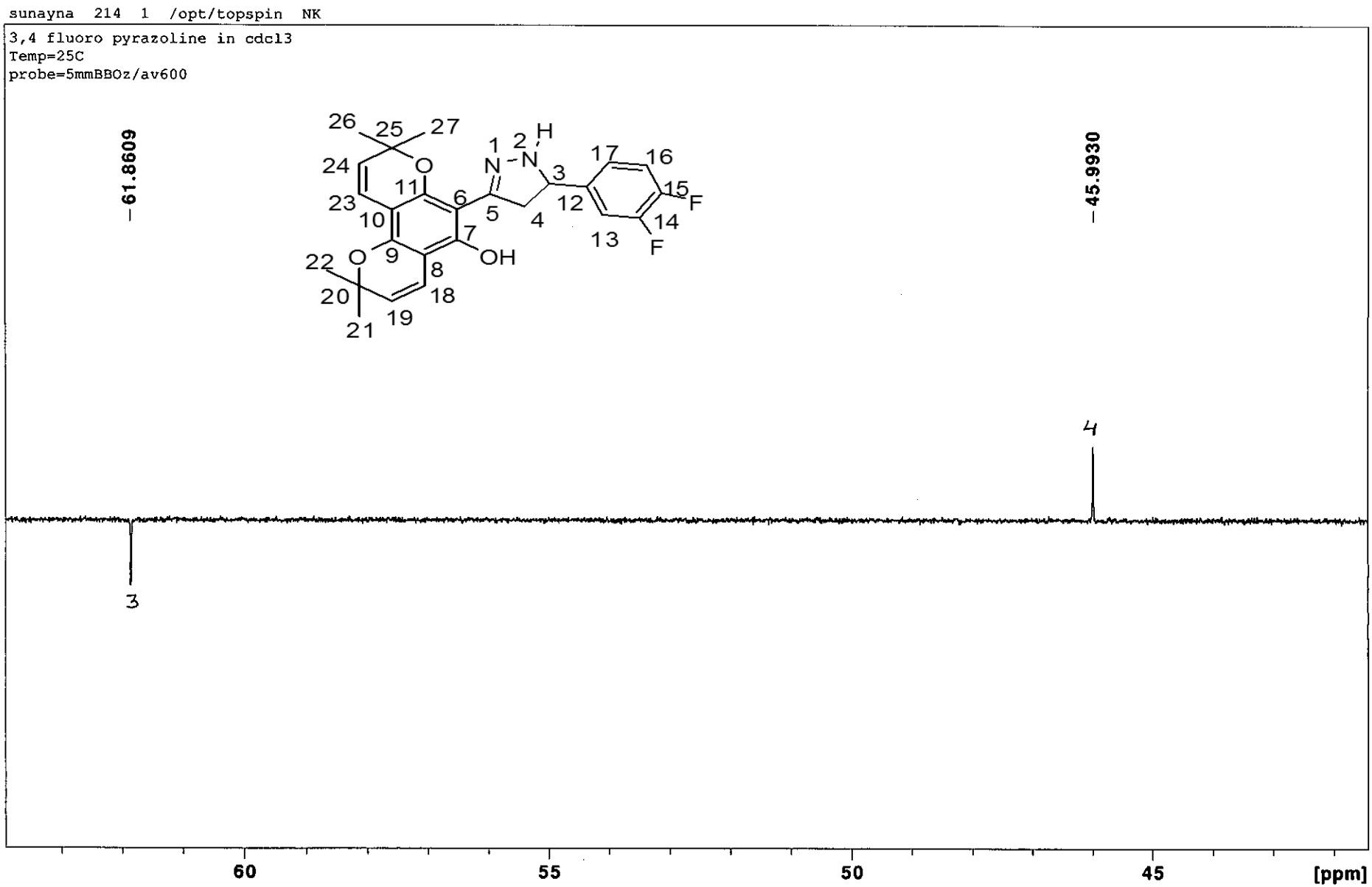
Expanded 13C NMR spectrum of 3-(3,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4h)



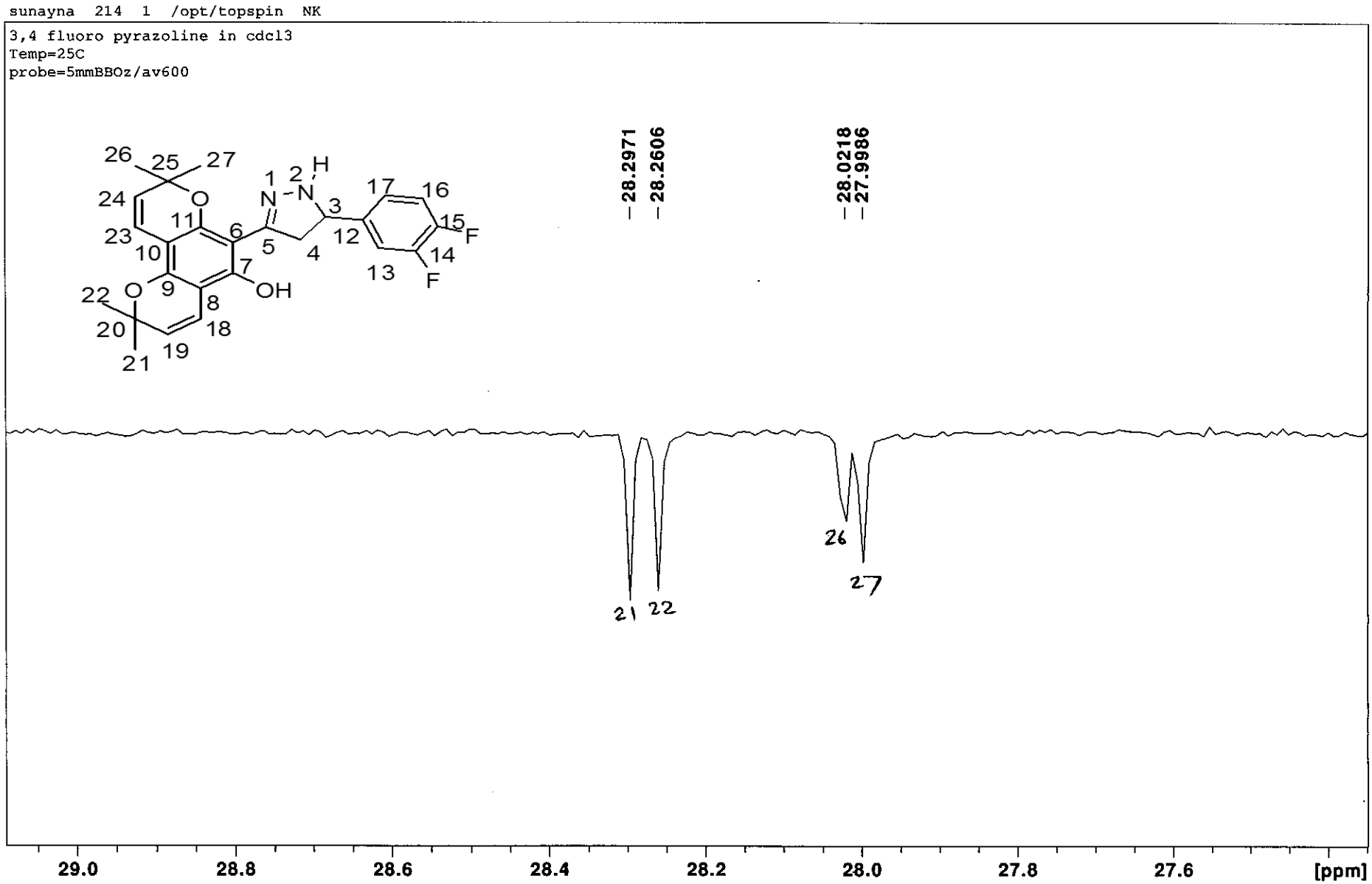
Expanded 13C NMR spectrum of 3-(3,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4h)



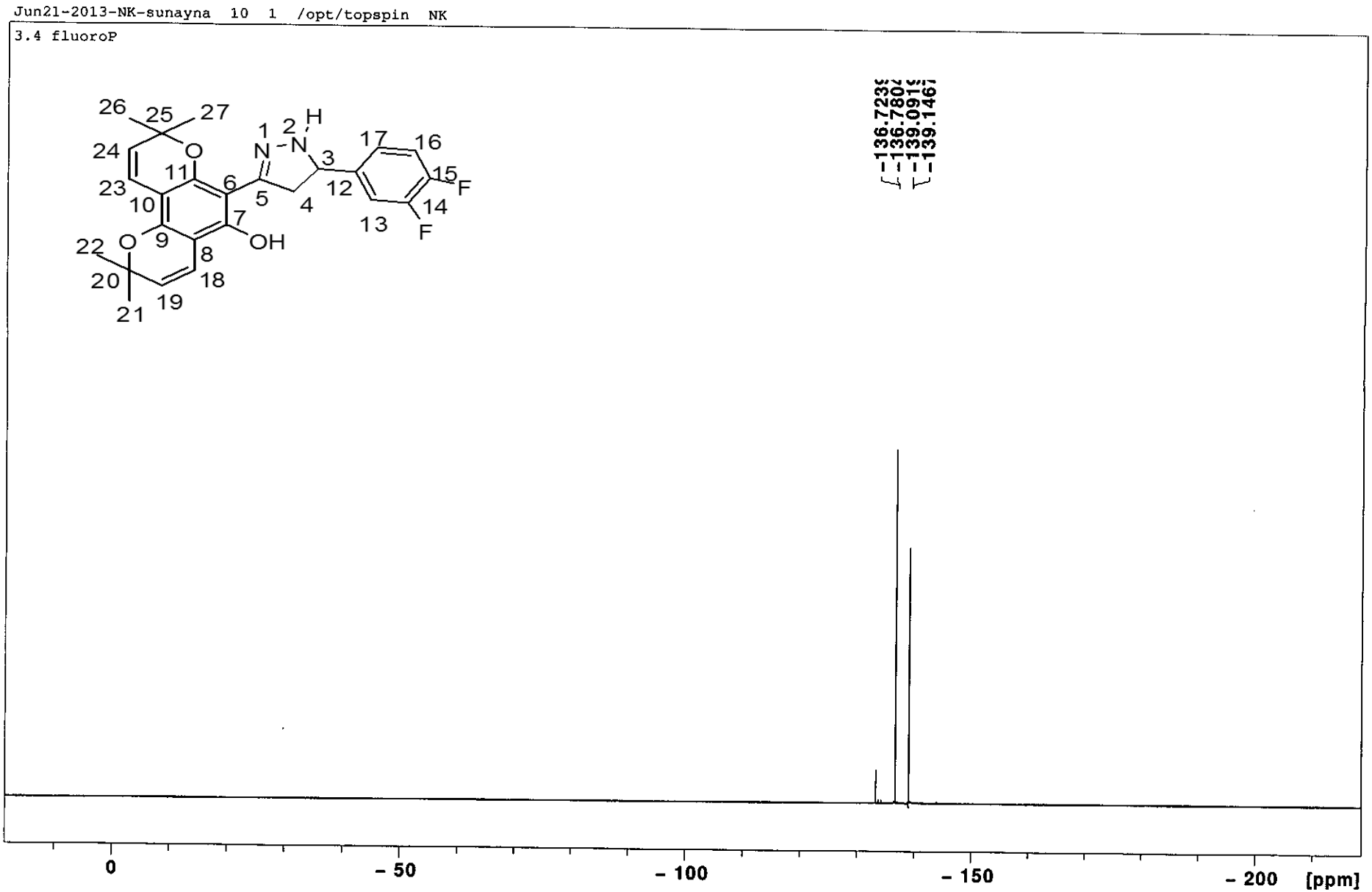
Expanded 13C NMR spectrum of 3-(3,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4h)



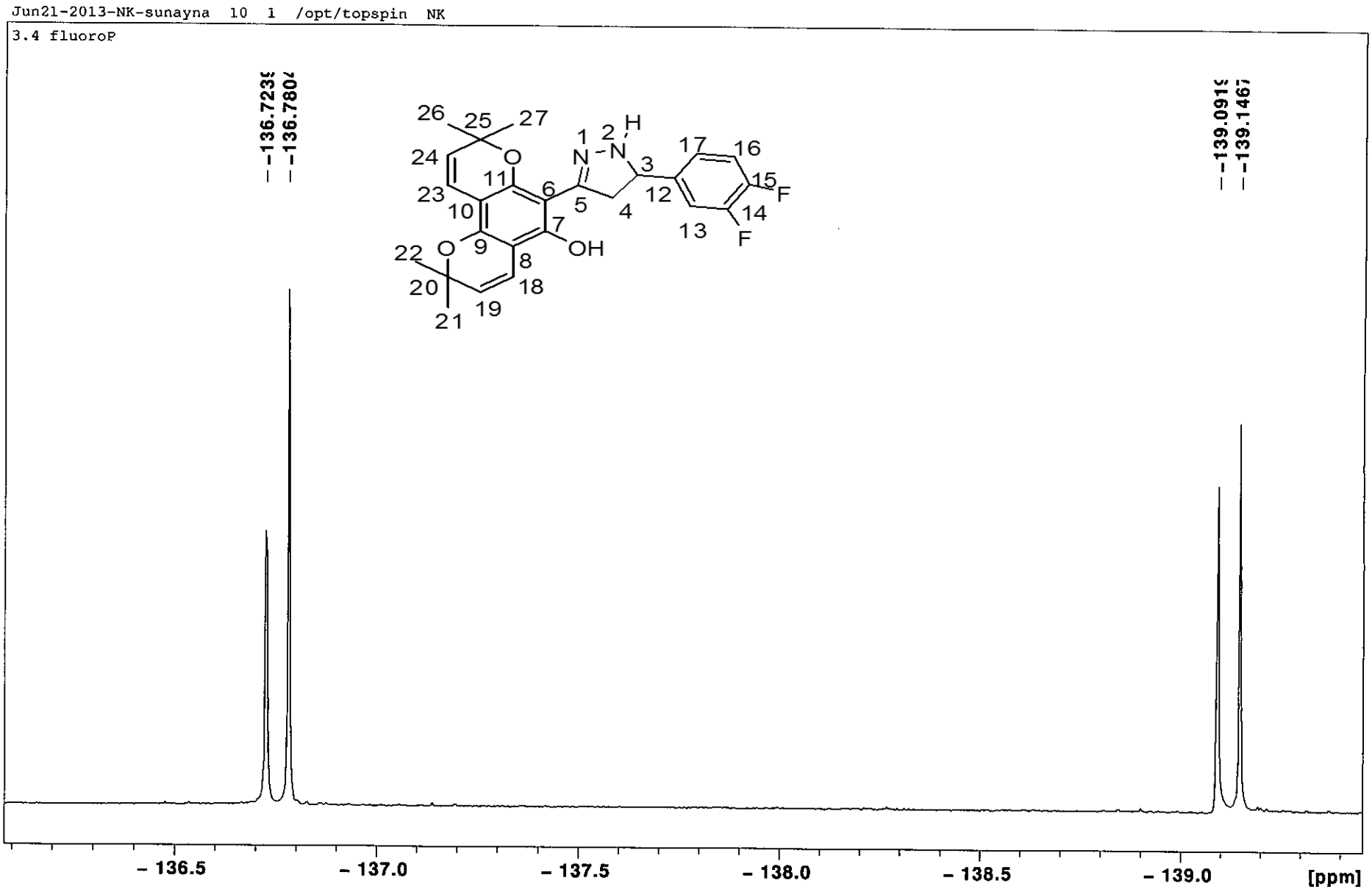
Expanded 13C NMR spectrum of 3-(3,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4h)



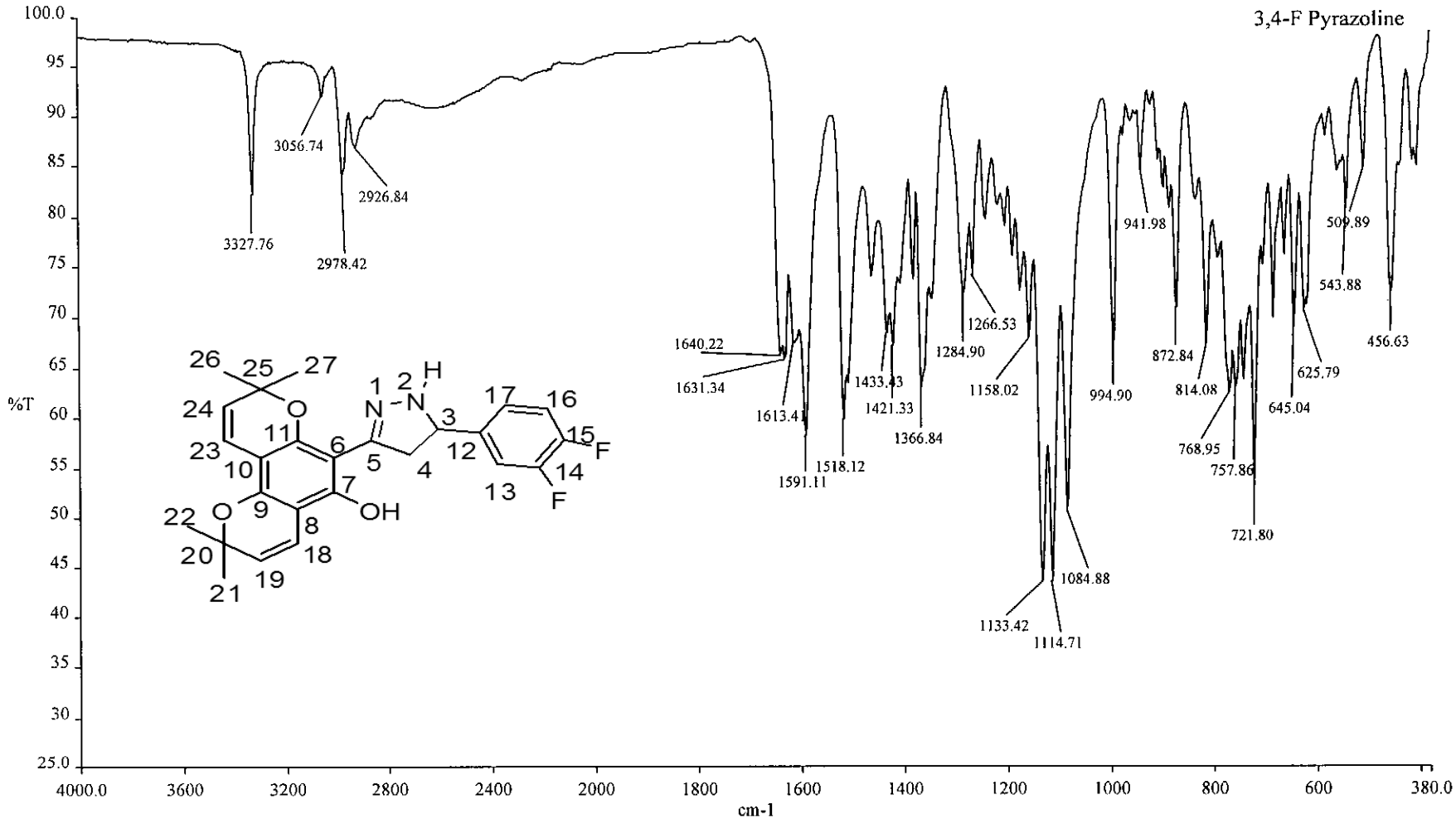
Expanded 13C NMR spectrum of 3-(3,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4h)



19F NMR spectrum of 3-(3,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4h)



Expanded 19F NMR spectrum of 3-(3,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4h)

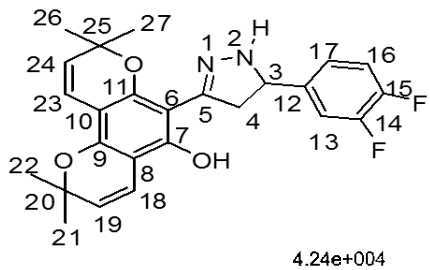


Infrared spectrum of 3-(3,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4h)

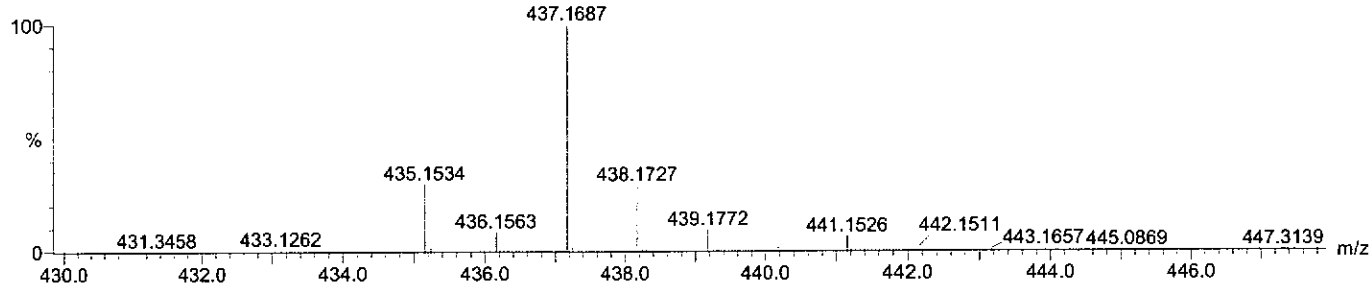
Elemental Composition Report **Page 1**

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

Monoisotopic Mass, Even Electron Ions
19 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-30 H: 20-30 N: 0-4 O: 0-4 F: 2-2
15 19 (0.307) Cm (1:30)
TOF MS ES-

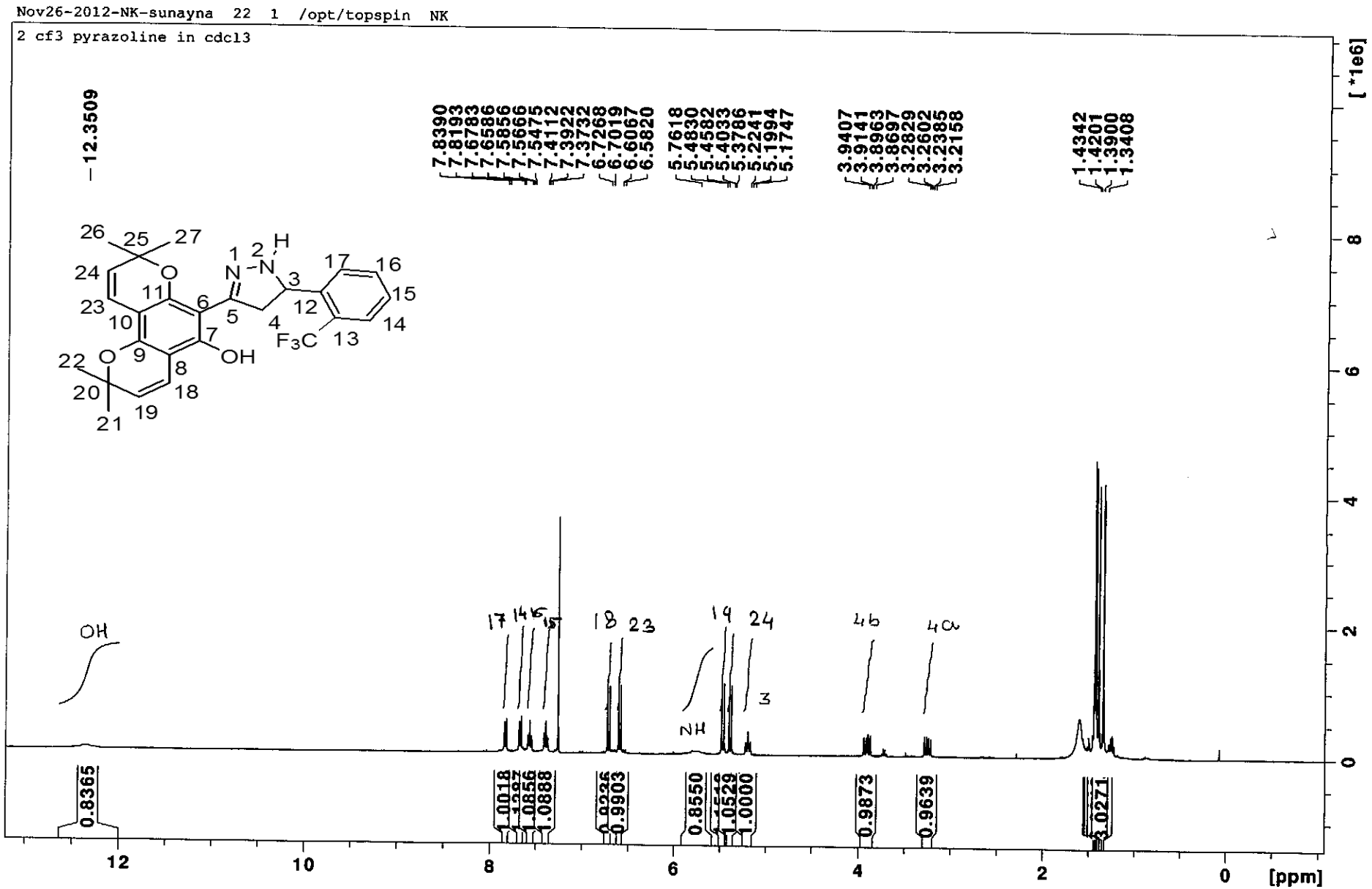


4.24e+004

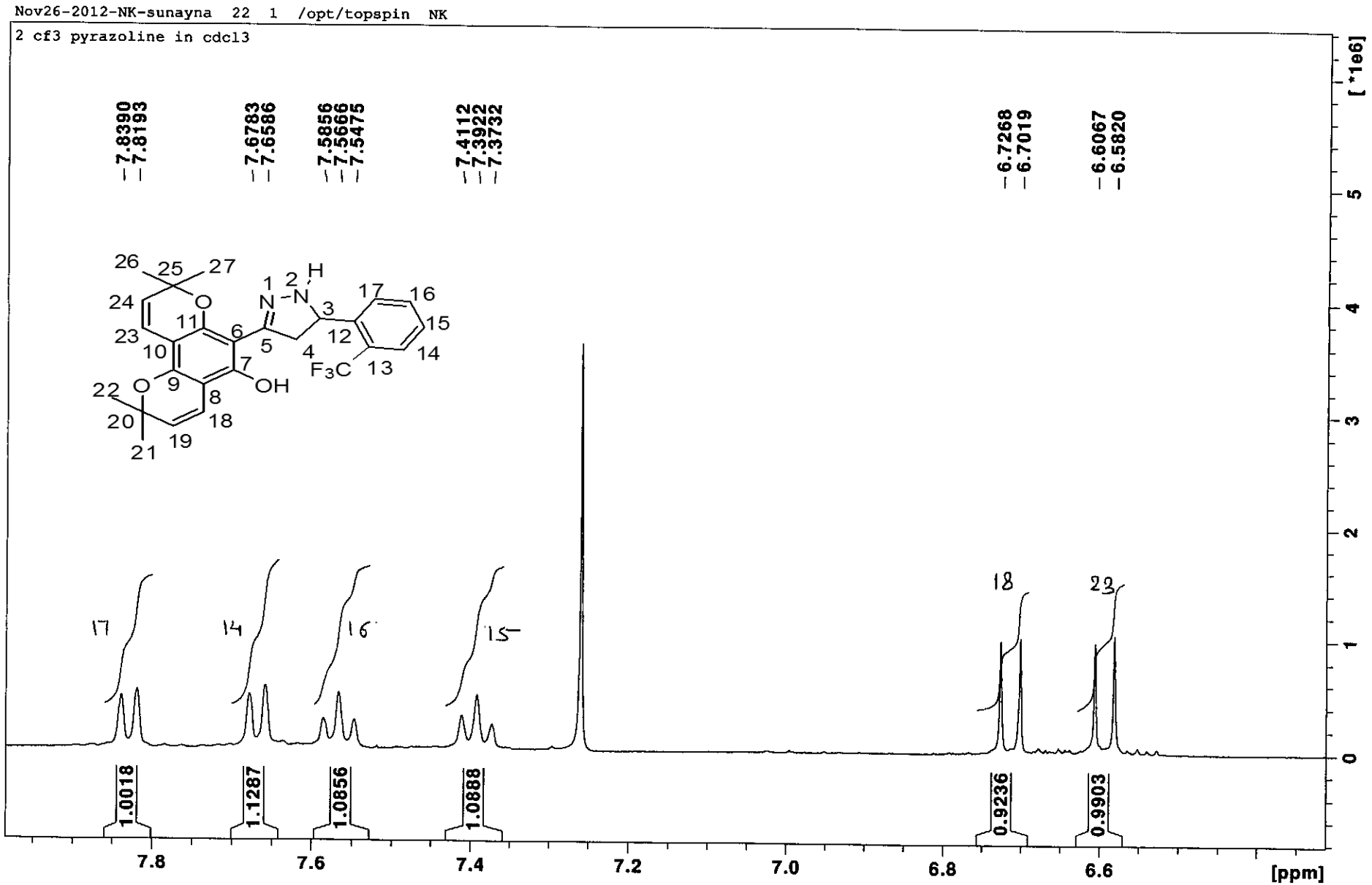


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
437.1687	437.1677	1.0	2.3	14.5	456.0	0.0	C25 H23 N2 O3 F2

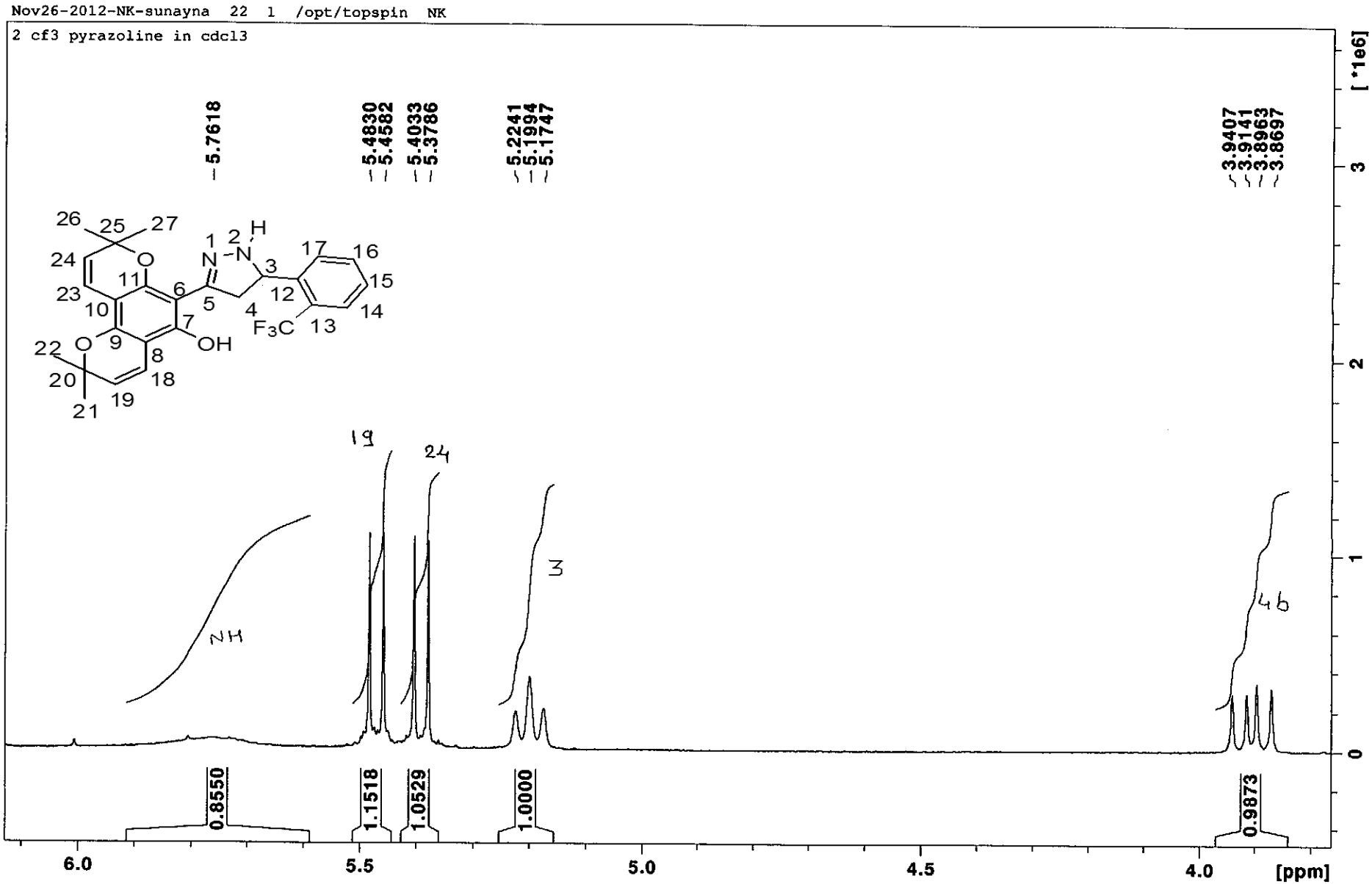
HRMS spectrum of 3-(3,4-difluorophenyl)-5-pyranochromene pyrazoline (C-4h)



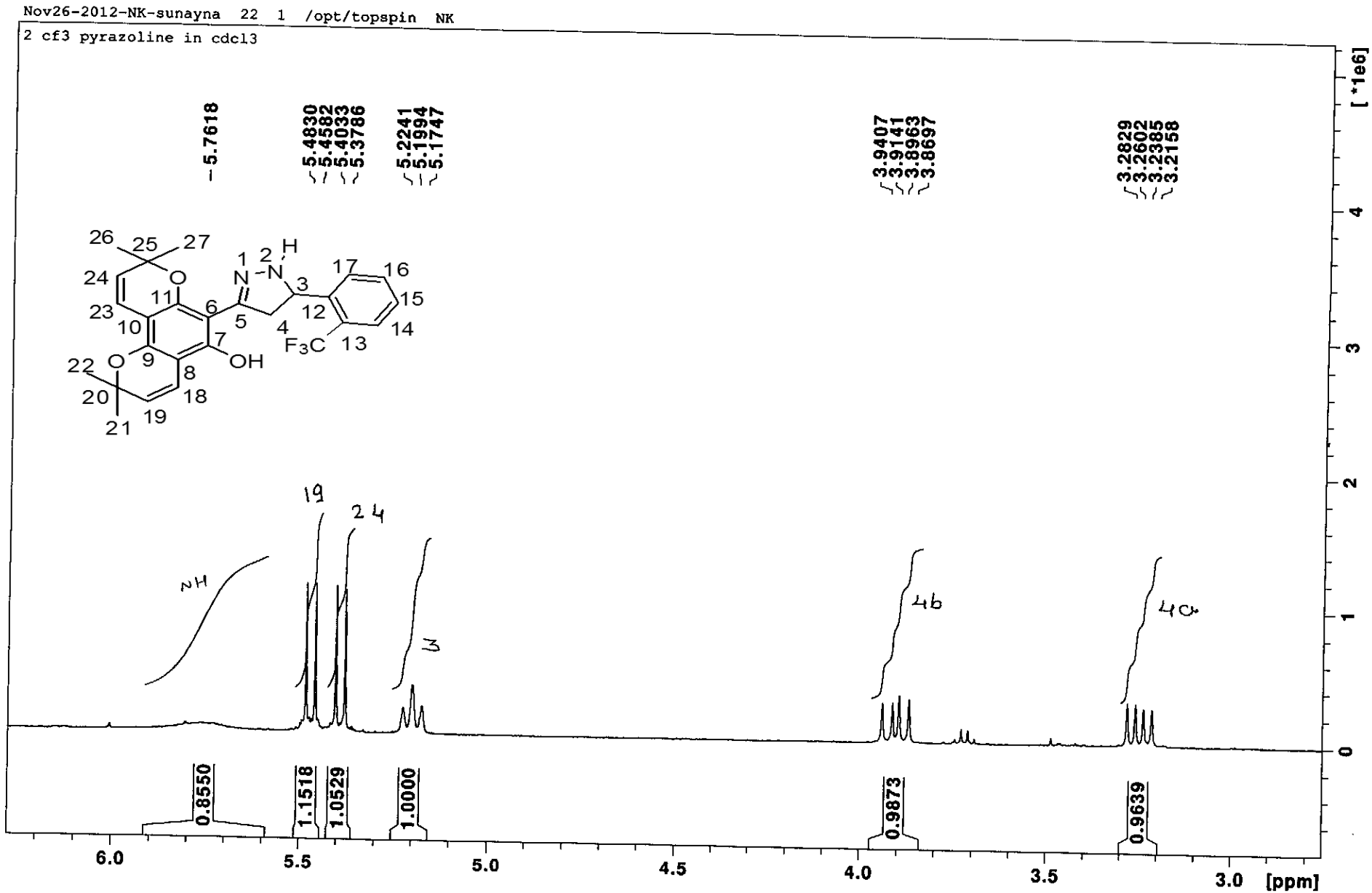
1H NMR spectrum of 3-(2-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4i)



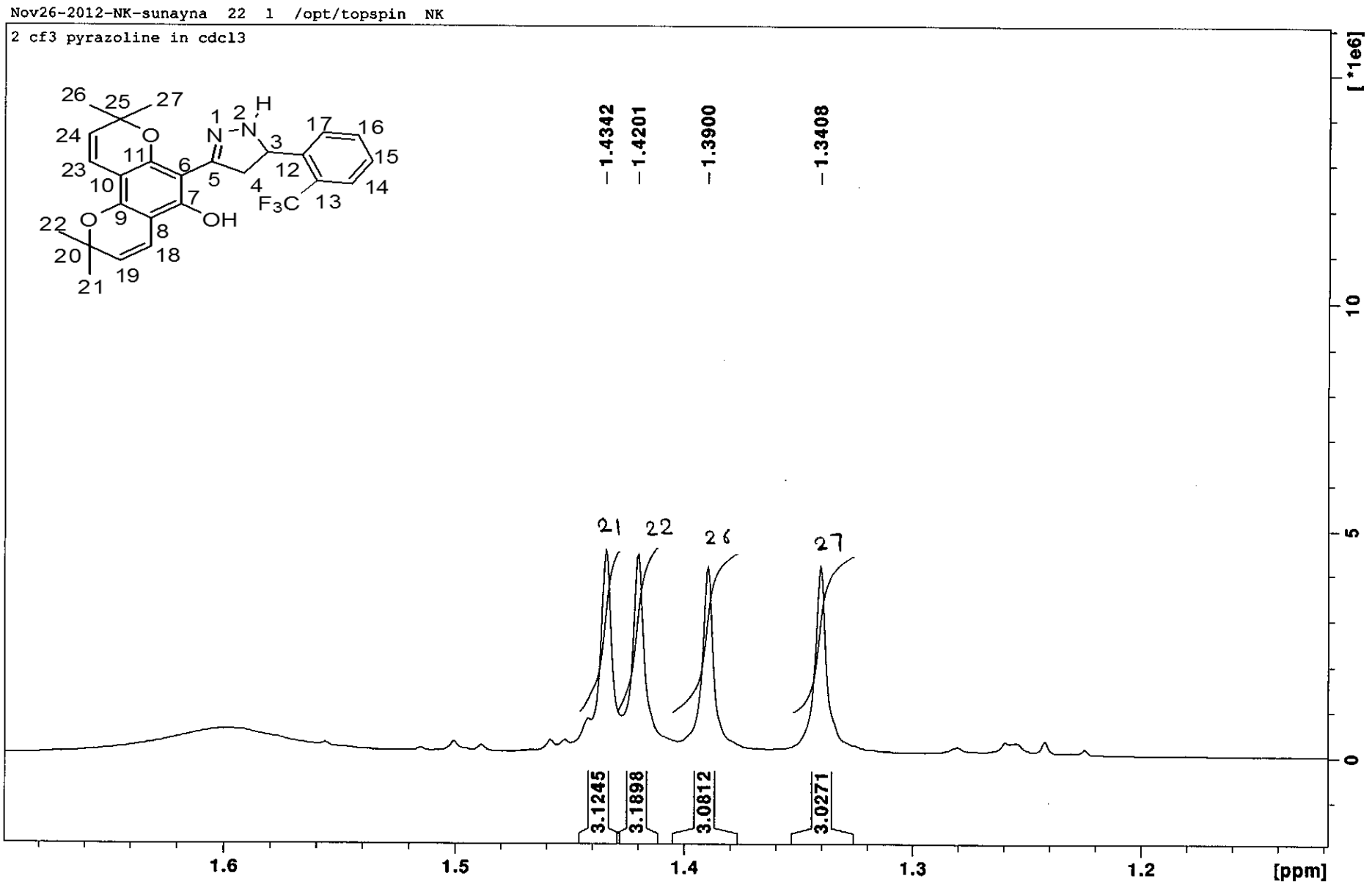
Expanded 1H NMR spectrum of 3-(2-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4i)



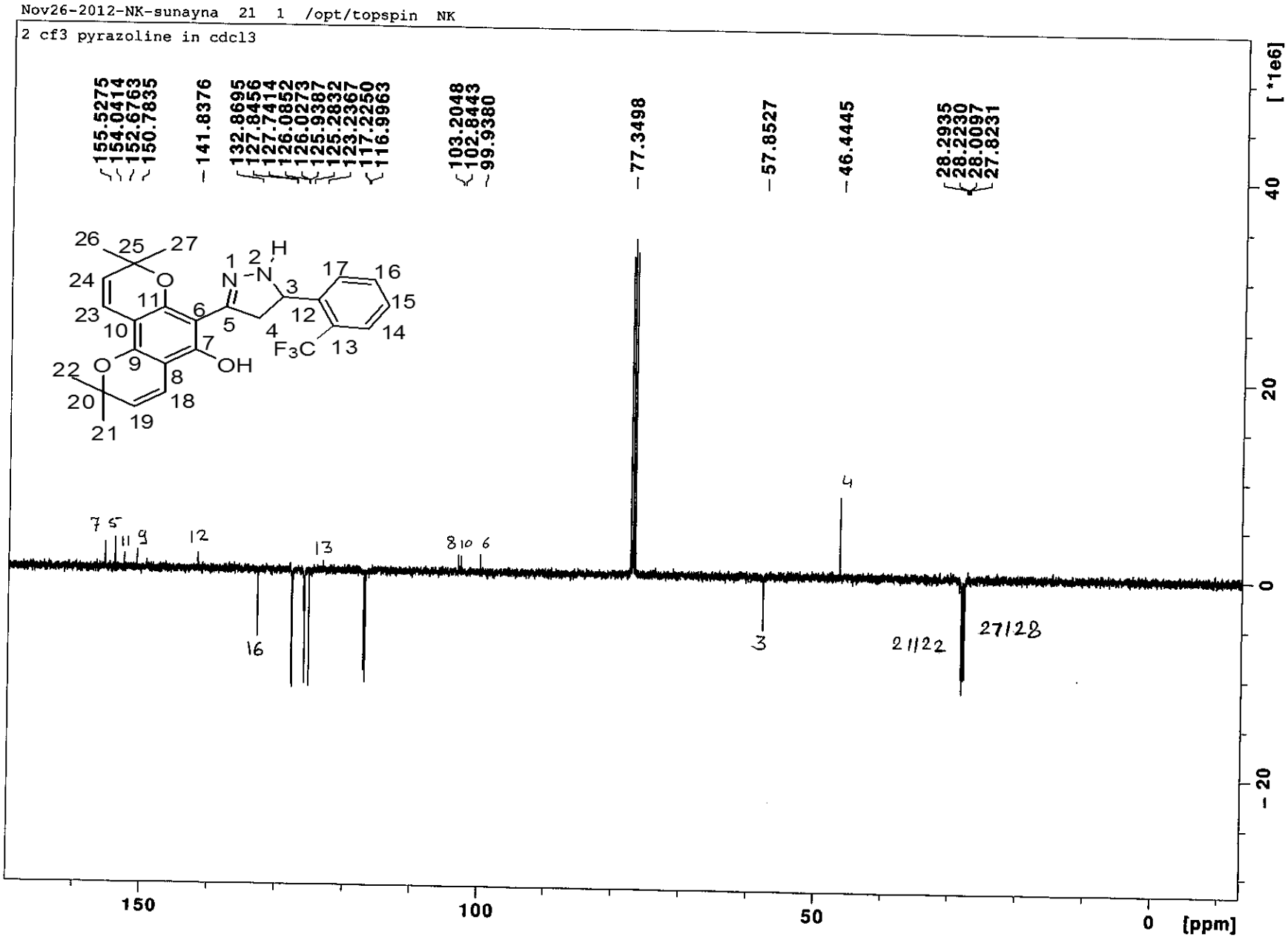
Expanded 1H NMR spectrum of 3-(2-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4i)



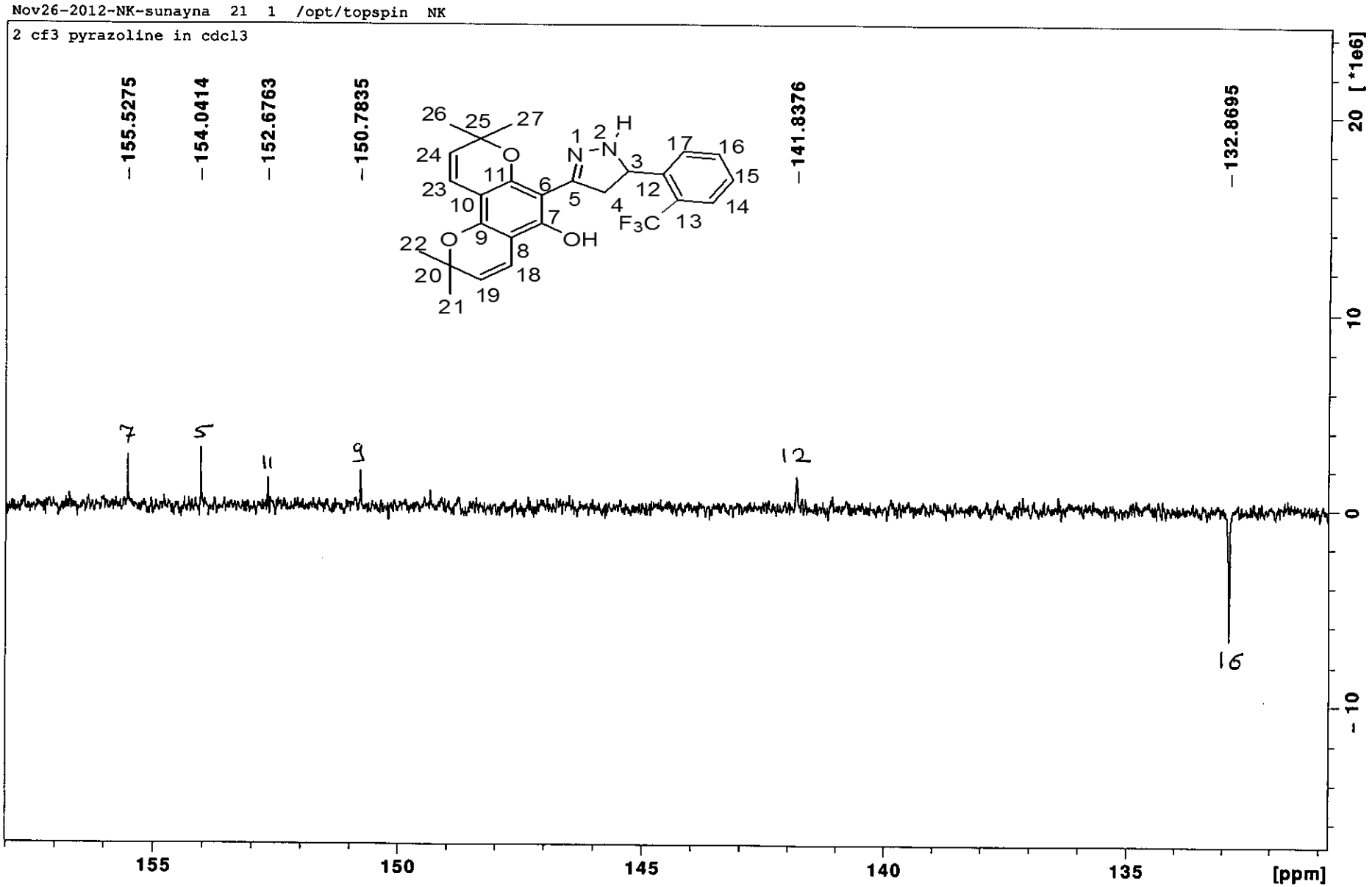
Expanded 1H NMR spectrum of 3-(2-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4i)



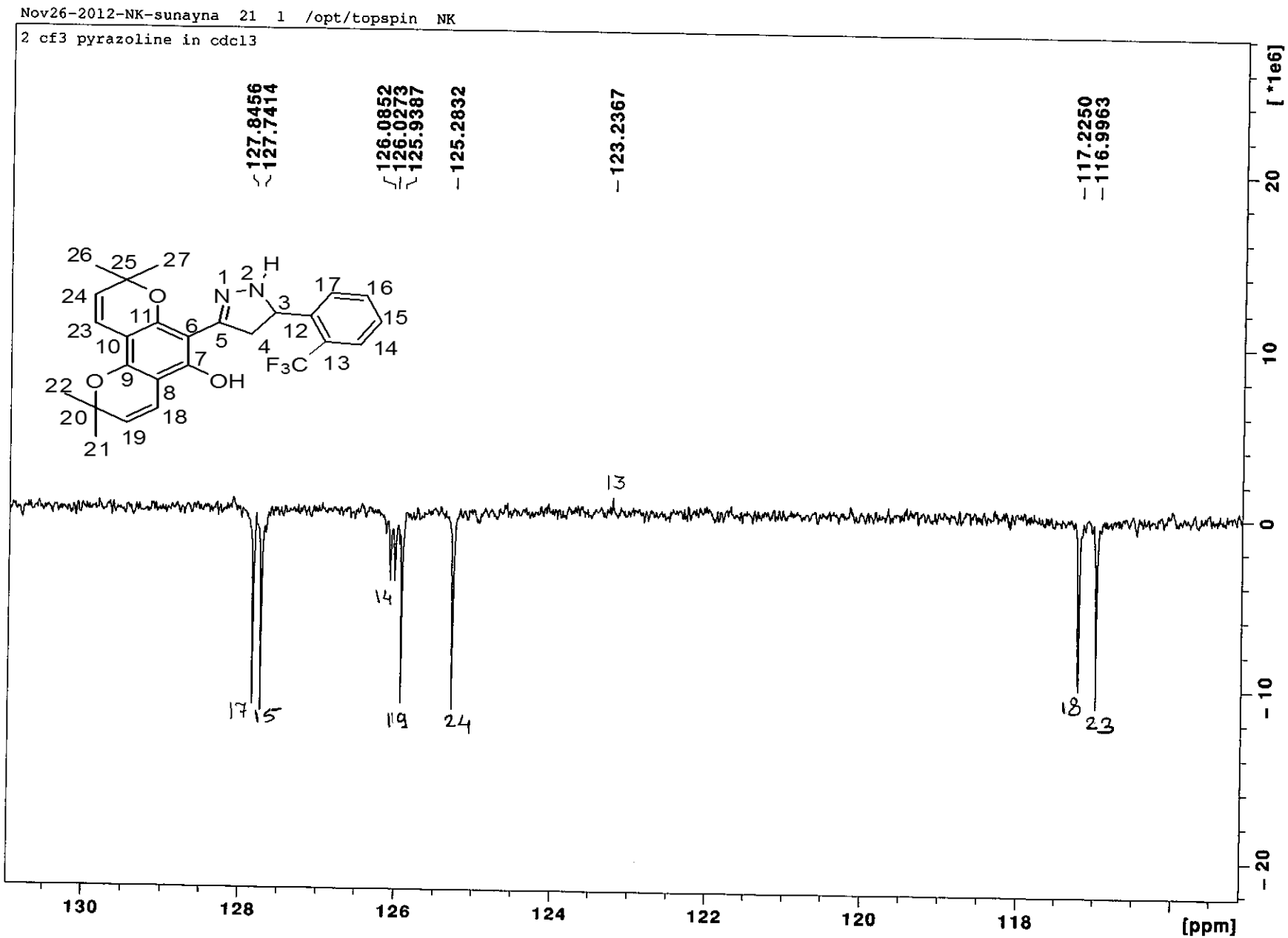
Expanded 1H NMR spectrum of 3-(2-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4i)



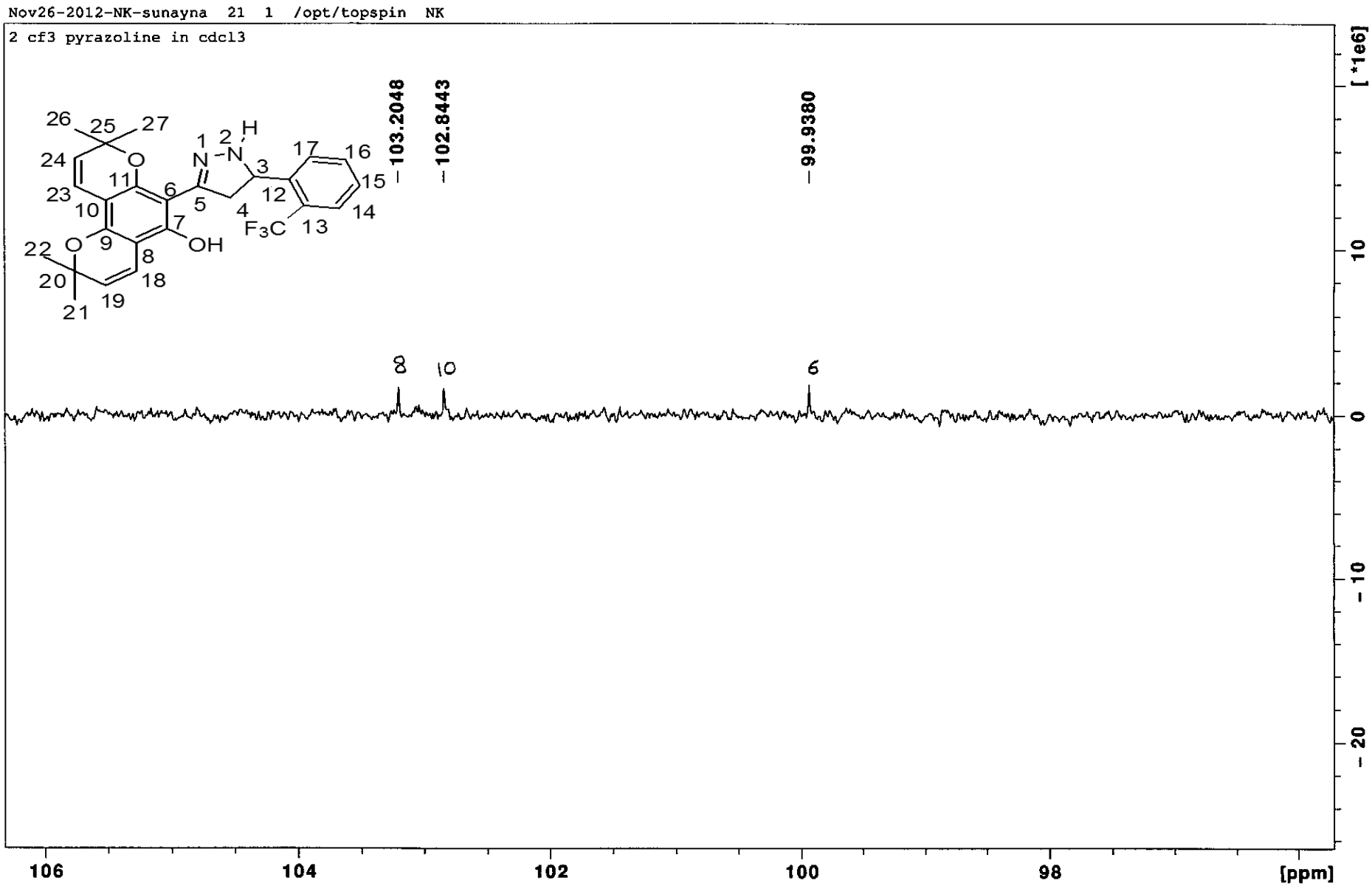
13C NMR spectrum of 3-(2-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4i)



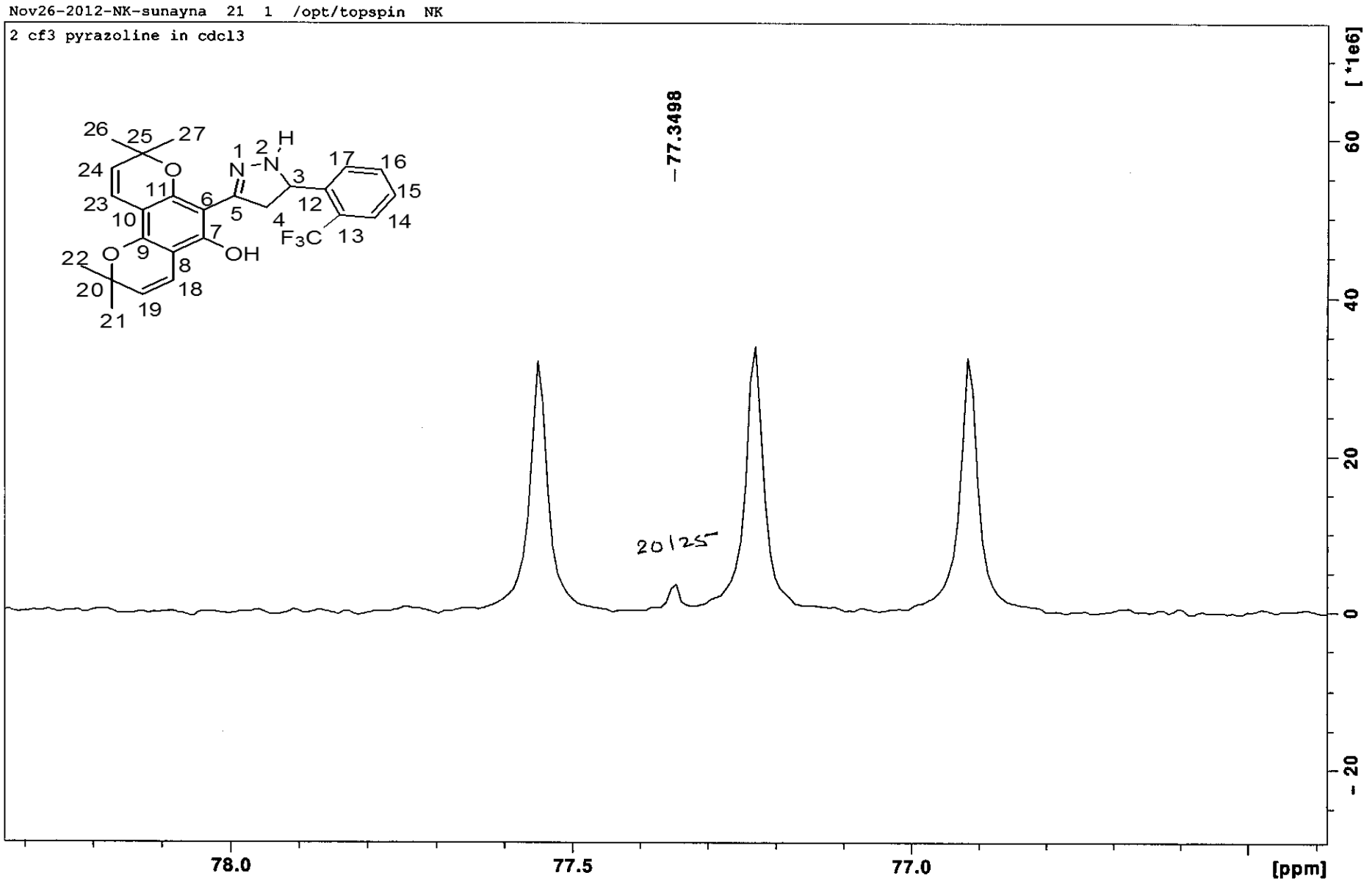
Expanded 13C NMR spectrum of 3-(2-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4i)



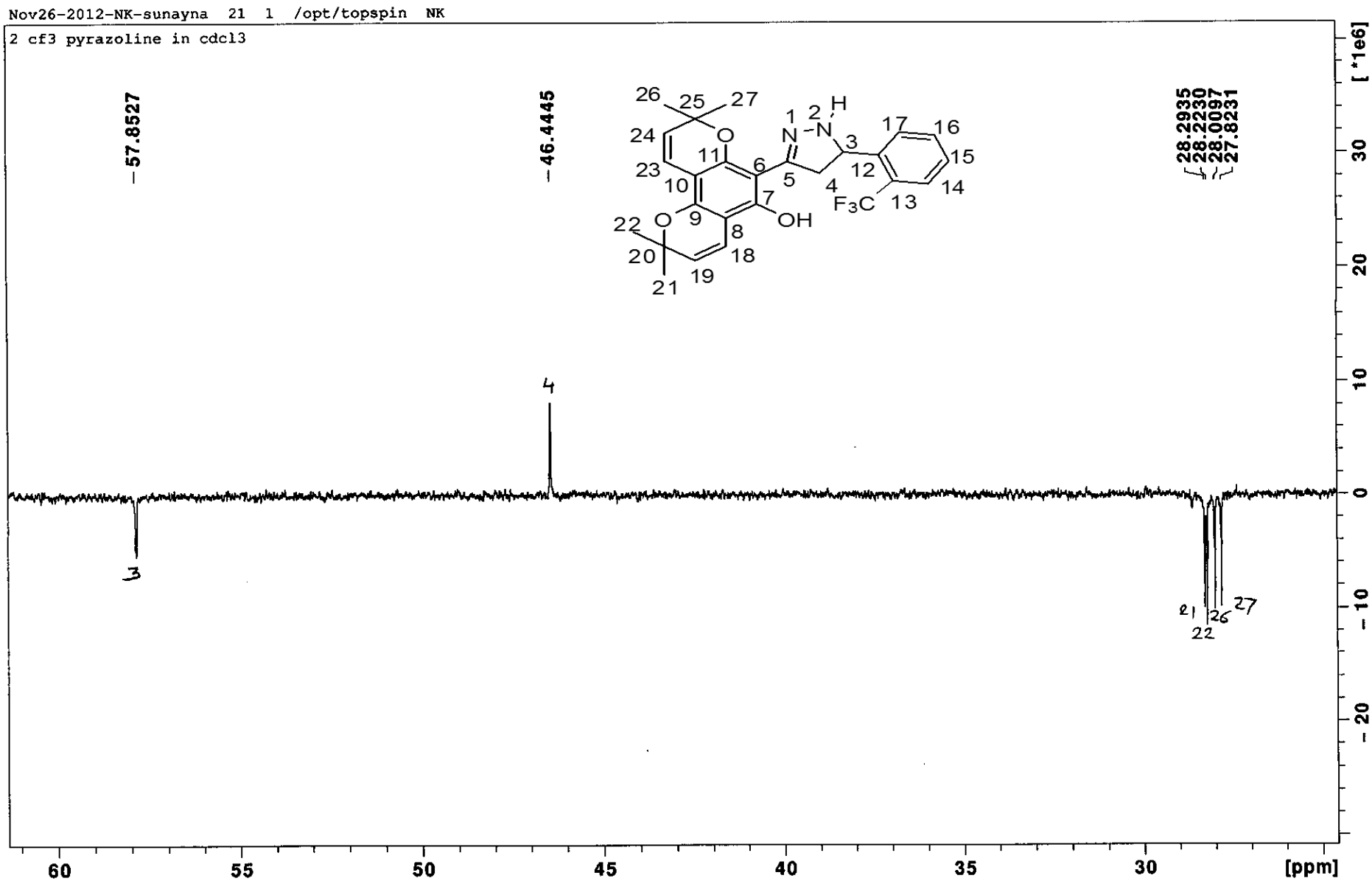
Expanded 13C NMR spectrum of 3-(2-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4i)



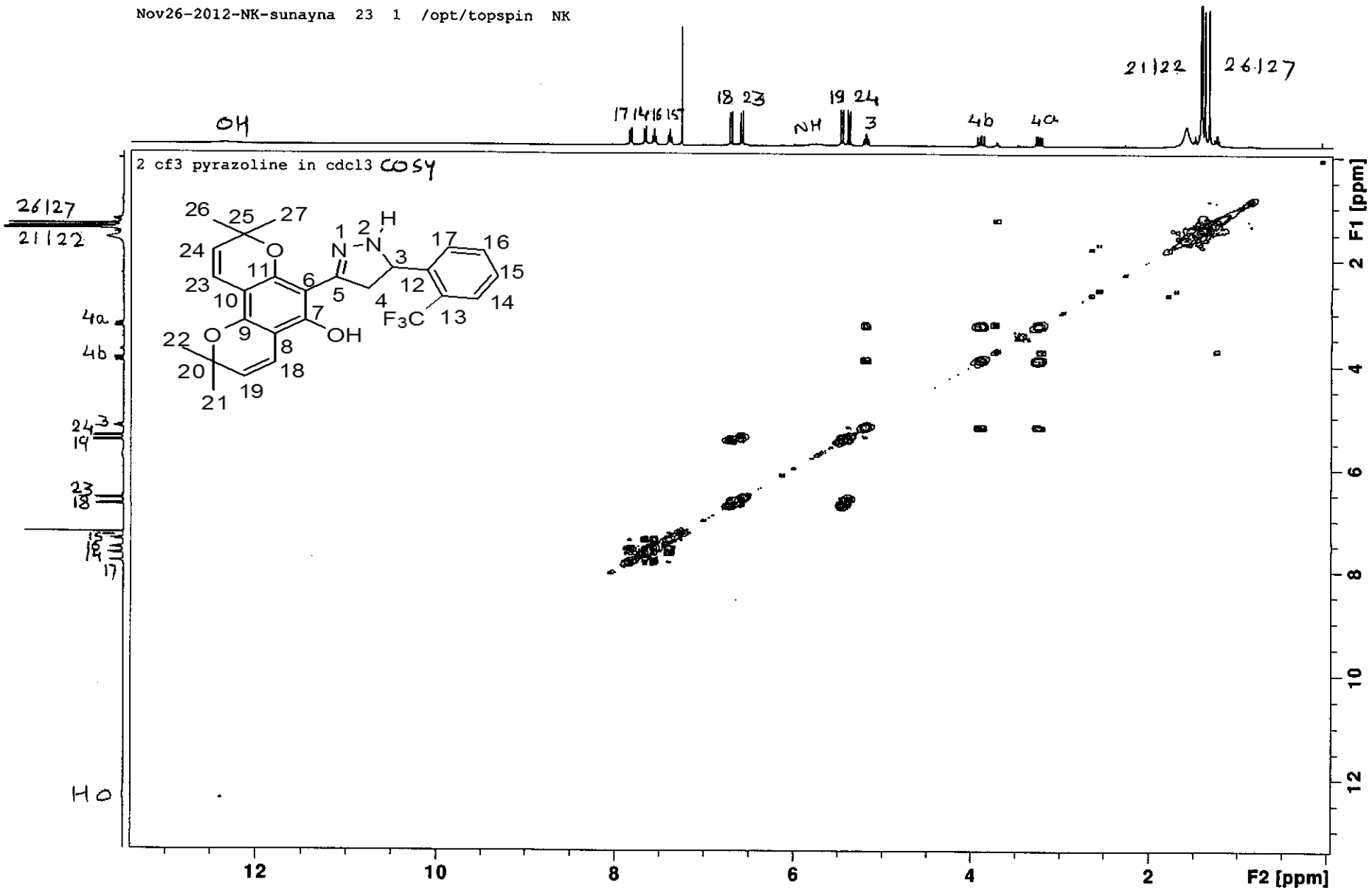
Expanded 13C NMR spectrum of 3-(2-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4i)



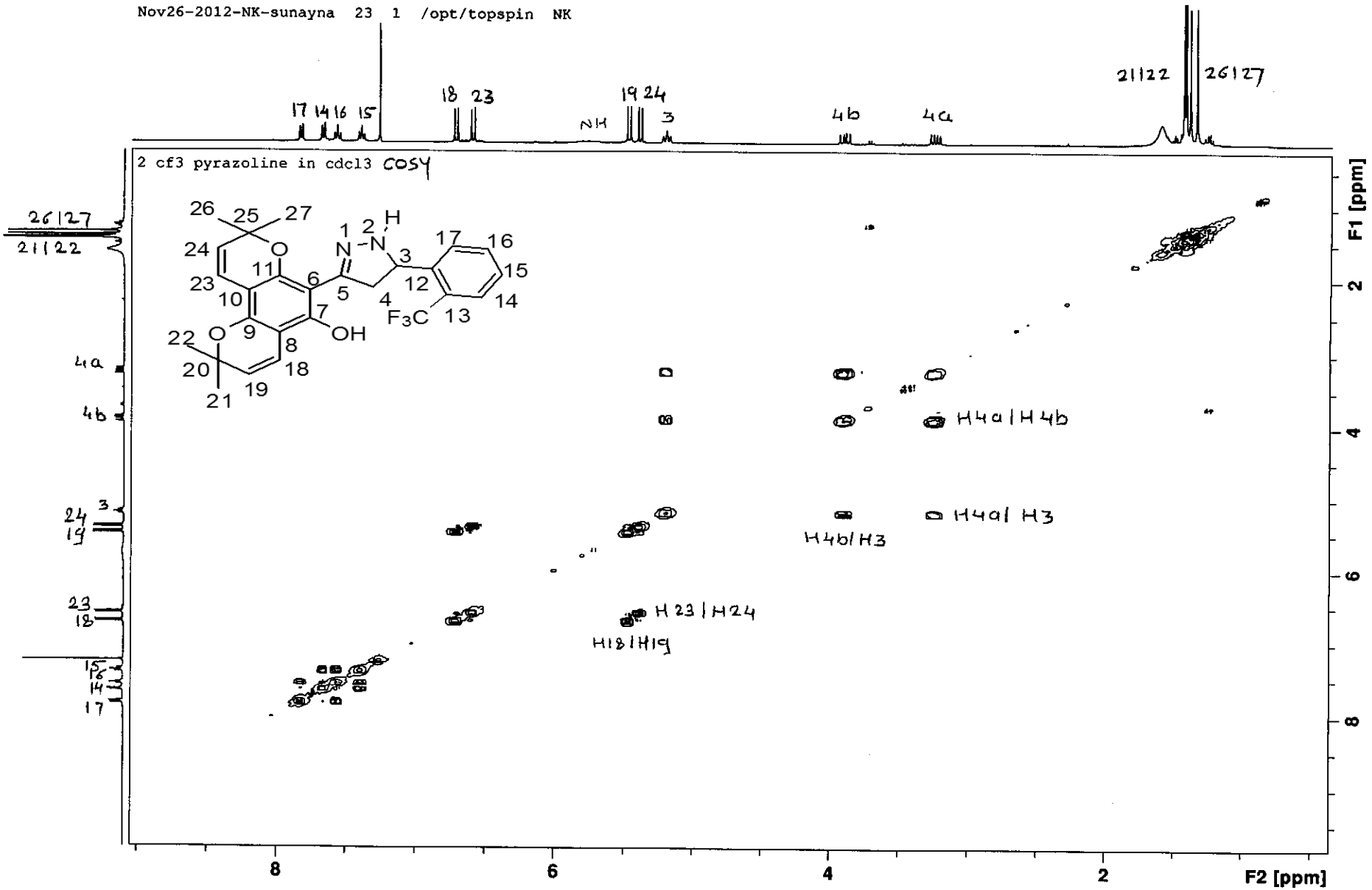
Expanded 13C NMR spectrum of 3-(2-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4i)



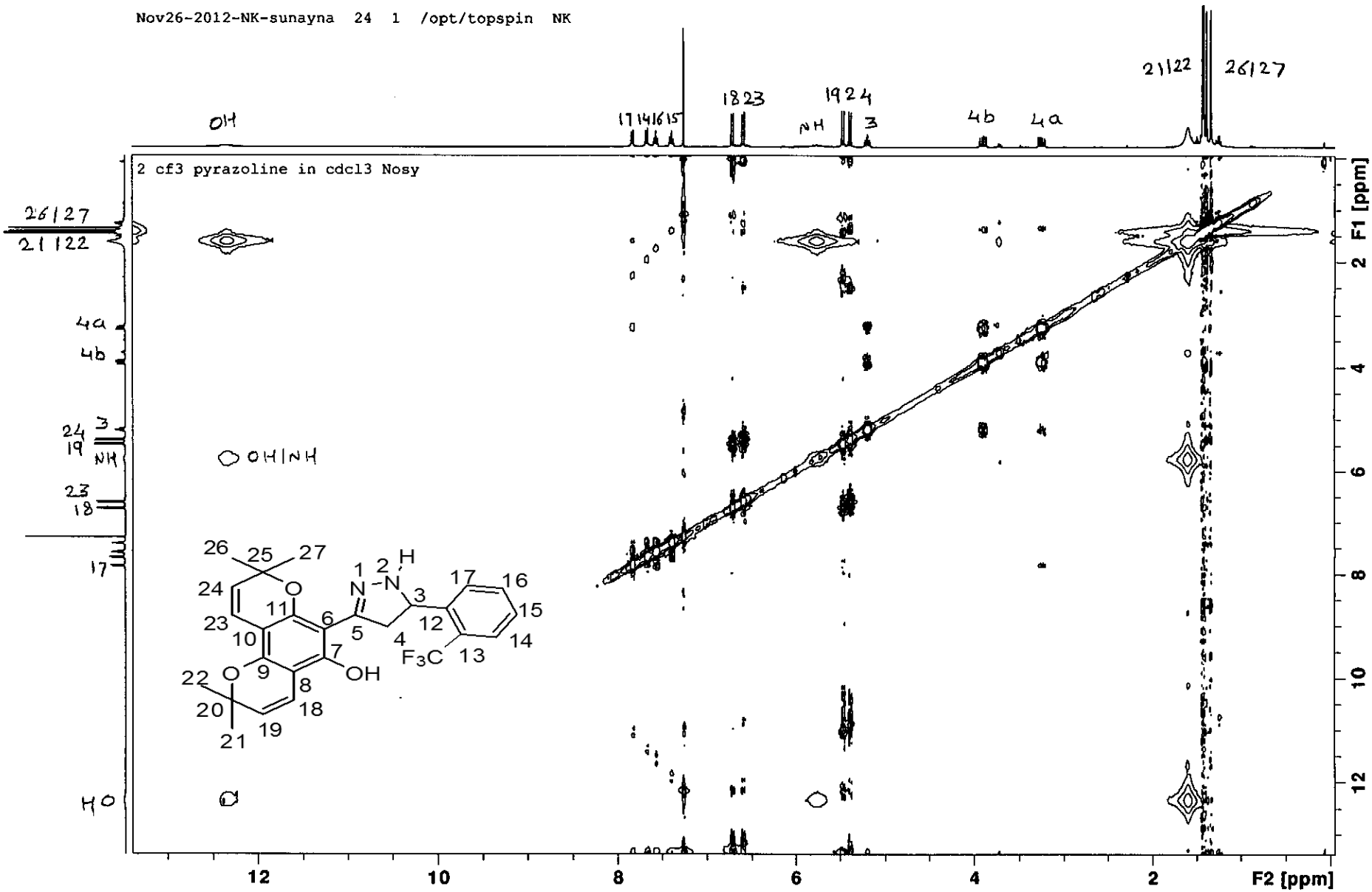
Expanded 13C NMR spectrum of 3-(2-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4i)



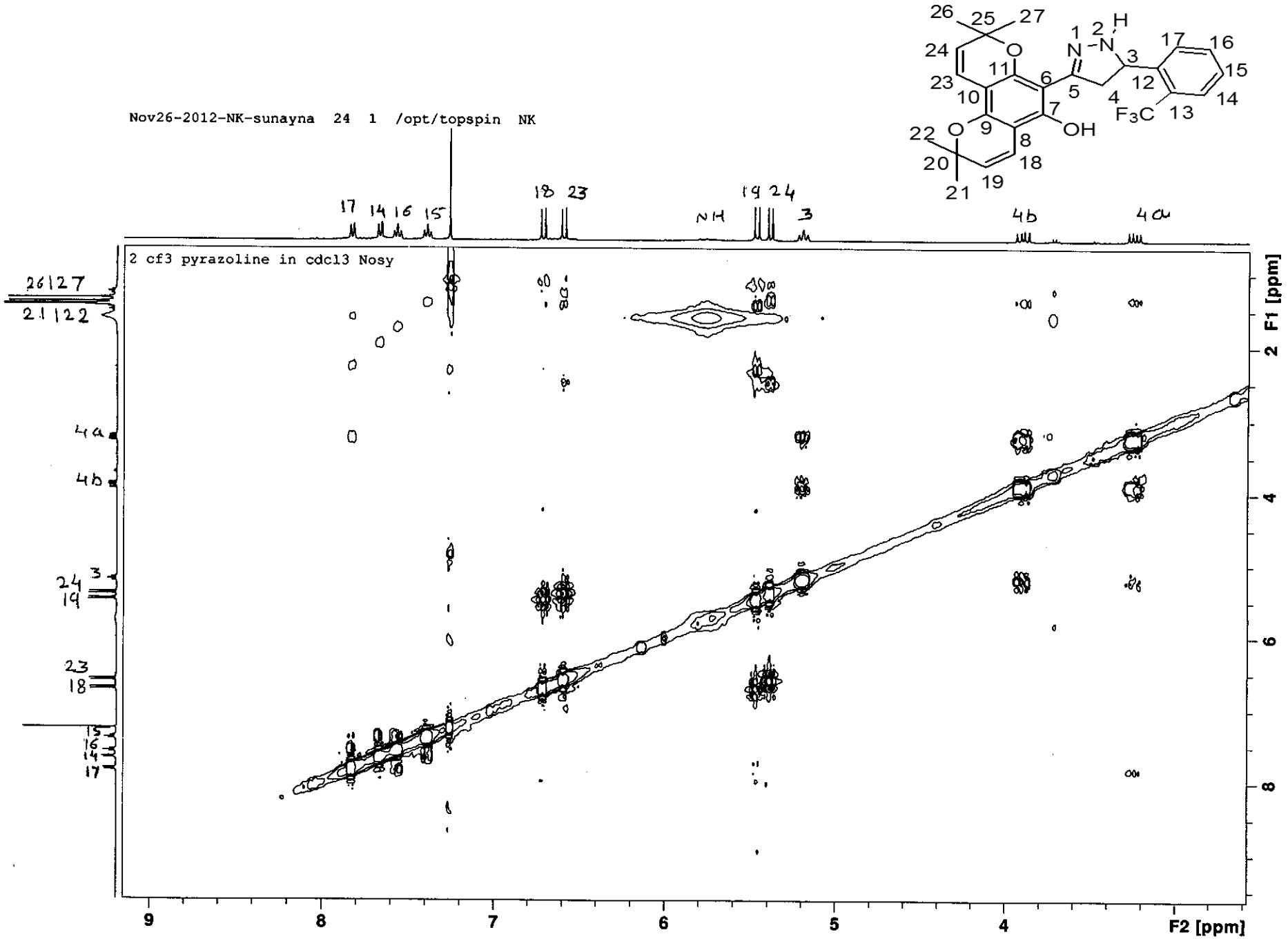
COSY NMR spectrum of 3-(2-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4i)



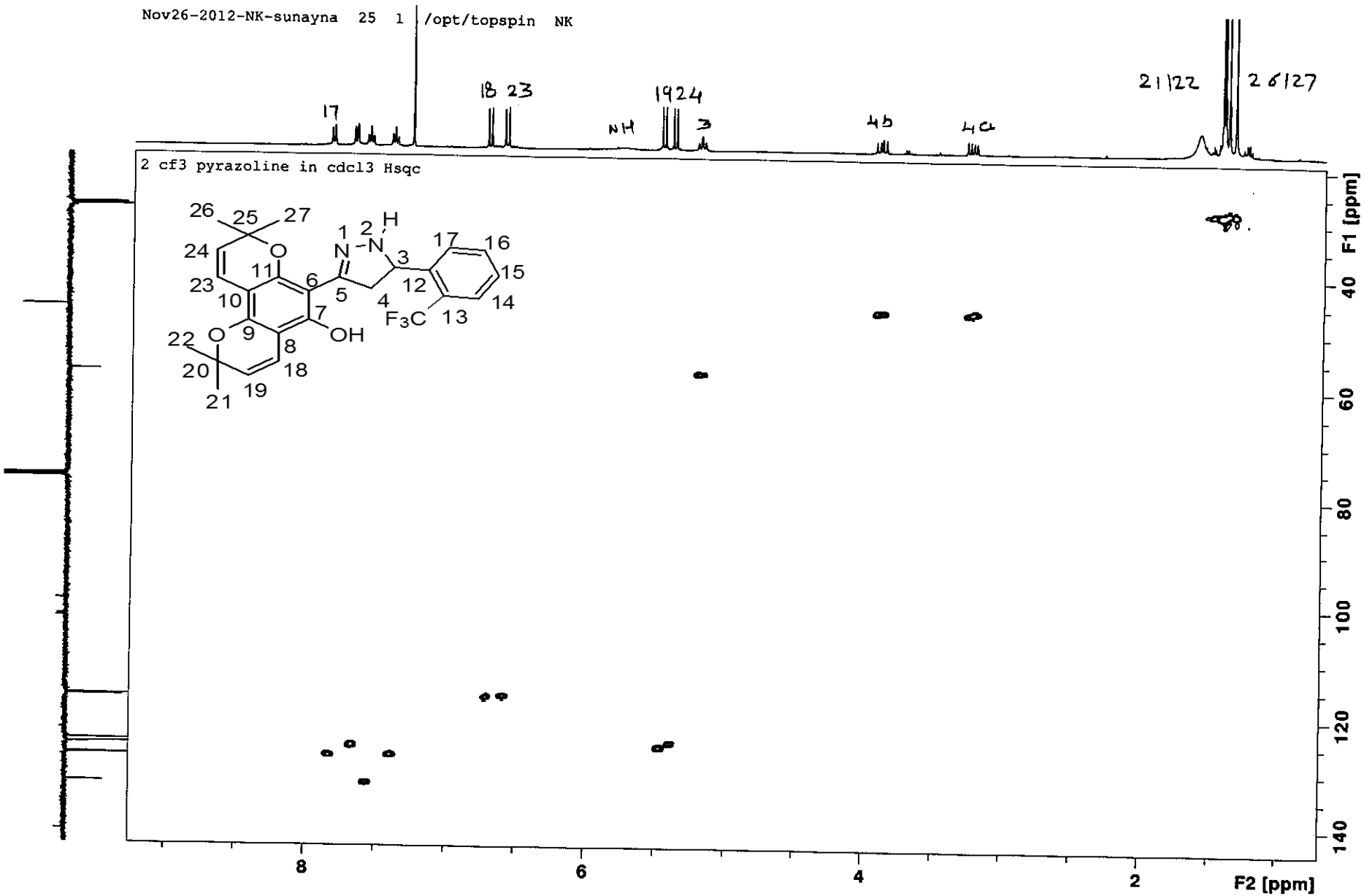
Expanded COSY spectrum of 3-(2-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4i)



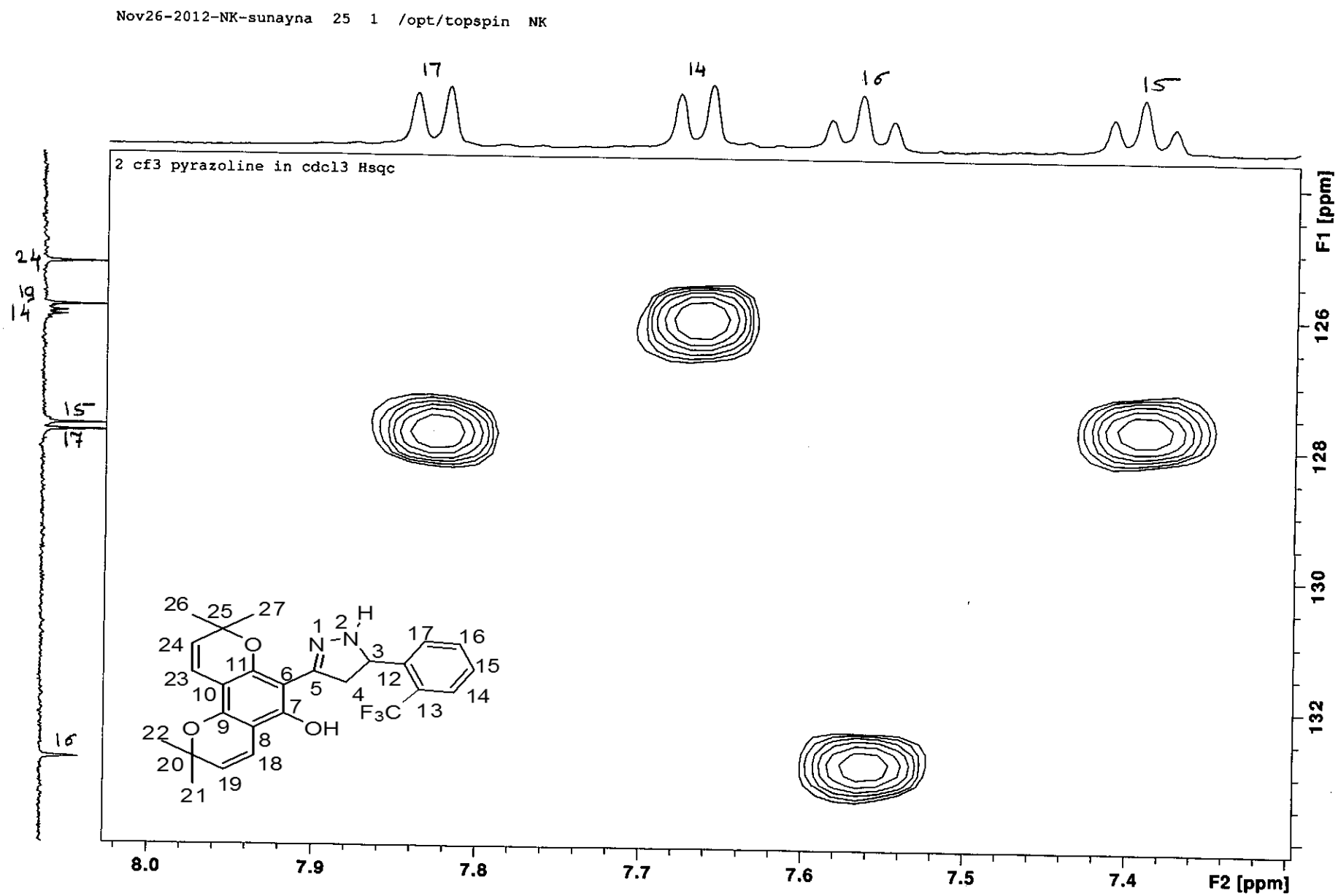
NOESY spectrum of 3-(2-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4i)



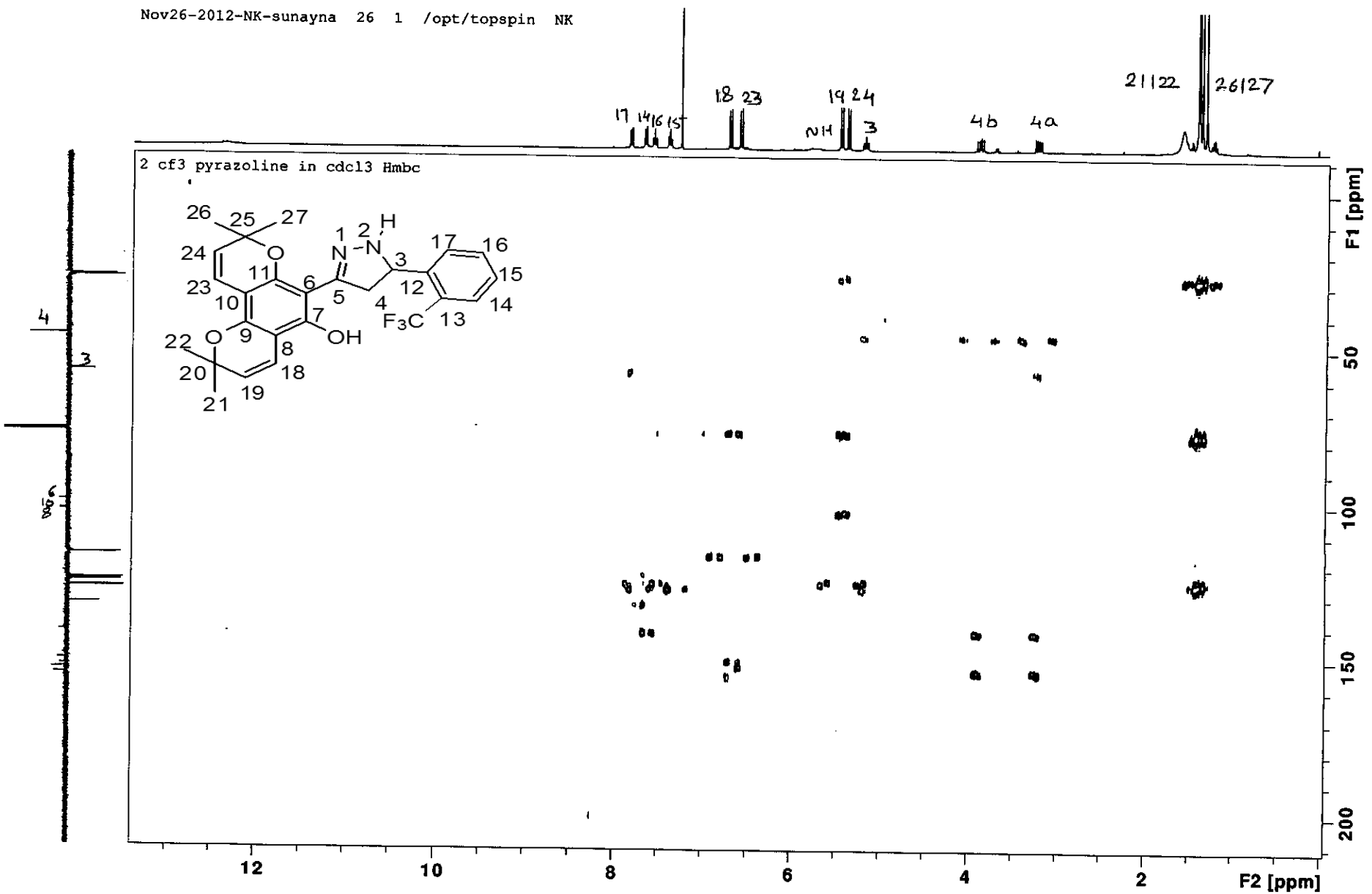
Expanded NOESY spectrum of 3-(2-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4i)



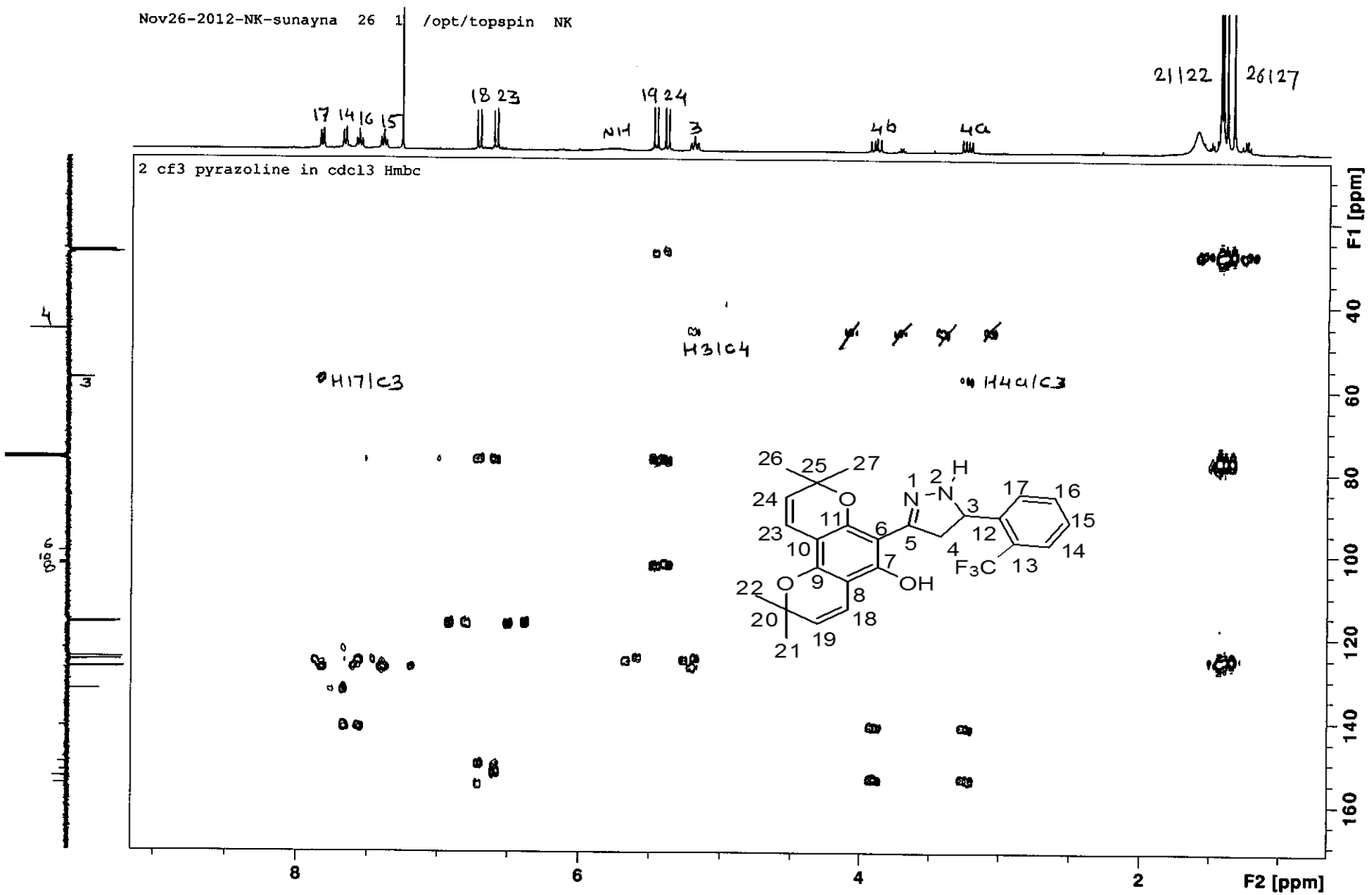
HSQC spectrum of 3-(2-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4i)



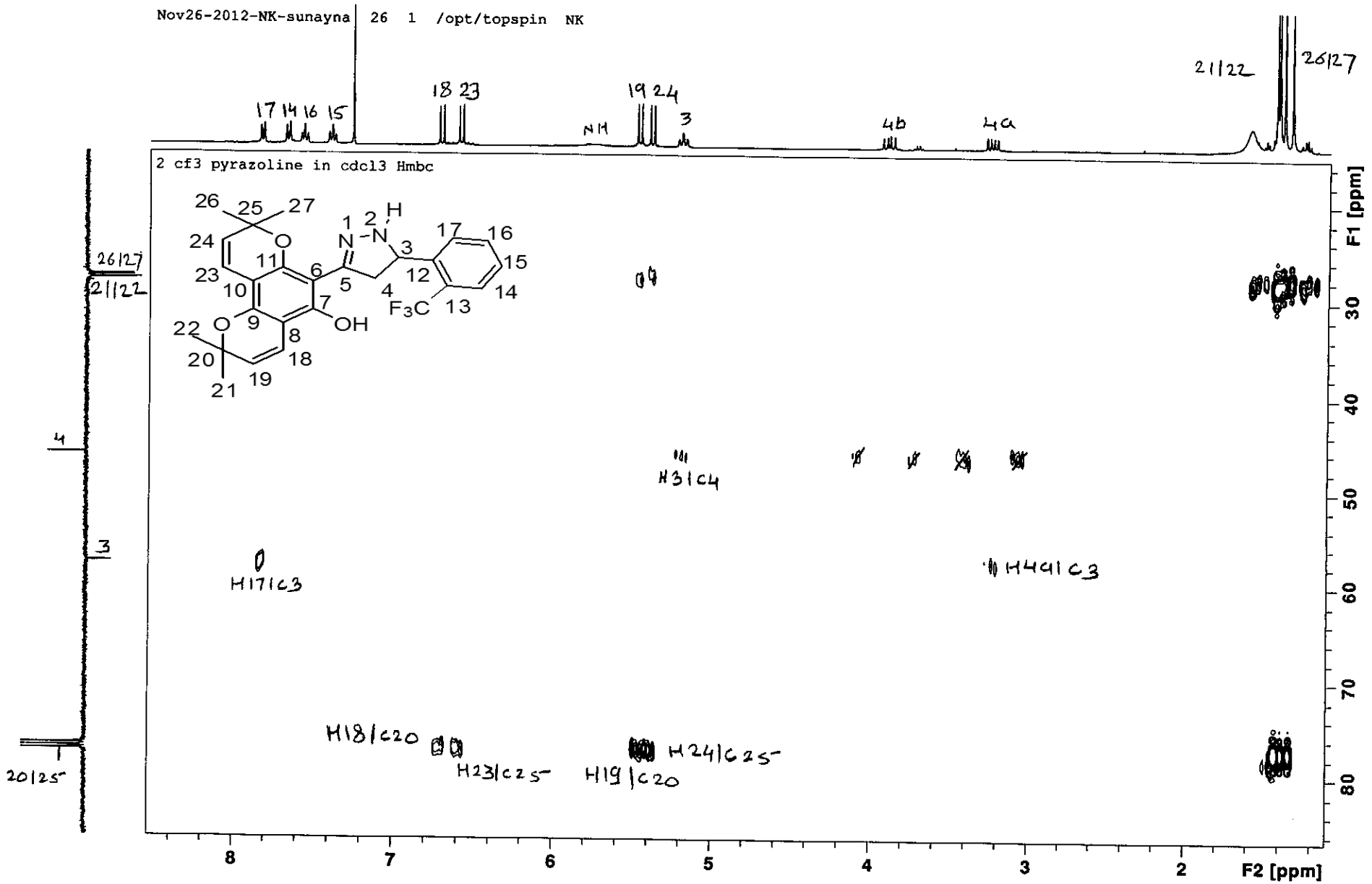
Expanded HSQC spectrum of 3-(2-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4i)



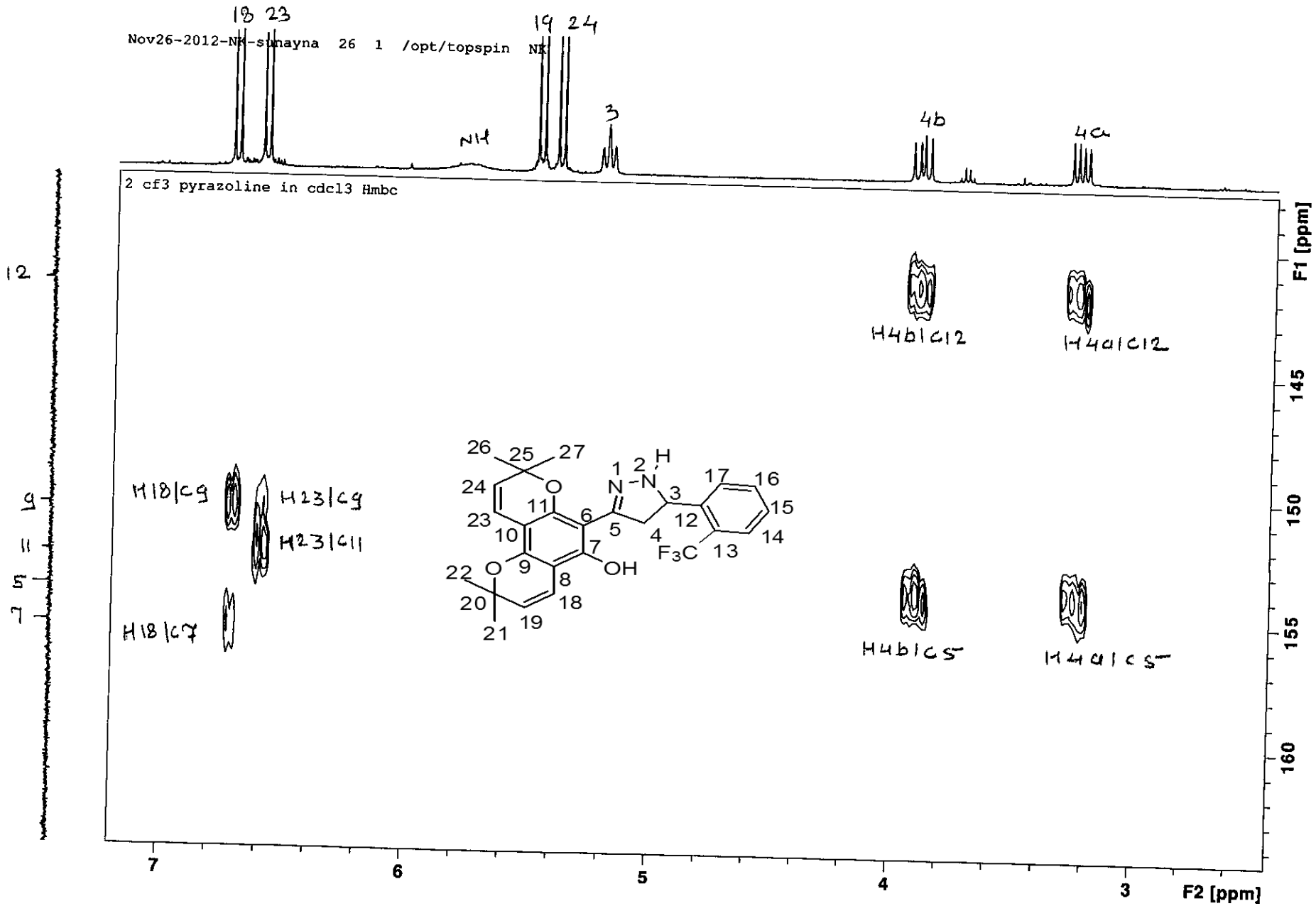
HMBC spectrum of 3-(2-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4i)



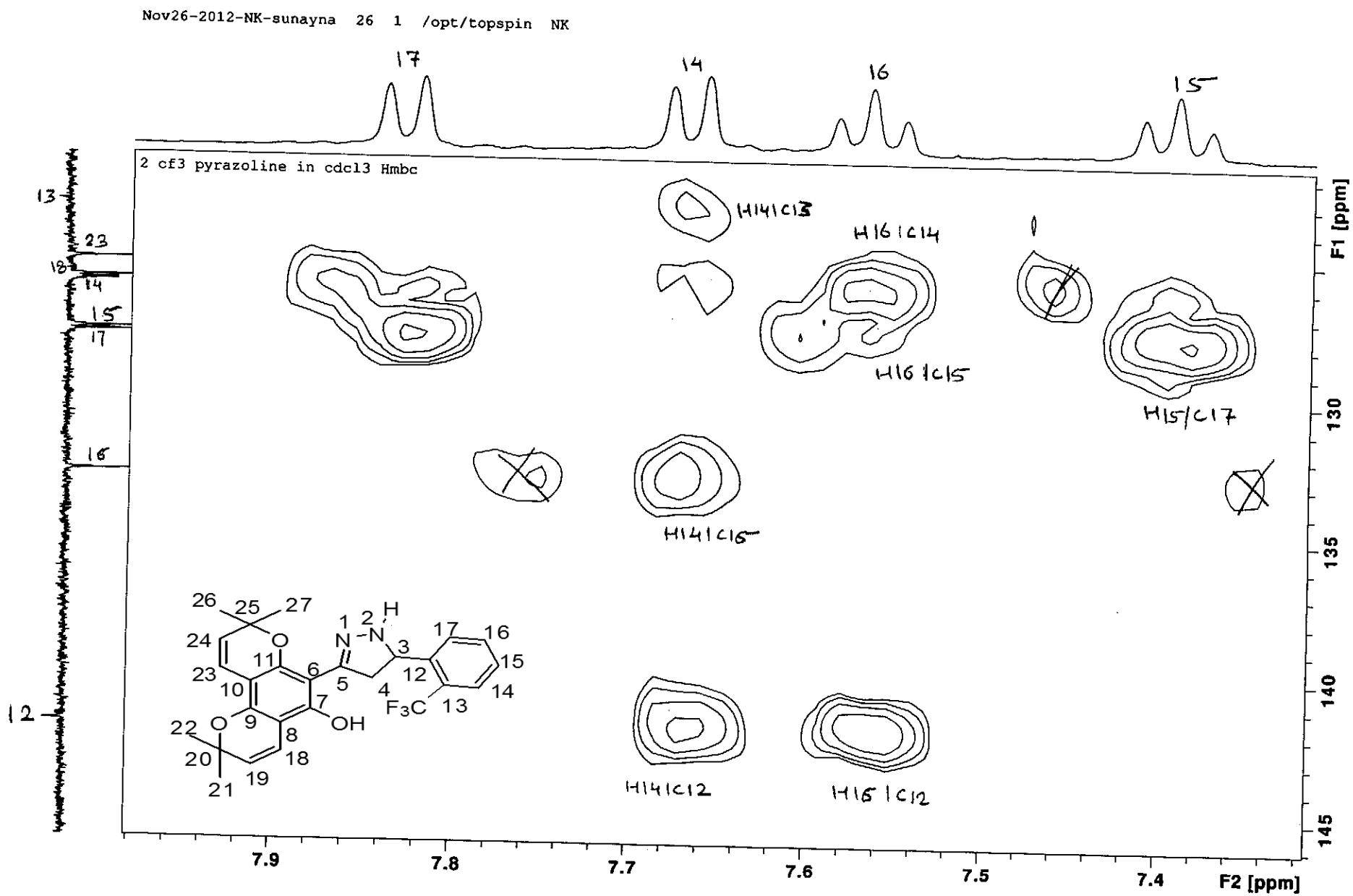
Expanded HMBC spectrum of 3-(2-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4i)



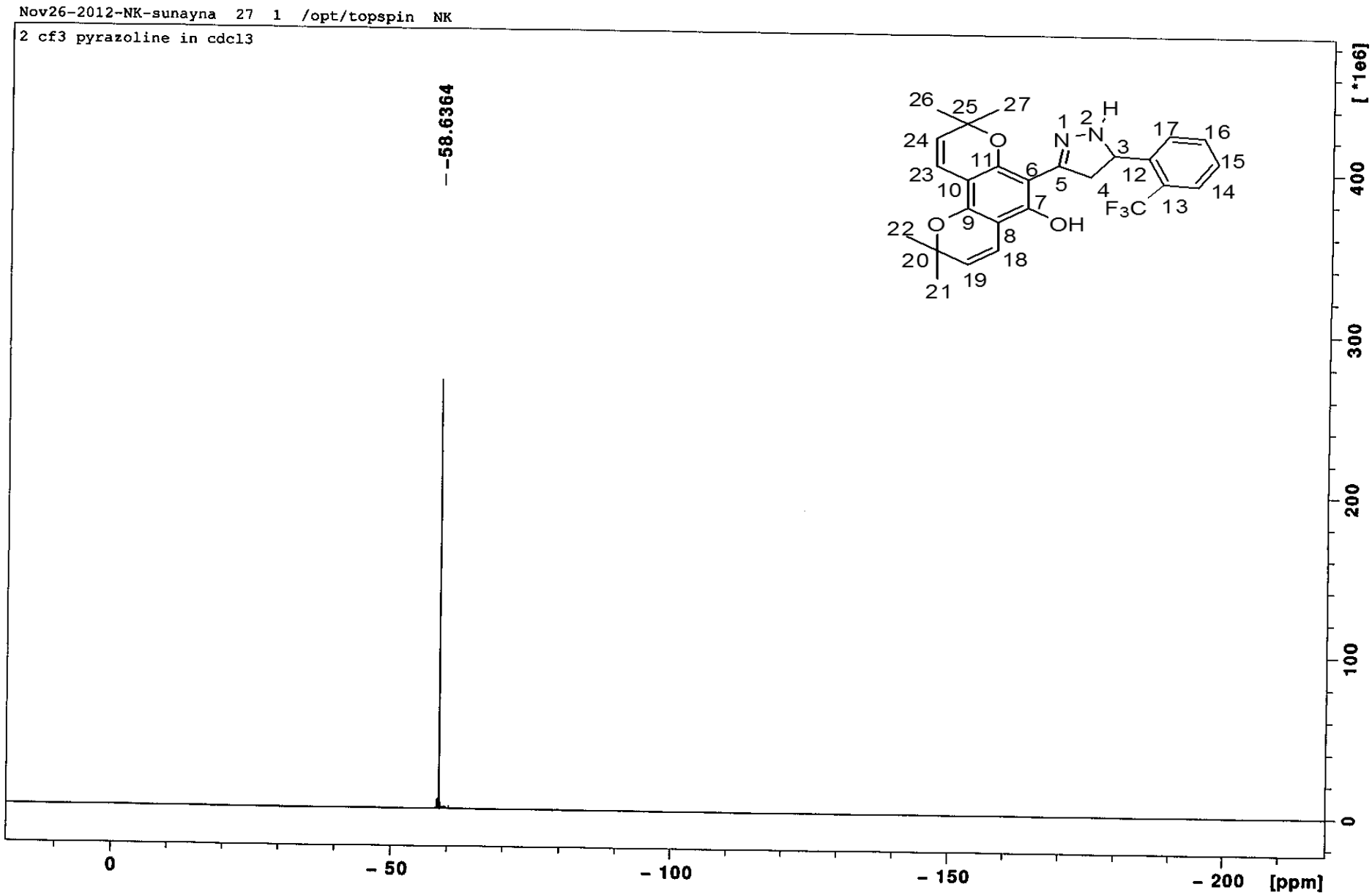
Expanded HMBC spectrum of 3-(2-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4i)



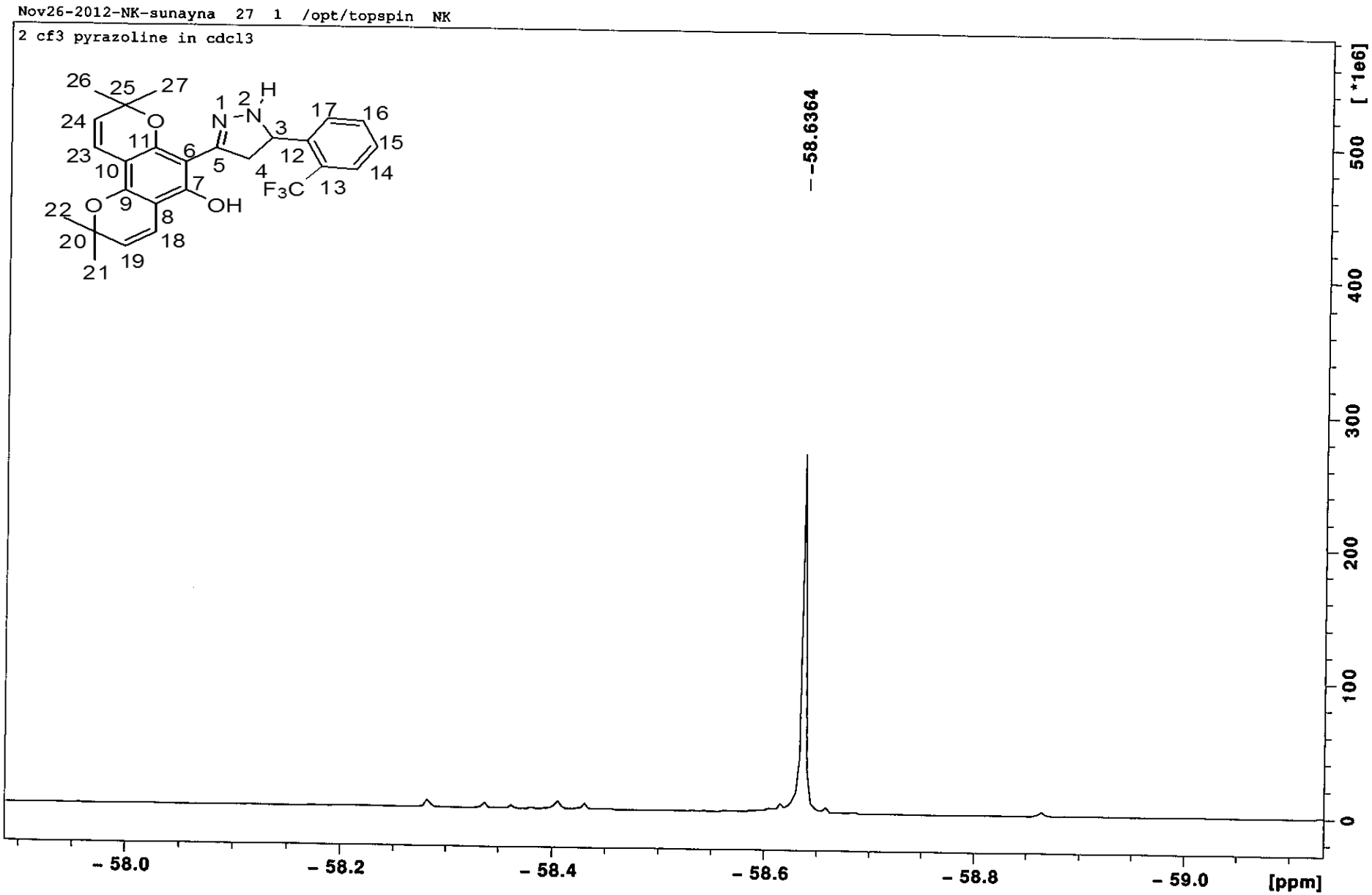
Expanded HMBC spectrum of 3-(2-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4i)



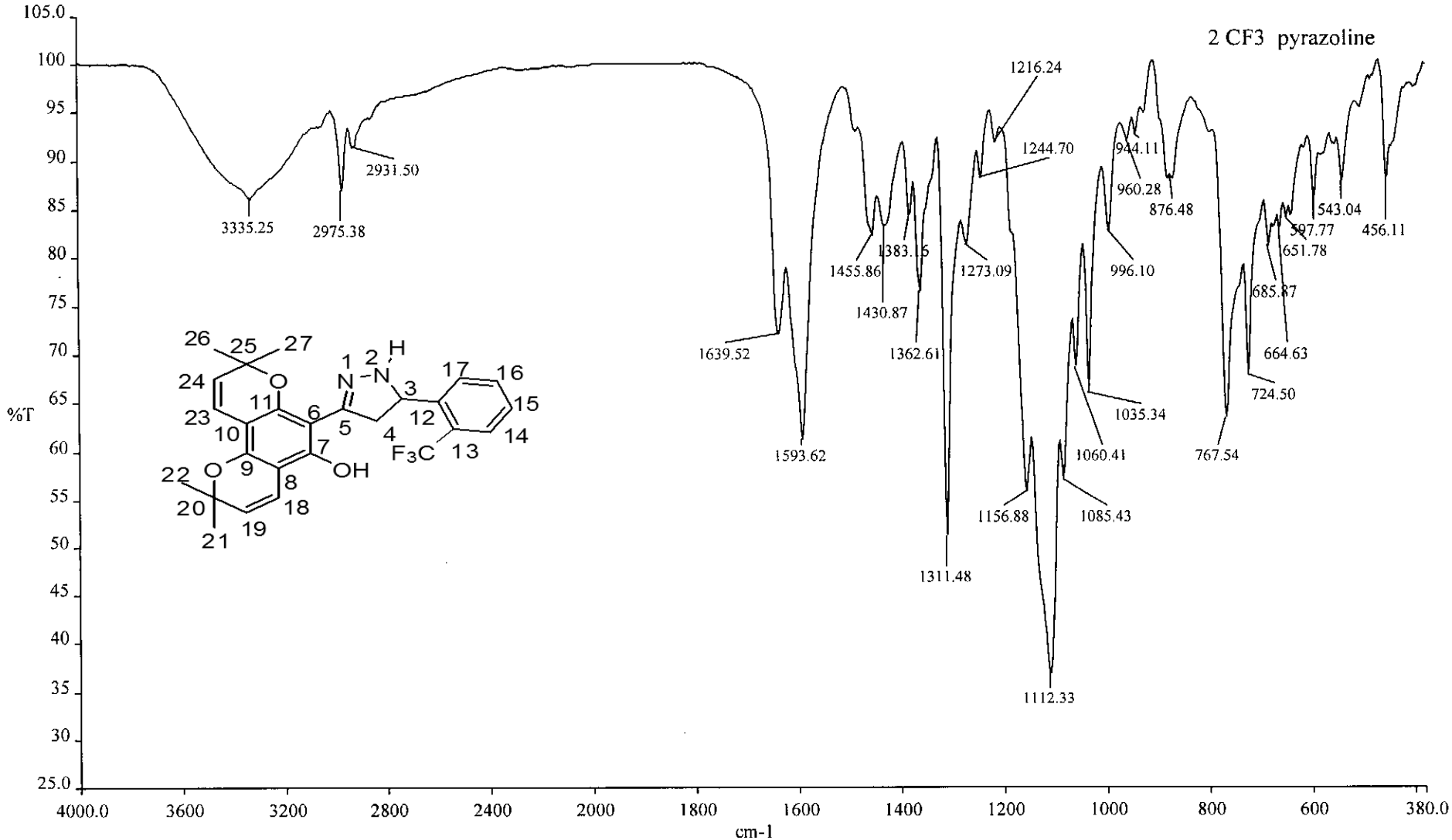
Expanded HMBC spectrum of 3-(2-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4i)



19F NMR spectrum of 3-(2-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4i)



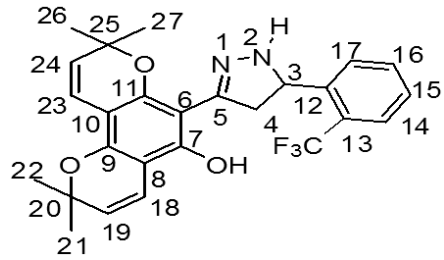
Expanded 19F NMR spectrum of 3-(2-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4i)



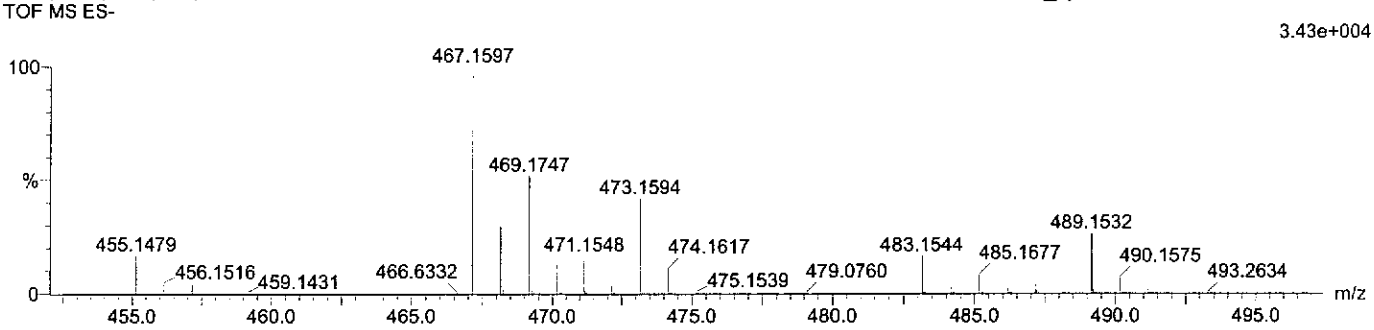
Infrared spectrum of 3-(2-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4i)

Elemental Composition Report **Page 1**

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

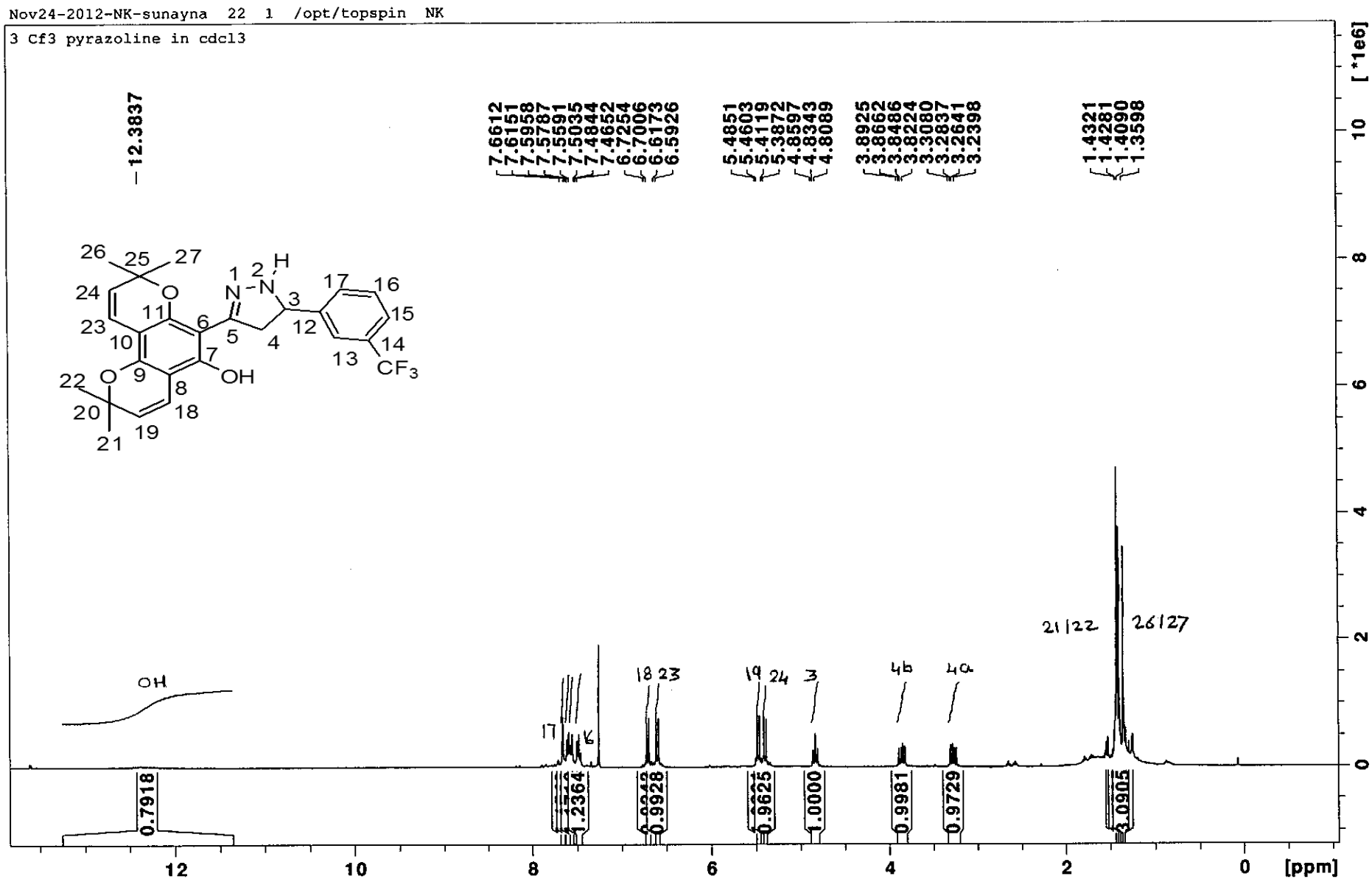


Monoisotopic Mass, Even Electron Ions
25 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-35 H: 20-30 N: 0-4 O: 0-4 F: 3-3
19 2 (0.017) Cm (1:30)
TOF MS ES-

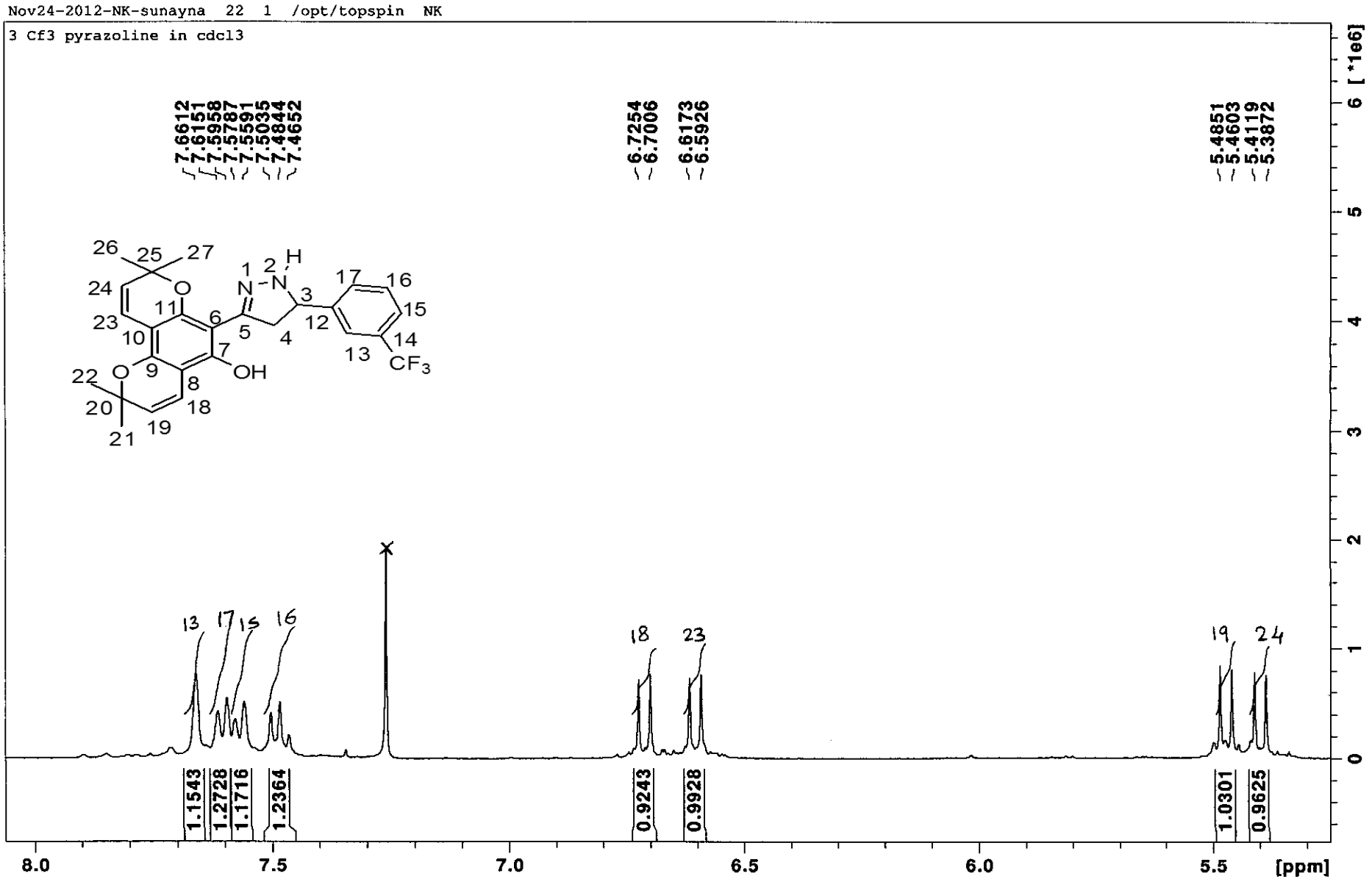


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
469.1747	469.1739	0.8	1.7	14.5	464.1	0.0	C26 H24 N2 O3 F3

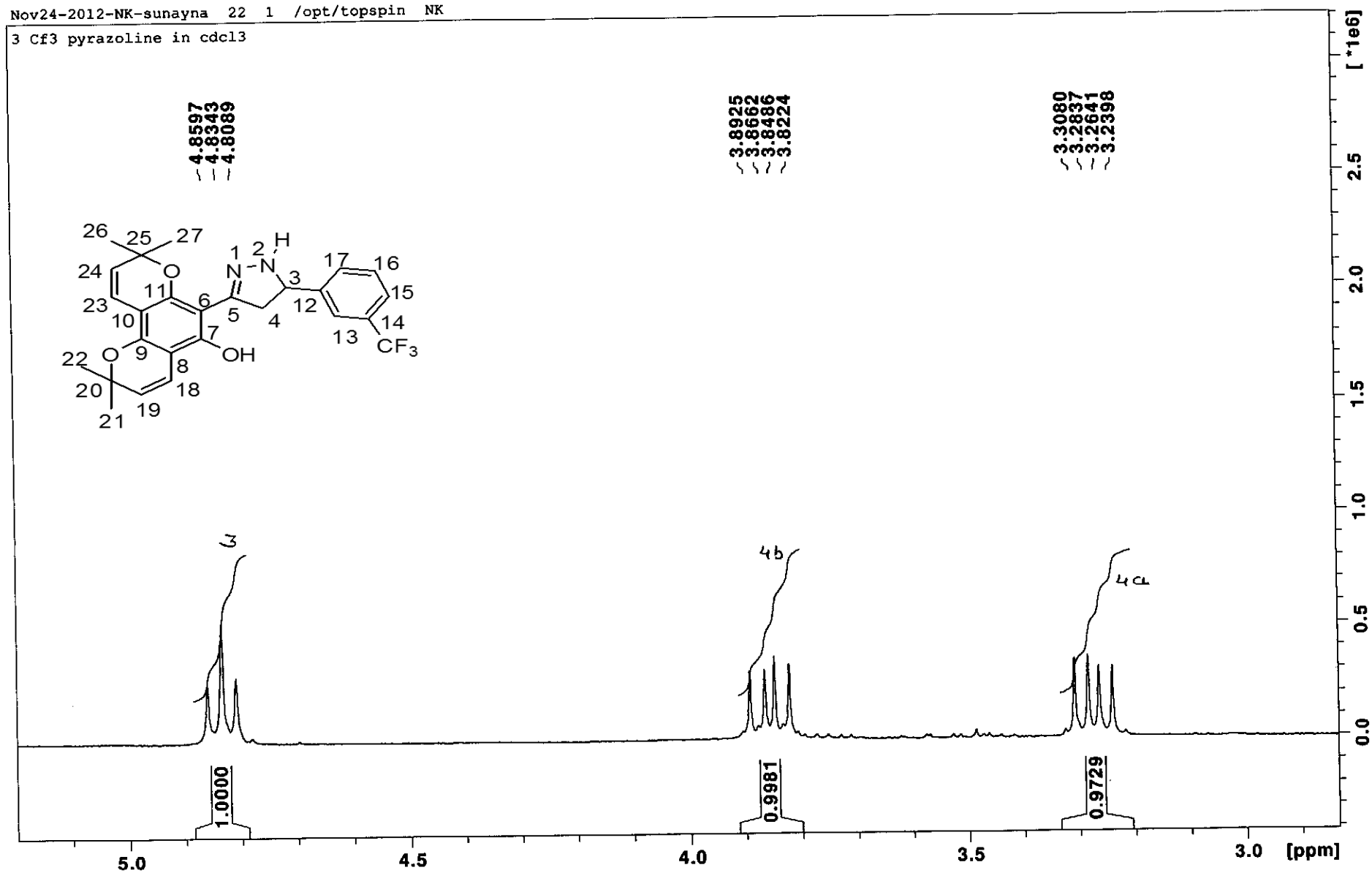
HRMS spectrum of 3-(2-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4i)



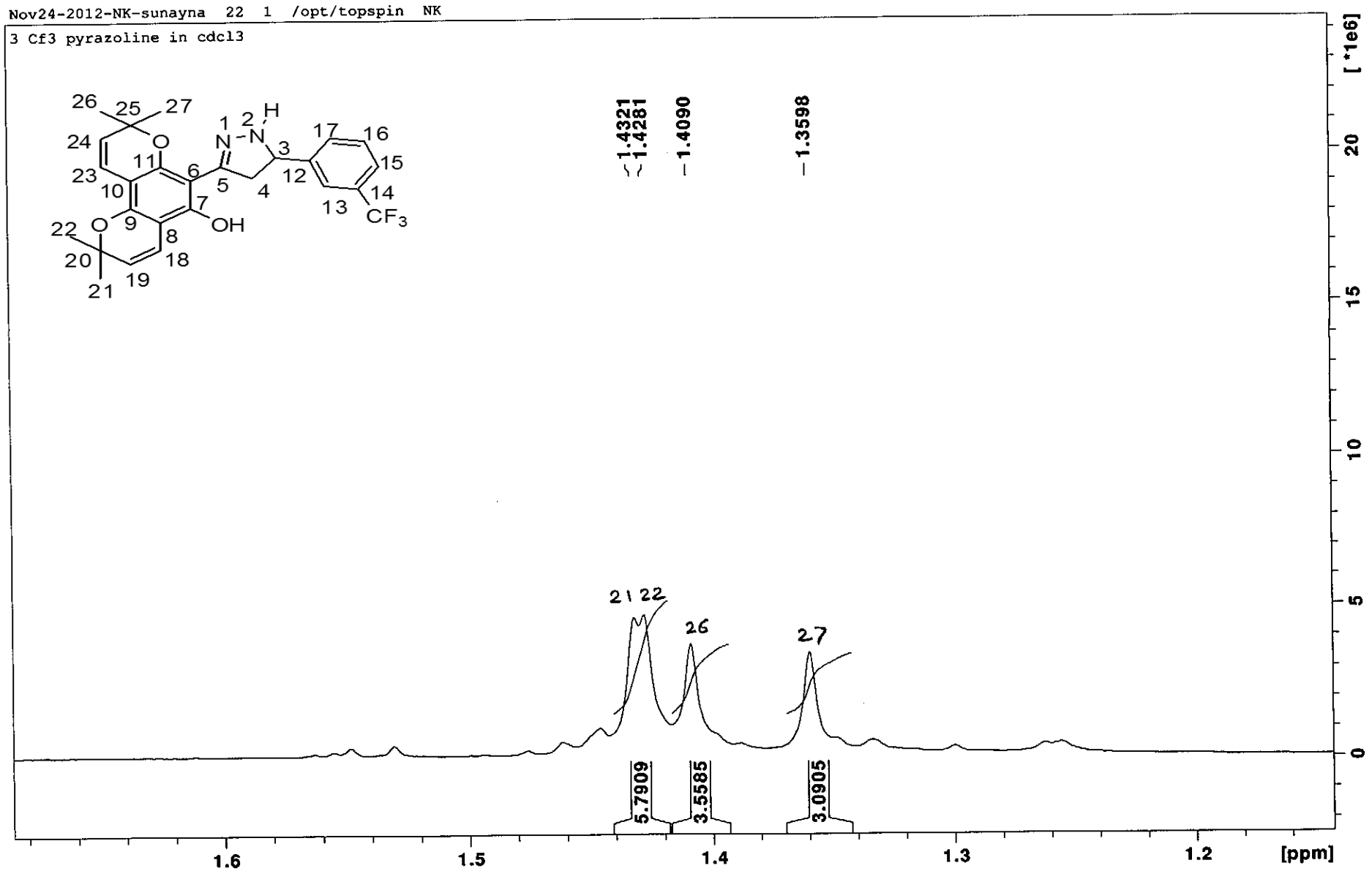
1H NMR spectrum of 3-(3-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4j)



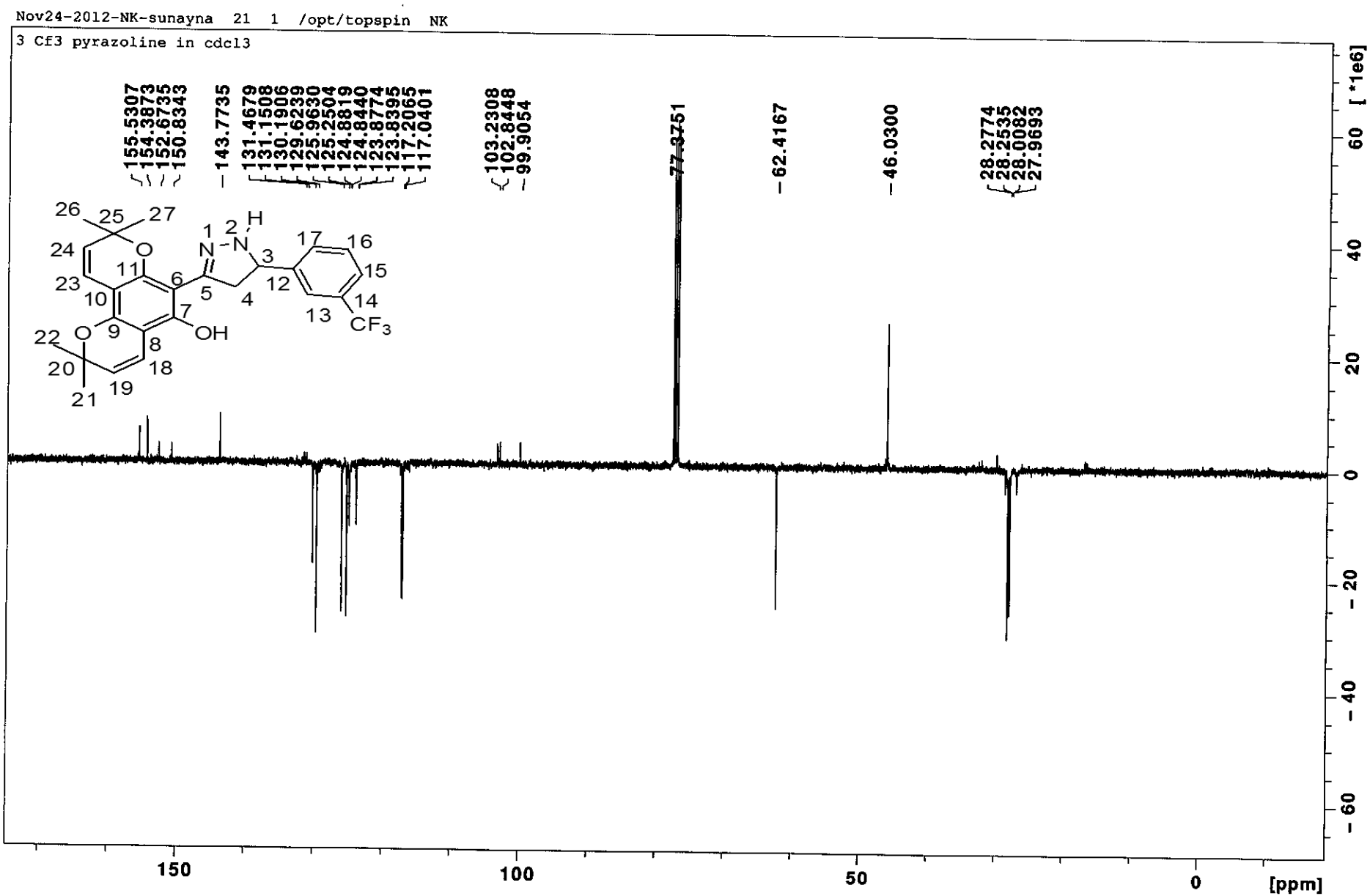
Expanded 1H NMR spectrum of 3-(3-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4j)



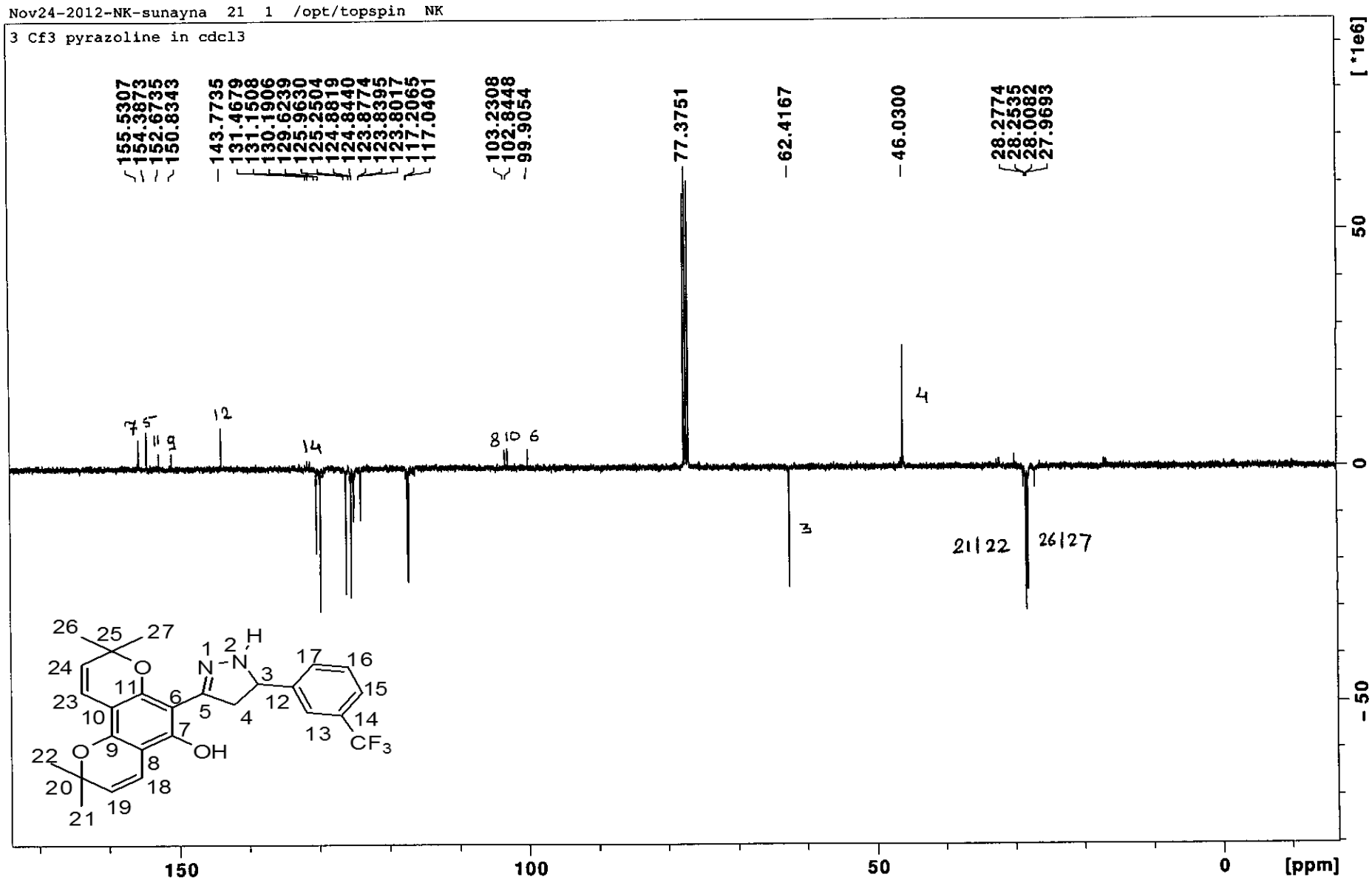
Expanded 1H NMR spectrum of 3-(3-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4j)



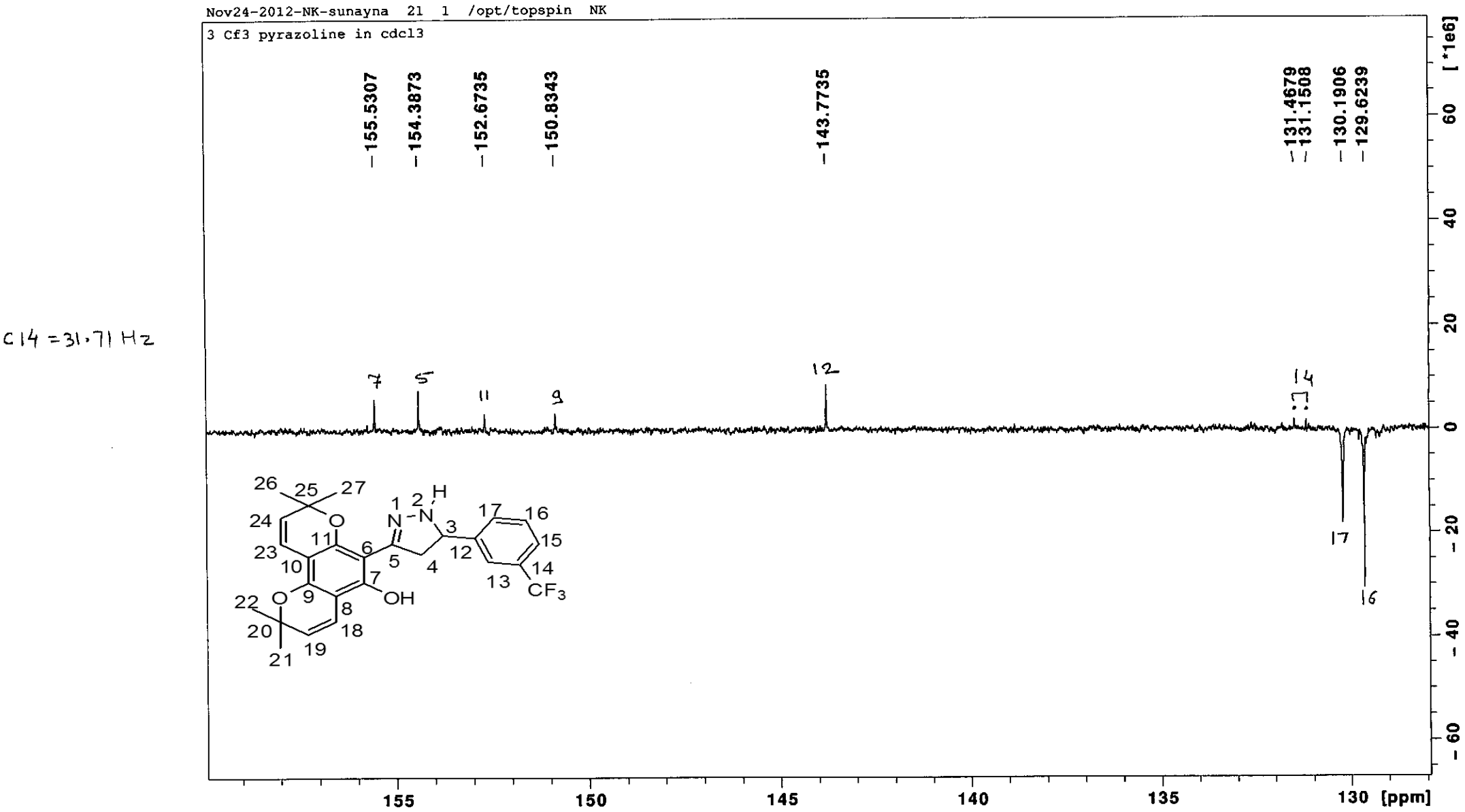
Expanded 1H NMR spectrum of 3-(3-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4j)



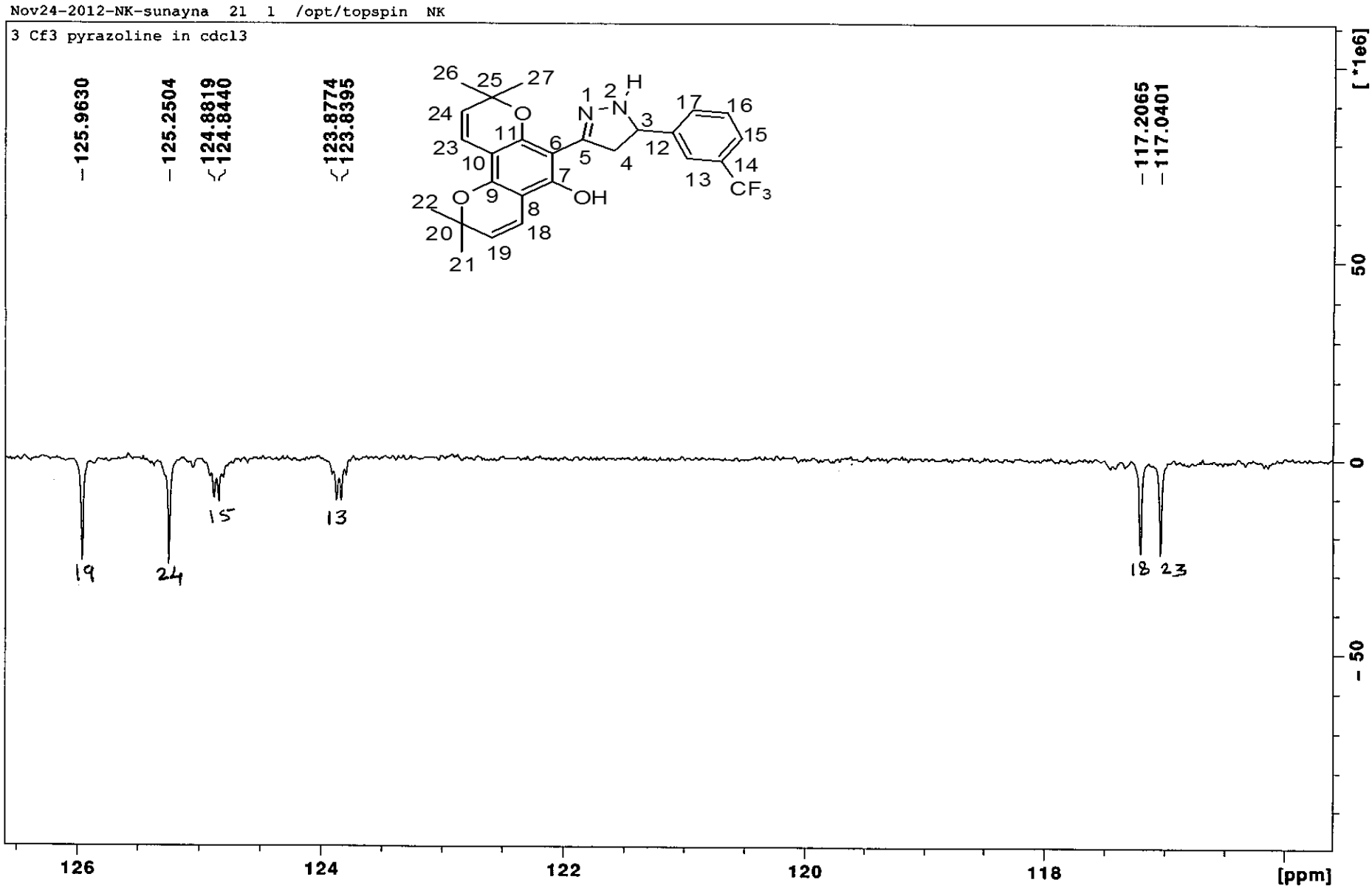
13C NMR spectrum of 3-(3-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4j)



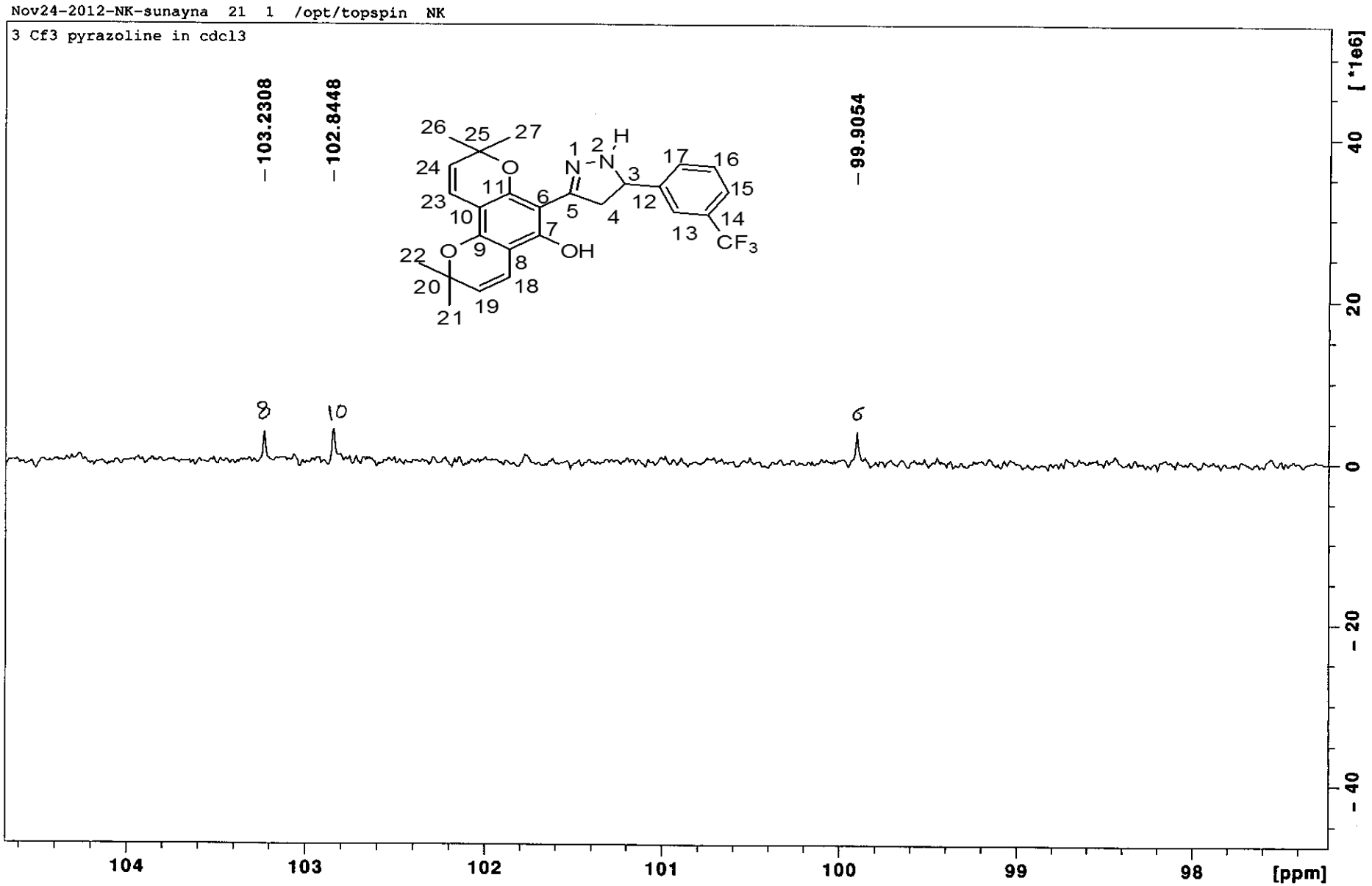
Expanded 13C NMR spectrum of 3-(3-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4j)



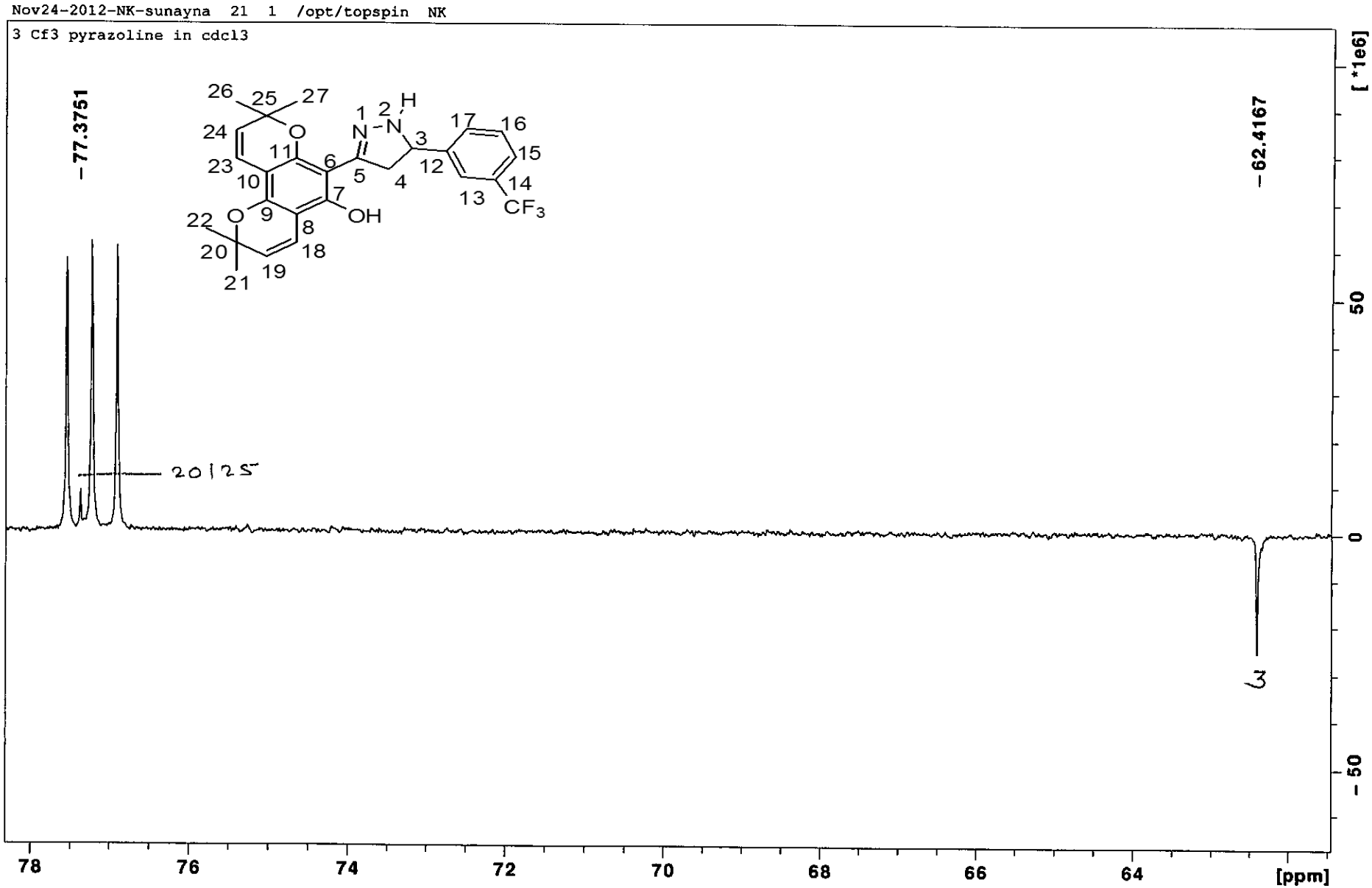
Expanded 13C NMR spectrum of 3-(3-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4j)



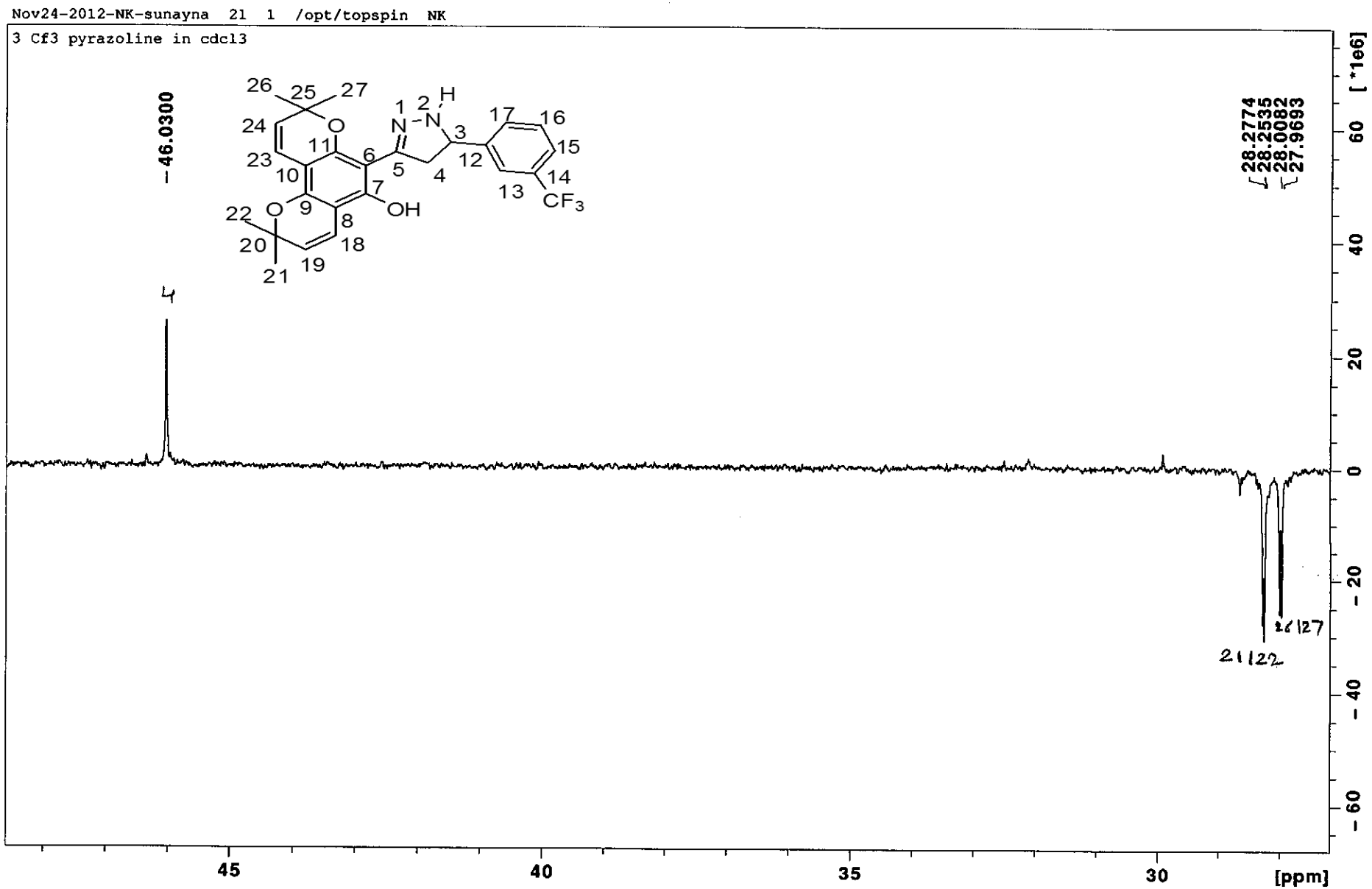
Expanded 13C NMR spectrum of 3-(3-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4j)



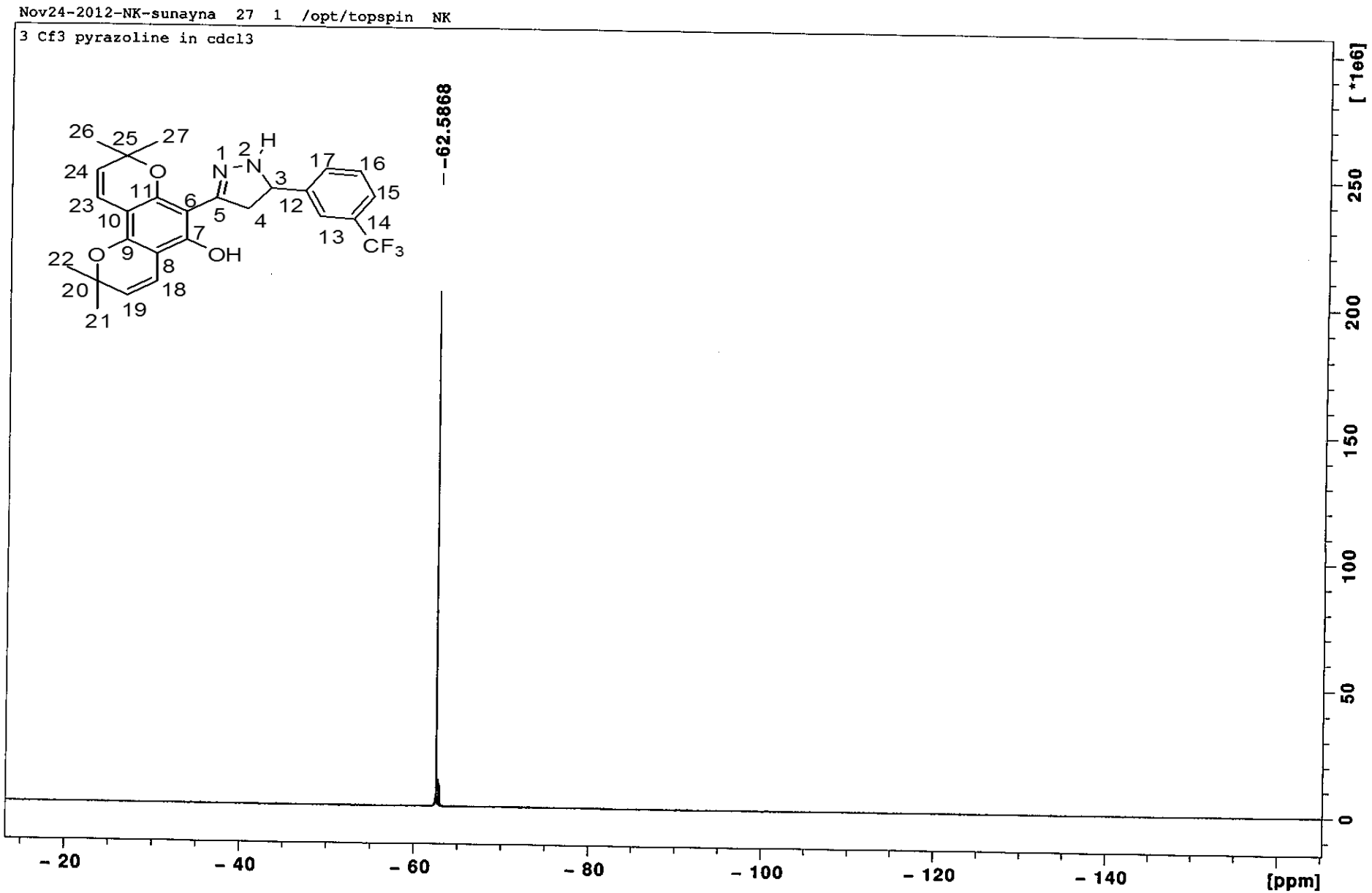
Expanded 13C NMR spectrum of 3-(3-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4j)



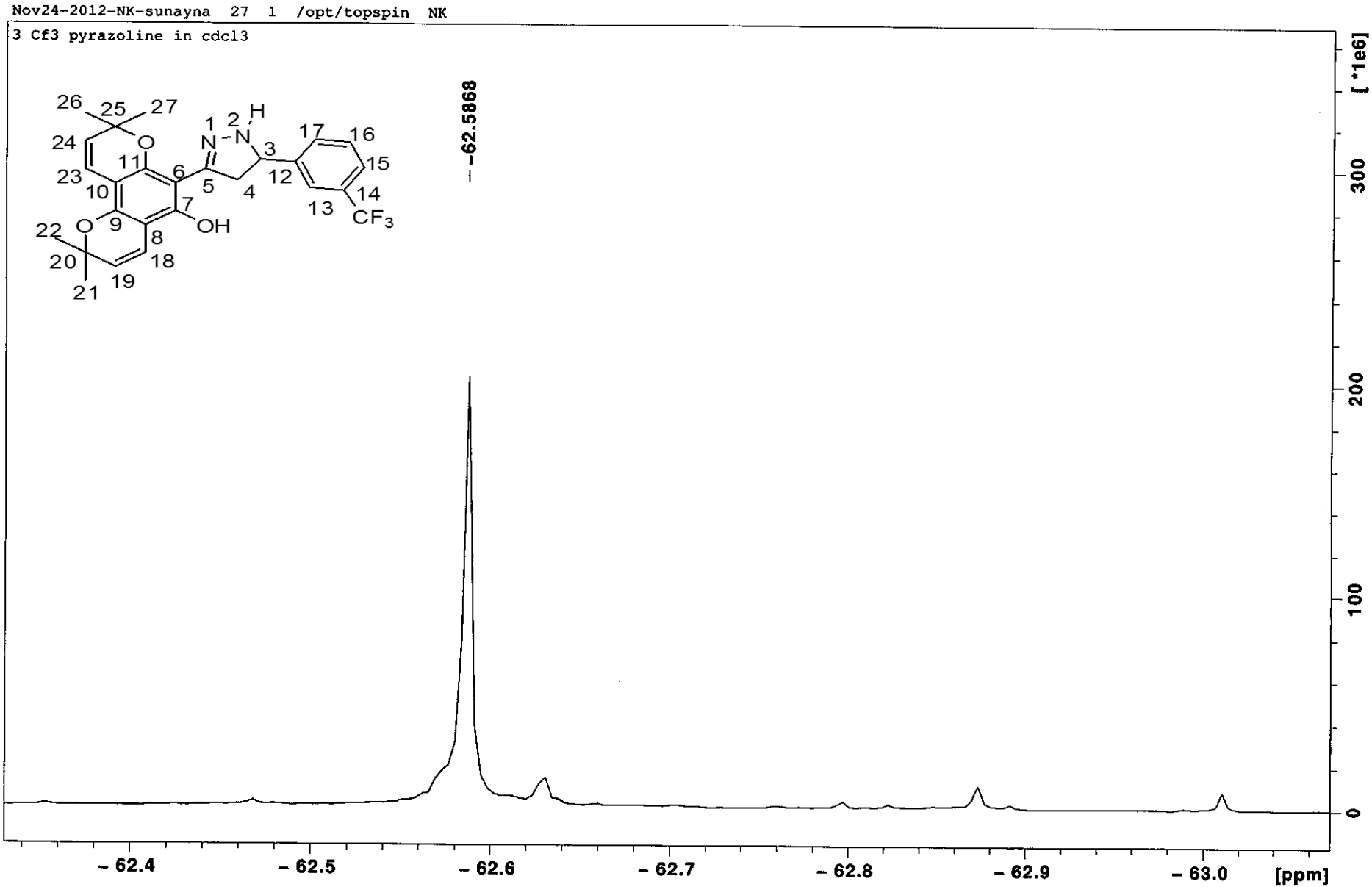
Expanded 13C NMR spectrum of 3-(3-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4j)



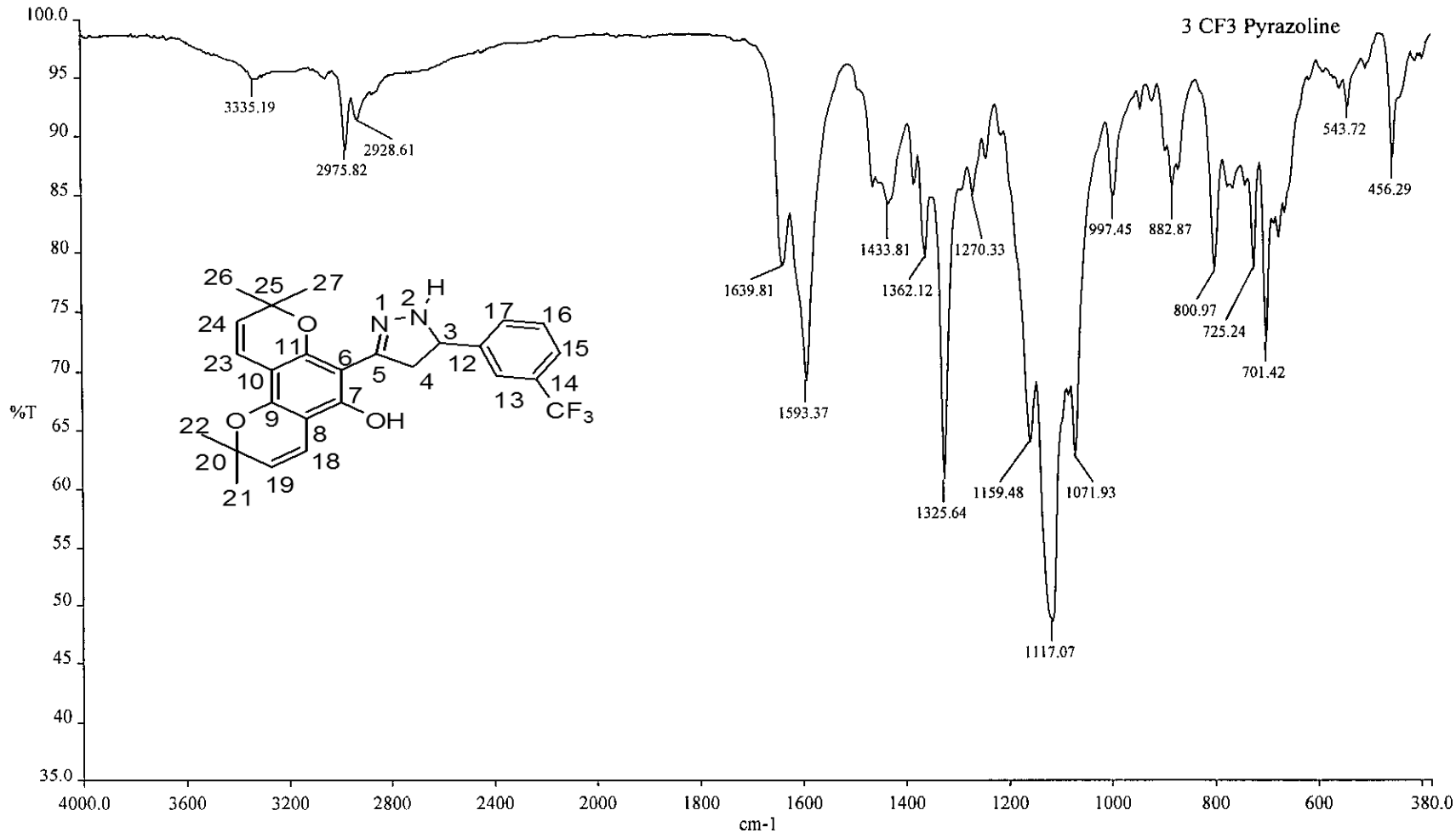
Expanded 13C NMR spectrum of 3-(3-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4j)



19F NMR spectrum of 3-(3-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4j)



Expanded 19F NMR spectrum of 3-(3-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4j)

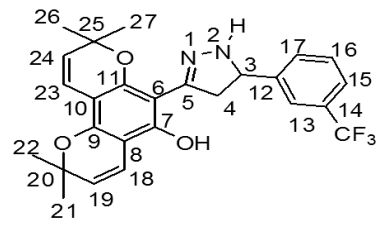


Infrared spectrum of 3-(3-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4j)

Elemental Composition Report

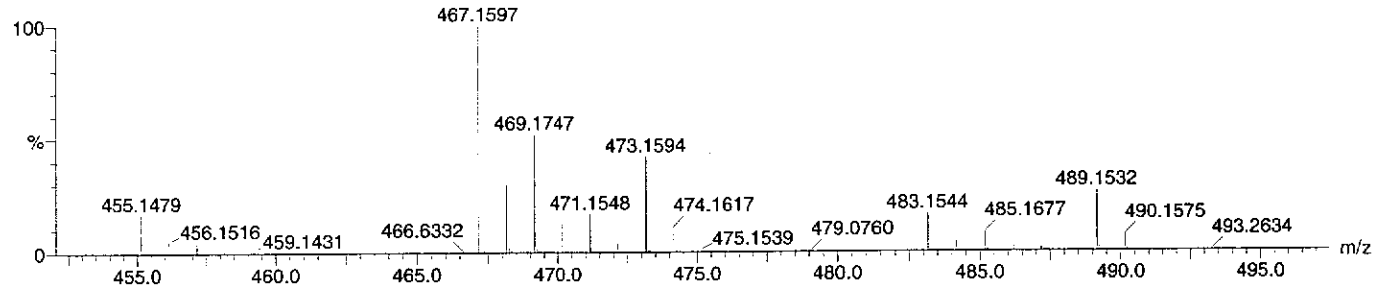
Single Mass Analysis

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



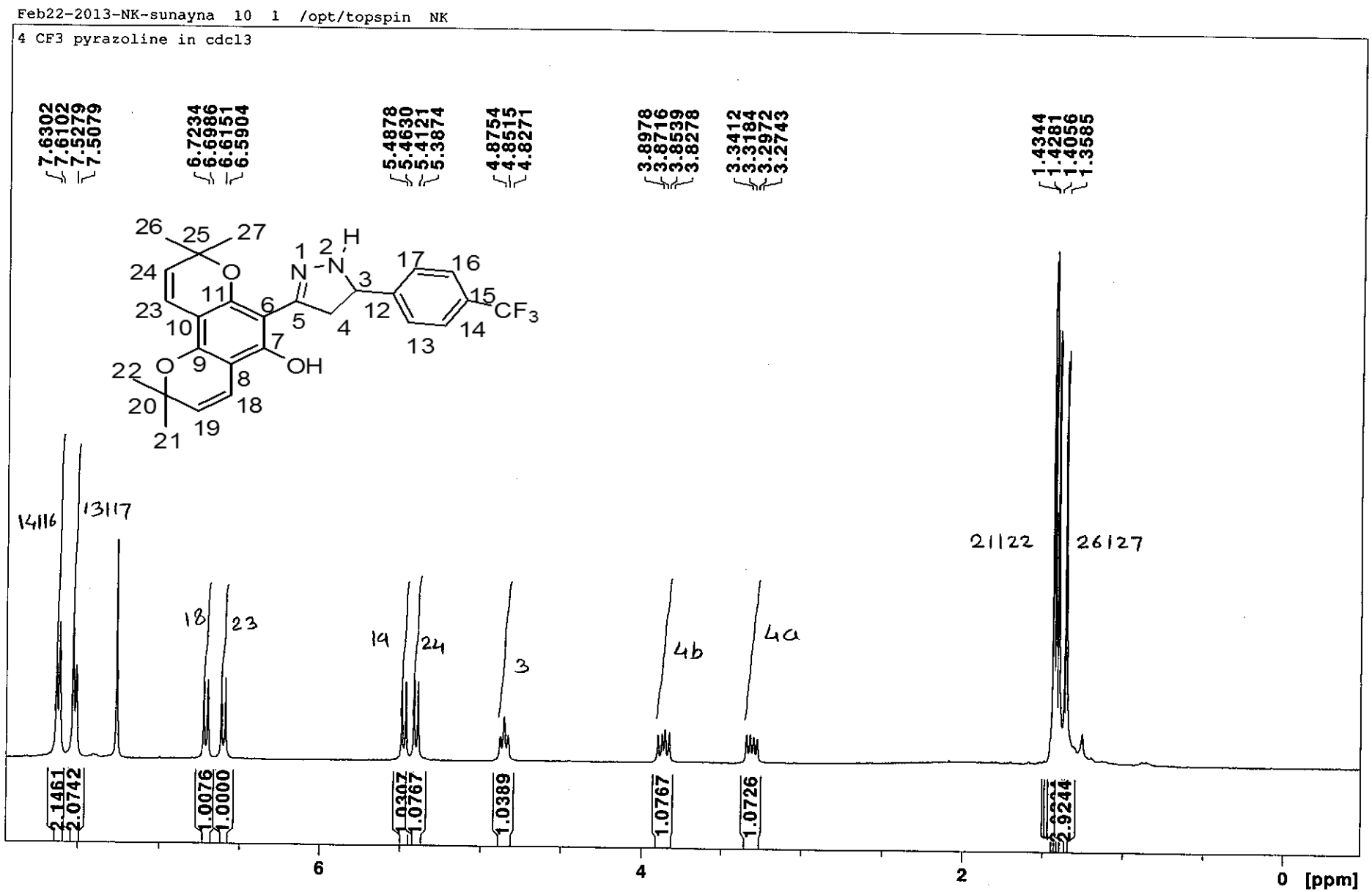
Monoisotopic Mass, Even Electron Ions
25 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-35 H: 20-30 N: 0-4 O: 0-4 F: 3-3
19 2 (0.017) Cm (1:30)
TOF MS ES-

3.43e+004

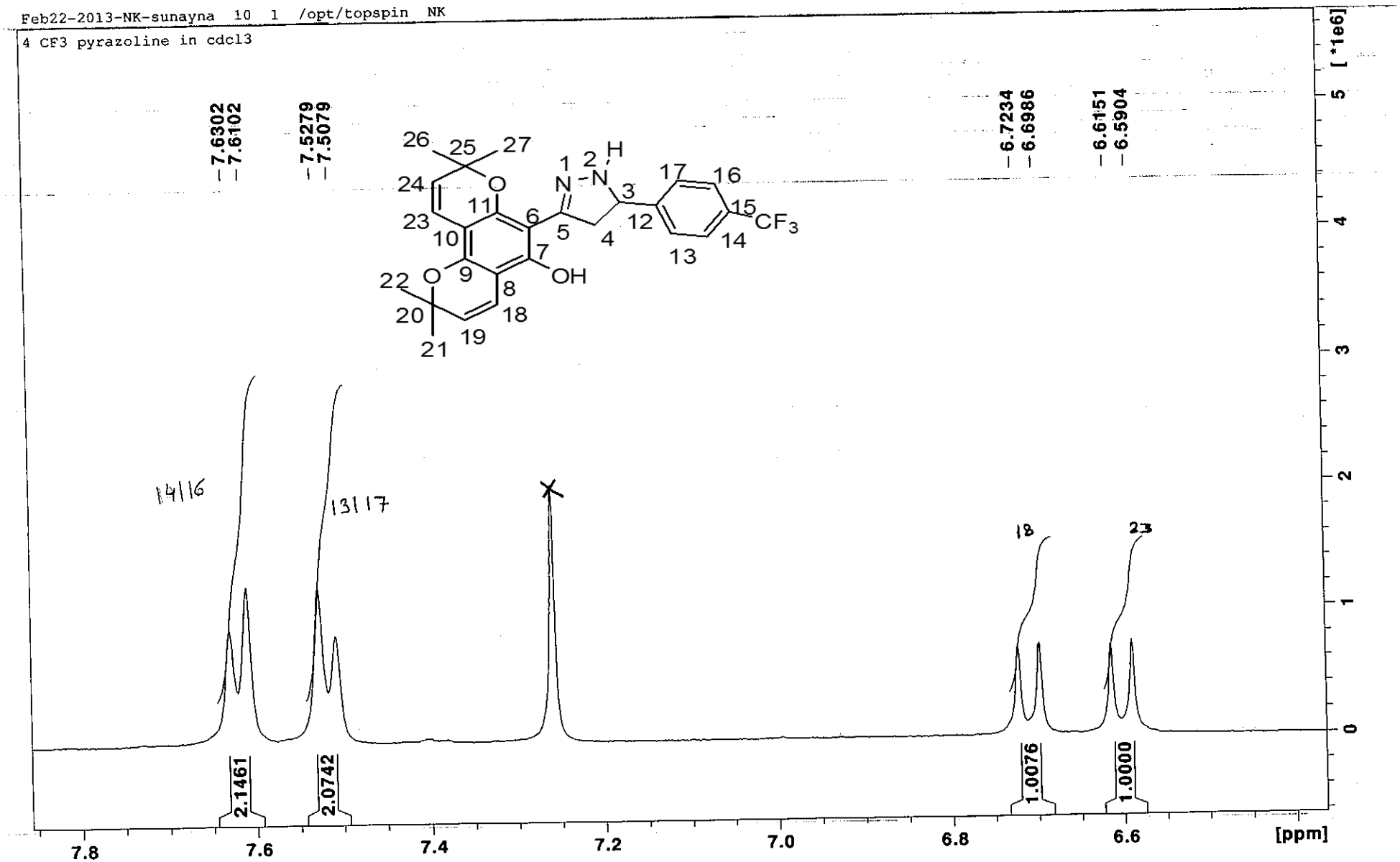


Minimum:				-1.5						
Maximum:		5.0	5.0	100.0						
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula			
469.1747	469.1739	0.8	1.7	14.5	464.1	0.0	C26	H24	N2	O3 F3

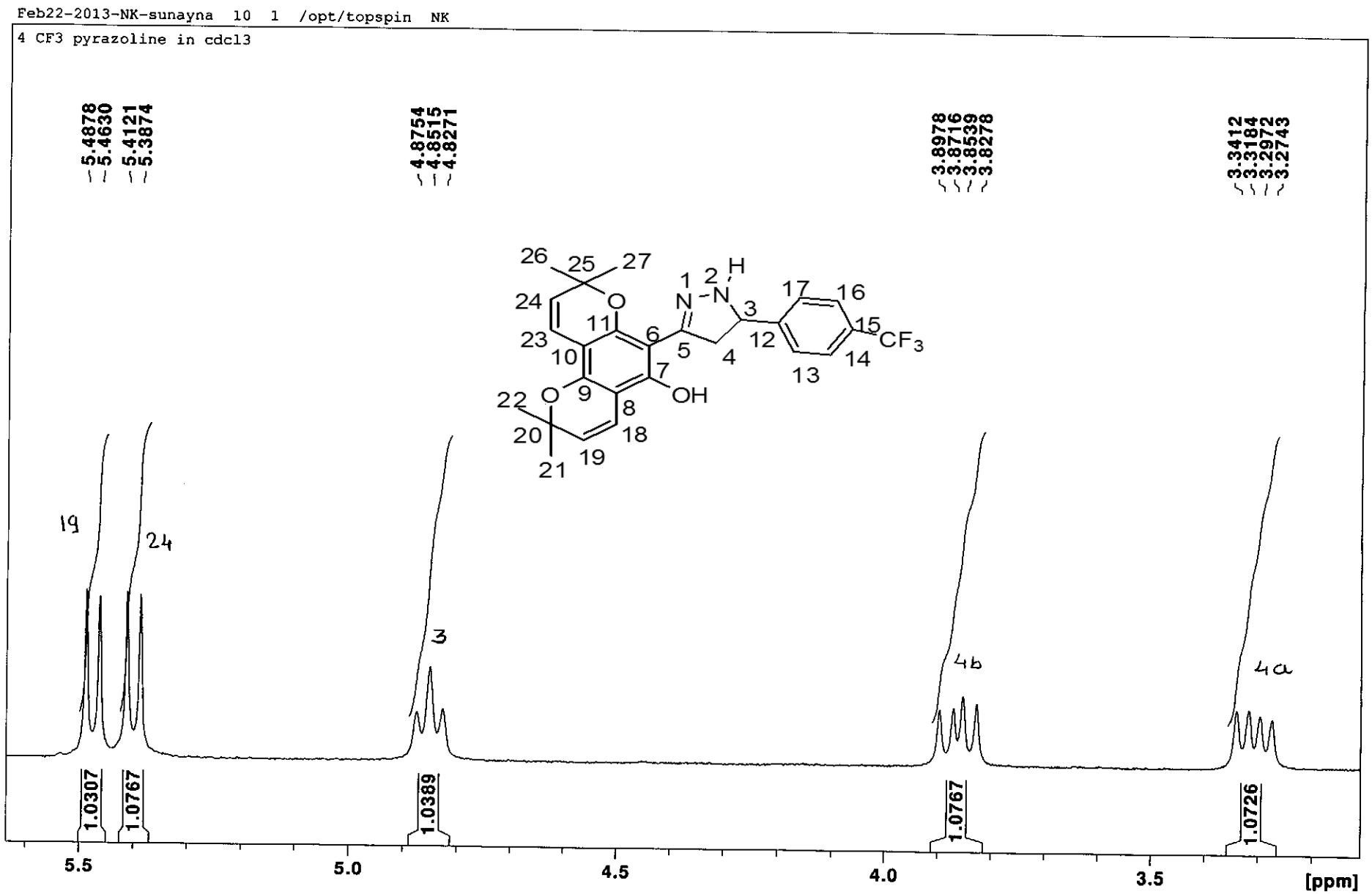
HRMS spectrum of 3-(3-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4j)



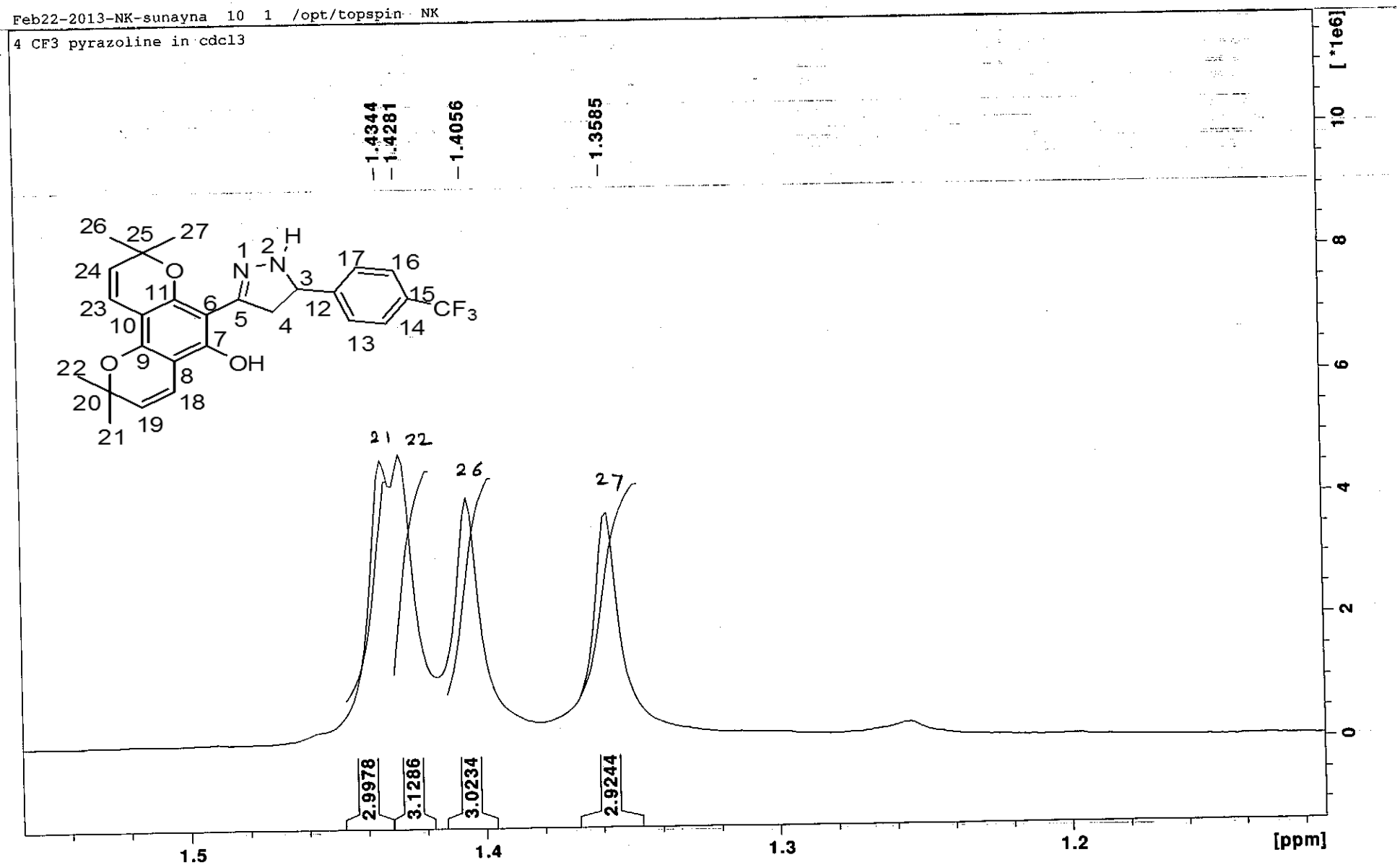
1H NMR spectrum of 3-(4-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4k)



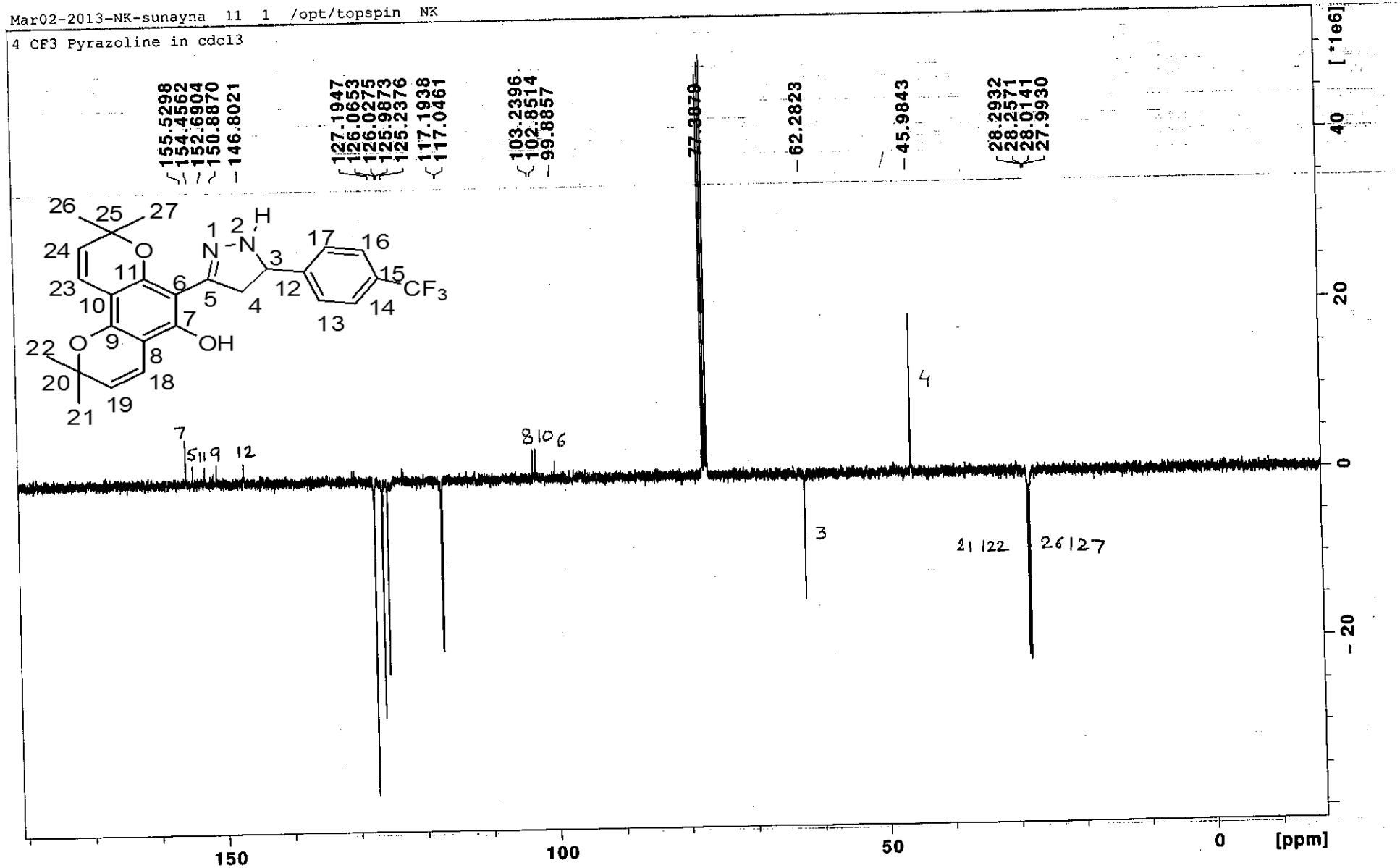
Expanded 1H NMR spectrum of 3-(4-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4k)



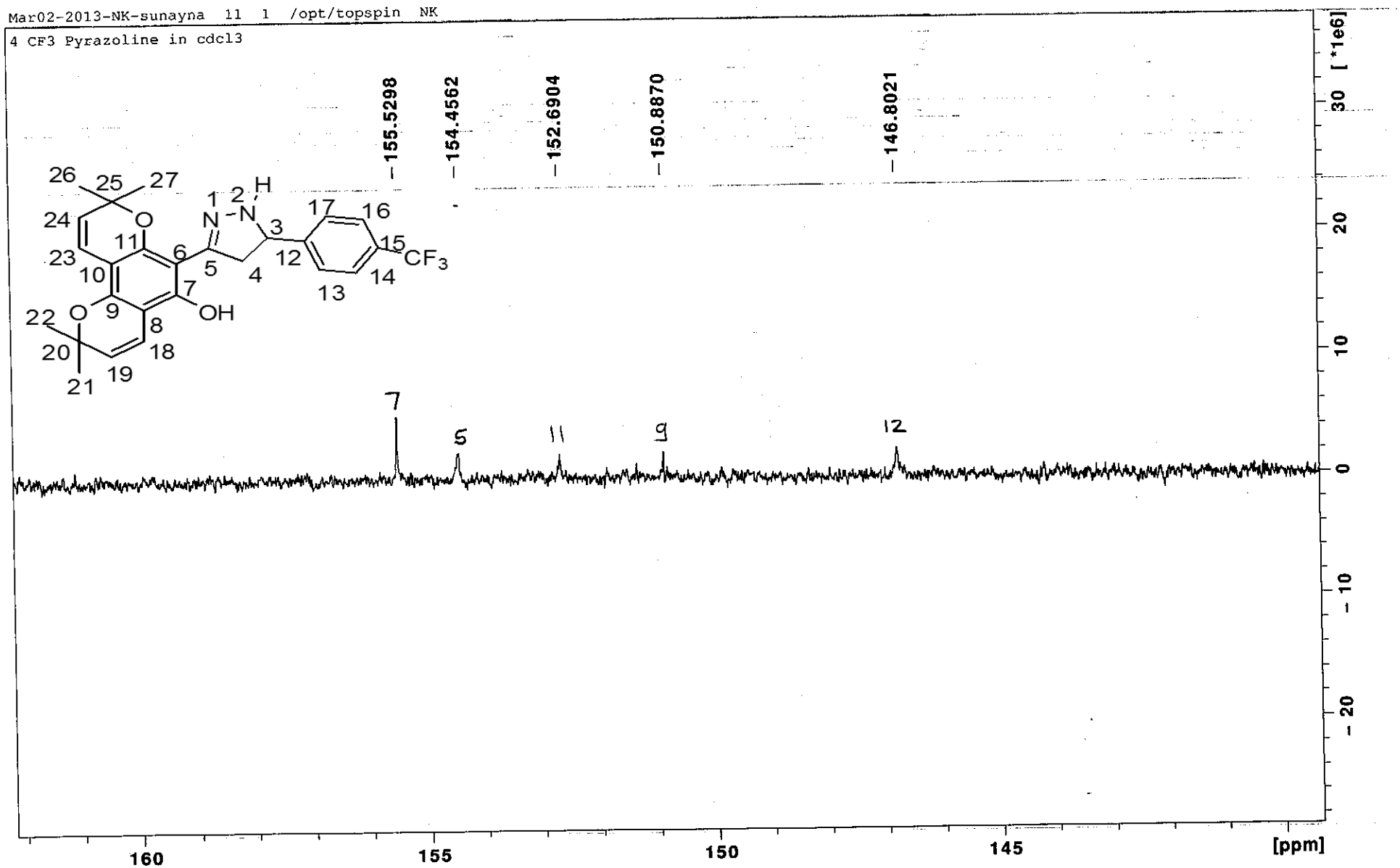
Expanded 1H NMR spectrum of 3-(4-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4k)



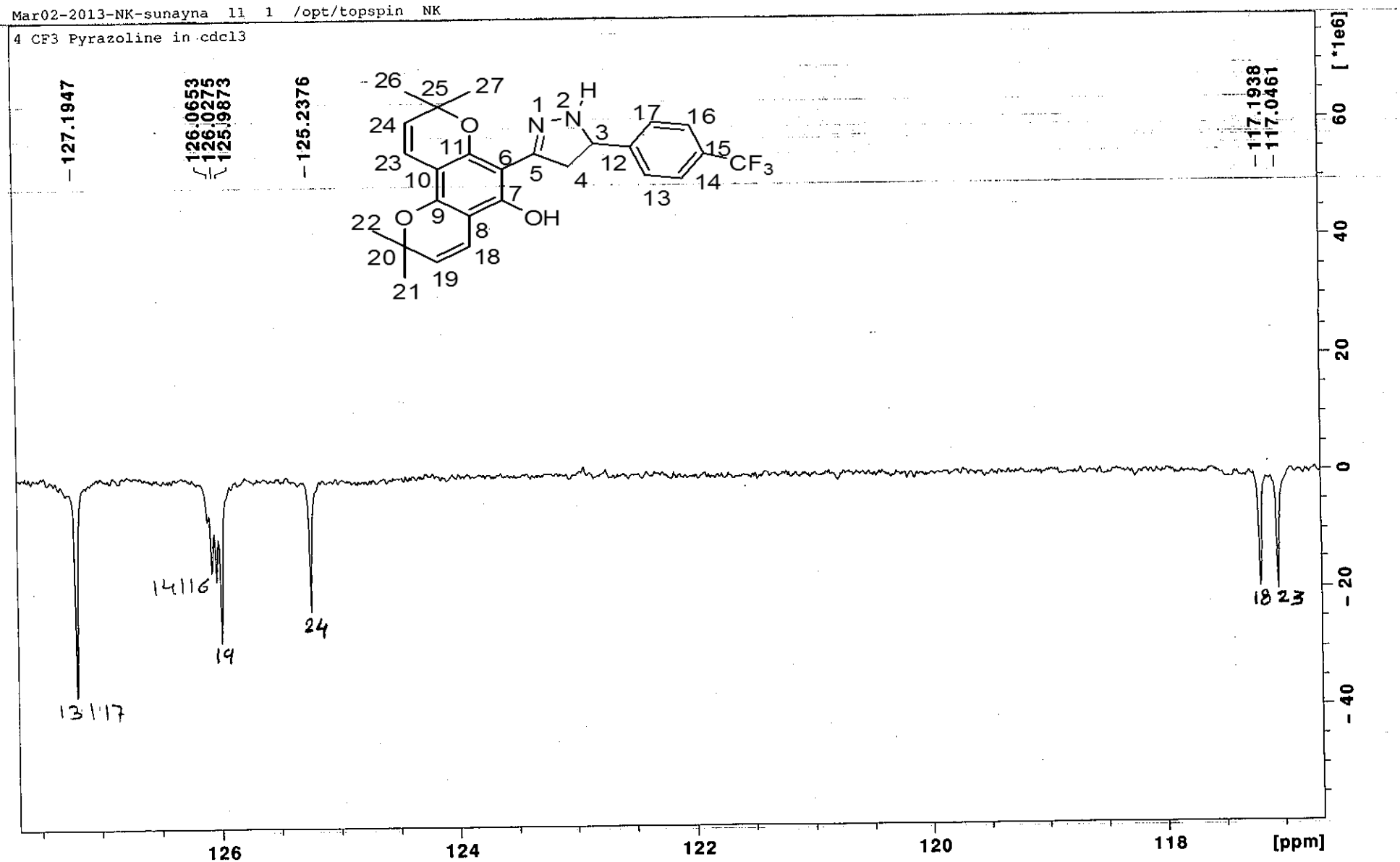
Expanded 1H NMR spectrum of 3-(4-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4k)



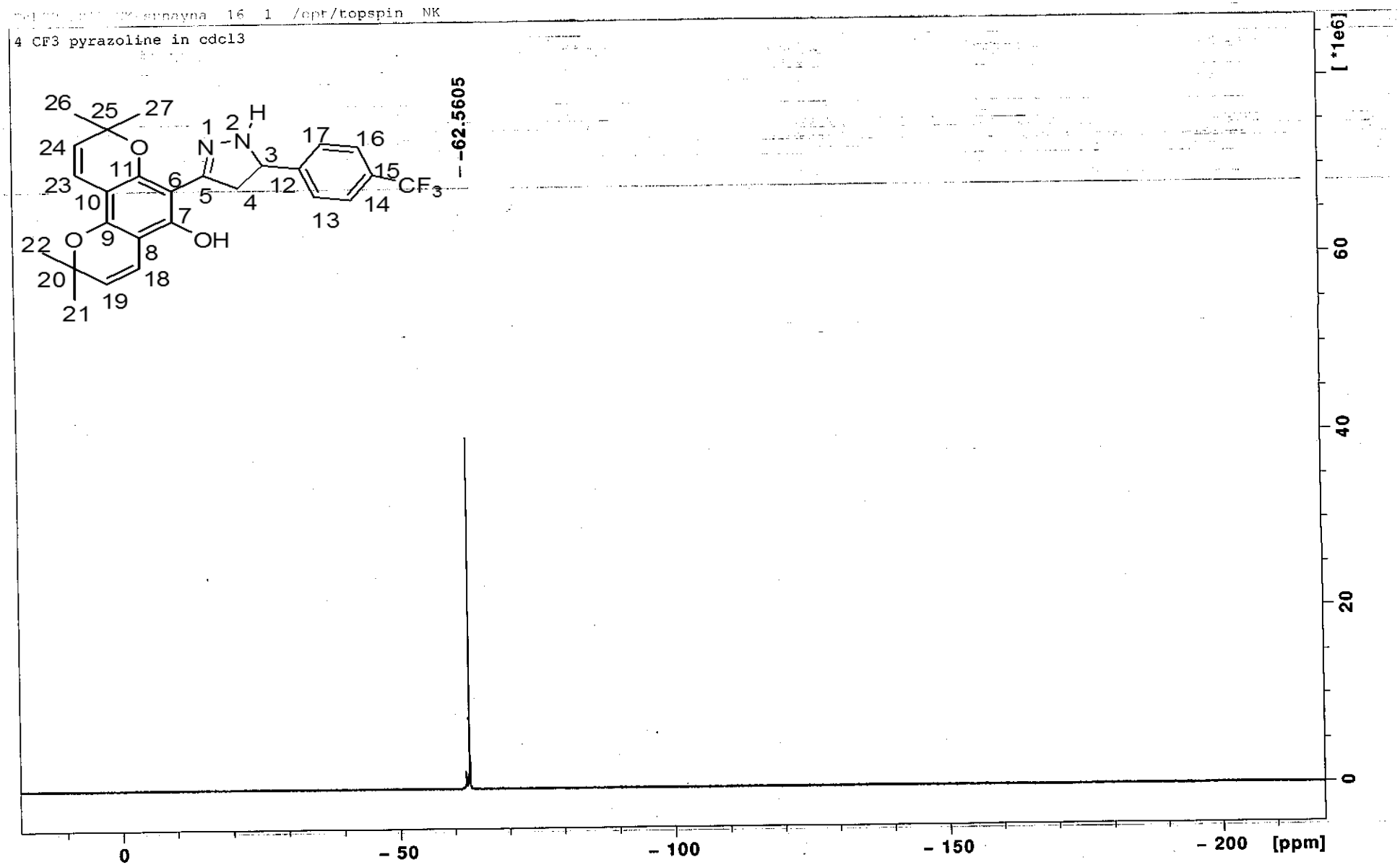
13C NMR spectrum of 3-(4-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4k)



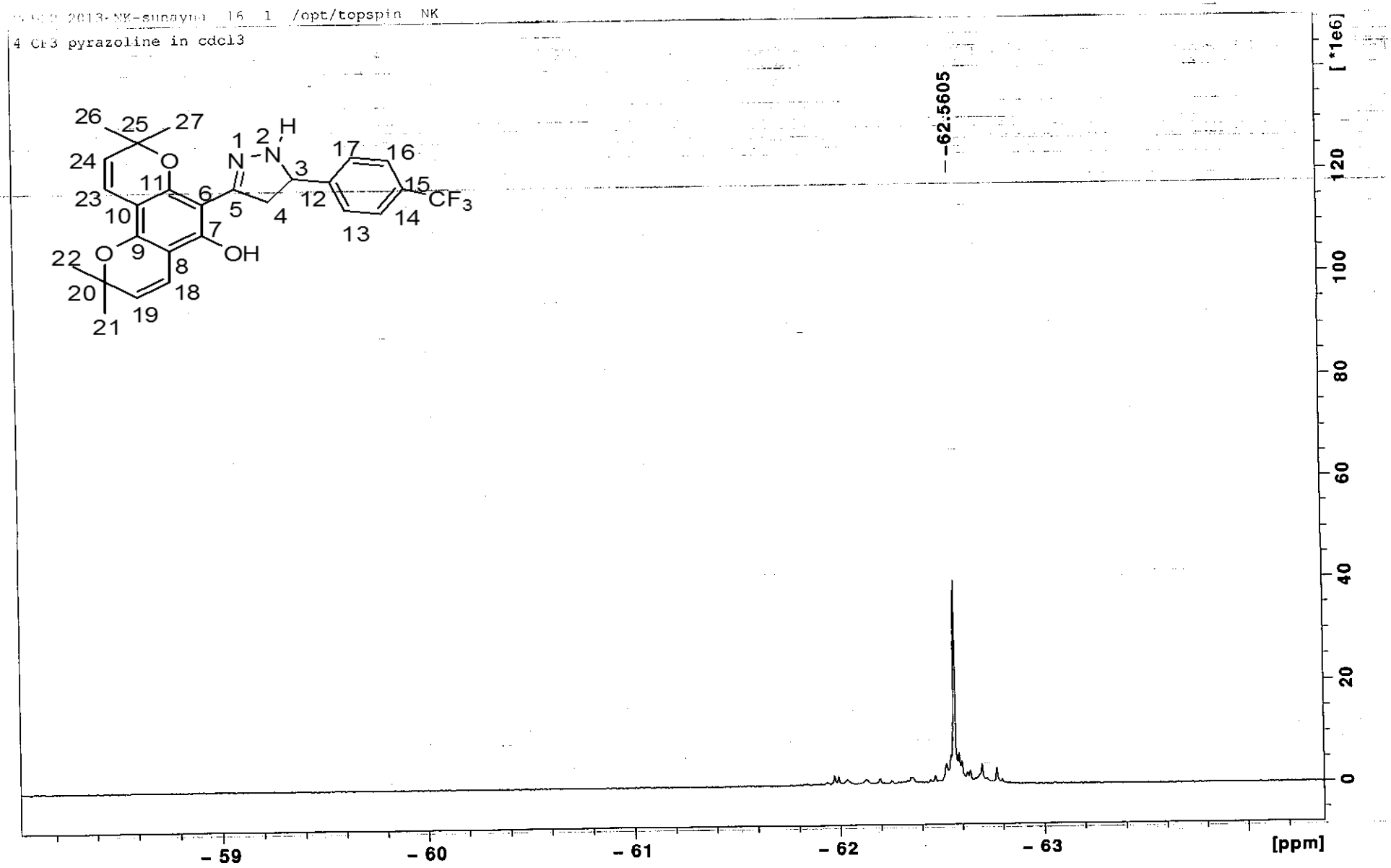
Expanded 13C NMR spectrum of 3-(4-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4k)



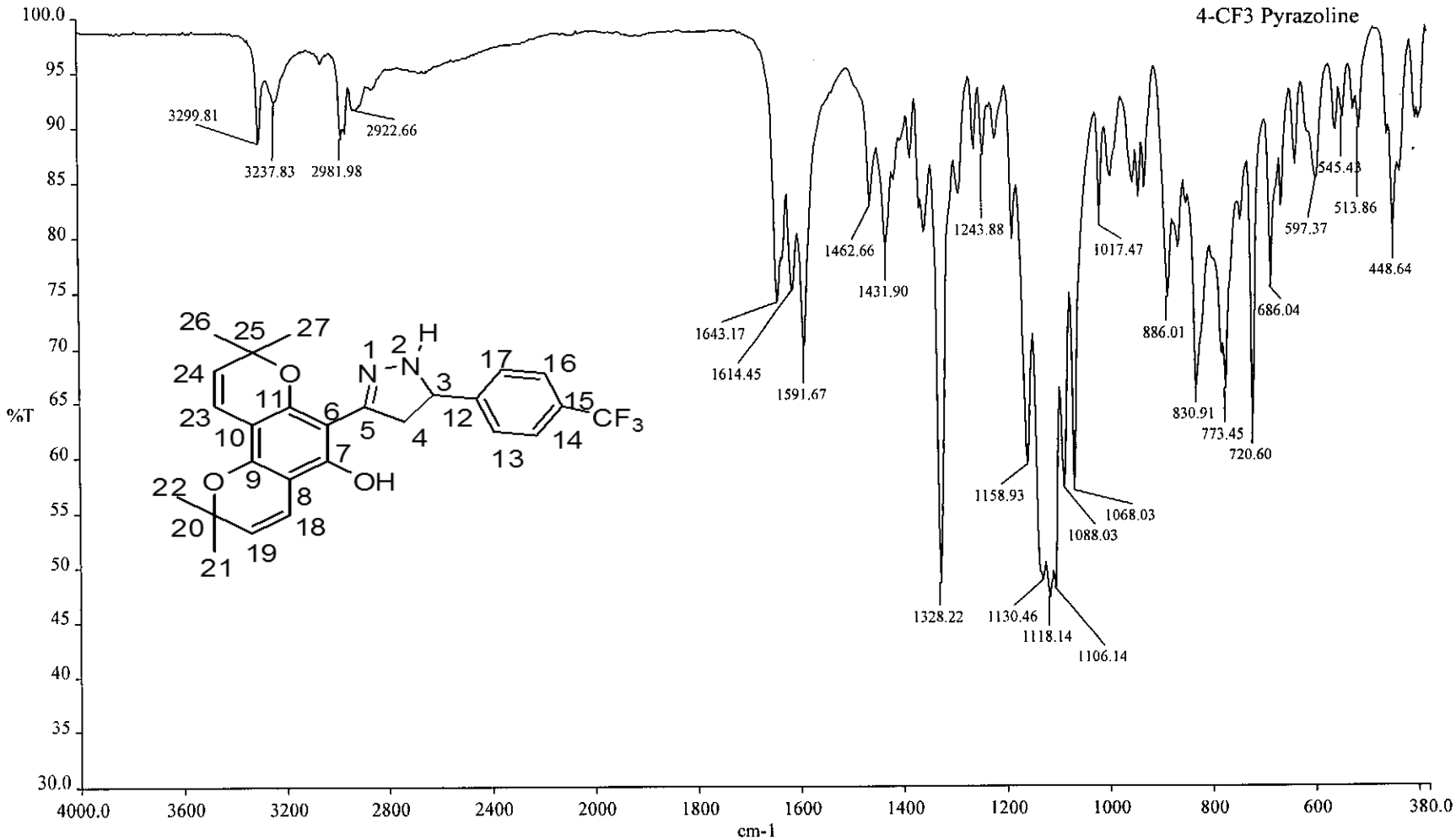
Expanded 13C NMR spectrum of 3-(4-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4k)



19F NMR spectrum of 3-(4-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4k)



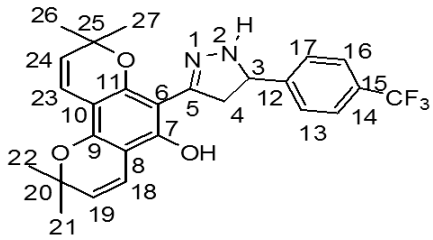
Expanded 19F NMR spectrum of 3-(4-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4k)



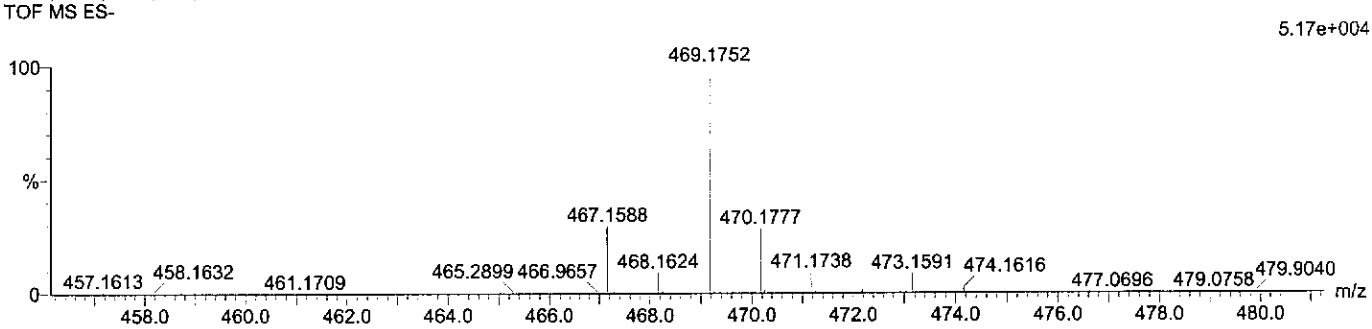
Infrared spectrum of 3-(4-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4k)

Elemental Composition Report **Page 1**

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

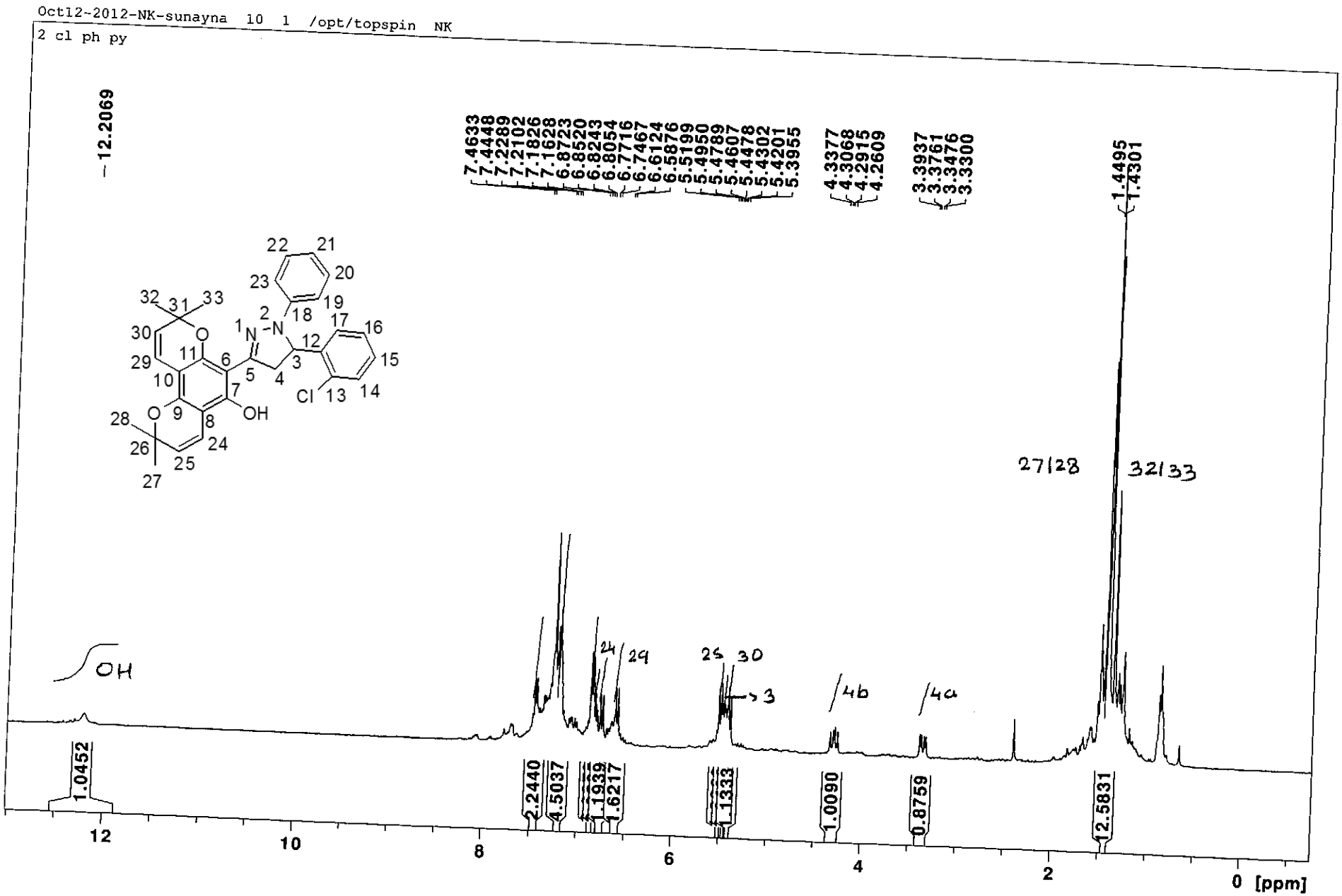


Monoisotopic Mass, Even Electron Ions
25 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-35 H: 20-30 N: 0-4 O: 0-4 F: 3-3
21 2 (0.017) Cm (1:31)
TOF MS ES-

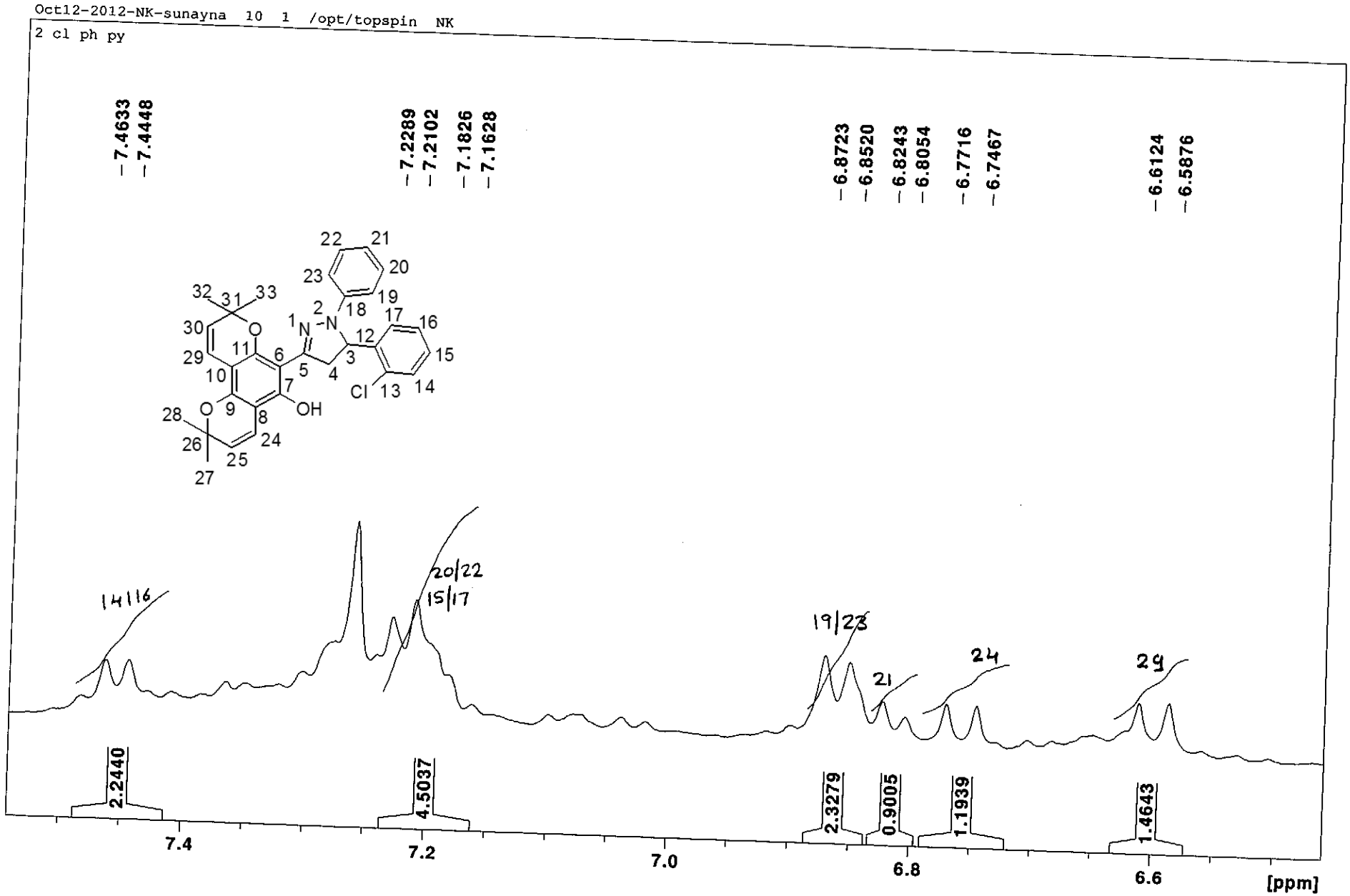


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
469.1752	469.1739	1.3	2.8	14.5	472.3	0.0	C26 H24 N2 O3 F3

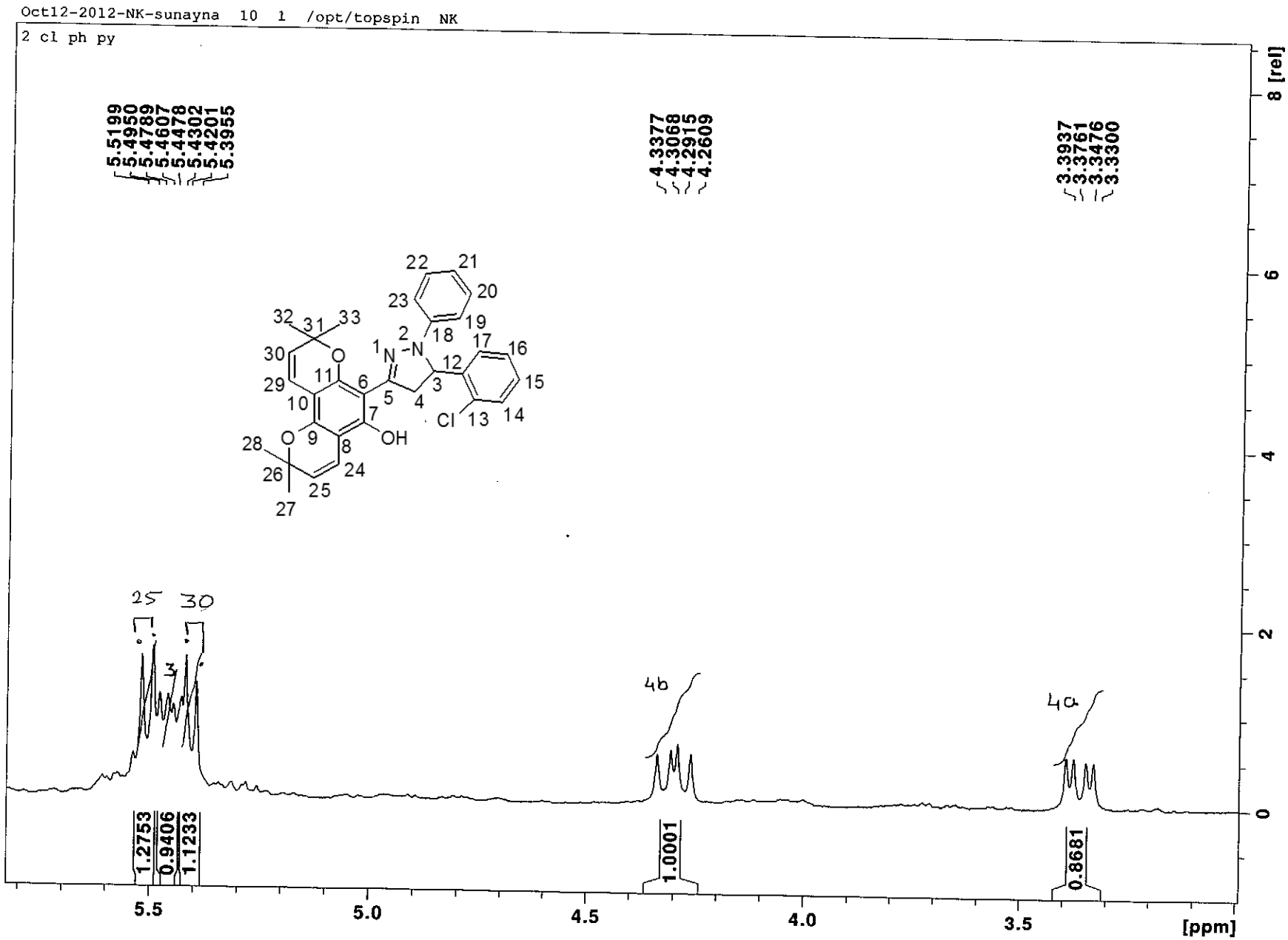
HRMS spectrum of 3-(4-trifluoromethylphenyl)-5-pyranochromene pyrazoline (C-4k)



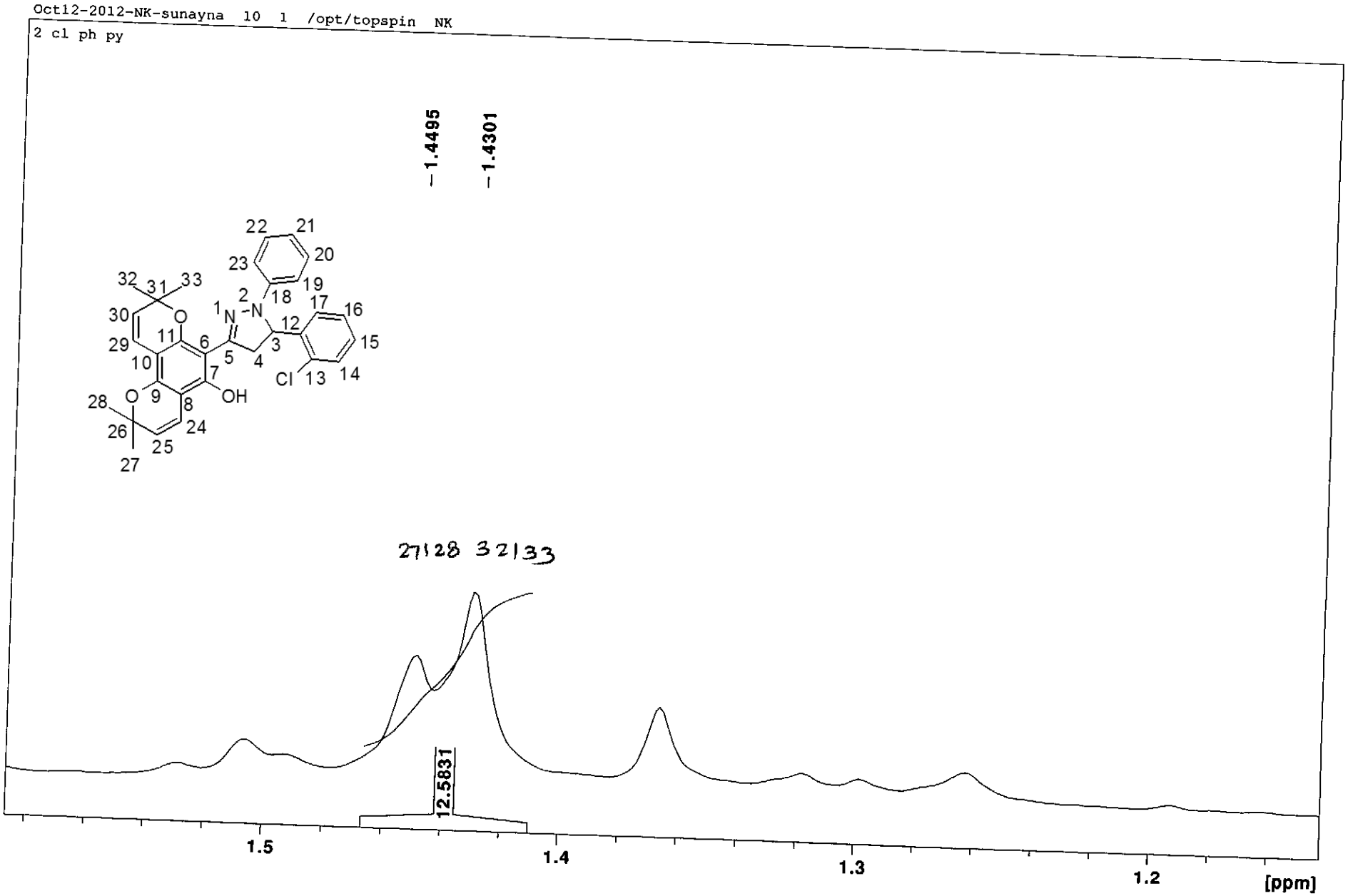
1H NMR spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)



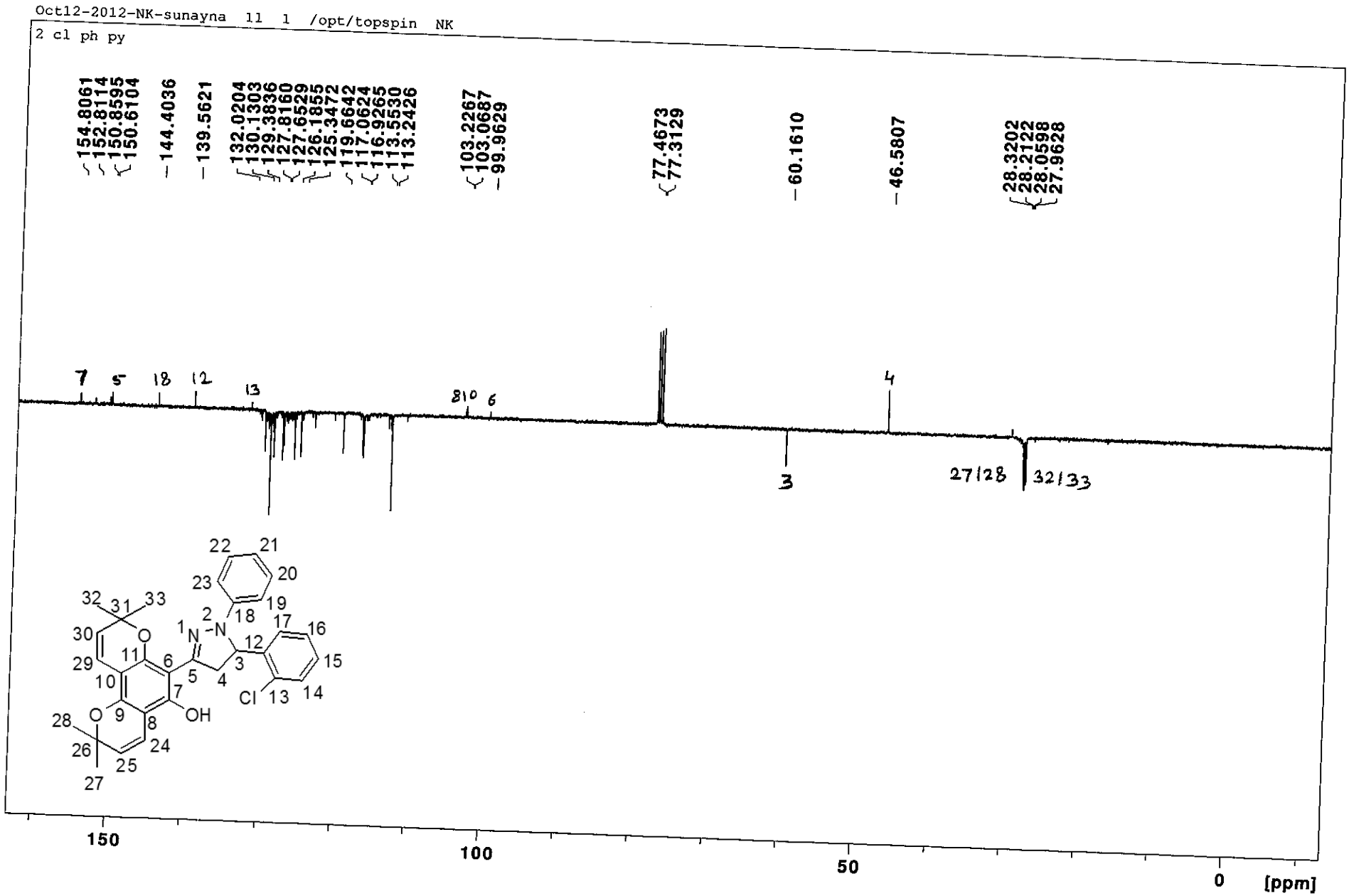
Expanded 1H NMR spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)



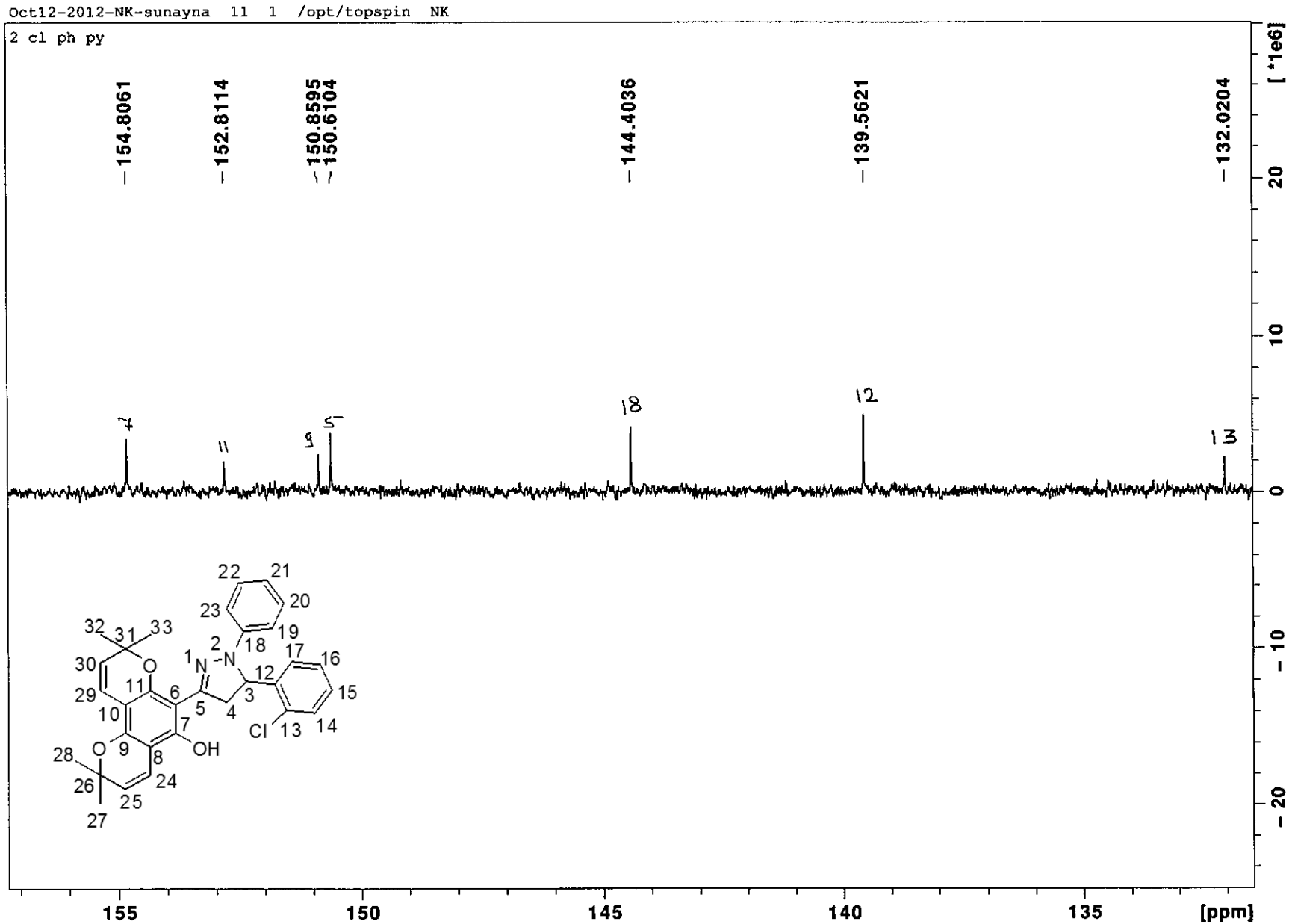
Expanded 1H NMR spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)



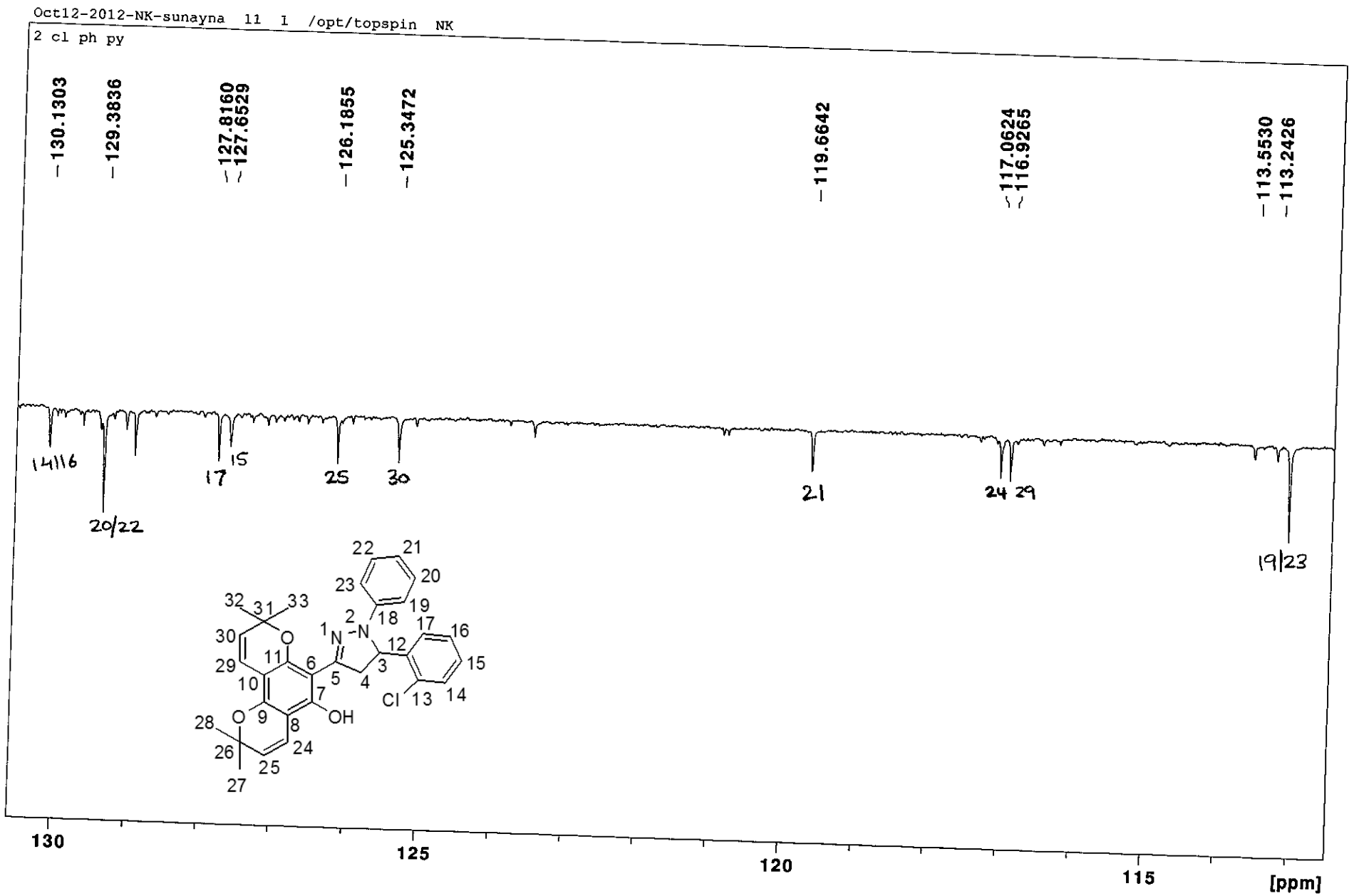
Expanded 1H NMR spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)



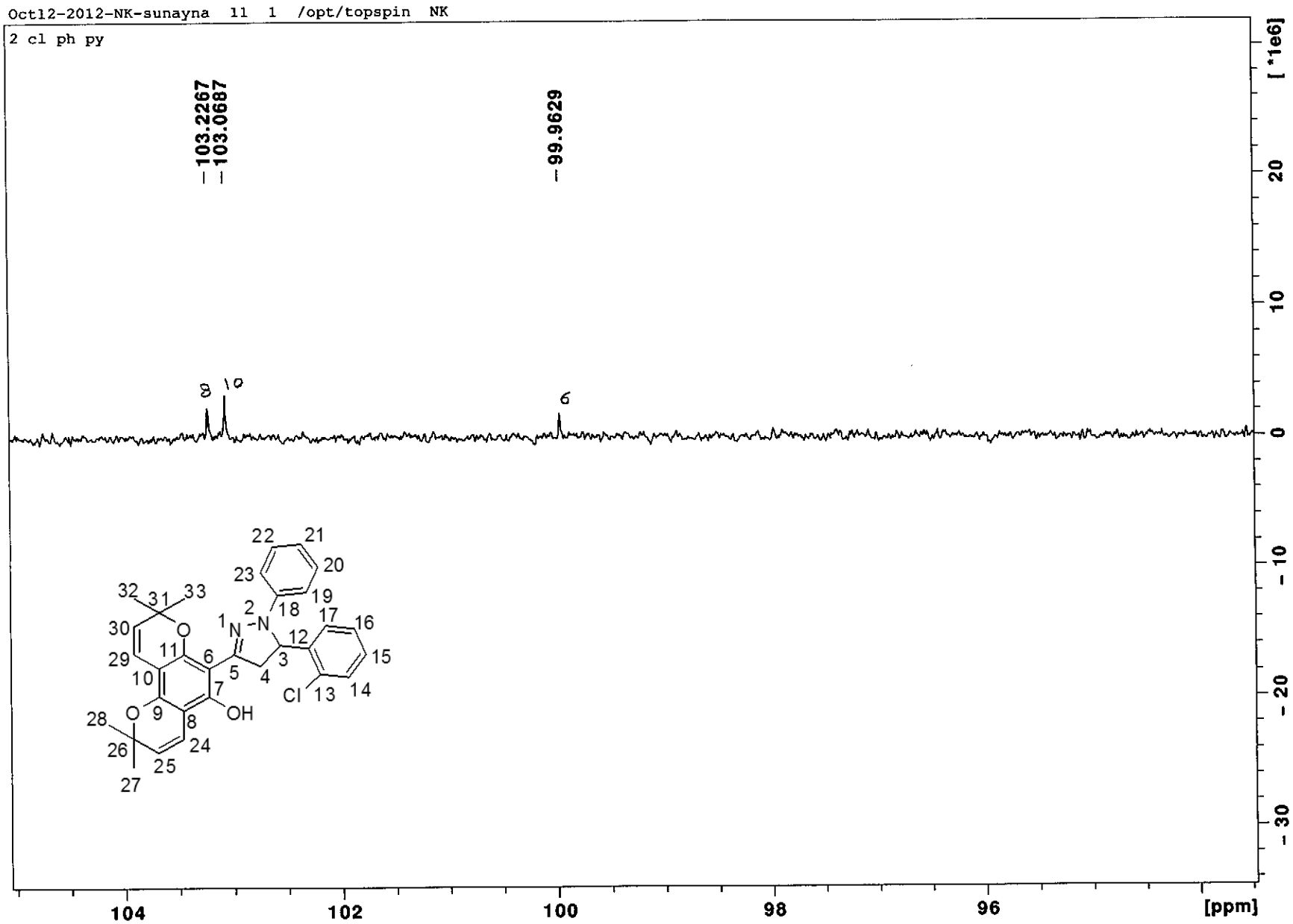
13C NMR spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)



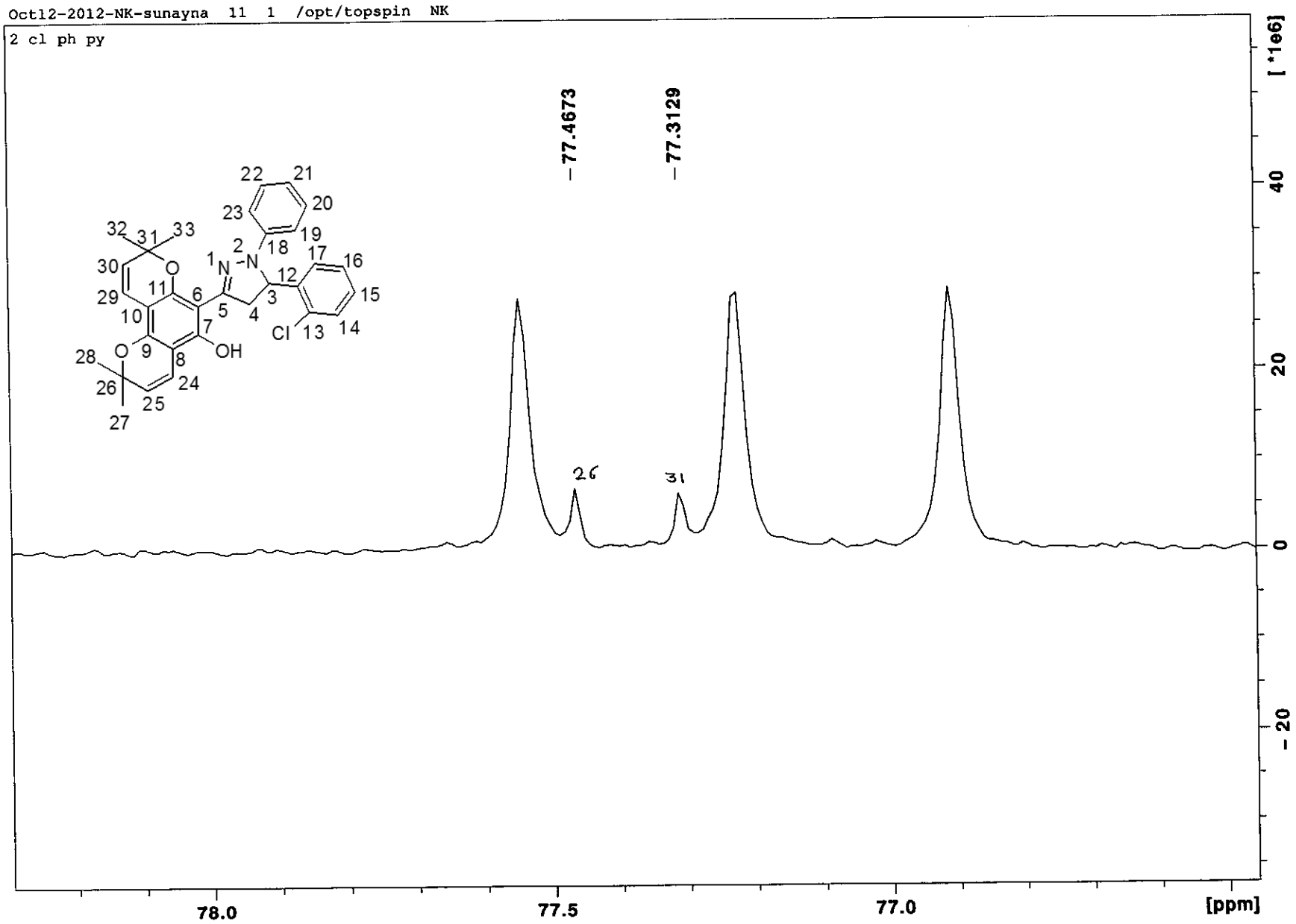
Expanded 13C NMR spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)



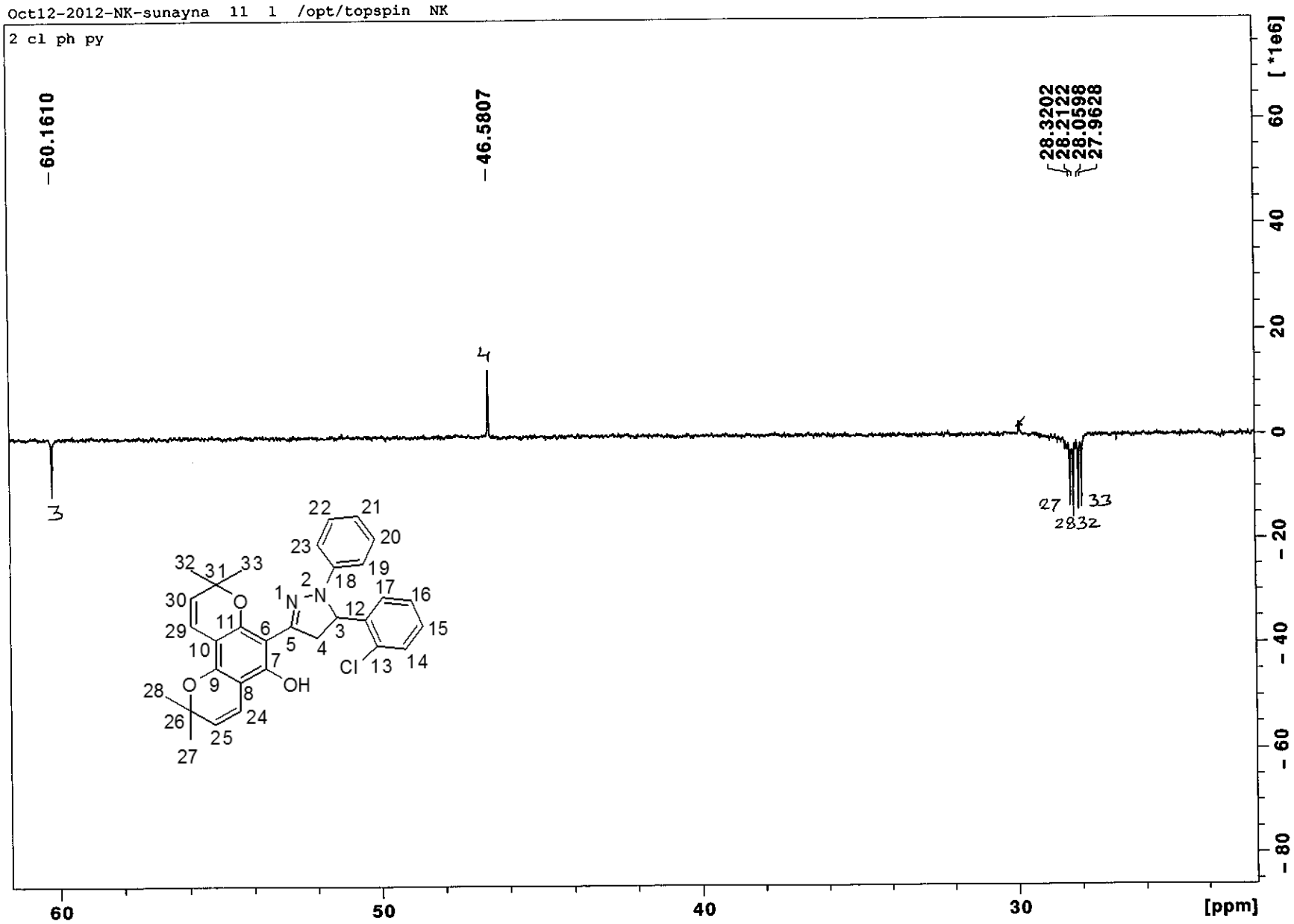
Expanded 13C NMR spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)



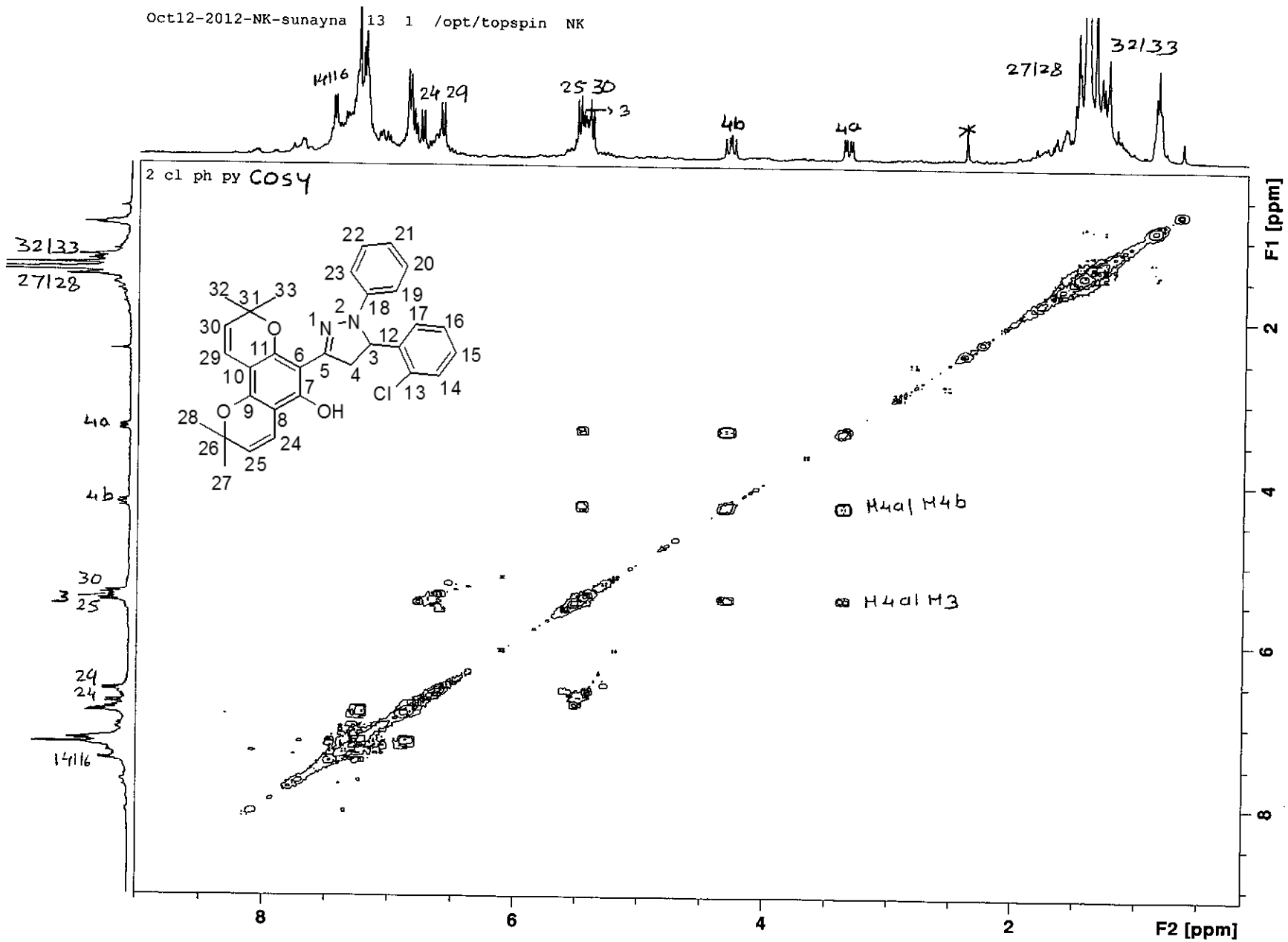
Expanded 13C NMR spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)



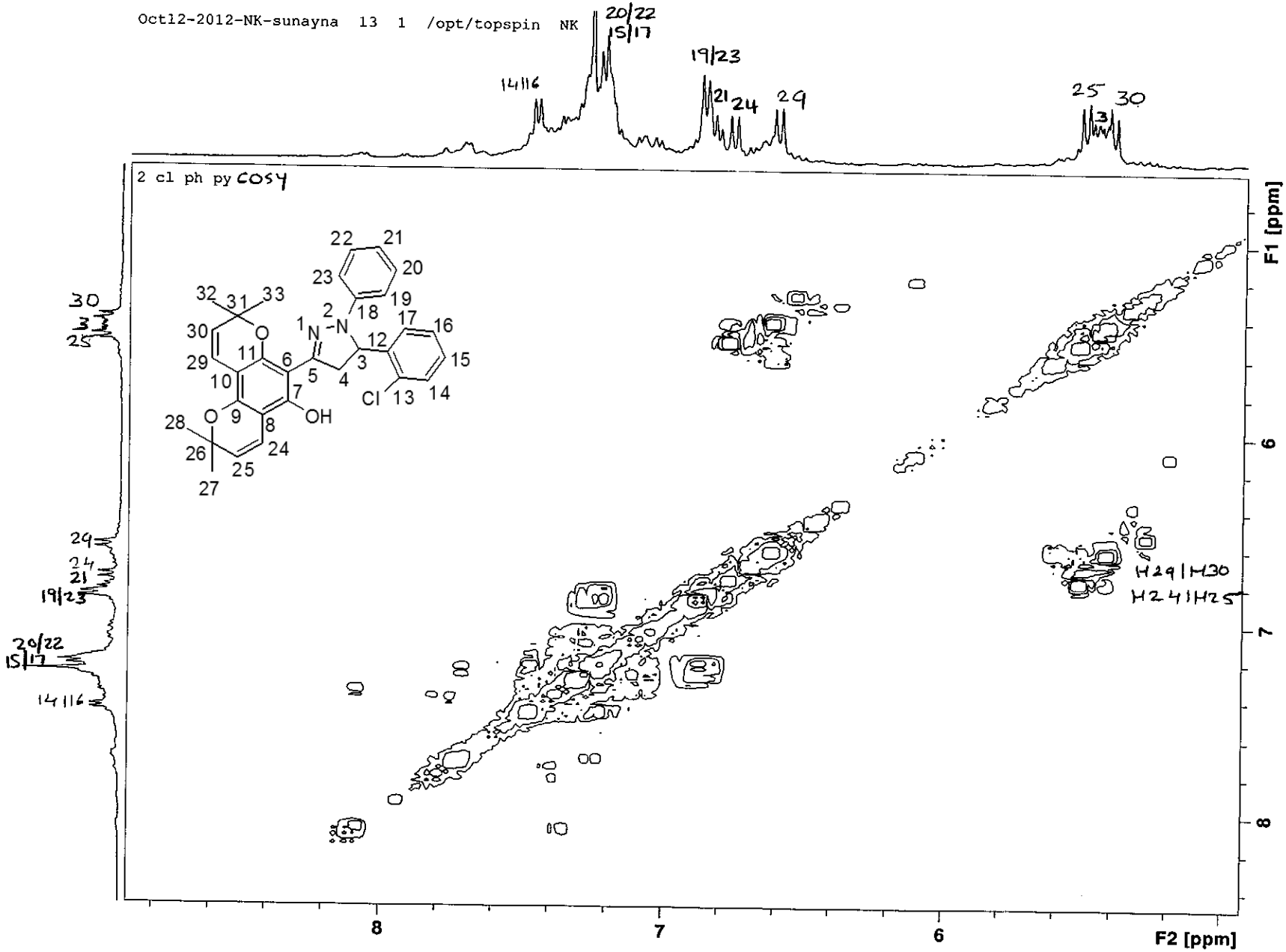
Expanded 13C NMR spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)



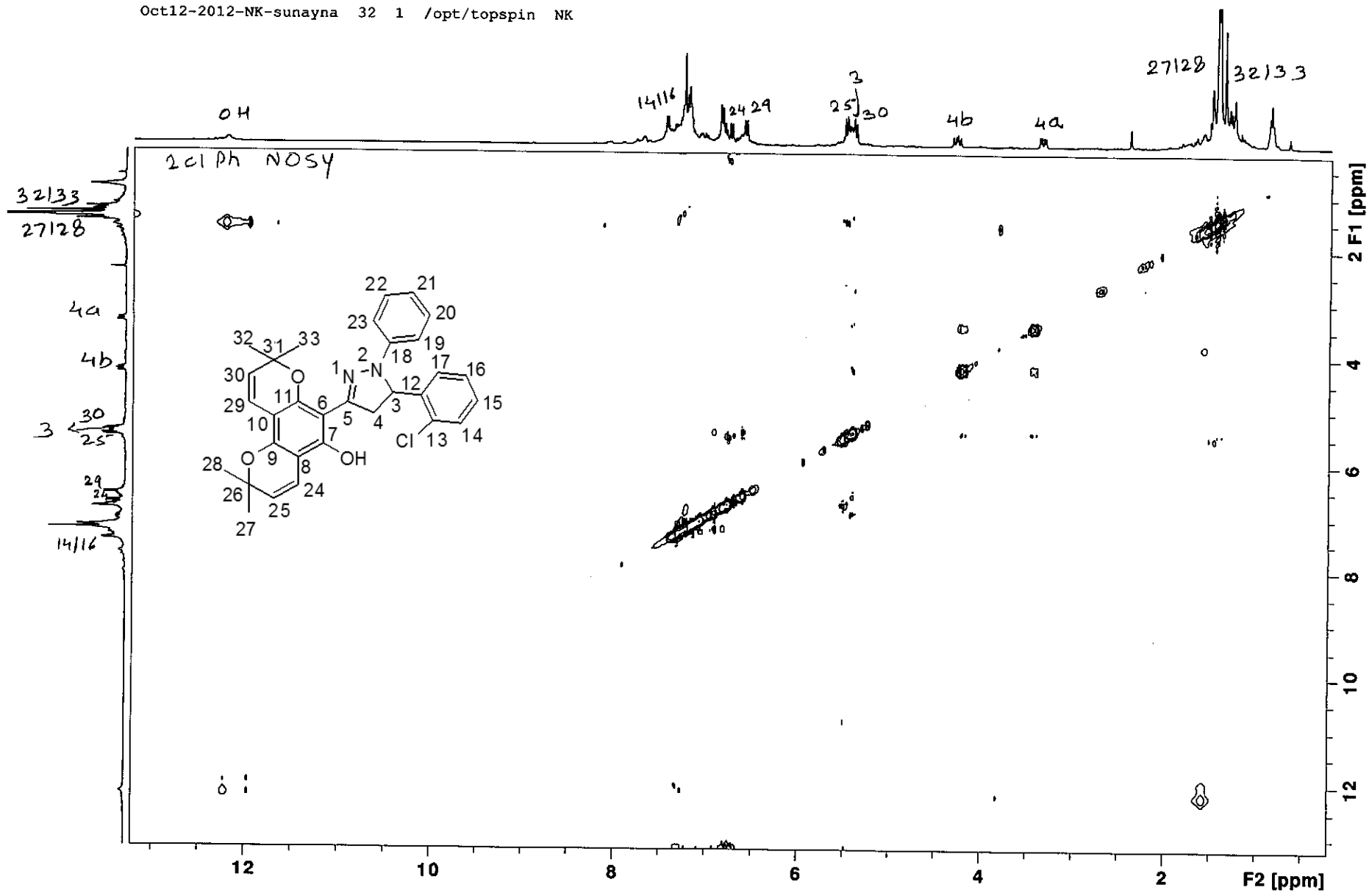
Expanded 13C NMR spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)



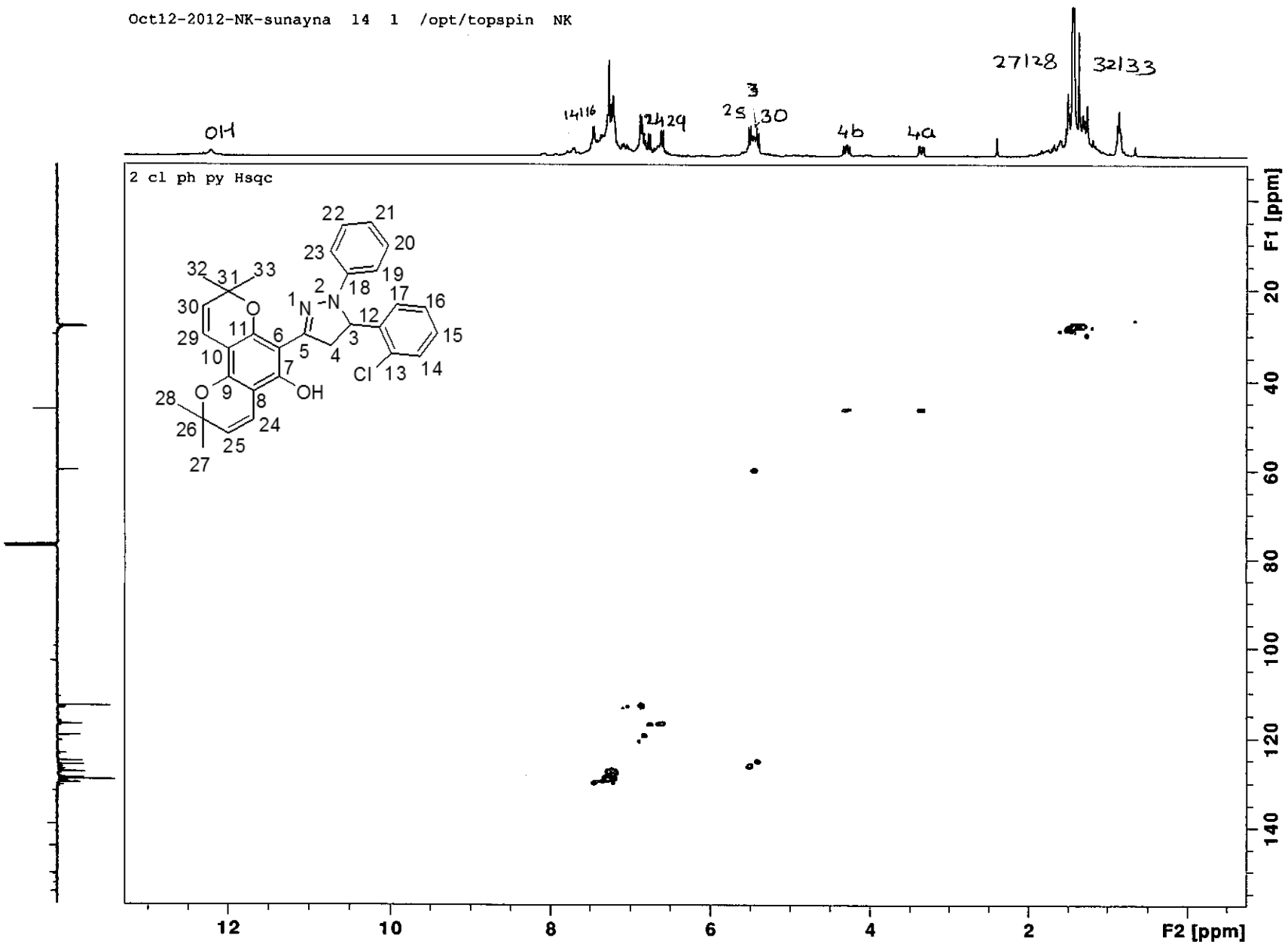
COSY NMR spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)



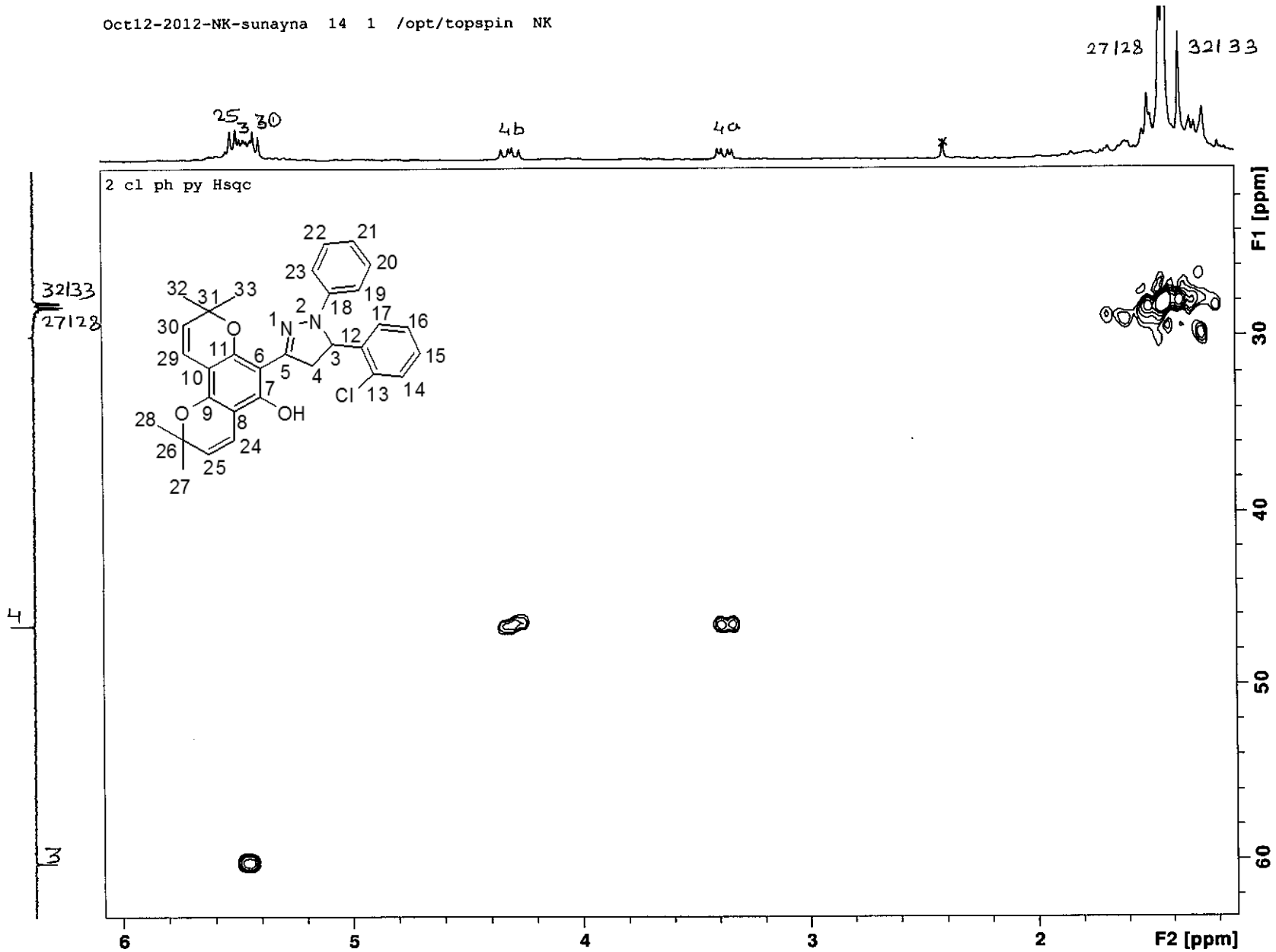
Expanded COSY NMR spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)



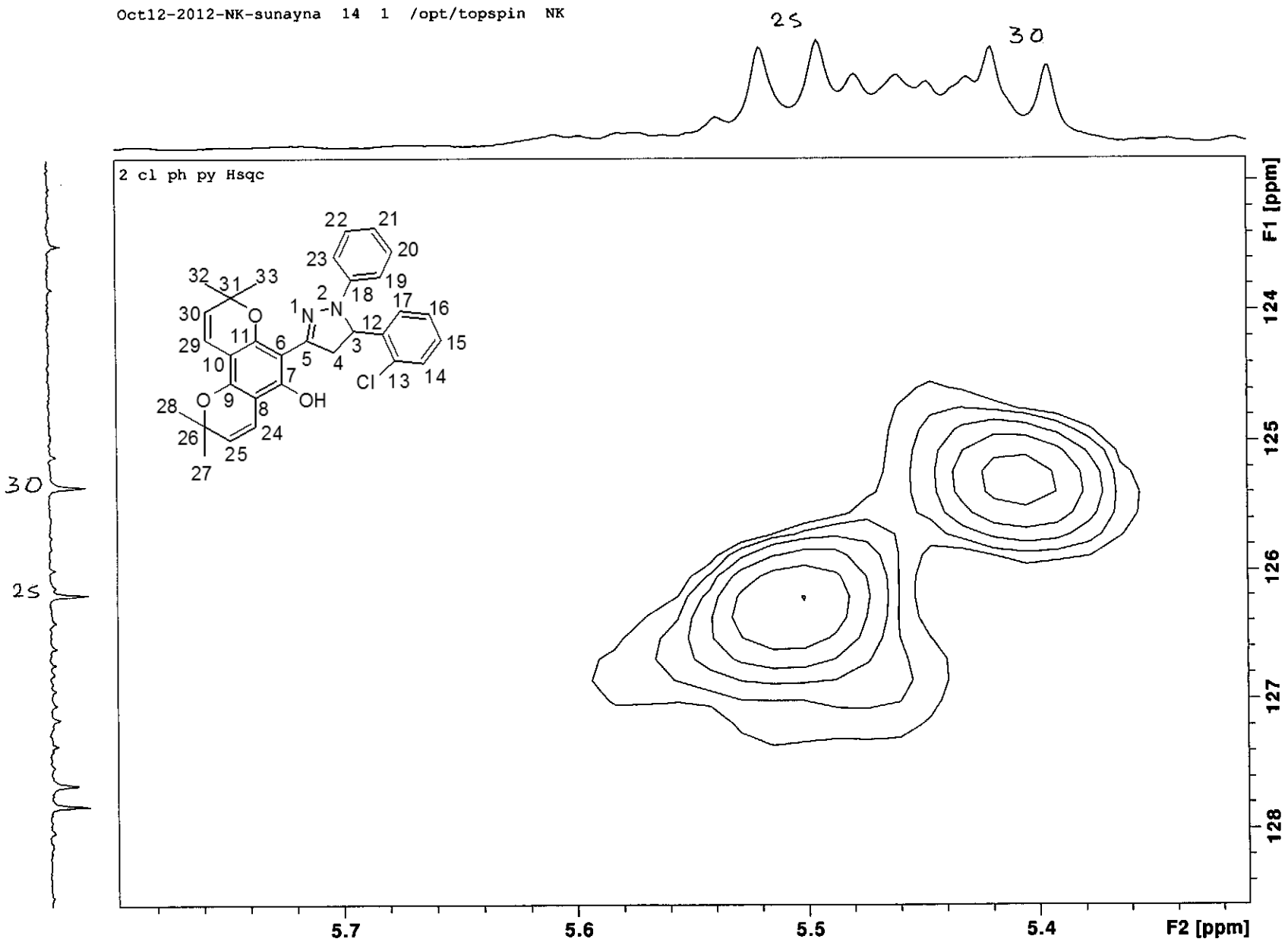
NOESY NMR spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)



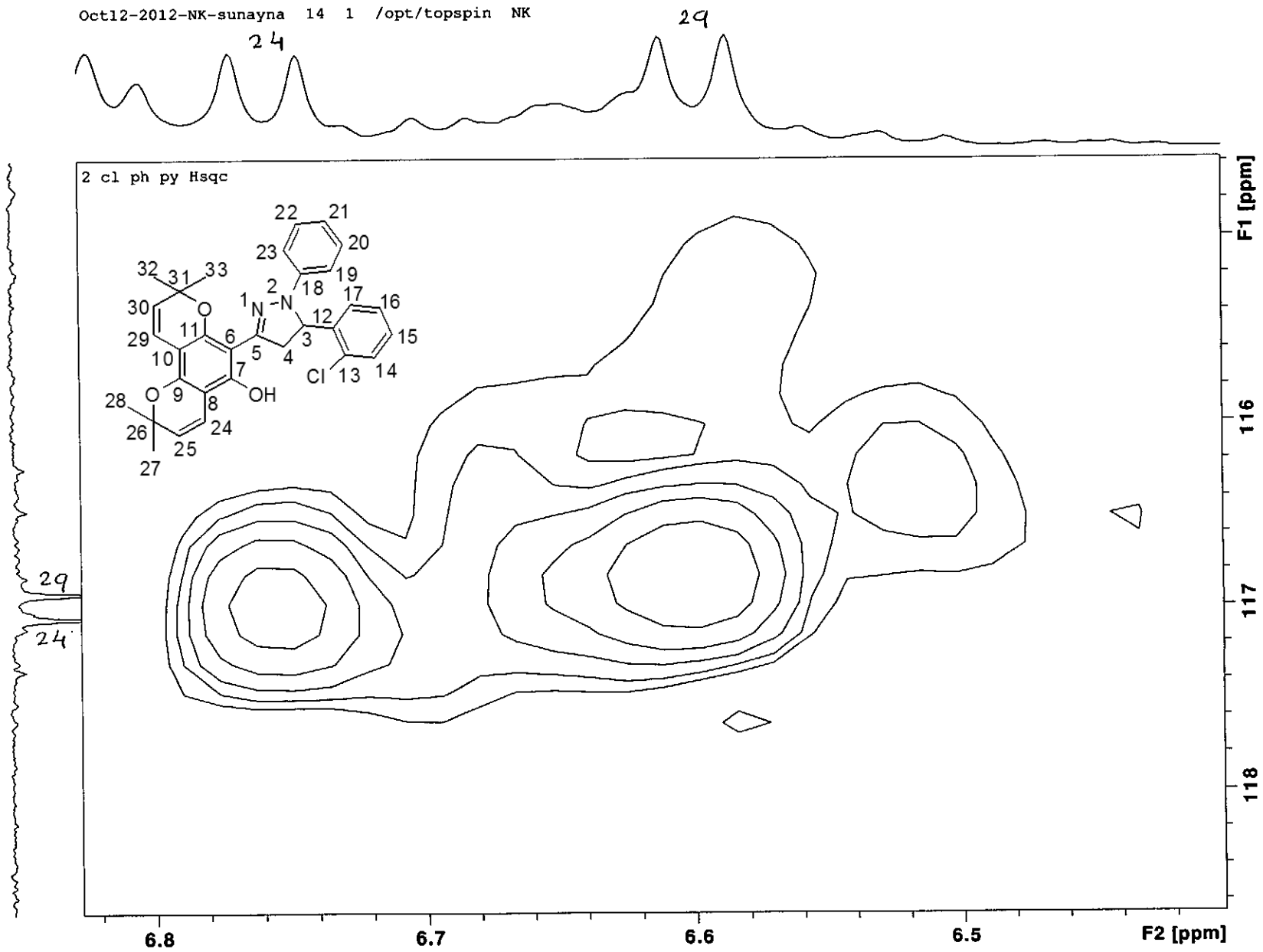
HSQC NMR spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)



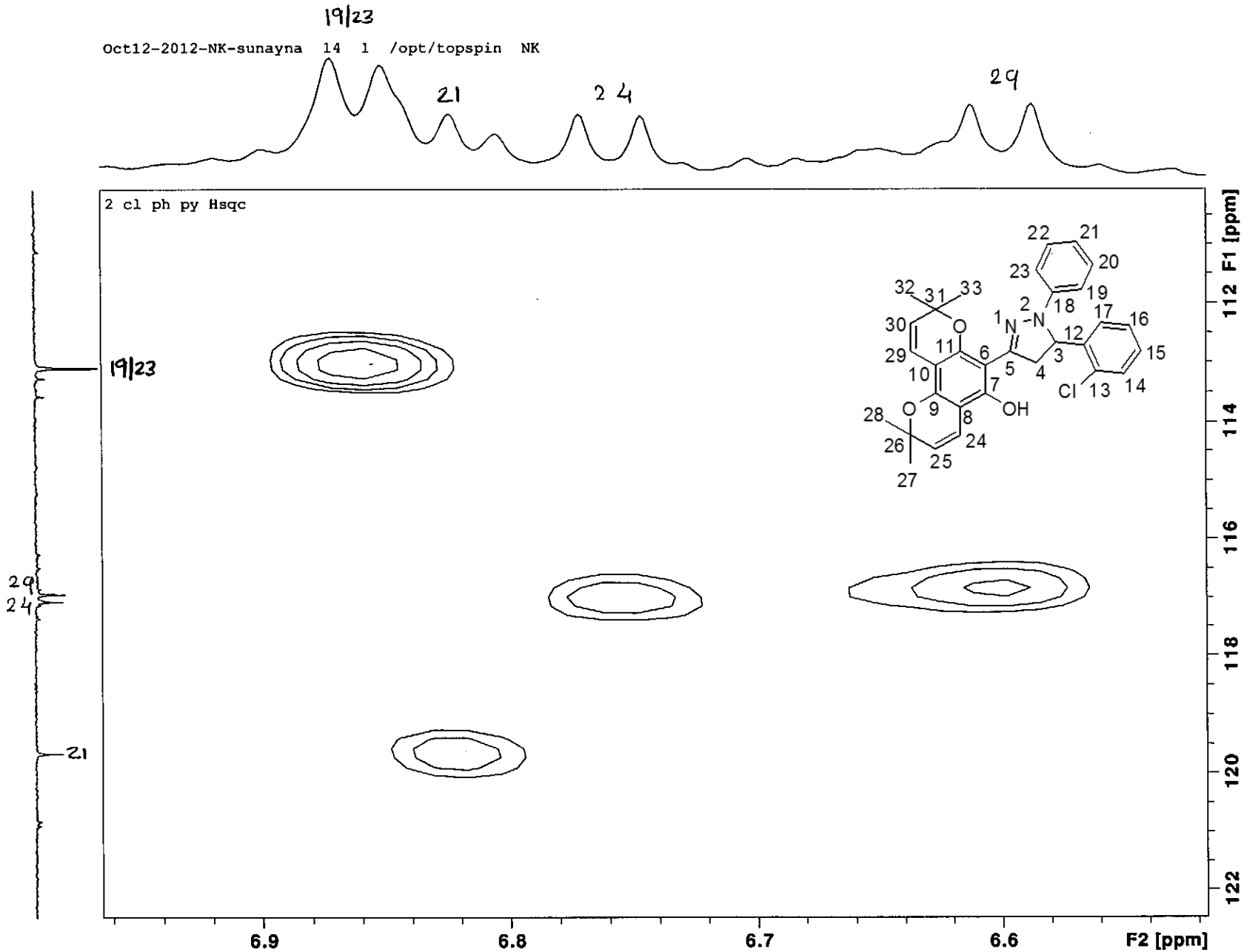
Expanded HSQC NMR spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)



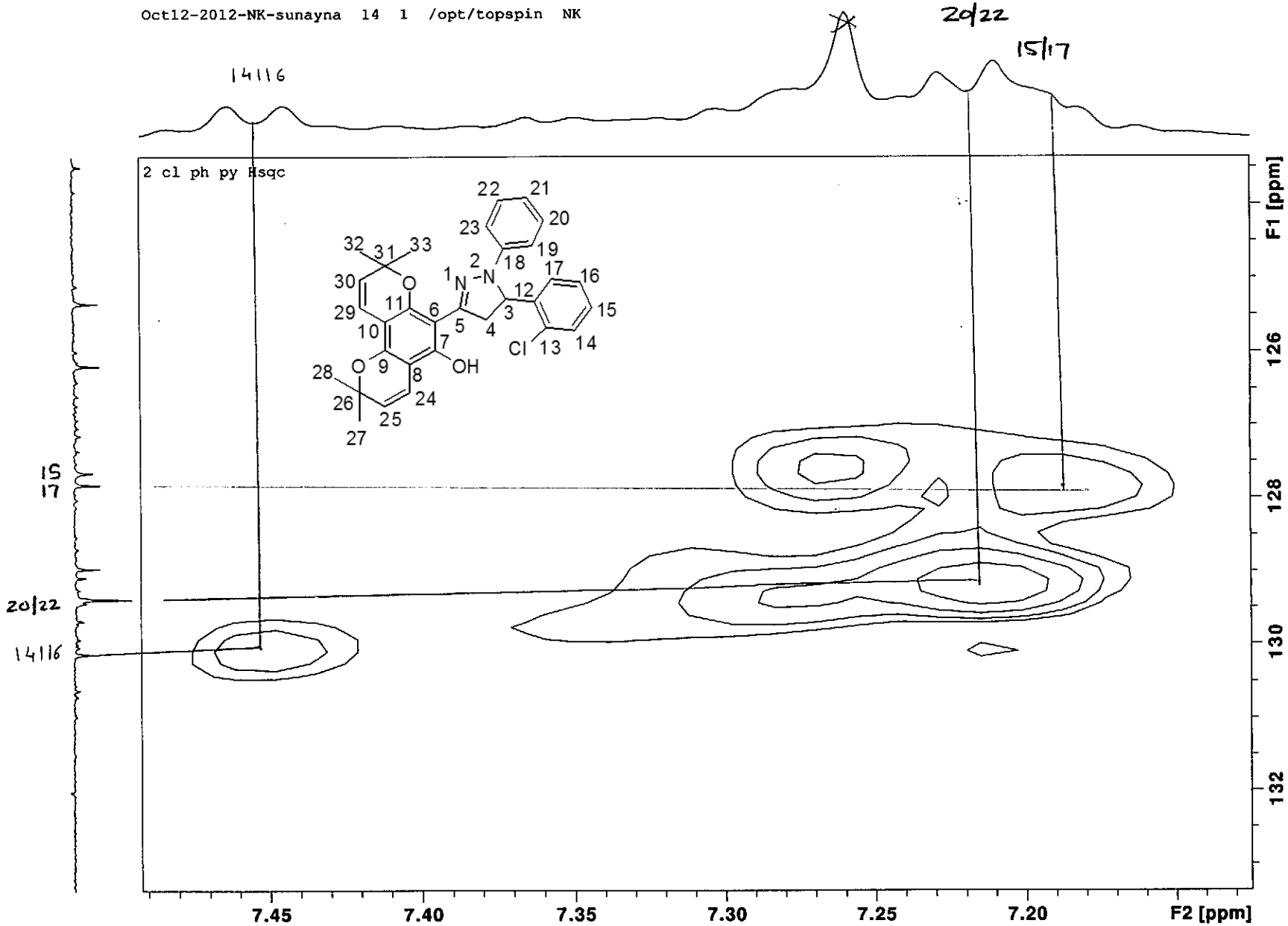
Expanded HSQC NMR spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)



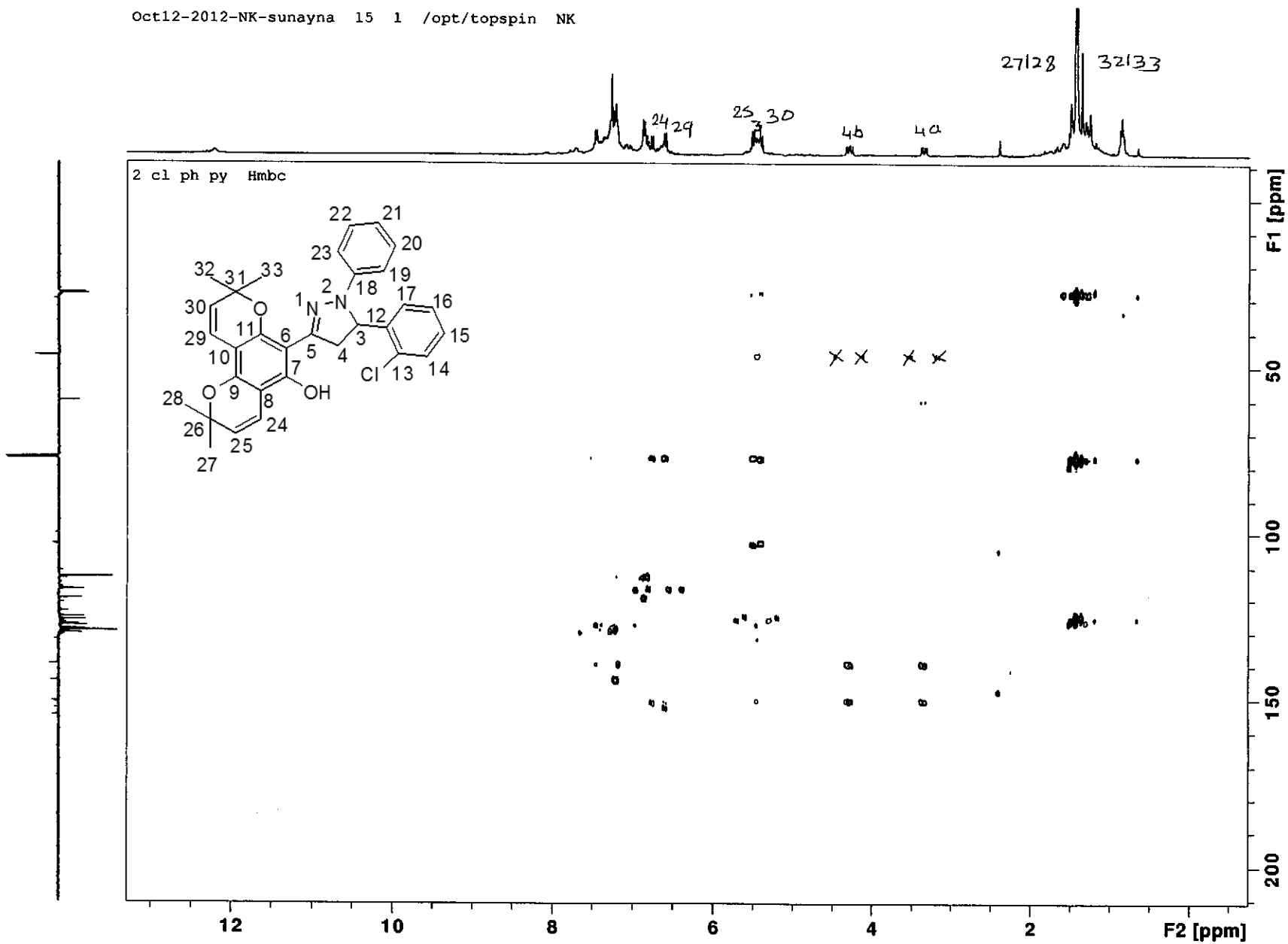
Expanded HSQC NMR spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)



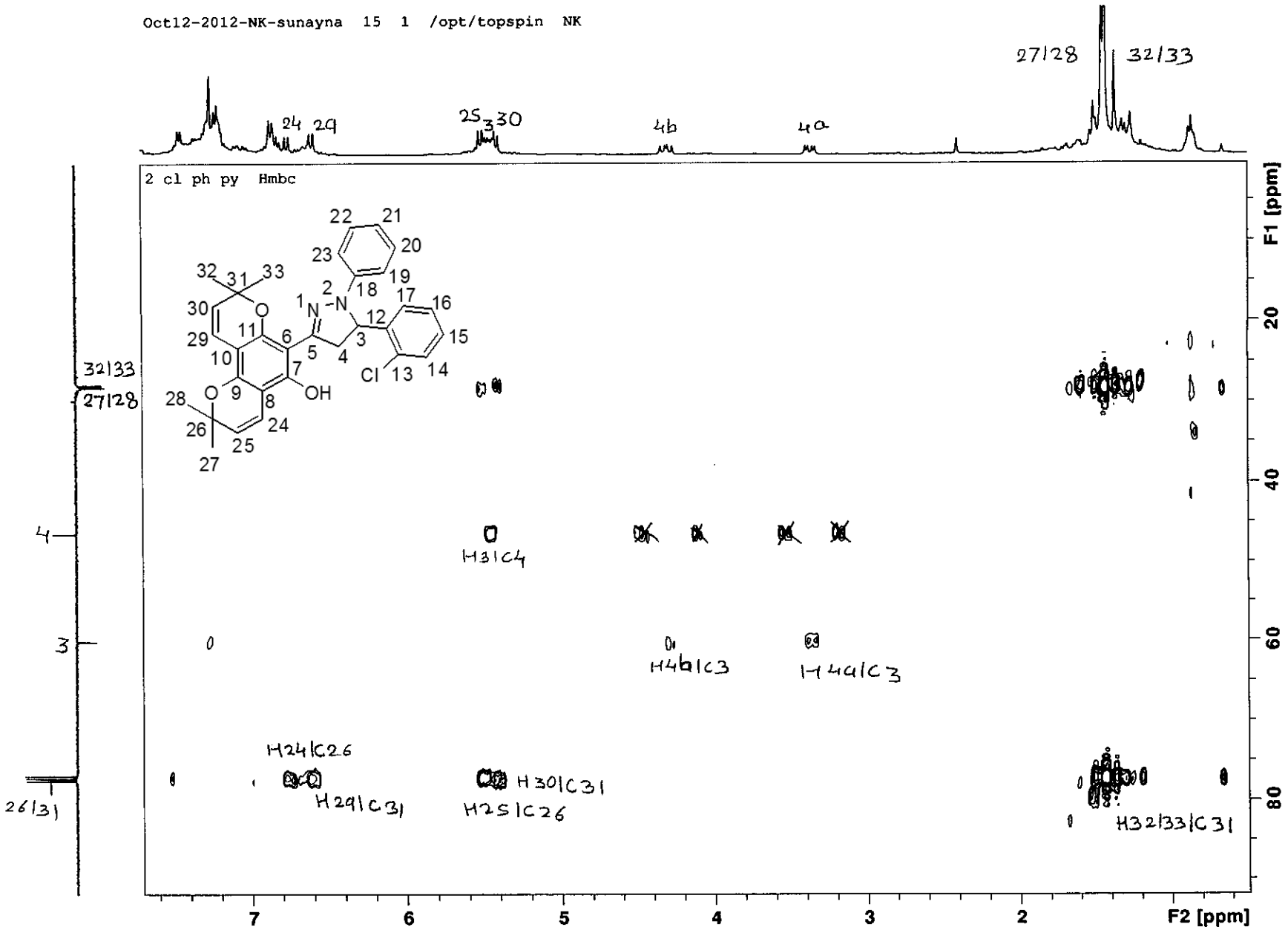
Expanded HSQC NMR spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)



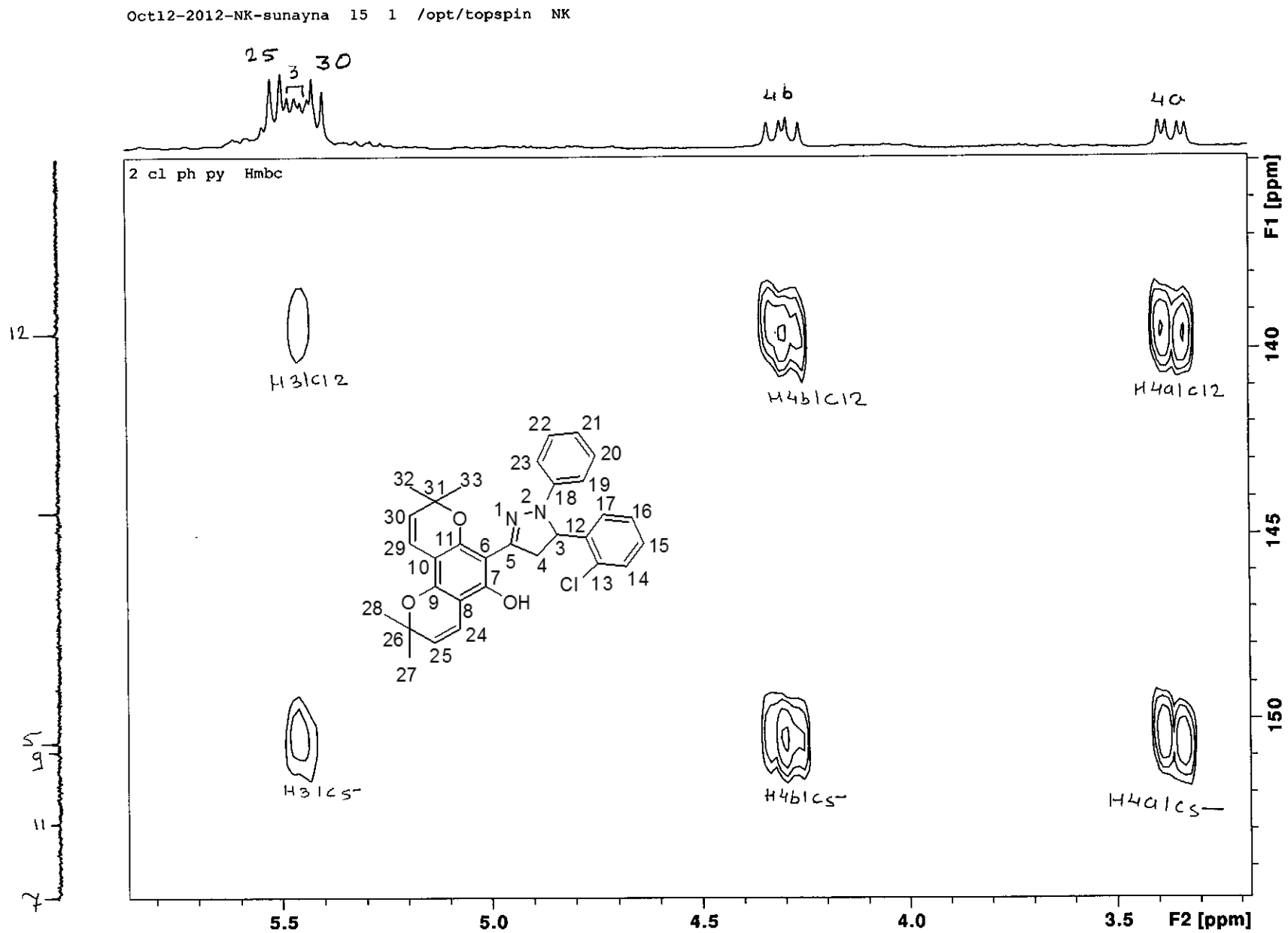
Expanded HSQC NMR spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)



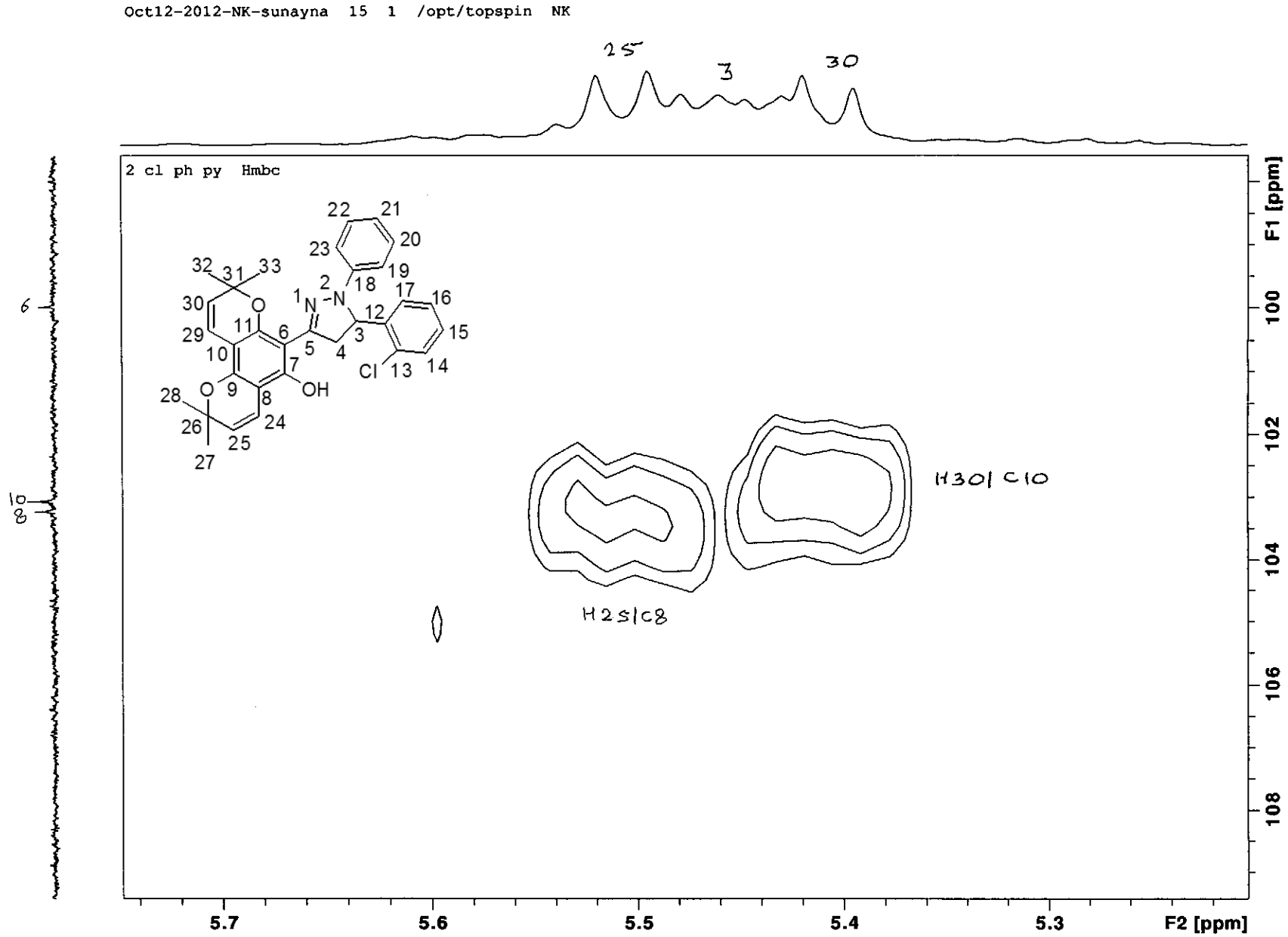
HMBC spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)



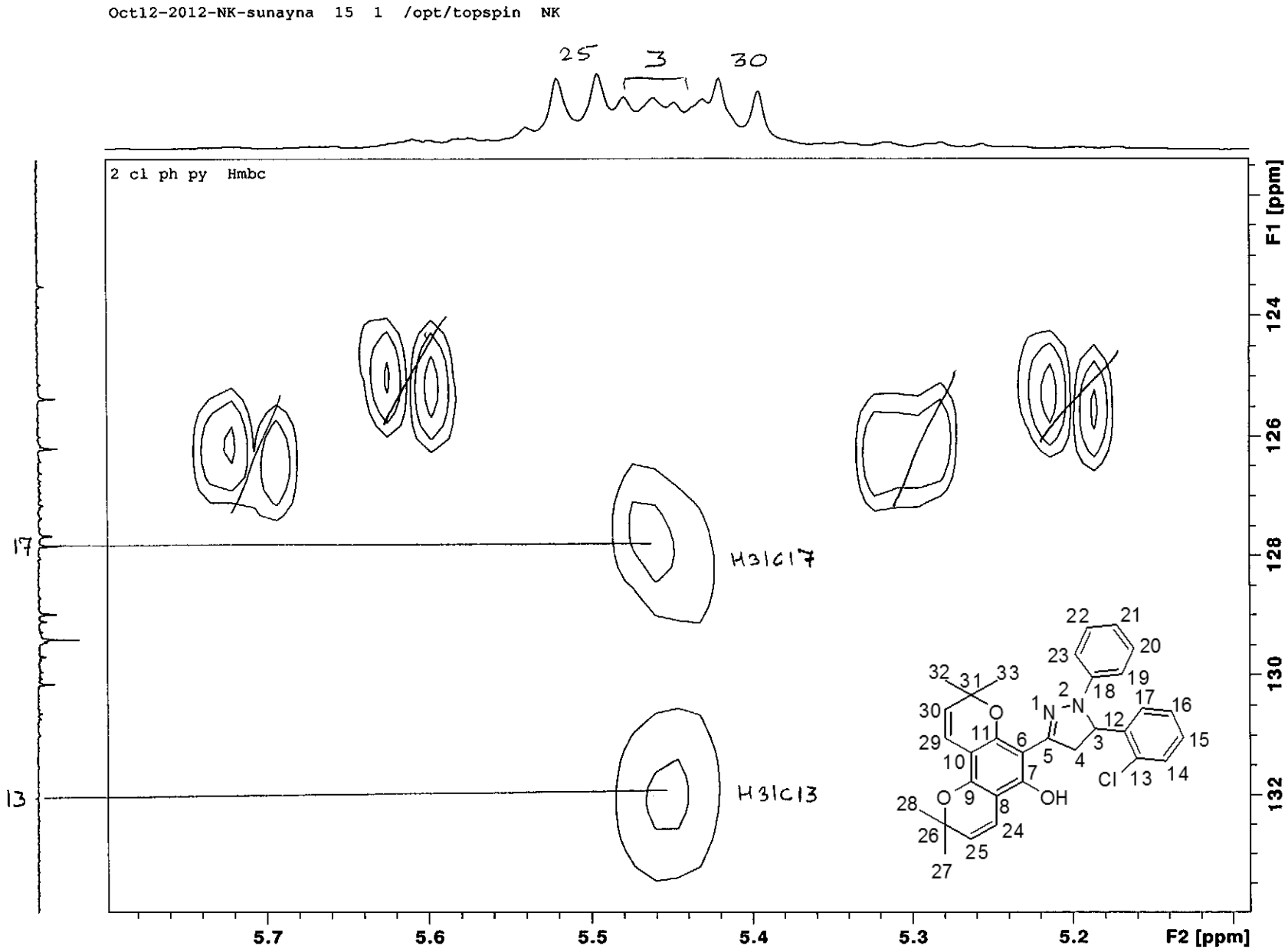
Expanded HMBC spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)



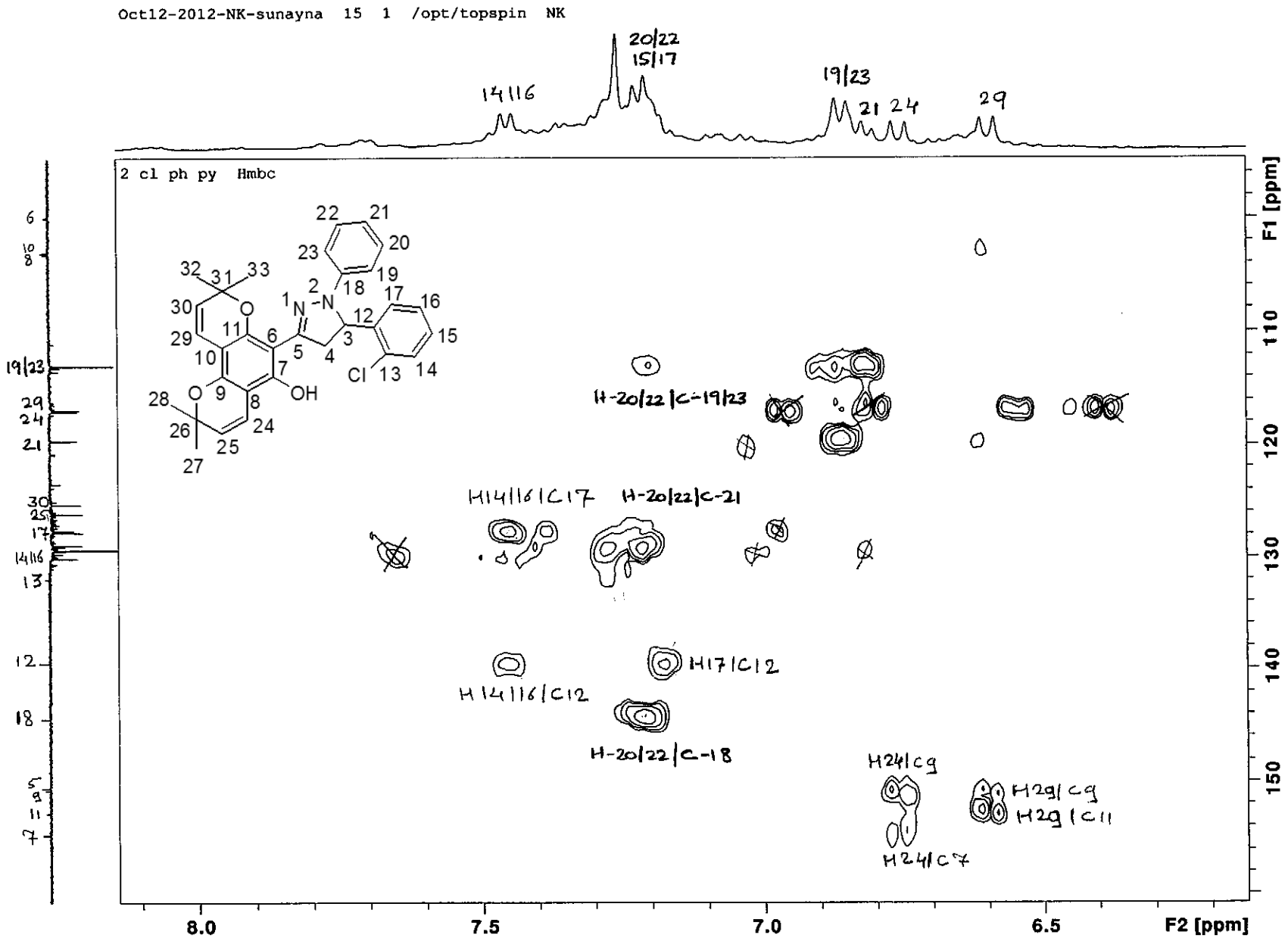
Expanded HMBC spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)



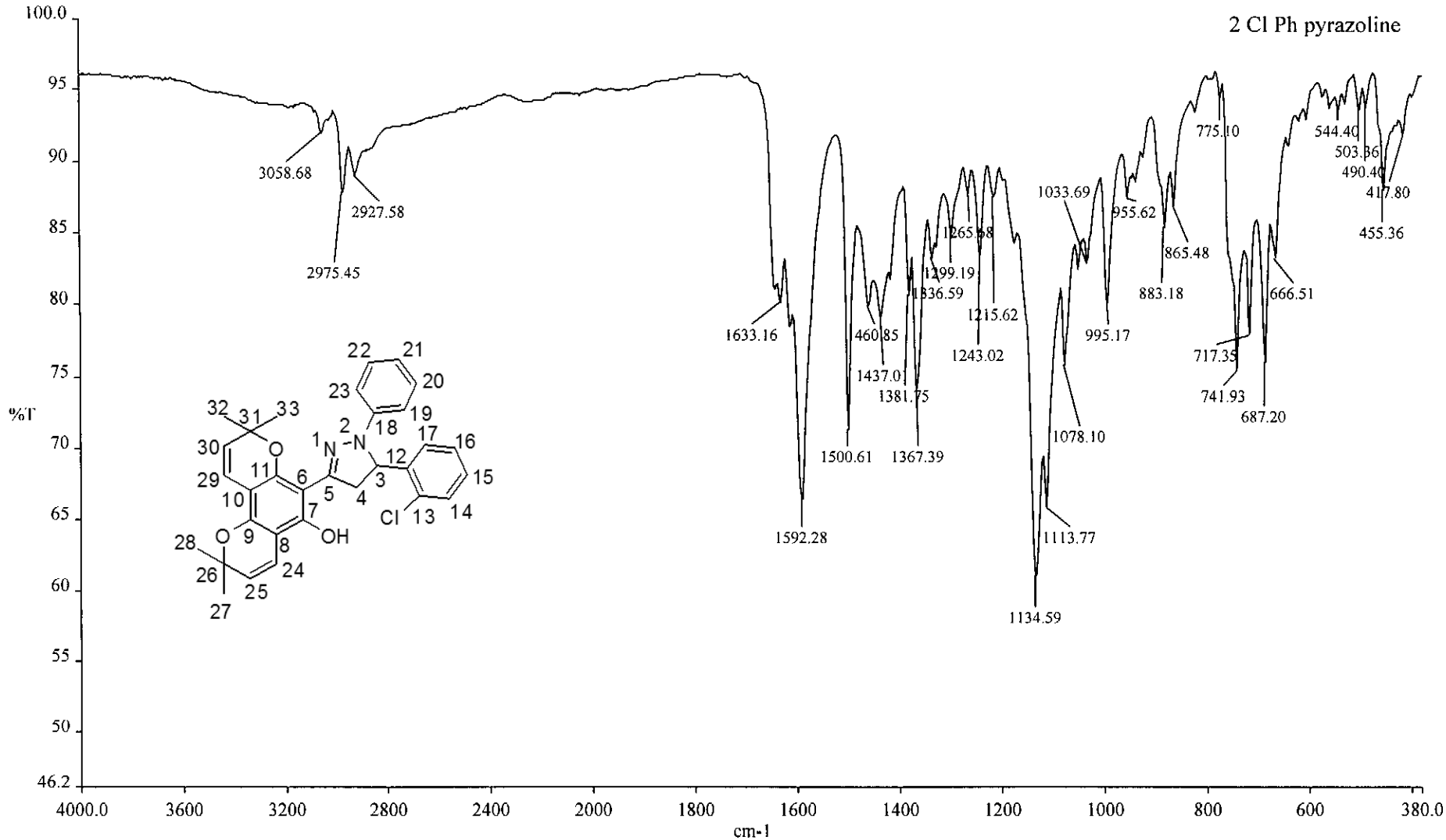
Expanded HMBC spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)



Expanded HMBC spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)



Expanded HMBC spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)

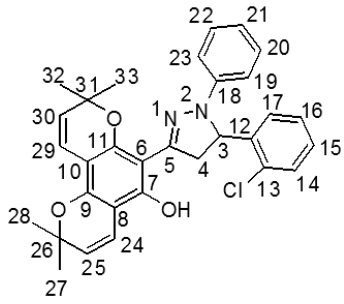


Infrared spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)

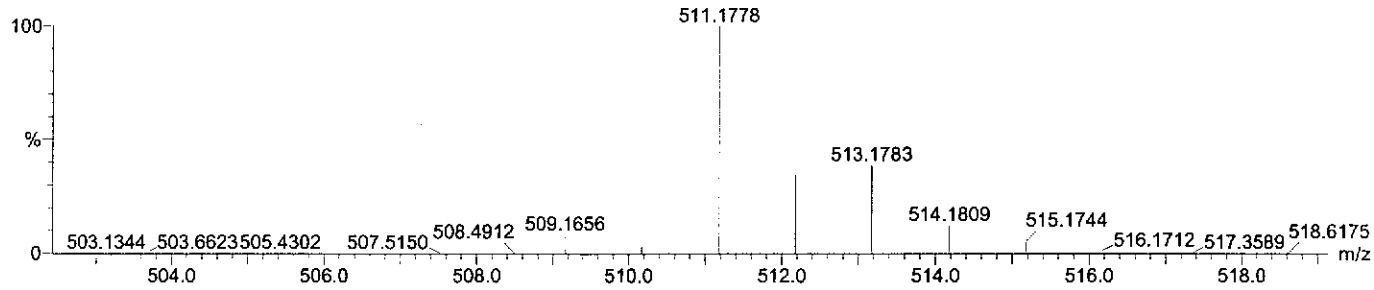
Elemental Composition Report **Page 1**

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

Monoisotopic Mass, Even Electron Ions
22 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-35 H: 20-30 N: 0-4 O: 0-4 Cl: 1-1
2 8 (0.137) Cm (1:30)
TOF MS ES-

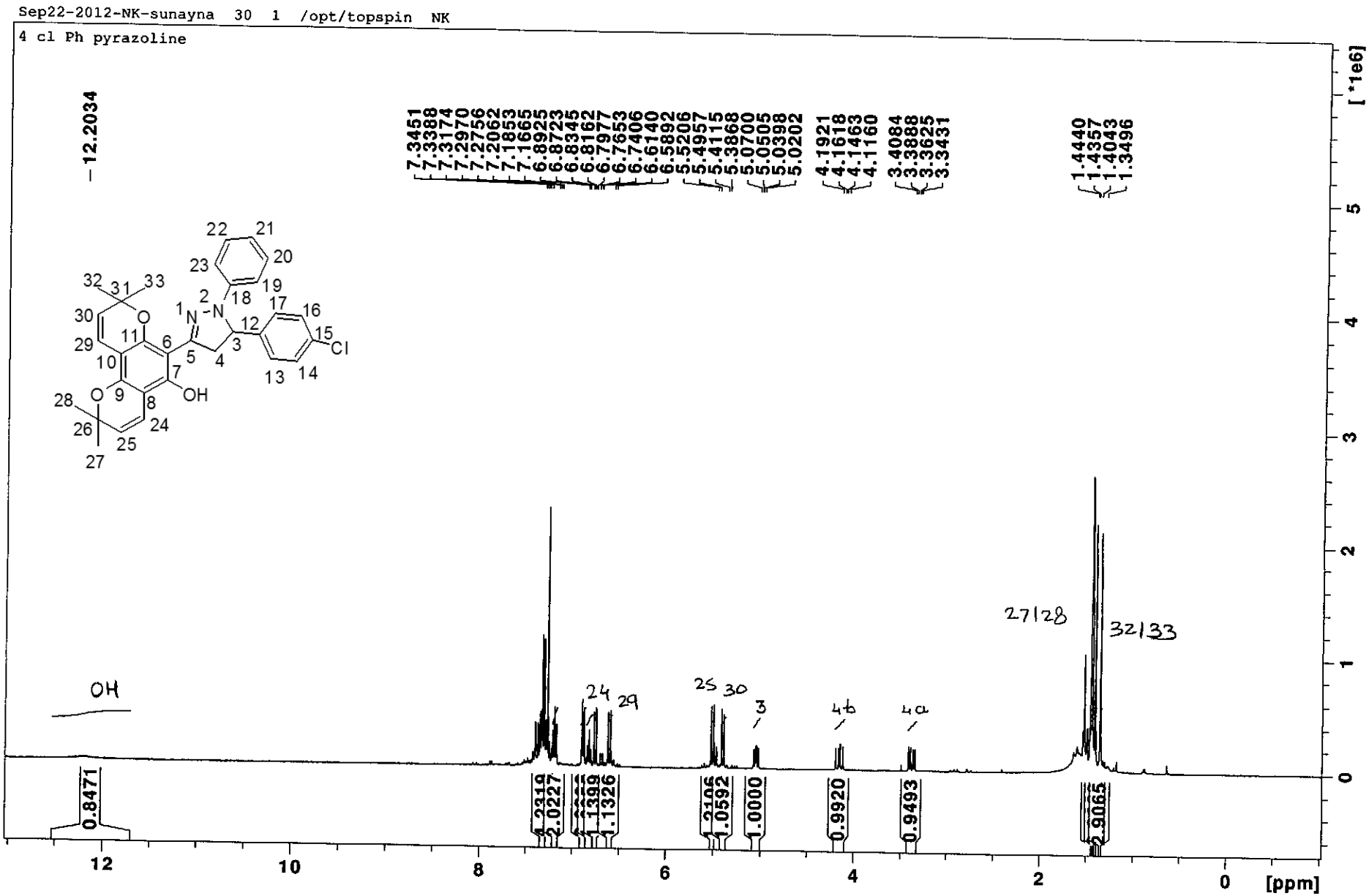


1.89e+005

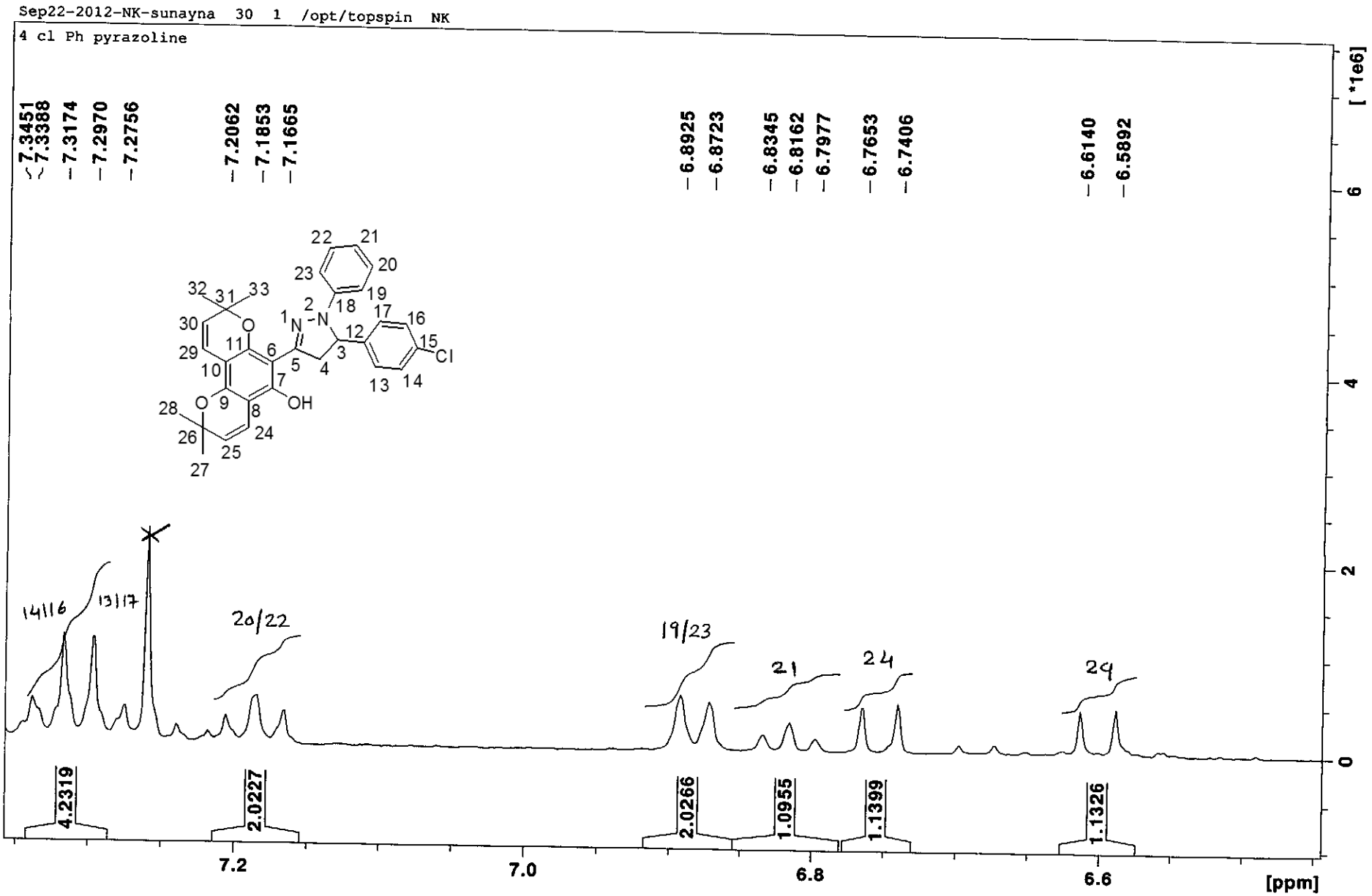


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
511.1778	511.1788	-1.0	-2.0	18.5	475.9	0.0	C31 H28 N2 O3 Cl

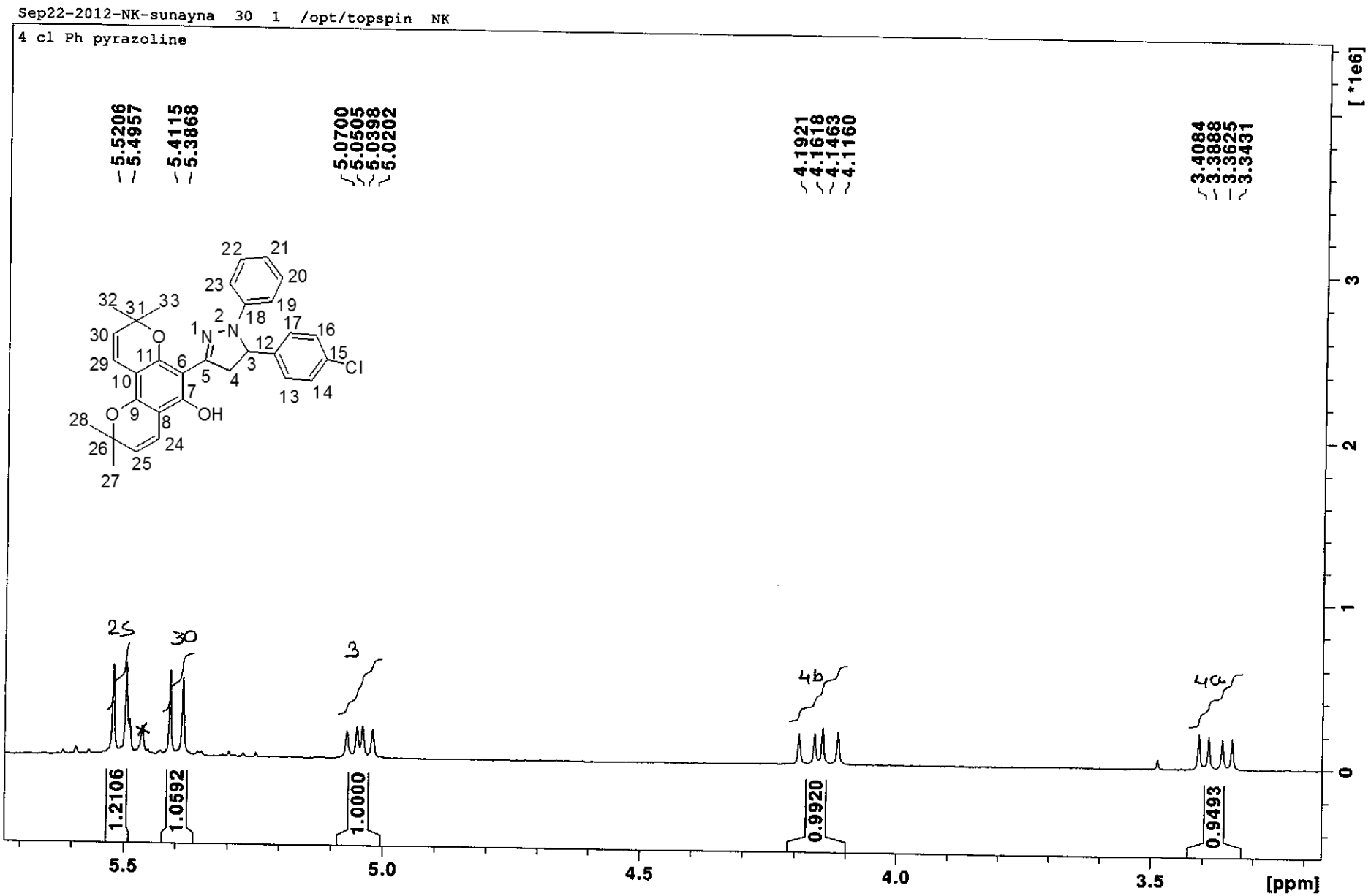
HRMS spectrum of 3-(2-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5a)



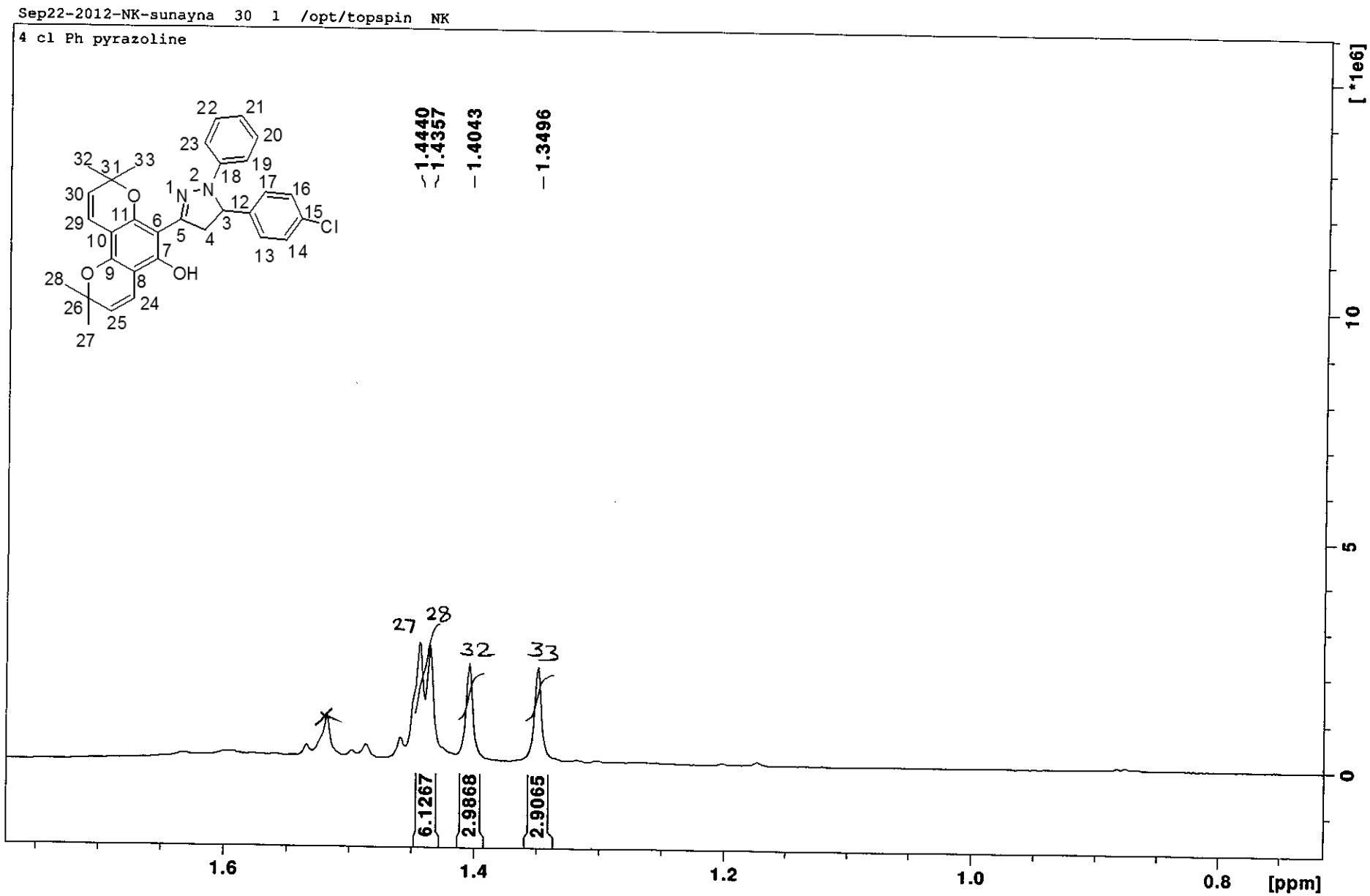
1H NMR spectrum of 3-(4-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5b)



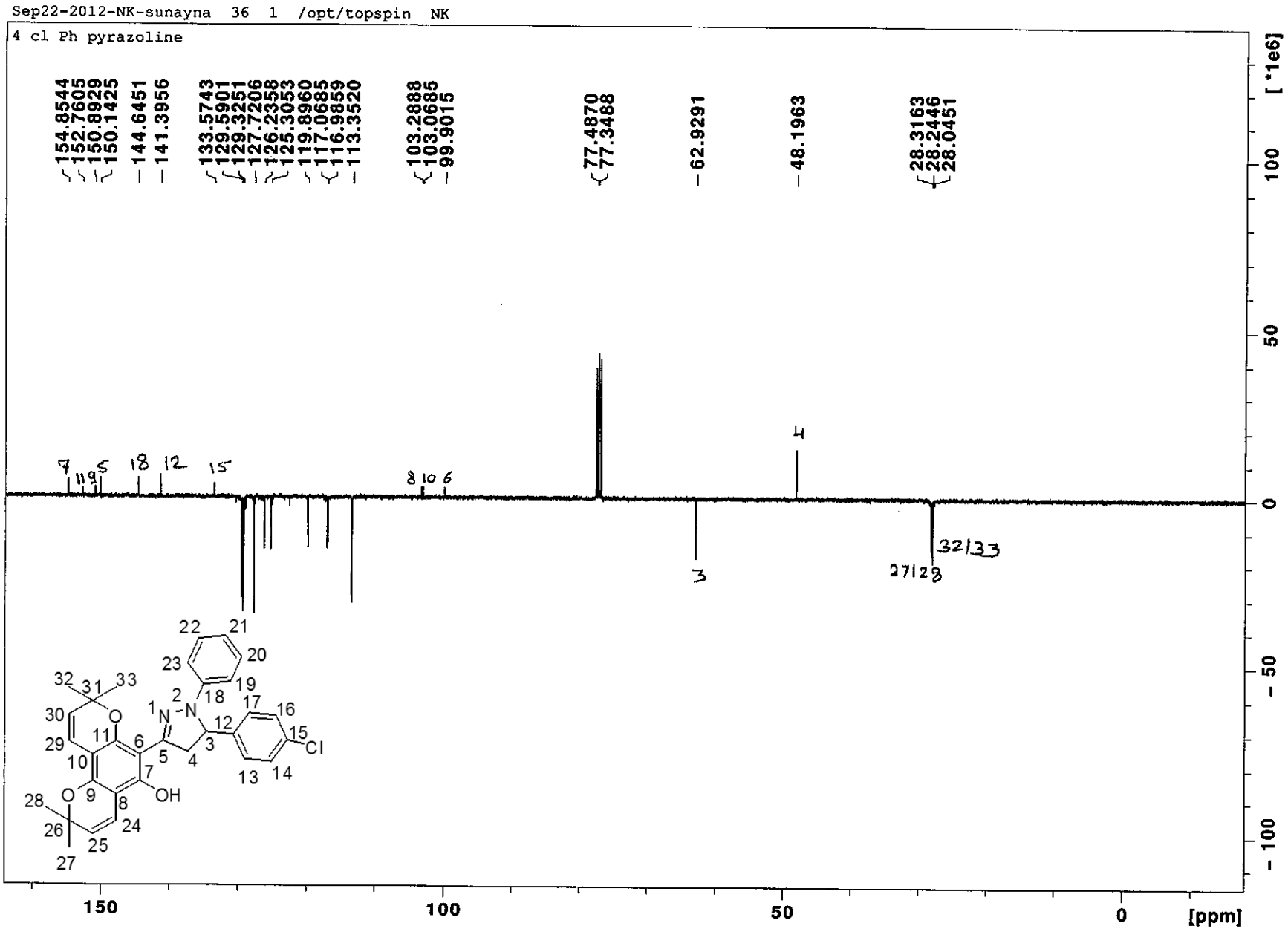
Expanded 1H NMR spectrum of 3-(4-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5b)



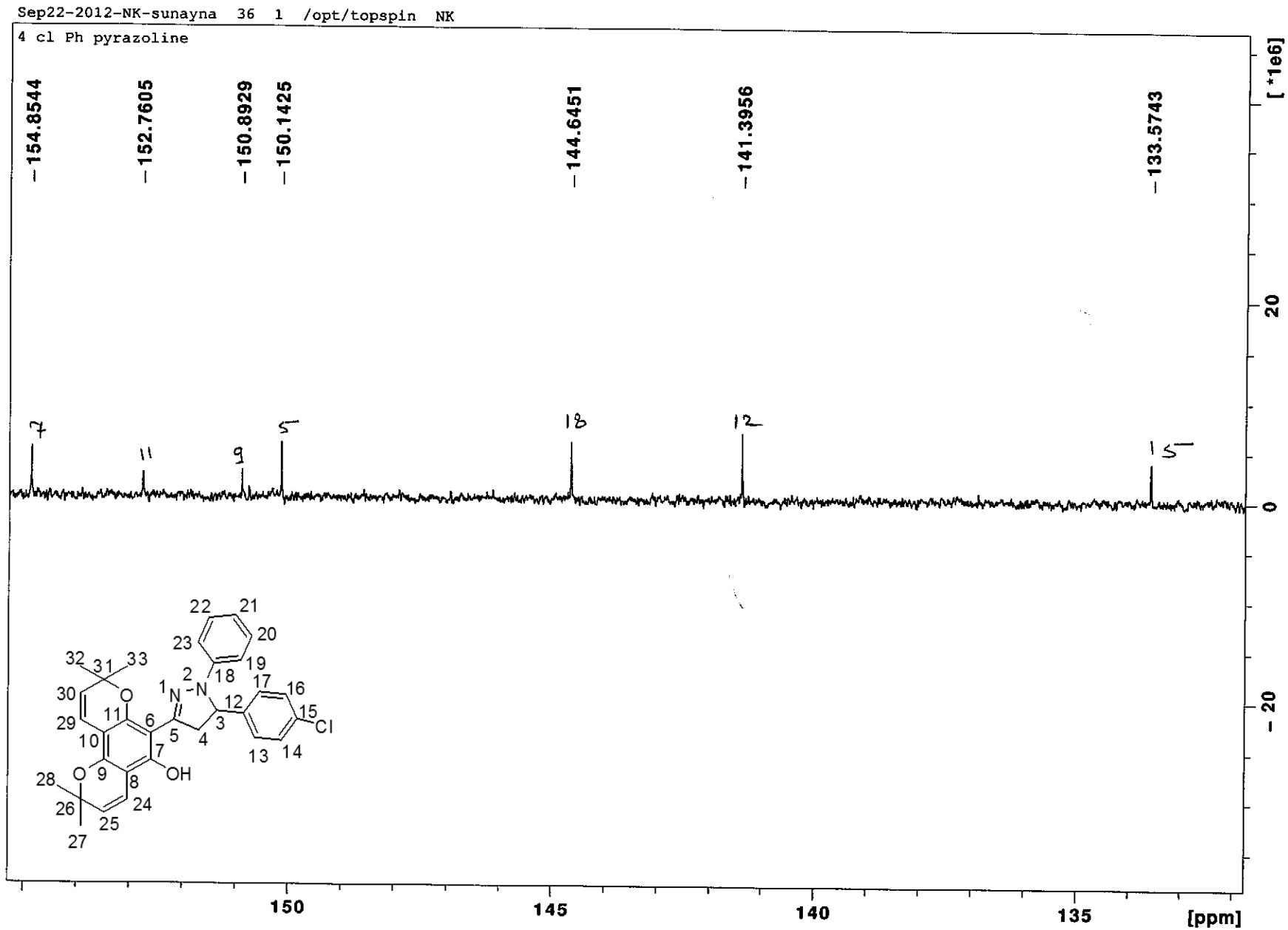
Expanded 1H NMR spectrum of 3-(4-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5b)



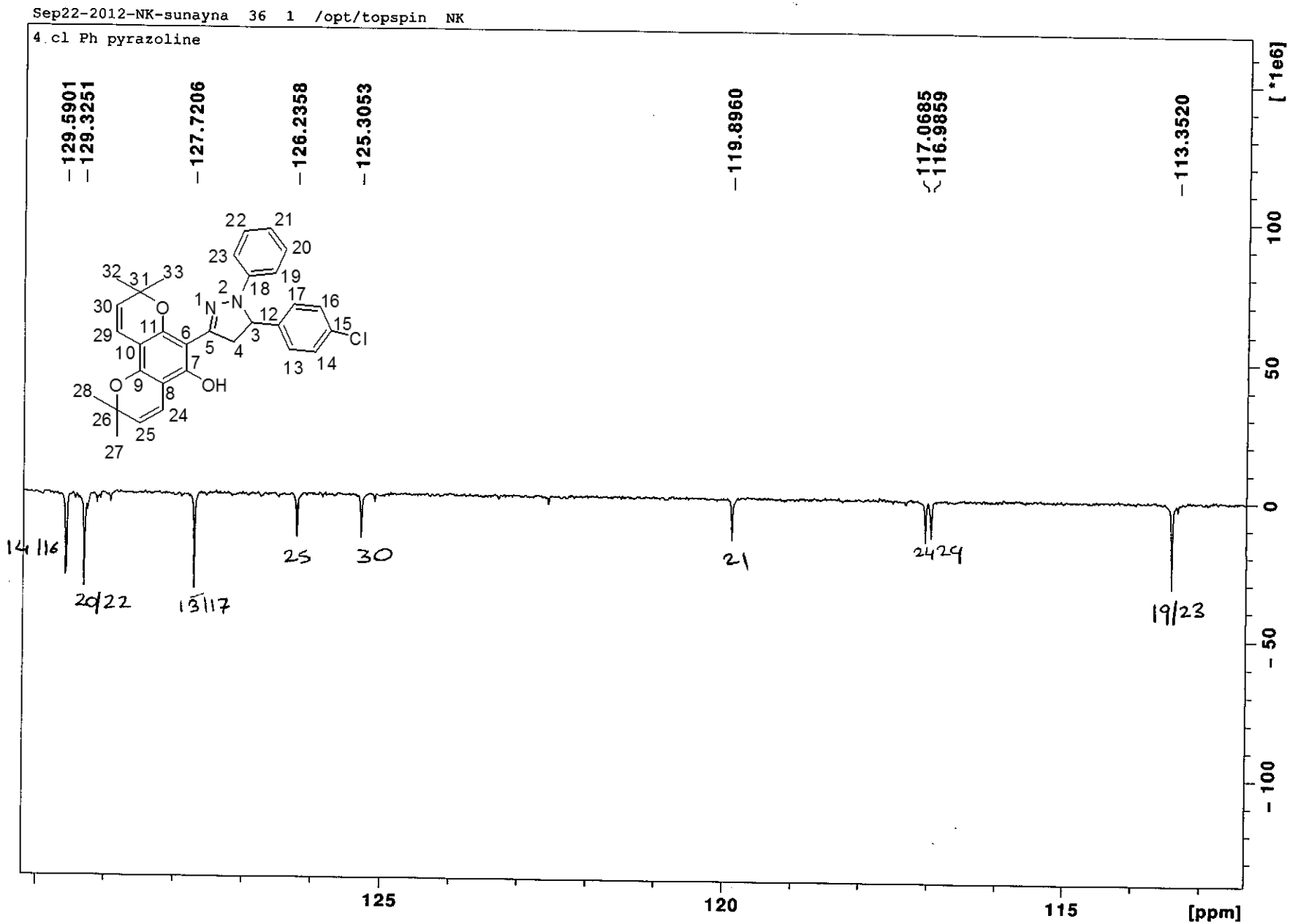
Expanded 1H NMR spectrum of 3-(4-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5b)



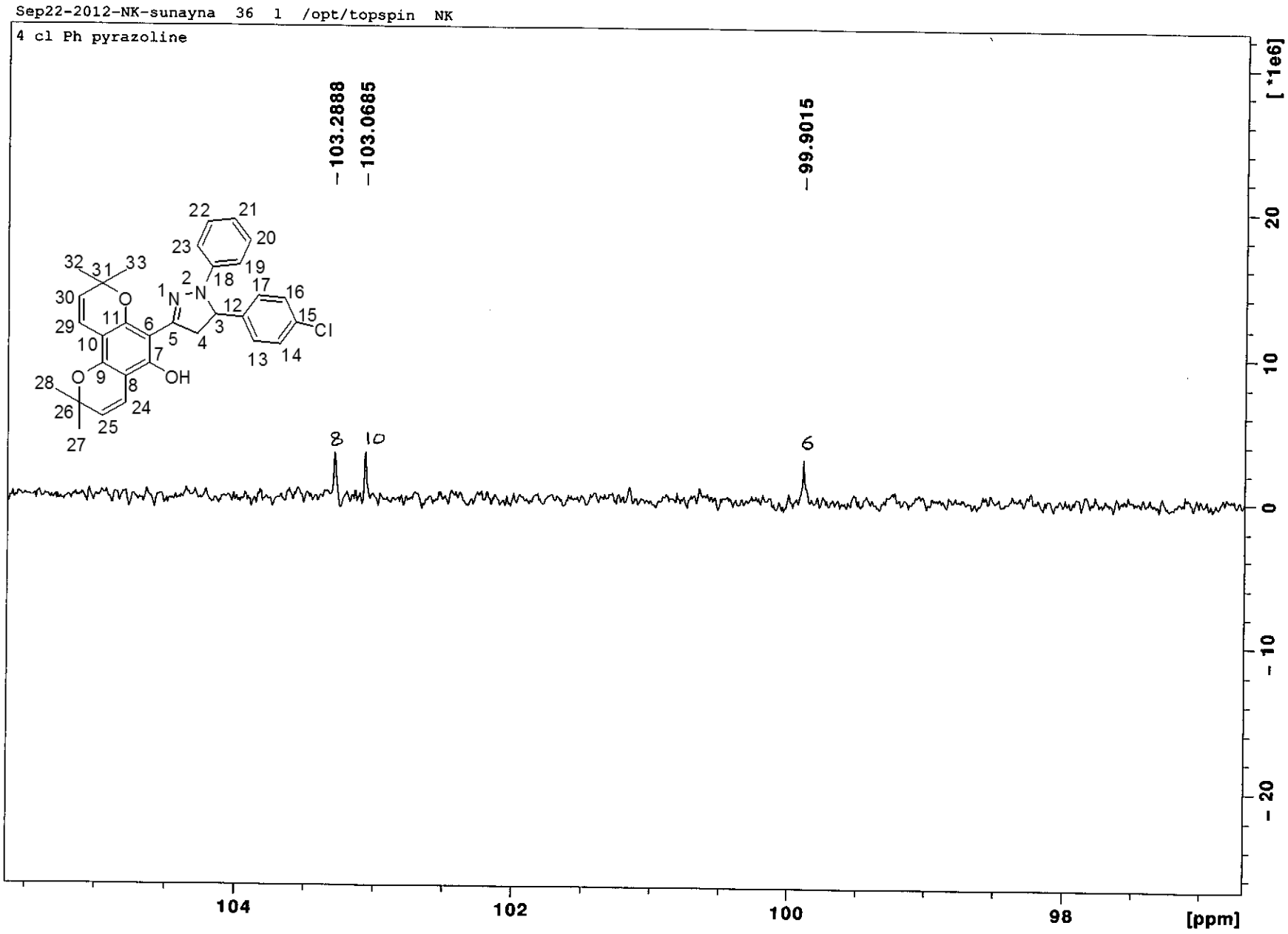
13C NMR spectrum of 3-(4-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5b)



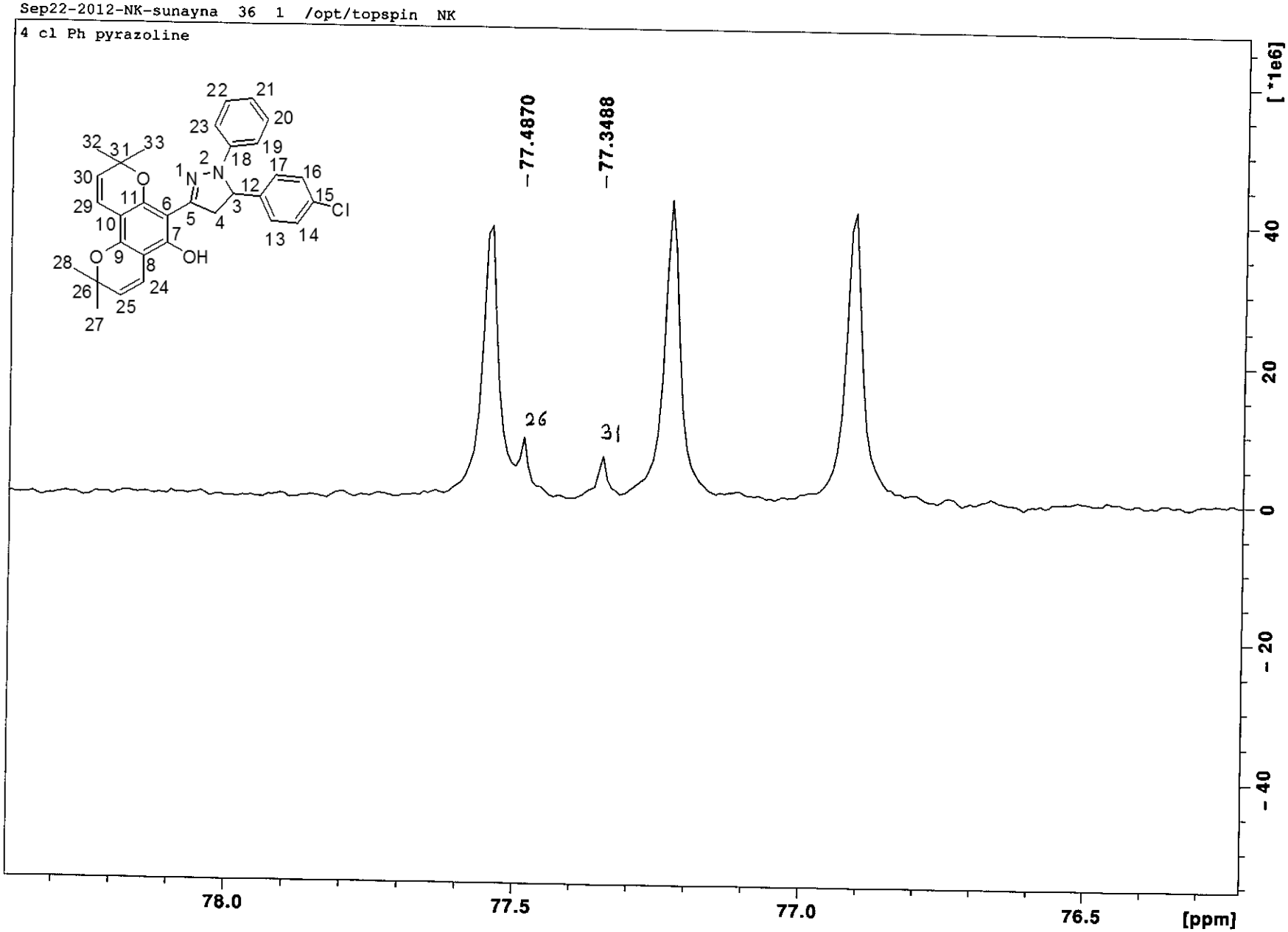
Expanded 13C NMR spectrum of 3-(4-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5b)



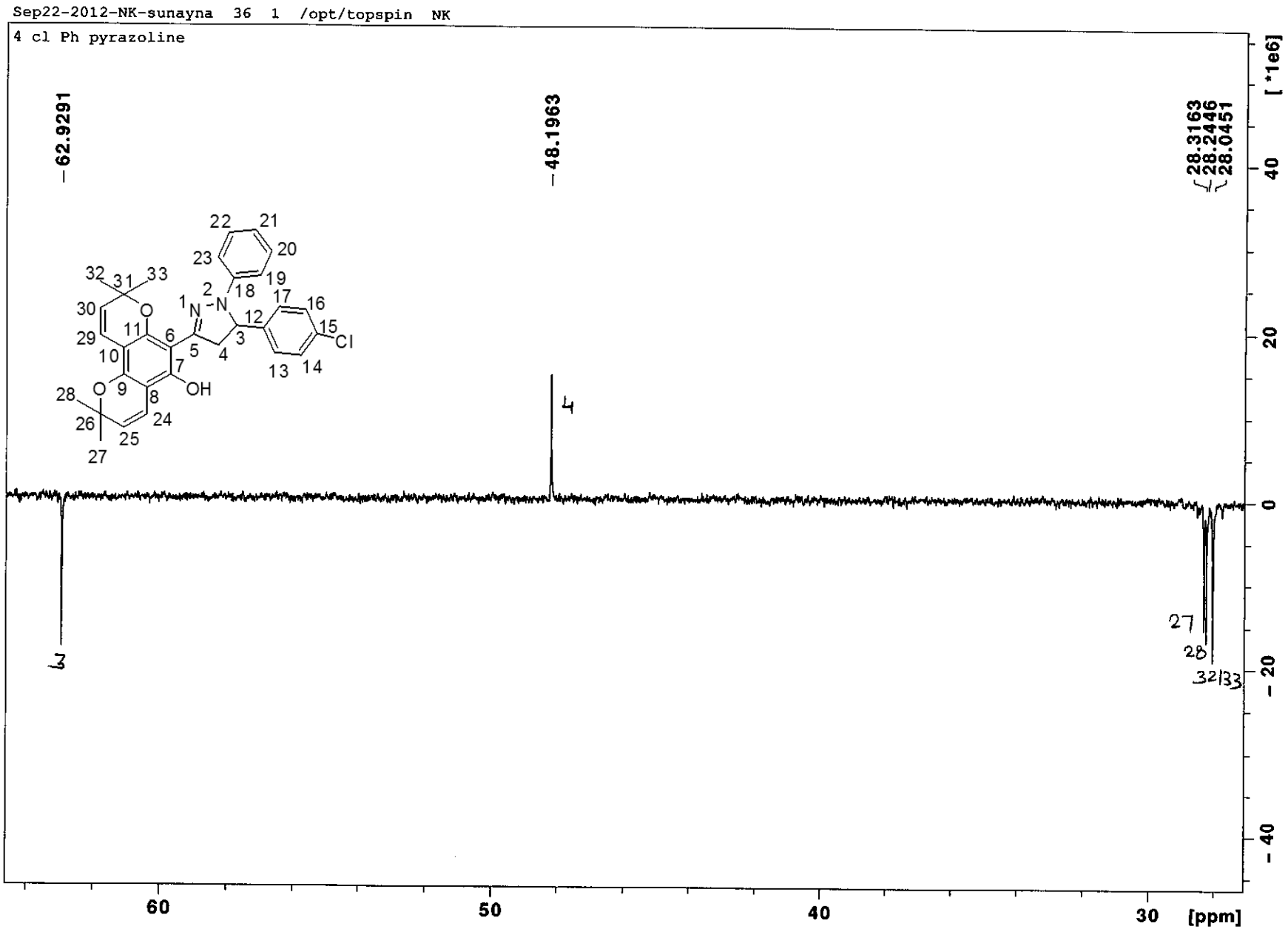
Expanded 13C NMR spectrum of 3-(4-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5b)



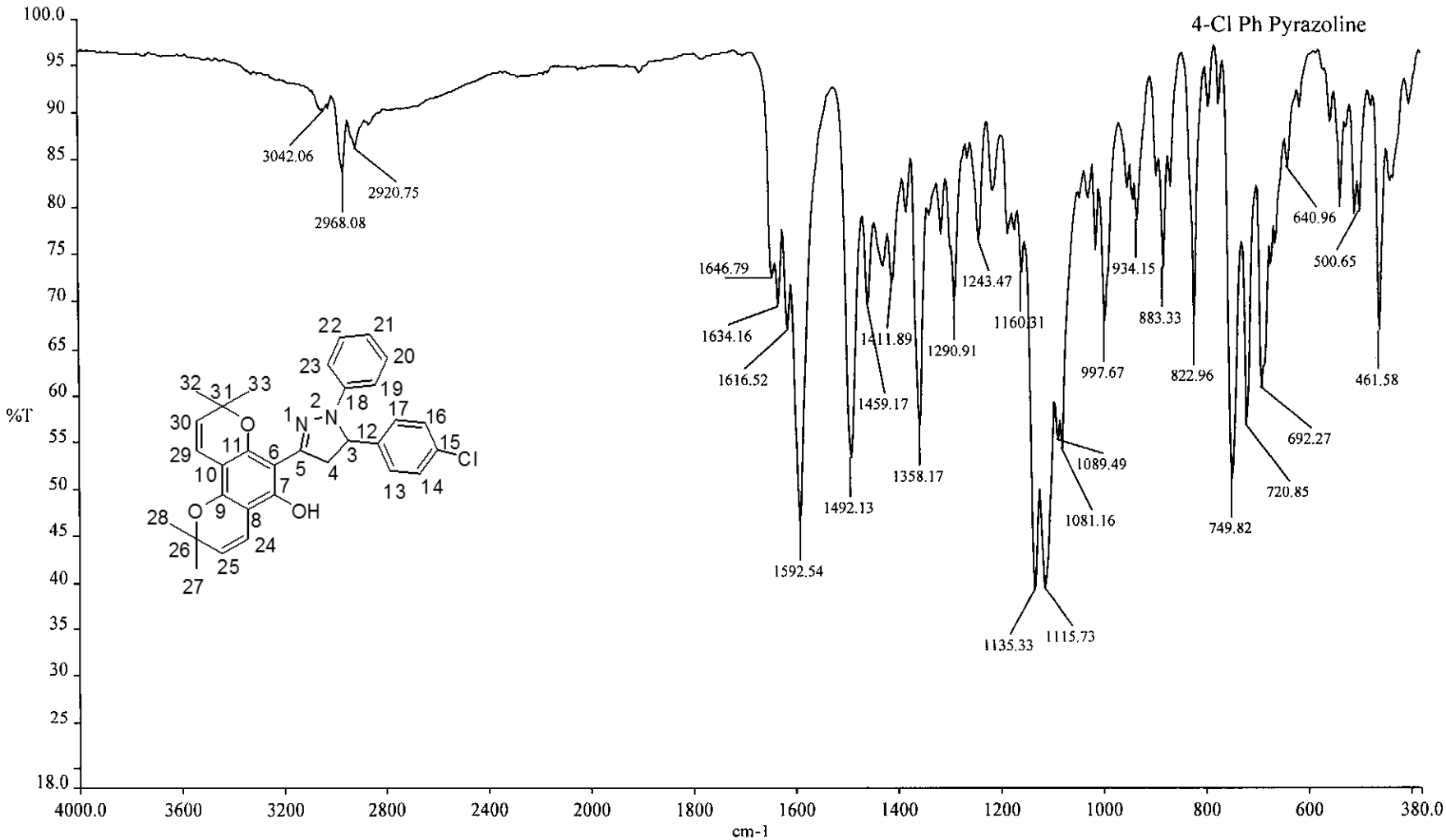
Expanded 13C NMR spectrum of 3-(4-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5b)



Expanded 13C NMR spectrum of 3-(4-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5b)



Expanded 13C NMR spectrum of 3-(4-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5b)

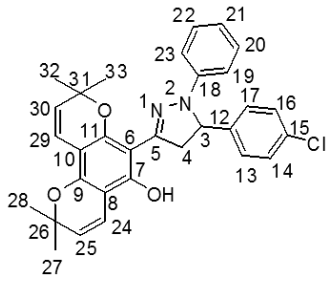


Infrared spectrum of 3-(4-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5b)

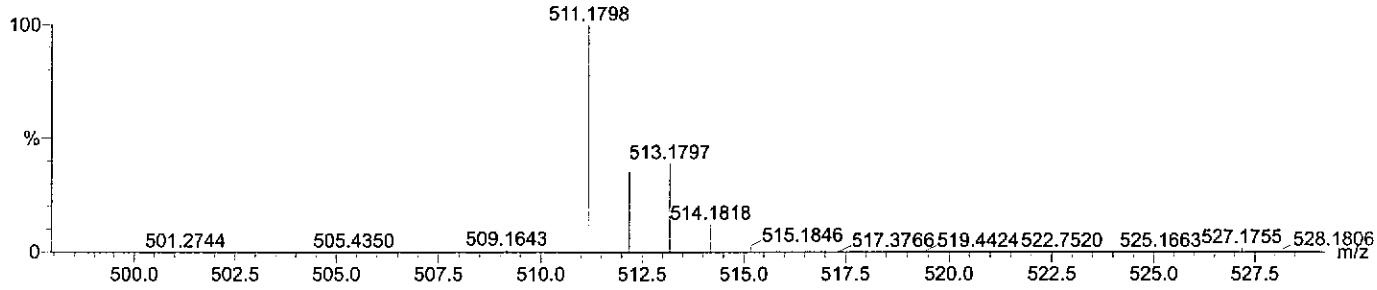
Elemental Composition Report **Page 1**

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

Monoisotopic Mass, Even Electron Ions
22 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-35 H: 20-30 N: 0-4 O: 0-4 Cl: 1-1
4 2 (0.017) Cm (1:31)
TOF MS ES-

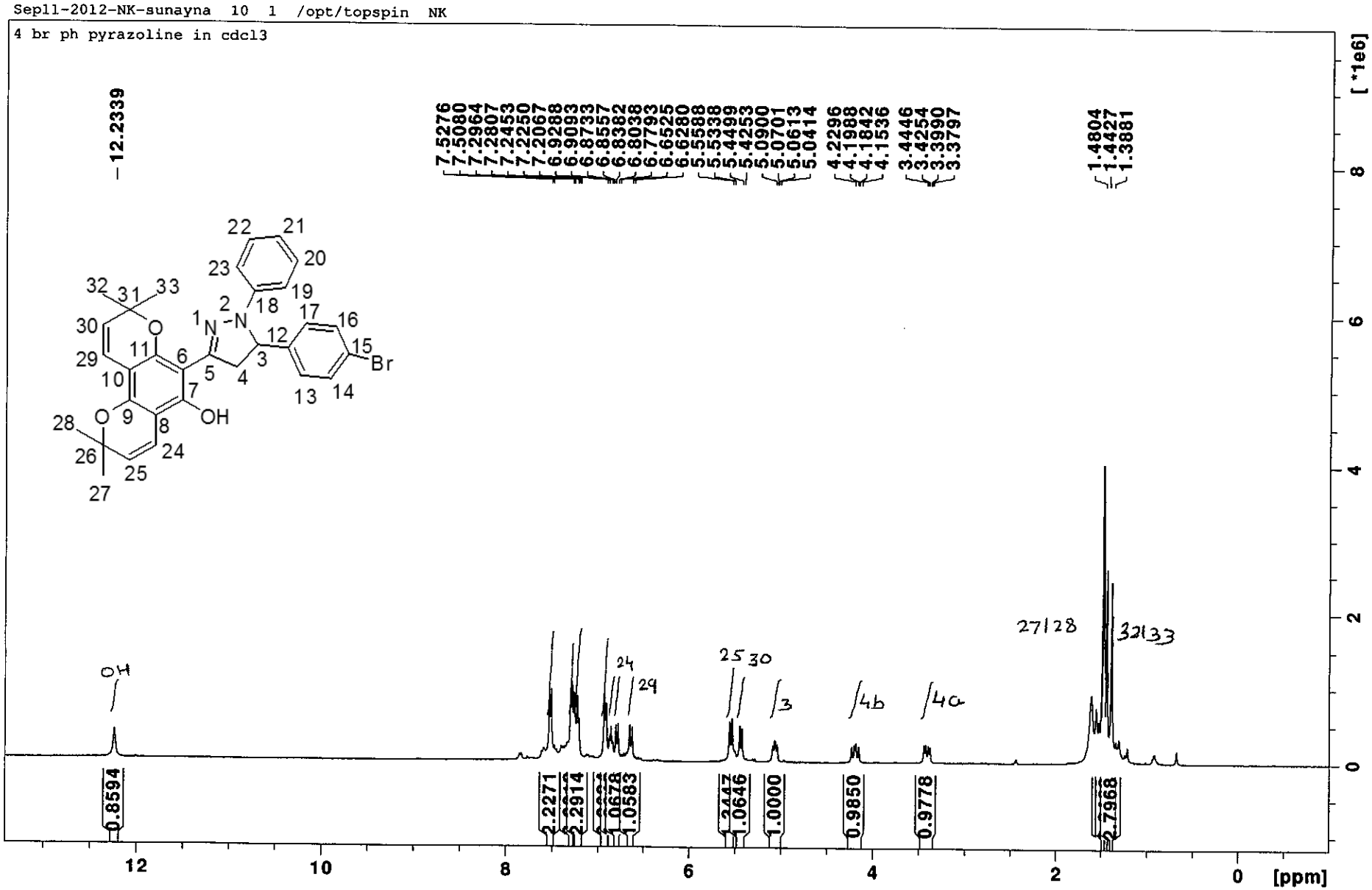


1.26e+005

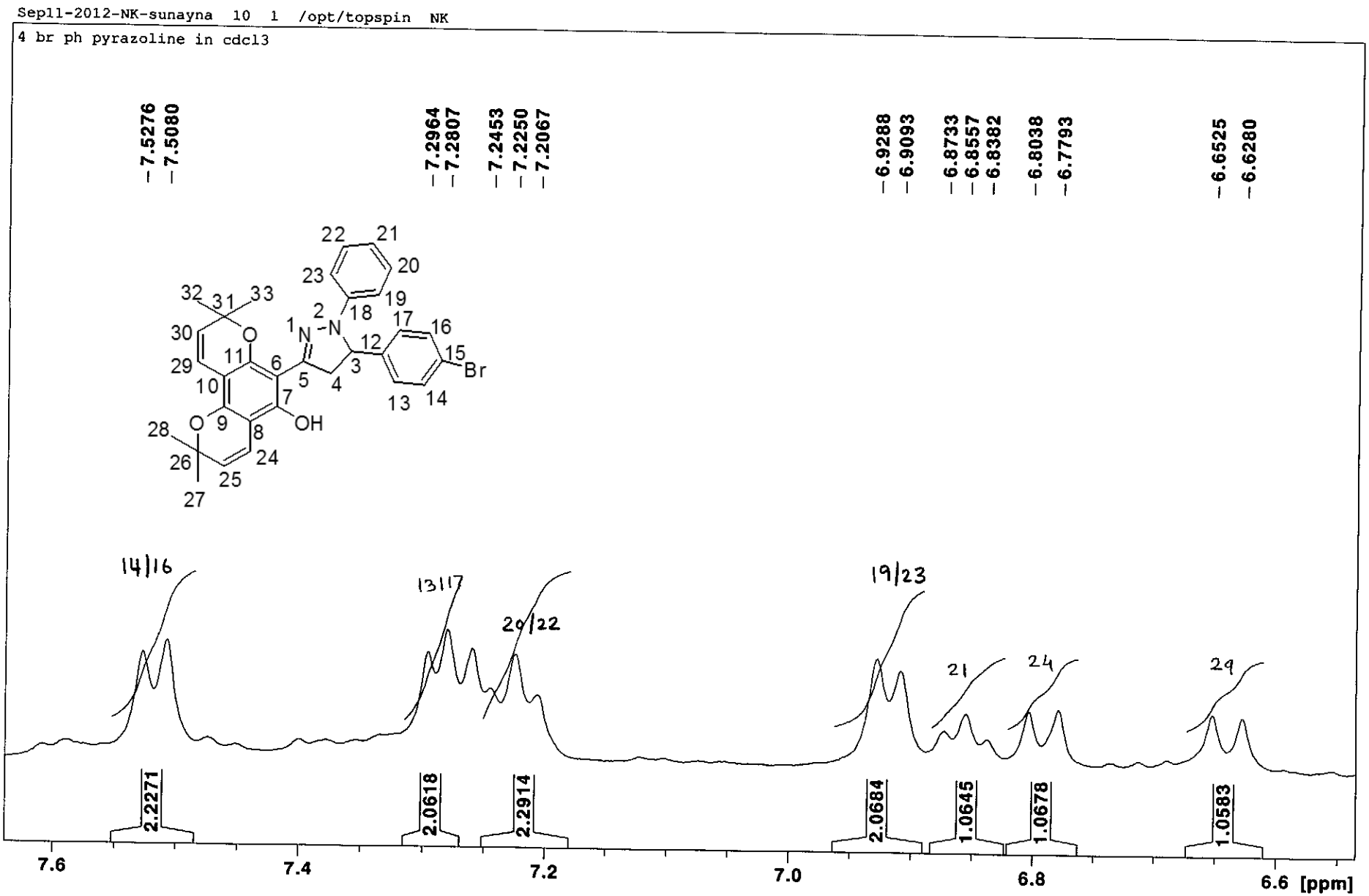


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
511.1798	511.1788	1.0	2.0	18.5	484.2	0.0	C31 H28 N2 O3 Cl

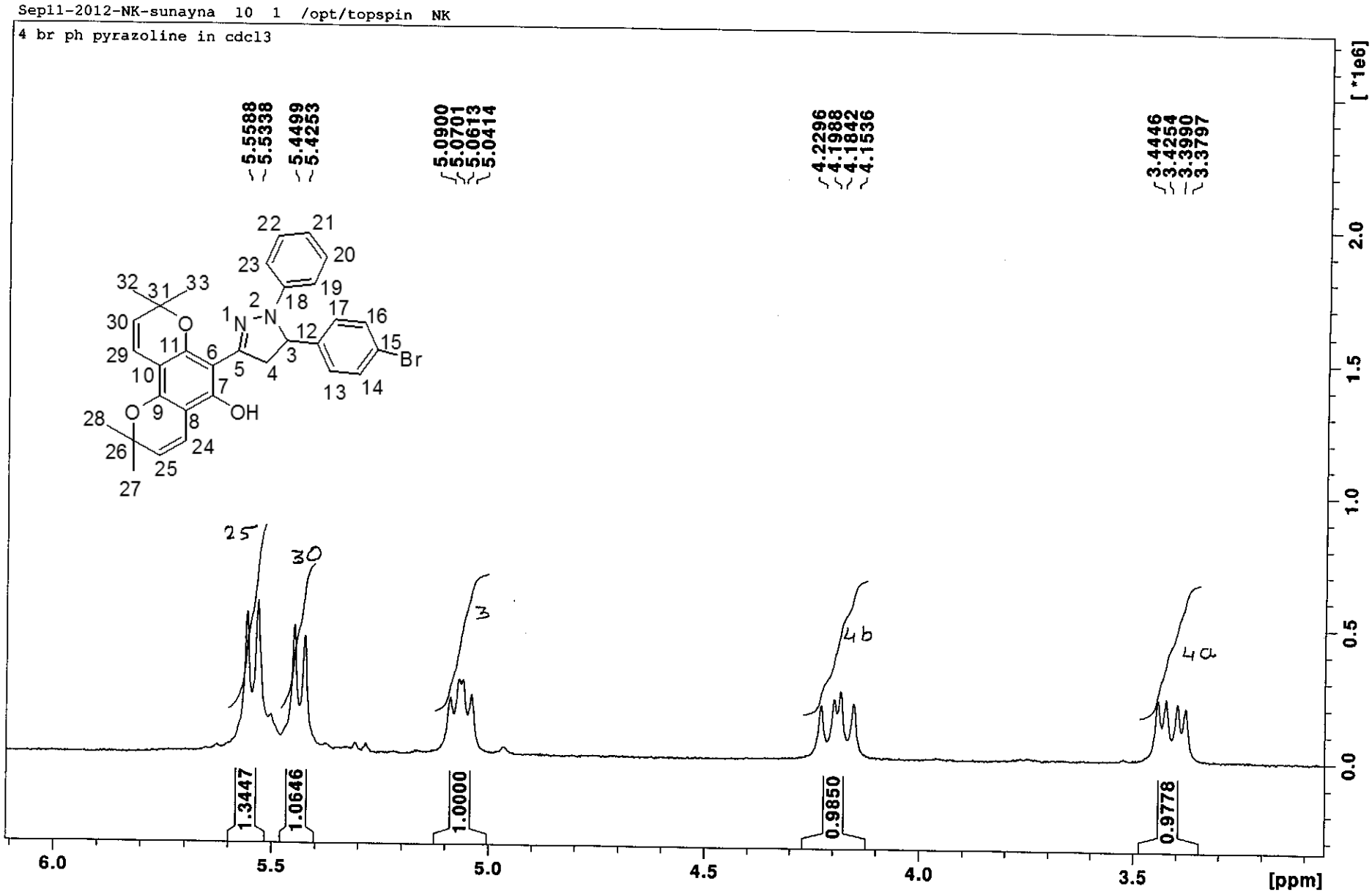
HRMS spectrum of 3-(4-chlorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5b)



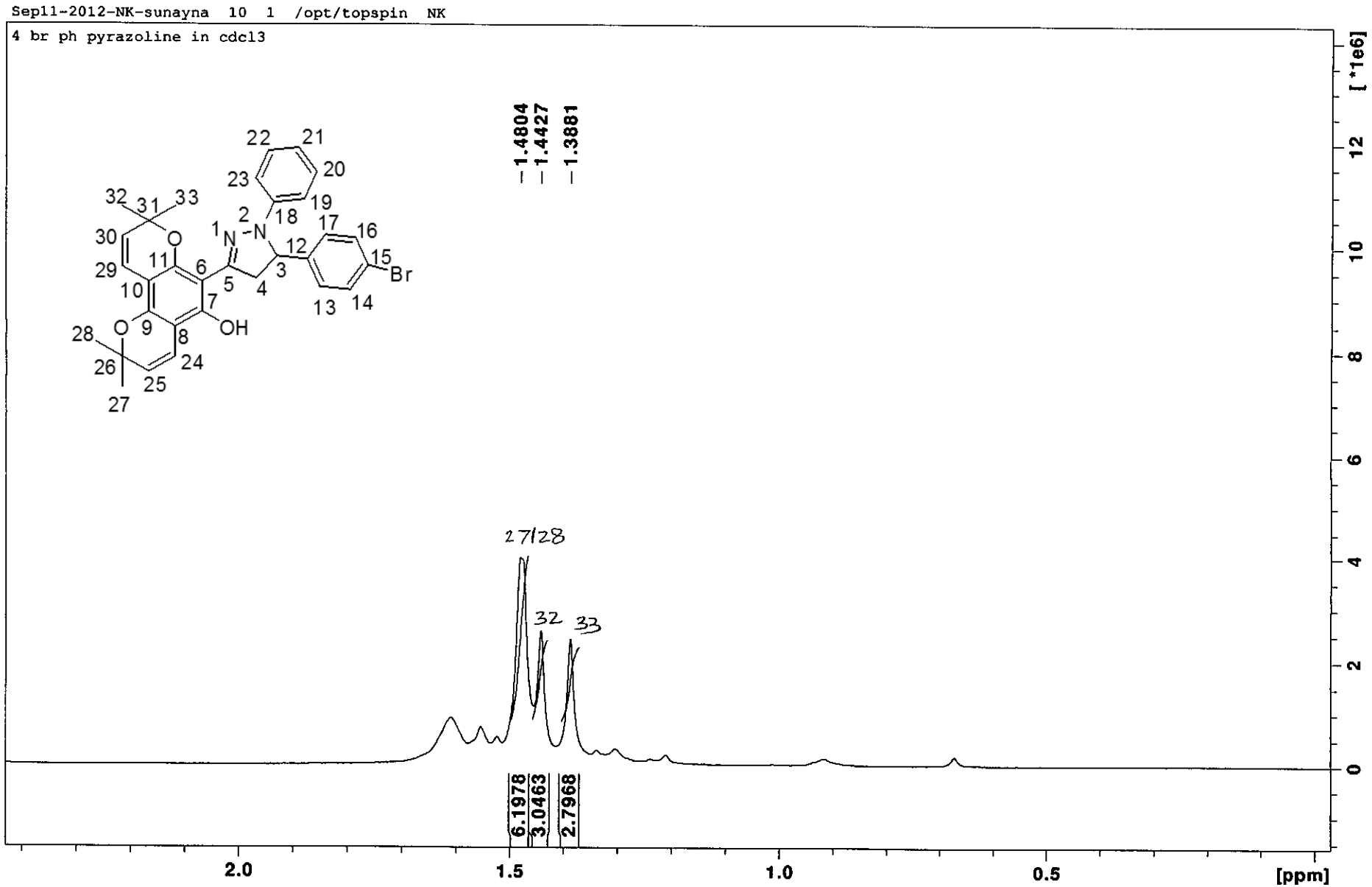
1H NMR spectrum of 3-(4-bromophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5c)



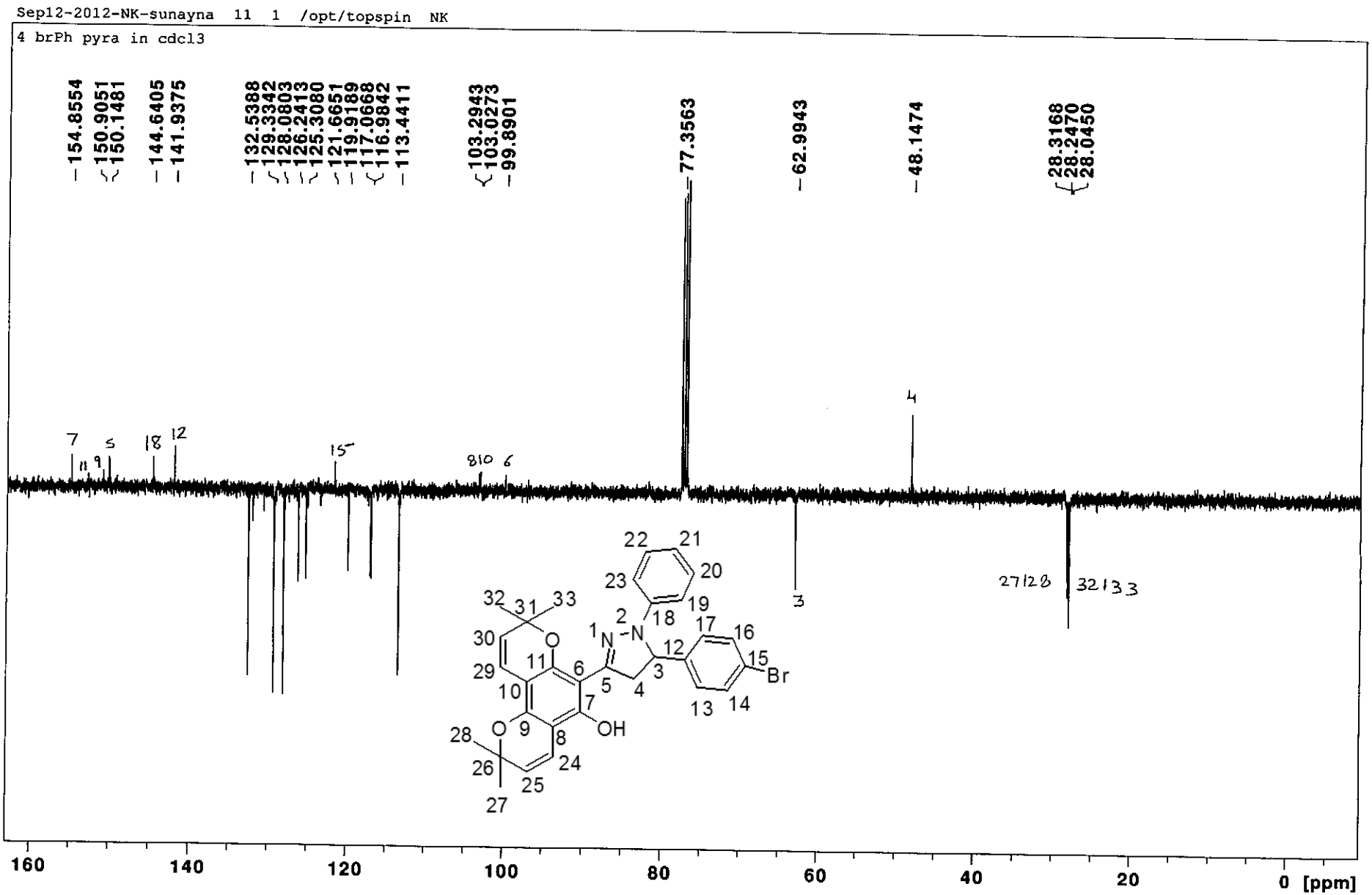
Expanded 1H NMR spectrum of 3-(4-bromophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5c)



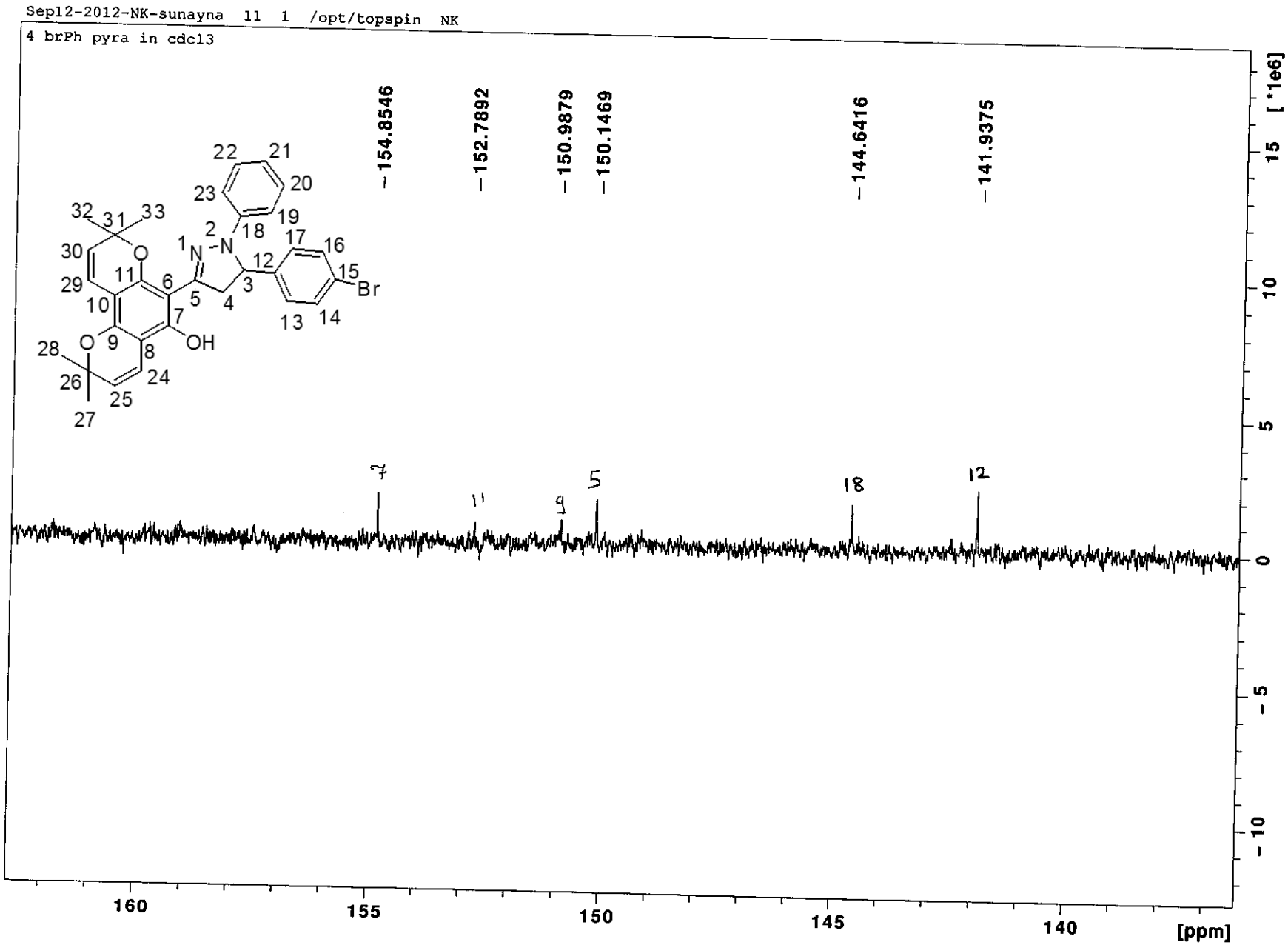
Expanded 1H NMR spectrum of 3-(4-bromophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5c)



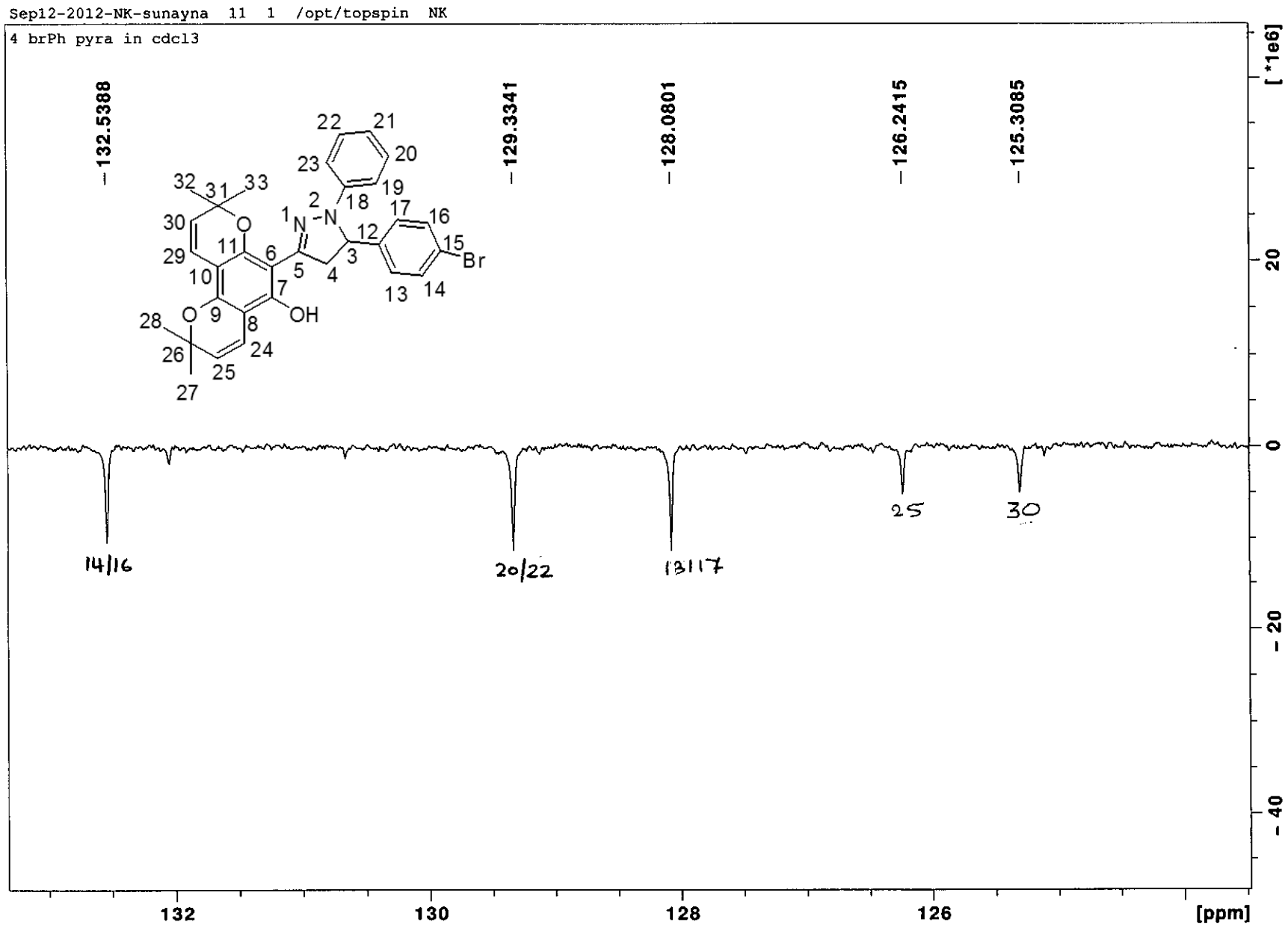
Expanded 1H NMR spectrum of 3-(4-bromophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5c)



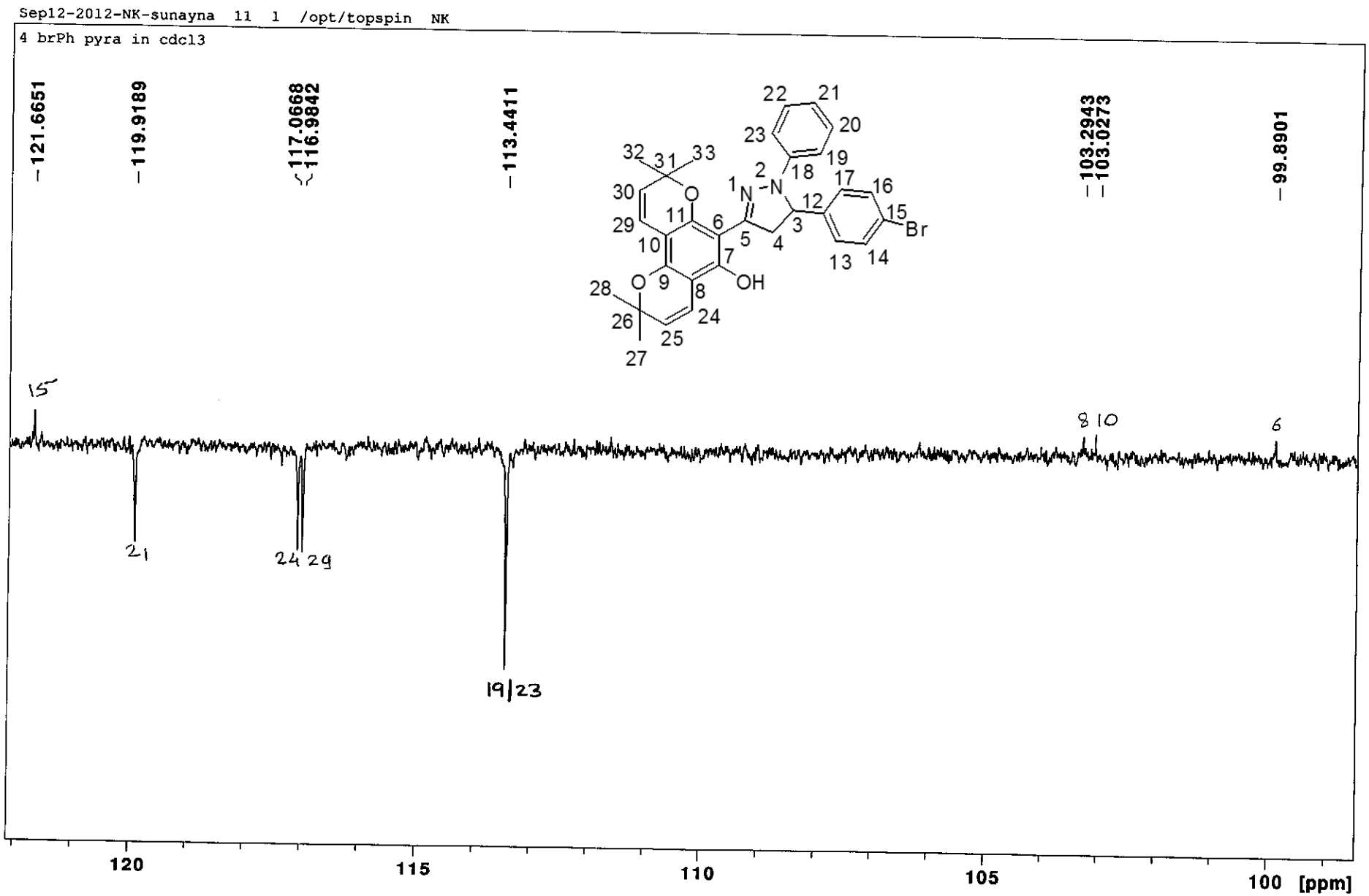
13C NMR spectrum of 3-(4-bromophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5c)



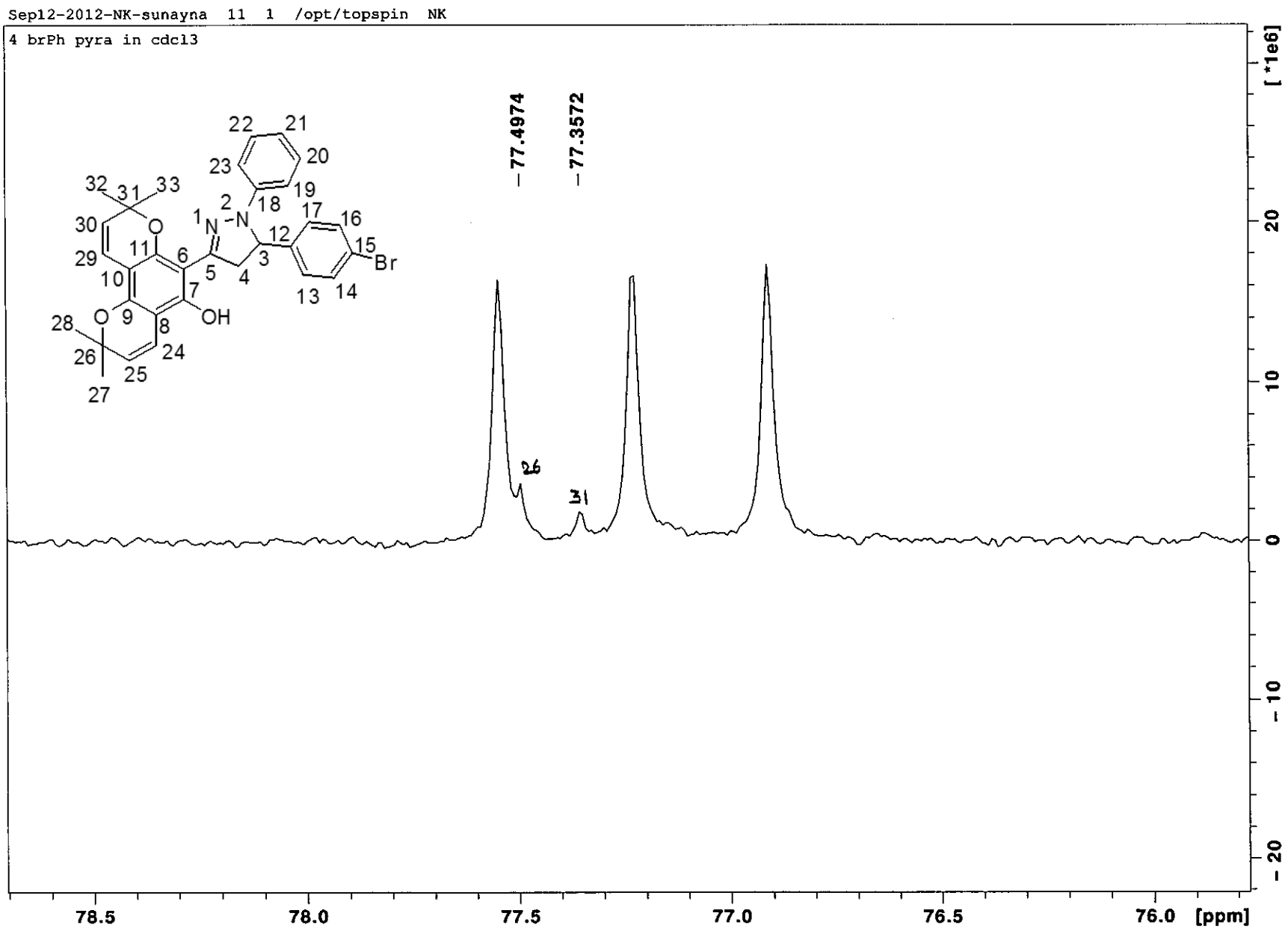
Expanded 13C NMR spectrum of 3-(4-bromophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5c)



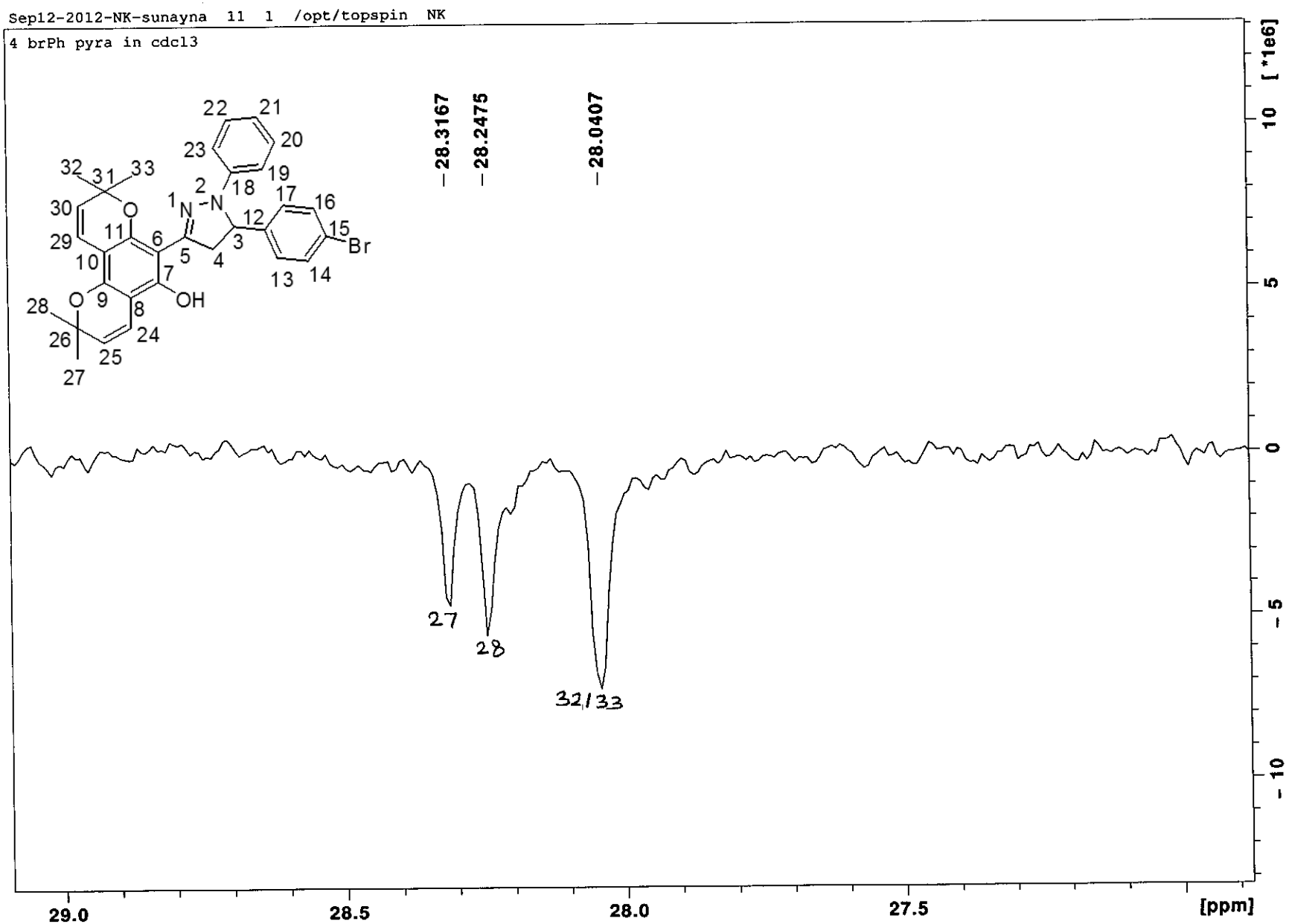
Expanded 13C NMR spectrum of 3-(4-bromophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5c)



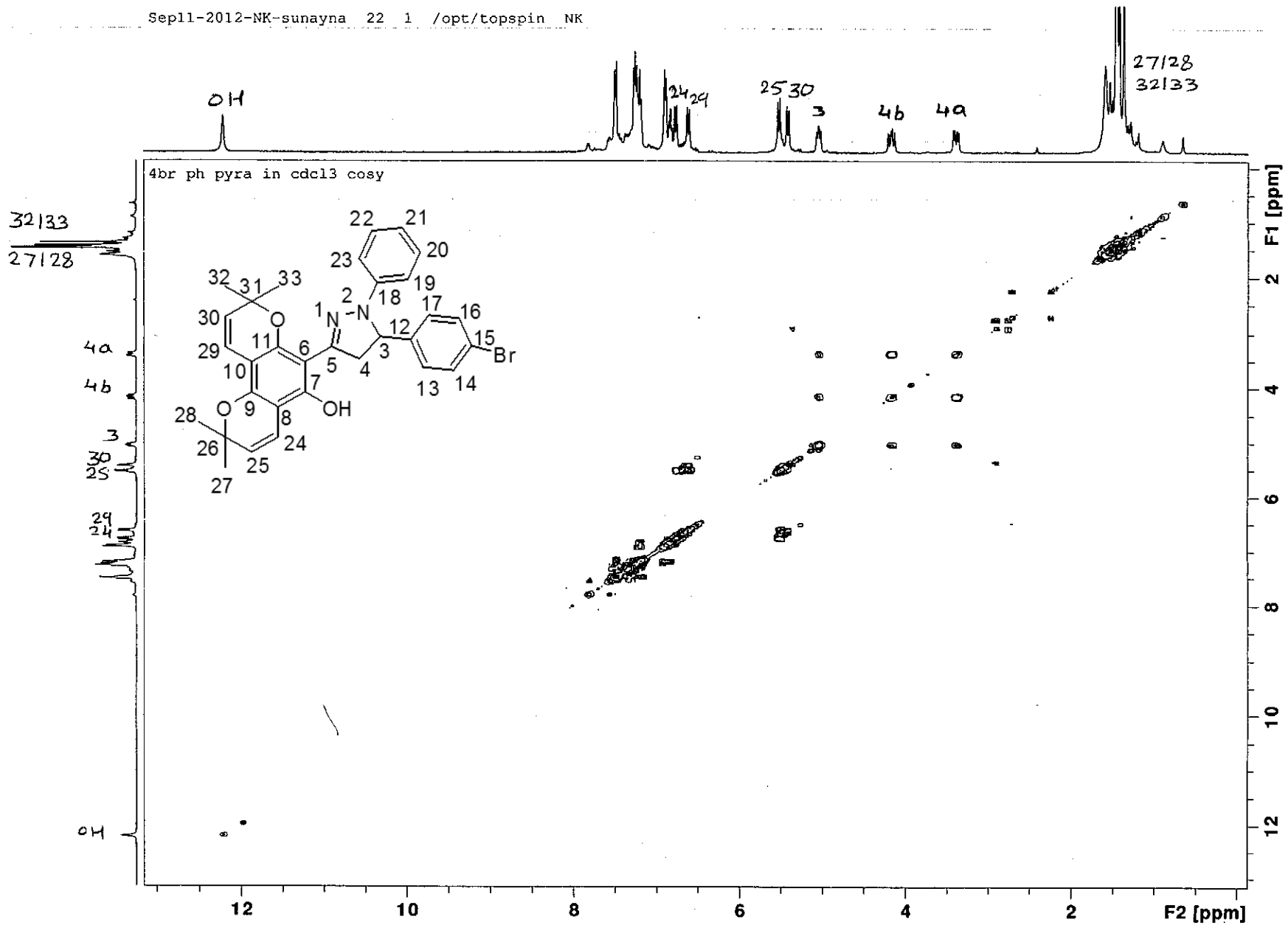
Expanded 13C NMR spectrum of 3-(4-bromophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5c)



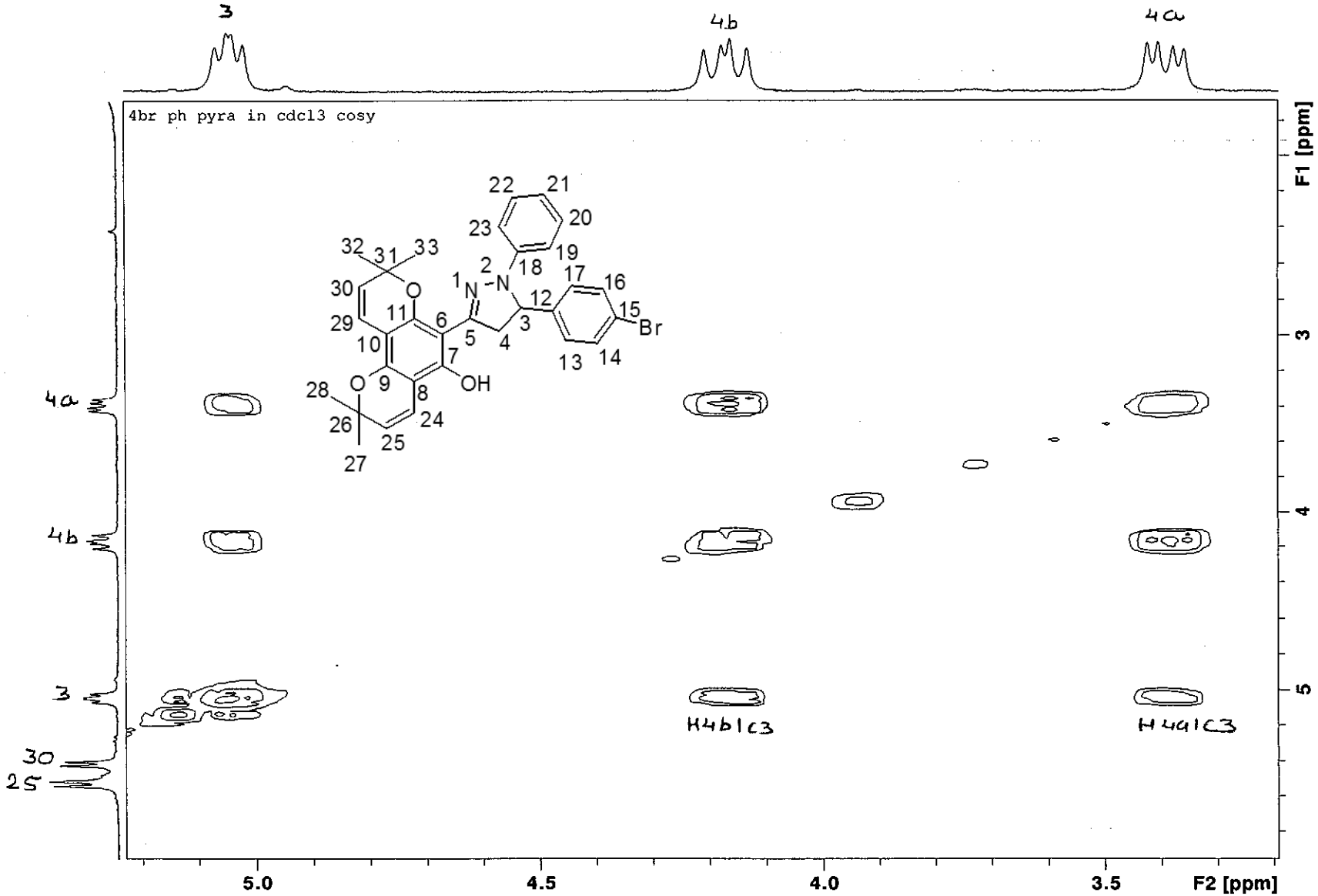
Expanded 13C NMR spectrum of 3-(4-bromophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5c)



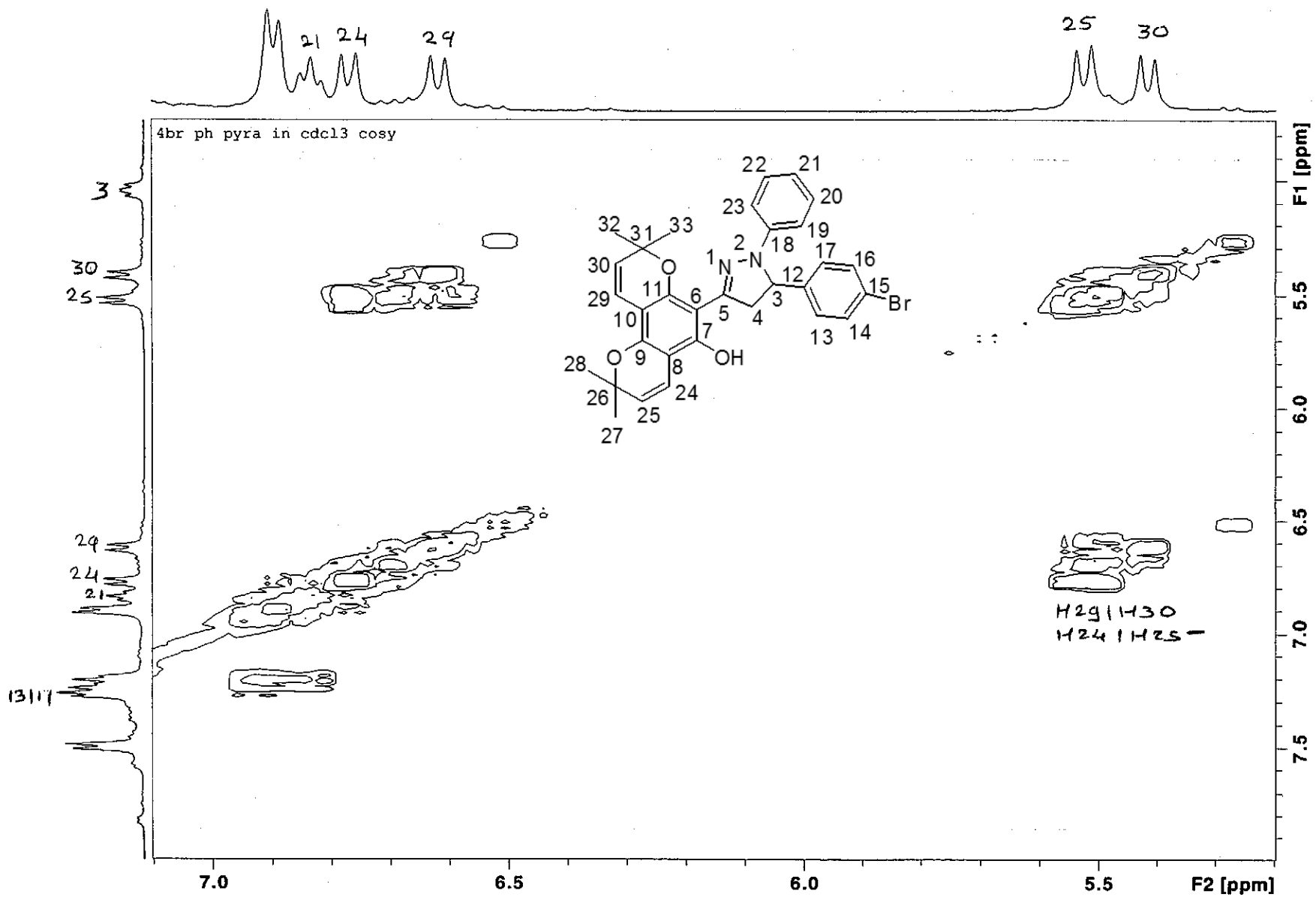
Expanded 13C NMR spectrum of 3-(4-bromophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5c)



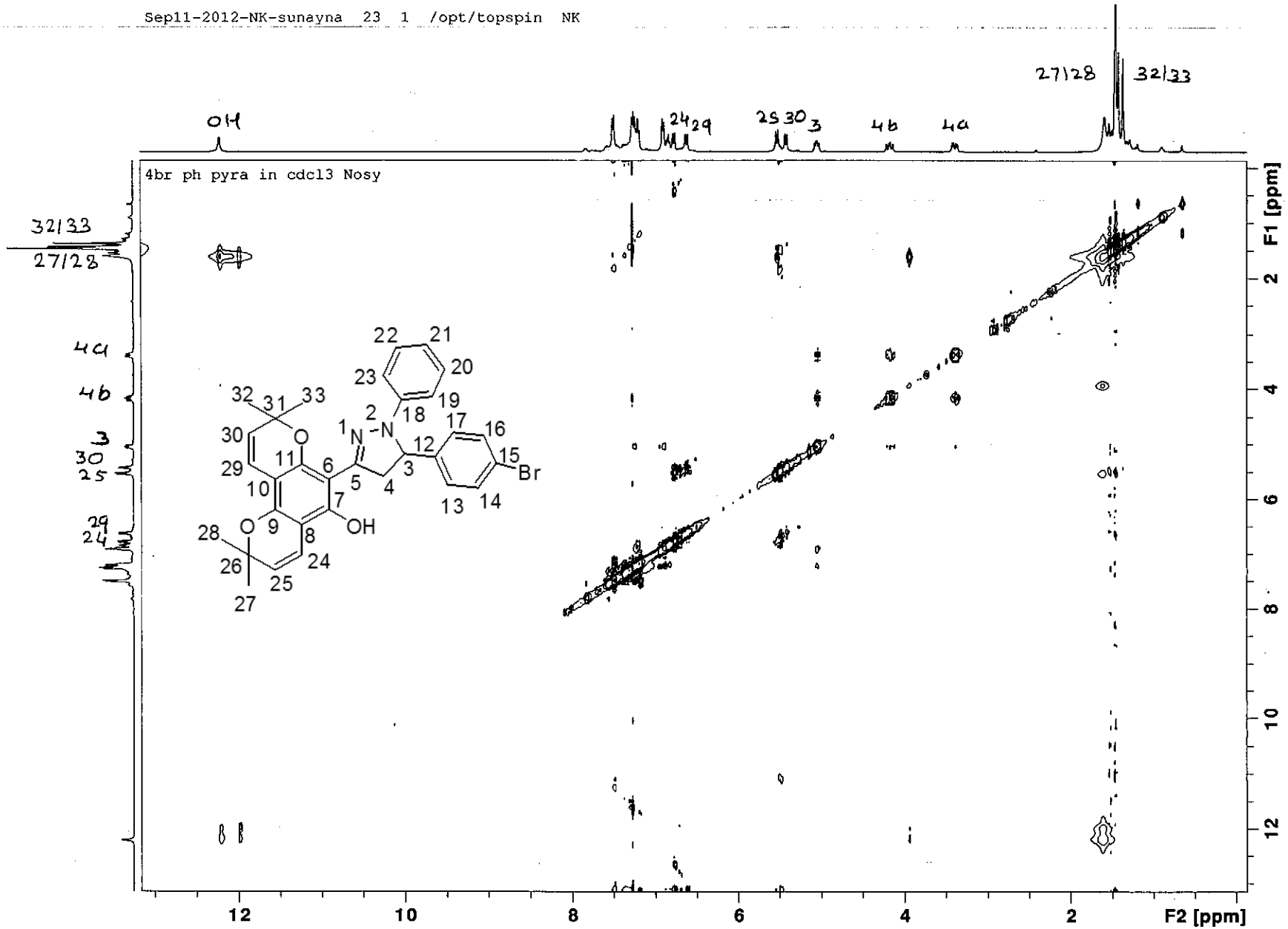
COSY NMR spectrum of 3-(4-bromophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5c)



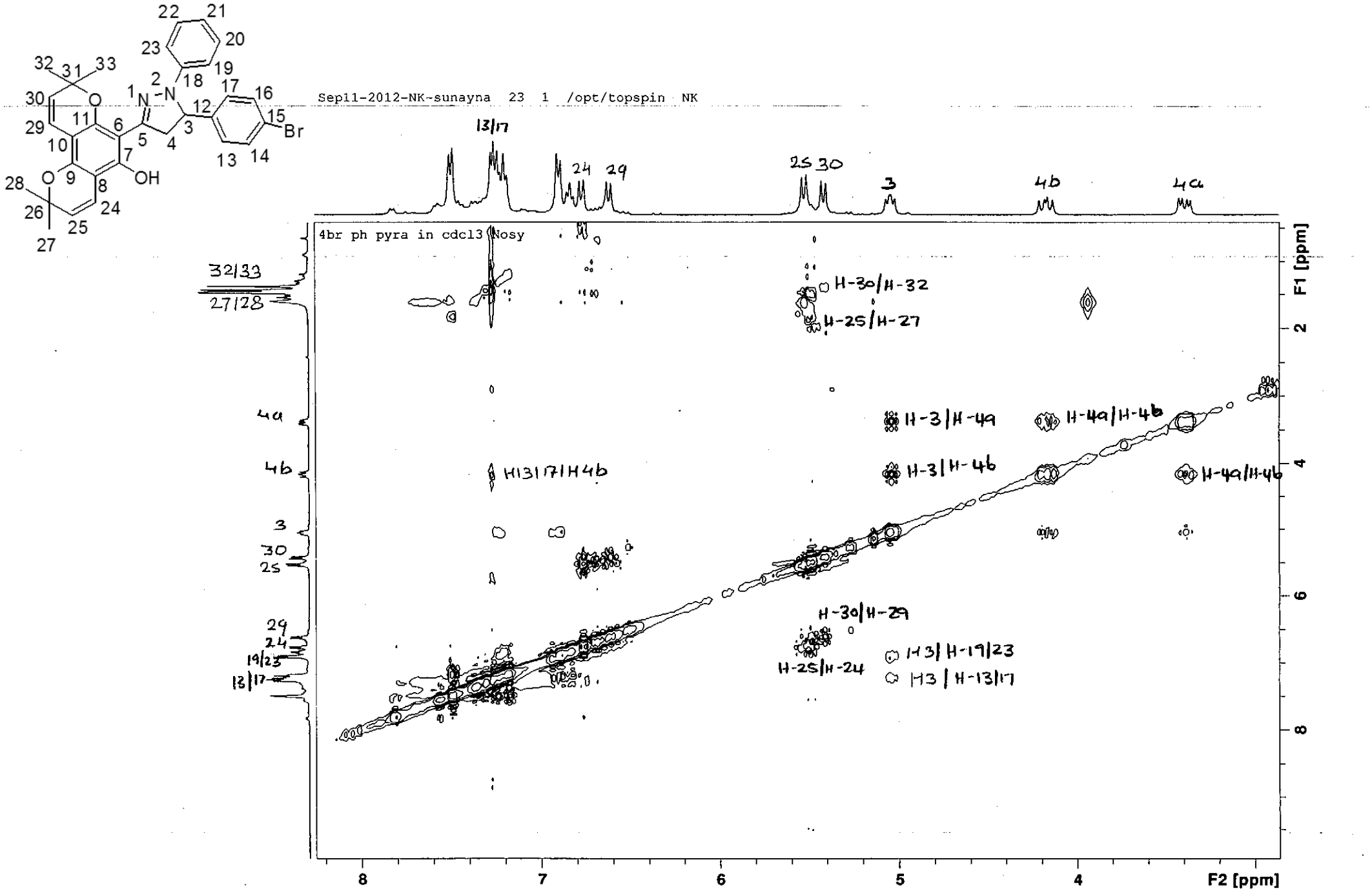
Expanded COSY NMR spectrum of 3-(4-bromophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5c)



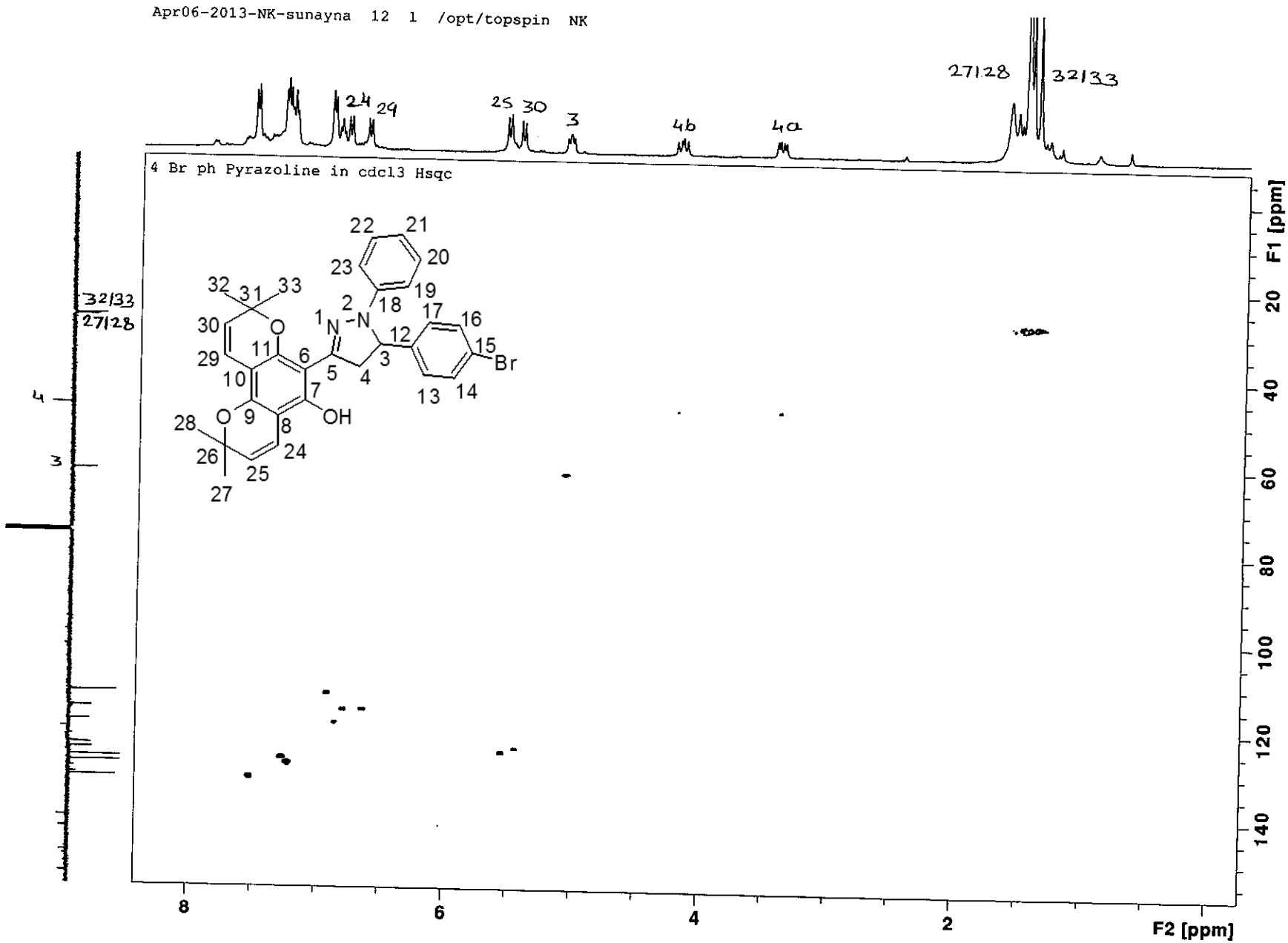
Expanded COSY NMR spectrum of 3-(4-bromophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5c)



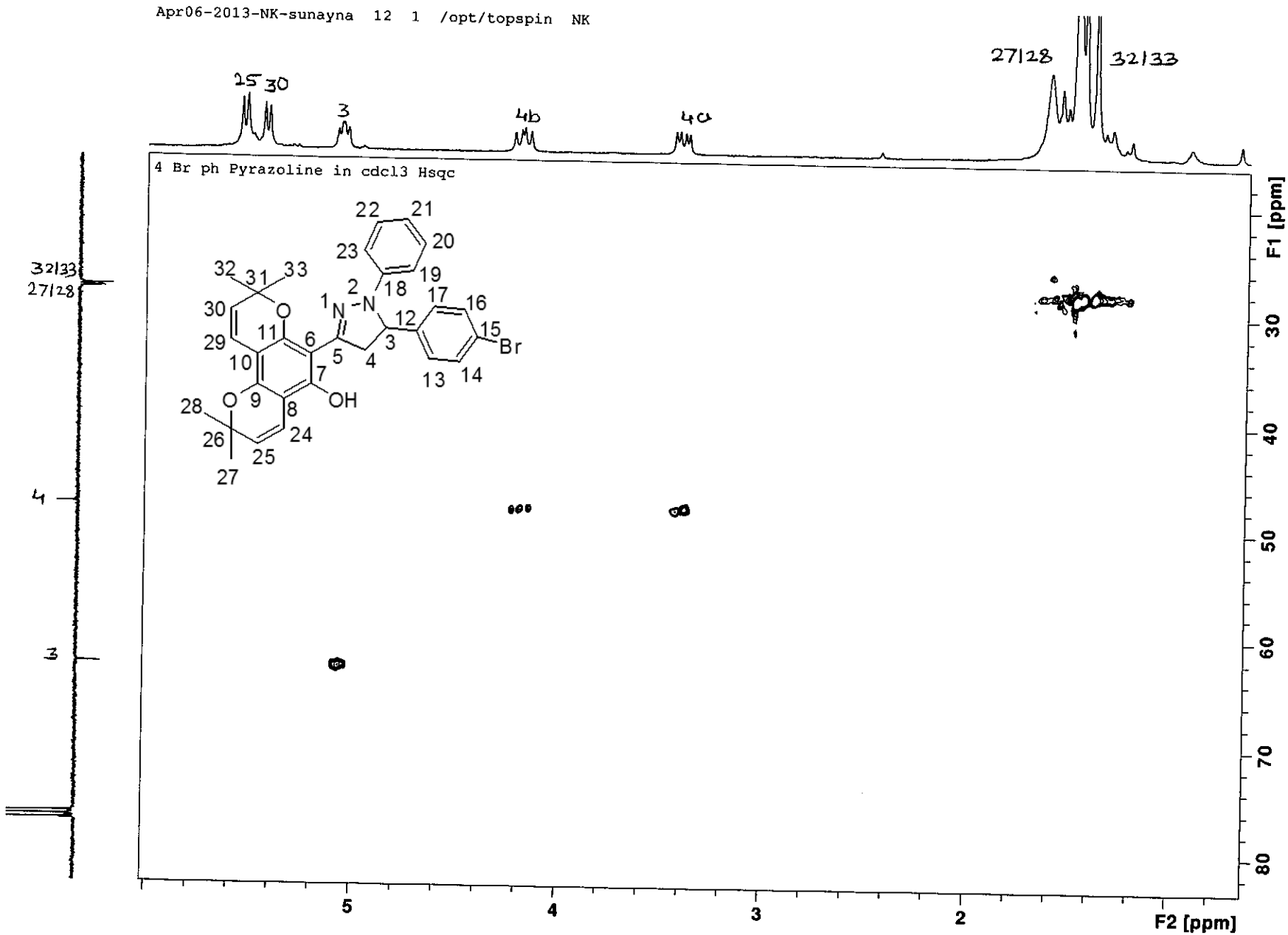
NOESY spectrum of 3-(4-bromophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5c)



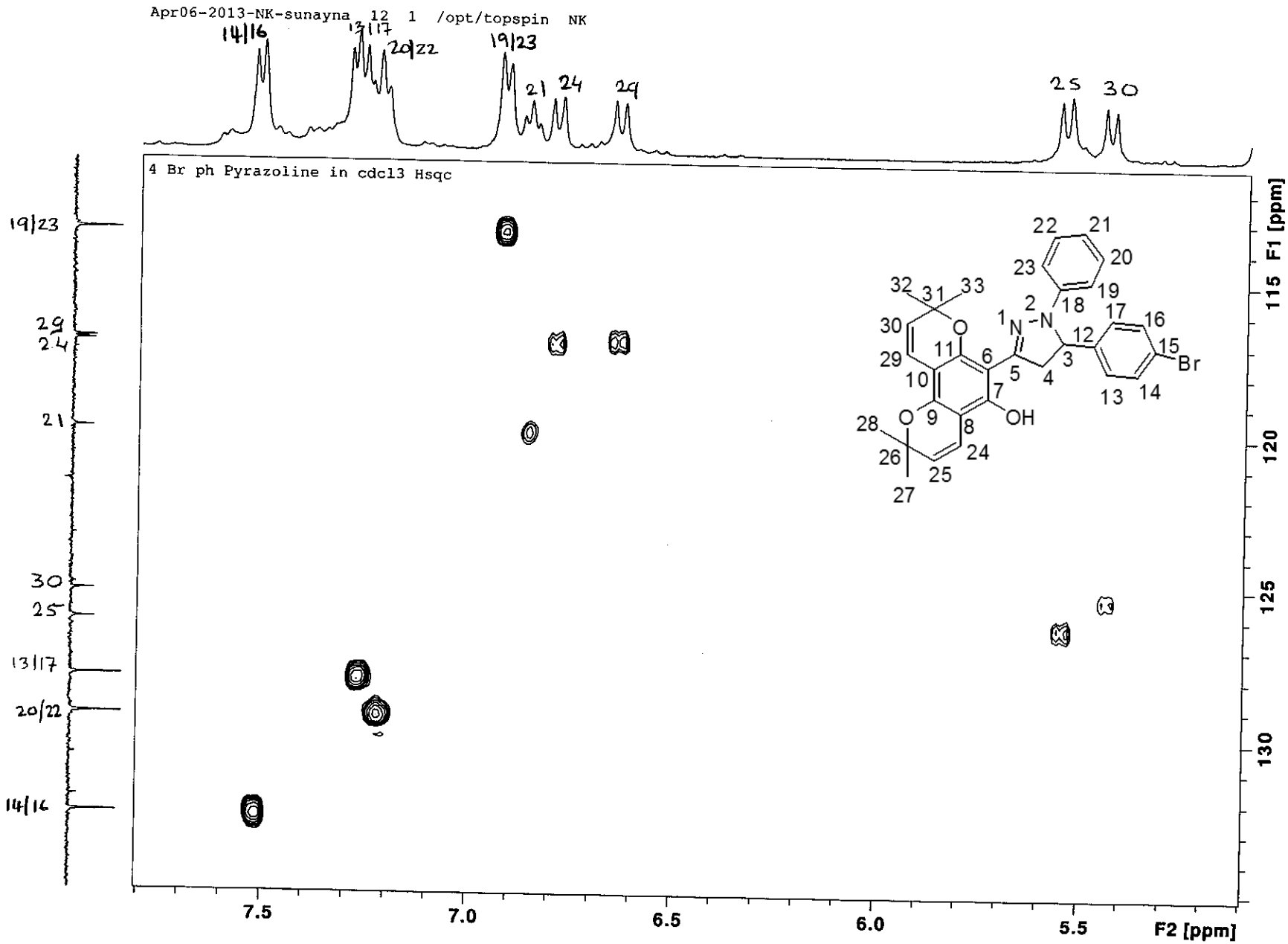
Expanded NOESY spectrum of 3-(4-bromophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5c)



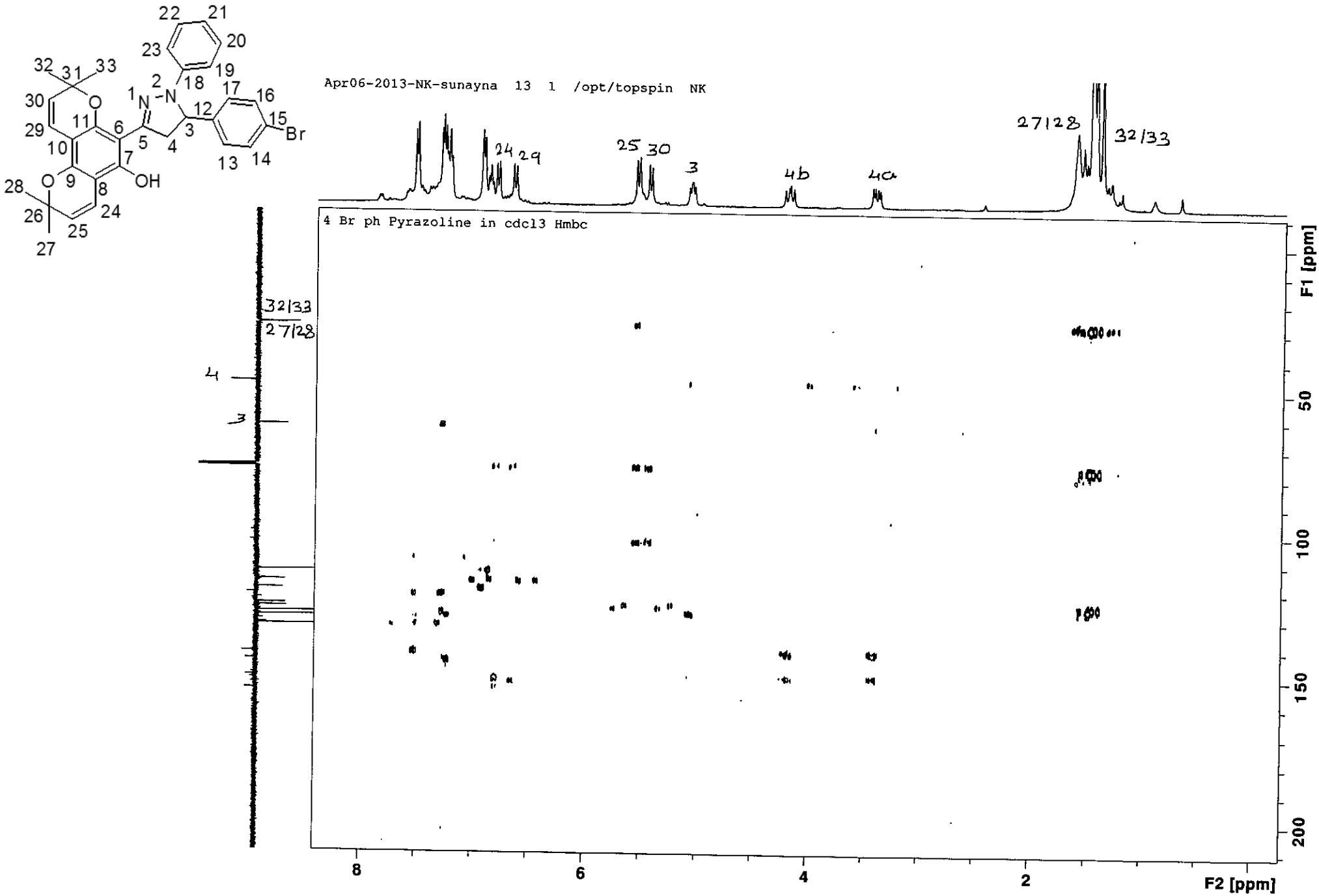
HSQC spectrum of 3-(4-bromophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5c)



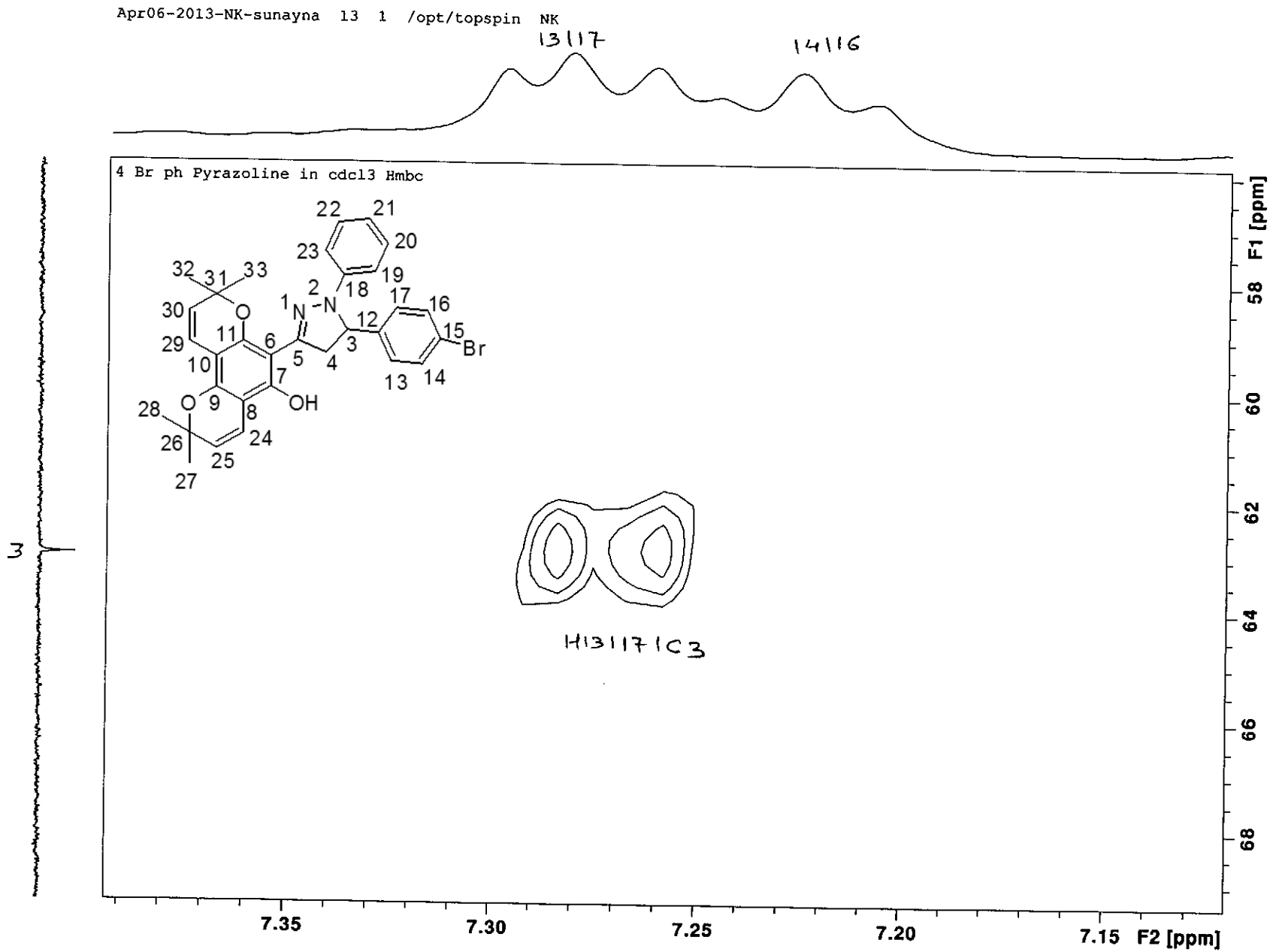
HSQC spectrum of 3-(4-bromophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5c)



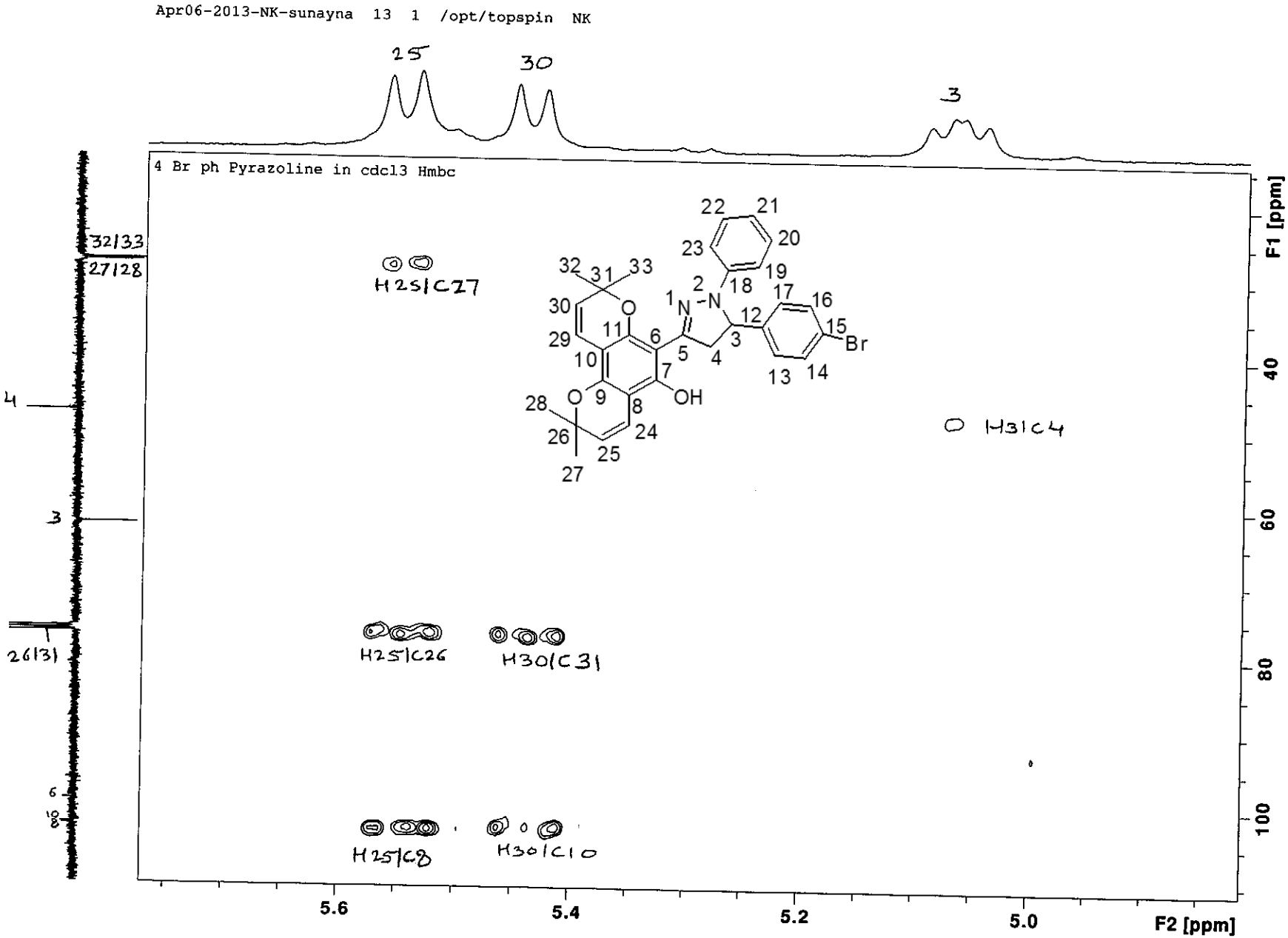
Expanded HSQC spectrum of 3-(4-bromophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5c)



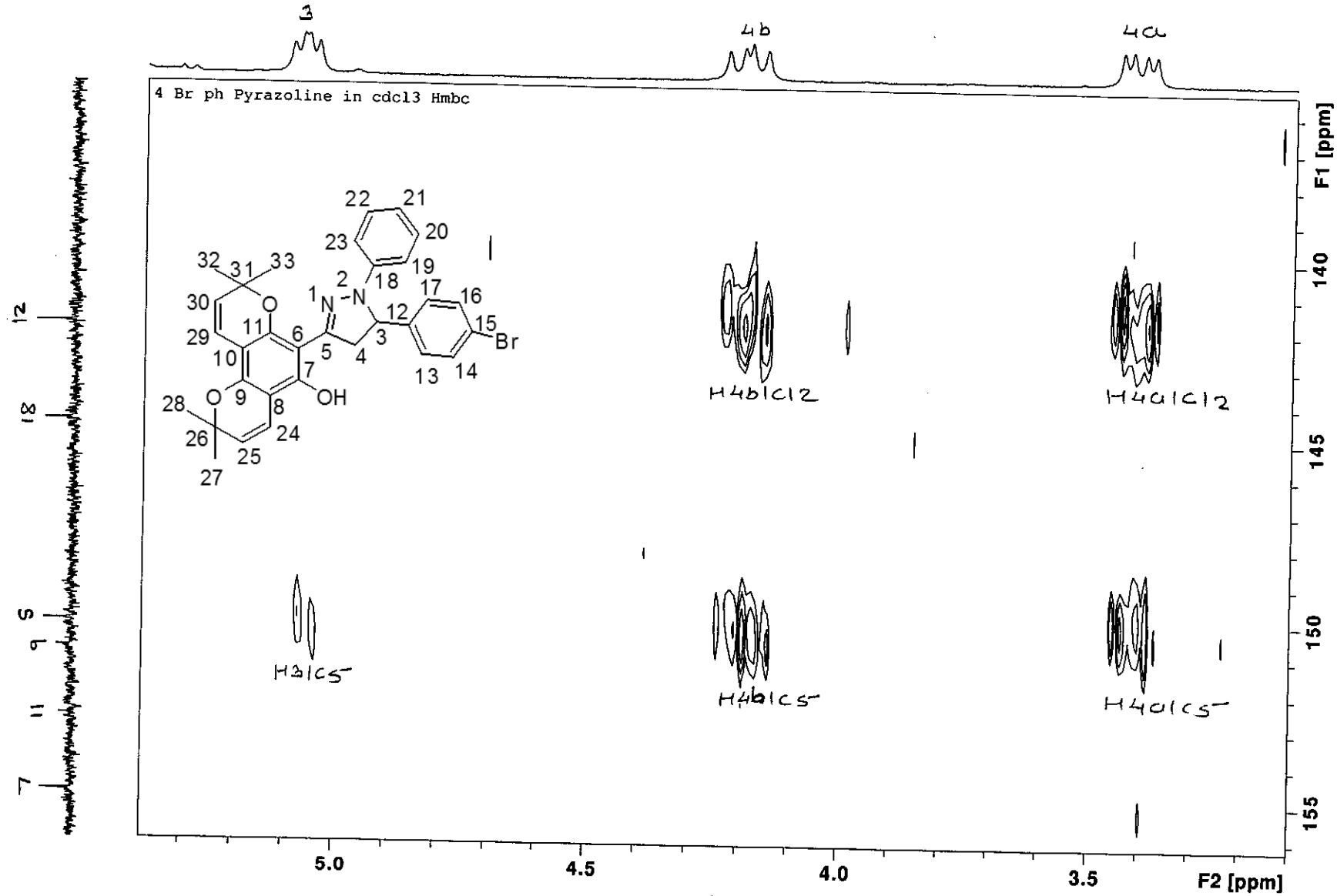
HMBC spectrum of 3-(4-bromophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5c)



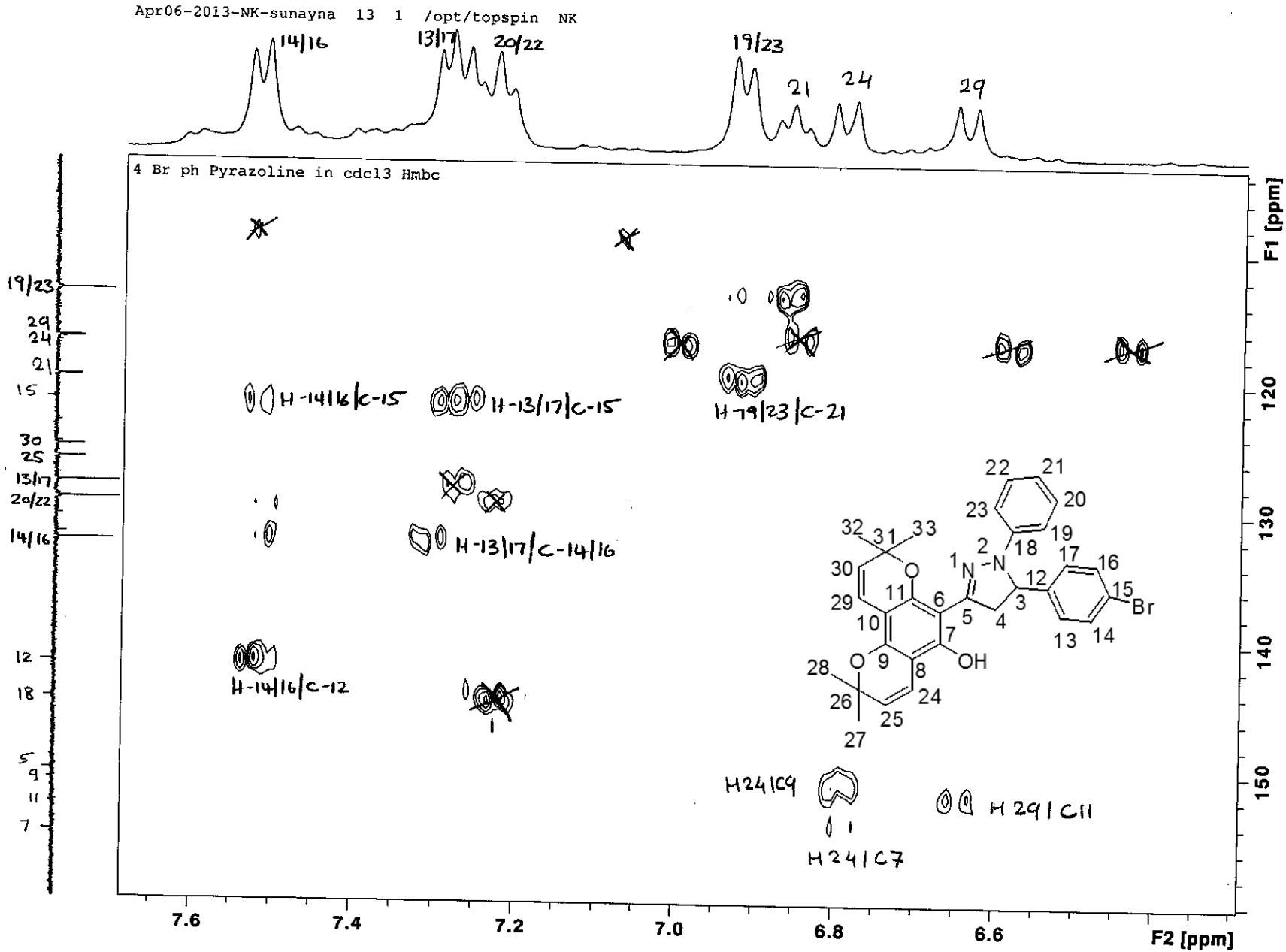
Expanded HMBC spectrum of 3-(4-bromophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5c)



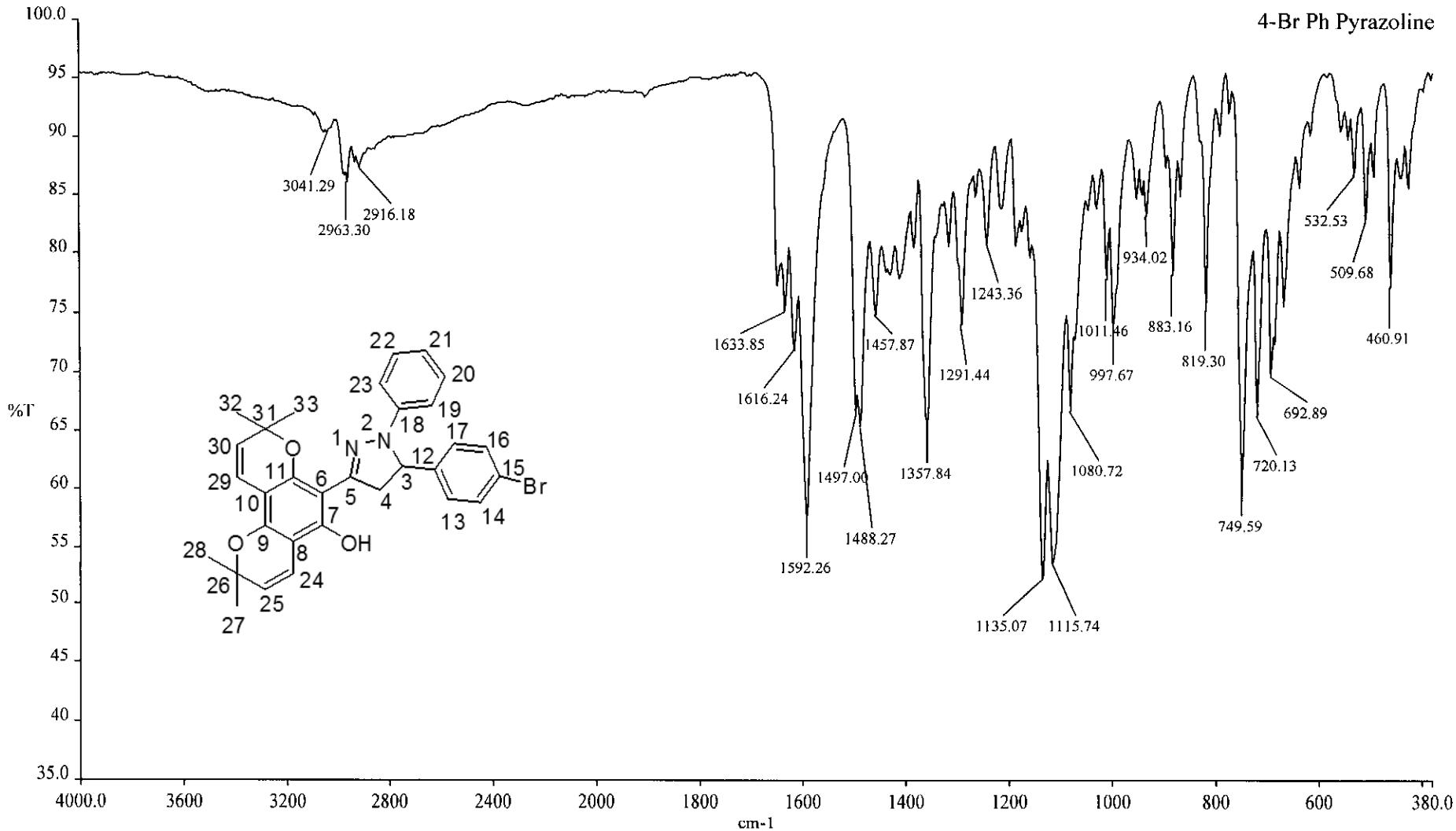
Expanded HMBC spectrum of 3-(4-bromophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5c)



Expanded HMBC spectrum of 3-(4-bromophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5c)



Expanded HMBC spectrum of 3-(4-bromophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5c)

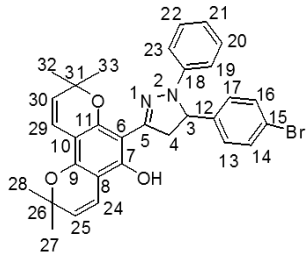


Infrared spectrum of 3-(4-bromophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5c)

Elemental Composition Report **Page 1**

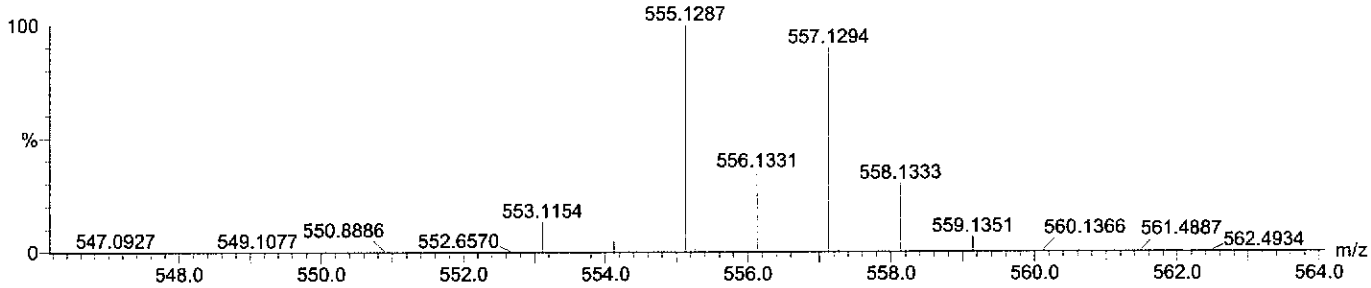
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
22 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-35 H: 20-30 N: 0-4 O: 0-4 Br: 1-1



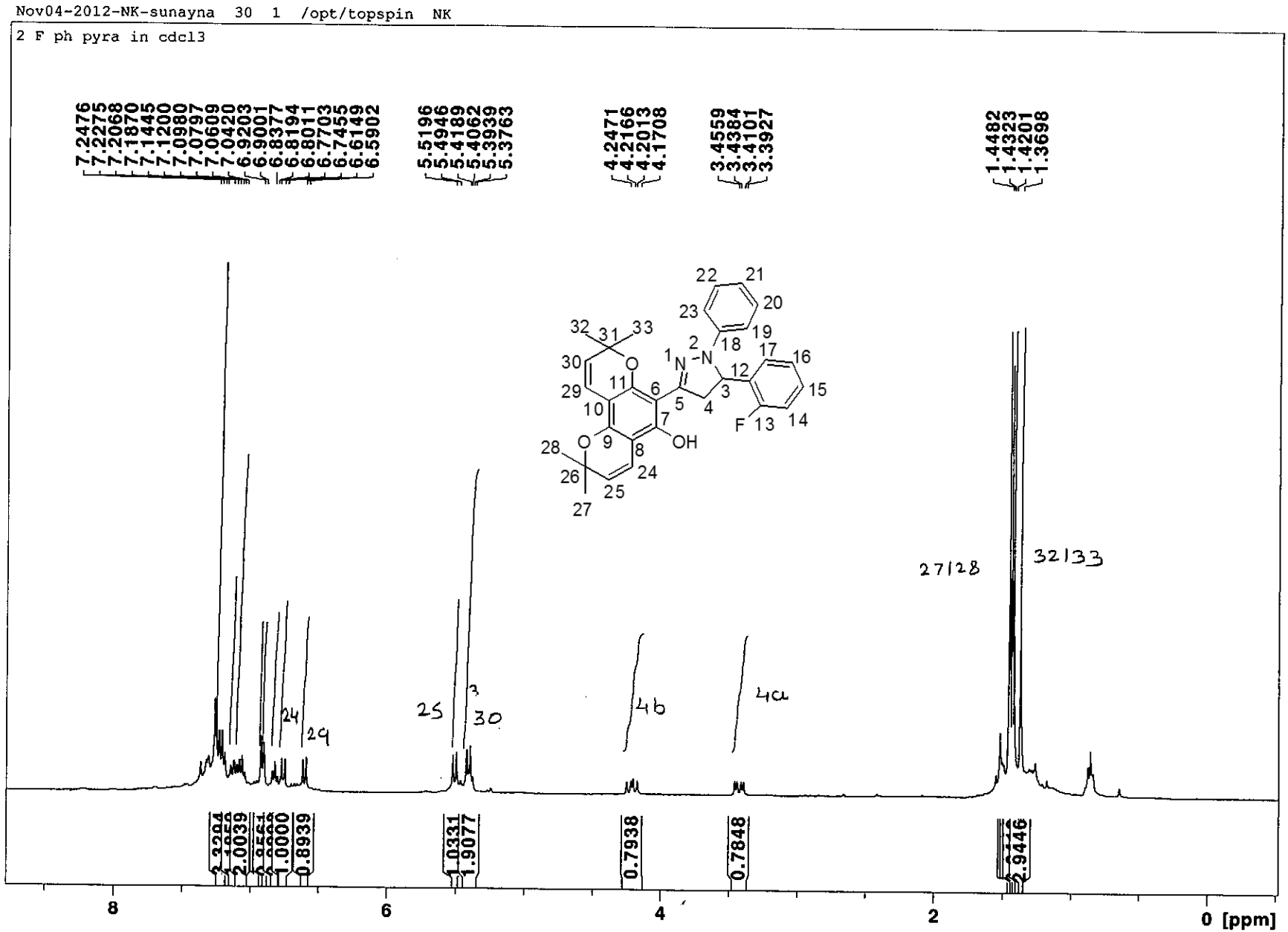
6 2 (0.017) Cm (1:31)
TOF MS ES-

1.71e+005

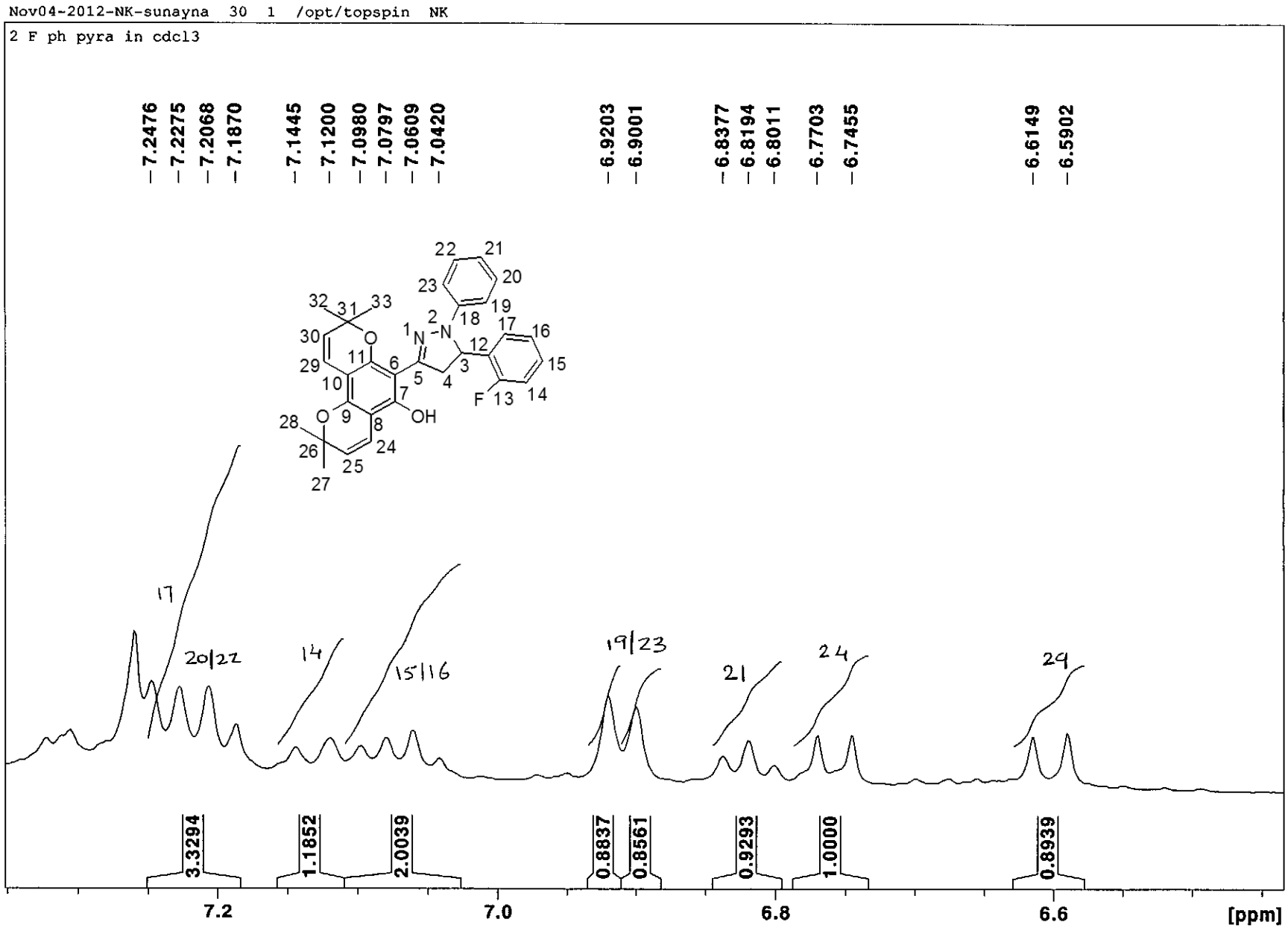


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
555.1287	555.1283	0.4	0.7	18.5	490.4	0.0	C31 H28 N2 O3 Br

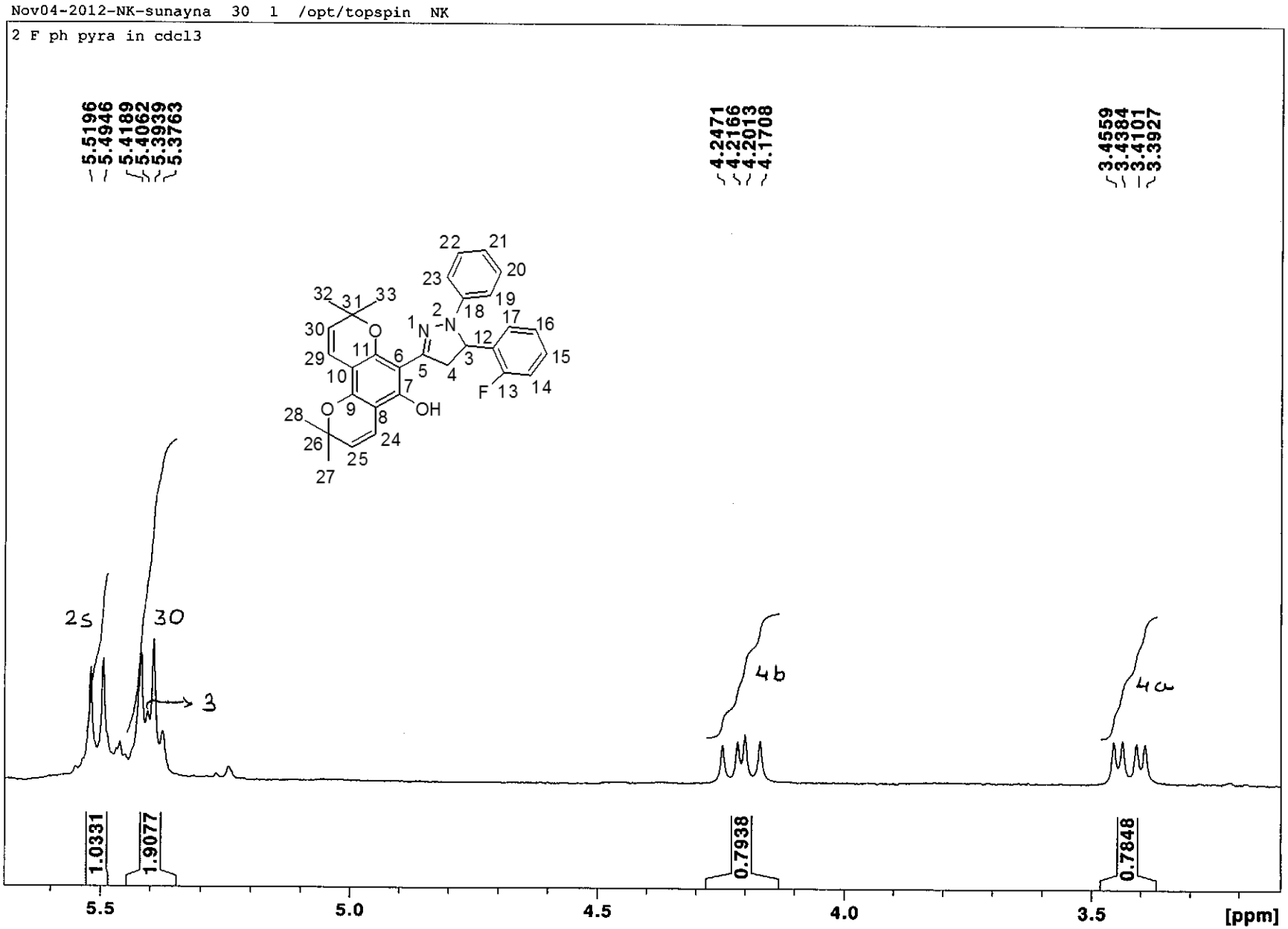
HRMS spectrum of 3-(4-bromophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5c)



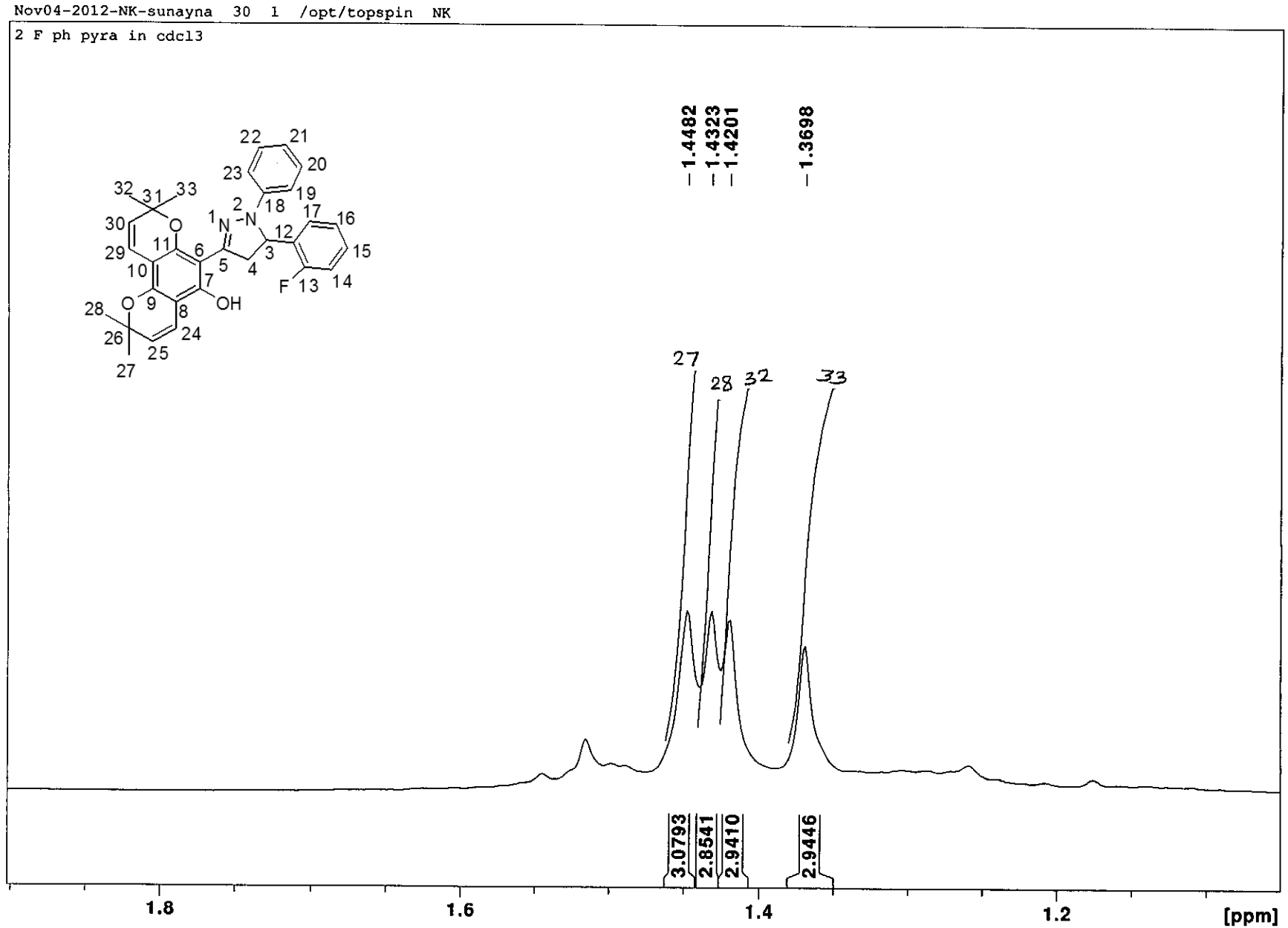
1H NMR spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



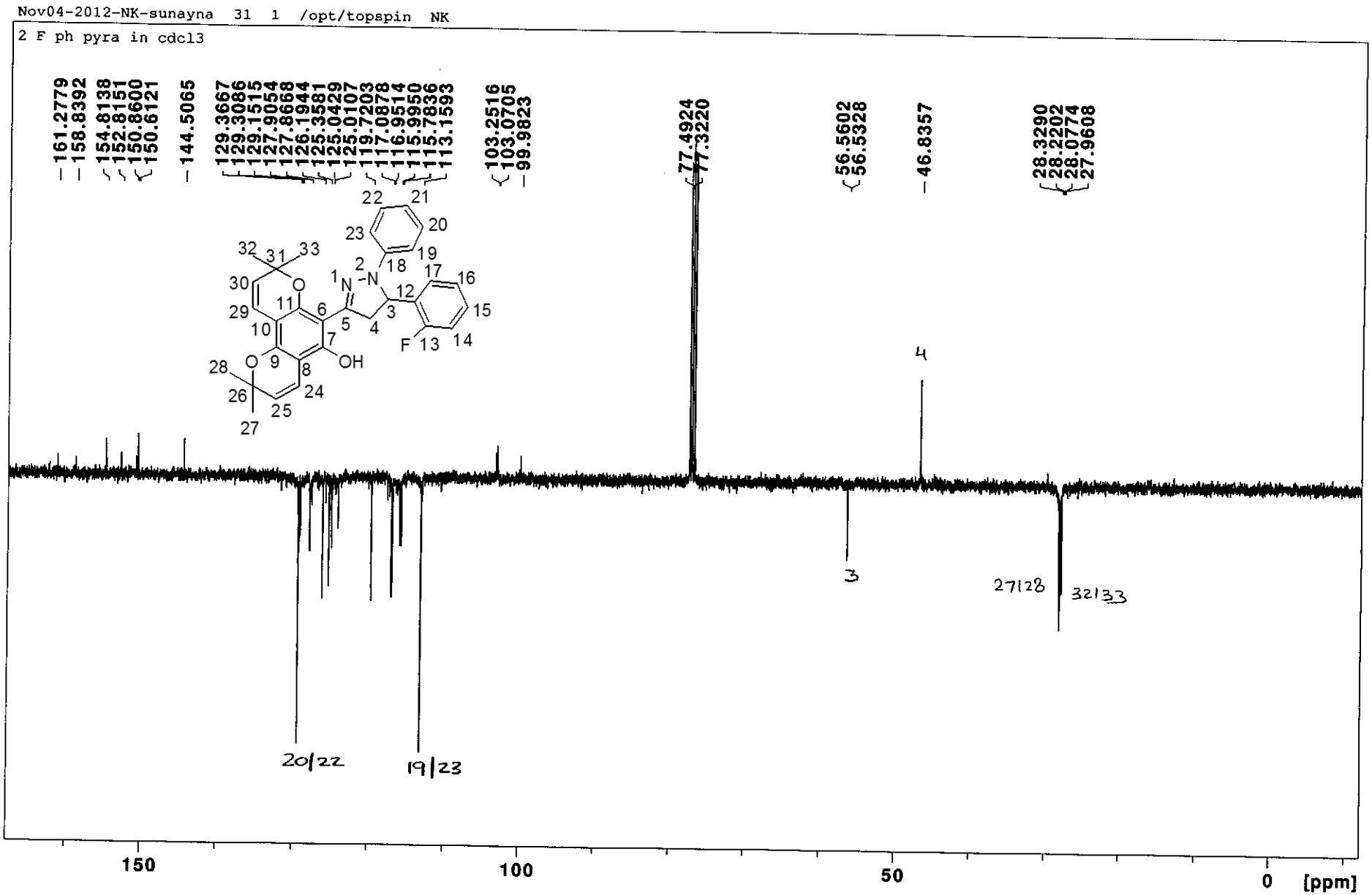
Expanded 1H NMR spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



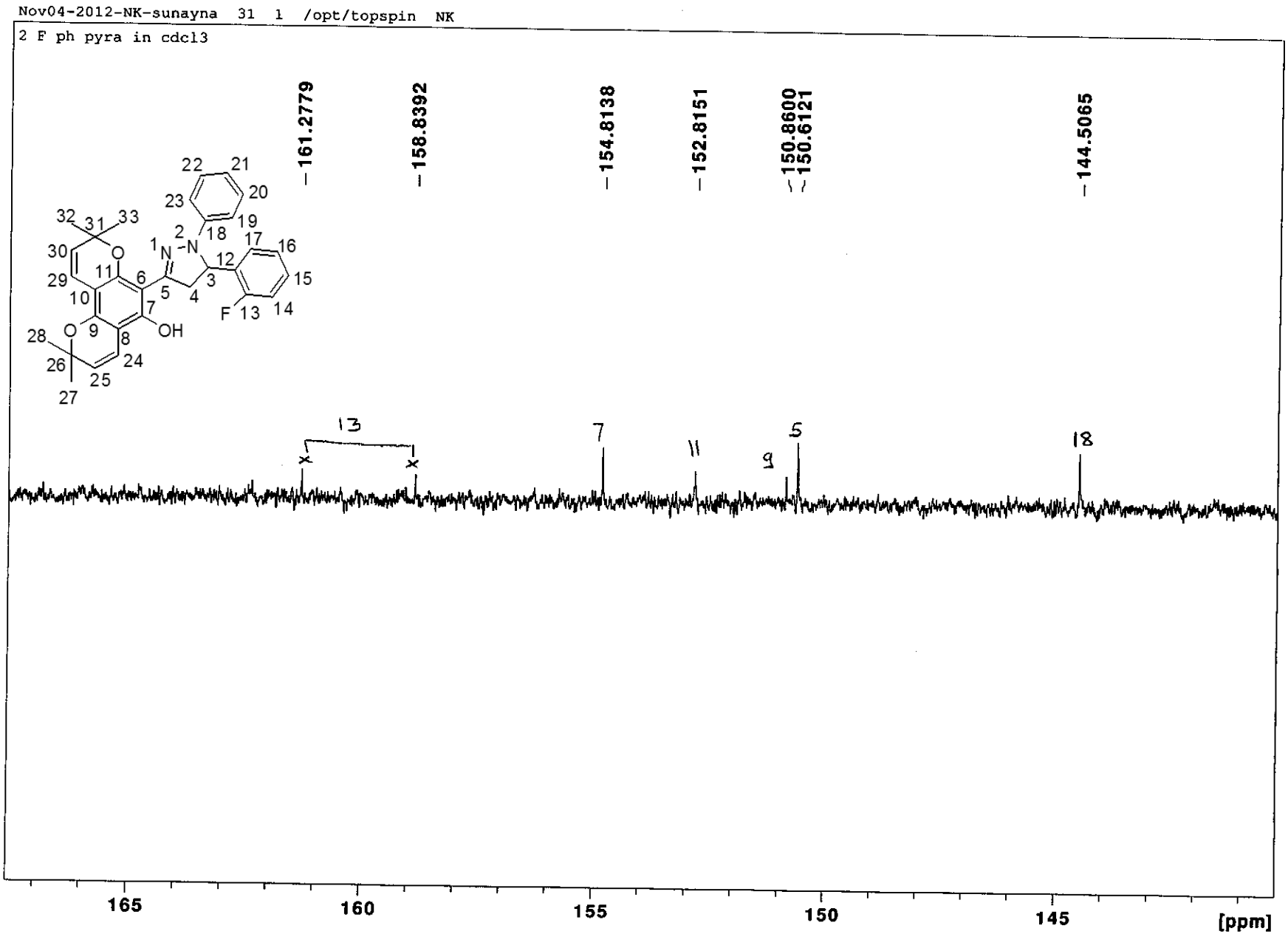
Expanded 1H NMR spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



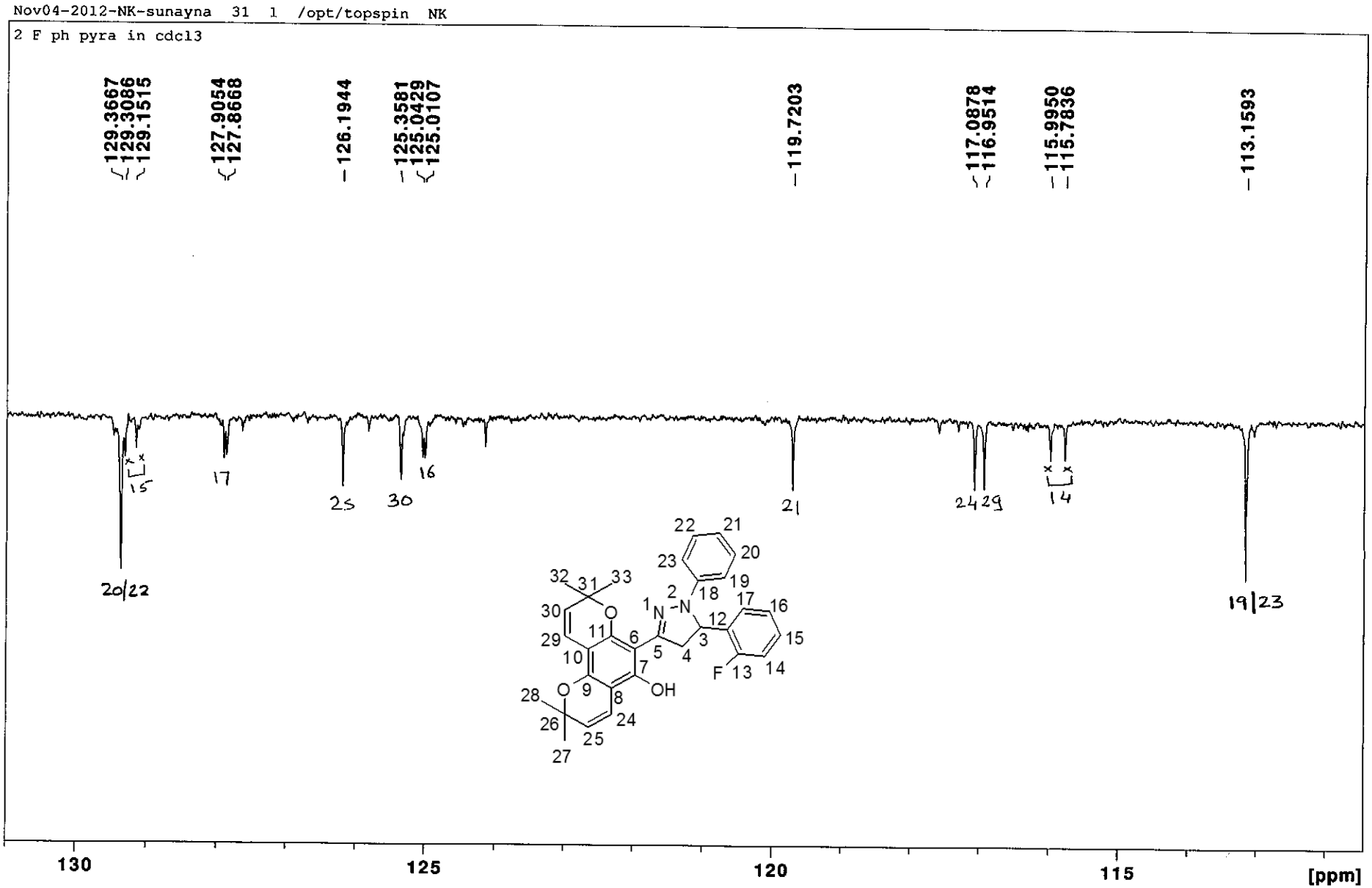
Expanded 1H NMR spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



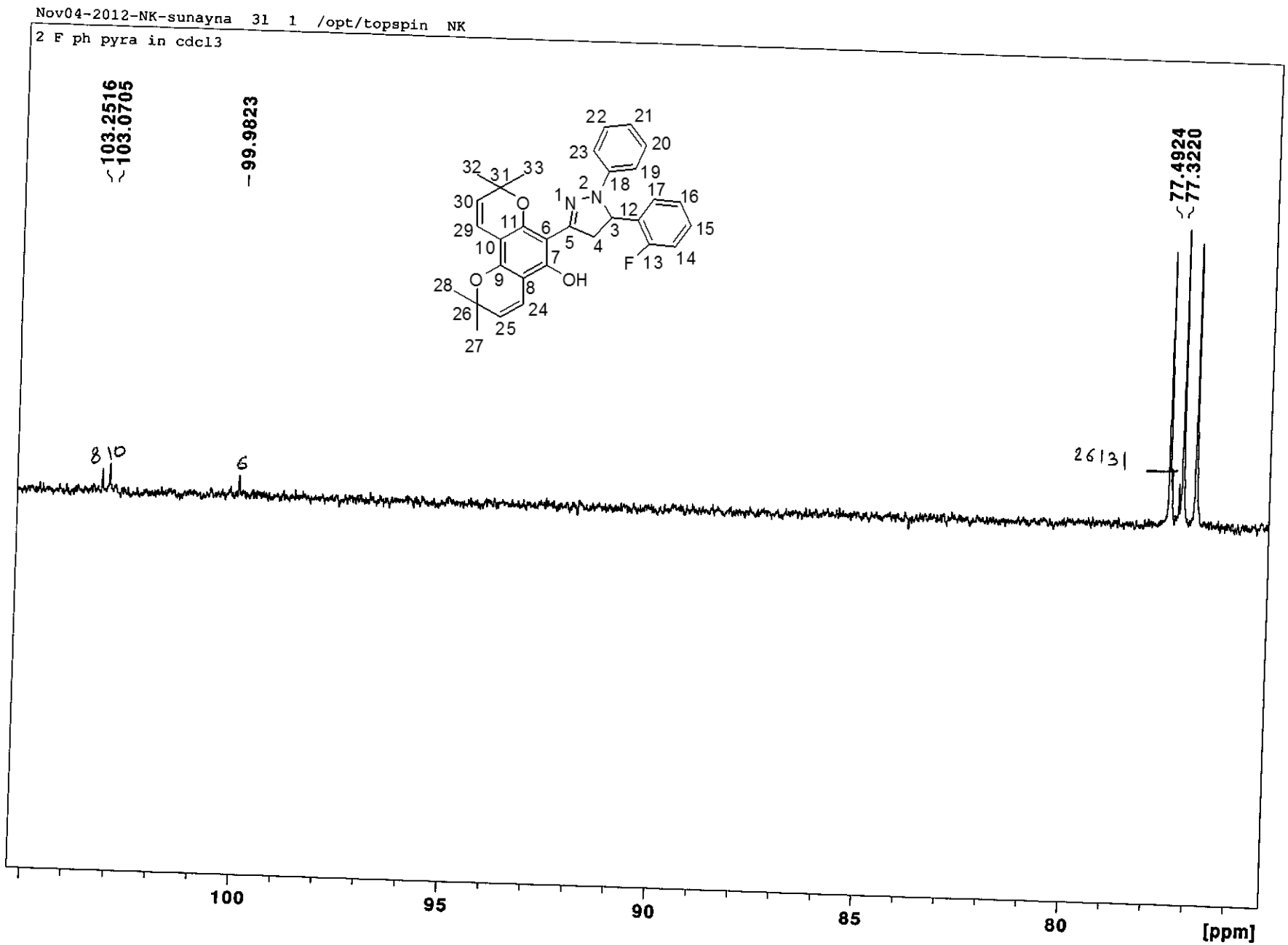
13C NMR spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



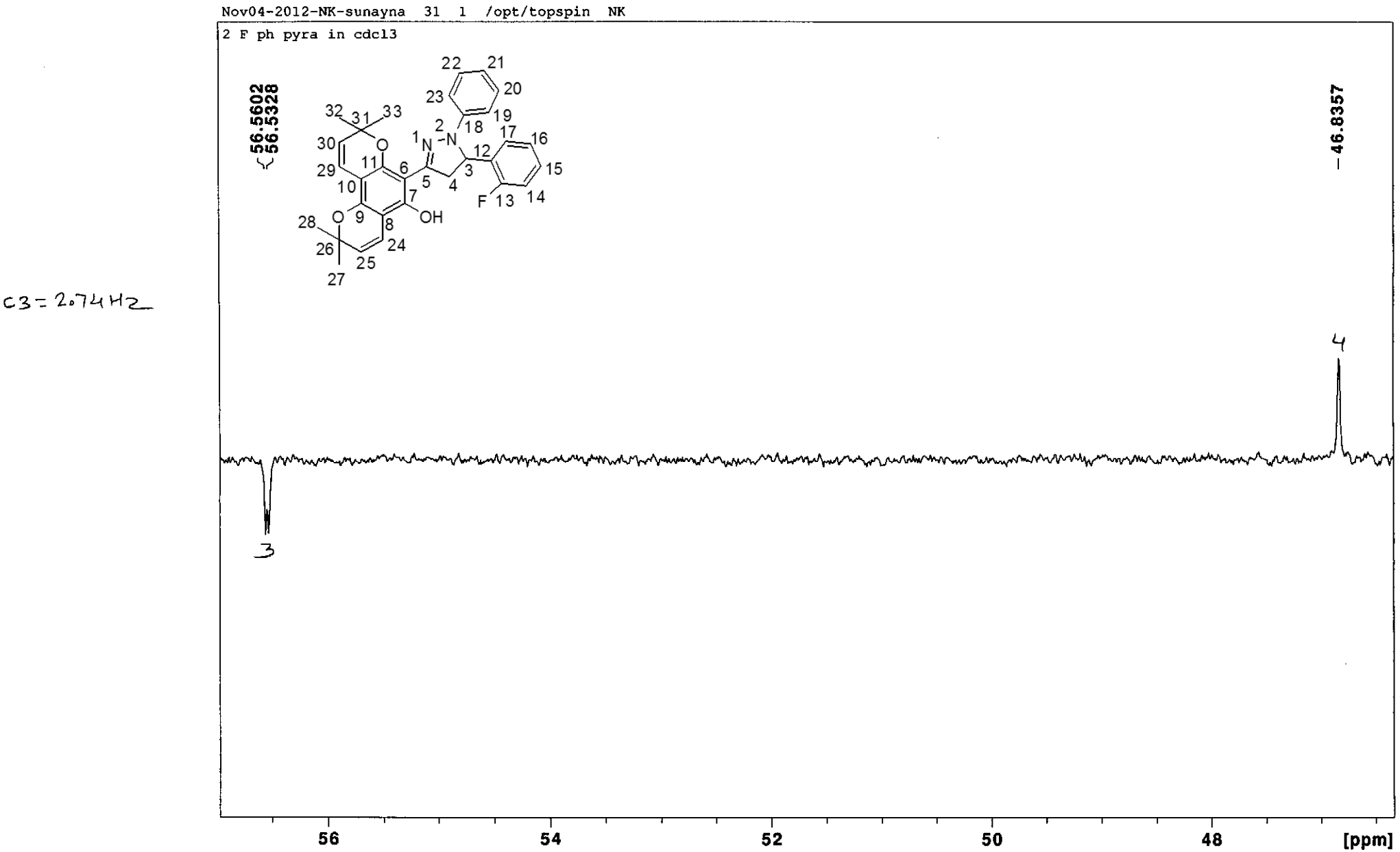
Expanded 13C NMR spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



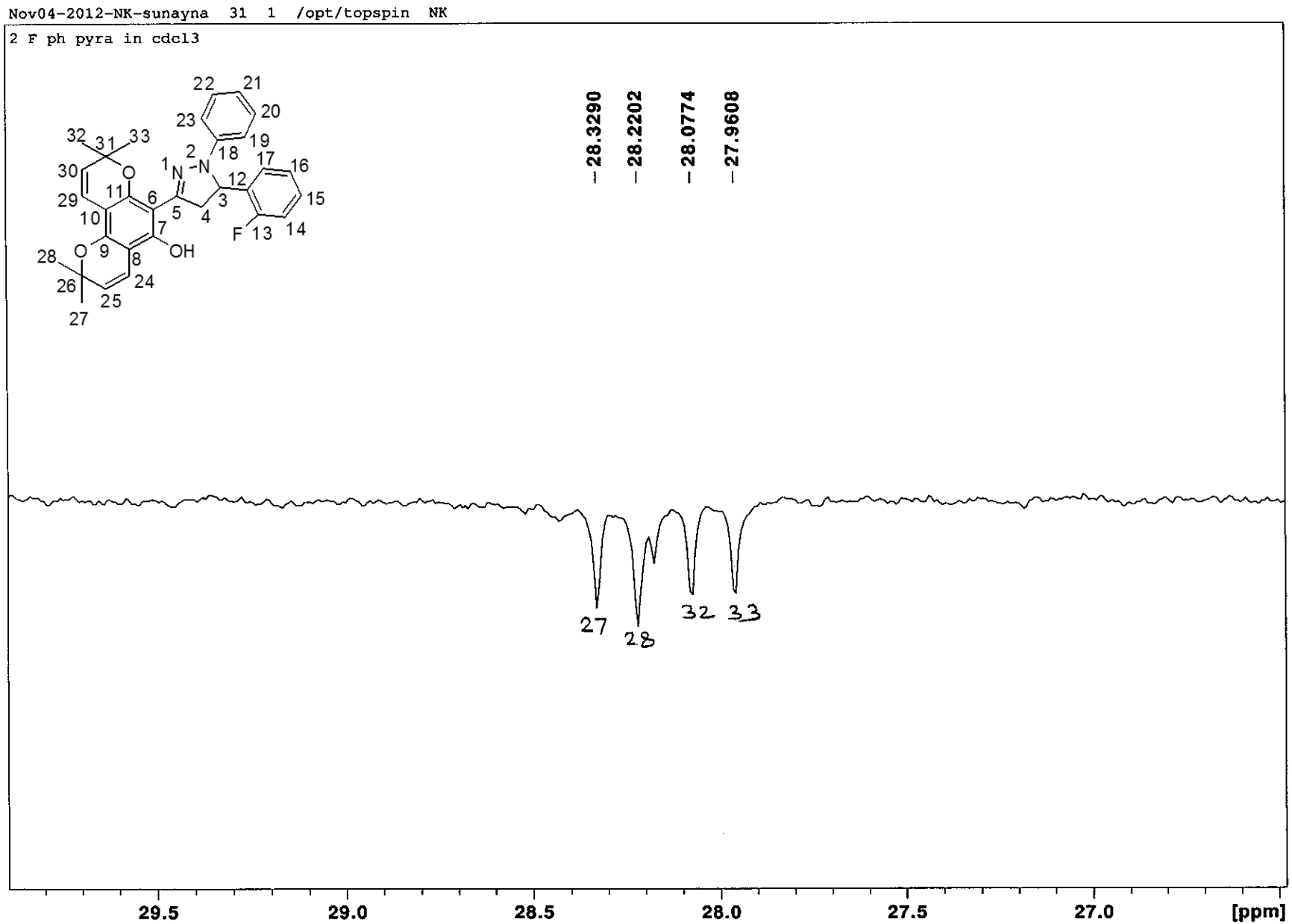
Expanded 13C NMR spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



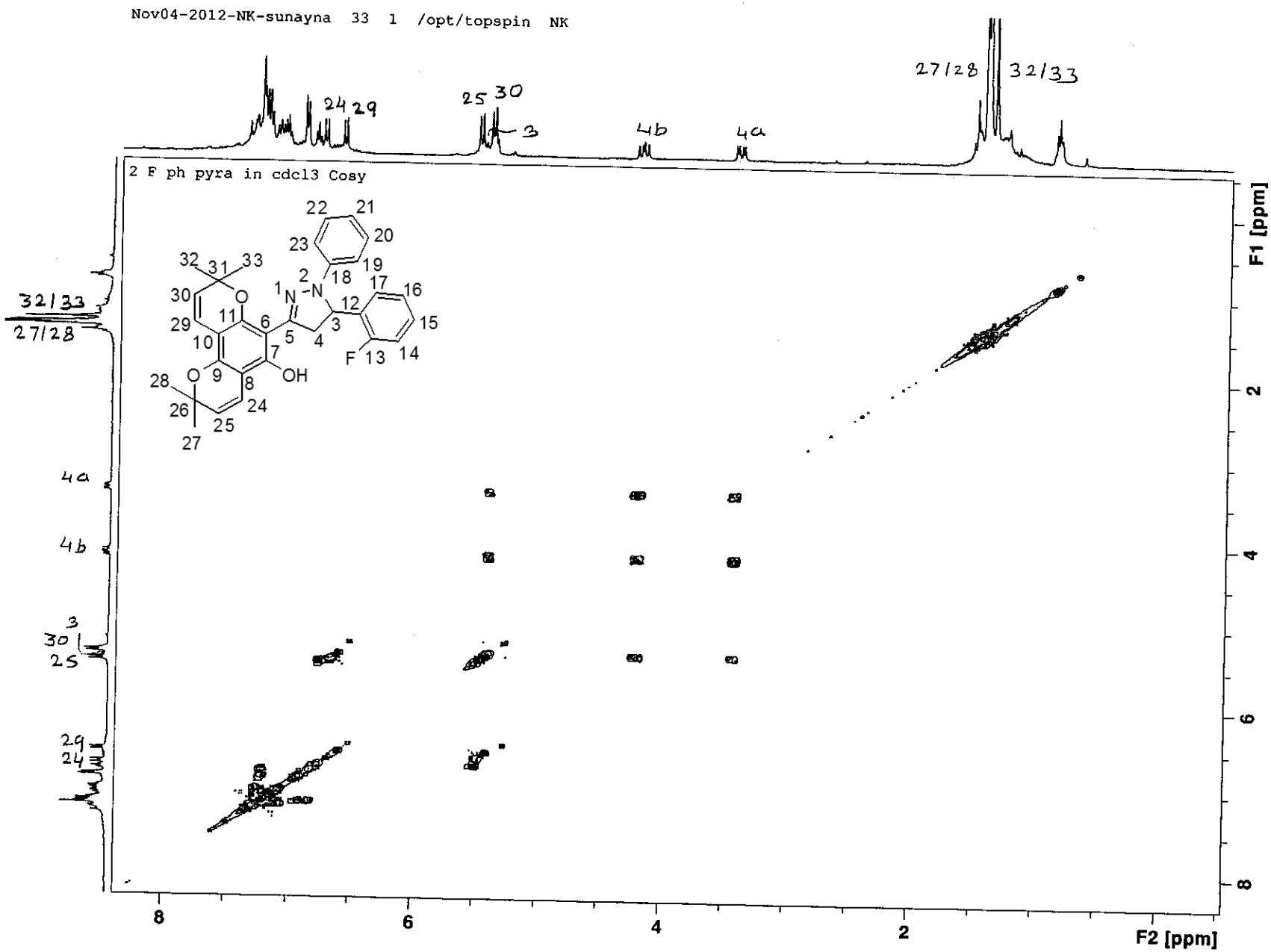
Expanded 13C NMR spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



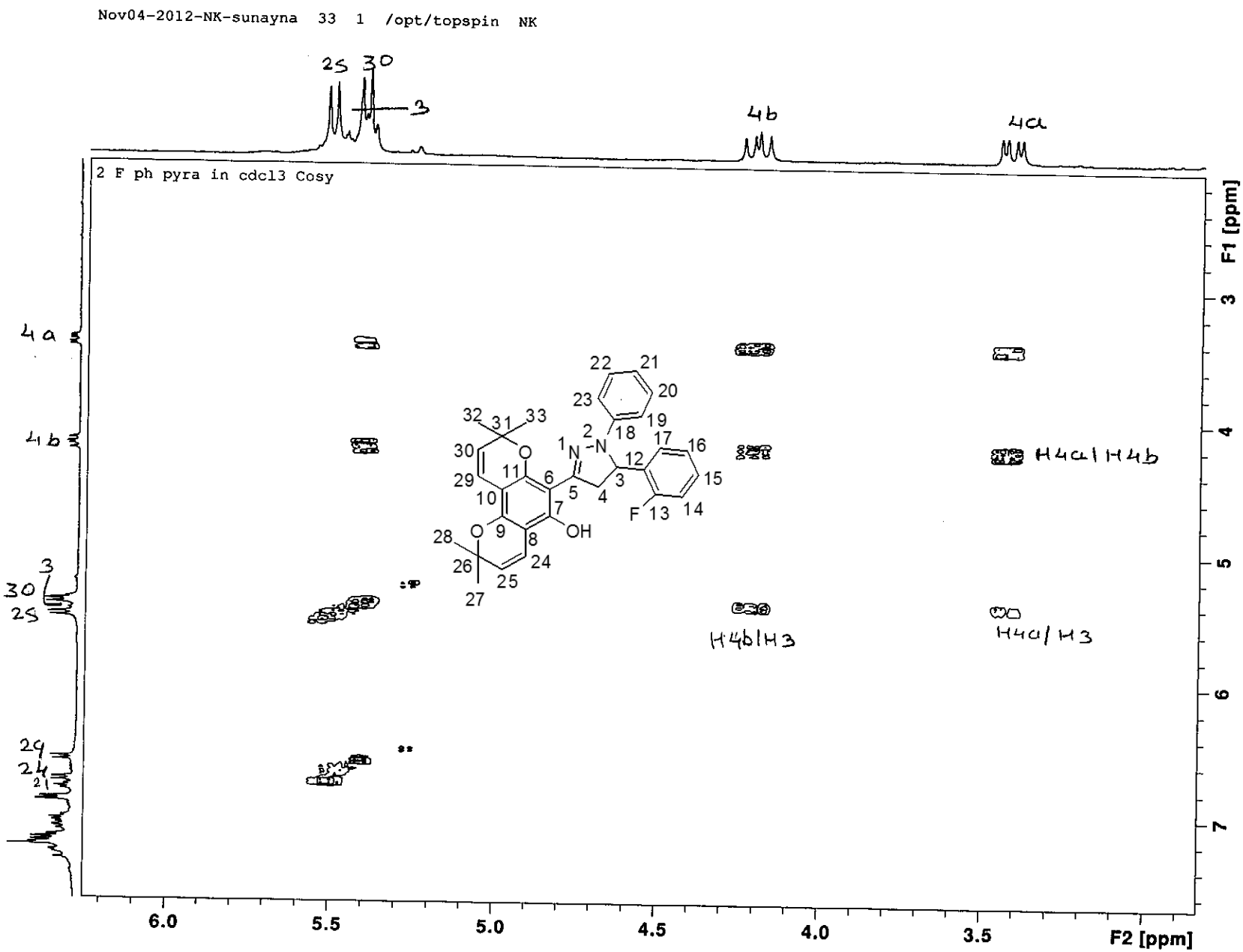
Expanded 13C NMR spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



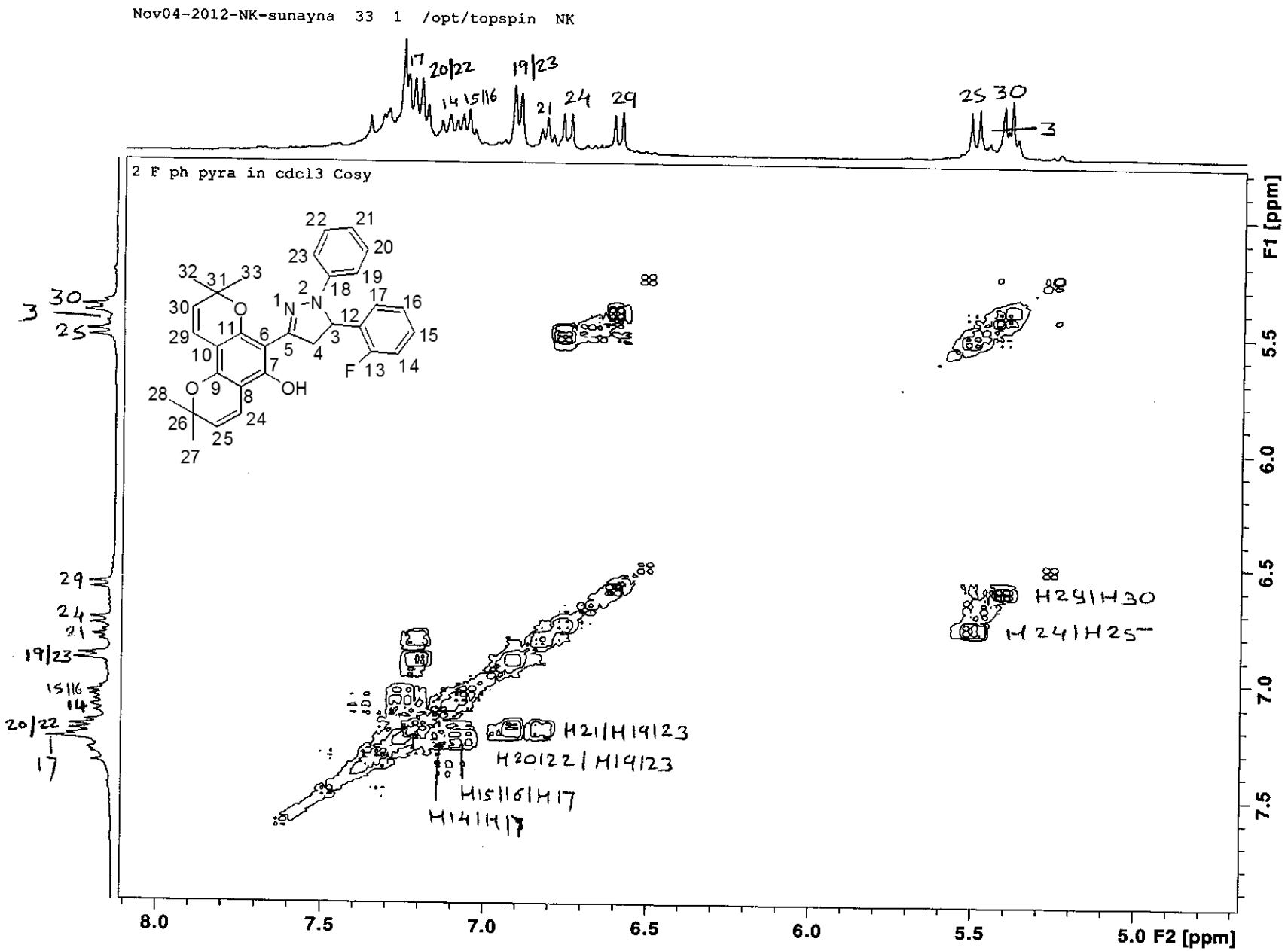
Expanded 13C NMR spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



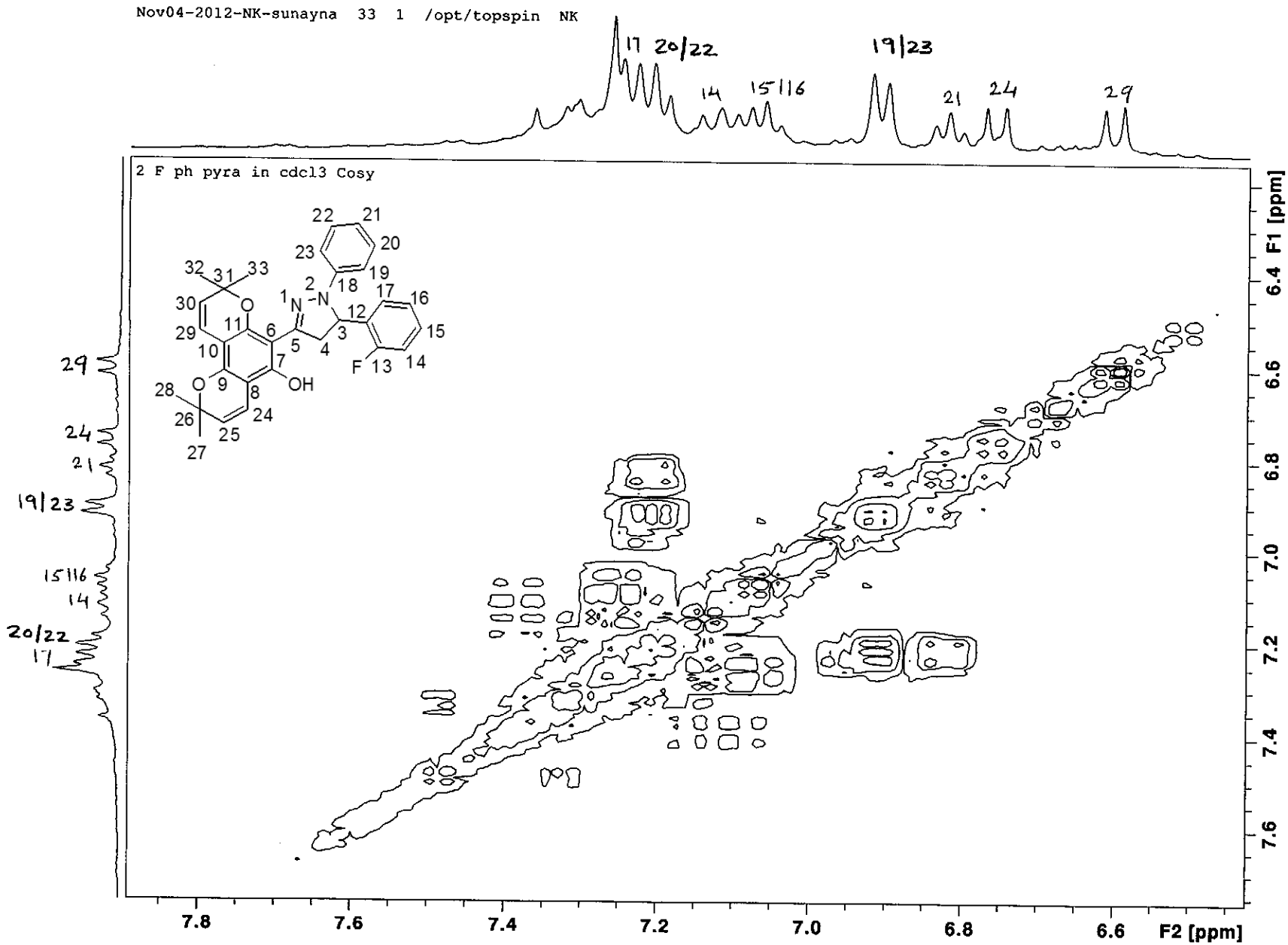
COSY NMR spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



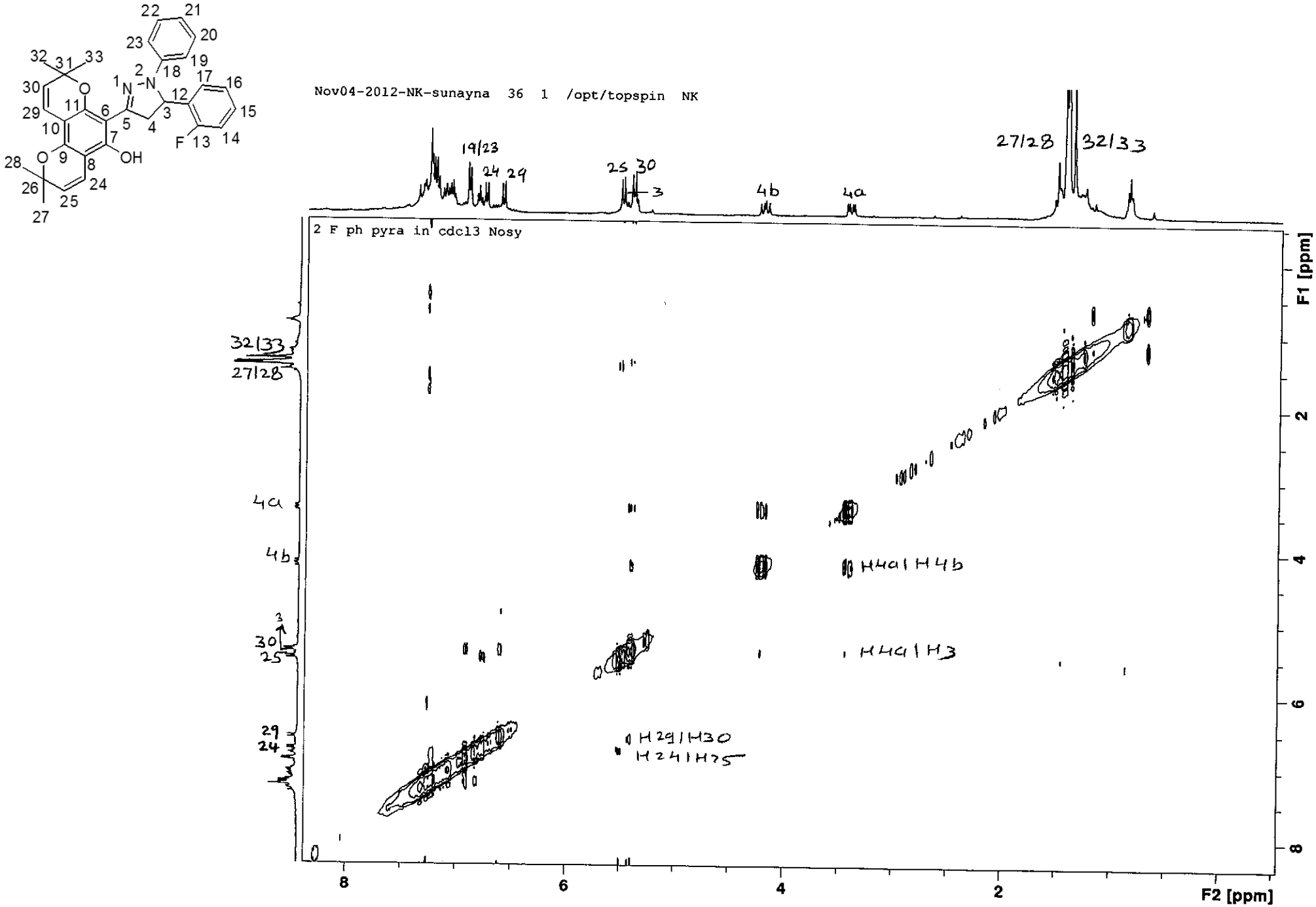
Expanded COSY NMR spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



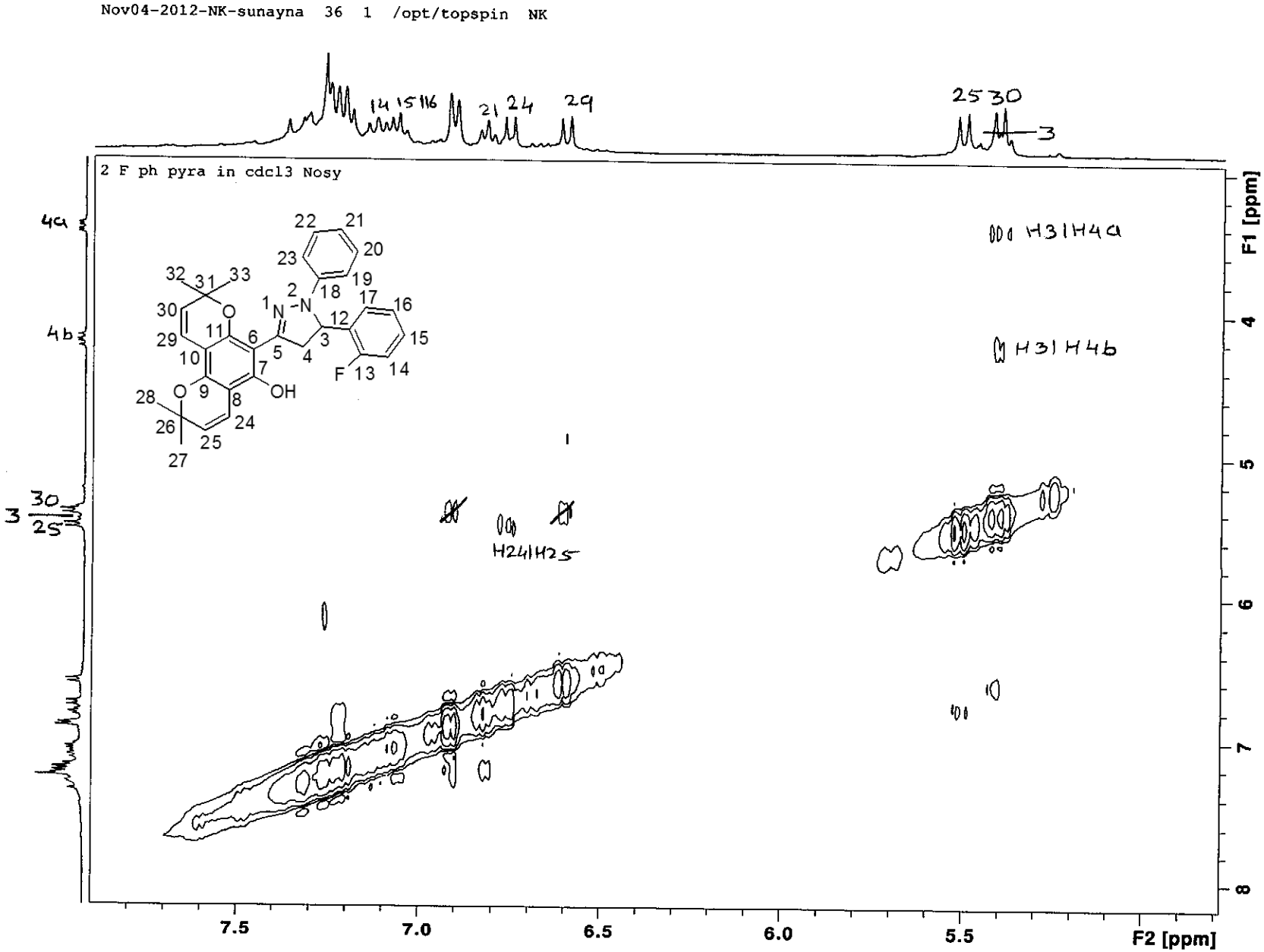
Expanded COSY NMR spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



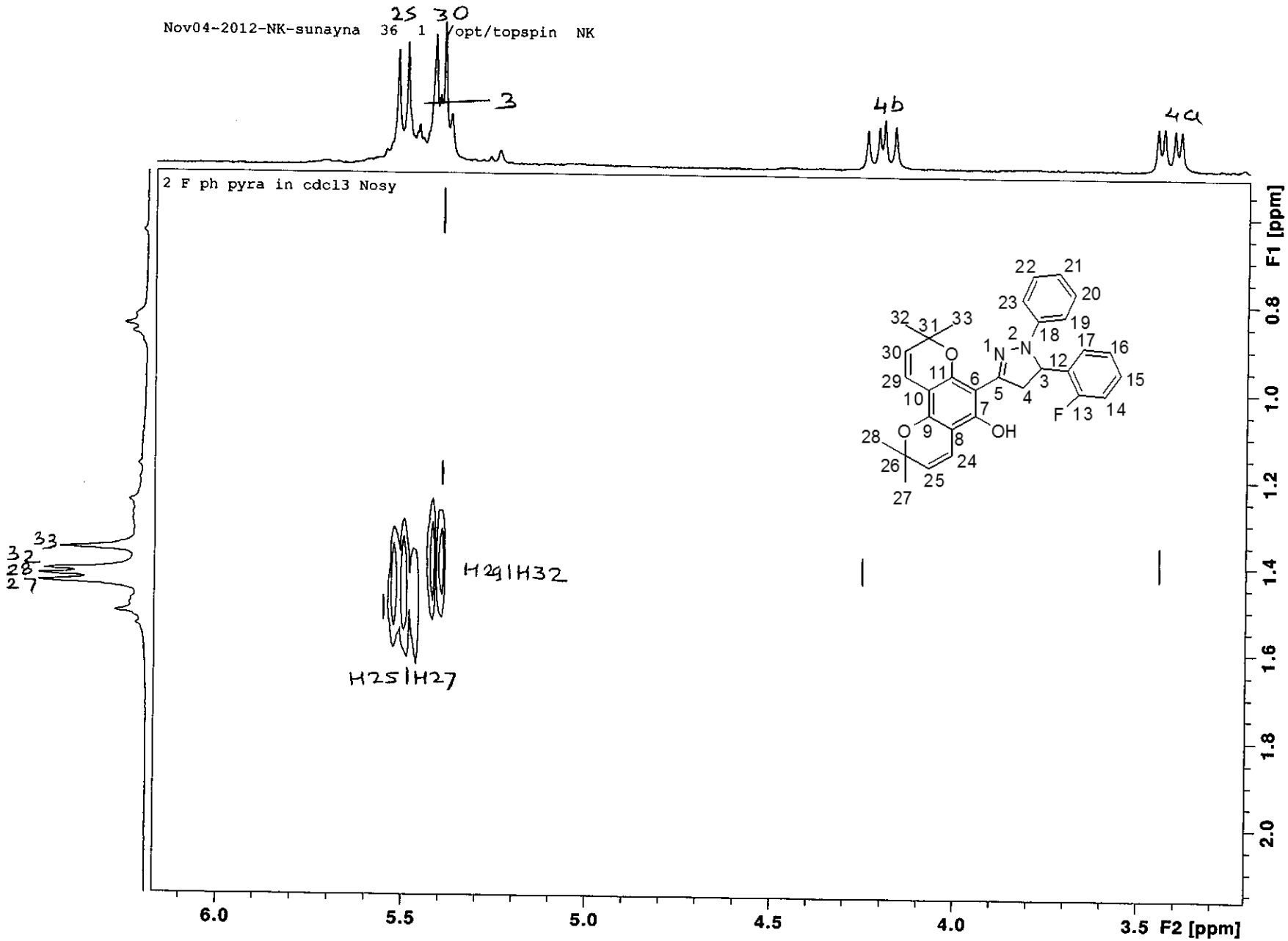
Expanded COSY NMR spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



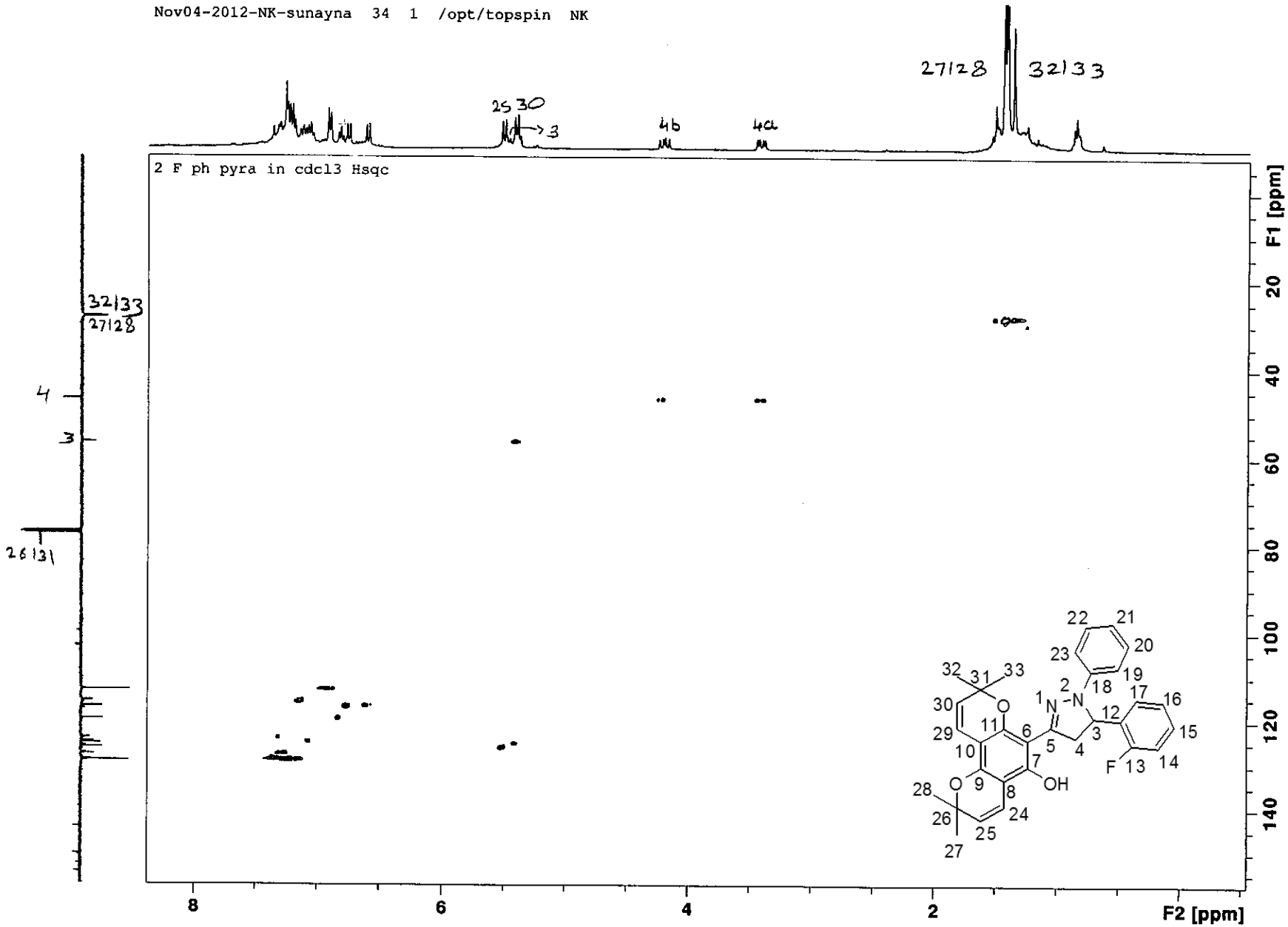
NOESY spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



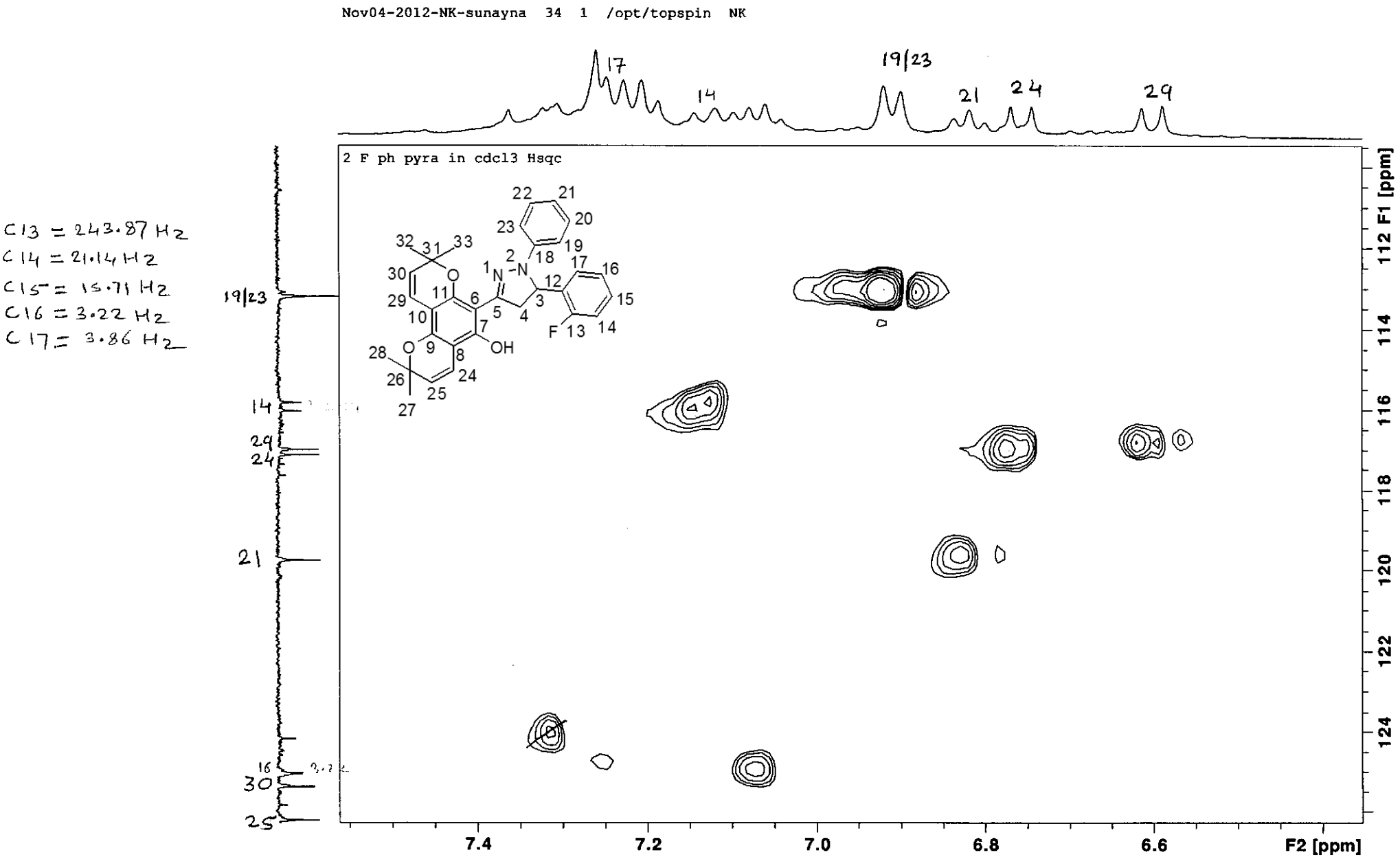
Expanded NOESY spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



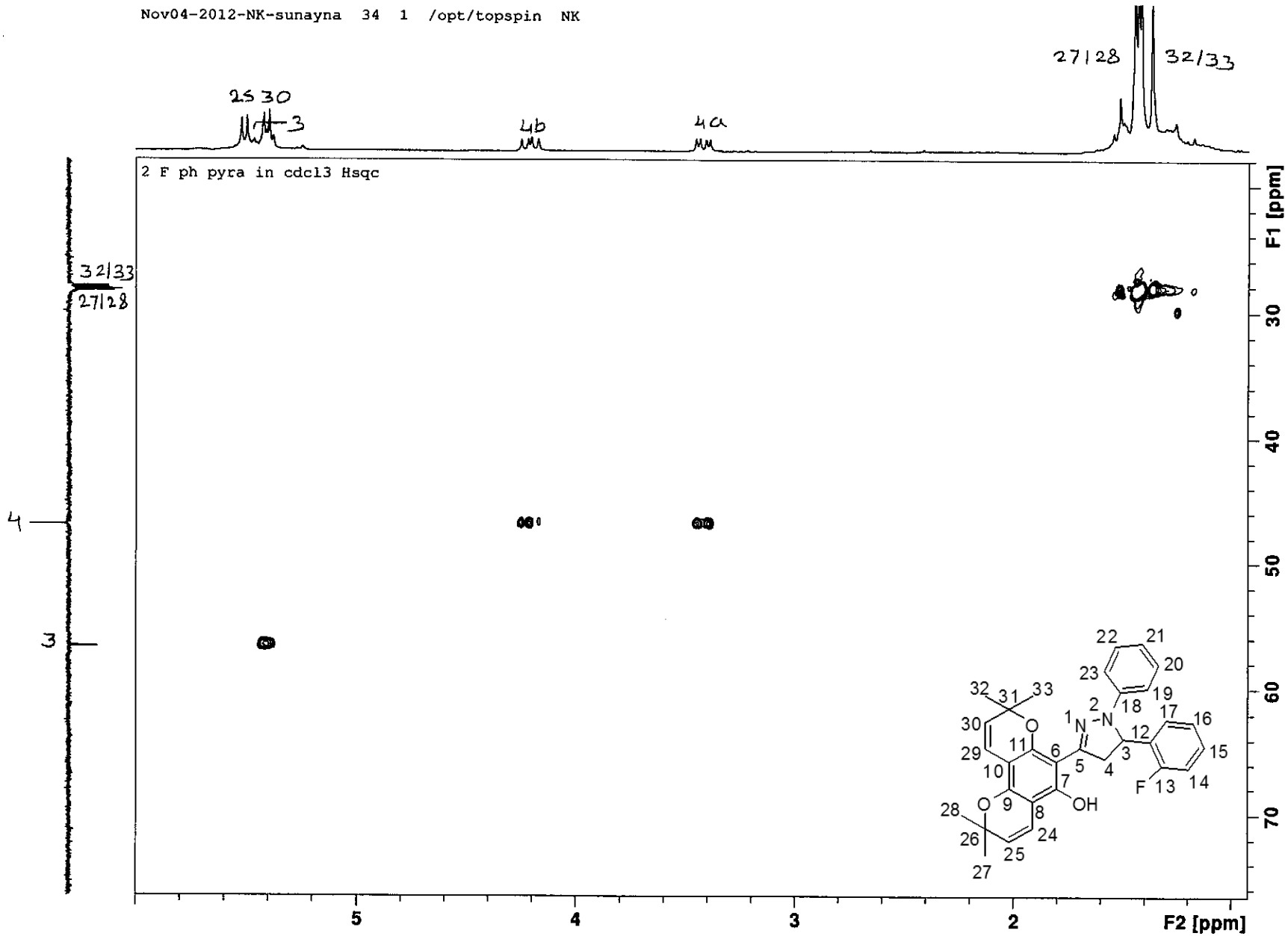
Expanded NOESY spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



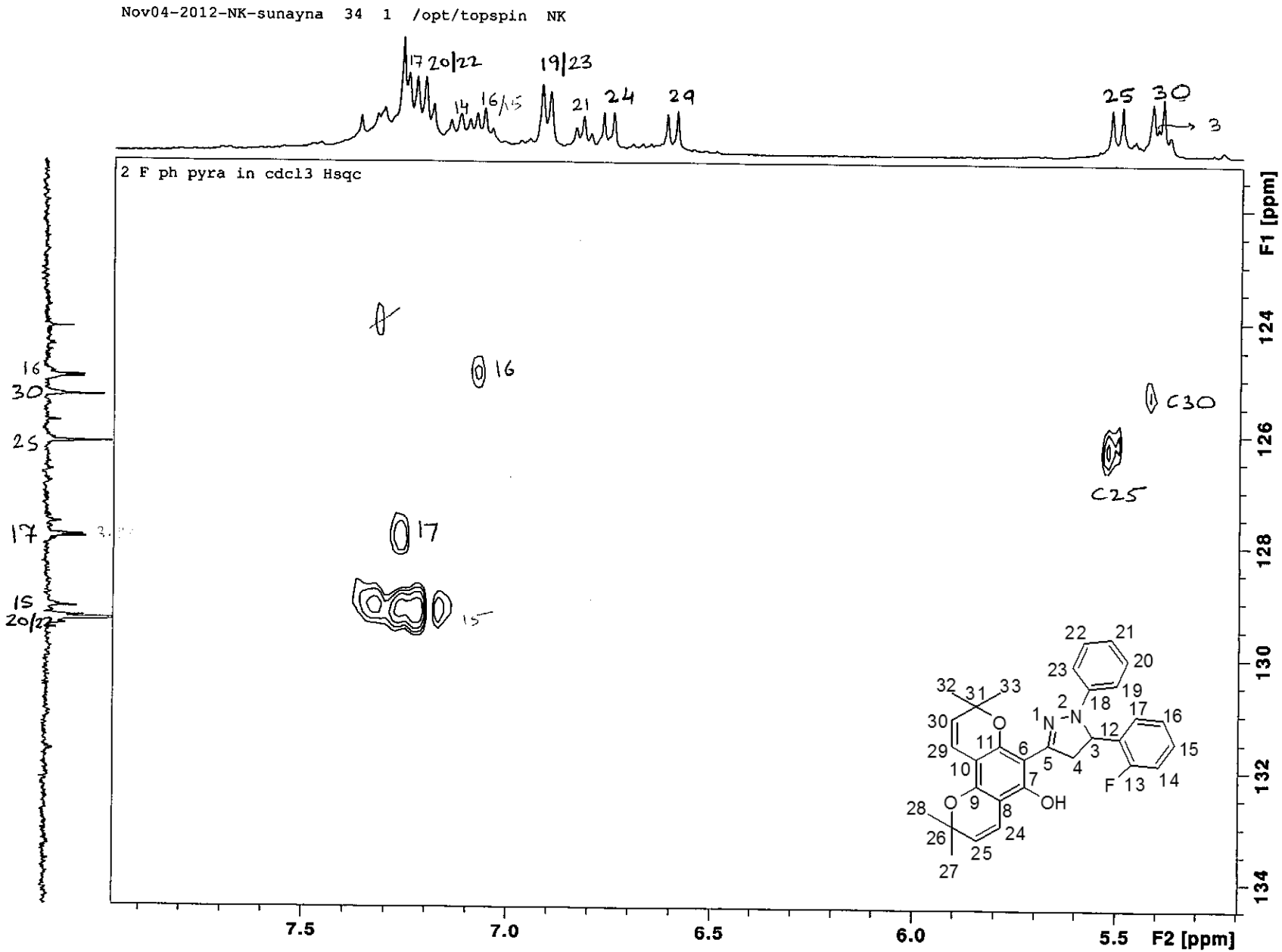
HSQC spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



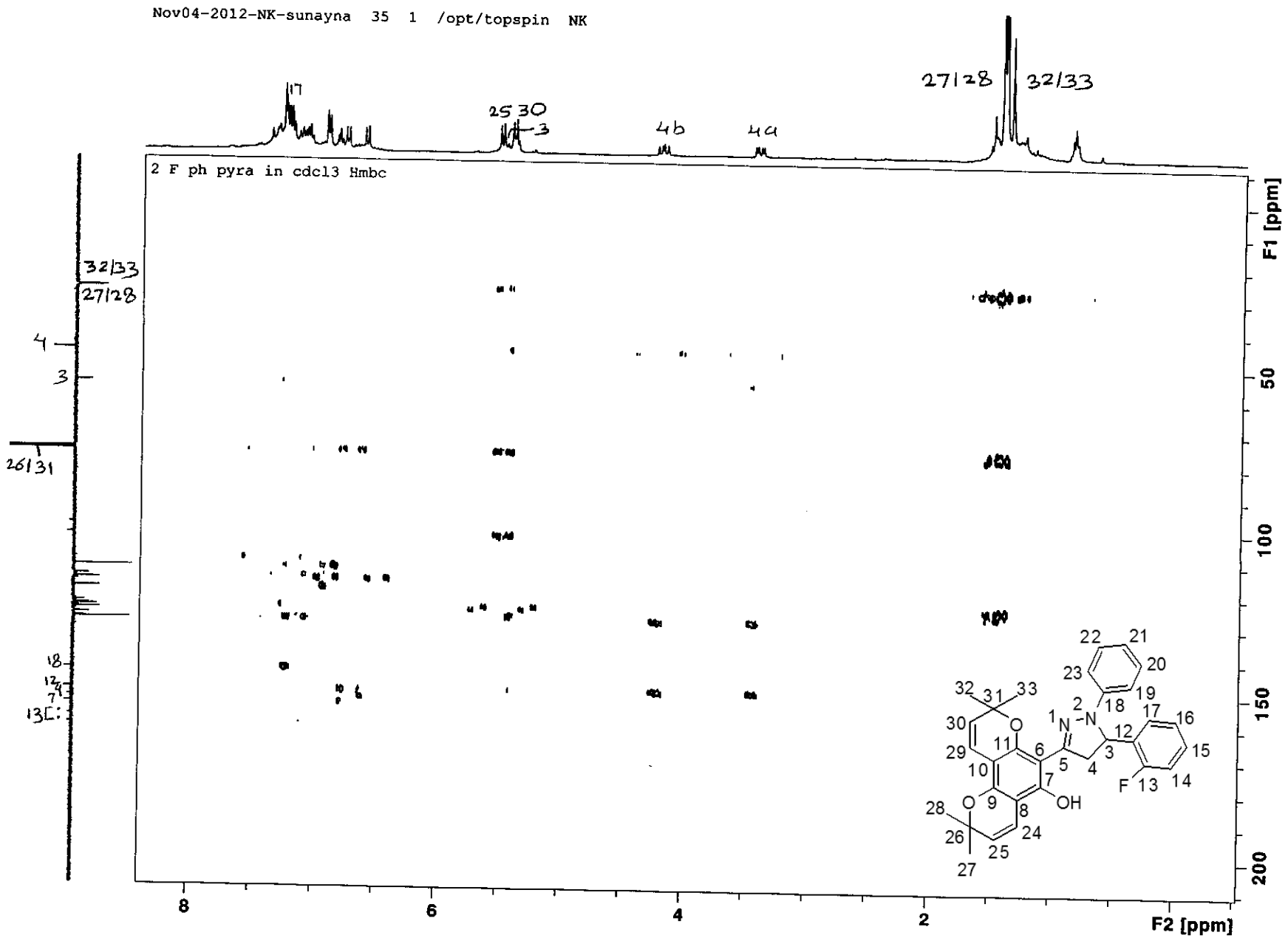
Expanded HSQC spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



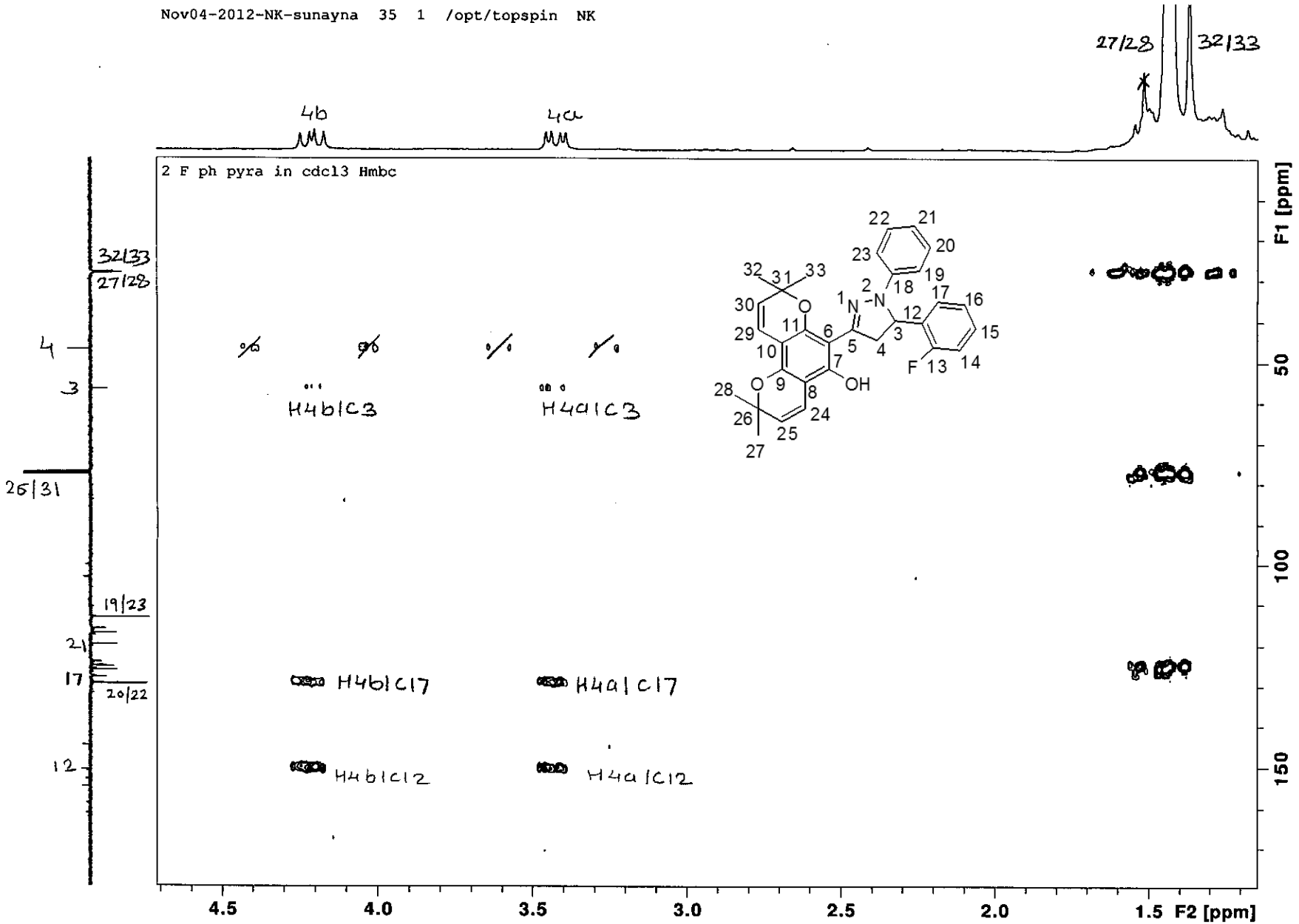
Expanded HSQC spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



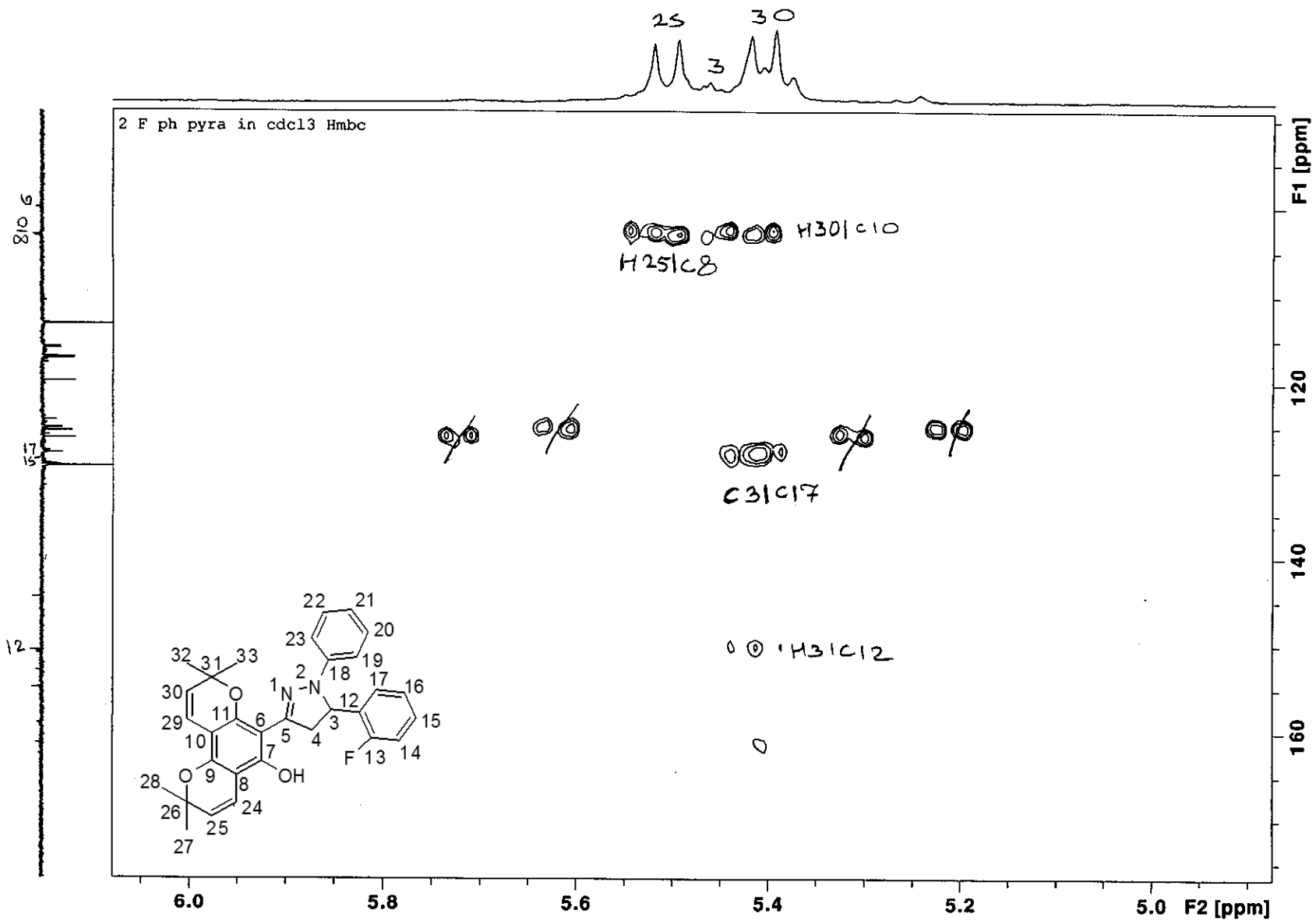
Expanded HSQC spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



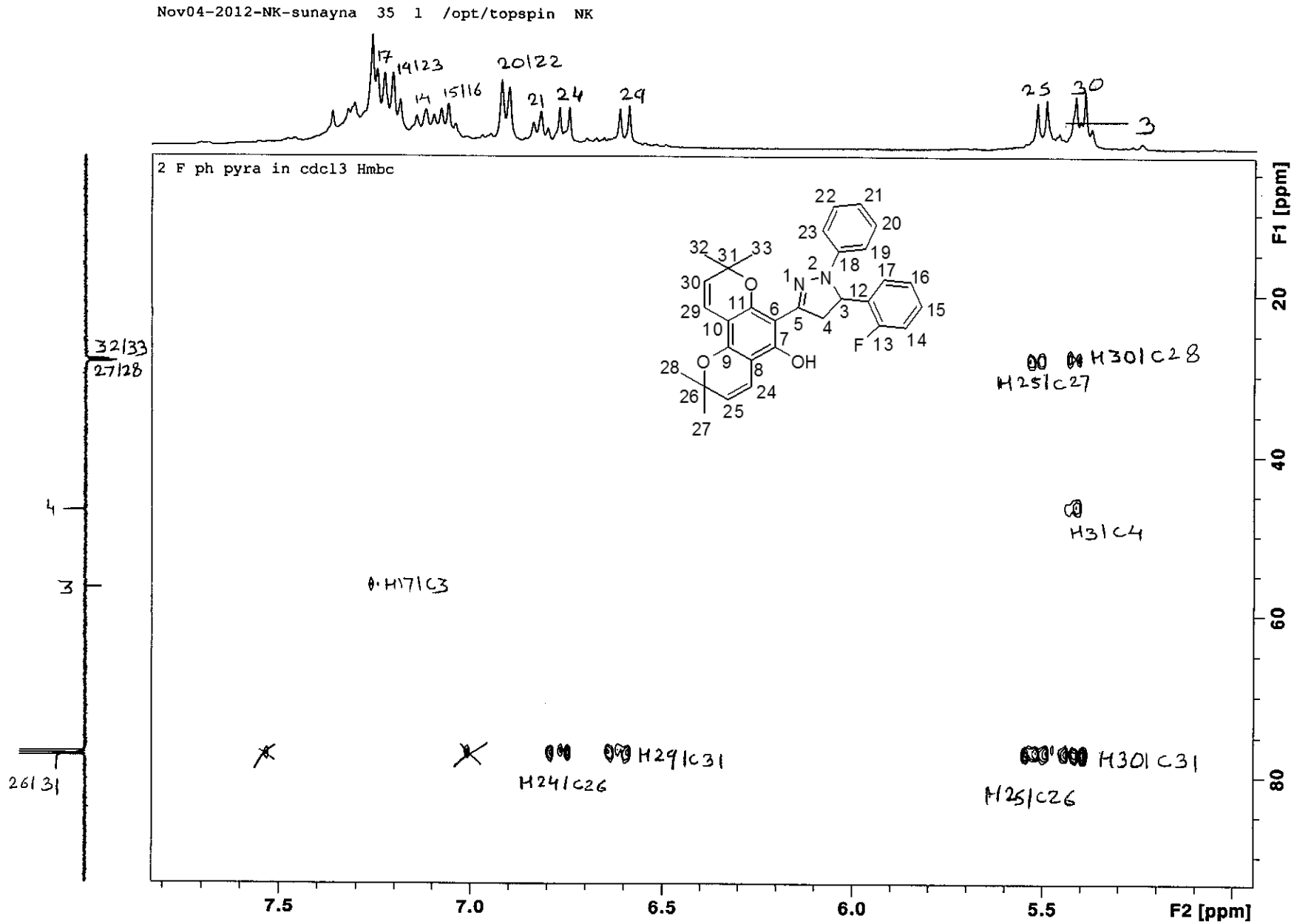
HMBC spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



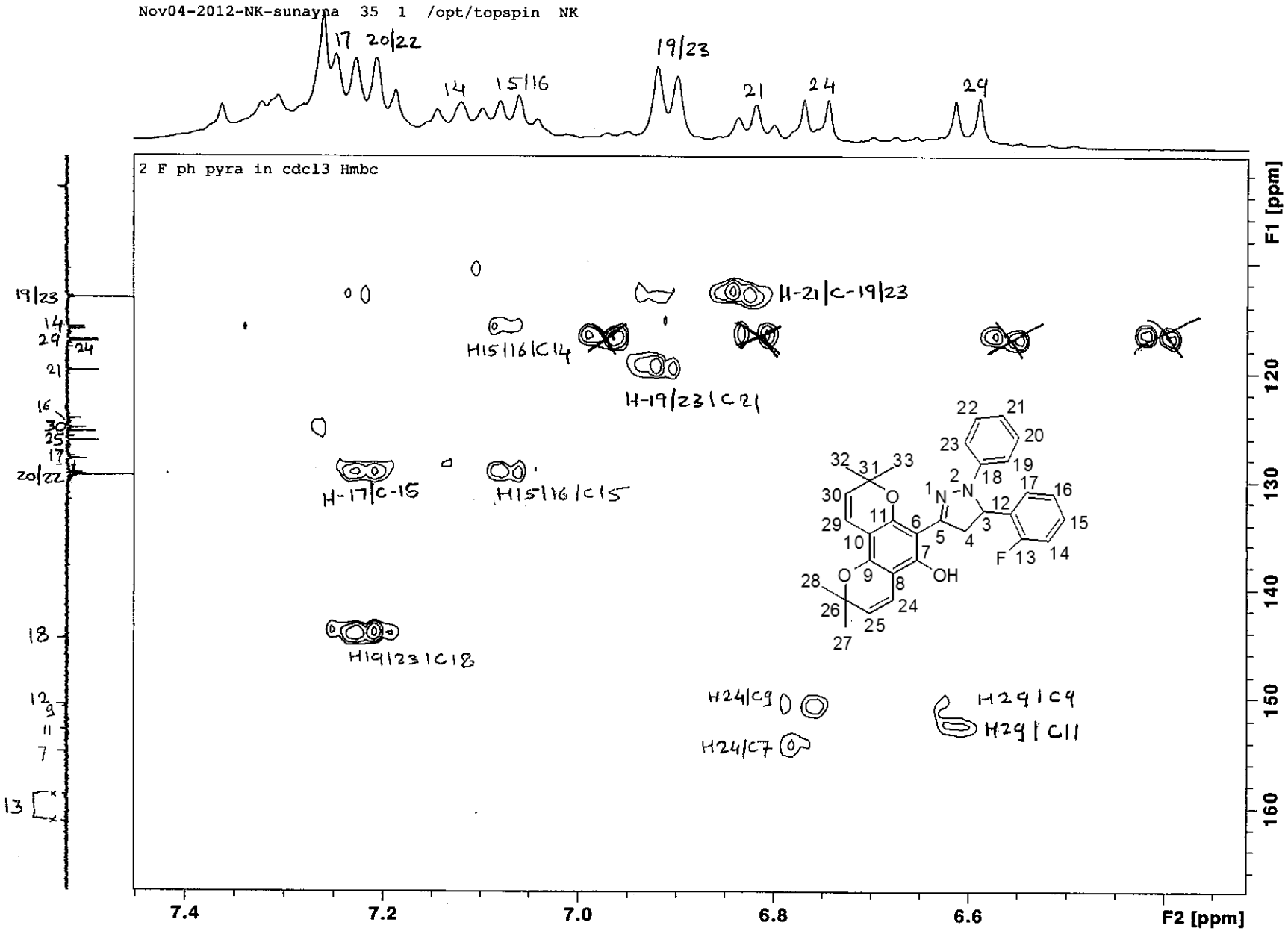
Expanded HMBC spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



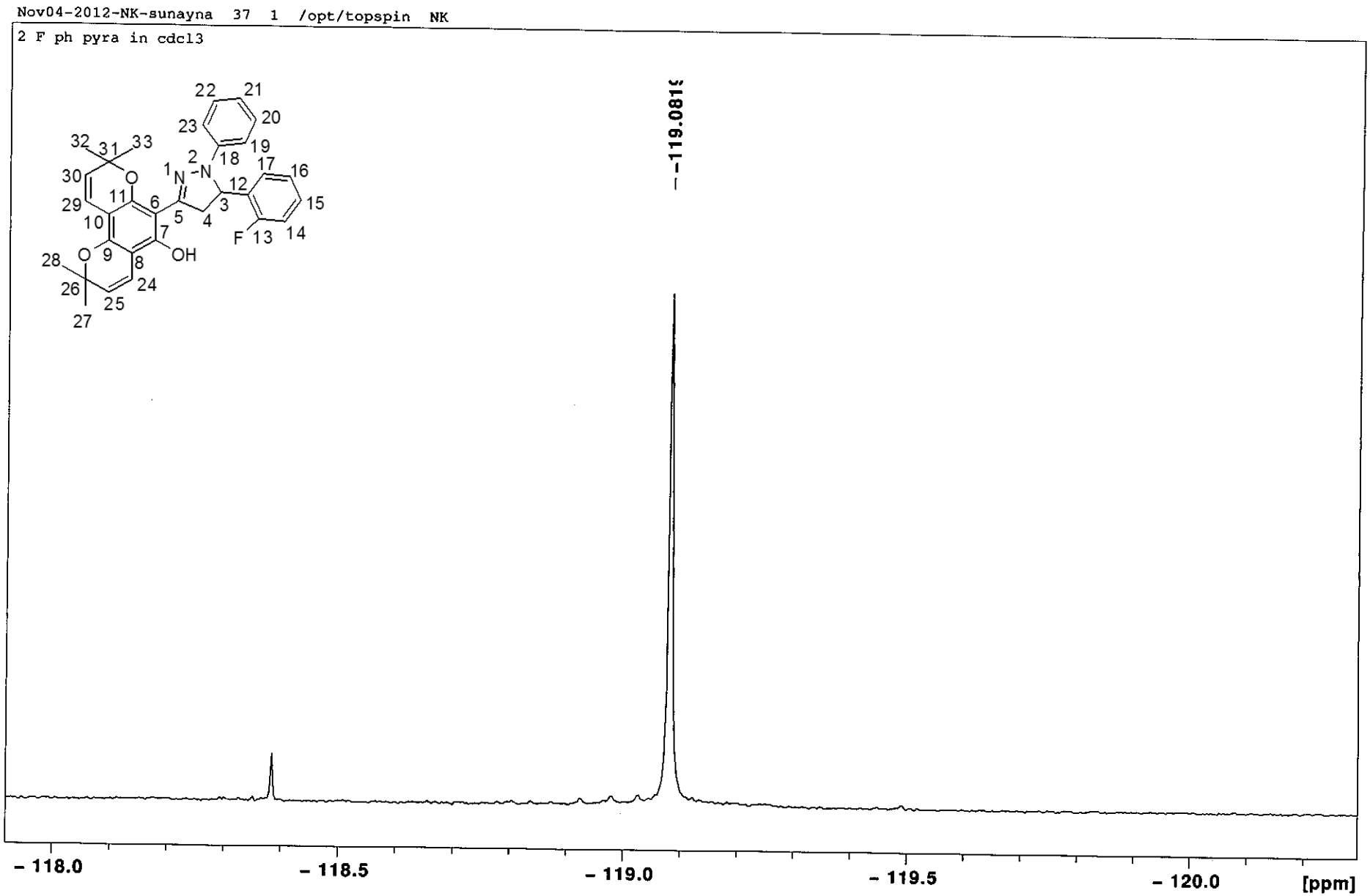
Expanded HMBC spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



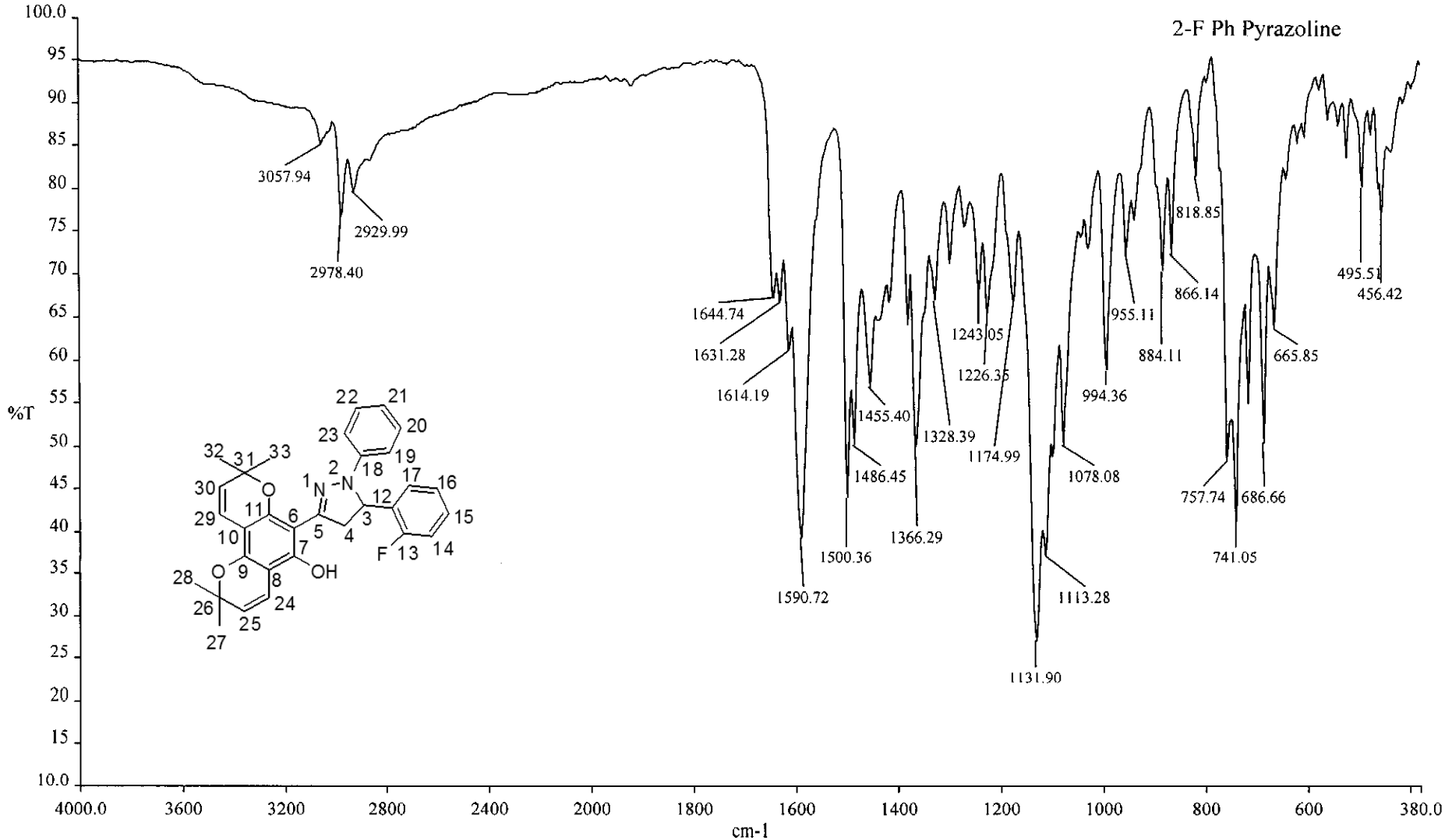
Expanded HMBC spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



Expanded HMBC spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



19F NMR spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)

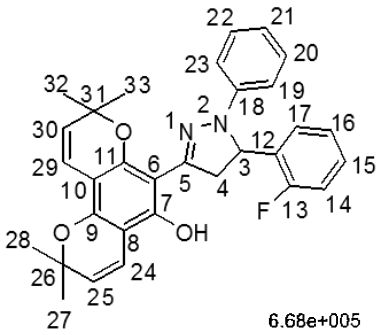


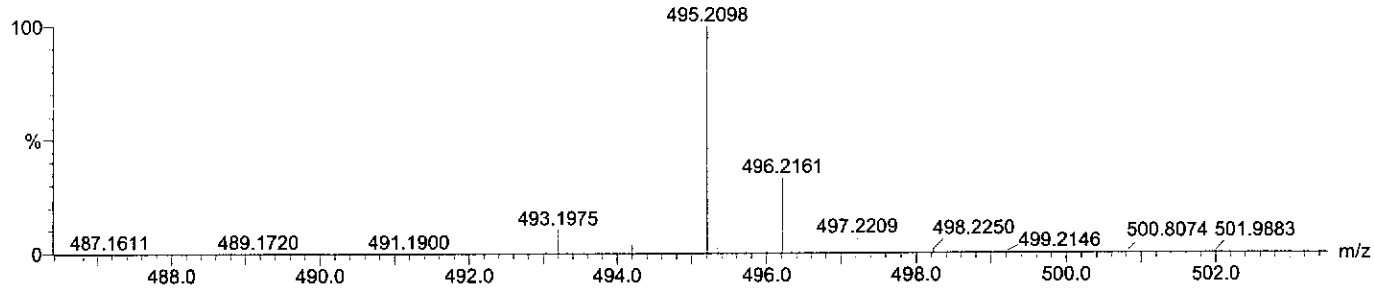
Infrared spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)

Elemental Composition Report **Page 1**

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

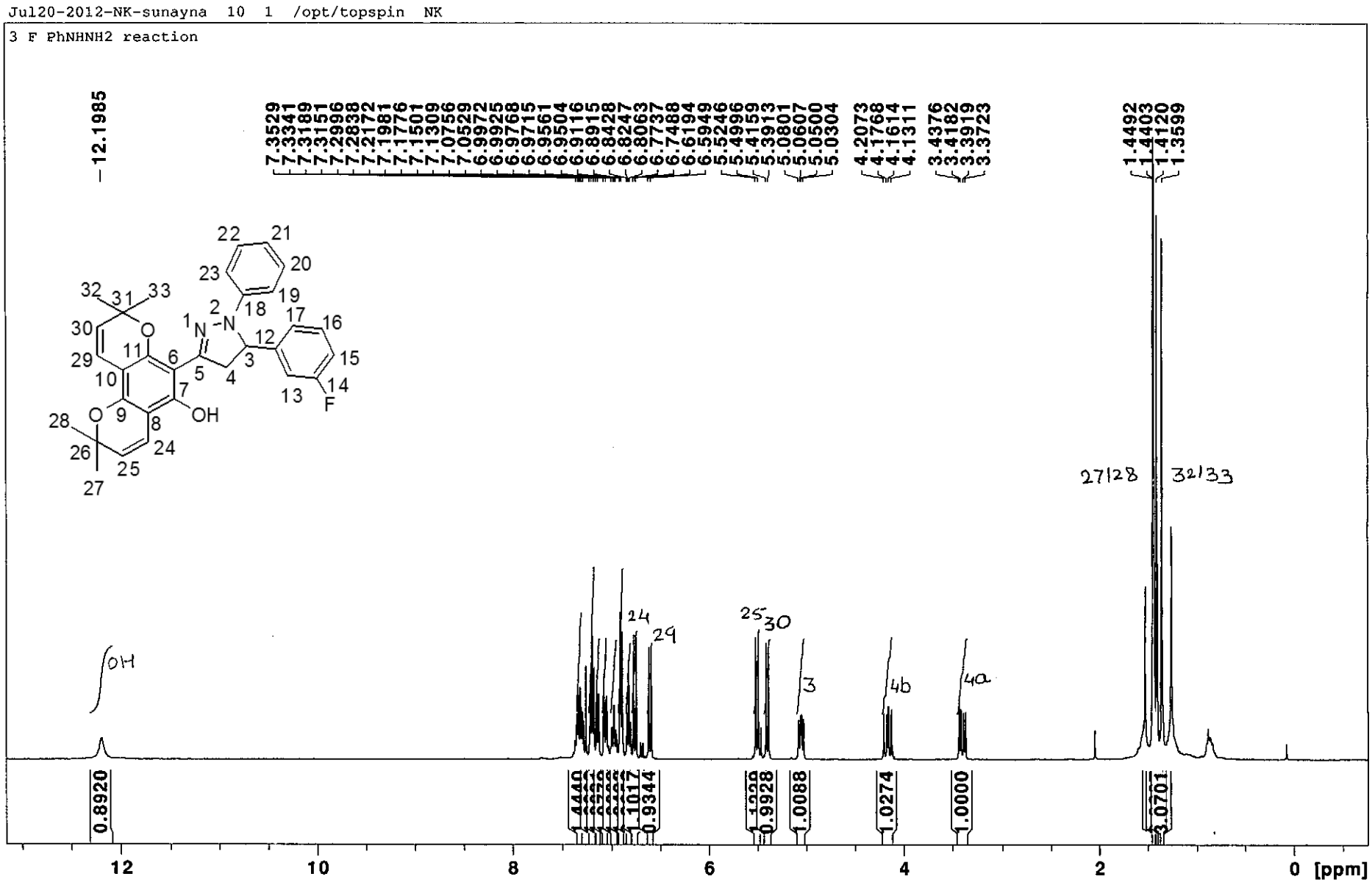
Monoisotopic Mass, Even Electron Ions
22 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-35 H: 20-30 N: 0-4 O: 0-4 F: 1-1
8 25 (0.409) Cm (1:31)
TOF MS ES-



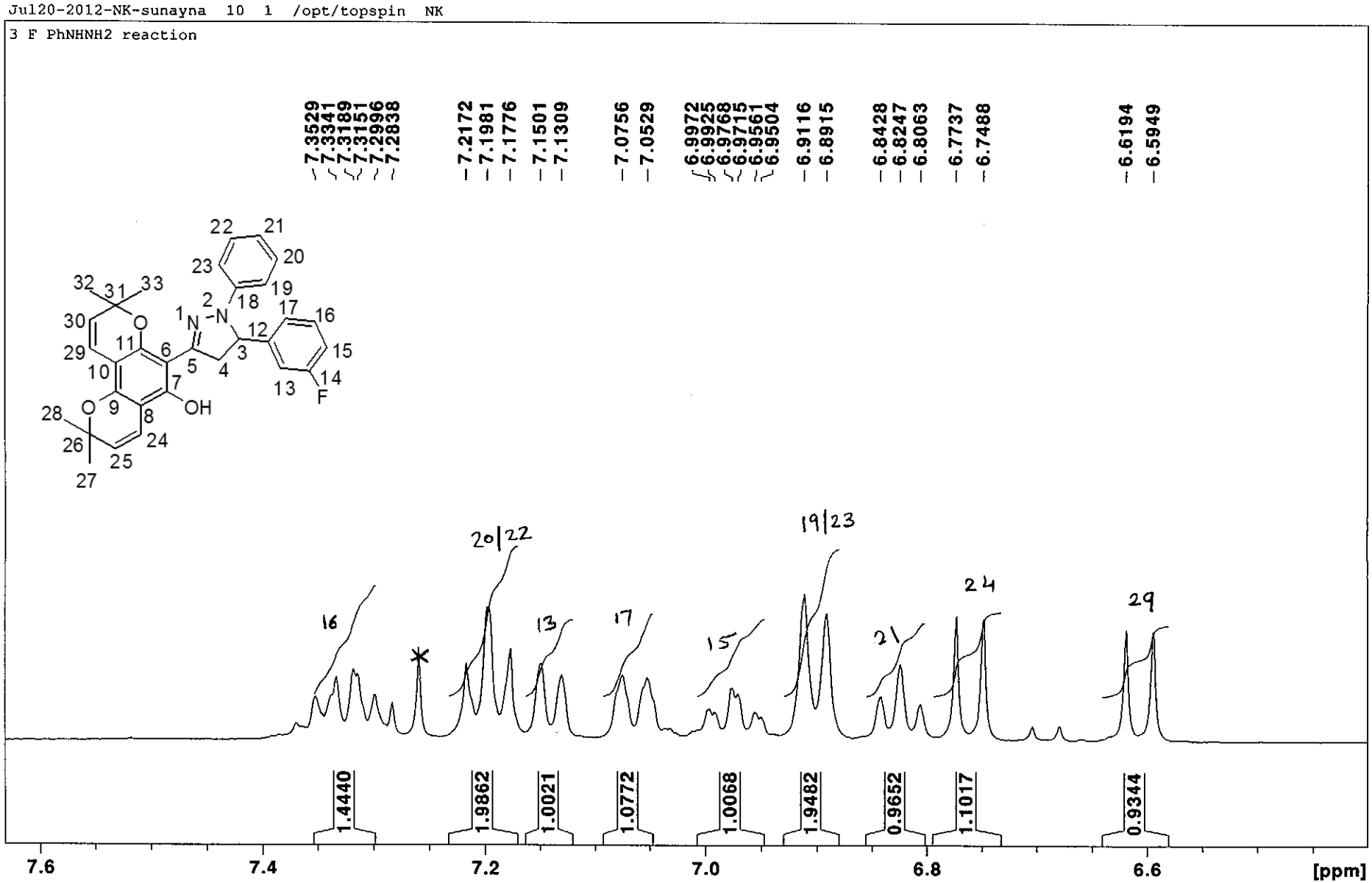


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
495.2098	495.2084	1.4	2.8	18.5	571.7	0.0	C31 H28 N2 O3 F

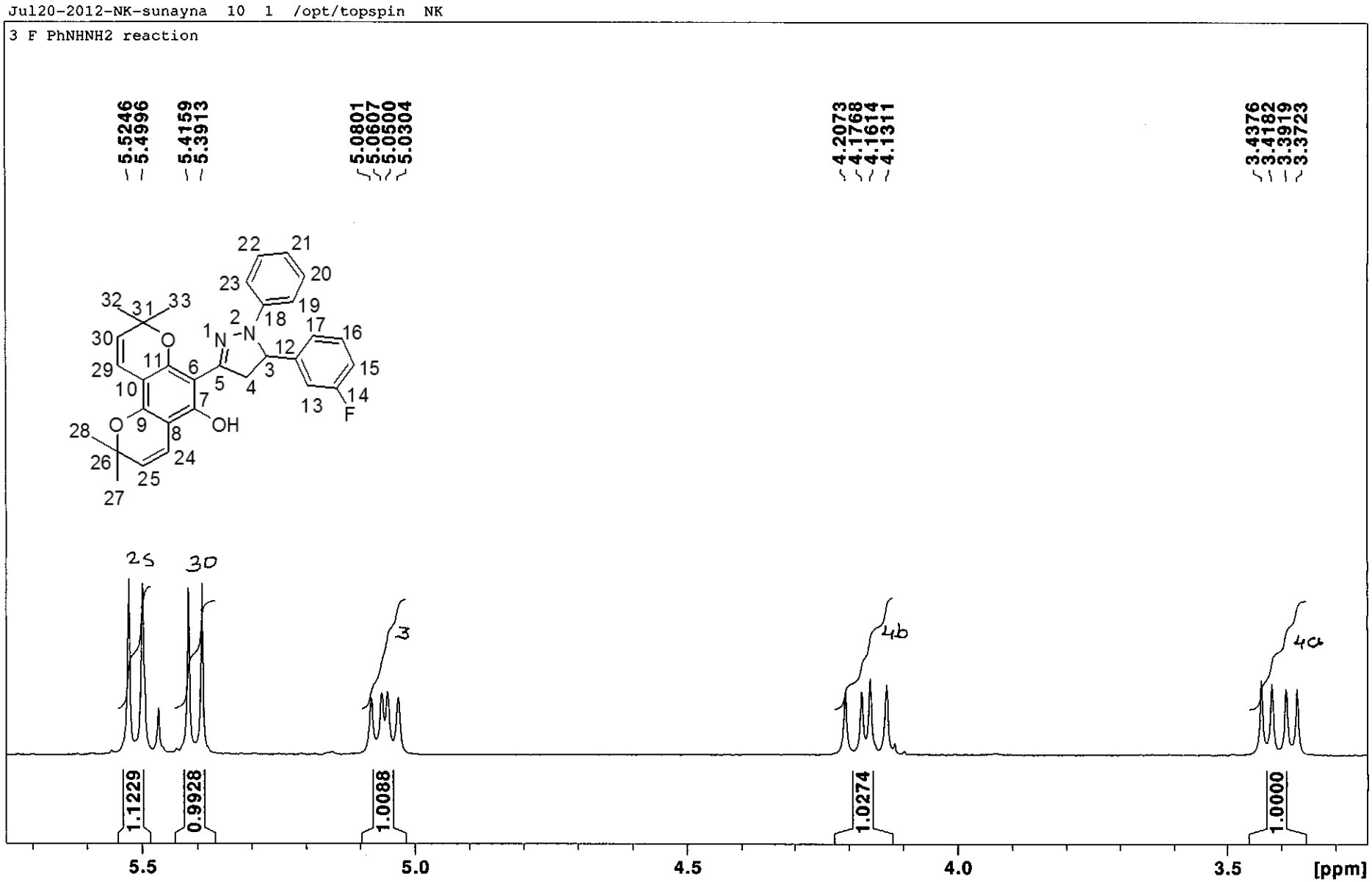
HRMS spectrum of 3-(2-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5d)



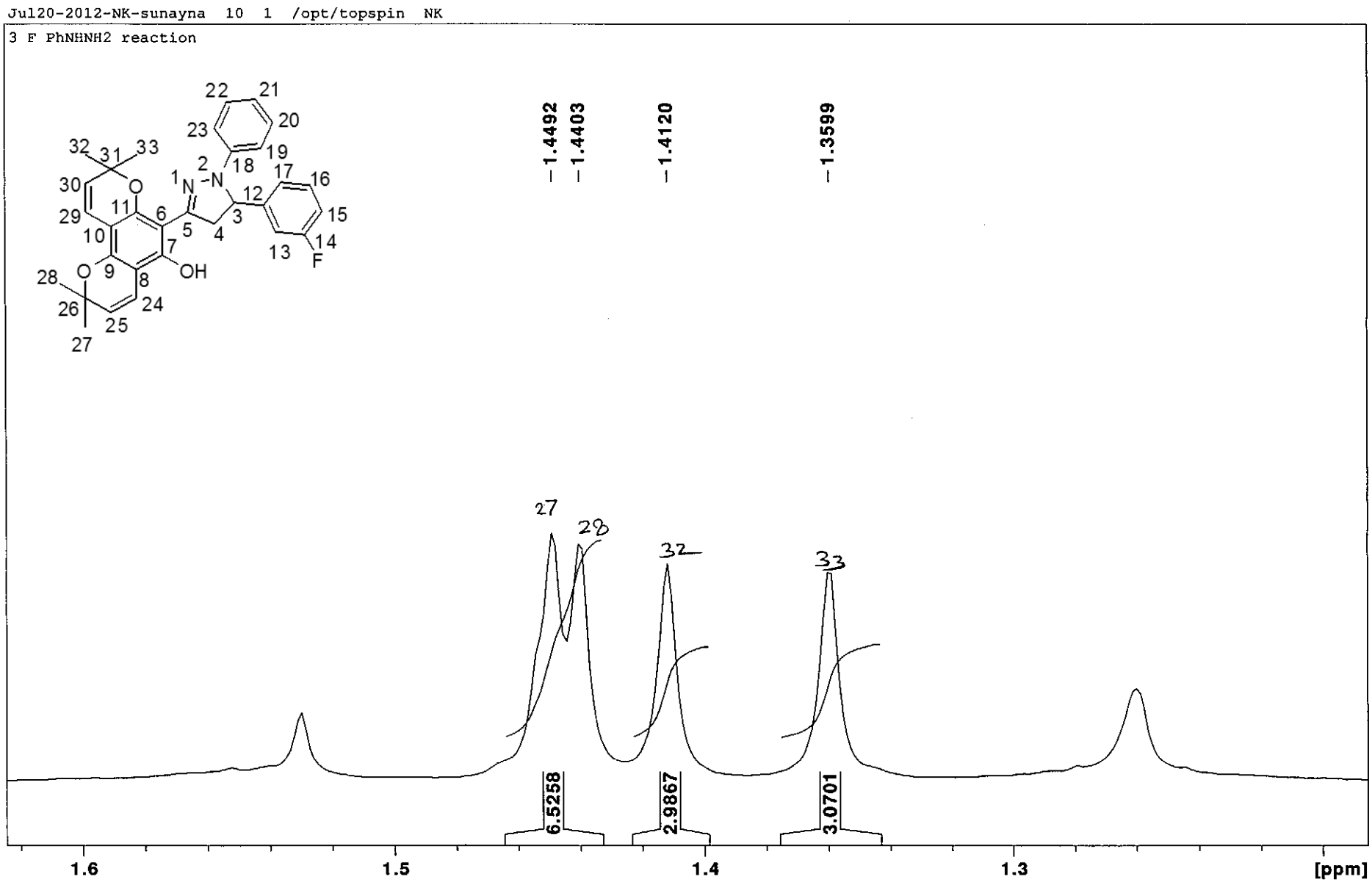
1H NMR spectrum of 3-(3-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5e)



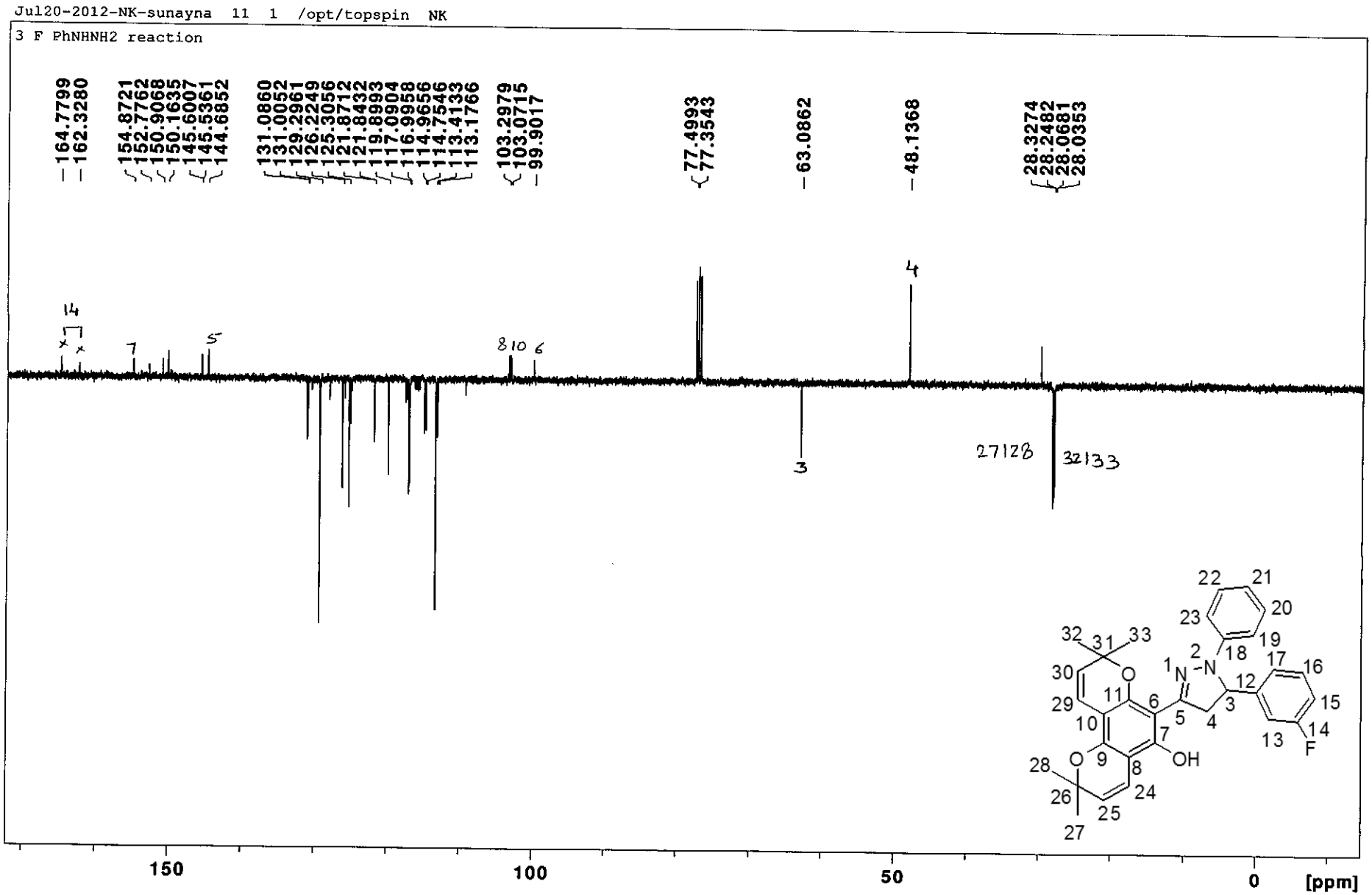
Expanded 1H NMR spectrum of 3-(3-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5e)



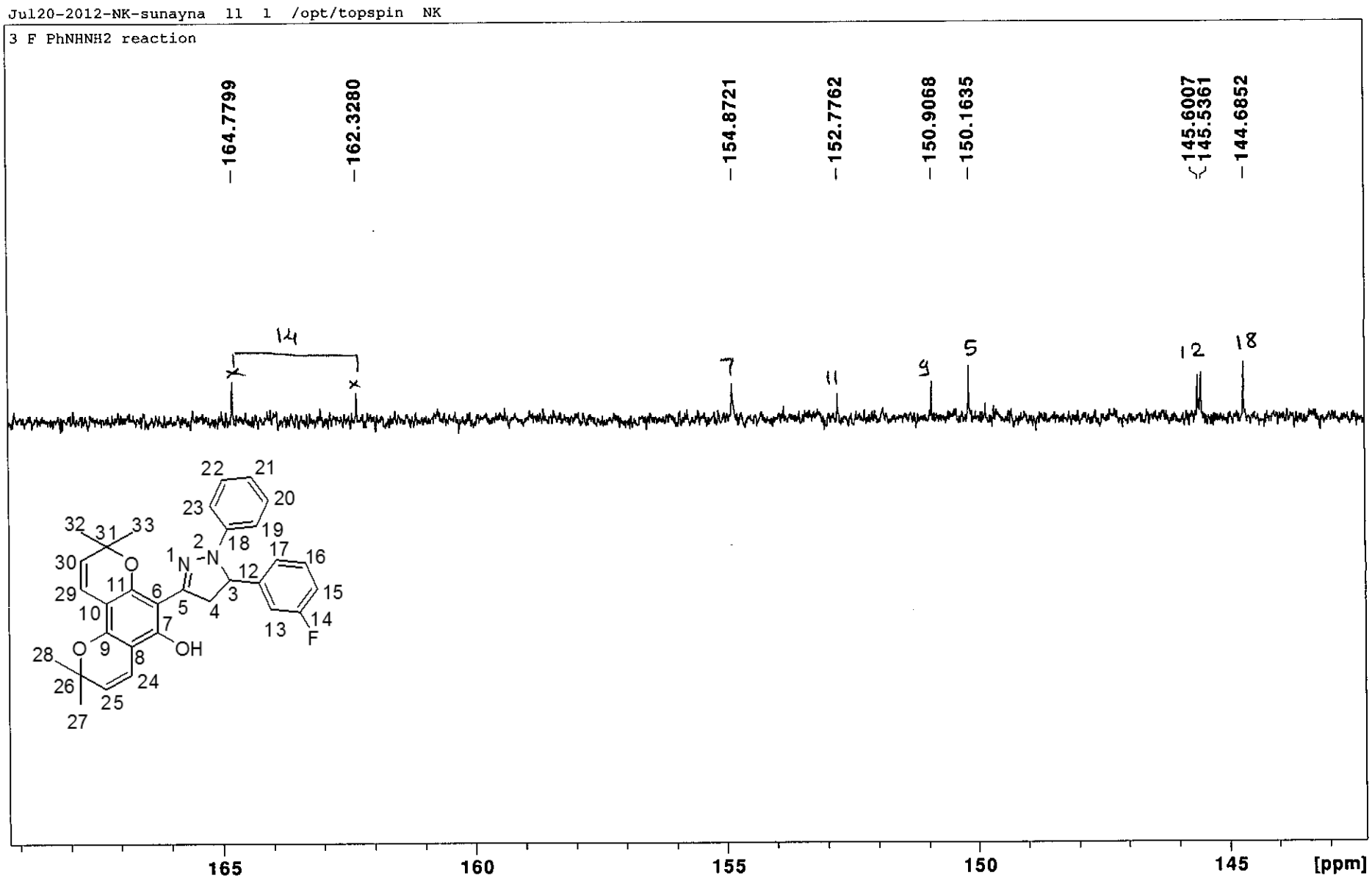
Expanded 1H NMR spectrum of 3-(3-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5e)



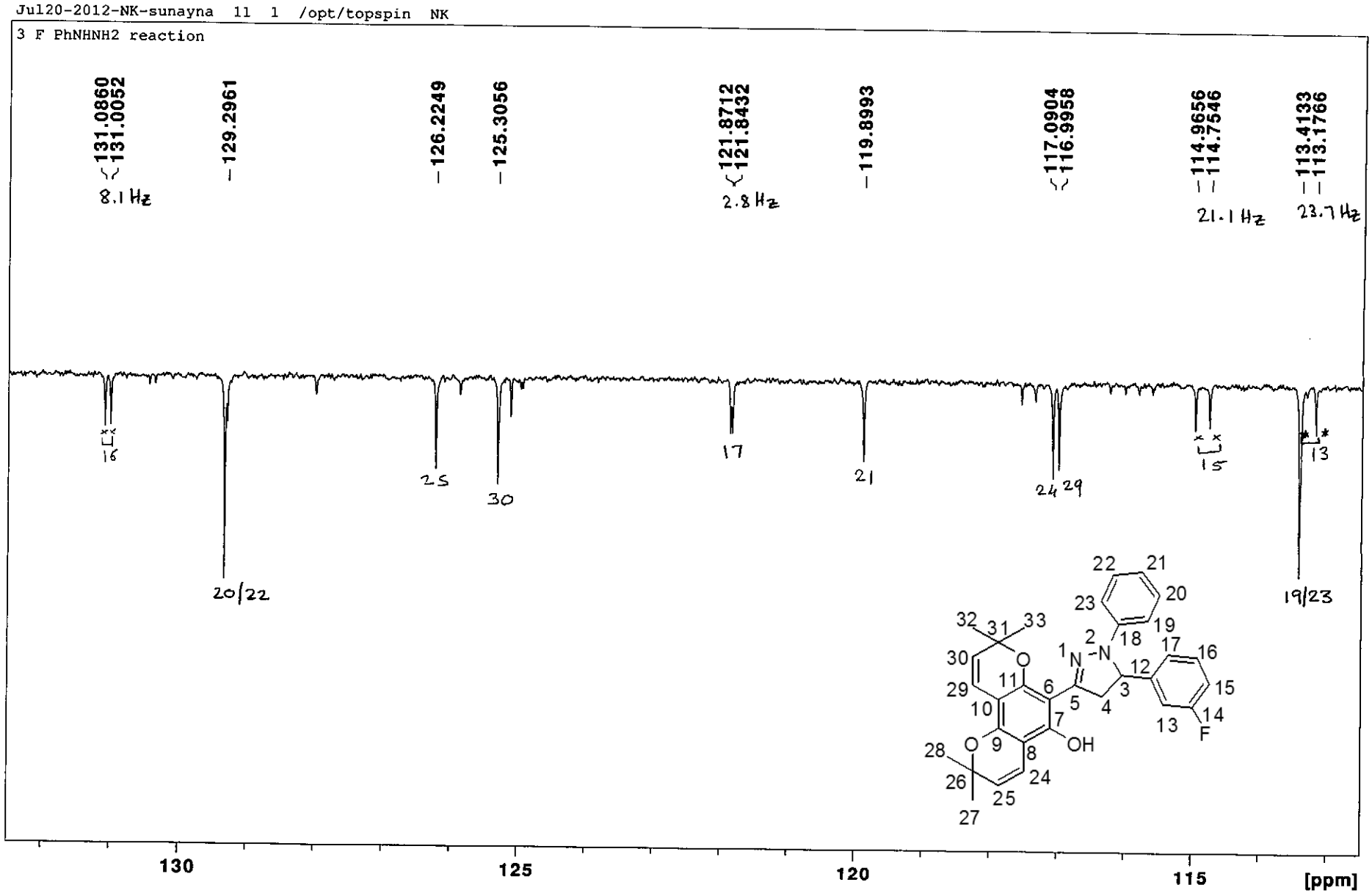
Expanded 1H NMR spectrum of 3-(3-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5e)



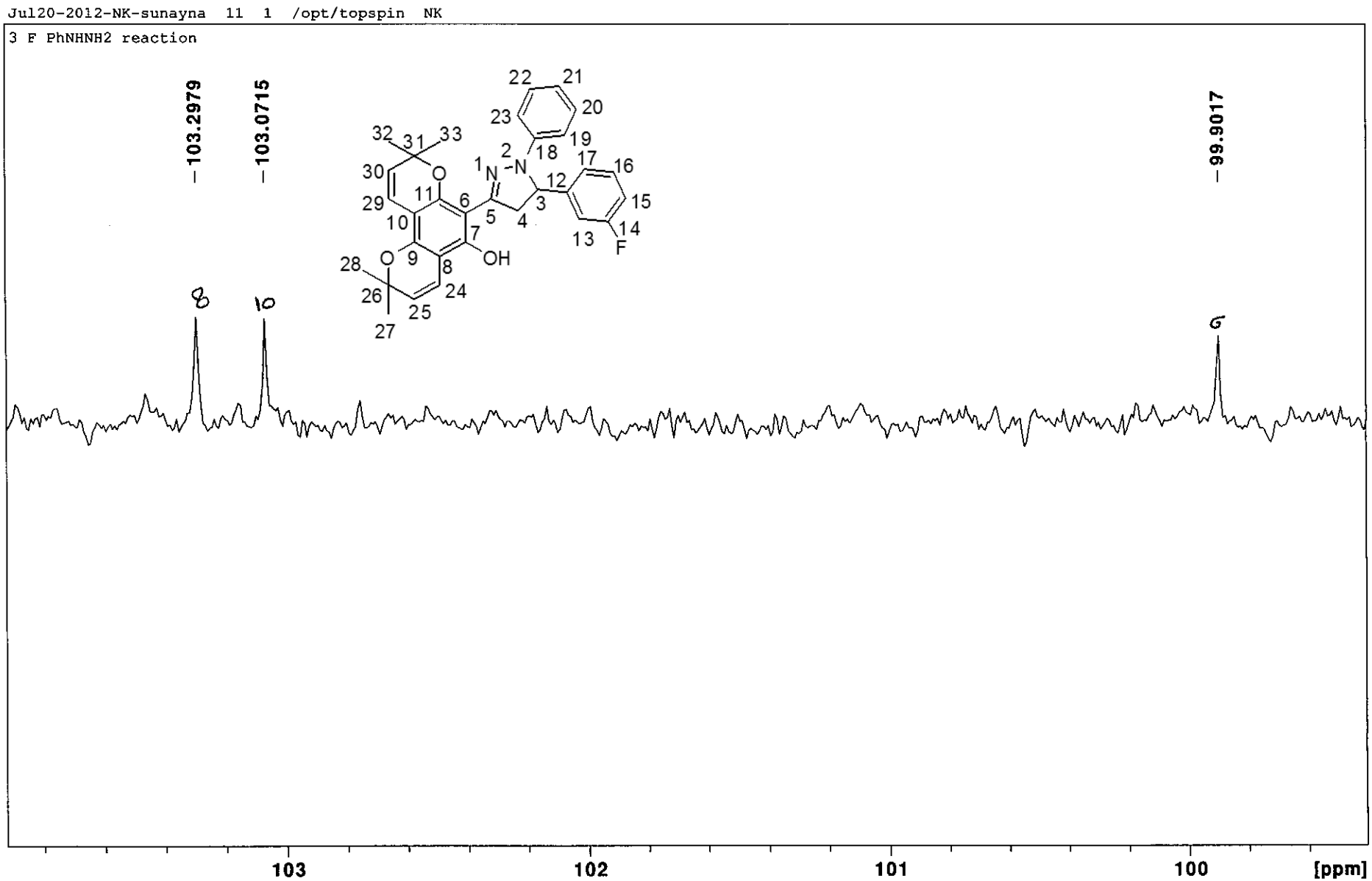
13C NMR spectrum of 3-(3-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5e)



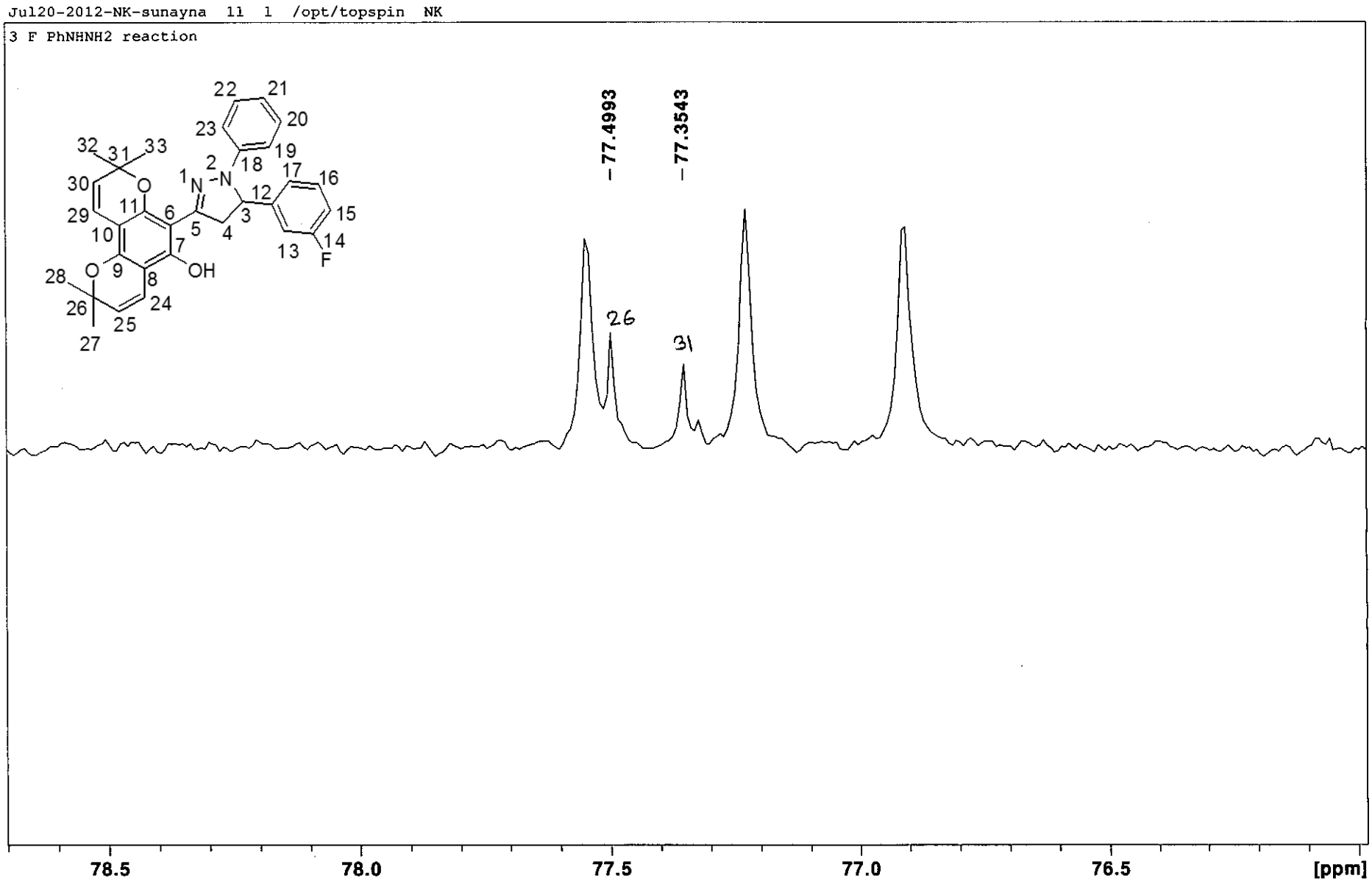
Expanded 13C NMR spectrum of 3-(3-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5e)



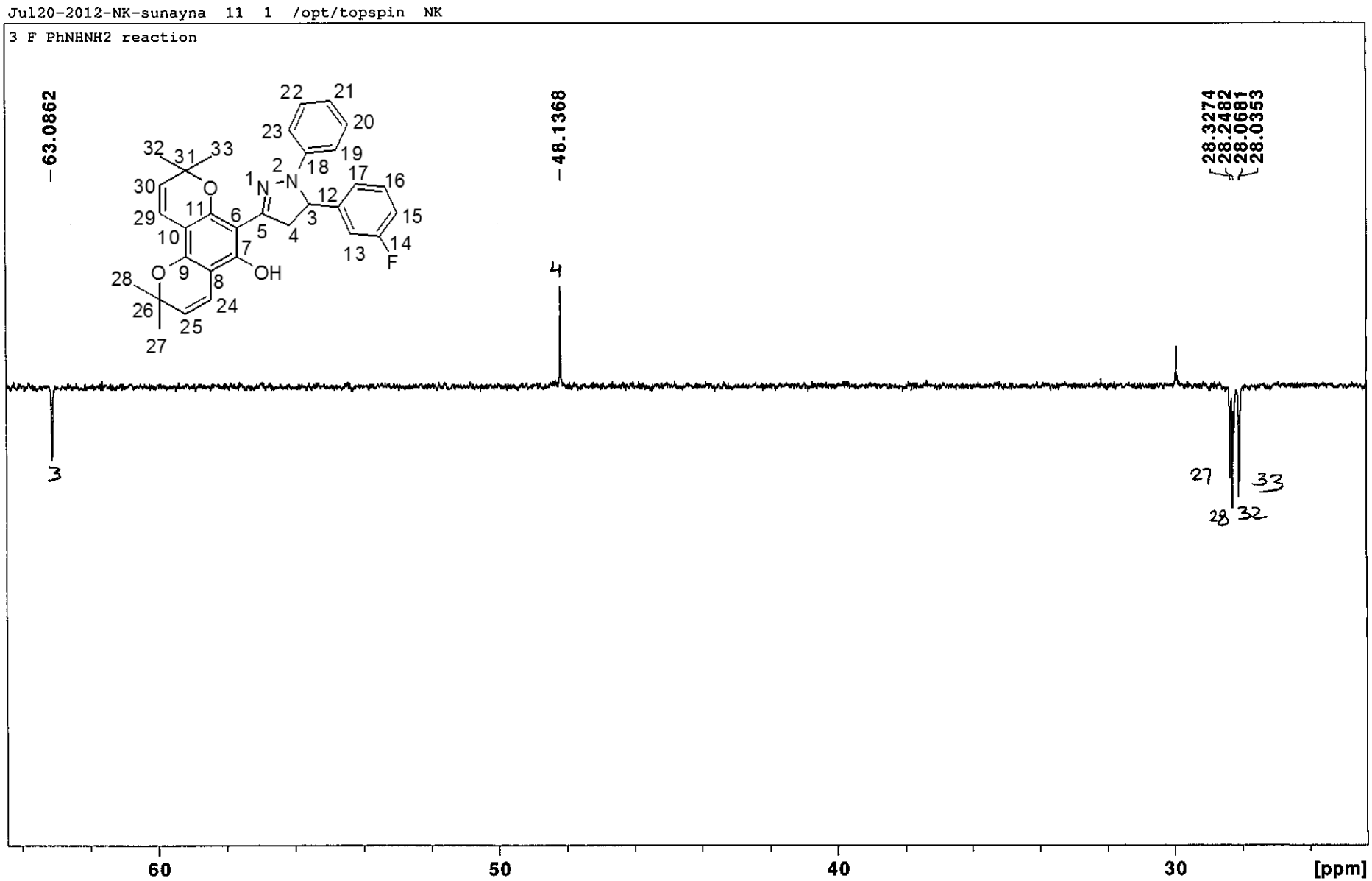
Expanded 13C NMR spectrum of 3-(3-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5e)



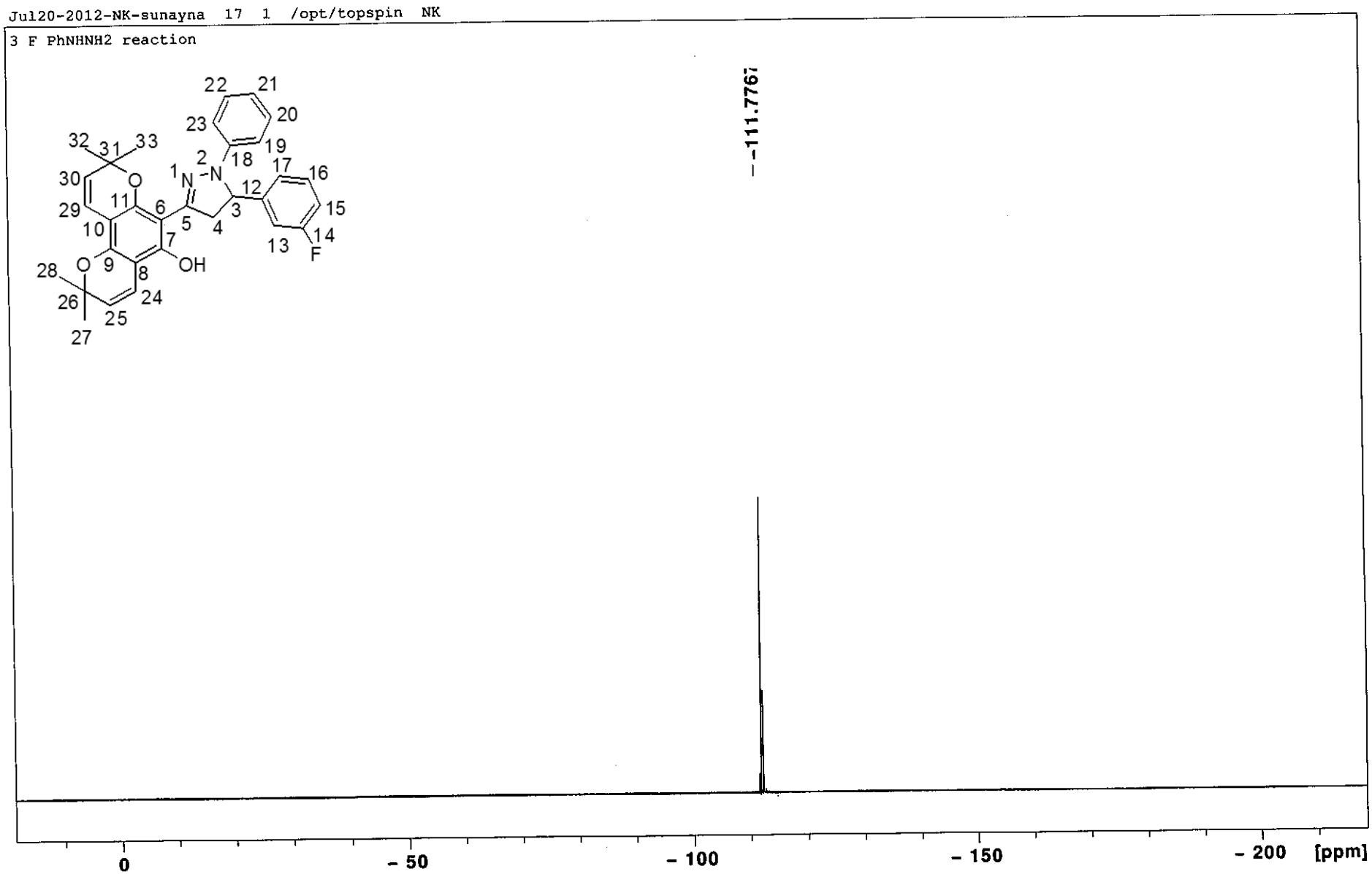
Expanded 13C NMR spectrum of 3-(3-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5e)



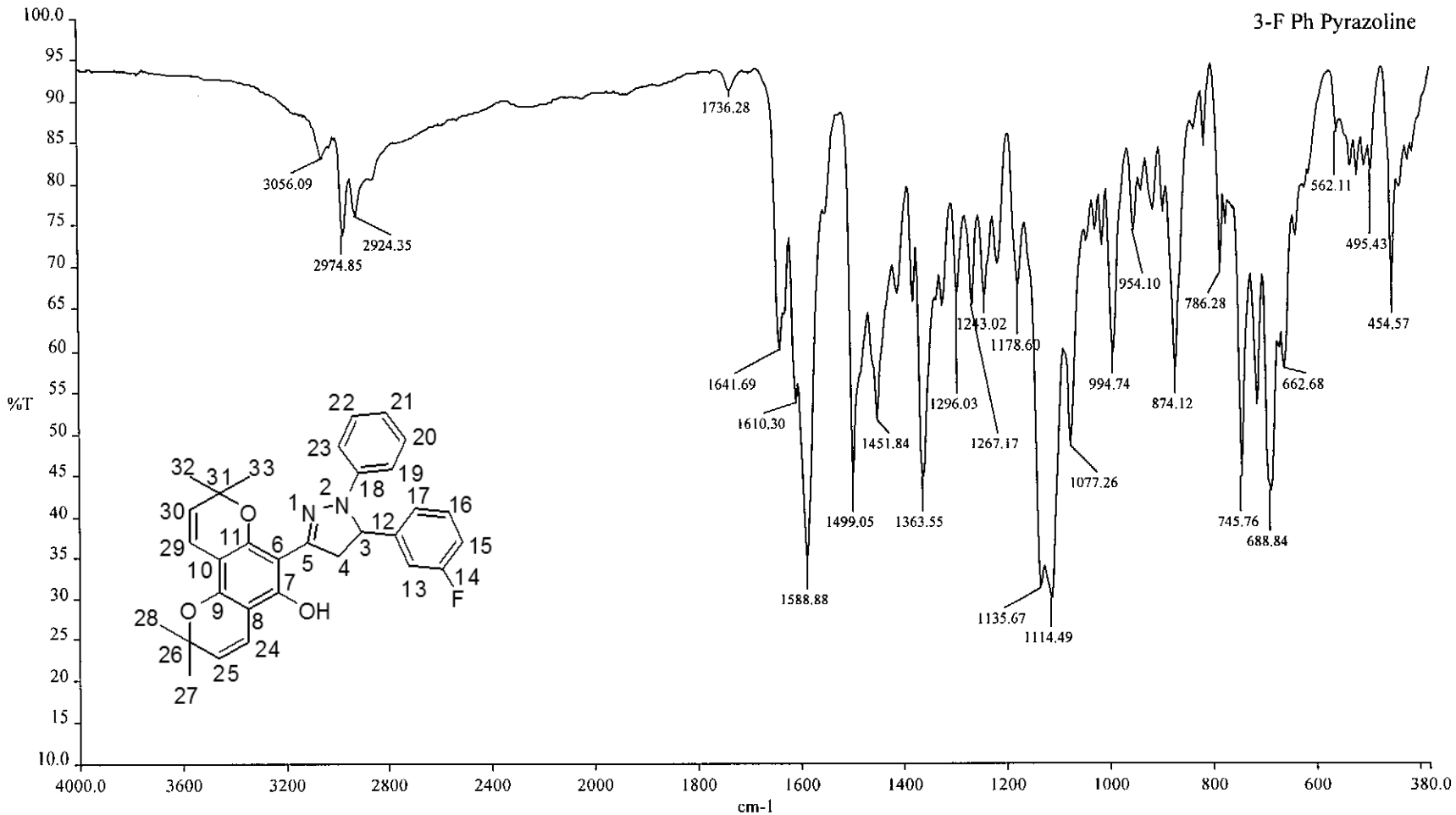
Expanded 13C NMR spectrum of 3-(3-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5e)



Expanded 13C NMR spectrum of 3-(3-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5e)



19F NMR spectrum of 3-(3-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5e)

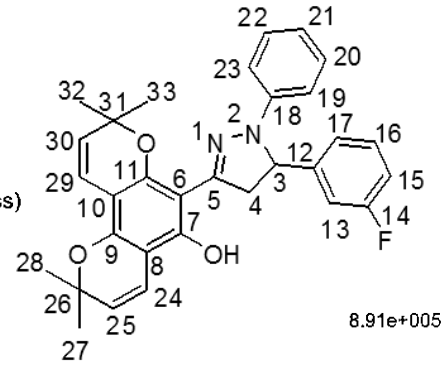


Infrared spectrum of 3-(3-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5e)

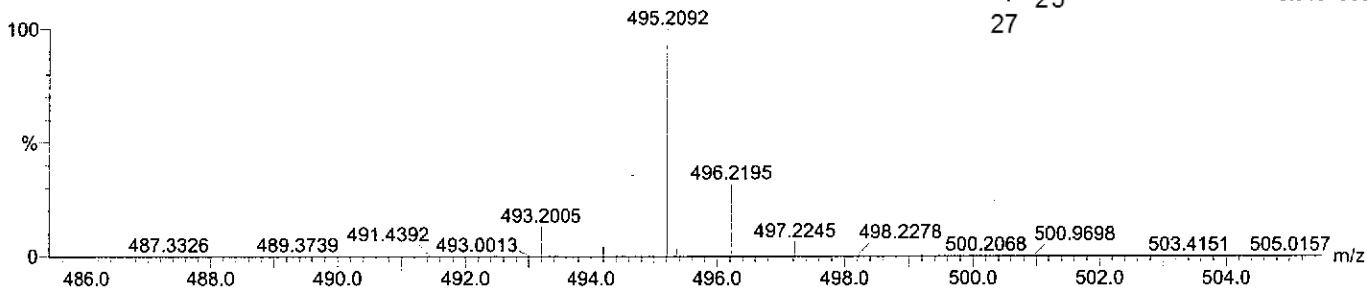
Elemental Composition Report **Page 1**

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

Monoisotopic Mass, Even Electron Ions
22 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-35 H: 20-30 N: 0-4 O: 0-4 F: 1-1
10 1 (0.017) Cm (1:29)
TOF MS ES-

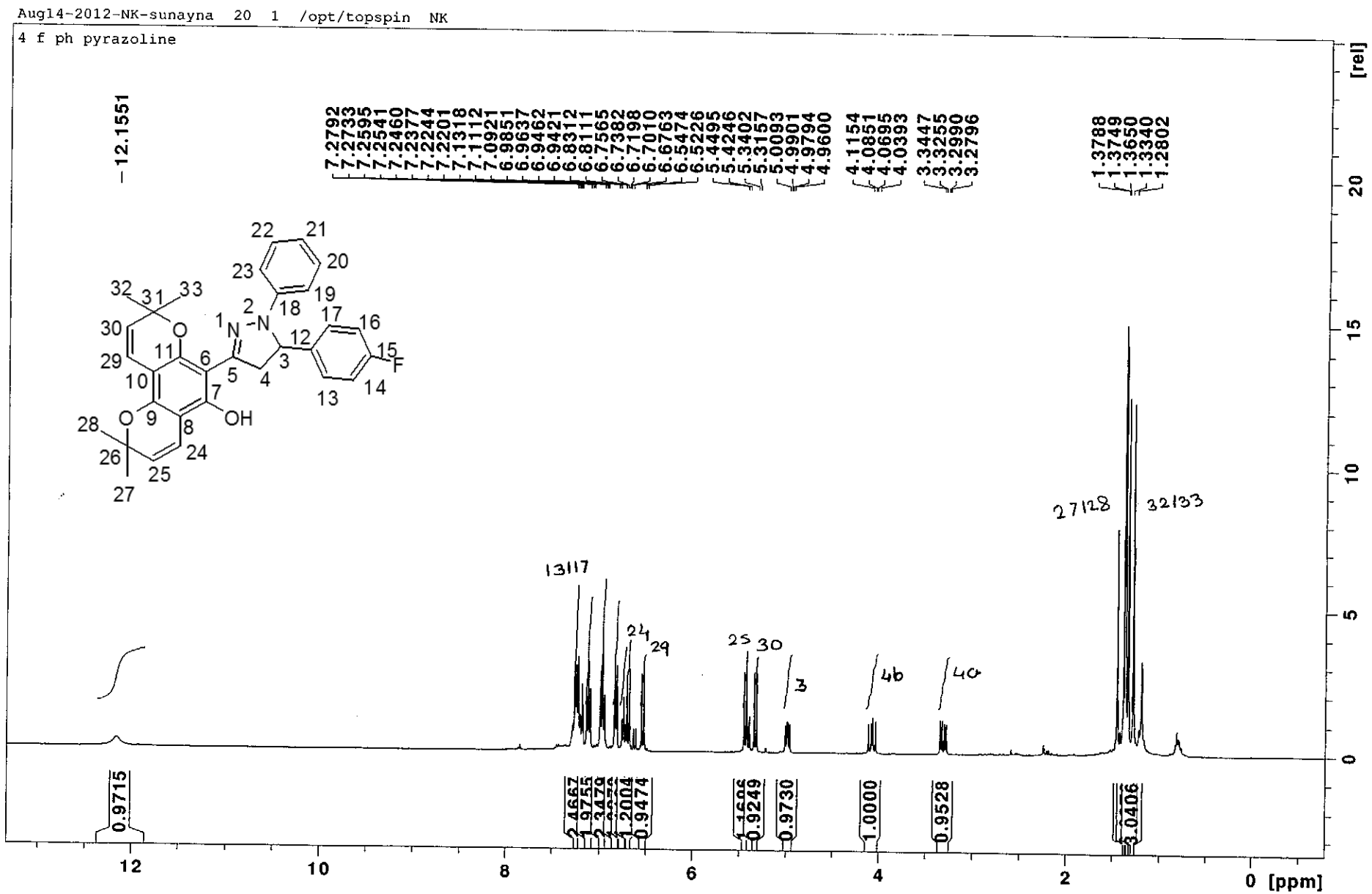


8.91e+005

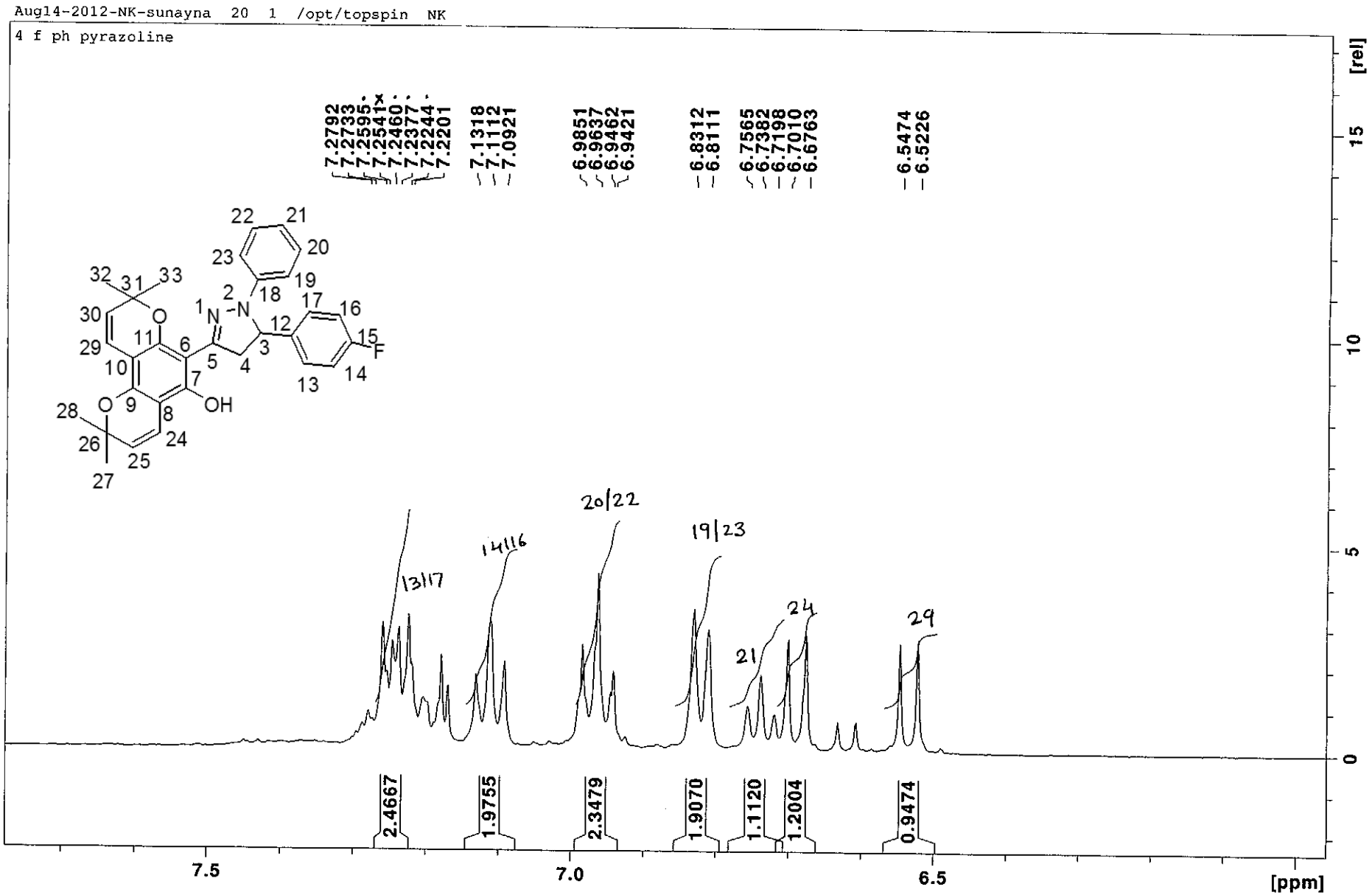


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
495.2092	495.2084	0.8	1.6	18.5	575.3	0.0	C31 H28 N2 O3 F

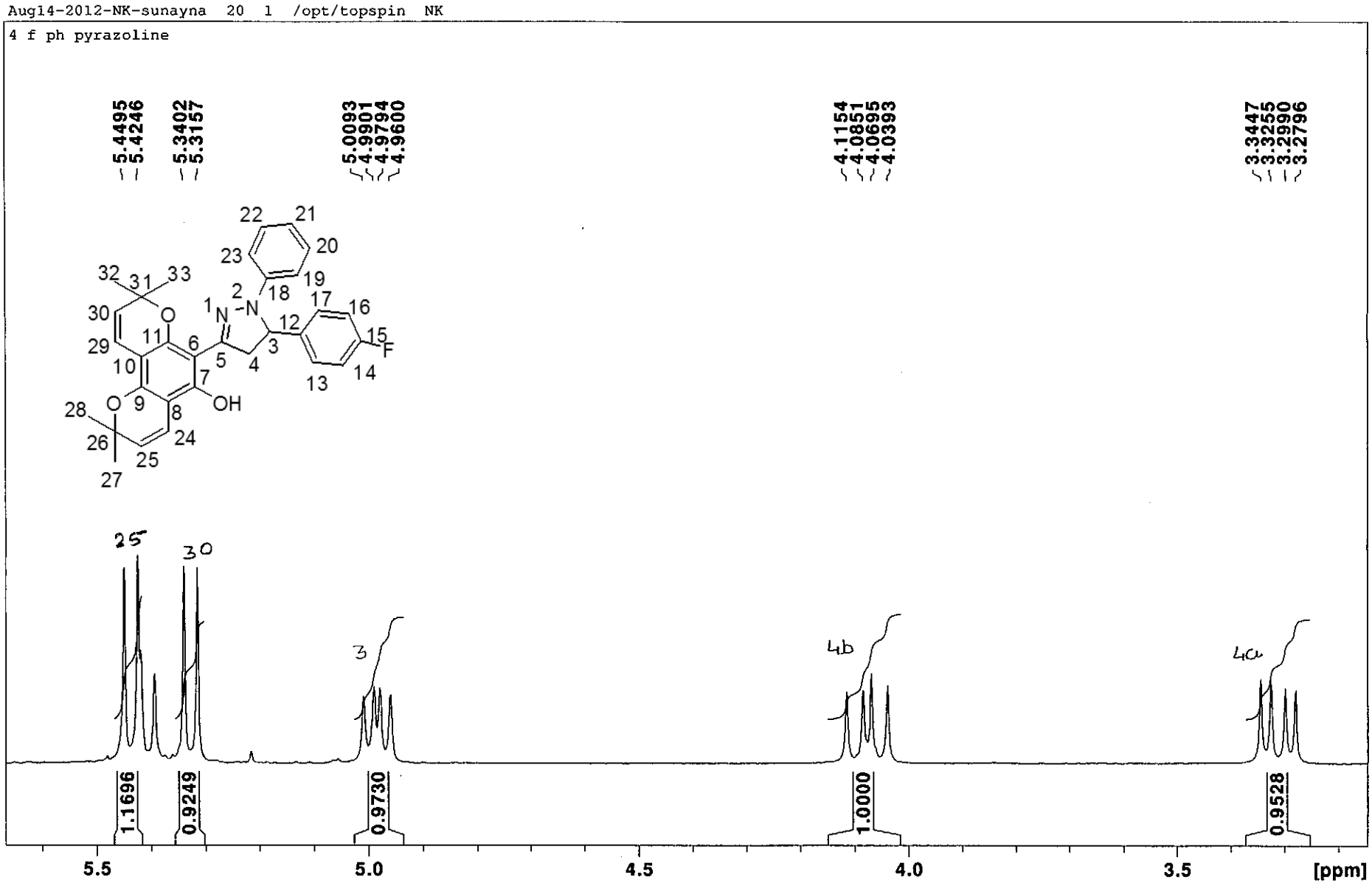
HRMS spectrum of 3-(3-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5e)



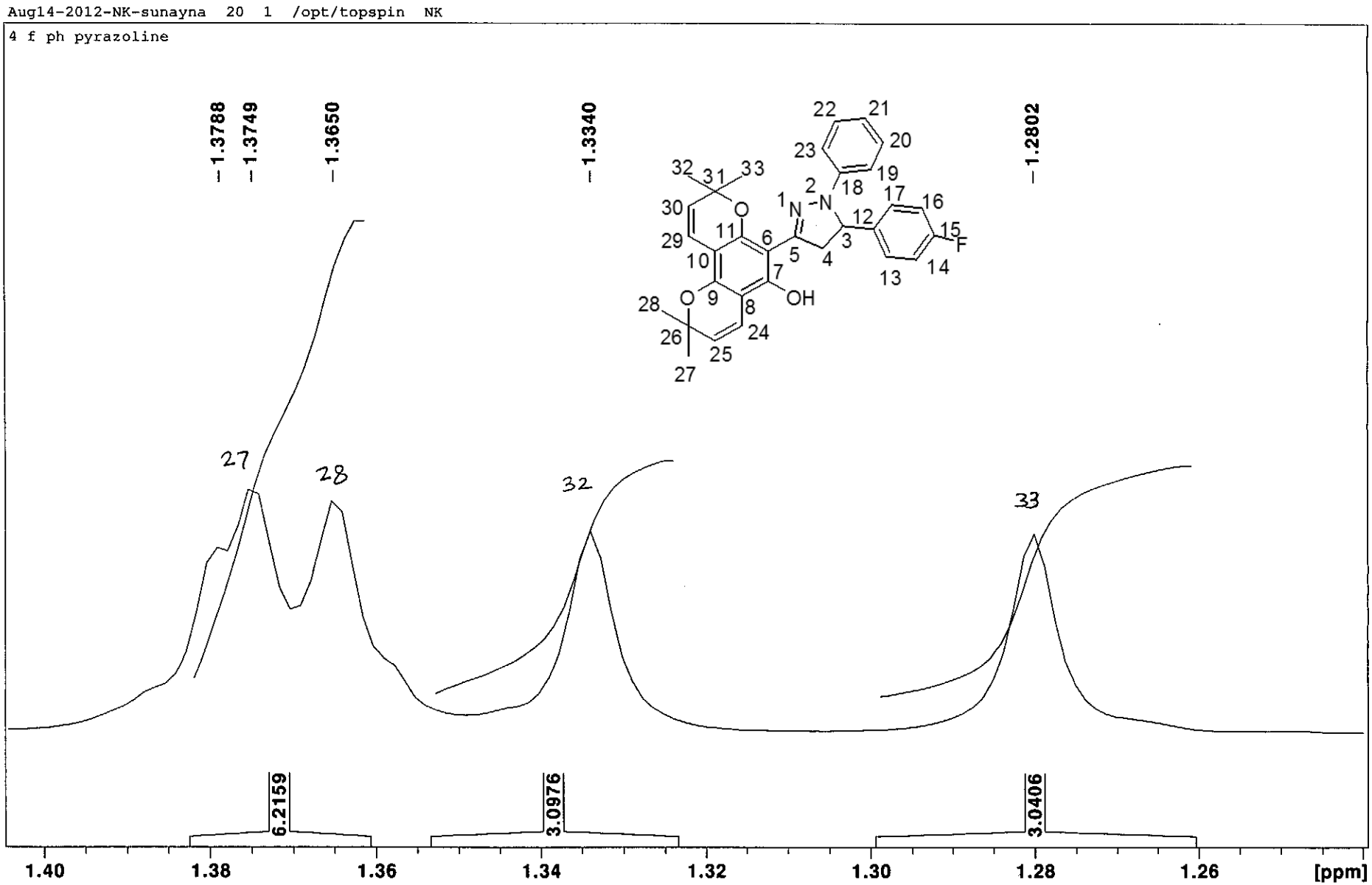
1H NMR spectrum of 3-(4-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5f)



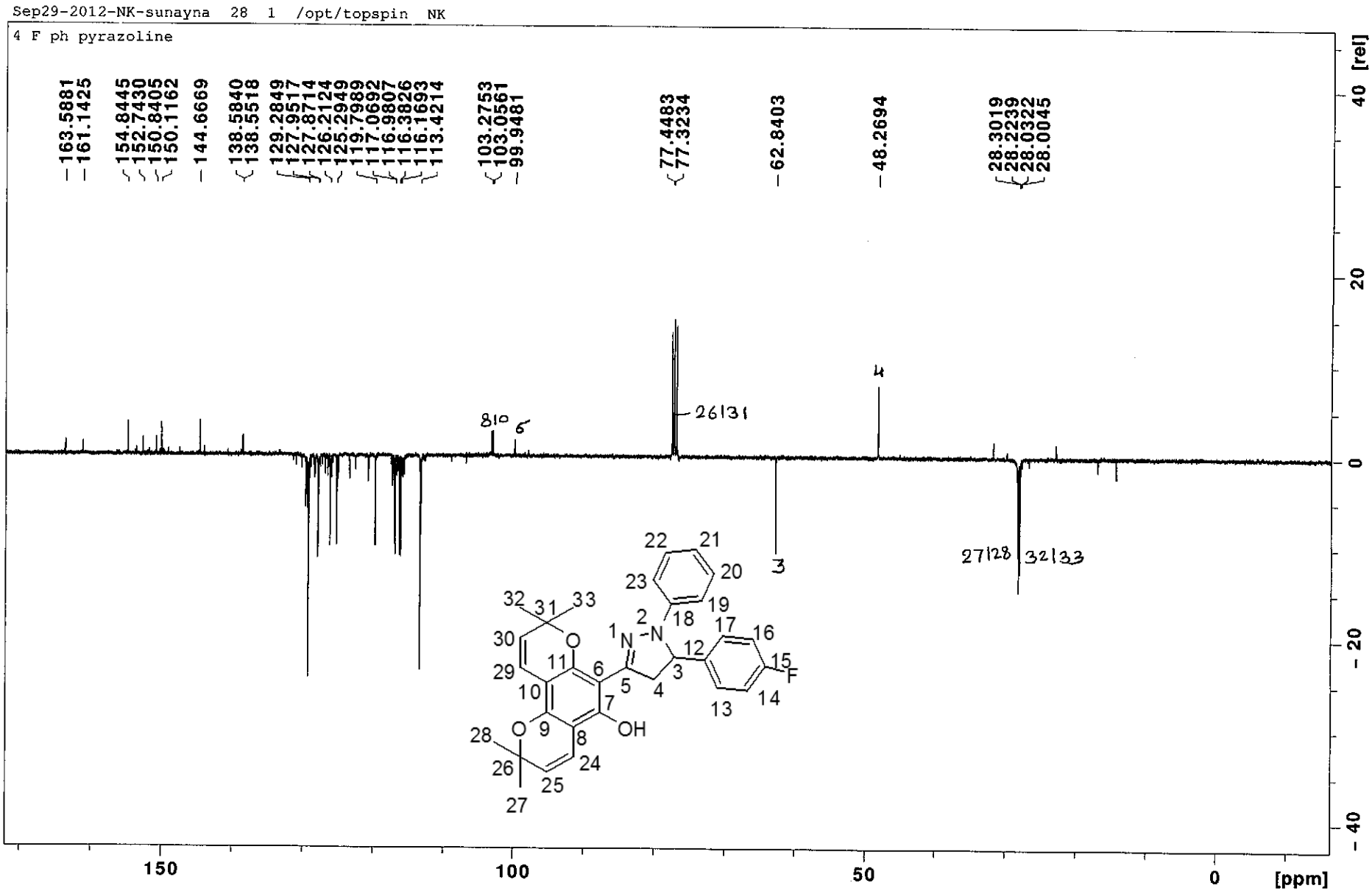
Expanded 1H NMR spectrum of 3-(4-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5f)



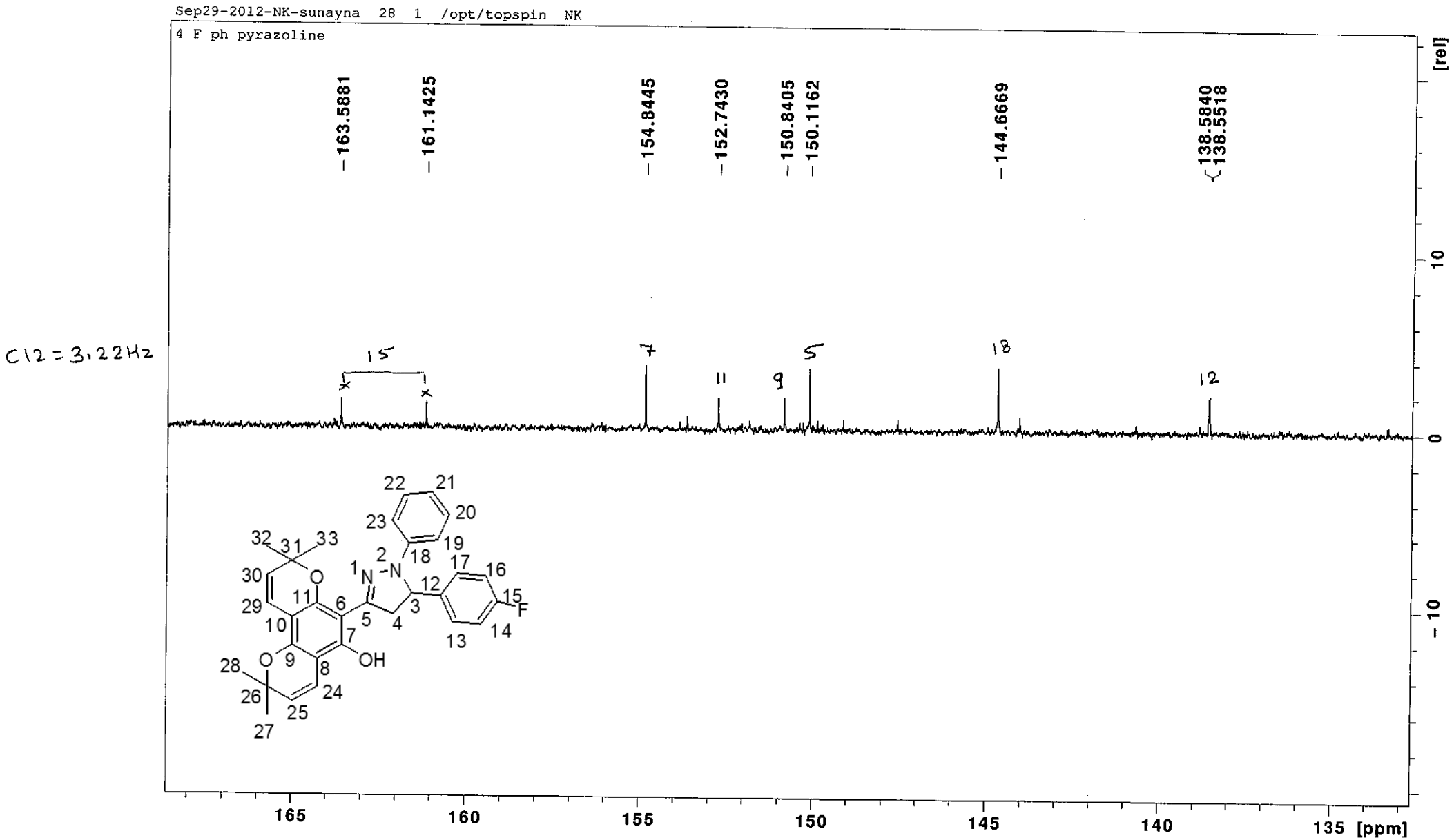
Expanded 1H NMR spectrum of 3-(4-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5f)



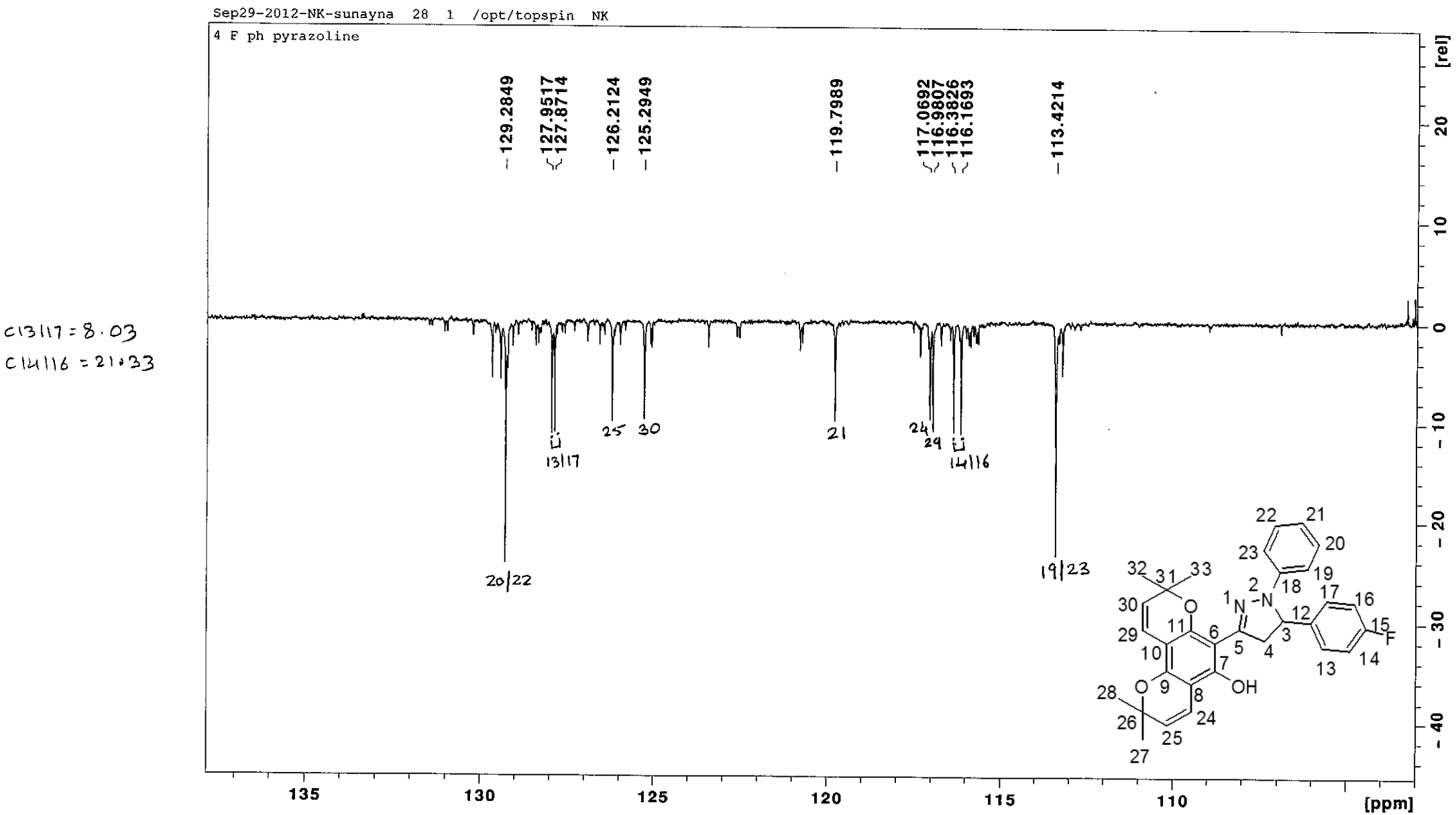
Expanded 1H NMR spectrum of 3-(4-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5f)



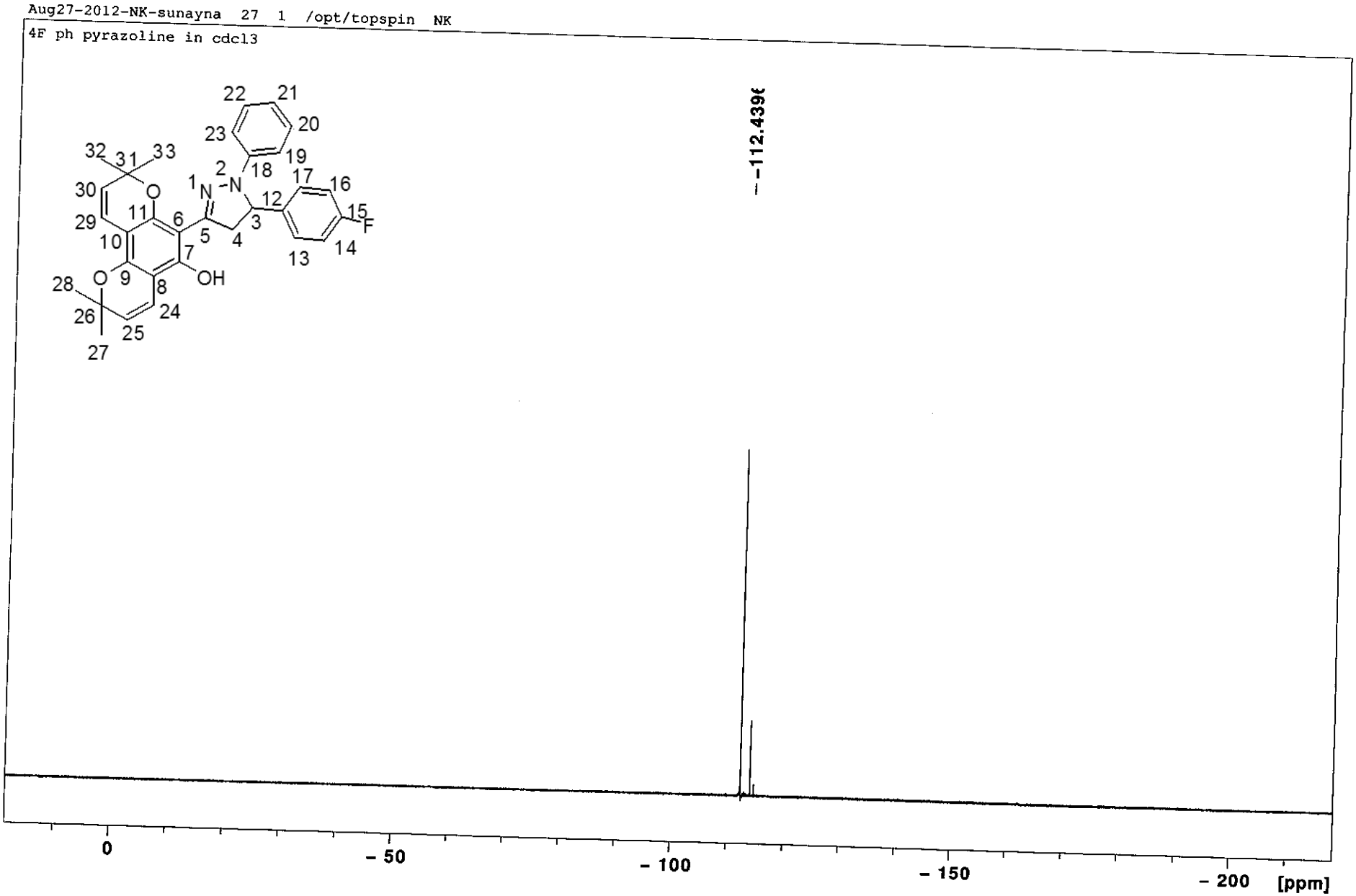
13C NMR spectrum of 3-(4-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5f)



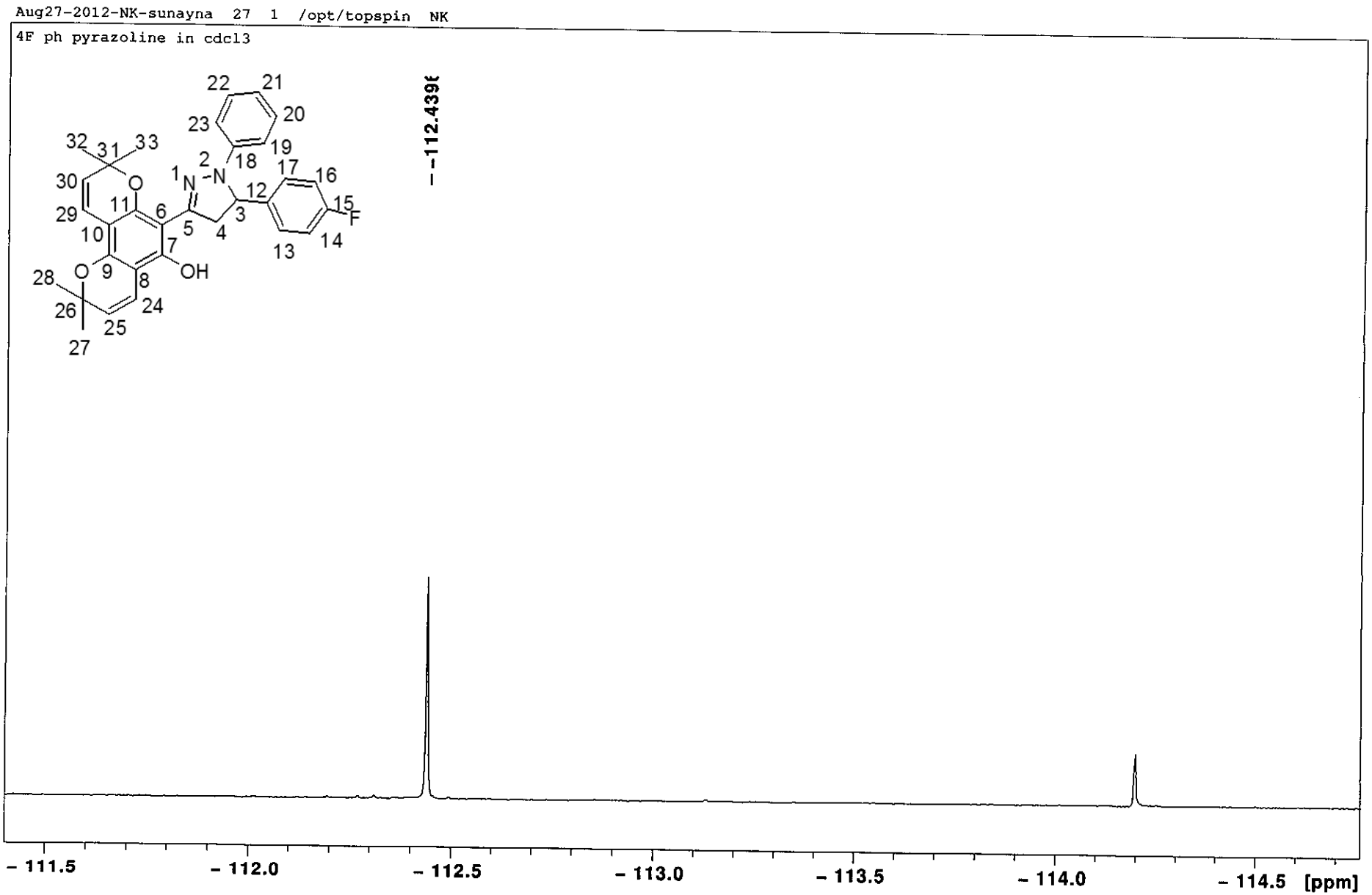
Expanded 13C NMR spectrum of 3-(4-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5f)



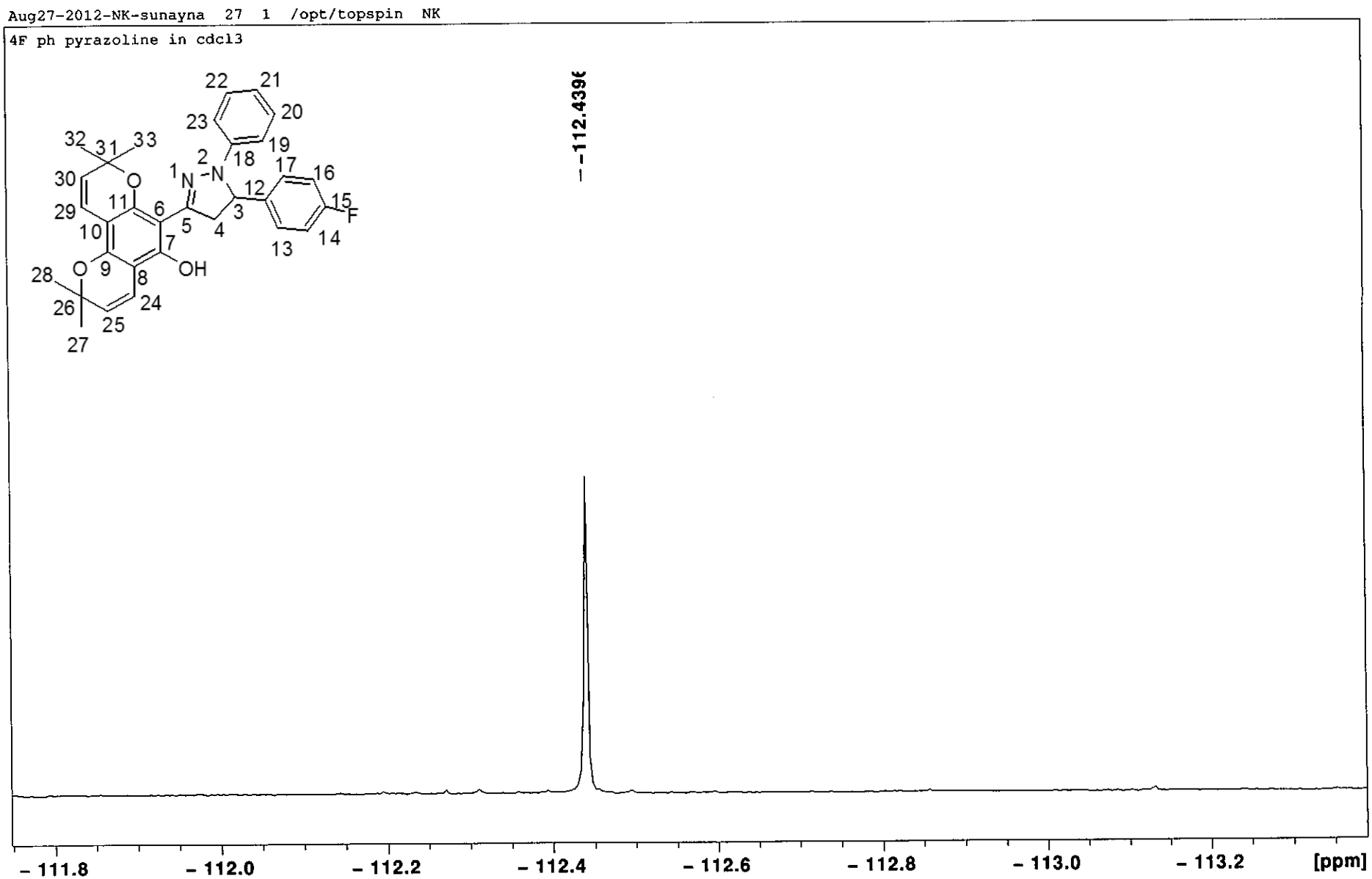
Expanded 13C NMR spectrum of 3-(4-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5f)



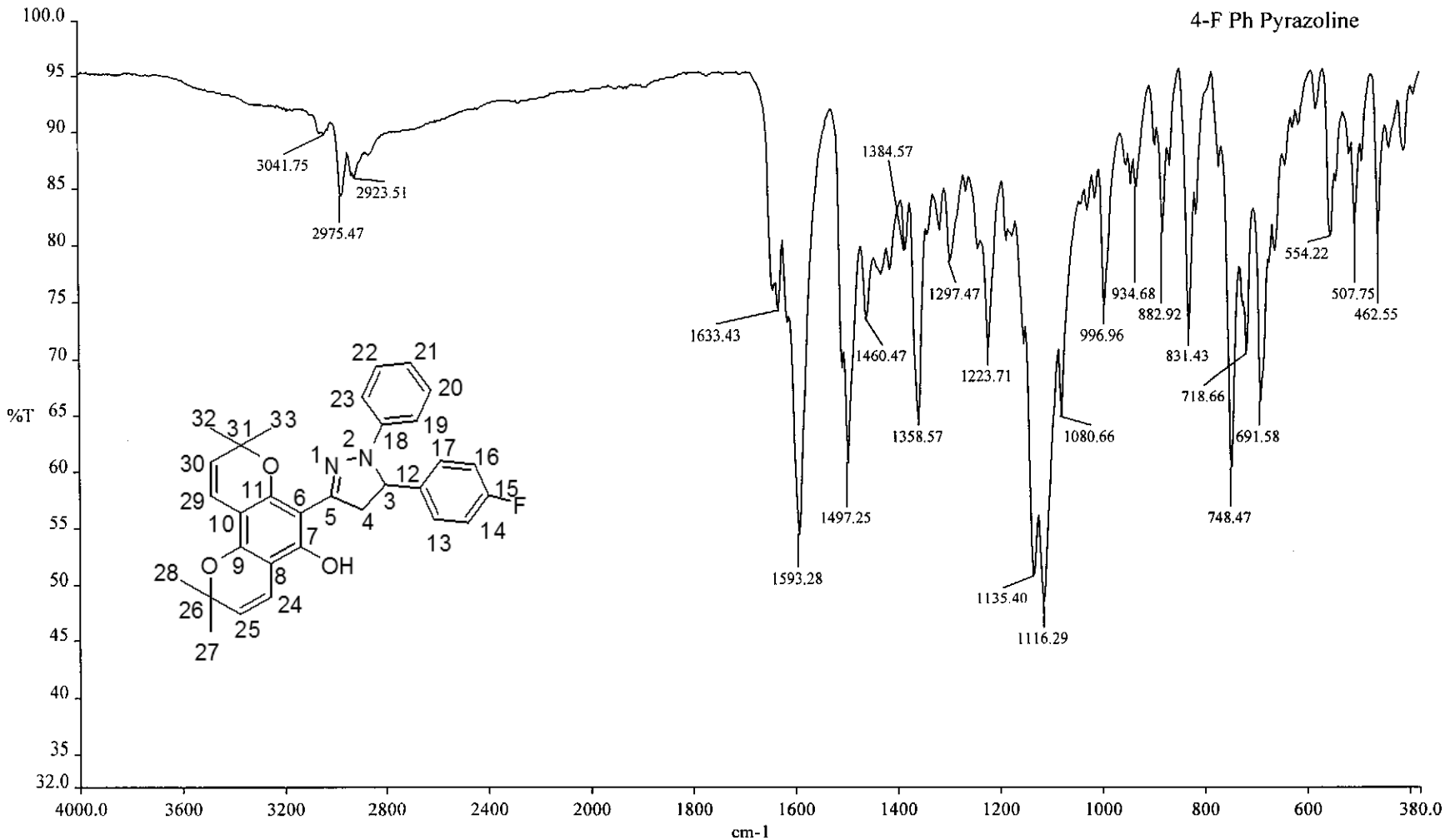
19F NMR spectrum of 3-(4-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5f)



Expanded 19F NMR spectrum of 3-(4-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5f)



Expanded 19F NMR spectrum of 3-(4-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5f)

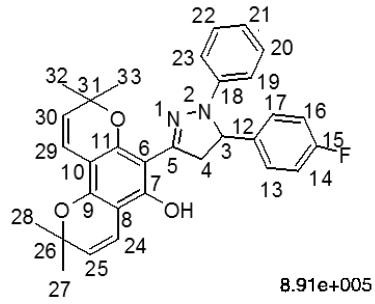


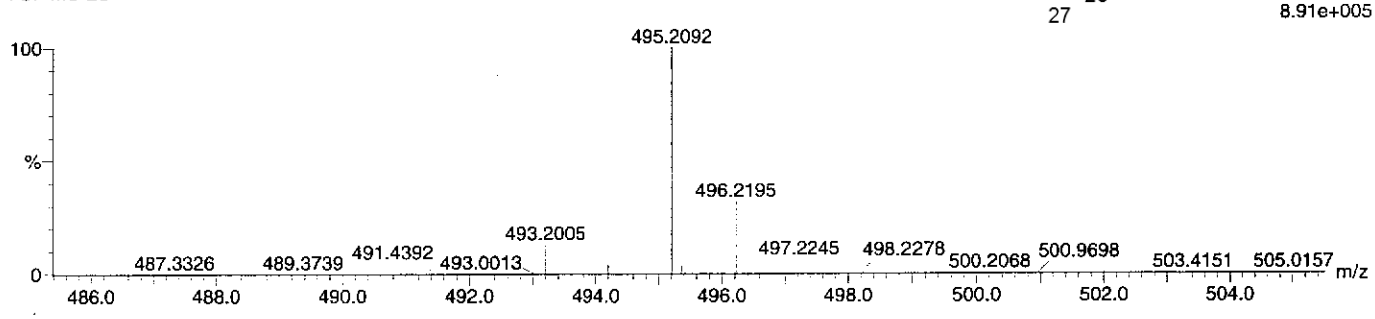
Infrared NMR spectrum of 3-(4-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5f)

Elemental Composition Report **Page 1**

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

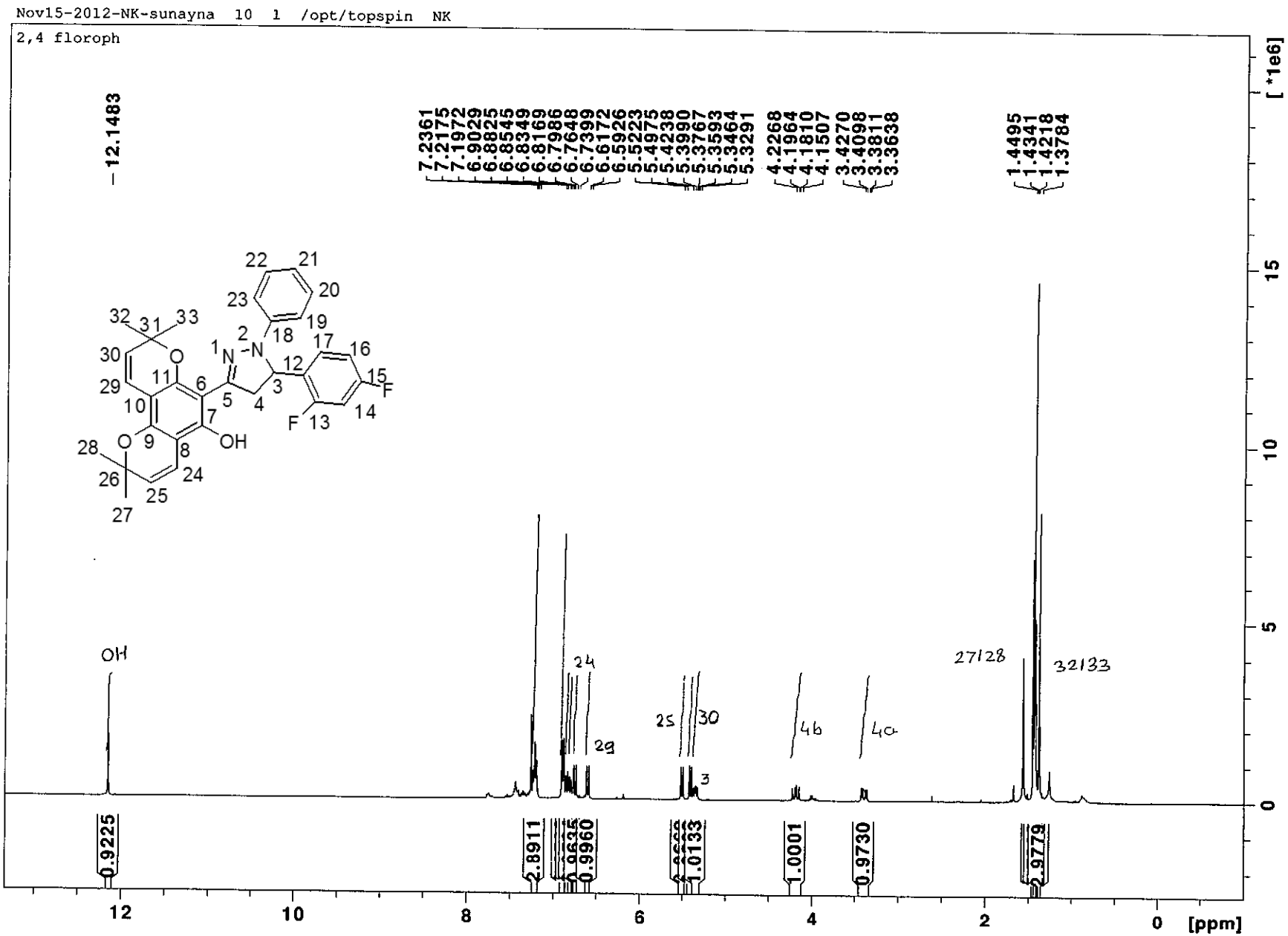
Monoisotopic Mass, Even Electron Ions
22 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-35 H: 20-30 N: 0-4 O: 0-4 F: 1-1
10 1 (0.017) Cm (1:29)
TOF MS ES-



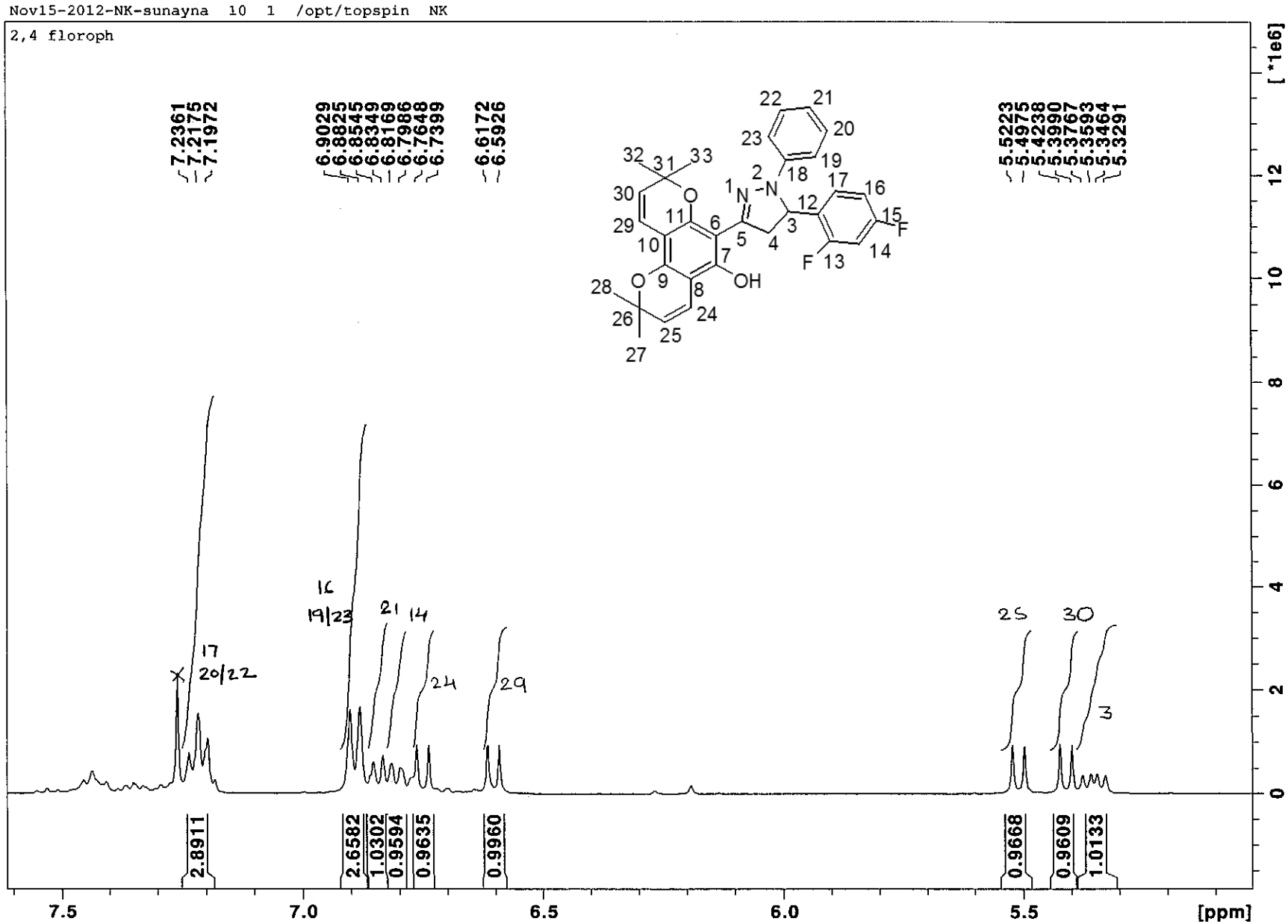


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
495.2092	495.2084	0.8	1.6	18.5	575.3	0.0	C31 H28 N2 O3 F

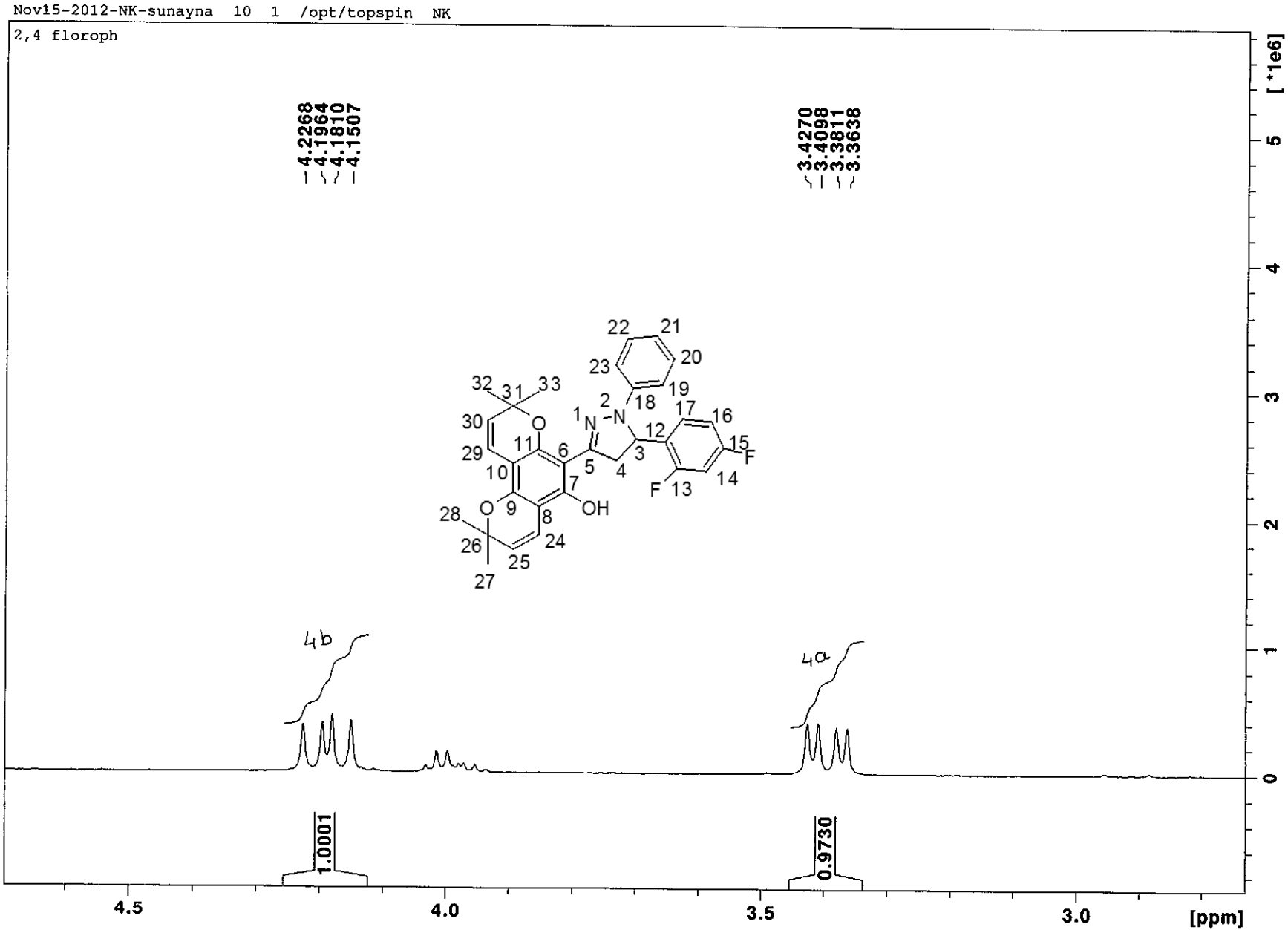
HRMS spectrum of 3-(4-fluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5f)



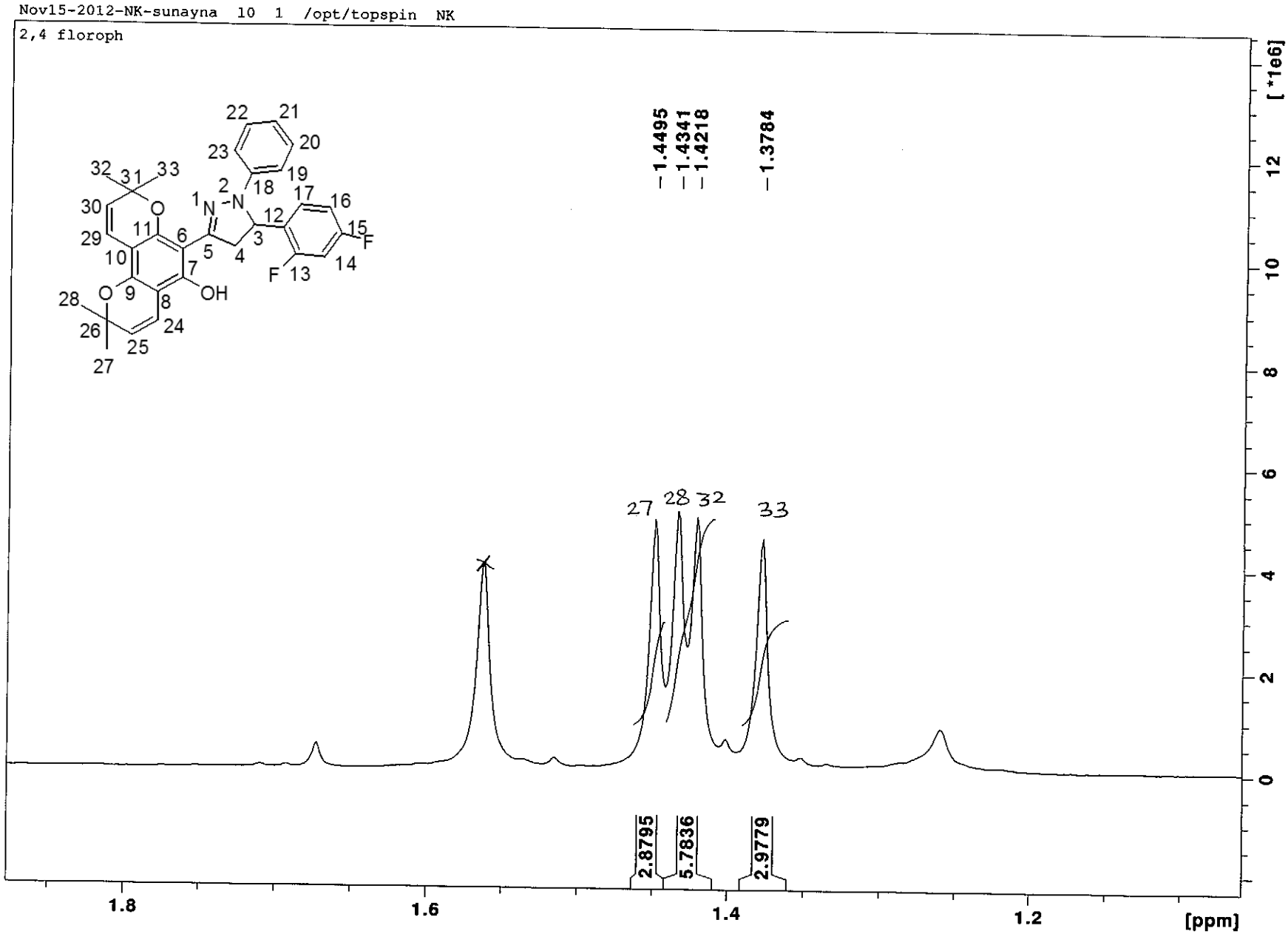
1H NMR spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



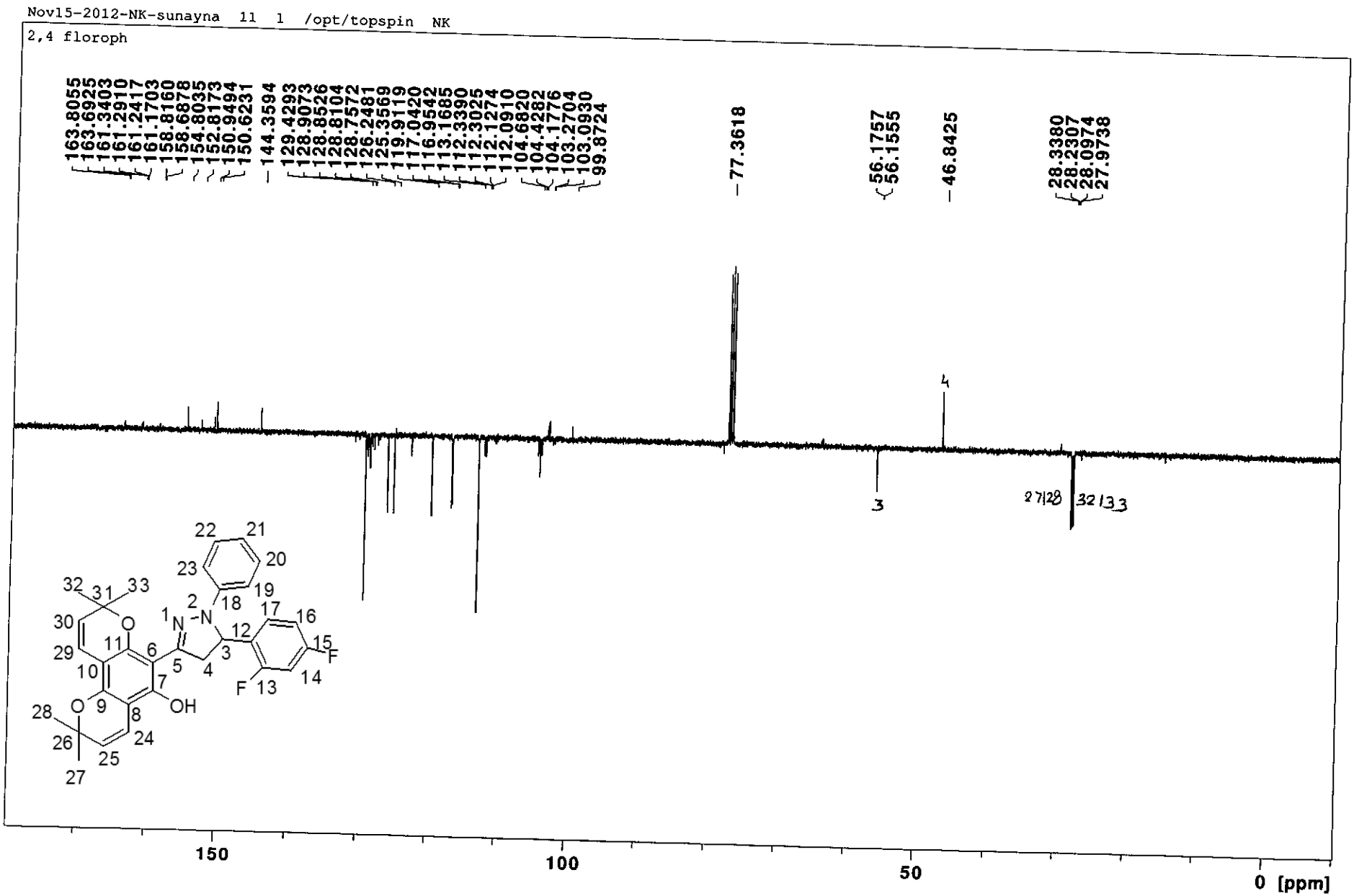
Expanded 1H NMR spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



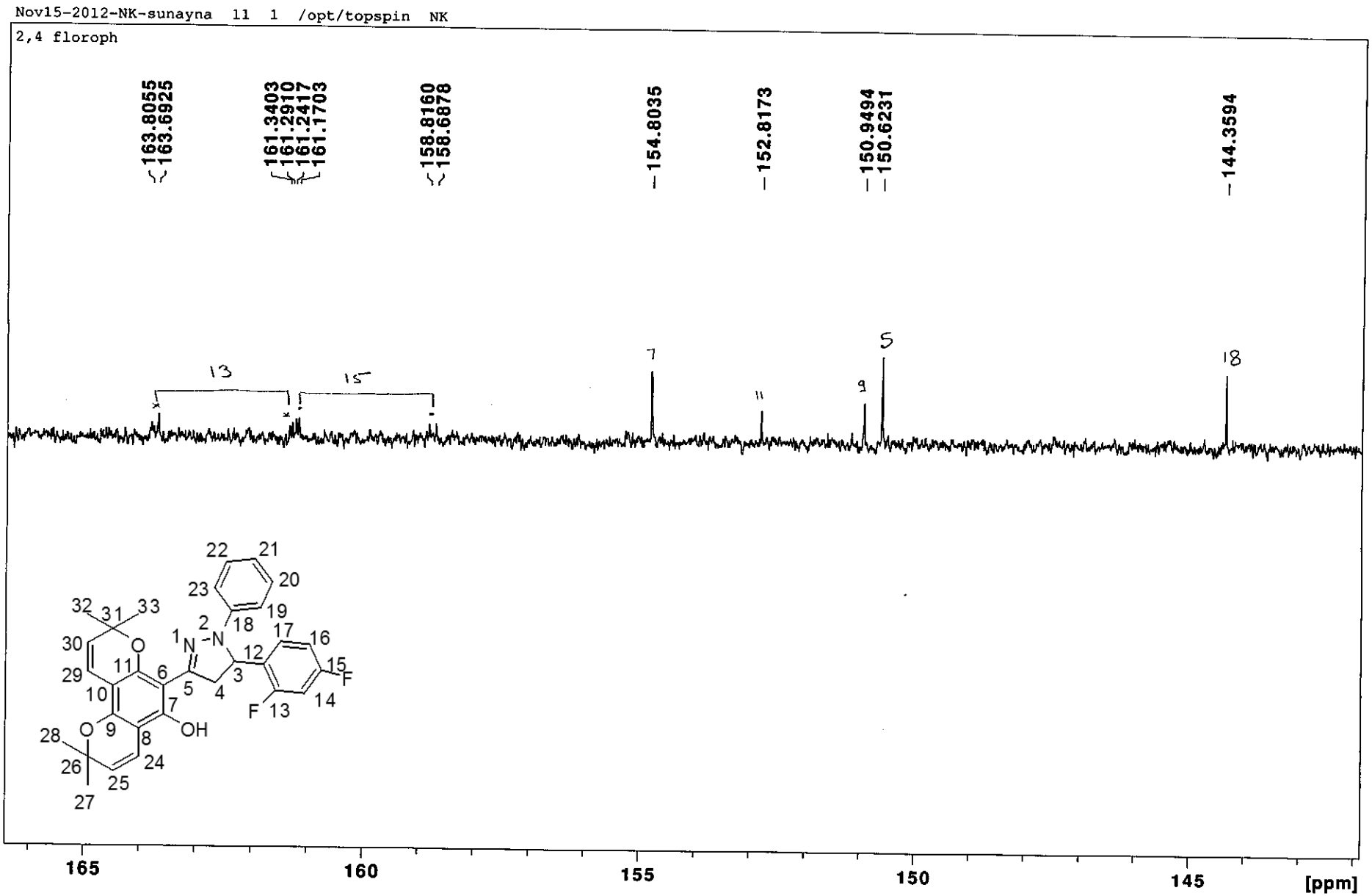
Expanded 1H NMR spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



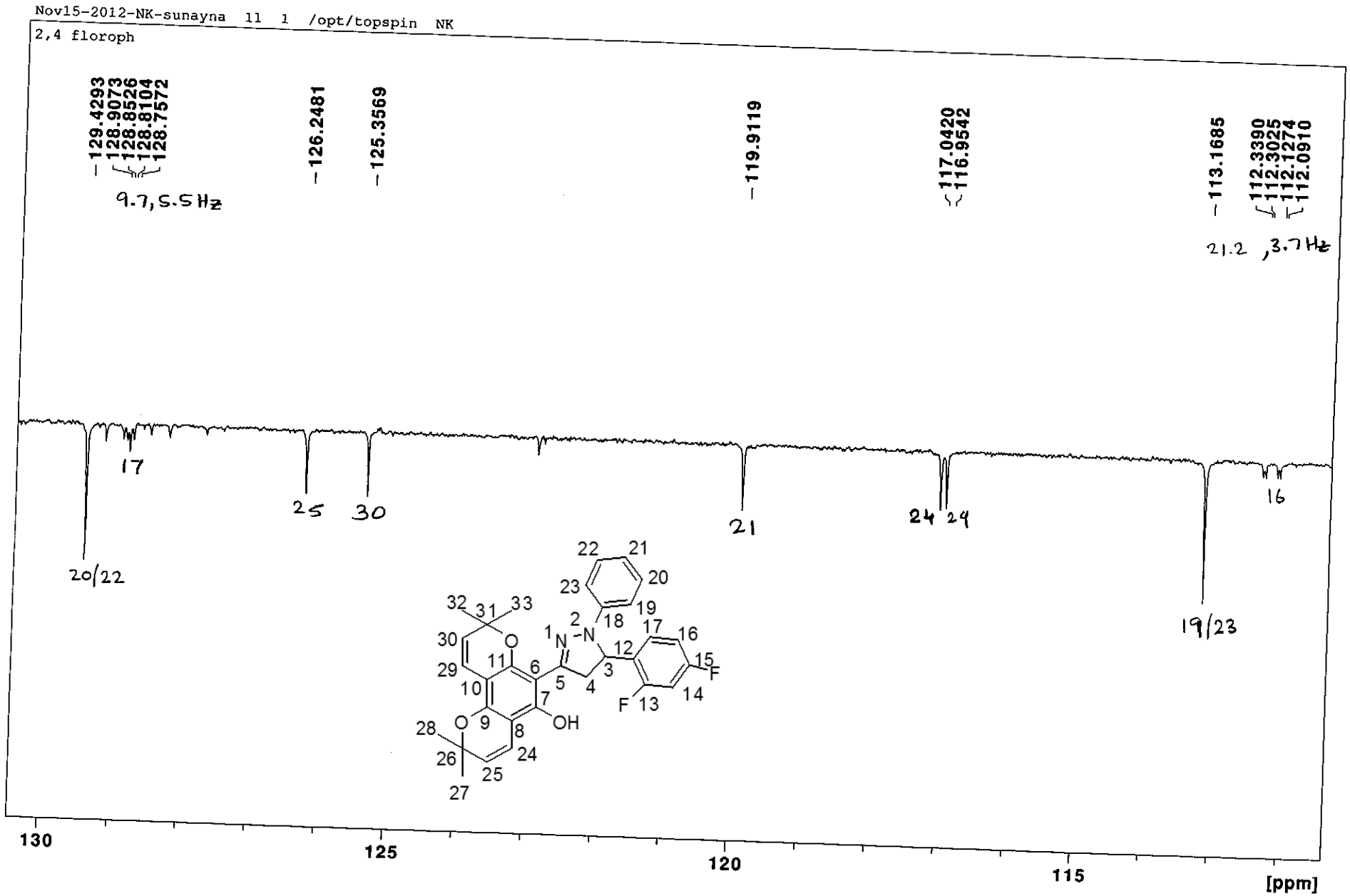
Expanded 1H NMR spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



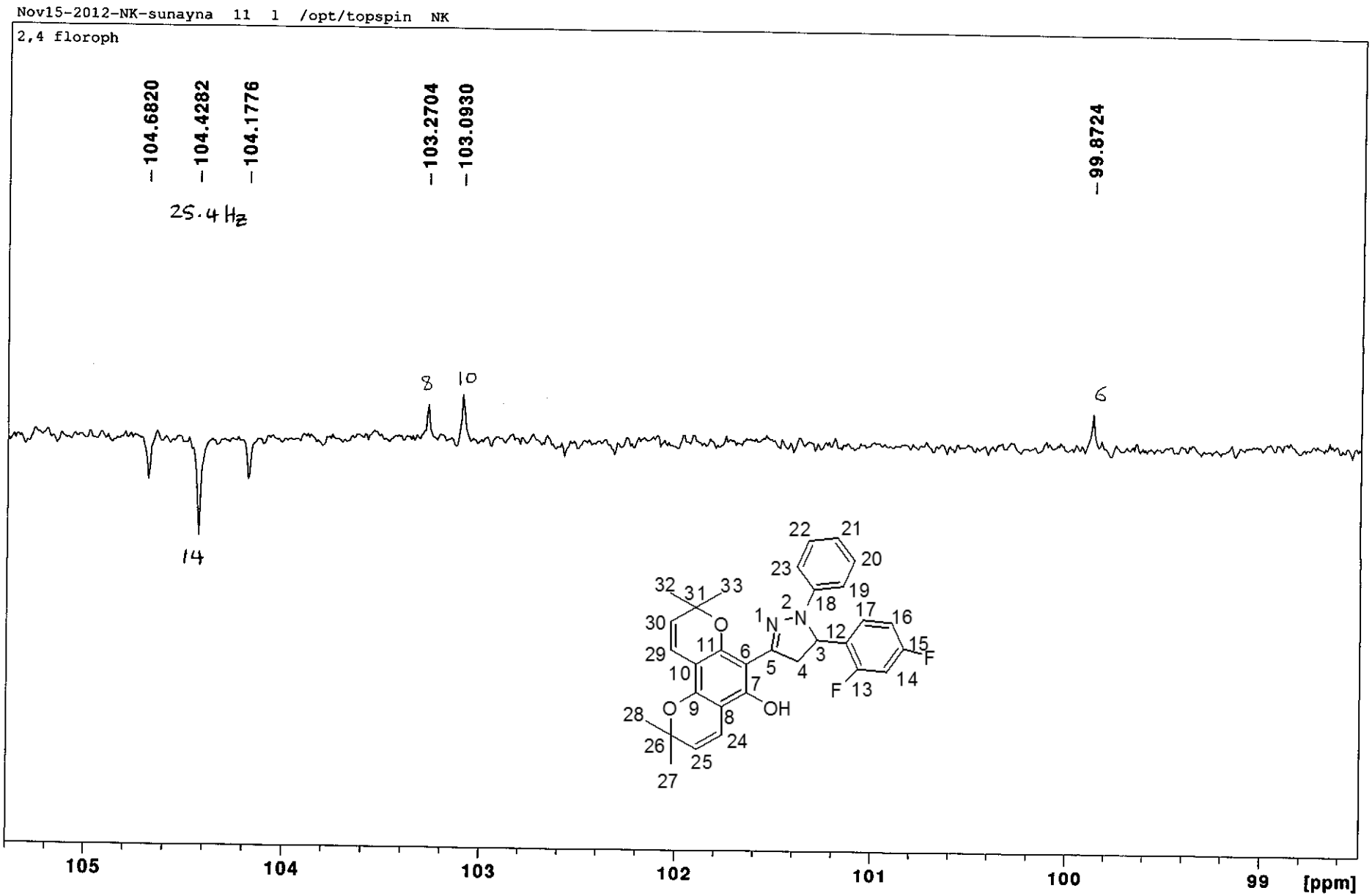
13C NMR spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



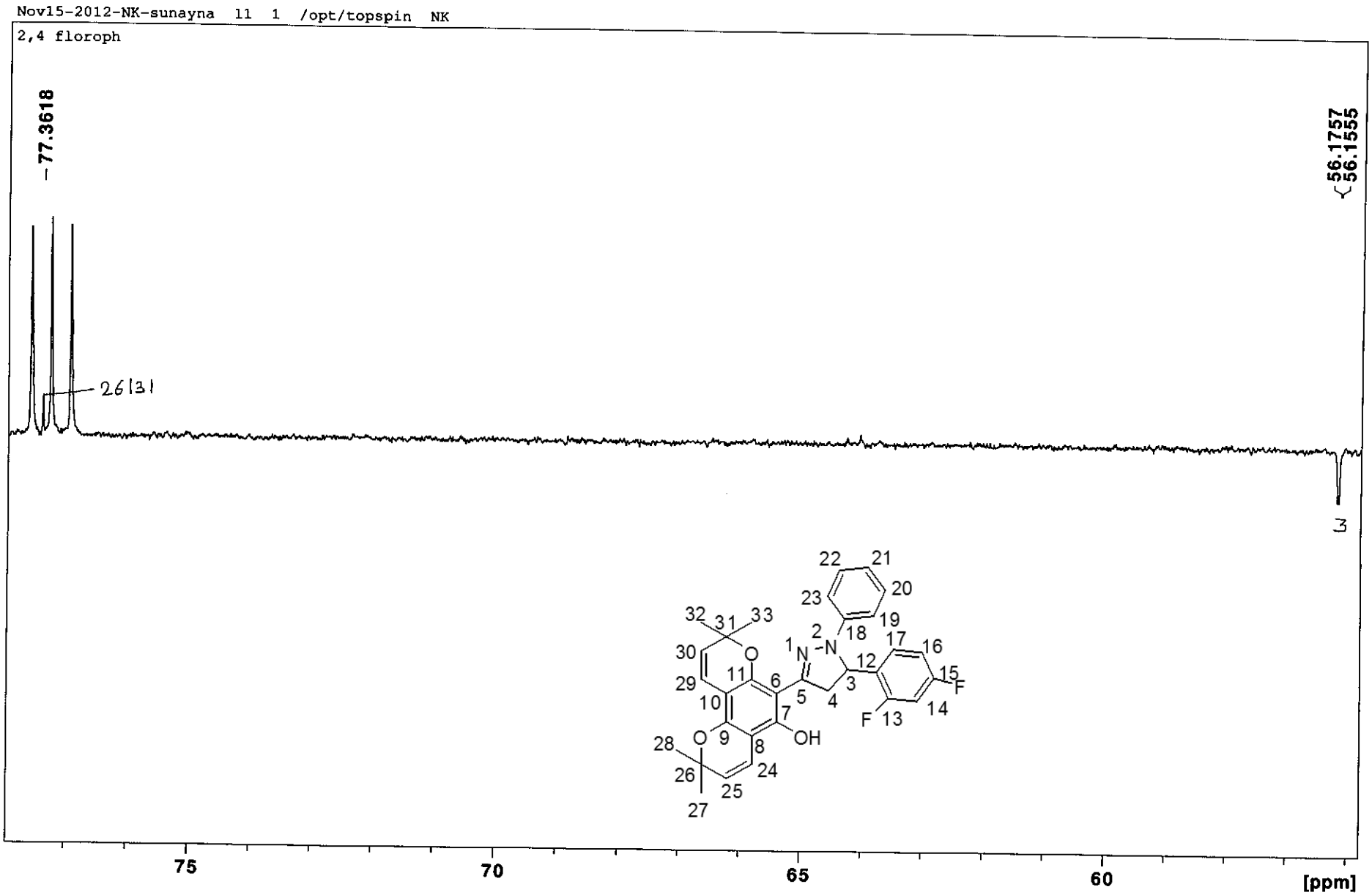
Expanded 13C NMR spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



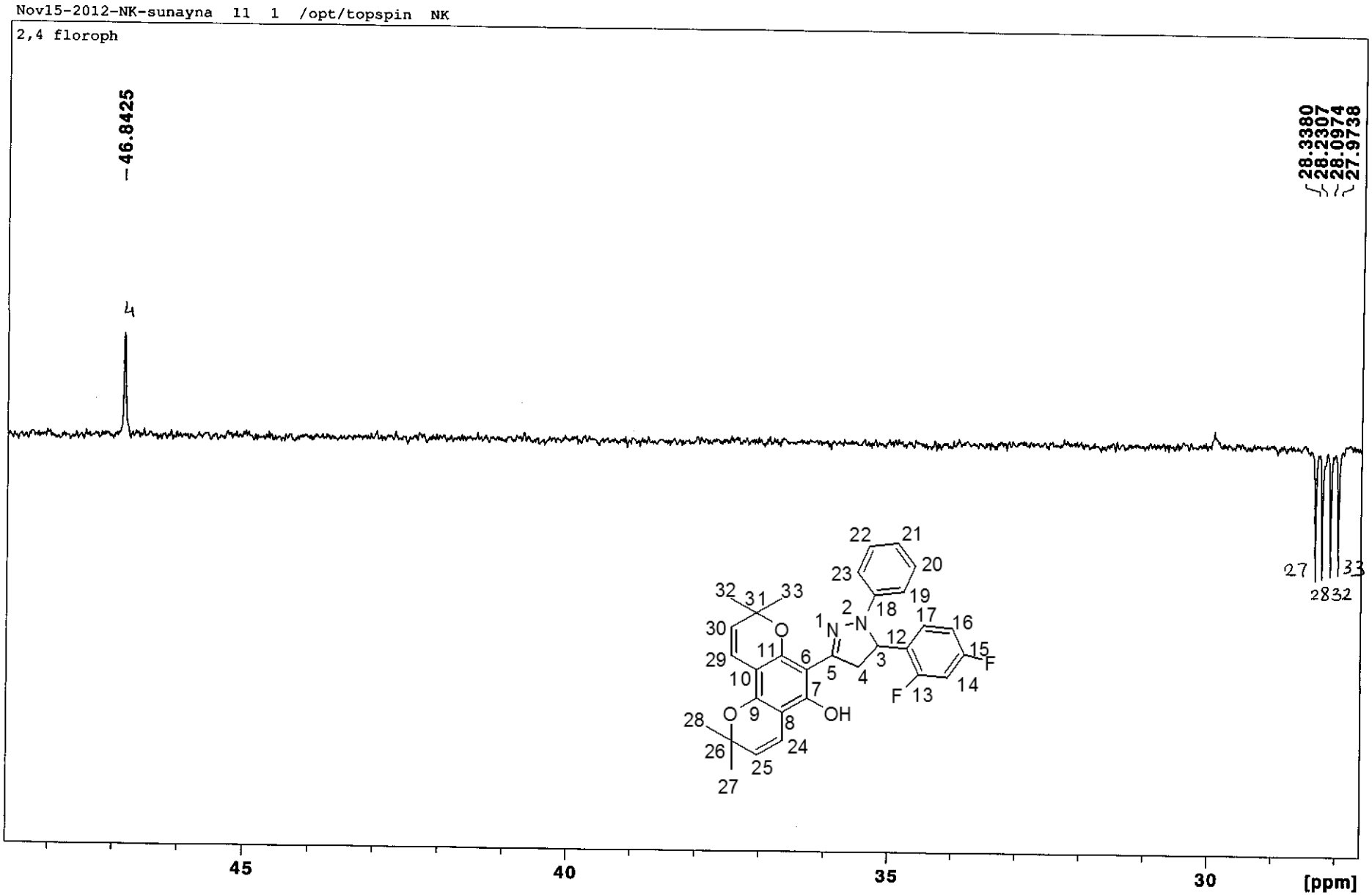
Expanded 13C NMR spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



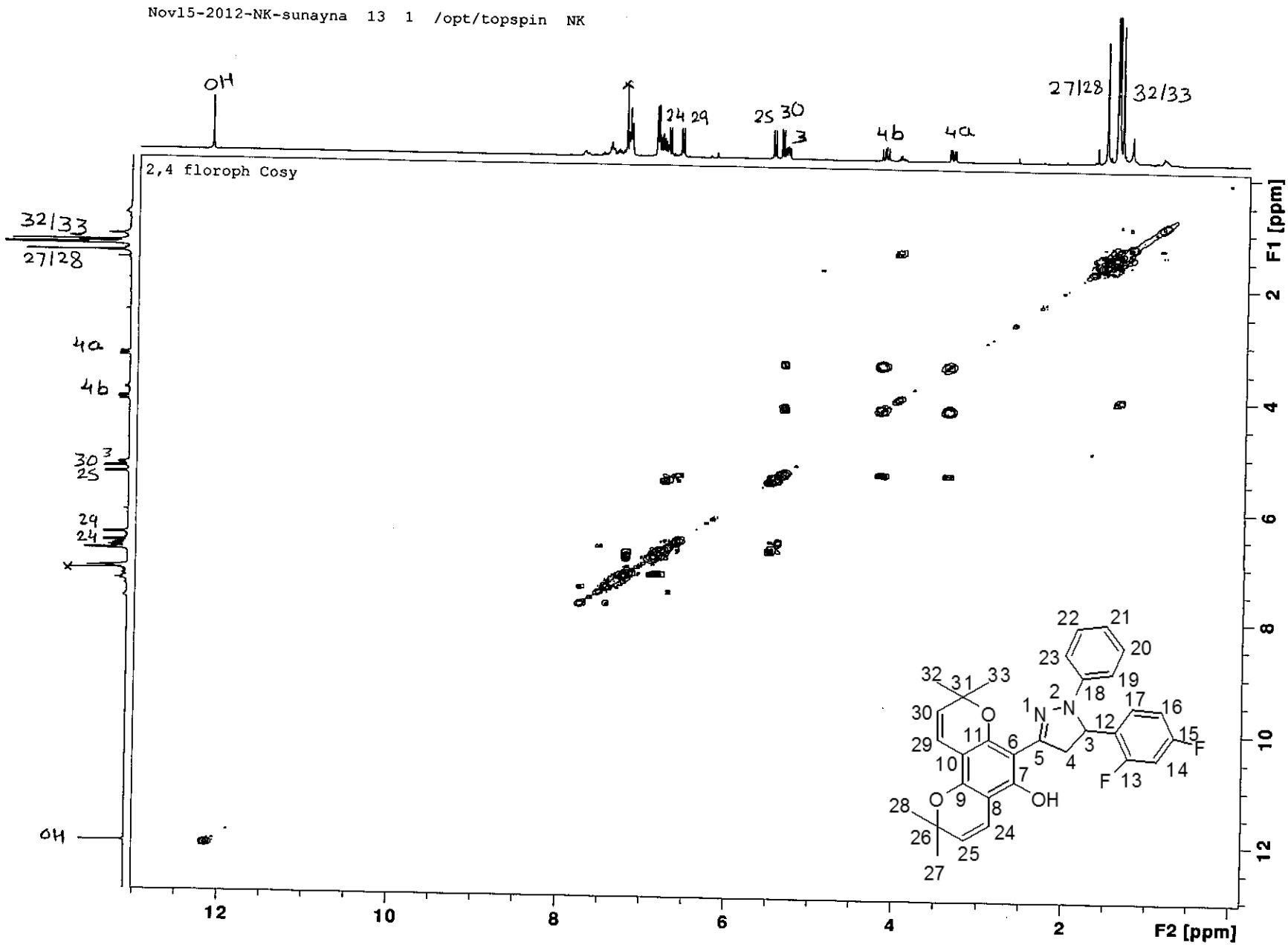
Expanded 13C NMR spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



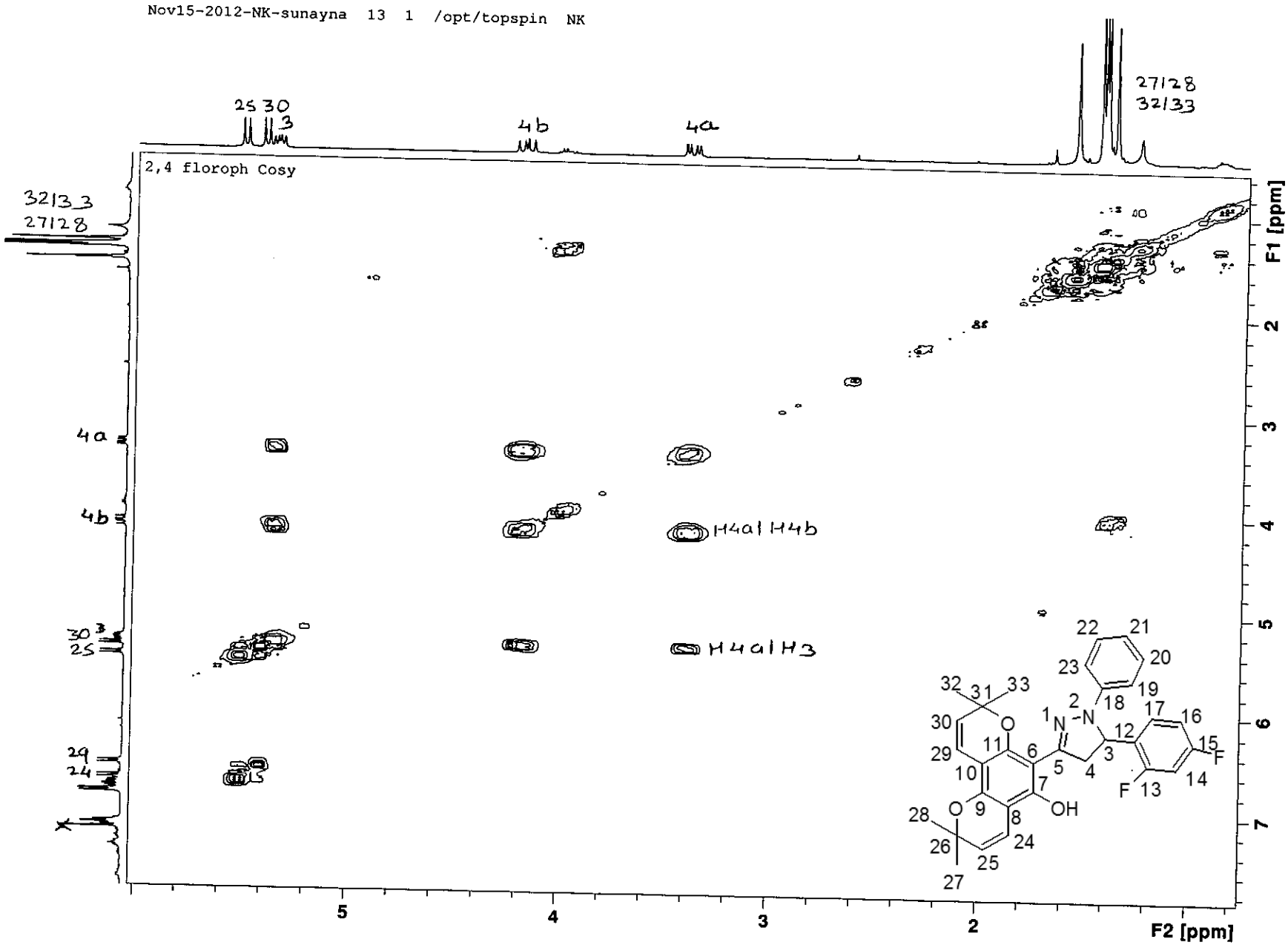
Expanded 13C NMR spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



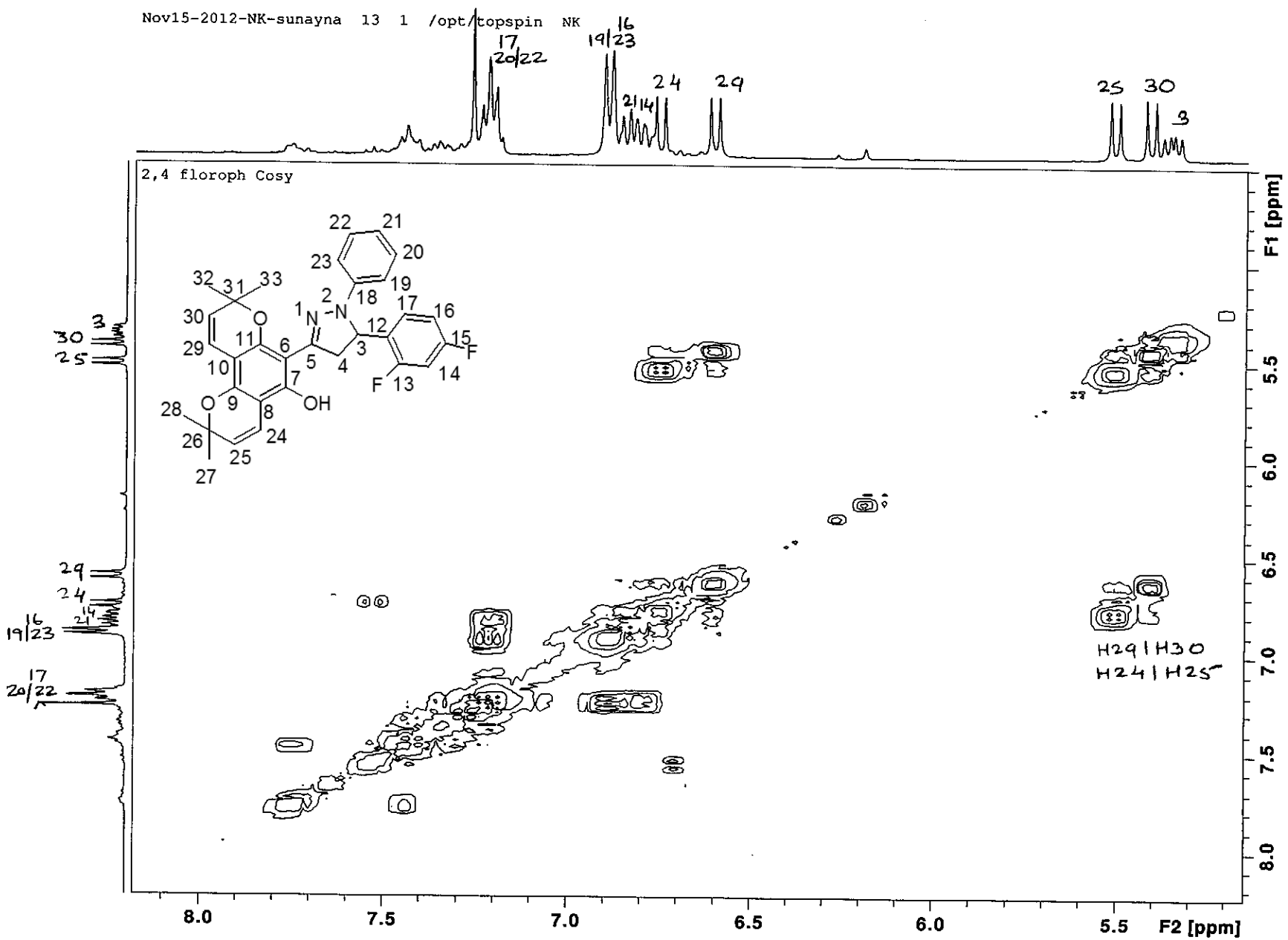
Expanded 13C NMR spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



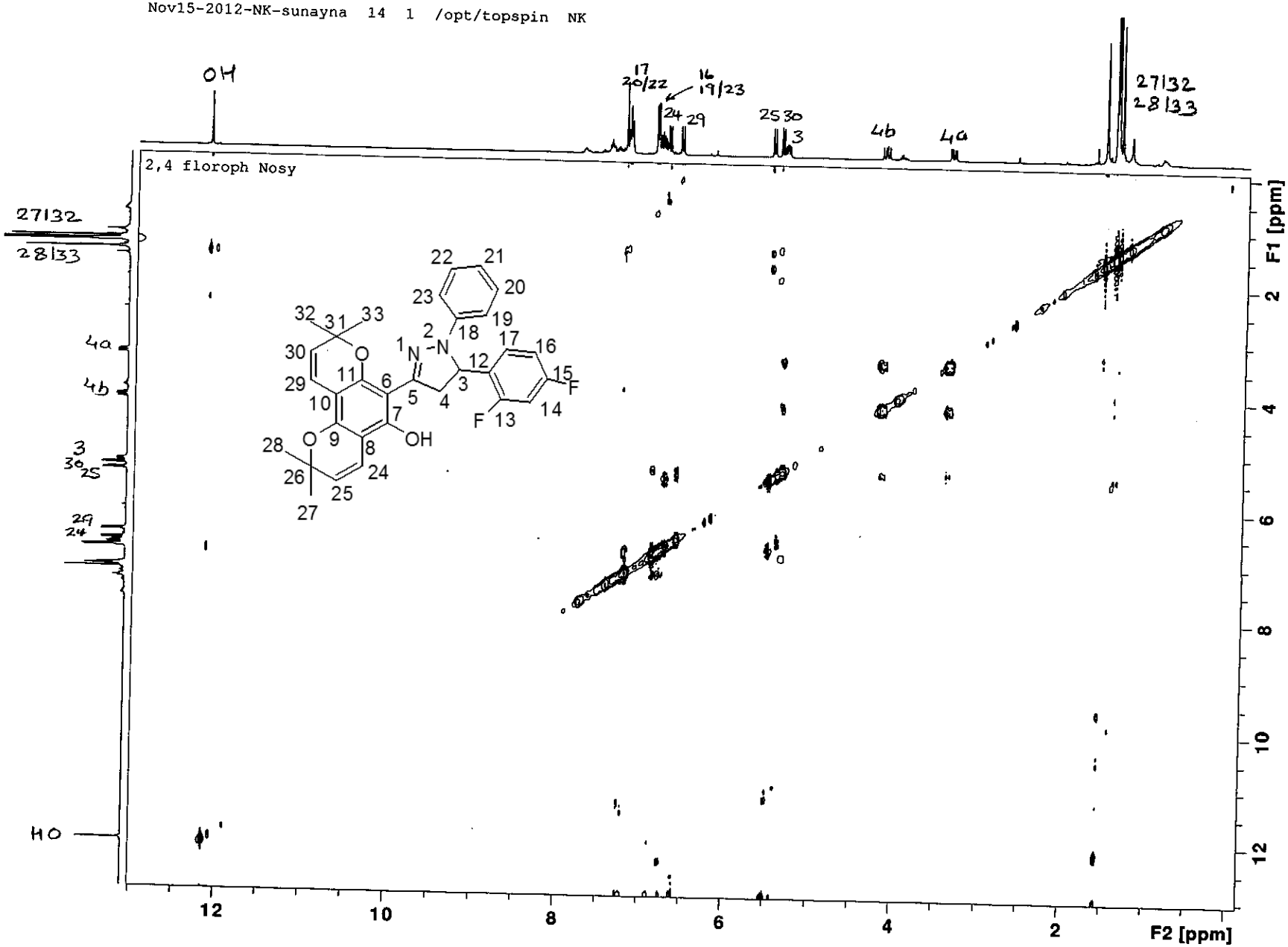
COSY NMR spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



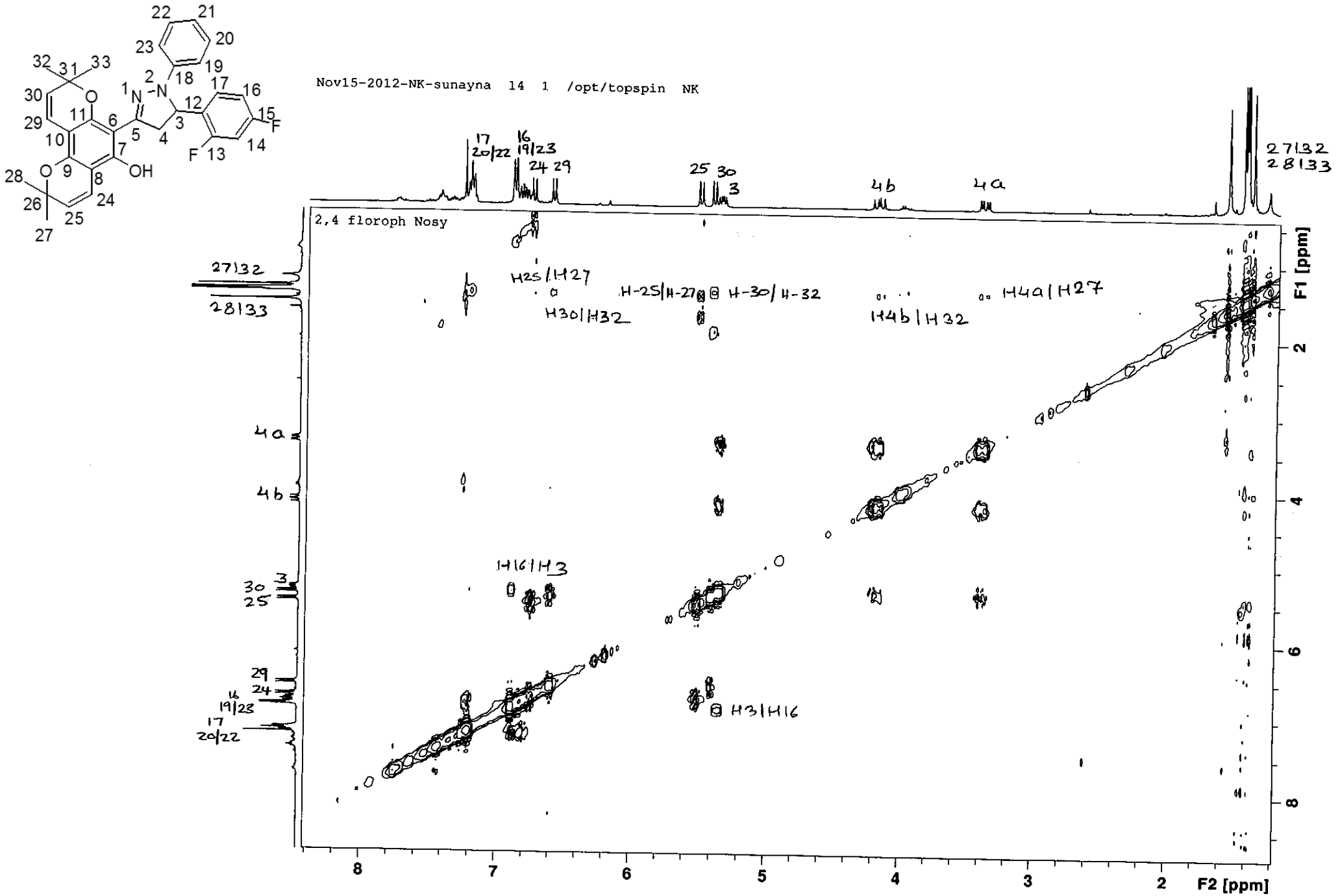
Expanded COSY NMR spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



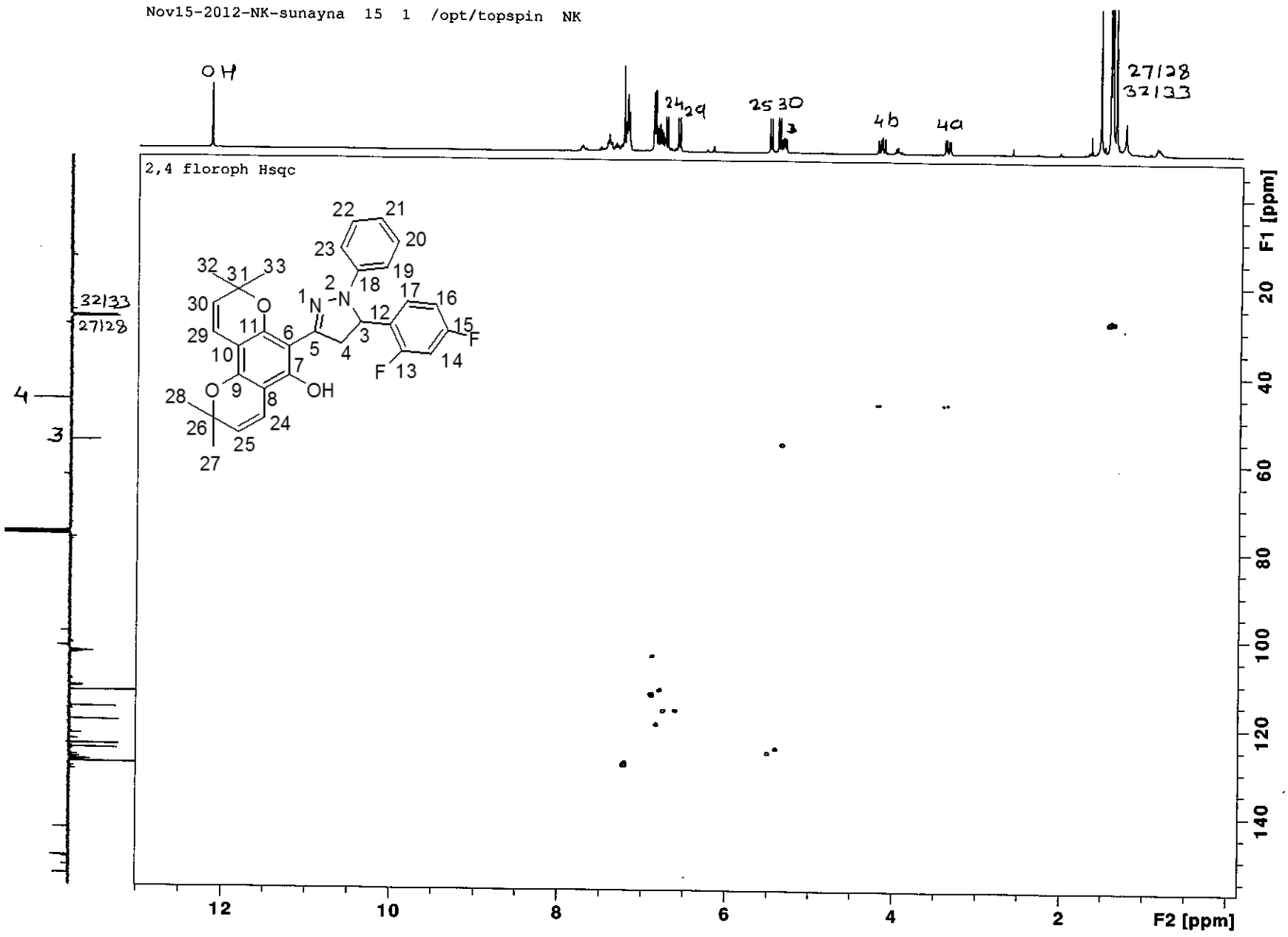
Expanded COSY NMR spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



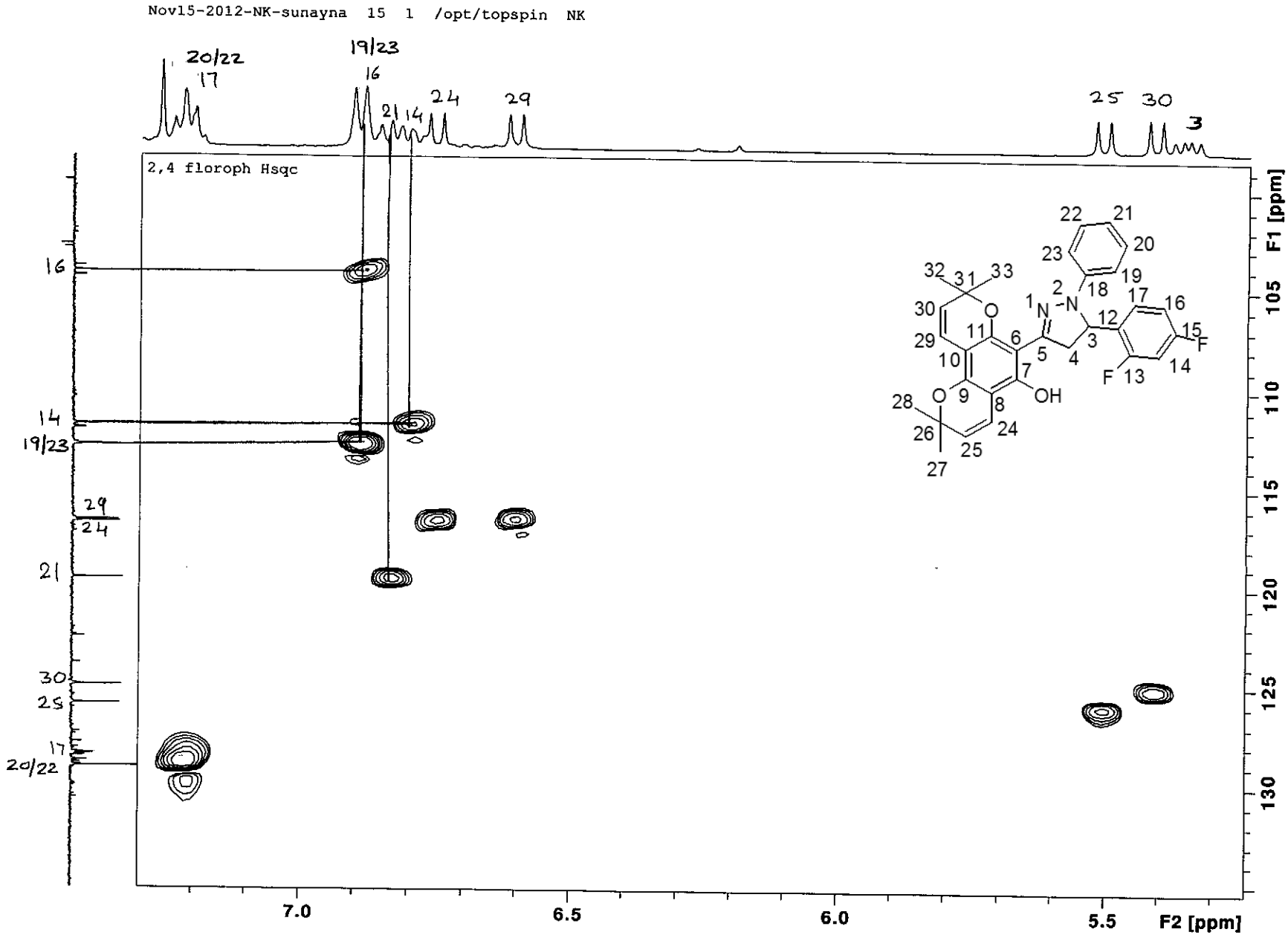
NOESY NMR spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



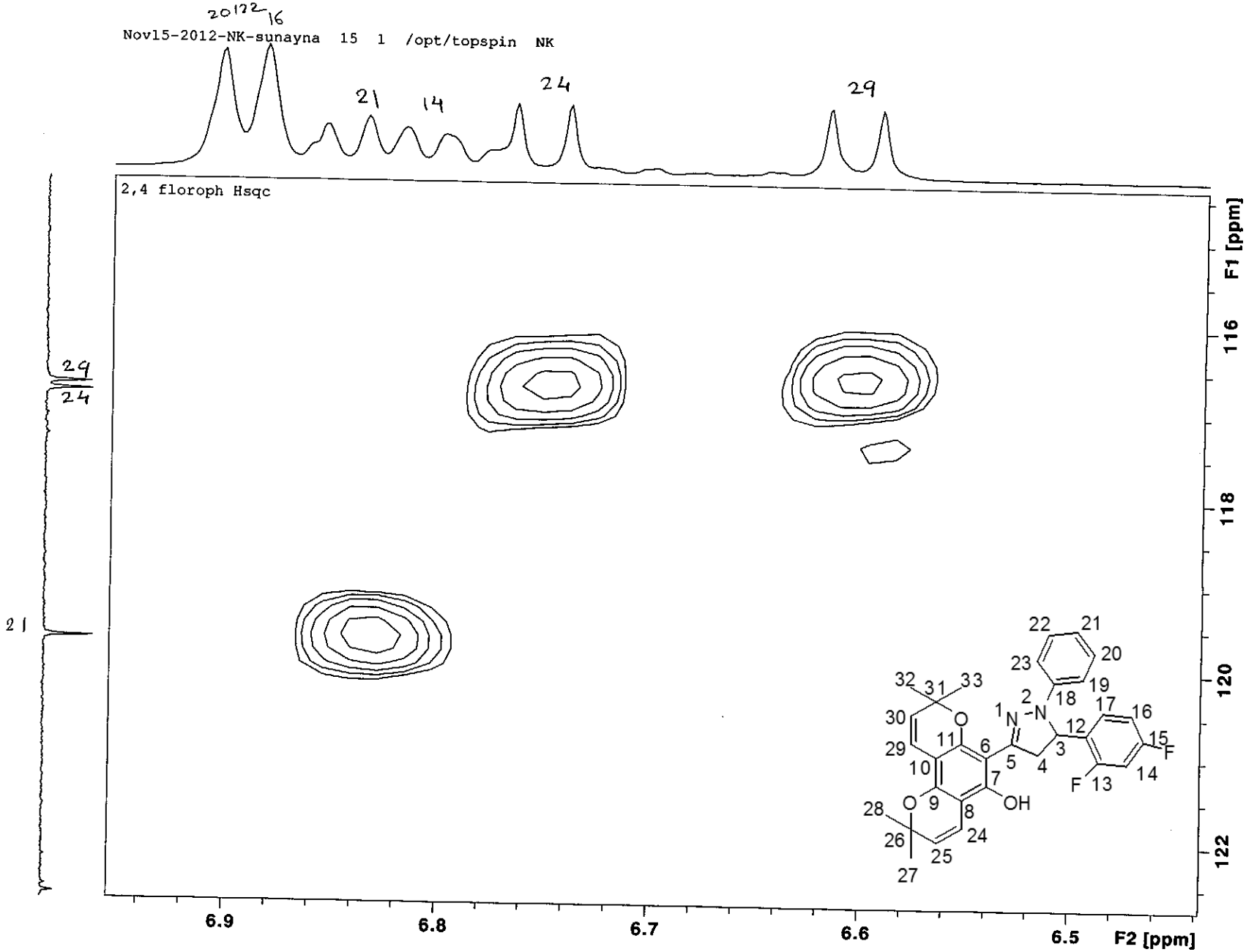
Expanded NOESY NMR spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



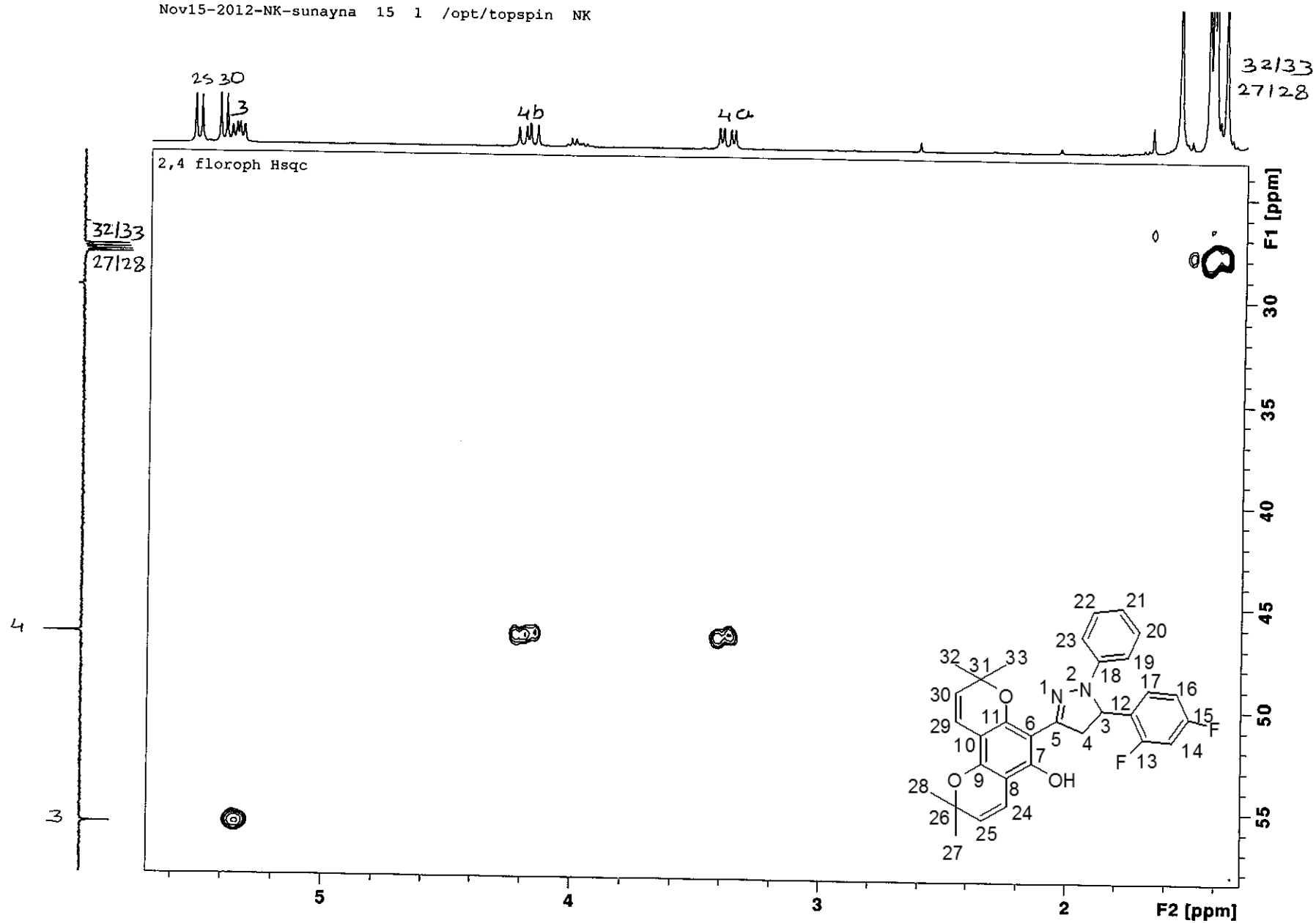
HSQC spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



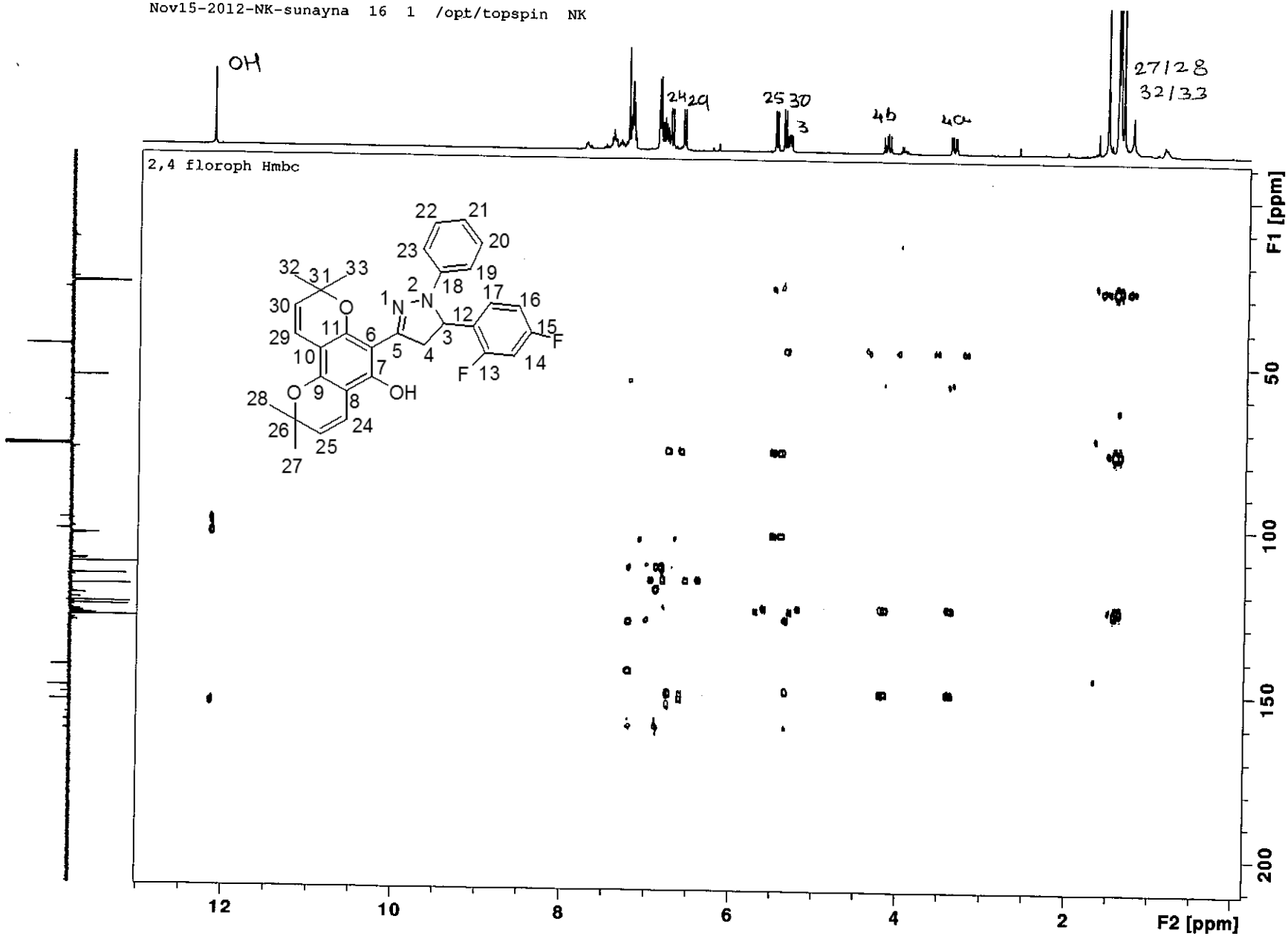
Expanded HSQC spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



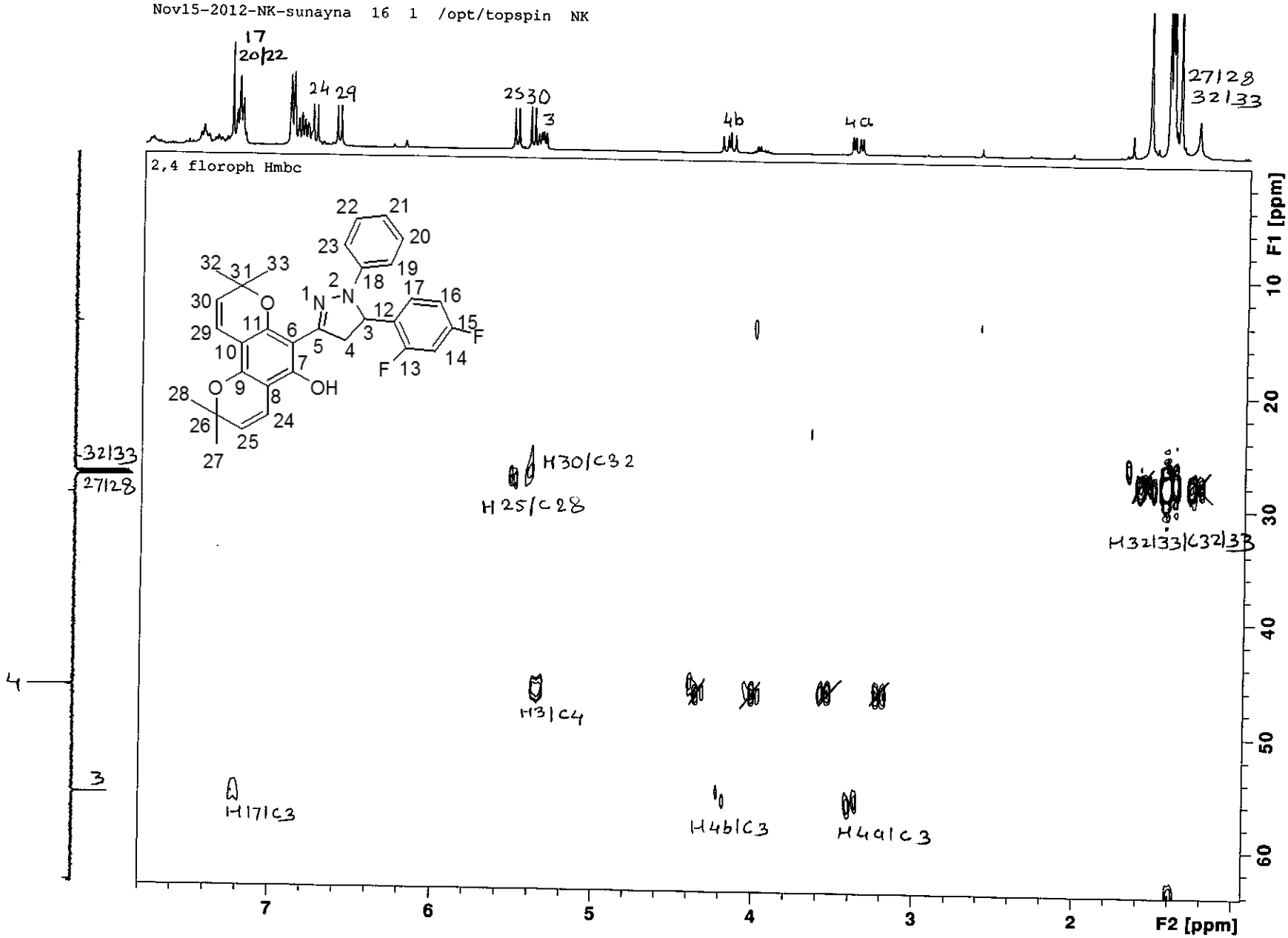
Expanded HSQC spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



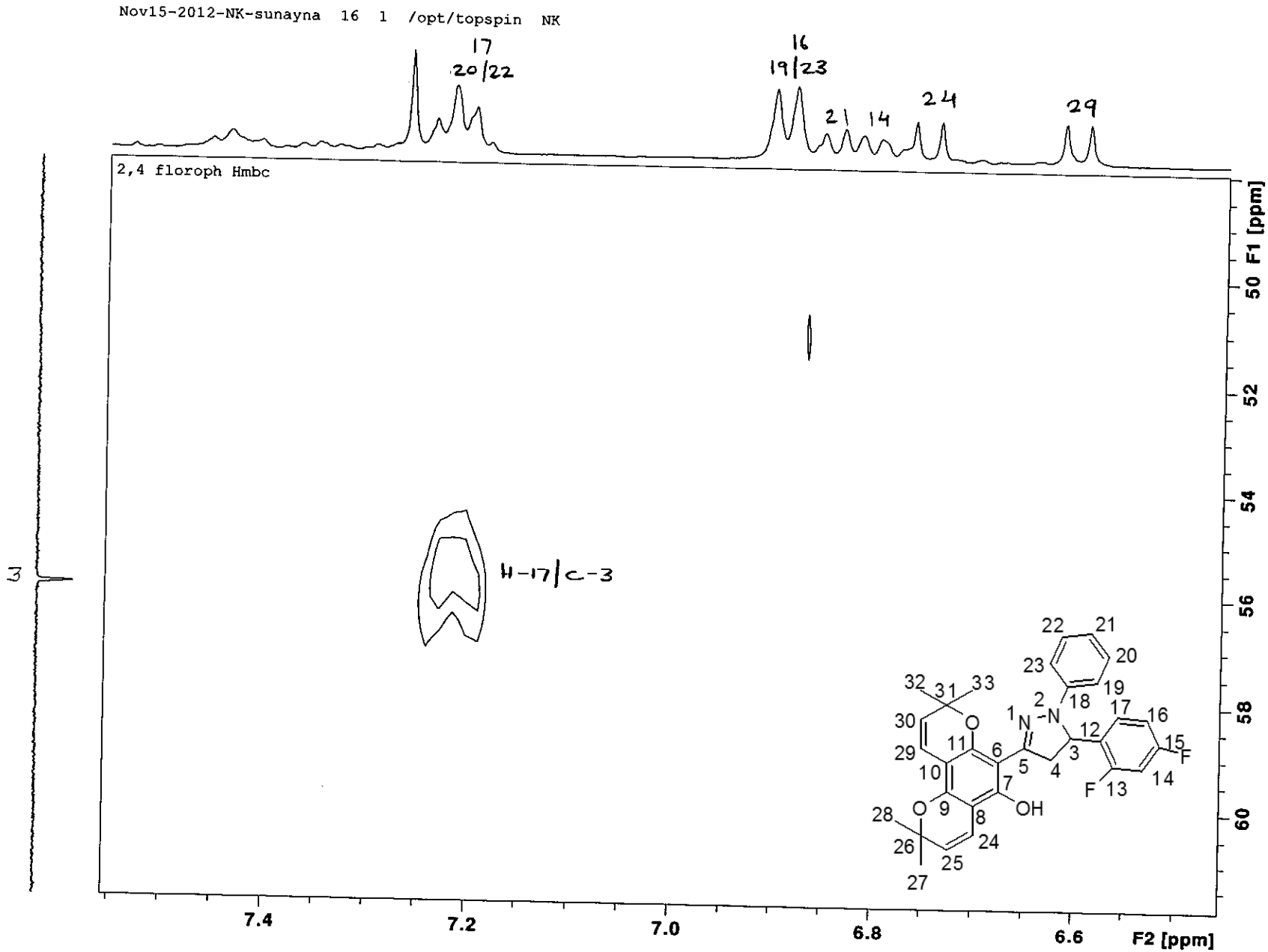
Expanded HSQC spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



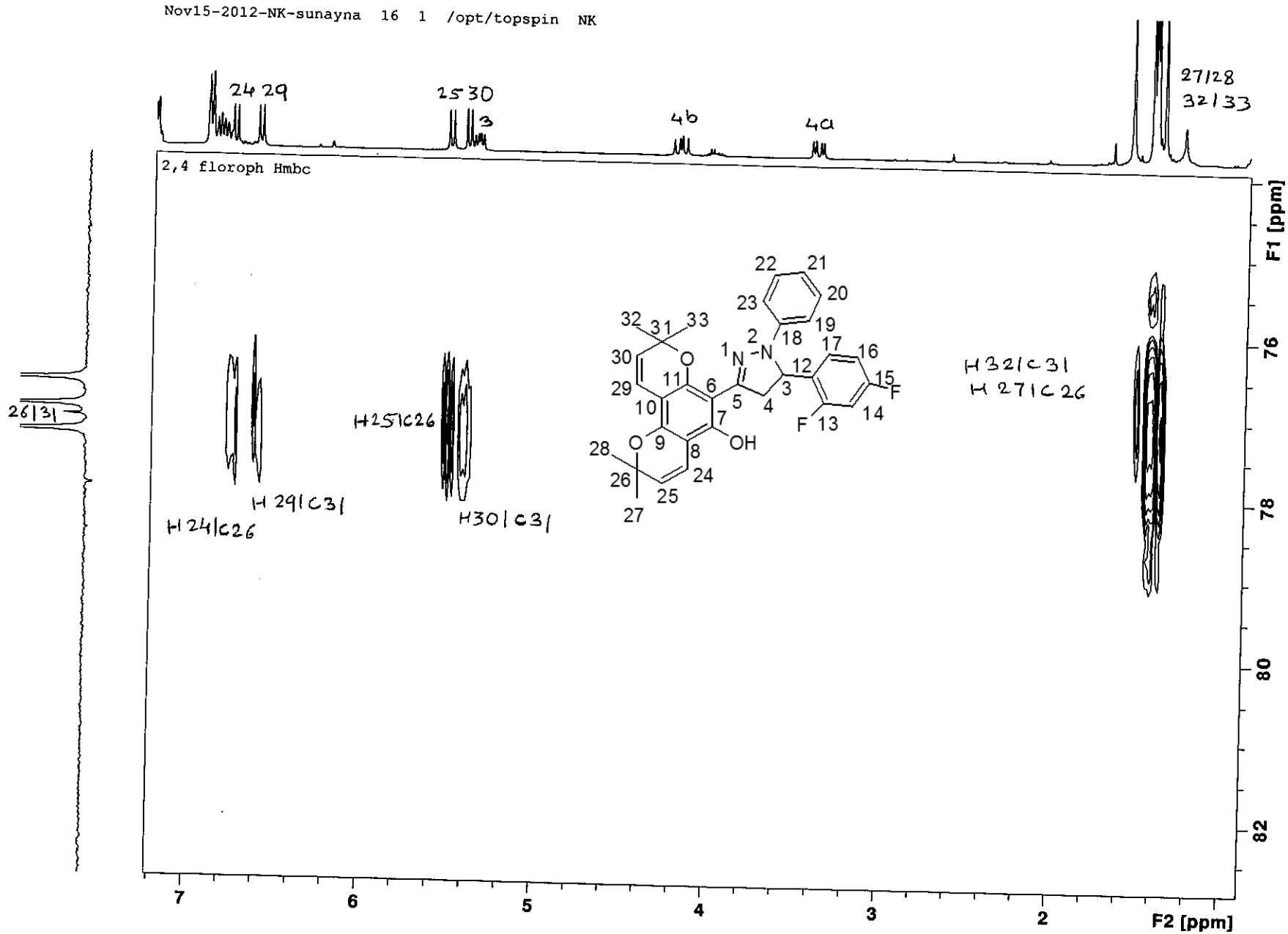
HMBC spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



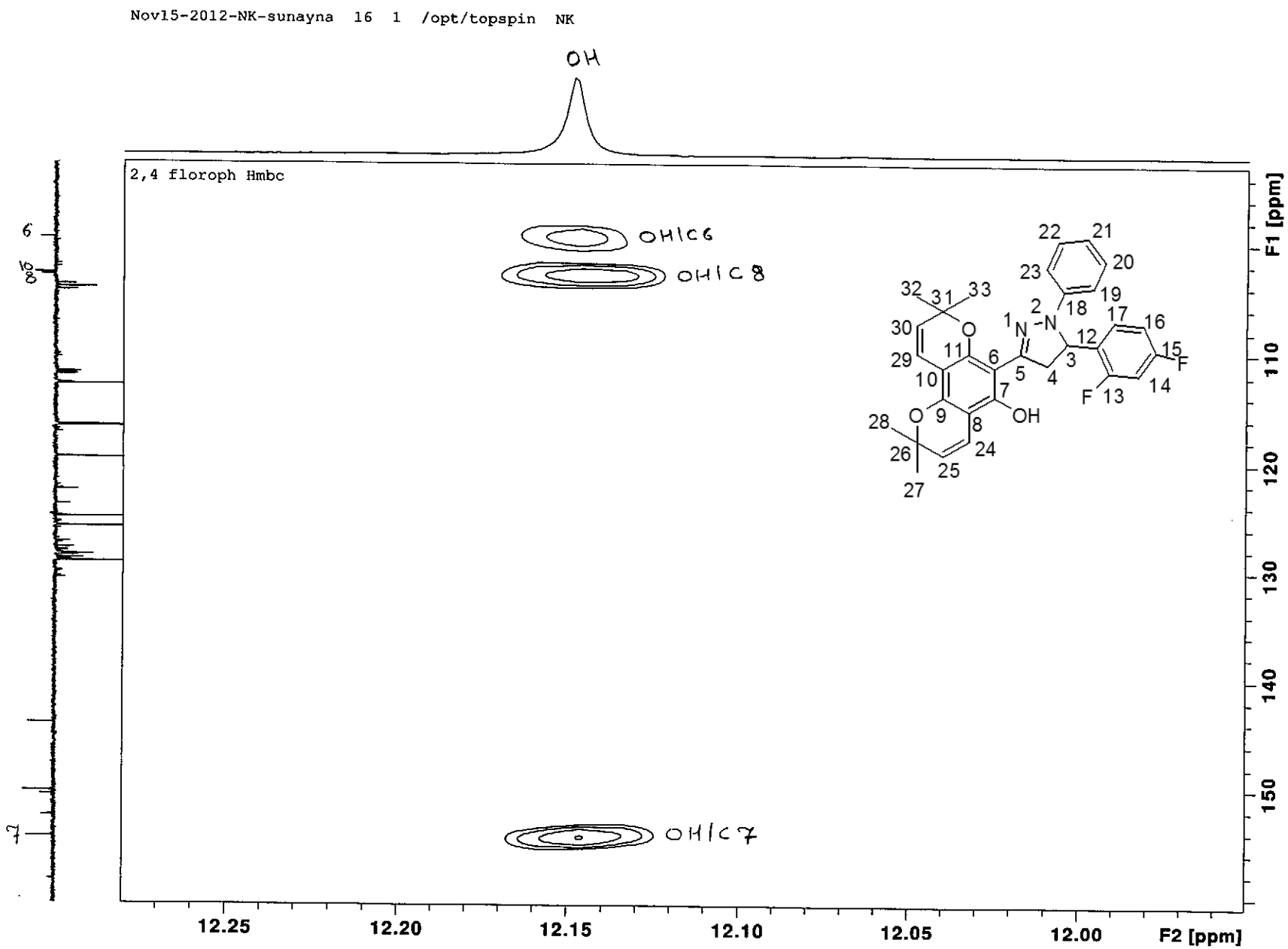
Expanded HMBC spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



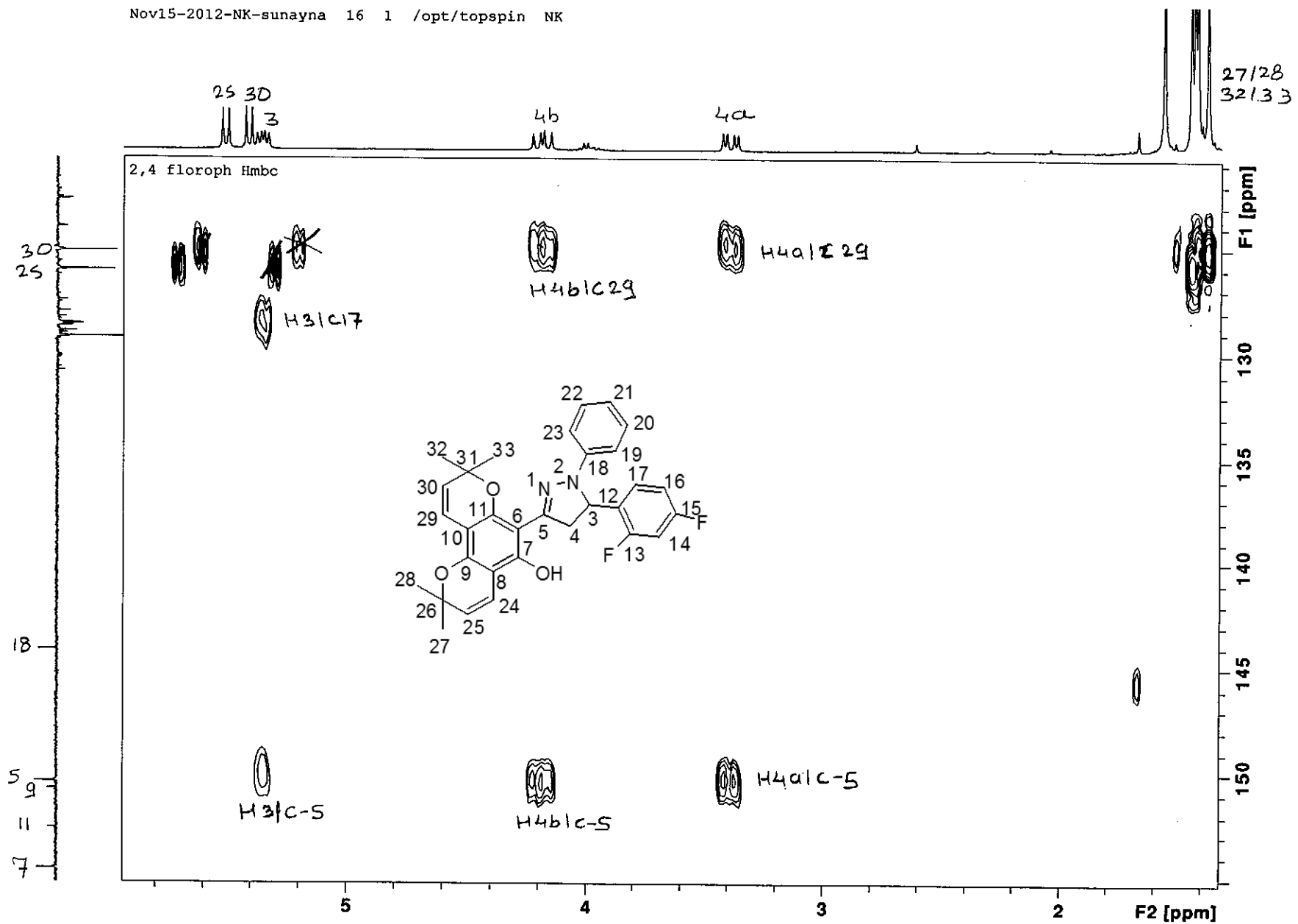
Expanded HMBC spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



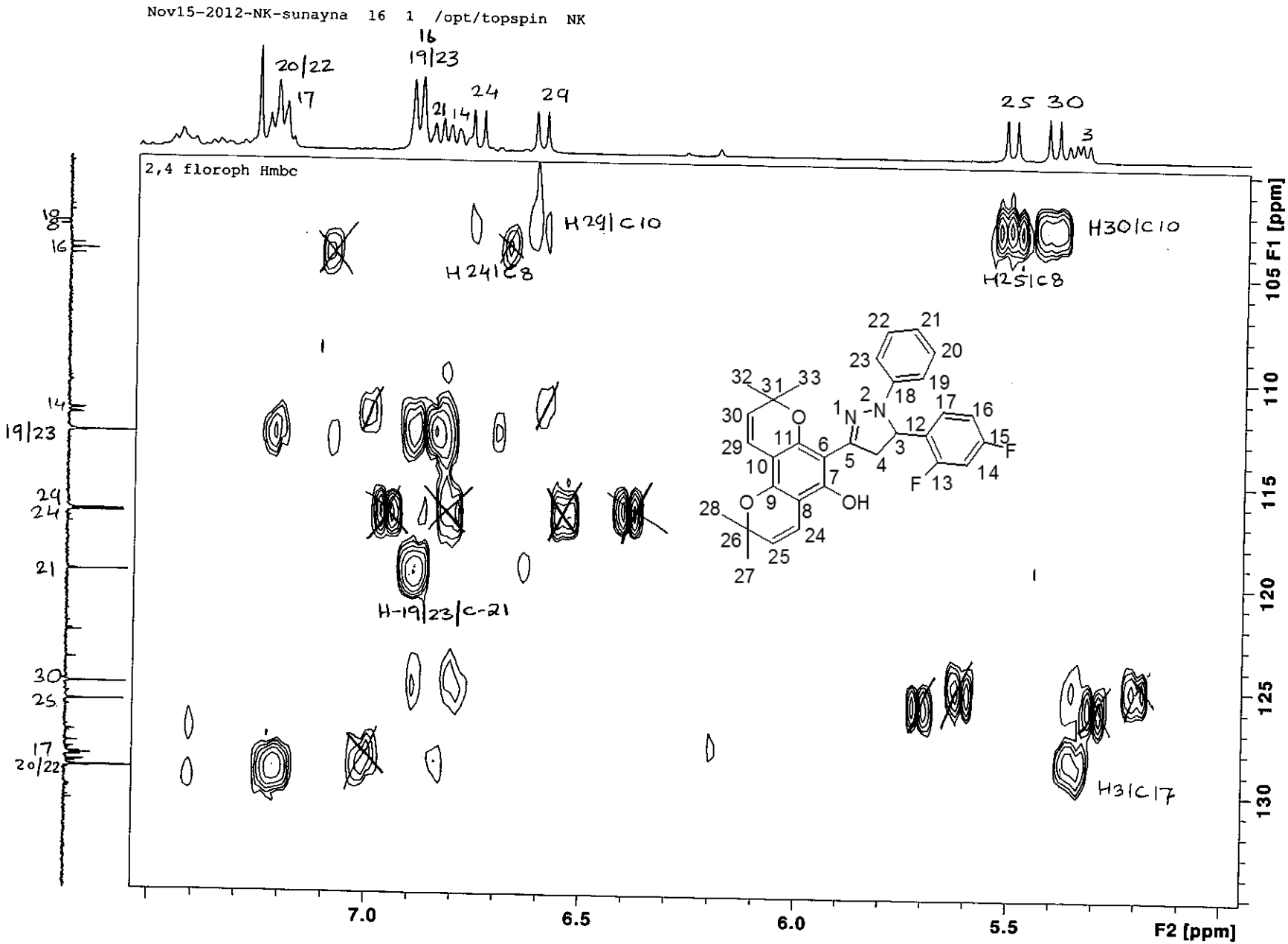
Expanded HMBC spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



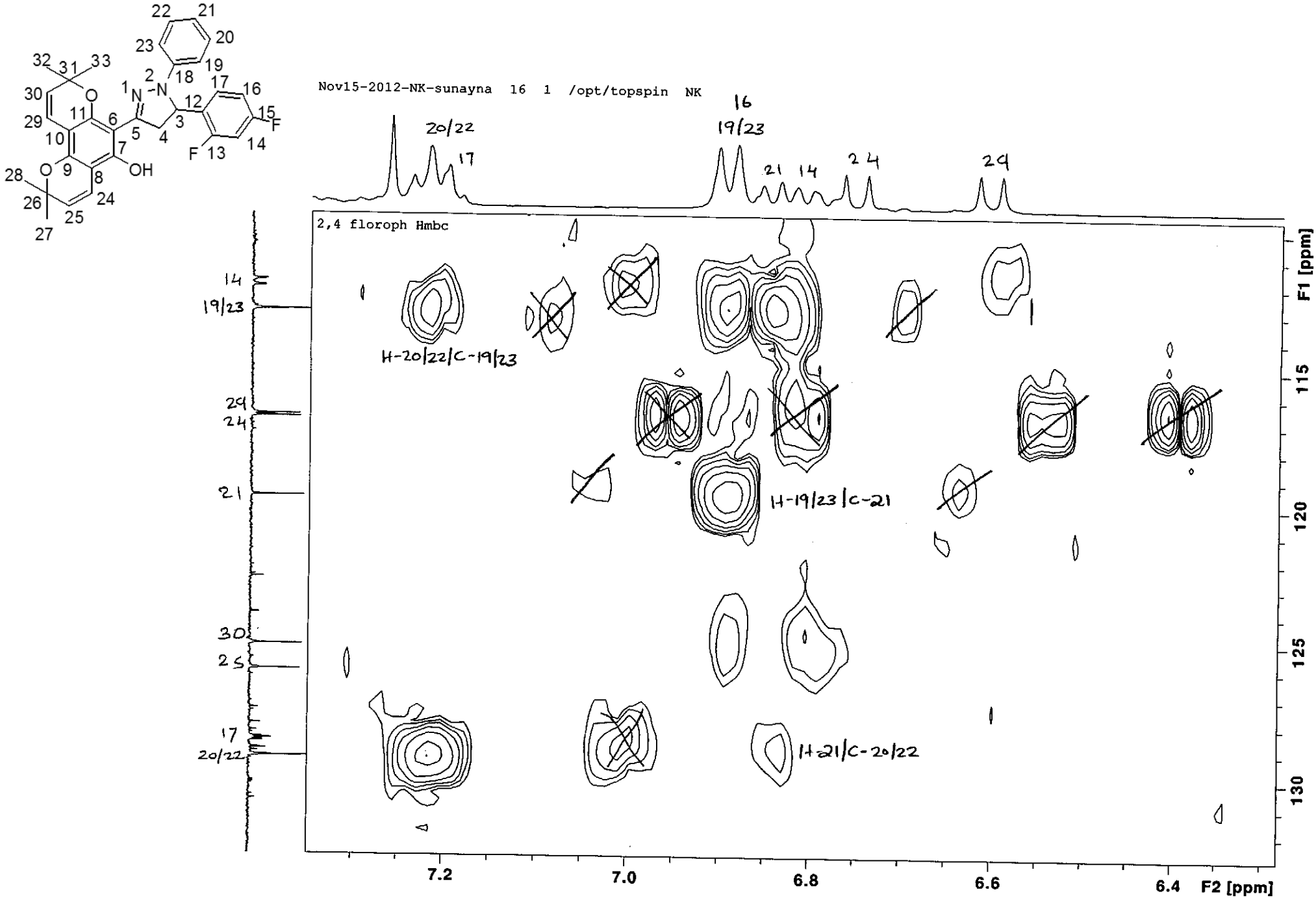
Expanded HMBC spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



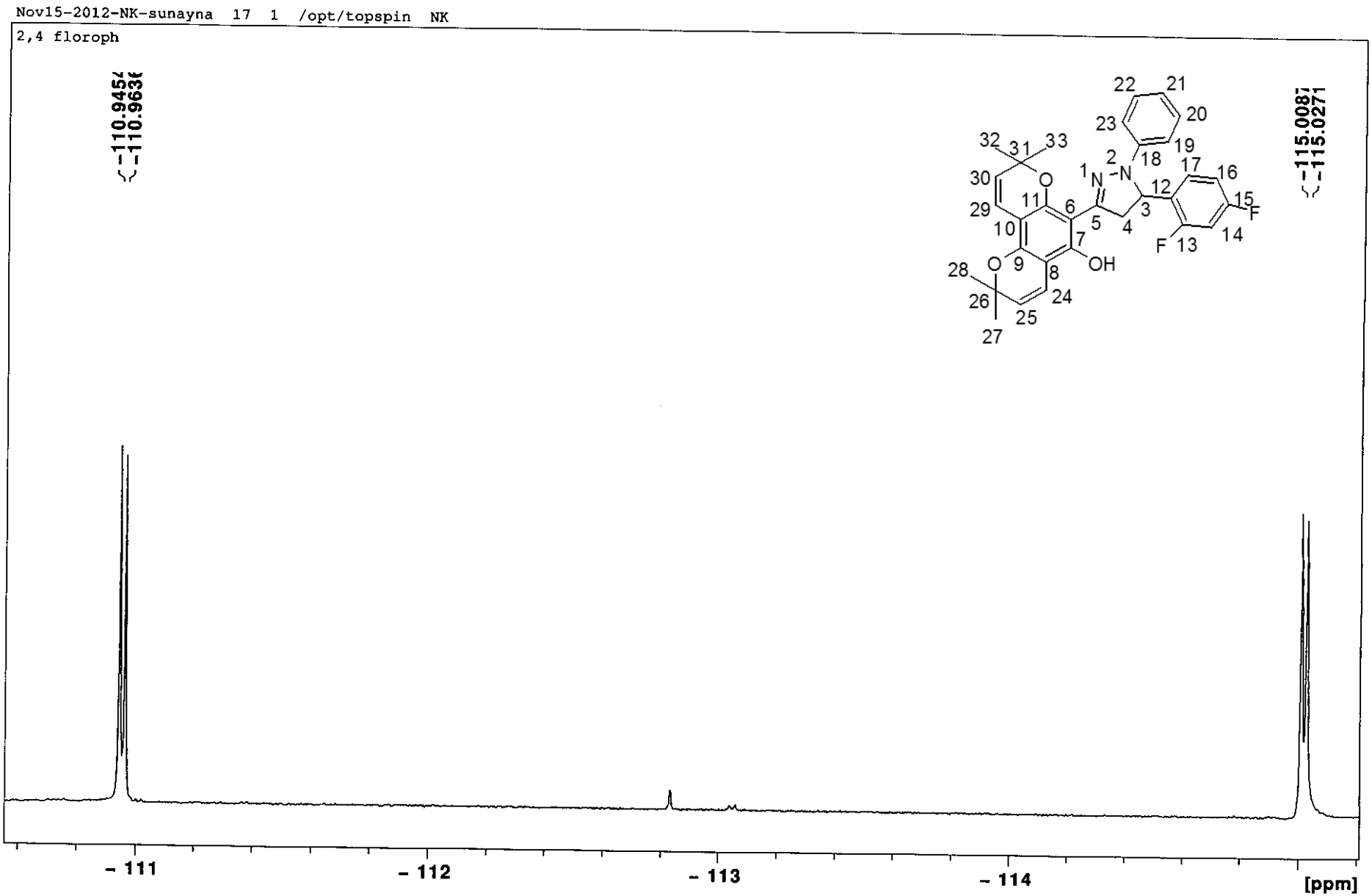
Expanded HMBC spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



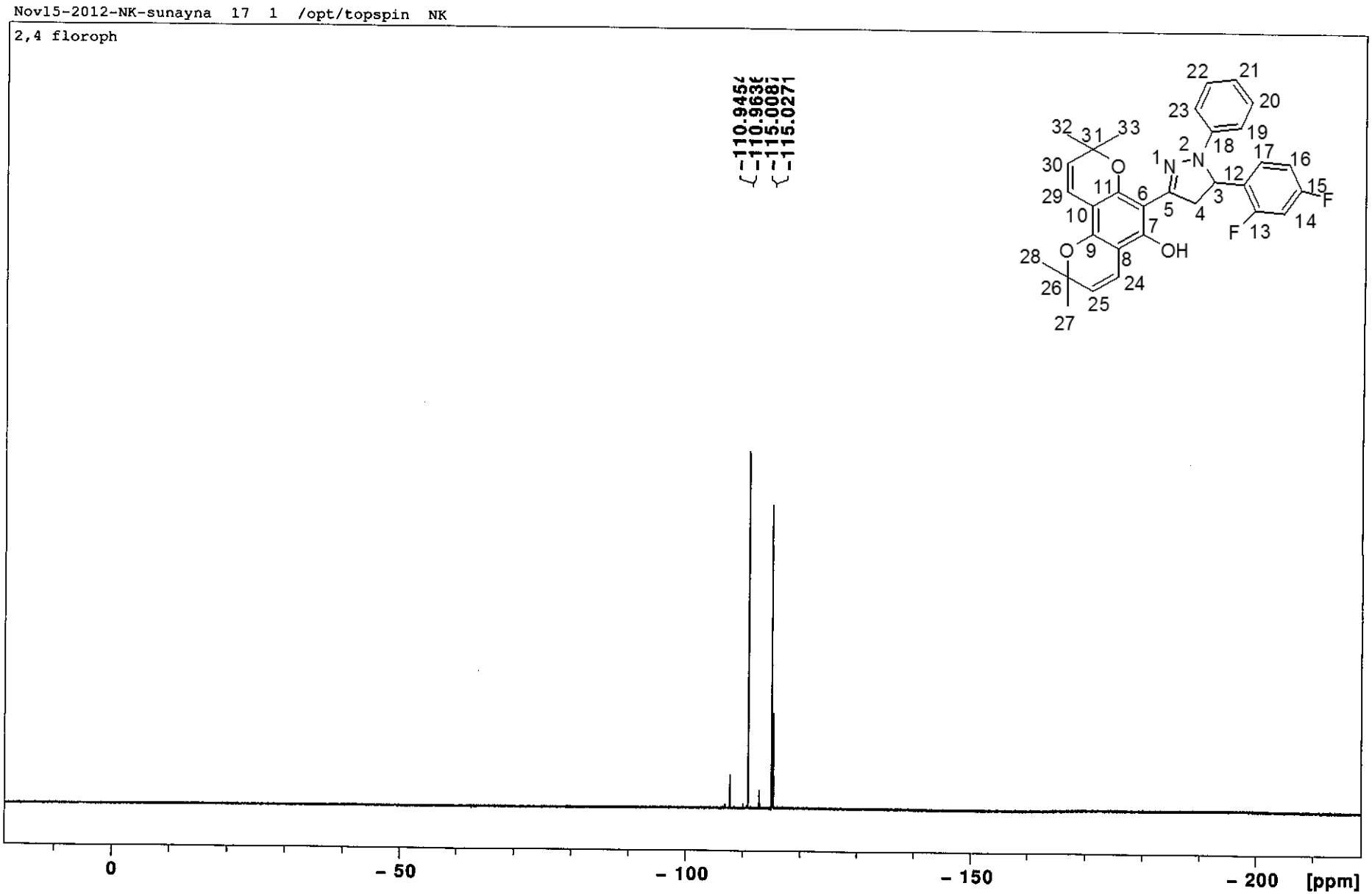
Expanded HMBC spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



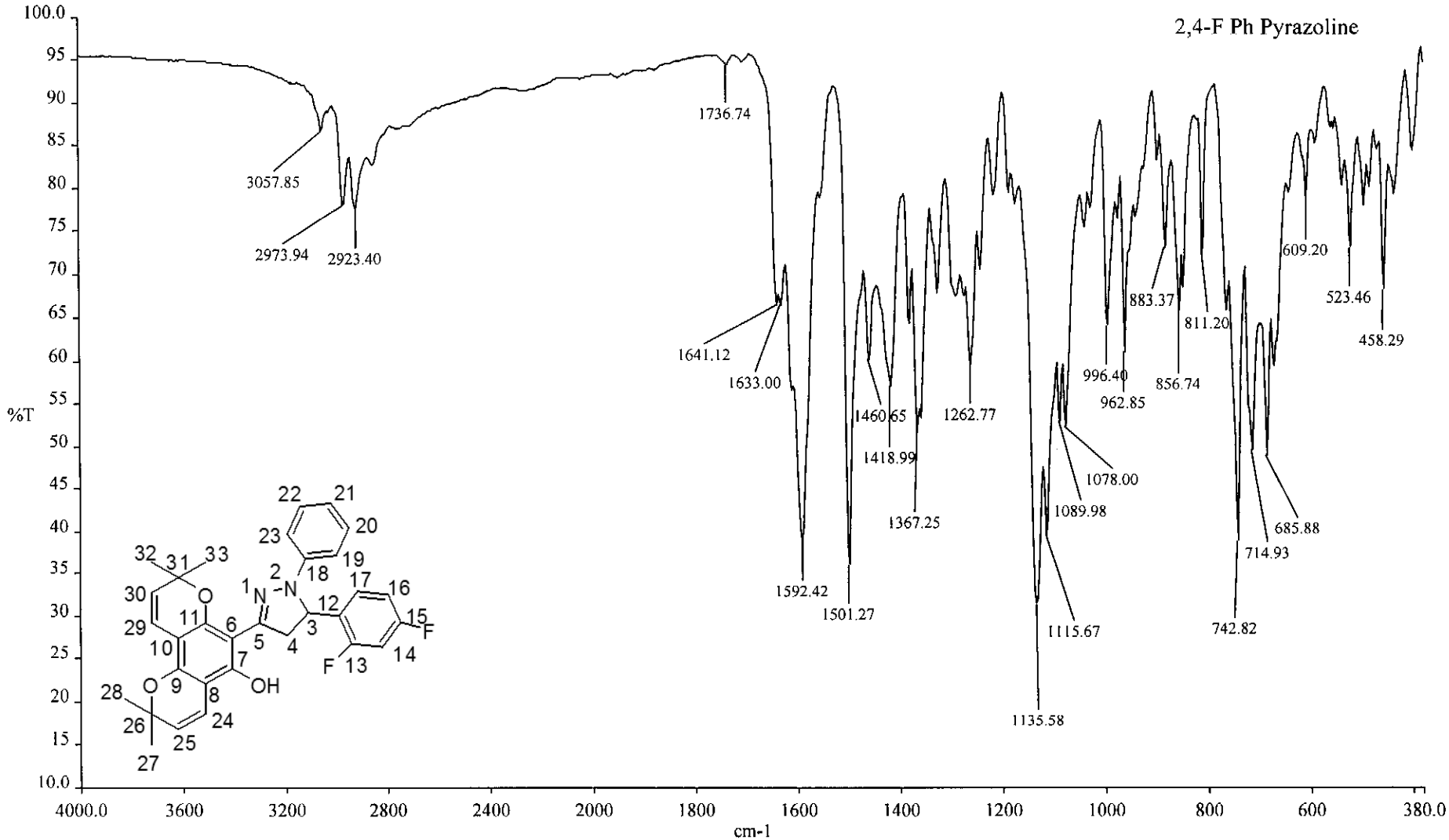
Expanded HMBC spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



Expanded 19F NMR spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)

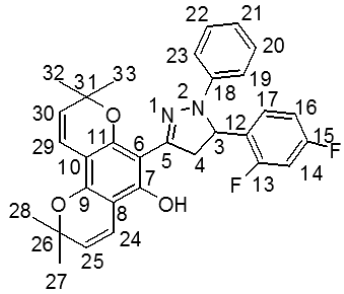


19F NMR spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



Infrared spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)

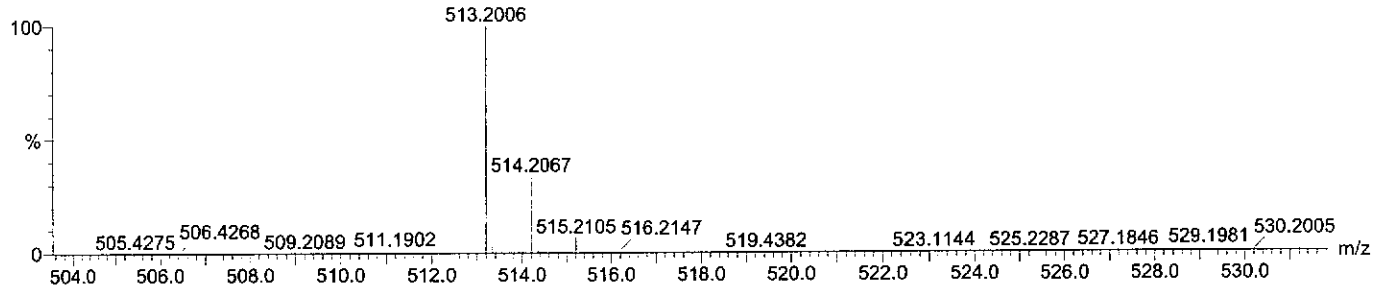
Elemental Composition Report **Page 1**



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

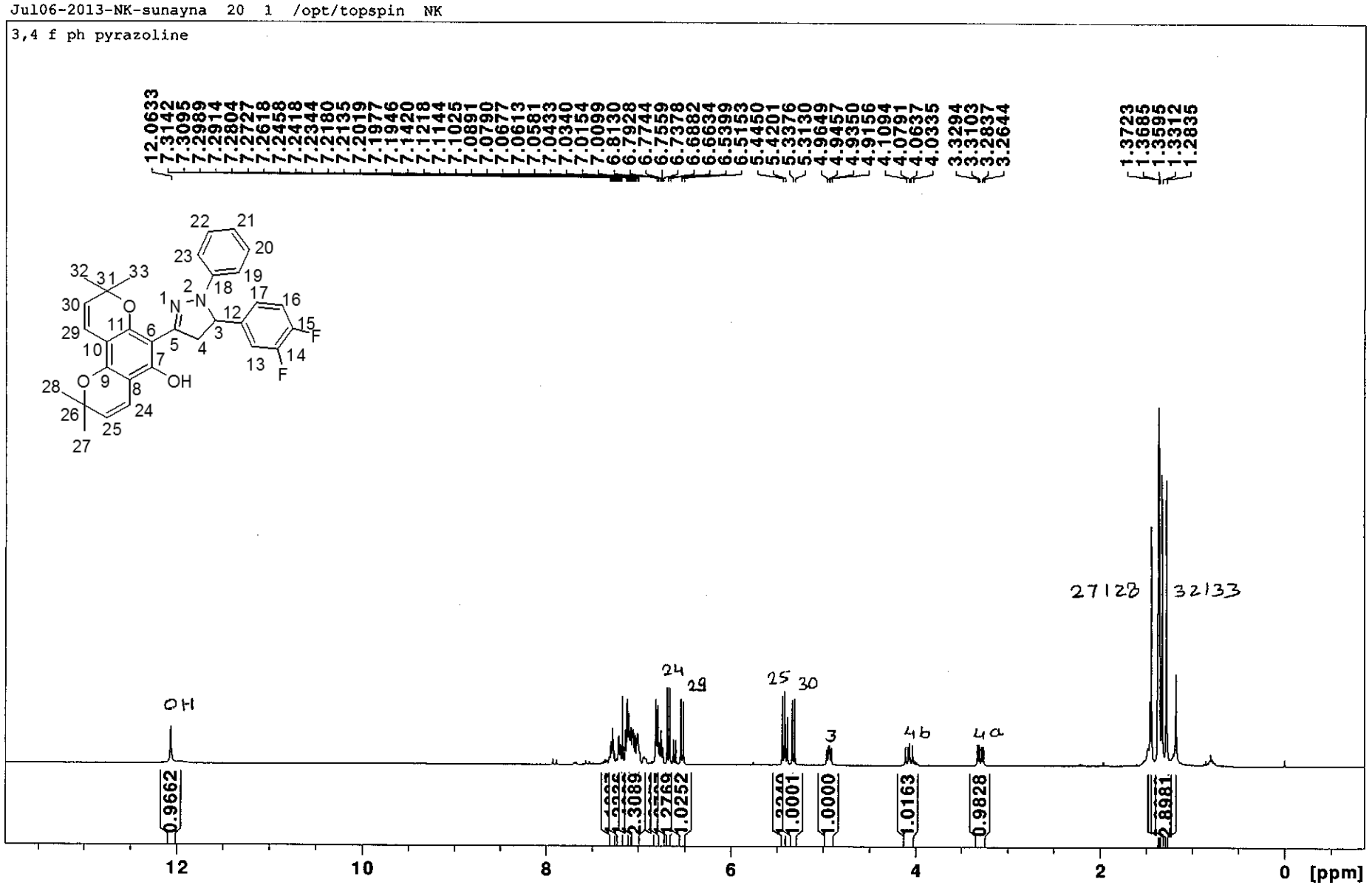
Monoisotopic Mass, Even Electron Ions
36 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-35 H: 20-30 N: 0-4 O: 0-4 F: 1-2
14 2 (0.017) Cm (1:31)
TOF MS ES-

5.95e+005

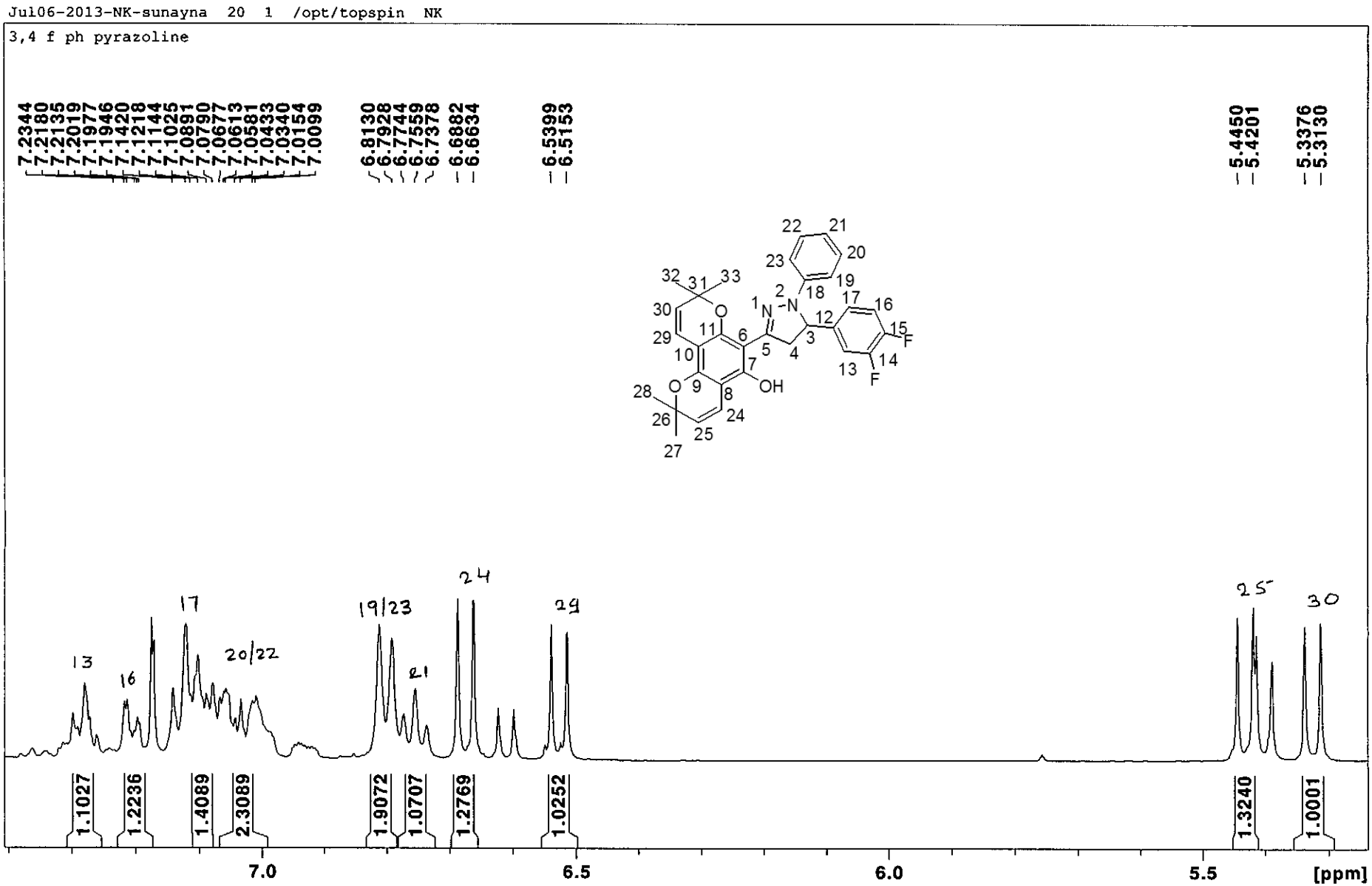


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
513.2006	513.1990	1.6	3.1	18.5	569.6	0.0	C31 H27 N2 O3 F2

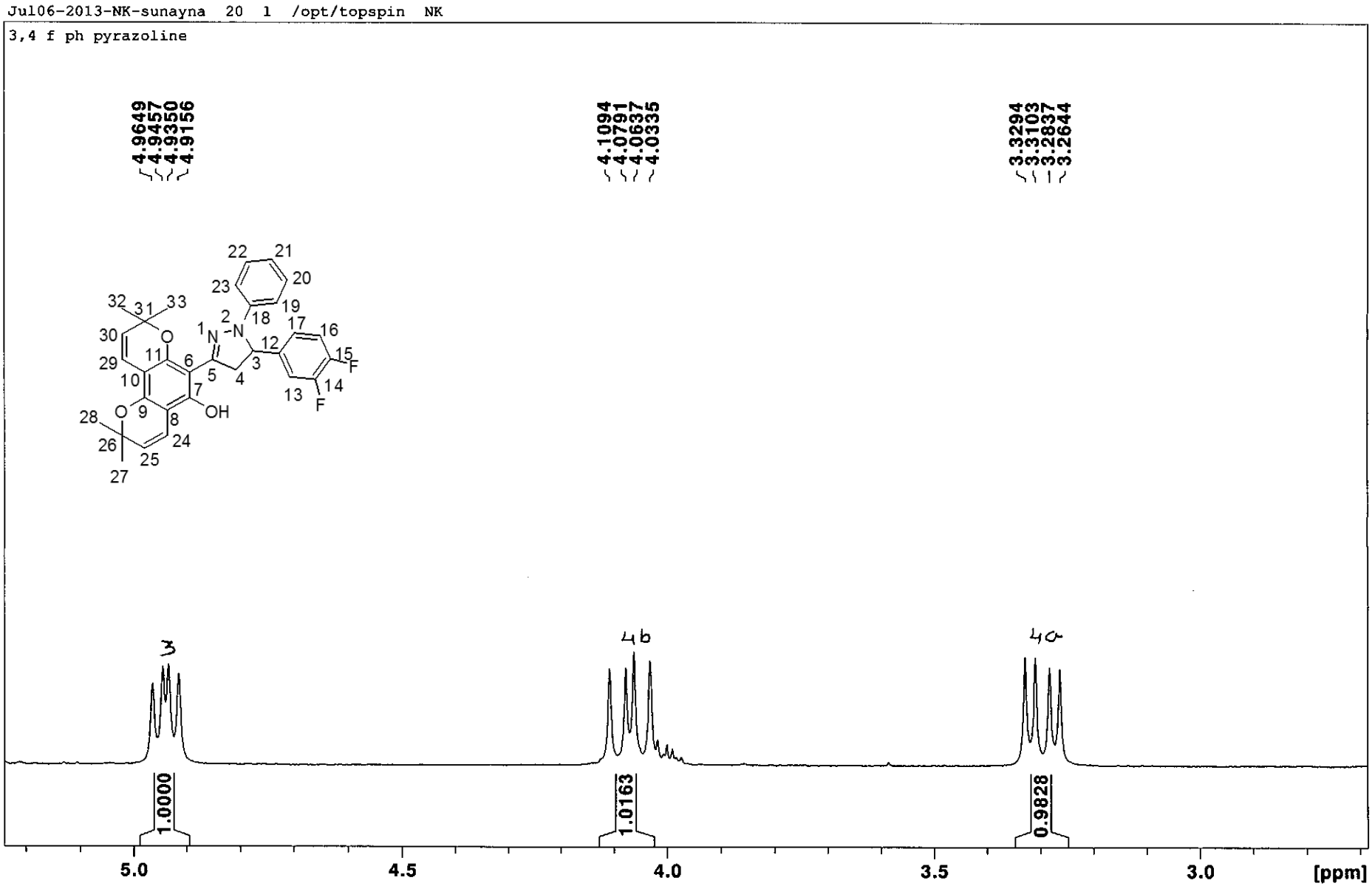
HRMS spectrum of 3-(2,4-difluorophenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5g)



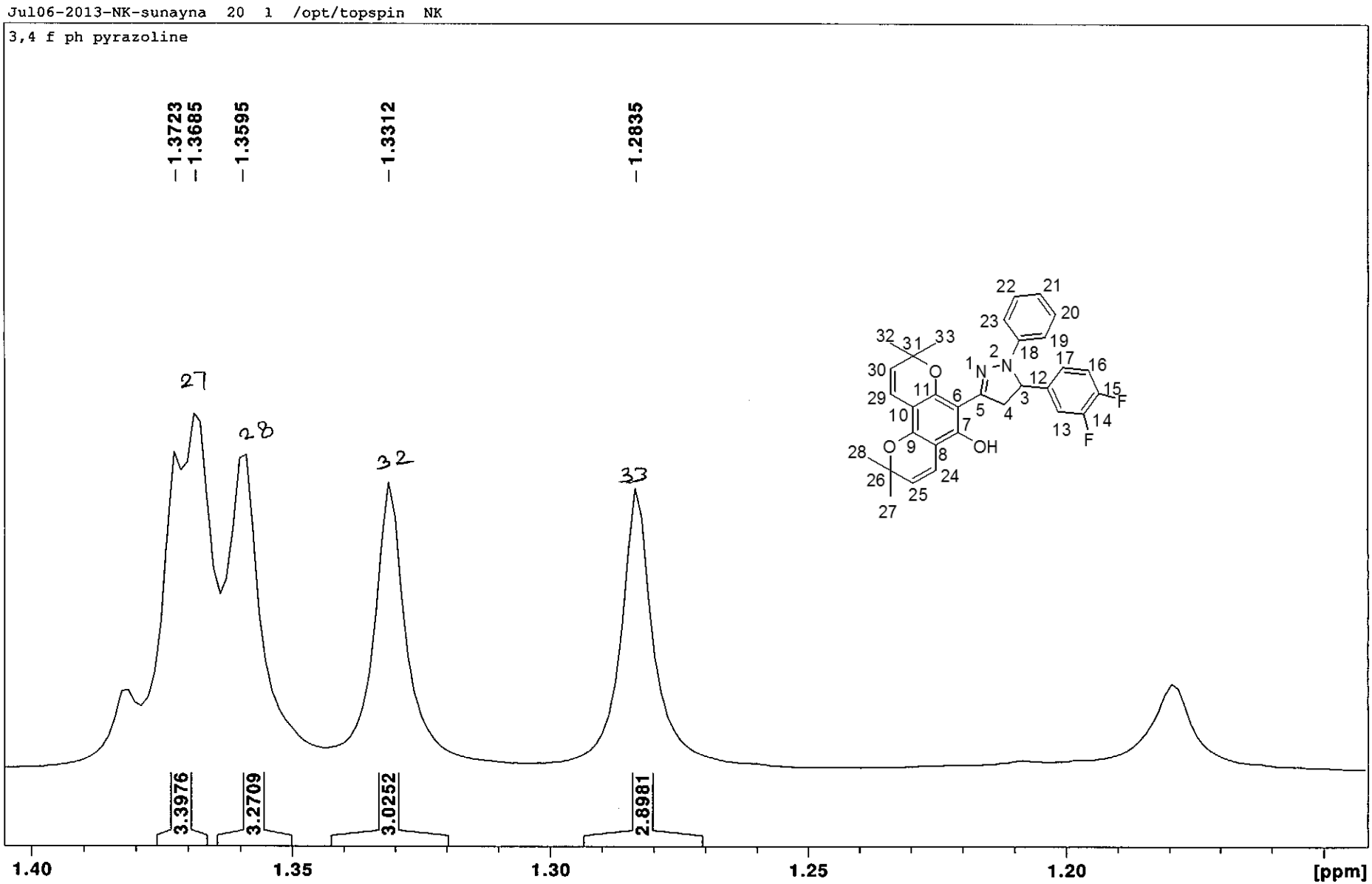
1H NMR spectrum of 3-(3,4-difluorophenyl)-2-phenyl-2-pyranochromene pyrazoline (C-5h)



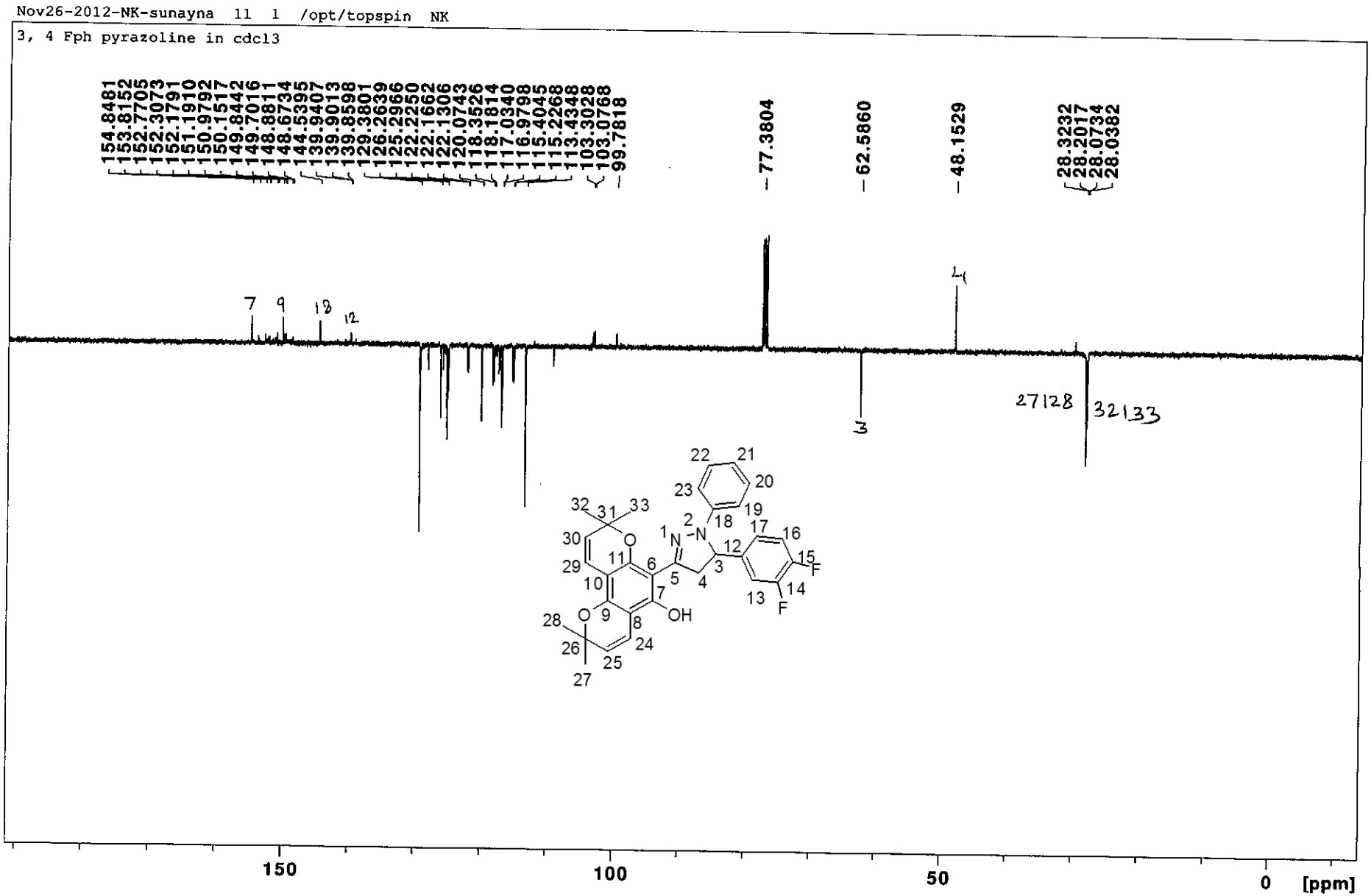
Expanded 1H NMR spectrum of 3-(3,4-difluorophenyl)-2-phenyl-2-pyranochromene pyrazoline (C-5h)



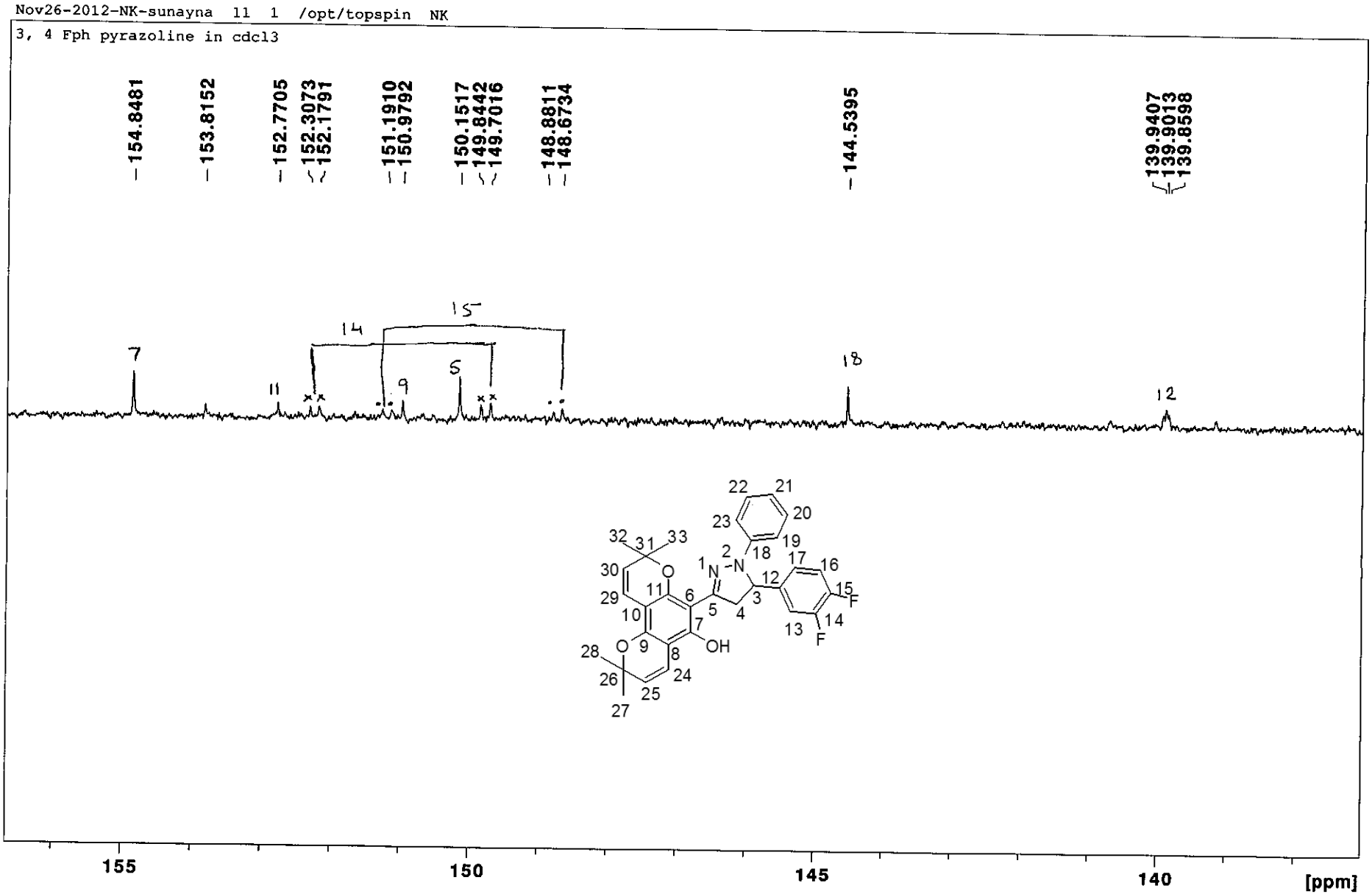
Expanded 1H NMR spectrum of 3-(3,4-difluorophenyl)-2-phenyl-2-pyranochromene pyrazoline (C-5h)



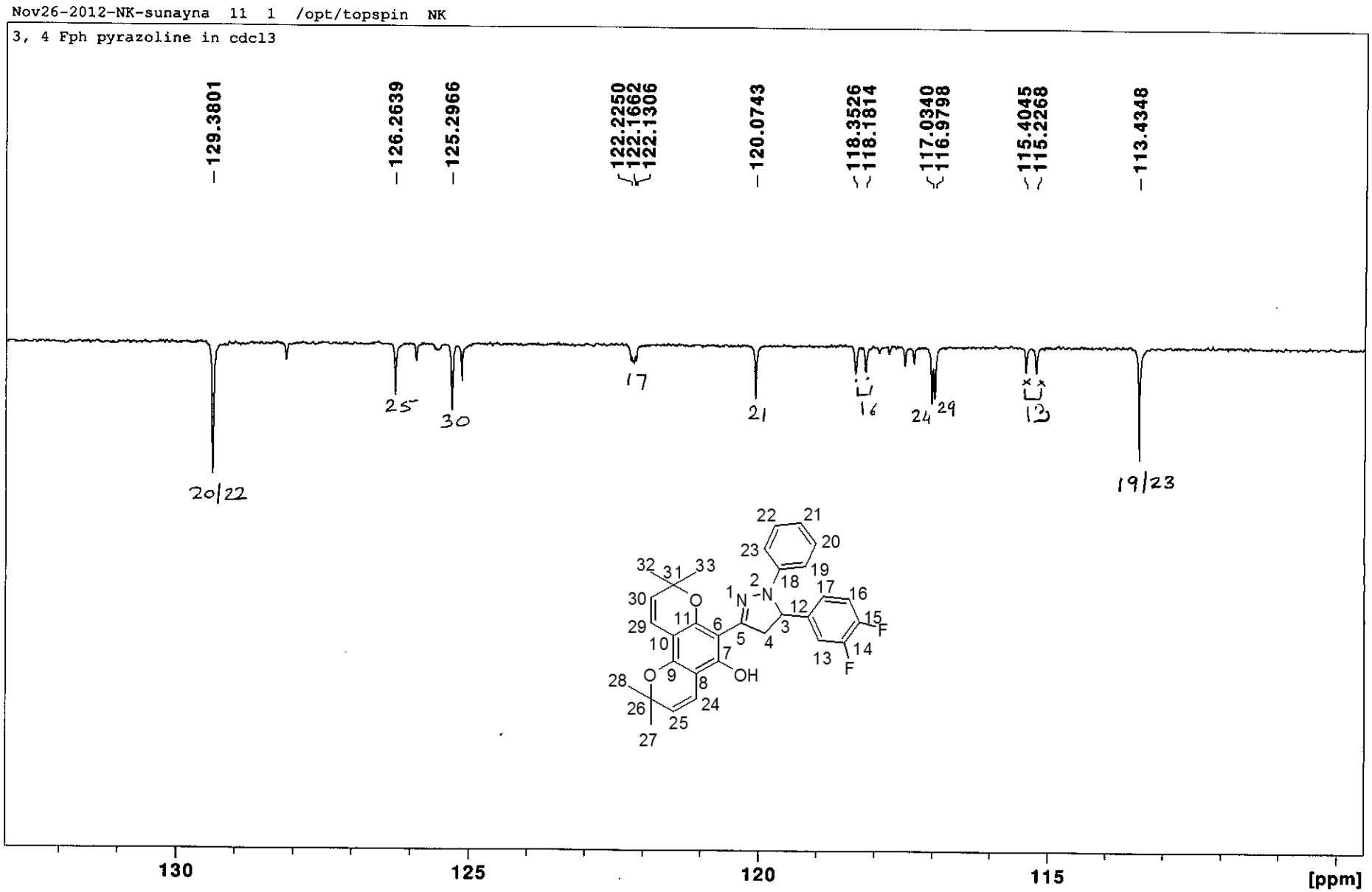
Expanded 1H NMR spectrum of 3-(3,4-difluorophenyl)-2-phenyl-2-pyranochromene pyrazoline (C-5h)



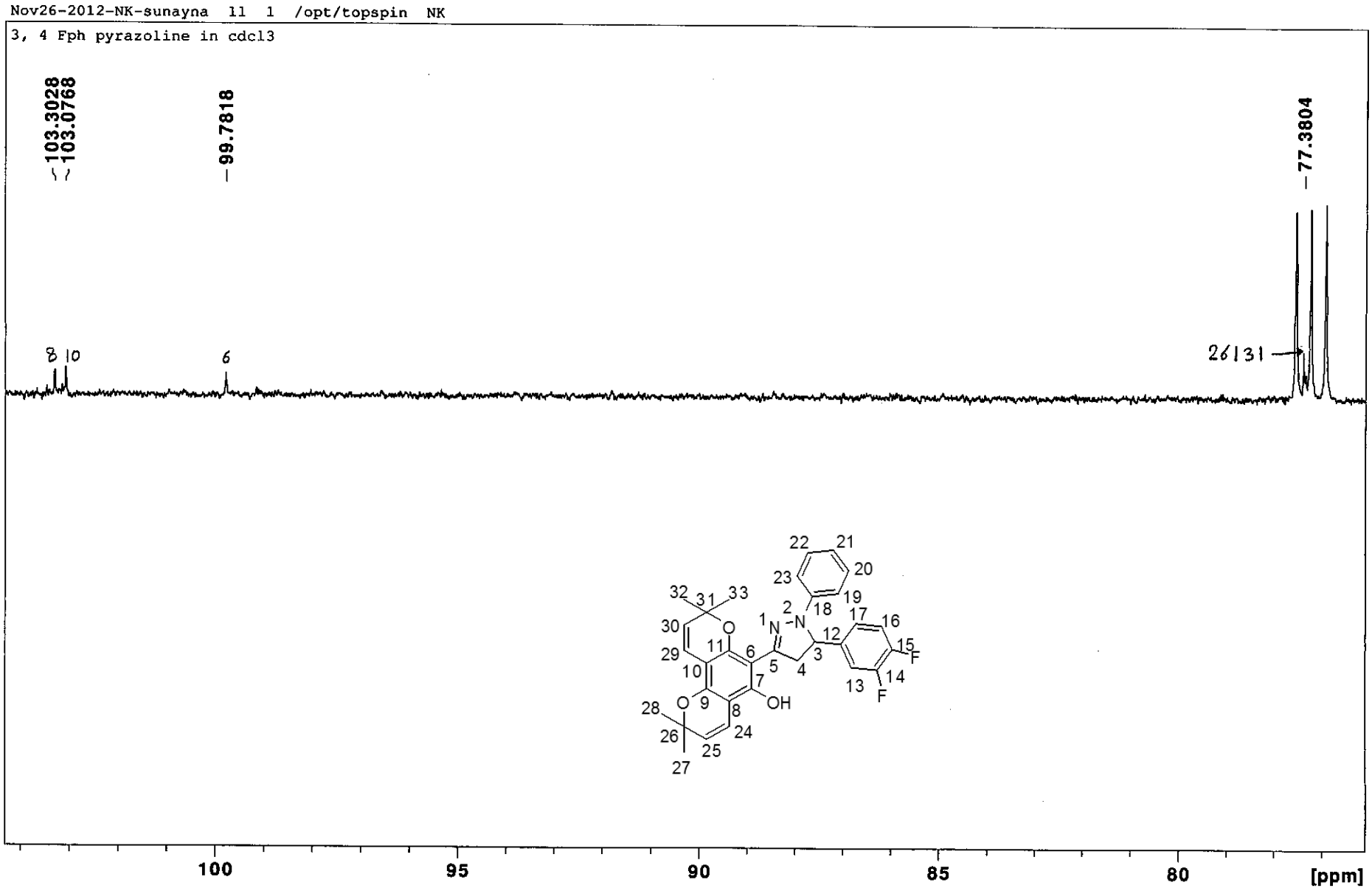
13C NMR spectrum of 3-(3,4-difluorophenyl)-2-phenyl-2-pyranochromene pyrazoline (C-5h)



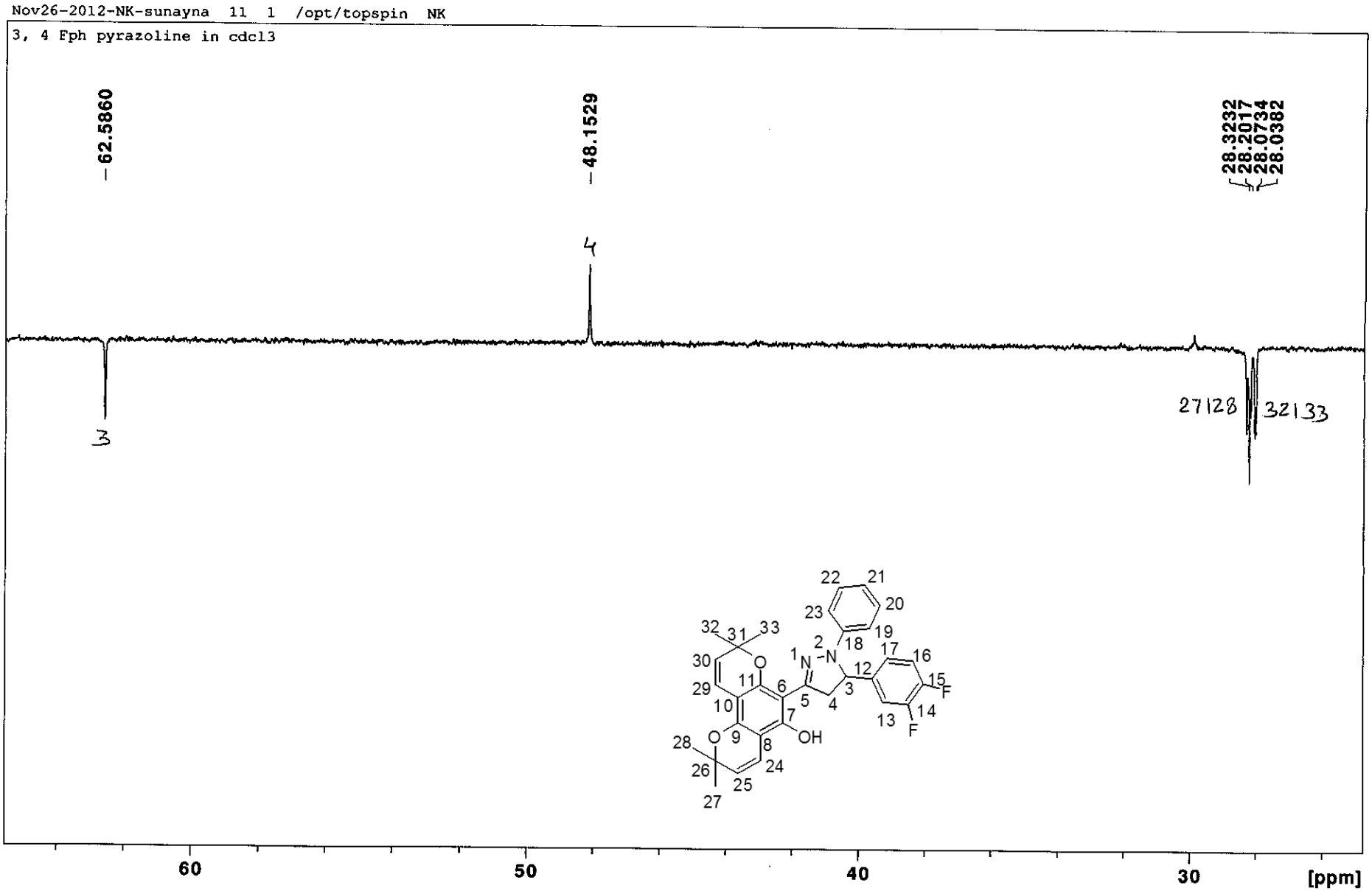
Expanded 13C NMR spectrum of 3-(3,4-difluorophenyl)-2-phenyl-2-pyranochromene pyrazoline (C-5h)



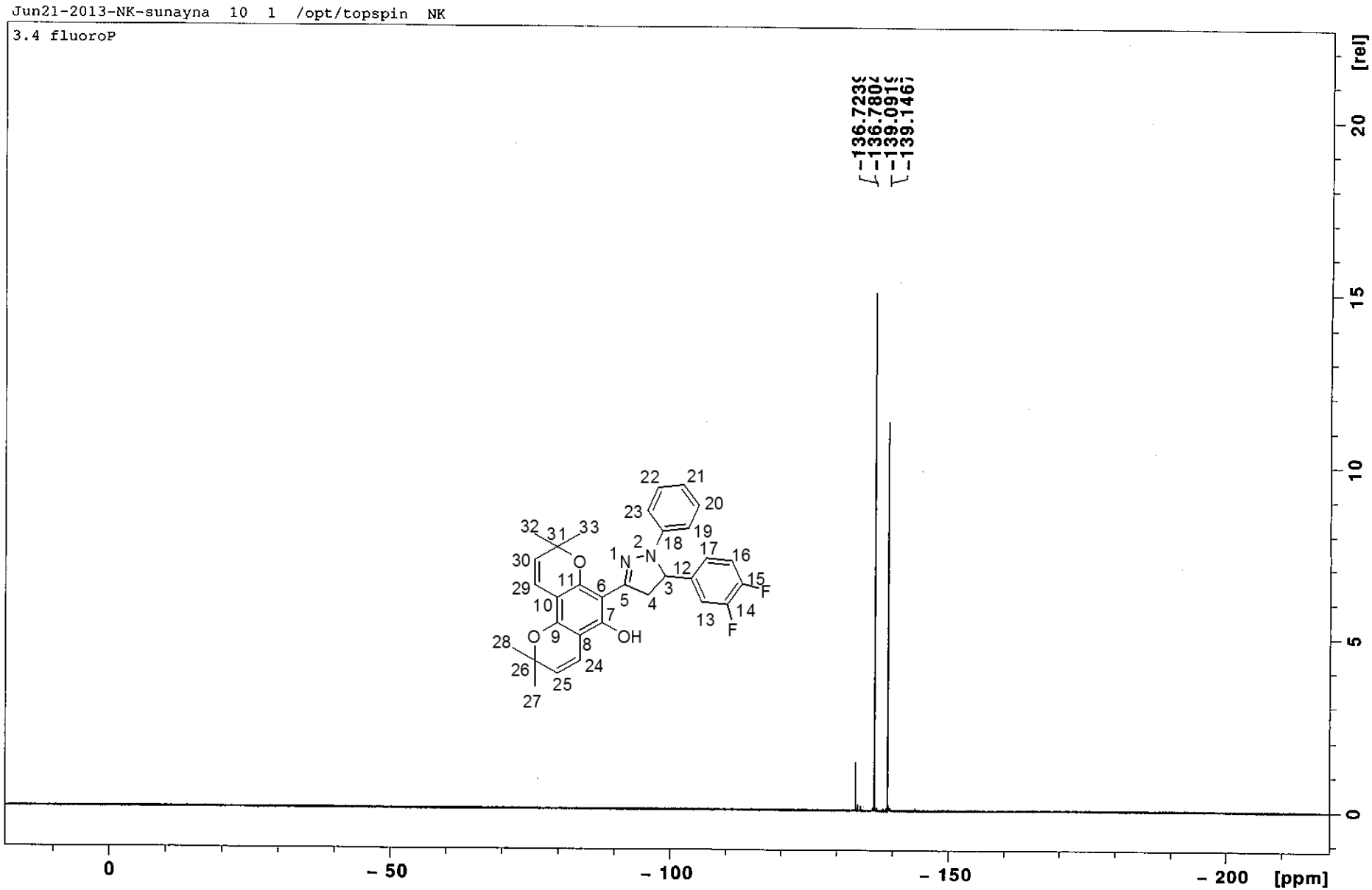
Expanded 13C NMR spectrum of 3-(3,4-difluorophenyl)-2-phenyl-2-pyranochromene pyrazoline (C-5h)



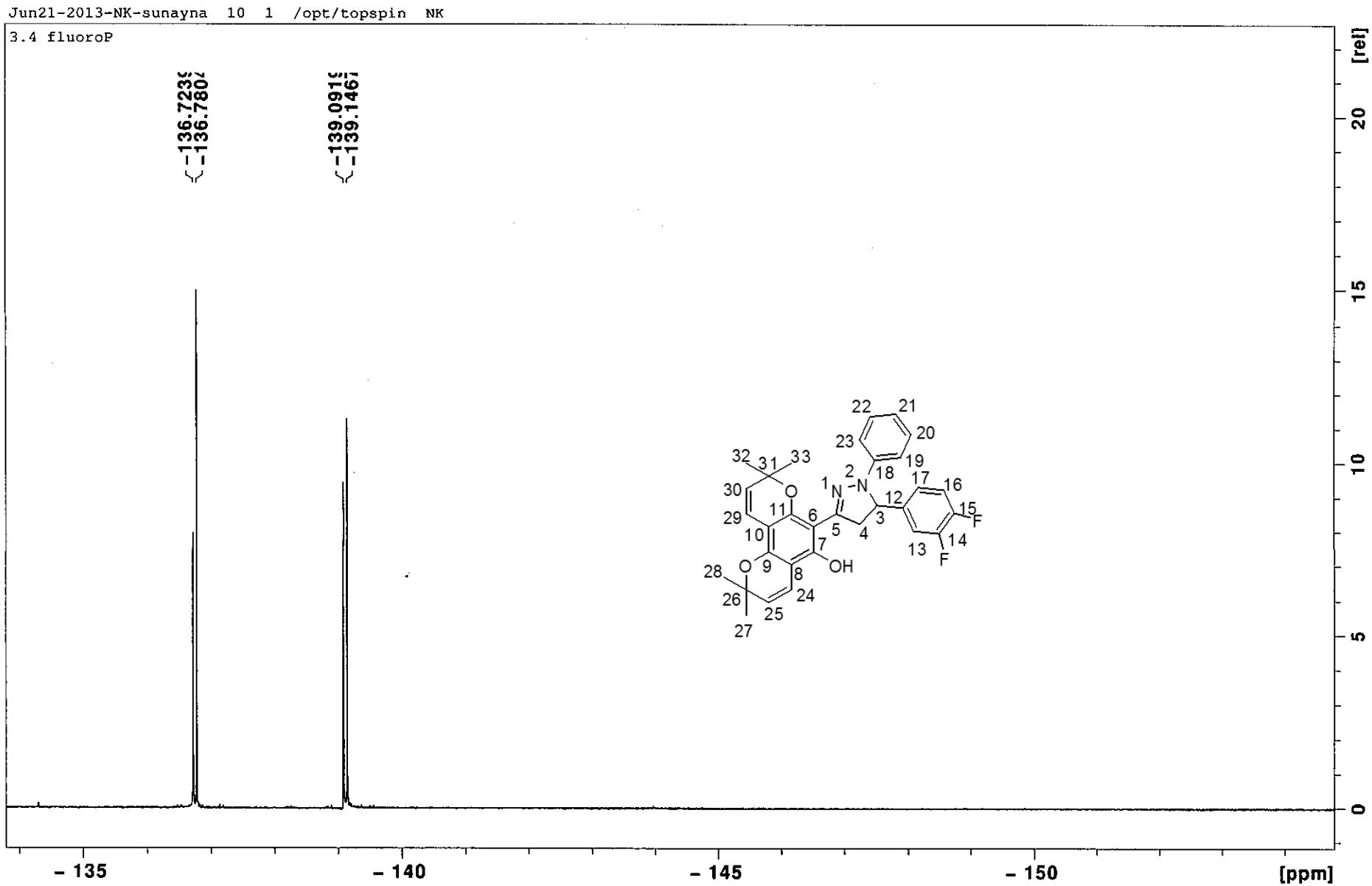
Expanded 13C NMR spectrum of 3-(3,4-difluorophenyl)-2-phenyl-2-pyranochromene pyrazoline (C-5h)



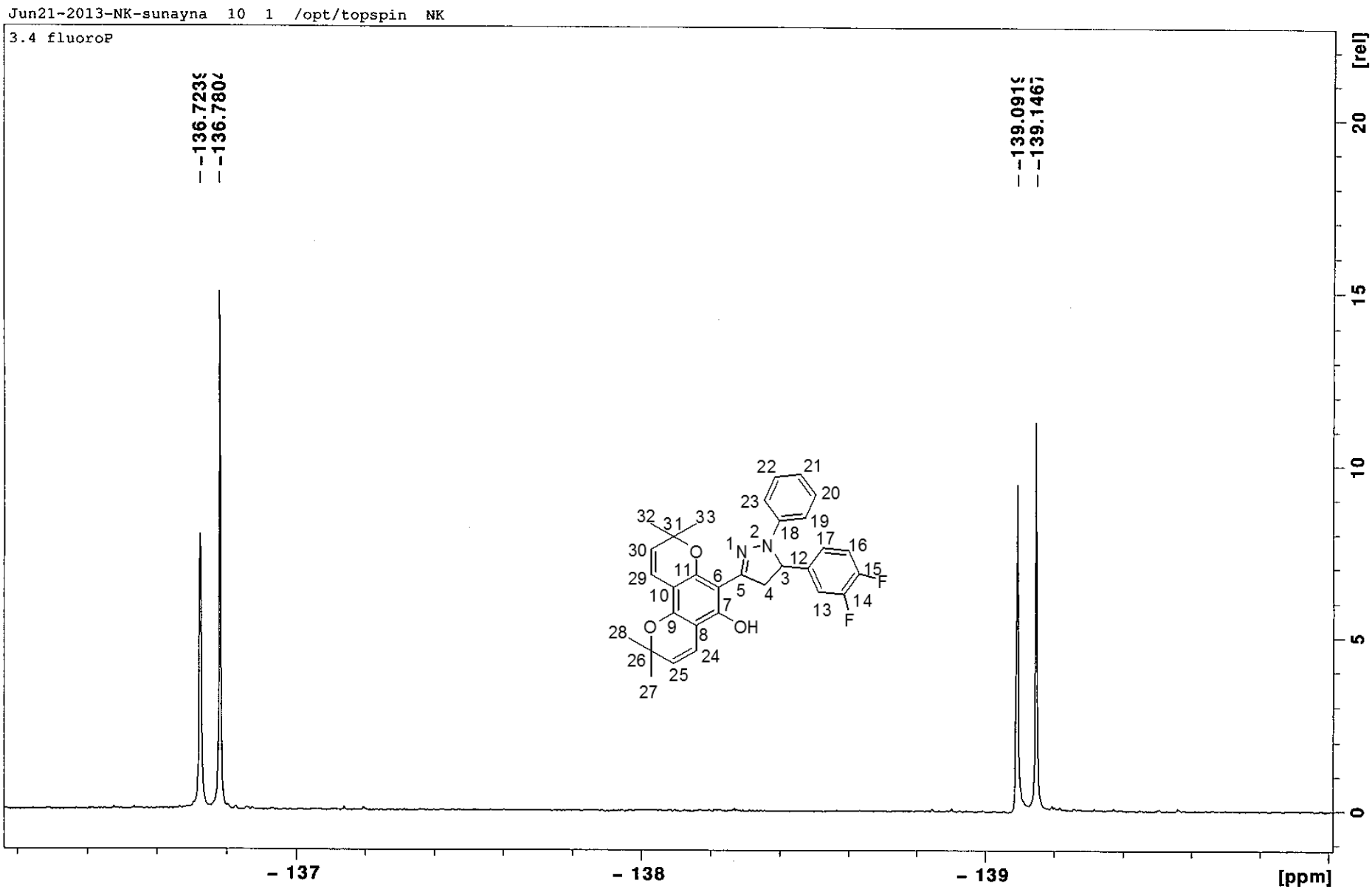
Expanded 13C NMR spectrum of 3-(3,4-difluorophenyl)-2-phenyl-2-pyranochromene pyrazoline (C-5h)



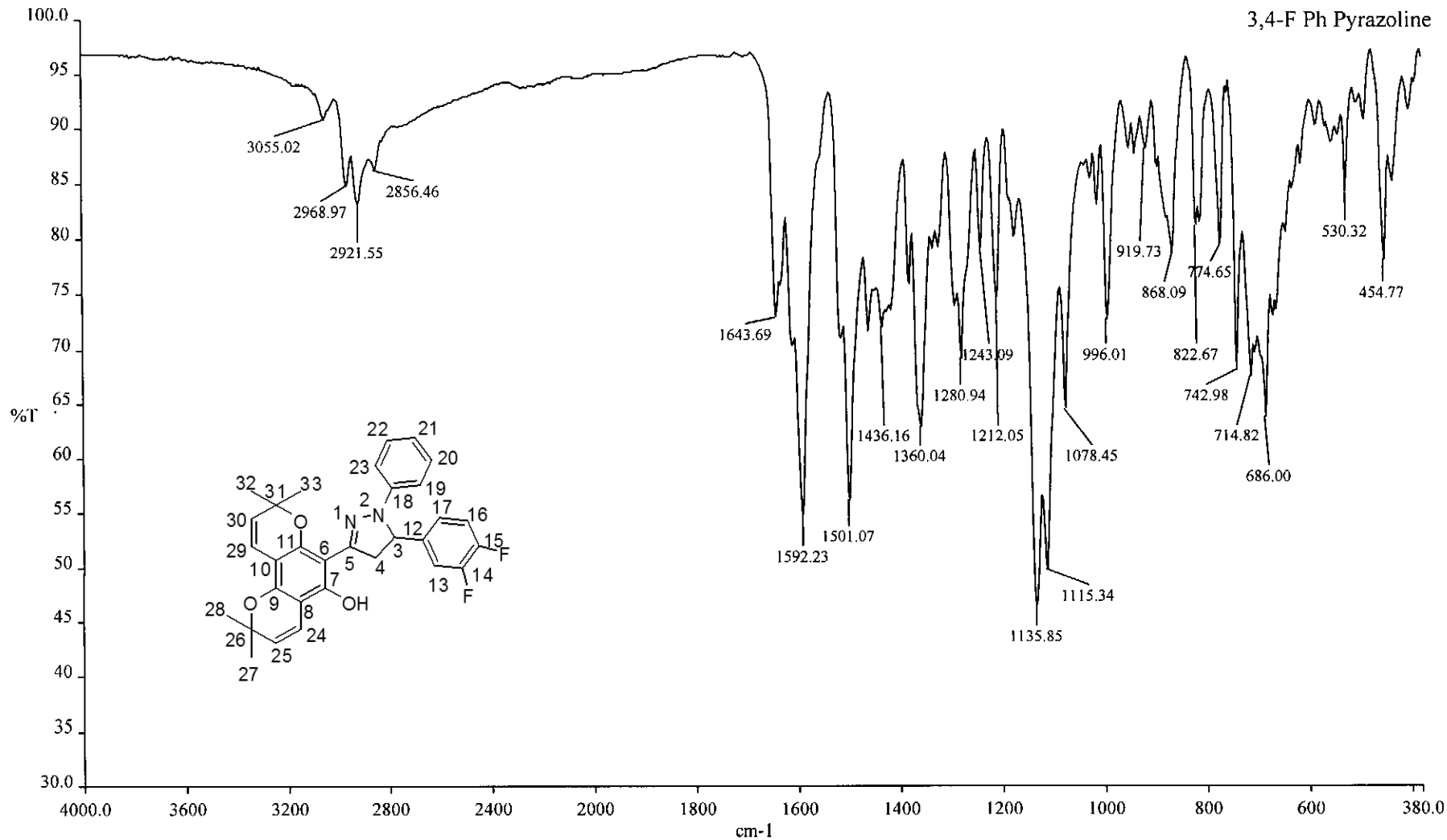
19F NMR spectrum of 3-(3,4-difluorophenyl)-2-phenyl-2-pyranochromene pyrazoline (C-5h)



Expanded 19F NMR spectrum of 3-(3,4-difluorophenyl)-2-phenyl-2-pyranochromene pyrazoline (C-5h)



Expanded 19F NMR spectrum of 3-(3,4-difluorophenyl)-2-phenyl-2-pyranochromene pyrazoline (C-5h)

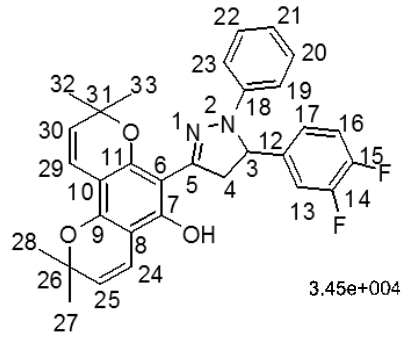


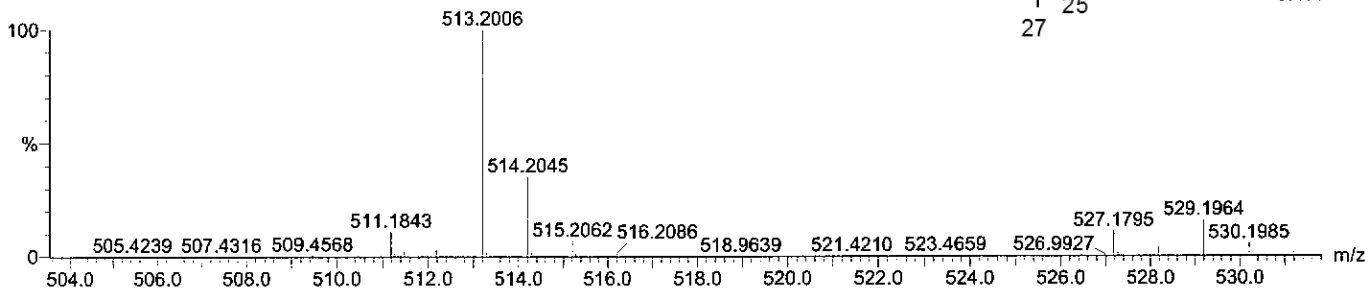
Infrared spectrum of 3-(3,4-difluorophenyl)-2-phenyl-2-pyranochromene pyrazoline (C-5h)

Elemental Composition Report **Page 1**

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

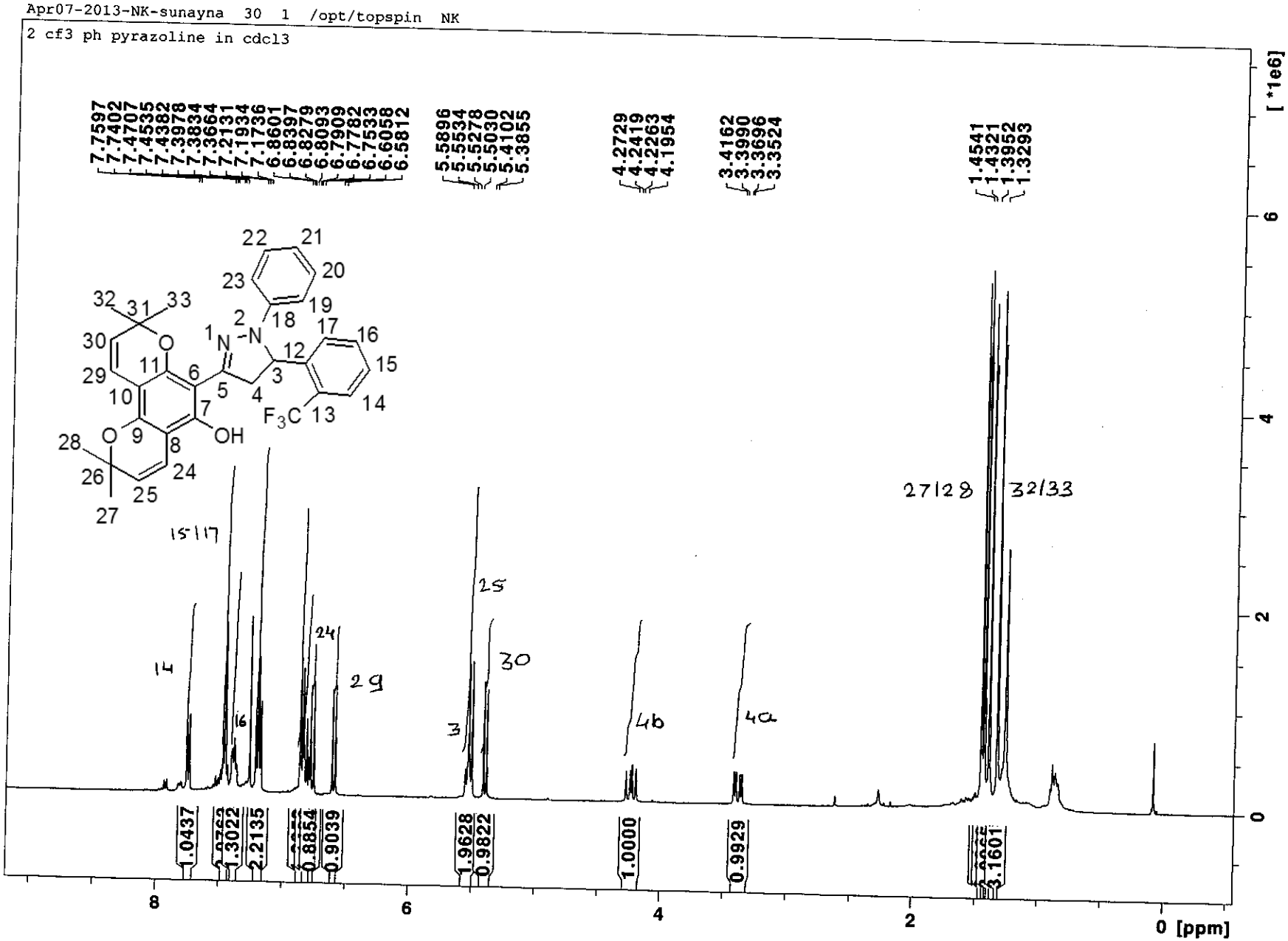
Monoisotopic Mass, Even Electron Ions
18 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-35 H: 20-30 N: 0-4 O: 0-4 F: 2-2
16 25 (0.410) Cm (1:30)
TOF MS ES-



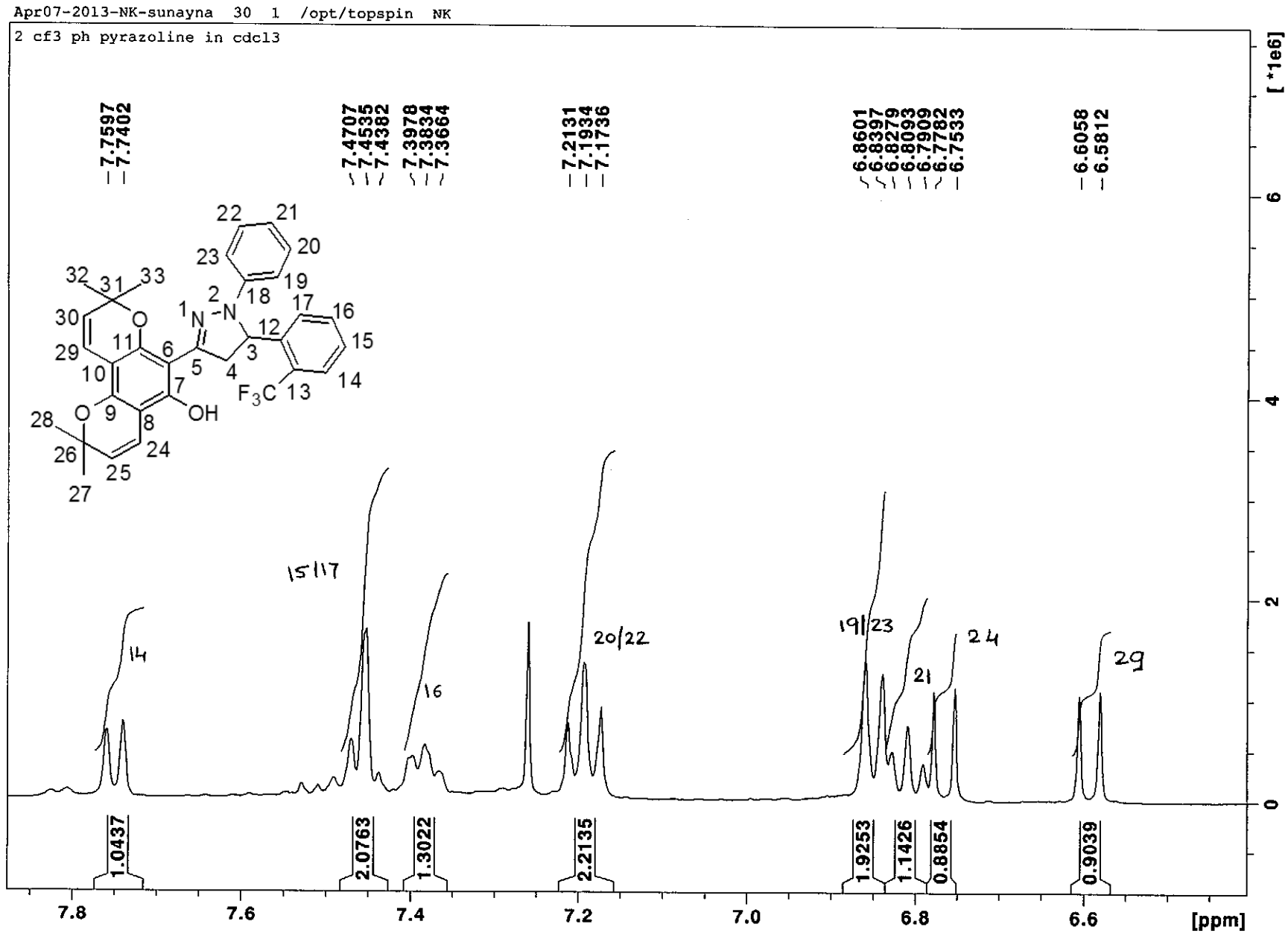


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
513.2006	513.1990	1.6	3.1	18.5	429.1	0.0	C31 H27 N2 O3 F2

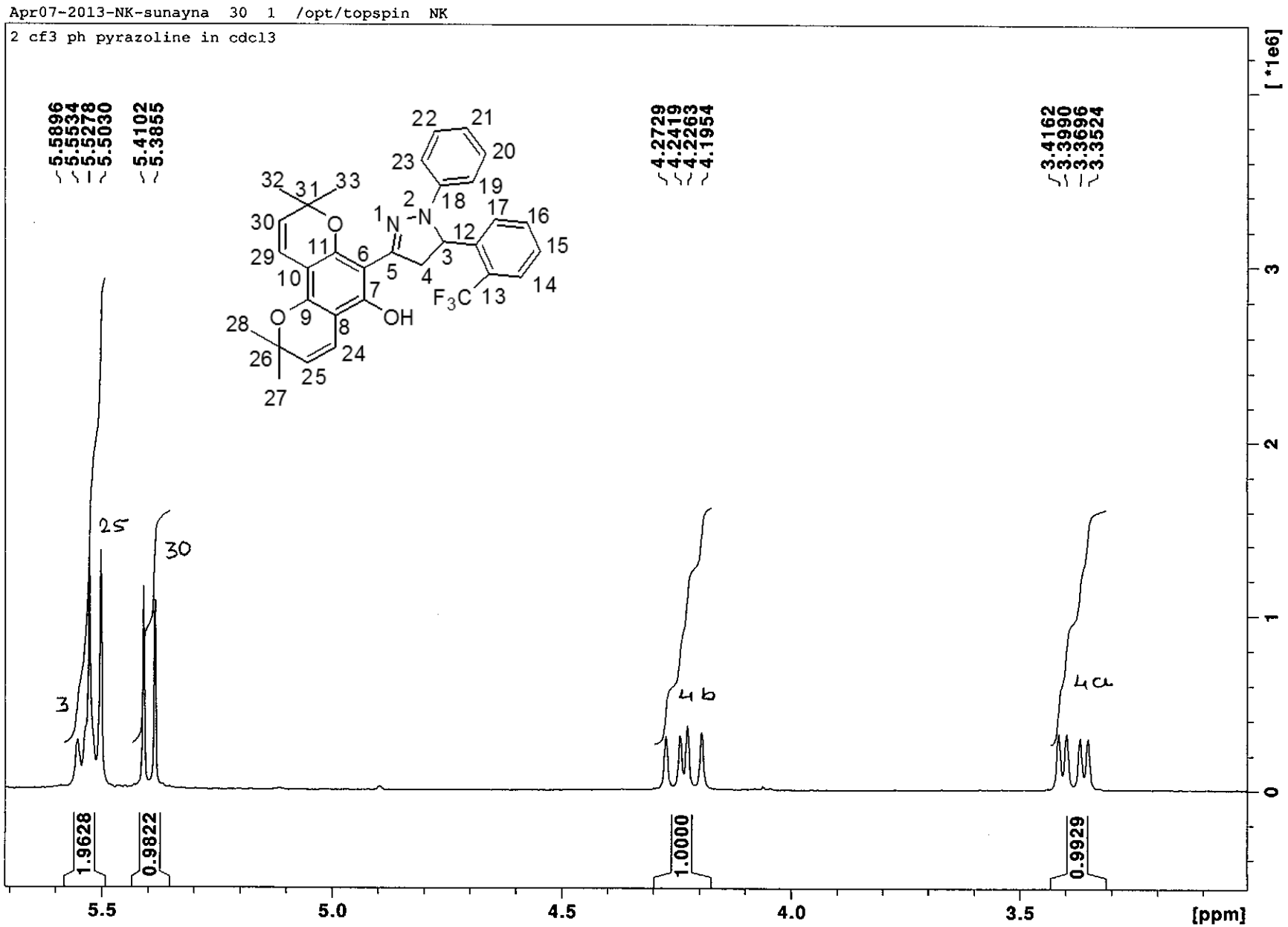
HRMS spectrum of 3-(3,4-difluorophenyl)-2-phenyl-2-pyranochromene pyrazoline (C-5h)



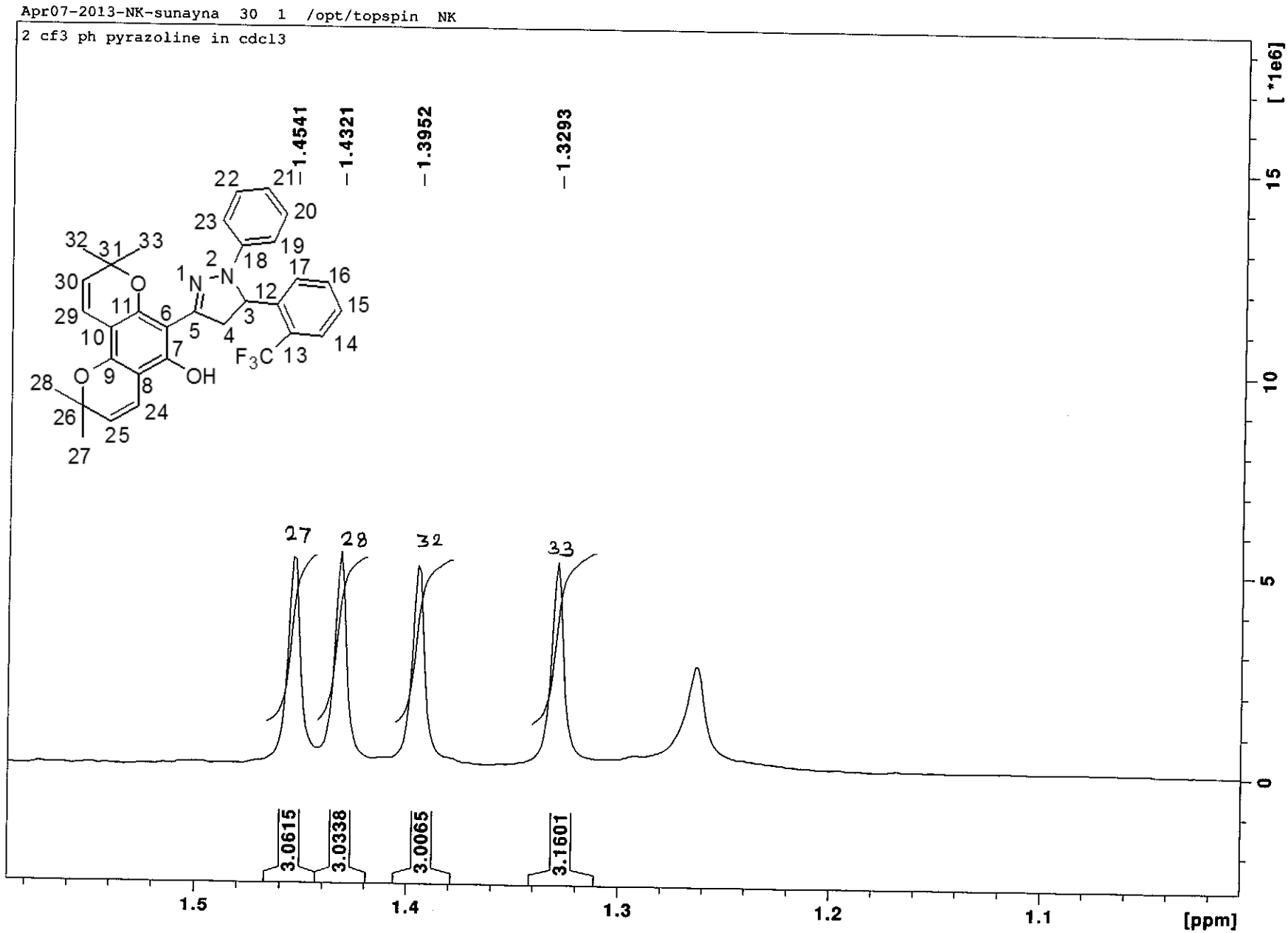
1H NMR spectrum of 3-(2-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5i)



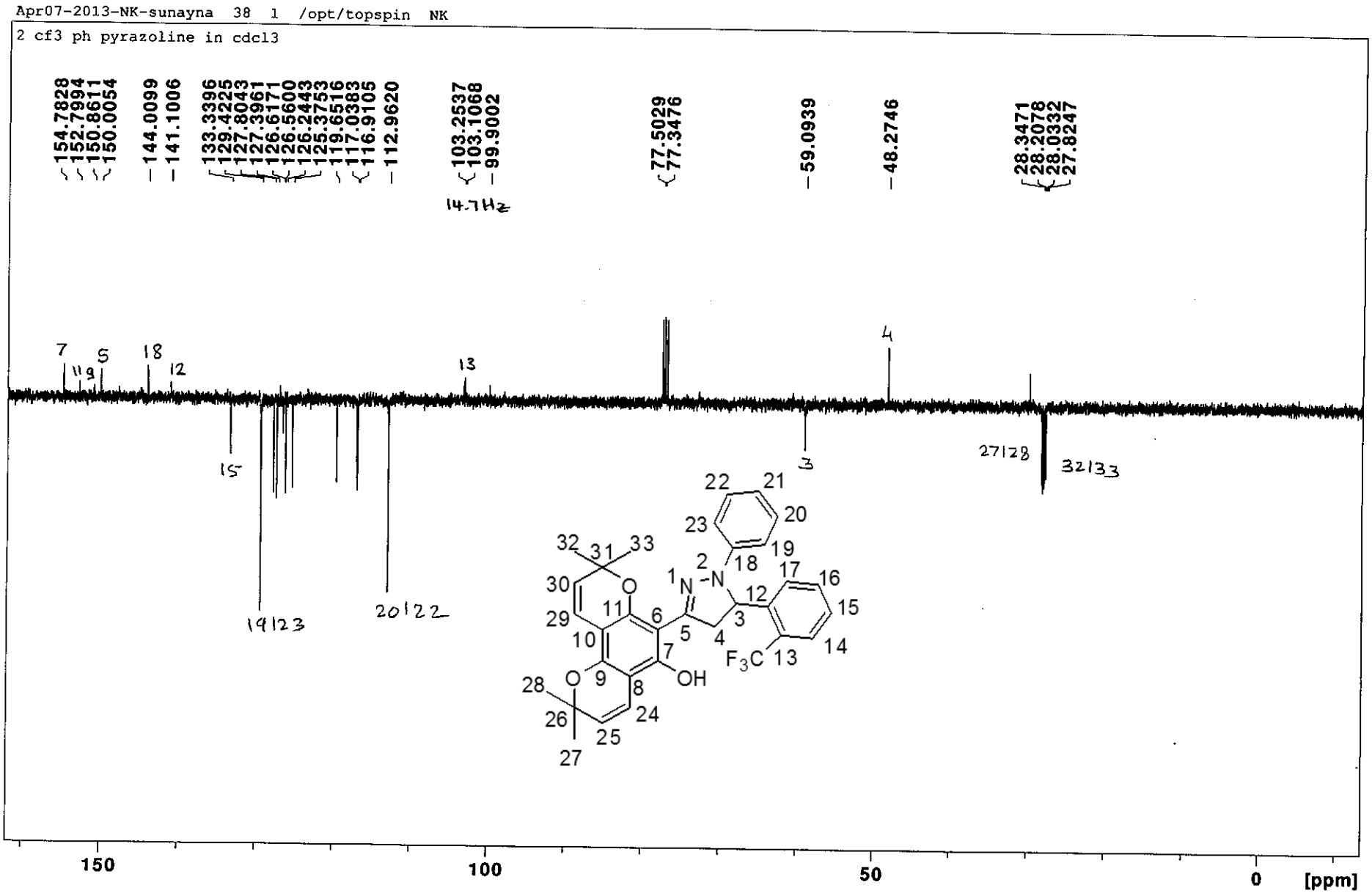
Expanded 1H NMR spectrum of 3-(2-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5i)



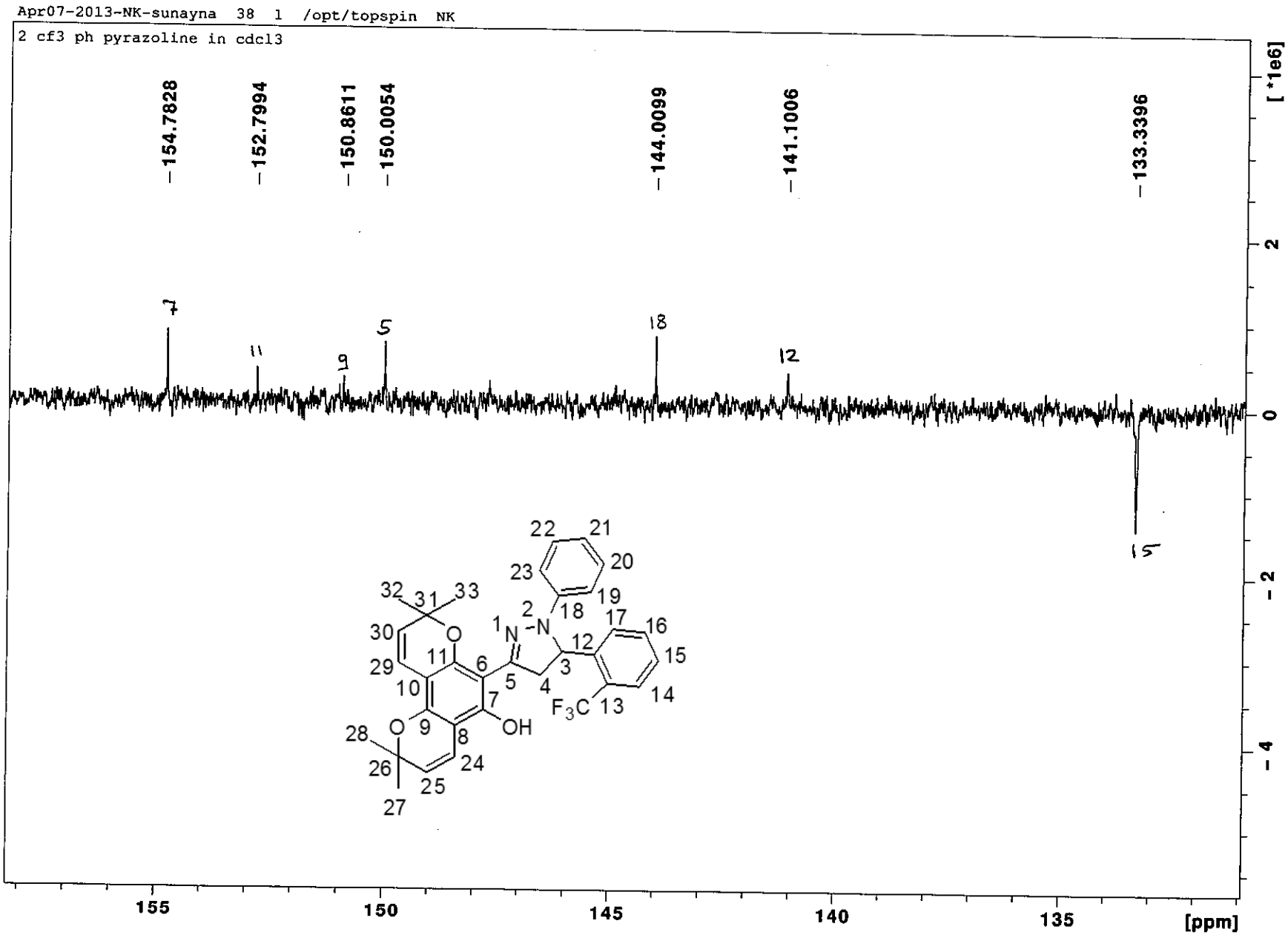
Expanded 1H NMR spectrum of 3-(2-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5i)



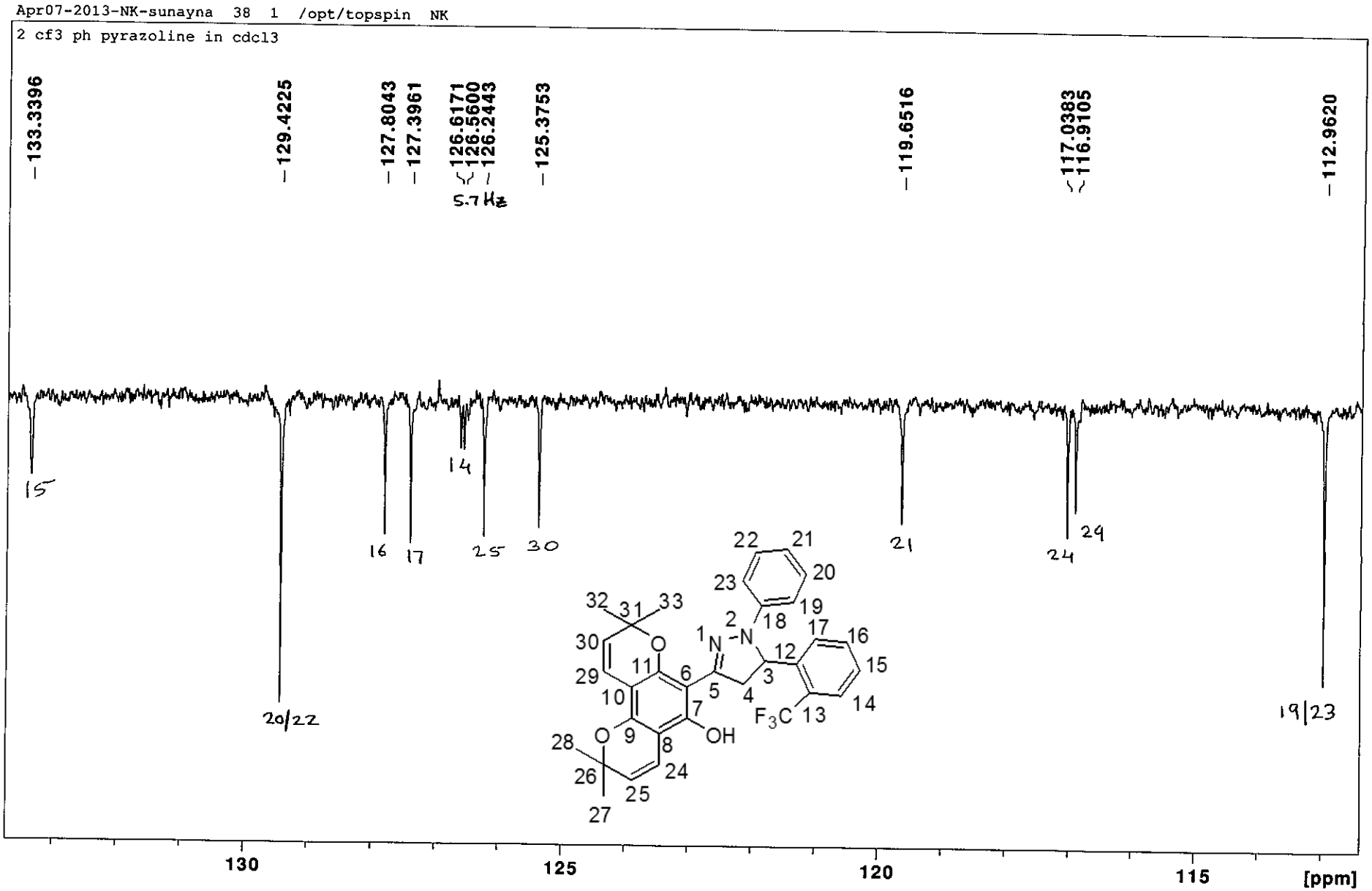
Expanded 1H NMR spectrum of 3-(2-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5i)



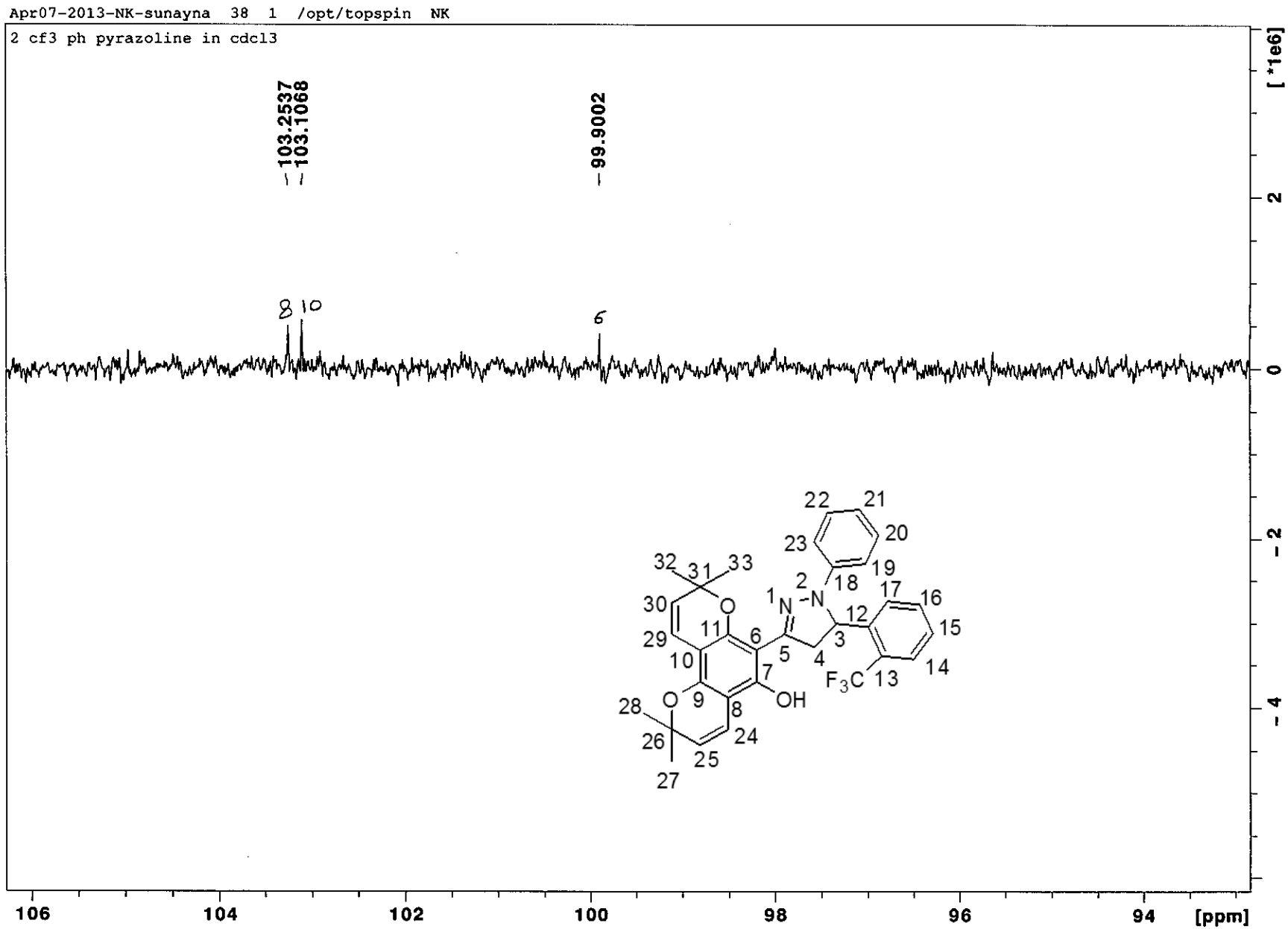
13C NMR spectrum of 3-(2-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5i)



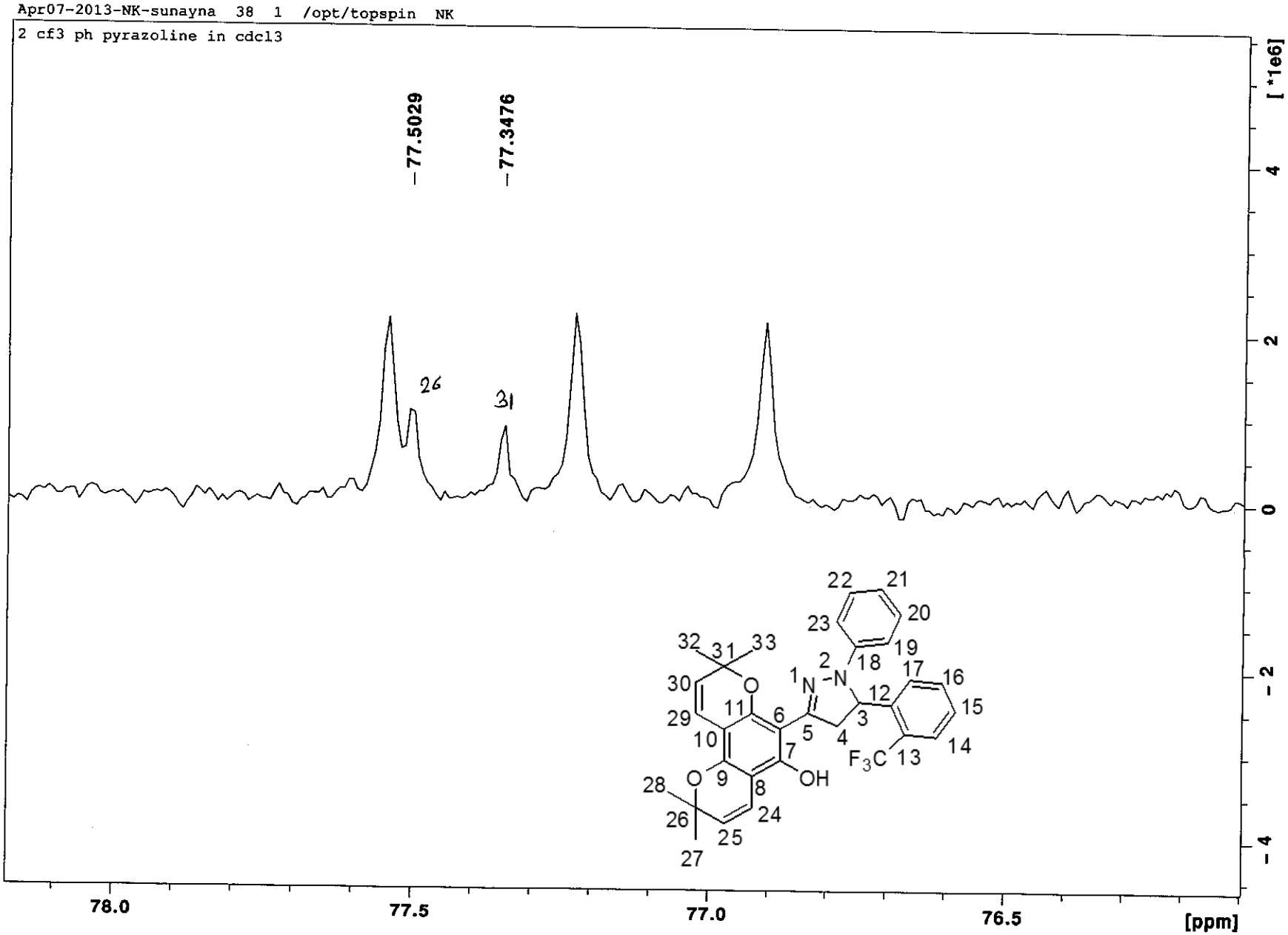
Expanded 13C NMR spectrum of 3-(2-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5i)



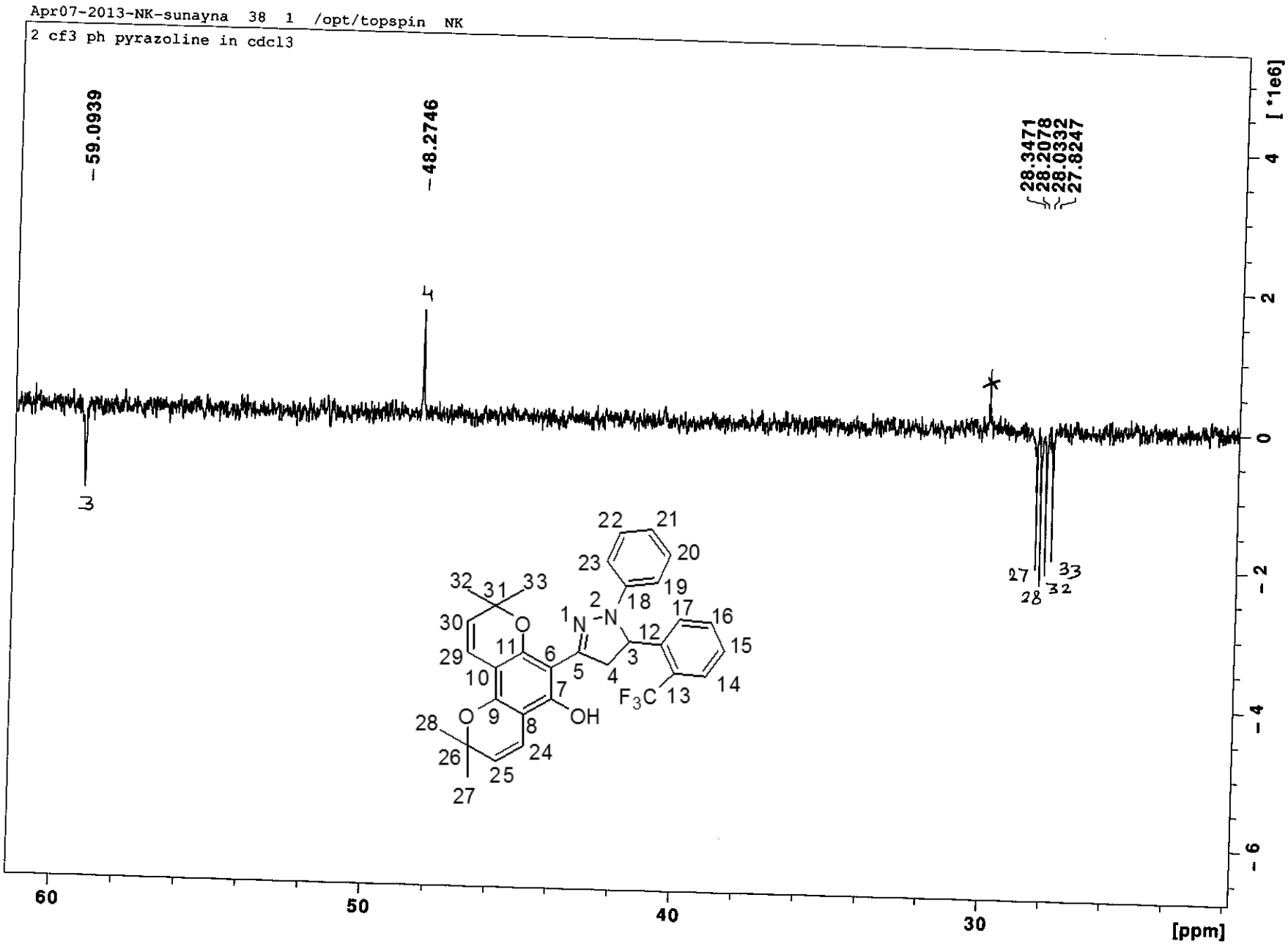
Expanded 13C NMR spectrum of 3-(2-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5i)



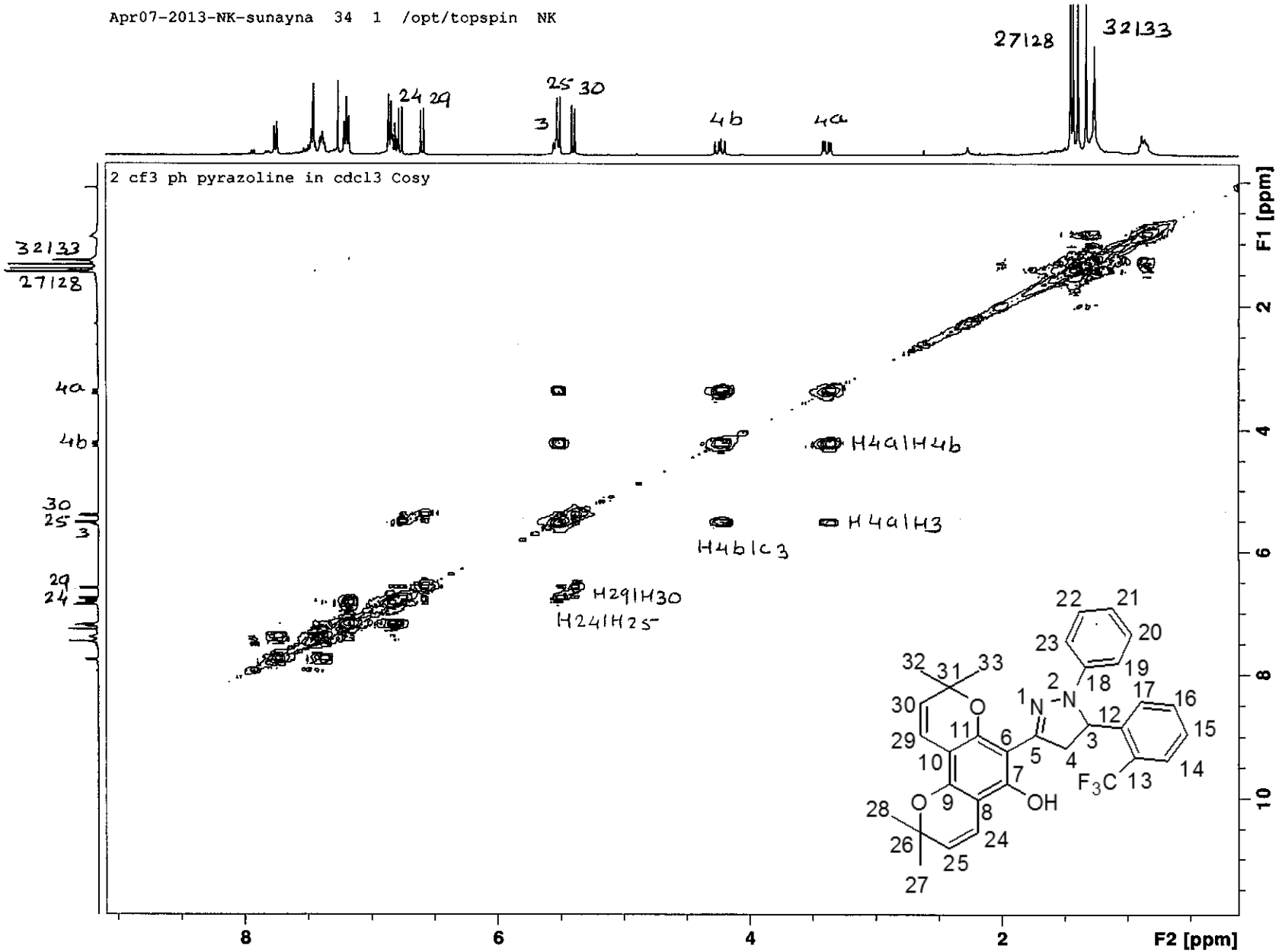
Expanded 13C NMR spectrum of 3-(2-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5i)



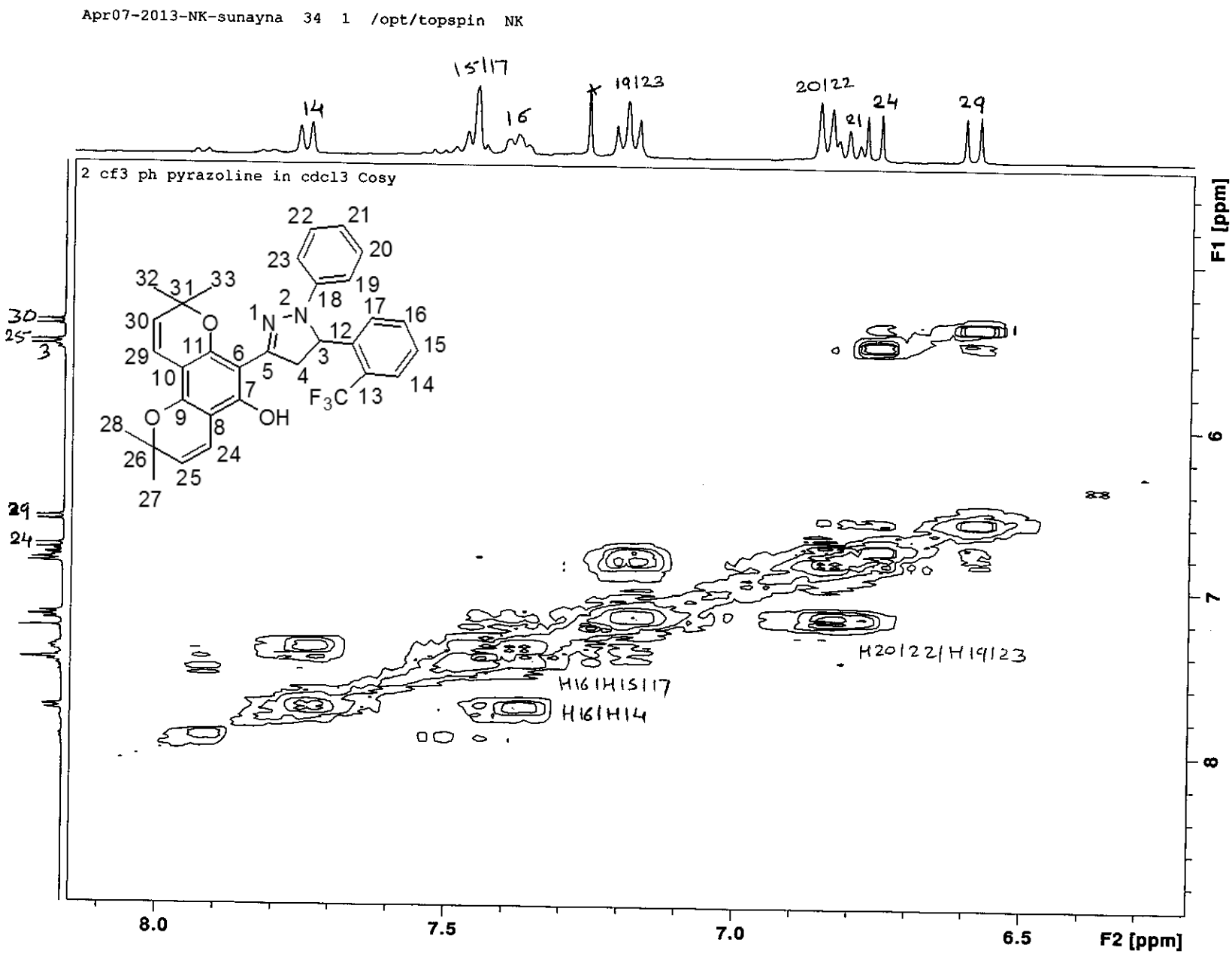
Expanded 13C NMR spectrum of 3-(2-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5i)



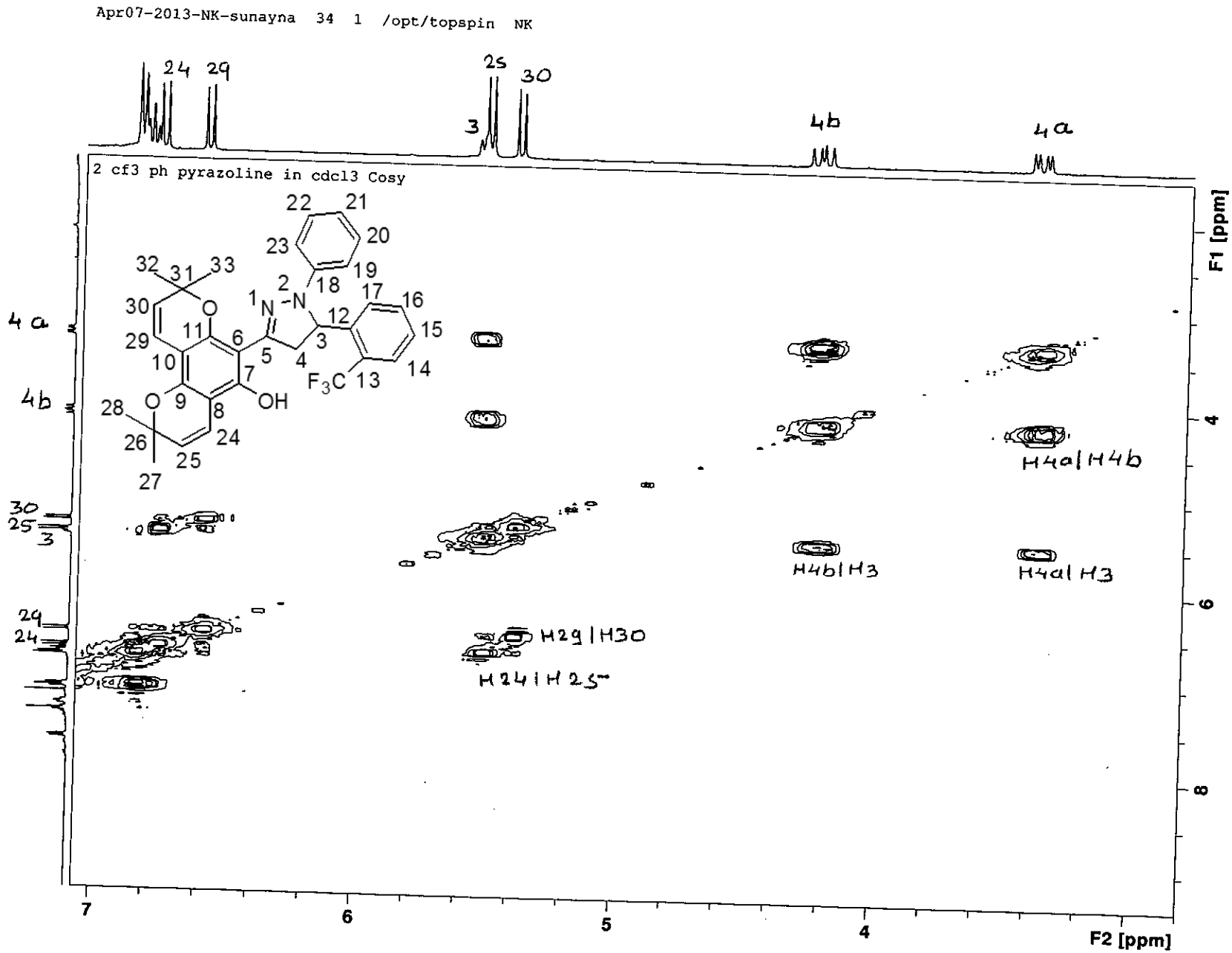
Expanded 13C NMR spectrum of 3-(2-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5i)



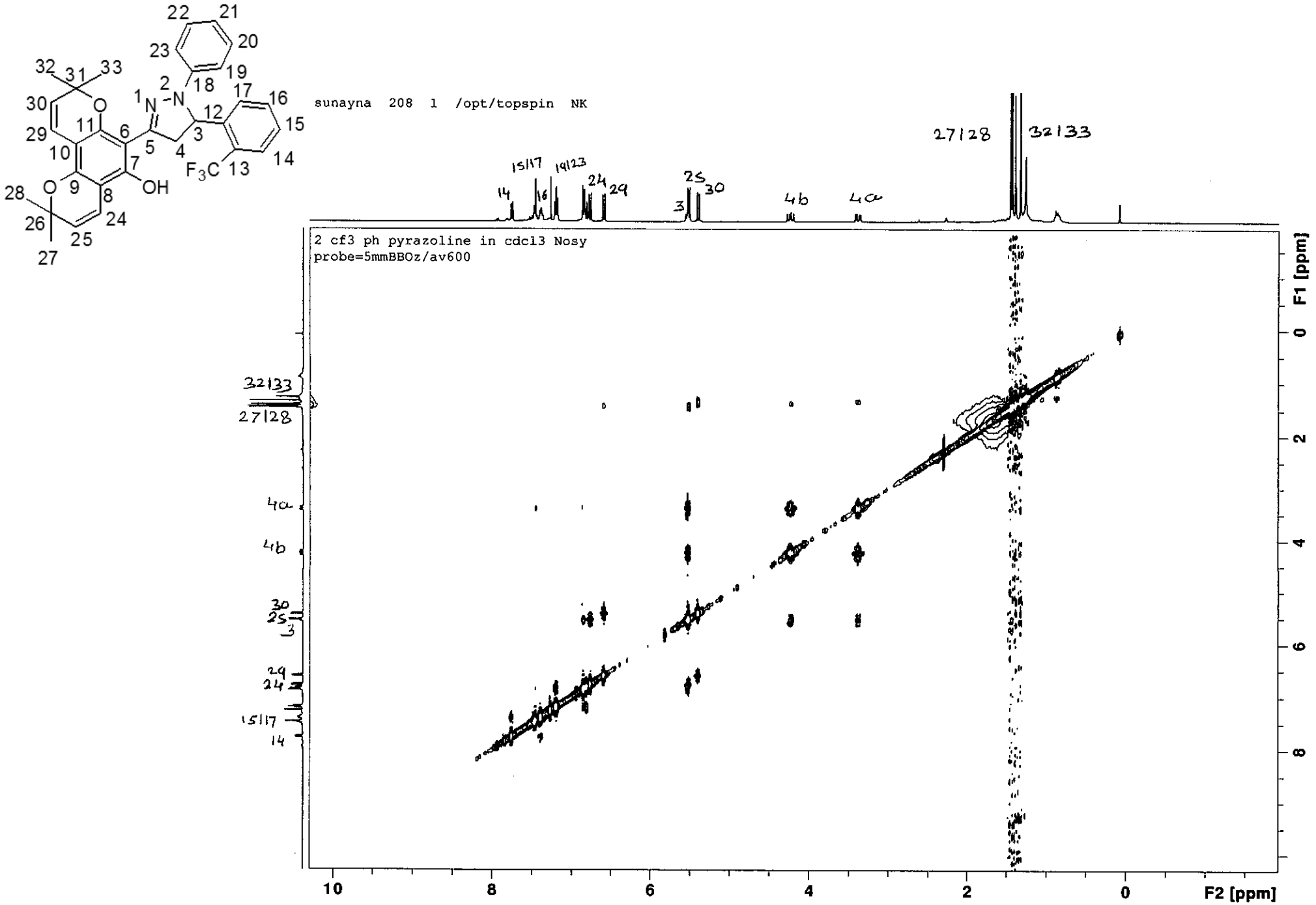
COSY NMR spectrum of 3-(2-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5i)



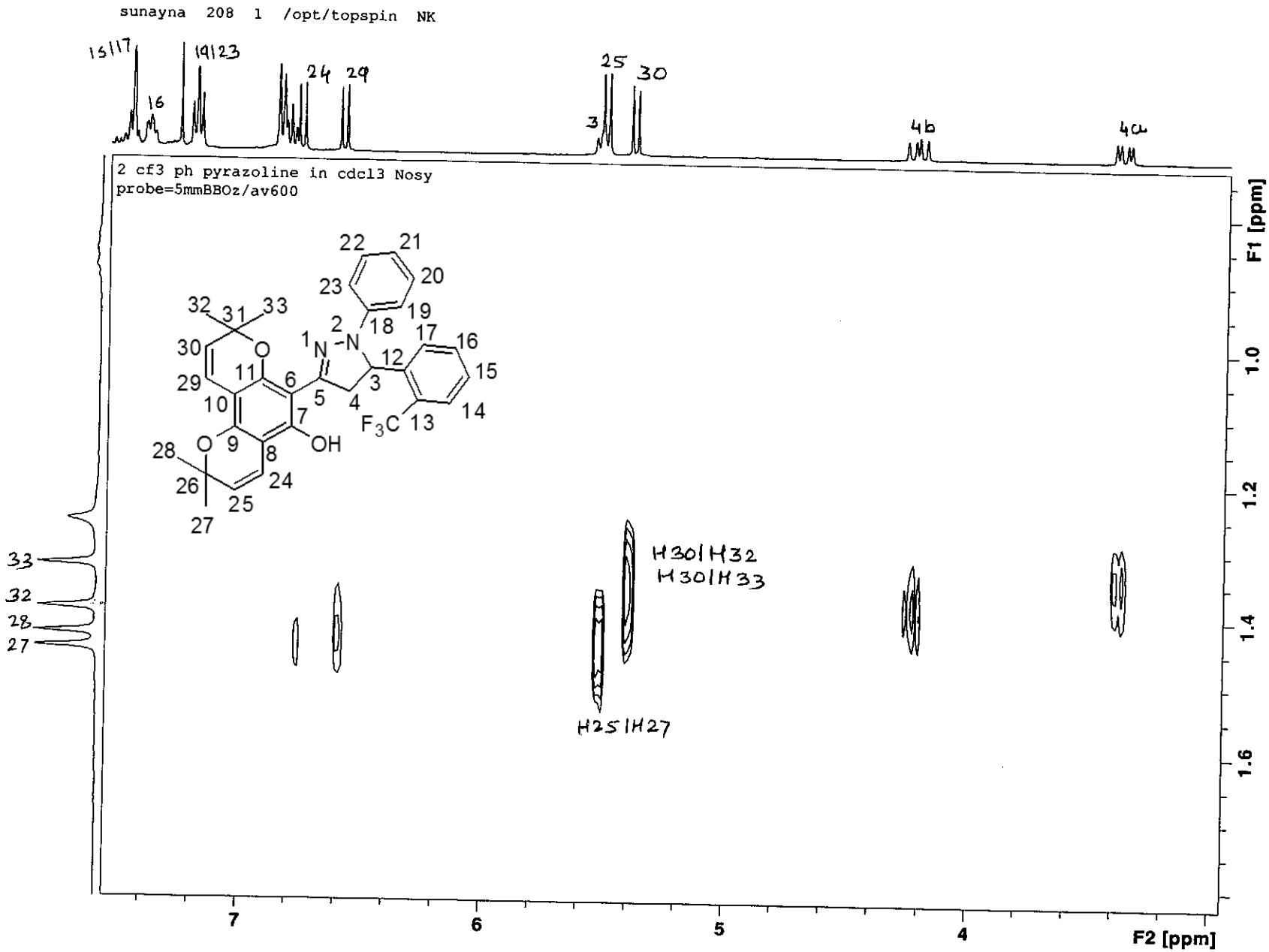
Expanded COSY NMR spectrum of 3-(2-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5i)



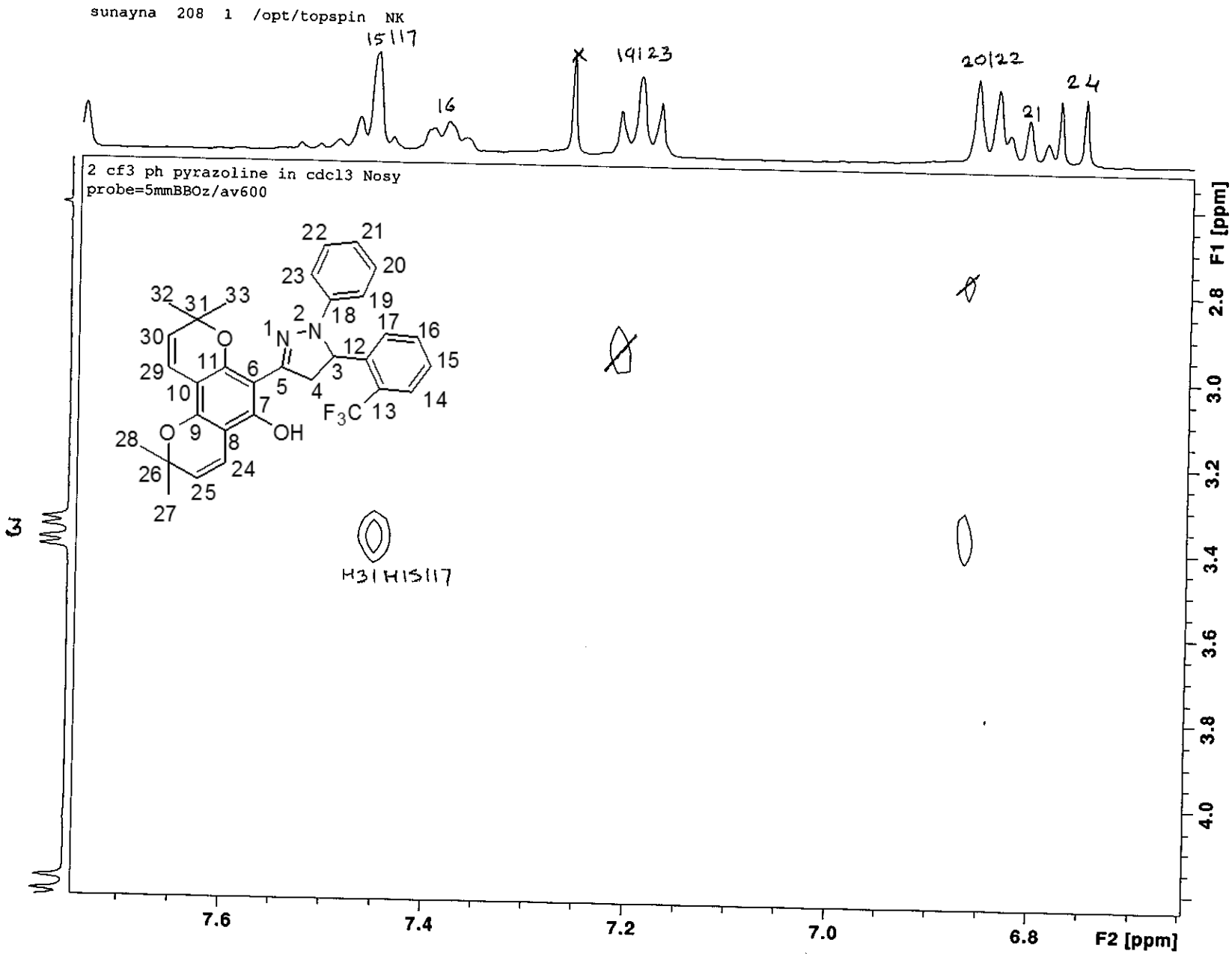
Expanded COSY NMR spectrum of 3-(2-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5i)



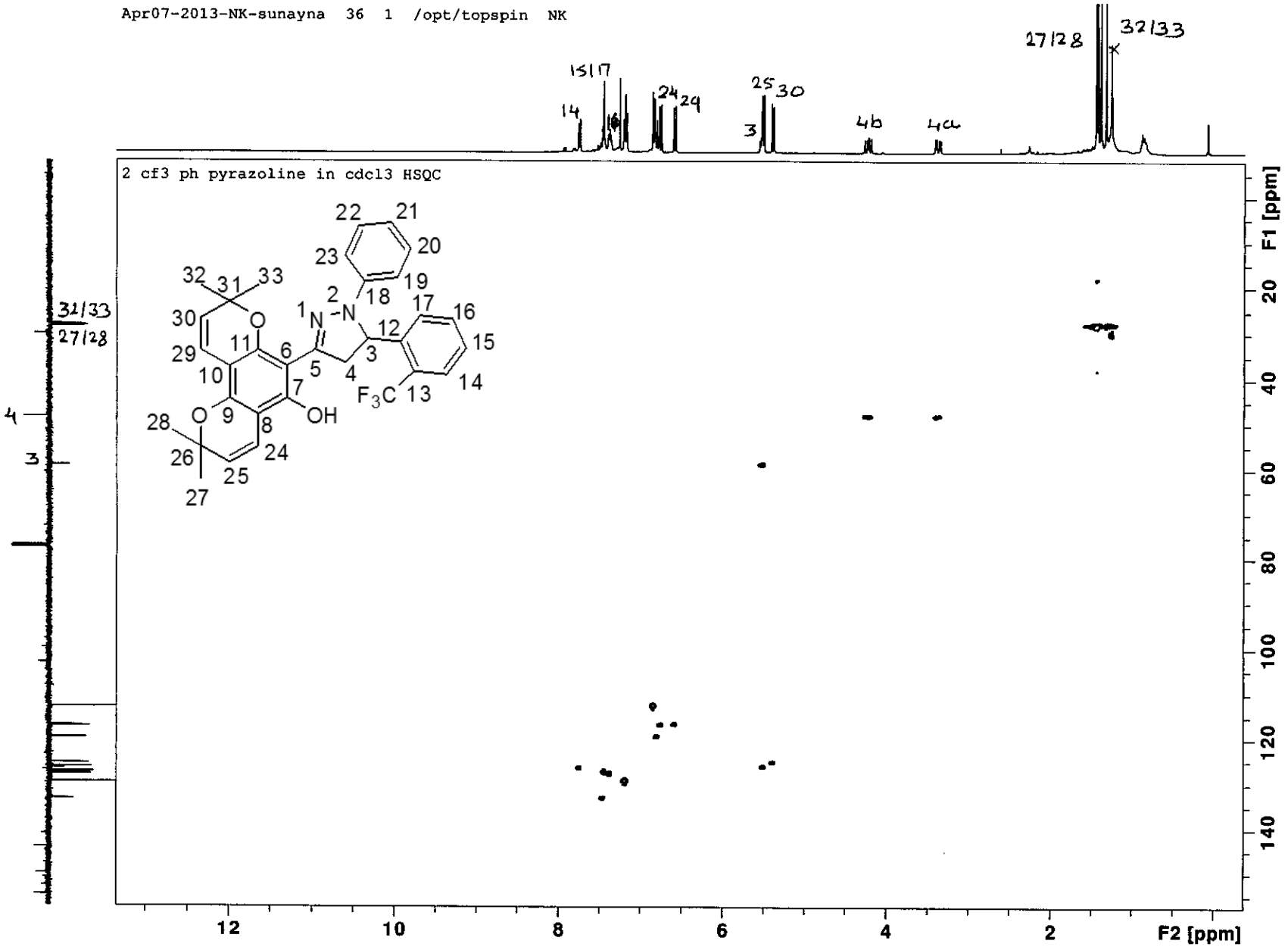
NOESY NMR spectrum of 3-(2-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5i)



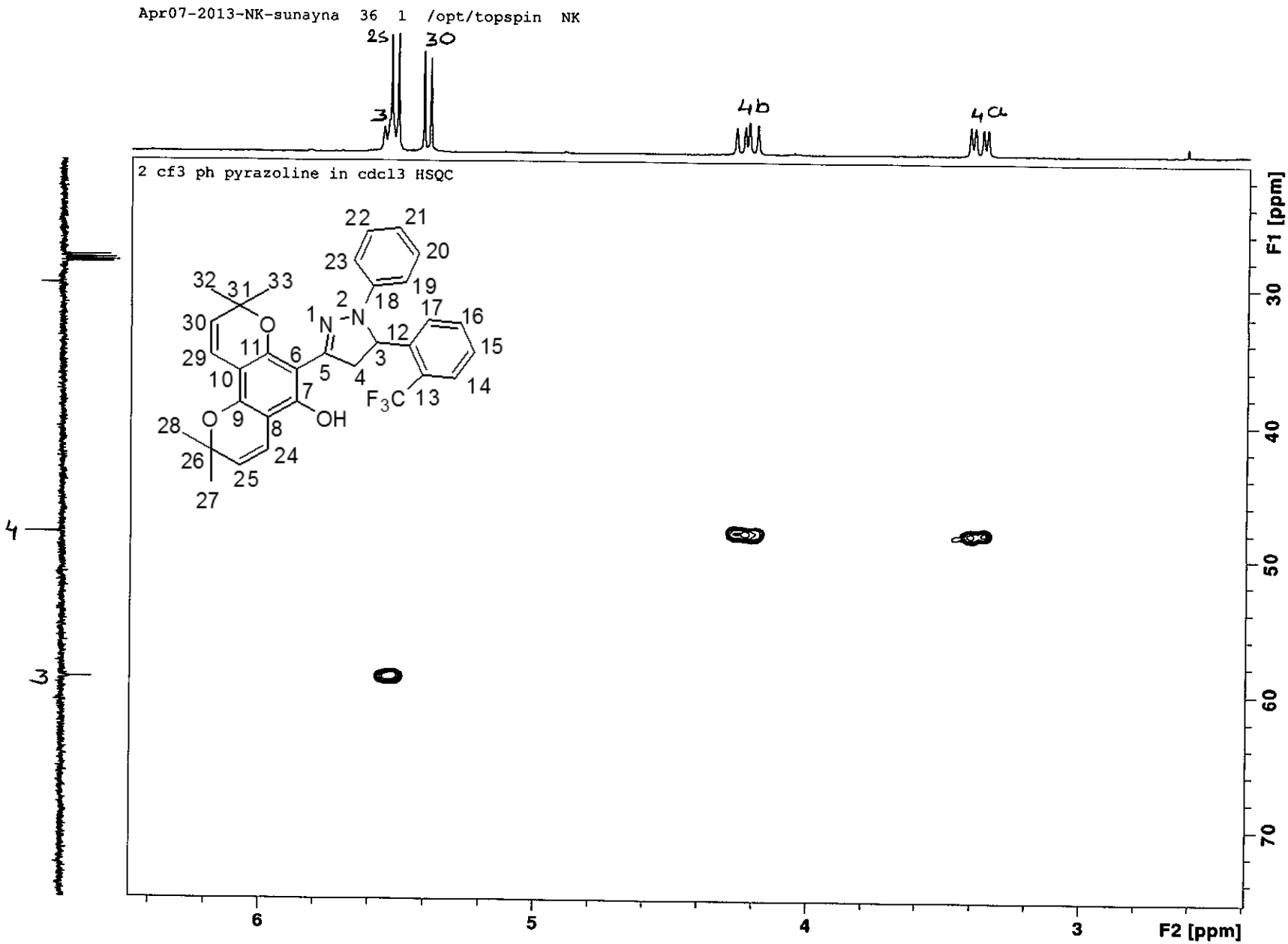
Expanded NOESY NMR spectrum of 3-(2-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5i)



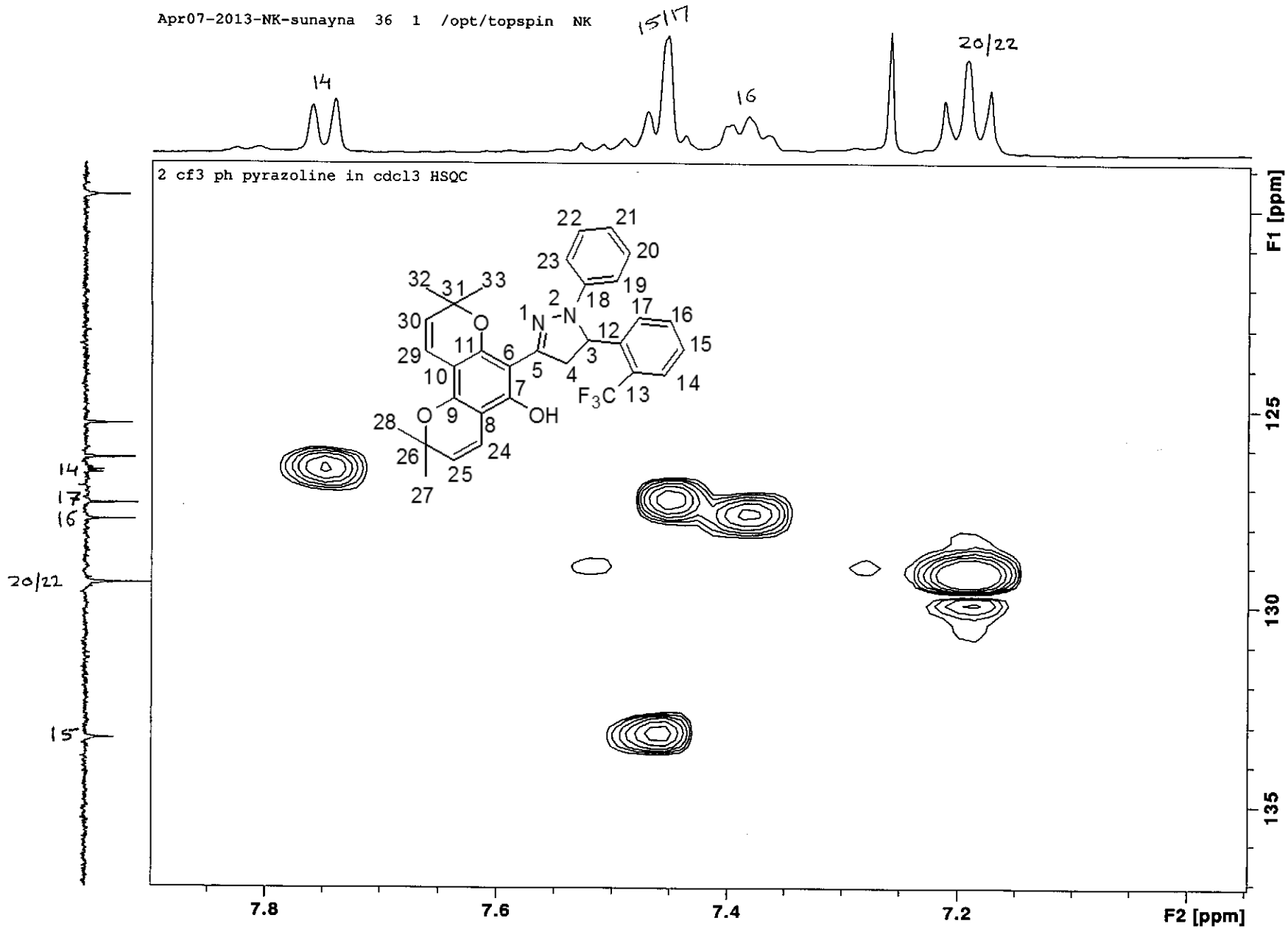
Expanded NOESY NMR spectrum of 3-(2-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5i)



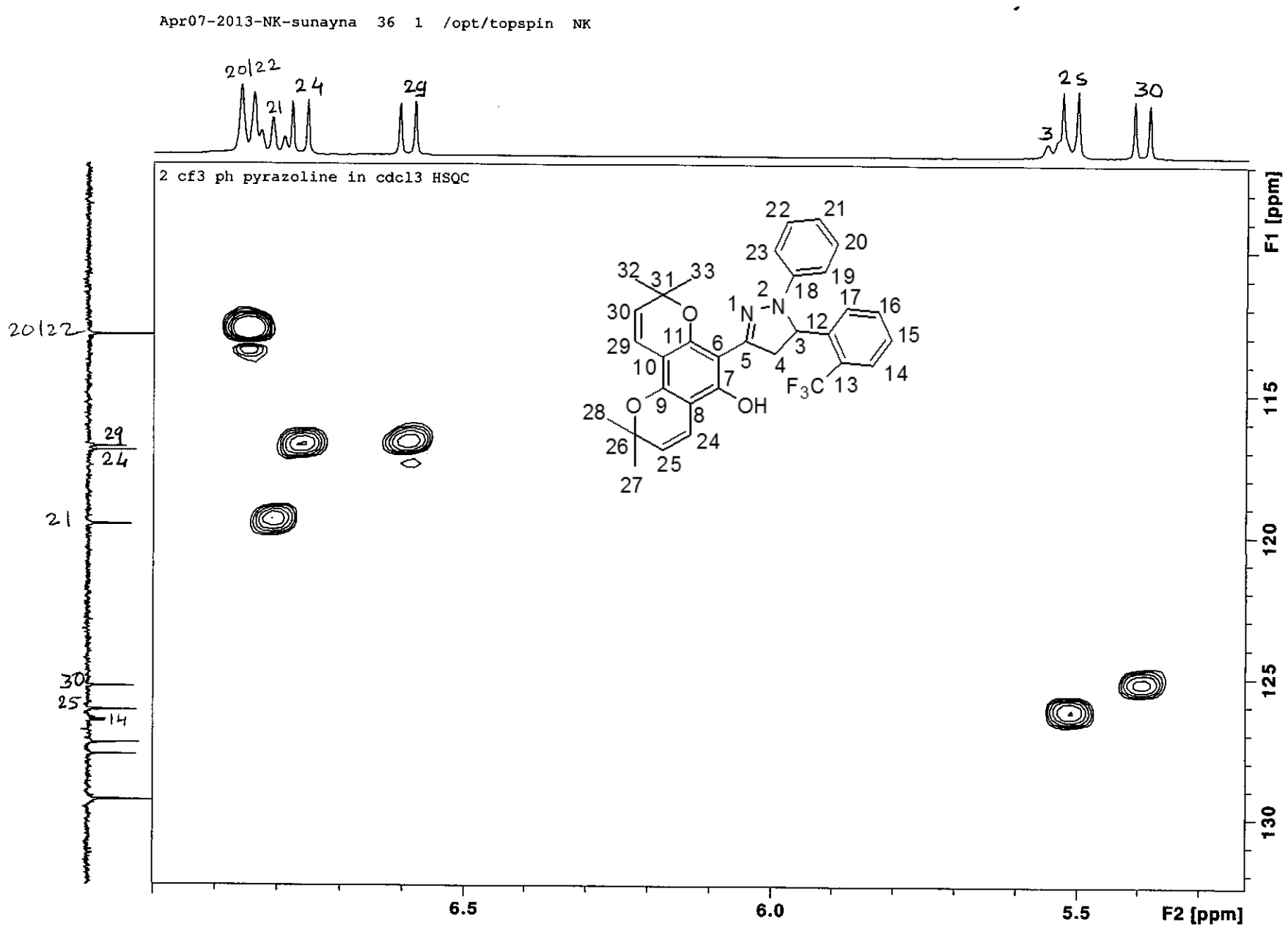
HSQC spectrum of 3-(2-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5i)



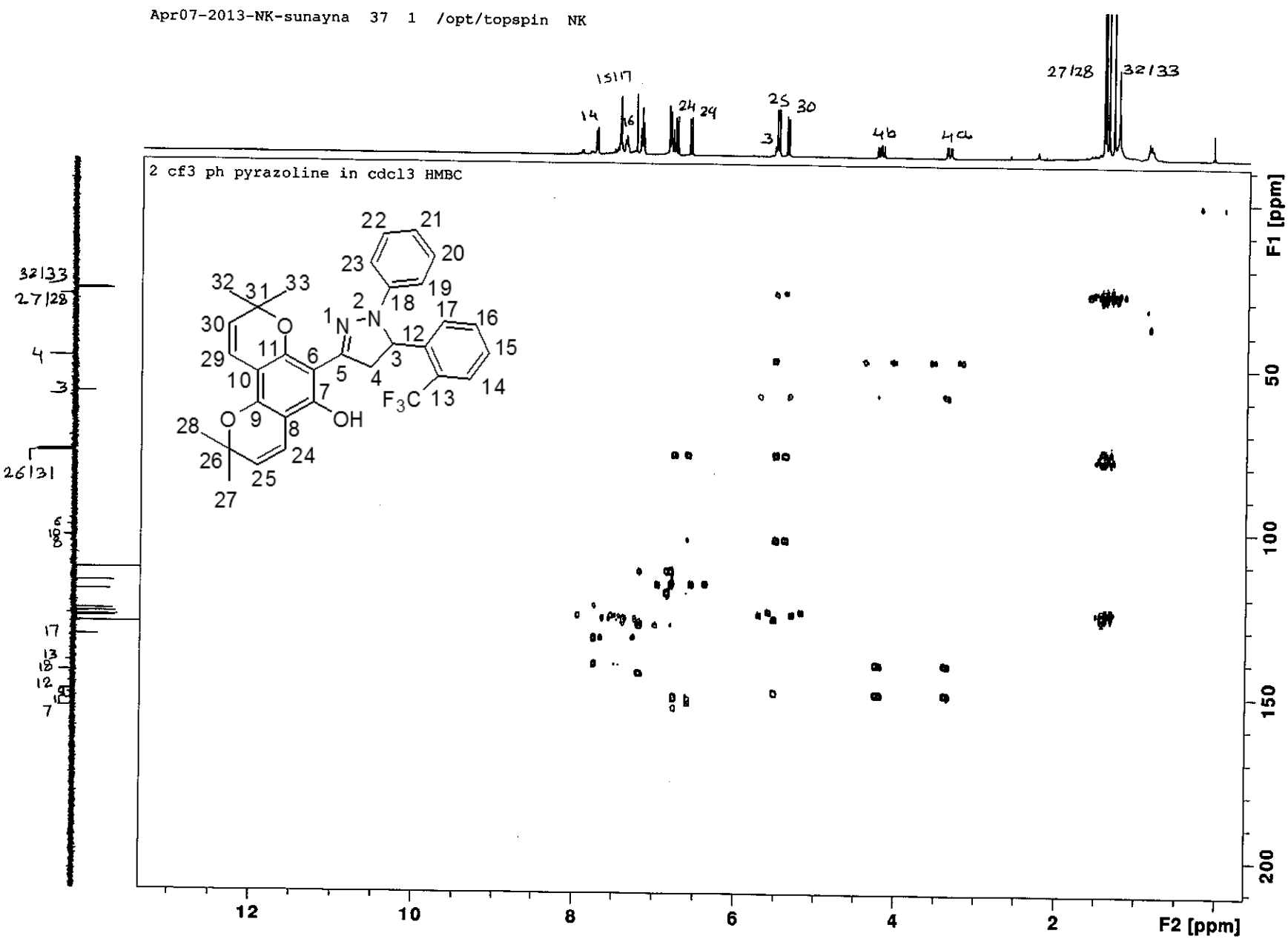
HSQC spectrum of 3-(2-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5i)



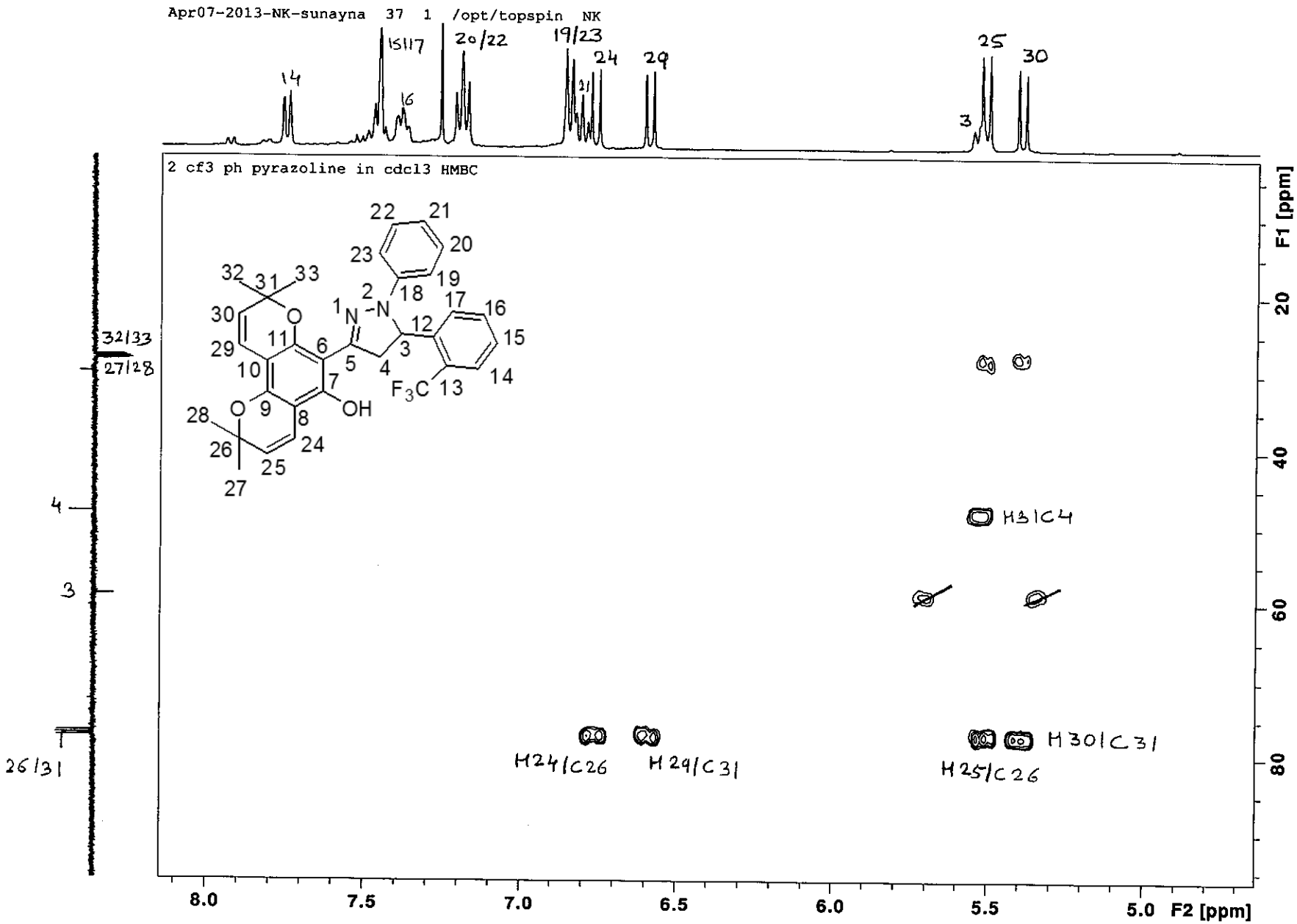
Expanded HSQC spectrum of 3-(2-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5i) 911



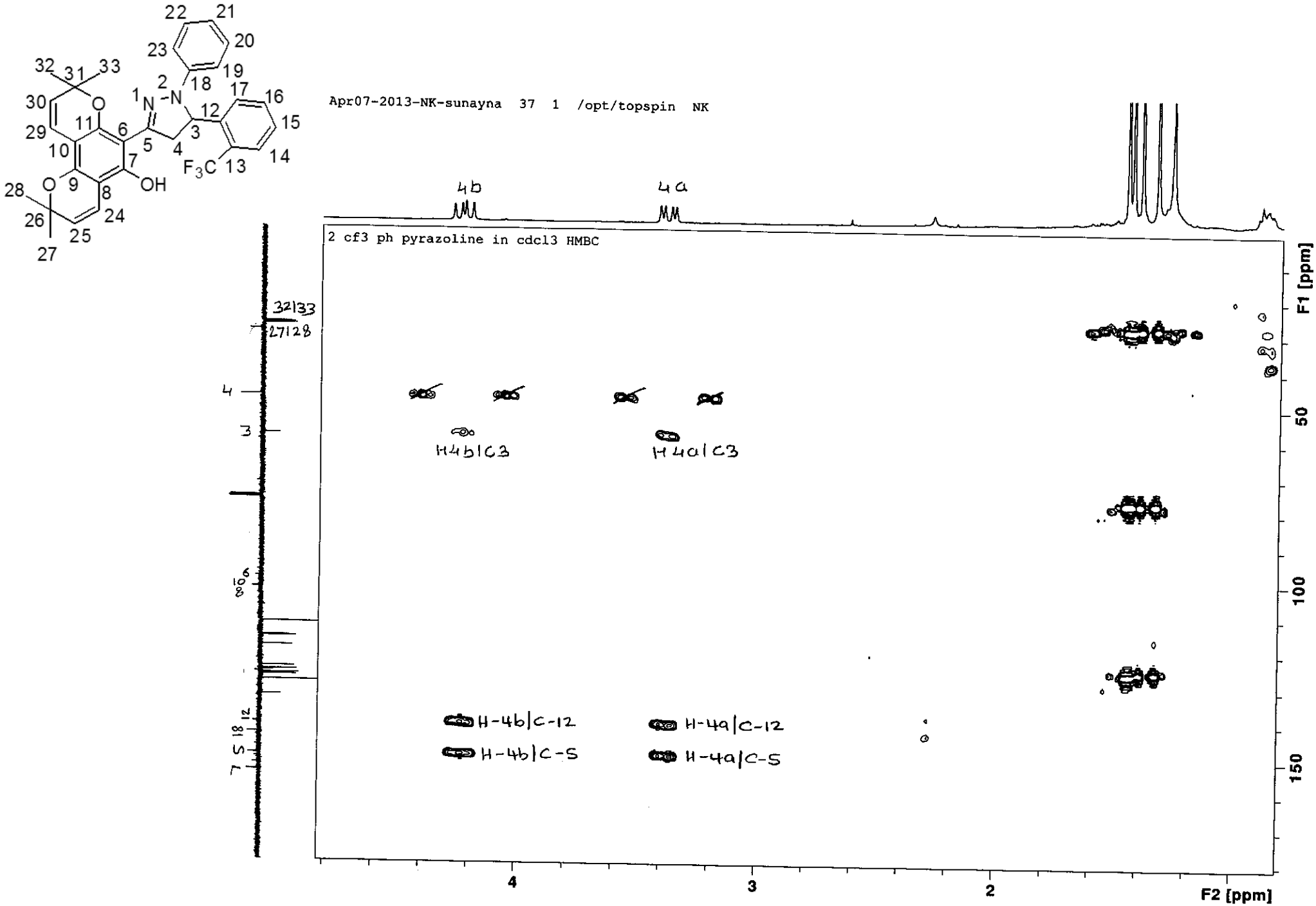
Expanded HSQC spectrum of 3-(2-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5i)



HMBC spectrum of 3-(2-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5i)



HMBC spectrum of 3-(2-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5i)



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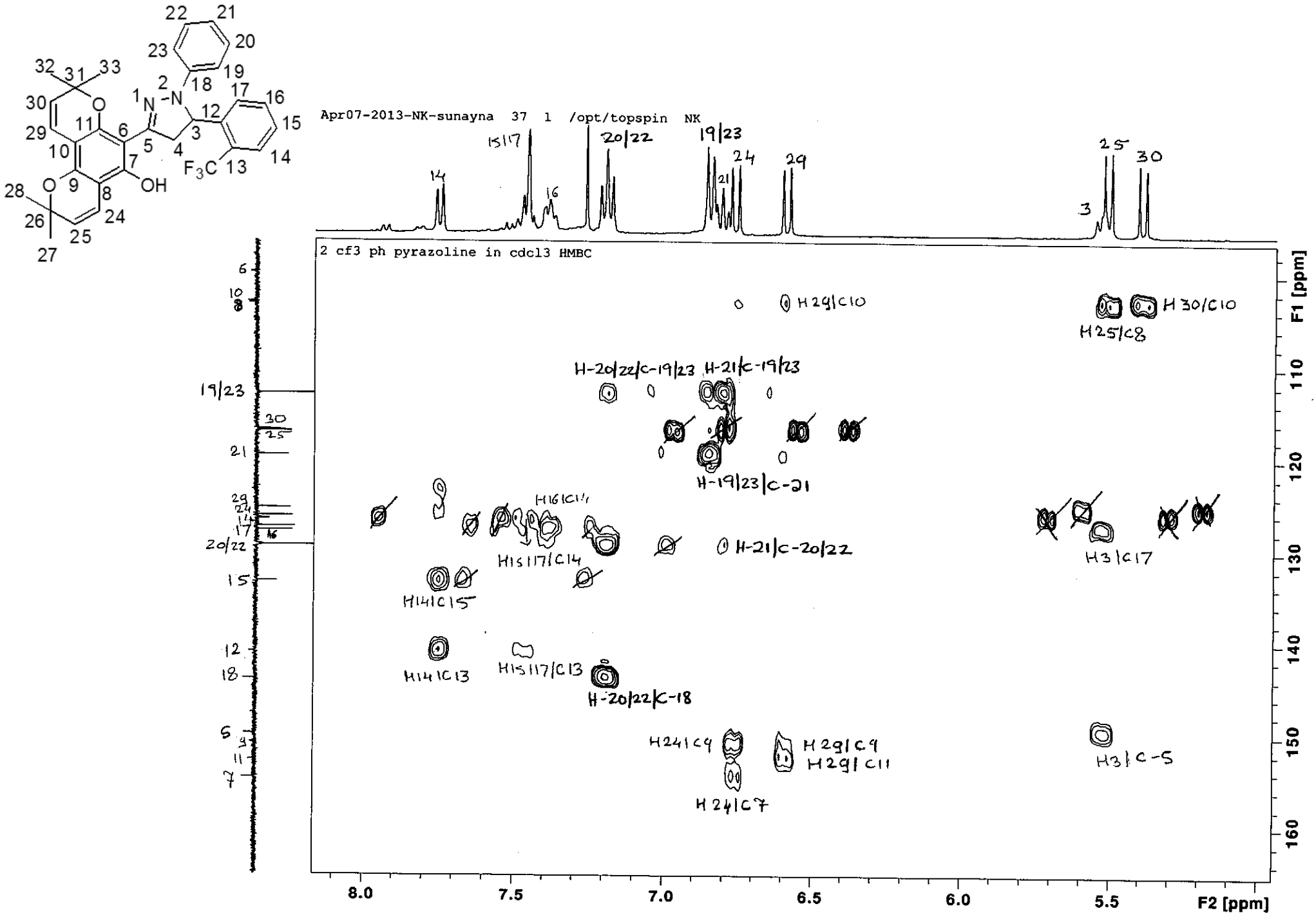
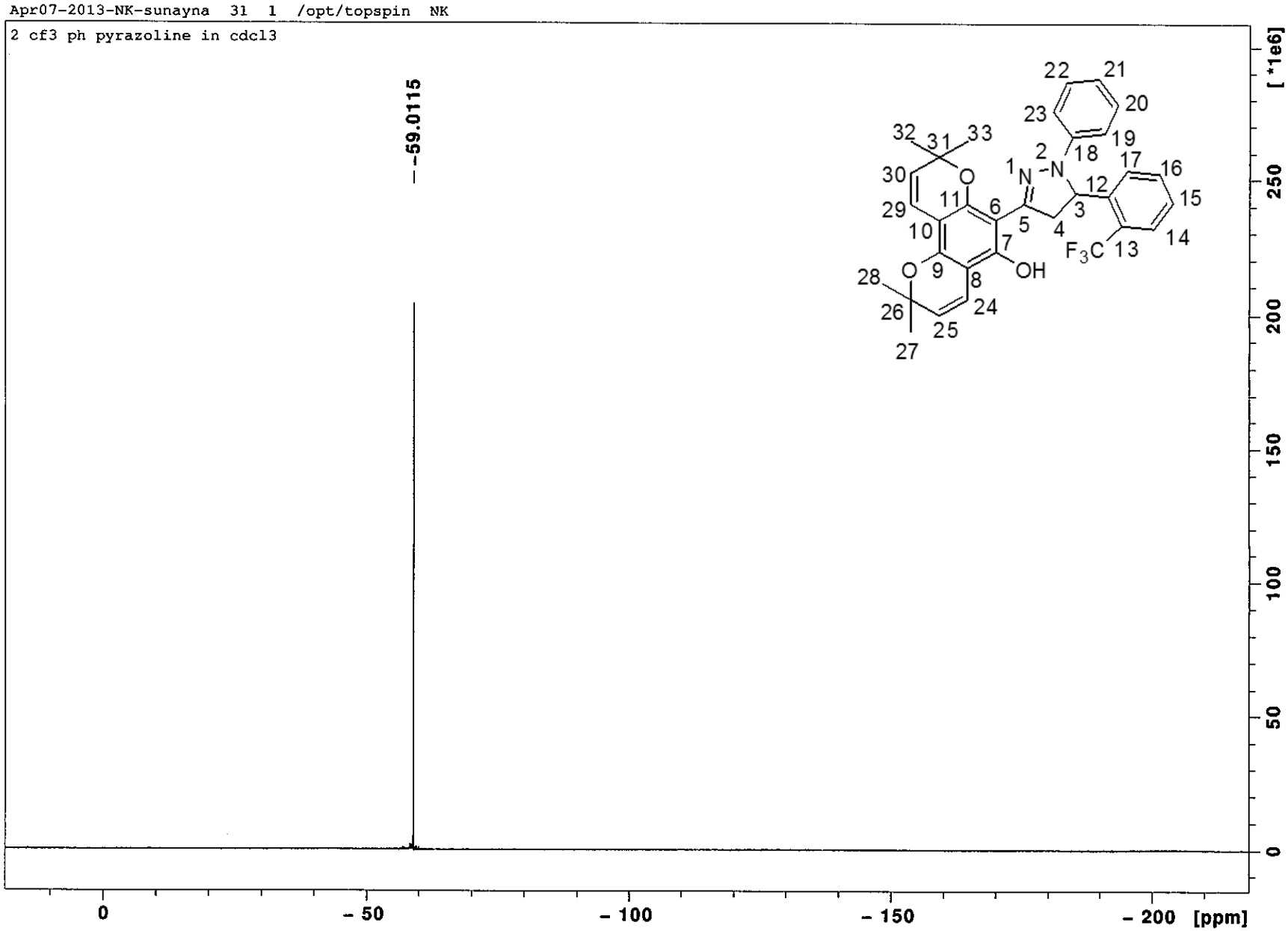
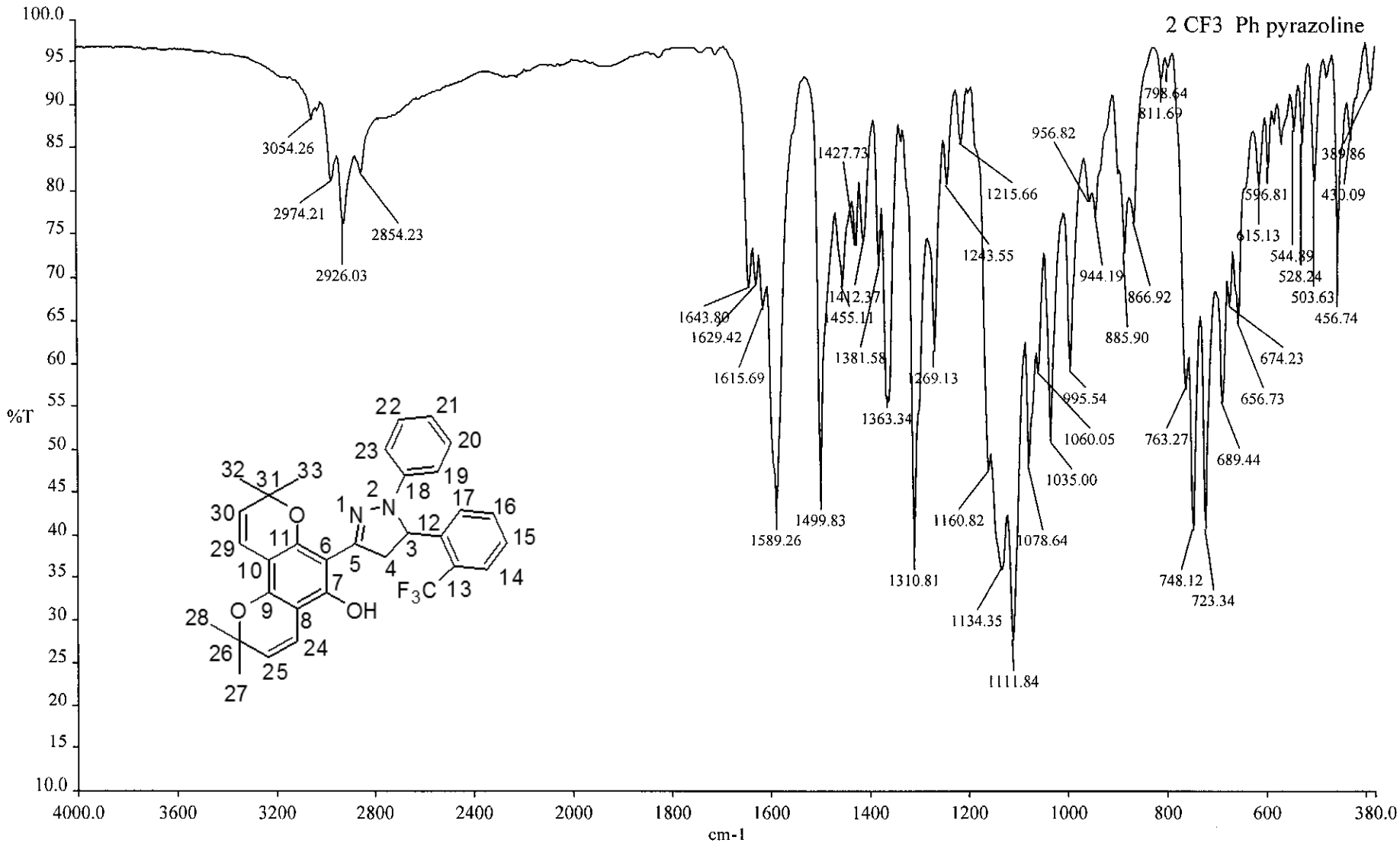


Expanded HMBC spectrum of 3-(2-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5i)



19F NMR spectrum of 3-(2-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5i)



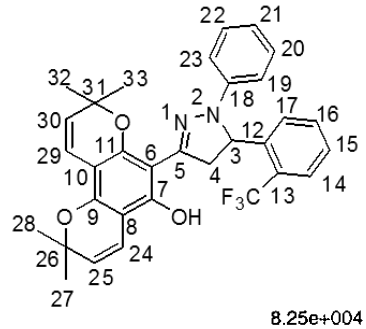
Infrared spectrum of 3-(2-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5i)

2 CF3 Ph

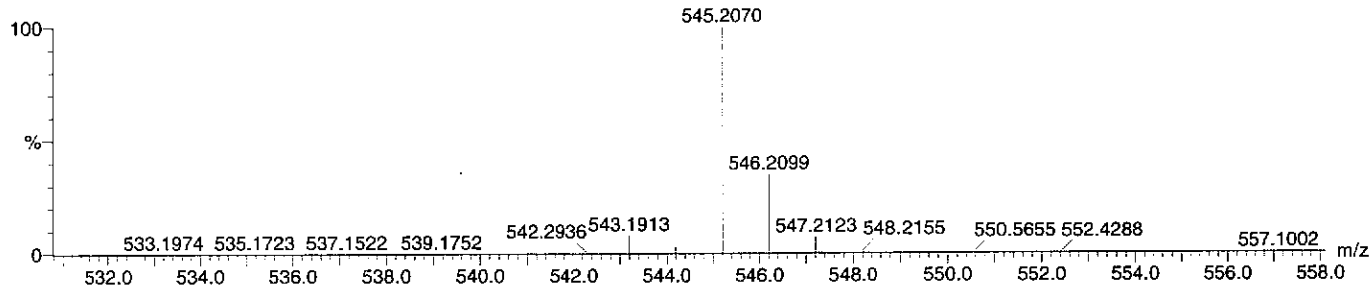
Elemental Composition Report **Page 1**

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

Monoisotopic Mass, Even Electron Ions
19 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-35 H: 20-30 N: 0-4 O: 0-4 F: 3-3
22 2 (0.017) Cm (1:31)
TOF MS ES-

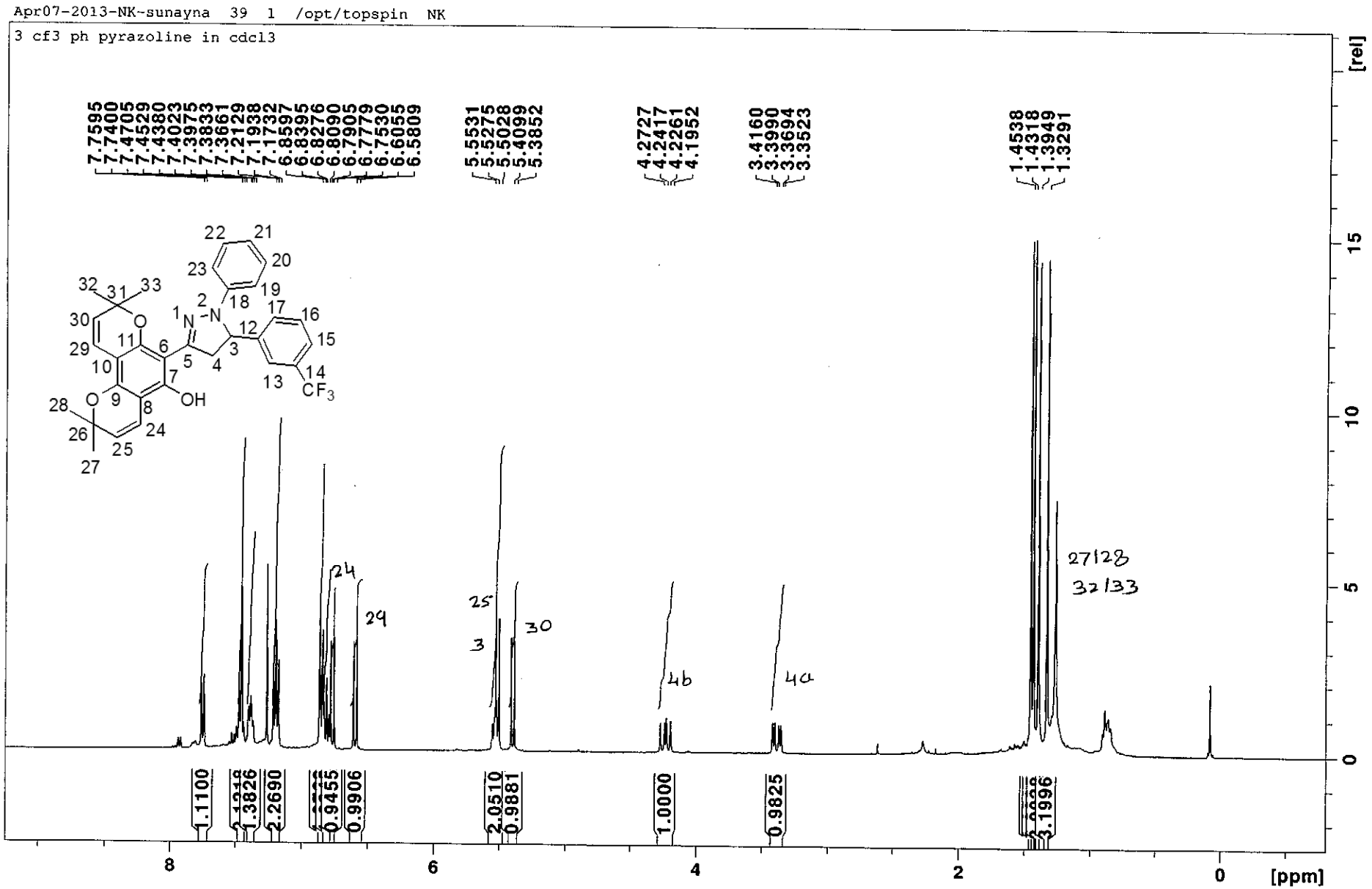


8.25e+004

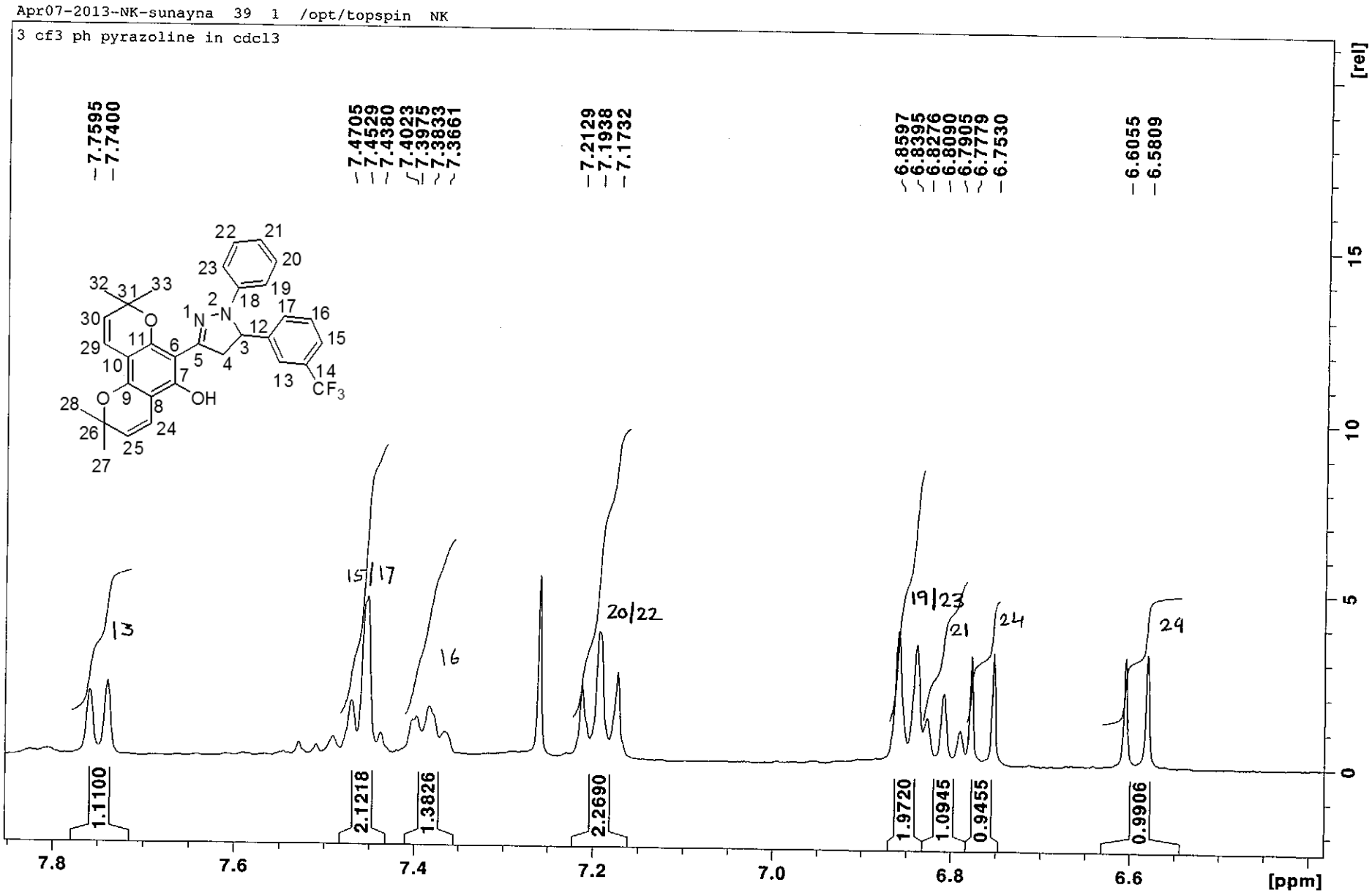


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
545.2070	545.2052	1.8	3.3	18.5	430.5	0.0	C32 H28 N2 O3 F3

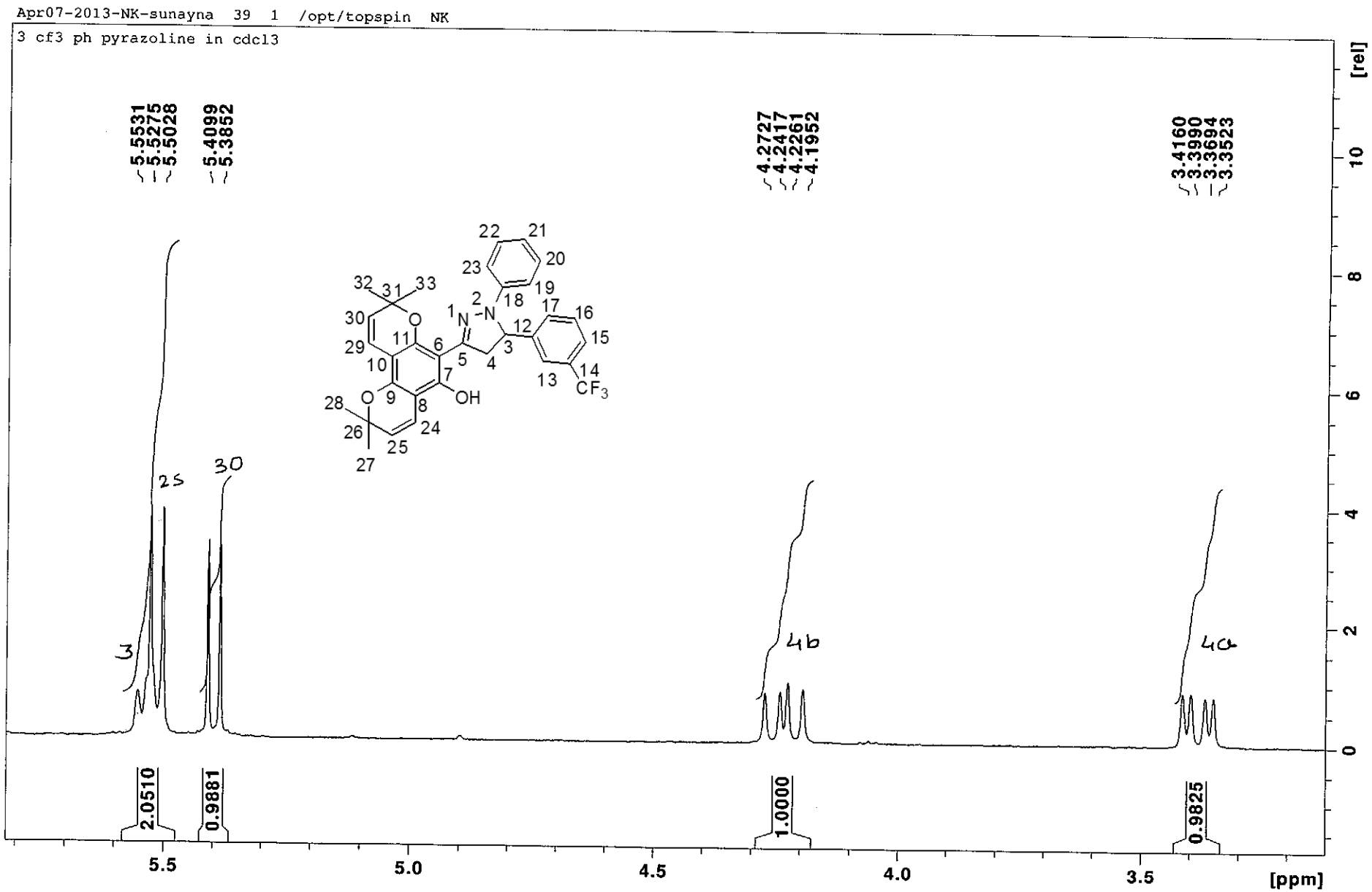
HRMS spectrum of 3-(2-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5i)



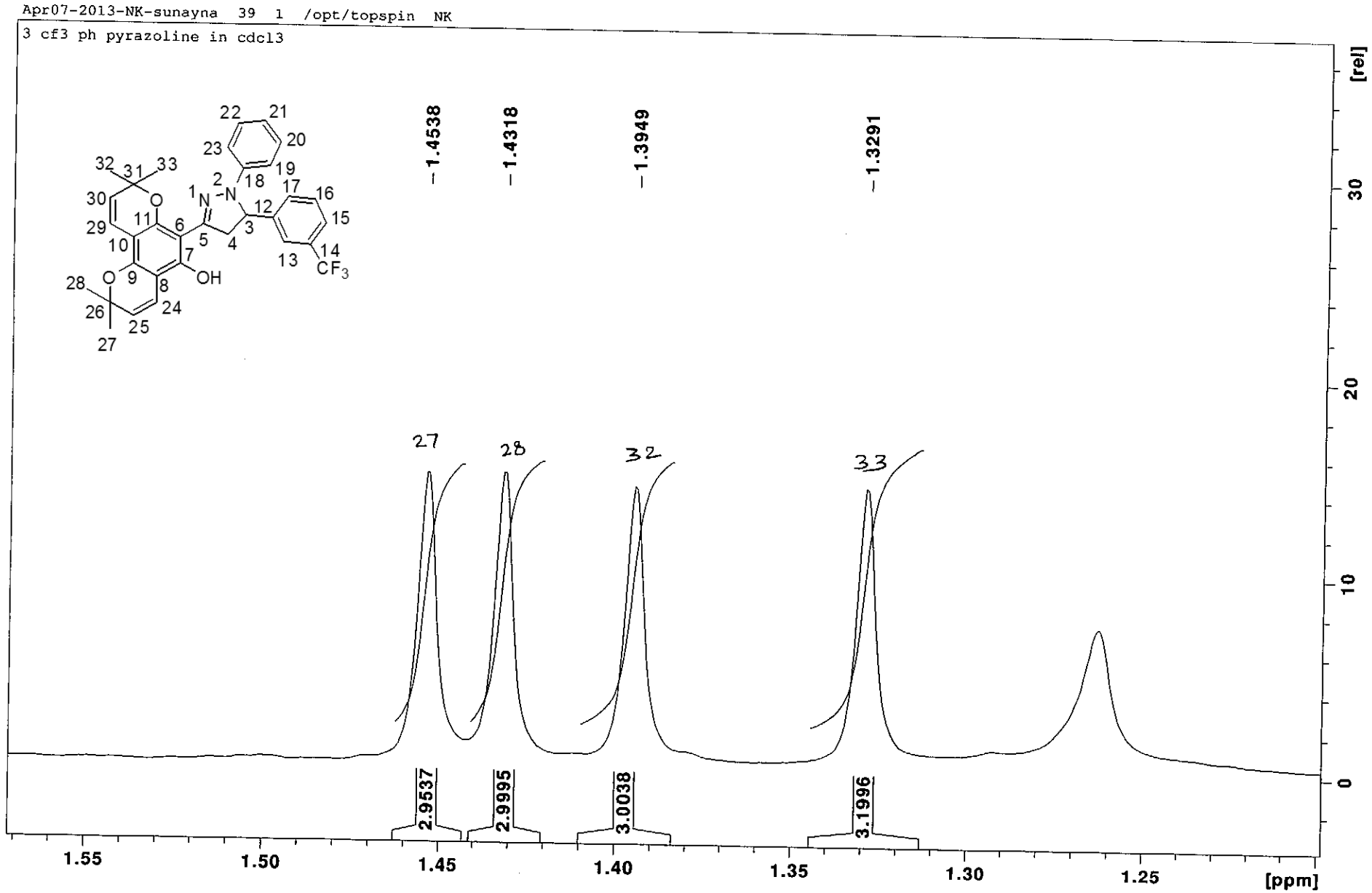
1H NMR spectrum of 3-(3-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5j)



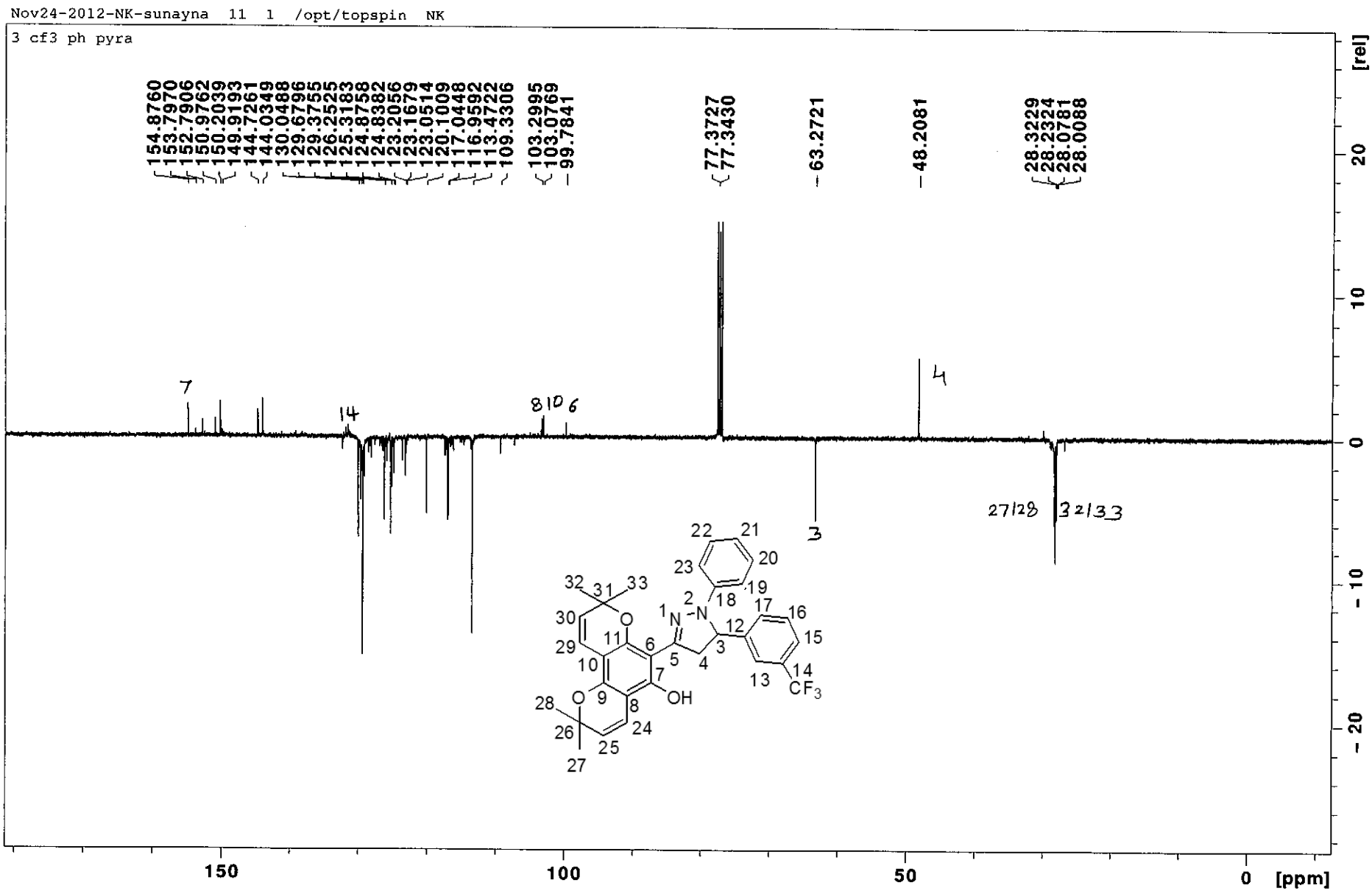
Expanded 1H NMR spectrum of 3-(3-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5j)



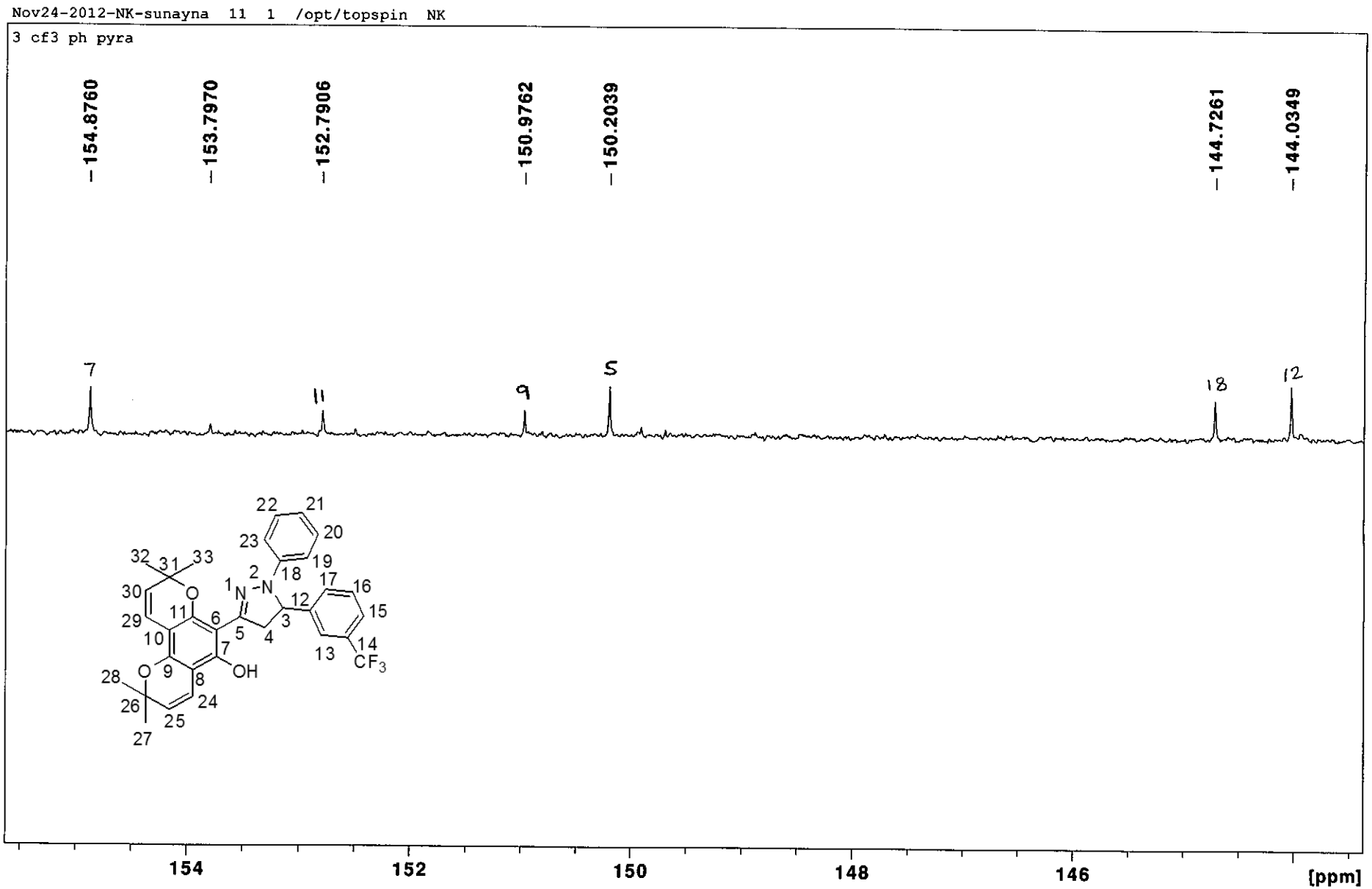
Expanded 1H NMR spectrum of 3-(3-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5j)



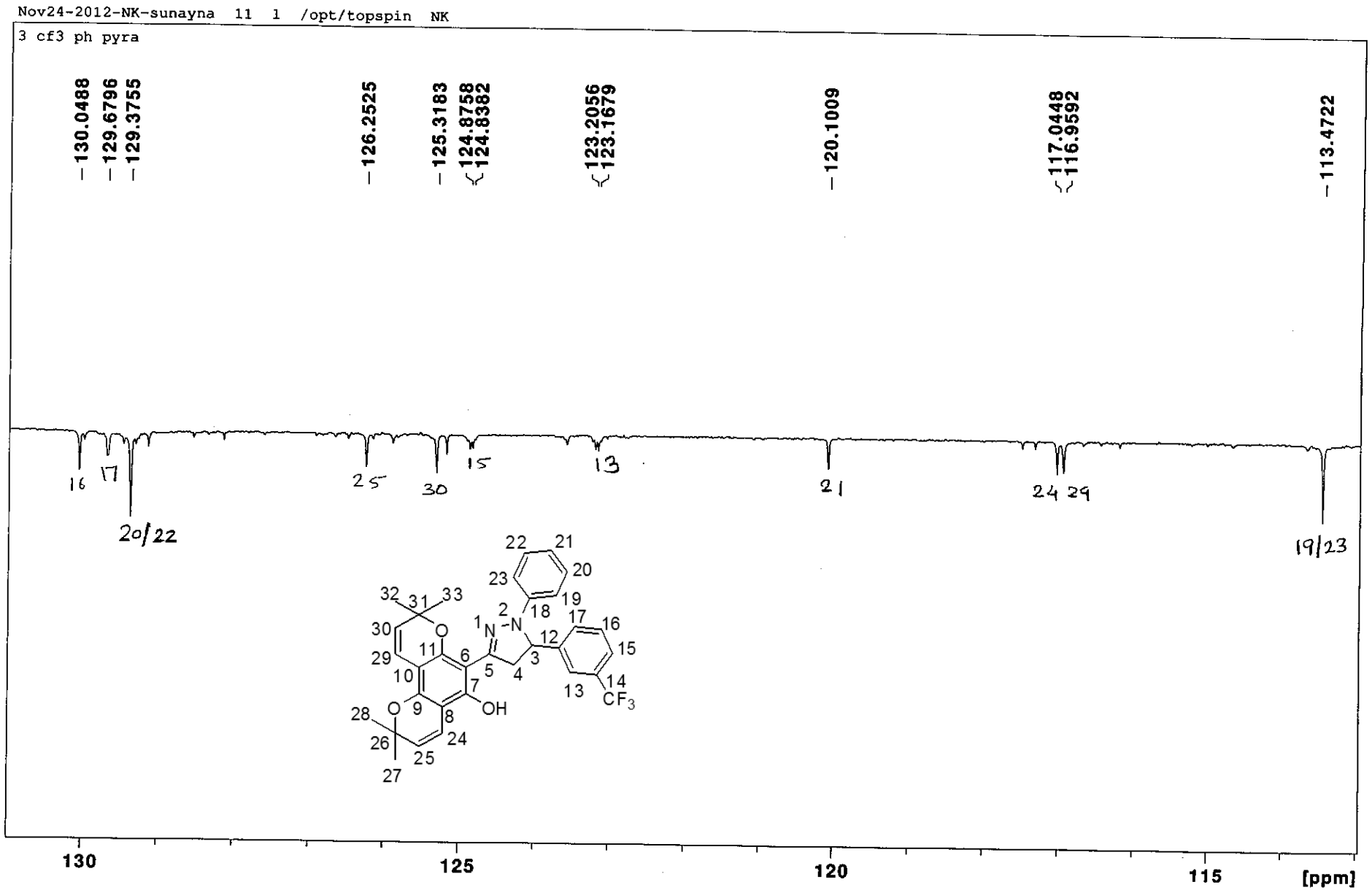
Expanded 1H NMR spectrum of 3-(3-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5j)



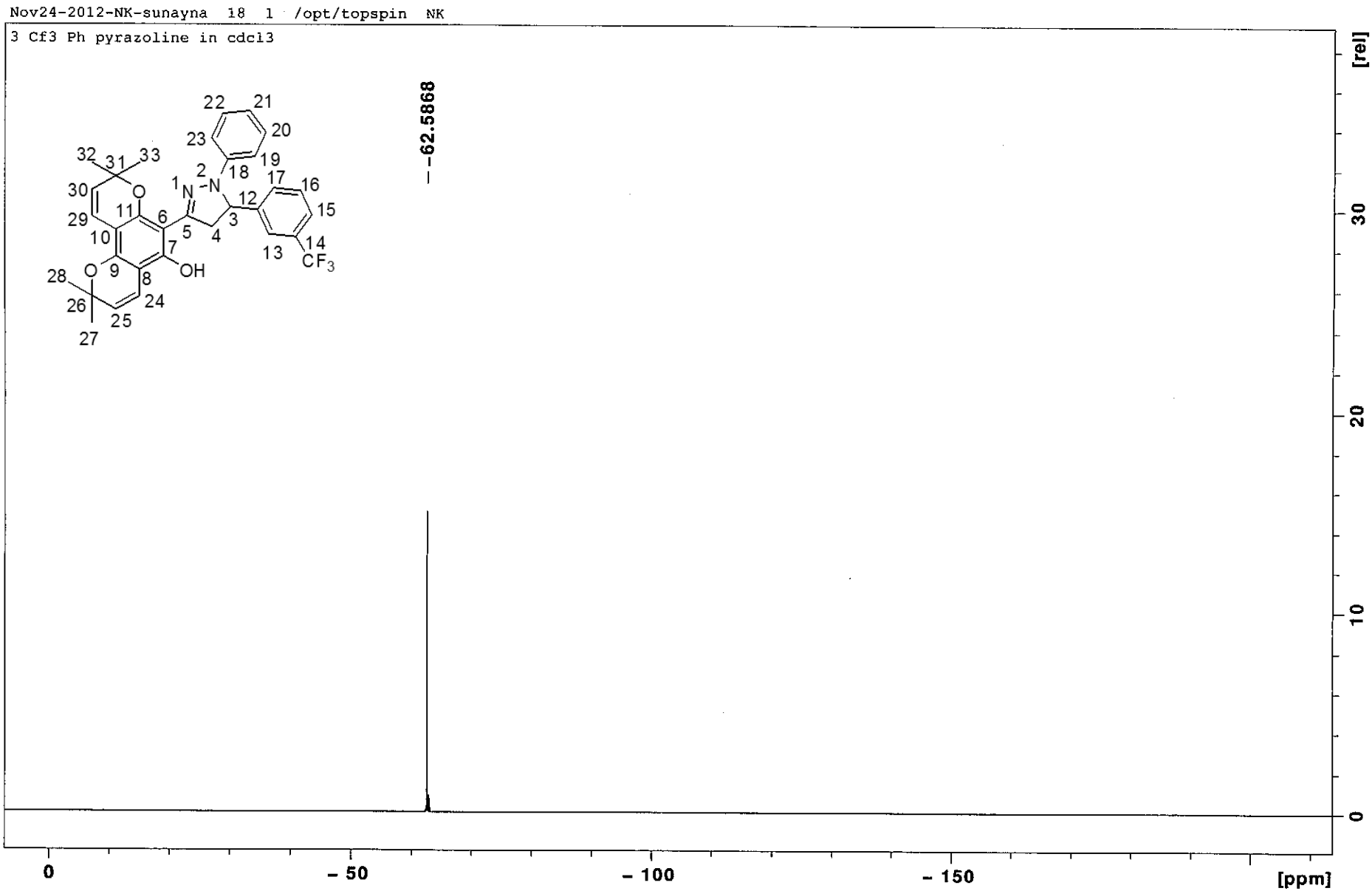
13C NMR spectrum of 3-(3-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5j)



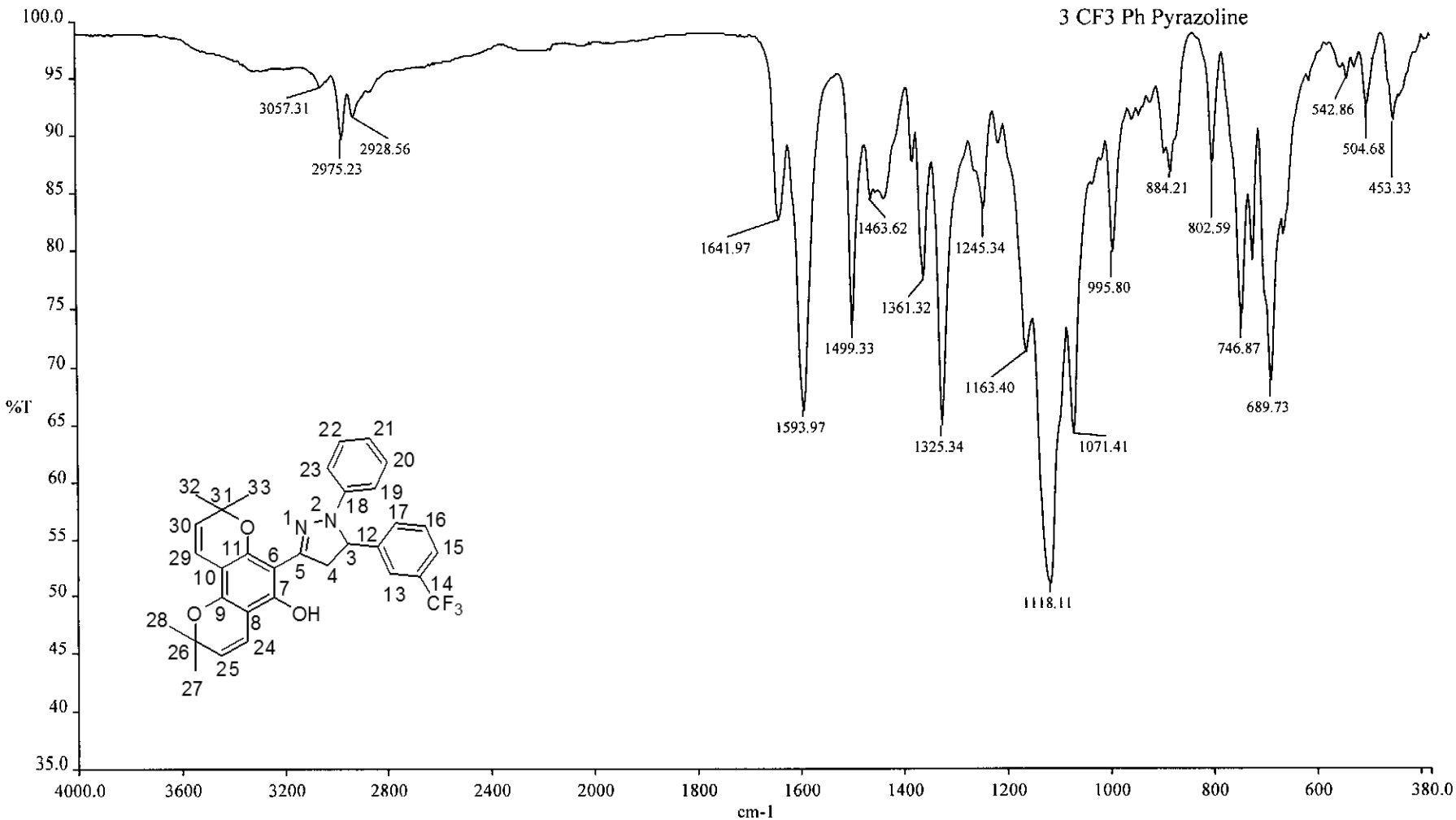
Expanded 13C NMR spectrum of 3-(3-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5j)



Expanded 13C NMR spectrum of 3-(3-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5j)



19F NMR spectrum of 3-(3-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5j)



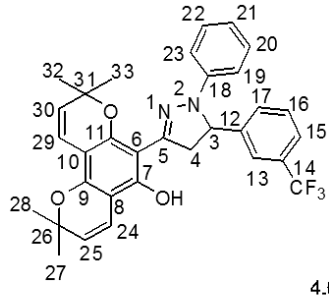
Infrared spectrum of 3-(3-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5j)

3CF3pA 18

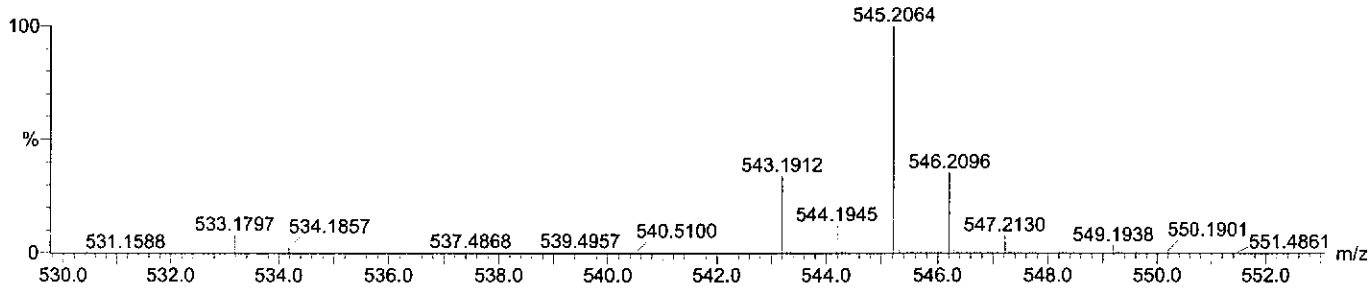
Elemental Composition Report **Page 1**

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

Monoisotopic Mass, Even Electron Ions
19 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-35 H: 20-30 N: 0-4 O: 0-4 F: 3-3
18 2 (0.017) Cm (1:31)
TOF MS ES-

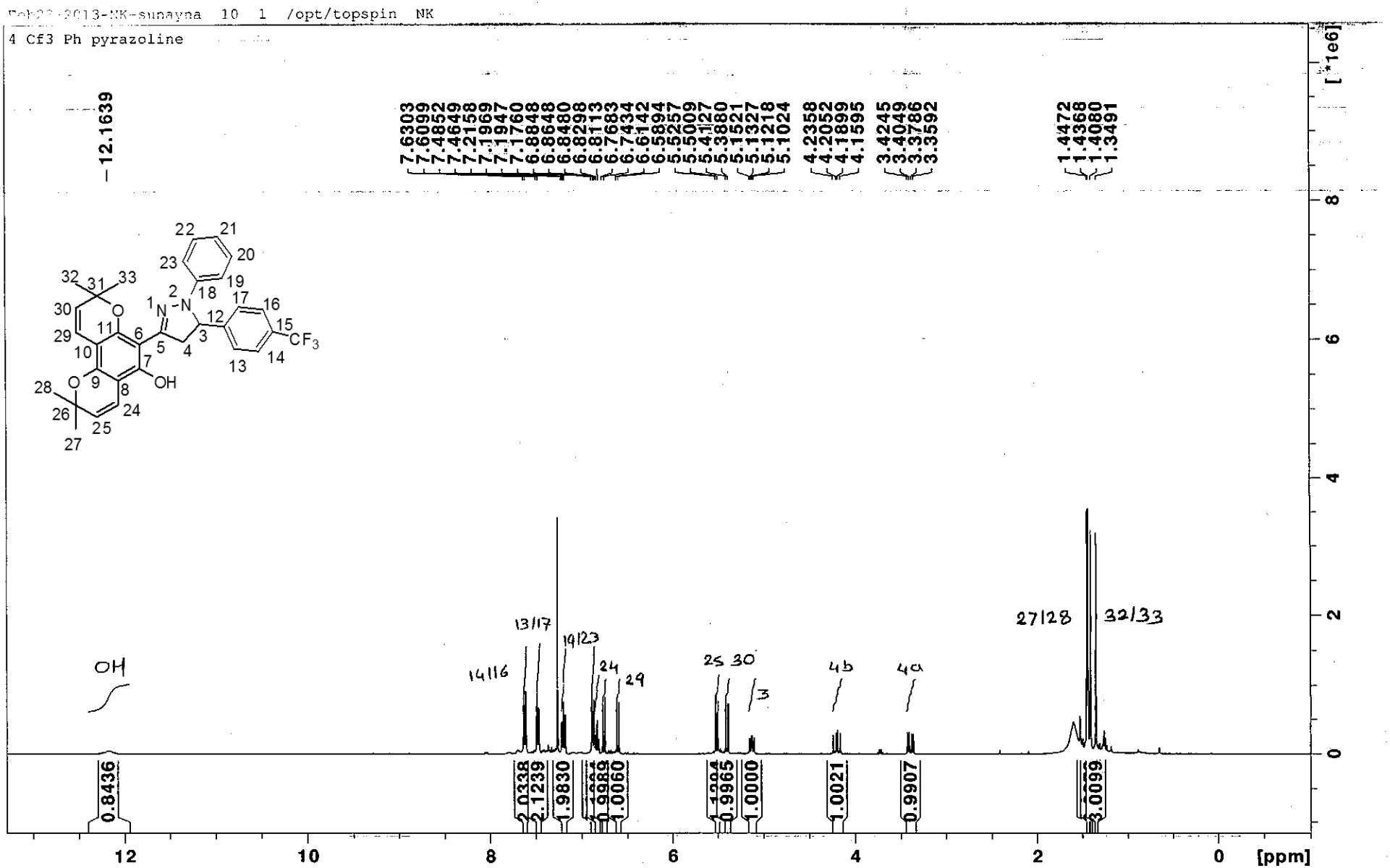


4.09e+004

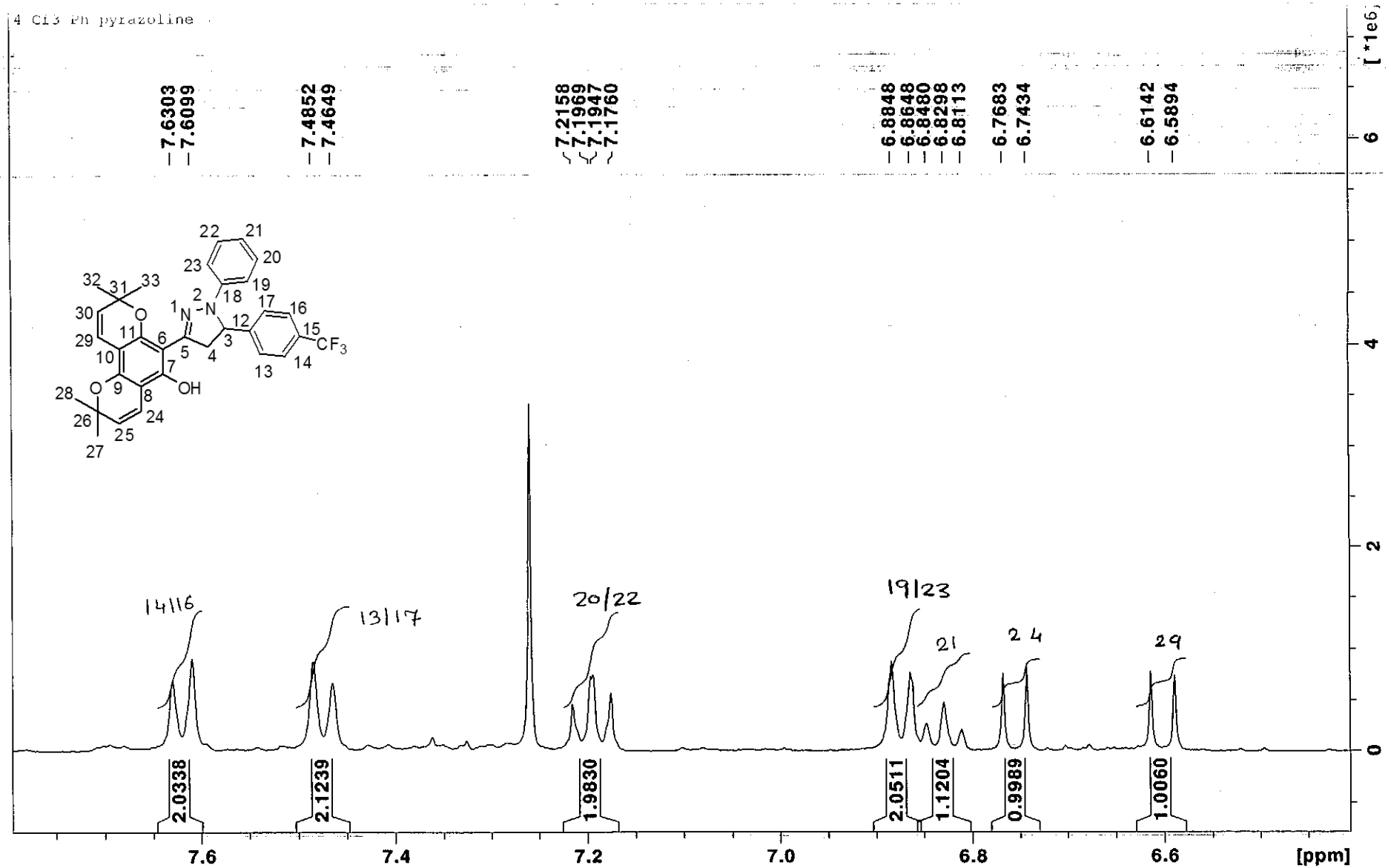


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
545.2064	545.2052	1.2	2.2	18.5	444.2	0.0	C32 H28 N2 O3 F3

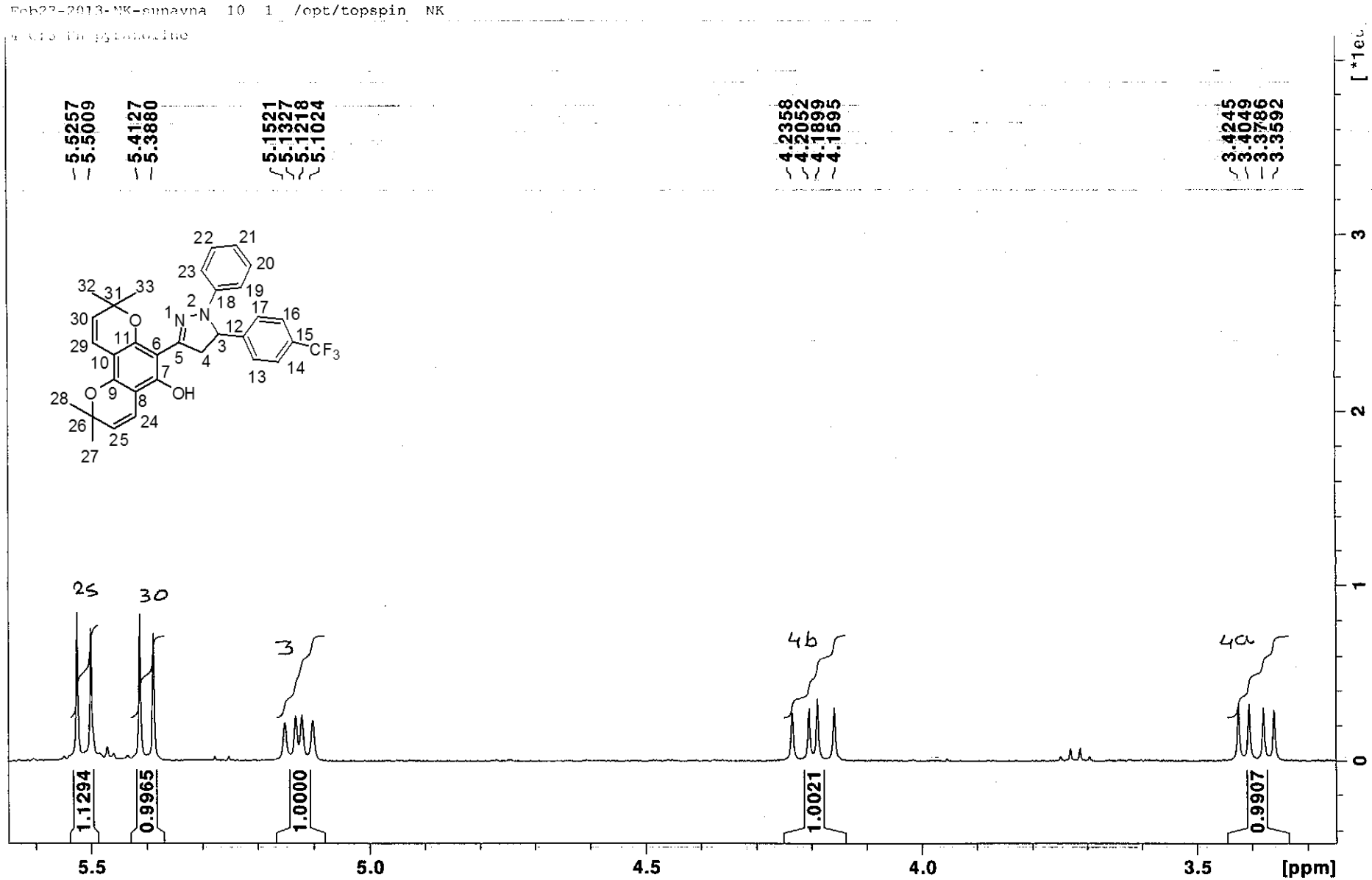
HRMS spectrum of 3-(3-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5j)



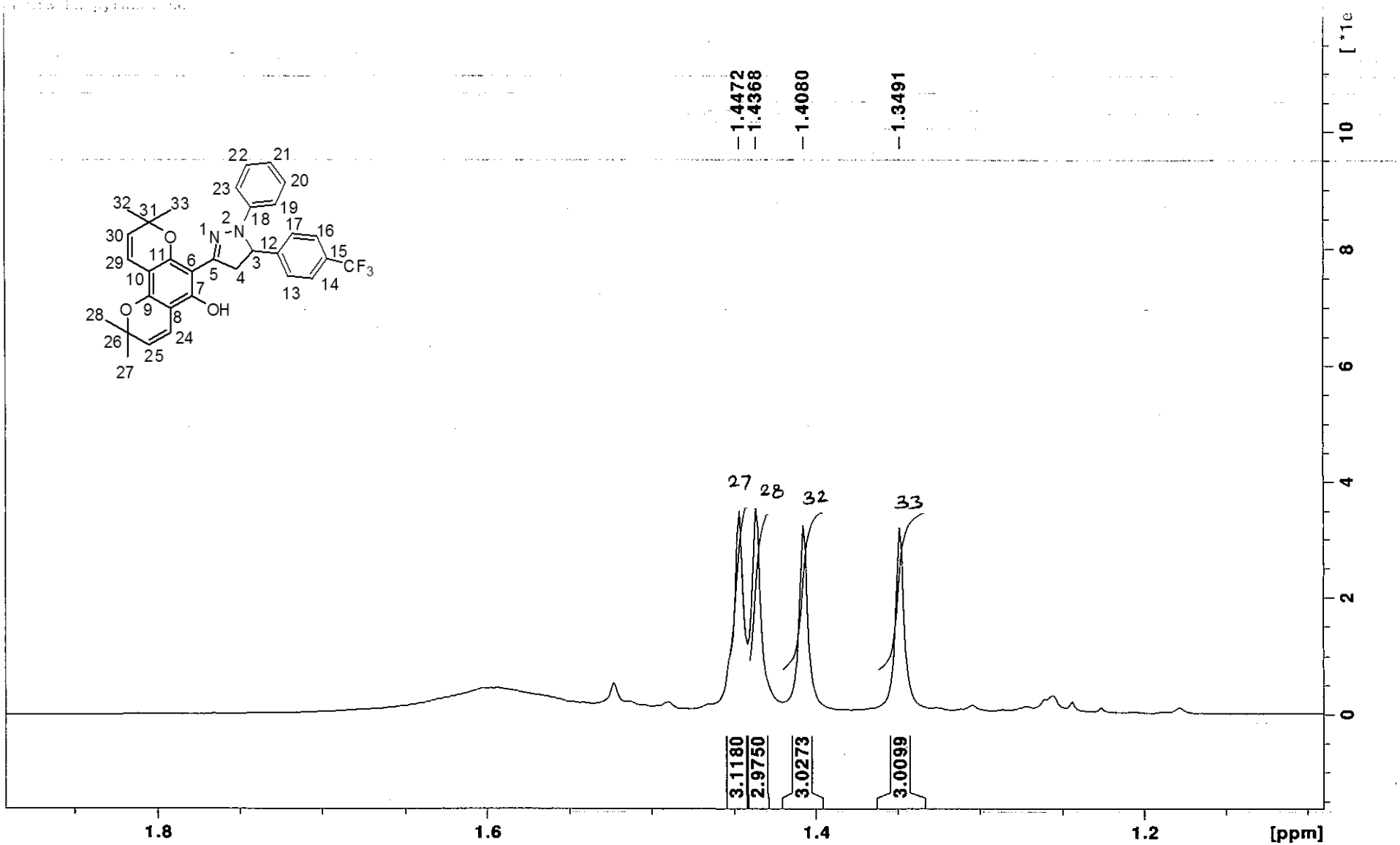
1H NMR spectrum of 3-(4-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5k)



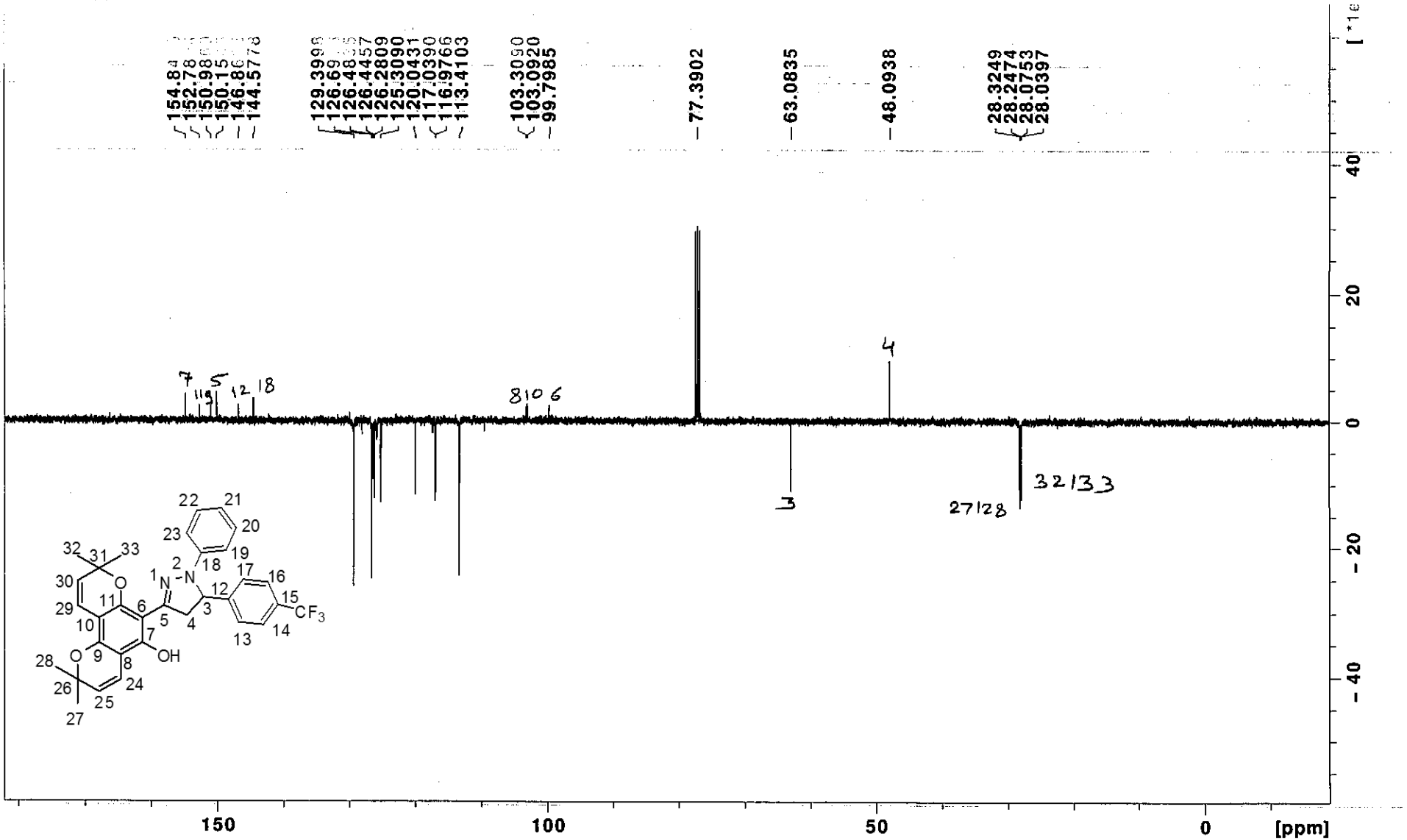
Expanded 1H NMR spectrum of 3-(4-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5k)



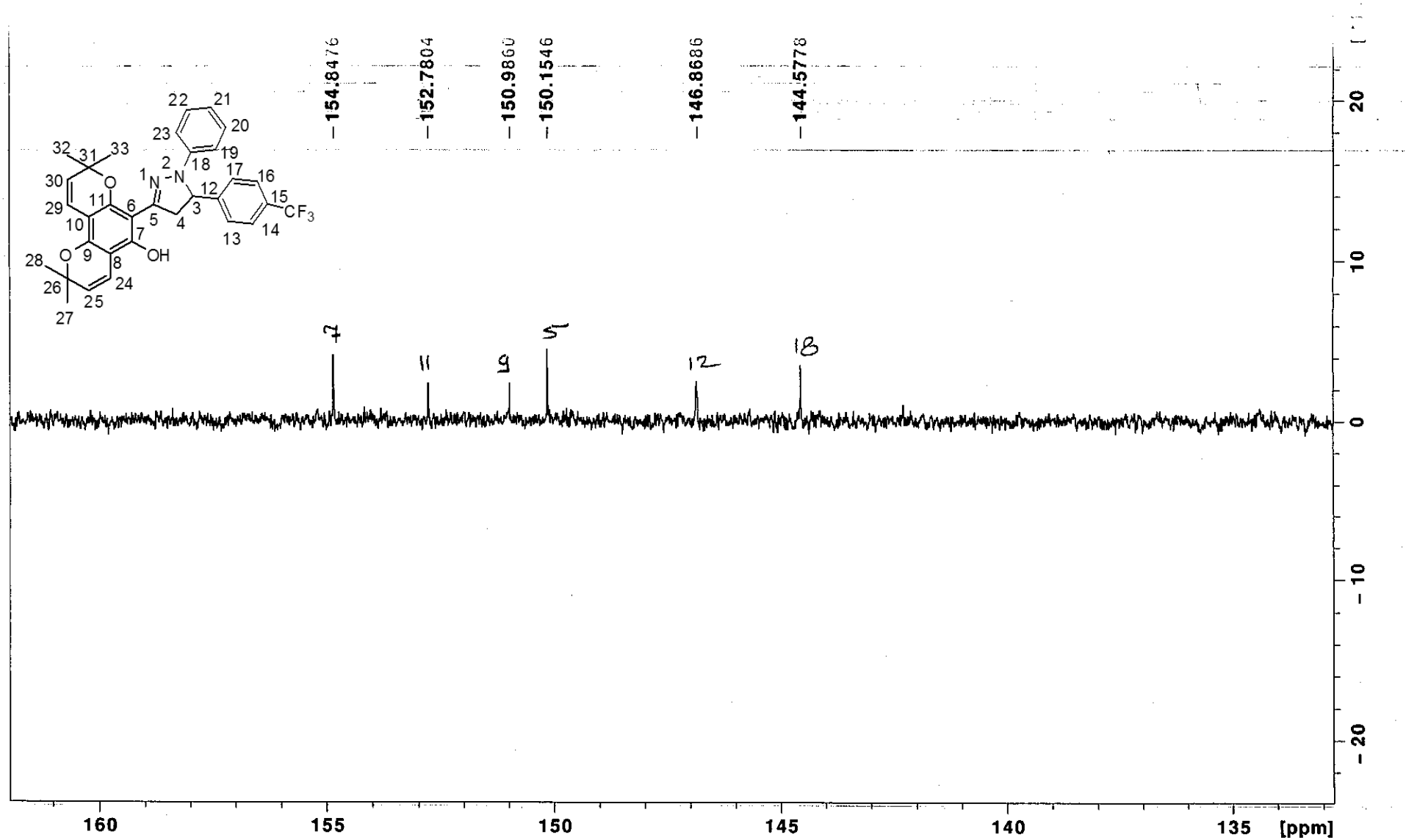
Expanded 1H NMR spectrum of 3-(4-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5k)



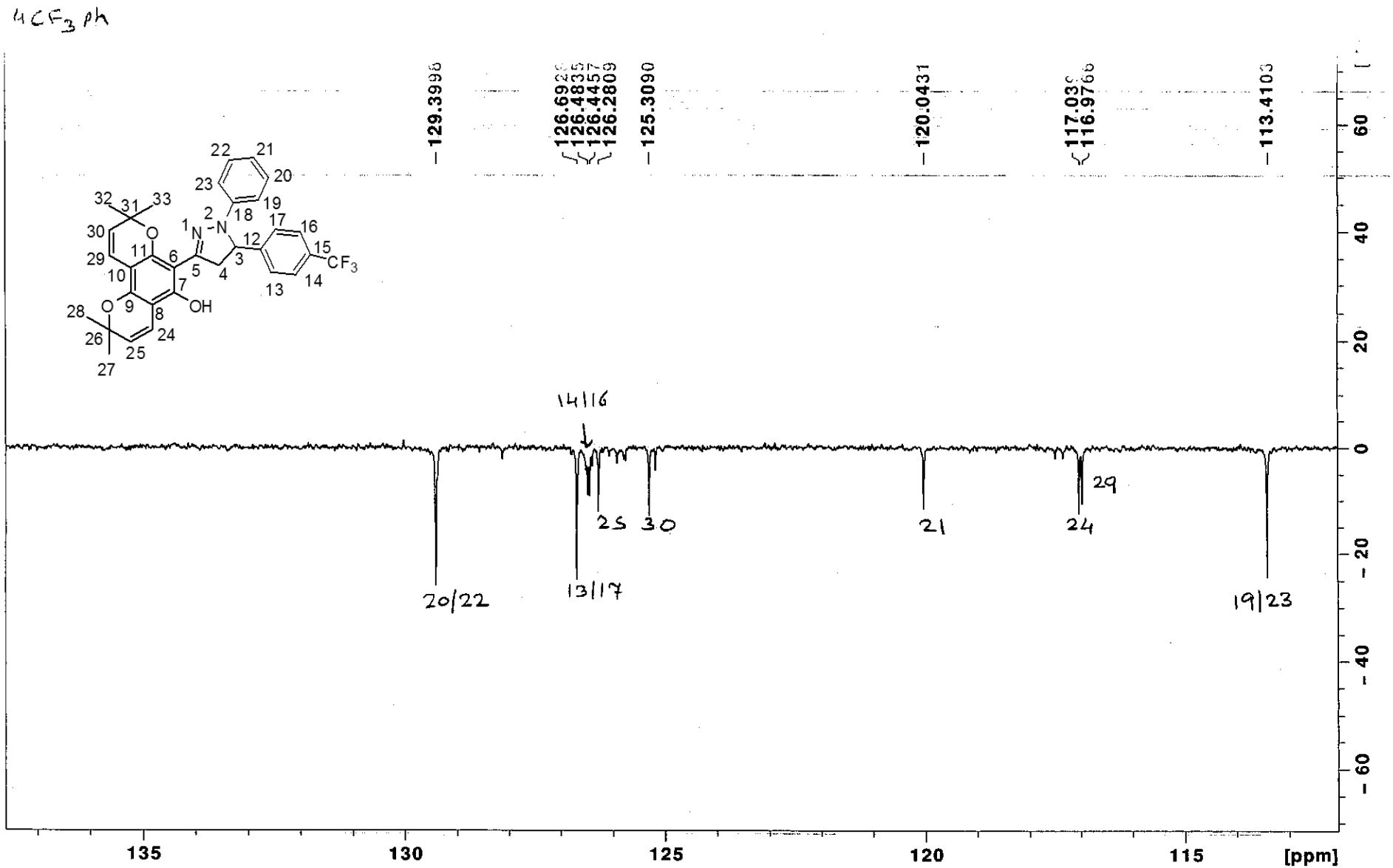
Expanded 1H NMR spectrum of 3-(4-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5k)



13C NMR spectrum of 3-(4-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5k)



Expanded 13C NMR spectrum of 3-(4-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5k)



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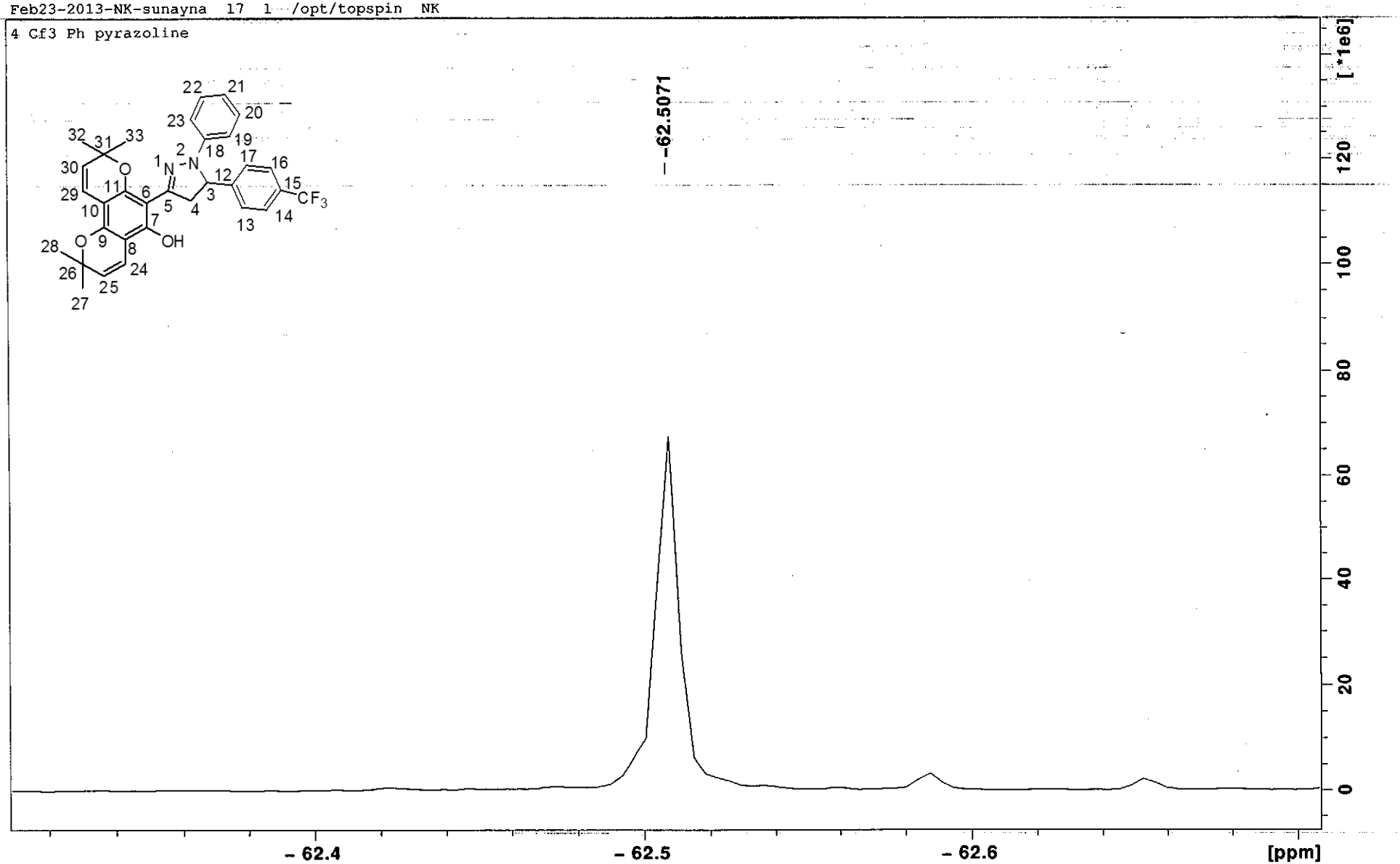

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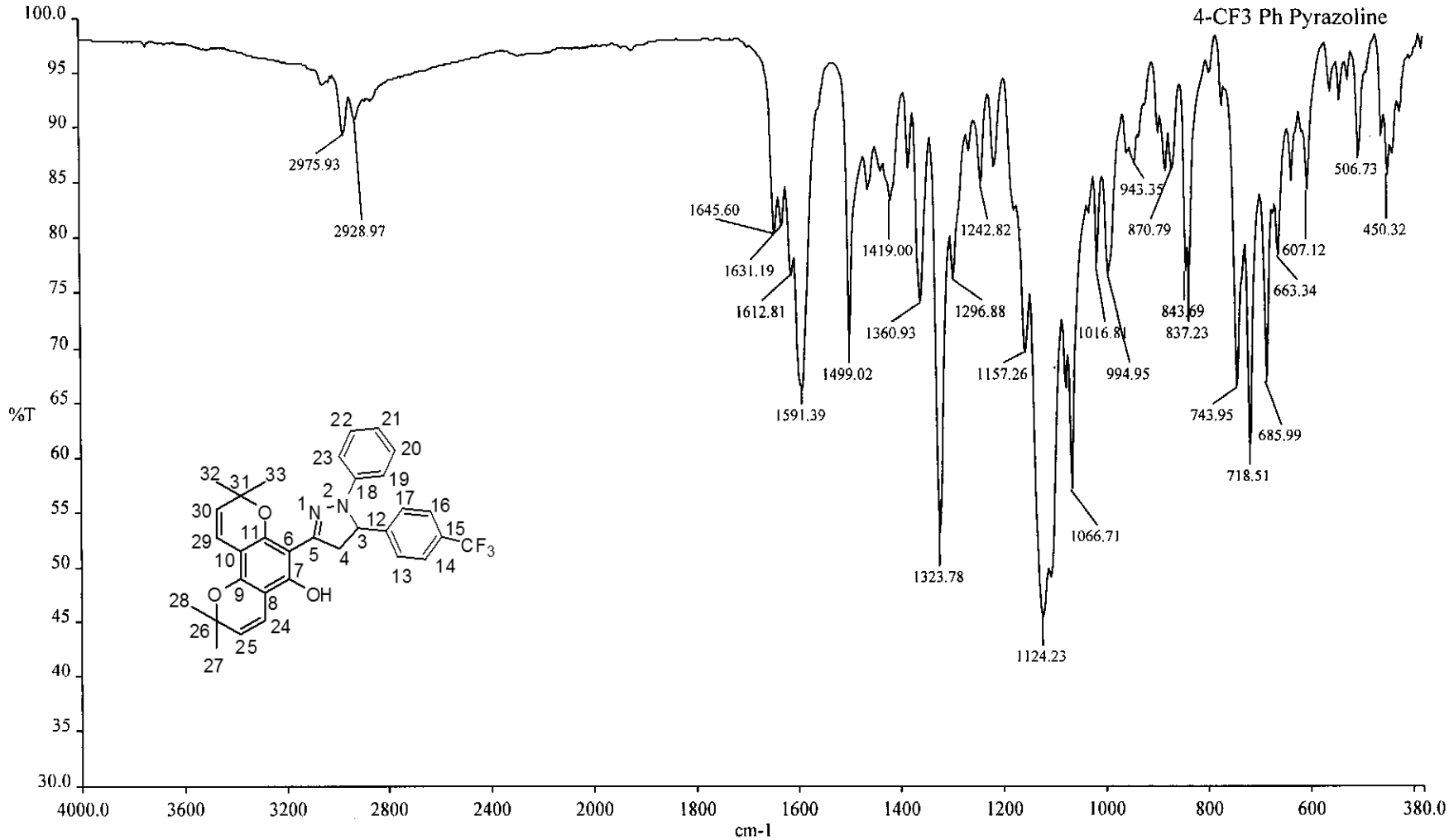

Infrared spectrum of 3-(4-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5k)

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Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
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Monoisotopic Mass, Even Electron Ions
19 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
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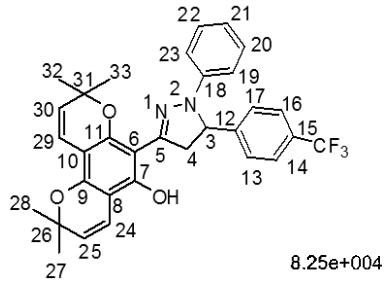
TOF MS ES-



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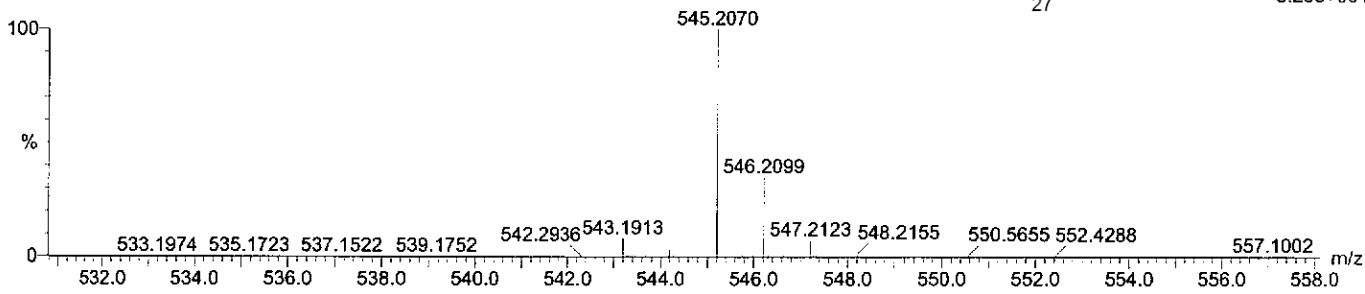

Minimum:				-1.5			
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Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
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HRMS spectrum of 3-(4-trifluoromethylphenyl)-2-phenyl-5-pyranochromene pyrazoline (C-5k)