UNIVERSITY OF KWAZULU-NATAL

PHYTOCHEMISTRY AND BIOACTIVE NATURAL PRODUCTS FROM *LANNEA ALATA*, *LANNEA RIVAE*, *LANNEA SCHIMPERI* AND *LANNEA SCHWEINFURTHII* (ANACARDIACEAE)

2014

OKOTH AKINYI DOROTHY

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2014

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 already been published. Typically these chapters will have been published in internationally recognized, peer- reviewed journals.

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

Supervisor: Prof. Neil A. Koorbanally

Signed: ----------Name: ---------- Date: ----------

ABSTRACT

Lannea plants belong to the family Anacardiaceae and are used in traditional medicine in the management of infectious diseases. Previous research on this genus focused on the biological activities of the plants. The Phytochemistry of most *Lannea* species is not reported. *Lannea alata*, *Lannea rivae*, *Lannea schimperi* and *Lannea schweinfurthii* were selected for this study. Previous studies illustrated antiplasmodial, cytotoxicity, antiviral, antioxidant and acetylcholinesterase inhibition of *Lannea schweinfurthii* extracts. *Lannea schimperi* extracts demonstrated antiulcer, antibacterial, cytotoxic and antifungal activities. However, no investigations were conducted to determine the compounds responsible for the observed activities. No prior work on *Lannea alata* and *Lannea rivae* has been reported. The present study aimed to isolate the biologically active phytochemicals occurring in the plants. This is the first phytochemical report of these *Lannea* species. In addition, pharmacological activities of the isolated compounds are discussed.

From the four *Lannea* species seven known terpenes were isolated; sitosterol (**A6**), sitosterol glycoside (**B6**), taraxerol (**B8**) and taraxerone (**B7**), lupeol (**A5**), lupenone (**D5**) and lutein (**B9**). Two novel prenylated flavonoids, lanneaflavonol (**A1**) and dihydrolanneaflavonol (**A2**), together with eight known flavonoids, myricetin (**B10**), myricetin-3-*O*-α-rhamnopyranoside (**A3**), myricetin-3-*O*-α-arabinofuranoside (**A4**), myricetin-3-*O*-β-galactopyranoside (**B11**), catechin (**D7**), epicatechin (**D6**), epicatechin gallate (**B12**) and rutin (**D8**) were also isolated.

Mixtures of phenolic lipids (cardanols) and its derivatives the alkyl cyclohexenols and alkyl cyclohexenones were isolated from *Lannea rivae*, *Lannea schimperi* and *Lannea schweinfurthii*. The non-isoprenyl aliphatic side chains of these compounds varied in length

with odd carbon chains of between 13 to 23 carbons and were either fully saturated or contained one or two double bonds. The cardanols and the phenolic derivatives were not observed in *Lannea alata*, possibly indicating a more distant chemotaxonomic relationship with the other three species. Although the core structures of the cardanols have been reported previously, there were novel side chains attached to **B1a** from *Lannea rivae*, **C1a** and **C1d** from *Lannea schimperii* and **D1c-D1e** from *Lannea schweinfurthii*. Novel alkylated furanocyclohex-2-enone (**B2**), trihydroxycyclohexanone (**B3**), dihydroxcyclohex-2-enones (**B4a** and **B4b**) and a trihydroxycyclohexane (**B5**) were isolated from *Lannea rivae*. While the core structures of 4,5-dihydroxycyclohex-2-enones (**C2a** and **C2d**) isolated from *Lannea schimperii* is known, the side chains in these molecules make them novel. Also novel from *Lannea schimperii* were the alkylated trihydroxycyclohexenes (**C3a-C3c**) and the dihydroxycyclohexenes (**C4a-C4c**). From *Lannea schweinfurthii*, the hydroxycyclohex-2-enones (**D2a-D2e**) with a novel core structure were isolated as well as novel compounds with the core structures of **C3** and **C4** but with novel alkylated side chaines (**D3a-D3b** and **D4a-Dg**).

The flavonoids, myricetin (**B10**), its glycosides (**B11** and **A3**) and epicatechin gallate (**B12**) showed good antioxidant activity with myricetin (**B10**) and epicatechin gallate (**B12**) exhibiting the best activity, comparable to the standards used. The novel dihydrolanneaflavonol (**A2**), betmidin (**A4**), myricetin (**B10**) and epicatechin gallate (**B12**) also showed good antibacterial activity. The pentacyclic triterpenes exhibited moderate to good cytotoxicity. The 5-[alkenyl]-4,5-dihydroxycyclohex-2-enone mixture (**C2a-C2d**) exhibited good *in vitro* cytotoxicity against the Chinese Hamster Ovarian mammalian cell-line. The furanocyclohex-2-enone (**B2**) and the trihydroxycyclohexanone (**B3**) showed good cytotoxic and antiplasmodial activity while the mixture of **B4a** and **B4b** also showed

antiplasmodial activity. The mixture where the core structure had a conjugated ketone (**D2a-D2e**) exhibited good antibacterial, antiplasmodial, larvicidal and cytotoxic activities.

Myricetin flavonoids are considered chemotaxonomic markers in the Anacardiaceae and their isolation in these *Lannea* species confirm their place within the Anarcadiaceae. The cardanols are also characteristic within the Anacardiaceae and their isolation like the myricetin flavonoids are expected in these species. The alkyl cyclohexenone and alkyl cyclohexenol derivatives of the types isolated in these *Lannea* species are however rare, only previously reported in *Lannea* and *Tapirira* and are not widespread in the Anarcadiaceae. Their occurrence within the species studied here points to the possibility of a unique set of enzymes only contained in the *Lannea* and *Tapirira* genera responsible for the biosynthesis of these secondary metabolites. These findings also suggest the possibility of a common evolutionary pathway in the *Lannea* and *Tapirira* genera. Further to this, isolation of similar cardanols, trihydroxycyclohexenes and dihydroxycyclohexenes from *Lannea schimperii* and *Lannea schweinfurthii* suggest a closer relationship between these two species.

The isolation of bioactive compounds from these four *Lannea* species indicates a rationale for their use in African traditional medicinal. These studies indicate that *Lannea* species are promising sources of new active phytochemicals that can be used as leads for the synthesis of potentially bioactive compounds.

List of isolated compounds

Novel compounds are indicated in bold

Chapter 2 Compounds from *Lannea alata*

A1 **lanneaflavonol**

A2 **dihydrolanneaflavonol**

A3 myricetin-3-*O*-α-rhamnopyranoside

A4 myricetin-3-*O*-α-arabinofuranoside (betmidin)

A5 lupeol

A6 sitosterol

Chapter 3[a] Compounds from *Lannea rivae*

B1a **3-nonadec-14'-*Z*-enyl phenol**

B1b 3-heptadec-12'-*Z*-enyl phenol

B1c 3-pentadec-10'-*Z*-enyl phenol

B1d 3-pentadecyl phenol

B2 **4,5-dihydroxy-4,5-furan-2'-[16'-(*Z*)-18'-(*E*)-heneicosenyldiene] cyclohex-2-enone**

B3 **2,4,5-trihydroxy-2-[16'-(*Z*)-heneicosenyl] cyclohexanone**

B4a **4*S*,6*R*-dihydroxy-6-(12'(*Z*)-heptadecenyl) 2-cyclohexenone**

B4b **4*S*,6*R*-dihydroxy-6-(14'(*Z*)-nonadecenyl) 2-cyclohexenone**

B5 **1,2,4-trihydroxy-4-[16'(*Z*)-heneicosenyl] cyclohexane**

B6 sitosterol glucoside

B7 taraxerone

B8 taraxerol

B9 E-lutein

B10 myricetin

B11 myricetin-3-*O*-β-galactopyranoside

B12 epicatechin gallate

[a] **A3** (myricetin-3-*O*-α-rhanmnopyranoside) and **A6** (sitosterol) were also featured in Chapter 3.

Chapter 4[b] Compounds from *Lannea schimperi*

C1a **3-[12'(*E*)-pentadecenyl] phenol**

C1b 3-[14'(*E*)-heptadecenyl] phenol

C1c 3-[16'(*E*)-nonadecenyl] phenol

C1d **3-[18'(*E*)-heneicosenyl] phenol**

C2a **5-[12'(*E*)-pentadecenyl] 4,5-dihydroxycyclohex-2-enone**

C2b 5-[14'(*E*)-heptadecenyl] 4,5-dihydroxycyclohex-2-enone

C2c 5-[16'(*E*)-nonadecenyl] 4,5-dihydroxycyclohex-2-enone

C2d **5-[18'(*E*)-heneicosenyl] 4,5-dihydroxycyclohex-2-enone**

C3a **1-[12'(*E*)-pentadecenyl] cyclohex-3-en-1,2,5-triol**

C3b **1-[14'(*E*)-heptadecenyl] cyclohex-3-en-1,2,5-triol**

C3c **1-[16'(*E*)-nonadecenyl] cyclohex-3-en-1,2,5-triol**

C4a **1-[14'(*E*)-heptadecenyl] 4-cyclohex-4-en-1,3-diol**

C4b **1-[16'(*E*)-nonadecenyl] 4-cyclohex-4-en-1,3-diol**

C4c **1-[18'(*E*)-heneicosenyl] 4-cyclohex-4-en-1,3-diol**

[b]**A6** (sitosterol), **(B7)** taraxerone and **(B8)** taraxerol were also featured in Chapter 4

Chapter 5[c] Compounds from *Lannea schweinfurthii*

D1a 3-[tridecyl] phenol

D1b 3-[heptadecyl] phenol

D1c 3-[heptadec-12'(*Z*),14'(*E*)-dienyl] phenol

D1d 3-[nonadec-14'(*Z*),16'(*E*)-dienyl] phenol

D1e 3-[heneicos-16'(*Z*),18'(*E*)-dienyl] phenol

D2a 5-hydroxy-5-[tridecyl] cyclohex-2-enone

D2b 5-hydroxy-5-[pentadecyl] cyclohex-2-enone

D2c 5-hydroxy-5-[heptadecyl] cyclohex-2-enone

D2d 5-hydroxy-5-[pentadec-12'(*E*)-enyl] cyclohex-2-enone

D2e 5-hydroxy-5-[heptadec-14'(*E*)-enyl] cyclohex-2-enone

D3a 1-[tridecyl] cyclohex-3-en-1,2,5-triol

D3b 1-[heptadecyl] cyclohex-3-en-1,2,5-triol

D4a 1-[tridecyl] cyclohex-4-en-1,3-diol

D4b 1-[nonadecyl] cyclohex-4-en-1,3-diol

D4c 1-[heneicosyl] cyclohex-4-en-1,3-diol

D4d 1-[tricosyl] cyclohex-4-en-1,3-diol

D4e 1-[pentadec-12'(*E*)-enyl] cyclohex-4-en-1,3-diol

D4f 1-[nonadec-14'(*Z*),16'(*E*)-dienyl] cyclohex-4-en-1,3-diol

D4g 1-[heneicosen-16'(*Z*),18'(*E*)-dienyl] cyclohex-4-en-1,3 diol

D5 lupenone

D6 epicatechin

D7 catechin

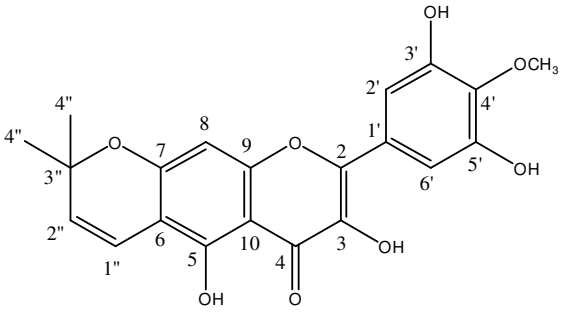
D8 Rutin

[c] **A6, B6, B1d, B12, C1a-C1d, C3a, C3c, C4a-C4c** were also featured in Chapter 5.

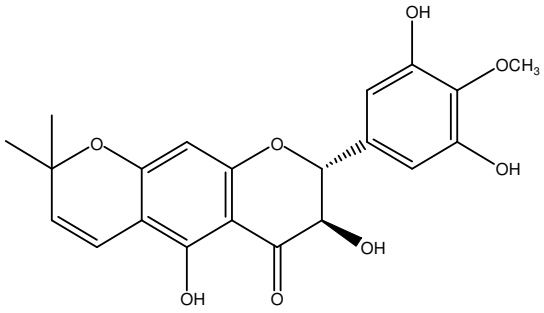
Structures of isolated compounds

Structures in subsequent chapters are not repeated

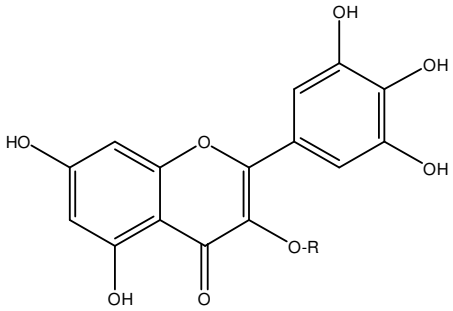
Chapter 2



A1

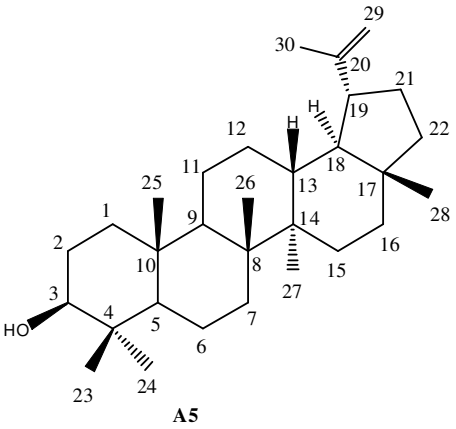


A2

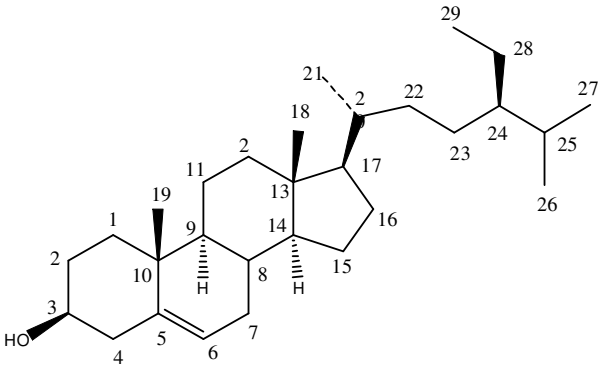


A3: R = rhamnose

A4: R = arabibofuranose

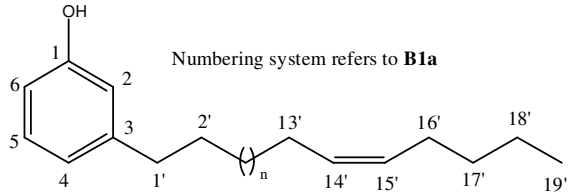


A5



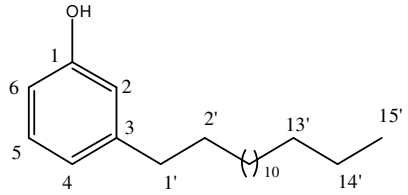
A6

Chapter 3



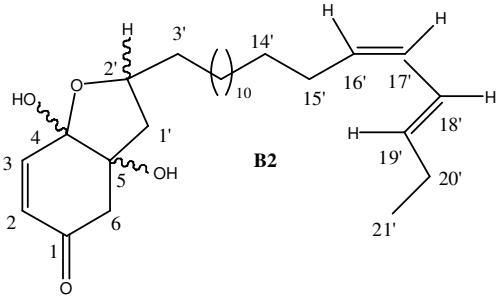
B1a : n = 10 **B1b** : n = 8 **B1c**: n = 6

M^+ 358 M^+ 330 M^+ 302

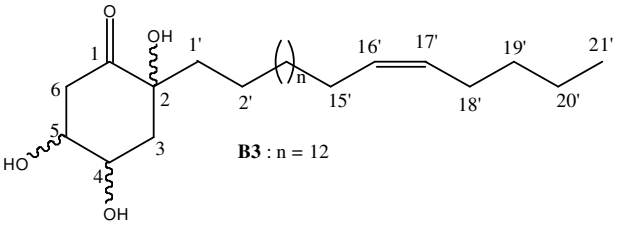


B1d

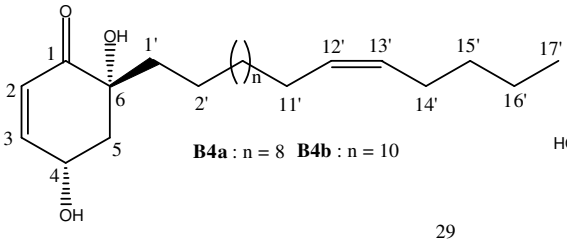
M^+ 304



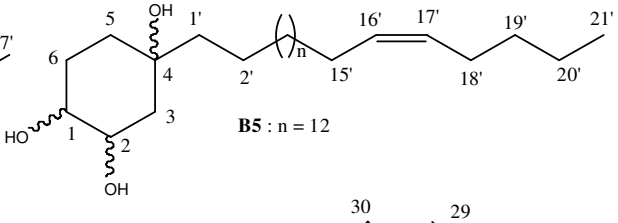
B2



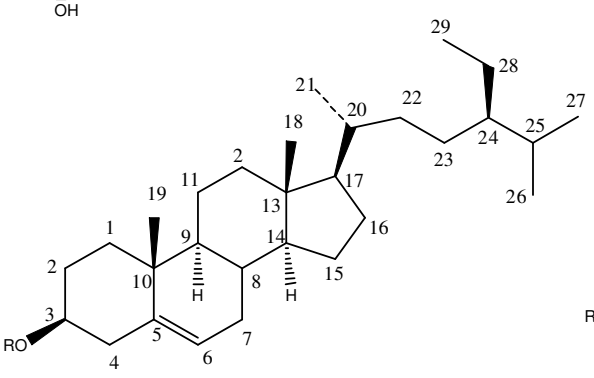
B3 : n = 12



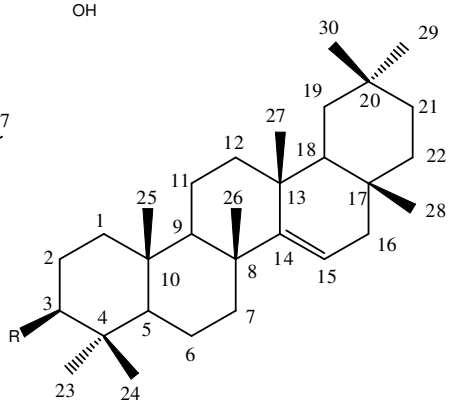
B4a : n = 8 **B4b** : n = 10



B5 : n = 12

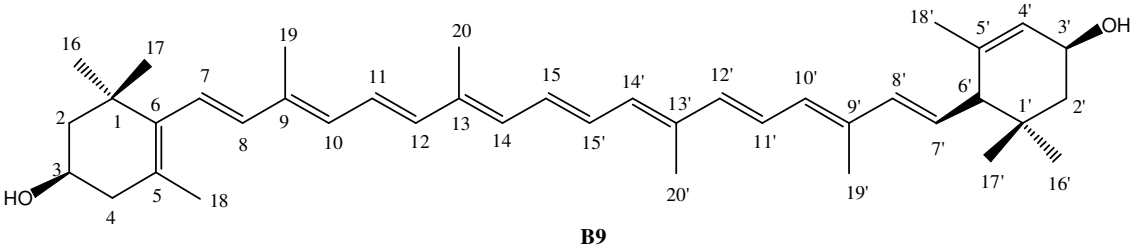


B6 R = glucose

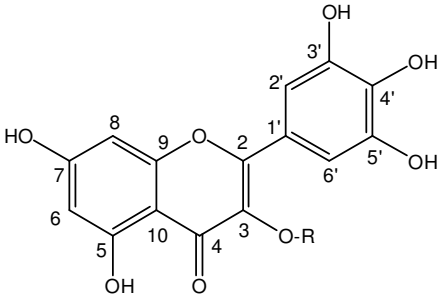


B7 R = =O
B8 R = -OH

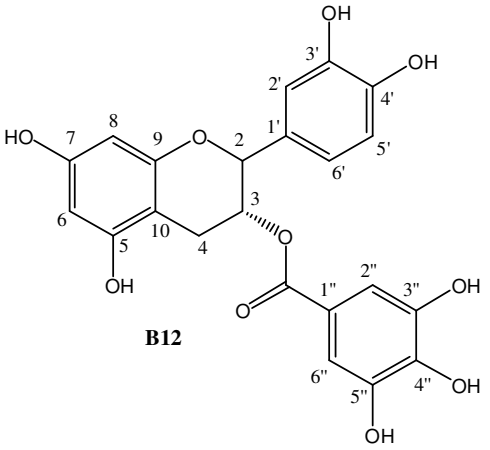
Chapter 3 continued…



B9

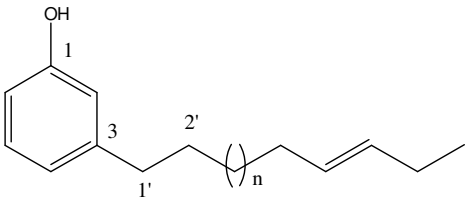


B10 R = rhamnose
B11 R = galactose

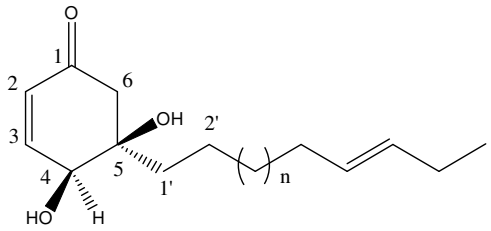


B12

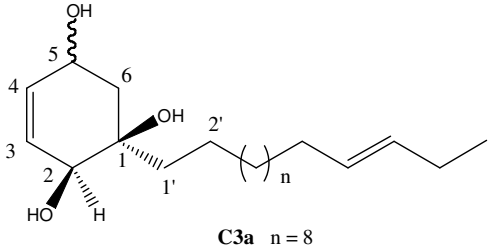
Chapter 4



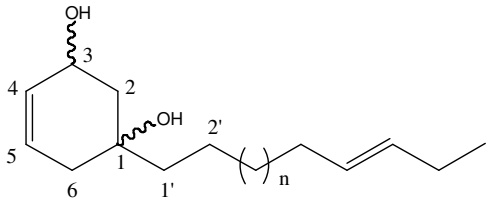
C1a n = 8
C1b n = 10
C1c n = 12
C1d n = 14



C2a n = 8
C2b n = 10
C2c n = 12
C2d n = 14

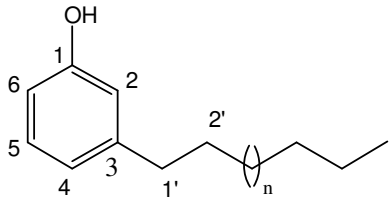


C3a n = 8
C3b n = 10
C3c n = 12

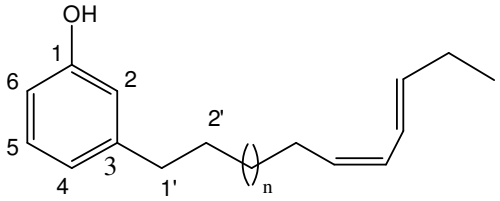


C4a n = 10
C4b n = 12
C4c n = 14

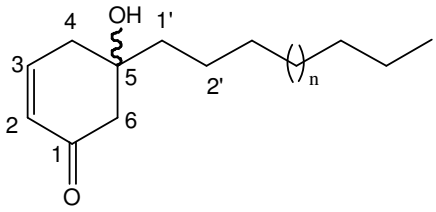
Chapter 5



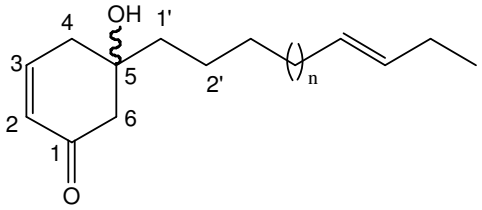
D1a: n = 8 **D1b**: n = 12



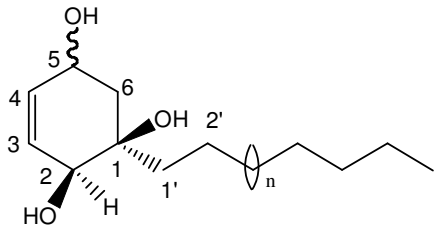
D1c: n = 8 **D1d**: n = 10 **D1e**: n = 12



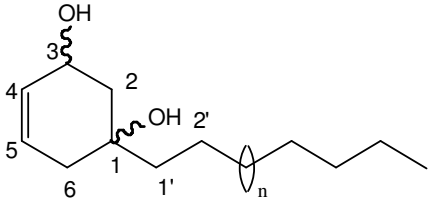
D2a: n = 7 **D2b**: n = 9 **D2c**: n = 11



D2d: n = 8 **D2e**: n = 10

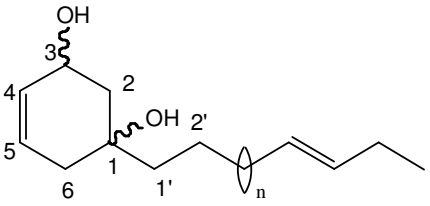


D3a: n = 7 **D3b**: n = 11

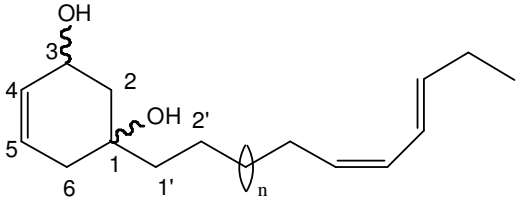


D4a: n = 7 **D4b**: n = 13

D4c: n = 15 **D4d**: n = 17

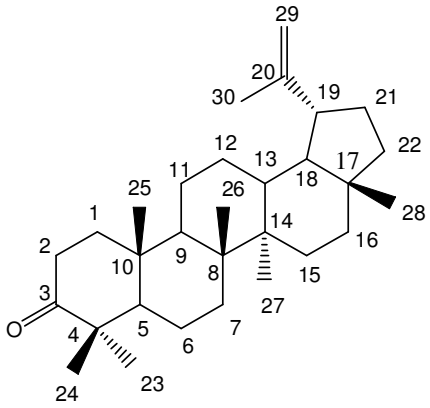


D4e: n = 9



D4f: n = 10 **D4g**: n = 12

Chapter 5 continued…



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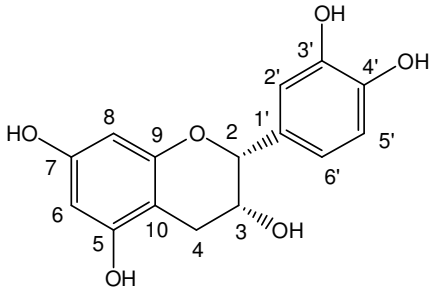

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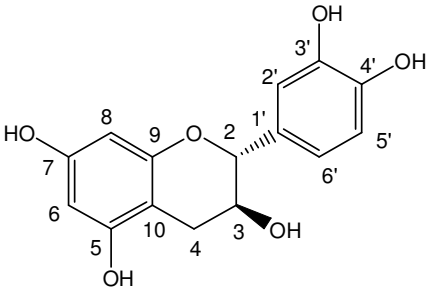
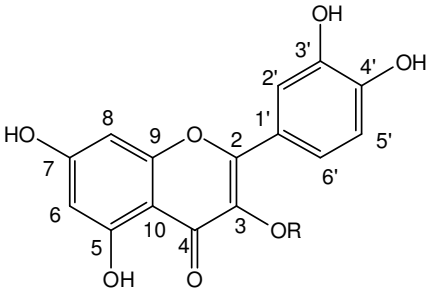


D7



D8: R = rutinose

LIST OF ABBREVIATIONS

^{13}C NMR	C-13 nuclear magnetic resonance spectroscopy
^{1}H NMR	proton nuclear magnetic resonance spectroscopy
ANOVA	analysis of variance
br	broad
CD	circular dichroism
CD_3OD	deuterated methanol
$CDCl_3$	deuterated chloroform
COSY	correlated spectroscopy
d	doublet
dd	double doublet
DEPT	distortionless enhancement by polarization transfer
DMSO-d_6	deuterated dimethyl sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
HMBC	heteronuclear multiple bond correlations
HPLC	high pressure liquid chromatography
HREIMS	high resolution electron impact mass spectroscopy
HSQC	heteronuclear single quantum coherence
Hz	hertz
IR	infrared
LCMS	liquid chromatography- mass spectrometry
m	multiplet
Me	methyl
MIC	minimum inhibitory concentration
Mp	melting point
MS	mass spectroscopy
MTT	(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NCAPD	national coordinating agency for population and development
NOESY	nuclear overhauser effect spectroscopy
s	singlet
t	triplet
td	triplet of doublets
TLC	thin layer chromatography
UV	ultraviolet

DECLARATIONS

DECLARATION 1 – PLAGIARISM

I, **Okoth Akinyi Dorothy** declare that

1. The research reported in this thesis is my original research, except where otherwise indicated.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
   a. Their words have been re-written but the general information attributed to them have been referenced
   b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.
5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

Signed 

DECLARATION 2 - PUBLICATIONS

DETAILS OF 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)

1. Okoth, D.A., Chenia, H.Y., and Koorbanally, N.A. (2013). Antibacterial and antioxidant activities of flavonoids from *Lannea alata* (Engl.) Engl. (Anacardiaceae). Phytochemistry Letters 6, 476-481.
2. Okoth, D.A., Akala, M.H., Johnson, J.D., and Koorbanally, N.A. Antibacterial, antioxidant, antiplasmodial and cytotoxic activities of *Lannea rivae* (Chiov) sacleux (Anacardiaceae), Manuscript in preparation
3. Okoth, D.A., and Koorbanally, N.A. Cardanols, long chain cyclohexenones and cyclohexenols from *Lannea schimperi* (Hochst. Ex. A.Rich) (Anacardiaceae), Manuscript in preparation
4. Okoth, D.A., Akala, M.H., Johnson, J.D., and Koorbanally, N.A. Alkyl phenols, alkyl cylohexenols and alkyl cyclohexenones isolated from *Lannea schweinfurthii* (Engl.) Engl. (Anacardiaceae) and their bioactivity, Manuscript in preparation

In the above publications/ manuscripts I carried out all the experimental work, interpreted the data and compiled the findings thereof. The co-authors contribution was to edit, check the scientific content and verify my interpretation of the data based on their areas of expertise.

Signed: ……..

DEDICATION

This thesis is dedicated to my beloved Dad, Mr. Daniel Okoth

Okwany and Son Neil-Antony Okoth

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to my supervisor, Prof. N.A Koorbanally for his assistance and advice throughout my PhD studies. Your kindness and dedication to assist your students is highly regarded.

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The Organization for Women in Science for the Developing World (OWSDW), formally TWOWS, is acknowledged for financial support.

Finally I wish to thank my family Dad, Mum, Sam, Edwine, Phelix, Bether, Emily, Judy, Liz, Tumaini and Maureen. Your love and support made me strong through it all.

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Chapter 1 Introduction

1.1 An introduction to traditional medicine

Traditional medicine plays an important role in the primary health care of a number of developing countries. In Kenya, conventional medicine provides for only 30% of the population. Thus, more than two thirds of the population relies on traditional medicine to meet their primary health care needs. Due to the high cost of modern medicine and drugs, most Kenyans turn to traditional medical practitioners since they are a cheaper alternative to modern medicine, are more accessible and the majority of the population are confident in their ability to manage debilitating, incurable disease (NCAPD, 2008).

Many people use herbal concoctions with the assumption that the medicine is safe and effective. For people with chronic diseases, combining herbal medicine with conventional medicine is a common practice. It is expected that there may be synergistic effects between the conventional drugs and the herbal remedies. The use of indigenous medicinal plants is not based on science and is mostly unregulated and as such varies considerably from place to place. There is often no standard dosage and the safety of such practice remains unclear.

In traditional practice, several plants are often used in combinations and the activity may be a result of either additive or synergistic effects. Many medicinal plants are the focus of biological screening and the crude extracts are screened for *in vitro* or *in vivo* activity or both. In some cases single active constituents for a particular action have been successfully identified. Phytochemical and toxicological studies can identify toxic components in order to assess and possibly eliminate or reduce harmful side effects of the extracts (Nyika, 2009). There is still a great need for phytochemical and pharmacological research of medicinal

plants in order to improve the efficacy and reduce potentially harmful side effects that may be associated with it.

Interest in medicinal plants arises from the desire to provide a scientific rationale for the plant uses and the possibility of discovering novel compounds of pharmaceutical value. Historically, compounds containing novel structures from plant sources were a major source for the discovery and development of new drugs for several diseases. These include quinine from *Cinchona* species and artemisinin (Artemisinin®) from *Artemisia annua.* Quinine was used as a template for the synthesis of chloroquine, which was the main antimalarial drug until recently. Semi-synthetic derivatives of artemisinine such as atreether (Artemotil®), artemether (Artemetheri®) and sodium artesunate (Arinate®) are often used more frequently for malaria treatment. Lapachol (a prenylnaphthoquinone), an antimalarial drug from *Tabebuia species* (Bignoniaceae) was a template for the synthesis of the newest antimalarial drug atovaquinone (Malarone®) (Kaur *et al.*, 2009; Newman and Cragg, 2007; Oliveira *et al*., 2009). Plant-derived antibacterials such as daptomycin (Cubicin®), fosfomycin tromentamol (Monuril®) and Isepamicin (Isepacin®) (isolated from plants) and Biapenem (Omegacin), cefuroxime axetil (Zinnat), dorifenem (Finibax®), Eriththromycin acistrate (Erasis®), Tigecyclin (Tyagcil®) (semi-synthesized from natural products) (Newnan and Cragg, 2007; 2012; Cragg and Newman, 2013) demonstrate the potential of finding new and better antibacterial agents with fewer side-effects, which could provide alternative antibacterial agents to resistant bacteria. Flavonoids such as epicatechin gallate, epigallocatechin gallate and gallocatechin gallate isolated from tea are examples of plant-derived compounds with potentially exploitable activities, including antibacterial activity (Hamilton-Miller and Shah, 2000), synergism with antibiotics (Shiota *et al*., 1999; Qin *et al*.,

2013), suppression of bacterial virulence (Shah *et al*., 2008) and suppression of resistance (Stapleton *et al.*, 2004; 2007).

A number of plant extracts and compounds have demonstrated activity against malarial pathogens. Different classes of compounds (alkaloids, flavonoids, quinones, xanthones and terpenes) with antiplasmodial activity have been isolated (Batista *et al.*, 2009; Caniato and Puricelli, 2003; Nogueira and Lopes, 2011; Oliveira *et al.*, 2009). Some of the compounds like galloylated catechins and procyanidins are reported to inhibit or reverse resistance of the most virulent malarial parasite *Plasmodium falciparum* (Park *et al*., 2010; Ramanandralbe *et al*., 2008; Sannella *et al*., 2007; Tasdemir *et al.*, 2006).

Anticancer agents derived from plants and their derivatives e.g. paclitaxel (Taxol®), vincristine (Oncovin®), vinorelbine (Navelbine®), teniposide (Vumon®) and camptothecin (e.g. Hycamtin®) have been proven to be effective for cancer prevention and therapeutics (Cragg *et al.*, 2009; Cragg and Newman, 2013; Pezzuto, 1997). Phytochemicals are used for the treatment of cancer related symptoms due to their safety, low toxicity, and general availability. Many active phytochemicals are in human clinical trials but there is the possibility of discovering novel and more effective anticancer phytochemicals (Pratheeshkumar *et al.*, 2012). Consuming phytochemicals as part of a daily diet have been shown to have cancer protective effects by inhibiting, delaying, or reversing carcinogenesis by inducing detoxifying and antioxidant enzyme systems, regulating inflammatory and proliferative signaling pathways, and inducing cell cycle arrest and apoptosis (Pratheeshkumar *et al*., 2012; Rajput and Mandal, 2012; Singh *et al.*, 2002).

1.2 Traditional medicinal uses of the genus *Lannea*

The genus *Lannea* belongs to the family Anacardiaceae and consists of about 40 species of shrubs or trees native to tropical Africa. They are used as sources of timber, as a source of fruit and for a number of purposes in indigenous medicine. Table 1-1 shows a selection of the more popular *Lannea* species and their use in indigenous medicine. The medicinal use varies with the species and community or tribe involved. In general, *Lannea* species are used in the management of mental disorders, gastrointestinal disorders, bacterial infections, viral infections, fungal infections and fever (Table **1-1**). The plants are used to manage an array of symptoms rather than a specific disease. It must be noted that the diagnosis is made by traditional healers with no scientific training. A disease symptom such as fever may be due to a number of causes such as bacterial or viral infections, malaria and food poisoning.

Lannea species are traditionally used in folk medicine as natural healing remedies with therapeutic effects such as the prevention of cardiovascular diseases such as hypertension, atherosclerosis and ischemia, inflammation disorders (Singh and Singh,1994; 1996) such as asthma, gingivitis, rheumatoid arthritis, cellulites, gastroenteritis and inflammatory bowel disease (Kone *et al*., 2004; 2011; Maiga *et al* 2006; Maregesi *et al*., 2007; Singh and Singh,1994), neurological diseases such as Alzheimer's disease, Parkinson's disease, memory loss and depression (Adewusi and Steenkamp, 2011) and pulmonary diseases such as chronic obstructive pulmonary diseases (Kerharo and Adams, 1974; Maiga *et al* 2006). They are also used to reduce the risk of cancer (Adewusi and Steenkamp, 2011; Atawodi, 2005).

A number of *Lannea* species have demonstrated antibacterial, anti-inflammatory, antioxidant and radical scavenging activities and lipoxygenase inhibition activities (Diallo *et al.*, 2001;

Koné *et al.*, 2011; Lamien-Meda *et al.*, 2008; Maiga *et al.*, 2006; 2007; Ouattara *et al.*, 2011b; Picerno *et al*., 2006; Saravanan, 2010; Singh and Singh, 1994). These activities have been associated with their polyphenol contents. The ability of some species to cure wounds, abrasions and sores are known (Agyare *et al*., 2013; Deji-Agboola and Olajubu 2010; Sathish, 2010). This could also be due to their polyphenol content since antioxidants are also involved in wound healing and tissue repairs (Sen *et al*., 2002; Shetty, 2013). The plants have also demonstrated their capacity to manage diabetes as well as hypertension and related symptoms (Deutschlander *et al*., 2009; Nyarko *et al.*, 2005; Okine *et al*., 2005; Rahmatullah *et al.*, 2012; Singh and Singh, 1996).

Lannea species are also used in the management of antibacterial, antifungal and viral diseases (HIV type 1 and II, herpes zoster and herpes simplex), which have been verified by pharmacological studies (Bationo *et al*., 2012; Diallo *et al*., 2001; Haule *et al.*, 2012; Kisangau *et al*., 2007; 2009; Kone *et al.*, 2004; 2011; Maregesi *et al.*, 2010; Maregesi *et al.*, 2007; Maregesi *et al.*, 2008; Ouattara *et al.*, 2011a; Ouattara *et al.*, 2011b, Runyoro *et al.*, 2006). These infections are often considered as HIV opportunistic infections. The use of *Lannea* species in the treatment of malaria and associated symptoms has been justified by the demonstrated antiplasmodial action of *Lannea disclor* and *Lannea schweinfurthii* extracts (Clarkson *et al.*, 2004; Gathirwa *et al*., 2008; 2011; Maregesi *et al*., 2010). The antigiardial, vibriocidal, antibacterial and antidiarrheal activity of some species demonstrate the ability of the plants to manage diarrhea and related infections (Akinsinde and Olukoya, 1995; Etuk *et al.*, 2009; Johns *et al*., 1995; Olatokunboh *et al.*, 2010).

Acetylcholinesterase inhibition activity has been observed in some *Lannea* species (Adewusi and Steenkamp, 2011; Koné *et al.*, 2011) and hence has the potential to be used for

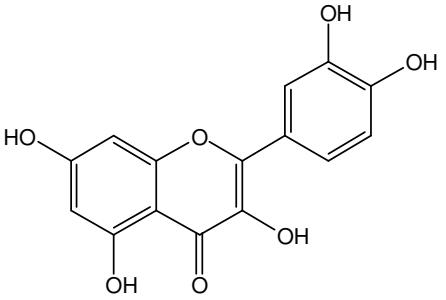
Alzheimer's disease and other neurological disorders. Cytotoxicity and anticancer activity has also been observed in *L. acida*, *L. coromandelica*, *L. humilis*, *L. schweinfurthii*, *L. schimperi*, *L. welwitschii* and *L. nigratiana* (Akter *et al*., 2013; Fadeyi *et al*., 2013; Gathirwa *et al*., 2011; George *et al*., 2010; Groweiss *et al*., 1997; Kapche *et al.*, 2007; Mothana *et al.*, 2009; Nibret *et al.*, 2010; Roy *et al.*, 2011; Sowemimo *et al.*, 2009). In most of the studies, no follow up isolation of the phytocompounds were carried out on the crude extracts, however these were done in a few cases where flavonoids, terpenes, alkyl phenols and their derivatives were identified as the compounds responsible for the activity.

1.3 Phytochemical studies of the genus Lannea

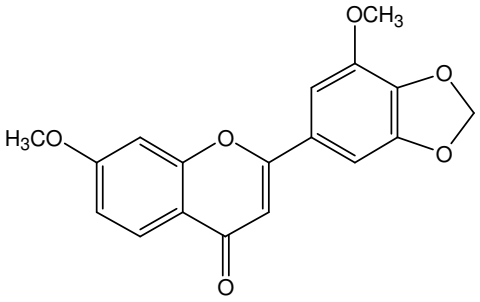
Phytochemical studies of the genus *Lannea* indicate various classes of compounds (Table **1-2**) such as tetracyclic and pentacyclic triterpenes, flavonoids (flavones, flavonols, dihydroflavonols, flavanones, flavanols, isoflavans, anthocyanidins) and phenolic lipids and cyclohexene derivatives (Govindac *et al.*, 1971; Groweiss *et al.*, 1997; Islam and Tahara, 2000; Kapche *et al.*, 2007; Nair *et al*., 1963; Picerno *et al*., 2006; Queiroz *et al*., 2003; Sankara and Nair, 1971; Sultana and Ilyas, 1986a; 1986b; Yun *et al*., 2012). Representative structures are shown in Figure **1-1** to Figure 1-5.

The most commonly isolated class of compounds found in the *Lannea* species are the flavonoids being found in seven of the eight species studied. These are *L. acida, L. coromandelica*, *L. edulis*, *L. grandis, L. microcarpa*, *L. nigratiana* and *L. velutina*. Examples of the flavonoids are quercetin (**1**) (flavonol), 7,2'-dimethoxy-4',5'-methylenedioxyflavone (**2**) (flavone), 6,7,2,2-dimethylchromeno-8γ,γ-dimethylallyl flavanone (flavanone) (**3**), 5-methoxyvestitol (**4**) (isoflavan), cyanidin 3-*O*-β-D-galactopyranoside (**5**) (anthocyanidin), (2*R*,3*R*)-(+)-4',5,7-trimethoxydihydroflavonol (**6**) (dihydroflavonol), epigallocatechin gallate

(**7**) (flavan) and physcion (**8**) (anthraquinone). This is followed by the triterpenes isolated from *L. grandis* and *L. coromandelica* species. These triterpenes have either tetracyclic or pentacyclic skeletons. Examples of tetracyclic triterpenes are lanosterol (**9**) β-sitosterol (**10**) stigmast-4-ene-6-β-ol-3-one (**11**) and 5-α-stigmastane-3,6-dione (**12**). Taraxerone (**13**), taraxerol (**14**) and taraxeryl acetate (**15**) are pentacyclic triterpenes all isolated from *L. coromandelica*. In addition phytochemical studies of *Lannea edulis*, *Lannea nigratiana* and *Lannea welwitschii* led to the isolation of phenolic lipids (alkyl/alkenyl hydroquinones and cardanols/3-alky/alkenyl phenols) and their derivatives (the alkyl/alkenyl cyclohexenones and alkyl/alkenyl cyclohexenols). Lanneaquinol (**16**) and 2-(*R*)-hydroxylanneaquinol (**17**) are examples of alkyl hydroquinones isolated from *Lannea welwitschii* (Groweiss *et al*., 1997). The cardanols, 3-[14'*E*-nonadecenyl]-phenol (**18**) and 3-[16'*E*-heptadecenyl]-phenol (**19**) were reported in *Lannea edulis* (Queiroz *et al*., 2003). The alkyl cycloxenones are represented by 4,5-dihydroxy-5-[14'(*E*)-heptadecenyl]-2-cyclohexenone (**20**) and the isomeric 4,5-dihydroxy-5-[8'(*Z*)-heptadecenyl]-2-cyclohexenones (**21** and **22**) (Groweiss *et al*. 1997; Queiroz *et al*., 2003). The alkylated cyclohexenetriol, lanneanol (**23**) was isolated from *Lannea nigratiana* (Kapche *et al.*, 2007). The alkyl phenols and their derivatives isolated from the different species vary in the number, position and configuration of the double bonds along the side chain, the number of carbons on the side chains as well as the number and position of hydroxyl functional groups. Alkyl cyclohexenols and alkyl cyclohexenones have only been isolated from the *Lannea* and *Tapirira* genera (Correia *et al*., 2001; David *et al*., 1998; Roumy *et al*., 2009). They are proposed to be the biogenic precursors of the alkyl phenols in these species (Correia *et al*., 2001). The long chain phenols are characteristic of the Anacardiaceae. Some of these compounds have demonstrated antioxidant, antibacterial, antiplasmodial, wound healing ability and cytotoxicity.



1



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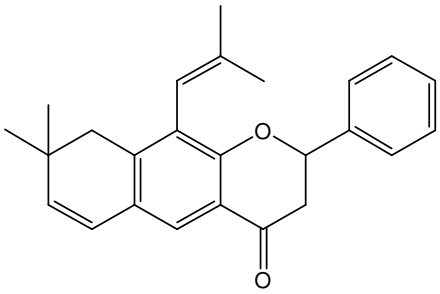

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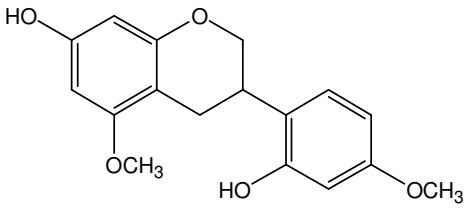

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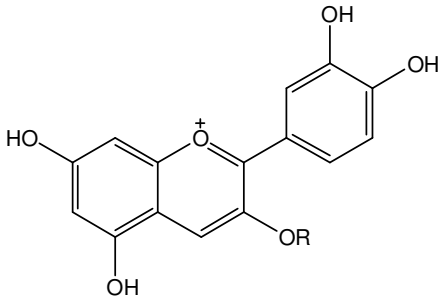
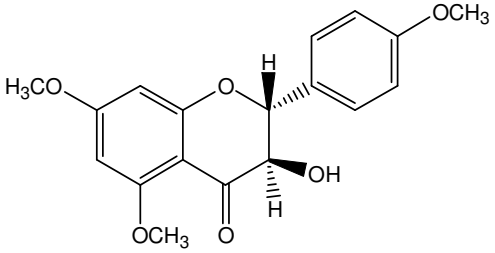


5 R = galactopyranose



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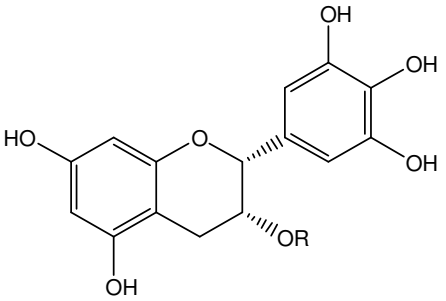
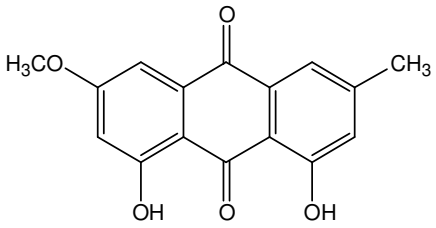


7 R = gallate



8

Figure 1-1 Representative structures of flavonoids from *Lannea* species

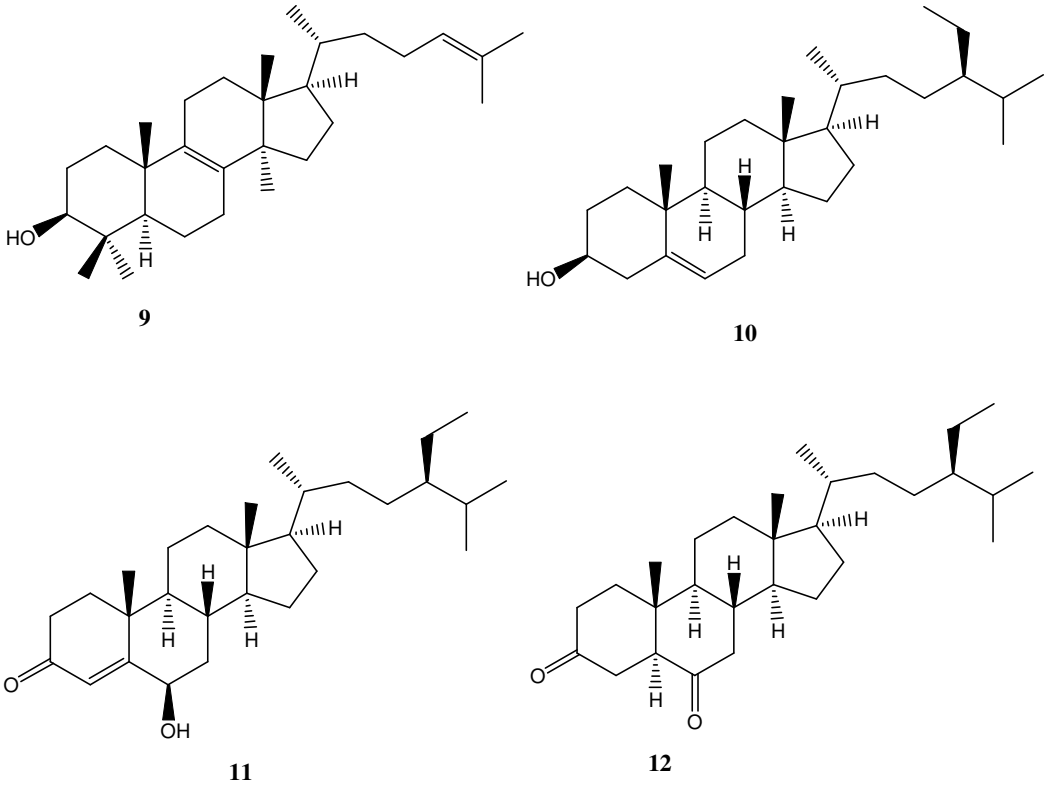


Figure 1-2 Representative tetracyclic triterpenes from *Lannea* species

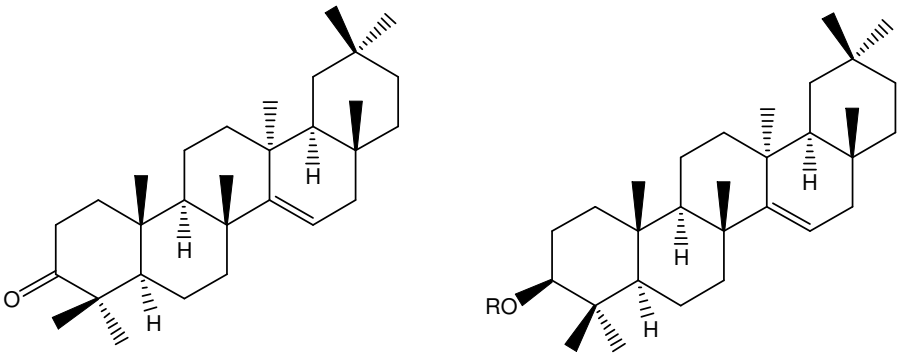
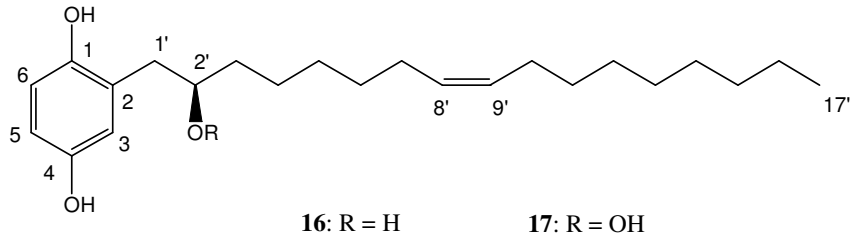
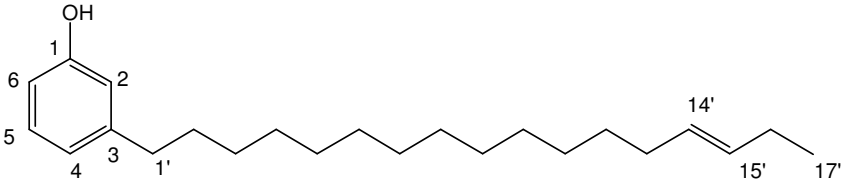


Figure 1-3 Pentacyclic triterpenes from *Lannea* species



16: R = H **17**: R = OH



18

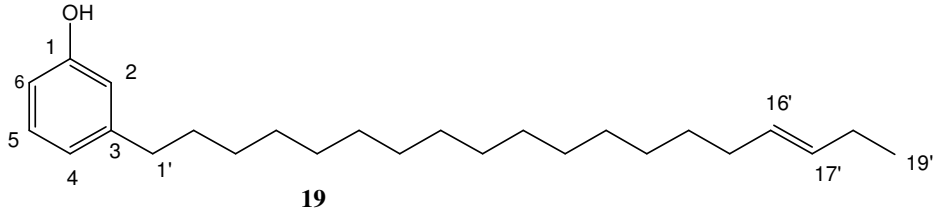


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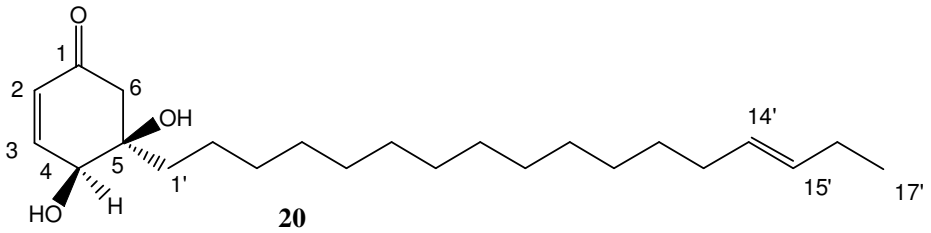
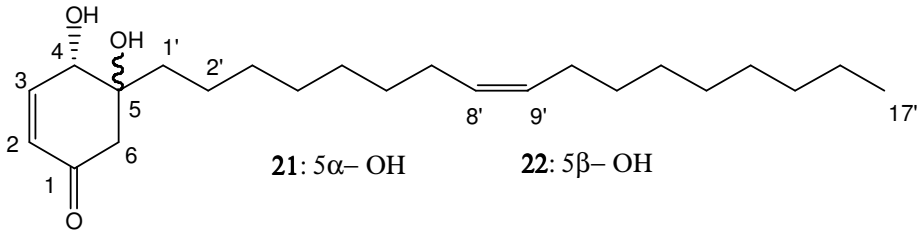
Figure 1-4 Phenolic lipids from *Lannea* species



20



21: 5α– OH **22**: 5β– OH

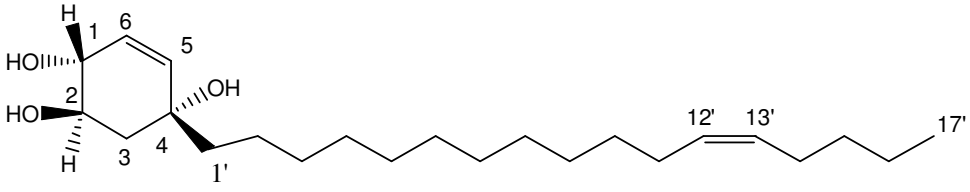


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Figure 1-5 Representative structures of cyclohexene lipid derivatives from *Lannea* species

1.4 An introduction to the plants studied in this work

Lannea schweinfurthii, *Lannea alata*, *Lannea rivae* and *Lannea schimperi* are widely distributed in Kenya. In East Africa root and stem bark concoctions of *Lannea schweinfurthii* is used in the treatment of boils, paludism/ high fever and chills related to malaria, gastrointestinal diseases, stomach pain, odema, diarrhea, constipation, abscesses, cellulitis (localized or diffused inflammation of connective tissues), oral candidiasis, amoebiasis, venereal diseases (syphilis) and opportunistic infections associated with HIV like herpes zoster and herpes simplex (Egunyomi *et al*., 2009; Gathirwa *et al.*, 2011; Geissler *et al*., 2002; Johns *et al*., 1995; Maregesi *et al*., 2007; Mothana *et al*., 2009; Tabuti *et al*., 2003).

The stem, branch and trunk juice or boiled decoction of *Lannea schimperi* is used to treat chronic diarrhea, chest problems, stomach disorders, stomach pain, coughs, opportunistic diseases (herpes simplex and herpes zoster), skin infections, anemia, mental disorders, swelling and snake bites and used as purgatives, laxatives, carminatives and anthelmintics (Batista *et al*., 2009; George *et al*., 2010; Johns *et al*., 1994; Kisangau *et al*., 2009; Nogueira and Lopes, 2011; Yun *et al*., 2012). The extracts from *Lannea schimperi* exhibited antiulcer, antibacterial, cytotoxicity and antifungal activities (Haule *et al*., 2012; Kisangau *et al*., 2009).

The bark decoction of *Lannea alata* is drunk for fever and malaria, whilst an infusion is taken for snake bite and a paste is applied on fractures, injuries and wounds (Pandey and Rizvi, 2009). It is also an excellent fruit tree for dry lands (Maundu *et al*., 2005). The inner bark of *Lannea rivae* is chewed for its sweet taste and as a source of water. The inner bark is chewed to treat colds, fever, coughs and stomachache (Maundu *et al*., 2005, Kokwaro *et al*., 2009).

Plant extracts from *Lannea schweinfurthii* have previously demonstrated antiplasmodial, antimalarial, antitrypanosomal, antigiardial, antioxidant, antibacterial antifungal, cytotoxic, acetylcholinesterase inhibition, anti-HIV and used for Herpes simplex (Adewusi and Steenkamp, 2011; Gathirwa *et al*., 2008; 2011; Johns *et al*., 1995; Maregesi *et al*., 2008; 2010; Nibret *et al*., 2010).

There are no reports of the phytochemistry or biological activity of *Lannea rivae* and *Lannea alata* in the literature even though their indigenous medicinal uses suggest antioxidant, antibacterial and antimalarial activities. Even though *Lannea schweinfurthii* and *Lannea schimperi* demonstrated biological activity that validate their medicinal roles, no phytochemical studies was performed to isolate the chemical constituents responsible for the observed activity. The aim of this study was to validate some of the medicinal uses of these four plants and to determine the compounds that are responsible for the activities observed.

1.5 Methodology used in this study

Lannea alata, *Lannea rivae*, *Lannea schimperi*, and *Lannea schweinfurthii* were identified for this study. They were collected, identified and voucher specimens deposited in reputable herbariums. The different parts of the plant were extracted with solvents of varying degrees of polarity and each extract purified using column chromatography. The aim of the separation was to isolate the components in the extract in as pure a form as possible. Fats, oils, sugars and amino acids were regarded as primary metabolites and were not targeted for isolation. Once isolated and purified, the secondary metabolites (normally small organic molecules) were subject to a range of spectroscopic techniques, NMR (both 1D and 2D), mass spectrometry, IR and UV spectroscopy to determine the structures of the isolated compounds. The known compounds were verified and compared with the data in the literature and new

compounds were verified by usually more than one set of spectroscopic evidence. Usually 2D NMR, COSY, HSQC, HMBC and NOESY data coupled with Electron Impact Mass Spectrometry (EIMS) and High Resolution Mass Spectrometry (HRMS) was used for the unequivocal elucidation of the structure.

Biological studies were conducted on the isolated compounds and in some cases crude extracts based on the traditional use of the plant. In addition, the type of activity tested was also dependent on particular compound types, for instance flavonoids, are known to be good antioxidants and therefore the flavonoids isolated were evaluated for their antioxidant activity.

This thesis reports on the new and known compounds isolated from *Lannea alata*, *Lannea rivae*, *Lannea schimperi* and *Lannea schweinfurthii* and their biological activities. This is the first phytochemical investigation of these four *Lannea* species. Compounds responsible for antioxidant, antibacterial, antiplasmodial, larvicidal and cytotoxic activity in these plants is reported here.

1.6 Hypothesis

The four species of *Lannea* are reported to have many medicinal uses and are used by traditional healers in Kenya for a range of illnesses. It is therefore hypothesized that there must be active chemical constituents either acting alone or in synergy to produce these effects.

1.7 Objectives

- To isolate the secondary metabolites present in extracts of the four *Lannea* species under investigation.
- To determine the identity of the compounds isolated if known.
- To elucidate the structures of the compounds if novel including an attempt to assign the stereochemistry in the molecule if possible.
- To determine the biological activity of the extracts of each of the species in line with what they are used for ethnomedicinally.
- To determine the biological activity of the isolated compounds in line with both the class of the compound and with what the plants are used for medicinally.
- To provide a rationale for the use of the plants in traditional medicine.
- To provide lead compounds for drug development.

Table 1-1 Ethnobotanical uses and biological activities of *Lannea* species

species	Plant part	Ethnobotanical uses	Biological activity	Reference
L. schweinfurthii Engl.	Stem and root bark, leaves	Stomachache, diarrhea, swelling of abdomen, skin rashes, oral infection, boils, febrifuge, malaria, syphilis, cellulitis, abscesses, oral candidiasis, gingivitis, nasal ulcers, asthma, neurological disorders, anaemia, coughs	Antibacterial, antifungal, antiviral (Semliki forest virus, HIV type I and II), antiplasmodial, antimalarial, toxicity, antigiardial, inhibition of acetylcholinesterase, antioxidant	Adewusi and Steenkamp, 2011; Gathirwa *et al*., 2008, 2011; Geissler *et al*., 2002; Johns *et al*., 1995; Kokwaro, 2009; Maregesi *et al*., 2007; 2008; 2010; Ribeiro *et al*., 2010
L. humilis Oliv.	roots	Anaemia, stomach pains, nausea, general body weakness	Cytotoxicity, antitrypanosomal	Kokwaro, 2009; Maregesi *et al*., 2007; Nibret *et al*., 2010
L. rivae	bark	colds, chewed for its sweet taste and as a source of water	none	Kokwaro, 2009
L.alata	Bark, roots	fever, malaria, snake bites, fractures and injuries	none	Maundu *et al*., 2005
L.triphylla	bark	Coughs, constipation, colds	none	Kokwaro, 2009
L. stuhlmanii Engl.	root	Tonic, antifungal, pain relief, herpes zoster, herpes simplex, skin infections, oral candiasis, anaemia	Cytotoxicity, antitrypanosomal, antifungal	Chinsembu and Hedimbi, 2010; Nibret *et al*., 2010; Iwu, 1993; Omolo *et al*., 1997; Runyoro *et al*., 2006
L. schimperi (A.	bark	Chronic diarrhea, pain, stomach	Antiulcer, antibacterial,	Chinsembu and Hedimbi, 2010;

Rich) Engl.		and chest problems, tuberculosis, skin problems, herpes zoster, herpes simplex	cytotoxicity, antifungal	Jeruto *et al*., 2008; Haule *et al*., 2012; Kisangau *et al*., 2007; 2009
L. microcarpa	Leaves, bark, root	Conjunctivitis, stomatitis, gingivitis, dressing wounds, skin eruptions, stomachache, beriberi, schistosomiasis and haemorroids; mouth blisters, rheumatism, sore throat, dysentery, as a cathartic and as a dressing on boils	Anti-inflammatory effect , antidiarrheoic activity, antioxidant	Bationo *et al*., 2012; Marquet and Jansen, 2005; Lamien-Meda *et al*., 2008; Ouattara *et al*., 2011b; Picerno *et al*., 2006; Tapsoba and Deschamps, 2006
L. velutina	roots and bark	diarrhoea, rachitic children, wounds and strained muscles, respiratory diseases, oedema, paralysis, epilepsy and insanity	Antioxidant and radical scavenging activities, larvicidal, molluscicidal, lipoxygenase inhibition	Diallo *et al*., 2001; Maiga *et al*., 2006; 2007; Ouattara *et al*., 2011b
L. acida	Stem bark, root	Diarrhea, stomach ache, gonorrhea, rheumatism, oral diseases, malaria	Antibacterial, antioxidant, vibriocidal, cytotoxicity	Akinsinde and Olukoya, 1995; Asase *et al*., 2005; Etuk *et al*., 2009; Kone *et al*., 2004; Ouattara *et al*., 2011a; 2011b; Sowemimo *et al*., 2009; Tapsoba and Deschamps, 2006
L.edulis	Root bark	Diarrhea, sore eyes, boils, abscesses, diabetes, schistosomiasis (bilhazia), gonorrhea, pre-hepatic	Mutagenic effects, antioxidant	Deutschlander *et al*., 2009; Maroyi, 2011, 2013; Queiroz *et al*., 2003; Sohni *et al*., 1995; Segawa and

		jaundice		Kasenene, 2007; Van Wyk *et al*., 1997
L. disclor		Malaria, fever, constipation, menorrhagia, infertility	antimalarial	Clarkson *et al*., 2004; Kazembe *et al*., 2012; Maroyi, 2013
L. welwitschii	Bark, seeds	Diarrhea, haemorroids, menstrual problems, abdominal pains, pain after birth, epilepsy, oedema, gout, swelling, palpitation, skin infections and ulcers, snake bites, wounds, diabetes	Cytotoxicity, antibacterial, anti-diarrheal, antidiabetic , antisickling activity	Deji-Agboola and Olajubu 2010; Egunyomi *et al*., 2009; Groweiss *et al*., 1997; Nyarko *et al*., 2005; Okine *et al*., 2005; Olatokunboh *et al*., 2010; Olukoya *et al*., 1993
L. coromandelica (Houtt) Merril	Bark, leaves	Bark used for treatment of diabetes, diarrhea, toothache, astringent, and lotion for leprous and other ulcers, impetigenious eruptions from contagious disease. Leaves are used for pain relief	Antioxidant, analgesic, cytotoxicity, hypotensive activity, hyperglycemic, wound healing activity, anti-atherothrombosis, antibacterial, antifungal, zoosporocidal, anti-inflammatory, anti-neuplasmic, anticancer	Abdul and Rahman, 2010; Akter *et al*., 2013; Alam *et al*., 2012; Basuri *et al*., 2011; George *et al*., 2010; Islam *et al*., 2002; Kadir *et al*., 2013; Rahmatullah *et al*., 2012; Reddy *et al*., 2011 Saravanan *et al*., 2010; Sathish, 2010; Singh and Singh, 1994; 1996
L. barteri	bark	Wounds, rheumatic, diarrhea, gastritis, sterility, intestinal	Antibacterial, antifungal, antioxidant,	Allabi *et al*., 2011; Jansen, 2005, Kone *et al*, 2005; 2011; Adoum, 2009

		helminthes, oedema, scurvy, epilepsy, malaria, anaemia	acetylcholinesterase inhibitor, brine shrimp toxicity	
L. transuta	Bark, flowers, leaves	Haemostatic for wounds, abrasion and sores	Anticancer, antioxidant, antimicrobial	Mothana *et al*., 2009
L. nigritana	Stem and root bark, leaves	diarrhea, dysentery; pain-killers, pulmonary troubles; skin, mucosae, paralysis, epilepsy, convulsions, spasm, laxatives, stomach troubles	Cytotoxic, anticancer	Burkill, 1985; Kapche *et al*., 2007; Fadeyi *et al*., 2013; Magassouba *et al*., 2007

Table 1-2 Compounds isolated from *Lannea* species

Plant species	**Isolated compounds**	**References**
L. acida	Flavonoids: *Flavanone*: 6,7,2,2-dimethylchromeno-8γ,γ-dimethylallyl *flavones*: Lanceolatin B and 7,2'-dimethoxy-4',5'-methylenedioxyflavone	Sultana and Ilyas, 1986a;1986b
L. coromandelica	Flavonoids: *dihydroflavonols*: (2*R*,3*S*)-(+)-3',5-dihydroxy-4',7-dimethoxydihydroflavonol, (2*R*,3*R*)-(+)-4',5,7-trimethoxydihydroflavonol, (2*R*,3*R*)-(+)-4',7-di-*O*-methyldihydroquercetin, (2*R*,3*R*)-(+)-4',7-di-*O*-methyldihydrokaempferol and (2*R*,3*R*)-(+)-4'-*O*-methyldihydroquercetin *flavanols*: quercetin, isoquercitrin, quercetin-3-*O*-arabinoside, quercetin-3-*O*-rutinoside, morin *Leucoanthocyanidins*: leucodelphinin, leucocyanidin *anthraquinones*; physcion and physcion anthranol B *Terpenes and terpenoids*:lanosterol, sitosterol, β-sitosterol glycoside, stigmast-4-ene-6-β-ol-3-one, 5-α-stigmastane-3-6-dione, taraxerone, taraxerol and taraxeryl acetate *Other phenolic compounds*: 4-hydroxy-3-methoxybenzaldehyde	Islam and Tahara, 2000; Nair *et al*., 1963; Sankara and Nair, 1971; Subramanian and Nair, 1971; Yun, 2012
L. edulis	Flavonoids: *isoflavans* : vestitol, 5-methoxyvestitol, lotisoflavan Phenolic lipids and their derivatives; 3-[14'(*E*)-nonadecenyl]-phenol, 3-[16'(*E*)-heptadecenyl], 4,5-dihydroxy-5[14'(*E*)-heptadecenyl]-2-cyclohexenone	Queiroz *et al*., 2003
L. grandis	Triterpenes: lanosterol Flavonoids: epicatechin, quercetin , rutin, (+) leucocyanidin Other phenolic compounds: cluytyl ferrulate	Govindac *et al*., 1971; Sulochana *et al*., 1967; 1970; Sulochana and Sastry, 1968

L. microcarpa	Flavonoids: *Flavonols*: 4'-methoxymyricetin-3-*O*-α-L-rhamnopyranoside, myricetin-3-*O*-α-L-rhamnopyranoside, myricetin-3-O-β-D-glucopyranoside *Flavones:* Vitexin, isovitexin *anthocyanidins* **:** cyanidin 3-*O*-(2-*O*-β-D-xylopyranosyl)-β-D-galactopyranoside and cyanidin 3-*O*-β-D-galactopyranoside	Pale *et al*., 1998; Picerno *et al*., 2006
L. nigratiana	Flavonoids: *Flavanols*: epicatechin gallate, epigallocatechin gallate Phenolic lipids and their derivatives: gallic acid, 3,4,5-trimethoxy phenol, 3,4,5 trimethyl-phenol glucoside, 4-hydroxy-3-methoxybenzoic acid, 3,4-dihydroxybenzoic acid, 3,4,5-trihydroxybenzoic acid, lanneanol (1,2,4 trihydroxy-4-[12'(*Z*)-heptadecenyl]cyclohexe-5-ene)	Kapche *et al*., 2007
L. welwitschii	Phenolic lipids and their derivatives; alkyl hydroquinones lanneaquinol and 2'(*R*)-hydroxyhydroquinones, alkyl cyclohexenones, 4,5-dihydroxy-5-[8'(*Z*)-heptadecenyl]-2-cyclohexenones	Groweiss *et al*., 1997
L. velutina	Catechin, procyanidin B-1 and a series of proanthocyanidins with different degrees of polymerization between 3-12	Maiga *et al*., 2007

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Chapter 2 Antibacterial and antioxidant activities of flavonoids from *Lannea alata* (Engl.) Engl. (Anacardiaceae)

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Abstract

Two new prenylated flavonoids, lanneaflavonol (**A1**) and dihydrolanneaflavonol (**A2**) together with the known compounds myricetin-3-*O*-α-rhamnopyranoside (myricitrin) (**A3**) and myricetin-3-*O*-α-arabinofuranoside (betmidin) (**A4**), lupeol (**A5**) and sitosterol (**A6**) were isolated from the roots of *Lannea alata*. Compounds **A1**-**A4** exhibited good antibacterial and radical scavenging activity with the glycosides **A3** and **A4** showing better antioxidant activity than the aglycones **A1** and **A2** and betmidin (**A4**) showing the best antimicrobial activity followed by the aglycones **A1** and **A2**. Betmidin (**A4**) with an arabinose moiety at the 3-*O*-position showed the best antibacterial activity against Gram-positive bacteria, followed by the prenylated dihydroflavonol (**A2**), whilst the prenylated linear flavonol (**A1**) showed limited activity against Gram-negative bacteria. The arabinofuranoside (**A4**) followed by the rhamnopyranoside (**A3**) showed the best antioxidant activity comparable to that of ascorbic acid. The biological activities justify the ethnomedicinal uses of the plant in the management of diseases associated with Gram-positive bacteria, such as being used to treat injuries and wounds.

Keywords: *Lannea alata*, prenylated flavonoids, lanneaflavonol, lanneadihydroflavonol, antibacterial activity, antioxidant activity.

2.1 Introduction

Lannea alata (Engl.) Engl. is a deciduous shrub about 1.5-4 m high, with drooping branches and a smooth grey bark. The leaves are clustered on short shoots and divided into tiny leaflets which are bluntly toothed towards the apex and the roots are covered by dense wool like hair (Maundu *et al*., 1999). The bark decoction is drunk for fever and malaria, whilst an infusion is taken for snake bite and a paste is applied on fractures, injuries and wounds (Maundu *et al*., 1999). It is also an excellent fruit tree for dry lands (Maundu *et al*., 1999).

There is no literature on the chemistry of the plant or biological activities, but based on its ethnomedicinal uses it potentially has anti-plasmodial (being used for malaria) and antimicrobial (being used for injuries and wounds) activities. Although there have been no phytochemical reports on *L. alata* itself, other related species of *Lannea* have been reported to contain alkylated hydroquinones and cyclohexenones (Groweiss *et al*., 1997), polyflavonoid tannins (Islam *et al*., 2002), polysaccharides (Ramachandran and Joshi, 1968), cyanidins (Sulochana *et al*., 1967), flavonoids (Sulochana and Sastry, 1968; Sultana and Ilyas, 1986), alkylphenols and dihydroalkylhexenones (Queiroz *et al*., 2003).

The phytochemical investigations of *L. alata* stem and root extracts led to the isolation of four flavonoids (**A1**-**A4**) and two common triterpenes, sitosterol (**A5**) and lupeol (**A6**). Based on the traditional use of the plant and nature of the isolated compounds, antibacterial and antioxidant activity testing was carried out on the isolated compounds and is reported herein.

2.2 Results and Discussion

The ethyl acetate and n-hexane extracts yielded two novel prenylated flavonoids **A1** and **A2** as well as two known flavonoid glycosides, myricetin-3-*O*-α-L-rhamnopyranoside

(myricitrin) (**A3**) (Fossen *et al*., 1999) and myricetin-3-*O*-α-L-arabinofuranoside (betmidin) (**A4**) (Kim *et al*., 1994) as well as two ubiquitous triterpenoids, lupeol (**A5**) (Burns *et al*., 2000) and sitosterol (**A6**) (Kovganko *et al*., 1999). The structures of **A1** and **A2** (Figure **2-1**) were elucidated by a combination of spectroscopic methods while compounds **A3**-**A6** were identified by comparison of their spectral data with that in the literature.

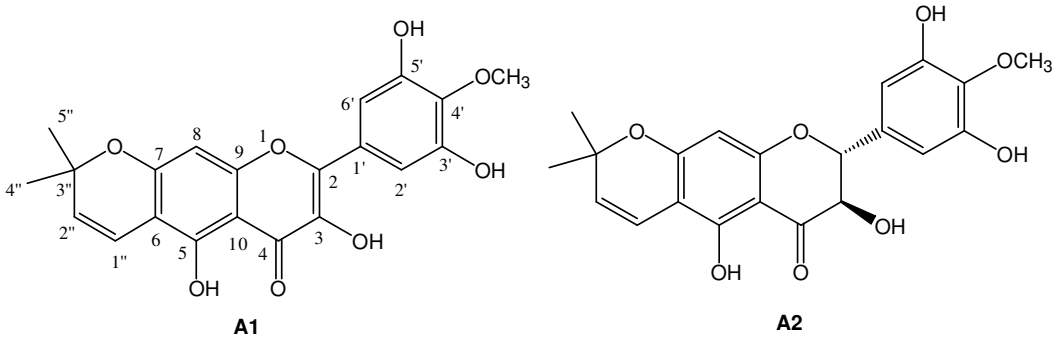

Figure 2-1 The structures of lanneaflavonol (**A1**) and dihydrolanneaflavonol (**A2**)

Compound **A1** was isolated as a yellow solid. The IR spectrum exhibited absorption bands suggesting the presence of hydroxyl (3323 cm^{-1}), chelated carbonyl (1655 cm^{-1}), geminal dimethyl (1380 cm^{-1}), methoxy groups (2985 cm^{-1}) and aromatic rings (1585 and 1478 cm^{-1}). The UV spectrum showed absorption bands at 290 and 357 nm indicative of a flavonol skeleton (Mabry *et al*., 1970). The mass spectrum showed a molecular ion peak at *m/z* 398 followed by the cleavage of two methyl groups, *m/z* 283 [M^+ -CH_3] and *m/z* 268 [M^+ -$2CH_3$].

The ^1H NMR spectrum showed the characteristic resonances of a cyclised prenyl group with a pair of doublets at δ_H 5.79 (H-2", J = 10.04 Hz) and 6.61 (H-1", J = 10.04 Hz) and a six-proton methyl resonance indicating two equivalent methyl groups at δ_H 1.43 (2 x CH_3-4"). In addition, a two-proton singlet aromatic resonance at δ_H 7.19, characteristic of phenyl protons

(H-2'/6') and a benzopyran aromatic resonance at δ_H 6.45 (H-8) was observed. A methoxy resonance at δ_H 3.74 (4'-OCH_3) was also present. Further to this, three exchangeable proton resonances at δ_H 12.83, 9.58 and 9.40 (2H) was present and ascribed to the phenolic protons 5-OH, 3-OH and 3'/5'-OH respectively.

The carbon spectrum consisted of seventeen carbon resonances with fourteen of these resonances being in the aromatic or double bond region and a chelated carbonyl resonance at δ_C 176.21, consistent with the structure of a flavonoid. Of the fourteen resonances, seven resonances were oxygenated between δ_C 136 and 158 with the resonance at δ_C 150.53 being due to two carbon resonances as indicated by the intensity of the resonance. These were attributed to the eight oxygenated carbon atoms, C-2, C-3, C-5, C-7, C-9, C-3'/5' and C-4'. A further six resonances in the olefinic and aromatic region between δ_C 94 and 129 were attributed to either the protonated or singlet carbon atoms of the olefinic bonds or the aromatic rings, C-6/10, C-8, C-1', C-2'/6', C-1" and C-2". The oxygenated carbon C-3" occurred at δ_C 77.96, the methoxy carbon at δ_C 59.74 and the equivalent geminal dimethyl resonance occurred at δ_C 27.84.

Both the olefinic proton resonances of the cyclised prenyl group, H-1" and H-2" were seen coupled in the COSY spectrum. H-2" showed HMBC correlations to C-4", the methyl carbon at δ_C 27.84 and the oxygenated carbon of the prenyl group at δ_C 77.96 as well as to the singlet aromatic carbon at δ_C 104.02. The fact that this resonance was also seen coupled to the chelated hydroxyl proton of 5-OH prompted us to form a bond between C-1" and C-6 rather than C-1" and C-8, resulting in a linear pyranoflavone rather than an angular one. Furthermore, C-10 at δ_C 103.98 was very close to that of C-6 and both these resonances showed HMBC correlations to H-8 at δ_H 6.45. H-1" showed HMBC correlations to C-6 (δ_C

104.02) and C-5 at δ_C 155.24 and C-7 at δ_C 158.61. The H-8 resonance showed a HMBC correlation to C-7 and C-9 at δ_C 154.67. The proton resonance at δ_H 7.19 was assigned to H-2'/6' of the phenyl ring based on HMBC correlations to C-2 at δ_C 146.56. Furthermore, the 3'/5'-OH proton resonance showed HMBC correlations to both C-4' and C-2'/6', confirming the substitution pattern on ring B. Finally, the resonance at δ_C 137.32 was assigned to C-4' due to a HMBC correlation with the methoxy group at δ_H 3.75. The compound was thus identified as 3,5,3',5'-tetrahydroxy-4'-methoxy-6,7-(2",2"-dimethylchromene)-flavonol and accorded the trivial name lanneaflavonol.

The ^{1}H NMR spectrum of compound **A2** (a light yellow solid) was very similar to that of **A1** with the proton resonances and splitting patterns of the 5-OH, 3'/5'-OH, H-8, H-2'/6', 4'-OCH_3, H-1", H-2" and 3H-4" all being very close to that of **A1**. However there were three new resonances, two doublets and a double doublet, that appeared in the ^{1}H NMR spectrum of **A2**, at δ_H 5.88 (J = 6.28 Hz), which did not correspond to any carbon in the HSQC spectrum, indicative of a hydroxyl proton resonance, and at δ_H 4.97 (J = 11.05 Hz) and δ_H 4.47 (J = 11.05, 6.20 Hz) which corresponded to aliphatic oxygenated carbon resonances at δ_C 82.98 and 71.51 respectively. These two carbon resonances were also not present in the ^{13}C NMR spectrum of compound **A1**. Furthermore, the C-2 and C-3 resonances of **A1** were absent in **A2**. This led to the conclusion that the Δ^2 double bond in **A1** had now been saturated in **A2** and the 2,3-dihydro derivative was now present. Based on their coupling constants and correlations in the COSY spectrum, δ_H 5.88 was attributed to the 3-O<u>H</u> resonance, δ_H 4.97 to H-2 and δ_H 4.47 to H-3. COSY correlations existed between H-2 and H-3 and H-3 and 3-O<u>H</u>. The three carbon resonances at δ_C 83.02 (C-2), 71.55 (C-3) and 198.32 (C-4) were characteristic of 3-hydroxyflavanones (Agrawal, 1989). The molecular

ion peak at *m/z* 400 in the MS confirmed this and there was also a peak at *m/z* 382 [M^+ - H_2O] typical for an aliphatic hydroxyl group.

Based on the large coupling constant of H-2 and H-3 (J = 11.05 Hz), the configuration at C-2 and C-3 was assigned as *trans* diaxial and together with a positive optical rotation, the absolute stereochemistry was of the *2R*, *3R* configuration, consistent with dihydroflavanols with a positive optical rotation (Bohm, 1975). This was supported by the CD curve of **A2**, which showed four cotton effects in the order (+), (-), (+), (+) from 400 to 200 nm (Markham and Mabry, 1968; Ruangrungsi *et al*., 1981), and a positive cotton effect at $[\Theta]_{320}$ due to the n-π^* transition and a negative effect at $[\Theta]_{300}$, due to the π - π^* transition, consistent with that of the *2R*:*3R* configuration (Gaffield, 1970; Slade *et al*., 2005; Cao *et al*., 2006).

Thus, **A2** was identified as (*2R*,*3R*)-3,5,3',5'-tetrahydroxy-4'-methoxy-6,7-(2'',2''-dimethylchromene)-dihydroflavonol and accorded the trivial name lanneadihydroflavonol.

Compound **A3**, identified as myricetin-3-*O*-α-L- rhamnopyranoside (myricetin) was previously isolated from *Nyphaea caerulae* (Fossen *et al*., 1999) and *Lannea microcarpa* (Picerno *et al*., 2006), while compound **A4** was identified as the flavonol glycoside, myricetin-3-*O*-α-L-arabinofuranoside (betmidin), which has been previously isolated from the leaves of *Polygonium aviculare* (Kim *et al*., 1994).

Antibacterial activity

The crude methanol and ethyl acetate extracts showed good activity against *Enterococcus faecium* ATCC 19434 with inhibition zones of 18 and 16 mm respectively and the hexane extract showed intermediate activity with a 10 mm inhibition zone. However, none of the

isolated compounds showed activity to *E. faecium* indicating that this activity must have either been due to some other compound or a synergistic effect of several compounds. Compound **A1** demonstrated activity comparable to that of tetracycline against both *Pseudomonas* strains (Table **2-1**). Interestingly the dihydroflavonol (**A2**) did not show the same activity, suggesting that the planar structure of the flavonol (**A1**) is more suited as a Gram-negative antibacterial agent and that the activity is probably associated with the 3-OH group which must be situated on a planar double bond.

All four isolated compounds demonstrated antimicrobial activity against several strains of Gram-positive bacteria, with compound **A4** (myricetin-3-*O*-α-arabinofuranoside) being the most effective, followed by compounds **A2**, **A1** and **A3**. *B. subtilis* ATCC 6633 demonstrated susceptibility to both compounds **A2** and **A4** and was intermediately susceptible to compound **A1**, while *S. pyogenes* ATCC 19615 was susceptible to compound **A4**, and intermediately susceptible to compounds **A2** and **A3**. All the compounds were generally less active compared to the standard antimicrobial agents, ampicillin and tetracycline, although compound **A4** demonstrated activity against *S. aureus* strains that were close to that of ampicillin. The activity of **A4** over **A3** indicates that the arabinose sugar attached to the 3-position has better activity than the rhamnose attached at the same position, demonstrating the importance that the type of sugar attached to the 3-oxygenated position has on antimicrobial activity. Results of the disc diffusion assay indicate that the presence of the double bond actually decreases activity against Gram-positive bacteria and that the chiral centres of C-2 and C-3 are important for activity. This contrasts with findings of Xu and Lee (2001), who demonstrated that quercetin (flavonol) with the Δ^2 double bond displayed better activity than catechin (flavanol) (Xu and Lee, 2001). However, the inactivity of catechin could also be due to the absence of the carbonyl group at C-4.

Table 2-1 Antimicrobial susceptibility: Mean zones of inhibition (mm) for isolated compounds 1-4 and standard antimicrobial agents, ampicillin and tetracycline

Bacteria[a]	**Compounds/Extracts**[b]								
	A1	**A2**	**A3**	**A4**	**H**	**E**	**M**	**A**	**T**
Gram negative									
P. aeruginosa ATCC 27853	$8^{(R)}$	$0^{(R)}$	$0^{(R)}$	$0^{(R)}$	$0^{(R)}$	$0^{(R)}$	$0^{(R)}$	$0^{(R)}$	$10^{(R)}$
P. aeruginosa ATCC 35032	$9^{(R)}$	$0^{(R)}$	$0^{(R)}$	$0^{(R)}$	$0^{(R)}$	$0^{(R)}$	$0^{(R)}$	$0^{(R)}$	$9^{(R)}$
Gram positive									
B. subtilis ATCC 6633	$\mathbf{11}^{(I)}$	$\mathbf{16}^{(S)}$	$10^{(R)}$	$\mathbf{20}^{(S)}$	$0^{(R)}$	$0^{(R)}$	$0^{(R)}$	$32^{(S)}$	$35^{(S)}$
E. faecium ATCC 19434	$0^{(R)}$	$0^{(R)}$	$0^{(R)}$	$0^{(R)}$	$10^{(I)}$	$\mathbf{16}^{(S)}$	$\mathbf{18}^{(S)}$	$16^{(S)}$	$9^{(R)}$
S. aureus ATCC 29213	$8^{(R)}$	$\mathbf{15}^{(S)}$	$9^{(R)}$	$\mathbf{20}^{(S)}$	$0^{(R)}$	$0^{(R)}$	$0^{(R)}$	$22^{(S)}$	$26^{(S)}$
S. aureus ATCC 43300	$9^{(R)}$	$\mathbf{14}^{(I)}$	$0^{(R)}$	$\mathbf{18}^{(S)}$	$0^{(R)}$	$0^{(R)}$	$0^{(R)}$	$22^{(S)}$	$26^{(S)}$
S. epidermidis ATCC14990	$8^{(R)}$	$\mathbf{11}^{(I)}$	$0^{(R)}$	$0^{(R)}$	$0^{(R)}$	$0^{(R)}$	$0^{(R)}$	$21^{(S)}$	$29^{(S)}$
S. saprophyticus ATCC 35552	$8^{(R)}$	$\mathbf{13}^{(I)}$	$8^{(R)}$	$7^{(R)}$	$0^{(R)}$	$0^{(R)}$	$0^{(R)}$	$36^{(S)}$	$26^{(S)}$
S. scuiri ATCC 29062	$9^{(R)}$	$\mathbf{12}^{(I)}$	$0^{(R)}$	$\mathbf{14}^{(I)}$	$0^{(R)}$	$0^{(R)}$	$0^{(R)}$	$21^{(S)}$	$29^{(S)}$
S. xylosus ATCC 35033	$8^{(R)}$	$\mathbf{14}^{(I)}$	$8^{(R)}$	$\mathbf{16}^{(S)}$	$0^{(R)}$	$0^{(R)}$	$0^{(R)}$	$23^{(S)}$	$26^{(S)}$
S. agalactiae ATCC 13813	$0^{(R)}$	$0^{(R)}$	$10^{(R)}$	$0^{(R)}$	$0^{(R)}$	$0^{(R)}$	$0^{(R)}$	$0^{(R)}$	$22^{(S)}$
S. pyogenes ATCC 19615	$10^{(R)}$	$\mathbf{14}^{(I)}$	$\mathbf{12}^{(I)}$	$\mathbf{22}^{(S)}$	$0^{(R)}$	$0^{(R)}$	$0^{(R)}$	$26^{(S)}$	$32^{(S)}$

[a]The Gram-positive *Enterococcus faecalis* ATCC 51299 and the Gram-negative *Escherichia coli* ATCC 25922 and ATCC 35218 and *Klebsiella pneumoniae* ATCC 700603 were resistant to compounds **A1**-**A4** and the crude extracts and exhibited no zones of inhibition.

[b]Compounds/Extracts: lanneaflavonol (**A1**), dihydrolanneaflavonol (**A2**), myricetin-3-*O*-α-rhamnopyranoside (myricitrin) (**A3**), myricetin-3-*O*-α-arabinofuranoside (**A4**), hexane extract (**H**), ethyl acetate extract (**E**), methanol extract (**M**), Ampicillin AMP10 (**A**); Tetracylcine TE30 (**T**).

(R) denotes resistance, (I) denotes intermediate susceptibility and (S) denotes complete susceptibility

The MIC values of the four compounds were determined on three *Staphylococcus* spp. strains (*S. aureus* ATCC 29213, *S. sciuri* ATCC 29062 and *S. xylosus* ATCC 35033), based on the disc diffusion results. However, the compounds were only active in the mM range with compounds **A3** and**A 4** showing slightly better activity than **A1** and **A2** (Table **2-2**).

However the MIC values of the test compounds were not comparable to the standard antibiotics ampicillin and tetracycline which was active against the same bacterial species in the μM range.

Table 2-2 Minimum inhibitory concentrations of compounds **A1-A4** against selected Gram-positive bacterial species

Bacteria	**Compounds (mM)**				**Antibiotics (μM)**	
	A1	**A2**	**A3**	**A4**	Amp[a]	Tet[a]
S. aureus ATCC 29213	2.01	2.00	0.86	0.89	1.47	0.58
S. sciuri ATCC 29062	2.01	2.00	0.86	0.89	0.37	0.58
S. xylosus ATCC 35033	2.01	2.00	3.45	0.89	1.47	1.15

[a] Standard antibiotics Ampicillin and Tetracycline

Antioxidant activity

When tested individually, each flavonoid (**A1-A4**) exhibited dose dependent radical-scavenging activity in the presence of the DPPH radical. The percentage radical scavenging activity for the isolated flavonol glycosides and ascorbic acid for comparison are given in Table **2-3**. The flavonoids exhibited radical scavenging activity in the order **A4>A3>A1>A2**. This is in keeping with the idea that the glycosides can generate extra hydrogen radicals due to the greater number of free hydroxyl groups, capable of quenching radicals better than the aglycones. Our studies are contrary to those reported in a review by Rice-Evans *et al*. (1996) which reports that blocking the 3-OH group with a glycoside reduces activity, however it must be noted that the compounds reported in the review by Rice-Evans *et al*. (1996) is based on compounds with free hydroxy groups at C-5 and C-7. Compounds **A1** and **A2** possess a cyclised prenyl group, which may be responsible for reduced antioxidant activity compared to the glycosides. Furthermore, the fact that the dihydroflavonol (**A2**) is a better antioxidant

than the flavonol (**A1**) is also contrary to reports that the unsaturated Δ^2 double bond is better for antioxidant activity (Rice-Evans *et al*., 1996). Since the two compounds **A1** and **A2** are the same in all respects except for the difference in saturation at C-2 and C-3, it must be concluded that this is specific for cyclised prenylated flavonols.

Table 2-3 Antioxidant activity of compounds isolated from *Lannea alata*: Mean % radical scavenging activity

	Concentrations					**Mean Comp.** *	**IC_{50}**
Comp.	**6.25 µg mL^{-1}**	**12.50 µg mL^{-1}**	**25.00 µg mL^{-1}**	**50.00 µg mL^{-1}**	**100 µg mL^{-1}**		
A1	23.80 ± 0.88	31.91 ± 1.74	48.86 ± 0.72	65.70 ± 2.83	75.02 ± 0.96	49.06[a]	25.96
A2	46.07 ± 1.03	53.36 ± 1.17	72.36 ± 2.16	80.37± 1.14	87.36 ± 1.40	68.77[b]	20.96
A3	62.68 ± 2.15	72.07 ± 1.42	80.46 ±1.40	86.46 ± 1.22	91.73 ± 2.08	78.69[c]	20.13
A4	65.49 ± 2.15	78.82 ± 1.95	86.72 ±0.38	93.64 ± 1.78	97.10 ± 0.98	84.35[d]	16.83
Ascorbic acid	87.26 ± 1.72	94.04 ± 1.13	96.66 ± 0.57	99.04 ± 0.16	99.97 ± 0.57	95.52[e]	11.43
Mean. Conc.*	57.26[a]	59.08[b]	68.40[c]	82.60[d]	88.29[e]		

*means that differ significantly have different letters, $p < 0.05$; mean conc. indicates the mean of all the compounds tested at a specific concentration; mean comp. indicates the mean of the compound tested at different concentrations

The flavonoids myricetin-3-*O*-α-rhamnopyranoside (**A3**) and myricetin-3-*O*-α-arabinofuranoside (**A4**) displayed antioxidant activity slightly lower than ascorbic acid at lower concentrations but comparable to ascorbic acid at higher concentrations. These compounds (**A3** and **A4**) are known to have strong antioxidant activity (Jayasinghe *et al*., 2012; Tung *et al*., 2009; Abd El-Kader *et al*., 2012; Yan *et al*., 2002).

2.3 Experimental

General Experimental Procedures

The ^{1}H, ^{13}C and all 2D NMR spectroscopy were recorded using a Bruker Avance^{III} 400 MHz spectrometer at 400.22 MHz for ^{1}H and 100.63 MHz for ^{13}C. 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 referenced to the internal standard, TMS, respectively. IR spectra were recorded using a Perkin Elmer Universal ATR spectrometer. Optical rotations were measured at 20 °C on a Perkin Elmer™, Model 341 Polarimeter with a 10 cm flow tube. Circular dichroism was performed on a Chirosan Plus spectropolarimeter (Applied Photophysics). UV spectra were obtained on a Hewlett Packard UV-3600 Spectrophotometer. The melting points were determined on an Ernst Leitz Wetziar micro-hot stage melting point apparatus and are uncorrected. Merck silica gel 60 (0.040–0.063 mm) was used for column chromatography and Merck 20 cm × 20 cm silica gel 60 F_{254} aluminium sheets were used for thin-layer chromatography. The TLC plates were analysed under UV (254 and 366 nm) before being sprayed and developed with a [1:2:97] anisaldehyde:concentrated sulphuric acid:methanol spray reagent and then heated.

Plant Material

The stem and roots of *Lannea alata* (Engl.) Engl. were collected in June 2011, at Wote (Makueni District) in Kenya and the specimen authenticated and deposited at the Maseno University, Botanic garden Herbarium in Kenya, voucher no MSU/ BG-3/13. The stem and roots of the plant were dried and ground using a Wiley mill.

Chromatographic Isolation

The powdered dry roots of *Lannea alata* (5 kg) were sequentially extracted with 10 L of hexane, ethyl acetate and methanol using an orbital shaker. For the root extraction, TLC of

the crude extracts before evaporation showed that the hexane and ethyl acetate extracts had a similar profile and these extracts were therefore combined. After evaporation of the solvents, 33.54 g of hexane-ethyl acetate extract and 43.43 g of methanol extract was obtained. The hexane-ethyl acetate extract (22.8 g) was chromatographed on silica gel with a hexane:ethyl acetate gradient mixture starting from 100% hexane and increasing the polarity stepwise by 10% after collection of every 1L of eluent, up to 100% ethyl acetate. In the last step, a 5% methanol in ethyl acetate mixture was used. Ten fractions of 100 mL were collected for each step and 120 fractions collected in total. Fractions were monitored on TLC plates using UV light and anisaldehyde spray reagent. Further purification of fraction 20-30 with 5% ethyl acetate in hexane led to the isolation of an amorphous white solid **A5** (35.42 mg) in fractions 6-13 and white crystals **A6** (98.56 mg) in fractions 16-20. Fraction 30-34 of the crude column was rechromatographed and eluted with 20% ethyl acetate in hexane. Fifteen fractions were collected of which fraction 7-10 yielded a yellow solid **A1** (379.80 mg). Fraction 41-48 was eluted with 30% ethyl acetate in hexane and resulted in a pale yellow solid **A2** (290.27 mg), which was eluted in fractions 19-26. TLC analysis of fractions 80-100 of the crude column indicated that it was made up of two compounds. Purification of this fraction with Sephadex LH20 using methanol as the solvent, resulted in two compounds **A3** (62.68 mg) and **A4** (29.50 mg) in fractions 7 and 10 respectively. The methanol extract of the roots (28.6 g) was chromatographed with 2L of ethyl acetate: methanol (95:5). Fraction 5-9 contained compounds **A3** and **A4**, which was purified as above yielding 239.6 mg of **A3** and 184.8 mg of **A4**.

The stem bark (1.58 kg) was extracted sequentially as above with hexane, ethyl acetate and methanol to yield 6.97 g of hexane, 10.15 g of ethyl acetate and 22.18 g of methanol extract. TLC analysis of the hexane and ethyl acetate extracts showed similar profiles and were

combined and separated as above, yielding 35.12 mg of **A1**, 72.13 mg of **A2**, 187.53 mg of **A5** and 283.21 mg of **A6**. The methanol extract yielded 45 mg of **A3** and 29.65 mg of **A4**. The compounds were isolated and purified as above.

3,5,3',5'-tetrahydroxy-4'-methoxy-6,7-(2'',2''-dimethylchromene)-flavonol (lanneaflavonol) **(A1)** yellow solid; m.p. 202-203 °C; UV (CH_3OH) λ_{max} (log ε) 290 (2.80), 357 (2.97) nm; IR υ_{max} (cm^{-1}) 3323 (O-H), 1655 (C=O), 1631, 1585, 1478, 1380, 1147, 1045; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.43 (6H, s, 2 x H-4''), 3.74 (3H, s, 4'-OCH_3), 5.80 (1H, d, *J* = 10.04 Hz, H-2''), 6.45 (1H, s, H-8), 6.61 (1H, d, *J* = 10.04 Hz, H-1''), 7.19 (2H, s, H-2'/6'), 9.40 (2H, s, 3'/5'-O<u>H</u>), 9.58 (1H, s, 3-O<u>H</u>), 12.83 (1H, s, 5-O<u>H</u>); ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.84 (2C, C-4''), 59.73 (4'-O<u>C</u>H_3), 77.96 (C-3''), 94.36 (C-8), 103.98 (C-10), 104.02 (C-6), 107.34 (2C, C-2'/6'), 114.44 (C-1''), 125.65 (C-1'), 129.00 (C-2''), 136.74 (C-3), 137.31 (C-4'), 146.56 (C-2), 150.53 (C-3'/5'), 154.67 (C-9), 155.24 (C-5), 158.61 (C-7), 176.21 (C-4); EIMS *m/z* (rel. int.): 398 [M^+] (34), 383 [M^+ -CH_3] (100), 368 [M^+ - (2 x CH_3)] (30); HREIMS *m/z* 398.1006 [M^+] (calcd for $C_{21}H_{18}O_8$, 398.1002).

(2R,3R)-3,5,3',5'-tetrahydroxy-4'-methoxy-6,7-(2'',2''-dimethylchromene)-dihydroflavonol (lanneadihydroflavonol) **(A2)** light yellow solid; m.p. 208-209 ° C; $[\alpha]_D^{20}$ +30.77° (c = 0.13, MeOH); CD (MeOH) [Θ] (deg cm^2 $decimole^{-1}$): $[\Theta]_{220}$ +3000, $[\Theta]_{300}$ -12180, $[\Theta]_{320}$ +9450, $[\Theta]_{370}$ +9450; UV (MeOH) λ_{max} (log ε) 204 (4.94), 272 (4.89), 295 (4.42) nm; IR υ_{max} (cm^{-1}) 3571, 3428 (O-H), 3362 (O-H), 2977, 1651 (C=O), 1634, 1574, 1463, 1360, 1126, 1035; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.39 (6H, s, 2 x H-4''), 3.68 (3H, s, 4'-OCH_3), 4.47 (1H, dd, *J* = 11.04, 6.20 Hz, H-3), 4.97 (1H, d, *J* = 11.04 Hz, H-2), 5.65 (1H, d, *J* = 10.08 Hz, H-2''), 5.88 (1H, d, *J* = 6.28 Hz, 3-O<u>H</u>), 5.91 (1H, s, H-8), 6.43 (2H, s, H-2'/6'), 9.16 (2H, s, 3'/5'-O<u>H</u>), 12.19 (1H, s, 5-O<u>H</u>); ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.90 (2 x C-4''), 59.60 (4'-

OCH_3), 71.51 (C-3), 78.26 (C-3''), 82.98 (C-2), 95.56 (C-8), 101.15 (C-10), 102.18 (C-6), 107.11 (C-2'/6'), 114.37 (C-1''), 126.99 (C-2''), 132.04 (C-1'), 135.67 (C-4'), 150.42 (C-3'/5'), 157.22 (C-5), 161.19 (C-9), 161.73 (C-7), 198.28 (C-4). EIMS *m/z* (rel. int.): 400 [M^+] (2), 382 [M^+-H_2O] (23), 367 [M^+-H_2O-CH_3] (7), 281 (21), 241 (21), 207 (48), 177 (100). HREIMS *m/z* 400.1162 [M^+] (calcd for $C_{21}H_{20}O_8$, 400.1158).

Antibacterial Activity

Bacterial strains

Eleven Gram-positive strains: *Bacillus subtilis* ATCC 6633, *Enterococcus faecium* ATCC 19434, *Enterococcus faecalis* ATCC 51299, *Staphylococcus aureus* ATCC 29213 and ATCC 43300, *Staphylococcus epidermidis* ATCC 14990, *Staphylococcus saprophyticus* ATCC 35552, *Staphylococcus sciuri* ATCC 29062, *Staphylococcus xylosus* ATCC 35033, *Streptococcus agalactiae* ATCC 13813, *Streptococcus pyogenes* ATCC 19615 and five Gram-negative strains, *Escherichia coli* ATCC 25922 and ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853 and ATCC 35032 and *Klebsiella pneumoniae* ATCC 700603 were used for the antibacterial assays. Isolates were maintained on brain heart infusion (BHI) agar or tryptic soy agar (TSA) plates at 4 °C and for long-term storage as 20% glycerol stocks at -70 °C.

Antimicrobial susceptibility testing

The antimicrobial susceptibility to the flavonoids isolated from *L. alata* was determined using the disc diffusion method (CLSI, 2007). A 20 mg mL^{-1} stock solution of each compound and crude extracts was made using DMSO. Blank discs (6 mm; MAST, UK) were impregnated with 20 µL of each compound (400 µg mL^{-1}) and crude extracts (400 µg mL^{-1}) and allowed to dry. The bacterial isolates, grown overnight on BHI or TSA agar plates, were resuspended in sterile distilled water and the turbidity of cell suspensions adjusted equivalent

to that of a 0.5 McFarland standard. These were used to inoculate Mueller-Hinton (MH) agar plates by streaking swabs over the entire agar surface followed by the application of the respective phytochemical extract discs (CLSI, 2007). Plates were then incubated for 24 h at 37 °C. Testing was done in duplicate and tetracycline (TE30) and ampicillin (AMP10) discs (Oxoid, UK) were used as standard antimicrobial agent controls, while DMSO-impregnated discs were used as negative controls. Zone diameters were determined and averaged. The following zone diameter criteria were used to assign susceptibility or resistance to phytochemicals tested: Susceptible (S) ≥ 15 mm, Intermediate (I) = 11 – 14 mm, and Resistant (R) ≤ 10 mm. The criteria for assigning susceptibility or resistance to AMP10 was as follows: (S) ≥ 17 mm, (I) = 14 – 16 mm, (R) ≤ 13 mm, while those for TE30 were: (S) ≥ 19 mm, (I) 15 – 18 mm, (R) ≤ 14 mm (CLSI, 2007).

Three *Staphylococcus* spp. strains (*S. aureus* ATCC 29213, *S. sciuri* ATCC 29062 and *S. xylosus* ATCC 35033) were selected for the determination of MICs based on their disc-diffusion susceptibility data. MICs of the four isolated compounds together with ampicillin and tetracycline were determined using the broth microdilution assay (Andrews, 2001). Cultures were grown overnight on TSA and diluted equivalent to a 0.5 McFarland standard (Andrews, 2001). Microtiter plate wells (final total volume of 200 µL), each containing 90 µL of Mueller-Hinton (MH) broth were inoculated with 10 µL of cell suspension and two-fold serial dilutions of compounds 1-4 (215.34 µM to 4.02 mM) and antimicrobial agents ampicillin and tetracycline (9.00 nM to 9.22 µM). The plates were incubated at 30 ºC for 24 h without shaking. The negative control wells contained MH broth only and the positive control wells contained the respective cell suspensions with no compound/antimicrobial agents added. This was done in triplicate (Andrews, 2001). The MIC was the lowest concentration of antimicrobial agent, which inhibited visible growth of organism.

Antioxidant Activity

A 10 mg mL^{-1} stock solution of each compound was made by dissolving the compounds in DMSO. Sample concentrations of 6.25, 12.50, 25.00, 50.00 and 100 μg mL^{-1} were made in methanol. The DPPH radical scavenging activity of the compounds was determined according to the method of Kumawat *et al*. (2012). Briefly, 1 mL of each compound was added to 1 mL of DPPH (1,1-diphenyl-2-picrylhydrazyl) solution (0.1 mM in methanol). The mixture was shaken and kept in darkness for 30 minutes at room temperature. The decrease of solution absorbance was determined at 517 nm. Vitamin C (ascorbic acid) was used as the positive control. The DPPH radical scavenging activity was calculated using the following formula:

DPPH Radical Scavenging Activity (%) = $[(A_0 - A_1 / A_0) \times 100]$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the compound or standard sample.

The statistical analysis was performed using a two-way analysis of variance (ANOVA) followed by the Tukey's multiple range post-hoc test to separate the means. SPPS version 12 software was used for analysis.

2.4 Conclusion

The occurrence of the new flavonol and dihydroflavonol (**A1** and **A2**), derived from myricetin add to the number of flavonoids already identified within *Lannea* species and in particular add prenylated flavonoids to the list. Compound **A4** was effective against certain strains of Gram-positive bacteria, while both compounds **A3** and **A4** had antioxidant activity

comparable to that of ascorbic acid. A prenylated flavanone (6,7[2",2"-dimethyl chromene]) has only once been reported in *Lannea acida* (Sultana and Ilyas, 1986). This is the second occurrence of prenylated flavonoids in a *Lannea* species and indicates a closer taxonomical link between *L. acida* and *L. alata*. The current finding justifies the ethno botanical use of the plant extracts to make a paste for the treatment of wounds and injuries as this paste may act as an antibacterial agent, preventing the wound from becoming infected and allowing it to heal.

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Chapter 3 Antibacterial, antioxidant, antiplasmodial and cytotoxic activities of *Lannea rivae* (chiov) Sacleux (Anacardiaceae)

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Abstract

Six novel compounds, 3-nonadec-14'-(*Z*)-enyl phenol (**B1a**); 4,5-dihydroxy-4,2'-epoxy-5-[16'-*Z*-18'-*E*-heneicosenyldiene]-cyclohex-2-enone (**B2**), 2,4,5-trihydroxy-2-[16'-*Z*-heneicosenyl]-cyclohexanone (**B3**), 4*S*,6*R*-dihydroxy-6-[12'-*Z*-heptadecenyl]-cyclohex-2-enone (**B4a**); 4*S*,6*R*-dihydroxy-6-[14'-*Z*-nonadecenyl]-cyclohex-2-enone (**B4b**); and 1,2,4-trihydroxy-4-[16'-*Z*-heneicosenyl]-cyclohexane (**B5**) were isolated from the roots and stems of *Lannea rivae* in addition to the known compounds; cardanols **B1b-B1d**, sitosterol (**A6**), sitosterol glucoside (**B6**), taraxerone (**B7**), taraxerol (**B8**), E-lutein (**B9**), myricetin (**B10**), myricetin-3-*O*-α-rhamnopyranoside (**A3**), myricetin-3-*O*-β-galactopyranoside (**B11**), and (-)-epicatechin-3-*O*-gallate (**B12**). Myricetin (**B10**), its glycosides (**B11** and **A3**) and epicatechin gallate (**B12**) showed good antioxidant activity, while **B10** and **B12** but not the glycosides showed good antibacterial activity. **B1a-B1d**, **B4a-B4b** and **B5** were all relatively non-toxic, whilst **B2** and **B3** showed more toxicity than the others. These two toxic compounds, **B2** and **B3** also showed good antiplasmodial activity. The mixture of **B4a** and **B4b**, which was far less toxic than **B2** and **B3** also showed promising antiplasmodial activity and is a good lead for an antiplasmodial drug.

Keywords: *Lannea rivae*, alkenyl phenols, alkenyl cyclohexenones, alkenyl cyclohexenols, cytotoxicity, antiplasmodial, antibacterial, antioxidant.

3.1 Introduction

In sub-Saharan Africa where access to healthcare facilities and medicine is difficult, the majority of the population, especially those living in rural areas far from cities turn to traditional medicines for medical disorders and disease (WHO, 2008). Although the safety and efficacy of the traditional remedies are not verified, most of the population in need of these medicines has no alternative but to use them. The majority of the remedies is herbal, with many remedies containing different parts of more than a few plants and is administered as concoctions or poultices. The three main diseases that plague most countries in Africa are malaria, tuberculosis and HIV and whilst other diseases are also rampant on the African continent, the majority of the population seeks relief and cure for these three diseases. The fact that traditional healers are so popularly used is evidence enough that the remedies must contain compounds which are probably active against either the symptoms or the causes of their diseases. The phytochemical study of plants used in African traditional medicine is therefore a good lead to finding active compounds against these diseases.

Lannea rivae (Chiov) Sacleux is one such plant where the inner bark is applied in traditional medicine in the management of colds, fever, coughs and stomach ache (Kokwaro, 2009). The bark is also chewed for its sweet taste and as a source of water. The fruits of the plant are also edible (Maundu *et al*., 1999). To the best of our knowledge, this plant has not been studied previously for its phytochemical constituents as there are no accounts reported in the literature. Phytochemical studies of other *Lannea* species reported the isolation of flavonoids, some with antibacterial and antioxidant activity (Sultana and Ilyas, 1986; Islam *et al*., 2002; Picerno *et al*., 2006; Okoth *et al.*, 2013), alkylphenols and dihydrocylcohexenones with antioxidant activity (Queiroz *et al*., 2003), and alkylated hydroquinones,

dihydrocylcohexenols and phenolic compounds with cytotoxic activity (Groweiss *et al*., 1997; Kapche *et al*., 2007).

Apart from these phytochemical investigations, extracts of other *Lannea* species were also reported to have antioxidant, lipoxygenase, anti-inflammatory, analgesic, acetylcholinesterase, anti-malarial, anti-HIV, antibacterial, antifungal and antiviral activity (Clarkson *et al*., 2004; Picerno *et al*., 2006; Maiga *et al*., 2006; 2007; Gathirwa *et al*., 2007; 2008; 2011; Maregesi *et al*., 2008; 2010; Deji-Agboola and Olajubu, 2010; Koné *et al*., 2011; Ouattara *et al*., 2011; Alam *et al*., 2012). In the present study, a phytochemical investigation of *Lannea rivae* was conducted and the isolated compounds assessed for their antioxidant, antibacterial, and antiplasmodial activity. Cytotoxicity studies are also reported for the isolated compounds.

3.2 Results and discussion

Purification of the leaf, root and stem bark extracts led to isolation of six new compounds, 3-nonadec-14'-(*Z*)-enyl phenol (**B1a**); 4,5-dihydroxy-4,2'-epoxy-5-[16'-*Z*-18'-*E*-heneicosenyldiene]-cyclohexe-2-enone (**B2**), 2,4,5-trihydroxy-2-[16'-*Z*-heneicosenyl]-cyclohexanone (**B3**), 4*S*,6*R*-dihydroxy-6-[12'-*Z*-heptadecenyl]-cyclohex-2-enone (**B4a**); 4*S*,6*R*-dihydroxy-6-[14'-*Z*-nonadecenyl]-cyclohex-2-enone (**B4b**); and 1,2,4-trihydroxy-4-[16'-*Z*-heneicosenyl]-cyclohexane (**B5**) (Figure **3-1**). The new compounds were elucidated mainly by 1D and 2D NMR, and mass spectral data.

In addition, several known compounds whose structures were determined from their ^{1}H, ^{13}C and 2D NMR spectroscopy were also isolated. Once identified, their structures were verified by comparison with the data from the literature. These were the alkenyl phenols (or

cardanols) 3-heptadec-12'-*Z*-enyl phenol (**B1b**) and 3-pentadec-10'-*Z*-enyl phenol (**B1c**) (Liu and Abreu, 2006), and the alkyl phenol, 3-pentadecyl phenol (**B1d**) (Lomonaco *et al*., 2009), which were isolated as a mixture along with **B1a**; the triterpenes sitosterol (**A6**) (Kovganko *et al*., 1999), sitosterol glucoside (**B6**) (Faizi *et al*., 2001), taraxerone (**B7**) (Sakurai *et al*., 1987), and taraxerol (**B8**) (Liu *et al*., 2010), a tetraterpene, *trans* lutein (**B9**) (Khachik and Chang, 2009) and the flavonoids myricetin (**B10**) (Liu *et al*., 2011), myricetin-3-*O*-α-*L*-rhanmopyranoside (**A3**) (Braca *et al*., 2003), myricetin-3-*O*-β-galactopyranoside (**B11**) (Lee *et al*., 2011) and (-)-epicatechin gallate (**B12**) (Braca *et al*., 2003).

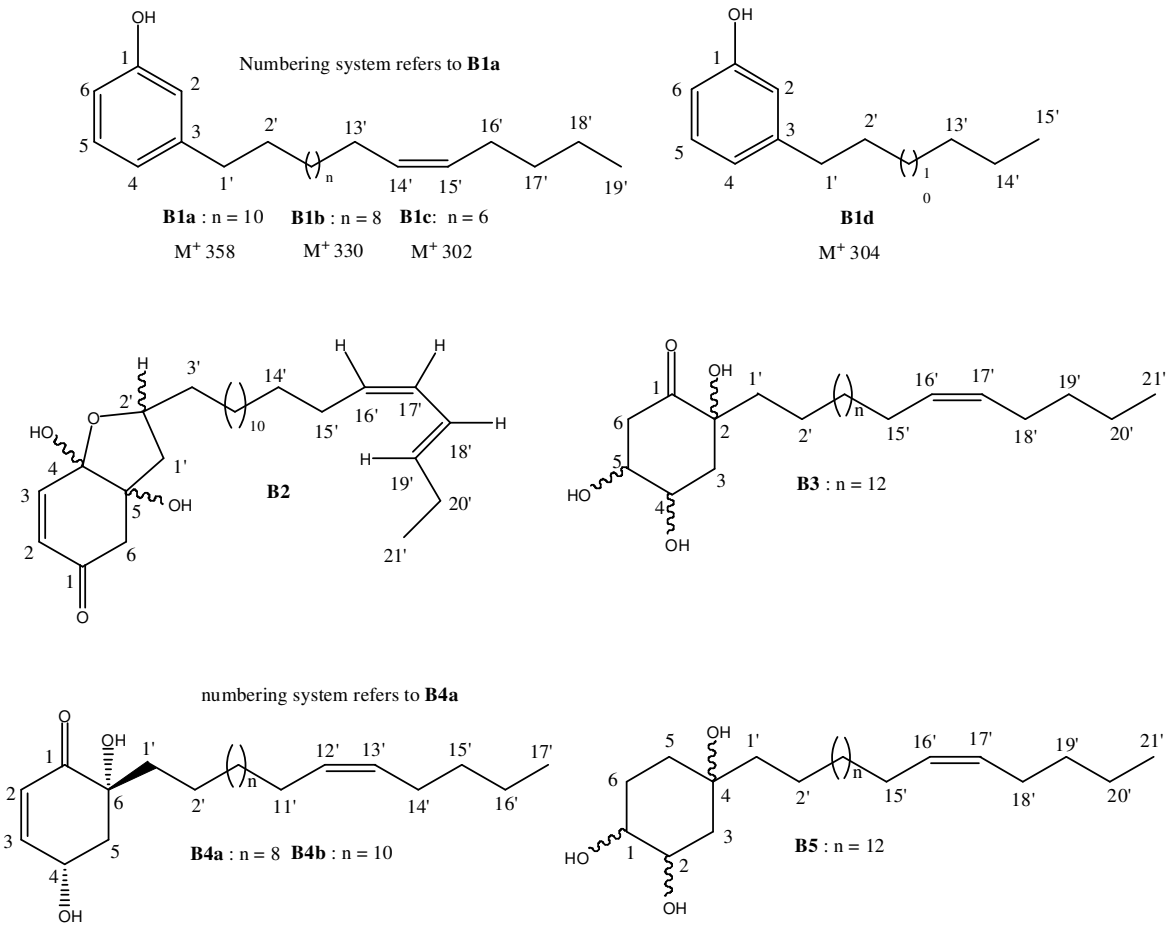

Figure 3-1 Novel compounds isolated from *Lannea Rivae*

The cardanols (**B1a**-**B1d)** were isolated as a mixture and despite several attempts could not be separated quantitatively. The NMR spectra of the mixture compare well with that in the literature for **B1b-B1d** (Liu and Abreu, 2006; Lomonaco *et al*., 2009). They were however qualitatively separated by GCMS, where the four compounds were identified as the novel cardanol **B1a** containing a C-19 side chain with M^+ at *m/z* 358, two known monounsaturated cardanols containing a C-17 side chain (M^+ at *m/z* 330) (**B1b**) and a C-15 side chain (M^+ at *m/z* 302) (**B1c**) and a saturated cardanol with a C-15 side chain (M^+ at *m/z* 304) (**B1d**). The mass spectra of all the compounds indicated an ion at *m/z* 108 due to the benzylic cleavage resulting in a 3-methylhydroxybenzene cation, confirming the presence of the aromatic substituent. The double bond in the monounsaturated long alkyl chain was characterized by the carbon resonances at δ_C 129.92 and 129.84 and the position of the double bond using **B1a** as an example was based on COSY correlations between H-13' and H-14' and between H-14' and H-15' as well as HMBC correlations between C-15' and H-17' and C-16' and H-17'. The fact that H-14' and H-15' appeared as overlapping resonances at δ 5.32 (*t*, *J* = 4.48 Hz) with corresponding allylic carbon resonances for C-13' and C-16' occurring at δ 26.91 and 27.20 indicate a *Z* configuration for the double bond in the side chain as the *E* configuration has slightly higher values for the allylic carbon resonances at approximately δ 32.0 (Roumy *et al.*, 2009). The C-17 and C-15 alkylated phenols, 3-heptadec-12'-(*Z*)-enyl phenol (**B1b**) and 3-pentadec-10'-(*Z*)-enyl phenol (**B1c**) besides being isolated in *Ozoroa insignus* (Liu and Abreu, 2006) have also been reported in *Ginko biloba* (Sun *et al*., 2012) and *Knema laurina* (Akhtar *et al*., 2011). A C-19 (3-nonadec-16'-(*E*)-enyl phenol), different to **B1a** in the geometry and position of the double bond in the side chain has been reported in *Lannea edulis* (Queiroz *et al*. 2003).

Compound **B2** showed IR absorption bands typical of a hydroxyl stretch at 3382 cm^{-1} and an α,β-unsaturated carbonyl stretch at 1679 cm^{-1}. The molecular ion peak at *m/z* 432 was evident in the mass spectrum. The ^1H NMR spectrum showed the presence of six olefinic proton resonances, including two which overlapped at δ_H 5.91. This indicated that three double bonds were present in the molecule. The H-2 and H-3 resonances occurred as a pair of doublets at δ_H 5.91 and 6.71 (J = 10.2 Hz), indicative of an isolated *cis* double bond without adjacent protons. The other two pairs of olefinic protons were located on the alkyl chain with H-16', H-17' and H-18' all being *cis* to each other as indicated by their coupling constants of approximately 10 Hz at δ_H 5.24-5.32 (m), δ_H 5.92 (dd) and δ_H 6.27 (dd) with coupling constants of $J_{16',17'}$ = 10.2 Hz and $J_{17',18'}$ = 10.8 Hz. The H-19' proton was located *trans* to H-18' at δ_H 5.67 (dt, J = 15.1, 6.6 Hz) due to $J_{18',19'}$ being approximately 15 Hz. The double bonds on the alkyl chain were located at $\Delta^{16'}$ and $\Delta^{18'}$ as H-19' showed COSY correlations with H-20' which in turn showed COSY correlations with the terminal methyl group in the alkyl chain. The length of the alkyl chain was determined by the mass spectrum taking into account the mass of the rest of the molecule.

The resonance at δ_H 6.71 showed HMBC correlations to the ketone peak at δ_c 196.9 and the other olefinic peak at δ_H 5.91 showed HMBC correlations to the methylene carbon peak at δ_C 47.0, which corresponded to the two non-quivalent doublet resonances at δ_H 2.82 and 2.68 (J = 16.0 Hz). Thus, δ_H 6.71, δ_H 5.91, δ_c 196.9 and δ_c 47.0 were attributed to H-3, H-2, C-1 and C-6 respectively. The H-6 proton resonances of δ_H 2.82 and 2.68 showed HMBC correlations to three other carbon resonances at δ_C 43.6, 78.7 and 99.5, the latter two being oxygenated with the last resonance being much more deshielded than the others, indicating a hemiacetal carbon atom. These resonances were therefore assigned to C-1', C-5 and C-4 respectively. There was an additional methine oxygenated resonance at δ_H 4.37-4.41 (m, H-

2'), which formed part of the furan ring fused to the cyclohexenone moiety. The stereochemistry at C-4, C-5 and C-2' could not be determined from the NOESY data available. Compound **B2** was thus identified as 4,5-dihydroxy-4,5-furan-2'-[16'-(*Z*)-18'-(*E*)-heneicosenyldiene]-cyclohex-2-enone.

Compound **B3** displayed an IR spectrum with a broad hydroxyl stretching absorption at 3384 cm^{-1} and a carbonyl stretching frequency at 1724 cm^{-1}. The molecular mass of the compound was deduced from the mass spectrum, where even though the M^+ ion was absent, an M^+-H_2O ion was present, quite common for secondary and tertiary alcohols. This M^+-H_2O peak was evident at *m/z* 420, for the compound with a molecular mass of 438 and a molecular formula of $C_{27}H_{50}O_4$.

The ^1H NMR spectrum indicated the presence of an alkenyl side chain with a *cis* double bond due to the triplet at δ_H 5.32 (J = 4.7 Hz) and the carbon resonances adjacent to the double bond being at a chemical shift of δ_C 27.2 and 26.9 (Roumy *et al*., 2009) similar to compounds **B1a-B1c**. The position of the double bond was ascertained by HMBC correlations between C-17' and H-19' as well as between H-19' and C-20', and C-20' and H-21'. Three pairs of non-equivalent proton resonances at δ_H 1.68 (td, J = 12.4, 4.1 Hz, H-1'a) and δ_H 1.94 (m, H-1'b); δ_H 2.16 (dd, J = 14.5, 4.0 Hz, H-3a) and δ_H 2.00 (m, H-3b); and δ_H 2.99 (dd, J = 13.9, 3.8 Hz, H-6a) and δ_H 2.62 (dd, J = 13.9, 5.7 Hz) as well as two deshielded oxygenated resonances at δ_H 4.09 (m, H-5) and δ_H 4.04 (m, H-4), the ketone resonance at δ_C 211.8 (C-1) and the oxygenated tertiary carbon resonance at δ_C 78.1 (C-2) make up the resonances of the cyclohexanone ring. Their positions on the cyclohexanone ring were established by COSY correlations between H-5 and H-6a and H-6b and between H-4 and H-3a and H-3b as well as the HMBC correlations of C-1 with H-5, H-6a, H-6b, H-3a, H-3b and

H-1'a and H-1'b; C-2 with H-4; and C-4 with H-6a and H-6b. Unfortunately, the stereochemistry again could not be established from NOESY correlations. Thus, compound **B3** was named 2,4,5-trihydroxy-2-[16'-(*Z*)-heneicosenyl]-cyclohexanone.

Compounds **B4a** and **B4b** were obtained as a mixture, exhibiting molecular ions of *m/z* 364 and 392 consistent with molecular formulae of $C_{23}H_{40}O_3$ and $C_{25}H_{44}O_3$ with C-17 and C-19 side chains respectively. In both cases, the M^+-H_2O peaks at *m/z* 346 (for **B4a**) and *m/z* 374 (**B4b**) are more prominent than the M^+ ions, typical for secondary and tertiary alcohols. The IR spectrum indicated the presence of hydroxyl groups (3392 cm^{-1}), aliphatic groups (2922 and 2852 cm^{-1}) and an α,β-unsaturated carbonyl group (1678 cm^{-1}). The ^1H and ^{13}C NMR spectral data of **B4a** and **B4b** were similar to both 4*S*,6*S*-dihydroxy-6-(14'(*Z*)-nonadecenyl)-2-cyclohexenone (relative configuration) (de Jesus Correia *et al*., 2001) and 4*R*,6*S*-dihydroxy-4-(10'(*Z*)-heptadecenyl)-2-cyclohexenone (absolute configuration) (David *et al*., 1998). The difference between **B4b** and its isomer published in de Jesus Correia *et al*. (2001) is the difference in stereochemistry at C-6. The difference between **B4a** and its isomer in David *et al*. (1998) is in the position of the alkyl chain in the molecule, the side chain in **B4a** occurring at C-6 instead of C-4 and the position of the double bond in the side chain, **B4a** occurring at $\Delta^{12'}$ and its isomer in David *et al*. (1998) occurring at $\Delta^{10'}$.

The position of the alkyl chain in **B4a** and **B4b** was determined to be at C-6 due to HMBC correlations between C-1 and 2H-1'. The configuration of H-4 could be determined as pseudoaxial (4*S*) from the coupling constants of its neighbouring H-5 proton resonances (δ_H 2.22 and δ_H 2.23 had *J* values of 4.6 and 5.3 Hz respectively) (Roumy *et al*., 2009). Irradition of the H-3 resonance in a 1D NOE experiment did not show a positive correlation with the H-1' resonances as seen in de Jesus Correia *et al*. (2001) and as such, the stereochemistry at this

position was made 6*R* in relation to the 4*S* stereocentre. Thus, compounds **B4a** and **B4b** had the relative configuration of 4*S*, 6*R* and were identified as 4*S*,6*R*-dihydroxy-6-(12'(*Z*)-heptadecenyl)-2-cyclohexenone and 4*S*,6*R*-dihydroxy-6-(14'(*Z*)-nonadecenyl)-2-cyclohexenone respectively.

In comparison to **B3**, one of the oxygenated methine resonances in compound **B5** moved more upfield to δ_H 3.41 (dd, J = 10.5, 4.6 Hz) and one pair of non-equivalent methylene resonances also moved more upfield in the region from δ_H 1.5-2.0 in comparison to δ_H 2.62 and 2.92 in **B3**. There was also an additional methylene carbon resonance in the ^{13}C NMR spectrum at δ_C 25.0 with a concomitant disappearance of the carbonyl resonance which was evident in the ^{13}C NMR spectrum in **B3** at δ 211.8. The absence of the carbonyl stretching band was also noted in the IR spectrum. Further to this, the H-1' methylene carbon resonance was also shifted upfield to δ 32.0 from δ 39.0 in **B3**. These critical changes in the ^{1}H and ^{13}C NMR spectra were indicative that the carbonyl group at C-1 in compound **B3** had now been reduced in compound **B5** to a methylene group (C-5). The ^{1}H NMR resonances of the alkenyl side chain (H-2'-14', H15'/18', H-16'/17' and H-21') remained similar to that of **B3** and was confirmed by mass spectrometry with a molecular ion peak at *m/z* 424. The structure was supported by HMBC correlations between C-4 and the two H-1' resonances and between C-1 and H-6. Similar to compound **B3**, the stereochemistry at C-1, C-3 and C-4 could not be determined from the NOESY data. Compound **B5** was thus identified as 1,2,4-trihydroxy-4-[16'(*Z*)-heneicosenyl]-cyclohexane.

Antioxidant activity

The antioxidant activity of the aromatic cardanols and the flavonoids were carried out and compared to ascorbic acid and was found to be in the order ascorbic acid > epicatechin

gallate (**B12**) > myricetin (**B10**) > myricetin-3-*O*-α-rhamnopyranoside (**A3**) = myricetin-3-*O*-β- galactopyranoside (**B11**) > cardanol (**B1a-B1d**) (

). In general the flavonoids had better activity than their glycosides. The cardanols had the worst activity. The most important features of flavonoids for optimum radical scavenging activity are the *ortho*-dihydroxy substitution of ring B, the Δ^2 double bond conjugated to a C-4 carbonyl and the additional presence of both C-3 and C-5 hydroxyl groups (Croft, 1998). In addition to these myricetin has hydroxyl groups at C-5' and C-7 which make it a good radical scavenger. The 3',4',5'-trihydroxy substitution pattern in ring B renders myricetin a more effective radical scavenging compound than other flavonoid aglycones with a different substitution pattern on ring B.

Table 3-1: Antioxidant activity of the flavonoids and flavonoid glycosides from *Lannea rivae*

Compound	**DPPH radical scavenging activity**					**Mean* Comp.**	**IC_{50}**
Conc.	**6.25 µg mL^{-1}**	**12.50 µg mL^{-1}**	**25.00 µg mL^{-1}**	**50.00 µg mL^{-1}**	**100.00 µg mL^{-1}**		
Cardanol (**B1a-d**)	13.18 ± 0.32	15.91±-0.08	26.63 ± 0.15	32.69±0.55	53.79 ± 0.50	28.37^{a}	96.23
Myricetin-3-*O*-α-rhamnoside (**A3**)	30.26 ±0.06	49.51 ±0.51	61.74 ±0.24	73.91 ±0.74	89.30 ±0.62	60.76^{b}	14.42
Myricetin-3-*O*-β-galactoside (**B11**)	31.26 ±0.16	49.41 ±0.41	60.84 ±0.03	74.81 ±0.64	89.50 ±0.52	61.24^{b}	14.25
Myricetin (**B10**)	42.76 ±0.54	62.40 ±1.21	83.03 ±0.02	94.82 ±0.71	97.34 ±0.48	76.81^{c}	8.08
Epicatechin gallate (**B12**)	45.93 ±0.95	66.09 ±0.88	86.72 ±0.36	98.31 ±0.83	99.49 ±0.49	79.30^{d}	7.30
Ascorbic acid	59.78 ±0.41	73.77 ±0.17	95.30 ±0.06	99.03 ±0.02	99.04 ±0.48	85.45^{e}	5.04
Mean* Conc.	48.77^{a}	53.90^{b}	70.77^{c}	79.68^{d}	87.97^{e}		

*means that differ significantly have different letters, $p < 0.05$; mean conc. indicates the mean of all the compounds tested at a specific concentration; mean comp. indicates the mean of the compound tested at different concentrations.

Glycosidation of the C-3 hydroxyl group, as in the case of myricetin-3-*O*-α-rhamnoside and myricetin-3-*O*-β-galactoside lowered the antioxidant activity in comparison to the aglycones. This was consistent with the literature (Rice-Evans *et al*., 1996). Antioxidant activity of myricetin and its glycosides are known (Lee *et al*., 2011, Chaabia *et al*., 2008). Although epicatechin gallate lacks the Δ^2 double bond and carbonyl group, the galloyl moiety at position 3 has a strong radical scavenging activity equal or superior to the *ortho*-dihydroxy group in the B-ring (Nanjo *et al*., 1996, Guo *et al*., 1999, Xu *et al*., 2004).

Antibacterial activity

Selected compounds including the flavonoids and flavonoid glycosides along with the crude hexane, dichloromethane (DCM), ethyl acetate (EtOAc) and methanol (MeOH) extracts of the stems and roots were tested for their antibacterial activity against both Gram positive and Gram negative bacterial strains. The hexane extracts exhibited intermediate antibacterial activity only against *E. faecalis* while the DCM extracts showed intermediate activity against both Gram positive bacteria *E. faecalis* and *S. aureus*, but no activity against gram negative bacteria (Table **3-2**). The EtOAc and MeOH extracts demonstrated a broader spectrum of activity with better activity being observed with the gram positive bacteria.

Myricetin (**B10**) and epicatechin gallate (**B12**) exhibited good activity in both gram positive and gram negative bacteria, again with better activity against Gram positive bacteria. The activity of both **B10** and **B12** were comparable to the standard antibiotic erythromycin. **B4a/B4b** and **B2** exhibited good antibacterial activity against the gram positive bacteria but weak activity against gram negative bacteria. None of the compounds or extracts tested demonstrated activity against *Salmonella typhimurium.*

Table 3-2: Antibacterial activity (zones of inhibition in mm) of extracts and active compounds isolated from *Lannea rivae*

	Gram positive bacteria		**Gram negative bacteria**	
	E. faecalis ATCC 29212	*S. aureus* 25923	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853
Extract/ standard				
Hexane stem	10	0	0	0
Hexane root	10	0	0	0
Dichloromethane stem	12	15	0	0
Dichloromethane root	11	13	0	0
Ethyl acetate stem	15	19	10	11
Ethyl acetate root	14	20	10	11
Methanol stem	10	15	11	9
Methanol root	10	15	12	10
B4a and **B4b**[a]	13	16	8	8
B2[b]	14	18	10	10
Myricetin (**B10**)	18	23	16	17
Epicatechin gallate (**B12**)	17	26	13	14
Penicillin	nd	34	nd	nd
Erythromycin	nd	23	nd	nd
Vancomycin	18	nd	nd	nd
Cefuroxime	nd	nd	23	nd
Ciprofloxacin	nd	nd	32	23
Nalidixic acid	nd	nd	23	nd

[a]4,6-dihyhydroxy-6-(alkyl)-2-cyclohexenone mixture; [b]4,5-dihydroxy-4,5-furan-2'-[16'-(*Z*)-18'-(*E*)-heneicosenyl diene]-cyclohex-2-enone; nd = not determined

The antibacterial properties of epicatechin gallate (**B12**) is known (Gibbons *et al*., 2004; Hamilton-Miller and Shah, 2000; Park *et al.,* 2004; Sakanaka *et al* 2000) and is associated with the presence of the gallic ester at C-3. There have been many reports of epicatechin gallate (**B12**) potentiating the effect of current antibiotics, especially β-lactams against multidrug resistant bacteria (Anderson *et al*., 2005; 2011; Gibbons, 2005; Park *et al* 2004; Shiota *et al*., 1999; Stapleton *et al*., 2004; 2006; 2007; Qin *et al*., 2013; Xiao *et al*., 2012). Galloylated catechins disrupts the bacterial membrane by intercalating within the hydrocarbon chains of the bacterial phospholipid palisade, thus affecting the physical properties of the membranes significantly (Shah *et al*., 2008; Caturla *et al*., 2003). The

flavan-3-*O*-gallates are more effective against gram positive than gram negative bacteria. The structure of the bacterial cell wall and different affinities of the catechin gallates with various cell wall components are responsible for the different susceptibilities (Ciu *et al*. 2012, Yoda *et al*. 2004).

Myricetin is active against several gram positive and gram negative bacteria, with MIC values ranging from 64-256 μg mL^{-1} (D'Souza *et al*., 2010; Griep *et al*., 2007; Jayaraman *et al*., 2010; Liu and Matsuzaki, 1995; Xu and Lee, 2001). In the literature, myricetin was also shown to be inactive against *Salmonella typhi* and *Vibrio cholerae* (D'Souza *et al*., 2010). The good antibacterial activity is associated with the presence of a free B ring with a 3',4',5'-trihydroxy substitution pattern in addition to a free C-3 hydroxyl group. The mechanism of action of myricetin is proposed to involve inhibition of bacterial protein synthesis (Xu and Lee 2001).

Cytotoxicity

The cardanols (**B1a**-**B1d**) and the alkylated dihydroxycyclohexenones (**B4a-B4b**) did not show cytotoxicity at the concentrations tested i.e IC_{50} > 100 μg mL^{-1} (Table **3-3**). The alkylated trihydroxycyclohexane (**B5**) and triterpenes taraxerone (**B7**) and taraxerol (**B8**) exhibited moderate cytotoxicity. However, 2,4,5-trihydroxy-2-[16'-(*Z*)-heneicosenyl]-cyclohexanone (**B3**) and 4,5-dihydroxy-4,5-furan-2'-[16'-(*Z*)-18'-(*E*)-heneicosenyldiene]-cyclohex-2-enone (**B2**) exhibited high cytotoxicity values. Cytotoxicity seems to be associated with the furan ring in **B2** as the mixture of **B4a** and **B4b** although having an α,β-unsaturated ketone moiety in the cyclohexenone ring similar to that in **B2**, did not show any toxicity at the concentrations tested. However, addition of water across the double bond in

B4a results in a more toxic compound **B3**, which could also be due to the added hydroxyl group.

Table 3-3: Cyctotoxicity of *Lannea rivae* compounds

Cytotoxicity	
Compound	**IC_{50} (µg/ml), n = 3**
Cardanol mixture (**B1a-B1d**)	>100
4,5-dihydroxy-4,5-furan-2'-[16'-(*Z*)-18'-(*E*)-heneicosenyldiene]-2-cyclohexenone (**B2**)	1.93
2,4,5-trihydroxy-2-[16'-(*Z*)-heneicosenyl]-cyclohexanone (**B3**)	6.52
4,6-dihyhydroxy-6-(alkyl)-2-cyclohexenone mixture (**B4a** and **B4b**)	>100
1,2,4-trihydroxy-4-[16'(*Z*)-heneicosenyl]-cyclohexane (**B5**)	56.5
Taraxerone (**B7**)	56.2
Taraxerol (**B8**)	42.2
Emetine	0.069

n = 3 indicates the number of replicates carried out.

Compounds related structurally to **B1a-B1d**, lanneaquinol and 2′(*R*)-hydroxylanneaquinol isolated from *Lannea welwitschii* exhibited modest cytotoxicity against human tumor cell lines (Groweiss *et al.*, 1997) and it may be worthwhile testing the mixture of **B1a-d** for anticancer activity. Compounds structurally similar to **B2** and **B4a** and **B4b**, 4,6,2'-trihydroxy-6-[10'(*Z*)-heptadecenyl]-1-cyclohexen-2-one (similar to **B4a-B4b**) and 1,4,6-trihydroxy-1,2'-epoxy-6-[10'(*Z*)-heptadecenyl]-2-cyclohexene (similar to **B2**) isolated from *Tapirira guianensis* (Roumy *et al*., 2009) were also found to be cytotoxic and 4*S*,6*S*-dihydroxy-6-(14'-nonadecenyl)-2-cyclohexenone (an isomer of **B4b**) from *Tapirira obtusa* demonstrated cytotoxic activity against human cancer cell lines (de Jesus Correia *et al.*, 2001). The fact that **B4a-B4b** did not show toxicity must have to do with the specific stereochemistry of **B4a-B4b** or the fact that it is a mixture of the two compounds. It would be interesting to see whether the mixture **B4a-b** shows the same anticancer activity as that reported for the isomer of **B4b** in de Jesus Correia *et al.* (2001).

Antiplasmodial activity

Compounds containing the cyclohexenone and cyclohexanone moieties, **B2**, **B3** and **B4a-B4b**, as well as the flavonoids, myricetin (**B10**) and epicatechin gallate (**B12**) exhibited good antiplasmodial activity against both chloroquine sensitive and chloroquine resistant strains, with the cylcohexenone compounds **B2** and **B4a-B4b** showing better activity than the cyclohexanone compound (**B3**) and the flavonoids **B10** and **B12** (Table **3-4**). The flavonoid glycosides, myricetin-3-*O*-α-rhamnoside (**A3**) and myricetin-3-*O*-β-galactoside (**B11**) did not exhibit any antiplasmodial activity. Even though **B2** and **B3** had good antiplasmodial activity, its cytotoxicity was higher than the other compounds, although it was not as toxic as the reference drug emetine. The most promising lead amongst these compounds is the mixture, **B4a-b**, which had good antiplasmodial activity as well as low cytotoxic values.

Compounds with structures closely resembling **B2** and **B4a-b** have also demonstrated good antiplasmodial activity (Roumy *et al*., 2009). The mode of activity of such compounds is not known but is thought to be related to the unsaturated ketone moiety, which acts as a Michael acceptor (Roumy *et al*., 2009). The antiplasmodial activity of epicatechin gallate (**B12**) against both chloroquine sensitive and resistant strains is consistent with that in the literature (Tasdemir *et al*., 2006; Sannella *et al*., 2007). This activity is associated with the galloyl ester substituent as catechin itself is not active. Myricetin's (**B10**) antiplasmodial activity is also consistent with the literature (Tasdemir *et al*., 2006; Lehane and Saliba, 2008). Hydroxy substitution at two or more sites on the phenyl ring B of a flavone structure increases the antiplasmodial activity with the pyrogallol moiety (3',4',5'-trihydroxy) in ring B increasing selectivity toward the FabI enzyme of *P. falciparum* (Tasdemir *et al*., 2006).

Table 3-4: Antiplasmodial activity of active compounds from *Lannea rivae*

Compounds	IC_{50} (µg/ml) D6 (CQ resistant clone)	IC_{50} (µg/ml) W2 (CQ sensitive clone)
4,5-dihydroxy-4,5-furan-2'-[16'-(*Z*)-18'-(*E*)-heneicosenyldiene]-cyclohex-2-enone (**B2**)	0.484±0.054	0.430±0.084
2,4,5-trihydroxy-2-[16'-(*Z*)-heneicosenyl]-cyclohexanone (**B3**)	2.055±0.124	1.406±0.173
4*S*,6*R*-dihydroxy-6-(12'(*Z*)-heptadecenyl)-2-cyclohexenone (**B4a/B4b**) (10'(*Z*) isomer)	1.039 ±0.139	0.826±0.066
Myricetin (**B10**)	4.638±0.280	7.503±0.517
Epicatechin gallate (**B12**)	2.787±0.341	2.106±1.97
Chloroquine	0.07626	0.00443
Mefloquine	0.0374	0.01217

3.3 Experimental

General experimental procedures

Reagents and chemicals used in this study were purchased from Merck, South Africa and all organic solvents were redistilled and dried according to standard procedures before being used. Optical rotation was recorded using a Perkin Elmer™, Model 341 Polarimeter with a 10 cm flow tube. The melting points of the isolated compounds were recorded on an Ernst Leitz Wetziar micro-hot stage melting point apparatus and are uncorrected. NMR spectra were recorded using $CDCl_3$, CD_3OD or DMSO-d_6 on a Bruker Avance^{III} 400 MHz spectrometer at room temperature with chemical shifts (δ) recorded against tetramethylsilane (TMS), the internal standard. IR spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrometer with universal ATR sampling accessory. UV spectra were obtained on a Varian Cary UV-VIS Spectrophotometer in chloroform. For GC-MS analyses, the samples were analysed on an Agilent MS 5973 instrument connected to a GC 6890 equipped with a DB-5SIL MS (30 m x 0.25 mm i.d., 0.25 µm film thickness) fused-silica capillary column using helium as a carrier gas with a flow rate of 2 mL min^{-1}. The MS was operated in the EI mode at 70 eV. Chromatographic separations were carried out by column chromatography

(CC) using silica gel 60 (40–63 μm, Merck 1.09385) and analytical TLC was performed on pre-coated silica gel 60 F_{254} plates (Merck 1.05554) and developed by spraying with anisaldehyde:H_2SO_4:MeOH (1:2:97 v/v) followed by heating.

Plant material

The leaves, roots and stem bark of *Lannea Rivae* (Chiov) Sacleux were collected from Wote town, Makueni District, Kenya, and was authenticated and deposited at Maseno University Botanic Gardens herbarium and assigned a voucher number MSU/BG-2/13. The plant material was air-dried under shade for 14 days and thereafter ground using a Wiley laboratory mill available at Kibos sugar company- Kisumu.

Extraction and Isolation

The n-hexane root extract (59.57 g) was packed on a silica gel open column (700 g, 80 mm column diameter) and eluted with a gradient solvent system from 100% n-hexane to 50:50 n-hexane:ethyl acetate, collecting 10 × 100 mL fractions before increasing the polarity by 10% at each stage. A total of 60 fractions were collected and combined based on their TLC profiles. Fraction 6-13 (356.91 mg) was repacked on a smaller column (40 mm diameter) and eluted with 500 mL of n-hexane followed by 5% ethyl acetate in hexane, collecting 50 mL fractions. Fraction 6-10 yielded a yellow liquid and contained a mixture of four cardanols (**B1a-B1d**) (167.93 mg), fraction 17-21 white crystals of taraxerone (**B7**) (112.91 mg), fractions 23-28 tiny crystals of taraxerol (**B8**) (131.20 mg) and fraction 30-45 white needle like crystals of sitosterol (**A6** – see chapter 2) (77.89 mg). All these compounds were purified using 5% ethyl acetate in n-hexane on smaller columns. Despite several attempts on silica and sephadex, the cardanols **B1a-B1d** could not be separated. The cardanols (**B1a-**

B1d) (34.63 mg), taraxerone (**B7**) (18.95 mg), taraxerol (**B8**) (32.76 mg) and sitosterol (**A6**) (38.66 mg) were also obtained from the hexane extract (20.08 g) of the leaves.

The ethyl acetate extract (69.95 g) was chromatographed as above using a n-hexane:ethyl acetate step gradient increasing the polarity by 10% for each step until 100% ethyl acetate was reached. At each stage 10 × 100 mL fractions were collected. Sitosterol (**A6**) (345.65 mg) was isolated from fraction 32-41 of the ethyl acetate crude extracts and needed no further purification. Fraction 44-76 (419.68 mg) from the crude column was rechromatographed with 20% and 30% ethyl acetate in n-hexane respectively collecting 20 × 50 mL fractions at each stage. Fraction 24-30 yielded a reddish brown paste which contained a mixture of two compounds (224.67 mg) (**B4a** and **B4b**). This too, despite several attempts on silica and sephadex, could not be separated. Fraction 80-90 (173.34 mg) of the crude column was further purified with 2L of n-hexane:ethyl acetate (1:1) collecting 40 × 50 mL fractions. A reddish brown paste (90.11 mg) (**B3**) was obtained from fraction 13-16 and purified with the same solvent system on silica gel.

The methanol extracts of the roots (64.21g) was chromatographed with 2 L of ethyl acetate (20 × 100 mL) followed by 2 L of 5% methanol in ethyl acetate (20 × 100 mL) on a silica gel column. Fraction 6-15 (589.45 mg) of this crude separation was packed on a LH-20 sephadex (30 g) column and eluted with 100% methanol, collecting 10 mL fractions. Fraction 8-18 contained sitosterol glucoside (**B6**), a white amorphous solid (295.06 mg) and a yellow crystalline solid, myricetin rhamnopyranoside (**A3** – see chapter 2) (111.18 mg). These two compounds (**B6** and **A3**) were obtained similarly from the stem bark extracts.

The ethyl acetate extract (56.33 g) from the leaves was chromatographed similarly to the ethyl acetate root extract above. Based on their TLC profiles, the fractions were combined into three larger fractions 30-60 (464.56 mg), 70-80 (217.67 mg) and 80-88 (274.44 mg). Fraction 30-60 was rechromatographed on sephadex LH-20 and eluted with methanol, collecting 10 mL fractions. Fractions 6-10 and 13-14 yielded 13.14 mg of red solid, all *E*-lutein (**B9**) and 216.22 mg of a yellowish brown paste (**B2**). Fraction 70-80, which was also eluted on sephadex in a similar way yielded an amorphous white solid, sitosterol glucoside (**B6**) (78.55 mg) and two yellow solids, myricetin-3-*O*-α-rhamnopyranoside (**A3**) (77.2 mg) and myricetin (**B10**) (98.92 mg) from fractions 16-24, 30-37 and 40-45 respectively. Purification of fraction 80-88 with sephadex resulted in the isolation of a reddish brown paste, myricetin-3-*O*-β-galactopyranoside (**B11**) (39.18 mg) and epicatechin gallate (**B12**) (149.89 mg).

3-[nonadec-14'(*Z*)-enyl]-phenol (**B1a**) isolated as a yellow liquid mixture with **B1b-B1d**; UV (MeOH) λ_{max} (log ε) 273, 216 nm; IR (υ_{max}) (neat) cm^{-1} 3354, 2922, 2852, 1589, 1456, 1265, 1154; EIMS *m/z* (rel. int.): **B1a** 358 (24) [M^+], 121 (12), 120 (17), 108 (100) [C_7H_8O]; ^1H NMR (400 MHz, $CDCl_3$) δ_H 7.11 (1H, t, *J* = 7.7 Hz, H-5), 6.73 (1H, d, *J* = 7.7 Hz, H-4), 6.63 (1H, s, H-2), 6.61 (1H, d, *J* = 2.1 Hz, H-6), 5.33 (2H, t, *J* = 4.5 Hz, H-14'/15'), 2.54 (2H, t, *J* = 5.6 Hz, H-1'), 1.99 (4H, m, H-13'/16'), 1.57 (2H, t, *J* = 6.7 Hz, H-2'), 1.29 (4H, m, H-17'/18'), 1.20-1.26 (20H, m, H-3'-12'), 0.86 (3H, t, *J* = 6.1 Hz, H-19'); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 155.5 (C-1), 145.0 (C-3), 129.9 (C-15'[a]), 129.8 (C-14'[a]), 129.3 (C-5), 120.9 (C-4), 115.3 (C-2), 112.5 (C-6), 35.8 (C-1'), 32.0 (C-17'), 31.3 (C-2'), 29.3-29.9 (C-3'-12'), 27.2 (C-13'), 26.9 (C-16'), 22.4 (C-18'), 14.0 (C-19'). [a]Assigments may be interchanged.

4,5-dihydroxy-4,5-furan-2'-[16'(*Z*),18'(*E*)-nonadecadienyl]-cyclohex-2-enone (**B2**) yellowish oil; $[\alpha]^{20}_{D}$ -42.86 ° (c = 1.05, CH_2Cl_2); UV ($CHCl_3$) λ_{max} (log ε) 268 (1.28), 221 (3.02) nm. IR (υ_{max}) (neat) cm^{-1} 3383, 2923, 2853, 1679, 1456, 1373, 1130, 1063; EIMS *m/z* (rel. int.): 432 (4), 360 (33), 342 (8), 124 (100); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 6.71 (1H, d, *J* = 10.2 Hz, H-3), 6.27 (1H, dt, *J* = 15.1, 10.8 Hz), 5.92 (1H, dd, *J* = 10.8, 10.2 Hz, H-17'), 5.91 (1H, d, *J* = 10.2 Hz, H-2), 5.69 (1H, dt, *J* = 15.1, 6.6 Hz, H-19'), 5.24-5.32 (1H, m, H-16'), 4.37-4.41 (1H, m, H-2'), 2.81 (1H, d, *J* = 16.0 Hz, H-6a), 2.66 (1H, d, *J* = 16.0 Hz, H-6b), 2.19 (1H, dd, *J* = 13.2, 6.1 Hz, H-1'a), 2.05-2.15 (4H, m, H-15'/20'), 1.66 (1H, dd, *J* = 13.2, 9.4 Hz, H-1'b), 1.57-1.61 (1H, m, H-3'a), 1.34-1.36 (1H, m, H-3'b), 1.20-1.40 (22H, m, H-4'-14'), 1.00 (3H, t, *J* = 7.5 Hz, H-21'); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 196.9 (C-1), 146.1 (C-3), 136.1 (C-19'), 130.1 (C-16'), 128.6 (C-17'), 126.8 (C-2), 124.7 (C-18'), 99.5 (C-4), 78.7 (C-5), 77.0 (C-2'), 47.0 (C-6), 43.6 (C-1'), 36.0 (C-3'), 29.2-29.7 (C-4'-C13'), 27.7 (C-15'), 25.9 (C-20'), 25.6 (C-14'), 13.7 (C-21').

2,4,5-trihydroxy-2-[heneicos-16'(*Z*)-enyl]-cyclohexanone (**B3**) reddish brown residue; $[\alpha]^{20}_{D}$ + 30.48 ° (c = 1.05, CH_2Cl_2); UV ($CHCl_3$) λ_{max} (log ε) 240 (3.28) nm. IR (υ_{max}) (neat) cm^{-1}: 3384, 2918, 2850, 1724, 1465, 1078; EIMS *m/z* (rel. int.): 420 (10) $[M\text{-}H_2O]^+$, 402 (8) $[M\text{-}2\times H_2O]^+$, 293 (18), 185 (30), 167 (34), 142 (76), 99 (100); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.32 (2H, t, *J* = 4.7 Hz, H-16'/17'), 4.07-4.09 (1H, m, H-5), 4.02-4.05 (1H, m, H-4), 2.99 (1H, dd, *J* = 13.9, 3.8 Hz, H-6a), 2.62 (1H, dd, *J* = 13.9, 5.7 Hz, H-6b), 2.16 (1H, dd, *J* = 14.5, 4.0 Hz, H-3a), 2.00 (1H, m, H-3b), 1.91-2.04 (4H, m, H-15'/18'), 1.92-1.94 (1H, m, H-1'a), 1.68 (1H, td, *J* = 12.4, 4.1 Hz, H-1'b), 1.27-1.29 (4H, m, H-19'/20'), 1.21-1.30 (26H, m, H-2'-H-14'), 0.86 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 211.8 (C-1), 129.9 (C-16'^{a}), 129.8 (C-17'^{a}), 78.1 (C-2), 74.2 (C-5), 69.8 (C-4), 42.0 (C-6), 41.4 (C-3), 38.9 (C-1'), 32.0

(C-19'), 29.3-29.9 (C-2'-13'), 27.2 (C-15'), 26.9 (C-18'), 23.2 (C-14'), 22.3 (C-20'), 14.0 (C-21'). [a]Assignments may be interchanged.

4*S*,6*R*-dihydroxy-6-[12'(*Z*)-heptadecenyl]-cyclohex-2-enone (**B4a**); isolated as a reddish brown oily mixture with **B4b**; $[\alpha]^{20}_{D}$ + 30.95° (c = 1.05, CH_2Cl_2); UV ($CHCl_3$) λ_{max} (log ε) 242 nm. IR (υ_{max}) (neat) cm^{-1}: 3393, 2922, 2852, 1678, 1464, 1377, 1033; EIMS *m/z* (rel. int.): 364(5) [M^+], 346 [M^+-H_2O] (14), 84 (100); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 6.83 (1H, dd, *J* = 10.1, 3.7 Hz, H-3), 6.01 (1H, dd, *J* = 10.1, 0.8 Hz, H-2), 5.33 (2H, t, *J* = 4.5 Hz, H-12'/13'), 4.65-4.67 (1H, m, H-4), 2.22 (1H, dd, *J* = 6.2, 4.7 Hz, H-5), 1.95-2.03 (4H, m, H-11'/14'), 1.73-1.77 (2H, m, H-1'), 1.27-1.32 (4H, m, H-15'/16'), 1.21-1.29 (18H, m, H-2'-H-10'), 0.86 (3H, t, *J* = 7.0 Hz, H-17'); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 201.2 (C-1), 149.2 (C-3), 129.9 (C-12'), 129.8 (C-13'), 126.7 (C-2), 74.6 (C-6), 64.2 (C-4), 41.1 (C-5), 39.2 (C-1'), 32.0 (C-15'), 29.3-29.9 (C-1'-C-10'), 27.2 (C-11'[a]), 26.9 (C-14'[a]), 23.0 (C-2'), 22.3 (C-16'), 14.0 (C-17'). The assignments refer to the numbering system of **B4a**; [a]Assignments may be interchanged.

1,2,4-trihydroxy-4-[16'(*Z*)-heneicosenyl]-cyclohexane (**B5**); reddish brown residue; $[\alpha]^{20}_{D}$ + 2.85° (c = 1.05, CH_2Cl_2); UV ($CHCl_3$) λ_{max} (log ε): 229 (2.12) nm. IR (υ_{max}) (neat) cm^{-1}: 3382, 2921, 2852, 1457, 1056; EIMS *m/z* (rel. int.): 424 (13) [M^+], 393 (8), 365 (100), 187 (42), 108 (57); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.32 (2H, t, *J* = 5.0 Hz, H-16'/17'), 4.01 (1H, bs, H-2), 3.41 (1H, dd, *J* = 10.5, 4.6 Hz, H-1), 1.98-2.02 (4H, m, H-15'/18'), 1.96-1.98 (1H, m, H-3), 1.86 (1H, m, H-5), 1.84 (1H, m, H-1'), 1.62 (1H, m, H-5), 1.53 (1H, m, H-6), 1.48 (1H, m, H-1'), 1.45 (1H, m, H-3), 1.20-1.30 (30H, m, H-2'-H-14', H-19'/20'), 0.86 (3H, t, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 129.84 (C-16'[a]), 129.90 (C-17'[a]), 75.4 (C-4), 72.8 (C-1), 66.9 (C-2), 25.0 (C-6), 38.8 (C-6), 38.3 (C-3), 32.0 (C-1'), 31.2 (C-19'), 29.3-30.2 (C-

2'-C-13'), 27.2 (C-15'), 26.9 (C-18'), 25.0 (C-5), 23.4 (C-14'), 22.3 (C-20'), 14.0 (C-21'). [a]Assignments may be interchanged.

Antioxidant activity

A 10 mg mL^{-1} stock solution of selected compounds (Table **3-1**) thought to have the potential to have antioxidant activity was made by dissolving the compounds in DMSO. Sample concentrations of 6.25, 12.50, 25.00, 50.00 and 100.00 μg mL^{-1} were made in methanol. The DPPH radical scavenging activity of the compounds was determined according to the method of Kumawat *et al*., (2012). Briefly, 1 mL of each compound was added to 1 mL of DPPH (1,1-diphenyl-2-picrylhydrazyl) solution (0.1 mM in methanol). The mixture was shaken and kept in the dark for 30 minutes at room temperature. The decrease in absorbance was determined at 517 nm. Vitamin C (ascorbic acid) was used as the positive control. The DPPH radical scavenging activity was calculated using the following formula:

DPPH Radical Scavenging Activity (%) = $[(A_0 - A_1 / A_0) \times 100]$,

where A_0 is the absorbance of the control and A_1 is the absorbance of the compound or standard sample.

All the tests were performed in triplicate. The results were given as means ± S.D. analysis of variance and significant differences among means were tested by two way ANOVA, using SPSS (Version 12.0 for Windows, SPSS Inc., Chicago, IL, USA). When significant main effects existed, differences were tested by Duncans test at 95% confidence. The IC_{50} values were calculated from dose-response curves, using non-linear dose-response curve fitting analysis GraphPad Prism (Version 5) software.

Disc diffusion antibacterial assays.

The antibacterial activity was conducted by the agar diffusion assay on all isolated compounds as well as the extracts of different parts of the plant. Briefly, filter paper discs (6 mm in diameter) impregnated with sample solutions were placed on Mueller Hinton agar plates (BBL, Becton Dickinson and Co., Cockeysville, MD), which have been inoculated with test organisms (*Staphylococcus aureus* ATCC 25923, *Enteroccoccus faecalis* 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhimurium* ATCC 13311) according to the standard protocol described by the National Committee of Clinical Laboratory Standards (2008). An amount of 100 µg of sample was tested on each disc. The plates were incubated at 35 °C and the diameters of the inhibition zones were measured after 24 h. Filter paper discs containing DMSO without any test compound served as a control and no inhibition was observed. Commercial penicillin (10 units), erythromycin (15 µg), cefuroxime (30 µg), nalidixic acid (30 µg), vancomycin (30 µg) and ciprofloxacin discs (5 µg) were included as a positive control.

SYBR Green I antiplasmodial assay

The SYBR Green I assay was used to screen the antiplasmodial activity of all isolated compounds against two *Plasmodium falciparum* strains, chloroquine sensitive (D6) from Sierra Leone and chloroquine resistant (W-2) from Vietnam. The cultures were maintained at the US Army Medical Research Unit in the Malaria Resistance Laboratories at the Kenya Medical Research Institute (KEMRI), Kisian-Kisumu, according to protocols in the literature (Smilkstein *et al*., 2004). Mefloquine and chloroquine reference drugs were used as a positive control. The culture medium was prepared as described by Johnson *et al*. (2007). Mefloquine was dissolved in 70% ethanol while the compounds and chloroquine were dissolved in 100% DMSO, to an initial concentration of 1 mg mL^{-1}. The *P. falciparum*

cultures were adjusted to 2% haematocrit and 1% parasitaemia for the assay (Akala *et al*. 2011), a modification of Desjardins *et al*. (1979) and Trager and Jensen (1976). After 72 h incubation, 100 µL of lysis buffer containing SYBR Green I dye was added to the 96-well plates prior to 1 h incubation in the dark. Five replicates were conducted for each test compound. The fluorescence was read using a Genios Tecan® micro-plate reader. IC_{50} values were then calculated by Graphpad Prism (Graphpad Prism for Windows, version 5.0; Graphpad Software, Inc., San Diego, CA).

Cytotoxicity assay

In vitro cytotoxicity of compounds **B1-B5**, **B7** and **B8** were tested against a mammalian cell-line, Chinese Hamster Ovarian (CHO). Only these compounds were chosen for this assay as compounds similar in structure demonstrated activity in similar cytotoxicity assays (Chaturvedula *et al*., 2004; David *et al*., 1998; Gachet *et al*., 2011; Groweiss *et al*., 1997; Kapche *et al*., 2007; Roumy *et al*., 2009). This was performed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-assay. The MTT-assay is used as a colorimetric assay for cellular growth and survival, and compares well with other available assays (Mosmann *et al*., 1983 and Rubinstein *et al*., 1990). The tetrazolium salt MTT was used to measure all growth and chemosensitivity. The test samples were tested in triplicate on one occasion. The test samples were prepared to a 20 mg mL^{-1} stock solution in 100% DMSO and were tested as a suspension if not properly dissolved. Test compounds were stored at -20 ºC until used. Emetine was used as the reference drug in all experiments. The initial concentration of test samples was 100 µg mL^{-1}. This was serially diluted in complete medium with 10-fold dilutions to give 6 concentrations, the lowest being 1ng mL^{-1}. The same dilution technique was applied to all the test samples. The highest concentration of solvent to which the cells were exposed to had no measurable effect on the cell viability. The

50% inhibitory concentration (IC_{50}) values were obtained from full dose-response curves, using a non-linear dose-response curve fitting analysis via GraphPad Prism v.4 software.

3.4 Conclusions

The *in vitro* activity of the isolated compounds and some crude extracts reported here support the reports by traditional healers that *Lannea rivae* extracts are effective in disease management. Nevertheless *in vivo* studies are recommended to further justify these claims. Six new cytotoxic compounds are reported here with **B1a** occurring in a mixture along with known compounds and **B4a** and **B4b**, two of the novel compounds, also being isolated as a mixture with each other. The cyclohexane moiety of compounds **B3**, **B4a-b** and **B5** can all be chemically transformed into each other, **B3** being dehydrated to **B4a-b** and reduced to **B5**. Some of the novel compounds showed good antibacterial and antiplasmodial activity in conjunction with low cytotoxicity and could be good lead compounds for antibiotics and antiplasmodial drugs. These types of novel compounds have only been reported in the genus *Tapirira*, which shows a close biosynthetic link between *Lannea* and *Tapirira*.

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Chapter 4 Cardanols, long chain cyclohexenones and cyclohexenols from *Lannea schimperi* (Hochst. Ex. A. Rich.) (Anacardiaceae)

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Abstract

Cardanols (**C1a-C1d**), alkenyl cyclohexenones (**C2a-C2d**) and alkenyl cyclohexenols (**C3a-C3c** and **C4a-C4c**) were isolated from the stem bark and root of *Lannea schimperi*. The cardanols **C1a** and **C1d** and the alkenyl cylcohexenols (**C2a** and **C2d**) have side chains which have not been reported previously in combination with the core skeletal structures. In addition, compounds **C3a-C3c** and **C4a-C4c** are all novel cylcohexenols. Also isolated were the triterpenes, sitosterol (**A6**), taraxerone (**B7**) and taraxerol (**B8**). The suite of compounds isolated (cylcohexenones and cyclohexenols) make up a nice biosynthetic pathway to the cardanols. The 5-[alkenyl]-4,5-dihydroxycyclohex-2-enone mixture (**C2a-C2d**) exhibited good *in vitro* cytotoxicity against the Chinese Hamster Ovarian mammalian cell-line. The compounds were identified by mainly GC-MS and NMR spectroscopic techniques.

Key words: *Lannea schimperi*, cardanols, alkenyl cyclohexenones, alkenyl cyclohexenols, taraxerol, taraxerone, sitosterol, cytotoxicity.

4.1 Introduction

The family Anacardiaceae is widely known for causing skin irritations, inflammation and blistering in sensitive individuals. Members of the family that are important in dermatology are the *Toxicodendron* genus (common poison ivy, poison oak, poison sumac), *Mangifera indica* L. (mango), *Toxicodendron vernicifluum* (lacquer tree) and *Anacardium occidentale* L. (cashew nut) (Rosen and Fordice, 1994; Hamilton and Zug, 1998; Oka *et al*., 2004; Gladman, 2006; Hirao *et al* 2008; Thoo and Freeman 2008). The active ingredients in these plants are mixtures of homologous long chain phenolic compounds collectively known as 'urushiol'. They are mono- or dihydroxybenzene derivatives of phenol (cardanols, catechols or resorcinols) or salicylic acid with a long alkyl or alkenyl carbon side chain (Evans and Schmidt, 1980; Stahl *et al.*, 1983). The phenols vary in the number and position of hydroxyl groups, in their alkyl chain length and in the number and position of double bonds in this chain. Phenolic lipids are also potentially useful in the treatment of cancer and skin diseases (Stasiuk and Kozubek, 2010). They can also be used as starting materials in the semi-synthesis of compounds for various biological activities such as long lasting hydrophobic anti-inflammatory drugs or analogues of cannabinoids (Kozubek and Tyman 1999). Cardanol is used in a wide range of technological applications such as in friction dusts for brake lining and clutch facings, and in polymer chemistry to form soft resins that are resistant to acids and bases. Derivatives of cardanol, cardol and anacardic acids have novel applications in dyes, pharmaceutical antioxidants and monomers for polymerization (Tyman, 1979). Plant species within the Anacardiaceae synthesize these toxic phenols to interact with their environment and ward off insect pests and microbes, to deter herbivores grazing from their leaves or to prevent seeds of other species from germinating in their proximity (Grayer, 2005).

Lannea schimperi occurs in tropical Africa. The bark of the roots and stem as well as the leaves of the plant are used medicinally to clean teeth and manage toothache, for diarrhea, chest infections, stomach pains, mental disorders, epilepsy, snake bites, tuberculosis, skin infections, herpes simplex, herpes zoster and other opportunistic infections resulting from HIV/AIDS (Verzar and Petrii, 1987; Ruffo, 1991; Kisangau *et al*., 2007; Jeruto *et al*., 2008; Lulekal *et al*., 2008; Kokwaro, 2009). Crude methanol and water extracts of the plant exhibited cytotoxic and antifungal activity (Moshi *et al*., 2006; Kisangau *et al*., 2009). No phytochemical studies appear to have been done on this plant. Therefore, the stem and root bark were analyzed for their phytochemical constituents.

4.2 Results and discussion

The hexane and ethyl acetate extracts led to isolation of the cardanols (**C1a-C1d**), the dihydroxycylcohexenones (**C2a-C2d)**, the cyclohexene triols (**C3a-C3c**) and the cyclohexene diols (**C4a-C4c**) (Table **4-1**; Figure **4-1**). All the compounds were alkylated on the cyclohexane ring and contained a double bond three bonds away from the end of the chain. Of these alkylated cyclohexenes and cardanols, only the cardanols **C1b** and **C1c** and the dihydroxycyclohexenones (**C2b** and **C2c**) were known (Queiroz *et al.*, 2003). All the other cyclohexenes and cardanols were novel. Also isolated from the plant were the known triterpenes sitosterol (**A6**), taraxerol (**B8**) and taraxerone (**B7**). The known triterpenes were identified by comparison of their spectral data with those in the literature (Sakurai *et al*., 1987; Kovganko *et al*., 1999; Mejin, 2009).

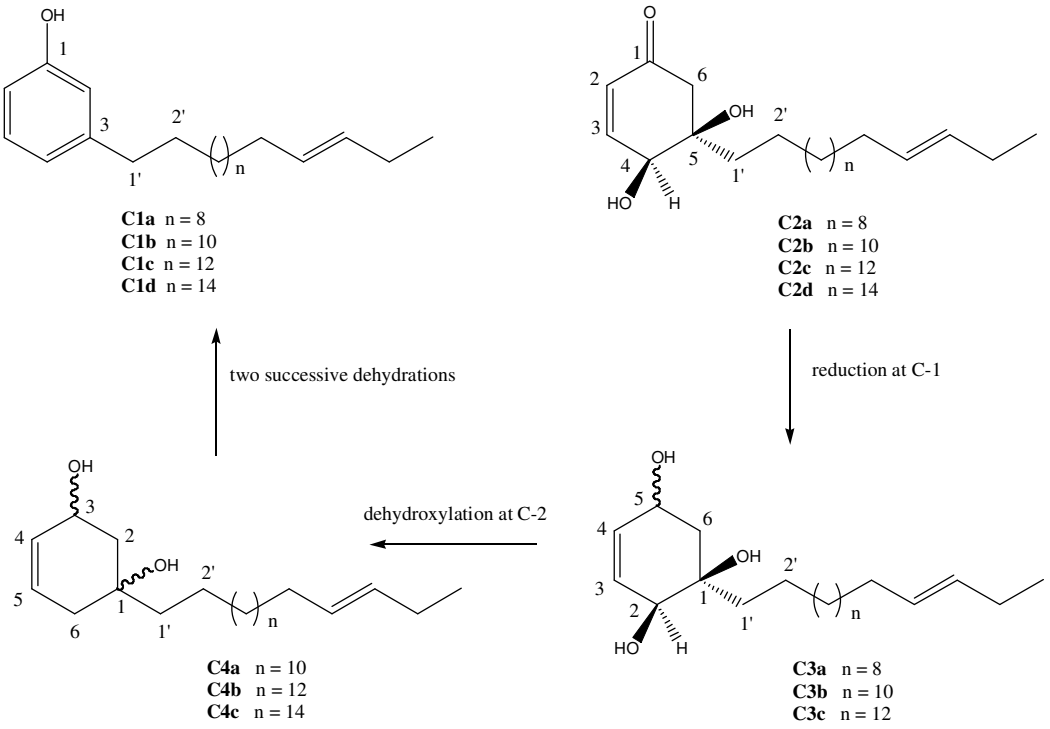

Figure 4-1 Biosynthetic sequence of the compounds isolated from *Lannea schimperi*

Compounds **C1a-C1d** all contained the same phenol structural moiety but differed in the length and nature of the alkyl chain. These compounds were isolated as a mixture of four compounds, with chain lengths varying from C-15 to C-21. Compounds **C1a-C1d** contained monounsaturated C-15, C-17, C-19 and C-21 alkyl chains. The structural elucidation was briefly discussed in Franke *et al*. (2001) and the ^{1}H and ^{13}C NMR data are the same as that published in Queiroz *et al*. (2003) for **C1b** and **C1c** isolated from *Lannea edulis*, however we have interchanged the assignments of C-18' and C-15' based on a HMBC correlation between C-18' and the methyl group H-19' (the numbering system refers to **C1c**). The C-18' resonance is now at δ_C 25.6 and the C-15' resonance at δ_C 32.6. We have also confirmed the assignments of the aromatic ring by HMBC correlations between H-1' and C-2, C-3 and C-4. The H-5, H-6 and H-2 proton resonances also showed HMBC correlations to C-1. The H-5 resonance, which appeared as a triplet (J = 7.7 Hz) due to coalescing of the dd resonance, was

placed between H-4 (d, J = 7.7 Hz) and H-6 (overlapping doublet with H-2) because of their splitting patterns. The names of the compounds, their molecular formulae and their molecular ion peaks as identified in the mass spectrum are contained in Table **4-1**. Both **C1a** and **C1d**, the pentadecenyl and the heneicosenyl derivative respectively are novel and have not been reported previously.

Compounds **C2a-d** occurred as a mixture of four 4-alkylated-4,5-dihydroxycyclohex-2-enones with C-15, C-17, C-19 and C-21 side chains. All the side chains had a double bond situated three bonds away from the terminal methyl group, since the olefinic carbon resonance in the side chain had a HMBC correlation with the terminal methyl group. Compounds **C2a-C2d** are listed in Table **4-1** and were able to be identified based on their molecular ion peaks as detected in the EIMS. The ^{1}H and ^{13}C NMR data for the mixture of compounds are the same as that published in Queiroz *et al*. (2003), where the structural elucidation is also discussed. We have however swopped around the assignments of H-2 and H-3 (now 6.88 for H-2 and 6.01 for H-3) as well as C-2 (now 152.8) and C-3 (now 125.8) based on HMBC correlations between C-2 and H-6_{ax}/H-6_{eq} as well as between C-5 and H-3. Compounds **C2a** and **C2d** are novel and have not been reported previously.

The ^{1}H NMR spectrum of compounds **C3a-C3c** showed a pair of olefinic resonances at δ_H 5.81 (dd, J = 10.2, 1.6 Hz) and 5.57 (dd, J = 10.2, 2.0 Hz) indicating a *cis* double bond. Two other oxygenated methine protons could be seen as broad singlets at δ_H 4.02 and δ_H 4.46 respectively and corresponded to the oxygenated methine carbon resonances at δ_C 65.5 and 70.0. There was also an additional oxygenated carbon resonance at δ_C 74.3. The double bond and the three oxygenated carbons takes up five carbon atoms of the cyclohexene ring with the last carbon in the ring being a methylene carbon atom at δ_C 40.8. This corresponded

to two non-equivalent proton resonances at δ_H 2.24 (dd, *J* = 5.5, 13.4 Hz, H-6a) and δ_H 1.42 (dd, *J* = 9.3, 13.2 Hz, H-6b).

Table 4-1 Compounds isolated from *Lannea schimperi*

	Name	**Molecular formula**	**EIMS (*m/z* (rel. int.))**
C1a	3-[12'(*E*)-pentadecenyl]phenol	$C_{21}H_{34}O$	302 [M^+] (11), 120 (16), 108 (100)
C1b[#]	3-[14'(*E*)-heptadecenyl]phenol	$C_{23}H_{38}O$	330 [M^+] (19), 147 (7), 120 (19), 108 (100)
C1c[#]	3-[16'(*E*)-nonadecenyl]phenol	$C_{25}H_{42}O$	358 [M^+] (17), 147 (6), 133 (5), 120 (18), 108 (100)
C1d	3-[18'(*E*)-heneicosenyl]phenol	$C_{27}H_{46}O$	386 [M^+] (19), 147 (5), 133 (4), 120 (14), 108 (100)
C2a	5-[12'(*E*)-pentadecenyl]-4,5-dihydroxycyclohex-2-enone	$C_{21}H_{36}O_3$	336 [M^+] (4), 318 (13), 237 (11), 123 (11), 95 (19), 84 (100)
C2b[#]	5-[14'(*E*)-heptadecenyl]-4,5-dihydroxycyclohex-2-enone	$C_{23}H_{40}O_3$	364 [M^+] (4), 346 (21), 265 (16), 123 (14), 95 (18), 84 (100)
C2c[#]	5-[16'(*E*)-nonadecenyl]-4,5-dihydroxycyclohex-2-enone	$C_{25}H_{44}O_3$	392 [M^+] (4), 374 (21), 293 (14), 123 (14), 95 (18), 84 (100)
C2d	5-[18'(*E*)-heneicosenyl]-4,5-dihydroxycyclohex-2-enone	$C_{27}H_{48}O_3$	420 [M^+] (2), 402 (10), 346 (20), 265 (7), 179 (13), 12 (46), 95 (48), 84 (100)
C3a	1-[12'(*E*)-pentadecenyl]-cyclohex-3-en-1,2,5-triol	$C_{21}H_{38}O_3$	320 [M^+-H_2O] (7), 237 (5), 111 (16), 95 (15), 86 (100)
C3b	1-[14'(*E*)-heptadecenyl]-cyclohex-3-en-1,2,5-triol	$C_{23}H_{42}O_3$	348 [M^+-H_2O] (8), 265 (6), 111 (18), 95 (22), 86 (100)
C3c	1-[16'(*E*)-nonadecenyl]-cyclohex-3-en-1,2,5-triol	$C_{25}H_{46}O_3$	376 [M^+-H_2O] (10), 293 (7), 111 (19), 95 (22), 86 (100)
C4a	1-[14'(*E*)-heptadecenyl]-4-cyclohex-4-en-1,3-diol	$C_{23}H_{42}O_2$	332 [M^+-H_2O] (7), 314 (5), 265 (11), 104 (33), 95 (100)
C4b	1-[16'(*E*)-nonadecenyl]-4-cyclohex-4-en-1,3-diol	$C_{25}H_{46}O_2$	360 [M^+-H_2O] (7), 342 (6), 293 (11), 123 (6), 104 (30), 95 (100)
C4c	1-[18'(*E*)-heneicosenyl]-4-cyclohex-4-en-1,3-diol	$C_{27}H_{50}O_2$	388 [M^+-H_2O] (8), 370 (6), 321 (11), 104 (31), 95 (100)

[#] ^1H and ^{13}C NMR data in Queiroz *et al*. (2003)

The two *cis* coupled olefinic protons showed strong coupling in the COSY spectrum. Another coupled system was seen between the two H-6 resonances and the oxygenated methine resonance at δ_H 4.46 (H-5). In the NOESY spectrum, this H-5 resonance was also seen coupled to the H-4 olefinic resonance at δ_H 5.81 and the other oxygenated methine resonance at δ_H 4.02 (H-2) showed NOESY correlations to the other olefinic doublet at δ_H 5.57 (H-3). The substitution pattern on the cyclohexene ring was supported by the HMBC correlations of H-4 with C-2, H-3 with C-5 and the other fully substituted oxygenated carbon resonance at δ_C 74.3 (C-1) as well as H-6a and H-6b showing HMBC correlations to C-1, C-2, C-4 and C-5.

The position of the alkyl chain was deduced from HMBC correlations between H-1' with C-1, C-2 and C-6. The double bond in the side chain was located three bonds away from the terminal methyl group since there was a HMBC correlation between one of the olefinic double bonds and the terminal methyl group of the alkyl chain. In the case of the heptadecenyl side chain, this was seen between C-15' and H-17'. The relative stereochemistry of H-2 and the alkyl chain was taken to be *cis*, since there was a NOESY correlation between H-2 and 2H-1'. However, due to a lack of NOESY correlations and coupling constants of H-5, the stereochemistry at C-5 could not be determined.

GCMS data of compound **C3a-C3c** (Table **4-1**) indicated that it was a mixture of three compounds, each with a double bond three bonds away from the terminal methyl group and with C-15, C-17 and C-19 alkyl chains. The molecular ion peaks for the three compounds were negligibly detected, but a more pronounced M^+-H_2O ion as is common for most secondary and tertiary alcohols is present in all three compounds. These ions together with

the molecular formulae and names of **3a-C3c** are given in Table **4-1**. Thus, **3a-C3c** were identified as their various 1-alkylated-1,2,5-trihydroxy-3-cyclohexenes.

In comparison to compound C**3a-C3c**, the ^{1}H NMR spectrum of **C4a-C4c** showed three changes; the multiplicity of the olefinic resonance H-5 changed to a multiplet and was shifted slightly downfield to δ_H 5.65-5.69, the oxymethine resonance at δ_H 4.00 disappeared and the 2-hydroxy resonance at δ_H 2.18 also disappeared. In the ^{13}C NMR spectrum, the oxymethine resonance at δ_C 70.0 disappeared and an additional methylene resonance at δ_C 37.4 was seen. Apart from these changes, all the other resonances of compound **C4a-C4c** were similar to that of **C3a-C3c**. Thus, apart from the resonances of the alkylated olefinic side chain, compound **C4a-C4c** had two hydroxyl groups, indicated by secondary and tertiary oxygenated carbon resonances at δ_C 66.0 and 72.4 respectively and two methylene groups at δ_C 42.9 and 37.4 in the ^{13}C NMR spectrum. The oxygenated methine proton was present as a broad singlet at δ_H 4.46. The ^{1}H NMR resonances of the methylene groups were present as non-equivalent resonances at δ_H 2.09 (dd, J = 12.8, 6.0 Hz, H-2a) and δ_H 1.39 (dd, J = 12.8, 9.6 Hz, H-2b). The other pair of non-eqivalent resonances for the second methylene group appeared at δ_H 2.16 (dd, J = 18.3, 2.7 Hz, H-6a) and a multiplet at δ_H 1.97 (H-6b). In addition, an olefinic double bond indicated by the doublet at δ_H 5.77 and the multiplet at δ_H 5.65-5.69 in the ^{1}H NMR spectrum was present. The olefinic carbon resonances were present at δ_C 130.6 and 125.9.

The positions of these groups on the cyclohexene ring were established by COSY correlations between H-2a and H-2b and H-3, and between H-6a and H-6b and H-5. This was supported by HMBC correlations; C-6 with H-4 and H-5; C-4 with H-2a and H-2b; C-3 with H-5; and C-1 with H-2a and H-2b. The position of the alkyl chain at C-1 was supported

by HMBC correlations between 2H-1' with C-1, C-2 and C-6 and the double bond on the side chain located three bonds away from the terminal methyl group due to a correlation between the terminal methyl proton resonance and the olefinic carbon resonance. Unfortunately, there were no NOESY correlations to determine the relative stereochemistry at C-1 and C-3.

Analysis of the GCMS data showed that compound C**4a-C4c** was a mixture of three compounds, each with a different alkyl chain length; C-17 (**C4a**); C-19 (**C4b**); and C-21 (**C4c**). Although the molecular ion peaks were not present in the mass spectra of the compounds, a prominent M^+-H_2O peak is seen for all three compounds, typical for tertiary alcohols. The names of **C4a-c** are presented in Table **4-1**.

Compounds **C2a-C2d**, **C3a-C3c and** C**4a-C4c** are biogenic precursors of the cardanols **C1a-C1d** (Walters *et al*., 1990). Reduction of **C2a-C2d** would lead to the formation of **C3a-C3c** and dehydroxylation of **C3a-C3c** would result in **C4a-C4c**. Dehydration of **C4a-C4c** results in the cardanols (Figure **4-1**). The 5-alkenyl cyclohexenone mixture **C2a-C2d** exhibited good *in vitro* cytotoxicity activity (IC_{50} = 7.95 μg mL^{-1}) against the Chinese Hamster Ovarian mammalian cell-line. The cardanols (**C1a-C1d**), the alkenyl cyclohexene triols (**C3a-C3c**) and the alkenyl cyclohexene diol mixtures (**C4a-C4c**) did not exhibit any cytotoxic activity (IC_{50} > 100 μg mL^{-1}). The standard drug emetine had an IC_{50} of 0.069 μg mL^{-1}. Alkenyl cyclohexenones have also previously demonstrated cytotoxicity (Correia *et al*., 2001., David *et al*., 1998, Groweiss *et al*., 1997, Roumy *et al*., 2009) and their activity is possibly related to the conjugated ketone, a Michael acceptor, often involved in pharmacological activities (Roumy *et al*., 2009).

Alkyl phenols that differ in chain length, degree of unsaturation and position of the double bonds occur in several genera within the Anacardiaceae family (Himejima and Kubo, 1991;

Correia *et al*., 2001; Franke *et al*., 2001; Masuda *et al*., 2002; Queiroz *et al*., 2003; Liu and Abreu, 2006; Saitta *et al*., 2009). The side chain usually has an odd number of carbons atoms ranging from C-15 to C-29, the most common chain length being C-15 and C-17 (Correia *et al*., 2006). The cardanols **C1b** and **C1c** with C-17 and C-19 side chains respectively were reported previously in *Lannea edulis* and *Rhus thyrsiflora* (Queiroz *et al*., 2003; Franke *et al*., 2001). Furthermore, the dihydroxy alkylated cyclohexenones (**C2b** and **C2c**) were also isolated in *L. edulis* (Queiroz *et al.*, 2003). As such, the isolation of these compounds in *Lannea schimperi* as well suggests a close chemotaxonomic relationship between both *L. edulis* and *L. schimperi.*

Compounds similar to **C2a-C2d** and **C3a-C3c** with the same cyclohexenone and cyclohexenol skeletons but with the functional groups arranged differently and the position of the double bond in the side chain being different, were isolated in *Lannea welwitschii* (Groweiss *et al.*, 1997), *Lannea nigritana* (Kapche *et al*., 2007) and from *Tapirira guianensis* (David *et al*. 1998; Roumy *et al*., 2009). This also suggests a chemotaxonomic link between the *Lannea* and *Tapirira* genera. The triterpenes taraxerol (**B8**) and taraxerone (**B7**) were also isolated in *Lannea rivae*.

4.3 Experimental

Plant material

The root and stem bark of *Lannea schimperi* (Engl.) Engl. were collected from Wote town, Makueni District, Kenya and were authenticated at the Maseno University Botanic garden Herbarium. This was assigned a voucher number (MSU/BG-3/13) and a sample specimen deposited at the museum for future reference. The plant material was air-dried under shade

for fourteen days and ground using a Wiley laboratory mill available at Kibos Sugar Company- Kisumu.

General experimental procedures

NMR spectra were recorded in $CDCl_3$ or CD_3OD on a Varian 400 MHz spectrometer. EIMS data were recorded on an Agilent MS 5973 instrument connected to a GC 6890. Ultraviolet absorption spectra were obtained on a Varian DMS 300 UV/visible spectrophotometer and Infrared spectra were recorded using a Nicolet Impact 400D Fourier-Transform Infra-Red (FT-IR) spectrometer. For column chromatography (CC), silica gel 60 (40–63 µm, Merck 1.09385) was used as the stationary phase. Analytical TLC was performed on precoated silica gel 60 F_{254} plates (Merck 1.05554) and was developed by spraying with anisaldehyde:H_2SO_4:MeOH (1:2:97 v/v) followed by heating. GC-MS data were recorded on an Agilent GC-MSD apparatus equipped with a DB-5SIL MS (30 m x 0.25 mm i.d., 0.25 µm film thickness) fused silica capillary column. He (2 mL min^{-1}) was used as a carrier gas and acetone or methanol (MeOH) was used to dissolve the sample. The injector was kept at 250 ºC and the transfer line at 280 ºC. The column temperature was held at 50 ºC for 2 min, and then ramped to 280 ºC at 20 ºC min^{-1} where it was held for 15 min. The MS was operated in the EI mode at 70 eV.

Extraction and Isolation

Two kilograms of the root was extracted in the cold on an orbital shaker sequentially with hexane and ethyl acetate for 72 hrs each to give crude extracts of 14.17 g and 34.56 g respectively. The TLC profiles of the extracts indicated that similar compounds were extracted by both solvents and therefore the extracts were combined. The crude extracts were

subjected to open column chromatography over silica gel and eluted with a stepwise gradient of n-hexane:ethyl acetate (1:0 to 0:1, increasing the amount of ethyl acetate gradually), followed by ethyl acetate:methanol (19:1). Fractions of 100 mL each were collected and monitored by TLC. TLC plates were observed under UV light at wavelengths of 254 nm and 365 nm as well as anisaldehyde spraying reagent. Similar fractions were combined and concentrated under vacuum using a Buchi rotary evaporator R-124.

Fractions 1-6 obtained from the crude column with hexane:ethyl acetate (95:5) was purified further with the same solvent system and resulted in a red liquid (4.44 g, cardanols C**1a**-C**1d**). The mixture could not be separated further and further attempts only resulted in the compounds co-eluting. Fractions 8-15 of the crude column was chromatographed with hexane:ethyl acetate (9:1), where the triterpenes, taraxerol (**B8**) (598 mg, fine colourless crystals) and taraxerone (**B7**) (799 mg, colourless crystals) were obtained from fractions 7-10 and 12-14 respectively. Sitosterol (**A6**) (1.69 g) was purified from fractions 19-30 of the crude column with 20% ethyl acetate in hexane. Fractions 30-39 from the crude column was separated with hexane:ethyl acetate (6:4 and 5:5) to yield two mixtures, alkenyl cyclohexenones (**C2a-C2d**) (34.60 mg) and alkenyl cyclohexene diols (**C4a-C4c**) (74.68 mg). The final mixture isolated was purified from fraction 45-55 from the crude column with ethyl acetate:hexane (6:4) to yield the alkenyl cyclohexene triols (**C3a-C3c**) (63.33 mg).

The stem bark extract was separated in a similar manner to the roots above where the same compounds were isolated from similar fractions.

Cytotoxicity assay

The MTT-assay was used as a colorimetric assay for cellular growth and survival. The method compares well with other assays (Mosmann, 1983; Rubinstein *et al.*, 1990). The

tetrazolium salt MTT was used to measure all growth and chemosensitivity. The test samples were tested in triplicate.

The test samples were prepared to a 20 mg mL^{-1} stock solution in 100% DMSO. Test compounds were stored at -20 ºC until used. Emetine was used as the reference drug in all experiments. The initial concentration of test samples was 100 µg mL^{-1}, which was serially diluted in complete medium with 10-fold dilutions to give 6 concentrations, the lowest being 0.001 µg mL^{-1}. The same dilution technique was applied to the all test samples. The highest concentration of solvent to which the cells were exposed to had no measurable effect on the cell viability (data not shown). The 50% inhibitory concentration (IC_{50}) values were obtained from full dose-response curves, using a non-linear dose-response curve fitting analysis via GraphPad Prism v.4 software.

*1-[12'(E)-pentadecenyl]-cyclohex-3-en-1,2,5-triol (***C3a***)*, *1-[14'(E)-heptadecenyl]-cyclohex-3-en-1,2,5-triol (***C3b***)*, *1-[16'(E)-nonadecenyl]-1,2,5-triol (***C3c***)* reddish brown liquid mixture; $[\alpha]^{20}_{D}$ 49.67° (c 0.25, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} 288 nm; IR υ_{max} cm^{-1}: 3364, 2920, 2851, 1461, 1282, 1017, 964, 833, 720 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.81 (1H, dd, *J* = 10.2, 1.6 Hz, H-4), 5.57 (1H, dd, *J* = 10.2, 2.0 Hz, H-3), 5.33-5.42 (2H, m, H-14', H-15'), 4.46 (1H, bs, H-5), 4.02 (1H, bs, H-2), 2.38 (bs, 2-OH), 2.24 (1H, dd, *J* = 13.4, 5.5 Hz, H-6a), 2.18 (1H, bs, OH), 1.90-2.01 (4H, m, H-13', H-16'), 1.83 (bs, OH), 1.57 (2H, m, H-1'), 1.42 (1H, dd, *J* = 13.2, 9.3 Hz, H-6b), 1.23-1.30 (m, H-2'-H-12'), 0.93 (3H, t, *J* = 7.4 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 132.7 (C-4), 131.8 (C-15'), 129.6 (C-3), 129.4 (C-14'), 74.3 (C-1), 70.0 (C-2), 65.5 (C-5), 40.8 (C-6), 39.4 (C-1'), 32.6 (C-13'), 29.2-29.7 (C-3'-C-12'), 25.6 (C-16'), 23.6 (C-2'), 14.0 (C-17'); The numbering system refers to **C3b**. EIMS see Table **4-1**.

1-[14'(E)-heptadecenyl]-cyclohex-4-en-1,3-diol **(4a)**, *1-[16'(E)-nonadecenyl]-cyclohex-4-en-1,3-diol* **(4b)**, *1-[18'(E)-heneicosenyl]-cyclohex-4-en-1,3-diol* **(4c)** reddish brown liquid mixture, $[\alpha]^{20}_{D}$ 14.20° (c 0.27, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} 289 nm, IR υ_{max} cm^{-1}: 3291, 2916, 2849, 1468, 1309, 1015, 963; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.77 (1H, d, *J* = 10.0 Hz, H-4), 5.67 (1H, m, H-5), 5.35-5.42 (2H, m, H-14', H-15'), 4.47 (1H, bs, H-3), 2.16 (1H, dd, *J* = 18.3, 2.7 Hz, H-6a), 2.09 (1H, dd, *J* = 12.8, 6.0 Hz, H-2a), 1.96-1.99 (4H, m, H-13', H-16'), 1.97 (1H, m, H-6b), 1.48 (2H, m, H-1'), 1.39 (1H, dd, *J* = 12.8, 9.6 Hz, H-2b), 1.23-1.31 (m, H-2'-H-12'), 0.94 (3H, t, *J* = 7.4 Hz, H-17'); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 131.8 (C-15'), 130.6 (C-4), 129.4 (C-14'), 125.9 (C-5), 72.4 (C-1), 66.0 (C-3), 43.3 (C-1'), 42.9 (C-2), 37.4 (C-6), 32.6 (C-13'), 29.2-29.7 (C-3'-C-12'), 25.6 (C-16'), 23.0 (C-2'), 14.0 (C-17'). Numbering system refers to **C-4a**. EIMS see Table **4-1**.

4.4 Conclusion

The isolation of the cardanols (**C1a-C1d**), cycohexenones (**C2a-C2d**) and cyclohexenols (**C3a-C3c** and **C4a-C4c**) confirm *Lannea schimperi's* place in the Anacardiaceae and its close relationship with *Lannea edulis*. Furthermore, the isolation of the cyclohexenols (**C3a-C3c** and **C4a-C4c**) also indicate its similarity to *Lannea welwitschii* and *Lannea nigritana* and to *Tapirira guianensis*, which also contained similar compounds albeit with different substitution patterns on the cyclohexenone and cyclohexenol rings. The isolation of taraxerone (**B7**) and taraxerol (**B8**) from *Lannea schimperi*, which we have also recently isolated from *Lannea rivae* indicate a further chemotaxonomic marker for the *Lannea*. The demonstrated cytotoxicity of the α,β-unsaturated cyclohexenone (**C2a-C2d**) indicates that this class of compound could be a lead compound for the discovery of anticancer compounds.

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Chapter 5 Alkyl phenols, alkyl cyclohexenols and alkyl cyclohexenones isolated from *Lannea schweinfurthii* (Engl.) Engl. and their bioactivity

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ABSTRACT

We have isolated six mixtures from the roots and stem bark of *Lannea schweinfurthii* which consisted of alkylated phenols (cardanols), cyclohexenones and cyclohexenols. Each of the mixtures contained novel compounds, which was novel by virtue of a novel alkyl side-chain with the core structure being isolated previously with other side chains with the exception of mixture 3 (**D2a-D2e**), which contained a novel α,β-unsaturated ketone moiety within the cyclohexenone structure. Among the novel compounds are unique conjugated diunsaturated side chains (**D1c-D1e** and **D4f-D4g**) which take on a *cis* and *trans* configuration. In addition, we have also isolated the known triterpenoids, sitosterol (**A6**), sitosterol glucoside (**B6**) and lupenone (**D5**), and flavonoids, epicatechin (**D6**), epicatechin gallate (**B12**), catechin (**D7**) and rutin (**D8**). The novel mixture 3 (**D2a-D2e**) showed good antibacterial, antiplasmodial, larvicidal and cytotoxic activity, while epicatechin gallate (**B12**) also showed good antibacterial, antiplasmodial and antioxidant activity.

5.1 Introduction

L. schweinfurthii (Engl.) Engl. (Anacardiaceae) is a tree which grows to 15 m high and has a rounded usually dense crown. These trees are widespread in East Africa. Its fruits are reddish brown when ripe and although edible, are not used as a food source. Like most other *Lannea* species, the bark is soft and fleshy and is generally used for tea and medicine, with the dye obtained from it being used to decorate baskets (Maundu *et al*., 1999). The stem and root bark decoction is traditionally used for stomach ailments such as stomach pain, gastrointestinal diseases, diarrhea and constipation, and to treat headaches and boils (Johns *et al*., 1990; 1995; Geissler *et al*., 2002; Arwa *et al*., 2010). In Tanzania, the Sukuma of the Bunda district boil the stem bark to treat abscesses, oral candidiasis and syphilis as well as being used to reduce cellulite (Maregesi *et al*., 2007). The bark infusion is also used in Bumalogi, Uganda to cure sterility (Tabuti *et al*., 2003).

The methanol extract of the stem bark was found to have anti-HIV-1 and 2 activity (Maregesi *et al*., 2010) as well as antigiardial activity (Johns *et al*., 1995). In addition, the aqueous extracts of the plant demonstrated cytotoxicity and antiplasmodial activities (Gathirwa *et al*., 2008; 2011). In our ongoing study of the genus *Lannea* and because of its medicinal use and bioactivity of the methanol and aqueous extracts, we have worked on the phytochemistry of the plant in order to determine what compounds were present and whether or not the isolated compounds were active in some of the assays in which the extracts of the plant were tested.

The phytochemistry of *L. schweinfurthii* has to our knowledge not been documented and this is the first report on the plant's chemistry. Previous studies of other *Lannea* species has led to isolation of alkylated hydroquinones (Groweiss *et al*., 1997), alkyl phenols and dihydroakylhexenones (Queiroz *et al*., 2003), a dihydroakylcyclohexenol (Kapche *et al*.,

2007), and flavonoids (Soluchana and Sastry, 1968; Sankara and Nair, 1971; Sultana and IIyas 1986a;1986b; Islam and Tahara, 2000). We report here on the phytochemistry of the root and stem bark of *L. schweinfurthii* and the antioxidant, cytotoxicity and larvicidal activity of the compounds isolated as well as include a discussion on the chemotaxonomic significance of these findings within the Anacardiaceae.

5.2 Results and discussion

The roots, stems and leaves of *Lannea schweinfurthii* (Engl.) Engl. led to the isolation of six mixtures of compounds, cardanols, cyclohexenones or cyclohexenols with either saturated, monounsaturated or diunsaturated alkyl side chains; **D1a-D1b** and **C1a-C1c** (mixture 1); **D1a-D1e**, **B1d** and **C1a-C1d** (mixture 2); **D2a-D2e** (mixture 3); **D3a-D3b**, **C3a** and **C3c** (mixture 4); **D4a**, **D4e** and **C4a-C4b** (mixture 5); and **D4b-D4d**, **D4f-D4g** and **C4b-C4c** (mixture 6). In addition to this, the known triterpenes, sitosterol (**A6**), sitosterol glucoside (**B6**) and lupenone (**D5**), and four known flavonoids, epicatechin (**D6**), epicatechin gallate (**B12**), catechin (**D7**) and rutin (**D8**) were also isolated. The known compounds were identified from their ^{1}H and ^{13}C NMR spectra as well as their 2D NMR spectra and their structures confirmed by comparison of their spectral data with those published in the literature (Queiroz, *et al.*, 2003; Sakurai *et al.*, 1987; Mahato and Kundu, 2004; Biabani *et al*., 2002; Kovganko *et al*., 1999; Faizi *et al*., 2001; Galotta *et al*., 2008; Fang and Ye, 2008; Demirezer *et al.*, 2006).

Mixture 1 contained five similar cardanols, **D1a-D1b**, *meta* alkylated phenols with saturated C-13 and C-17 chains respectively and **C1a-C1c** with C-15, C-17 and C-19 monounsaturated alkyl chains where the double bond is situated three bonds away from the terminal methyl group. The structural elucidation of **C1a-C1c** was discussed in Chapter 4. Compounds **D1a-**

D1b contained the same aromatic moiety compared to **C1a-C1c** in Chapter 4, however the GC-MS spectrum indicated two additional compounds, **D1a**, $C_{19}H_{32}O$ with a saturated C-13 side chain and which indicated a molecular ion peak at M^+ 276 amu, and **D1b**, $C_{23}H_{40}O$ with a saturated C-17 side chain indicated by a molecular ion peak at M^+ 332 amu in the mass spectrum. Compounds **D1a** and **D1b** have been reported previously (Franke *et al.*, 2001; Liu and Abreu, 2006).

The ^1H and ^{13}C NMR spectra of mixture 2 (**D1a-D1e**, **B1d** and **C1a-C1d)** showed resonances that were similar to the spectra in mixture 1 and **C1a-C1c** in Chapter 4. In particular, the aromatic resonances of H-5, H-4, H-2 and H-6 were all the same as well as H-1' (a triplet at δ_H 2.53, J = 7.6 Hz) and H-2' (a multiplet at δ_H 1.55-1.59) and the olefinic resonances of the double bond in the monounsaturated side chain, a multiplet at δ_H 5.36-5.41. However, there were four additional olefinic resonances in the ^1H NMR spectrum, a multiplet at δ_H 5.25-5.35 (H-14'), a doublet of triplets at δ_H 5.68 (1H, J = 15.1, 6.7 Hz, H-17'), a triplet (actually a dd which resembles a triplet due to the same coupling constant values for each of the coupled protons) at δ_H 5.93 (J = 10.9 Hz, H-15') and a double doublet at δ_H 6.28 (1H, J = 15.0, 10.9 Hz, H-16'). The coupling constants of these resonances indicated that H-14', H-15' and H-16' were all *cis* to each other and that H-17' was *trans* to H-16' and formed part of a conjugated double bond system that was three bonds away from the terminal methyl group, due to a HMBC correlation between H-19' and C-17' (this refers to the numbering system of **D1d**). The ^1H NMR spectrum also indicated that three different chains were present by having three different methyl triplet resonances, two were the monounsaturated and diunsaturated chain methyl groups at δ_H 1.00 (3H, t, J = 7.5 Hz, terminal methyl group of the diunsaturated chain), δ_H 0.94 (3H, t, J = 7.4 Hz, terminal methyl group of the monounsaturated chain) and

δ_H 0.86 (3H, t, *J* = 7.0 Hz, terminal methyl group of the saturated chain). The NMR data for **D1d** is shown in Table **5-1**.

From the GCMS analysis, ten compounds could be identified in this mixture, three of which had saturated side chains (**D1a**, **D1b** and **B1d**, all isolated earlier), four had monounsaturated side chains (**C1a-C1d**, also isolated earlier) and **D1c-D1e**, novel compounds with diunsaturated side chains with molecular masses at M^+ 328, M^+ 356 and M^+ 384 respectively. In all cases, the mass spectra displayed a base peak at *m/z* 108 due to benzylic cleavage of the 1-hydroxy-3-alkyl-benzene. The ions at *m/z* 77, 91 and 108 suggested the presence of a benzene ring with a phenolic hydroxyl and the ions at *m/z* 133, 147, 161, 175, and 206, 220 and 234 suggested the presence of an alkyl chain.

The ^{1}H NMR spectrum of the mixture 3 (**D2a-D2e)** contained a pair of doublets at δ_H 6.07 (1H, d, *J* = 10.1 Hz, H-2) and δ_H 6.83-6.87 (1H, m, H-3). Both these resonances were coupled to each other in the COSY spectrum and to the aliphatic methylene resonance at δ_H 2.47-2.50 (2H, H-4). The fact that H-2 was a doublet with 10.1 Hz indicated that it was part of a *cis* double bond. In addition, H-3 showed HMBC correlations to the carbonyl resonance at δ_C 198.5 (C-1), which in turn showed HMBC correlations to another methylene resonance at δ_H 2.53 (1H, s, H-6). The H-3 proton resonance also showed HMBC correlations to another oxygenated tertiary carbon resonance at δ_C 74.0. The remainder of the ^{1}H NMR resonances was assigned to the aliphatic side chain. Thus, at the tertiary carbon atom (C-5) of the α,β unsaturated cyclohexenone ring, both a hydroxy group and a long aliphatic side chain was placed.

Table 5-1 ^{1}H and ^{13}C NMR data of the cardanol **D1d**, the cyclohexenone **D2e** and the cyclohexenediol **D4f** (400 MHz, $CDCl_3$) (*J* values in Hz are in parenthesis)

Pos.	D1d		D2e		D4f	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	-	155.5	-	198.5	-	72.4
2	6.64 *s*	115.3	6.07 *dt* (10.1, 2.0)	129.4	2.10-2.15 *m* 1.42 *dd* (12.7, 9.6)	42.9
3	-	145.0	6.83-6.87 *m*	146.7	4.45-4.49 *m*	66.0
4	6.74 *d* (7.6)	120.9	2.47-2.50 *m*	38.2	5.78 *d* (10.2)	130.6
5	7.12 *t* (7.6)	129.4	-	74.0	5.67-5.72 *m*	125.9
6	6.61 *d* (2.4)	112.5	2.53 *s*	50.3	2.17-2.20 *m* 2.08-2.12 *m*	37.4
1'	2.53 *t* (7.6)	35.8	1.53-1.57 *m*	42.0	1.45-1.50 *m*	43.3
2'	1.55-1.59 *m*	31.3	1.22-1.36 *m*	22.7	1.22-1.33 *m*	23.0
3'-12'	1.23-1.33 *m*	29.2-29.8		29.2-29.9		29.3-29.7
13'	2.08-2.15 *m*	27.8	1.91-1.98 *m*	32.6	2.10-2.15 *m*	27.7
14'	5.25-5.35 *m*	130.2	5.36-5.41 *m*	129.4	5.24-5.34 *m*	130.2
15'	5.93 *t* (10.9)	128.6	5.36-5.41 *m*	131.9	5.92 *t* (10.9)	128.5
16'	6.28 *dd* (15.1,10.9)	124.7	1.91-1.98 *m*	25.4	6.27 *dd* (15.1, 10.9)	124.7
17'	5.68 *dt* (15.1, 6.7)	136.1	0.94 *t* (7.4)	14.0	5.63-5.71 *m*	136.1
18'	2.08-2.15 *m*	25.9			2.08-2.12 *m*	25.9
19'	1.00 *t* (7.5)	13.7			1.00 *t* (7.4)	13.7

The ^{1}H NMR spectrum contained a mixture of compounds with the same core skeleton and the two-proton multiplet resonance at δ_H 5.36-5.41 indicated that some of the side chains contained a double bond. This double bond (H-14'/15') had a *trans* configuration and was coupled to another aliphatic multiplet resonance at δ_H 1.91-1.98 (4H, m, H-13'/16'). The numbering refers to **D2e**. One of the terminal methyl groups at δ_H 0.94 (3H, t, *J* = 7.4 Hz, H-

17') showed an HMBC correlation to C-15' indicating that the double bond was three bonds away from the terminal methyl group. The other triplet at δ_H 0.85 (J = 7.0 Hz) was assigned to C-17$^{\#}$, the methyl group of the fully saturated alkyl chain. The multiplet at δ_H 1.53-1.57 was attributed to the methylene group (H-1') as this resonance was seen coupled to C-4, C-5 and C-6 in the HMBC spectrum.

From the GCMS spectrum it was possible to identify five major compounds, **D2a**, **D2b** and **D2c**, with the same core skeleton and saturated side chains with C-13, C-15 and C-17 carbon atoms respectively. These compounds showed ion fragment peaks at M^+ - H_2O, *m/z* 276, M^+ - H_2O, *m/z* 304 and M^+ - H_2O, *m/z* 332 amu respectively as expected in compounds with tertiary alcohols. Two further monounsaturated C-15 and C-17 side chains, **D2d** and **D2e** were also identified in the mixture with M^+ - H_2O, *m/z* 302 and M^+ - H_2O, *m/z* 330 respectively. The saturated and unsaturated derivatives with C-15 side chains co-eluted at almost the same time as did the saturated and unsaturated derivatives with C-17 side chains.

The ^1H and ^{13}C NMR spectra of mixture 4 (**D3a-D3b**, **C3a** and **C3c**) showed resonances similar to that of the mixture that contained **C3a-C3c**. In particular, the *cis* olefinic proton resonances at δ_H 5.81 (H-4) and δ_H 5.57 (H-3), each double doublets, the broad singlets at δ_H 4.02 (H-2) and δ_H 4.46 (H-5) and the non-equivalent double doublet resonances of H-6 at δ_H 2.24 and δ_H 1.42 were the same as in the mixture of **C3a-C3c** discussed in Chapter 4. The monounsaturated side chain resonances at δ_H 5.33-5.42 (2H, m), the methylene resonances attributed to the methylene groups on either side of the double bond at δ_H 1.90-2.01 (4H, m) and the terminal methyl group of the monounsaturated methyl group at δ_H 0.93 were also all the same as that for **C3a-C3c**. The ^1H NMR spectrum however indicated that another alkyl chain was present δ_H 0.86 (3H, t, J = 6.9 Hz, terminal methyl group of the saturated chain).

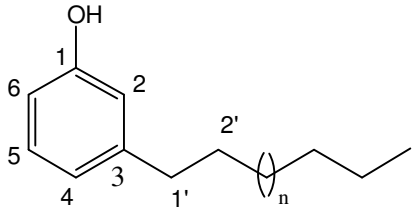
This was supported by the analysis of the GCMS data which indicated the presence of four compounds, two saturated, **D3a**, with a C-13 saturated alkyl chain (M^+ - H_2O at *m/z* 294) and **D3b** with a C-17 saturated alkyl chain (M^+ at 368 amu), and two unsaturated, **C3a** (M^+ - H_2O at *m/z* 320) and **C3c** (M^+ at 394 amu).

The ^1H and ^{13}C NMR spectra of the mixture 5 (**D4a**, **D4e** and **C4a-C4b**) were very similar to that of the mixture of **C4a-C4c** in chapter 4. The only differences were the extra triplet methyl resonance at δ_H 0.83-0.87 indicating a saturated methyl group and in the ^{13}C NMR spectrum, two methylene resonances, one and two bonds away from the terminal methyl group marked as C-11$^{\#}$ and C-12$^{\#}$ using the numbering system for **D4a**. Thus, this mixture contained a higher proportion of the core skeleton with a saturated aliphatic side chain than the mixture **C4a-C4c** from *Lannea schimperi*. GCMS analysis indicated the presence of four compounds with this core skeleton, one with a saturated C-13 aliphatic side chain **D4a** with M^+ - H_2O at 278 amu and three with a monounsaturated side chain, **D4e** with a C-15 side chain and M^+ - H_2O at 304 amu, **C4a** with a C-17 side chain (M^+- H_2O at 332 amu) and **C4b** with a C-19 side chain (M^+ - H_2O at 360 amu).

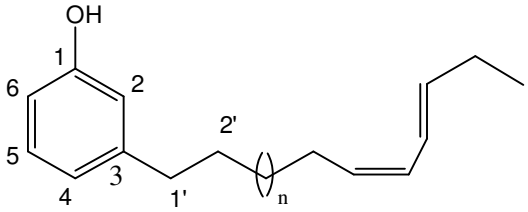
The ^1H and ^{13}C NMR spectrum of mixture 6 (**D4b-D4d**, **D4f-D4g** and **C4b-C4c**) was very similar to mixture 5 and the mixture of **C4a-C4c** in chapter 4 in that it had the same H-4 and H-5 proton resonances of the double bond, the H-3 methine resonance and the non-equivalent H-2 and H-6 methylene resonances. The ^{13}C NMR spectrum also indicated the same olefinic C-4 and C-5 resonances, the oxygenated C-1 and C-3 resonances and the methylene C-2 and C-6 resonances. With regard to the side chain, the monounsaturated olefinic protons were the same as that for the other mixtures with a multiplet at δ_H 5.36-5.41. The olefinic carbon resonances of the monounsaturated aliphatic chain were also the same on either side of the C-

4 resonance at δ_C 129.4 and δ_C 131.9. The olefinic region of the ^{1}H NMR spectrum also indicated four other olefinic resonances, two multiplets at δ_H 5.24-5.34 (H-14') and δ_H 5.63-5.71 (H-17') overlapping with the H-5 resonance and two more distinct olefinic resonances at δ_H 5.92 (H-15'), a double doublet appearing as a triplet due to the coalescing of resonances since the same coupling was observed to each proton on either side of it. This *J* value was 10.9 Hz indicating a *cis* relationship to H-14' and H-16'. The numbering system refers to **D4f**. The remaining oelfinic resonance at δ_H 6.27 (1H, dd, *J* = 15.1, 10.9 Hz, H-16') was *cis* to H-15' and *trans* to H-17' as indicated by the coupling constants of the double doublet. Thus, another unsaturated side chain with two conjugated double bonds where H-14', H-15' and H-16' had a *cis* relationship and H-16' and H-17' had a *trans* relationship was present.

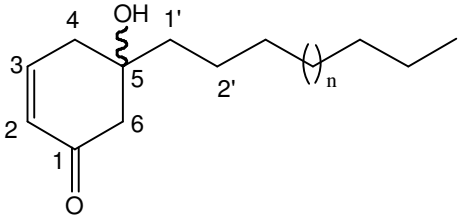
The ^{1}H NMR spectrum of the mixture 6 also indicated that three types of side chains were present since three terminal methyl group resonances could be seen at δ_H 0.99 (3H, t, *J* = 7.5 Hz, terminal methyl group of the diunsaturated chain), δ_H 0.93 (3H, t, *J* = 7.4 Hz, terminal methyl group of the monounsaturated chain) and δ_H 0.85 (3H, t, *J* = 7.0 Hz, terminal methyl group of the saturated chain). From the GCMS analysis of the mixture, seven compounds were identified, three with saturated C-19 (**D4b**, M^+ - H_2O at 362), C-21 (**D4c**, M^+ - H_2O at 390) and C-23 (**D4d**, M^+ - H_2O at 418) side chains, two with monounsaturated C-19 (**C4b**, M^+ - H_2O at 360) and C-21 (**C4c**, M^+ - H_2O at 388) side chains which were also isolated in *Lannea schimperi* (Chapter 4) and two with diunsaturated C-19 (**D4f**, M^+ - H_2O at 358) and C-21 (**D4g**, M^+ - H_2O at 386) side chains. The mass spectral data and molecular formulae of the novel compounds are indicated in Table **5-2** and the structures of the novel compounds are shown in Figure **5-1**. Novel compounds discussed in other chapters do not appear in Figure **5-1**.



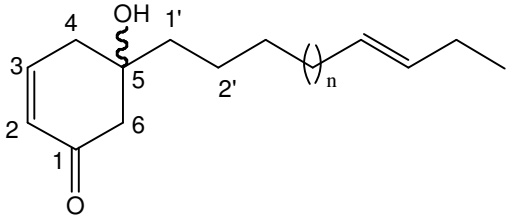
D1a: n = 8 **D1b**: n = 12



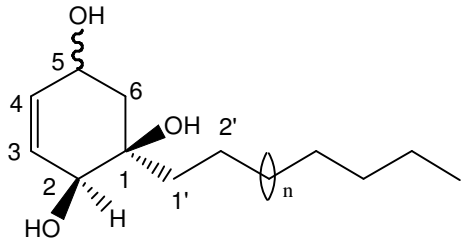
D1c: n = 8 **D1d**: n = 10 **D1e**: n = 12



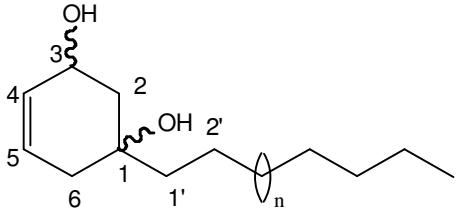
D2a: n = 7 **D2b**: n = 9 **D2c**: n = 11



D2d: n = 8 **D2e**: n = 10

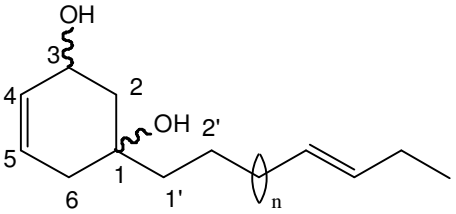


D3a: n = 7 **D3b**: n = 11

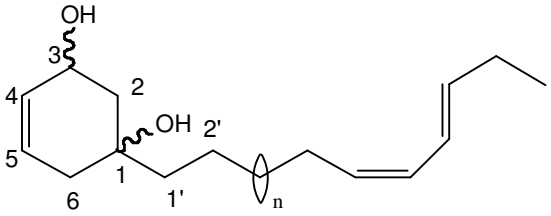


D4a: n = 7 **D4b**: n = 13

D4c: n = 15 **D4d**: n = 17



D4e: n = 9



D4f: n = 10 **D4g**: n = 12

Figure 5-1 Novel alkylated cardanols, cyclohexenones and cyclohexenols isolated from *Lannea schweinfurthii* (**D1a** and **D1b** has been isolated previously (Franke *et al*., 2001))

Table 5-2 Mass spectral data for novel compounds isolated from *Lannea schweinfurthii*

	Name	**Molecular formula**	**EIMS (*m/z* rel. int.)**
D1c	3-[heptadec-12'(*Z*),14'(*E*)-dienyl] phenol	$C_{23}H_{36}O$	328 [M^+] (18), 147 (35), 133 (17), 120 (46), 108 (100)
D1d	3-[nonadec-14'(*Z*),16'(*E*)-dienyl] phenol	$C_{25}H_{40}O$	356 [M^+] (26), 147 (21), 133 (13), 120 (26), 108 (100)
D1e	3-[heneicos-16'(*Z*),18'(*E*)-dienyl] phenol	$C_{27}H_{44}O$	384 [M^+] (29), 147 (19), 133 (12), 120 (20), 108 (100)
D2a	5-hydroxy-5-[tridecyl] cyclohex-2-enone	$C_{19}H_{34}O$	276 [M^+-H_2O] (7), 211 (14), 111 (100), 95 (8), 83 (8), 68 (15), 55 (12), 42 (23)
D2b	5-hydroxy-5-[pentadecyl] cyclohex-2-enone	$C_{21}H_{38}O$	304 [M^+-H_2O] (8), 239 (8), 111 (100), 95 (12), 83 (9), 68 (14), 55 (14), 43 (18)
D2c	5-hydroxy-5-[heptadecyl] cyclohex-2-enone	$C_{23}H_{42}O$	332 [M^+-H_2O] (9), 267 (4), 111 (100), 95 (14), 83 (8), 68 (10), 55 (11), 43 (18)
D2d	5-hydroxy-5-[pentadec-12'(*E*)-enyl] cyclohex-2-enone	$C_{25}H_{46}O$	302 [M^+-H_2O] (9), 121(12), 111 (100), 95 (30), 83 (19), 69 (23), 55 (34), 41 (23)
D2e	5-hydroxy-5-[heptadec-14'(*E*)-enyl] cyclohex-2-enone	$C_{23}H_{40}O$	330 [M^+-H_2O] (9), 111 (100), 95 (29), 83 (18), 69 (23), 55 (29), 41 (25)
D3a	1-[tridecyl] cyclohex-3-en-1,2,5-triol	$C_{19}H_{36}O_3$	294 [M^+-H_2O] (5), 227 (6), 211 (13), 111 (16), 86 (100), 57 (29), 43 (22)
D3b	1-[heptadecyl] cyclohex-3-en-1,2,5-triol	$C_{23}H_{44}O_3$	368 [M^+] (5), 185 (31), 167 (9), 142 (100), 75 (18), 43 (8)
D4a	1-[tridecyl] cyclohex-4-en-1,3-diol	$C_{19}H_{36}O_2$	278 [M^+-H_2O] (4), 255 (5), 227 (10), 115 (15), 95 (100)
D4b	1-[nonadecyl] cyclohex-4-en-1,3-diol	$C_{25}H_{48}O_2$	362 [M^+-H_2O] (4), 311 (12), 295 (9), 128 (8), 104 (12), 95 (100)
D4c	1-[heneicosyl] cyclohex-4-en-1,3-diol	$C_{27}H_{52}O_2$	390 [M^+- H_2O] (11), 372 (10), 354 (7), 305 (15), 123 (13), 109 (100), 95 (38), 57 (12)
D4d	1-[tricosyl] cyclohex-4-en-1,3-diol	$C_{29}H_{56}O_2$	418 [M^+-H_2O] (12), 400 (12), 382 (9), 333 (16), 123 (15), 109 (100), 95 (44)
D4e	1-[pentadec-12'(*E*)-enyl] cyclohex-4-en-1,3-diol	$C_{21}H_{38}O_2$	304 [M^+-H_2O] (3), 286 (4), 255 (6), 237 (7), 108 (11), 95 (100)
D4f	1-[nonadec-14'(*Z*),16'(*E*)-dienyl] cyclohex-4-en-1,3-diol	$C_{25}H_{44}O_2$	358 [M^+-H_2O] (11), 340 (11), 291 (5), 131 (8), 108 (34), 95 (100), 67 (54)
D4g	1-[heneicosen-16'(*Z*),18'(*E*)-dienyl] cyclohex-4-en-1,3 diol	$C_{27}H_{48}O_2$	386 [M^+-H_2O] (12), 368 (11), 319 (5), 151 (14), 108 (34), 95 (100), 81 (38), 67 (55)

Antibacterial activity

The hexane extract showed a narrow spectrum of activity against *E. faecium* and *E. faecalis* only (Table **5-3**). A broader spectrum of activity was observed with the methanol extracts of both the stem and the roots, with the highest activity being recorded by the methanol extract of the root against *S. aureus* and *E. faecium* (15 mm each). A previous study indicated that the water and methanol extracts of the same plant had moderate activity against other *S. aureus* strains (Maregesi *et al* 2008). Lower activities of the methanol extracts were observed against *P. aeruginosa* and *S. typhimurium* (8 mm each), while no activity was seen with *E. coli*. Of all the mixtures tested, only mixture 3, which contained the alkylated (both saturated and monounsaturated) hydroxycylcohexenones (**D2a-D2e**) was active against *S. aureus*, *E. faecium* and *E. faecalis* with zones of inhibition of 10 mm for each of the strains. This indicates the importance of the α,β-unsaturated ketone with regard to antibacterial activity as this was the only mixture that contained compounds with this core skeleton.

The flavonoids epicatechin (**D6**), catechin (**D7**), rutin (**D8**) and the triterpenoid sitosterol glucoside (**B6**) were also inactive. This was also indicated in previous studies (Kajiya *et al*., 2004). Epicatechin gallate (**B12**) had the best and broadest spectrum of antibacterial activity. Its activity toward *S. aureus* is comparable to erythromycin, a well known antibiotic. This compound is known to be active against *S. aureus* and several other bacterial strains and enhances the activity of antibiotics even against resistant strains (Hamilton-Miller and Shah, 2000; Stapleton *et al*., 2004; Hatano *et al*., 2005; Anderson *et al*., 2011; Qin *et al*., 2013). It is also known to inhibit bacterial DNA enzymes and cell wall synthesis and to disrupt virulence related proteins (Stepleton *et al*., 2007; Shah *et al*., 2008).

The plant is traditionally used to treat dysentery and severe diarrhea but none of the mixtures or compounds were active against *E. coli* except for epicatechin gallate, which had moderate activity against *E. coli.* Moderate activity was also seen with the methanol extracts and epicatechin gallate (**B12**) against *S. typhimurium.* This may imply that the plant could be treating diarrhea and dysentery resulting from other parasites such as amoeba, giardia or even viruses. Johns *et al*. (1995) demonstrated antigiardial activity of *L. schweinfurthii* extracts.

Table 5-3 Antibacterial activity of extracts, mixtures and compounds isolated from *Lannea schweinfurthii*

Sample	**Zones of inhibition (mm)**					
	S. aureus	*E. faecium*	*E. faecalis*	*P. aeroginosa*	*S. typhimurium*	*E. coli*
hexane root extract	0	10	10	0	0	0
Methanol stem extract	12	12	14	8	8	0
Methanol root extract	15	15	13	8	8	0
Mixture 3: **D2a-D2e**	10	10	10	0	0	0
Epicatechin gallate (**B12**)	22	14	14	12	8	9
Penicillin	34	-	-	-	-	-
Erythromycin	23	-	-	-	-	-
vancomycin	-	18	18	-	-	-
Cefuroxime	-	-		27		23
ciproflacin	-	-	-	-	33	32
chloraphenicol	-	-	-	-	26	

Data reported as an average of three readings; "-" indicates no testing was carried out

Antiplasmodial activity

The methanol and water extracts of *L. schweinfurthii* were not tested in this work, but demonstrated good antiplasmodial and antimalarial activity previously (Gathirwa *et al*., 2007; 2008; Maregesi *et al*., 2010). All isolated compounds were tested for their antiplasmodial

activity, however only mixture 3 (**D2a-D2e**) and epicatechin gallate (**B12**) showed antiplasmodial activity (Table **5-4**). The mixture **D2a-D2e** was moderately active whilst epicatechin gallate (**B12**) showed good antiplasmodial activity with IC_{50} values of 2.79 and 2.11 µg mL^{-1} against the chloroquine sensitive D6 and chloroquine resistant W2 *Plasmodium falciparum* strains respectively. This is indicative of the importance of the galloyl moiety of flavan-3-ol compounds (Ramanandralbe *et al*., 2008, Gibbons *et al*., 2004). This compound also inhibits important plasmodium enzymes (Tasdemir *et al*. 2006).

Table 5-4 Antiplasmodial activity of the active mixtures and compounds isolated from *Lannea schweinfurthii*

Sample	**D6 IC_{50} (µg mL^{-1})**	**W2 IC_{50} (µg mL^{-1})**
mixture 3: **D2a-D2e**	30.00 ± 1.34	24.12 ± 0.96
epicatechin gallate (**B12**)	2.79 ± 0.34	2.11 ± 1.9
chloroquine	0.0762	0.00443
mefloquine	0.0374	0.0122

Antioxidant activity

The antioxidant activity of the hexane and ethyl acetate extracts were not determined as the hexane extract contained mainly fatty acids and the ethyl acetate extract was reported to have low antioxidant potential (Adewusi and Steenkamp, 2011). The methanol extract and the flavonoids, known to have good antioxidant activity were tested using the DPPH radical scavenging method. At lower concentrations (6.25-25.0 µg mL^{-1}), only epicatechin gallate (**B12**) showed antioxidant activity comparable to ascorbic acid (

sample	**DPPH radical scavenging activity**	**Mean Comp.***	**IC50**

	6.25 µg mL-1	12.5 µg mL-1	25.0 µg mL-1	50.0 µg mL-1	100 µg mL-1		
rutin (**D8**)	24.88 ± 0.85	32.76 ± 1.23	53.40 ± 0.55	73.03 ± 0.05	77.17 ± 0.83	52.29a	22.48
epicatechin (**D6**)	32.80 ± 0.42	42.80 ± 0.42	60.72 ± 0.64	77.23 ± 0.44	85.51 ± 0.87	59.07b	15.15
catechin (**D7**)	30.26 ± 0.07	40.26 ± 0.06	61.18 ± 0.97	79.77 ± 0.74	83.92 ± 0.74	59.88b	16.04
epicatechin gallate (**B12**)	45.93 ± 0.95	66.09 ± 0.88	86.71 ± 0.60	98.31 ± 0.83	99.49 ± 0.49	74.42c	7.30
methanol extract (roots)	28.33 ± 0.82	42.51 ± 1.03	53.58 ± 0.45	59.67 ± 0.61	72.56 ± 0.95	51.29a	22.76
ascorbic acid	59.78 ± 0.41	73.77 ± 0.17	95.30 ± 0.10	99.03 ± 0.03	99.04 ± 0.48	84.64d	5.04
Mean Conc.*	36.49a	47.41b	68.54c	81.23d	86.20e		

Table 5-5). Epicatechin (**D6**), catechin (**D7**) and rutin (**D8**), as well as the methanol extract of the roots all showed moderate antioxidant activity at low concentrations, but good activity at higher concentrations (50.0-100 µg mL^{-1}). At higher concentrations the best activity was displayed by epicatechin gallate (**B12**) with antioxidant activity comparable to ascorbic acid. The gallate substitution at C-3 is responsible for the higher activity of epicatechin gallate. The antioxidant properties of phenolic compounds are due to their ability to donate an electron, quenching free radicals. The resultant radical anion is stabilized by the aromatic system of the phenols. In the same manner, phenols are also capable of donating hydrogen atoms to quench radicals. Studies have shown that the use of antioxidants in combination with chloroquine at the first signs of cerebral malaria prevents both inflammatory and vascular changes in the tissues of the brain as well as development of cognitive damage (Reis *et al*, 2010). This was seen in the use of artesunate, which when taken in conjunction with antioxidants was effective in the treatment of cerebral malaria and in preventing subsequent cognitive impairment in mice (Zimmerman and Castro, 2010; Percario *et al*., 2012).

sample	**DPPH radical scavenging activity**					**Mean Comp.***	**IC_{50}**
	6.25 µg mL^{-1}	12.5 µg mL^{-1}	25.0 µg mL^{-1}	50.0 µg mL^{-1}	100 µg mL^{-1}		

rutin (**D8**)	24.88 ± 0.85	32.76 ± 1.23	53.40 ± 0.55	73.03 ± 0.05	77.17 ± 0.83	52.29[a]	22.48
epicatechin (**D6**)	32.80 ± 0.42	42.80 ± 0.42	60.72 ± 0.64	77.23 ± 0.44	85.51 ± 0.87	59.07[b]	15.15
catechin (**D7**)	30.26 ± 0.07	40.26 ± 0.06	61.18 ± 0.97	79.77 ± 0.74	83.92 ± 0.74	59.88[b]	16.04
epicatechin gallate (**B12**)	45.93 ± 0.95	66.09 ± 0.88	86.71 ± 0.60	98.31 ± 0.83	99.49 ± 0.49	74.42[c]	7.30
methanol extract (roots)	28.33 ± 0.82	42.51 ± 1.03	53.58 ± 0.45	59.67 ± 0.61	72.56 ± 0.95	51.29[a]	22.76
ascorbic acid	59.78 ± 0.41	73.77 ± 0.17	95.30 ± 0.10	99.03 ± 0.03	99.04 ± 0.48	84.64[d]	5.04
Mean Conc.*	36.49[a]	47.41[b]	68.54[c]	81.23[d]	86.20[e]		

Table 5-5 Antioxidant activity of mixtures and compounds isolated from *Lannea schweinfurthii*

*means that differ significantly have different letters, $p < 0.05$; mean conc. indicates the mean of all the compounds tested at a specific concentration; mean comp. indicates the mean of the compound tested at different concentrations

Larvicidal activity

All the mixtures 1-6 showed good larvicidal activity (between 6.34 - 16.04 μg mL^{-1}) in comparison to the standard, pylarvex (3.12 μg mL^{-1}) (Table **5-6**). The best activity was seen by mixture 3, the α,β-unsaturated cylcohexenones (**D2a-D2e**) (6.34 μg mL^{-1}), followed closely by the cardanol mixture 2 (**D1a-D1e, B1d, C1a-C1d**) (8.17 μg mL^{-1}) and then the cyclohexene triol mixture 4 (**D3a**, **D3b**, **C3a**, **C3c**) (9.66 μg mL^{-1}). The high activity of mixture 3 could be attributed to the α,β-unsaturated cyclohexenone moiety. The cardanol mixture 1 was not as active as the cardanol mixture 2, possibly because mixture 1 (16.04 μg mL^{-1}) contained more cardanols with a saturated alkyl chain than cardanols with an unsaturated alkyl chain.

Table 5-6 Larvicidal activity of extracts and mixtures isolated from *Lannea schweinfurthii*

Extracts	**LC_{50} at 95% confidence limit (μg mL^{-1})**	**Mixtures**	**LC_{50} at 95% confidence limit (μg mL^{-1})**

Leaves (hexane)	51.57 ± 2.16[a]	mixture 1: **D1a**, **D1b**, **C1b** and **C1c**	16.04 ± 0.28[b]
Leaves (EtOAc)	47.89 ± 2.94[b]	mixture 2: **D1a-D1e**, **B1d**, **C1a-C1d**	8.17 ± 0.86[h]
Leaves (MeOH)	240.37 ± 1.31[c]	mixture 3: **D2a-D2e**	6.34 ± 0.51[h]
root (hexane)	73.36 ± 1.57[d]	mixture 4: **D3a**, **D3b**, **C3a**, **C3c**	9.66 ± 1.07[h]
root (EtOAc)	73.66 ± 1.59[d]	mixture 5: **D4a**, **D4e**, **C4a** and **C4b**	11.19 ± 1.03[i]
root (MeOH)	147.09 ± 1.11[e]	mixture 6: **D4b-D4d**, **D4f**, **D4g**, **C4b** and **C4c**	14.34 ± 1.01[i]
stem (hexane)	46.04 ± 1.32[b]	Pylarvex	3.12 ± 0.26
stem (EtOAc)	59.66 ± 0.76[f]		
stem (MeOH)	139.06 ± 2.90[g]		

Different superscript letters indicate significant differences in activity in the compounds ($p < 0.05$)

Larvicidal activity of cardanols is known (Lomonaco *et al*., 2009; Costa Oliveira *et al*., 2011; Souza *et al*., 2012). A higher degree of unsaturation was also shown to increase lipophilicity, which contributed to increased activity (Lomonaco *et al*., 2009). The cyclohexene diol mixtures 5 (**D4a**, **D4e**, **C4a** and **C4b)** and 6 (**D4b-D4d**, **D4f**, **D4g**, **C4b** and **C4c**), were the least active of the cyclohexenes. In comparison to the mixtures, the crude extracts had no significant larvicidal activity (Table **5-6**). The mode of action of many insecticides is by inhibition of the enzyme acetylcholinesterase (Finkelstein *et al*., 2002). The ethyl acetate crude root extract of *Lannea schweinfurthii* has been shown to have acetylcholinesterase inhibitory properties (Adewusi and Steenkamp, 2011) and hence this could be due to the alkylated cyclohexene derivatives. Furthermore, this must also be the mode of action for larvicidal activity of these compounds

Cytotoxicity

Mixtures 1, 2, 4 and 5 were non-toxic with IC_{50} values of between 80.5 to >100 μg mL^{-1} relative to the standard emetine (0.069 μg mL^{-1}) and hence could be considered safe to use for medicinal purposes (Table **5-7**). However, mixtures 3 (the α,β-unsaturated

cyclohexenones) and 6 (cyclohexene diols) could be considered as potentially toxic with IC_{50} values of 5.11 and 2.12 μg mL^{-1} respectively. In a structure-activity relationship study of lupane triterpenes on the induction of B16 cell 2F2 cell differentiation and apoptosis, it was demonstrated that the keto function at C-3 enhanced their differentiation inducing activities (Hata *et al*., 2003). Lupenone (**D5**) also showed cytotoxic activity with an IC_{50} value of 1.03 μg mL^{-1}. Previous studies have shown lupenone to have moderate cytotoxic activity (Gachet *et al*., 2011; Villareal *et al*., 2013). Mixtures 5 and 6 contain compounds with the same core structure, both cyclohexene diols and the fact that mixture 6 is quite toxic whereas mixture 5 is non-toxic could have to do with either the degree of unsaturation in the side chain (mixture 6 having compounds with a diunsaturated alkyl chain) or the particular *Z* and *E* configuration of the conjugated double bond in conjunction with the core structure. Previous cytotoxicity studies on *Lannea schweinfurthii* leaf extracts showed them to be cytotoxic against MT-4 cells and Vero E6 cells (Maregesi *et al*., 2010; Gathirwa *et al*., 2011). Moderate cytotoxicity of phenolic lipid derivatives isolated from *Lannea welwitschii* and *Lannea nigritana* were reported (Groweiss *et al*. 1997; Kapche *et al*., 2007). The mixtures 3 and 6 isolated from *Lannea schweinfurthii* could be leads to alternative antiproliferative agents for future development as anti-cancer drugs.

Table 5-7 Cytotoxicity of the mixtures and some of the compounds isolated from *Lannea schweinfurthii*

Sample	**IC_{50} (μg mL^{-1}) n = 3**	**Sample**	**IC_{50} (μg mL^{-1}) n = 3**
mixture 1: **D1a**, **D1b**, **C1b** and **C1c**	100	taraxerol (**B8**)	42.2
mixture 2: **D1a-D1e**, **B1d**, **C1a-C1d**	80.5	taraxerone (**B7**)	56.2
mixture 3: **D2a-D2e**	5.11	lupenone (**D5**)	1.03
mixture 4: **D3a**, **D3b**, **C3a**, **C3c**	> 100	emetine	0.069
mixture 5: **D4a**, **D4e**,	> 100		

C4a and **C4b**			
mixture 6: **D4b-D4d**, **D4f**, **D4g**, **C4b** and **C4c**	2.12		

Chemotaxonomic significance of the study

Cardanols, alkylcyclohexenones, alkylcyclohexenols, triterpenes and flavonoids are widely distributed in the Anacardiaceae family. The alkylcyclohexenones are thought to be the biosynthetic precursors of cardanols and have been previously isolated from *Lannea edulis*, *Lannea welwitschii*, *Tapirira guianensis* and *Tapirira obtusa*, the genus *Tapirira* also belonging to the Anacardiaceae (Queiroz *et al*., 2003, Roumy *et al*., 2009, Groweiss *et al*., 1997, David *et al*., 1998, Correia *et al*., 2001). A dihydroxyalkylcyclohexenol and a trihydroxyalkylcyclohexenol were isolated from *Lannea nigratina* and *Tapirira guianensis* respectively (Kapche *et al*., 2007; Roumy *et al*., 2009). In addition, a benzene derivative of a hydroxycyclohexenol was reported from *Tapirira obtusa* (Correia *et al*., 2001). Therefore, the occurrence of the cardanols, alkylcyclohexenols and alkylcyclohexenones confirm *Lannea schweinfurthii's* place in the Anacardiaceae and its close relationship to the other *Lannea* species and to the genus *Tapirira*.

Several cardanols where the alkyl chain differs in length, degree of unsaturation and position of double bonds occur in several genera within the Anacardiaceae family. Usually, the side chain is with an odd number of carbons C-15 to C-29, the most common chain length being C-15 and C-17. Cardanols with C-19 and C-17 side chains were reported in *Lannea edulis* and *Rhus thyrsiflora* (Franke *et al*., 2001; Queiroz *et al*., 2003; Liu and Abreu, 2006). We also report compounds with rare C-13 alkyl chains previously reported in *Ozoroa insignis* (Liu and Abreu, 2006).

The triterpenes, sitosterol (**A6**) and sitosterol glucoside (**B6**) are ubiquitous within the Plant Kingdom, however, the suite of compounds comprising sitosterol glucoside (**B6**), taraxerone (**B7**), taraxerol (**B8**) and epicatechin gallate (**B12**) were also reported in *Lannea rivae* (Chapter 3), indicating an even closer relationship between these two plant species than the other *Lannea* species. The flavonoid rutin (**D8**) has been reported previously in *Lannea grandis* (Soluchana and Sastry, 1968) while the flavonoids catechin (**D7**), epicatechin (**D6**) and epicatechin gallate (**B12**) reported here have also been reported to occur in *Lannea velutina*, *Lannea microcarpa* and *Lannea nigratina* respectively (Kapche *et al*., 2007; Maiga *et al*., 2007; Picerno *et al*., 2006). This is the first report of lupenone (**D5**) in the genus *Lannea*.

5.3 Materials and methods

Plant collection, identification and preparation

The plant material was collected at Seme- Kit Mikayi, Kombewa division, Kisumu West district in Kenya in June, 2010 and a voucher specimen deposited at the Maseno University Botanic Garden Herbarium (MSU/BG-1/13). The leaves, roots and stem bark of the plant were air dried away from direct sunlight for two weeks followed by grinding to a fine powder using a Wiley mill at Kibos Sugar Research Institute in Kisumu.

General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded in either $CDCl_3$ or CD_3OD on a Bruker Avance^{III} 400 MHz spectrometer at room temperature. GC-MS analysis was carried out using a Hewlett-Packard GC 5890 and a Hewlett-Packard 5970 mass selective detector on an HP-1 ultra-2 column, where the temperature was programmed from 50 to 250 °C at 10 °C min^{-1} and

maintained at 250 °C for 30 min. Optical rotations were recorded using a Perkin Elmer model 341 Polarimeter. UV absorption spectra were obtained on a UV/VIS 3600 Shimadzu spectrophotometer and Infrared spectra were recorded using a Perkin Elmer Universal Attenuated Total Reflectance (ATR) 100 series spectrophotometer. Chromatographic separations were achieved by column chromatography (CC) using silica gel 60 (40–63 μm, Merck 1.09385) and analytical TLC was performed on precoated silica gel 60 F_{254} plates (Merck 1.05554) and was developed by spraying with anisaldehyde:H_2SO_4:MeOH (1:2:97) followed by heating.

Extraction and isolation

The powdered root material (5 kg) was exhaustively extracted with n-hexane for 72 hours followed by ethyl acetate (EtOAc) and methanol (MeOH) and concentrated in vacuo to give 27.0 g of hexane extract, 25.8 g of EtOAc extract and 60.9 g of MeOH extract. The hexane extract was fractionated by open column chromatography on silica gel with a stepwise gradient of hexane:EtOAc (19:1 to 4:1). Sixty 100 mL fractions were collected. The following fractions were combined based on their TLC analysis: 1-8, 10-14, 20-26, 30-40, 50-56. Fraction 1-8 (3.78 g) was rechromatographed on a silica gel column (2cm diameter) and eluted with 19:1 hexane:EtOAc, collecting 20 mL fractions to yield a red liquid (2.07 g), a mixture of cardanols (mixture 1: **D1a, D1b, C1b and C1c)** from fraction 18-34. Fraction 10-14 (3.06 g) was rechromatographed on a silica gel column and eluted with 9:1 and 8:2 hexane:EtOAc. Thirteen × 20 mL fractions were collected. Fractions 6-10 and 12-14 of this column yielded 343 mg of taraxerone (**B7**) (white plates) and 193 mg of taraxerol (**B8**) (fine white crystals) respectively. Fraction 20-26 was purified with hexane:EtOAc (8:2) where a colourless paste 160.2 mg (mixture 3: **D2a-D2e)** was obtained from fractions 6-13. Purification of fraction 30-40 with hexane:EtOAc (7:3) yielded 1.04 g of sitosterol (**A6**)

(white needlelike crystals). Fraction 50-56 (6.87 g) was purified on a silica gel column and eluted with 7:3 and 6:4 hexane:EtOAc. Fractions of 20 mL were collected. Fraction 5-19 resulted in a yellow amorphous solid (4.23 g) (mixture 5: **D4a, D4e, C4a** and **C4b**).

The EtOAc extract of the roots was subject to the same chromatographic procedure, but with a stepwise gradient of hexane:dichloromethane (DCM) (19:1 to 100 % DCM). A total of 140 fractions of 100 mL each were collected. From these, four sets of fractions were combined (3-20, 50-60, 85-100, 106-139) based on similarities in their TLC profiles. Purification of fractions 3-20 with 19:1 hexane:EtOAc yielded more of mixture 1 (56.3 mg), the cardanols (**D1a, D1b, C1b** and **C1c**). Fraction 50-60 was purified with 30% EtOAc in hexane and a light yellow paste was obtained from fractions 10-29 (34.6 mg) (more of mixture 3: **D2a-D2e**). A 1:1 ethyl acetate:hexane was used to purify fraction 85-100. A yellow solid (45.3 mg) (mixture 5: **D4a, D4e, C4a** and **C4b)** was obtained from fractions 14-18. Fractions 106-139 was purified with 6:4 hexane:EtOAc and yielded a reddish brown solid (67.8 mg) (mixture 4: **D3a**, **D3b**, **C3a** and **C3c**) from fractions 17-37.

The methanol extracts of the roots, stems and leaves were combined and separated as above with a stepwise gradient of DCM:MeOH (19:1 to 7:3) to give three fractions, 50-56, 60-76 and 80-86. Fraction 50-56 was separated on a LH-20 sephadex column using 1:1 methanol:acetone to yield a reddish brown solid, and pale white solid, catechin (**D7**) (56.9 mg) and epicatechin (**D6**) (30.5 mg) respectively. Using 100% methanol, fraction 60-76 was purified on a LH-20 sephadex column to yield a reddish brown solid, epicatechin gallate (**B12**) (72.4 mg). A similar column was used to purify sitosterol glucoside (**B6**) (93.4 mg) (amorphous white solid) and rutin (**D8**) (45.1 mg) (yellow solid) from fraction 80-86.

The hexane stem bark extract was subjected to the same protocol as the hexane root extracts and yielded the same compounds as that contained in the root. In addition, the ethyl acetate extract which was separated with hexane:DCM (19:1) yielded a further mixture of cardanols (82.7 mg) (mixture 2: **D1a-D1e, B1d**, **C1a-C1d)** which was purified from fraction 85-105. Lupenone (**D5**) (38.4 mg) was obtained from fraction 70-76 as a colourless solid. Fraction 180-188 resulted in a yellow solid mixture (56.8 mg) (mixture 6: **D4b-D4d**, **D4f**, **D4g**, **C4b** and **C4c**). These were purified with hexane:EtOAc solvent mixtures (9:1 to 100% ethyl acetate) as in the root above.

mixture 1: *3-[tridecyl] phenol* **(D1a)**, *3-[heptadecyl] phenol* **(D1b)**, *3-[heptadec-14'(E)-enyl] phenol* **(C1b)** and *3-[nonadec-16'(E)-enyl] phenol* **(C1c)** red liquid; UV λ_{max} (MeOH) 273, 220 nm; IR υ_{max} cm^{-1}: 3369, 2922, 2852, 1588, 1485, 1265, 1154. EIMS see chapter 4; ^{1}H and ^{13}C NMR see Queiroz *et al*. (2003).

mixture 2: *3-[tridecyl] phenol* **(D1a)**, *3-[heptadecyl] phenol* **(D1b)**, *3-[heptadec-12'(Z),14'(E)-dienyl] phenol* **(D1c)**, *3-[nonadec-14'(Z),16'(E)-dienyl] phenol* **(D1d)**, *3-[heneicos-16'(Z),18'(E)-dienyl] phenol* **(D1e)**, *3-[pentadecyl] phenol* **(B1d)**, *3-[12'(E)-pentadecenyl]phenol* **(C1a)**, *3-[14'(E)-heptadecenyl]phenol* **(C1b)**, 3-[16'(*E*)-nonadecenyl]phenol **(C1c)**, *3-[18'(E)-heneicosenyl]phenol* **(C1d)** colorless oil; UV λ_{max} (MeOH) 310, 273 nm; IR υ_{max} cm^{-1}: 3371, 2922, 2852, 1589, 1456,1268, 1154; EIMS see chapters 3 and 4 and Table **5-2**; ^{1}H and ^{13}C NMR see Queiroz *et al.* (2003) and Table **5-1** for **D1c, D1d** and **D1e**.

mixture 3: *5-hydroxy-5-[tridecyl] cyclohex-2-enone* **(D2a)**, *5-hydroxy-5-[pentadecyl] cyclohex-2-enone* **(D2b)**, *5-hydroxy-5-[heptadecyl] cyclohex-2-enone* **(D2c)**, *5-hydroxy-5-*

[pentadec-12'(E)-enyl] cyclohex-2-enone **(D2d)**, *5-hydroxy-5-[heptadec-14'(E)-enyl] cyclohex-2-enone* **(D2e)** colourless paste; $[\alpha]_D^{20}$ -7.62 (c = 1.05, $CHCl_2$); UV λ_{max} (CH_2Cl_2) 273, 231 nm; IR υ_{max} cm^{-1}: 3390, 2921, 2851, 1668, 1463, 1387, 1255; EIMS see Table **5-2**; ^1H and ^{13}C NMR see Table **5-1**.

mixture 4: *1-[tridecyl] cyclohex-3-en-1,2,5-triol* **(D3a)**, *1-[heptadecyl] cyclohex-3-en-1,2,5-triol* **(D3b)**, *1-[12'(E)-pentadecenyl]-cyclohex-3-en-1,2,5-triol* **(C3a)**, *1-[16'(E)-nonadecenyl]-cyclohex-3-en-1,2,5-triol* **(C3c)** reddish brown paste; $[\alpha]_D^{20}$ +30.48 (c = 1.05, $CHCl_2$); UV λ_{max} (CH_2Cl_2) 232 nm; IR υ_{max} cm^{-1}: 3390, 2919, 2851, 1465, 1376, 1277; EIMS see chapter 4 and Table **5-2**; ^1H and ^{13}C NMR see chapter 4.

mixture 5: *1-[tridecyl] cyclohex-4-en-1,3-diol* **(D4a)**, *1-[pentadec-12'(E)-enyl] cyclohex-4-en-1,3-diol* **(D4e)**, *1-[14'(E)-heptadecenyl]-cyclohex-4-en-1,3-diol* **(C4a)**, *1-[16'(E)-nonadecenyl]-cyclohex-4-en-1,3-diol* **(C4b)** yellow solid; $[\alpha]_D^{20}$ +17.14 (c = 1.05, $CHCl_2$); UV λ_{max} ($CHCl_3$) 239 nm; IR υ_{max} cm^{-1}: 3269, 2916, 2850, 1468, 1310; EIMS see chapter 4 and Table **5-2**; ^1H and ^{13}C NMR see chapter 4.

mixture 6: *1-[nonadecyl] cyclohex-4-en-1,3-diol* **(D4b)**, *1-[heneicosyl] cyclohex-4-en-1,3-diol* **(D4c)**, *1-[tricosyl] cyclohex-4-en-1,3-diol* **(D4d)**, *1-[nonadec-14'(Z),16'(E)-dienyl] cyclohex-4-en-1,3-diol* **(D4f)**, *1-[heneicosen-16'(Z),18'(E)-dienyl] cyclohex-4-en-1,3-diol* **(D4g)**, *1-[16'(E)-nonadecenyl]-4-cyclohex-4-en-1,3-diol* **(C4b),** *1-[18'(E)-heneicosenyl]-4-cyclohex-4-en-1,3-diol* **(C4c)** colourless solid; $[\alpha]_D^{20}$ +20.07 (c = 1.05, $CHCl_2$); UV λ_{max} (CH_2Cl_2) 207, 242 nm; IR υ_{max} cm^{-1}: 3314, 2918, 2850, 1467, 1380, 1310; EIMS see chapter 4 and Table **5-2**.. ^1H and ^{13}C NMR see Table **5-1**.

Antibacterial assay

Microorganisms used in this study were isolated from clinical samples at CDC Kombewa, Kenya. Commercially available antibiotic diffusion discs were used. The bacteria tested were *Salmonella typhimurium* ATCC 14028*, Escherichia coli* ATCC 25922*, Enterococcus faecalis* ATCC 29212*, Enterococcus faecium* ATCC 19434*, Pseudomonus aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923.

The stock solutions of compounds and extracts were dissolved in 10% dimethylsulfoxide (DMSO) in water to a final concentration of 100 μg mL^{-1}. The sterile discs (6 mm) were impregnated with 10 μL of the compounds (1 μg). Petri plates were prepared with 20 mL of a base layer of molten Mueller Hinton agar. The inoculum of ATCC used was adjusted to 0.5 McFarland standard (10^6 CFU mL^{-1}). The plates were incubated for 24 h at 37 °C (CLSI, 2007). Negative controls were prepared using discs impregnated with 10% DMSO in water and commercially available antibiotic diffusion discs, penicillin (10 units), erythromycin (15 μg mL^{-1}), vancomycin (30 μg mL^{-1}) and cefuroxime (30 μg mL^{-1}) were used as positive reference standards. The diameters of the inhibition zones were evaluated in mm. The extracts or compounds inducing inhibition zones of ≥ 9 mm were considered as potential antibacterial compounds. All tests were performed in triplicate and the zone of inhibition (bacterial activity) was expressed as an average of the three readings in mm.

Cytotoxicity assay

In vitro cytotoxicity tests of the compounds were performed on a Chinese Hamster Ovarian mammalian cell-line using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-assay. The MTT-assay is used as a colorimetric assay for cellular growth and survival, and compares well with other available assays (Mosmann *et al*., 1983; Rubinstein *et*

al., 1990). The tetrazolium salt MTT was used to measure all growth and chemosensitivity. The samples were tested in triplicate on one occasion.

The test samples were prepared to a 20 mg mL^{-1} stock solution in 100% DMSO and were tested as a suspension if not properly dissolved. Test compounds were stored at -20 ºC until used. Emetine was used as the reference drug in all experiments. The initial concentration of test samples was 100 µg mL^{-1}, which was serially diluted in complete medium with 10-fold dilutions to give 6 concentrations, the lowest being 0.001 µg mL^{-1}. The same dilution technique was applied to all the test samples. The highest concentration of solvent to which the cells were exposed to had no measurable effect on the cell viability (data not shown). The 50% inhibitory concentration (IC_{50}) values were obtained from full dose-response curves, using a non-linear dose-response curve fitting analysis via GraphPad Prism v.4 software.

Larvicidal assays

The compounds were solubilized in dimethyl sulphoxide (DMSO, analytical reagent, Lobarchemi) and diluted to the required concentration with spring water. The concentration of DMSO was kept below 1%. The bioassay experiments were conducted according to standard procedure (WHO, 2005) with slight modifications. The bioassays were conducted at the Kenya Medical Research Institute (KEMRI), Insect Unit, Kisumu, Kenya, where the insects were reared in plastic and enamel trays in spring river water. They were maintained and all experiments were carried out at 26 ± 3 °C and the humidity ranged between 70% and 75%. The bioassays were performed with late third and early fourth instar larvae of *A. gambiae* and carried out in triplicate using 25 larvae for each replicate assay according to established procedures (WHO, 2005). The replicates were run simultaneously yielding a final total of 75 larvae for each dosage. The larvae were placed in 40 mL disposable plastic

cups containing 20 mL of test solution and fed on tetramin fish feed during all testing. Mortality and survival was established after 24 h of exposure. Larvae were considered dead if they were unresponsive within a period of time, even when gently prodded. The dead larvae in the three replicates were recorded after the 24 hr exposure. The negative control was 1% DMSO in spring river water while the positive control was the pyrethrum based larvicide pylarvex® (Pyrethrum Board of Kenya). The probit analysis of the concentration mortality data was conducted using IBM SPSS statistics version 19 to obtain LD_{50} values and the associated 95% confidence limits.

SYBR Green I assay antiplasmodial assay

The plant extracts were screened against two *Plasmodium falciparum* strains, chloroquine sensitive (D6) from Sierra Leone and chloroquine resistant (W2) from Vietnam. The cultures were maintained at the US Army Medical Research Unit—Kenya, Malaria Resistance Laboratories at the Kenya Medical Research Institute (KEMRI), Kisian-Kisumu, according to literature (Smilkstein *et al*., 2004). Mefloquine and chloroquine reference drugs were used as a positive control. The culture medium was prepared as described by Johnson *et al*. (2007). Mefloquine was dissolved in 70% ethanol while the compounds and chloroquine were dissolved in 100% DMSO to an initial concentration of 1 mg mL^{-1}. The *P. falciparum* cultures were adjusted to 2% haematocrit and 1% parasitemia for the assay (Akala *et al*., 2011) a modification of Desjardins *et al*. (1979) and Trager and Jensen (1976). After 72 h incubation, 100 µL of lysis buffer containing SYBR Green I dye was added to the 96-well plates prior to 1 h incubation in the dark. The fluorescence was then measured using a Genios Tecan® micro-plate reader. IC_{50} values were then calculated by Graphpad Prism (Graphpad Prism for Windows, version 5.0; Graphpad Software, Inc., San Diego, CA).

Antioxidant assay

A 10 mg mL^{-1} stock solution of each compound was made by dissolving the compounds in DMSO. Sample concentrations of 6.25, 12.50, 25.00, 50.00 and 100 μg mL^{-1} were made in methanol. The DPPH radical scavenging activity of the compounds was determined according to the method of Kumawat *et al.* (2012). Briefly, 1 mL of each compound was added to 1 mL of DPPH (1,1-diphenyl-2-picrylhydrazyl) solution (0.1 mM in methanol). The mixture was shaken and kept in the darkness for 30 minutes at room temperature. The decrease of solution absorbance was determined at 517 nm. Vitamin C (ascorbic acid) was used as the positive control. The DPPH radical scavenging activity was calculated using the following formula:

DPPH Radical Scavenging Activity (%) = $[(A_0 - A_1 / A_0) \times 100]$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the compound or standard sample.

All the tests were performed in triplicate. The results were given as means ± S.D. Analysis of variance and significant differences among means were tested by two way ANOVA, using SPSS (Version 12.0 for Windows, SPSS Inc., Chicago, IL, USA). When significant main effects existed, differences were tested by Duncans test at 95% confidence. The IC_{50} values were calculated using dose-response curves, using a non-linear dose-response curve fitting analysis GraphPad Prism (Version 5) software.

5.4 Conclusion

We have found six mixtures in the roots and stem bark of *L. schweinfurthii*, one which had a novel core structure with an α,β-unsaturated ketone moiety (mixture 3; **D2a-D2e**) and which which was found to be active in antibacterial, antiplasmodial, larvicidal and cytotoxicity assays along with epicatechin gallate (**B12**), which did not show larvicidal activity, but

showed antioxidant activity in addition to the antibacterial, antiplasmodial and cytotoxic activity. It is highly likely that the medicinal uses and bioactivity of the extracts is a result of these phytochemical constituents present in the plant. While epicatechin gallate (**B12**) has been studied extensively, the novel structures in mixture 3 can provide leads for antibacterial, antiplasmodial, larvicidal and anti-cancer drugs. The fact that mixture 6, which is more cytotoxic is not active in the any of the assays above indicates that the toxicity of mixture 3 is not responsible for the antibacterial, antiplasmodial and larvicidal activity, but rather the α,β-unsaturated ketone moiety could play a role in the activity of the compounds in mixture 3.

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Chapter 6 Conclusion

This thesis describes the phytochemistry of *Lannea alata*, *Lannea rivae*, *Lannea schweinfurthii* and *Lannea schimperi* and the bioactivities of the extracts, mixtures and compounds from these four species. The plants are used in traditional medicine to treat a variety of disease symptoms like fever, malaria, diarrhea and wounds.

Prior to this study, *Lannea alata* was only known to be used to treat fever, malaria, fractures, wounds and snakebites. This investigation led to the isolation of two known and two new flavonoids (myricetin-3-*O*-α-rhamnopyranoside (**A3**), myricetin-3-*O*-α-arabinofuranoside (betmidine) (**A4**), lanneaflavonol (**A1**) and lanneadihyroflavonol (**A2**)), along with lupeol (**A5**) and sitosterol (**A6**). The flavonoids were shown to exhibit antibacterial and antioxidant activities and lupeol (**A5**) is reported to have numerous biological activities including antibacterial, antifungal, antiplasmodial, anti-trypanosomal, antitumor/anticancer, anti-inflammatory and anti-arthritic activities. Sitosterol (**A6**) is most commonly known for its immune boosting properties. The antibacterial and antiplasmodial activity of the compounds isolated from *L. alata* could therefore be responsible for the antipyretic and antimalarial action of the plant extracts and its use to treat snakebites, wounds and fractures, could be due to the anti-inflammatory action of lupeol (**A5**). The immunostimulant properties of sitosterol (**A6**) may also contribute generally to the plant being used for medicinal purposes. The new flavonoids, lanneaflavonol (**A1**) and dihydroflavonol (**A2**) could be leads for the development of antibacterial and antioxidant pharmaceuticals.

Lannea rivae is a medicinal plant used for the treatment of colds, coughs, fever and stomachache. Its medicinal uses suggest the presence of antibacterial compounds in its

extracts. The phytochemical investigation led to isolation of the triterpenes, sitosterol (**A6**), sitosterol glucoside (**B6**), taraxerone (**B7**) and taraxerol (**B8**), the flavonoids, myricetin (**B10**), myricetin-3-*O*-α-rhamnopyranoside (**A3**), myricetin-3-*O*-β-galactopyranoside (**B11**), (-)-epicatechin-3-*O*-gallate (**B12**), and a mixture of four cardanols (**B1a-B1d**), a furanocyclohex-2-enone (**B2**), a trihydroxycyclohexanone (**B3**), a mixture of dihydroxycyclohex-2-enones (**B4a** and **B4b**) and a trihydroxycyclohexane (**B5**). A tetratraterpene, *trans* lutein (**B9**) was also isolated. Myricetin (**B10**), its glycosides (**B11** and **A3**) and epicatechin gallate (**B12**) showed good antioxidant activity and myricetin (**B10**) and epicatechin gallate (**B12**) showed good antibacterial activity. The furanocyclohex-2-enone (**B2**) and trihydroxycyclohexanone (**B3**) showed good cytotoxic activity as well as good antiplasmodial activity. The mixture of **B4a** and **B4b** was much less toxic than **B2** and **B3** and showed promising antiplasmodial activity, suggesting a good lead for a non-toxic antiplasmodial drug. The isolation of these active compounds in the plant extracts provides a rationale for the use of the plant to treat coughs, colds, fever and stomachache. The novel compounds **B2**, **B3** and **B4a-B4b** could be pursued as leads for antiplasmodial and anticancer drugs.

The bark of the roots and stem as well as the leaves of *Lannea schimperi* is used medicinally to clean teeth and manage toothache, for diarrhea, chest infections, stomach pains, mental disorders, epilepsy, snake bites, tuberculosis, skin infections, herpes simplex, herpes zoster and other opportunistic infections from HIV/AIDS. This study led to the isolation of moderately cytotoxic triterpenoids (taraxerol (**B8**) and taraxerone (**B7**)) as well as cardanols (**C1a-C1d**), dihydroxycyclohex-2-enones (**C2a-C2d**), 1,2,5-trihydroxycyclohex-3-enes (**C3a-C3c**) and 1,3-dihydroxycyclohex-4-enes (**C4a-C4c**). The 5-[alkenyl]-4,5-dihydroxycyclohex-2-enone mixture (**C2a-C2d**) exhibited good *in vitro* cytotoxicity. We were not able to test these particular extracts for antibacterial activity, however **D2a-D2e**

isolated from *Lannea schweinfurthii* below, with structures similar to **C2a-C2d** showed moderate antibacterial activity. The compounds in the other mixtures **C3a-C3c** and **C4a-C4c** were also present in the mixtures from *L. schweinfurthii* below, but showed no demonstrable antibacterial activity. Future work could involve the reisolation of the mixture **C2a-C2d** to see if the antibacterial activity is better than **D2a-D2e**. Although the isolates from *L. schimperii* do not necessarily support the reported medicinal uses of the plant, the mixture of **C2a-C2d** could be a good lead for an anticancer drug.

Previous studies on *Lannea schweinfurthii* crude extracts indicated moderate cytotoxicity, antioxidant, antiplasmodial and acetylcholinesterase inhibition activities but no active constituents were reported. The stem and root bark decoction is traditionally used for stomach ailments such as stomach pain, gastrointestinal diseases, diarrhea and constipation, and to treat headaches and boils. We report the isolation of antioxidant flavonoids (epicatechin (**D6**), epicatechin gallate (**B12**), catechin (**D7**) and rutin (**D8**)) from the plant. Of these only epicatechin gallate (**B12**) demonstrated good antibacterial and antiplasmodial activity. Six mixtures of alkylated cardanols, cyclohexenones and cyclohexenols were also isolated. Of these mixtures, mixture 3 (**D2a-D2e**) showed good antibacterial, antiplasmodial, larvicidal and cytotoxic activity. In addition, the triterpenoids, sitosterol (**A6**), sitosterol glucoside (**B6**) and lupenone (**D5**) was also isolated. Sitosterol (**A6**) and sitosterol glucoside (**B6**) are known immune boosters and could well contribute to the many medicinal uses of the plant. The many compounds and mixtures, each with their own bioactivity could play a role in the bioactivity of the extracts of the plant as well as the medicinal effects of the plant extracts administered as traditional medicine.

Phenolic lipids are derivatives of mono and dihydroxyphenols, namely catechol, resorcinol and hydroquinones. Plants within the Anacardiaceae are the main source of phenolic lipids. The cardanols were isolated from the three *Lannea* species, *alata*, *schimperi* and *schweinfurthii*. The alkyl/alkenyl cyclohexenones and alkyl/alkenyl cyclohexenols have only been isolated from *Tapirira* (*Tapirira obtusa, Tapirira guiainensis*) and *Lannea* (*Lannea welwitschii, Lannea nigratina* and *Lannea edulis*) within the Anacardiaceae family. They are thought to be precursors in the biosynthetic pathway of phenolic lipids. Isolation of these kinds of compounds in three out of four *Lannea* species studied is indicative of the close phylogenetic relationship between these three *Lannea* species and the two species from *Tapirira*. These findings agree with the classification of both *Lannea* and *Tapirira* in the Spondieae tribe of the Anacardiaceae family as proposed by Engler. Thus, phenolic lipids can be considered as chemotaxonomic markers of the *Lannea* and *Tapirira* genera. Phenolic lipids are also known to exhibit antibacterial, antifungal, antigenotoxic, antioxidant, cytostatic, cytotoxic and antiprotozoal activities. These activities are related to the ability of phenolic lipids to interact with proteins on cell membranes. Phenolic lipids are potentially useful for the treatment of cancer, viral and skin diseases. The *Lannea* genus promises to be a source of new bioactive substances.

Limitations of the study and recommendations for future work

Attempts to isolate the individual cardanols, cyclohexenenones and cyclohexenols using silica gel, sephadex, and silica gel coated with silver nitrate packed columns were unsuccessful. Future work could involve the investigation of how to separate these compounds with very similar structures and polarity and which only differ in the size and nature of the side chain present. Preparative silver ion HPLC can be explored as one alternative and derivatisation, followed by separation as another alternative, however it must

be possible to convert the derivatised compounds back to their original compounds so that they could be tested in its orginal form.

The absolute stereochemistry of some compounds could not be established since they were isolated as mixtures. It would be good to explore possibilities of determining the absolute stereochemistry of the compounds. One could try to derivatise the molecule without affecting the stereochemical centres and then try to crystallise the derivatised compounds and get an X-ray structure of the molecule.

Alkyl phenols and their derivatives have previously demonstrated cytotoxicity against tumor cells. In this study, their cytotoxicity was evaluated against a mammalian cell-line, Chinese Hamster Ovarian (CHO). We did not test the isolates from the *Lannea* species against tumor cell lines as we did not have access to these cell lines. Future work could involve testing of the compounds against tumor cell lines to identify possible leads for anticancer chemotherapy. Some of the compounds that were cytotoxic also exhibited good antiplasmodial and antibacterial activities. A further study could assess the cytotoxic selectivity of these compounds i.e whether they are more toxic towards infected cells compared to normal cells.

In vitro activity was carried out in all the bioassays undertaken in this work. *In vivo* experiments need to be carried out on the active compounds to determine whether or not they have the potential to be developed into drugs.

The MTT assay used to evaluate cytotoxicity. Though fast and reliable, it does not differentiate whether these compounds kill cells or merely inhibit their growth. The mode of action of these compounds could be determined by clonogenic assays.

This thesis demonstrated some biological activities of individual compounds isolated from *Lannea* species. Studies using combinations of the isolated compounds can be carried out to determine their synergistic or antagonistic effects.

In general, we have successfully carried out the phytochemistry of four *Lannea* species and the compounds isolated exhibited cytotoxicity, antibacterial, antiplasmodial, larvicidal and antioxidant activities and supported their use in traditional medicine.

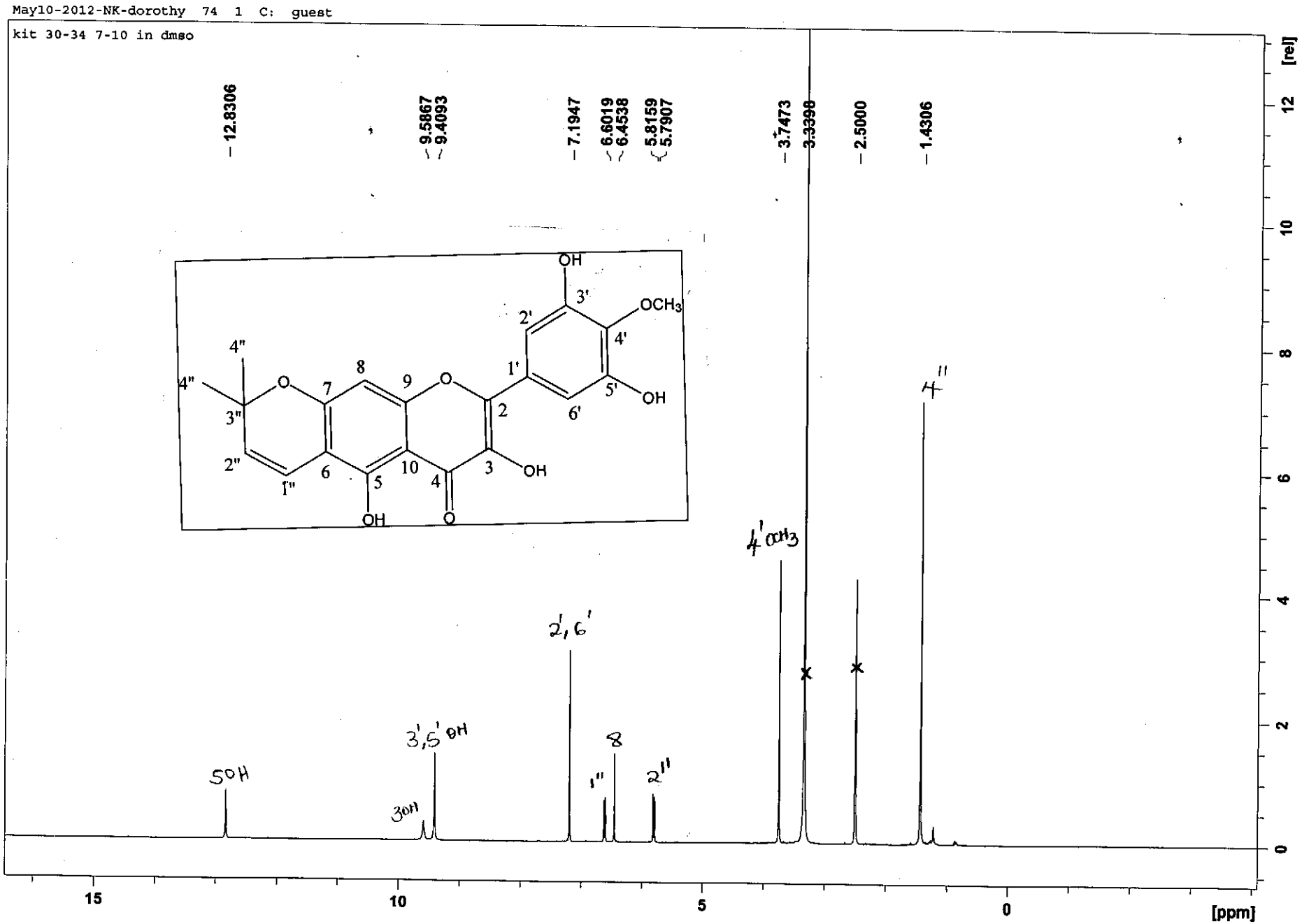
UNIVERSITY OF KWAZULU-NATAL

Appendix 1

PHYTOCHEMISTRY AND BIOACTIVE NATURAL PRODUCTS FROM *LANNEA ALATA*, *LANNEA RIVAE*, *LANNEA SCHIMPERI* AND *LANNEA SCHWEINFURTHII* (ANACARDIACEAE)

2014

OKOTH AKINYI DOROTHY



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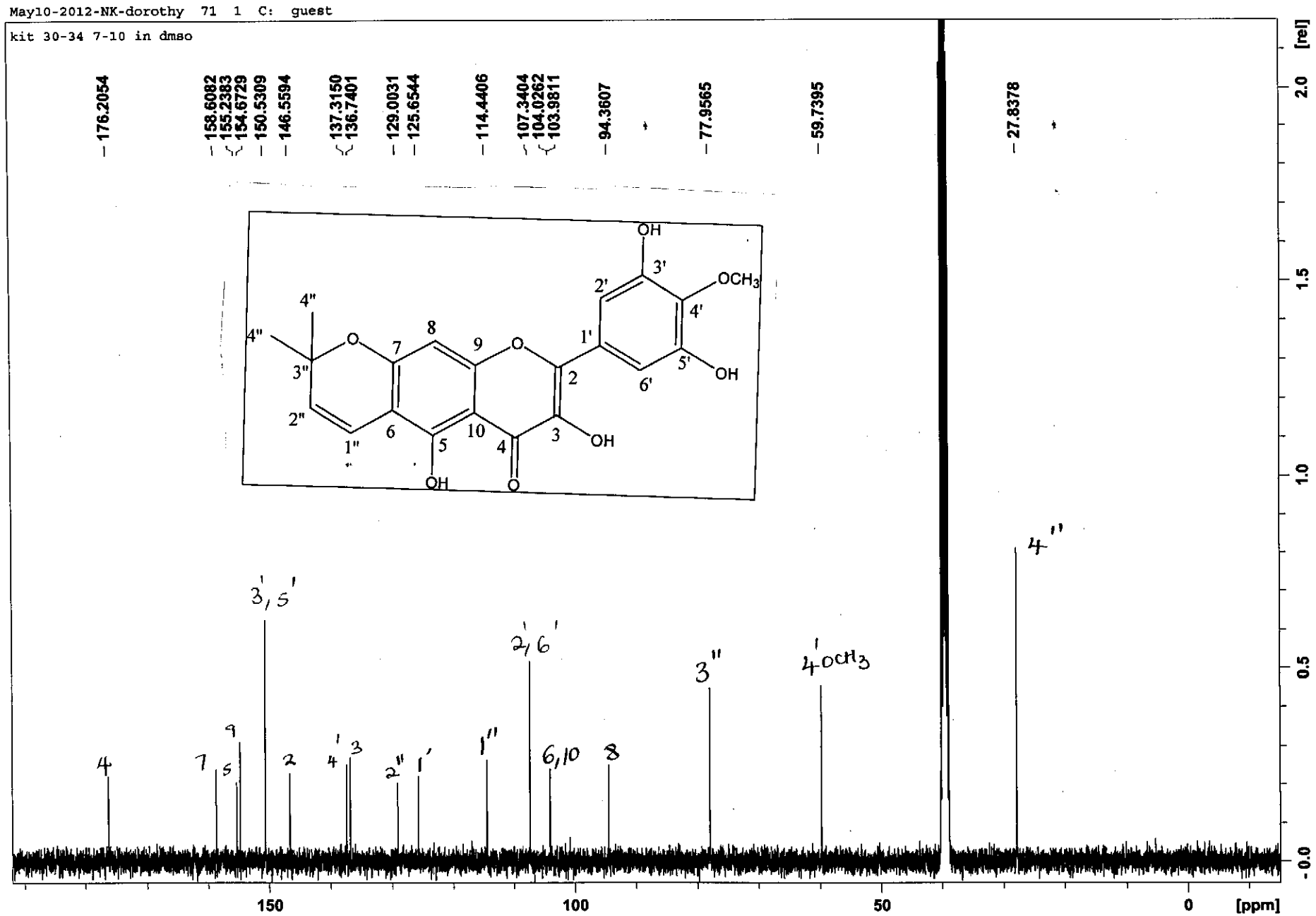

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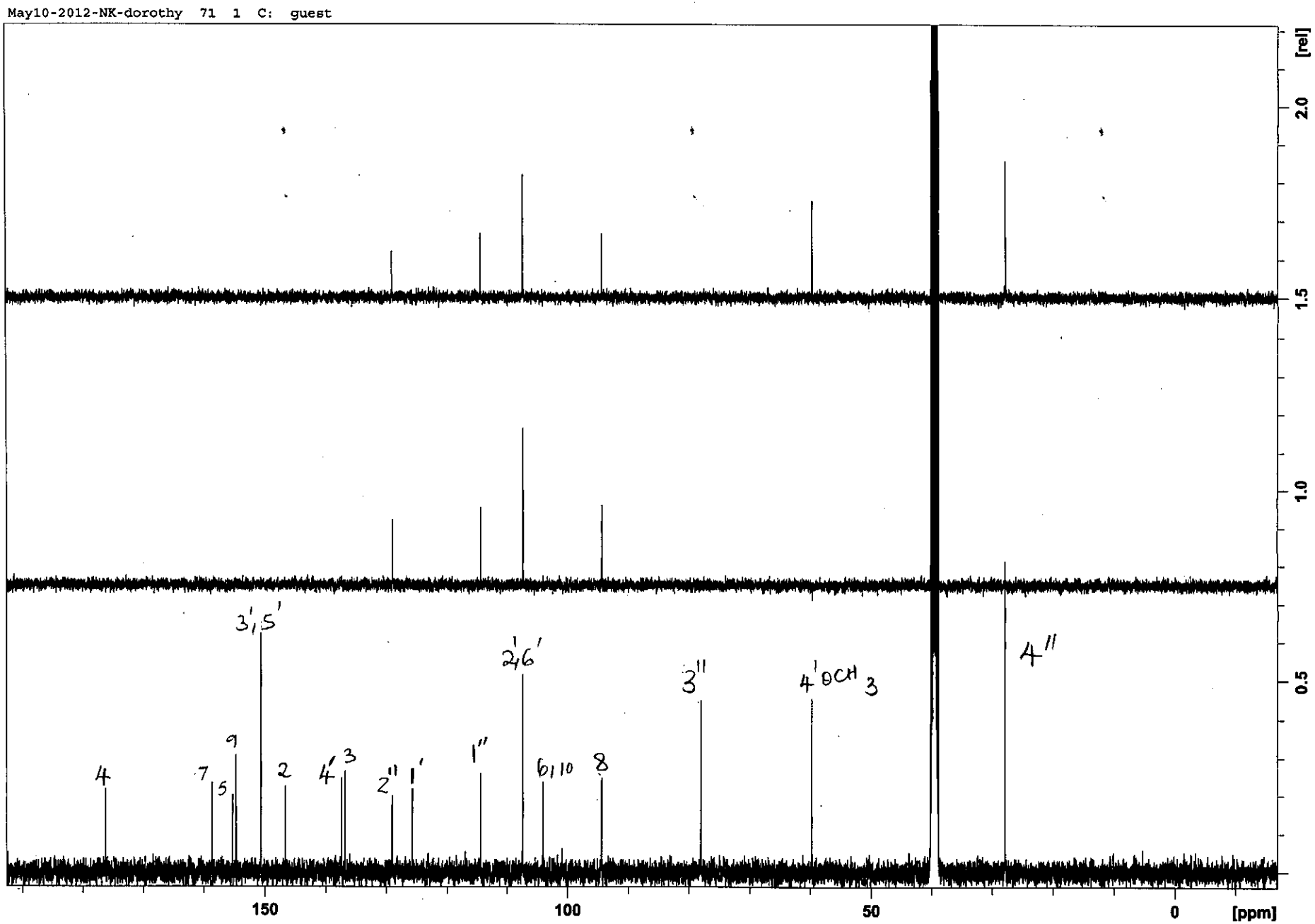
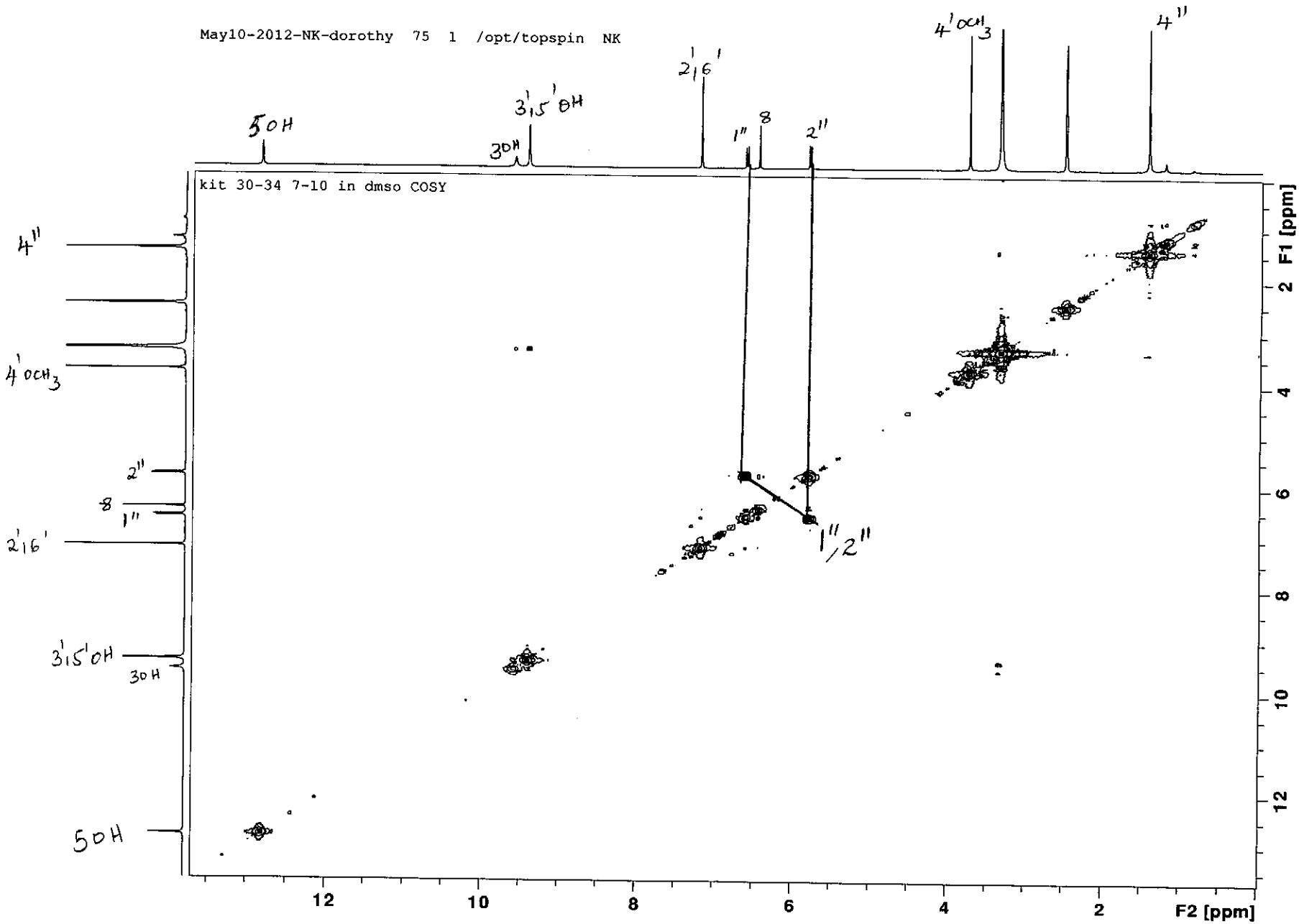


DEPT spectrum of A1



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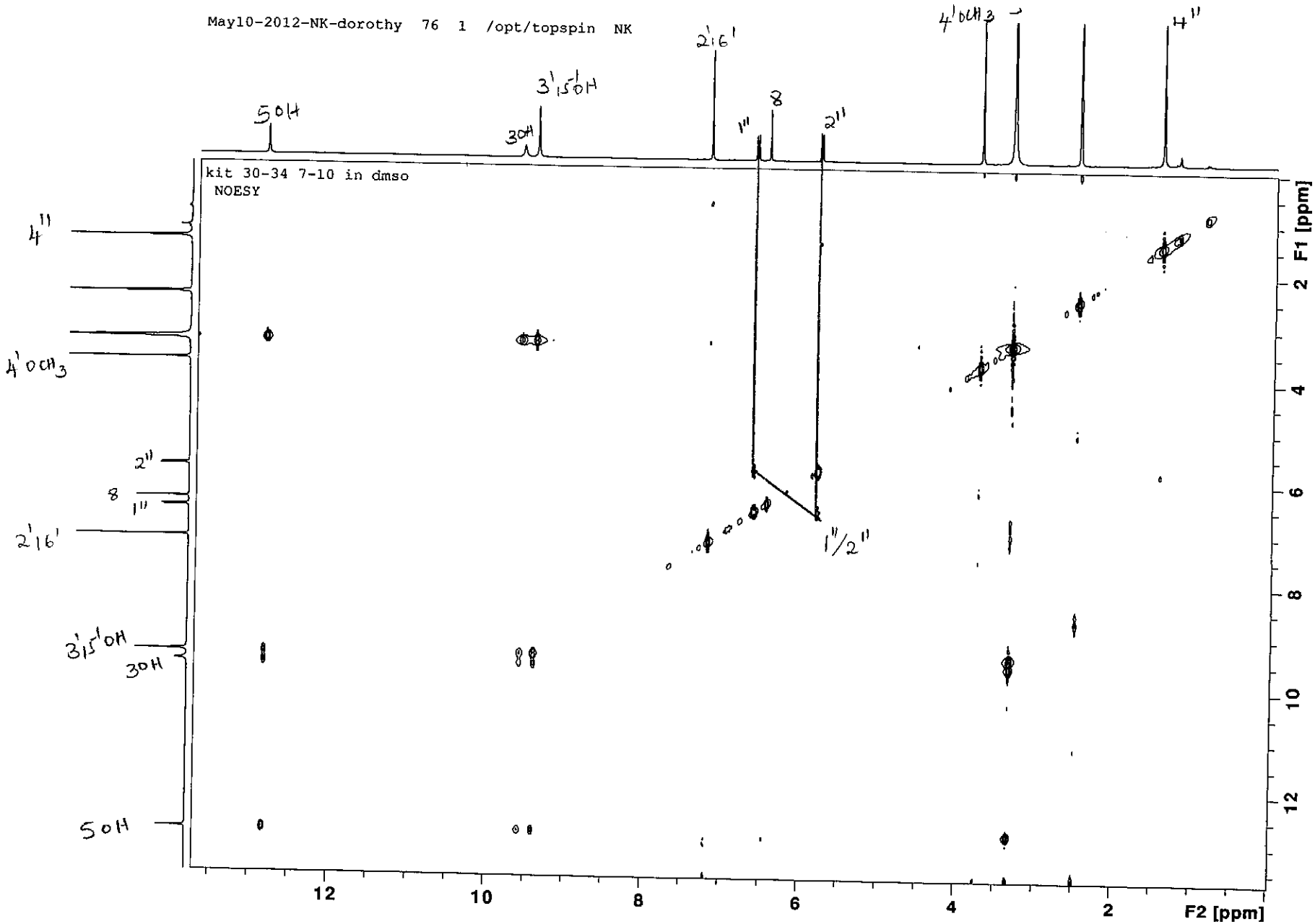

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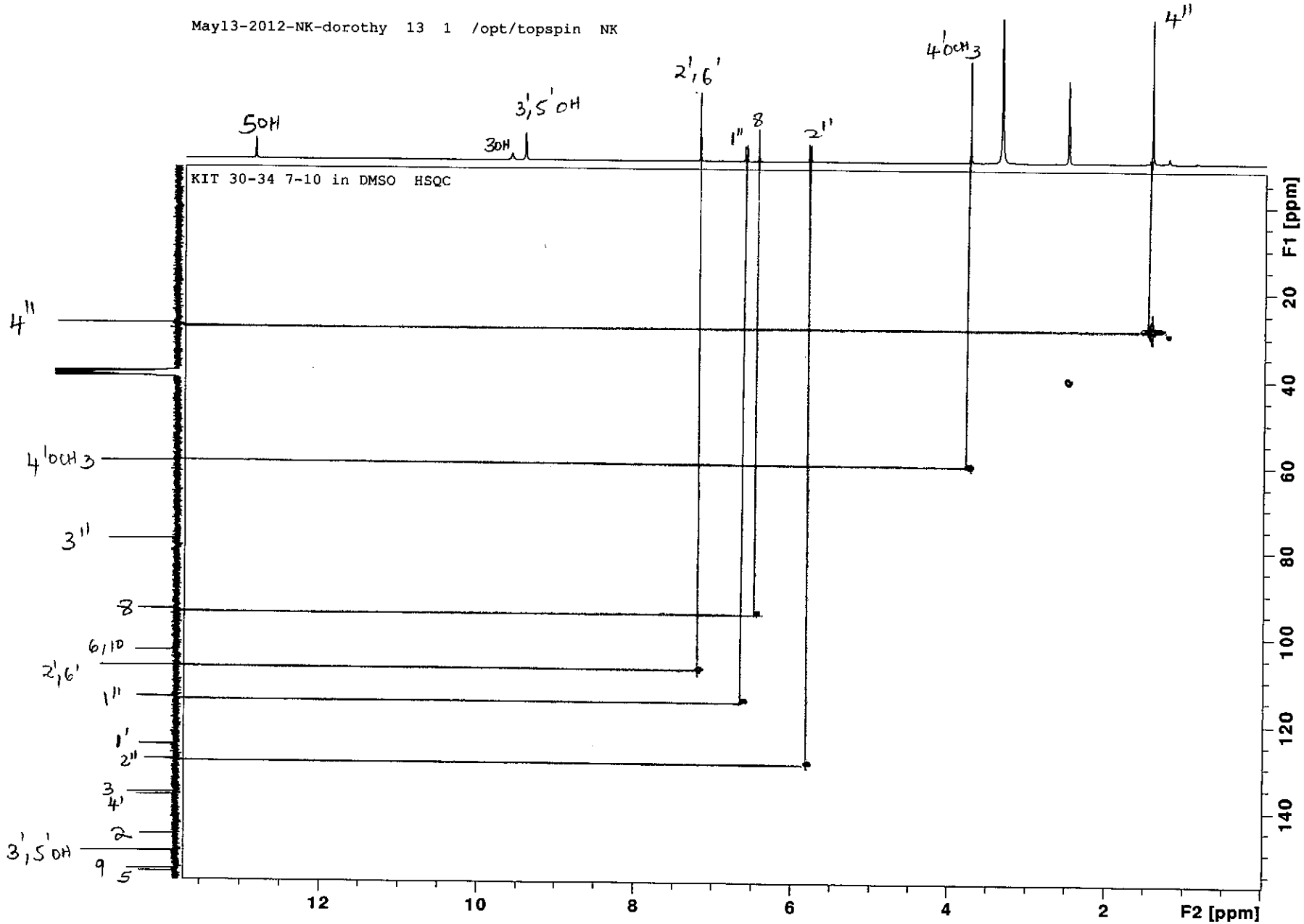
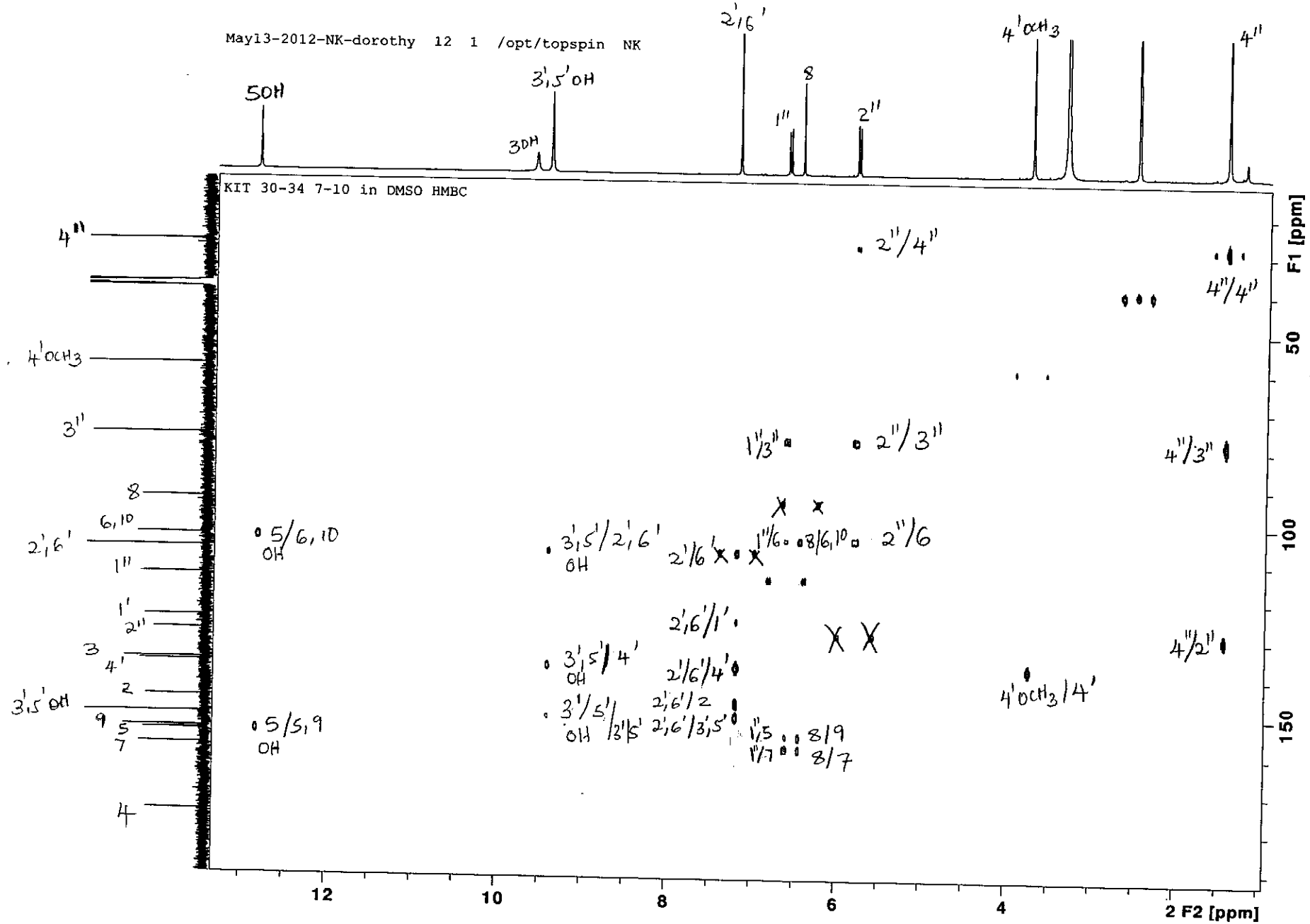
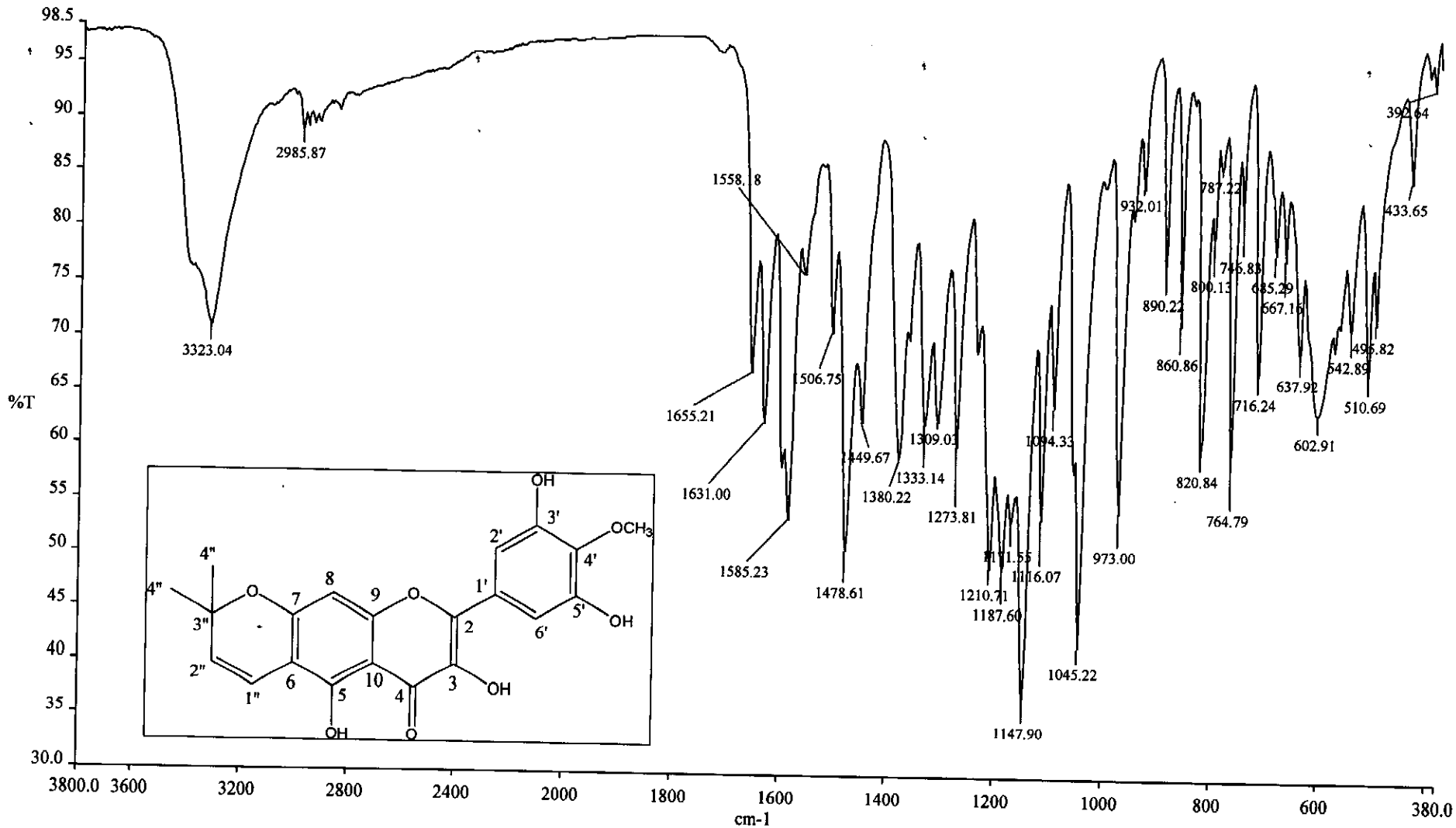


HSQC spectrum of A1



HMBC spectrum of A1

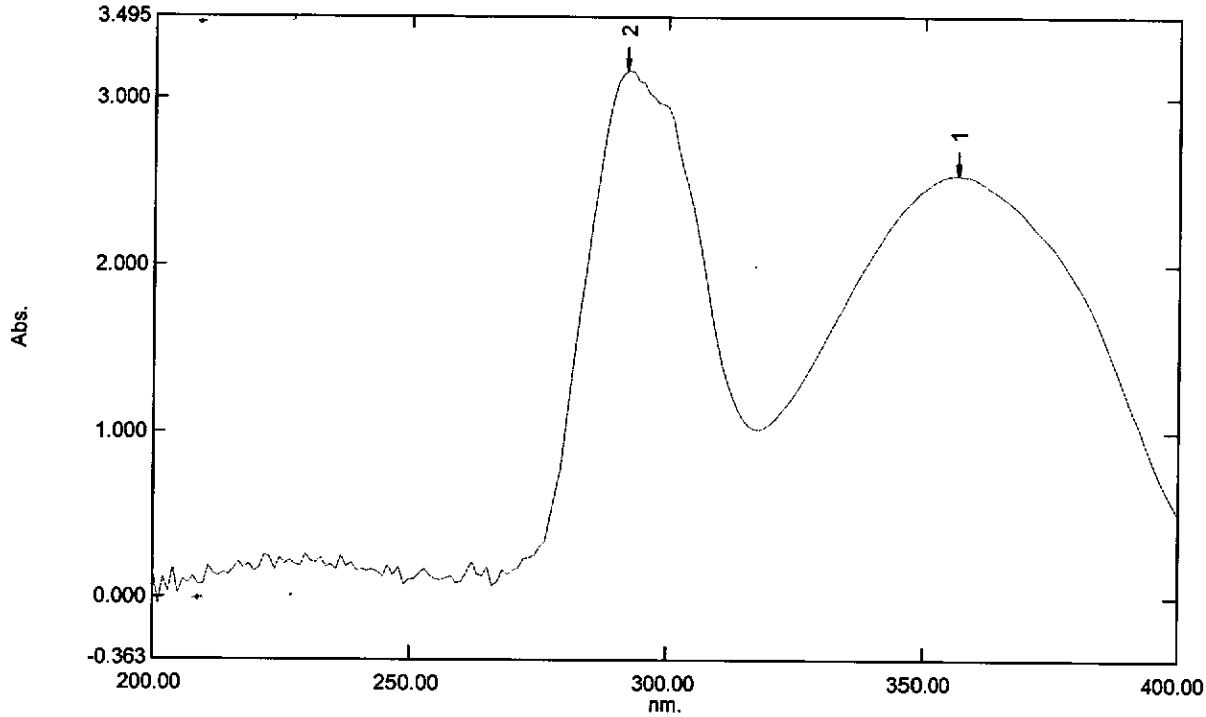


IR spectrum of A1

Spectrum Peak Pick Report

17/04/2012 01:59:51 PM

Data Set: kit 30-34 7-10 D .spc - Storage 135829



No.	P/V	Wavelength	Abs.	Description
1		357.00	2.543	
2		292.00	3.174	
3		318.00	1.012	

Measurement Properties

Wavelength Range (nm.):	200.00 to 400.00
Scan Speed:	Medium
Sampling Interval:	1.0
Auto Sampling Interval:	Disabled
Scan Mode:	Single

Instrument Properties

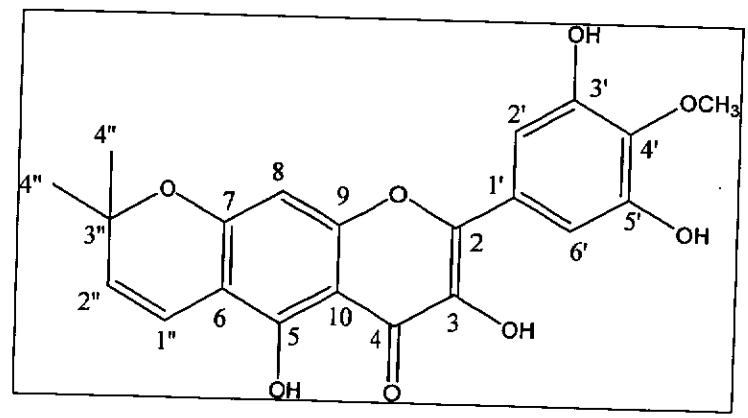
Instrument Type:	UV-3600 Series
Measuring Mode:	Absorbance
Slit Width:	2.0 nm
Time Constant:	0.1 sec.
Source Lamp:	Auto
Light Source Change Wavelength:	310.00 nm
Detector Unit:	Direct
Detector Change Wavelength:	830.00 nm 1800.00 nm
Grating Change Wavelength:	720.00 nm
S/R Exchange:	Normal
Detector Lock:	Auto
Slit Program:	Normal
Beam Mode:	Double
Stair Correction:	Disable

Attachment Properties

Attachment:	None

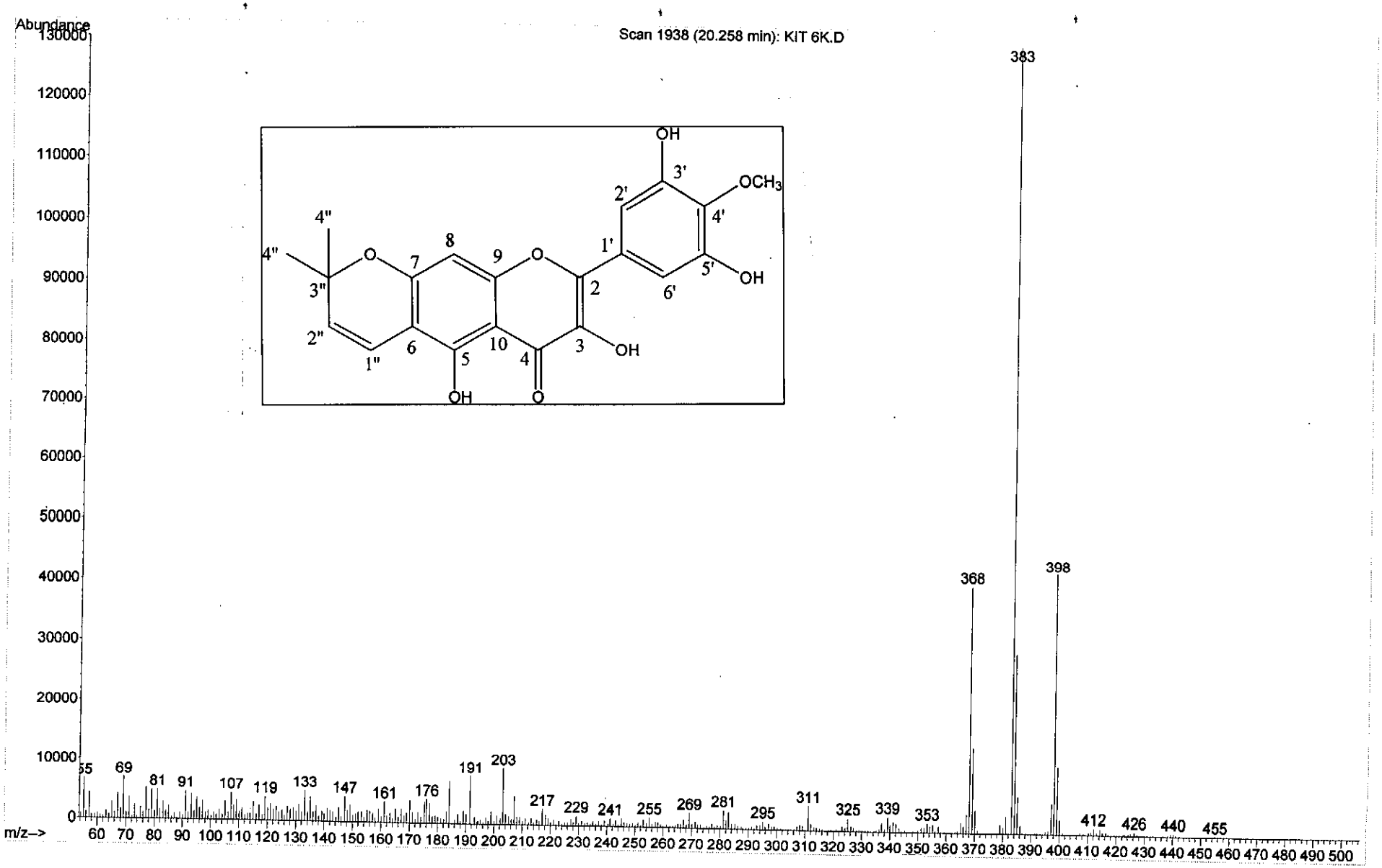
Sample Preparation Properties

Weight:	1mg
Volume:	15ml
Dilution:	
Path Length:	
Additional Information:	



UV spectrum of A1

File : C:\MSDCHEM\1\DATA\DOROTHY RAW DATA KITHOKIT\KIT 6K.D
Operator : Dorothy
Acquired : 18 May 2012 15:39 using AcqMethod NATPRODUCTS MANUAL
Instrument : 5973N
Sample Name: kit 30-34 ee
Misc Info :
Vial Number: 1



MS spectrum of A1

Elemental Composition Report

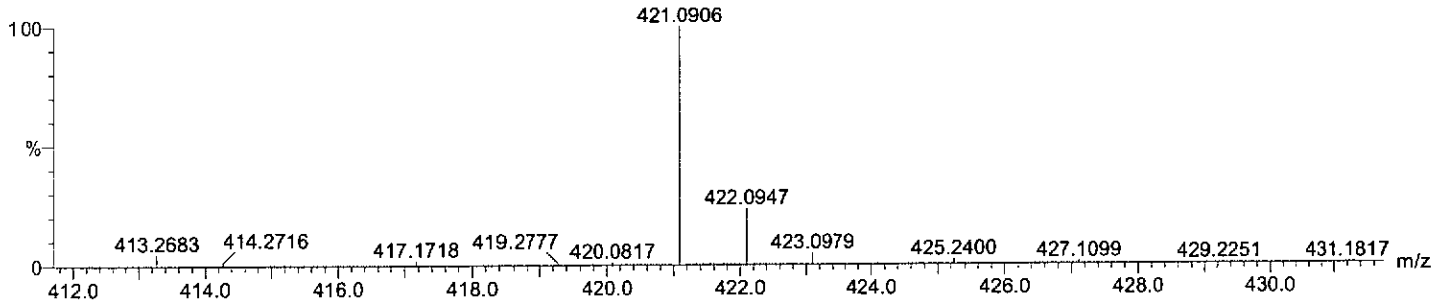
Single Mass Analysis

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

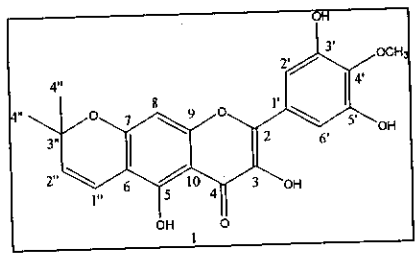
Monoisotopic Mass, Even Electron Ions
2 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-22 H: 15-20 O: 5-10 Na: 0-1

KIT 30-34 (Sample1) 25 (0.410) Cm (1:31)
TOF MS ES+

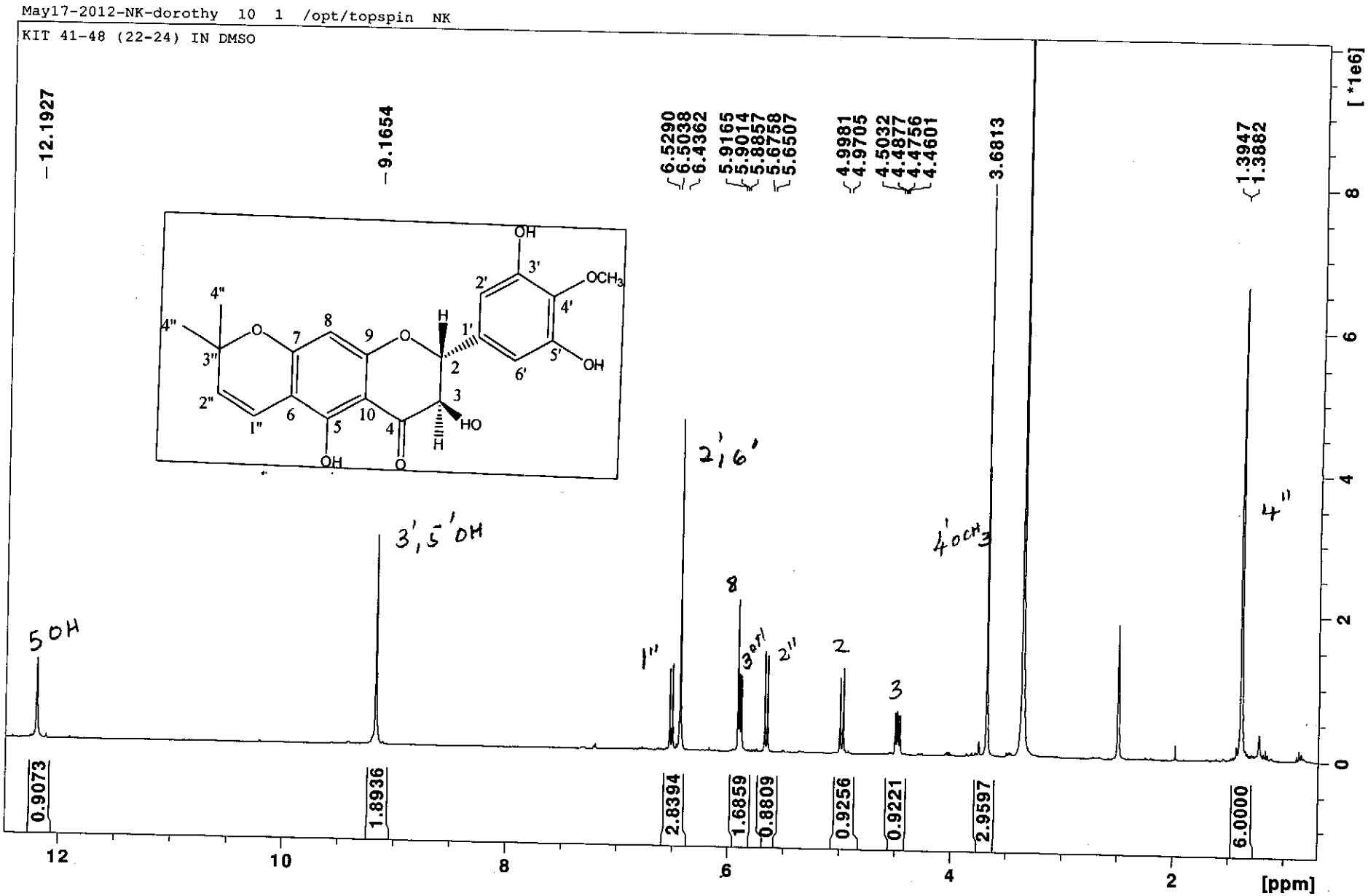
8.31e+004



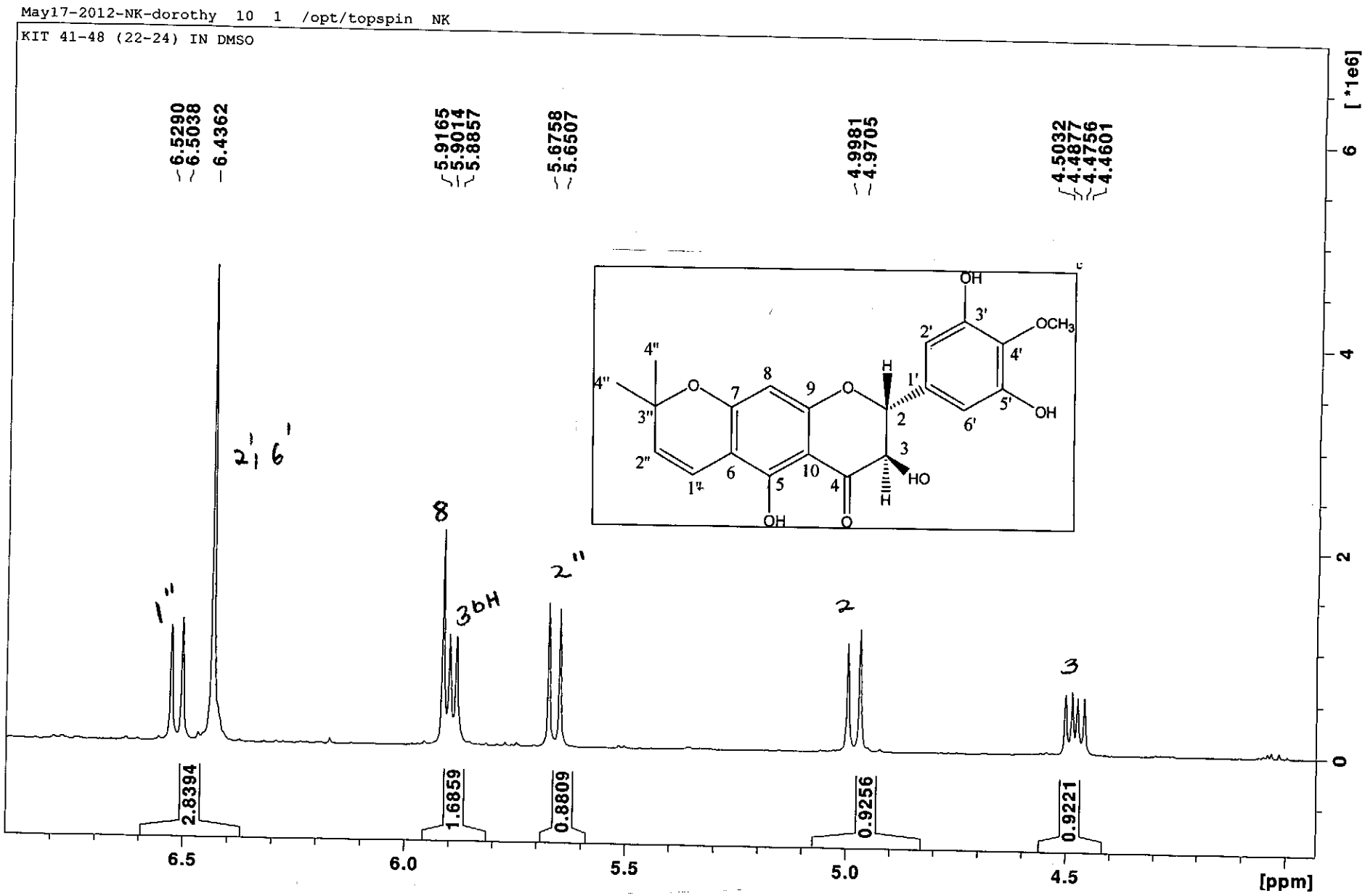
Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
421.0906	421.0899	0.7	1.7	12.5	509.0	0.0	C21 H18 O8 Na



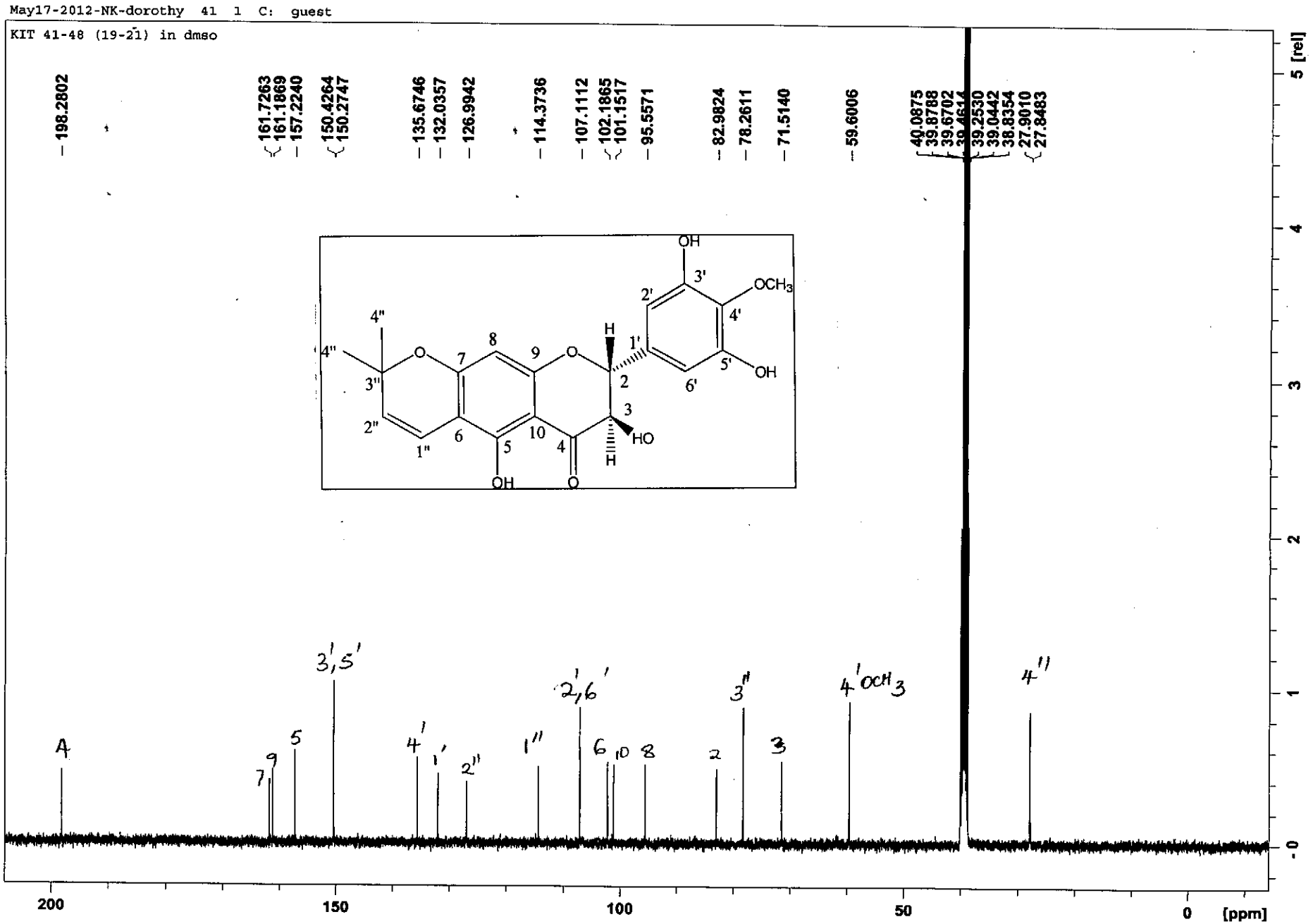
HREIMS spectrum of A1



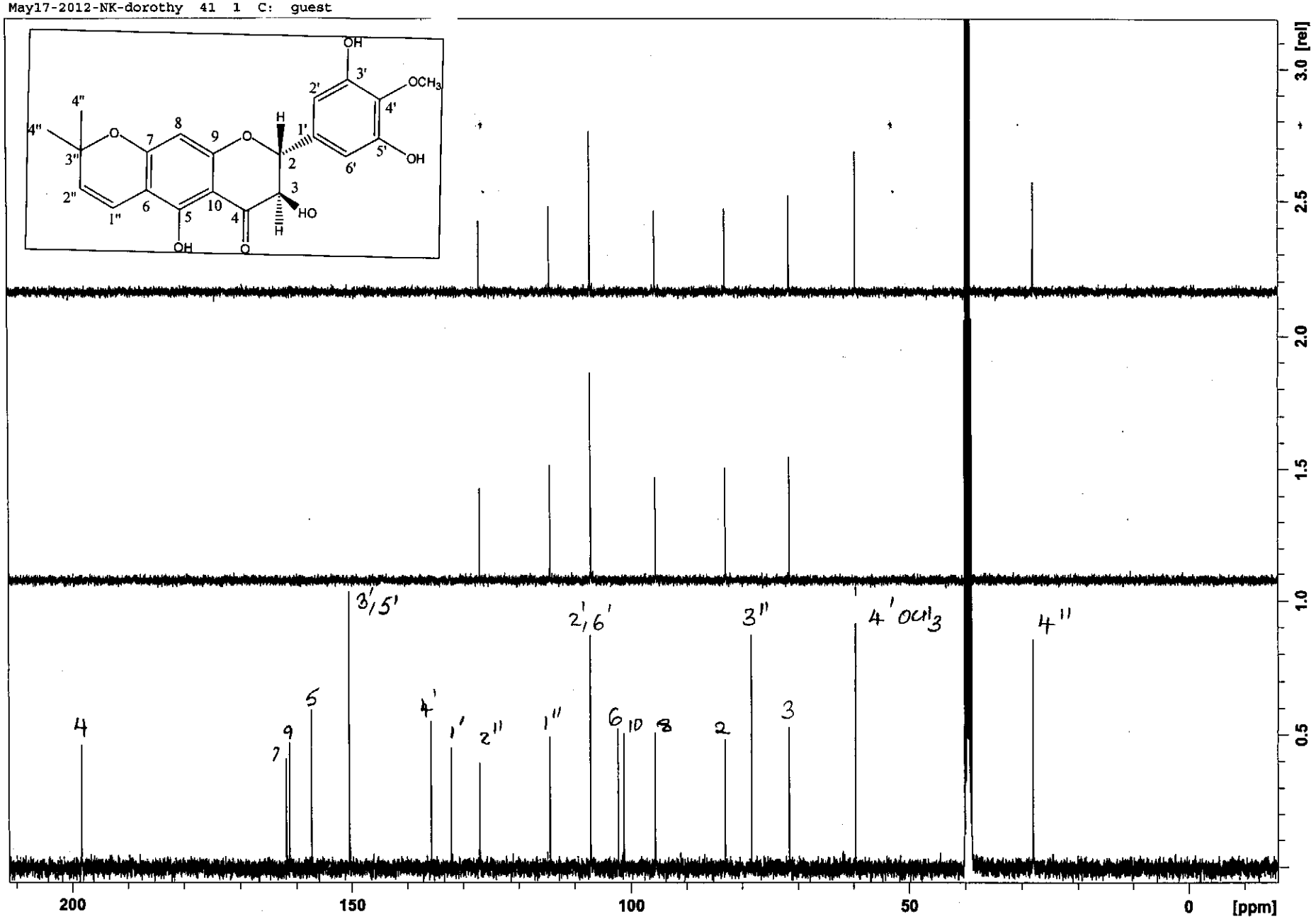
^{1}H NMR spectrum of A2



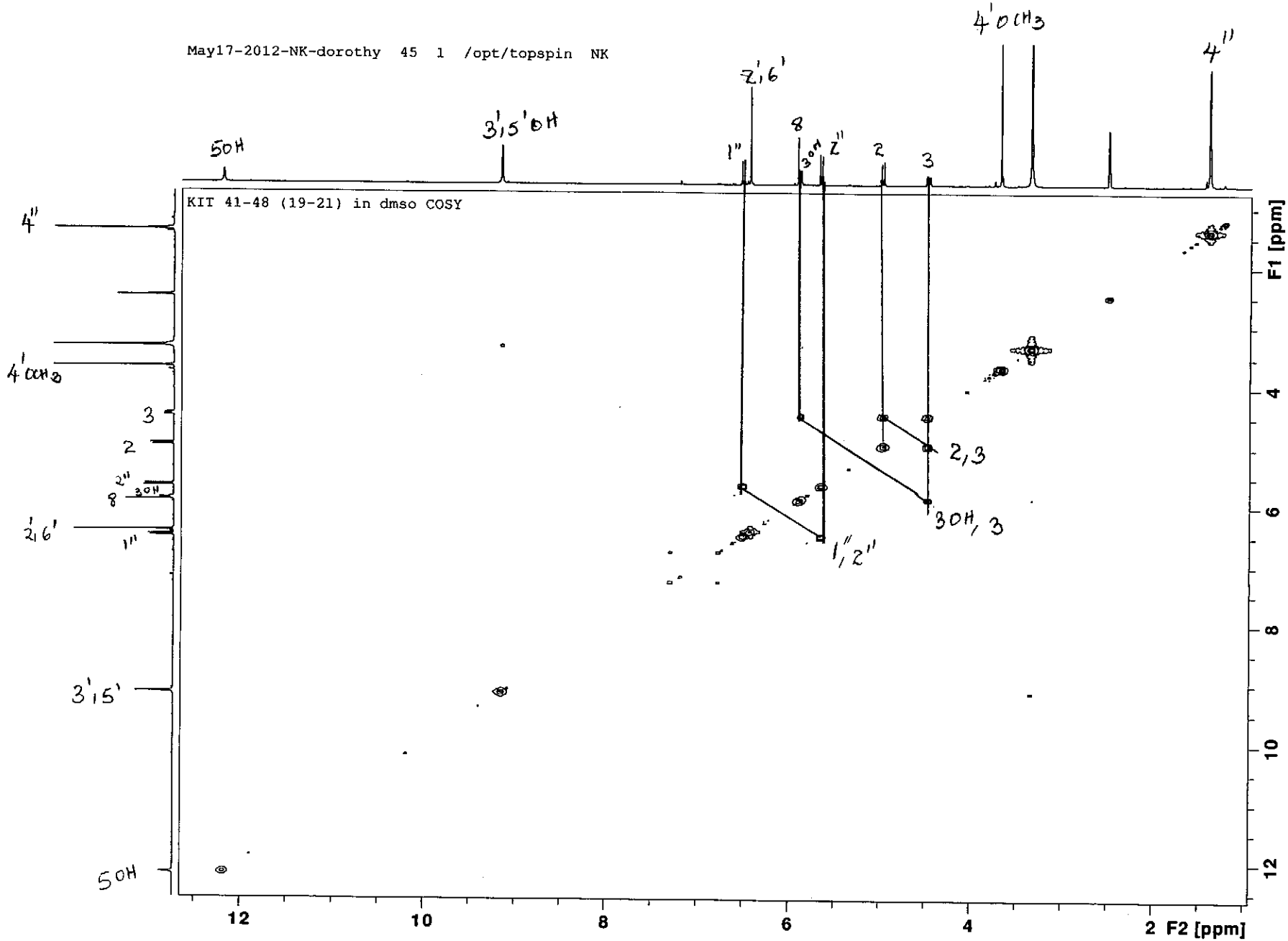
^{1}H NMR spectrum of A2 expanded (4-7 ppm)



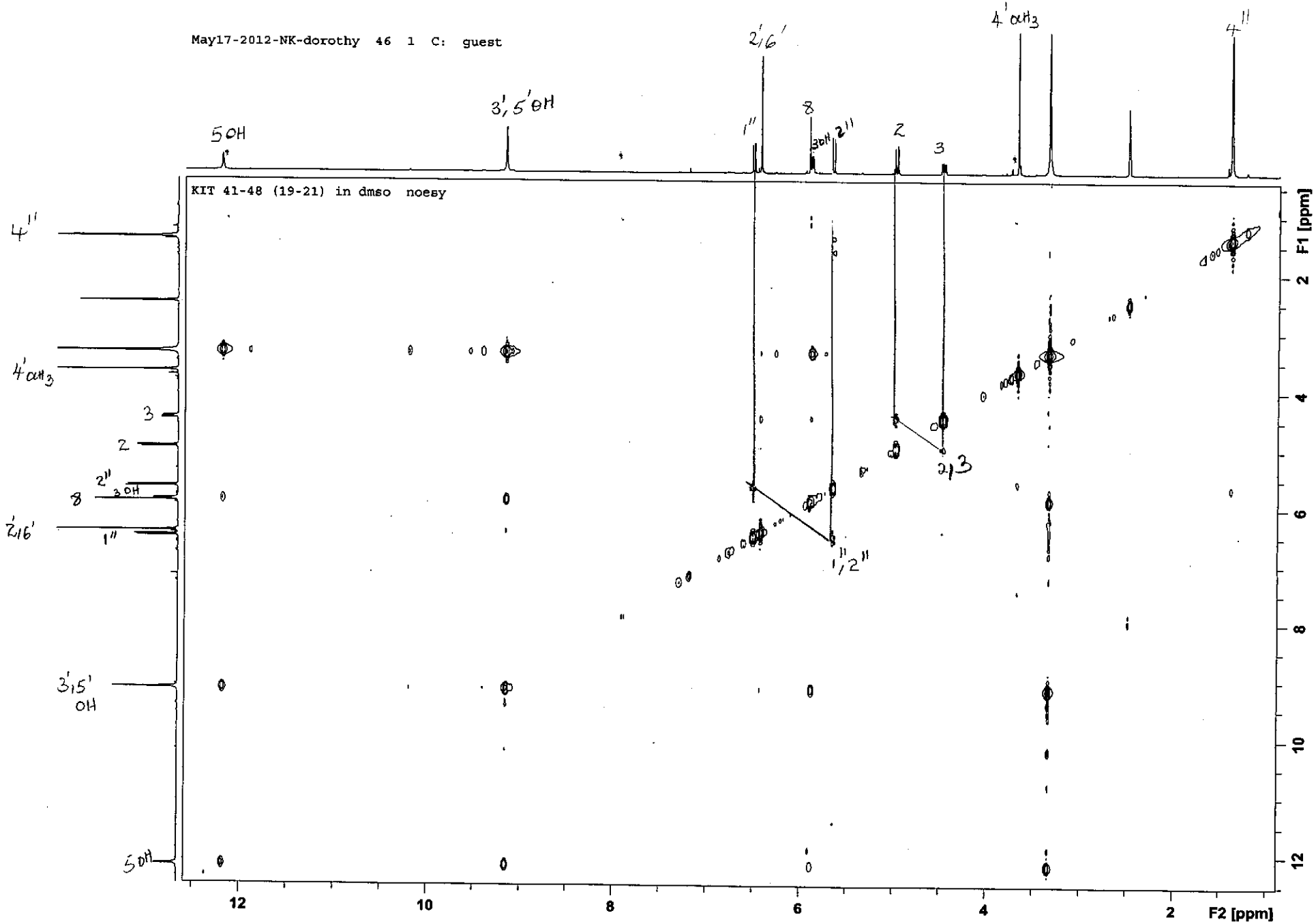
^{13}C NMR spectrum of A2



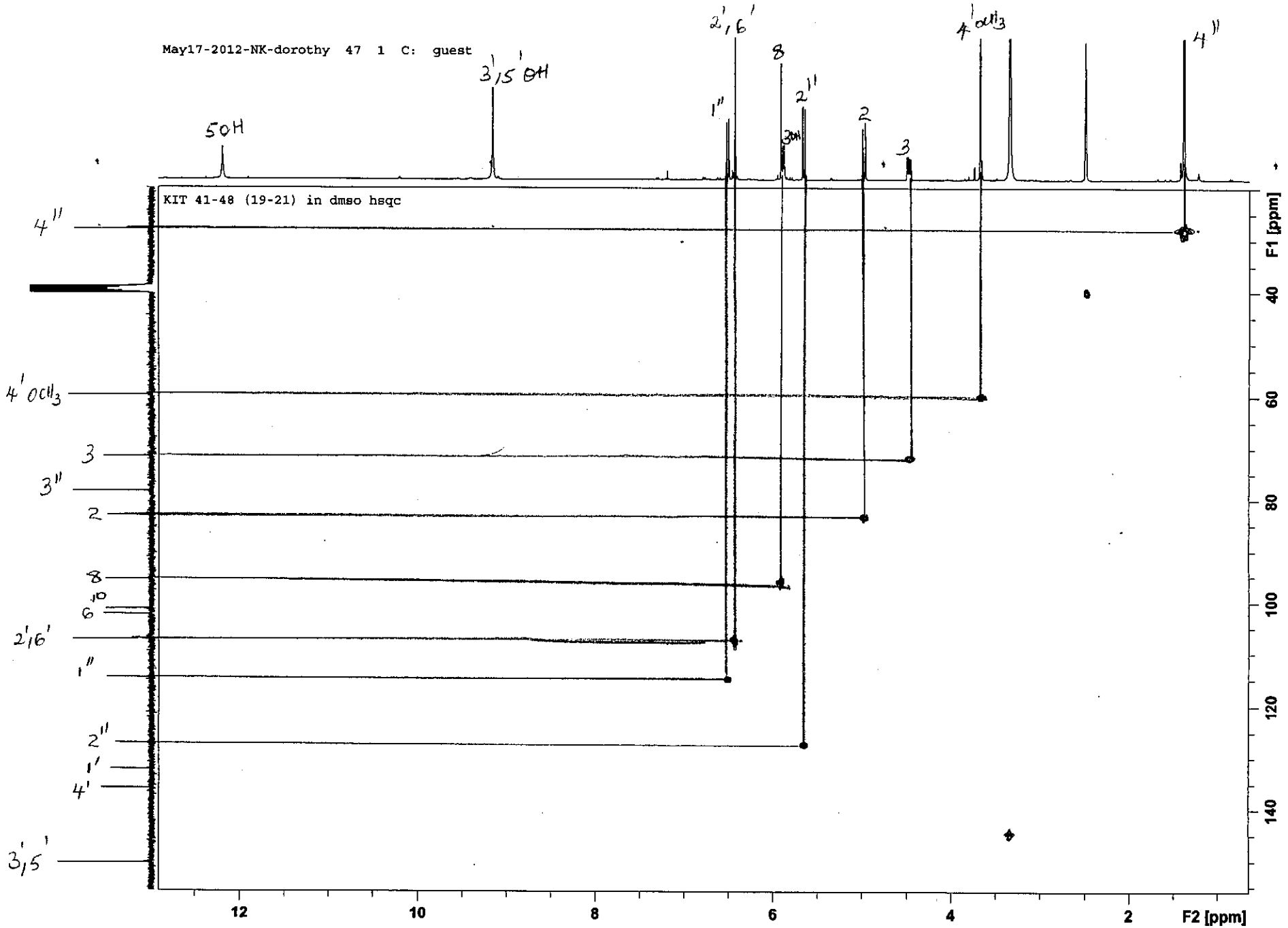
DEPT spectrum of A2



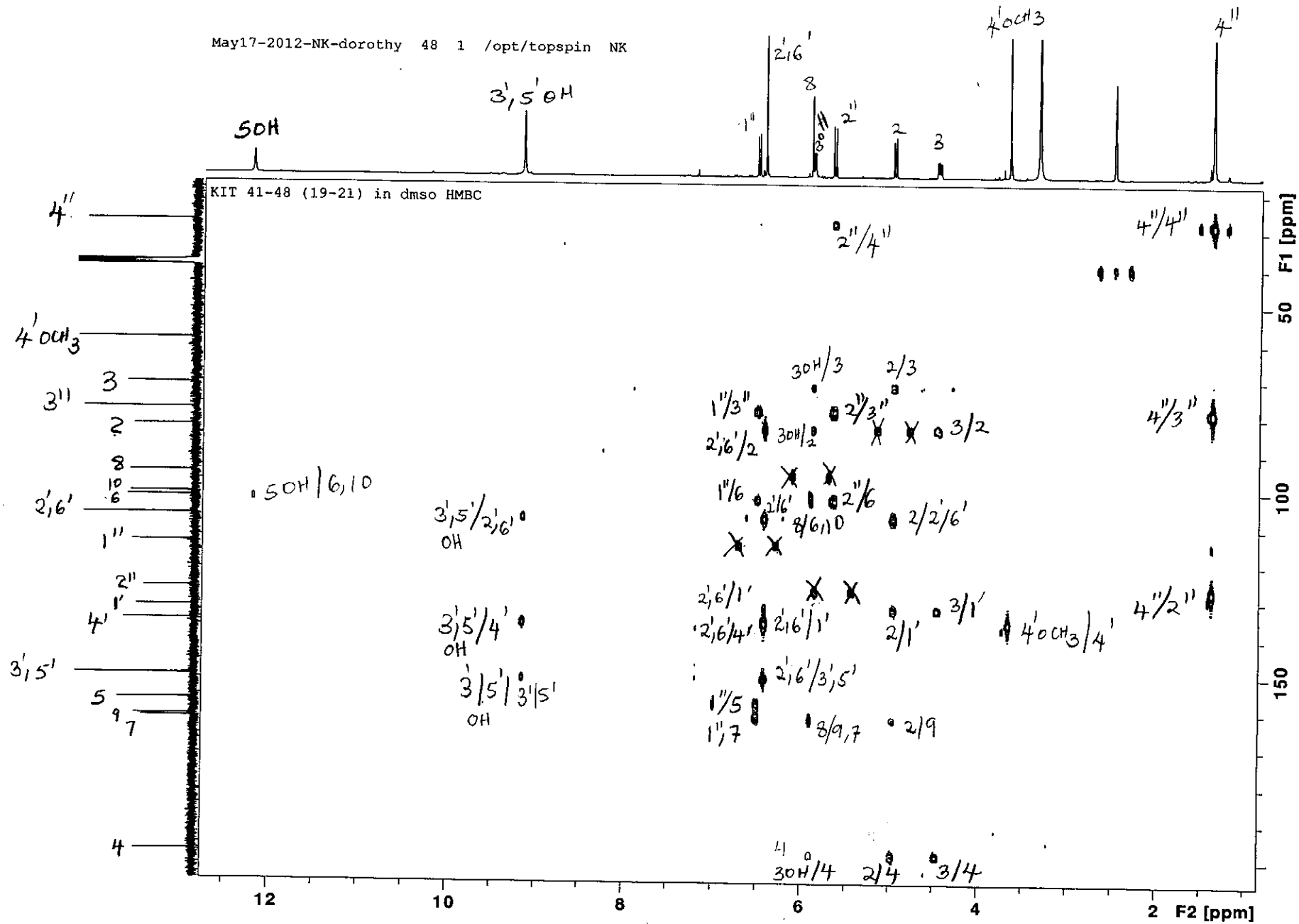
COSY spectrum of A2



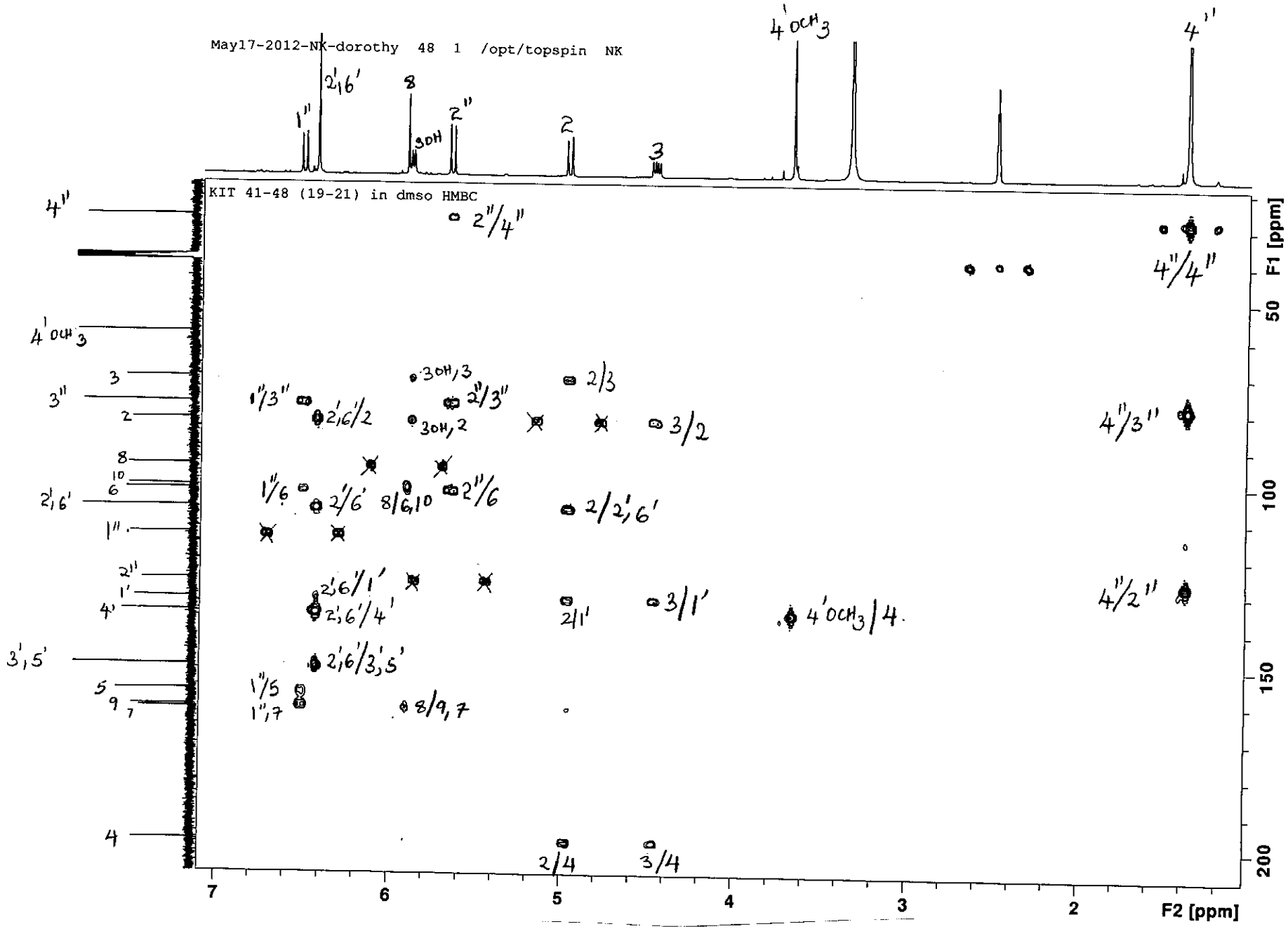
NOESY spectrum of A2



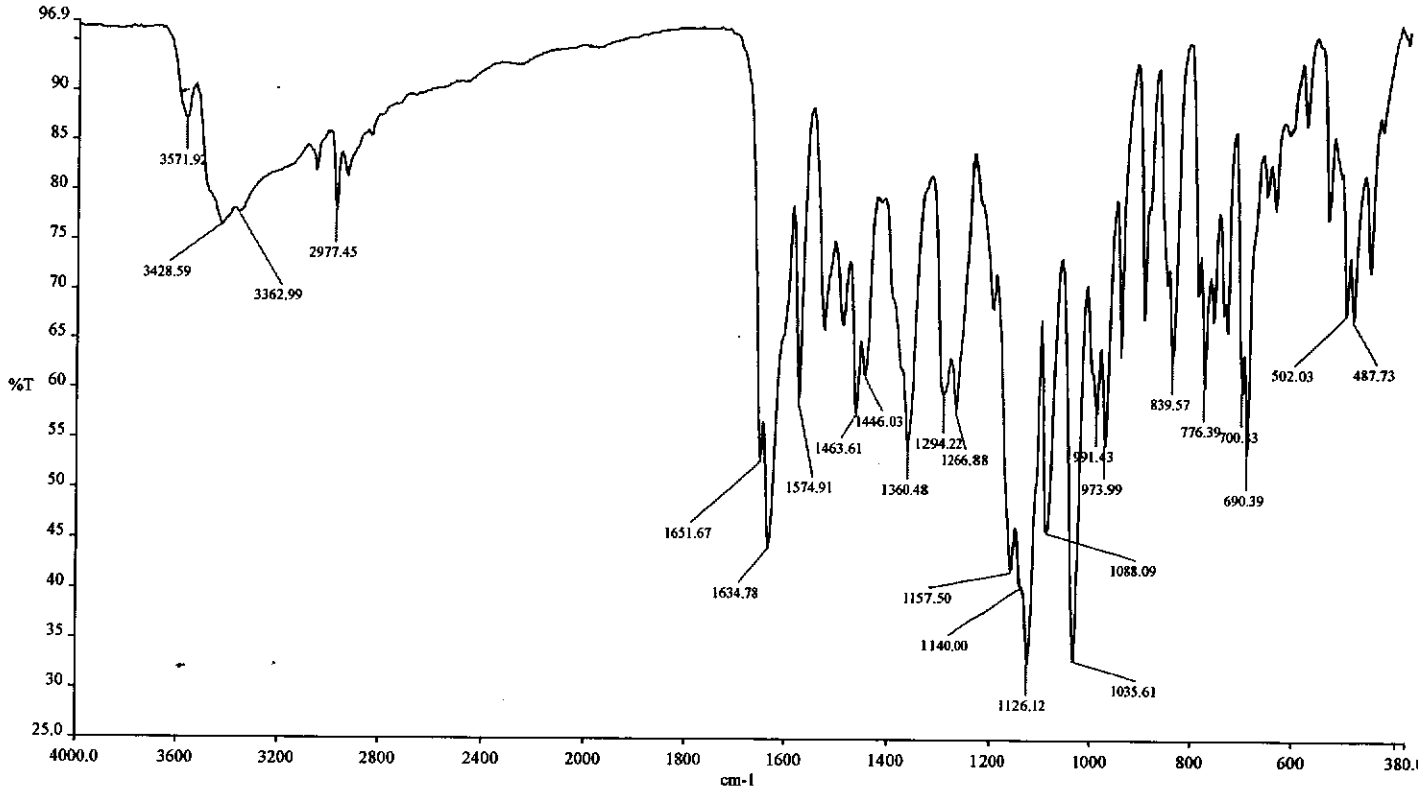
HSQC spectrum of A2

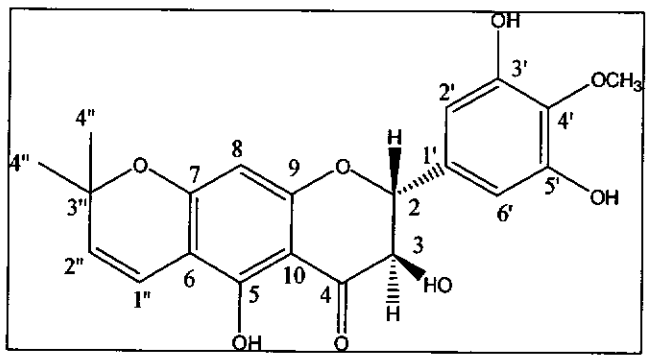


HMBC spectrum of A2



HMBC spectrum of A2 expanded (1-7 ppm)



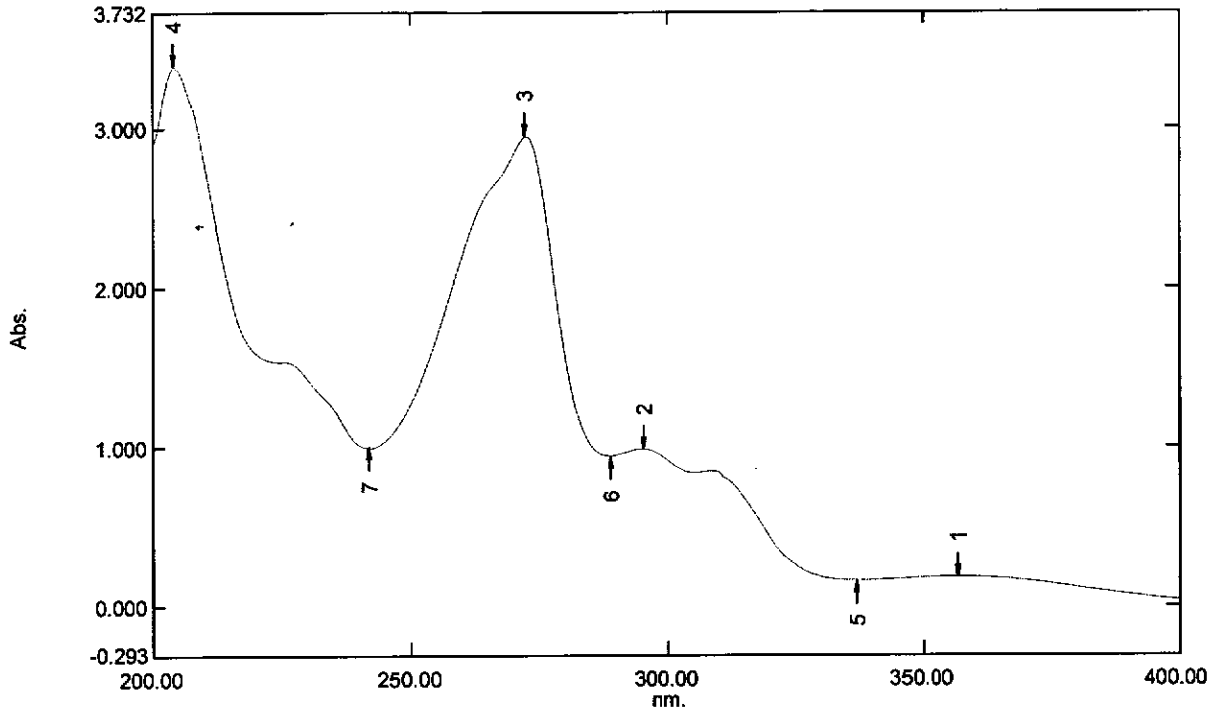


IR spectrum of A2

Spectrum Peak Pick Report

01/05/2012 01:53:48 PM

Data Set: KIT 41-48 (19-20).spc - Storage 135002



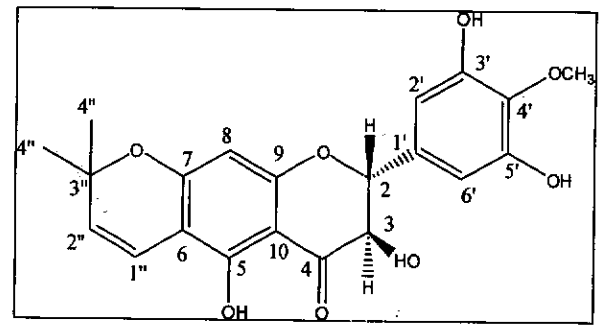
No.	P/V	Wavelength	Abs.	Description
1	●	357.00	0.187	
2	●	295.00	0.993	
3	●	272.00	2.955	
4	●	204.00	3.397	
5	▼	337.00	0.164	
6	▼	289.00	0.950	
7	▼	242.00	1.000	

Measurement Properties
Wavelength Range (nm.): 200.00 to 400.00
Scan Speed: Medium
Sampling Interval: 1.0
Auto Sampling Interval: Disabled
Scan Mode: Single

Instrument Properties
Instrument Type: UV-3600 Series
Measuring Mode: Absorbance
Slit Width: 2.0 nm
Time Constant: 0.1 sec.
Source Lamp: Auto
Light Source Change Wavelength: 310.00 nm
Detector Unit: Direct
Detector Change Wavelength: 830.00 nm 1800.00 nm
Grating Change Wavelength: 720.00 nm
S/R Exchange: Normal
Detector Lock: Auto
Slit Program: Normal
Beam Mode: Double
Stair Correction: Disable

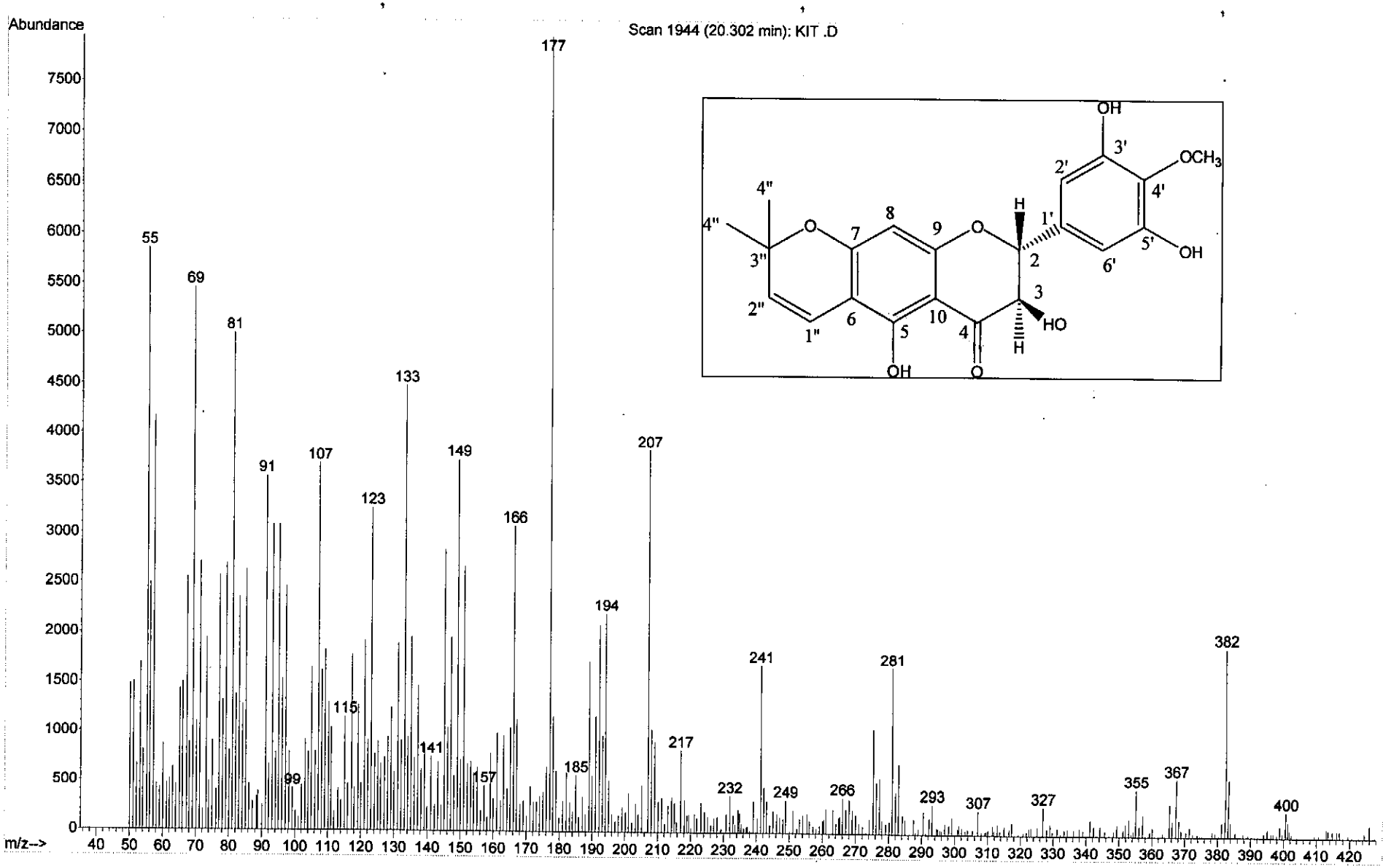
Attachment Properties
Attachment: None

Sample Preparation Properties
Weight: 0.3mg
Volume: 20ml
Dilution:
Path Length:
Additional Information:



UV spectrum of A2

File : C:\MSDCHEM\1\DATA\DOROTHY RAW DATA KITHOKIT\KIT .D
Operator : dororhy
Acquired : 31 May 2012 11:42 using AcqMethod NATPRODUCTS MANUAL
Instrument : 5973N
Sample Name: KIT 41-48 (19-21)
Misc Info :
Vial Number: 1



MS spectrum of A2

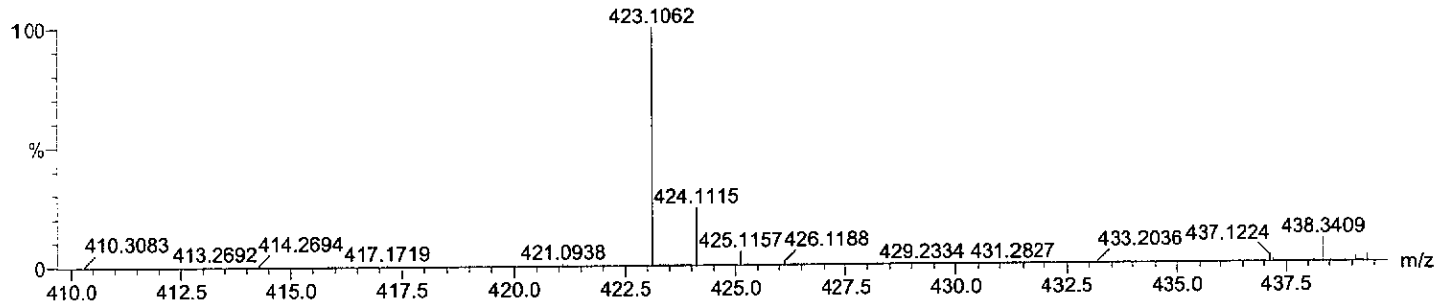
Elemental Composition Report

Single Mass Analysis

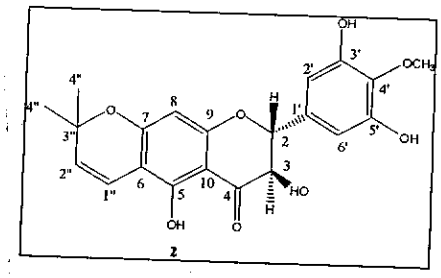
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
4 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 20-22 H: 15-20 O: 5-10 Na: 0-1
KIT 41-48 (Sample2) 2 (0.017) Cm (1:31)
TOF MS ES+

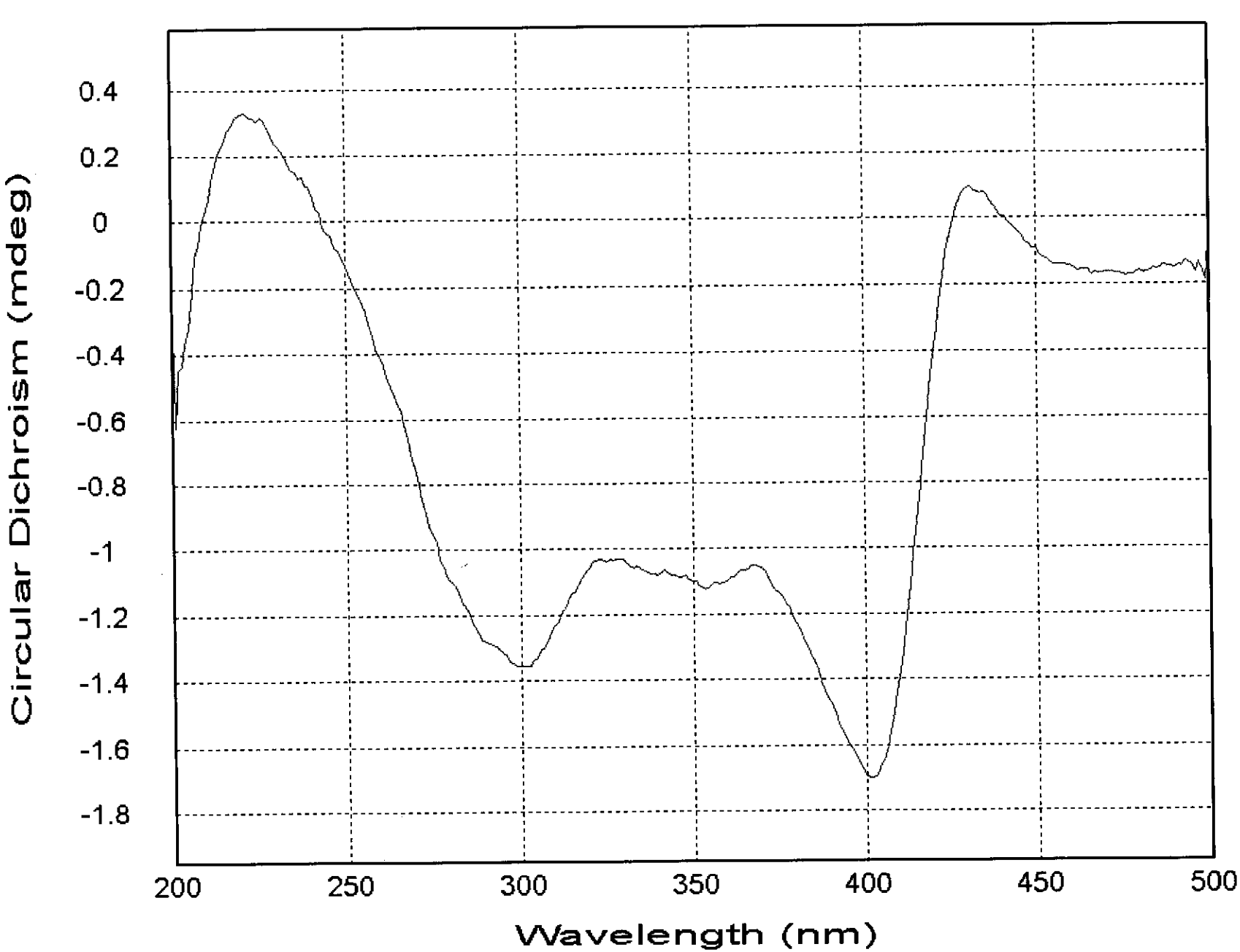
1.93e+005



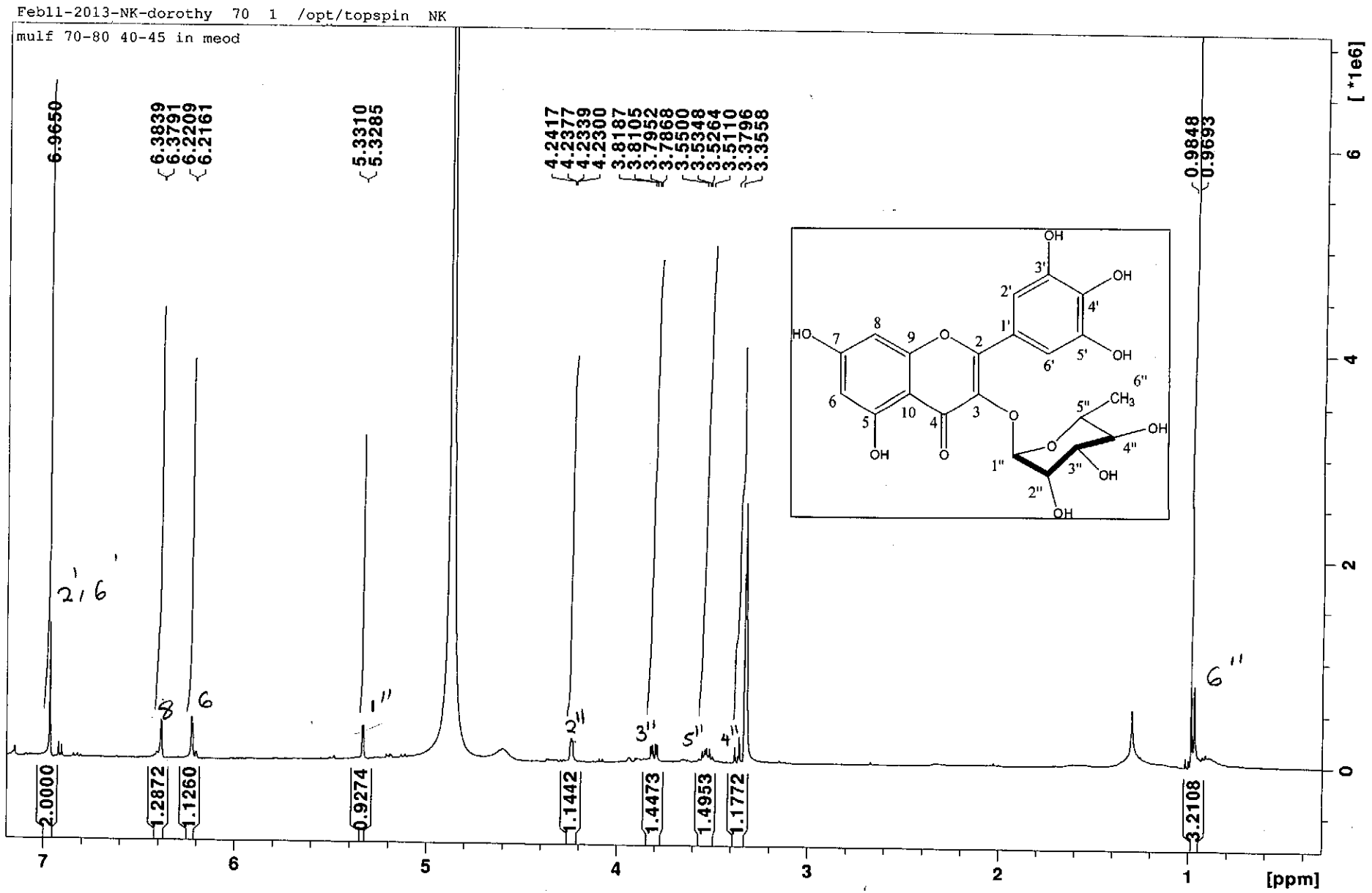
Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
423.1062	423.1056	0.6	1.4	11.5	564.8	0.0	C21 H20 O8 Na



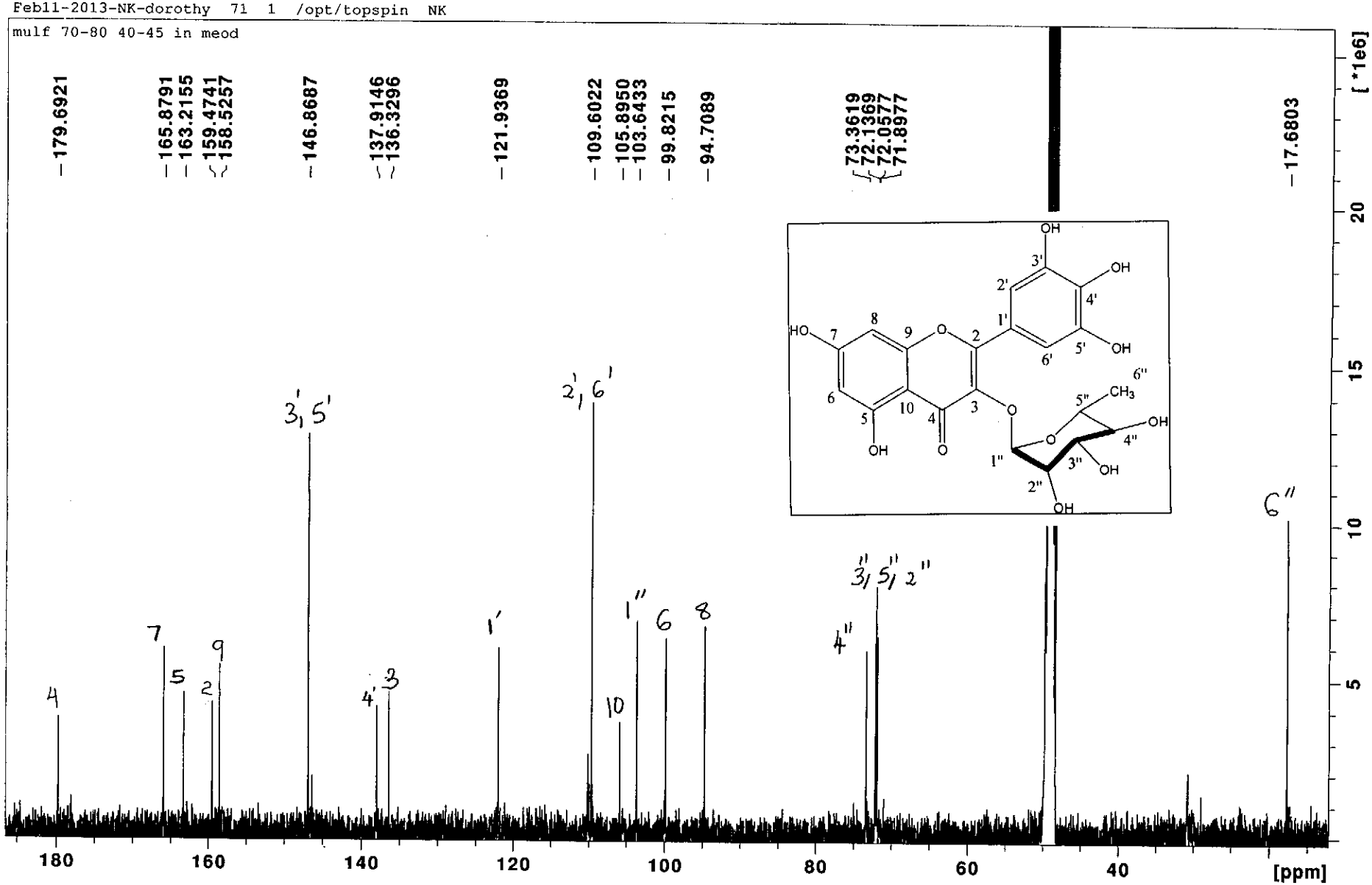
HREIMS spectrum of A2



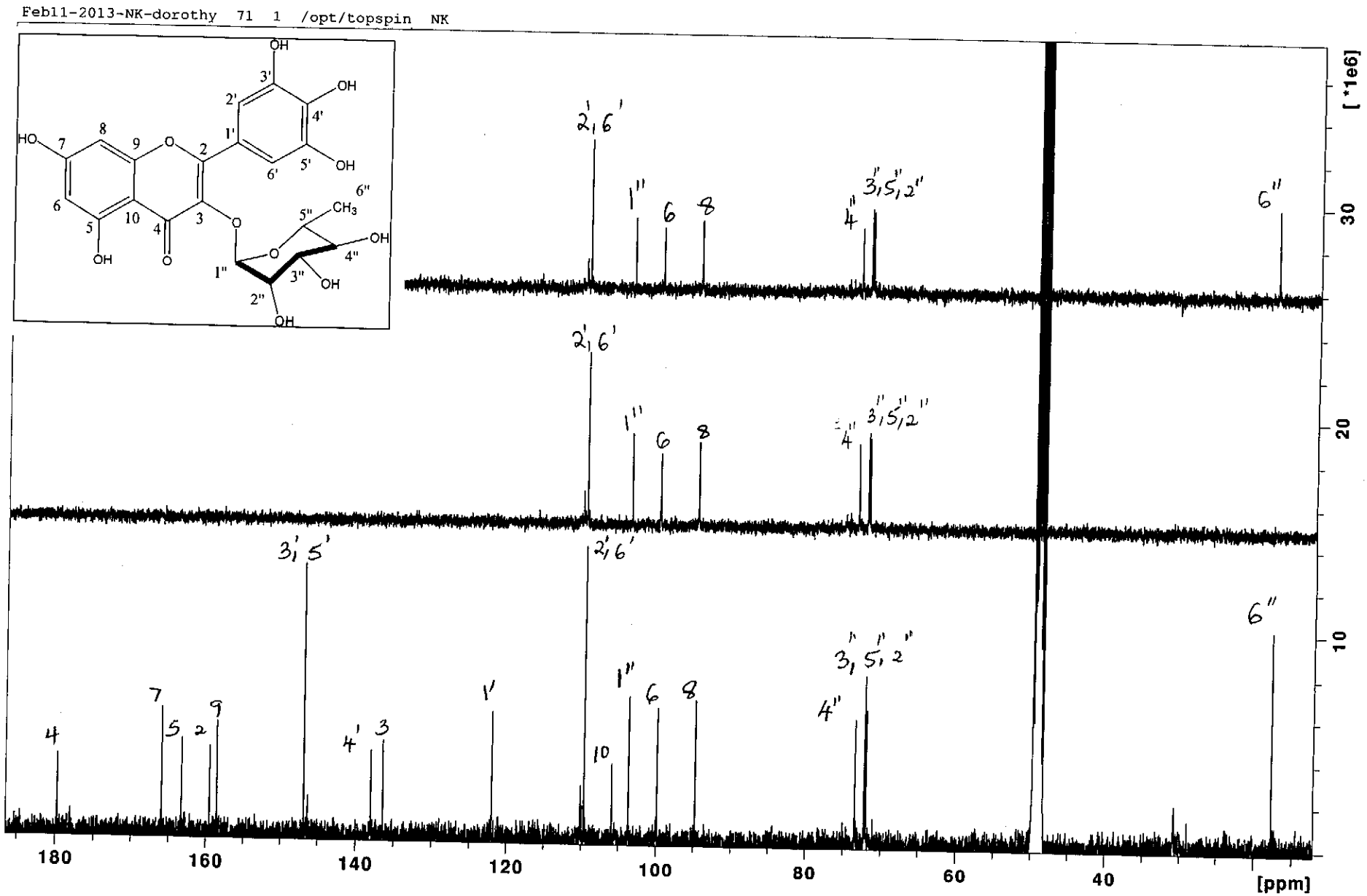
CD spectrum of A2



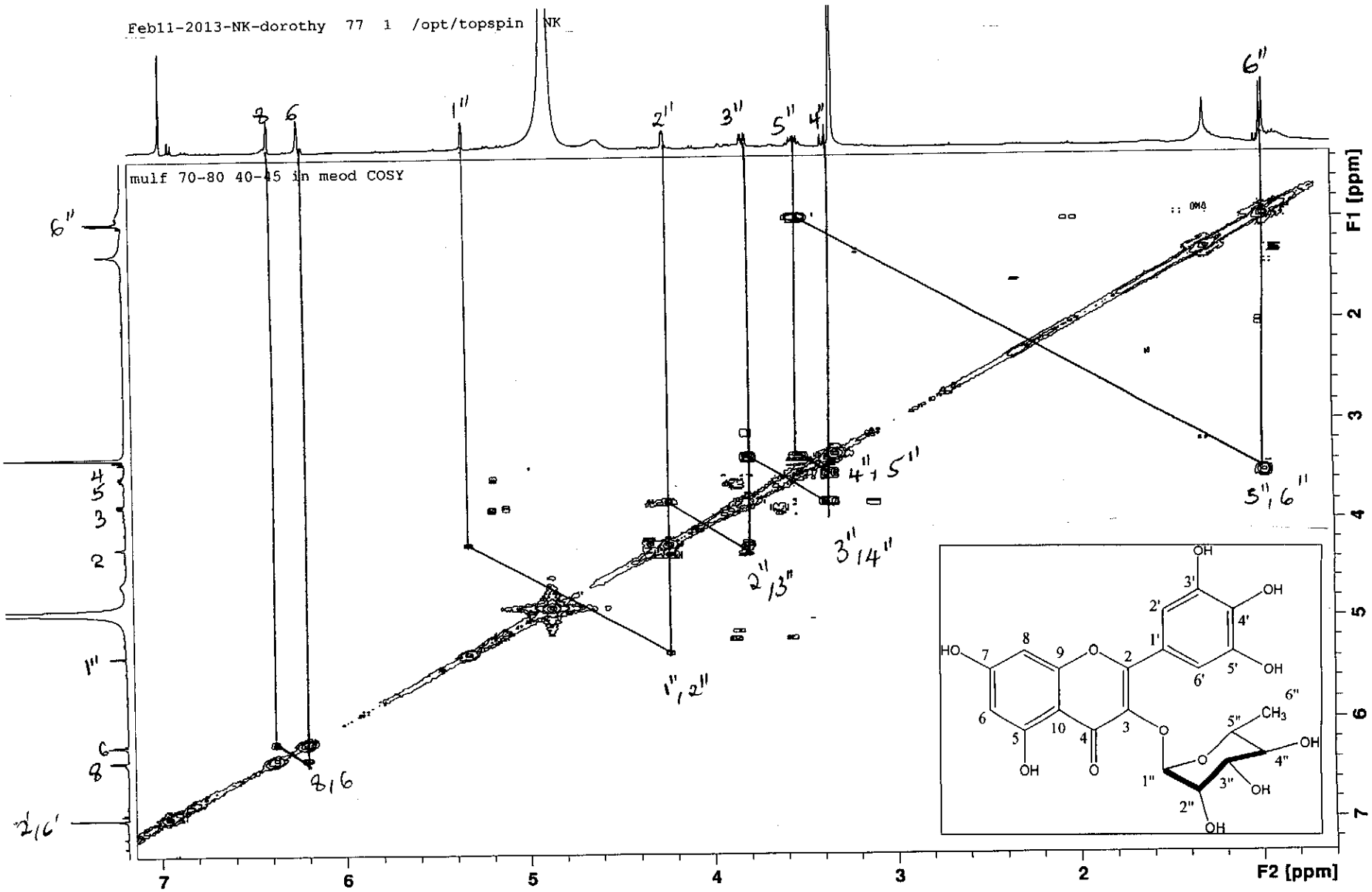
^{1}H NMR spectrum of A3



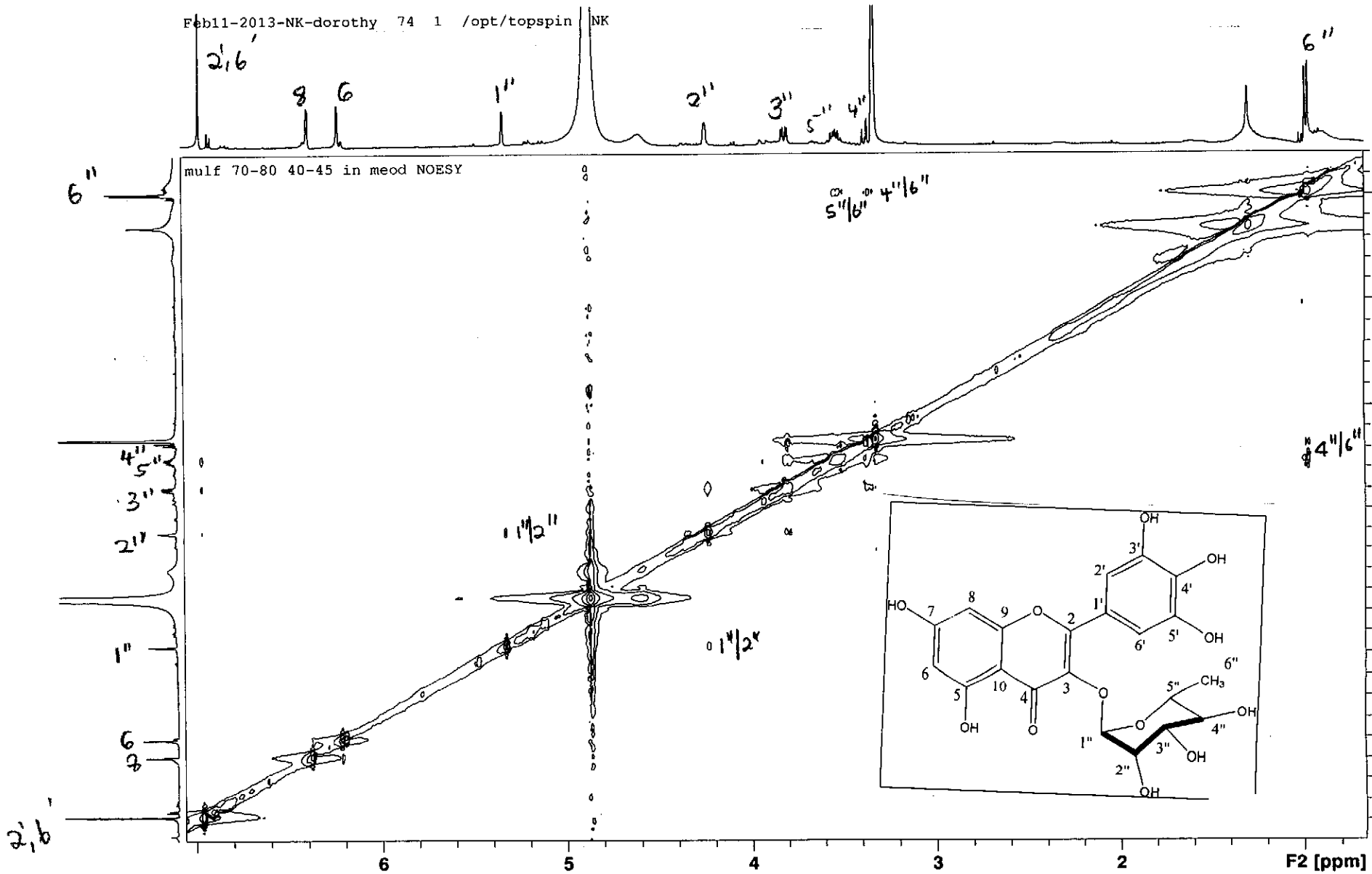
^{13}C NMR spectrum of A3



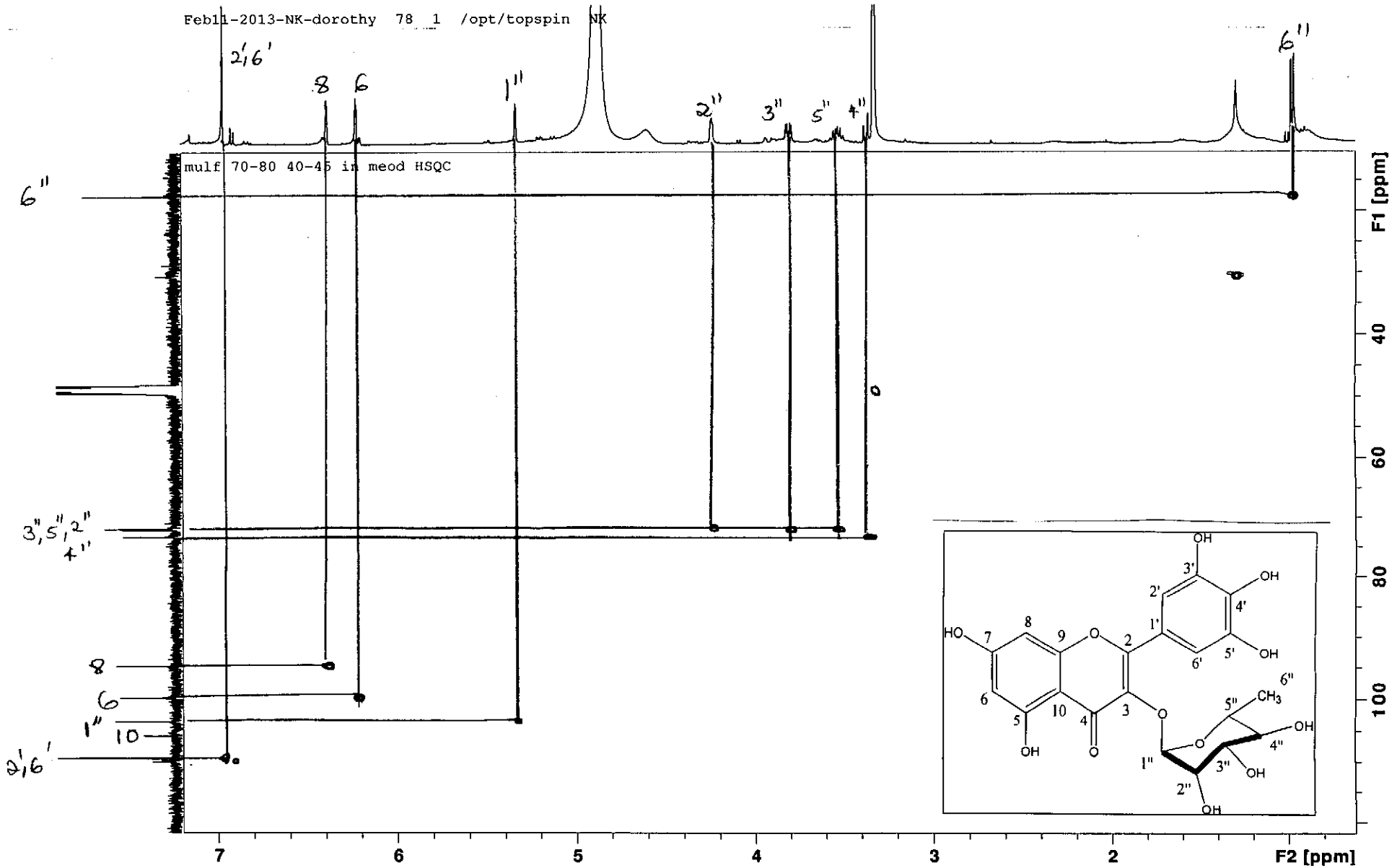
DEPT spectrum of A3



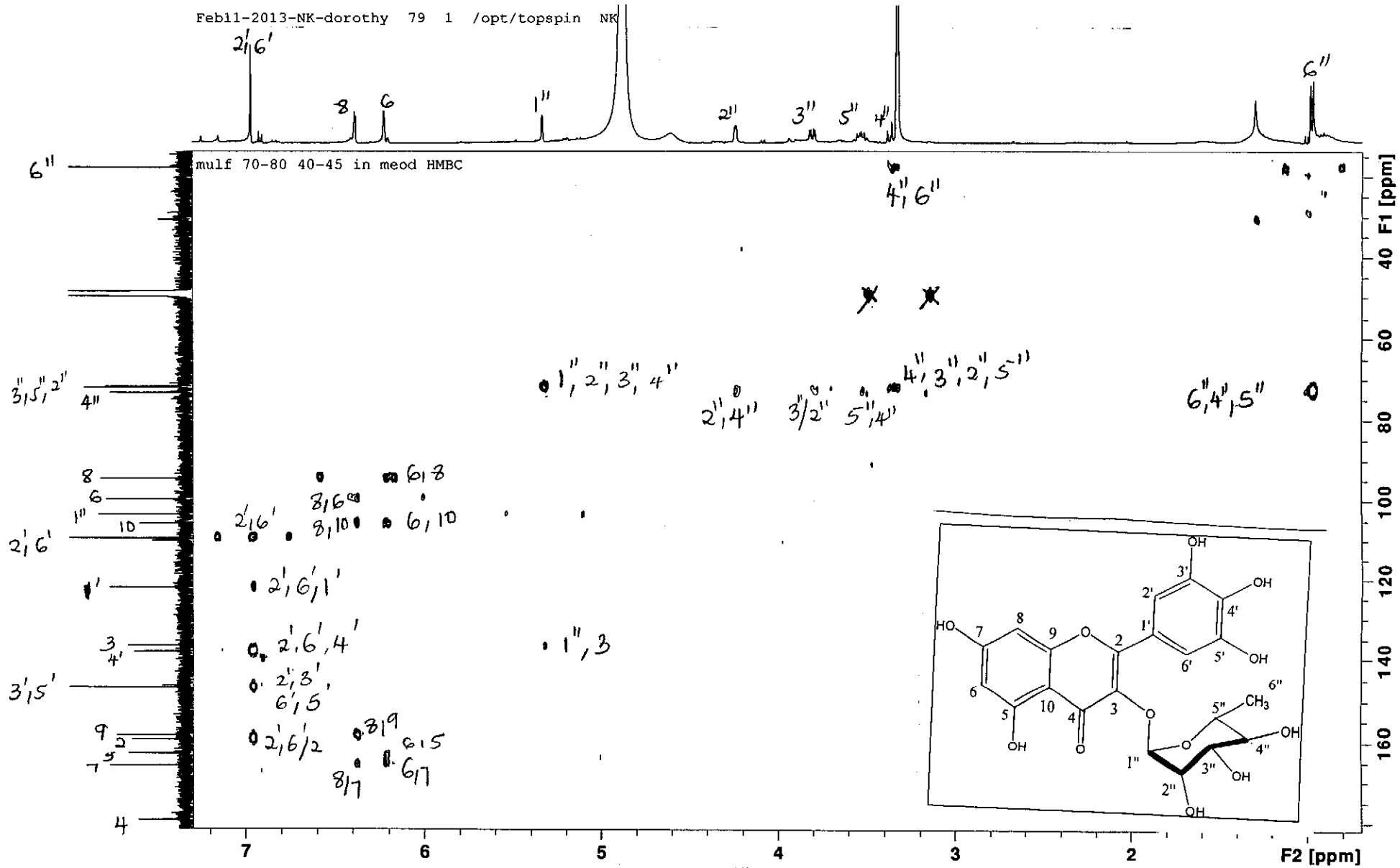
COSY spectrum of A3



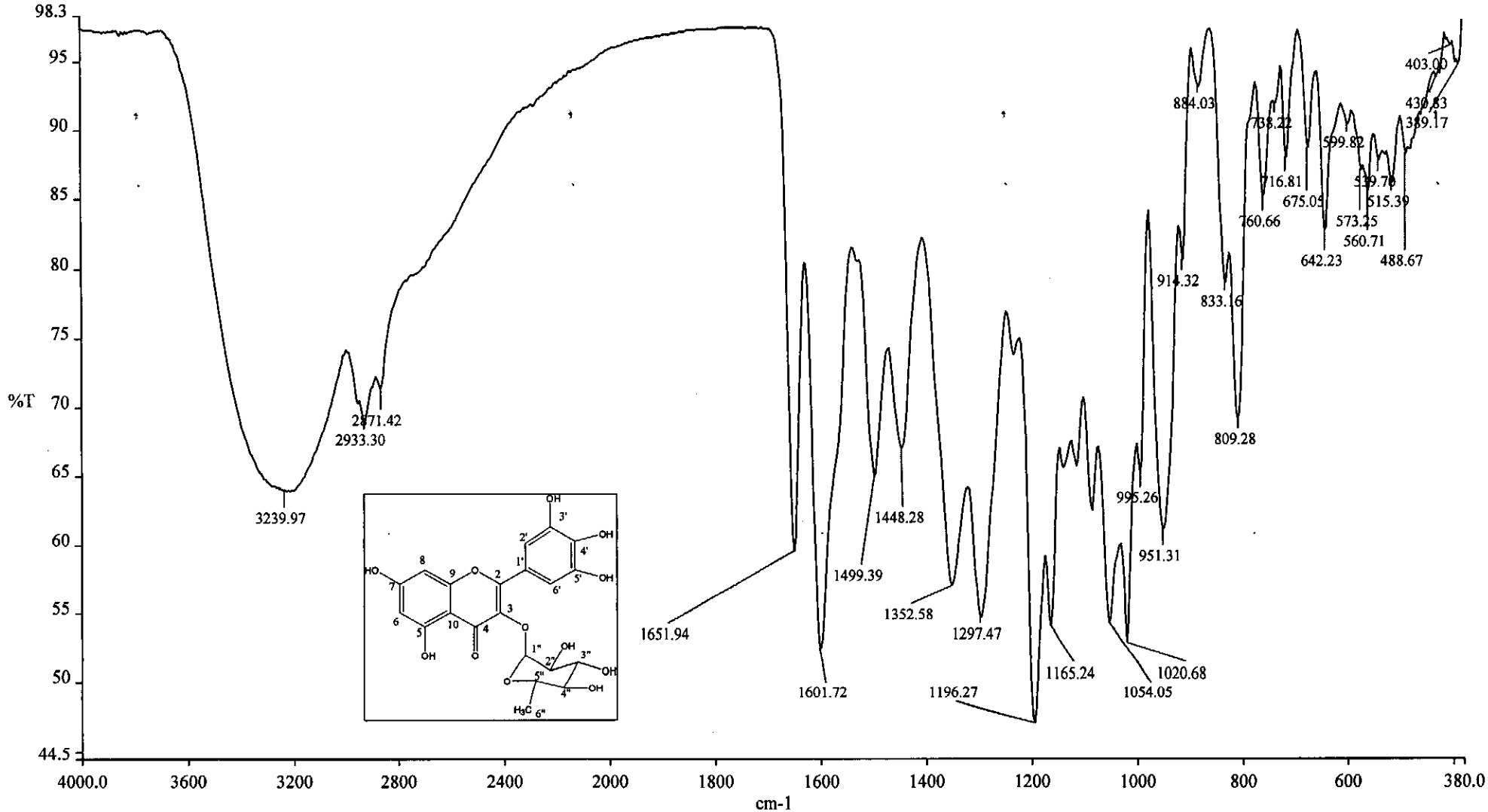
NOESY spectrum of A3



HSQC spectrum of A3



HMBC spectrum of A3

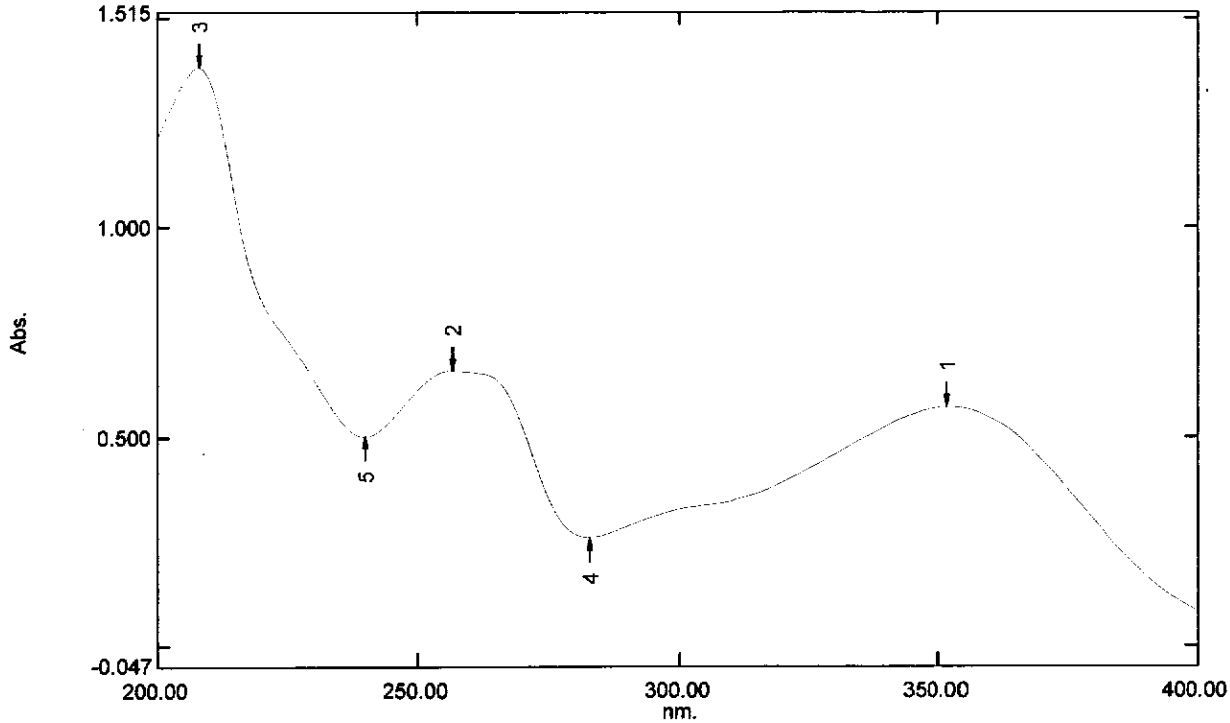


IR spectrum of A3

Spectrum Peak Pick Report

19/05/2012 12:06:38 PM

Data Set: kit 114-119 (7).spc - Storage 115508



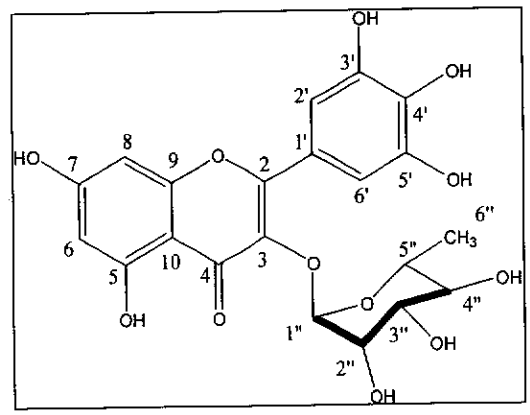
Measurement Properties	
Wavelength Range (nm.):	200.00 to 400.00
Scan Speed:	Medium
Sampling Interval:	1.0
Auto Sampling Interval:	Disabled
Scan Mode:	Single

Instrument Properties	
Instrument Type:	UV-3600 Series
Measuring Mode:	Absorbance
Slit Width:	2.0 nm
Time Constant:	0.1 sec.
Source Lamp:	Auto
Light Source Change Wavelength:	310.00 nm
Detector Unit:	Direct
Detector Change Wavelength:	830.00 nm 1800.00 nm
Grating Change Wavelength:	720.00 nm
S/R Exchange:	Normal
Detector Lock:	Auto
Slit Program:	Normal
Beam Mode:	Double
Stair Correction:	Disable

Attachment Properties	
Attachment:	None

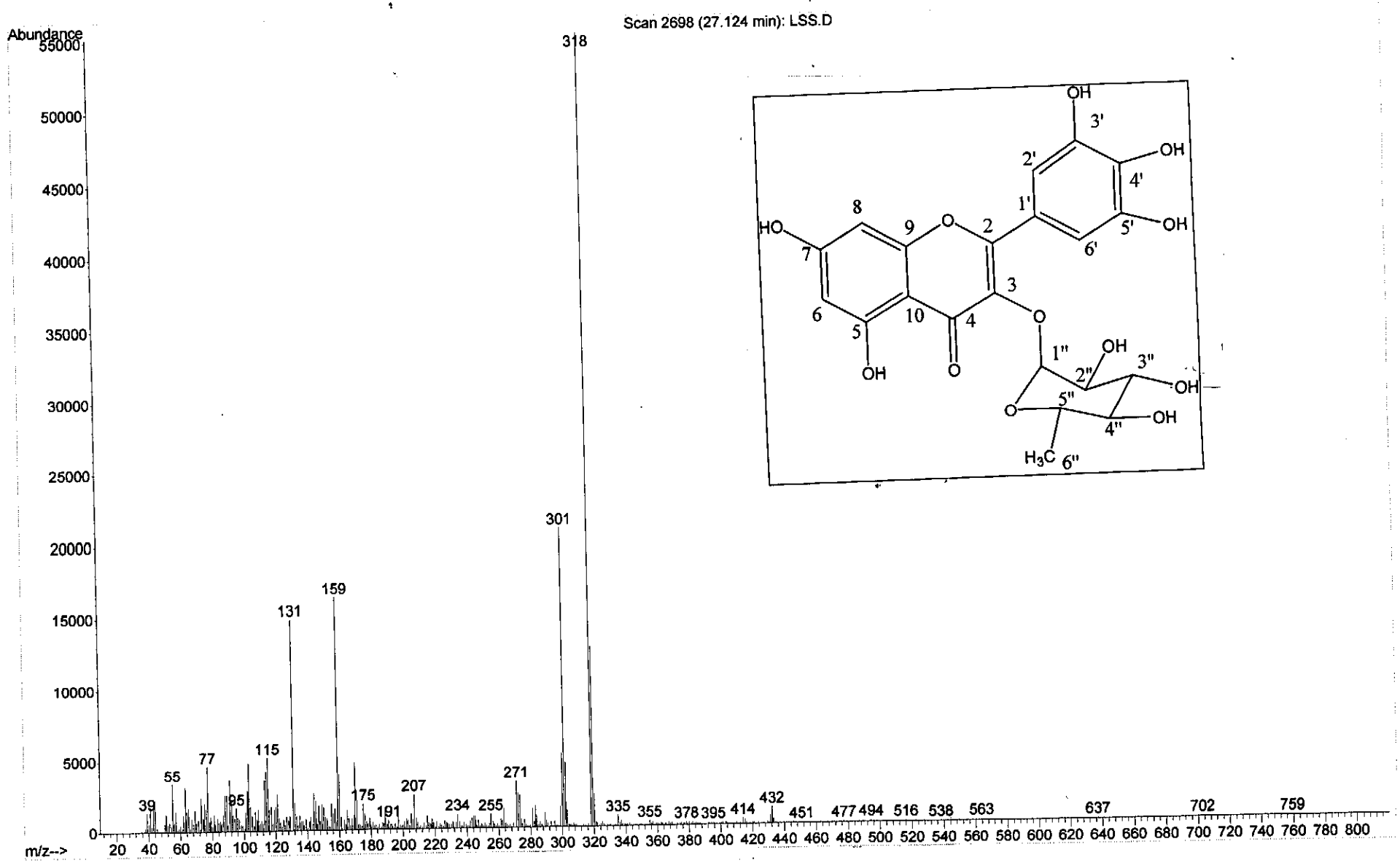
Sample Preparation Properties	
Weight:	0.3mg
Volume:	20ml
Dilution:	
Path Length:	
Additional Information:	

No.	P/V	Wavelength	Abs.	Description
1	●	352.00	0.570	
2	●	257.00	0.659	
3	●	208.00	1.385	
4	◐	283.00	0.260	
5	◐	240.00	0.501	



UV spectrum of A3

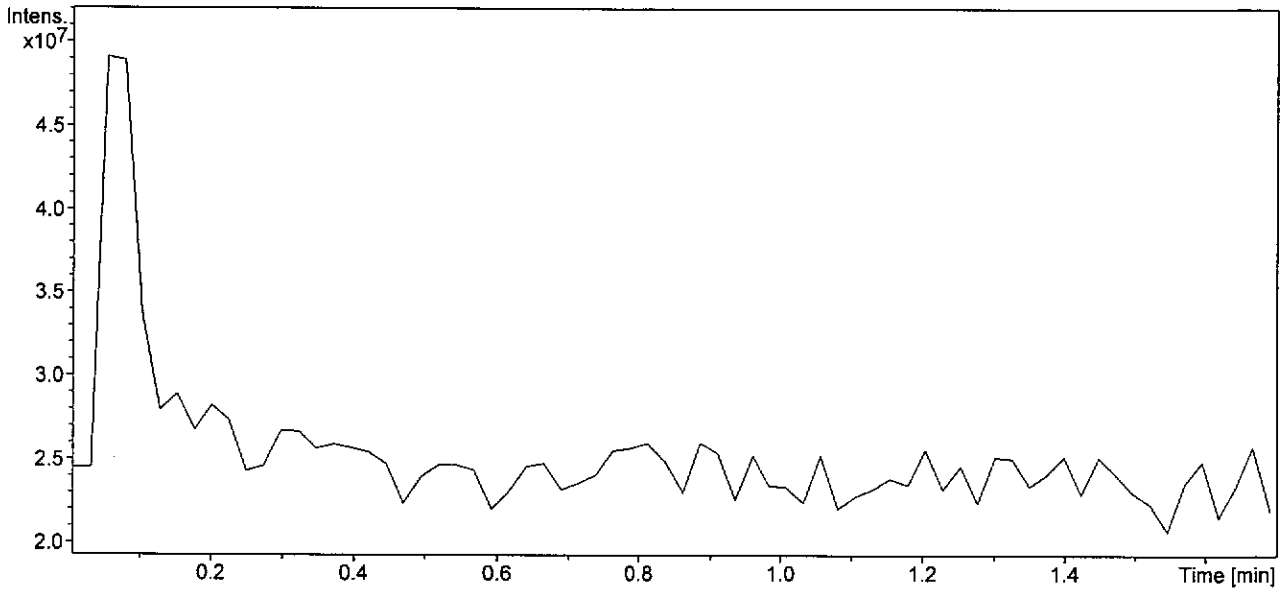
File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\DORTHY\LSS.D
Operator : DOROTHY
Acquired : 19 Jun 2011 10:38 using AcqMethod NATURAL
Instrument : Instrumen
Sample Name: LSS 70-76
Misc Info :
Vial Number: 1



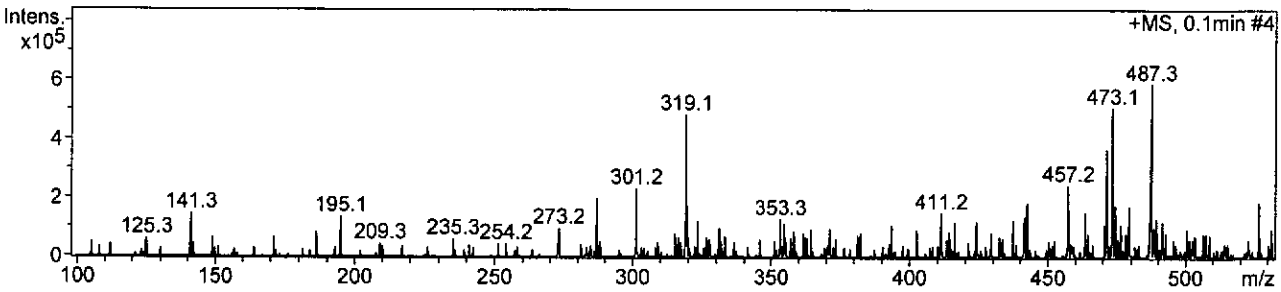
MS spectrum of A3

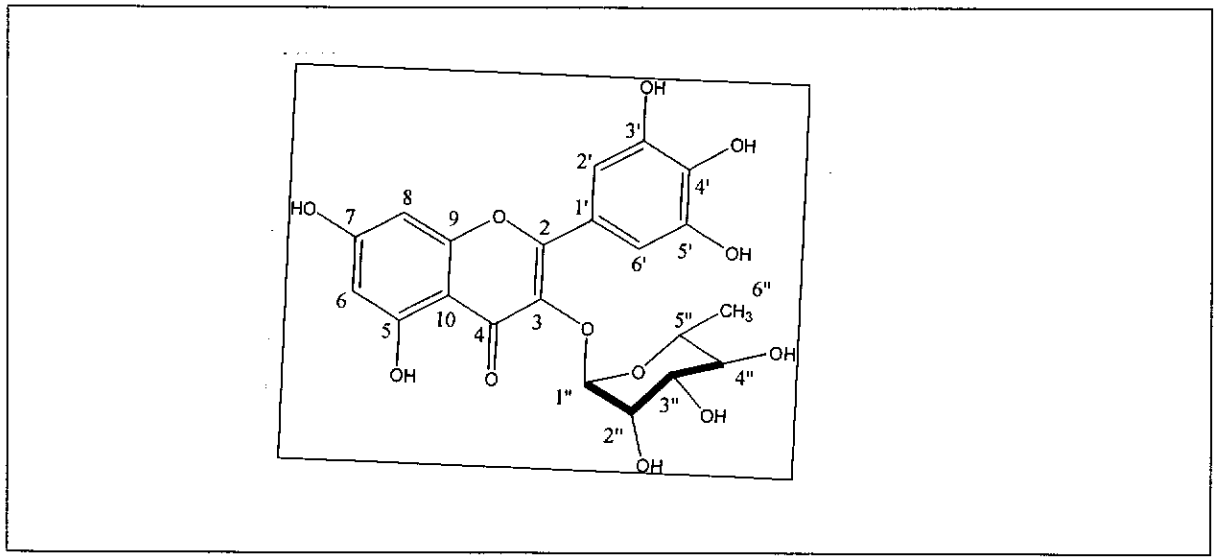
Display Report - All Windows Selected Analysis

Analysis Name: MRR000002.D **Instrument:** LC-MSD-Trap-VL **Print Date:** 1/15/2013 12:12:32 PM
Method: GUMBI2.M **Operator:** Operator **Acq. Date:** 6/20/2012 11:09:39 AM
Sample Name: Default
Analysis Info:

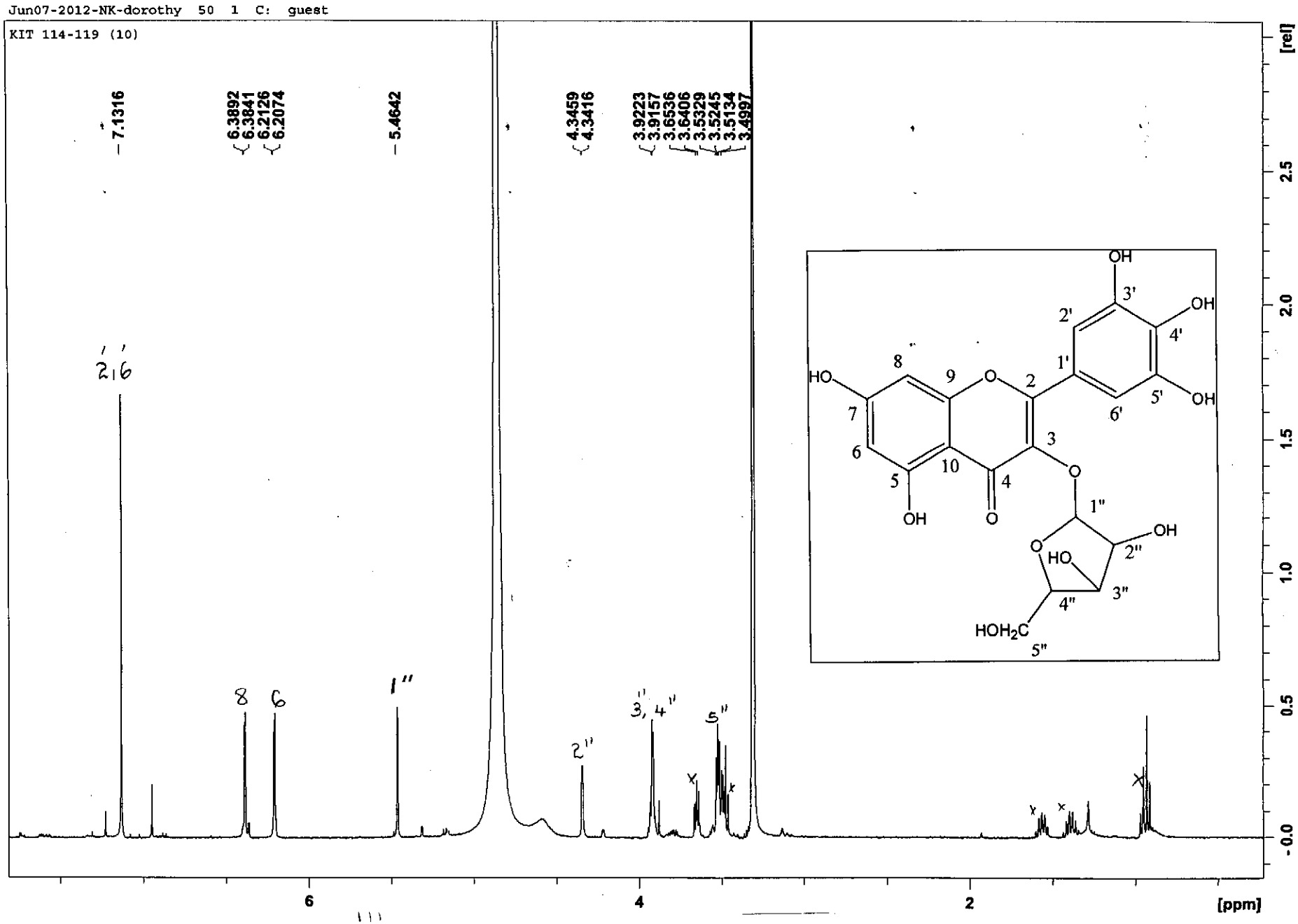


TIC +All MS

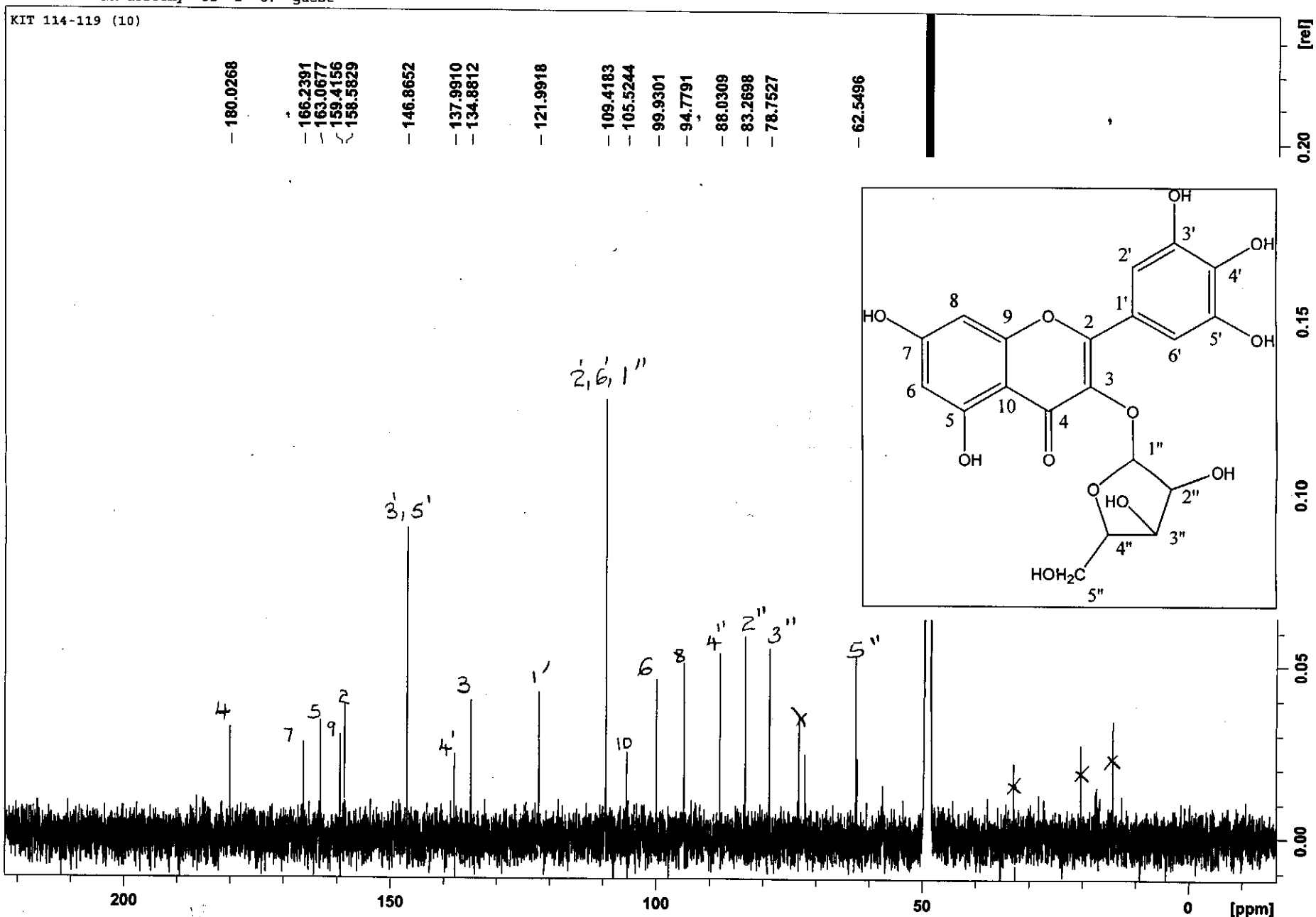




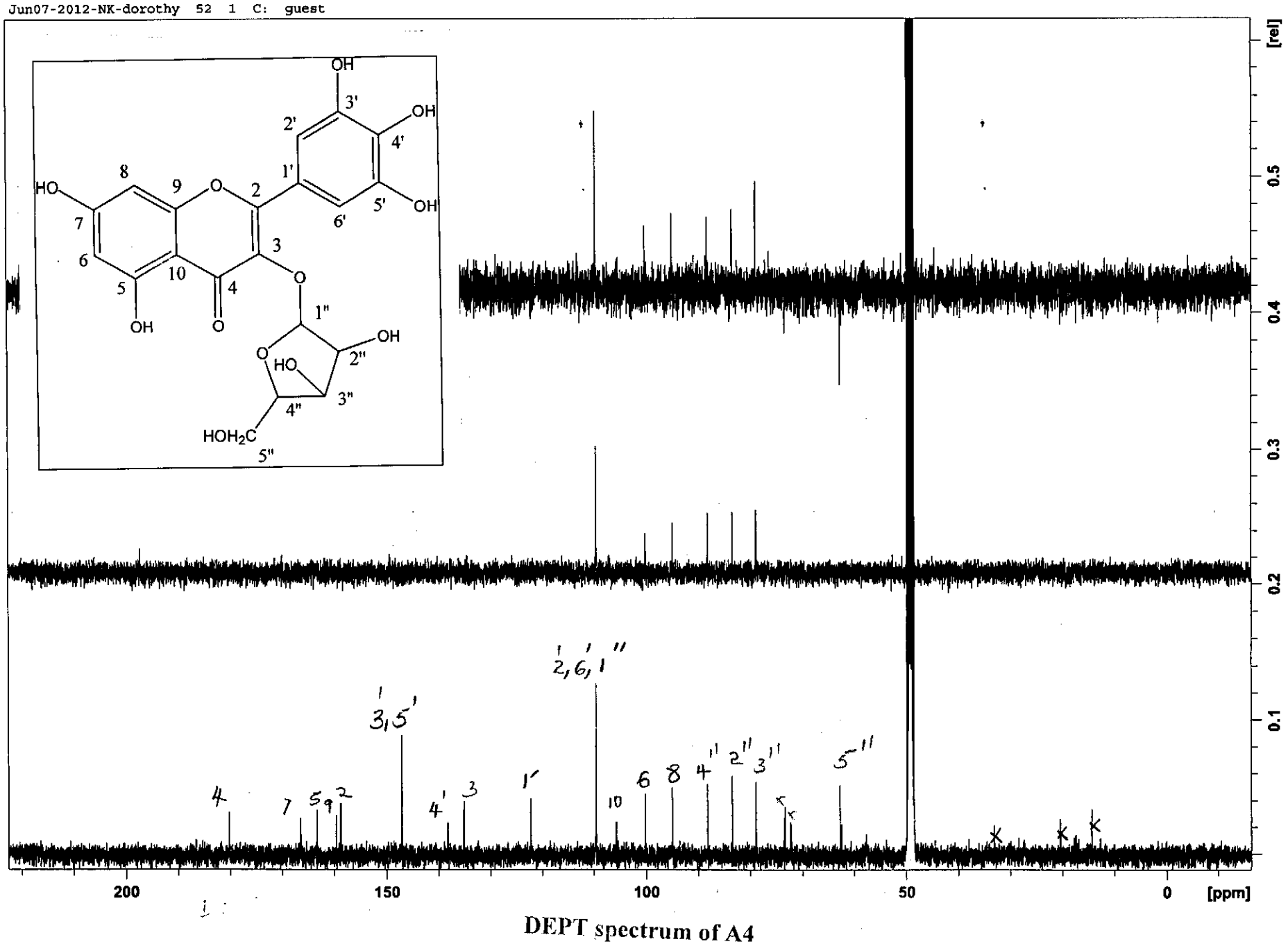
LC-MS spectrum of A3



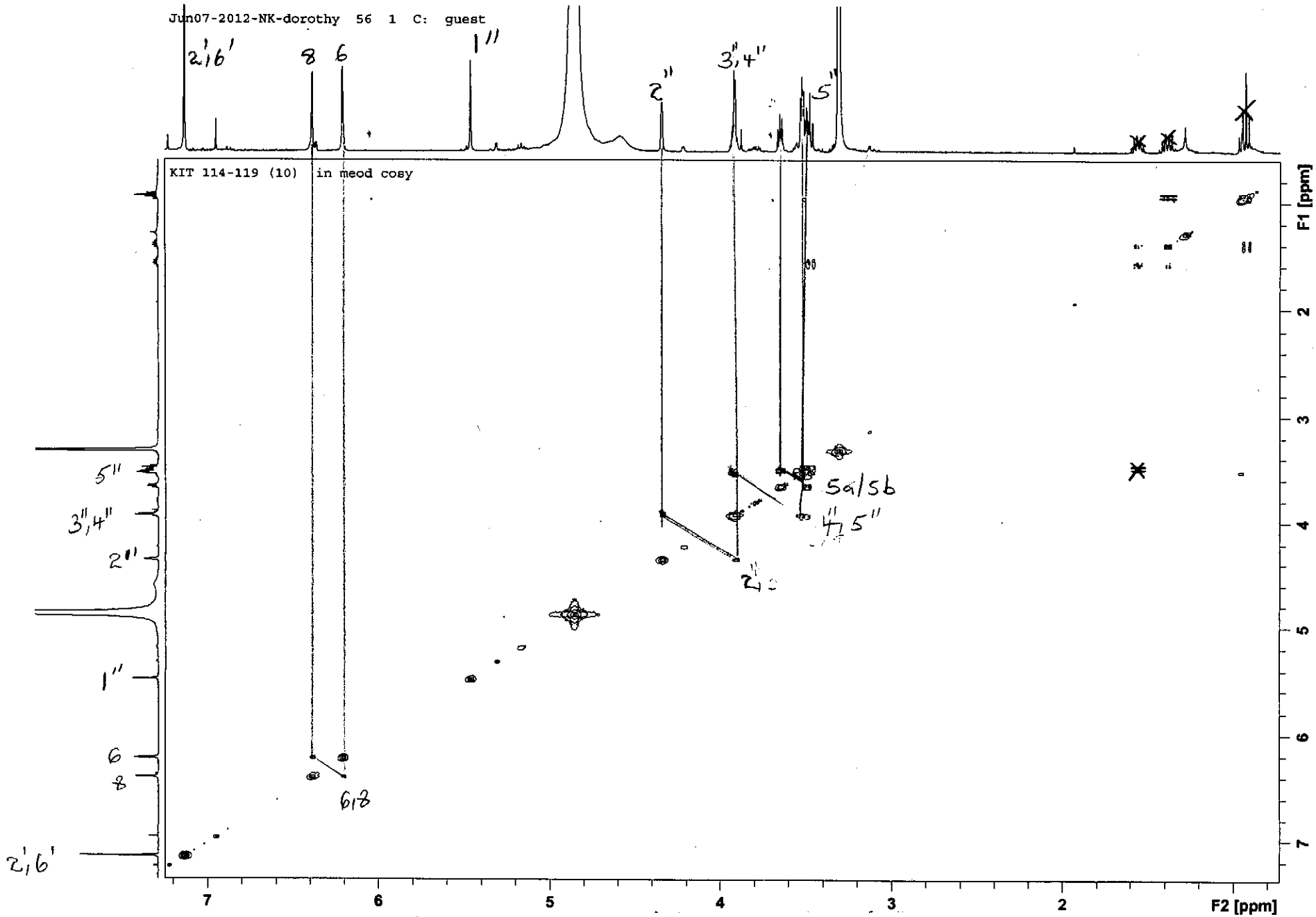
^{1}H NMR spectrum of A4



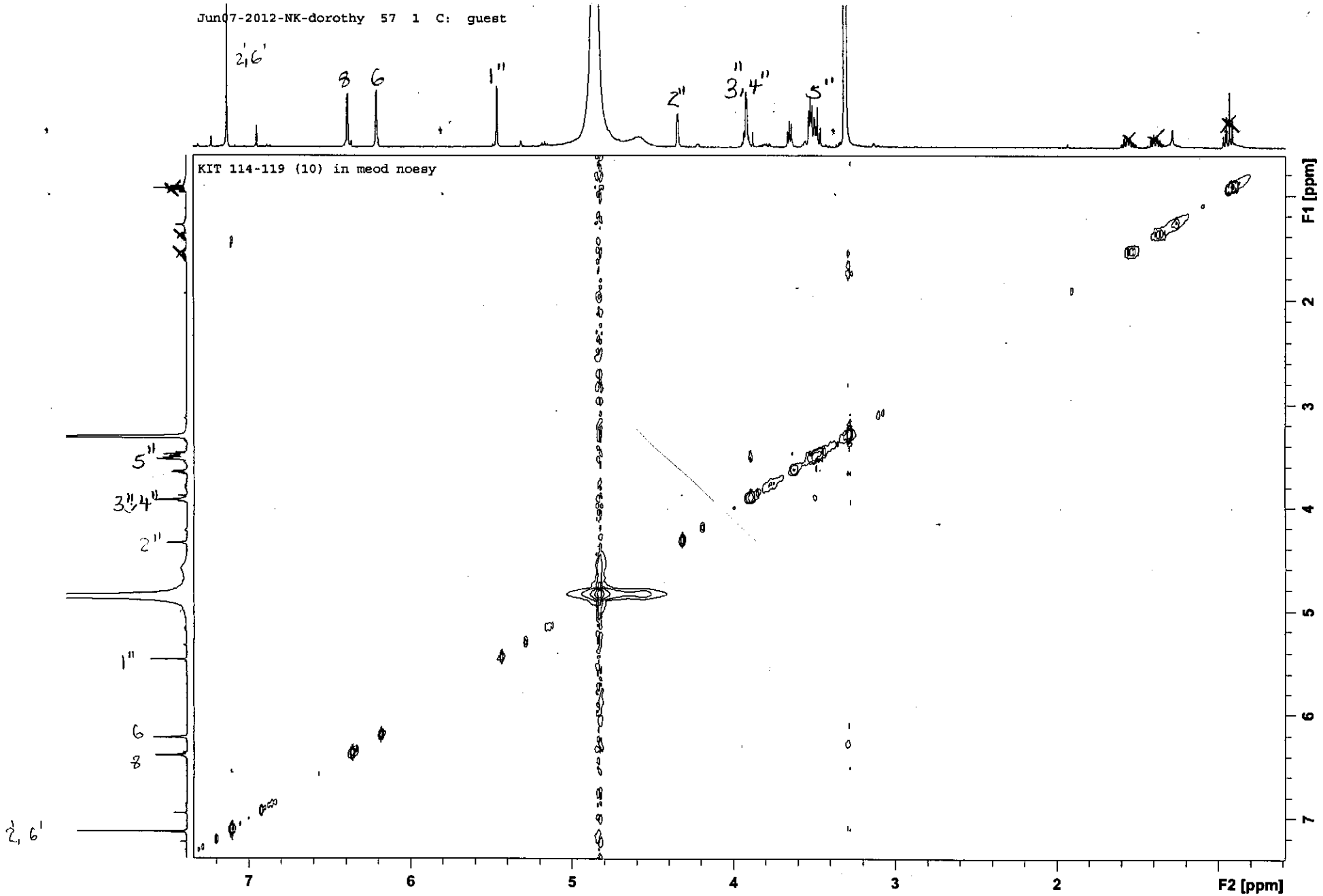
^{13}C NMR spectrum of A4



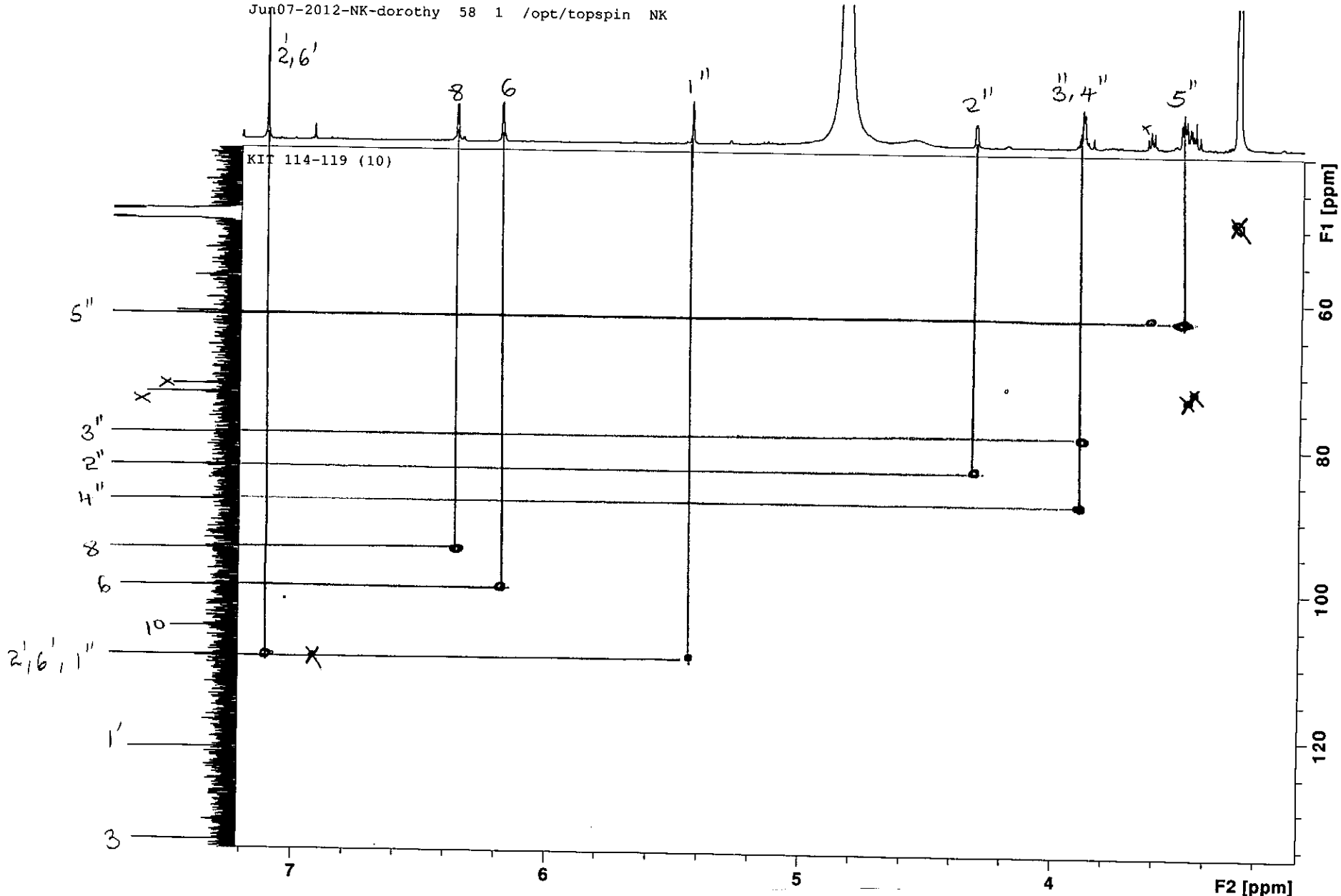
DEPT spectrum of A4



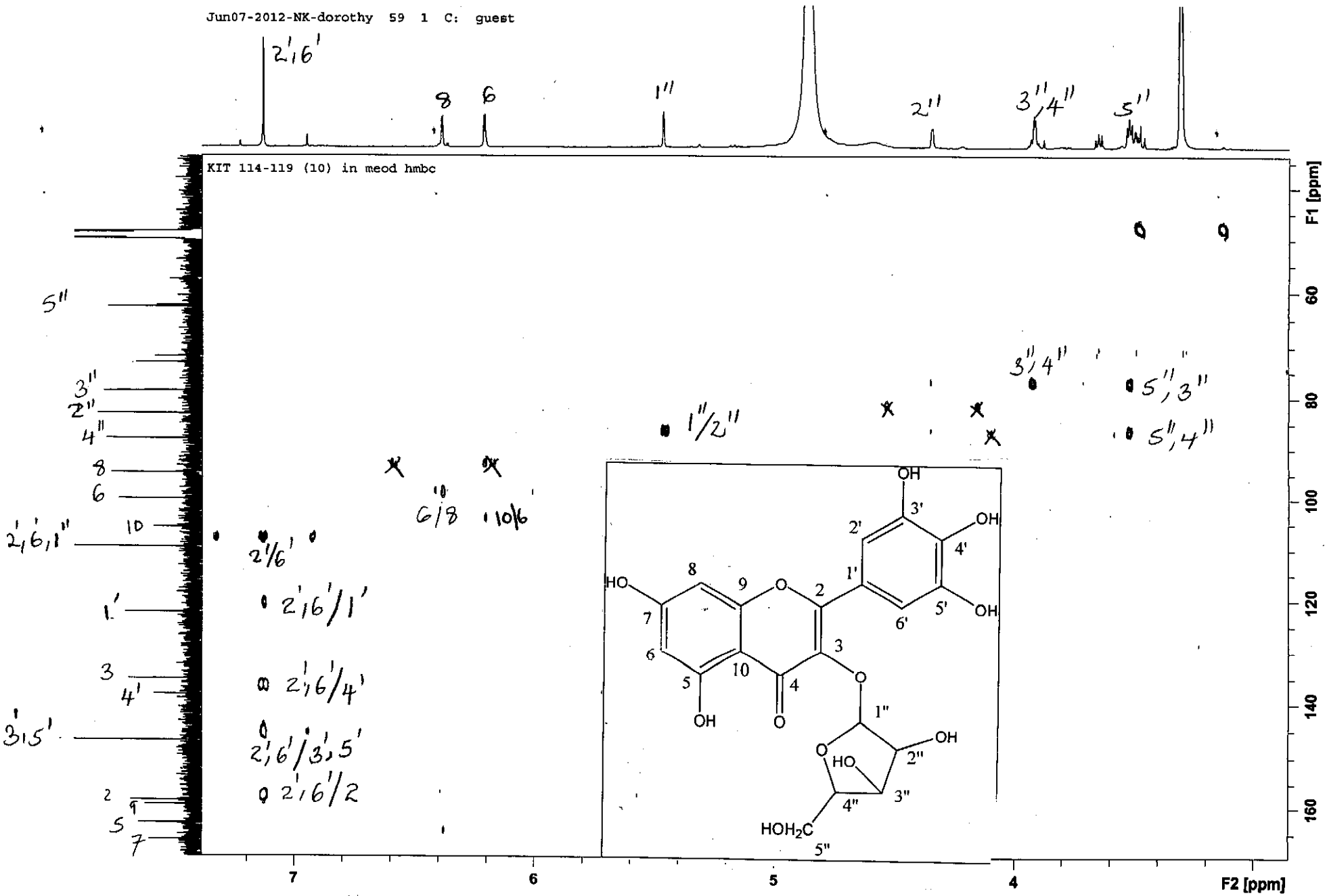
COSY spectrum of A4



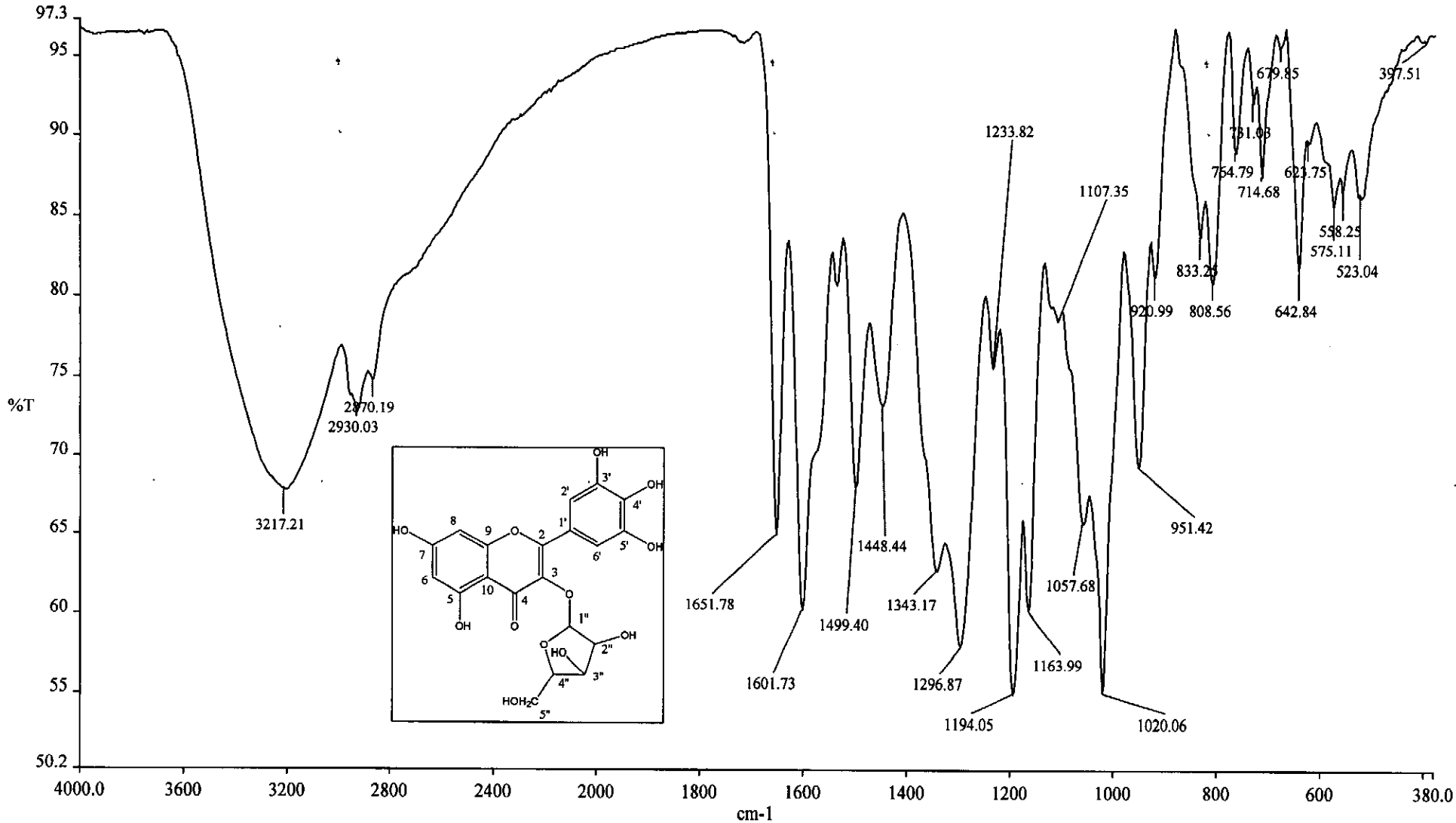
NOESY spectrum of A4



HSQC spectrum of A4



HMBC spectrum of A4

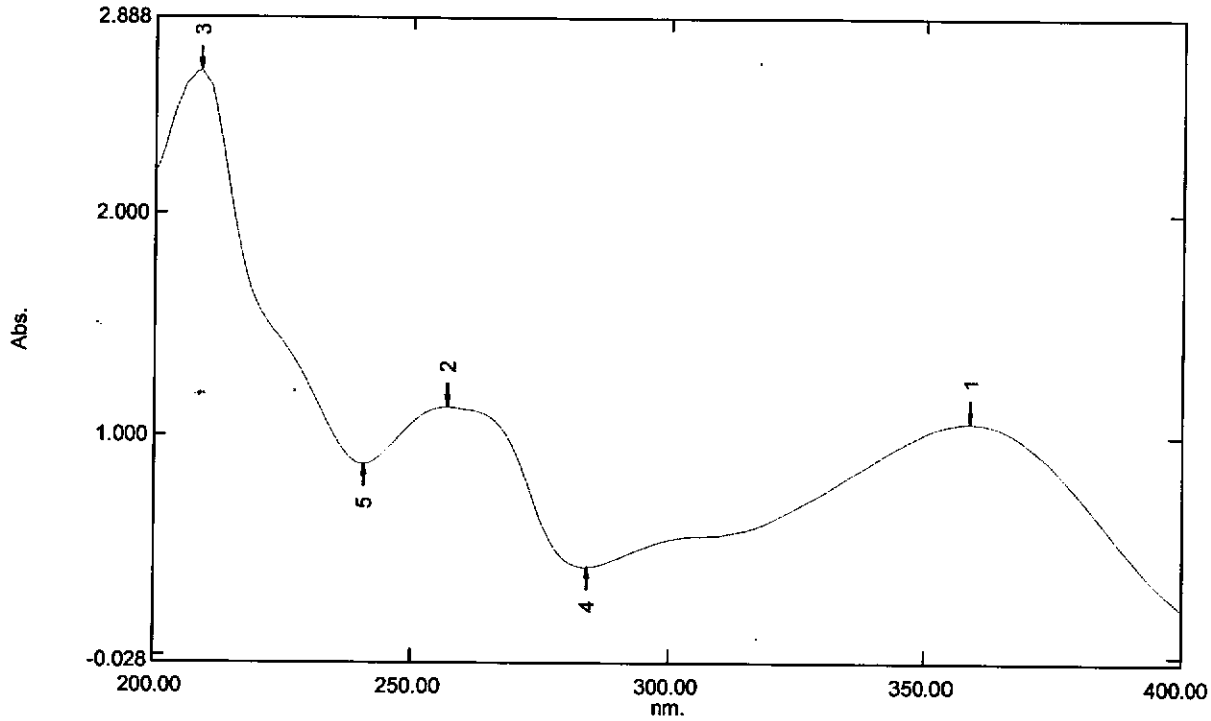


IR spectrum of A4

Spectrum Peak Pick Report

19/05/2012 03:55:27 PM

Data Set: kit 114-119 (10).spc - Storage 115937



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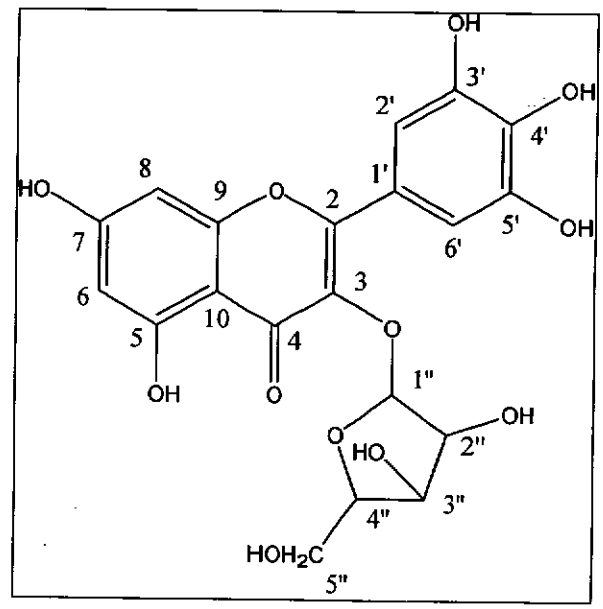
2		257.00	1.125	
3		209.00	2.645	
4		284.00	0.400	
5		241.00	0.867	

Measurement Properties
Wavelength Range (nm.): 200.00 to 400.00
Scan Speed: Medium
Sampling Interval: 1.0
Auto Sampling Interval: Disabled
Scan Mode: Single

Instrument Properties
Instrument Type: UV-3600 Series
Measuring Mode: Absorbance
Slit Width: 2.0 nm
Time Constant: 0.1 sec.
Source Lamp: Auto
Light Source Change Wavelength: 310.00 nm
Detector Unit: Direct
Detector Change Wavelength: 830.00 nm 1800.00 nm
Grating Change Wavelength: 720.00 nm
S/R Exchange: Normal
Detector Lock: Auto
Slit Program: Normal
Beam Mode: Double
Stair Correction: Disable

Attachment Properties
Attachment: None

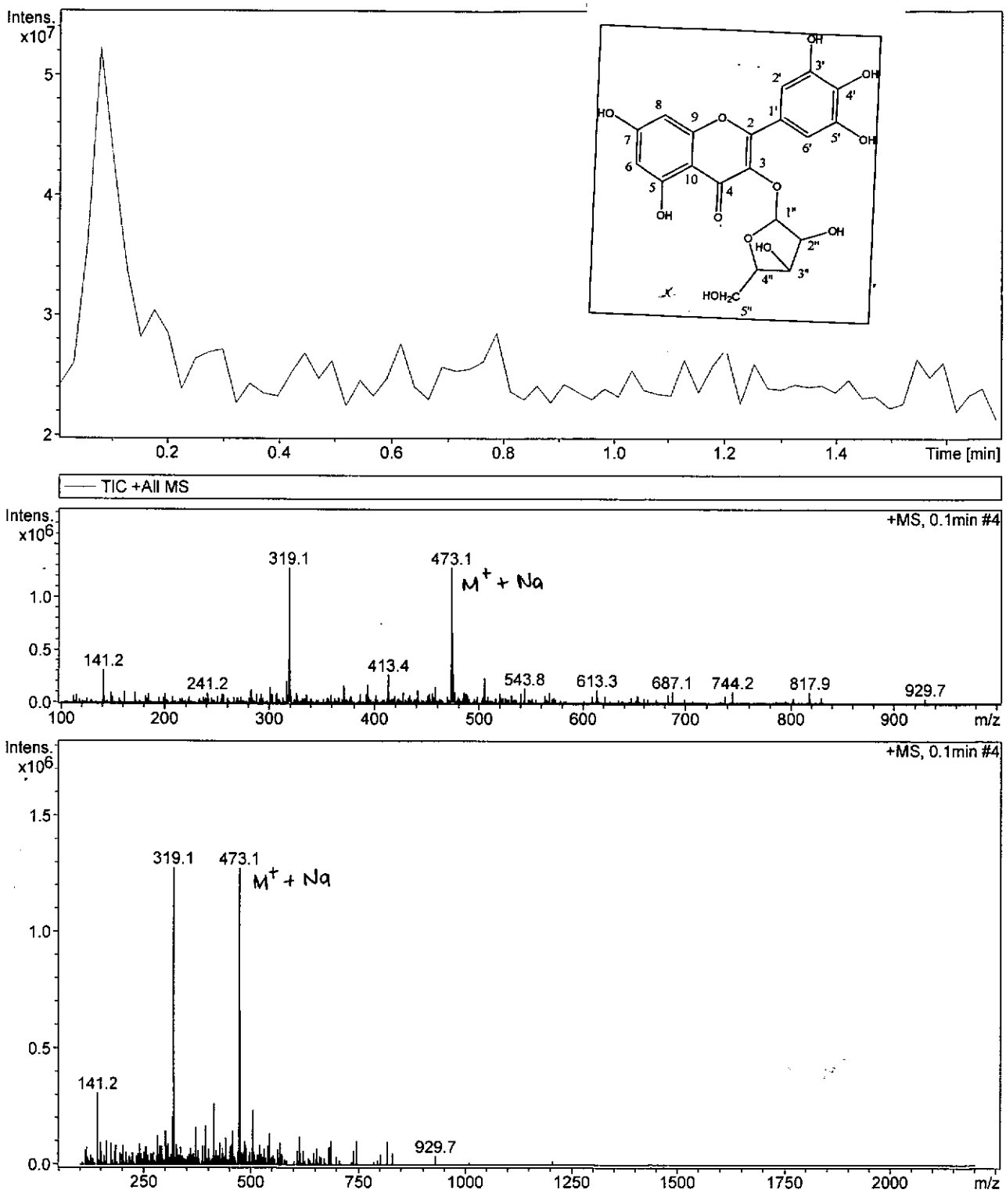
Sample Preparation Properties
Weight: 0.3mg
Volume: 20ml
Dilution:
Path Length:
Additional Information:



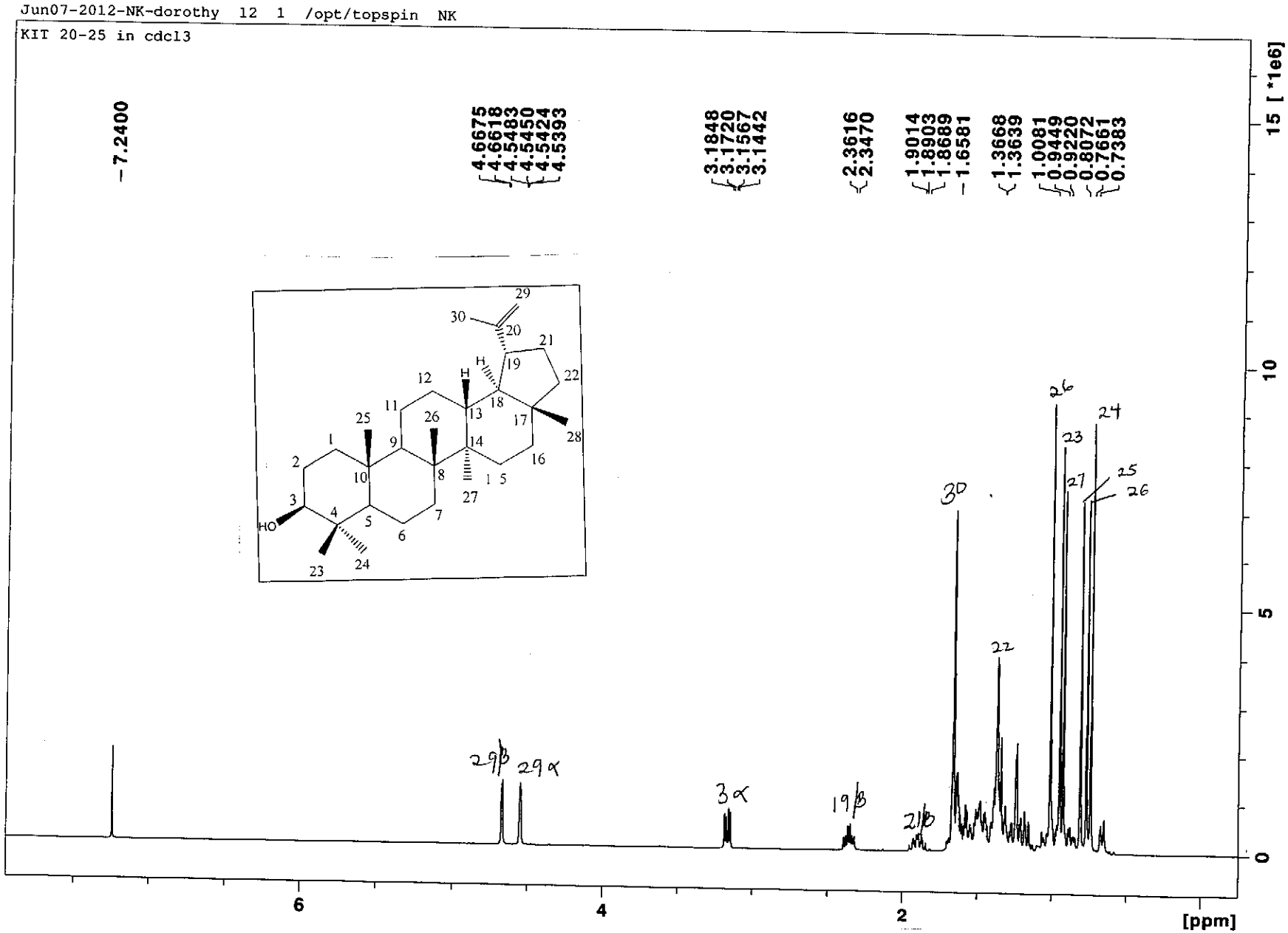
UV spectrum of A4

Display Report - All Windows Selected Analysis

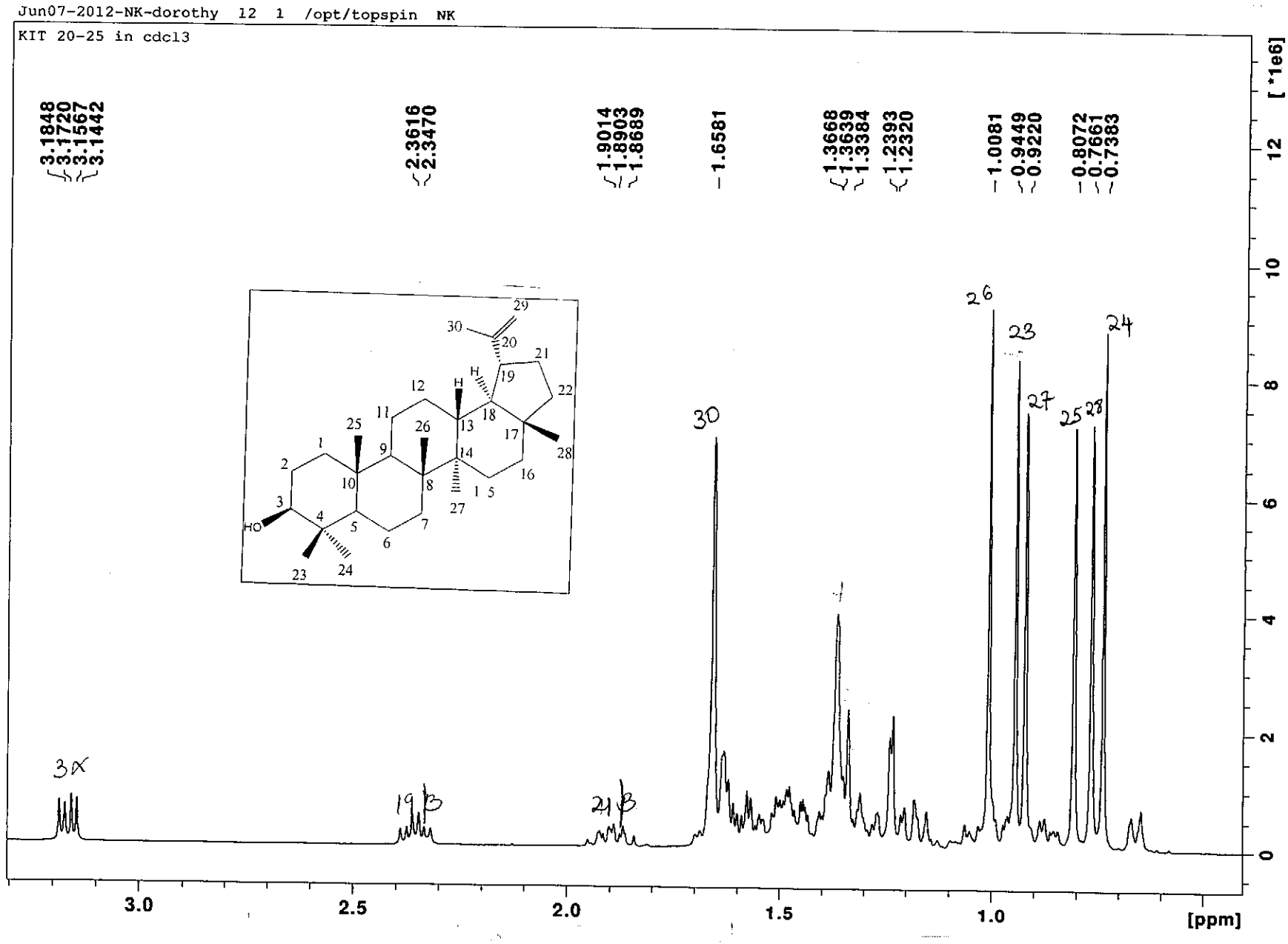
Analysis Name: MA000001.D **Instrument:** LC-MSD-Trap-VL **Print Date:** 1/15/2013 12:09:10 PM
Method: GUMBI2.M **Operator:** Operator **Acq. Date:** 6/20/2012 10:49:33 AM
Sample Name: Default
Analysis Info:



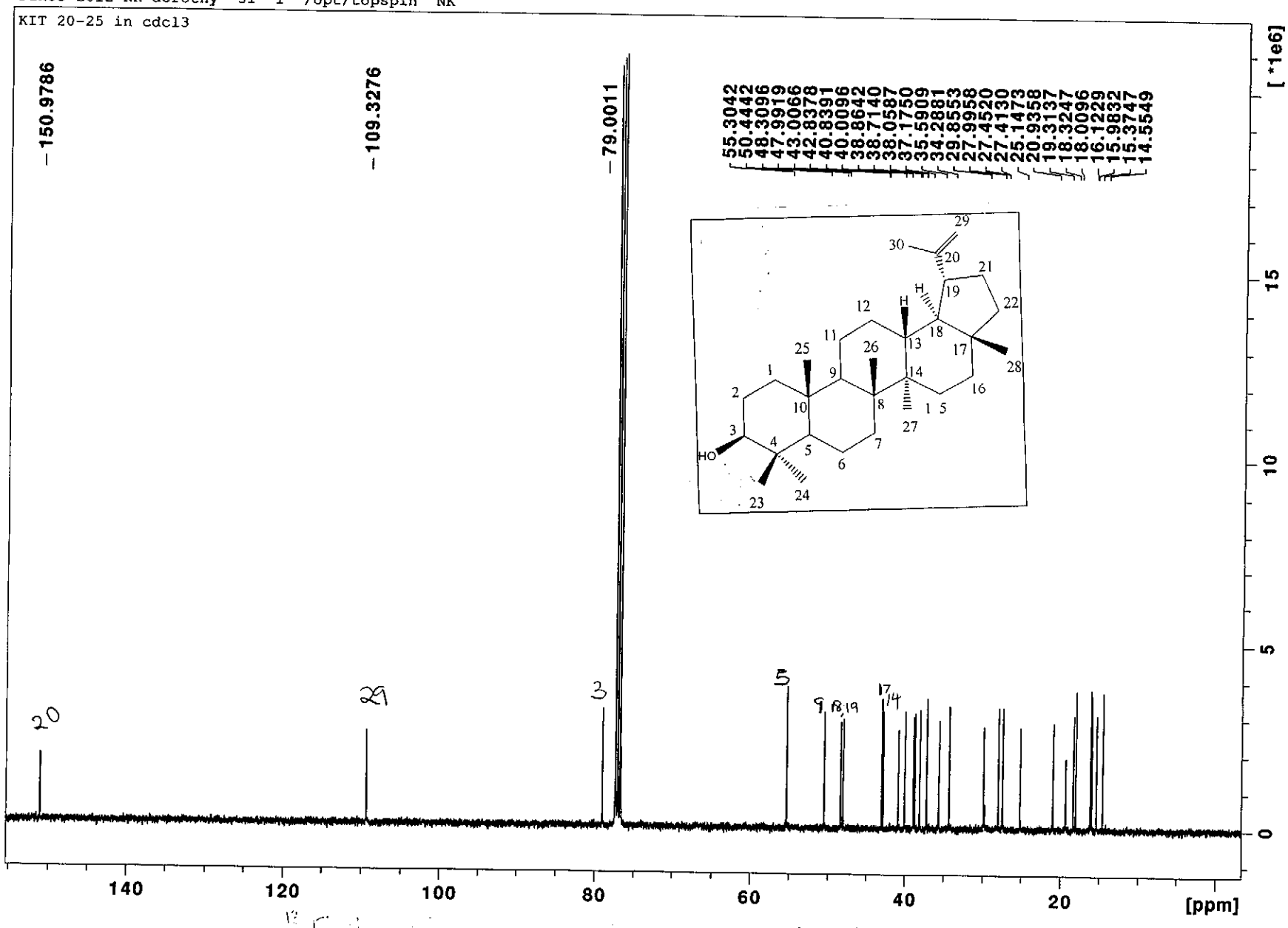
LC-MS spectrum of A4



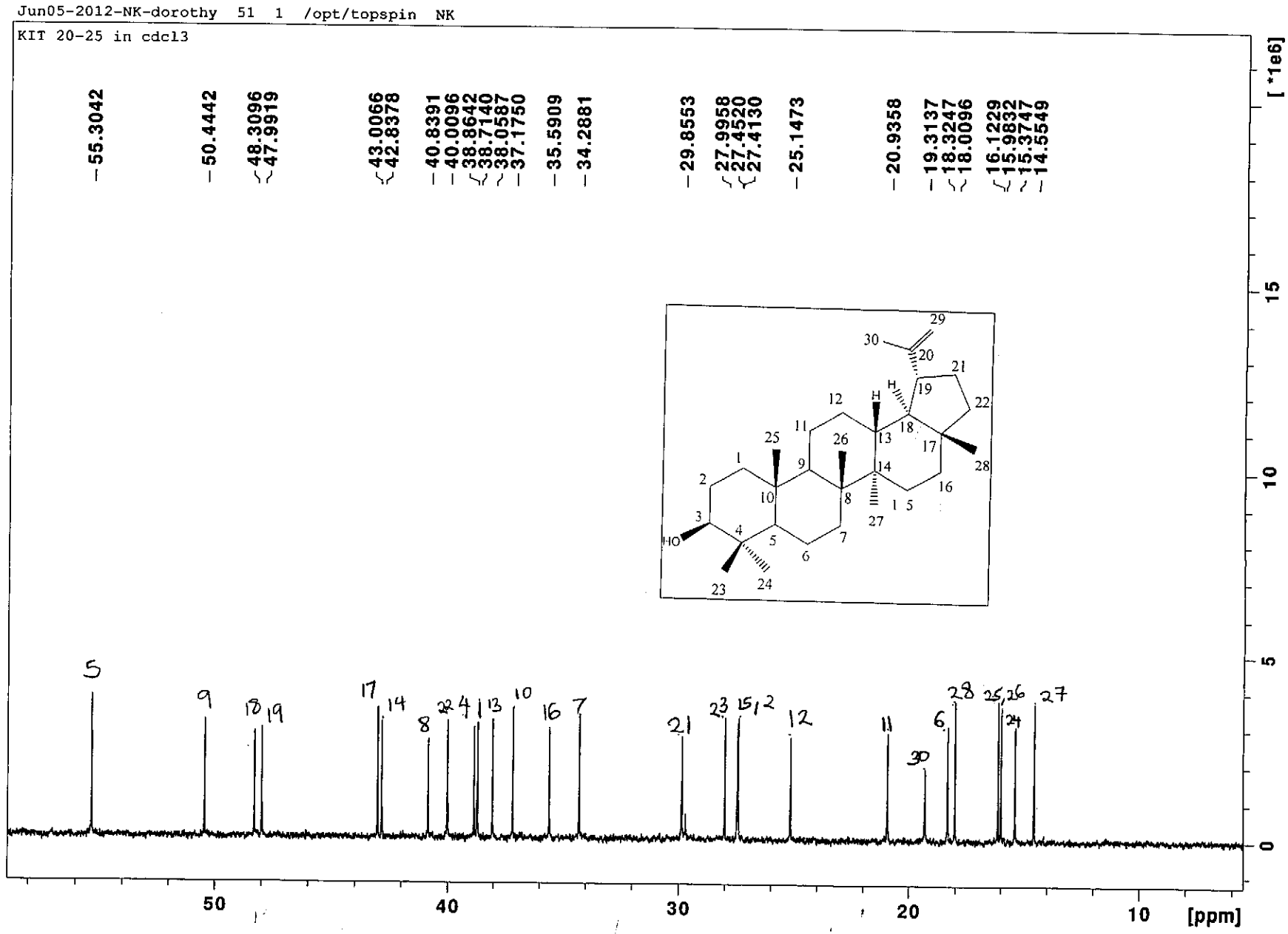
^{1}H NMR spectrum of A5



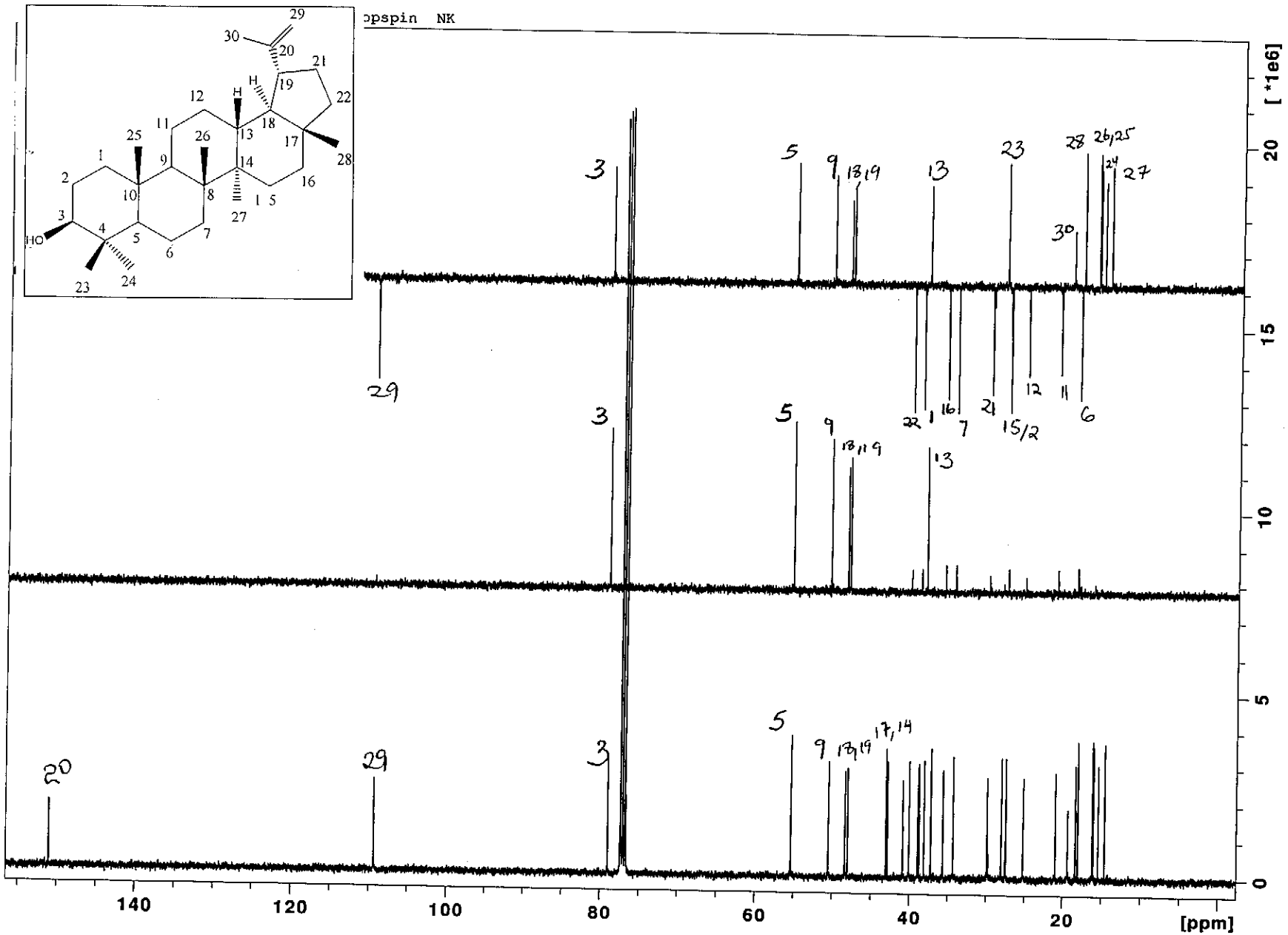
^{1}H NMR spectrum of A5 expanded (0-3.5 ppm)



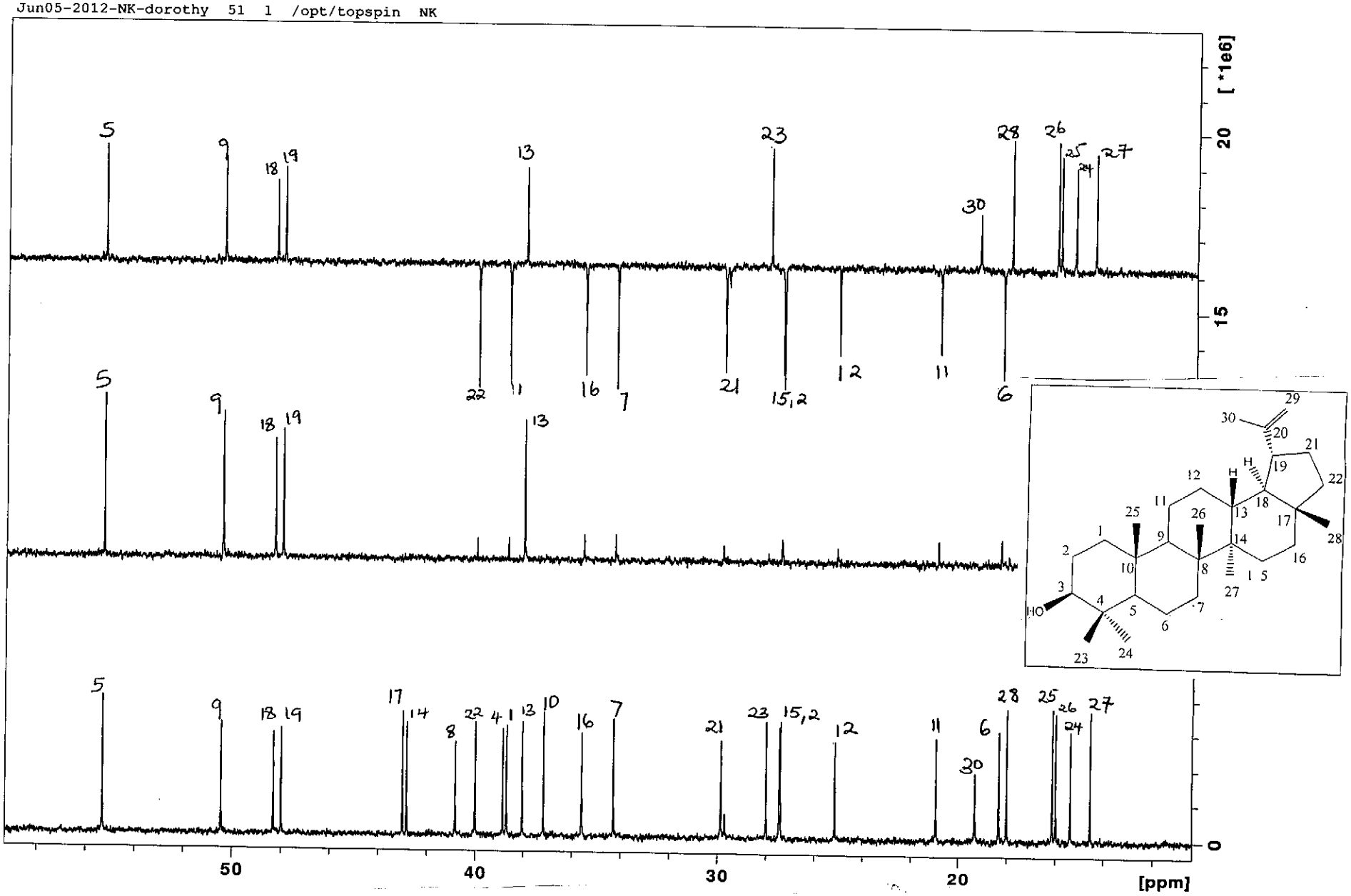
^{13}C NMR spectrum of A5



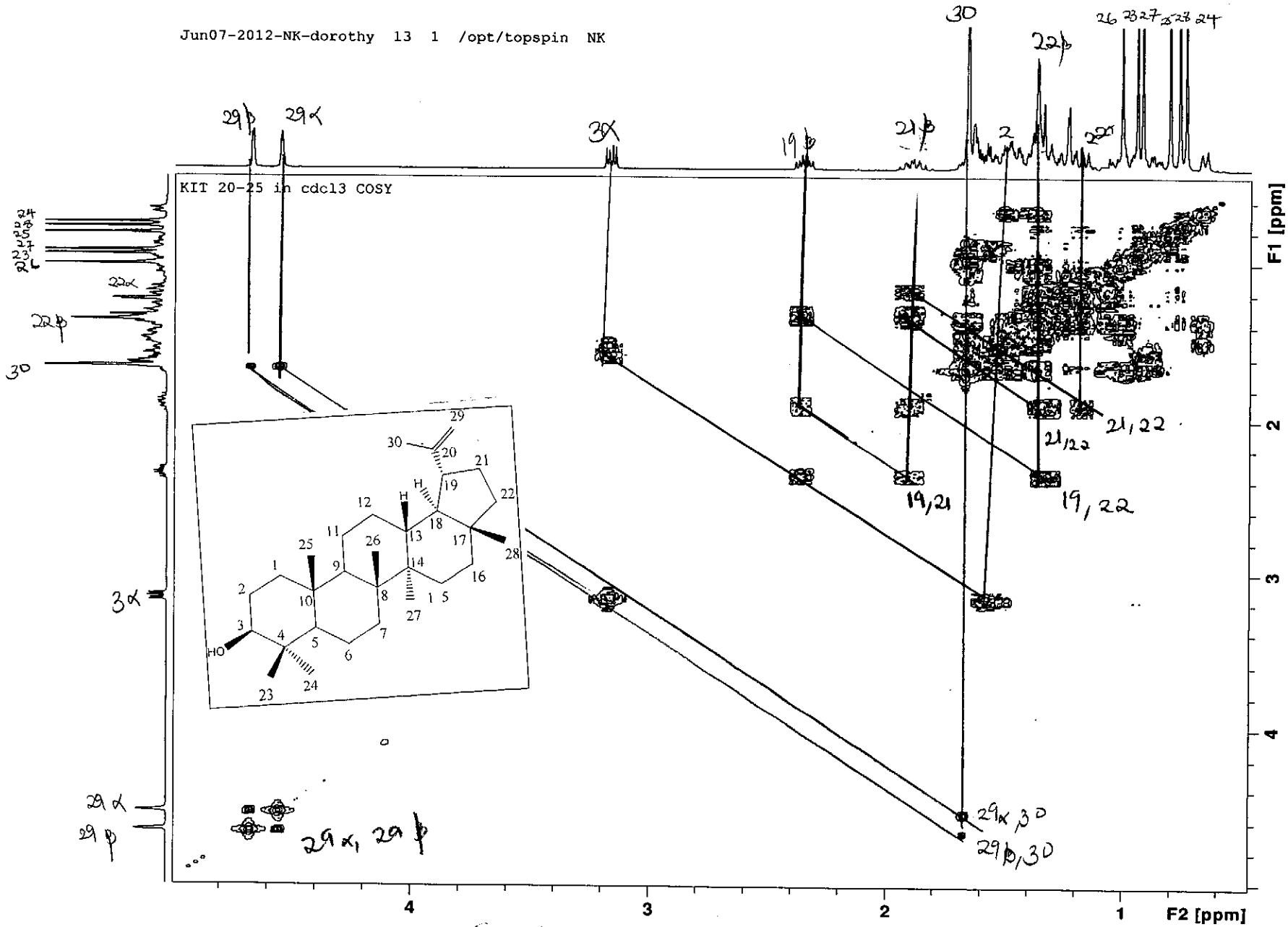
^{13}C NMR spectrum of A5 expanded (0-60 ppm)



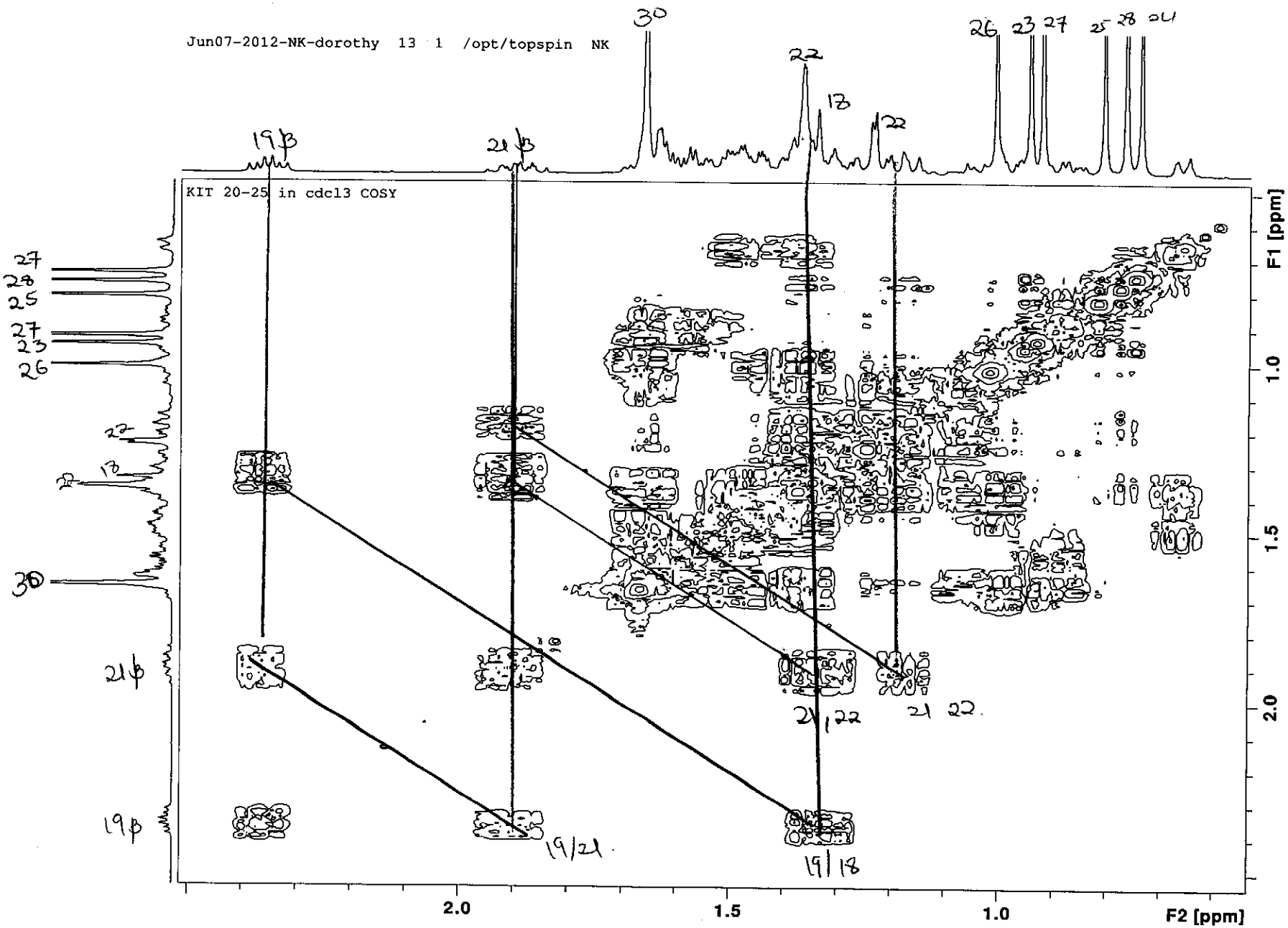
DEPT spectrum of A5



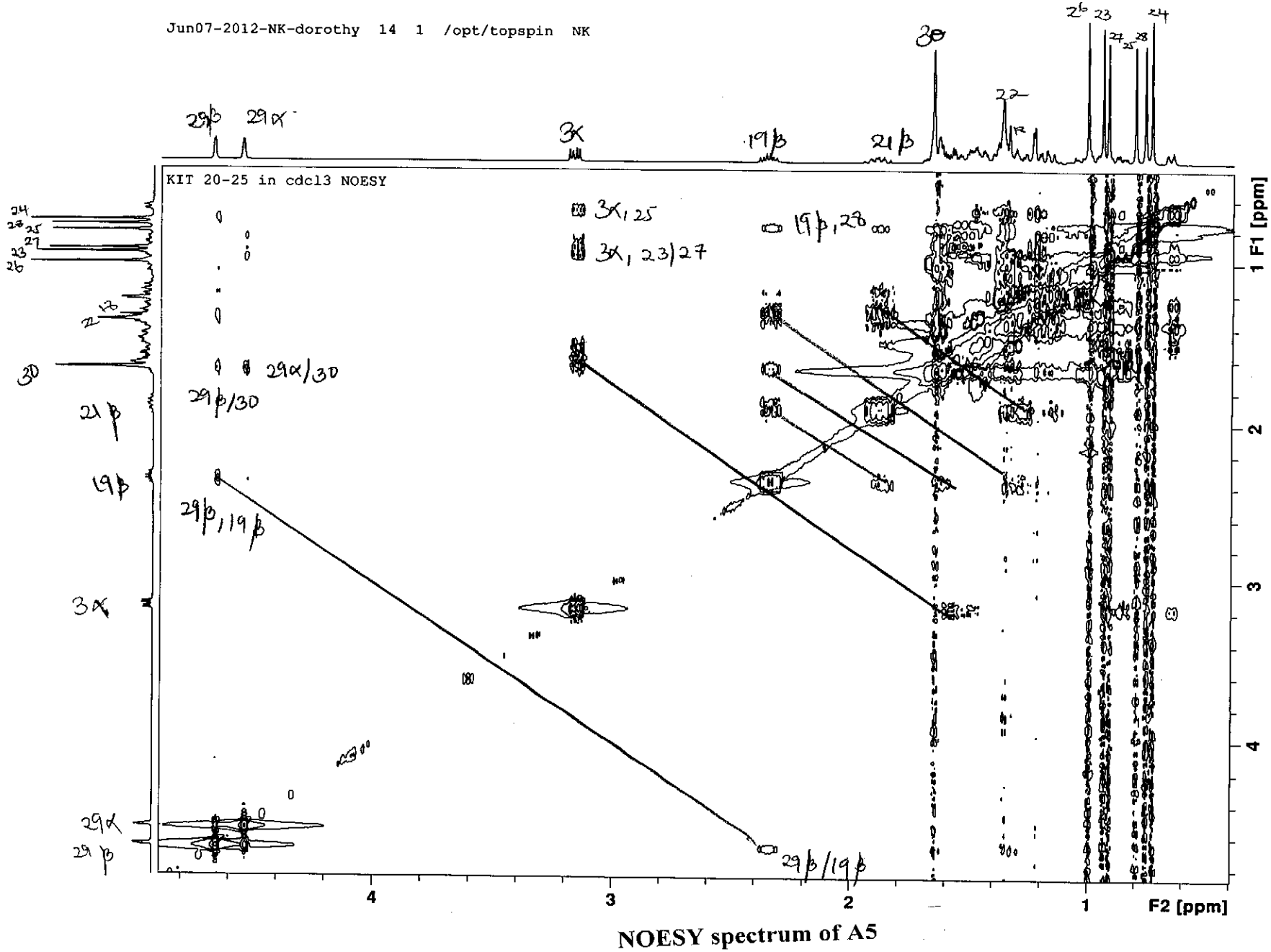
DEPT spectrum of A5 expanded (0-60 ppm)



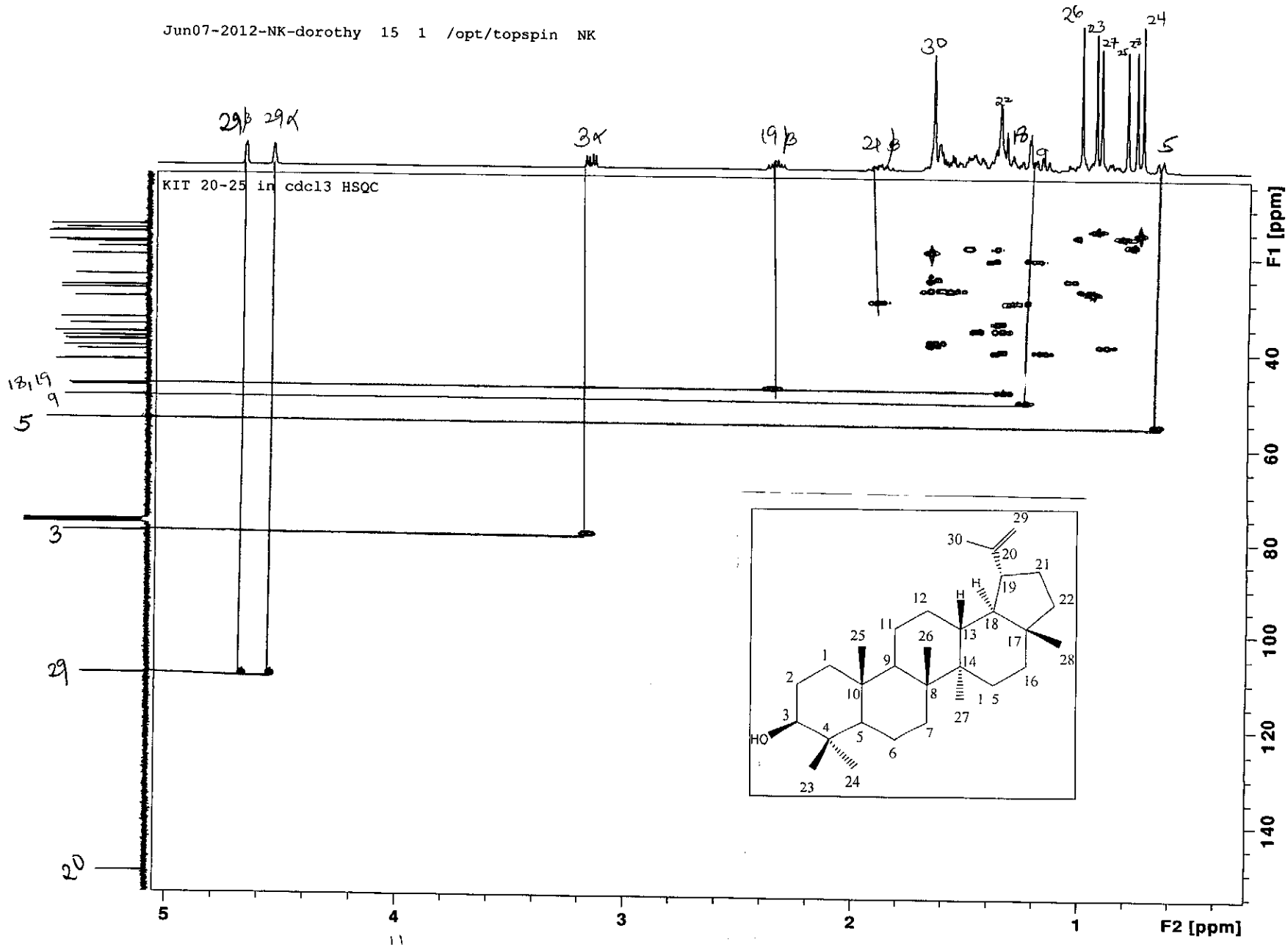
COSY spectrum of A5



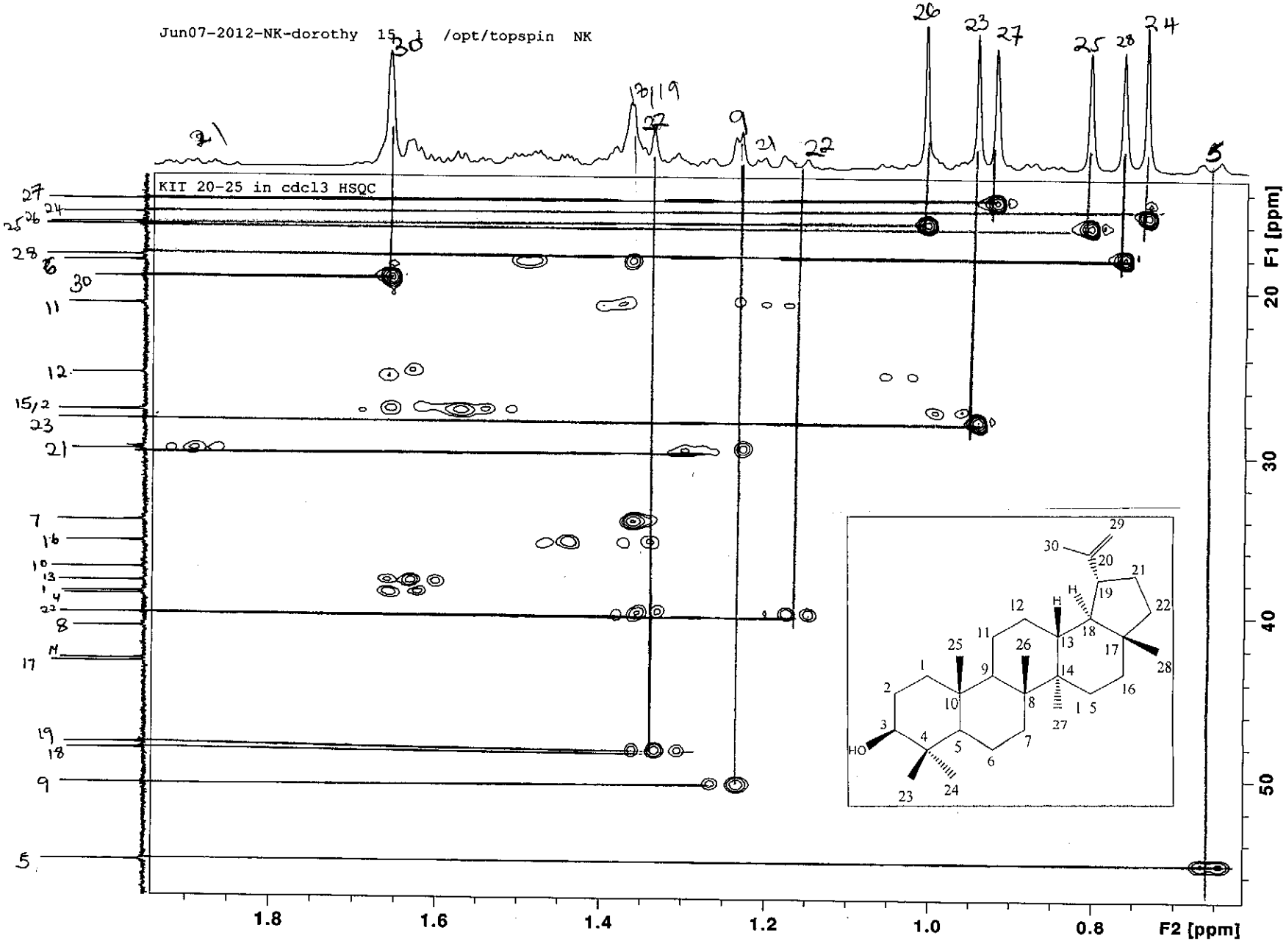
COSY spectrum of A5 expanded (0-2.5 ppm)



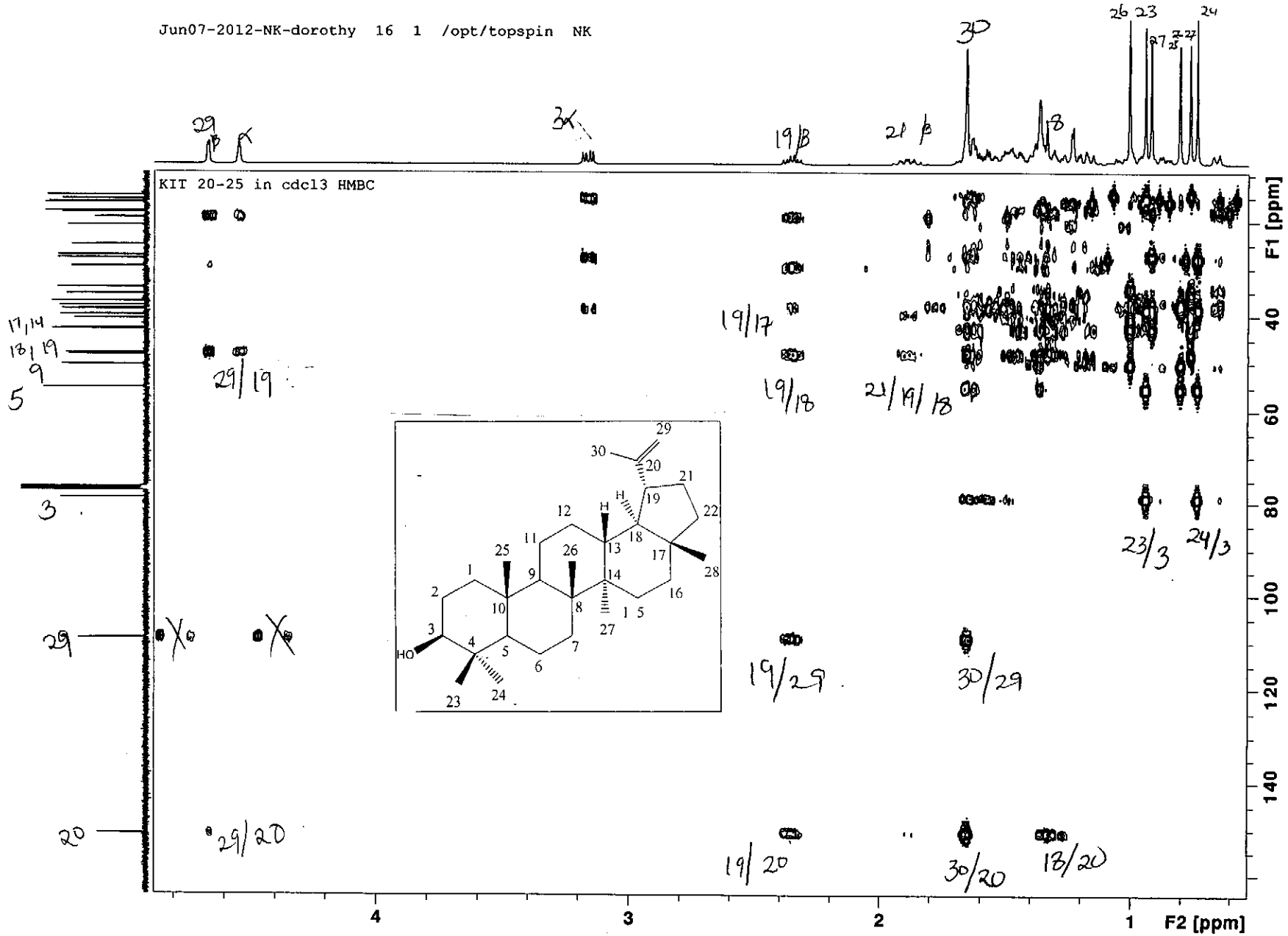
NOESY spectrum of A5



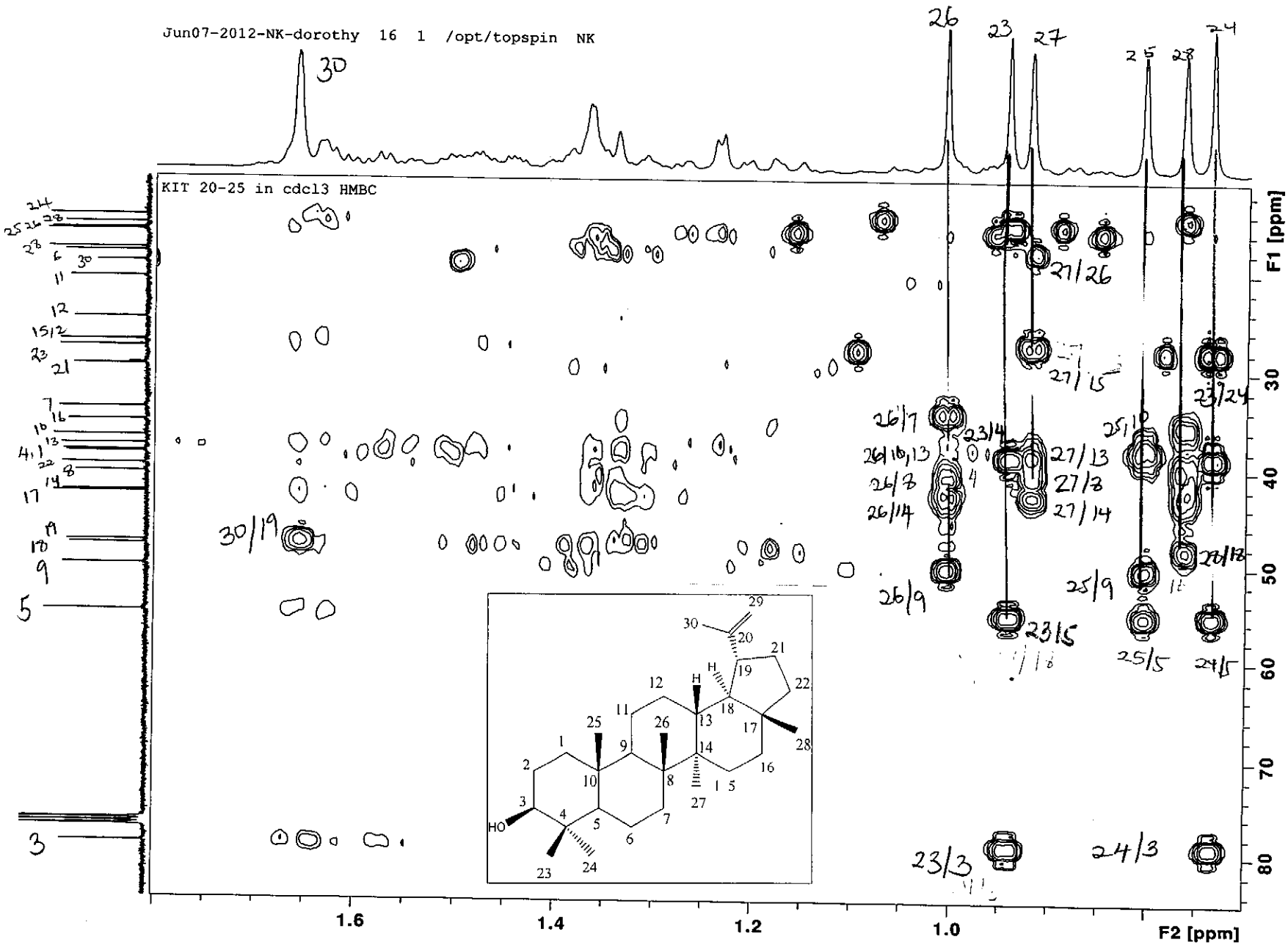
HSQC spectrum of A5



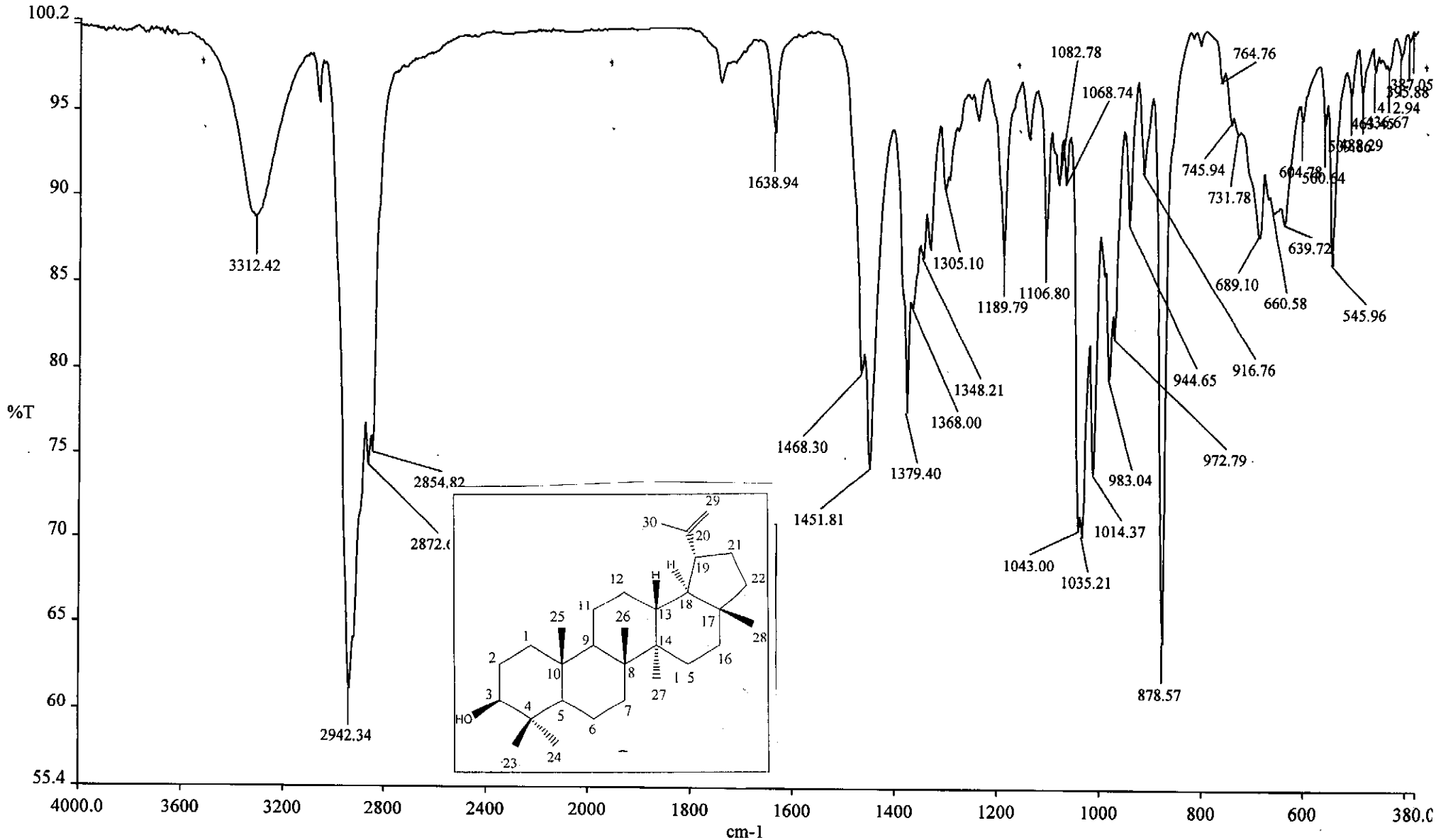
HSQC spectrum of A5 expanded (F1, 0-60 ppm, F2, 0-2 ppm)



HMBC spectrum of A5



HMBC spectrum of A5 expanded (F1, 0-85 ppm, F2, 0-2 ppm)


100.2
95
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70
65
60
55.4
%T
4000.0
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
380.0
cm-1
3312.42
2942.34
2854.82
2872.6
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1468.30
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1379.40
1368.00
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1082.78
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983.04
972.79
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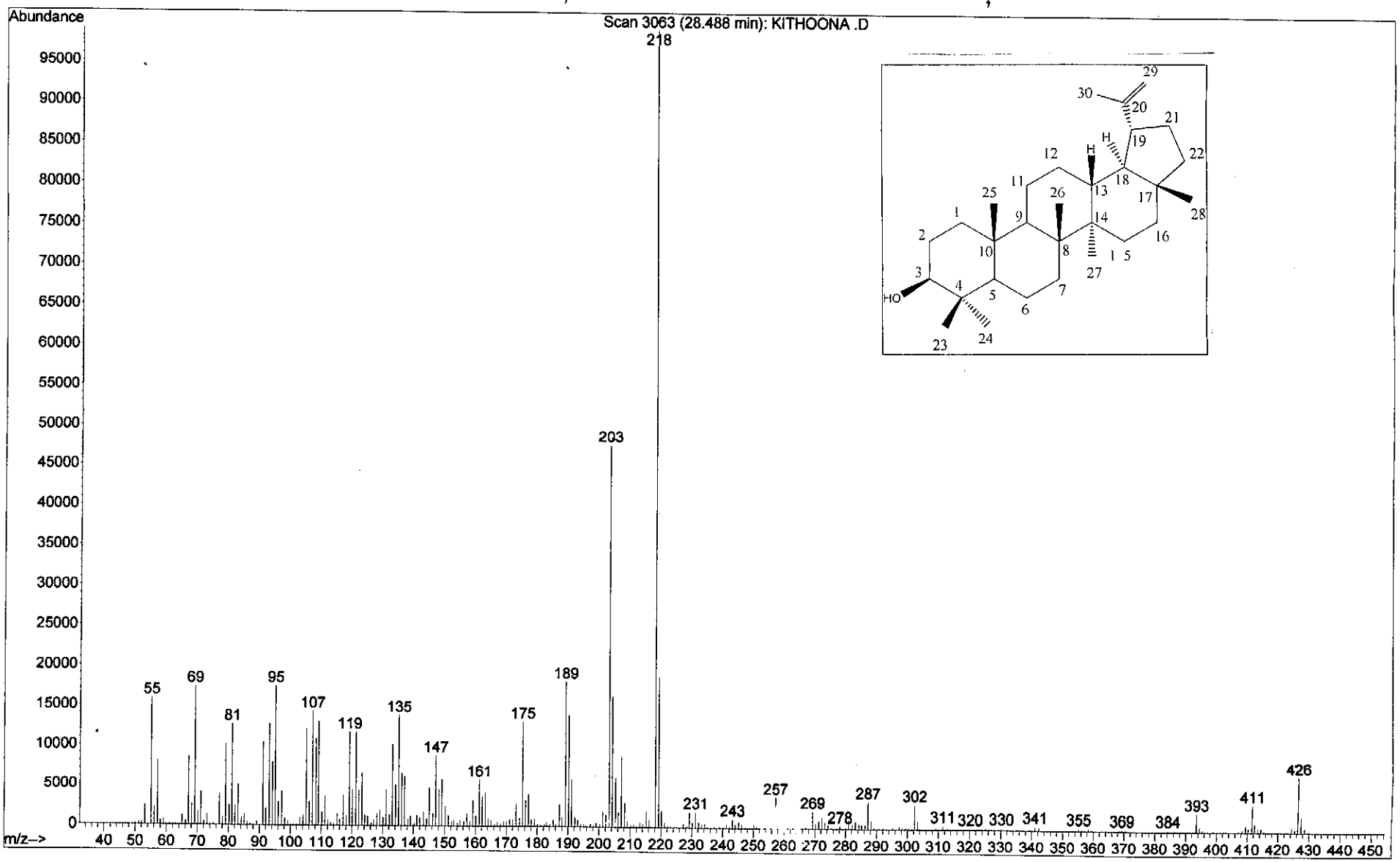
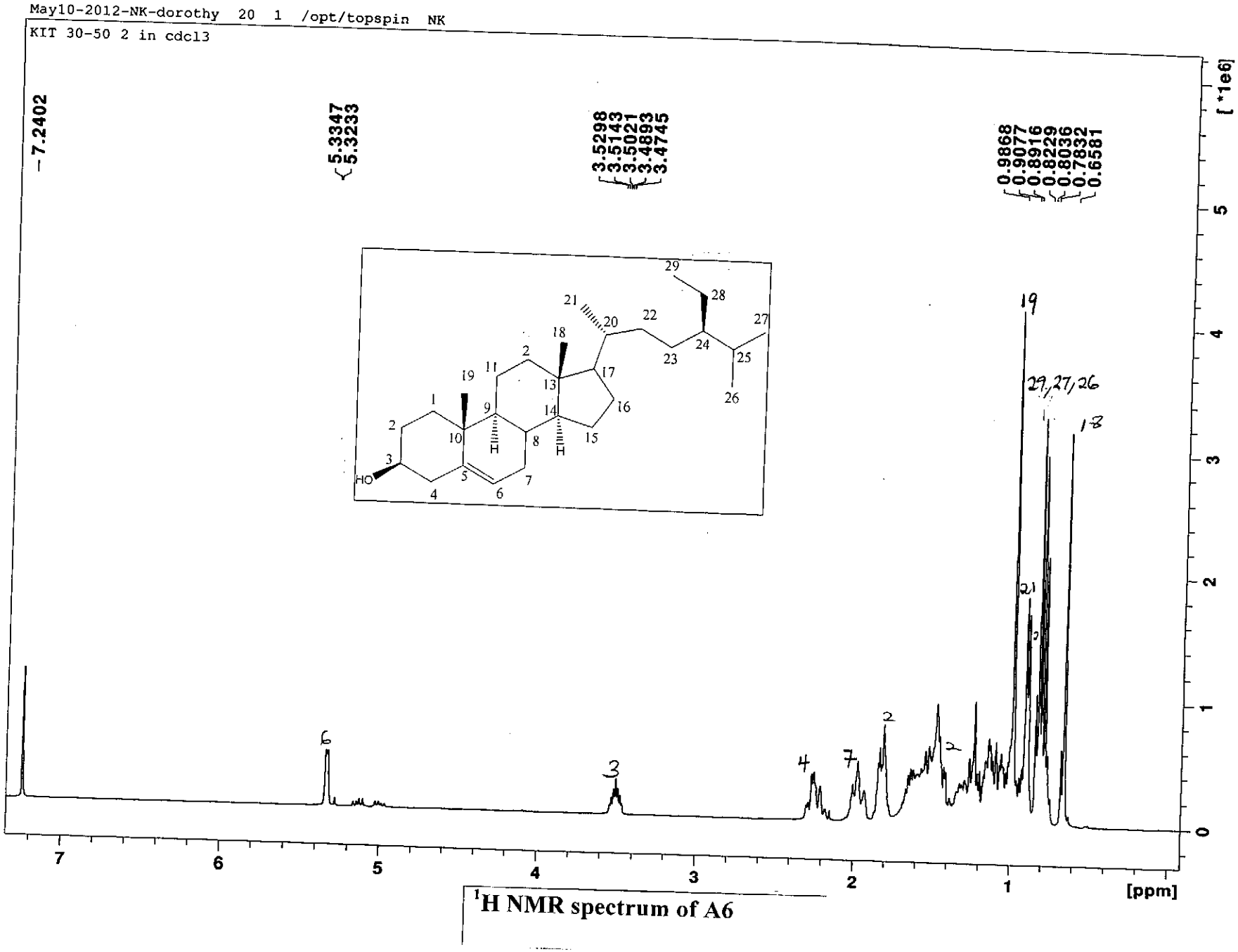
745.94
731.78
689.10
660.58
639.72
604.78
560.54
545.96
HO

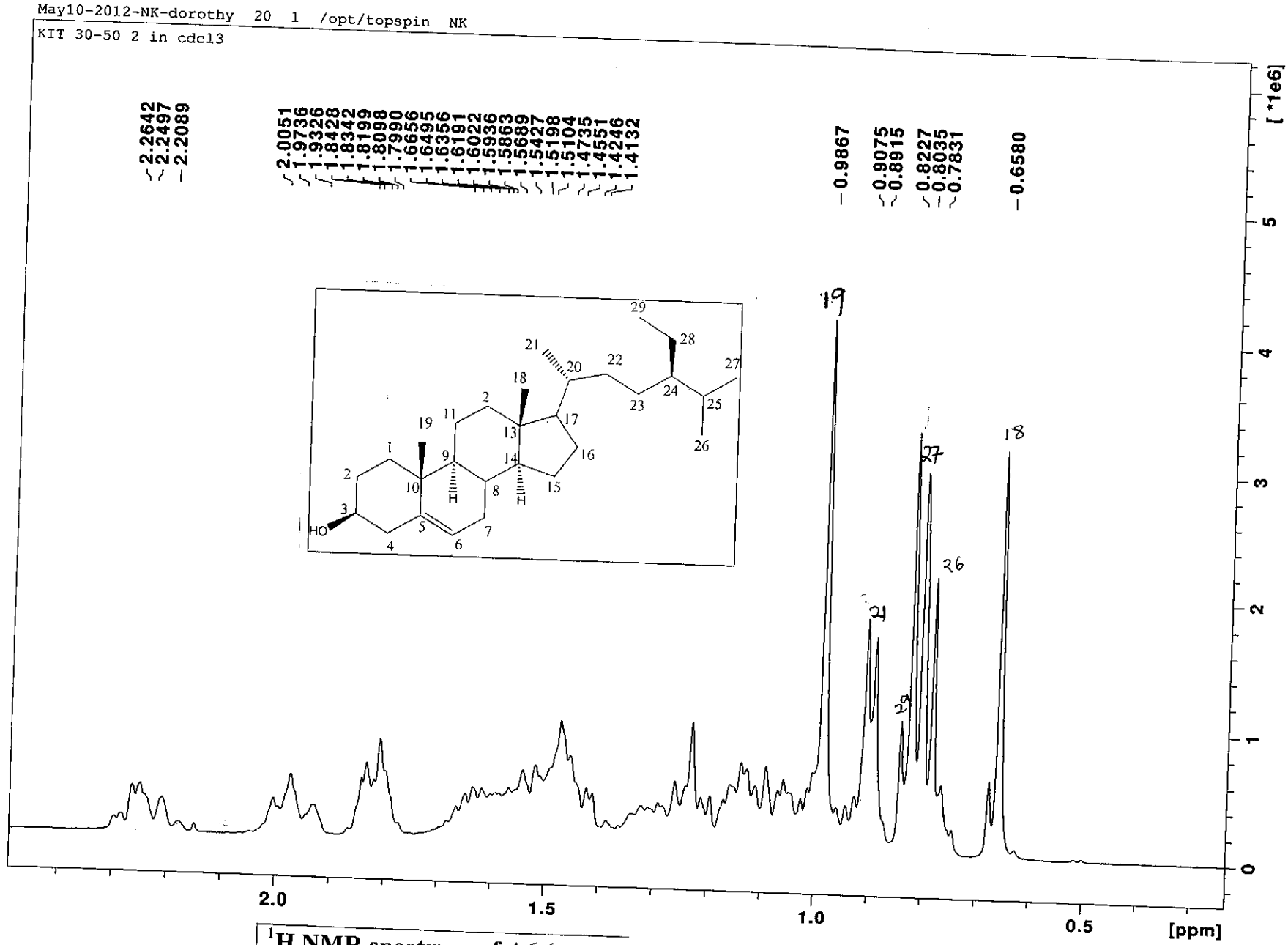
File : C:\MSDCHEM\1\DATA\DOROTHY RAW DATA KITHOKIT\KITHOONA .D
Operator : Dorothy
Acquired : 5 May 2012 18:53 using AcqMethod NATPRODUCTS MANUAL
Instrument : 5973N
Sample Name: kithoona 1-6 (3,4)
Misc Info :
Vial Number: 1



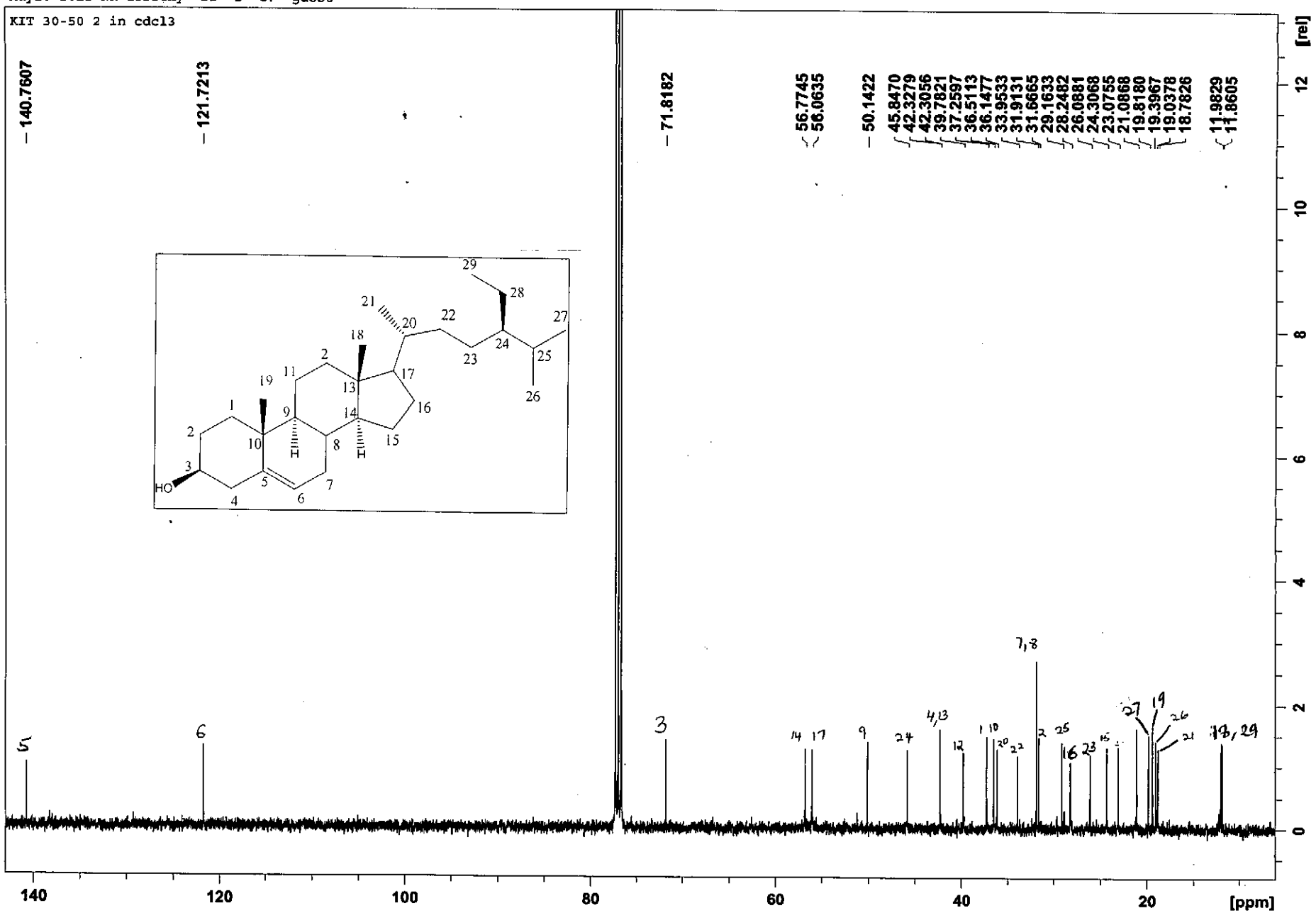
MS spectrum of A5



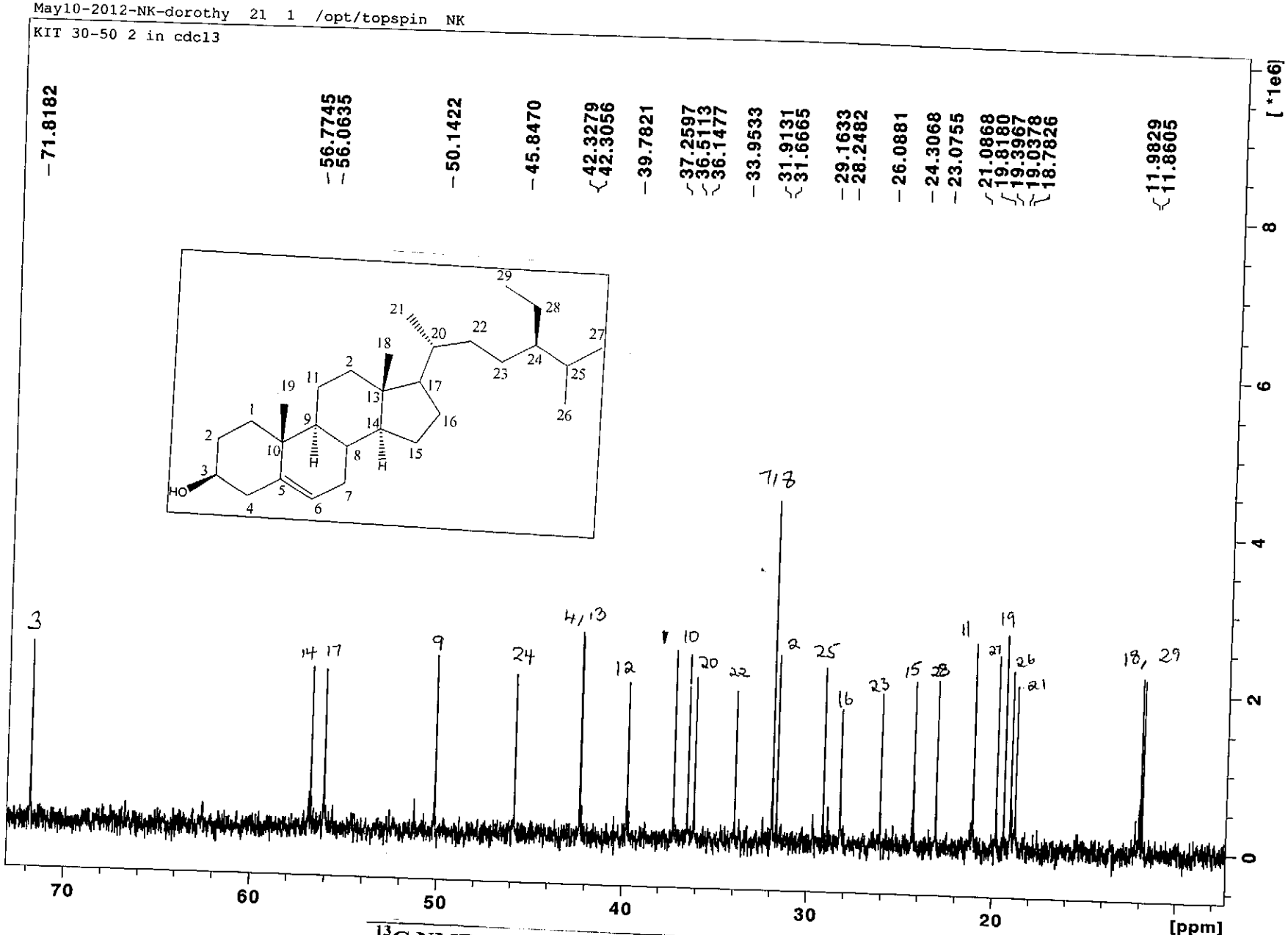
^{1}H NMR spectrum of A6



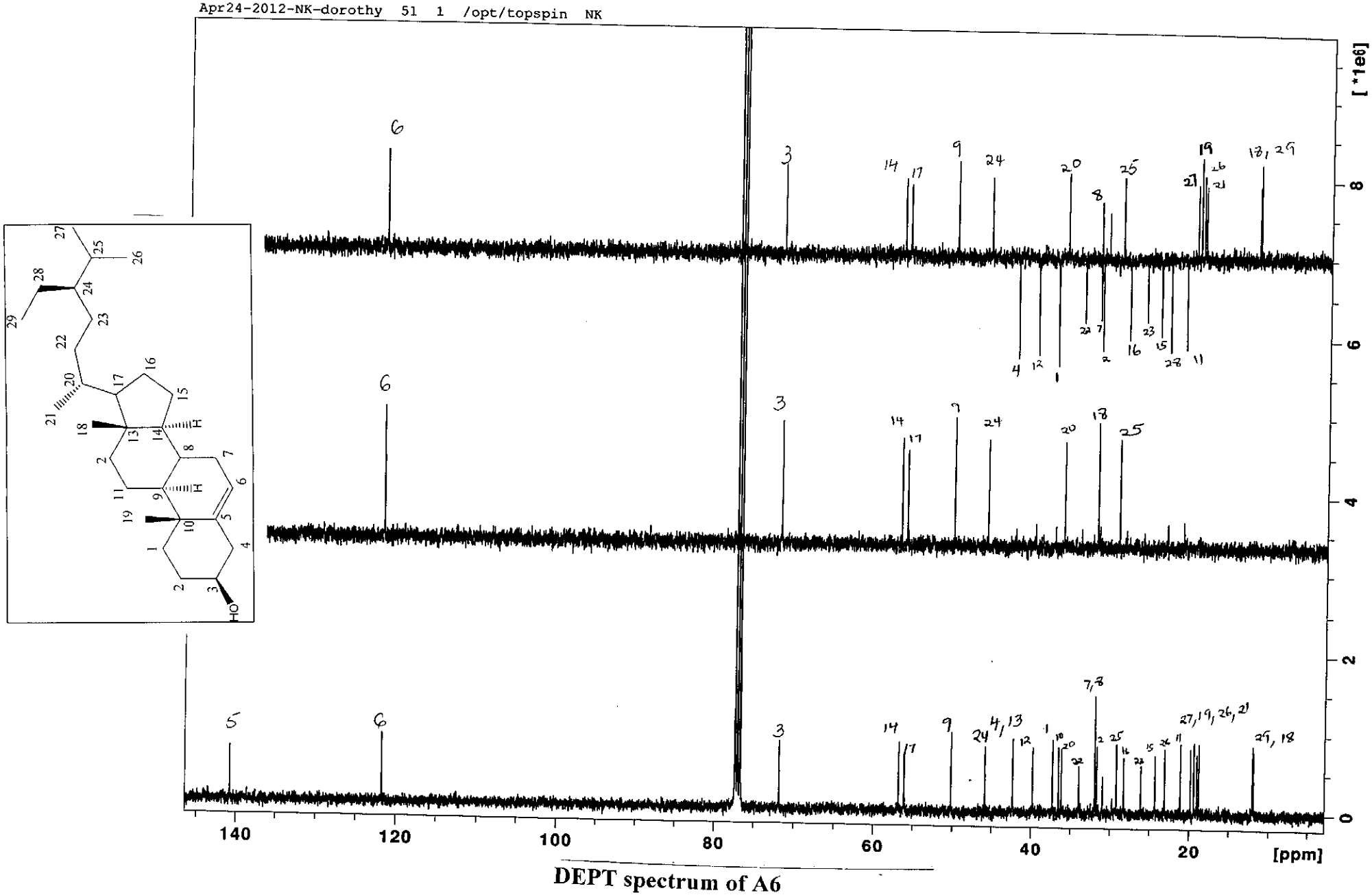
^{1}H NMR spectrum of A6 (expanded 0-2.5 ppm)



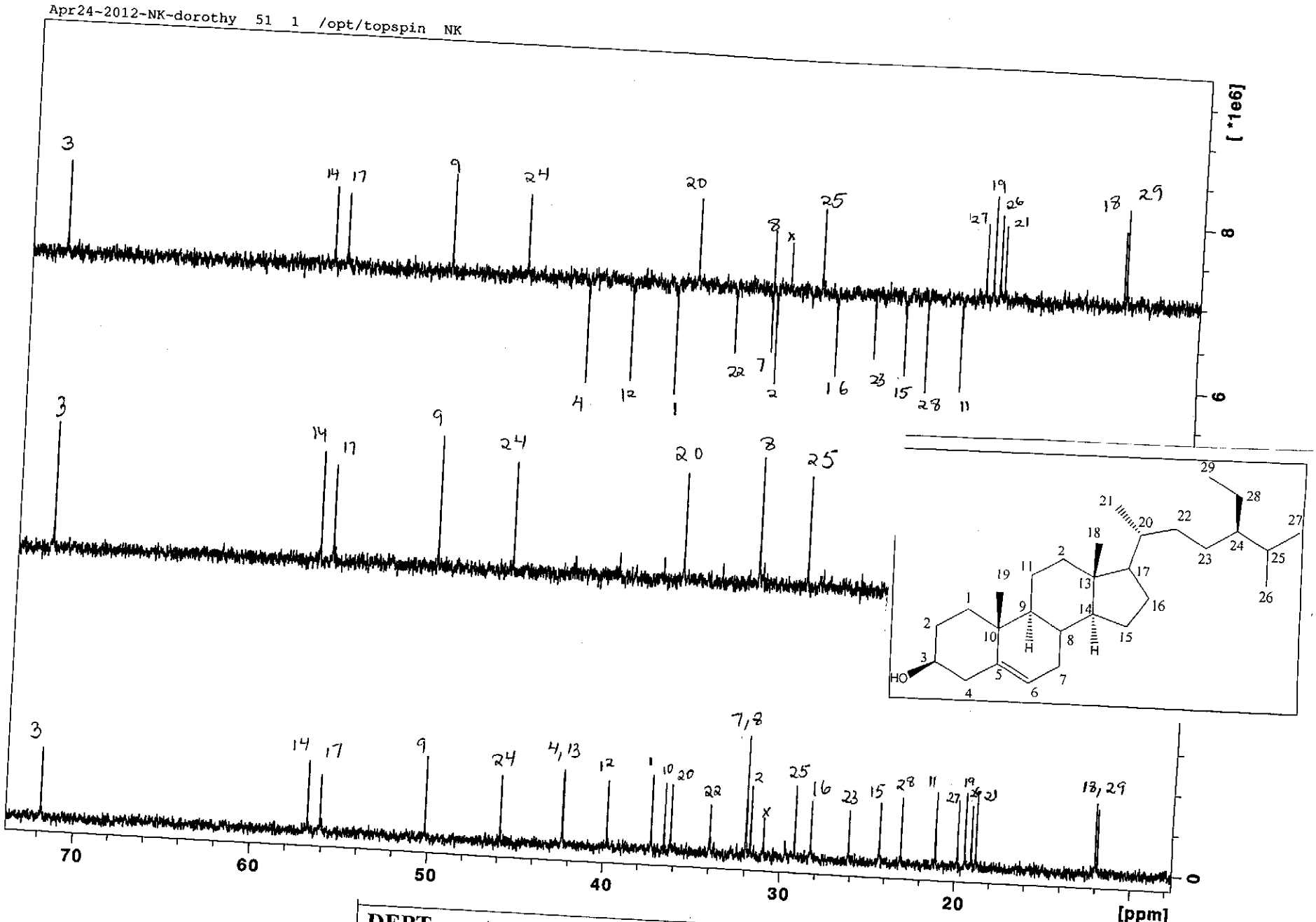
^{13}C NMR spectrum of A6



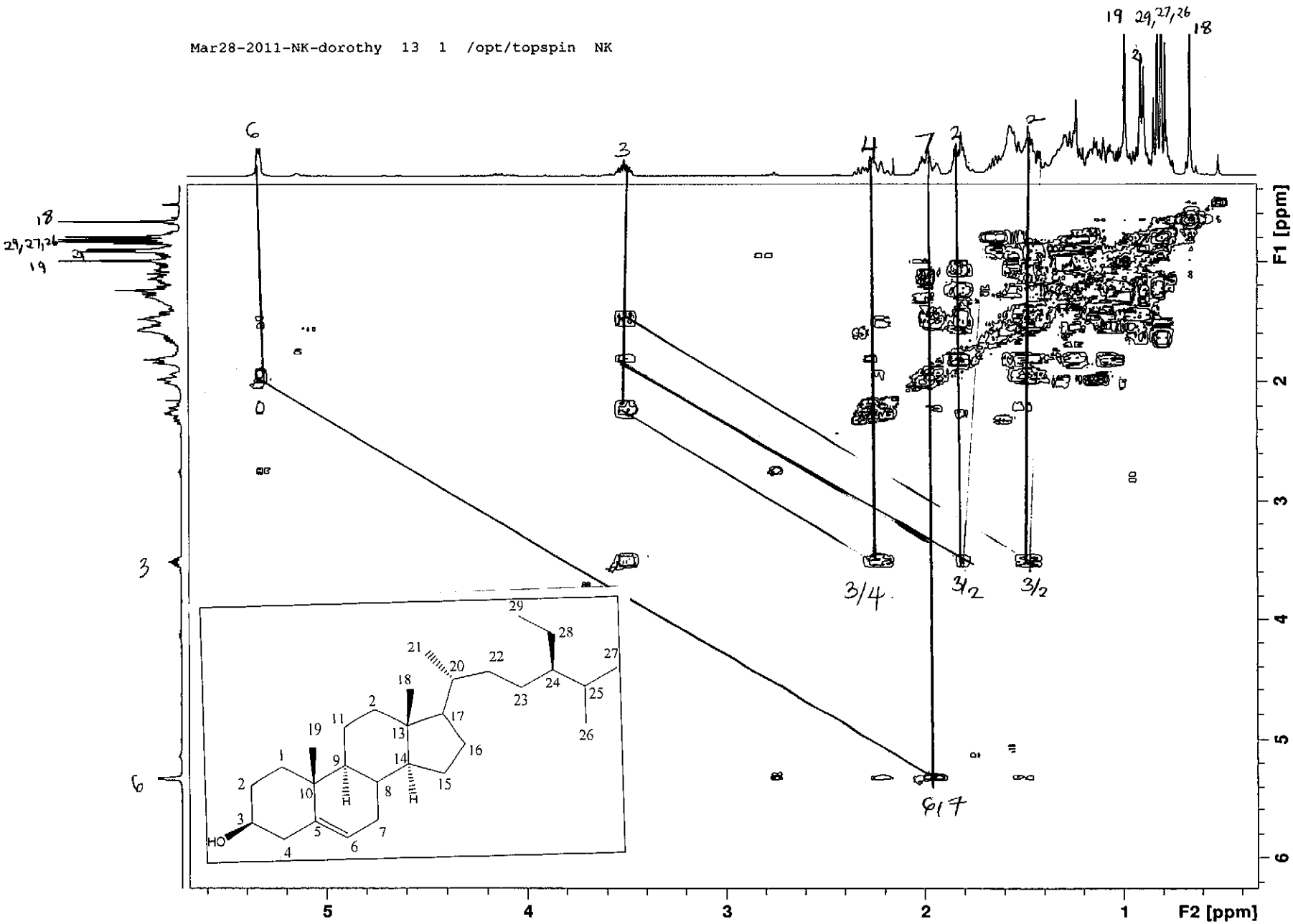
^{13}C NMR spectrum of A6 (expanded 0–72 ppm)



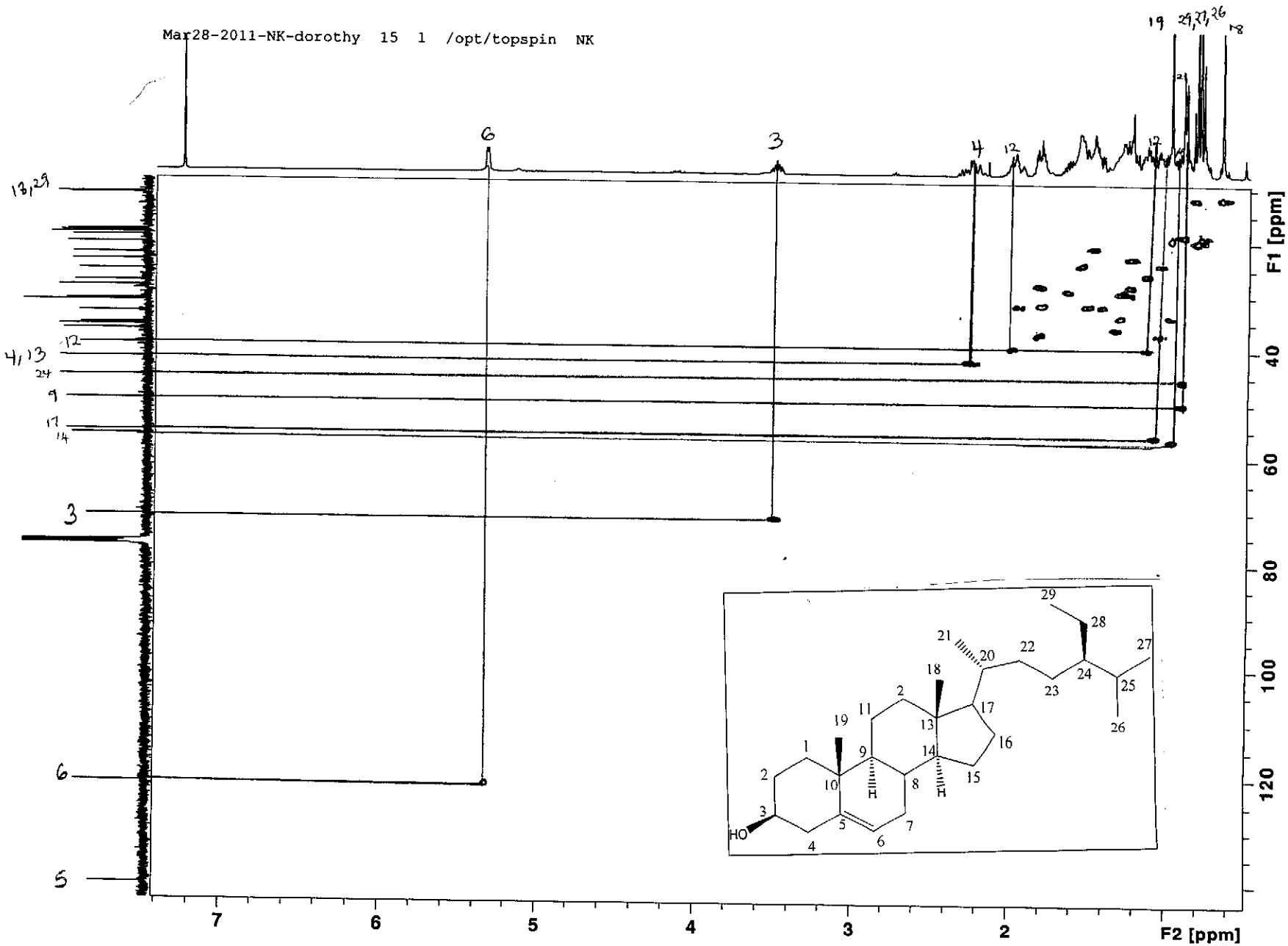
DEPT spectrum of A6



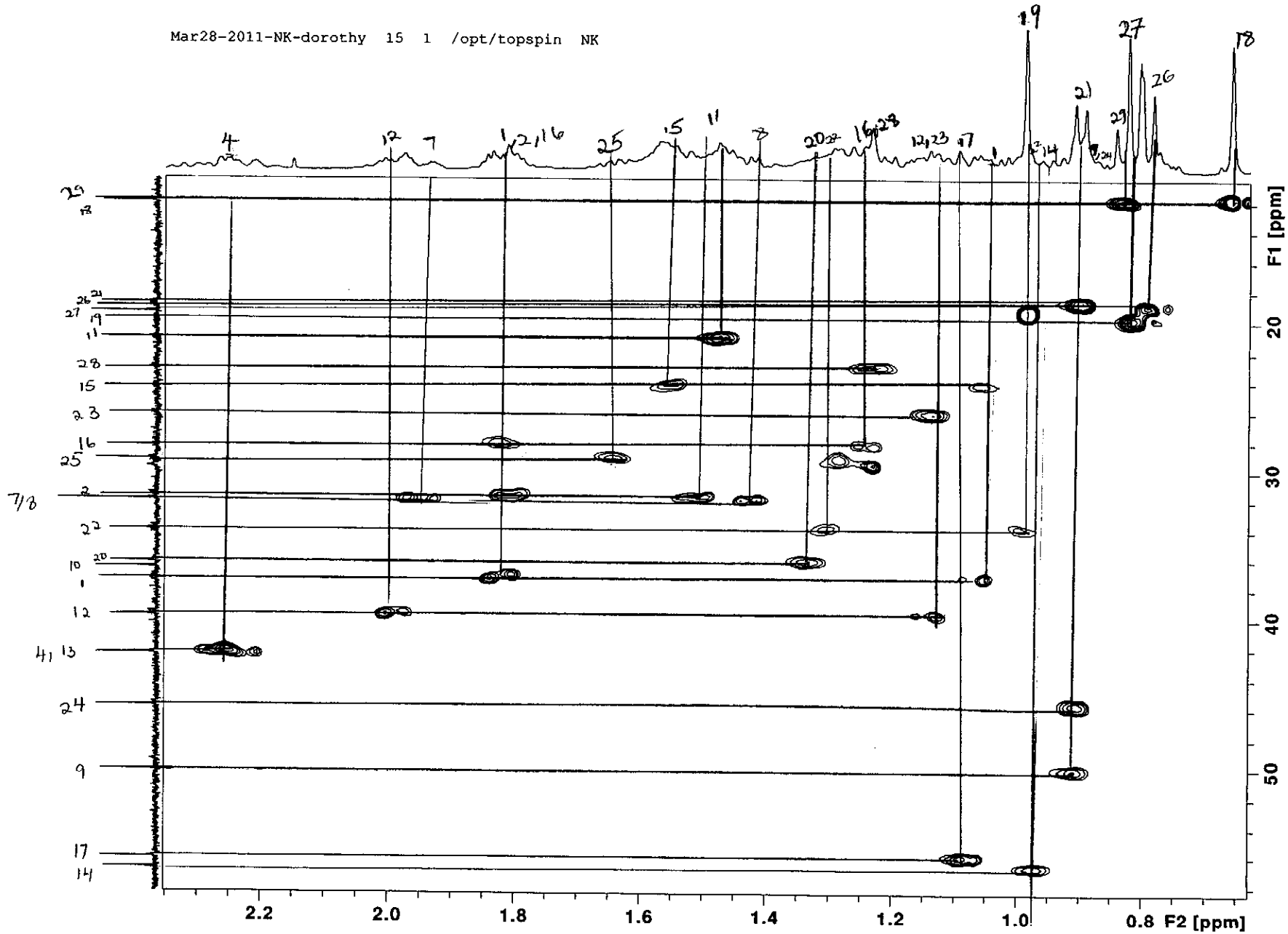
DEPT spectrum of A6 (expanded 0-72 ppm)



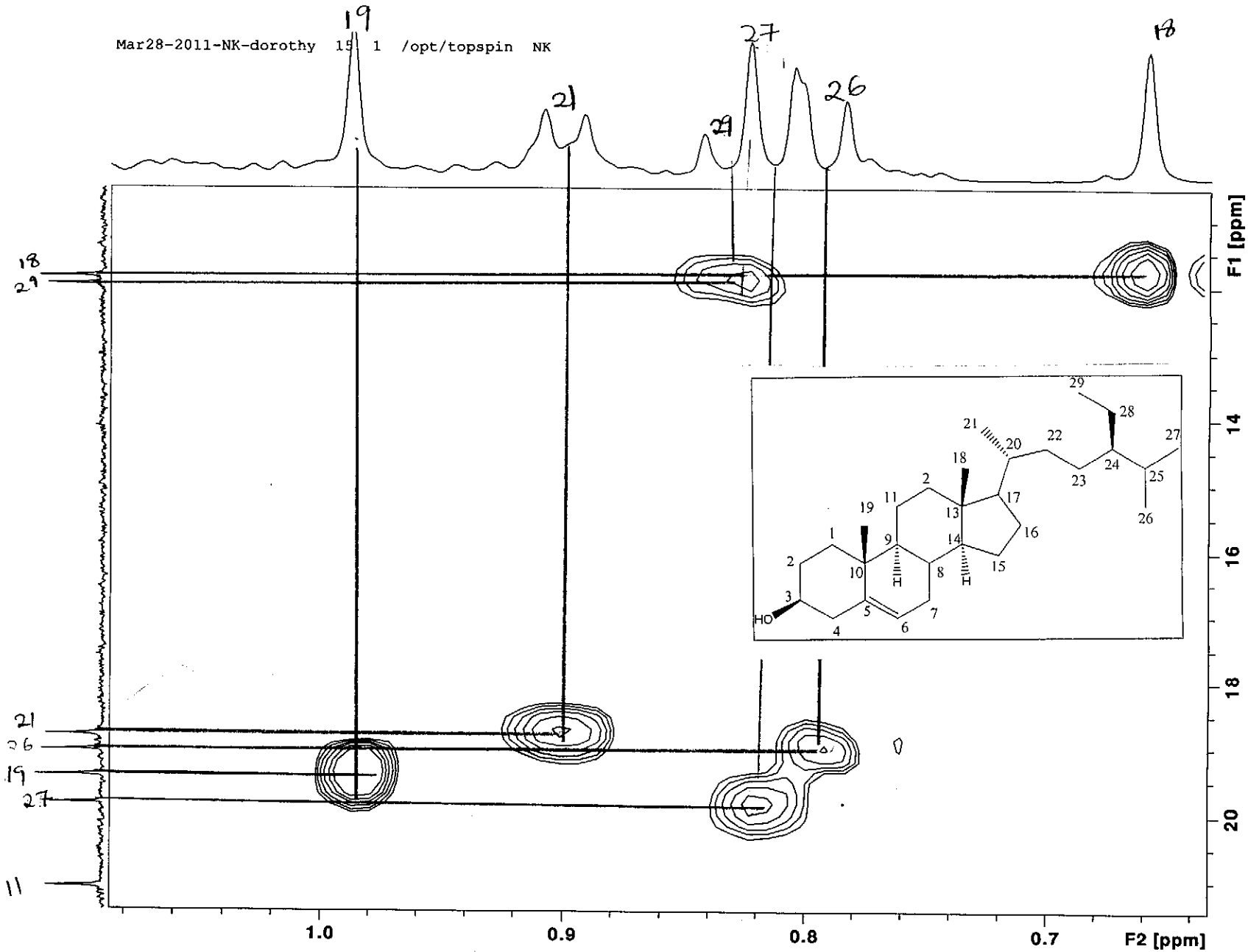
COSY spectrum of A6



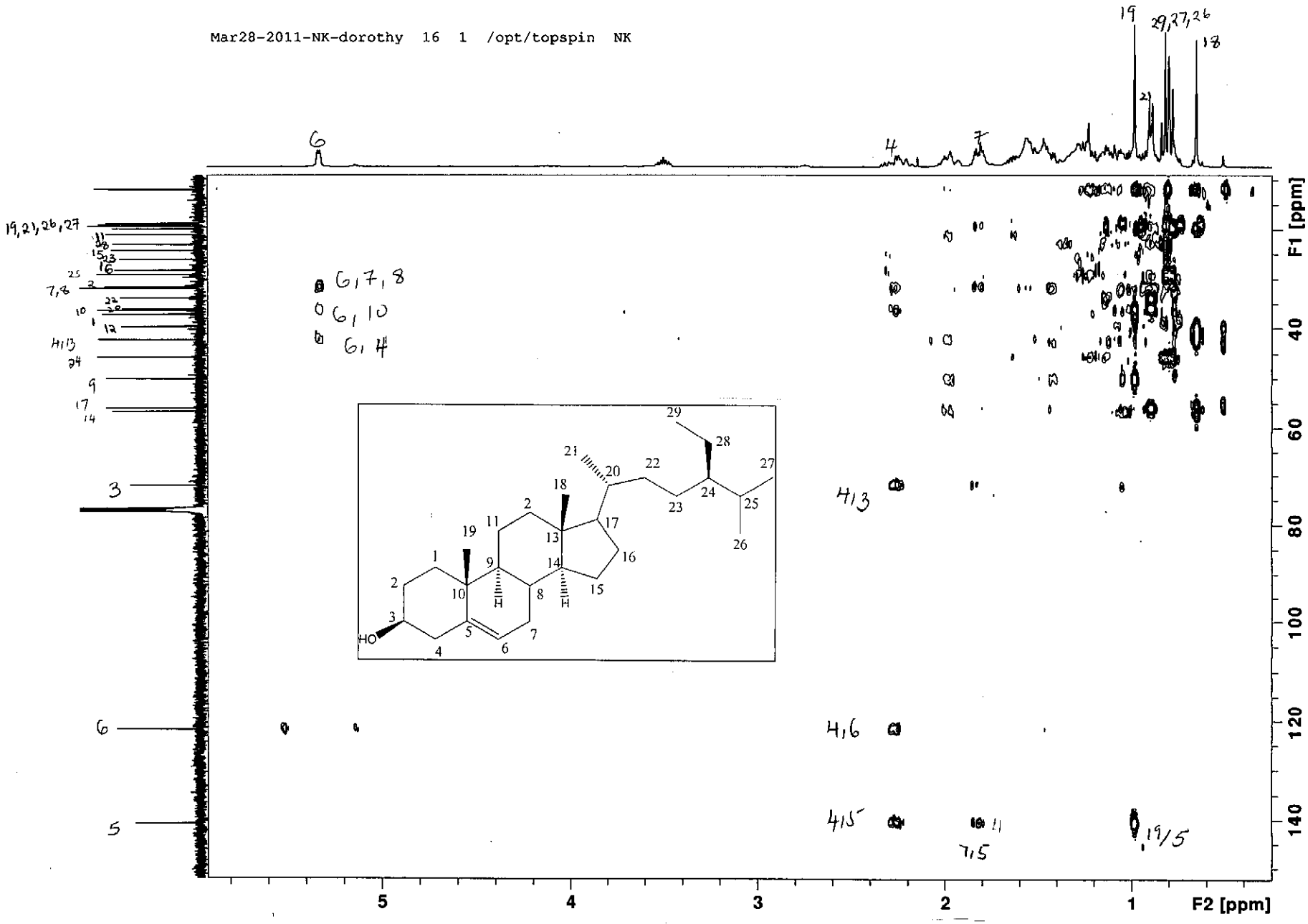
HSQC spectrum of A6



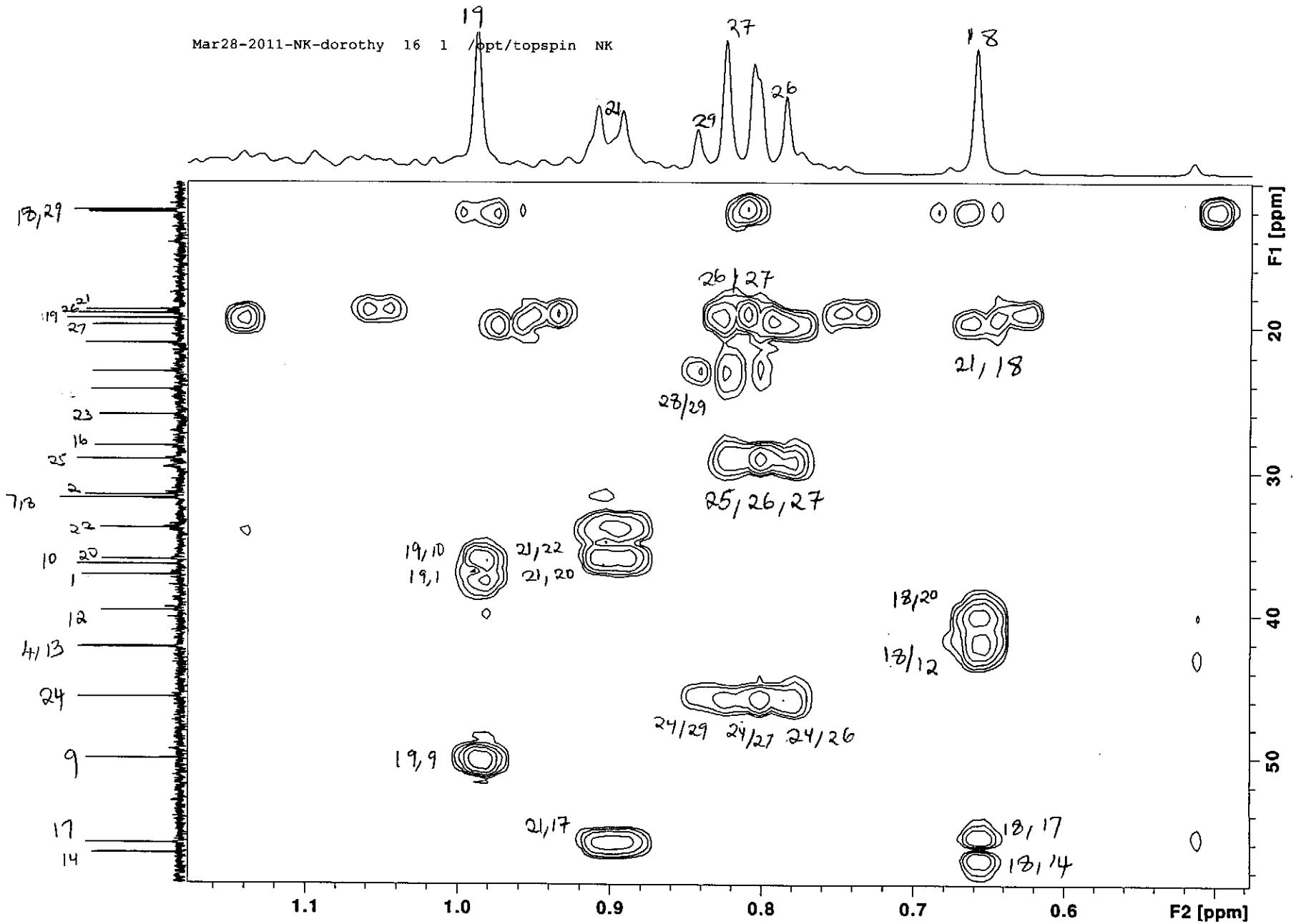
HSQC spectrum of A6 (expanded 0-57 ppm)



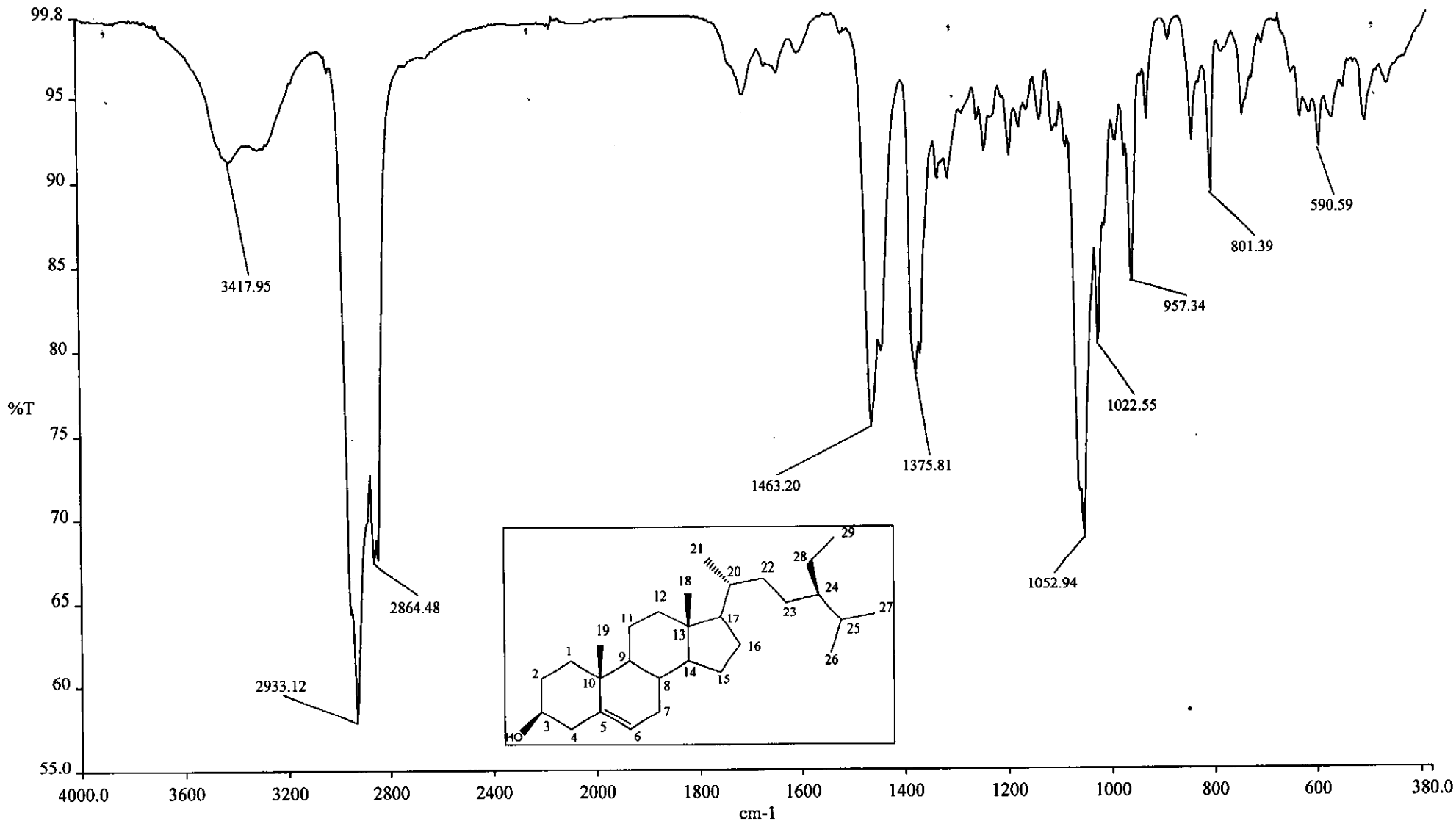
HSQC spectrum of A6 (expanded 0-25 ppm)



HMBC spectrum of A6



HMBC spectrum of A6 (expanded 0-57 ppm)

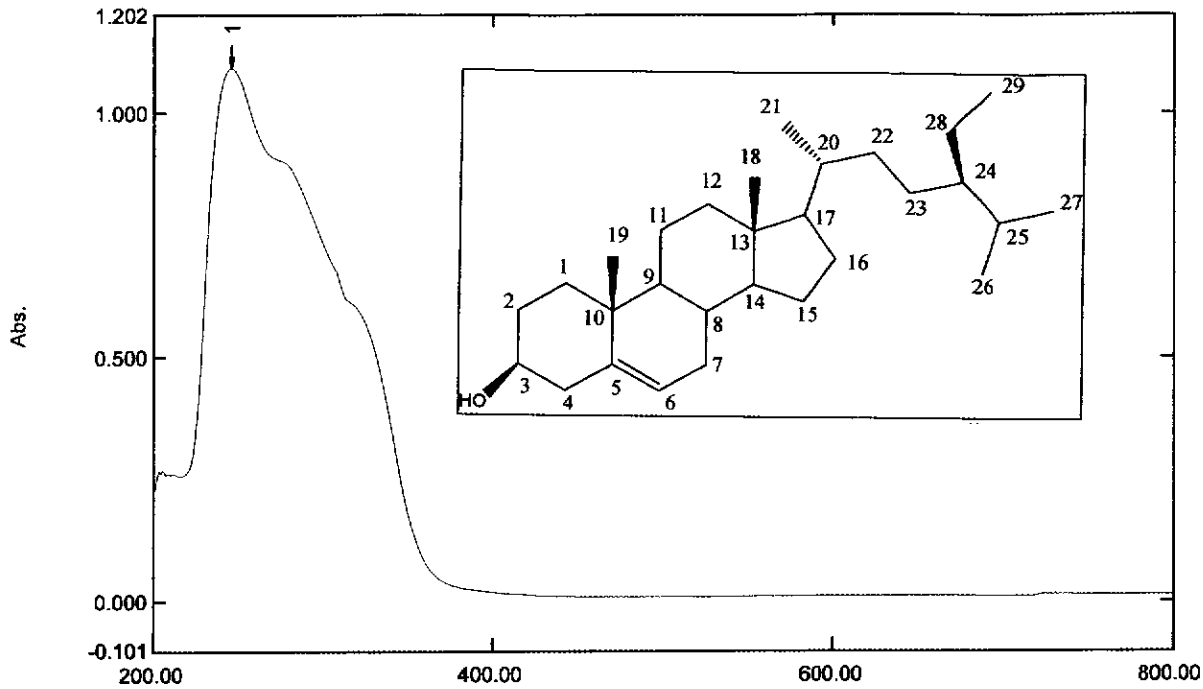


IR spectrum of A6

Spectrum Peak Pick Report

04/10/2011 12:11:36 PM

Data Set: LSS 120-150.spc - Storage 143827



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Measurement Properties
Wavelength Range (nm.): 200.00 to 800.00
Scan Speed: Medium
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Auto Sampling Interval: Disabled
Scan Mode: Single

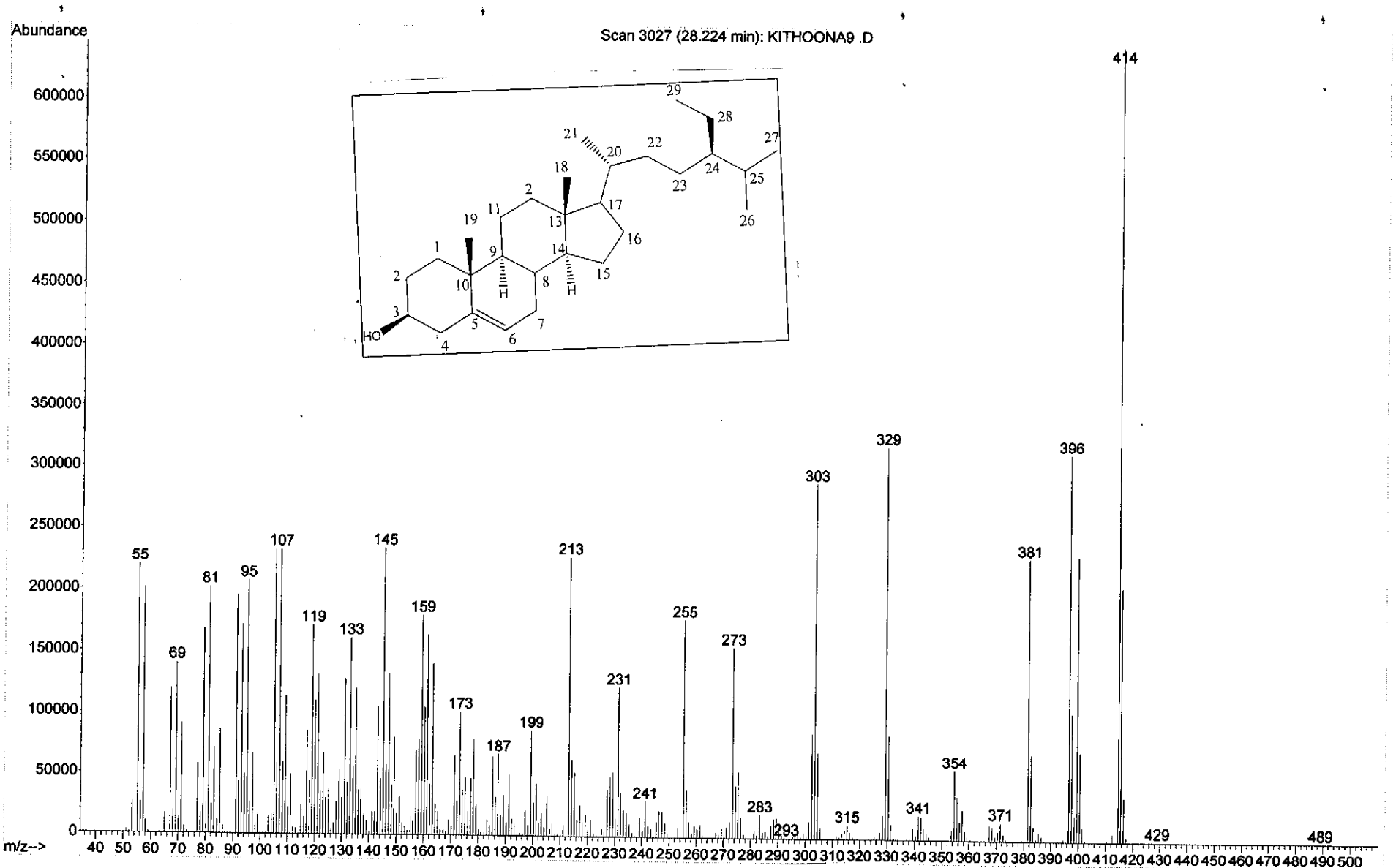
Instrument Properties
Instrument Type: UV-3600 Series
Measuring Mode: Absorbance
Slit Width: 8.0 nm
Time Constant: 0.1 sec.
Source Lamp: Auto
Light Source Change Wavelength: 310.00 nm
Detector Unit: Direct
Detector Change Wavelength: 830.00 nm 1800.00 nm
Grating Change Wavelength: 720.00 nm
S/R Exchange: Normal
Detector Lock: Auto
Slit Program: Normal
Beam Mode: Double
Stair Correction: Disable

Attachment Properties
Attachment: None

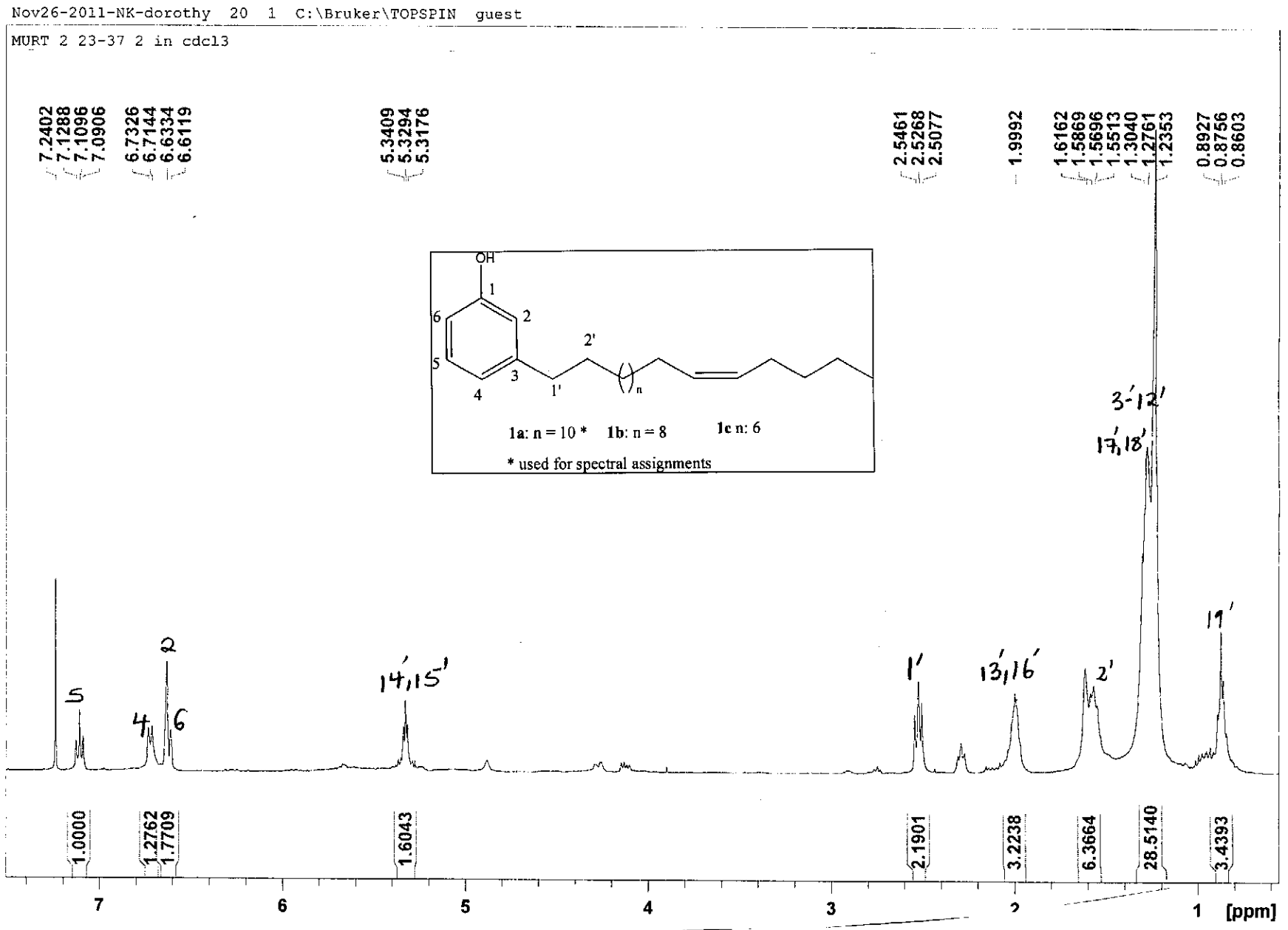
Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

UV spectrum of A6

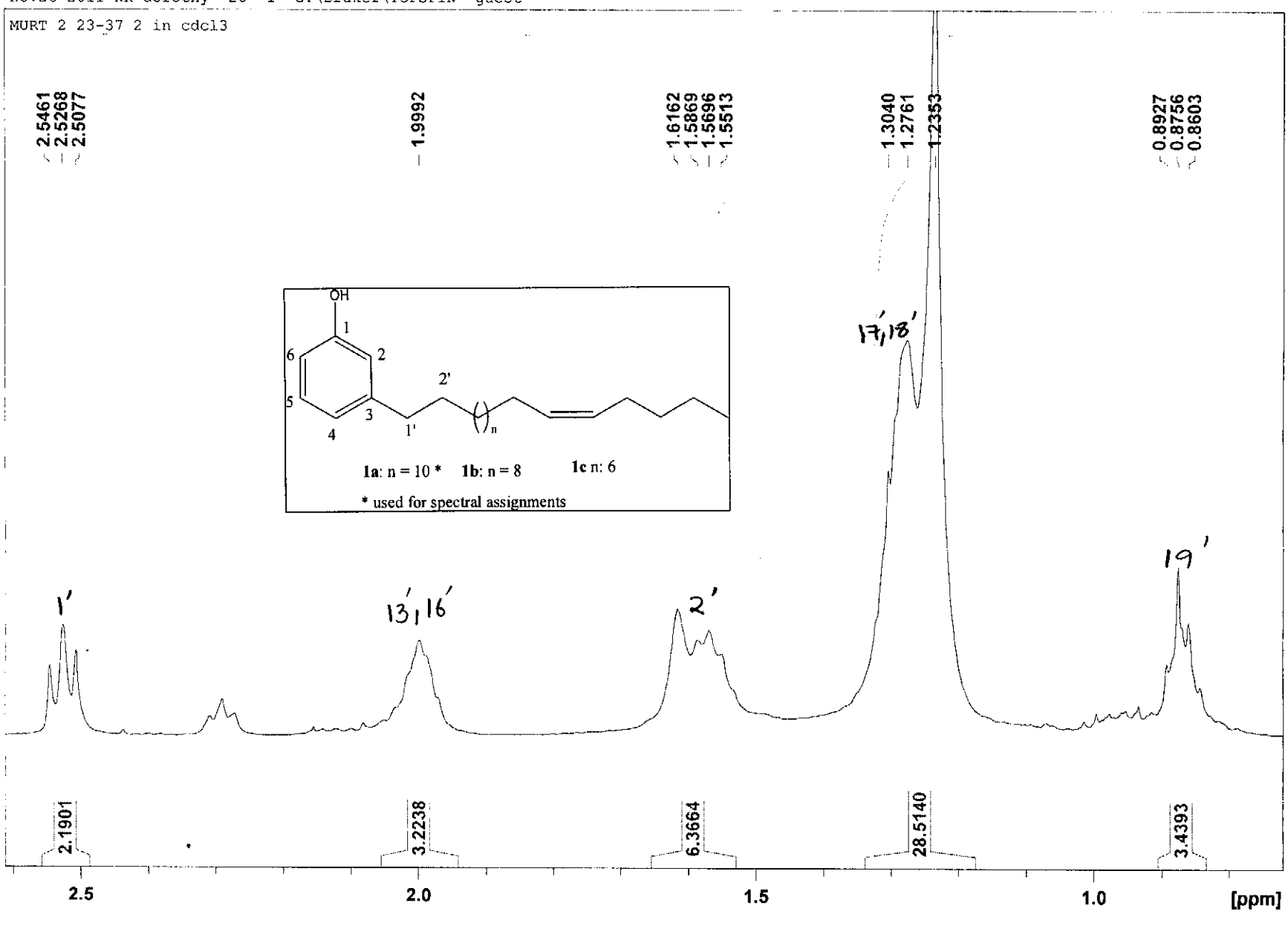
File : C:\MSDCHEM\1\DATA\DOROTHY RAW DATA KITHOKIT\KITHOONA9 .D
Operator : Dorothy
Acquired : 6 May 2012 1:36 using AcqMethod NATPRODUCTS MANUAL
Instrument : 5973N
Sample Name: kithoona 12-13 6-16
Misc Info :
Vial Number: 1



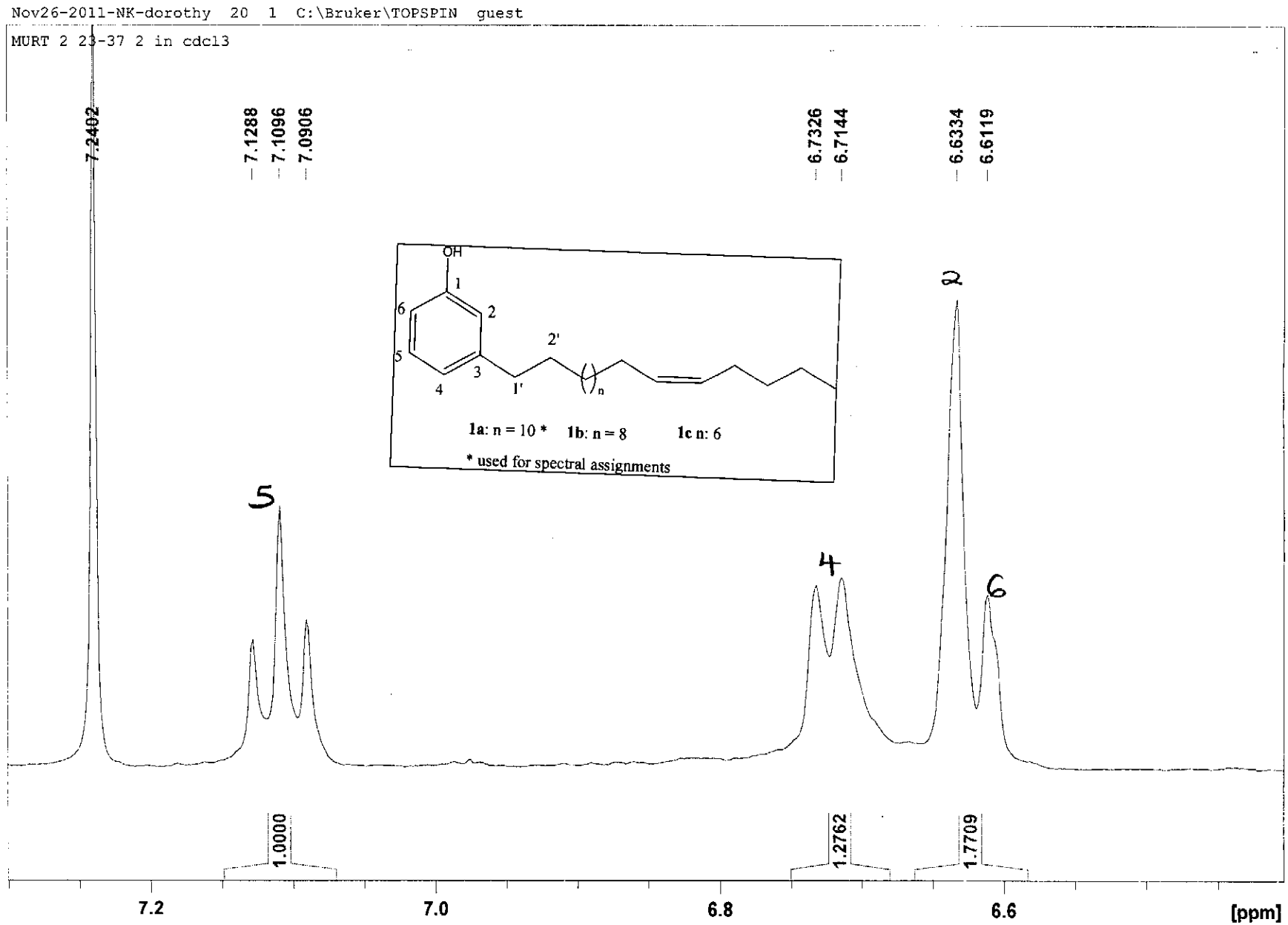
MS spectrum of A6



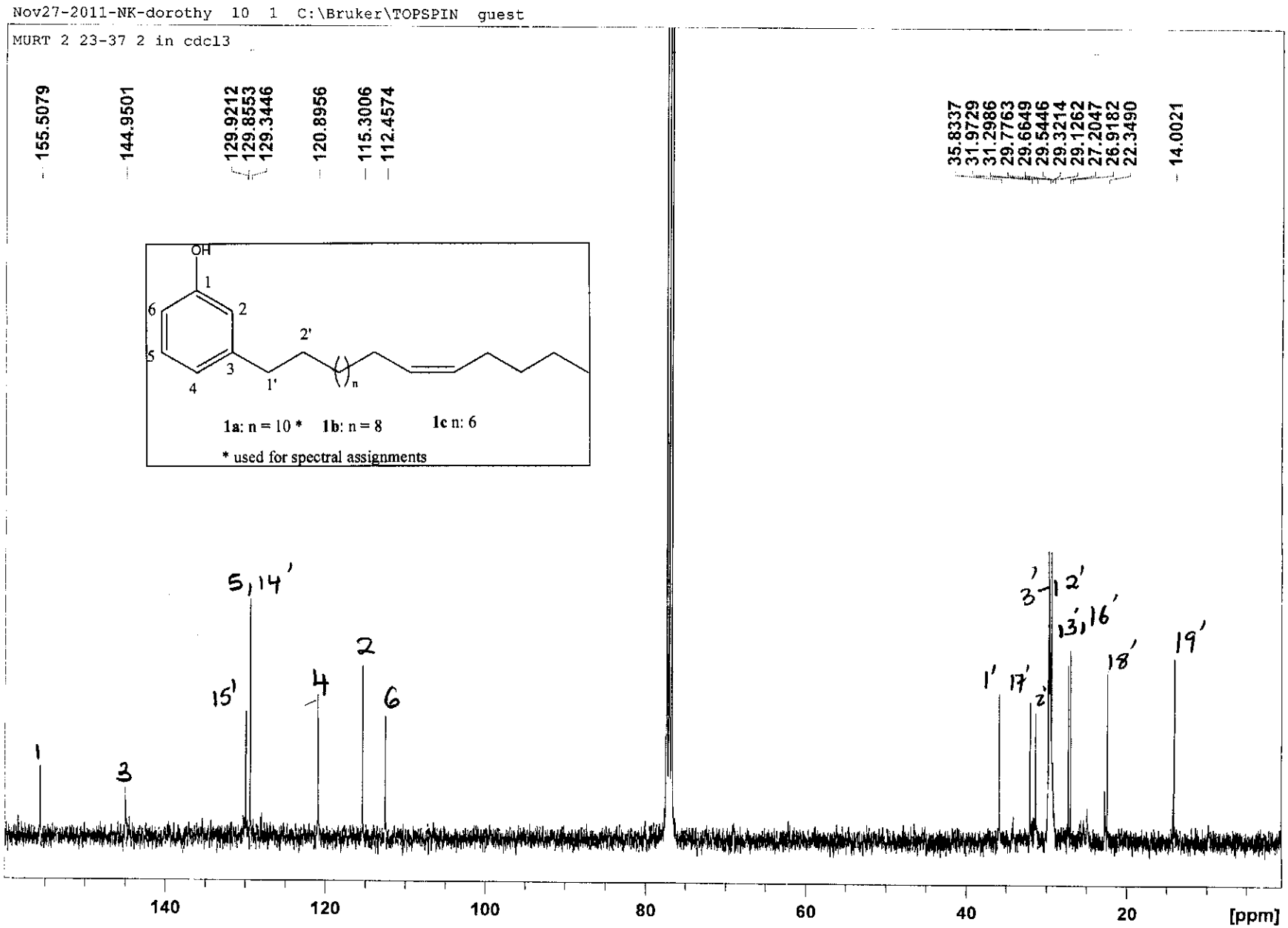
^{1}H NMR spectrum of B1 (mixture a, b, c and d)



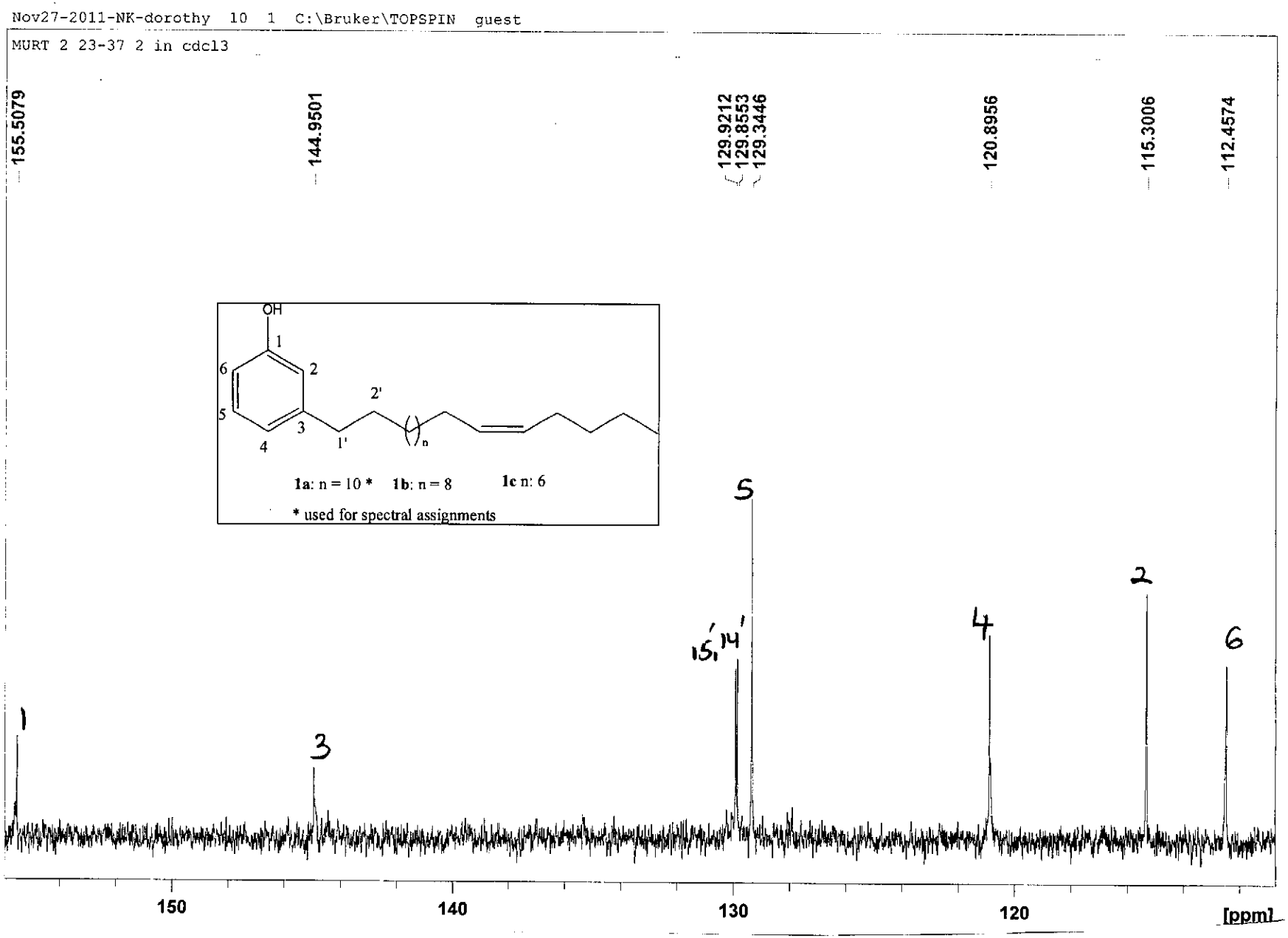
^{1}H NMR spectrum of B1 expanded 0.6-2.5 ppm (mixture a, b, c and d)



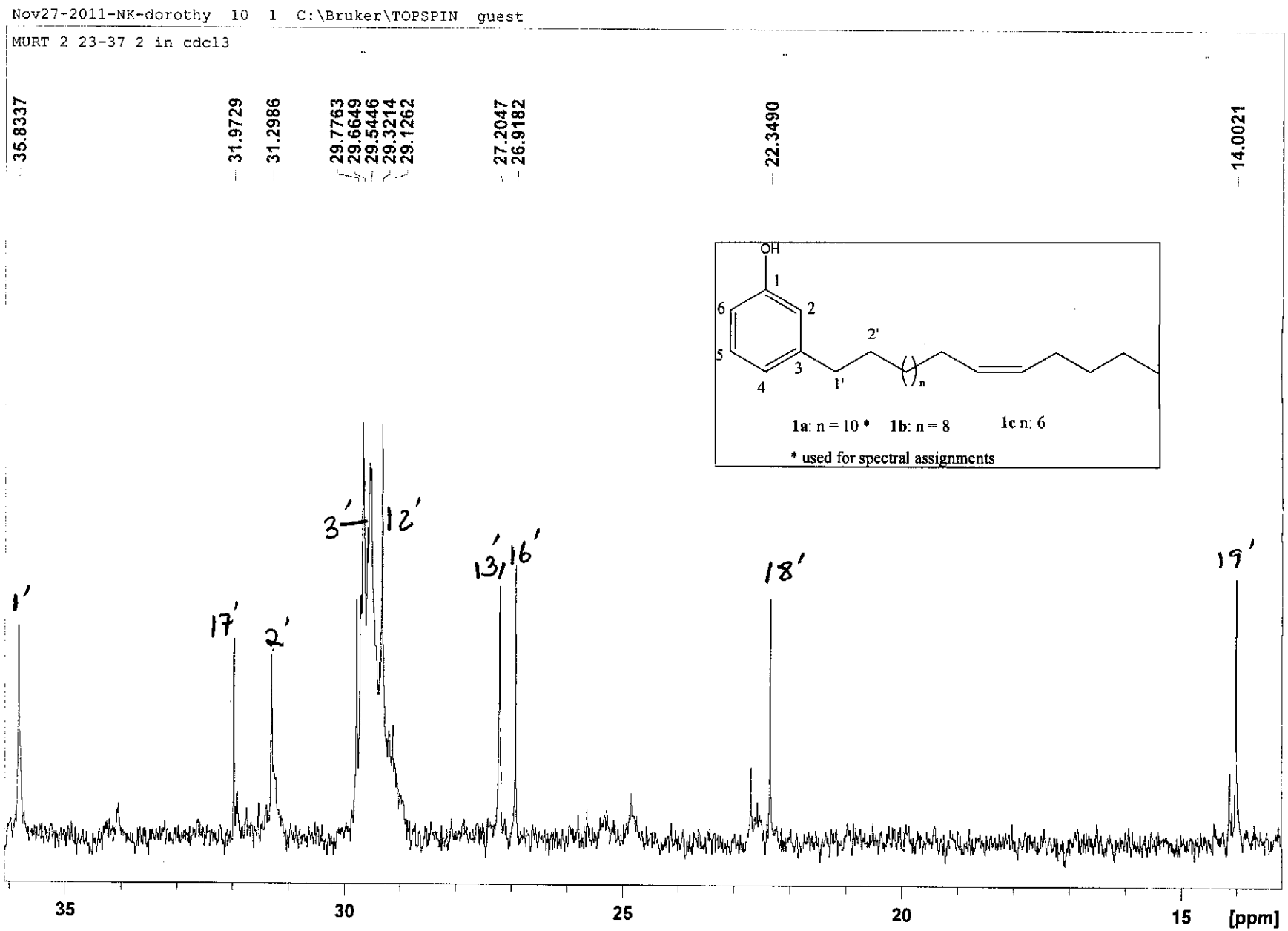
^{1}H NMR spectrum of B1 expanded 6.4-7.3 ppm (mixture a, b, c and d)



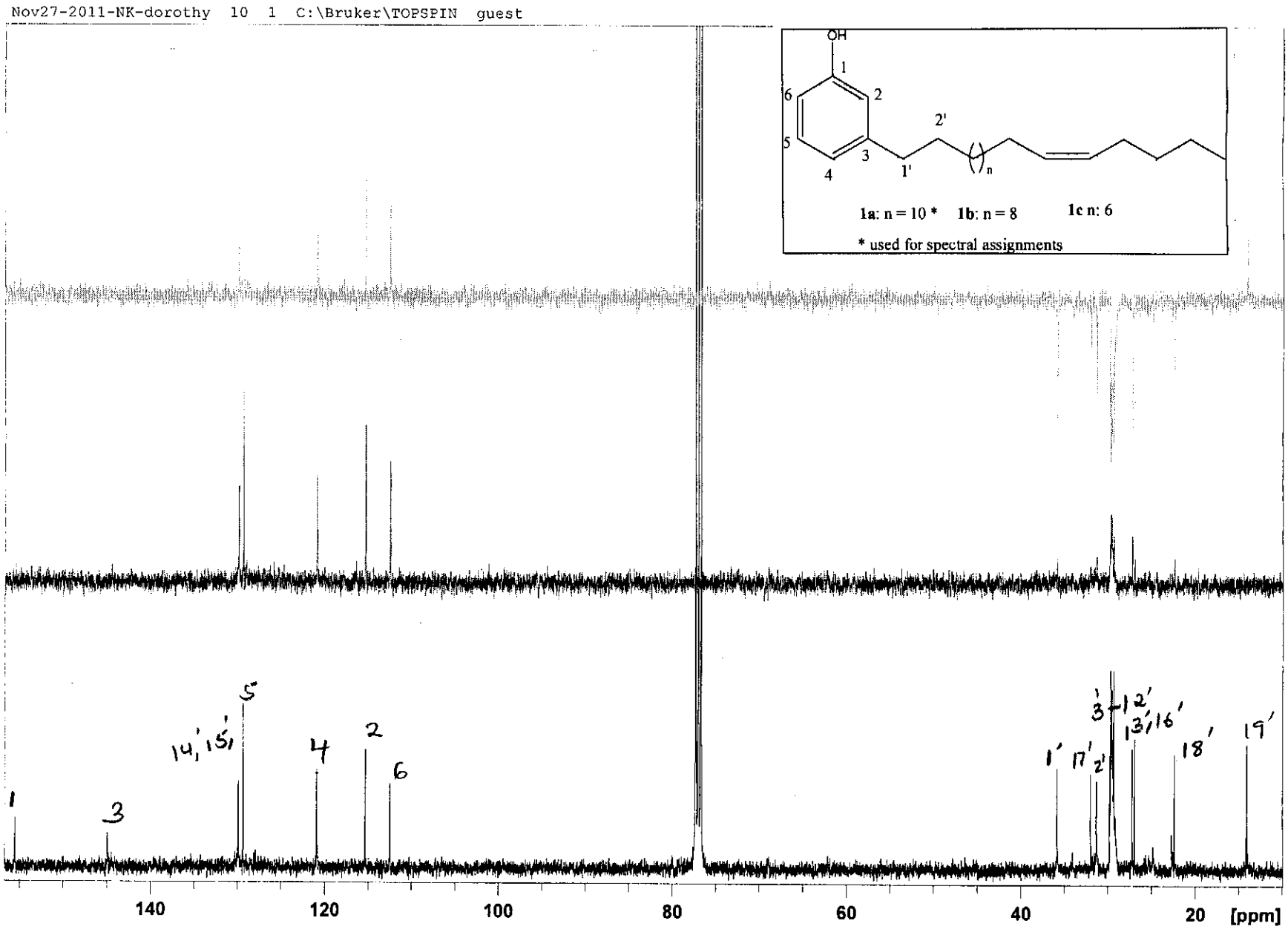
^{13}C NMR spectrum of B1 (mixture a, b, c and d)



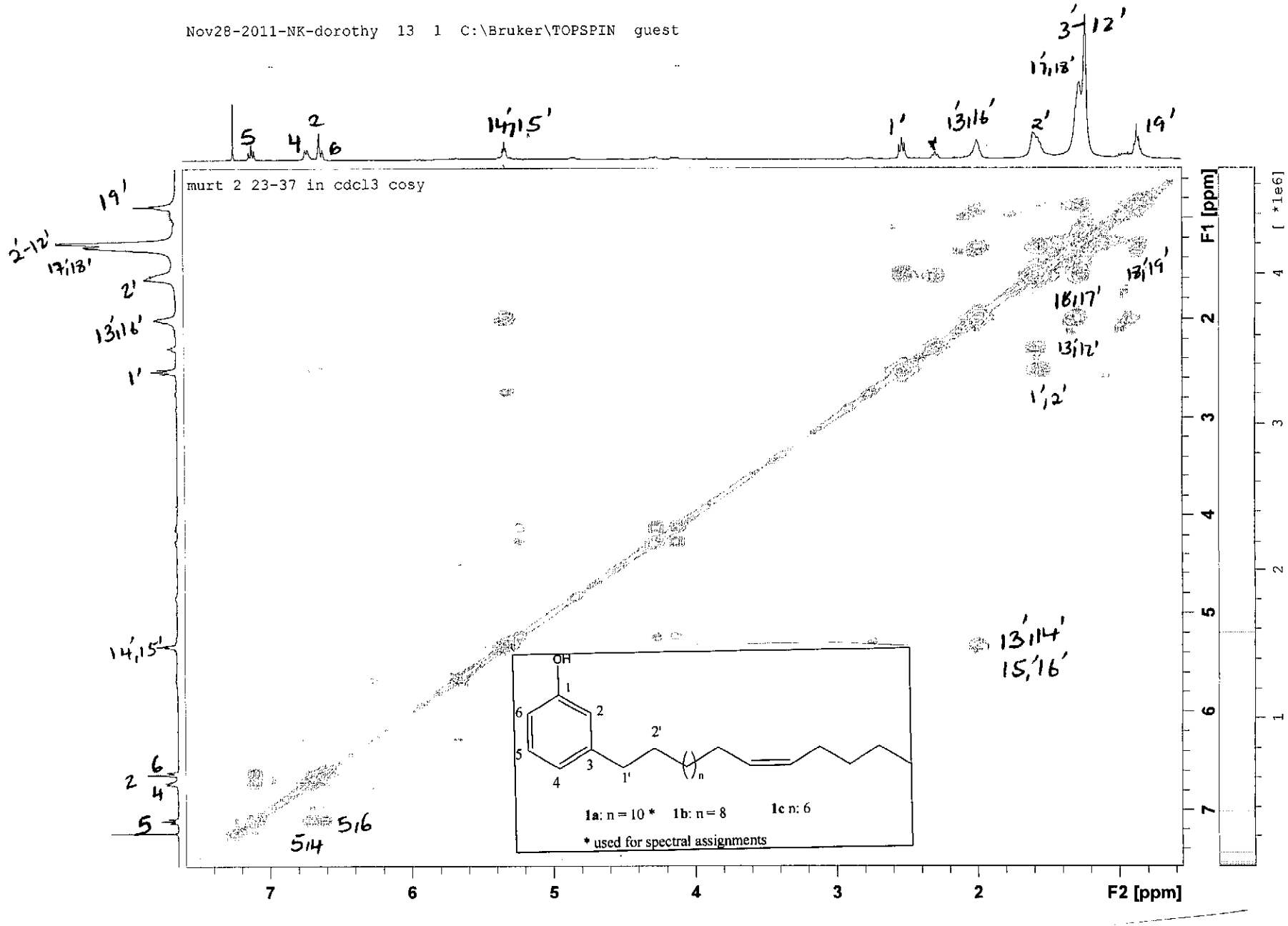
^{13}C NMR spectrum of B1expanded 110-156 ppm (mixture a, b, c and d)



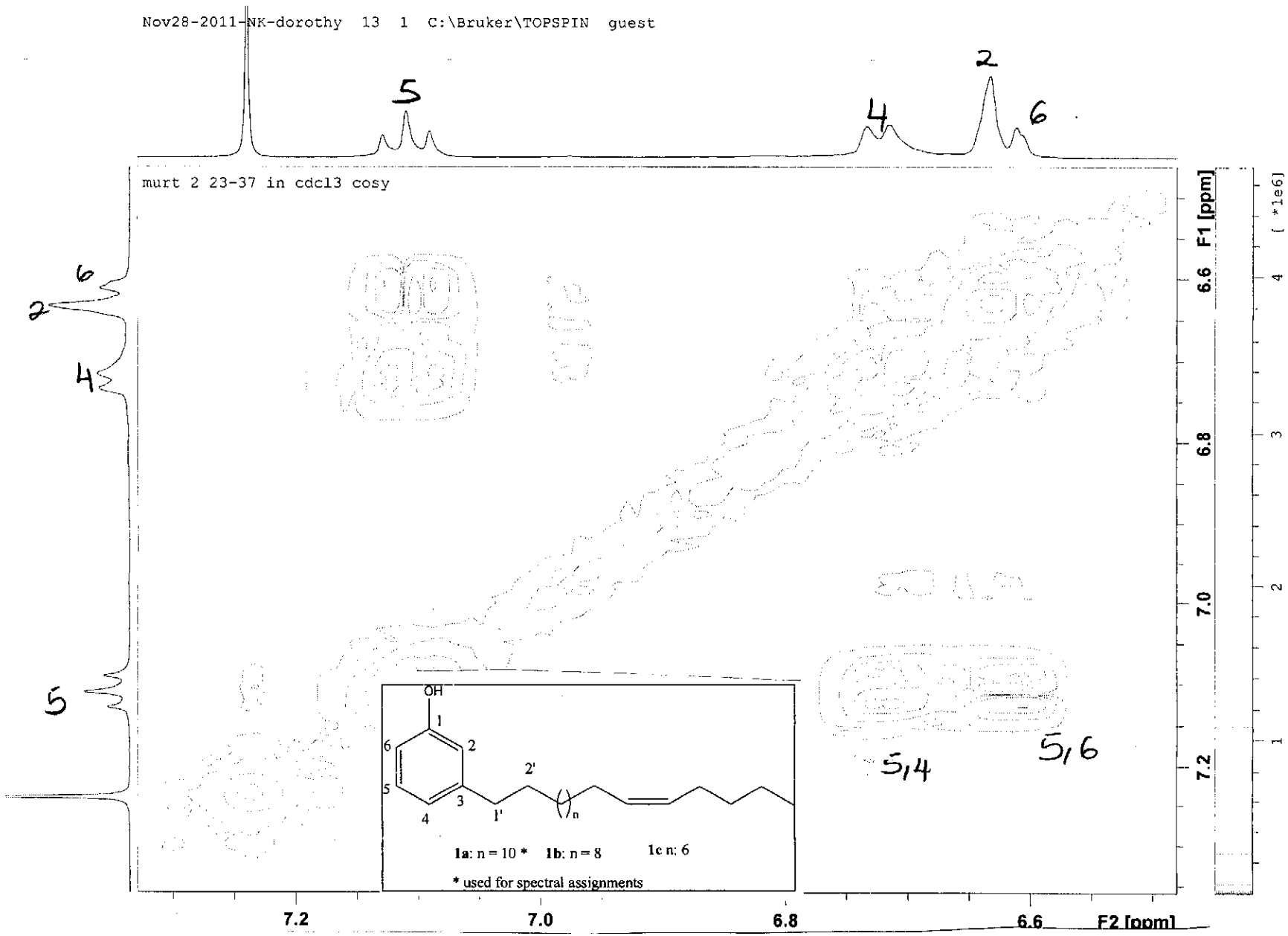
^{13}C NMR spectrum of B1expanded 13.8-36 ppm (mixture a, b, c and d)



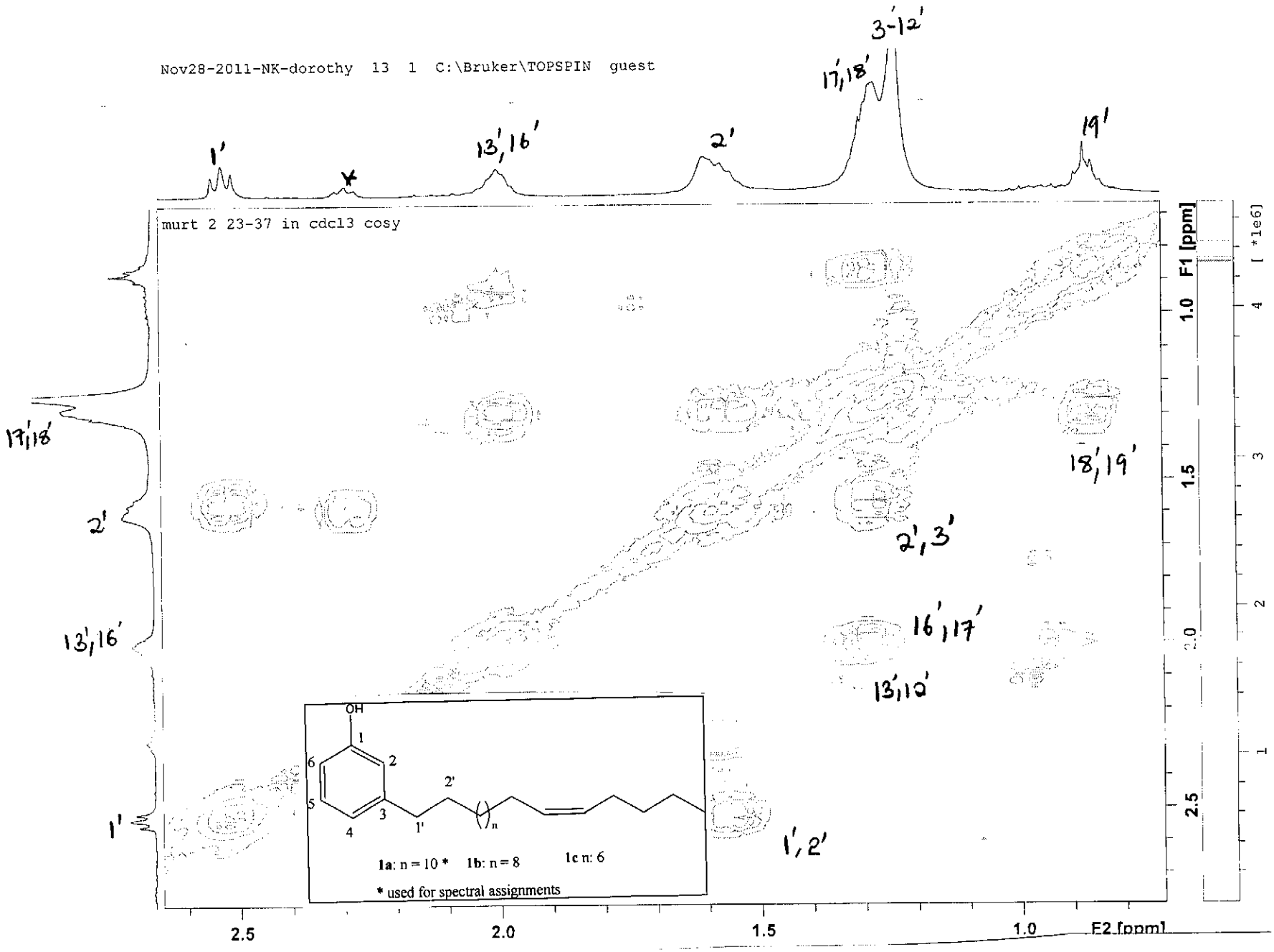
DEPT spectrum of B1 (mixture a, b, c and d)



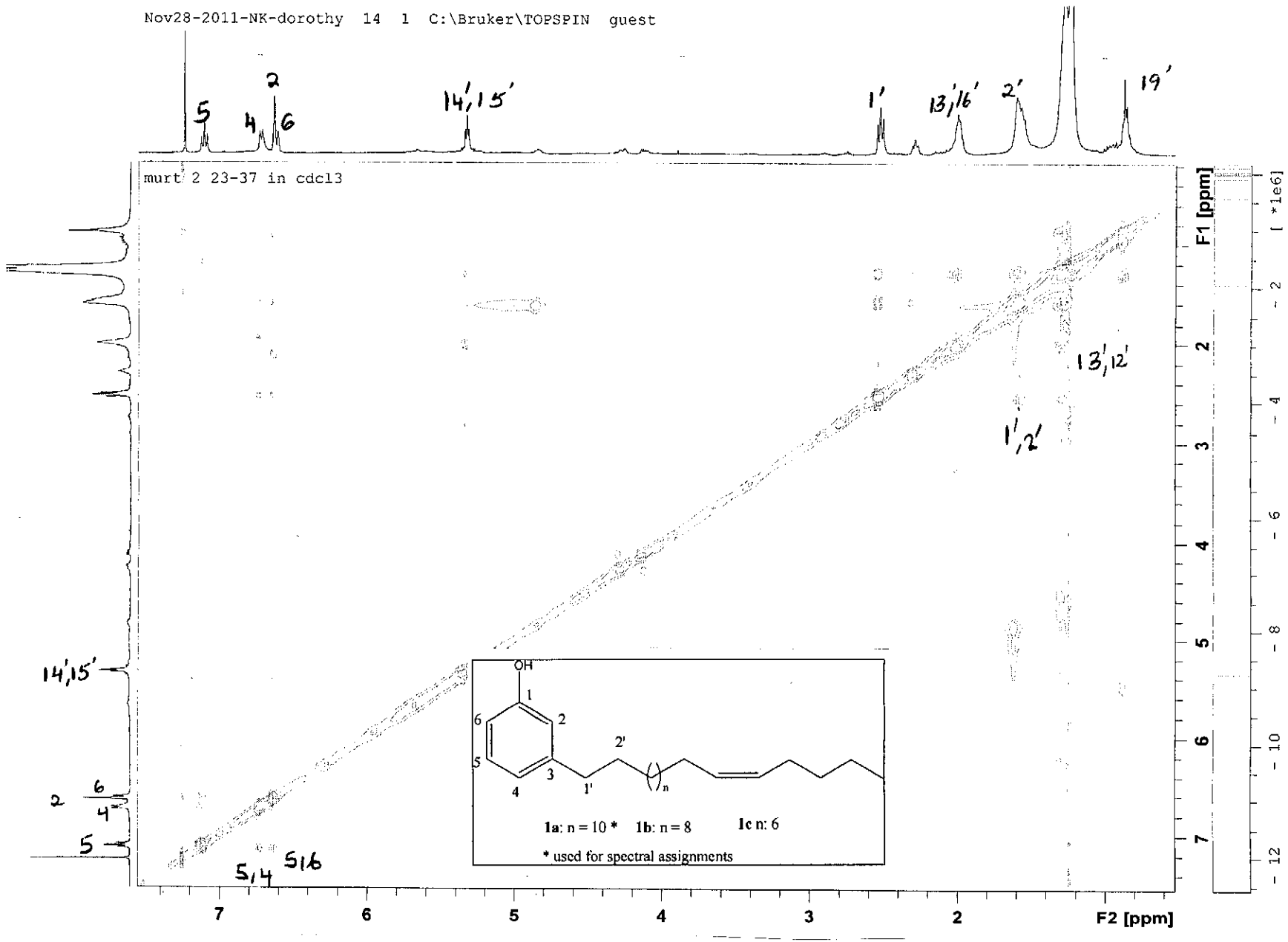
COSY spectrum of B1 (mixture a, b, c and d)



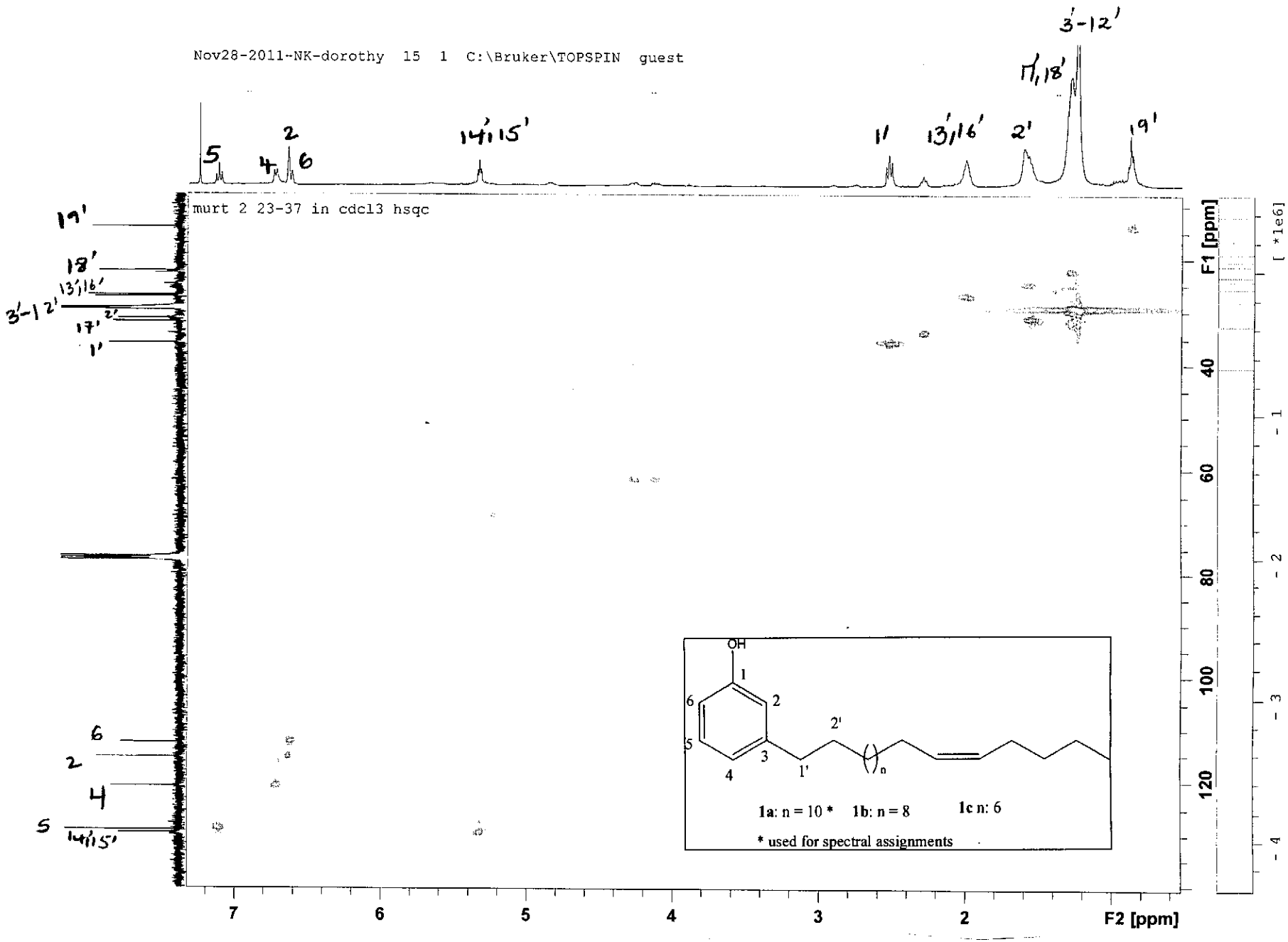
COSY spectrum of B1 expanded section F2 6.5-7.3 ppm (mixture a, b, c and d)



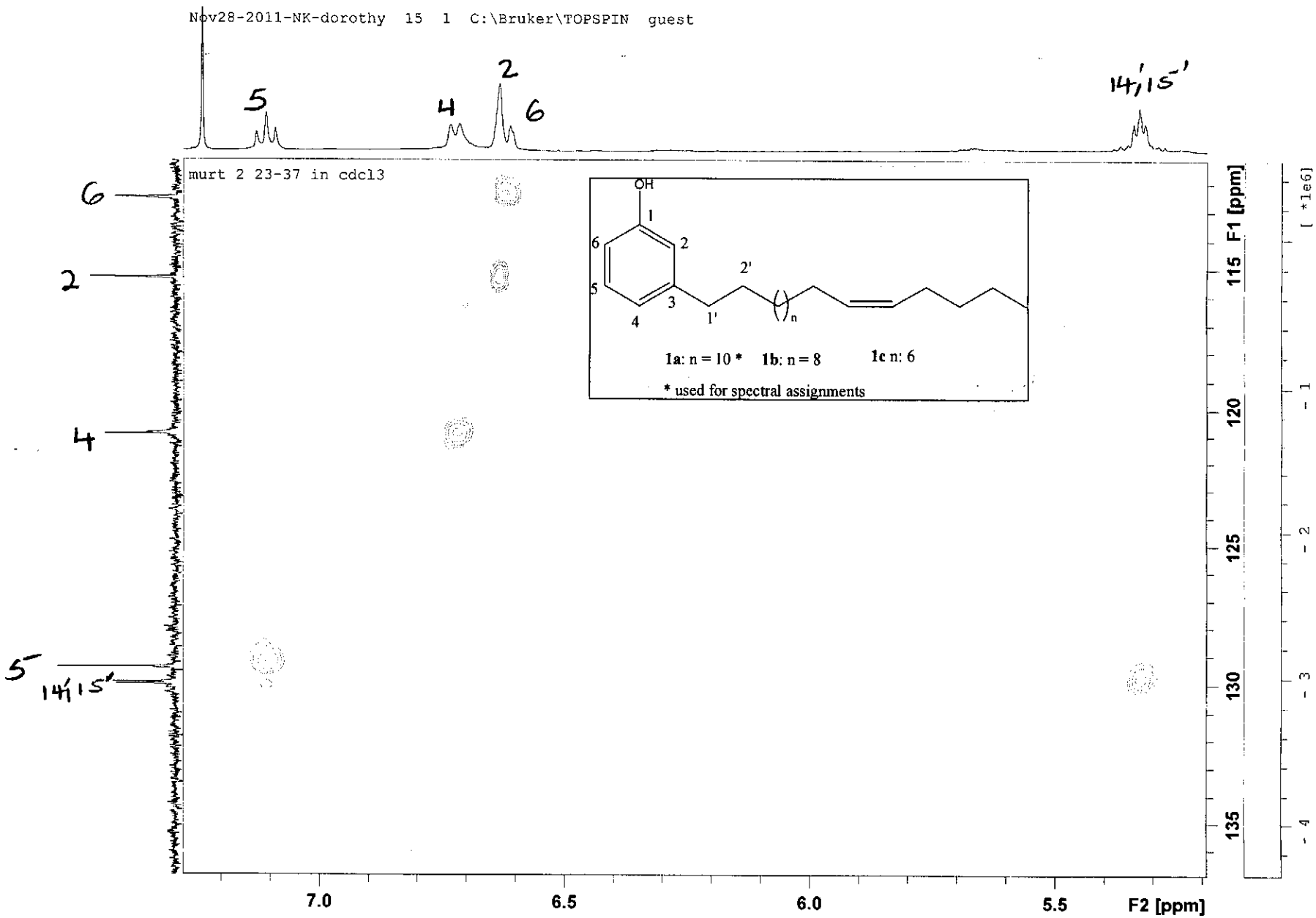
COSY spectrum of B1 expanded section F2 0.6 - 2.6 ppm (mixture a, b, c and d)



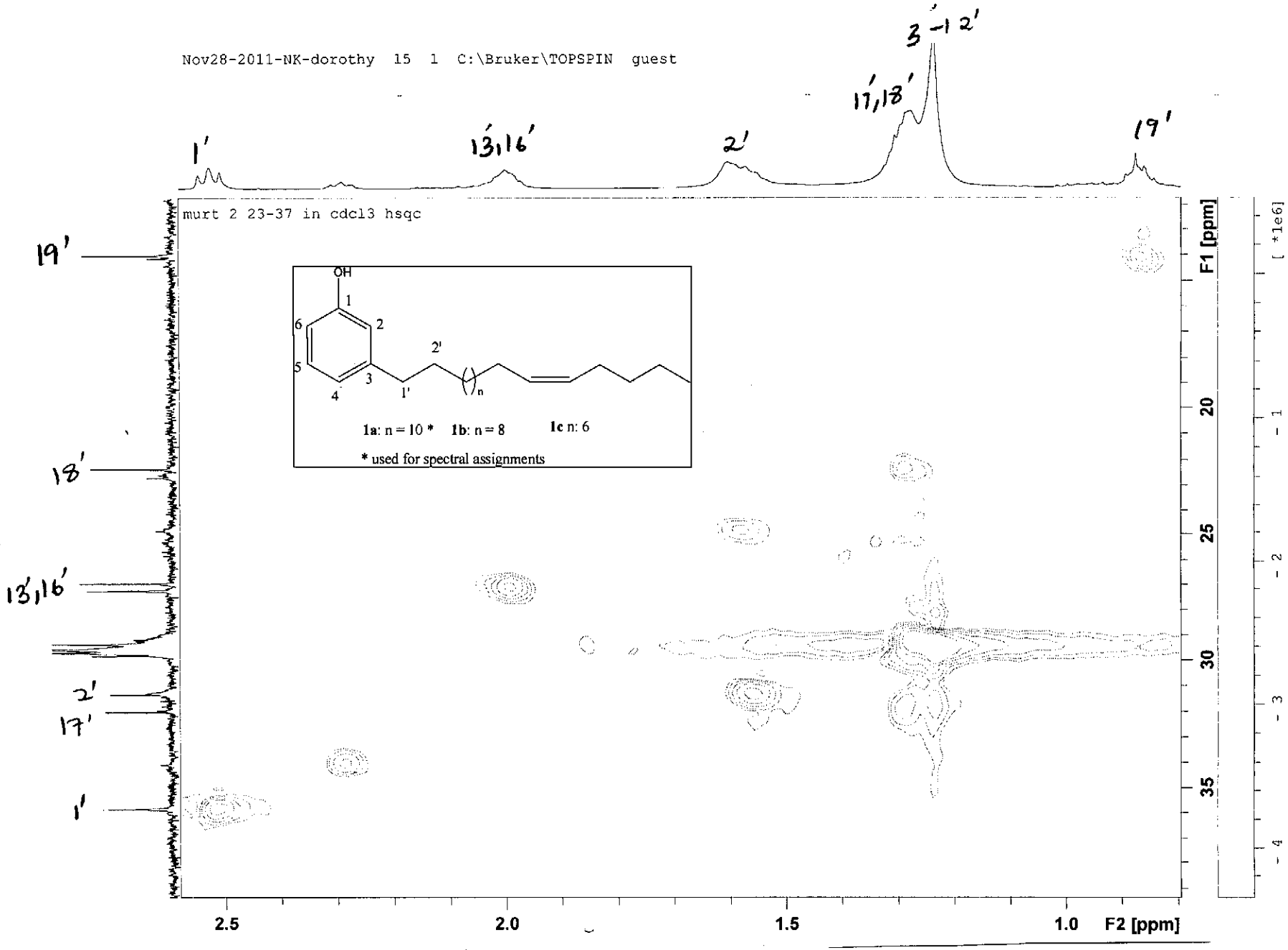
NOESY spectrum of B1 (mixture a, b, c and d)



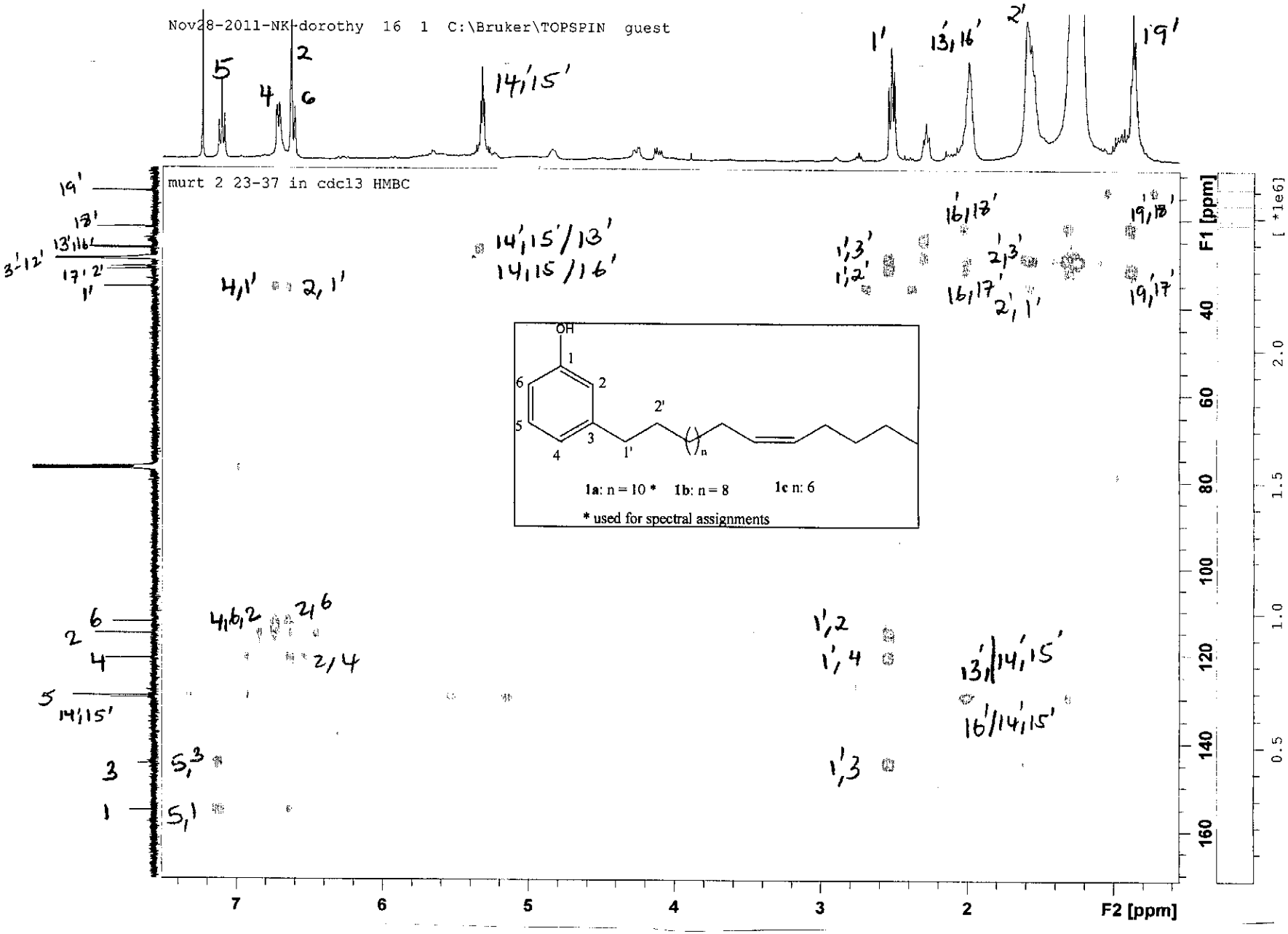
HSQC spectrum of B1 (mixture a, b, c and d)



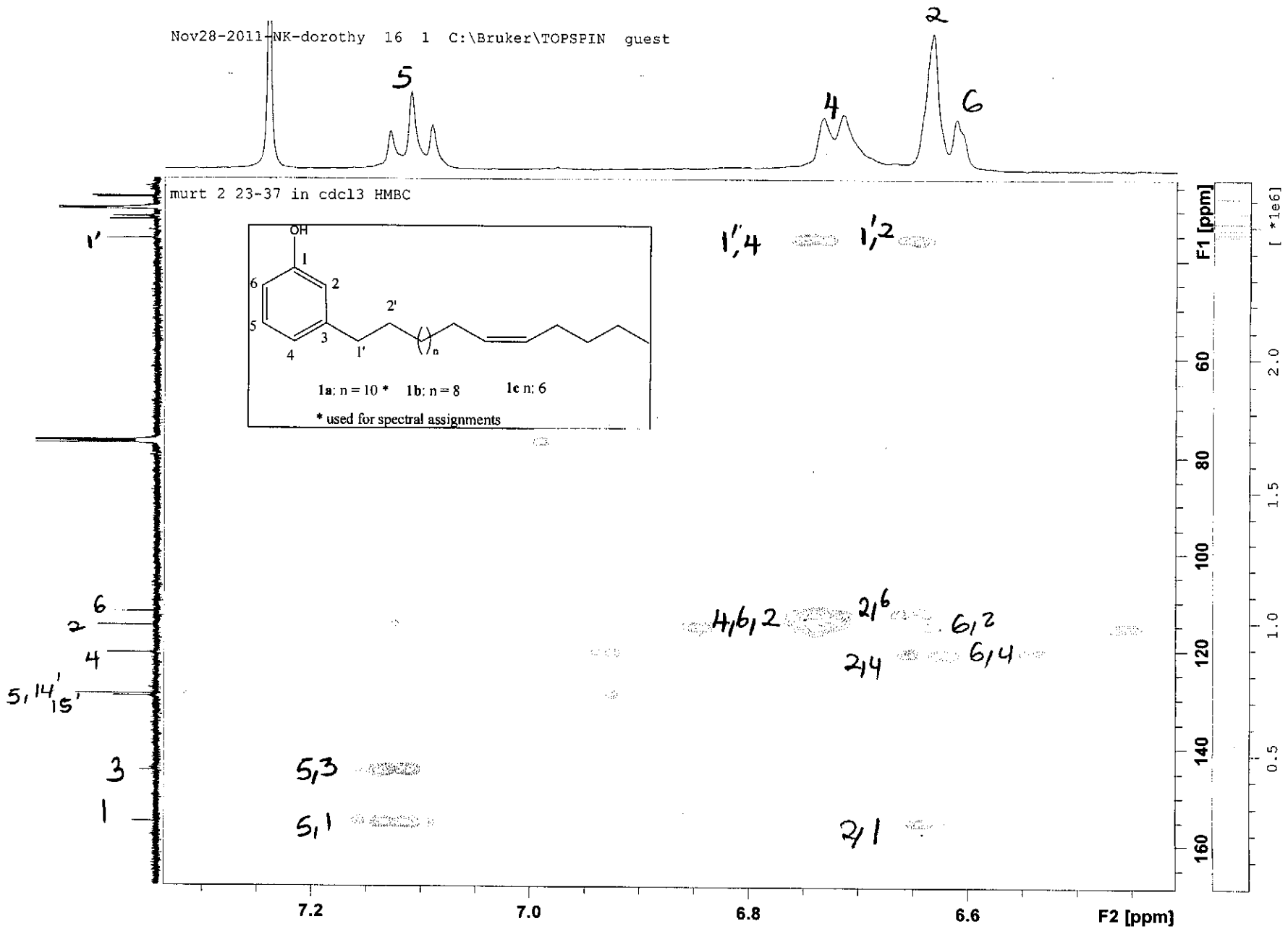
HSQC spectrum of B1 expanded F2 5.2-7.3 ppm (mixture a, b, c and d)



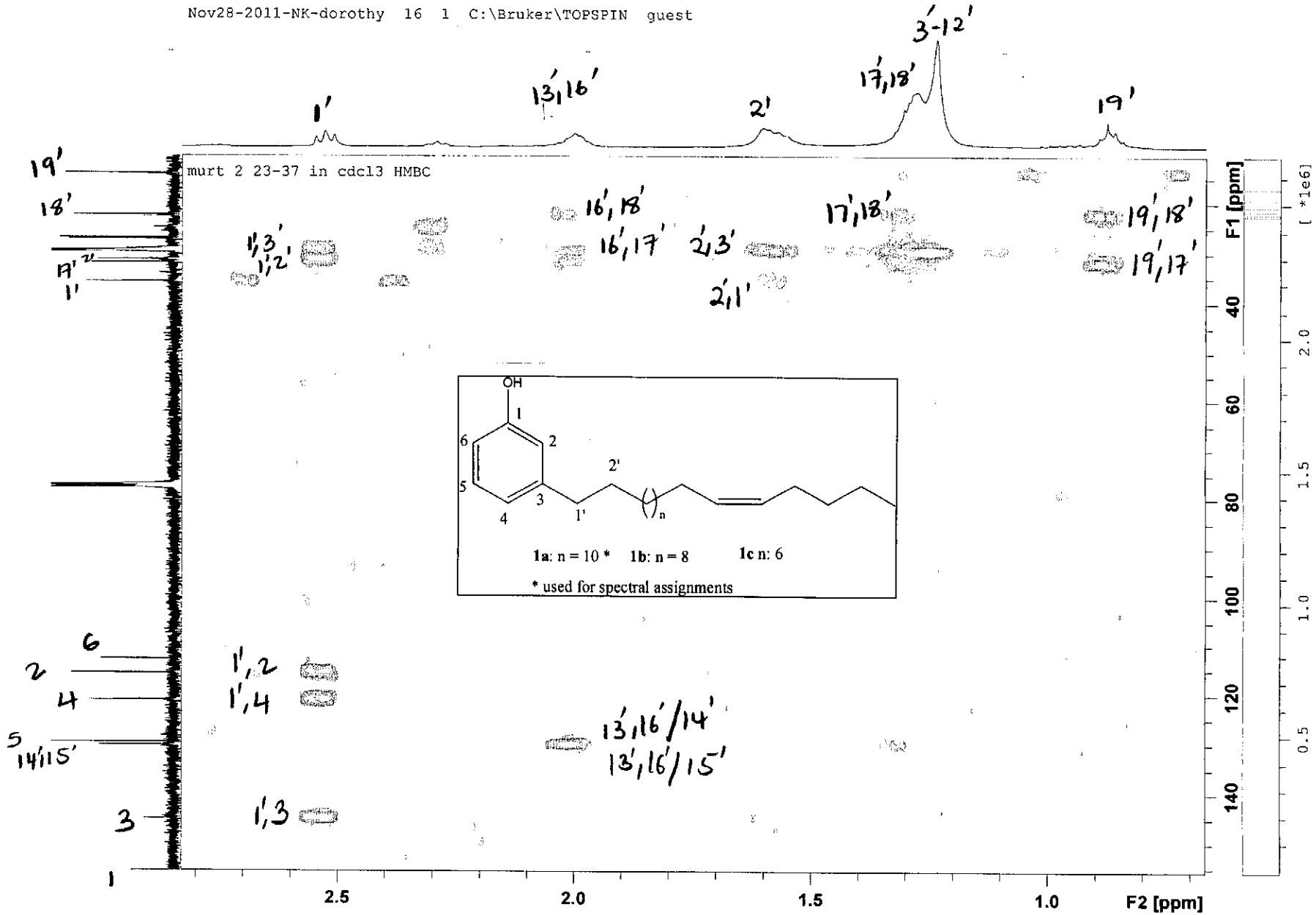
HSQC spectrum of B1 expanded F2 0.6 - 2.6 ppm (mixture a, b, c and d)



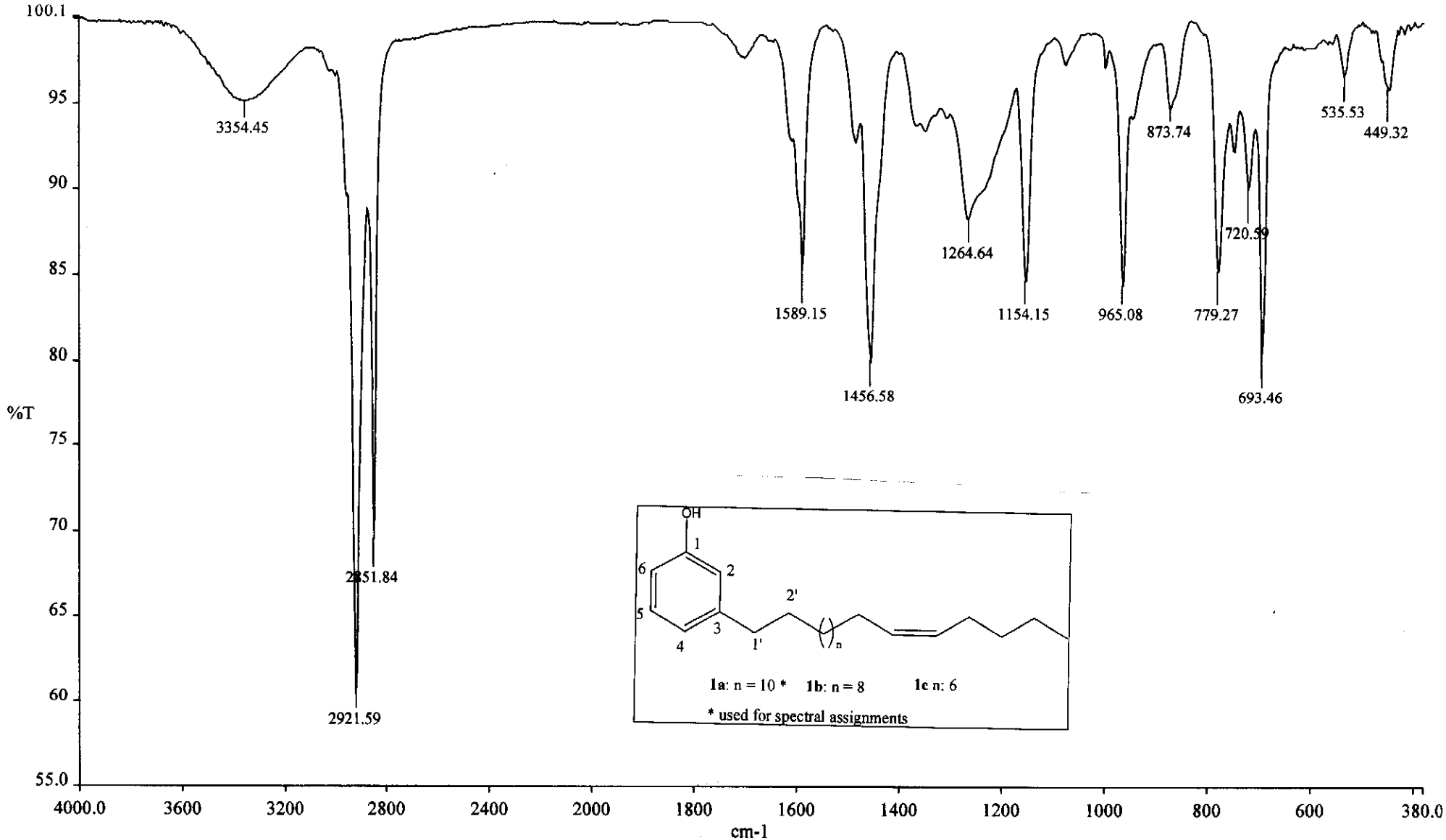
HMBC spectrum of B1 (mixture a, b, c and d)



HMBC spectrum of B1 expanded F2 6.4 – 7.3 ppm (mixture a, b, c and d)



HMBC spectrum of B1 expanded F2 0.7 – 2.7 ppm (mixture a, b, c and d)

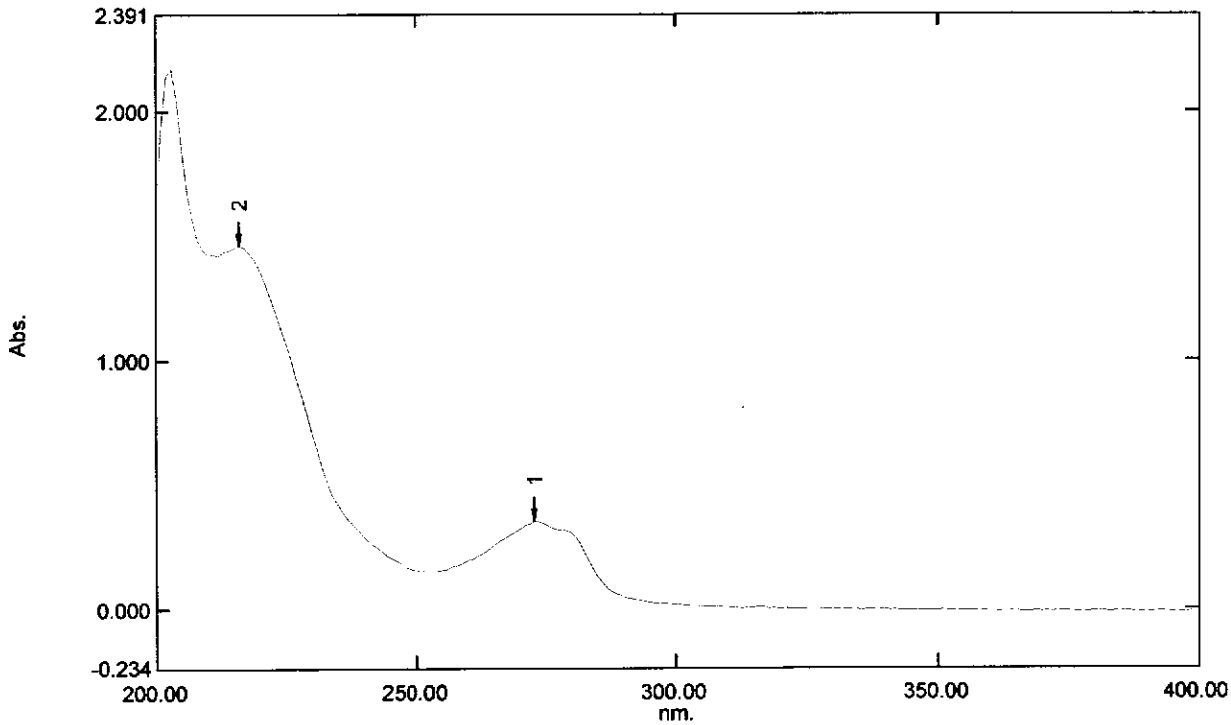


IR spectrum of B1 (mixture a, b, c and d)

Spectrum Peak Pick Report

23/05/2012 06:16:45 PM

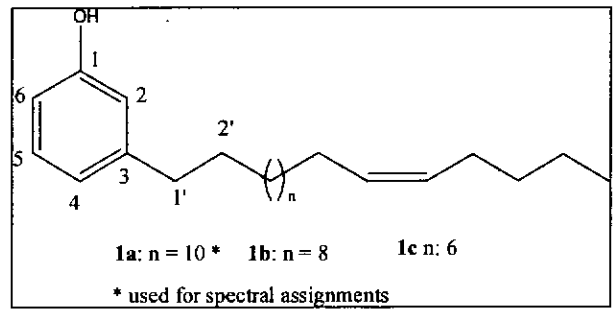
Data Set: murt 2 28-38 in methanol.spc - Storage 175326



No.	P/V	Wavelength	Abs.	Description
1	●	273.00	0.353	
2	●	216.00	1.465	

Measurement Properties
Wavelength Range (nm.): 200.00 to 400.00
Scan Speed: Medium
Sampling Interval: 1.0
Auto Sampling Interval: Disabled
Scan Mode: Single

Instrument Properties
Instrument Type: UV-3600 Series
Measuring Mode: Absorbance
Slit Width: 2.0 nm
Time Constant: 0.1 sec.
Source Lamp: Auto
Light Source Change Wavelength: 310.00 nm
Detector Unit: Direct
Detector Change Wavelength: 830.00 nm 1800.00 nm
Grating Change Wavelength: 720.00 nm
S/R Exchange: Normal
Detector Lock: Auto
Slit Program: Normal
Beam Mode: Double
Stair Correction: Disable

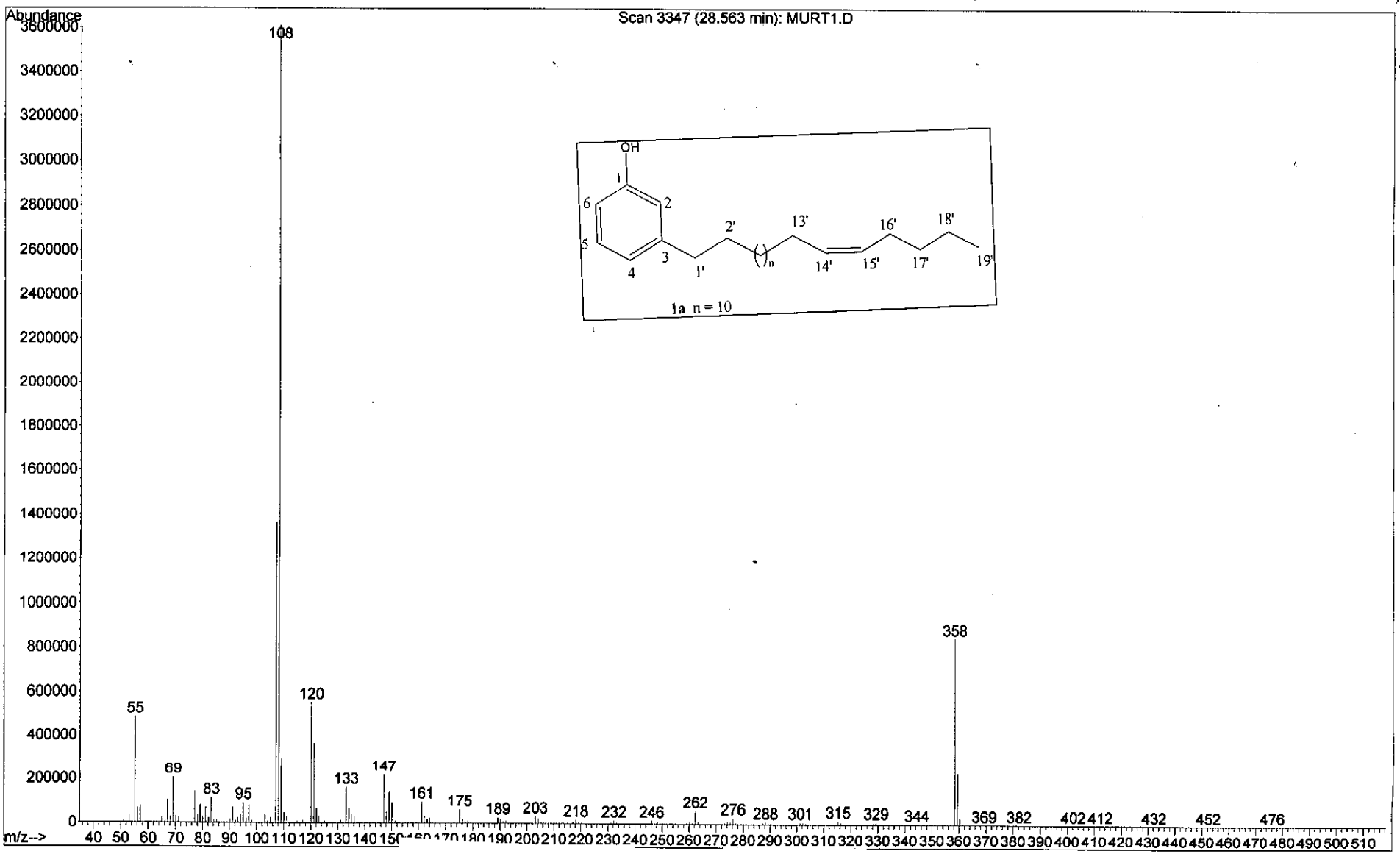


Attachment Properties
Attachment: None

Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

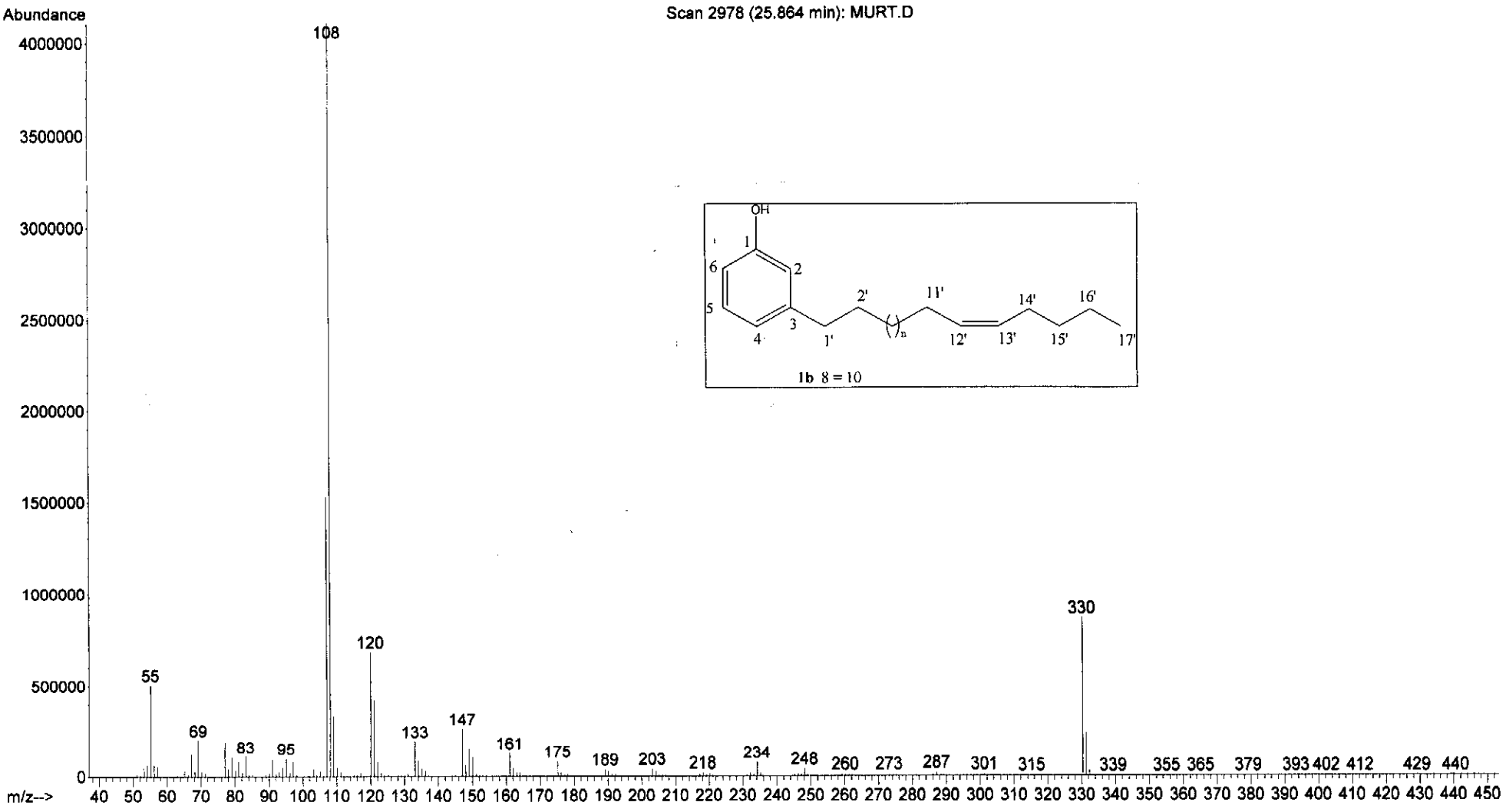
UV spectrum of B1 (mixture a, b, c and d)

File : C:\MSDCHEM\1\DATA\DOROTHY GCMS TWO\DOROTHY GCMS TWO\MURT1.D
Operator : dorothy
Acquired : 26 Nov 2011 20:09 using AcqMethod DOROTHY
Instrument : Instrumen
Sample Name: murt 2 43-48 (2)
Misc Info :
Vial Number: 1



MS spectrum of B1 (a)

File : C:\MSDCHEM\1\DATA\DOROTHY GCMS TWO\DOROTHY GCMS TWO\MURT.D
Operator : dorothy
Acquired : 26 Nov 2011 18:35 using AcqMethod DOROTHY
Instrument : Instrumen
Sample Name: murt 2 43-48
Misc Info :
Vial Number: 1



MS spectrum of B1 (b)

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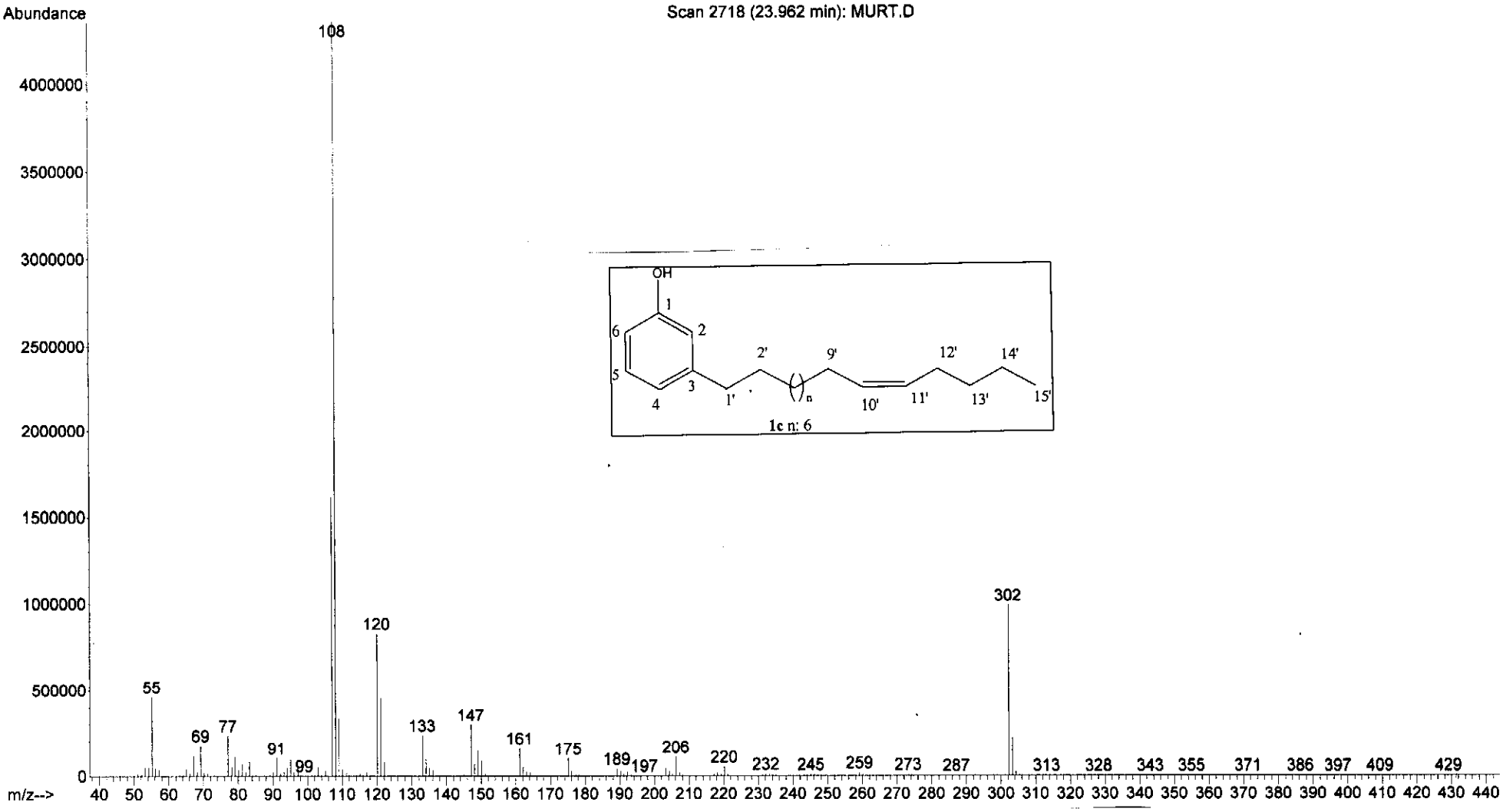
Operator : dorothy
Acquired : 26 Nov 2011 18:35 using AcqMethod DOROTHY
Instrument : Instrumen
Sample Name: murt 2 43-48
Misc Info :
Vial Number: 1



MS spectrum of B1 (c)

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Operator : dorothy
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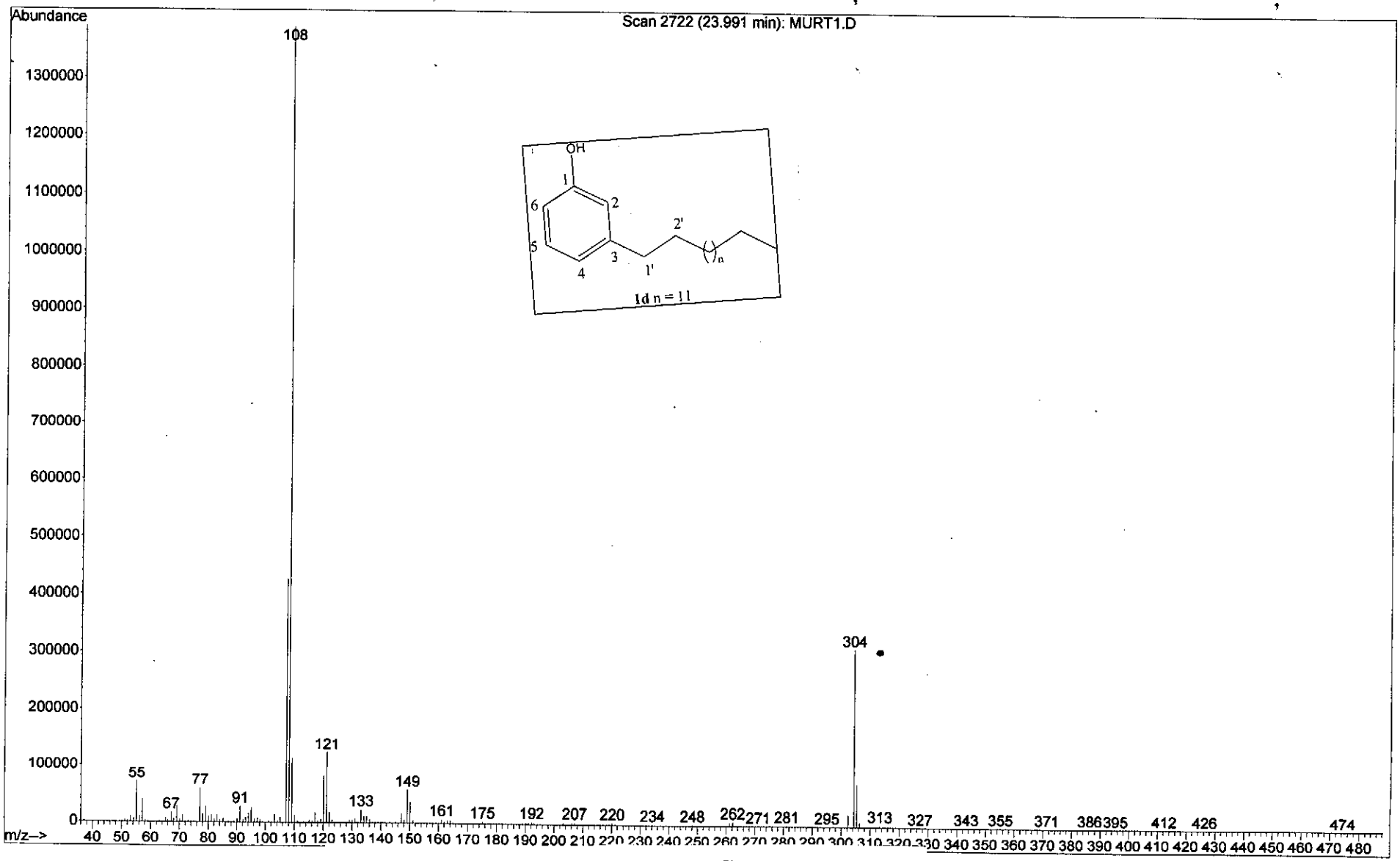
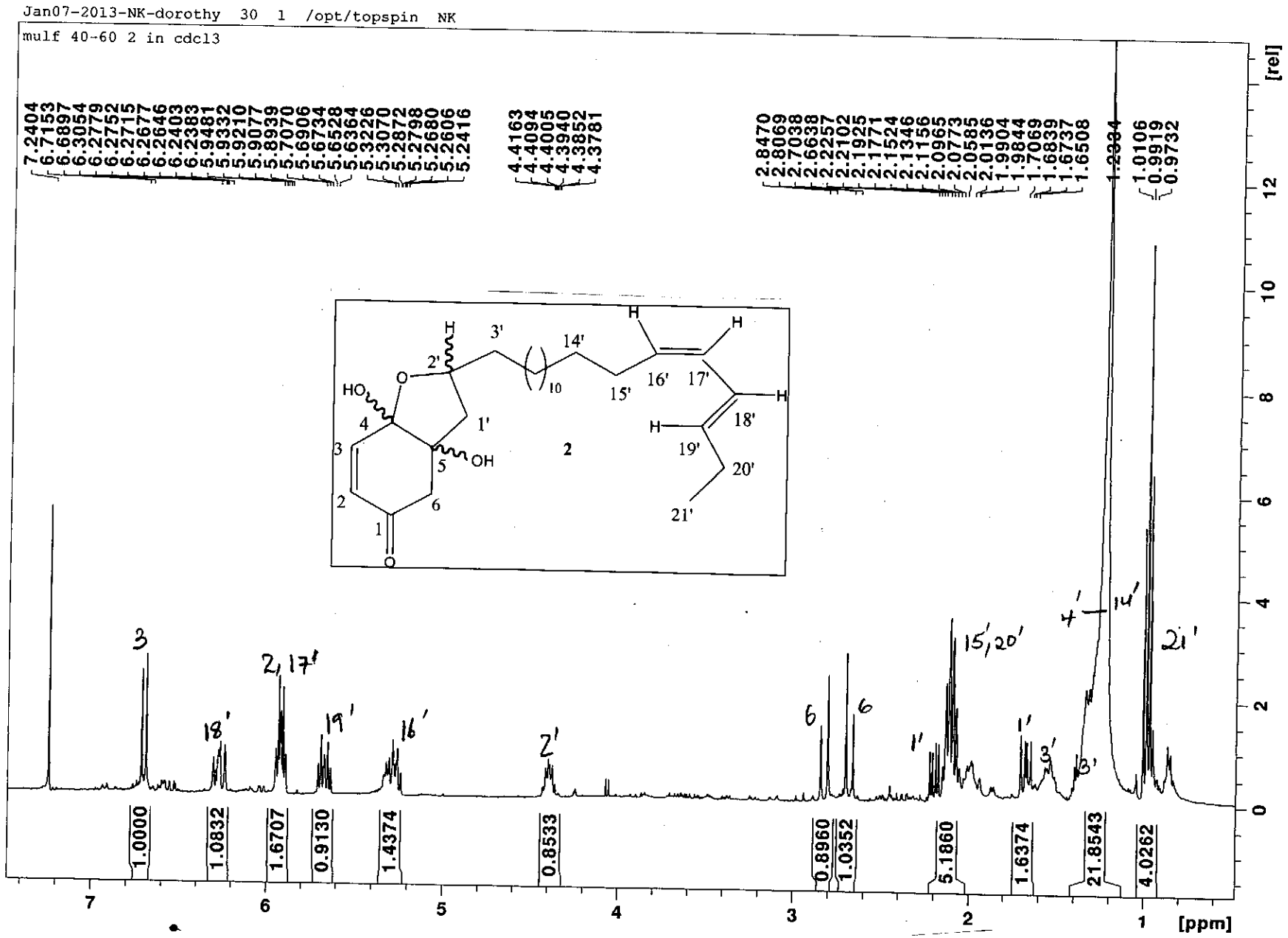
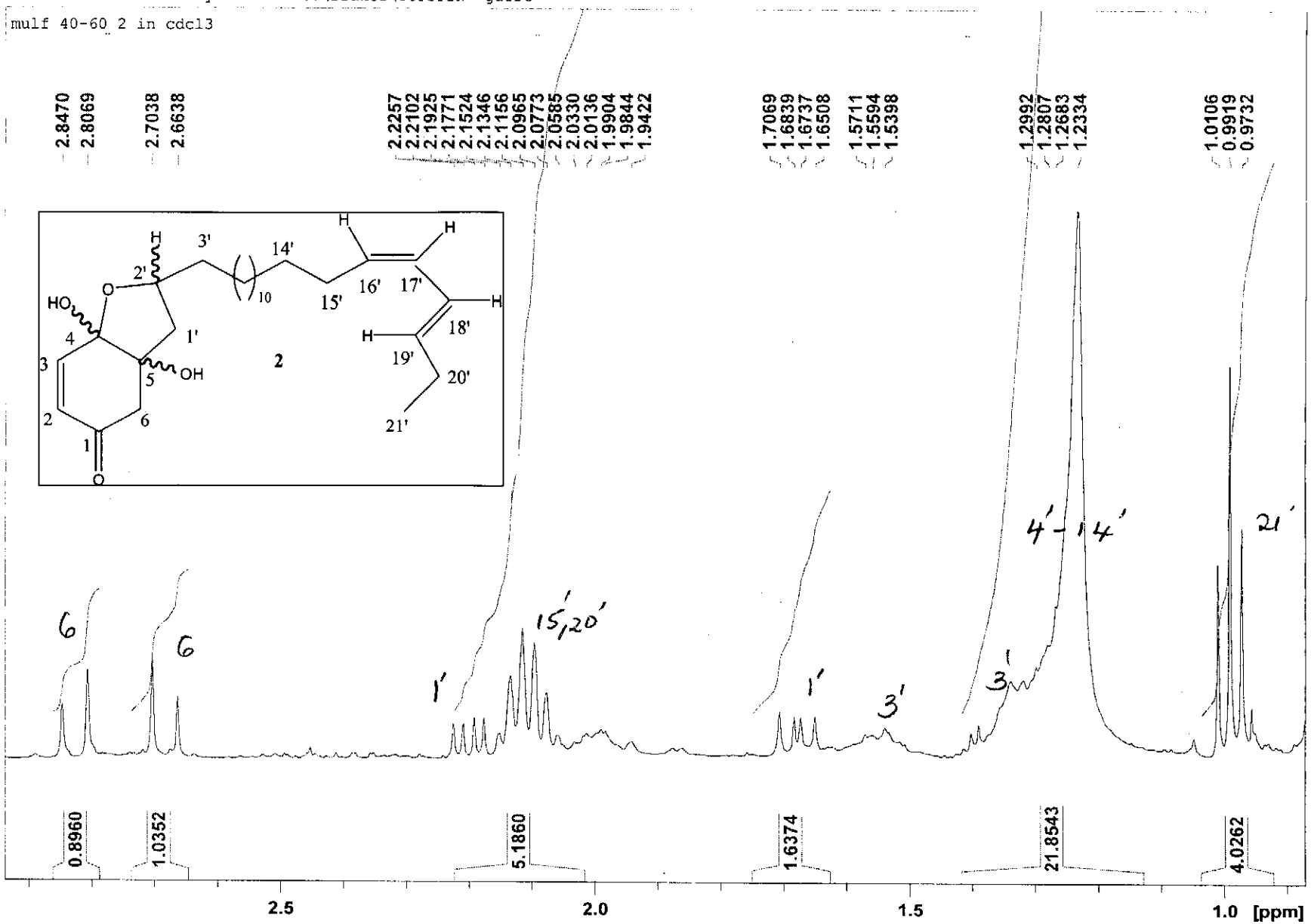
Instrument : Instrumen
Sample Name: murt 2 43-48 (2)
Misc Info :
Vial Number: 1



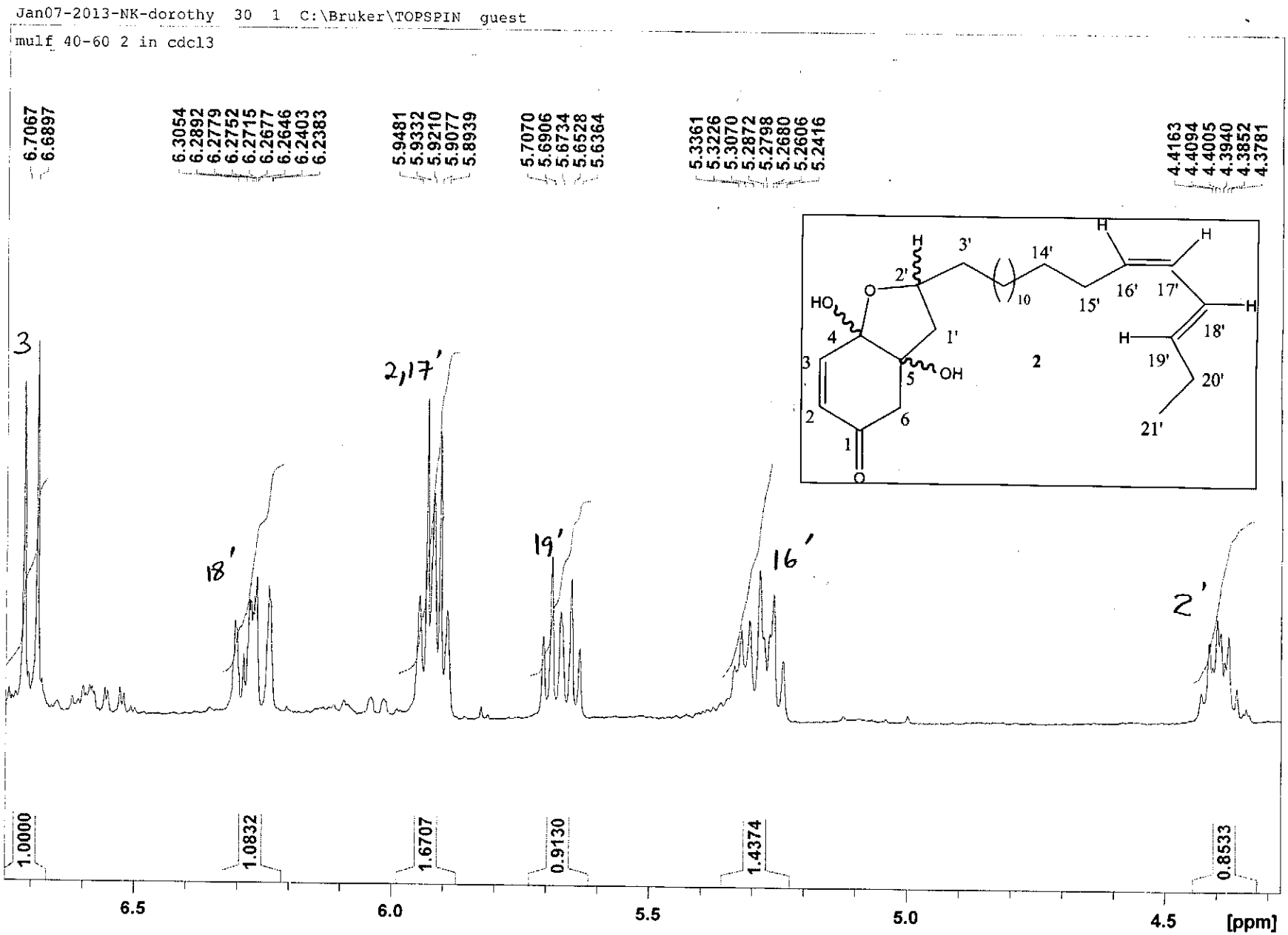
MS spectrum of B1 (d)



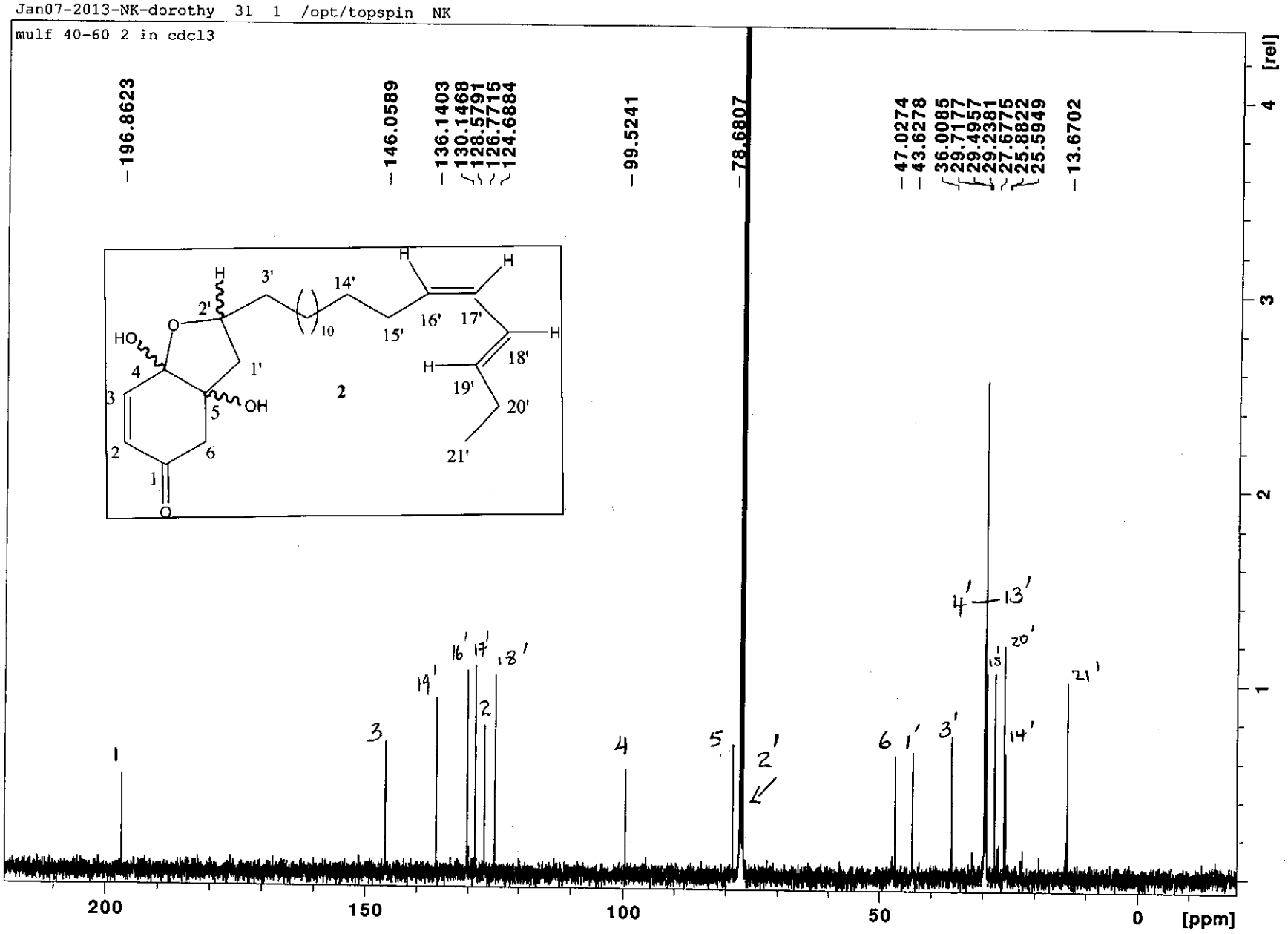
^{1}H NMR spectrum of B2



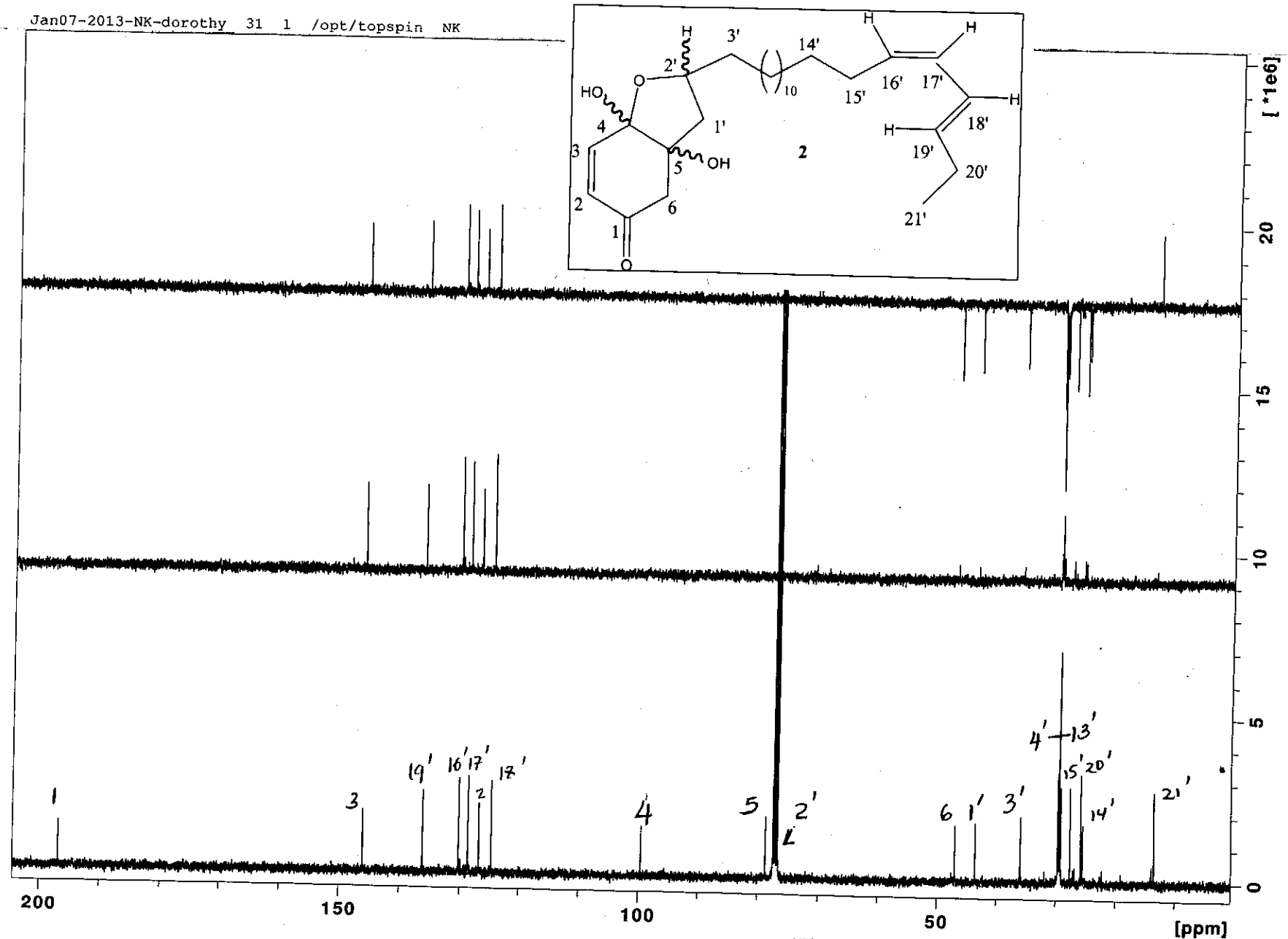
^{1}H NMR spectrum of B2 expanded (0.8-2.9 ppm)



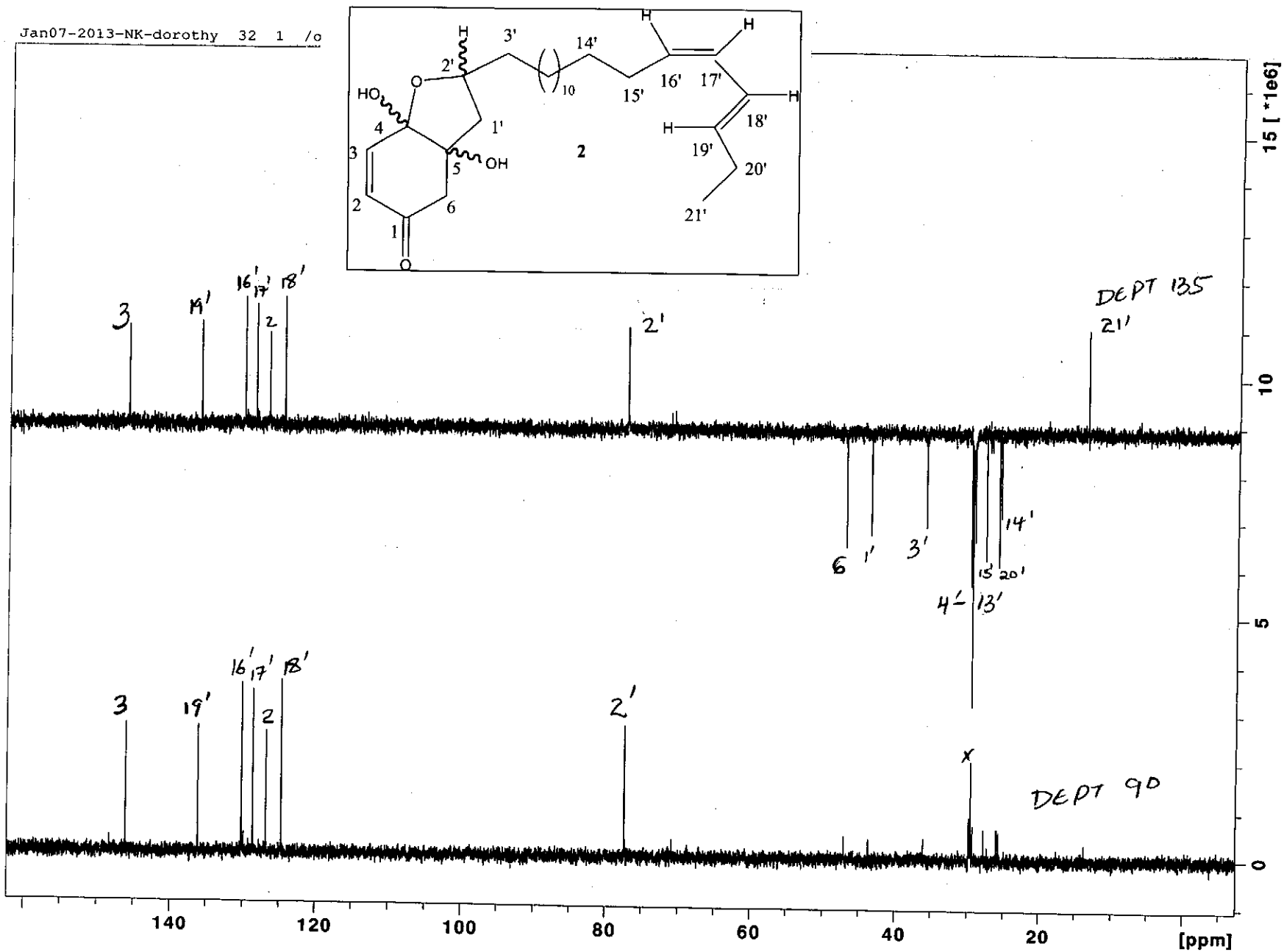
^{1}H NMR spectrum of B2 expanded (4.3-6.7 ppm)



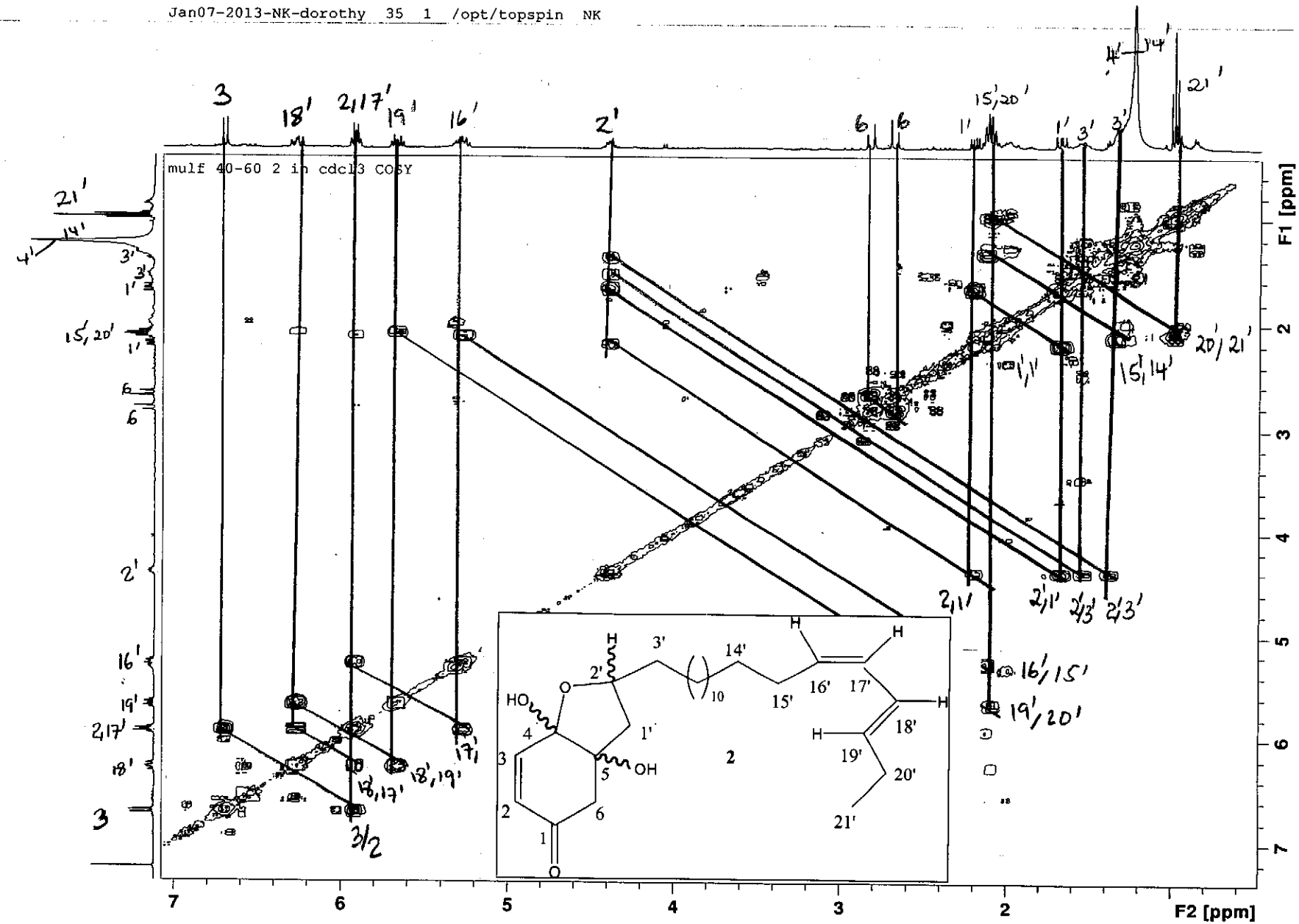
^{13}C NMR spectrum of B2



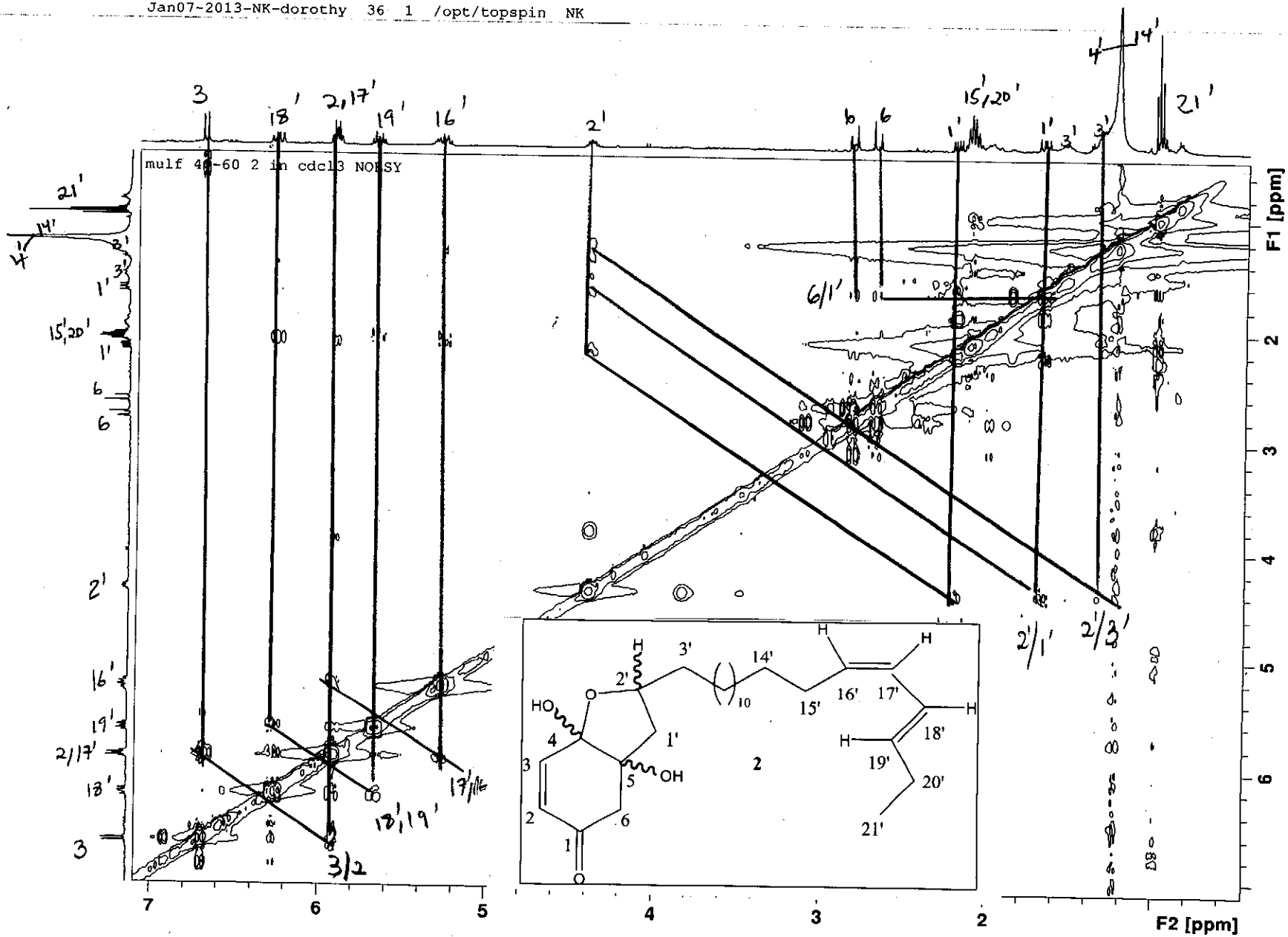
DEPT spectrum of B2 (^{13}C NMR, DEPT 90 and 135)



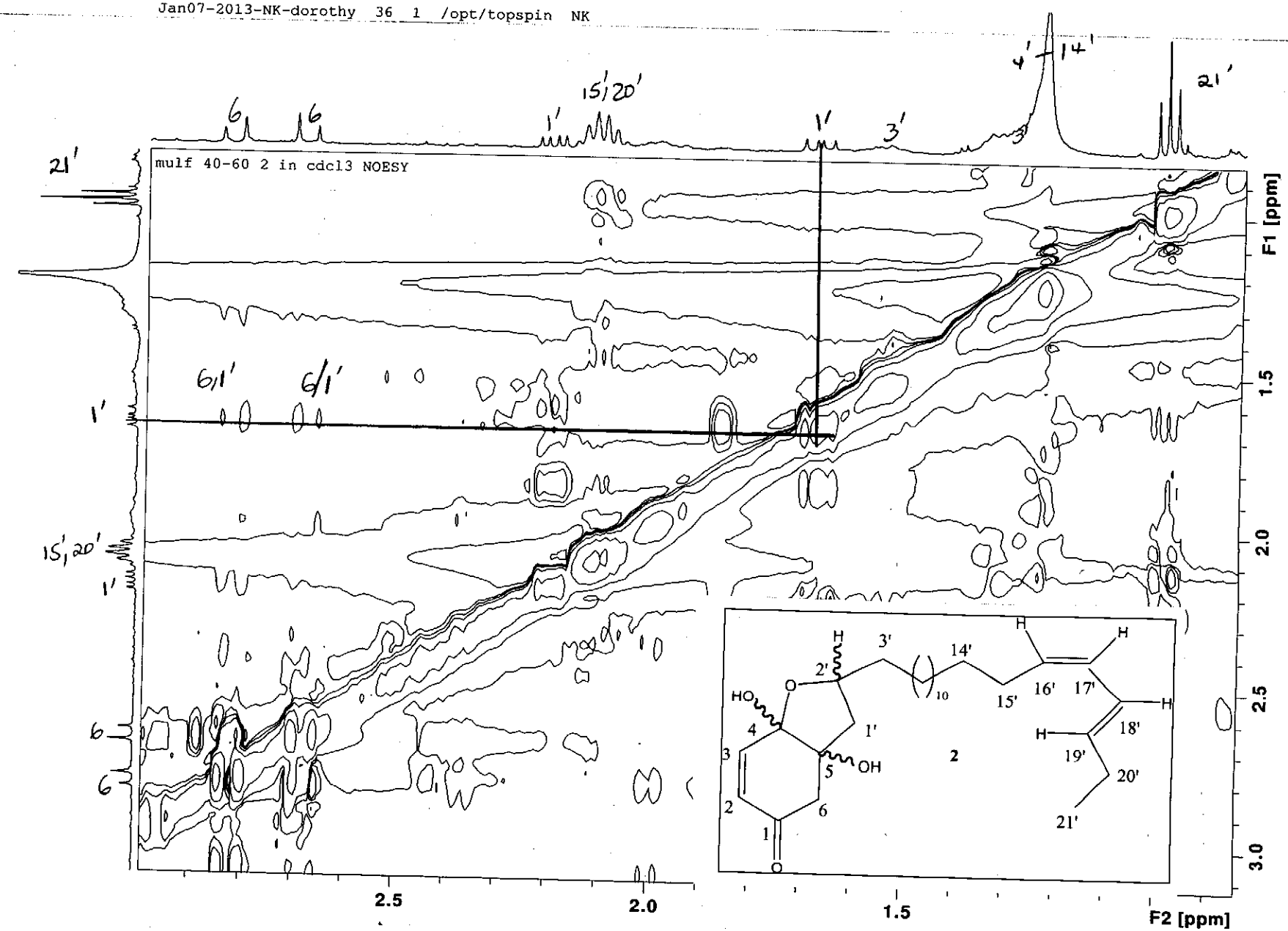
DEPT spectrum of B2 (DEPT 90 and 135)



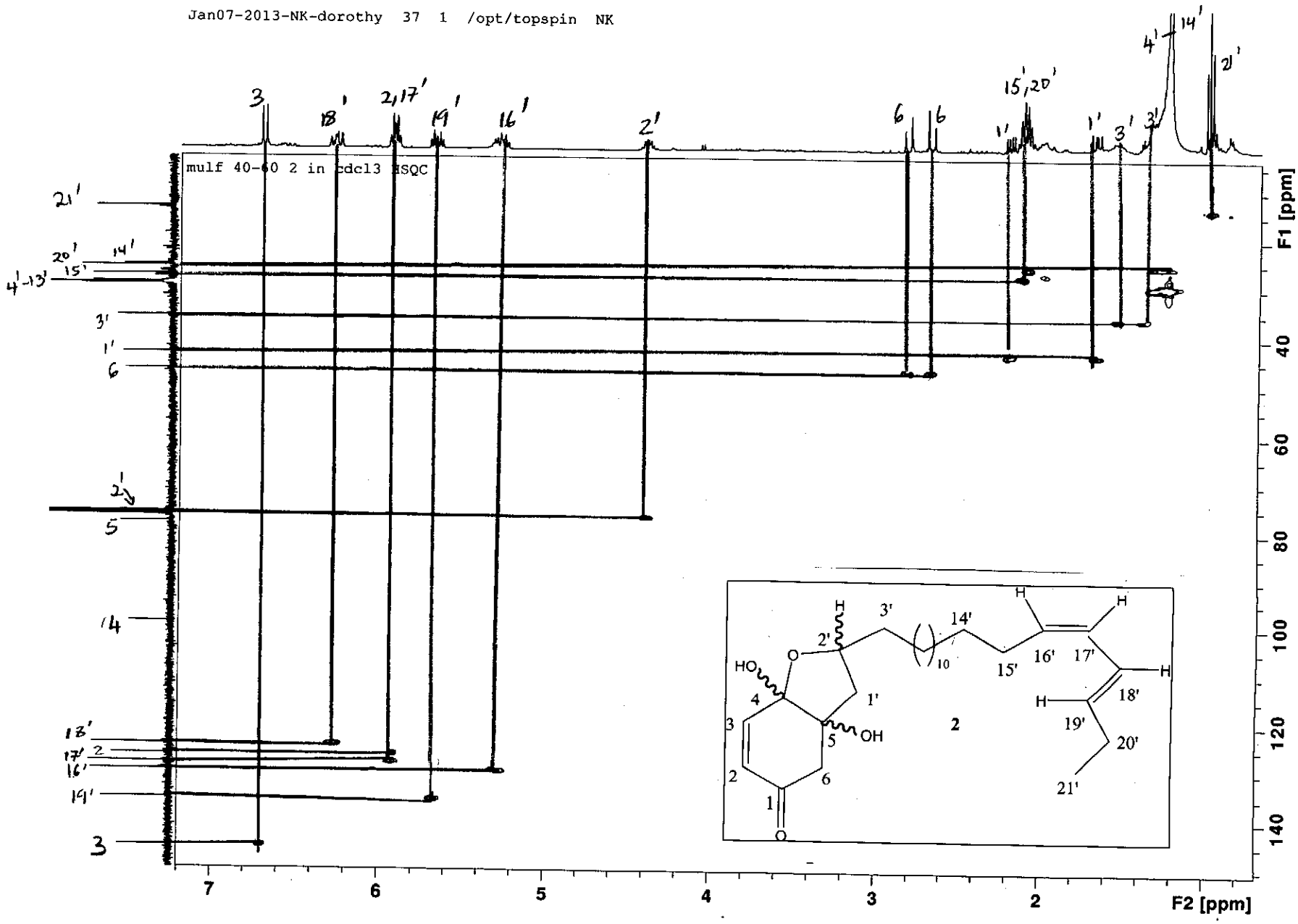
COSY spectrum of B2



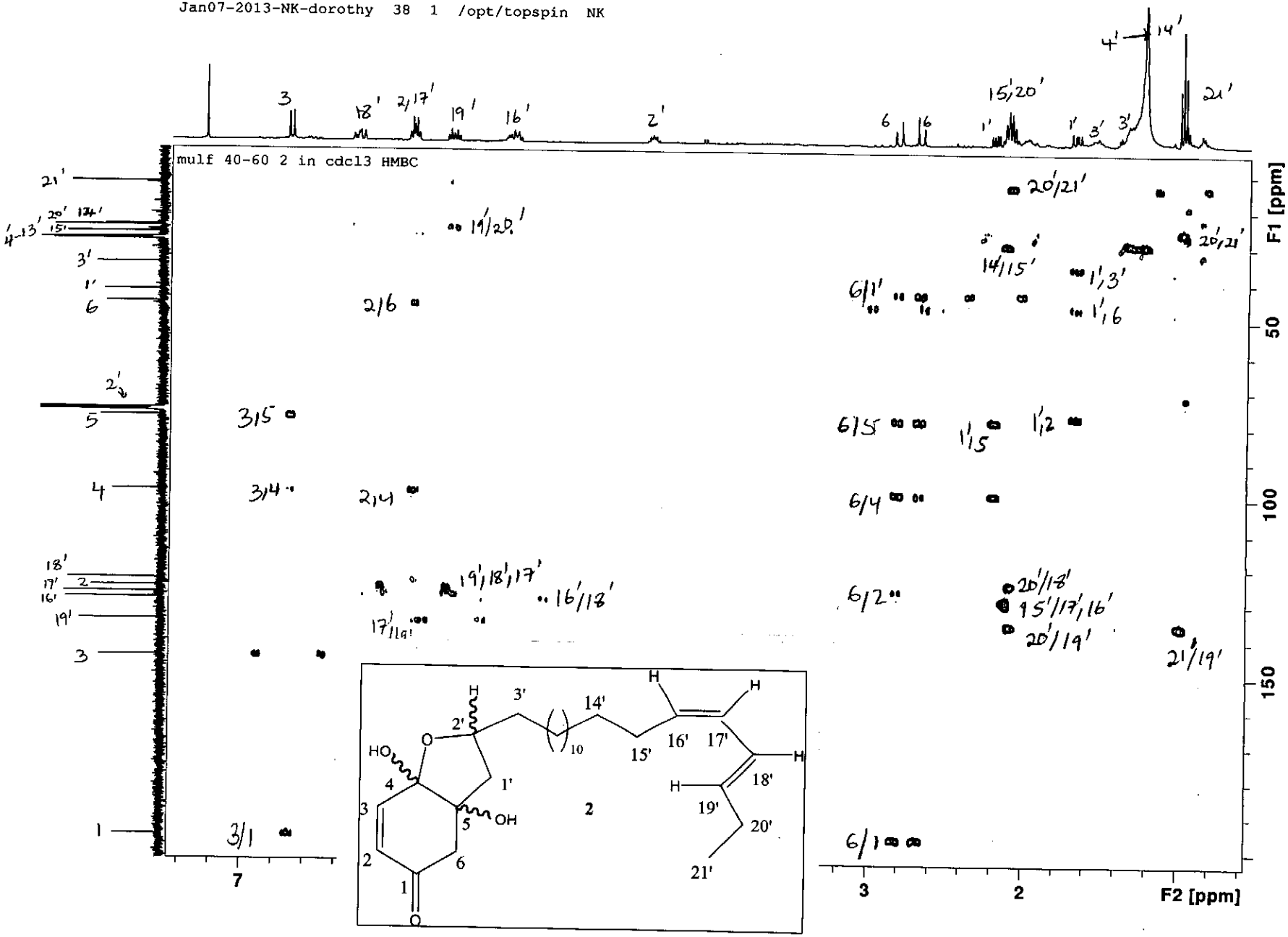
NOESY spectrum of B2



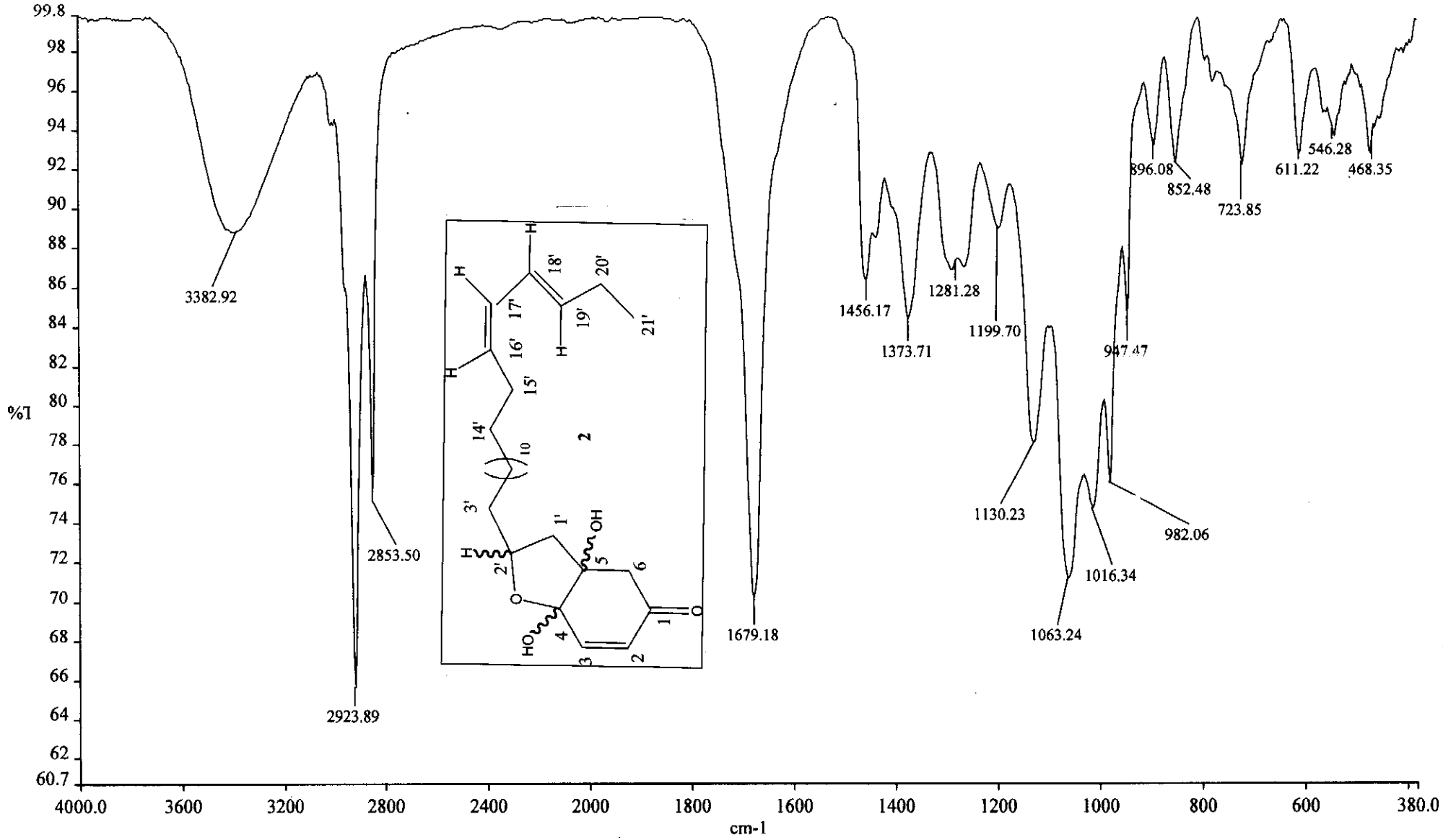
NOESY spectrum of B2 expanded (F1 0-3.1, F2 0-2.9 ppm)



HSQC spectrum of B2



HMBC spectrum of B2

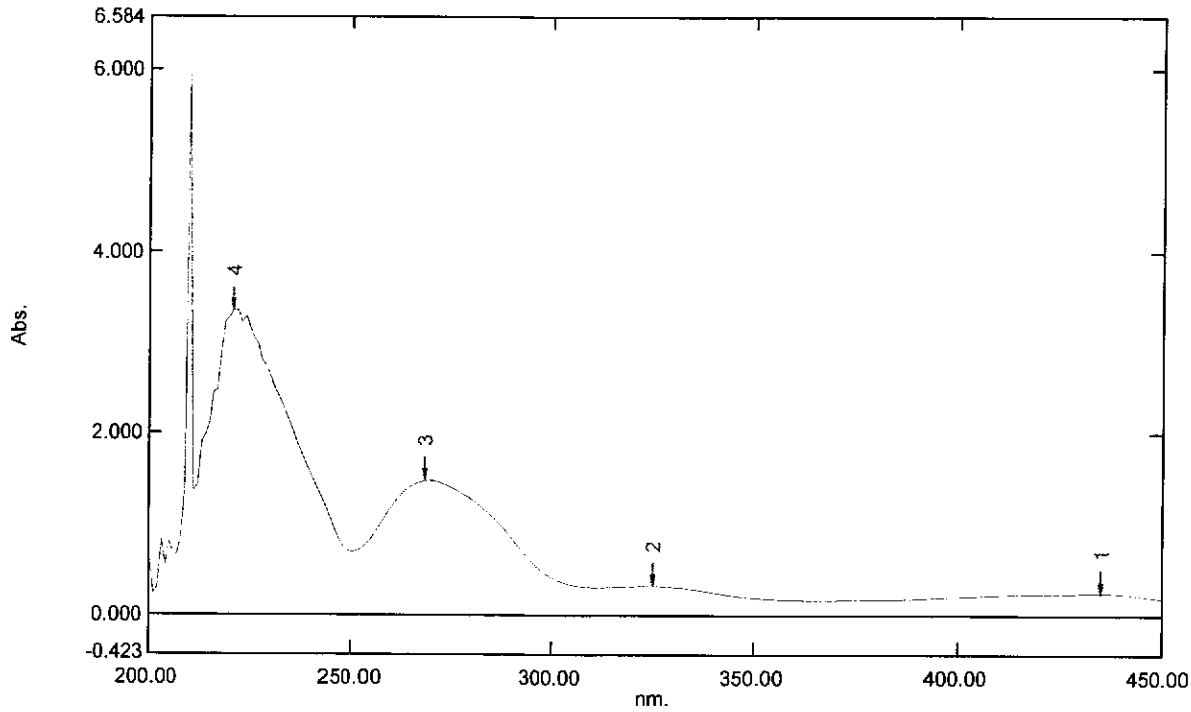


IR spectrum of B2

Spectrum Peak Pick Report

26/02/2013 03:57:02 PM

Data Set: 3.spc - Storage 155411



Measurement Properties	
Wavelength Range (nm.):	200.00 to 450.00
Scan Speed:	Medium
Sampling Interval:	1.0
Auto Sampling Interval:	Disabled
Scan Mode:	Single
Instrument Properties	
Instrument Type:	UV-3600 Series
Measuring Mode:	Absorbance
Slit Width:	1.0 nm
Time Constant:	0.1 sec.
Source Lamp:	Auto
Light Source Change Wavelength:	310.00 nm
Detector Unit:	Direct
Detector Change Wavelength:	830.00 nm 1800.00 nm
Grating Change Wavelength:	720.00 nm
S/R Exchange:	Normal
Detector Lock:	Auto
Slit Program:	Normal
Beam Mode:	Double
Stair Correction:	Disable
Attachment Properties	
Attachment:	None
Sample Preparation Properties	
Weight:	
Volume:	
Dilution:	
Path Length:	1 cm
Additional Information:	

No.	P/V	Wavelength	Abs.	Description
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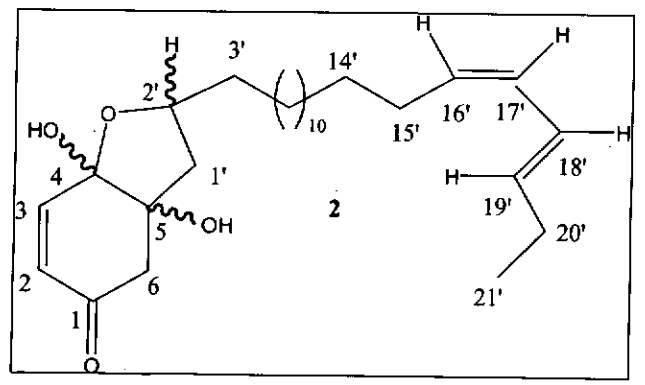
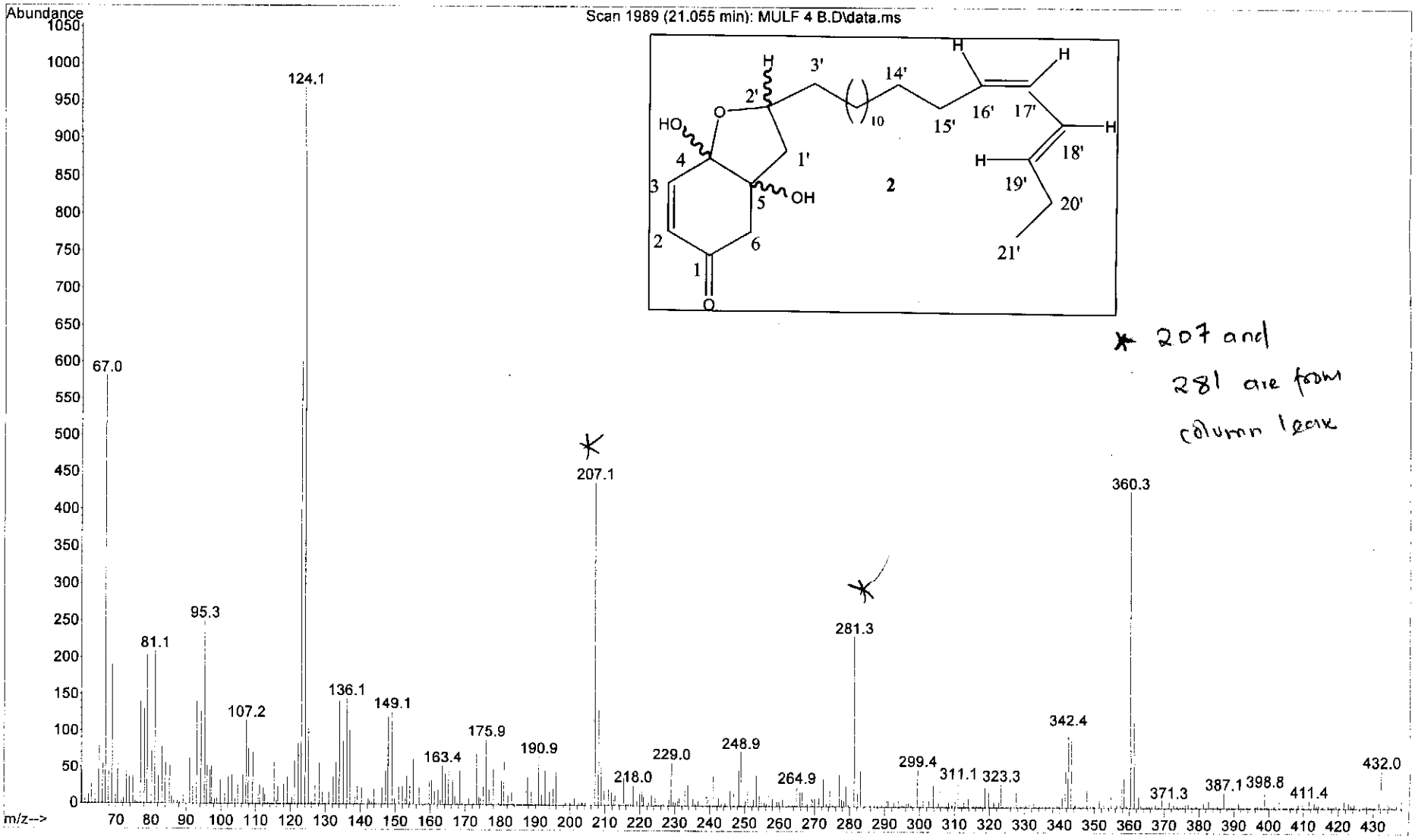
2	●	325.00	0.317	
3	●	268.00	1.476	
4	●	221.00	3.359	
5	◆	366.00	0.161	
6	◆	312.00	0.304	
7	◆	250.00	0.692	

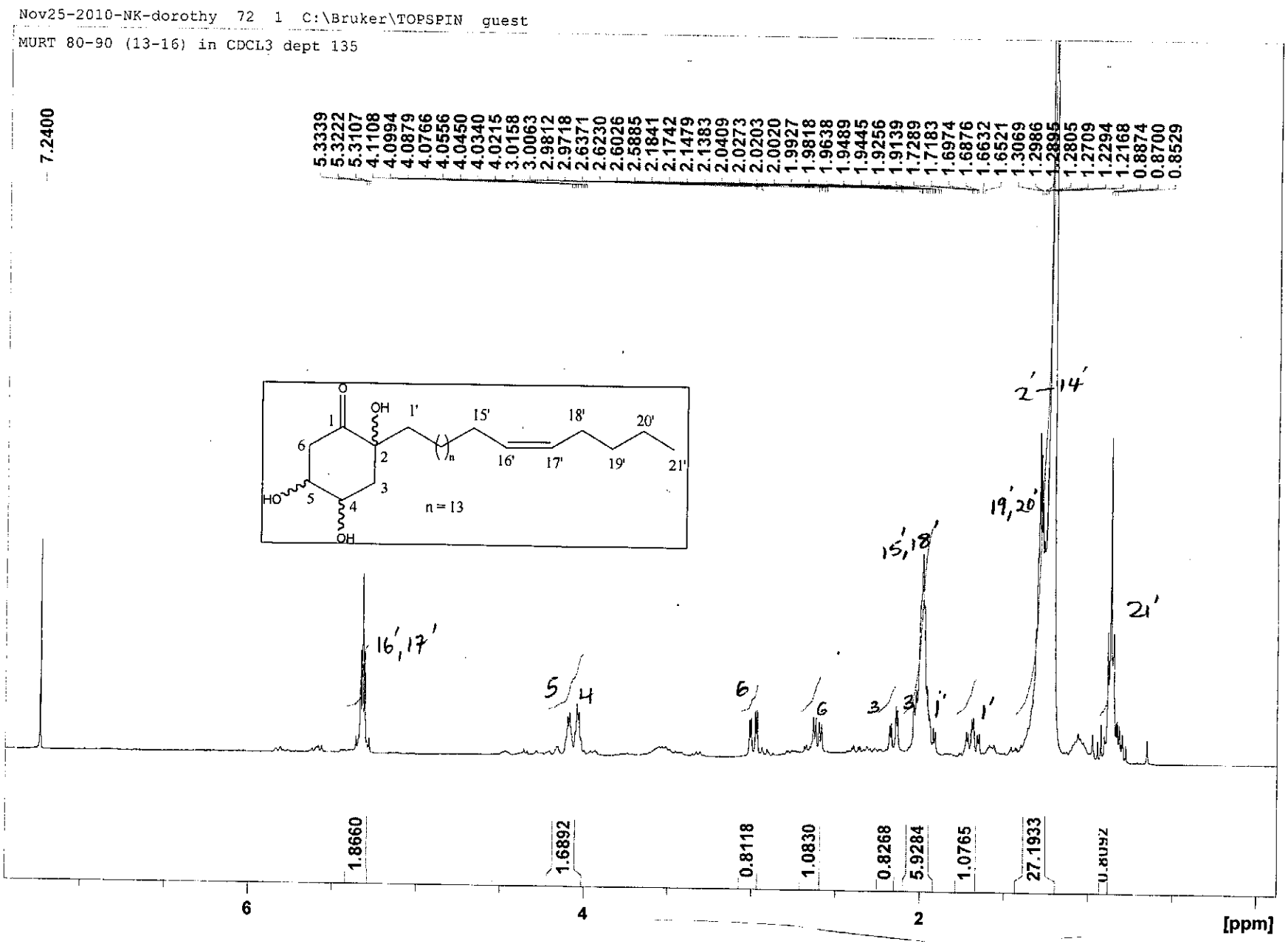


UV spectrum of B2

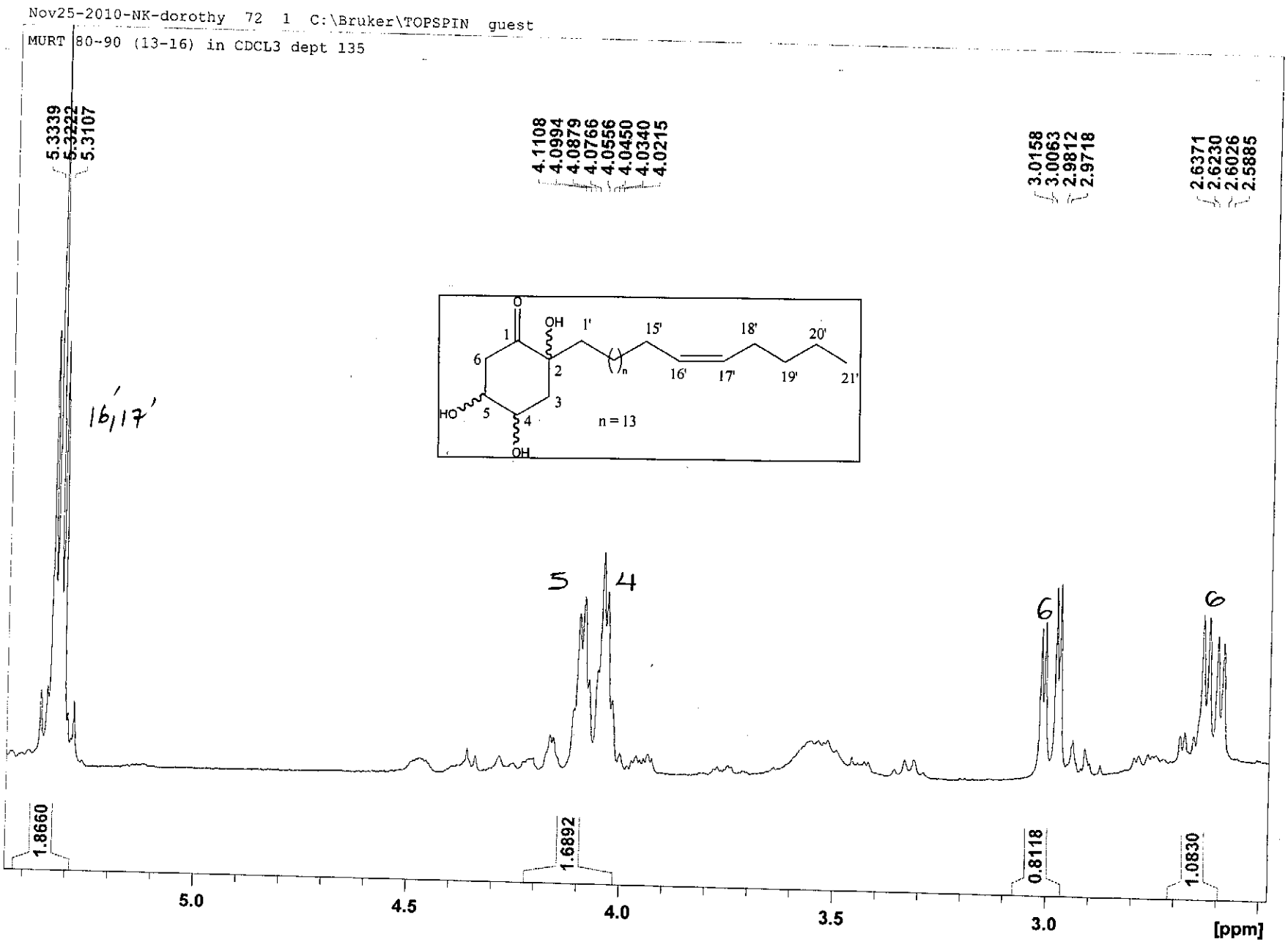
File :C:\msdchem\1\data\dorothy\MULF 4 B.D
Operator : Dorothy
Acquired : 27 Feb 2013 13:16 using AcqMethod NATPRODUCTS MANUAL INJ SPLIT.M
Instrument : 5973N
Sample Name: mulf 40-60 2a
Misc Info :
Vial Number: 1



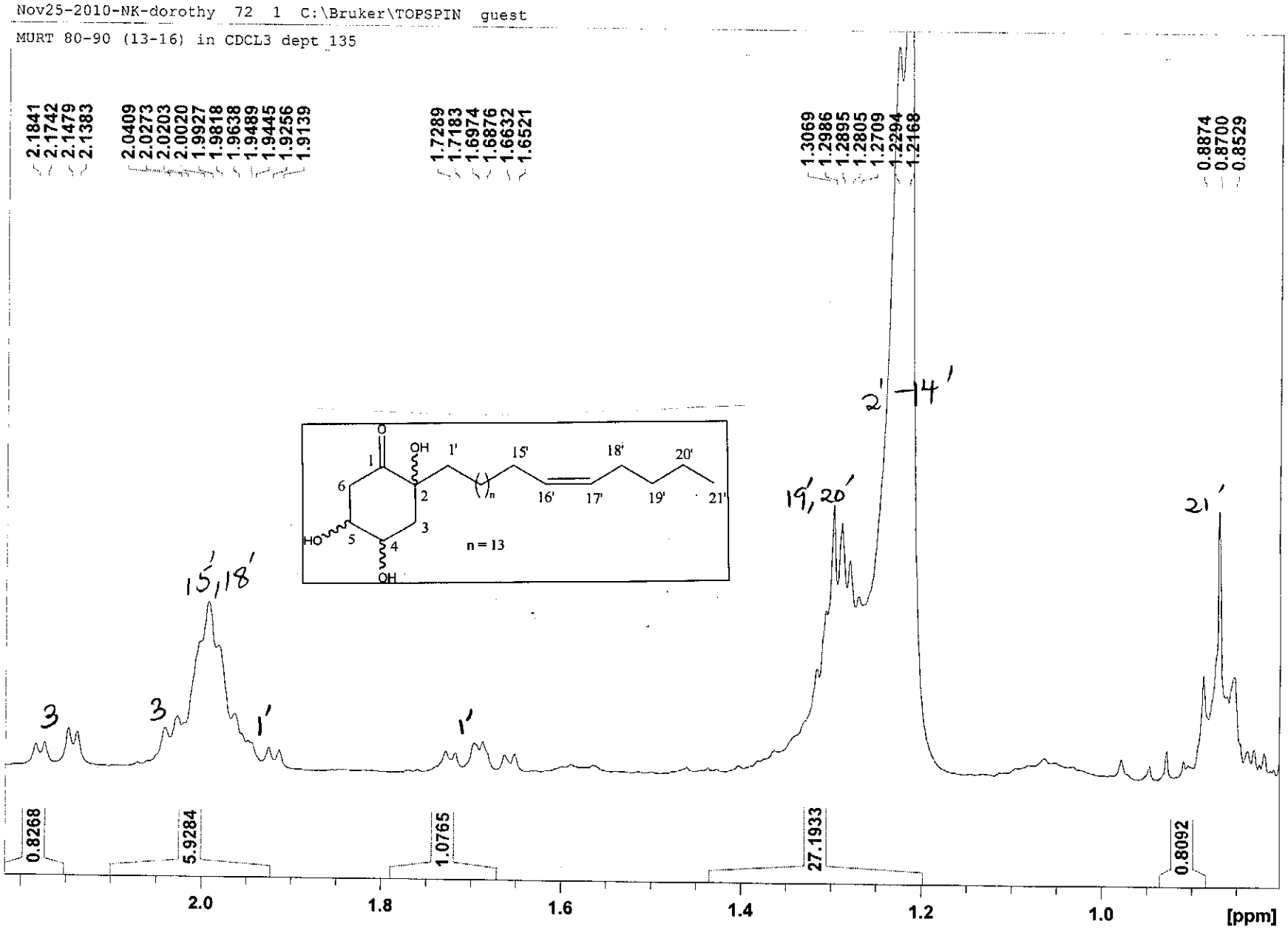
MS spectrum of B2



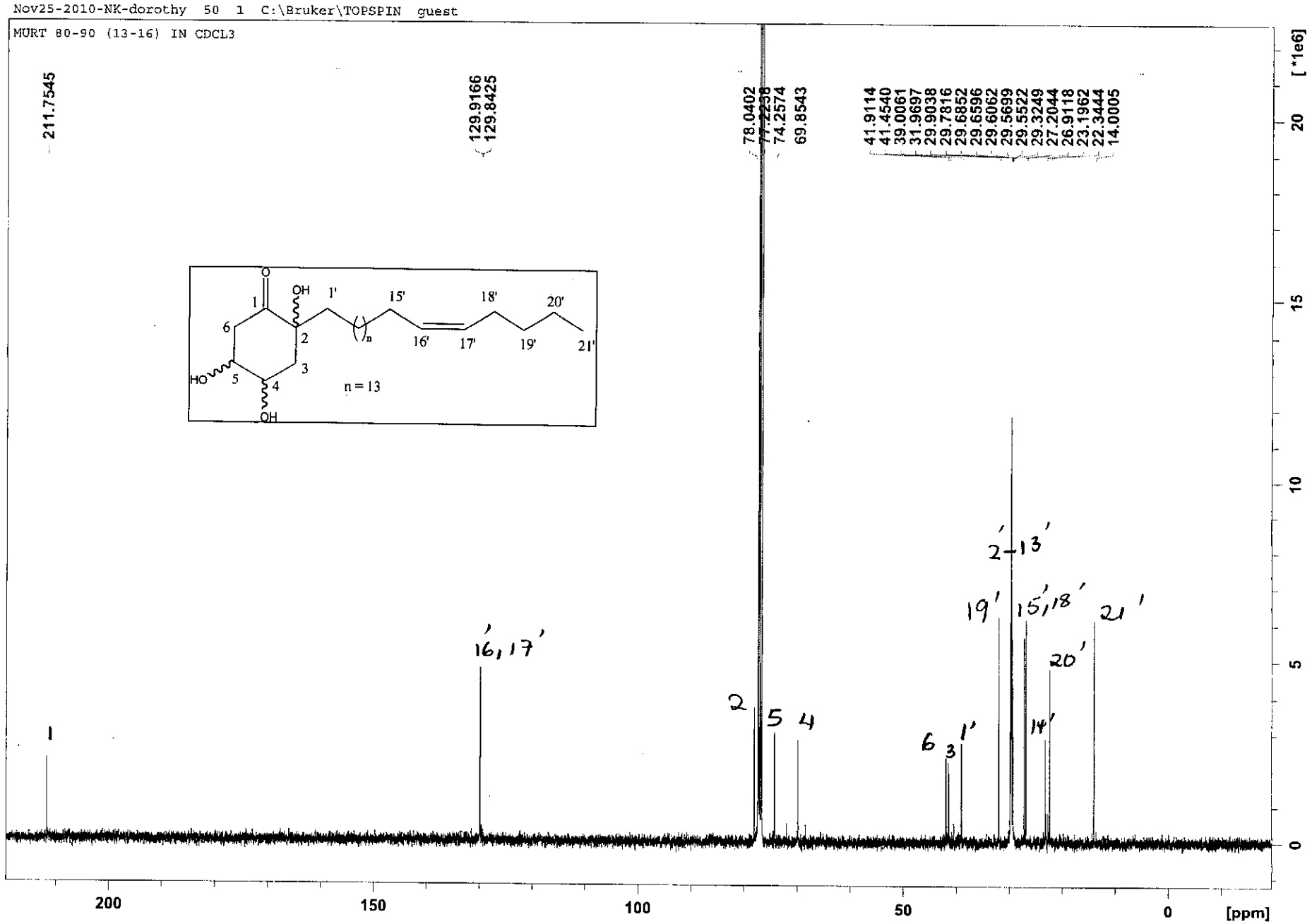
^{1}H NMR spectrum of B3



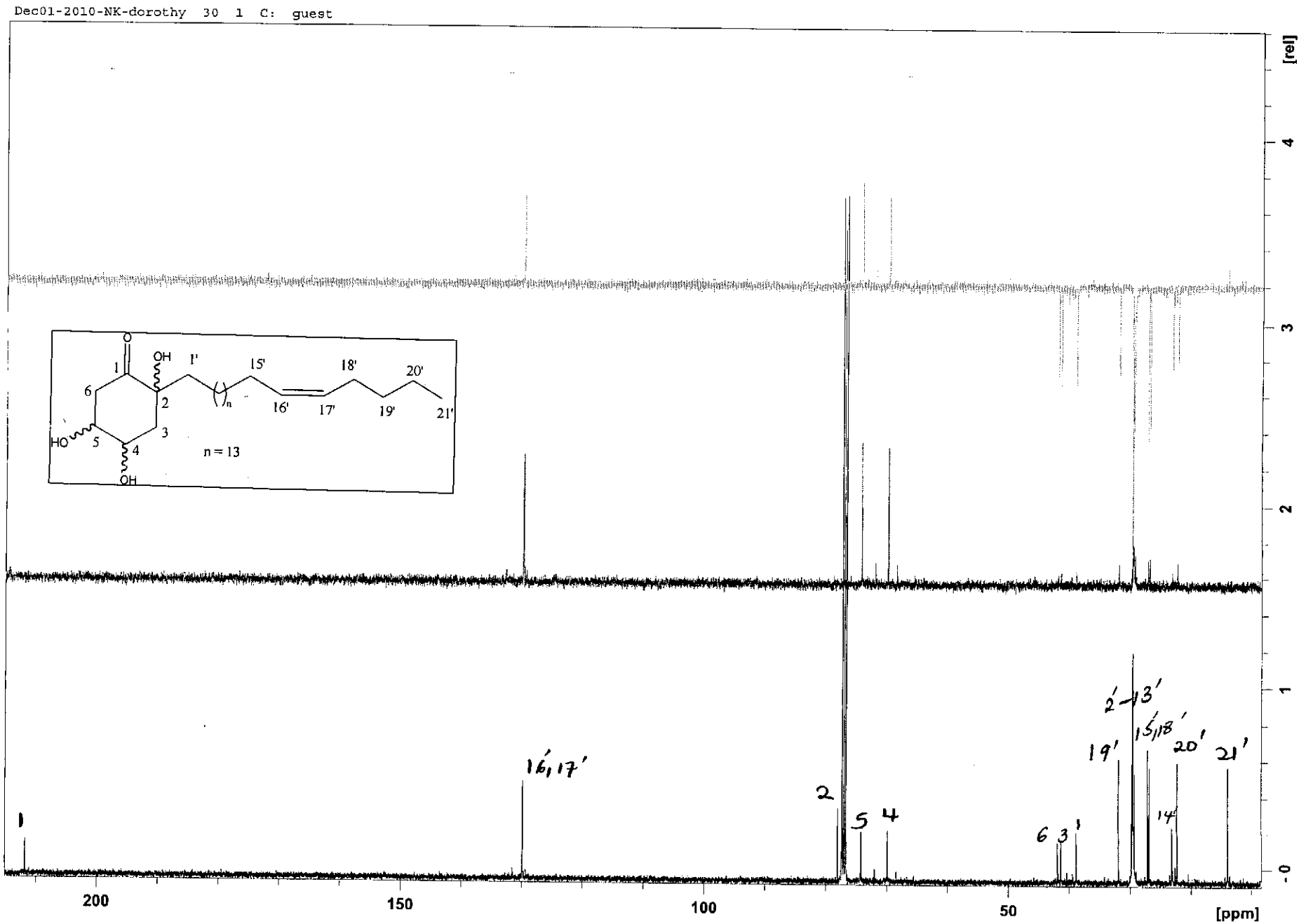
^{1}H NMR spectrum of B3 expanded (2.5-5.4 ppm)



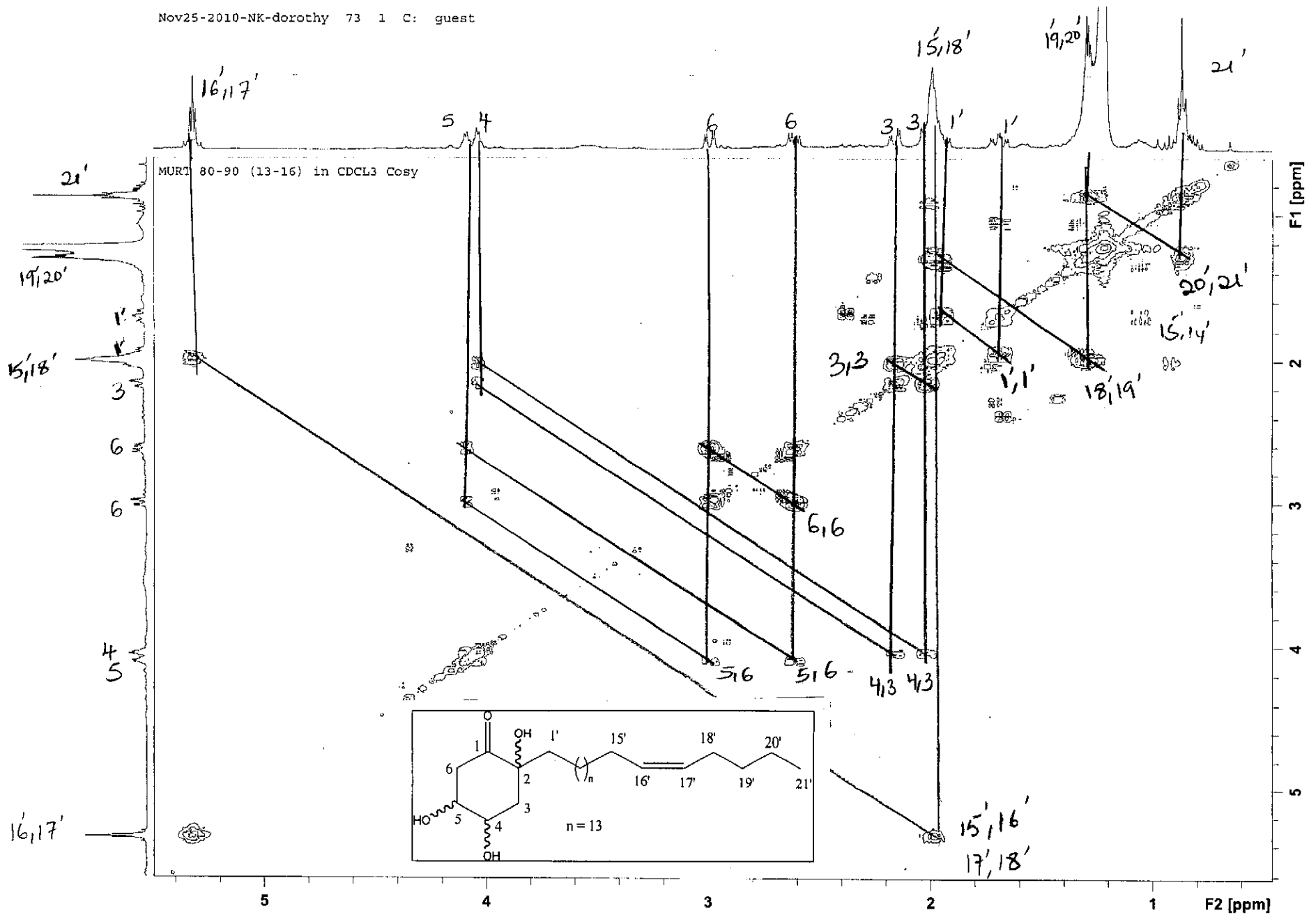
^{1}H NMR spectrum of B3 expanded (0.6-2.4 ppm)



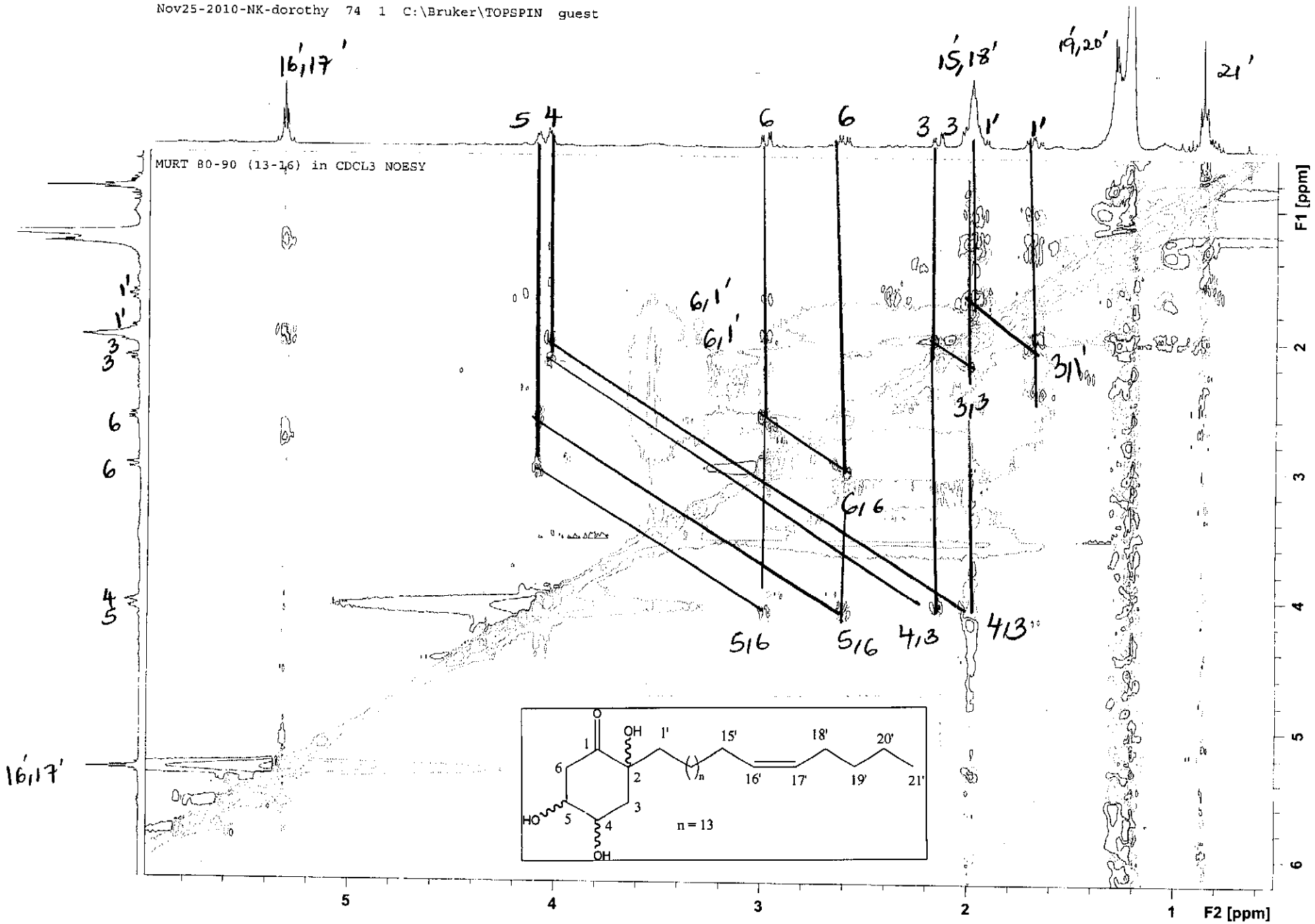
^{13}C NMR spectrum of B3



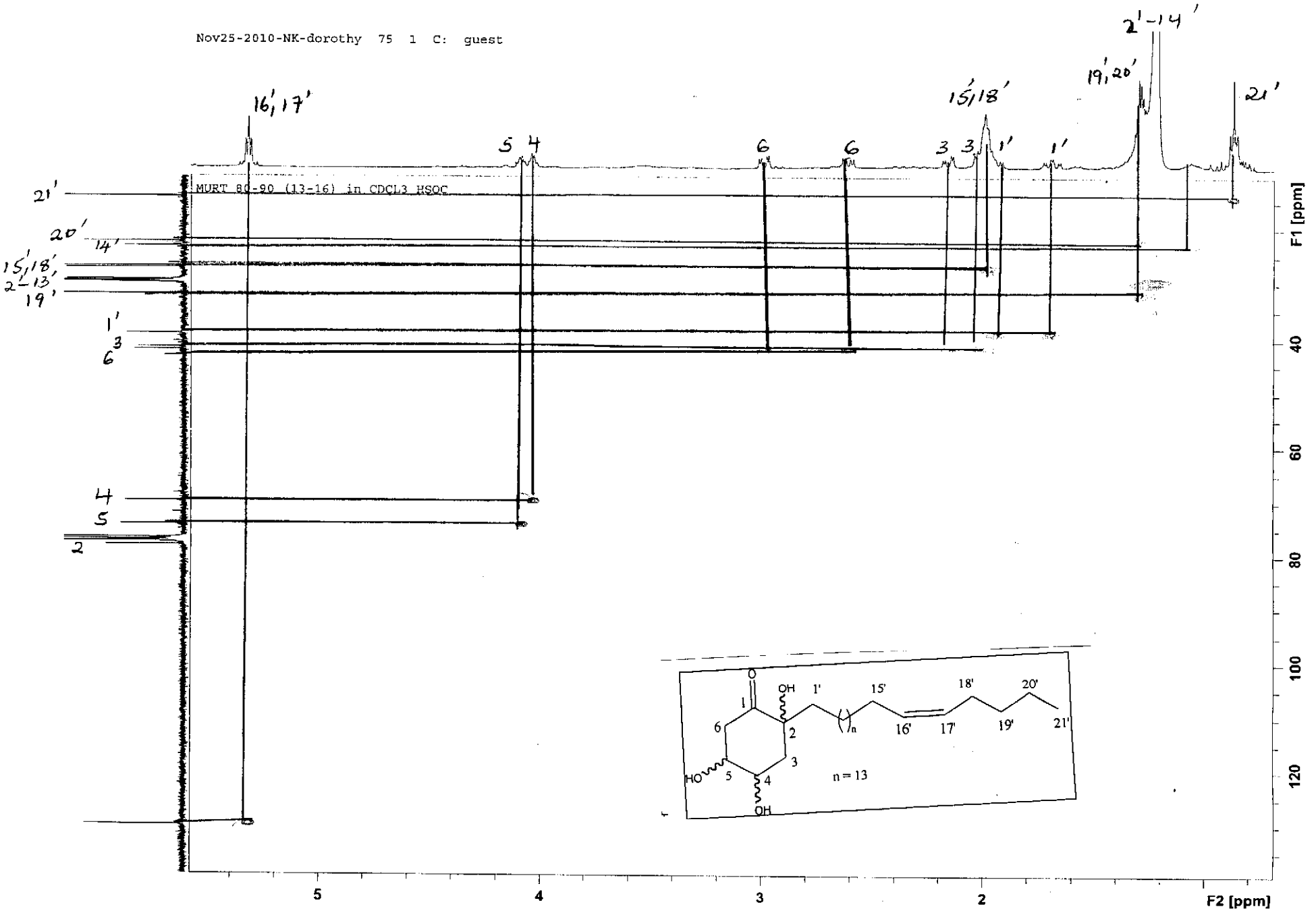
DEPT spectrum of B3



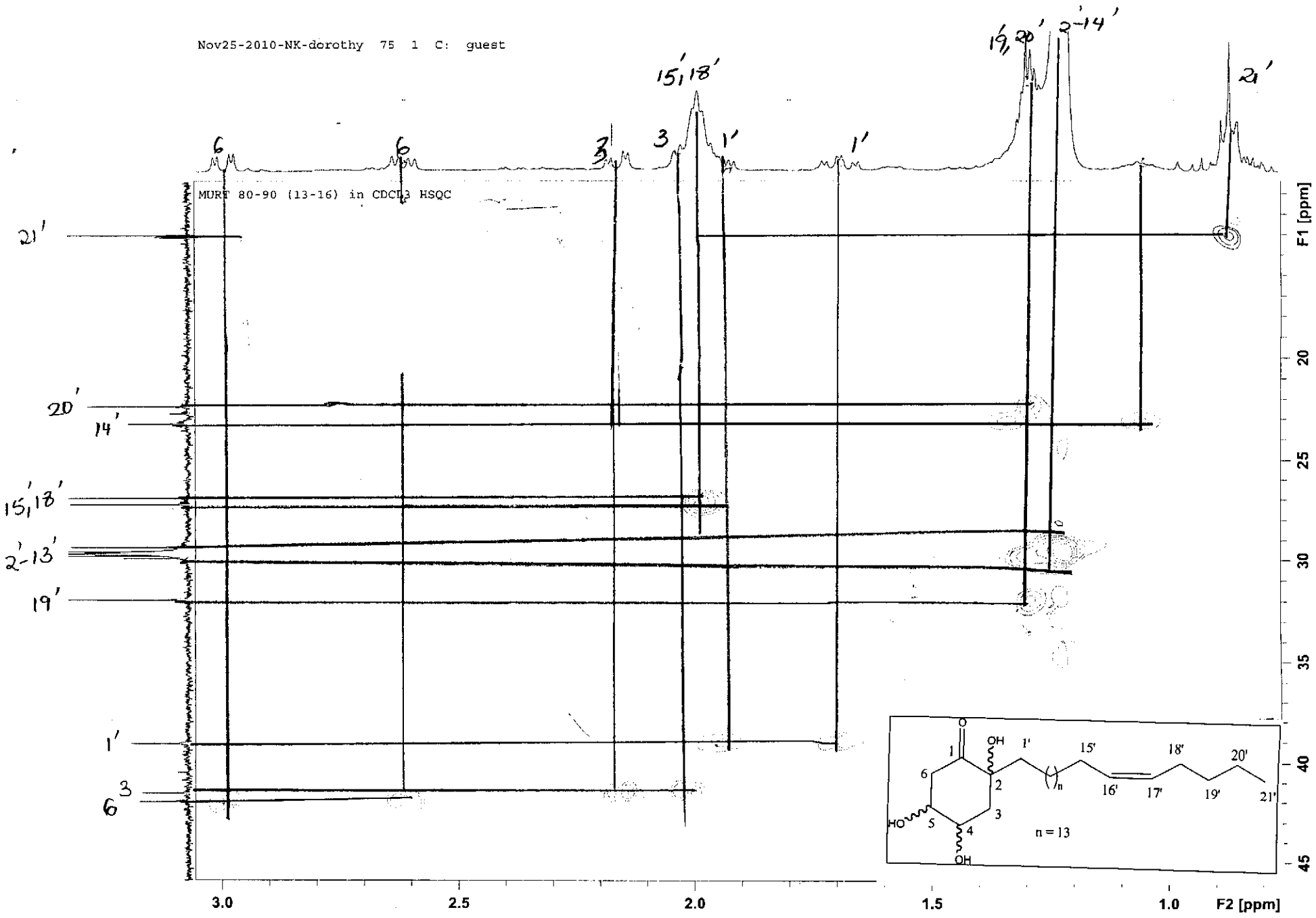
COSY spectrum of B3



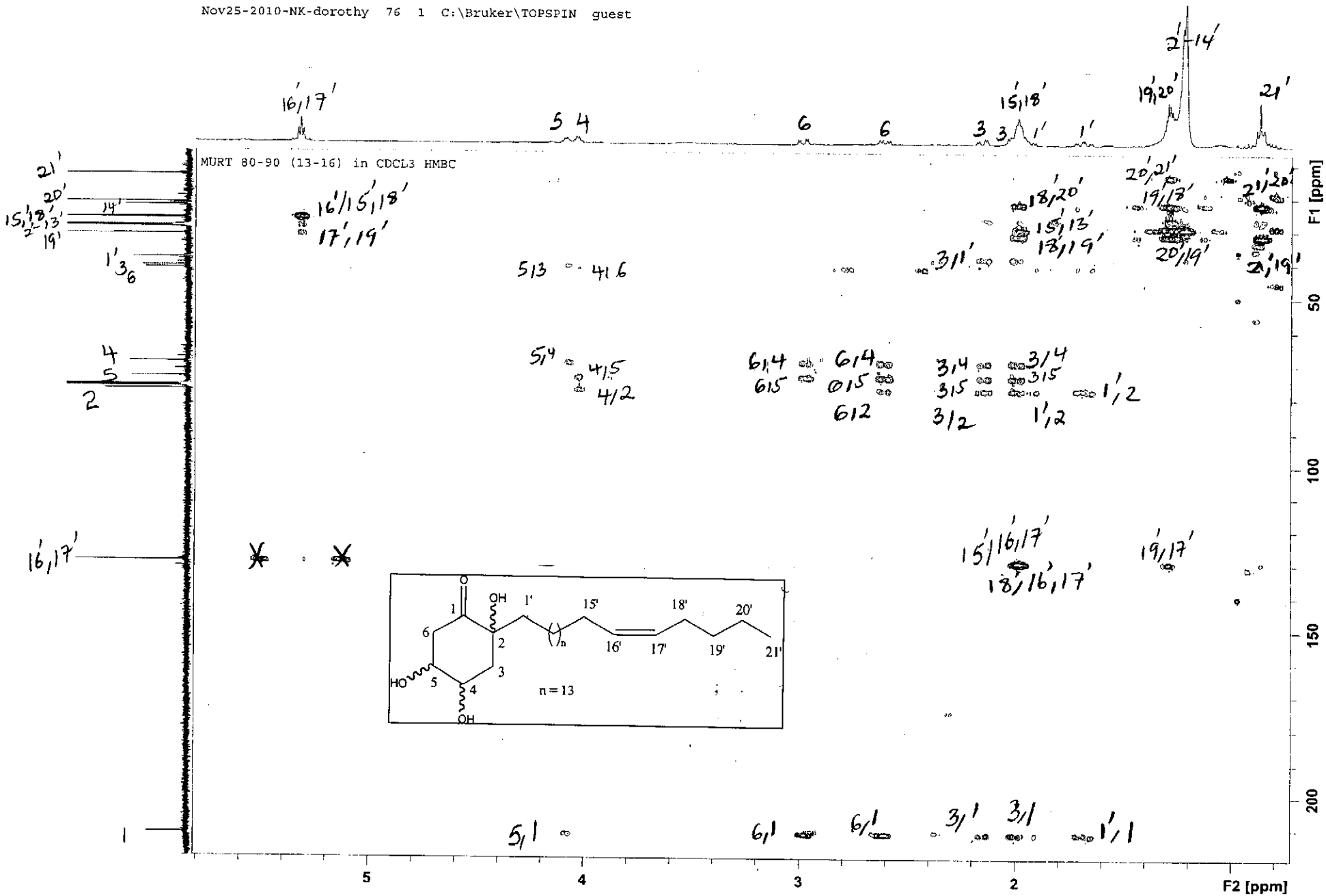
NOESY spectrum of B3



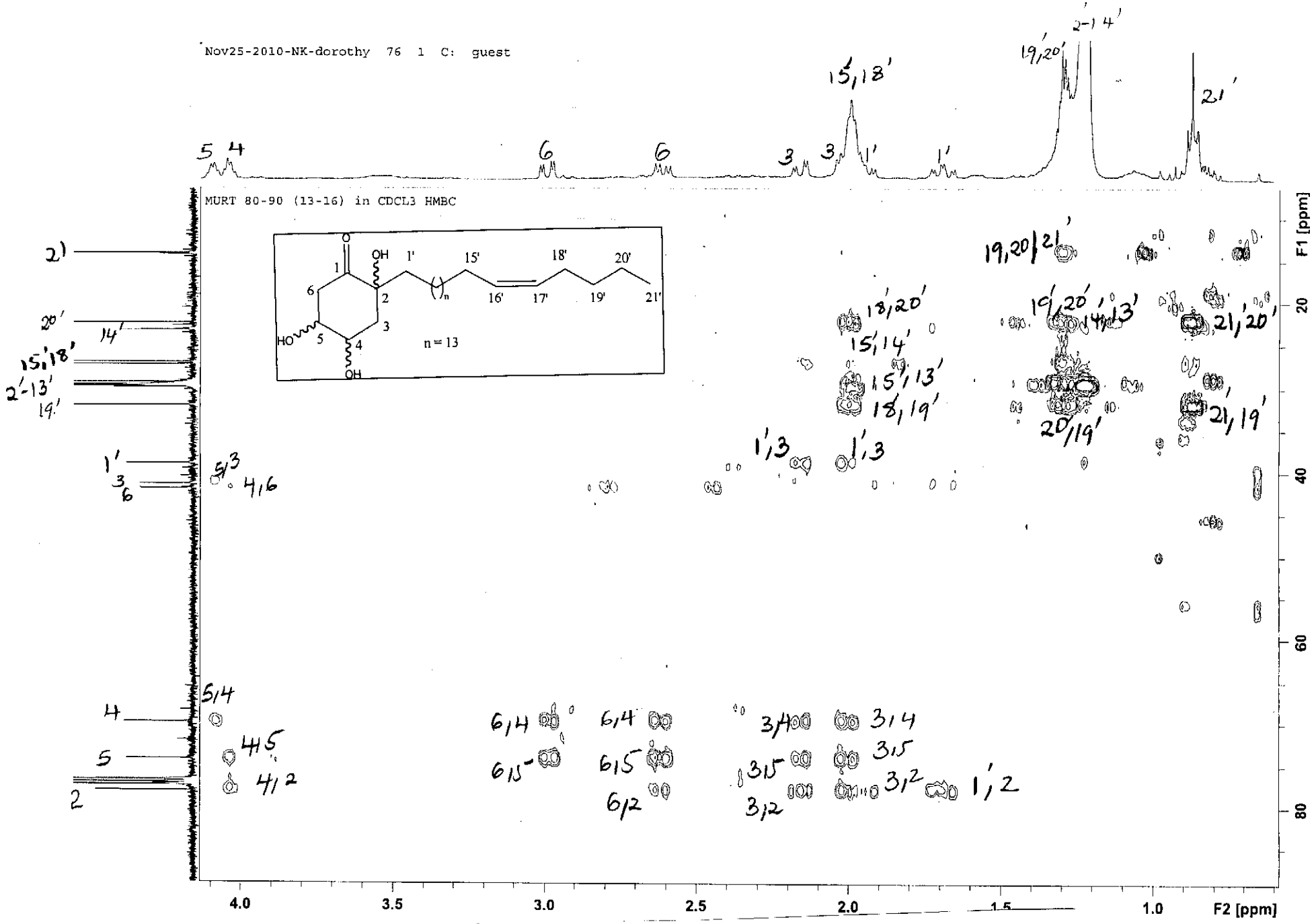
HSQC spectrum of B3



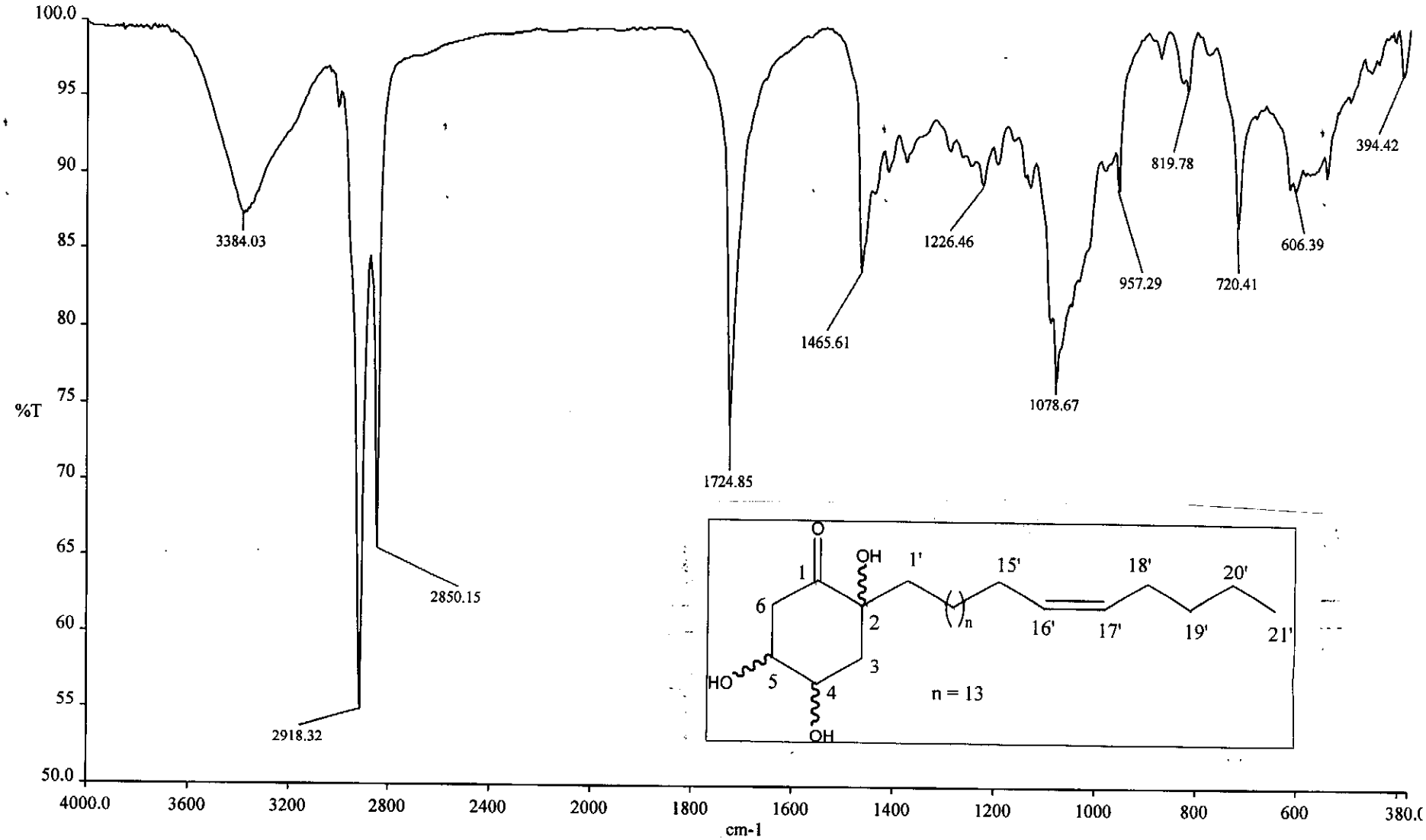
HSQC spectrum of B3 (F1 15-45 ppm, F2 0.8-3 ppm)



HMBC spectrum of B3



HMBC spectrum of B3 (F1 15-85 ppm, F2 0.5-4.1 ppm)

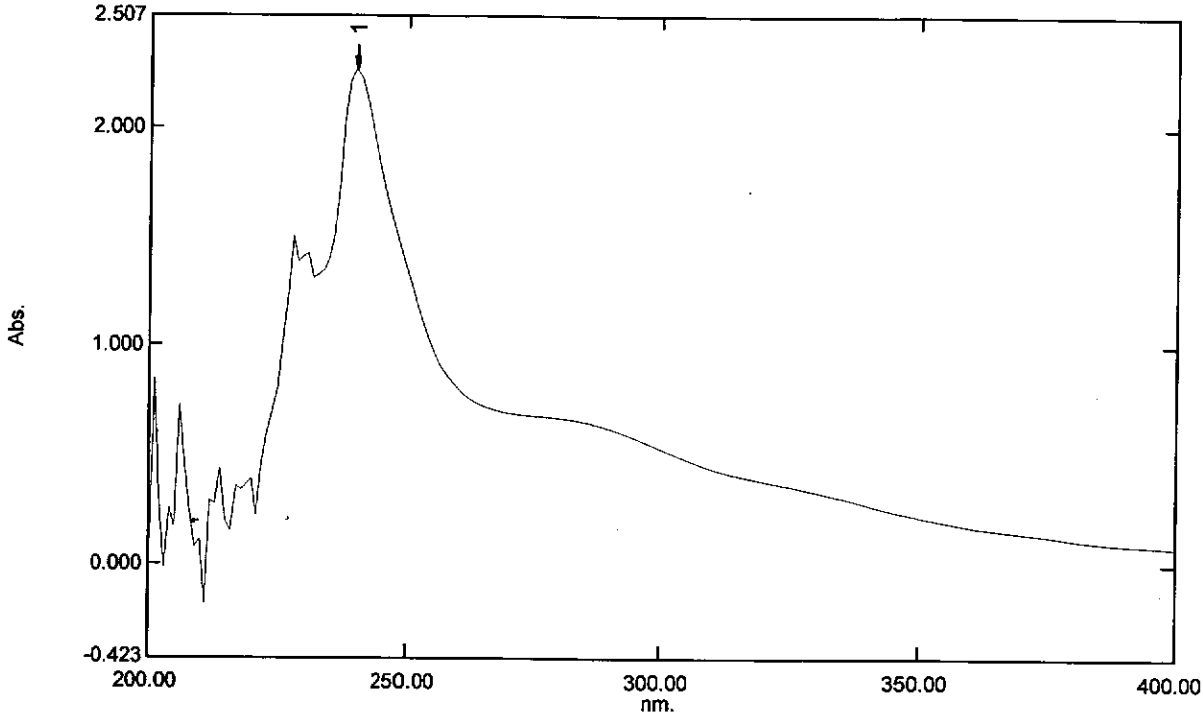


IR spectrum of B3

Spectrum Peak Pick Report

23/05/2012 06:22:28 PM

Data Set: murt 80-90 2.spc - Storage 165117



No.	P/V	Wavelength	Abs.	Description
1		240.00	2.263	

Measurement Properties

Wavelength Range (nm.):	200.00 to 400.00
Scan Speed:	Medium
Sampling Interval:	1.0
Auto Sampling Interval:	Disabled
Scan Mode:	Single

Instrument Properties

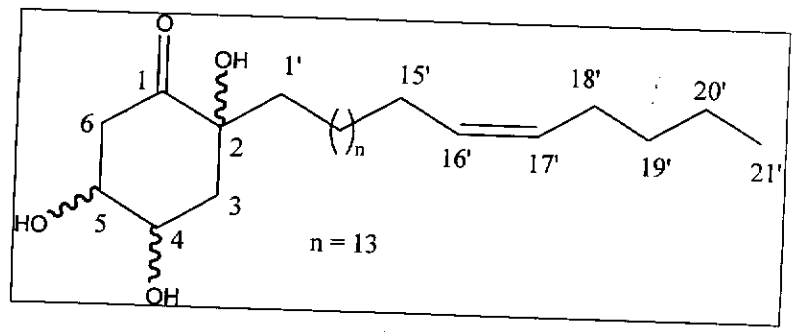
Instrument Type:	UV-3600 Series
Measuring Mode:	Absorbance
Slit Width:	2.0 nm
Time Constant:	0.1 sec.
Source Lamp:	Auto
Light Source Change Wavelength:	310.00 nm
Detector Unit:	Direct
Detector Change Wavelength:	830.00 nm 1800.00 nm
Grating Change Wavelength:	720.00 nm
S/R Exchange:	Normal
Detector Lock:	Auto
Slit Program:	Normal
Beam Mode:	Double
Stair Correction:	Disable

Attachment Properties

Attachment:	None

Sample Preparation Properties

Weight:	
Volume:	
Dilution:	
Path Length:	
Additional Information:	



UV spectrum of B3

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Operator   : DOROTHY
Acquired   : 19 Jun 2011  19:41     using AcqMethod NATURAL
Instrument :   Instrumen
Sample Name: murt 80-90 8-12
Misc Info  :
Vial Number: 1
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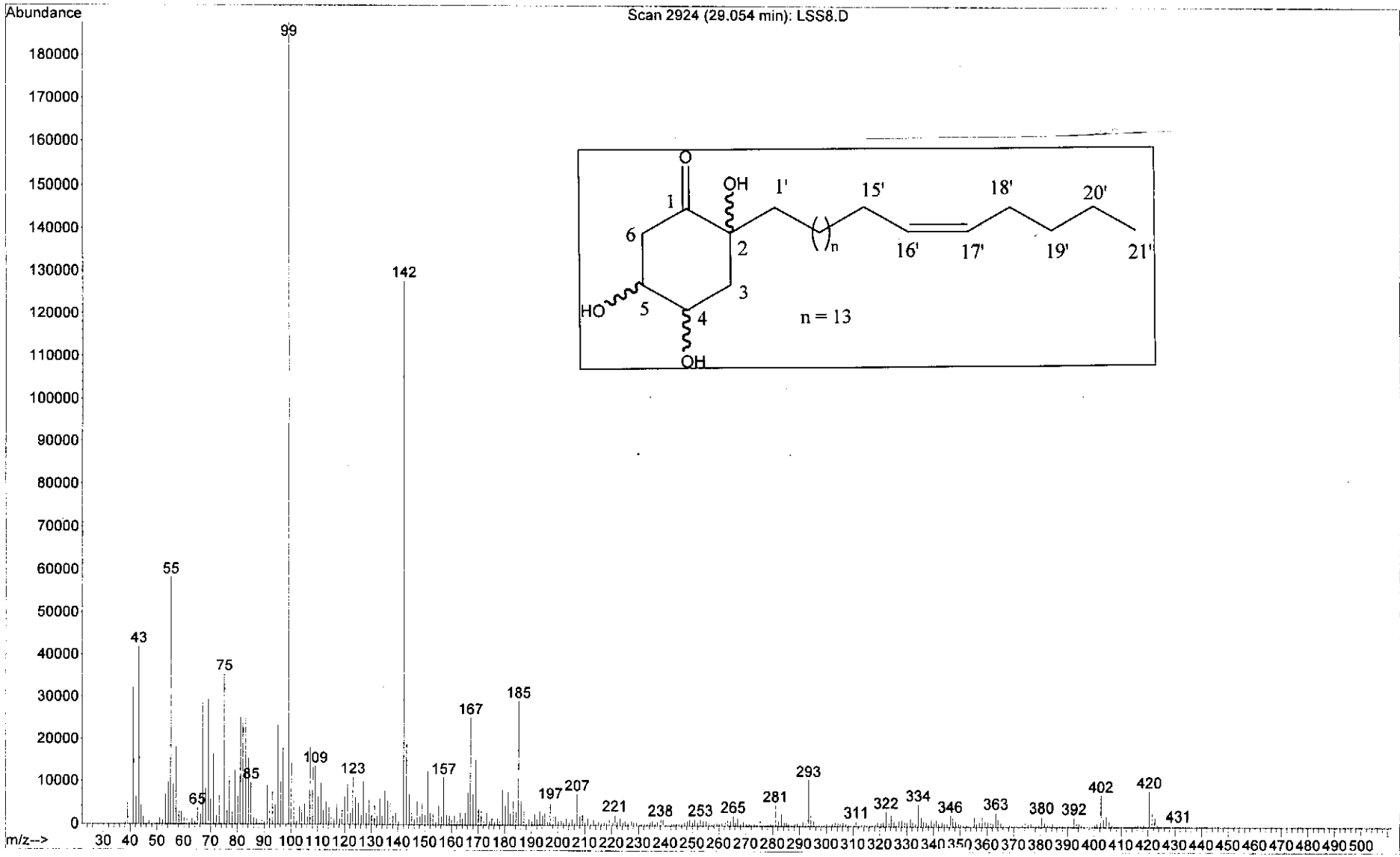
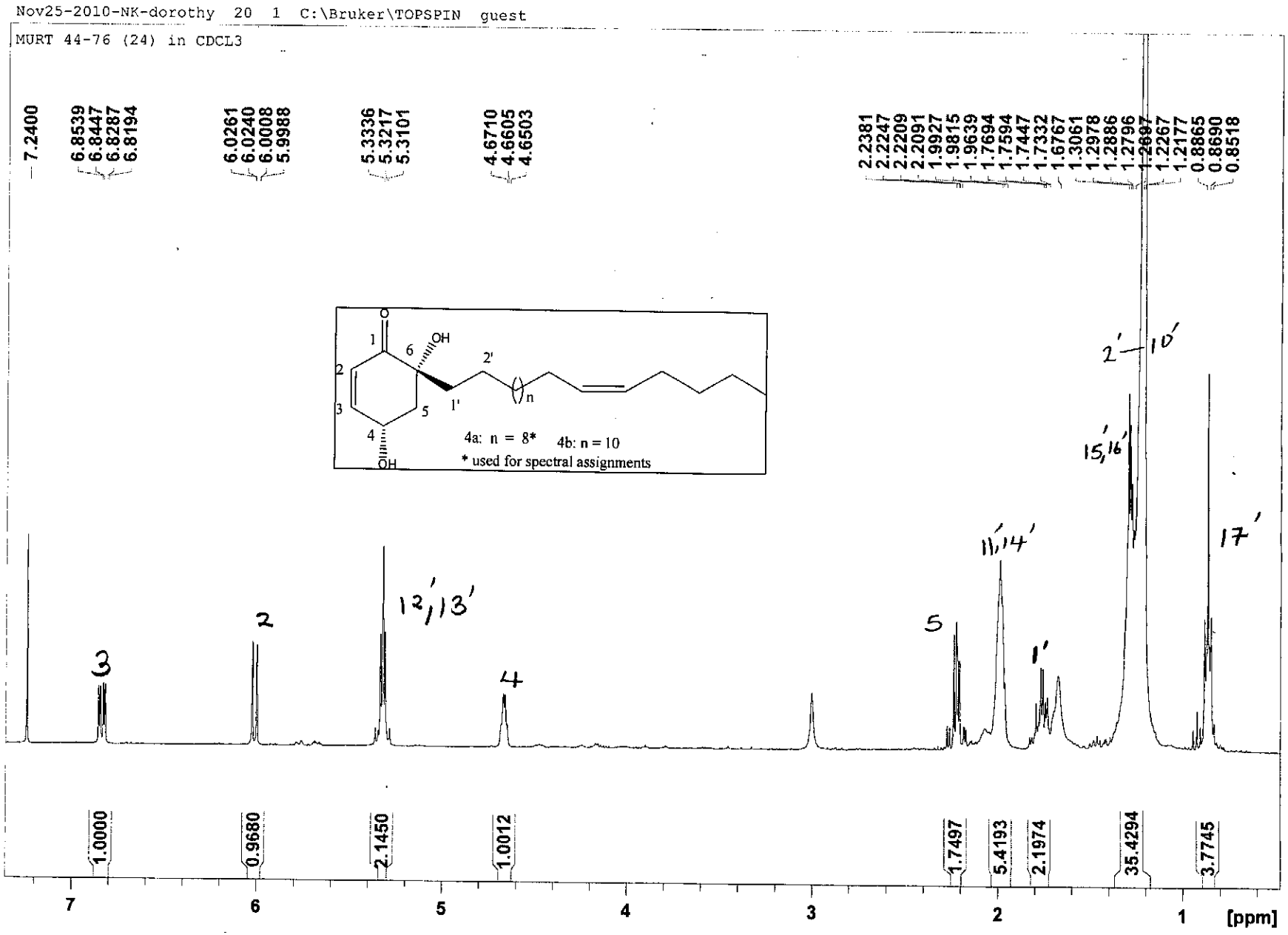
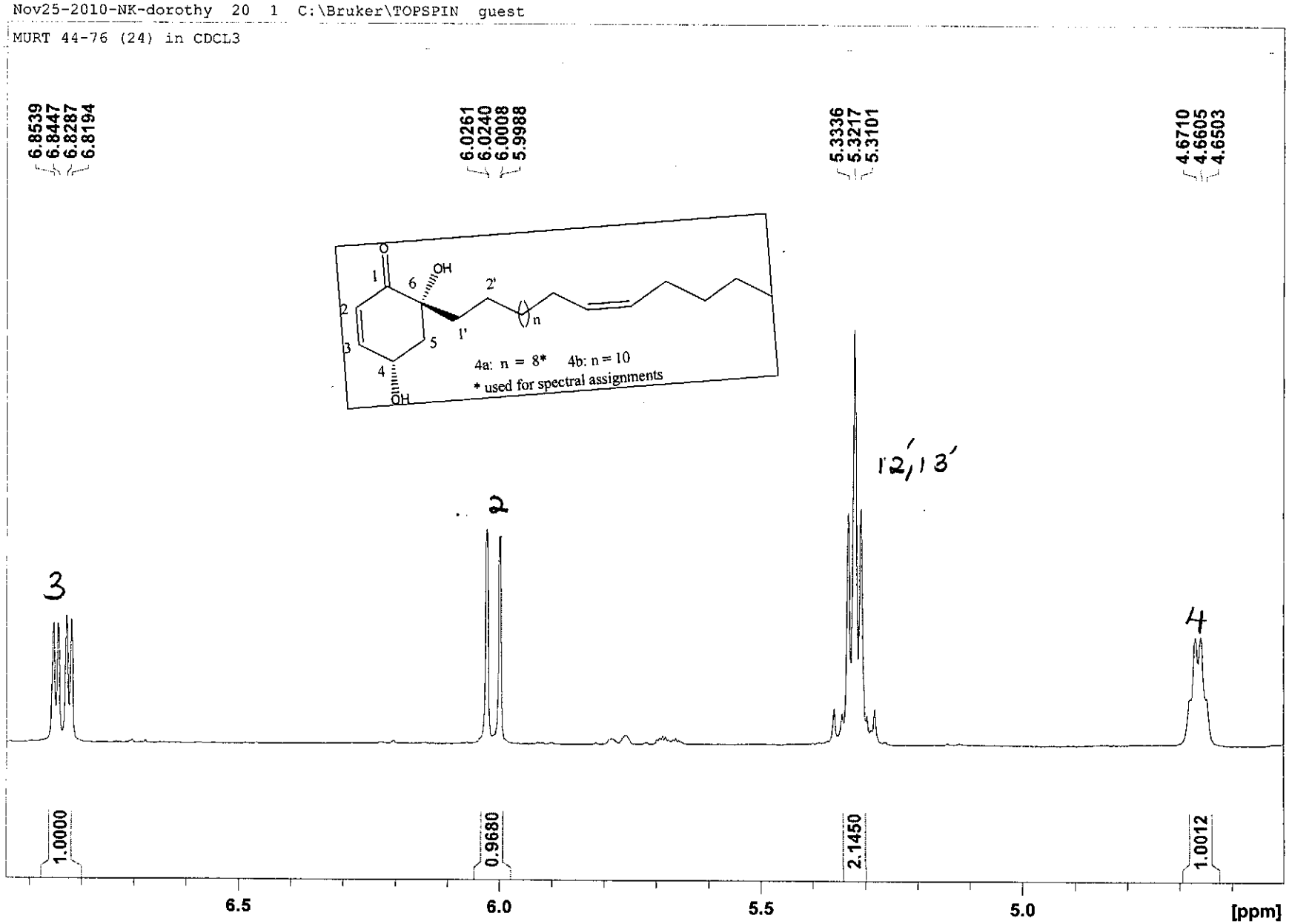


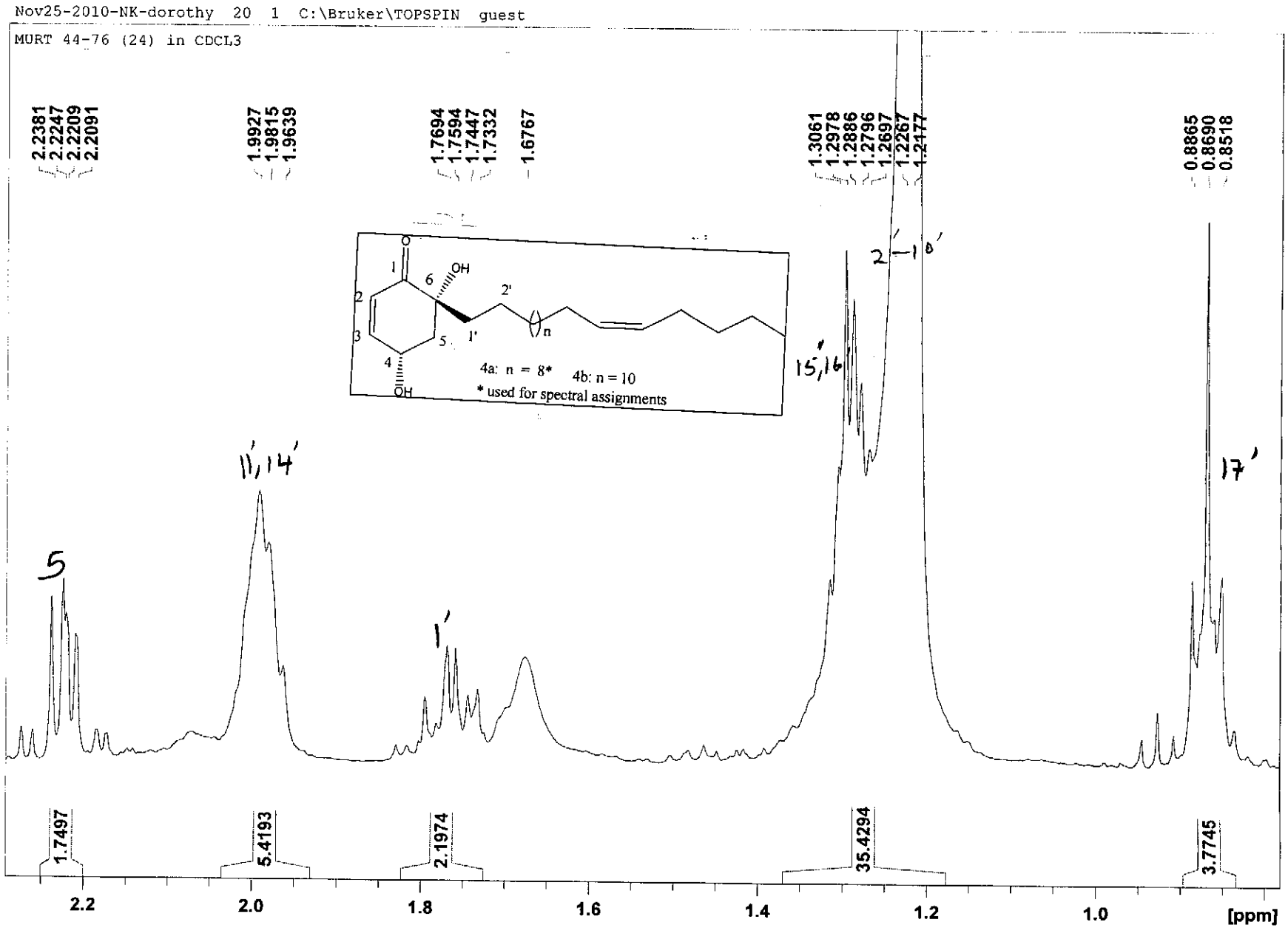
MS spectrum of B3



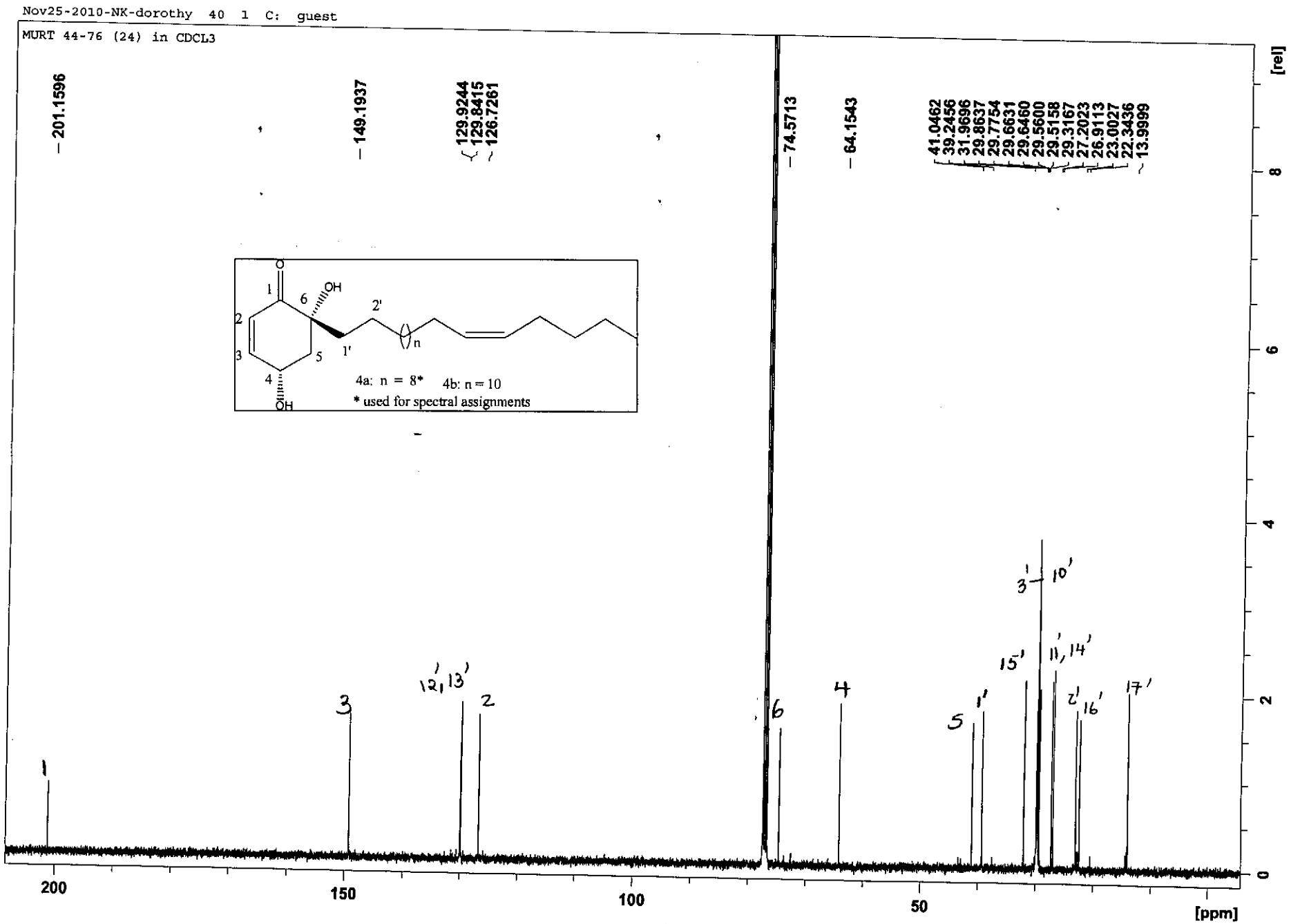
^{1}H NMR spectrum of B4 (mixture of a and b)



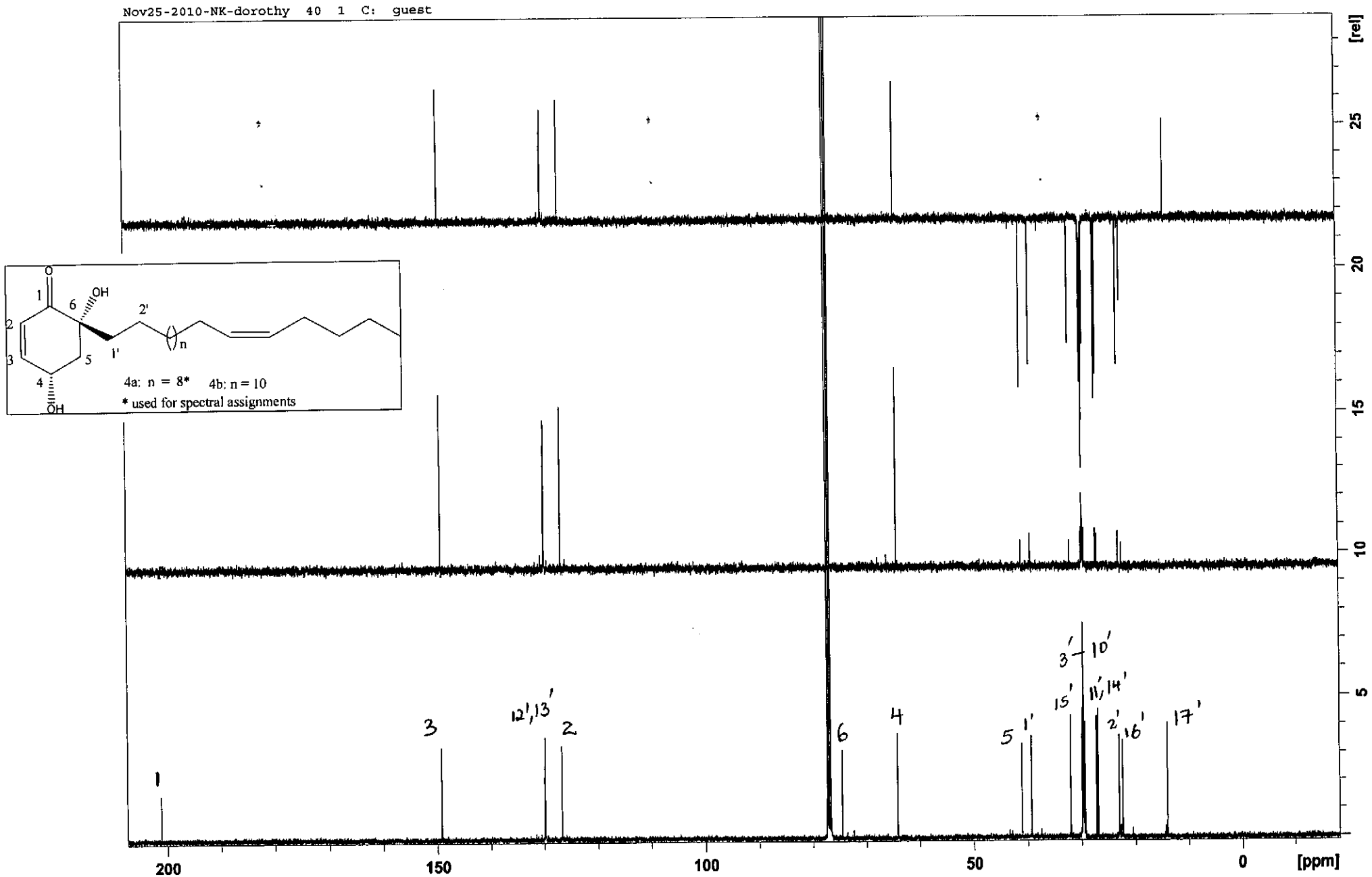
^{1}H NMR spectrum of B4 expanded 4.6-6.9 ppm (mixture of a and b)



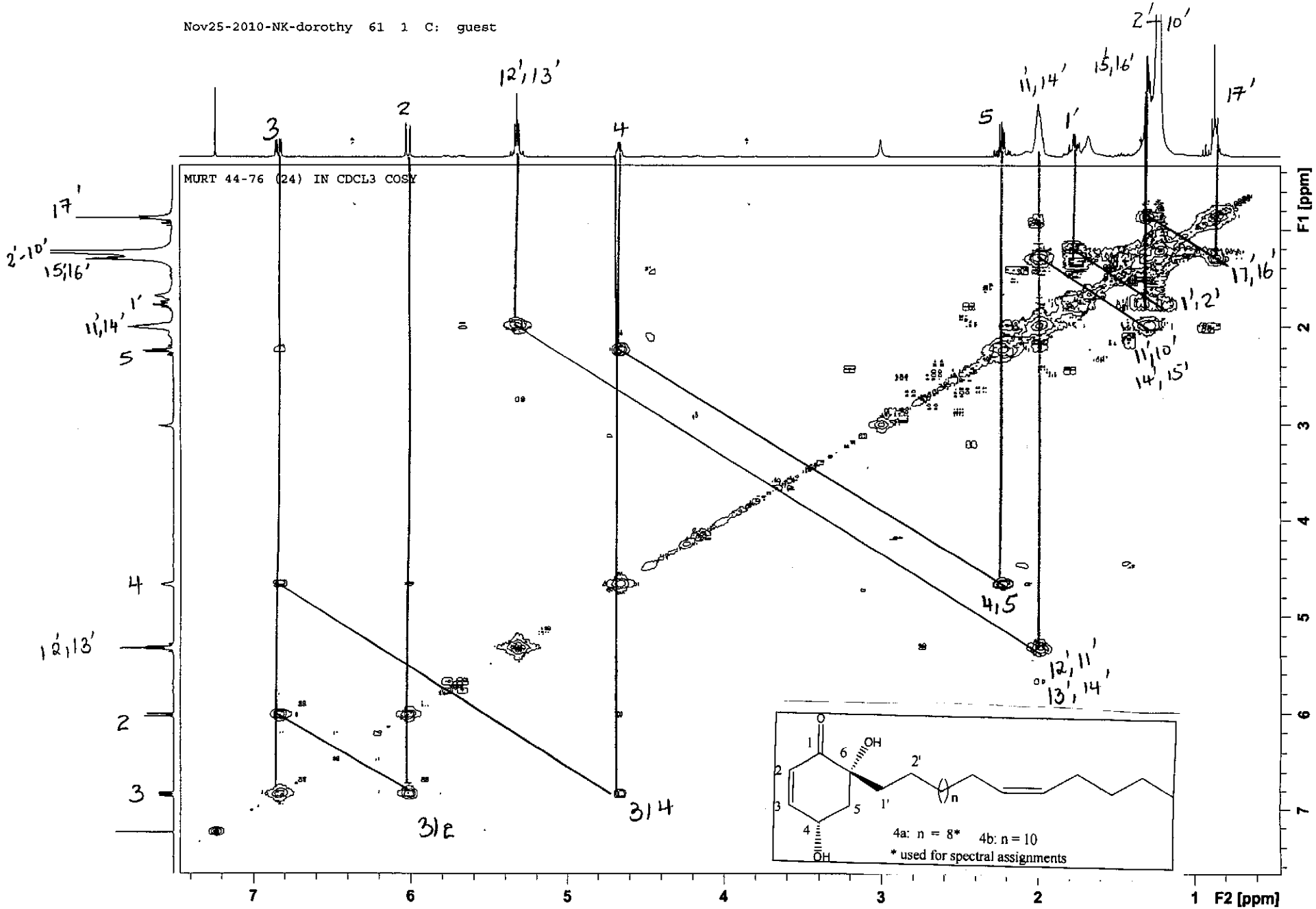
^{1}H NMR spectrum of B4 expanded 0.6-2.3 ppm (mixture of a and b)



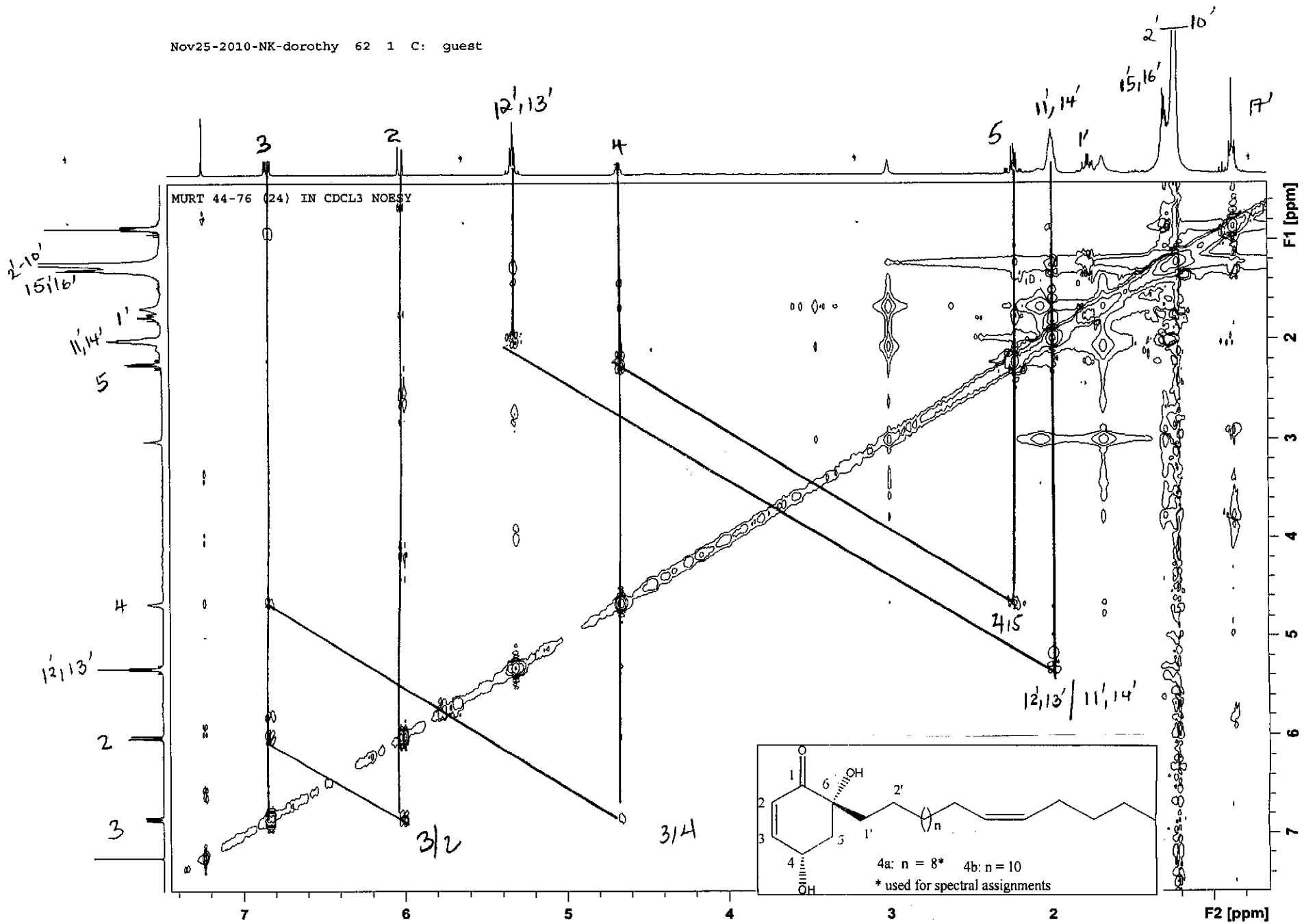
^{13}C NMR spectrum of B4 (mixture of a and b)



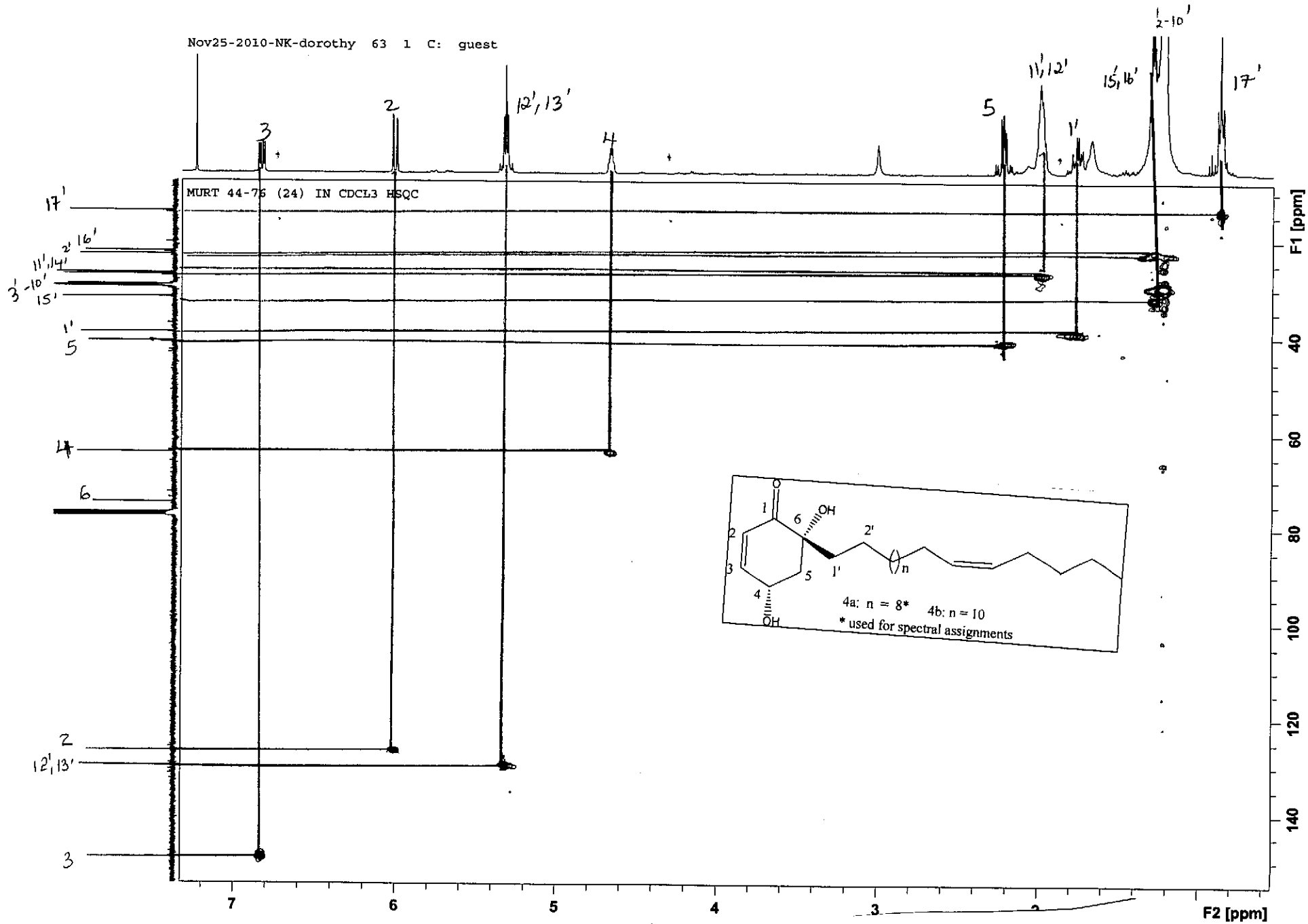
DEPT spectrum of B4 (mixture of a and b)



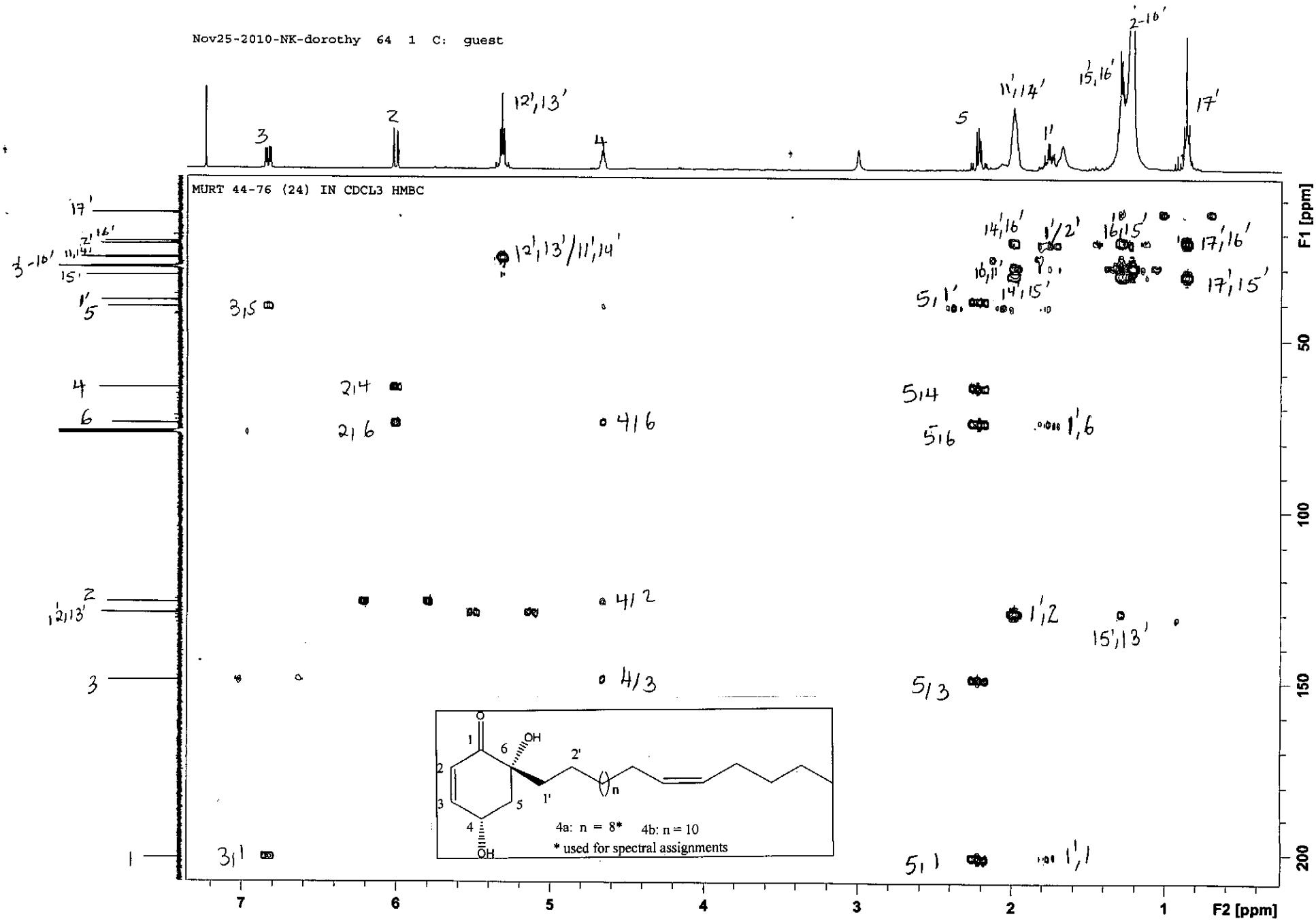
COSY spectrum of B4 (mixture of a and b)



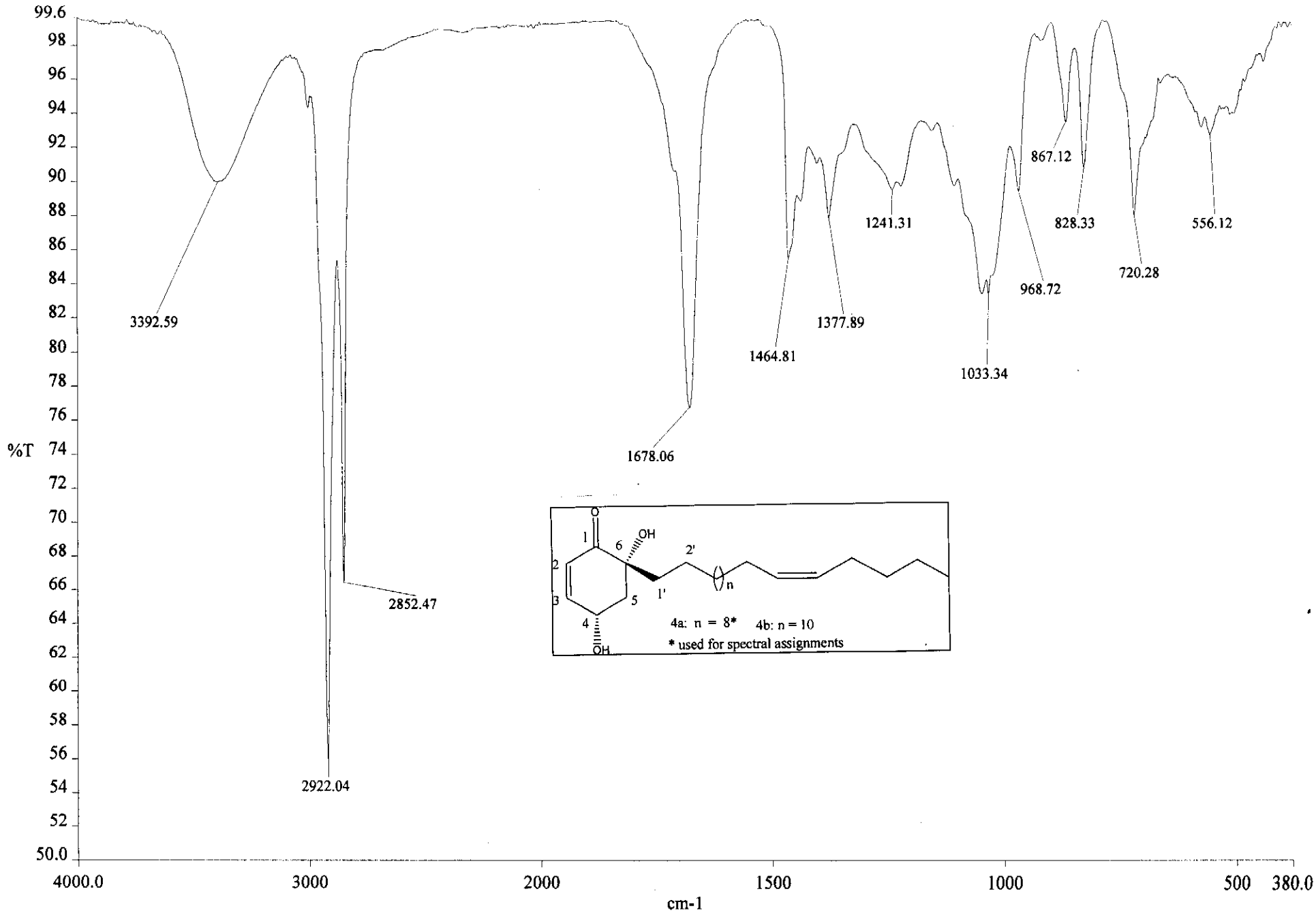
NOESY spectrum of B4 (mixture of a and b)



HSQC spectrum of B4 (mixture of a and b)



HMBC spectrum of B4 (mixture of a and b)

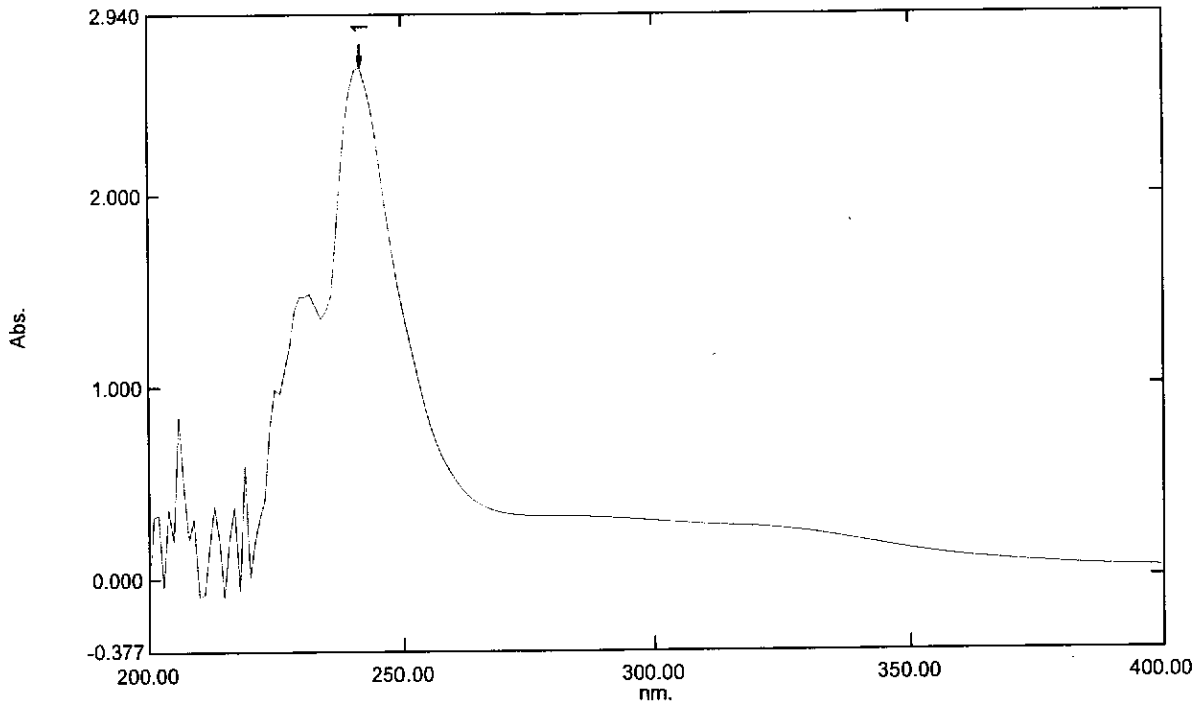


IR spectrum of B4 (mixture of a and b)

Spectrum Peak Pick Report

23/05/2012 06:20:58 PM

Data Set: murt 44-76 (24).spc - Storage 164510



No.	P/V	Wavelength	Abs.	Description
1		242.00	2.664	

Measurement Properties

Wavelength Range (nm.):	200.00 to 400.00
Scan Speed:	Medium
Sampling Interval:	1.0
Auto Sampling Interval:	Disabled
Scan Mode:	Single

Instrument Properties

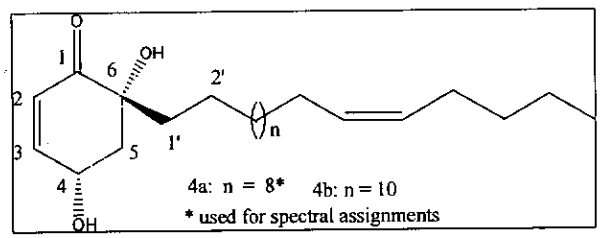
Instrument Type:	UV-3600 Series
Measuring Mode:	Absorbance
Slit Width:	2.0 nm
Time Constant:	0.1 sec.
Source Lamp:	Auto
Light Source Change Wavelength:	310.00 nm
Detector Unit:	Direct
Detector Change Wavelength:	830.00 nm 1800.00 nm
Grating Change Wavelength:	720.00 nm
S/R Exchange:	Normal
Detector Lock:	Auto
Slit Program:	Normal
Beam Mode:	Double
Stair Correction:	Disable

Attachment Properties

Attachment:	None

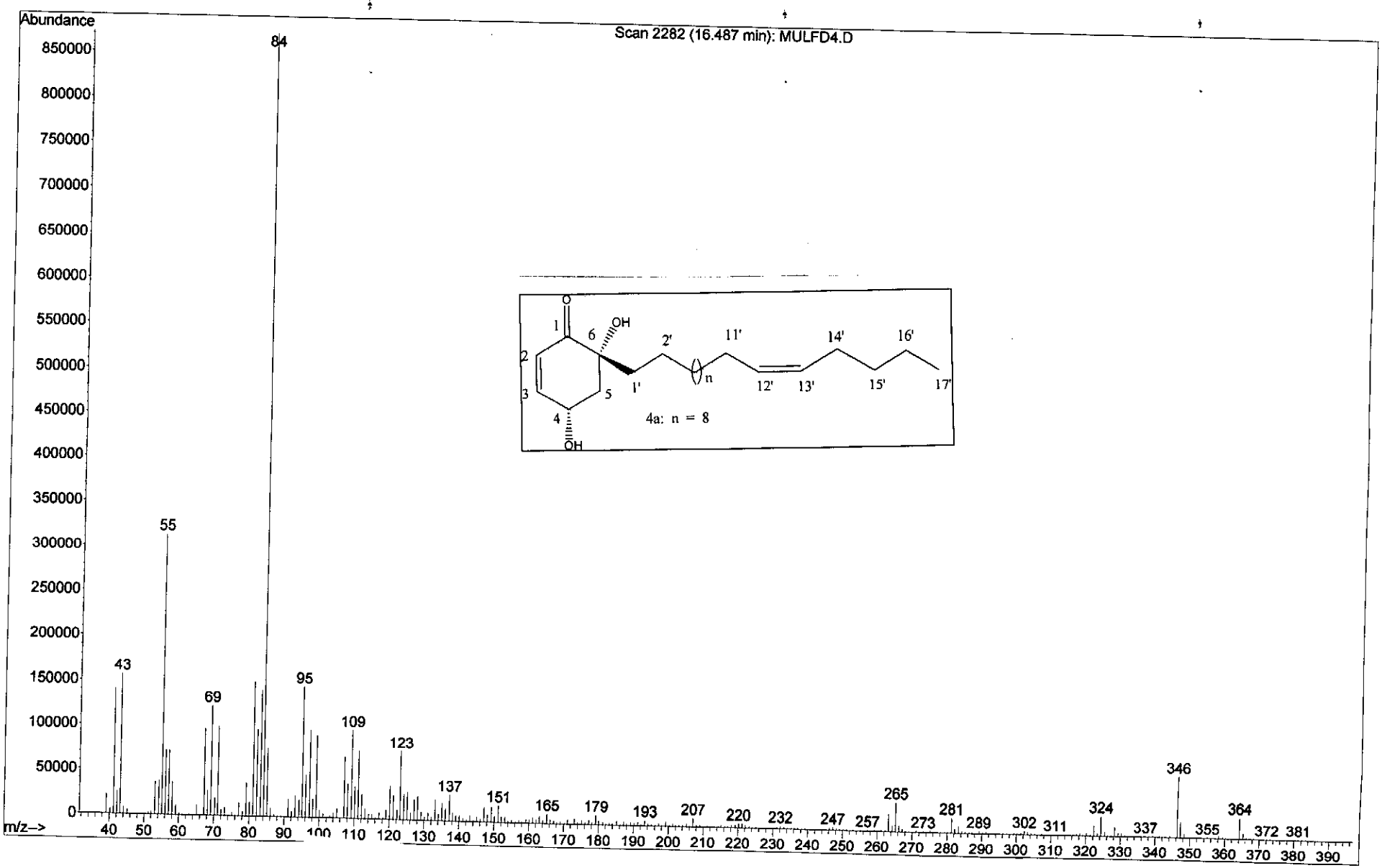
Sample Preparation Properties

Weight:	
Volume:	
Dilution:	
Path Length:	
Additional Information:	



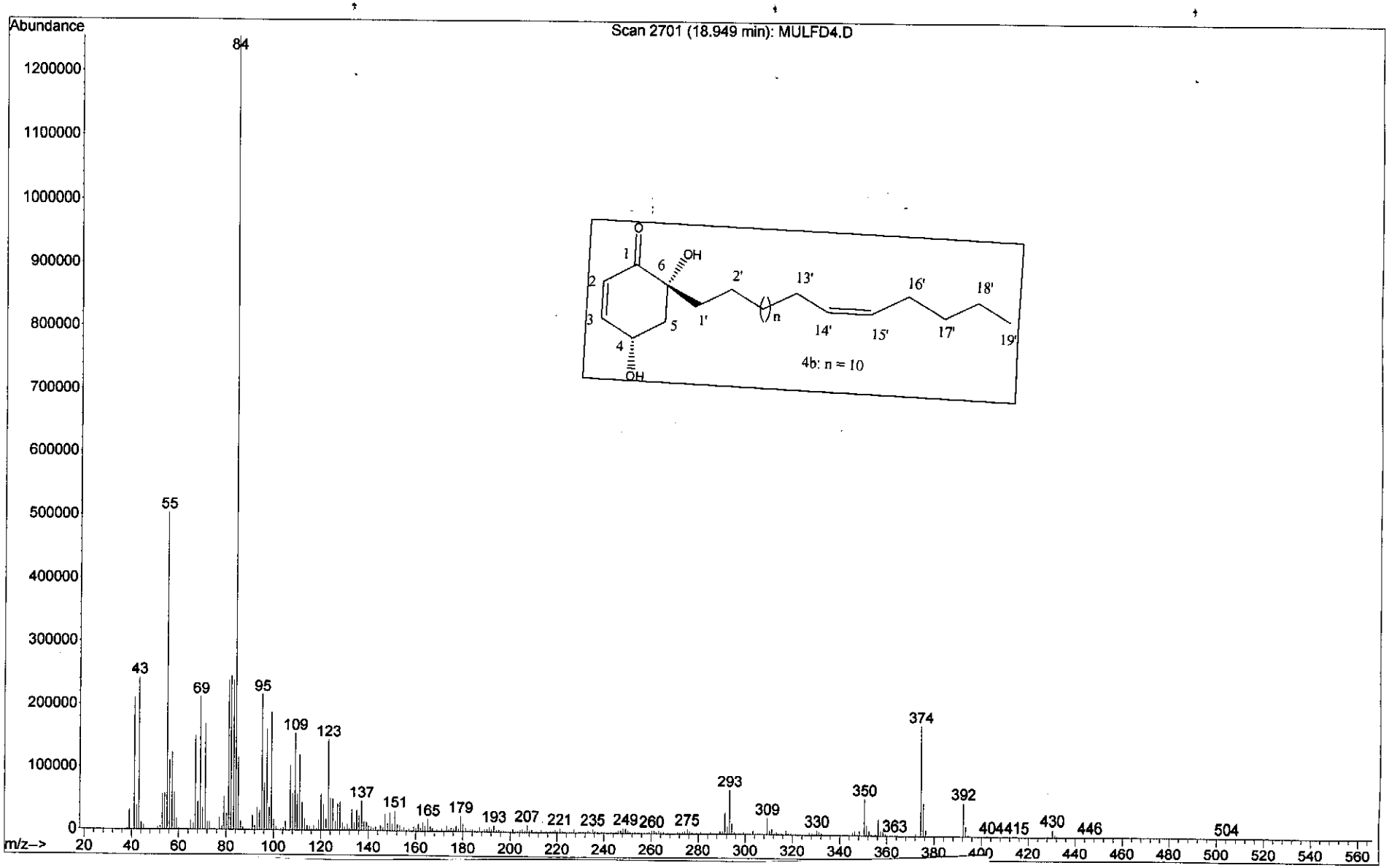
UV spectrum of B4 (mixture of a and b)

File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\MULFD4.D
Operator : Dorothy
Acquired : 29 Apr 2011 00:45 using AcqMethod NATURALJ
Instrument : Instrumen
Sample Name: MURT 44-76 (24)
Misc Info :
Vial Number: 1

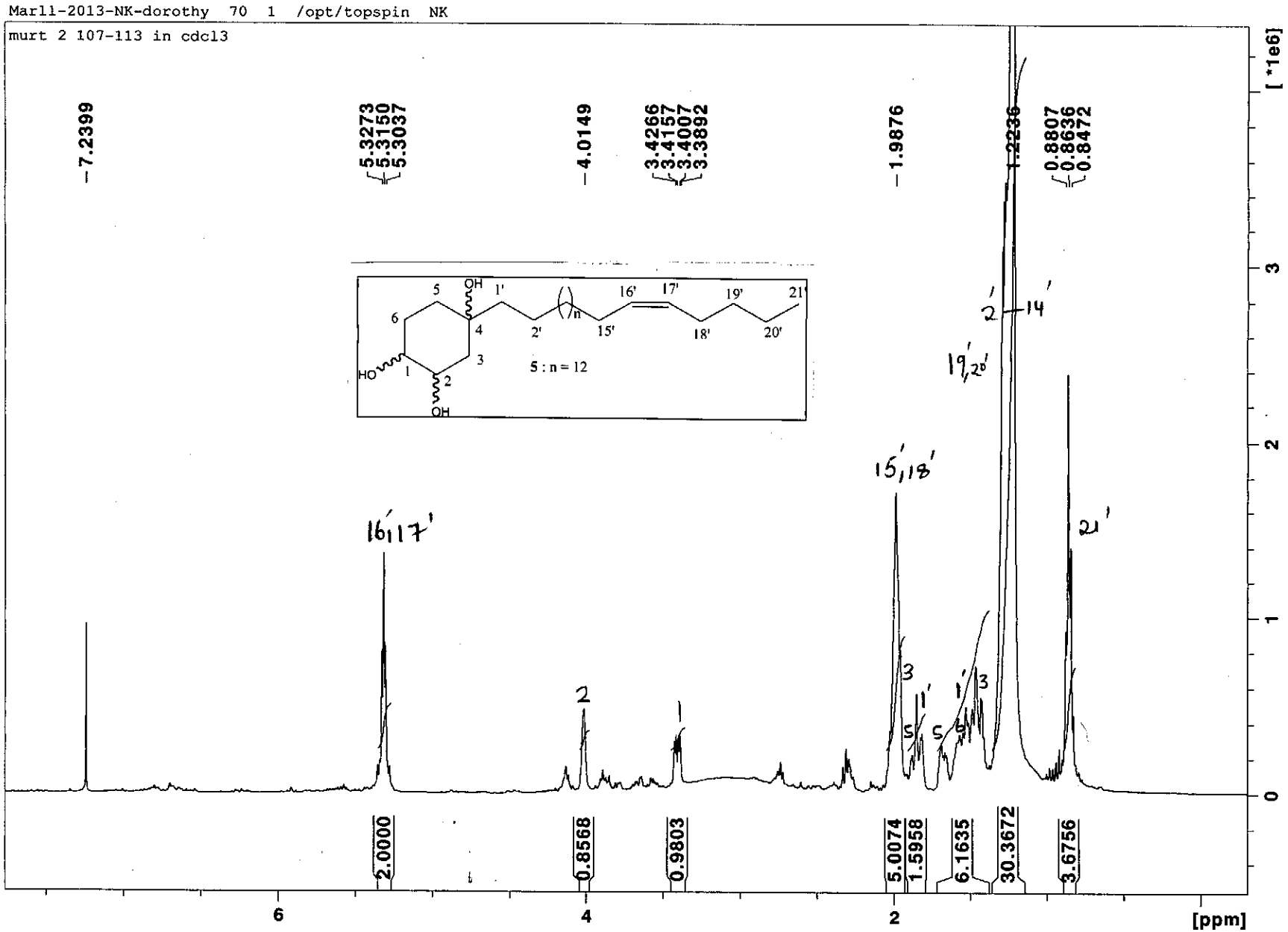


MS spectrum of B4 (a)

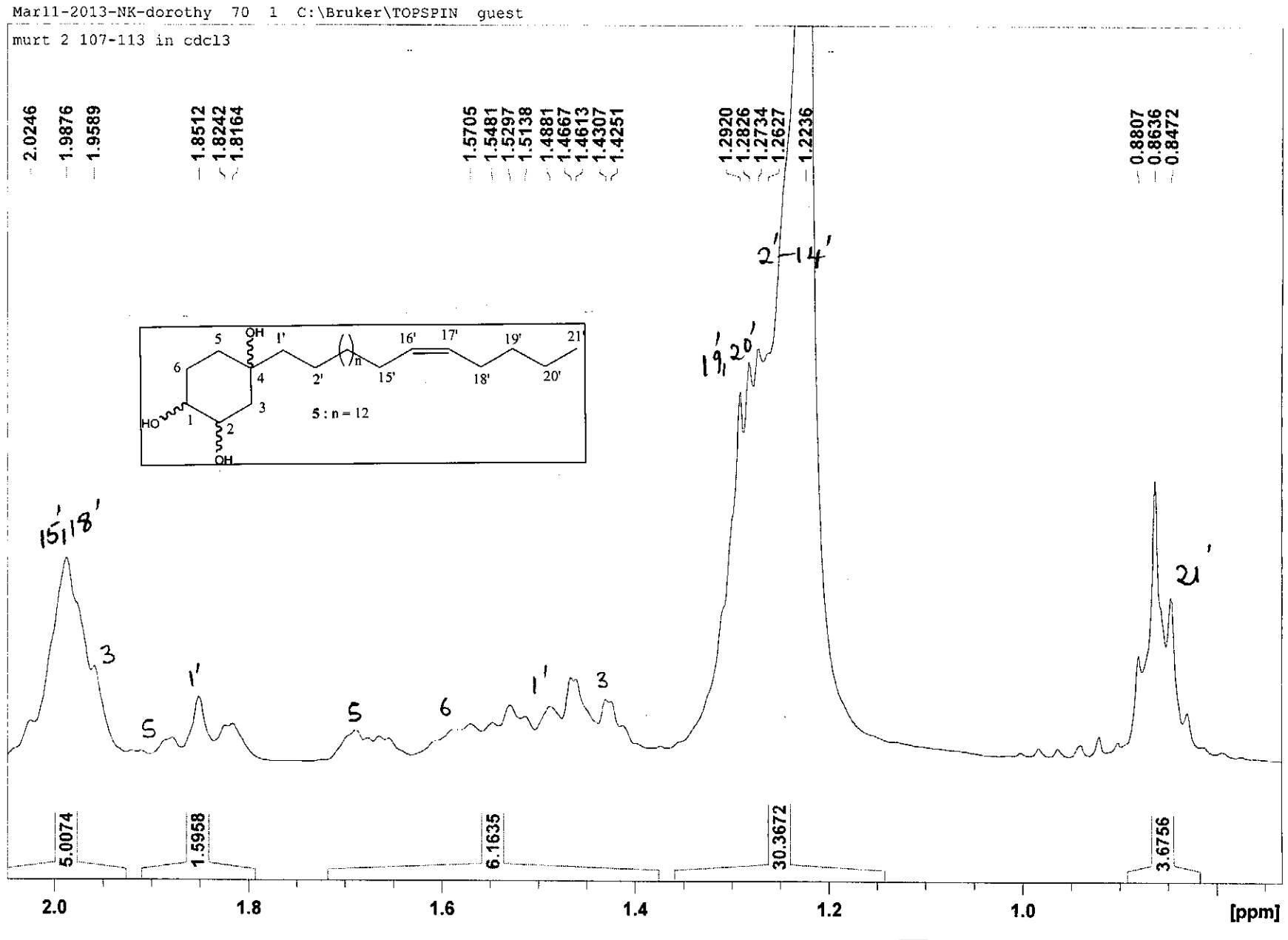
File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\MULFD4.D
Operator : Dorothy
Acquired : 29 Apr 2011 00:45 using AcqMethod NATURALJ
Instrument : Instrumen
Sample Name: MURT 44-76 (24)
Misc Info :
Vial Number: 1



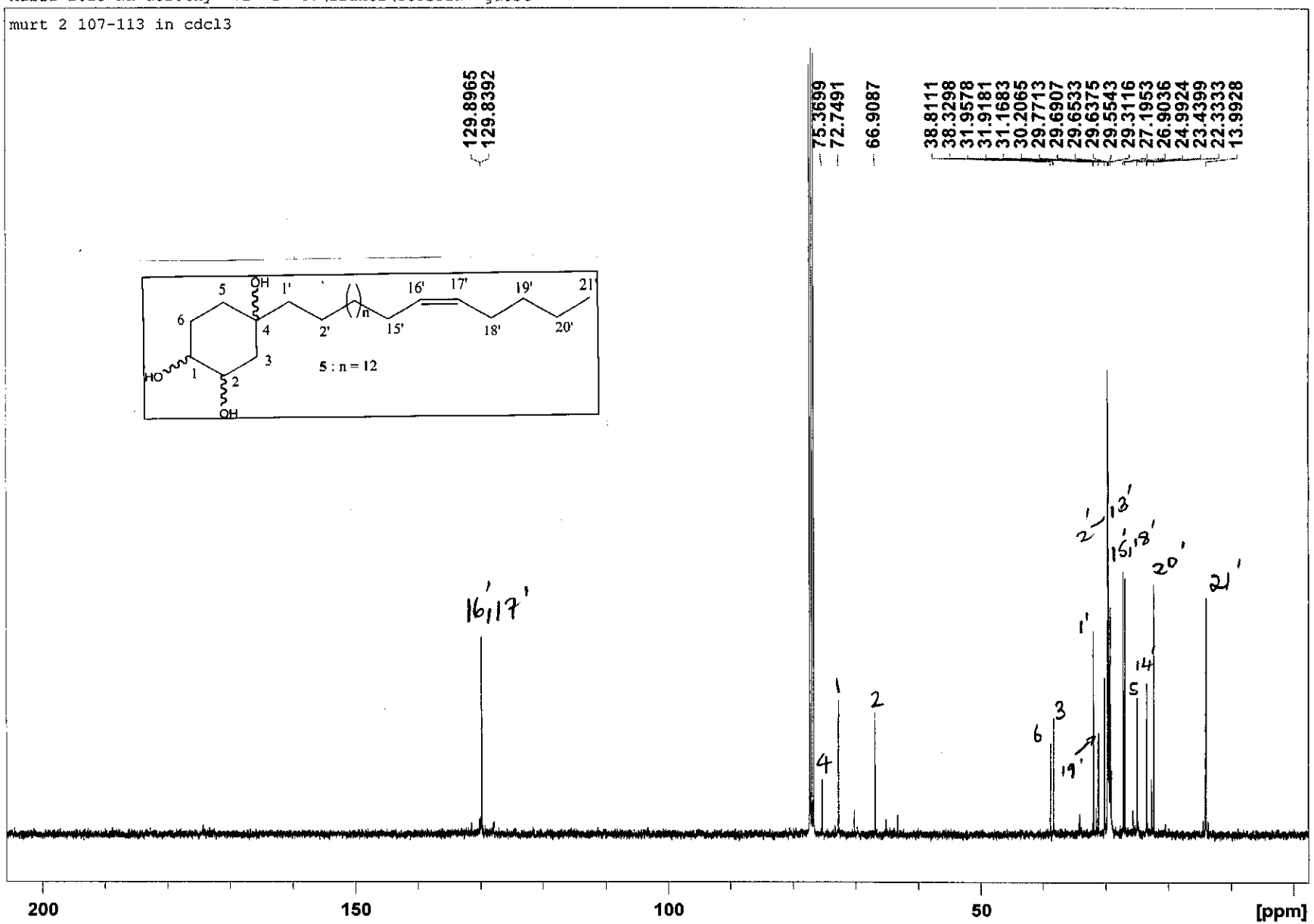
MS spectrum of B4 (b)



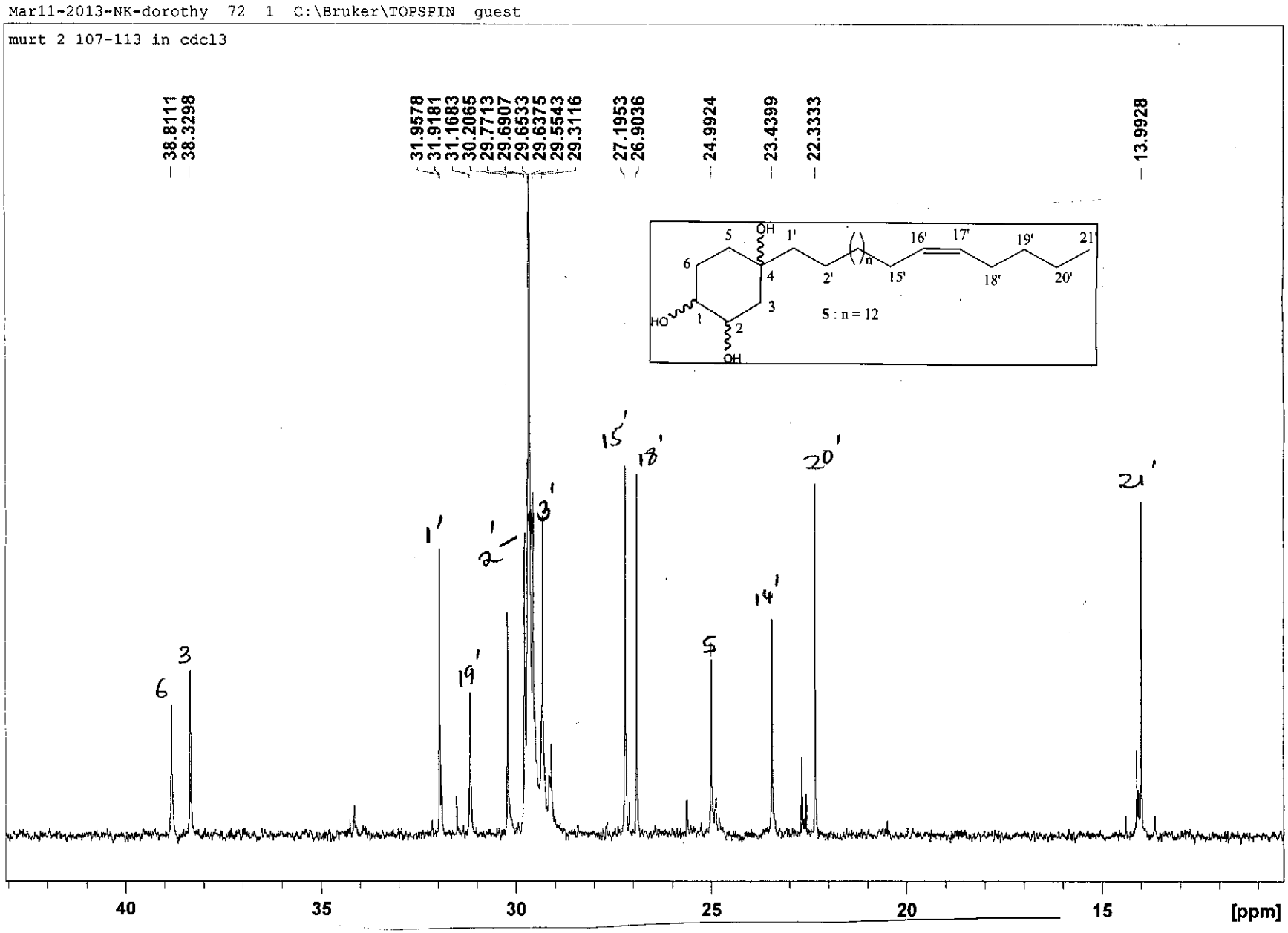
^{1}H NMR spectrum of B5



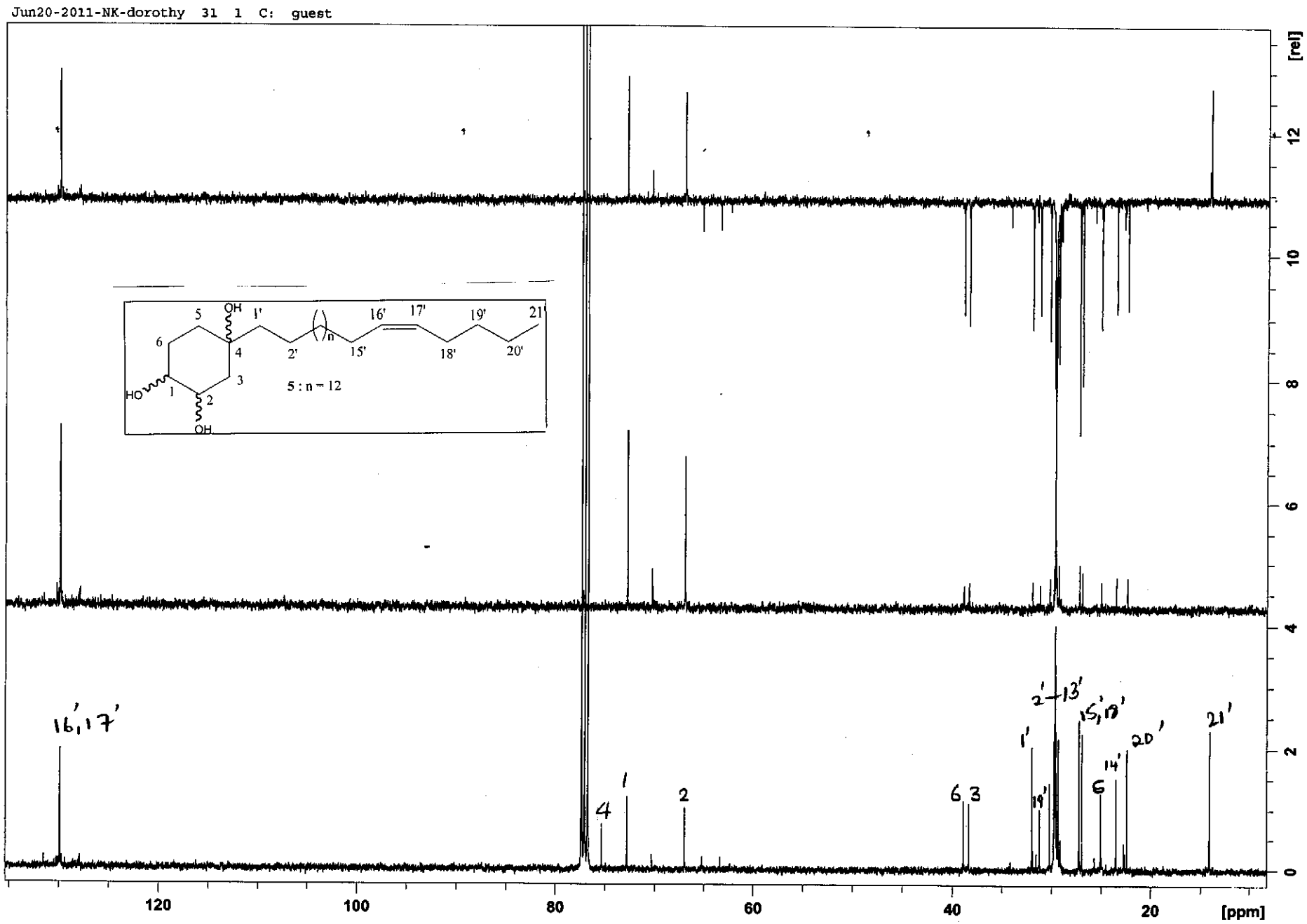
^{1}H NMR spectrum of B5 expanded (F1 0.6-2.1 ppm)



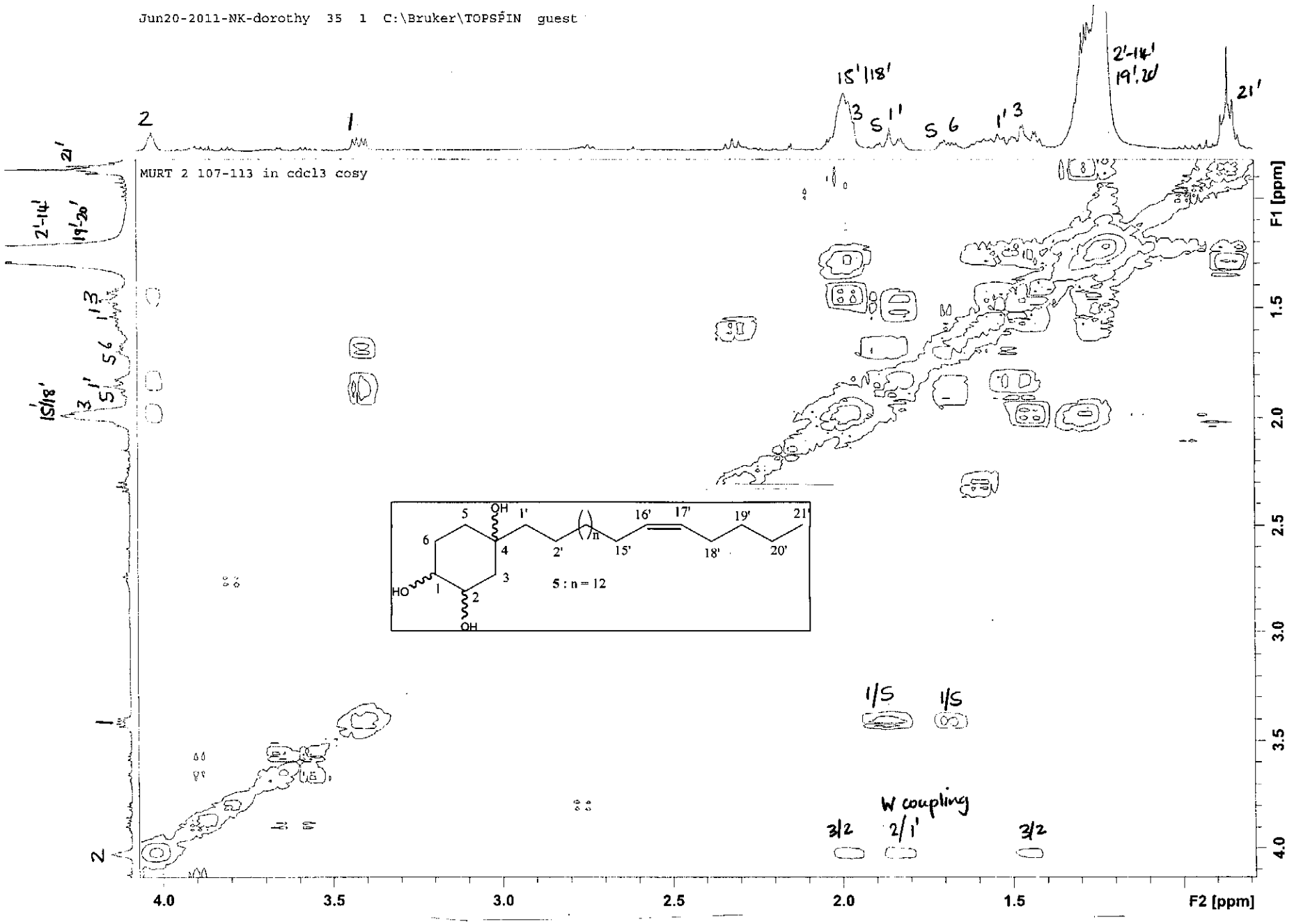
^{13}C NMR spectrum of B5



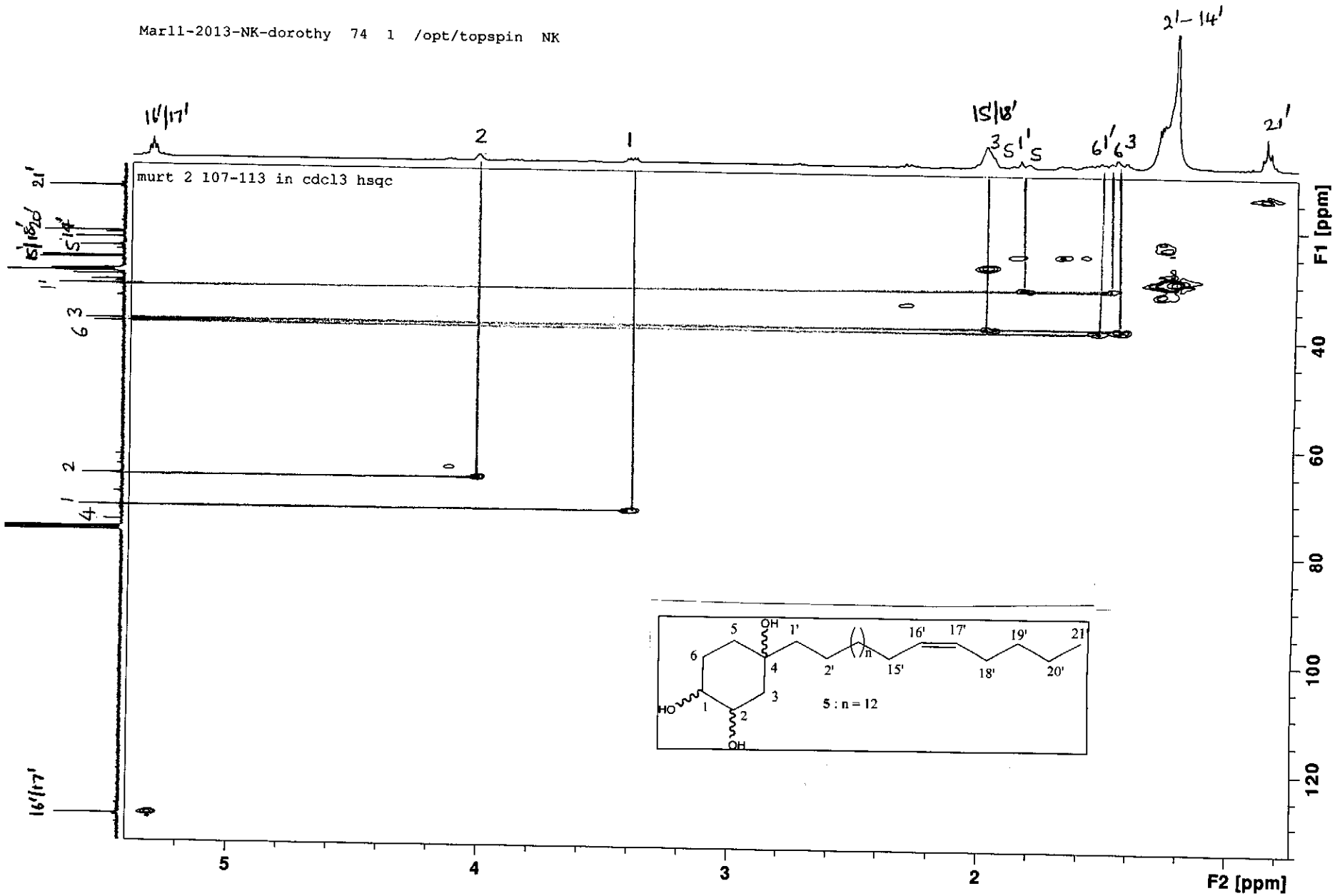
^{13}C NMR spectrum of B5 expanded (10-44 ppm)



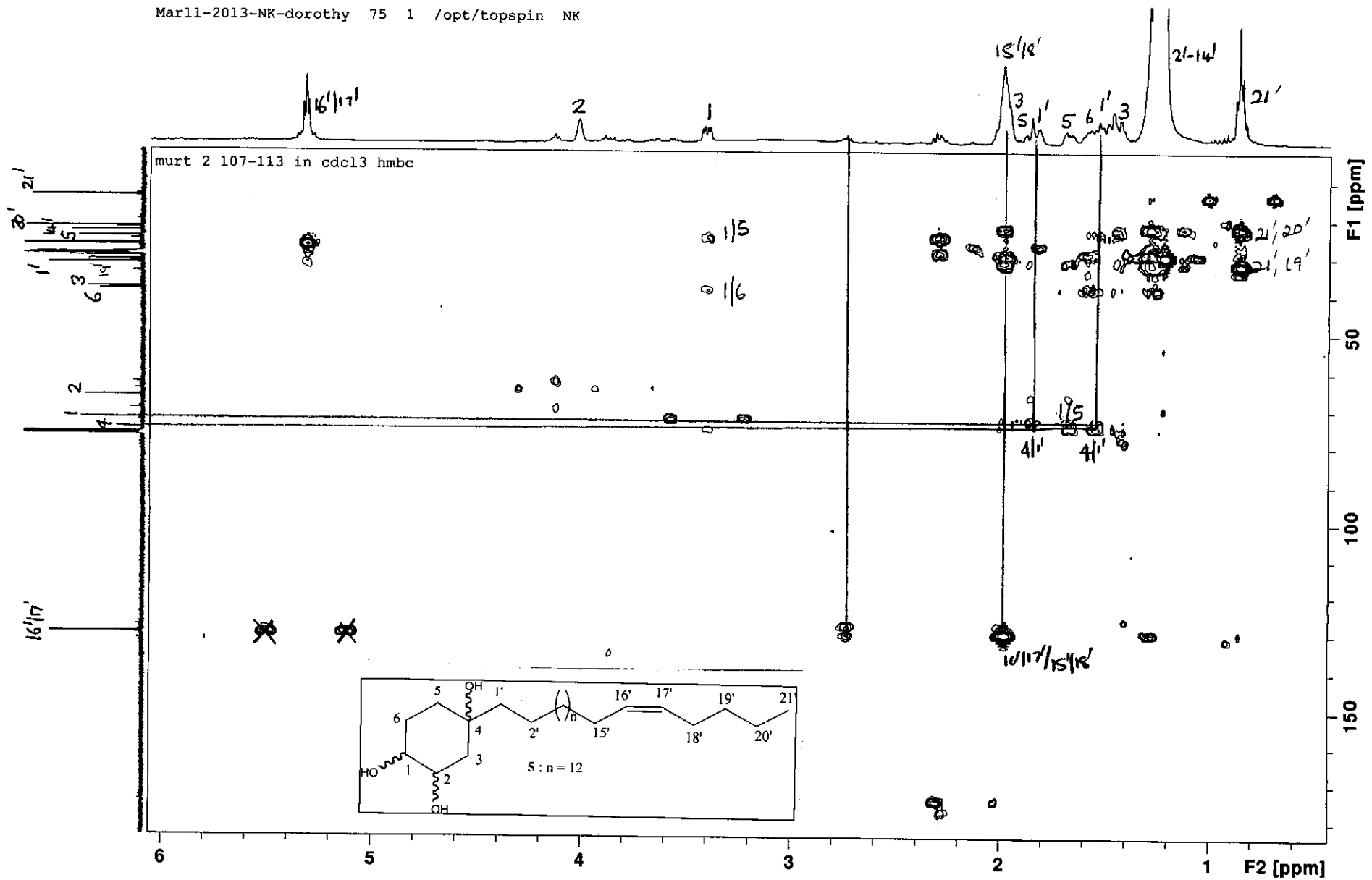
DEPT spectrum of B5



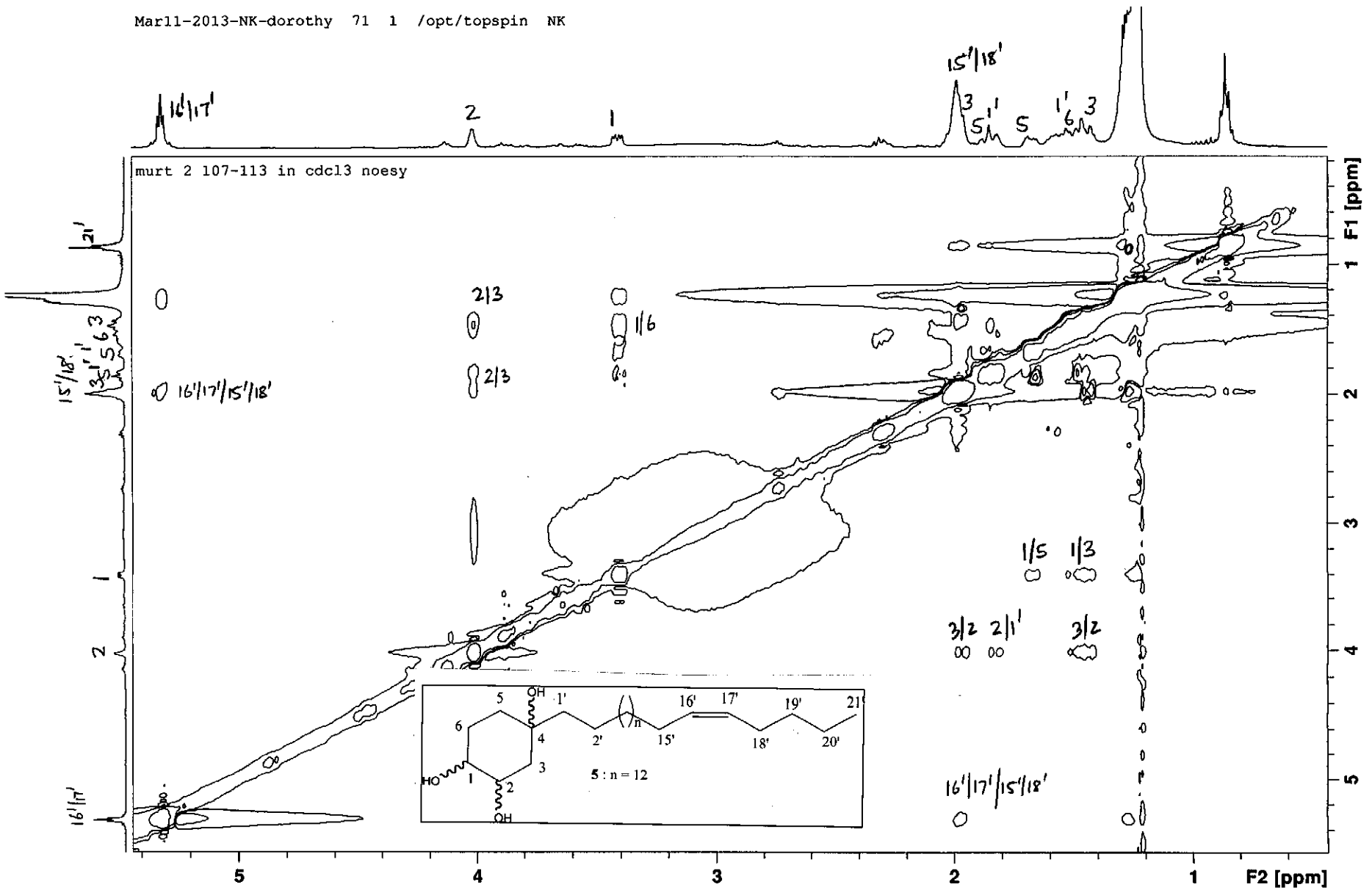
COSY spectrum of B5 expanded (F1, F2 0.6 -4.1 ppm)



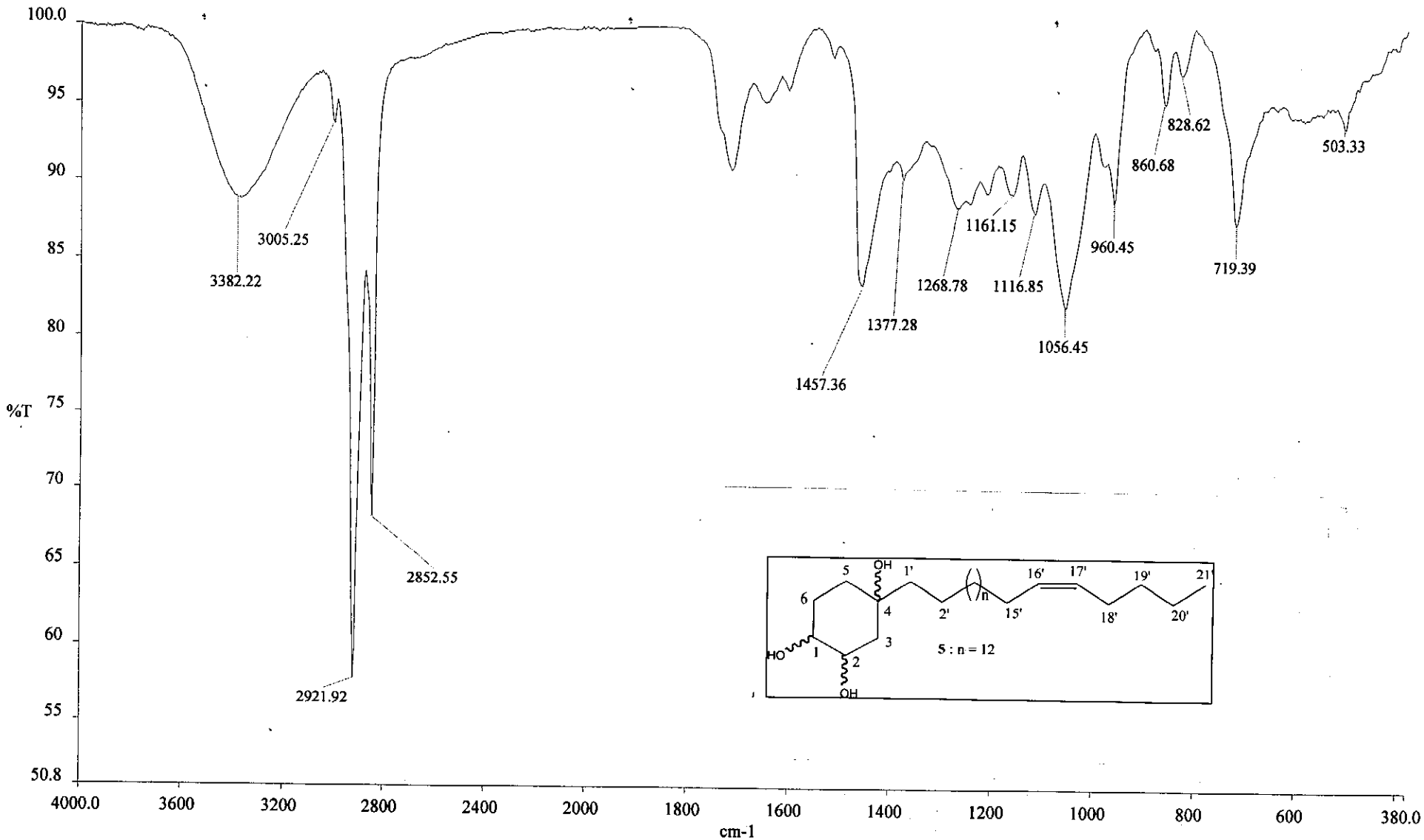
HSQC spectrum of B5



HMBC spectrum of B5



NOESY spectrum of B5

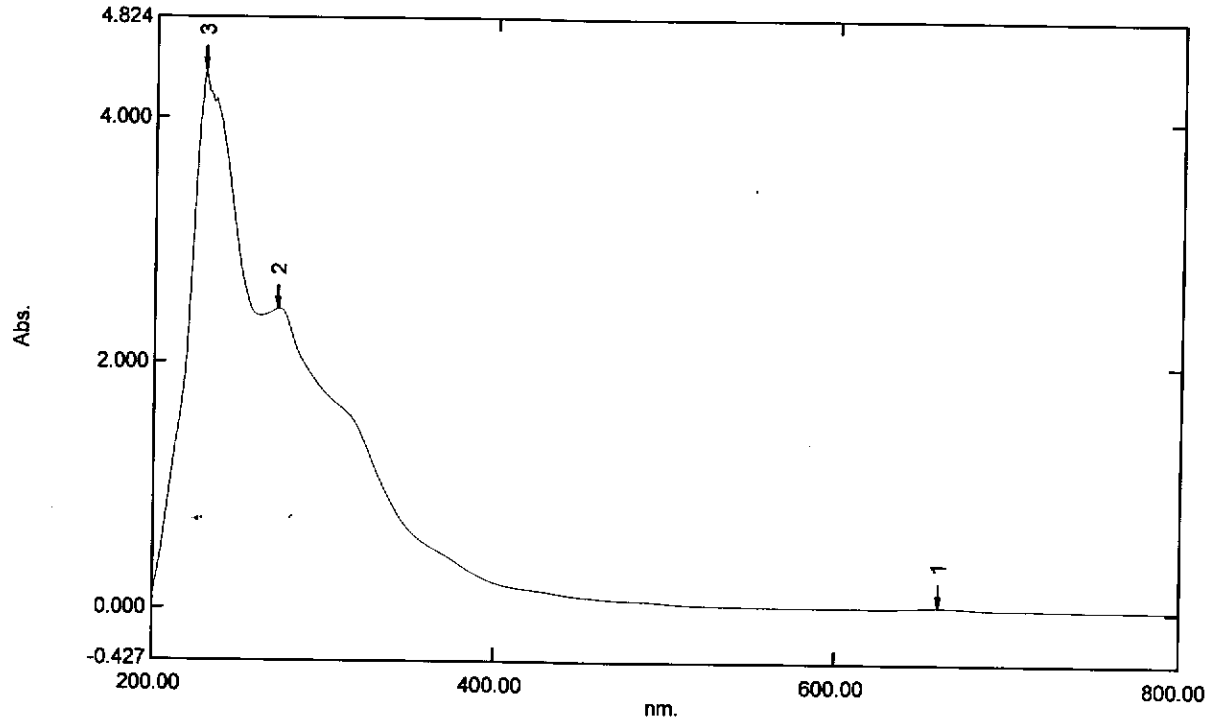


IR spectrum of B5

Spectrum Peak Pick Report

04/10/2011 05:12:04 PM

Data Set: MURT 2 107-113 GOOD.spc - Storage 161821



No.	P/V	Wavelength	Abs.	Description
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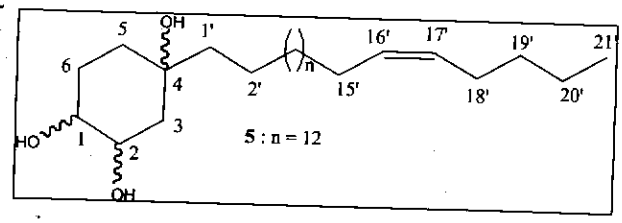
2	●	273.00	2.434	
3	●	229.00	4.387	
4	◆	570.00	0.025	
5	◆	262.00	2.373	

Measurement Properties
Wavelength Range (nm.): 200.00 to 800.00
Scan Speed: Medium
Sampling Interval: 1.0
Auto Sampling Interval: Disabled
Scan Mode: Single

Instrument Properties
Instrument Type: UV-3600 Series
Measuring Mode: Absorbance
Slit Width: 8.0 nm
Time Constant: 0.1 sec.
Source Lamp: Auto
Light Source Change Wavelength: 310.00 nm
Detector Unit: Direct
Detector Change Wavelength: 830.00 nm 1800.00
Grating Change Wavelength: 720.00 nm
S/R Exchange: Normal
Detector Lock: Auto
Slit Program: Normal
Beam Mode: Double
Stair Correction: Disable

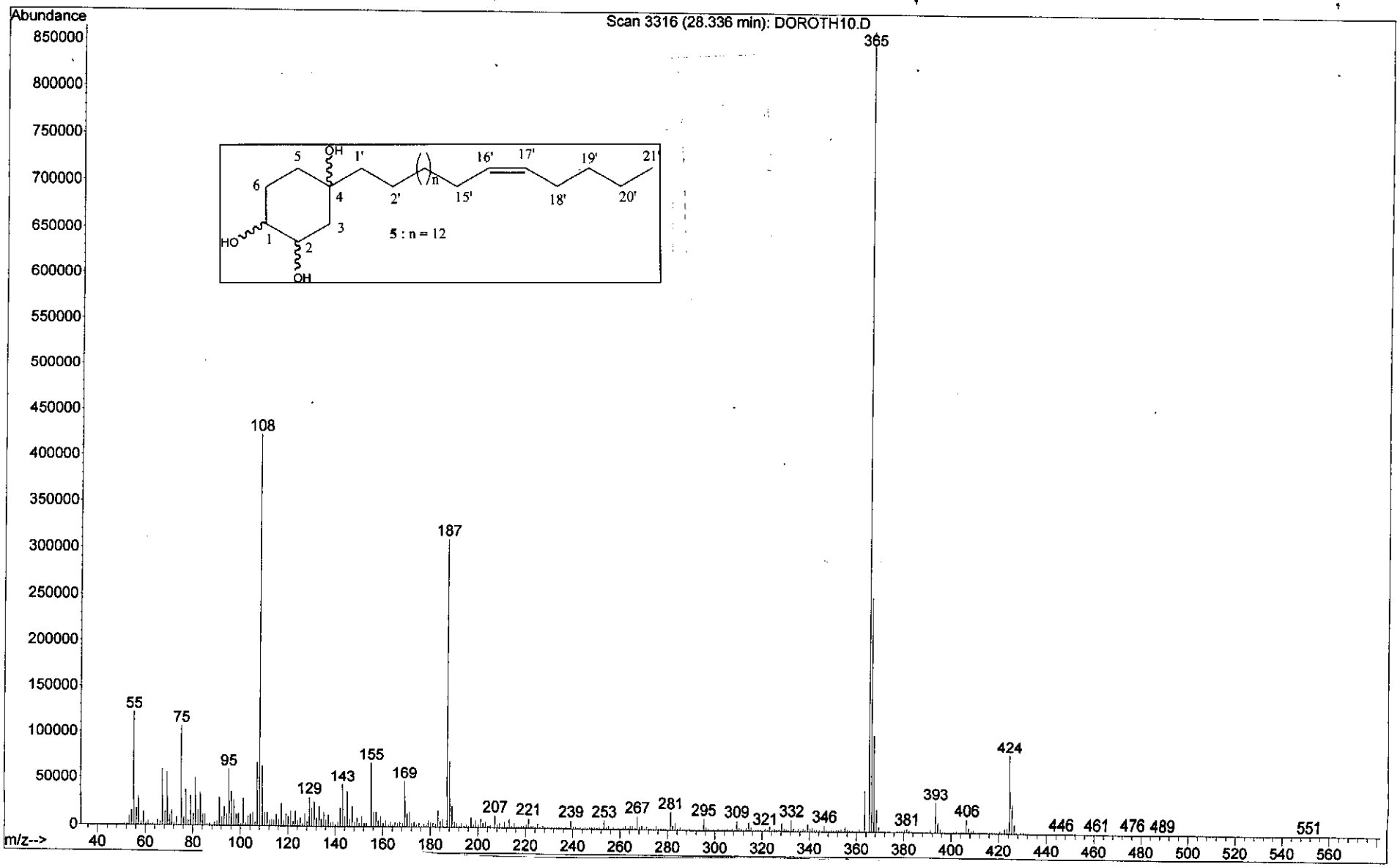


Attachment Properties
Attachment: None

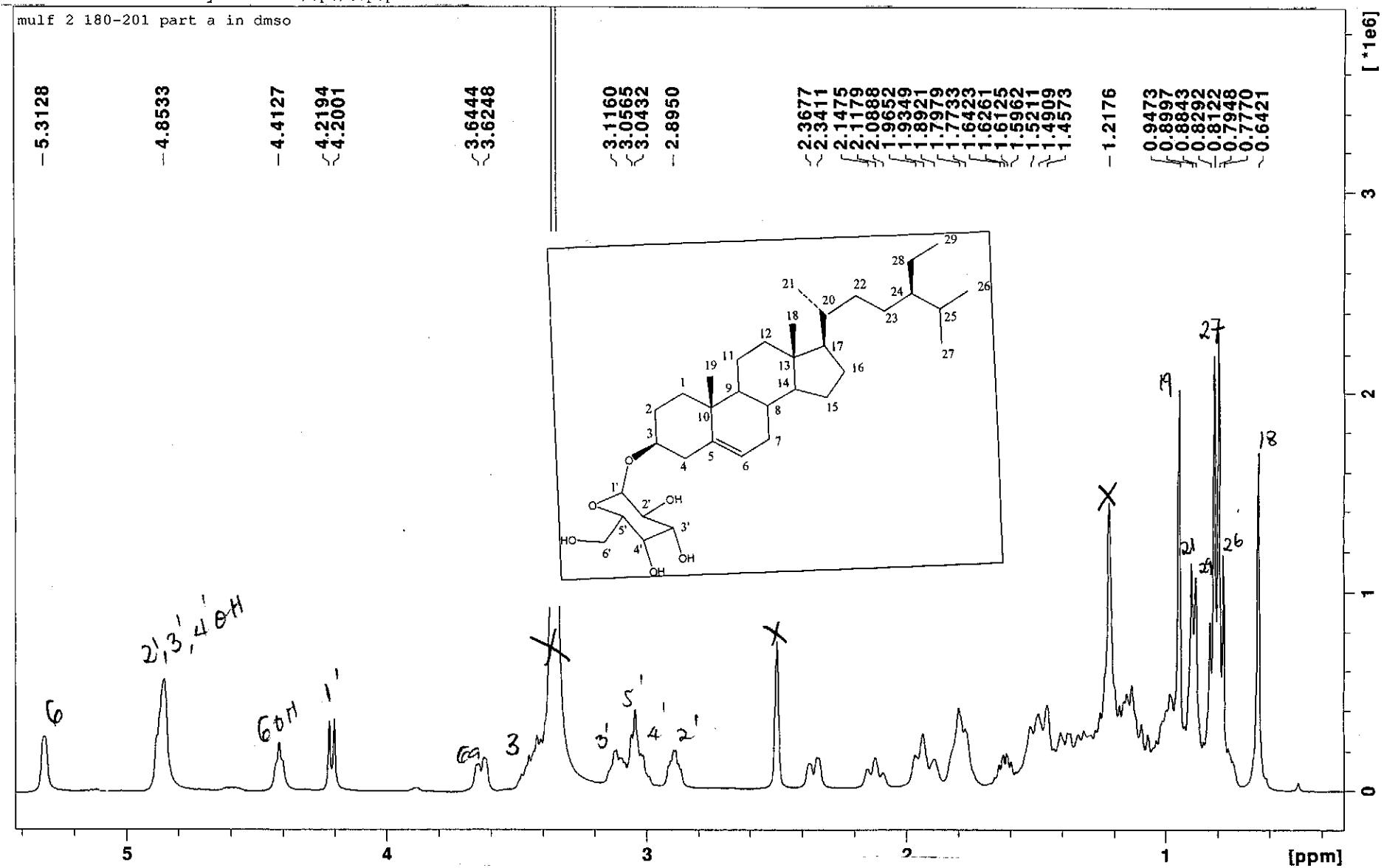
Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

UV spectrum of B5

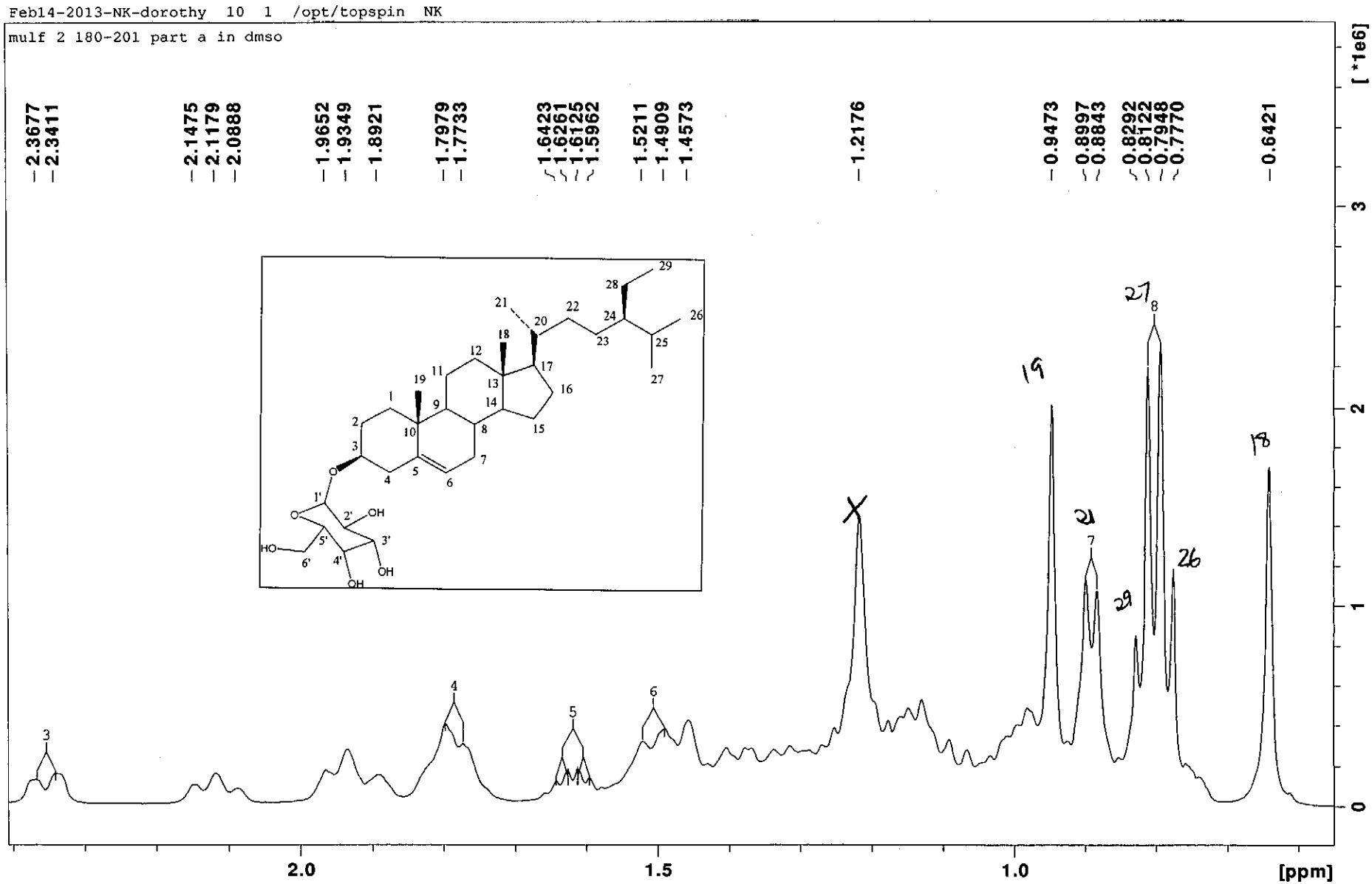
File : C:\MSDCHEM\1\DATA\DOROTHY GCMS TWO\DOROTHY GCMS TWO\DOROTH10.D
Operator : dorothy
Acquired : 27 Nov 2011 23:02 using AcqMethod DOROTHY
Instrument : Instrumen
Sample Name: MURT 2 107-113
Misc Info :
Vial Number: 1



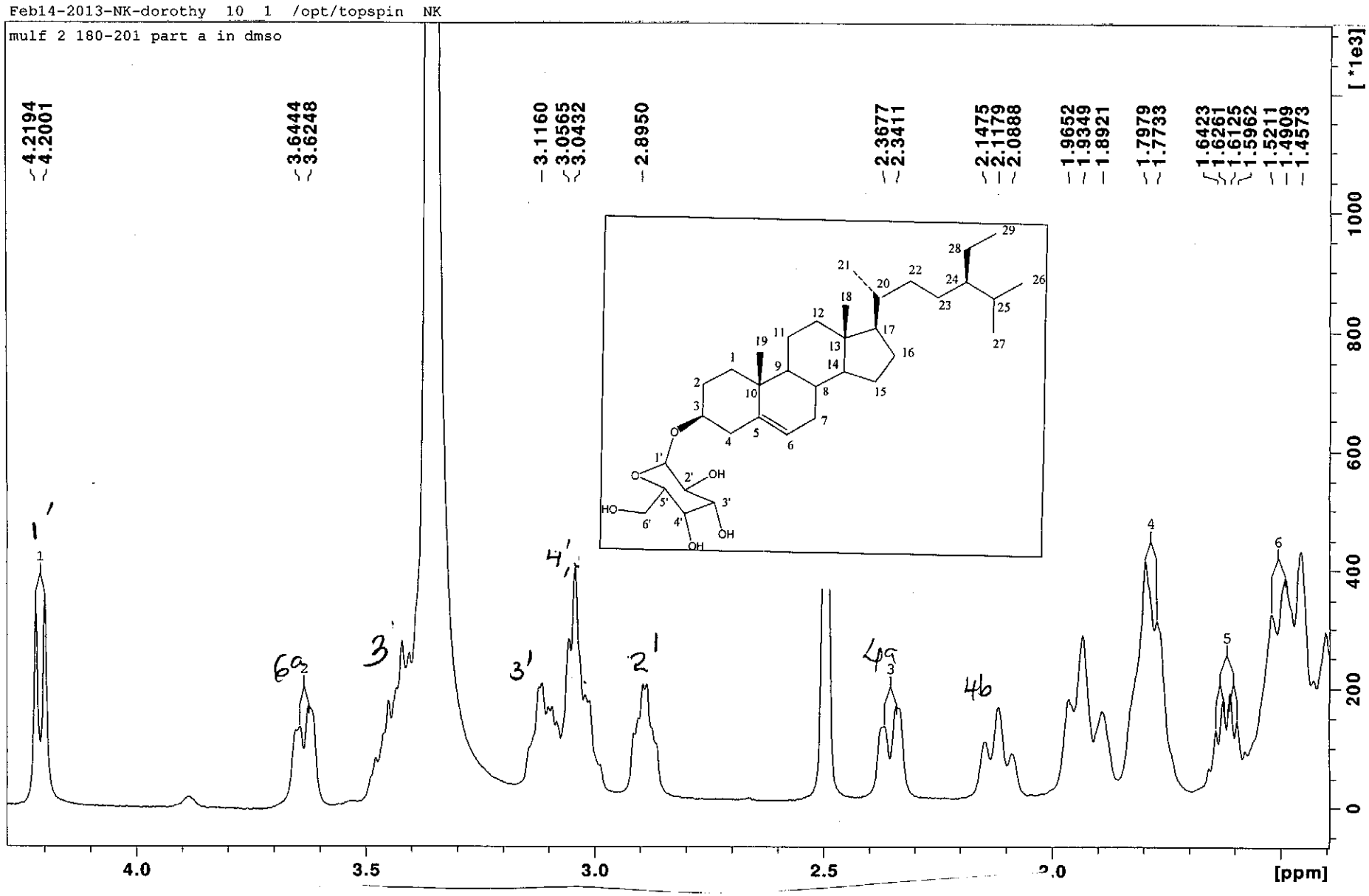
MS spectrum of B5



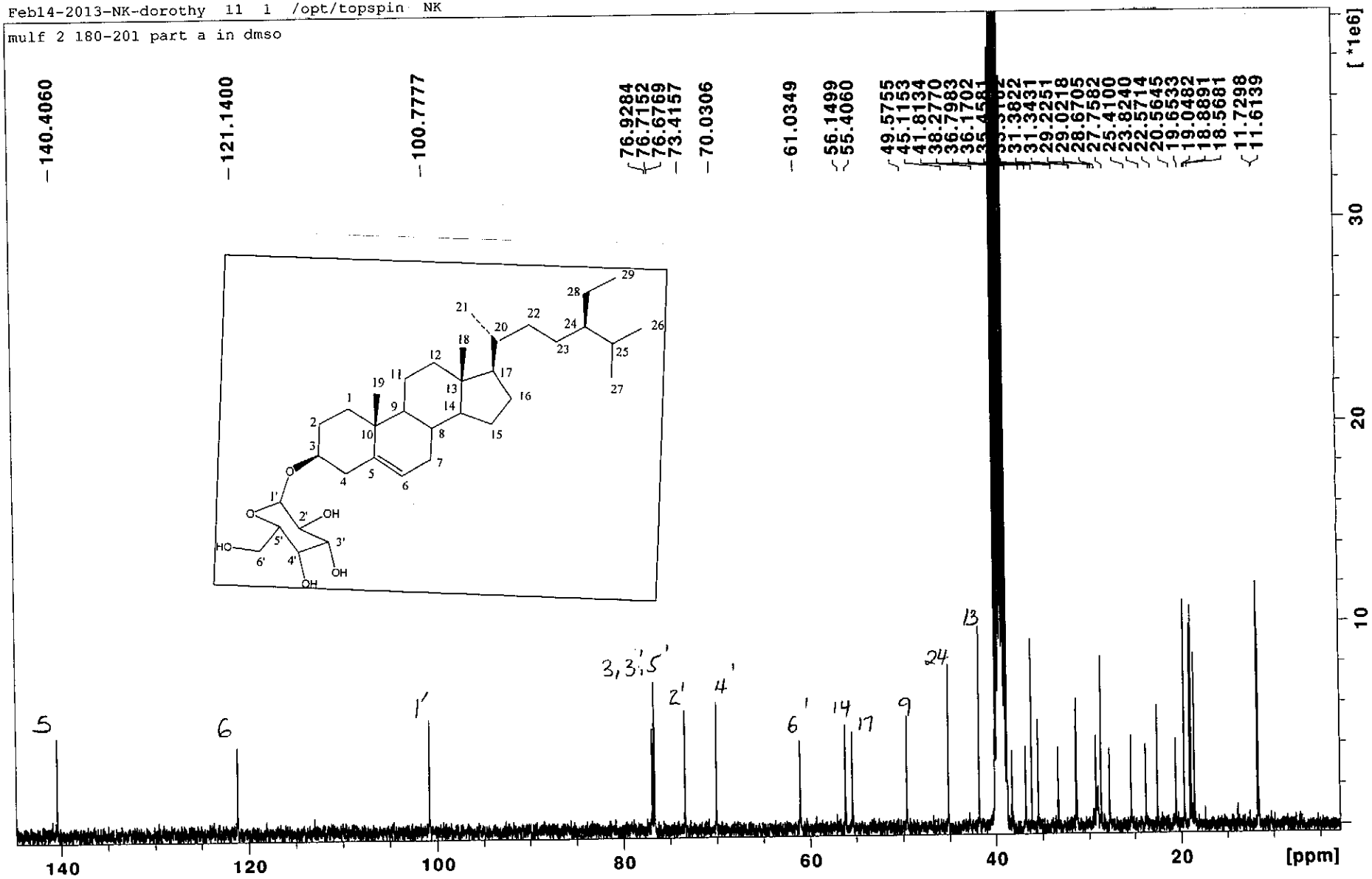
^{1}H NMR spectrum of B6



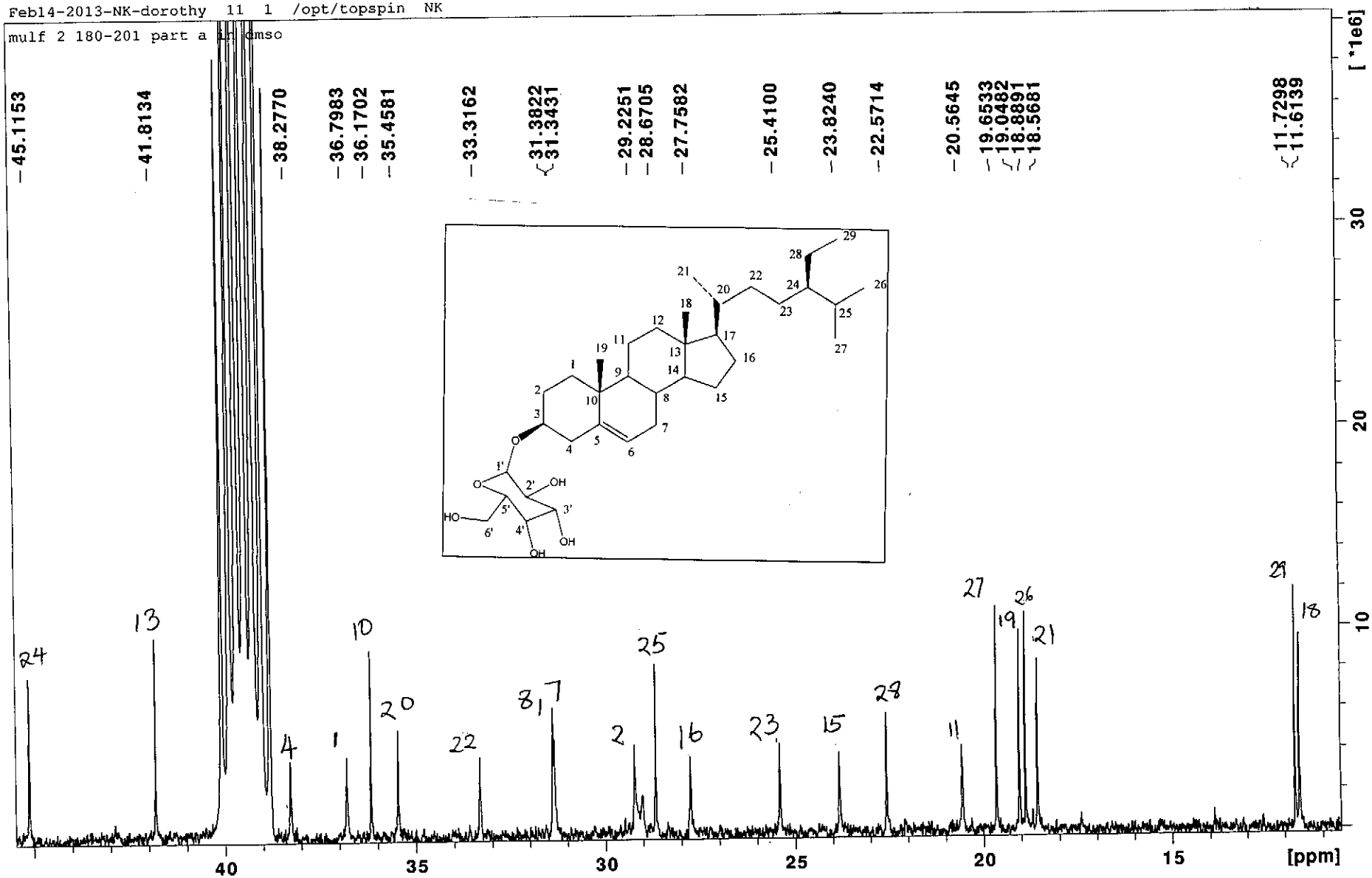
^{1}H NMR spectrum of B6 expanded (0-2.5 ppm)



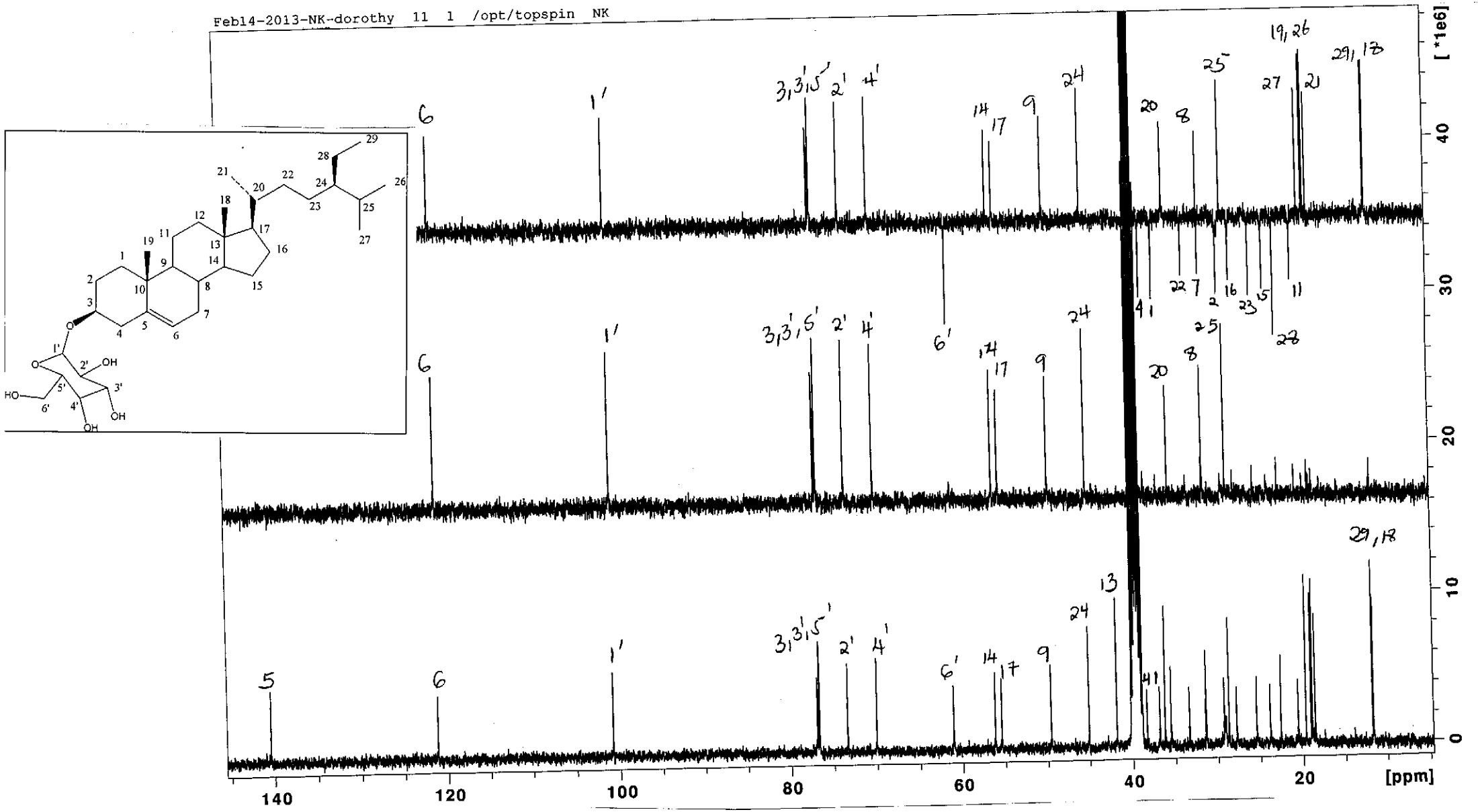
^{1}H NMR spectrum of B6 expanded (1.0-4.4 ppm)



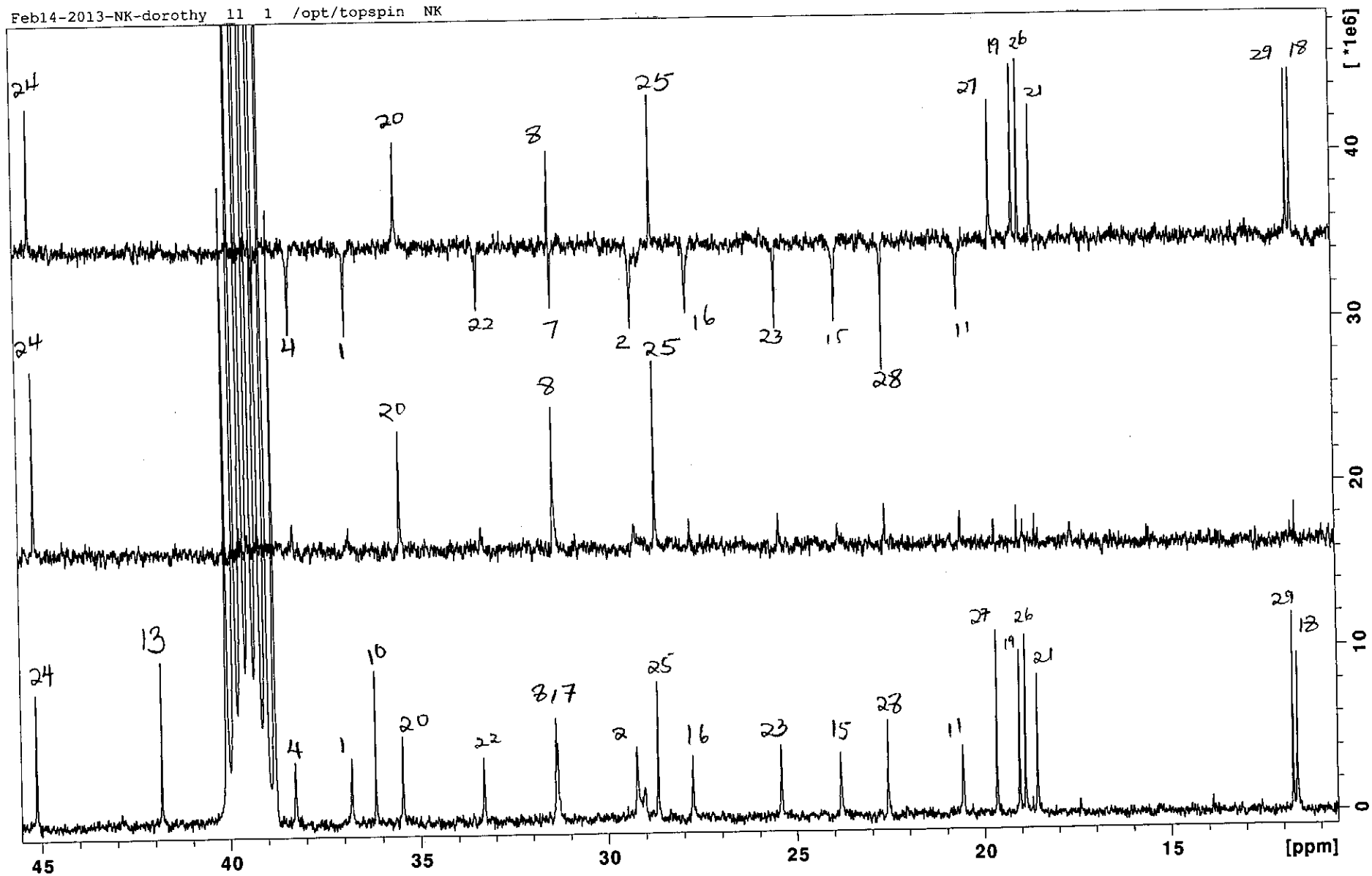
^{13}C NMR spectrum of B6



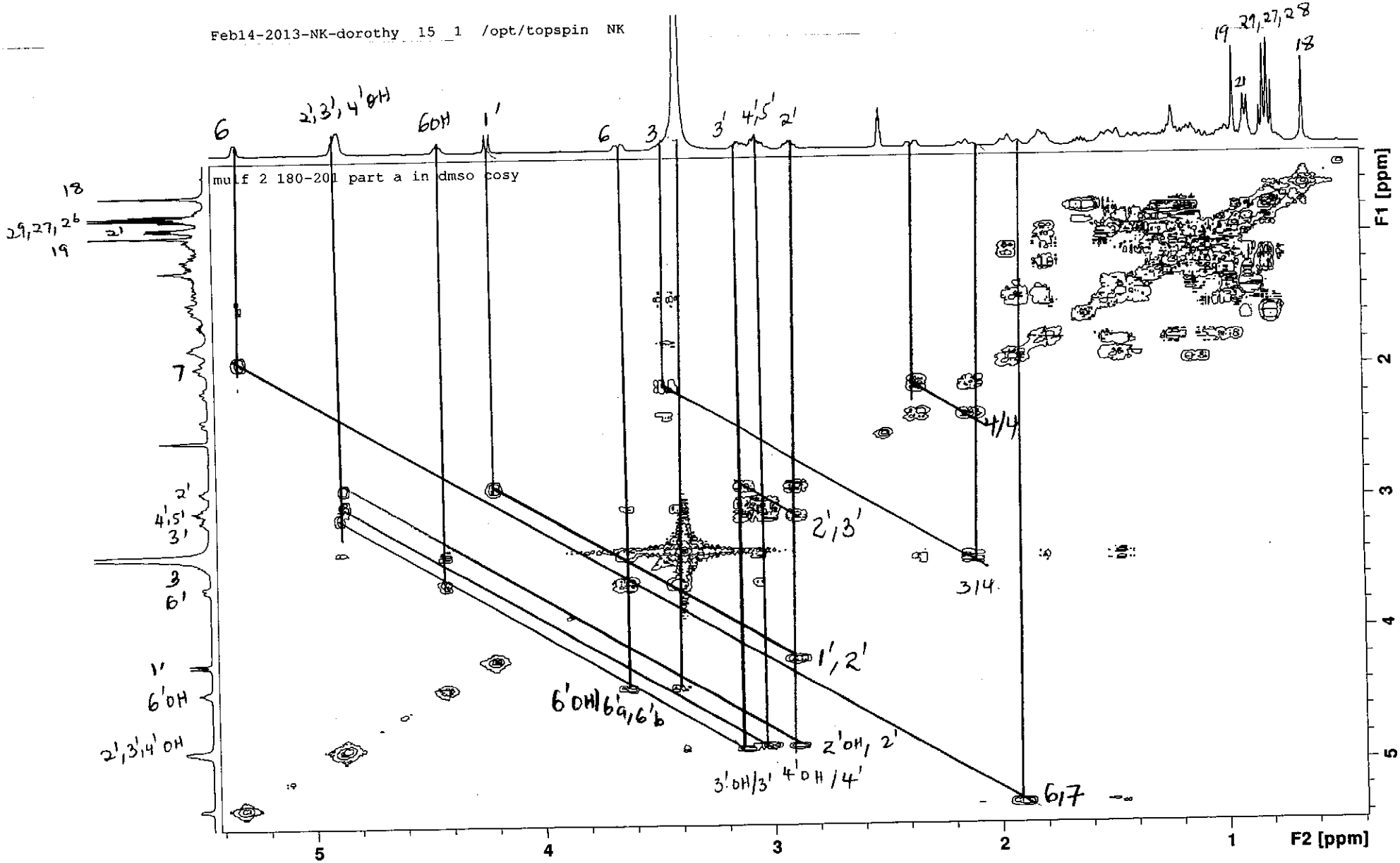
^{13}C NMR spectrum of B6 expanded (10-46 ppm)



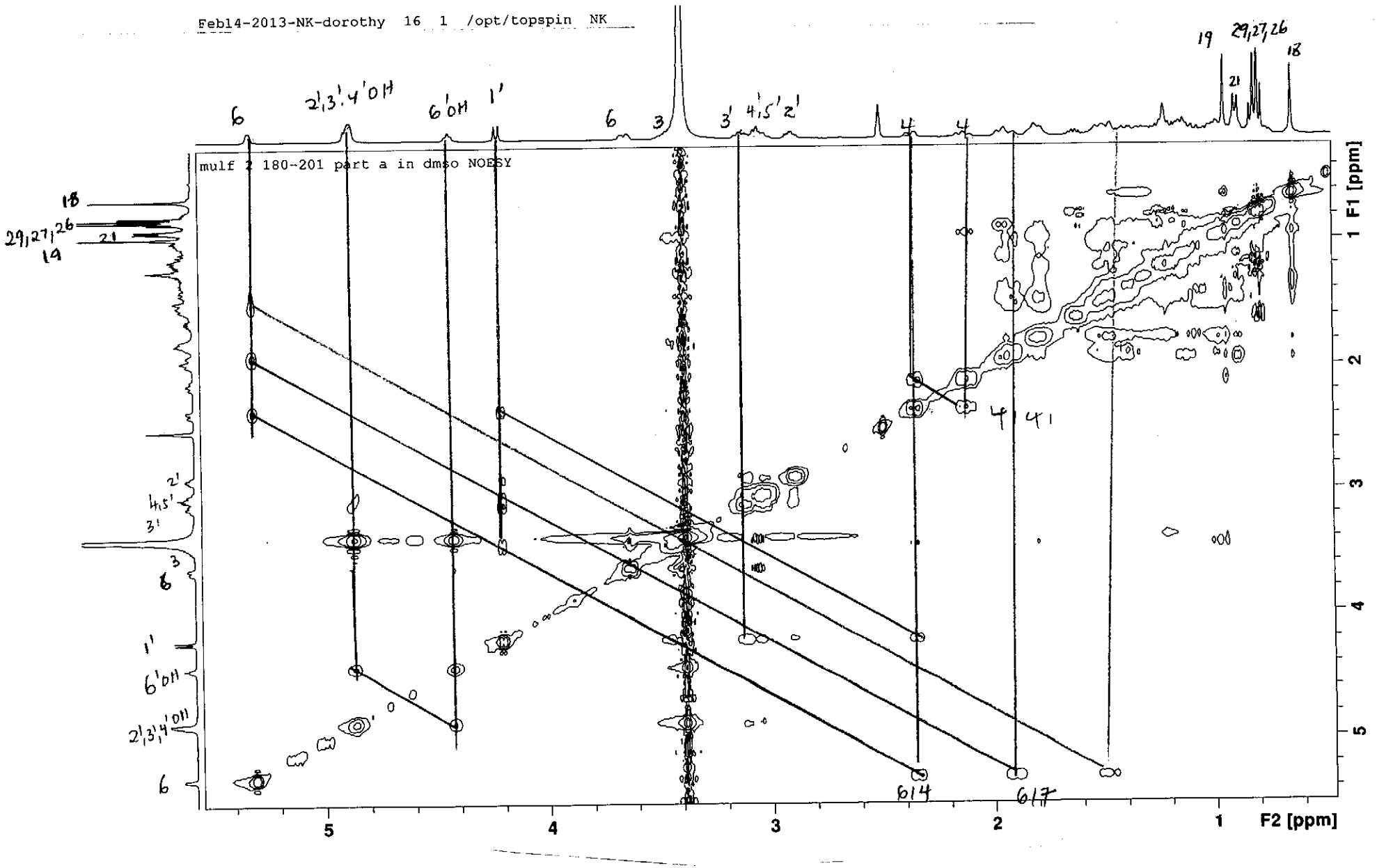
DEPT spectrum of B6



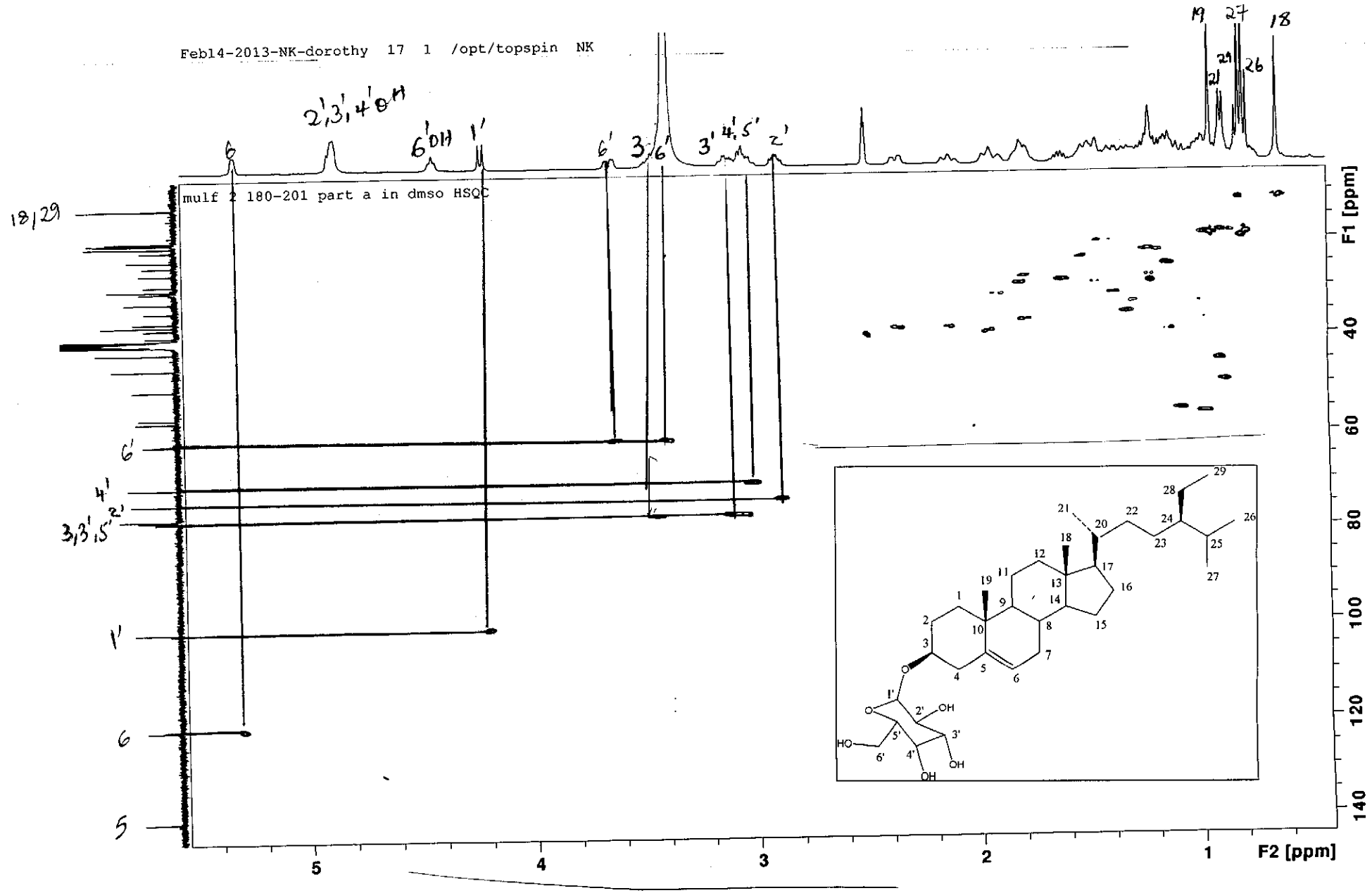
DEPT spectrum of B6 expanded (10-46 ppm)



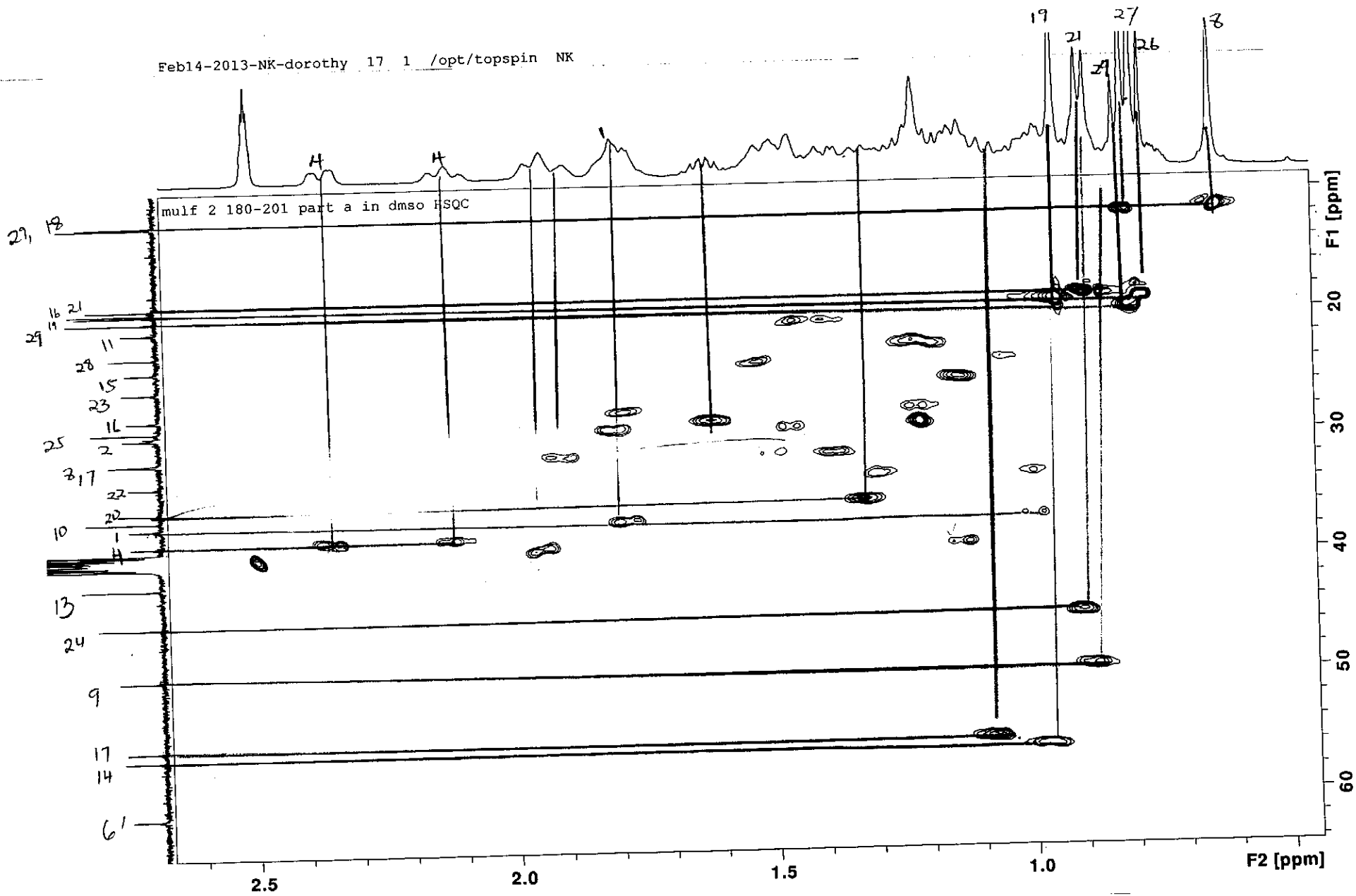
COSY spectrum of B6



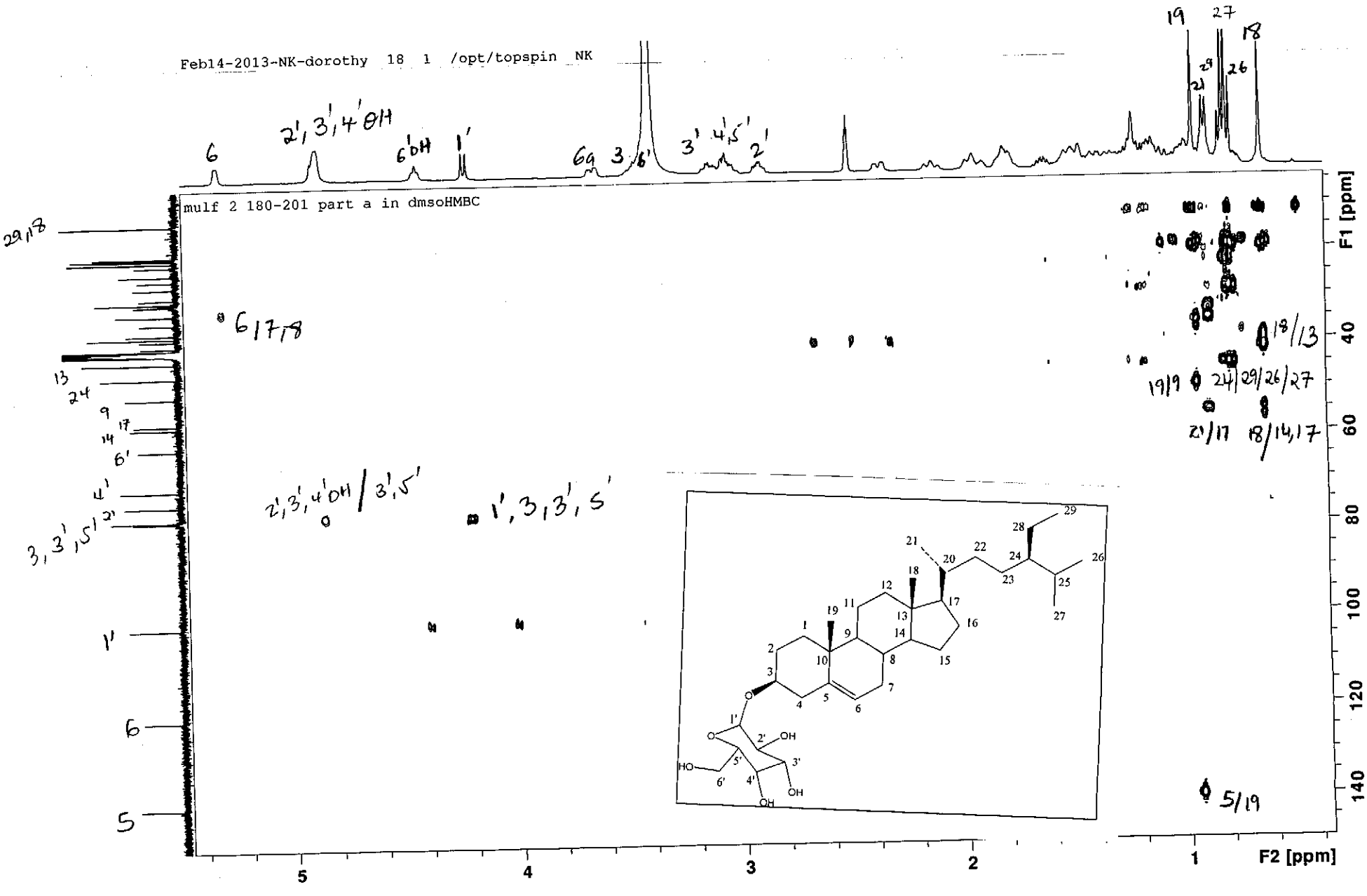
NOESY spectrum of B6



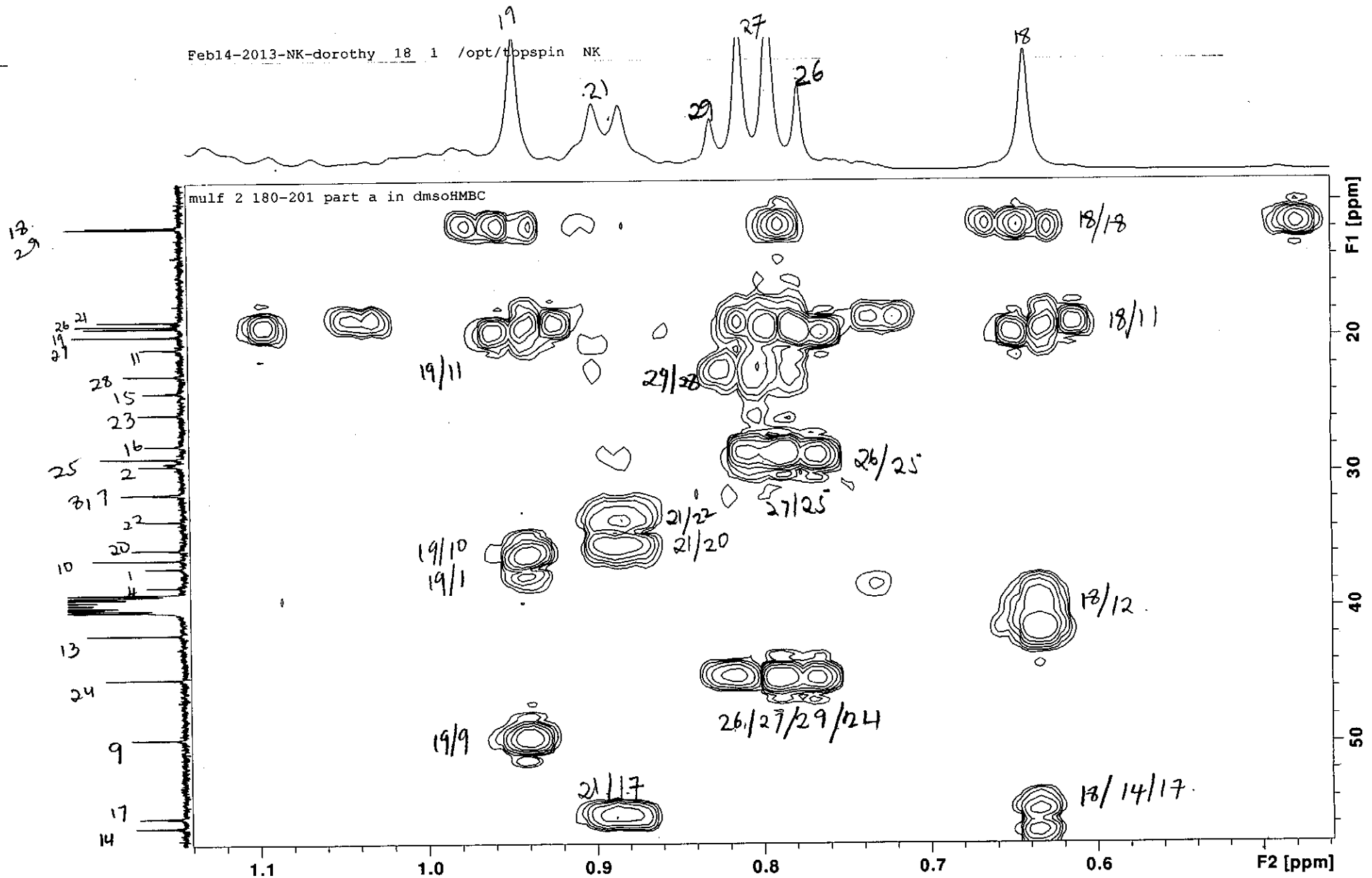
HSQC spectrum of B6



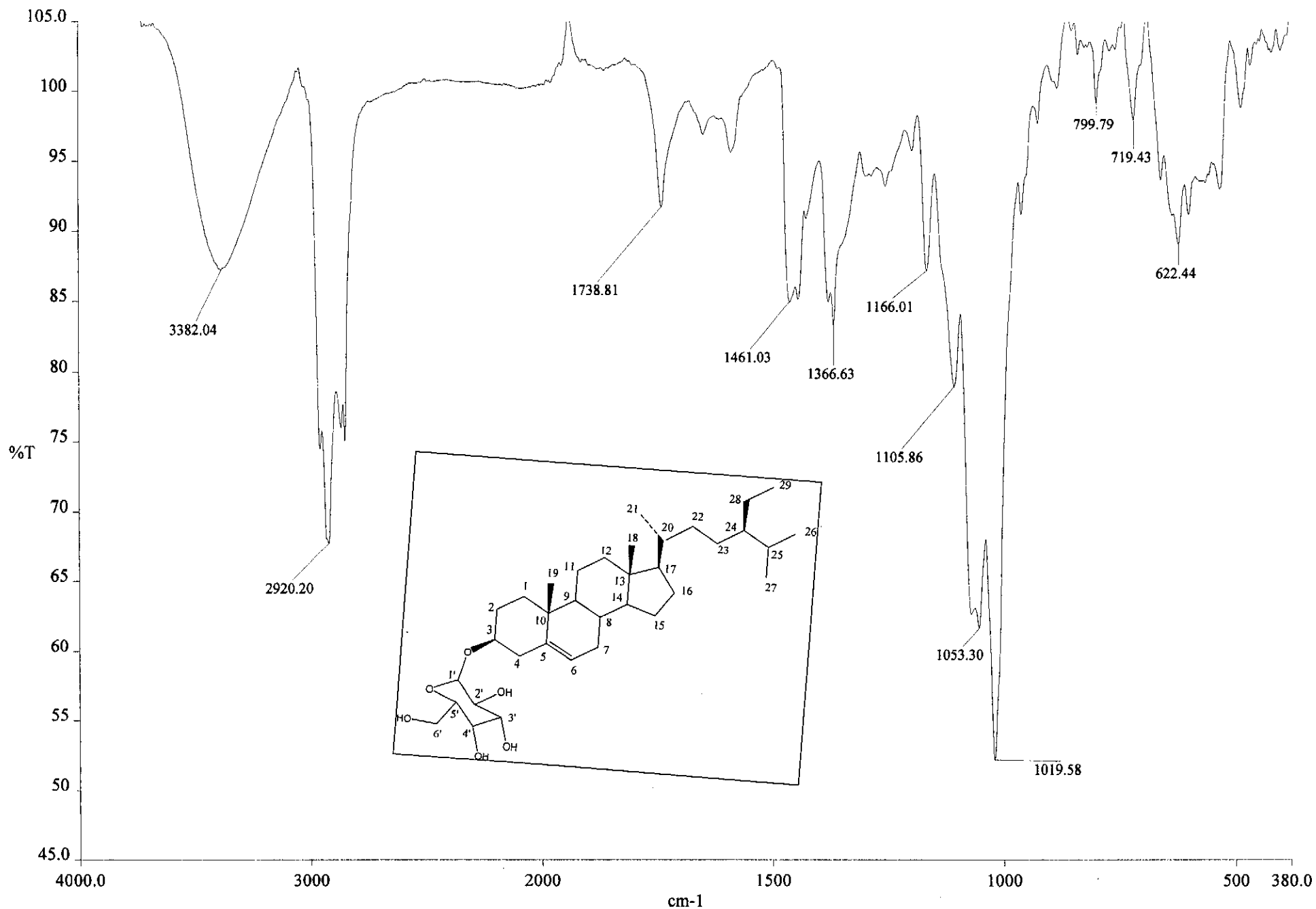
HSQC spectrum of B6 (F1 10-64 ppm, F2 0.0- 2.6 ppm)



HMBC spectrum of B6



HMBC spectrum of B6 expanded (F1 0-56 ppm, F2 0.5-1.2 ppm)

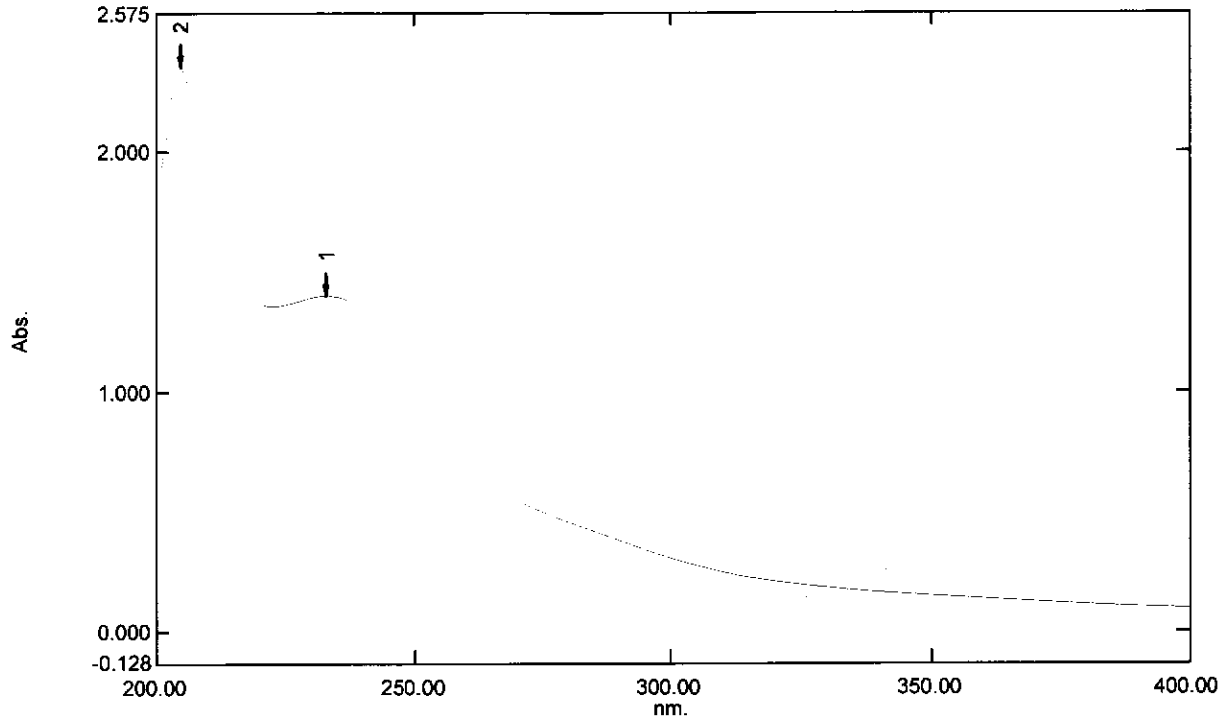


IR spectrum of B6

Spectrum Peak Pick Report

04/12/2011 06:58:47 PM

Data Set: MULF 80--88 23-38 18-31.spc - Storage 175323



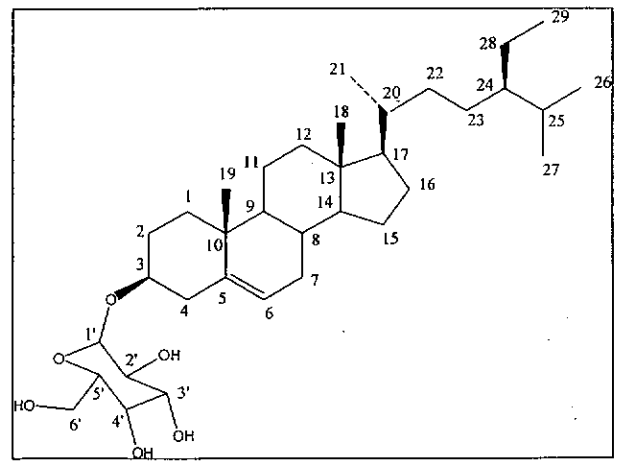
Measurement Properties

Wavelength Range (nm.):	200.00 to 400.00
Scan Speed:	Medium
Sampling Interval:	1.0
Auto Sampling Interval:	Disabled
Scan Mode:	Single

No.	P/V	Wavelength	Abs.	Description
1		233.00	1.400	
2		205.00	2.350	
3		223.00	1.356	

Instrument Properties

Instrument Type:	UV-3600 Series
Measuring Mode:	Absorbance
Slit Width:	8.0 nm
Time Constant:	0.1 sec.
Source Lamp:	Auto
Light Source Change Wavelength:	310.00 nm
Detector Unit:	Direct
Detector Change Wavelength:	830.00 nm 1800.00 nm
Grating Change Wavelength:	720.00 nm
S/R Exchange:	Normal
Detector Lock:	Auto
Slit Program:	Normal
Beam Mode:	Double
Stair Correction:	Disable



Attachment Properties

Attachment:	None

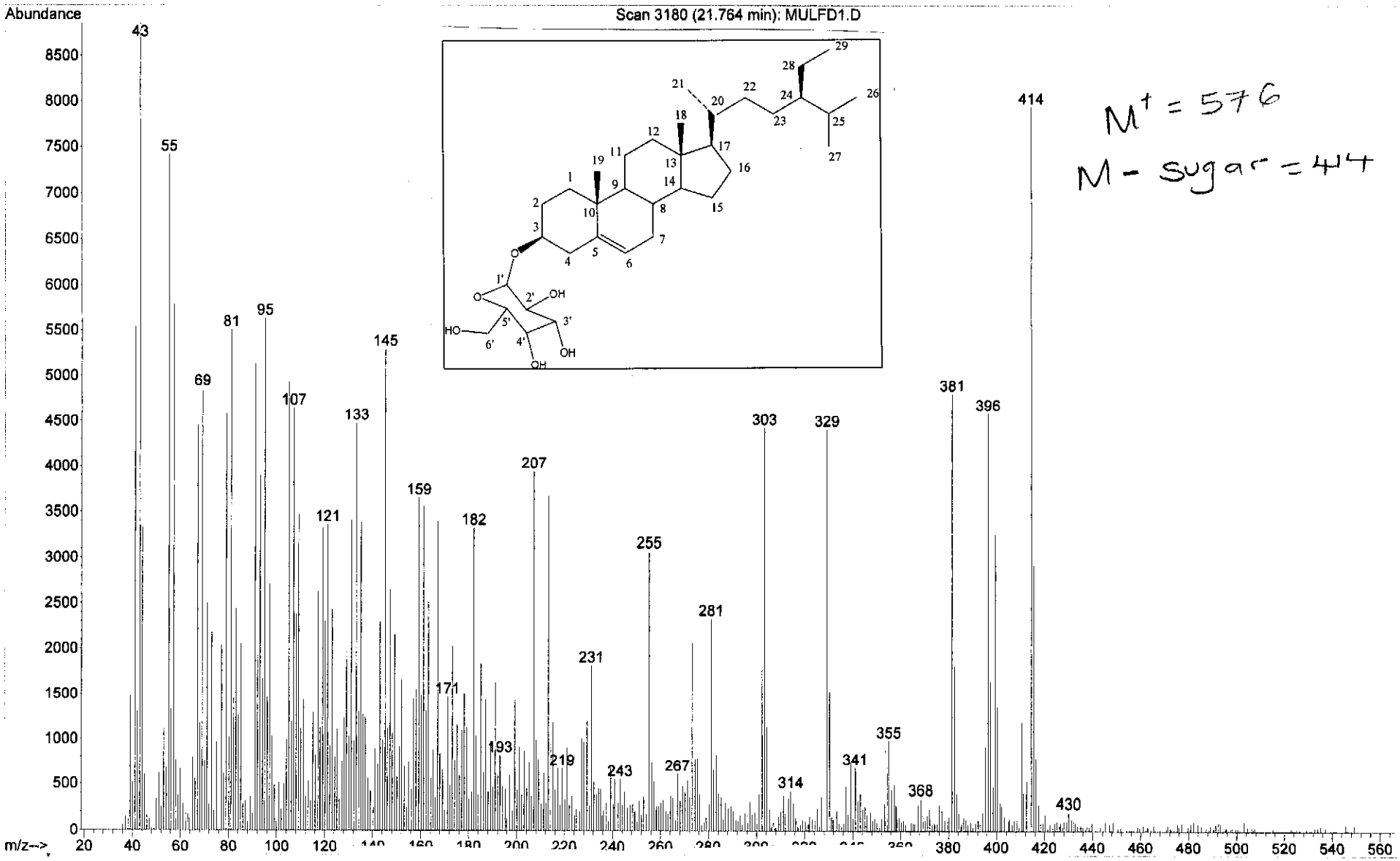
Sample Preparation Properties

Weight:	
Volume:	
Dilution:	
Path Length:	
Additional Information:	

UV spectrum of B6

File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\MULFD1.D
Operator : Dorothy
Acquired : 28 Apr 2011 18:47 using AcqMethod NATURALJ
Instrument : Instrumen
Sample Name: MULF 71-78 (33-40) 3
Misc Info :
Vial Number: 1

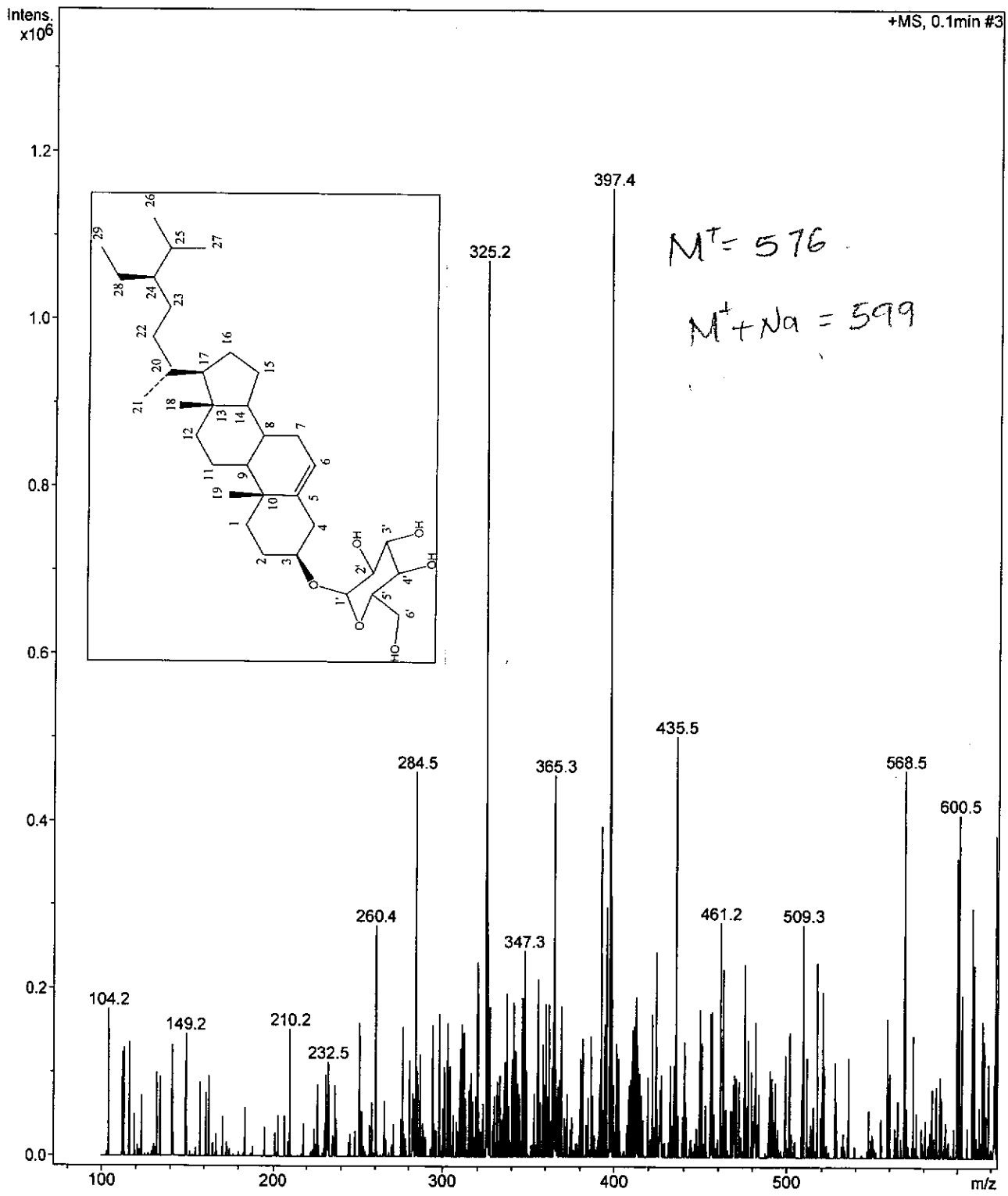
Sitosterol-β-glucoside



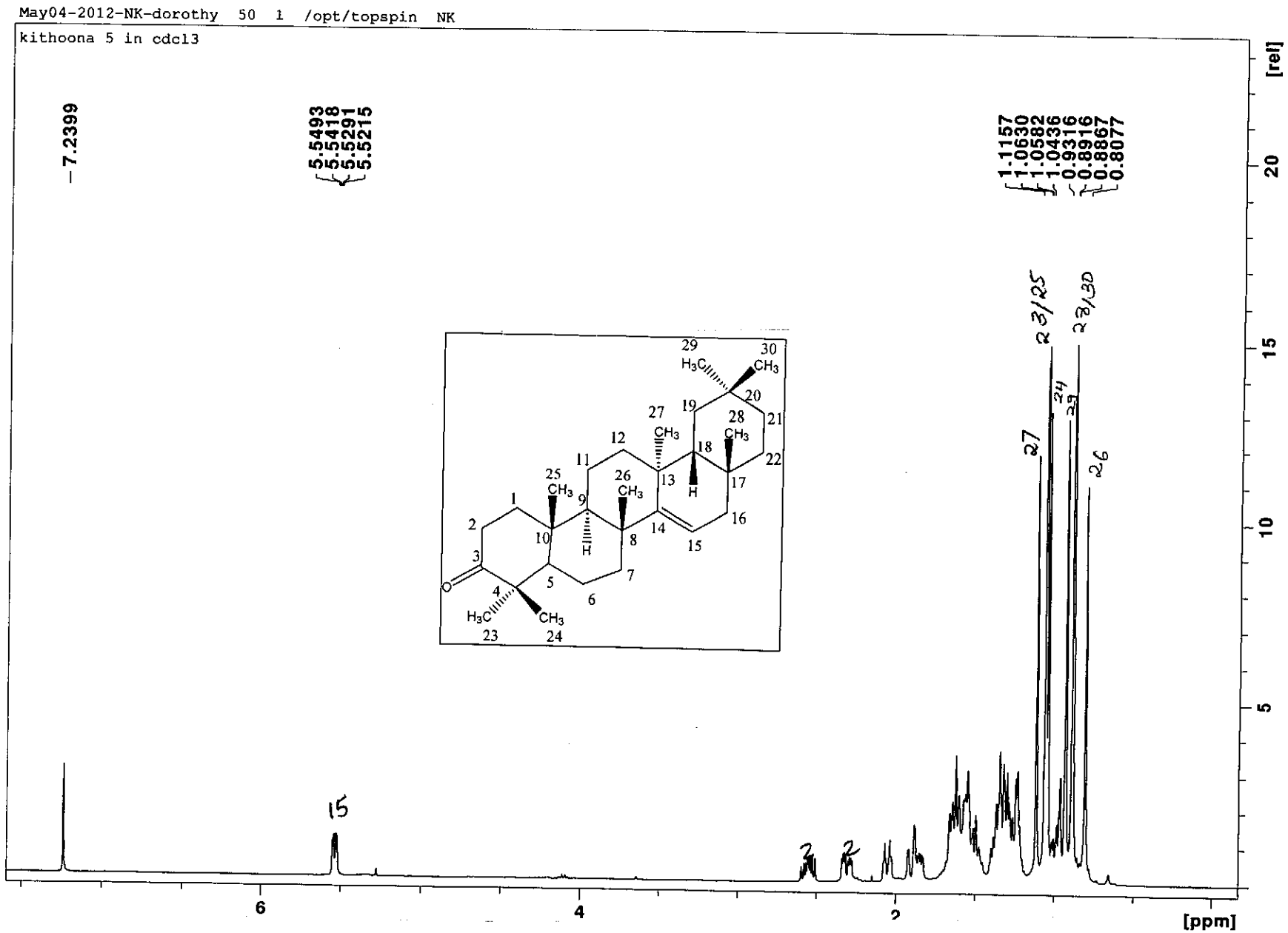
MS spectrum of B6

Display Report - Selected Window Selected Analysis

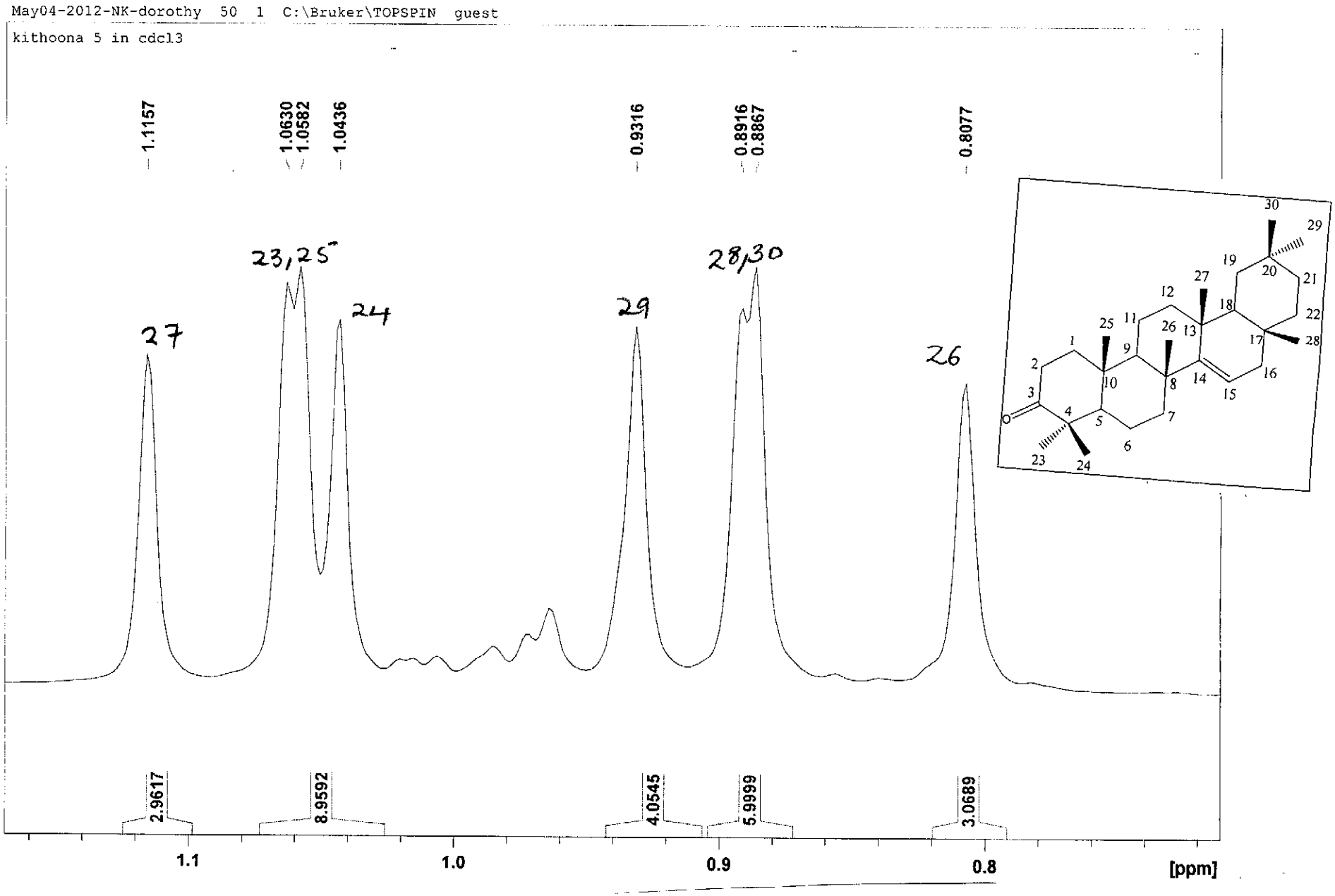
Analysis Name: SG000003.D **Instrument:** LC-MSD-Trap-VL **Print Date:** 6/20/2012 11:15:50 AM
Method: GUMBI2.M **Operator:** Operator **Acq. Date:** 6/20/2012 11:13:14 AM
Sample Name: Default
Analysis Info:



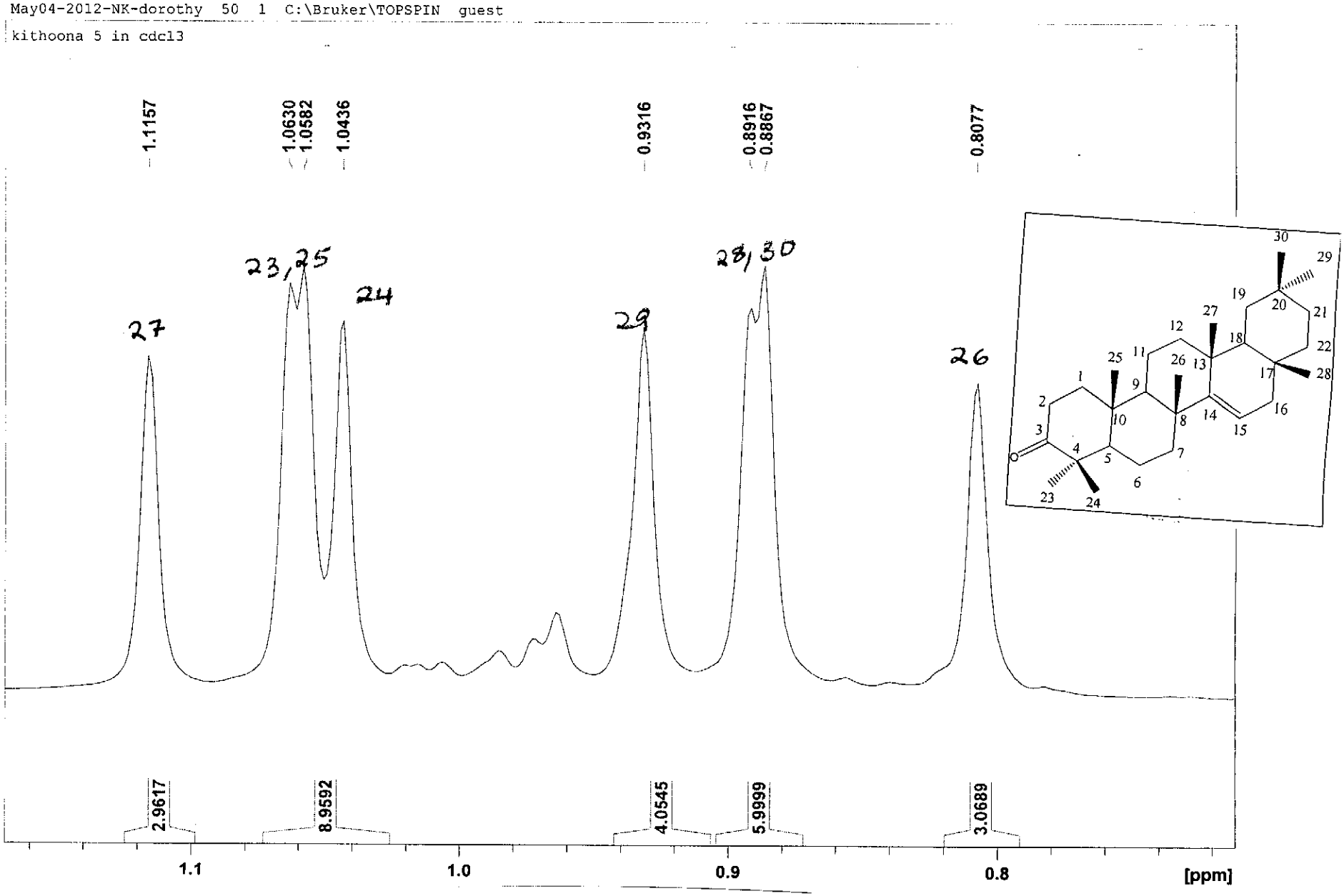
LC-MS spectrum of B6



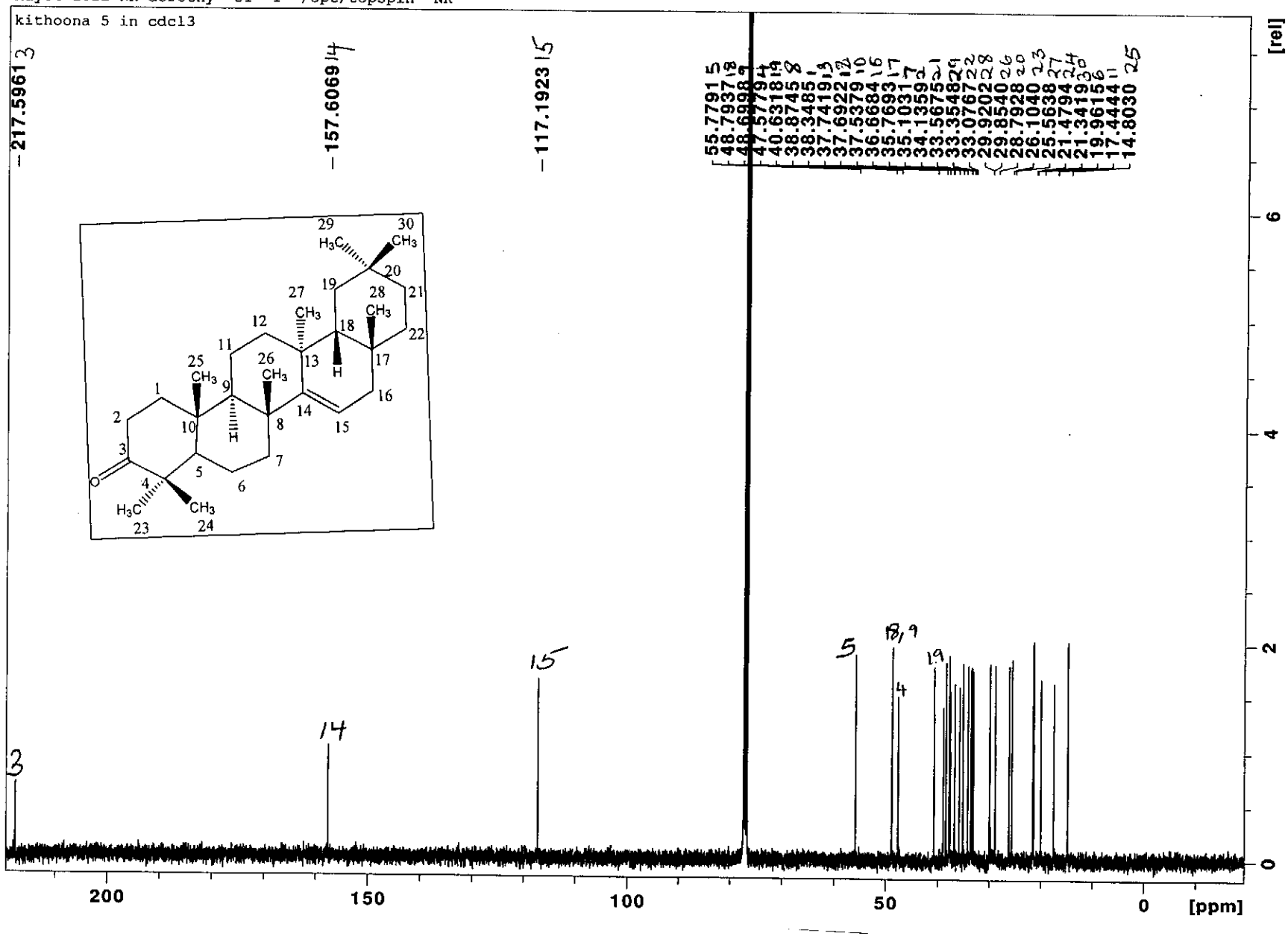
^{1}H NMR spectrum of B7



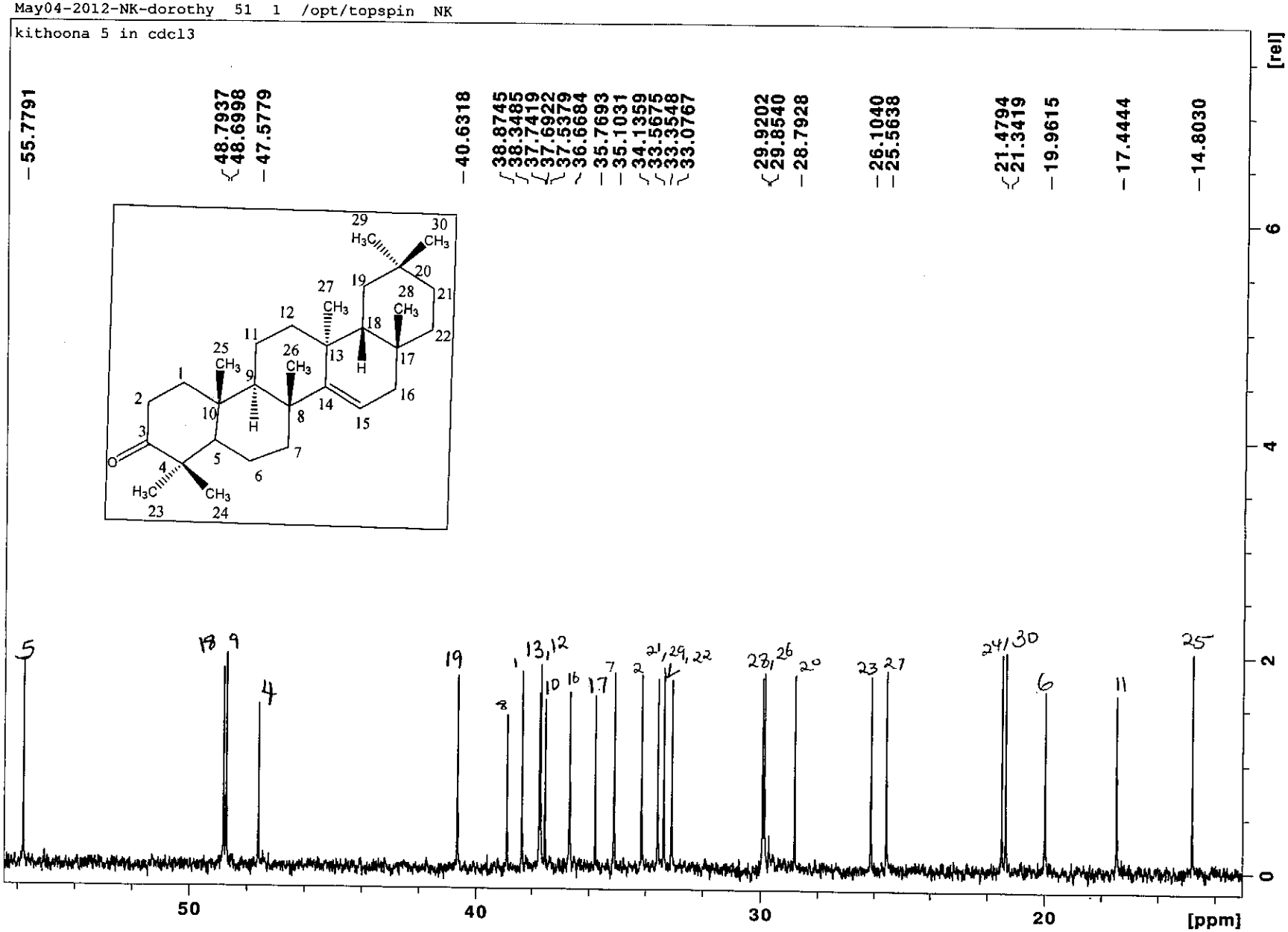
^{1}H NMR spectrum of B7 expanded (0.6-1.2 ppm)



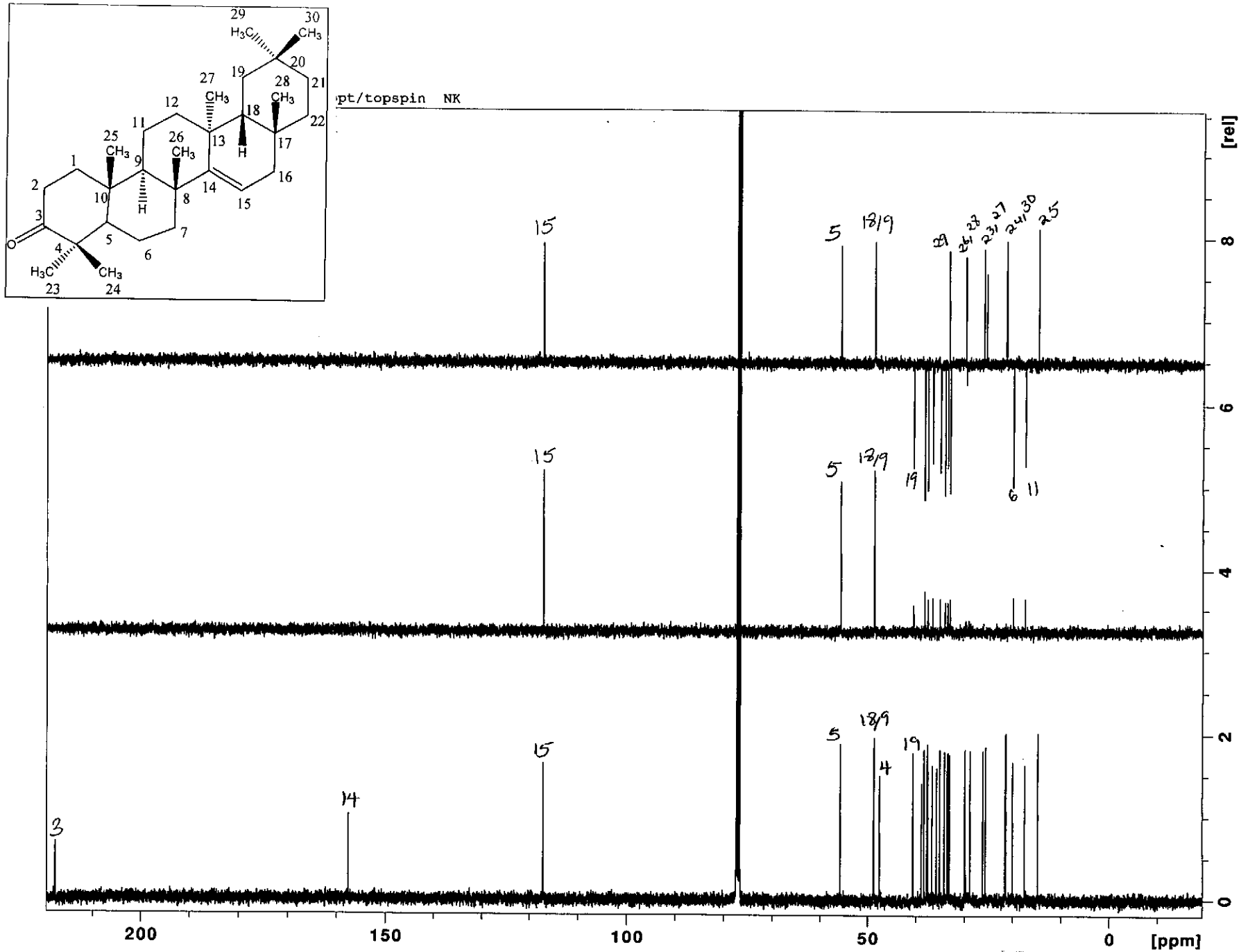
^{1}H NMR spectrum of B7 expanded (0.6-1.2 ppm)



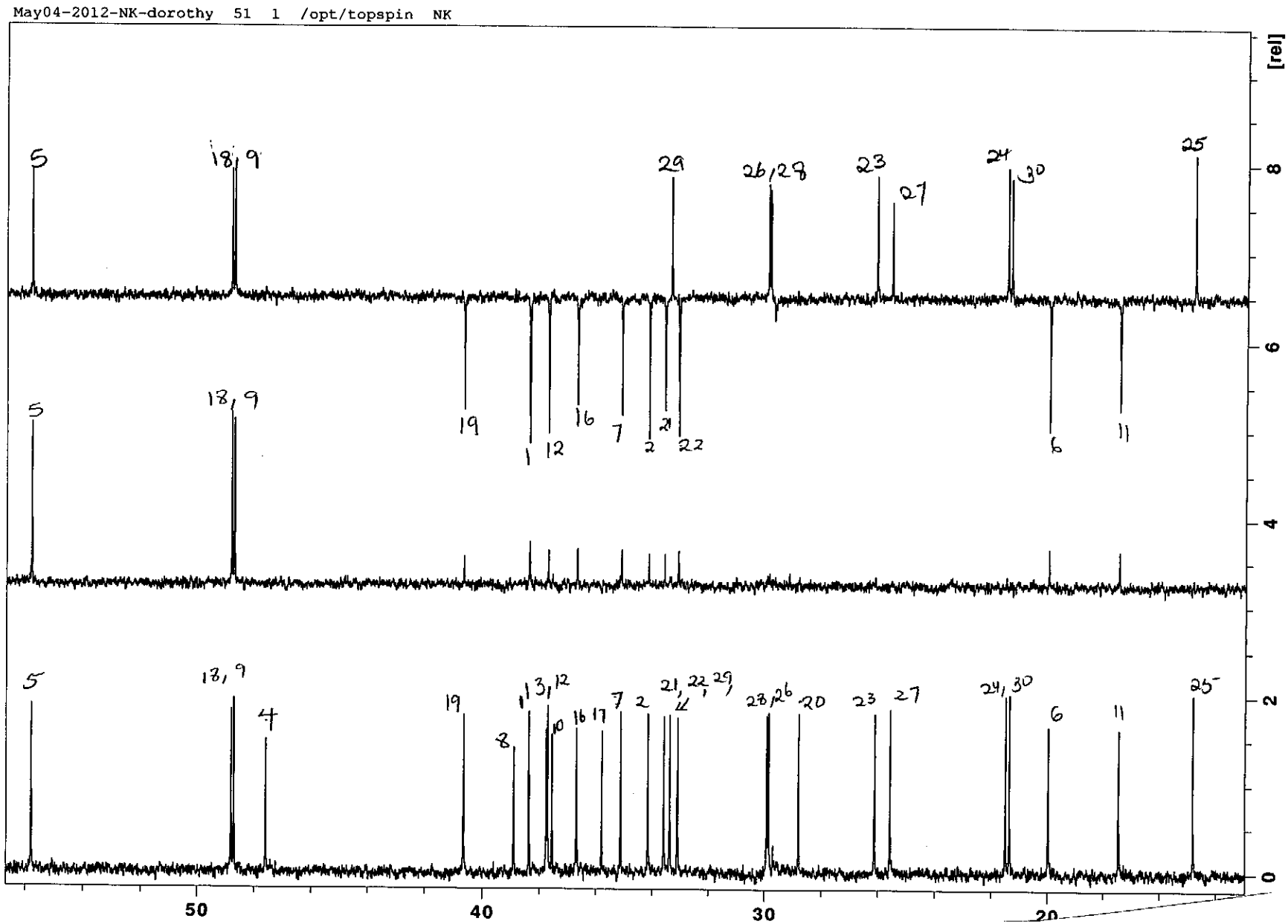
^{13}C NMR spectrum of B7



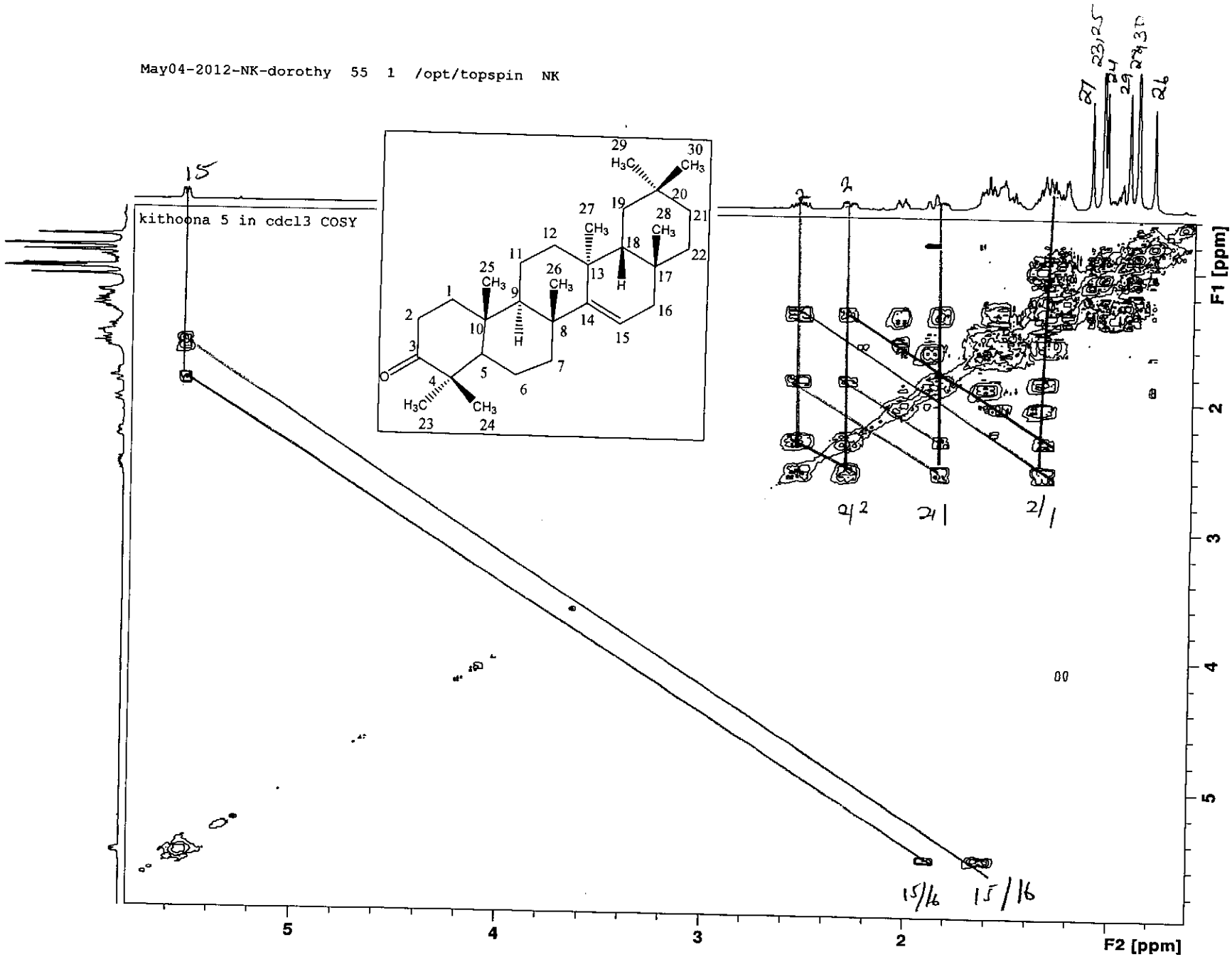
^{13}C NMR spectrum of B7 expanded (14.0-56.0 ppm)



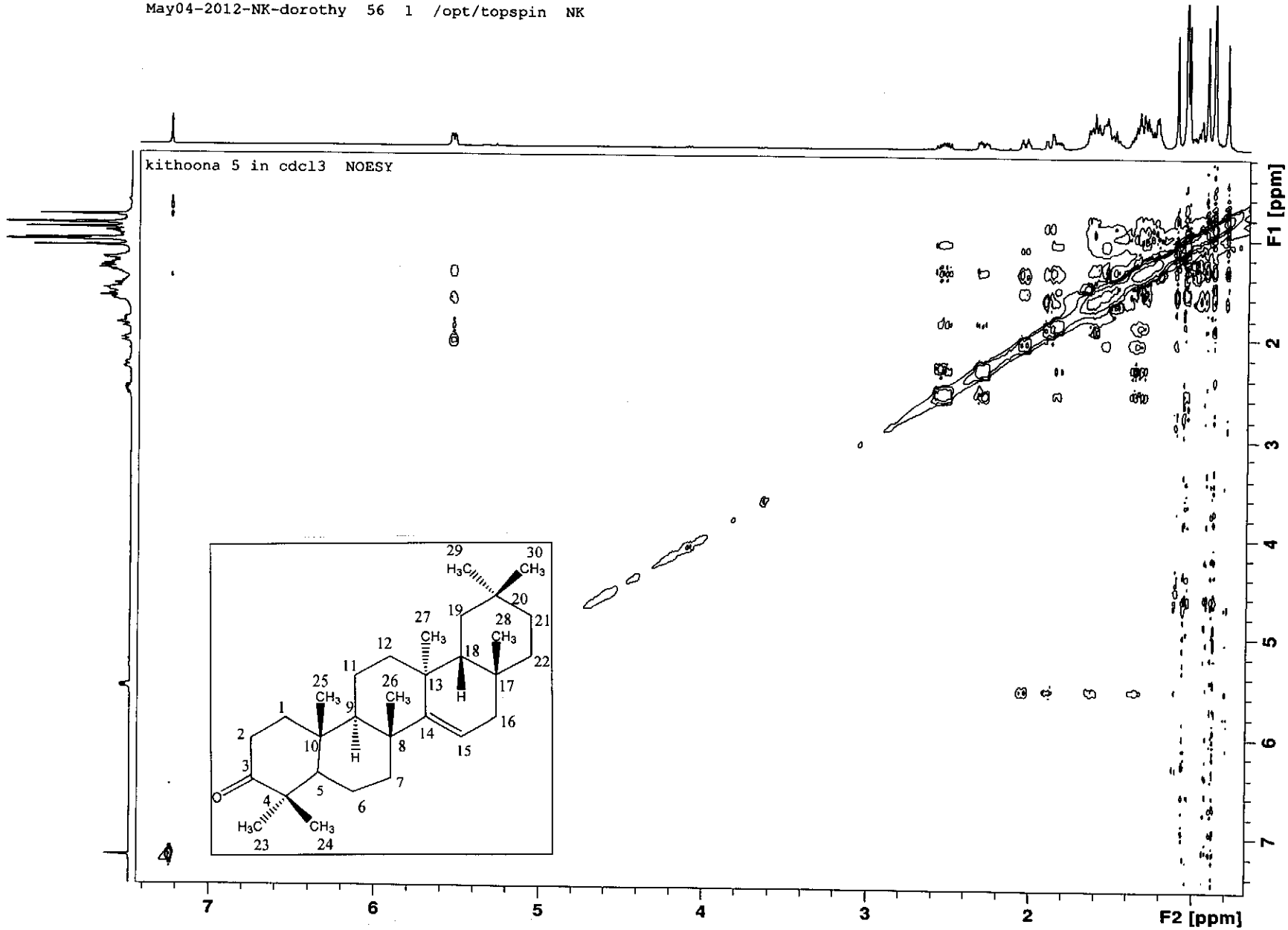
DEPT spectrum of B7



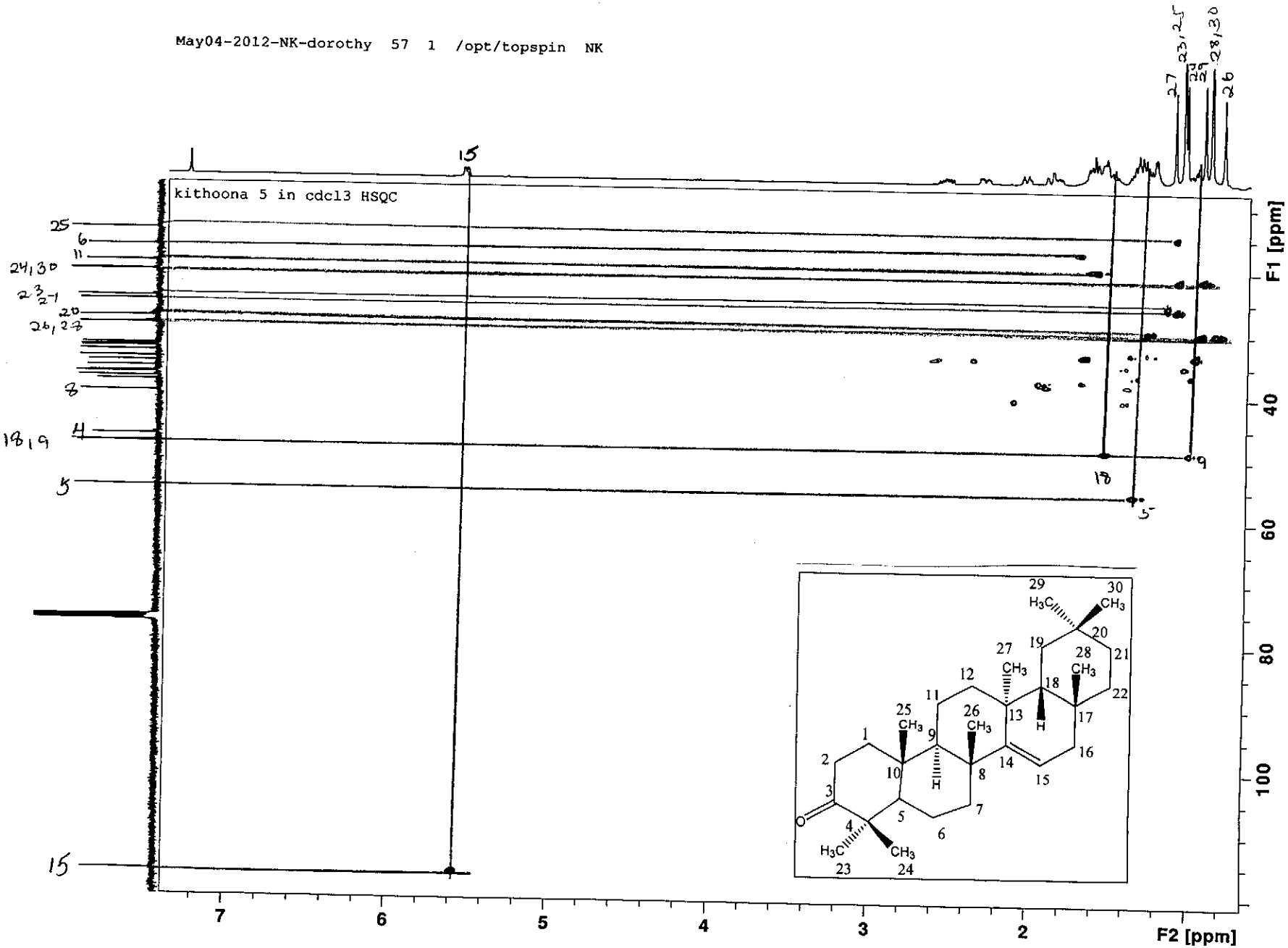
DEPT spectrum of B7 expanded (14.0-56.0 ppm)



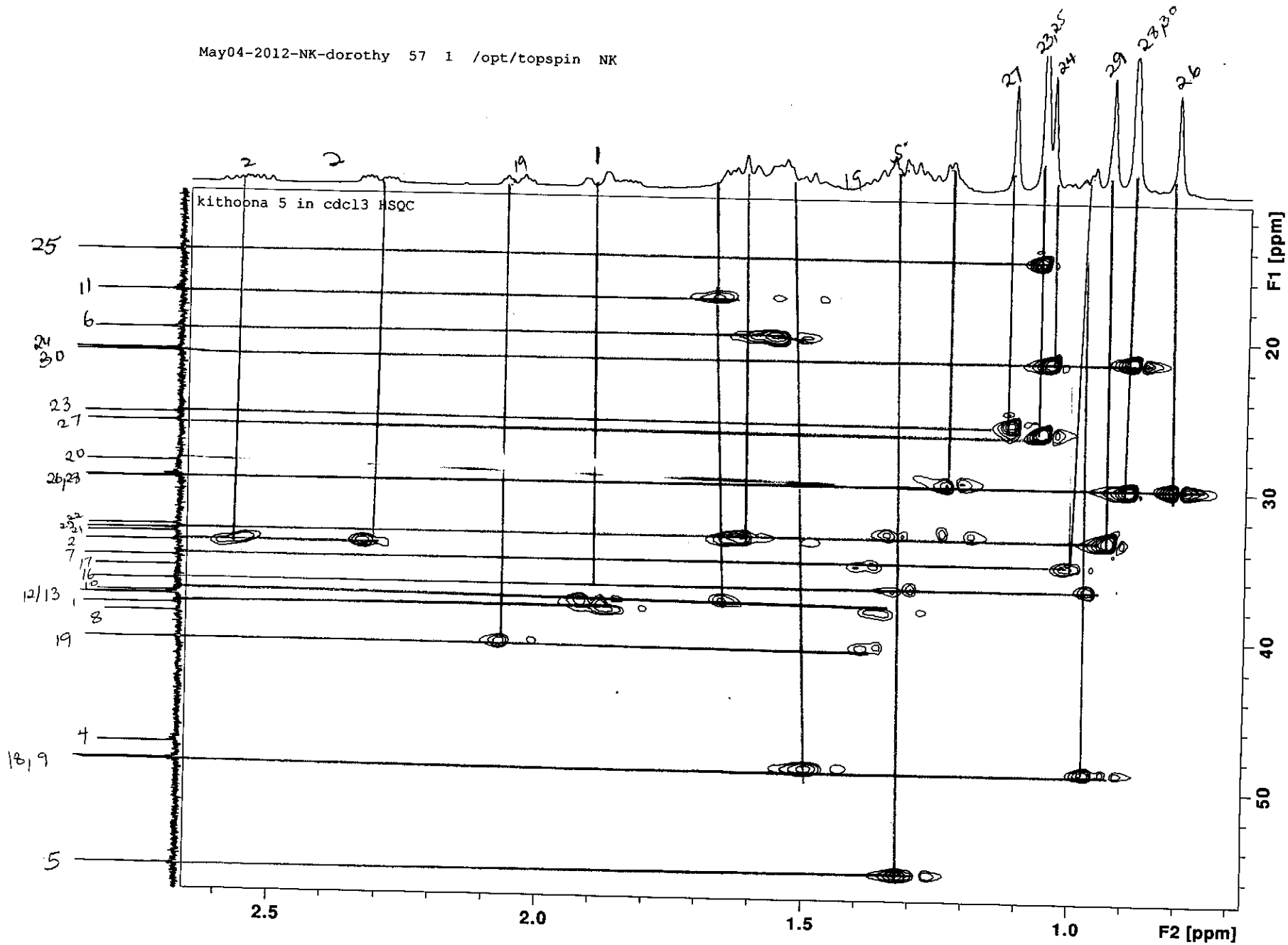
COSY spectrum of B7



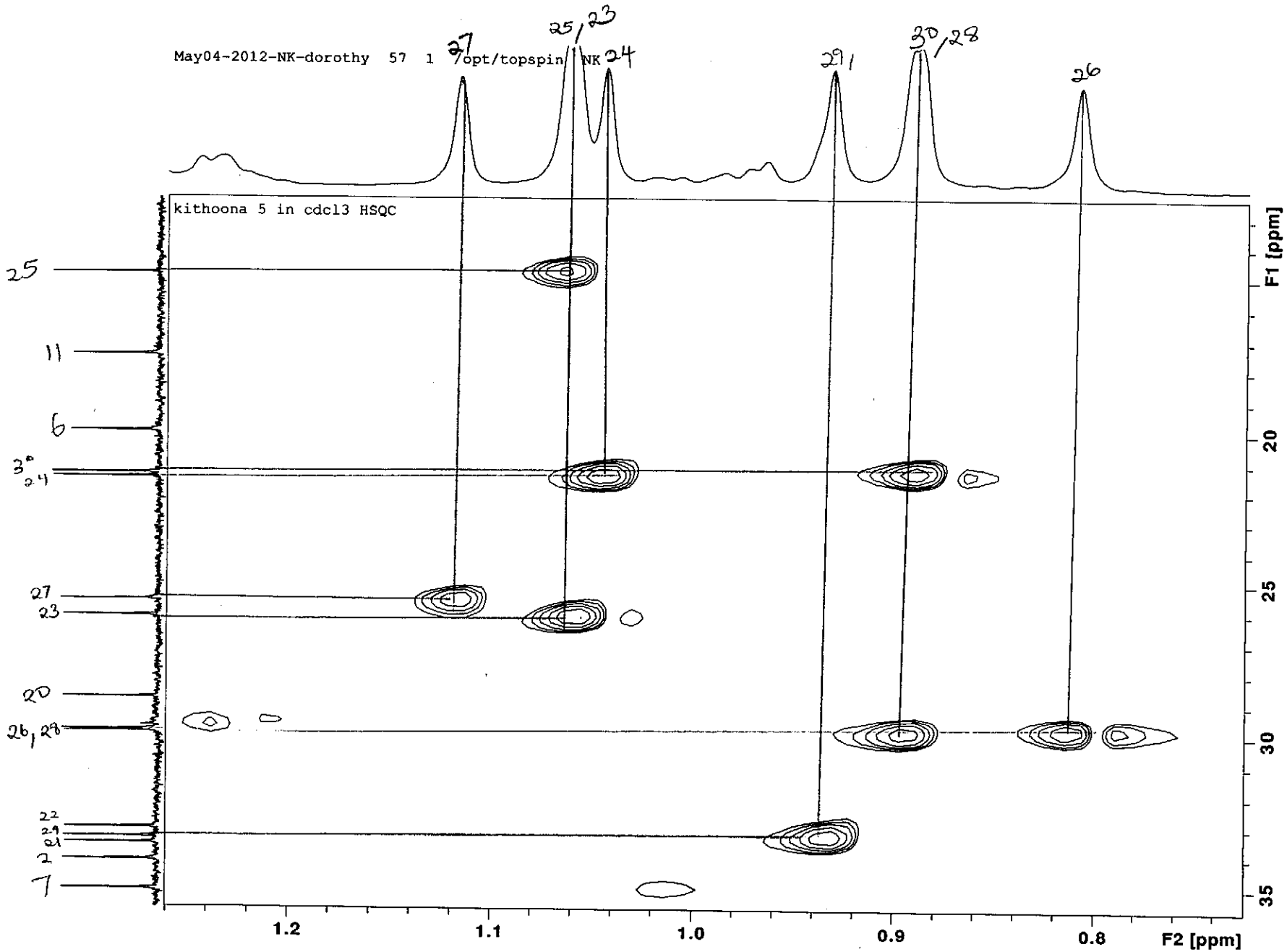
NOESY spectrum of B7



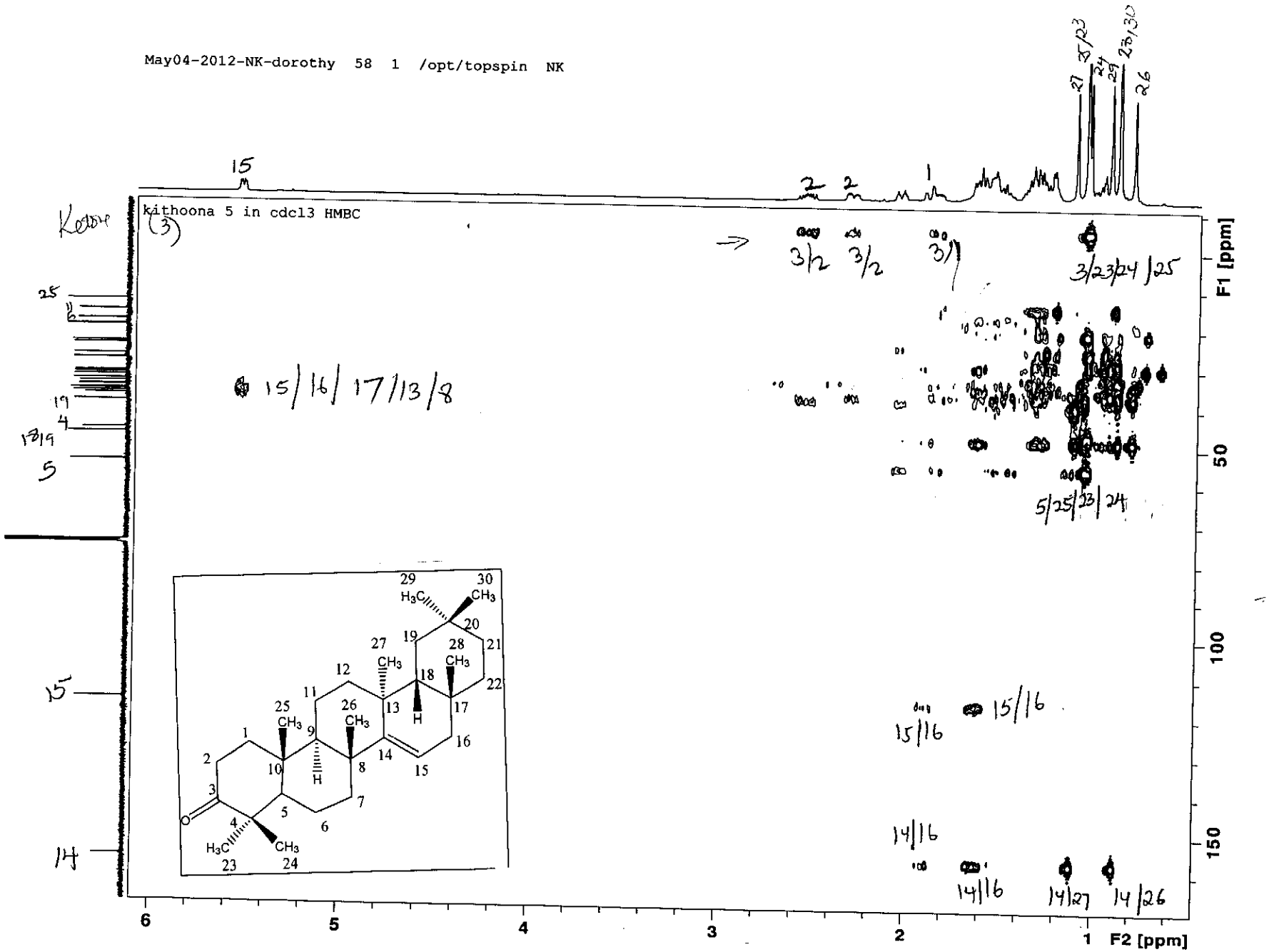
HSQC spectrum of B7



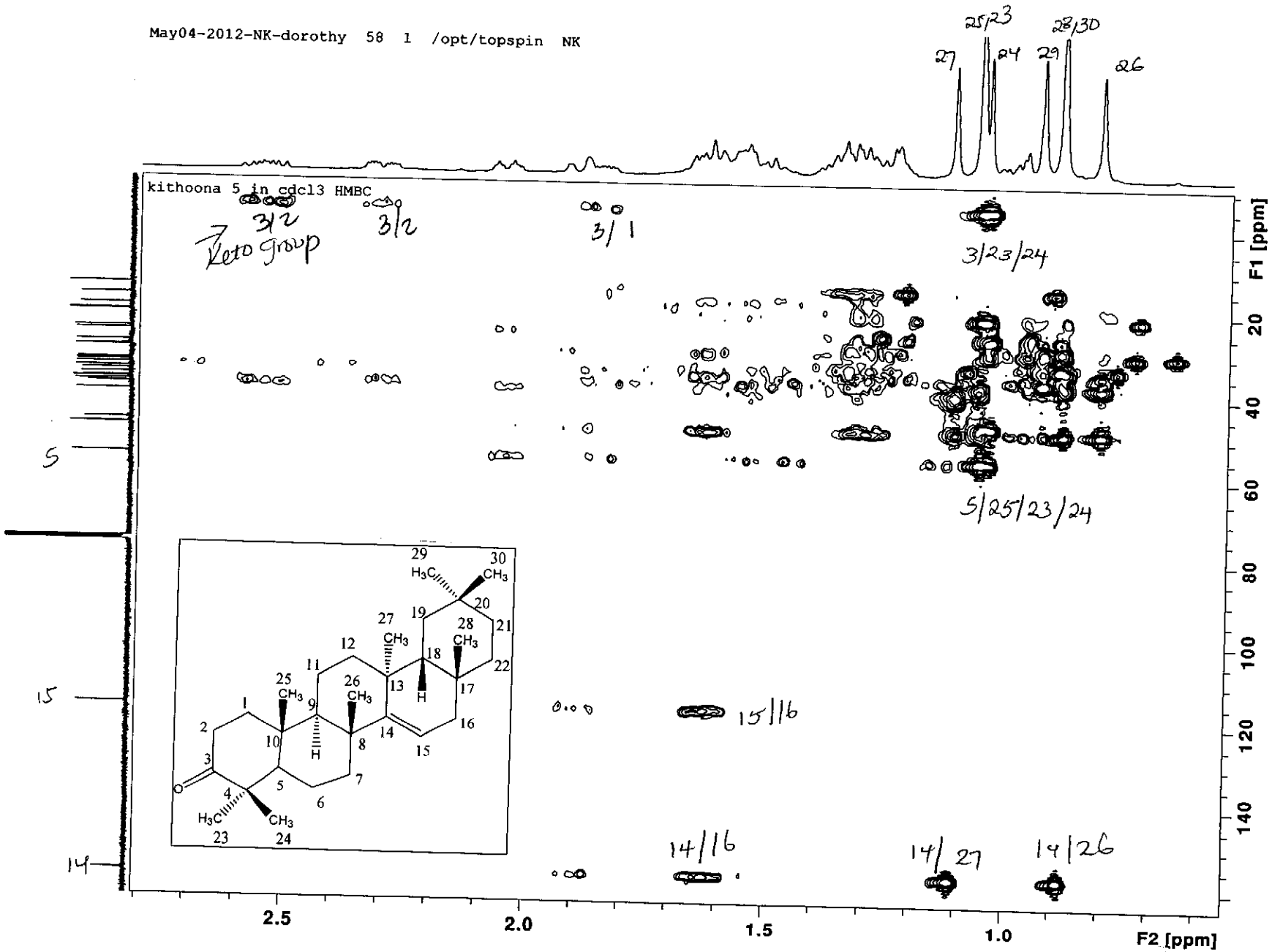
HSQC spectrum of B7 (F1 10-56 ppm, F2 0.7- 2.6 ppm)



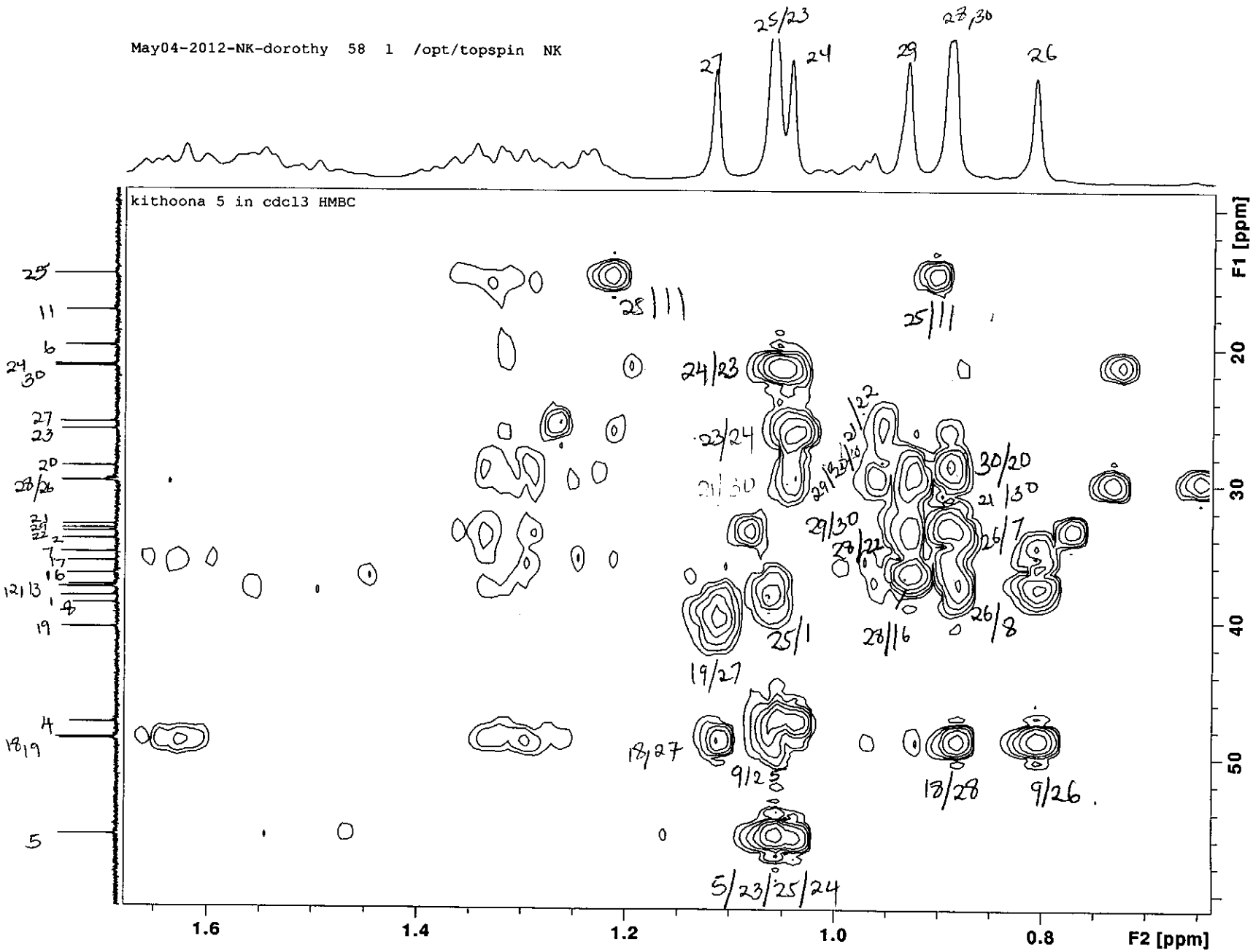
HSQC spectrum of B7 (F1 10-35 ppm, F2 0.7- 1.25 ppm)



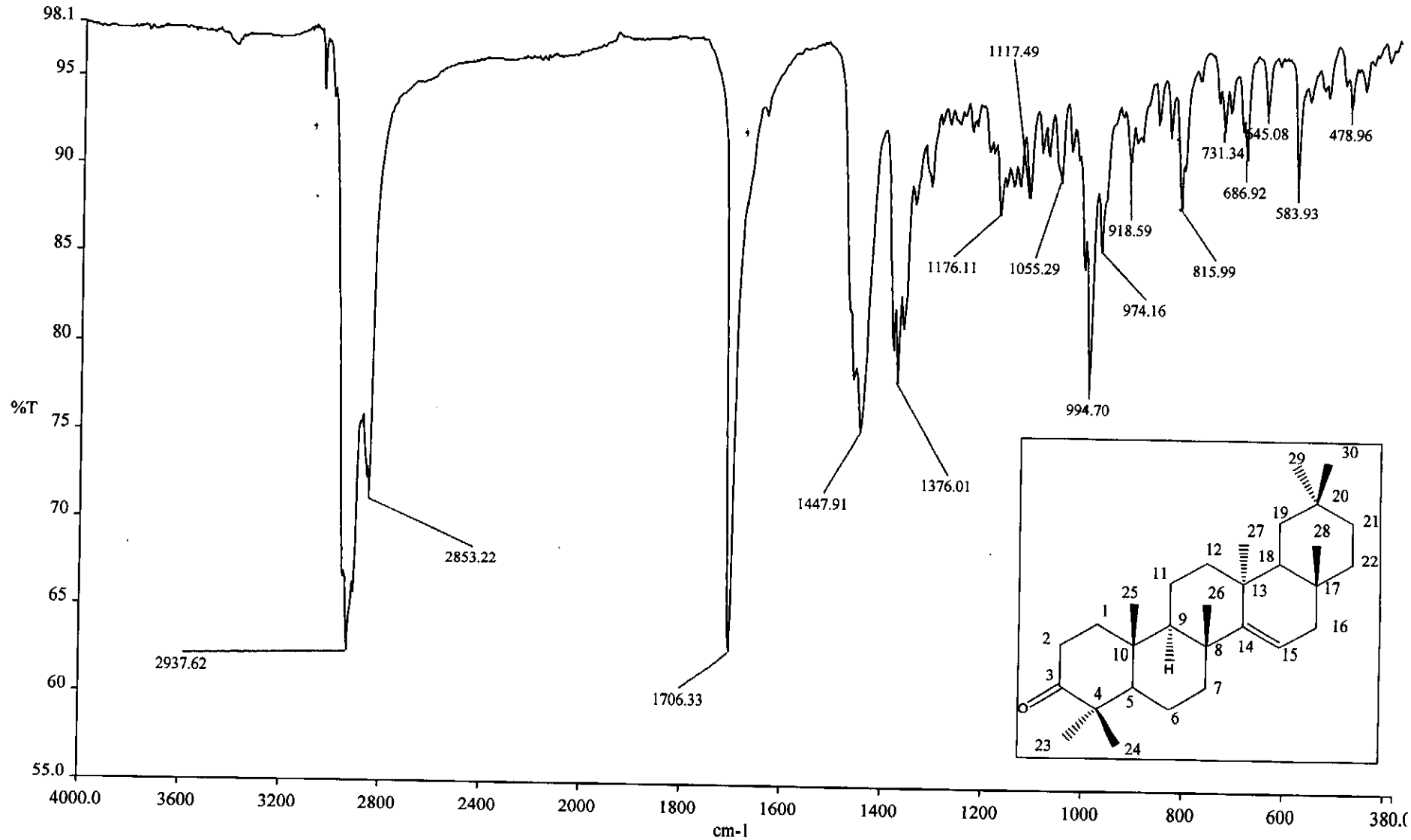
HMBC spectrum of B7



HMBC spectrum of B7 expanded (F1 0-158 ppm, F2 0-2.7 ppm)



HMBC spectrum of B7 expanded (F1 0-56 ppm, F2 0-1.6 ppm)

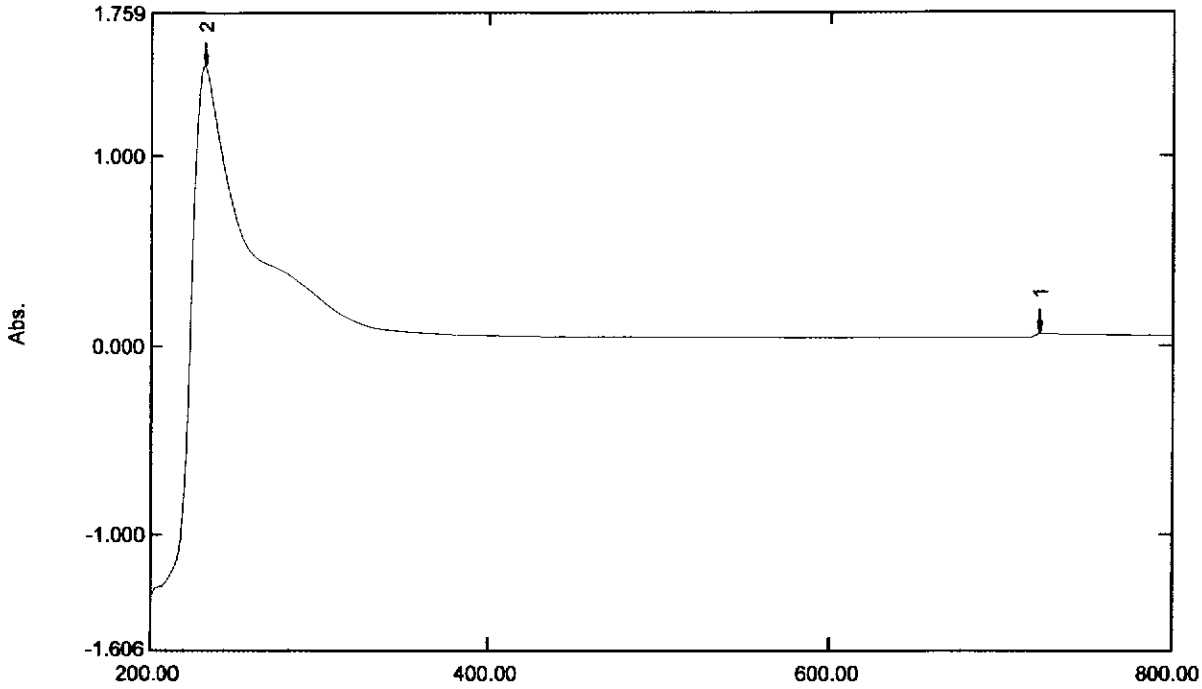


IR spectrum of B7

Spectrum Peak Pick Report

04/10/2011 12:20:29 PM

Data Set: MURT 6 (17-44).spc - Storage 133352



No.	P/V	Wavelength	Abs.	Description
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2	●	232.00	1.479	
3	◔	718.00	0.040	

Measurement Properties

Wavelength Range (nm.):	200.00 to 800.00
Scan Speed:	Medium
Sampling Interval:	1.0
Auto Sampling Interval:	Disabled
Scan Mode:	Single

Instrument Properties

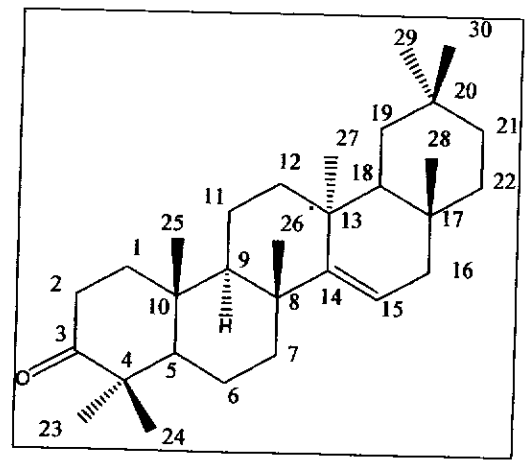
Instrument Type:	UV-3600 Series
Measuring Mode:	Absorbance
Slit Width:	8.0 nm
Time Constant:	0.1 sec.
Source Lamp:	Auto
Light Source Change Wavelength:	310.00 nm
Detector Unit:	Direct
Detector Change Wavelength:	830.00 nm 1800.00 nm
Grating Change Wavelength:	720.00 nm
S/R Exchange:	Normal
Detector Lock:	Auto
Slit Program:	Normal
Beam Mode:	Double
Stair Correction:	Disable

Attachment Properties

Attachment:	None

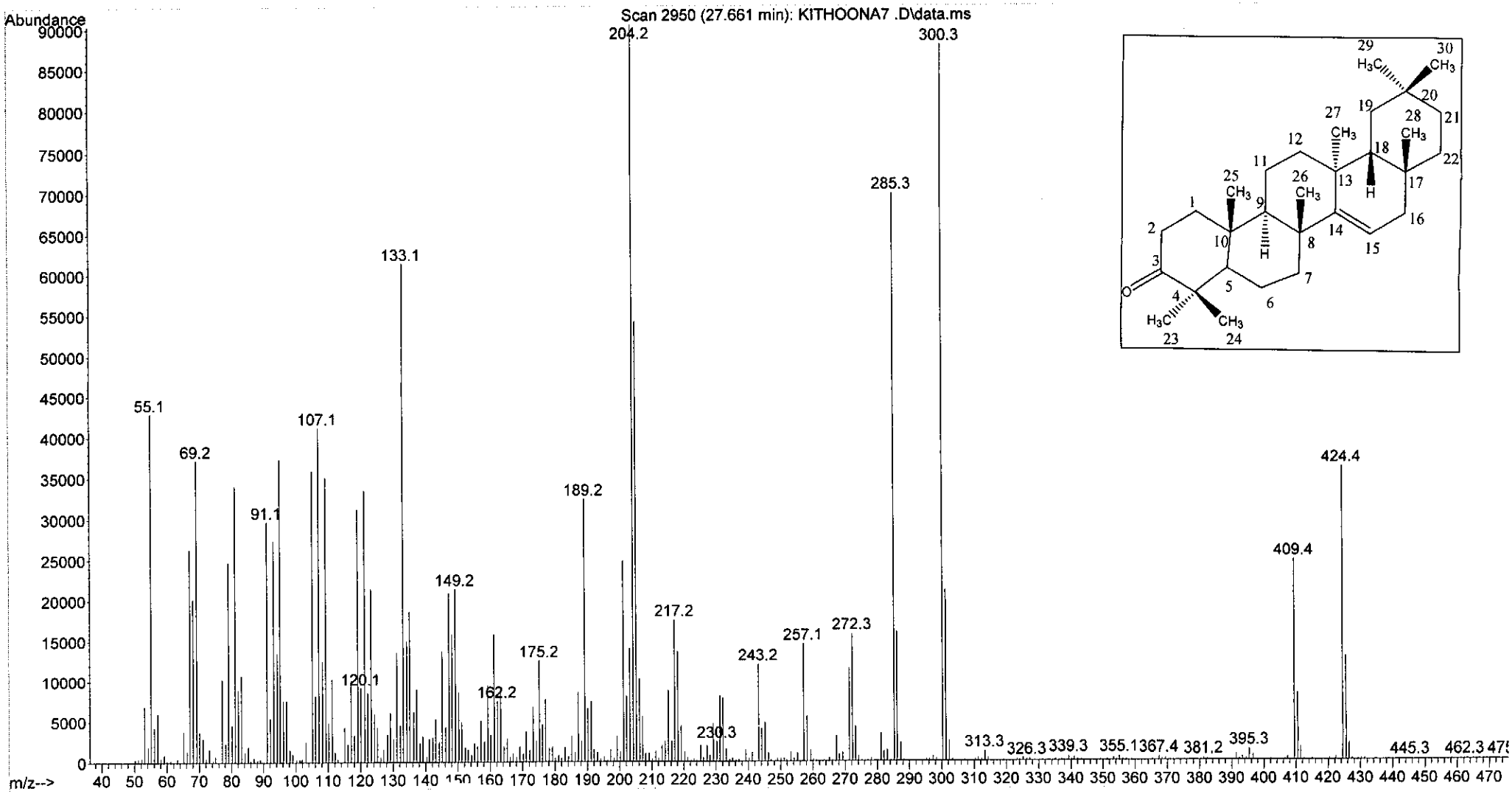
Sample Preparation Properties

Weight:	
Volume:	
Dilution:	
Path Length:	
Additional Information:	

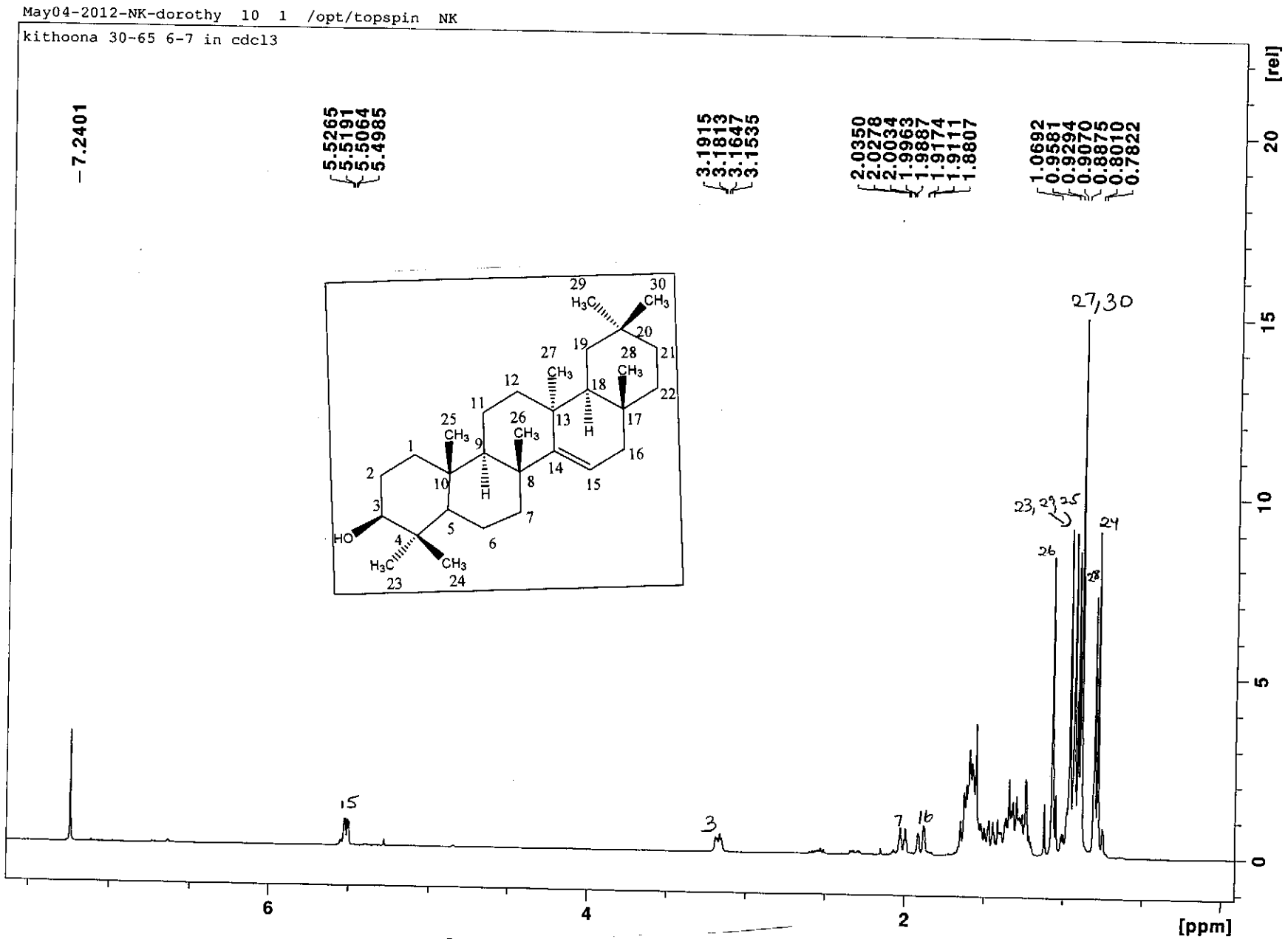


UV spectrum of B7

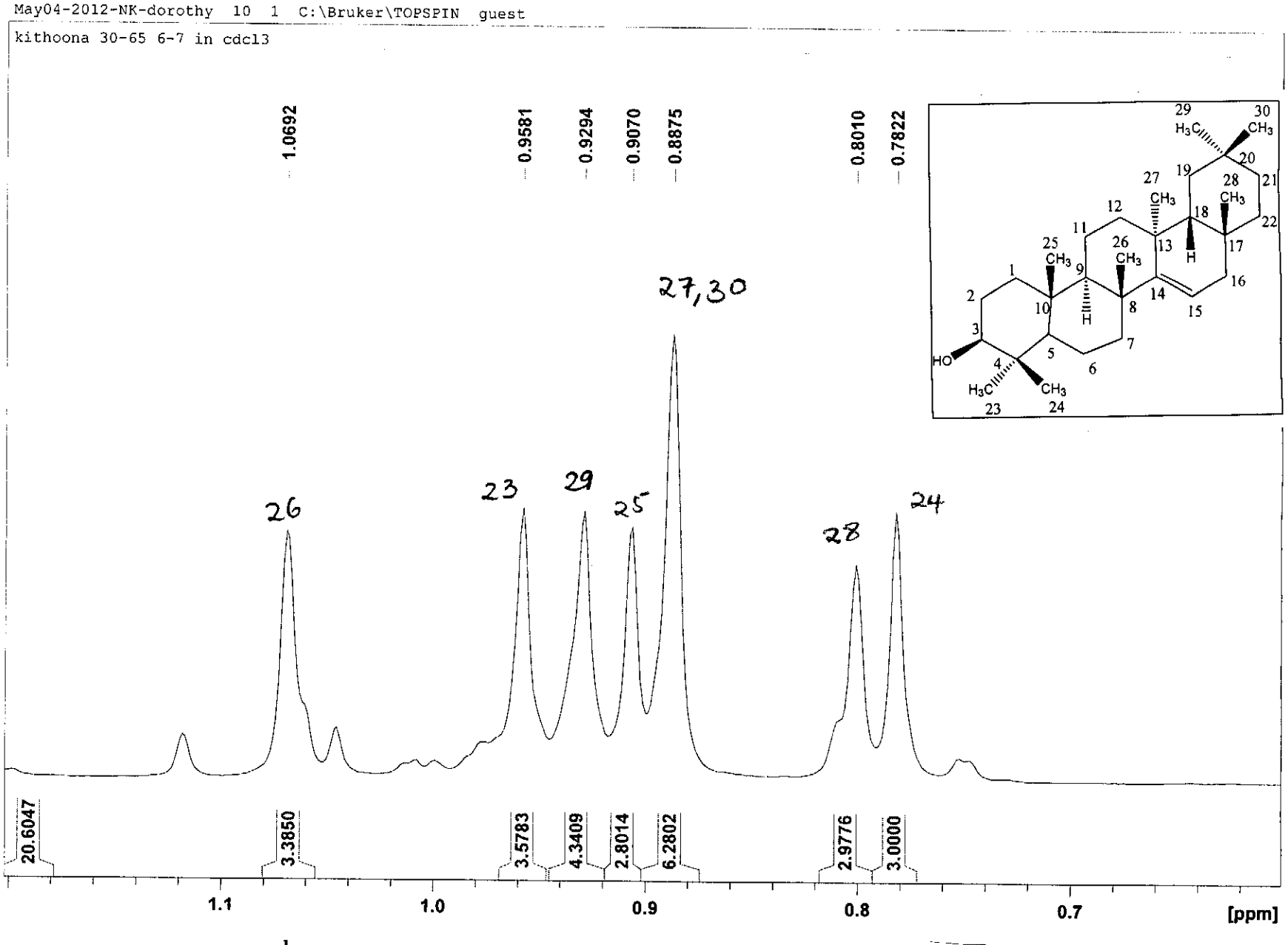
File :C:\msdchem\1\data\dorothy\KITHOONA7 .D
Operator : Dorothy
Acquired : 5 May 2012 23:57 using AcqMethod NATPRODUCTS MANUAL INJ SPLIT.M
Instrument : 5973N
Sample Name: kithoona 8-12 (6-7)
Misc Info :
Vial Number: 1



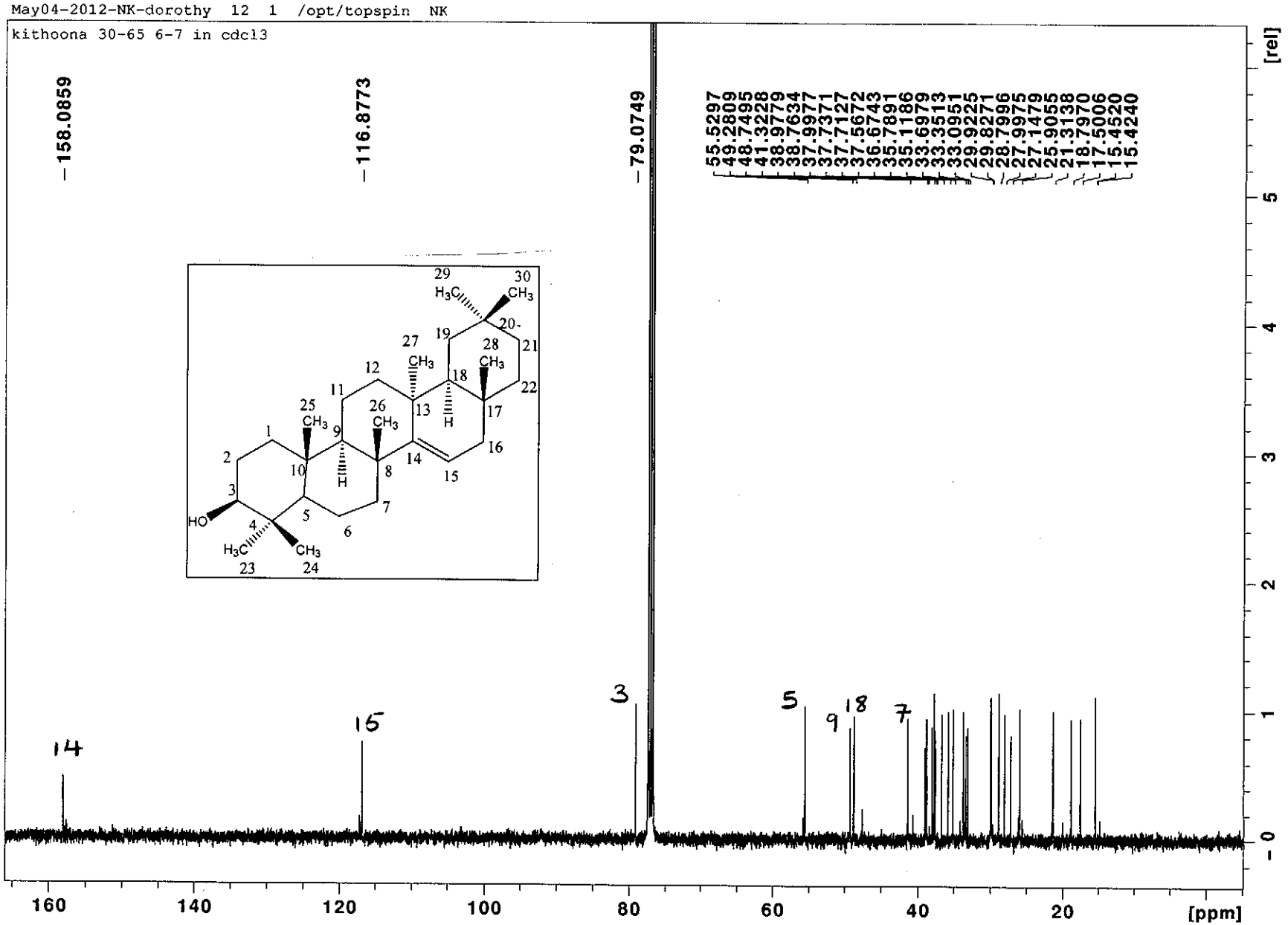
MS spectrum of B7



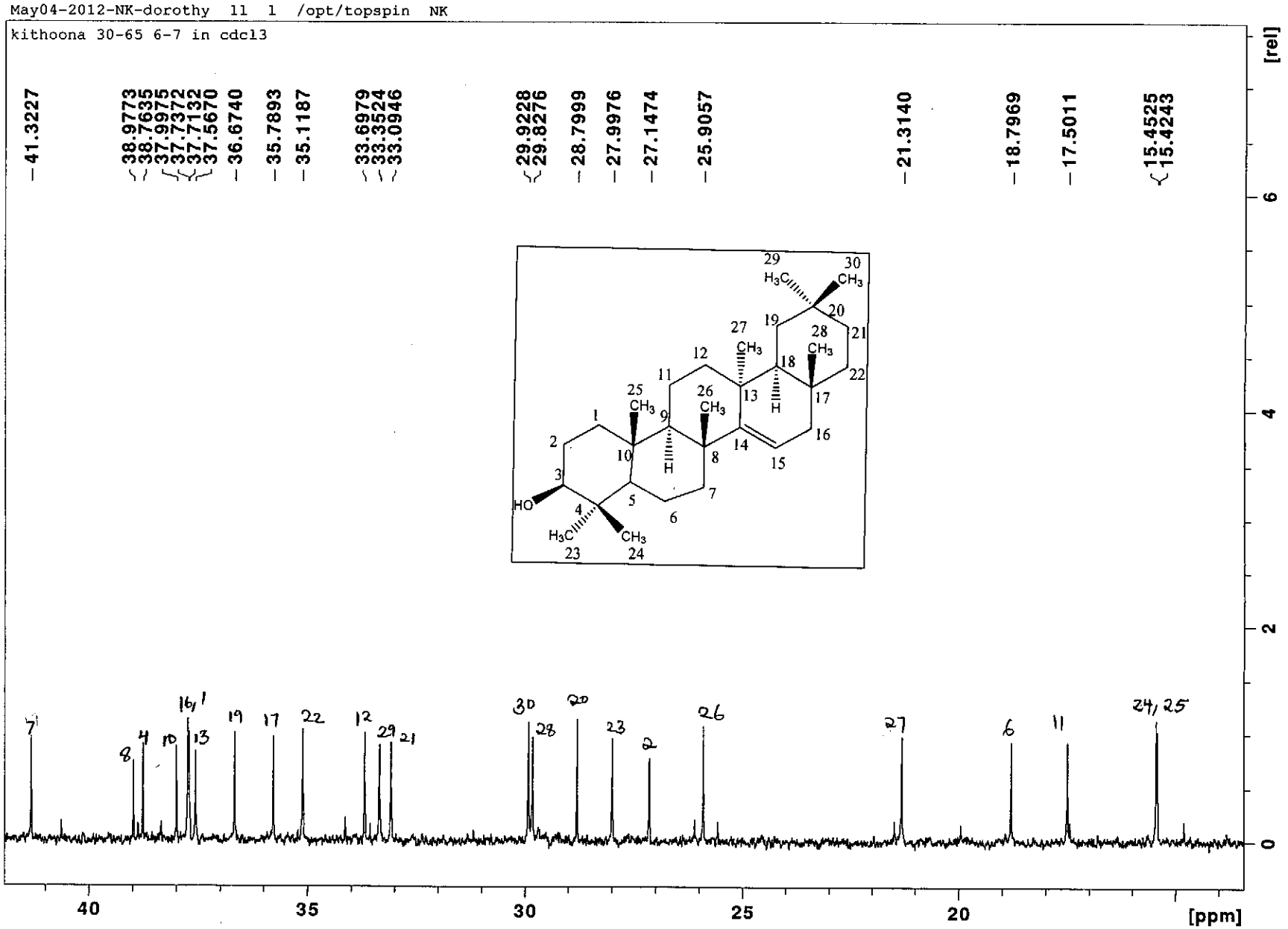
^{1}H NMR spectrum of B8



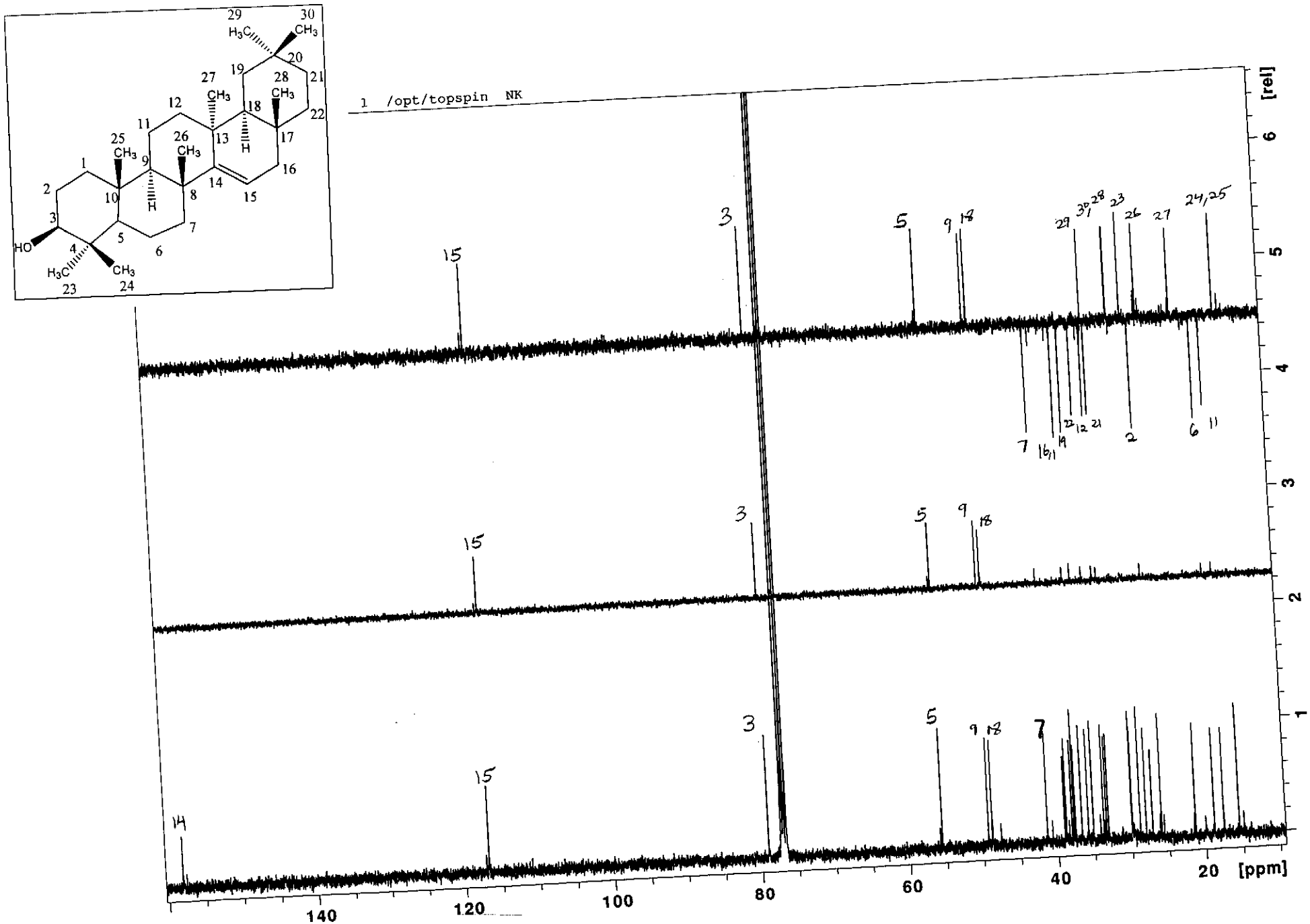
^{1}H NMR spectrum of B8 expanded (0.6-1.2 ppm)



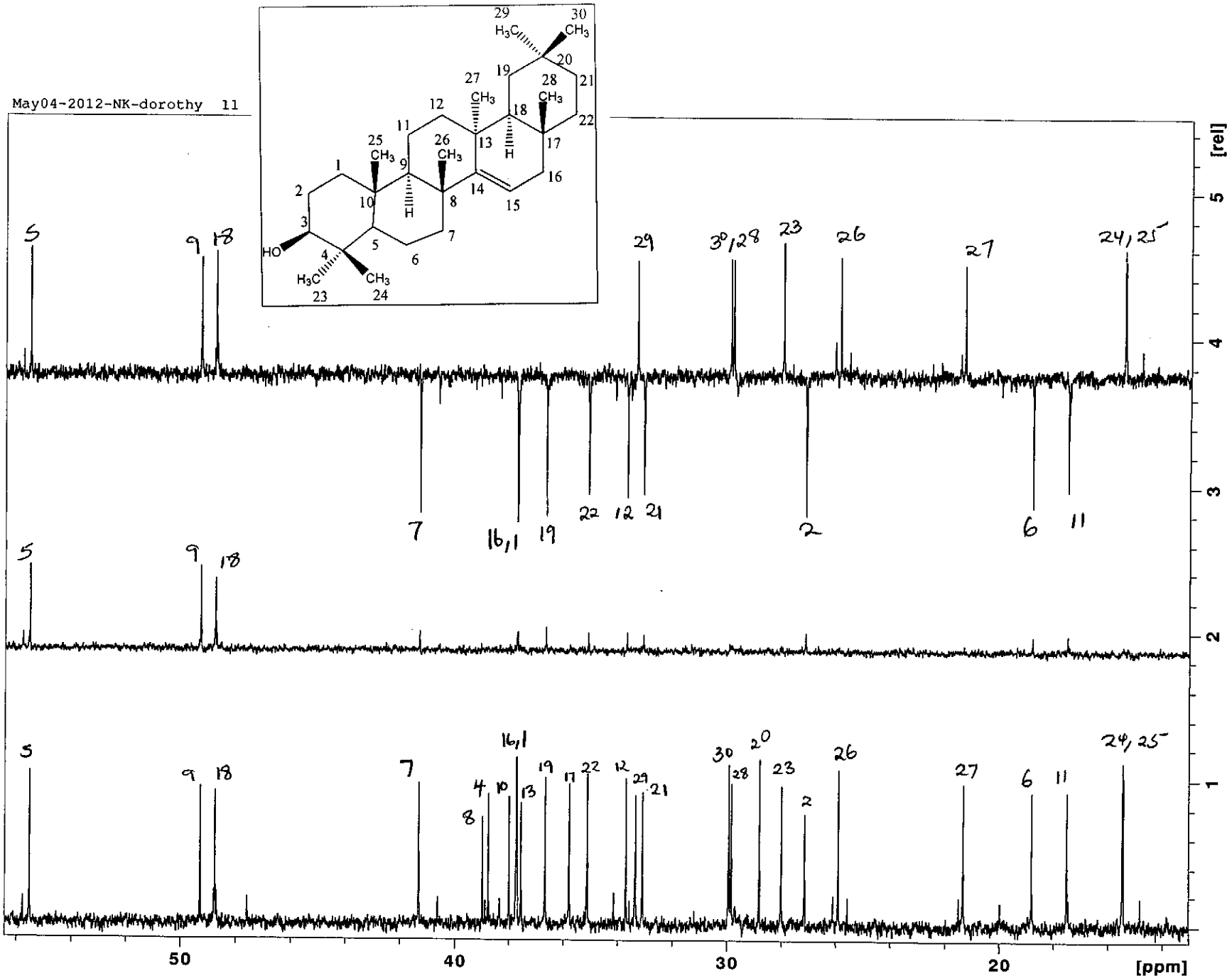
^{13}C NMR spectrum of B8



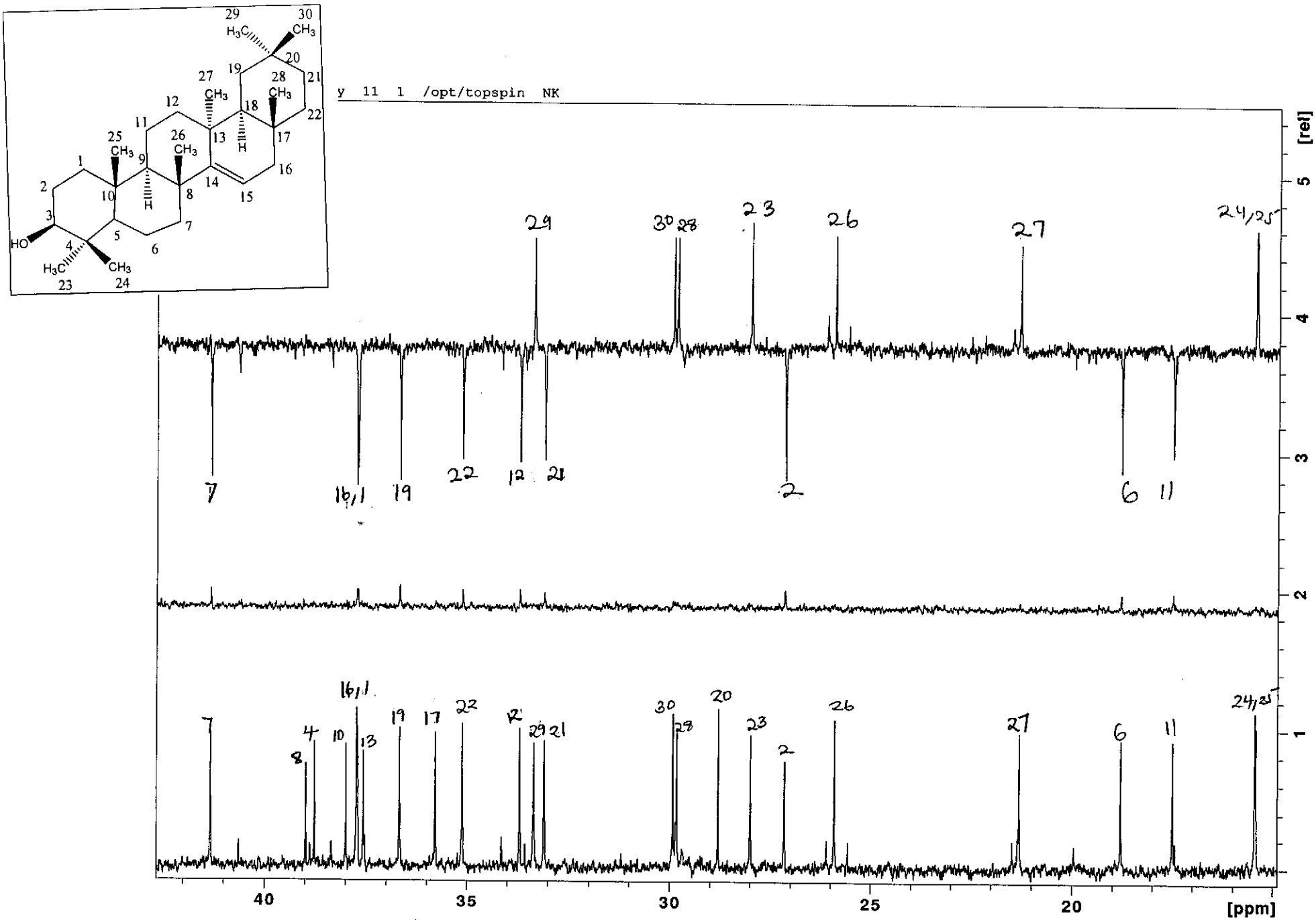
^{13}C NMR spectrum of B8 expanded (14.0-42.0 ppm)



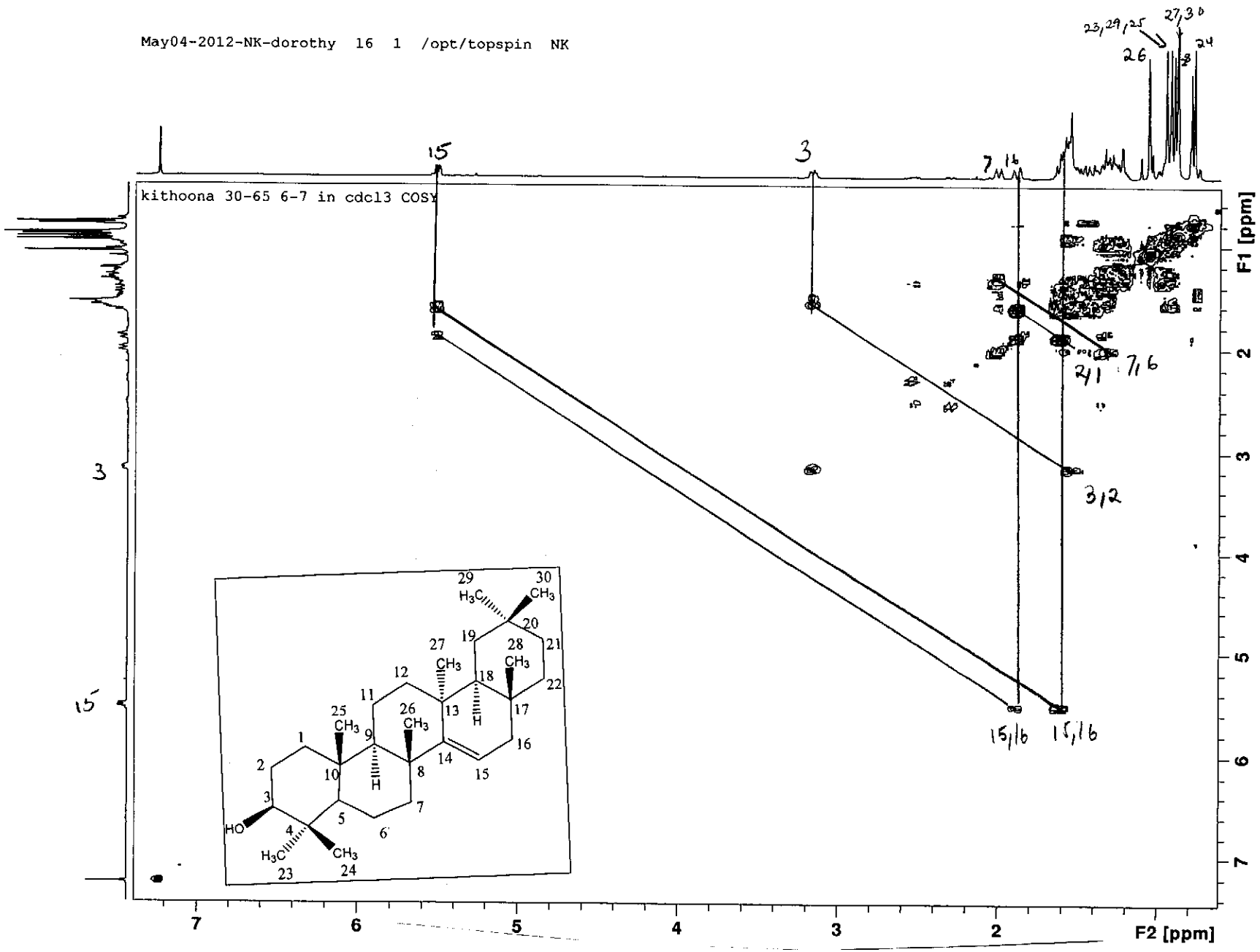
DEPT spectrum of B8



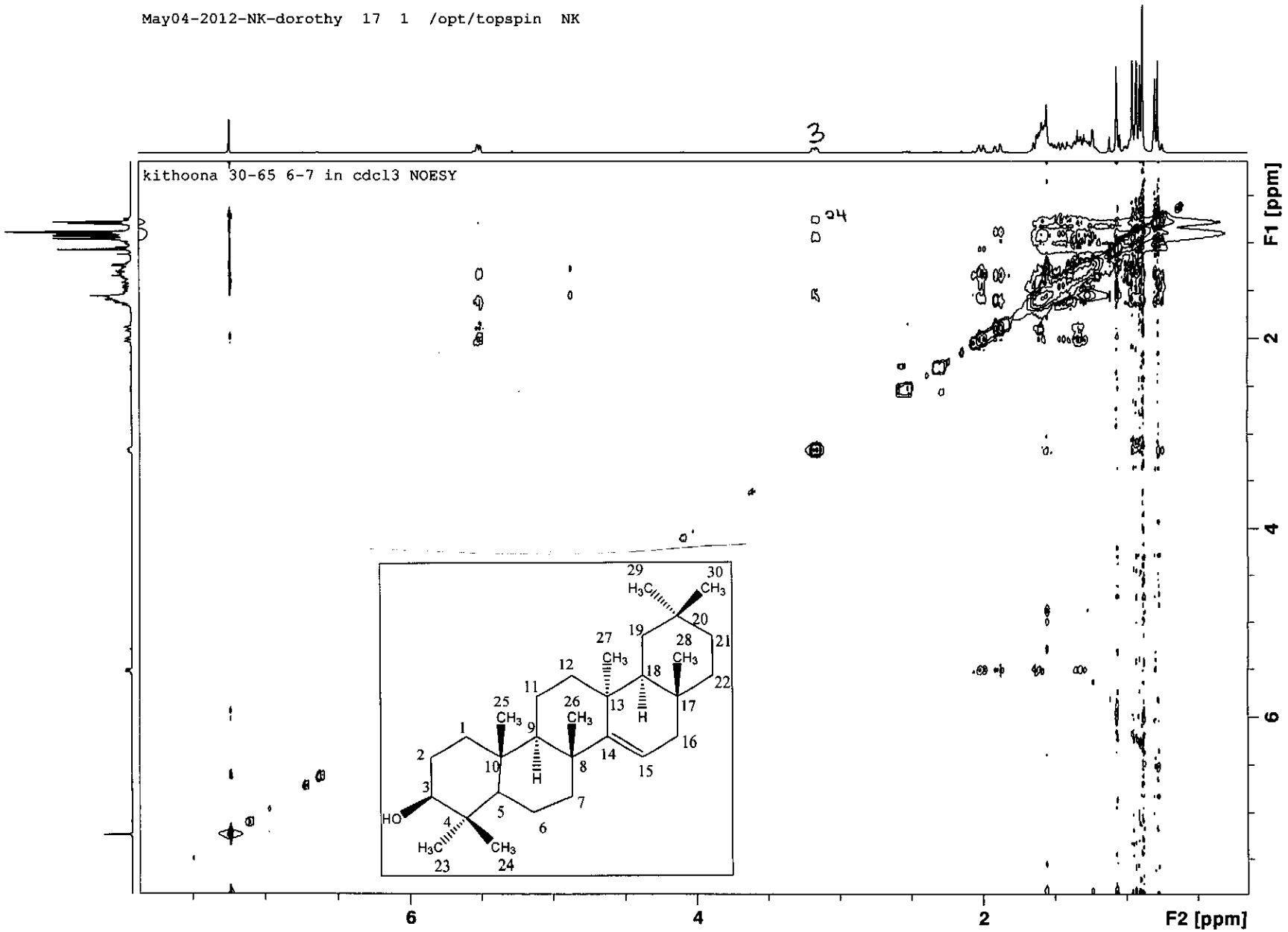
^{13}C NMR spectrum of B8 expanded (14.0-56.0 ppm)



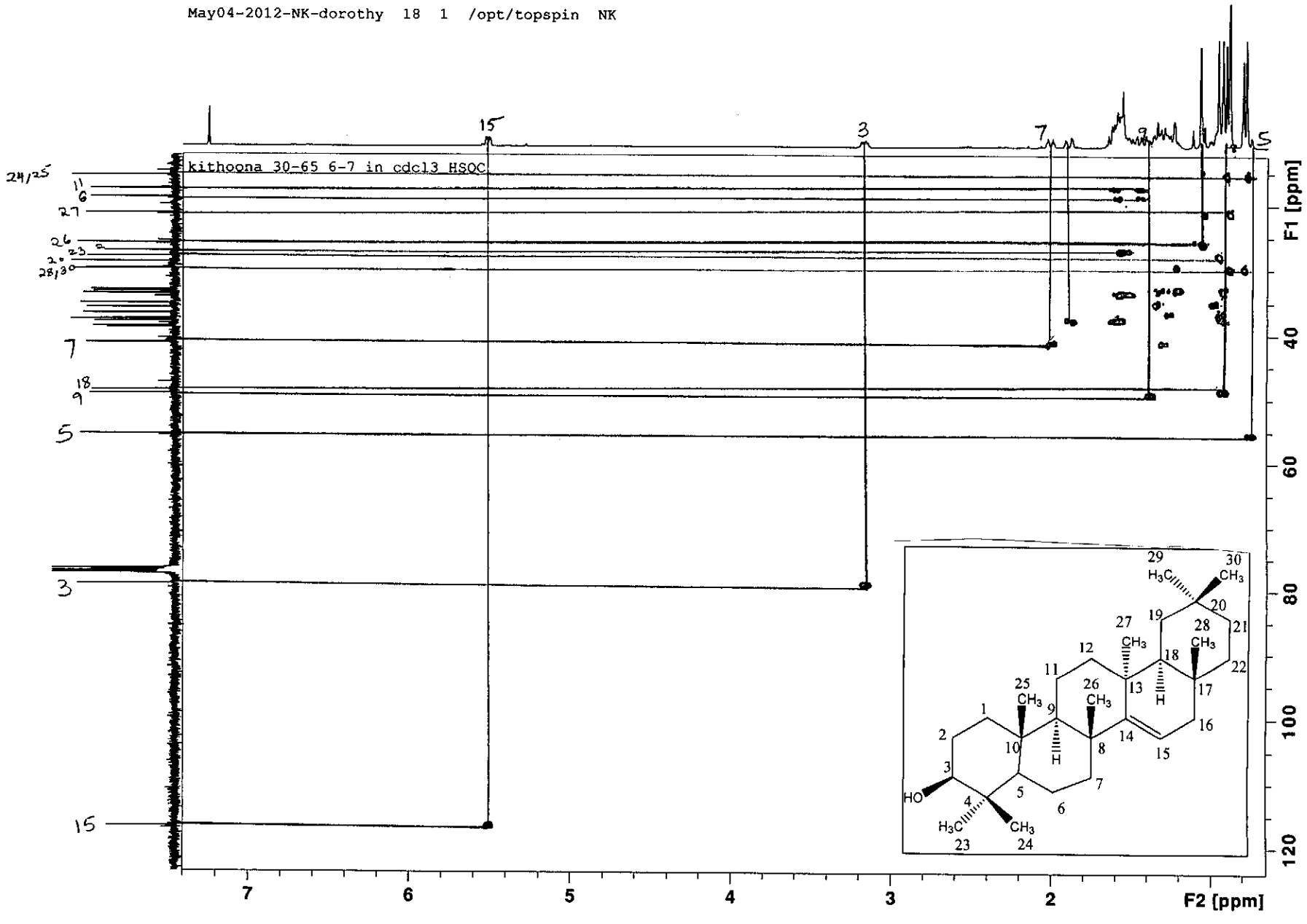
DEPT spectrum of B8 expanded (14.0-42.0 ppm)



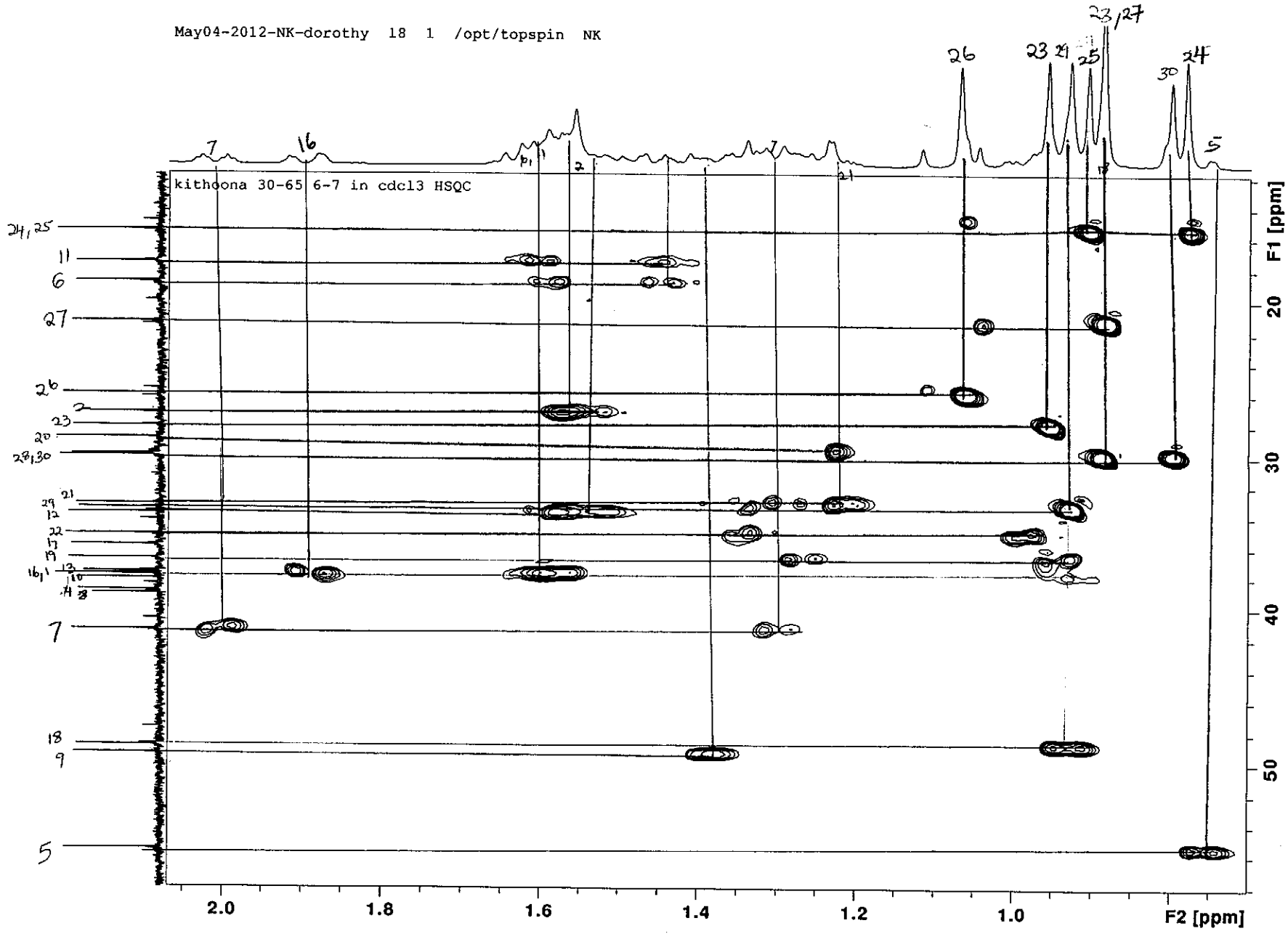
COSY spectrum of B8



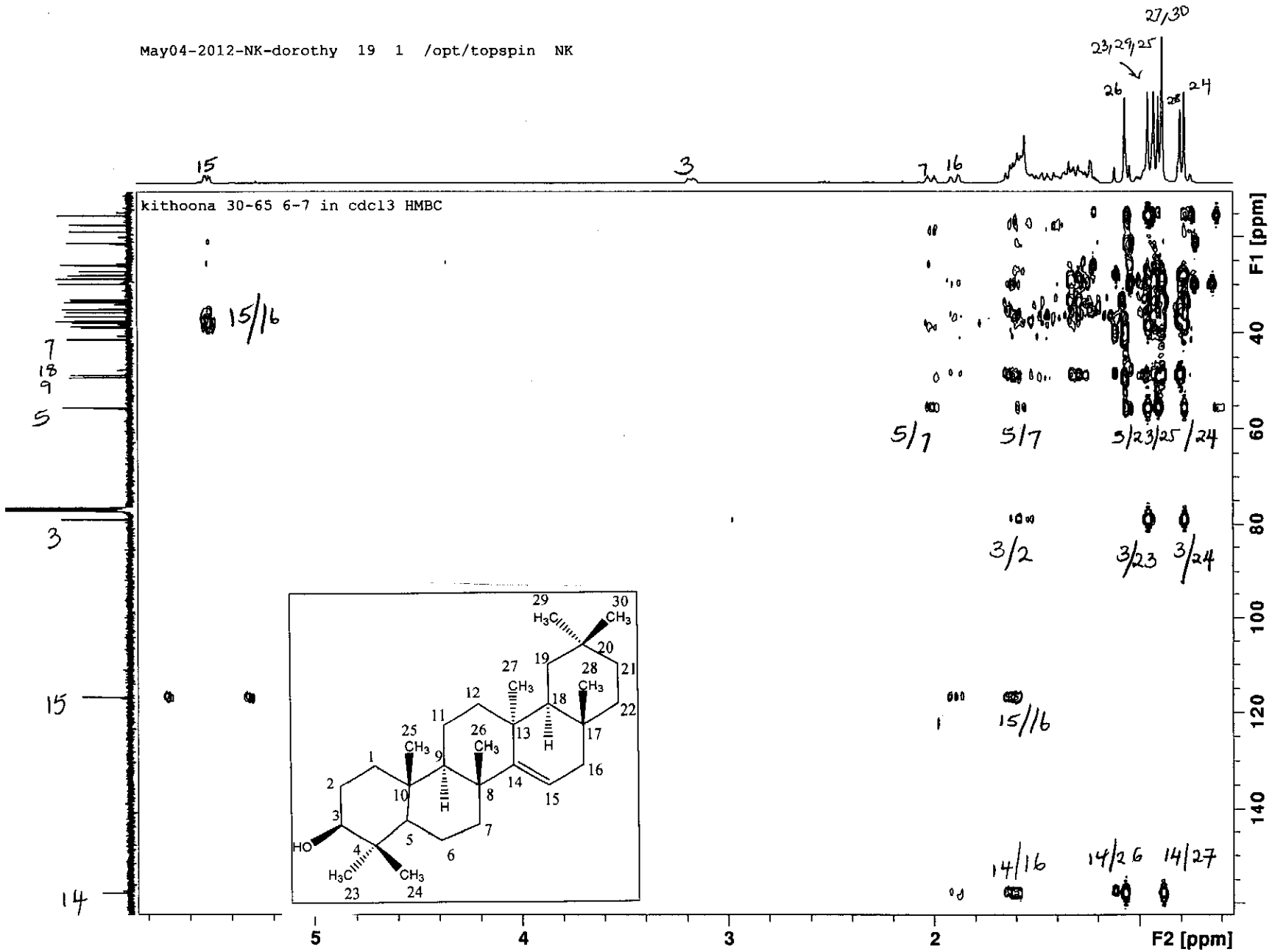
NOESY spectrum of B8



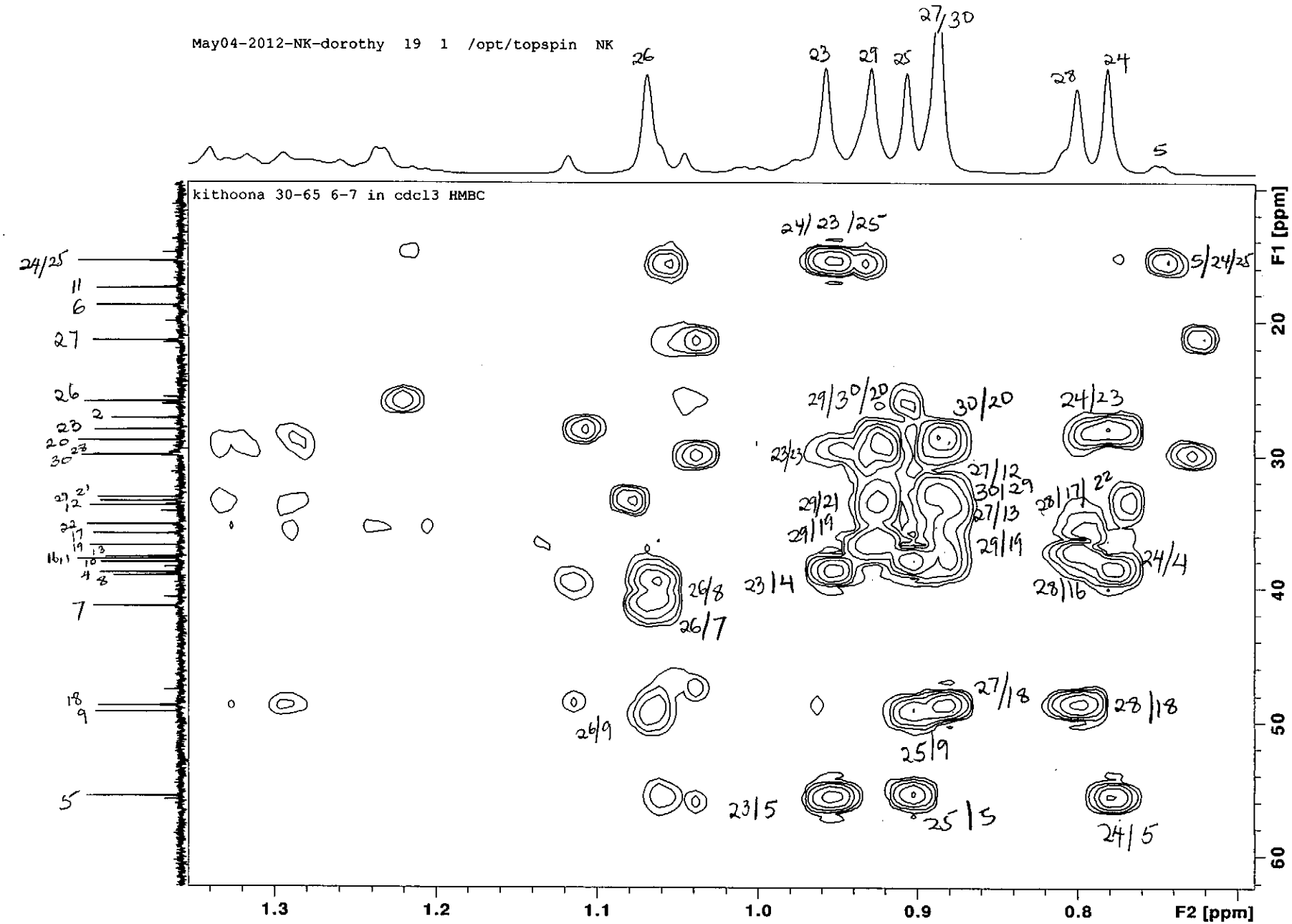
HSQC spectrum of B8



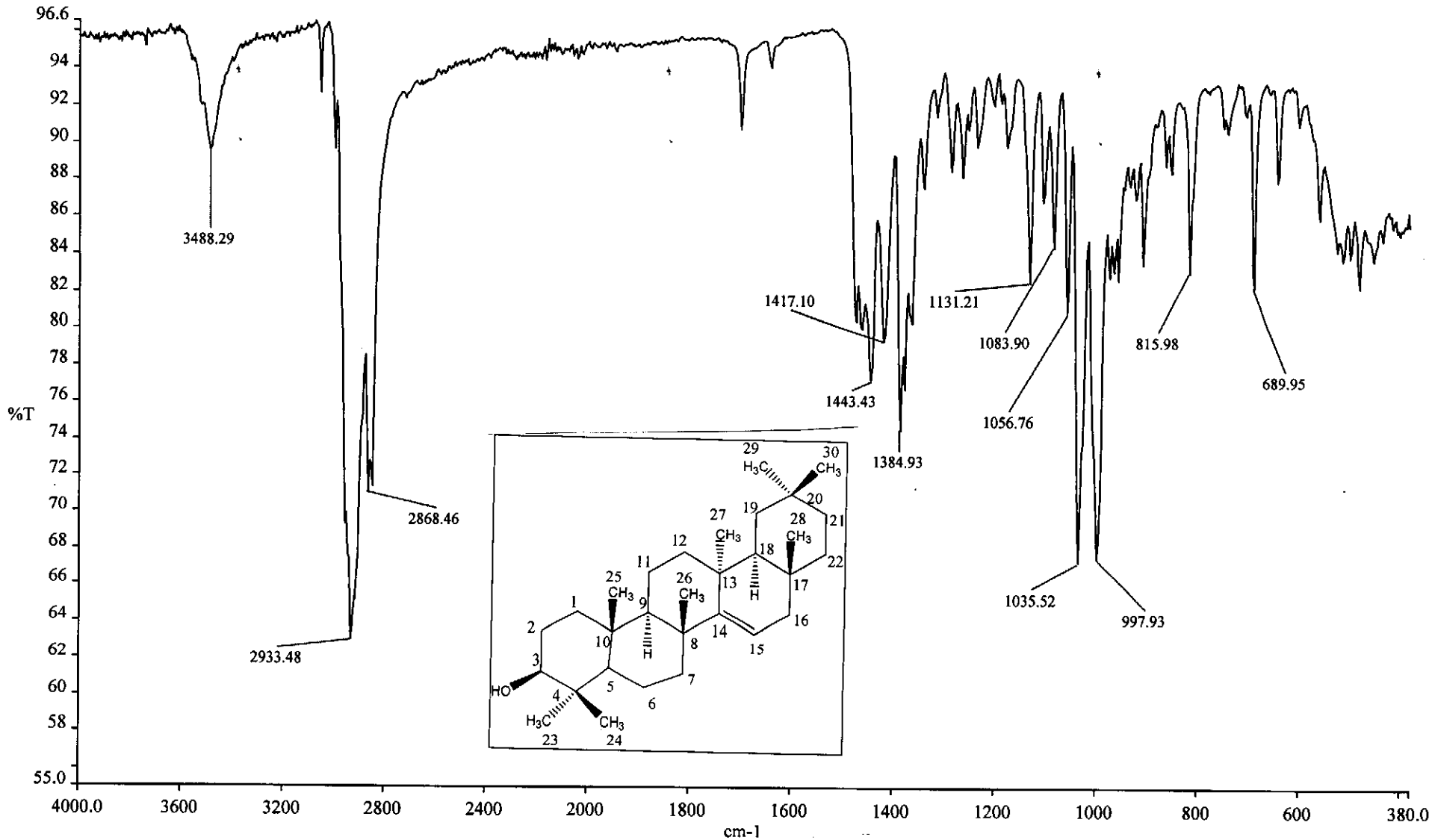
HSQC spectrum of B8 (F1 17-56 ppm, F2 0.7- 2.1 ppm)



HMBC spectrum of B8



HMBC spectrum of B8 expanded (F1 14-60 ppm, F2 0.7-1.3 ppm)



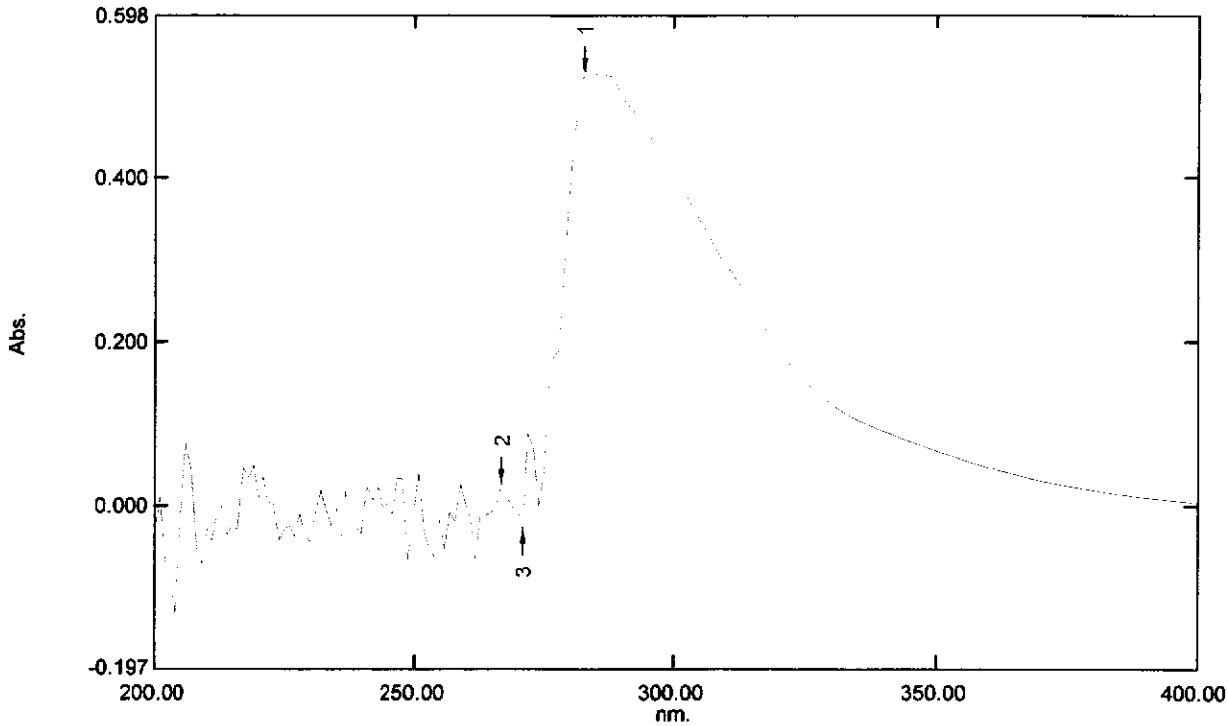
IR spectrum of B8

Spectrum Peak Pick Report

08/04/2012 06:49:40 PM

Data Set: kithoona 5.spc - Storage 180228

Not good



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2	●	267.00	0.031	
3	▼	271.00	-0.029	

Measurement Properties

Wavelength Range (nm.):	200.00 to 400.00
Scan Speed:	Medium
Sampling Interval:	1.0
Auto Sampling Interval:	Disabled
Scan Mode:	Single

Instrument Properties

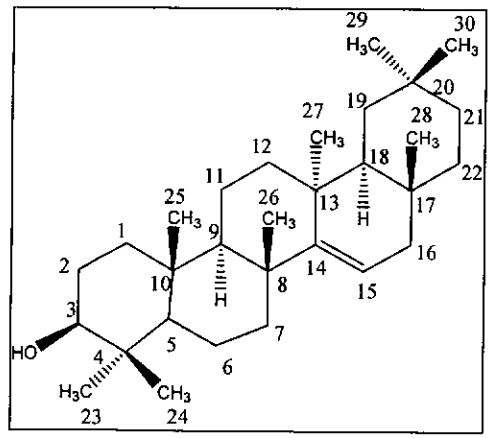
Instrument Type:	UV-3600 Series
Measuring Mode:	Absorbance
Slit Width:	2.0 nm
Time Constant:	0.1 sec.
Source Lamp:	Auto
Light Source Change Wavelength:	310.00 nm
Detector Unit:	Direct
Detector Change Wavelength:	830.00 nm 1800.00 nm
Grating Change Wavelength:	720.00 nm
S/R Exchange:	Normal
Detector Lock:	Auto
Slit Program:	Normal
Beam Mode:	Double
Stair Correction:	Disable

Attachment Properties

Attachment:	None

Sample Preparation Properties

Weight:	
Volume:	
Dilution:	
Path Length:	
Additional Information:	



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Operator : Dorothy
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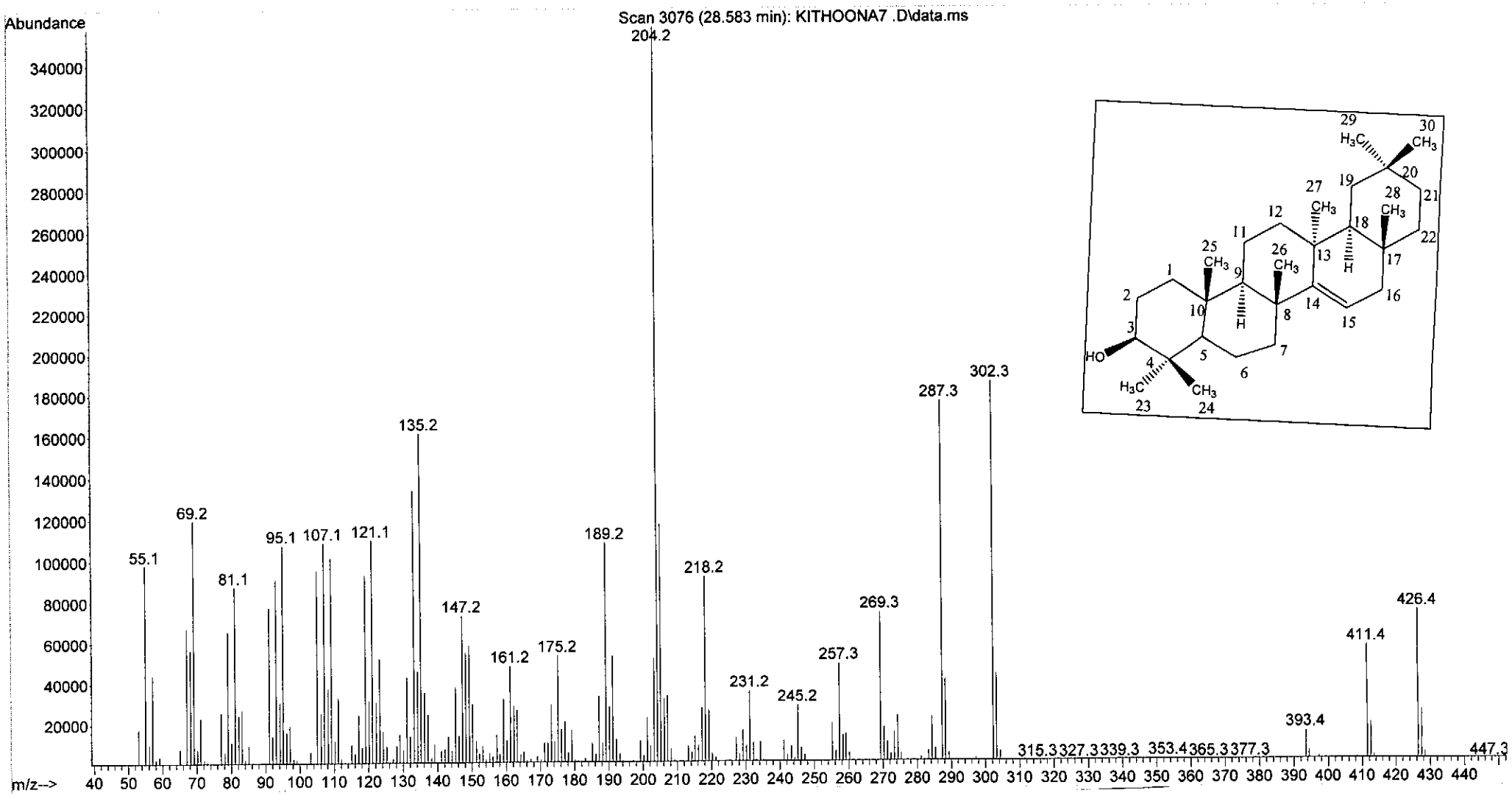
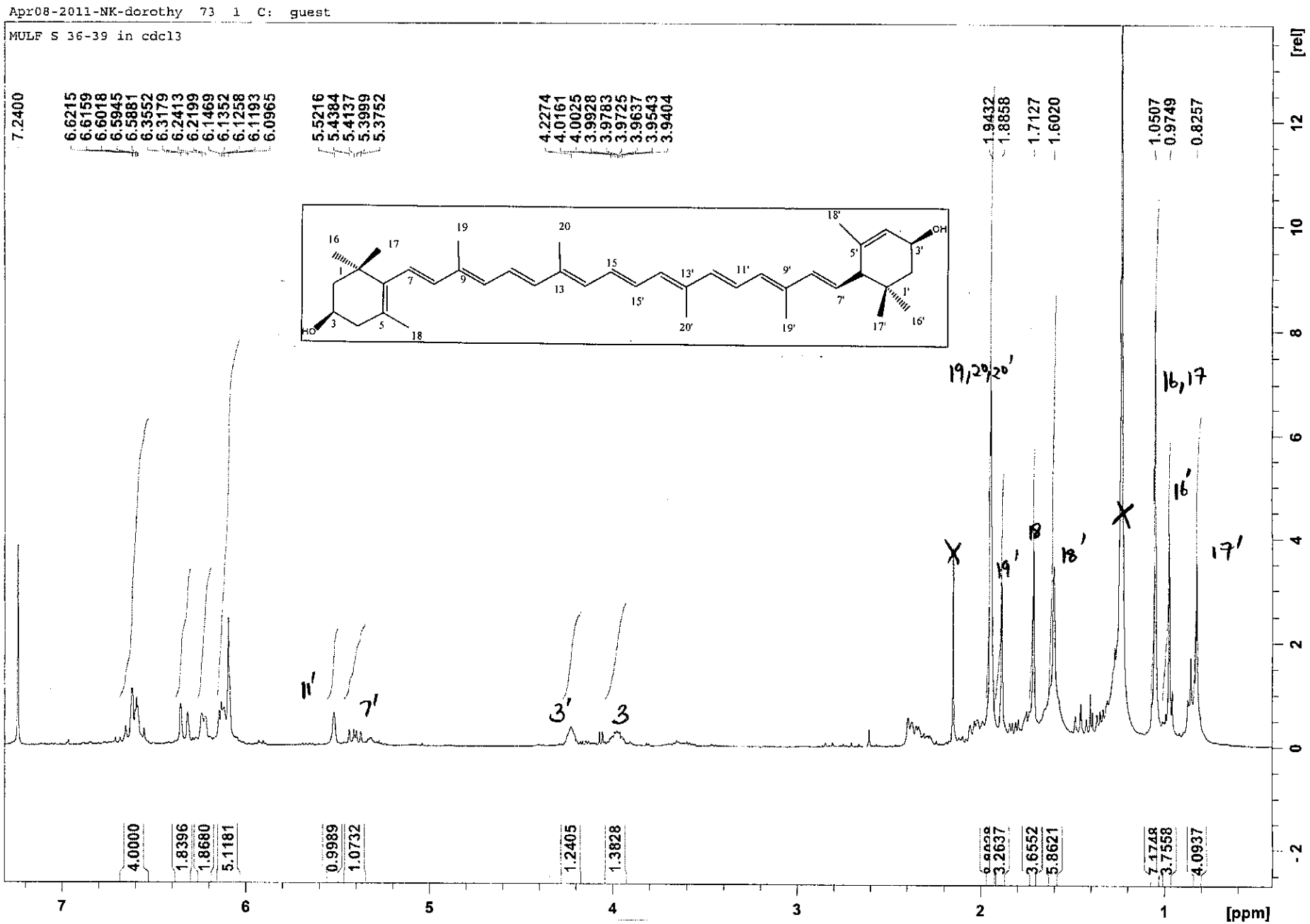
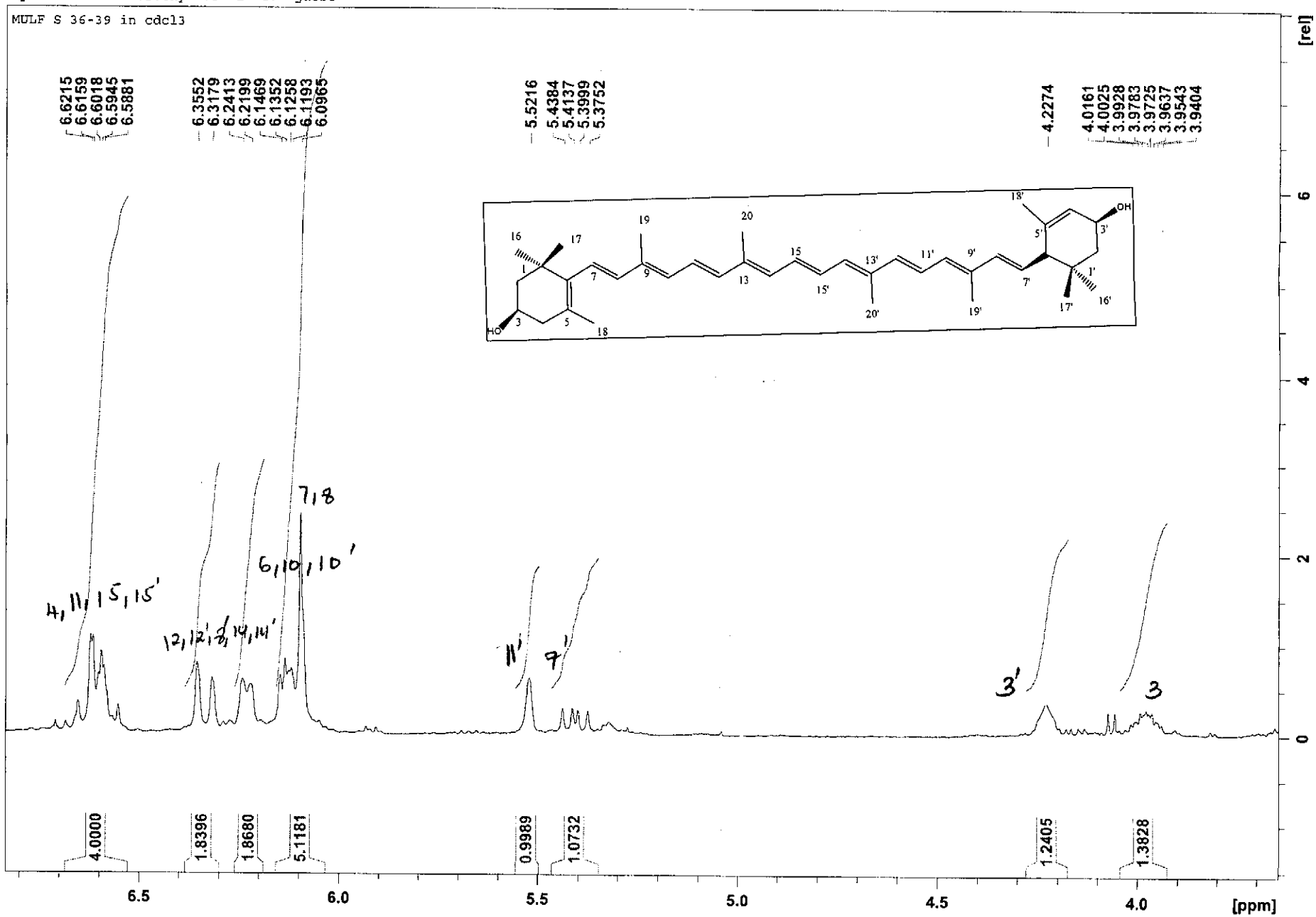
Instrument : 5973N
Sample Name: kithoona 8-12 (6-7)
Misc Info :
Vial Number: 1



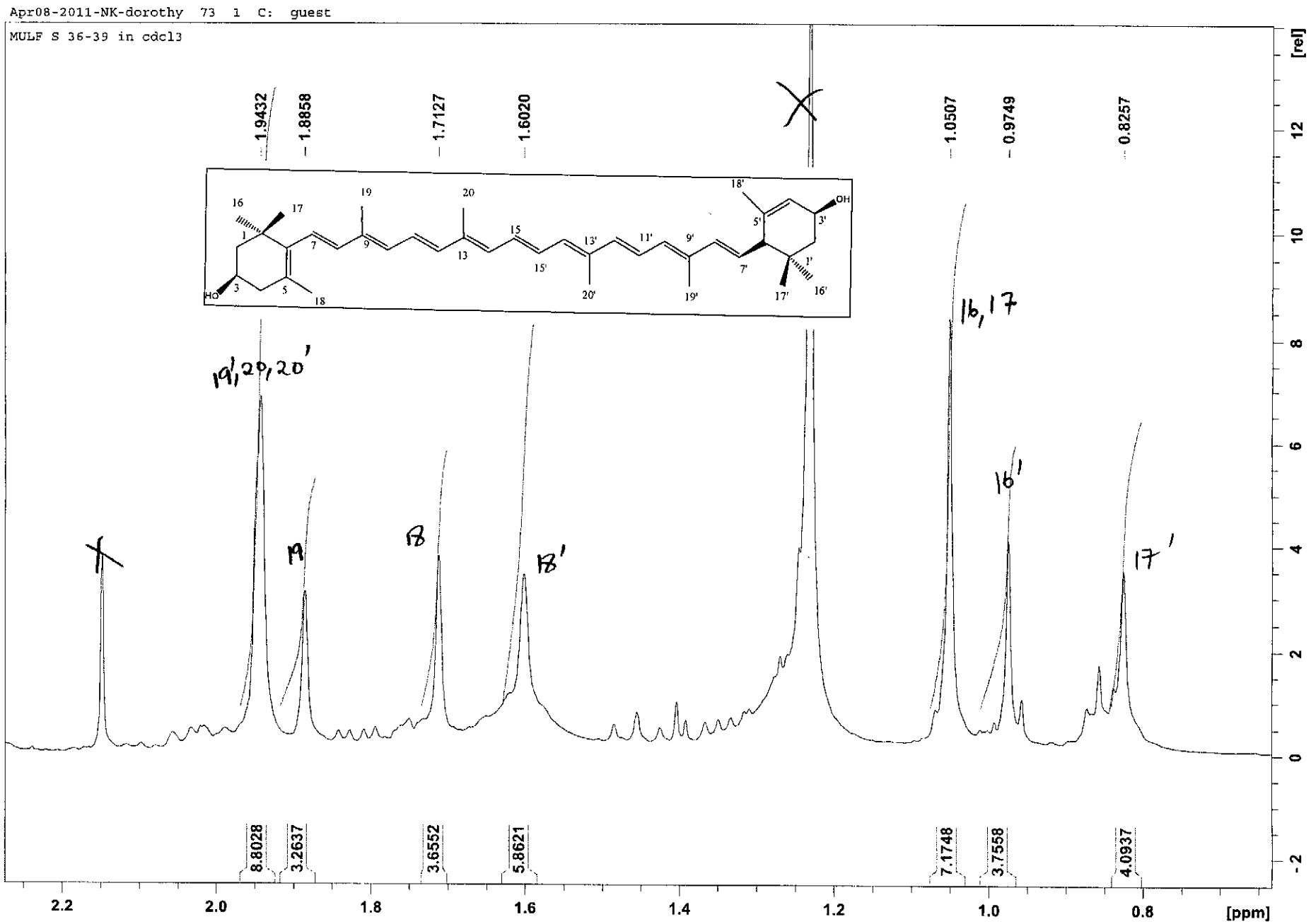
MS spectrum of B8



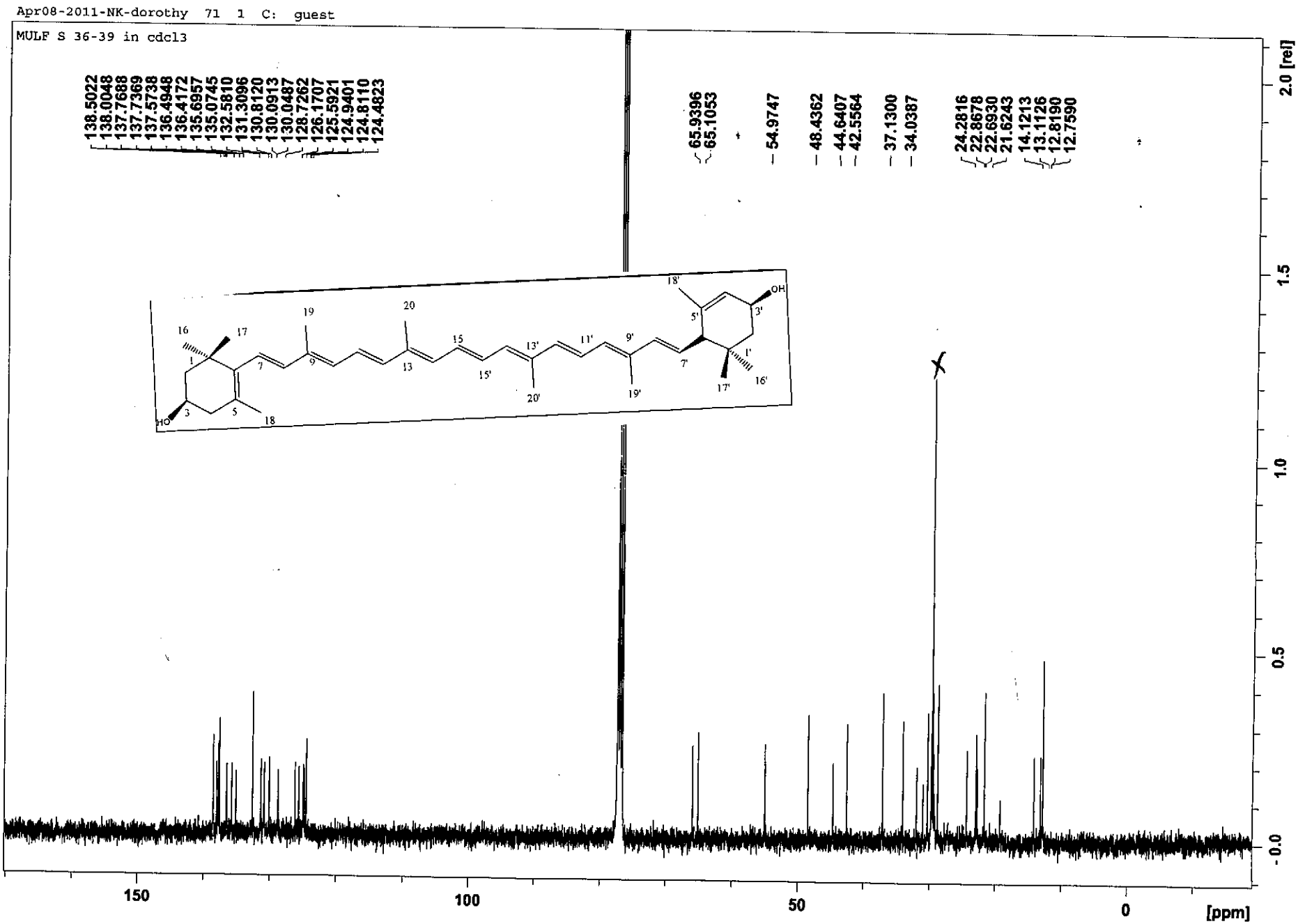
^{1}H NMR spectrum of B9



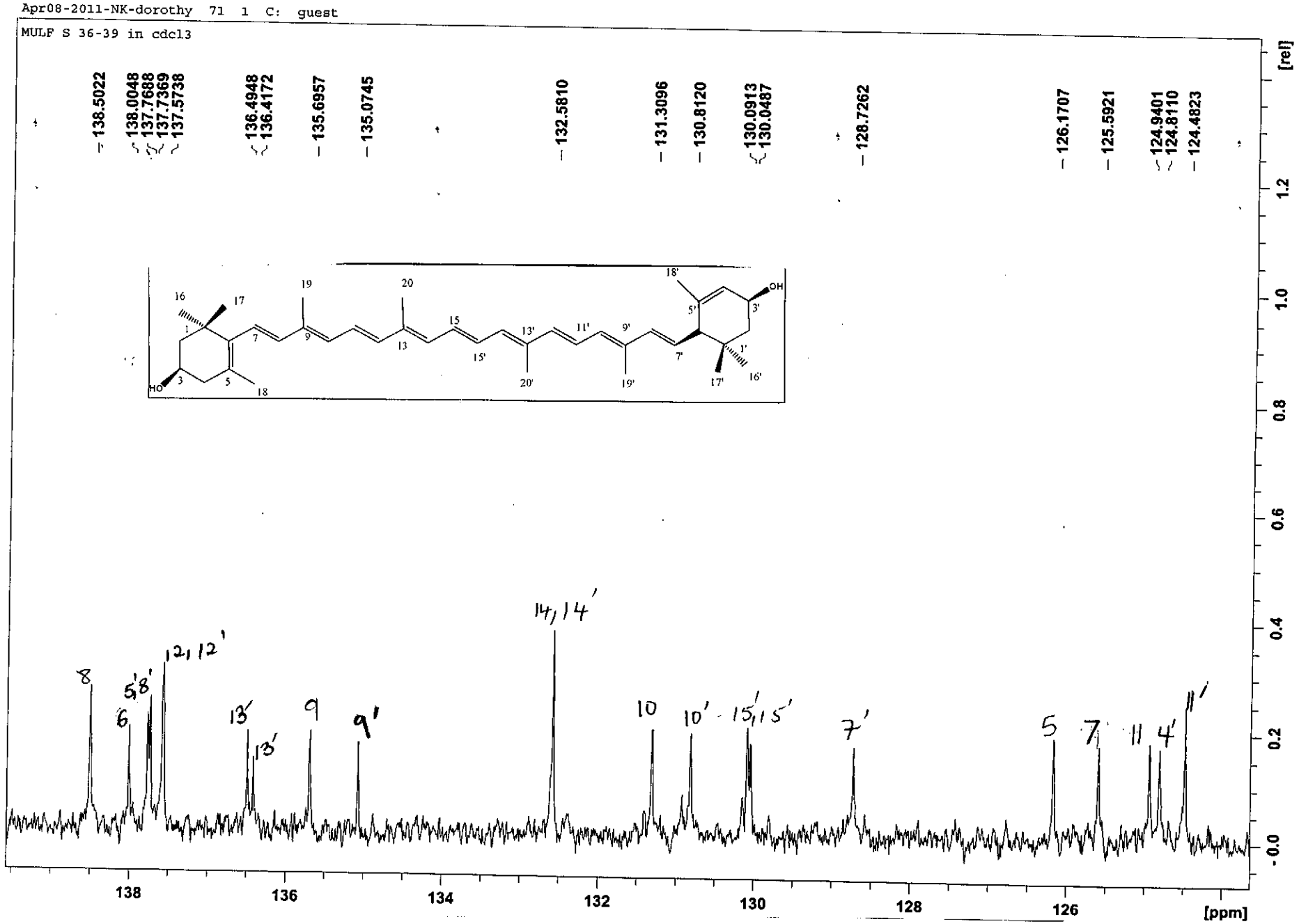
^{1}H NMR spectrum of B9 expanded (3.7-6.8 ppm)



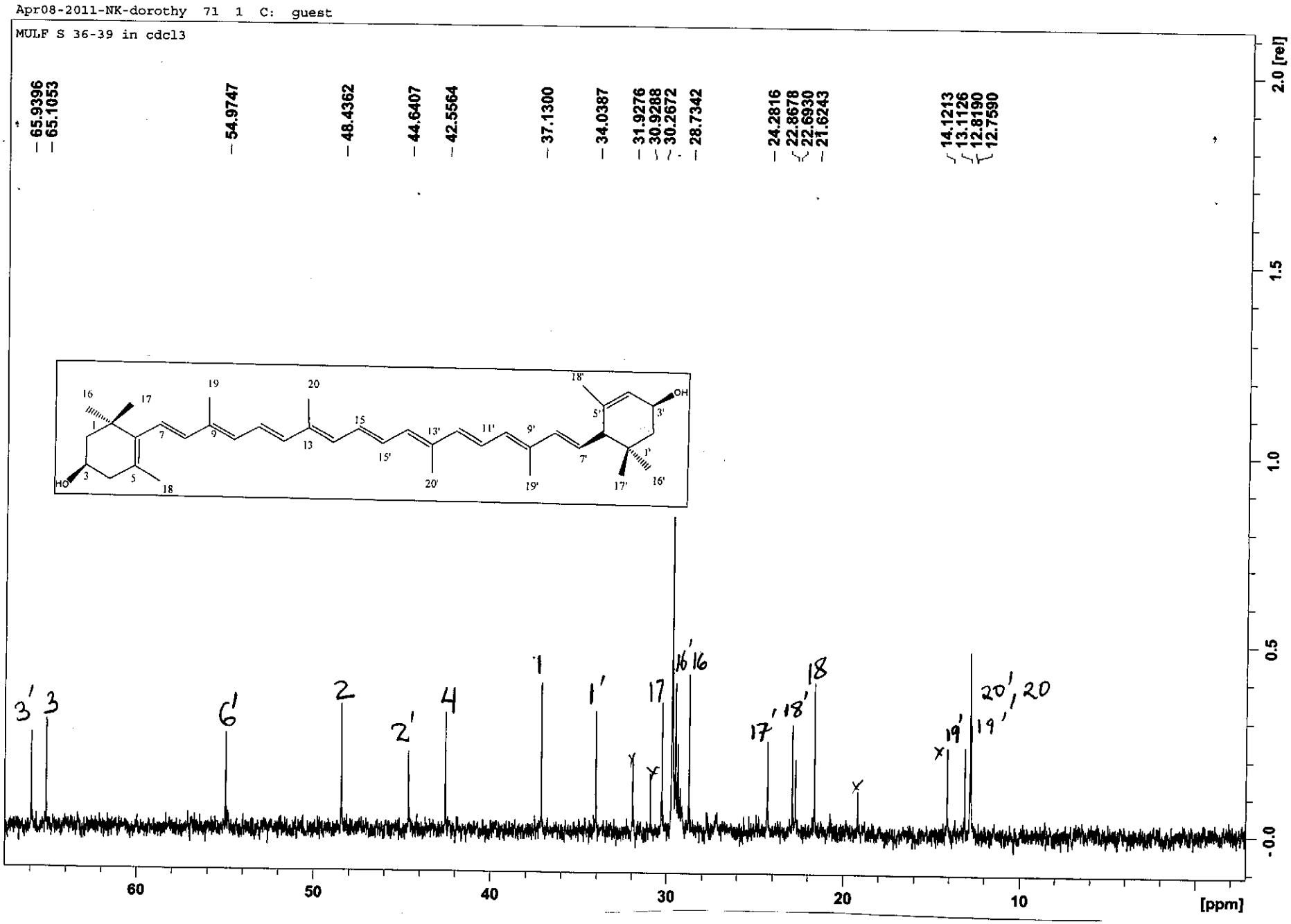
^{1}H NMR spectrum of B9 expanded (0.7-2.2 ppm)



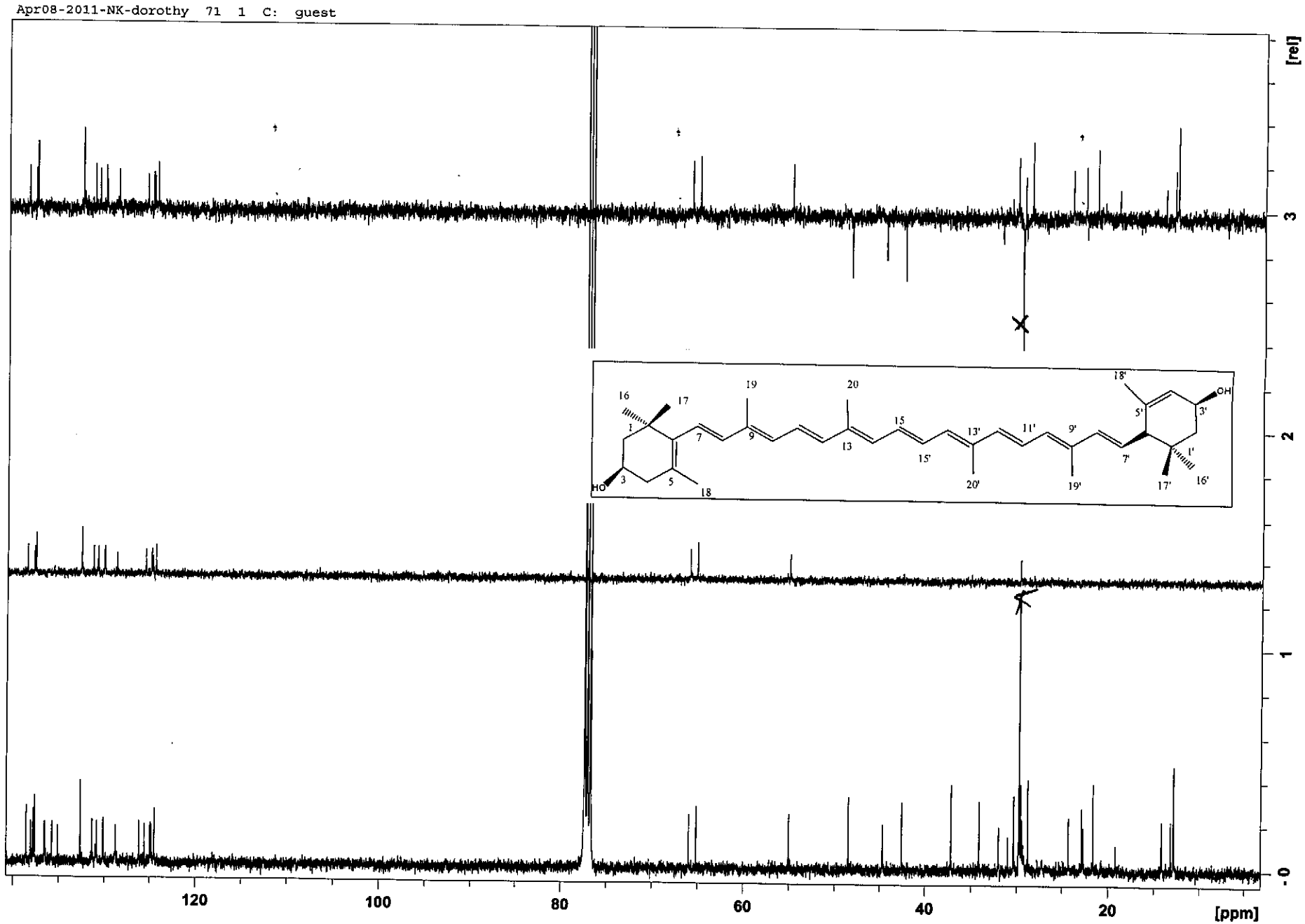
^{13}C NMR spectrum of B9



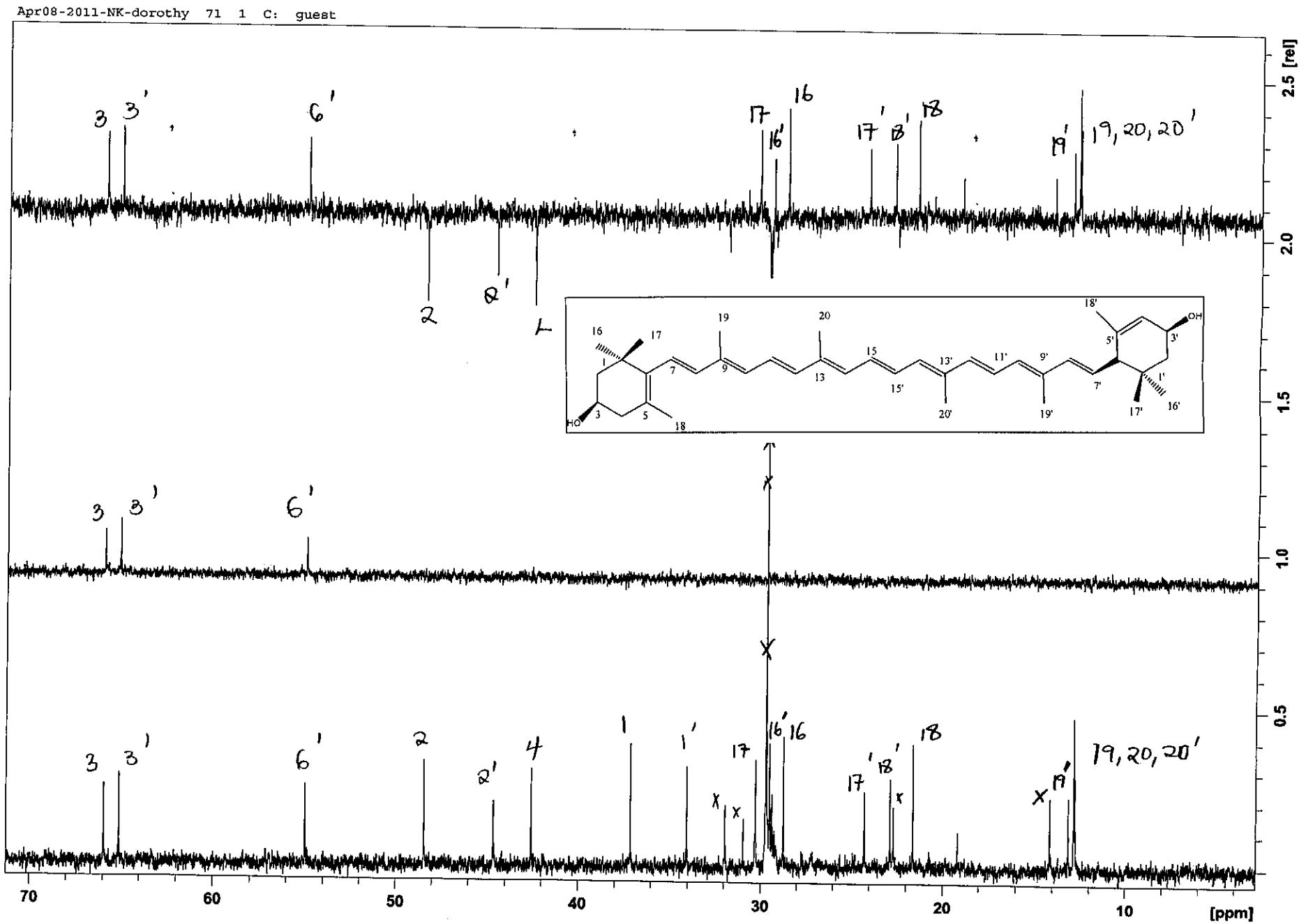
^{13}C NMR spectrum of B9 expanded (124-139 ppm)



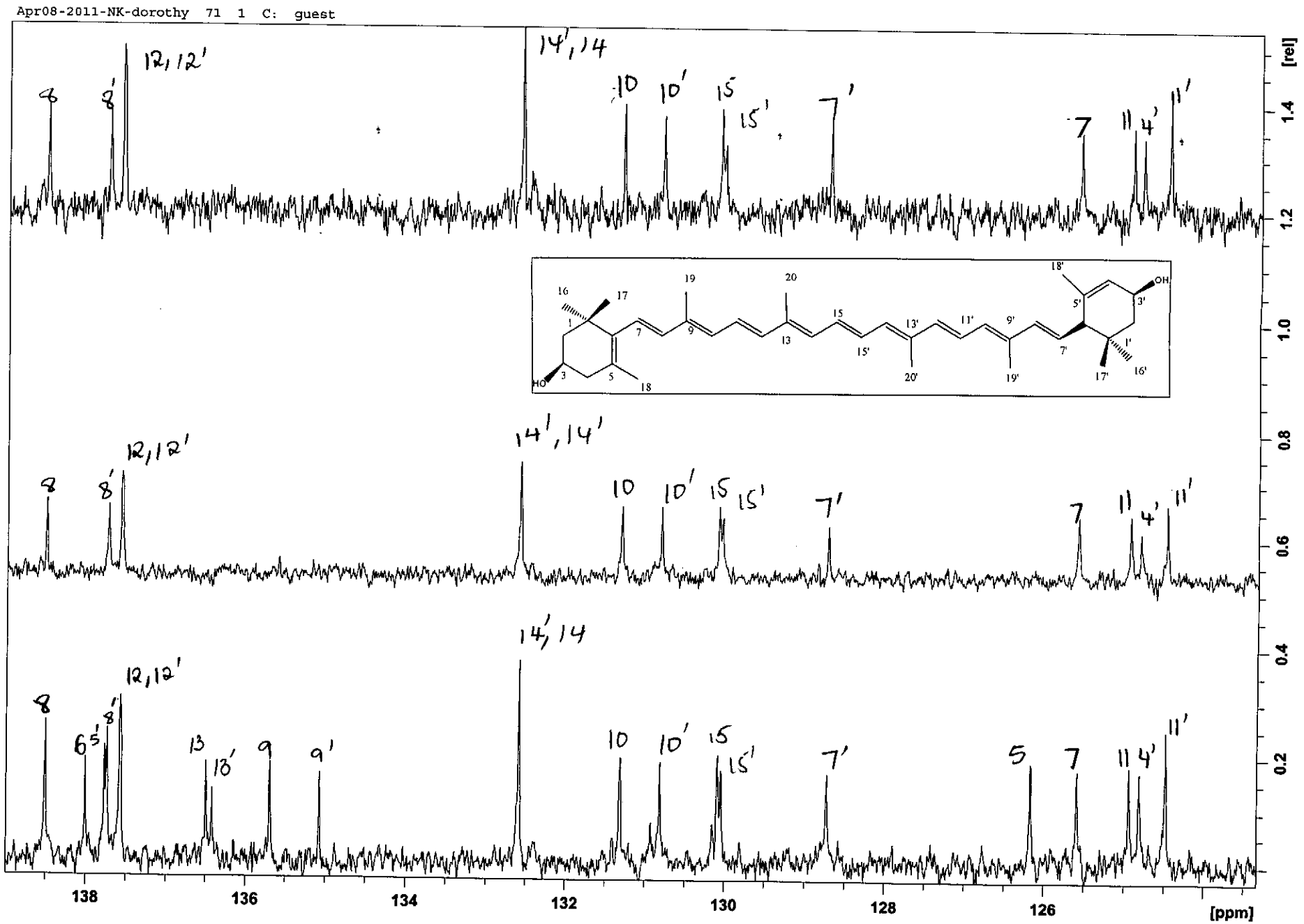
^{13}C NMR spectrum of B9 expanded (0-66 ppm)



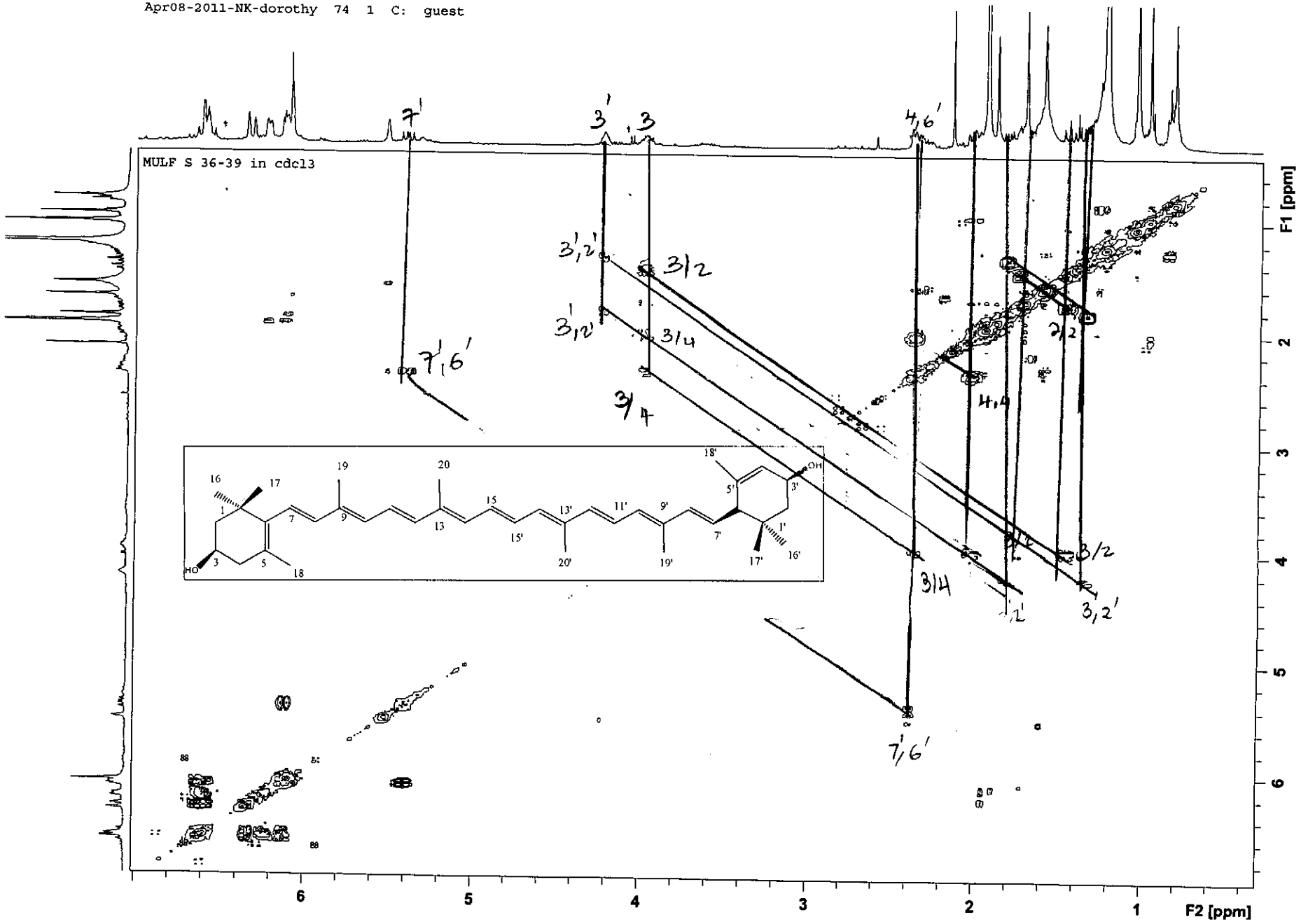
DEPT spectrum of B9



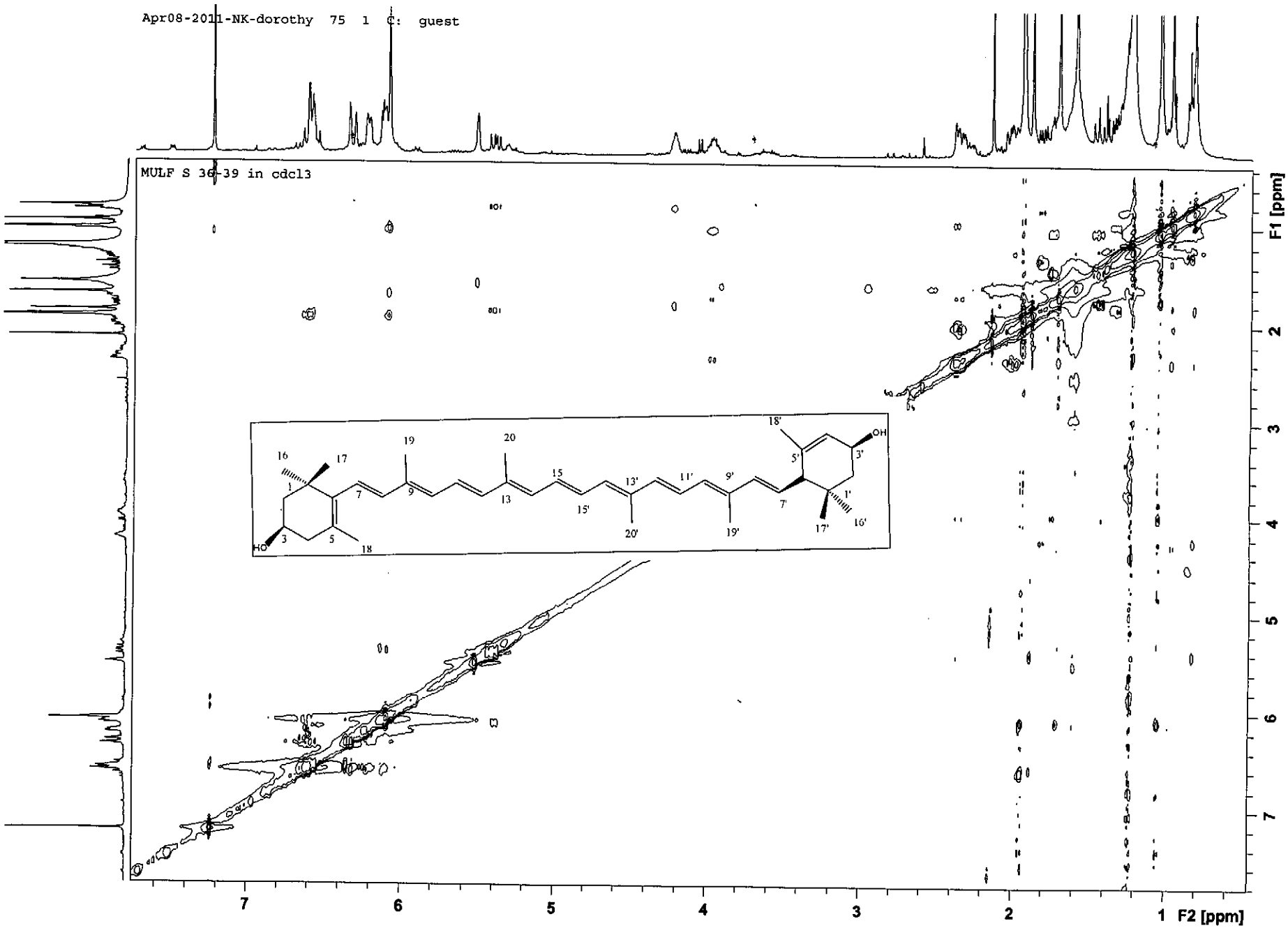
DEPT spectrum of B9 expanded (0-70 ppm)



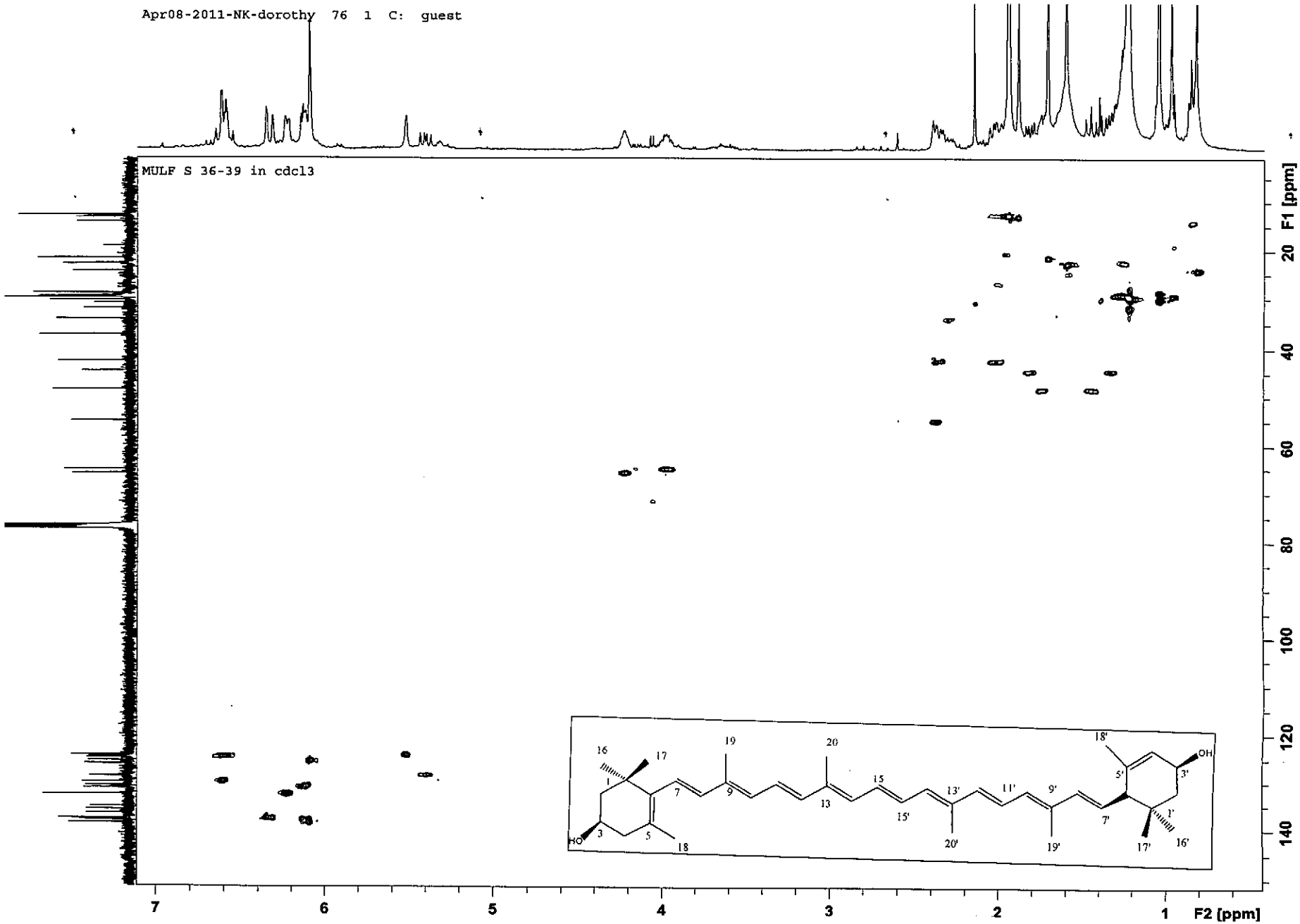
DEPT spectrum of B9 expanded (124-139 ppm)



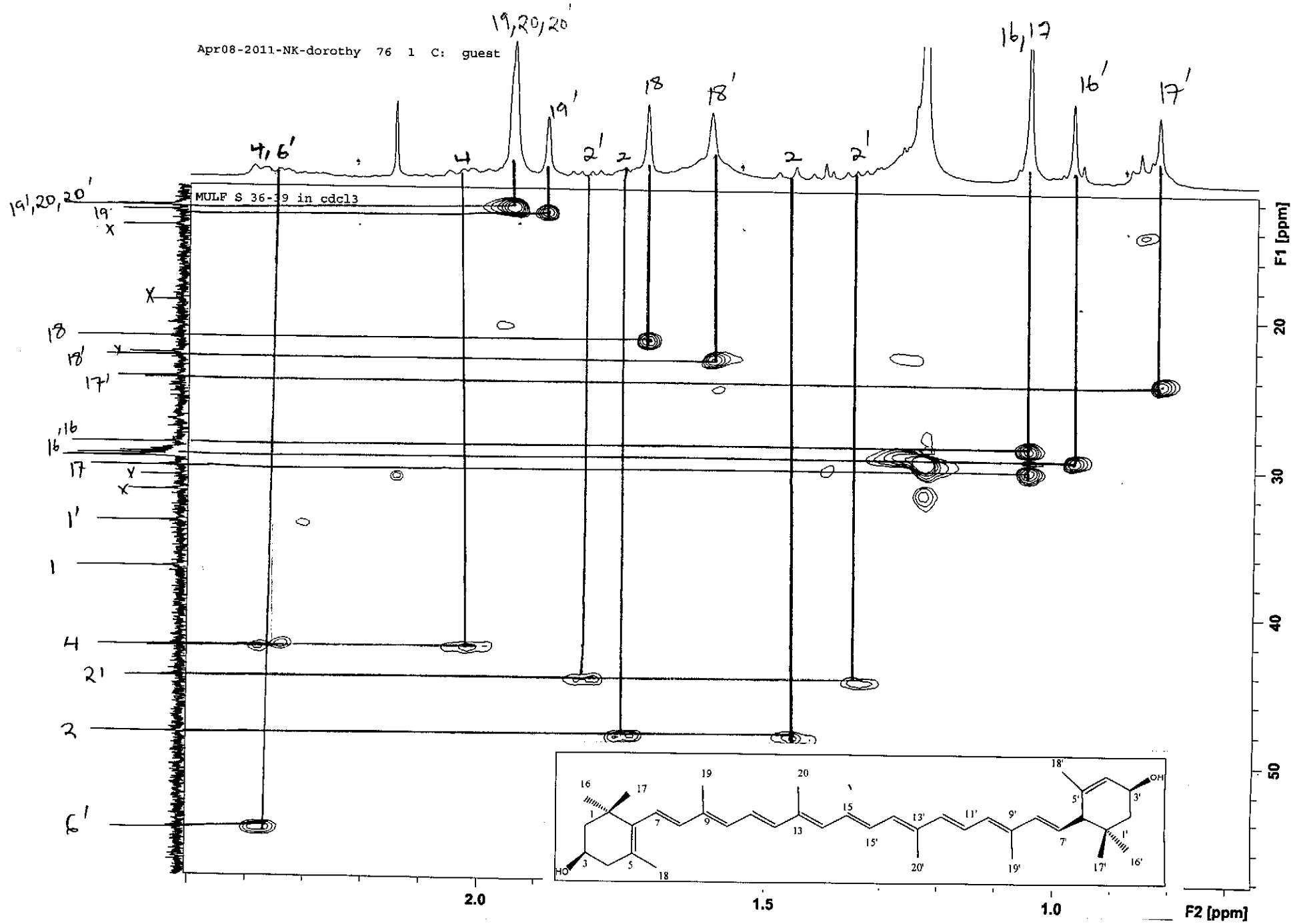
COSY spectrum of B9



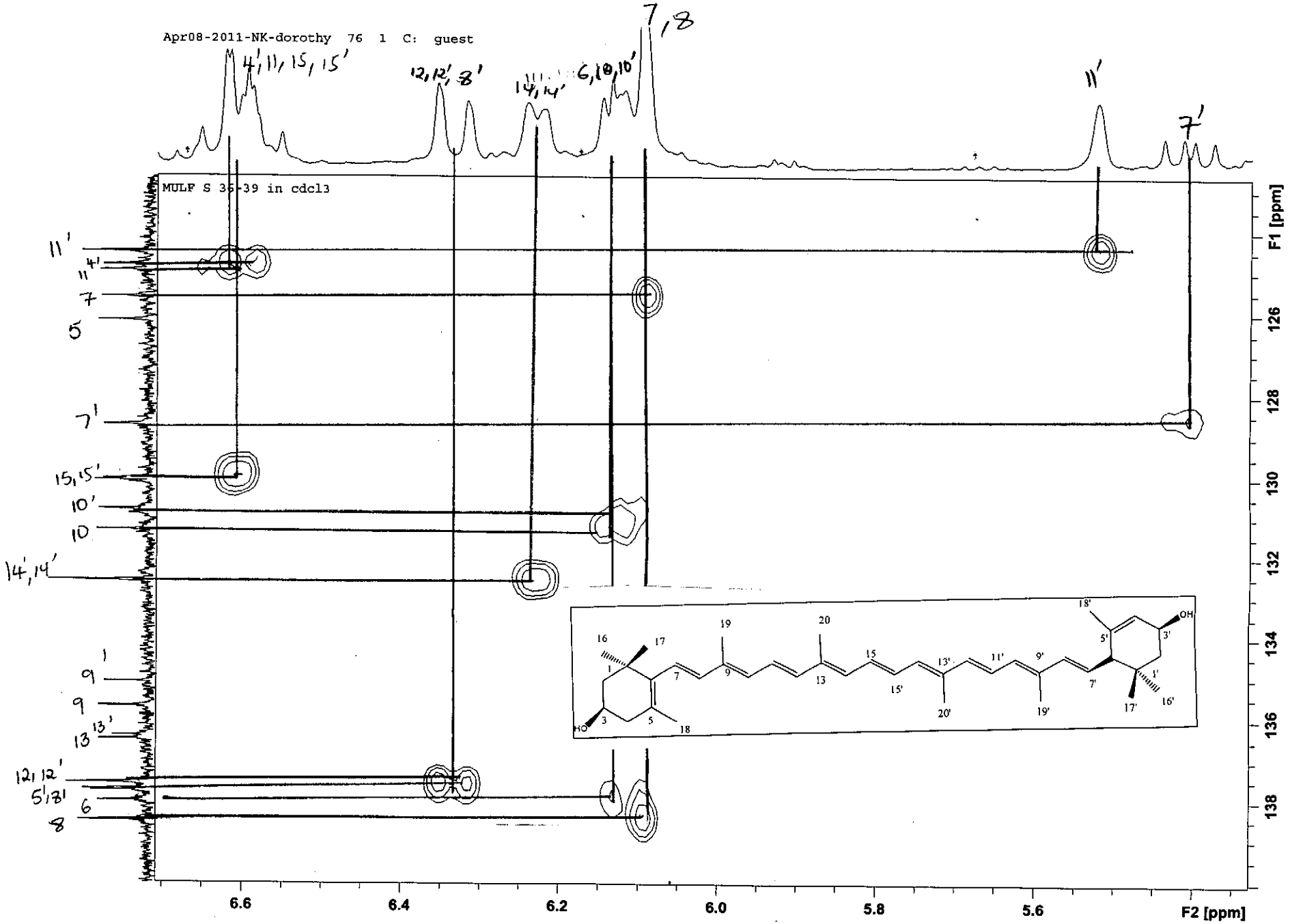
NOESY spectrum of B9



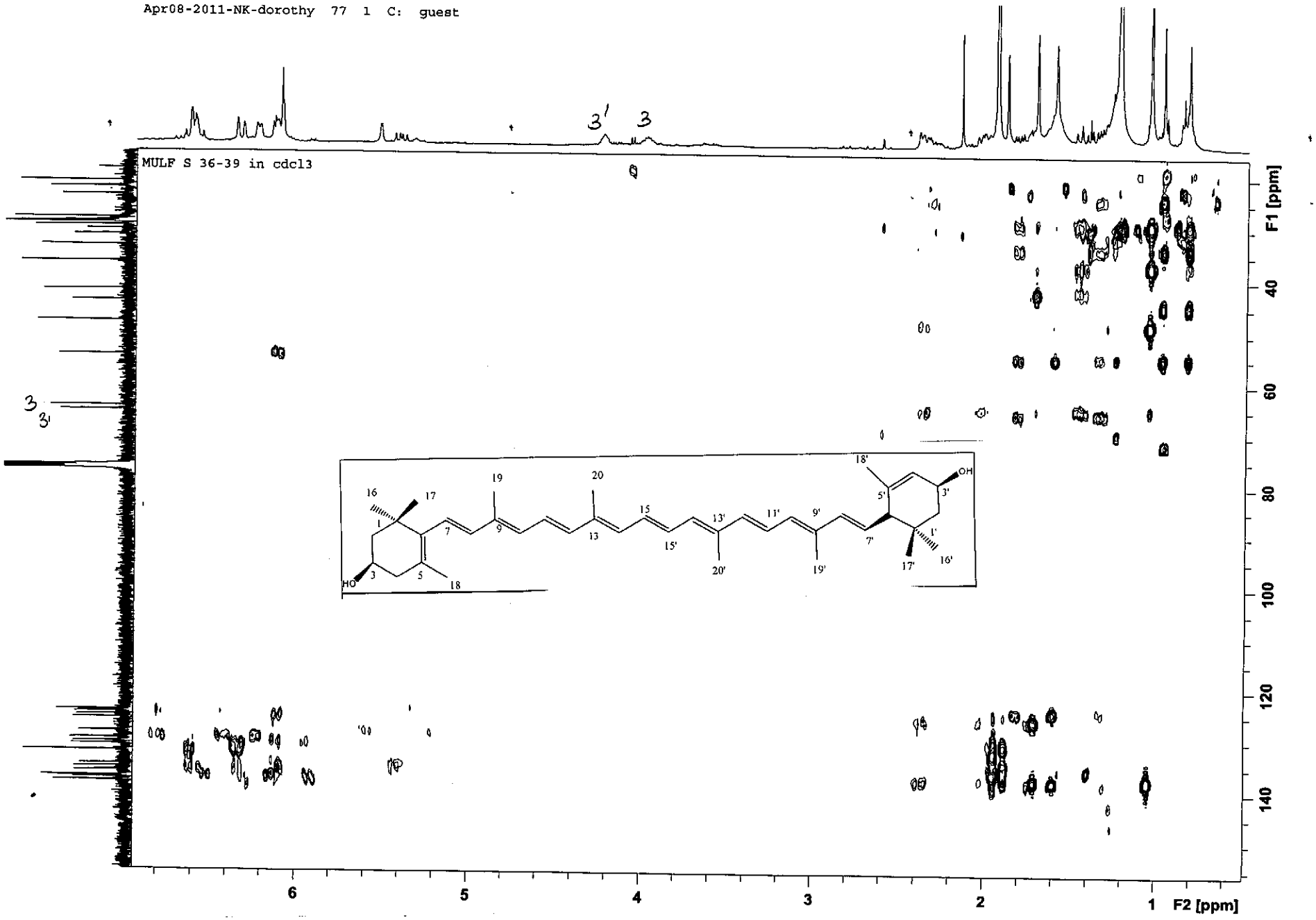
HSQC spectrum of B9



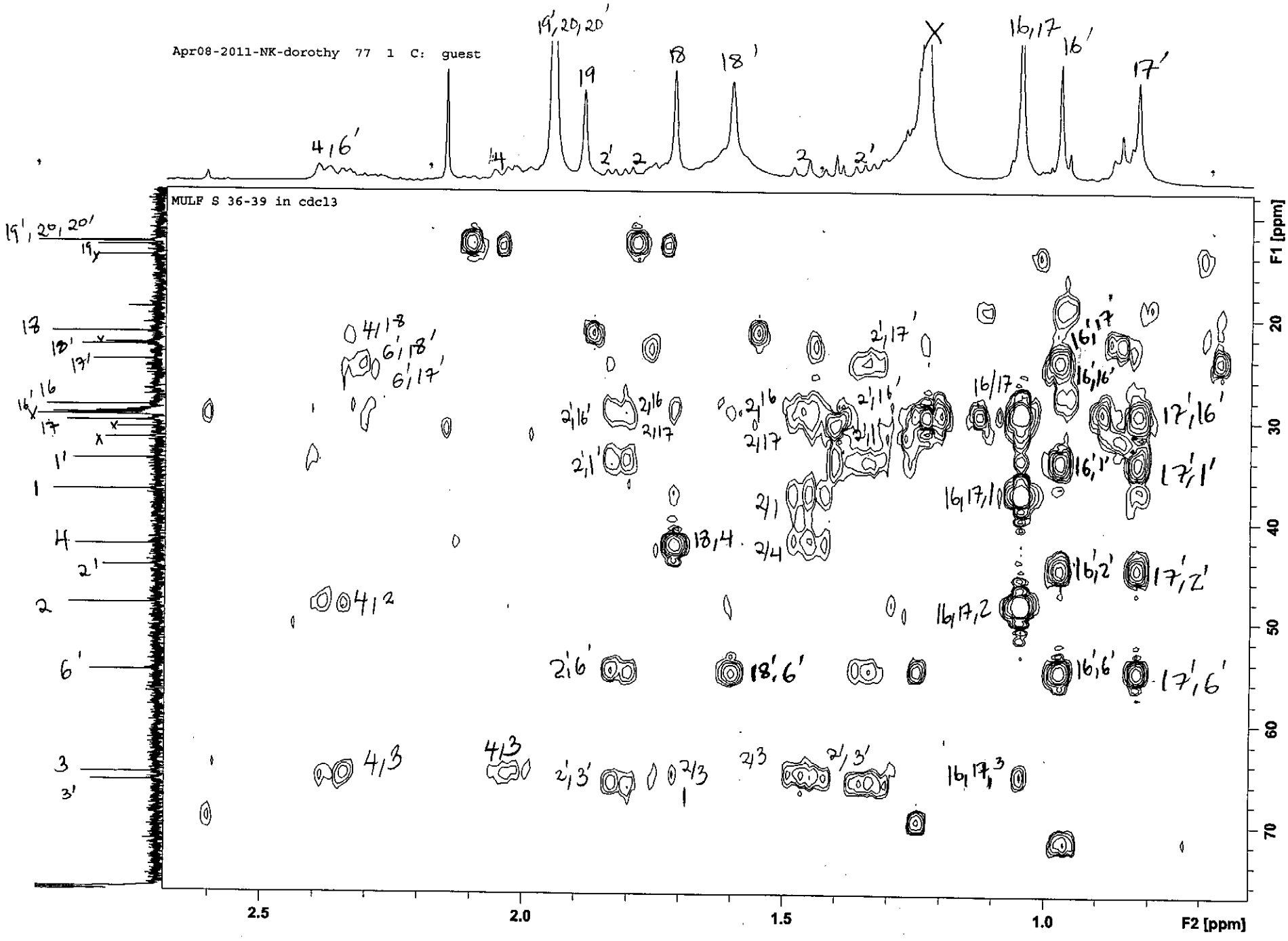
HSQC spectrum of B9 (F1 10-54 ppm, F2 0.5- 2.6 ppm)



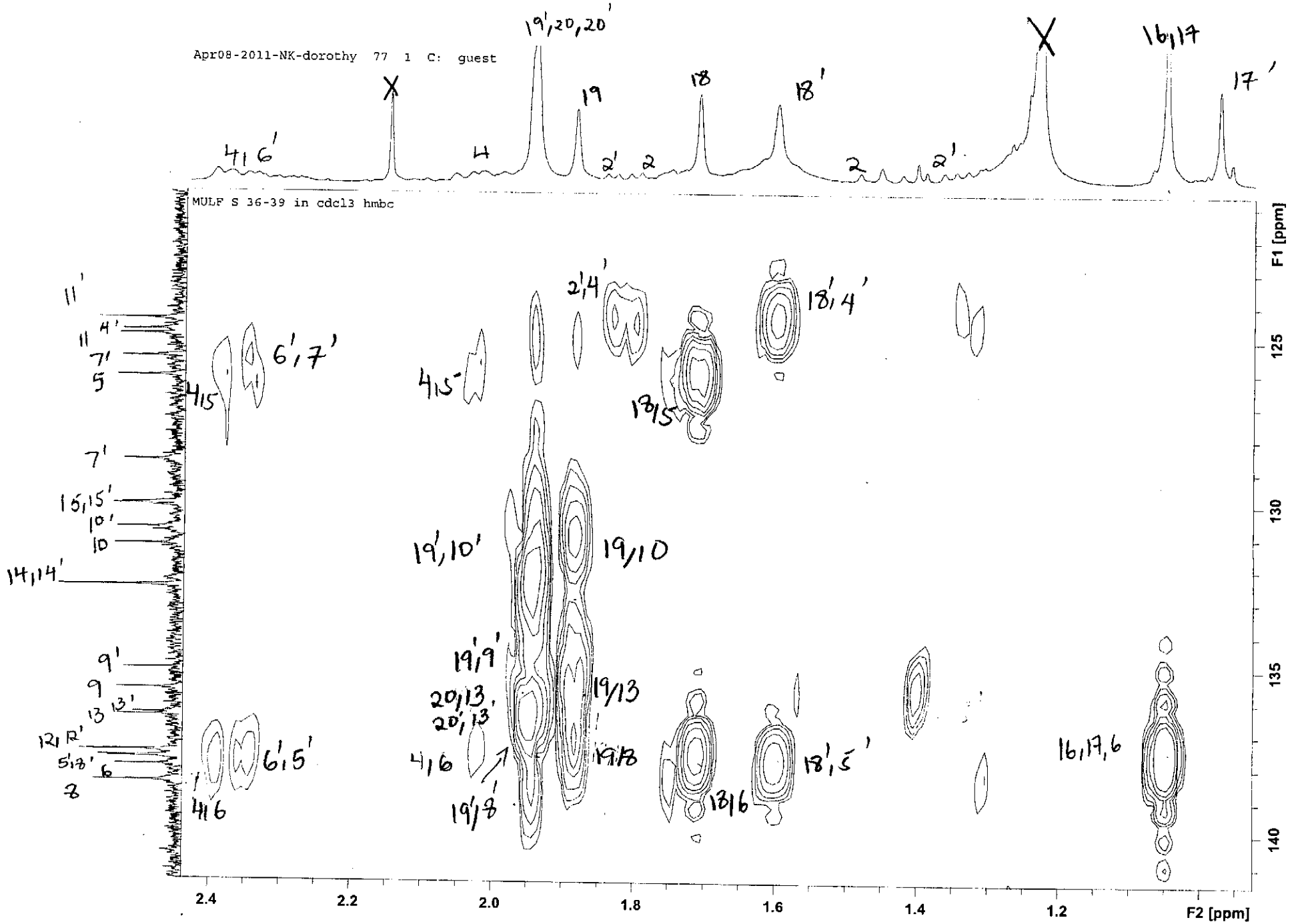
HSQC spectrum of B9 (F1 124-139 ppm, F2 5.4-6.6 ppm)



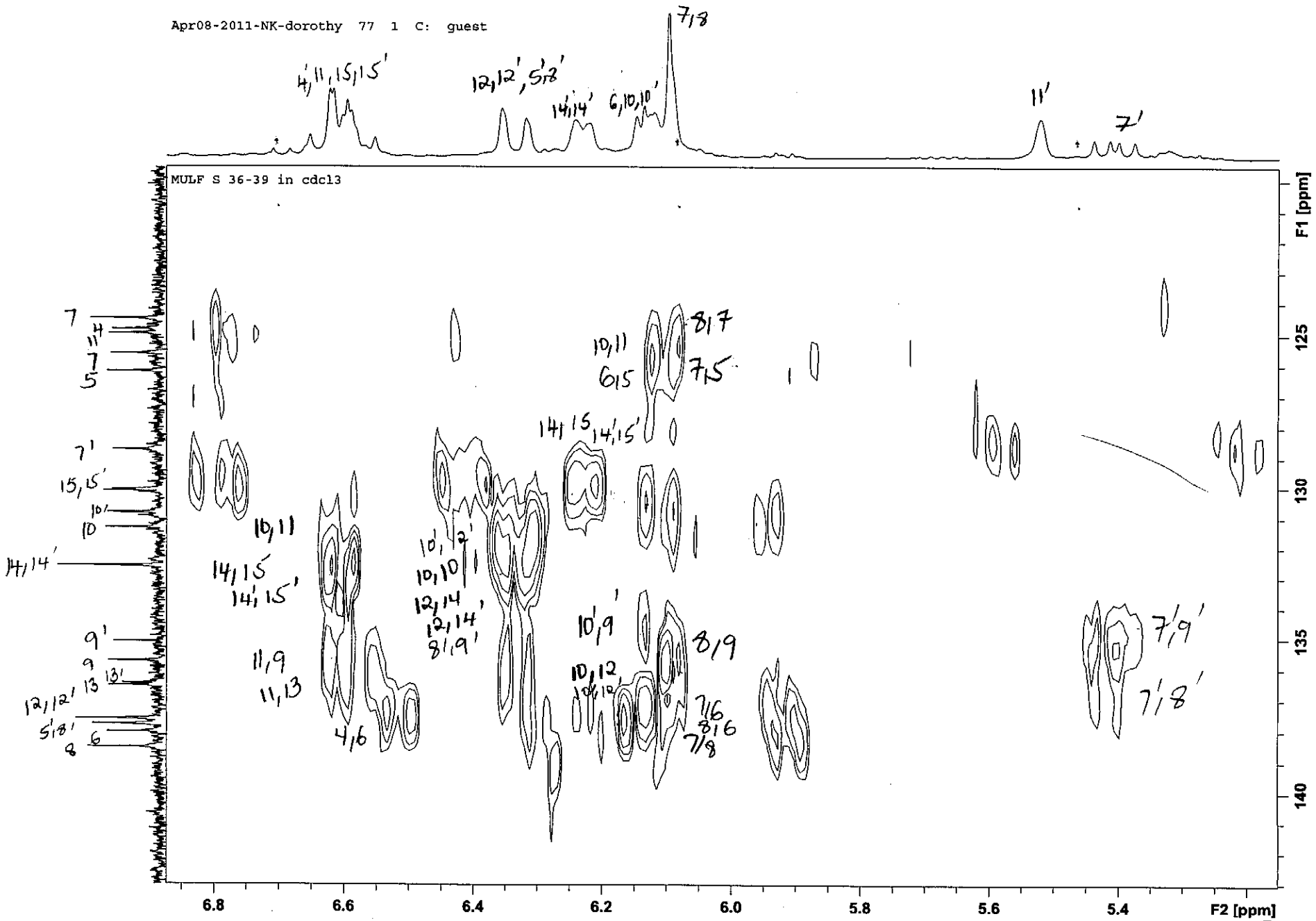
HMBC spectrum of B9



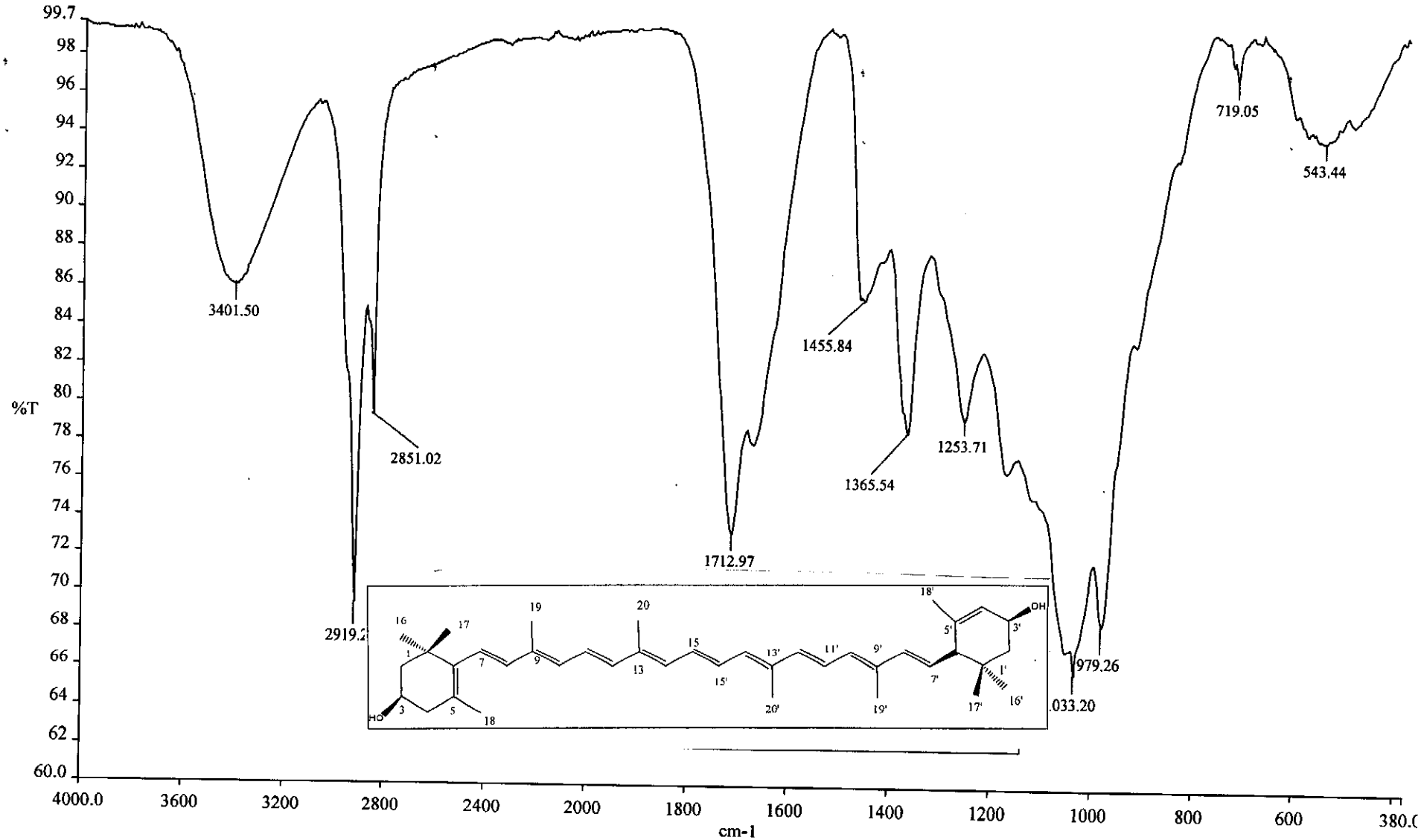
HMBC spectrum of B9 expanded (F1 10-75 ppm, F2 0.6-2.5 ppm)



HMBC spectrum of B9 expanded (F1 124-140 ppm, F2 1.0-2.4 ppm)



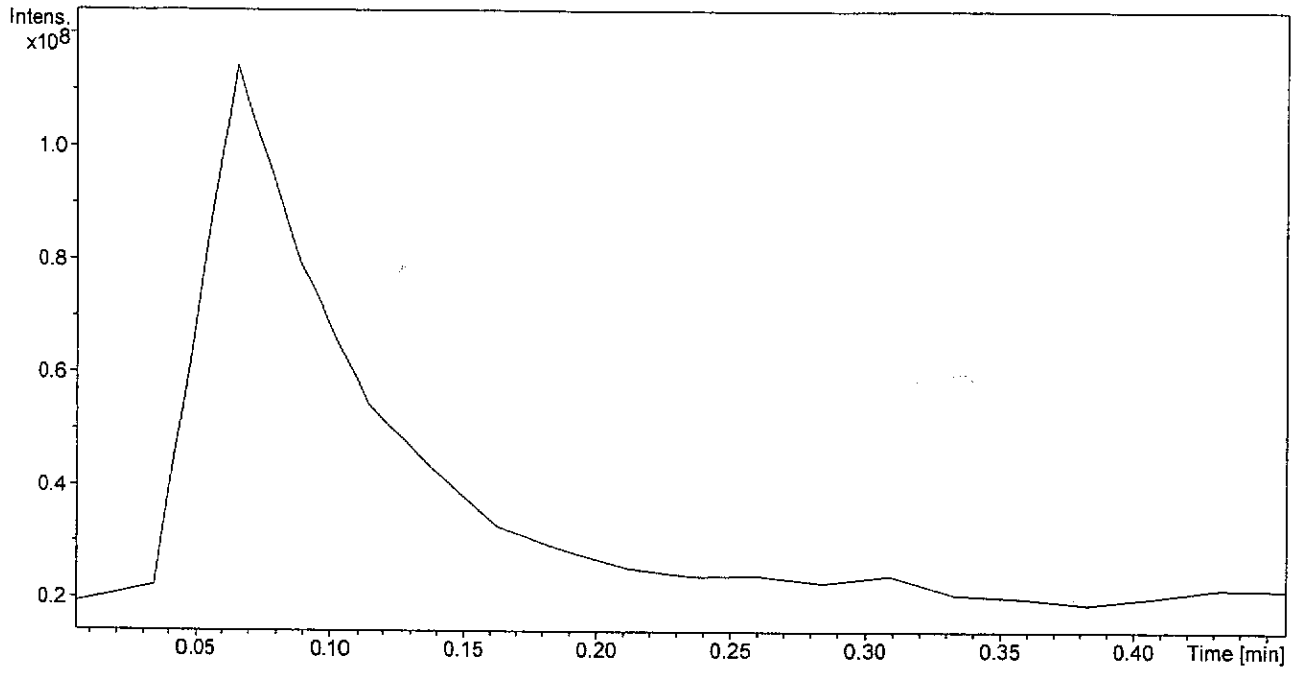
HMBC spectrum of B9 expanded (F1 120-143 ppm, F2 5.2-6.8 ppm)



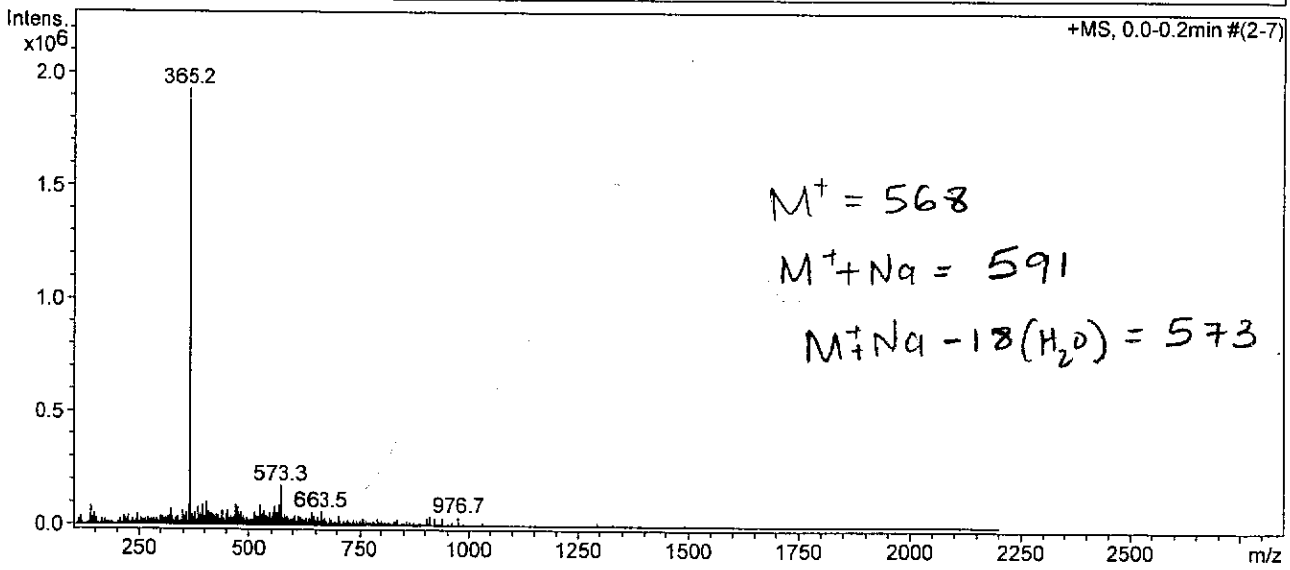
IR spectrum of B9

Display Report - All Windows All Analyses

Operator: Operator **Instrument:** LC-MSD-Trap-VL **Print Date:** 7/27/2012 2:33:13 PM



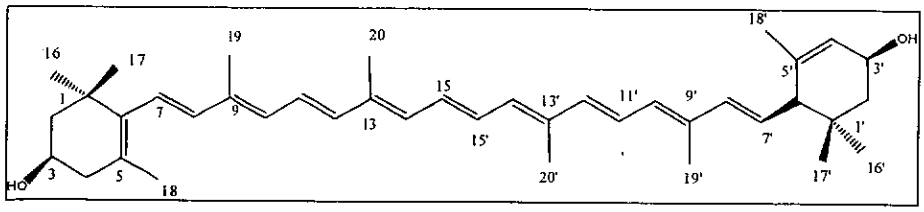
2000000.D: TIC +All MS



$M^+ = 568$

$M^+ + Na = 591$

$M^+ Na - 18(H_2O) = 573$



LC-MS spectrum of B9

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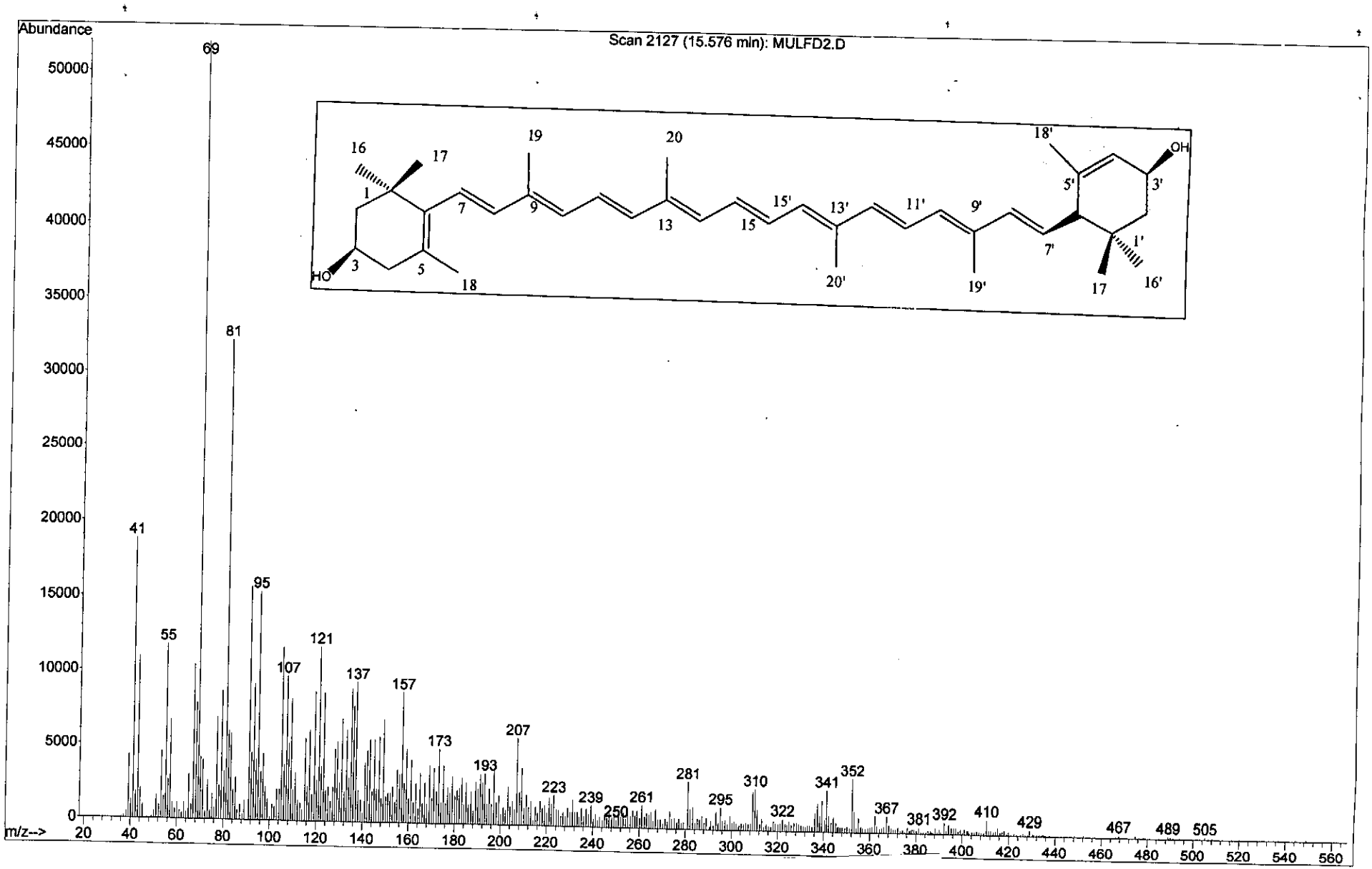
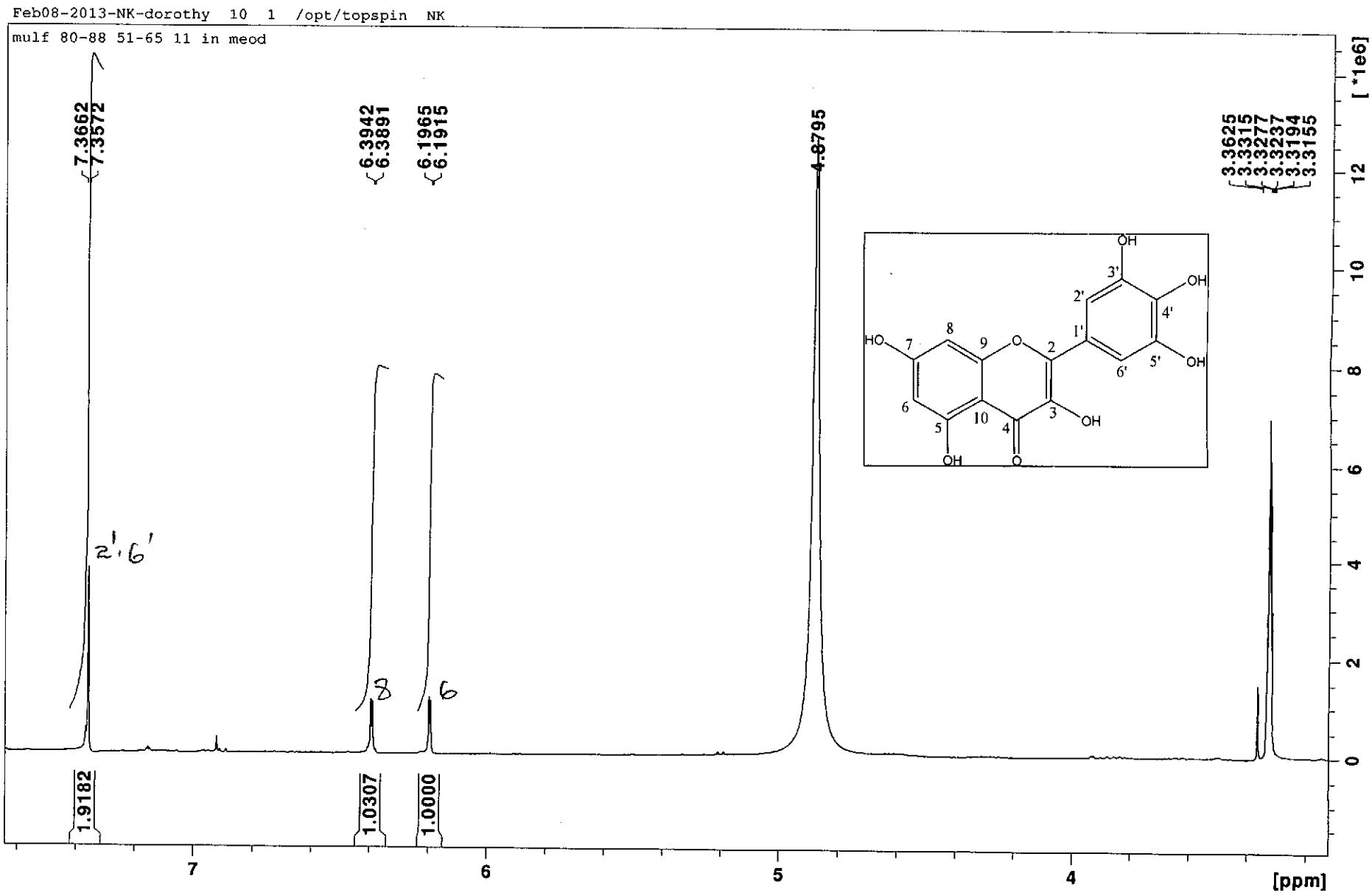
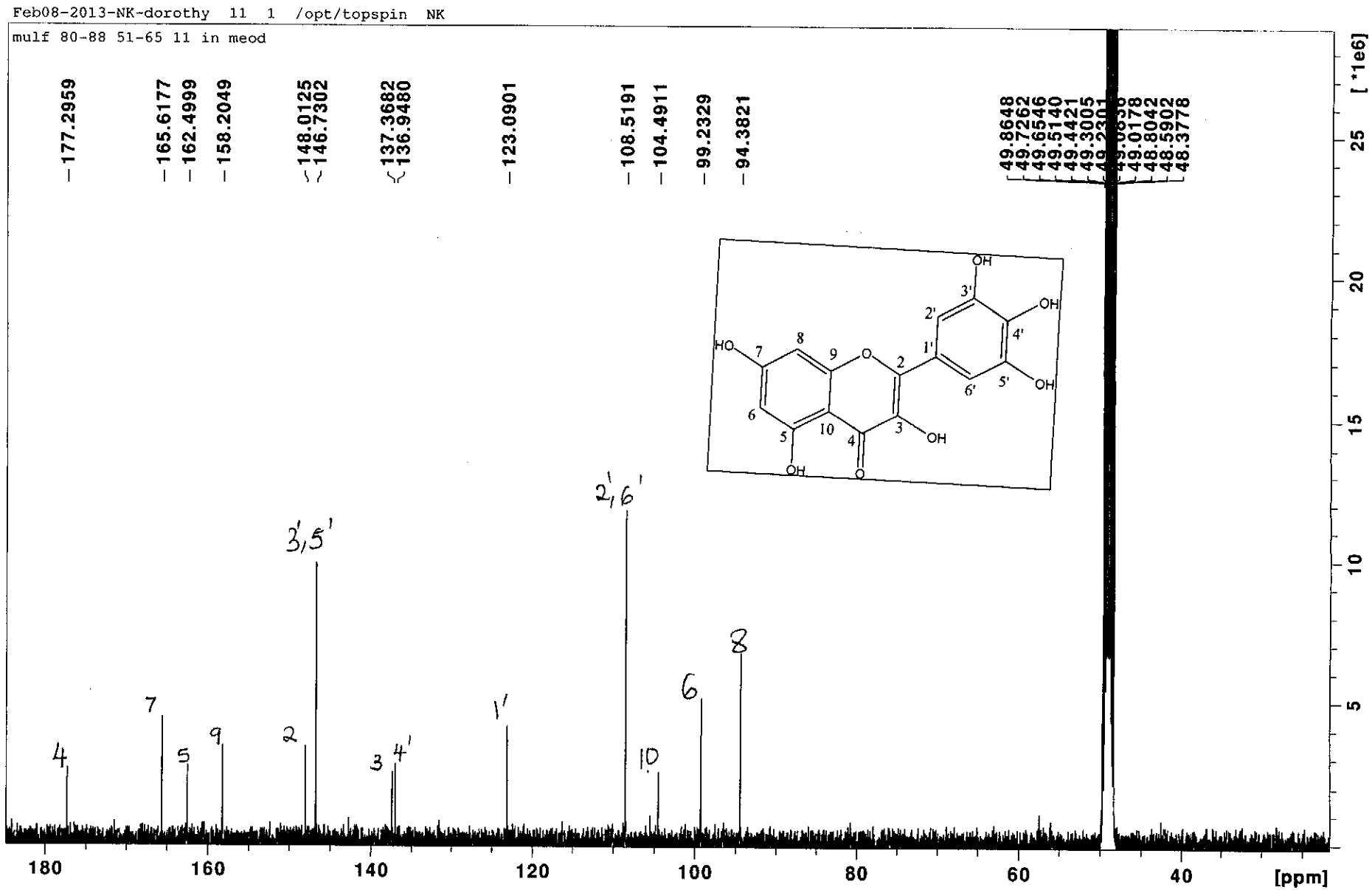
Operator : Dorothy
Acquired : 28 Apr 2011 21:33 using AcqMethod NATURALJ
Instrument : Instrumen
Sample Name: MULF 30-39 (S)
Misc Info :
Vial Number: 1



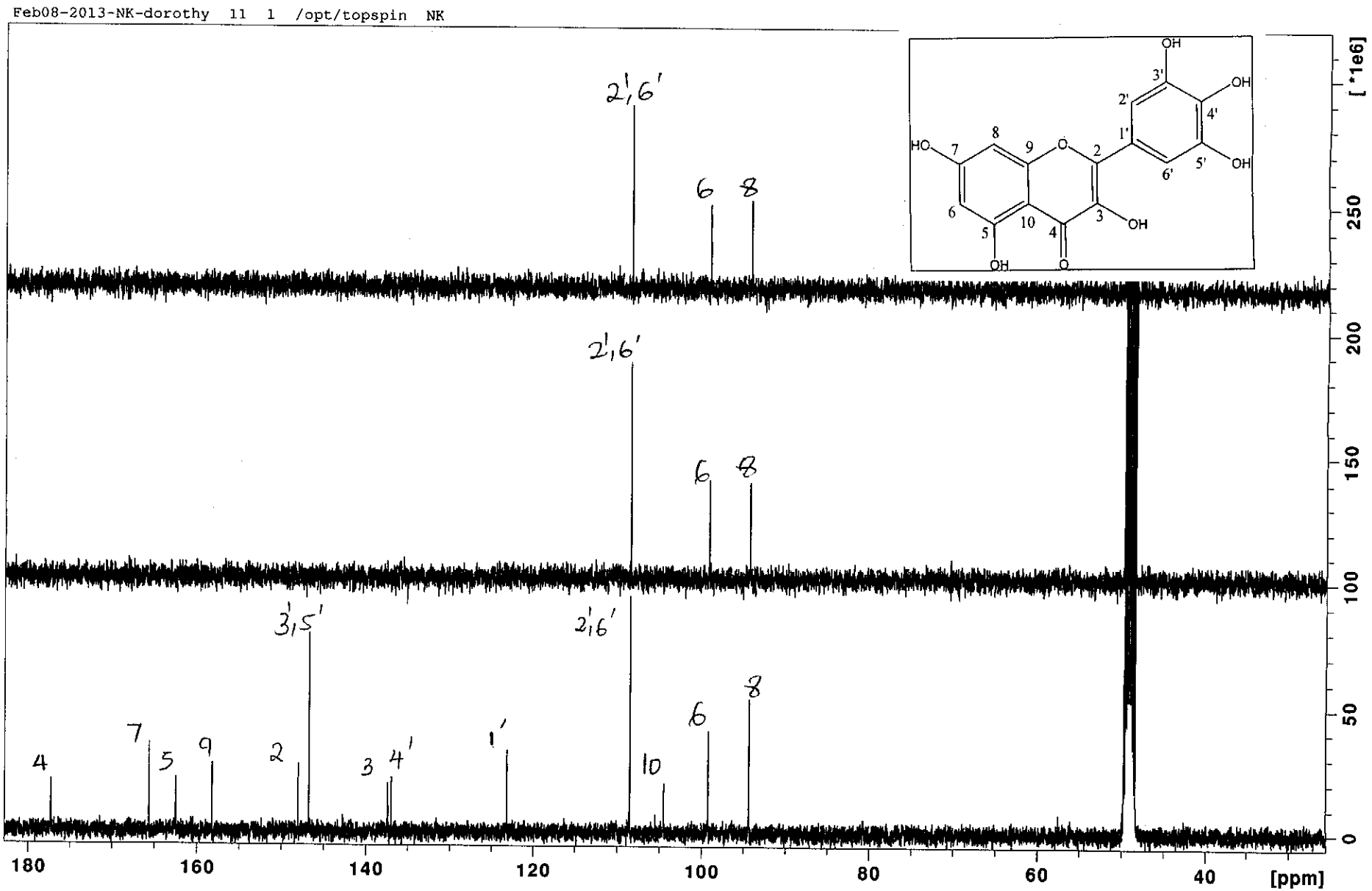
MS spectrum of B9



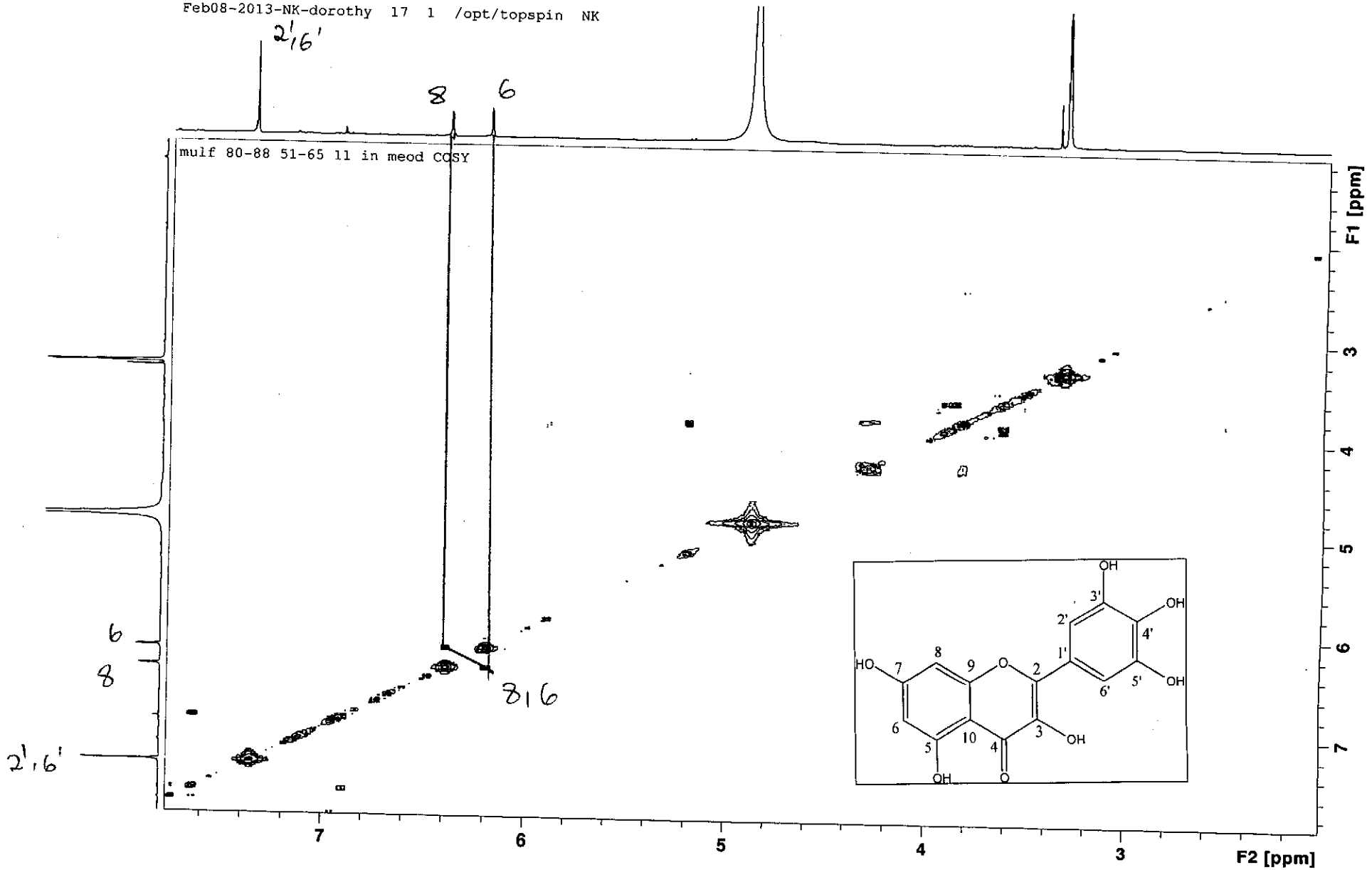
^{1}H NMR spectrum of B10



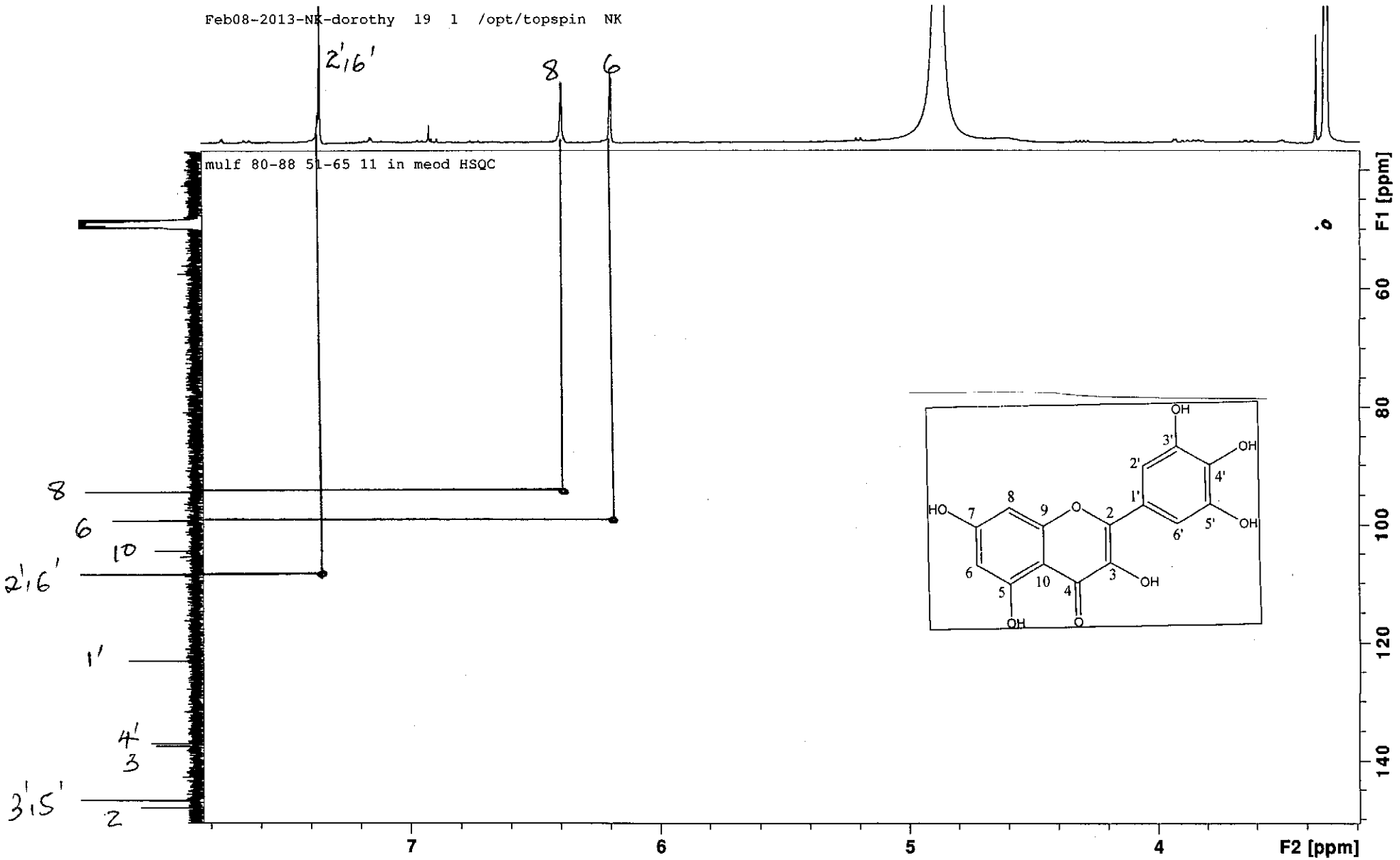
^{13}C NMR spectrum of B10



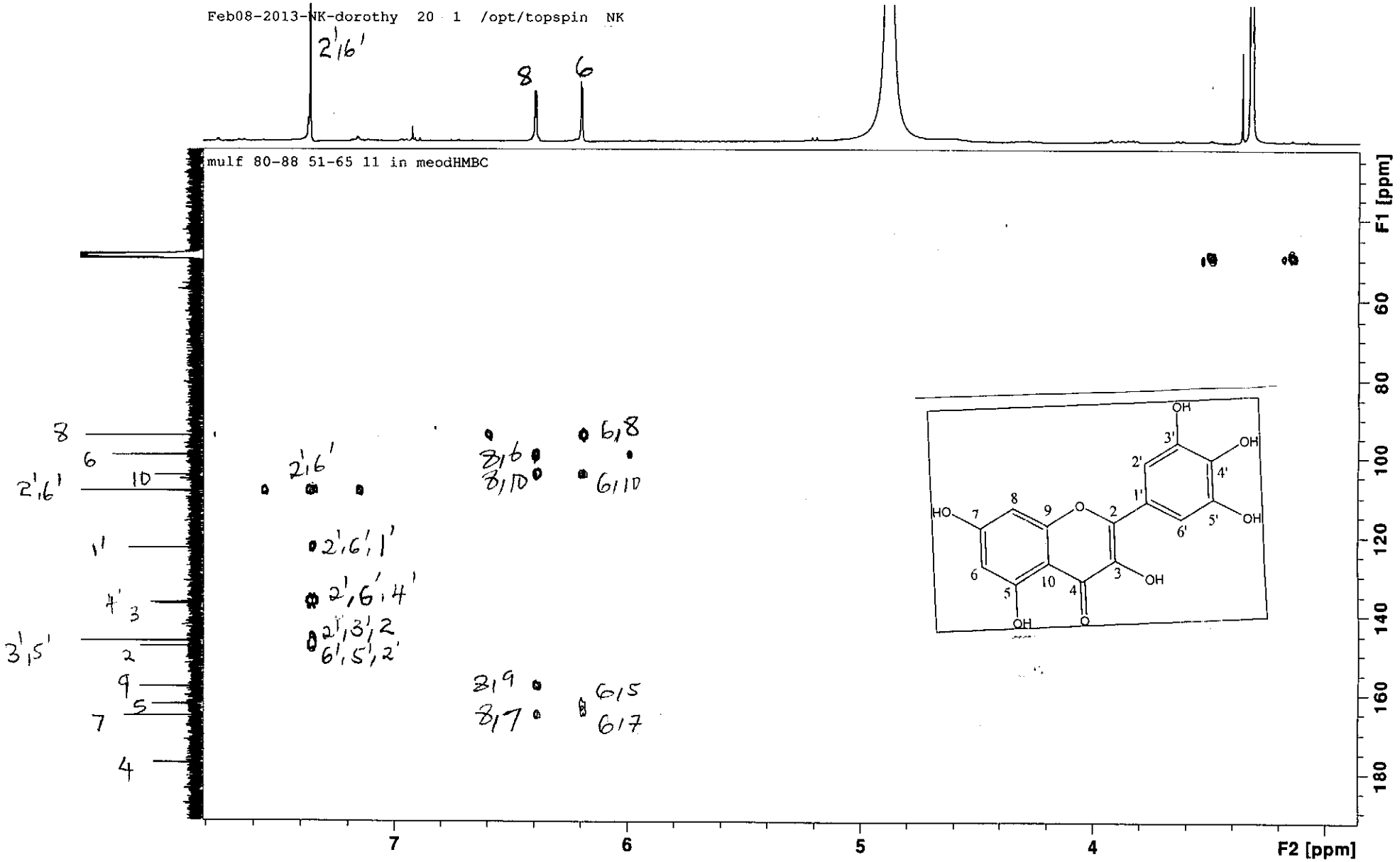
DEPT spectrum of B10



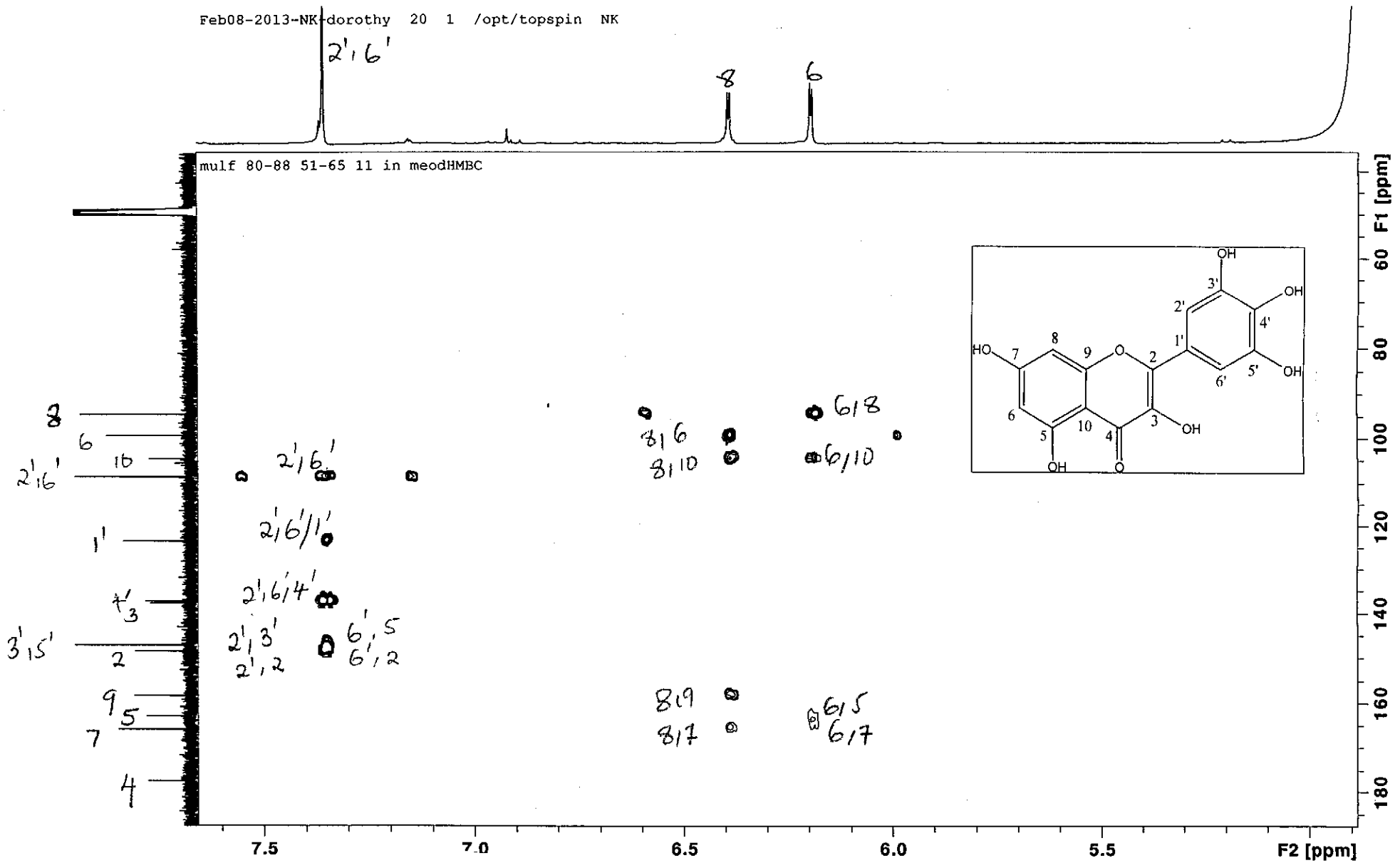
COSY spectrum of B10



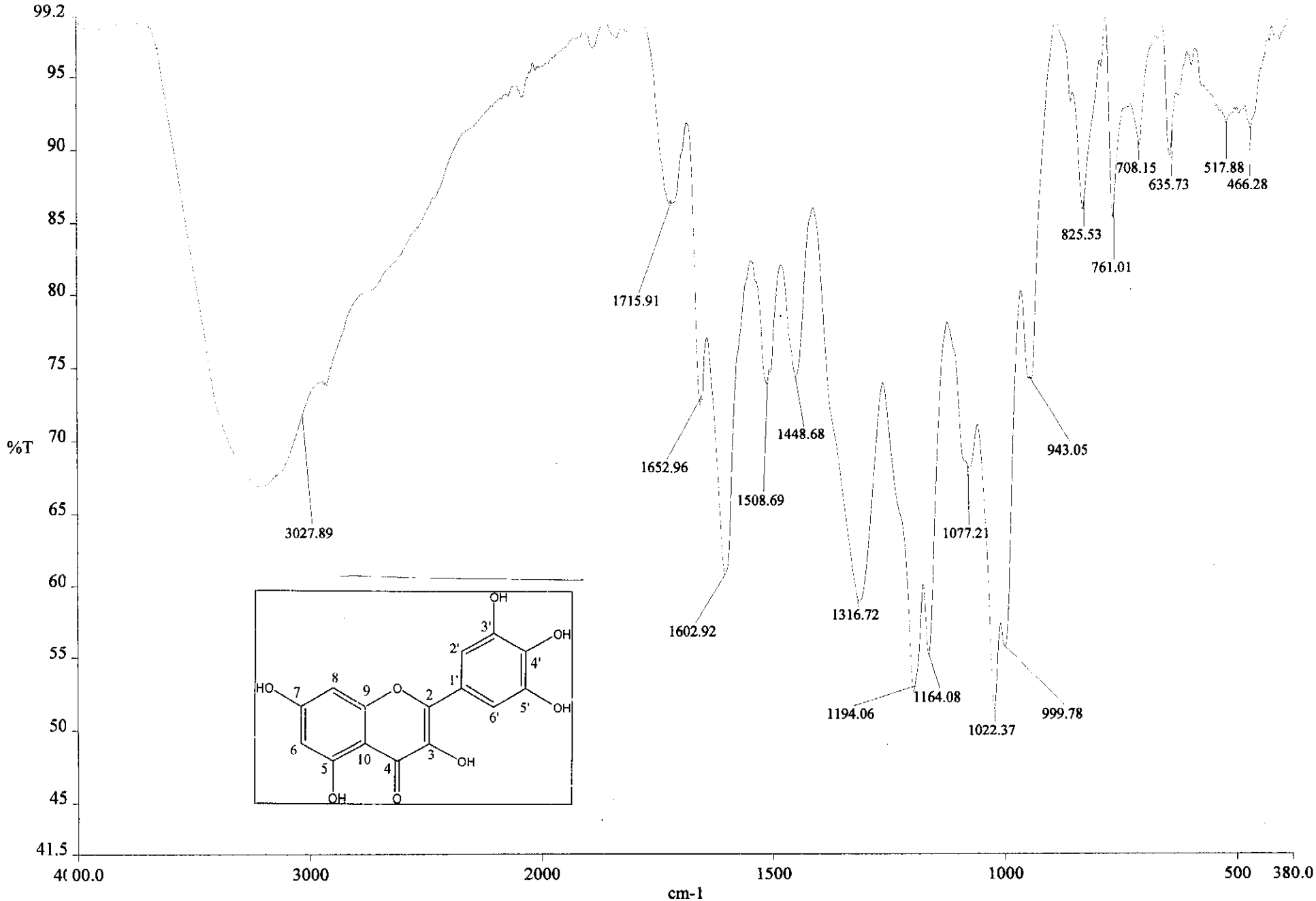
HSQC spectrum of B10



HMBC spectrum of B10

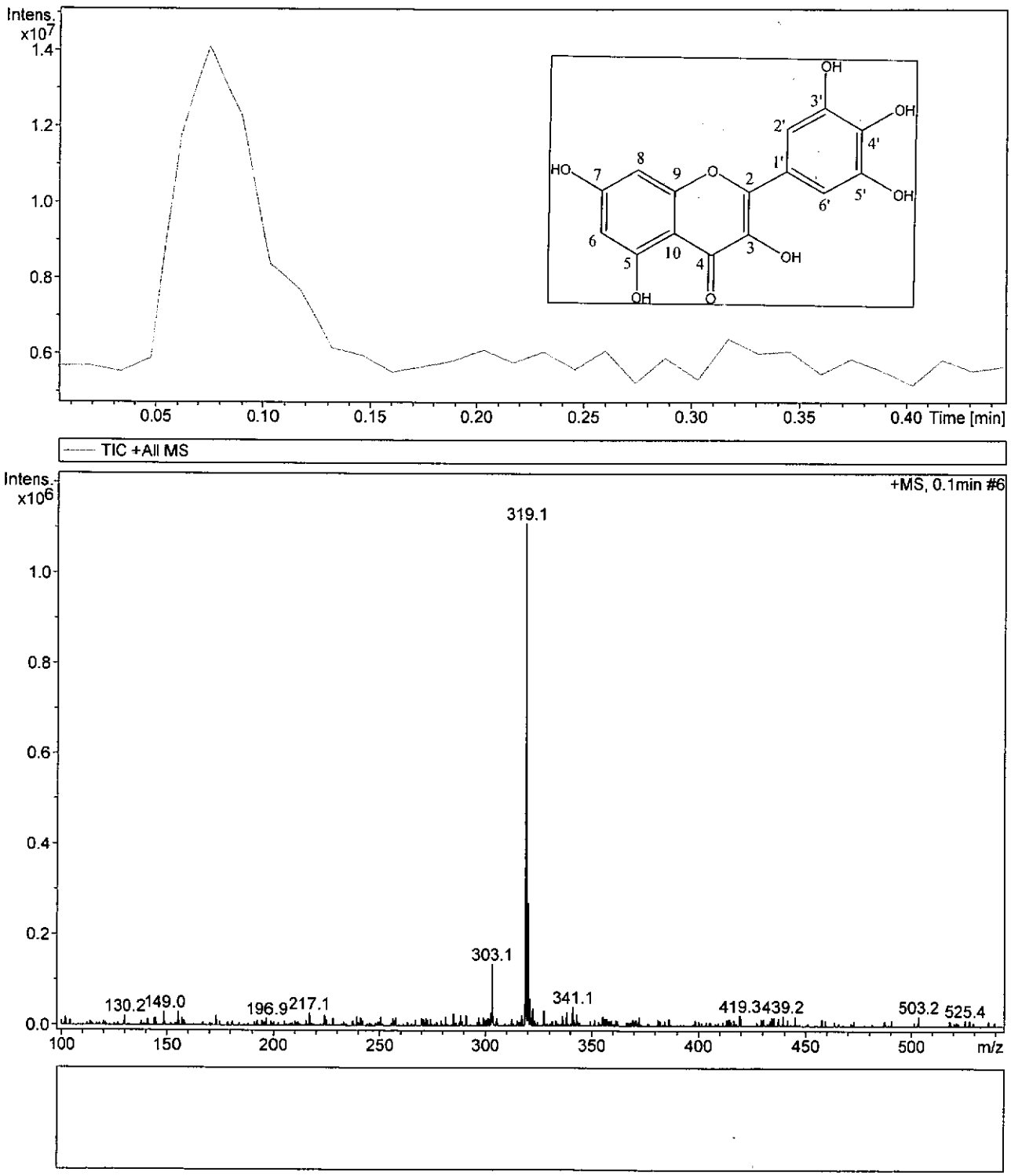


HMBC spectrum of B10 Expanded


99.2
95
90
85
80
75
70
65
60
55
50
45
41.5
%T
4(00.0
3000
2000
1500
1000
500
380.0
cm-1
3027.89
1715.91
1652.96
1602.92
1508.69
1448.68
1316.72
1194.06
1164.08
1077.21
1022.37
999.78
943.05
825.53
761.01
708.15
635.73
517.88
466.28
OH
HO
O
c:\pel_data\spectra\dorothy\dorothy myri 3a.sp - myricetin

Display Report - All Windows Selected Analysis

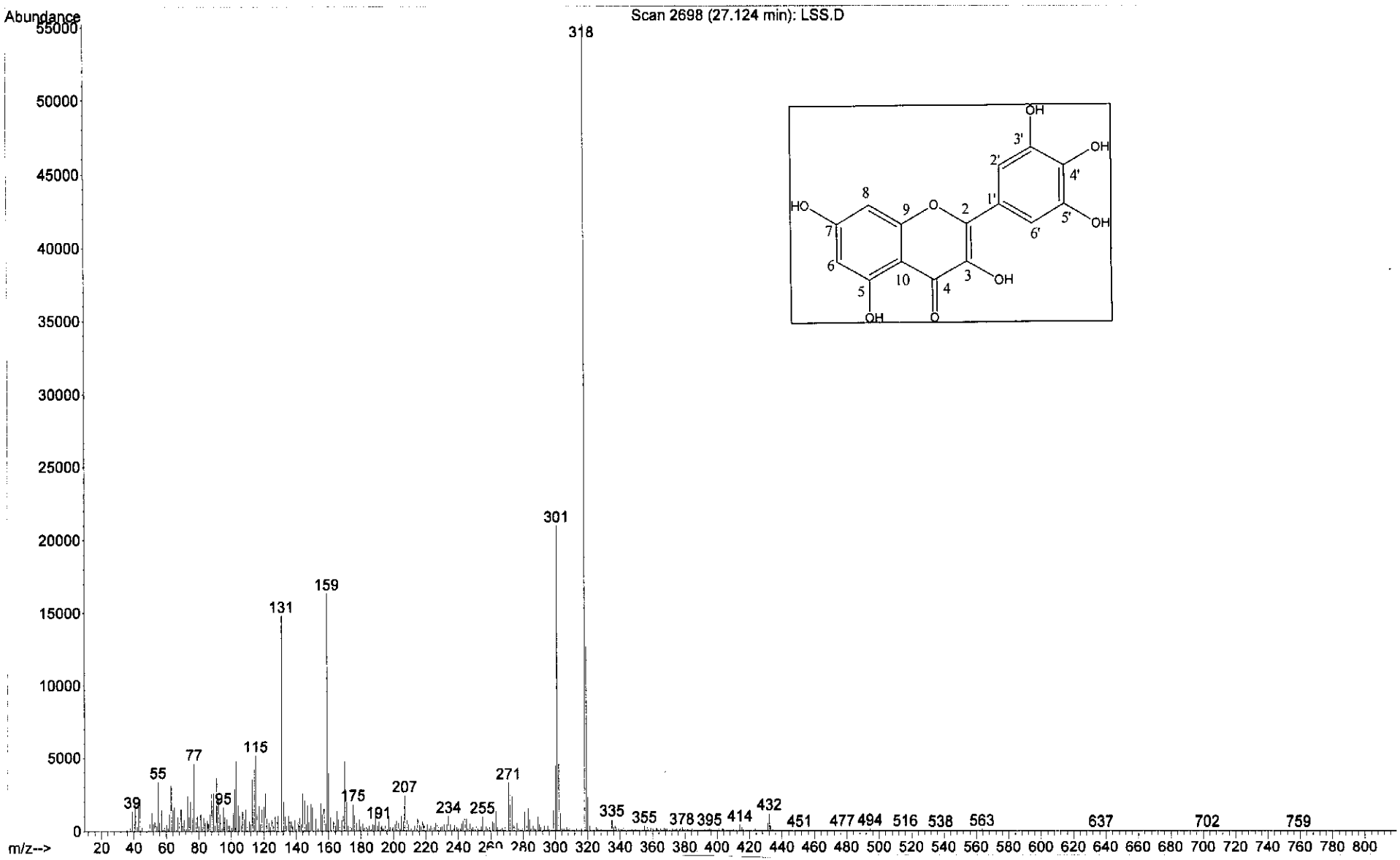
Analysis Name: MYRICETIN000 **Instrument:** LC-MSD-Trap-VL **Print Date:** 2/18/2013 11:20:09 AM
Method: AN 2MIN.M **Operator:** Operator **Acq. Date:** 2/18/2013 11:19:05 AM
Sample Name: Default
Analysis Info:



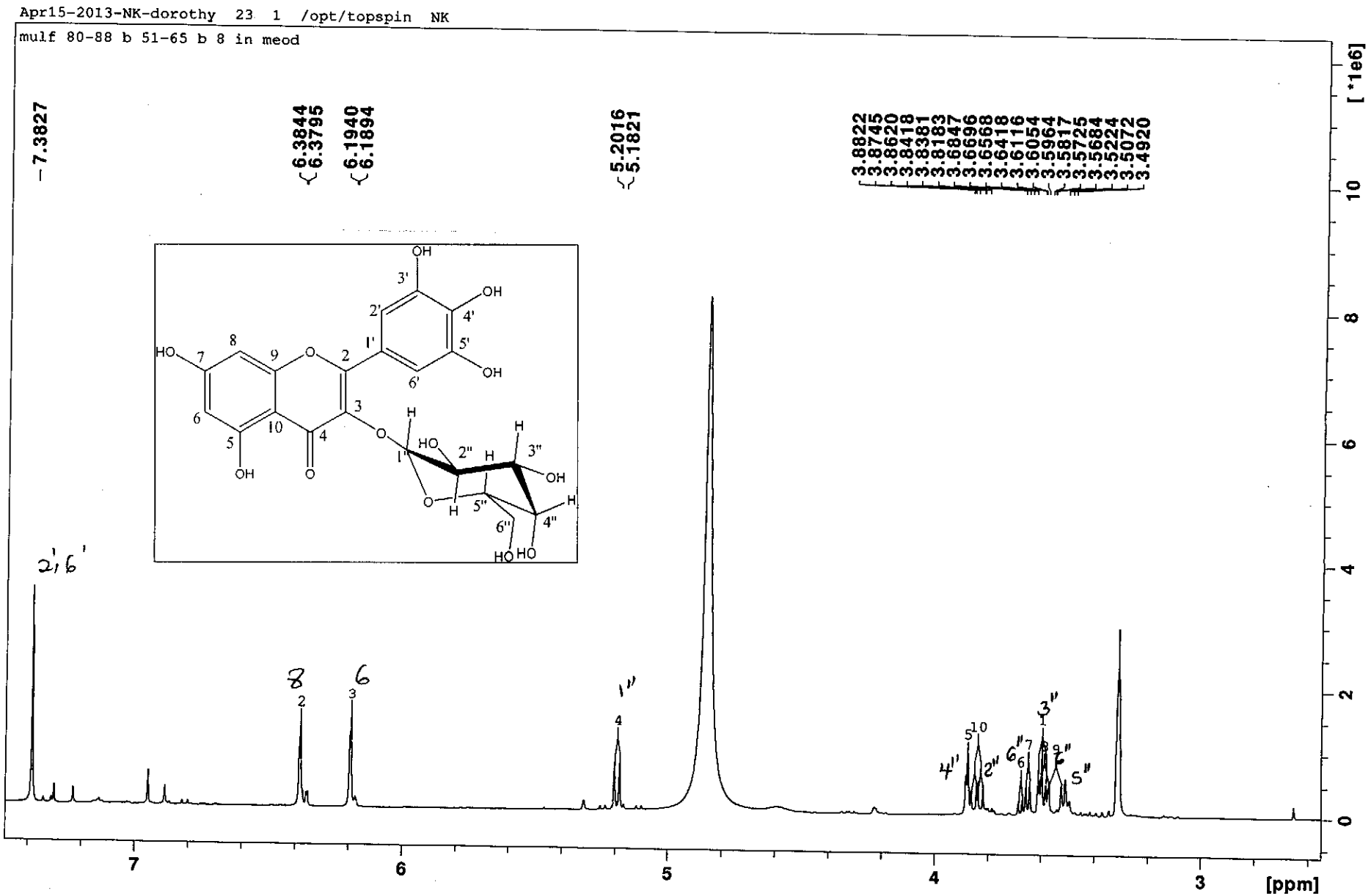
LC-MS spectrum of B10

Agilent Technologies

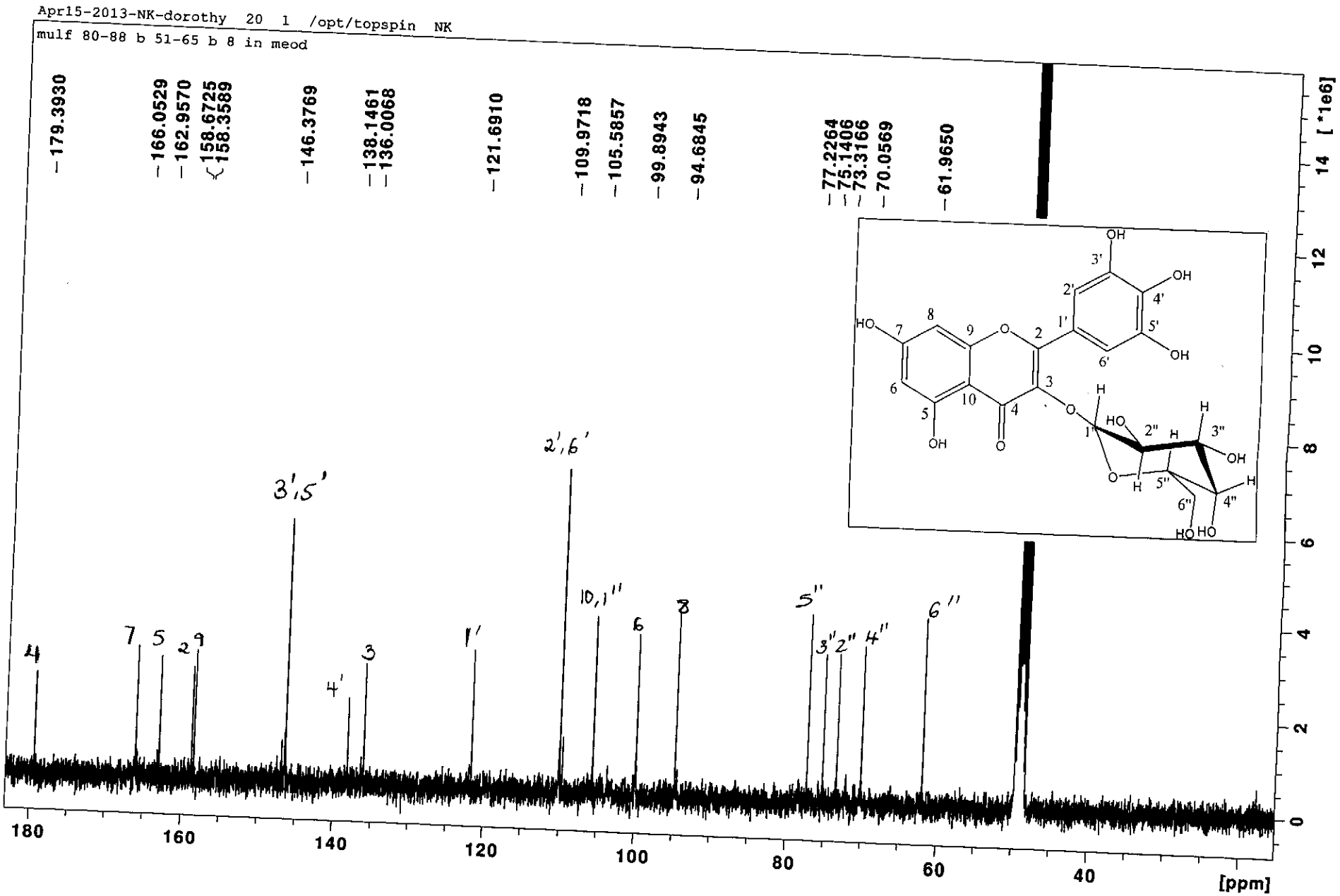
File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\DORTHY\LSS.D
Operator : DOROTHY
Acquired : 19 Jun 2011 10:38 using AcqMethod NATURAL
Instrument : Instrumen
Sample Name: LSS 70-76
Misc Info :
Vial Number: 1



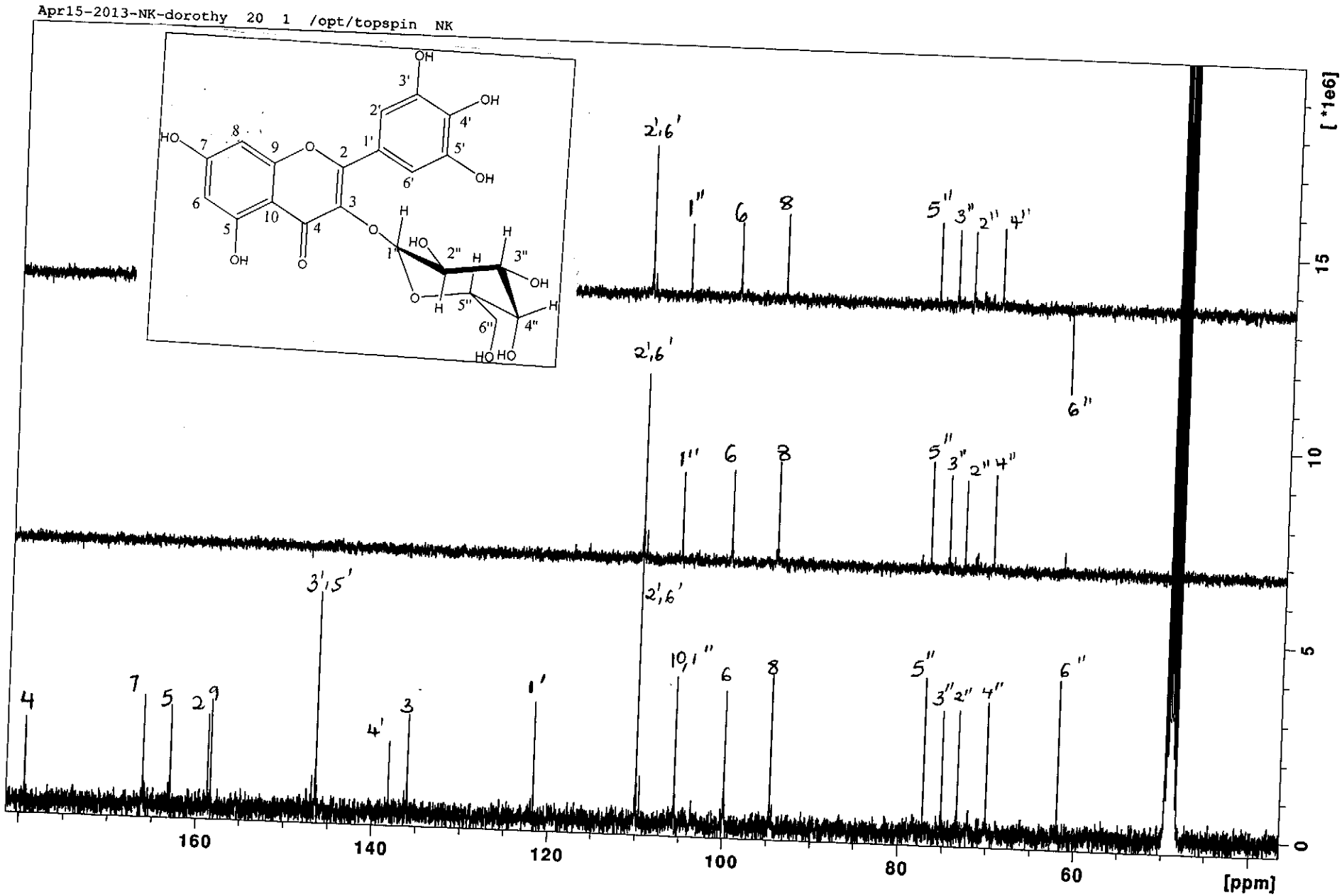
MS spectrum of B10



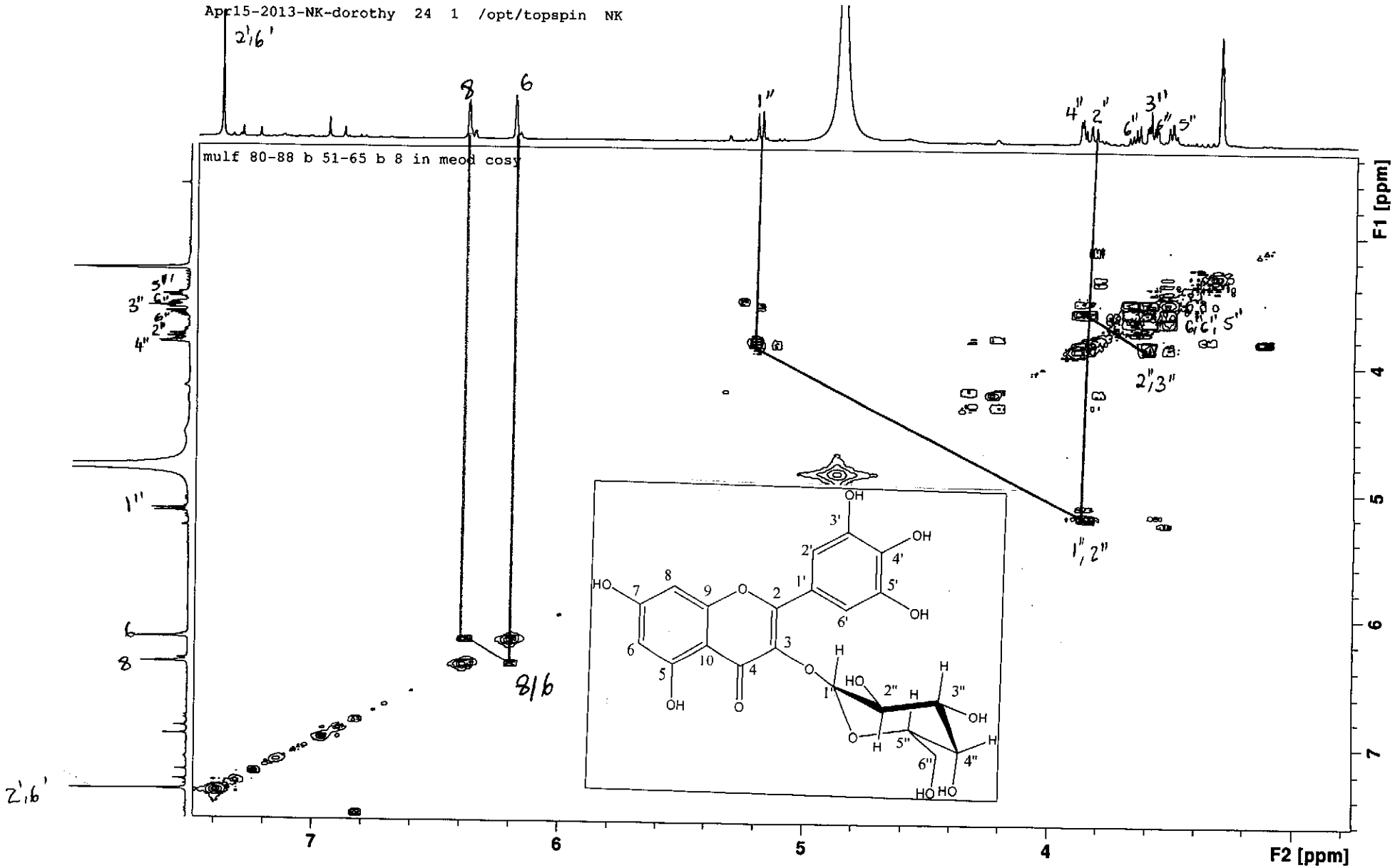
^{1}H NMR spectrum of B11



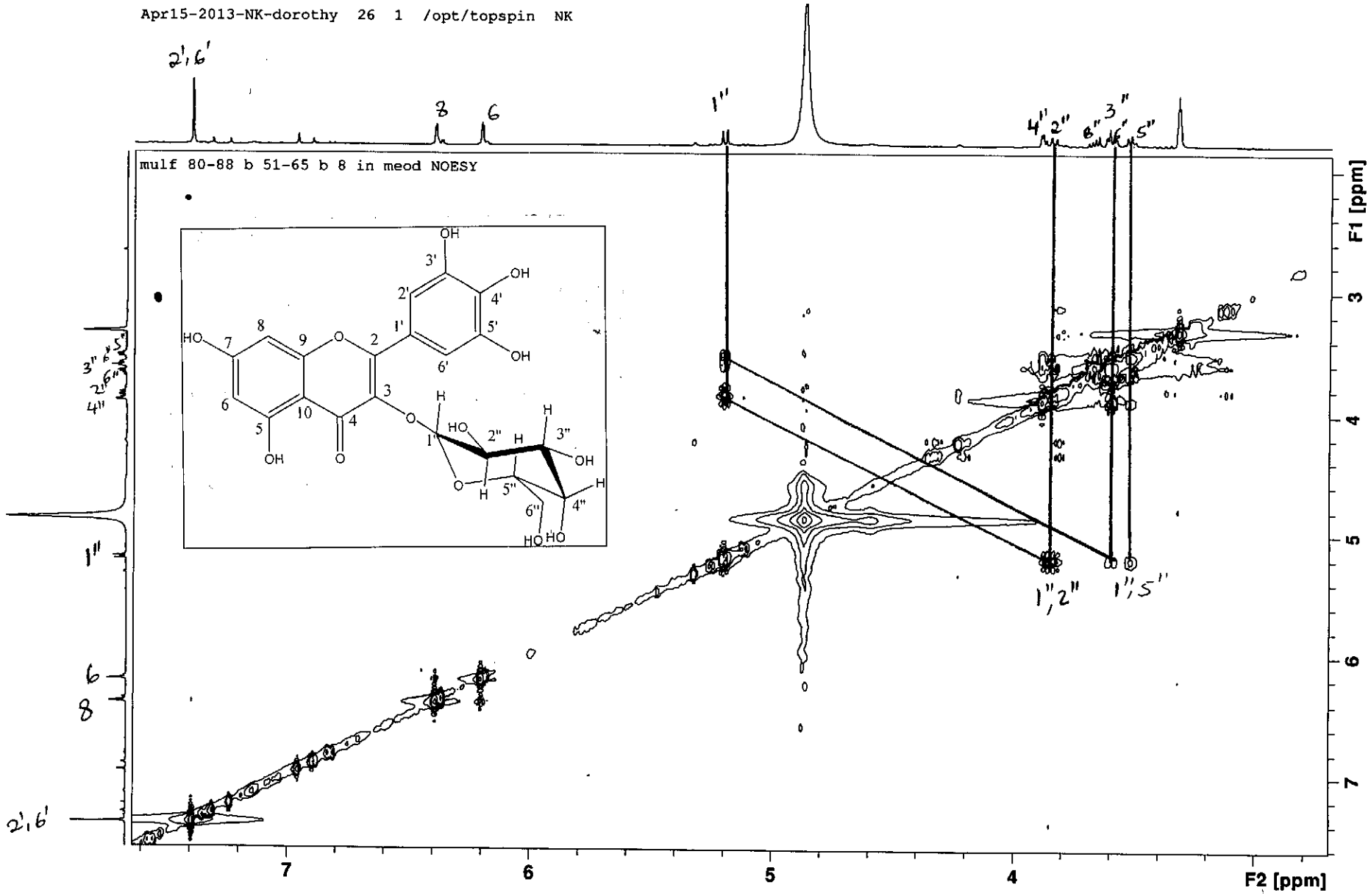
^{13}C NMR spectrum of B1



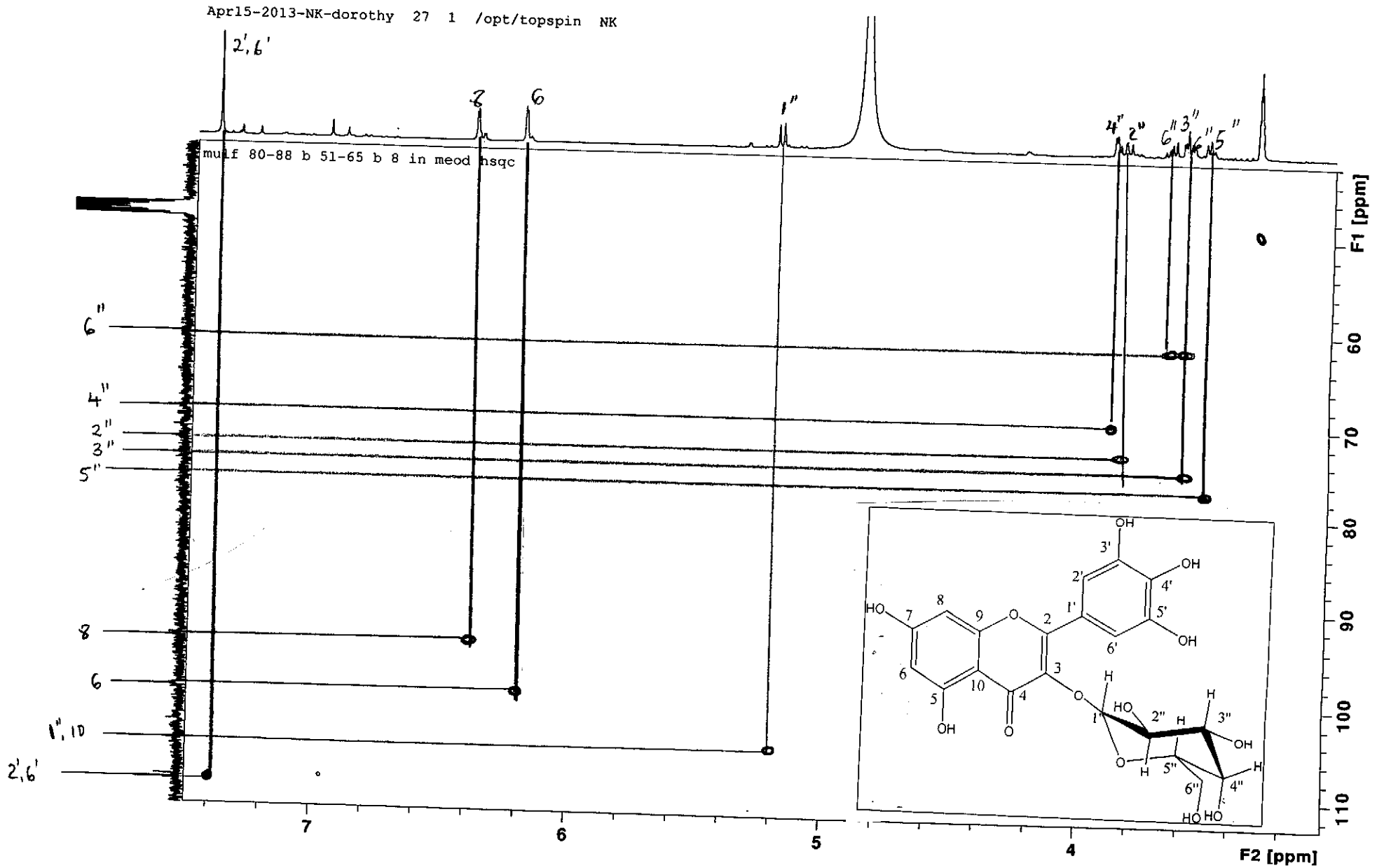
DEPT spectrum of B11



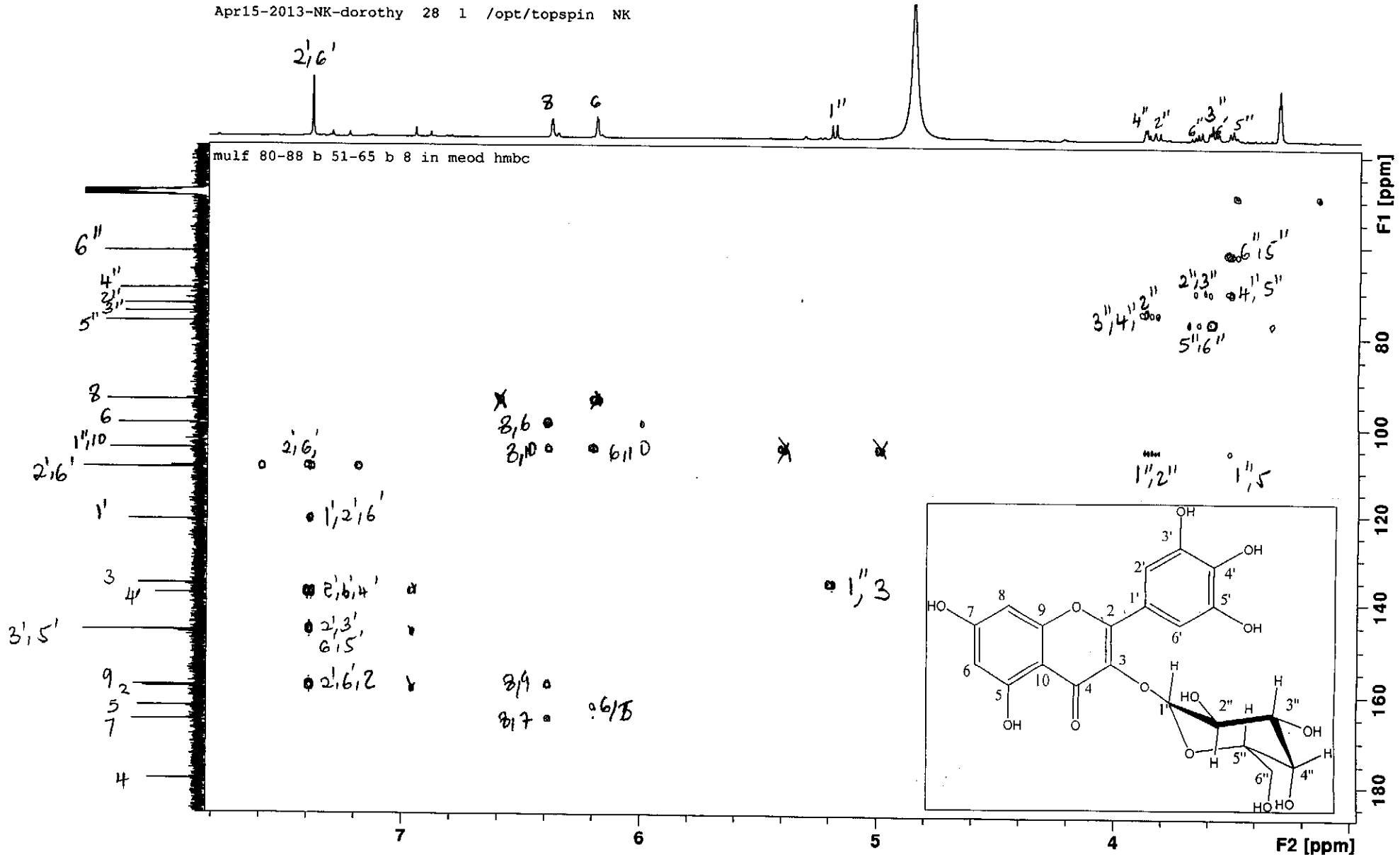
COSY spectrum of B11



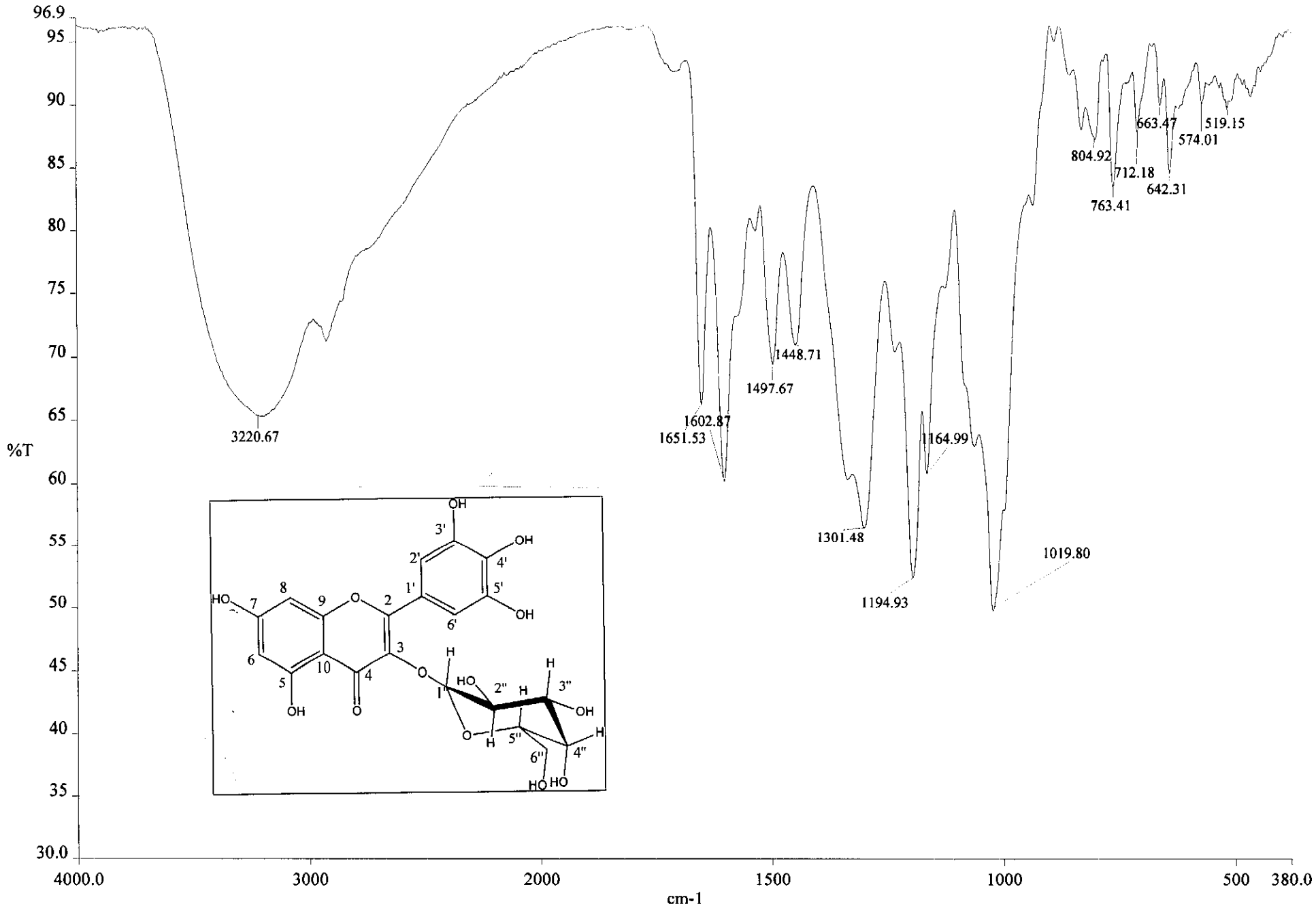
NOESY spectrum of B11



HSQC spectrum of B11



HMBC spectrum of B11

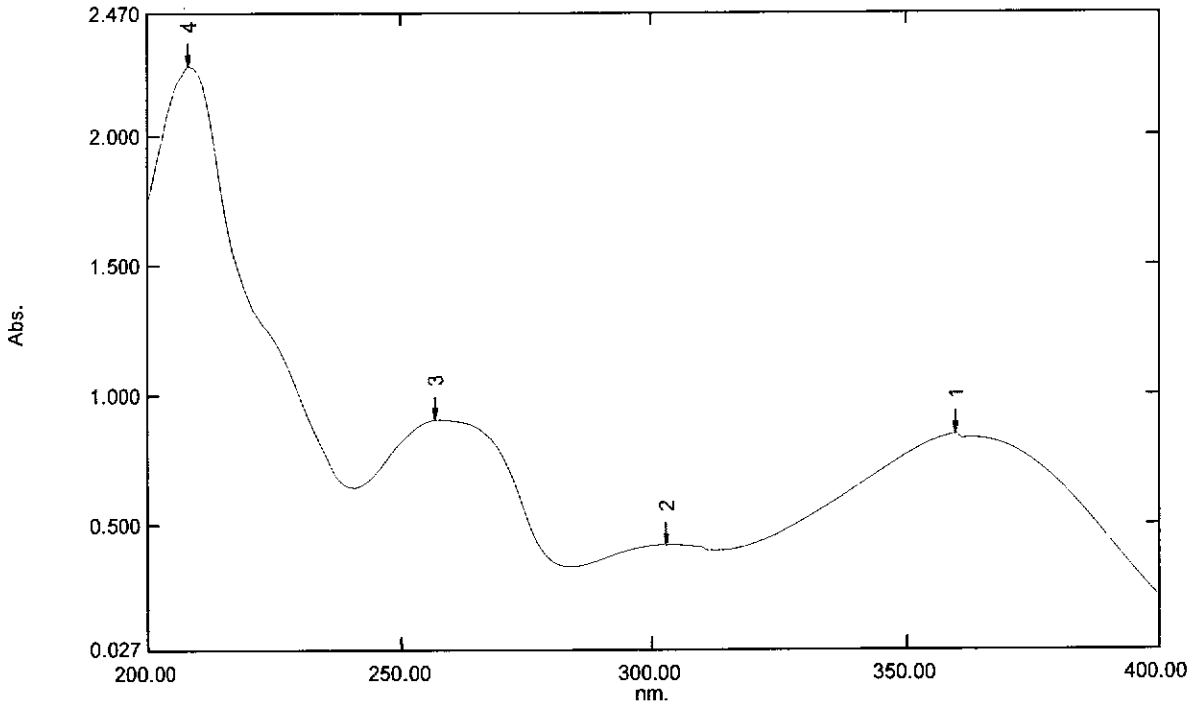


IR spectrum of B11

Spectrum Peak Pick Report

18/04/2013 10:27:58 AM

Data Set: myricetin galactoside batter.spc - Storage 102515



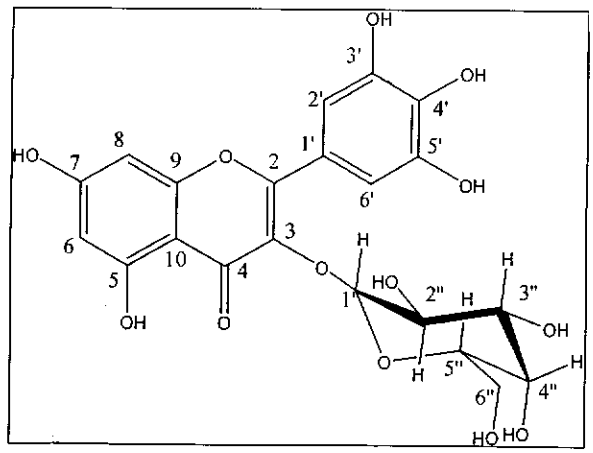
Measurement Properties	
Wavelength Range (nm.):	200.00 to 400.00
Scan Speed:	Medium
Sampling Interval:	1.0
Auto Sampling Interval:	Disabled
Scan Mode:	Single

Instrument Properties	
Instrument Type:	UV-3600 Series
Measuring Mode:	Absorbance
Slit Width:	1.0 nm
Time Constant:	1.0 sec.
Source Lamp:	Auto
Light Source Change Wavelength:	310.00 nm
Detector Unit:	Direct
Detector Change Wavelength:	830.00 nm 1800.00 nm
Grating Change Wavelength:	720.00 nm
S/R Exchange:	Normal
Detector Lock:	Auto
Slit Program:	Normal
Beam Mode:	Double
Stair Correction:	Disable

Attachment Properties	
Attachment:	None

Sample Preparation Properties	
Weight:	
Volume:	
Dilution:	
Path Length:	
Additional Information:	

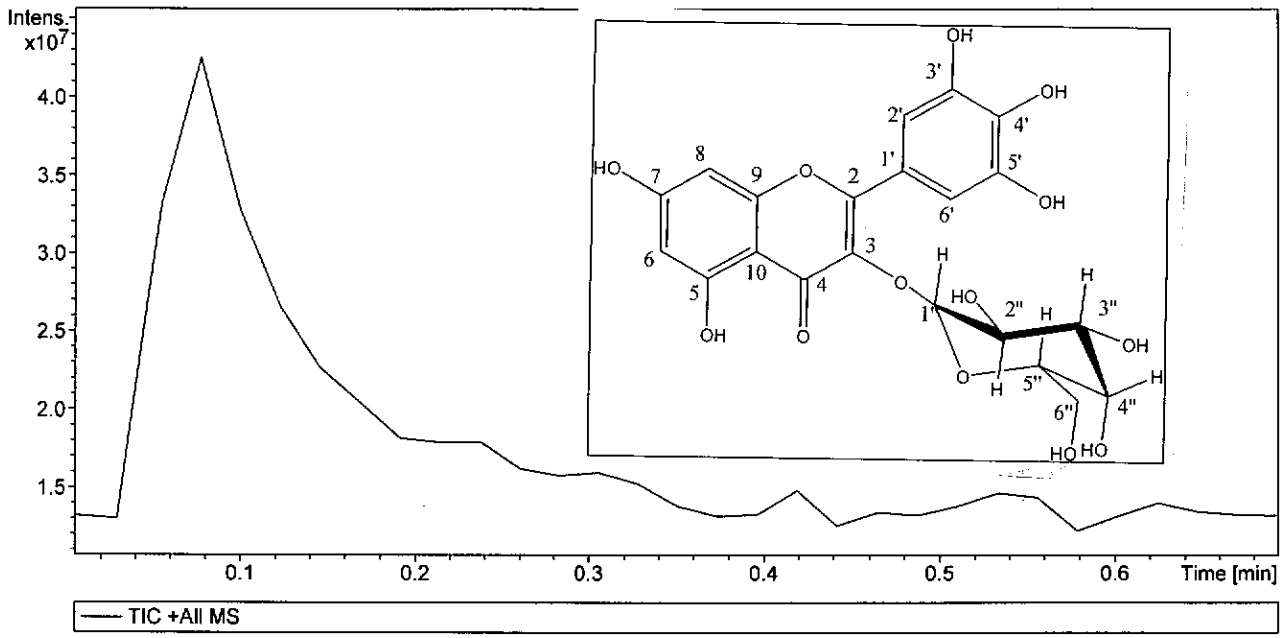
No.	P/V	Wavelength	Abs.	Description
1	●	360.00	0.851	
2	●	303.00	0.426	
3	●	257.00	0.907	
4	●	208.00	2.266	
5	▼	314.00	0.403	
6	▼	284.00	0.341	
7	▼	241.00	0.646	



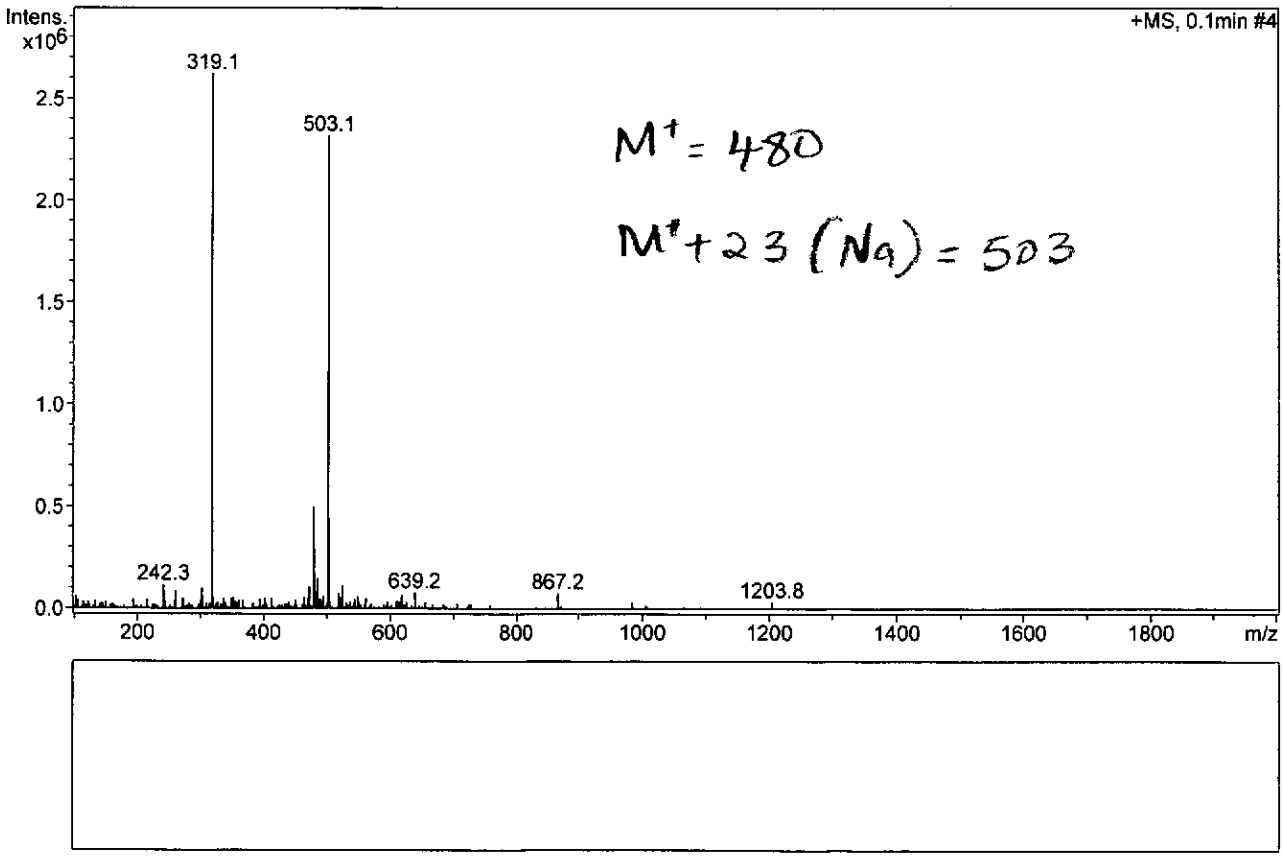
UV spectrum of B11

Display Report - All Windows Selected Analysis

Analysis Name: MRG000000.D **Instrument:** LC-MSD-Trap-VL **Print Date:** 4/18/2013 9:46:06 AM
Method: AN 2MIN.M **Operator:** Operator **Acq. Date:** 4/18/2013 9:44:42 AM
Sample Name: Default
Analysis Info: pos



TIC +All MS

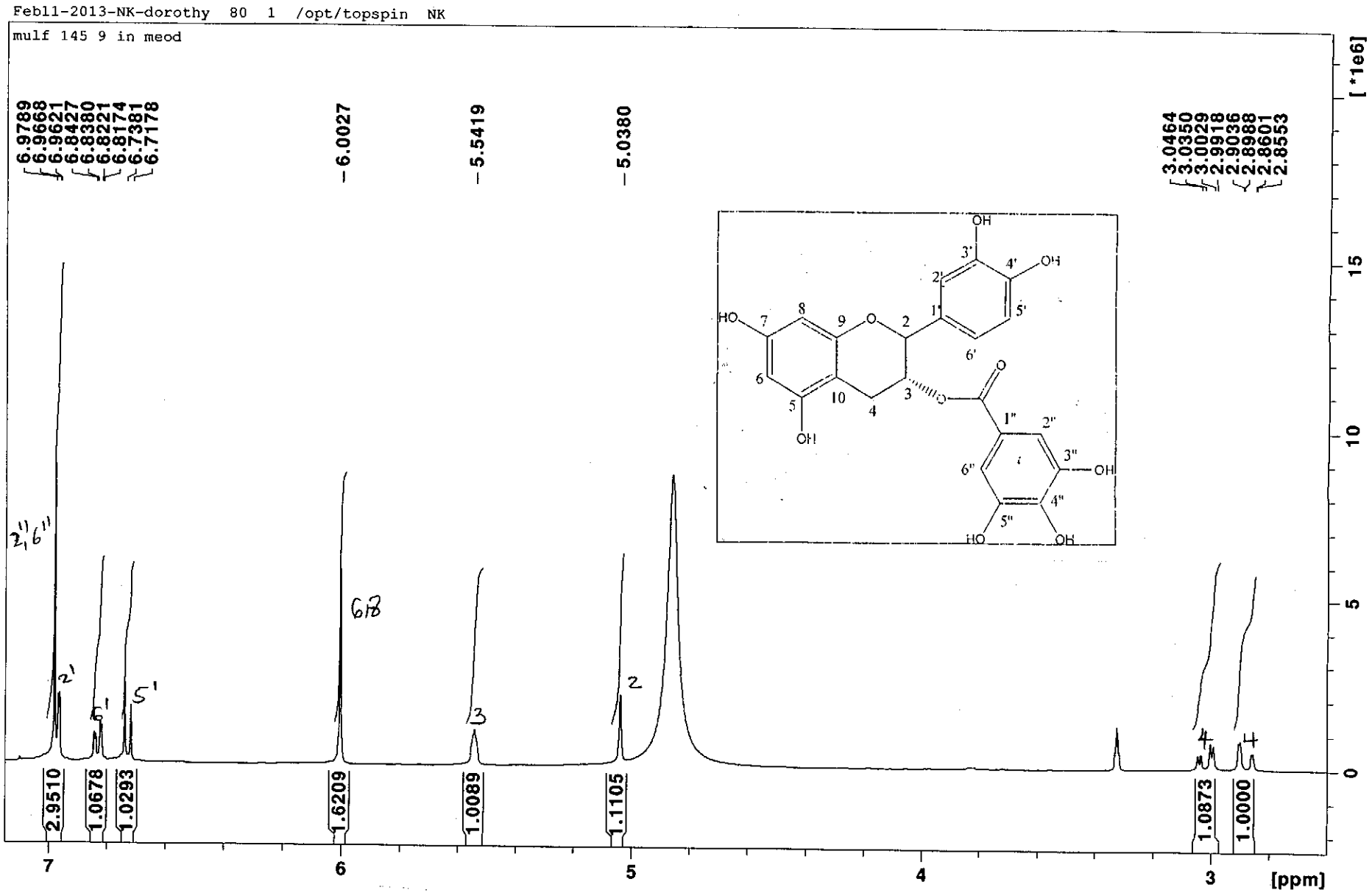


$M^+ = 480$

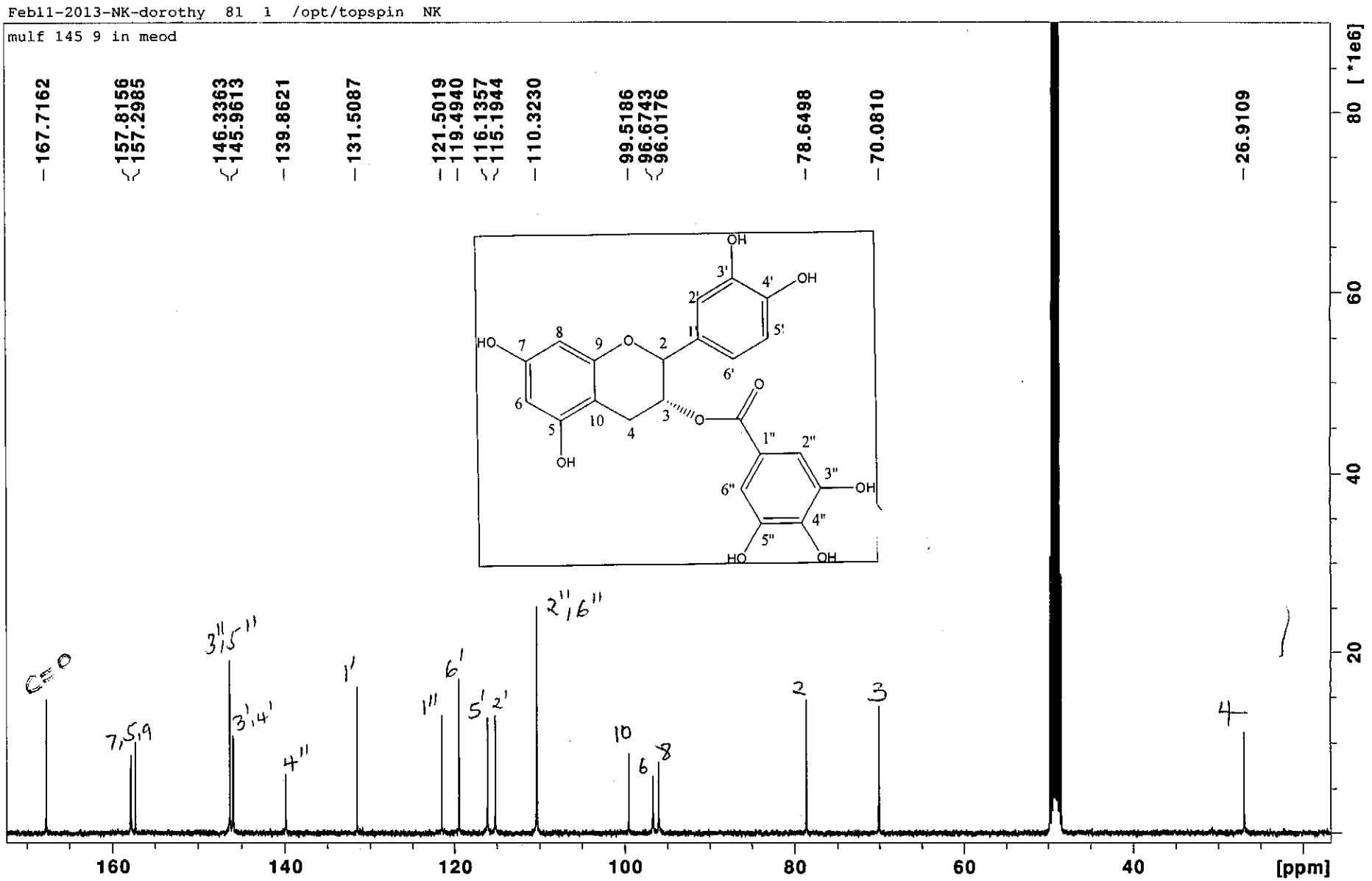
$M^+ + 23\ (Na) = 503$

LC-MS spectrum of B11

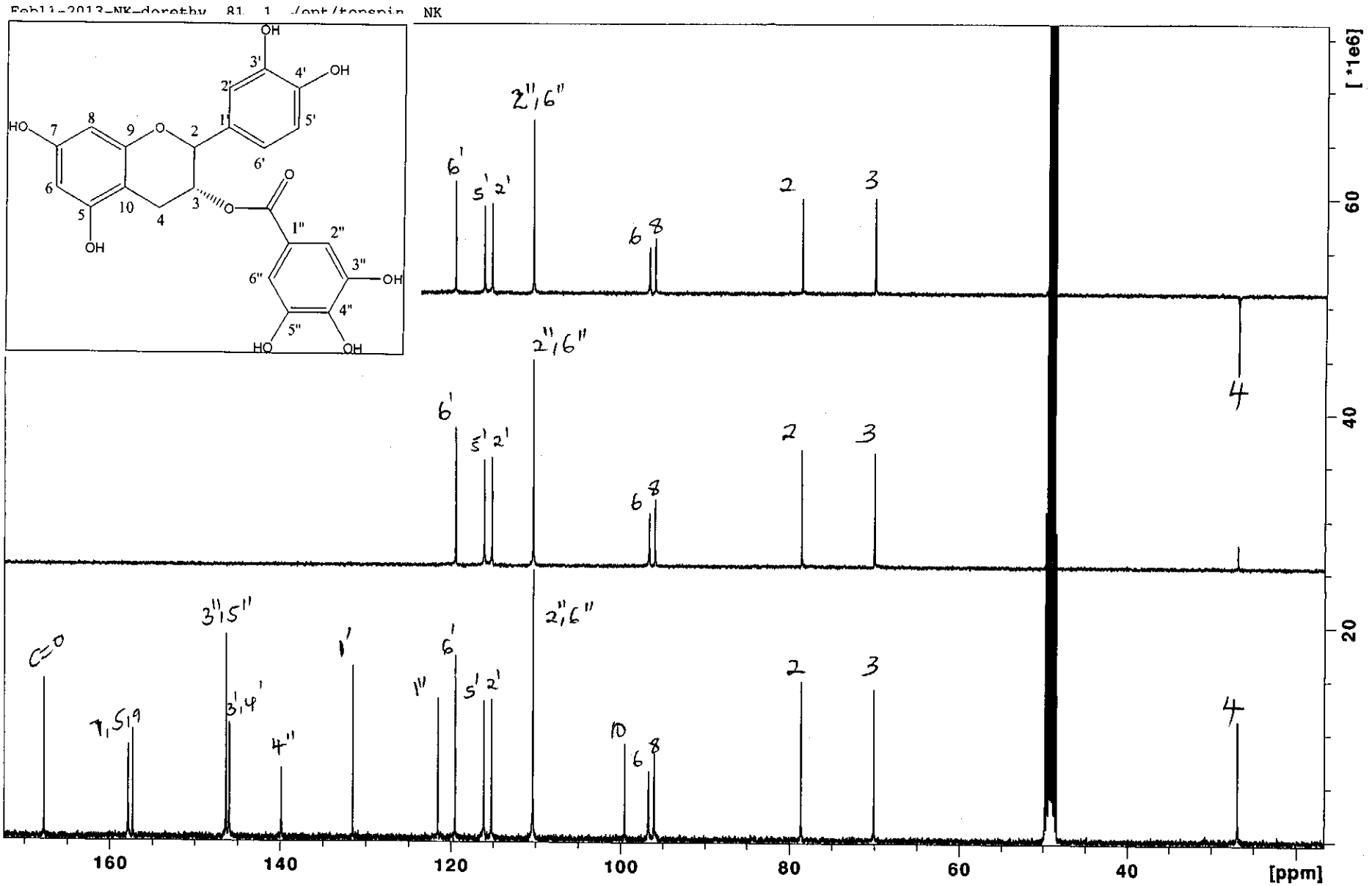
Agilent Technologies



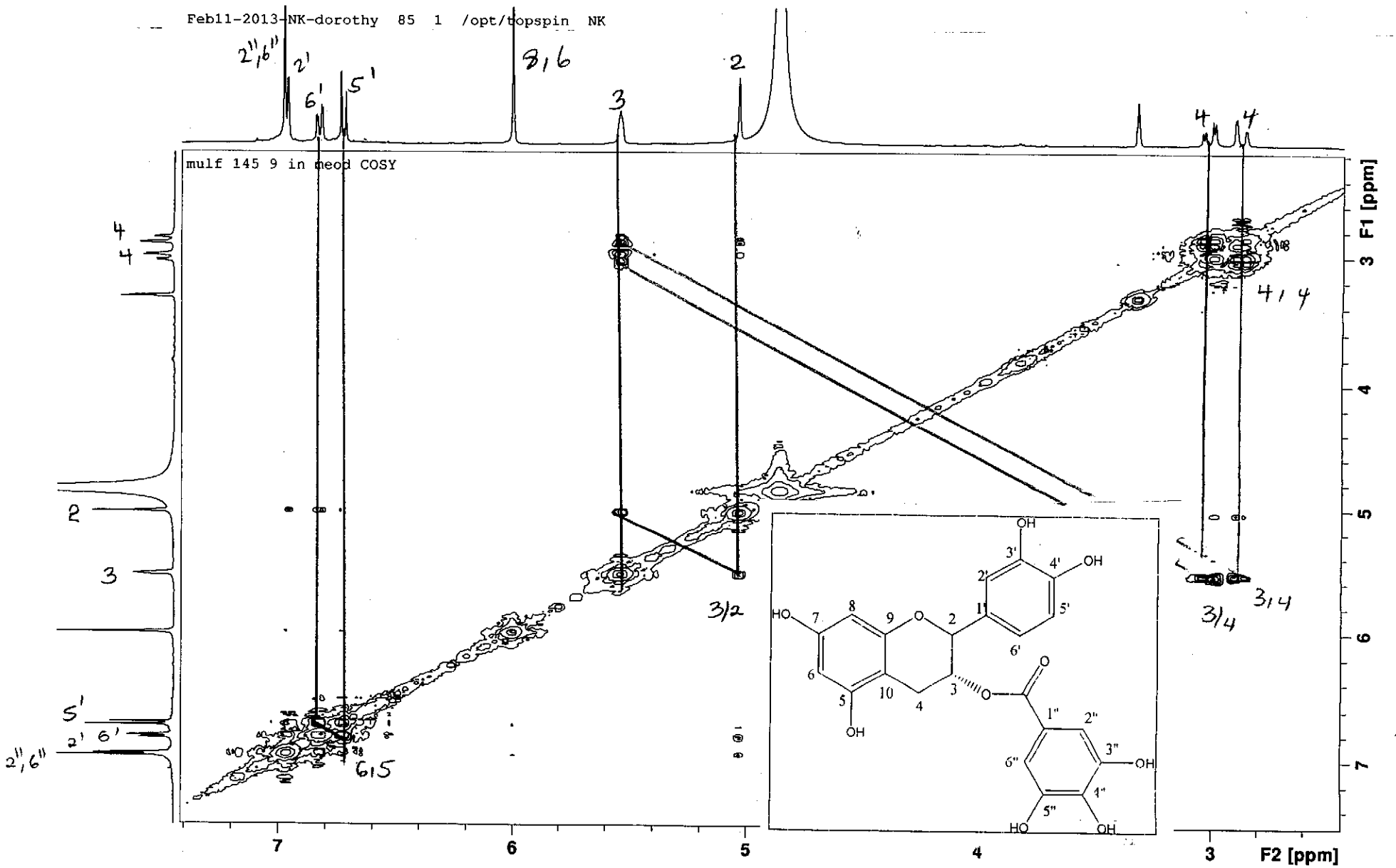
^{1}H NMR spectrum of B12



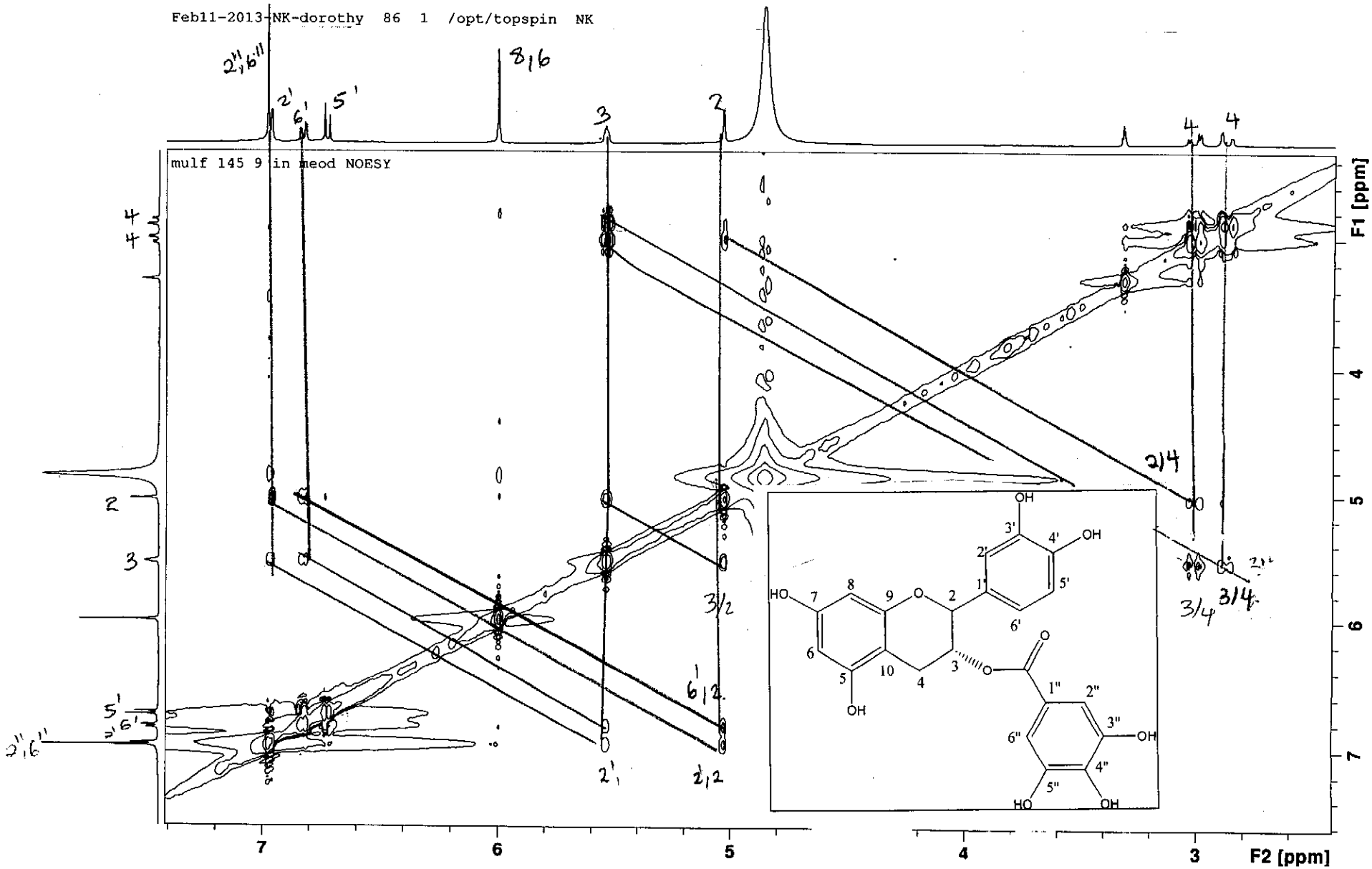
^{13}C NMR spectrum of B12



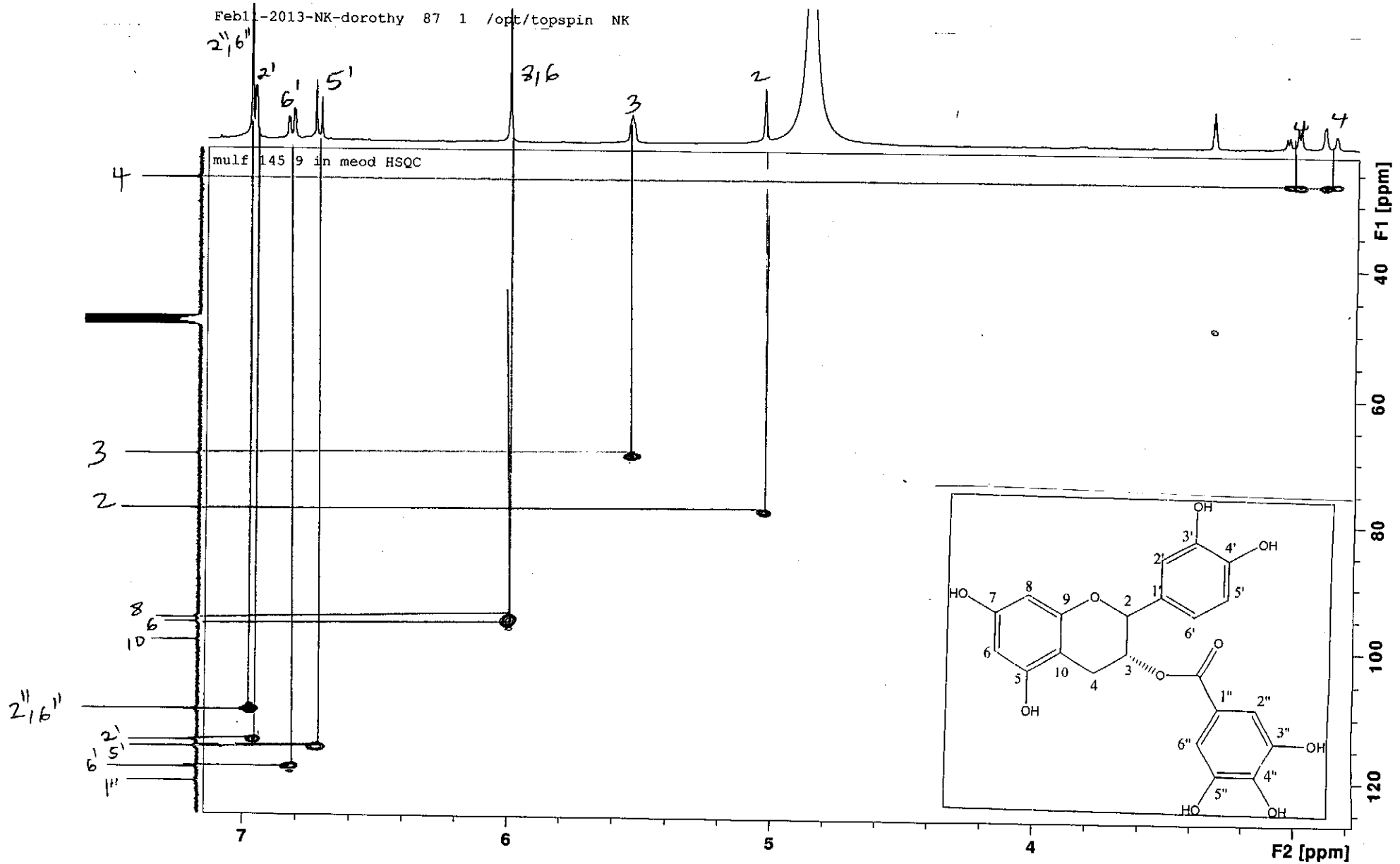
DEPT spectrum of B12



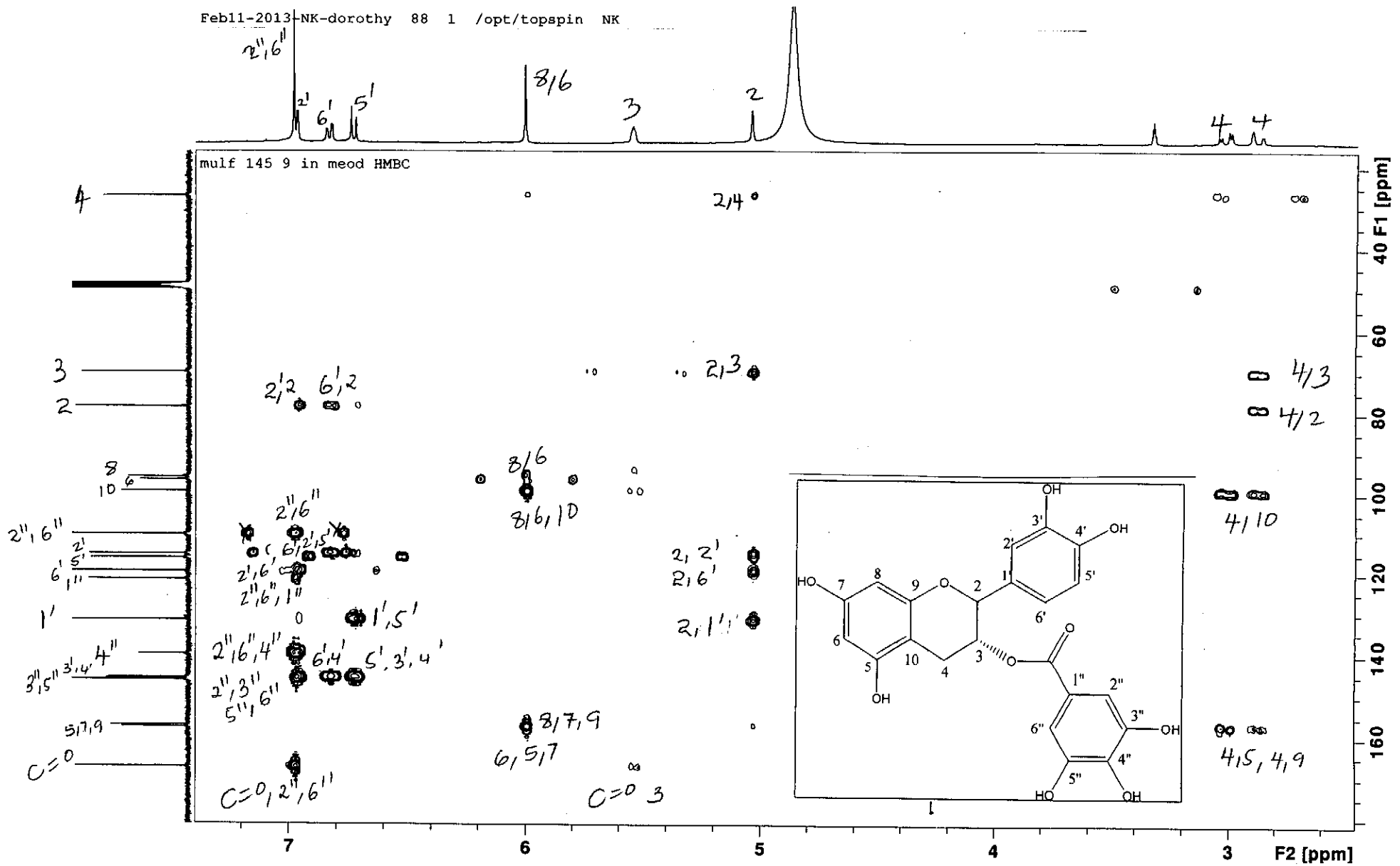
COSY spectrum of B12



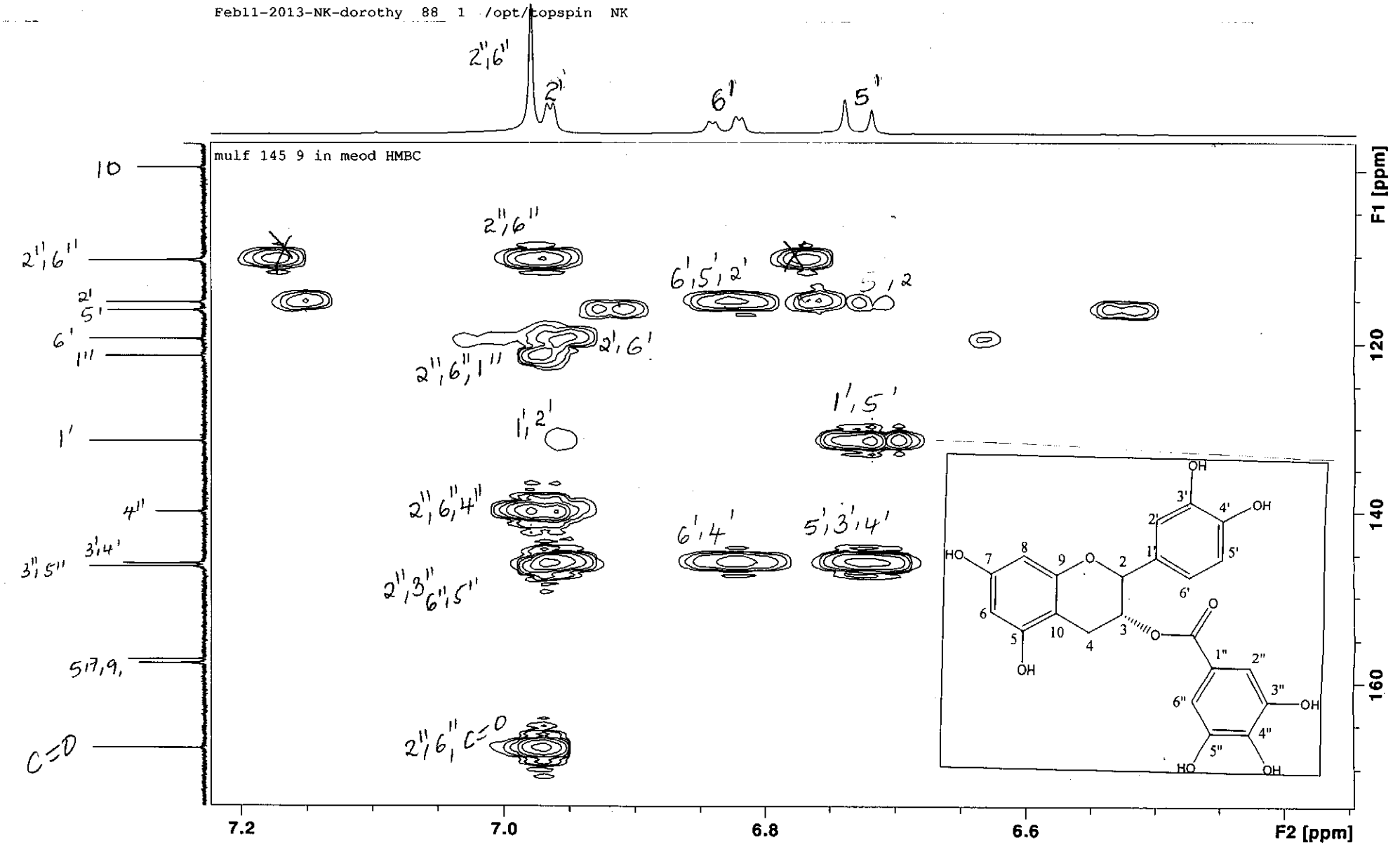
NOESY spectrum of B12



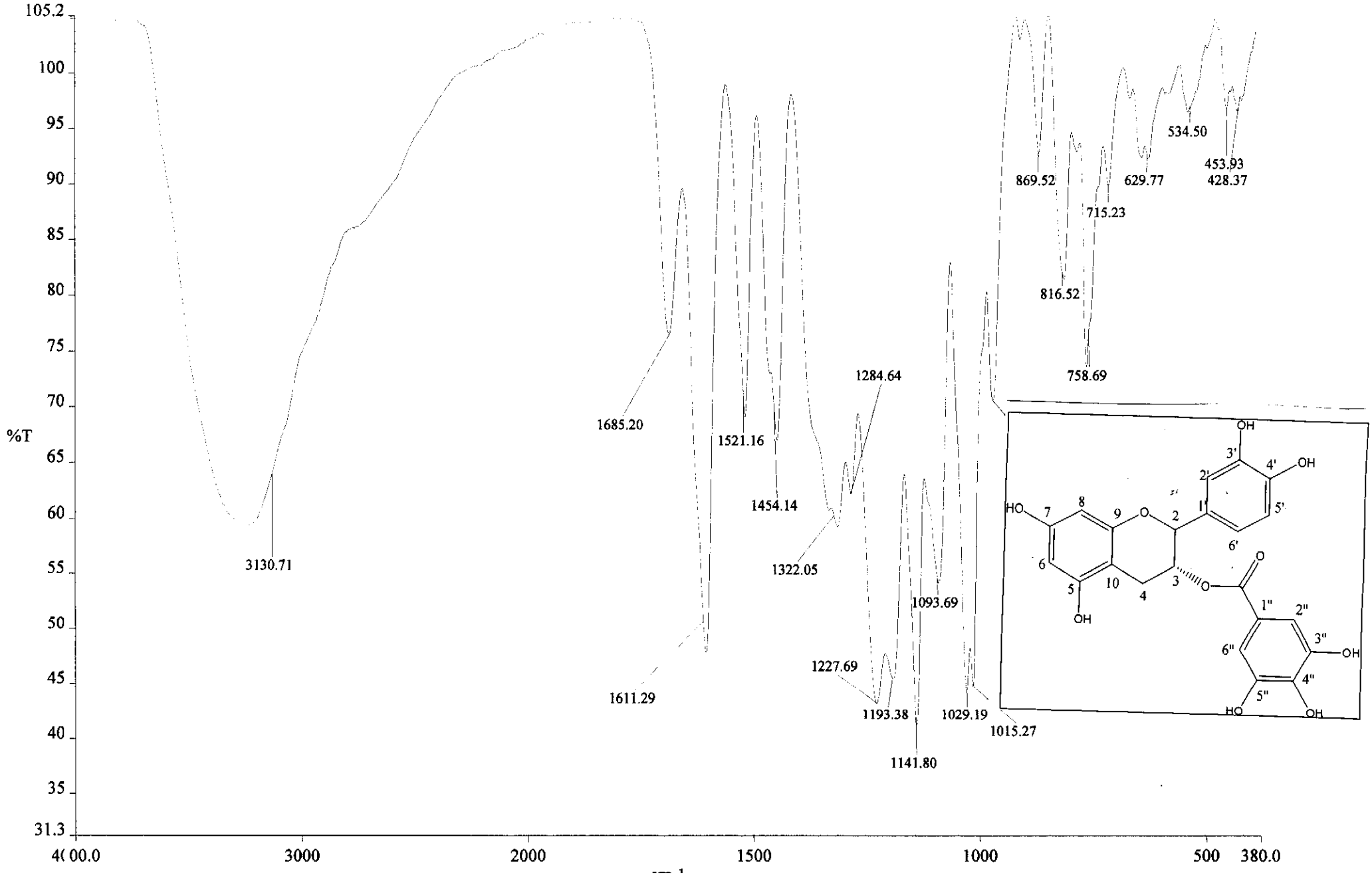
HSQC spectrum of B12



HMBC spectrum of B12



HMBC spectrum of B12 (expanded F1 95- 175 ppm , F2 6.4 -7.2 ppm)

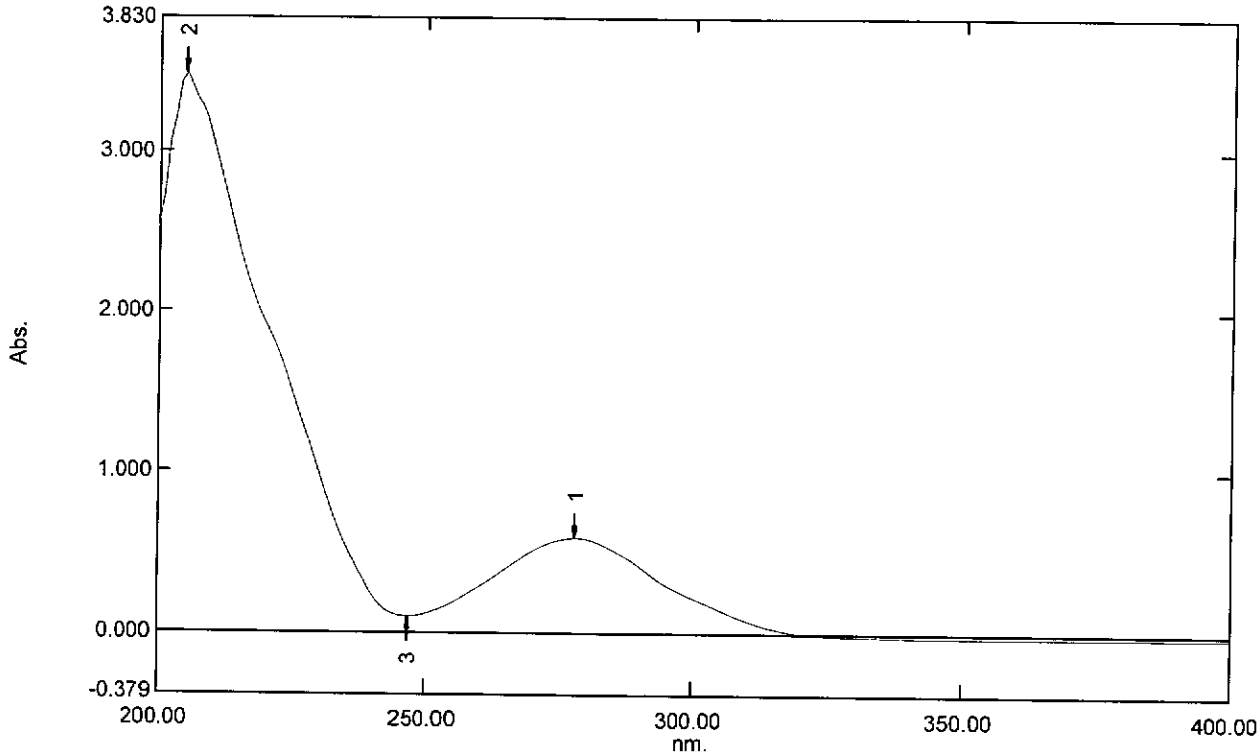


IR spectrum of B12

Spectrum Peak Pick Report

14/02/2013 04:10:42 PM

Data Set: EPICATECHIN GALLATE.spc - Storage 160621



No.	P/V	Wavelength	Abs.	Description
1		278.00	0.591	
2		205.00	3.480	
3		247.00	0.096	

Measurement Properties

Wavelength Range (nm.):	200.00 to 400.00
Scan Speed:	Medium
Sampling Interval:	1.0
Auto Sampling Interval:	Disabled
Scan Mode:	Single

Instrument Properties

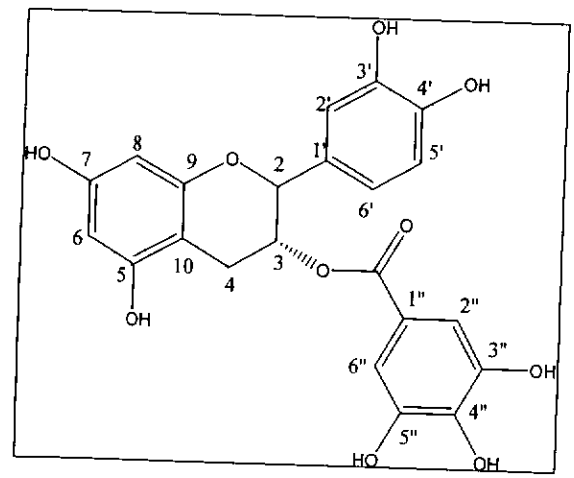
Instrument Type:	UV-3600 Series
Measuring Mode:	Absorbance
Slit Width:	1.0 nm
Time Constant:	0.1 sec.
Source Lamp:	Auto
Light Source Change Wavelength:	310.00 nm
Detector Unit:	Direct
Detector Change Wavelength:	830.00 nm 1800.00 nm
Grating Change Wavelength:	720.00 nm
S/R Exchange:	Normal
Detector Lock:	Auto
Slit Program:	Normal
Beam Mode:	Double
Stair Correction:	Disable

Attachment Properties

Attachment:	None

Sample Preparation Properties

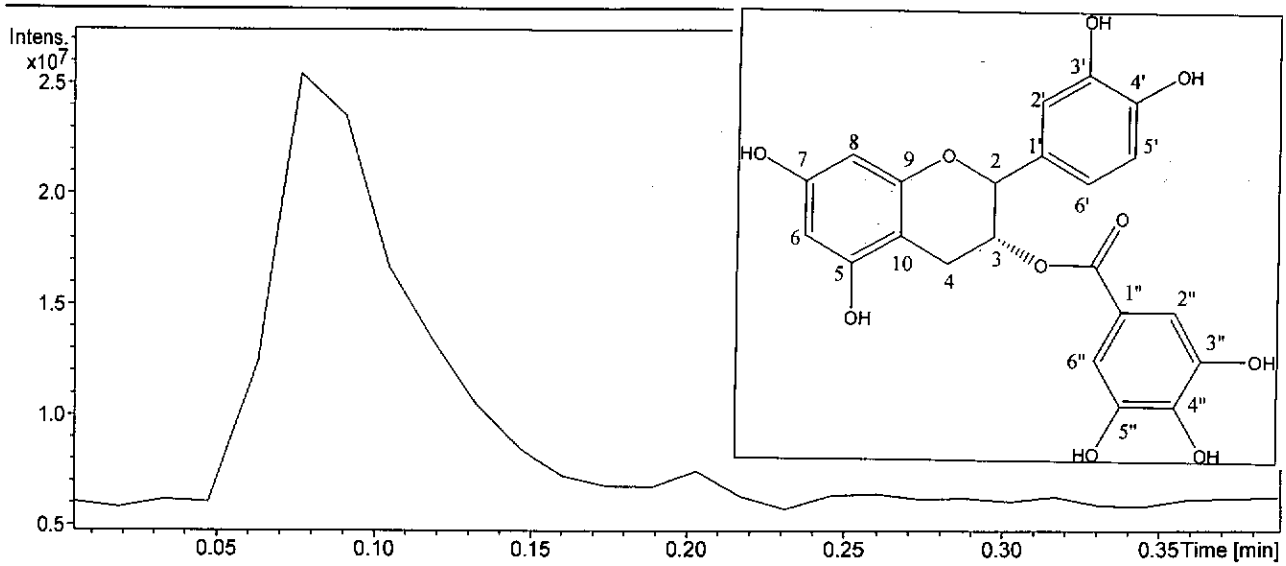
Weight:	
Volume:	
Dilution:	
Path Length:	1 cm
Additional Information:	

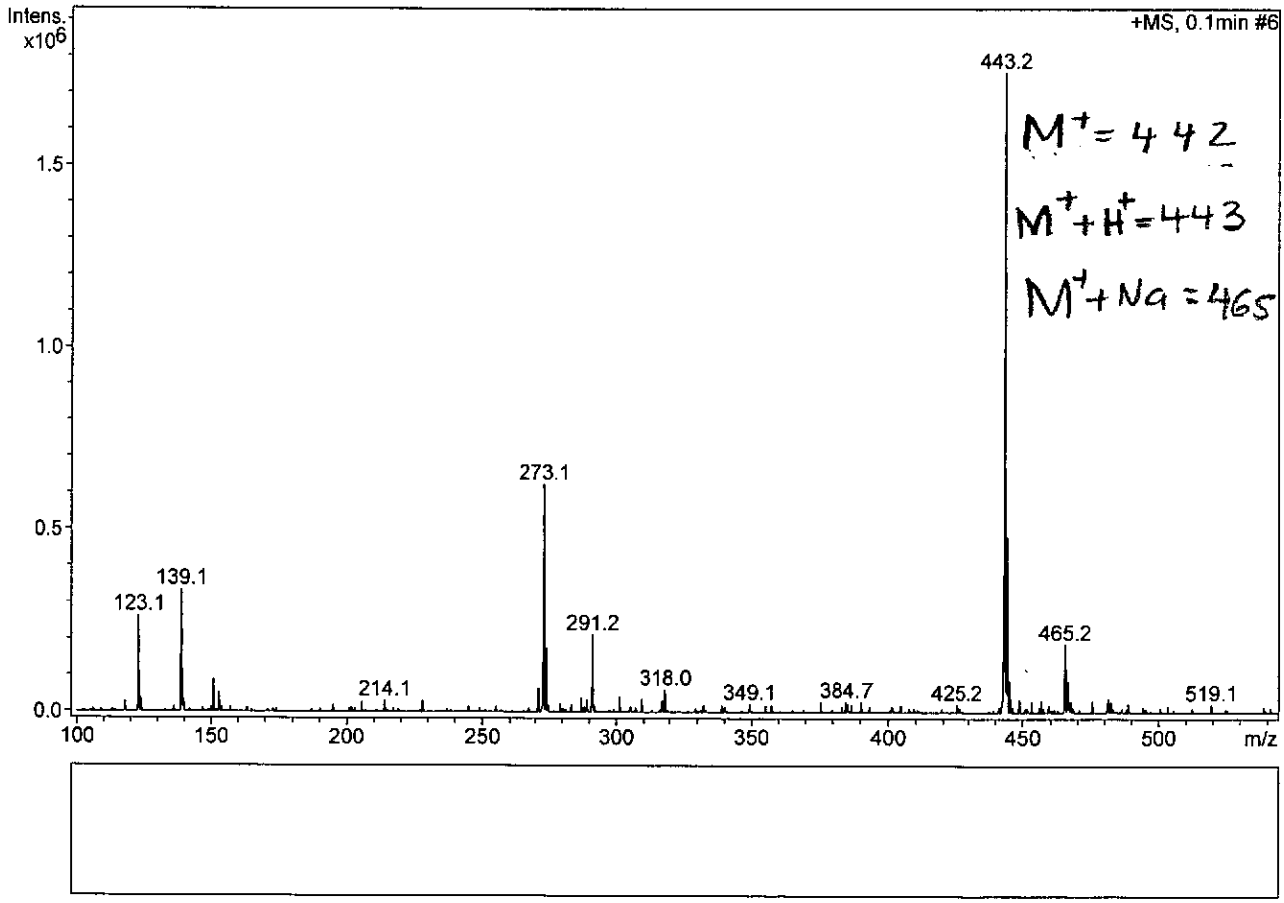


UV spectrum of B12

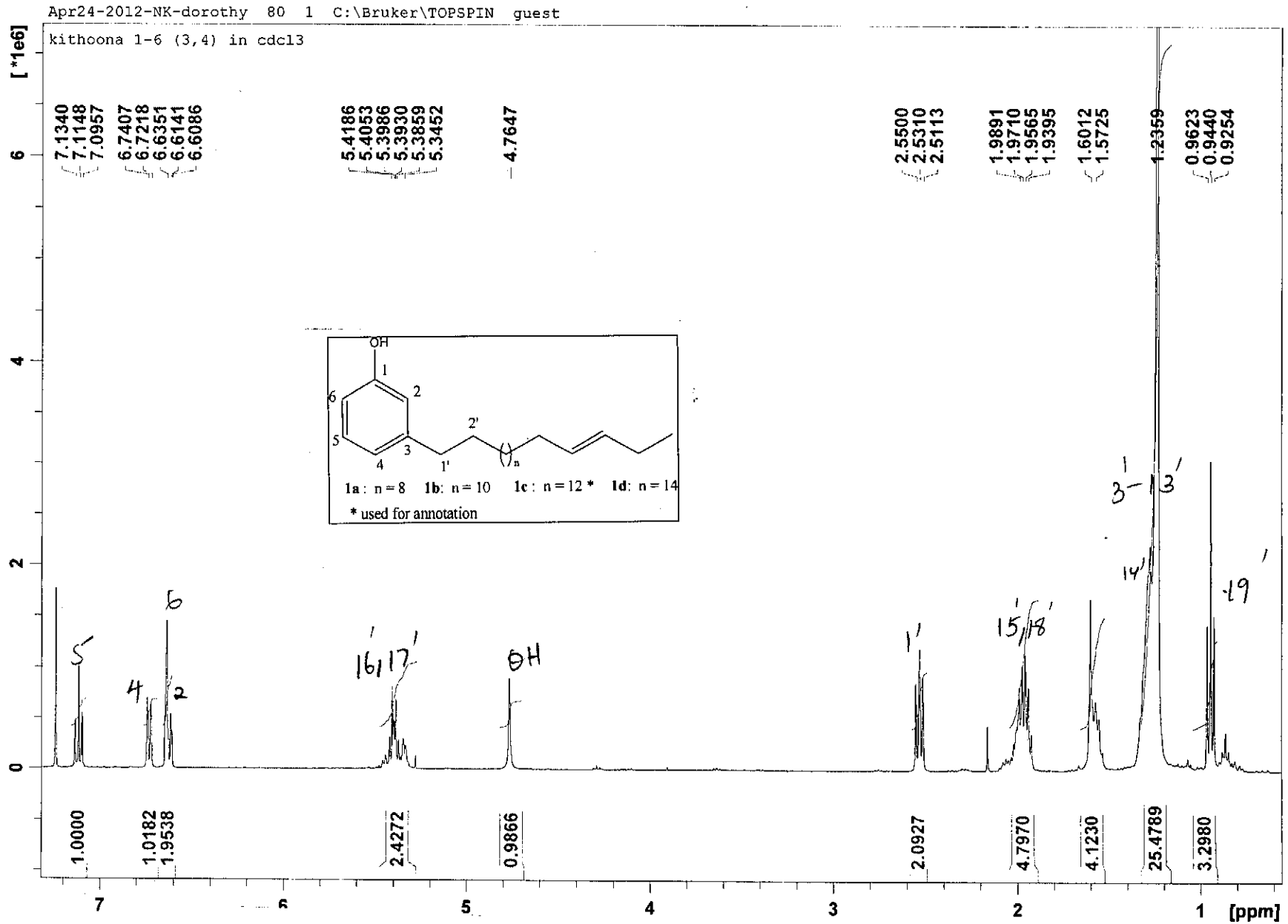
Display Report - All Windows Selected Analysis

Analysis Name: EPPICATECHIN
Method: AN 2MIN.M
Sample Name: Default
Analysis Info:
Instrument: LC-MSD-Trap-VL
Operator: Operator
Print Date: 2/18/2013 11:24:09 AM
Acq. Date: 2/18/2013 11:23:35 AM

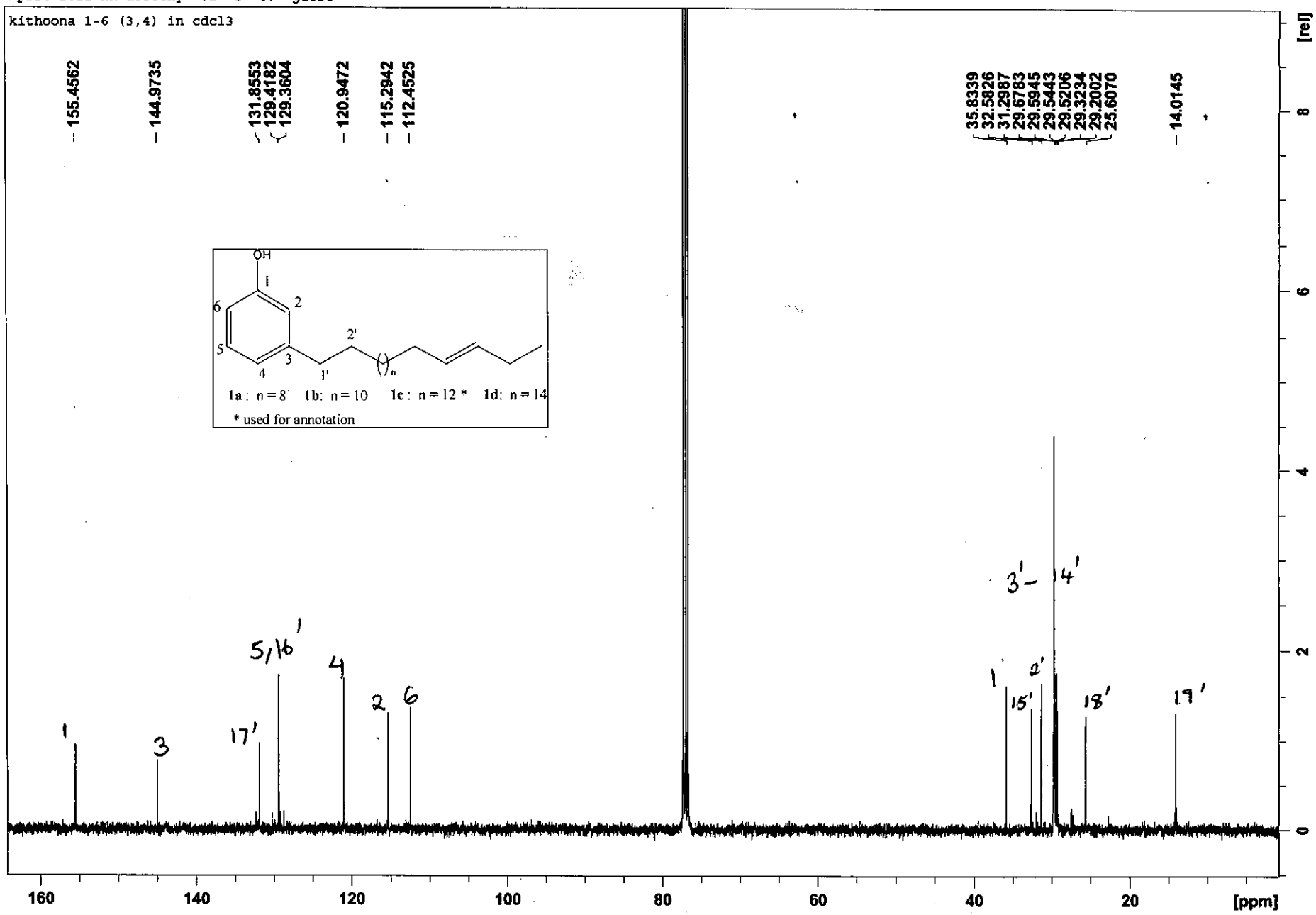




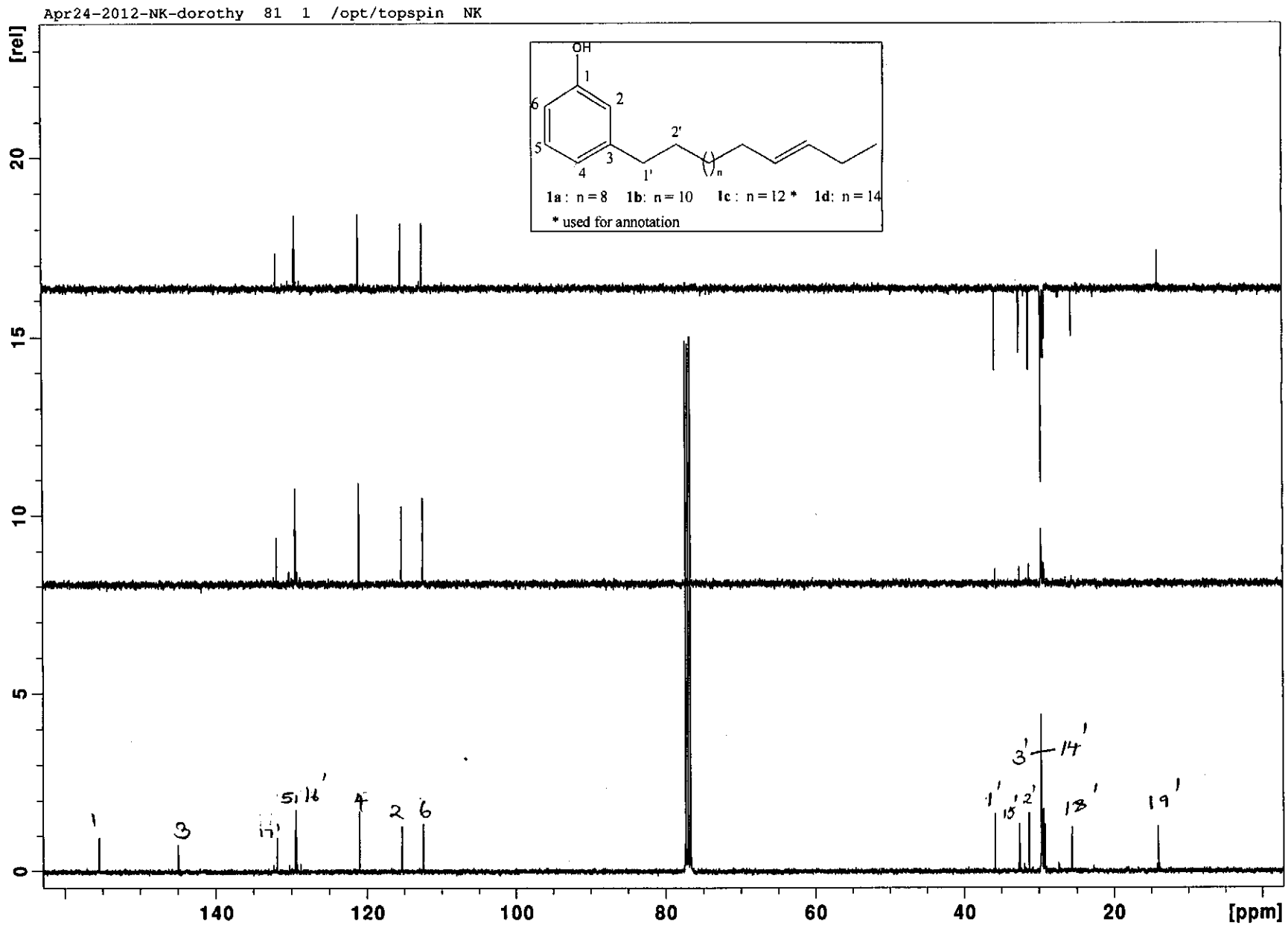
LC-MS spectrum of B12



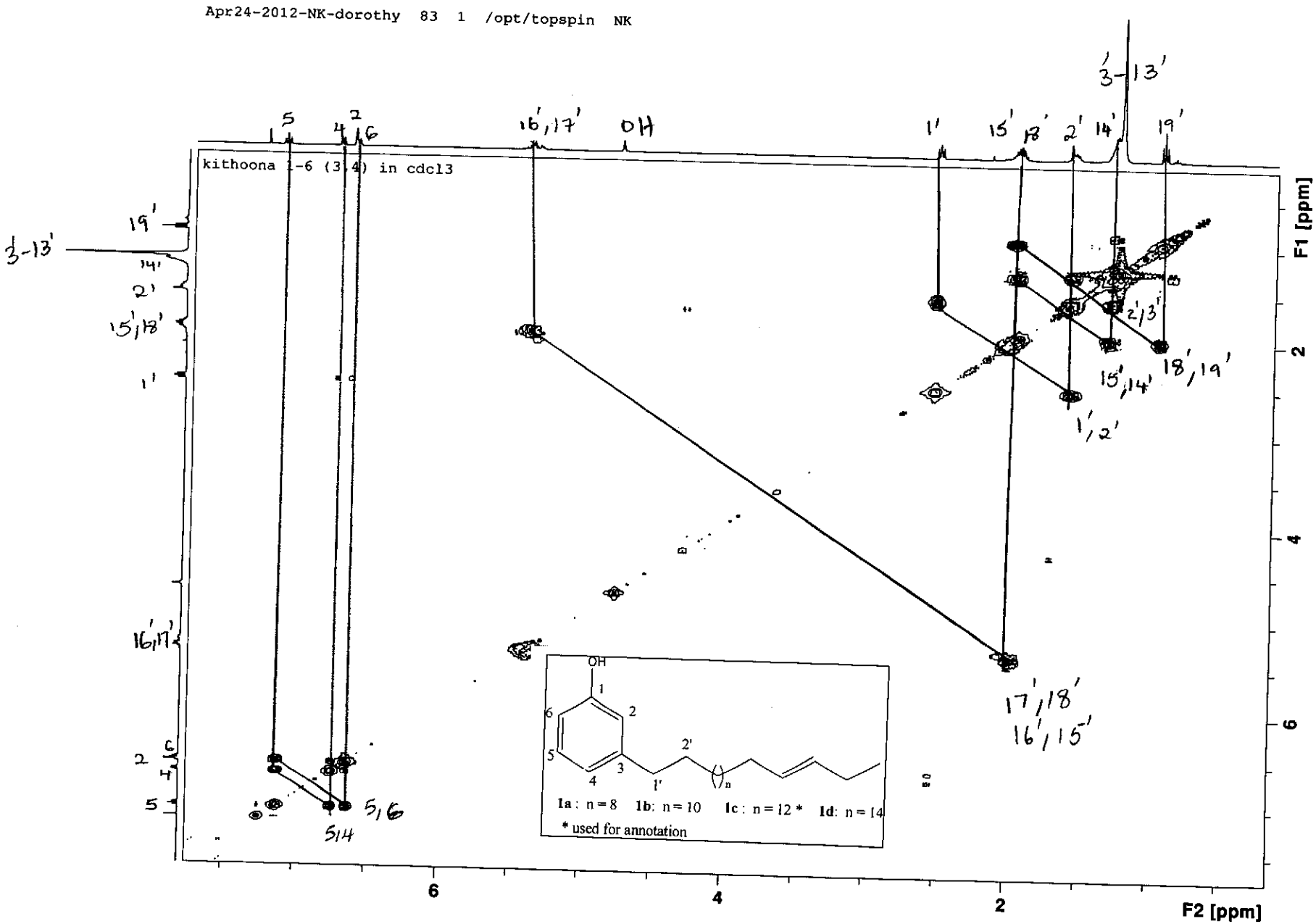
^{1}H NMR spectrum of C-1 (a-d)



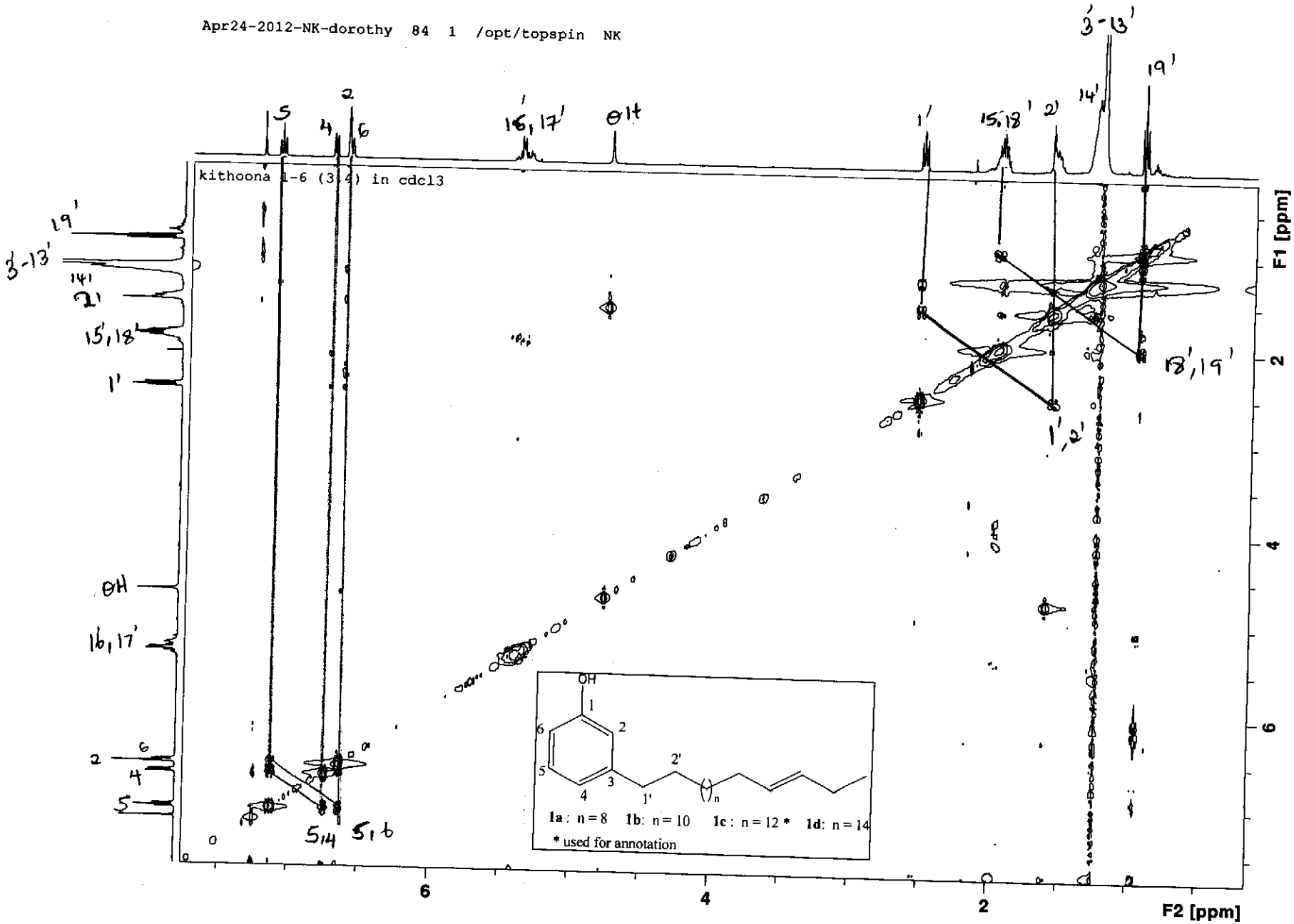
^{13}C NMR spectrum of C-1 (a-d)



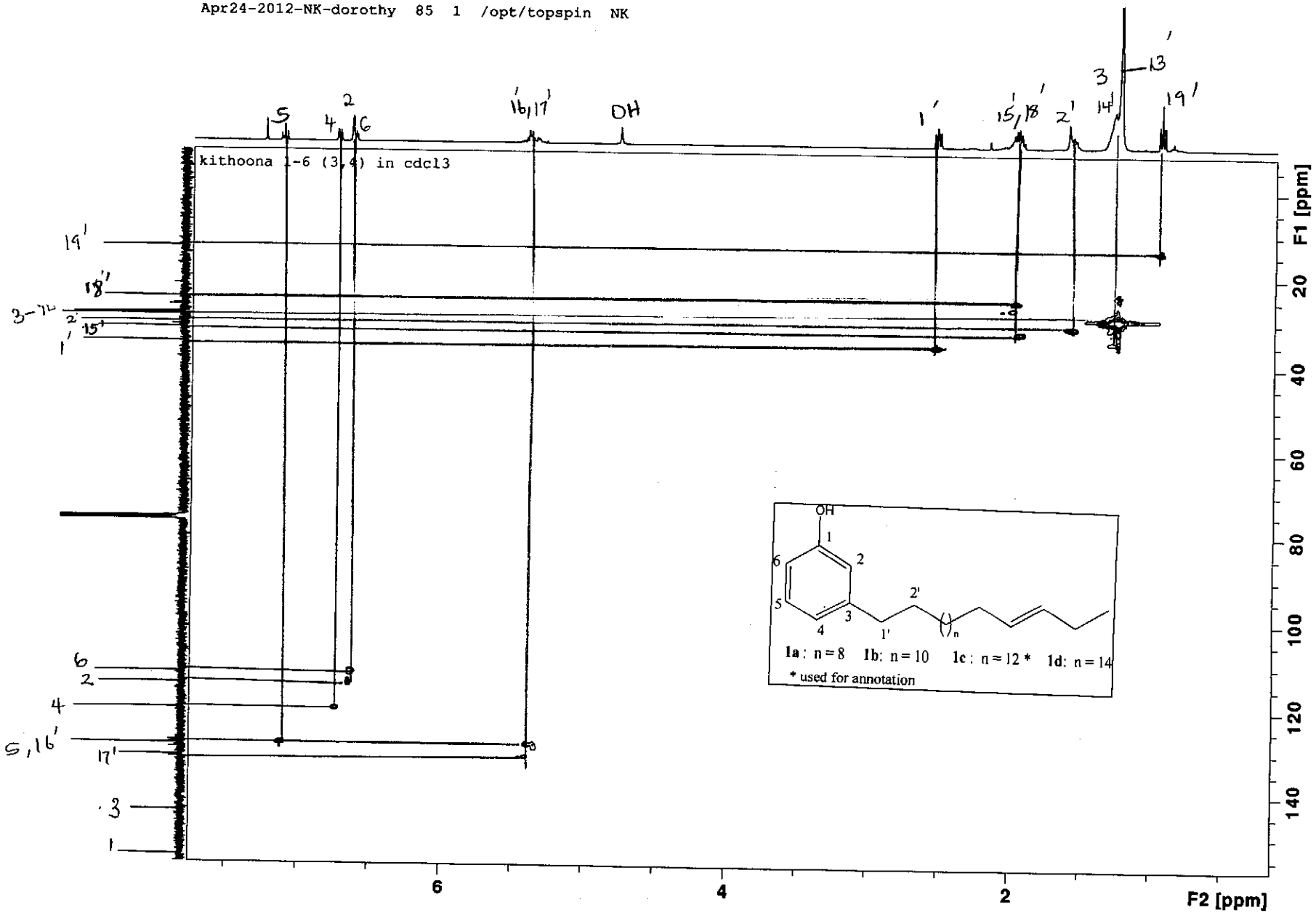
DEPT spectrum of C-1 (a-d)



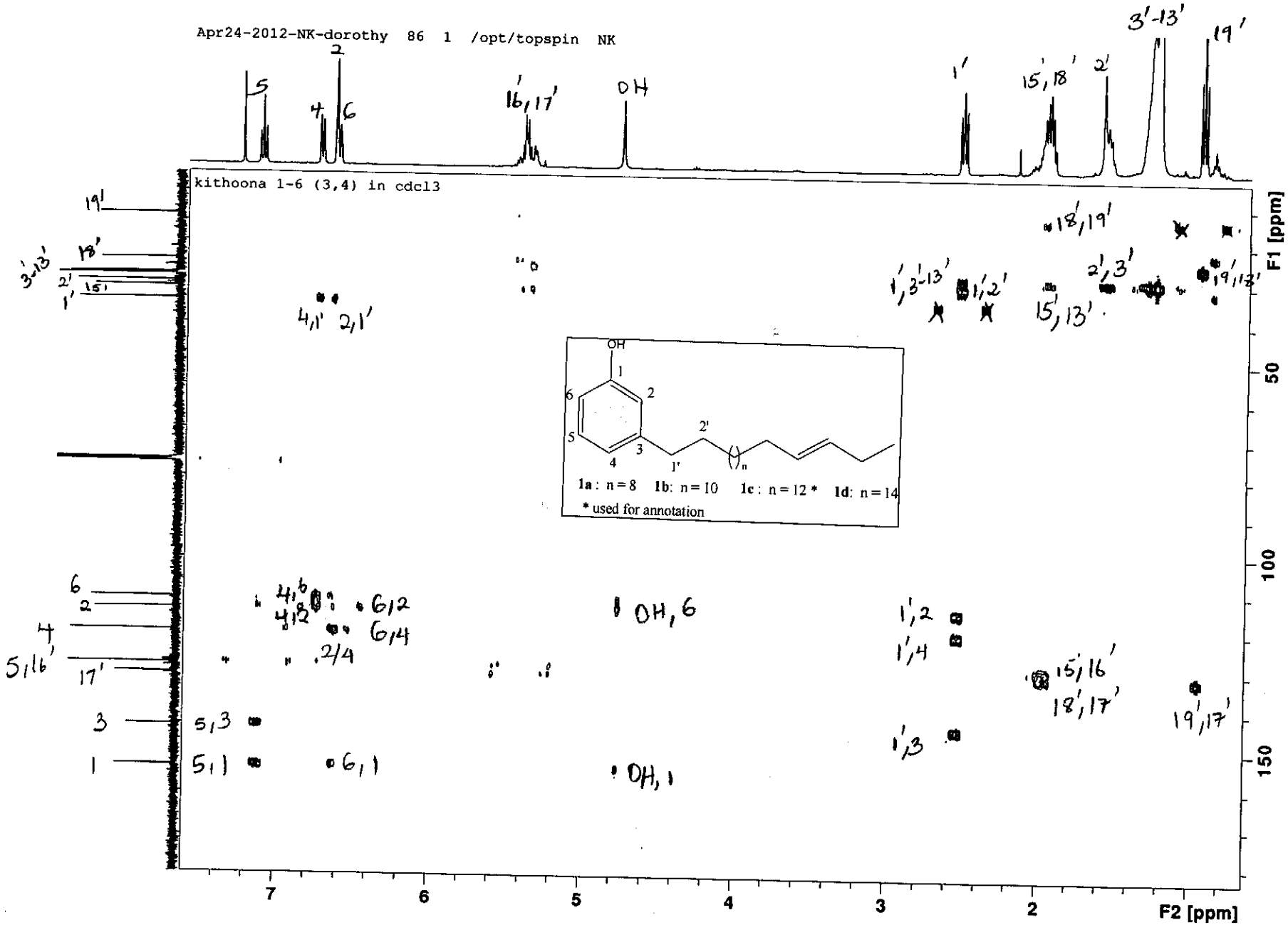
COSY spectrum of C-1 (a-d)



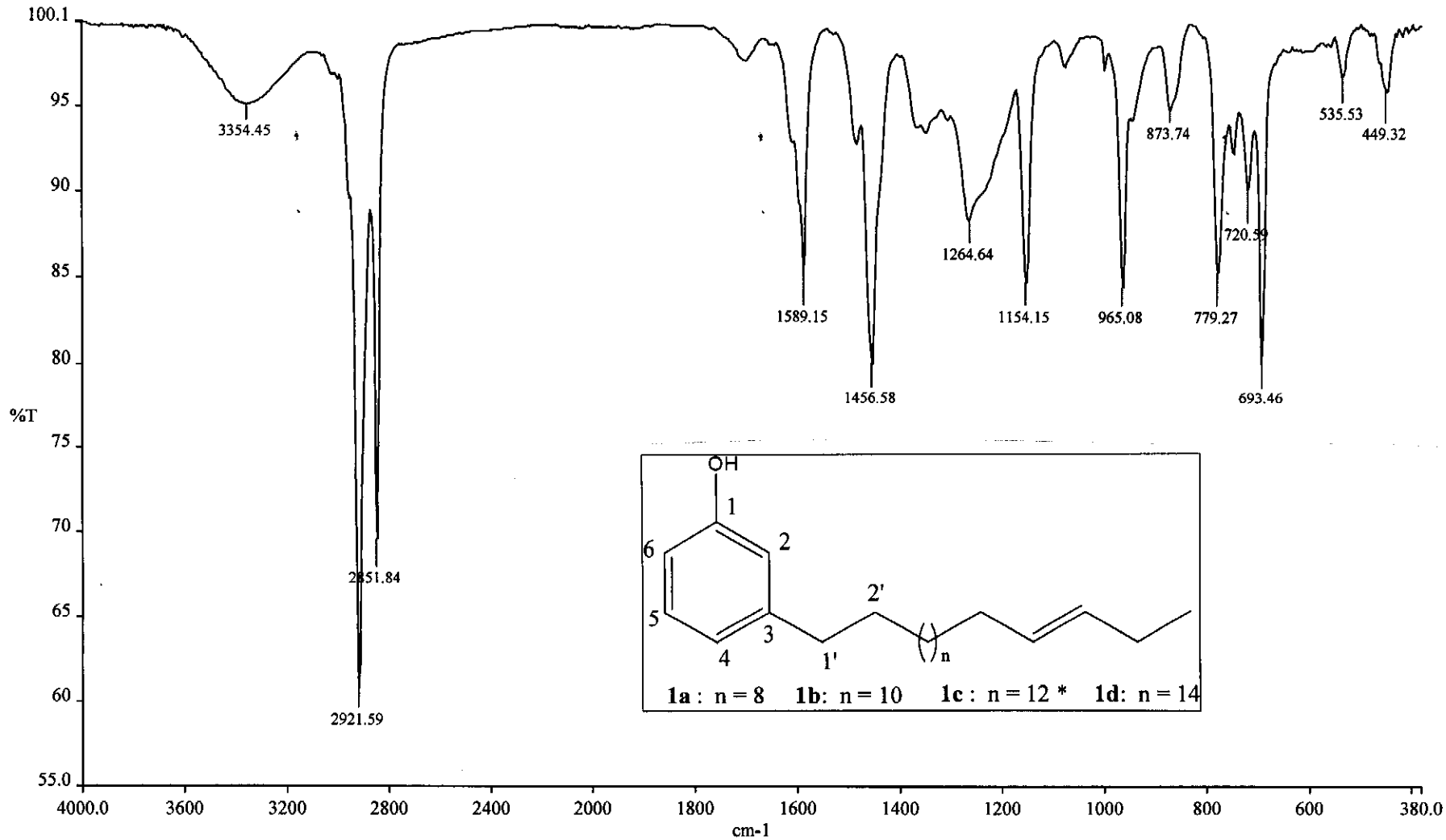
NOESY spectrum of C-1 (a-d)



HSQC spectrum of C-1 (a-d)



HMBC spectrum of C-1 (a-d)

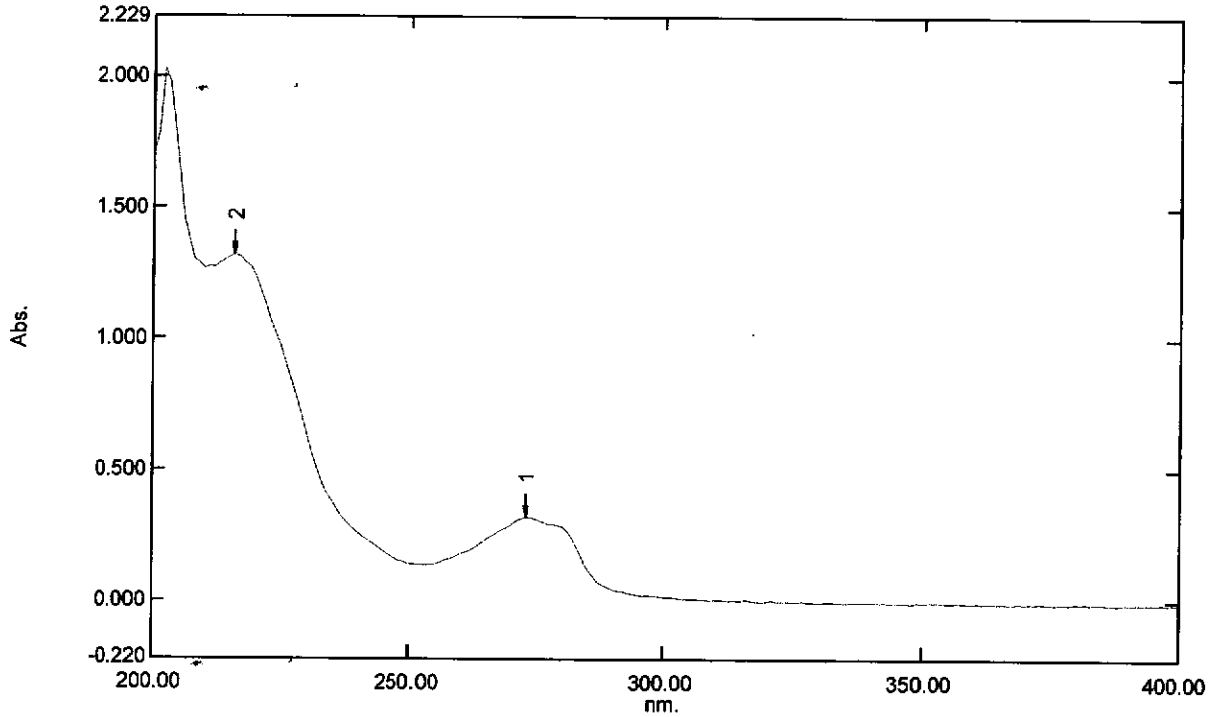


IR spectrum of C-1 (a-d)

Spectrum Peak Pick Report

23/05/2012 06:16:05 PM

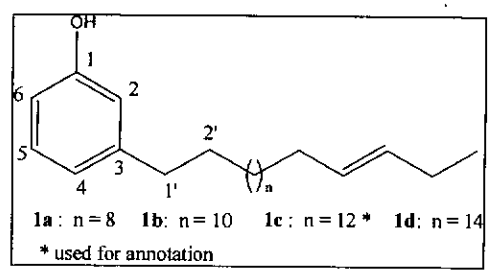
Data Set: kitheona1-6 (3,4) a.spc - Storage 171123



No.	P/V	Wavelength	Abs.	Description
1		273.00	0.317	
2		216.00	1.315	

Measurement Properties
Wavelength Range (nm.): 200.00 to 400.00
Scan Speed: Medium
Sampling Interval: 1.0
Auto Sampling Interval: Disabled
Scan Mode: Single

Instrument Properties
Instrument Type: UV-3600 Series
Measuring Mode: Absorbance
Slit Width: 2.0 nm
Time Constant: 0.1 sec.
Source Lamp: Auto
Light Source Change Wavelength: 310.00 nm
Detector Unit: Direct
Detector Change Wavelength: 830.00 nm 1800.00 nm
Grating Change Wavelength: 720.00 nm
S/R Exchange: Normal
Detector Lock: Auto
Slit Program: Normal
Beam Mode: Double
Stair Correction: Disable



1a: n = 8 **1b**: n = 10 **1c**: n = 12 * **1d**: n = 14

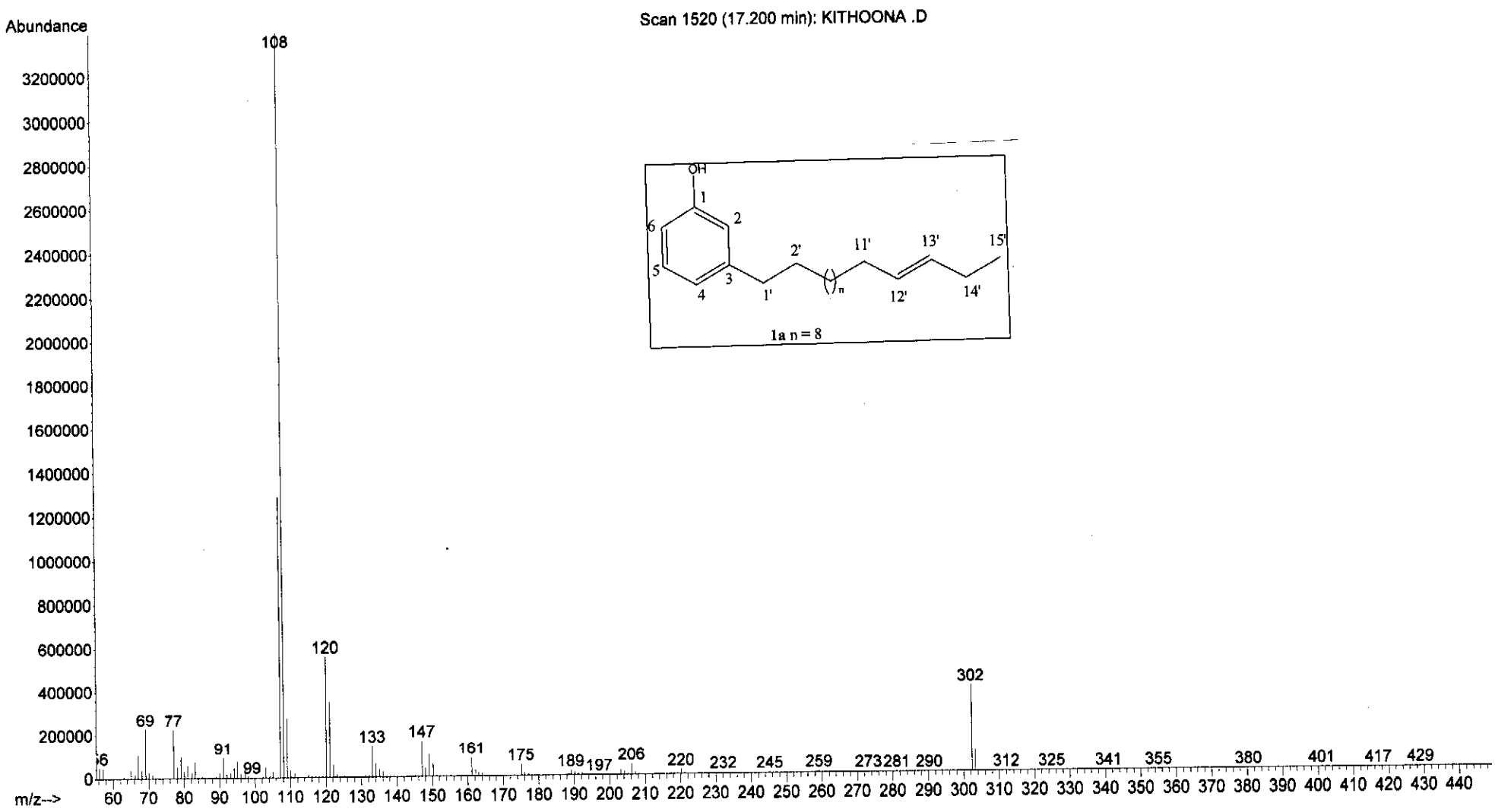
* used for annotation

Attachment Properties
Attachment: None

Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

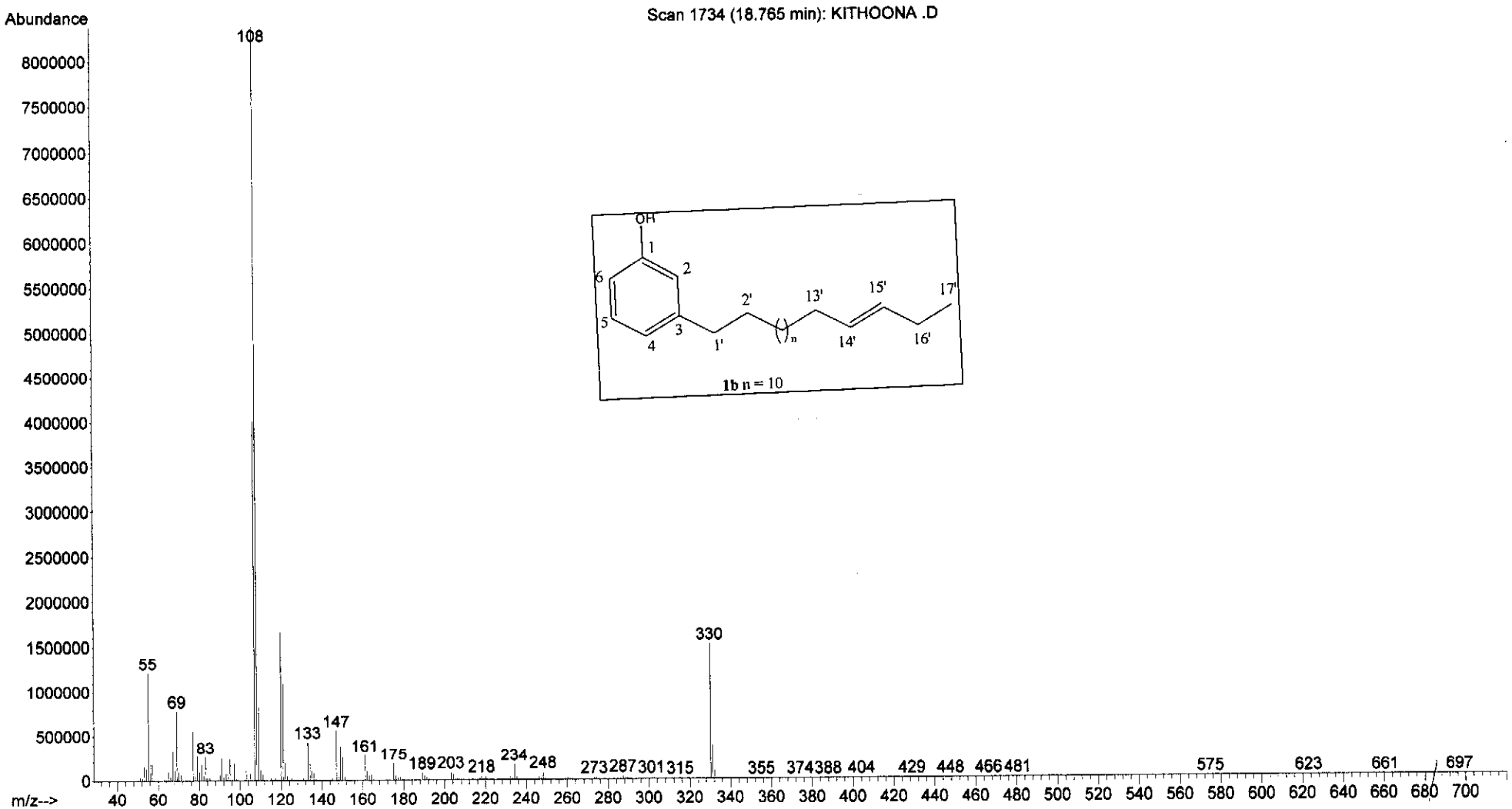
UV spectrum of C-1 (a-d)

File : C:\MSDCHEM\1\DATA\DOROTHY RAW DATA KITHOKIT\KITHOONA .D
Operator : Dorothy
Acquired : 5 May 2012 18:53 using AcqMethod NATPRODUCTS MANUAL
Instrument : 5973N
Sample Name: kithoona 1-6 (3,4)
Misc Info :
Vial Number: 1



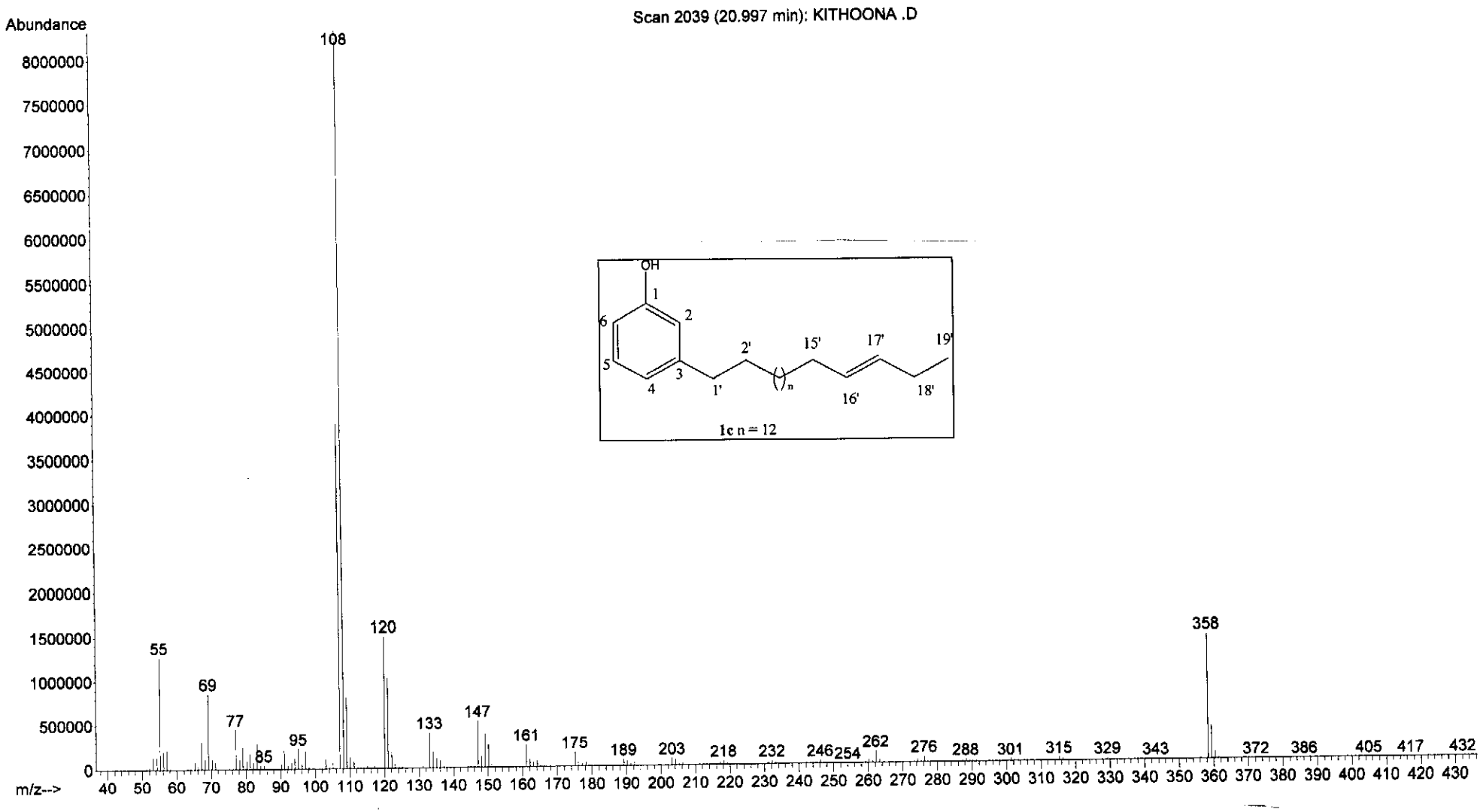
MS spectrum of C-1 a

File : C:\MSDCHEM\1\DATA\DOROTHY RAW DATA KITHOKIT\KITHOONA .D
Operator : Dorothy
Acquired : 5 May 2012 18:53 using AcqMethod NATPRODUCTS MANUAL
Instrument : 5973N
Sample Name: kithoona 1-6 (3,4)
Misc Info :
Vial Number: 1



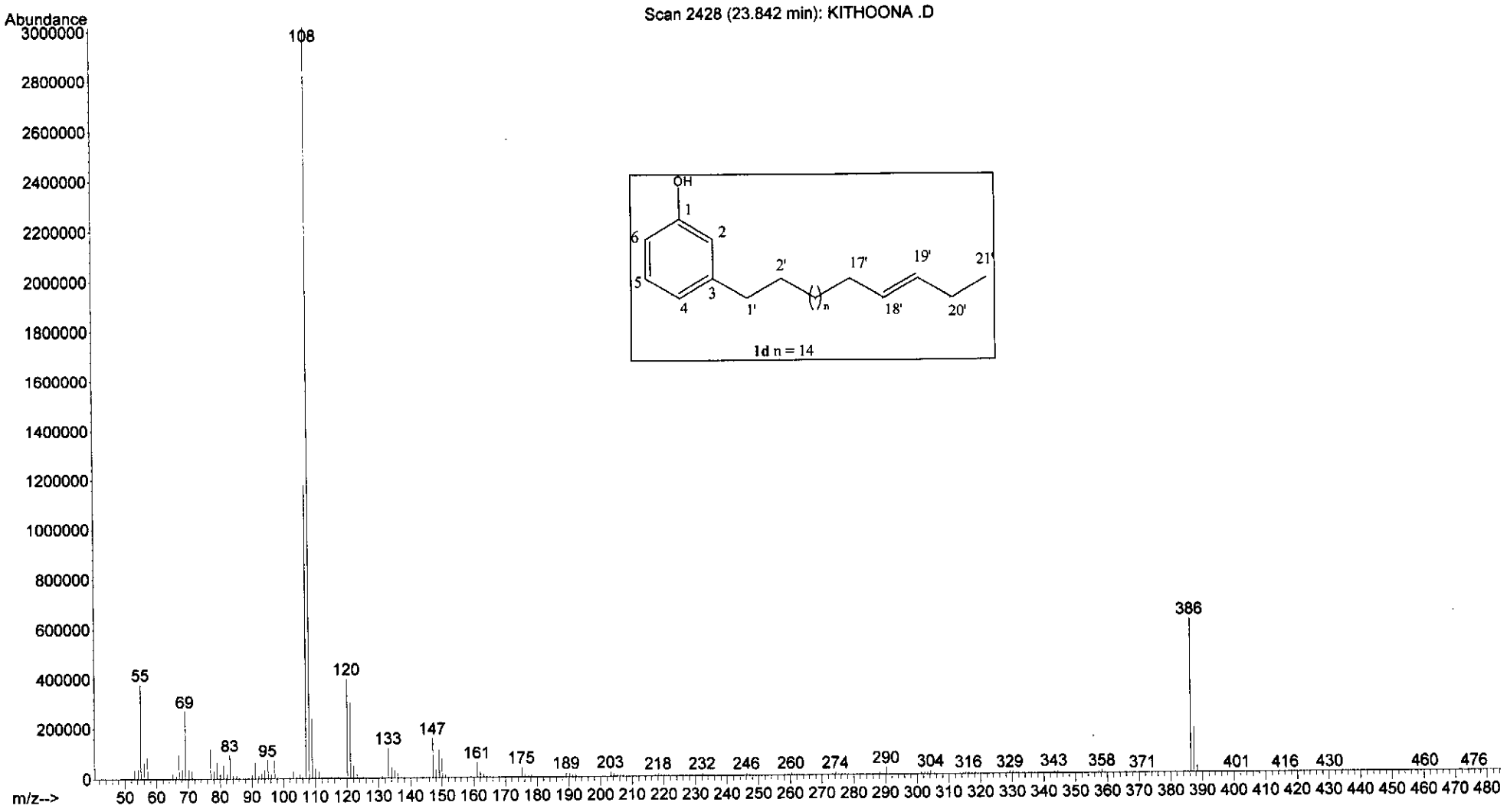
MS spectrum of C-1 b

File : C:\MSDCHEM\1\DATA\DOROTHY RAW DATA KITHOKIT\KITHOONA .D
Operator : Dorothy
Acquired : 5 May 2012 18:53 using AcqMethod NATPRODUCTS MANUAL
Instrument : 5973N
Sample Name: kithoona 1-6 (3,4)
Misc Info :
Vial Number: 1

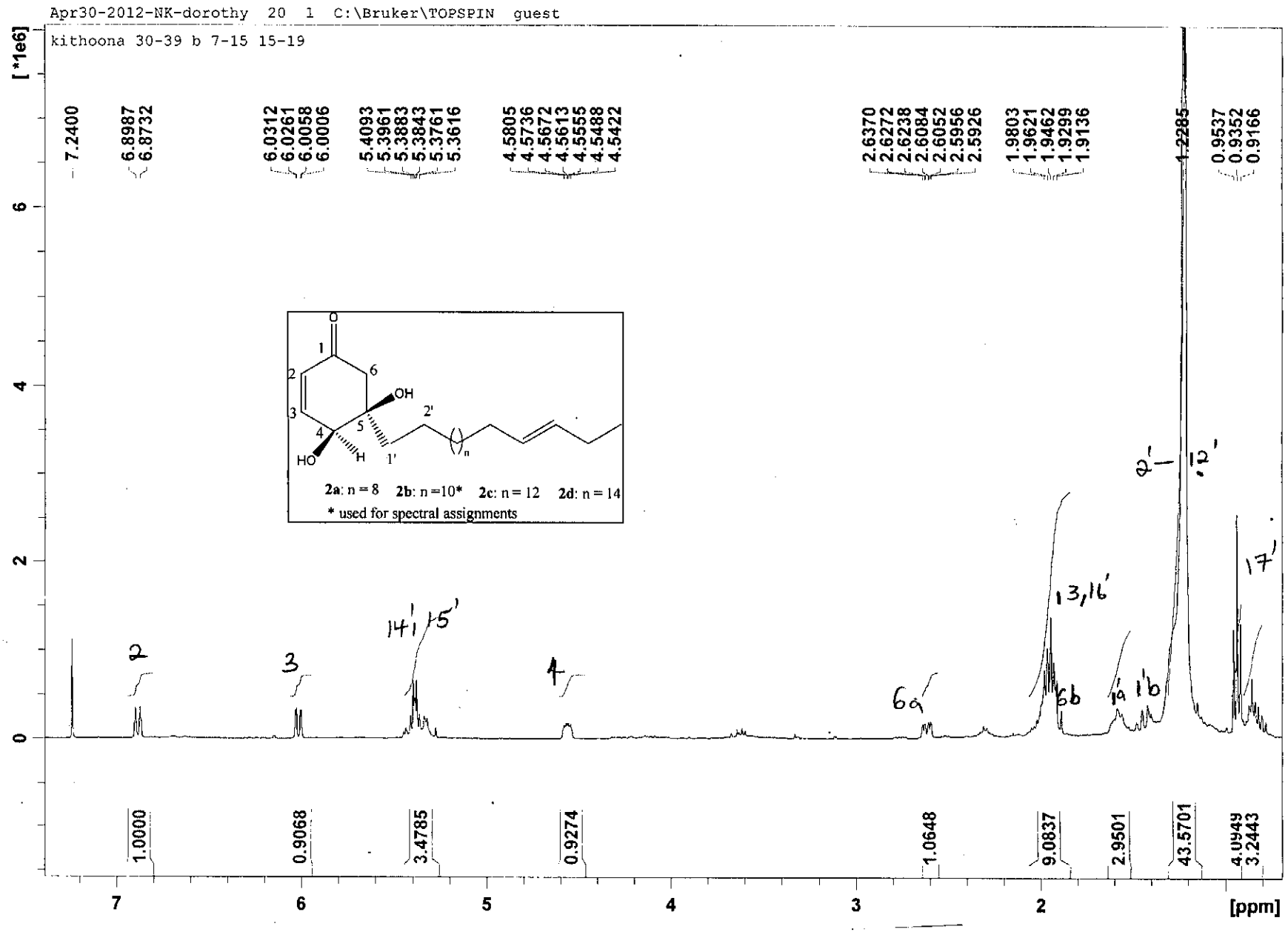


MS spectrum of C-1 c

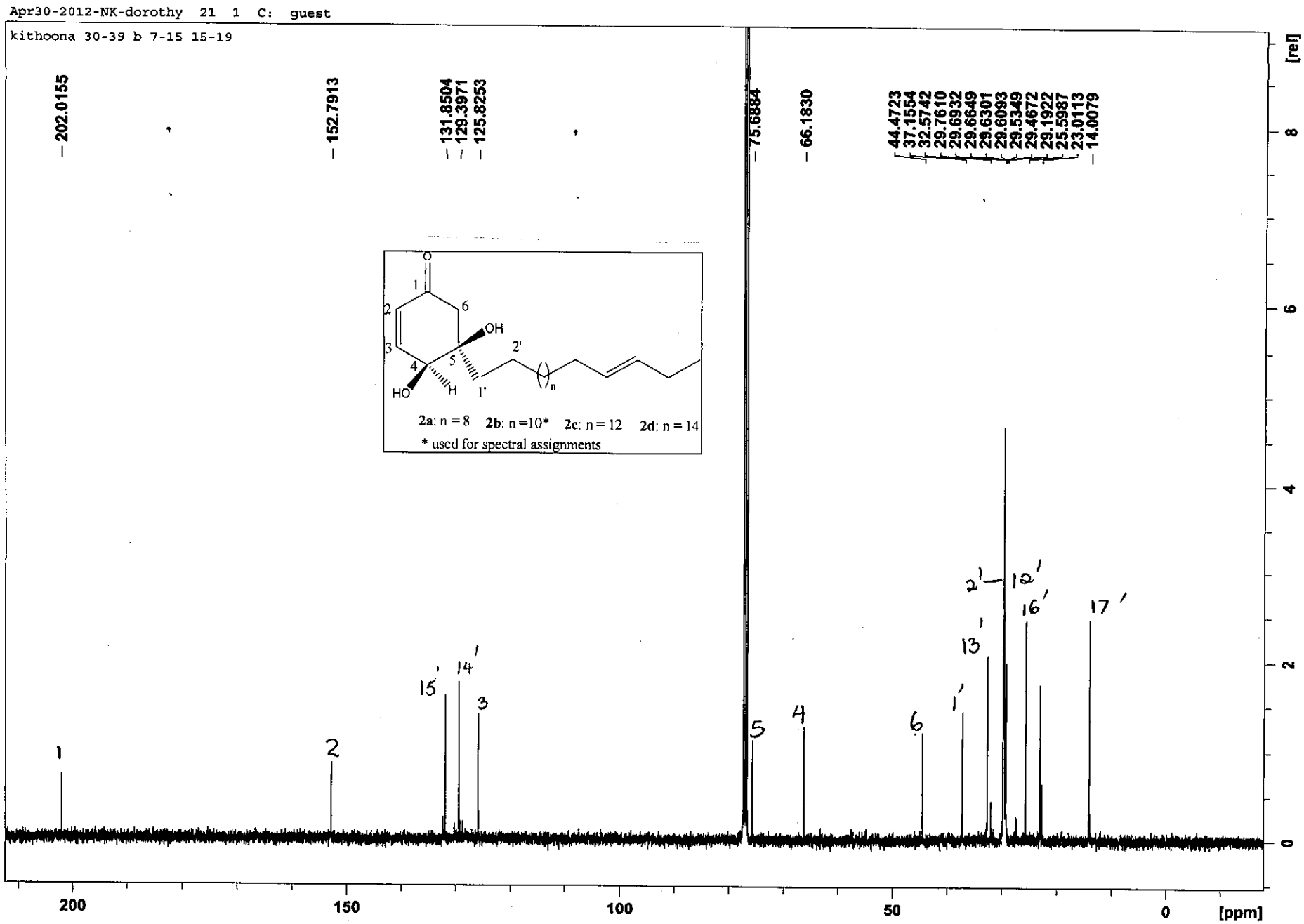
File : C:\MSDCHEM\1\DATA\DOROTHY RAW DATA KITHOKIT\KITHOONA .D
Operator : Dorothy
Acquired : 5 May 2012 18:53 using AcqMethod NATPRODUCTS MANUAL
Instrument : 5973N
Sample Name: kithoona 1-6 (3,4)
Misc Info :
Vial Number: 1



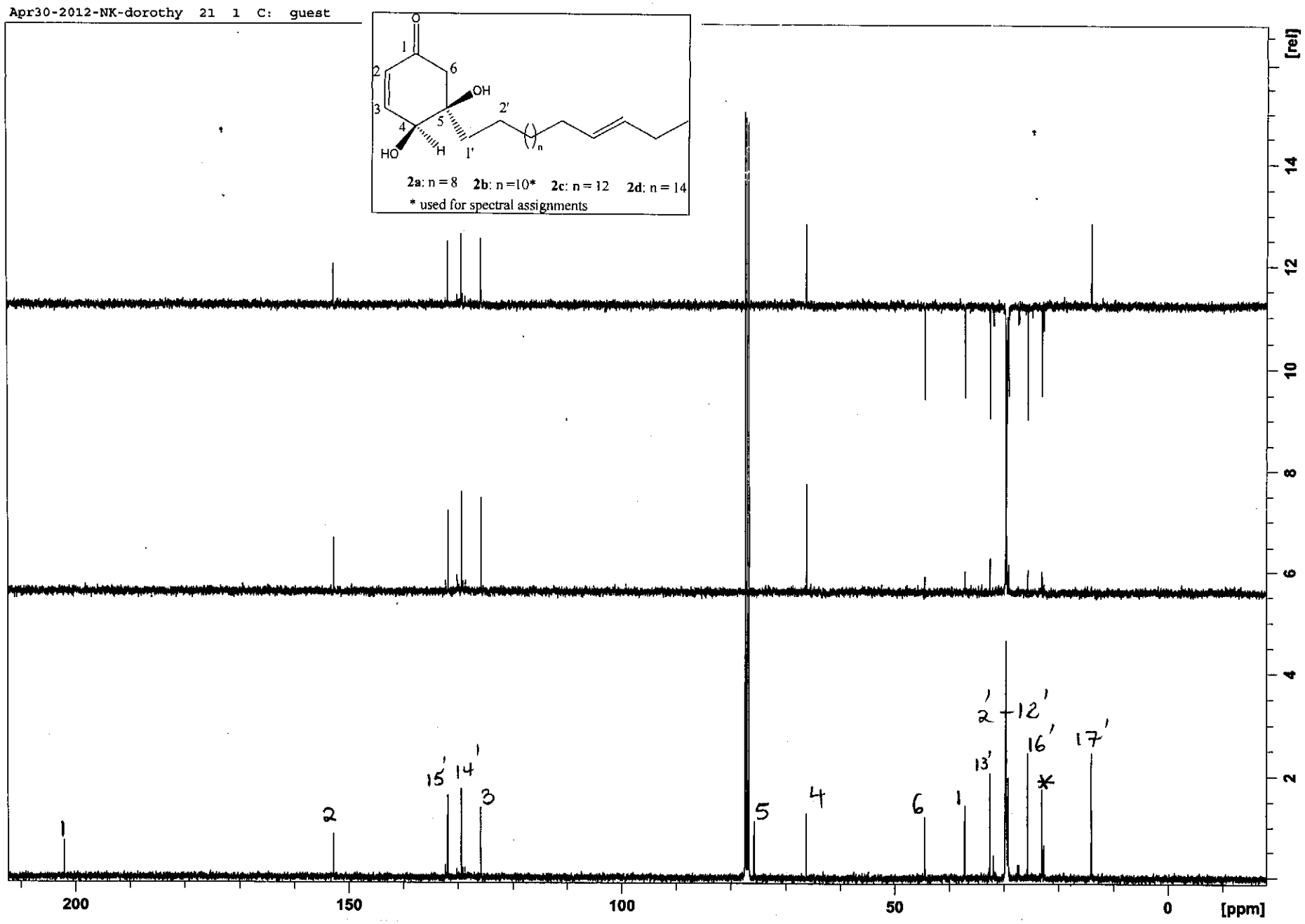
MS spectrum of C-1 d



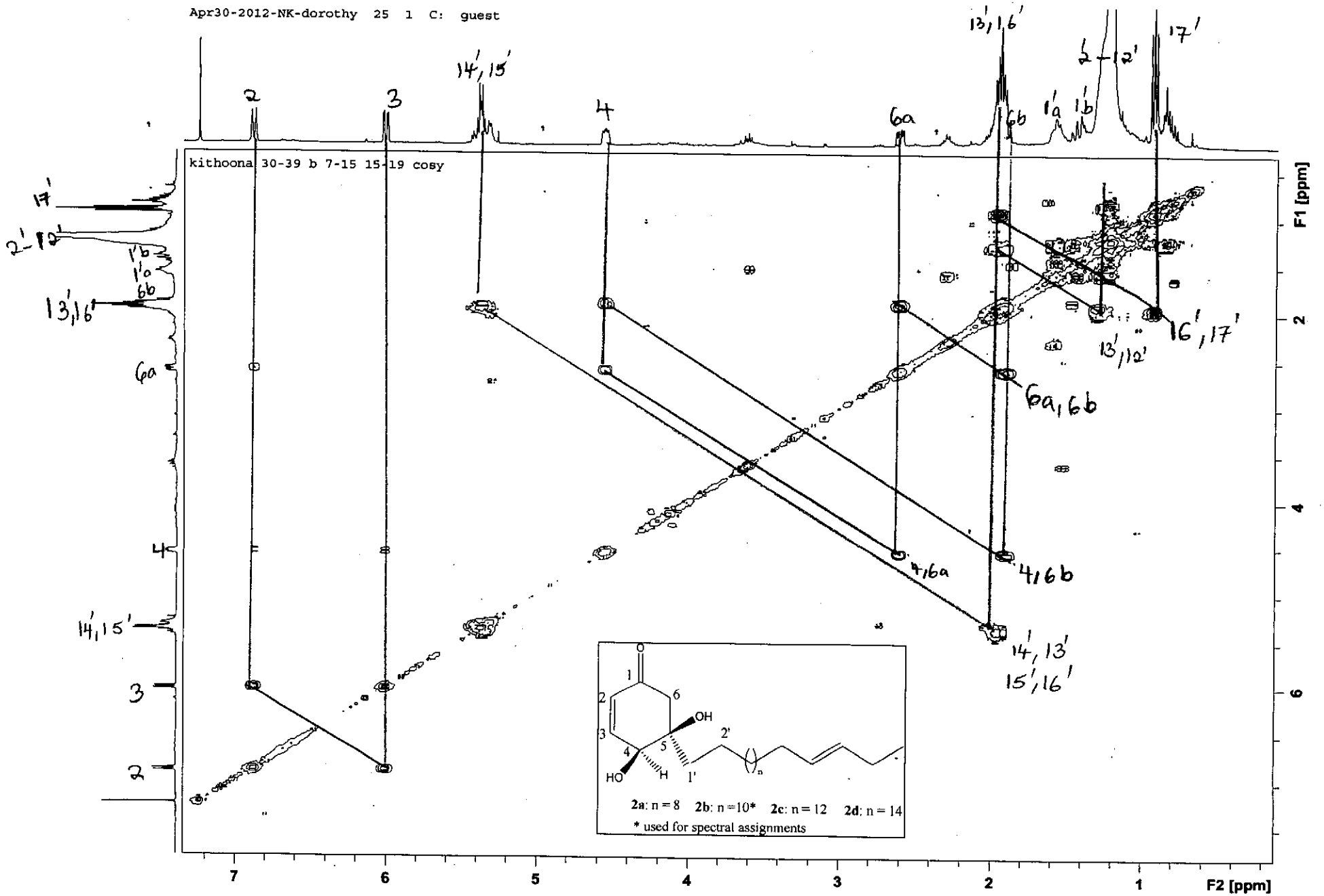
^{1}H NMR spectrum of C-2 (a-d)



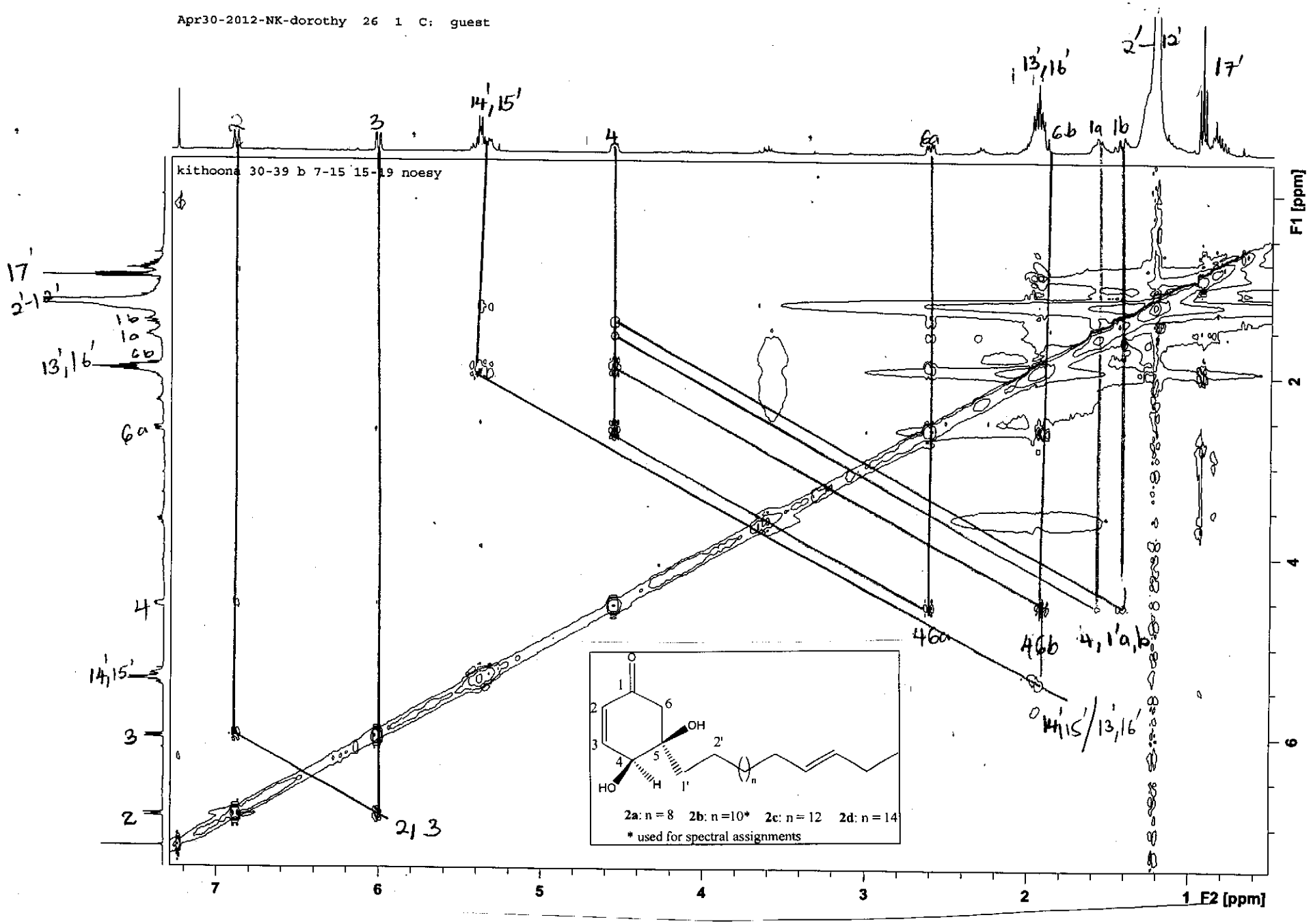
^{13}C NMR spectrum of C-2 (a-d)



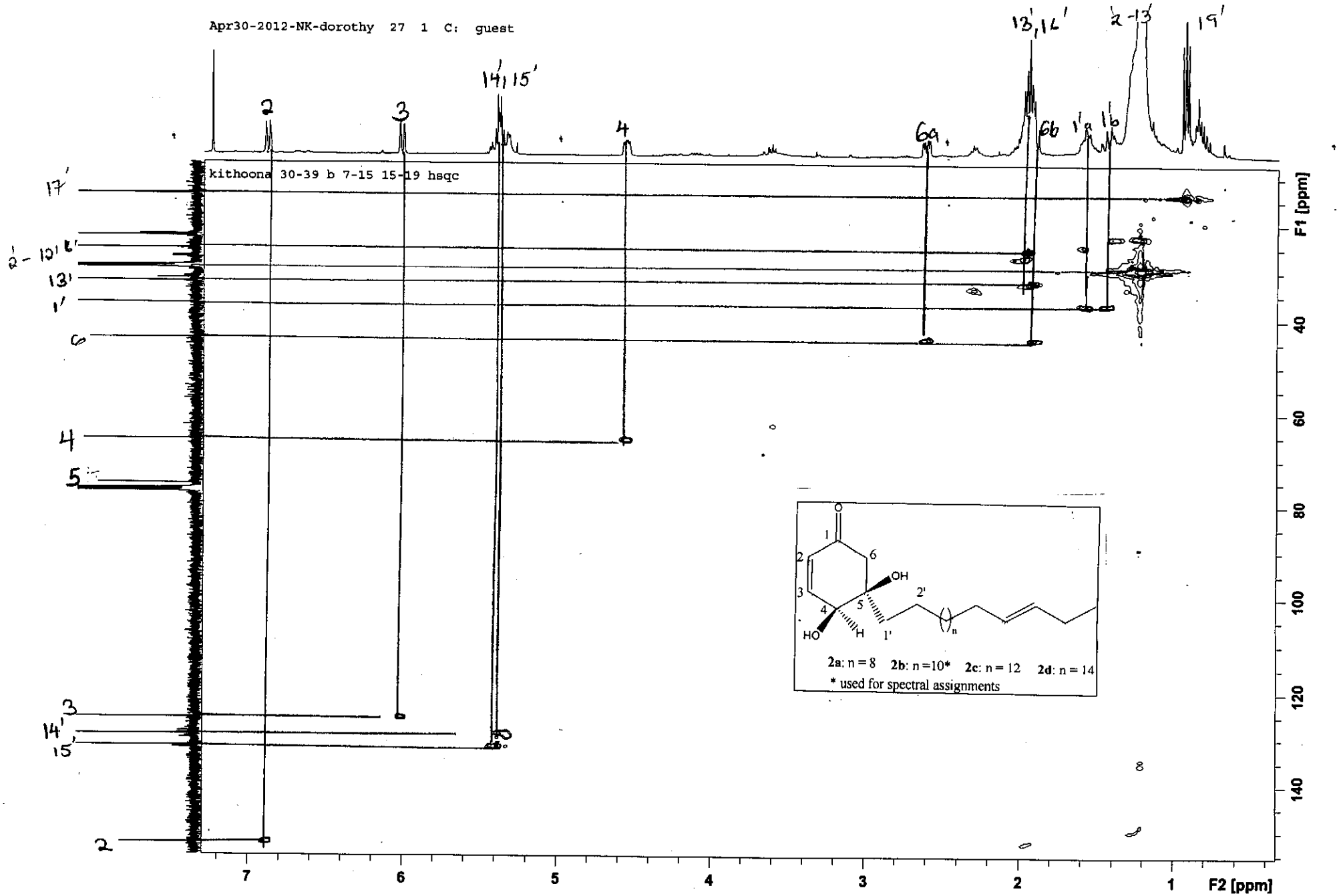
DEPT spectrum of C-2 (a-d)



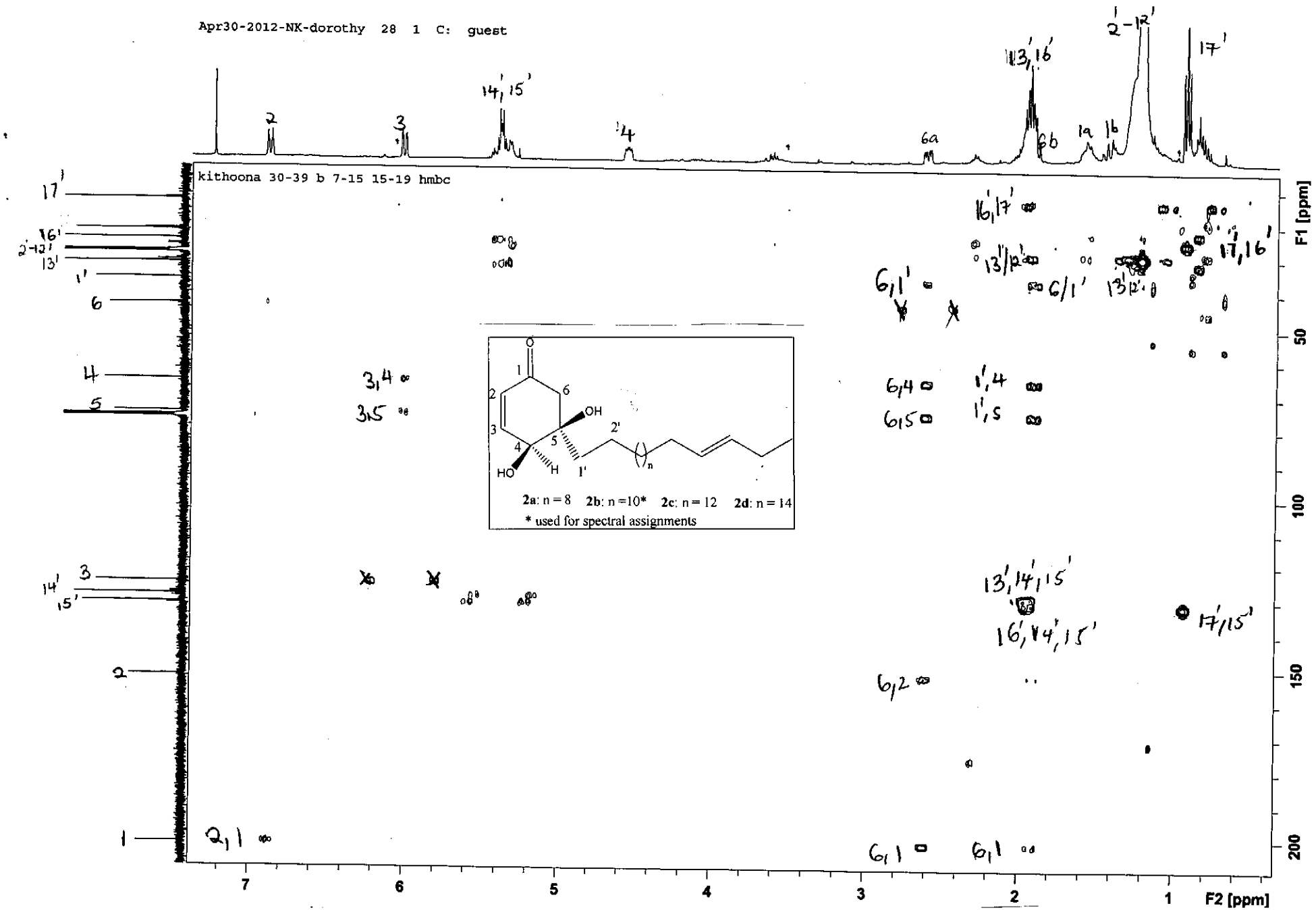
COSY spectrum of C-2 (a-d)



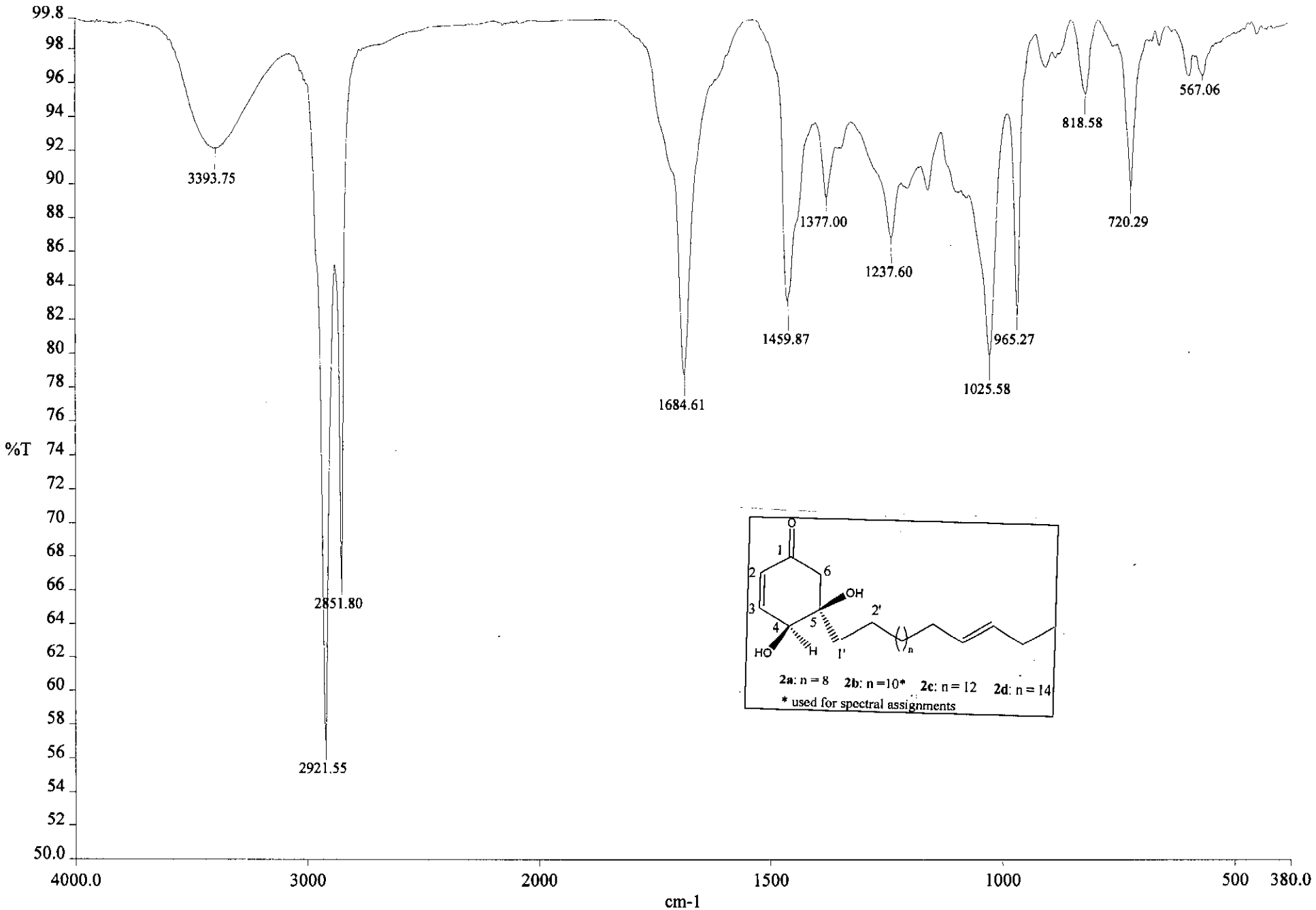
NOESY spectrum of C-2 (a-d)



HSQC spectrum of C-2 (a-d)



HMBC spectrum of C-2 (a-d)

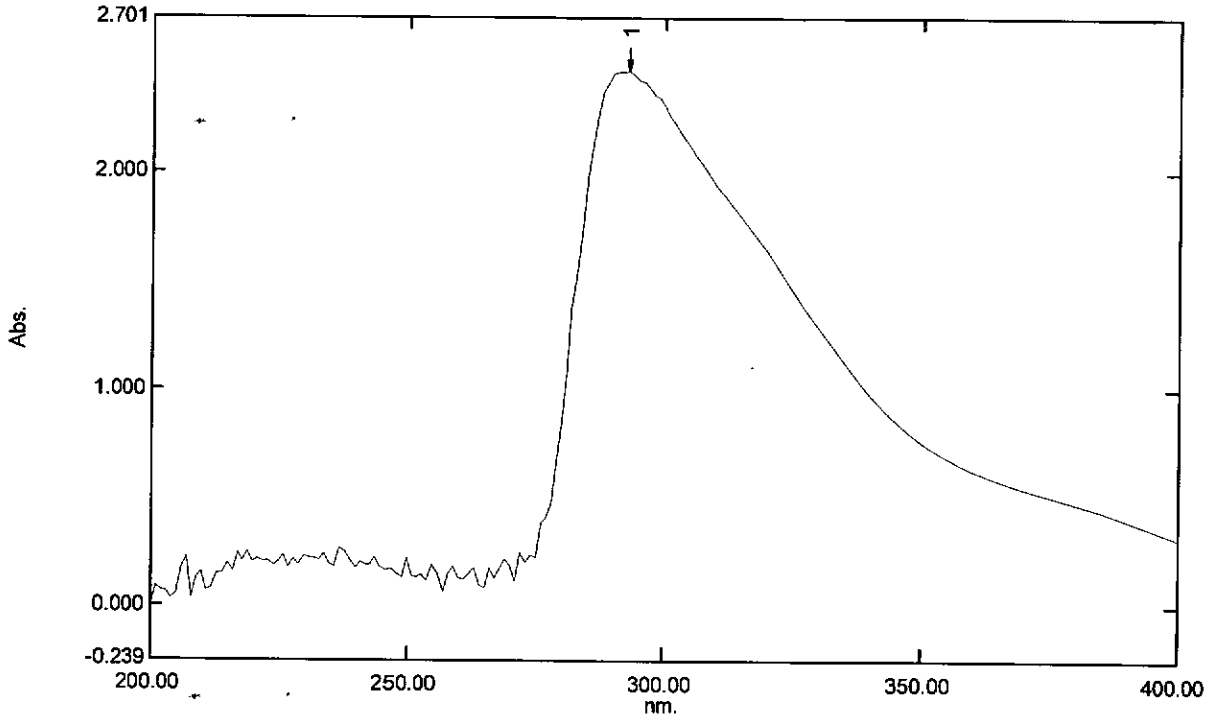


IR spectrum of C-2 (a-d)

Spectrum Peak Pick Report

08/04/2012 06:58:38 PM

Data Set: kithoona 30-39B 7-15 (15-19) b.spc - Storage 173554



No.	P/V	Wavelength	Abs.	Description
1	●	293.00	2.456	

Measurement Properties

Wavelength Range (nm.):	200.00 to 400.00
Scan Speed:	Medium
Sampling Interval:	1.0
Auto Sampling Interval:	Disabled
Scan Mode:	Single

Instrument Properties

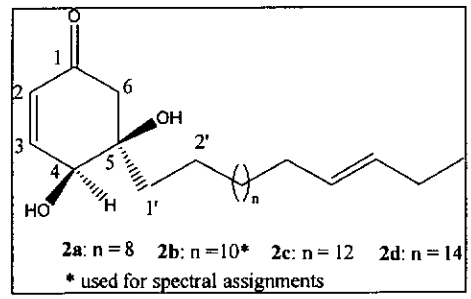
Instrument Type:	UV-3600 Series
Measuring Mode:	Absorbance
Slit Width:	2.0 nm
Time Constant:	0.1 sec.
Source Lamp:	Auto
Light Source Change Wavelength:	310.00 nm
Detector Unit:	Direct
Detector Change Wavelength:	830.00 nm 1800.00 nm
Grating Change Wavelength:	720.00 nm
S/R Exchange:	Normal
Detector Lock:	Auto
Slit Program:	Normal
Beam Mode:	Double
Stair Correction:	Disable

Attachment Properties

Attachment:	None

Sample Preparation Properties

Weight:	
Volume:	
Dilution:	
Path Length:	
Additional Information:	

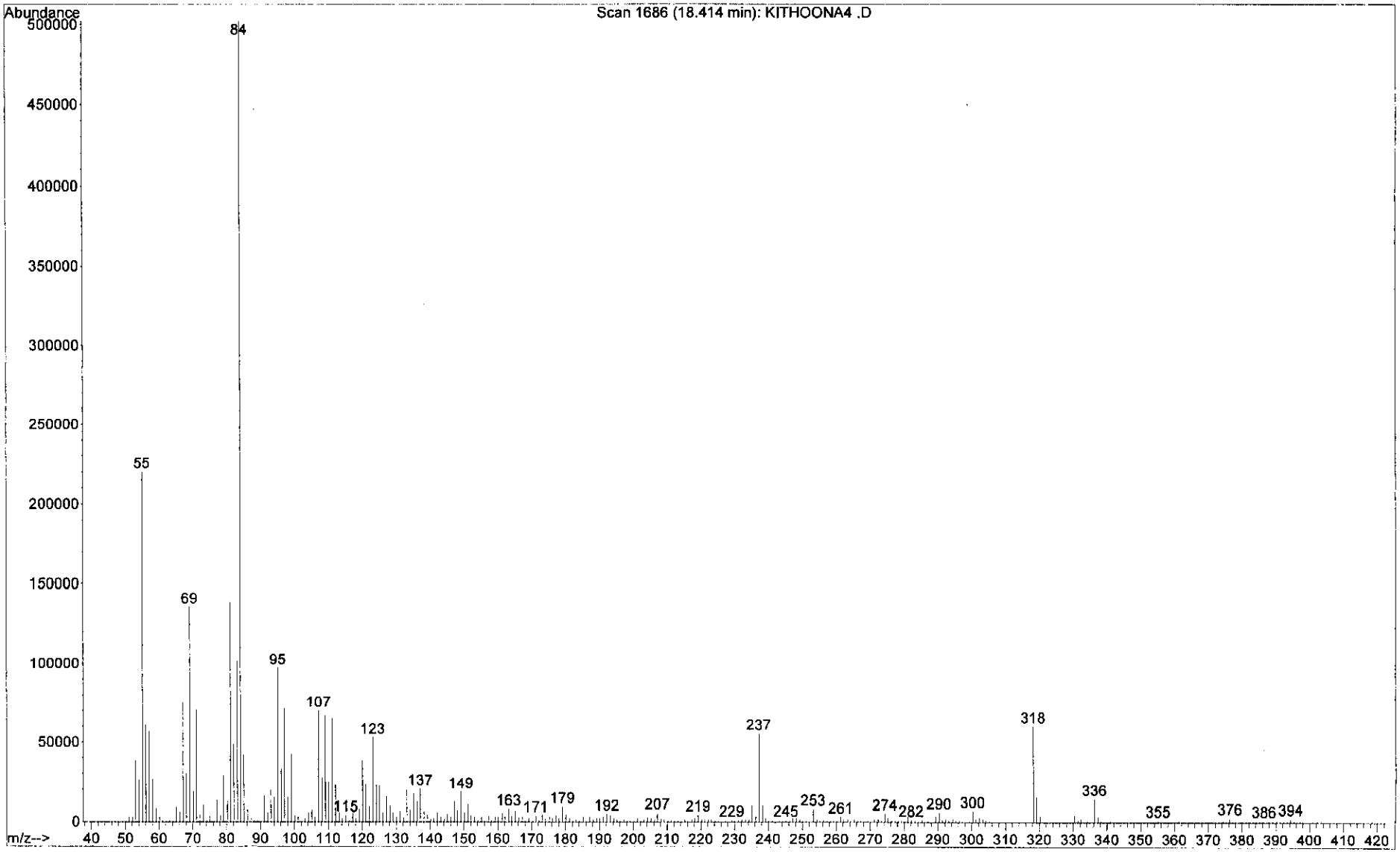


2a: n = 8 **2b**: n = 10* **2c**: n = 12 **2d**: n = 14

* used for spectral assignments

UV spectrum of C-2 (a-d)

File : C:\MSDCHEM\1\DATA\DOROTHY RAW DATA KITHOKIT\KITHOONA4 .D
Operator : Dorothy
Acquired : 5 May 2012 21:28 using AcqMethod NATPRODUCTS MANUAL
Instrument : 5973N
Sample Name: kithoona 30-39B 7-15 (15-19)
Misc Info :
Vial Number: 1



MS spectrum of C-2 a

```
File       : C:\MSDCHEM\1\DATA\DOROTHY RAW DATA KITHOKIT\KITHOONA4 .D
Operator   : Dorothy
Acquired   :  5 May 2012  21:28     using AcqMethod NATPRODUCTS MANUAL
Instrument :   5973N
Sample Name: kithoona 30-39B 7-15 (15-19)
Misc Info  :
Vial Number: 1
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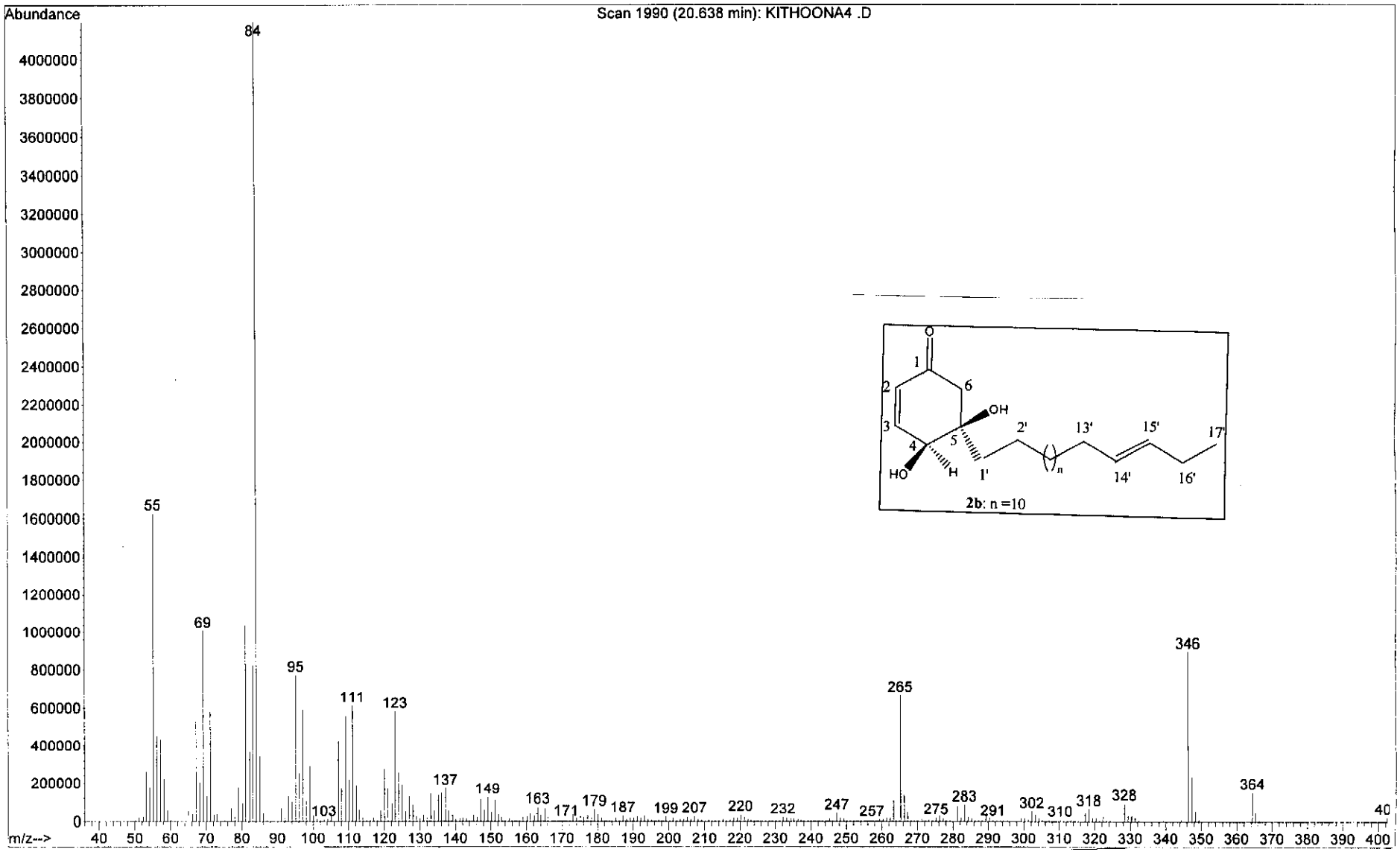
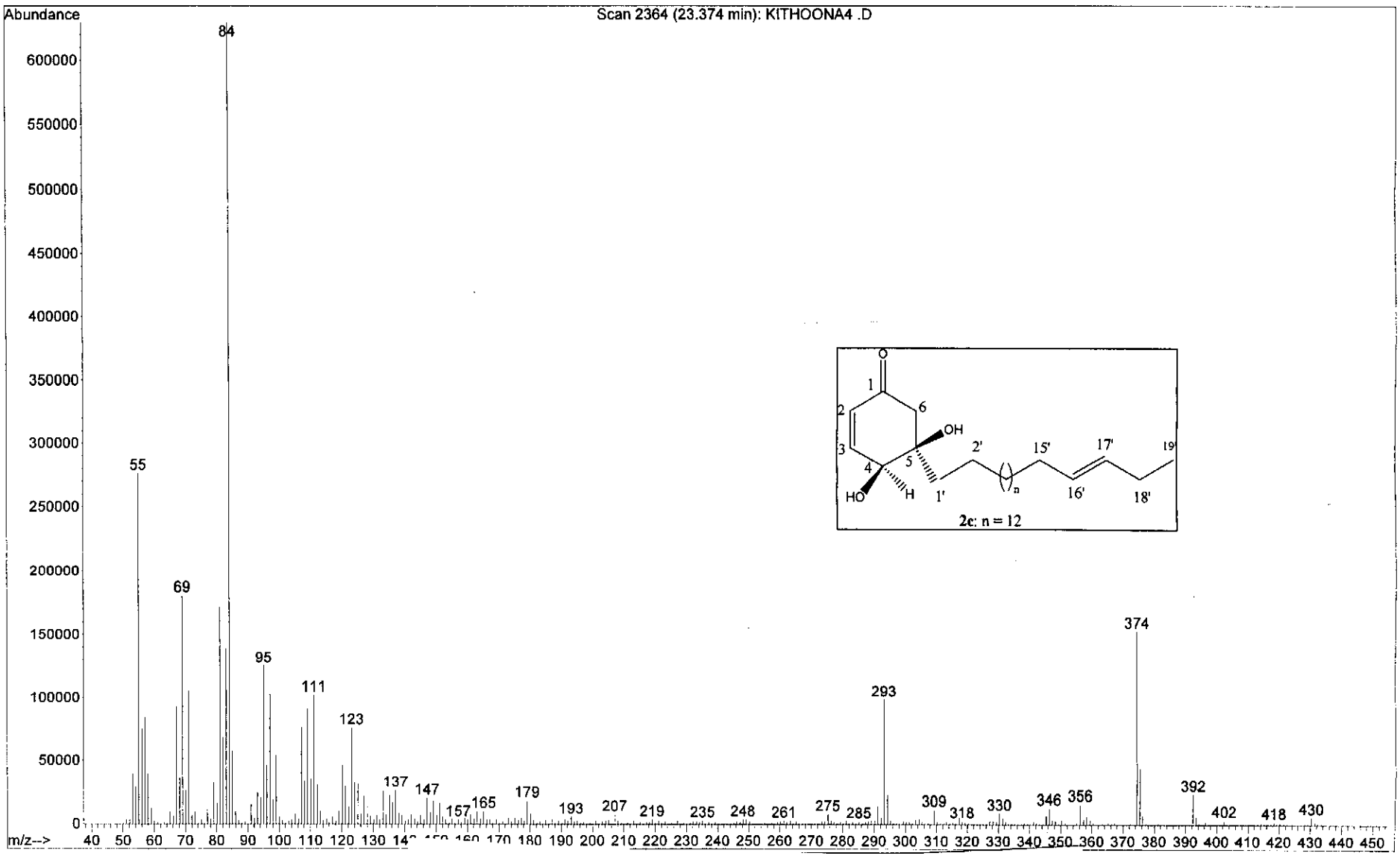


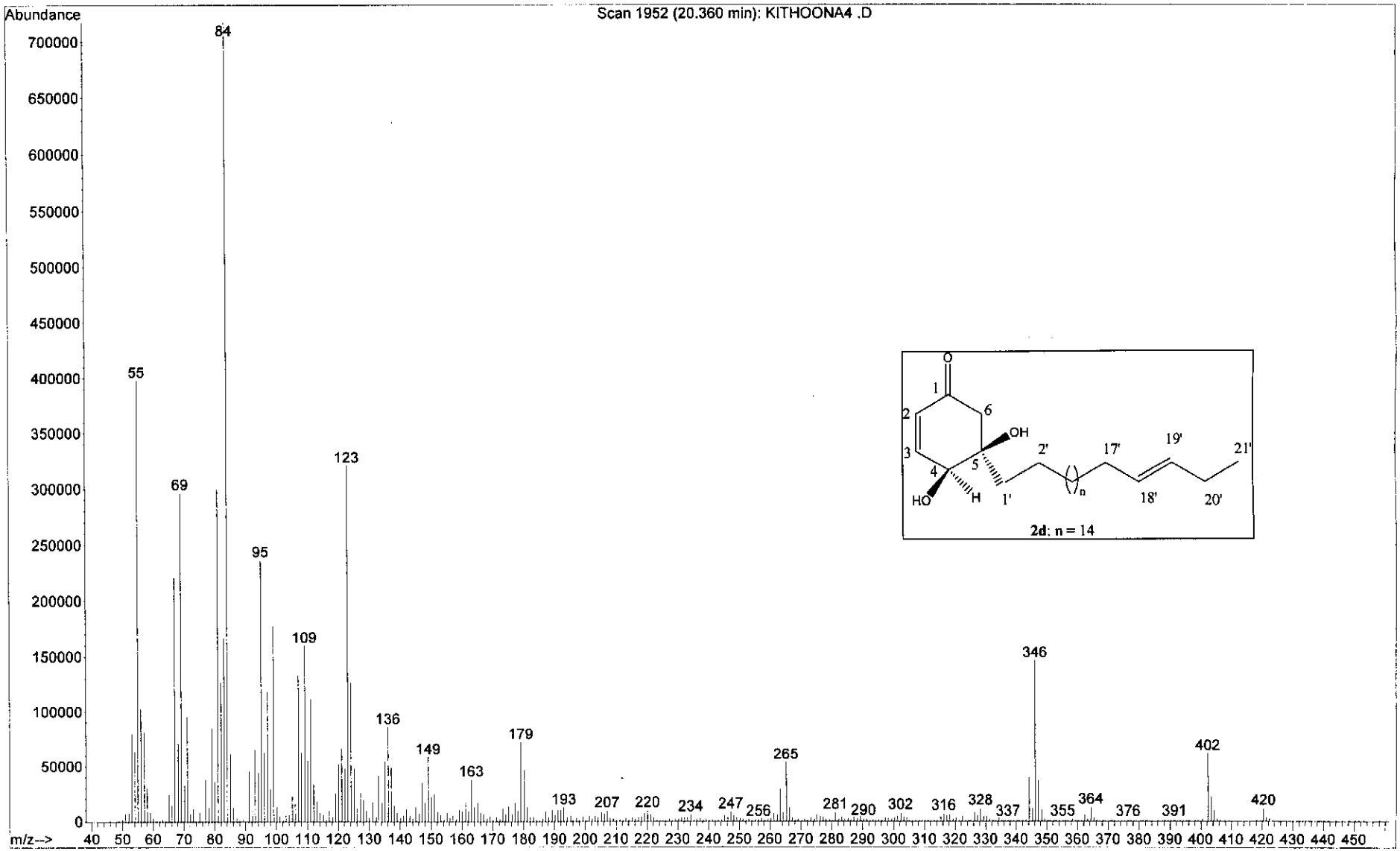
MS spectrum of C-2 b

File : C:\MSDCHEM\1\DATA\DOROTHY RAW DATA KITHOKIT\KITHOONA4 .D
Operator : Dorothy
Acquired : 5 May 2012 21:28 using AcqMethod NATPRODUCTS MANUAL
Instrument : 5973N
Sample Name: kithoona 30-39B 7-15 (15-19)
Misc Info :
Vial Number: 1

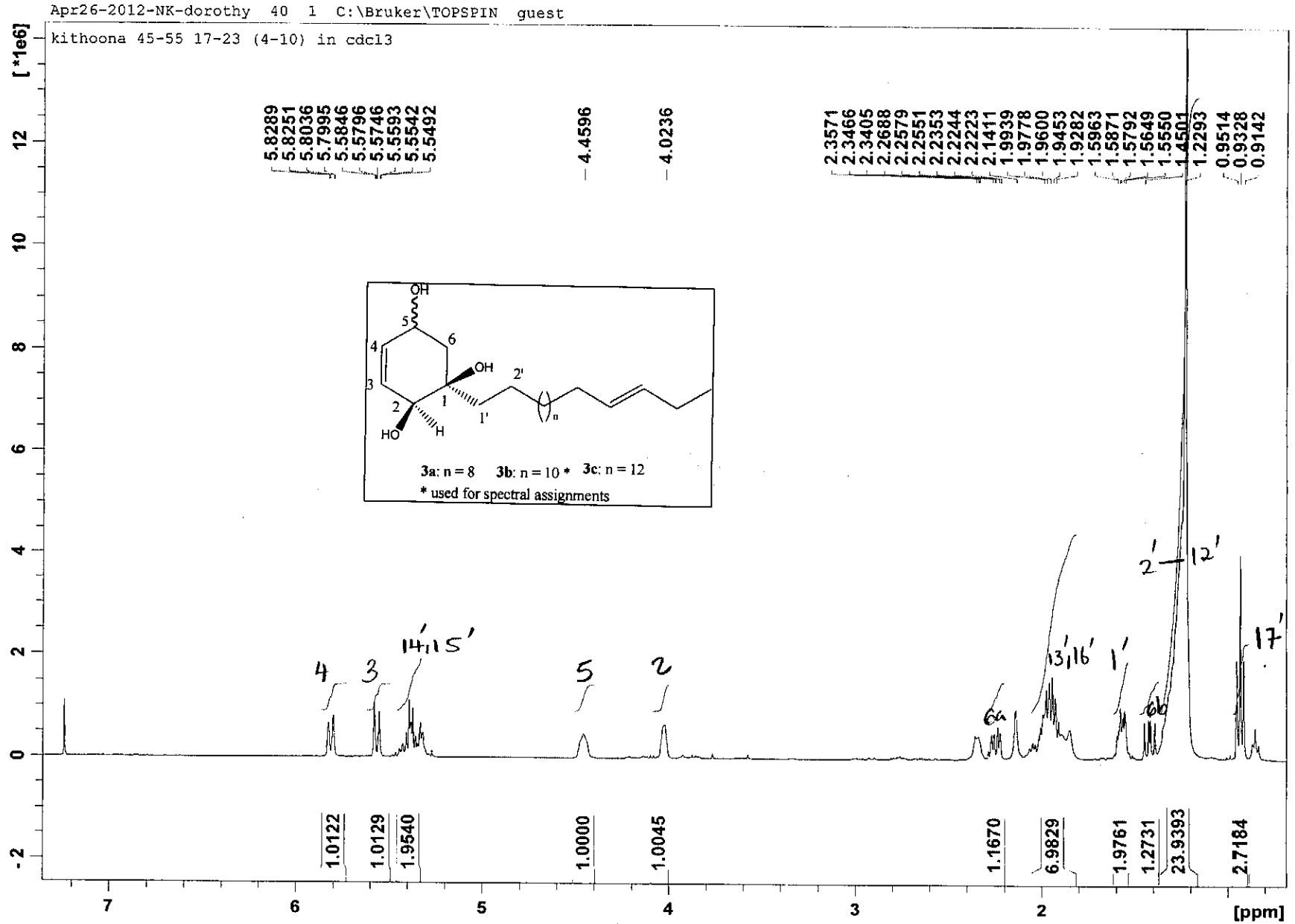


MS spectrum of C-2 c

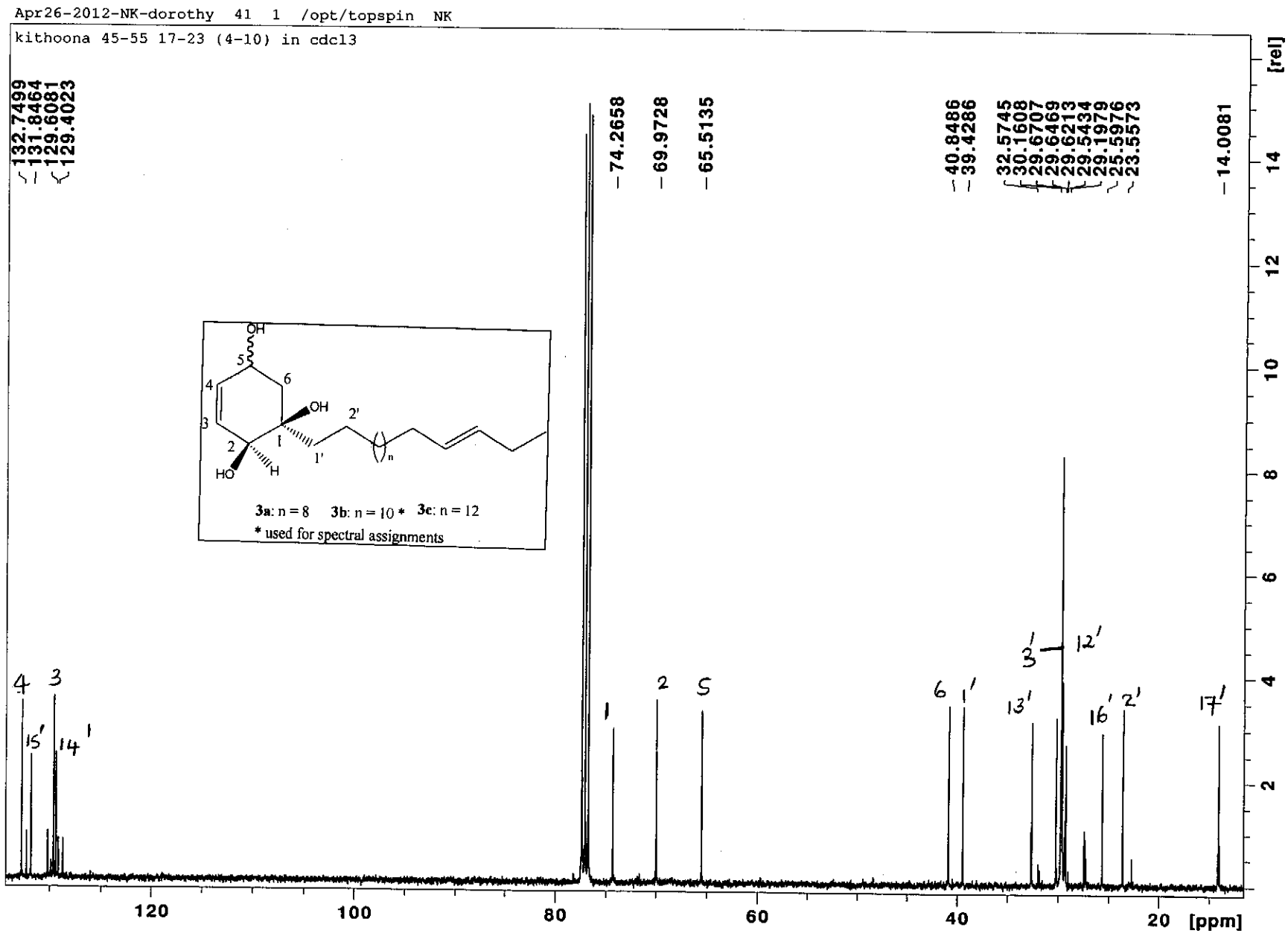
```
File       : C:\MSDCHEM\1\DATA\DOROTHY RAW DATA KITHOKIT\KITHOONA4 .D
Operator   : Dorothy
Acquired   :  5 May 2012  21:28     using AcqMethod NATPRODUCTS MANUAL
Instrument :   5973N
Sample Name: kithoona 30-39B 7-15 (15-19)
Misc Info  :
Vial Number: 1
```



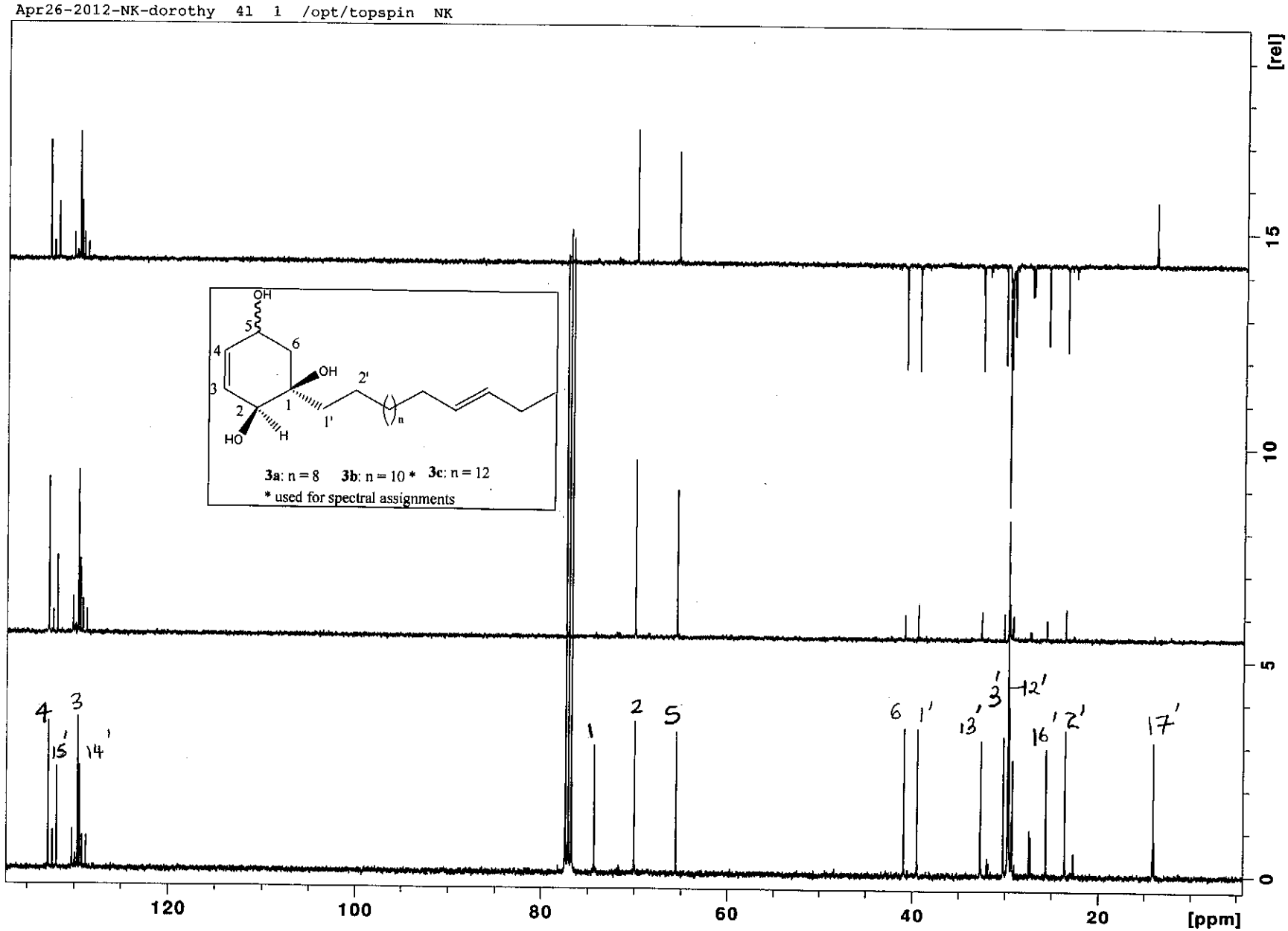
MS spectrum of C-2 d



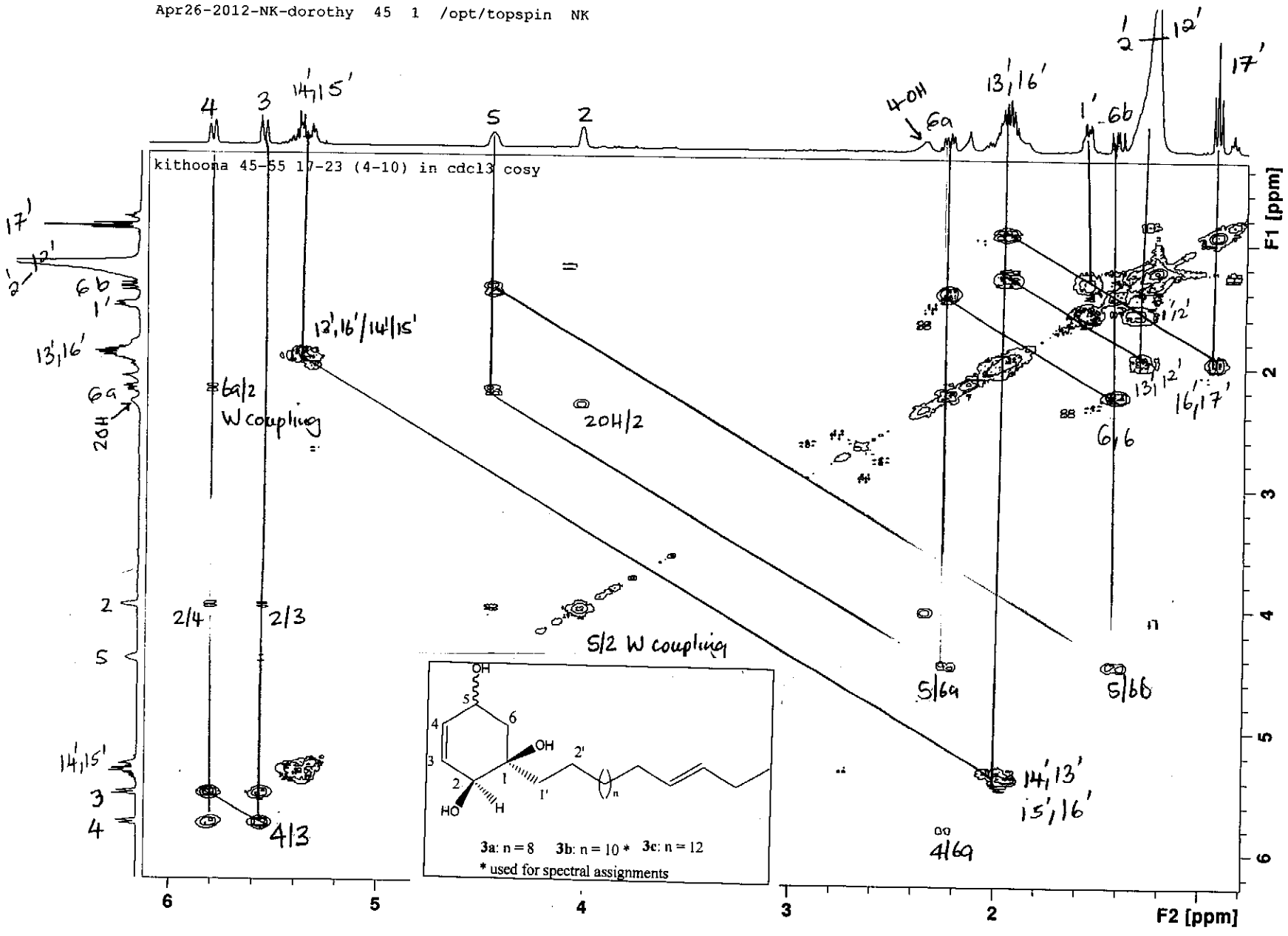
^{1}H NMR spectrum of C-3 (a-c)



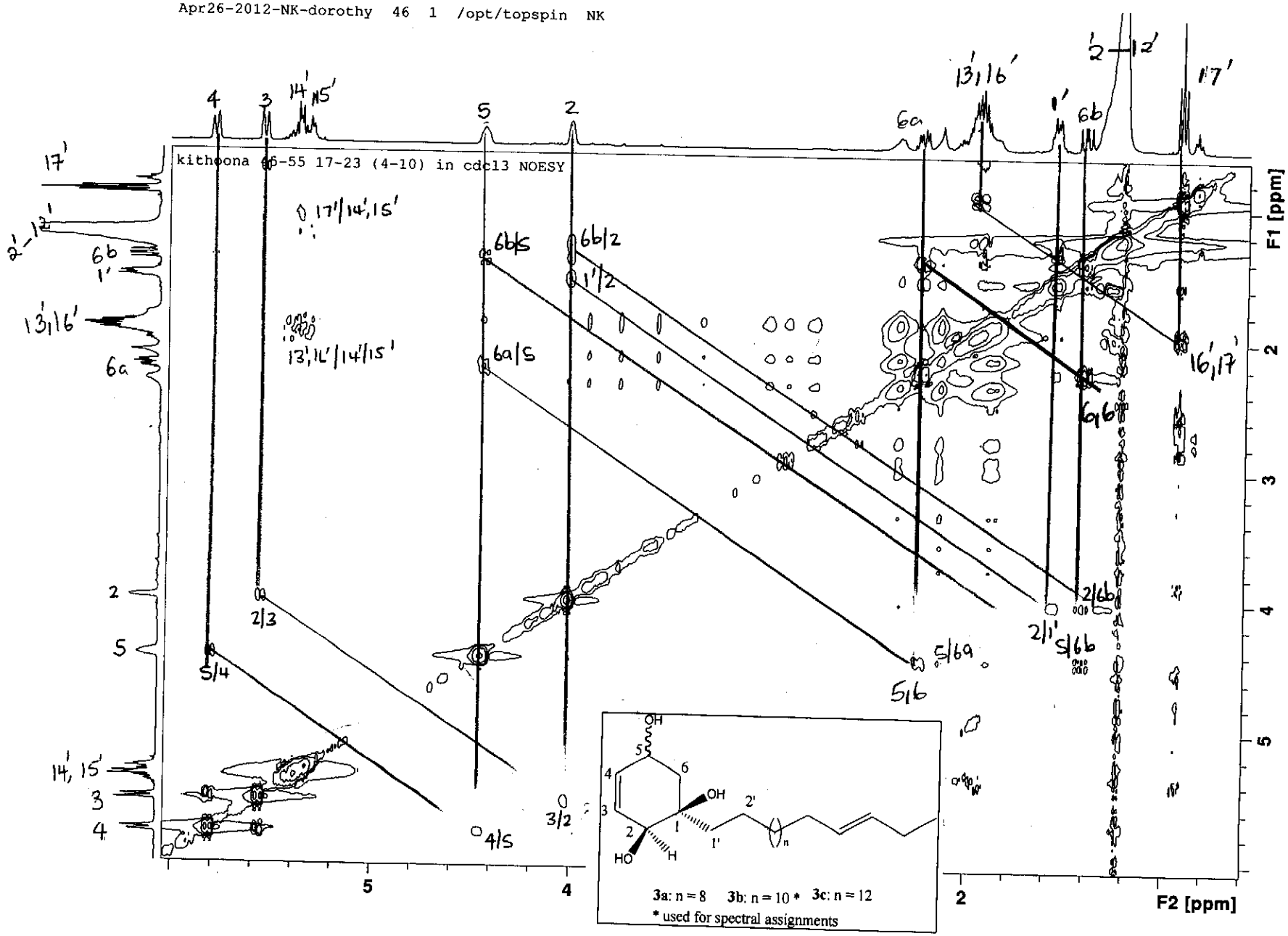
^{13}C NMR spectrum of C-3 (a-c)



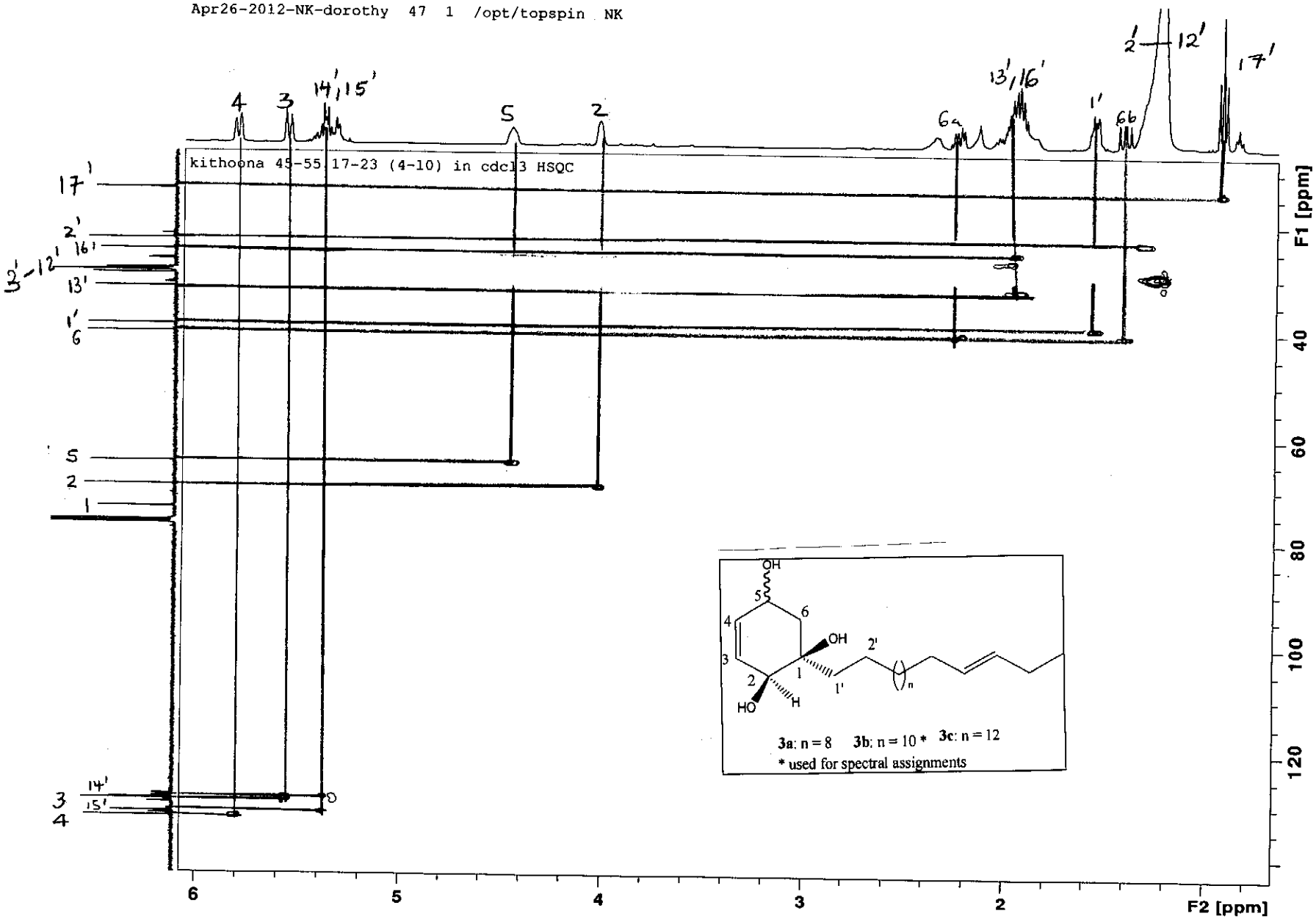
DEPT spectrum of C-3 (a-c)



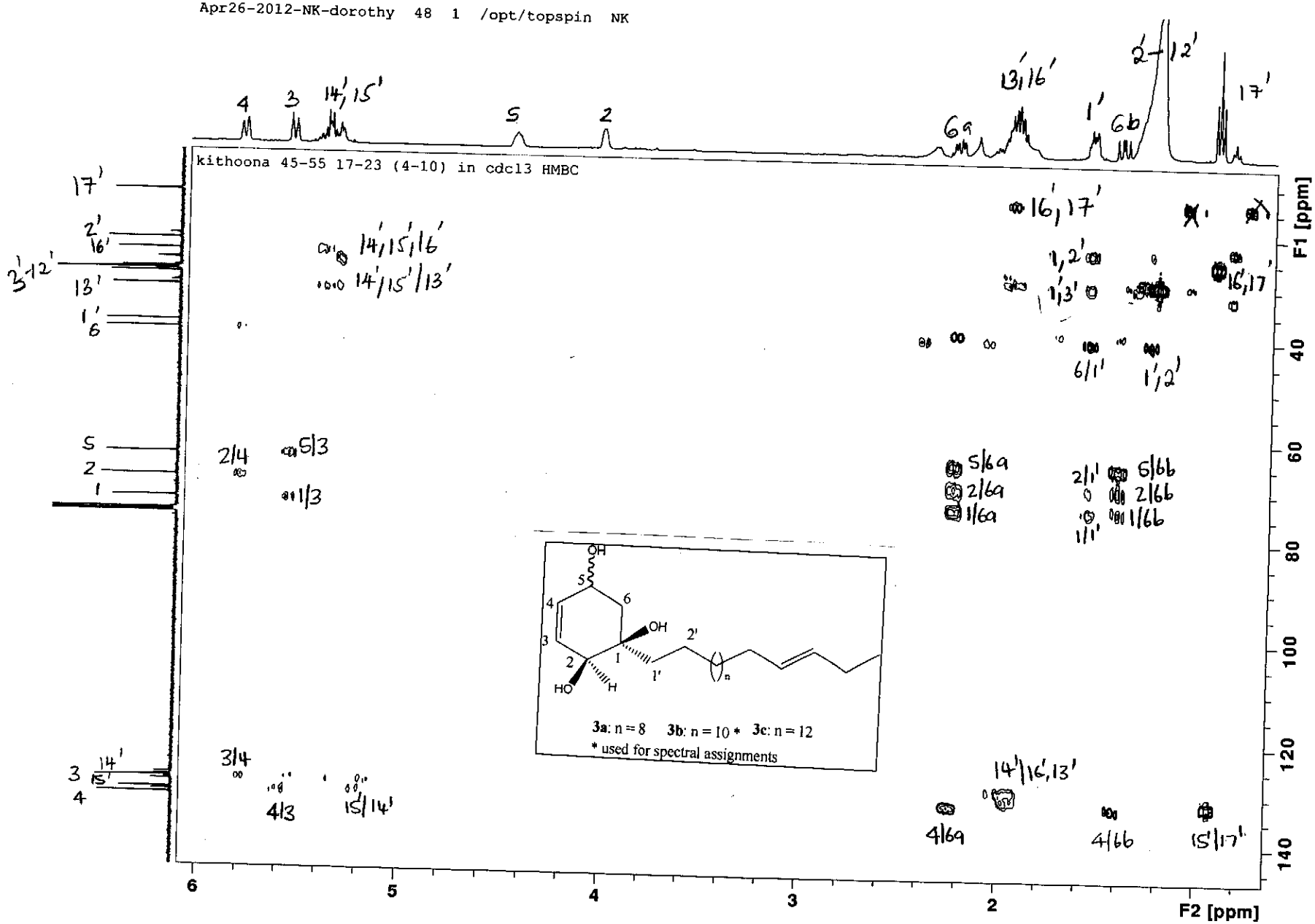
COSY spectrum of C-3 (a-c)



NOESY spectrum of C-3 (a-c)



HSQC spectrum of C-3 (a-c)

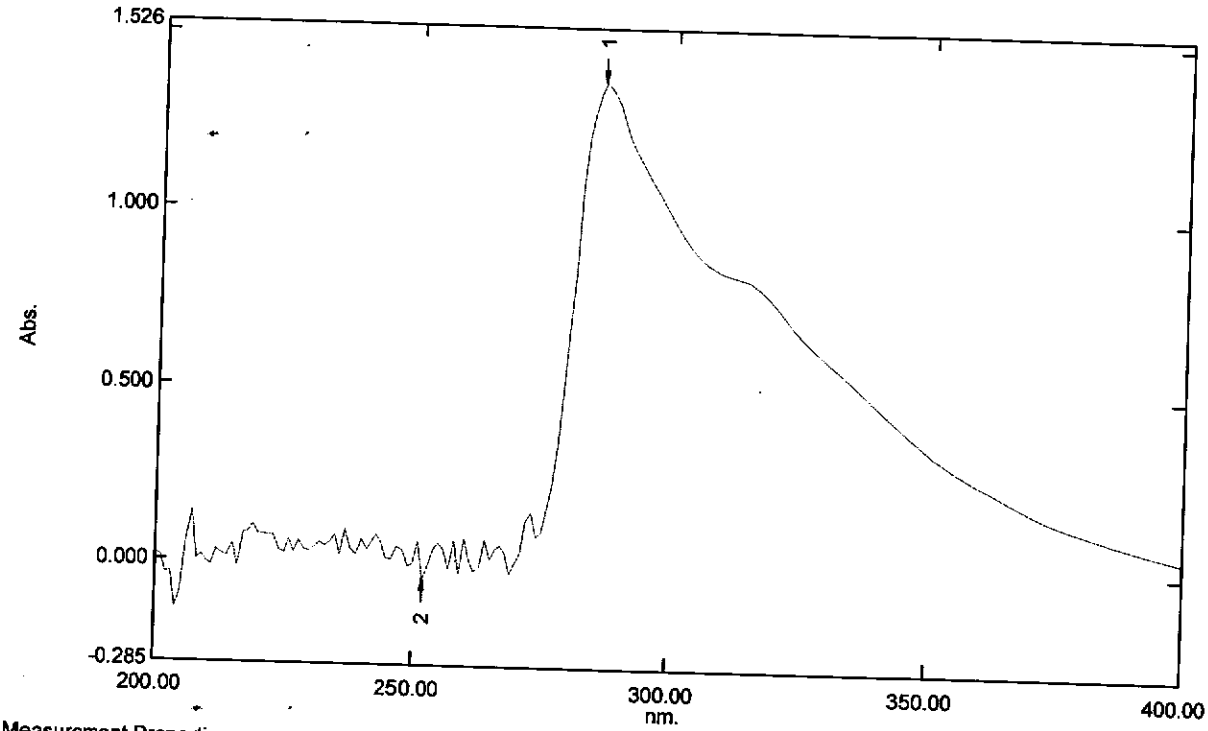


HMBC spectrum of C-3 (a-c)

Spectrum Peak Pick Report

08/04/2012 07:01:15 PM

Data Set: kithoona 45-55 17-23 (4-10).spc - Storage 174127



Measurement Properties

Wavelength Range (nm.):	200.00 to 400.00
Scan Speed:	Medium
Sampling Interval:	1.0
Auto Sampling Interval:	Disabled
Scan Mode:	Single

No.	P/V	Wavelength	Abs.	Description
1		286.00	1.375	
2		252.00	-0.043	

Instrument Properties

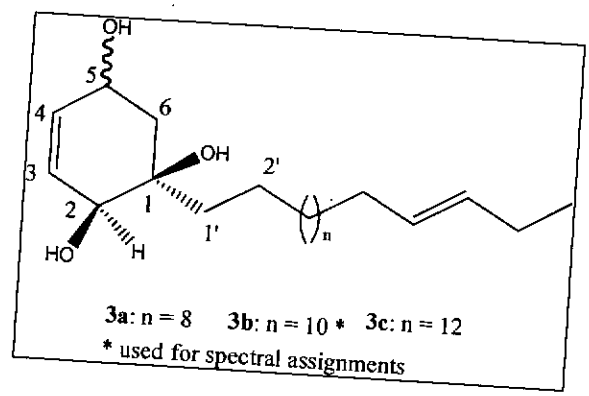
Instrument Type:	UV-3600 Series
Measuring Mode:	Absorbance
Slit Width:	2.0 nm
Time Constant:	0.1 sec.
Source Lamp:	Auto
Light Source Change Wavelength:	310.00 nm
Detector Unit:	Direct
Detector Change Wavelength:	830.00 nm 1800.00 nm
Grating Change Wavelength:	720.00 nm
S/R Exchange:	Normal
Detector Lock:	Auto
Slit Program:	Normal
Beam Mode:	Double
Stair Correction:	Disable

Attachment Properties

Attachment:	None

Sample Preparation Properties

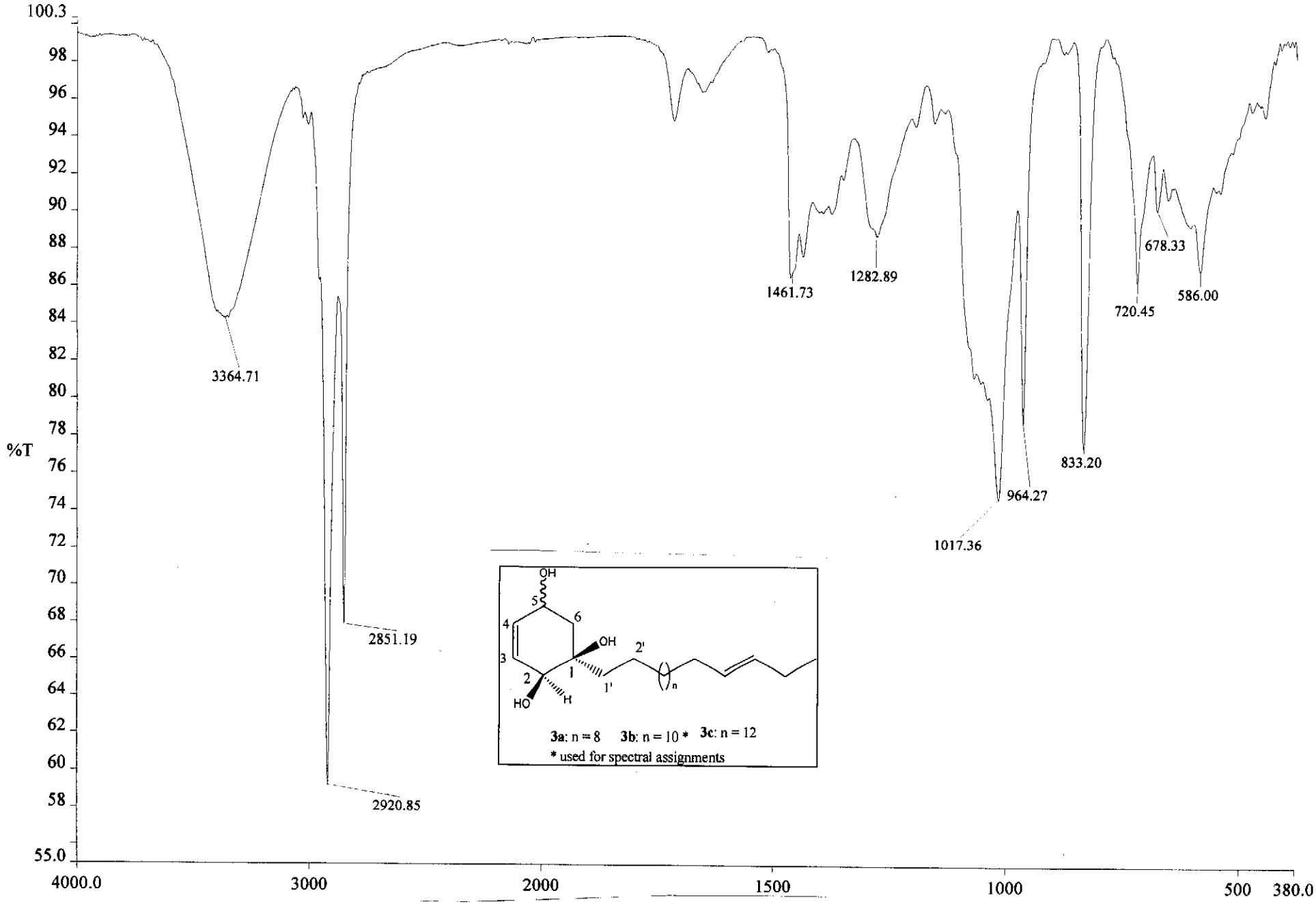
Weight:	
Volume:	
Dilution:	
Path Length:	
Additional Information:	



3a: n = 8 **3b**: n = 10 * **3c**: n = 12

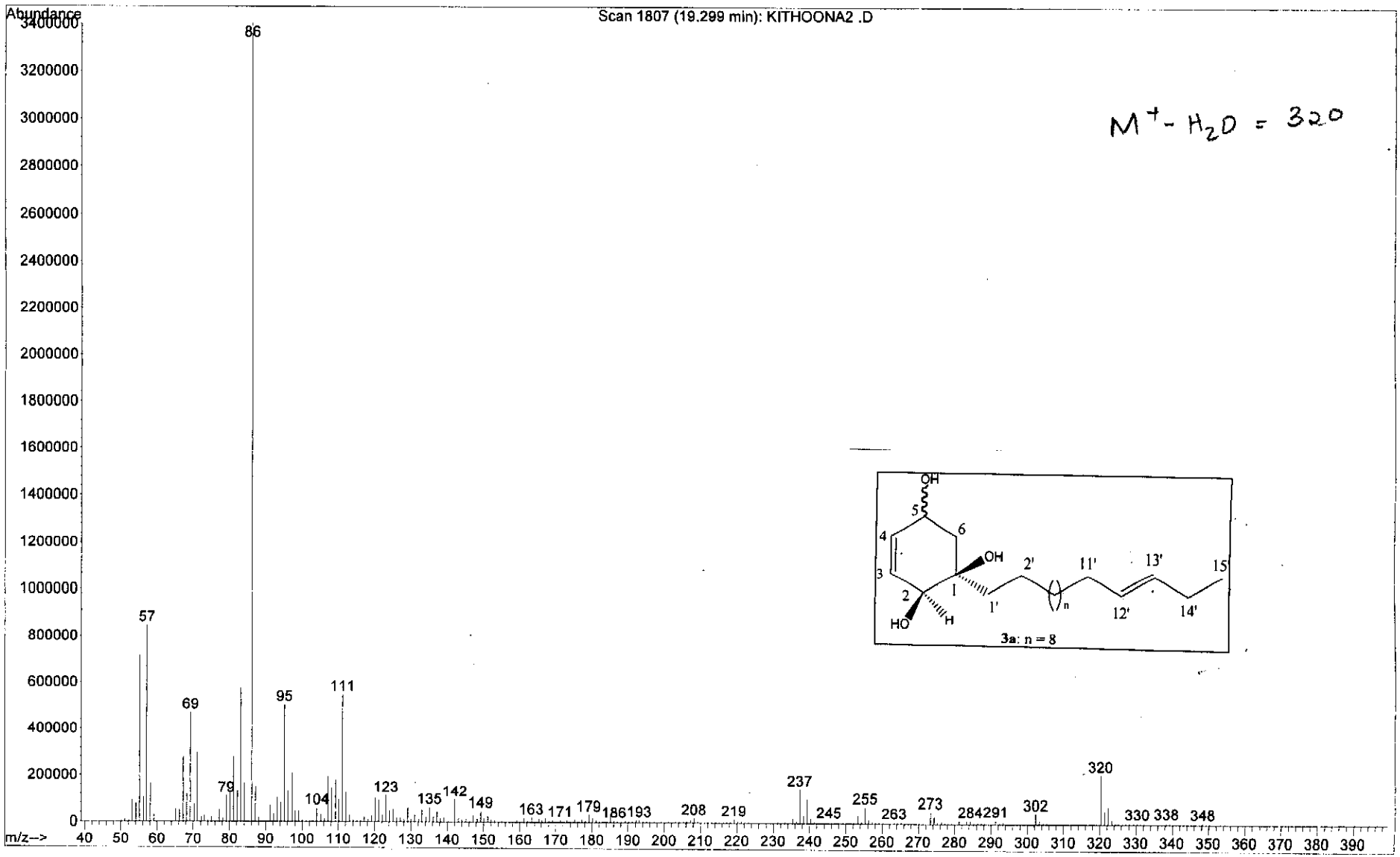
* used for spectral assignments

UV spectrum of C-3 (a-c)



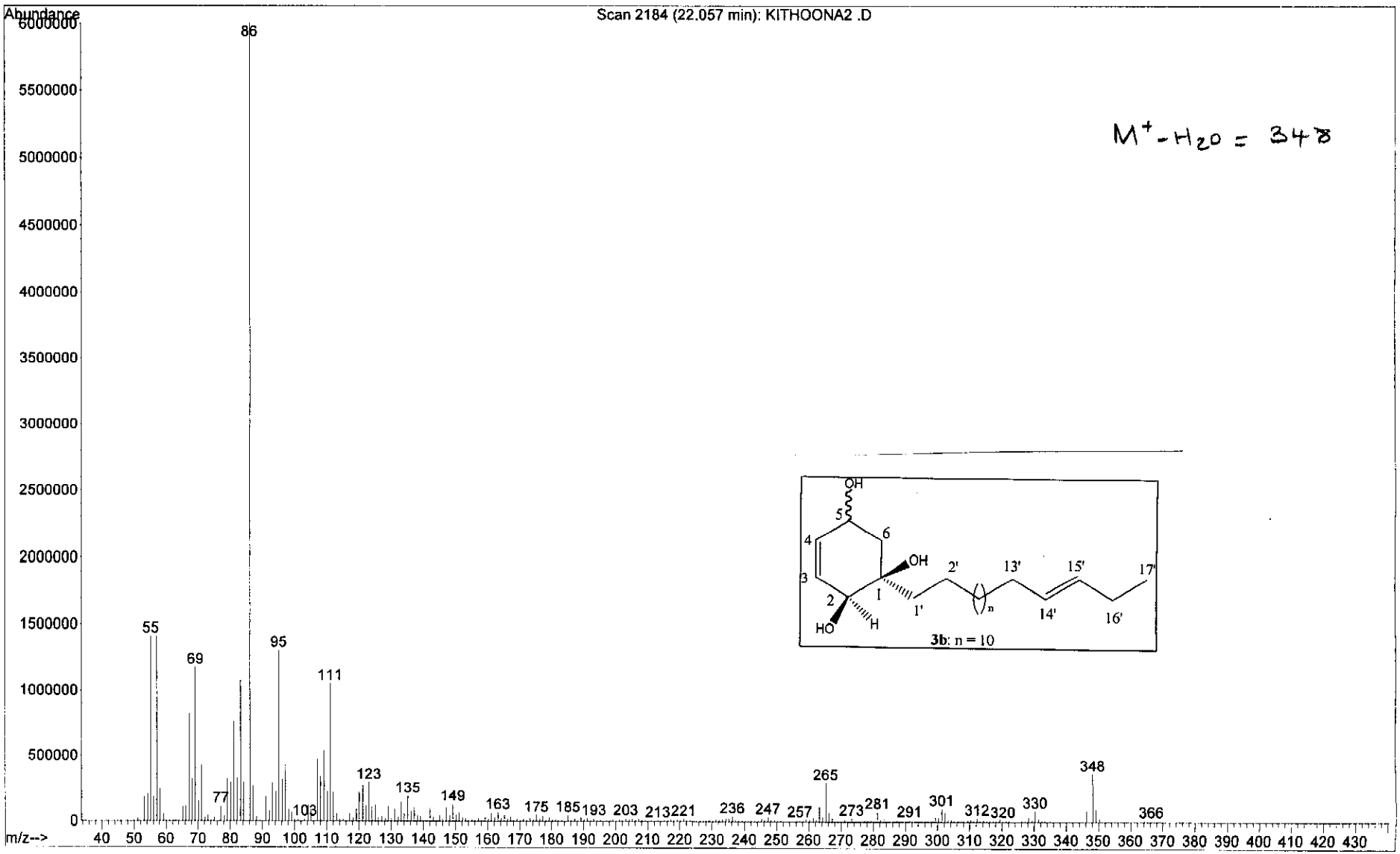
IR spectrum of C-3 (a-c)

File : C:\MSDCHEM\1\DATA\DOROTHY RAW DATA KITHOKIT\KITHOONA2 .D
Operator : Dorothy
Acquired : 5 May 2012 19:52 using AcqMethod NATPRODUCTS MANUAL
Instrument : 5973N
Sample Name: kithoona 45-55 17-23 (4-10)
Misc Info :
Vial Number: 1



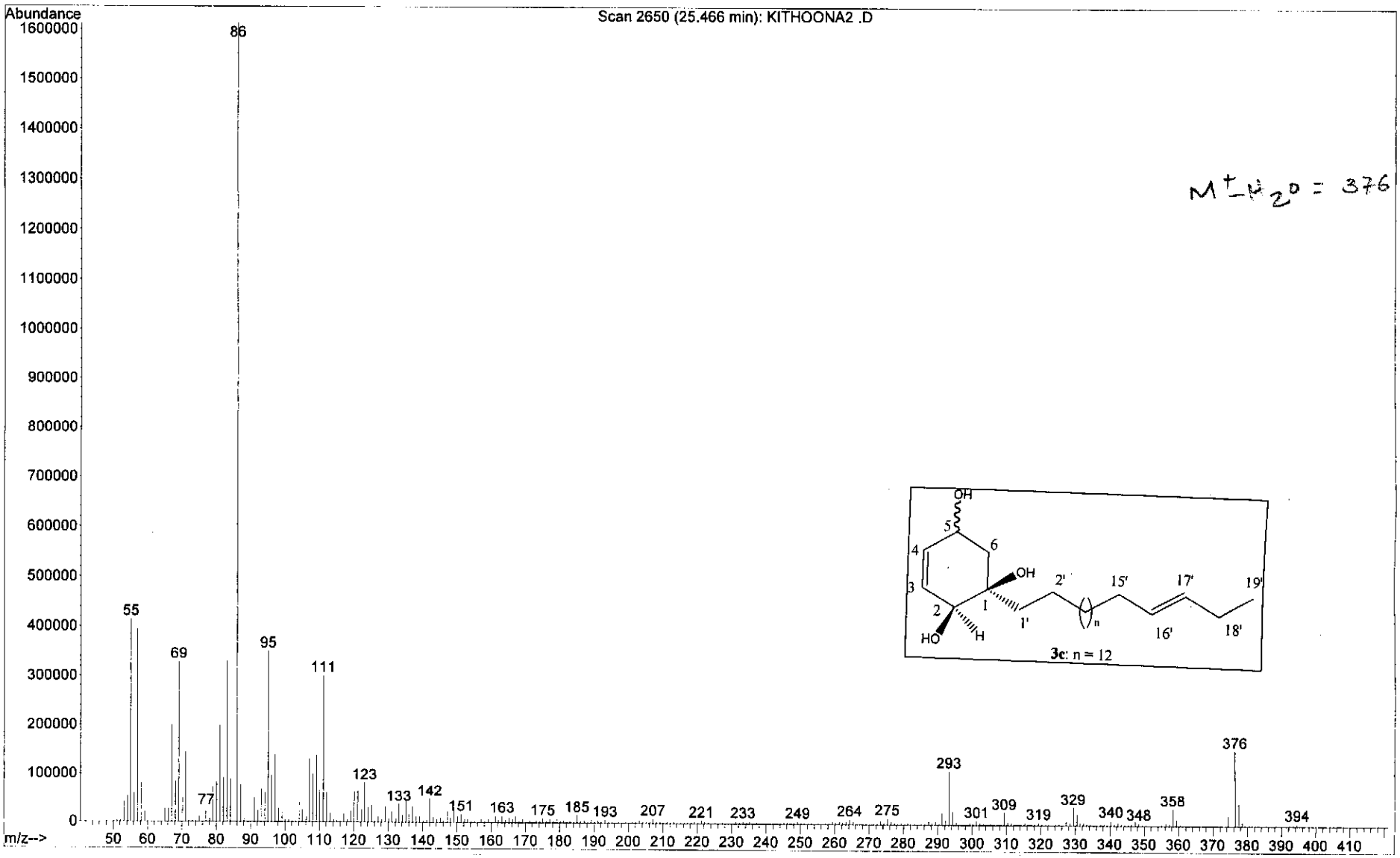
MS spectrum of C-3 a

File : C:\MSDCHEM\1\DATA\DOROTHY RAW DATA KITHOKIT\KITHOONA2 .D
Operator : Dorothy
Acquired : 5 May 2012 19:52 using AcqMethod NATPRODUCTS MANUAL
Instrument : 5973N
Sample Name: kithoona 45-55 17-23 (4-10)
Misc Info :
Vial Number: 1

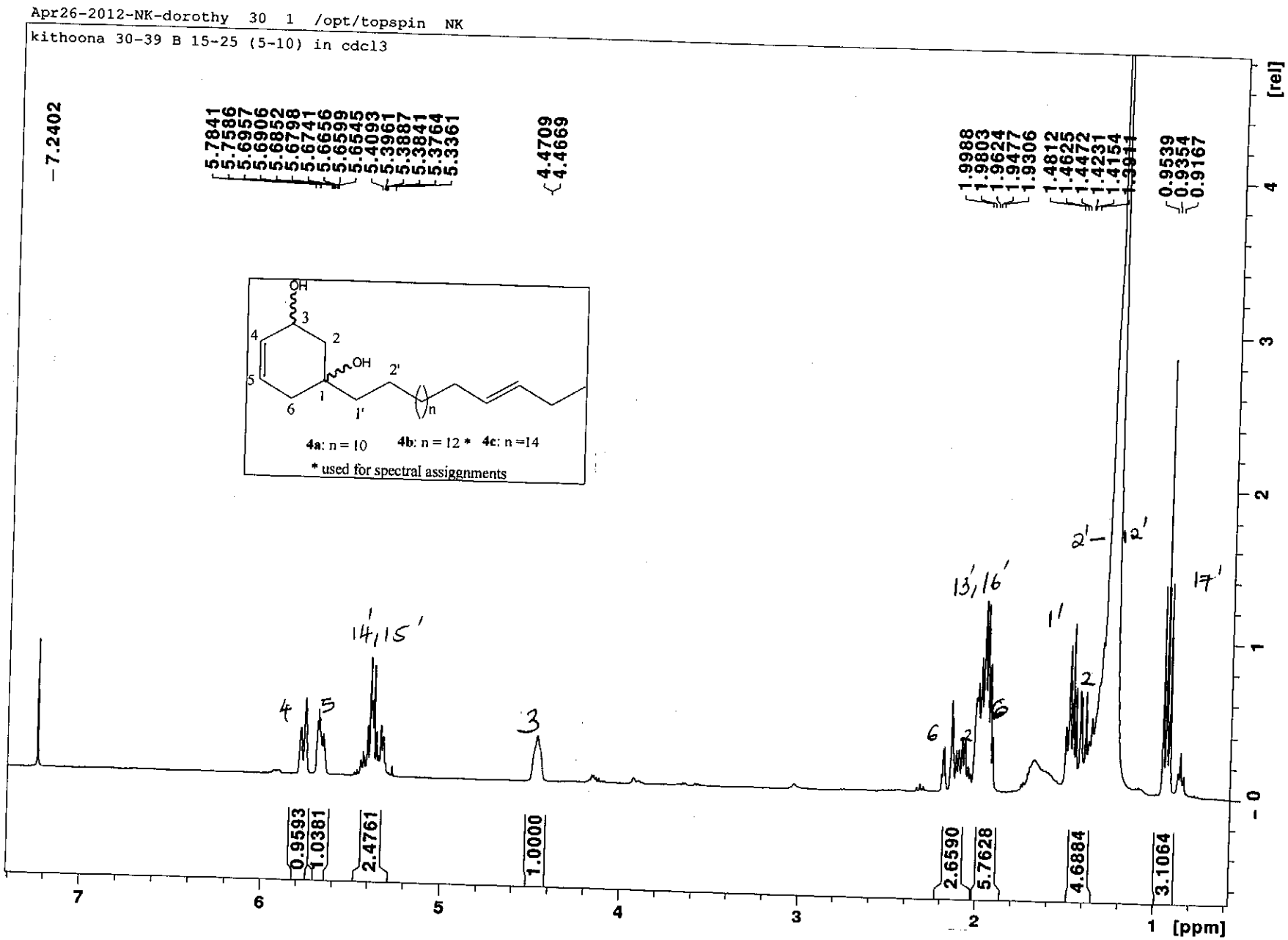


MS spectrum of C-3 b

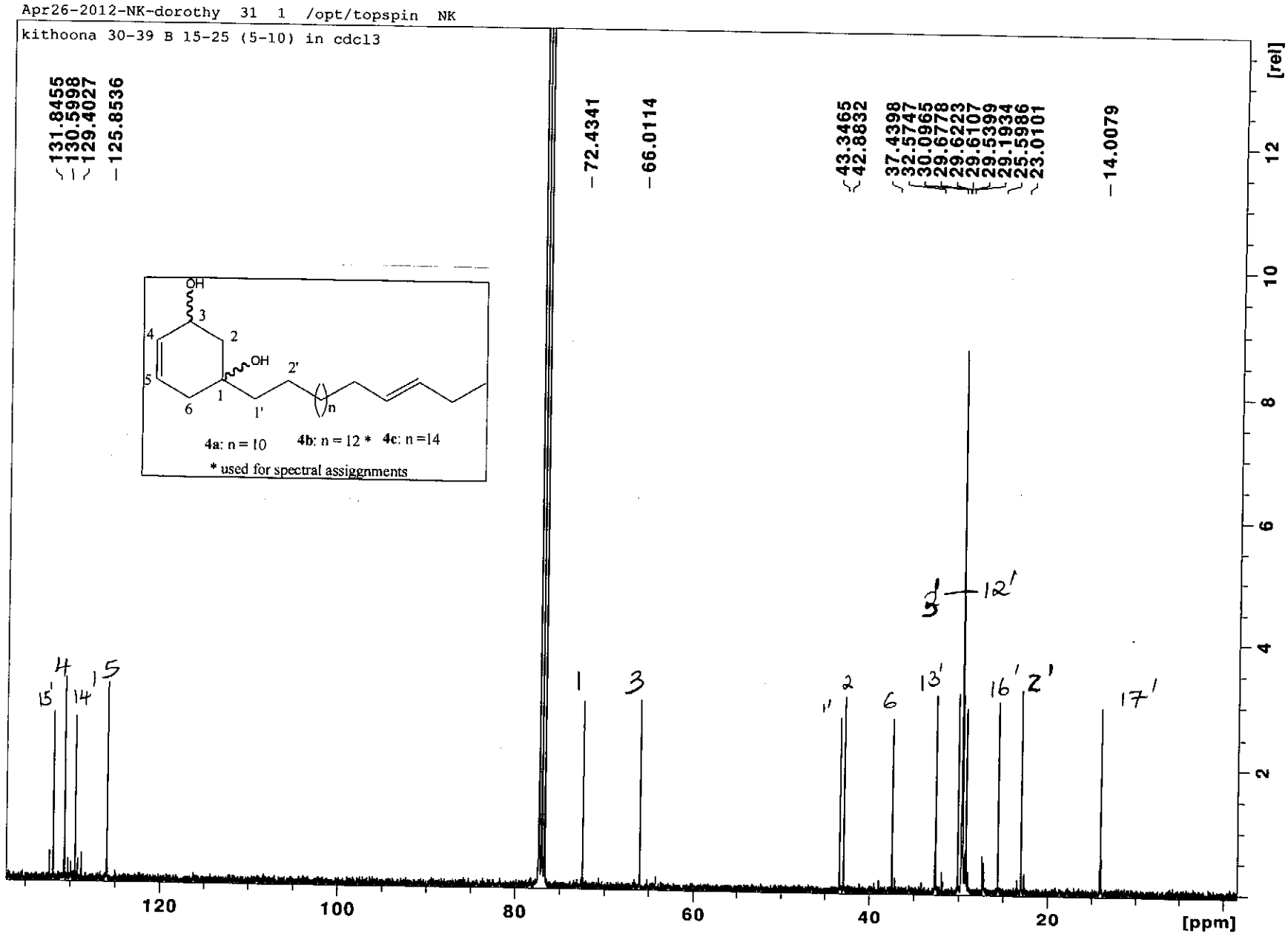
File : C:\MSDCHEM\1\DATA\DOROTHY RAW DATA KITHOKIT\KITHOONA2 .D
Operator : Dorothy
Acquired : 5 May 2012 19:52 using AcqMethod NATPRODUCTS MANUAL
Instrument : 5973N
Sample Name: kithoona 45-55 17-23 (4-10)
Misc Info :
Vial Number: 1



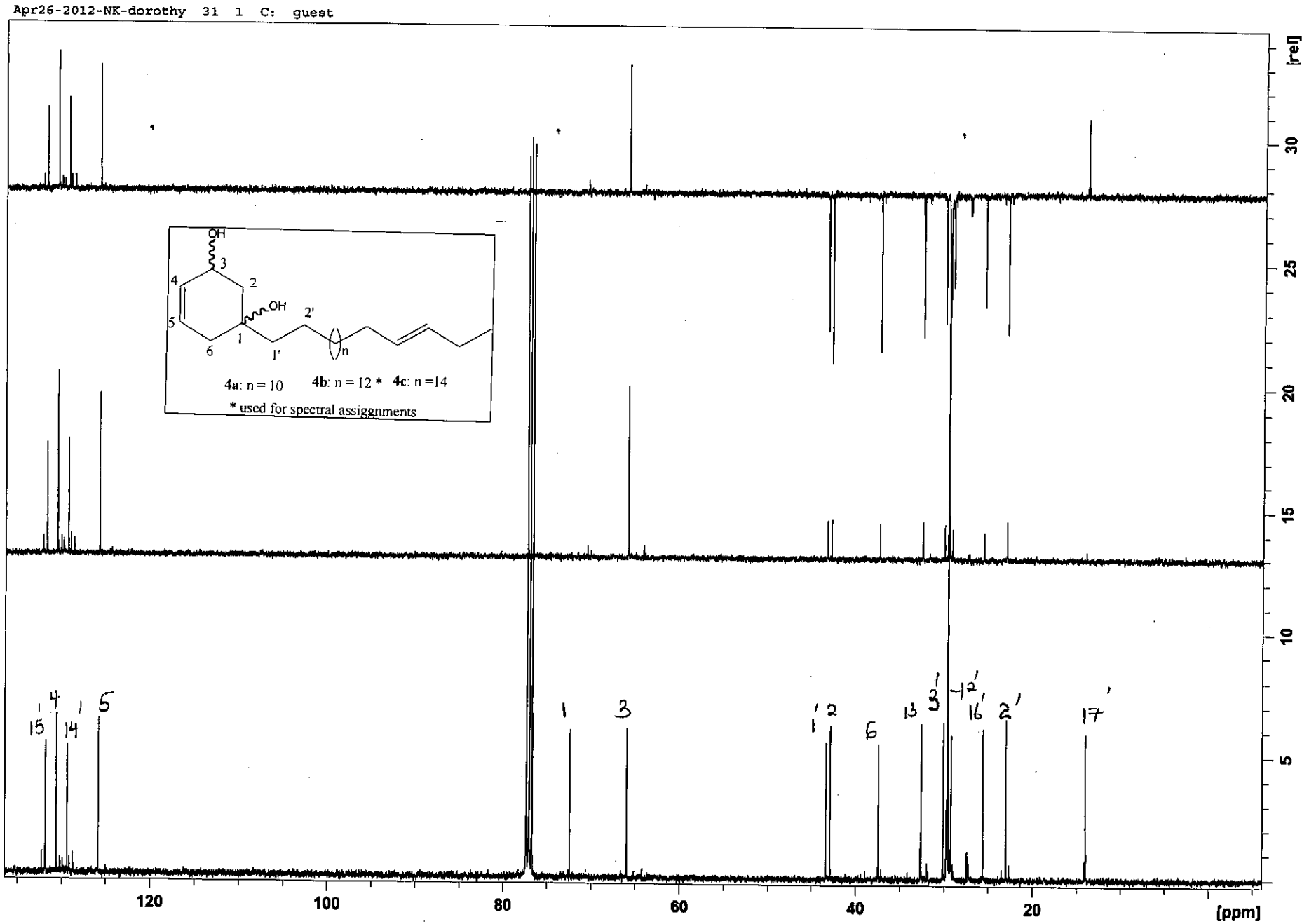
MS spectrum of C-3 c



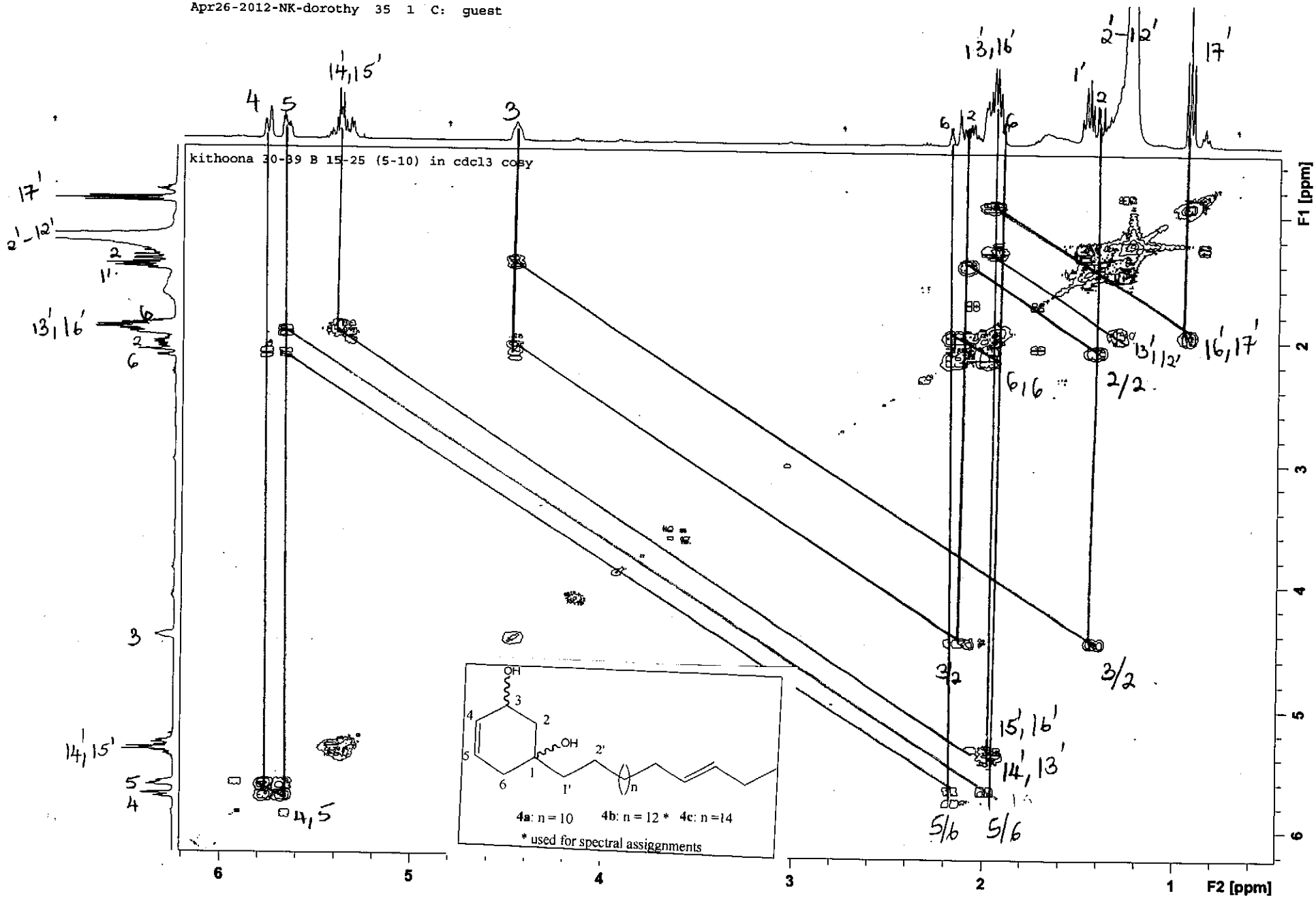
^{1}H NMR spectrum of C-4 (a-c)



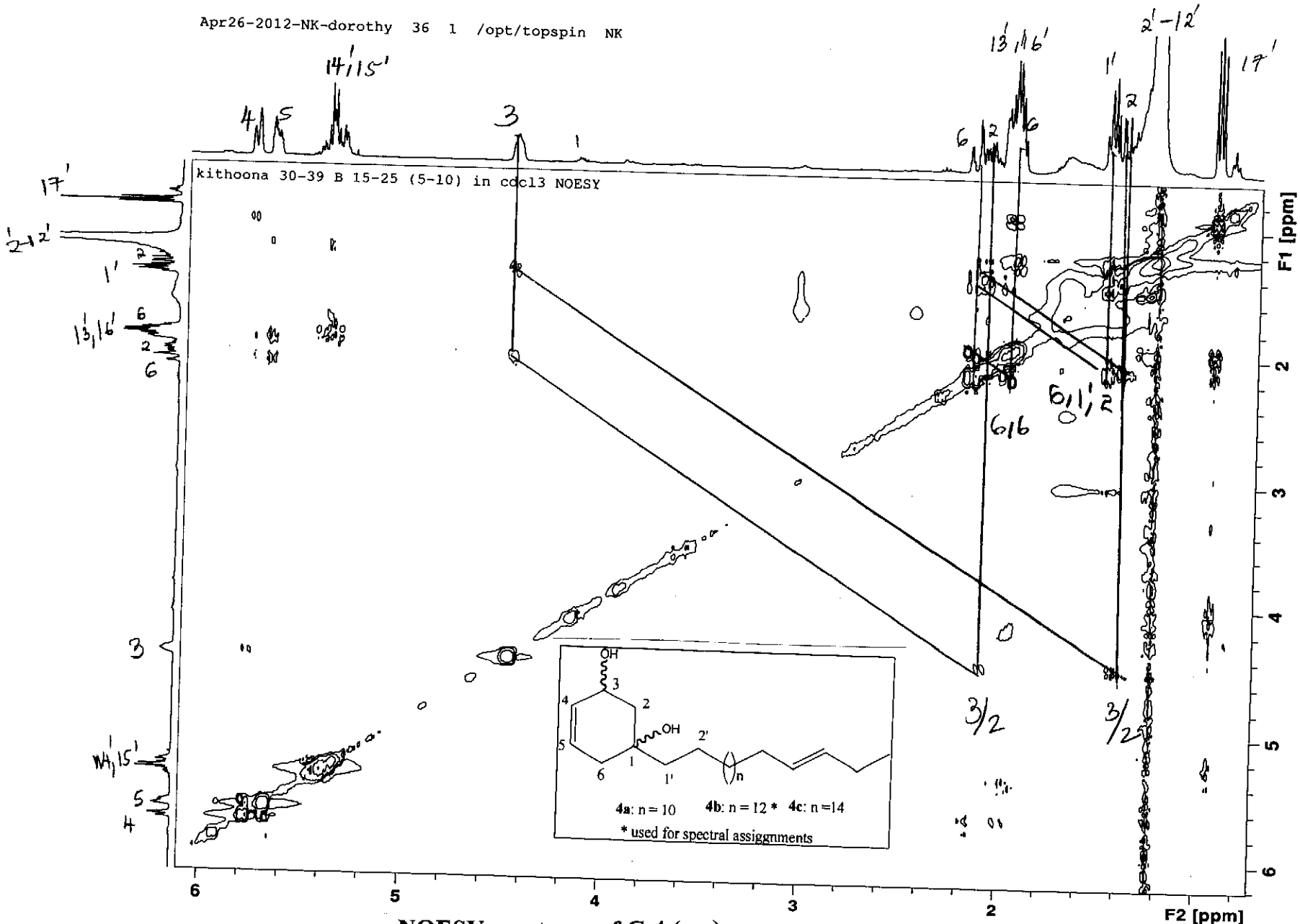
^{13}C NMR spectrum of C-4 (a-c)



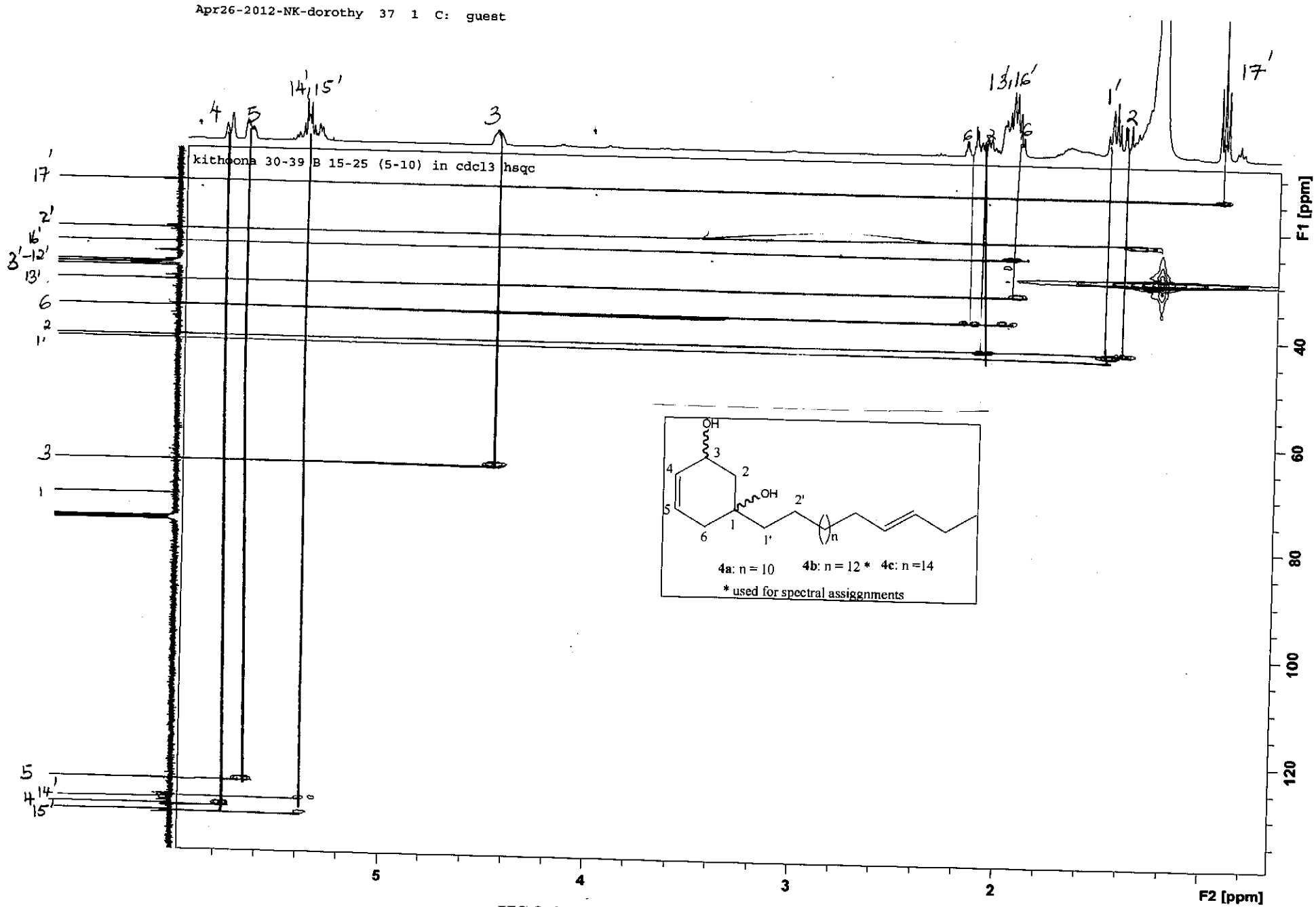
DEPT spectrum of C-4 (a-c)



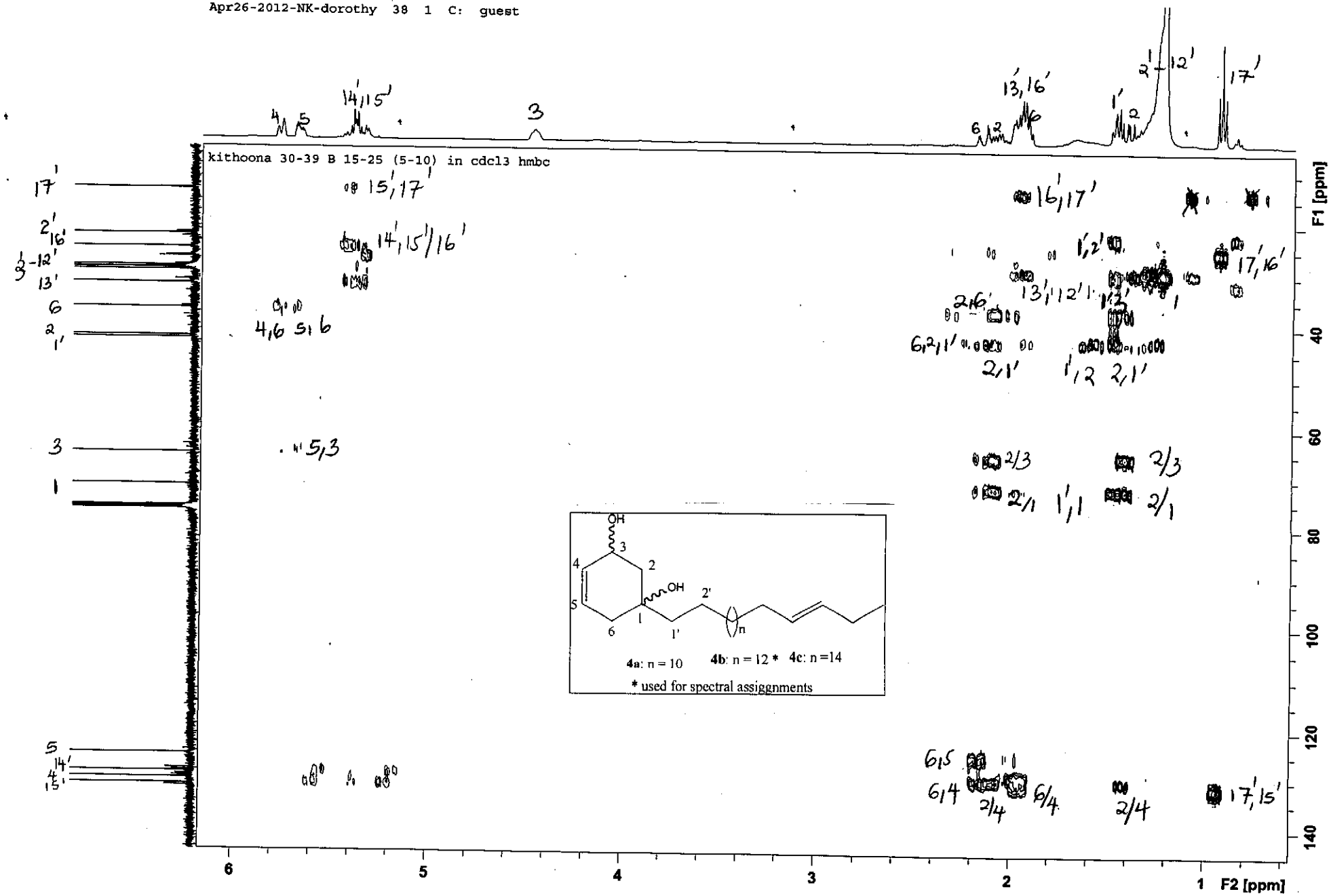
COSY spectrum of C-4 (a-c)



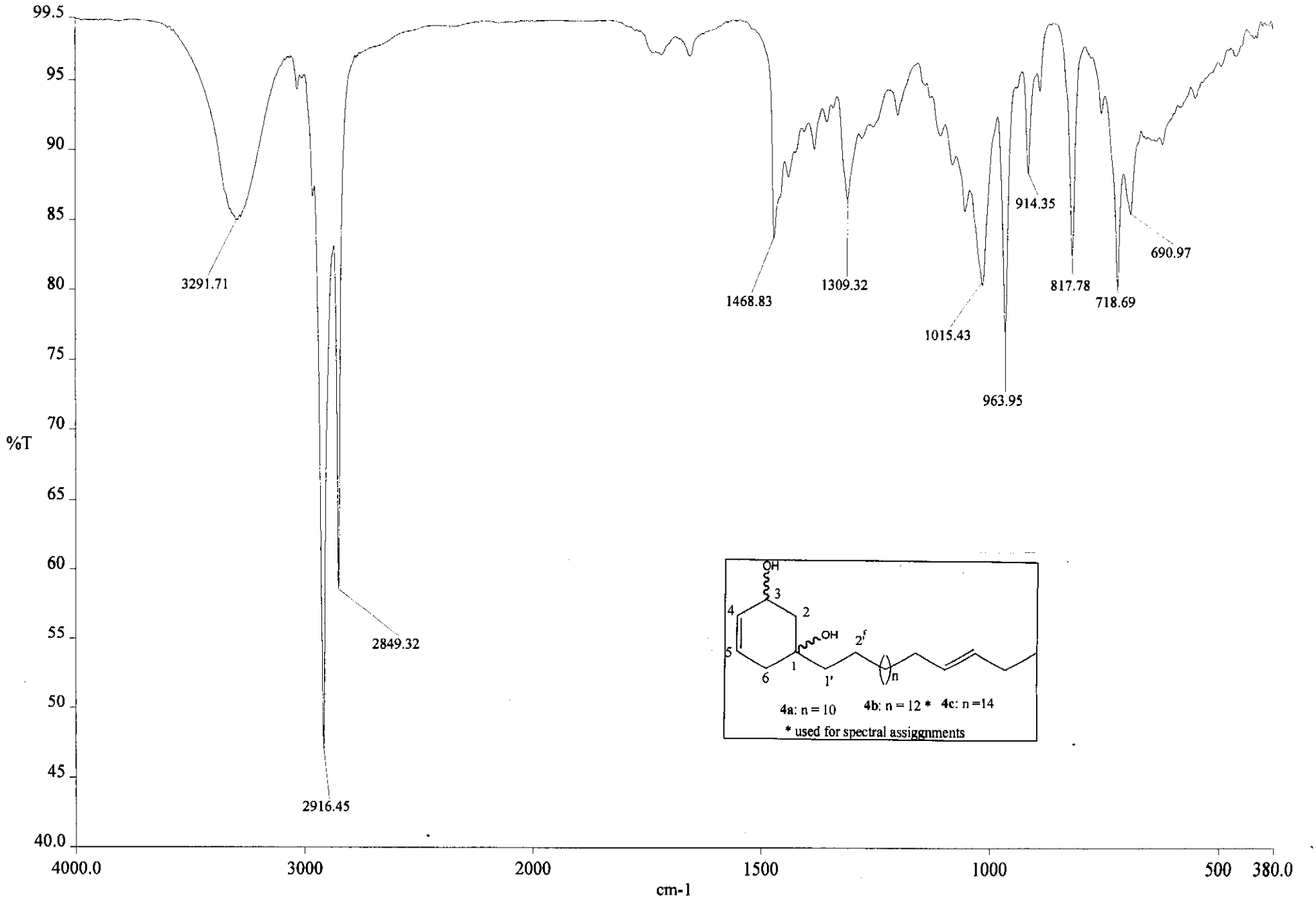
NOESY spectrum of C-4 (a-c)



HSQC spectrum of C-4 (a-c)



HMBC spectrum of C-4 (a-c)

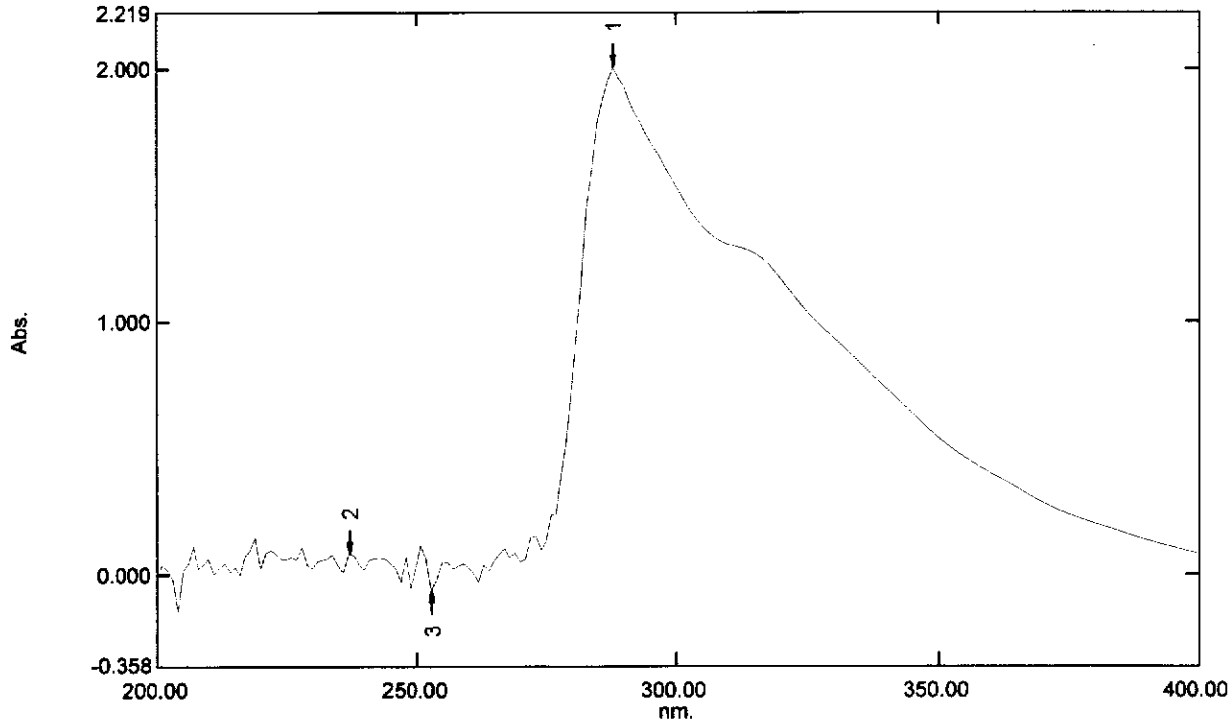


IR spectrum of C-4 (a-c)

Spectrum Peak Pick Report

08/04/2012 06:57:18 PM

Data Set: kithoona 30-39 B 45-55 (17-23) 4-10.spc - Storage 174453



No.	P/V	Wavelength	Abs.	Description
1	●	288.00	2.004	
2	●	237.00	0.087	
3	▼	253.00	-0.057	

Measurement Properties

Wavelength Range (nm.):	200.00 to 400.00
Scan Speed:	Medium
Sampling Interval:	1.0
Auto Sampling Interval:	Disabled
Scan Mode:	Single

Instrument Properties

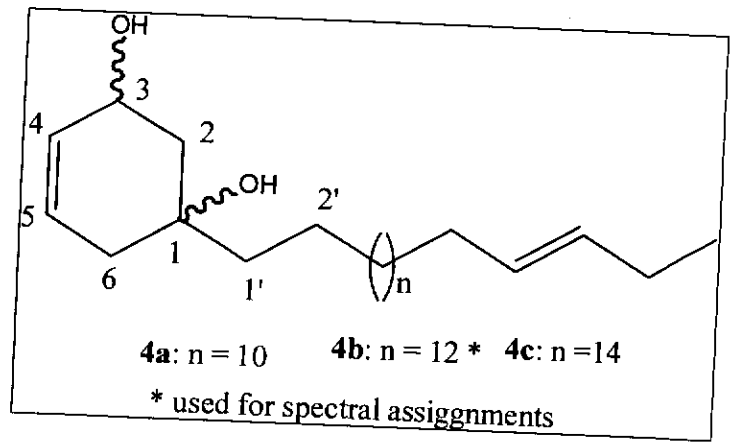
Instrument Type:	UV-3600 Series
Measuring Mode:	Absorbance
Slit Width:	2.0 nm
Time Constant:	0.1 sec.
Source Lamp:	Auto
Light Source Change Wavelength:	310.00 nm
Detector Unit:	Direct
Detector Change Wavelength:	830.00 nm 1800.00 nm
Grating Change Wavelength:	720.00 nm
S/R Exchange:	Normal
Detector Lock:	Auto
Slit Program:	Normal
Beam Mode:	Double
Stair Correction:	Disable

Attachment Properties

Attachment:	None

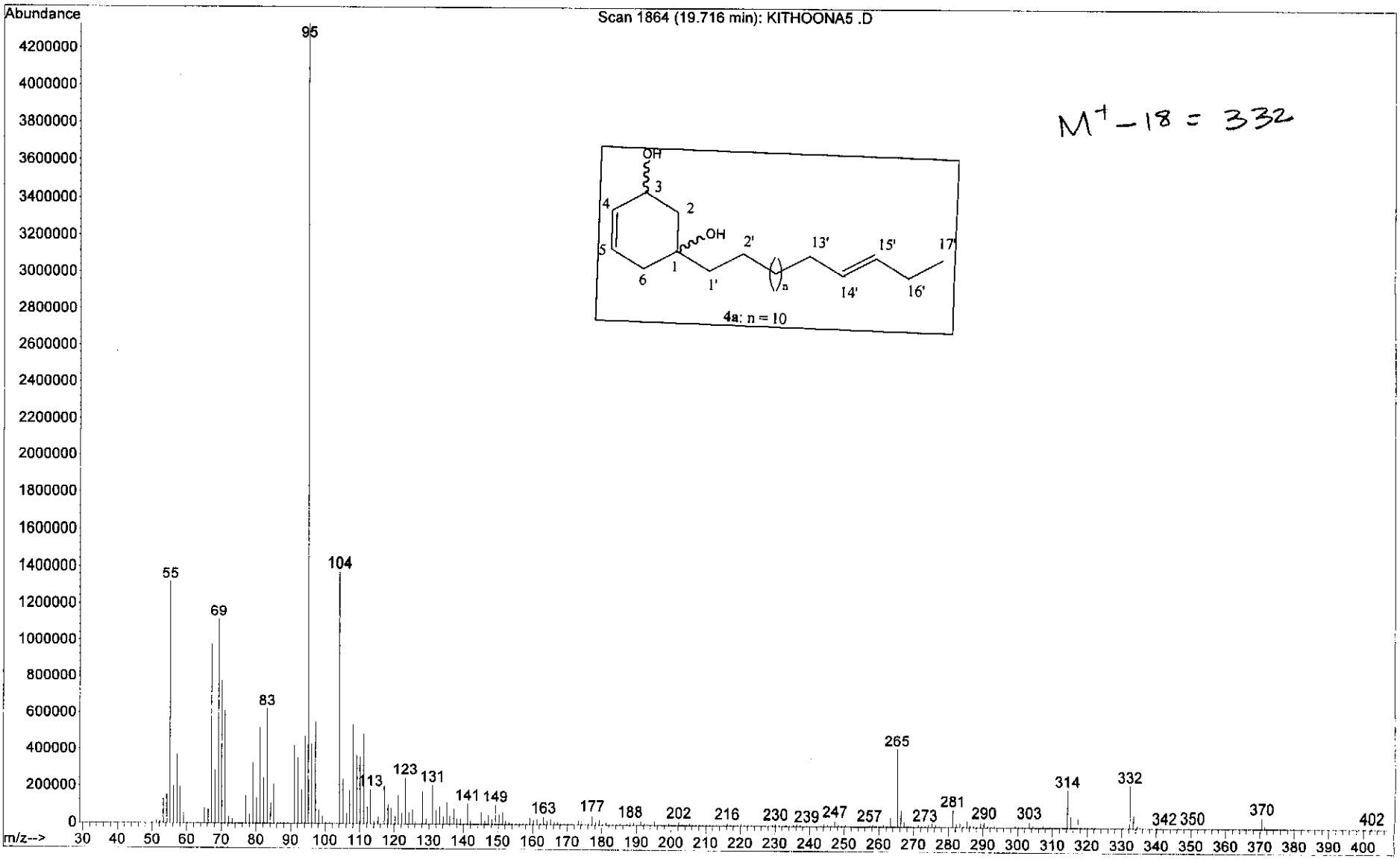
Sample Preparation Properties

Weight:	
Volume:	
Dilution:	
Path Length:	
Additional Information:	



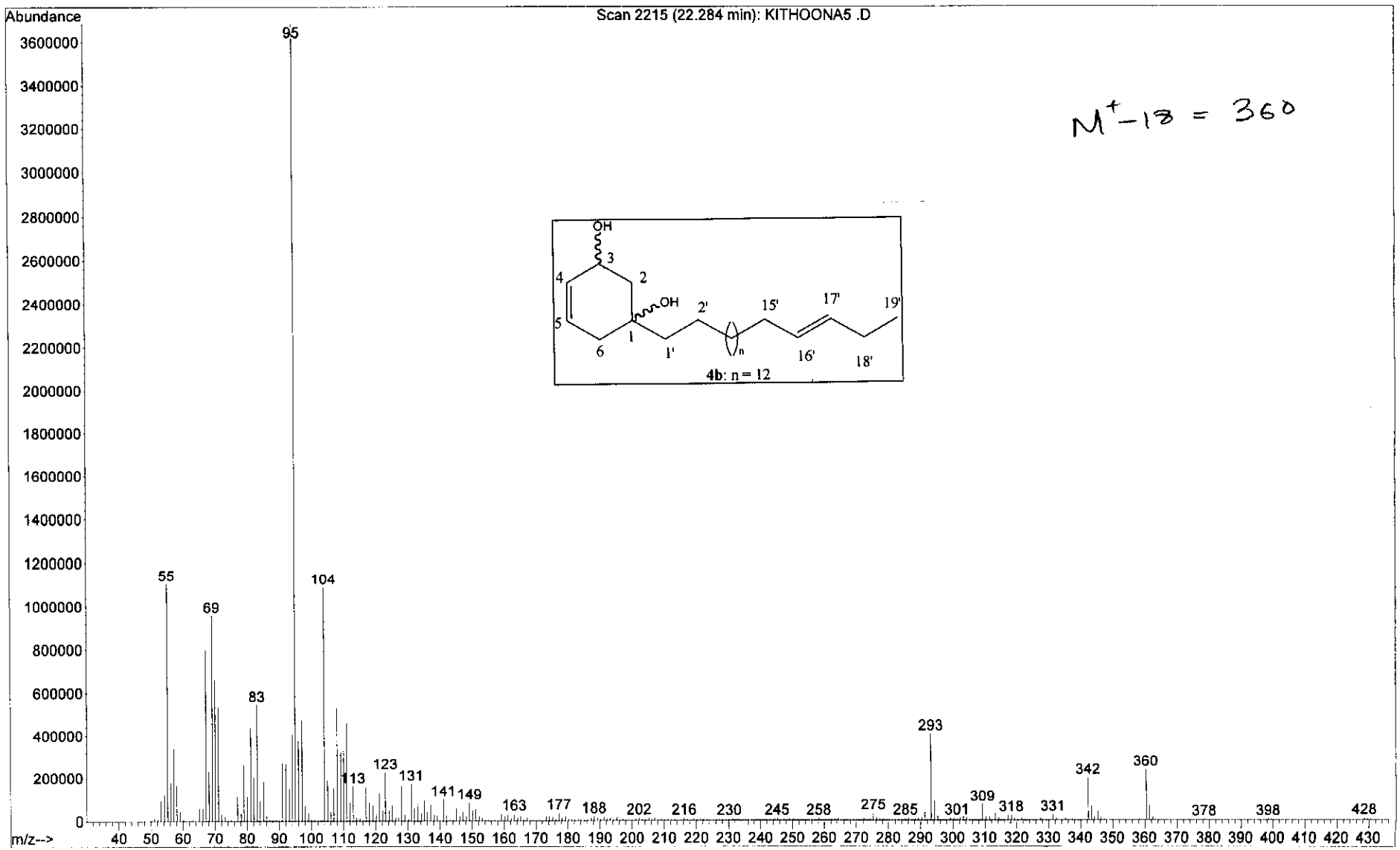
UV spectrum of C-4 (a-c)

File : C:\MSDCHEM\1\DATA\DOROTHY RAW DATA KITHOKIT\KITHOONA5 .D
Operator : Dorothy
Acquired : 5 May 2012 22:17 using AcqMethod NATPRODUCTS MANUAL
Instrument : 5973N
Sample Name: kithoona 30-39B 15-25 5-10
Misc Info :
Vial Number: 1



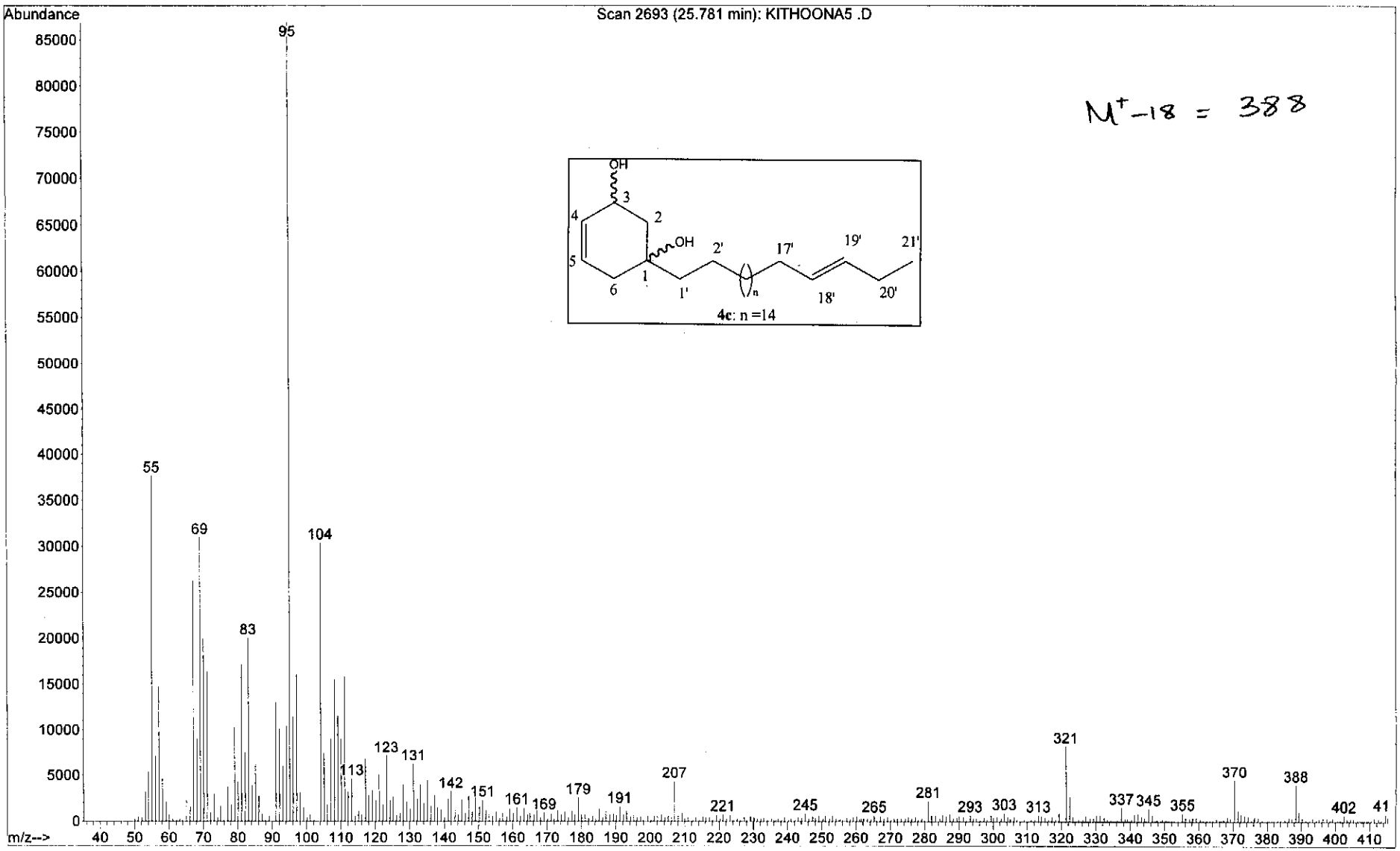
MS spectrum of C-4 a

File : C:\MSDCHEM\1\DATA\DOROTHY RAW DATA KITHOKIT\KITHOONA5 .D
Operator : Dorothy
Acquired : 5 May 2012 22:17 using AcqMethod NATPRODUCTS MANUAL
Instrument : 5973N
Sample Name: kithoona 30-39B 15-25 5-10
Misc Info :
Vial Number: 1

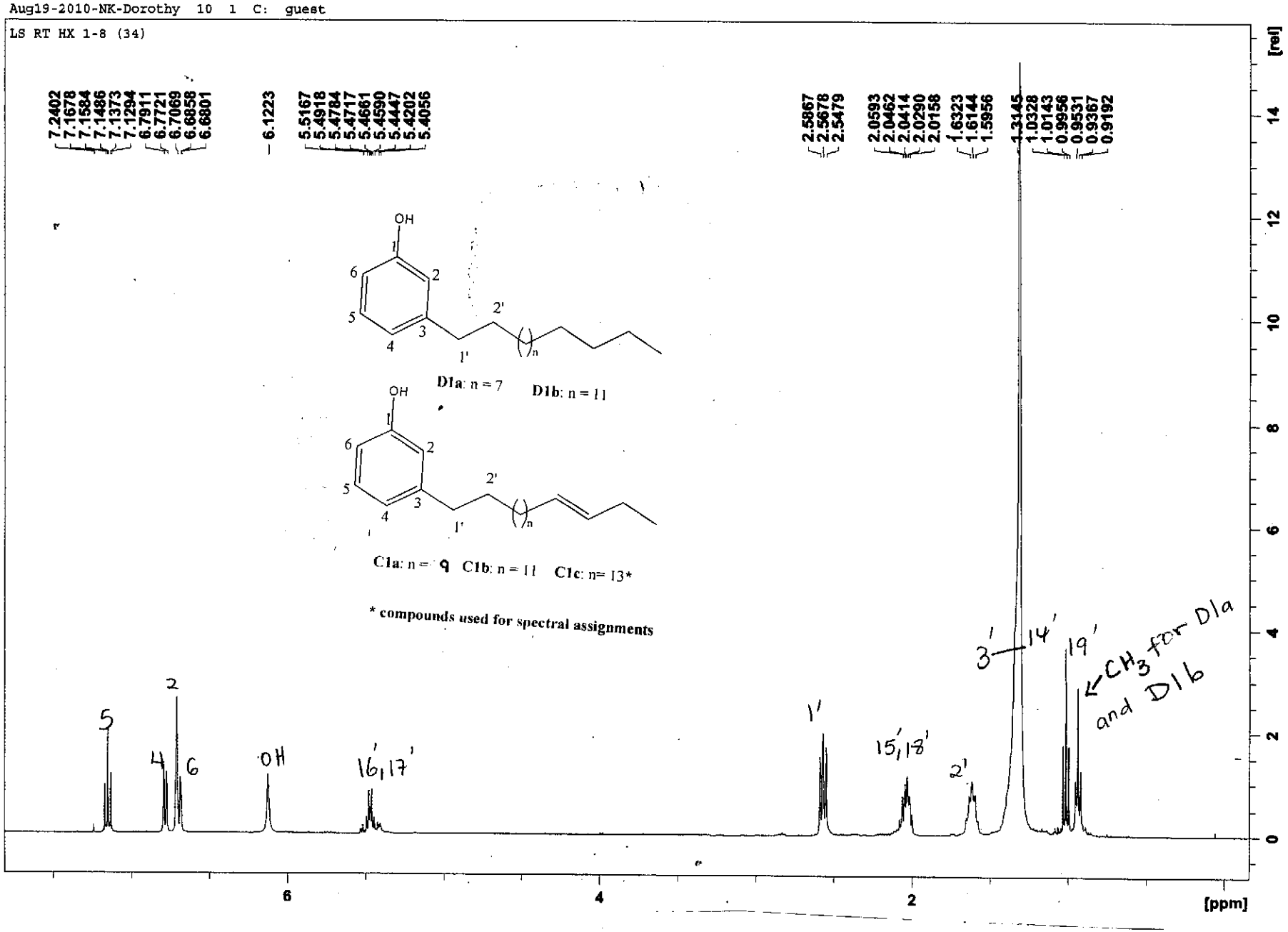


MS spectrum of C-4 b

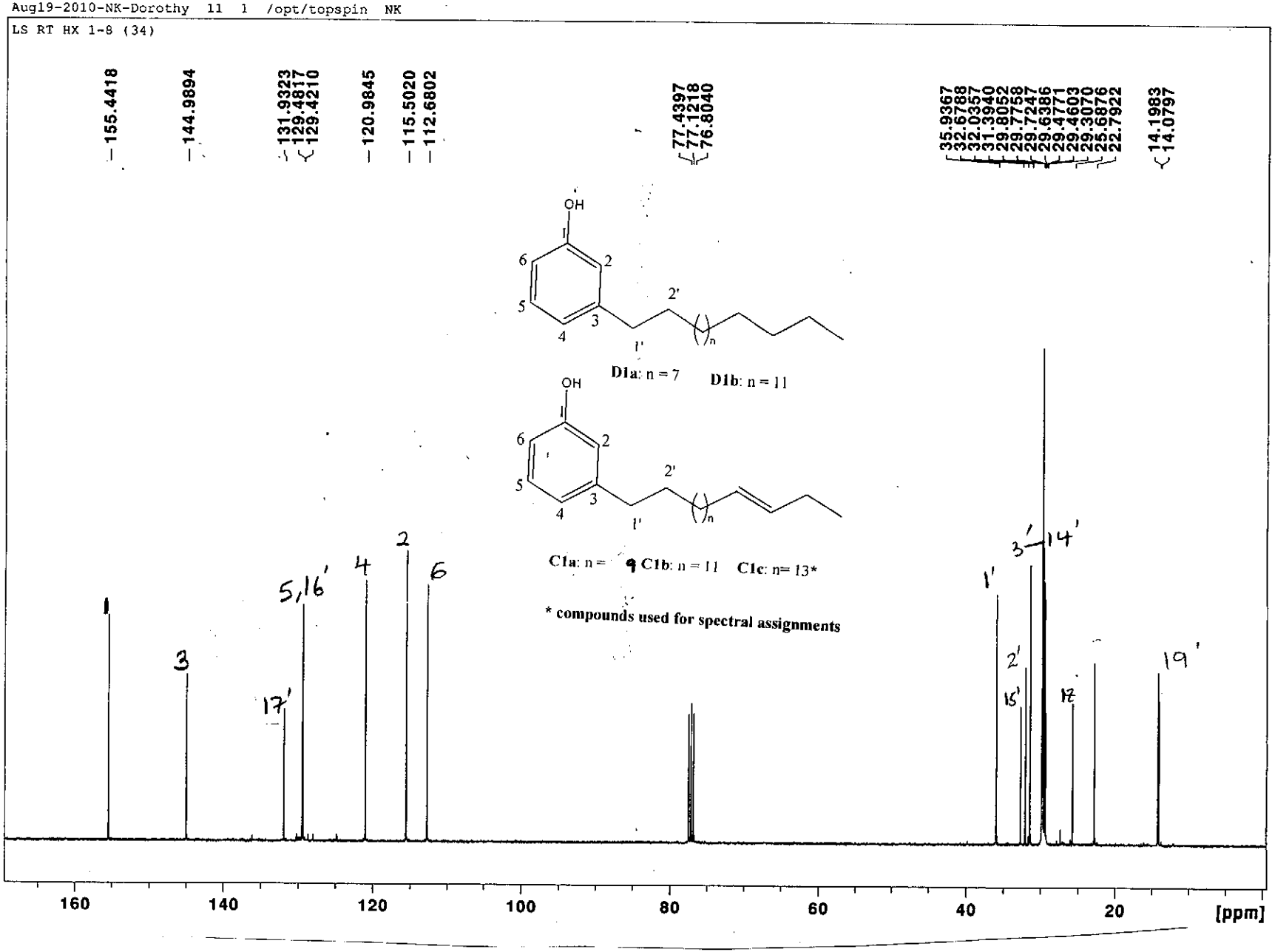
File : C:\MSDCHEM\1\DATA\DOROTHY RAW DATA KITHOKIT\KITHOONA5 .D
Operator : Dorothy
Acquired : 5 May 2012 22:17 using AcqMethod NATPRODUCTS MANUAL
Instrument : 5973N
Sample Name: kithoona 30-39B 15-25 5-10
Misc Info :
Vial Number: 1



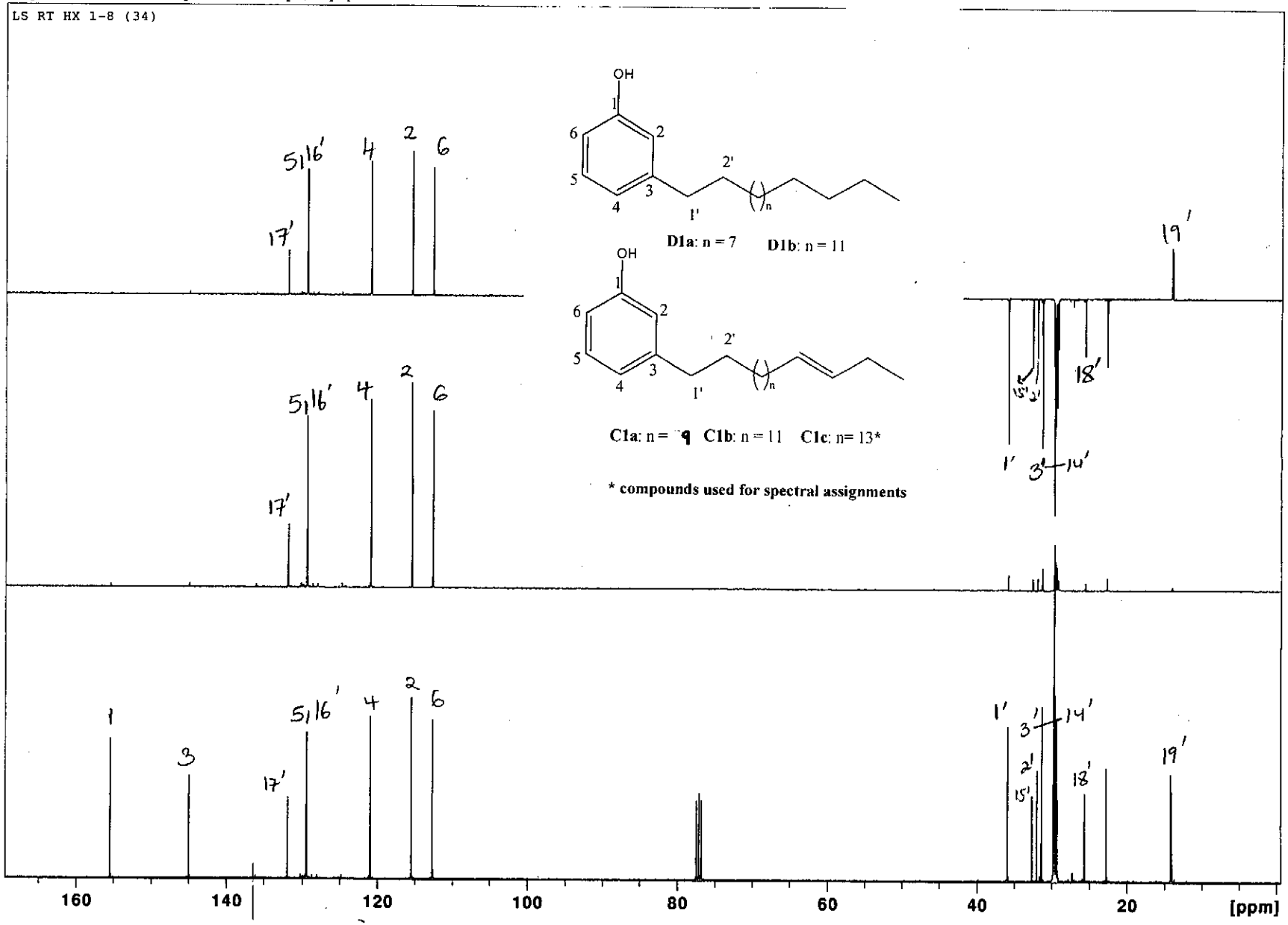
MS spectrum of C-4 c



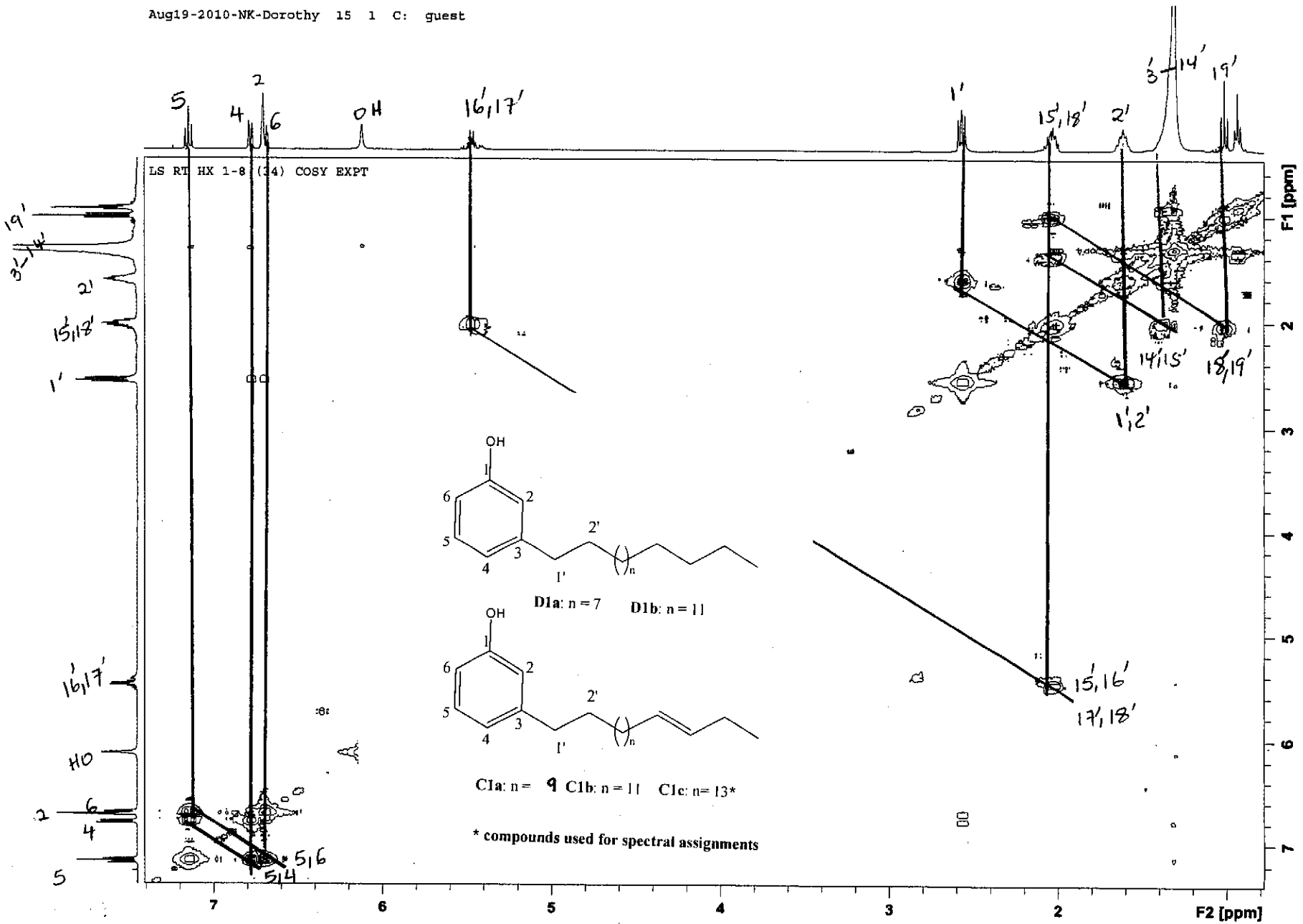
^{1}H NMR spectrum of a mixture of D1a, D1b, C1a, C1b and C1c



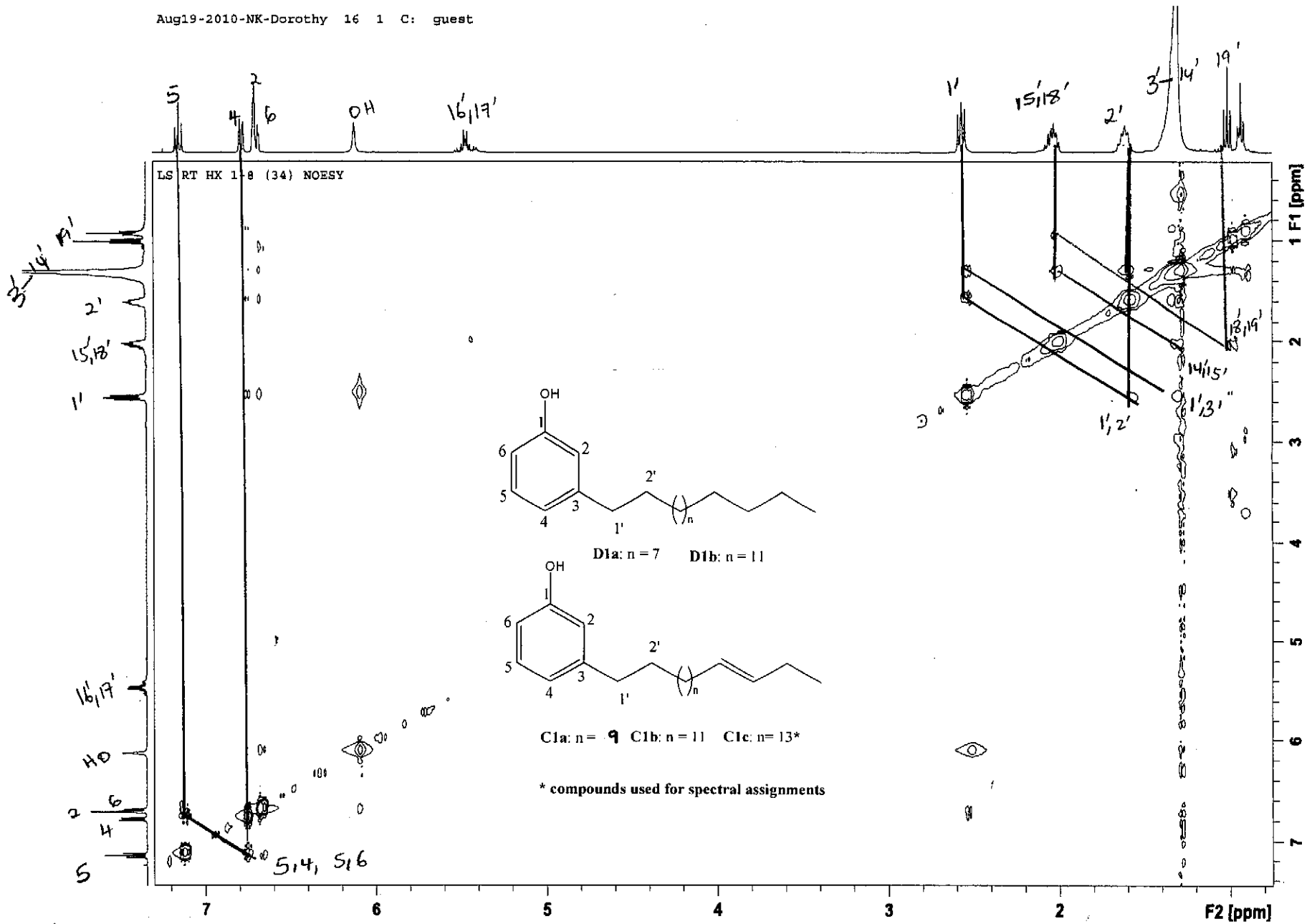
^{13}C NMR spectrum of a mixture of D1a, D1b, C1a, C1b and C1c



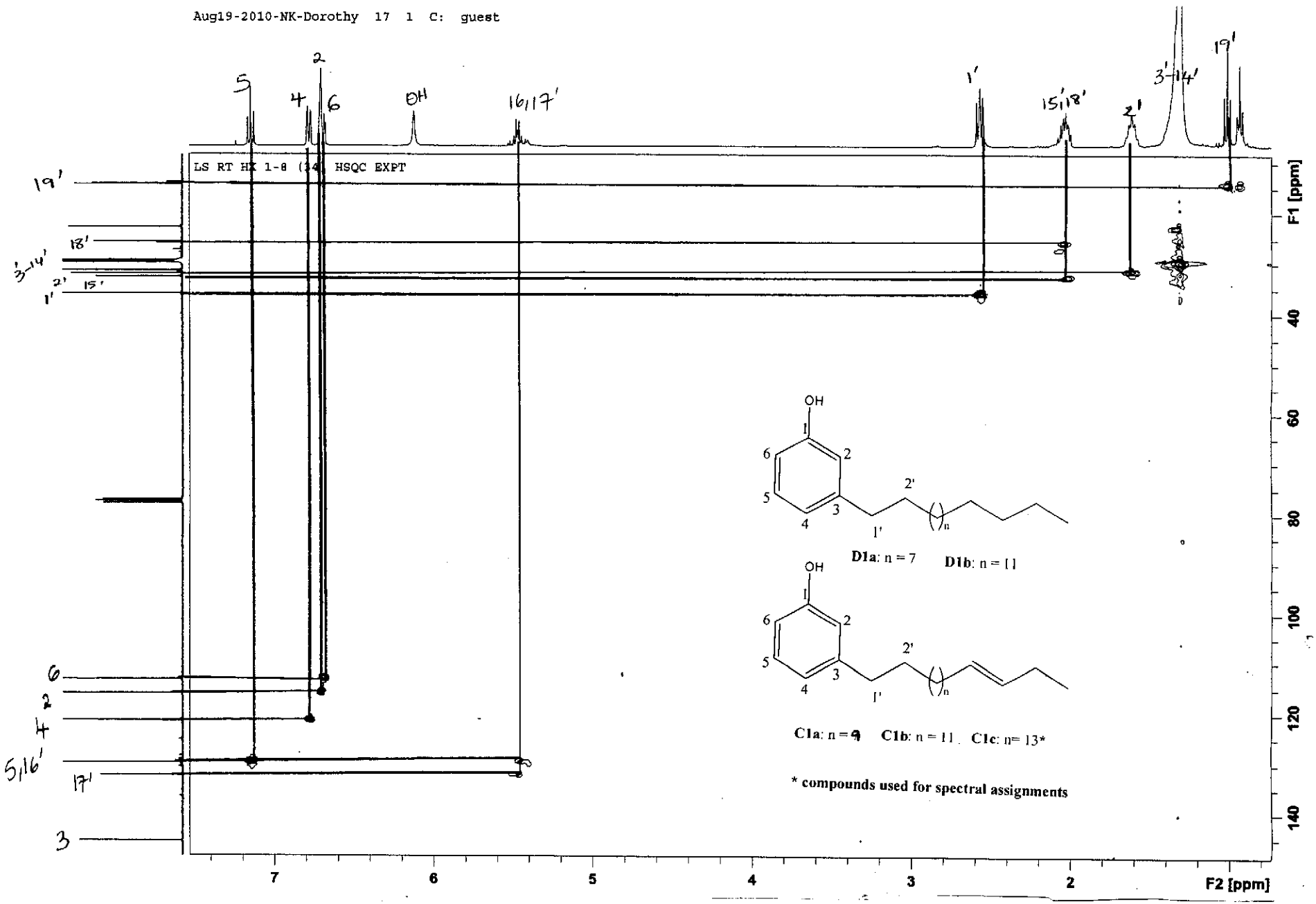
DEPT spectrum of a mixture of D1a, D1b, C1a, C1b and C1c



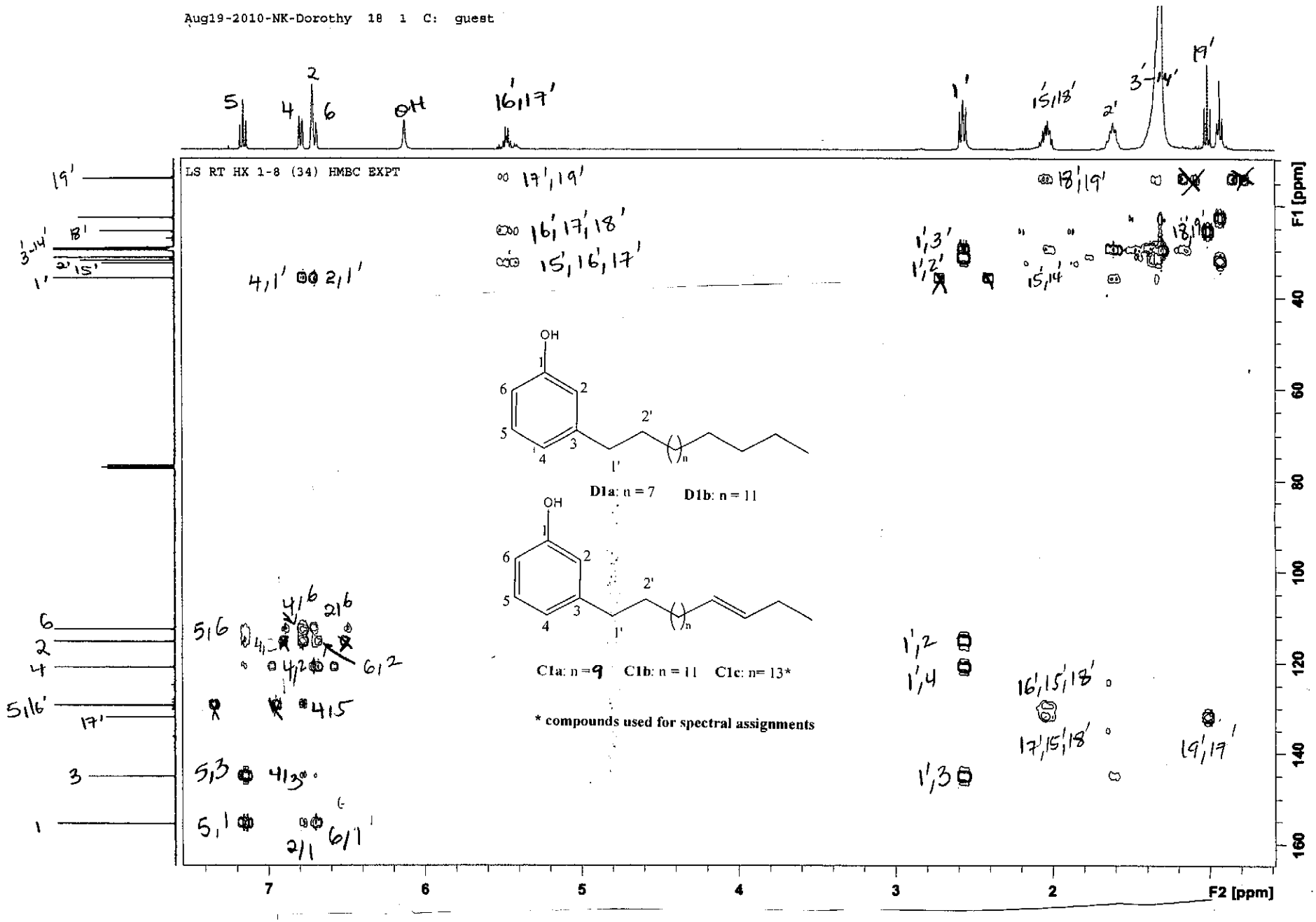
COSY spectrum of a mixture of D1a, D1b, C1a, C1b and C1c



NOESY spectrum of a mixture of D1a, D1b, C1a, C1b and C1c



HSQC spectrum of a mixture of D1a, D1b, C1a, C1b and C1c

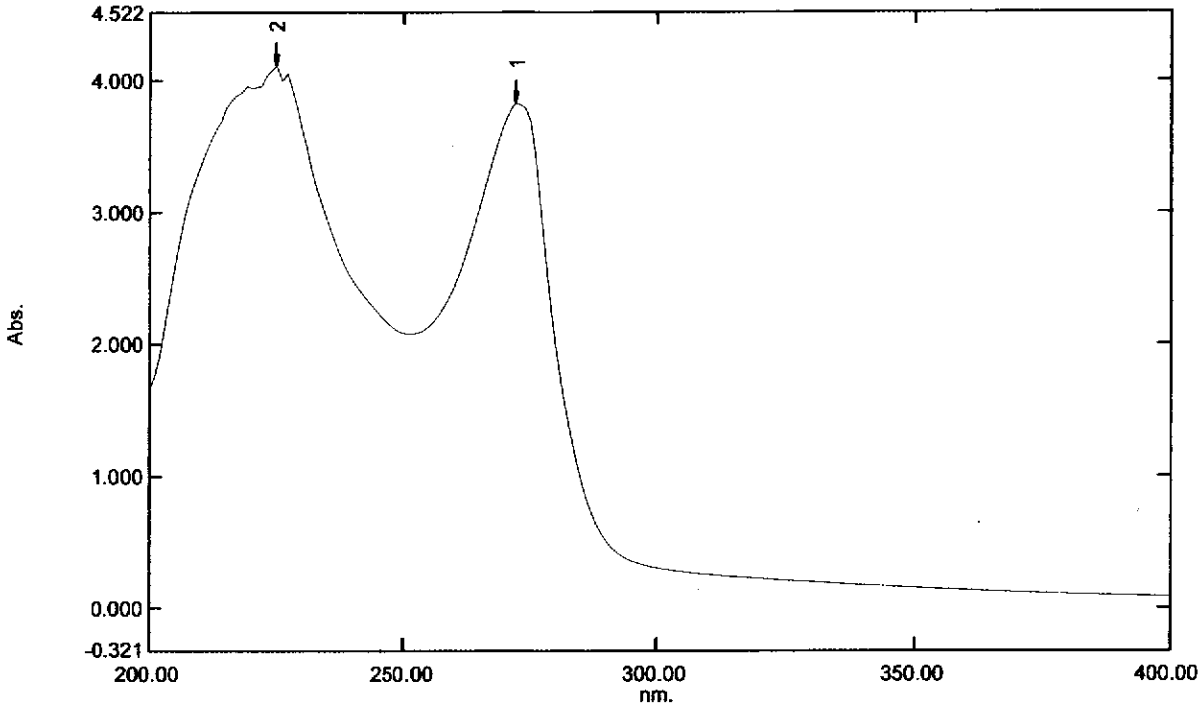


HMBC spectrum of a mixture of D1a, D1b, C1a, C1b and C1c

Spectrum Peak Pick Report

04/12/2011 08:32:12 PM

Data Set: EALSRT 42-57 CARDANOL.spc - Storage 203127



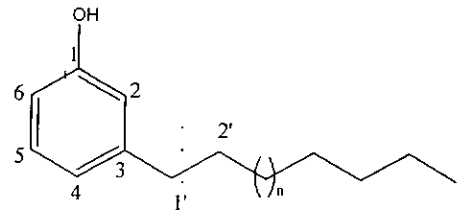
No.	P/V	Wavelength	Abs.	Description
1	●	272.00	3.828	
2	●	225.00	4.118	
3	●	251.00	2.077	

Measurement Properties
Wavelength Range (nm.): 200.00 to 400.00
Scan Speed: Medium
Sampling Interval: 1.0
Auto Sampling Interval: Disabled
Scan Mode: Single

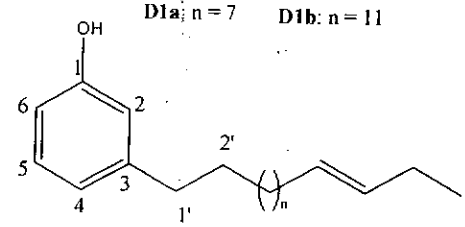
Instrument Properties
Instrument Type: UV-3600 Series
Measuring Mode: Absorbance
Slit Width: 8.0 nm
Time Constant: 0.1 sec.
Source Lamp: Auto
Light Source Change Wavelength: 310.00 nm
Detector Unit: Direct
Detector Change Wavelength: 830.00 nm 1800.00 nm
Grating Change Wavelength: 720.00 nm
S/R Exchange: Normal
Detector Lock: Auto
Slit Program: Normal
Beam Mode: Double
Stair Correction: Disable

Attachment Properties
Attachment: None

Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length:
Additional Information:



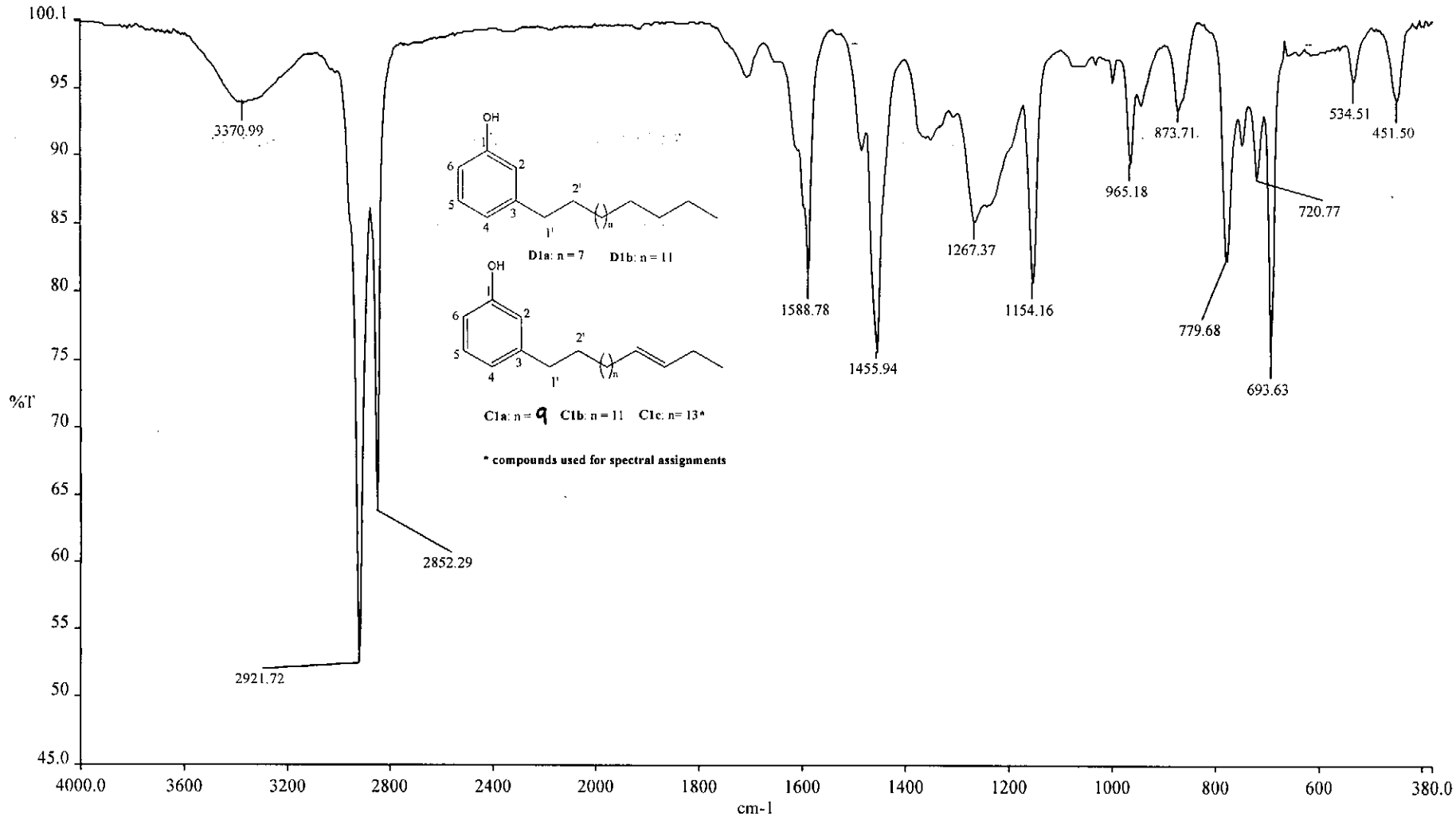
D1a: n = 7 **D1b**: n = 11



C1a: n = 9 **C1b**: n = 11 **C1c**: n= 13*

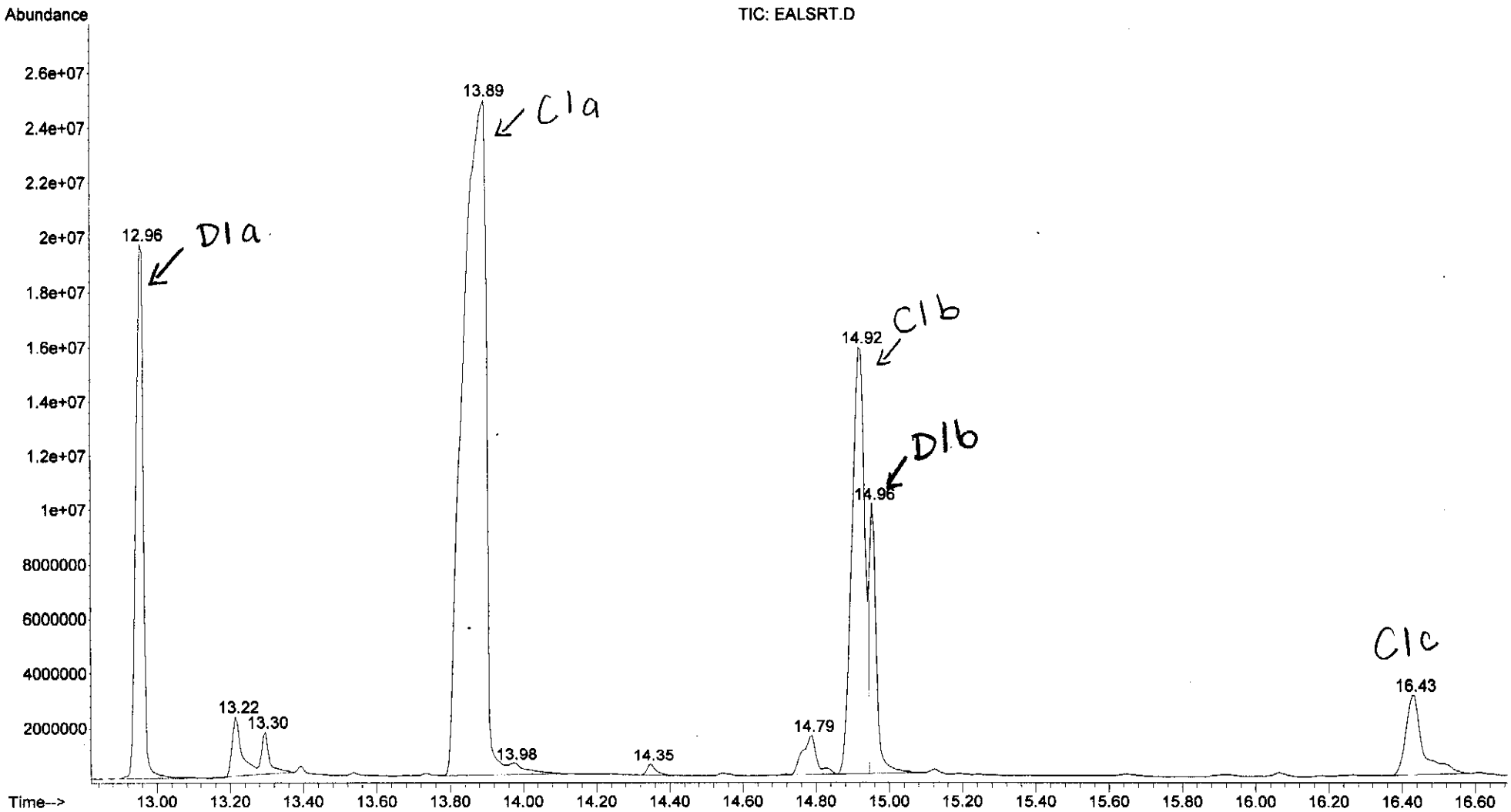
* compounds used for spectral assignments

UV spectrum of a mixture of D1a, D1b, C1a, C1b and C1c



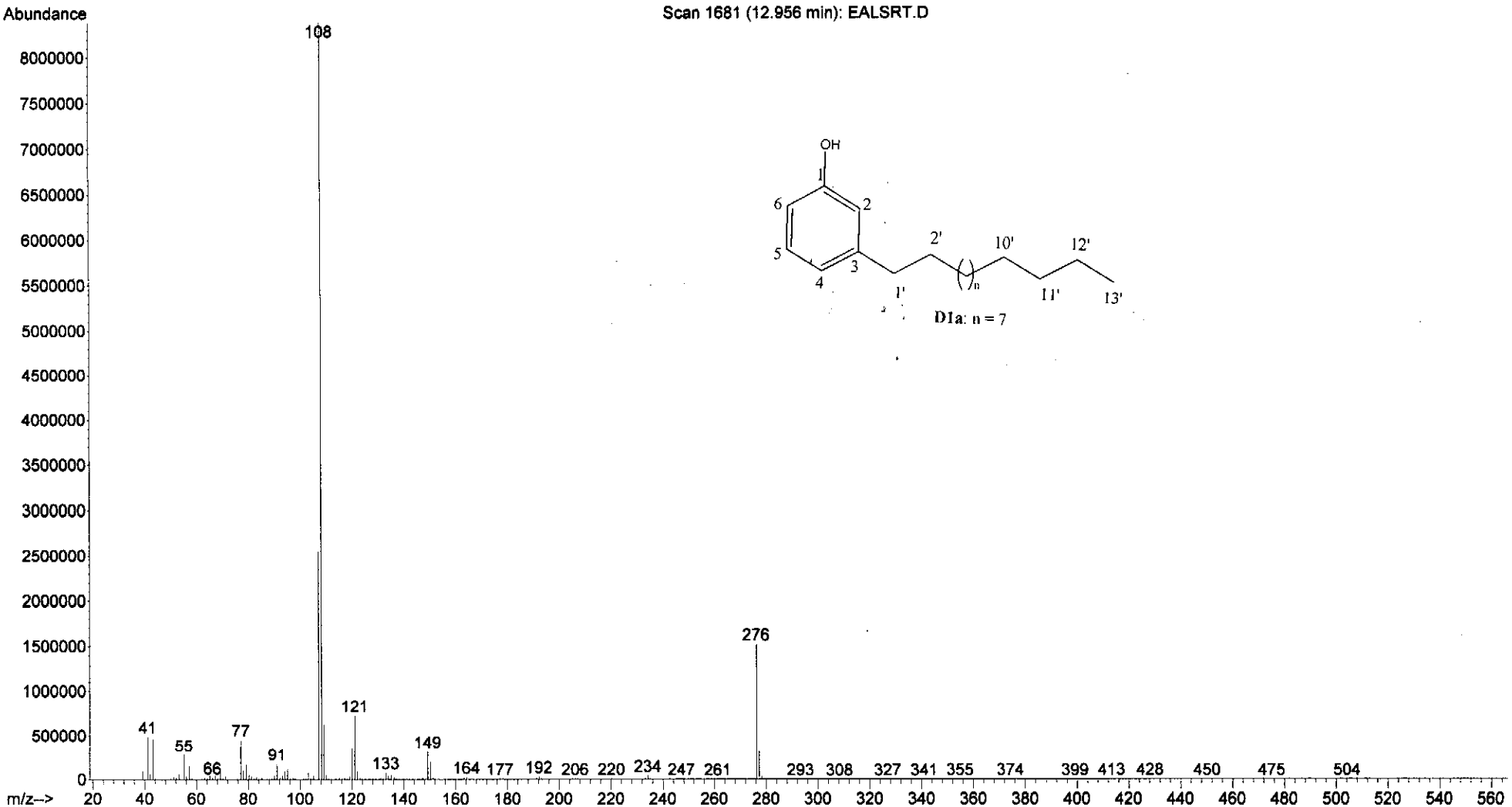
IR spectrum of a mixture of D1a, D1b, C1a, C1b and C1c

File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\EALSRT.D
Operator : Dorothy
Acquired : 2 May 2011 11:14 using AcqMethod NATURALJ
Instrument : Instrumen
Sample Name: EALSRT 1
Misc Info :
Vial Number: 1



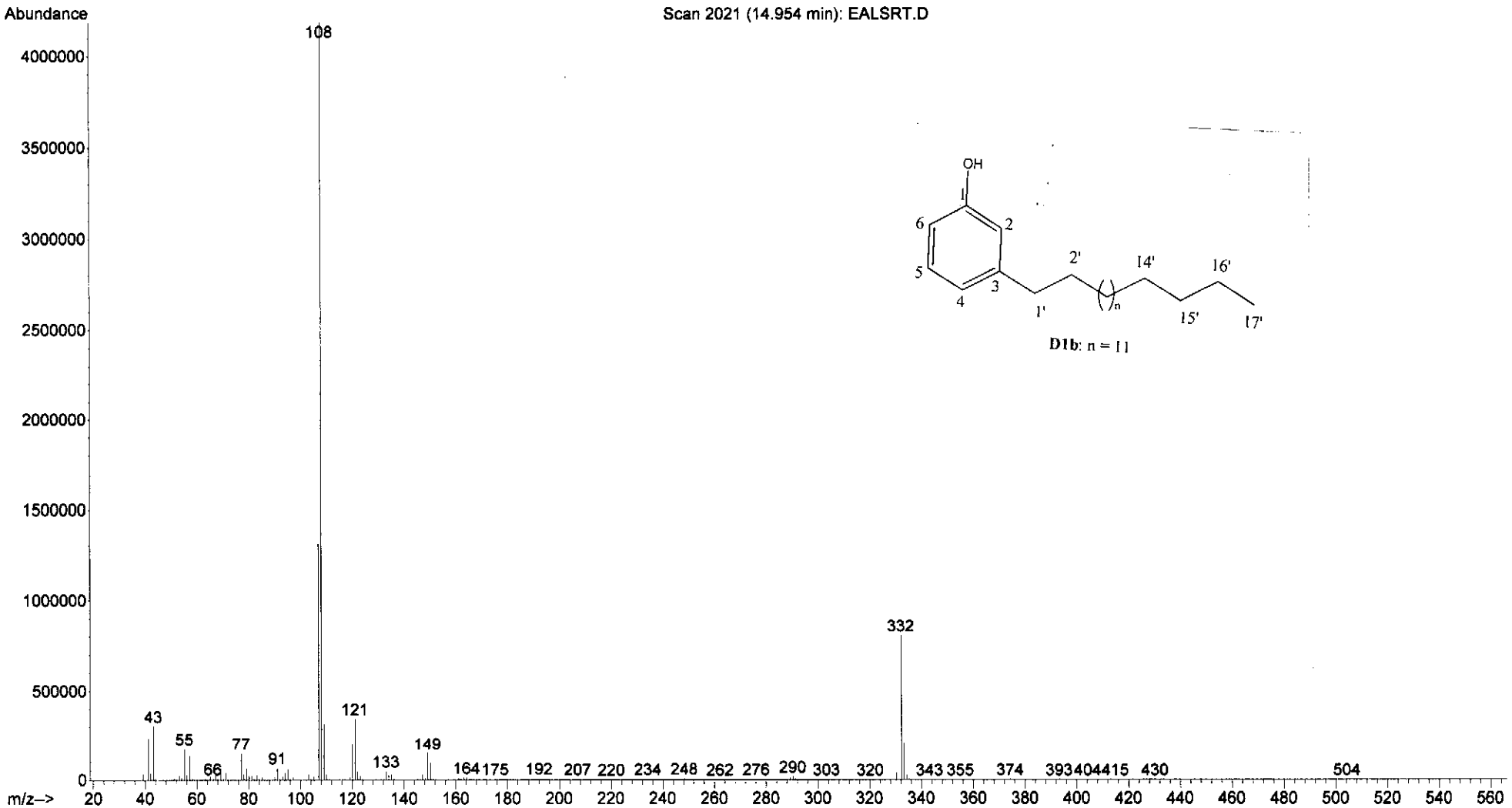
GC chromatogram of a mixture of D1a, D1b, C1a, C1b and C1c

File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\DORTHY\EALSRT.D
Operator : Dorothy
Acquired : 2 May 2011 11:14 using AcqMethod NATURALJ
Instrument : Instrumen
Sample Name: EALSRT 1
Misc Info :
Vial Number: 1



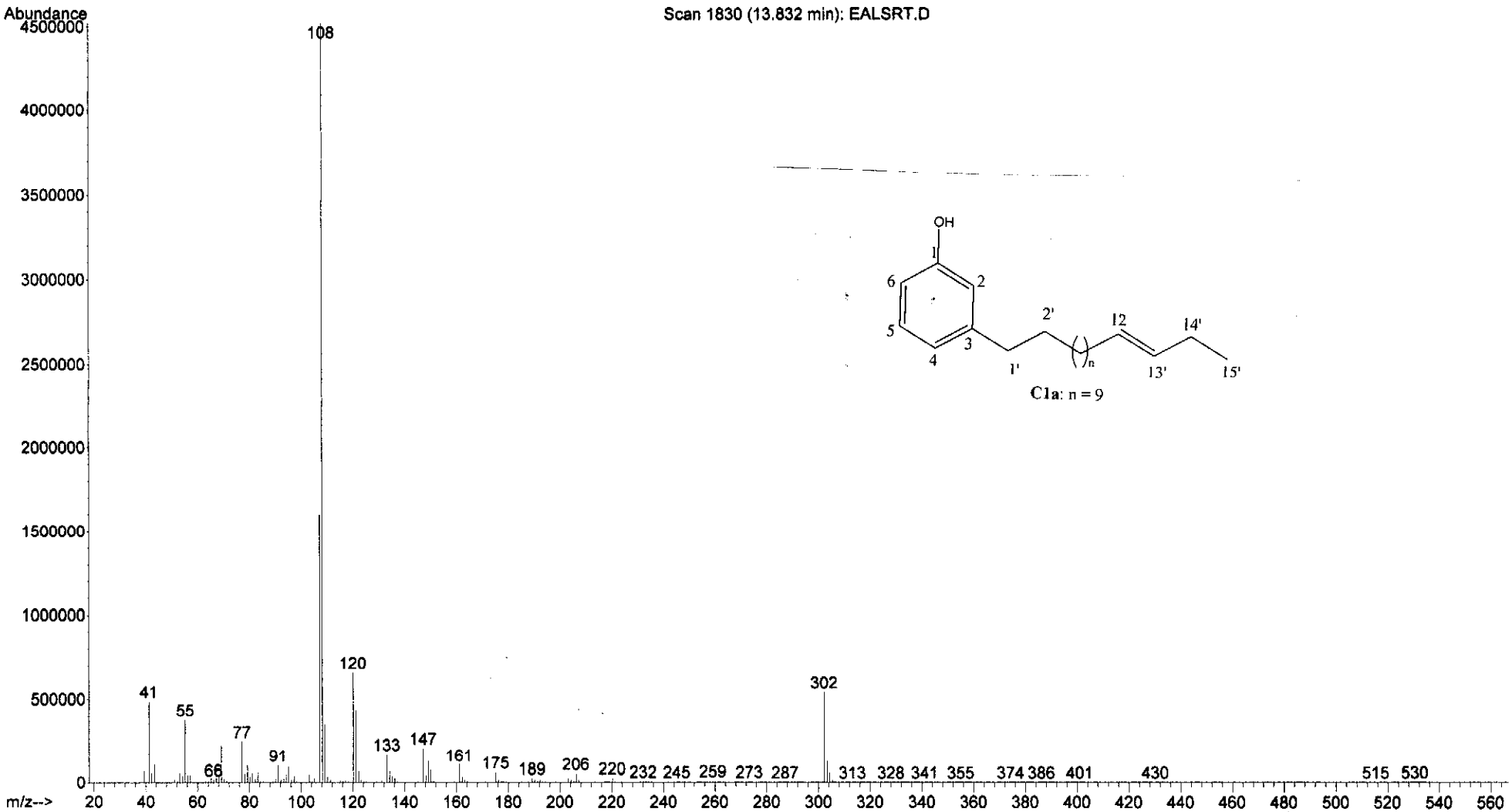
Mass spectrum of D1a

File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\EALSRT.D
Operator : Dorothy
Acquired : 2 May 2011 11:14 using AcqMethod NATURALJ
Instrument : Instrumen
Sample Name: EALSRT 1
Misc Info :
Vial Number: 1



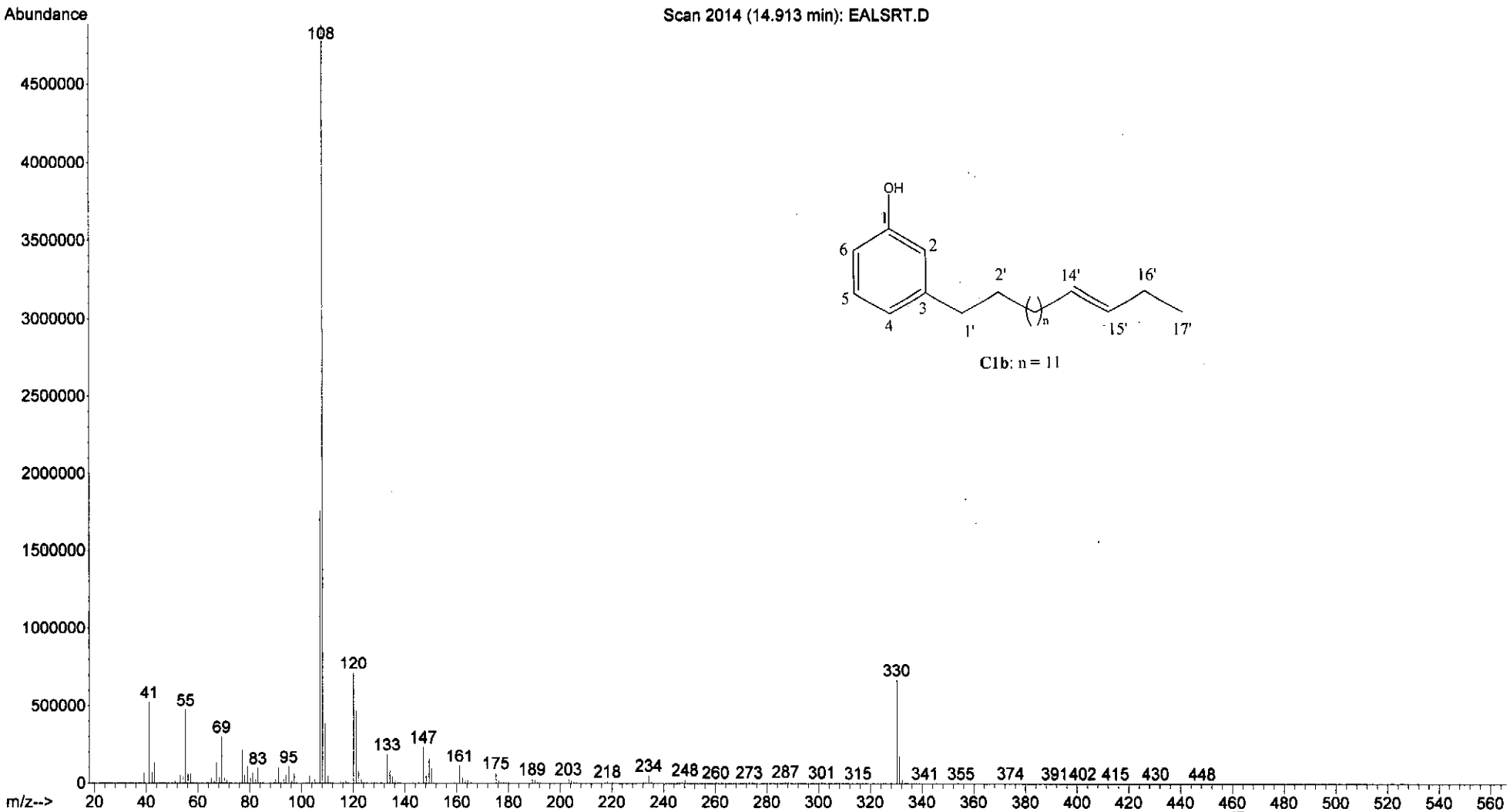
Mass spectrum of D1b

File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\EALSRT.D
Operator : Dorothy
Acquired : 2 May 2011 11:14 using AcqMethod NATURALJ
Instrument : Instrumen
Sample Name: EALSRT 1
Misc Info :
Vial Number: 1



Mass spectrum of C1a

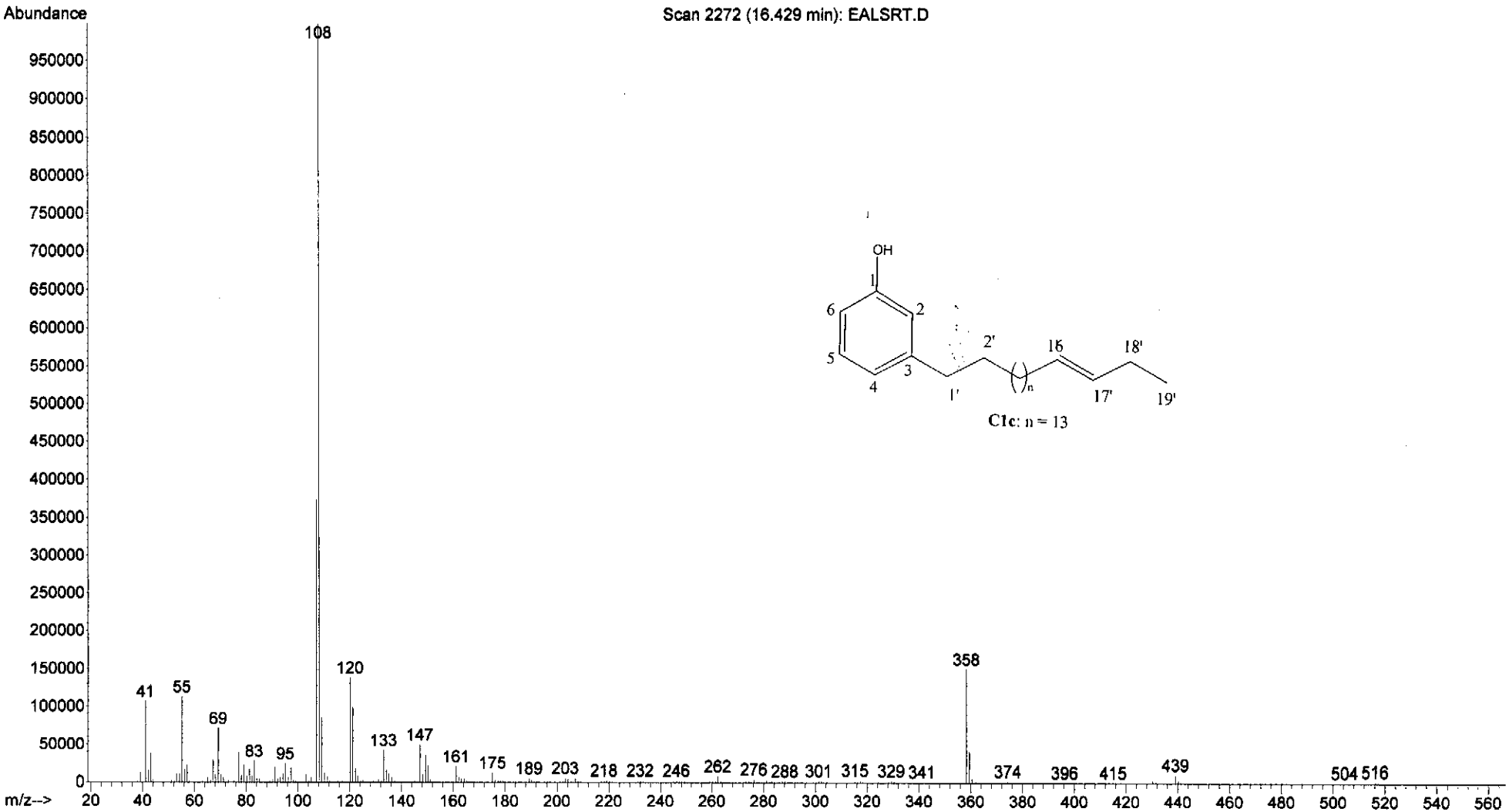
File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\EALSRT.D
Operator : Dorothy
Acquired : 2 May 2011 11:14 using AcqMethod NATURALJ
Instrument : Instrumen
Sample Name: EALSRT 1
Misc Info :
Vial Number: 1



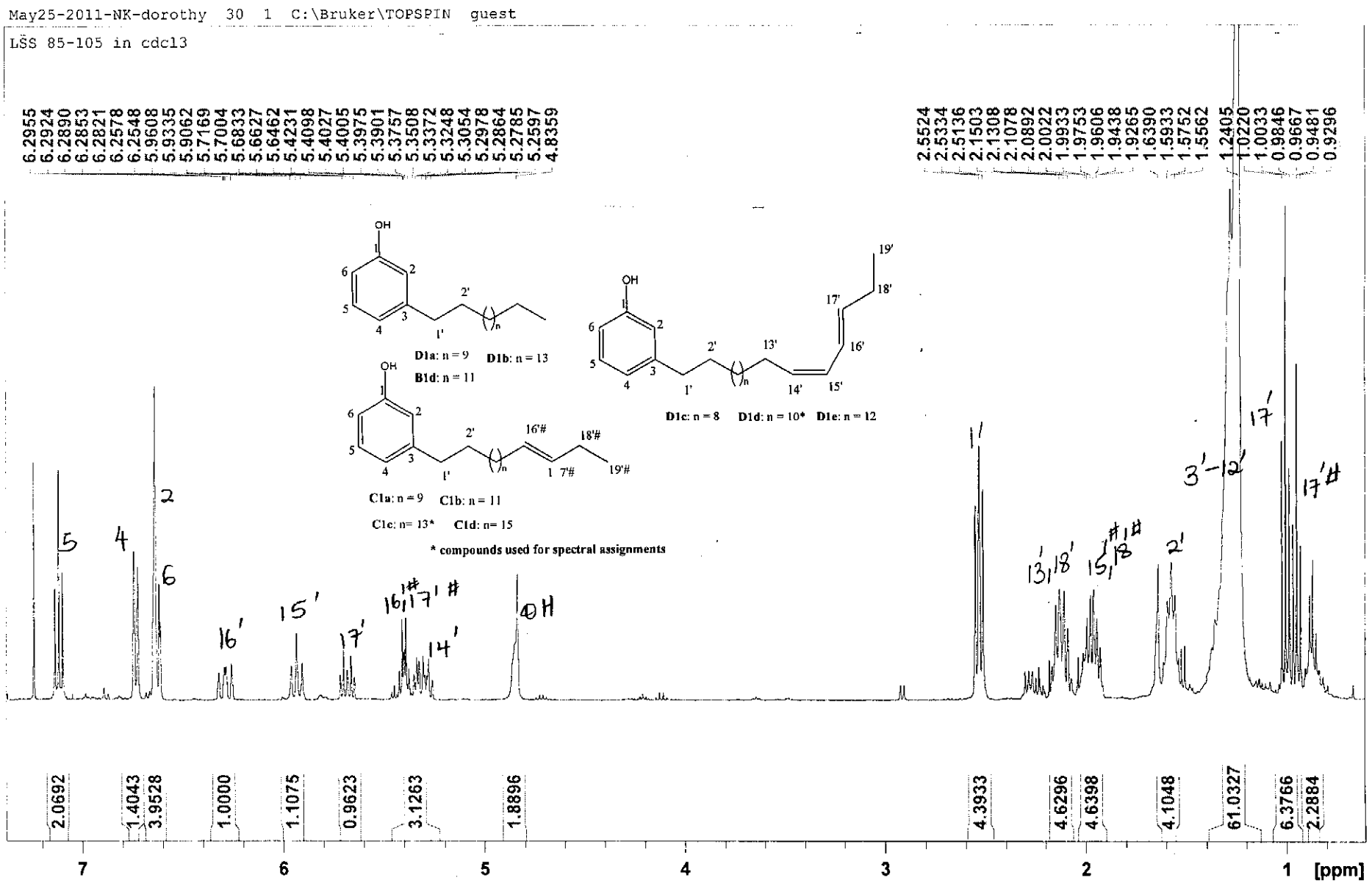
C1b: n = 11

Mass spectrum of C1b

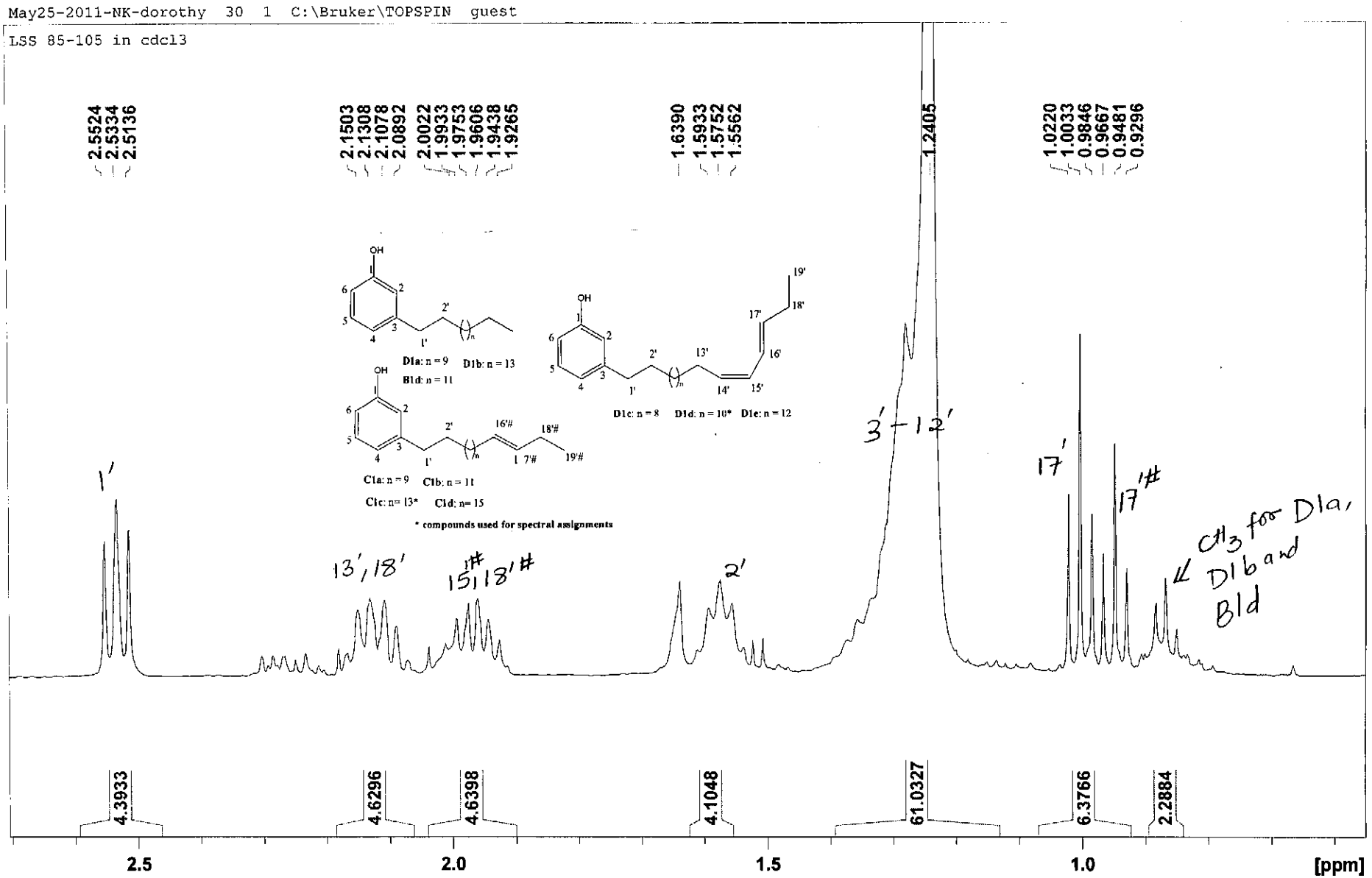
File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\EALSRT.D
Operator : Dorothy
Acquired : 2 May 2011 11:14 using AcqMethod NATURALJ
Instrument : Instrumen
Sample Name: EALSRT 1
Misc Info :
Vial Number: 1



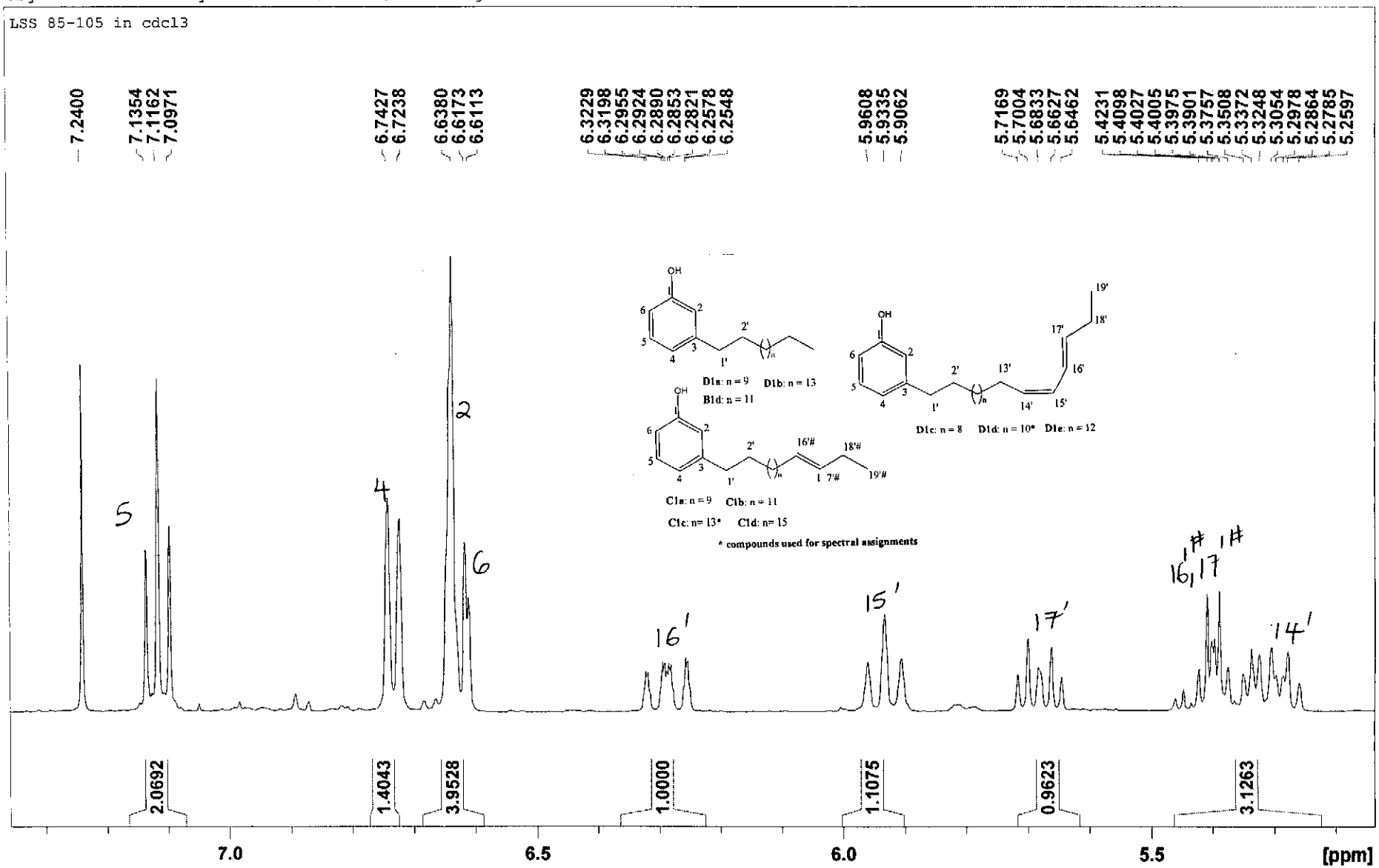
Mass spectrum of C1c



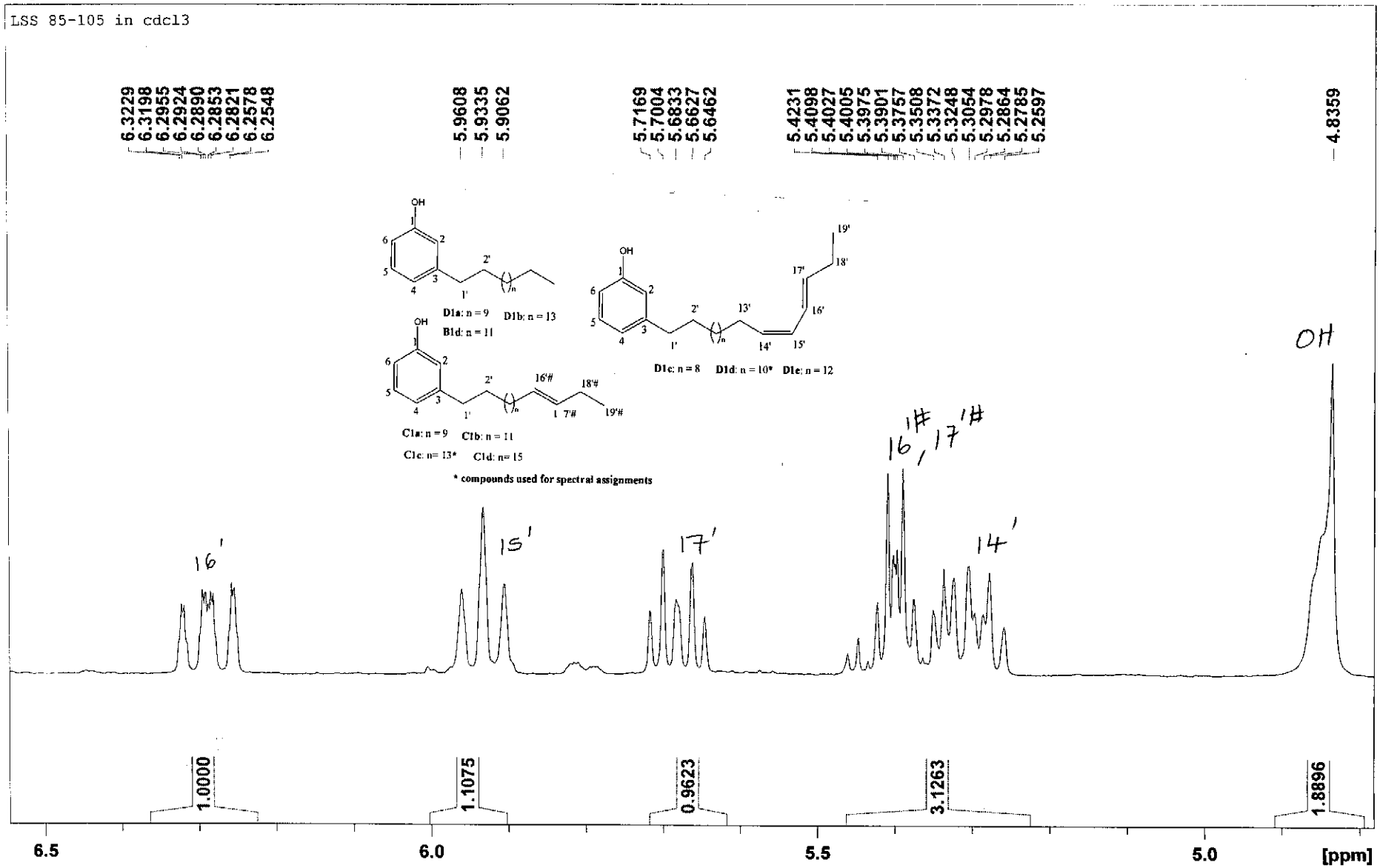
^{1}H NMR spectrum of a mixture of D1a, D1b, D1c, D1d, D1e, B1d, C1a, C1b, C1c and C1d



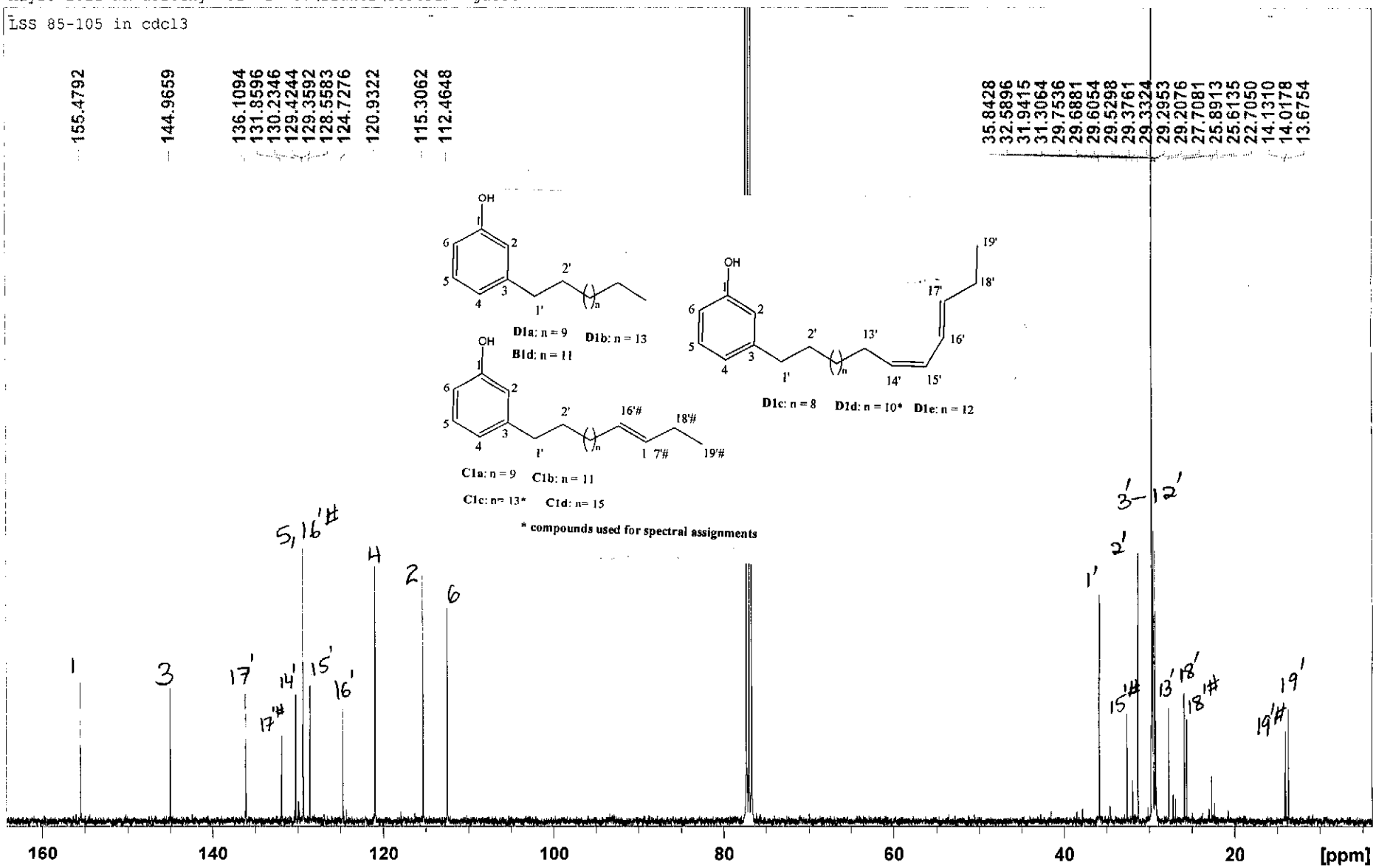
^{1}H NMR spectrum of a mixture of D1a, D1b, D1c, D1d, D1e, B1d, C1a, C1b, C1c and C1d (expanded)



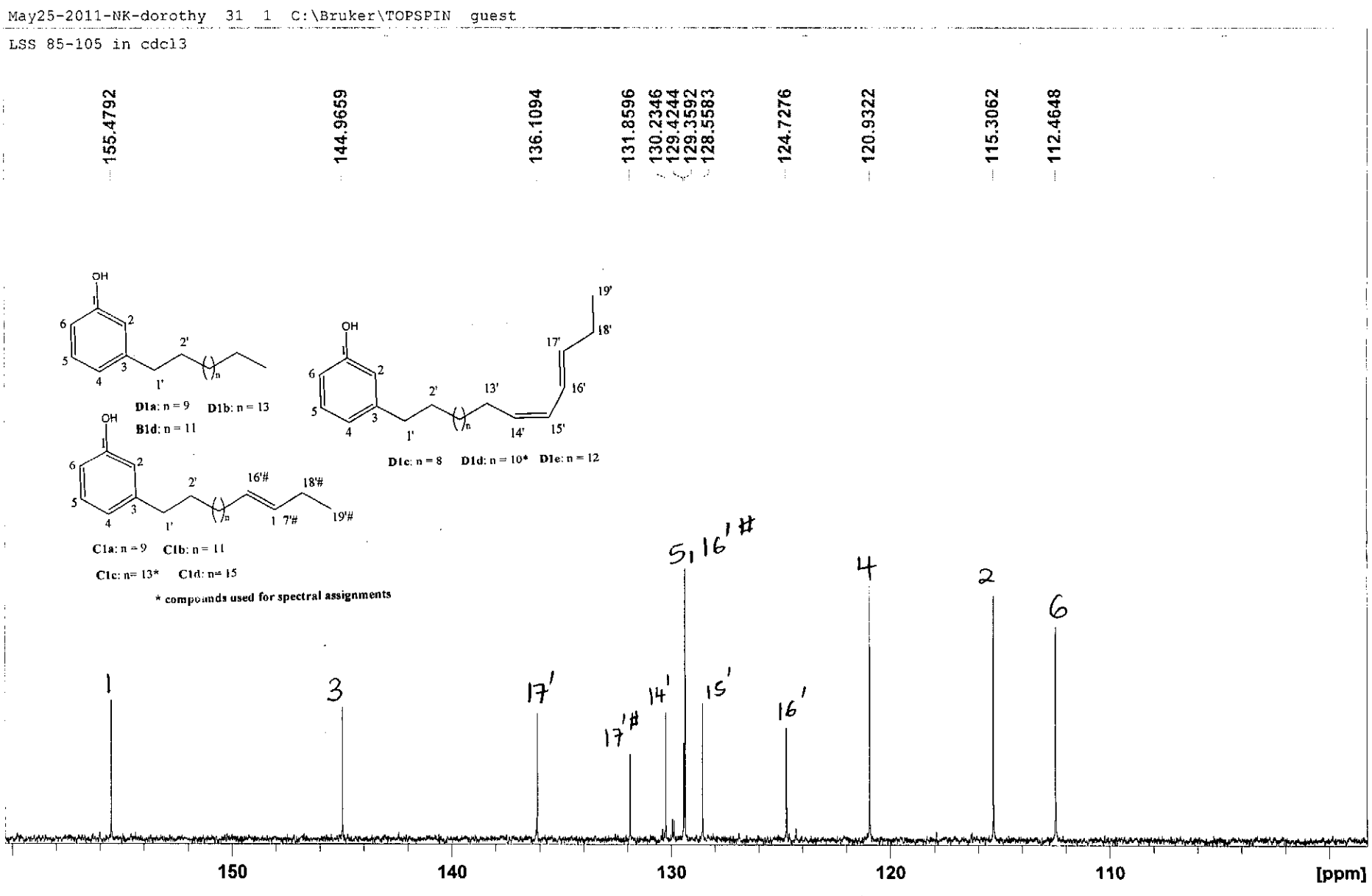
^{1}H NMR spectrum of a mixture of D1a, D1b, D1c, D1d, D1e, B1d, C1a, C1b, C1c and C1d (expanded)



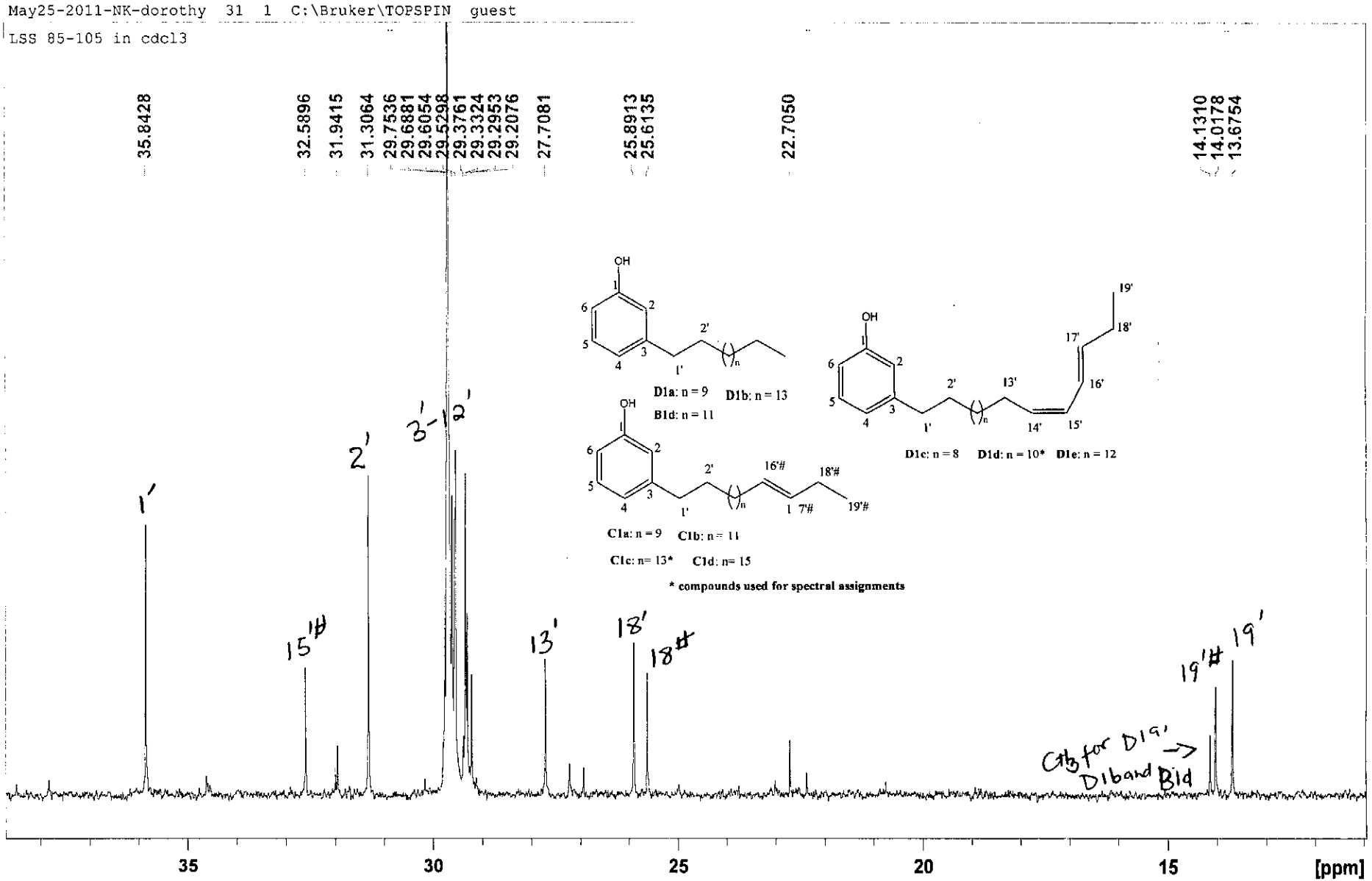
^{1}H NMR spectrum of a mixture of D1a, D1b, D1c, D1d, D1e, B1d, C1a, C1b, C1c and C1d (expanded)



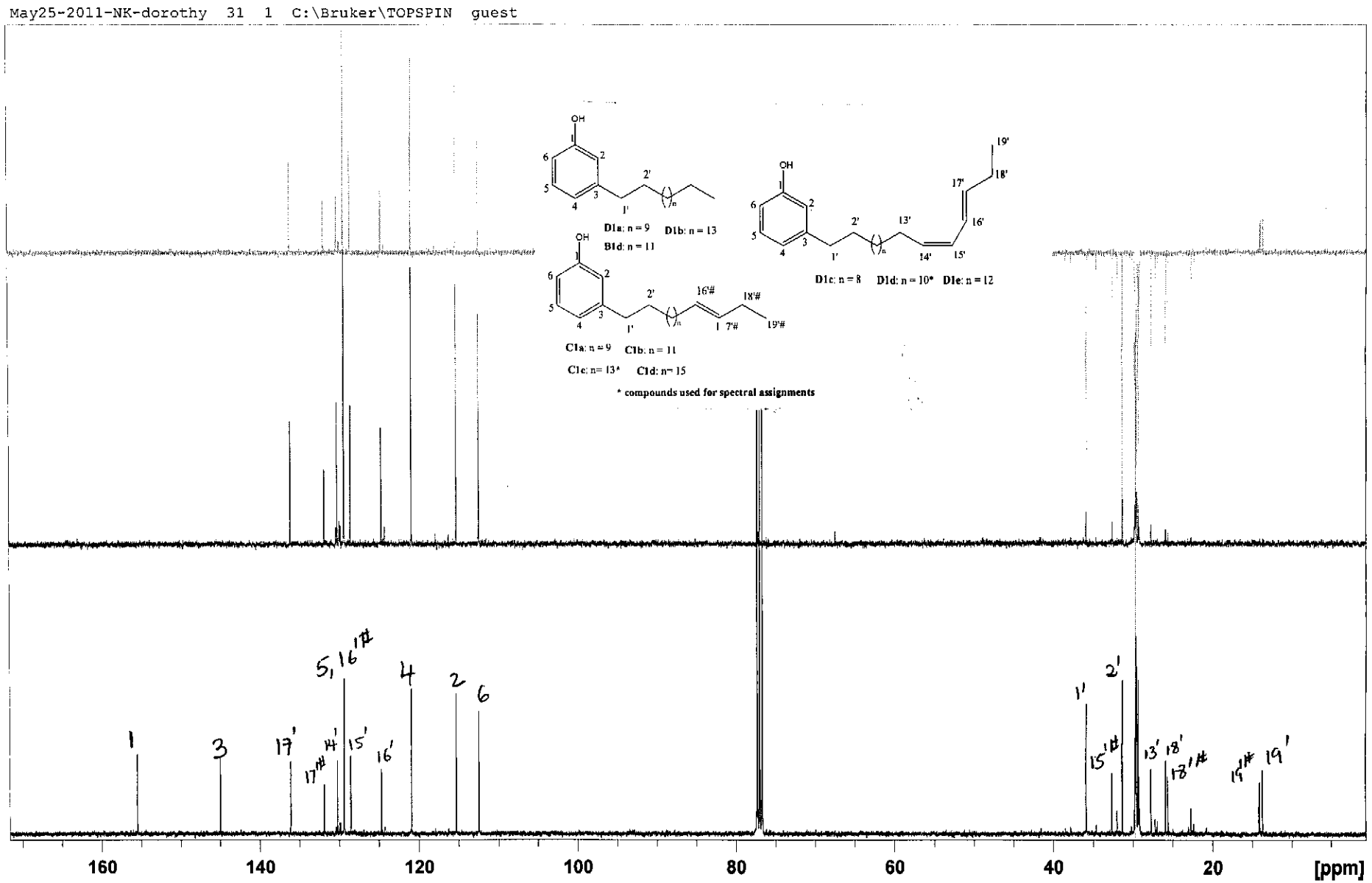
^{13}C NMR spectrum of a mixture of D1a, D1b, D1c, D1d, D1e, B1d, C1a, C1b, C1c and C1d



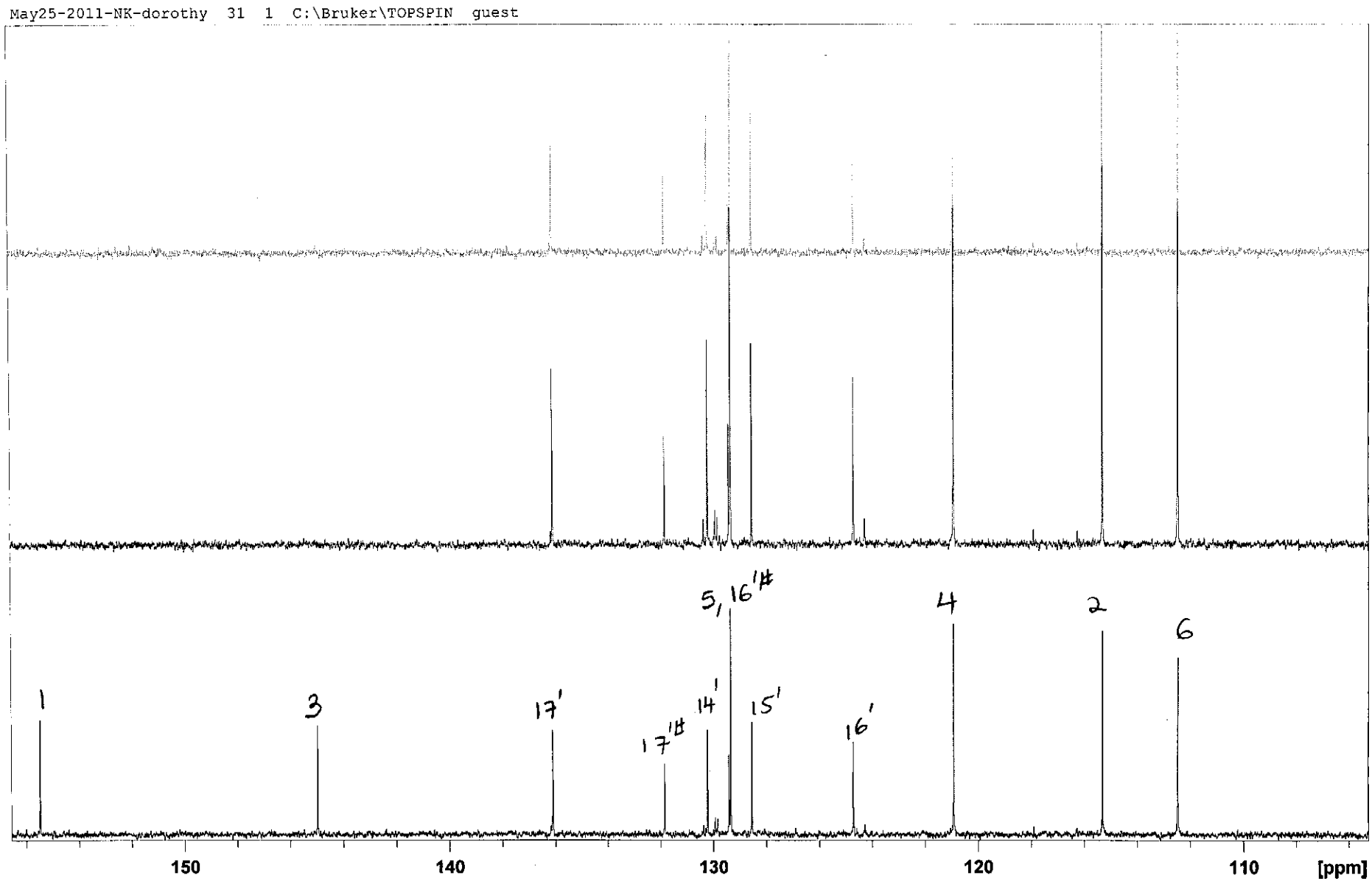
^{13}C NMR spectrum of a mixture of D1a, D1b, D1c, D1d, D1e, B1d, C1a, C1b, C1c and C1d (expanded)



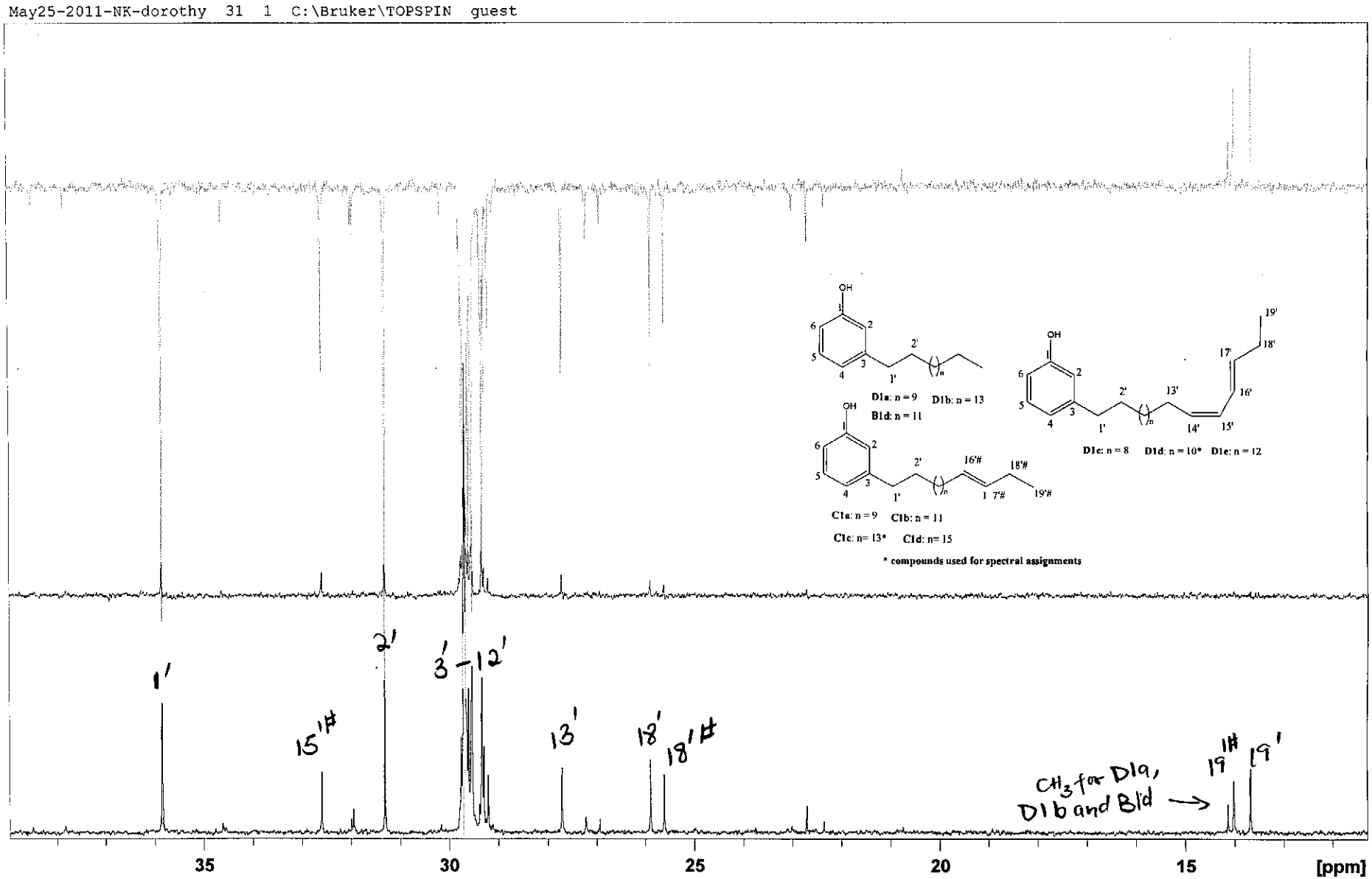
^{13}C NMR spectrum of a mixture of D1a, D1b, D1c, D1d, D1e, B1d, C1a, C1b, C1c and C1d (expanded)



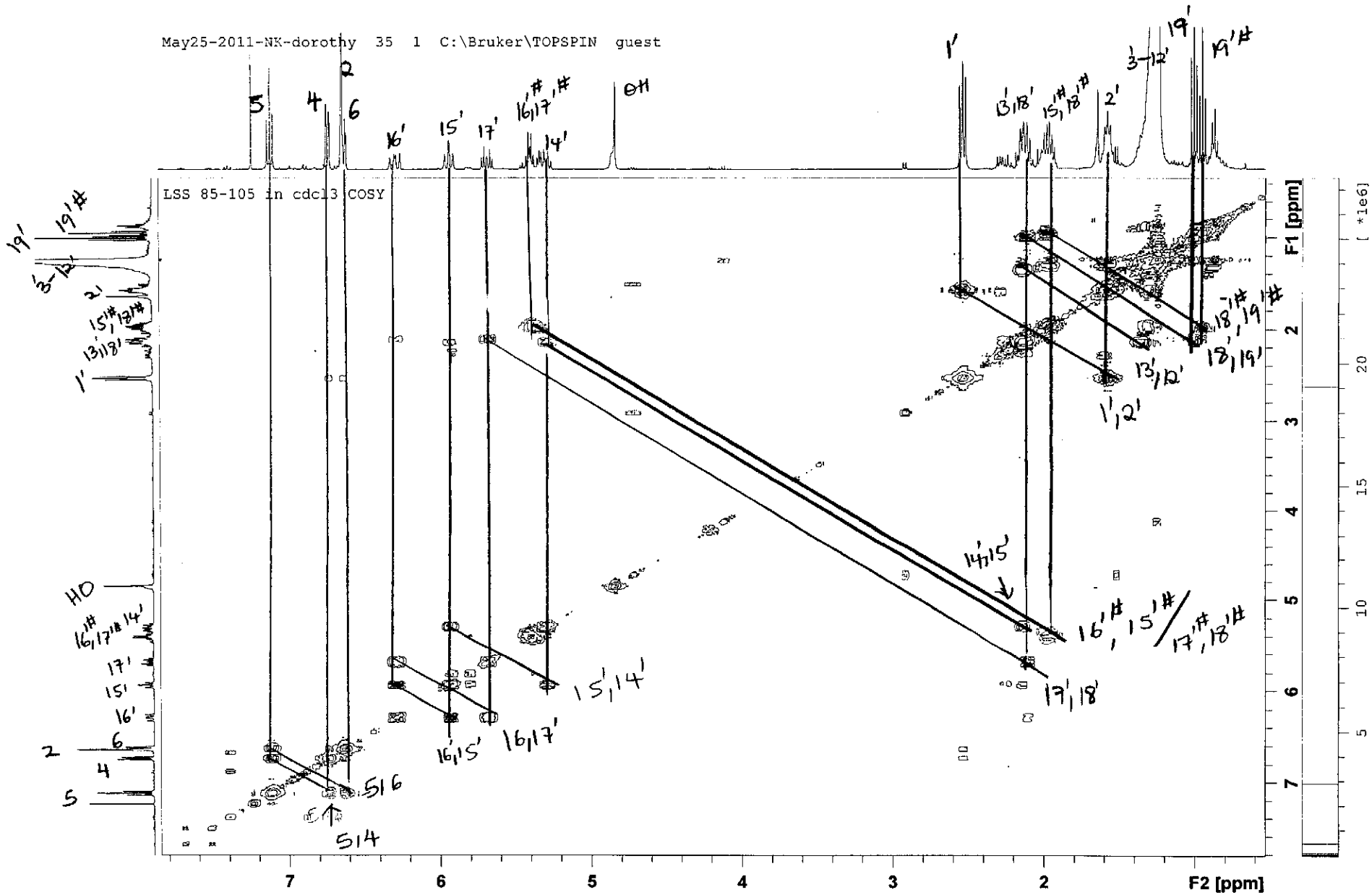
DEPT spectrum of a mixture of D1a, D1b, D1c, D1d, D1e, B1d, C1a, C1b, C1c and C1d



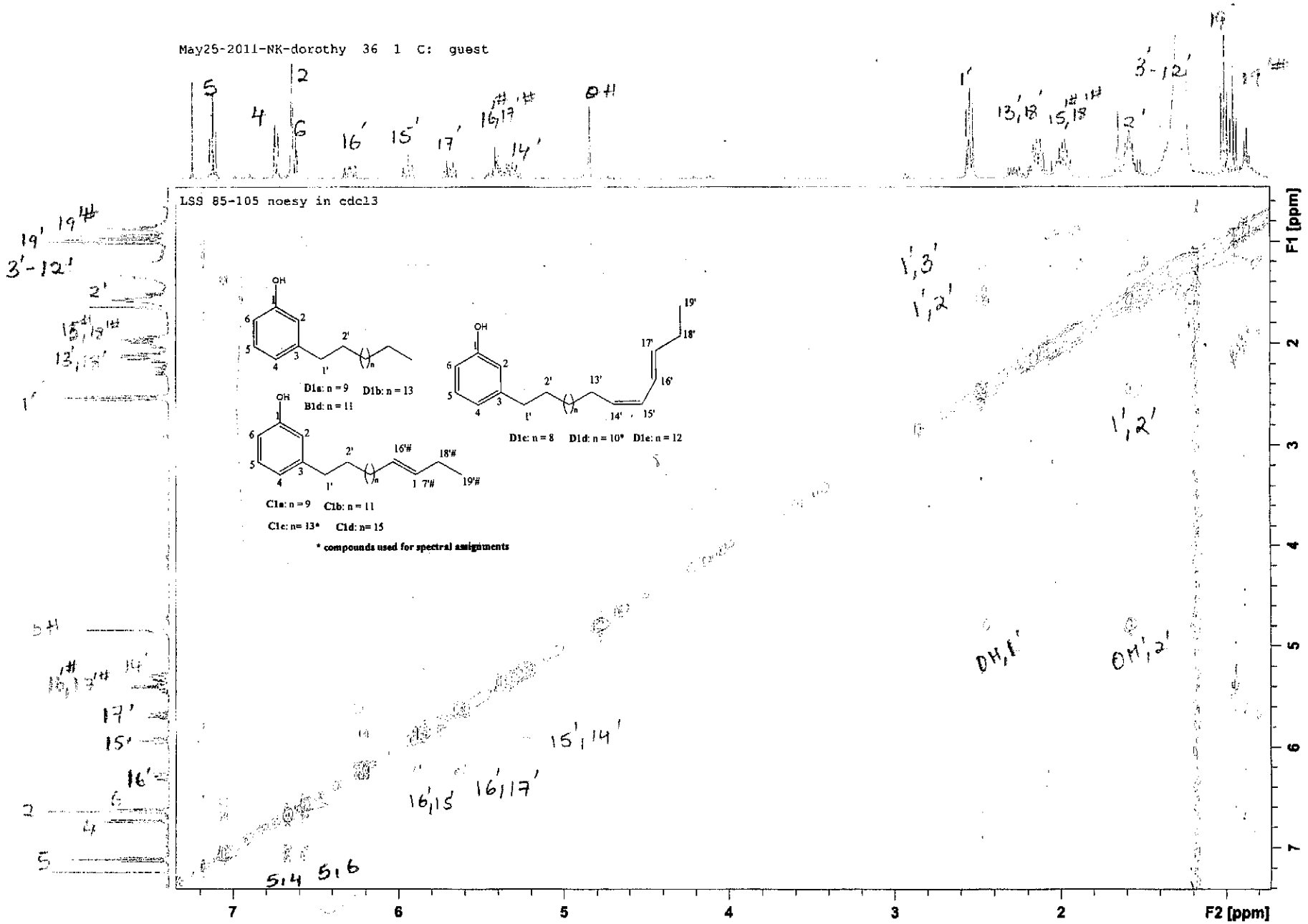
DEPT spectrum of a mixture of D1a, D1b, D1c, D1d, D1e, B1d, C1a, C1b, C1c and C1d (expanded)



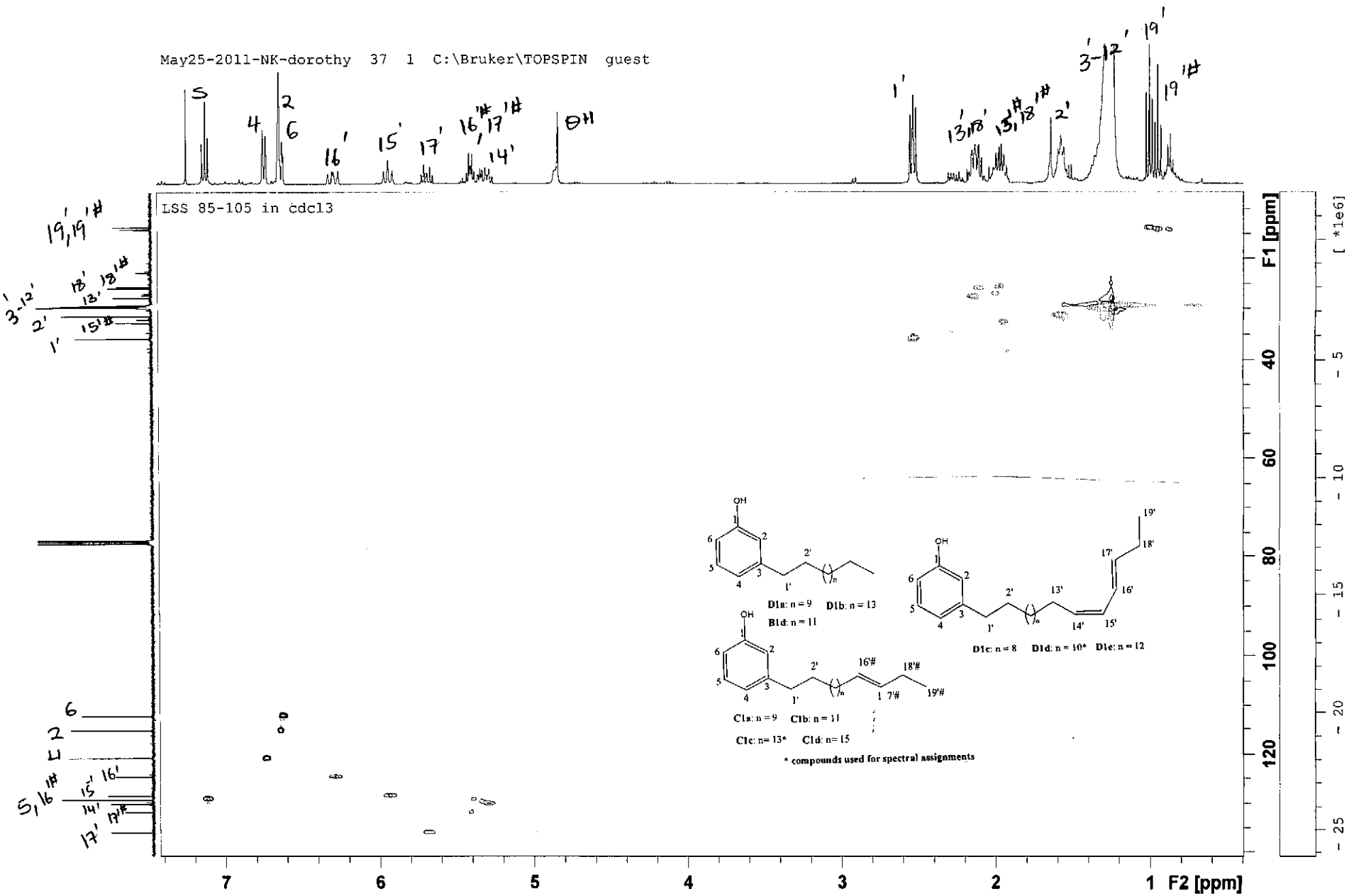
DEPT spectrum of a mixture of D1a, D1b, D1c, D1d, D1e, B1d, C1a, C1b, C1c and C1d (expanded)



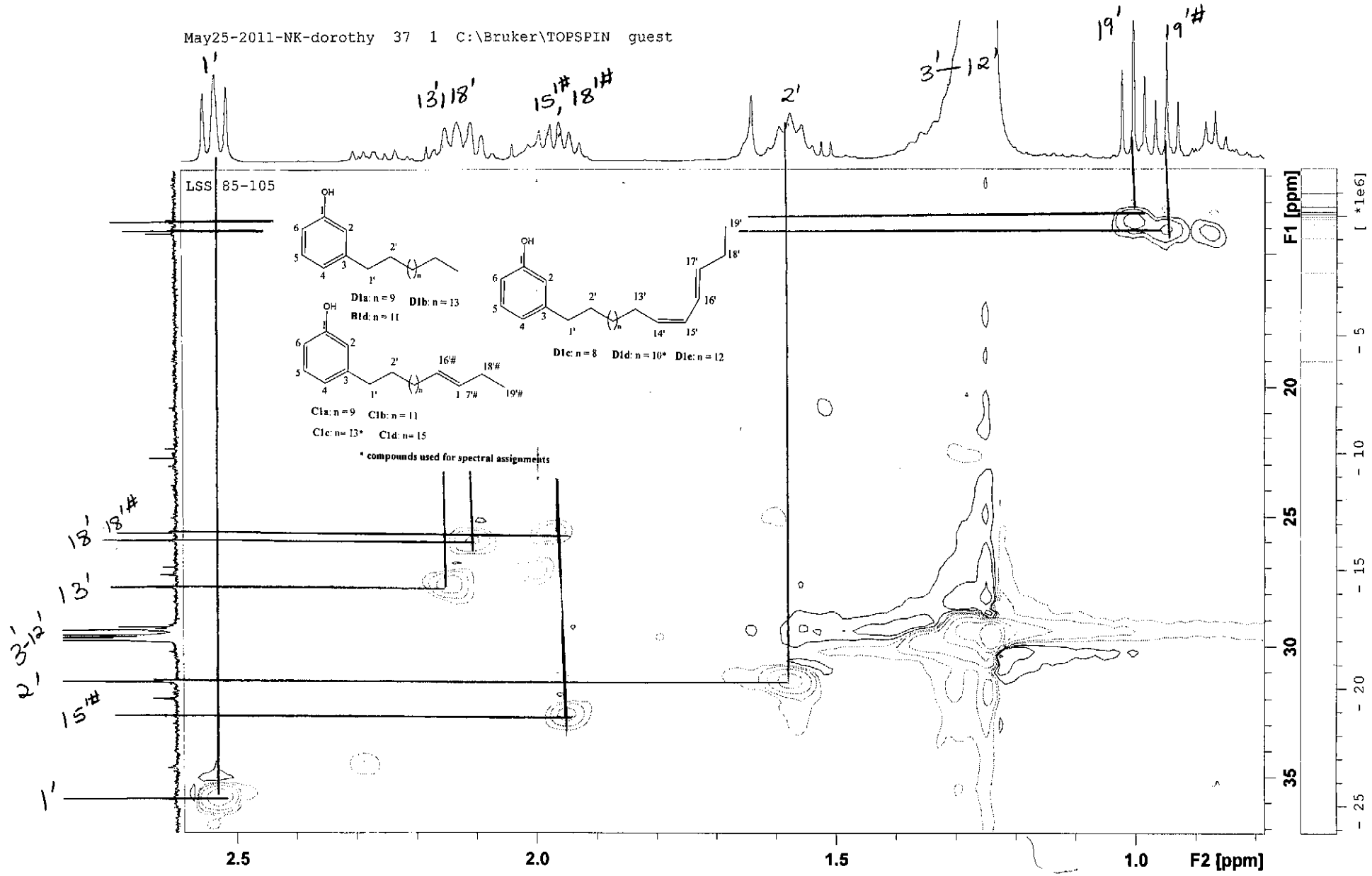
COSY spectrum of a mixture of D1a, D1b, D1c, D1d, D1e, B1d, C1a, C1b, C1c and C1d



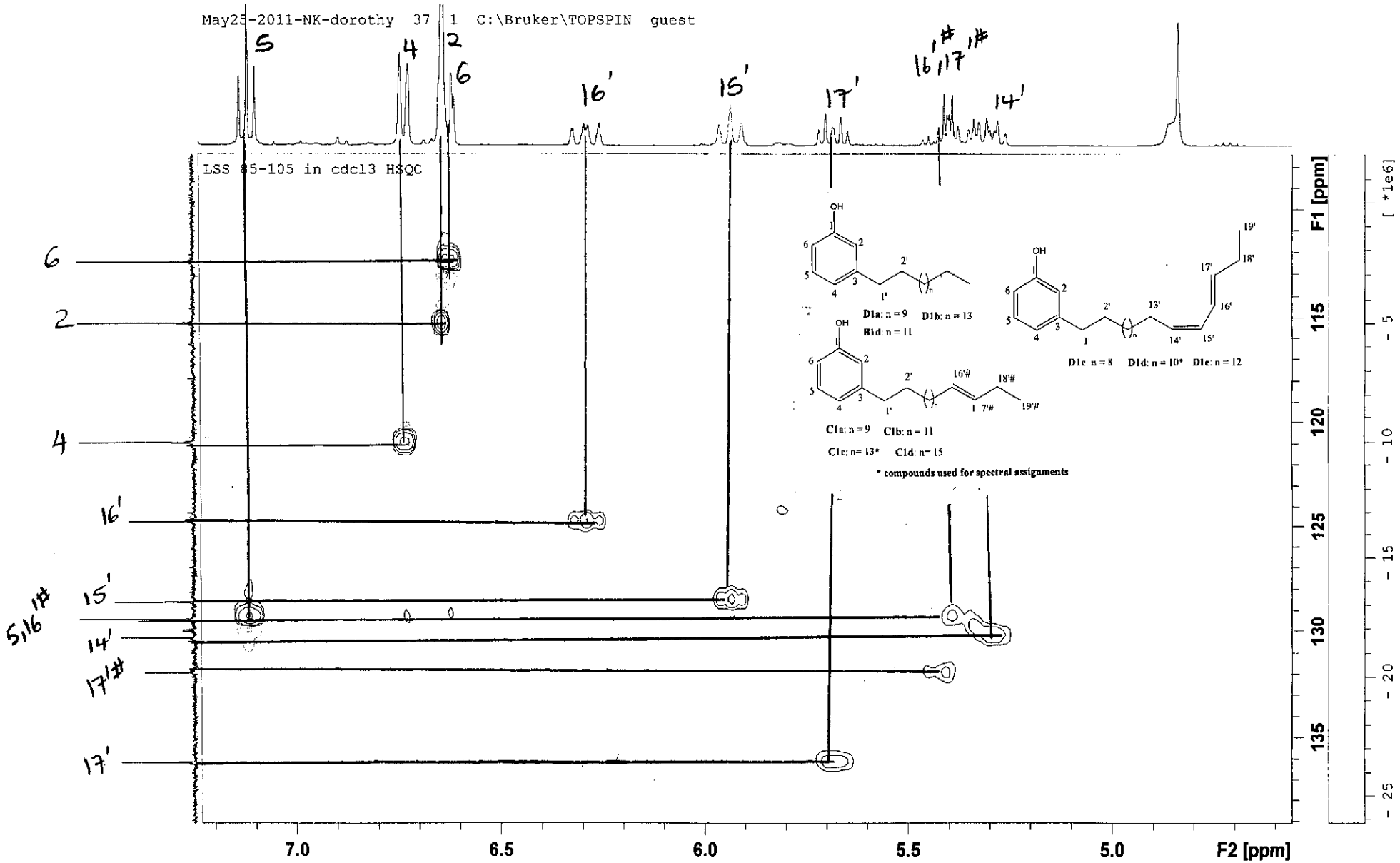
NOESY spectrum of a mixture of D1a. D1B, D1c, D1d, D1e, B1d, C1a, C1b, C1c and C1d



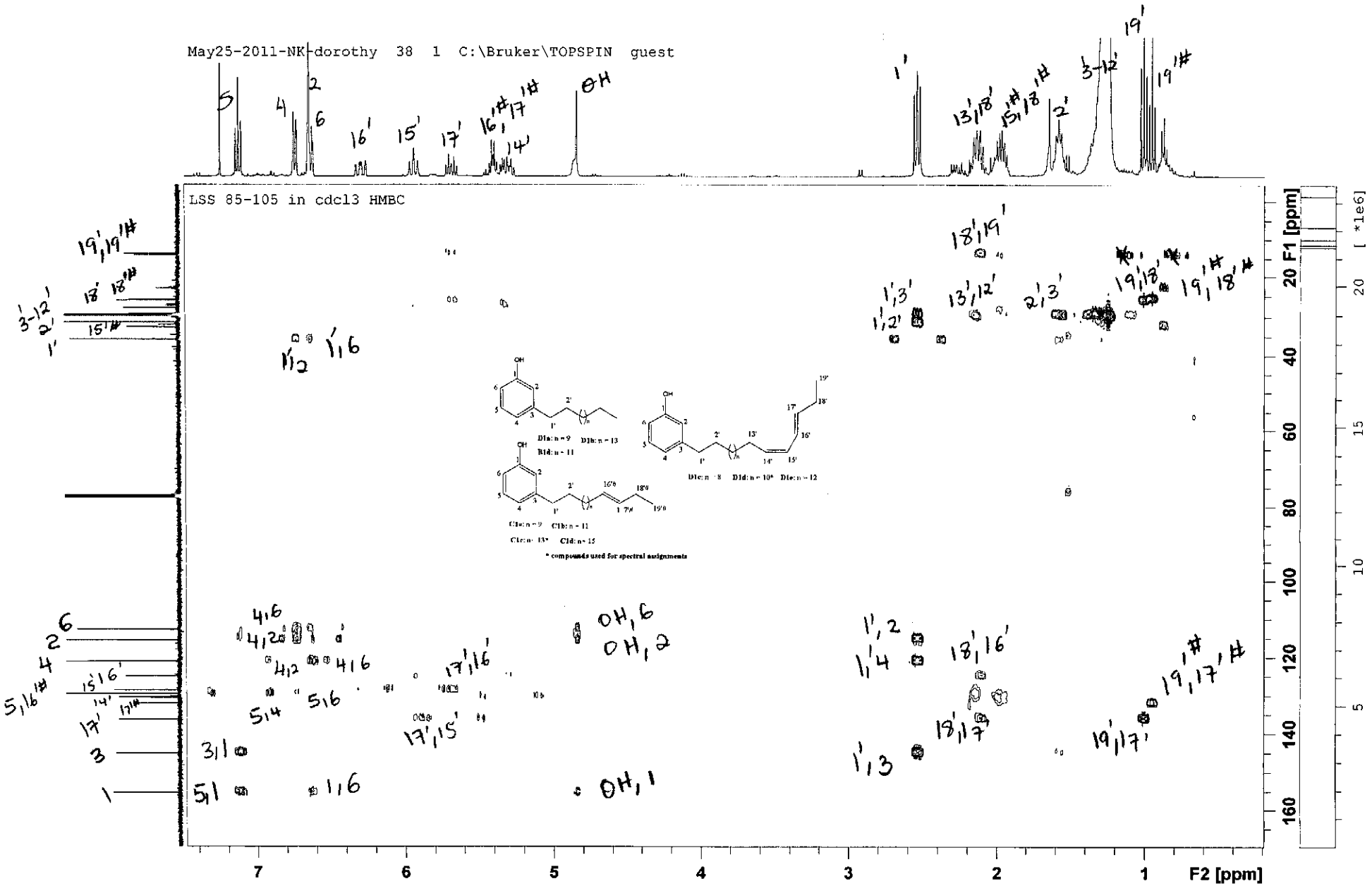
HSQC spectrum of a mixture of D1a, D1b, D1c, D1d, D1e, B1d, C1a, C1b, C1c and C1d



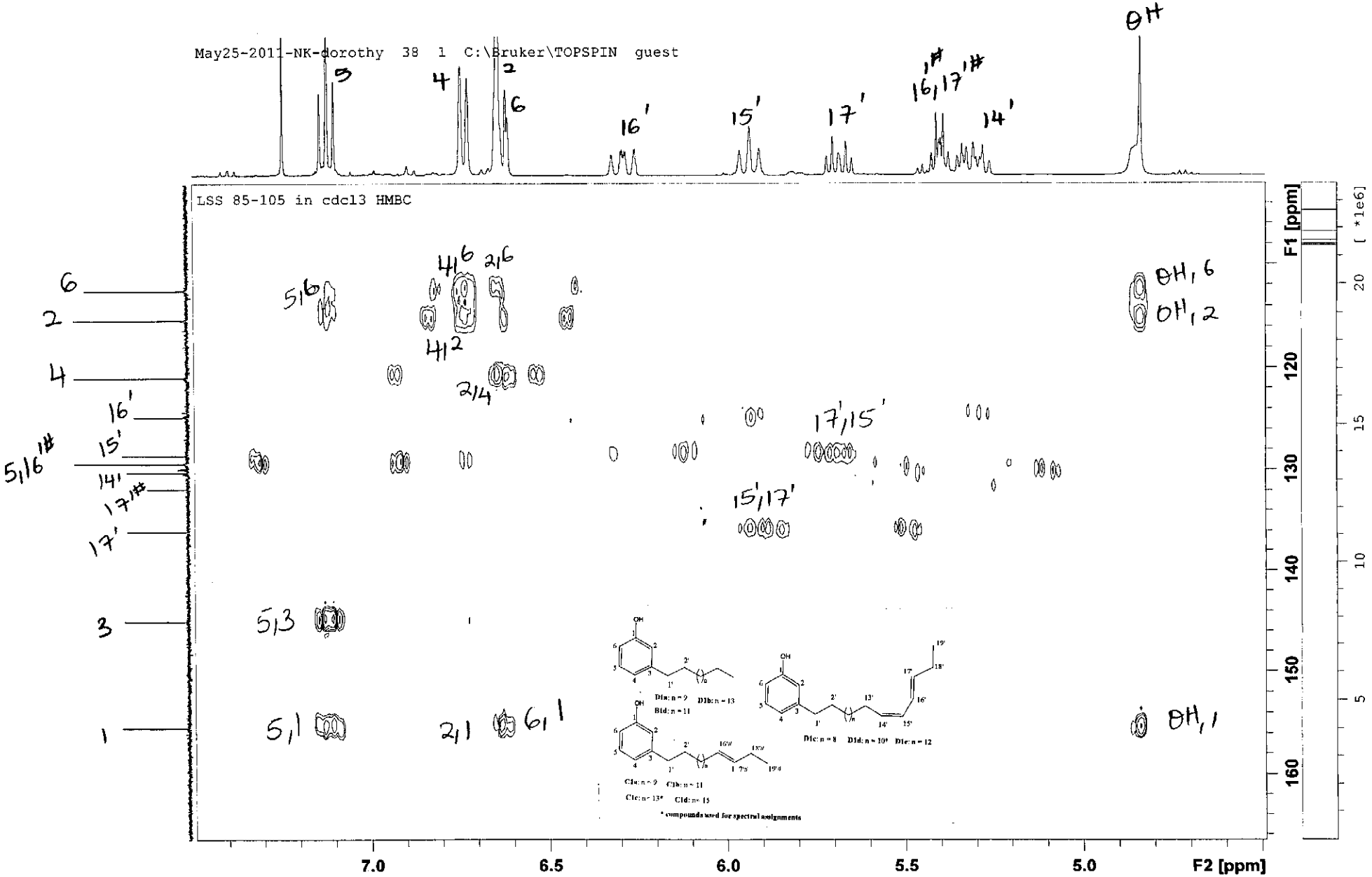
HSQC spectrum of a mixture of D1a, D1b, D1c, D1d, D1e, B1d, C1a, C1b, C1c and C1d (expanded)



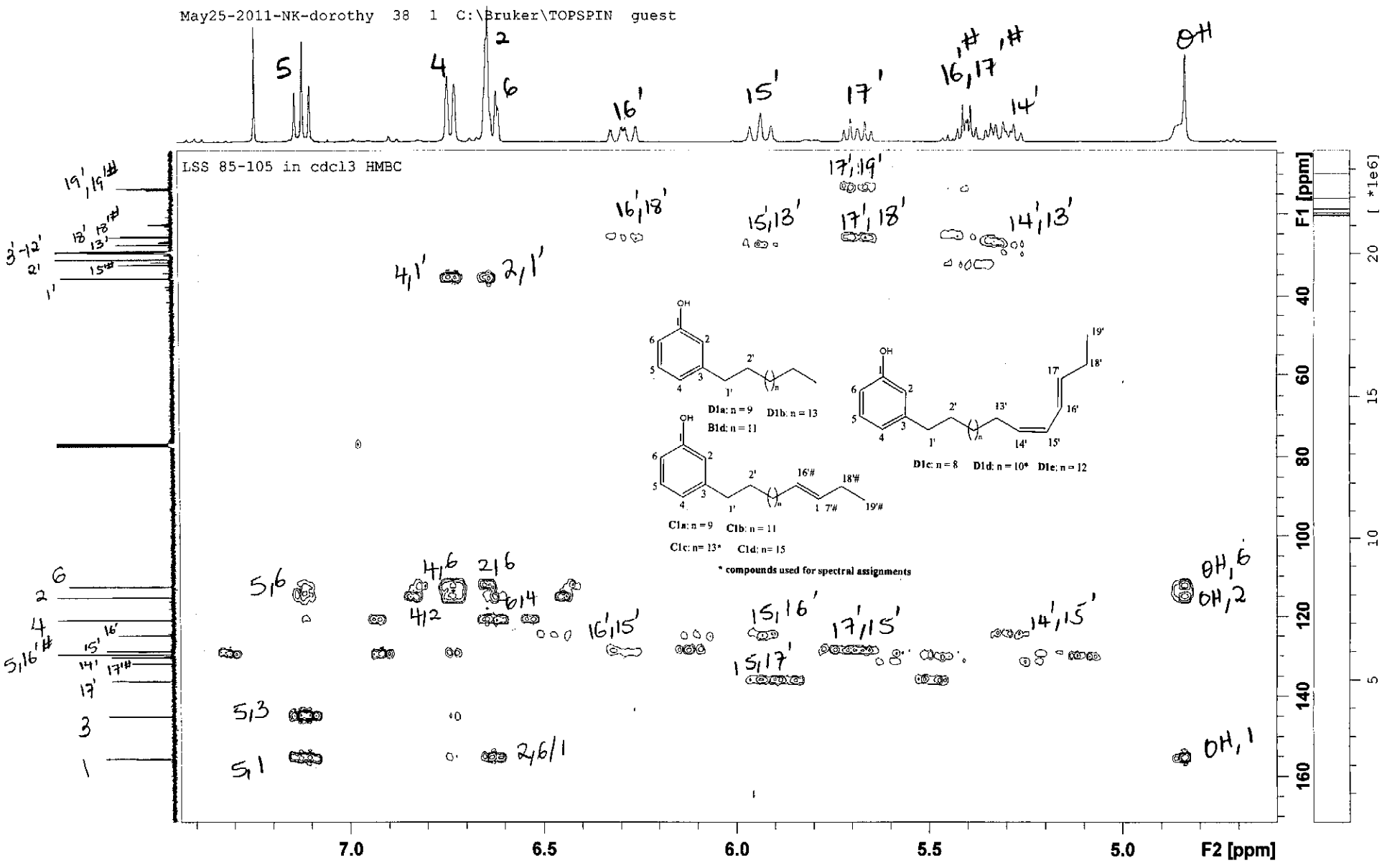
HSQC spectrum of a mixture of D1a, D1b, D1c, D1d, D1e, B1d, C1a, C1b, C1c and C1d (expanded)



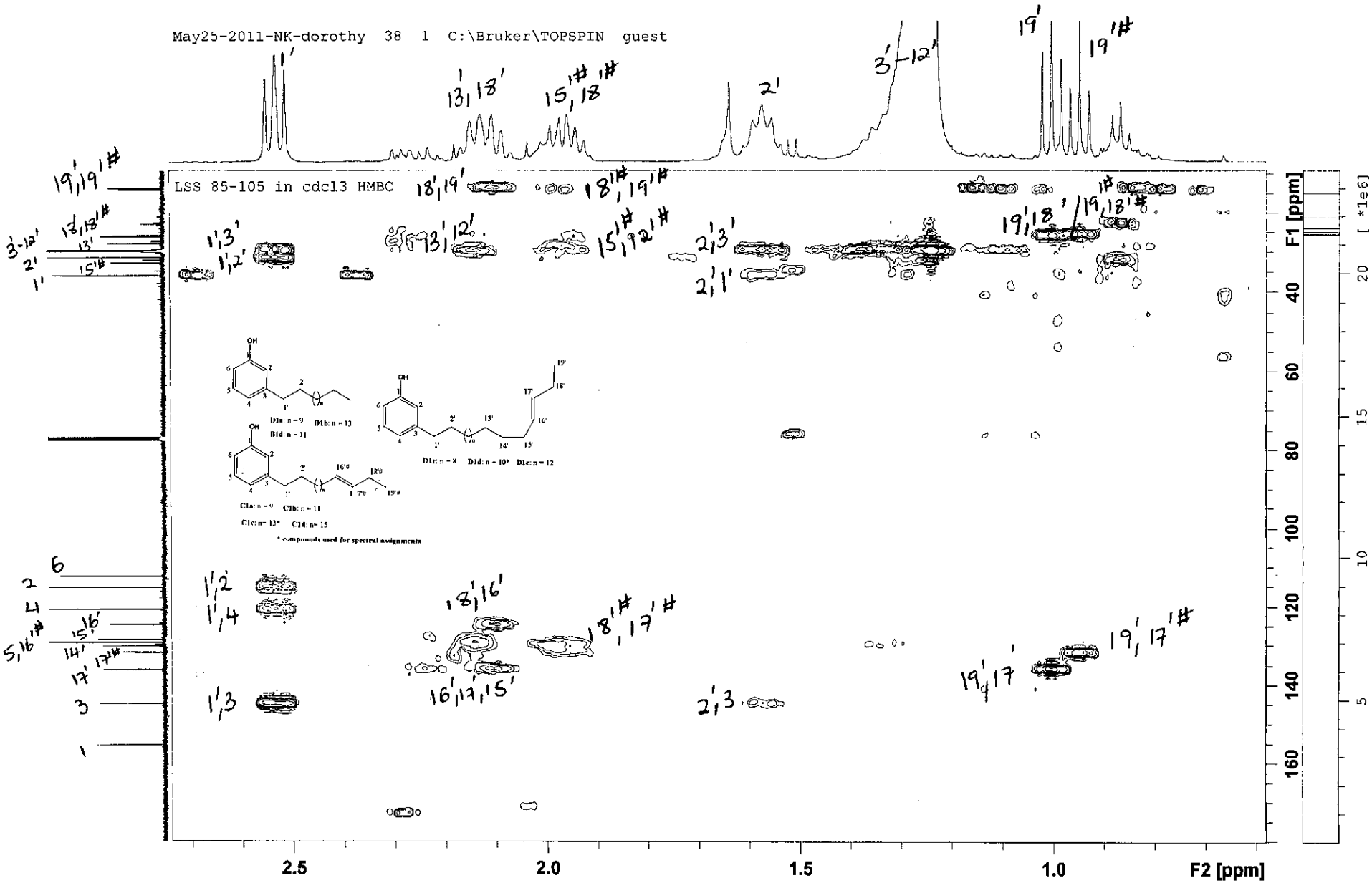
HMBC spectrum of a mixture of D1a, D1b, D1c, D1d, D1e, B1d, C1a, C1b, C1c and C1d



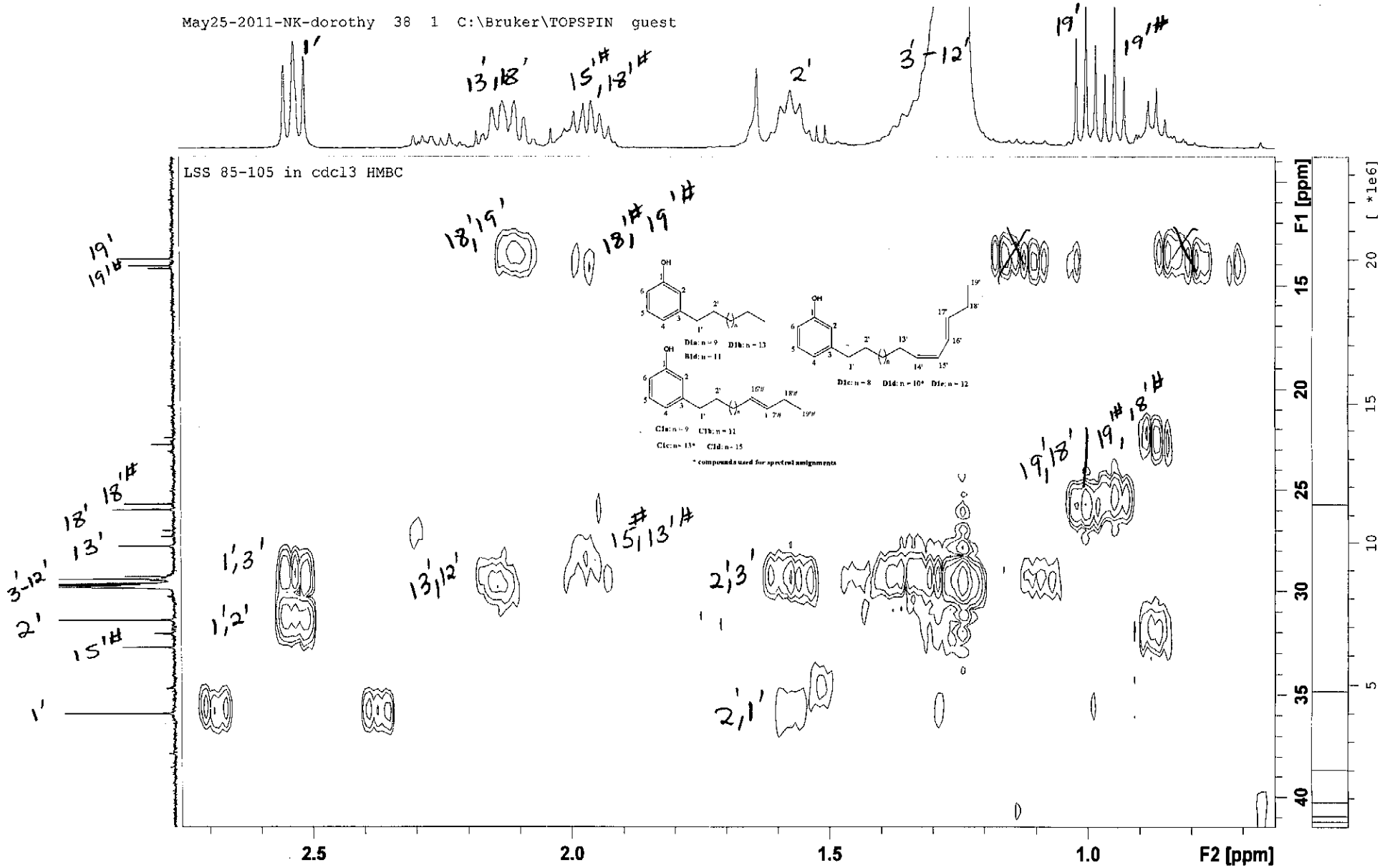
HMBC spectrum of a mixture of D1a, D1b, D1c, D1d, D1e, B1d, C1a, C1b, C1c and C1d (expanded)



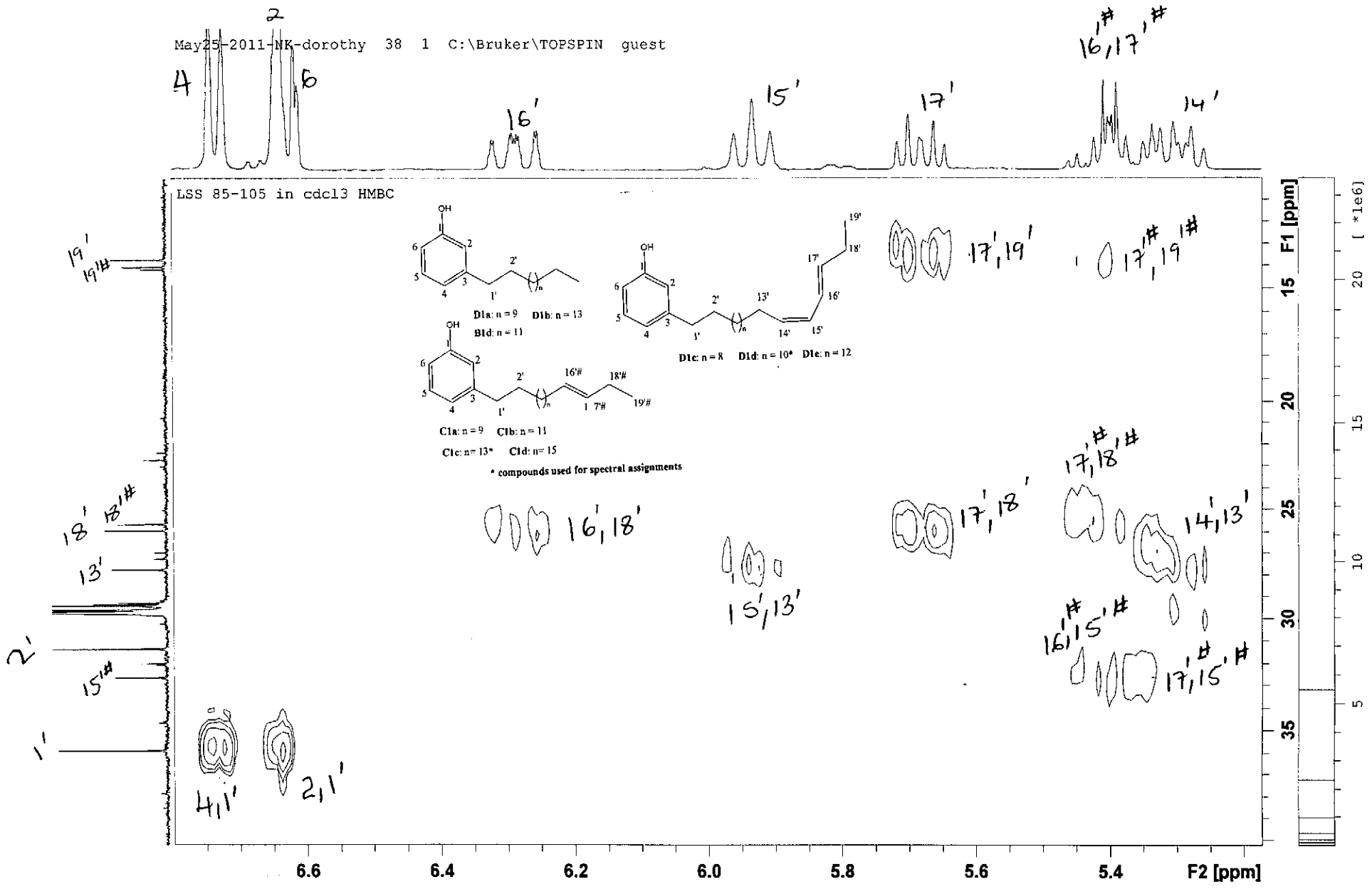
HMBC spectrum of a mixture of D1a, D1b, D1c, D1d, D1e, B1d, C1a, C1b, C1c and C1d (expanded)



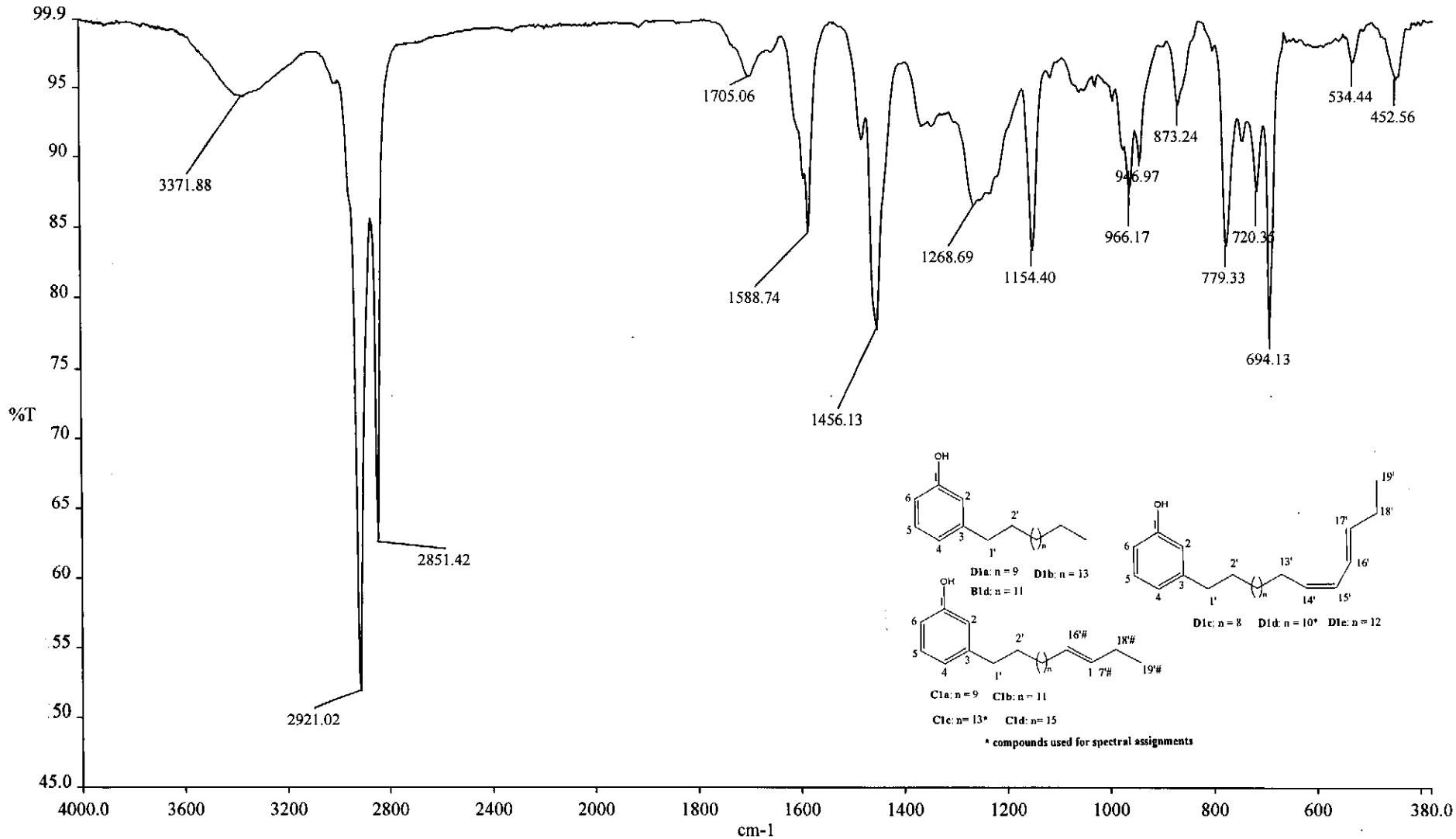
HMBC spectrum of a mixture of D1a, D1b, D1c, D1d, D1e, B1d, C1a, C1b, C1c and C1d (expanded)



HMBC spectrum of a mixture of D1a, D1b, D1c, D1d, D1e, B1d, C1a, C1b, C1c and C1d (expanded)



HMBC spectrum of a mixture of D1a, D1b, D1c, D1d, D1e, B1d, C1a, C1b, C1c and C1d (expanded)

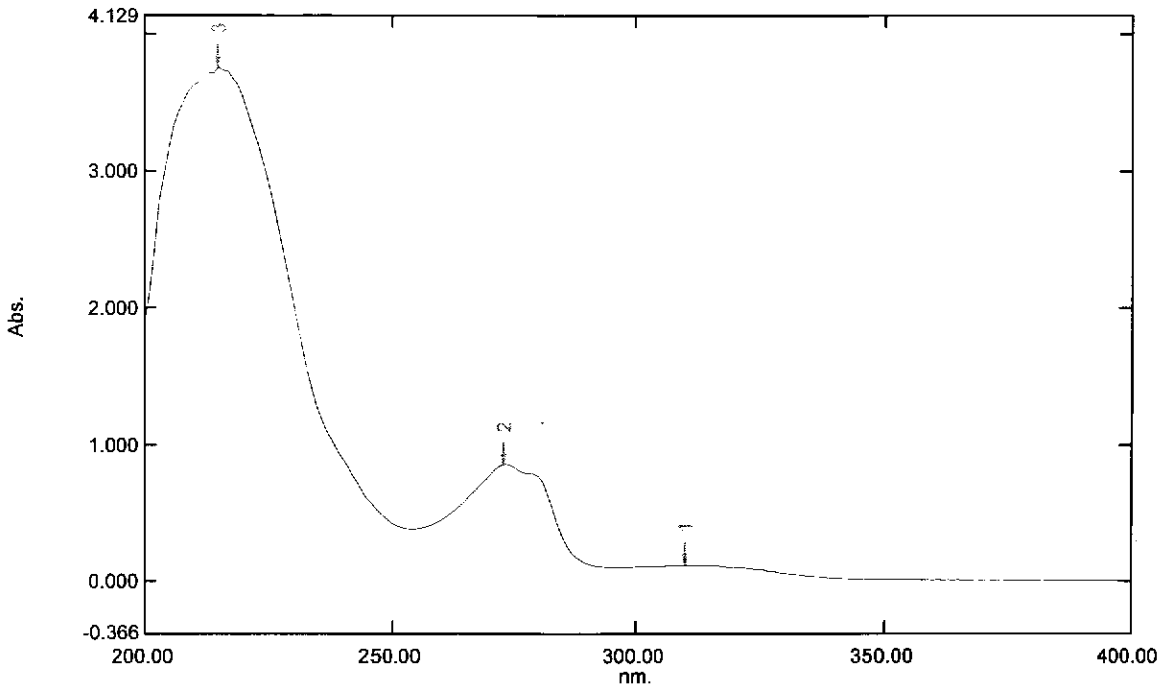


IR spectrum of a mixture of D1a, D1b, D1c, D1d, D1e, B1d, C1a, C1b, C1c and C1d

Spectrum Peak Pick Report

23/05/2012 06:07:48 PM

Data Set: Iss 85-105 (met).spc - Storage 173452



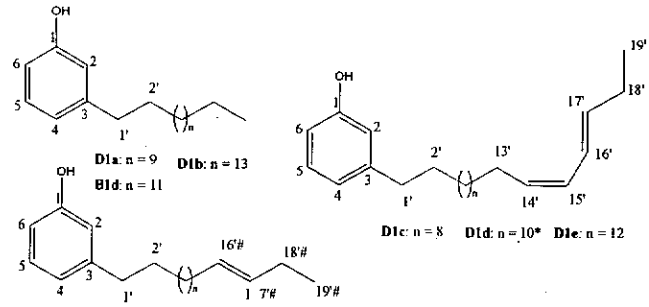
No.	P/V	Wavelength	Abs.	Description
1	⊕	310.00	0.123	
2	⊕	273.00	0.864	
3	⊕	215.00	3.755	

Measurement Properties
Wavelength Range (nm.): 200.00 to 400.00
Scan Speed: Medium
Sampling Interval: 1.0
Auto Sampling Interval: Disabled
Scan Mode: Single

Instrument Properties
Instrument Type: UV-3600 Series
Measuring Mode: Absorbance
Slit Width: 2.0 nm
Time Constant: 0.1 sec.
Source Lamp: Auto
Light Source Change Wavelength: 310.00 nm
Detector Unit: Direct
Detector Change Wavelength: 830.00 nm 1800.00 nm
Grating Change Wavelength: 720.00 nm
S/R Exchange: Normal
Detector Lock: Auto
Slit Program: Normal
Beam Mode: Double
Stair Correction: Disable

Attachment Properties
Attachment: None

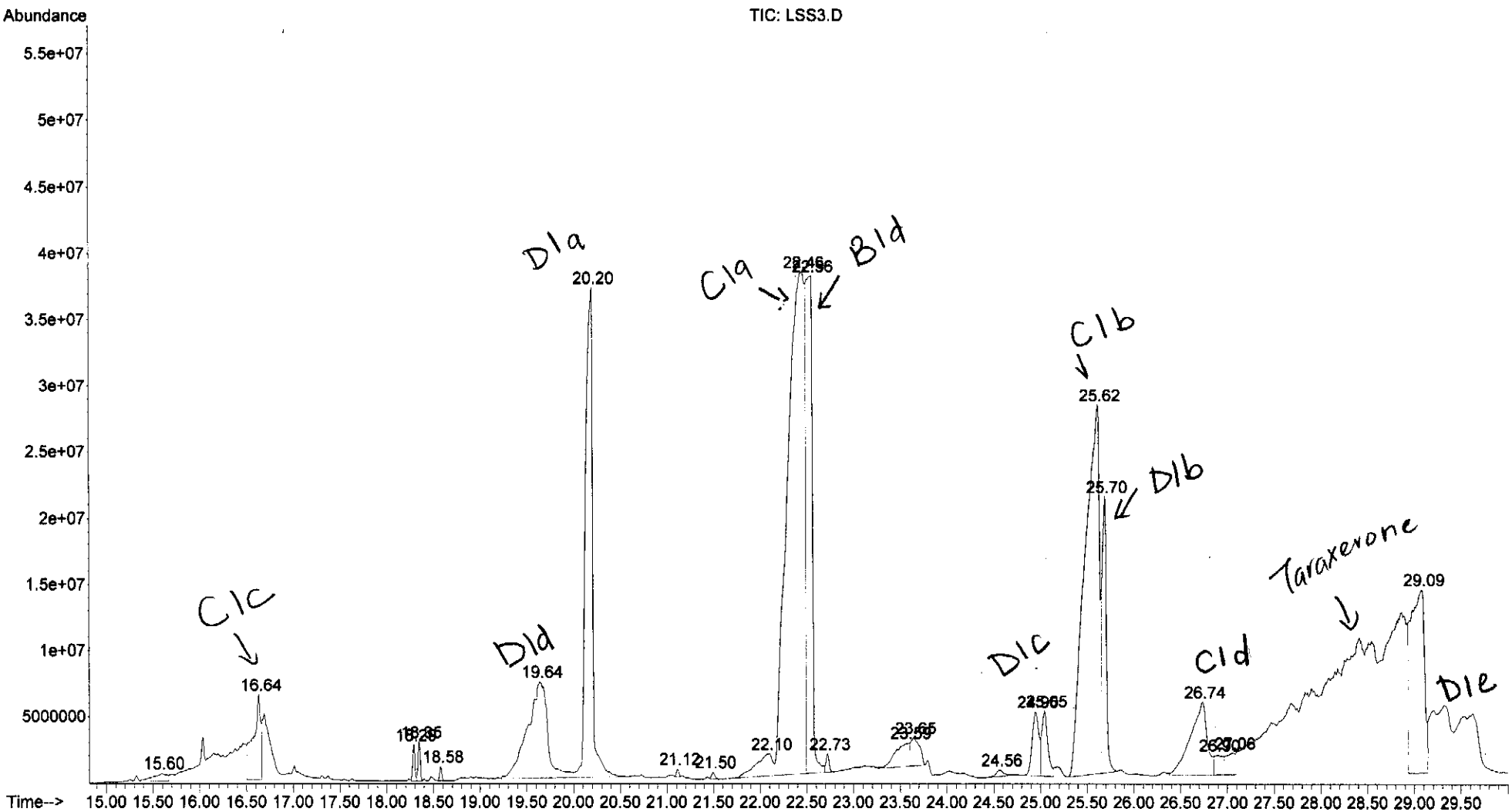
Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length:
Additional Information:



* compounds used for spectral assignments

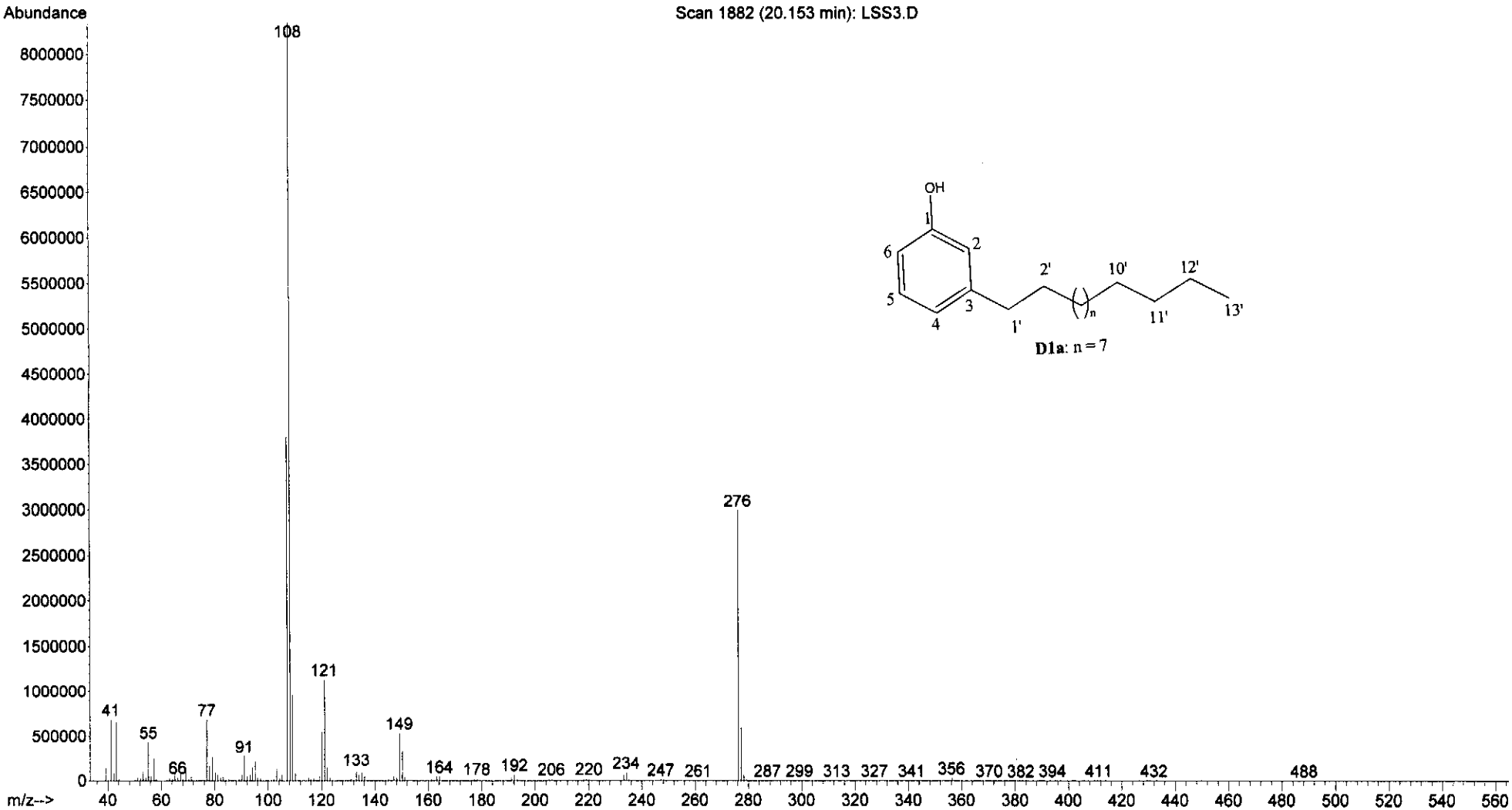
UV spectrum of a mixture of D1a, D1b, D1c, D1d, D1e, B1d, C1a, C1b, C1c and C1d

File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\DORTHY\LSS3.D
Operator : DOROTHY
Acquired : 19 Jun 2011 12:38 using AcqMethod NATURAL
Instrument : Instrumen
Sample Name: MEOH LSRT 8-13
Misc Info :
Vial Number: 1



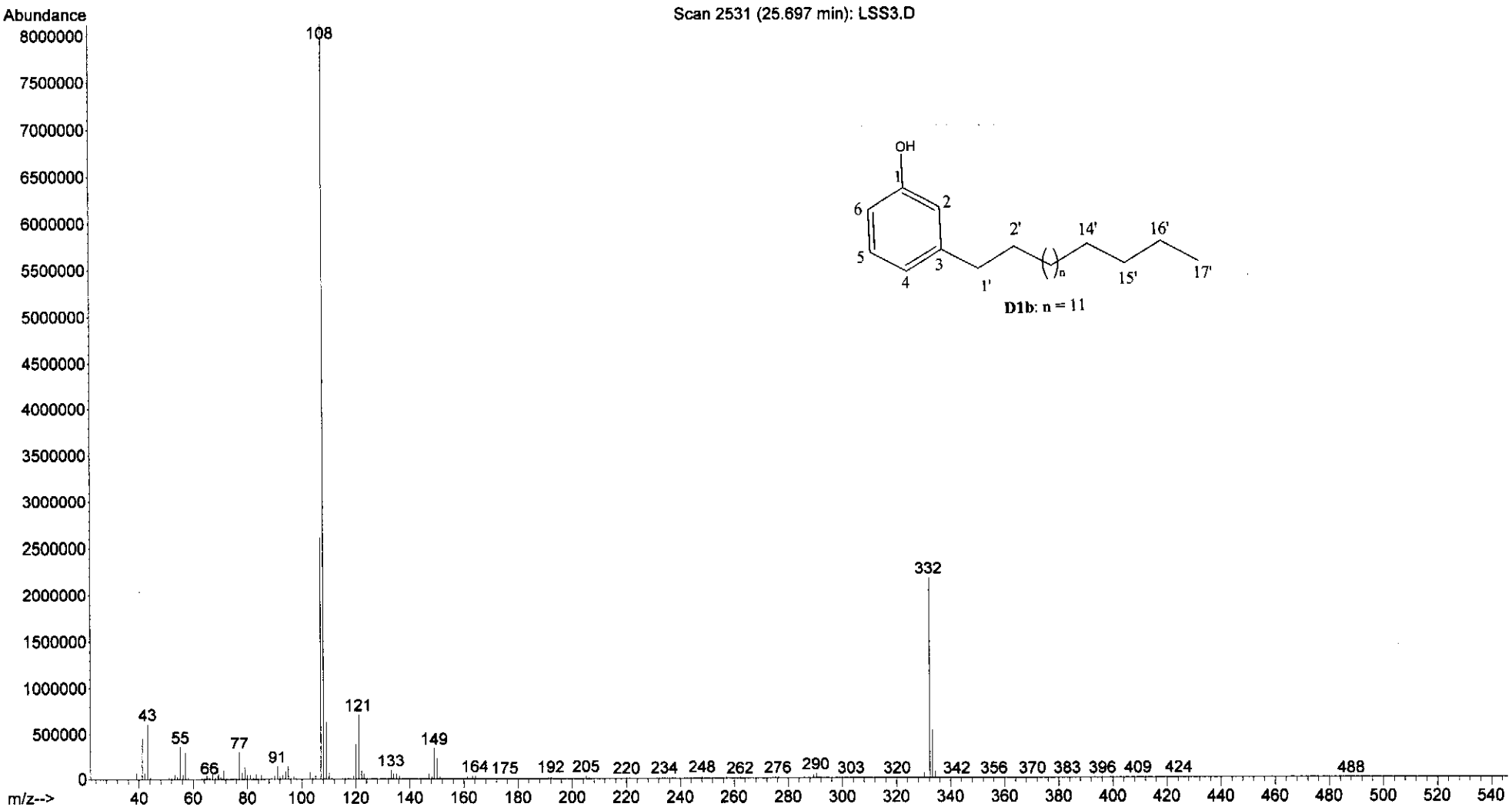
GC chromatogram of a mixture of D1a, D1b, D1c, D1d, D1e, B1d, C1a, C1b, C1c and C1d

File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\DORTHY\LSS3.D
Operator : DOROTHY
Acquired : 19 Jun 2011 12:38 using AcqMethod NATURAL
Instrument : Instrumen
Sample Name: MEOH LSRT 8-13
Misc Info :
Vial Number: 1



Mass spectrum of D1a

File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\DORTHY\LSS3.D
Operator : DOROTHY
Acquired : 19 Jun 2011 12:38 using AcqMethod NATURAL
Instrument : Instrumen
Sample Name: MEOH LSRT 8-13
Misc Info :
Vial Number: 1



D1b: n = 11

Mass spectrum of D1b

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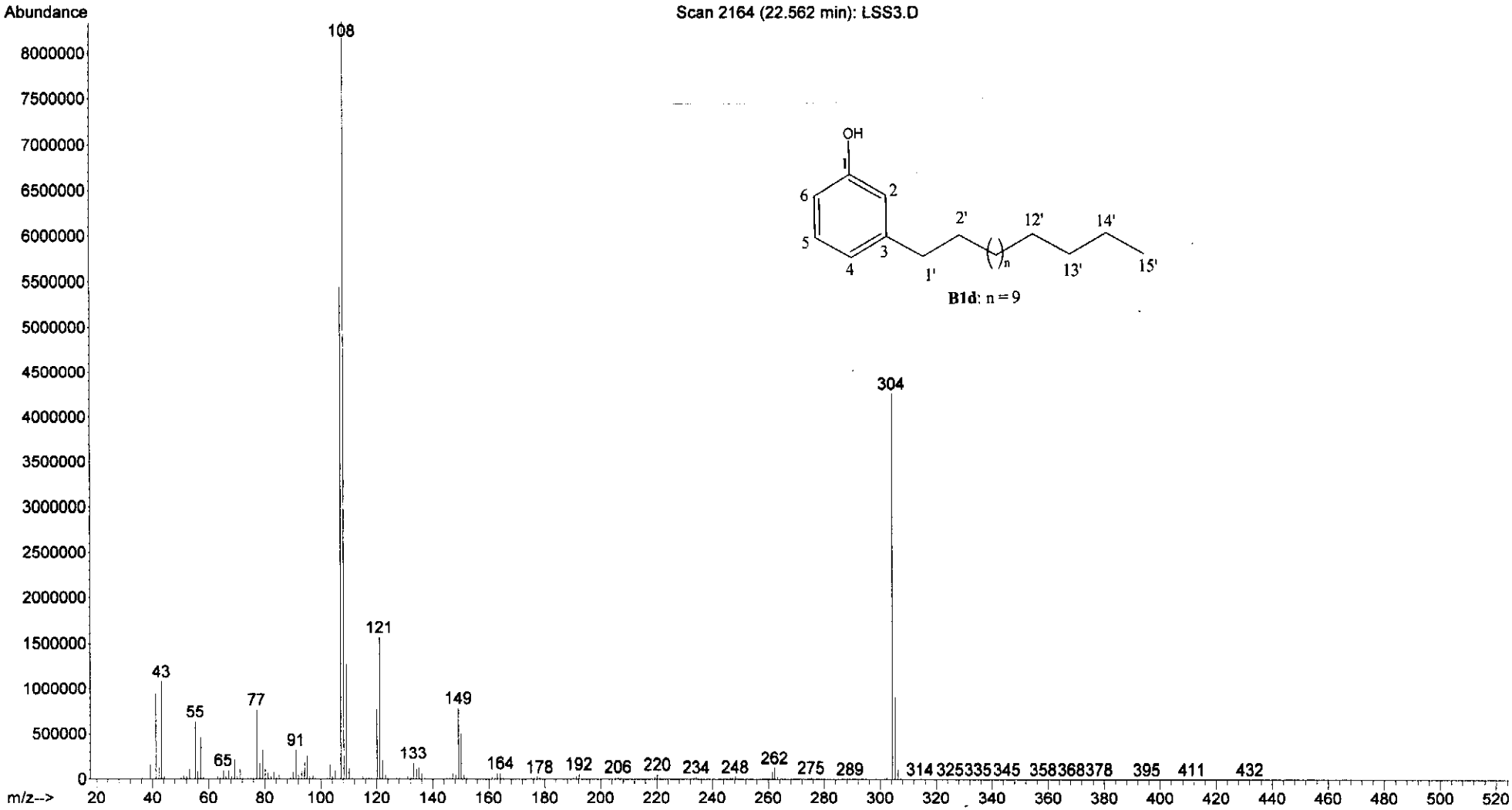
Operator : DOROTHY
Acquired : 19 Jun 2011 12:38 using AcqMethod NATURAL
Instrument : Instrumen
Sample Name: MEOH LSRT 8-13
Misc Info :
Vial Number: 1



Mass spectrum of B1d

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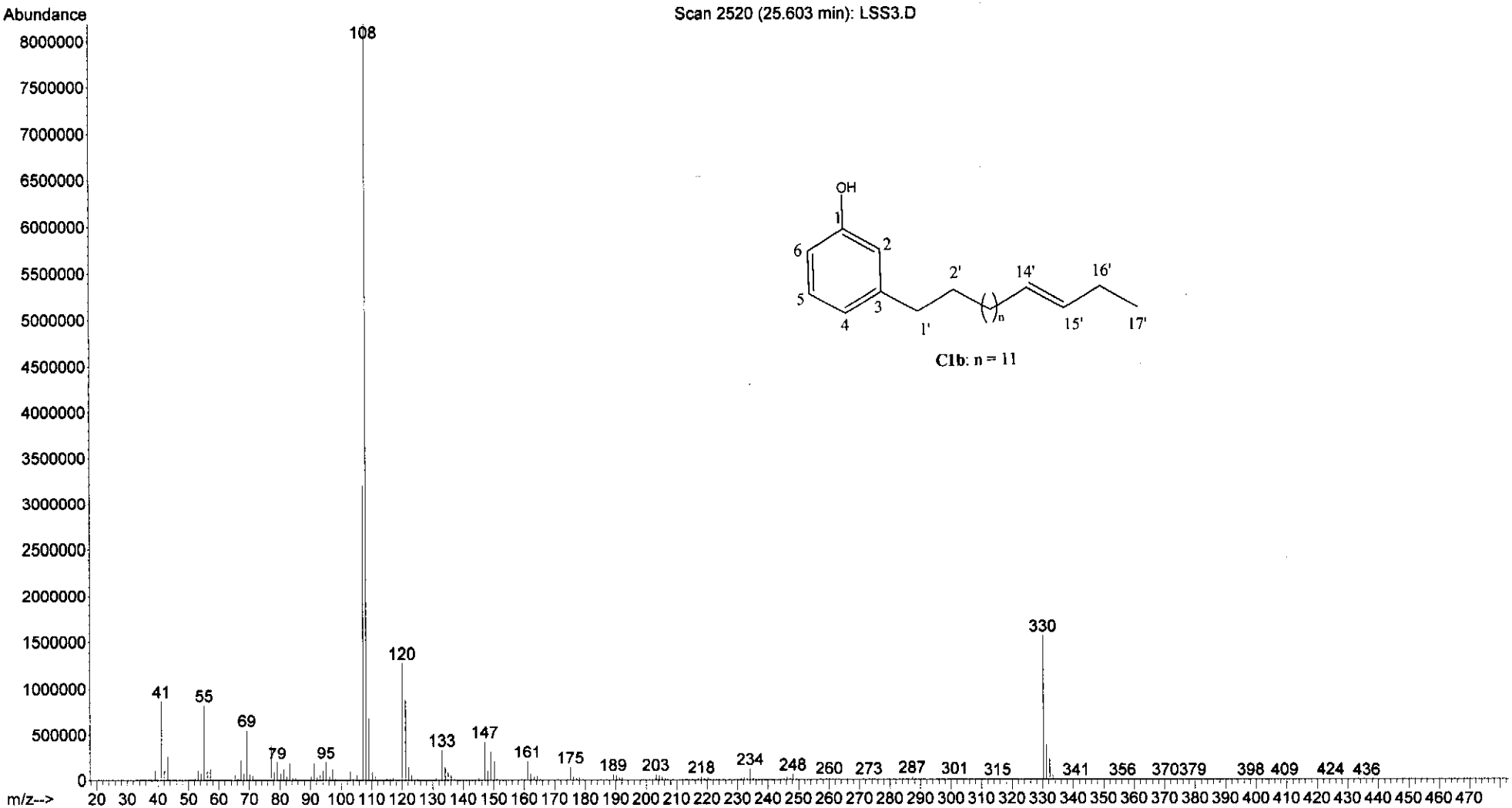
Operator : DOROTHY
Acquired : 19 Jun 2011 12:38 using AcqMethod NATURAL
Instrument : Instrumen
Sample Name: MEOH LSRT 8-13
Misc Info :
Vial Number: 1



Mass spectrum of C1b

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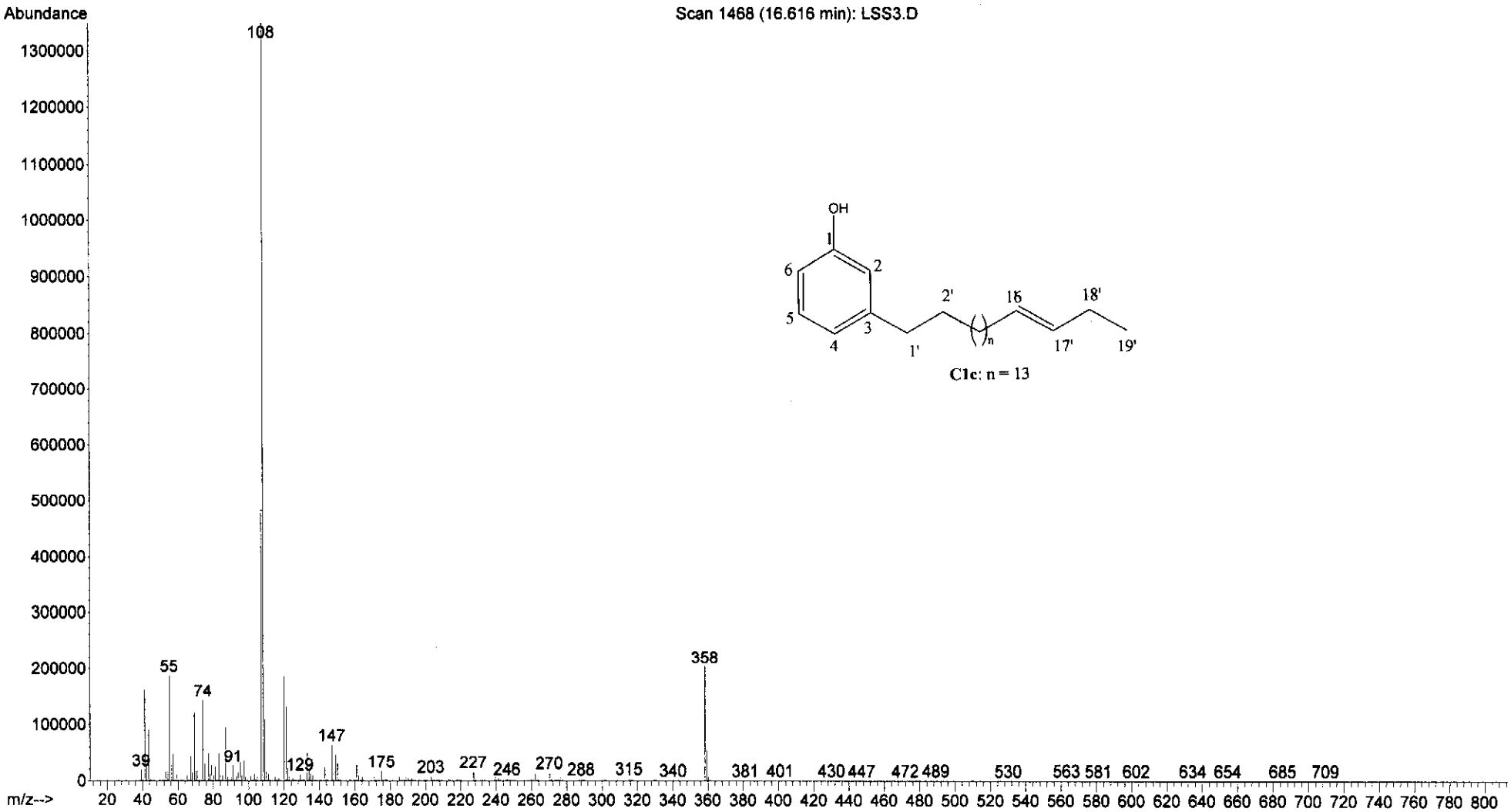
Operator : DOROTHY
Acquired : 19 Jun 2011 12:38 using AcqMethod NATURAL
Instrument : Instrumen
Sample Name: MEOH LSRT 8-13
Misc Info :
Vial Number: 1



C1c: n = 13

Mass spectrum of C1c

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Operator : DOROTHY
Acquired : 19 Jun 2011 12:38 using AcqMethod NATURAL
Instrument : Instrumen
Sample Name: MEOH LSRT 8-13
Misc Info :
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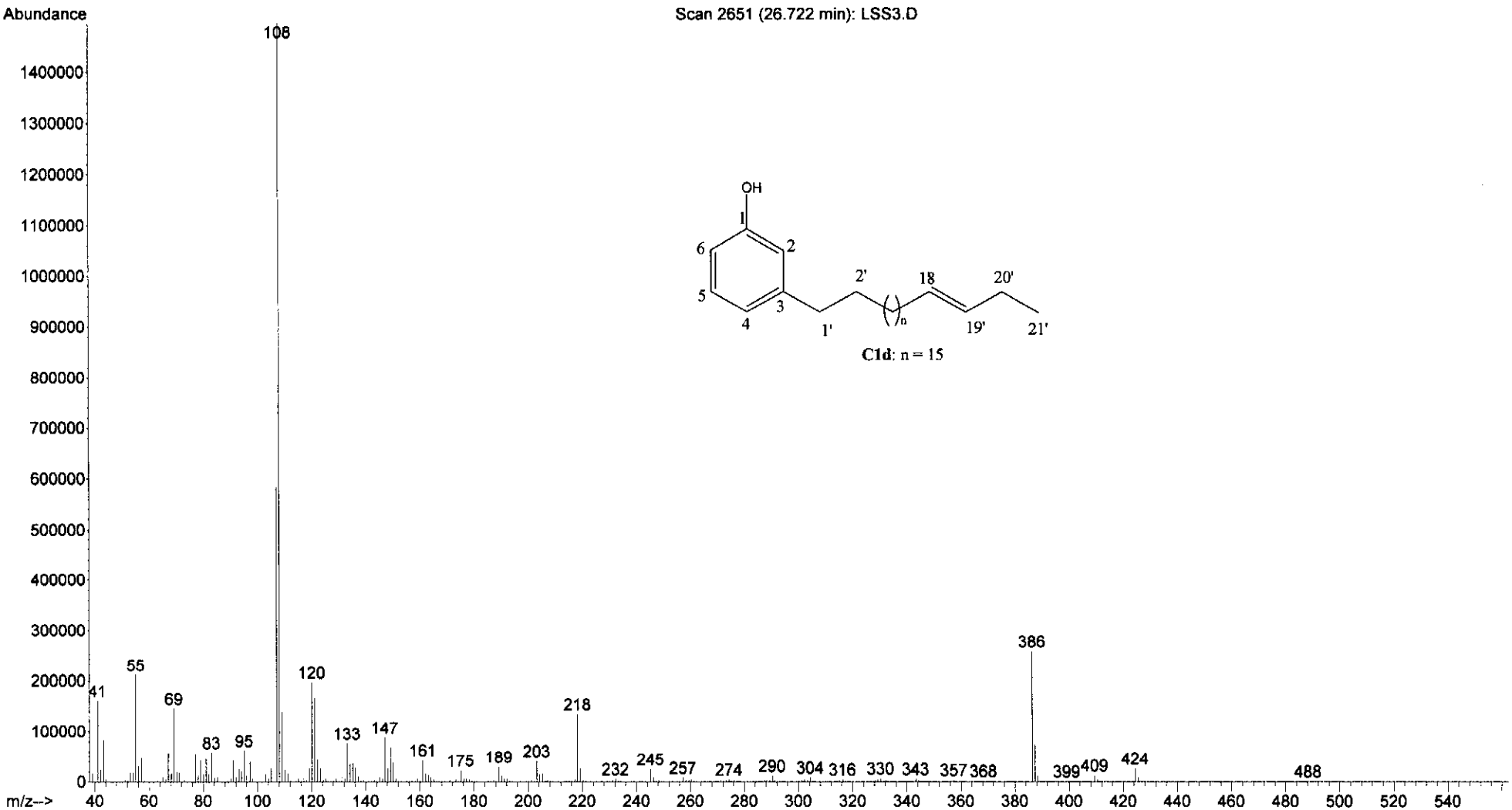

Mass spectrum of C1d

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Operator : DOROTHY
Acquired : 19 Jun 2011 12:38 using AcqMethod NATURAL
Instrument : Instrumen
Sample Name: MEOH LSRT 8-13
Misc Info :
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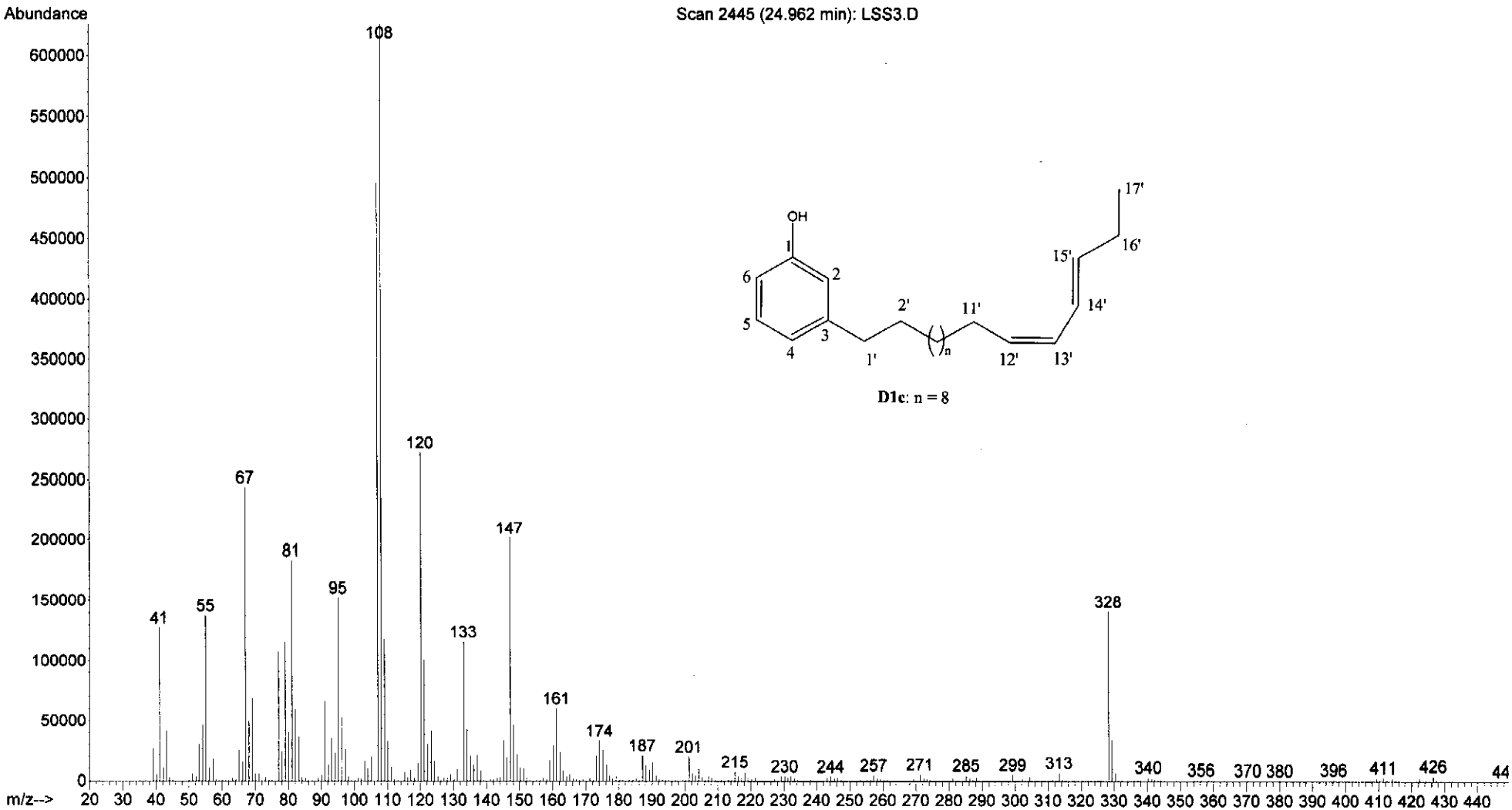

D1c: n = 8

Mass spectrum of D1c

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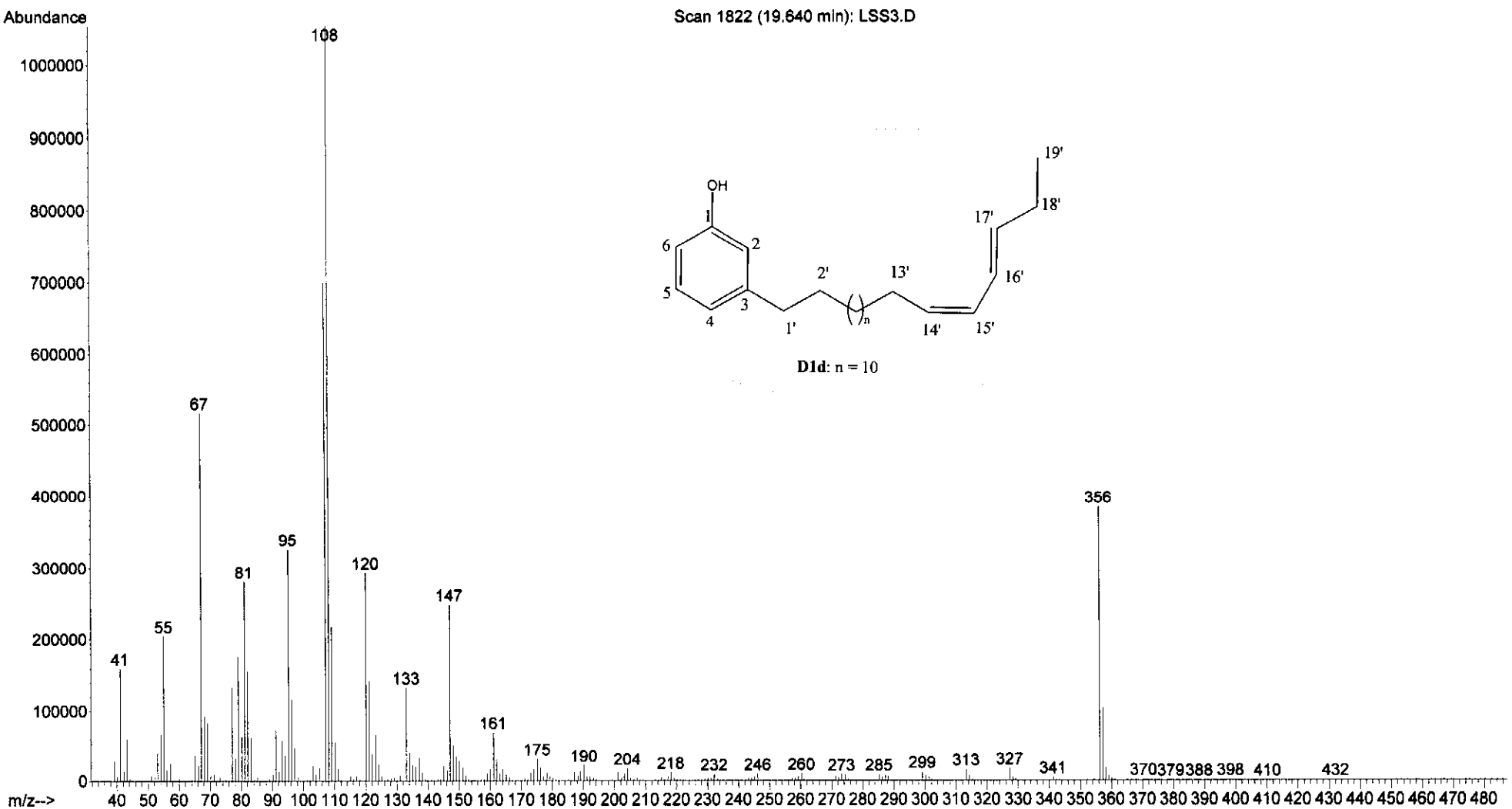
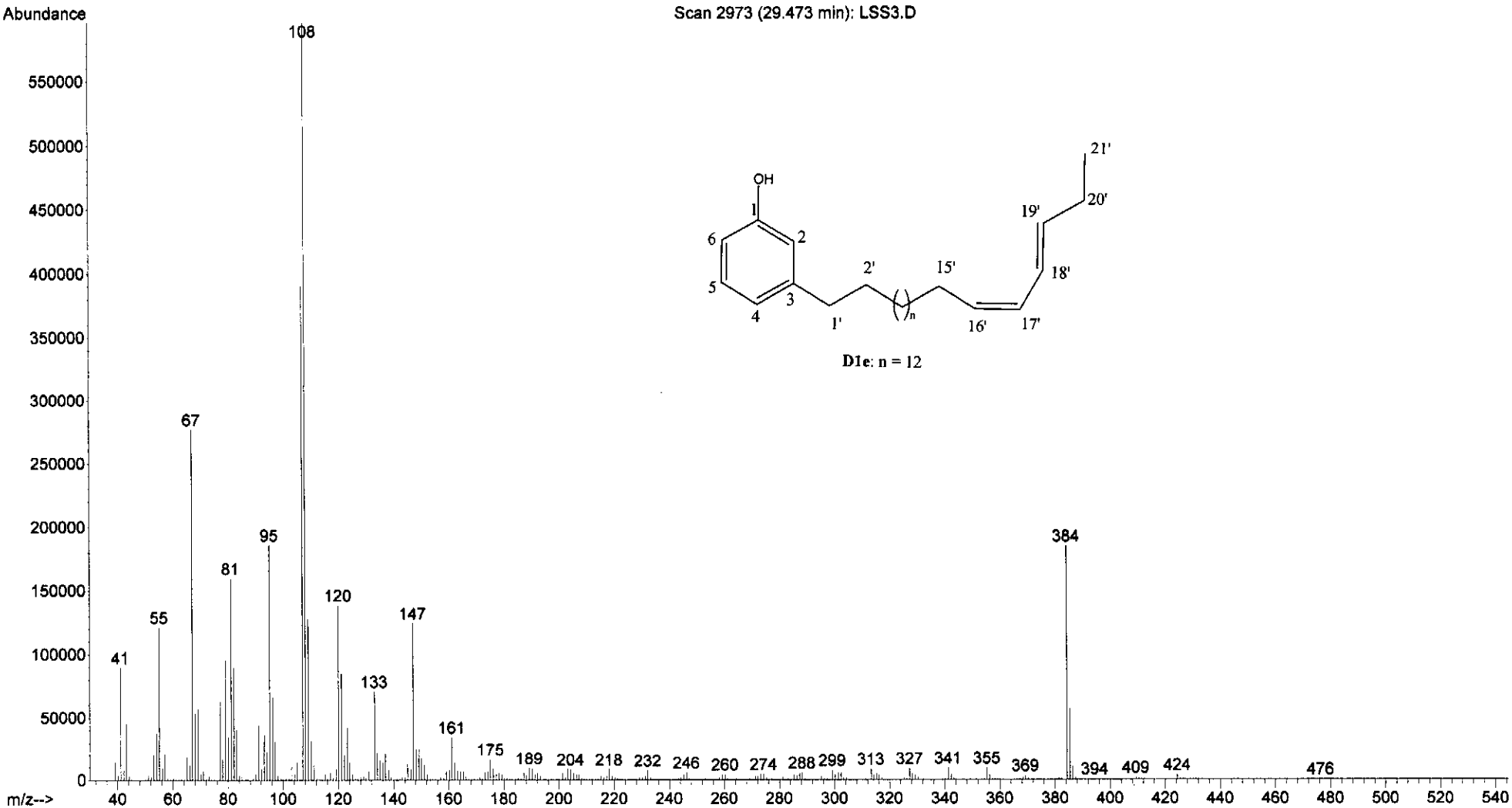
Operator : DOROTHY
Acquired : 19 Jun 2011 12:38 using AcqMethod NATURAL
Instrument : Instrumen
Sample Name: MEOH LSRT 8-13
Misc Info :
Vial Number: 1



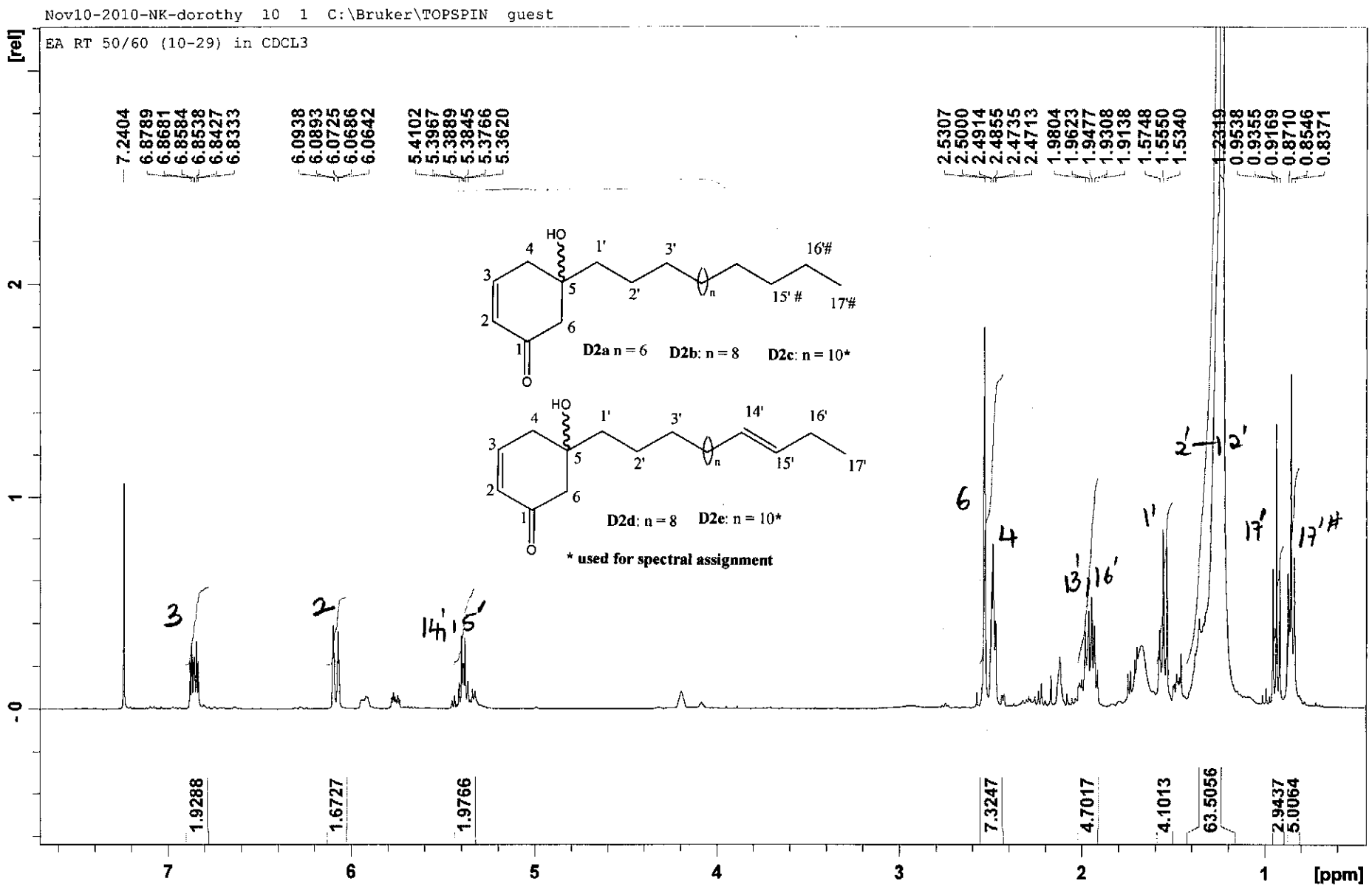
D1d: n = 10

Mass spectrum of D1d

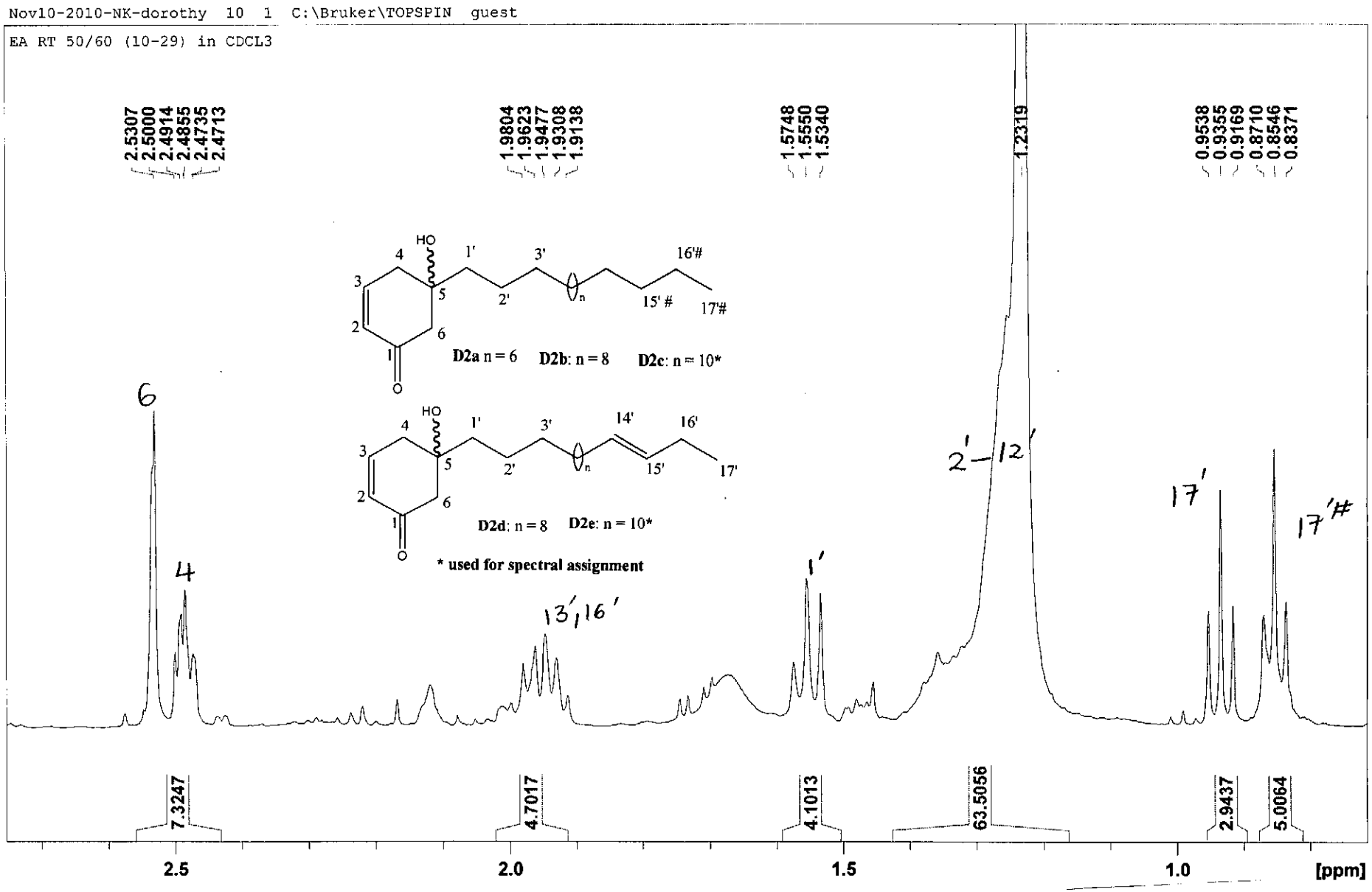
File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\DORTHY\LSS3.D
Operator : DOROTHY
Acquired : 19 Jun 2011 12:38 using AcqMethod NATURAL
Instrument : Instrumen
Sample Name: MEOH LSRT 8-13
Misc Info :
Vial Number: 1



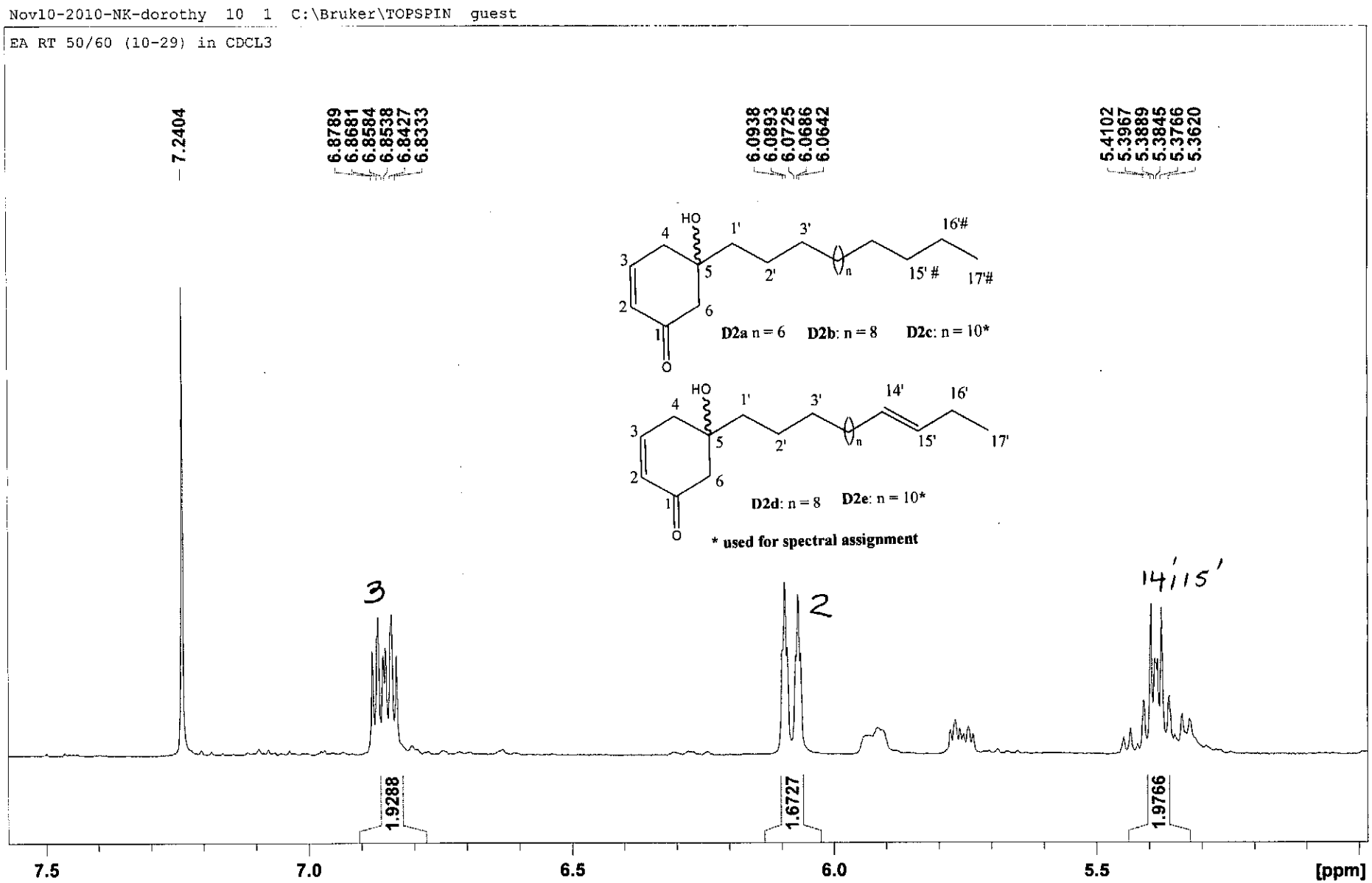
Mass spectrum of D1e



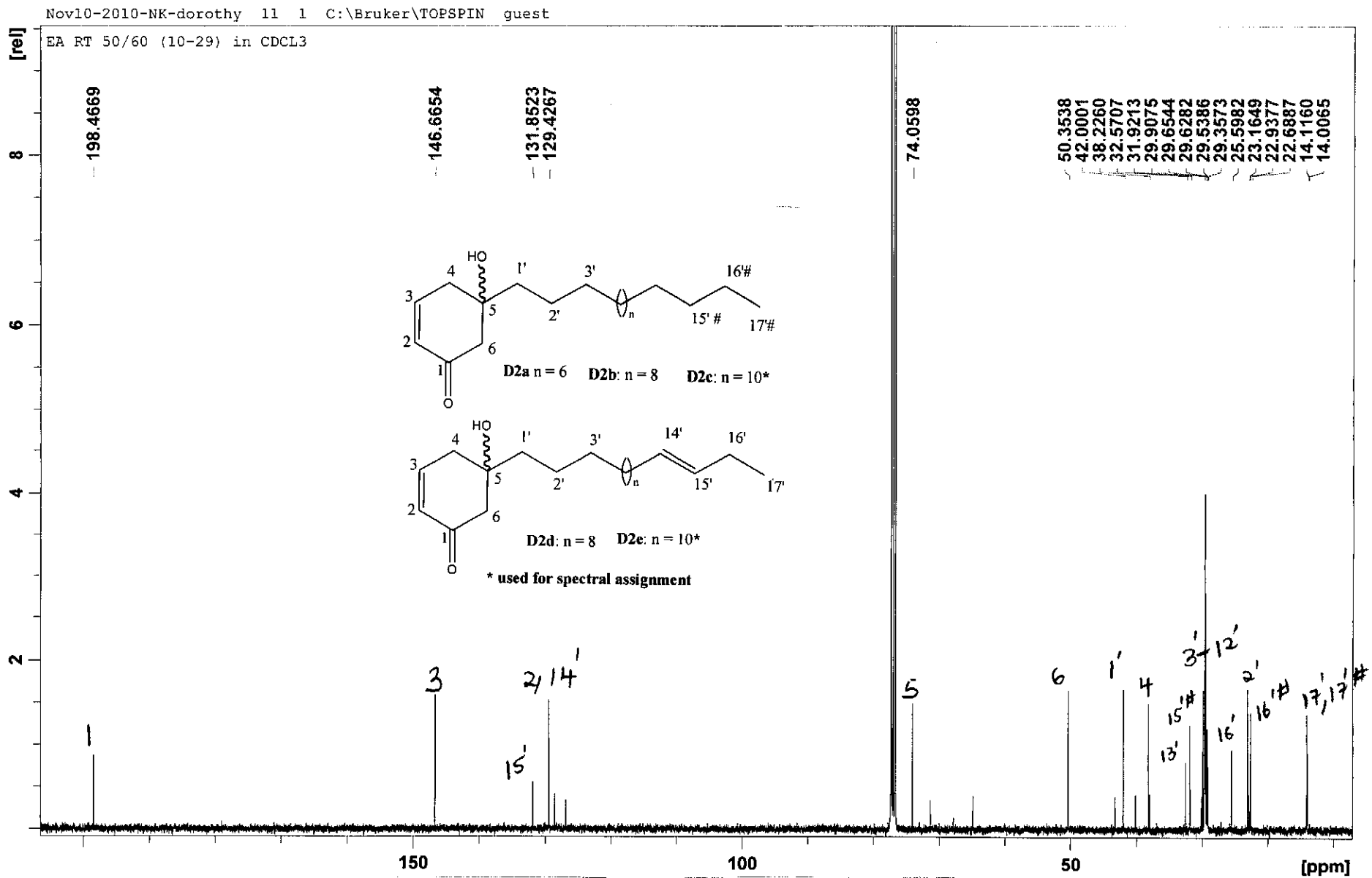
^{1}H NMR spectrum of a mixture of D2a, D2b, D2c, D2d and D2e



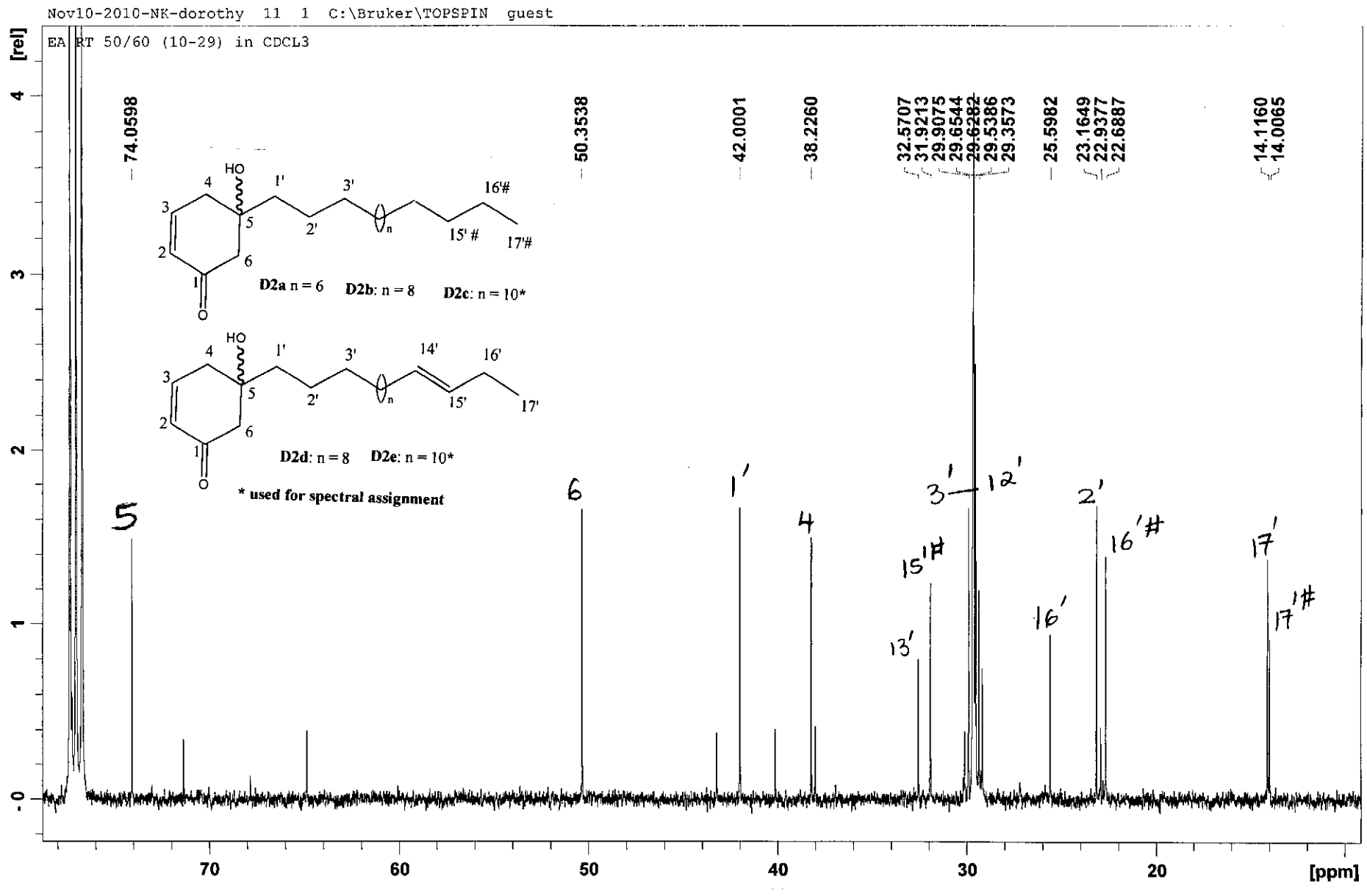
^{1}H NMR spectrum of a mixture of D2a, D2b, D2c, D2d and D2e (expanded)



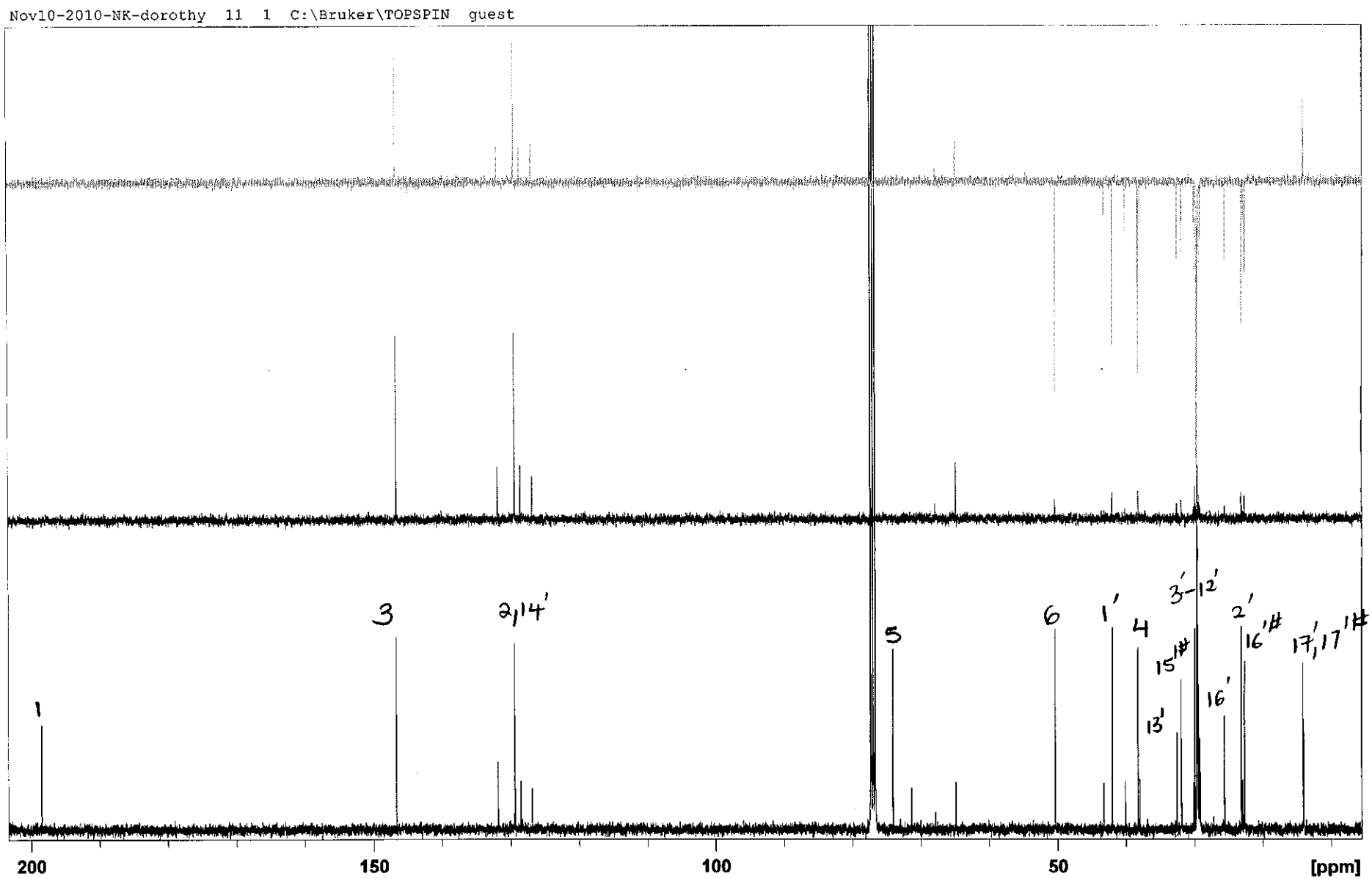
^{1}H NMR spectrum of a mixture of D2a, D2b, D2c, D2d and D2e (expanded)



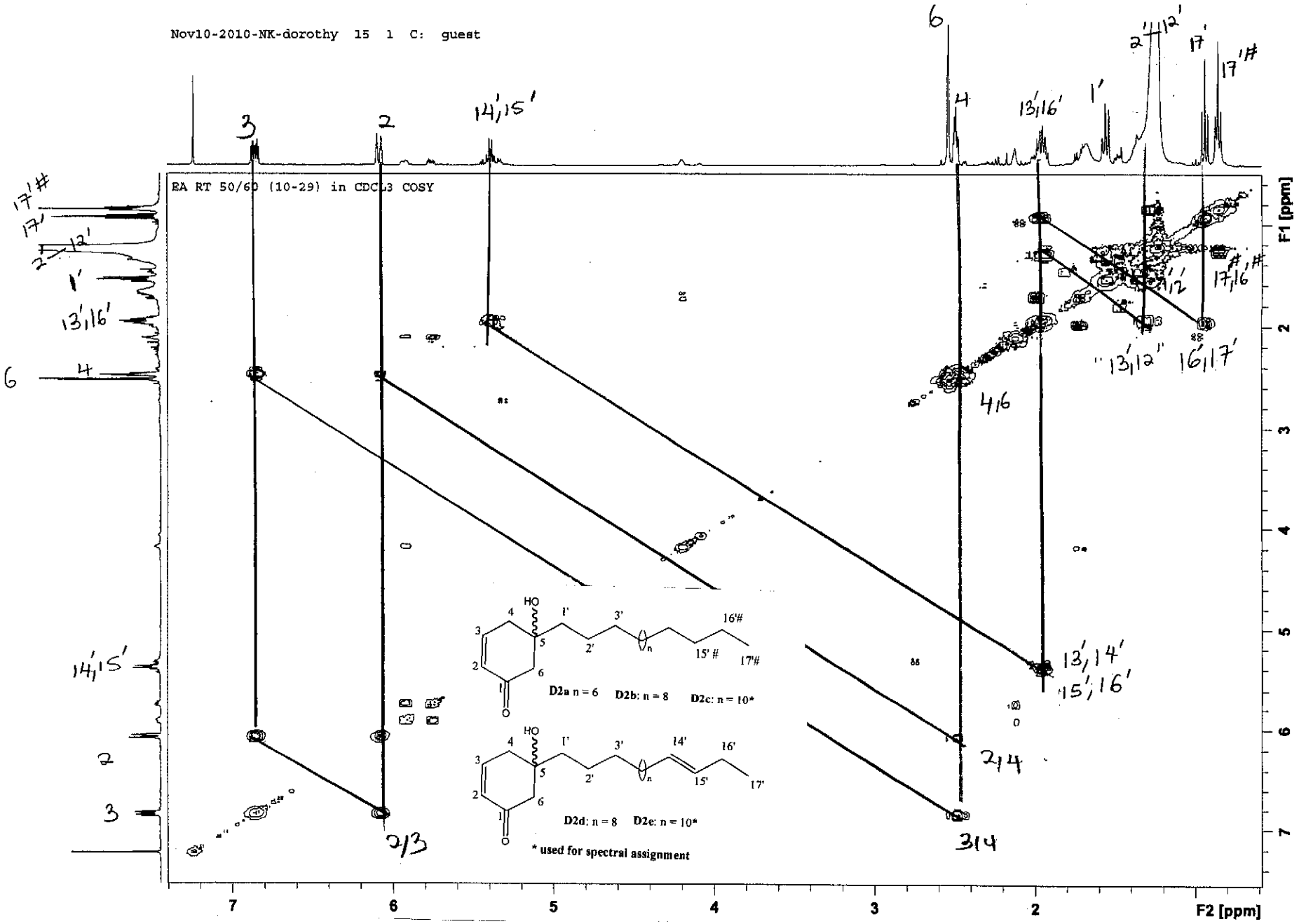
^{13}C NMR spectrum of a mixture of D2a, D2b, D2c, D2d and D2e



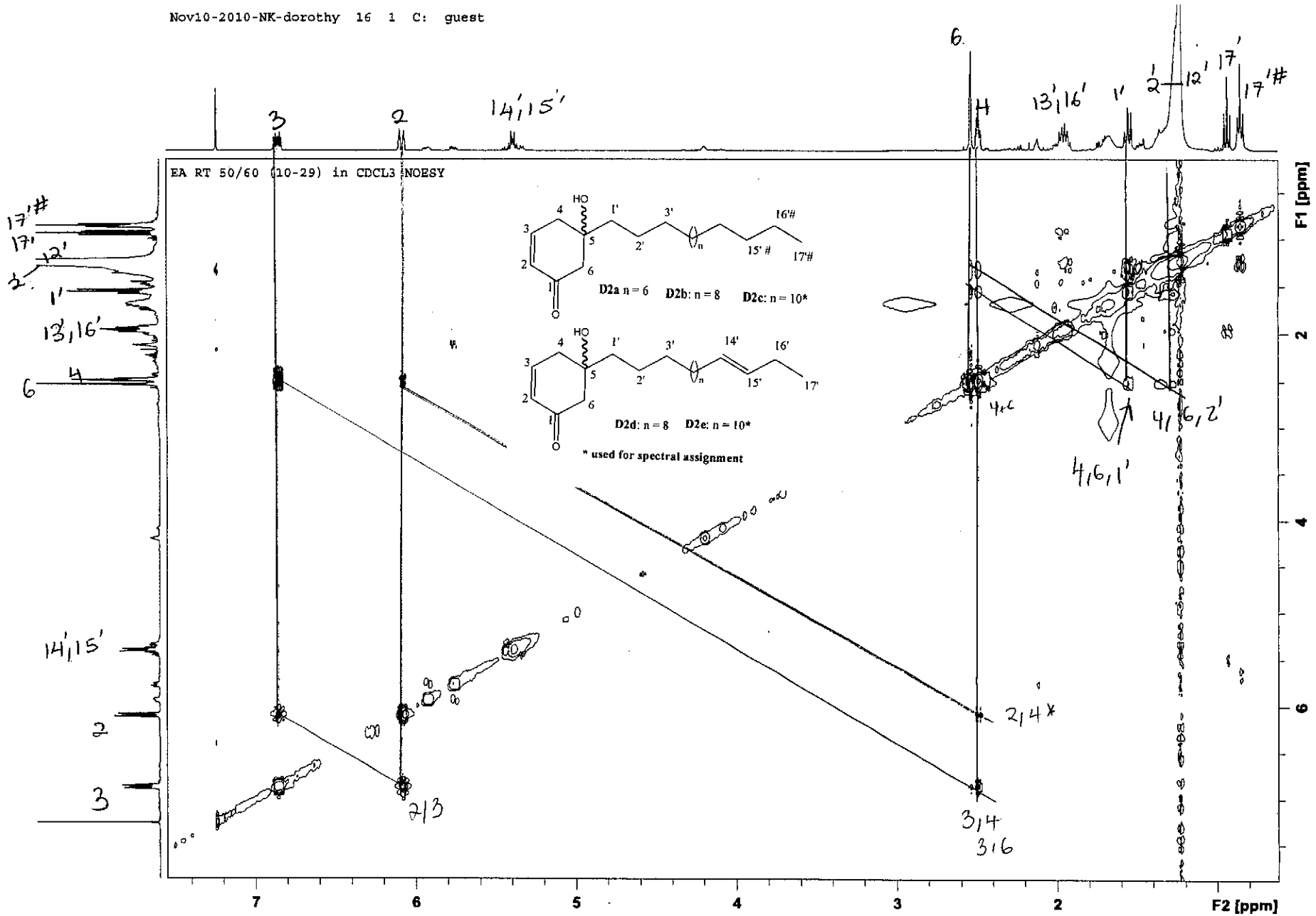
^{13}C NMR spectrum of a mixture of D2a, D2b, D2c, D2d and D2e (expanded)



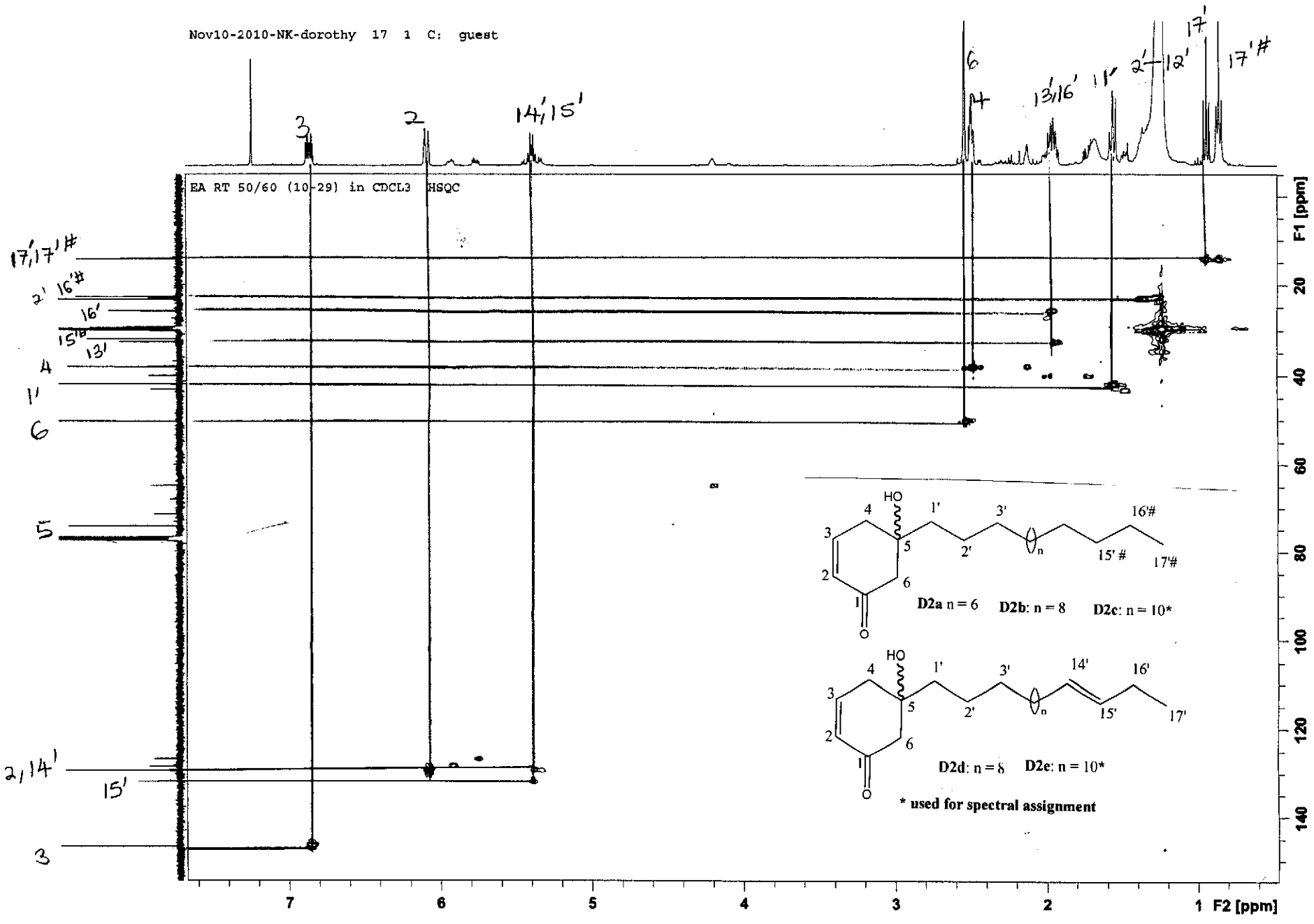
DEPT spectrum of a mixture of D2a, D2b, D2c, D2d and D2e



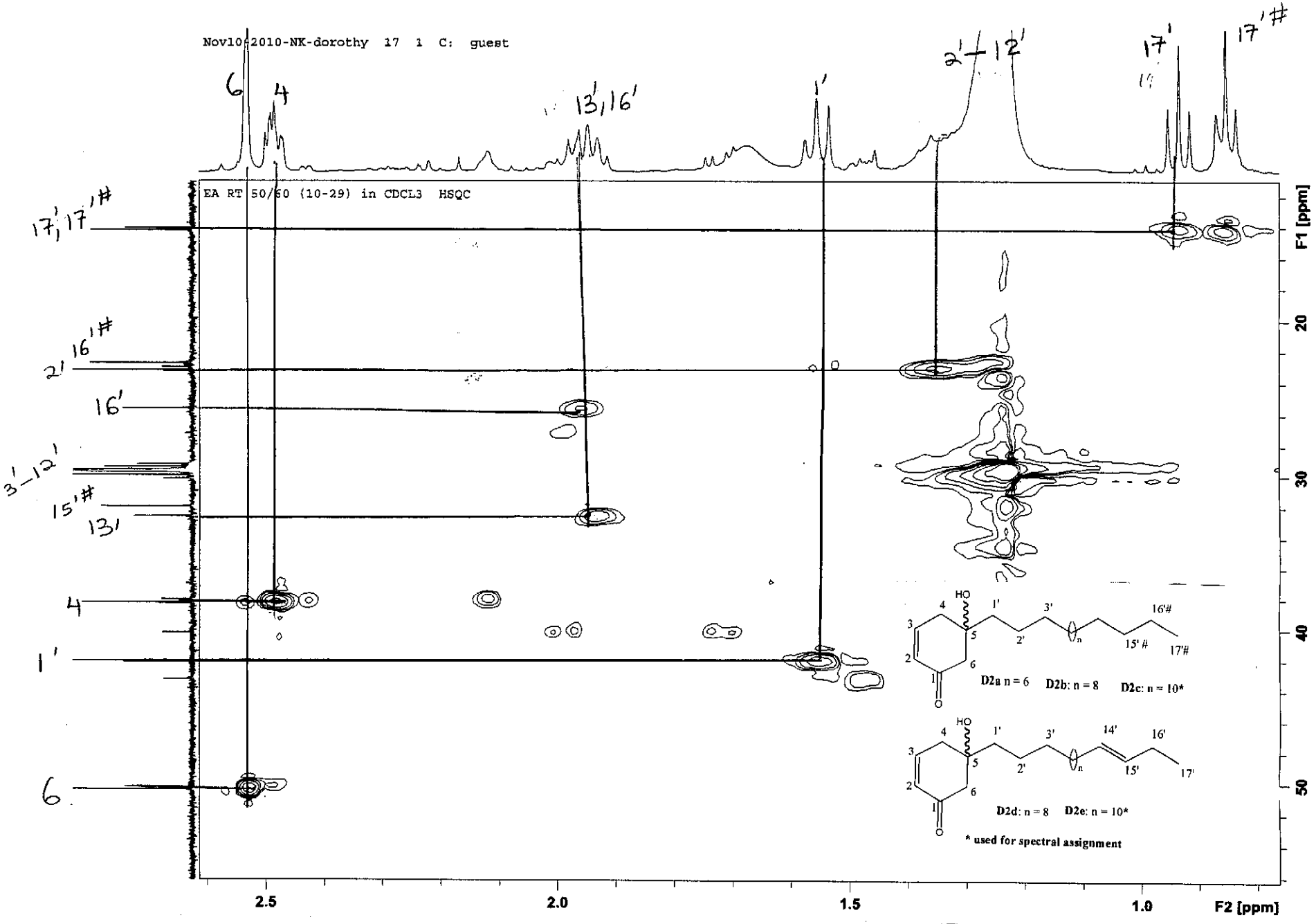
COSY spectrum of a mixture of D2a, D2b, D2c, D2d and D2e



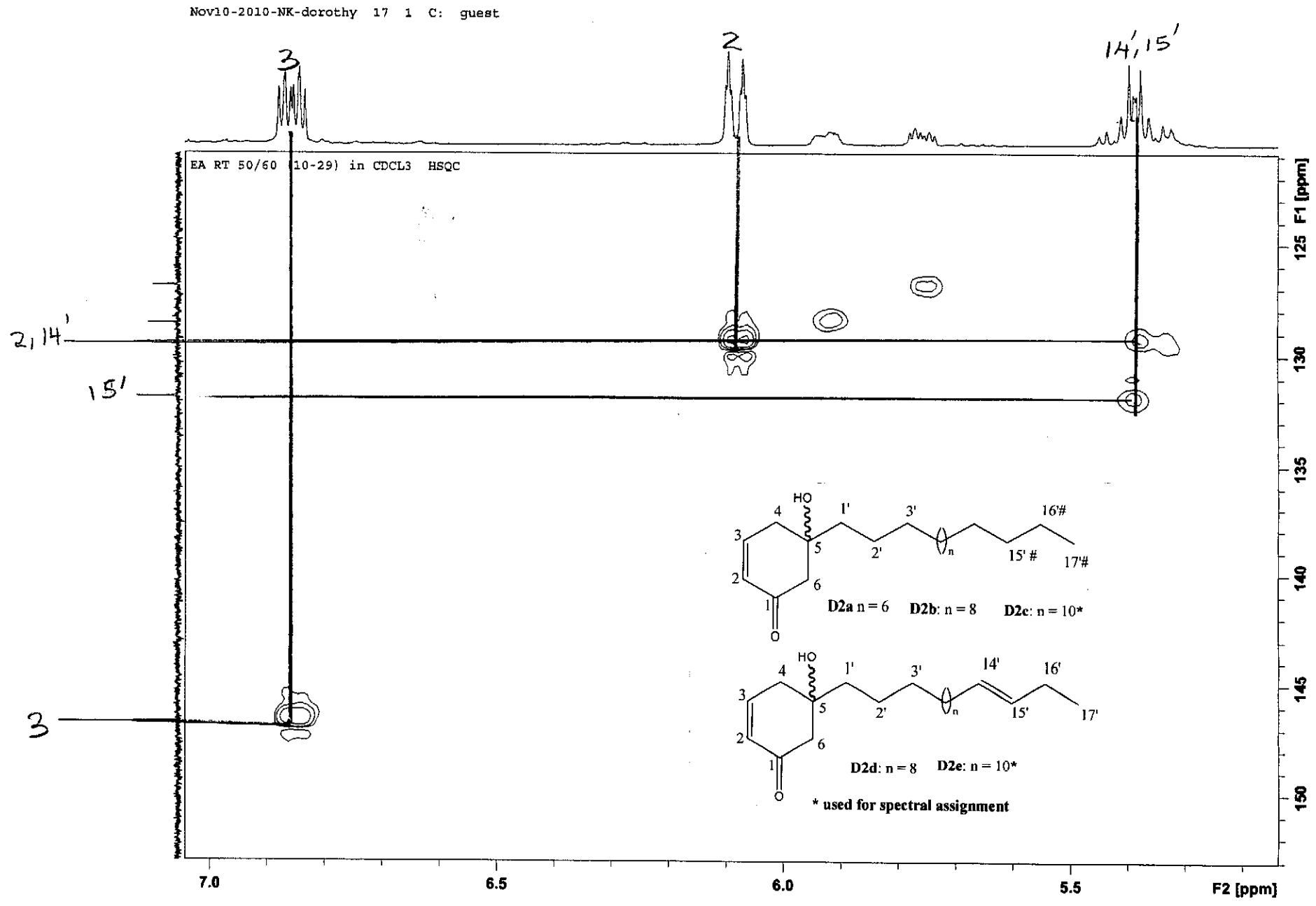
NOESY spectrum of a mixture of D2a, D2b, D2c, D2d and D2e



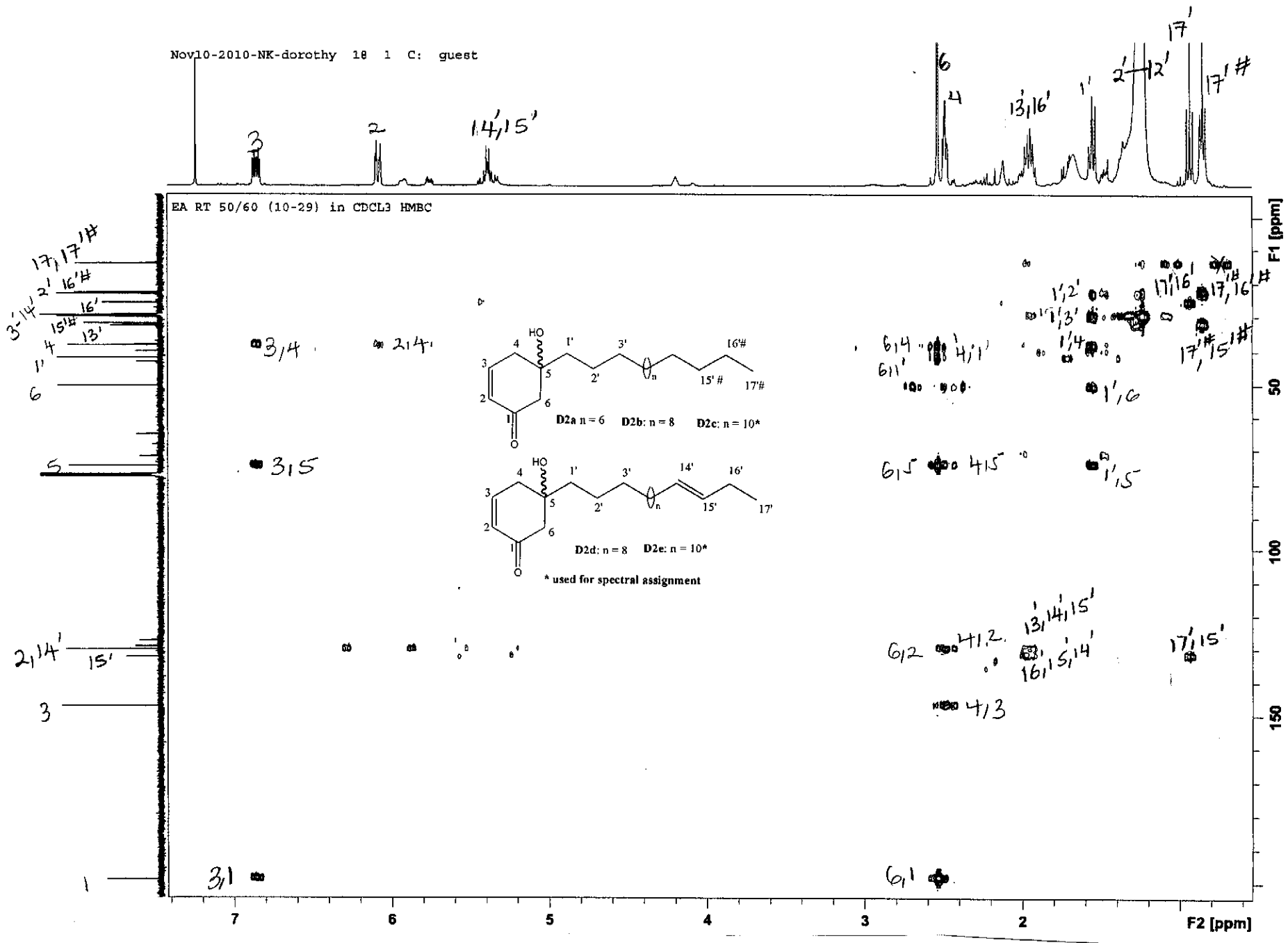
HSQC spectrum of a mixture of D2a, D2b, D2c, D2d and D2e



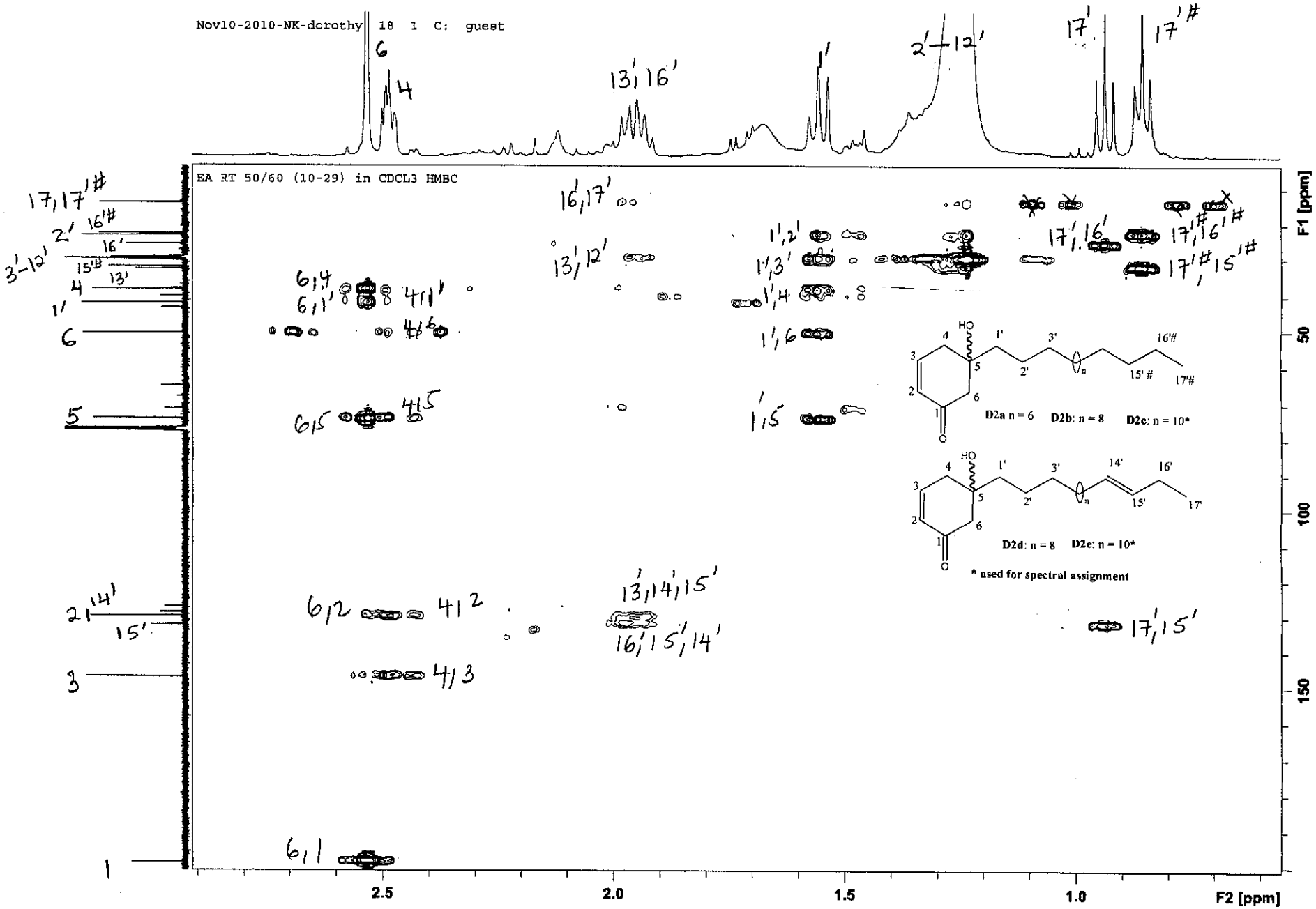
HSQC spectrum of a mixture of D2a, D2b, D2c, D2d and D2e (expanded)



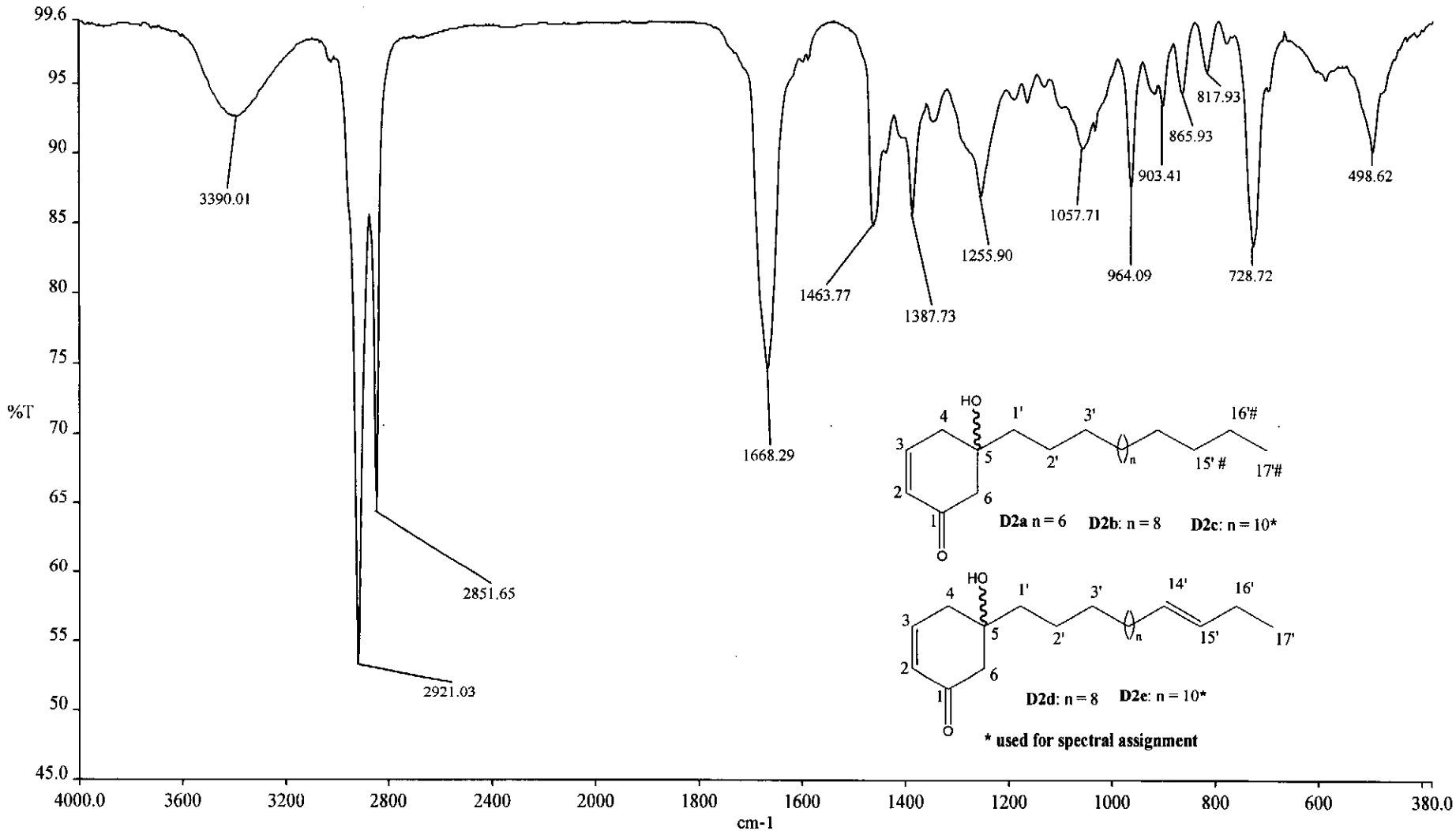
HSQC spectrum of a mixture of D2a, D2b, D2c, D2d and D2e (expanded)



HMBC spectrum of a mixture of D2a, D2b, D2c, D2d and D2e



HMBC spectrum of a mixture of D2a, D2b, D2c, D2d and D2e (expanded)

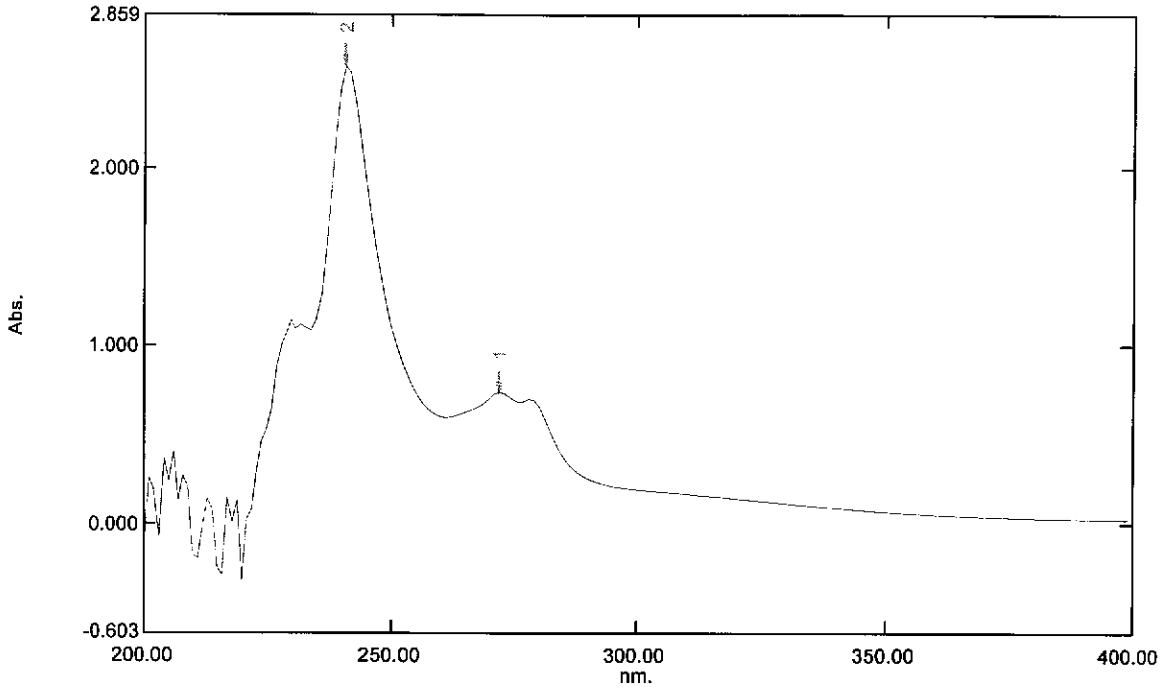


IR spectrum of a mixture of D2a, D2b, D2c, D2d and D2e

Spectrum Peak Pick Report

23/05/2012 06:03:06 PM

Data Set: EA RT 50-60 10-29.spc - Storage 170736



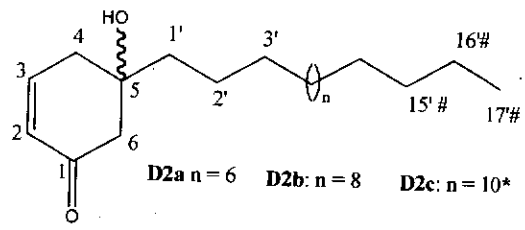
No.	P/V	Wavelength	Abs.	Description
1	↑	272.00	0.743	
2	↑	241.00	2.570	
3	↓	261.00	0.599	

Measurement Properties
Wavelength Range (nm.): 200.00 to 400.00
Scan Speed: Medium
Sampling Interval: 1.0
Auto Sampling Interval: Disabled
Scan Mode: Single

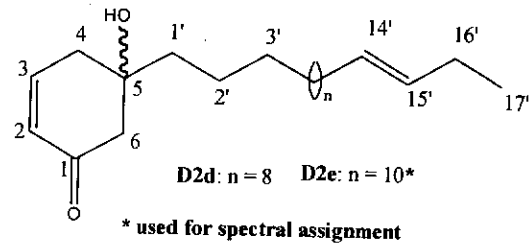
Instrument Properties
Instrument Type: UV-3600 Series
Measuring Mode: Absorbance
Slit Width: 2.0 nm
Time Constant: 0.1 sec.
Source Lamp: Auto
Light Source Change Wavelength: 310.00 nm
Detector Unit: Direct
Detector Change Wavelength: 830.00 nm 1800.00 nm
Grating Change Wavelength: 720.00 nm
S/R Exchange: Normal
Detector Lock: Auto
Slit Program: Normal
Beam Mode: Double
Stair Correction: Disable

Attachment Properties
Attachment: None

Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length:
Additional Information:



D2a n = 6 **D2b**: n = 8 **D2c**: n = 10*

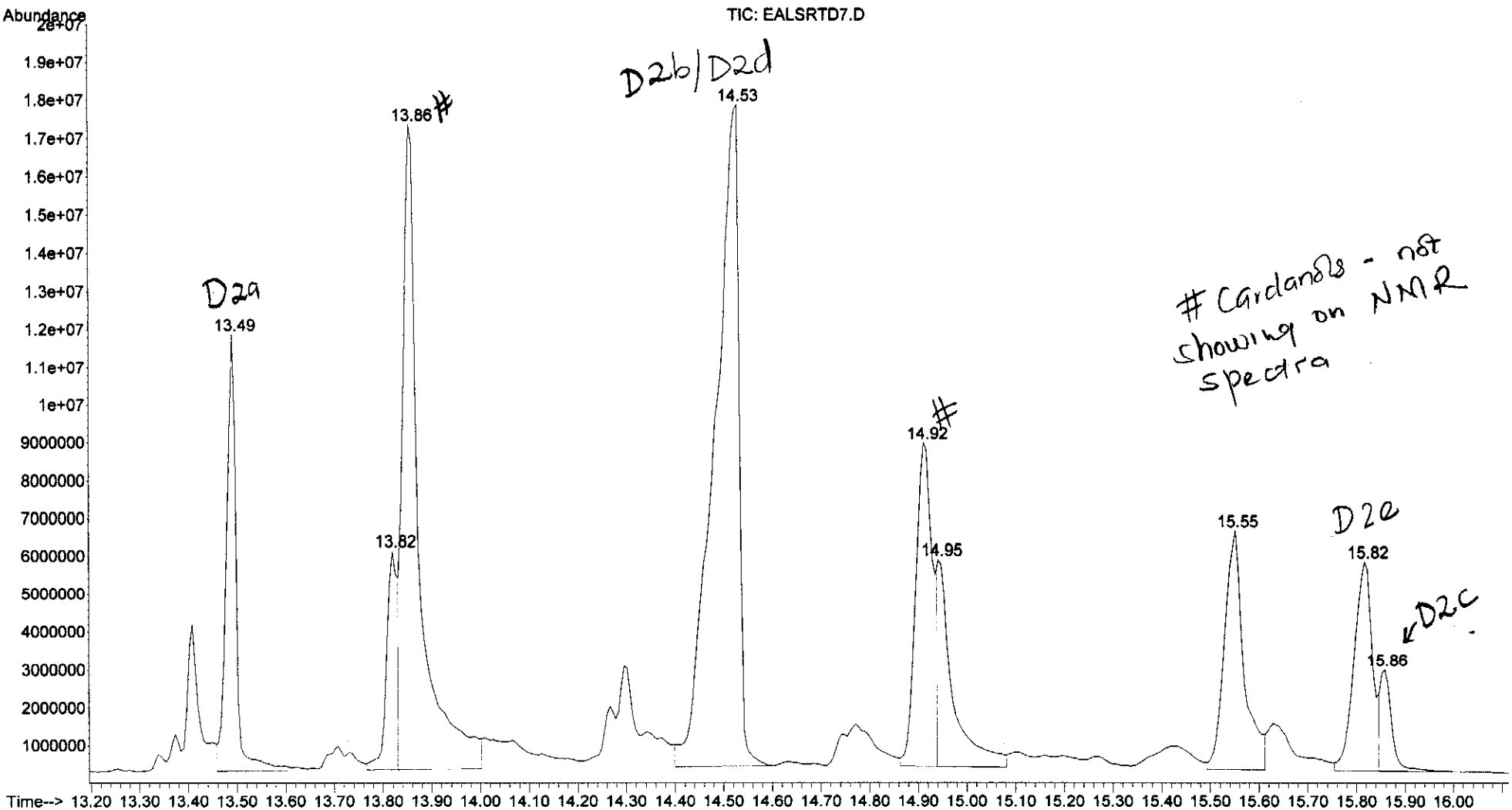


D2d: n = 8 **D2e**: n = 10*

*** used for spectral assignment**

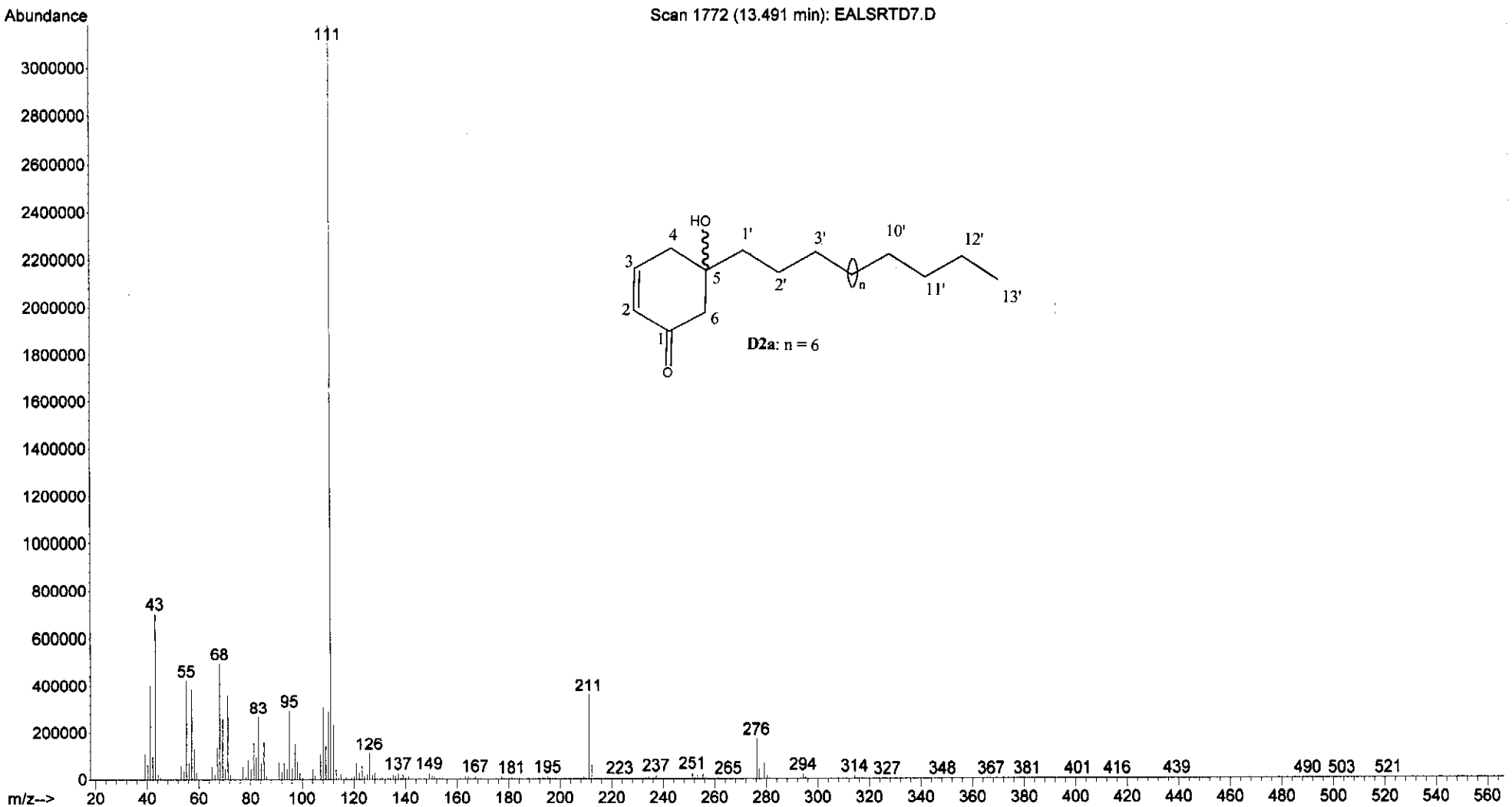
UV spectrum of a mixture of D2a, D2b, D2c, D2d and D2e

File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\EALSRTD7.D
Operator : Dorothy
Acquired : 28 Apr 2011 9:44 using AcqMethod NATURALJ
Instrument : Instrumen
Sample Name: EA LS RT50/60 (10-29)
Misc Info :
Vial Number: 1



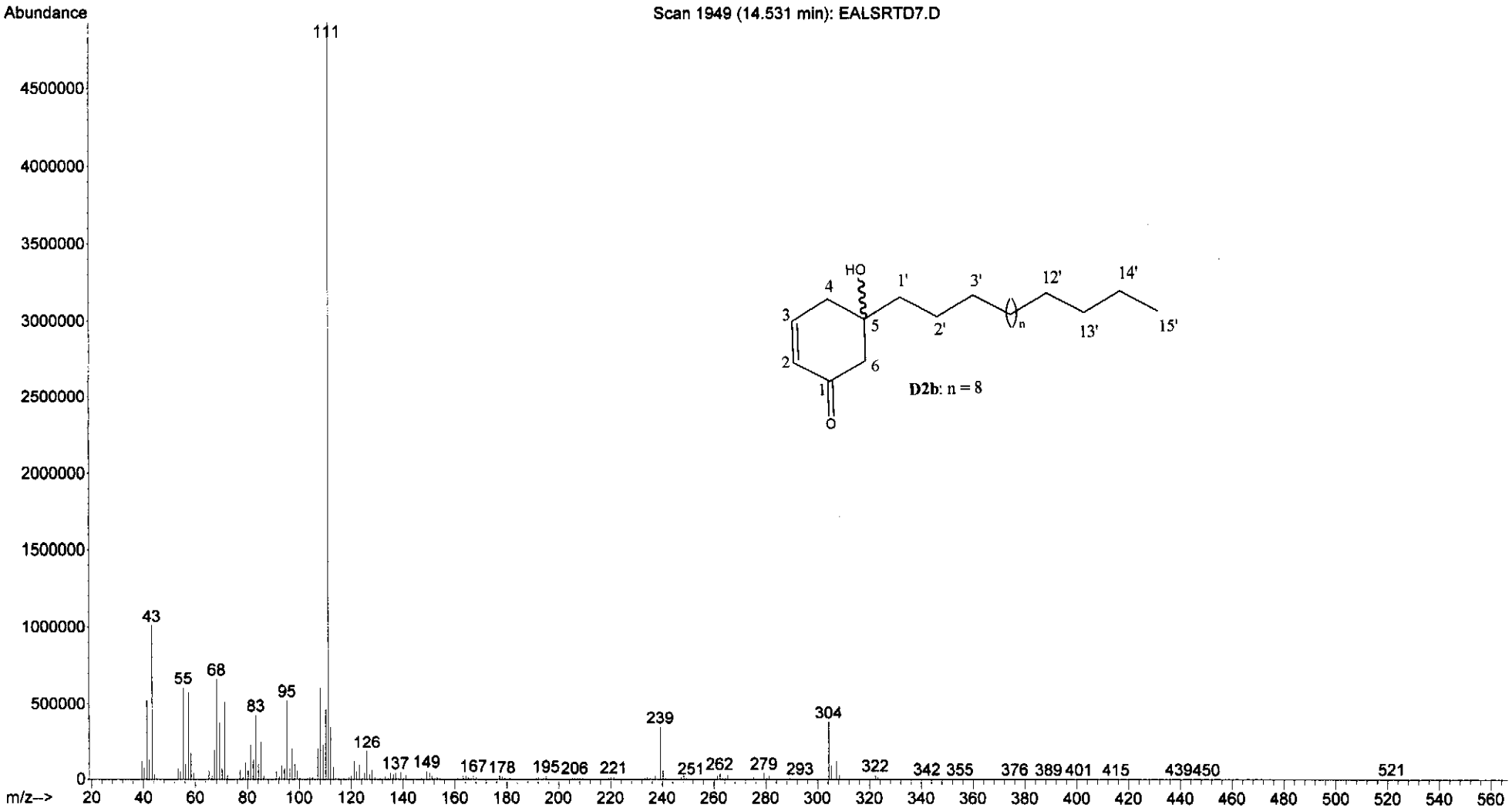
GC chromatogram of a mixture of D2a, D2b, D2c, D2d and D2e

File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\EALSRTD7.D
Operator : Dorothy
Acquired : 28 Apr 2011 9:44 using AcqMethod NATURALJ
Instrument : Instrumen
Sample Name: EA LS RT50/60 (10-29)
Misc Info :
Vial Number: 1



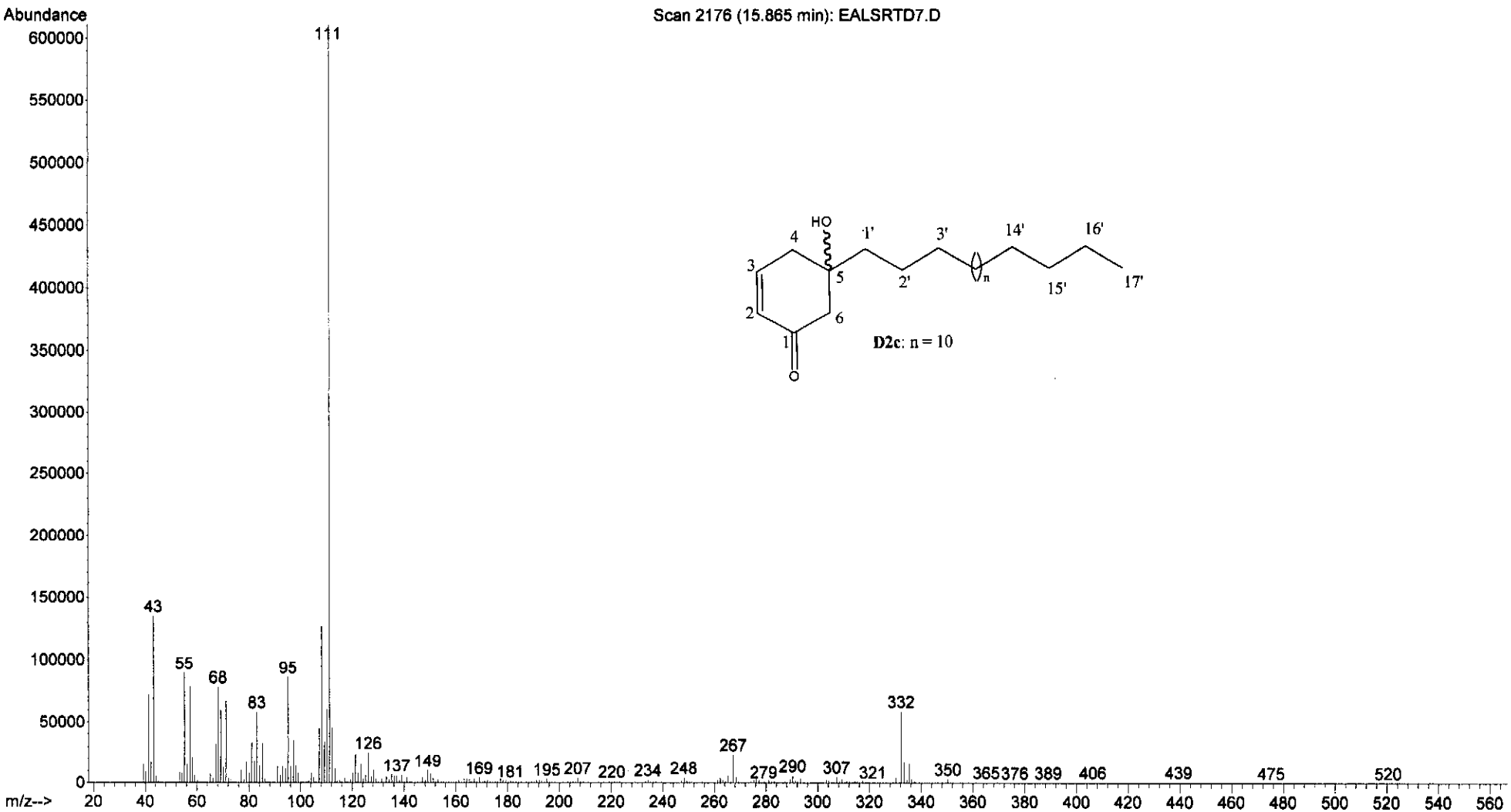
Mass spectrum of D2a

File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\EALSRTD7.D
Operator : Dorothy
Acquired : 28 Apr 2011 9:44 using AcqMethod NATURALJ
Instrument : Instrumen
Sample Name: EA LS RT50/60 (10-29)
Misc Info :
Vial Number: 1



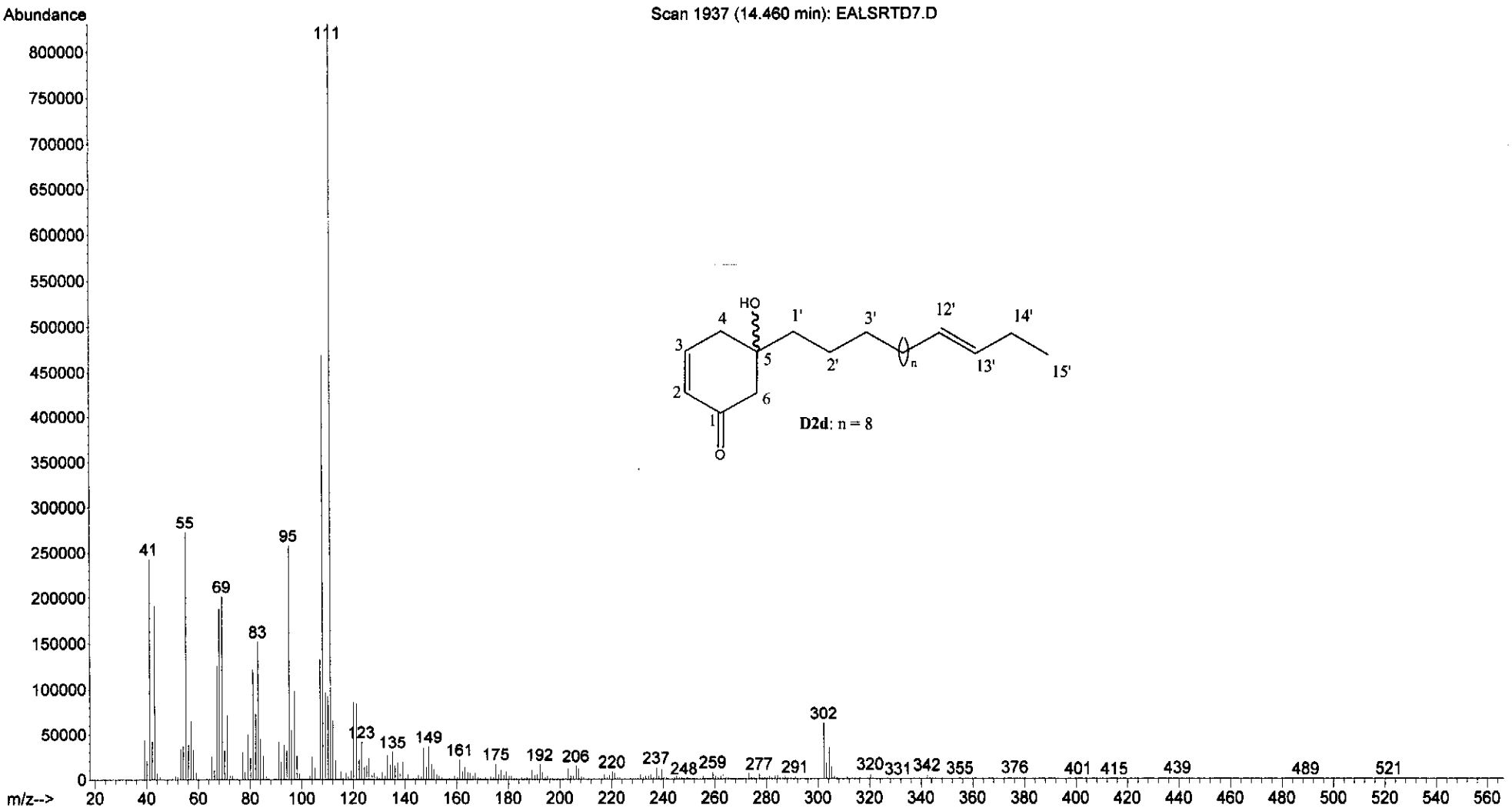
Mass spectrum of D2b

File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\EALSRTD7.D
Operator : Dorothy
Acquired : 28 Apr 2011 9:44 using AcqMethod NATURALJ
Instrument : Instrumen
Sample Name: EA LS RT50/60 (10-29)
Misc Info :
Vial Number: 1



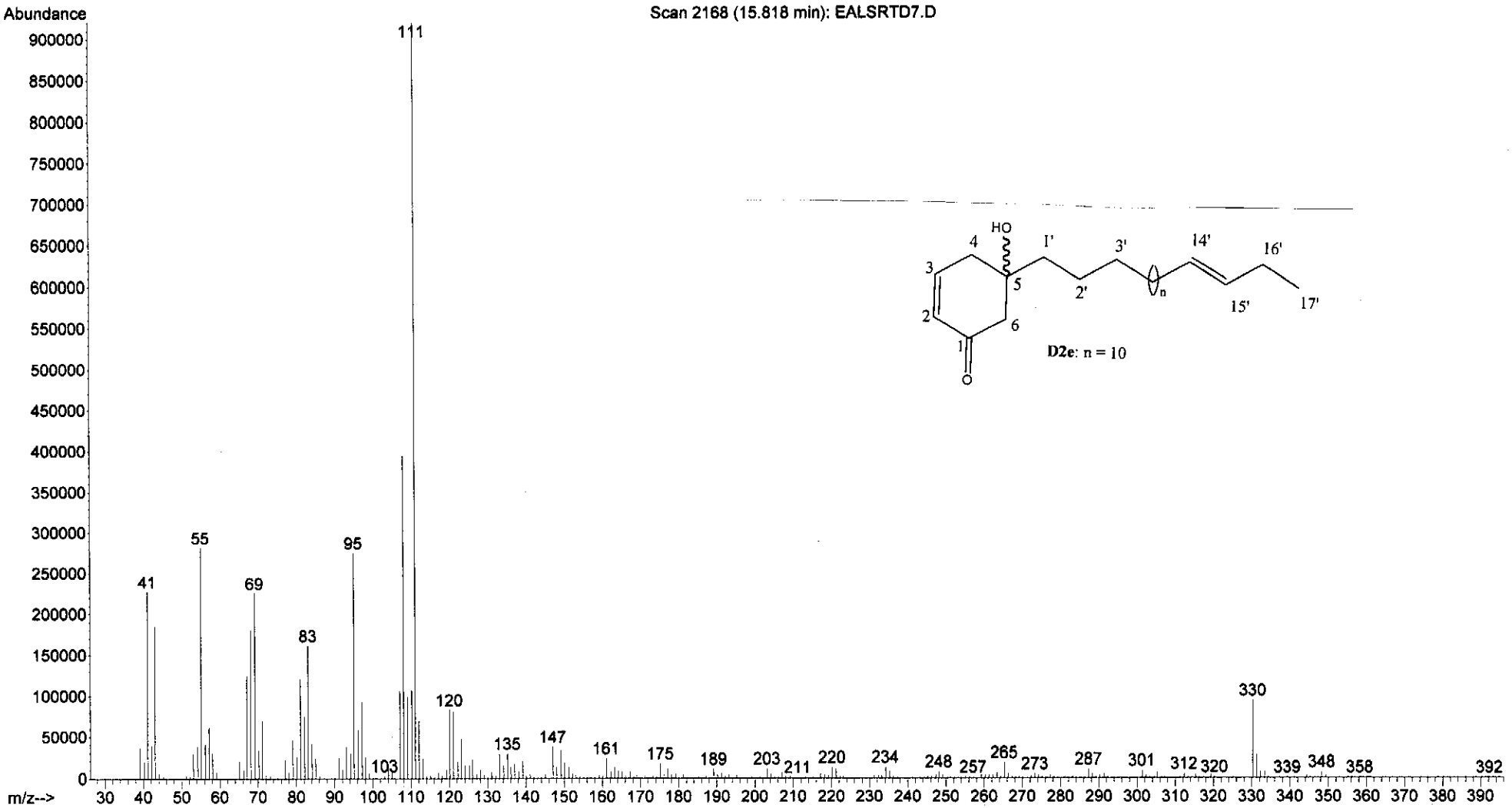
Mass spectrum of D2c

File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\EALSRTD7.D
Operator : Dorothy
Acquired : 28 Apr 2011 9:44 using AcqMethod NATURALJ
Instrument : Instrumen
Sample Name: EA LS RT50/60 (10-29)
Misc Info :
Vial Number: 1

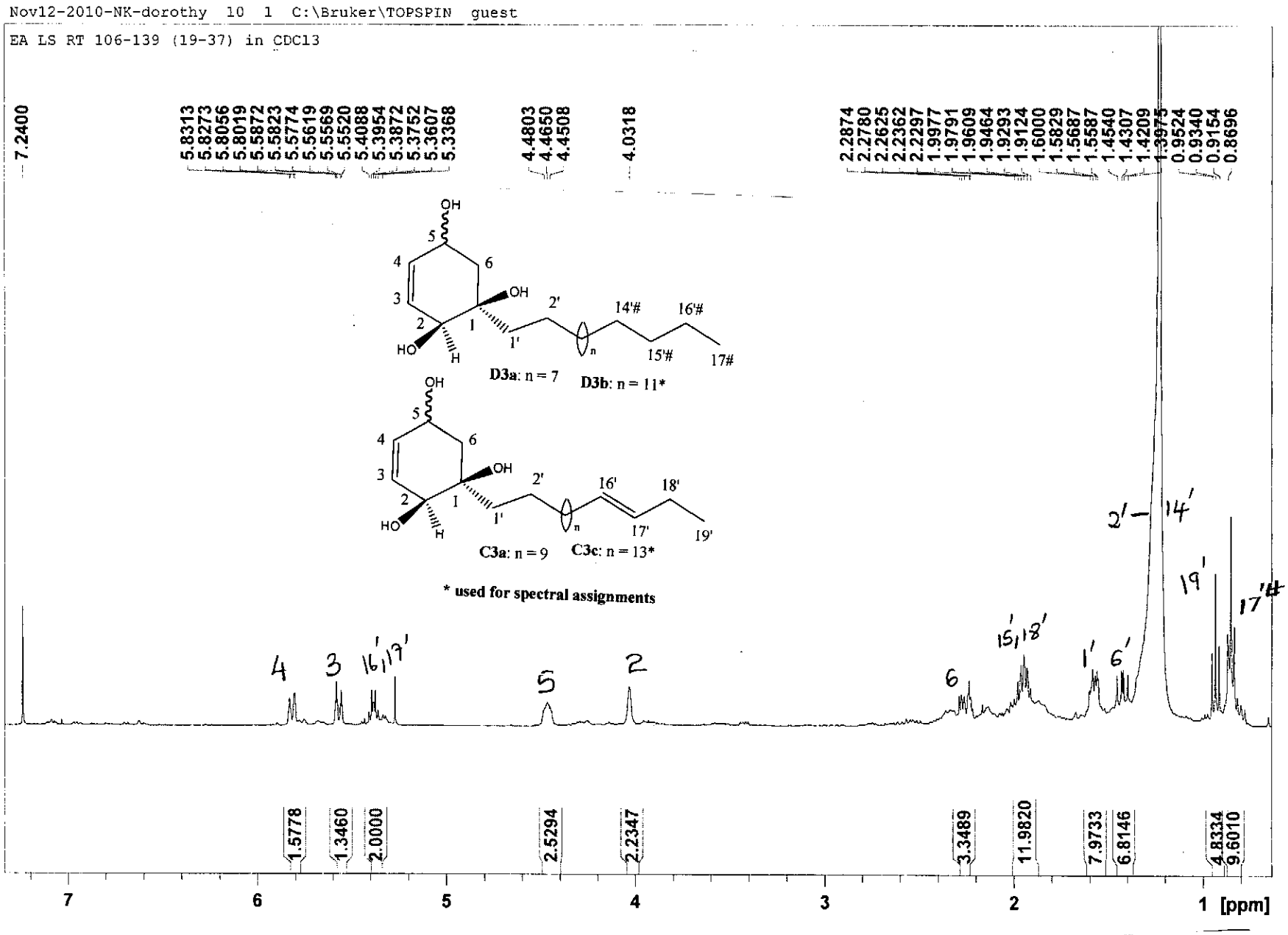


Mass spectrum of D2d

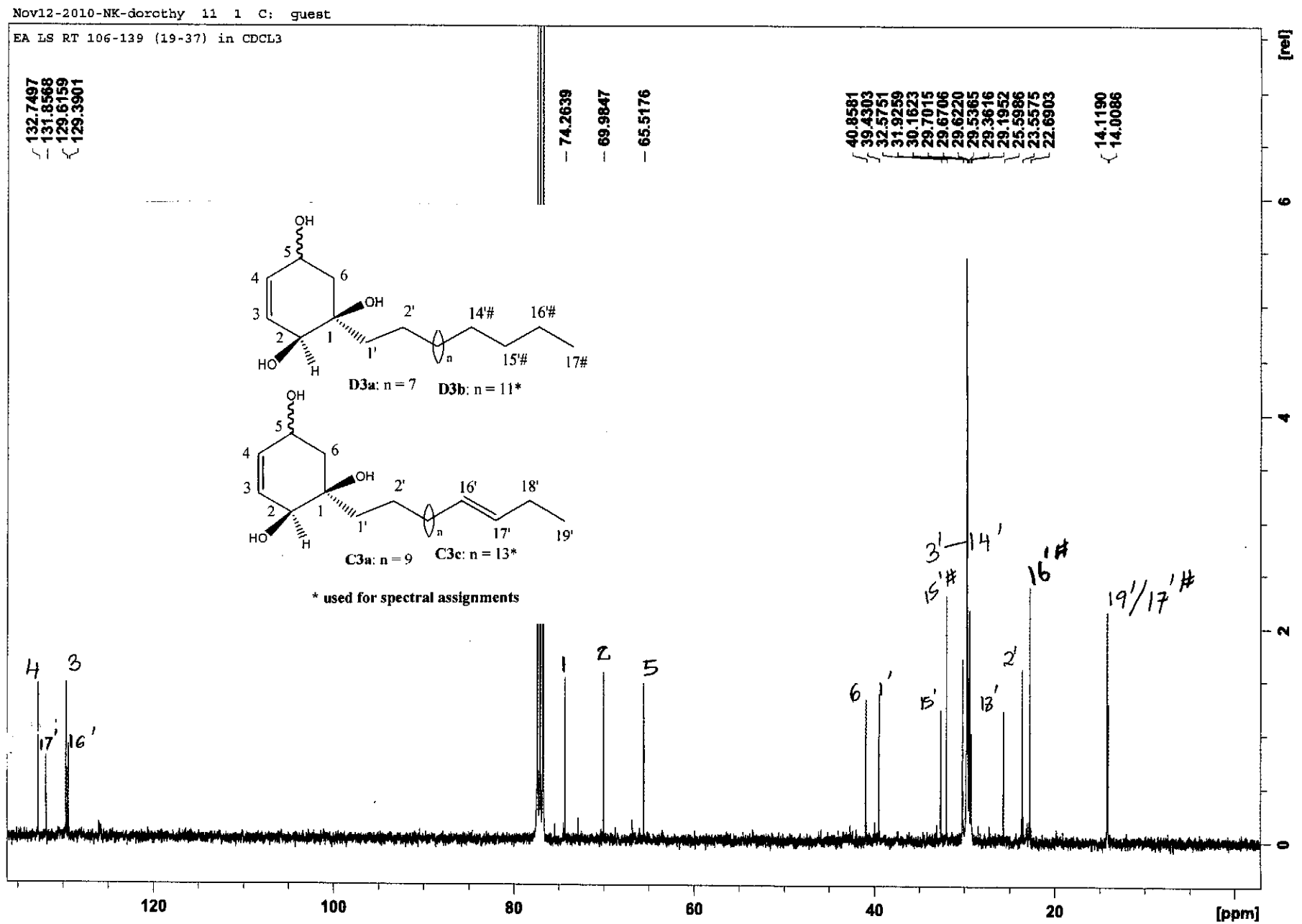
File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\EALSRTD7.D
Operator : Dorothy
Acquired : 28 Apr 2011 9:44 using AcqMethod NATURALJ
Instrument : Instrumen
Sample Name: EA LS RT50/60 (10-29)
Misc Info :
Vial Number: 1



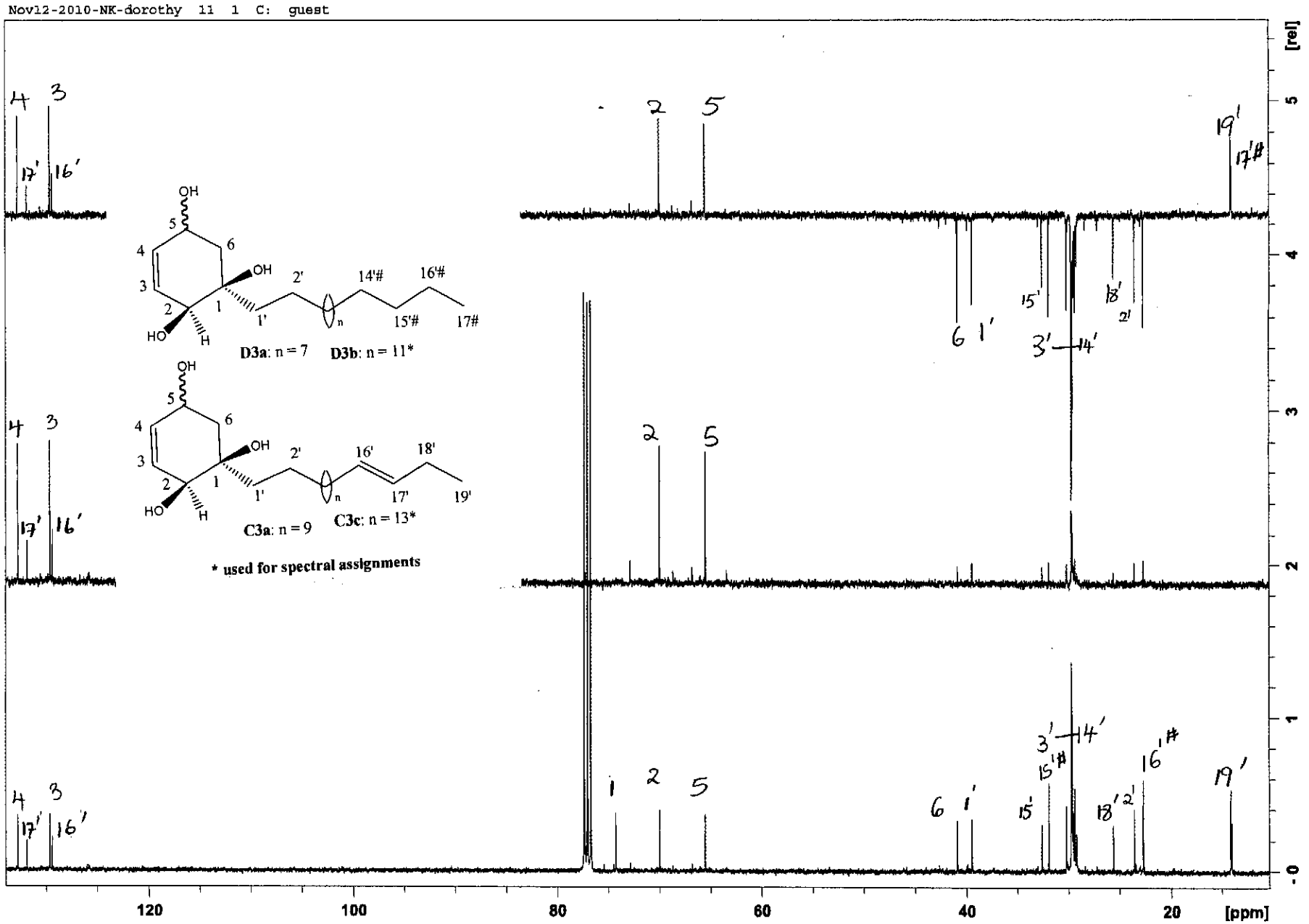
Mass spectrum of D2e



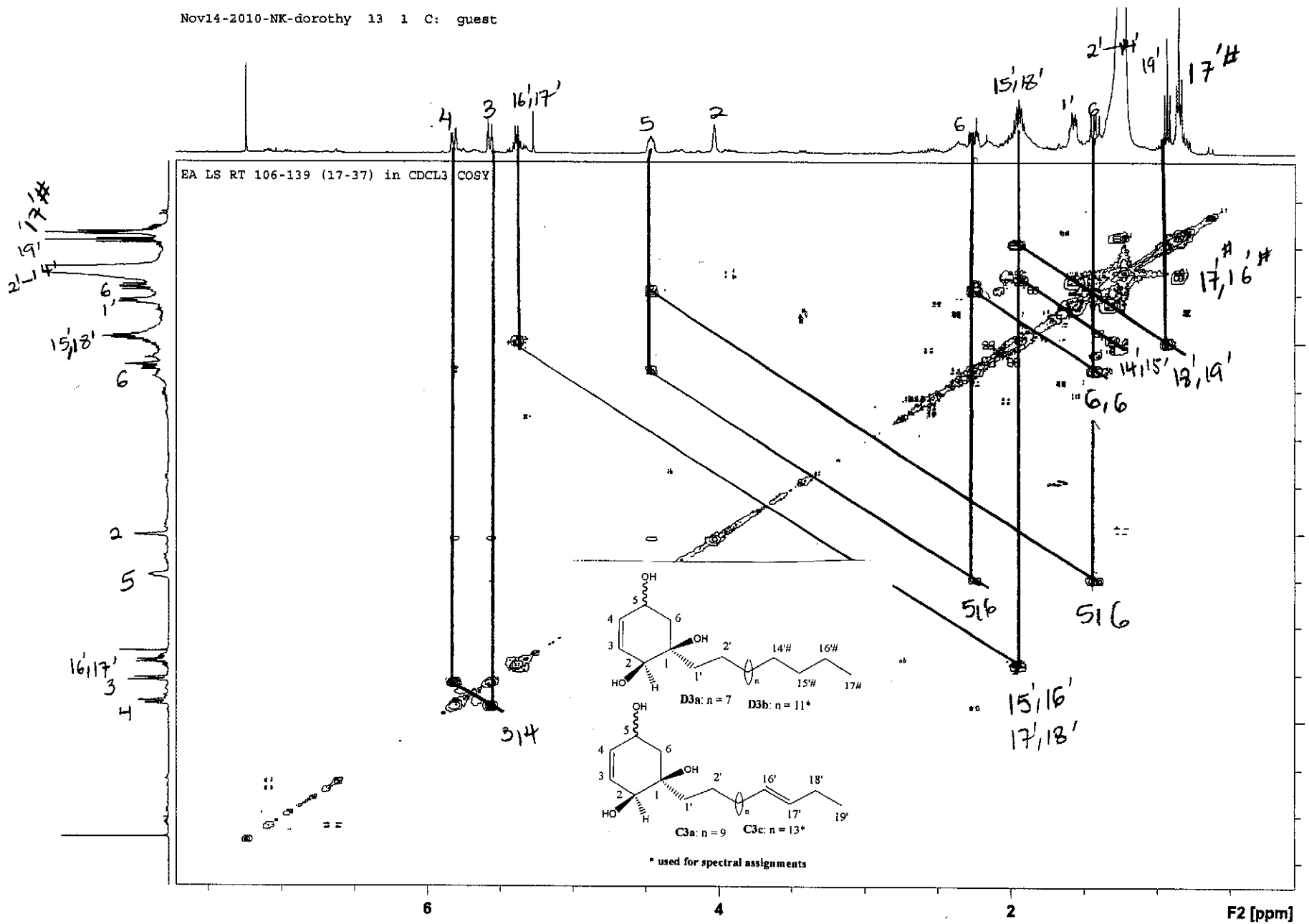
^{1}H NMR spectrum of a mixture of D3a, D3b, C3a, and C3c



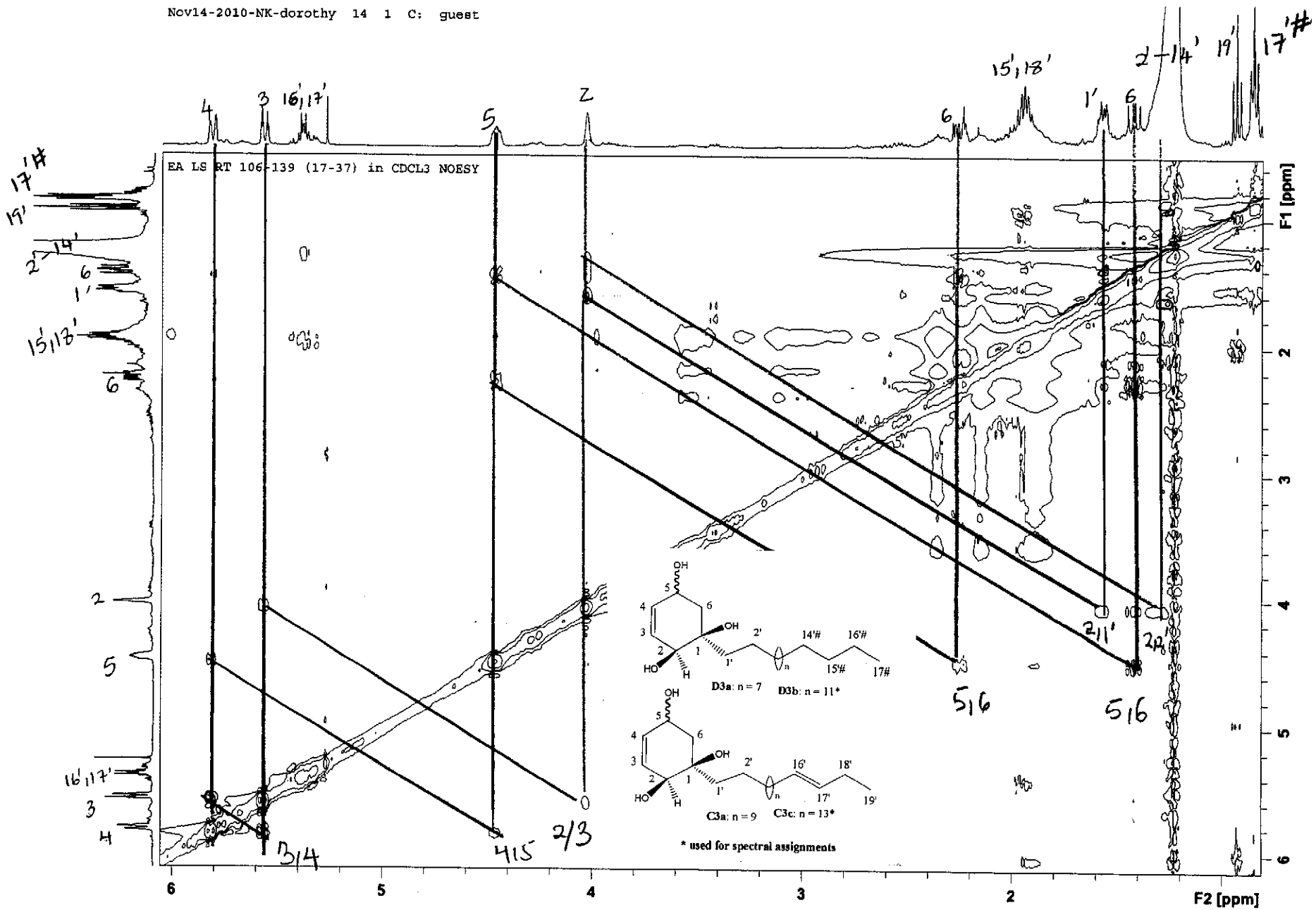
^{13}C NMR spectrum of a mixture of D3a, D3b, C3a, and C3c



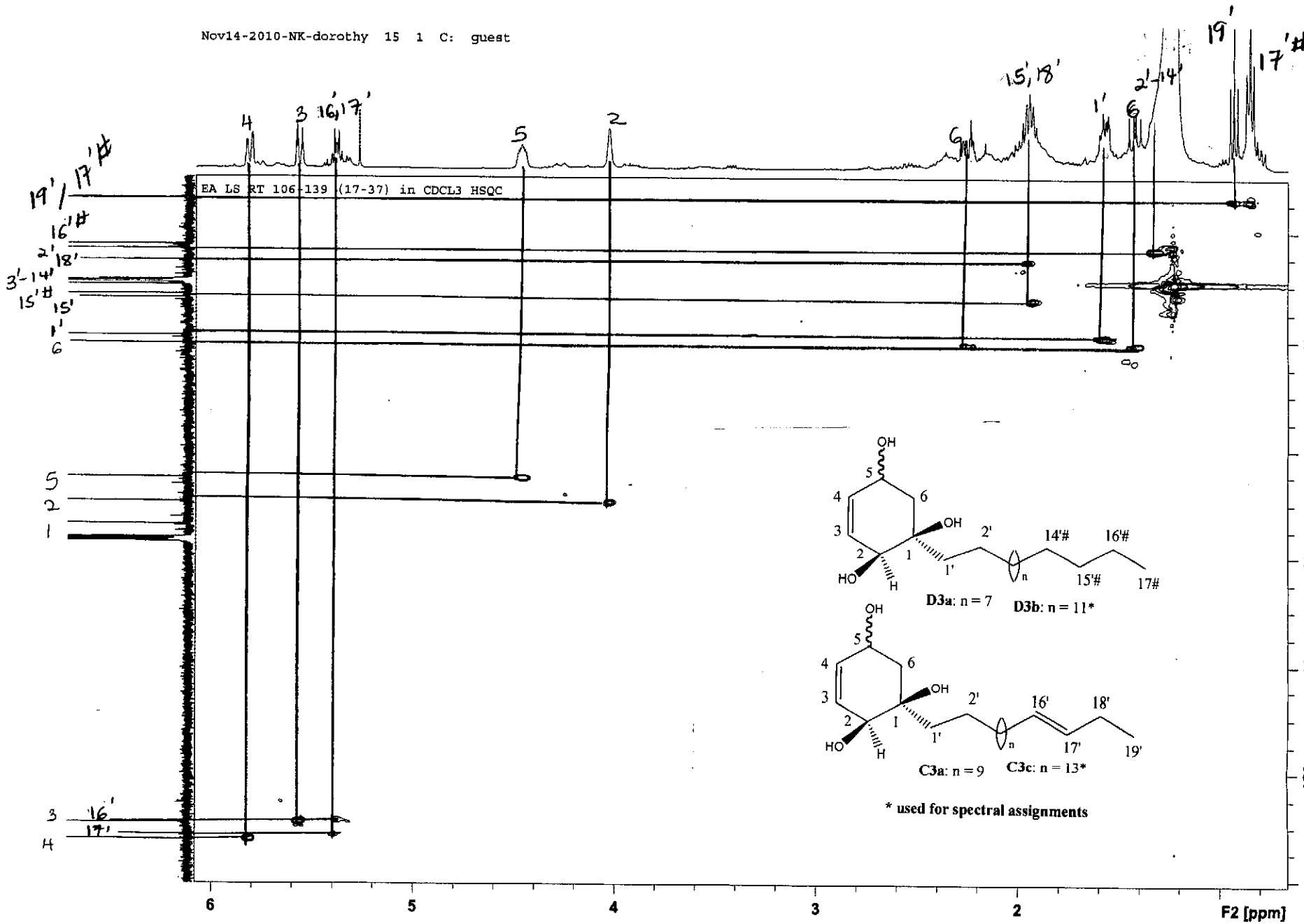
DEPT spectrum of a mixture of D3a, D3b, C3a, and C3c



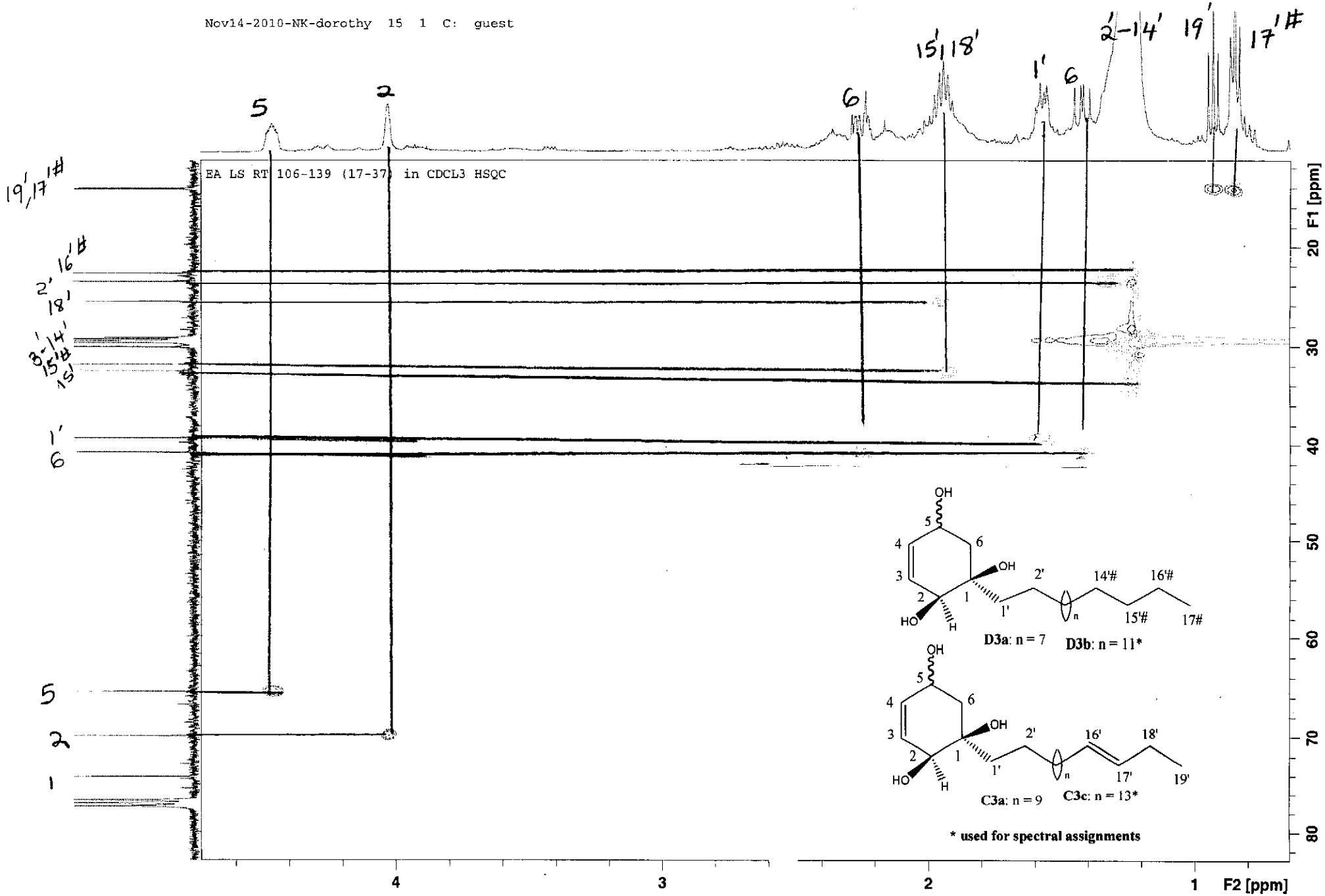
COSY spectrum of a mixture of D3a, D3b, C3a, and C3c



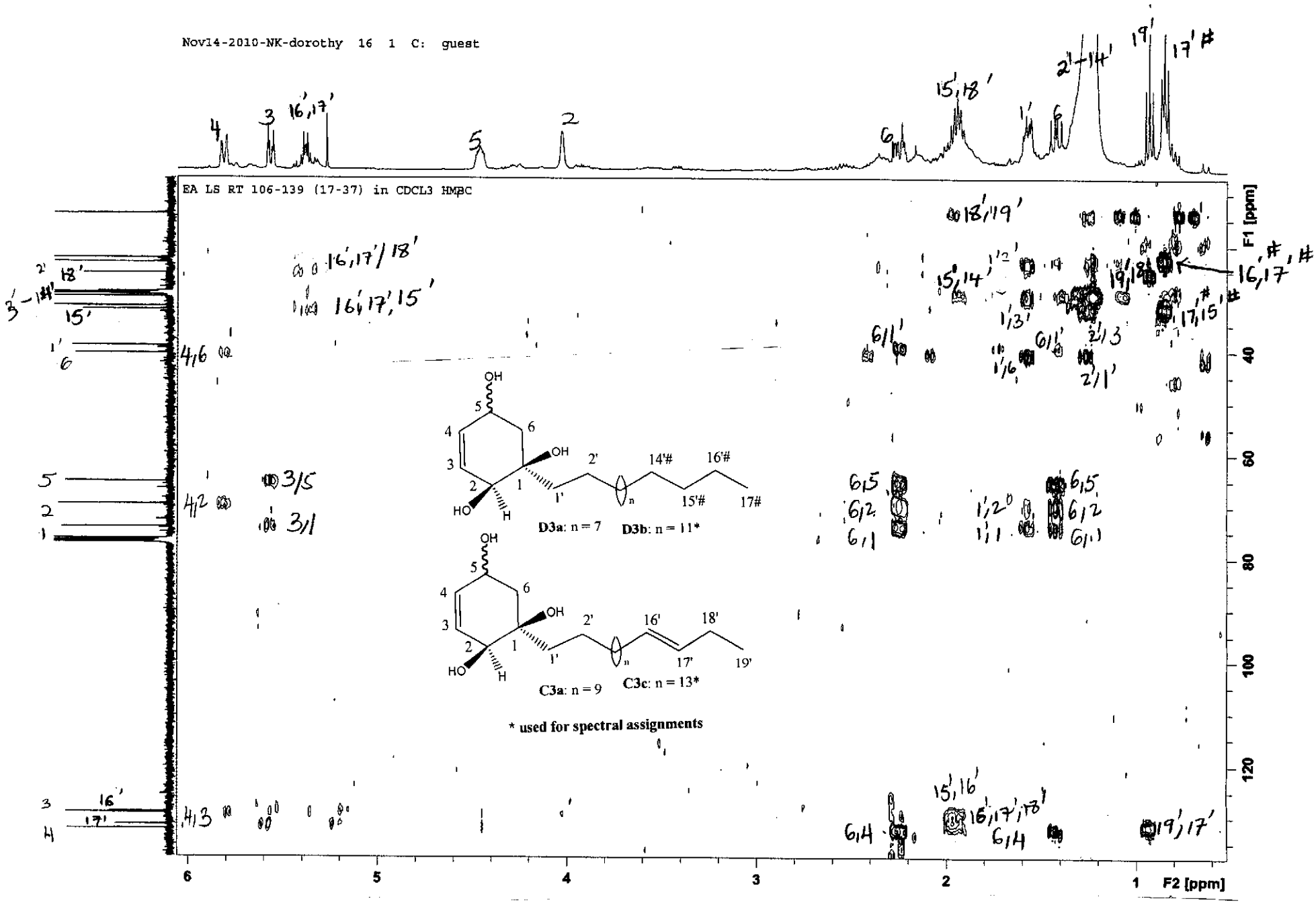
NOESY spectrum of a mixture of D3a, D3b, C3a, and C3c



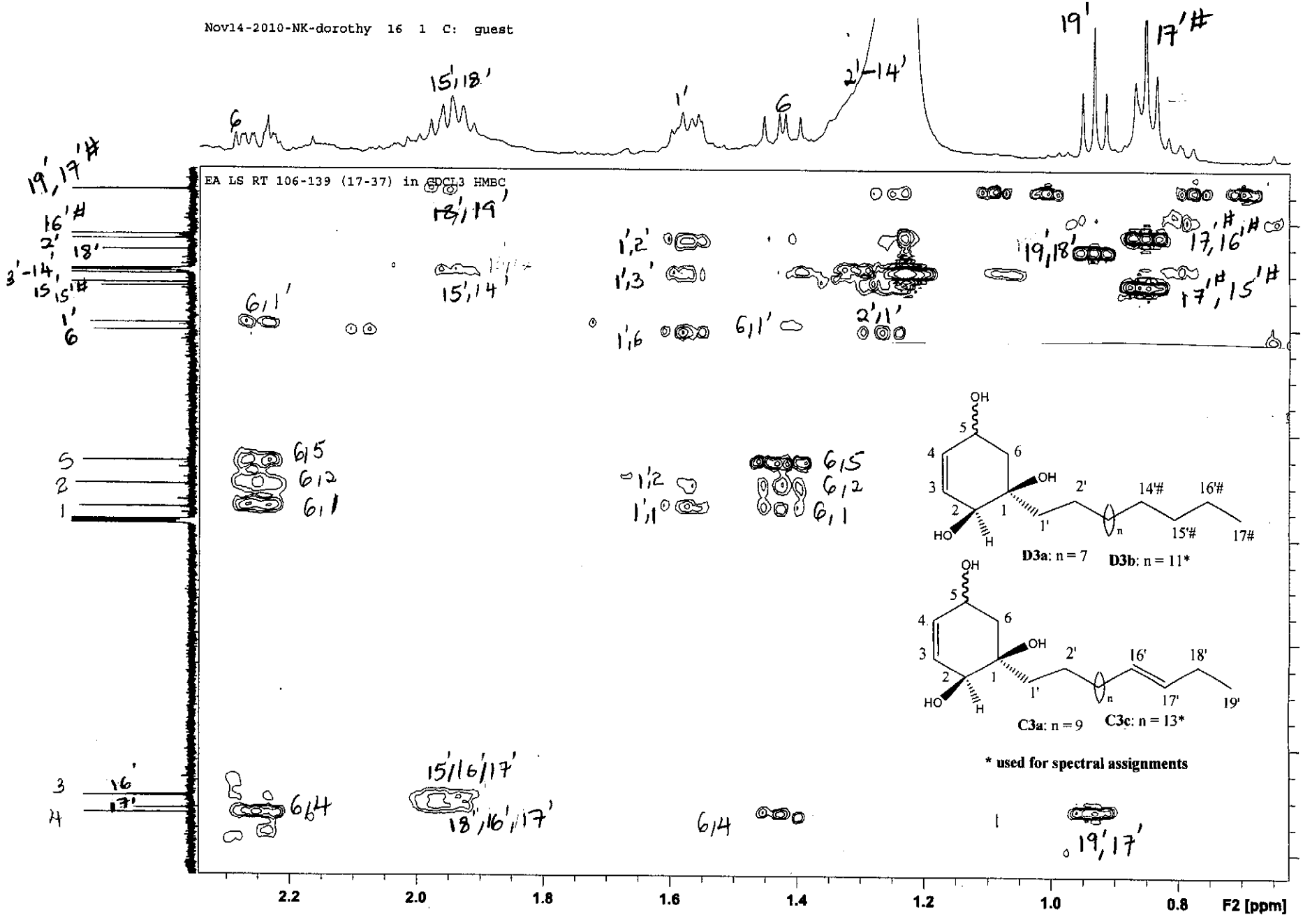
HSQC spectrum of a mixture of D3a, D3b, C3a, and C3c



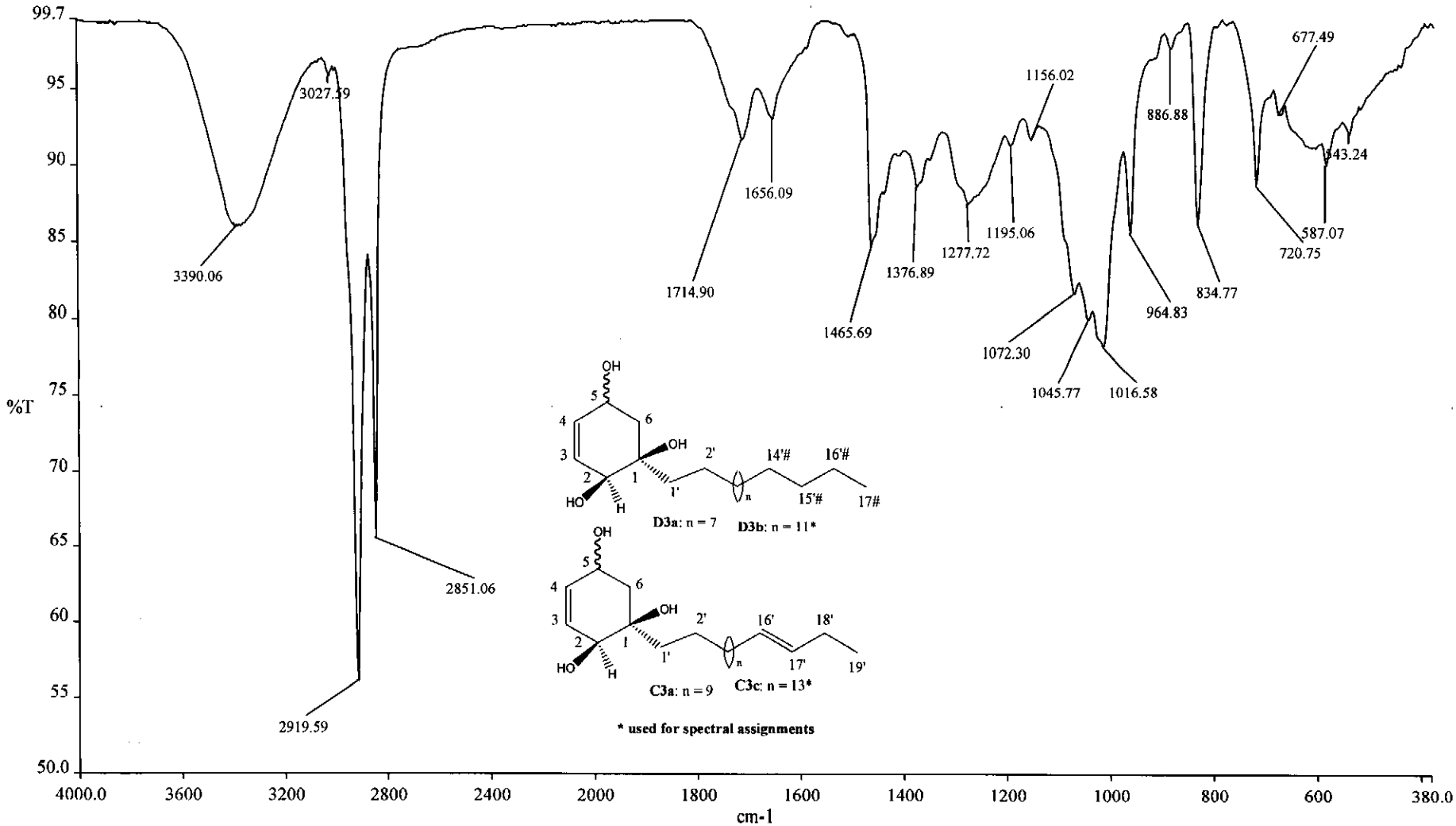
HSQC spectrum of a mixture of D3a, D3b, C3a, and C3c (expanded)



HMBC spectrum of a mixture of D3a, D3b, C3a, and C3c



HMBC spectrum of a mixture of D3a, D3b, C3a, and C3c (expanded)

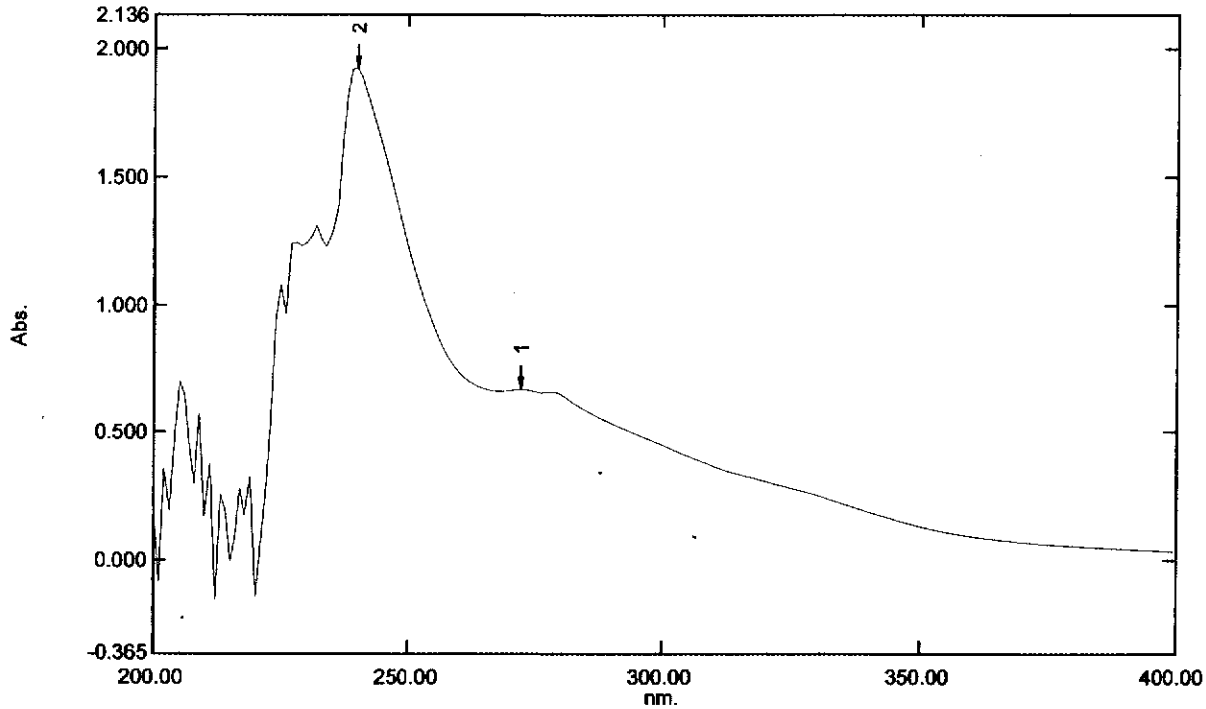


IR spectrum of a mixture of D3a, D3b, C3a, and C3c

Spectrum Peak Pick Report

23/05/2012 06:05:16 PM

Data Set: eart 106-139 17-37 2.spc - Storage 172237



No.	P/V	Wavelength	Abs.	Description
1		272.00	0.668	
2		240.00	1.928	
3		268.00	0.660	

Measurement Properties

Wavelength Range (nm.):	200.00 to 400.00
Scan Speed:	Medium
Sampling Interval:	1.0
Auto Sampling Interval:	Disabled
Scan Mode:	Single

Instrument Properties

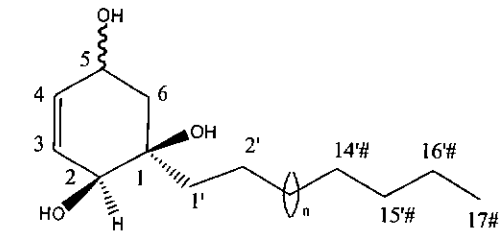
Instrument Type:	UV-3600 Series
Measuring Mode:	Absorbance
Slit Width:	2.0 nm
Time Constant:	0.1 sec.
Source Lamp:	Auto
Light Source Change Wavelength:	310.00 nm
Detector Unit:	Direct
Detector Change Wavelength:	830.00 nm 1800.00 nm
Grating Change Wavelength:	720.00 nm
S/R Exchange:	Normal
Detector Lock:	Auto
Slit Program:	Normal
Beam Mode:	Double
Stair Correction:	Disable

Attachment Properties

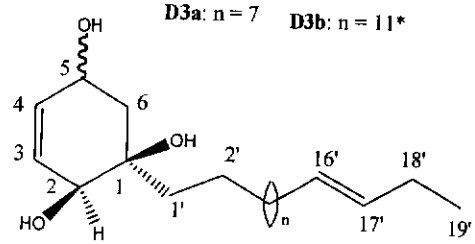
Attachment:	None

Sample Preparation Properties

Weight:	
Volume:	
Dilution:	
Path Length:	
Additional Information:	



D3a: n = 7 **D3b**: n = 11*

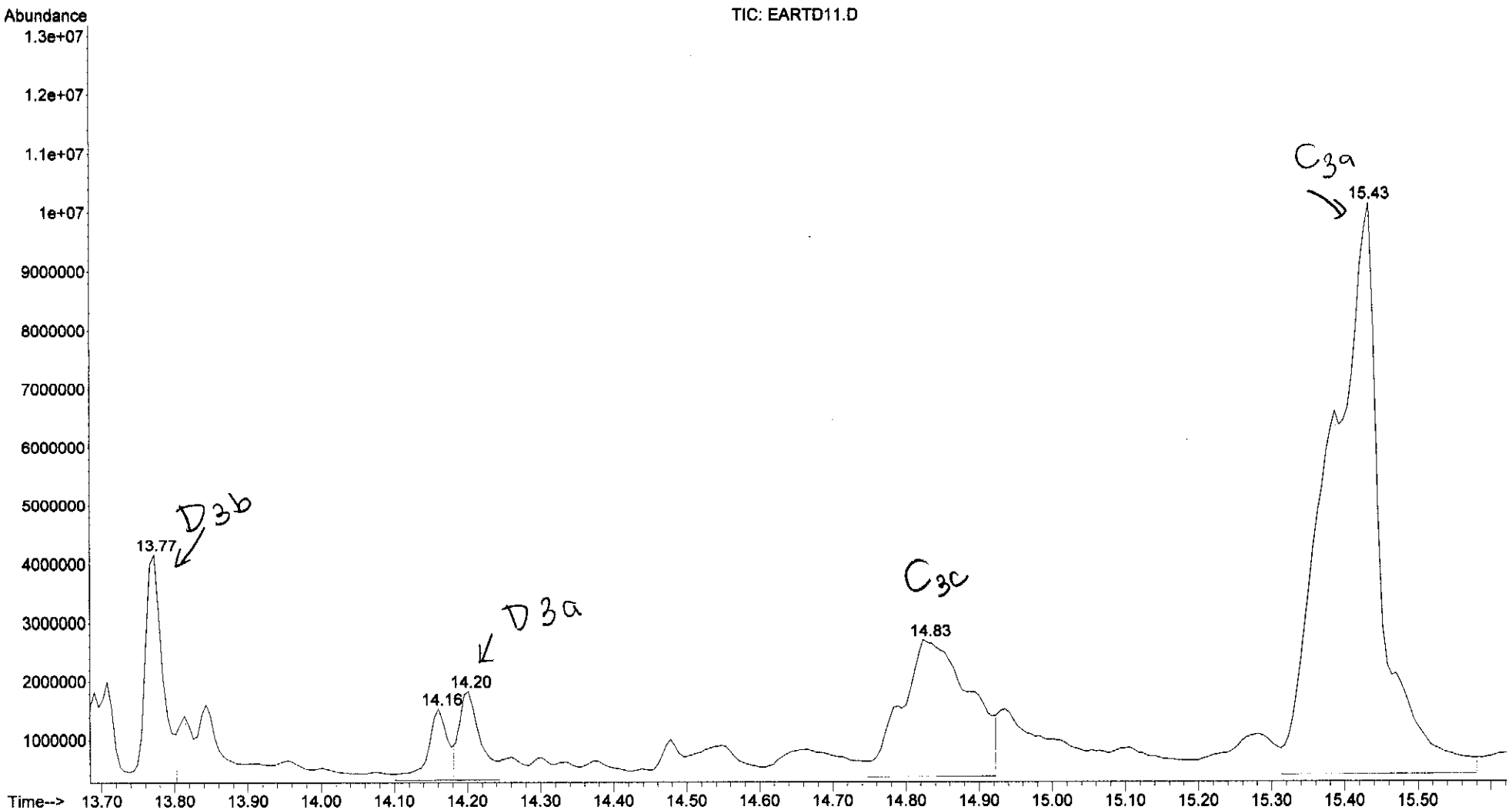


C3a: n = 9 **C3c**: n = 13*

*** used for spectral assignments**

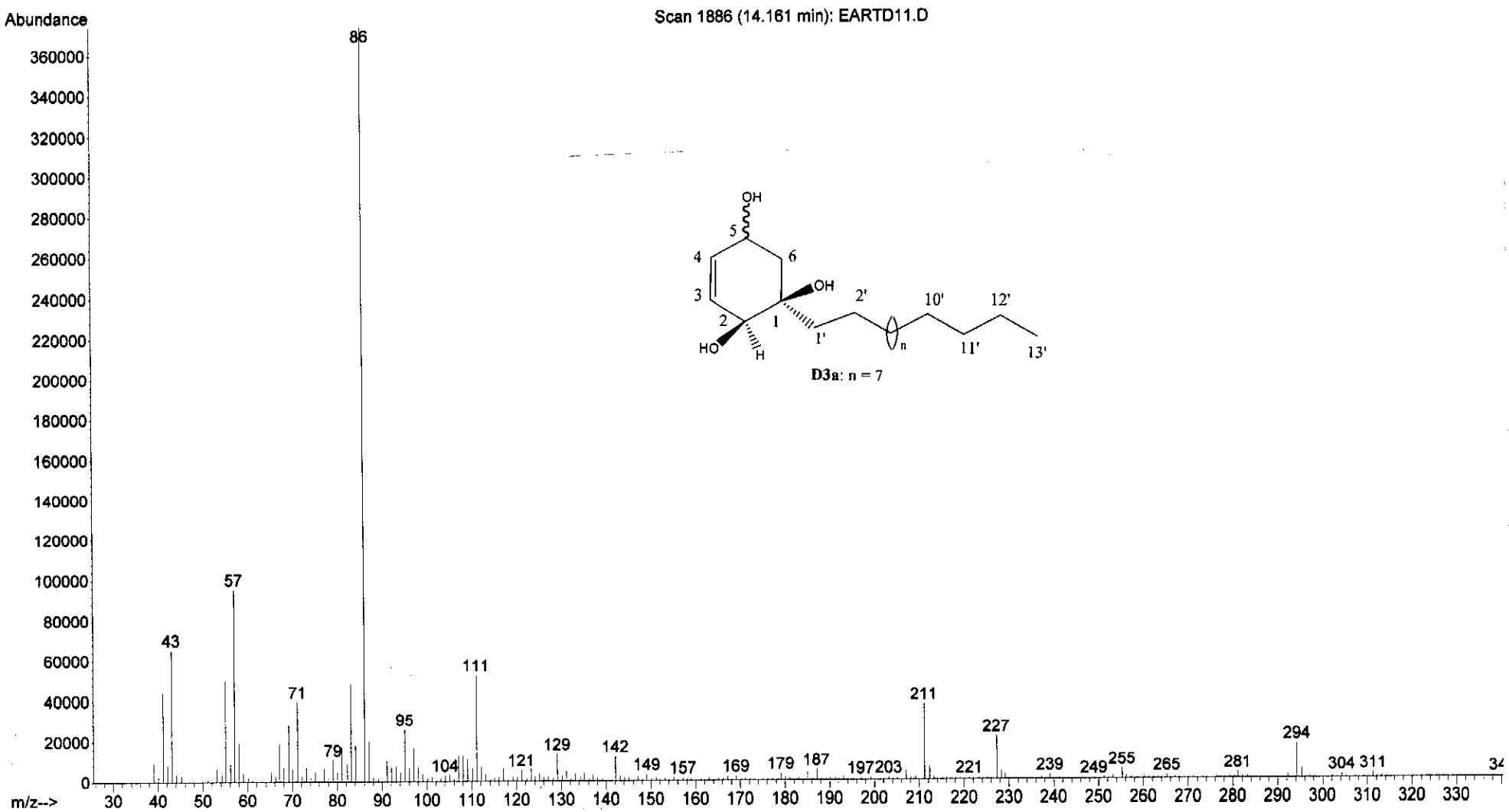
UV spectrum of a mixture of D3a, D3b, C3a, and C3c

File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\EARTD11.D
Operator : Dorothy
Acquired : 28 Apr 2011 15:21 using AcqMethod NATURALJ
Instrument : Instrumen
Sample Name: EA LS RT 106-139(19-37)
Misc Info :
Vial Number: 1



GC chromatogram of a mixture of D3a, D3b, C3a, and C3c

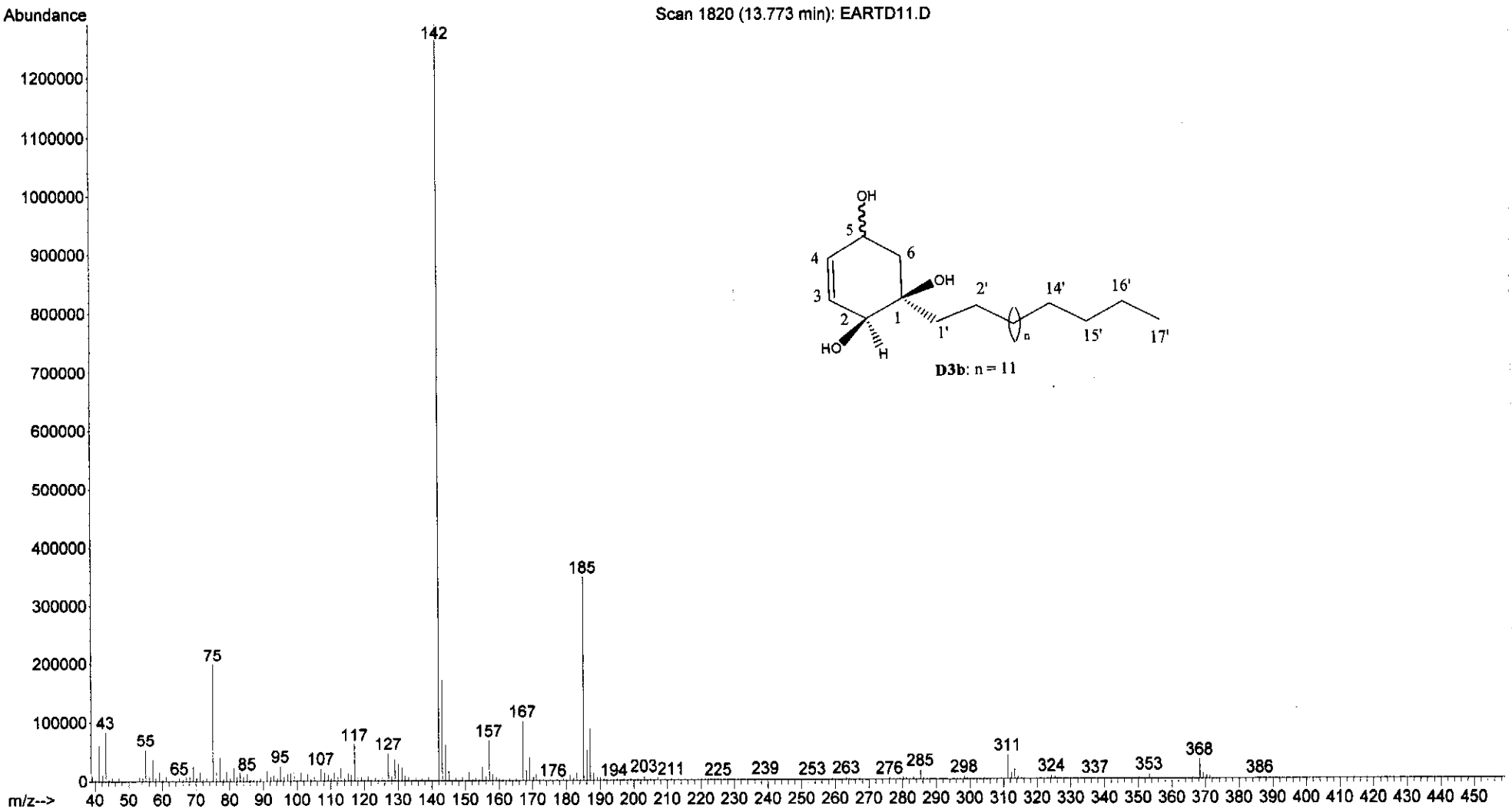
File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\EARTD11.D
Operator : Dorothy
Acquired : 28 Apr 2011 15:21 using AcqMethod NATURALJ
Instrument : Instrumen
Sample Name: EA LS RT 106-139(19-37)
Misc Info :
Vial Number: 1



D3a: n = 7

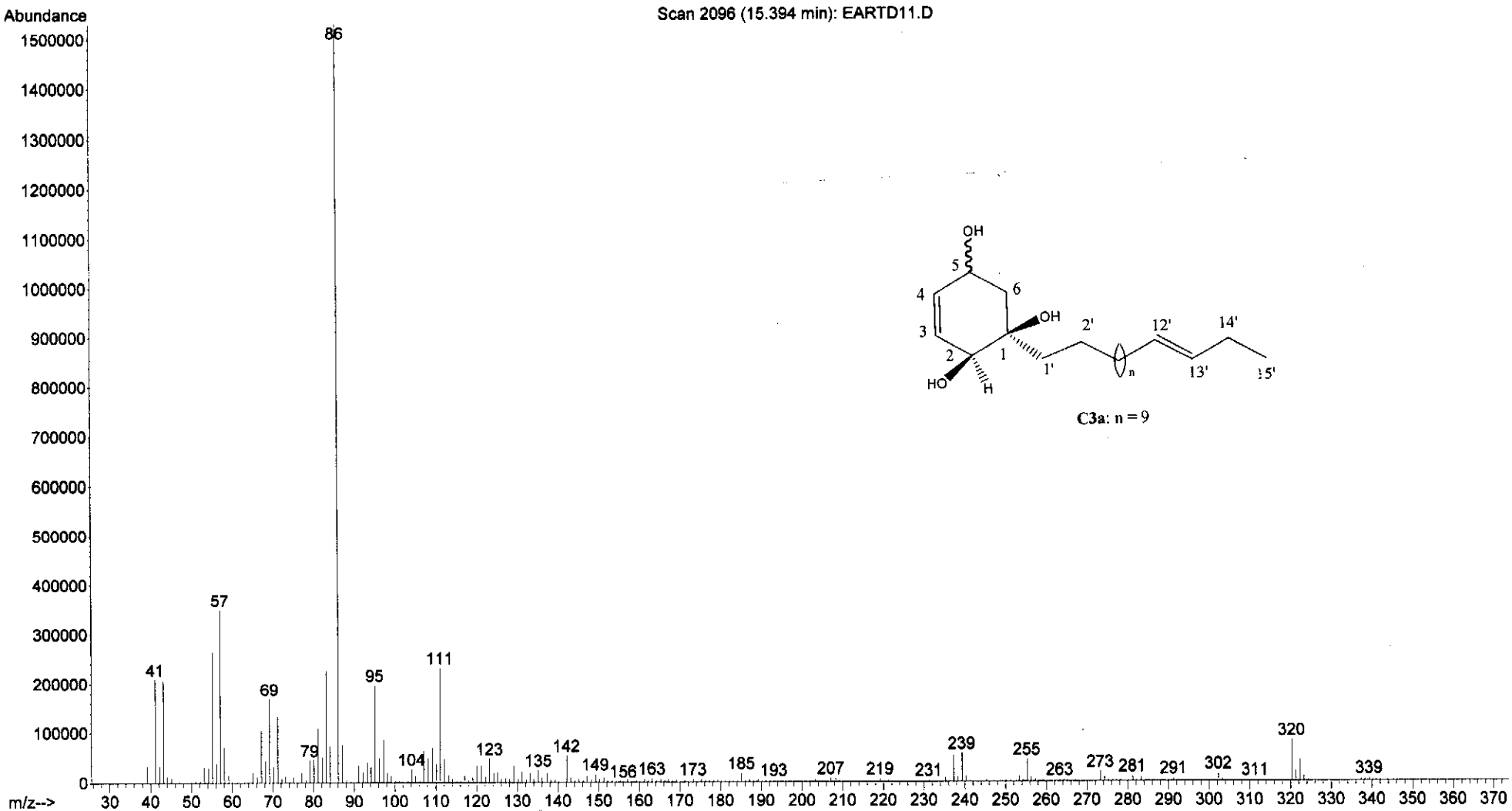
Mass spectrum of D3a

File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\EARTD11.D
Operator : Dorothy
Acquired : 28 Apr 2011 15:21 using AcqMethod NATURALJ
Instrument : Instrumen
Sample Name: EA LS RT 106-139(19-37)
Misc Info :
Vial Number: 1



Mass spectrum of D3b

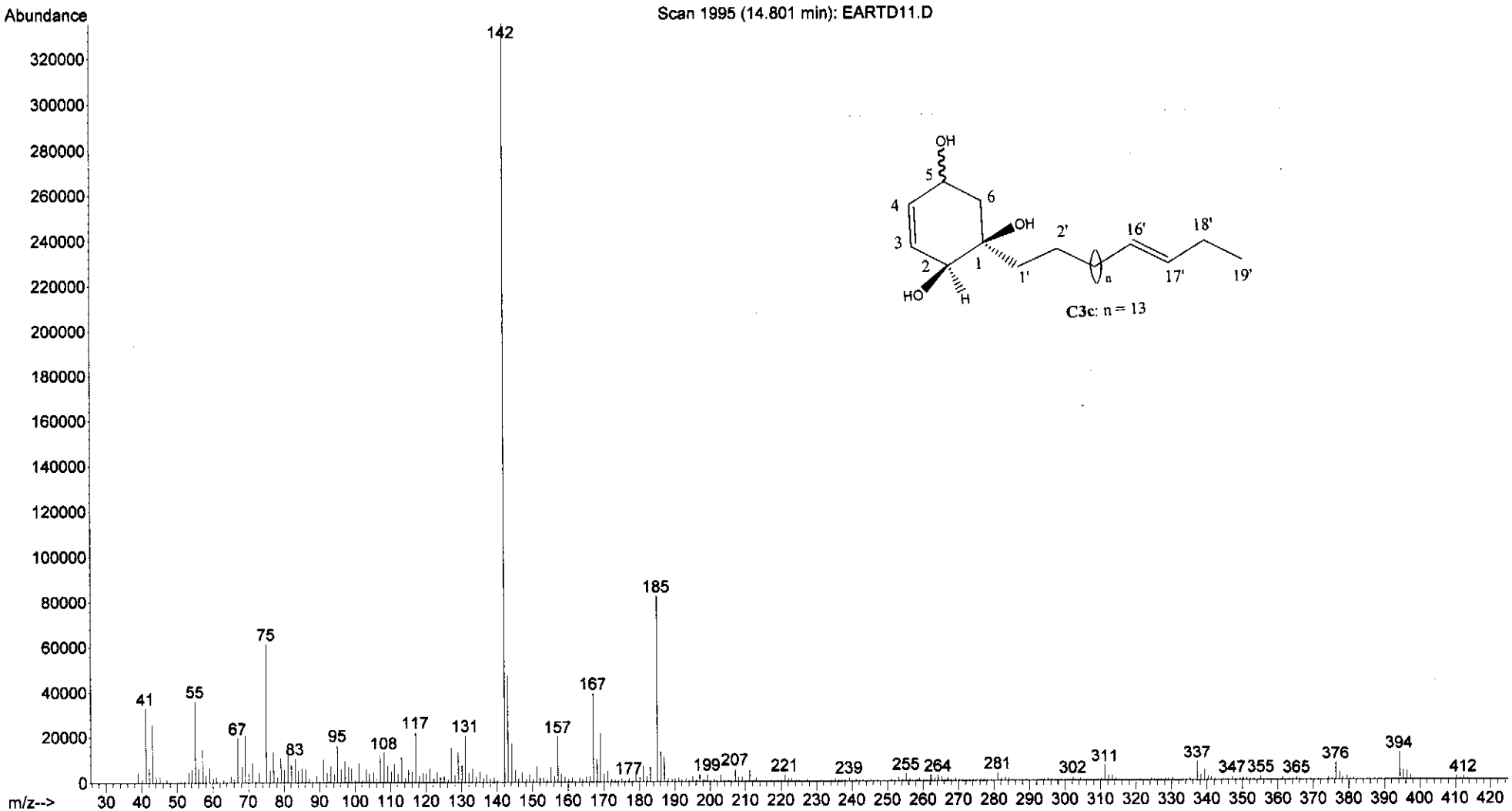
File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\EARTD11.D
Operator : Dorothy
Acquired : 28 Apr 2011 15:21 using AcqMethod NATURALJ
Instrument : Instrumen
Sample Name: EA LS RT 106-139(19-37)
Misc Info :
Vial Number: 1



C3a: n = 9

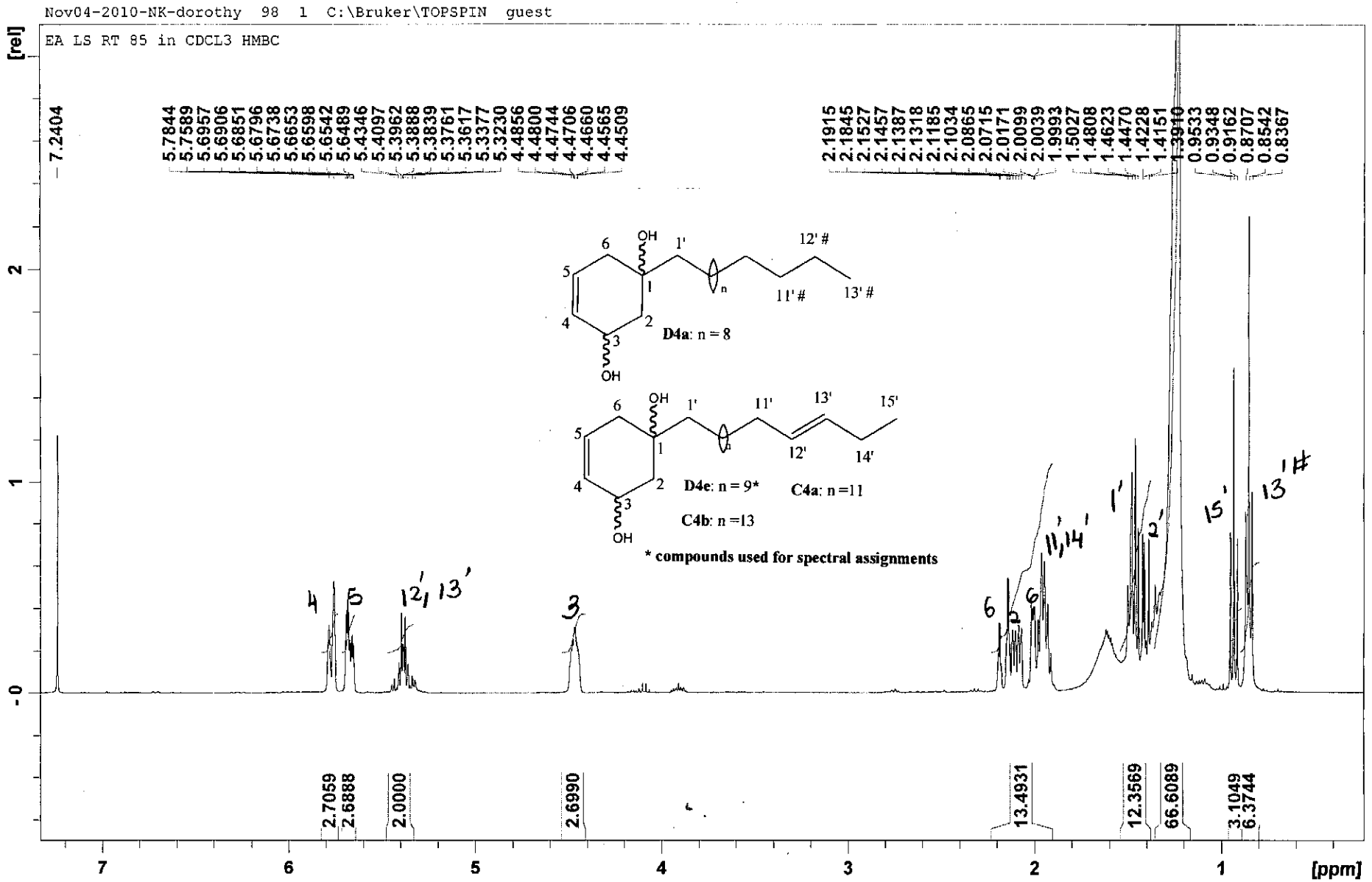
Mass spectrum of C3a

File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\EARTD11.D
Operator : Dorothy
Acquired : 28 Apr 2011 15:21 using AcqMethod NATURALJ
Instrument : Instrumen
Sample Name: EA LS RT 106-139(19-37)
Misc Info :
Vial Number: 1

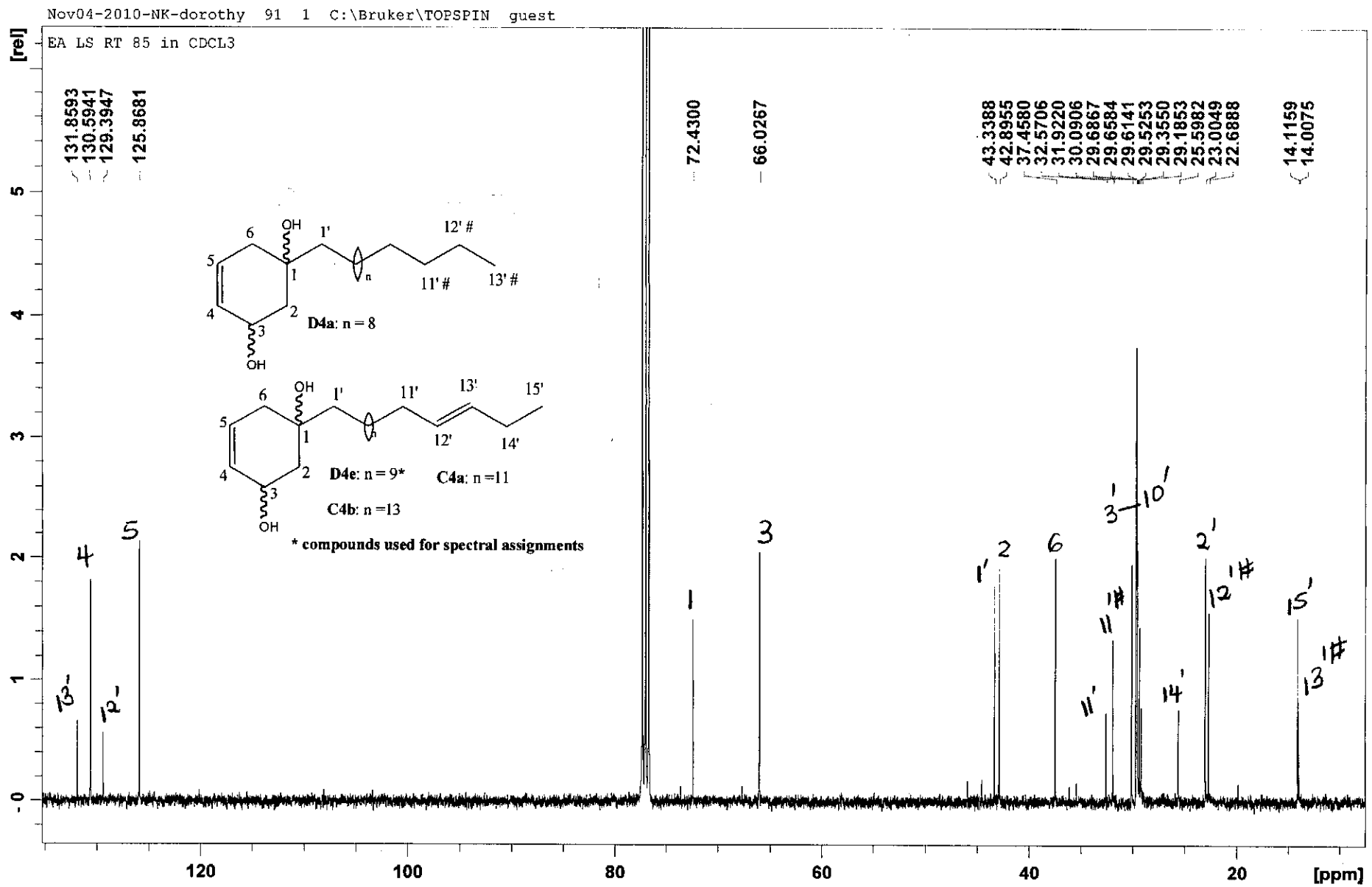


C3c: n = 13

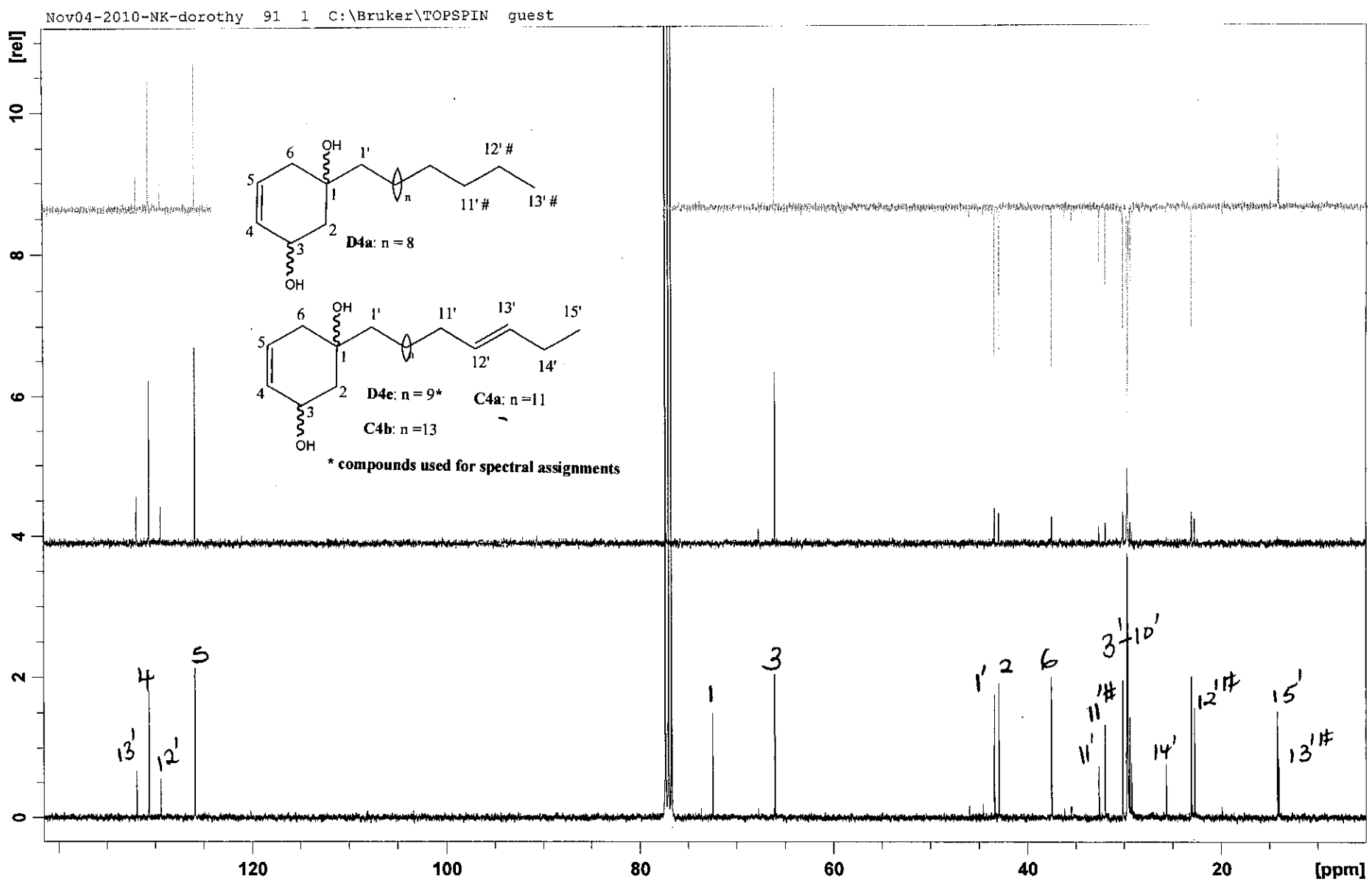
Mass spectrum of C3c



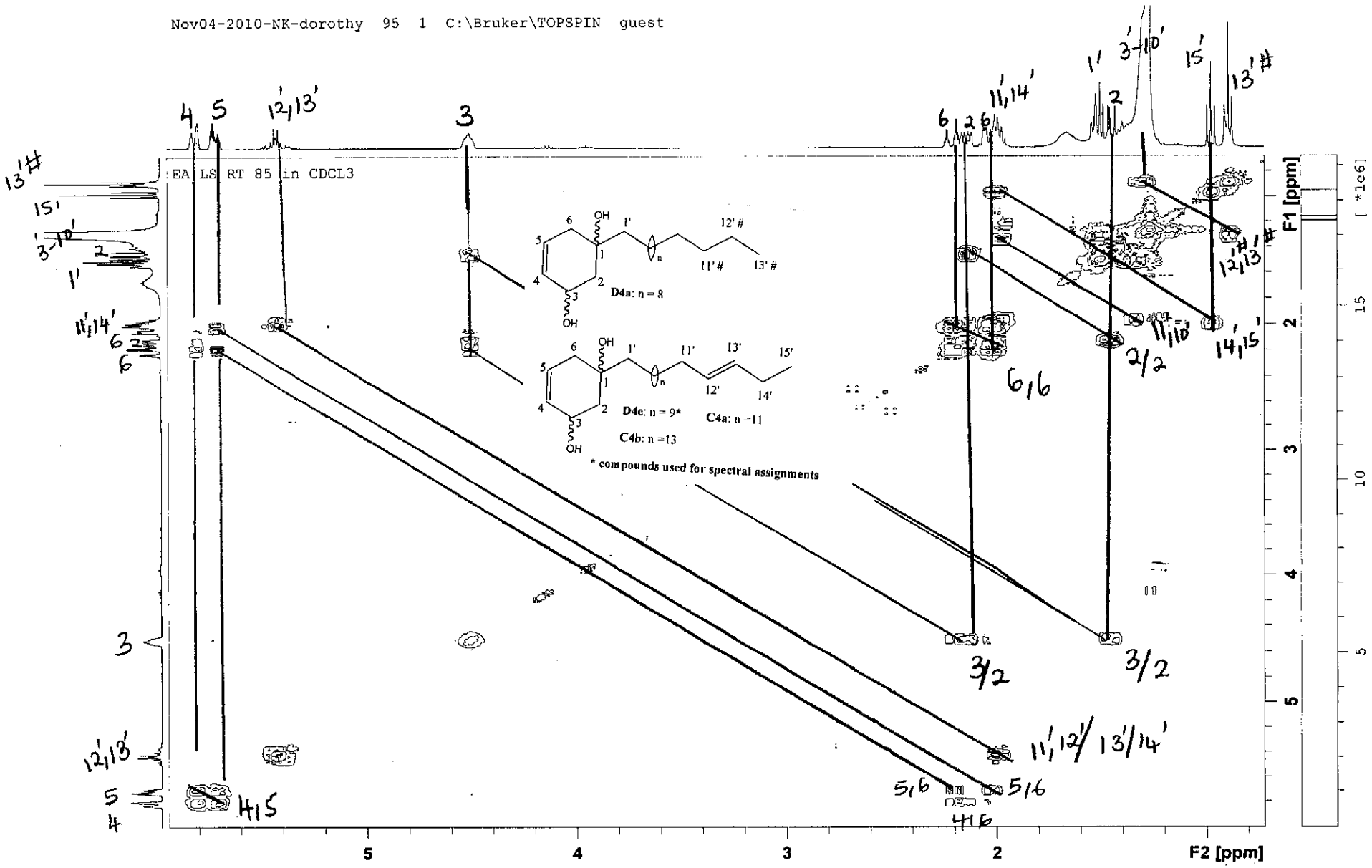
^{1}H NMR spectrum of a mixture of D4a, D4e, C4a, and C4b



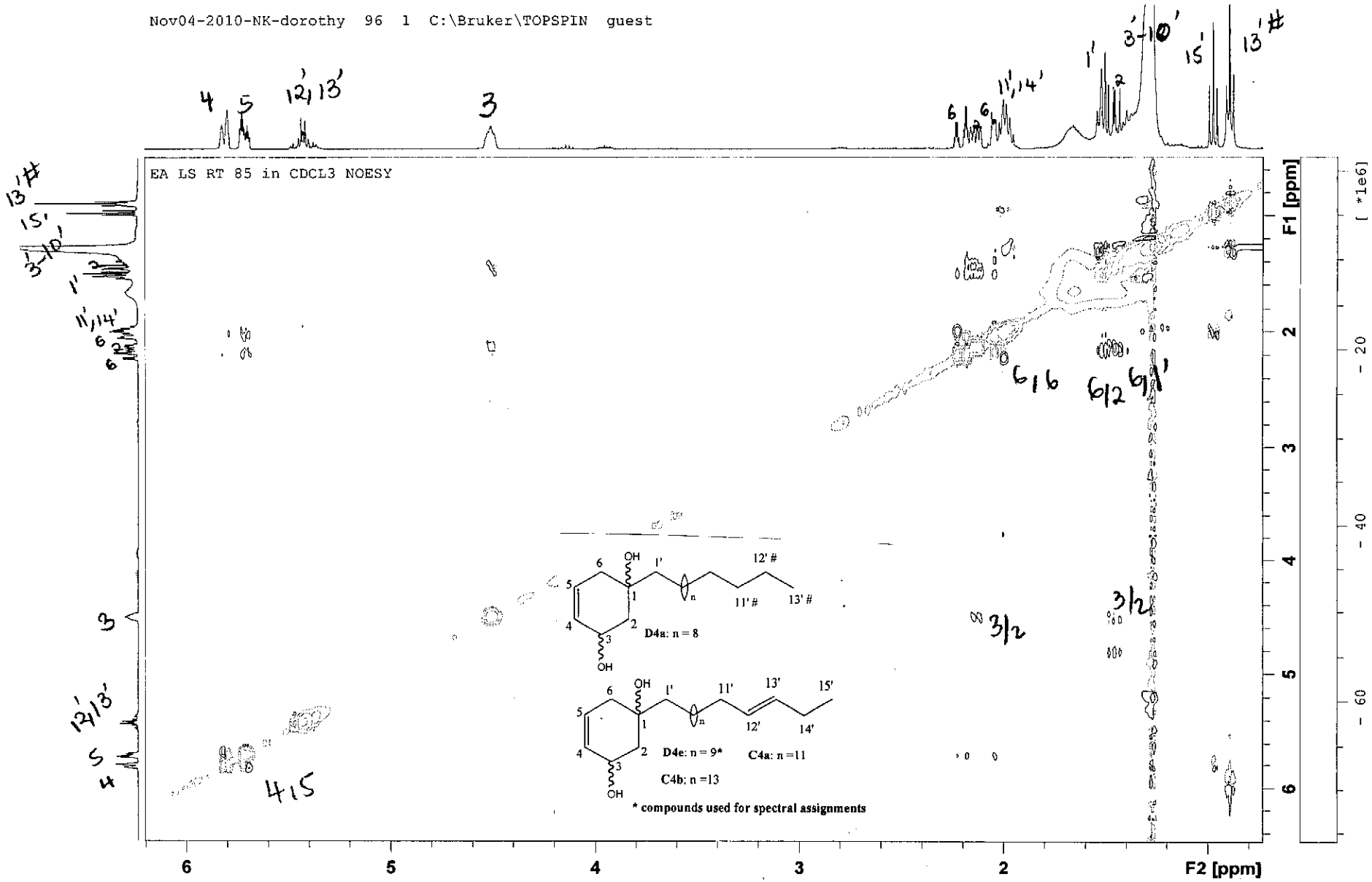
^{13}C NMR spectrum of a mixture of D4a, D4e, C4a, and C4b



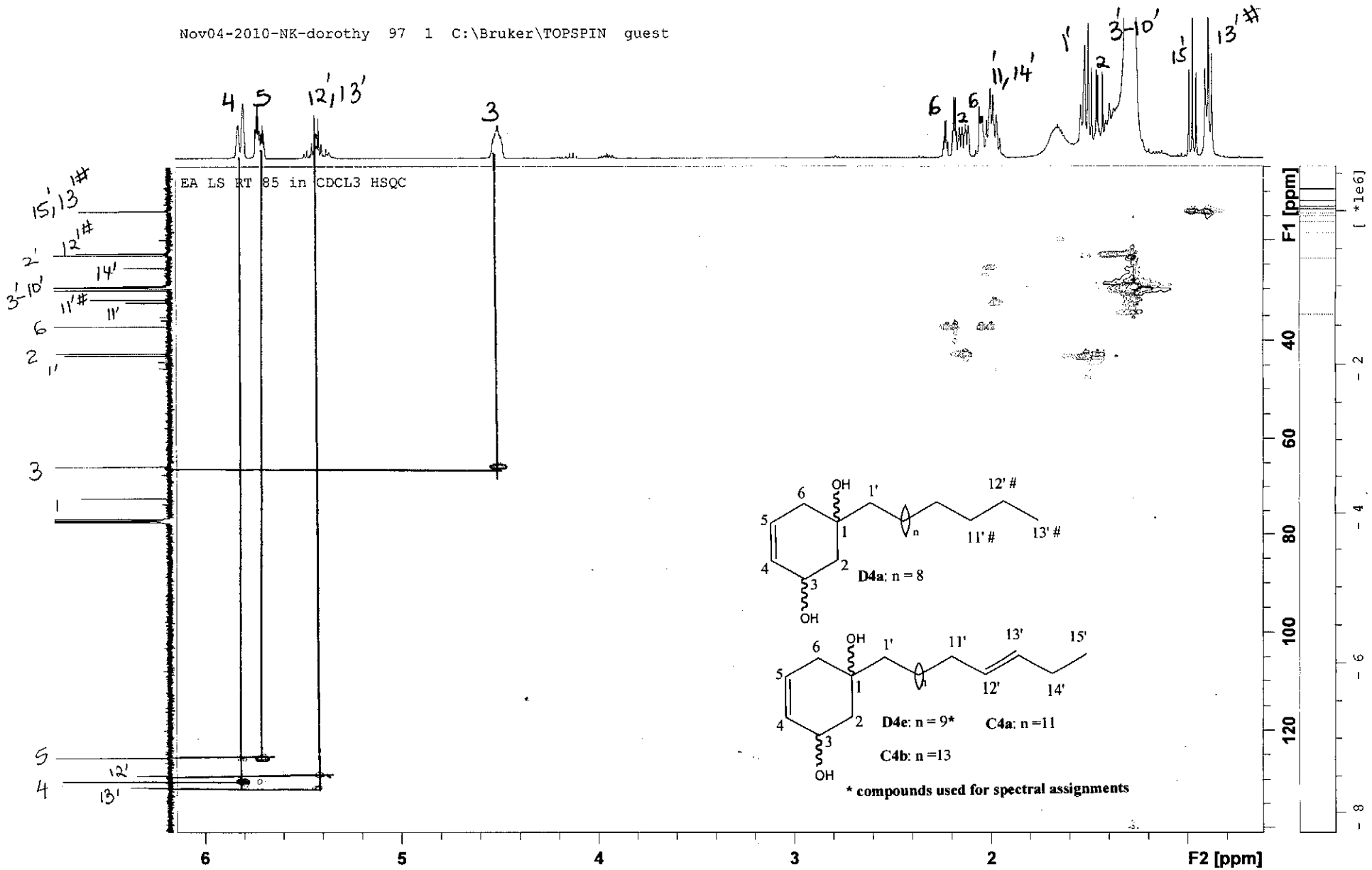
DEPT spectrum of a mixture of D4a, D4e, C4a, and C4b



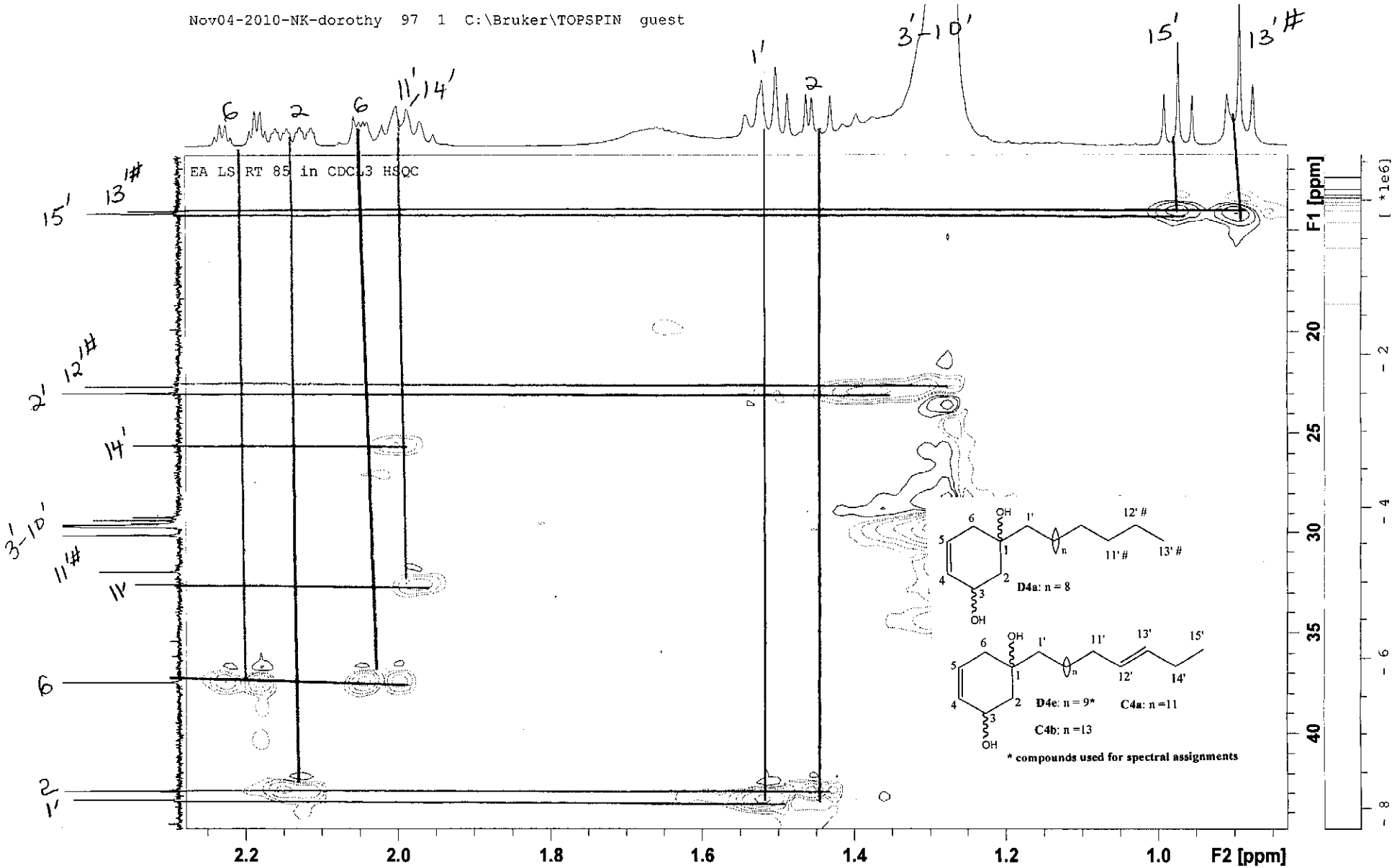
COSY spectrum of a mixture of D4a, D4e, C4a, and C4b



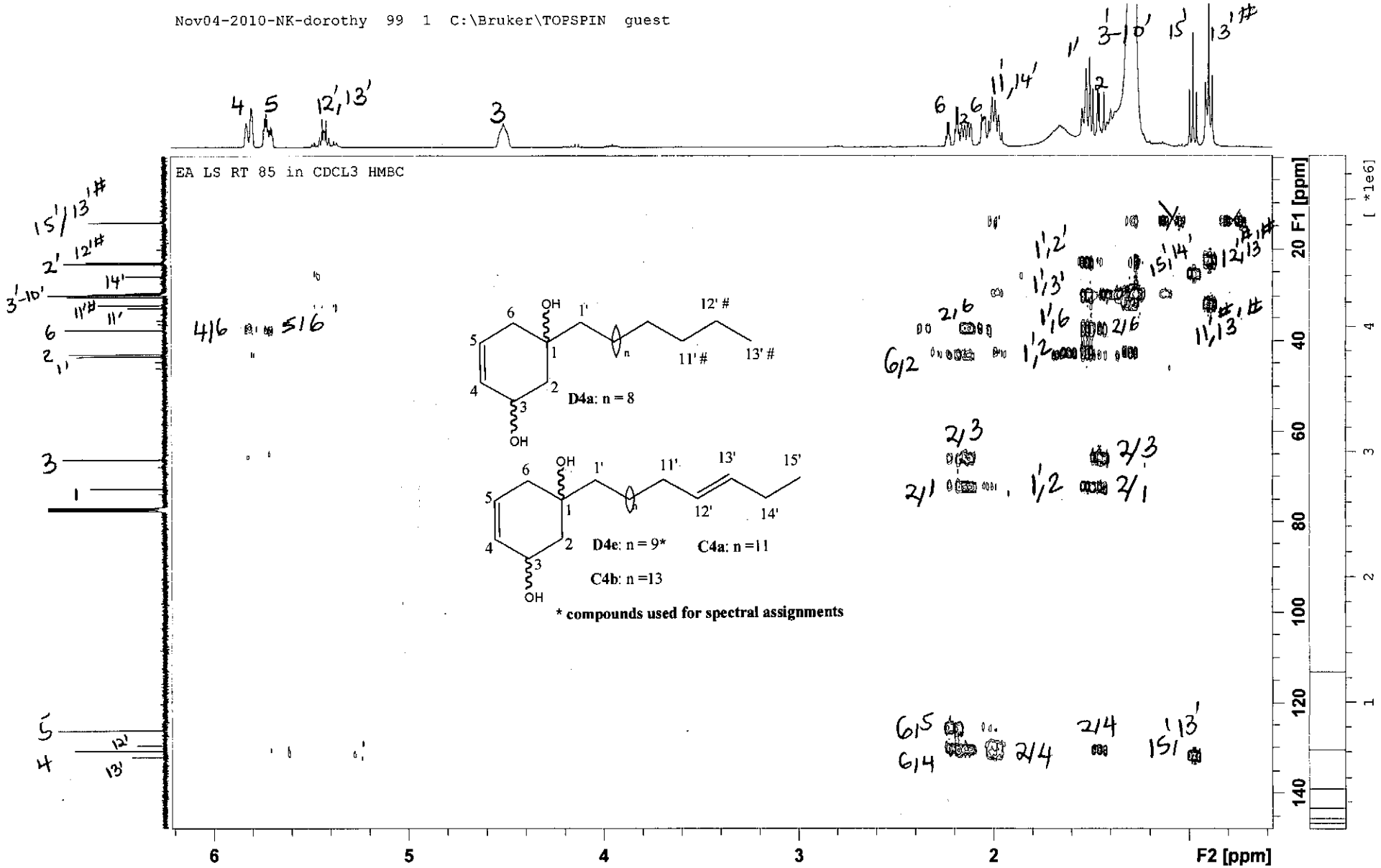
NOESY spectrum of a mixture of D4a, D4e, C4a, and C4b



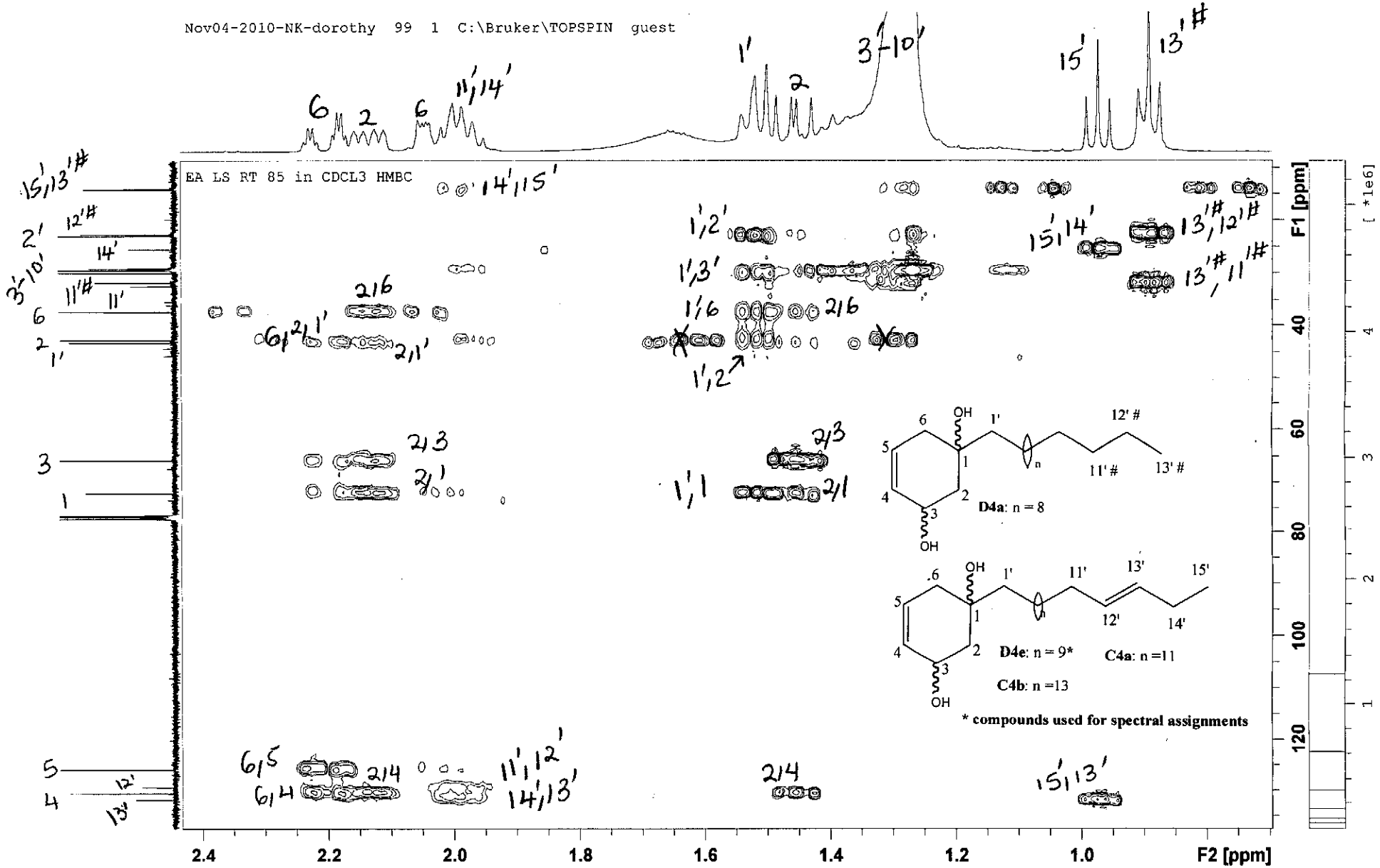
HSQC spectrum of a mixture of D4a, D4e, C4a, and C4b



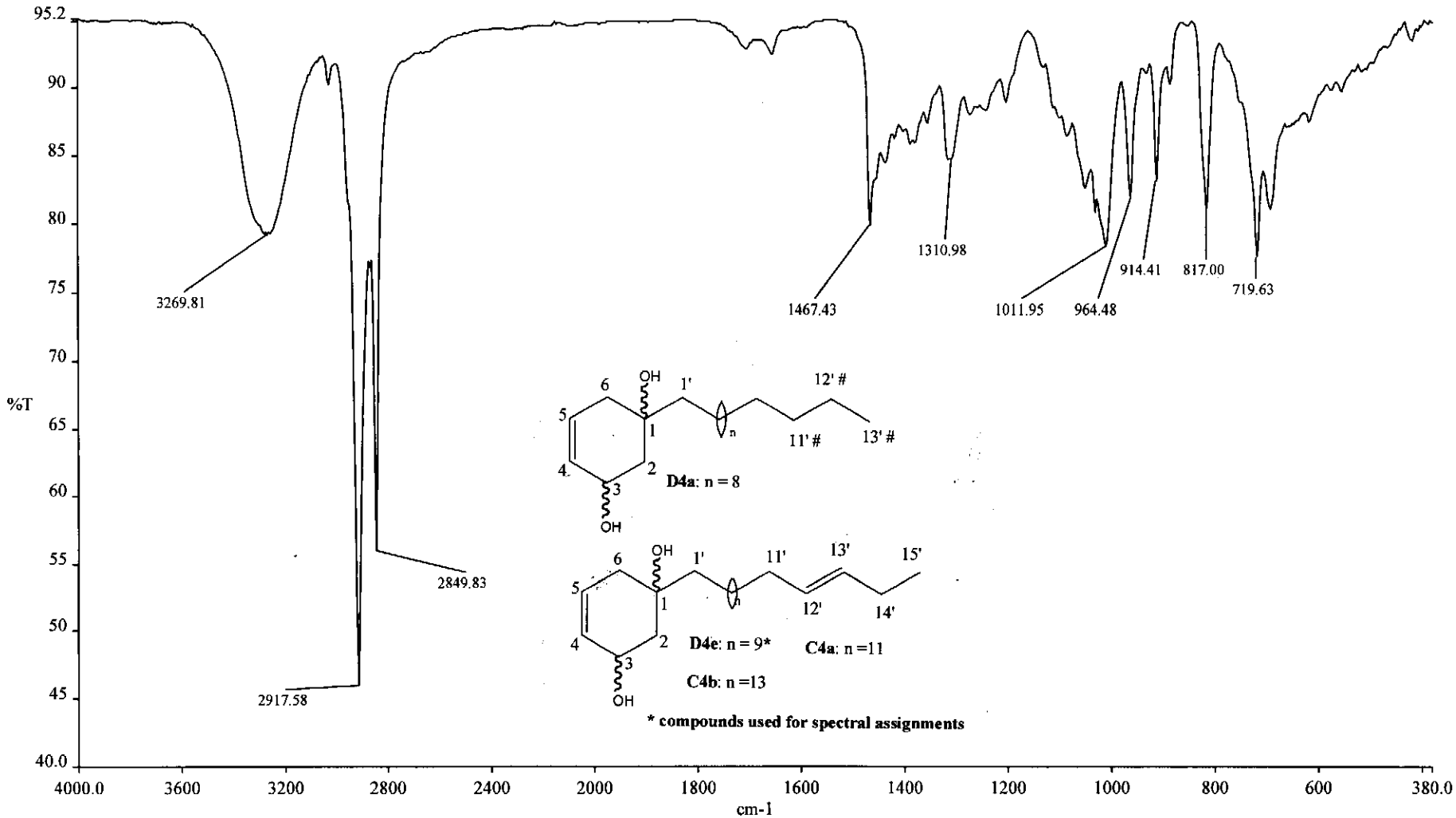
HSQC spectrum of a mixture of D4a, D4e, C4a, and C4b (expanded)



HMBC spectrum of a mixture of D4a, D4e, C4a, and C4b



HMBC spectrum of a mixture of D4a, D4e, C4a, and C4b (expanded)

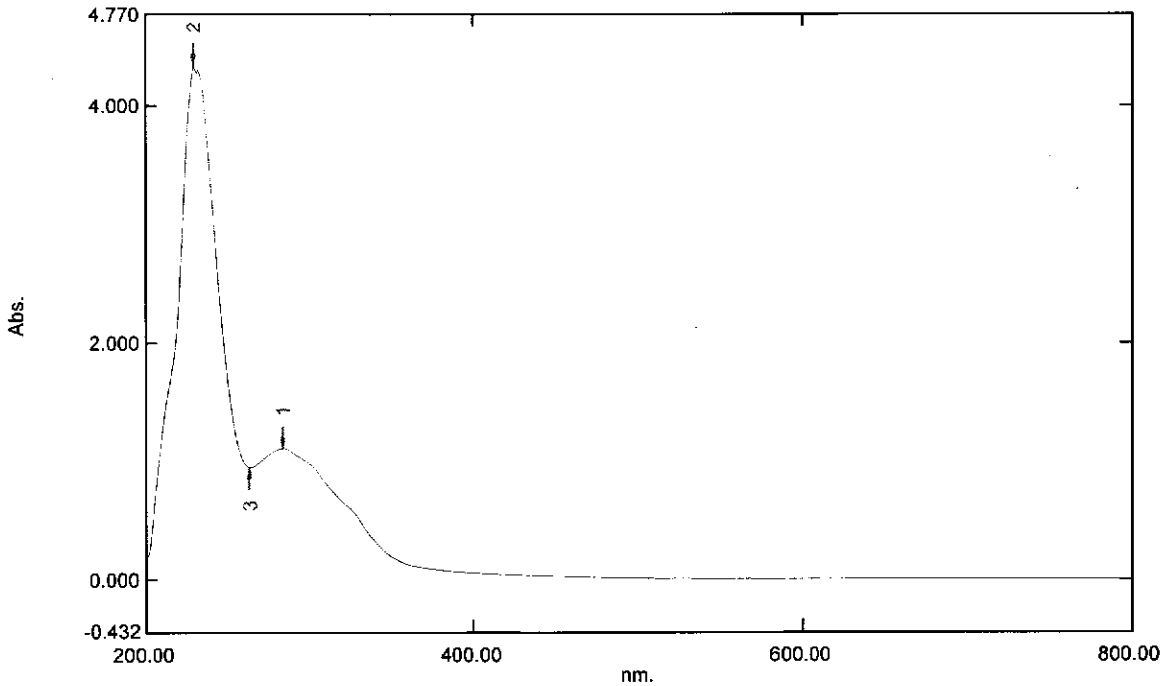


IR spectrum of a mixture of D4a, D4e, C4a, and C4b

Spectrum Peak Pick Report

04/10/2011 12:09:11 PM

Data Set: LS EA RT 851.spc - Storage 111641



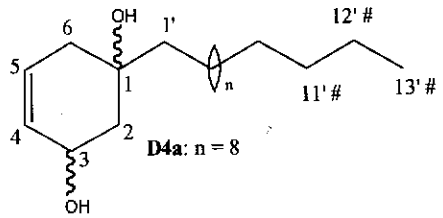
No.	P/V	Wavelength	Abs.	Description
1	⬆	284.00	1.111	
2	⬆	229.00	4.336	
3	⬇	264.00	0.955	

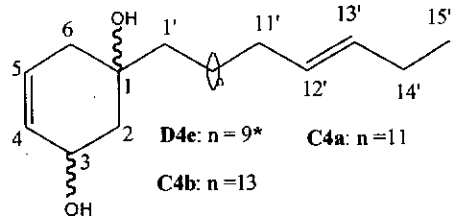
Measurement Properties
Wavelength Range (nm.): 200.00 to 800.00
Scan Speed: Medium
Sampling Interval: 1.0
Auto Sampling Interval: Disabled
Scan Mode: Single

Instrument Properties
Instrument Type: UV-3600 Series
Measuring Mode: Absorbance
Slit Width: 8.0 nm
Time Constant: 0.1 sec.
Source Lamp: Auto
Light Source Change Wavelength: 310.00 nm
Detector Unit: Direct
Detector Change Wavelength: 830.00 nm 1800.00 nm
Grating Change Wavelength: 720.00 nm
S/R Exchange: Normal
Detector Lock: Auto
Slit Program: Normal
Beam Mode: Double
Stair Correction: Disable

Attachment Properties
Attachment: None

Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

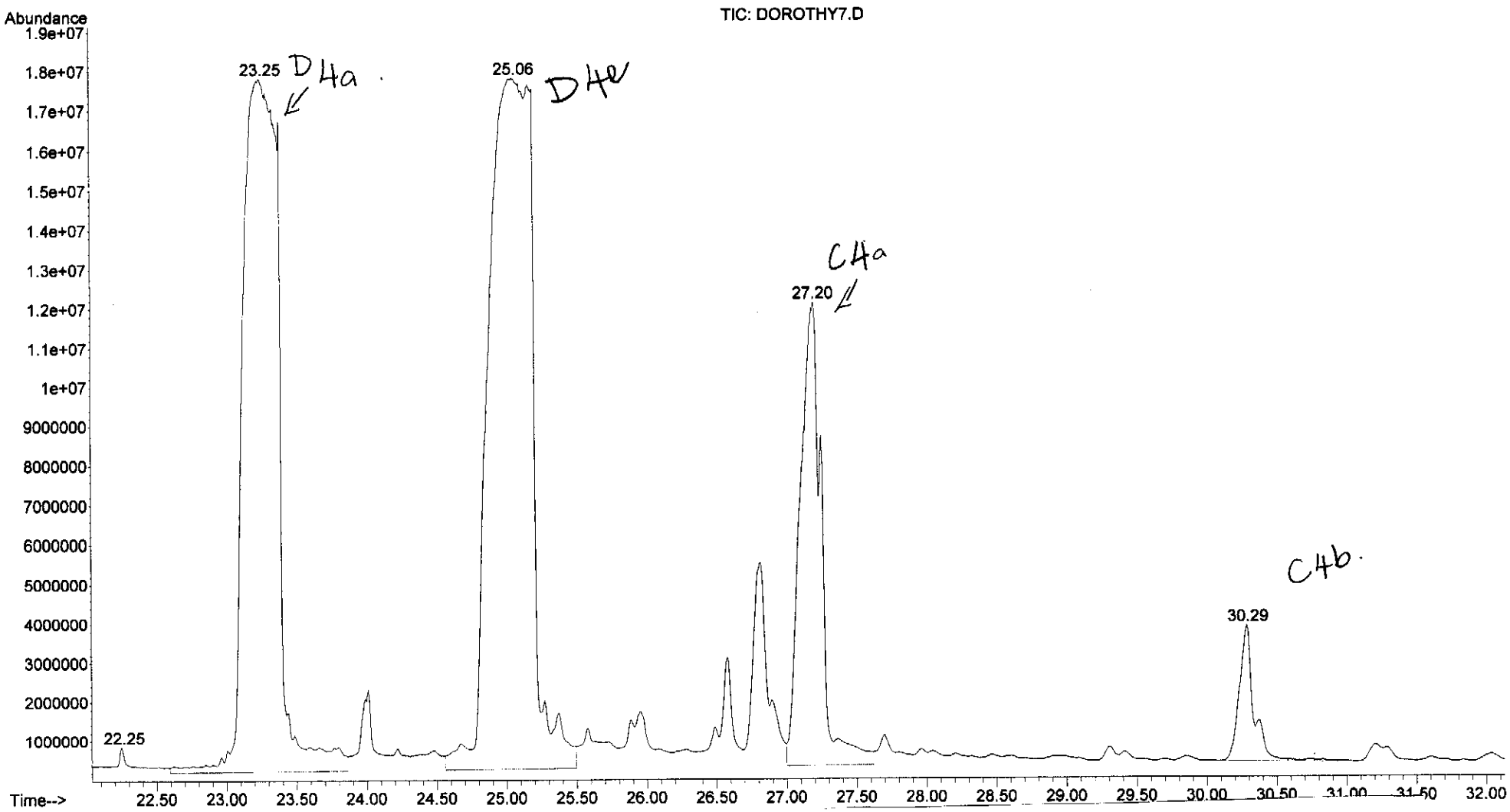




*** compounds used for spectral assignments**

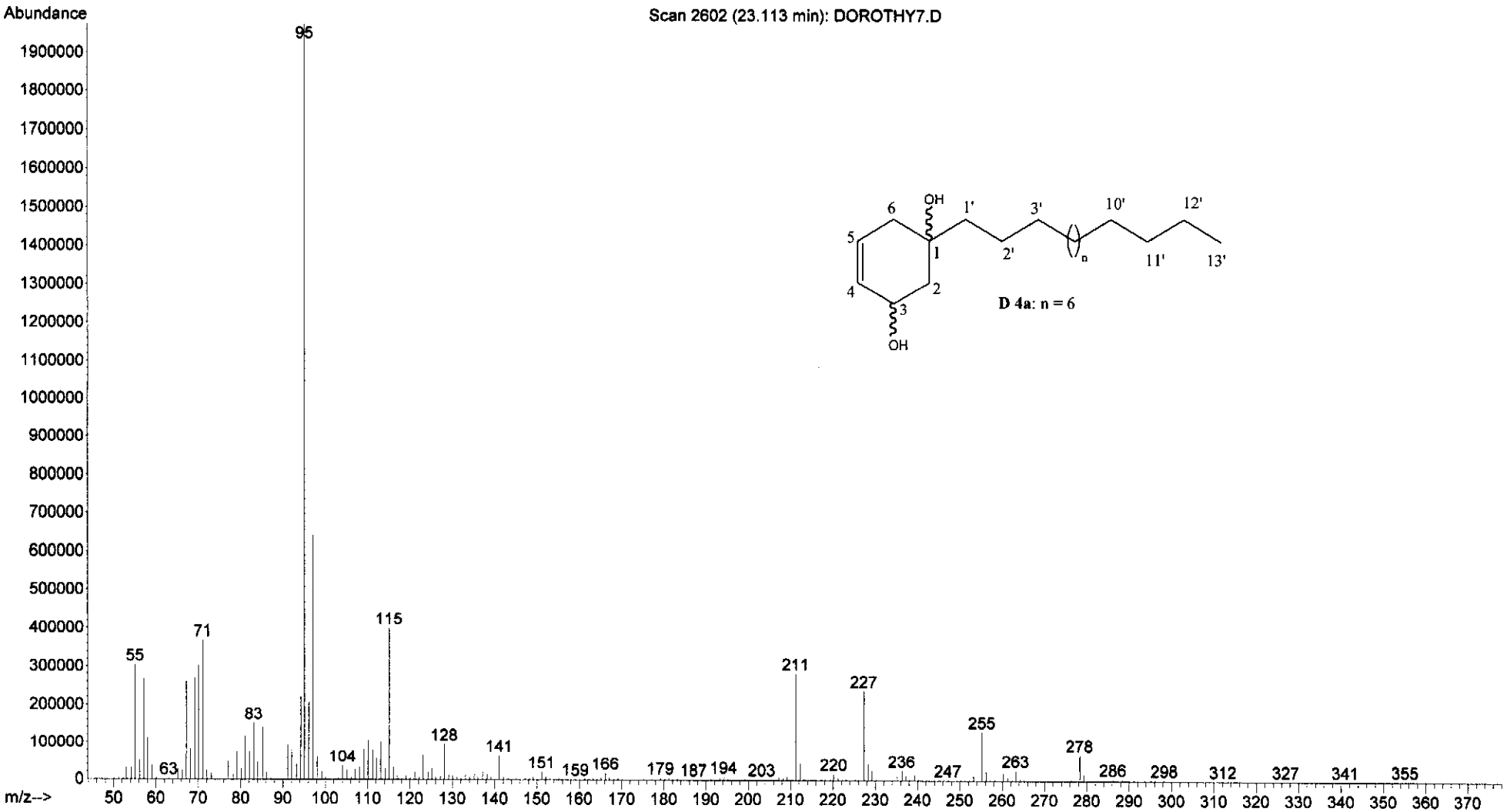
UV spectrum of a mixture of D4a, D4e, C4a, and C4b

File : C:\MSDCHEM\1\DATA\DOROTHY GCMS TWO\DOROTHY GCMS TWO\DOROTHY7.D
Operator : dorothy
Acquired : 27 Nov 2011 19:53 using AcqMethod DOROTHY
Instrument : Instrumen
Sample Name: EALS RT 85
Misc Info :
Vial Number: 1



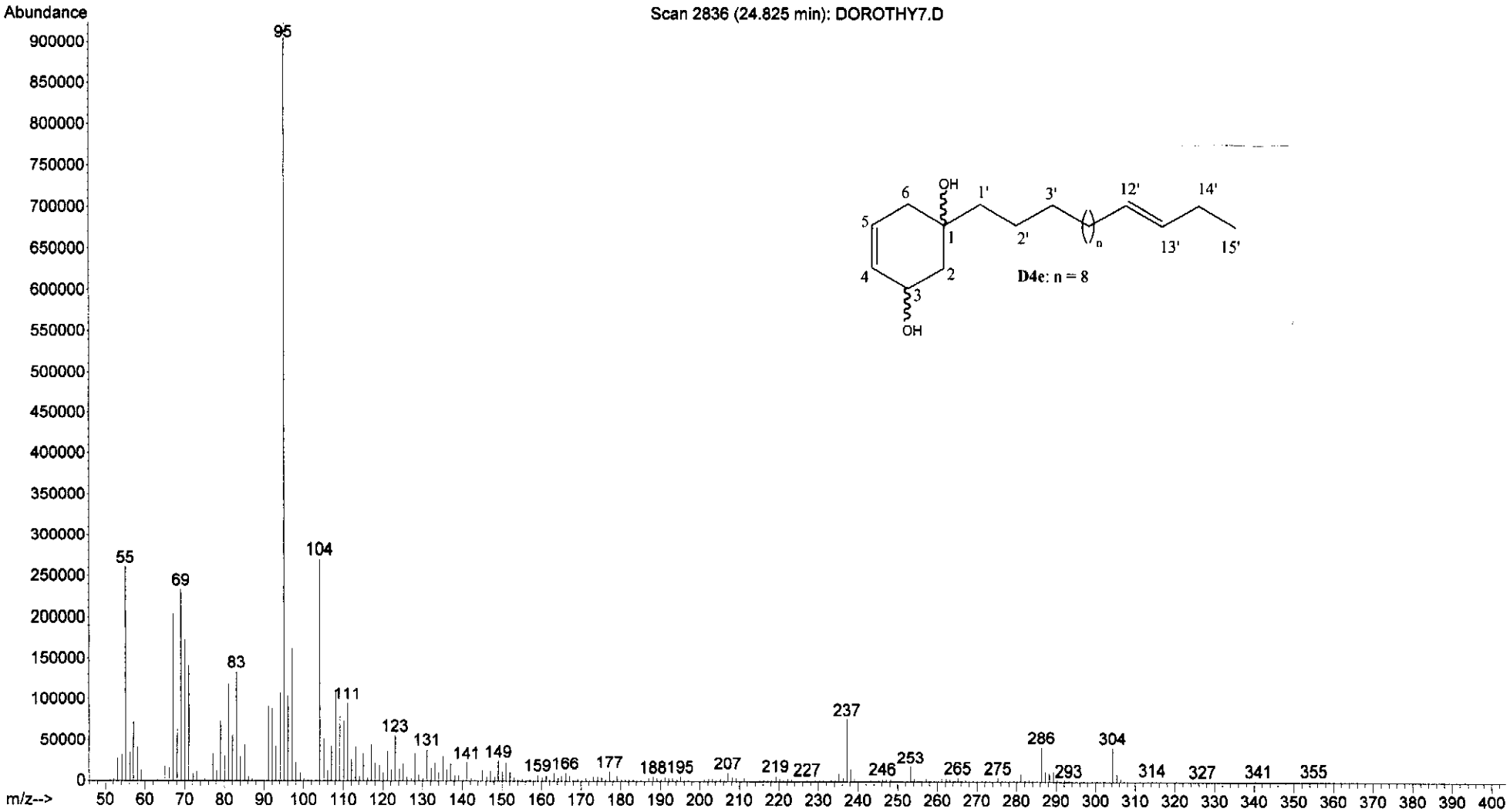
GC chromatogram of a mixture of D4a, D4e, C4a, and C4b

File : C:\MSDCHEM\1\DATA\DOROTHY GCMS TWO\DOROTHY GCMS TWO\DOROTHY7.D
Operator : dorothy
Acquired : 27 Nov 2011 19:53 using AcqMethod DOROTHY
Instrument : Instrumen
Sample Name: EALS RT 85
Misc Info :
Vial Number: 1



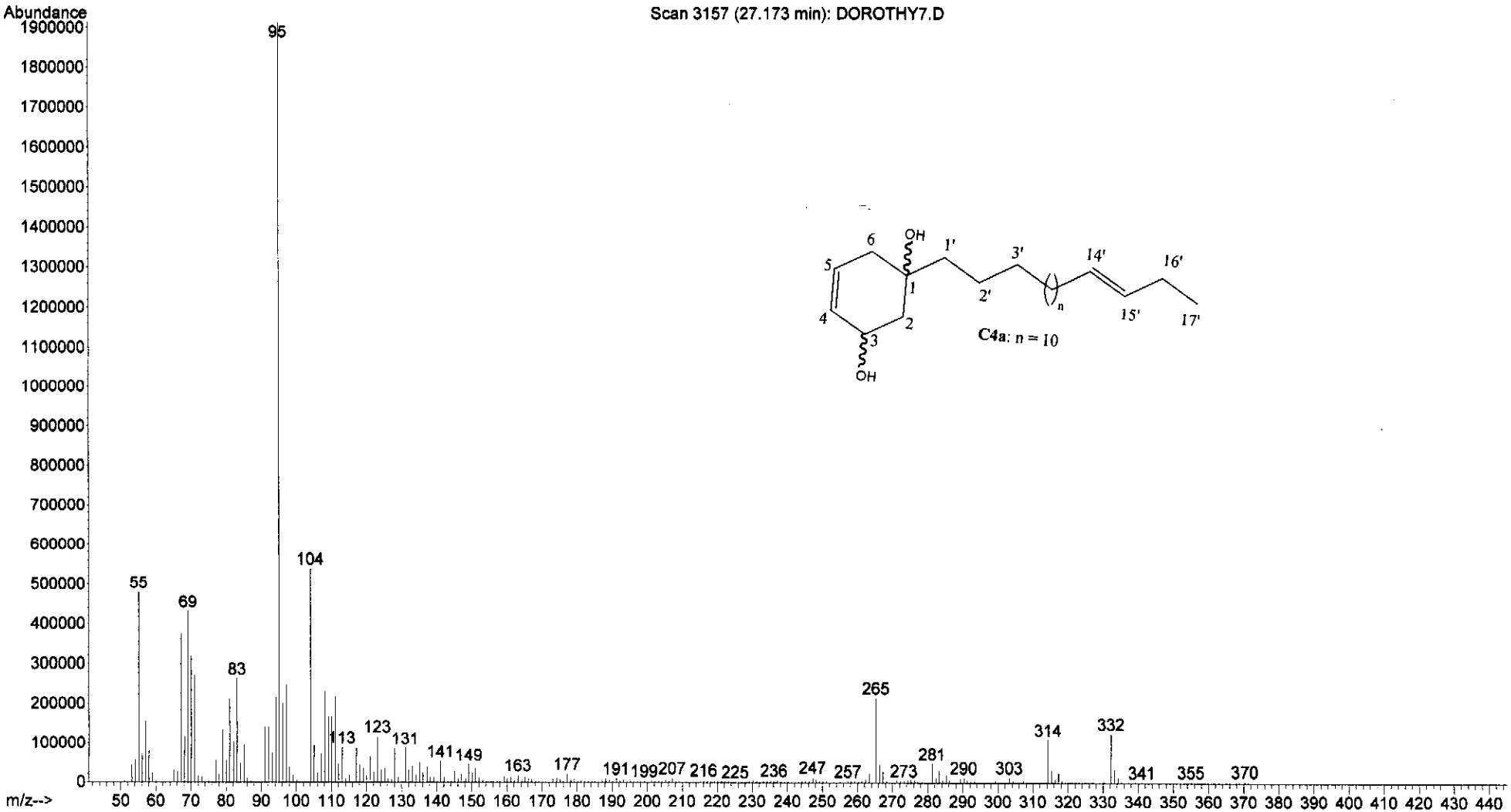
Mass spectrum of D4a

File : C:\MSDCHEM\1\DATA\DOROTHY GCMS TWO\DOROTHY GCMS TWO\DOROTHY7.D
Operator : dorothy
Acquired : 27 Nov 2011 19:53 using AcqMethod DOROTHY
Instrument : Instrumen
Sample Name: EALS RT 85
Misc Info :
Vial Number: 1



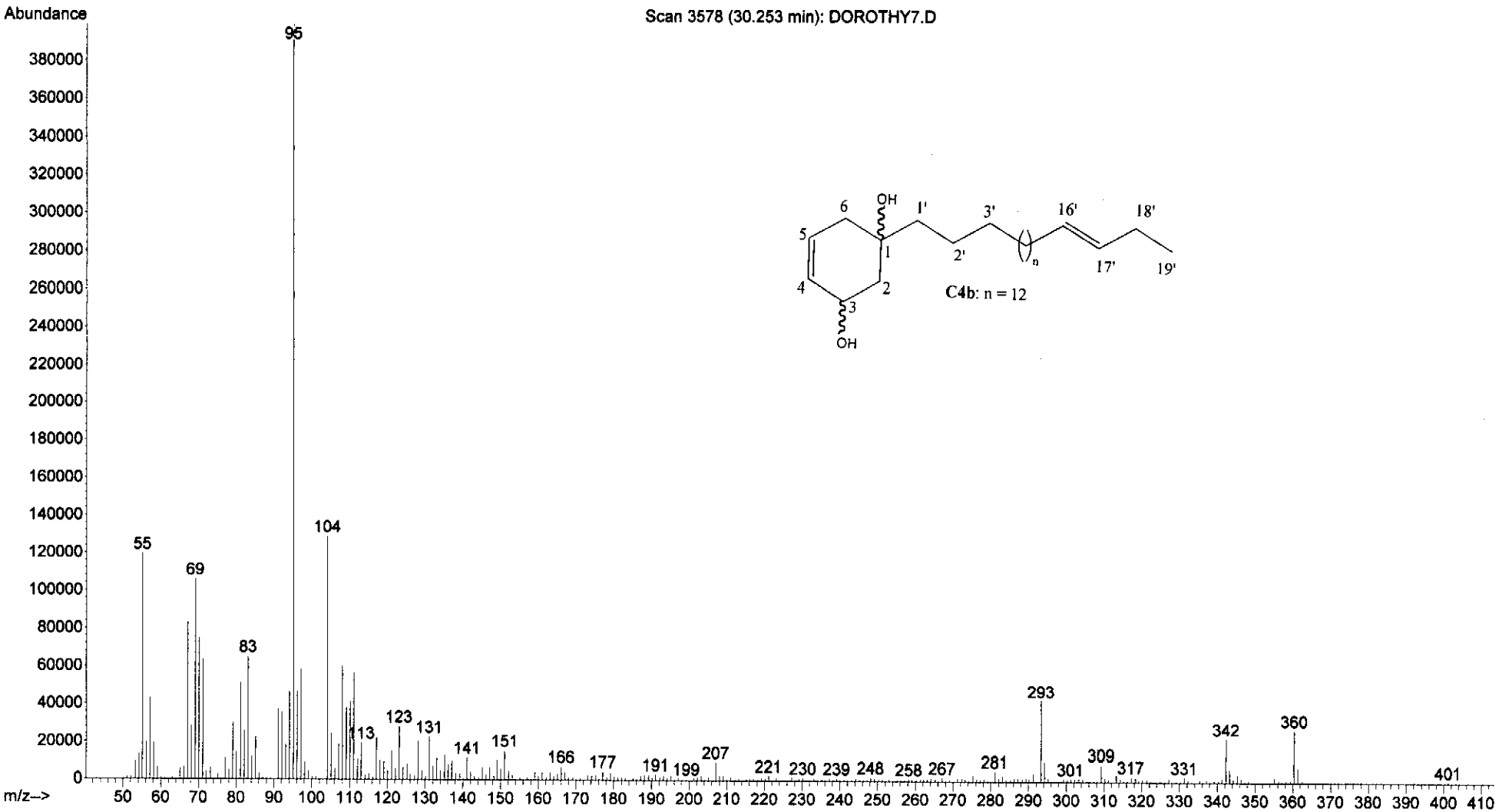
Mass spectrum of D4e

File : C:\MSDCHEM\1\DATA\DOROTHY GCMS TWO\DOROTHY GCMS TWO\DOROTHY7.D
Operator : dorothy
Acquired : 27 Nov 2011 19:53 using AcqMethod DOROTHY
Instrument : Instrumen
Sample Name: EALS RT 85
Misc Info :
Vial Number: 1

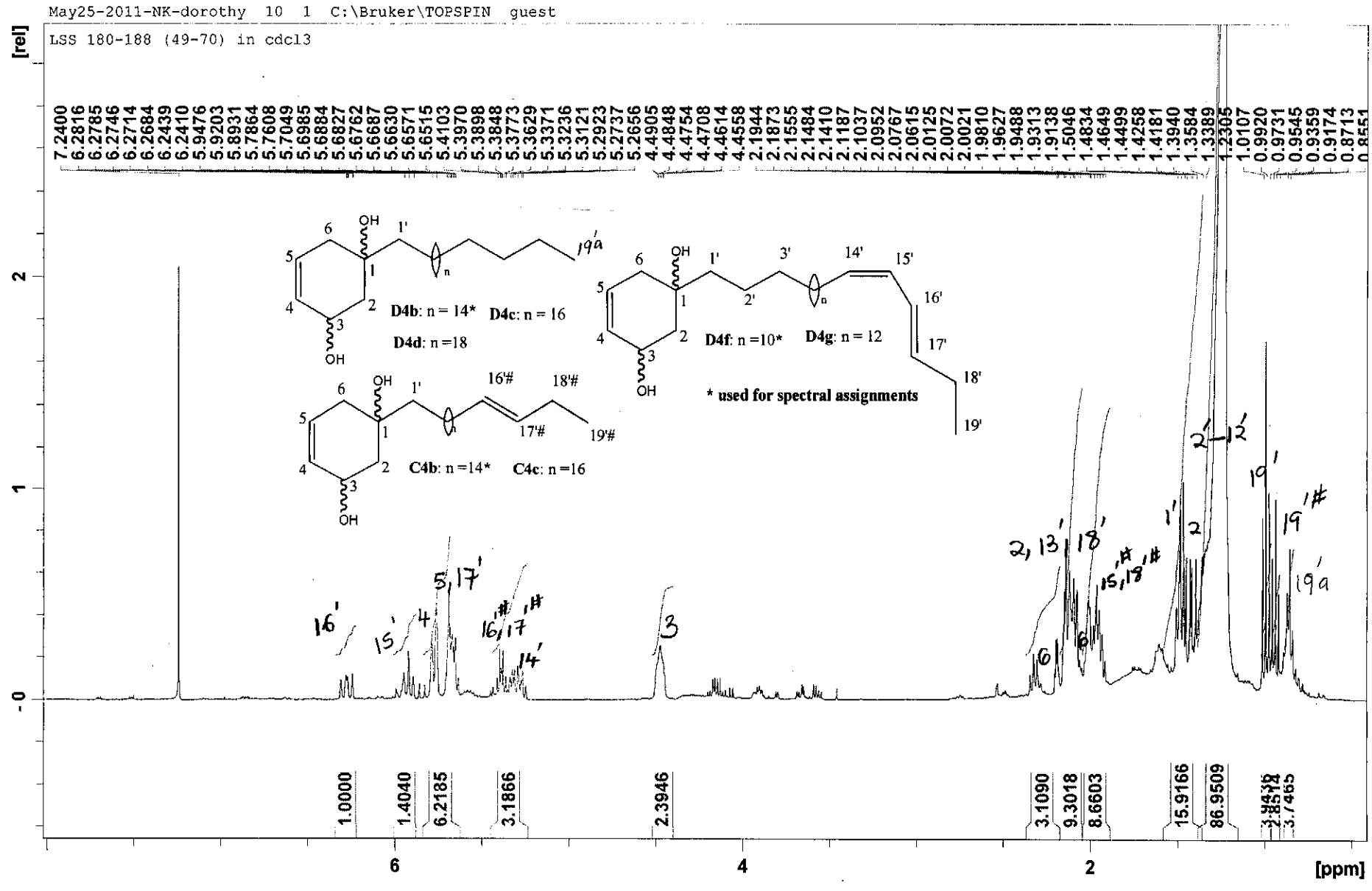


Mass spectrum of C4a

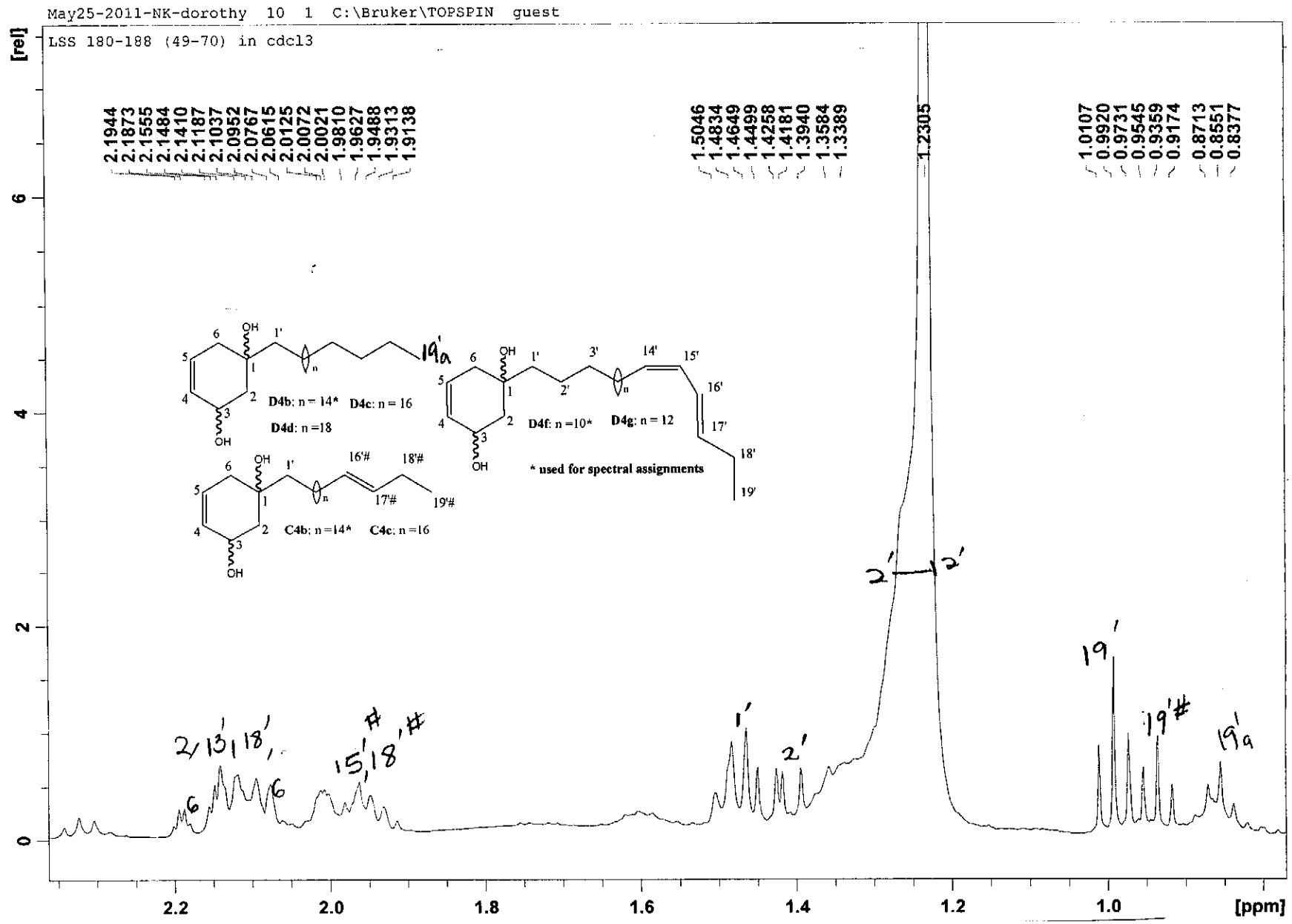
File : C:\MSDCHEM\1\DATA\DOROTHY GCMS TWO\DOROTHY GCMS TWO\DOROTHY7.D
Operator : dorothy
Acquired : 27 Nov 2011 19:53 using AcqMethod DOROTHY
Instrument : Instrumen
Sample Name: EALS RT 85
Misc Info :
Vial Number: 1



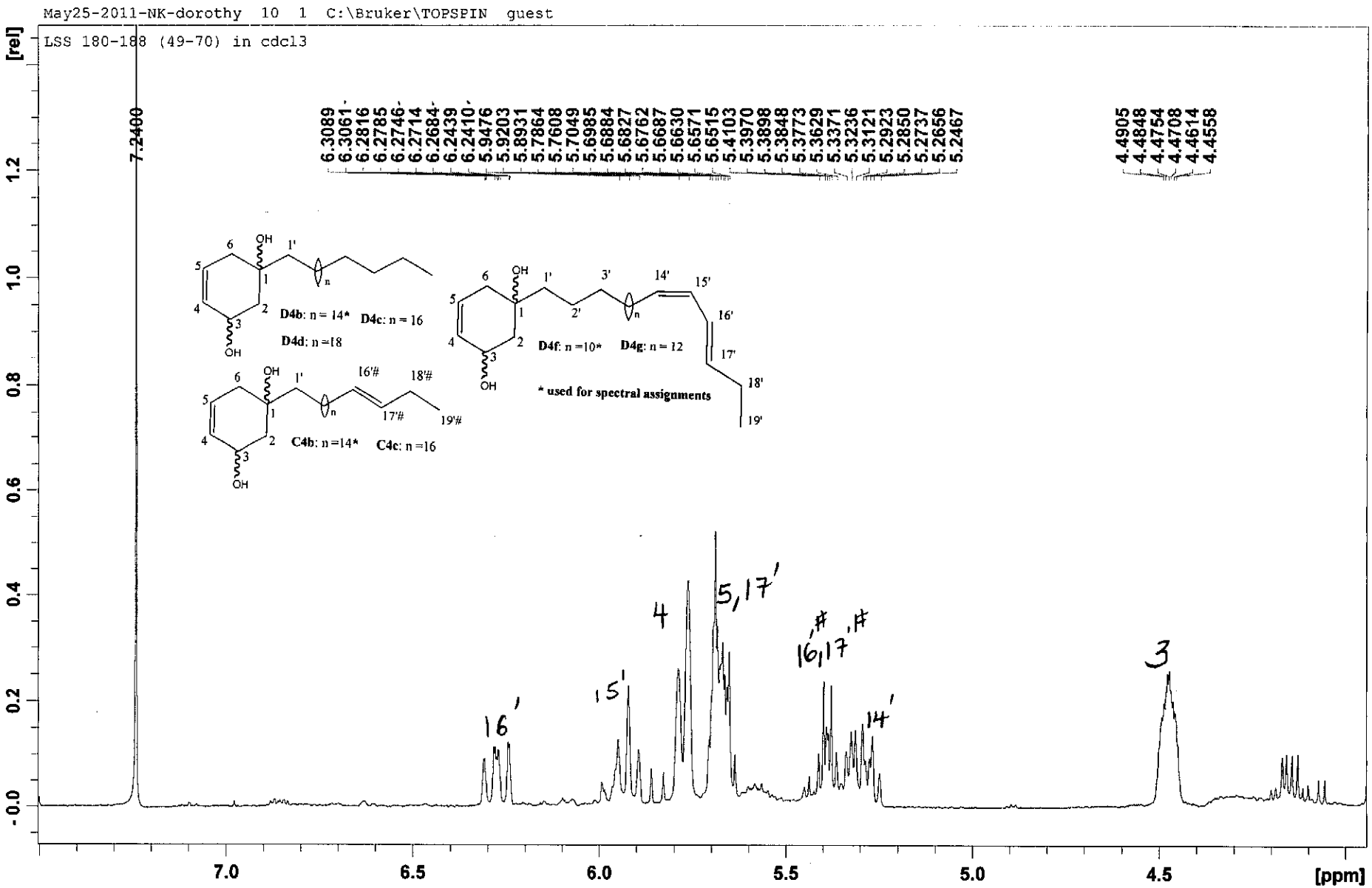
Mass spectrum of C4b



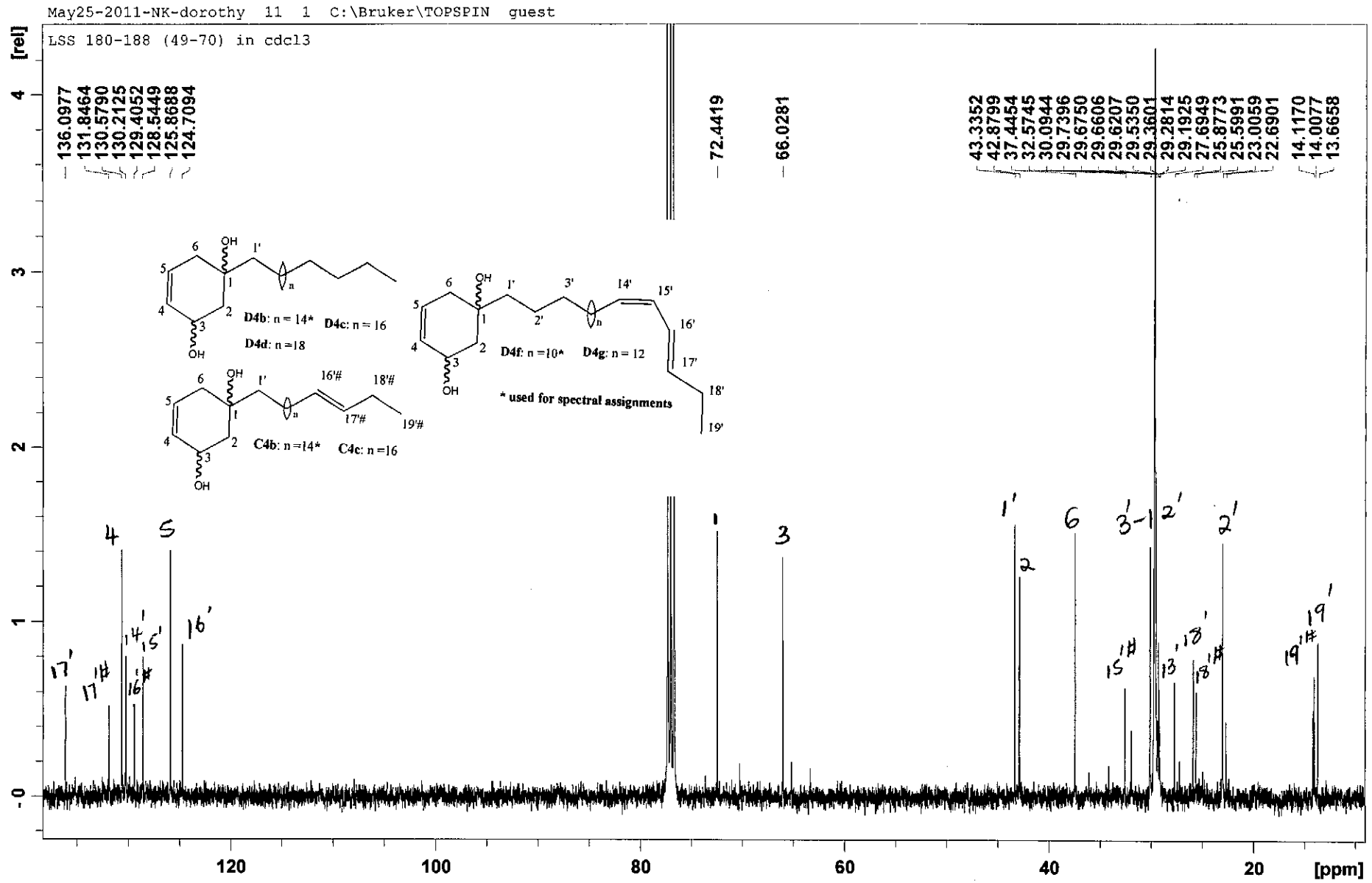
^{1}H NMR spectrum of a mixture of D4b, D4c, D4d, D4f, D4g, C4b and C4c



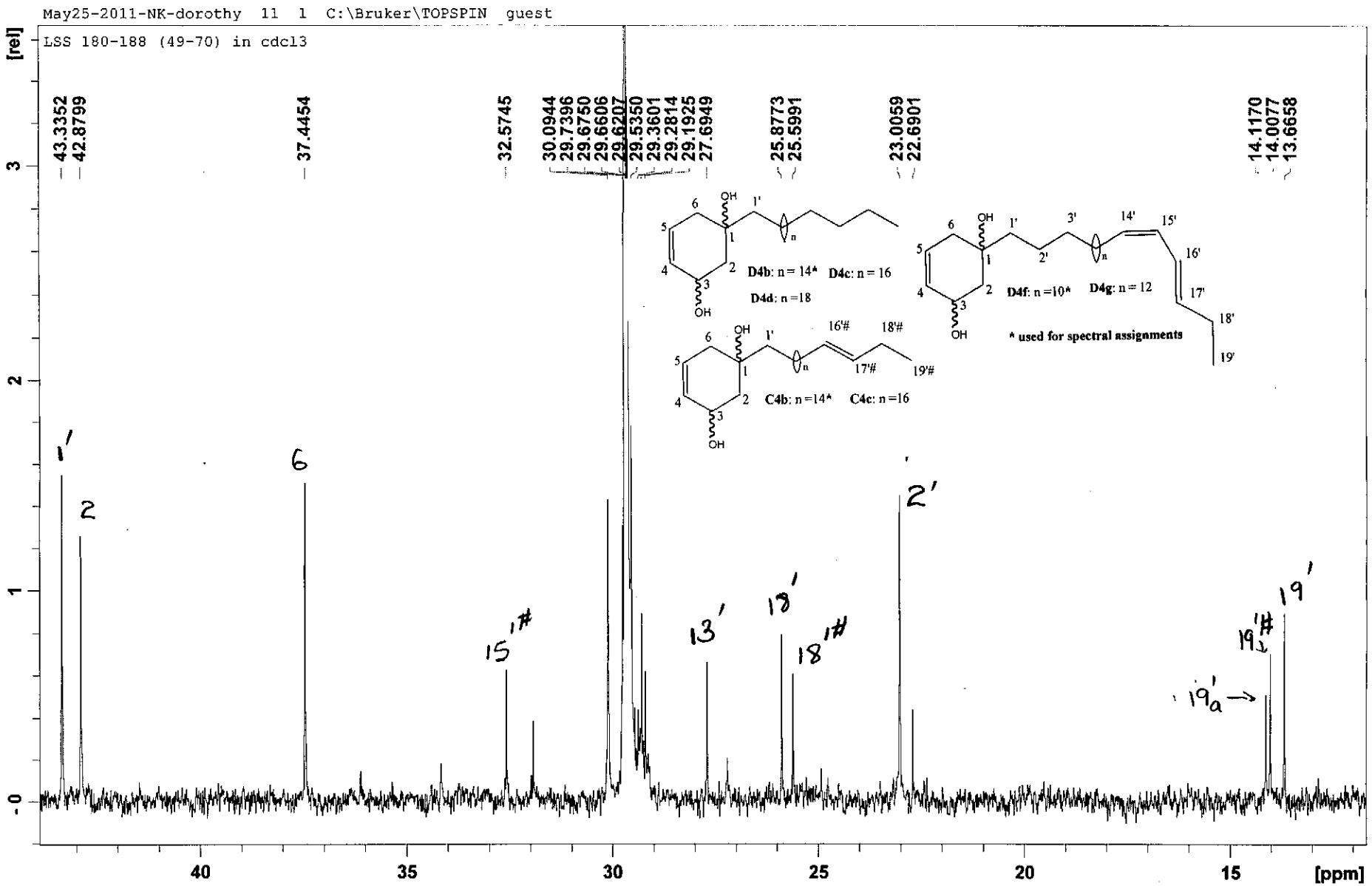
^{1}H NMR spectrum of a mixture of D4b, D4c, D4d, D4f, D4g, C4b and C4c (expanded)



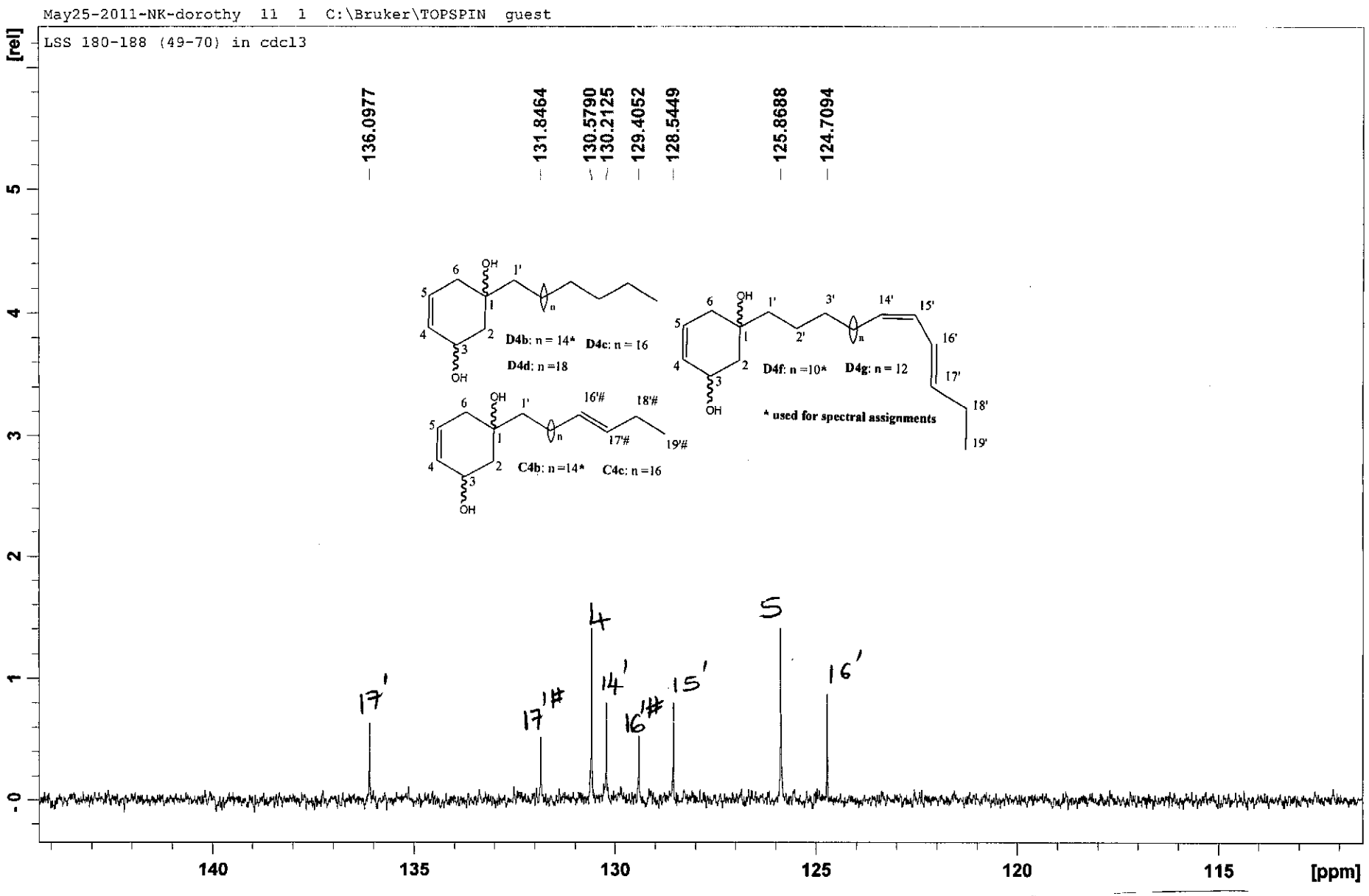
^{1}H NMR spectrum of a mixture of D4b, D4c, D4d, D4f, D4g, C4b and C4c (expanded)



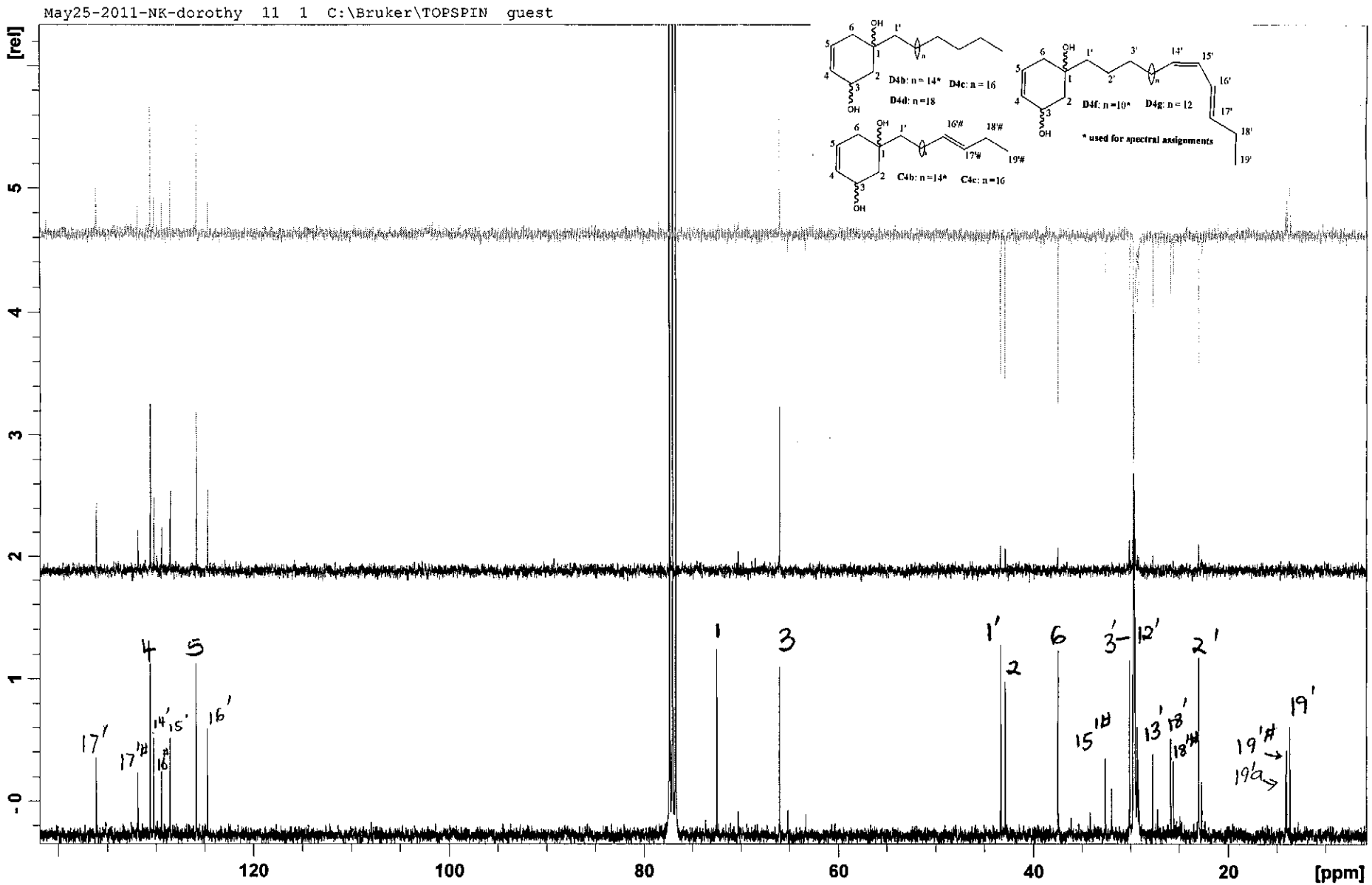
^{13}C NMR spectrum of a mixture of D4b, D4c, D4d, D4f, D4g, C4b and C4c



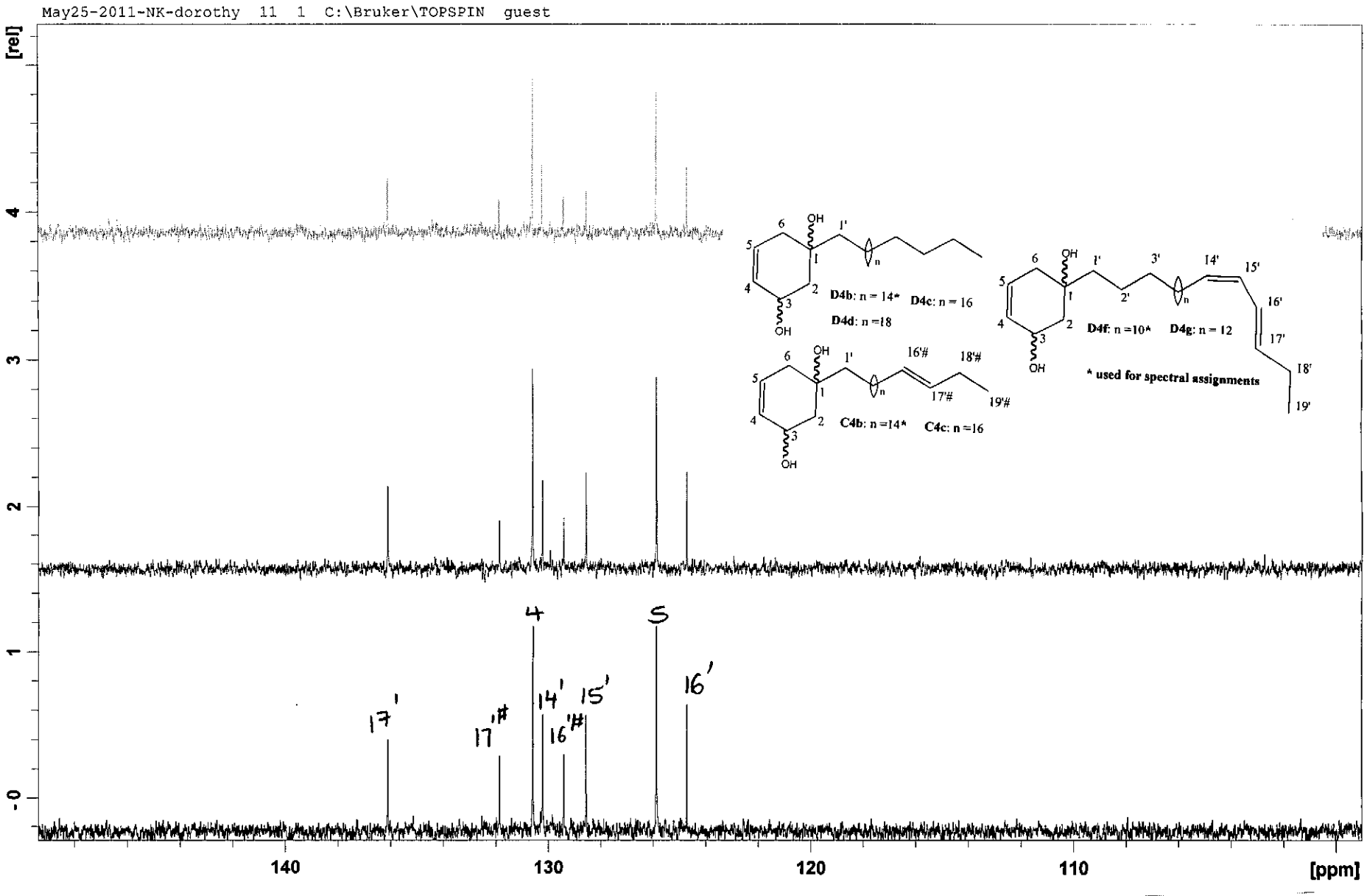
^{13}C NMR spectrum of a mixture of D4b, D4c, D4d, D4f, D4g, C4b and C4c (expanded)



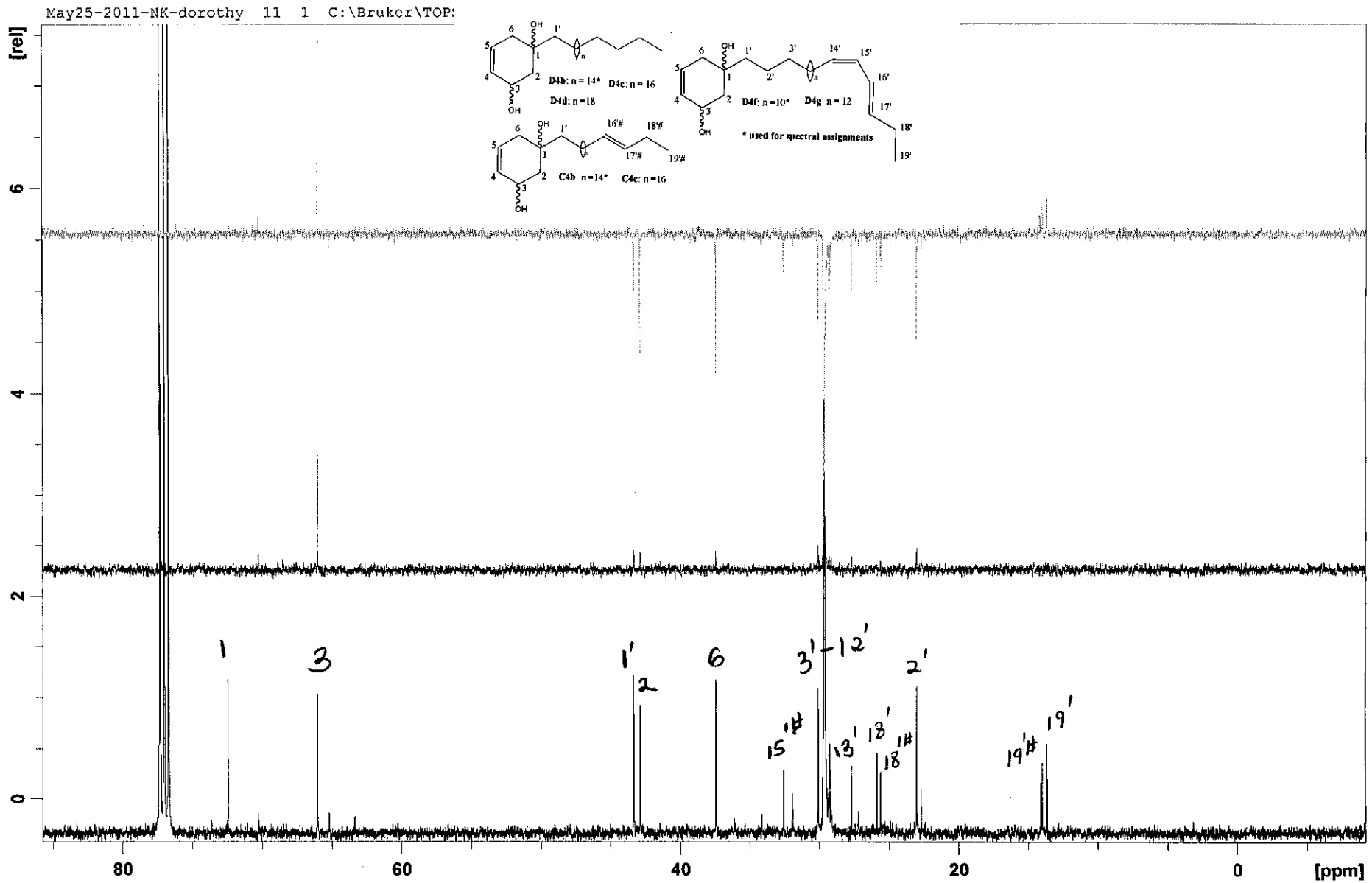
^{13}C NMR spectrum of a mixture of D4b, D4c, D4d, D4f, D4g, C4b and C4c (expanded)



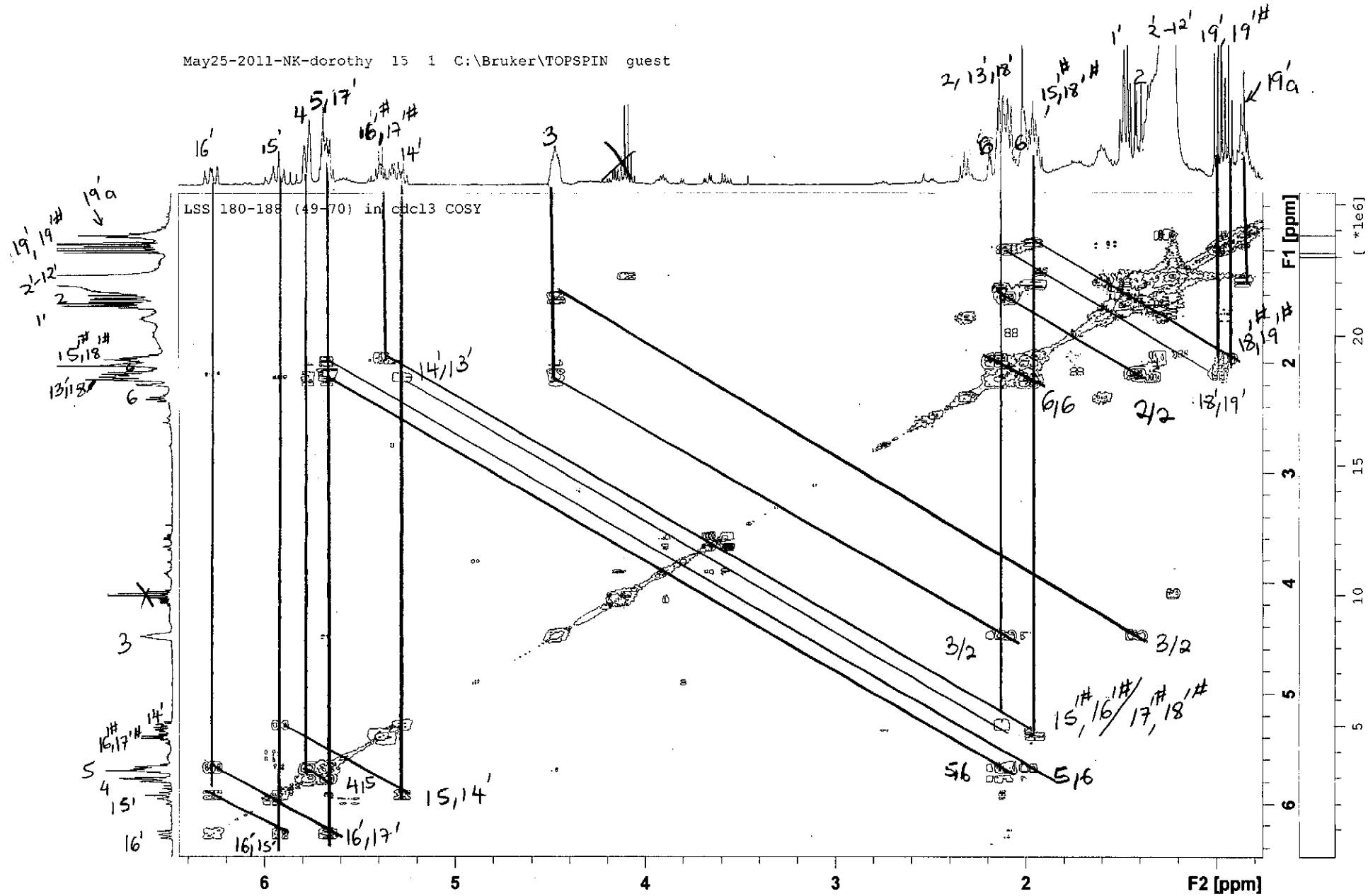
DEPT spectrum of a mixture of D4b, D4c, D4d, D4f, D4g, C4b and C4c



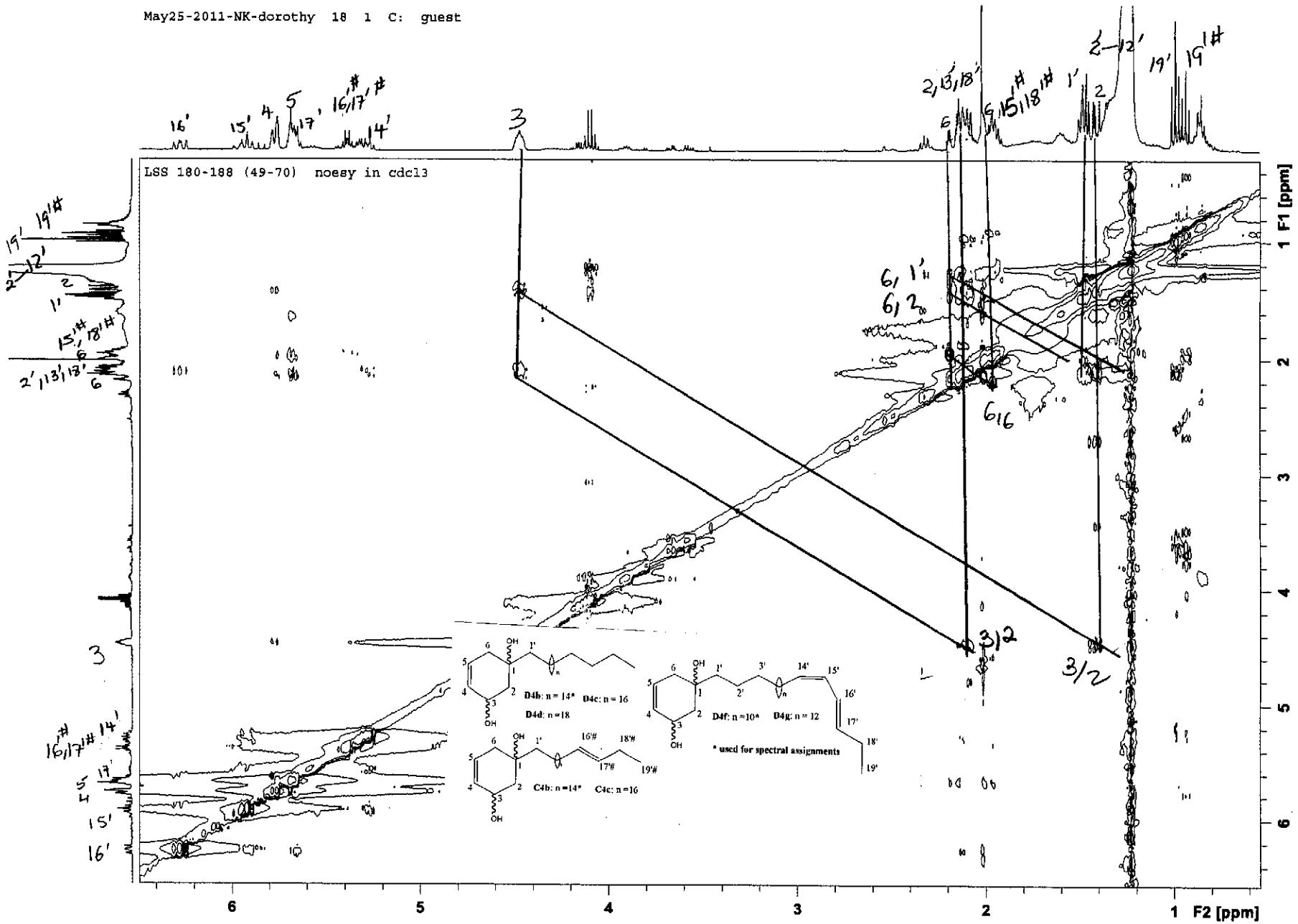
DEPT spectrum of a mixture of D4b, D4c, D4d, D4f, D4g, C4b and C4c (expanded)



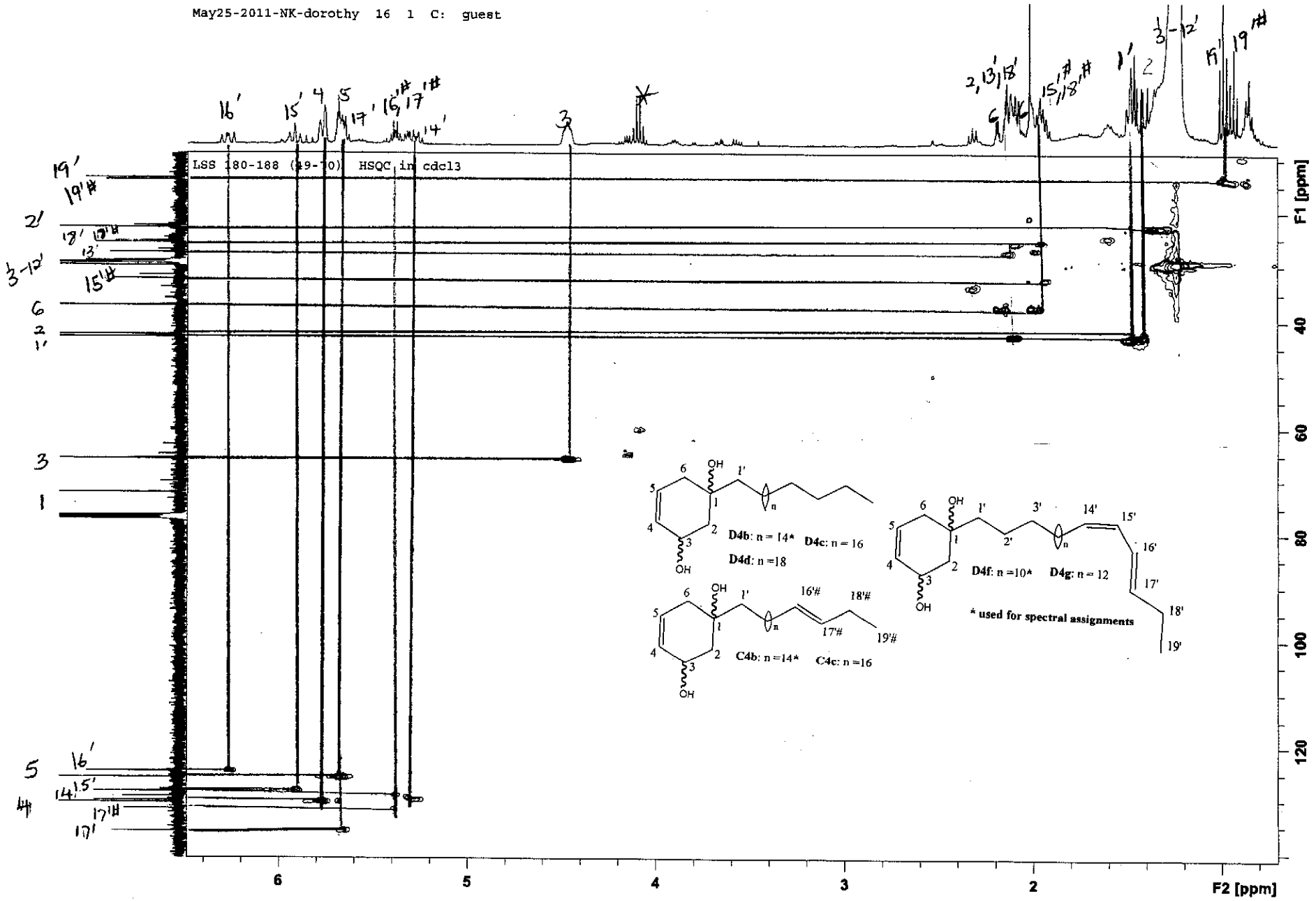
DEPT spectrum of a mixture of D4b, D4c, D4d, D4f, D4g, C4b and C4c (expanded)



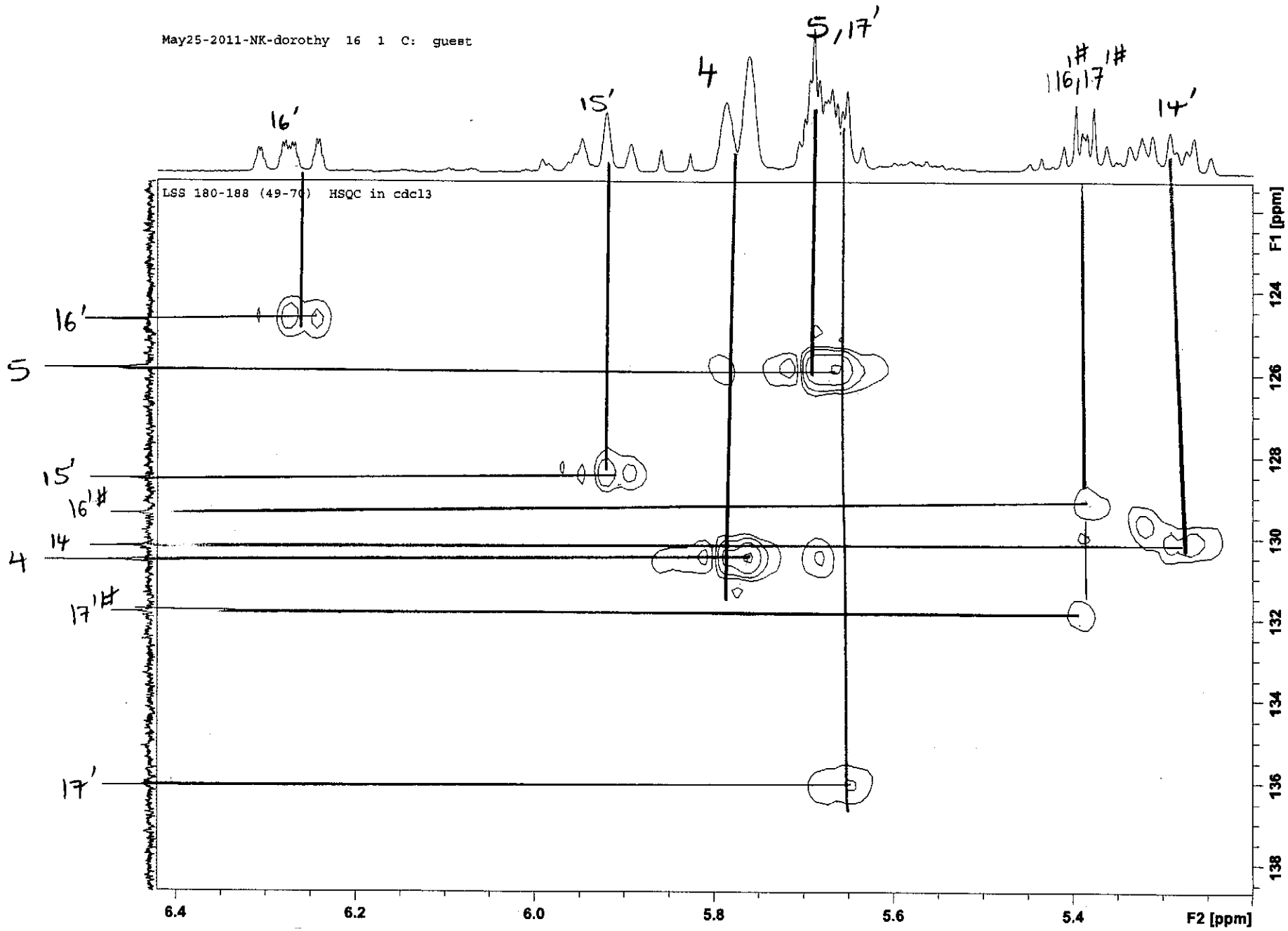
COSY spectrum of a mixture of D4b, D4c, D4d, D4f, D4g, C4b and C4c



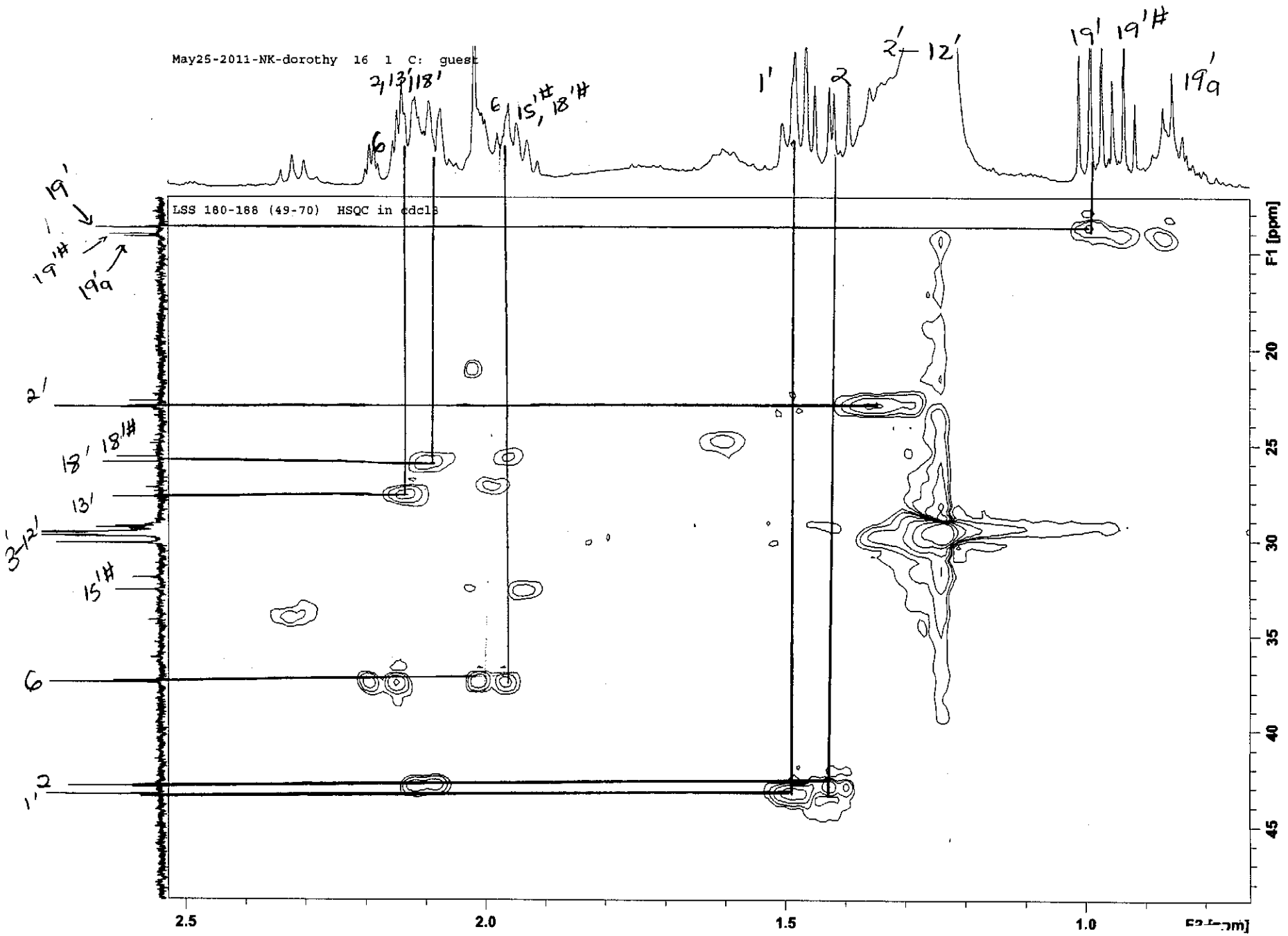
NOESY spectrum of a mixture of D4b, D4c, D4d, D4f, D4g, C4b and C4c



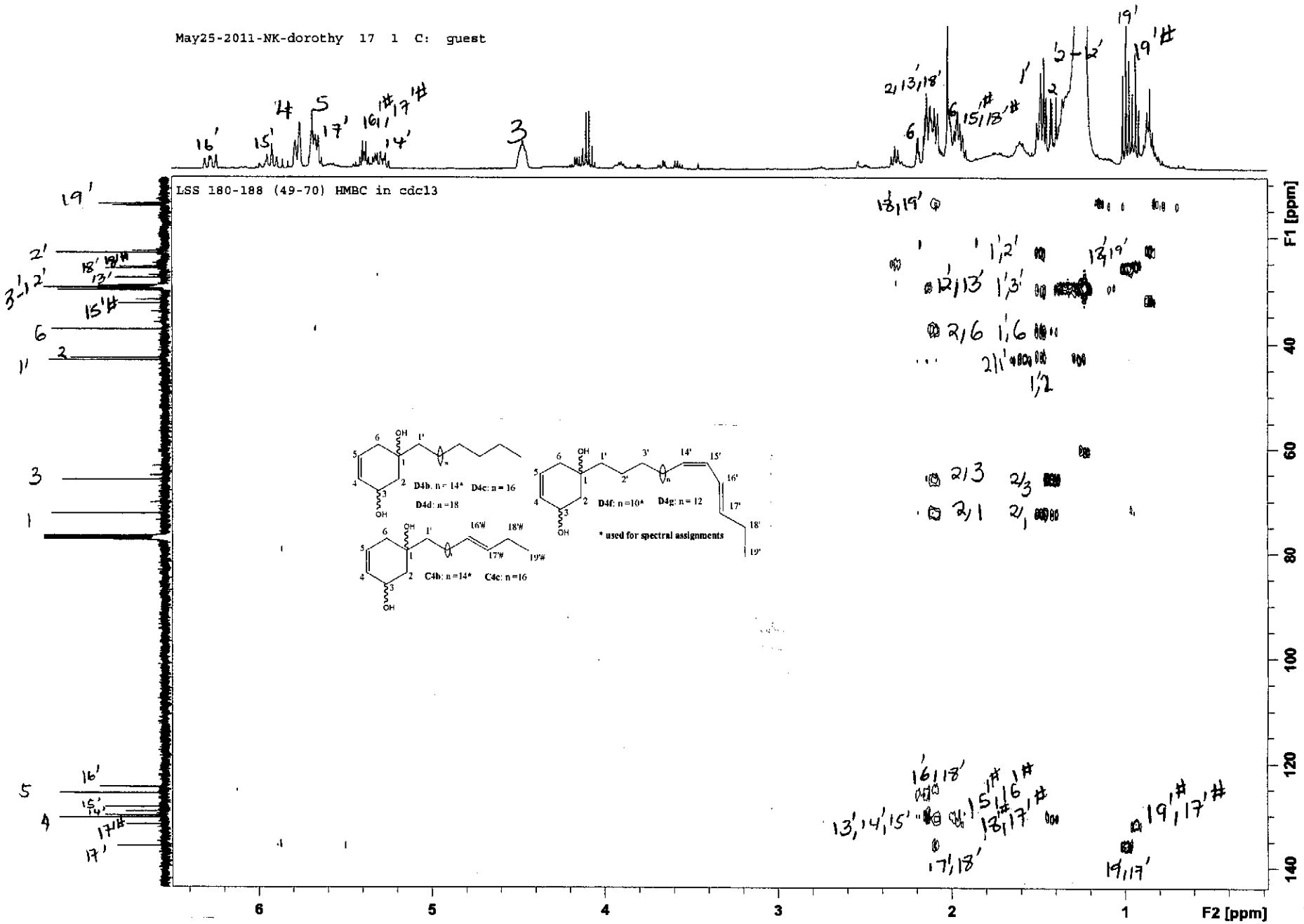
HSQC spectrum of a mixture of D4b, D4c, D4d, D4f, D4g, C4b and C4c



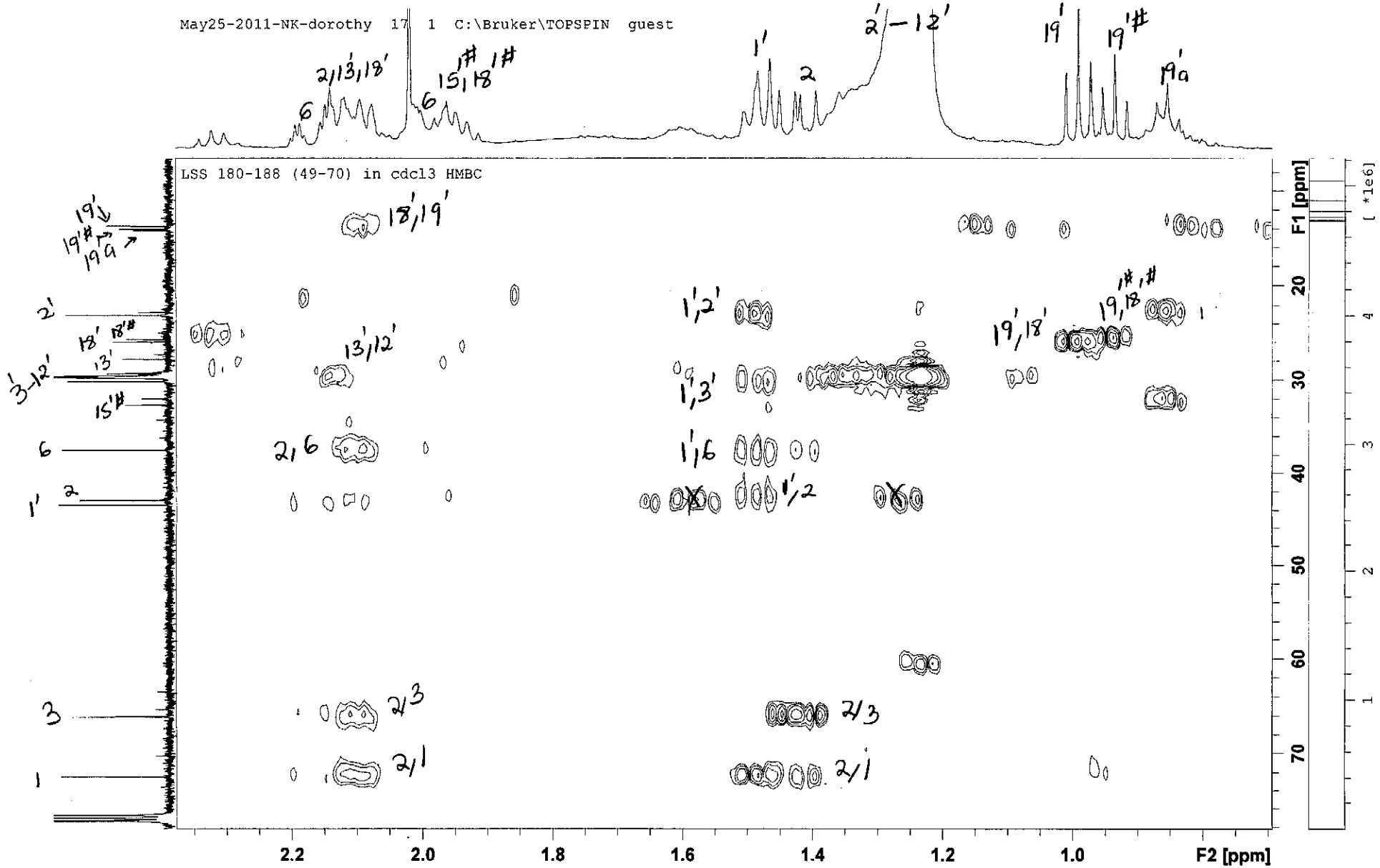
HSQC spectrum of a mixture of D4b, D4c, D4d, D4f, D4g, C4b and C4c (expanded)



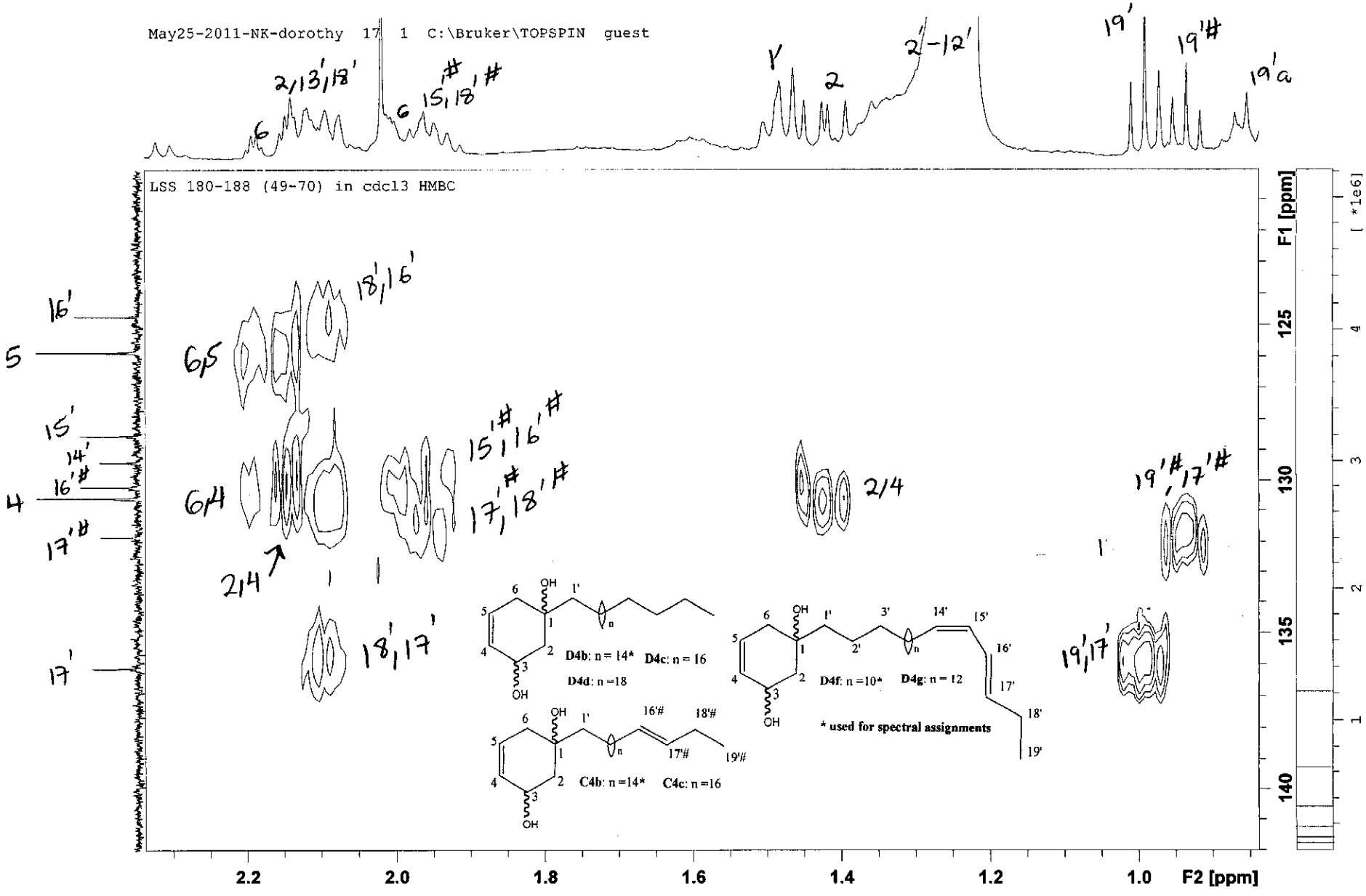
HSQC spectrum of a mixture of D4b, D4c, D4d, D4f, D4g, C4b and C4c (expanded)



HMBC spectrum of a mixture of D4b, D4c, D4d, D4f, D4g, C4b and C4c



HMBC spectrum of a mixture of D4b, D4c, D4d, D4f, D4g, C4b and C4c (expanded)

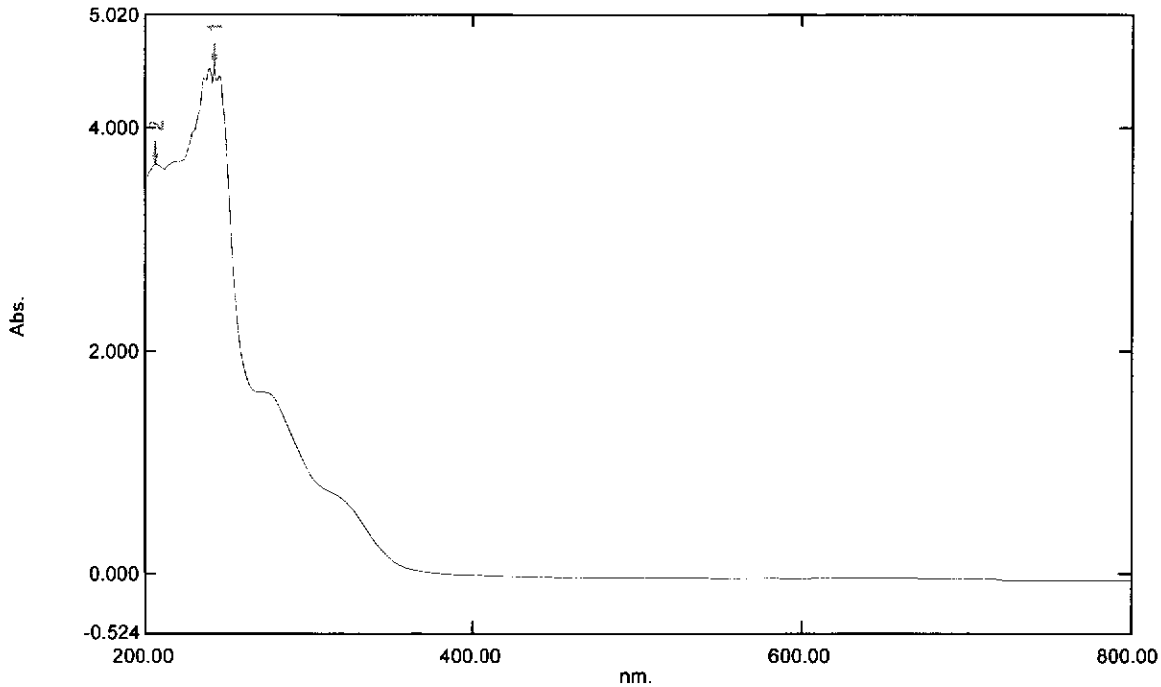


HMBC spectrum of a mixture of D4b, D4c, D4d, D4f, D4g, C4b and C4c (expanded)

Spectrum Peak Pick Report

04/10/2011 12:12:38 PM

Data Set: LSS 180-189.spc - Storage 144559



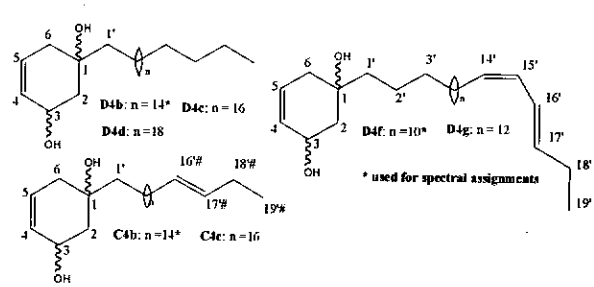
No.	P/V	Wavelength	Abs.	Description
1	↑	242.00	4.558	
2	↑	207.00	3.676	
3	↓	466.00	-0.035	
4	↓	212.00	3.631	

Measurement Properties
Wavelength Range (nm.): 200.00 to 800.00
Scan Speed: Medium
Sampling Interval: 1.0
Auto Sampling Interval: Disabled
Scan Mode: Single

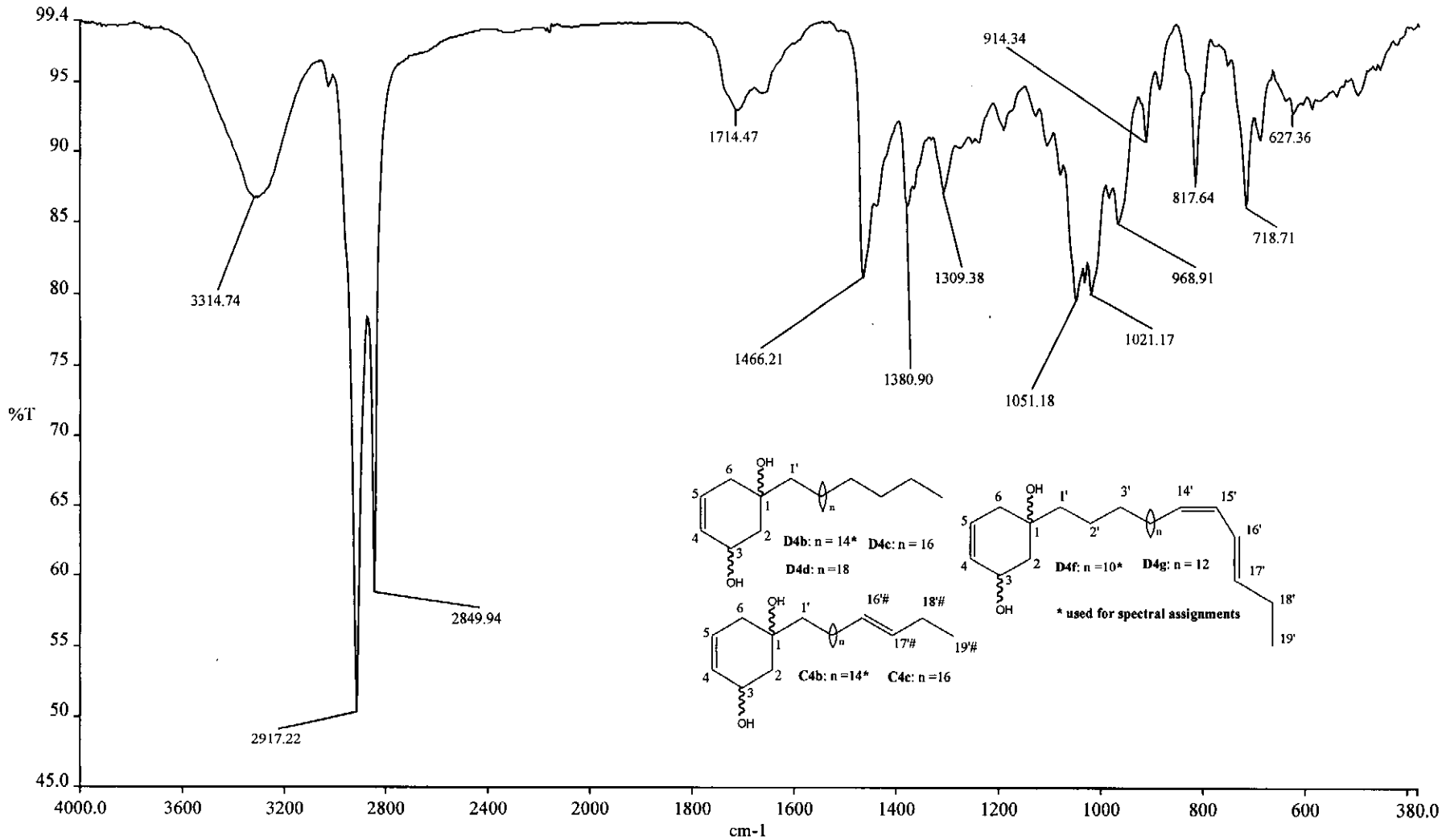
Instrument Properties
Instrument Type: UV-3600 Series
Measuring Mode: Absorbance
Slit Width: 8.0 nm
Time Constant: 0.1 sec.
Source Lamp: Auto
Light Source Change Wavelength: 310.00 nm
Detector Unit: Direct
Detector Change Wavelength: 830.00 nm 1800.00 nm
Grating Change Wavelength: 720.00 nm
S/R Exchange: Normal
Detector Lock: Auto
Slit Program: Normal
Beam Mode: Double
Stair Correction: Disable

Attachment Properties
Attachment: None

Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

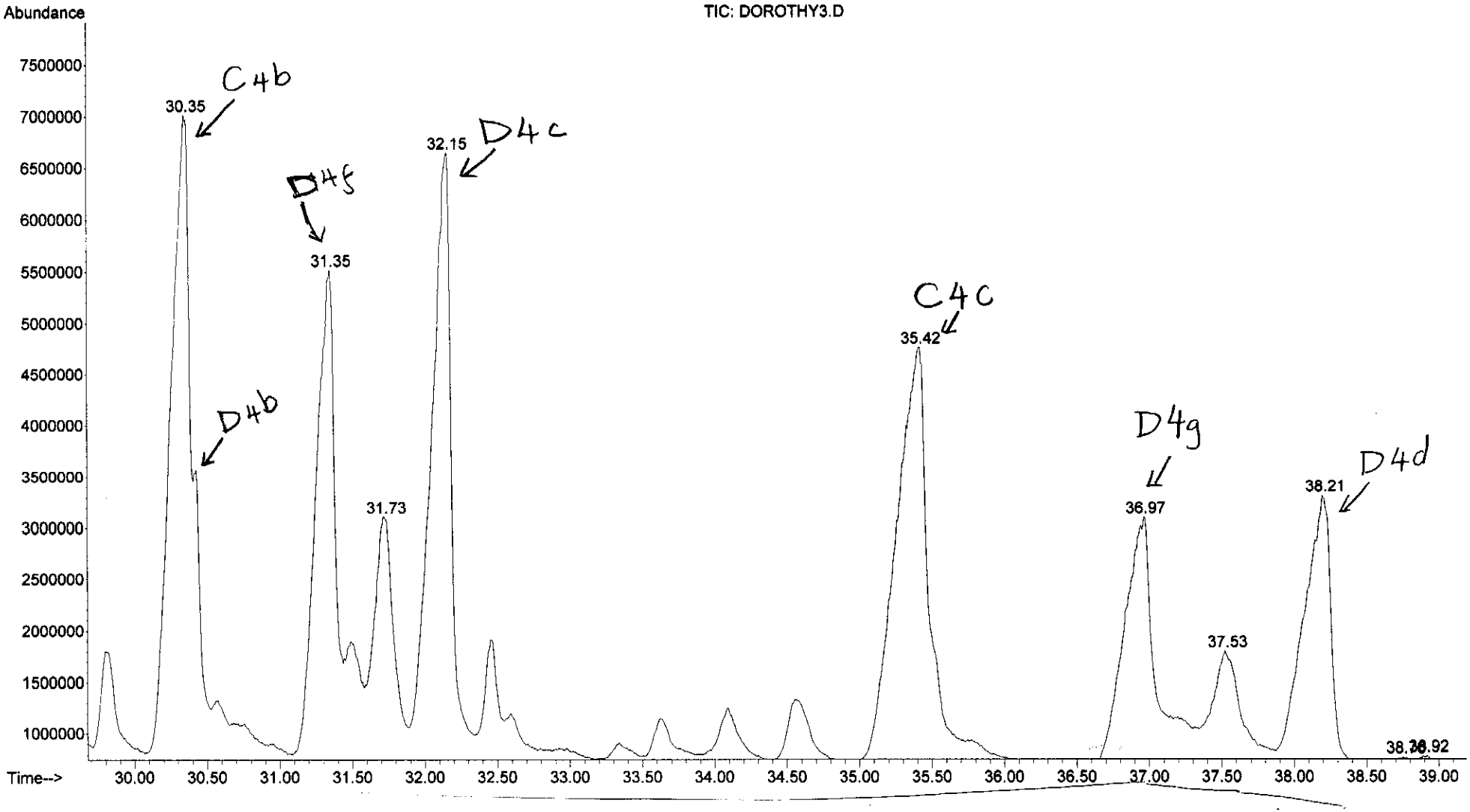


UV spectrum of a mixture of D4b, D4c, D4d, D4f, D4g, C4b and C4c



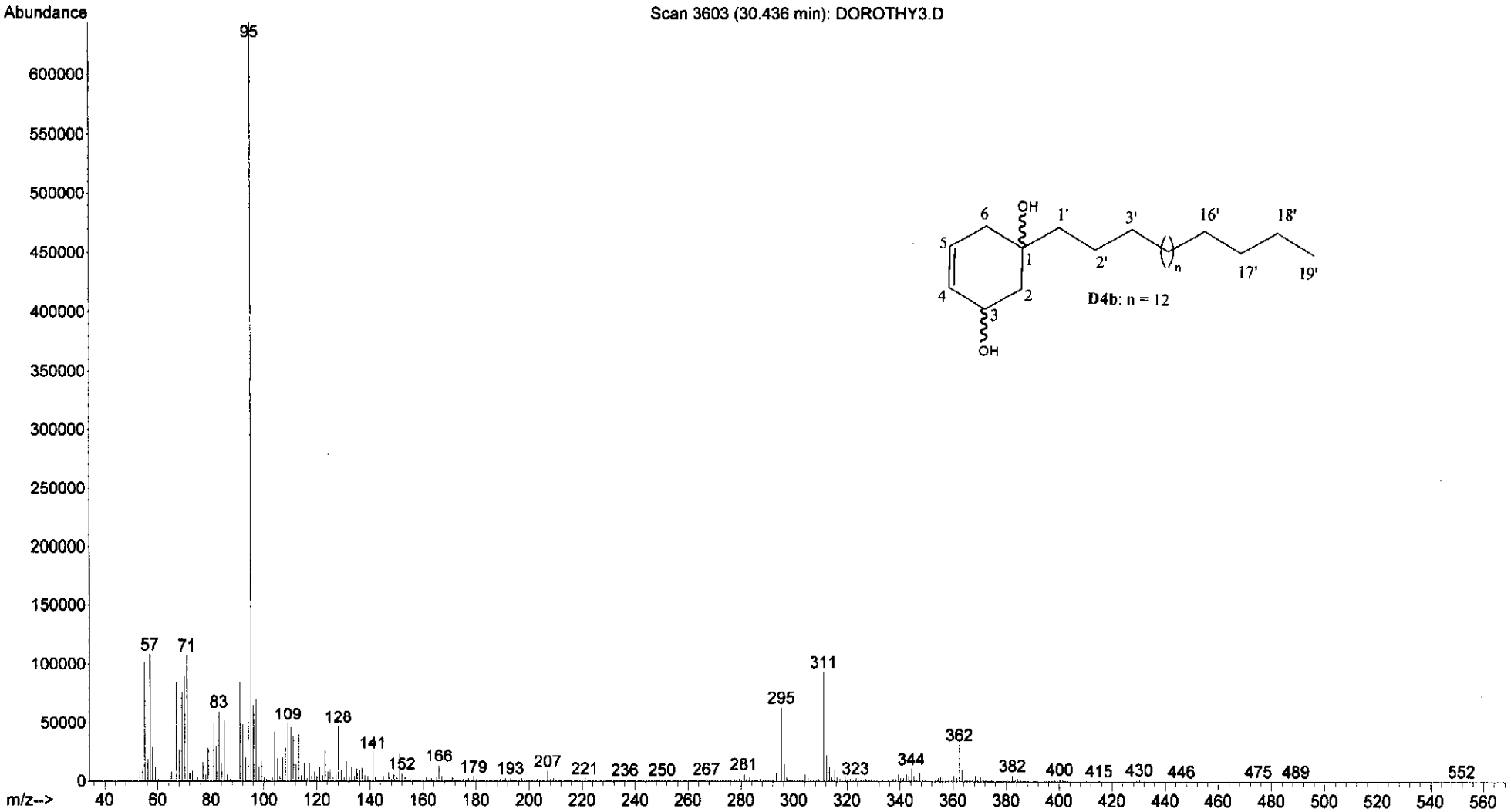
IR spectrum of a mixture of D4b, D4c, D4d, D4f, D4g, C4b and C4c

File : C:\MSDCHEM\1\DATA\DOROTHY GCMS TWO\DOROTHY GCMS TWO\DOROTHY3.D
Operator : dorothy
Acquired : 27 Nov 2011 14:33 using AcqMethod DOROTHY
Instrument : Instrumen
Sample Name: LSS 180-188 34-48B
Misc Info :
Vial Number: 1



GC chromatogram of a mixture of D4b, D4c, D4d, D4f, D4g, C4b and C4c

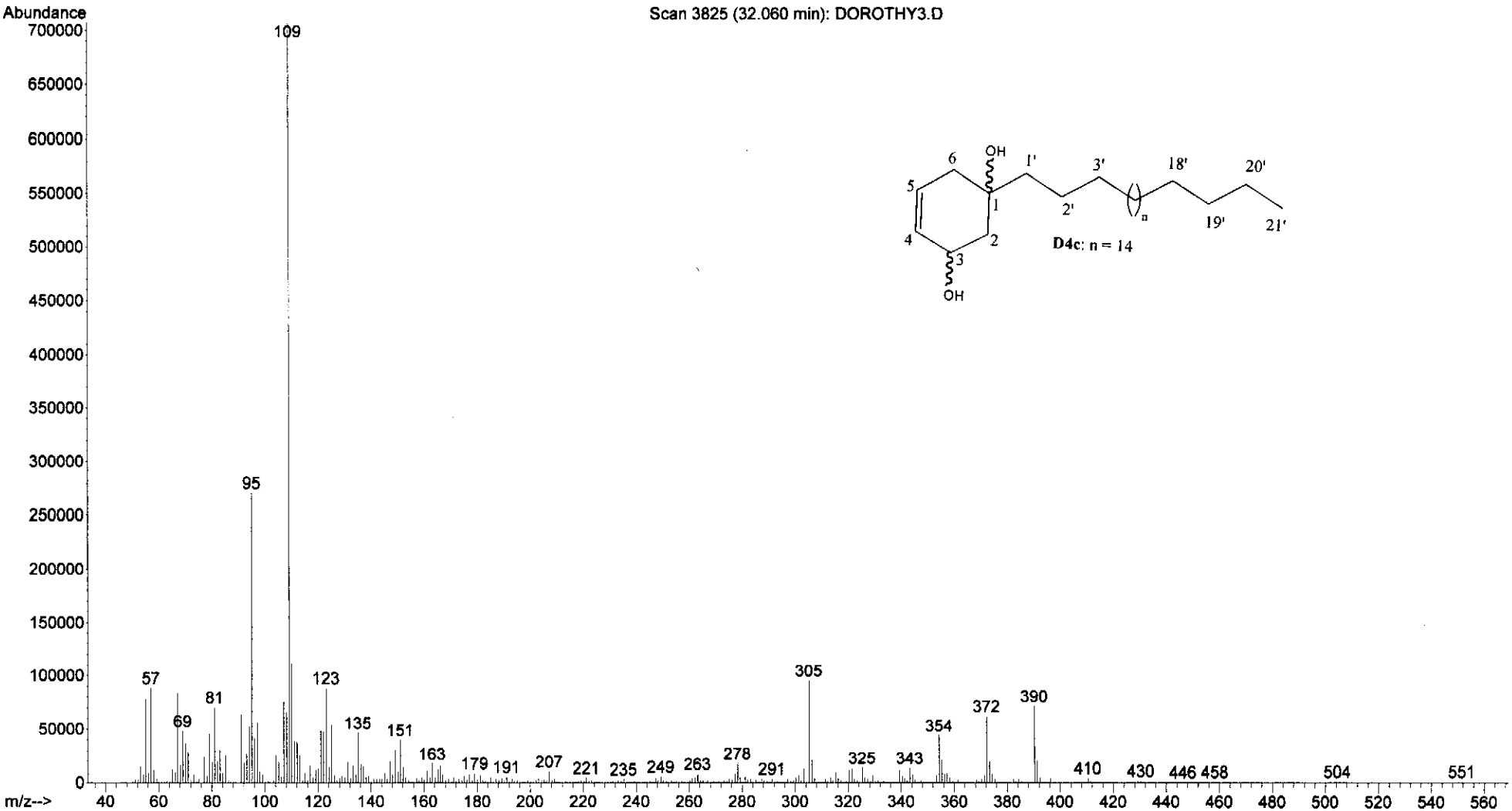
File : C:\MSDCHEM\1\DATA\DOROTHY GCMS TWO\DOROTHY GCMS TWO\DOROTHY3.D
Operator : dorothy
Acquired : 27 Nov 2011 14:33 using AcqMethod DOROTHY
Instrument : Instrumen
Sample Name: LSS 180-188 34-48B
Misc Info :
Vial Number: 1



D4b: n = 12

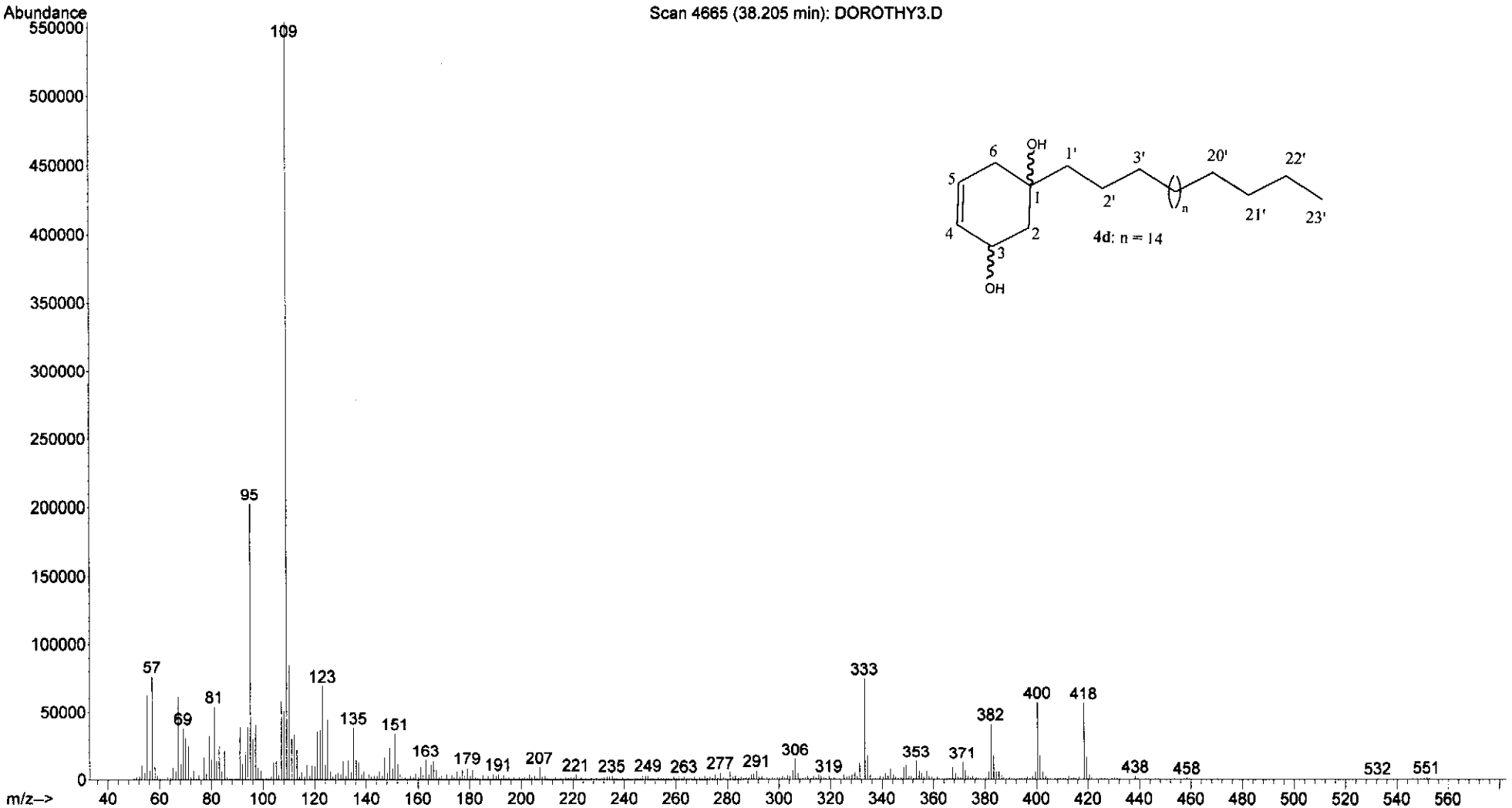
Mass spectrum of D4b

File : C:\MSDCHEM\1\DATA\DOROTHY GCMS TWO\DOROTHY GCMS TWO\DOROTHY3.D
Operator : dorothy
Acquired : 27 Nov 2011 14:33 using AcqMethod DOROTHY
Instrument : Instrumen
Sample Name: LSS 180-188 34-48B
Misc Info :
Vial Number: 1



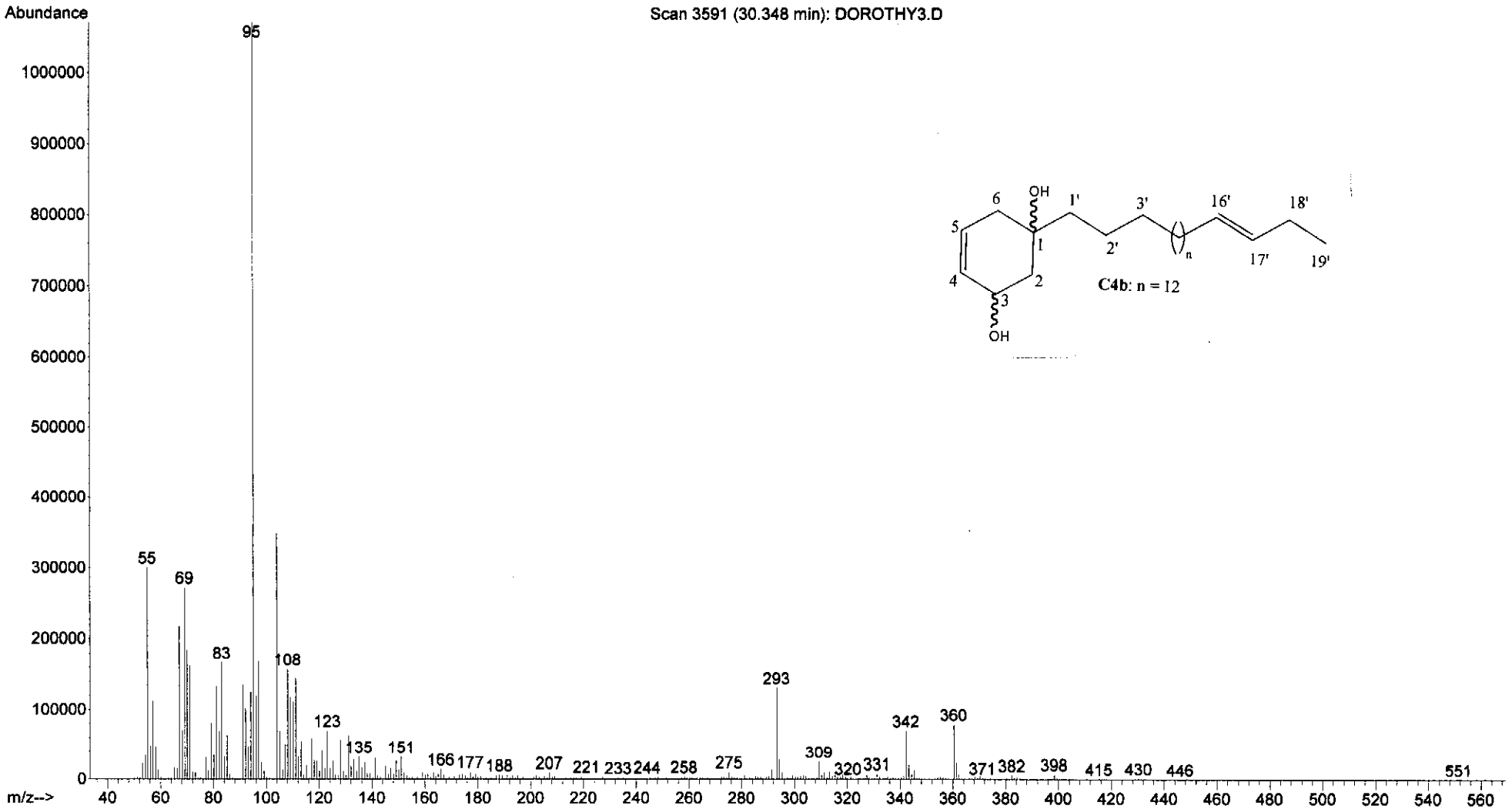
Mass spectrum of D4c

File : C:\MSDCHEM\1\DATA\DOROTHY GCMS TWO\DOROTHY GCMS TWO\DOROTHY3.D
Operator : dorothy
Acquired : 27 Nov 2011 14:33 using AcqMethod DOROTHY
Instrument : Instrumen
Sample Name: LSS 180-188 34-48B
Misc Info :
Vial Number: 1



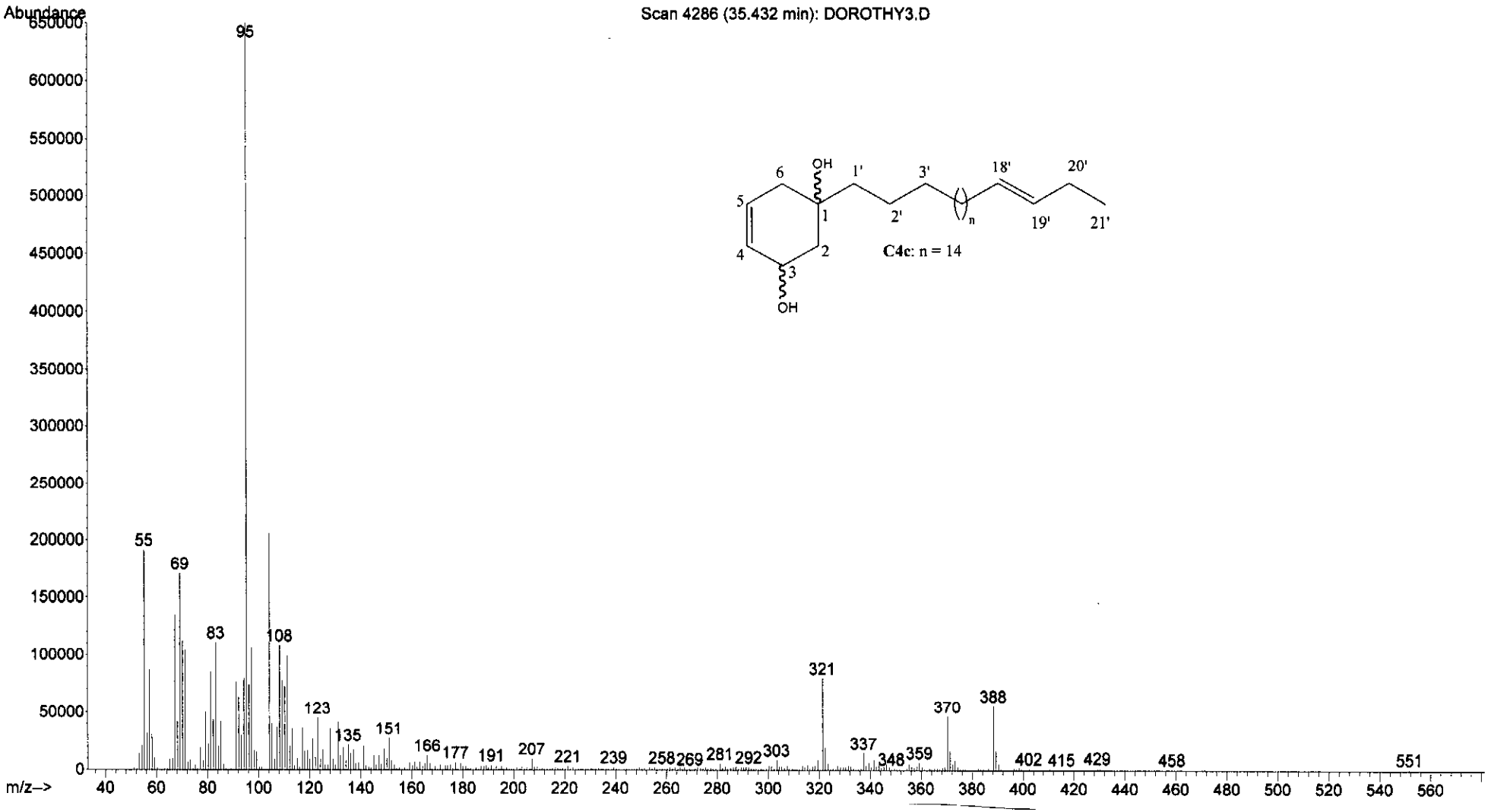
Mass spectrum of D4d

File : C:\MSDCHEM\1\DATA\DOROTHY GCMS TWO\DOROTHY GCMS TWO\DOROTHY3.D
Operator : dorothy
Acquired : 27 Nov 2011 14:33 using AcqMethod DOROTHY
Instrument : Instrumen
Sample Name: LSS 180-188 34-48B
Misc Info :
Vial Number: 1



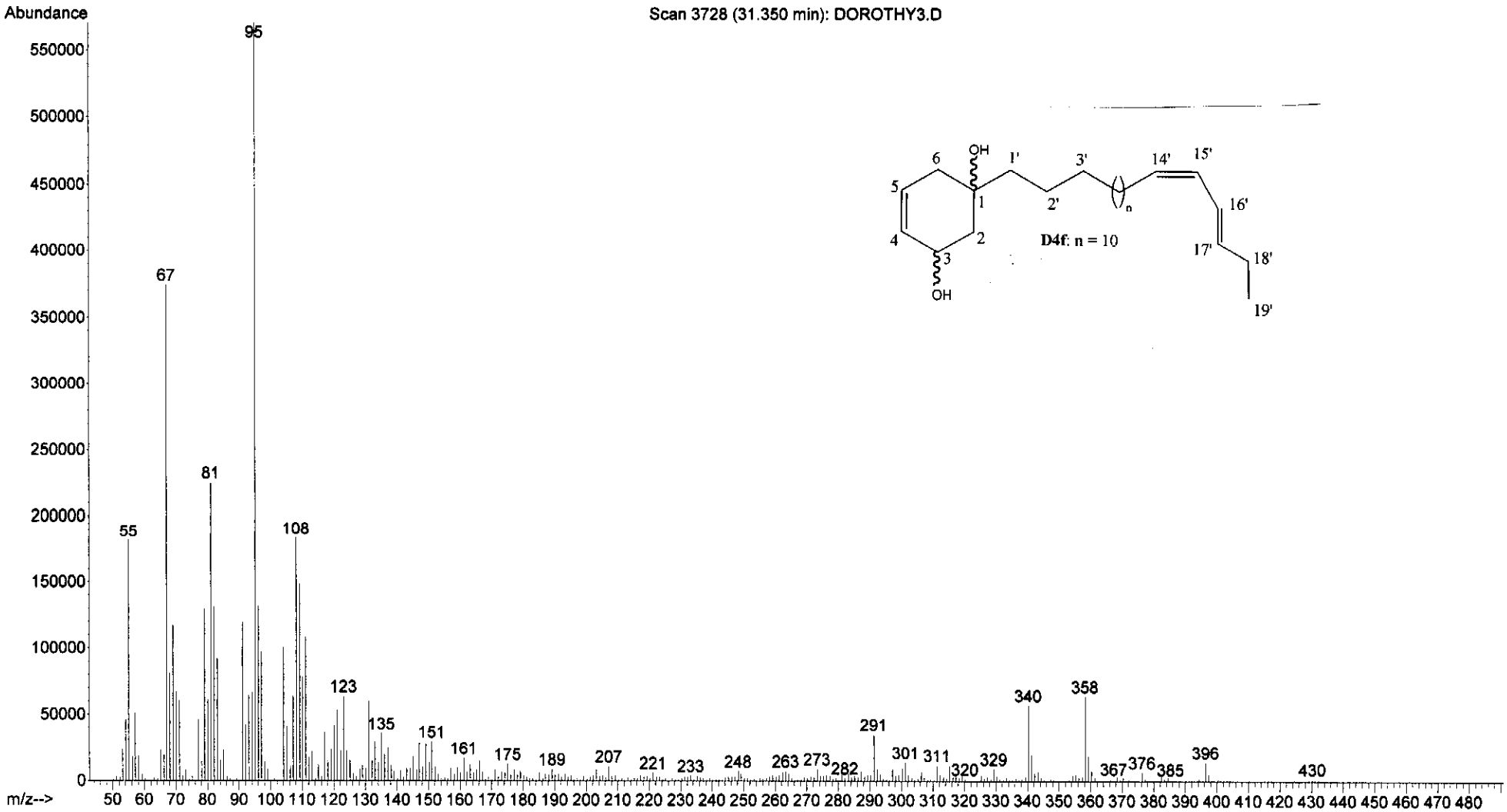
Mass spectrum of C4b

File : C:\MSDCHEM\1\DATA\DOROTHY GCMS TWO\DOROTHY GCMS TWO\DOROTHY3.D
Operator : dorothy
Acquired : 27 Nov 2011 14:33 using AcqMethod DOROTHY
Instrument : Instrumen
Sample Name: LSS 180-188 34-48B
Misc Info :
Vial Number: 1



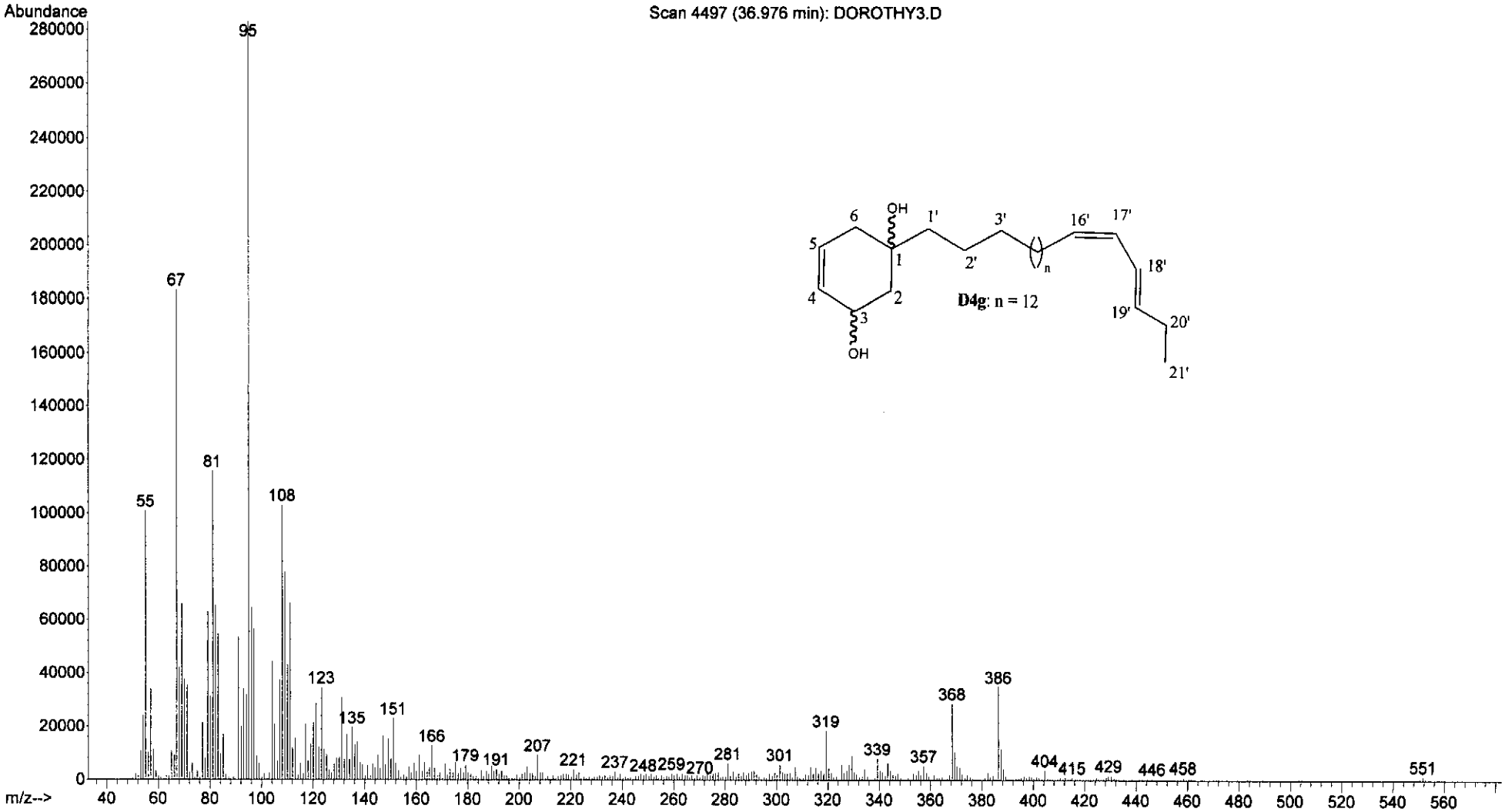
Mass spectrum of C4c

File : C:\MSDCHEM\1\DATA\DOROTHY GCMS TWO\DOROTHY GCMS TWO\DOROTHY3.D
Operator : dorothy
Acquired : 27 Nov 2011 14:33 using AcqMethod DOROTHY
Instrument : Instrumen
Sample Name: LSS 180-188 34-48B
Misc Info :
Vial Number: 1

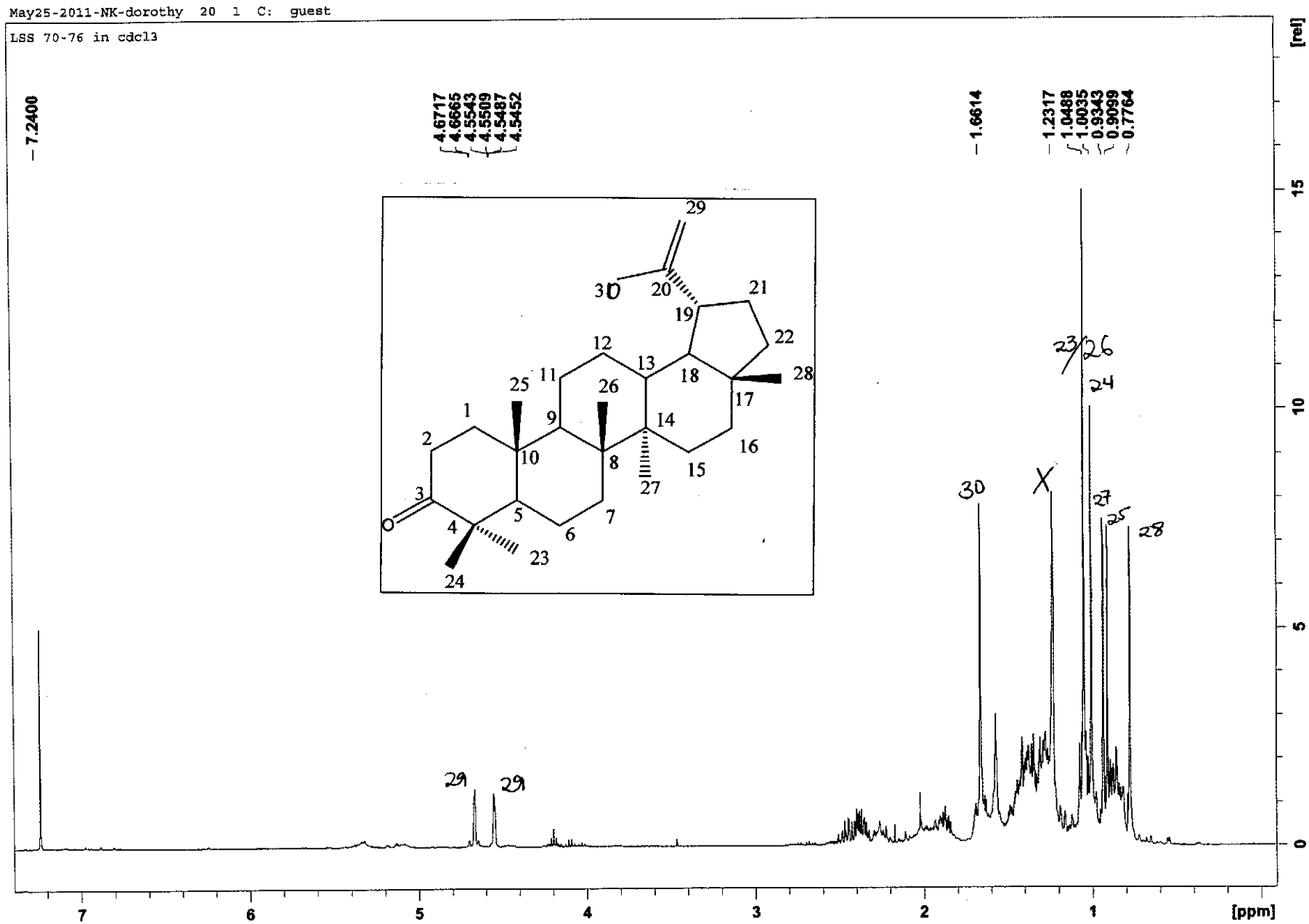


Mass spectrum of D4f

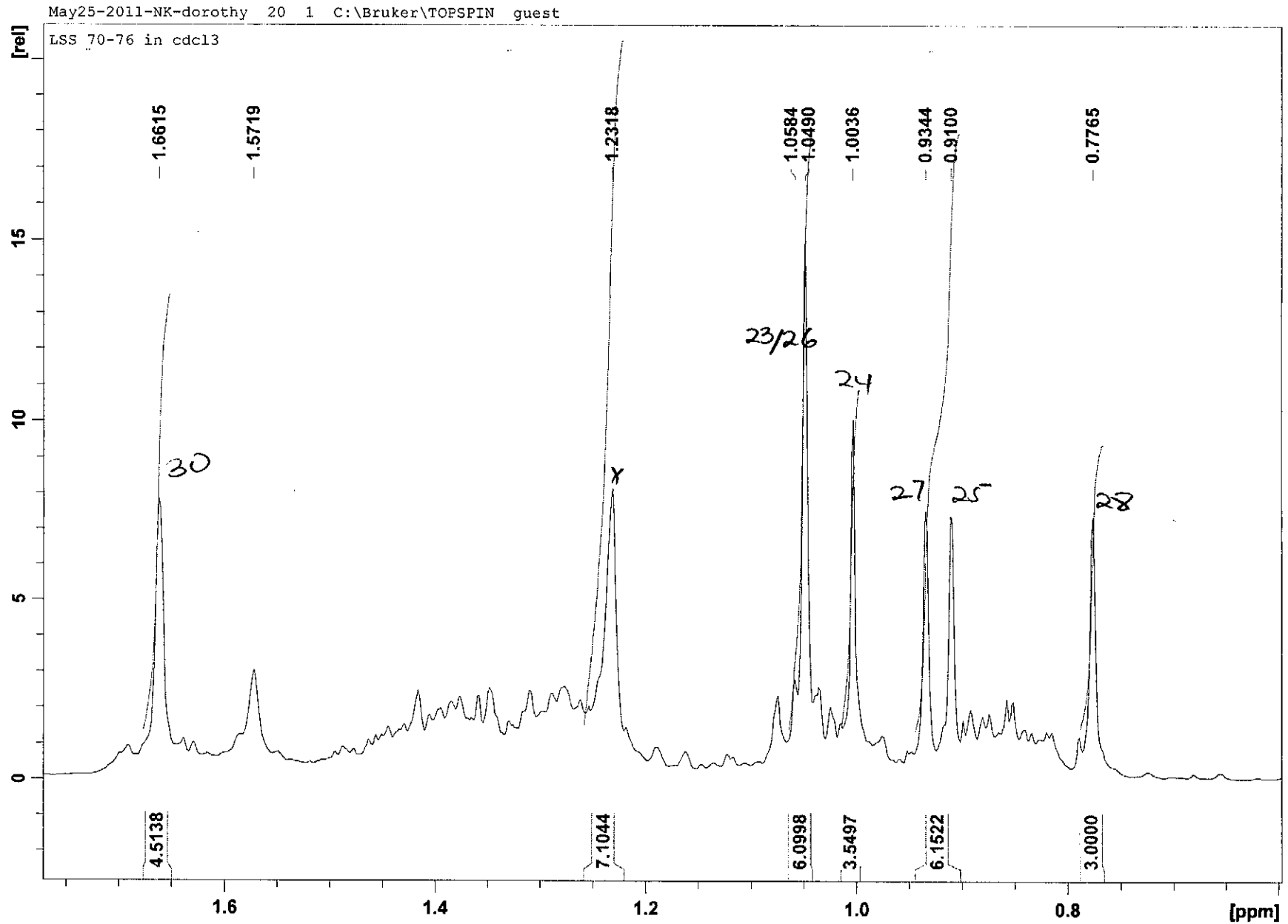
File : C:\MSDCHEM\1\DATA\DOROTHY GCMS TWO\DOROTHY GCMS TWO\DOROTHY3.D
Operator : dorothy
Acquired : 27 Nov 2011 14:33 using AcqMethod DOROTHY
Instrument : Instrumen
Sample Name: LSS 180-188 34-48B
Misc Info :
Vial Number: 1



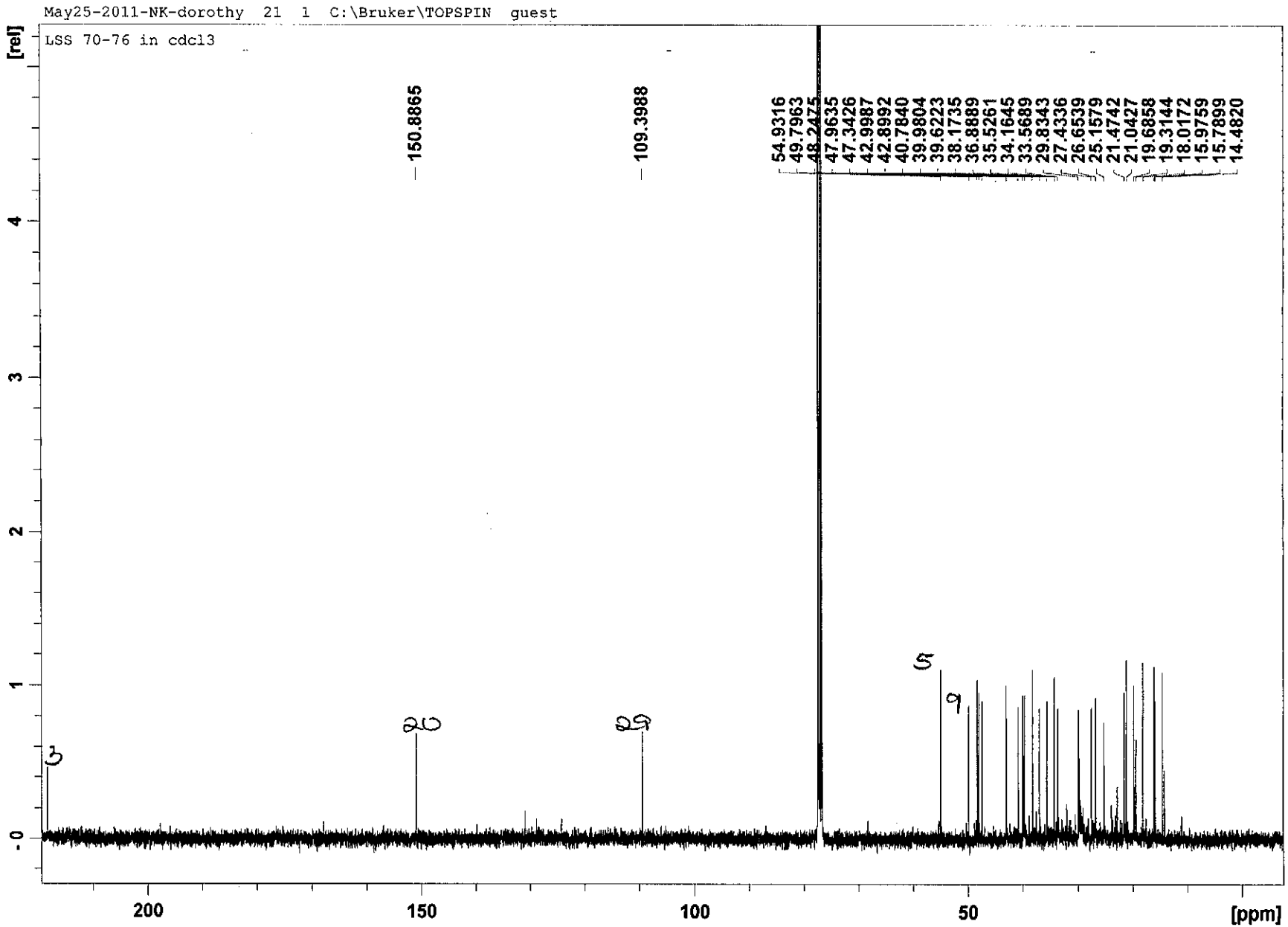
Mass spectrum of D4g



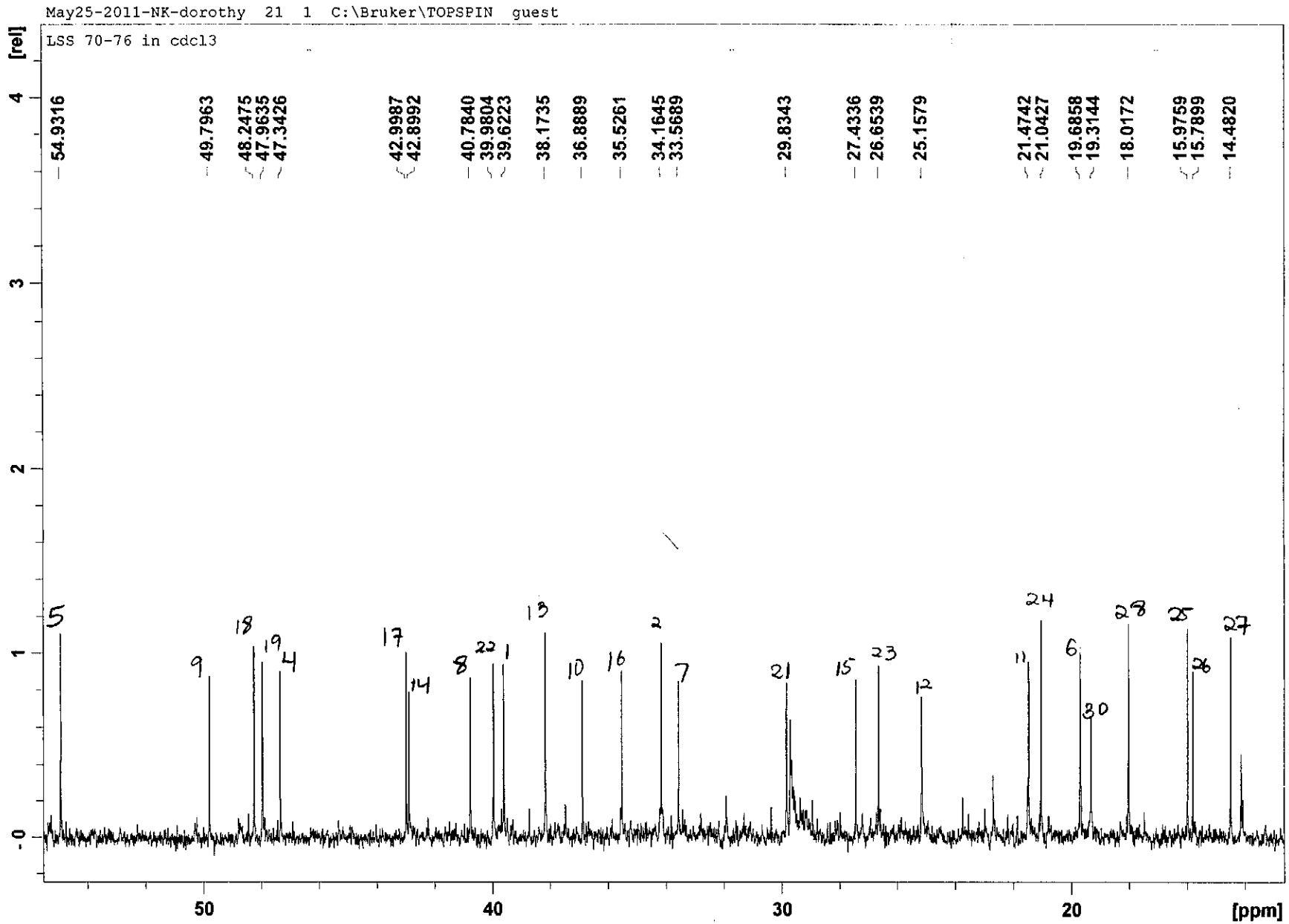
^{1}H NMR spectrum of D5



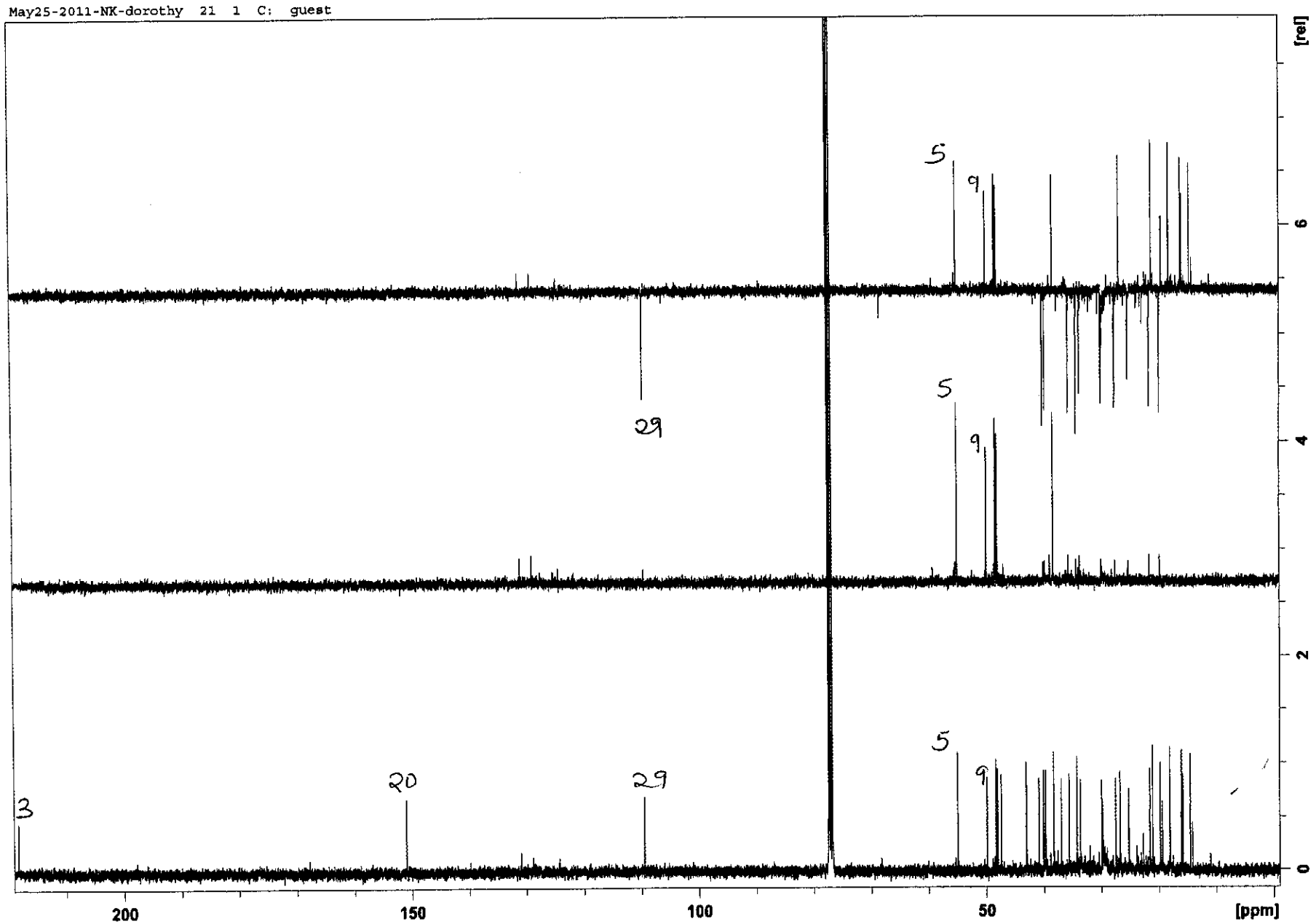
^{1}H NMR spectrum of D5 (expanded 0-1.7 ppm)



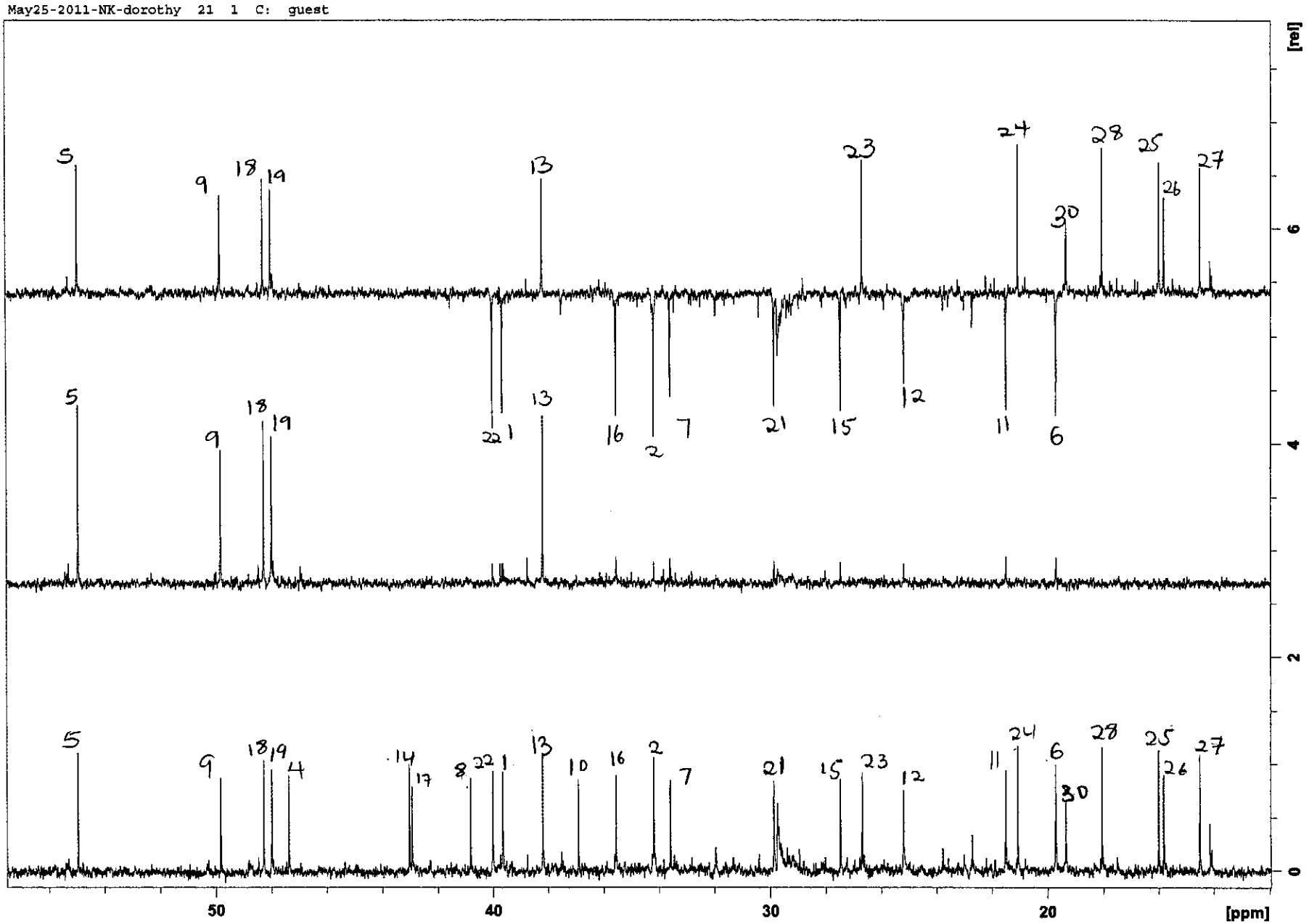
^{13}C NMR spectrum of D5



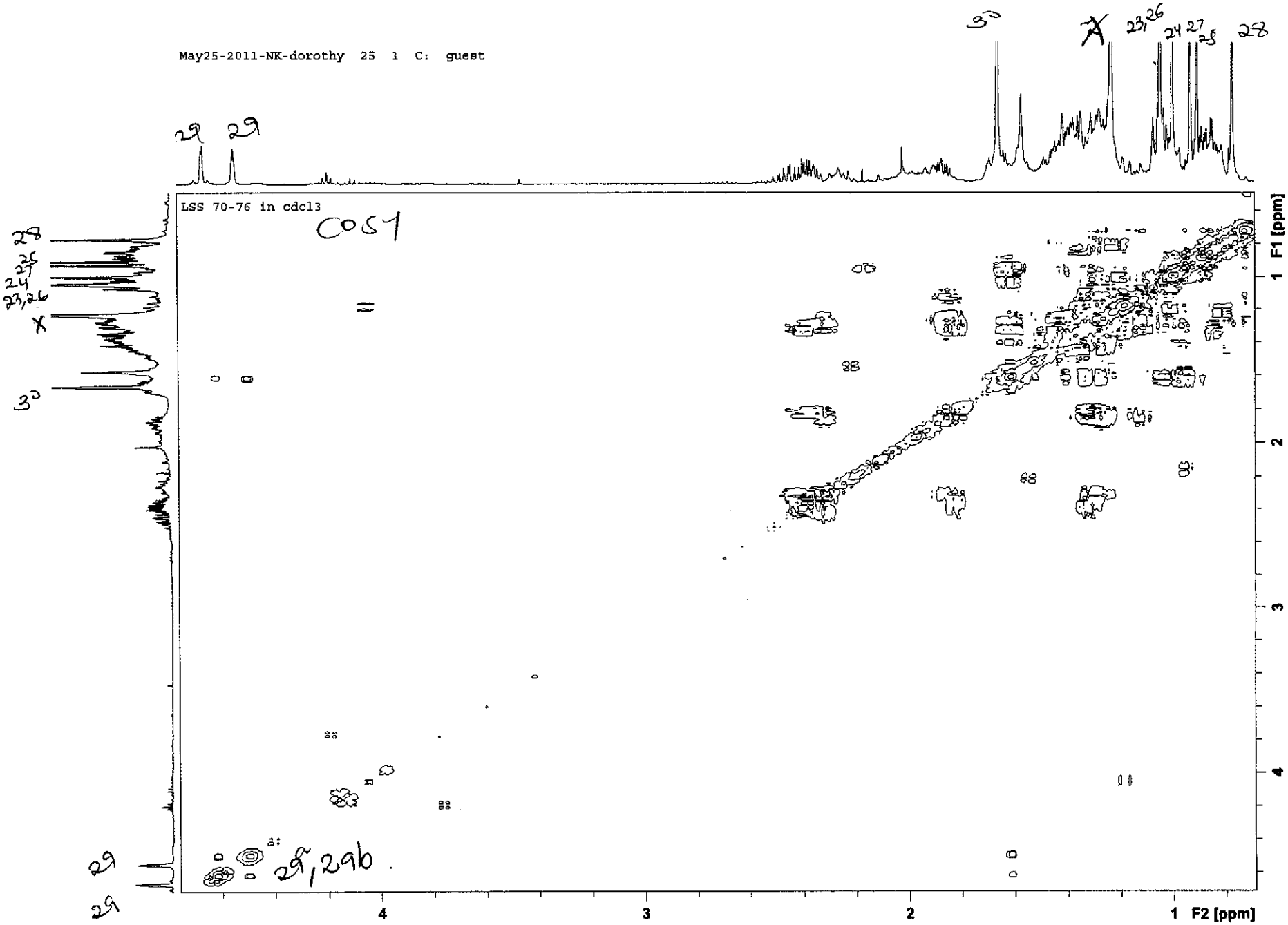
^{13}C NMR spectrum of D5 (expanded 0-55 ppm)



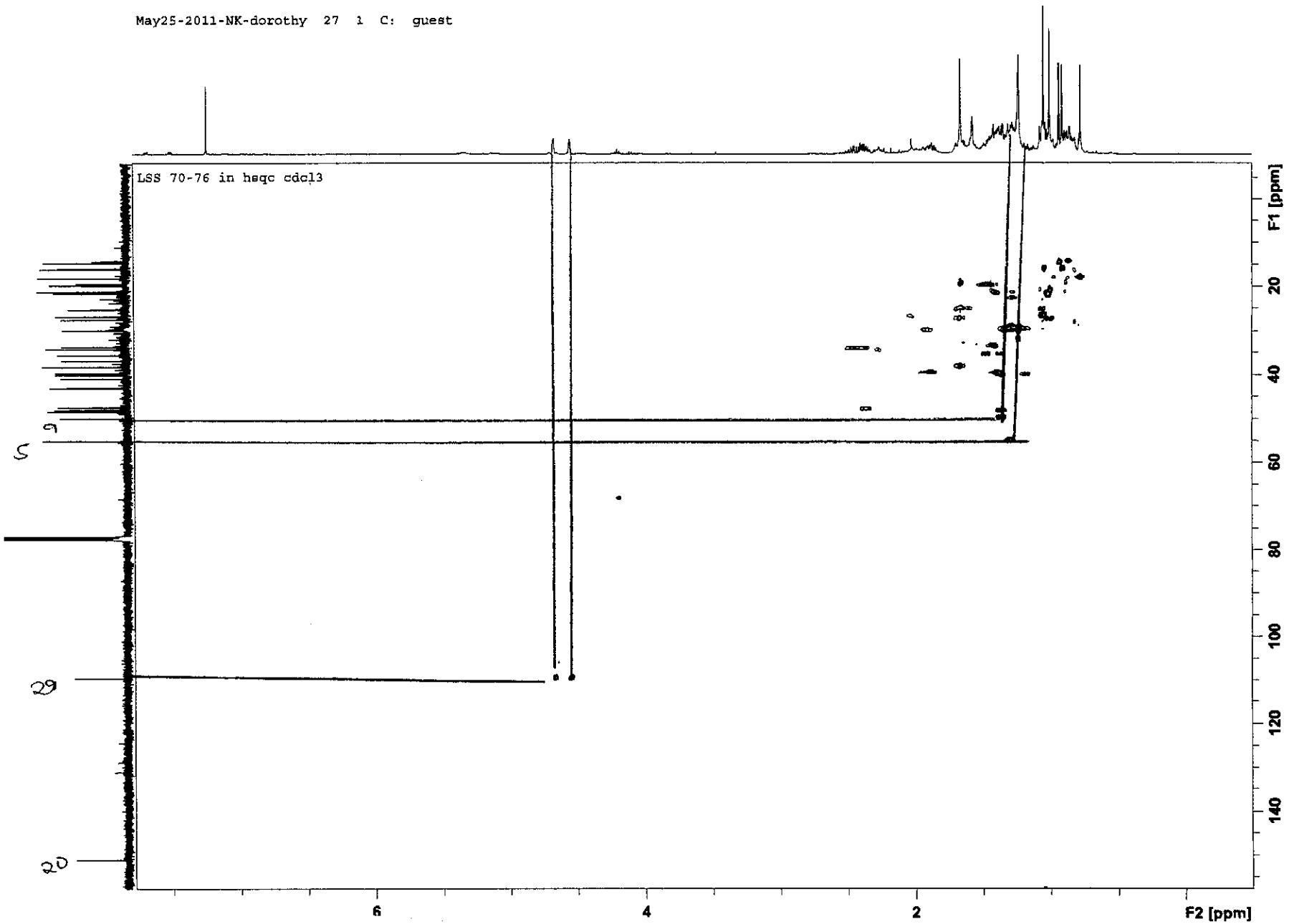
DEPT spectrum of D5



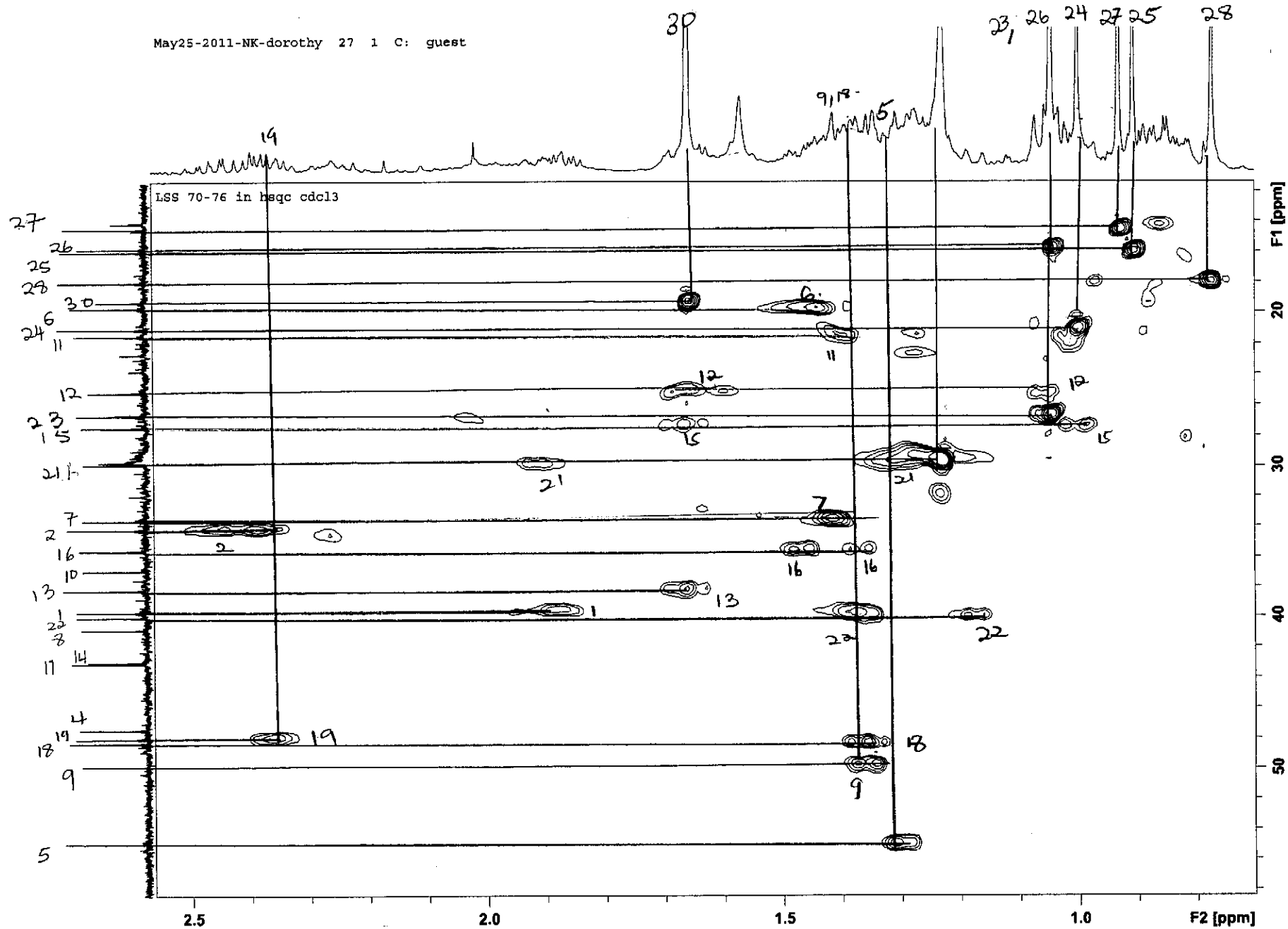
DEPT spectrum of D5 (expanded 0-55 ppm)



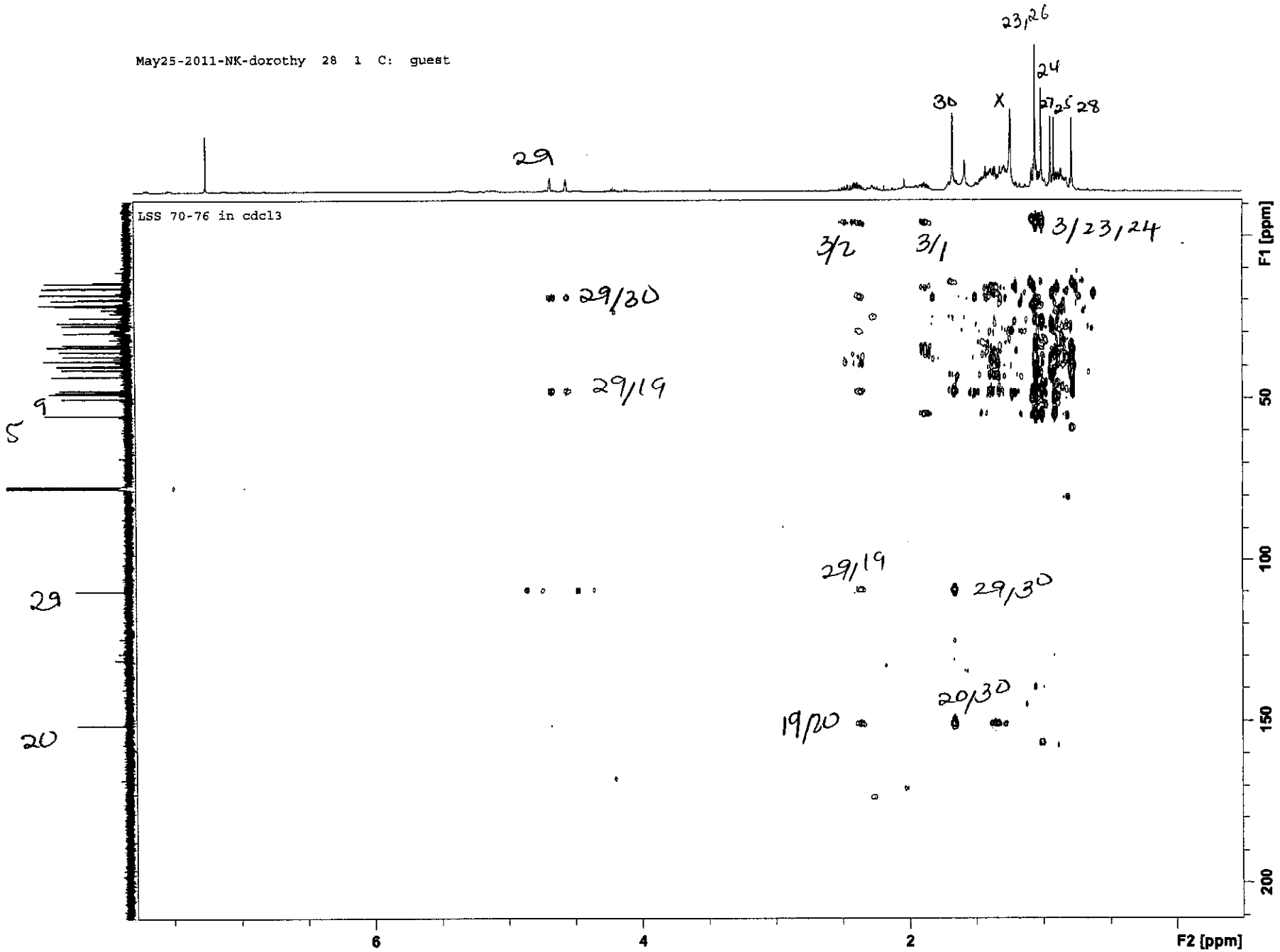
COSY spectrum of D5



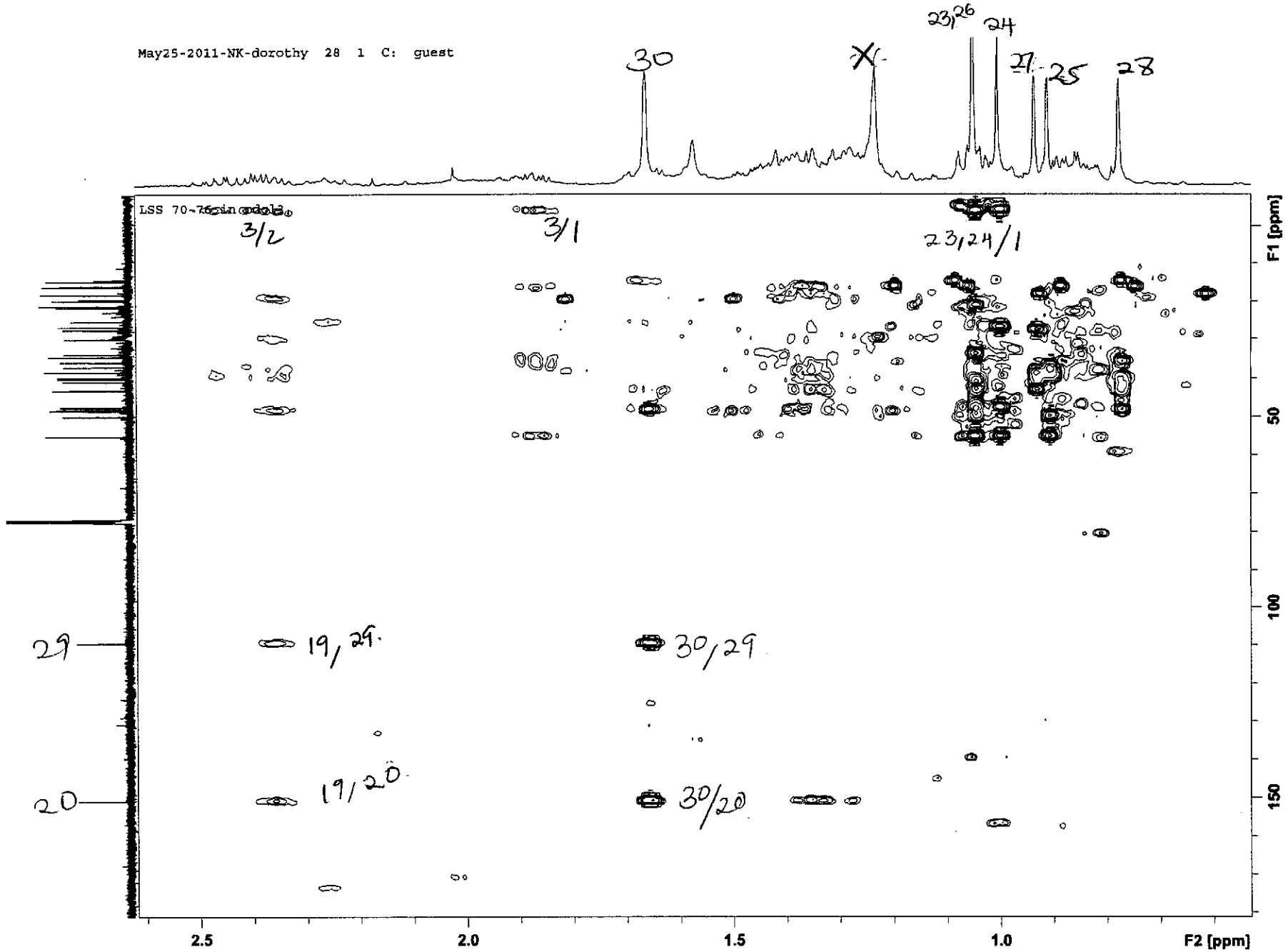
HSQC spectrum of D5



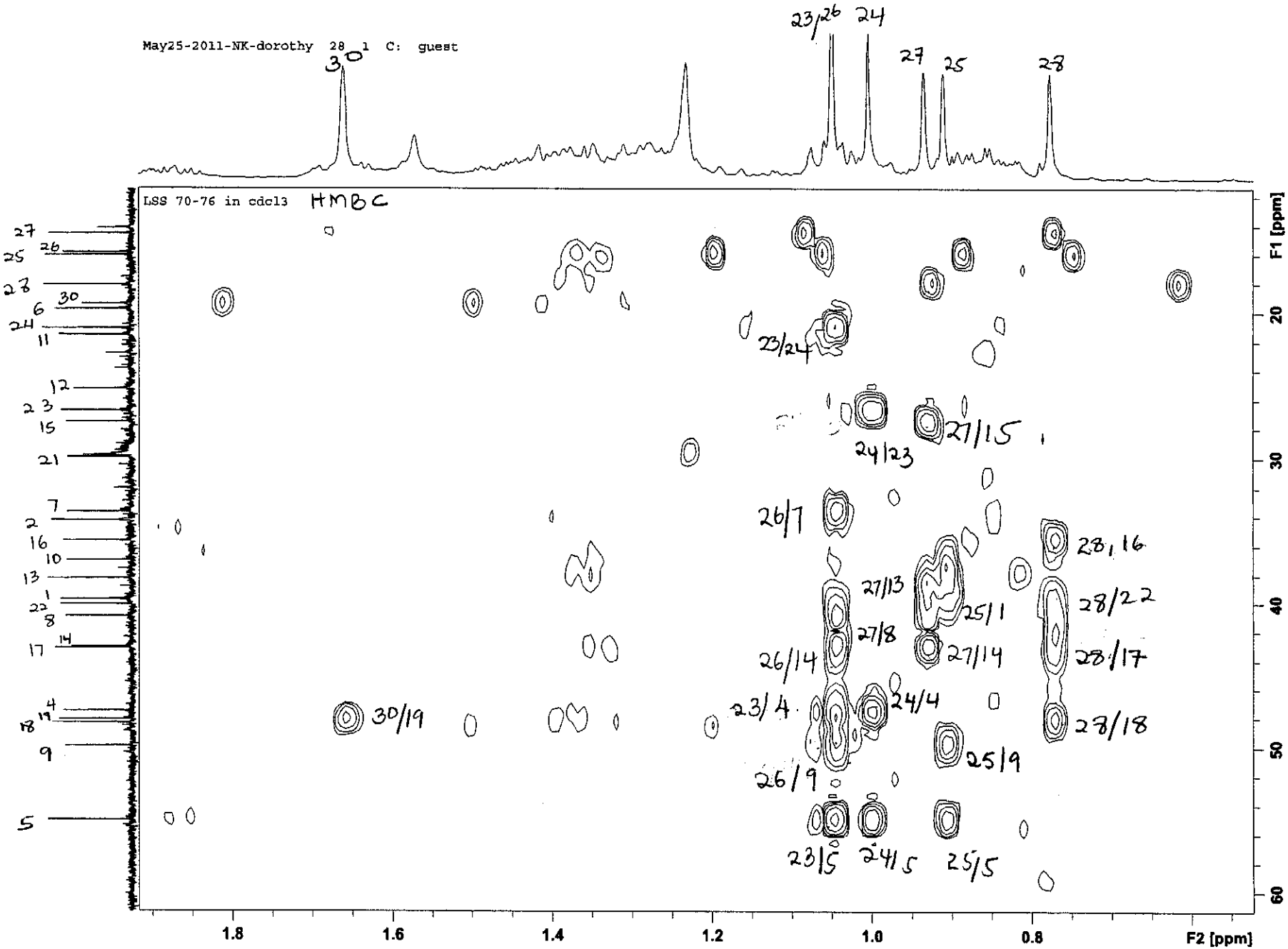
HSQC spectrum of D5 (expanded F1 0-2.5 ppm)



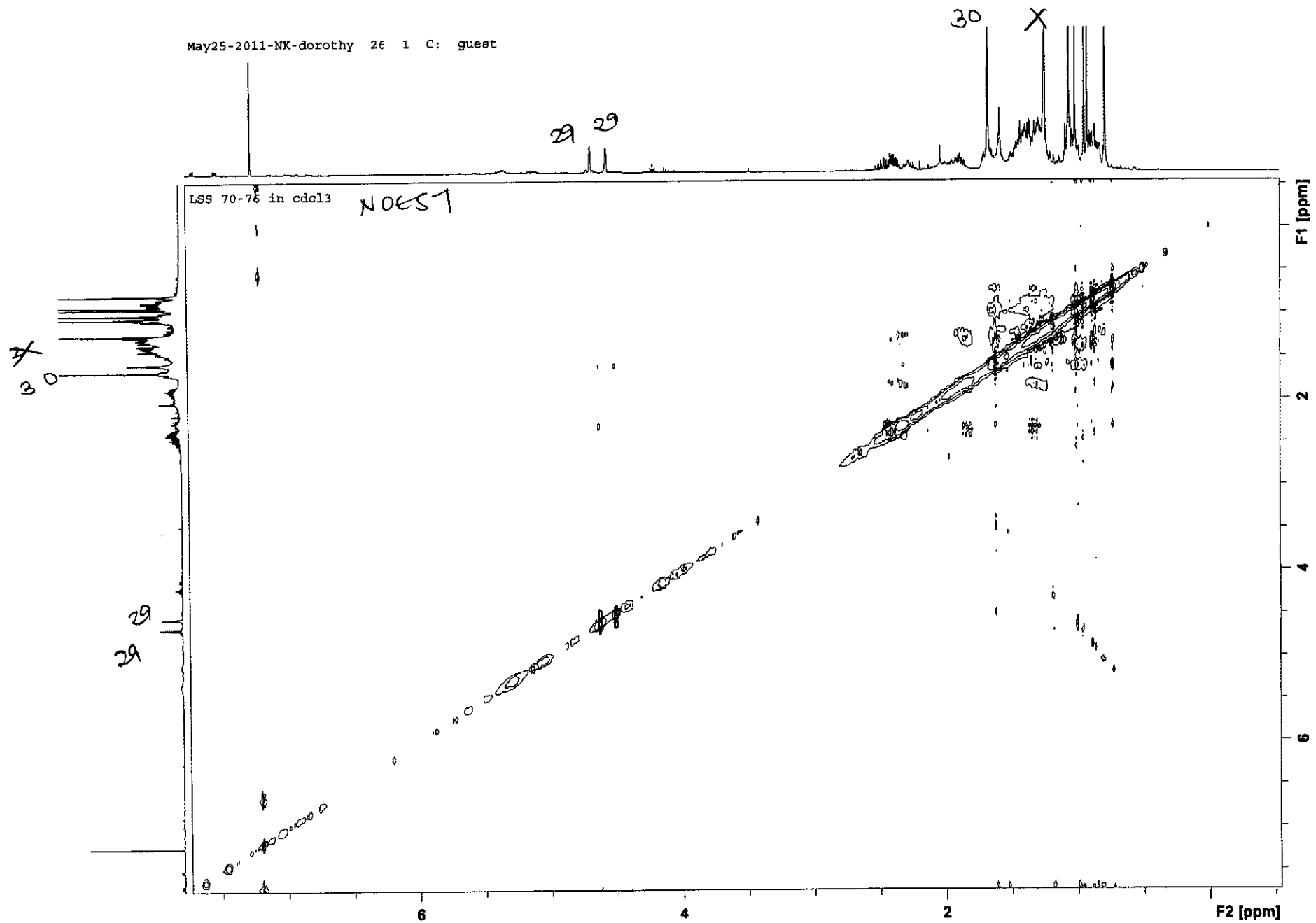
HMBC spectrum of D5



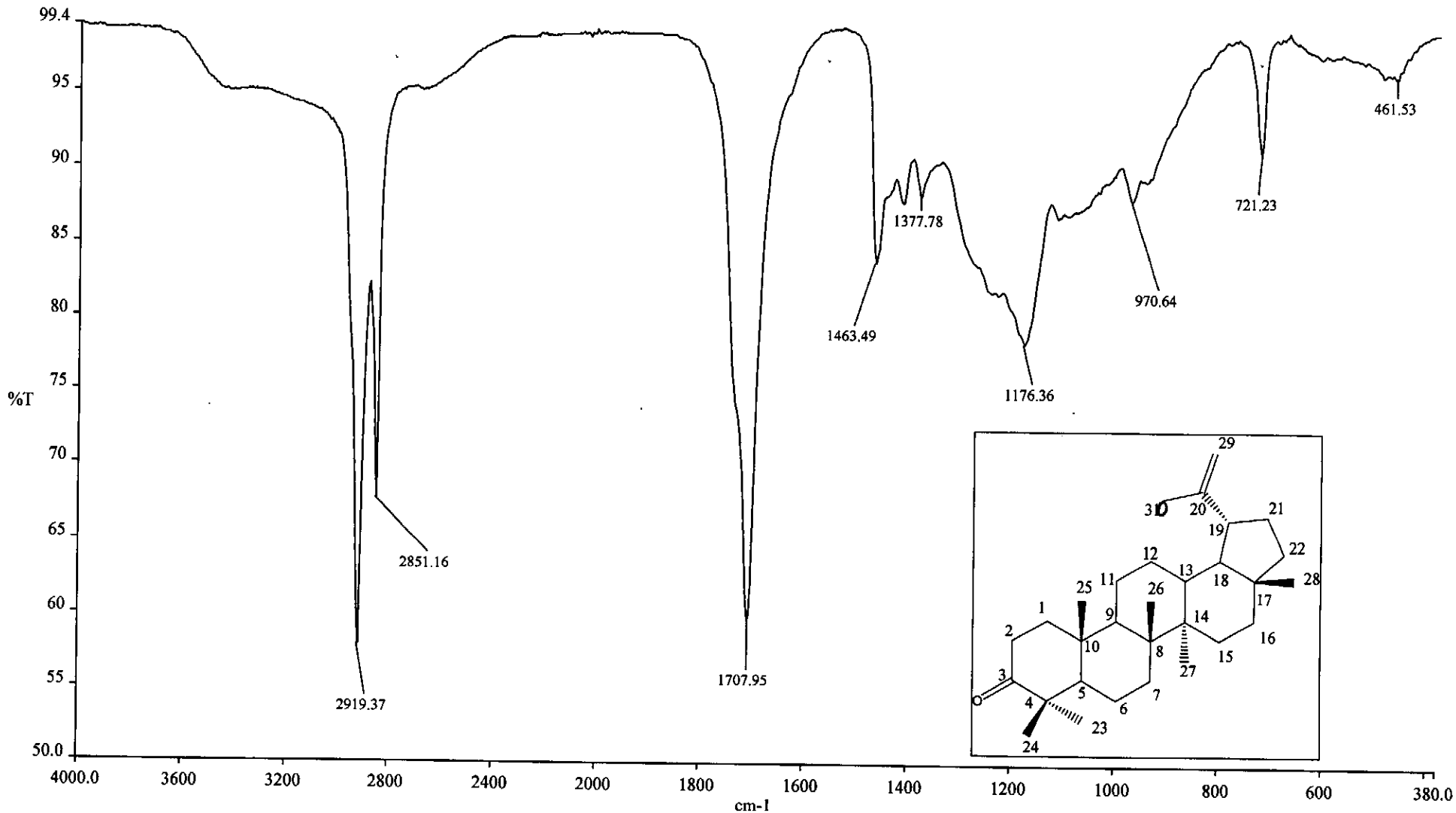
HMBC spectrum of D5 (expanded F1 0-2.5 ppm)



HMBC spectrum of D5 (expanded 0-1.8 ppm)



NOESY spectrum of D5

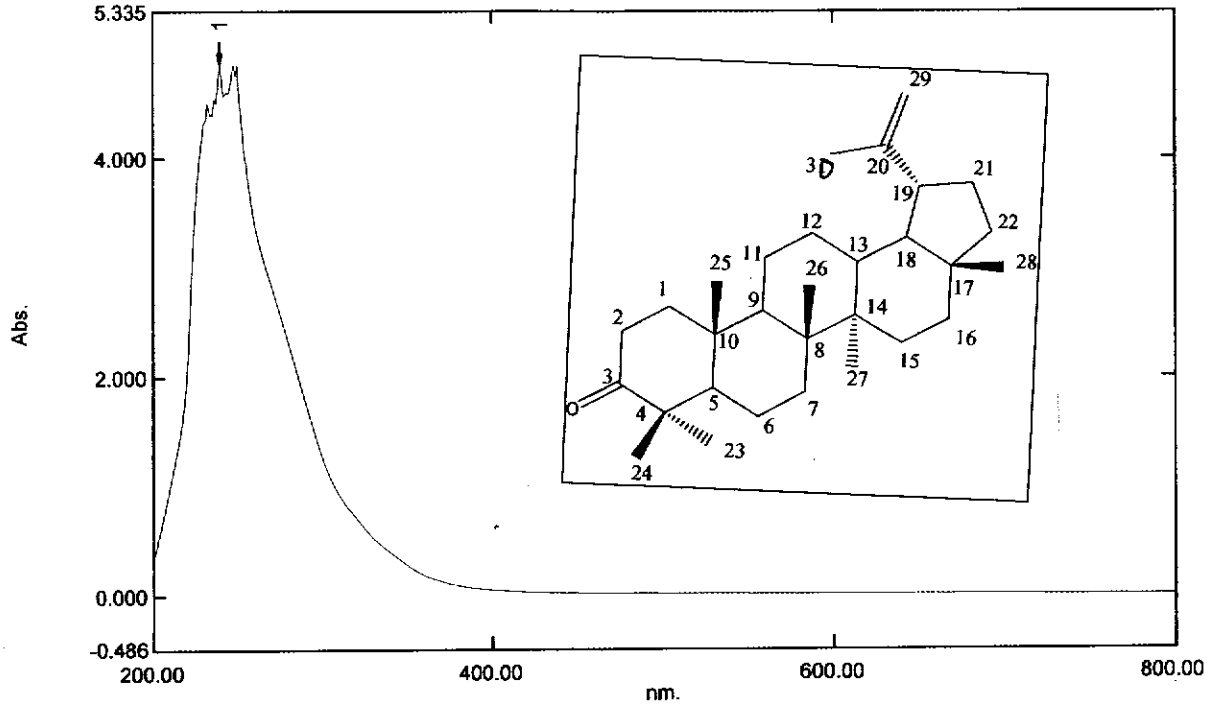


IR spectrum of D5

Spectrum Peak Pick Report

04/10/2011 12:10:28 PM

Data Set: LSS 70-77.spc - Storage 130711



No.	P/V	Wavelength	Abs.	Description
1	●	239.00	4.850	

Measurement Properties
Wavelength Range (nm.): 200.00 to 800.00
Scan Speed: Medium
Sampling Interval: 1.0
Auto Sampling Interval: Disabled
Scan Mode: Single

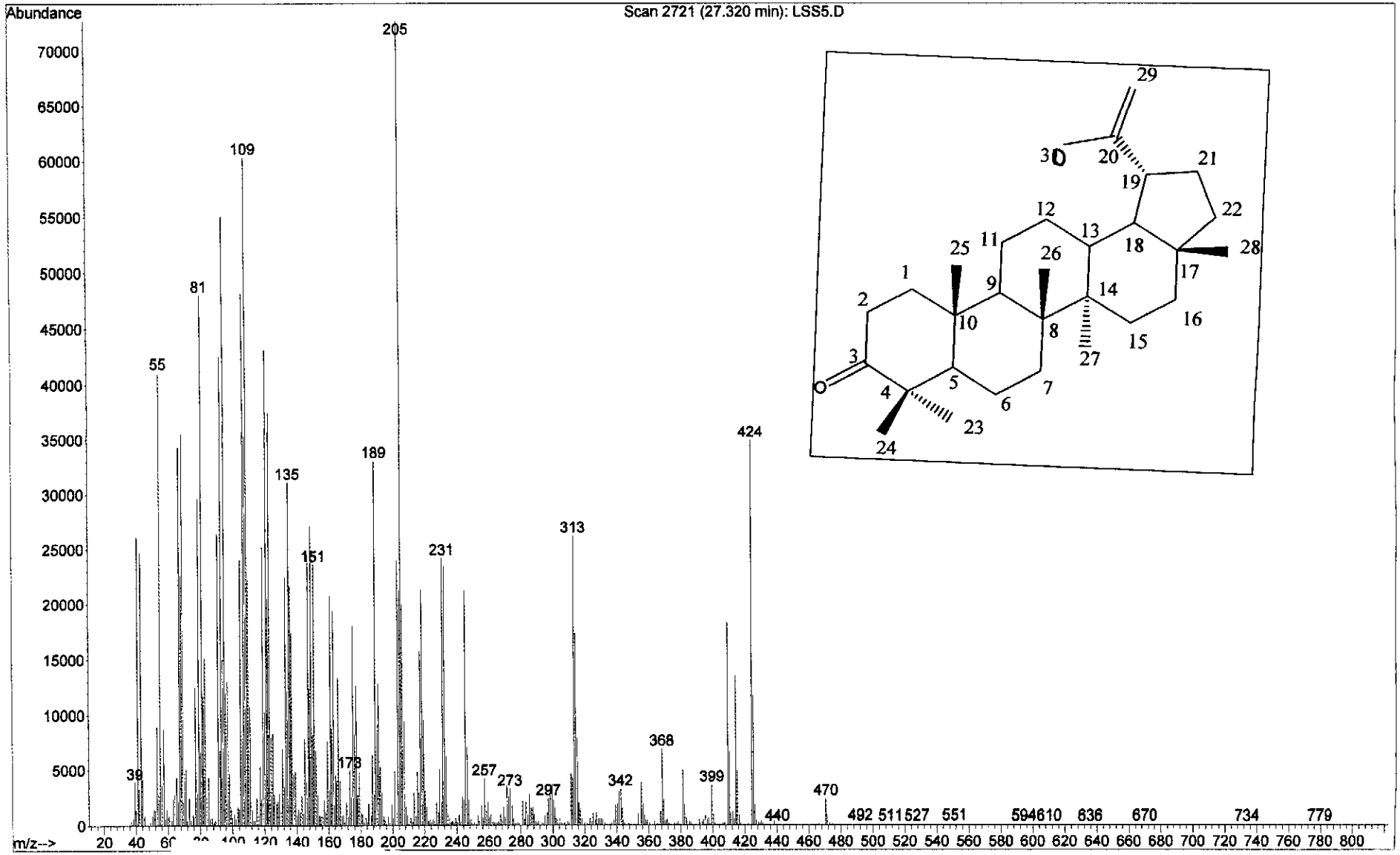
Instrument Properties
Instrument Type: UV-3600 Series
Measuring Mode: Absorbance
Slit Width: 8.0 nm
Time Constant: 0.1 sec.
Source Lamp: Auto
Light Source Change Wavelength: 310.00 nm
Detector Unit: Direct
Detector Change Wavelength: 830.00 nm 1800.00 nm
Grating Change Wavelength: 720.00 nm
S/R Exchange: Normal
Detector Lock: Auto
Slit Program: Normal
Beam Mode: Double
Stair Correction: Disable

Attachment Properties
Attachment: None

Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

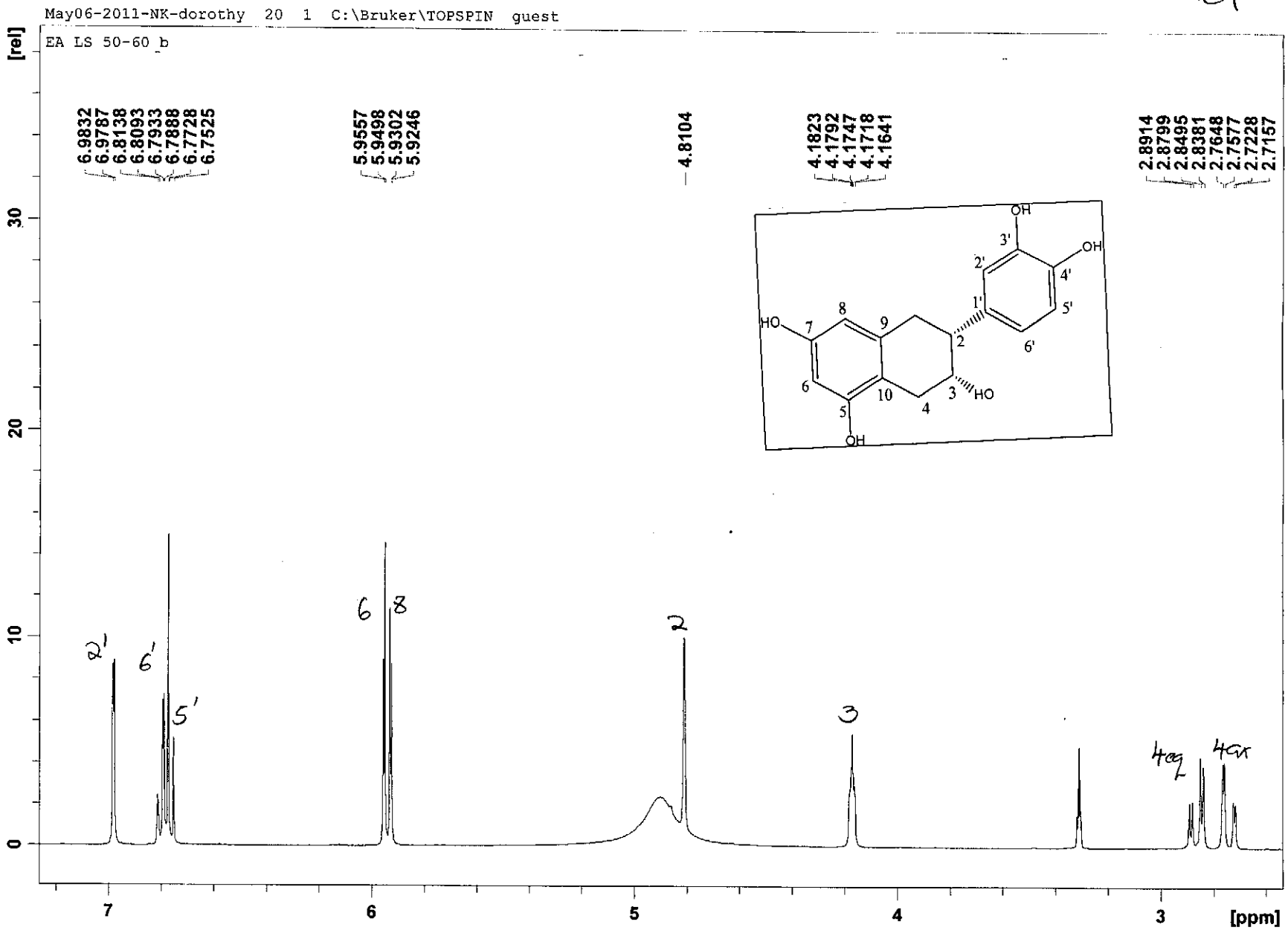
UV spectrum of D5

File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\DORTHY\LSS5.D
Operator : DOROTHY
Acquired : 19 Jun 2011 14:20 using AcqMethod NATURAL
Instrument : Instrumen
Sample Name: EA ls 55-60 B
Misc Info :
Vial Number: 1

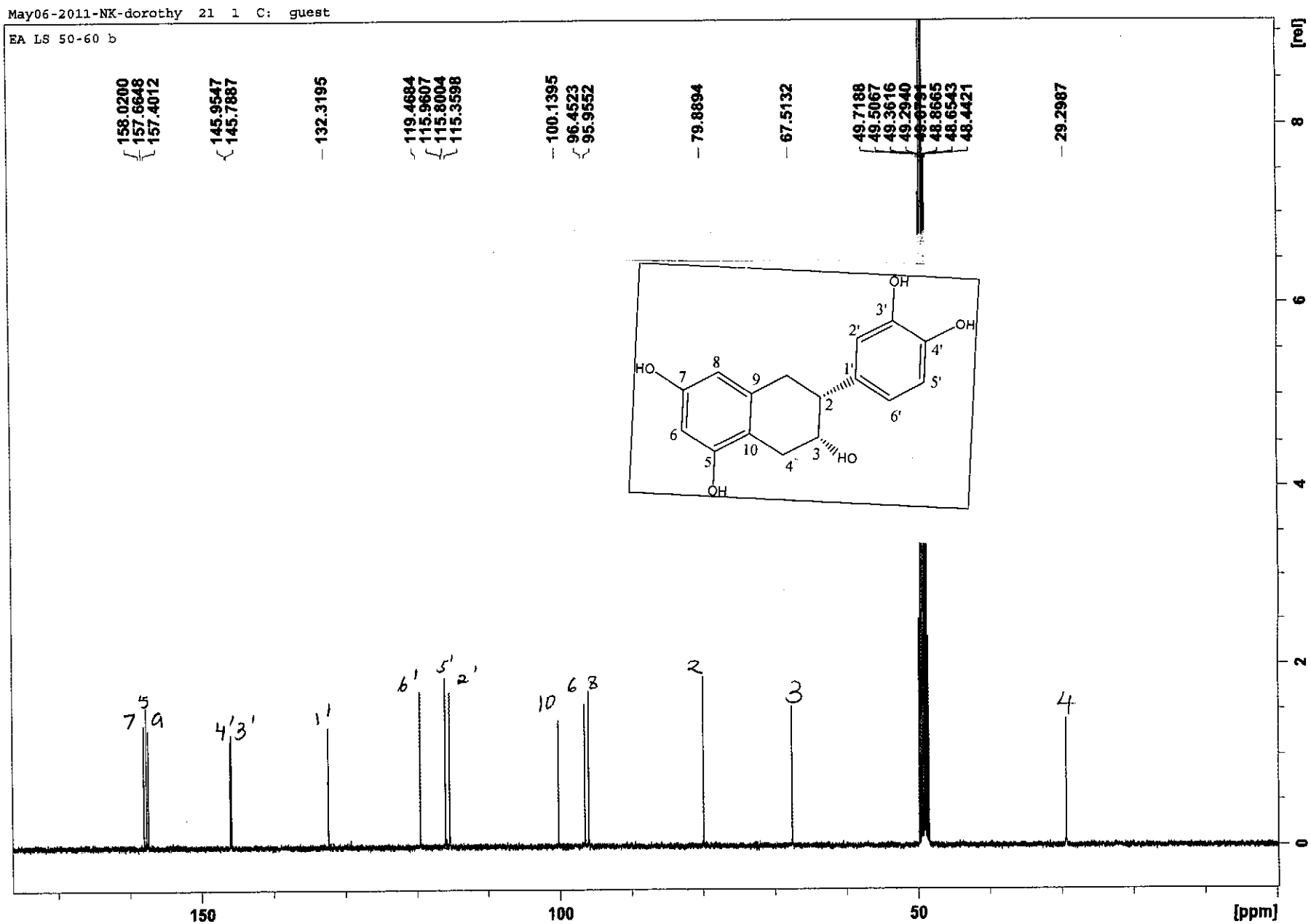


MS spectrum of D5

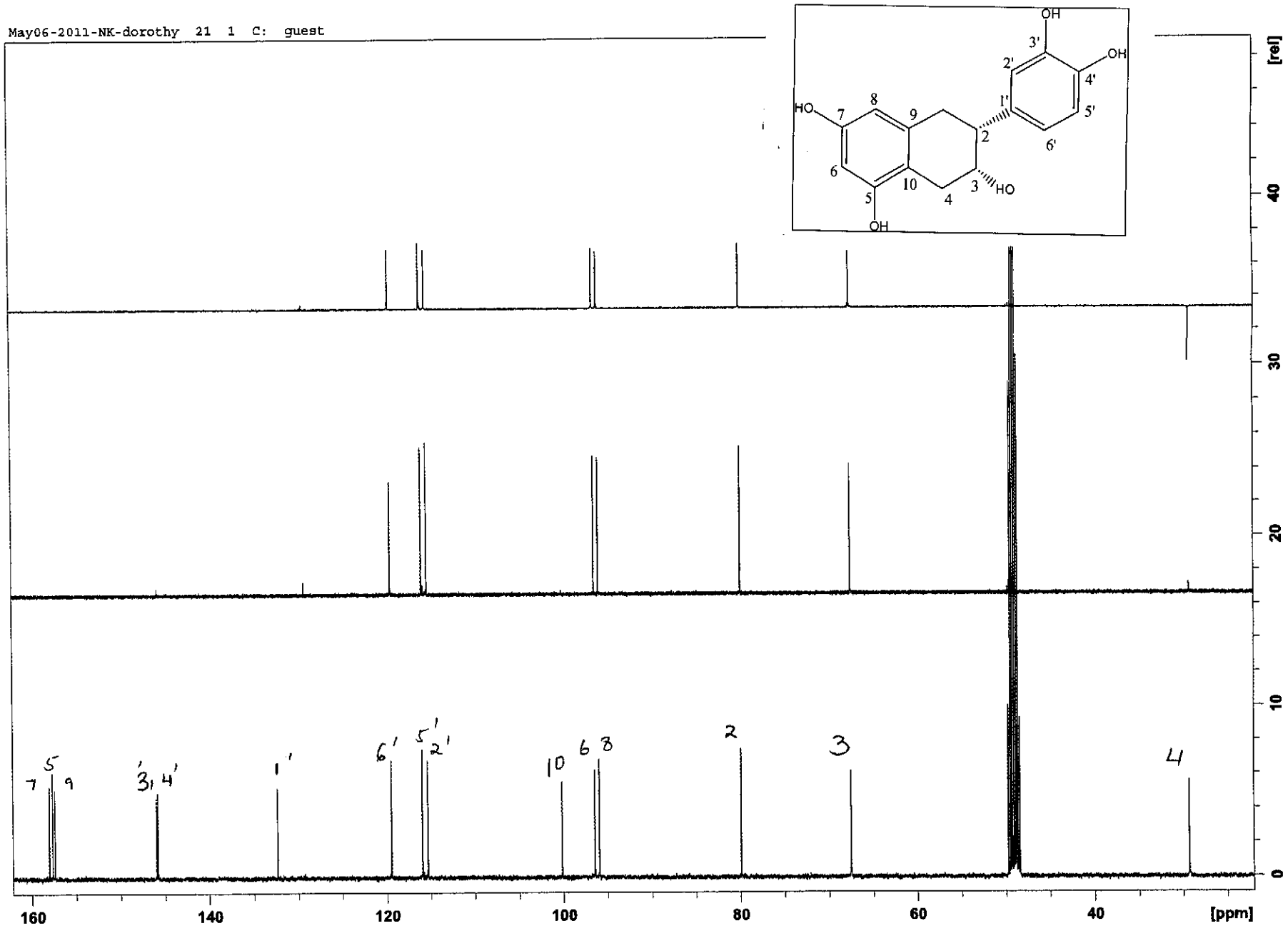
epicatechin



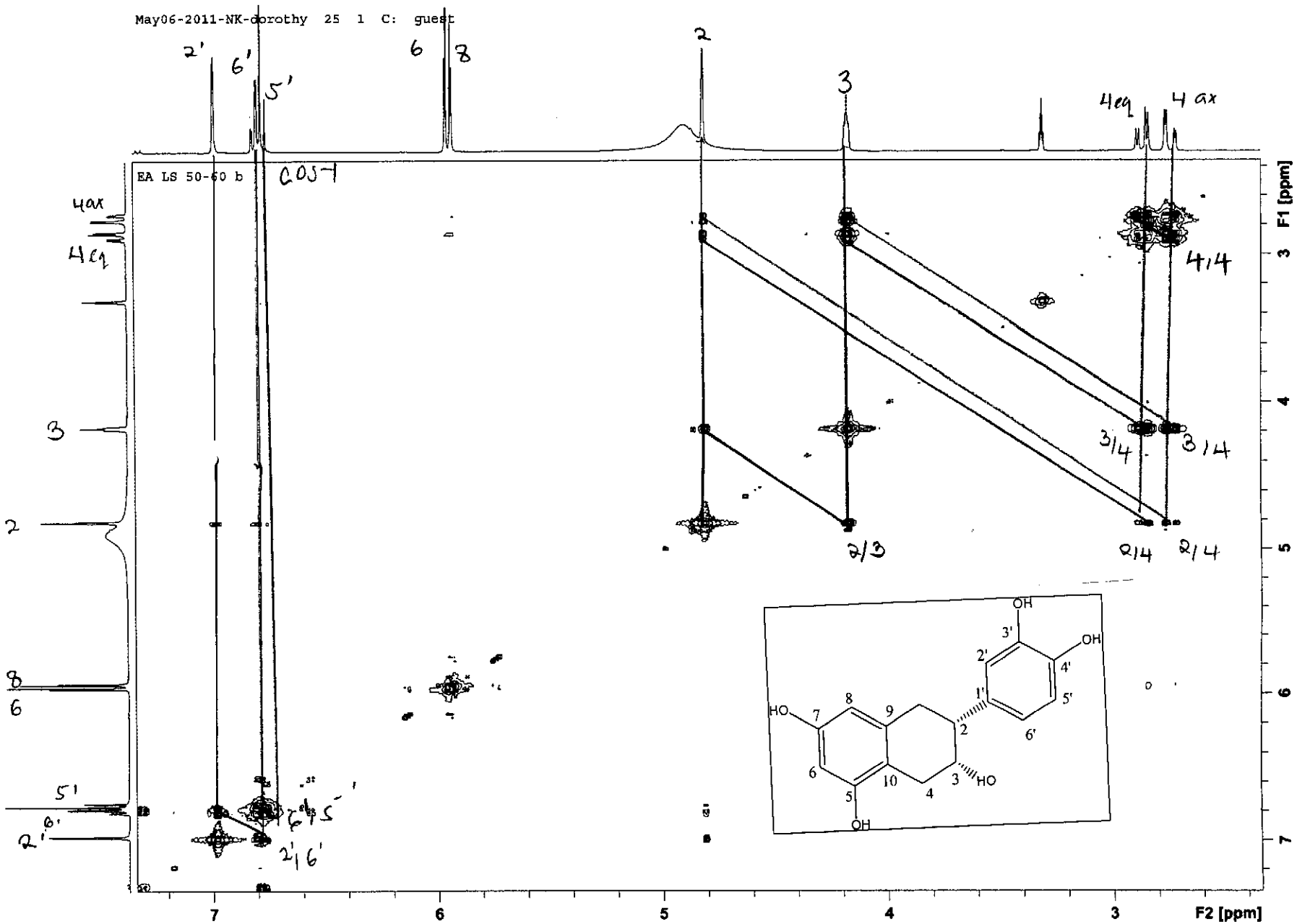
^{1}H NMR spectrum of D6



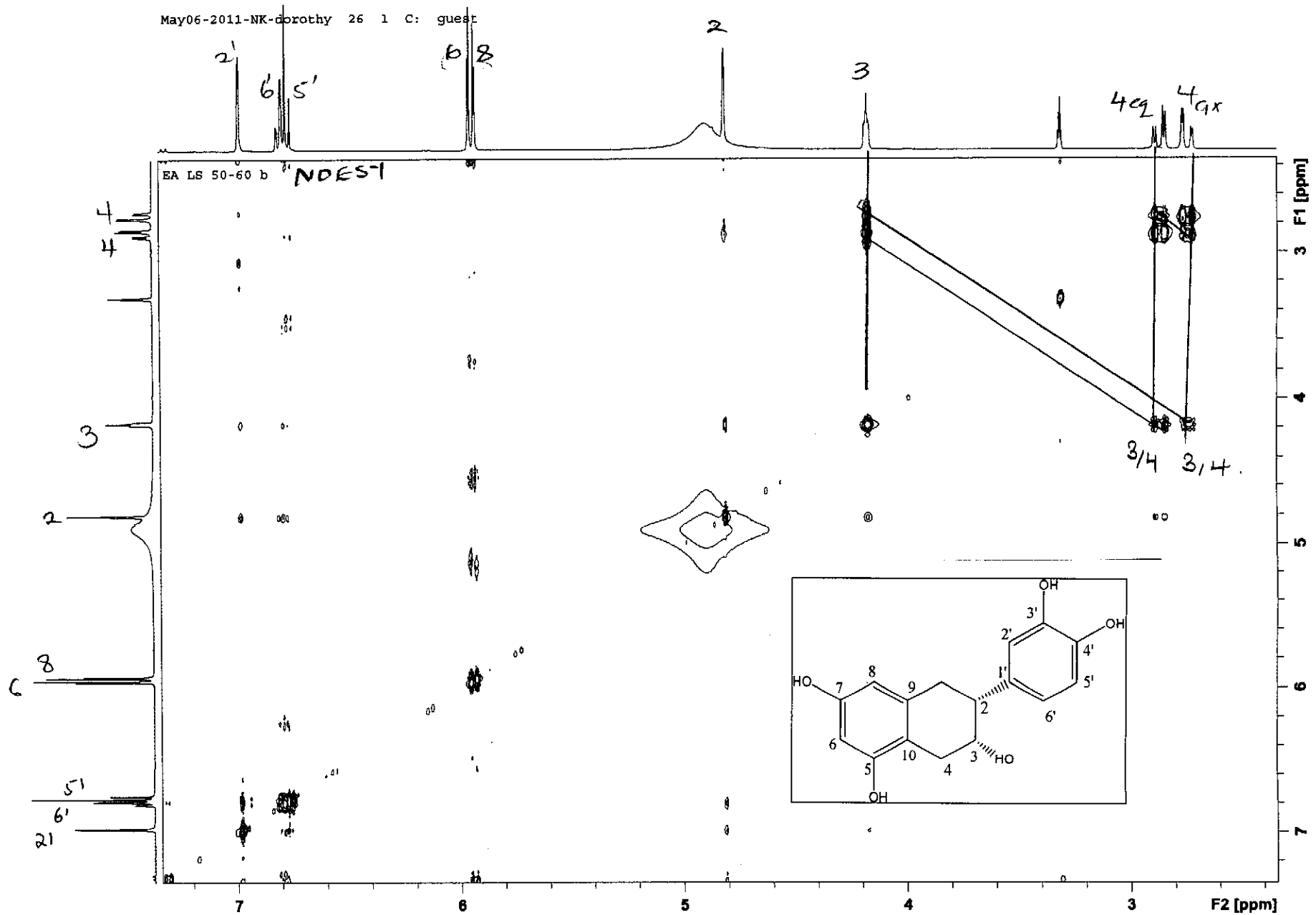
^{13}C NMR spectrum of D6



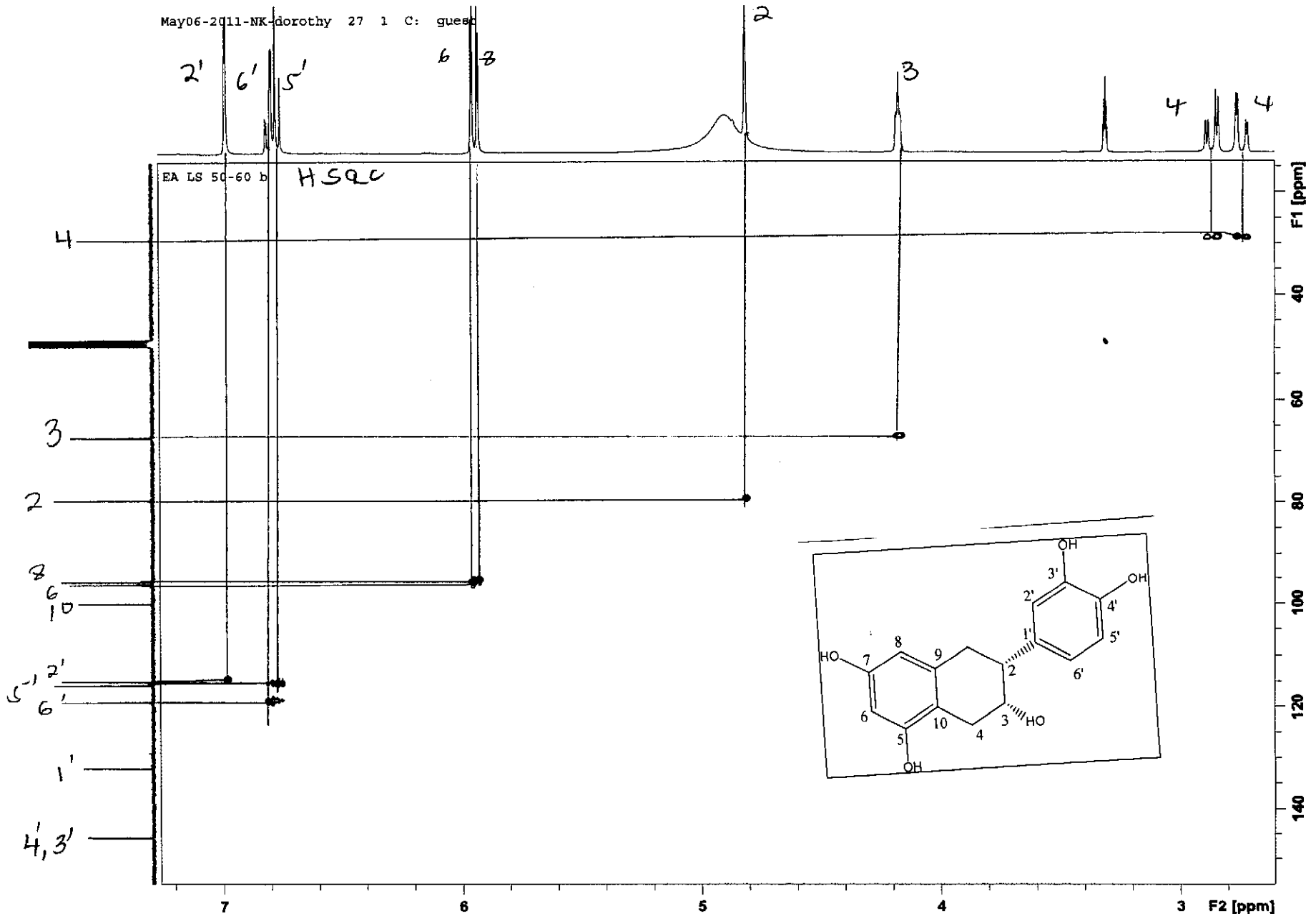
DEPT spectrum of D6



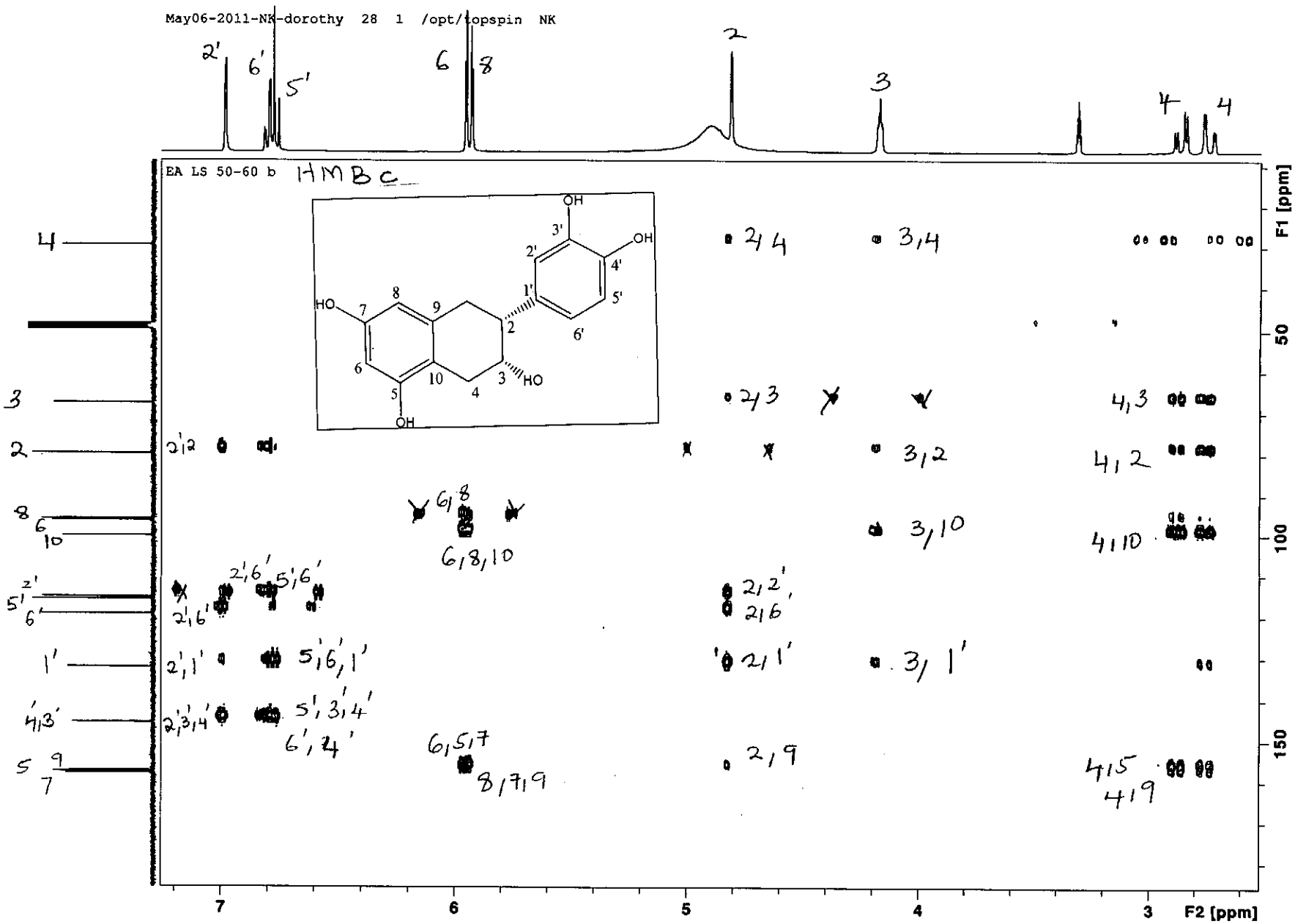
COSY spectrum of D6



NOESY spectrum of D6



HSQC spectrum of D6

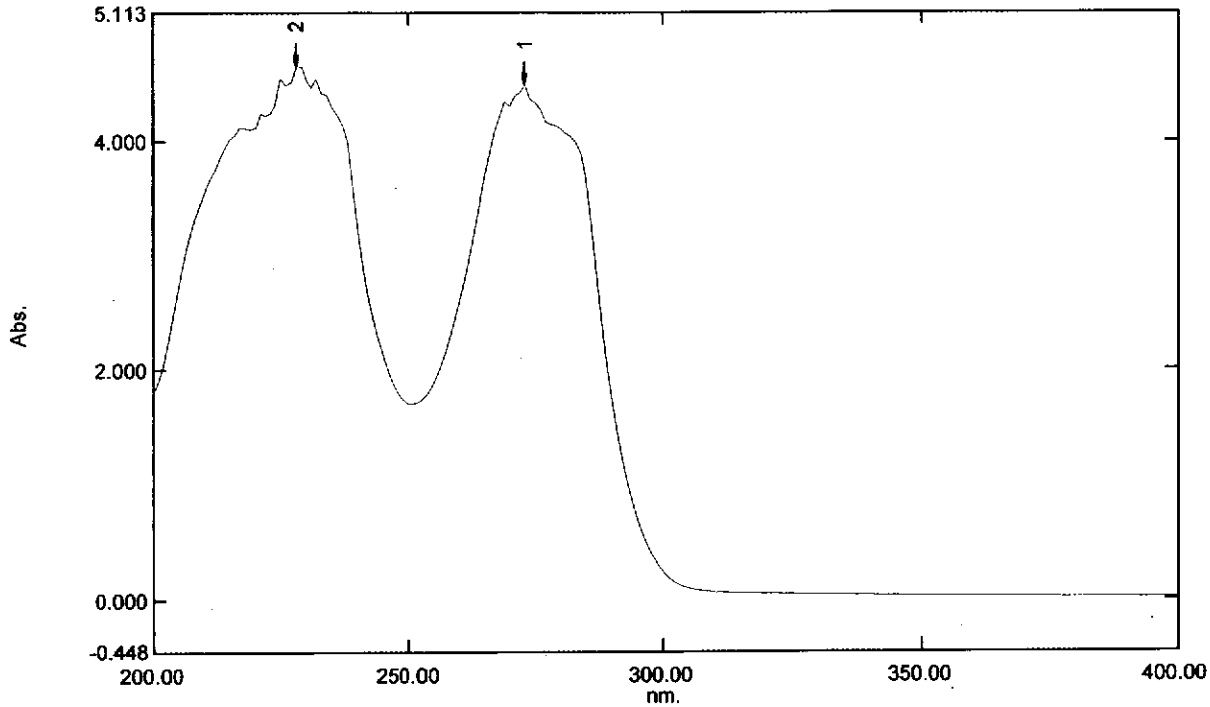


HMBC spectrum of D6

Spectrum Peak Pick Report

04/12/2011 08:00:08 PM

Data Set: EALSRT 50-60 BC.spc - Storage 172030



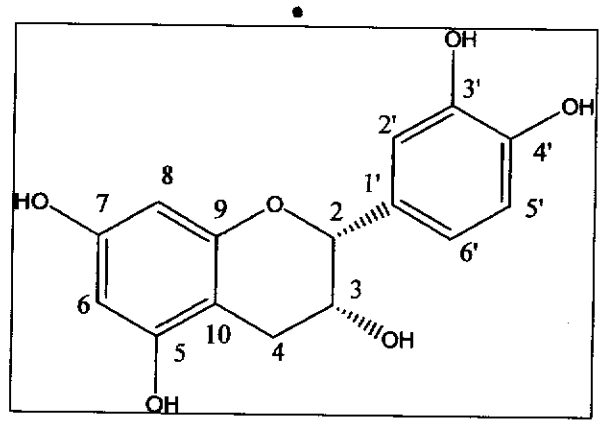
No.	P/V	Wavelength	Abs.	Description
1	●	273.00	4.485	
2	●	228.00	4.650	
3	●	251.00	1.705	

Measurement Properties
Wavelength Range (nm.): 200.00 to 400.00
Scan Speed: Medium
Sampling Interval: 1.0
Auto Sampling Interval: Disabled
Scan Mode: Single

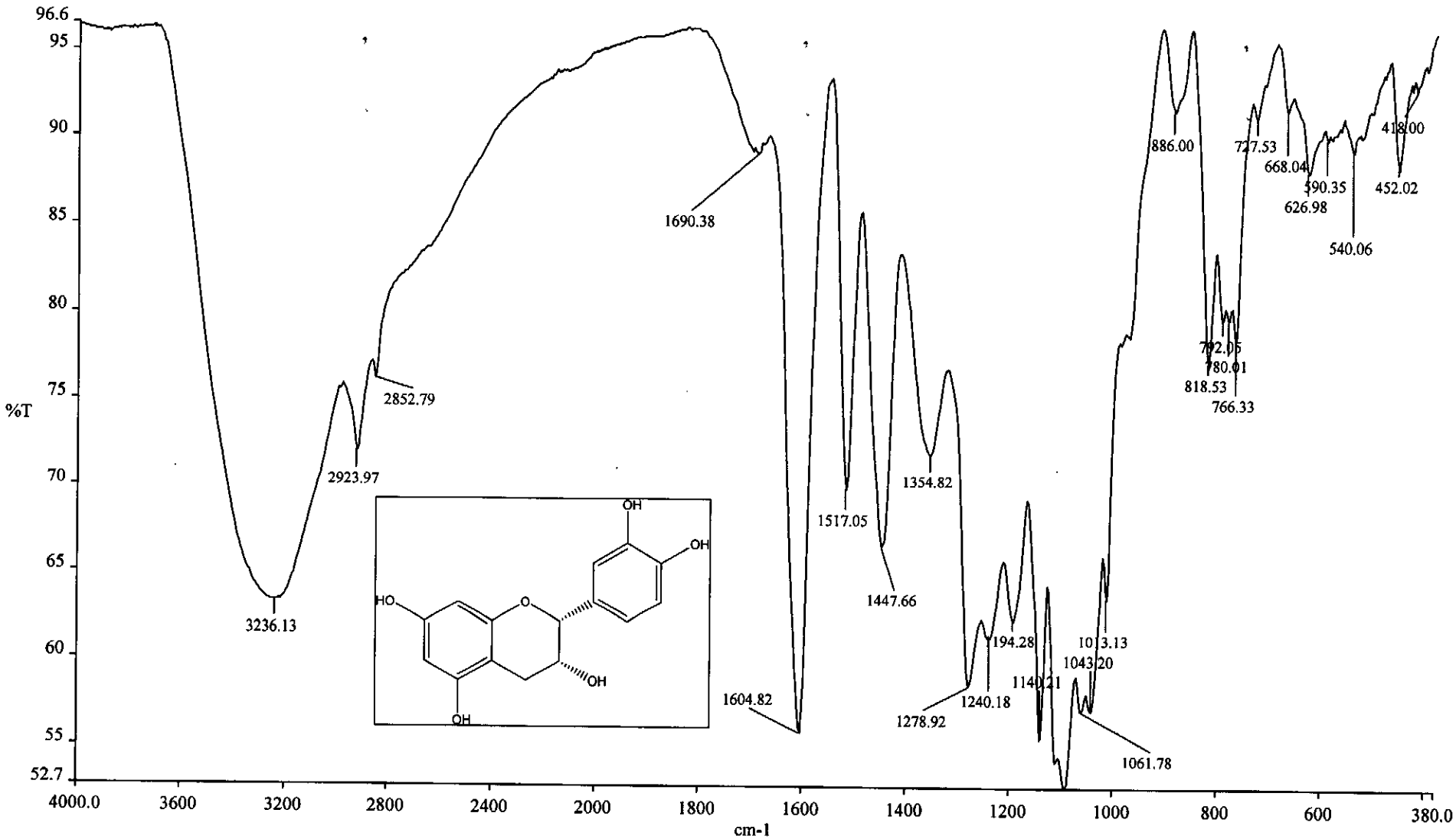
Instrument Properties
Instrument Type: UV-3600 Series
Measuring Mode: Absorbance
Slit Width: 8.0 nm
Time Constant: 0.1 sec.
Source Lamp: Auto
Light Source Change Wavelength: 310.00 nm
Detector Unit: Direct
Detector Change Wavelength: 830.00 nm 1800.00 nm
Grating Change Wavelength: 720.00 nm
S/R Exchange: Normal
Detector Lock: Auto
Slit Program: Normal
Beam Mode: Double
Stair Correction: Disable

Attachment Properties
Attachment: None

Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

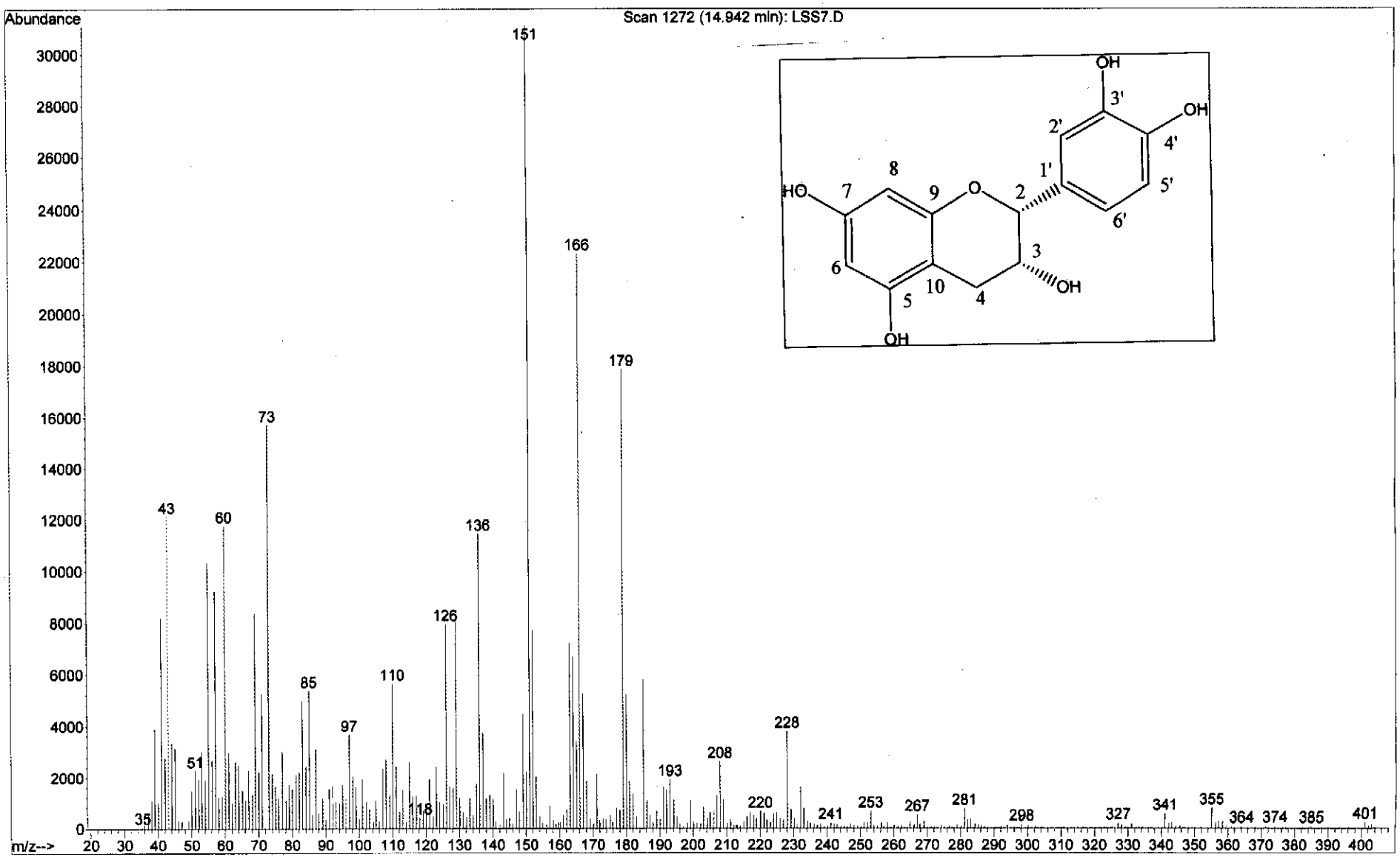


UV spectrum of D6



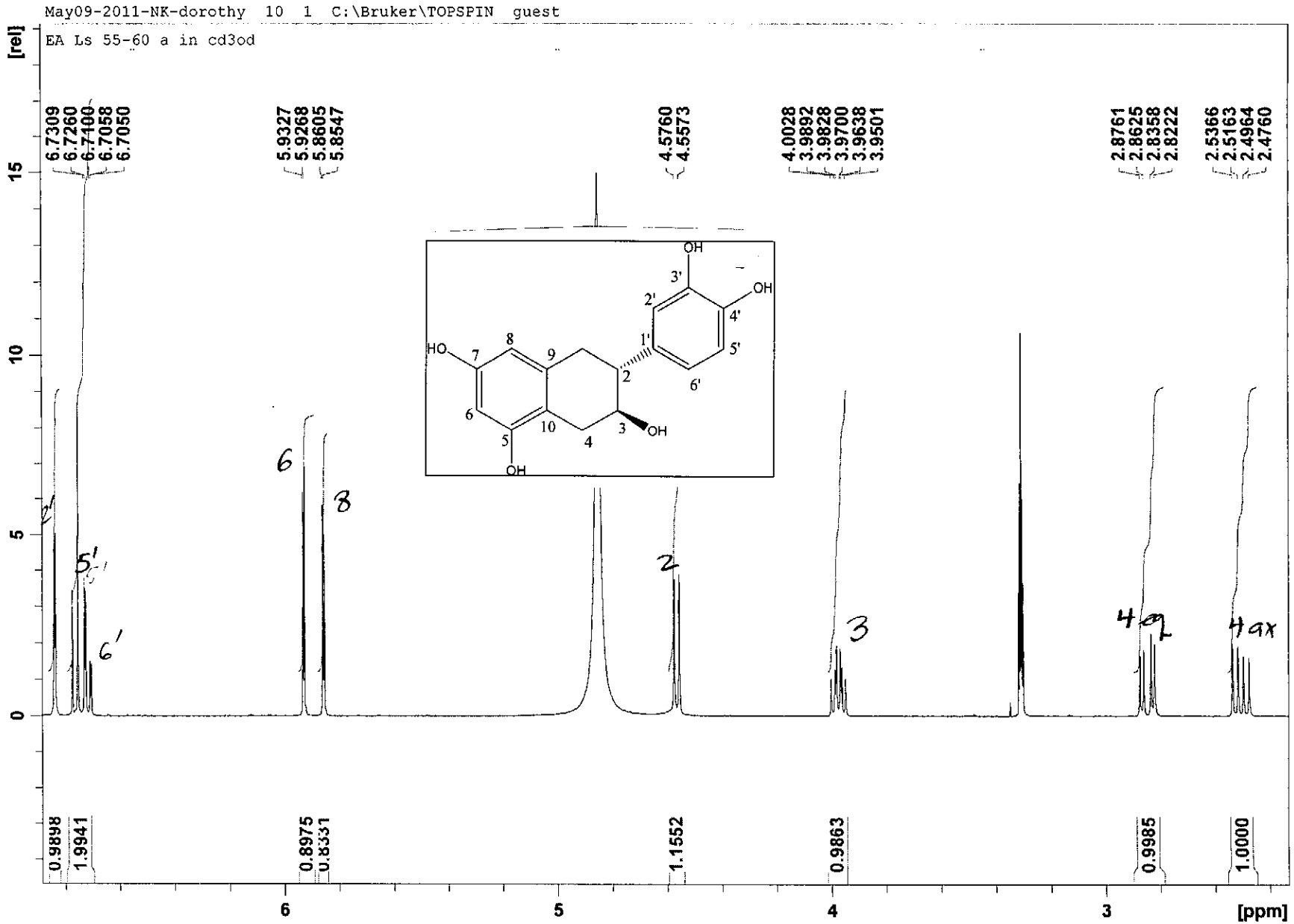
IR spectrum of D6

File : C:\MSDCHEM\1\DATA\DOROTHY GC MS PROCESSED\DORTHY\LSS7.D
Operator : DOROTHY
Acquired : 19 Jun 2011 18:40 using AcqMethod NATURAL
Instrument : Instrumen
Sample Name: EA LS 57-76
Misc Info :
Vial Number: 1

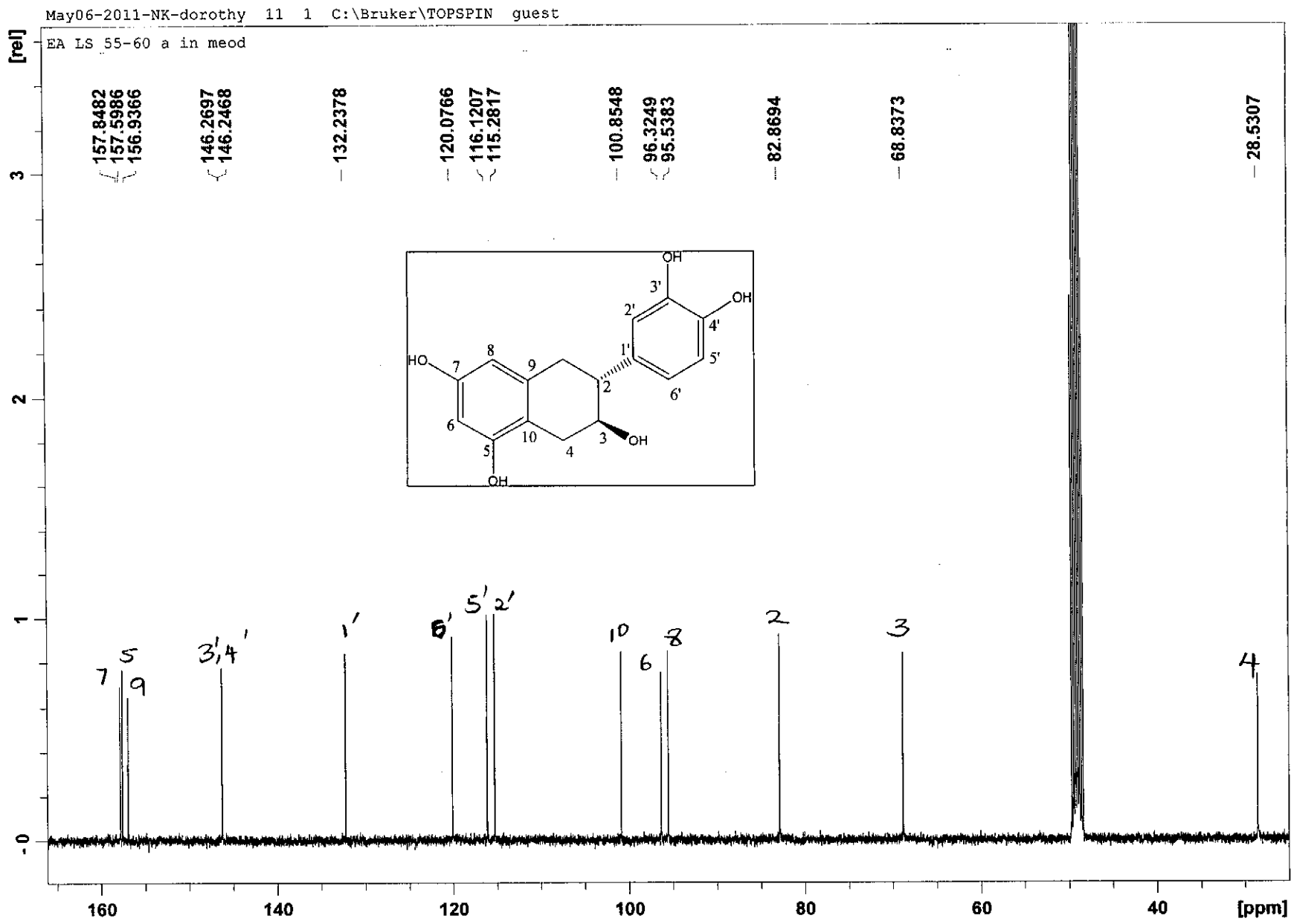


MS spectrum of D6

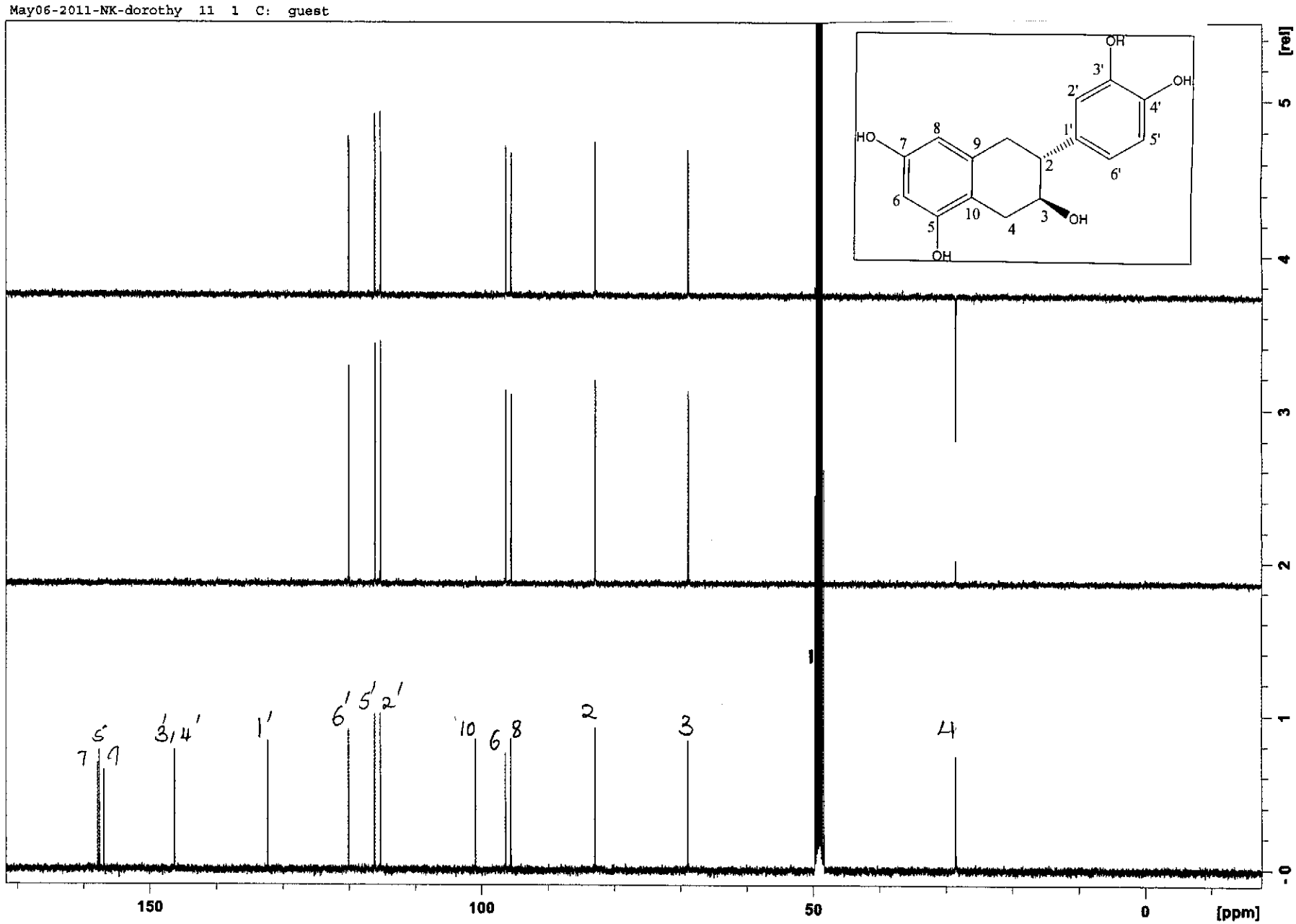
Catechin



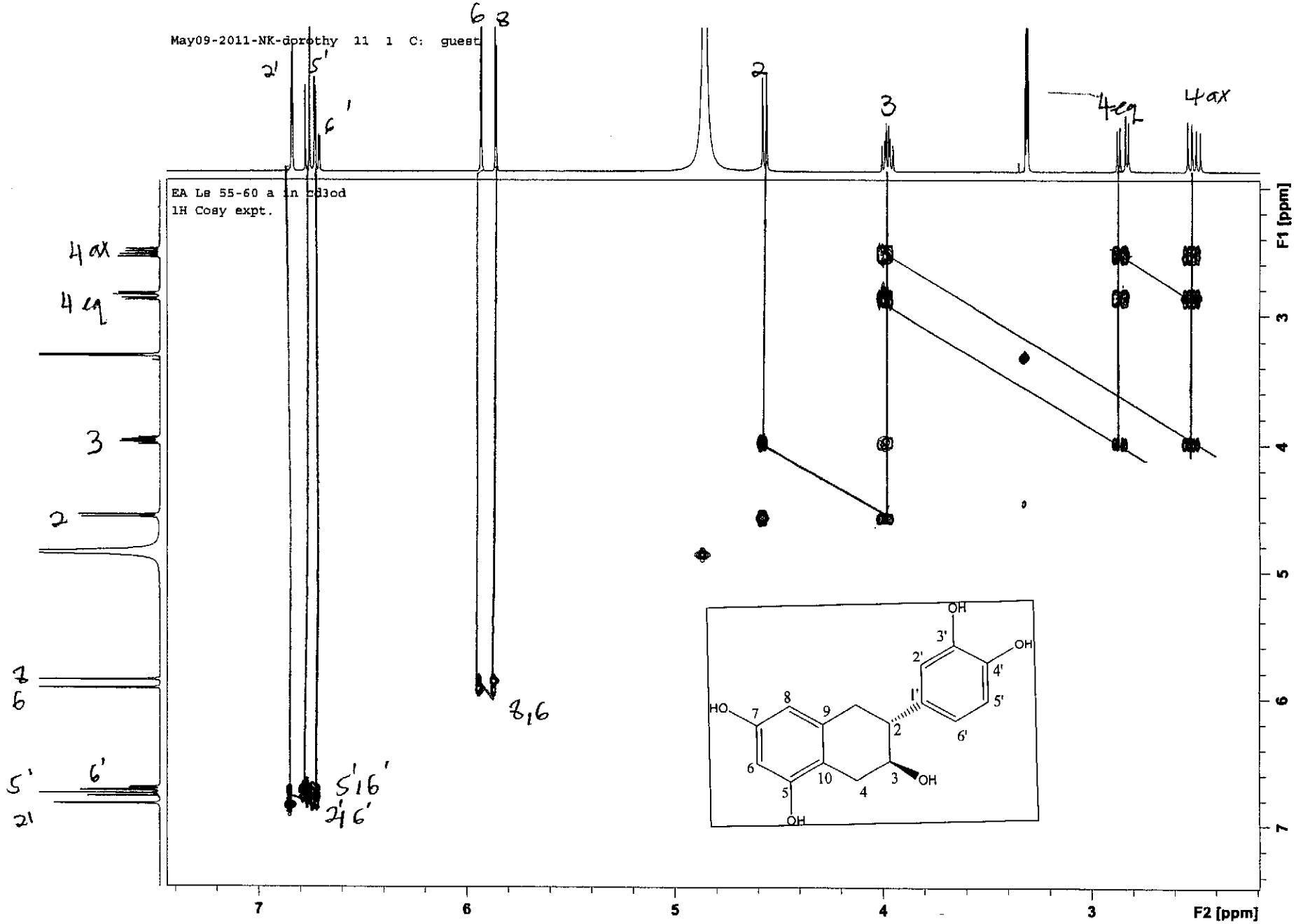
^{1}H NMR spectrum of D7



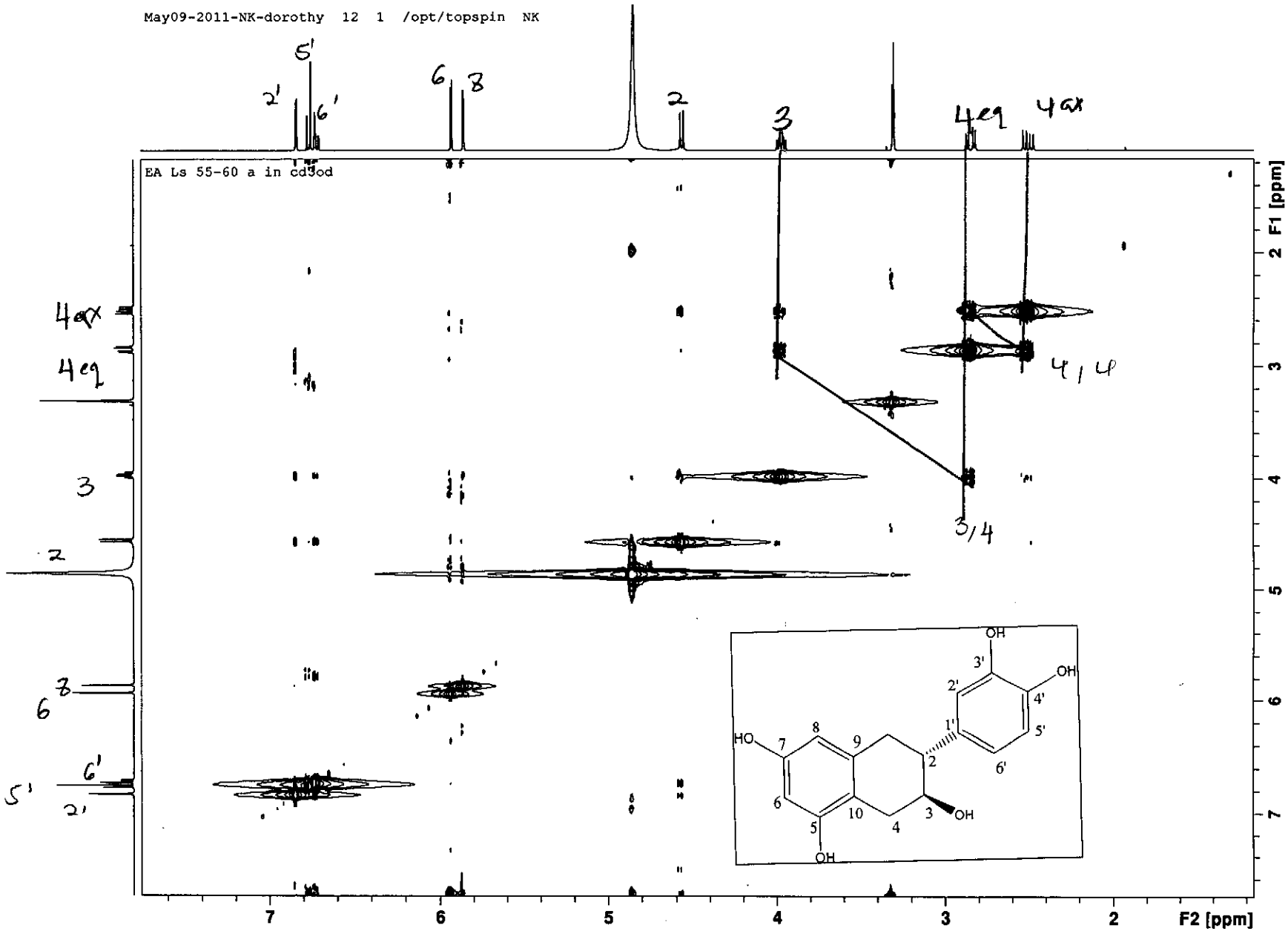
^{13}C NMR spectrum of D7



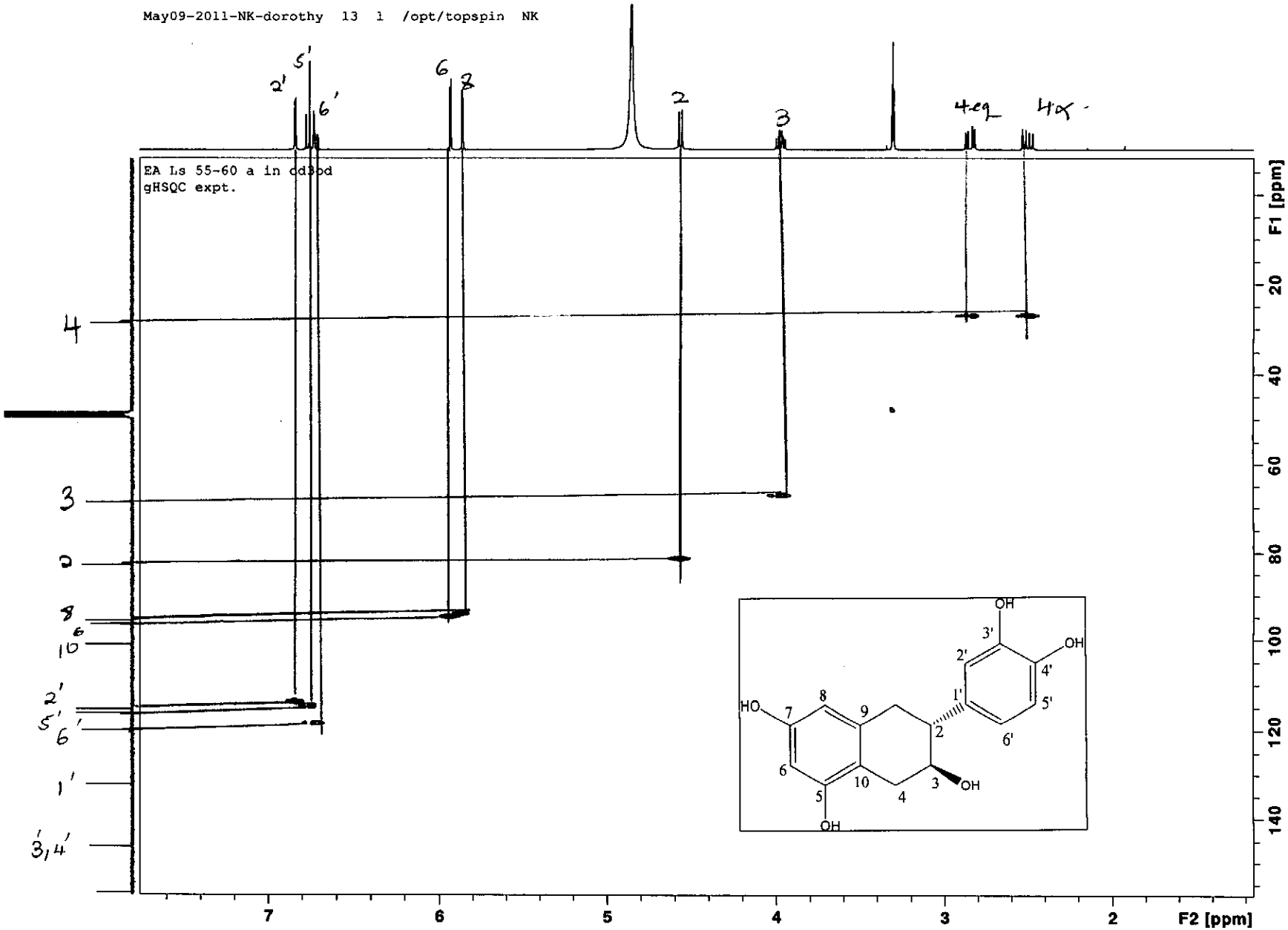
DEPT spectrum of D7



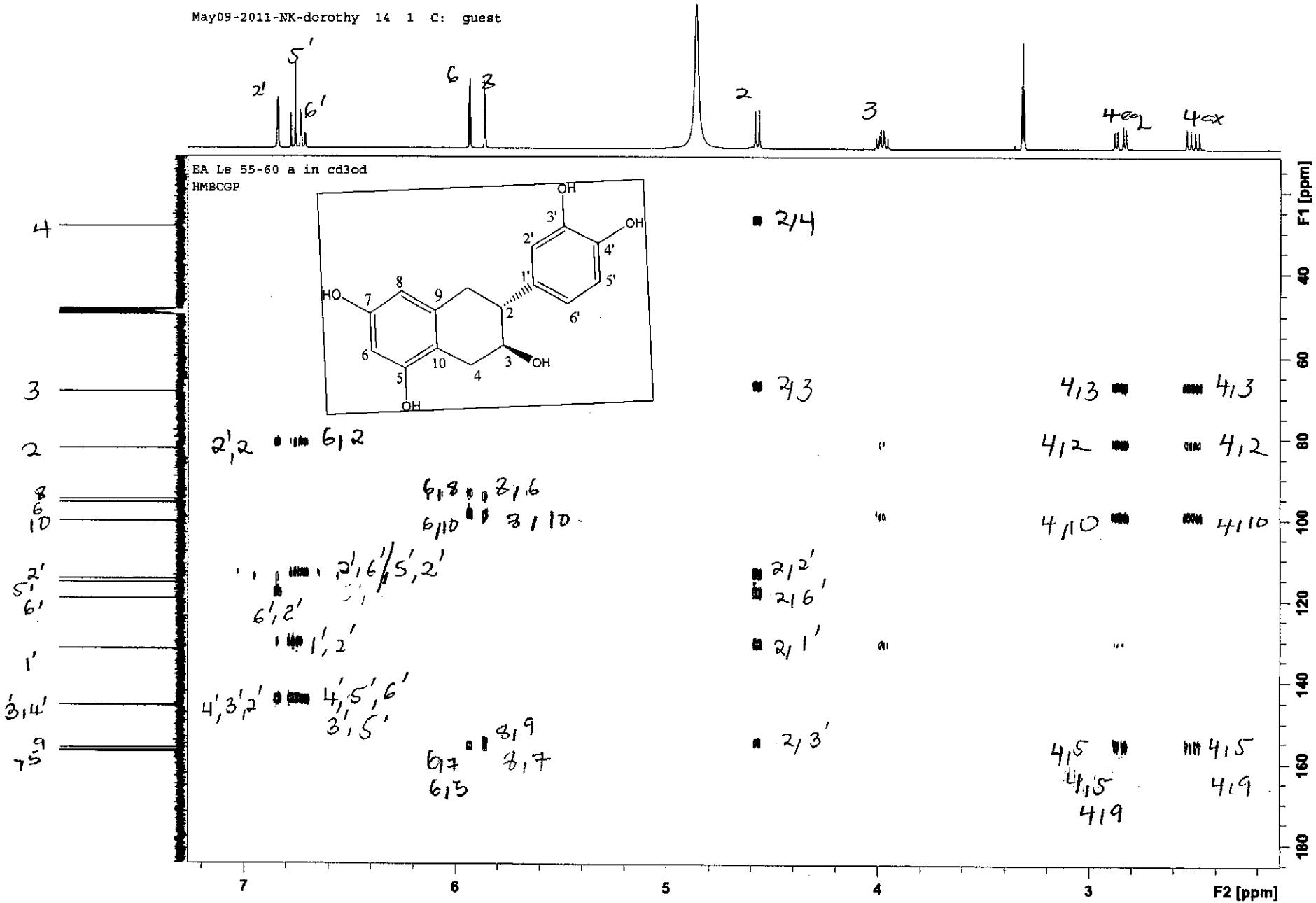
COSY spectrum of D7



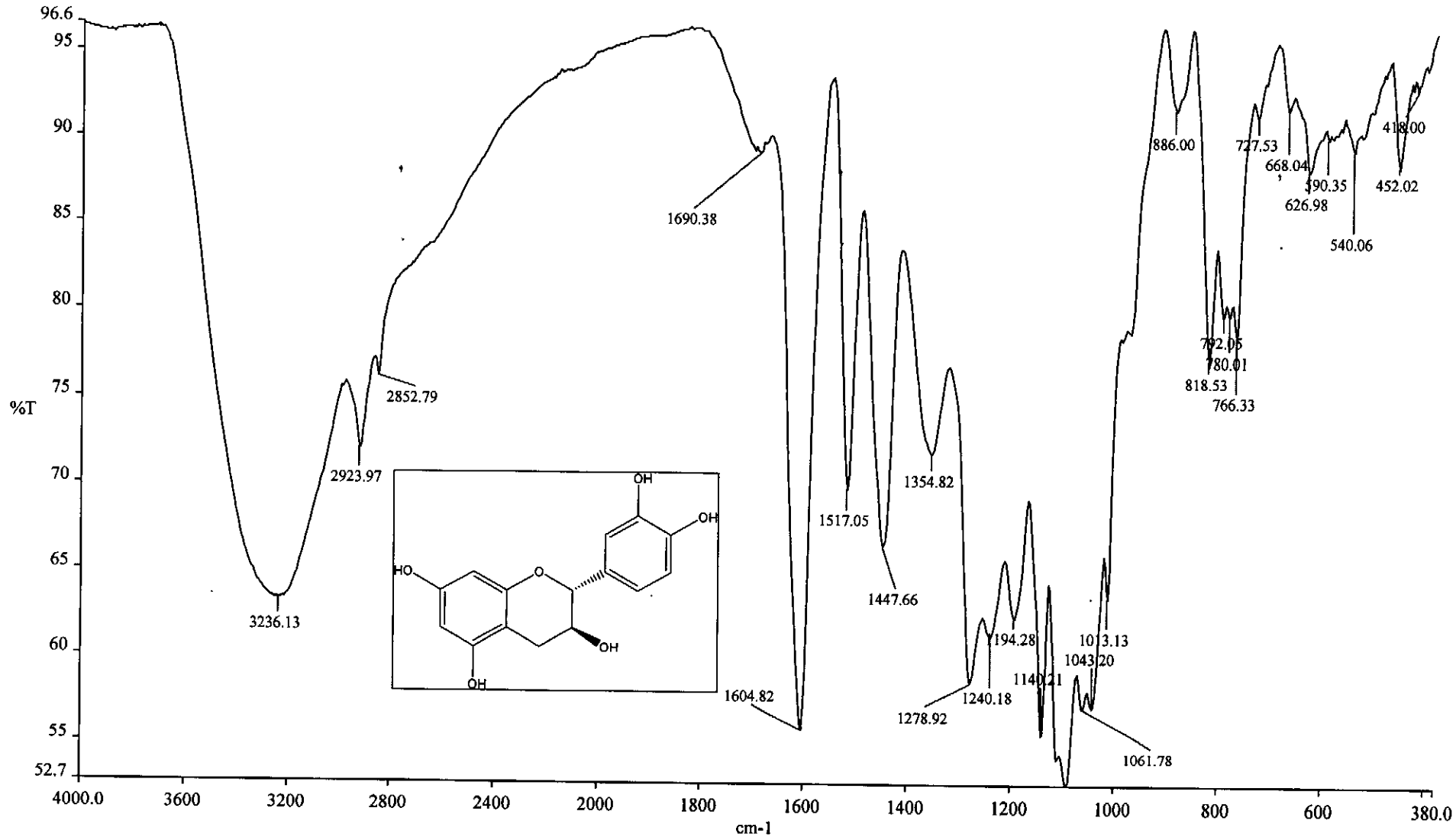
NOESY spectrum of D7



HSQC spectrum of D7



HMBC spectrum of D7

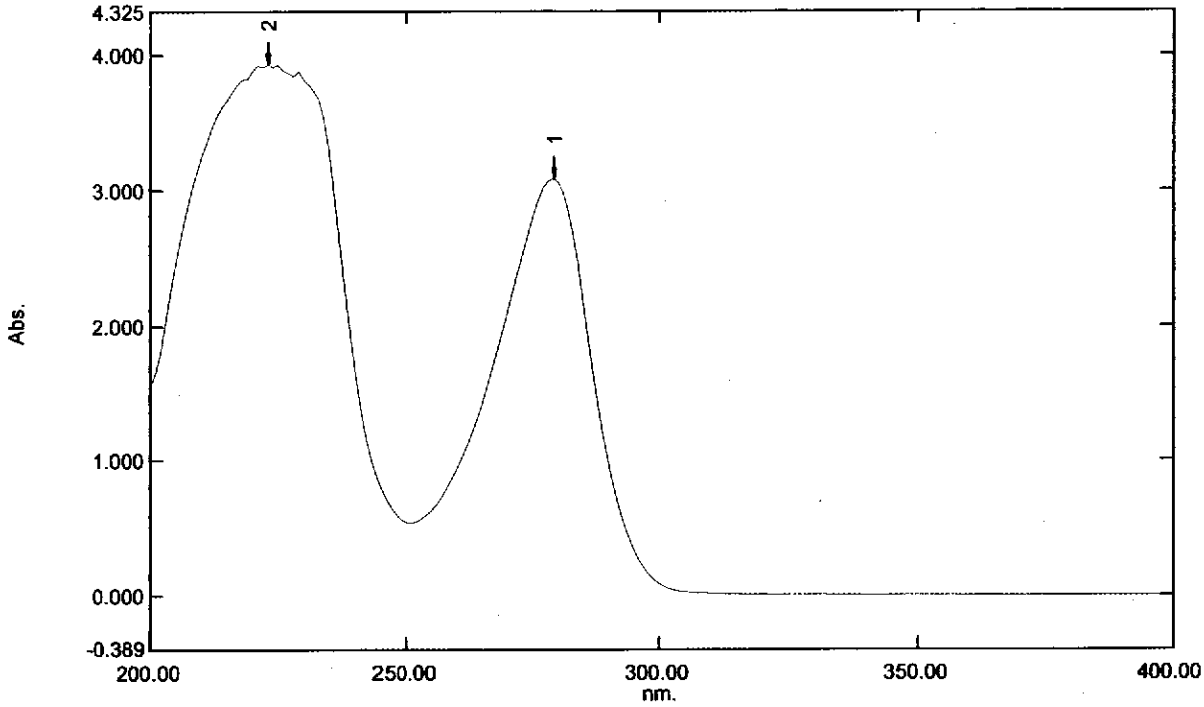


IR spectrum of D7

Spectrum Peak Pick Report

04/12/2011 08:27:18 PM

Data Set: EALSRT 50-60 A CAT BETTER.spc - Storage 202614



No.	P/V	Wavelength	Abs.	Description
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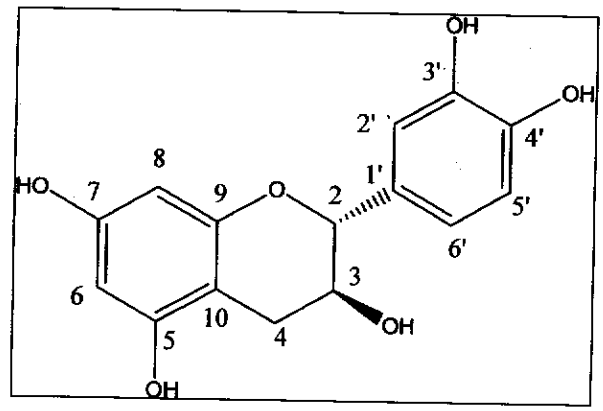
2	●	223.00	3.932	
3	●	251.00	0.541	

Measurement Properties
Wavelength Range (nm.): 200.00 to 400.00
Scan Speed: Medium
Sampling Interval: 1.0
Auto Sampling Interval: Disabled
Scan Mode: Single

Instrument Properties
Instrument Type: UV-3600 Series
Measuring Mode: Absorbance
Slit Width: 8.0 nm
Time Constant: 0.1 sec.
Source Lamp: Auto
Light Source Change Wavelength: 310.00 nm
Detector Unit: Direct
Detector Change Wavelength: 830.00 nm 1800.00 nm
Grating Change Wavelength: 720.00 nm
S/R Exchange: Normal
Detector Lock: Auto
Slit Program: Normal
Beam Mode: Double
Stair Correction: Disable

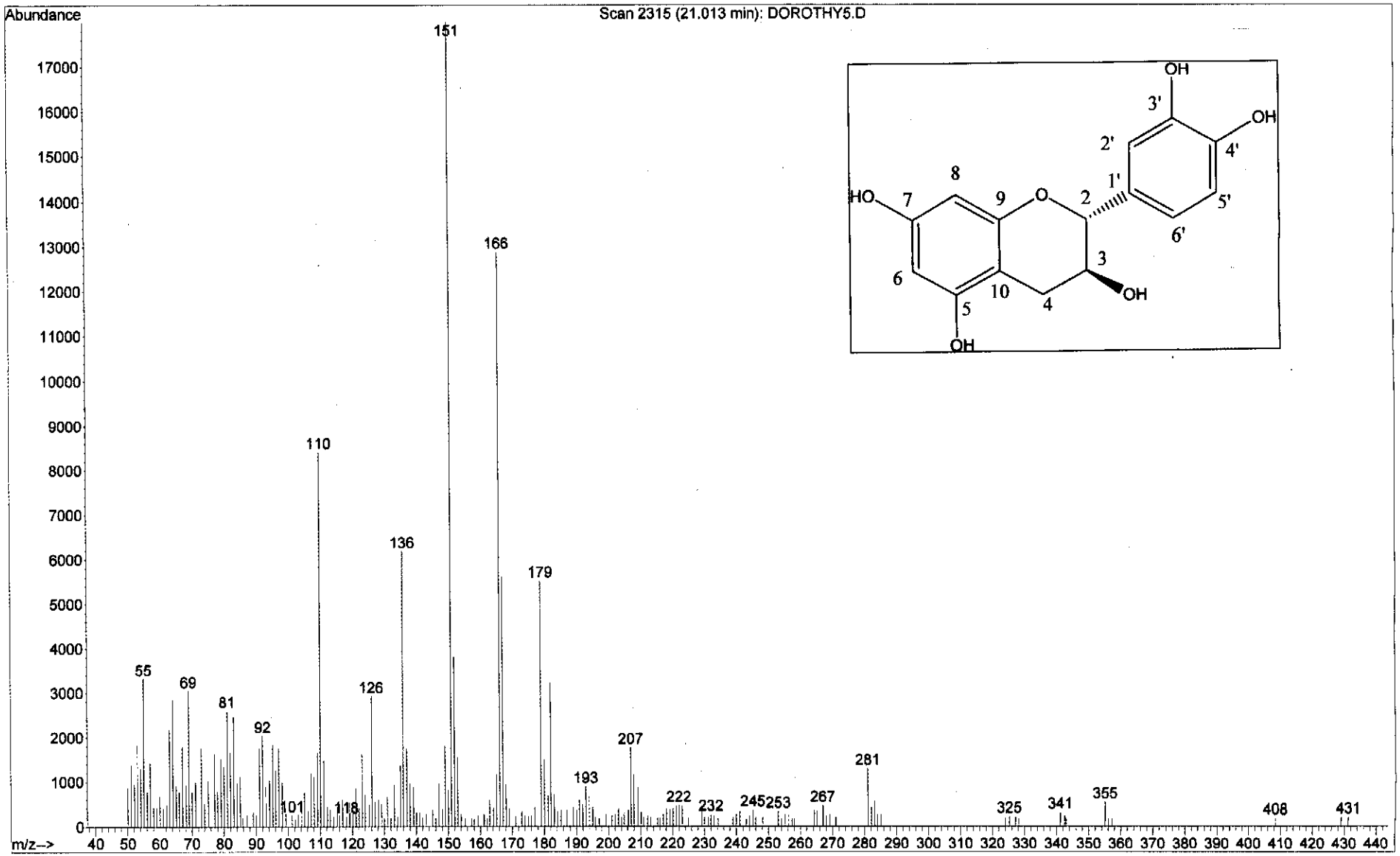


Attachment Properties
Attachment: None

Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

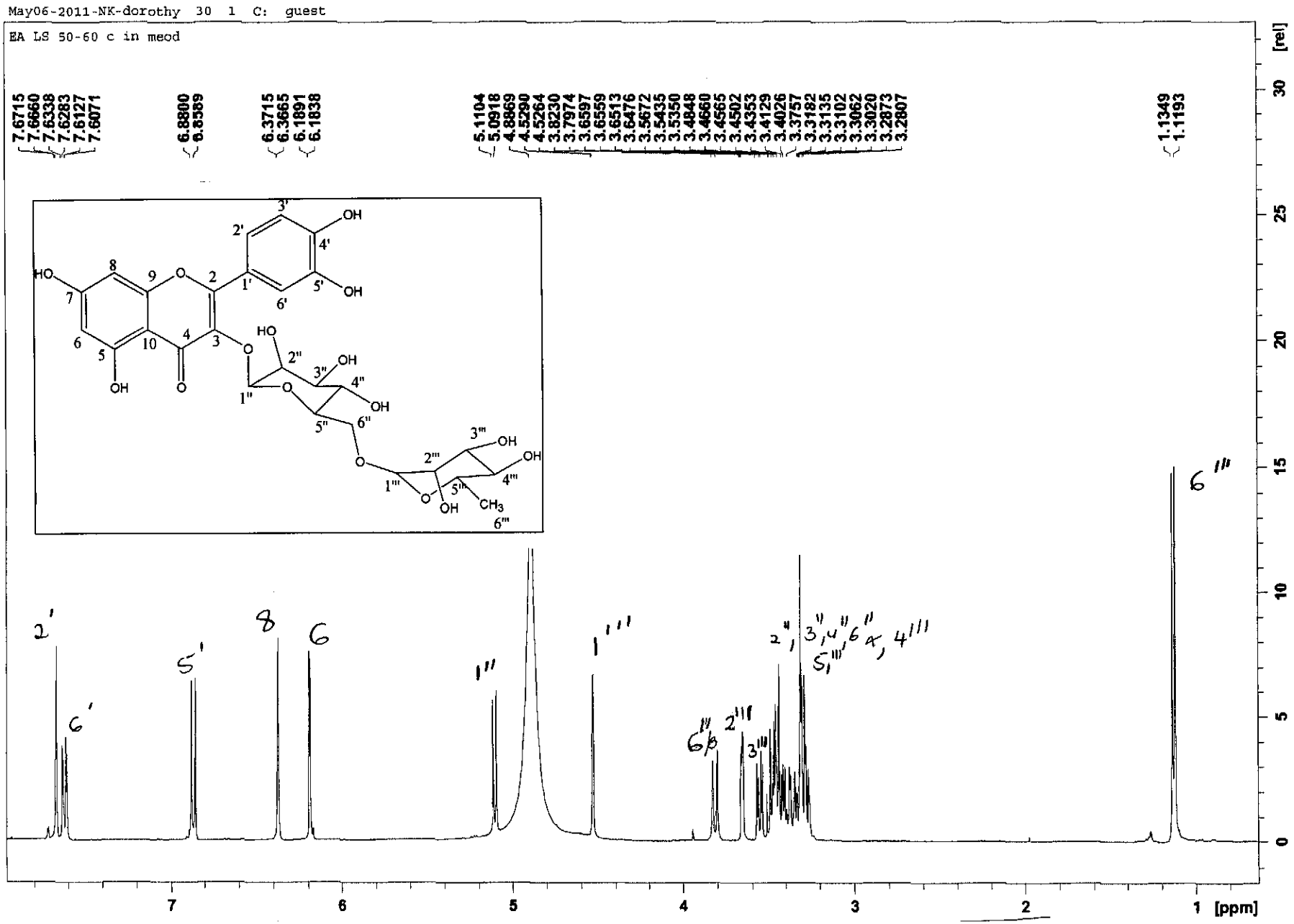
UV spectrum of D7

File : C:\MSDCHEM\1\DATA\DOROTHY GCMS TWO\DOROTHY GCMS TWO\DOROTHY5.D
Operator : dorothy
Acquired : 27 Nov 2011 17:46 using AcqMethod DOROTHY
Instrument : Instrumen
Sample Name: EA LS 50-60 A
Misc Info :
Vial Number: 1

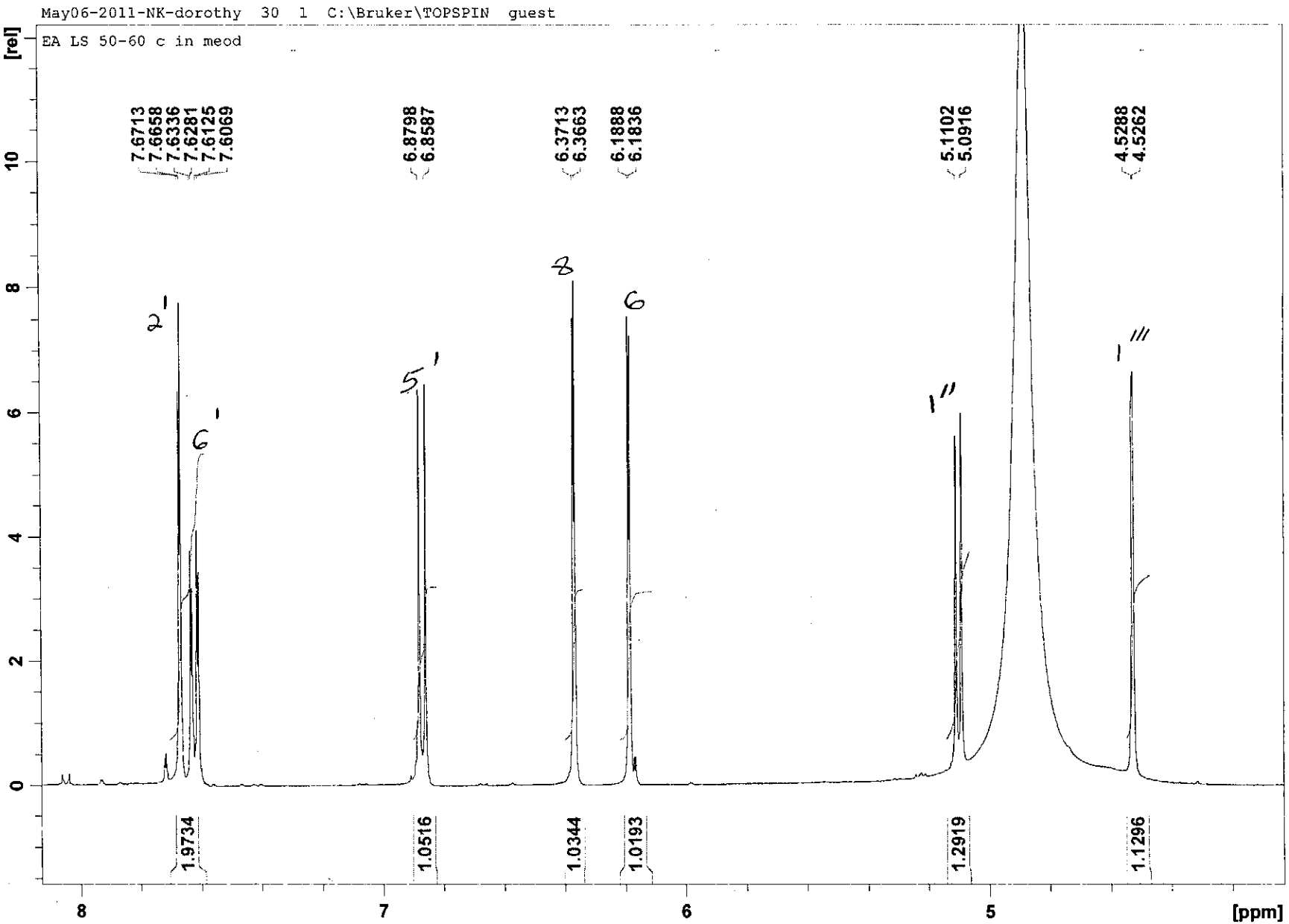


MS spectrum of D7

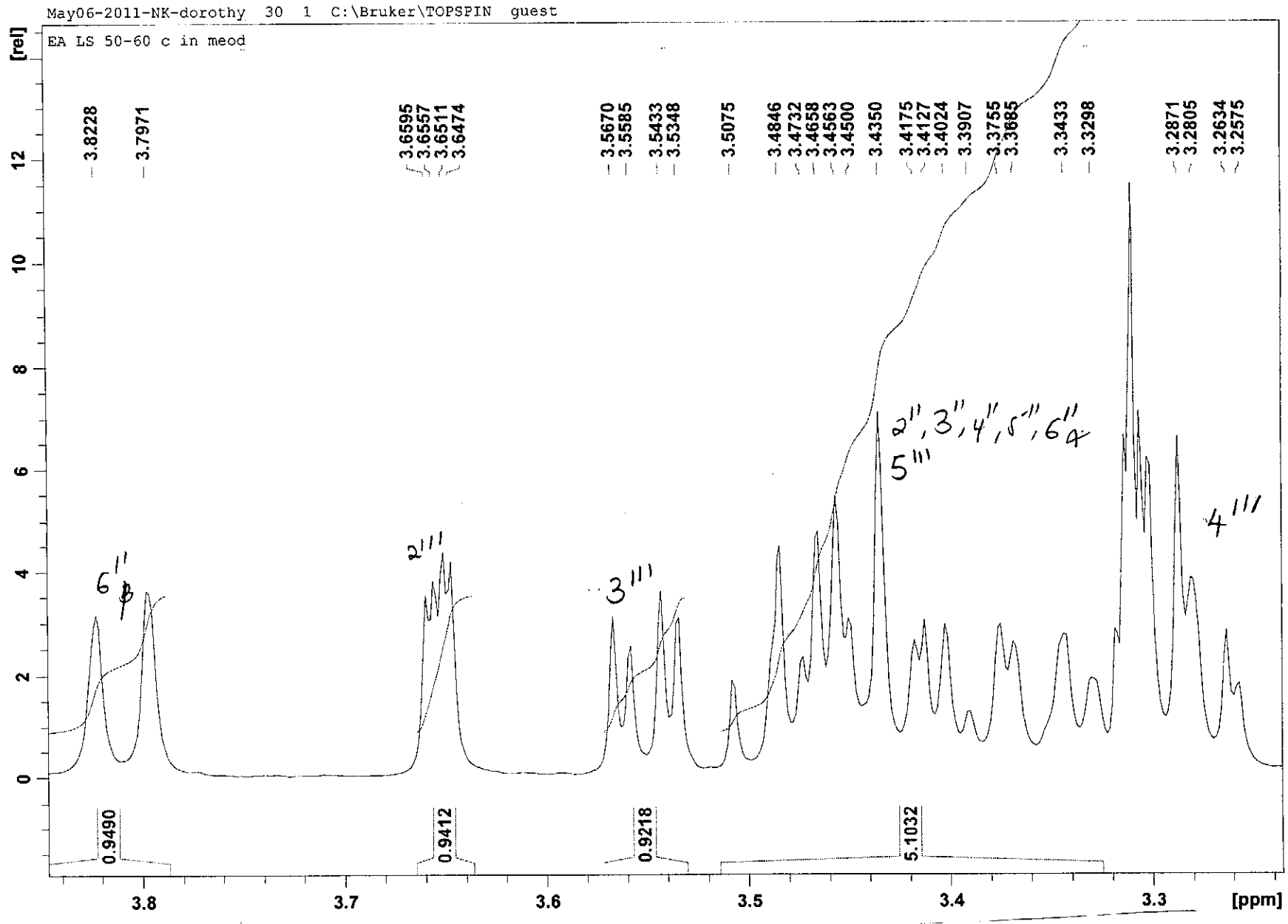
Rutin



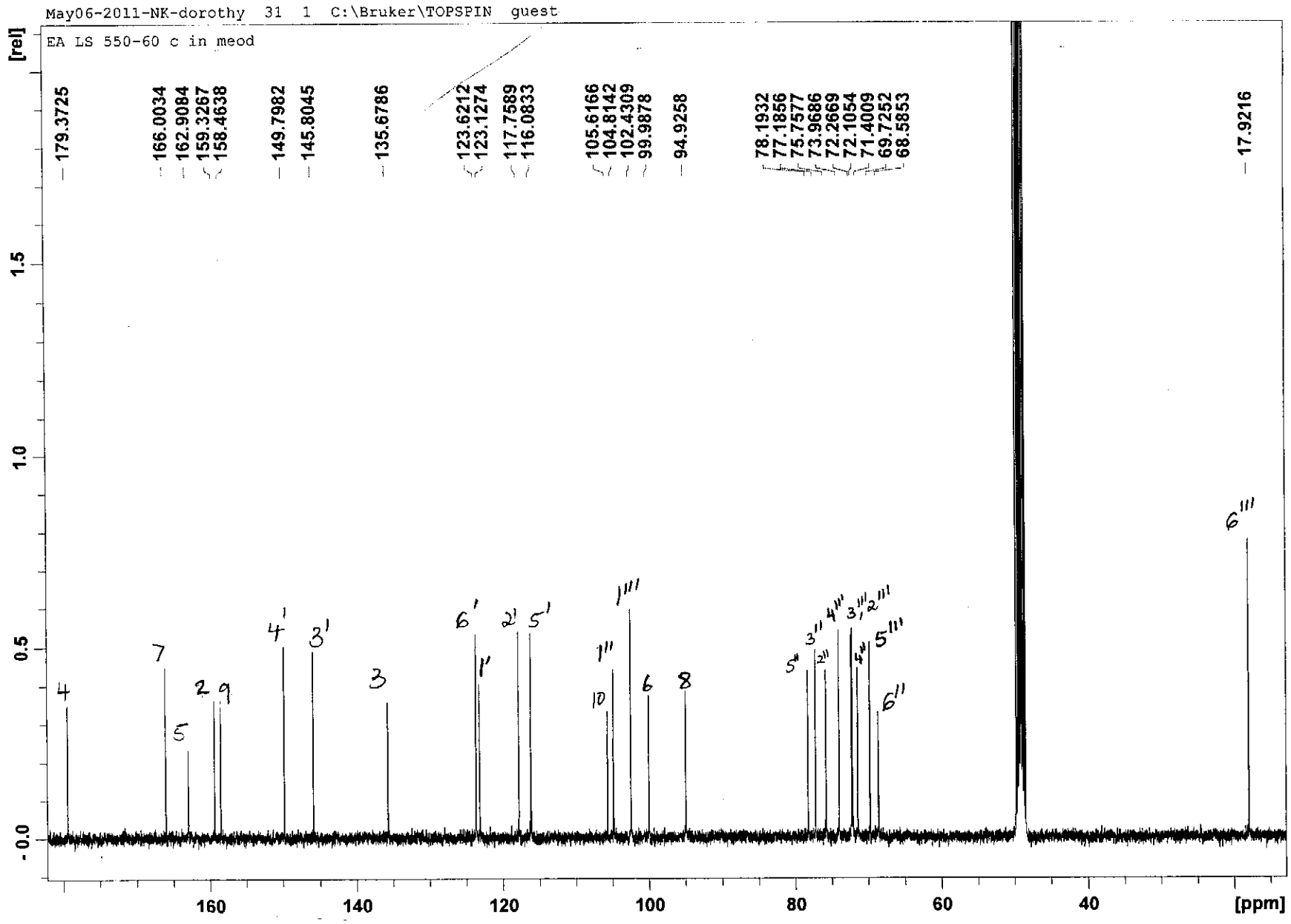
^{1}H NMR spectrum of D8



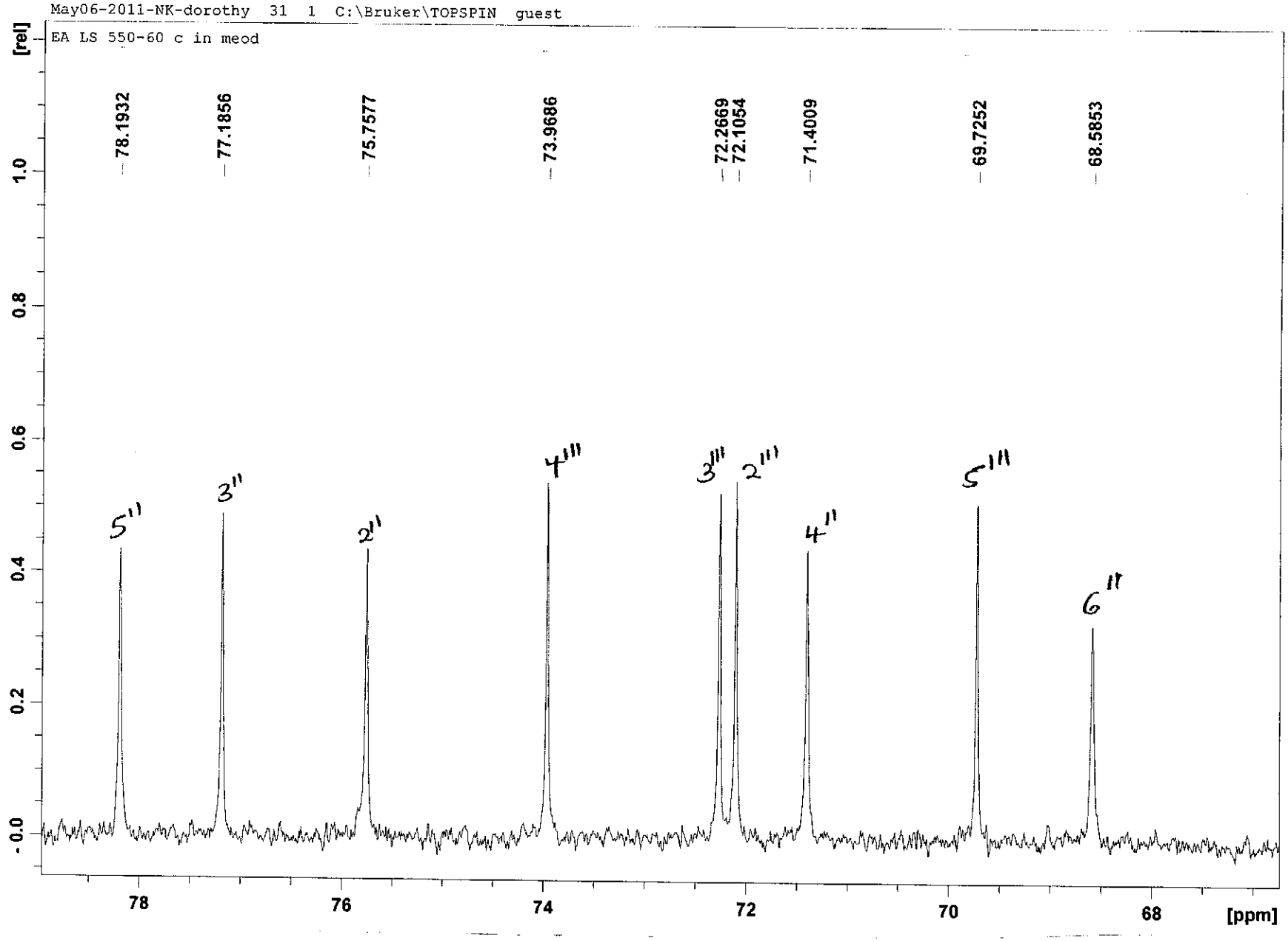
^{1}H NMR spectrum of D8 (expanded 4.0-8 ppm)



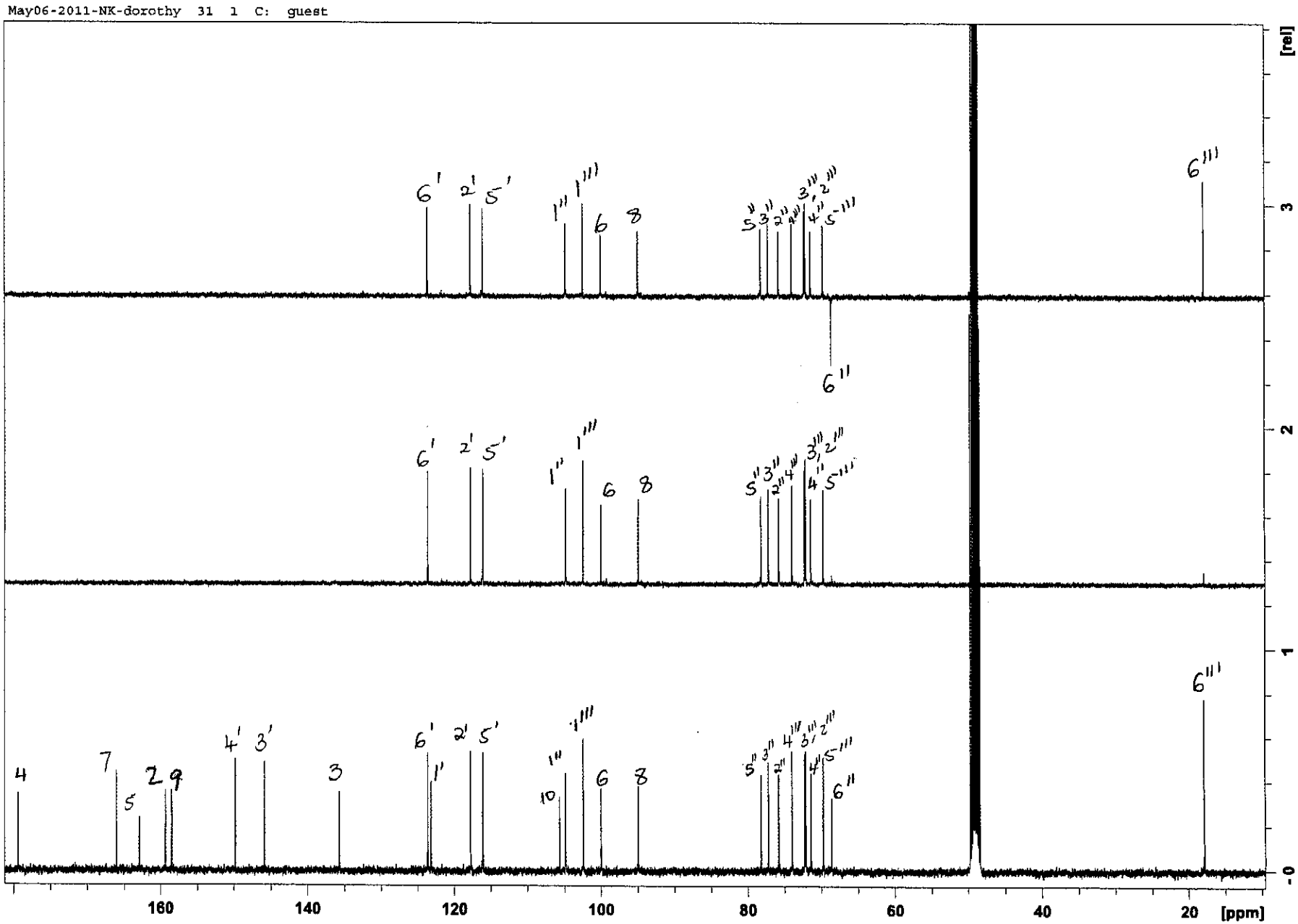
^{1}H NMR spectrum of D8 (expanded 3.2-3.9 ppm)



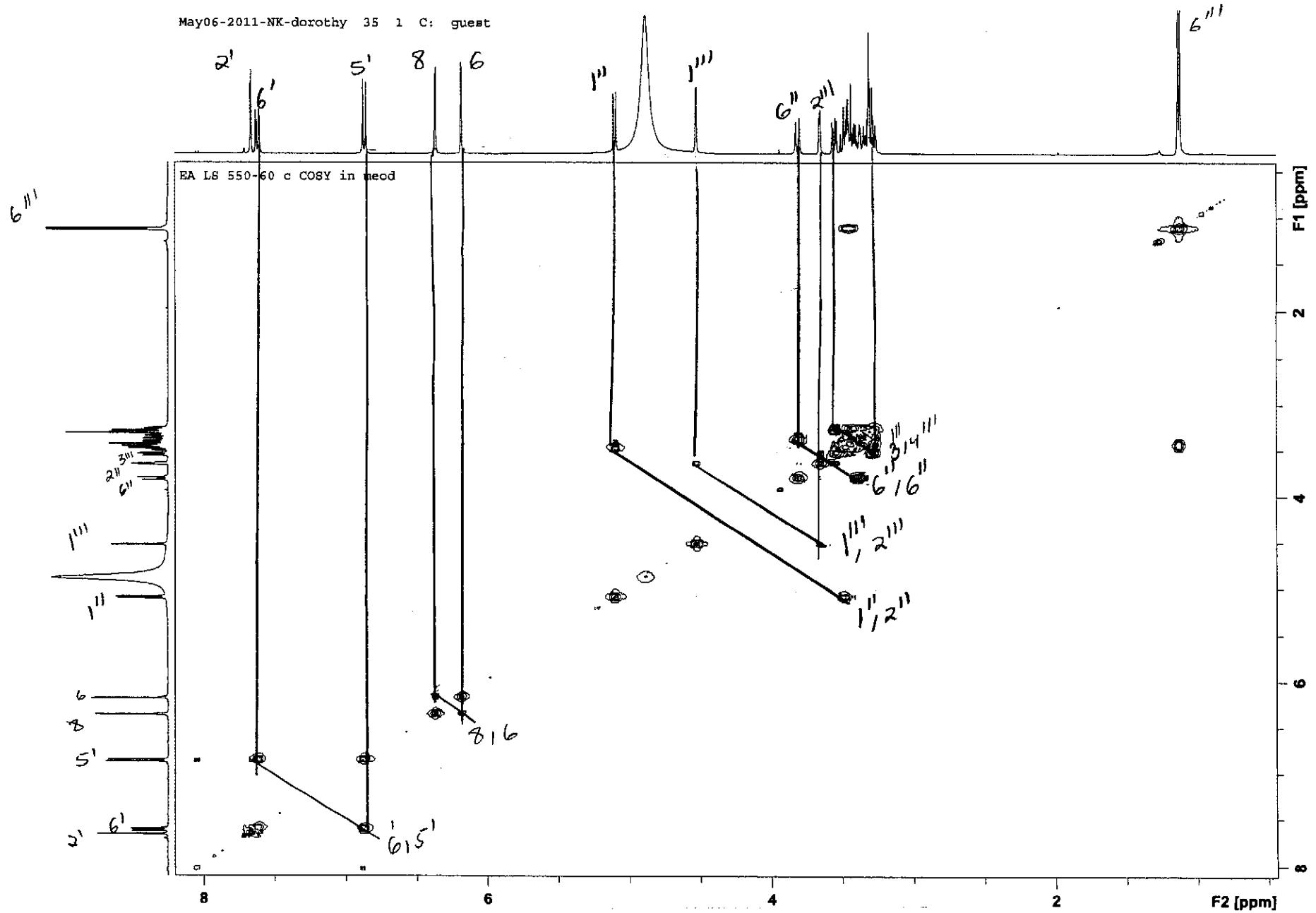
^{13}C NMR spectrum of D8



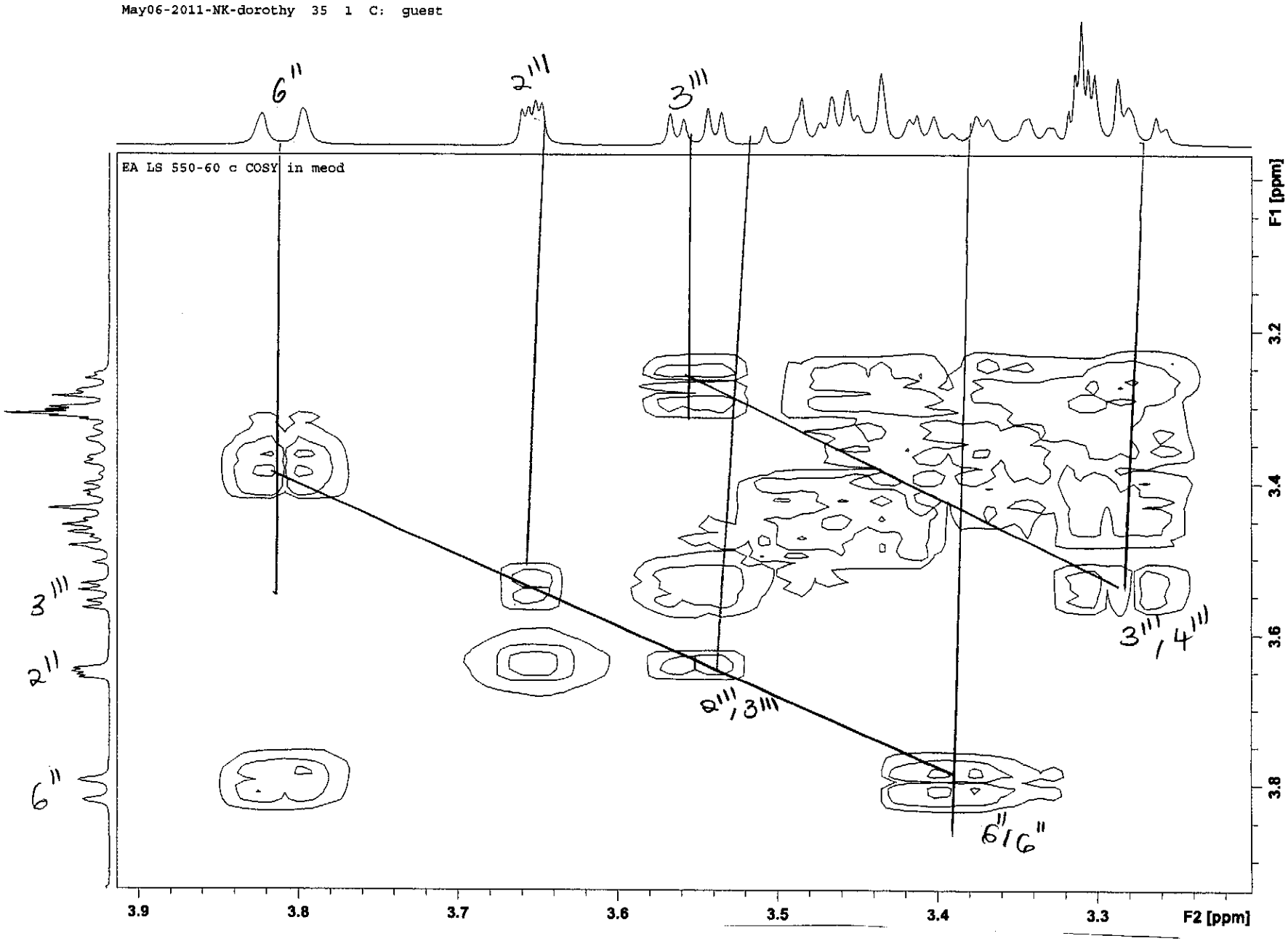
^{13}C NMR spectrum of D8 (expanded 67-79 ppm)



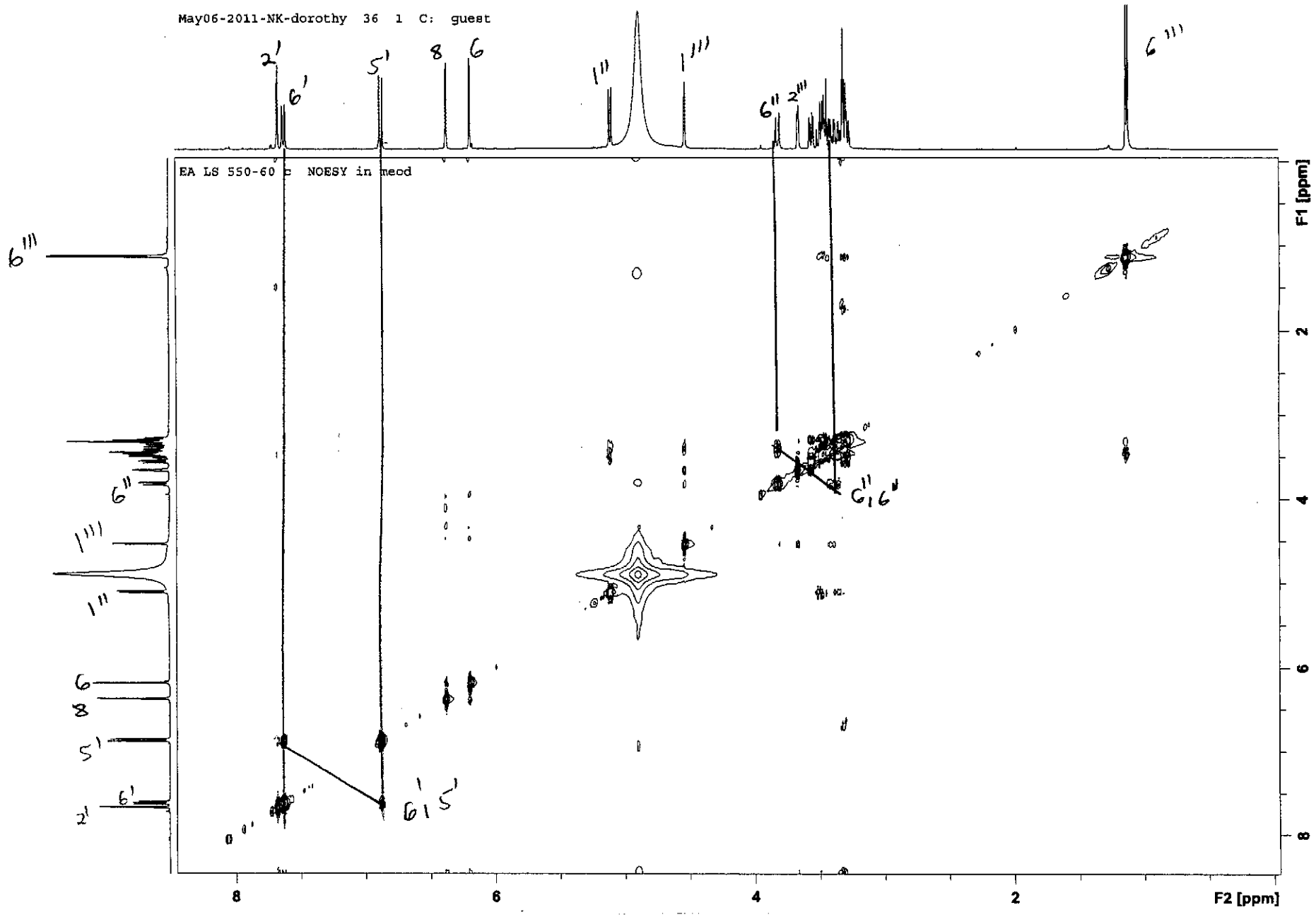
DEPT spectrum of D8



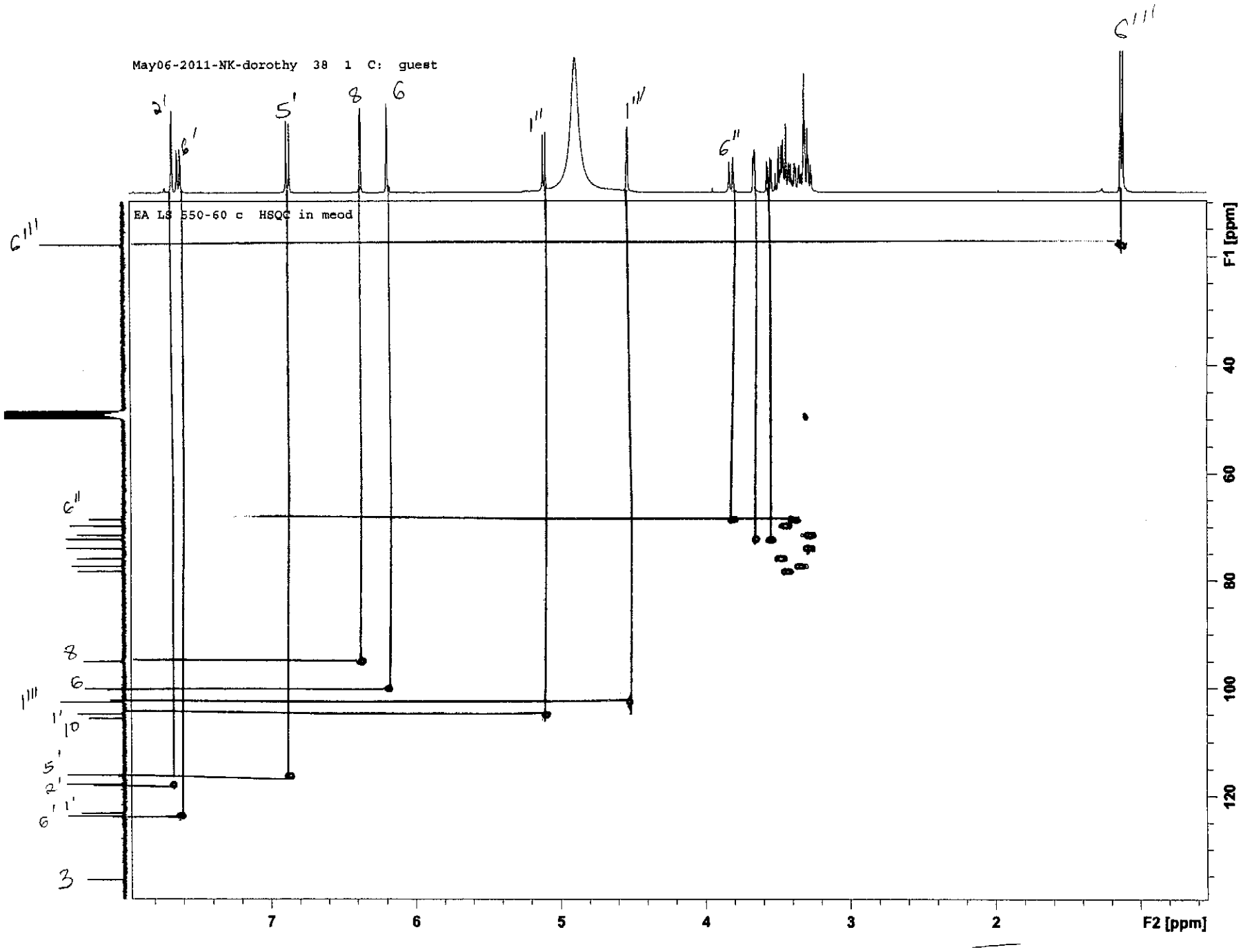
COSY spectrum of D8



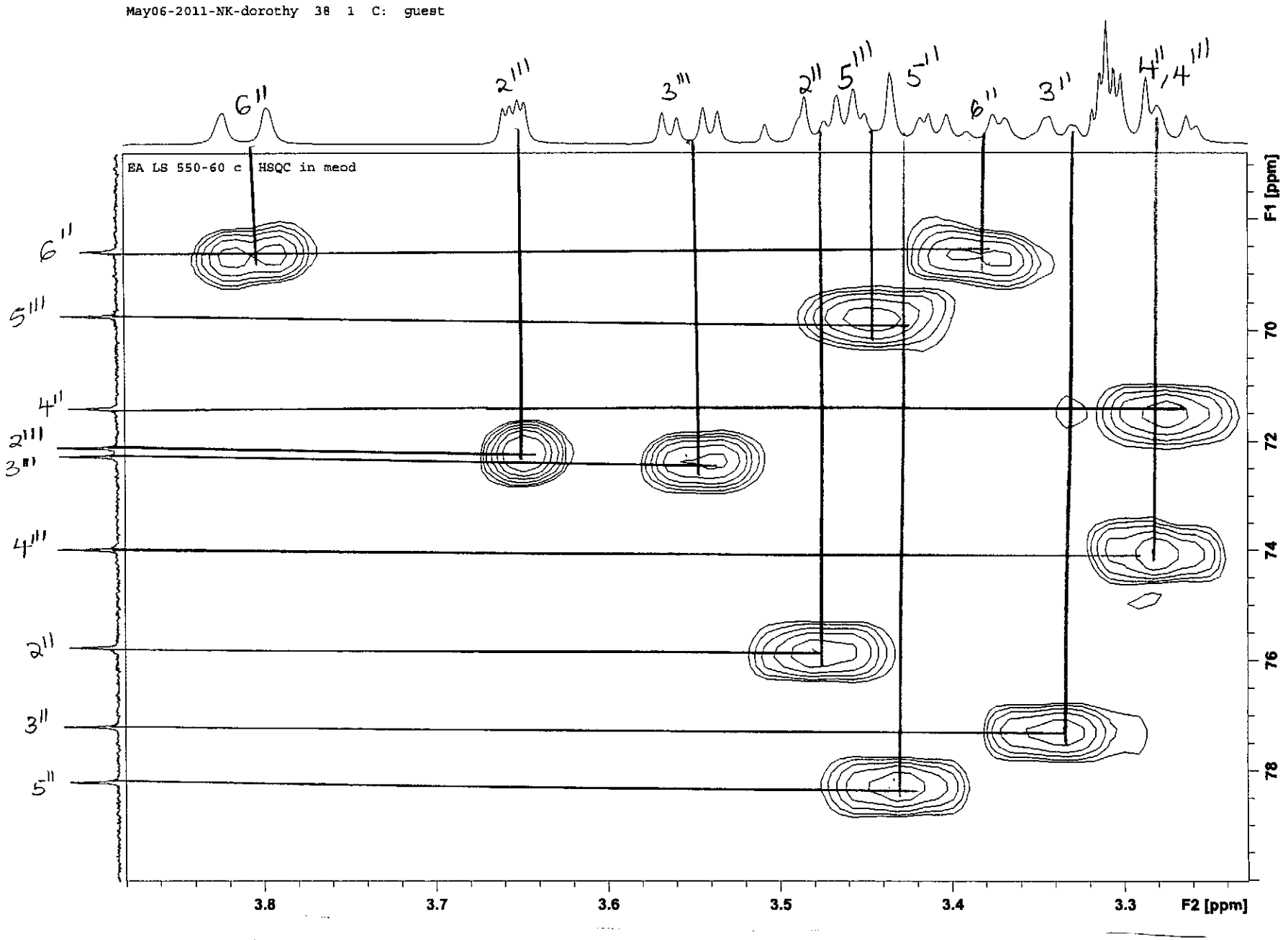
COSY spectrum of D8 (expanded 3.2-3.9 ppm)



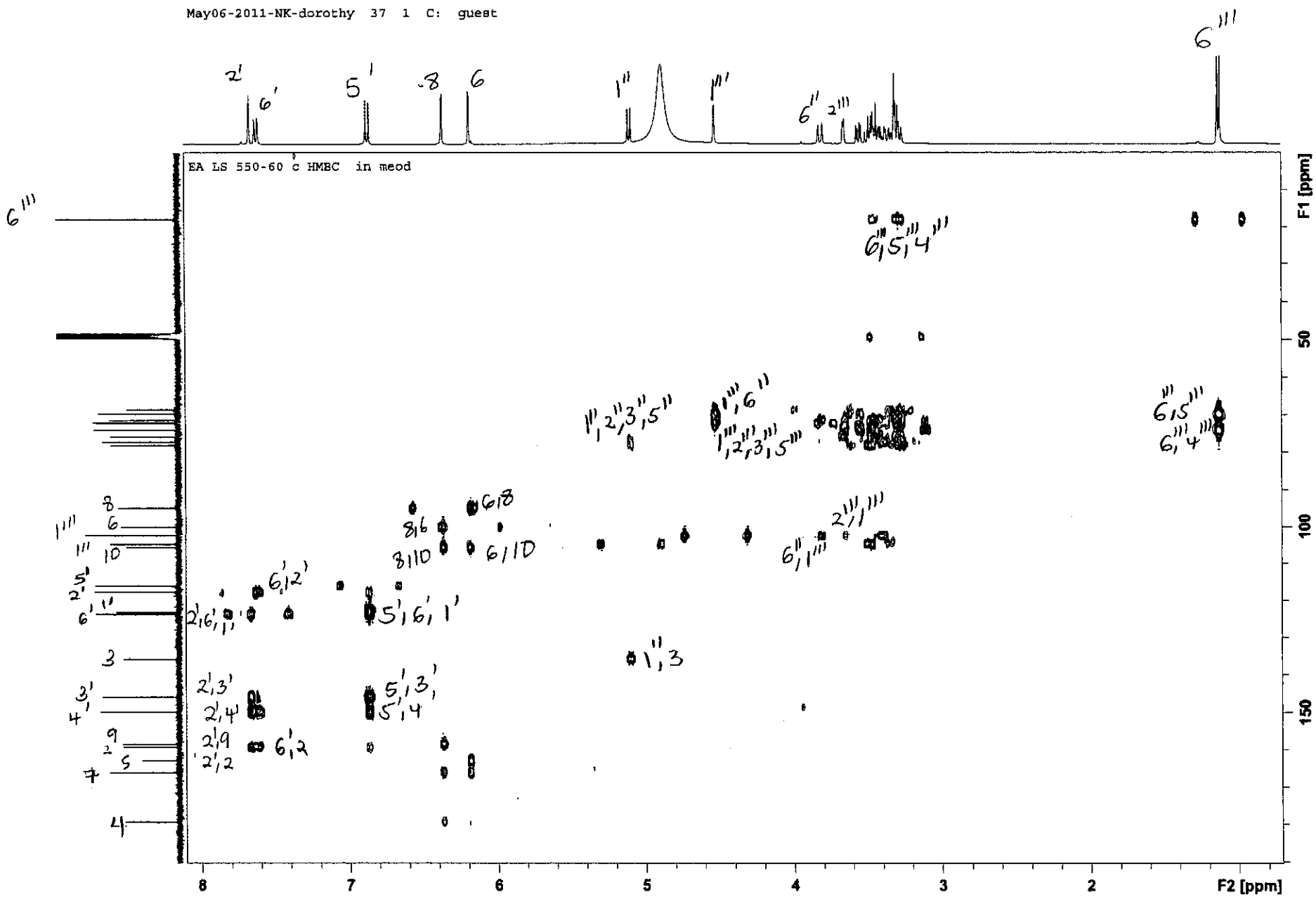
NOESY spectrum of D8



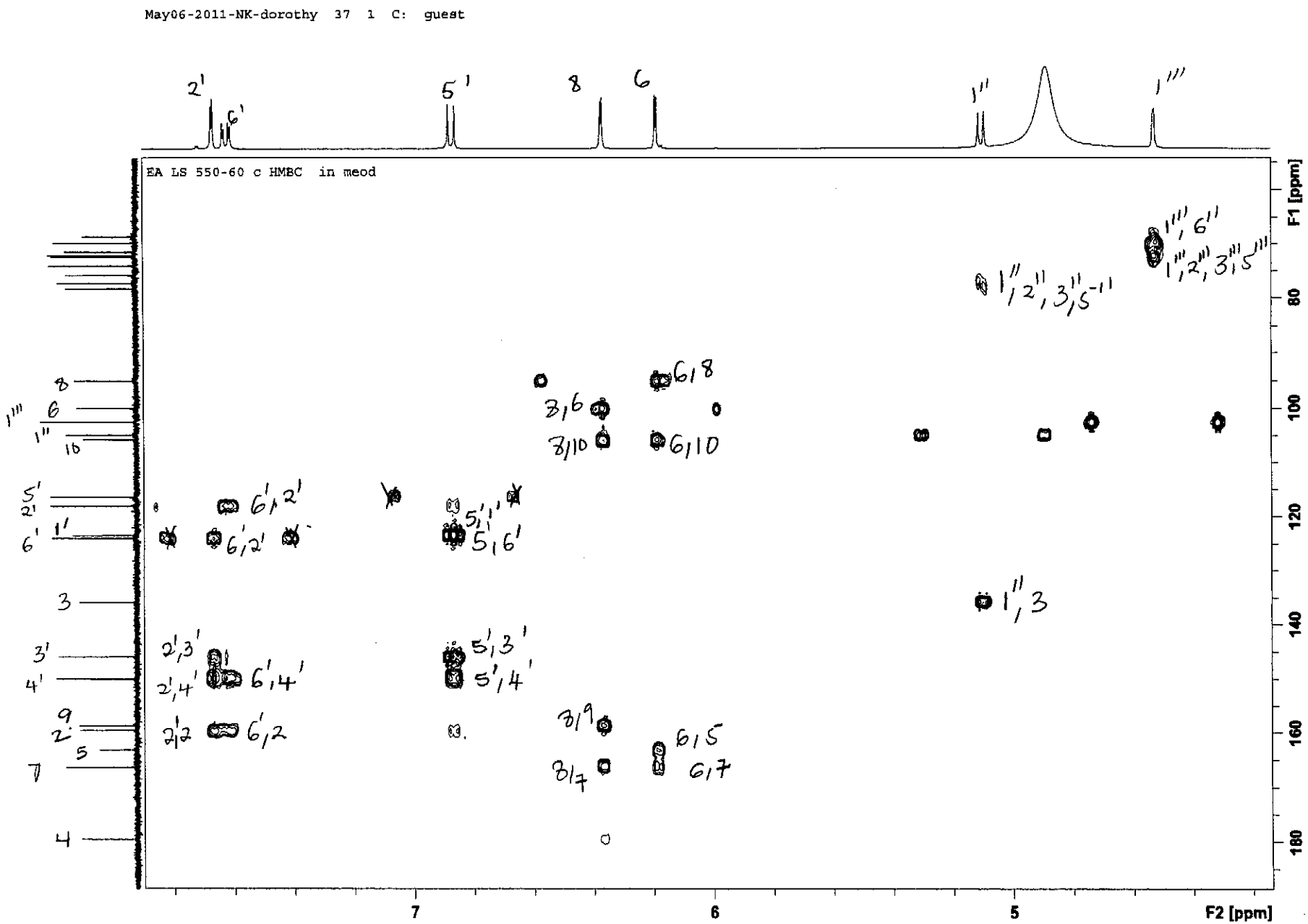
HSQC spectrum of D8



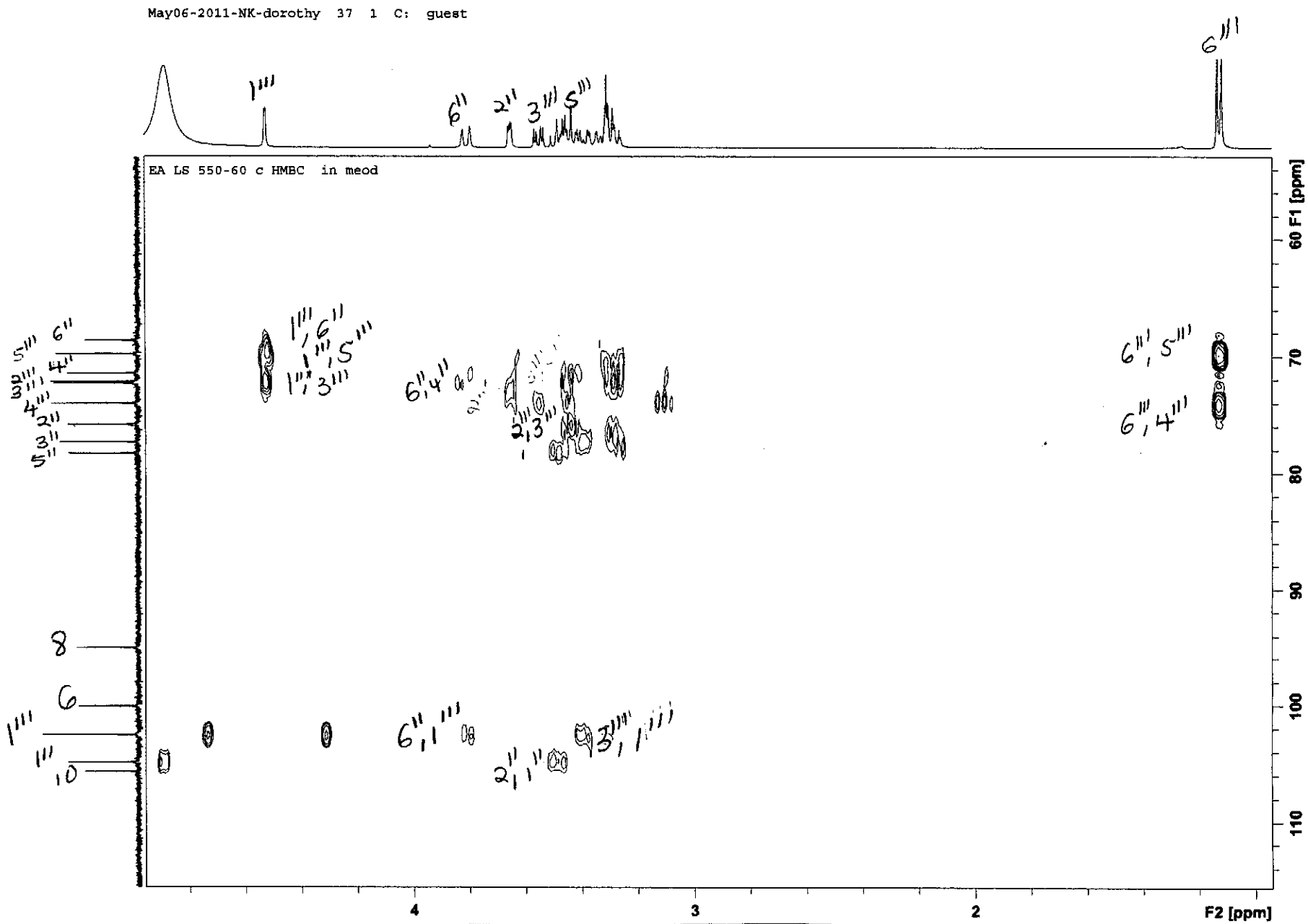
HSQC spectrum of D8 (expanded F1 3.2-3.9 ppm)



HMBC spectrum of D8



HMBC spectrum of D8 (expanded F1 4.0-8 ppm)

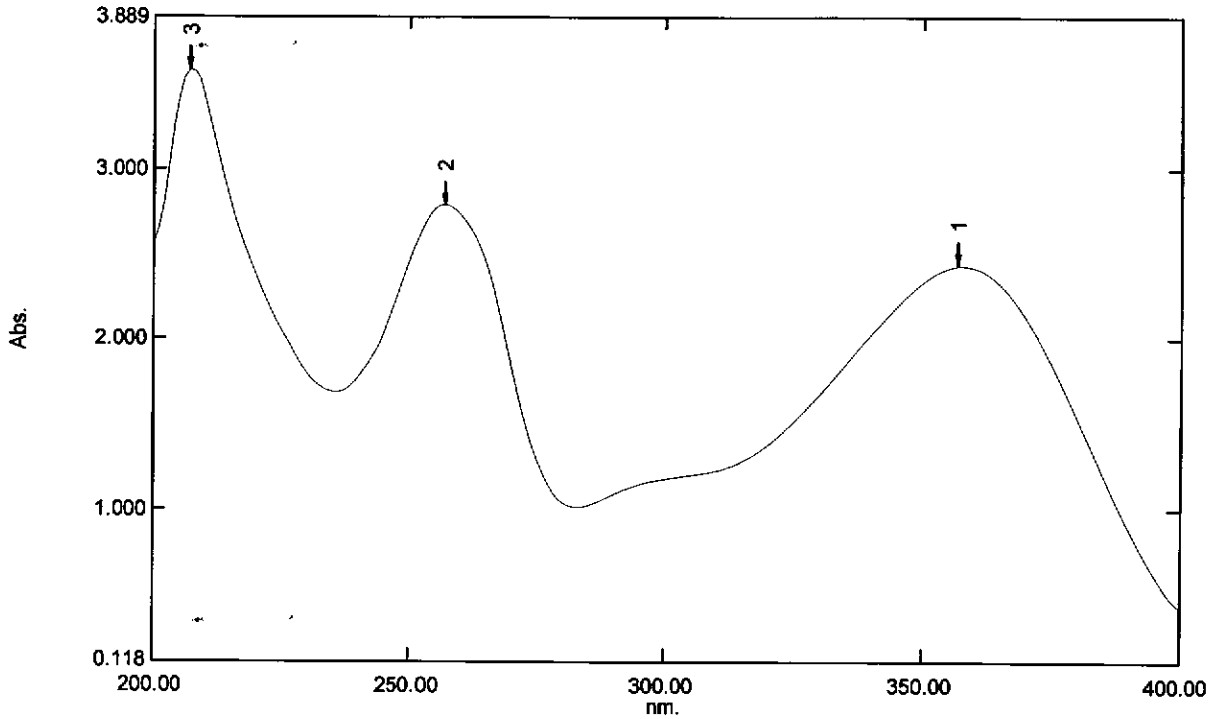


HMBC spectrum of D8 (expanded F1 1.0-5 ppm)

Spectrum Peak Pick Report

04/12/2011 07:54:15 PM

Data Set: EALSRT 50-60 C GOOD.spc - Storage 174824



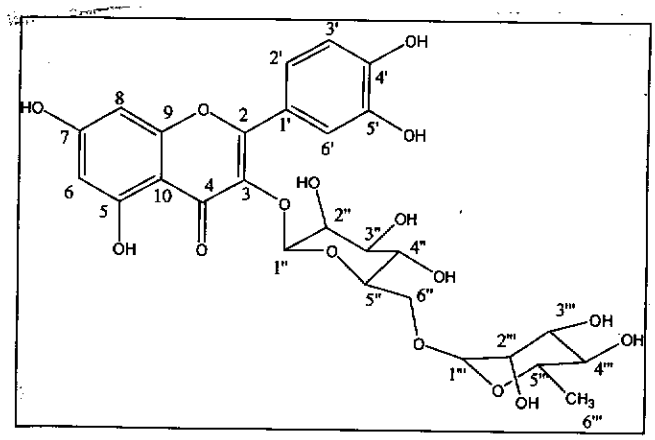
Measurement Properties	
Wavelength Range (nm.):	200.00 to 400.00
Scan Speed:	Medium
Sampling Interval:	1.0
Auto Sampling Interval:	Disabled
Scan Mode:	Single

No.	P/V	Wavelength	Abs.	Description
1	⬢	357.00	2.435	
2	⬢	257.00	2.790	
3	⬢	207.00	3.575	
4	⬢	283.00	1.015	
5	⬢	236.00	1.688	

Instrument Properties	
Instrument Type:	UV-3600 Series
Measuring Mode:	Absorbance
Slit Width:	8.0 nm
Time Constant:	0.1 sec.
Source Lamp:	Auto
Light Source Change Wavelength:	310.00 nm
Detector Unit:	Direct
Detector Change Wavelength:	830.00 nm 1800.00 nm
Grating Change Wavelength:	720.00 nm
S/R Exchange:	Normal
Detector Lock:	Auto
Slit Program:	Normal
Beam Mode:	Double
Stair Correction:	Disable

Attachment Properties	
Attachment:	None

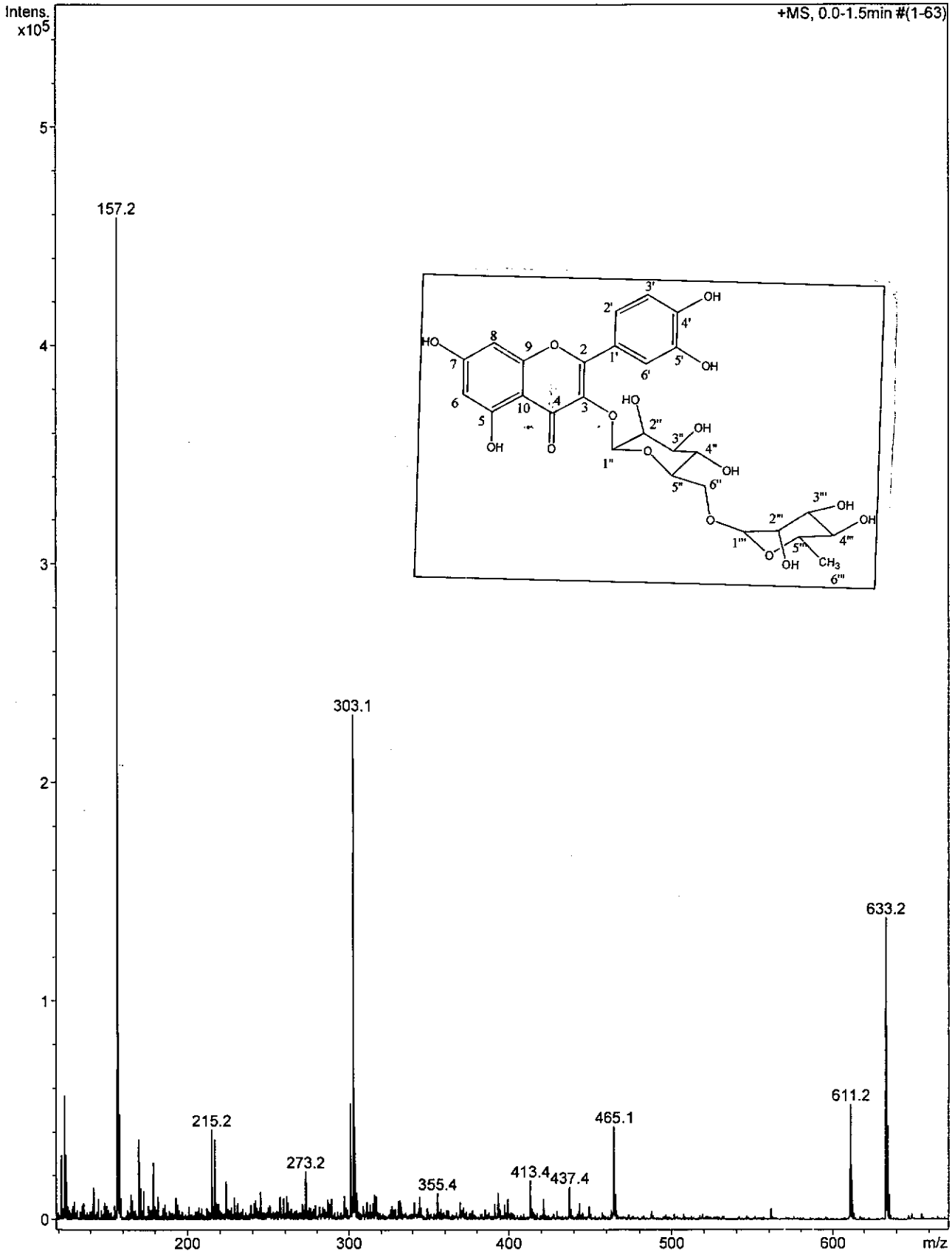
Sample Preparation Properties	
Weight:	
Volume:	
Dilution:	
Path Length:	
Additional Information:	



UV spectrum of D8

Display Report - Selected Window All Analyses

Operator: Operator **Instrument:** LC-MSD-Trap-VL **Print Date:** 6/5/2012 12:40:09 PM



LCMS spectrum of D5

Agilent Technologies