

**EXTRACTIVES OF THE PHYLLANTHACEAE, EUPHORBIACEAE AND  
THE MELIACEAE FAMILIES**

**BY**

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## **DEDICATION**

*To my parents Mr. and Mrs. Ndlebe*

*Who have worked tirelessly to ensure that I have a sense of belonging and*

*appreciation in the*

*Society at large*

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## ABSTRACT

Historically, plants (fruits, vegetables, medicinal herbs) have provided a good source of a wide variety of compounds, such as phenolic compounds, nitrogen containing compounds, vitamins, terpenoids and some other secondary metabolites, which are rich in valuable bioactivities e.g. antioxidant, anti-inflammatory, larvicidal, anti-mutagenic, anti-carcinogenic, anti-malarial, anti-bacterial or anti-viral activities. Recently artemesinin from the Chinese herb *Artemisia annua* has been formulated into drugs for antimalarial activity.

In many countries, the traditional herbal medicine has been widely used for thousands of years. Herbal plants have become the main object of chemists, biochemists and pharmacists. Their research has played an important role for discovering and developing new drugs. Natural products from plants have been used for centuries to prevent or cure diseases, those plant extracts from the family Phyllanthaceae, Euphorbiaceae and Meliaceae have been used traditionally for the treatment of diseases such as kidney disorders, urinary bladder infections, intestinal infections, jaundice, gonorrhea, diabetes (internal use) as well as poultices, skin ulcer and other skin problems (external use). Infusions have been made from young shoots as a treatment of chronic dysentery. Many plant extracts have been evaluated for their biological activities. However, most of the plants from the families have not been investigated before for their chemical composition, therefore the rationale of this study.

The study sought to identify, extract, isolate, purify and characterize compounds from the following plants: *Phyllanthus cedrelifolius* and *Phyllanthus reticulatus* of the Phyllanthaceae family, *Sapium integrerrimum* of the Euphorbiaceae and *Malleastrum rakotozafayi* of the Meliaceae family. By use of various isolation and purification techniques, a total of twenty-six compounds were isolated from the four plants, of which nine were unreported previously. The compounds belonged to four main classes: - Triterpenoids, diterpenoids, coumarin types and a tocopherol. 1D and 2D NMR, IR, MS, optical rotation and melting points were used in determining the structures of the compounds.

## **LIST OF ABBREVIATIONS**

<sup>1</sup> H NMR spectroscopy	Proton nuclear magnetic resonance spectroscopy
<sup>13</sup> C NMR spectroscopy	Carbon 13- nuclear magnetic resonance spectroscopy
COSY	Correlated spectroscopy
NOESY	Nuclear Overhauser effect spectroscopy
DEPT	Distortionless enhancement by polarisation transfer
HSQC	Heteronuclear multiple quantum coherence
HMBC	Heteronuclear multiple bond coherence
EIMS	Electron impact mass spectroscopy
HRMS	High resolution mass spectroscopy
FTIR	Fourier transformed infrared spectroscopy
UV	Ultra violet spectroscopy
FABMS	Fast atom bombardment mass spectrometry
TLC	Thin Layer Chromatography
Mp	Melting point
[α] <sub>D</sub>	Optical rotation
brs	Broad singlet
s	Singlet
d	Doublet
t	Triplet
q	Quartet
m	Multiplet
dd	Doublets of Doublets
Hz	Hertz
MHz	Mega Hertz
ppm	Parts per million
HBV	Hepatitis B virus
DPPH	2,2-diphenyl-1-picryl-hydrazyl
BHT	butylated hydroxyl toulene
RT	reverse transcriptase

W-M	Wagner- Meerwein
SAM	S-adenosylmethionine
Ad	adenosyl
PKC	protein kinase C
MIC	minimum inhibitory concentration
TPA	12- <i>O</i> -tetradecanoylphorbol -13-acetate
GGPP	geranylgeranyl pyrophosphate
DMAPP	dimethyl allyl diphosphate
IPP	isopentenyl diphosphate
GPP	geranyl diphosphate
FPP	farnesyl diphosphate
PP	diphosphate
TOF	time of flight
ES	electro spray
MeOH	methanol
DCM	dichloromethane
EtOAc	ethyl acetate
CDCl <sub>3</sub>	deuteriochloroform
<i>d</i> -DMSO	deuterio- dimethyl sulfoxide

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## **CHAPTER 1: A REVIEW OF THE PHYTOCHEMISTRY AND BIOACTIVITY OF PLANTS OF THE PHYLLANTHACEAE FAMILY**

### **1.1: The Phyllanthaceae family**

The Phyllanthaceae family was previously included in the Euphorbiaceae family as the Phyllanthoideae subfamily.<sup>1</sup> The Phyllanthaceae family was removed from the Euphorbiaceae family based on plastid *rbcL* DNA analysis. The Phyllanthaceae family comprises 60 genera and 2000 species and is scattered worldwide, mainly in the tropical and subtropical regions.<sup>1,2</sup>

### **1.2: The genus *Phyllanthus***

The genus *Phyllanthus* (usually referred to as the Leaf Flowers) consists of about 700 species which are distributed in the pantropics.<sup>1,3</sup> Plants of this family consist of trees, shrubs, annual or biennial herbs.<sup>4</sup> Four species from the *Phyllanthus* genus of the Phyllanthaceae family, namely *Phyllanthus myrtaceus*, *P. pinnatus*, *P. reticulatus* and *P. cedrelifolius* (also known *P. polyanthus*) are reported to occur in South Africa.<sup>5</sup> *P. cedrelifolius* and *P. reticulatus* were investigated in this study.

#### **1.2.1: Ethnobotany and biological screening of the genus *Phyllanthus***

Pharmacological, biochemical and clinical studies of plant extracts and compounds isolated from different species of the genus *Phyllanthus* have been undertaken. It is reported that the biological activities of the plant extracts and the pure compounds of this genus generally support their ethnopharmacological uses.<sup>6</sup> Plants of this genus have been used in folk medicine to cure, amongst others, kidney disorders, urinary bladder infections, intestinal infections, diabetes and hepatitis B.<sup>6</sup> *P. amarus* is reported to protect the liver from hepatocarcinogenesis that is induced by *N*-nitrosodiethylamine in animal models.<sup>8</sup> The roots of *P. acuminatus* were found to inhibit the growth of murine P-388 lymphocytic leukemia and B-16 melanoma cell lines.<sup>9,10</sup>

Muthu *et al.* reported that the aerial parts of *P. amarus* have been used ethnomedicinally in India and other tropical countries to treat various diseases and disorders such as jaundice, for which the

leaves of the plant are ground and mixed with a cup of cow or goat's milk and taken internally to treat jaundice.<sup>11</sup> The extracts of *P. amarus* cease the development of the hepatitis B virus (HBV) by blocking RNA polymerase, the enzyme needed for the virus to reproduce, therefore it possess antiviral properties.<sup>11</sup>

The whole plant of *P. emblica*, particularly its fruits, are widely used to treat diseases associated with atherosclerosis.<sup>12</sup> *P. urinaria* is often used traditionally in Asian countries for liver protecting, diabetes, hepatitis, jaundice and dropsy.<sup>13,65</sup> Sheng *et al.* reported that *P. urinaria* is widely used in Asia. It was tested *in vivo* in mice for its anti-tumour and anti-angiogenic effects. Oral administration showed significant inhibition of tumour development with lower occurrence and a remarked reduction in tumour size.<sup>14</sup> *P. urinaria* has been reported to have anti-HBV effects.<sup>7</sup>

*P. niruri* is a medicinal plant used to treat various diseases in different regions, and is used as a laxative, to treat diuretic disorders, hepatitis B, tuberculosis, gonorrhoea and diarrhoea, as an antispasmodic, against constipation, against fever including malaria, and to treat tuberculosis, vaginitis and stomach ache.<sup>15</sup>

The hot water leaf decoction of *P. oxyphyllus* is used to bath new born babies, as an anti-infective and also to relieve fever and gonorrhoea.<sup>15,57</sup> The dichloromethane extracts of the root of *P. oxyphyllus* caused 100% death to *Artemia salina* nauplii (brine shrimps) at 100 ppm concentration and was found to exhibit radical scavenging properties when tested against the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical.<sup>16,57</sup>

Plant extracts of *P. maderaspatensis* are used by traditional healers in India to treat liver disorders and the hexane extract of the same plant has been screened and showed significant hepatoprotection against the toxins carbon tetrachloride and thioacetamide that induce liver damage in rats.<sup>17</sup> Kuman *et al.* evaluated the methanol extracts of *P. debilis*, *P. urinaria*, *P. virgatus*, *P. maderaspatensis* and *P. amarus* for anti-oxidative effects and the results were compared to the

standard antioxidants, butylated hydroxy toluene (BHT) and ascorbic acid. *P. debilis* was found to show strong antioxidant activity.<sup>18</sup> *P. piscatorum* is used by ethnic groups of the Amazon for its piscicidal activity. Its methanol extract showed weak activity against *Plasmodium falciparum*.<sup>19</sup>

### **1.3: Review of previous phytochemical investigations of plants of the genus *Phyllanthus***

Plants of the genus *Phyllanthus* accumulate secondary metabolites belonging to various classes of compounds. The phytochemical analysis of several species of the genus *Phyllanthus*, such as *P. niruri*, *P. urinaria*, *P. emblica*, *P. flexuosus*, *P. sellowianus* and *P. reticulatus* has yielded a wide range of alkaloids, coumarins, phenolics (such as flavonoids, lignans, and tannins), mono-, di- and triterpenoids and other simple aromatic compounds.<sup>6</sup>

#### **1.3.1: Alkaloids isolated from *Phyllanthus* species**

Few alkaloids have been isolated from the genus *Phyllanthus*, and they belong to the indolizidine and pyrrolizidine classes as shown in Table 1.5.1 and Table 1.5.2. The alkaloids viroallosecurinine and securinine from *P. discoideus* were found to exhibit antibacterial activity.<sup>20</sup>

Phyllathine, securinine, norsecurinine, *epibubbialine* and *bubbialine* were isolated from *P. amarus*.<sup>24</sup> 14,15-Dihydroallosecurinine, viroallosecurinine and norsecurinine alkaloids were isolated from *P. discoideus*.<sup>20</sup> From the plant *P. simplex*, a pyrrolizidine alkaloid simplexine was isolated.<sup>23</sup> The pyrrolizidine alkaloid nirurine was isolated from *P. niruri*<sup>22</sup> and the pyrrolizidine alkaloid niruroide was isolated from *P. niruroides*.<sup>21</sup>

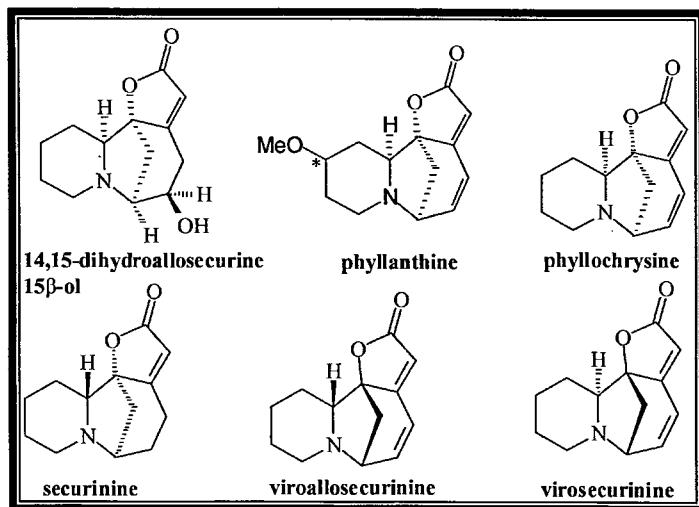


Figure 1.1: Structures of indolizidine alkaloids isolated from *Phyllanthus* species

Table 1.1: Indolizidine alkaloids isolated from *Phyllanthus* species

Plant name	Name of compound
<i>P. discoideus</i> <sup>20</sup>	14,15-dihydroallosecurinine <sup>20</sup>
<i>P. simplex</i> , <sup>23</sup> <i>P. amarus</i> <sup>24</sup>	phyllanthine
<i>P. discoideus</i> , <sup>20</sup> <i>P. amarus</i> <sup>24</sup>	securinine
<i>P. discoideus</i> <sup>20</sup>	viroallosecurinine <sup>20</sup>

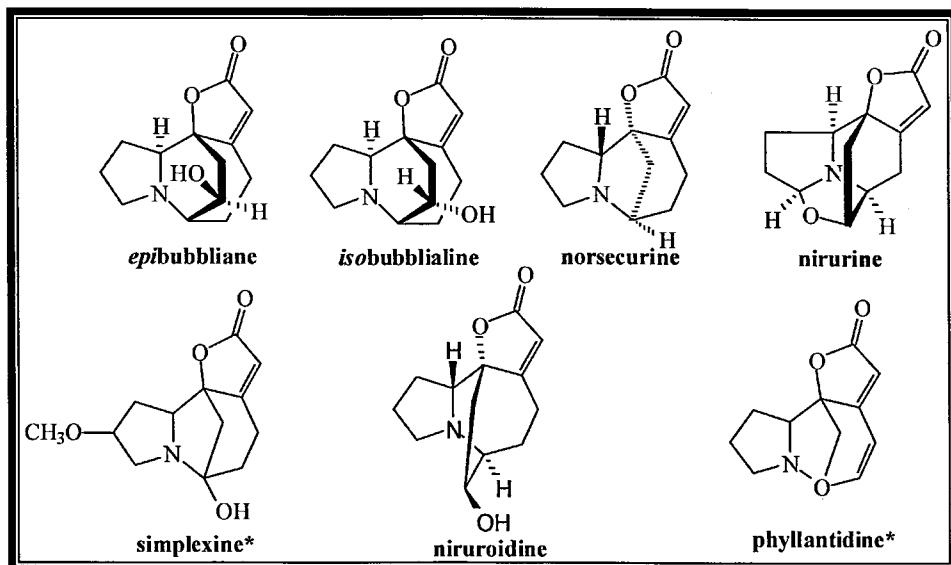


Figure 1.2: Structures of pyrrolizidine alkaloids isolated from *Phyllanthus* species. (\* authors did not indicate stereochemistry)

**Table 1.2: Pyrrolizidine alkaloids isolated from *Phyllanthus* species**

Plant Name	Name of compound
<i>P. amarus</i> <sup>24</sup> , <i>P. discoideus</i> <sup>20</sup>	norsecurinine
<i>P. amarus</i> <sup>24</sup>	epibubbialine
<i>P. amarus</i> <sup>24</sup>	isobubbialine
<i>P. niruri</i> <sup>22</sup>	nirurine <sup>22</sup>
<i>P. niruroides</i> <sup>21</sup>	niruroidine <sup>21</sup>
<i>P. simplex</i> <sup>23</sup>	simplexine <sup>23</sup>

The alkaloid, phyllanthimide was isolated from the *Phyllanthus sellowianus*.

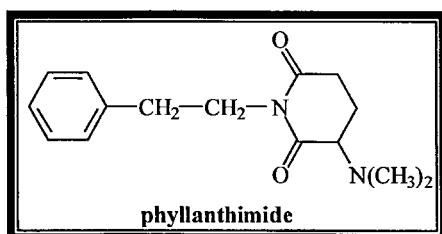


Figure 1.3: Structure of phyllanthimide isolated from *Phyllanthus sellowianus*<sup>25</sup>

#### 1.4: Aromatic compounds

Coumarins have been isolated from *Phyllanthus* species. Bergenin, a glycoside of 4-O-methyl gallic acid was isolated from *P. ussuriensis*<sup>26</sup> and was found to show anti-arthritis activity,<sup>27</sup> moderate anti-oxidant properties,<sup>28</sup> anti-HIV activity,<sup>29</sup> hepatoprotective<sup>30</sup> and anti-inflammatory effects.<sup>31</sup> Bergenin was also found to show antihepatotoxic properties against CCl<sub>4</sub>-induced cytotoxicity in primary cultured rat hepatocytes.<sup>32</sup>

Scopoletin (6-methoxy-7-hydroxycoumarin), a common coumarin, was isolated from *P. sellowianus*.<sup>33</sup> Scopoletin was found to exhibit anti-inflammatory activity.<sup>33,34</sup> The coumarins, brevifolin carboxylic methyl ester and ellagic acid, were isolated from *P. urinaria*.<sup>35,37</sup> *P. emblica* yielded chebulic acid.<sup>36</sup>

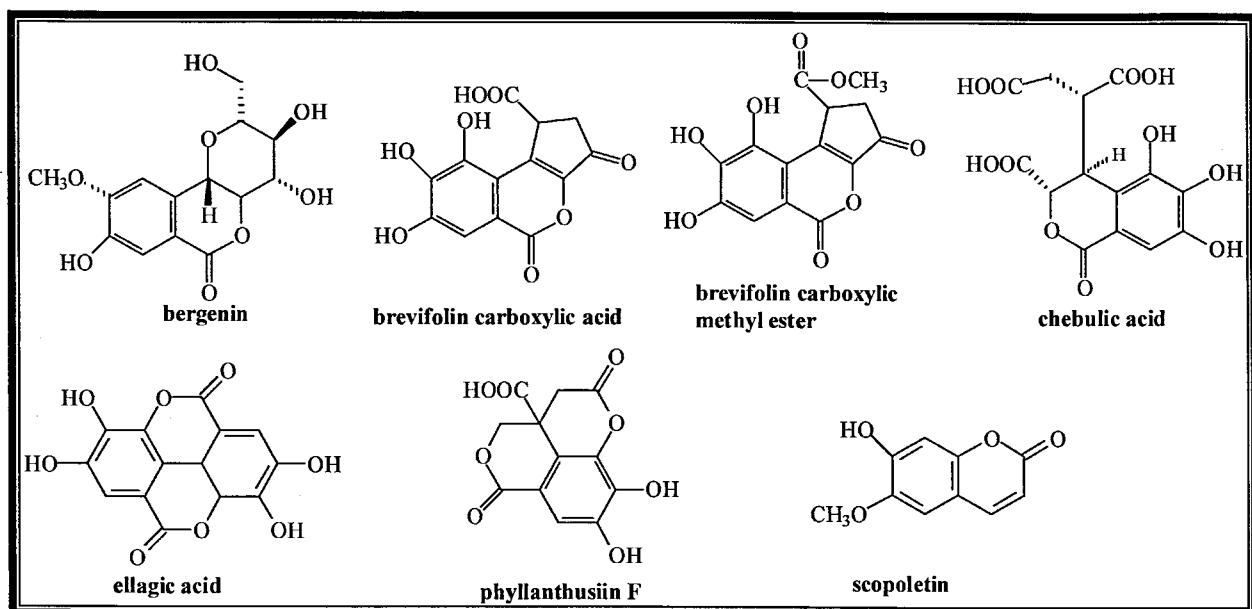


Figure 1.4: Coumarins isolated from *Phyllanthus* species.

**Table 1.3: Aromatic compounds from *Phyllanthus* species**

Plant name	Name of compound
<i>P. ussuriensis</i>	bergenin <sup>26</sup>
<i>P. urinaria</i> <sup>35</sup>	brevifolin carboxylic acid <sup>35</sup>
<i>P. emblica</i>	chebulic acid <sup>36</sup>
<i>P. urinaria</i>	ellagic acid <sup>37</sup>
<i>P. sellowianus</i>	scopoletin <sup>33</sup>
<i>P. flexuosus</i>	phyllanthusiin F <sup>36</sup>

## 1.5: Flavonoids

Flavonoids are known to contribute to colour in plants: yellow colouration due to chalcones and flavonols, red, blue and violet from anthocyanidins.<sup>39</sup> There are more than 6000 flavonoids identified. The flavonoids show antioxidative effects *in vitro* and are also involved in protection against cardiovascular diseases.<sup>38,40</sup> Flavonoids are also reported to neutralize free radicals in various models.<sup>39</sup> The plants of the Phyllanthaceae family have yielded different classes of flavonoids such as flavonols, flavones and anthocyanins.

### 1.5.1: Flavones

A prenylated flavone aglycone, nirurinetin, was isolated from *Phyllanthus niruri*.<sup>41</sup>

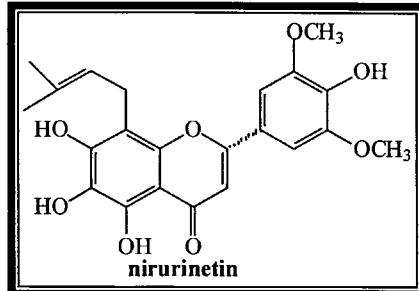


Figure 1.5: Structure of flavones isolated from *Phyllanthus niruri*.

### 1.5.2: Flavonols

The flavonol quercetin has been isolated from a number of *Phyllanthus* species, such as *P. emblica*.<sup>42</sup> This compound is known to be a powerful anti-oxidant, chelating metals and scavenging free radicals. It is found in red wine and studies suggest that it gives protection against cardiovascular disease<sup>46,39</sup> and has anti-inflammatory properties.<sup>47</sup> Kaempferol is also found in red wine and has been found to exhibit antioxidant and anti-inflammatory properties.<sup>39,48</sup>

Rutin (quercetin3-rhamnosyl glucoside), a common flavonoid which was also isolated from *P. virgatus*,<sup>43</sup> was found to exhibit antioxidant properties *in vitro*.<sup>51</sup> The flavonols astragalin, galangin-8-sulfonate, galangin-3- $\beta$ -glucoside-8-sulfonate, kaempferol-8-sulfonate, kaempferol-3-*O*-rhamnoside, quecetin, quercetin, rutin and myricitrin, were isolated from *P. embica* and *P. virgatus*.<sup>42,43</sup>

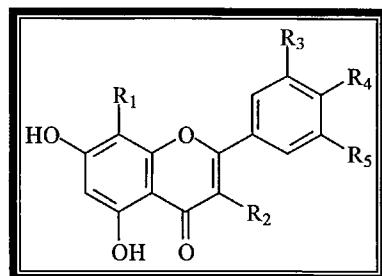


Figure 1.6: Flavonols isolated from *Phyllanthus* species

**Table 1.4: Flavonols from *Phyllanthus* species**

Plant source	Compound name	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>
<i>P. tenellus, P. virgatus</i>	astragalin <sup>43, 44</sup>	H	Glu	OH	H	H
<i>P. virgatus</i>	galangin-8-sulfonate <sup>43</sup>	SO <sub>3</sub> Na	OH	H	H	H
<i>P. virgatus</i>	galangin-3-O-β-glucoside-8-sulfonate <sup>43</sup>	SO <sub>3</sub> Na	Glu	H	H	H
<i>P. virgatus P. emblica</i>	kaempferol <sup>42, 43</sup>	H	OH	H	OH	H
<i>P. matsumare</i>	hyperoside	H	RhaGlu	OH	OH	H
<i>P. tenellus, P. virgatus</i>	myricitrin <sup>43, 44</sup>	H	Glu	OH	OH	OH
<i>P. takaoensis</i>	nicotiflorin	H	Rha	H	OH	H
<i>P. emblica P. virgatus</i>	isoquercetin <sup>43</sup>	H	Glu	OH	OH	H
<i>P. emblica, P. virgatus</i>	quercetin <sup>42, 43</sup>	H	OH	H	H	OH
<i>P. emblica, P. tenellus, P. virgatus</i>	quecetrin <sup>42, 43, 44</sup>	H	Rha	OH	OH	H
<i>P. virgatus</i>	rutin <sup>43</sup>	H	RhaGlu	OH	OH	H

### 1.5.3: Dihydroflavonols

The dihydroflavonols, catechin, gallicatechin and epicatechin were isolated from *P. emblica*.<sup>50</sup>

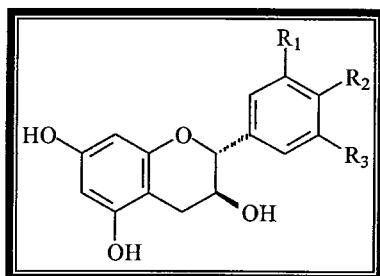


Figure 1.7: Dihydroflavonols isolated from *Phyllanthus* species

**Table 1.5: Dihydroflavonols from *Phyllanthus* species**

Plant name	Compound name	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
<i>P. emblica</i>	catechin <sup>50</sup>	OH	OH	H
<i>P. emblica</i>	gallicatechin <sup>50</sup>	OH	OH	OH

### 1.5.4: Epidihydroflavonols

The epidihydroflavonols, epicatechin, epiafzalechin, epigallocatechin and epigallocatechin-3-O-gallate were isolated from the plant species, *P. emblica* and *P. urinaria*.<sup>45,50</sup> Epigallocatechin which was isolated from *P. emblica*<sup>50</sup> was found to exhibit anti-allergic properties.<sup>53</sup>

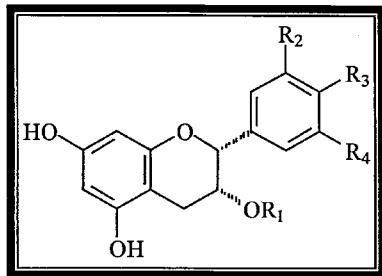


Figure 1.8: Epidihydroflavonols isolated from *Phyllanthus* species

Table 1.6: Epidihydroflavonols from *Phyllanthus* species

Plant name	Compound name	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>
<i>P. emblica, P. niruri</i>	epicatechin <sup>45, 50</sup>	H	OH	OH	H
<i>P. emblica</i>	epiafzalechin <sup>45</sup>	H	H	OH	OH
<i>P. emblica, P. niruri</i>	epigallocatechin <sup>45, 50</sup>	H	OH	OH	OH
<i>P. emblica, P. niruri</i>	epigallocatechin-3-O-gallate <sup>50</sup>	gallate	OH	OH	OH

### 1.5.5: Flavanones

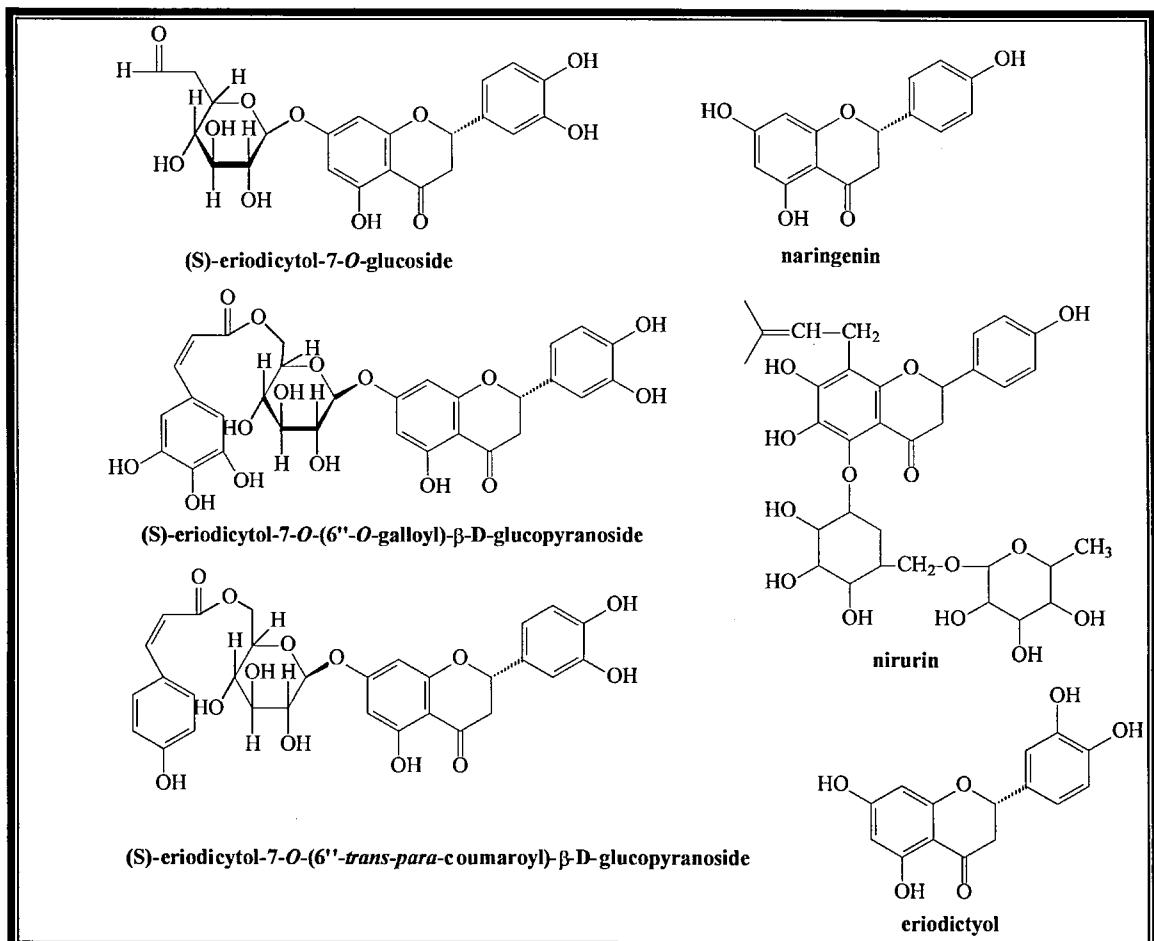


Figure 1.9: Flavanones isolated from *Phyllanthus* species.

(S)-Eriodictyol-7-O-(6''-O-galloyl)- $\beta$ -D-glucopyranoside, (S)-eriodictyol-7-O-(6''-trans-para-coumaroyl)- $\beta$ -D-glucopyranoside, eriodictyol-7-O- $\beta$ -D-glucoside and naringenin were isolated from *P. emblica*.<sup>42</sup> Naringenin was found to have anti-inflammatory properties.<sup>49</sup>

**Table 1.7: Flavanones isolated from *Phyllanthus* species**

Plant source	Name of compound
<i>P. emblica</i>	eriodictyol <sup>42</sup>
<i>P. emblica</i>	(S)-eriodictyol-7-O-(6''-O-galloyl)- $\beta$ -D-glucopyranoside <sup>42</sup>
<i>P. emblica</i>	(S)-eriodictyol-7-O-(6''-trans-para-coumaroyl)- $\beta$ -D-glucopyranoside <sup>42</sup>
<i>P. emblica</i>	eriodictyol-7-O- $\beta$ -D-glucoside <sup>42</sup>
<i>P. emblica</i>	naringenin <sup>42</sup>
<i>P. niruri</i>	nirurin <sup>41</sup>

### 1.5.5: Dihydroflavonols

Zhang *et al.* isolated dihydroflavonols, proanthocyanidin gallates, prodelphinidin B-1 and B-2, epicatechin(4 $\beta$ →8) epigallocatechin and prodelphinidin β-2-3'O-gallate from *P. emblica*<sup>45</sup> and prodelphinidin A-1 was isolated from *P. emblica*.<sup>50</sup> Prodelphinidin β-2,3'-O-gallate was found to possess anti-viral activity.<sup>52</sup>

**Table 1.8: Flavanoids isolated from *Phyllanthus* species.**

Plant source	Name of compound
<i>P. emblica</i>	catechin-(4-β-8) epi-gallocatechin <sup>50</sup>
<i>P. emblica</i>	epicatechin-(4-β→8) epigallocatechin-3-O-gallate <sup>50</sup>
<i>P. emblica</i>	prodelphinidin A-1 <sup>50</sup>
<i>P. emblica</i>	prodelphinidin B-1 <sup>45</sup>
<i>P. emblica</i>	prodelphinidin B-2 <sup>45</sup>
<i>P. emblica</i>	prodelphinidin B-2,3'-O-gallate <sup>45</sup>

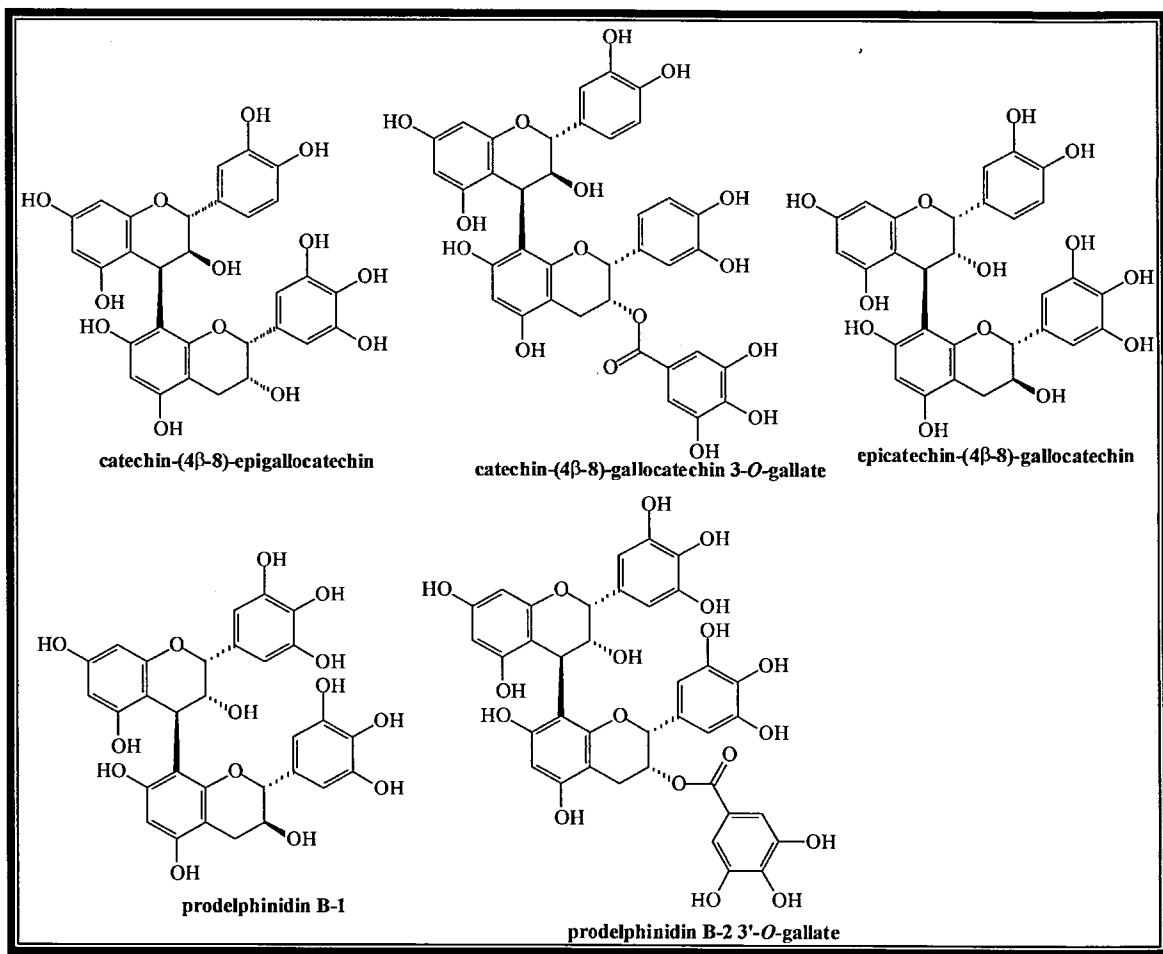


Figure 1.10: Flavanoids isolated from *Phyllanthus* species

## 1.6: Lignans

Lignans are derived biosynthetically from the aromatic amino acid phenylalanine.<sup>54</sup> Plants of the genus *Phyllanthus* have yielded a series of bioactive lignans. Many lignans isolated from plants have been reported to exhibit antitumour and antiviral activities. These activities include reverse transcriptase, integrase, and topoisomerase inhibition.<sup>57,64</sup> Furthermore some lignans show anti-hepatitis<sup>69</sup> and antioxidant properties.<sup>70</sup> Phyllamycin A, B, C, D, E and F were isolated from *P. myrtifolius*.<sup>58,66</sup> Phyllamycoside A, B and C were isolated from the same source.<sup>58</sup> Phyllanthin, a common lignan has been found in many *Phyllanthus* species, such as *P. urinaria*, *P. niruri* and *P. amarus*.<sup>59,62,65</sup>

*P. urinaria* yielded the lignans, 5-demethoxyniranthin, dextrobursehernin, heliophthalmin lactone, urinaligran, virgatusin and urinatetralin.<sup>65</sup> The lignans, retrojusticidin, isolintetralin, niranthin, and nirtetralin, were isolated from *P. urinaria*, *P. virgatus* and *P. niruri*.<sup>59,60,65</sup> The lignans retrojusticidin B and phyllamyricin B, which were isolated from *P. myrtifolius* were found to have high selectivity towards the HIV-1 reverse transcriptase enzyme.<sup>91</sup> Seco-isolariciresinol ( $I_{50} = 0.017 \pm 0.001$  Mm), isolated from *P. oxyphyllus*, was tested for its anti-oxidative properties using DPPH stable radicals and was found to show stronger anti-oxidative effects than the standard 2,6-di-tert-butyl-4-methylphenol (BHT) ( $I_{50} = 0.031 \pm 0.001$  mM).<sup>57</sup>

Phyllanthin and hypophyllanthin, isolated from *P. amarus*, were found to enhance the cytotoxic response against cultured multi-drug resistant cells.<sup>62</sup> Phyllanthostatin A, a lignan glycoside, was isolated from the roots of *P. acuminatus* and was found to prevent growth of PS leukemia cells *in vitro* at a concentration of  $ED_{50} = 4$  µg/ml.<sup>64</sup>

Phyllamyricin, isolated from *P. myrtifolius*, showed a weak anti-HIV 1 reverse transcriptase (RT) activity. Phyllamyricoside B and C were found to be inactive, however, phyllamyricoside A was found to increase the activity of HIV 1 RT by 65% at a concentration of 1.89 µM.<sup>58</sup> Isolintetralin was isolated from *P. niruri*.<sup>59</sup> Piscatorin and justicidin B, isolated from *P. piscatorum*, were found to have antifungal, antiprotozoal, cytotoxic and piscicidal properties.<sup>61</sup> The lignans niranthin, nirtetralin, hinokinin and phyltetralin showed significant anti-hepatitis activities.<sup>69</sup> Hinokinin was found to exhibit strong anti-oxidant activity.<sup>70</sup>

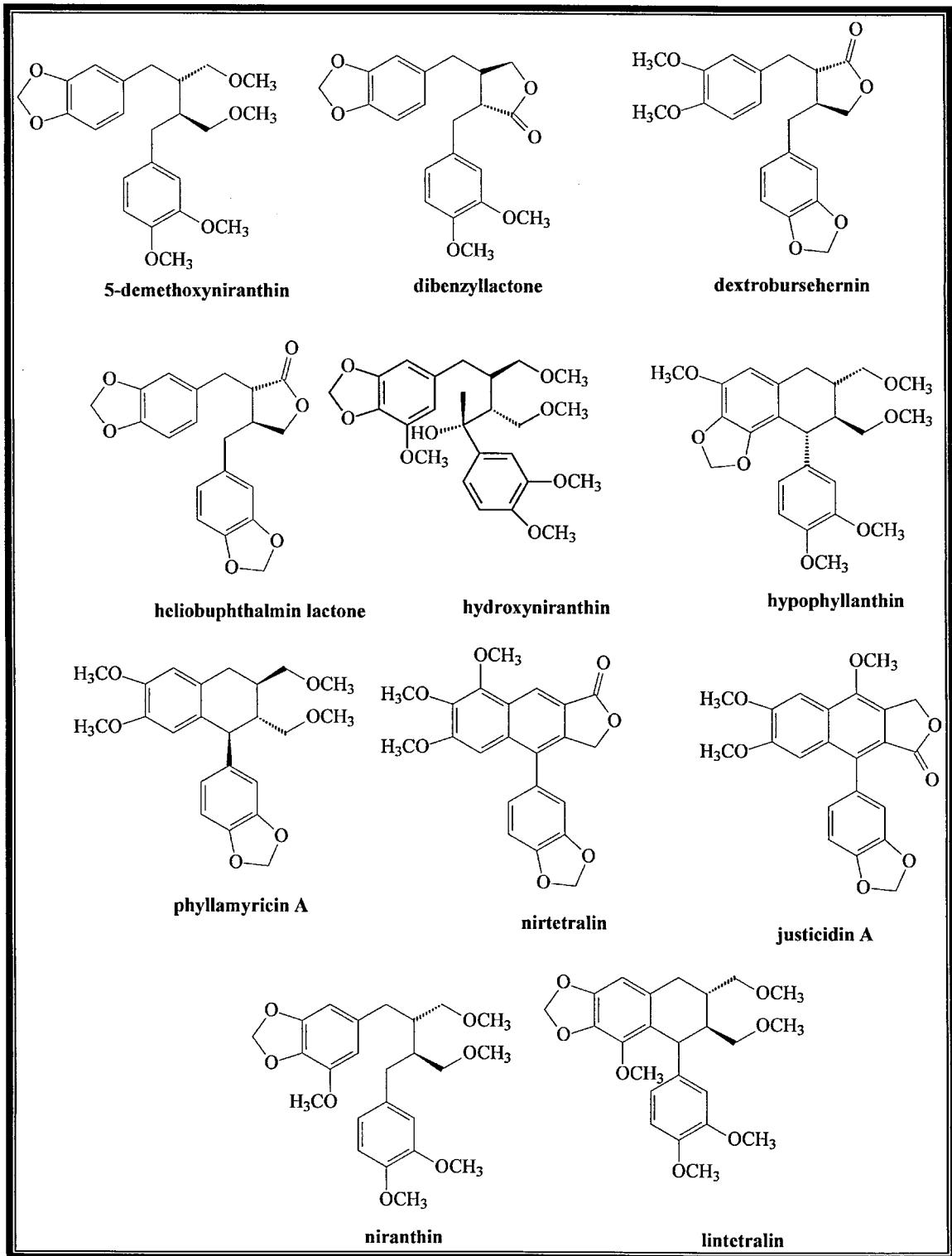


Figure 1.11: Lignans isolated from *Phyllanthus* species

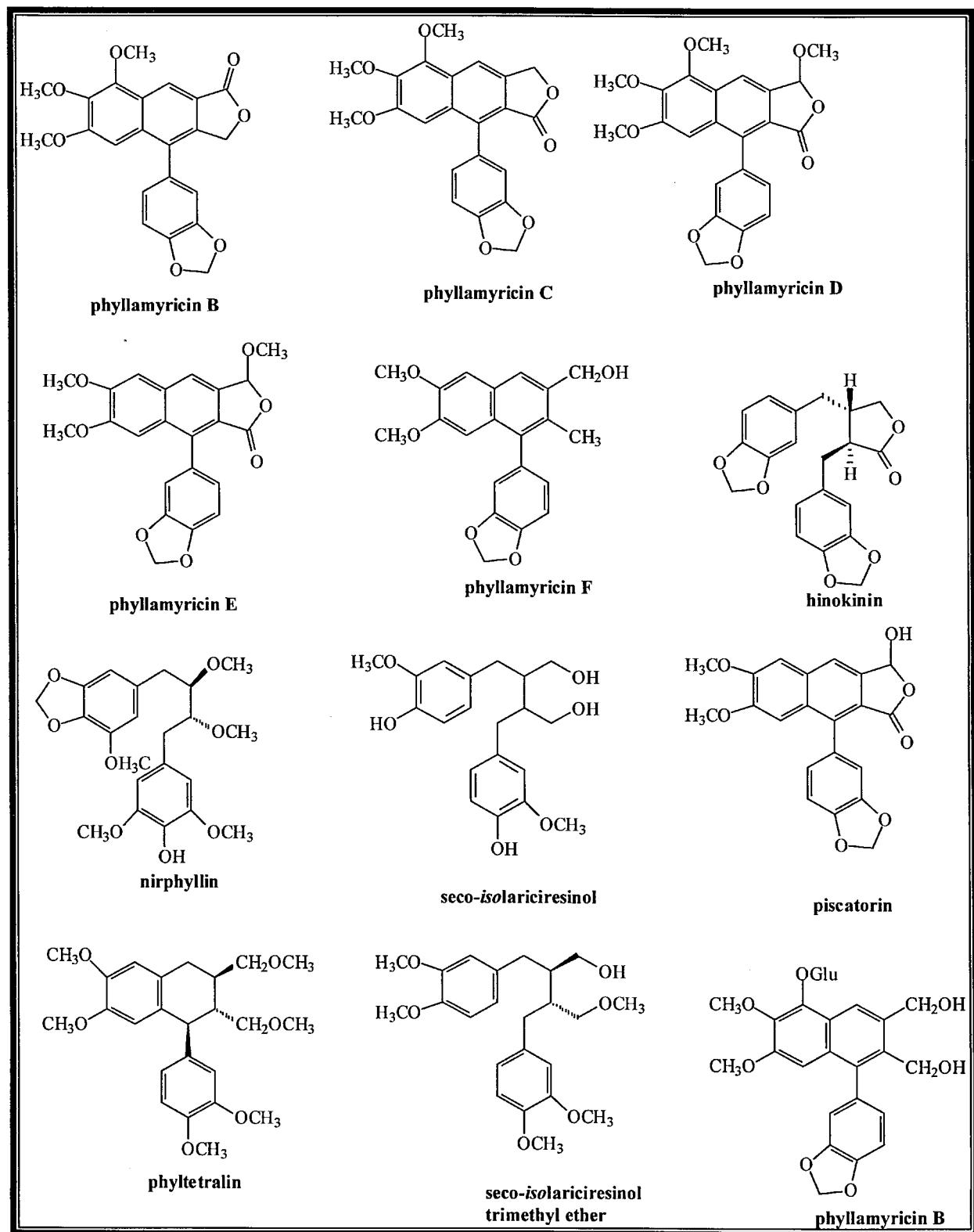


Figure 1.12: Lignans isolated from *Phyllanthus* species

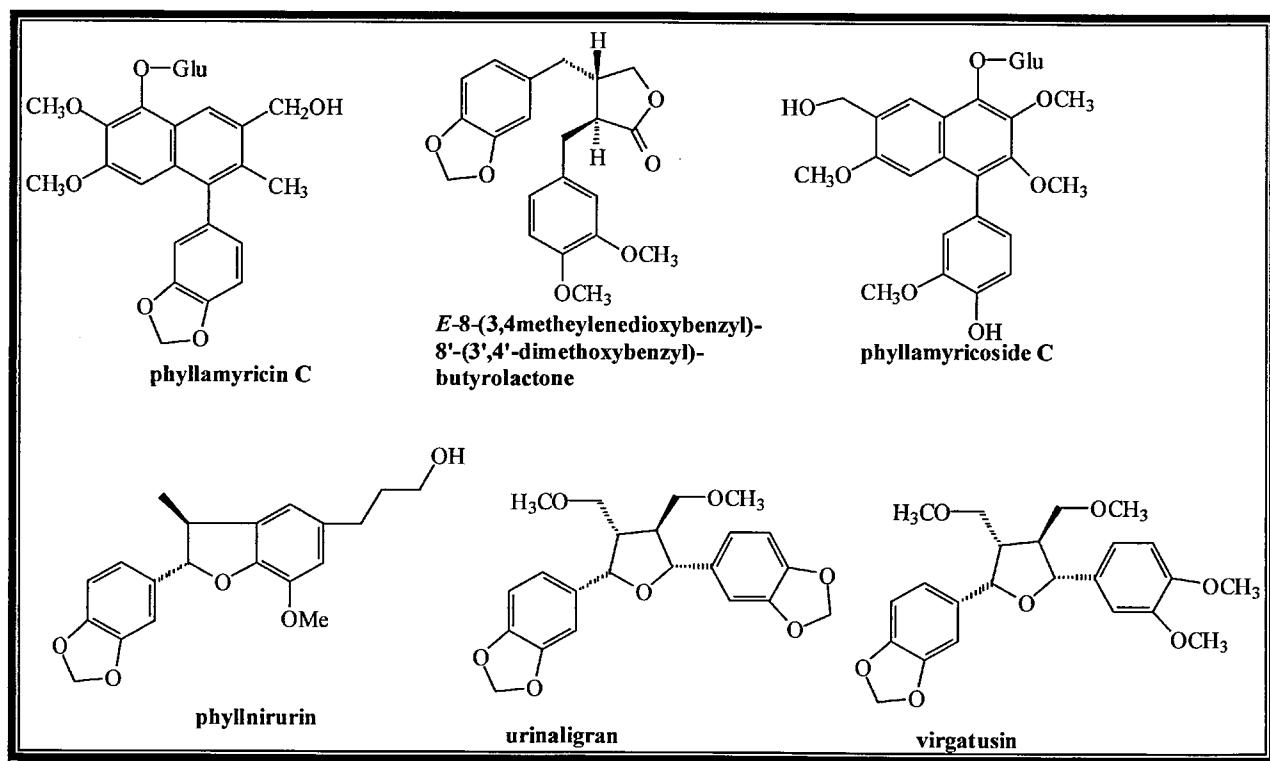


Figure 1.13: Lignans isolated from *Phyllanthus* species

Table 1.9: Lignans isolated from *Phyllanthus* species

Plant source	Name of compound
<i>P. urinaria</i> <sup>65</sup>	5-demethoxyniranthin
<i>P. urinaria</i> <sup>65</sup>	dextrobursehernin
<i>P. urinaria</i> <sup>65</sup>	heliothupthalmin lactone
<i>P. amarus</i> , <sup>62</sup> <i>P. niruri</i> , <sup>59</sup> <i>P. virgatus</i> <sup>60</sup>	hypophyllanthin
<i>P. myrtifolius</i> <sup>66</sup>	justicidin A
<i>P. myrtifolius</i> , <sup>66</sup> <i>P. anisolobus</i> , <sup>55</sup> <i>P. piscatorum</i> , <sup>61</sup> <i>P. acuminatus</i> <sup>63, 64</sup>	justicidin B
<i>P. myrtifolius</i> <sup>66</sup>	justicidin
<i>P. urinaria</i> , <sup>65</sup> <i>P. virgatus</i> , <sup>60</sup> <i>P. niruri</i> <sup>59</sup>	lintetralin
<i>P. urinaria</i> , <sup>65</sup> <i>P. virgatus</i> , <sup>60</sup> <i>P. niruri</i> <sup>59</sup>	niranthin
<i>P. urinaria</i> , <sup>65</sup> <i>P. niruri</i> , <sup>59</sup> <i>P. virgatus</i> <sup>60</sup>	nirtetralin
<i>P. niruri</i> , <sup>59</sup> <i>P. virgatus</i> <sup>60</sup>	hinokin
<i>P. myrtifolius</i> <sup>66</sup>	phyllamyrycin A
<i>P. myrtifolius</i> <sup>66</sup>	phyllamyrycin B
<i>P. myrtifolius</i> <sup>66</sup>	phyllamyrycin C
<i>P. myrtifolius</i> <sup>58</sup>	phyllamyrycin D
<i>P. myrtifolius</i> <sup>58</sup>	phyllamyrycin E
<i>P. myrtifolius</i> <sup>58</sup>	phyllamyrycin F

<i>P. myrtifolius</i> <sup>58</sup>	phyllamyricoside A
<i>P. myrtifolius</i> <sup>58</sup>	phyllamyricoside B
<i>P. myrtifolius</i> <sup>58</sup>	phyllamyricoside C
<i>P. urinaria</i> , <sup>65</sup> <i>P. niruri</i> , <sup>59</sup> <i>P. amarus</i> <sup>62</sup>	phyllanthin
<i>P. anisolobus</i> , <i>P. acuminatus</i> <sup>64</sup>	phyllanthostatin A
<i>P. urinaria</i> <sup>65</sup>	phytetalrin
<i>P. niruri</i> <sup>68</sup>	<i>seco</i> -4-hydroxyltetralin, hydroxyniranthin
<i>P. niruri</i> <sup>56</sup>	<i>seco</i> -isolariciresinol trimethyl ether
<i>P. urinaria</i> <sup>65</sup>	urinaligran
<i>P. piscatorum</i> <sup>61</sup>	piscatorum
<i>P. virgatus</i> , <sup>60</sup> <i>P. urinaria</i> <sup>65</sup>	virgatusin
<i>P. urinaria</i> <sup>65</sup>	urinatetralin
<i>P. oxyphyllus</i> <sup>57</sup>	<i>seco</i> -isolariciresinol
<i>P. oxyphyllus</i> <sup>57</sup>	pinoresinol

## 1.7: Terpenoids

A wide variety of terpenoids have been isolated from the Phyllanthaceae family.

### 1.7.1: Diterpenoids

Diterpenoids belonging to the cleistanthane and kaurane classes have been isolated from the *Phyllanthus* genus.

#### 1.7.1.1: Cleistanthane class

*P. oxyphyllus* yielded the rare cleinstanthane diterpenoids, spruceanol and cleistanthol. The two compounds have significant antioxidant properties.<sup>57</sup>

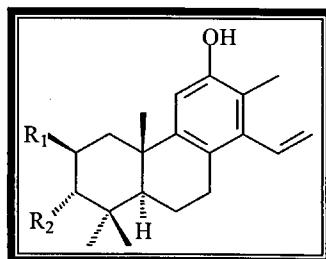


Figure 1.14: Cleistanthane diterpenoids isolated from *Phyllanthus oxyphyllus*

**Table 1.10: Cleistanthane diterpenoids isolated from *Phyllanthus oxyphyllus***

Plant source	Name of compound	R <sub>1</sub>	R <sub>2</sub>
<i>P. oxyphyllus</i> <sup>57</sup>	spruceanol	OH	OH
<i>P. oxyphyllus</i> <sup>57</sup>	cleistanthol	H	OH

### 1.7.2: Sesquiterpenes

Zhang *et al.* isolated phyllaemblicin A, B, C and phyllaemblic acid methyl ester, a highly oxygenated norbisabolane sesquiterpenoid, from the roots of *P. emblica*.<sup>50</sup> The bisabolane type sesquiterpenoids phyllaemblic acids B, C and D, were isolated from *P. emblica* and possesses an aglycone like that of the antineoplastic phyllanthostatins 1- 6, but showed no cytotoxic activity.<sup>71</sup> The antineoplastic agents phyllanthostatin 1 and phyllanthoside were isolated from *P. acuminatus* and were found to inhibit the growth of murine P388 lymphocytic leukemia and were also seen to significantly retard the progression of murine B16 melanoma.<sup>63</sup>

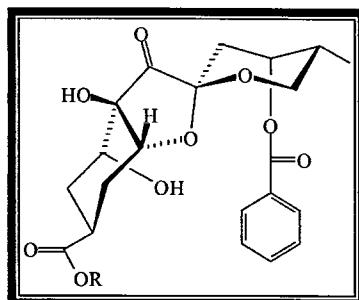


Figure 1.16: Sesquiterpenoids isolated from *Phyllanthus emblica*

**Table 1.11: Sesquiterpenes isolated from *Phyllanthus emblica***

Plant name	Compound name	R
<i>P. emblica</i> <sup>50,72</sup>	phyllaemblic acid	H
<i>P. emblica</i> <sup>50</sup>	phyllaemblic methyl ester	CH <sub>3</sub>
<i>P. emblica</i> <sup>50</sup>	phyllaemblicin A	Glu
<i>P. emblica</i> <sup>50</sup>	phyllaemblicin B	3xGlu acetate
<i>P. emblica</i> <sup>50</sup>	phyllaemblicin C	2xGlu

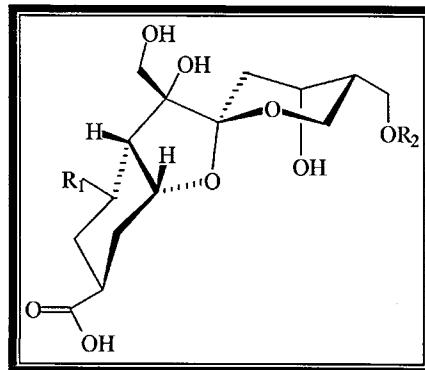


Figure 1.17: Sequiterpenoids isolated from *Phyllanthus emblica*

**Table 1.12: Sesquiterpenes isolated from *Phyllanthus emblica***

Plant source	Compound name	R <sub>1</sub>	R <sub>2</sub>
<i>P. emblica</i> <sup>71</sup>	phyllaemblic acid B	OH	H
<i>P. emblica</i> <sup>71</sup>	phyllaemblic acid C	H	H
<i>P. emblica</i> <sup>71</sup>	phyllaemblic acid D	H	Glu

The sesquiterpenoids phyllanthoside, dideacetylphyllanthoside, phyllanthostatin 1, 2, 3, 4, 5, 6 and didephyllanthostatin 3, were isolated from *P. acuminatus*.<sup>63,73,74</sup> Phyllanthostatin 6, a sesquiterpene glycoside which was isolated from the root of *P. acuminatus*, was found to inhibit growth of the murine P 388 lymphocytic leukaemia cell line ( $ED_{50} = 0.354 \mu\text{g/ml}$ ).<sup>74</sup> It was reported that phyllanthusol A and B, isolated from *P. acidus*, were cytotoxic.<sup>72</sup> Phyllanthoside and phyllanthostatin 1, 2, and 3 were found to possess antineoplastic activity, inhibiting growth of the murine P388 lymphocytic leukaemia.<sup>75</sup>

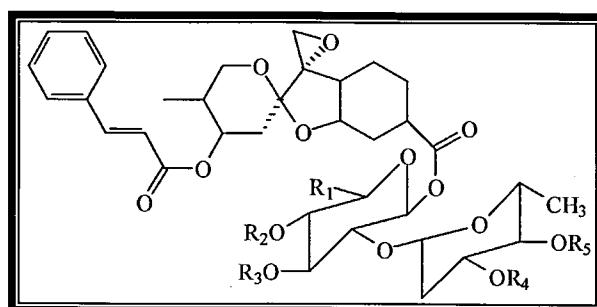


Figure 1.18: Sequiterpenoids isolated from *Phyllanthus* species

**Table 1.13: Sesquiterpenes isolated from *Phyllanthus* species**

Plant source	Name of compound	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>
<i>P. acuminatus</i> , <sup>63, 74, 75</sup> <i>P. brasiliensis</i>	phyllanthoside	CH <sub>3</sub>	H	Ac	Ac	H
<i>P. acuminatus</i> , <sup>63,74</sup>	phyllanthostatin 1	CH <sub>3</sub>	Ac	H	Ac	H
<i>P. acuminatus</i> , <sup>63,74</sup>	phyllanthostatin 2	CH <sub>2</sub> OH	H	Ac	Ac	H
<i>P. acuminatus</i> , <sup>63,74</sup>	phyllanthostatin 3	CH <sub>2</sub> OH	H	H	H	H
<i>P. acuminatus</i> <sup>74</sup>	phyllanthostatin 4	CH <sub>3</sub>	H	Ac	H	Ac
<i>P. acuminatus</i> <sup>74</sup>	phyllanthostatin 5	CH <sub>3</sub>	Ac	H	H	Ac
<i>P. acuminatus</i> <sup>74</sup>	phyllanthostatin 6	CH <sub>2</sub> OH	H	H	H	H
<i>P. acuminatus</i> <sup>74</sup>	didephyllanthostatin 3	CH <sub>2</sub> OH	H	Ac	Ac	H

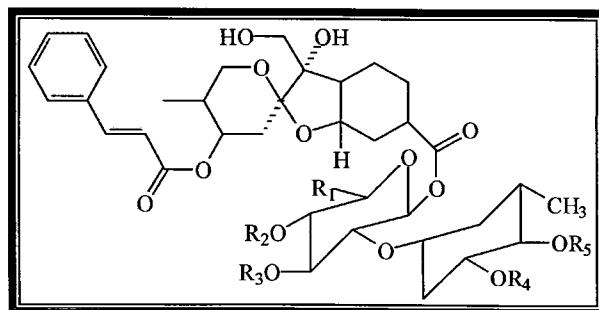


Figure 1.19: Sesquiterpenoid isolated from *Phyllanthus acuminatus*

**Table 1.14: Sesquiterpenes isolated from *Phyllanthus acuminatus***

Plant source	Name of compound	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>
<i>P. acuminatus</i> <sup>75</sup>	didephyllanthostatin 3	CH <sub>2</sub> OH	H	Ac	Ac	H

Phyllanthusol A and B, which are norbisabolane glycosides, were isolated from *P. acidus* and were found to have cytotoxic activity.<sup>72</sup>

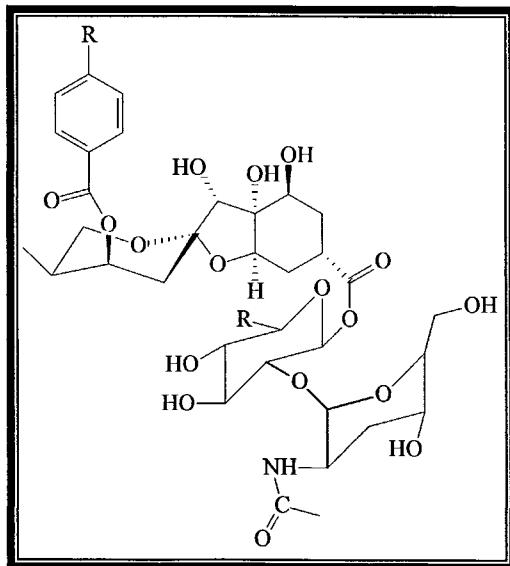


Figure 1.20: Sesquiterpenoids isolated from *Phyllanthus acidus*.<sup>72</sup>

**Table 1.15:** Sesquiterpenes isolated from *Phyllanthus acidus*

Plant source	Name of compound	R <sub>1</sub>
<i>P. acidus</i> <sup>73</sup>	phyllanthusol A	OH
<i>P. acidus</i> <sup>73</sup>	phyllanthusol A	H

Phyllanthocin, a bisabolane aglycone, was isolated from *P. brasiliensis*.<sup>73</sup> and descinnamoylphyllanthocindiol was isolated from *P. acuminatus*.<sup>75</sup>

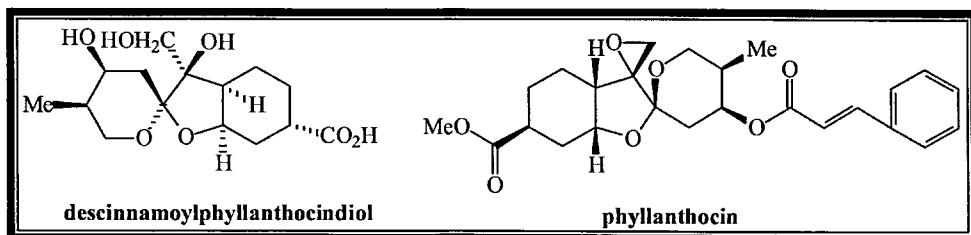


Figure 1.21: Sesquiterpenoids isolated from *Phyllanthus* species<sup>73,74</sup>

**Table 1.16:** Sesquiterpenes isolated from *Phyllanthus* species

Plant source	Name of compound
<i>P. acuminatus</i> <sup>75</sup>	descinnamoylphyllanthocindiol
<i>P. brasiliensis</i> <sup>74,75</sup>	phyllanthocin

### 1.7.3: Triterpenoids

Pentacyclic triterpenoids are known to exhibit a wide range of pharmacological and other biological activities, which include antioxidant, anti-allergic, anti-inflammatory, anti-tumour, gastroprotective, antibacterial, and hepatoprotective effects.<sup>76</sup> Lupane, oleanane, fridelane, glutinane and ursane triterpenoids have been isolated from the genus *Phyllanthus* of the Phyllanthaceae family.<sup>94</sup>

#### 1.7.3.1: Friedelane class

A friedelane triterpenoid, friedelin, isolated from different sources of the genus *Phyllanthus*, such *P. watsonii* and *P. flexuosus* was reported to have diuretic activity.<sup>89</sup> 3-Oxo-friedelan-29-oic acid was isolated from the plant *P. oxyphyllus*.<sup>57</sup>

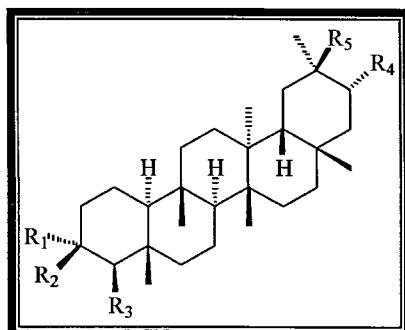


Figure 1.22: Friedelane triterpenoids isolated from the *Phyllanthus* species

**Table 1.17: Friedelane triterpenoids isolated from *Phyllanthus* species**

Plant source	Name of compound	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>
<i>P. flexuosus</i> , <sup>77</sup> <i>P. watsonii</i> , <sup>79</sup> <i>P. acidus</i> , <sup>85</sup> <i>P. reticulatus</i> <sup>78</sup>	friedelin	-	=O	CH <sub>3</sub>	H	CH <sub>3</sub>
<i>P. watsonii</i> <sup>79</sup>	<i>epi</i> -friedelanol	H	OH	CH <sub>3</sub>	H	CH <sub>3</sub>
<i>P. reticulatus</i> <sup>78</sup>	21α-hydroxyfriedelan-4(23)-en-3-one	-	=O	=CH <sub>2</sub>	OH	CH <sub>3</sub>
<i>P. oxyphyllus</i> <sup>57</sup>	3-oxofriedelan-29-oic acid	-	=O	CH <sub>3</sub>	H	COOH

*P. watsonii* afforded 26-nor-D:A-friedoolean-14-en-3-one and 26-nor-D:A-friedoolean-14-en-3β-ol.<sup>79</sup>

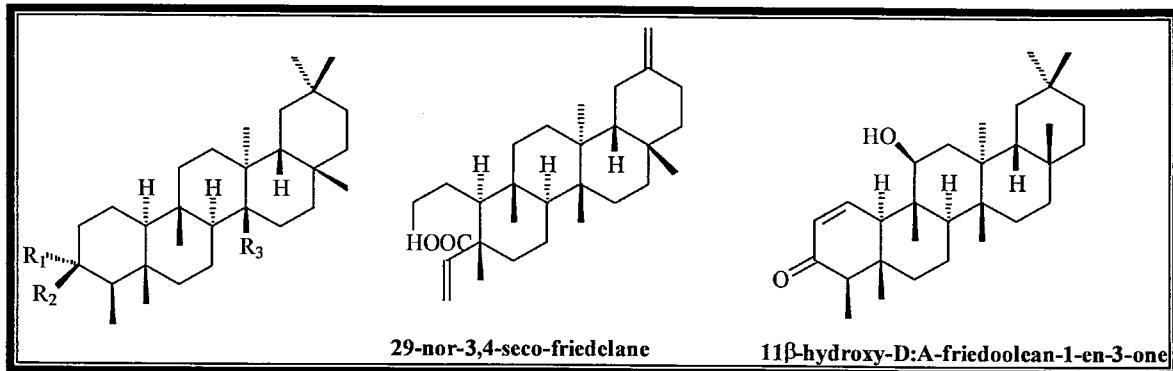


Figure 1.23: Friedelane triterpenoids isolated from *Phyllanthus* species

**Table 1.18: Friedelane triterpenoids isolated from *Phyllanthus* species**

Plant name	Compound name	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
<i>P. watsonii</i> <sup>79</sup>	26-nor-D:A-friedoolean-14-en-3-one	-	=O	CH <sub>3</sub>
<i>P. watsonii</i> <sup>79</sup>	26-nor-D:A-friedoolean-14-en-3 $\beta$ -ol	H	OH	CH <sub>3</sub>
<i>P. oxyphyllus</i> <sup>57</sup>	29-nor-3,4-seco-friedelane	-	-	-
<i>P. flexuosus</i> <sup>81</sup>	11 $\beta$ -hydroxy-D:A-friedoolean-1-en-3-one	-	=O	-
<i>P. flexuosus</i> <sup>83</sup>	trichadenic acid B	H	OH	COOH

### 1.7.3.2: Lupane class

Lupeol has been isolated from different plant species of the genus *Phyllanthus* such as *P. flexuosus*,<sup>77</sup> *P. watsonii*<sup>79</sup> and *P. oxyphyllus*.<sup>57</sup> Betulin, lupeol, and lup-20(29)-ene-3 $\beta$ ,24-diol,<sup>80</sup> were isolated from the stem bark of *P. flexuosus*. These were found to be selective inhibitors of human Topo II activity with an IC<sub>50</sub> value in the range 10-39  $\mu$ M.<sup>84</sup> Lupeol has been found to have antiangiogenic<sup>85</sup> and anti-inflammatory activities.<sup>86</sup> Betulinic acid has been found to show anti-allergic activities *in vitro*.<sup>87</sup> Betulinic acid, a common pentacyclic triterpenoid isolated from *P. discoideus*, was reported to have anti-HIV activity and specific cytotoxicity against a variety of tumour cell lines.<sup>88</sup>

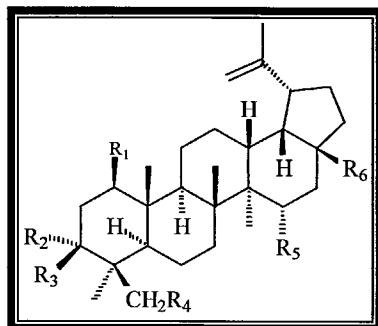


Figure 1.24 Lupane triterpenoids from *Phyllanthus* species

**Table 1.19: Lupane triterpenoids isolated from *Phyllanthus* species**

Plant source	Name	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>
<i>P. flexuosus</i> <sup>80</sup>	betulin	H	H	OH	H	H	CH <sub>2</sub> OH
<i>P. flexuosus</i> , <sup>76</sup> <i>P. watsonii</i> , <sup>79</sup> <i>P. oxyphyllus</i> <sup>57</sup>	lupeol	H	H	OH	H	H	H
<i>P. watsonii</i> , <sup>79</sup> <i>P. reticulatus</i> <sup>78</sup>	glochidonol	OH	=O	-	H	H	H
<i>P. watsonii</i> , <sup>79</sup>	glochidiol	OH	H	OH	H	H	H
<i>P. flexuosus</i> , <sup>77</sup> <i>P. watsonii</i> , <sup>79</sup>	glochidone	H	=O	-	H	H	H
<i>P. flexuosus</i> <sup>81</sup>	lup-20(29)-ene-3 $\beta$ , 15 $\alpha$ -diol	H	H	OH	H	OH	H
<i>P. flexuosus</i> <sup>80</sup>	lup-20(29)-ene-3 $\beta$ , 24-diol	H	H	OH	OH	H	H

### 1.7.3.3: Oleanane class

The oleanane pentacyclic triterpenoid, olean-12-ene-3 $\beta$ -,15 $\alpha$ -diol, was found to possess *in vitro* anti-tumour promoting activity. Olean-12-ene-3 $\beta$ -24-diol and olean-12-ene-3 $\beta$ ,15 $\alpha$ ,24-triol were identified as selective inhibitors of human Topo II<sup>84</sup> and were isolated from the bark of *P. flexuosus*.<sup>80,81</sup>  $\beta$ -Amyrin was found to suppress the scratching behaviour in a mouse model of pruritus, the mouse itching was induced by dextran T40 and results showed clearly the anti-pruritic effects.<sup>76</sup>

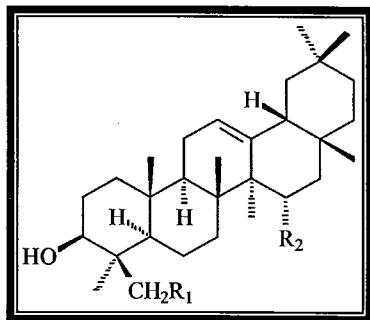


Figure 1.25: Oleanane triterpenoids isolated from *Phyllanthus* species

**Table 1.20:** Oleanane triterpenoids isolated from *Phyllanthus* species

Plant source	Name of compound	R <sub>1</sub>	R <sub>2</sub>
<i>P. flexuosus</i> <sup>77</sup>	β-amyrin	H	H
<i>P. flexuosus</i> <sup>79,81</sup>	olean-12-ene-3β,15α-diol	H	OH
<i>P. flexuosus</i> <sup>80,81</sup>	olean-12-ene-3β,24-diol	OH	H
<i>P. flexuosus</i> <sup>80,81</sup>	olean-12-ene-3β,15α-24-triol	OH	OH

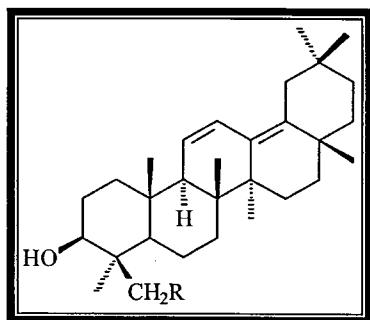


Figure 1.26: Oleanane triterpenoids isolated from *Phyllanthus* species

**Table 1.21:** Oleanane triterpenoids isolated from *Phyllanthus flexuosus*

Plant name	Name of compound	R
<i>P. flexuosus</i> <sup>77</sup>	oleana-11:13 (18)-diene-3β, 24-diol	OH
<i>P. flexuosus</i> <sup>77</sup>	olean-11:13 (18)-dien-3β-ol	H

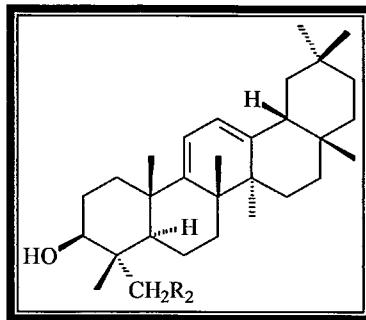


Figure 1.27: Oleanane triterpenoids isolated from *Phyllanthus flexuosus*

**Table 1.22:** Oleanane triterpenoids isolated from *Phyllanthus flexuosus*

Plant source	Name of compound	R
<i>P. flexuosus</i> <sup>81</sup>	olean-9(11),12-dien-3 $\beta$ -ol	H
<i>P. flexuosus</i> <sup>81</sup>	olean-9(11),12-diene-3 $\beta$ , 24-diol	OH

#### 1.7.3.4: Ursane class

The ursane triterpenoid, phyllanthol, was isolated from *P. engleri* and *P. acidus*.<sup>90,91</sup> No biological activity was reported.

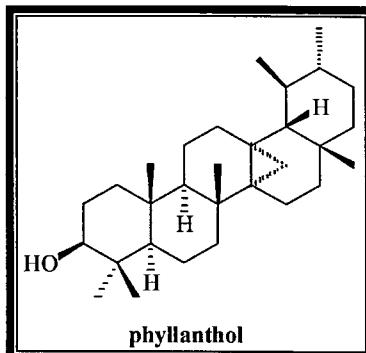


Figure 1.28: Phyllanthol from *Phyllanthus* species

**Table 1.23:** Ursane triterpenoids isolated from *Phyllanthus* species

Plant source	Name of compound
<i>P. engleri, P. acidus</i> <sup>90,91</sup>	phyllanthol

### 1.7.3.5: Glutinane class

Three glutinane type triterpenoids were isolated from *P. myrtifolius*.<sup>92</sup> No biological activities were reported.

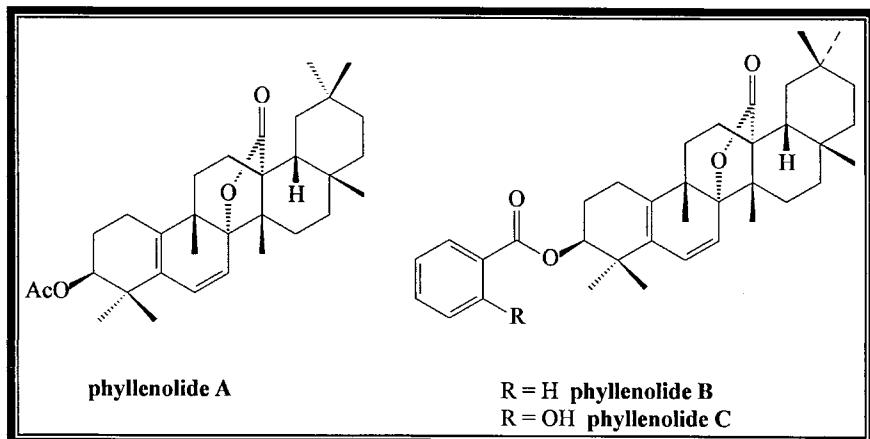


Figure 1.29: Glutinane triterpenoids isolated from *Phyllanthus* species

**Table 1.24: Glutinane triterpenoids isolated from *Phyllanthus* species**

Plant name	Name of compound
<i>P. myrtifolius</i> <sup>92</sup>	phyllenolide A
<i>P. myrtifolius</i> <sup>92</sup>	phyllenolide B
<i>P. myrtifolius</i> <sup>92</sup>	phyllenolide C

### 1.7.3.6: Other triterpenoids isolated from *Phyllanthus* species

An acyclic triterpenoid was isolated from *P. niruri*.<sup>93</sup> No bioactivities were reported. Ocotillo-II was isolated from *P. flexuosus*.<sup>82</sup>

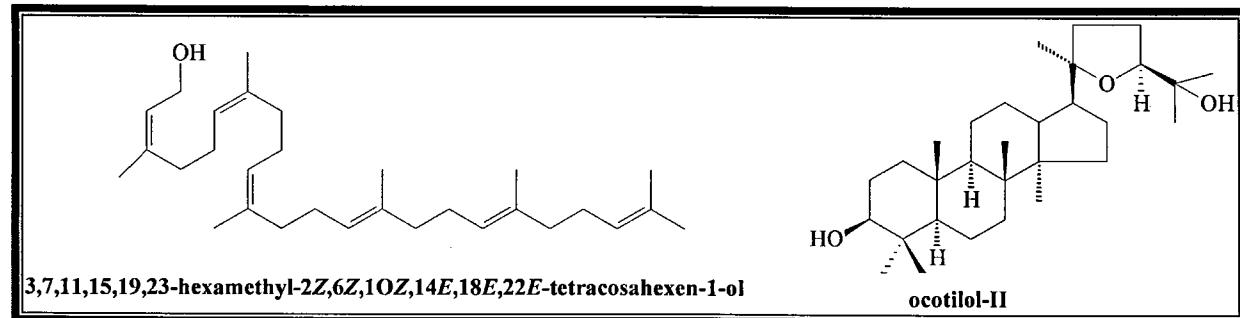


Figure 1.30: Triterpenoids isolated from *Phyllanthus* species

**Table 1.25: Triterpenoids isolated from *Phyllanthus* species**

Plant source	Name of compound
<i>P. flexuosus</i> <sup>82</sup>	ocotilol-II
<i>P. niruri</i> <sup>93</sup>	3,7,11,15,19,23-hexamethyl-2Z,6Z,10Z,14E,18E,22E-tetracosahexen-1-ol

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## **CHAPTER 2: A PHYTOCHEMICAL INVESTIGATION OF THREE MEMBERS OF THE PHYLLANTHACEAE FAMILY. *PHYLLANTHUS CEDRELIFOLIUS*, *PHYLLANTHUS RETICULATUS* AND *HEYWOODIA LUCENS***

### **2.1: A PHYTOCHEMICAL INVESTIGATION OF *PHYLLANTHUS CEDRELIFOLIUS* (PHYLLANTHACEAE FAMILY)**

#### **2.1.1: Introduction**

*Phyllanthus cedrelifolius* Verdoorn (syn *P. polyanthus*), also known as the forest potato bush, is a small deciduous plant, 3-5 m in height, but sometimes growing to 9 m. The stem bark is dark, rough and warty. Leaves are alternate and grow along slender branchlets up to 60 cm long, they occur as leaflets of large pinnate leaves and are hairless, thin textured, shiny and fresh green in colour. The flowers are small, yellowish green, growing in clusters which are made up of one female flower and several male flowers. Fruits are almost spherical or a rather flattened capsule which are up to 5 cm in diameter.<sup>1</sup>



Picture 2.1 *Phyllanthus cedrelifolius* (Photo: Prof. Neil Crouch)

The main objective of this study was to investigate the phytochemistry of *P. cedrelifolius*.

The hexane extract of the stem bark of *P. cedrelifolius* yielded compounds **2.3** (13, 27-cycloursan-3 $\beta$ -ol (phyllanthol)), **2.4** (13,27-cycloursan-3-one (phyllanthone)) and **2.5** (3 $\beta$ -acetoxyolean-13(18)-ene ( $\delta$ -amyrin acetate)), and the dichloromethane extract yielded compounds **2.1** ((20*S*)-3 $\beta$ -acetoxy-24-methylidenedammaran-20-ol) and **2.6** lupenone (3-oxolup-20(29)-ene). The combined hexane and dichloromethane extracts of the leaves of *P. cedrelifolius* yielded compounds **2.2** ((20*S*)-3 $\alpha$ -acetoxy-24-methylidenedammaran-20-ol), **2.3** (phyllanthol) and **2.6** (lupenone).

The ethyl acetate extract of the leaves yielded compound **2.2** ((20*S*)-3 $\alpha$ -acetoxy-24-methylidenedammaran-20-ol).

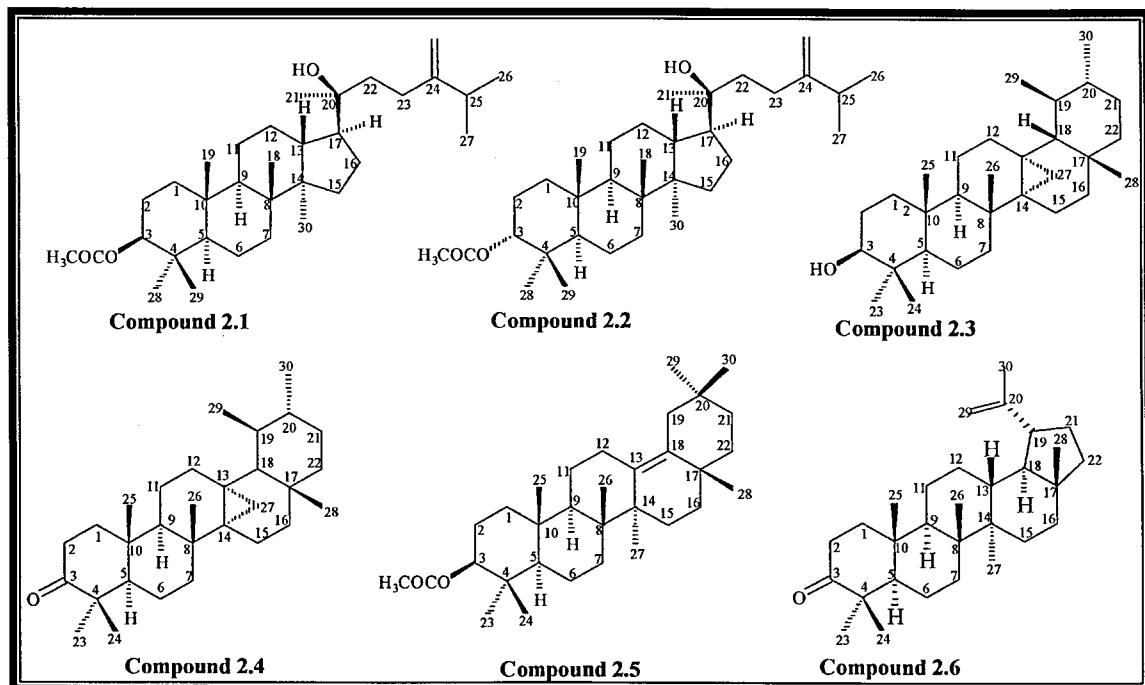
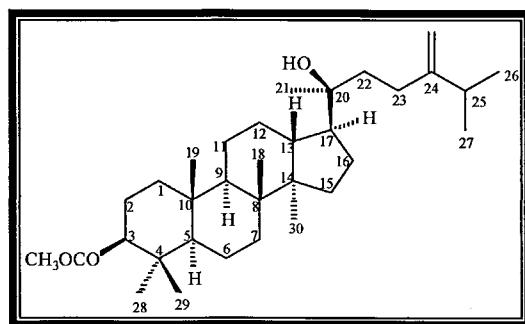


Figure 2.1: Triterpenoids from *P. cedrelifolius*

## 2.1.2: Triterpenoids from *Phyllanthus cedrelifolius*

### 2.1.2.1: Structural elucidation of compound 2.1: (20S)-3 $\beta$ -acetoxy-24-methylidenedammaran-20-ol



Compound 2.1

The TOF-MS ES<sup>+</sup> spectrum of compound 2.1 showed a [M+H]<sup>+</sup> ion peak at *m/z* 501.4303, which corresponded to a molecular formula of C<sub>33</sub>H<sub>57</sub>O<sub>3</sub> [M+H]<sup>+</sup>, and C<sub>33</sub>H<sub>56</sub>O<sub>3</sub> for the compound. A double bond equivalence of six was deduced. The low resolution mass spectrum showed fragment ions at [M-15]<sup>+</sup> (loss of CH<sub>3</sub>), [M-18]<sup>+</sup> (loss of H<sub>2</sub>O) and [M-60]<sup>+</sup> (loss of CH<sub>3</sub>COOH). The FTIR spectrum showed the carbonyl stretch of an acetate group at 1726 cm<sup>-1</sup>, a hydroxyl stretch at 3298 cm<sup>-1</sup> and CH stretches at 2923 and 2858 cm<sup>-1</sup>.<sup>2,3</sup>

The <sup>1</sup>H NMR spectrum indicated that compound 2.1 had an acetate ester present by the presence of a three proton resonance seen at  $\delta$  2.04 in the <sup>1</sup>H NMR spectrum. Subtracting two carbons for the acetate group from the thirty-three carbons shown in the mass spectrum left a thirty-one carbon molecule, suggesting a methylated triterpenoid. The presence of the eight methyl groups and a terminal methylene group in the molecule supported the presence of a methylated triterpenoid.

A resonance at  $\delta$  4.45 (dd, *J* = 10.65, *J* = 5.75 Hz) in the <sup>1</sup>H NMR spectrum was assigned as H-3 and corresponded to the C-3 resonance  $\delta$  81.1 in the <sup>13</sup>C NMR spectrum. The coupling constants indicated that H-3 was in the  $\alpha$ -orientation.<sup>4</sup> The acetate group was placed at C-3 due to the correlation seen in the HMBC spectrum between the acetate group carbonyl

carbon resonance at  $\delta$  171.2 and the H-3 resonance. The C-3 carbon resonance showed correlations in the HMBC spectrum with the 3H-28 and 3H-29 methyl group proton resonances ( $\delta$  0.82,  $\delta$  0.85), the H-5 methine proton resonance ( $\delta$  0.83) and with the two H-1 methylene proton resonances ( $\delta$  1.66,  $\delta$  1.02), which showed coupling with the superimposed H-2 methylene proton resonances at  $\delta$  1.61 in the COSY spectrum.

The H-1 proton resonances showed correlations with the carbon resonances ascribed to the C-19 methyl group carbon ( $\delta$  15.7), the C-9 methine carbon ( $\delta$  50.8), the C-5 methine carbon ( $\delta$  56.2), the C-8 fully substituted carbon ( $\delta$  40.6) and the C-3 carbon resonance, previously assigned, in the HMBC spectrum. The C-9 carbon resonance showed correlations in the HMBC spectrum with the two methylene proton resonances at  $\delta$  1.72 and  $\delta$  1.49, which were ascribed to 2H-12, which showed coupling with the two H-11 methylene proton resonances at  $\delta$  1.49 and  $\delta$  1.25 in the COSY spectrum and also with the H-13 methine proton resonance at  $\delta$  1.63. More correlations were observed in the COSY spectrum between the H-13 methine resonance and the H-17 methine proton resonance at  $\delta$  1.76, which was seen to be further coupled with the two H-16 methylene proton resonances ( $\delta$  1.81,  $\delta$  1.27), which, in turn, were seen to be coupled with the two H-15 methylene proton resonances at  $\delta$  1.45 and  $\delta$  1.07. The H-15 resonances were not further coupled.

The H-17 proton resonance showed correlations in the HMBC spectrum with a methyl group carbon resonance ascribed to C-21 ( $\delta$  25.6), the C-22 methylene carbon resonance ( $\delta$  39.7), and a fully substituted carbon resonance which could be ascribed to C-20 ( $\delta$  75.5).<sup>7</sup> The two H-22 proton resonances ( $\delta$  1.59) showed coupling in the COSY spectrum with the 2H-23 ( $\delta$  2.09) proton resonances. The corresponding C-23 carbon resonance at  $\delta$  28.6, showed correlations with the two terminal methylene proton resonances H-24A and H-24B ( $\delta$  4.72,  $\delta$  4.66) and with the H-25 methine proton resonance at  $\delta$  2.27 in the HMBC spectrum.

The H-25 methine resonance showed coupling in the COSY spectrum with the 3H-27 and 3H-26 methyl group proton resonances ( $\delta$  1.04, d,  $J$  = 6.82 Hz). A literature search indicated that compound **2.1** is similar to (20S)-5 $\alpha$ -dammar-24-en-3 $\beta$ -20-diol isolated by Asakawa from *Gingseng*<sup>5</sup> and the two related dammaranes previously isolated from *Securinega*

*melanthesoides* (Eurphorbiaceae) by Schutz *et al.*,<sup>6</sup> namely *trans*-securinegin [(20*S*)-24-methylidenedammaran-3 $\alpha$ -yl (2*E*)-3-(4-hydroxyphenyl)-2-propenoate ( $[\alpha]_D = +9.7^\circ$ ) and *cis*-securinegin [(20*S*)-24-methylidenedammaran-3 $\alpha$ -yl (2*Z*)-3-(4-hydroxyphenyl)-2-propenoate ( $[\alpha]_D = -8.8^\circ$ )].<sup>5,6</sup>

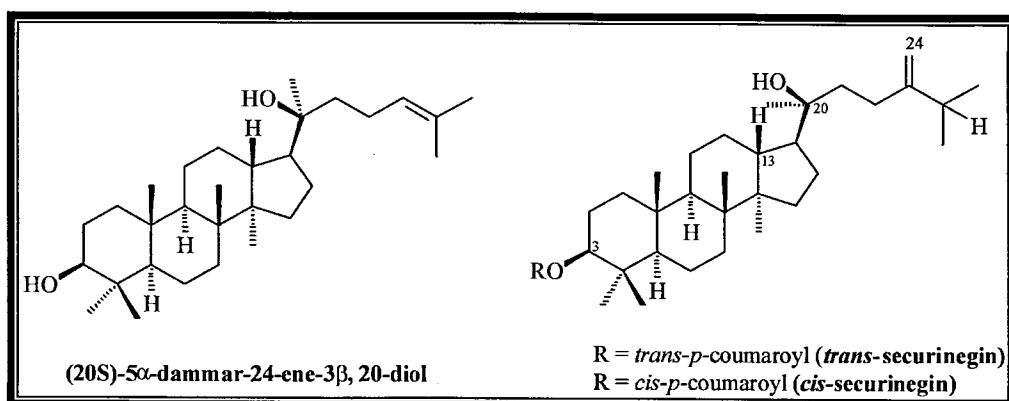


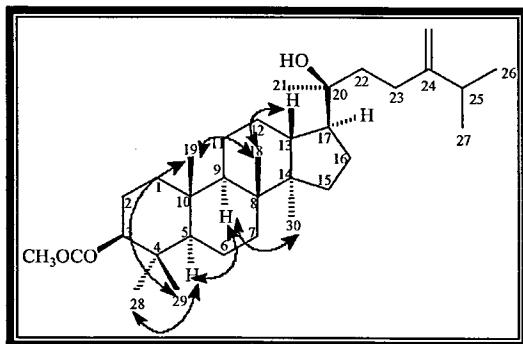
Figure 2.2 Related dammaranes from literature<sup>6,7</sup>

Asakawa *et al.*<sup>5</sup> and Schutz *et al.*<sup>6</sup> have investigated the effects of the stereochemistry at C-20 on the  $^{13}\text{C}$  shifts of the C-21 and C-22 resonances. Asakawa *et al.* compared the shifts of C-21 and C-22 for C-20 epimers of dammaranes with either 3 $\beta$ -hydroxy or 3-keto groups. Schutz *et al.* used Asakawa's conclusion to determine the stereochemistry at C-20 as *S* for *cis* and *trans*-securinegin as shown in the Table 2.1 below.

**Table 2.1: Comparison of  $^{13}\text{C}$  NMR values for C-21 and C-22 of 20*R* and 20*S* 20-hydroxy-dammaranes.<sup>5,6</sup>**

	20 <i>S</i> <sup>5</sup>	20 <i>S</i> <sup>5</sup>	20 <i>R</i> <sup>5</sup>	20 <i>R</i> <sup>5</sup>	20 <i>S</i> - <i>trans</i> -securinegin <sup>6</sup>	Compound 2.1
	3 $\beta$ -OH	3=O	3 $\beta$ -OH	3=O		
C-21	24.9	24.7	23.5	23.5	25.3	25.6
C-22	40.5	40.5	41.8	41.8	39.4	39.7

By comparing values of compound 2.1 against those of Asakawa *et al.*<sup>5</sup> and Schutz *et al.*<sup>6</sup> It was concluded that the 20*S* stereochemistry was present in compound 2.1.



### Correlations seen in the NOESY spectrum of compound 2.1

The NOESY spectrum confirmed the dammarane stereochemistry. A correlation was seen in the NOESY spectrum between the H-5 methine proton resonance and the H-9 methine resonance and the 3H-28 resonance. The H-9 resonance showed a further correlation with the 3H-30 proton resonance establishing this methyl group as  $\alpha$ . The 3H-29 resonance showed a correlation with the 3H-19 resonance which showed a correlation with the 3H-18 resonance, which, in turn, showed a correlation with the H-13 resonance establishing all these to be in the  $\beta$ -orientation. As expected, no correlations were seen between the H-13 and the 3H-30 resonances, nor between the H-13 and the H-17 resonances. A NOESY correlation was observed between the H-17 methine and 3H-21 methyl group proton resonances. Compound 2.1, (20*S*)-3 $\beta$ -acetoxy-24-methylidenedammarane-20-ol has not been reported previously.

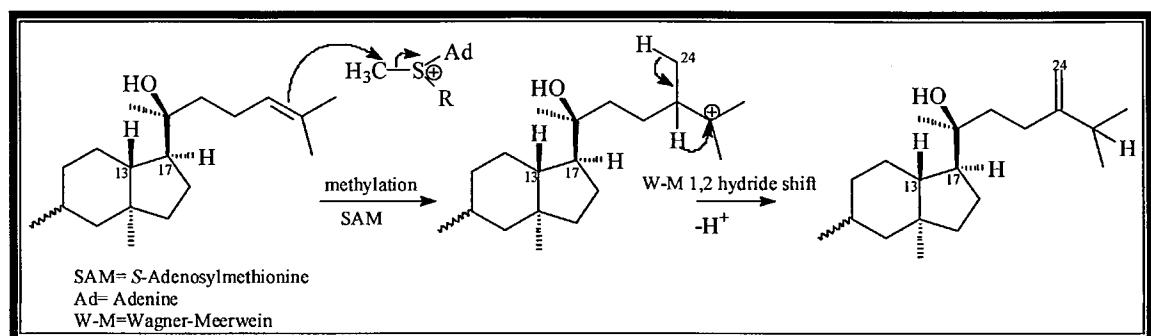
**Table 2.2: NMR data for compound 2.1 in CDCl<sub>3</sub>**

C	<sup>1</sup> H NMR (500 MHz)	<sup>13</sup> C NMR (125 MHz)	HMBC (H→C)	COSY	NOESY
1 $\alpha$	1.05 (1H, m)	38.9 (CH <sub>2</sub> )	C-2,C-3, C-5, C-10 , C-19	H-1 $\beta$ , 2H-2	
1 $\beta$	1.68 (1H, m)		C-4, C-9	H-1 $\alpha$	3H-19
2 $\alpha$	1.62 (1H, m)	23.9 (CH <sub>2</sub> )	C-3	H-3, H-1 $\alpha$	3H-19
2 $\beta$	1.62 (1H, m)		C-3	H-3, H-1 $\alpha$	
3	4.46 (dd, <i>J</i> = 10.65, 5.75 Hz)	81.1 (CH)	C-1, C-4, C-5, C-28, C-29, O-CO-CH <sub>3</sub>	H-2 $\alpha$ , H-2 $\beta$	
4	-	38.1 (C)		-	-
5	0.83 (1H, m)	56.2 (CH)	C-1, C-3, C-7, C-28, C-29	H-6 $\alpha$	H-6 $\alpha$ , H-9
6 $\alpha$	152 (1H, m)	18.4 (CH <sub>2</sub> )	C-7	H-5, H-7 $\beta$	H-5
6 $\beta$	1.44 (1H, m)		C-7, C-29		
7 $\alpha$	153 (1H, m)	35.4 (CH <sub>2</sub> )	C-6, C-9	H-7 $\beta$	
7 $\beta$	1.27 (1H, m)		C-5, C-9	H-6 $\alpha$ , H-7 $\alpha$	
8	-	40.6 (C)	-	-	-
9	1.35 (1H, m)	50.8 (CH)	C-1, C-11	2H-11,	H-5, H-11 $\alpha$ , 3H-28, 3H-30
10	-	37.2 (C)	-	-	-
11 $\alpha$	1.25 (1H, m)	21.8 (CH <sub>2</sub> )	C-9	H-9, 2H-12, H-	H-9

				$11\beta$	
$11\beta$	1.49 (1H, m)		C-9	H-11 $\alpha$	
$12\alpha$	1.74 (1H, m)	25.0 (CH <sub>2</sub> )	C-17	H-12 $\beta$ , H-13	
$12\beta$	1.52 (1H, m)		C-9, C-17	H-12 <sup>a</sup> , H-13	
13	1.63 (1H, m)	42.6 (CH)	C-8, C-22	2H-12, H-17	3H-18
14	-	50.6 (C)	-	-	-
$15\alpha$	1.45 (1H, m)	31.4 (CH <sub>2</sub> )	C-12	H-15 $\beta$ , 2H-16	
$15\beta$	1.07 (1H, m)			H-15 <sup>a</sup> , 2H-16	
$16\alpha$	1.81 (1H, m)	27.7 (CH <sub>2</sub> )	C-17	H-16 $\beta$ , 2H-15	
$16\beta$	1.27 (1H, m)		C-17	H-16 <sup>a</sup> , 2H-15	
17	1.76 (1H, m)	50.0 (CH)	C-12, C-13, C-15, C-16, C-17, C-21, C-20	H-13, 2H-16	3H-21
18	0.94 (3H, s)	15.7 (CH <sub>3</sub> )		-	H-13, H-19
19	0.84 (3H, s)	16.5 (CH <sub>3</sub> )	C-1, C-9	-	3H-18, 3H-29
20	-	75.5 (C)	-	-	-
21	1.16 (1H, m)	25.6 (CH <sub>3</sub> )	C-17, C-20, C-22, C-23	-	H-17
22 $\alpha$	1.59 (1H, m)	39.7 (CH <sub>2</sub> )	C-17, C-20, C-21, C-23, C-24	2H-23	
22 $\beta$	1.59 (1H, m)		C-20, C-24	2H-23	
23 $\alpha$	2.09 (1H, m)	28.6 (CH <sub>2</sub> )	C-20, C-24	2H-22	
23 $\beta$	2.09 (1H, m)		C-20, C-24	2H-22	
24	-	156.7 (C)	-	-	-
24A	4.74 (1H, brs)	106.4 (CH <sub>2</sub> )	C-23, C-24, C-25, C-26,	H-24B	H-24B
24B	4.66 (d, $J = 0.95$ Hz)		C-27	H-24A	H-24A
25	2.27 (1H, sept, $J = 6.82$ Hz)	34.2 (CH)	C-23, C-24, C-24A, C-26, C-27	3H-26, 3H-27	
26	1.04 (1H, d, $J = 6.82$ )	22.0 (CH <sub>3</sub> )	C-24	H-25	
27	1.04 (1H, d, $J = 6.82$ )	22.0 (CH <sub>3</sub> )	C-24	H-25	
28	0.82 (3H, s)	28.2 (CH <sub>3</sub> )	C-3, C-4, C-5, C-29	-	H-9
29	0.82 (3H, s)	16.7 (CH <sub>3</sub> )	C-3, C-4, C-5, C-29	-	H-19
30	0.85 (3H, s)	16.7 (CH <sub>3</sub> )	C-8, C-15	-	H-9
O-CO-CH <sub>3</sub>	-	171.2 (C)	-	-	-
O-CO-CH <sub>3</sub>	2.04 (3H, s)	21.5 (CH <sub>3</sub> )	C-1, C-3	-	

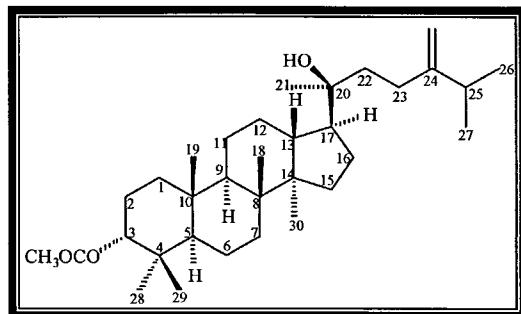
Methylation at C-24 of the sidechain as shown in Scheme 2.1 is fairly common in triterpenoids such as 24-methylenecycloartenol, gramisterol, ergosterol and campesterol.<sup>30</sup>

### Proposed biosynthesis of side chain of compound 2.1 and 2.2



Scheme 2.1: Proposed biosynthesis of side chain of compound 2.1.<sup>29</sup>

### 2.1.2.2: Structural elucidation of compound 2.2: (20S)-3 $\alpha$ -acetoxy-24-methylidenedammaran-20-ol



Compound 2.2

The TOF MS ES<sup>+</sup> mass spectrum of compound 2.2 gave a [M+H]<sup>+</sup> peak at *m/z* 501.4308, which corresponded to a molecular formula for the compound of C<sub>33</sub>H<sub>56</sub>O<sub>3</sub>. A double bond equivalence of six was calculated. The FTIR spectrum showed a carbonyl stretch at 1728 cm<sup>-1</sup>, CH stretches 2933 and 2851 cm<sup>-1</sup> and a hydroxyl stretch at 3415 cm<sup>-1</sup>.<sup>2,3</sup>

The <sup>13</sup>C NMR spectrum of compound 2.2 showed an acetate carbonyl resonance at  $\delta$  171.1 similar to that of compound 2.1. The H-3 resonance was seen to occur as a triplet (*t*, *J* = 2.75 Hz) at  $\delta$  4.61. The coupling constant showed that H-3 was in an equatorial ( $\beta$ ) position, hence the acetate was in an axial ( $\alpha$ ) position.<sup>7</sup> The corresponding C-3 carbon resonance at  $\delta$  78.5 showed correlations with the 3H-28 and 3H-29 methyl group proton resonances ( $\delta$  0.82,  $\delta$  0.86) and with the H-5 methine proton resonance at  $\delta$  1.21. The <sup>13</sup>C NMR spectrum showed a significant difference in the chemical shift of the C-5 resonance of compound 2.1 ( $\delta$  56.2) compared to compound 2.2 ( $\delta$  50.9) due to the effect of the acetate carbonyl group, which caused the C-5 carbon resonance of compound 2.2 to be more shielded in comparison to the C-5 carbon resonance of compound 2.1.

Compound 2.2 was found to differ from compound 2.1 in the stereochemistry at C-3. The C-5 carbon resonance showed correlations in the HMBC spectrum with the H-9 methine proton resonance ( $\delta$  1.45), with the 3H-19 methyl group proton resonance ( $\delta$  0.96) and the previously assigned 3H-28 and 3H-29 methyl group proton resonances. The H-9 methine resonance showed correlations in the HMBC spectrum with the carbon resonances C-5, previously assigned, C-14

( $\delta$  50.6), C-18 ( $\delta$  16.8), and C-12 ( $\delta$  24.9). The two H-12 methylene proton resonances were seen to be coupled in the COSY spectrum with the H-13 methine resonance at  $\delta$  1.65, and in turn, with the H-17 methine resonance at  $\delta$  1.77. The same side chain as in compound **2.1** was also present in compound **2.2**, and correlations were done in same manner to compound **2.1**. Compound **2.2**, (20*S*)-3 $\alpha$ -acetoxy-24-methylidene-dammaran-20-ol, has not been reported previously.

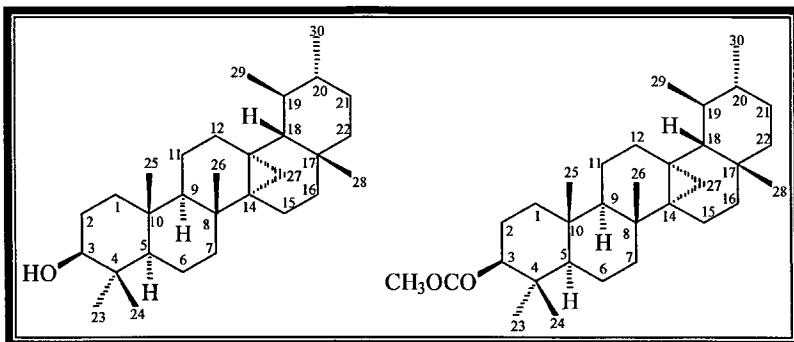
**Table 2.3:** NMR data for compound **2.2** in  $\text{CDCl}_3$

C	$^1\text{H}$ NMR (500 MHz)	$^{13}\text{C}$ NMR (125 MHz)	HMBC (H $\rightarrow$ C)	COSY	NOESY
1 $\alpha$	1.17 (1H, m)	34.5 (CH <sub>2</sub> )	C-4/C-10, C-5, C-9	2H-2	
1 $\beta$	1.43 (1H, m)		C-2, C-9		3H-19
2 $\alpha$	1.88 (1H, m)	23.1 (CH <sub>2</sub> )	-	2H-1, H-3	
2 $\beta$	1.57 (1H, m)		C-3		3H-19
3	4.61 (1H, t, $J$ =2.75 Hz)	78.6 (CH <sub>2</sub> )	C-1, C-2, C-28, C-29, C-31, C-32	H-3, 2H-2	
4	-	37.3 (C)	-	-	-
5	1.21 (1H, m)	50.9 (CH)	C-4, C-9, C-19	H-6 $\alpha$	H-9, H-6 $\alpha$
6 $\alpha$	1.41 (1H, m)*	18.2 (CH <sub>2</sub> )	C-5, C-7	2H-7, H-5	
6 $\beta$	1.41 (1H, m)*		-		
7 $\alpha$	1.58 (1H, m)	35.2 (CH <sub>2</sub> )	C-5, C-8	2H-6, H-7 $\beta$	
7 $\beta$	1.28 (1H, m)		C-5, C-6	2H-6, H-7 $\alpha$	
8	-	40.7 (C)	-	-	-
9	1.45 (1H, m)	50.6 (CH)	C-5, C4, 10, C-12, C-18	2H-11	H-5, 3H-30
10	-	37.4 (C)	-	-	-
11 $\alpha$	1.53 (1H, m)	21.5 (CH <sub>2</sub> )	H-11	2H-12	
11 $\beta$	1.24 (1H, m)		H-11	2H-12	
12 $\alpha$	1.52 (1H, m)	24.9 (CH <sub>2</sub> )	C-9	2H-11, H-12 $\beta$	
12 $\beta$	1.72 (1H, m)		C-9	2H-11, H-12 $\alpha$	3H-19
13	1.65	42.5 (CH)	-	H-17	3H-18
14	-	50.6 (C)	-	-	-
15 $\alpha$	1.47 (1H, m)	31.5 (CH <sub>2</sub> )	C-16	H-16 $\beta$	
15 $\beta$	1.08 (1H, m)		C-16	H-16 $\alpha$	
16 $\alpha$	1.81 (1H, m)	27.8 (CH <sub>2</sub> )	C-15	H-15 $\beta$	
16 $\beta$	1.27 (1H, m)		C-15	H-15 $\alpha$	
17	1.77	49.9 (CH)	C-13, C-20, C-21	H-16 $\beta$ , H-13	H-21
18	0.92 (3H, s)	16.8 (CH <sub>3</sub> )	C-8, C-14, C-15	-	H-13
19	0.96 (3H, s)	15.7 (CH <sub>3</sub> )	C-1, C-9	-	
20	-	75.6 (C)	-	-	-
21	1.16 (3H, s)	25.5 (CH <sub>3</sub> )	C-17, C-20, C-22	-	H-13
22 $\alpha$	1.59 (1H, m)	39.6 (CH <sub>2</sub> )	C-17, C-20, C-21,	2H-23	
22 $\beta$	1.59 (1H, m)		C-23, C-24	2H-23	
23 $\alpha$	2.09 (1H, m)	28.6 (CH <sub>2</sub> )	C-20, C-22, C-24,	2H-22	
23 $\beta$	2.09 (1H, m)		C-24', C-25	2H-22	
24	-	156.7 (C)	-	-	-
24A	4.74 (1H, brs)	106.4 (CH <sub>2</sub> )	C-23, C-24, C-25	H-24B	H-24B
24B	4.68 (1H, $J$ =0.55 Hz)		C-23, C-24, C-25	H-24A	H-24A
25	2.24 (1H, sept, $J$ =6.82 Hz)	34.2 (CH)	C-23, C-24, C-24', C-26, C-27	3H-26, 3H-27	
26	1.03 (1H, d, $J$ =	22.1 (CH <sub>3</sub> )	C-24, C-25	H-25	

	6.82)				
27	103 (1H, d, $J = 6.82$ )	22.1 (CH <sub>3</sub> )	C-24, C-25	H-25	
28	0.82 (3H, s)	28.1 (CH <sub>3</sub> )	C-3, C-5, C-10, C-19, C-29	-	
29	0.86 (3H, s)	22.1 (CH <sub>3</sub> )	C-3, C-5	-	
30	0.92 (3H, s)	16.9 (CH <sub>3</sub> )	C-14, C-15	-	
O-CO-CH <sub>3</sub>	-	171.0 (C)	-	-	-
O-CO-CH <sub>3</sub>	2.08 (3H, s)	21.9 (CH <sub>3</sub> )	-	-	

Resonance superimposed  $\delta 1.41^*$

### 2.1.2.3: Structural elucidation of compound 2.3: 13, 27-cycloursan-3 $\beta$ -ol (phyllanthol) and phyllanthol acetate.



Compound 2.3 and its 3 $\beta$ -acetyl derivative

Compound 2.3 was isolated as white crystals which were identified as phyllanthol. The high resolution mass spectrum indicated a molecular ion peak at  $m/z$  426.3849, which corresponded to the molecular formula of C<sub>30</sub>H<sub>50</sub>O and indicated a double bond equivalence of six. The FTIR spectrum of the compound showed an absorbance band at 3235 cm<sup>-1</sup> due to a hydroxyl stretch, and a cyclopropane ring stretch at 1449 cm<sup>-1</sup>.<sup>2,8,9</sup>

Phyllanthol was first isolated in 1951 by Alberman and Kipping from the root bark of *Phyllanthus engleri*. They prepared the acetate, benzoate and *p*-nitrobenzoate derivatives. They deduced it was an alcohol of high melting point which contained no double bonds, with a melting point of 233-234°. Using elemental analysis, they determined the molar mass as 426 g.mol<sup>-1</sup>, from which they deduced a molecular formula of C<sub>30</sub>H<sub>50</sub>O. From these results they concluded that phyllanthol was a triterpenoid, probably related to  $\psi$ -taraxasterol.<sup>10,12</sup> In 1953, Barton and Mayo confirmed that no double bonds were present and from the molecular formula

deduced the presence of six rings. They were able to convert phyllanthol into  $\alpha$ -amyrin by refluxing with HCl in acetic acid. They interpreted these results as phyllanthol has a cyclopropane ring with one apex of the cyclopropane ring terminating at C-12 or C-13. There were seven possible structures, which could be narrowed down to a compound with a C-12,13, 27-cyclopropane ring, based on characteristic infra-red peaks at 3042-3052  $\text{cm}^{-1}$ , which are characteristic of the C-H stretching frequency of a methylene group within a cyclopropane ring.<sup>9</sup> Sengupta *et al.* isolated phyllanthol, which they identified by comparison against an authentic sample from *Phyllanthus acidus* in 1966.<sup>11</sup> The next report of this compound was by Hnatyszyn and Ferraro in 1985 from *Phyllanthus sellowianus*. They reported  $^1\text{H}$  NMR data for the compound and used IR data in their structural determination.<sup>13</sup> They further reported the compound in 1996 with mass spectrometry results, UV and  $^1\text{H}$  NMR data.<sup>14</sup> The  $^1\text{H}$  NMR data reported by these researchers is clearly incorrect. They report that the two H-27 cyclopropane ring protons occur at  $\delta$  3.87. They report no upfield peaks that could be assigned to the cyclopropane methylene group protons.<sup>14, 15</sup> In addition, they assigned no stereochemistry to their structure. This led us to undertake a complete assignment of the NMR spectra for this compound.

The  $^1\text{H}$  NMR spectrum showed a proton resonance at  $\delta$  3.18 (dd,  $J = 5.75, J = 10.91 \text{ Hz}$ ) which was seen to correspond to the carbon resonance at  $\delta$  79.3 in the HSQC spectrum. This resonance showed correlations in the HMBC spectrum with the 3H-23 and 3H-24 methyl group proton resonances at  $\delta$  0.96 and  $\delta$  0.77 and with the methine proton resonance at  $\delta$  0.72 which was ascribed to H-5. The splitting pattern established that H-3 was in an axial ( $\alpha$ ) configuration and hydroxyl was then in the  $\beta$  or equatorial configuration.<sup>22</sup> The COSY spectrum showed coupling between the H-3 resonance and the superimposed H-2 methylene proton resonances at  $\delta$  1.57, which, in turn, were seen to be coupled with the H-1 methylene proton resonances at  $\delta$  0.89 and  $\delta$  1.54.

The corresponding C-1 carbon resonance at  $\delta$  38.7 showed correlations in the HMBC spectrum with the H-9 methine proton resonance at  $\delta$  0.75 and the 3H-25 methyl group proton resonance at  $\delta$  0.86. These correlations confirmed that compound **2.3** was not a cycloartane. In addition, the H-9 methine resonance showed coupling in the COSY spectrum with the superimposed H-11

proton resonances ( $\delta$  1.27), which, in turn, were seen to be coupled with the two H-12 methylene proton resonances at  $\delta$  1.77 and  $\delta$  1.85.

The corresponding C-12 carbon resonance ( $\delta$  35.4) showed correlations in the HMBC spectrum with the H-18 methine proton resonance at  $\delta$  0.99 and the cyclopropane ring proton resonances at  $\delta$  0.004 (d,  $J = 5.55$  Hz) and  $\delta$  0.66 (d,  $J = 5.55$  Hz), which were ascribed to H-27A and H-27B. The H-27 methylene proton resonances were seen to correlate in the HMBC spectrum with the carbon resonances previously assigned to C-9 and C-12, and the two fully substituted carbon resonances at  $\delta$  37.2 (C-8),  $\delta$  32.4 (C-14) and the C-15 carbon resonance ( $\delta$  21.5). The COSY spectrum showed coupling between the H-15 proton resonances at  $\delta$  1.42 and  $\delta$  1.82 and the two H-16 proton resonances ( $\delta$  1.32 and  $\delta$  0.73).

The HMBC spectrum showed correlations between the C-16 carbon resonance ( $\delta$  27.5) and the 3H-28 methyl group proton resonance at  $\delta$  0.89 and with the previously assigned H-18 methine proton resonance. The COSY spectrum showed coupling between the H-18 and H-19 ( $\delta$  0.85) methine proton resonances, which, in turn, were seen to be coupled in the COSY spectrum with the H-20 methine proton resonance at  $\delta$  0.96. The H-20 methine resonance was seen to be coupled with the methyl group proton resonance  $\delta$  0.87, which was ascribed to 3H-30. The H-20 resonance was seen to be coupled with the two H-21 resonances ( $\delta$  0.98,  $\delta$  1.30) and these were further coupled with the H-22 proton resonances ( $\delta$  1.24,  $\delta$  1.32).

The NOESY spectrum showed correlations between the 3H-23 methyl group proton resonance and the H-5 methine resonance, which, in turn, was seen to be coupled with the H-9 methine proton resonance. The H-27B resonance was seen to correlate with the 3H-30 methyl group proton resonance and with H-22 $\alpha$  resonance in the NOESY spectrum. These correlations were also seen in the NOESY spectrum of phyllanthol acetate.

Further correlations were seen in the NOESY spectrum between the 3H-24 and 3H-25 methyl group proton resonances, which, in turn, showed correlation with the methyl group resonance 3H-26. The H-18 methine proton resonance and the 3H-28 methyl group proton resonance were

seen to correlate in the NOESY spectrum. The structure of compound **2.3** was confirmed as 13, 27-cycloursan-3 $\beta$ -ol. Phyllanthol was acetylated to give phyllanthol acetate.

From the literature only two other derivatives of this rare class have been isolated from the aerial roots of *Ficus microcarpa* (Moraceae), namely 3 $\beta$ -acetoxy-13,27-cycloursan-11-en-15 $\alpha$ -ol ( $[\alpha]_D = +16.8^\circ$ ) and 3 $\beta$ -acetoxy-12 $\alpha$ -formyloxy-13, 27-cycloursan-11 $\alpha$ -ol ( $[\alpha]_D = +43.9^\circ$ )<sup>16</sup> as shown in Figure 2.2. No biological activities were reported for the two compounds, however the plant extract of *Ficus microcarpa* was found to inhibit antiplatelet activity.<sup>15</sup>

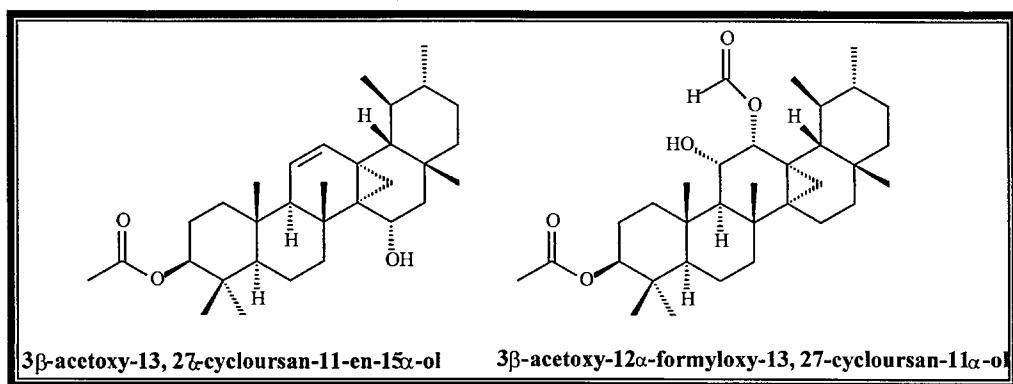


Figure 2.3: 13, 27-Ursane triterpenoids from *Ficus microcarpa*<sup>15</sup>

**Table 2.4: NMR data of compound 2.3 (phyllanthol) in  $\text{CDCl}_3$  and acetylated phyllanthol in  $\text{CDCl}_3$**

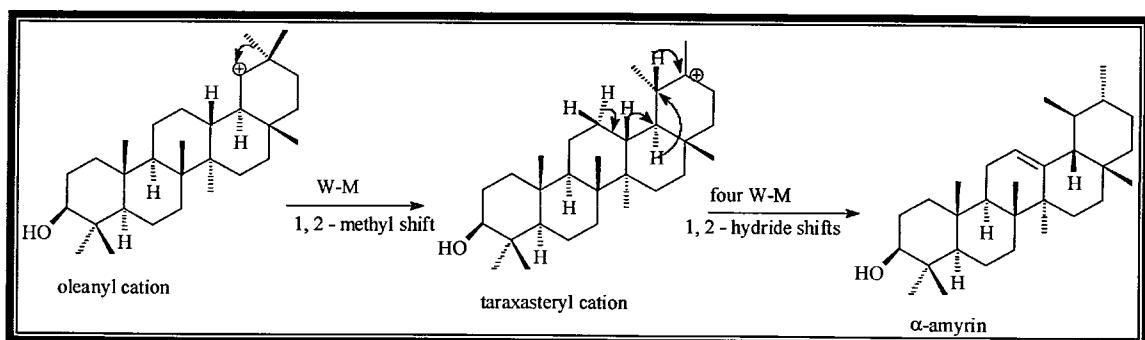
No	$^1\text{H}$ NMR MHz)	$^1\text{H}$ NMR (500 MHz)	$^{13}\text{C}$ NMR (125 MHz)	HMBC (H $\rightarrow$ C)	COSY	NOESY	$^1\text{H}$ NMR (phyllanthol Ac)	$^{13}\text{C}$ NMR (phyllanthol Ac)
1 $\alpha$	0.89 (1H, m)	38.7 (CH <sub>2</sub> )	C-2, C-9, C-25	H-1 $\beta$ , 2H-1	H-2 $\alpha$ , H-5	0.96 (1H, m)	38.2 (CH <sub>2</sub> )	
1 $\beta$	1.54 (1H, m)		C-3, C-5, C-25	H-1 $\alpha$	3H-25	1.55 (1H, m)		
2 $\alpha$	1.57 (1H, m)	27.5 (CH <sub>2</sub> )	C-1, C-3, C-10	H-3, 2H-1		1.60 (1H, m)	23.7 (CH <sub>2</sub> )	
2 $\beta$	1.57 (1H, m)			H-3, 2H-1	3H-25			
3	3.18 ( $J = 5.75, J = 10.91$ Hz)	79.3 (CH)	C-2,C-5, C-23, C-25	2H-2	H-2 $\alpha$ , H-5, 3H-23	4.49 (1H, $J=5.70$ , 11.0 Hz)	81.2 (CH)	
4	-	39.1 (C)	-	-	-	-	40.0 (C)	
5	0.72 (1H, m)	55.9 (CH)	C-6, C-23,C-9, C-25	H-6 $\beta$	H-3,H-9, H-27B	0.83 (1H, m)	56.0 (CH)	
6 $\alpha$	1.54 (1H, m)	18.3 (CH <sub>2</sub> )	C-3, C-5, C-25	C-7		1.52 (1H, m)	18.2 (CH <sub>2</sub> )	
6 $\beta$	1.37 (1H, m)			H-5		1.37 (1H, m)		
7 $\alpha$	1.72 (1H, m)	38.7 (CH <sub>2</sub> )	C-6, C-9	H-7 $\beta$		1.72 (1H, m)	38.6 (CH <sub>2</sub> )	
7 $\beta$	1.21 (1H, m)			H-7 $\alpha$		1.20 (1H, m)		
8	-	37.2 (C)	-	-	-	-	37.2 (C)	
9	0.75 (1H, m)	54.2 (CH)	C-1,C-5, C-8, C-11, C-12,C-14, C-25		H-5	0.75 (1H, m)	54.2 (CH)	
10	-	37.5 (C)	-	-	-	-	37.4 (C)	
11 $\alpha$	1.27 (1H, m)	17.8 (CH <sub>2</sub> )	C-12, C-13, C-18	2H-12		1.27 (1H, m)		
11 $\beta$	1.27 (1H, m)			2H-12		1.27 (1H, m)	17.8 (CH <sub>2</sub> )	
12 $\alpha$	1.77 (1H, m)	35.4 (CH <sub>2</sub> )	C-9, C-27	H-12 $\beta$ , 2H-11		1.77 (1H, m)		
12 $\beta$	1.85 (1H, m)		C-11, C-13, C-14, C-18, C-27	H-12 $\alpha$ , 2H-11	3H-29	1.86 (1H, m)	35.3 (CH <sub>2</sub> )	
13	-	26.6 (C)	-	-	•	-		
14	-	32.4 (C)	-	-	•	-	26.2 (C)	
15 $\alpha$	1.42 (1H, m)	21.5 (CH <sub>2</sub> )	C-14, C-16, C-27	H-15 $\beta$		1.42 (1H, m)		32.4 (C)
15 $\beta$	1.82 (1H, m)		C-13, C-17, C-28	H-15 $\alpha$ , H-16 $\beta$ , H-16 $\alpha$	3H-26, 3H-28	1.81 (1H, m)		21.5 (CH <sub>2</sub> )
16 $\alpha$	1.32 (1H, m)	27.5 (CH <sub>2</sub> )	C-15, C-28	H-15 $\beta$ , H-16 $\beta$		1.32 (1H, m)	27.5 (CH <sub>2</sub> )	
16 $\beta$	0.73 (1H, m)		C-18	H-15 $\beta$ , H-16 $\alpha$		0.74 (1H, m)		
17	-	32.1 (C)	-	-	-	-	32.1 (C)	
18	0.99 (1H, m)	50.3 (CH)	C-22, C-28, C-29, C-30	H-19, H-20	3H-26, 3H-28,	1.01 (1H, m)		
19	0.85 (1H, m)	41.0 (CH)	C-18, C-21	H-18, 3H-29	H-27B	0.84 (1H, m)		
20	0.96 (1H, m)	38.7 (CH)	C-18			0.96 (1H, m)	40.9 (CH)	
21 $\alpha$	0.98 (1H, m)	31.3 (CH <sub>2</sub> )				0.98 (1H, m)	38.6 (CH)	
21 $\beta$	1.30 (1H, m)					1.29 (1H, m)	31.3 (CH <sub>2</sub> )	
22 $\alpha$	1.24 (1H, m)	42.3 (CH <sub>2</sub> )	C-13, C-18	2H-21	H-27B	1.23 (1H, m)	42.3 (C)	

22 $\beta$	1.32 (1H, m)	28.1 (CH <sub>3</sub> )	C-3, C-5, C-24	2H-21	H-3, H-5, H-9	1.30 (1H, m)
23	0.96 (3H, s)	15.5 (CH <sub>3</sub> )	C-3	-	0.84 (3H, s)	28.1 (CH <sub>3</sub> )
24	0.77 (3H, s)	15.5 (CH <sub>3</sub> )	C-3	-	0.84	16.6 (CH <sub>3</sub> )
25	0.86 (3H, s)	16.2 (CH <sub>3</sub> )	C-9	-	0.89	16.3 (CH <sub>3</sub> )
26	1.11 (3H, s)	18.2 (CH <sub>3</sub> )	C-4, C-8, C-11, C-14	-	H-15 $\beta$ , 3H-25, 3H-28	18.2 (CH <sub>3</sub> )
27A	0.66 (d, <i>J</i> = 5.55 Hz)	13.5 (CH <sub>2</sub> )	C-8, C-9, C-12, C-14, C-15, C-16	H-27B	H-27B	0.65 (1H, d, <i>J</i> = 5.55 Hz)
27B	0.0004 (d, <i>J</i> = 5.55 Hz)		C-8, C-9, C-12, C-14,	H-27A	H-27A, H-22 $\alpha$ , H-19, H-30	0.013 (1H,d, <i>J</i> = 5.55 Hz)
28	0.89 (3H, s)	28.4 (CH <sub>3</sub> )	C-16, C-18	-	H-18, 3H-26	28.4 (CH <sub>3</sub> )
29	0.93 (1H, d, <i>J</i> = 6.00 Hz)	18.3 (CH <sub>3</sub> )	C-20	H-12	H-12 $\beta$ , H-21, H-18	18.1
30	0.87 (1H, d, <i>J</i> = 5.60 Hz)	20.9 (CH <sub>3</sub> )	C-19	H-20	H-27B	0.86
31	COCH <sub>3</sub>	-	-	-	-	171.2 (C)
32	COCH <sub>3</sub>	-	-	-	-	21.5 (CH <sub>3</sub> )

#### 2.1.2.4: Biosynthesis of phyllanthol from *Phyllanthus cedrelifolius*

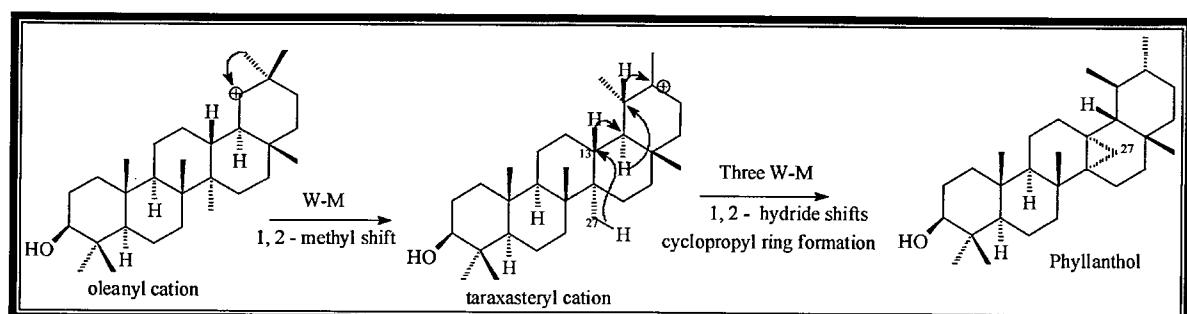
##### Proposed biosynthesis of Phyllanthol

In the biosynthesis of ursane-type compounds such as  $\alpha$ -amyrin, it is proposed that the oleanyl cation undergoes a Wagner-Meerwein, 1,2-methyl shift to produce the taraxasteryl cation, which then undergoes four Wagner-Meerwein 1,2-hydride shifts to produce  $\alpha$ -amyrin as shown below.<sup>30</sup>



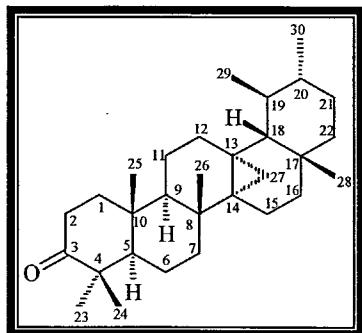
Scheme 2.2: Proposed biosynthesis of  $\alpha$ -amyrin<sup>29</sup>

In the biosynthesis of phyllanthol, it is proposed that the 1,2-methyl shift is followed by three 1,2-hydride shifts, followed by the loss of one of the methyl group protons and bond formation between C-27 and C-13, to form the cyclopropyl ring, as shown below.



Scheme 2.3: Proposed biosynthesis of Phyllanthol

#### 2.1.2.5: Structural elucidation of compound 2.4: 13, 27-cycloursan-3-one (phyllanthone)



Compound 2.4

The high resolution mass spectrum of compound 2.4 gave a molecular ion peak at  $m/z$  424.3707, which corresponded to the molecular formula of  $C_{30}H_{48}O$ . The double bond equivalence of seven was deduced. Compound 2.4 was found to be a derivative of compound 2.3, only differing at position 3. The FTIR spectrum showed a carbonyl absorption band at  $1708\text{ cm}^{-1}$ , and CH stretches at  $2920$  and  $2858\text{ cm}^{-1}$ , however no hydroxyl stretch was observed. This indicated that the hydroxyl group at C-3 may have been replaced by a ketone.

The  $^{13}\text{C}$  NMR spectrum revealed the C-3 ketonic carbonyl resonance at  $\delta$  218.2. The corresponding C-3 resonance showed correlations in the HMBC spectrum with the methyl group proton resonances at  $\delta$  1.07 and  $\delta$  1.03, which were ascribed to the 3H-23 and 3H-24 resonances, and the H-5 methine proton resonance at  $\delta$  1.35. Therefore this confirmed the position of the ketone. The H-5 methine resonance was seen to be coupled with the H-6 proton resonances ( $\delta$  1.77 and  $\delta$  1.25), which, in turn, were seen to be coupled with the superimposed H-7 proton resonances at  $\delta$  1.31.

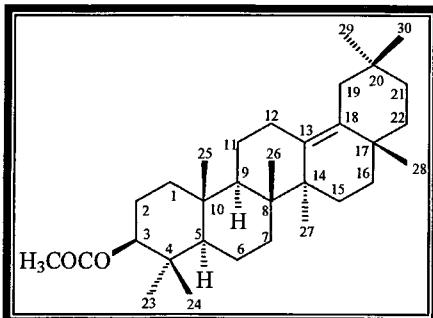
The corresponding C-7 carbon resonance at  $\delta$  18.1 showed correlations in the HMBC spectrum with the 3H-26 methyl group proton resonances at  $\delta$  1.18 and the fully substituted C-8 ( $\delta$  37.0) resonance. The C-8 carbon resonance showed correlations in the HMBC spectrum with cyclopropane ring proton resonances at  $\delta$  0.05 (d,  $J = 5.40$ ) and  $\delta$  0.63 (d,  $J = 5.40\text{ Hz}$ ). These correlations confirmed the position of the cyclopropane ring.

Further correlations were done in the same manner as those of phyllanthol. Compound **2.4**. was identified as 13, 27-cycloursan-3-one and has not been isolated previously from a natural source.

**Table 2.5: NMR data for compound 2.4 (phyllanthone) in CDCl<sub>3</sub>**

C	<sup>1</sup> H NMR (500 MHz)	<sup>13</sup> C NMR (125 MHz)	HMBC (H→C)	COSY	NOESY
1 $\alpha$	1.78 (1H, m)	39.5 (CH <sub>2</sub> )	C-3, C-5	H-1 $\beta$ , H-2 $\alpha$ , H-2 $\beta$	H-9 $\alpha$ , H-5 $\alpha$ , H-2 $\alpha$
1 $\beta$	1.36 (1H, m)		C-10	H-1 $\alpha$ , H-2 $\alpha$ , H-2 $\beta$	H-2 $\beta$
2 $\alpha$	2.48 (1H, m)	34.3 (CH <sub>2</sub> )	C-1, C-3, C-10	H-1 $\alpha$ , H-1 $\beta$ , H-2 $\alpha$	H-1 $\alpha$
2 $\beta$	2.38 (1H, m)		C-1,C-2, C-3, C-10	H-1 $\alpha$ , H-1 $\beta$ , H-2 $\beta$	
3	-	218.2 (C)	-	-	-
4	-	47.7 (C)	-	-	-
5	1.35 (1H, m)	55.5 (CH)	C-3, C-4	2H-6	H-1 $\alpha$ , 2H-6 $\alpha$ , H-9 $\alpha$ , H-7 $\alpha$
6 $\alpha$	1.77 (1H, m)	37.9 (CH <sub>2</sub> )	C-7	H-6 $\beta$ , H-7 $\alpha$ , H-7 $\beta$	H-5 $\alpha$
6 $\beta$	1.25 (1H, m)			H-5, H-6 $\alpha$ H-7 $\alpha$ , H-7 $\beta$	
7 $\alpha$	1.31 (1H, m)	18.1 (CH <sub>2</sub> )	C-8	H-6 $\alpha$ , H-6 $\beta$	H-5 $\alpha$
7 $\beta$	1.31 (1H, m)		C-8	H-6 $\alpha$ , H-6 $\beta$	
8	-	37.0 (C)	-	-	-
9	0.78 (1H, m)	54.6 (CH)	C-25	H-11 $\alpha$ , H-11 $\beta$	H-1 $\alpha$ , H-5 $\alpha$
10	-	37.2 (C)	-	-	-
11 $\alpha$	1.47(1H, m)	19.6 (CH <sub>2</sub> )	C-12	H-9, H-12 $\alpha$ , H-12 $\beta$	H-12 $\alpha$ , H-27b
11 $\beta$	1.47 (1H, m)		C-12	H-12 $\alpha$ , H-12 $\beta$	H-12 $\beta$
12 $\alpha$	1.87 (1H, m)	35.8 (CH <sub>2</sub> )	C-18, C-27	H-11 $\alpha$ , H-11 $\beta$ , H-12 $\beta$	H-11 $\alpha$ , H-9 $\alpha$
12 $\beta$	1.80 (1H, m)		C-9, C-18, C-27	H-11 $\alpha$ , H-11 $\beta$ , H-12 $\alpha$	H-11 $\beta$
13	-	19.9 (C)	-	-	-
14	-	32.7 (C)	-	-	-
15 $\alpha$	1.84 (1H, m)	21.5 (CH <sub>2</sub> )	C-16, C-17, C-27	H-15 $\beta$ , H-16 $\beta$	H-12 $\alpha$ , H-16 $\alpha$
15 $\beta$	1.47 (1H, m)		C-14, C-16, C-27	H-15 $\alpha$	H-12 $\beta$ , H-16 $\beta$
16 $\alpha$	1.34 (1H, m)	27.4 (CH <sub>2</sub> )	C-18	H-16 $\beta$	H-15 $\alpha$
16 $\beta$	0.75 (1H, m)			H-15 $\alpha$ , H-16 $\alpha$	H-15 $\beta$ , 3H-28
17	-	32.1 (C)	-	-	
18	1.08 (1H, m)	49.8 (CH)	C-14, C-17, C-20	H-19	Not seen
19	0.86 (1H, m)	41.0 (CH)	C-13	H-18, H-20, H-29,	H-27B
20	0.98 (1H, m)	38.6 (CH)	C-22	H-19, 3H-30	
21 $\alpha$	1.32 (1H, m)	31.3 (CH <sub>2</sub> )	C-17	H-21 $\beta$ , H-22 $\alpha$ , H-22 $\beta$	3H-30
21 $\beta$	0.98 (1H, m)		C-17	H-21 $\alpha$ , H-22 $\alpha$ , H-22 $\beta$	
22 $\alpha$	1.33 (1H, m)	42.2 (CH <sub>2</sub> )	C-17, C-20	H-21 $\alpha$ , H-21 $\beta$	
22 $\beta$	1.33 (1H, m)		C-17, C-20	H-21 $\alpha$ , H-21 $\beta$	
23	1.07 (3H, s)	26.7 (CH <sub>3</sub> )	C-3, C-5, C-4, C-24	-	
24	1.03 (3H, s)	21.0 (CH <sub>3</sub> )	C-3, C-4, C-5, C-24	-	3H-25
25	0.96 (3H, s)	15.8 (CH <sub>3</sub> )	C-1, C-9, C-10	-	3H-26, 3H-26
26	1.18 (3H, s)	17.9 (CH <sub>3</sub> )	C-8, C-14	-	3H-27
27A	0.63 (1H, d, <i>J</i> = 5.40 Hz)	13.8 (CH <sub>2</sub> )	C-9, C-12, C-15, C-16	H-27B	H-27B
27B	0.05 (1H, d, <i>J</i> = 5.40 Hz)			H-27A	H-18, H-27A H-21 $\alpha$ , 3H-30
28	0.90 (3H, s)	28.5 (CH <sub>3</sub> )	C-16,C-17, C-22	-	H-16 $\beta$
29	0.93 (1H, d, <i>J</i> = 6.00 Hz)	18.1 (CH <sub>3</sub> )	C-18	H-19	
30	0.87 (1H, d, <i>J</i> = 5.60 Hz)	21.2 (CH <sub>3</sub> )	C-20, C-21	H-20	H-21 $\alpha$

### 2.1.2.6: Structural elucidation of compound 2.5: 3 $\beta$ -acetoxyolean-13(18)-ene ( $\delta$ -amyrin acetate)



Compound 2.5

The needle-like crystals of compound 2.5 gave a molecular ion peak at  $m/z$  468 from the low resolution mass spectrum, which was consistent with the molecular formula of  $C_{32}H_{52}O_2$ . A double bond equivalence of seven was calculated. The FTIR spectrum showed a carbonyl stretch at  $1726\text{ cm}^{-1}$ .<sup>4</sup>

The  $^1\text{H}$  NMR spectrum revealed a resonance at  $\delta$  4.48 (dd,  $J = 6.00, J = 10.5$  Hz), which corresponded to the carbon resonance at  $\delta$  81.2 in the HSQC spectrum. These coupling constants established the configuration of H-3 as  $\alpha$  with a  $\beta$ -acetate group.<sup>4</sup> This proton resonance showed correlations in the HMBC spectrum with the carbonyl carbon resonance of an ester at  $\delta$  171.2, with the C-23 and C-24 methyl group proton resonances at  $\delta$  28.3 and  $\delta$  17.9 and with the C-5 methine carbon resonance at  $\delta$  55.6. The C-5 carbon resonance showed correlations with the H-9 methine proton resonance at  $\delta$  1.45 and the 3H-25 methyl group proton resonance at  $\delta$  0.81 in the HMBC spectrum.

The H-9 methine proton resonance was seen to be coupled in the COSY spectrum with two H-11 methylene proton resonances at  $\delta$  1.42 and  $\delta$  1.20 and these, in turn, were seen to be coupled with two H-12 methylene proton resonances at  $\delta$  1.73 and  $\delta$  1.04. The H-11 proton resonances showed correlations in the HMBC spectrum with the C-13 fully substituted carbon resonance at  $\delta$  134.5, indicating the presence of a double bond at this position. The H-12 proton resonances

showed correlations with the two fully substituted carbon resonances assigned to C-18 ( $\delta$  133.4) and C-14 ( $\delta$  44.9) in the HMBC spectrum.

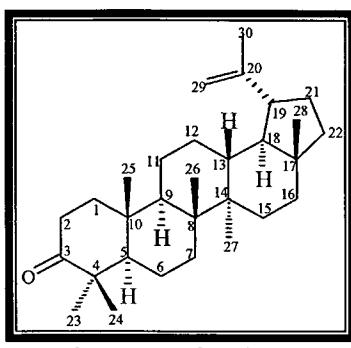
Compound **2.5** was identified as  $3\beta$ -acetoxyolean-13(18)-ene, commonly known as  $\delta$ -amyrin acetate. The compound was previously isolated from *Vernonia cinerea* Less (Asteraceae) by Misra *et al.* in 1984 who deduced the structure to be  $3\beta$ -acetoxyolean-13(18)-ene, based on mass spectrometry and  $^1\text{H}$  NMR spectroscopy.<sup>16</sup> The next report was by Ansari, in 2000, who reported the  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR data, however the  $^{13}\text{C}$  NMR shifts were different from those found for compound **2.5** as shown in Table 2.6.<sup>18</sup> They reported a melting point of 85-87° and an optical rotation of 0° for the compound, in contrast to several other authors who reported values ranging from 207-222° for the compound and optical rotation values between -36 and -44.<sup>16,18</sup> Values of 210-211° and -41° were found for the melting point and optical rotation respectively for compound **2.5**. No other  $^{13}\text{C}$  NMR data could be found in the literature for comparison, so data of  $3\beta$ -formyoxyolean-13(18)-ene was used for comparison.<sup>24</sup>

**Table 2.6:** NMR data for compound **2.5**:  $\delta$ -amyrin acetate, data reported by Ansari *et al.*,<sup>18</sup> and for  $3\beta$ -formyoxyolean-13(18)-ene.<sup>24</sup>

C	$^1\text{H}$ NMR (500 MHz) $\text{CDCl}_3$	$^{13}\text{C}$ NMR (125 MHz) $\text{CDCl}_3$	$^{13}\text{C}$ NMR (75 MHz) $\text{CDCl}_3$ <sup>18</sup>	$^{13}\text{C}$ NMR (100 MHz) $\text{CDCl}_3$ <sup>24</sup>
1 $\alpha$	1.05 (1H, m)	39.6 (CH <sub>2</sub> )	38.6 (CH <sub>2</sub> )	38.7 (CH <sub>2</sub> )
1 $\beta$	1.70 (1H, m)			
2 $\alpha$	1.62 (1H, m)	23.9 (CH <sub>2</sub> )	26.2 (CH <sub>2</sub> )	23.9 (CH <sub>2</sub> )
2 $\beta$	1.62 (1H, m)			
3	4.48 (1H, dd, $J = 10.5, J = 6.00$ Hz)	81.2 (CH)	80.6 (CH)	81.1 (CH)
4	-	37.7(C)	37.9 (C)	37.7 (C)
5	0.82 (1H, m)	55.6 (CH)	55.2 (CH)	55.4 (CH)
6 $\alpha$	1.49 (1H, m)	18.6 (CH <sub>2</sub> )	18.3 (CH <sub>2</sub> )	18.3 (CH <sub>2</sub> )
6 $\beta$	1.30 (1H, m)			
7 $\alpha$	1.41 (1H, m)	35.0 (CH <sub>2</sub> )	33.1 (CH <sub>2</sub> )	34.8 (CH <sub>2</sub> )
7 $\beta$	1.41 (1H, m)			
8	-	41.2 (C)	42.6 (C)	41.0 (C)
9	1.45 (1H, m)	50.8 (CH)	50.3 (CH)	50.6 (CH)
10	-	37.1 (C)	36.9 (C)	37.2 (C)
11 $\alpha$	1.42 (1H, m)	21.9 (CH <sub>2</sub> )	21.2 (CH <sub>2</sub> )	21.7 (CH <sub>2</sub> )
11 $\beta$	1.20 (1H, m)			
12 $\alpha$	1.73 (1H, m)	26.7 (CH <sub>2</sub> )	26.3 (CH <sub>2</sub> )	26.5 (CH <sub>2</sub> )
12 $\beta$	1.04 (1H, m)			
13	-	134.5 (C)	132.6 (C)	134.2 (C)
14	-	44.9 (C)	42.2 (C)	44.6 (C)
15 $\alpha$	2.62 (1H, m)	25.2 (CH <sub>2</sub> )	25.3 (CH <sub>2</sub> )	25.0 (CH <sub>2</sub> )
15 $\beta$	1.80 (1H, m)			

16 $\alpha$	1.39 (1H, m)	36.9 (CH <sub>2</sub> )	33.6 (CH <sub>2</sub> )	36.6 (CH <sub>2</sub> )
16 $\beta$	1.27 (1H, m)			
17	- (C)	34.5	41.6 (C)	34.6 (C)
18	- (C)	133.4	133.7 (C)	133.3 (C)
19 $\alpha$	1.30 (1H, m)	39.6 (CH <sub>2</sub> )	37.9 (CH <sub>2</sub> )	39.4 (CH <sub>2</sub> )
19 $\beta$	1.27 (1H, m)			
20	-	33.3 (C)	32.1(C)	33.4 (C)
21 $\alpha$	1.43 (1H, m)	35.6 (CH <sub>2</sub> )	43.6 (CH <sub>2</sub> )	35.4 (CH <sub>2</sub> )
21 $\beta$	1.10 (1H, m)			
22 $\alpha$	1.62 (1H, m)	38.9 (CH <sub>2</sub> )	37.6 (CH <sub>2</sub> )	38.5 (CH <sub>2</sub> )
22 $\beta$	2.24 (1H, dd, <i>J</i> = 13.92, <i>J</i> = 12.1 Hz)			
23	0.84 (3H, s)	28.3 (CH <sub>3</sub> )	28.0 (CH <sub>3</sub> )	28.0 (CH <sub>3</sub> )
24	0.83 (3H, s)	17.9 (CH <sub>3</sub> )	15.6 (CH <sub>3</sub> )	17.7 (CH <sub>3</sub> )
25	0.81 (3H, s)	16.8 (CH <sub>3</sub> )	16.3 (CH <sub>3</sub> )	16.6 (CH <sub>3</sub> )
26	0.85 (3H, s)	16.7 (CH <sub>3</sub> )	18.6 (CH <sub>3</sub> )	16.4 (CH <sub>3</sub> )
27	1.13 (3H, s)	21.5 (CH <sub>3</sub> )	20.3 (CH <sub>3</sub> )	21.3 (CH <sub>3</sub> )
28	0.67 (3H, s)	24.3 (CH <sub>3</sub> )	16.5 (CH <sub>3</sub> )	32.3 (CH <sub>3</sub> )
29	0.90 (3H, s)	32.6 (CH <sub>3</sub> )	32.0 (CH <sub>3</sub> )	23.8 (CH <sub>3</sub> )
30	0.98 (3H, s)	24.0 (CH <sub>3</sub> )	24.1 (CH <sub>3</sub> )	24.1 (CH <sub>3</sub> )
COCH <sub>3</sub>	2.02 (3H, s)	21.5 (CH <sub>3</sub> )	21.2	
COCH <sub>3</sub> -		171.2	171.1	161 (formate)

### 2.1.2.7: Structural elucidation of compound 2.6: lupenone (3-oxolup-20(29)-ene)



Compound 2.6 was found to be the common lupane triterpenoid, lupenone, which has been isolated from many plant sources.<sup>19</sup> The low resolution mass spectrum gave a molecular ion peak at *m/z* 424, which was consistent with a molecular formula of C<sub>30</sub>H<sub>48</sub>O. A double bond equivalence of seven was deduced. The FTIR spectrum showed a carbonyl stretch at 1707 cm<sup>-1</sup> and CH stretches at 2922 and 2858 cm<sup>-1</sup>.<sup>2,3</sup>

The <sup>1</sup>H NMR spectrum revealed seven methyl group proton resonances at  $\delta$  1.07, 1.02, 1.07, 0.93, 0.96, 0.79 and 1.68 and these resonances were seen to correspond with the carbon

resonances at  $\delta$  26.9 (C-23),  $\delta$  21.3 (C-24),  $\delta$  16.0 (C-25),  $\delta$  16.2 (26),  $\delta$  14.7 (C-27),  $\delta$  18.2 (C-28) and  $\delta$  19.5 (C-30) in the  $^{13}\text{C}$  NMR spectrum.

The  $^{13}\text{C}$  NMR spectrum showed a ketonic carbon resonance at  $\delta$  218.6 which was seen to correlate in the HMBC spectrum with the methyl group proton resonances at  $\delta$  1.07,  $\delta$  1.02, which were ascribed to 3H-23 and 3H-24 and hence was assigned to C-3. In addition the resonance was seen to correlate with the H-5 methine proton resonance at  $\delta$  1.34 in the HMBC spectrum.

The C-30 carbon resonance at  $\delta$  19.5 corresponded with the downfield three proton resonance ascribed to 3H-30 at  $\delta$  1.68 in the HSQC spectrum. This carbon resonance showed correlation in the HMBC spectrum with the two non-equivalent methylene group proton resonances at  $\delta$  4.69 and  $\delta$  4.57 which were ascribed to H-29A and H-29B and also with H-19 methine proton resonance at  $\delta$  2.39 indicating the presence of an isopropenyl group.

Compound **2.6** belongs to the lupane class triterpenoids. The above data of compound **2.6** was compared to that of literature<sup>19</sup> and confirmed that compound **2.6** was the common lupenone.

**Table 2.7: NMR data for compound 2.6: lupenone**

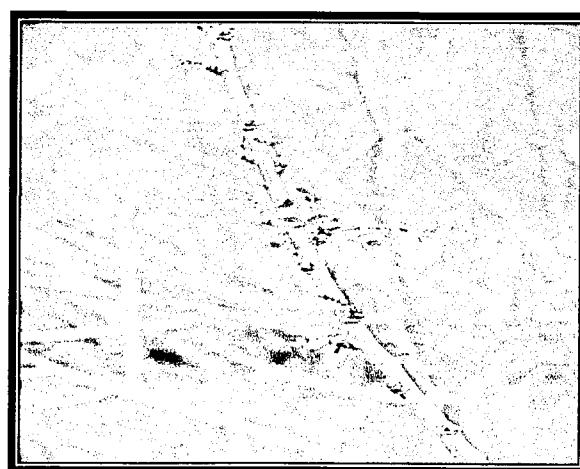
C	$^1\text{H}$ NMR (500 MHz) $\text{CDCl}_3$	$^{13}\text{C}$ NMR (125 MHz) $\text{CDCl}_3$	$^{13}\text{C}$ NMR (67.9 MHz) $\text{CDCl}_3$ <sup>19</sup>
1 $\alpha$	1.90 (1H, m)	39.8 (CH <sub>2</sub> )	39.6 (CH <sub>2</sub> )
1 $\beta$	1.42 (1H, m)		
2 $\alpha$	2.48 (1H, m)	34.4 (CH <sub>2</sub> )	34.1 (CH <sub>2</sub> )
2 $\beta$	2.42 (1H, m)		
3	-	218.6 (C)	217.9 (C)
4	-	47.5 (C)	47.3 (C)
5	1.34 (1H, m)	55.2 (CH)	55.0 (CH)
6 $\alpha$	1.47 (1H, m)	19.9 (CH <sub>2</sub> )	19.6 (CH <sub>2</sub> )
6 $\beta$	1.47 (1H, m)		
7 $\alpha$	1.43 (1H, m)	33.8 (CH <sub>2</sub> )	33.6 (CH <sub>2</sub> )
7 $\beta$	1.43 (1H, m)		
8	-	40.8 (C)	40.9 (C)
9	1.39 (1H, m)	50.0 (CH)	49.8 (CH)
10	-	37.1 (C)	36.9 (C)
11 $\alpha$	1.43 (1H, m)	21.7 (CH <sub>2</sub> )	21.5 (CH <sub>2</sub> )
11 $\beta$	1.43 (1H, m)		
12 $\alpha$	1.69 (1H, m)	25.4 (CH <sub>2</sub> )	25.2 (CH <sub>2</sub> )
12 $\beta$	1.10 (1H, m)		
13	1.68 (1H, m)	38.4 (CH)	38.2 (CH)
14	-	42.9 (C)	42.9 (C)
15	1.68 (1H, m)	27.7 (CH <sub>2</sub> )	27.4 (CH <sub>2</sub> )

15	1.02 (1H, m)		
16 $\alpha$	1.50 (1H, m)	35.7 (CH <sub>2</sub> )	35.6 (CH <sub>2</sub> )
16 $\beta$	1.41 (1H, m)		
17	-	42.9 (C)	42.9 (C)
18	1.39 (1H, m)	48.5 (CH)	48.3 (CH)
19	2.39 (1H, m)	47.9 (CH)	47.9 (CH)
20	-	151.5 (C)	150.7 (C)
21 $\alpha$	1.93 (1H, m)	30.1 (CH <sub>2</sub> )	29.9 (CH <sub>2</sub> )
21 $\beta$	1.27 (1H, m)		
22 $\alpha$	1.42 (1H, m)	40.2 (CH <sub>2</sub> )	40.0 (CH <sub>2</sub> )
22 $\beta$	1.21 (1H, m)		
23	1.07 (3H, s)	26.9 (CH <sub>3</sub> )	26.6 (CH <sub>3</sub> )
24	1.02 (3H, s)	21.3 (CH <sub>3</sub> )	21.0 (CH <sub>3</sub> )
25	1.07 (3H, s)	16.0 (CH <sub>3</sub> )	15.8 (CH <sub>3</sub> )
26	0.93 (3H, s)	16.2 (CH <sub>3</sub> )	15.9 (CH <sub>3</sub> )
27	0.96 (3H, s)	14.7 (CH <sub>3</sub> )	14.4 (CH <sub>3</sub> )
28	0.79 (3H, s)	18.2 (CH <sub>3</sub> )	18.0 (CH <sub>3</sub> )
29A	4.69 (1H, brs)	109.6 (CH <sub>2</sub> )	109.2 (CH <sub>2</sub> )
29B	4.57 (1H, brs)		
30	1.68 (3H, s)	19.5 (CH <sub>3</sub> )	19.3 (CH <sub>3</sub> )

## 2.2: A PHYTOCHEMICAL INVESTIGATION OF *PHYLLANTHUS RETICULATUS* (PHYLLANTHACEAE FAMILY)

### 2.2.1: Introduction

*Phyllanthus reticulatus* Poir is a small branched shrub usually 1-5 m high or a small, twiggy tree which grows up to 8 m in height and is often partially scrambling. The stem bark is light-reddish brown or grey-brown. The leaves alternate along slender branchlets up to 25 cm in length, appearing as leaflets of large pinnate leaves. Flowers are very small, and yellow. Fruits are berry-like, about 4-6 mm in diameter, which are black when mature.<sup>1</sup>



Picture 2.2 *Phyllanthus reticulatus* (Photo: Prof Neil Crouch)

*Phyllanthus reticulatus* was investigated previously by Hiu *et al.*<sup>20</sup> The petrol extracts of the stem bark and leaves yielded friedelan-3 $\beta$ -ol, friedelin, sitosterol, glochidionol, 21 $\alpha$ -hydroxyfriedelan-3-one, 21 $\alpha$ -hydroxyfriedel-4(23)-en-3-one and the ethanol extract yielded betulinic acid.<sup>20</sup> In 1997, Omulokoli *et al.* tested the leaf extracts of *P. reticulatus* *in vitro* for antiplasmodial activity against chloroquine-sensitive (K67) and chloroquine resistant (ENT 36) strains of *Plasmodium falciparum*, and they were found to be active.<sup>21</sup>

Re-investigation of the leaves and stem bark of *Phyllanthus reticulatus* has yielded the following compounds: the hexane and dichloromethane extracts of the stem bark afforded compound **2.7** friedelin, **2.8** glochidone, **2.9** lupeol, **2.10** *n*-tetraicosanyl-*trans*-4-hydroxy-3-methoxycinnamate and the common stigmasterol.

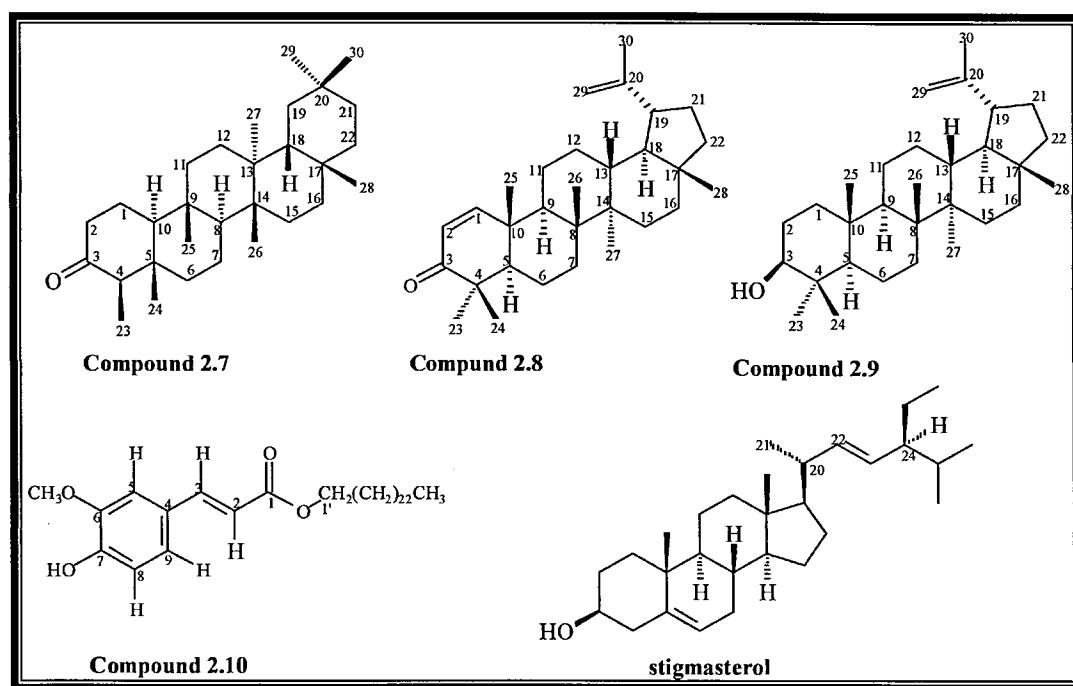
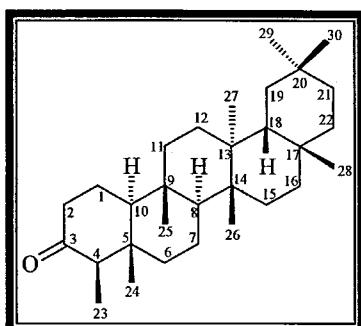


Figure 2.4: Compounds isolated from *Phyllanthus reticulatus*

## 2.2.2: Structural elucidation of compound 2.7: friedelan-3-one



Compound 2.7

Compound 2.7 was obtained as colourless needle-like crystals. The low resolution mass spectrum gave the molecular ion peak at  $m/z$  426 which is consistent with a molecular formula of  $C_{30}H_{50}O$  indicating a double bond equivalence of six. The FTIR mass spectrum showed the presence of a carbonyl group stretch at  $1709\text{ cm}^{-1}$ .<sup>2,3</sup> Compound 2.7 was identified as friedelin, which was isolated previously from other plant species of different families. Friedelin was isolated previously from *P. flexuosus* and *P. reticulatus*.<sup>20,22</sup>

The  $^1\text{H}$  NMR spectrum of compound 2.7 shows eight methyl group proton singlet resonances at  $\delta$  0.72, 0.87, 0.95, 1.00, 1.03, 1.05, 1.16 and 1.18 and a resonance at  $\delta$  0.87 occurred as a doublet ( $d, J= 6.6\text{ Hz}$ ). The  $^{13}\text{C}$  NMR spectrum showed the presence of a ketonic carbon resonance at  $\delta$  213.5 which was assigned to C-3. The C-3 carbon resonance showed correlations in the HMBC spectrum with the methyl proton resonance at  $\delta$  0.87, which was ascribed to 3H-23, and with the methine proton resonance at  $\delta$  2.25, assigned to H-4.

The corresponding C-4 carbon resonance at  $\delta$  58.4 showed correlations with the 3H-23 methyl group proton resonance previously assigned and with the 3H-24 methyl group proton resonance at  $\delta$  0.72 in the HMBC spectrum. In addition, the C-4 resonance was seen to correlate with the two H-6 proton resonances ( $\delta$  1.74,  $\delta$  1.29). These resonances were seen to be coupled with the two H-7 proton resonances ( $\delta$  1.48,  $\delta$  1.39) in the COSY spectrum. An additional correlation

was seen between the C-4 carbon resonance and the methine resonance at  $\delta$  1.53, which was assigned to H-10.

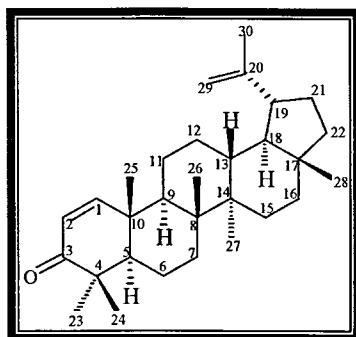
This information established the class of tritepenoid as a friedelane and a literature search indicated that compound 2.7 was the known friedelin.<sup>22</sup>

**Table 2.8: NMR data for compound 2.7: friedelan-3-one**

no	<sup>1</sup> H NMR (500 MHz) CDCl <sub>3</sub>	<sup>13</sup> C NMR (125 MHz) CDCl <sub>3</sub>	<sup>1</sup> H NMR (CDCl <sub>3</sub> ) <sup>22</sup>	<sup>13</sup> C NMR (CDCl <sub>3</sub> ) <sup>22</sup>
1 $\alpha$	1.95 (1H, m)	22.5 (CH <sub>2</sub> )	1.96	22.3 (CH <sub>2</sub> )
1 $\beta$	1.67 (1H, m)		1.68	
2 $\alpha$	2.39 (1H, m)	41.7 (CH <sub>2</sub> )	2.39	41.5 (CH <sub>2</sub> )
2 $\beta$	2.29 (1H, m)		2.28	
3	-	213.5 (C)	-	213.2 (C)
4	2.25 (1H, quartet, <i>J</i> =6.6 Hz))	58.2 (CH)	2.25	58.2 (CH)
5	-	42.4 (C)	-	42.1 (CH)
6 $\alpha$	1.29 (1H, m)	41.5 (CH <sub>2</sub> )	1.28	41.3 (CH <sub>2</sub> )
6 $\beta$	1.74 (1H, m)		1.75	
7 $\alpha$	1.48 (1H, m)	18.2 (CH <sub>2</sub> )	1.49	18.2 (CH <sub>2</sub> )
7 $\beta$	1.39 (1H, m)		1.37	
8	1.38 (1H, m)	53.3 (CH)	1.39	53.1 (CH)
9	-	37.7 (C)	-	37.4 (C)
10	1.53 (1H,d, <i>J</i> =1.3 Hz)	59.7 (CH)	1.53	59.5 (CH)
11 $\alpha$	1.24 (1H, m)	35.8 (CH <sub>2</sub> )	1.26	35.6 (CH <sub>2</sub> )
11 $\beta$	1.44 (1H, m)		1.46	
12 $\alpha$	1.34 (1H, m)	30.5 (CH <sub>2</sub> )	1.34	30.5 (CH <sub>2</sub> )
12 $\beta$	1.34 (1H, m)		1.34	
13	-	39.9 (C)	-	39.7 (C)
14	-	38.5 (C)	-	38.3 (C)
15 $\alpha$	1.48 (1H, m)	32.4 (CH <sub>2</sub> )	1.46	32.5 (CH <sub>2</sub> )
15 $\beta$	1.28 (1H, m)		1.27	
16 $\alpha$	1.55 (1H, m)	36.2 (CH <sub>2</sub> )	1.57	36.0 (CH <sub>2</sub> )
16 $\beta$	1.44 (1H, m)		1.46	
17	-	29.9 (C)	-	29.9 (C)
18	1.54 (1H, m)	43.0 (CH)	1.56	42.8 (CH)
19 $\alpha$	1.38 (1H, m)	35.5 (CH <sub>2</sub> )	1.38	35.3 (CH <sub>2</sub> )
19 $\beta$	1.21 (1H, m)		1.20	
20	-	28.4 (C)	-	28.2 (C)
21 $\alpha$	1.51 (1H, m)	32.9 (CH <sub>2</sub> )	1.51	32.4 (CH <sub>2</sub> )
21 $\beta$	1.29 (1H, m)		1.30	

22 $\alpha$	1.50 (1H,m)	39.5 (CH <sub>2</sub> )	1.50	39.4 (CH <sub>2</sub> )
22 $\beta$	0.93 (1H, m)		0.94	
23	0.87 (1H,d, <i>J</i> =6.6 Hz)	7.0 (CH <sub>3</sub> )	0.87	6.8 (CH <sub>3</sub> )
24	0.72 (3H, s)	14.9 (CH <sub>3</sub> )	0.71 (3H, s)	14.7 (CH <sub>3</sub> )
25	0.87 (3H, s)	18.2 (CH <sub>3</sub> )	0.86 (3H, s)	17.9 (CH <sub>3</sub> )
26	1.00 (3H, s)	20.5 (CH <sub>3</sub> )	1.00 (3H, s)	20.3 (CH <sub>3</sub> )
27	1.05 (3H, s)	18.9 (CH <sub>3</sub> )	1.05 (3H, s)	18.7 (CH <sub>3</sub> )
28	1.18 (3H, s)	32.1 (CH <sub>3</sub> )	1.17 (3H, s)	32.1 (CH <sub>3</sub> )
29	0.98 (3H, s)	31.8 (CH <sub>3</sub> )	1.00 (3H, s)	31.8 (CH <sub>3</sub> )
30	0.95 (3H, s)	35.2 (CH <sub>3</sub> )	0.95 (3H, s)	35.0 (CH <sub>3</sub> )

### 2.2.3: Structural elucidation of compound 2.8: 3-oxolup-1:20 (29)-diene (glochidone)



Compound 2.8

The low resolution mass spectrum of compound **2.8** gave a molecular ion peak at *m/z* 422, which is consistent with a molecular formula of C<sub>30</sub>H<sub>46</sub>O. A double bond equivalence of eight was calculated. The FTIR spectrum revealed a carbonyl stretch at 1671 cm<sup>-1</sup>. Compound **2.8** was identified as glochidone, which was previously isolated from *Glochidion* and *Phyllanthus* species.<sup>23</sup> The structure determination was determined based on <sup>1</sup>H, <sup>13</sup>C NMR, IR and MS analysis.<sup>24,25</sup>

The <sup>13</sup>C NMR spectrum revealed that compound **2.8** had an  $\alpha$ ,  $\beta$ -unsaturated ketone in ring A, with C-1, C-2 and C-3 occurring at  $\delta$  159.9, 125.1 and 205.6 respectively. The H-1 and H-2 resonances appeared as a pair of coupled doublets at  $\delta$  7.09 and  $\delta$  5.79 (*J* = 10.25 Hz) respectively. The C-3 carbon resonance showed correlations with the methyl group proton resonances at  $\delta$  1.13 and  $\delta$  1.10 which were assigned to the 3H-23 and 3H-24 proton resonances

and with the H-5 methine proton resonance at  $\delta$  1.54, which showed coupling with the superimposed H-6 proton resonances at  $\delta$  1.53. In addition, the C-3 resonance showed a correlation with the H-1 methine proton resonance at  $\delta$  7.09 (d,  $J = 10.25$  Hz). The H-1 proton resonance showed correlations with the methyl group carbon resonances at C-25, C-9 ( $\delta$  44.4) and C-3 ( $\delta$  205.6).

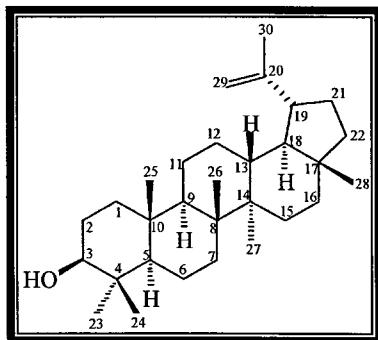
The  $^1\text{H}$  NMR spectrum showed a downfield methyl group proton resonance at  $\delta$  1.69 (3H-30), which was seen to correspond with the carbon resonance at  $\delta$  19.2 in the HSQC spectrum. The C-30 carbon resonance was seen to correlate in the HMBC spectrum with the non-equivalent methylene proton resonances  $\delta$  4.71 and  $\delta$  4.59 which were assigned to H-29A and H-29B. This indicated the presence of an isopropenyl group, suggesting that compound **2.8** belonged to the lupane class of triterpenoids. Therefore the data above confirmed that compound **2.8** was the known glochidone.

**Table 2.9:** NMR data for compound **2.8** glochidone in  $\text{CDCl}_3$

C	$^1\text{H}$ NMR (500 MHz)	$^{13}\text{C}$ NMR (125 MHz)	$^1\text{H}$ NMR ( $\text{CDCl}_3$ ) <sup>24,25</sup>	$^{13}\text{C}$ NMR ( $\text{CDCl}_3$ ) <sup>24,25</sup>
1	7.09 (1H, d, $J = 10.25$ Hz)	159.9 (CH)	7.02 (d, $J = 10.0$ Hz)	159.8 (CH)
2	5.79 (1H, d, $J = 10.25$ Hz)	125.1 (CH)	5.73 (d, $J = 10.0$ Hz)	125.1 (CH)
3	-	205.6 (C)		205.5 (C)
4	-	44.6 (C)		44.6 (C)
5	1.54 (1H, m)	53.4 (CH)		53.4 (CH)
6 $\alpha$	1.53 (1H, m)	19.0 (CH <sub>2</sub> )		19.0 (CH <sub>2</sub> )
6 $\beta$	1.53 (1H, m)			
7 $\alpha$	1.47 (1H, m)	33.7 (CH <sub>2</sub> )		33.8 (CH <sub>2</sub> )
7 $\beta$	1.47 (1H, m)			
8	-	39.5 (C)		39.5 (C)
9	1.57 (1H, m)	44.4 (CH)		44.4 (CH)
10	-	41.7 (C)		41.7 (C)
11 $\alpha$	1.63 (1H, m)	21.5 (CH <sub>2</sub> )		21.4 (CH <sub>2</sub> )
11 $\beta$	1.39 (1H, m)			
12 $\alpha$	1.76 (1H, m)	25.4 (CH <sub>2</sub> )		25.1 (CH <sub>2</sub> )
12 $\beta$	1.13 (1H, m)			
13	1.76 (1H, m)	38.2 (CH)		38.2 (CH)
14	-	43.1 (C)		43.1 (C)
15 $\alpha$	1.69 (1H, m)	27.3 (CH <sub>2</sub> )		27.3 (CH <sub>2</sub> )
15 $\beta$	1.04 (1H, m)			
16 $\alpha$	1.51 (1H, m)	35.5 (CH <sub>2</sub> )		35.5 (CH <sub>2</sub> )
16 $\beta$	1.40 (1H, m)			
17	-	43.0 (C)		43.1 (C)
18	1.38 (1H, m)	48.1 (CH)		48.1 (CH)
19 $\alpha$	2.41 (1H, m)	47.9 (CH)		47.9 (CH)
20	-	150.8 (C)		150.7 (C)
21 $\alpha$	1.93 (1H, m)	29.7 (CH <sub>2</sub> )		29.8 (CH <sub>2</sub> )

21 $\beta$	1.33 (1H, m)			
22 $\alpha$	1.41 (1H, m)	40.0 (CH <sub>2</sub> )		40.0 (CH <sub>2</sub> )
22 $\beta$	1.19 (1H, m)			
23	1.13 (3H, s)	27.8 (CH <sub>3</sub> )		27.8 (CH <sub>3</sub> )
24	1.11 (3H, s)	21.4 (CH <sub>3</sub> )		21.3 (C)
25		19.3 (CH <sub>3</sub> )		19.0 (CH <sub>3</sub> )
26	1.11 (3H, s)	16.4 (CH <sub>3</sub> )		16.5 (CH <sub>3</sub> )
27	0.96 (3H, s)	14.4 (CH <sub>3</sub> )	0.90	14.4 (CH <sub>3</sub> )
28	0.81 (3H, s)	18.0 (CH <sub>3</sub> )		18.1 (CH <sub>3</sub> )
29 A	4.71 (1H, brs)	109.5 (CH <sub>2</sub> )	4.60 (1H, brs)	109.5 (CH <sub>2</sub> )
29 B	4.59 (1H, brs)		4.50 (1H, brs)	
30	1.69 (3H, s)	19.2 (CH <sub>3</sub> )	1.60 (3H, s)	19.2 (CH <sub>3</sub> )

#### 2.2.4: Structural elucidation of compound 2.9: 3 $\beta$ -hydroxylup-20(29)-ene: lupeol



Compound 2.9

Compound 2.9 was isolated as a white crystalline solid. The low resolution mass spectrum showed a molecular ion peak at *m/z* 426, which corresponded to a molecular formula of C<sub>30</sub>H<sub>50</sub>O. The FTIR spectrum showed an OH group stretch band at 3437 cm<sup>-1</sup>. Compound 2.9 was identified as lupeol, previously isolated from a number of sources.<sup>24</sup> The structure of the compound was determined based on the <sup>1</sup>H NMR, <sup>13</sup>C NMR, and IR spectroscopy.<sup>26</sup>

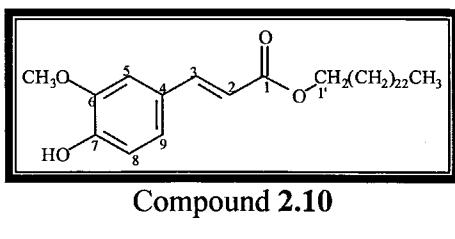
The <sup>1</sup>H NMR spectrum showed the presence of seven methyl group proton resonances at  $\delta$  0.77, 0.83, 0.97, 0.98, 1.02, and 1.69, which corresponded to the carbon resonances at 15.6 (C-24), 16.3 (C-25),  $\delta$  28.2 (C-23), 14.8 (C-29), 16.2 (C-27), and 19.5 (C-30) in the <sup>13</sup>C NMR spectrum. The <sup>1</sup>H NMR spectrum revealed a proton resonance at  $\delta$  3.18 (1H, dd, *J* = 11.35, *J* = 4.85 Hz) which was seen to correspond to a carbon resonance at  $\delta$  79.3 in the HSQC spectrum. This resonance was seen to correlate in the HMBC spectrum with the 3H-23 and 3H-24 methyl group resonances at  $\delta$  0.97 and 0.77 and with the H-5 proton resonance at  $\delta$  0.69 in the HMBC spectrum. An isopropenyl group was present in compound 2.9, as in compound 2.8. The HMBC

spectrum showed correlation between the downfield methyl group resonance at  $\delta$  1.69 and the two non-equivalent methylene proton resonances at  $\delta$  4.68 and 4.56 which were ascribed to H-29A and H-29B. Compound **2.9** was identified as the known  $3\beta$ -hydroxylup-20(29)-ene, commonly known as the lupeol. Lupeol has been evaluated for its anti-inflammatory and antiangiogenic activities.<sup>27,28</sup>

**Table 2.10: NMR data for compound 2.9: lupeol**

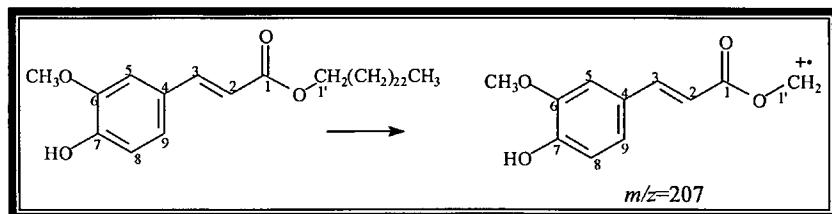
C	<sup>1</sup> H NMR (500 MHz) (CDCl <sub>3</sub> )	<sup>13</sup> C NMR (125 MHz) (CDCl <sub>3</sub> )	<sup>1</sup> HMR (500 MHz) (CDCl <sub>3</sub> ) <sup>26</sup>	<sup>13</sup> C NMR (125 MHz) (CDCl <sub>3</sub> ) <sup>26</sup>
1 $\alpha$	0.91 (1H, m)	38.9 (CH <sub>2</sub> )	0.90 (1H, d)	38.7 (CH <sub>2</sub> )
1 $\beta$	1.65 (1H, m)		1.67 (1H, t)	
2 $\alpha$	1.60 (1H, m)	27.6 (CH <sub>2</sub> )	1.60 (1H, d)	27.4 (CH <sub>2</sub> )
2 $\beta$	1.56 (1H, m)		1.56 (1H, q)	
3	3.18 (1H, dd, <i>J</i> = 11.35, 4.85 Hz)	79.2 (CH)	3.19 (1H, dd)	79.0 (CH)
4	-	38.9 (C)	-	38.8 (C)
5	0.69 (1H, d, <i>J</i> = 9.25 Hz)	55.5 (CH)	0.68 (1H, d)	55.3 (CH)
6 $\alpha$	1.51 (1H, m)	18.5 (CH <sub>2</sub> )	1.51 (1H, d)	18.3 (CH <sub>2</sub> )
6 $\beta$	1.39 (1H, m)		1.39 (1H, q)	
7 $\alpha$	1.38 (1H, m)	34.5 (CH <sub>2</sub> )	1.39 (1H, m)	34.3 (CH <sub>2</sub> )
7 $\beta$	1.38 (1H, m)		1.39 (1H, m)	
8	-	40.1 (C)	-	40.8 (C)
9	1.27 (1H, m)	50.7 (CH)	1.27 (1H, d)	50.4 (CH)
10	-	37.4 (C)	-	37.2 (C)
11 $\alpha$	1.41 (1H, m)	21.2 (CH <sub>2</sub> )	1.41 (1H, d)	20.9 (CH <sub>2</sub> )
11 $\beta$	1.21 (1H, m)		1.23 (1H, q)	
12 $\alpha$	1.06 (1H, m)	25.4 (CH <sub>2</sub> )	1.07 (1H, q)	25.2 (CH <sub>2</sub> )
12 $\beta$	1.67 (1H, m)		1.67 (1H, d)	
13	1.64 (1H, m)	38.3 (CH)	1.66 (1H, t)	38.1 (CH)
14	-	43.1 (C)	-	42.9 (C)
15 $\alpha$	1.00 (1H, m)	27.6 (CH <sub>2</sub> )	1.00 (1H, d)	27.5 (CH <sub>2</sub> )
15 $\beta$	1.67 (1H, m)		1.68 (1H, t)	
16 $\alpha$	1.38 (1H, m)	35.8 (CH <sub>2</sub> )	1.37 (1H, t)	35.6 (CH <sub>2</sub> )
16 $\beta$	1.47 (1H, d)		1.47	
17	-	43.2 (C)	-	43.0 (C)
18	1.35 (1H, m)	48.5 (CH)	1.36 (1H, t)	48.0 (CH)
19	2.38 (1H, m)	48.2 (CH)	2.39 (1H, m)	47.9 (CH)
20	-	151.2 (C)	-	151.0 (C)
21 $\alpha$	1.32 (1H, m)	29.9 (CH <sub>2</sub> )	1.32 (1H, m)	29.9 (CH <sub>2</sub> )
21 $\beta$	1.92 (1H, m)		1.92 (1H, m)	
22 $\alpha$	1.19 (1H, m)	40.2 (CH <sub>2</sub> )	1.19 (1H, m)	40.0 (CH <sub>2</sub> )
22 $\beta$	1.38 (1H, m)		1.38 (1H, m)	
23	0.97 (3H, s)	28.2 (CH <sub>3</sub> )	0.97 (3H, s)	28.00 (CH <sub>3</sub> )
24	0.77 (3H, s)	15.6 (CH <sub>3</sub> )	0.77 (3H, s)	15.4 (CH <sub>3</sub> )
25	0.83 (3H, s)	16.3 (CH <sub>3</sub> )	0.83 (3H, s)	16.1 (CH <sub>3</sub> )
26	1.02 (3H, s)	16.2 (CH <sub>3</sub> )	1.03 (3H, s)	16.0 (CH <sub>3</sub> )
27	0.98 (3H, s)	14.7 (CH <sub>3</sub> )	0.95 (3H, s)	14.6 (CH <sub>3</sub> )
28	0.79 (3H, s)	18.2 (CH <sub>3</sub> )	0.79 (3H, s)	18.0 (CH <sub>3</sub> )
29A	4.56 (1H, brs)	109.5 (CH <sub>2</sub> )	4.56 (1H, m)	109.3 (CH <sub>2</sub> )
29B	4.68 (1H, brs)		4.69 (1H, m)	
30	1.69 (3H, s)	19.5 (CH <sub>3</sub> )	1.68 (3H, s)	19.3 (CH <sub>3</sub> )

**2.2.5: Structural elucidation of compound 2.10: *n*-Tetraicosanyl-*trans*-4-hydroxy-3-methoxycinnamoate.**



Compound 2.10 was isolated as a white amorphous solid. The low resolution mass spectrum gave the highest peak at *m/z* 530, a base peak at *m/z* 529 and a fragment ion peak at *m/z* 207. The IR spectrum showed a carbonyl group stretch at 1708 cm<sup>-1</sup>.

The NMR spectra revealed that the compound was an alkylated cinnamic ester. The <sup>13</sup>C NMR spectrum revealed a C-1 ester carbonyl resonance at  $\delta$  167.6. This resonance showed correlations in the HMBC spectrum with the resonances at  $\delta$  6.27 (d, *J* = 15.9 Hz) and 7.59 (d, *J* = 15.9 Hz) which were assigned to H-2 and H-3 of a *trans*-double bond. The aromatic ring showed an ABX system with the H-5 proton resonance occurring as a doublet at  $\delta$  7.02 (d, *J* = 1.9 Hz), the H-9 proton resonances occurring as a double doublet at  $\delta$  7.06 (dd, *J* = 8.24, 1.9 Hz), and H-8 occurring at  $\delta$  6.90 (d, *J* = 8.24 Hz). A methoxy group proton resonance occurred at  $\delta$  3.91 and was seen to correlate with the H-5 resonance in the NOESY spectrum, hence the methoxy group was placed at C-6. A hydroxyl group was placed in the remaining C-7 position. The peak at *m/z* 207 corresponds to the fragment ion shown below. The difference between the molecular ion peak and this fragment at *m/z* 207 indicated a -(CH<sub>2</sub>)<sub>22</sub>-CH<sub>3</sub> fragment had been lost and that the esterifying alcohol was *n*-tetraicosanol. Thus compound 2.10 was identified as *n*-tetraicosanyl-*trans*-4-hydroxy-3-methoxycinnamoate.



Fragmentation of Compound 2.10

Table 2.11: NMR data for compound 2.10: n-Tetraeicosanyl-*trans*-4-hydroxy-3-methoxycinnamoate.

no	<sup>1</sup> H NMR (125 MHz) (CDCl <sub>3</sub> )	<sup>13</sup> C NMR (400 MHz) (CDCl <sub>3</sub> )
1	-	167.6 (C)
2	6.27 (1H, d, <i>J</i> = 15.93)	115.9 (CH)
3	7.59 (1H, d, <i>J</i> = 15.93)	144.8 (CH)
4	-	127.3 (C)
5	7.02 ((1H, d, <i>J</i> = 1.90)	109.5 (CH)
6	-	146.9 (C)
7	-	148.1 (C)
8	6.90 (1H, d, <i>J</i> = 8.24)	114.9 (CH)
9	7.06 (1H, dd, <i>J</i> = 8.24, <i>J</i> = 1.90)	123.3 (CH)
OCH <sub>3</sub>	3.91 (3H, s)	56.2 (CH <sub>3</sub> )
1'	4.17 (2H, t, <i>J</i> = 6.68)	64.8
2'	1.69 (pentet, <i>J</i> = 6.80)	29.0
3'-23'	1.3-1.4 (overlapping)	22.9, 26.2, 29.5, 29.7, 29.9, 32.2*
24'	(3H, t, <i>J</i> = 6.87)	14.3

\* Some peaks overlapping.

## 2.3: A PHYTOCHEMICAL INVESTIGATION OF *HEYWOODIA LUCENS* (PHYLLANTHEACEAE FAMILY)

### 2.3.1: Introduction

The stem bark of *Heywoodia lucens* yielded three known compounds, compounds **2.11** ( $\alpha$ -glutinol), lupeol and **2.8**, (*n*-tetraeicosanyl-*trans*-4-hydroxy-3-methoxycinnamoate).



Picture 2.2: *Heywoodia lucens* (Photo: Prof. Neil Crouch)

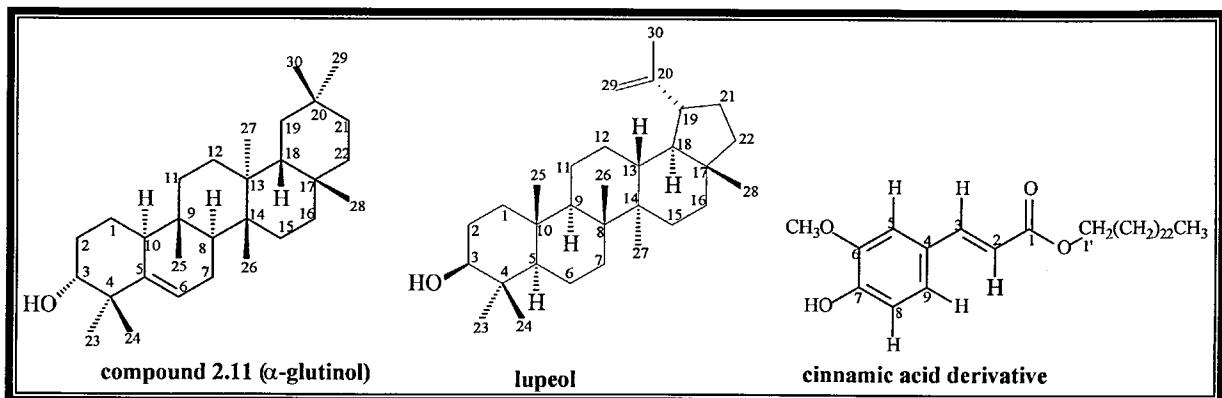
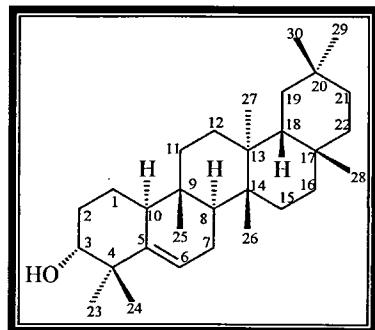


Figure 2.5: Compounds isolated from *Heywoodia lucens*

### 2.3.2: Structural elucidation of compound 2.11 ( $\alpha$ -glutinol): 3 $\alpha$ -hydroxy-D:B:friedoolean-5-ene



Compound 2.11

The low resolution mass spectrum of compound **2.11** gave a molecular ion peak at  $m/z$  426, which is consistent with a molecular formula of  $C_{30}H_{50}O$ . The FTIR spectrum showed an OH group stretch band at  $3451\text{ cm}^{-1}$ . Compound **2.11** was found to be  $\alpha$ -glutinol.<sup>24</sup> The structure of compound **2.11** was determined based on  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR analysis and IR analysis.

The  $^1\text{H}$  NMR spectrum revealed the resonance at  $\delta$  3.47 (t,  $J=3.00\text{ Hz}$ ) and which was seen to correspond to the carbon resonance at  $\delta$  76.6 in the HSQC spectrum. This resonance was assigned to H-3 $\beta$  due to the coupling constant and correlation between C-3 carbon resonance and the 3H-23 and 3H-24 methyl group proton resonances ( $\delta$  1.04,  $\delta$  1.14) in the HMBC spectrum. In addition the resonance was seen to correlate with the H-1 methylene proton resonances at  $\delta$

1.55 and  $\delta$  1.47 and these were seen, in turn, to be coupled with the two H-2 methylene proton resonances at  $\delta$  1.85 and  $\delta$  1.69. Furthermore the H-1 proton resonances were seen to be coupled with the H-10 methine proton resonance at  $\delta$  2.02 in the COSY spectrum. In addition, the H-1 resonance was seen to correlate in the HMBC spectrum with the C-5 carbon resonance at  $\delta$  141.8.

The H-10 methine proton showed correlations in the HMBC spectrum with carbon resonance ascribed to C-6 ( $\delta$  122.3), the C-25 methyl carbon resonance ( $\delta$  16.4), and the C-11 methylene carbon resonance ( $\delta$  38.8).

The above data confirmed compound **2.11** is  $\alpha$ -glutinol.

**Table 2.12:** NMR data of compound **2.11:**  $\alpha$ -glutinol

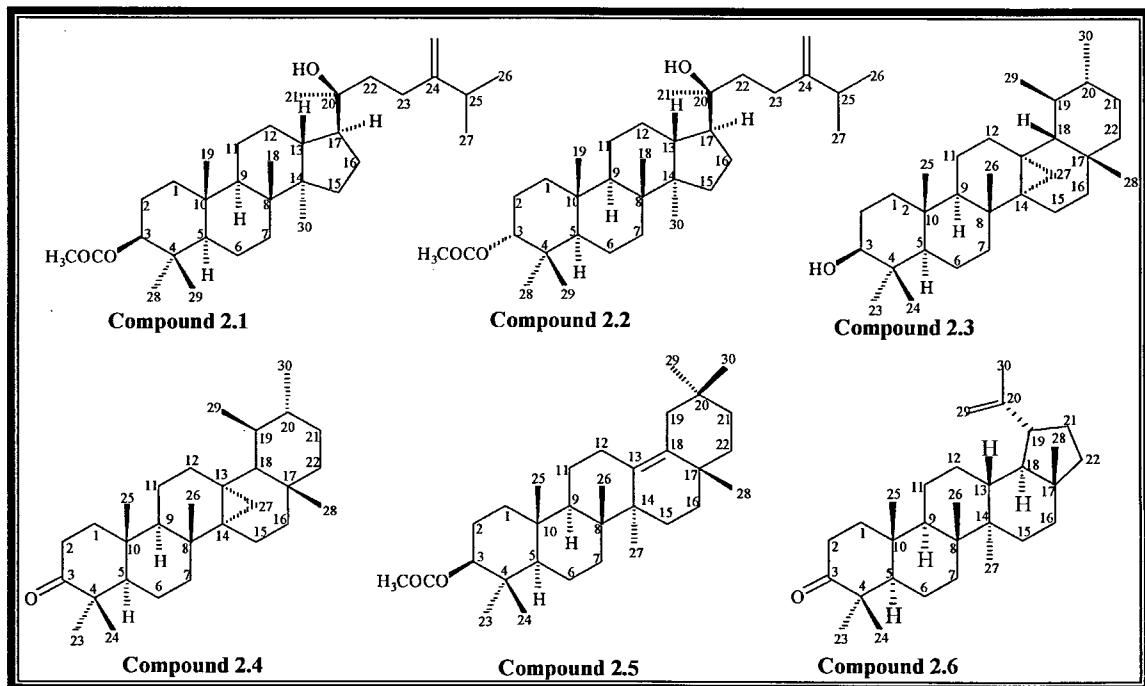
C	$^1\text{H}$ NMR (500 MHz) ( $\text{CDCl}_3$ )	$^{13}\text{C}$ NMR (125 MHz) ( $\text{CDCl}_3$ )	$^{13}\text{C}$ NMR ( $\text{CDCl}_3$ ) <sup>24</sup>
1 $\alpha$	1.55 (1H,m)	18.4 (CH <sub>2</sub> )	18.2 (CH <sub>2</sub> )
1 $\beta$	1.47 (1H,m)		
2 $\alpha$	1.85 (1H,m)	28.1 (CH <sub>2</sub> )	27.8 (CH <sub>2</sub> )
2 $\beta$	1.69 (1H,m)		
3	3.47 (1H, t, $J$ = 3.00 Hz)	76.6 (CH)	76.3 (CH)
4	-	41.1 (C)	40.8 (C)
5	-	141.8 (C)	141.6 (C)
6	5.64	122.3 (CH)	122.1 (CH)
7 $\alpha$	1.96 (1H, m)	23.9 (CH <sub>2</sub> )	23.6 (CH <sub>2</sub> )
7 $\beta$	1.85 (1H, m)		
8	1.52(1H, m)	47.7 (CH)	47.4 (CH)
9	-	35.1 (C)	34.8 (C)
10	2.02(1H, m)	50.0 (CH)	49.6 (CH)
11 $\alpha$	1.55 (1H, m)	34.8 (CH <sub>2</sub> )	34.6 (CH <sub>2</sub> )
11 $\beta$	1.40(1H, m)		
12 $\alpha$	<1.34 > (1H, m)	30.6 (CH <sub>2</sub> )	30.3 (CH <sub>2</sub> )
12 $\beta$	<1.34> (1H, m)		
13	-	38.1 (C)	37.8 (C)
14	-	39.5 (C)	39.3 (C)
15 $\alpha$	1.46 (1H, m)	32.3(CH <sub>2</sub> )	32.0 (CH <sub>2</sub> )
15 $\beta$	1.30 (1H, m)		
16 $\alpha$	1.53 (1H, m)	36.2 (CH <sub>2</sub> )	36.0 (CH <sub>2</sub> )

16 $\beta$	1.38 (1H, m)		
17	-	30.3 (C)	30.1 (C)
18	1.58 (1H, m)	43.3 (CH)	43.0 (CH)
19	1.40 (1H, m)	35.3 (CH <sub>2</sub> )	35.0 (CH <sub>2</sub> )
20	-	28.5 (C)	28.2 (C)
21 $\alpha$	1.28 (1H, m)	33.3 (CH <sub>2</sub> )	33.1 (CH <sub>2</sub> )
21 $\beta$	1.26 (1H, m)		
22 $\alpha$	1.54 (1H, m)	39.2 (CH <sub>2</sub> )	38.9 (CH <sub>2</sub> )
22 $\beta$	0.92 (1H, m)		
23	1.04 (3H, s)	29.2 (CH <sub>3</sub> )	28.9 (CH <sub>3</sub> )
24	1.14 (3H, s)	25.7 (CH <sub>3</sub> )	25.4 (CH <sub>3</sub> )
25	0.85 (3H, s)	16.5 (CH <sub>3</sub> )	16.2 (CH <sub>3</sub> )
26	1.11 (3H, s)	19.8 (CH <sub>3</sub> )	19.6 (CH <sub>3</sub> )
27	1.01 (3H, s)	18.6 (CH <sub>3</sub> )	18.4 (CH <sub>3</sub> )
28	1.16 (3H, s)	32.3 (CH <sub>3</sub> )	32.0 (CH <sub>3</sub> )
29	0.99(3H, s)	32.6 (CH <sub>3</sub> )	32.4 (CH <sub>3</sub> )
30	0.95(3H, s)	34.7 (CH <sub>3</sub> )	34.5 (CH <sub>3</sub> )

## 2.4: Conclusions

The examination of the stem bark and the leaves of *Phyllanthus cedrelifolius* yielded six compounds. The hexane extract of the stem bark of *P. cedrelifolius* yielded compounds **2.3** (13, 27-cycloursan-3 $\beta$ -ol (phyllanthol)), **2.4** (13, 27-cycloursan-3-one (phyllanthone)) and **2.5** (3 $\beta$ -acetoxyolean-13(18)-ene ( $\delta$ -amyrin acetate)). The dichloromethane extract yielded compounds **2.1** ((20S)-3 $\beta$ -acetoxy-24-methylidenedammarane-20-ol) and **2.6** lupenone (3-oxolup-20(29)-ene). The combined hexane and dichloromethane extracts of the leaves yielded compounds **2.2** (20S)-3 $\alpha$ -acetoxy-24-methylidenedammarane-20-ol, **2.3** (phyllanthol) and **2.6** (lupenone).

The ethyl acetate extract of the leaves yielded compound **2.2** (20S)- 3 $\alpha$ -acetoxy, 24-methylidenedammarane-20-ol.

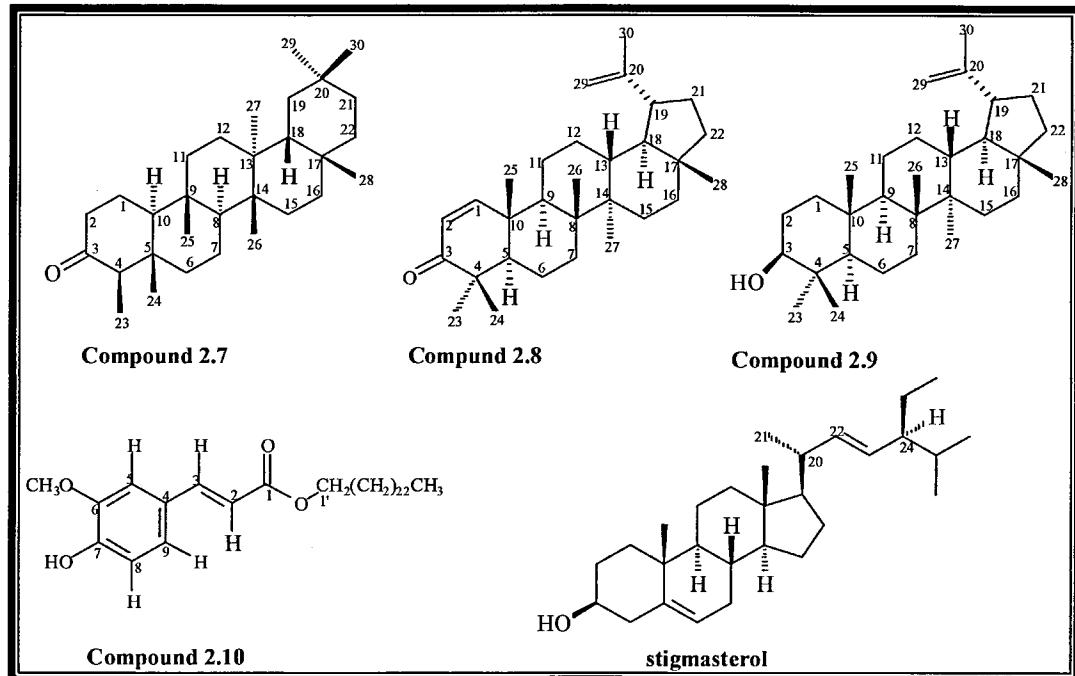


This plant has yielded triterpenoids of the dammarane, phyllanthane (modified ursane), oleanane and lupane classes. The dammaranes, (20*S*)-3 $\alpha$ -acetoxy-24-methylidenedammaran-20-ol and (20*S*)-3 $\beta$ -acetoxy-24-methylidenedammaran-20-ol, are the first dammaranes to be reported from the Phyllanthaceae family.

Phyllanthol has been reported previously from *Phyllanthus engleri* and *Phyllanthus acidus*. The report of Hnatyszyn and Ferraro of the isolation of this compound from *P. sellowianus* must be in doubt, as the NMR data did not support the phyllanthol structure. Complete assignment of the the NMR data for compounds **2.1** ((20*S*)-3 $\beta$ -acetoxy-24-methylidenedammaran-20-ol), compounds **2.2** (20*S*)-3 $\alpha$ -acetoxy-24-methylidenedammaran-20-ol, compounds **2.3** (13, 27-cycloursan-3 $\beta$ -ol (phyllanthol)) and **2.4** (13, 27-cycloursan-3-one (phyllanthone)) were undertaken.  $\delta$ -Amyrin acetate and lupenone are common triterpenoids. The six compounds isolated are now available for anti-microbial and anti-cancer screening.

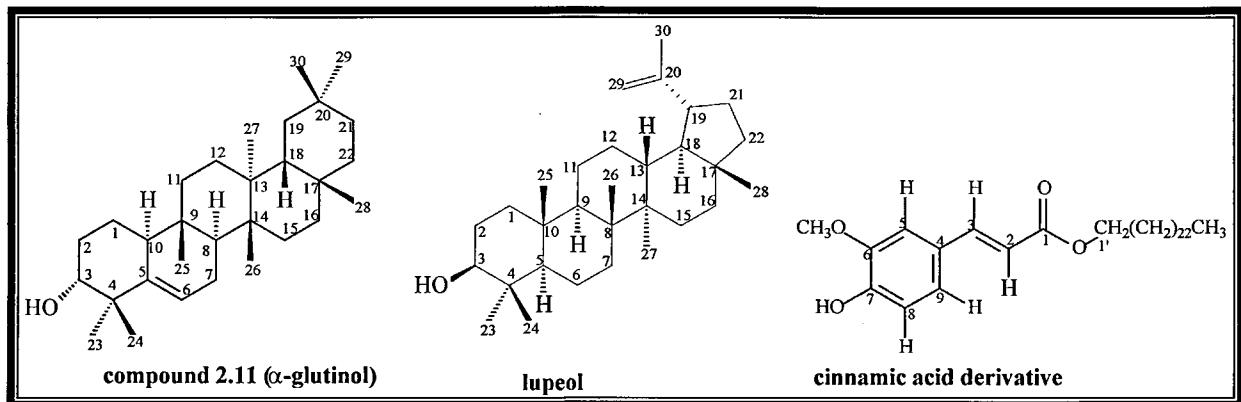
The examination of the leaves and stem bark of *Phyllanthus reticulatus* has yielded the common compound **2.7** friedelin, **2.8** glochidone, **2.9** lupeol, **2.10** *n*-tetraicosanyl-*trans*-4-hydroxy-3-

methoxycinnamate and the common stigmasterol. This plant has yielded the friedelane and lupane classes and a cinnamic acid derivative.



Compounds isolated from *Phyllanthus reticulatus*

The stem bark of *Heywoodia lucens* yielded three known compounds, compounds **2.11** ( $\alpha$ -glutinol), lupeol and **2.10**, (alkyl-*trans*-4-hydroxy-3-methoxycinnamate).



Compounds isolated from *Heywoodia lucens*

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## **CHAPTER 3: A REVIEW OF THE PHYTOCHEMISTRY AND BIOACTIVITY OF PLANTS OF THE GENUS *SAPIUM* OF THE EUPHORBIACEAE FAMILY**

### **3.1: The Euphorbiaceae family**

The Euphorbiaceae is a large diverse family, which is comprised of 340 genera and 8000-9000 species.<sup>1</sup> Plants of the Euphorbiaceae family may be herbs, shrubs or trees.<sup>2,3</sup> The plants are frequently characterized by a milky latex that is irritating or toxic.<sup>4,5</sup> The latex can cause severe irritation, with mucous membranes and eyes being affected. Plants of the Euphorbiaceae are rich in phenolic compounds, especially tannins and flavonoids.<sup>5</sup>

### **3.2: The genus *Sapium***

The genus *Sapium* of the Euphorbiaceae family is widely distributed in the coastal areas of South East Asia and Africa bordering the Indian Ocean tropics and subtropics. The genus comprises one hundred and twenty-one species.<sup>6</sup> Plant species of the genus *Sapium*, have been phytochemically investigated, and have yielded mono, di- and triterpenoids and also phenolic compounds such as flavonoids, coumarins, and tannins.<sup>7</sup> This genus is known to be rich in the highly active diterpenoid phorbol esters which produce a variety of biological effects such as lymphocyte mitogenesis, platelet aggregation and inflammation.<sup>5,8,47</sup> Phorbol esters activate protein kinase C (PKC). This PKC enzyme is responsible for the phosphorylation of many biochemical entities. The activation of PKC is thought to lead to uncontrolled cancerous growth.<sup>5</sup>

#### **3.2.1: Ethnobotany and biological screening of the genus *Sapium***

Plants of this genus are traditionally employed to treat tuberculosis, cancer and other ailments.<sup>9</sup> *Sapium indicum* is known as a poisonous plant and it is used traditionally as a piscicidal agent, or fish poison.<sup>10</sup> When crushed leaves are spread over water, most fish will die and float on the surface of the water.<sup>11</sup>

The roots of *S. sebiferum*, the Chinese tallow tree, are used as a purgative and as a diuretic agent and are effective against *Schistosoma japonicum*.<sup>12</sup> It is used also as a ‘blood purifying agent’ (as a depurative) and a laxative.<sup>12</sup> The chloroform soluble fraction of the methanol extract of *Sapium sebiferum* was tested and exhibited significant activity against P-388 lymphocytic leukemia.<sup>13</sup> The leaves of *S. sebiferum* are known to be used as an anti-inflammatory drug with diuretic and parasiticidal action in Chinese traditional medicine.<sup>14</sup> The methanol extract showed anti-fungal and anti-bacterial activity.<sup>17</sup>

The entire plant of *S. haematospermum* is used to treat toothache. In Argentina it is used as an anti-inflammatory agent,<sup>15</sup> and to treat gastrointestinal pain and boils.<sup>15</sup> The stem bark of *S. insigne* is used to treat wounds and dispel worms and germs.<sup>16</sup> The fresh fruits are used to treat psoriasis in India and also to treat indigestion. The paste made out of the stem bark is used to treat pimples.<sup>17</sup> *S. marmieri* is traditionally used as a purgative.<sup>18</sup> The methanol extract of *S. japonicum* was tested and showed cytotoxic and anti-tumour activities.<sup>19</sup> The methanol extract of *S. melanostictum* showed antiviral activity.<sup>20</sup> *S. baccatum* is used in folk medicine to treat bronchial asthma and to ease pain.<sup>21</sup> Compounds from the tallow tree, *S. sebiferum*, showed anti-carcinogenic properties.<sup>22</sup>

### **3.3: A review of previous phytochemical investigation of plants of the genus *Sapium***

The phytochemical investigations of the species of the genus *Sapium* have led to the identification of an anti-inflammatory alkaloid, antihypertensive phenolic compounds, toxic phorbol esters, hydrolysable tannins and flavonoids.<sup>24,33</sup>

#### **3.3.1: Alkaloids isolated from the genus *Sapium***

Only a few alkaloids have been isolated from the genus *Sapium*. An alkaloid, bukittingine, isolated from *S. baccatum*,<sup>23</sup> was found to exhibit anti-inflammatory activity. Bukittingine also showed an ability to reduce fever significantly in yeast-induced hyperthermic rats and possessed analgesic activity comparable of that of acetyl salicyclic acid. This suggested that bukittingine

possesses a mechanism of activity which is similar to that of acetyl salicyclic acid.<sup>24,25</sup> Anabellamide was isolated from *S. rigidifloium*.<sup>26</sup>

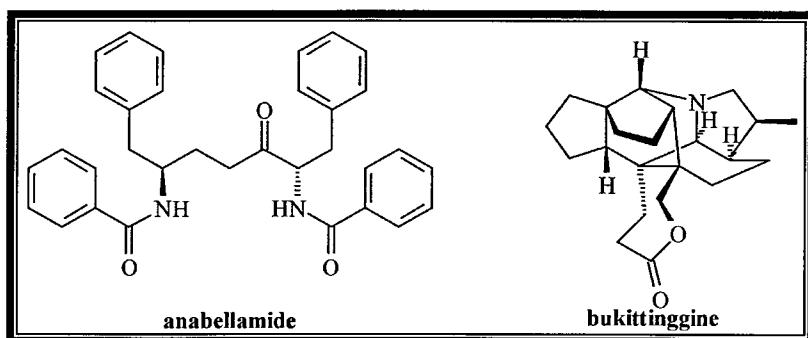


Figure 3.1: Alkaloids isolated from *Sapium* species

**Table 3.1: Alkaloids isolated from *Sapium* species**

Plant source	Name of compound
<i>S. baccatum</i>	bukittinggine <sup>24,25</sup>
<i>S. rigidifolium</i>	anabellamide <sup>26</sup>

### 3.3.2: Coumarins isolated from the genus *Sapium*

Coumarins have been isolated previously from the genus *Sapium*. Ellagic acid is widely distributed in many plants species. Ellagic acid was isolated from *S. sebiferum* and has been found to possess antioxidant, antineoplastic and anti-mutagenic properties.<sup>27</sup> Scopoletin, 5,6,7,8-tetramethoxy-coumarin and 6,7,8-trimethoxycoumarin were identified from the root bark of *S. sebiferum*.<sup>27</sup>

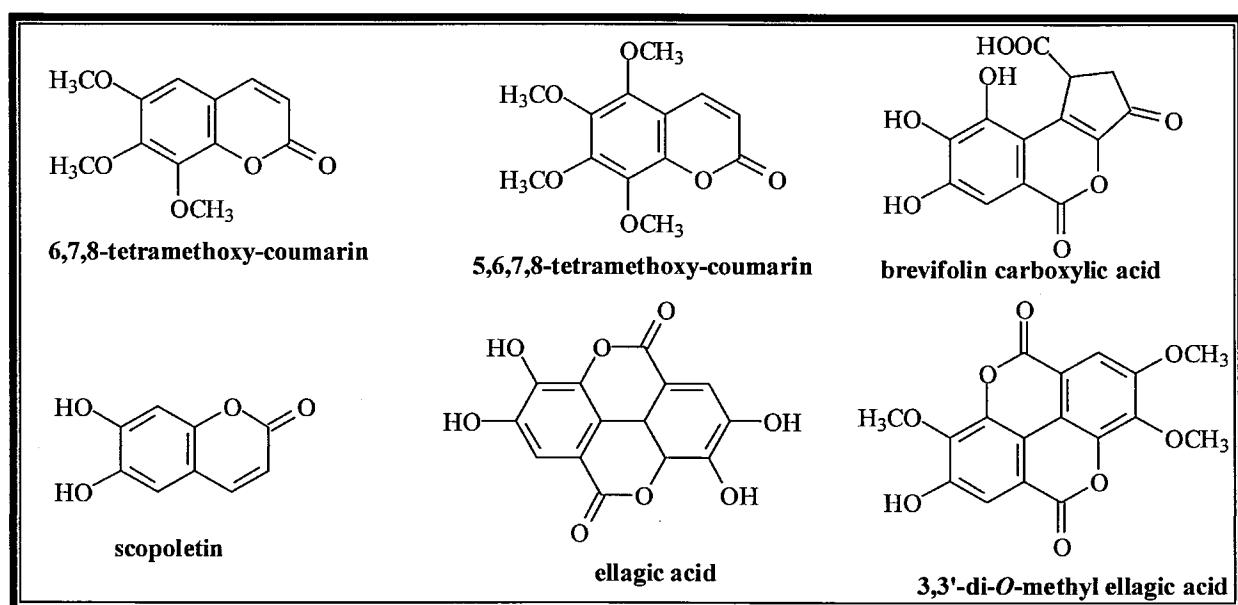


Figure 3.2: Aromatic compounds isolated from *Sapium* species

Table 3.2: Aromatic compounds isolated from *Sapium* species

Plant name	Name of compound
<i>S. sebiferum</i>	5,6,7,8-tetramethoxy-coumarin <sup>27</sup>
<i>S. sebiferum</i>	6,7,8-trimethoxy-coumarin <sup>27</sup>
<i>S. aucuparium</i> , <i>S. discolor</i> , <i>S. indicum</i> , <i>S. sebiferum</i>	ellagic acid <sup>27</sup>
<i>S. eugeniaefolium</i>	3-3'-di-O-methylellagic acid <sup>28</sup>
<i>S. sebiferum</i>	Scopoletin <sup>27</sup>

### 3.3.3: Flavonoids isolated from the genus *Sapium*

Astragalin, was isolated from *S. sebiferum* and was found to possess anti-dermatitis activity, the results showed reduction in existing dermatitis when administered orally in mice.<sup>32,33,39</sup> Astragalin (flavonoid glycoside) is found in white flowers.<sup>30</sup> The flavonol glycoside astragalin (kaempferol-3-*O*-β-D-glucopyranoside) and isoquercitrin (quercetin, 3-*O*-D-β-glucopyranoside) exhibit glycation inhibitory activity comparable to the known glycation inhibitor, aminoguanidine.<sup>29</sup>

Kaempferol is known as an effective antioxidant.<sup>5</sup> Rutin, a flavonol glycoside, has been reported to be included as a dietary supplement as vitamin P.<sup>5</sup>

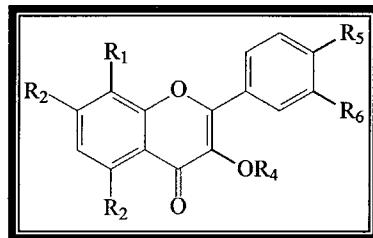


Figure 3.3: Flavonoids isolated from *Sapium* species

**Table 3.3: Flavonols isolated from the *Sapium* species**

Plant name	Compound name	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>
<i>S. insigne</i>	3-hydroxy-5,7,8-trimethoxy-2-phenyl flavone <sup>31</sup>	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	H	H	H
<i>S. sebiferum</i>	kaempferol <sup>43</sup>	H	OH	OH	H	OH	H
<i>S. japonicum</i>	trifolin <sup>32</sup>	H	OH	OH	Glu	OH	H
<i>S. japonicum</i>	quercetin <sup>32</sup>	H	OH	OH	H	OH	OH
<i>S. japonicum</i> , <i>S. sebiferum</i>	rutin <sup>32</sup>	H	OH	OH	Ru	OH	OH
<i>S. haematospermum</i> , <i>S. japonicum</i> , <i>S. sebiferum</i>	astragalin <sup>32,33</sup>	H	OH	OH	3-O-β-D-Glu	H	OH
<i>S. haematospermum</i> <i>S. japonicum</i> , <i>S. sebiferum</i>	isoquercitrin <sup>32,33</sup>	H	OH	OH	3-O-β-D-Glu	OH	OH

The flavonols afzelechin and 6-hydroxykaempferol-7-rutinoside have been isolated from *Sapium species*.<sup>32,34</sup>

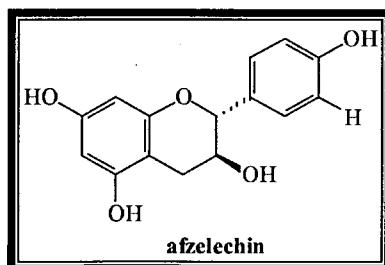


Figure 3.4: Flavanols isolated from *Sapium* species

**Table 3.4: Flavan-3-ols isolated from *Sapium* species**

Plant source	Name of compound
<i>S. japonicum</i> <sup>32</sup>	afzelechin
<i>S. eugeniaefolium</i> <sup>34</sup>	6-hydroxykaempferol-7-rutinoside

### **3.3.4: Diterpenes**

Phorbol esters, pimaradiene and kaurane diterpenoids have been isolated from the genus *Sapium* of the Euphorbiaceae family.

#### **Phorbol esters**

Species of the genus *Sapium* are known to yield phorbol ester diterpenoids, which are reported to be highly toxic and bioactive. Phorbol esters are of interest as pharmacological tools because of their activities. The diterpene esters of the tigiane type isolated from *S. sebiferum* were found to be skin irritants and tumour promoters.<sup>35,36</sup>

The phorbol esters isolated from fruits of *S. indicum* were evaluated for their antimycobacterial activity against *Mycobacterium tuberculosis*.<sup>36</sup> 12-(2-N-methylaminobenzoyl)-4β, 5, 20-trideoxyphorbol-13-acetate, sapintoxin A, sapintoxin B, sapintoxin C and 12-(2-methylaminobenzoyl)-4-deoxyphorbolaldehyde-13-acetate showed antimycobacterial activity with minimum inhibitory concentrations (MIC) between 3.12 and 200 µg/ml. However, α-sapinine and 12-(2-N-methylaminobenzoyl)-4α,20-dideoxy-5-hydroxyphorbol-13-acetate were inactive.<sup>36</sup>

Sapintoxin A, a highly fluorescent phorbol ester, was found to be a potent activator of protein kinase C, however not a tumour promoter.<sup>37</sup> Sapintoxin A, has been found to possess similar properties to promoters such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA), because it induces erythema *in vivo*, and also induces lymphocyte mitogenesis *in vitro* and also causes aggregation of human and rabbit platelets.<sup>37</sup>

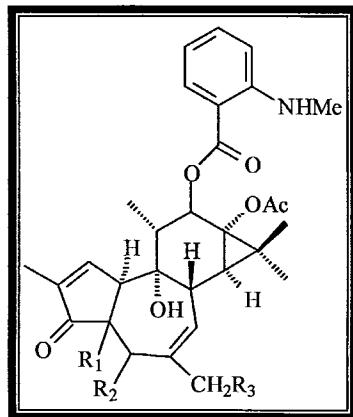


Figure 3.5: Phorbol ester diterpenoids isolated from *Sapium indicum*

**Table 3.5: Phorbol esters isolated from *Sapium* species**

Plant source	Name of the compound	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
<i>S. indicum</i>	12-(2-N-methylaminobenzoyl)-4β, 5, 20-trideoxyphorbol-13-acetate <sup>36</sup>	H	H	H-β
<i>S. indicum</i>	12-(2-N-methylaminobenzoyl)-4β, 5, 20-trideoxyphorbol-13-acetate <sup>36</sup>	H	H	H-α
<i>S. indicum</i>	sapintoxin A <sup>36,44</sup>	H	OH	H-β
<i>S. indicum</i>	α-sapinine <sup>36,44</sup>	H	OH- β	H-α
<i>S. indicum</i>	sapintoxin C <sup>36,40</sup>	OH	H	H-β
<i>S. indicum</i>	12-(2-N-methylaminobenzoyl)-4α, 20-dideoxy-5-hydroxyphorbol-13-acetate <sup>36</sup>			
<i>S. indicum</i>	sapintoxin B <sup>36,44</sup>	OH	OH	H-β
<i>S. indicum</i>	12-(2'-N-methylaminobenzoyl)-4α-deoxy-5, 20-dihydroxyphorbol-13-acetate <sup>40</sup>	OH	OH	H-α
<i>S. indicum</i>	12-(2-methylaminobenzoyl)-4-deoxyphorbolaldehyde-13-acetate <sup>36</sup>	H	CHO	H-β

In 1981, Taylor *et al.* isolated a phorbol ester, sapintoxin D, from *S. indicum*.<sup>38</sup> McLean, *et al.* reported that sapintoxin D was found to be a weak tumour promoter in Sencar mouse skin.<sup>39</sup> 4α-Sapinine a diterpenoid ester alkaloid of the tigliane type was isolated from *S. indicum*.<sup>40</sup> Sapintoxin D, was found to be hyperplasiogenic, sapintoxin C and 4α-sapinine were inactive as tumour-promoter and hyperplasiogenic.<sup>41</sup>

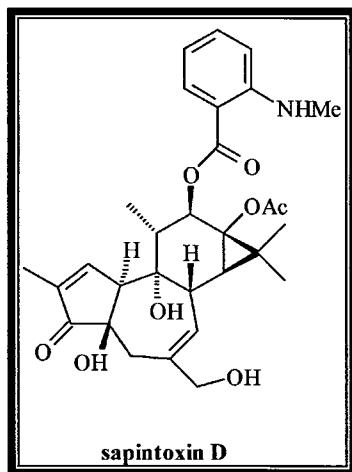


Figure 3.6: Phorbol ester diterpenoids isolated from *Sapium indicum*

From the dried fruits of *S. indicum*, Taylor *et al.* have isolated 12-[2-methylaminobenzoyl]-4 $\beta$ -deoxyphorbaldehyde-13-acetate and its isomer 12-[2-methylaminobenzoyl]-4 $\alpha$ -deoxyphorbaldehyde-13-acetate.<sup>42</sup>

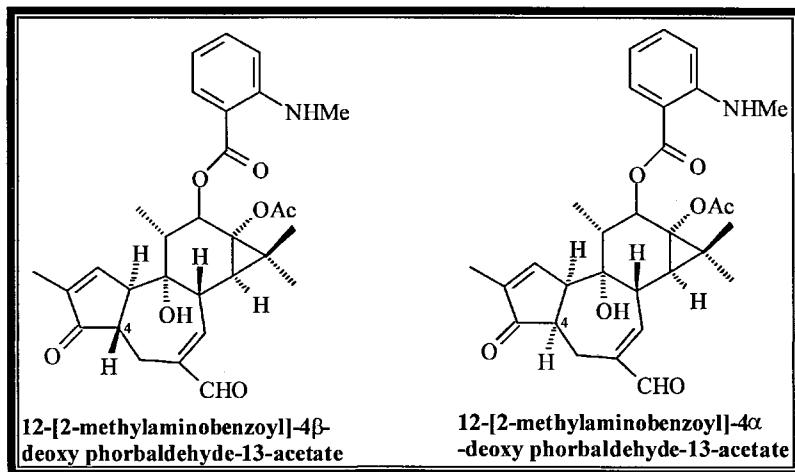


Figure 3.7: Phorbol ester diterpenoids isolated from *Sapium* species

Taylor *et al.* have further isolated the aliphatic esters of the tiglane-type from the unripe fruits of *S. indicum*. Sapantoxin A (12-*O*-[*n*-deca-2, 4, 6-trienoyl] 4-deoxyphorbol-13-acetate), sapantoxin B (12-*O*-[*n*-deca-2, 4, 6-trienoyl] 4-deoxy-5-hydroxyphorbol-13-acetate and sapantoxin C (12-*O*-[*n*-deca-2, 4, 6-trienoyl]-4, 20-dideoxy-5-hydroxyphorbol-13-acetate).<sup>45</sup>

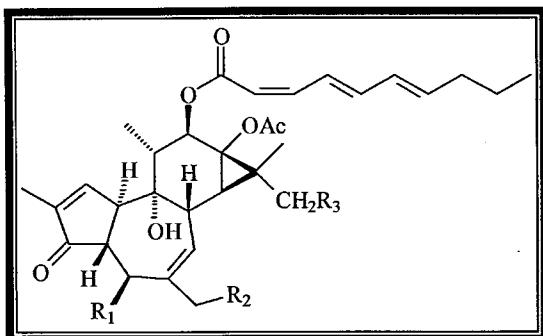


Figure 3.8: Phorbol ester diterpenoids isolated from *Sapium indicum*<sup>45</sup>

**Table 3.6: Aliphatic phorbol esters isolated from *Sapium indicum***

Plant name	Name of compound	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
<i>S. indicum</i>	sapatoxin A <sup>45</sup>	H	OH	H
<i>S. indicum</i>	sapatoxin B <sup>45</sup>	OH	OH	H
<i>S. indicum</i>	sapatoxin C <sup>45</sup>	OH	H	H
<i>S. indicum</i>	12- <i>O</i> -deca- <i>cis</i> -2- <i>cis</i> -4-dienyl)-4-deoxy-16-hydroxy phorbol 13-acetate <sup>45</sup>	H	OH	OH

From the twigs and leaves of *S. insignis*, Taylor *et al.* have isolated four phorbol esters, 12-*O*-hexanoyl-4*α*-deoxy-16-hydroxy-phorbol-13-acetate, 12-*O*-hexanoyl-4*α*-deoxy-16-hydroxyphorbol-13-acetate, 12-*O*-(2'*E*,4'*E*-decadienoyl)-4-deoxy-16-hydroxyphorbol-13-acetate and 12-*O*-(2'*E*,4'*E*-decadienoyl)-4-deoxy-16-hydroxyphorbol-13-acetate.<sup>46</sup> No bioactivity was reported.

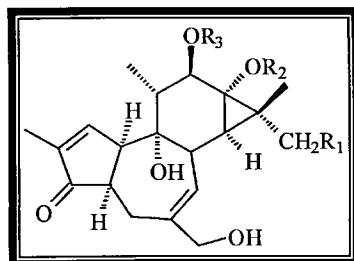


Figure 3.10: Phorbol ester diterpenoids isolated from *Sapium insignis*<sup>46</sup>

**Table 3.7: Phorbol ester diterpenoids isolated from *Sapium insigne***

Plant source	Name of compound	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
<i>S. insigne</i>	12-O-hexanoyl-4 $\alpha$ -deoxy-16-hydroxy-phorbol-13-acetate <sup>46</sup>	H	-COCH <sub>3</sub>	CO(CH <sub>2</sub> ) <sub>4</sub> CH <sub>3</sub>
<i>S. insigne</i>	12-O-dodecanoyl-4 $\alpha$ -deoxy-16-hydroxy-phorbol-13-acetate <sup>46</sup>	OH	-COCH <sub>3</sub>	CO(CH <sub>2</sub> ) <sub>10</sub> CH <sub>3</sub>

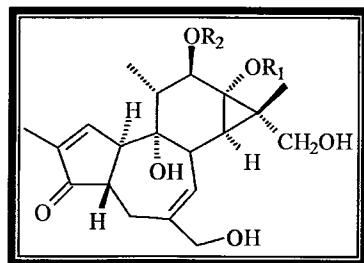


Figure 3.11: Phorbol ester diterpenoids isolated from *Sapium insigne*<sup>46</sup>

**Table 3.8: Phorbol ester diterpenoids isolated from *Sapium insigne***

Plant name	Name of compound	R <sub>1</sub>	R <sub>2</sub>
<i>S. insigne</i>	12-O-(2'E,4'E-decadienoyl)-4-deoxy-16-hydroxyphorbol-13-acetate <sup>46</sup>	COCH <sub>3</sub>	CO—CH=CH—CH=CH—(CH <sub>2</sub> ) <sub>4</sub> CH <sub>3</sub>

A phorbol ester, 12-O-hexadecanoyl-phorbol-13-acetate, was isolated from the soil collected from under *S. sebiferum* and has been found to show cocarcinogenicity in terms of tumour promoting activity compared to TPA (12-O-tetradecanoylphorbol acetate).<sup>47</sup>

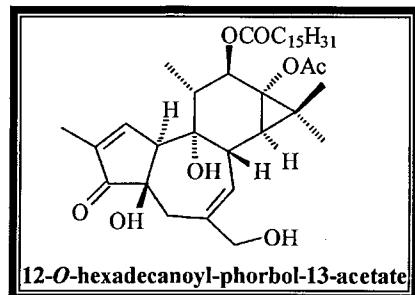


Figure 3.12: Phorbol ester diterpenoid isolated from *Sapium sebiferum*

## Kaurane diterpenoids

Three kaurane diterpenoids were isolated from *S. rigidifolium*.<sup>26</sup> Kaurane diterpenoids are found in many different plant species, belonging to several families.<sup>48</sup> These can occur as either the normal or *ent*-series.<sup>5,48</sup> Kaurenoic acid is known to have a wide range of bioactivities such as anti-bacterial, anti-fungal and anti-inflammatory activities.<sup>48</sup>

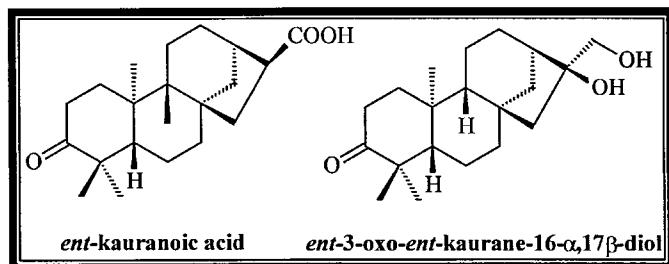


Figure 3.13: Kaurane diterpenoids isolated from *Sapium rigidifolium*<sup>26</sup>

**Table 3.9: Kaurane diterpenoids isolated from *Sapium rigidifolium***

Plant source	Name of compound
<i>S. rigidifolium</i>	<i>ent</i> -kaurenoic acid <sup>26</sup>
<i>S. rigidifolium</i>	3-oxo- <i>ent</i> -kaurane-16 $\alpha$ ,17 $\beta$ -diol <sup>26</sup>

## Isopimaradiene diterpenoids

Two isopimaradiene diterpenoids were isolated from *S. haematosperum*, 1 $\beta$ , 12 $\beta$ -dihydroxy-(5 $\alpha$ , 9 $\alpha$ , 20 $\alpha$ )-13-*epipimara*-8(14), 15-dien-3-one. (lecheronol A) and 12 $\beta$ , 19-dihydroxy-(5 $\alpha$ , 9 $\alpha$ , 20 $\alpha$ )-13-*epipimara*-8(14), 15-dien-3-one (lecheronol B). Lecheronol A was found to have cytotoxic activity.<sup>33</sup>

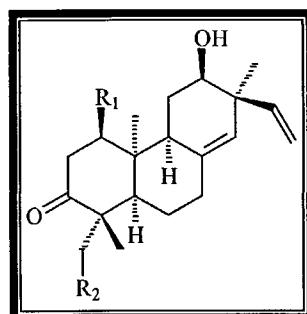


Figure 3.14: Pimaradiene diterpenoids isolated from *Sapium haematosperum*

**Table 3.10: Pimaradiene diterpenoids isolated from *S. haematosperum***

Plant source	Name of compound	R <sub>1</sub>	R <sub>2</sub>
<i>S. haematosperum</i>	lecheronol A <sup>33</sup>	OH	H
<i>S. haematosperum</i>	lecheronol B <sup>33</sup>	H	OH

A diterpenoid, rigidol, was isolated from *S. rigidifolium*.<sup>26</sup> No bioactivity was reported.

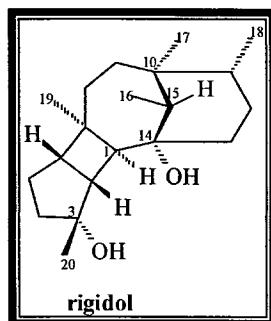


Figure 3.15: Diterpenoid isolated from *Sapium rigidifolium*<sup>26</sup>

### 3.3.5: Triterpenoids

Taraxerane, lupane, oleanane, hopane and ursane classes of pentacyclic triterpenoids have been isolated from the genus *Sapium* of the Euphorbiaceae family.

#### Taraxerane triterpenoids

The triterpenoid sebiferenic acid, was isolated from *S. sebiferum*.<sup>49</sup> Baccatin was isolated from *S. baccatum*. Aleuritolic acid shows skin anti-oxidation activity.<sup>50</sup>

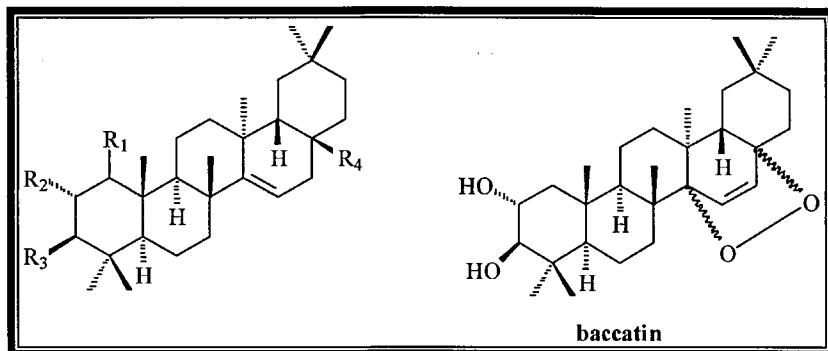


Figure 3.16: Taraxerane triterpenoids isolated from *Sapium* species

**Table 3.11: Taraxerane triterpenoids isolated from *Sapium* species**

Plant name	Compound name	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>
<i>S. rigidifolium</i>	3- <i>O</i> -acetylaleuritolic acid <sup>26</sup>	H	H	AcO	COOH
<i>S. sebiferum</i>	sebiferenic acid <sup>49</sup>	H	OH	OH	COOH
<i>S. sebiferum</i>	sebiferic acid <sup>49</sup>	H	OH	OH	COOCH <sub>3</sub>
<i>S. eugeniaefolium</i>	taraxerone <sup>51</sup>	H	-	=O	CH <sub>3</sub>
<i>S. eugeniaefolium</i>	taraxerol <sup>51</sup>	H	H	OH	CH <sub>3</sub>
<i>S. sebiferum</i>	aleuritolic acid <sup>49</sup>	H	H	OH	COOH
<i>S. sebiferum</i>	sebiferone <sup>52</sup>	=O	H	AcO	COOH
<i>S. baccatum</i>	baccatin <sup>50</sup>	-	-	-	-

### Oleanane triterpenoids

$\beta$ -amyrin has been isolated from different plant species of different families, including the genus *Sapium*.  $\beta$ -amyrin was isolated from *S. eugeniaefolium*<sup>51</sup> and showed anti-pruritic activity.<sup>54</sup>

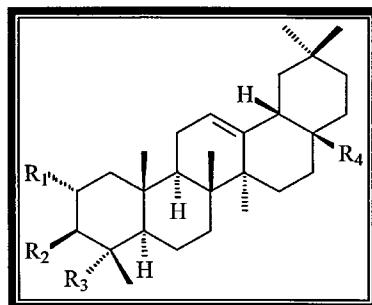


Figure 3.17: Oleanane triterpenoids isolated from *Sapium* species

**Table 3.12: Oleanane tritepenoids isolated from *Sapium* species**

Plant source	Name of compound	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>
<i>S. haematospermum</i>	$\beta$ -amyrin <sup>33</sup>	H <sub>2</sub>	OH	CH <sub>3</sub>	CH <sub>3</sub>
<i>S. haematospermum</i>	3 $\alpha$ -hydroxyolean-12-ene <sup>33</sup>	H	OH	CH <sub>3</sub>	CH <sub>3</sub>
<i>S. haematospermum</i>	2 $\alpha$ , 3 $\beta$ , 23-trihydroxyolean-12-en-28-oic acid <sup>33</sup>	OH	OH	CH <sub>2</sub> OH	COOH

## Hopane triterpenoids

The hopane triterpenoid moretenol, and its 3-keto derivative, moretenone were isolated from *Sapium eugeniaefolium*.<sup>51</sup> However, bioactivity was not reported.

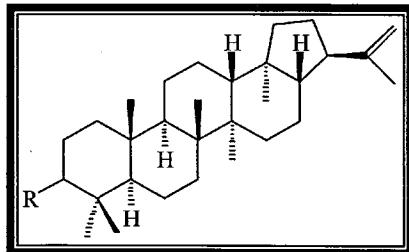


Figure 3.18: Hopane triterpenoids isolated from *Sapium eugeniaefolium*<sup>51</sup>

Table 3.13: Hopane class triterpenoids isolated from *Sapium* species

Plant source	Name of compound	R
<i>S. eugeniaefolium</i>	moretenone <sup>51</sup>	=O

## Lupane triterpenoids

Lupeol and epilupeol were isolated from *S. haematospermum*<sup>51</sup> and *S. rigidifolium*. Lupenone was isolated from *S. rigidifolium*.<sup>26</sup> 3 $\alpha$ -Lupeol was found to be cytotoxic.<sup>53</sup>

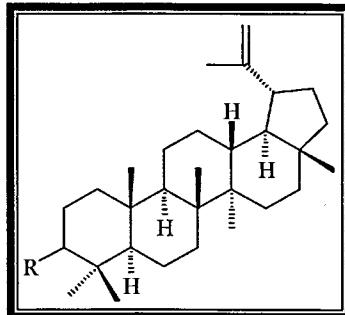


Figure 3.19 Lupane triterpenoids isolated from *Sapium* species

Table 3.14: Lupane triterpenoids isolated from *Sapium* species

Plant source	Name of compound	R
<i>S. haematospermum</i> , <i>S. rigidifolium</i>	3 $\beta$ -hydroxylup-20(29)-ene <sup>26,33</sup>	$\beta$ -OH
<i>S. haematospermum</i> , <i>S. rigidifolium</i>	3 $\alpha$ -hydroxylup-20(29)-ene <sup>26,33</sup>	$\alpha$ -OH
<i>S. rigidifolium</i>	lupenone <sup>26</sup>	=O

## Ursane triterpenoids

Ursane triterpenoids have been isolated from the genus *Sapium*. *S. haematospernum* yielded 3 $\alpha$ -hydroxyurs-12-ene.<sup>33</sup>

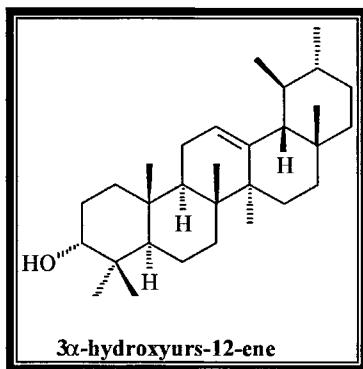


Figure 3.20: Ursane triterpenoids isolated from *Sapium* species

### 3.3.6: Tannins

6-*O*-Galloyl-D-glucose, a phenolic glycoside was isolated from *S. sebiferum*, and was found to have antihypertensive activity.<sup>14</sup> The compound was found to inhibit noradrenaline release.<sup>14</sup> Geraniin, exhibited anti-viral properties.<sup>9,32</sup>

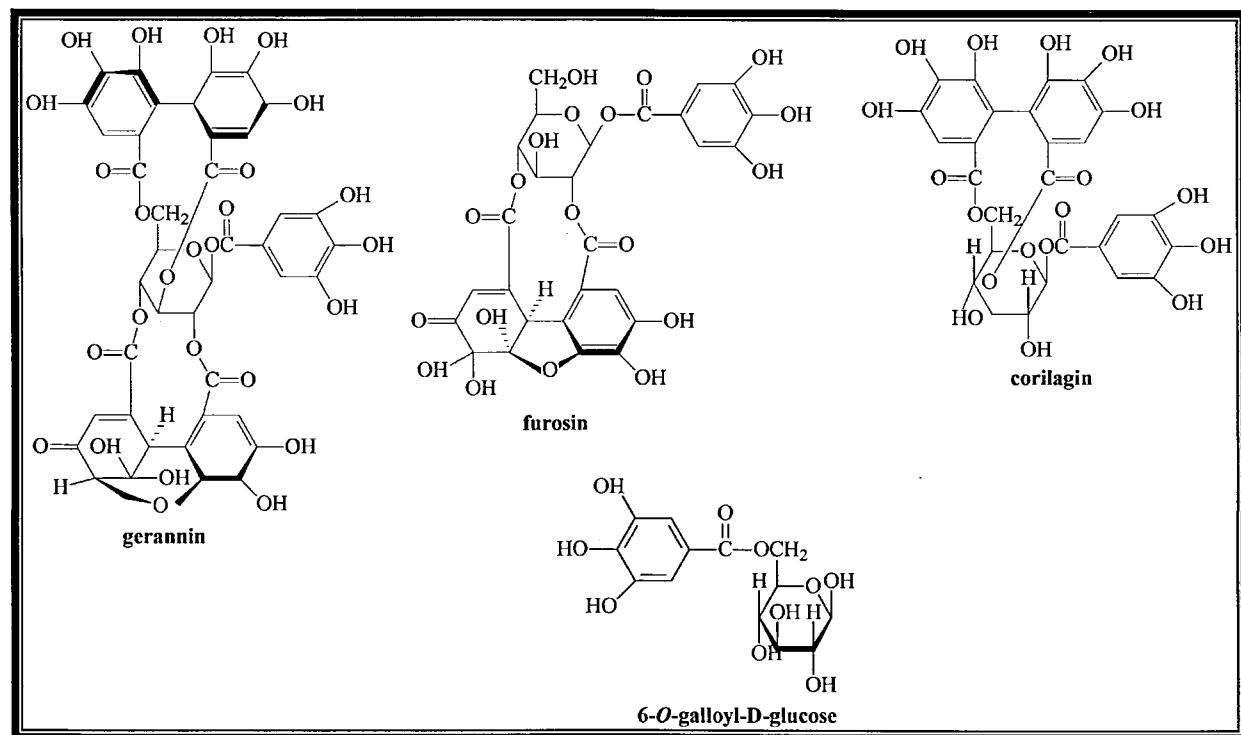


Figure 3.21: Tannins isolated from *Sapium* species

**Table 3.15: Tannins isolated from *Sapium* species**

Plant source	Name of compound
<i>S. sebiferum</i>	corilagin <sup>32</sup>
<i>S. sebiferum, S. japonicum</i>	geraniin <sup>9,32</sup>
<i>S. sebiferum</i>	1,2,3,4,6-penta-O-galloyl-β-D-glucose <sup>9</sup>
<i>S. sebiferum</i>	1-3-4-6-tetra-O-galloyl-β-D-glucose <sup>9</sup>
<i>S. sebiferum, S. japonicum</i>	furosin <sup>9,32</sup>
<i>S. sebiferum</i>	6-O-galloyl- β-D-glucose <sup>9,14</sup>
<i>S. sebiferum</i>	chebulagic acid <sup>7,9</sup>
<i>S. sebiferum</i>	chlorogenic acid <sup>9</sup>
<i>S. sebiferum</i>	β-glucogallin <sup>7,9</sup>
<i>S. sebiferum</i>	gallic acid <sup>7</sup>

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## CHAPTER 4: A PHYTOCHEMICAL INVESTIGATION OF *SAPIUM INTEGERRIMUM* OF THE EUPHORBIACEAE

### 4.1: Introduction

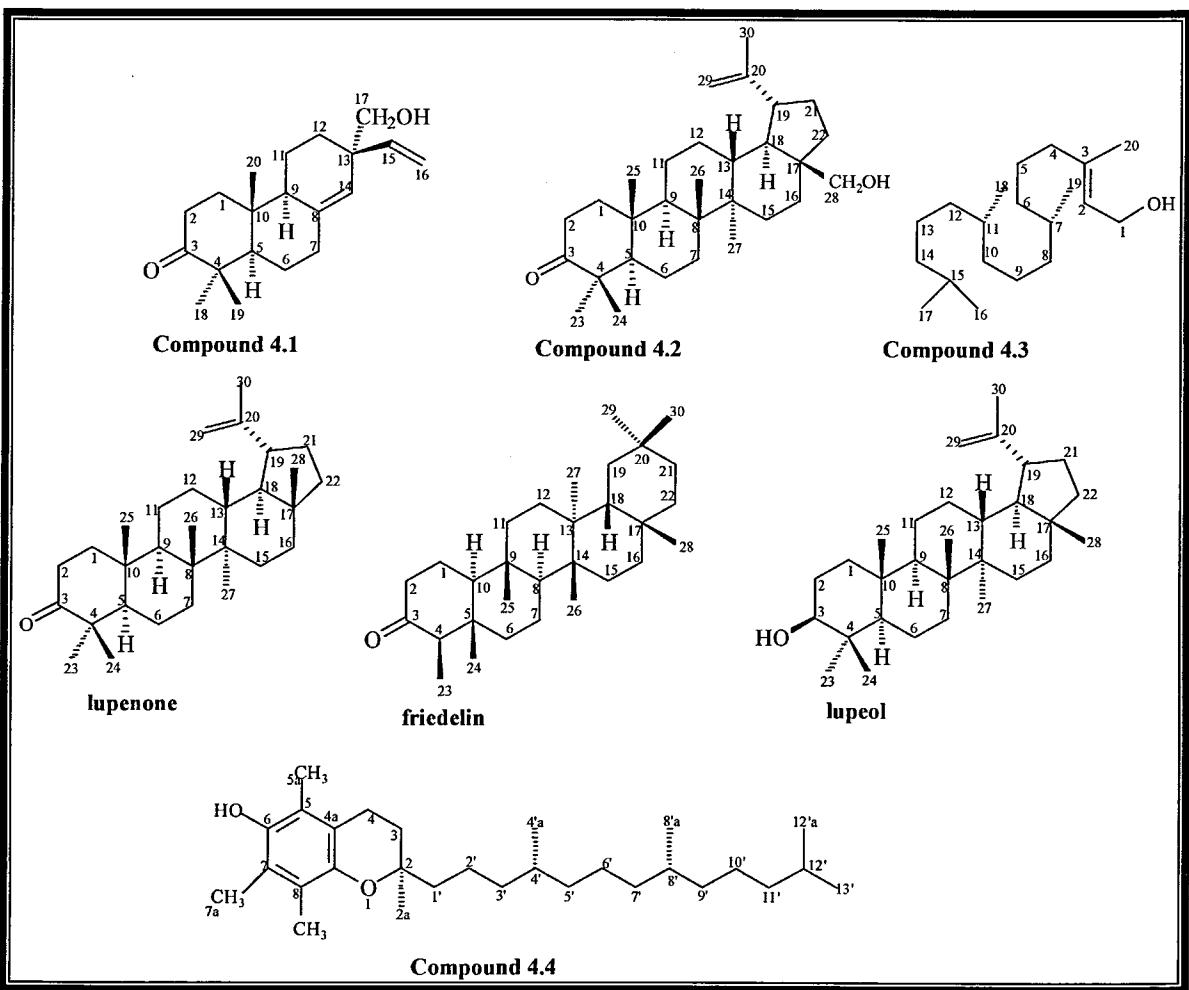
*Sapium integerrimum* is a shrub or a small tree 2-8 m tall and is a rare species found in the forests of KwaZulu-Natal Province, South Africa.<sup>1</sup> The plant is glabrous and monoecious, the twigs are reddish-brown, flaking at first and eventually turning grey-brown. They are lanceolate to ovate-oblong and shortly obtuse at the apex.<sup>1</sup> *S. integerrimum* has not been investigated phytochemically, therefore prompting the present study. Unspecified parts are used as traditional medicine and root decoctions are used as a mouth wash to treat toothache.<sup>2</sup>



**Picture 3: *Sapium integerrimum* (Photo: Professor Neil Crouch)**

*S. integerrimum* yielded eight compounds. The stem bark of the combined hexane and dichloromethane extracts afforded the previously unreported compound **4.1**, and the known betulone, **4.2**, lupenone, lupeol, friedelin and *trans*-phytol **4.3**.

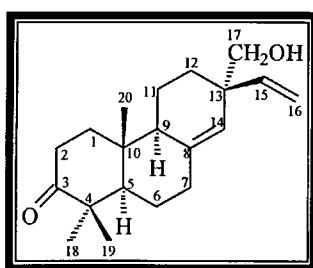
The combined hexane and dichloromethane extracts of the leaves yielded the known compounds  $\alpha$ -tocopherol, **4.4**, friedelin, **2.7**, lupenone, **2.6** and stigmasterol.



**Figure 4.1:** Compounds from the stem bark of *Sapium integrerrimum*

#### 4.2: Structural elucidation of compounds from the *Sapium integrerrimum*

##### 4.2.1: Structural elucidation of compound 4.1: 17-hydroxypimara-8(14),15-dien-3-one



Compound 4.1

The high resolution mass spectrum of compound **4.1** showed a molecular ion peak at *m/z* 302.2298, which corresponded to the molecular formula of C<sub>20</sub>H<sub>30</sub>O<sub>2</sub>. A double bond equivalence of six was calculated. The FTIR spectrum showed a carbonyl stretch at 1702 cm<sup>-1</sup> and an OH group stretch at 3446 cm<sup>-1</sup>.<sup>3,4</sup>

The <sup>13</sup>C NMR spectrum showed a resonance of a ketone carbon at δ 216.8. This resonance, which was assigned to C-3, showed correlations in the HMBC spectrum with the methyl group proton resonances at δ 1.07 and δ 1.04 which were ascribed to 3H-18 and 3H-19 and also with the H-1 proton resonances (δ 1.46, δ 1.95). These resonances were seen to be coupled with the H-2 proton resonances (δ 2.25, δ 2.62). In addition, the C-3 carbon resonance was seen to correlate with the H-5 methine proton resonance at δ 1.46. The H-5 methine proton resonance was seen to be coupled with the H-6 proton resonances (δ 1.38, 1.57), which, in turn, were seen to be coupled with two H-7 methylene proton resonances at δ 2.10 and δ 2.40 in the COSY spectrum.

The corresponding C-5 carbon resonance at δ 55.3 showed correlations with the 3H-20 methyl group proton resonance. In addition, the resonance was seen to correlate with the H-9 methine proton resonance at δ 1.78 in the HMBC spectrum. The COSY spectrum showed coupling between the H-9 methine resonance and the superimposed H-11 proton resonances (δ 1.54), which, in turn, were seen to be coupled with the H-12 proton resonances at δ 1.31 and δ 1.51. The C-12 carbon resonance at δ 29.7 showed correlations in the HMBC spectrum with the H-14 methine proton resonance at δ 5.41 (d, *J*=1.83 Hz), and with the pair of doublets at δ 3.28 (1H, d, *J*=10.46 Hz) and δ 3.39 (1H, d, *J*=10.46 Hz) which were assigned to 2H-17, this indicating that the C-17 methyl group was oxidized to an alcohol. In addition, the C-12 resonance was seen to correlate with the H-15 methine proton resonance δ 5.69 (dd, *J*= 17.40, 10.43 Hz), and this resonance were seen to be coupled with the H-16 proton resonances at δ 5.27 (dd, *J*= 10.43, 1.83 Hz), and δ 5.11 (dd, *J*= 17.40, 1.83 Hz). These correlations confirmed the presence of an AMX system.

The positive value of +19.2 in the optical rotation suggested the compound belonged to the pimara-8(14), 15-diene series with H-5 and H-9 in the α-orientation and the C-20 methyl group

in the  $\beta$ -orientation.<sup>10</sup> The NOESY spectrum showed a correlation between the 3H-18 methyl group proton resonance and the H-5 proton resonance, which, in turn, showed correlation with the H-9 methine proton resonance. The 3H-19 methyl group proton resonance was seen to correlate with the 3H-20 methyl group proton resonance. Furthermore, the 3H-20 proton resonance was seen to correlate with the H-16B proton resonance.

Compound **4. 1** has not been reported previously.

Two pimaranes have been isolated previously from *Sapium haematospermum* (Euphorbiaceae family) related to compound **4.1**. There are lecheronol A ( $[\alpha]_D = +8^\circ$ ) and lecheronol B ( $[\alpha]_D = +3^\circ$ ) as shown in Figure 4.2. The *Sapium haematospermum* extract was found to be active against *Mycobacterium tuberculosis*. Lechenerol A was found to be the most active. The cytotoxicity against Vero cells showed an IC<sub>50</sub> value of 104.8  $\mu\text{g}/\text{mL}$ .

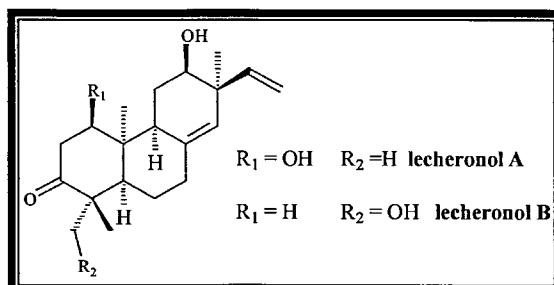


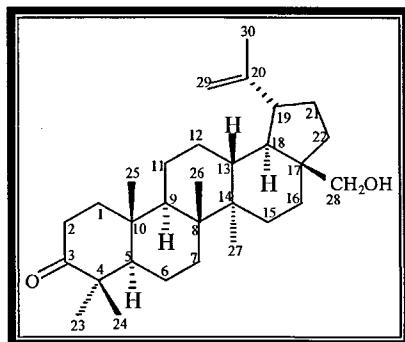
Figure 4.2: Pimarane diterpenoids from *Sapium haematospermum*<sup>5</sup>

**Table 4.1:** NMR data for compound **4.1**: 17-hydroxy-pimara-8(14),15-dien-3-one in  $\text{CDCl}_3$

no	<sup>1</sup> H NMR (400 MHz)	<sup>13</sup> C NMR (100 MHz)	HMBC (H → C)	COSY	NOESY
1 $\alpha$	1.46 (1H, m)	37.6 (CH <sub>2</sub> )	C-3, C-9, C-20	2H-2	H-2 $\alpha$
1 $\beta$	1.95 (1H, m)		C-3, C-5,C-10, C-20		3H-20, H-11 $\beta$
2 $\alpha$	2.25 (1H, m)	34.8 (CH <sub>2</sub> )	C-3, C-2	2H-1	
2 $\beta$	2.62 (1H, td, $J=14.60, J=5.74$ Hz)		C-3, C-1		3H-20
3	-	216.8 (C)	-	-	-
4	-	48.1 (C)	-	-	-
5	1.46 (1H, m)	55.3 (CH)	C-3, C-9, C-18, C-19, C-20	2H-6	3H-18, H-7 $\alpha$ , H-9
6 $\alpha$	1.38 (1H, m)	18.6 (CH <sub>2</sub> )	C-9, C-10	H-5, 2H-7	H-5 $\alpha$ , H-7 $\alpha$
6 $\beta$	1.57 (1H, m)		C-5, C-10, C-9		
7 $\alpha$	2.10 (1H, m)	35.2 (CH <sub>2</sub> )	C-8, C-9	2H-6	H-6 $\alpha$ , H-5 $\alpha$
7 $\beta$	2.40 (1H, m)		C-8, C-9		
8	-	140.4 (C)	-	-	-
9	1.78 (1H, m)	50.8 (CH)	C-1, C-6	2H-11	H-11 $\alpha$ , H-5
10	-	38.1 (C)	-	-	-

11 $\alpha$	1.54 (1H,m)	23.5 (CH <sub>2</sub> )	C-5, C-9, C-10	H-9, 2H-12	H-9 $\alpha$
11 $\beta$	1.54 (1H,m)		C-5, C-9, C-10		H-1 $\beta$
12 $\alpha$	1.31 (1H, m)	29.7 (CH <sub>2</sub> )	C-9, C-13 C-17	2H-11	
12 $\beta$	1.51 (1H, m)		C-9, C-13 C-17		H-17a, H-17b
13	-	45.1 (C)	-	-	-
14	5.41 (1H, s)	123.7 (CH)	C-7, C-9, C-17		H-17A, H-17B
15	5.69 (1H, dd, <i>J</i> =10.44, 17.40 Hz)	142.9 (CH)	C-12, C-17	2H-16	H-16A, H-16B
16A	5.27 (1H, dd, <i>J</i> =10.44, 1.83 Hz)	117.6 (CH <sub>2</sub> )	C-13	H-15	H-15
16B	5.11 (1H, dd, <i>J</i> =17.40, 1.83 Hz)		C-13		H-15, 3H-20
17A	3.28 (1H, d, <i>J</i> =10.46 Hz)	70.4 (CH <sub>2</sub> )	C-12, C-13	H-17b	H-17B, H-14
17B	3.39 (1H, d, <i>J</i> =10.46 Hz)		C-12, C-13	H-17a	H-17A, H-14
18	1.07 (3H, s)	25.6 (CH <sub>3</sub> )	C-4, C-5, C-19	-	H-5
19	1.04 (3H, s)	22.3 (CH <sub>3</sub> )	C-4, C-5, C-18	-	3H-20
20	0.94 (3H, s)	14.4 (CH <sub>3</sub> )	C-1, C-5, C-9	-	H-2 $\beta$ , H-1 $\beta$ , 3H-19, H-16B

#### 4.2.2: Structural elucidation of compound 4.2: Betulone (3-oxo-28-hydroxylup-20(29)-ene)



Compound 4.2

The low resolution mass spectrum of compound 4.2 gave a molecular ion peak at *m/z* 440 which is consistent with a molecular formula of C<sub>30</sub>H<sub>48</sub>O<sub>2</sub>. A double bond equivalence of seven was calculated. The FTIR spectrum showed a carbonyl stretch at 1702 cm<sup>-1</sup> and a primary OH stretch 3446 cm<sup>-1</sup>.<sup>2,3</sup> This compound was found to be betulone, which belongs to the lupane triterpenoid class. Betulone was previously isolated from *Salacia cordata* and other sources, and its structure was determined using <sup>1</sup>H NMR and <sup>13</sup>C NMR spectroscopy.<sup>6</sup>

The <sup>13</sup>C NMR spectrum showed a resonance at  $\delta$  218.3 which was assigned to C-3. The C-3 carbon resonance showed correlations in the HMBC spectrum with 3H-23 and 3H-24 methyl group proton resonances ( $\delta$  1.06 and  $\delta$  1.02) and with the H-5 methine proton resonance at  $\delta$  1.37. The H-5 methine proton showed correlations with the C-7 ( $\delta$  33.7) and C-25 ( $\delta$  16.2) carbon resonances.

The  $^1\text{H}$  NMR spectrum revealed a pair of doublets at  $\delta$  3.34 (d,  $J = 10.80$  Hz),  $\delta$  3.79 (d,  $J = 10.80$  Hz) which corresponded to the C-28 carbon resonance at  $\delta$  60.7 in the HSQC spectrum. The C-28 carbon resonance showed a correlation in the HMBC spectrum with the H-18 methine proton resonance at  $\delta$  1.60. The H-18 methine resonance was seen to be coupled with the H-19 proton resonance at  $\delta$  2.39 in the COSY spectrum.

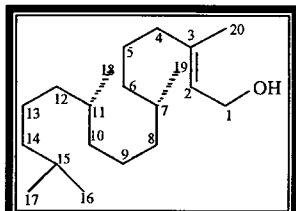
The HMBC spectrum showed a correlation between the C-19 carbon resonance ( $\delta$  48.0) and the downfield methyl group proton resonance at  $\delta$  1.68, which was ascribed to 3H-30 and with the non-equivalent methylene proton resonances at  $\delta$  4.68 and  $\delta$  4.57, which were assigned to H-29A and H-29B, indicating the presence of an isopropenyl group and confirming the position of the primary alcohol. Therefore the structure of compound **4.2** was confirmed as the known compound, betulone, a common triterpenoid isolated from several sources.<sup>5</sup>

**Table 4.2: NMR data of compound 4.2: betulone in  $\text{CDCl}_3$  and literature in  $\text{CDCl}_3$**

C	$^1\text{H}$ NMR (400 MHz)	$^{13}\text{C}$ NMR (100 MHz)	$^1\text{H}$ MNR ( $\text{CDCl}_3$ ) <sup>6</sup>	$^{13}\text{C}$ NMR ( $\text{CDCl}_3$ ) <sup>6</sup>
1 $\alpha$	1.87 (1H, m)	39.8 (CH <sub>2</sub> )	1.91	39.6 (CH <sub>2</sub> )
1 $\beta$	1.36 (1H, m)		1.39	
2 $\alpha$	2.44 (1H, m)	34.3 (CH <sub>2</sub> )	2.46	34.1 (CH <sub>2</sub> )
2 $\beta$	2.37 (1H, m)			
3	-	218.3 (C)	-	218.2 (C)
4	-	47.6 (C)	-	47.3 (C)
5	1.37 (1H, m)	55.1 (CH)	1.35	54.9 (CH)
6 $\alpha$	1.47 (1H, m)	19.9 (CH <sub>2</sub> )	1.49	19.7 (CH <sub>2</sub> )
6 $\beta$	1.47 (1H, m)			
7 $\alpha$	1.45 (1H, m)	33.7 (CH <sub>2</sub> )	1.46	33.5 (CH <sub>2</sub> )
7 $\beta$	1.45 (1H, m)			
8	-	41.0 (C)	-	40.8 (C)
9	1.38 (dd, $J=11.5, 14.8$ Hz)	50.0 (CH)	1.39	49.7 (CH)
10	-	37.1 (C)	-	36.9 (C)
11 $\alpha$	1.42 (1H, m)	21.6 (CH <sub>2</sub> )	1.44	21.4 (CH <sub>2</sub> )
11 $\beta$	1.26 (1H, m)		1.28	
12 $\alpha$	1.67 (1H, m)	25.4 (CH <sub>2</sub> )	1.67	25.2 (CH <sub>2</sub> )
12 $\beta$	1.07 (1H, m)		1.07	
13	1.68 (1H, m)	37.6 (CH)	1.69	37.4 (CH)
14	-	43.0 (C)	-	42.8 (C)
15 $\alpha$	1.72 (1H, m)	26.9 (CH <sub>2</sub> )	1.74	27.0 (CH <sub>2</sub> )
15 $\beta$	1.08 (1H, m)		1.09	
16 $\alpha$	1.94 (1H, m)	29.3 (CH <sub>2</sub> )	1.98	29.1(CH <sub>2</sub> )
16 $\beta$	1.22 (1H, m)		1.22	
17	-	48.0 (C)		47.8 (C)
18	1.60 (1H, m)	49.0 (CH)	1.61	48.9 (CH)
19 $\alpha$	2.39 (1H, m)	48.0 (CH <sub>2</sub> )	2.41	47.7 (CH <sub>2</sub> )
19 $\beta$	2.39 (1H, m)		2.41	
20	-	150.6 (C)	-	150.4 (C)
21 $\alpha$	1.94 (1H, m)	30.0 (CH <sub>2</sub> )	1.98	29.7 (CH <sub>2</sub> )

21 $\beta$	1.41 (1H, m)		1.42	
22 $\alpha$	1.87 (1H, m)	34.2 (CH <sub>2</sub> )	1.91	33.9 (CH <sub>2</sub> )
22 $\beta$	1.05 (1H, m)		1.05	
23	1.06 (3H, s)	26.9 (CH <sub>3</sub> )	1.08	26.6 (CH <sub>3</sub> )
24	1.02 (3H, s)	21.3 (CH <sub>3</sub> )	1.04	21.0 (CH <sub>3</sub> )
25	0.92 (3H, s)	16.2 (CH <sub>3</sub> )	0.94	15.9 (CH <sub>3</sub> )
26	1.07 (3H, s)	16.0 (CH <sub>3</sub> )	1.07	15.8 (CH <sub>3</sub> )
27	0.99 (3H, s)	14.9 (CH <sub>3</sub> )	1.00	14.7 (CH <sub>3</sub> )
28A	3.79 (1H,d, <i>J</i> =10.80 Hz)	60.7 (CH <sub>2</sub> )	3.80	60.4 (CH <sub>2</sub> )
28B	3.34 (1H,d, <i>J</i> = 10.80 Hz)		3.37	
29A	4.68 (1H, brs)	110.0 (CH <sub>2</sub> )	4.71	109.7 (CH <sub>2</sub> )
29B	4.57 (1H, brs)		4.61	
30	1.68 (3H, s)	19.3 (CH <sub>3</sub> )	1.69	19.1 (CH <sub>3</sub> )

#### 4.2.3: Structural elucidation of compound 4.3: *trans*-phytol



Compound 4.3

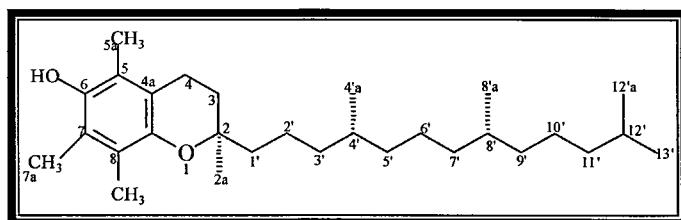
The low resolution mass spectrum of compound 4.3 gave a molecular ion peak at *m/z* 296, which is consistent with a molecular formula of C<sub>20</sub>H<sub>40</sub>O. The FTIR spectrum showed an OH stretch band at 3298 cm<sup>-1</sup>. The compound was identified as the known *trans*-phytol, a diterpenoid previously isolated from the red algae *Gracilaria andersoniana*,<sup>9</sup> and many other sources.

The <sup>13</sup>C NMR spectrum showed the presence of five methyl group proton resonances at  $\delta$  22.8 (C-16), 22.9 (C-17), 19.9 (C-18), 19.9 (C-19) and 16.4 (C-20), eight methylene carbon resonances and one fully substituted carbon resonance at  $\delta$  140.6 which was ascribed to C-3. The NMR data for the compound was compared to <sup>13</sup>C NMR data for *trans*-phytol from the literature. The data confirmed that compound 4.3 was the known *trans*-phytol.<sup>8,9</sup>

**Table 4.3: NMR data of compound 4.3 *trans*-phytol**

no	$^{13}\text{C}$ NMR (125 MHz) $\text{CDCl}_3$	$^{13}\text{C}$ NMR (75 MHz) in $\text{CDCl}_3$
1	59.7 ( $\text{CH}_2$ )	59.4 ( $\text{CH}_2$ )
2	123.6 ( $\text{CH}$ )	123.1 ( $\text{CH}$ )
3	140.6 (C)	140.2 (C)
4	40.1 ( $\text{CH}_2$ )	39.9 ( $\text{CH}_2$ )
5	25.4 ( $\text{CH}_2$ )	25.1 ( $\text{CH}_2$ )
6	36.9 ( $\text{CH}_2$ )	36.7 ( $\text{CH}_2$ )
7	33.0 ( $\text{CH}$ )	32.7 ( $\text{CH}$ )
8	37.6 ( $\text{CH}_2$ )	37.4 ( $\text{CH}_2$ )
9	24.7 ( $\text{CH}_2$ )	24.5 ( $\text{CH}_2$ )
10	37.7 ( $\text{CH}_2$ )	37.4 ( $\text{CH}_2$ )
11	32.9 ( $\text{CH}$ )	32.8 ( $\text{CH}$ )
12	37.5 ( $\text{CH}$ )	37.3 ( $\text{CH}$ )
13	250 ( $\text{CH}_2$ )	24.8 ( $\text{CH}_2$ )
14	39.7 ( $\text{CH}_2$ )	39.4 ( $\text{CH}_2$ )
15	28.2 ( $\text{CH}$ )	27.9 ( $\text{CH}$ )
16	22.8 ( $\text{CH}_3$ )	22.6 ( $\text{CH}_3$ )
17	22.9 ( $\text{CH}_3$ )	22.7 ( $\text{CH}_3$ )
18	19.9 ( $\text{CH}_3$ )	19.7 ( $\text{CH}_3$ )
19	19.9 ( $\text{CH}_3$ )	19.7 ( $\text{CH}_3$ )
20	16.4 ( $\text{CH}_3$ )	16.1 ( $\text{CH}_3$ )

#### 4.2.4: Structural elucidation of compound 2.11: $\alpha$ -tocopherol (Vitamin E)



Compound 4.4

Compound 4.4 was identified as the known  $\alpha$ -tocopherol. The low resolution mass spectrum gave a molecular ion peak at  $m/z$  430 corresponding to the molecular formula of  $\text{C}_{29}\text{H}_{50}\text{O}_2$ . The

FTIR spectrum showed CH stretches absorption bands at 2925 and 2854 cm<sup>-1</sup>.<sup>3,4</sup> Compound 4.4 was identified as  $\alpha$ -tocopherol.

The <sup>13</sup>C and DEPT NMR spectra showed the presence of an unsubstituted carbinolic carbon and six fully substituted aromatic carbons. Three proton resonances at  $\delta$  2.10, 2.10 and 2.14 suggested that three methyl groups were attached to an aromatic ring. Four doublet methyl proton resonances occurred at  $\delta$  0.81, 0.81, 0.82 and 0.82, and a singlet methyl group proton resonance occurred at  $\delta$  1.21. The <sup>13</sup>C NMR spectrum showed resonances due to C-2 (74.5), C-2a (23.8), C-3 (31.5), C-4 (20.7), C-6 (144.5) and C-8a (145.5). The presence of an isoprenyl side chain suggested that the compound 4.4 was  $\alpha$ -tocopherol. This was confirmed by comparison of the <sup>13</sup>C NMR signals with those reported in the literature.<sup>7</sup>

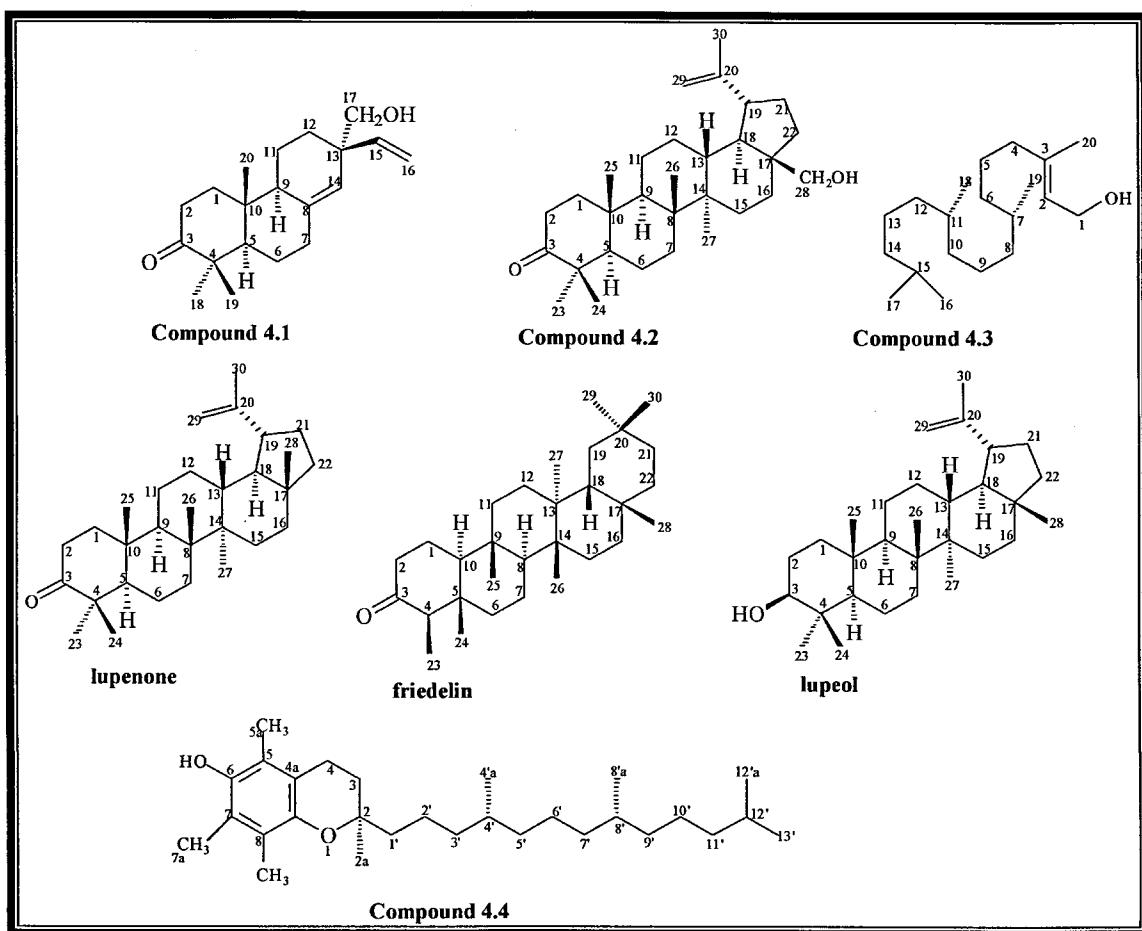
**Table 4.4: NMR data for compound 4.4:  $\alpha$ -tocopherol and literature in CDCl<sub>3</sub>.**

no	<sup>1</sup> H NMR (400 MHz)	<sup>13</sup> C NMR (100 MHz)	<sup>13</sup> C NMR (25.2 MHz) in CDCl <sub>3</sub> <sup>7</sup>
2	-	74.5 (C)	74.3 (C)
2a	1.21 (3H, s)	23.8 (CH <sub>3</sub> )	23.7 (CH <sub>3</sub> )
3 $\alpha$	1.77 (1H, dt, <i>J</i> = 7.0, 13.2 Hz)	31.5 (CH <sub>2</sub> )	31.6 (CH <sub>2</sub> )
3 $\beta$	1.83 (1H, m)		
4	2.59 (2H, br t, <i>J</i> = 7.0 Hz)	20.7 (CH <sub>2</sub> )	20.8 (CH <sub>2</sub> )
4a	-	117.3 (C)	117.0 (C)
5	-	118.4 (C)	118.5 (C)
6	-	144.5 (C)	144.4 (C)
7	-	120.9 (C)	121.0 (C)
8	-	122.6 (C)	122.3 (C)
8a	-	145.5 (C)	145.4 (C)
5	2.10 (3H, s)	11.2 (CH <sub>3</sub> )	11.2 (CH <sub>3</sub> )
7	2.14 (3H, s)	12.2 (CH <sub>3</sub> )	12.1 (CH <sub>3</sub> )
8	2.10 (3H, s)	11.7 (CH <sub>3</sub> )	11.8 (CH <sub>3</sub> )
6-OH	4.18 (1H, s)	-	-
1'	1.51 (2H, dd, <i>J</i> = 6.1, 7.2 Hz)	39.8 (CH <sub>2</sub> )	39.8 (CH <sub>2</sub> )
2'	1.38 (2H, m)	21.0 (CH <sub>2</sub> )	21.0 (CH <sub>2</sub> )
3'	1.22 (2H, m)	37.4 (CH <sub>2</sub> )	37.5 (CH <sub>2</sub> )
4'	1.36 (1H, m)	32.8 (CH)	32.7 (CH)
4'a	0.81 (3H, d, <i>J</i> = 6.5 Hz)	19.7 (CH <sub>3</sub> )	19.7 (CH <sub>3</sub> )
5'	1.22 (2H, m)	37.4 (CH <sub>2</sub> )	37.5 (CH <sub>2</sub> )
6'	1.26 (2H, m)	24.3 (CH <sub>2</sub> )	24.5 (CH <sub>2</sub> )
7'	1.08 (2H, m)	37.4 (CH <sub>2</sub> )	37.5 (CH <sub>2</sub> )
8'	1.36 (1H, m)	32.7 (CH)	32.7 (CH)
8'a	0.81 (3H, d, <i>J</i> = 6.5 Hz)	19.6 (CH <sub>3</sub> )	19.7 (CH <sub>3</sub> )
9'	1.08 (2H, m)	37.4 (CH <sub>2</sub> )	37.5 (CH <sub>2</sub> )
10'	1.90 (2H, m)	24.8 (CH <sub>2</sub> )	24.8 (CH <sub>2</sub> )
11'	1.10 (2H, m)	39.4 (CH <sub>2</sub> )	39.4 (CH <sub>2</sub> )
12'	1.50 (1H, m)	27.9 (CH)	28.0 (CH)
12'a	0.82 (3H, d, <i>J</i> = 6.6 Hz)	22.7 (CH <sub>3</sub> )	22.6 (CH <sub>3</sub> )
13'	0.82 (3H, d, <i>J</i> = 6.6 Hz)	22.6 (CH <sub>3</sub> )	22.6 (CH <sub>3</sub> )

#### 4.3: Conclusions

The examination of the stem bark and leaves of *Sapium integerrimum* of the Euphorbiaceae yielded eight compounds. The stem bark of the combined hexane and dichloromethane extracts afforded the unreported compound 4.1 (17-hydroxy-pimara-8(14),15-dien-3-one), and the known betulone, 4.2, lupenone, lupeol, friedelin and *trans*-phytol 4.3.

The combined hexane and dichloromethane extracts of the leaves yielded the known compounds  $\alpha$ -tocopherol, 4.4, friedelin, lupenone and the common stigmasterol.



Compounds from the stem bark of *Sapium integerrimum*

This plant has yielded triterpenoids of the friedelane and lupane classes and diterpenoids of the pimarane and phytane classes. Vitamin E, ( $\alpha$ -tocopherol), was also isolated from this plant.

#### **4.4: References**

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## CHAPTER 5: A REVIEW OF THE GENUS *MALLEASTRUM* (MELIACEAE) AND DITERPENOIDS ISOLATED FROM THE MELIACEAE FAMILY

### 5.1: Introduction to the phytochemistry of the genus *Malleastrum*

The genus *Malleastrum* is one of the fifty-one genera of the Meliaceae family and occurs in Madagascar and the Comores.<sup>1</sup> It belongs to the Trichilieae tribe alongside *Trichilia*, *Pseudobersama*, *Lepidotrichilia*, *Ekebergia* and *Astrotrichilia*.<sup>1</sup> *Malleastrum* is a small genus consisting of twenty-three species, including *M. rakotozafyi*, *M. gracile* and *M. antsingyense*.<sup>2</sup>

### 5.2: Phytochemistry of the genus *Malleastrum*

So far only *M. antsingyense* from this genus has been investigated phytochemically by Coombes *et al.*. The stem bark yielded two known limonoids, namely, 1,3-diacetylvilasinin and 1,3-diacetyl-12 $\alpha$ -hydroxy-7-tigloylvilasinin.<sup>2</sup> The two limonoids were previously isolated from an Indian specimen of *Melia volkensii*.<sup>3</sup>

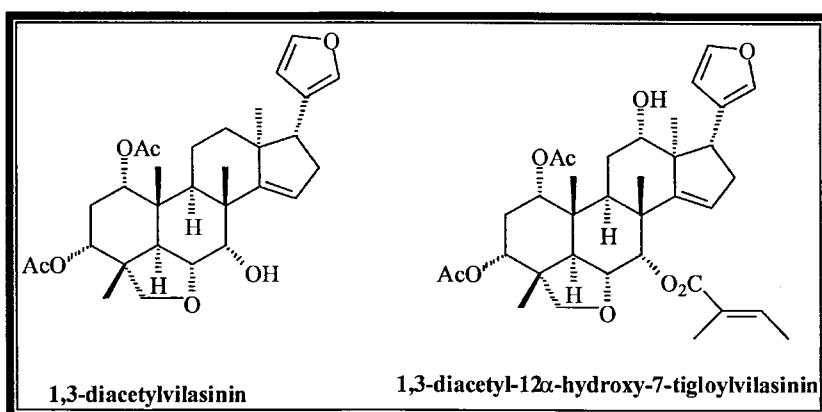


Figure 5.1: Limonoids from *M. antsingyense*<sup>2</sup>

In the present investigation, diterpenoids belonging to the *ent*-labdane and *ent*-isopimarane classes were isolated.

### 5.3: Diterpenoids from the Meliaceae family.

Diterpenoids possessing both normal and *ent*- skeleton are widespread in the Meliaceae family [Figure 5.2 – 5.4]. Normal and *ent*-isopimaranes have also been isolated.

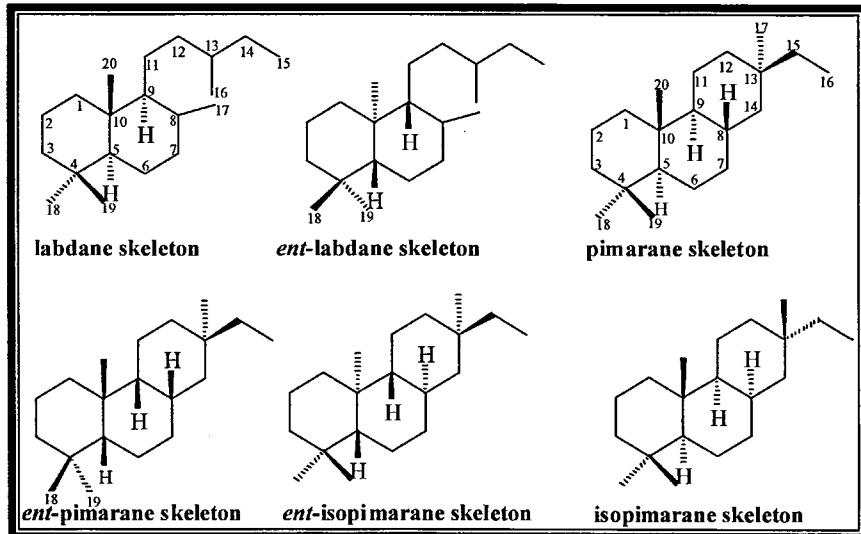


Figure 5.2: Normal and *ent*-classes of diterpenoids

Two isopimara-8(14)15-diene type diterpenoids, isopimara-8(14),15-diene and 7 $\alpha$ -hydroxyisopimara-8(14),15-diene are reported to have been isolated from *Dysoxylum spectabile*, however, no biological activities were reported for the two diterpenoids.<sup>4</sup>

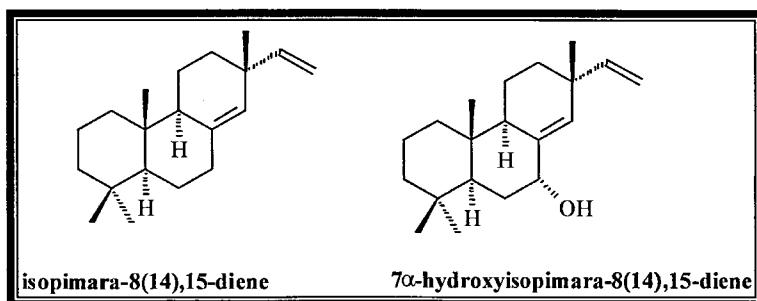


Figure 5.3: Isopimara-8(14)-diene diterpenoids from *Dysoxylum spectabile*<sup>4</sup>

A phytochemical investigation of *Dysoxylum hainanense* yielded four *ent*-pimarene diterpenoids, namely *ent*-18-acetoxy-8(14)-pimarene-15S,16-diol ( $[\alpha]_D = -3.5^\circ$ ), *ent*-18-acetoxy-16-hydroxy-8(14)-pimaren-15-one ( $[\alpha]_D = -0.4^\circ$ ), *ent*-16,18-dihydroxy-8(14)-pimaren-15-one ( $[\alpha]_D = -9.1^\circ$ )

and *ent*-19-nor-4,16,18-trihydroxy-8(14)-pimaren-15-one ( $[\alpha]_D = -25.6^\circ$ ).<sup>5</sup> The compounds have not been screened for their biological activities.

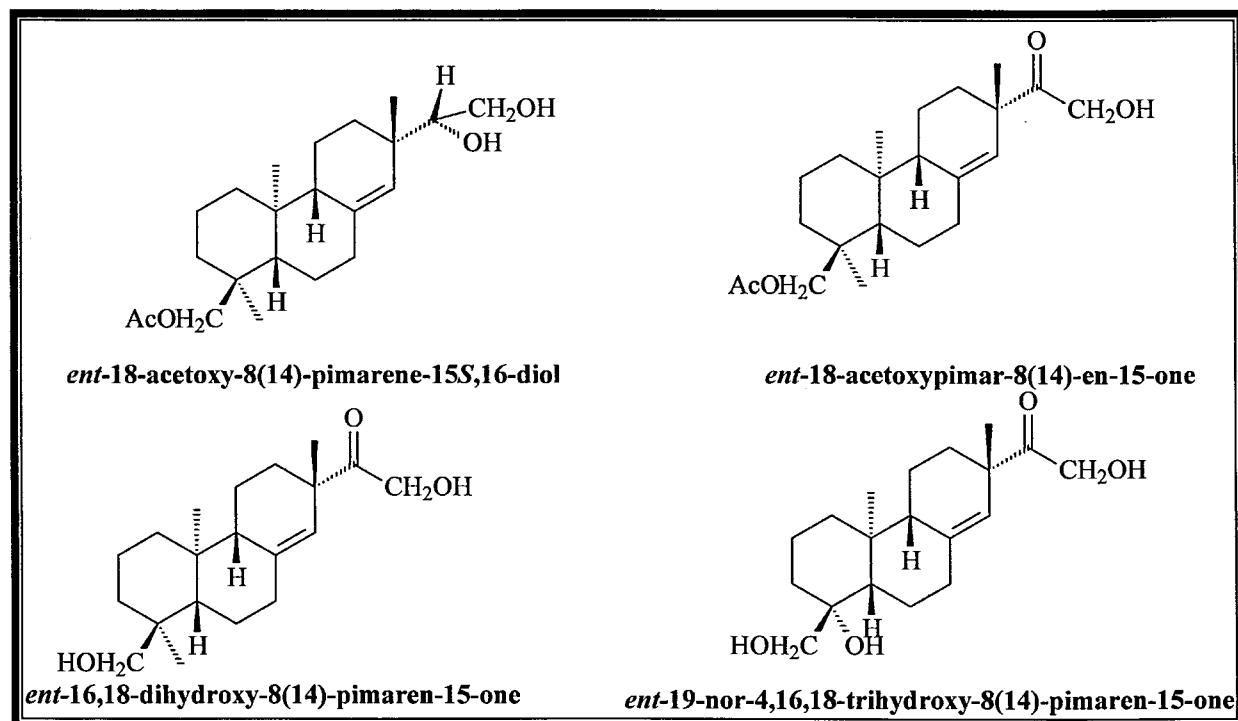


Figure 5.4: *Ent*-pimarane diterpenoids from *Dysoxylum hainanense*<sup>5</sup>

The methanol extract of *Guarea rhophalocarpa* was found to exhibit activity against *Leishmania donovani* promastigotes [ $IC_{50} = 62.5 \mu\text{g/ml}$  ( $IC_{95\%} = 70.7, 54.3$ )], *Trypanosoma brucei brucei* blood stream trypomastigotes [ $IC_{50} = 16 \mu\text{g/ml}$  ( $IC_{95\%} = 27.5$ )], *Plasmodium falciparum* [ $IC_{50} = 89 \mu\text{g/ml}$  ( $IC_{95\%} = 103, 75$ )] and KB cells [ $IC_{50} = 32.9 \mu\text{g/ml}$  ( $IC_{95\%} = 41.3, 24.1$ )]. Two diterpenoids were isolated and identified as *ent*-8(14),15-sandaracopimaradiene-2 $\alpha$ ,18-diol ( $[\alpha]_D = +8.77^\circ$ ) and *ent*-8(14),15-sandaracopimaradiene-2 $\beta$ ,18-diol ( $[\alpha]_D = +11.36^\circ$ ). The two diterpenoids were found to have antileishmanial activity, however the second compound was 3.3 times more active than the first against *L. donovani* promastigotes, but was found inactive against *Trypanosoma brucei brucei* blood stream trypomastigotes.<sup>6</sup> These compounds were named as *ent* by the authors, but structures were drawn as the “normal” series.

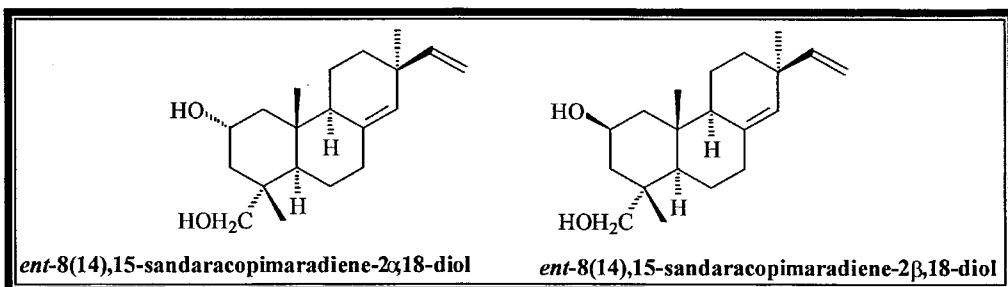


Figure 5.5: *Ent*-pimarane diterpenoids from *Guarea rhophalocarpa*<sup>6</sup> (Note: these compounds were named as ‘*ent*’ but structures were drawn as ‘normal’ in the literature.<sup>6</sup>)

A phytochemical investigation of *Guarea macrophylla* yielded isopimarane diterpenoids from the dichloromethane leaf extract, namely, isopimara-7,15-dien-3-one, isopimara-7,15-dien-3 $\beta$ -ol, isopimara-7,15-dien-2 $\beta$ -ol and a labdane, labda-8,13E-dien-15-ol, among other monoterpenes and triterpenoids.<sup>7</sup>

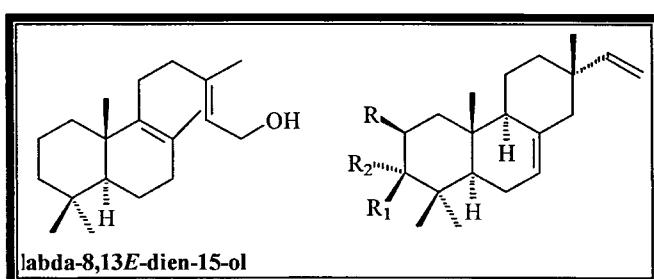


Figure 5.6: Labdane and isopimarane diterpenoids from *Guarea macrophylla*<sup>7</sup>

Table 5.1: Isopimarane diterpenoid class from *Guarea macrophylla*<sup>7</sup>

Compound	R	R <sub>1</sub>	R <sub>2</sub>
isopimara-7,15-dien-3-one	H	=O	-
isopimara-7,15-dien-3 $\beta$ -ol	H	OH	H
isopimara-7,15-dien-2 $\beta$ -ol	OH	H	H

In 2005, Lago *et al.* investigated the hexane phase from the methanol extract of the leaves of *Guarea macrophylla* and isolated the isopimarane diterpenoids, 7 $\alpha$ -hydroperoxy-isopimara-8(14),15-diene-2 $\alpha$ ,3 $\beta$ -diol and 19-nor-isopimara-4(18),7,15-trien-3-one.<sup>8</sup>

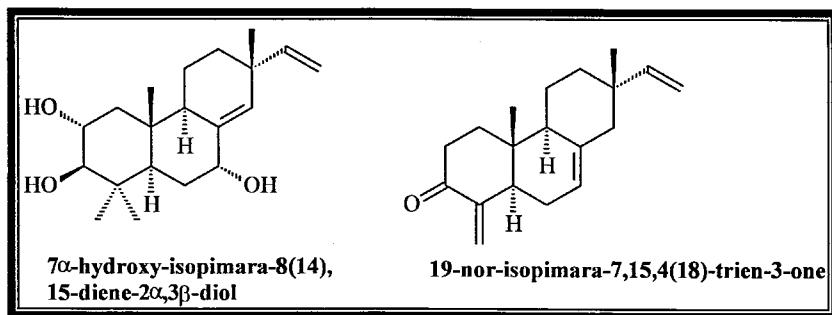


Figure 5.7: Isopimarane diterpenoids from *Guarea macrophylla*<sup>8</sup>

The acetone extract of the seeds of *Turraeanthus africanus* yielded two labdane diterpenoids, namely 12, 15-epoxylabda-8(17),12,14-trien-16-al ( $[\alpha]_D = +35.0^\circ$ ), 16-acetoxy-12(*R*), 15-epoxy-15 $\beta$ -hydroxylabda-8 (17), 13 (16)-diene, ( $[\alpha]_D = -190.1^\circ$ ).<sup>9</sup>

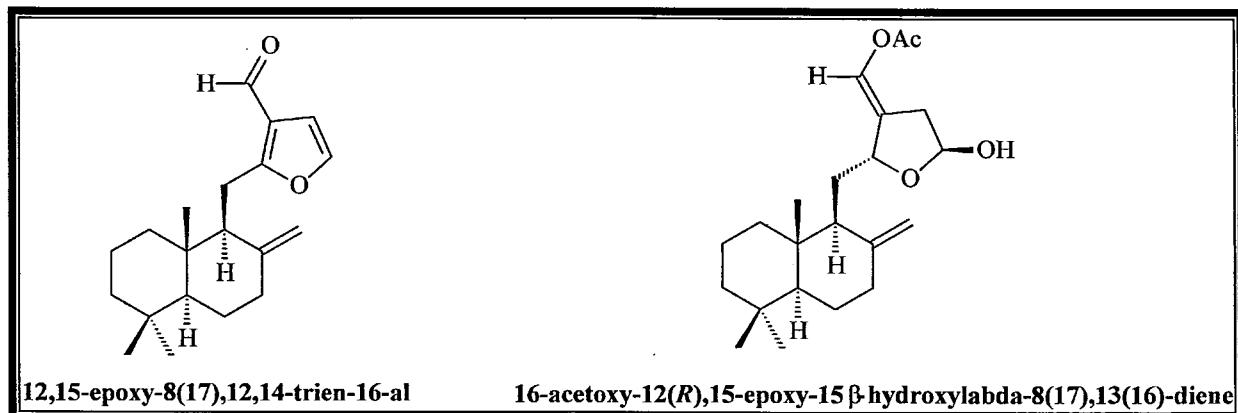


Figure 5.8: Labdane diterpenoids from *Turraeanthus africanus*<sup>9</sup>

The phytochemical investigation of *Aglaia odorata* yielded dolabellane-type diterpenoids, namely, (1*R*,3*R*,7*E*,11*S*,12*R*)-dolabella-4(16),7-diene-3,18-diol, (1*R*,3*E*,7*R*,11*S*,12*R*)-dolabella-3,8(17)-diene-7,18-diol and (1*R*,3*E*,7*E*,11*S*,12*R*)-dolabella-3,7-diene-18-ol.<sup>10</sup> No biological activities were reported.

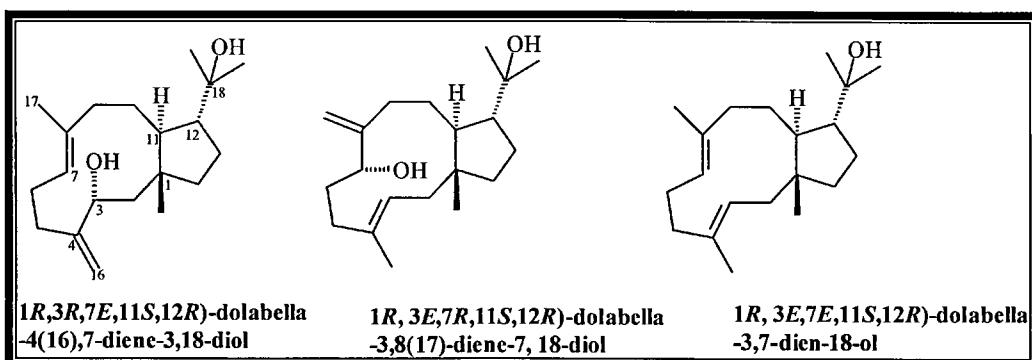


Figure 5.9: Dolabellane-type diterpenoids from *Aglaia odorata*<sup>10</sup>

Garcez *et al.* isolated different types of diterpenoids from *Guarea kunthiana* from the ethanol extract.<sup>11</sup> The authors reported that no limonoids were isolated from the plant.

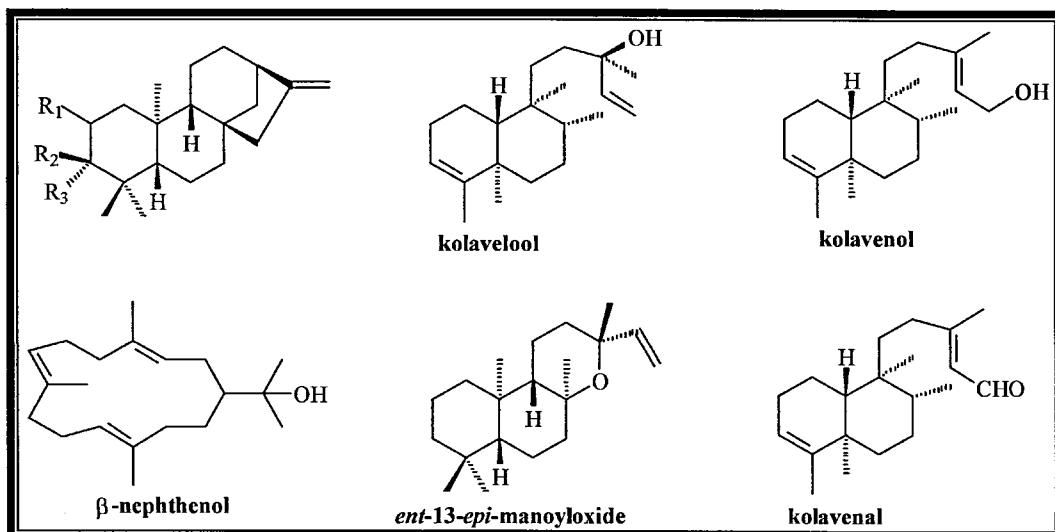


Figure 5.10: Cembrene, *ent*-kaurane, clerodane and *ent*-labdane diterpenoid types from *Guarea kunthiana*<sup>11</sup>

Table 5.2: *Ent*-kaurane diterpenoids from *Guarea kunthiana*<sup>11</sup>

Compound	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
<i>ent</i> -kaur-16-ene	H	H	H
<i>ent</i> -3α-hydroxykaur-16-ene	H	H	OH
<i>ent</i> -3β-hydroxykaur-16-ene	H	OH	H
<i>ent</i> -kaur-16-en-2-one	=O	H	H

The stem bark of *Turraeanthus africanus* exhibited significant anti-microbial activity against *Cryptococcus neoformans*, *Staphylococcus aureus* and methicillin-resistant *S. aureus*. (+)-Pumiloxide, (+)-16-acetoxy-12,15-epoxylabda-8(17),12,14-triene, *ent*-labda-8(17),12(*E*)-diene-15,16-dial, turraeanin A, B, C, D and F, 15β,16-diacetoxy-12(R),15-epoxylabda-8(17),13(16)-diene showed remarkable activities against *Cryptococcus neoformans* and methicillin-resistant *S. aureus*.<sup>12</sup>

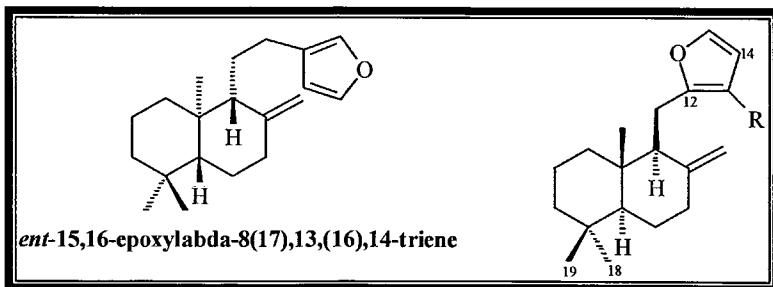


Figure 5.11a: *Ent* and normal labdane diterpenoids from *Turraeanthus africanus*<sup>12</sup>

Table 5.3: Labdane diterpenoids from *Turraeanthus africanus*

Compound	R
(+)-pumiloxide	CH <sub>3</sub>
(+)-16-acetoxy-12,15-epoxylabda-8(17),12,14-triene	CH <sub>2</sub> OAc

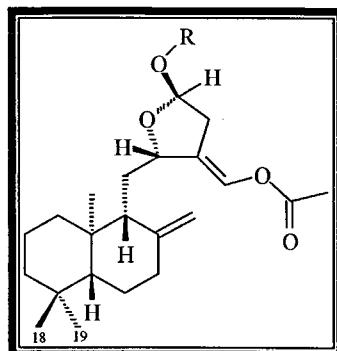


Figure 5.11b: *Ent* labdane diterpenoids isolated from *Turraeanthus africanus*<sup>12</sup>

Table 5.4: *Ent*-labdane diterpenoids from *Turraeanthus africanus*

Compound name	R
<i>ent</i> -(12S,15R,16E),16-acetoxy-12,15-epoxy-15-isopropoxylabda-8(17),13(16)-diene (turraeanin A)	CH(CH <sub>3</sub> ) <sub>2</sub>
<i>ent</i> -(12S,15R,16E),16-acetoxy-12,15-epoxy-15-methoxylabda-8(17),13(16)-diene (turraeanin C)	CH <sub>3</sub>

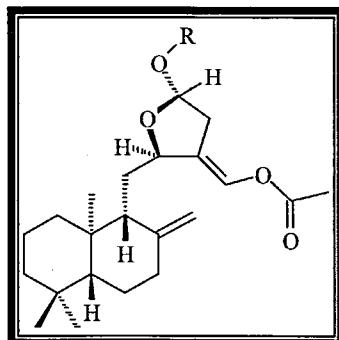


Figure 5.11c: *Ent-labdane diterpenoids from Turraeanthus africanus*<sup>12</sup>

Table 5.5: *Ent-labdane diterpenoids from Turraeanthus africanus*

Compound name	R
<i>ent</i> -[12 <i>R</i> ,15 <i>S</i> ,16 <i>E</i> ]-16-acetoxy-12,15-epoxy-15-isopropoxy-labda-8(17),13(16)-diene (turraeanin B)	CH(CH <sub>3</sub> ) <sub>2</sub>
<i>ent</i> -[16 <i>E</i> ,12 <i>R</i> ,15 <i>S</i> ]-16-acetoxy-12,15-epoxy-15-methoxy-labda-8(17),13(16)-diene (turraeanin D)	CH <sub>3</sub>

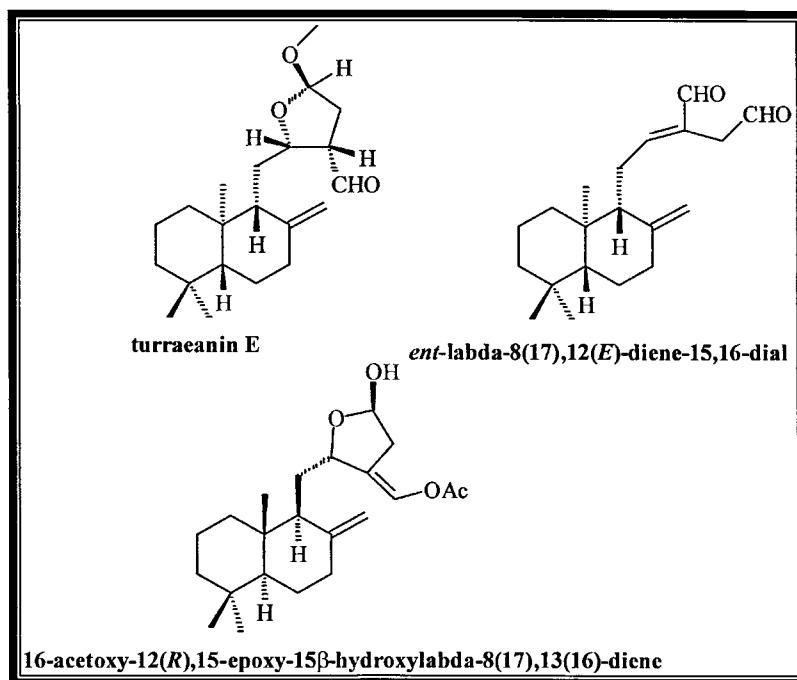


Figure 5.11d: Labdane diterpenoids from *Turraeanthus africanus*<sup>12</sup>

The leaves of *Guarea guidonia* yielded cneorubin related diterpenoids, cneorubin A, B, C, X, Y and emmottene. No bioactivity of the compounds was reported.<sup>13</sup>

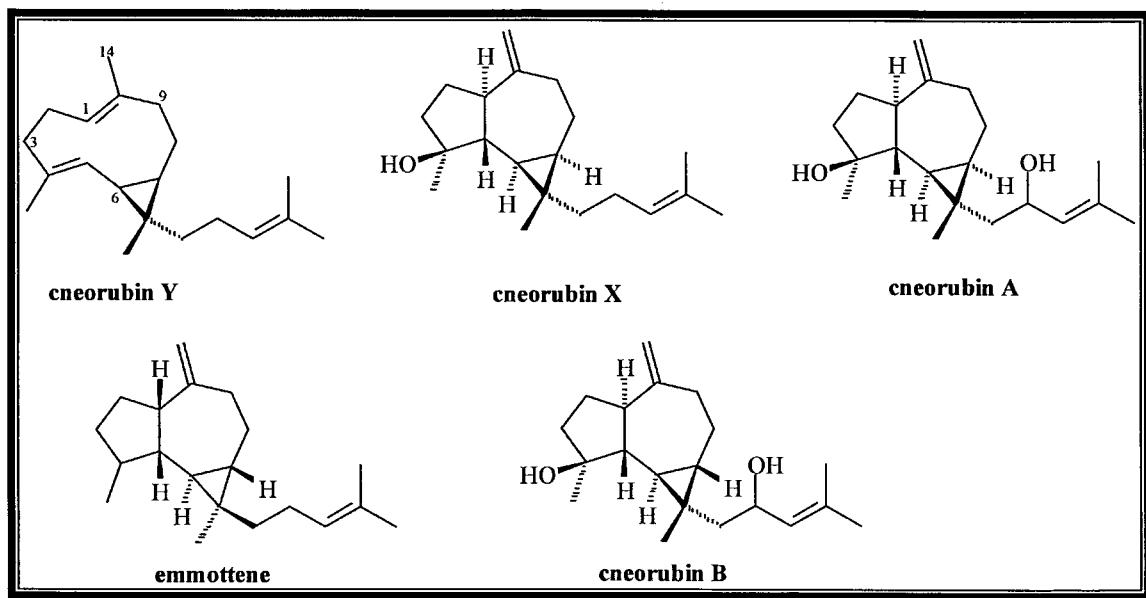


Figure 5.12: Cneorubin diterpenoids from *Guarea guidonia*<sup>13</sup>

#### 5.4: Biosynthesis of diterpenoids

Diterpenoids are classified as C-20 compounds, consisting of four isoprene units. Diterpenoids comprise a large group of isoprenoid compounds derived biosynthetically from geranylgeranyl pyrophosphate (GGPP).<sup>14,16</sup> They can be found in higher plants, fungi, insects and marine organisms.<sup>15</sup> They are categorized into acyclic and cyclic compounds. Bicyclic diterpenoids include the labdane and clerodane classes, tricyclic diterpenoids [Figure 5.12] include pimarane, abietane, cassane and rosane classes, tetracyclic diterpenoids include kaurane, beyerene and gibberellin classes, pentacyclic diterpenoids include the trachylobane class and monocyclic and macrocyclic diterpenoids include cembrane, casbene and taxane classes [Figure 5.13].<sup>16,17</sup>

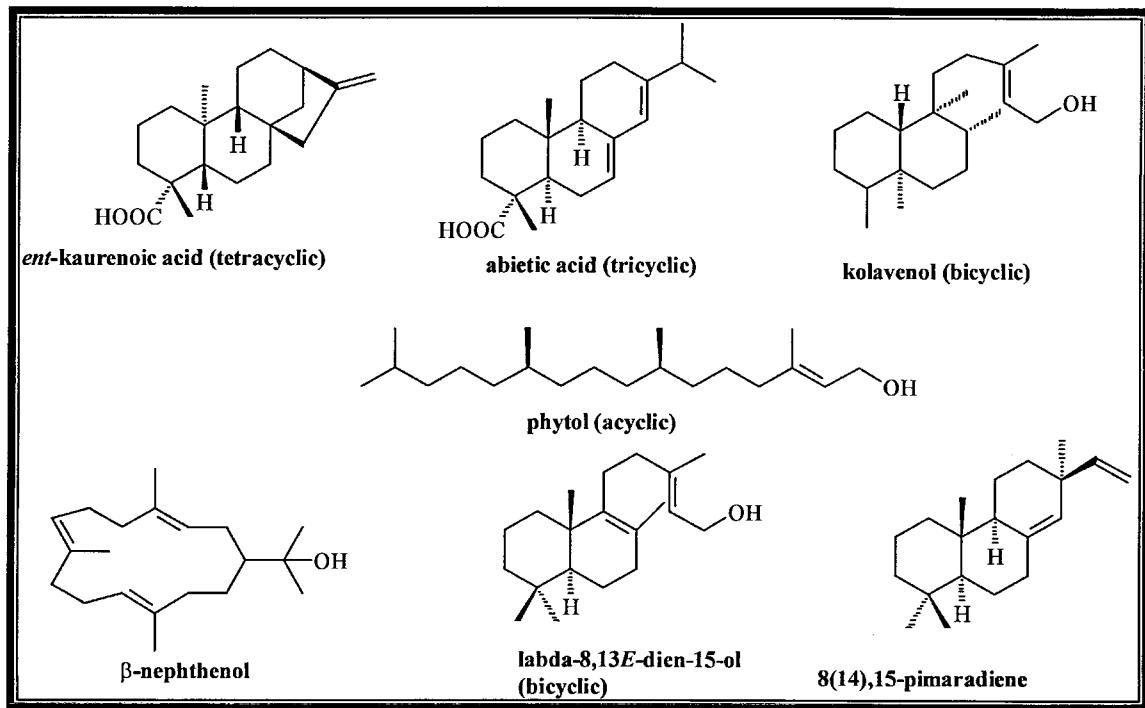
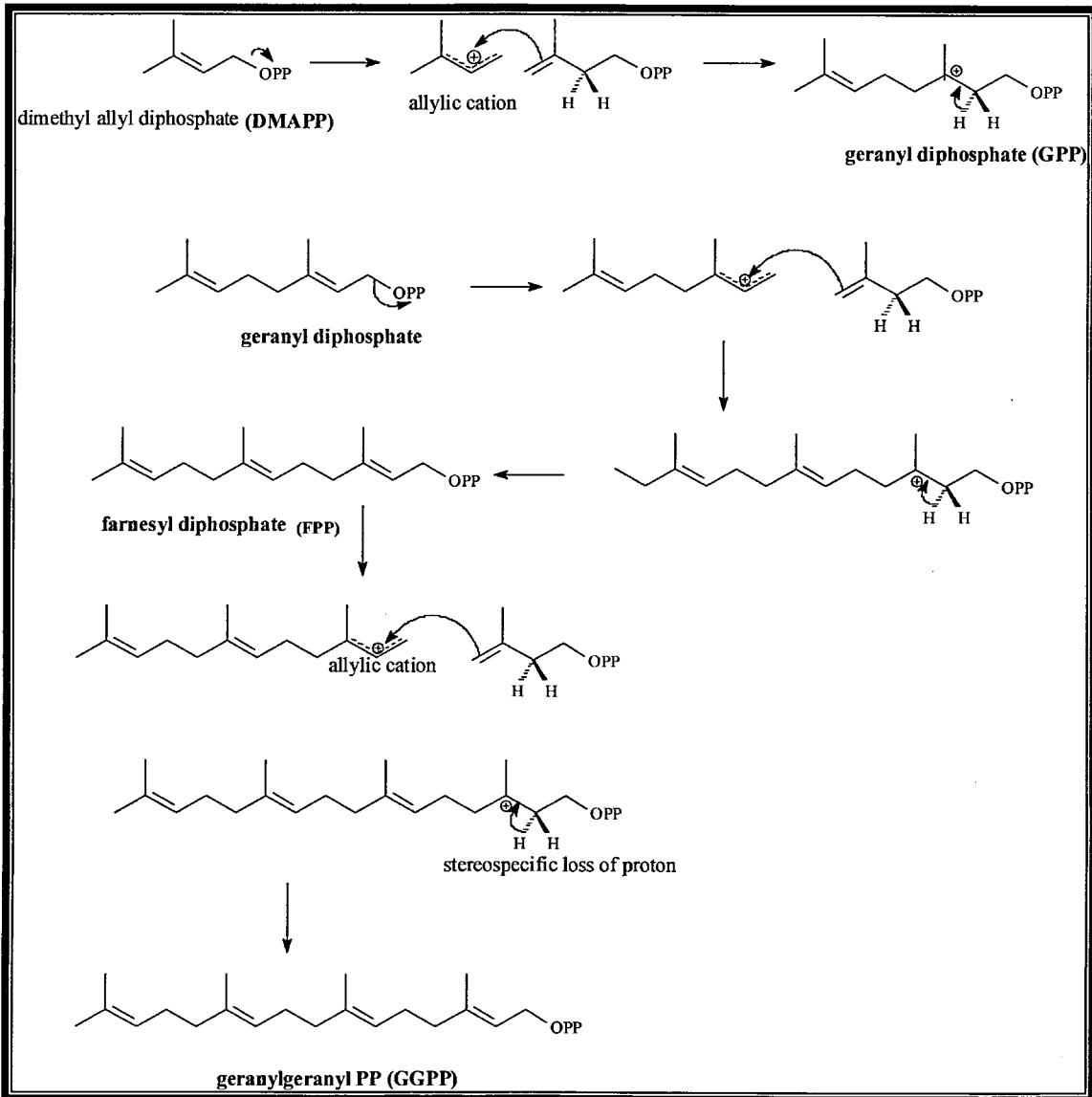
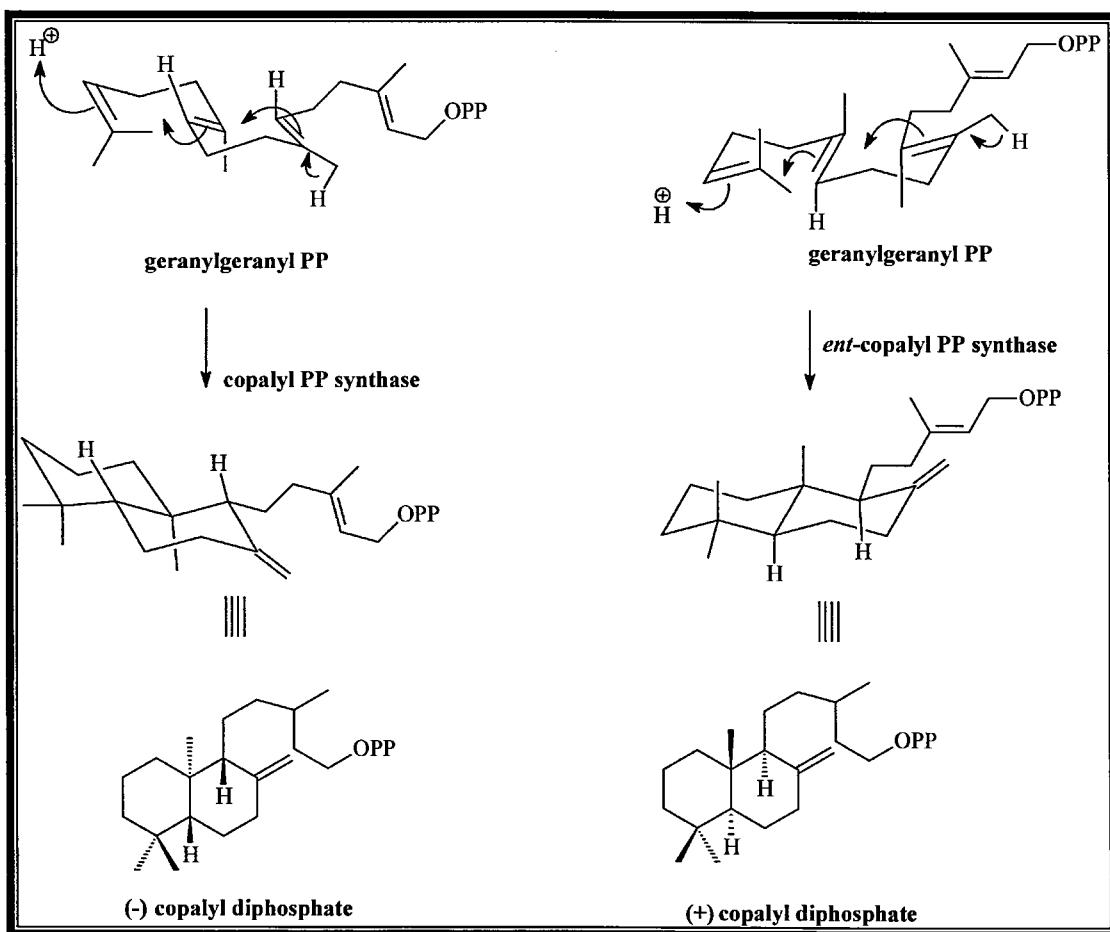


Figure 5.13: Examples of different types of diterpenoids

Biosynthesis of diterpenoids follows the biogenetic isoprene rule where dimethyl allyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP) are combined *via* the enzyme prenyl transferase to yield geranyl diphosphate (GPP) [Scheme 5.1].<sup>14</sup> An addition of a C<sub>5</sub> IPP unit to GPP forms farnesyl diphosphate [FPP] [Scheme 5.1]. Geranylgeranyl diphosphate (GGPP) is formed when an extra IPP molecule is added to FPP [Scheme 5.1].<sup>14</sup>

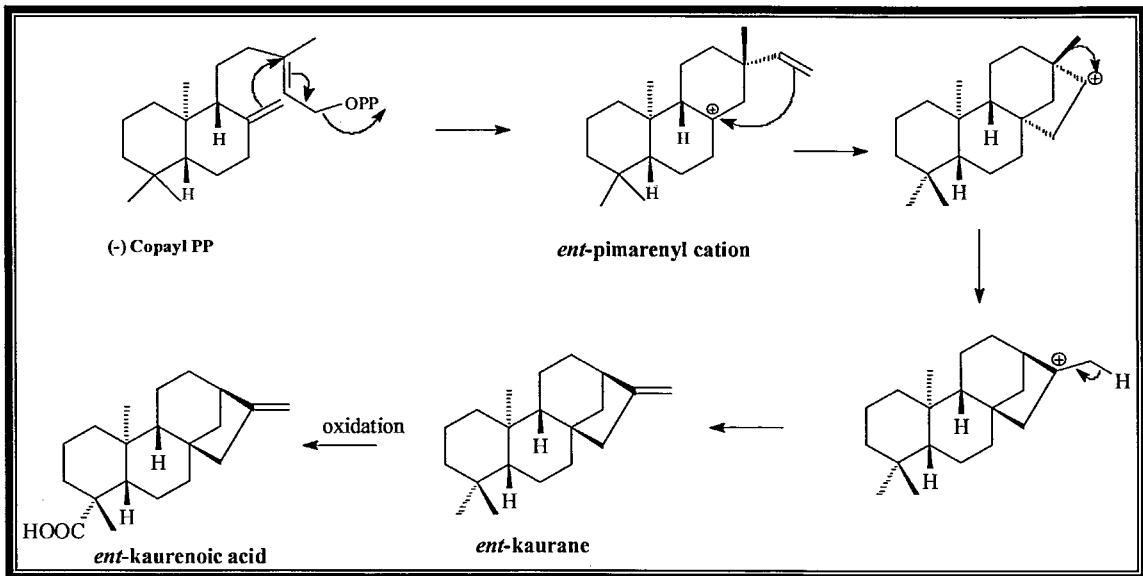


Scheme 5.1: Formation of geranylgeranyl diphosphate (GGPP).<sup>14</sup>



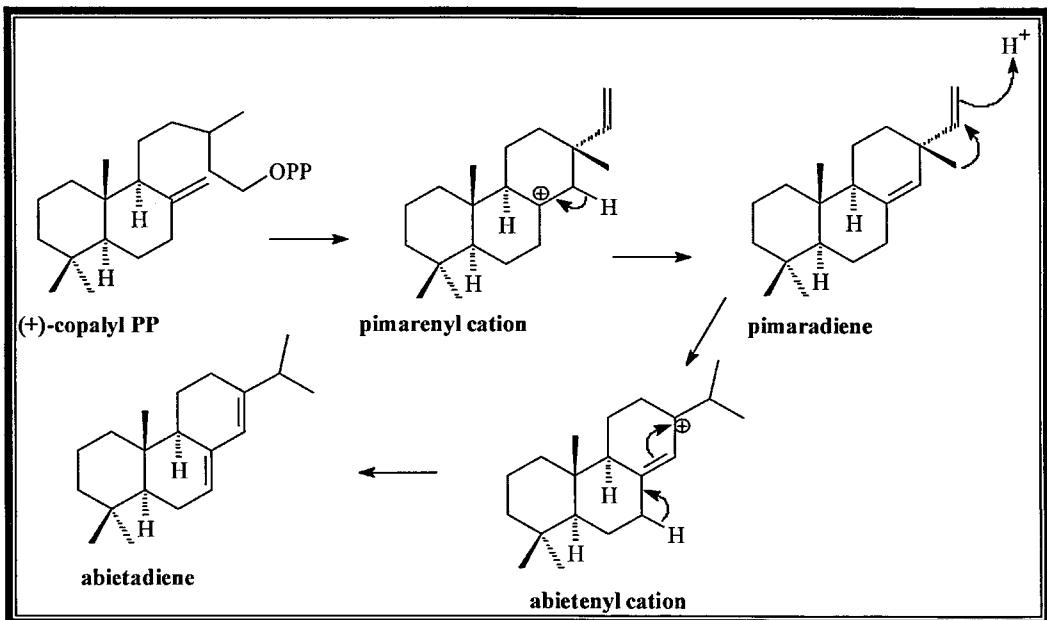
Scheme 5.2: Formation of bicyclic diterpenes (+)-copalyl and (-)-copalyl diphosphates<sup>14</sup>

The cyclization reactions of GGPP are mediated by carbocation formation and Wagner-Meerwein rearrangements determine the many structural variants of diterpenoids that are generated.<sup>14</sup> The two bicyclic enantiomers (+) copalyl PP and (-) copalyl PP are formed via the two different cyclization routes [Scheme 5.2]. The two enantiomers differ in the configuration at the C-5, C-9 and C-10 chiral centers. The folding of the geranylgeranyl diphosphate into different conformers is regulated enzymatically by copalyl and *ent*-copalyl diphosphate synthase.<sup>14</sup>



Scheme 5.3: Formation of the tricyclic and tetracyclic diterpenoids.<sup>14</sup>

*(-)*-Coparyl PP is the precursor for the biosynthesis of *ent*-kauranes. The cyclization of *ent*-coparyl PP is catalysed by a single enzyme (*ent*-kaurane synthase) resulting in the formation of *ent*-kaurane and this is initiated by the loss of a diphosphate (PP) leaving group resulting in the carbocation-mediated formation of the third ring. This is followed by the cyclization of the alkene on to a cation that produces a secondary carbocation and leads to a fourth ring (*ent*-kaurane). The loss of a proton from the methyl group of the tertiary cation leads to the formation of the exocyclic double bond of the *ent*-kaurene as shown in Scheme 5.3. Sequential oxidation of the methyl group results in the formation of *ent*-kaurenoic acid.<sup>14</sup>



Scheme 5.4: Formation of the tricyclic diterpene (-) abietadiene <sup>14</sup>

Cyclization of (+) copalyl disphosphate is catalysed by abietadiene synthase. The loss of the diphosphate allows for a carbocation mediated formation of a third ring, forming a tertiary cation, the pimarenyl cation. The loss of a proton from the pimarenyl cation, gives pimaradiene, and this is followed by a Wagner-Meerwein methyl shift to the side chain, thereafter the loss of a proton results in the formation of abietadiene.<sup>14</sup>

## 5.5: Biological properties of diterpenoids

Diterpenoids have been isolated from many plant species of different families. They are abundant and are well known for their biological activities.<sup>19</sup> Some diterpenoids, particularly the phorbol esters, are recognized as irritating, poisonous and carcinogenic.<sup>14</sup> Some compounds are found to exhibit antihypertensive, antiretroviral, anti-inflammatory, and anti-bacterial activities.<sup>20,21</sup>

## Taxane

Taxol (Paclitaxel), a diterpenoid ester, was isolated in 1971 from *Taxus brevifolia*. The compound is utilized commercially as an anticancer agent.<sup>14</sup>

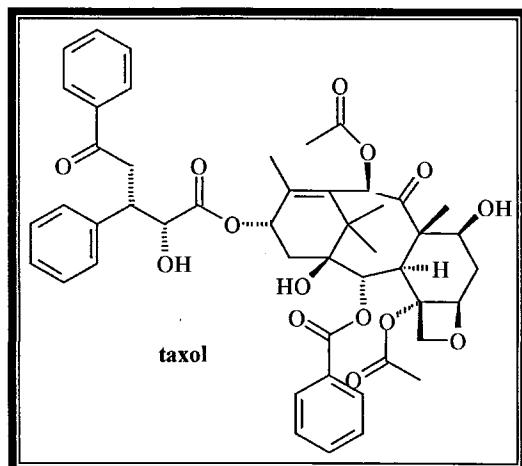


Figure 5.14: Taxane diterpenoid isolated from *Taxus brevifolia*<sup>14</sup>

## Pimaranes

The pimaranes  $7\beta$ -hydroxyisopimara-8,15-dien-14-one and  $14\alpha$ -hydroxyisopimara-7,15-dien-1-one which were isolated from *Hypoestes serpens*, showed antifungal activity against *Cladiosponium cucumerinum* and the yeast, *Candida albicans*.<sup>22</sup>

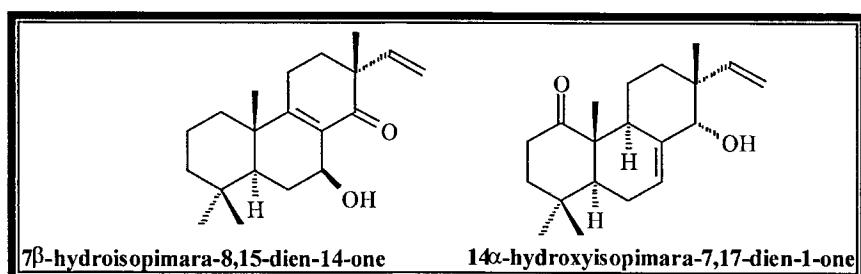


Figure 5.15: Isopimaranes isolated from *Hypoestes serpens*<sup>22</sup>

## Labdanes

The labdane diterpenoids from terrestrial and marine plants exhibit antibacterial, antifungal, anti-inflammatory, antileishmanial, and cytotoxic properties.<sup>24, 26</sup>

The labdane diterpenoid, 13-*epi*-clareol, isolated from the root bark of *Coleus forskohlii*, was found to exhibit antiproliferative activity.<sup>23</sup>

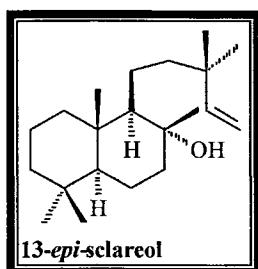


Figure 5.16: Labdane diterpenoids isolated from *Coleus forskohlii*.<sup>23</sup>

An *ent*-labdane, andanusol, isolated from *Sideritis foetens* (Lamiaceae), exhibited *in vivo* anti-inflammatory activity.<sup>25</sup>

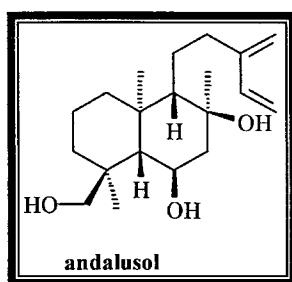


Figure 5.17: *Ent*-labdane diterpenoid isolated from *Sideritis foetens*.<sup>25</sup>

The labdanes isolated from the seeds of *Aframomum zambesiacum*, aulacocarpin A, aulacocarpin B, 3-deoxyaulacocarpin A, methyl 14 $\xi$ ,15-epoxy-3 $\beta$ -hydroxy-8(17),12E-labdadien-16-oate, 8 $\beta$ ,17-epoxy-12E-labdene-14 $\xi$ ,15,16-triol, galanolactone, zambesiadolactone A and zambesiadolactone B were tested for their *in vitro* antiplasmodial activity against *Plasmodium falciparum*. 3-Deoxyaulacocarpin A was the most active with an IC<sub>50</sub> of 4.97  $\mu$ M.<sup>26</sup>

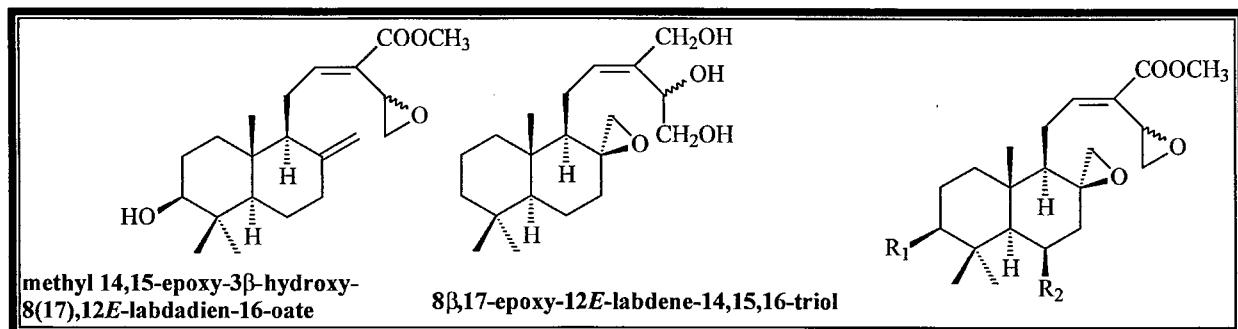


Figure 5.18: Labdane diterpenoids isolated from *Aframomum zambesiacum*<sup>26</sup>

Table 5.6: Labdanes from *Aframomum zambesiacum*<sup>26</sup>

Name	R <sub>1</sub>	R <sub>2</sub>
aulacocarpin A	OH	H
aulacocarpin B	OH	OH
3-deoxyaulacocarpin A	H	H

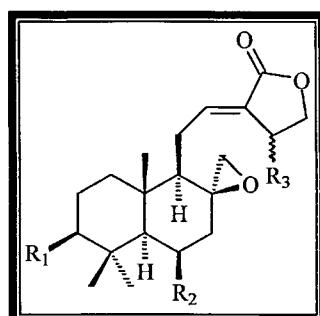


Figure 5.19: Labdane diterpenoids from *Aframomum zambesiacum*<sup>26</sup>

Table 5.7: Labdanes from *Aframomum zambesiacum*<sup>26</sup>

Name	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
Galanolactone	H	H	H
zambesiadolactone A	OH	H	OH
zambesiadolactone B	OH	OH	OH

The furano-*ent*-labdane *ent*-15,16-epoxy-12-oxo-8(17),13(16),14-labdatrien-19-oate, isolated from *Potamogeton pectinatus*, exhibited strong algicidal activity against *Raphidocelis subcapitata*.<sup>27</sup>

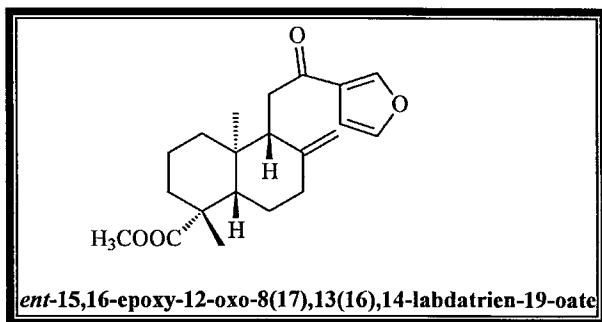


Figure 5.20: A furano-*ent*-labdane diterpenoid from *Potamogeton pectinatus*<sup>27</sup>

Methyl 14,15-epoxylabda-8(17), 12E-diene-16-oate, which was isolated from *Turraeanthus africanus*, was found to show antiplasmodial activity against a chloroquine-resistant strain of *Plasmodium falciparum*.<sup>31</sup>

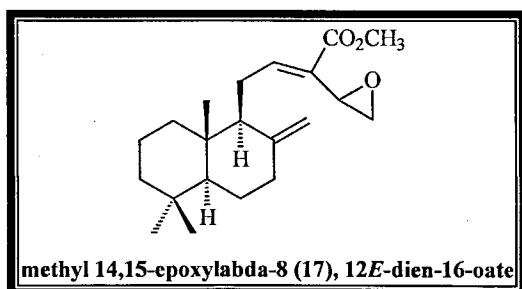


Figure 5.21: Labdane diterpenoid from *Turraeanthus africanus*<sup>31</sup>

The diverse biological properties of kaurane diterpenes have been demonstrated and these included plant growth regulation, antimicrobial, anti-parasitic, insect antifeedant, cytotoxic, antitumour, anti-HIV, anti-infertility and anti-inflammatory properties.<sup>28</sup> Kaurane glycosides have been studied for their biological activities.<sup>29</sup>

The *ent*-kaurane diterpenoid, kaurenoic acid, that is abundant in many plant species,<sup>29</sup> has been found to exhibit anti-inflammatory, antibacterial and antifungal properties.<sup>29</sup> A kaurane glycoside stevioside which was isolated from *Stevia rebaudiana* was found to have sweetening properties 250 to 300 times more than saccharose, though it was found to have carcinogenic and toxic properties. Steviol, an aglycone which was obtained from the hydrolysis of stevioside, exhibited high toxicity.<sup>14,29</sup>

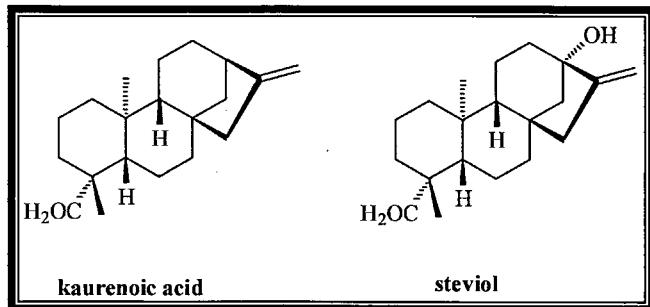


Figure 5.22: *Ent*-kaurane diterpenoids from *Stevia rebaudiana*<sup>14,29</sup>

Abietic acid which was isolated from *Pimenta racemosa* (Myrtaceae) has anti-inflammatory activity.<sup>30</sup>

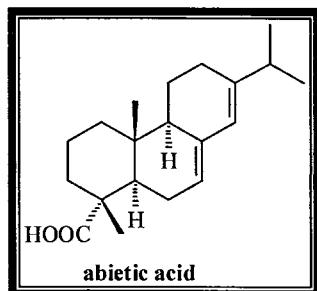


Figure 5.23: Abietic acid isolated from *Pimenta racemosa*<sup>30</sup>

## 5.6: References

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**CHAPTER 6: A PHYTOCHEMICAL INVESTIGATION OF *MALLEASTRUM RAKOTOZAFYI* M. Cheek (MELIACEAE)**

**6.1: INTRODUCTION**

*Malleastrum rakotozafyi*, a member of the Meliaceae family, is endemic to Madagascar. The leaves and stem bark were collected from the Angavo site in Madagascar. This plant is not used ethnomedicinally.



**Picture 6.1:** *Malleastrum rakotozafyi* (Photograph by Dr M. Randrianarivelojosia)

Eleven diterpenoids were isolated from *M. rakotozafyi*, eight were *ent*-labdanes and three were *ent*-pimaranes. Compounds **6.1-6.11** were isolated from the combined hexane and dichloromethane extracts of the leaves. Compounds **6.1, 6.2, 6.4, 6.5, 6.10** and **6.11** were isolated from the hexane extract of the stem bark. The structures of the *ent*-labdanes isolated are shown in Figure 6.1 and the *ent*-pimaranes are shown in Figure 6.2.

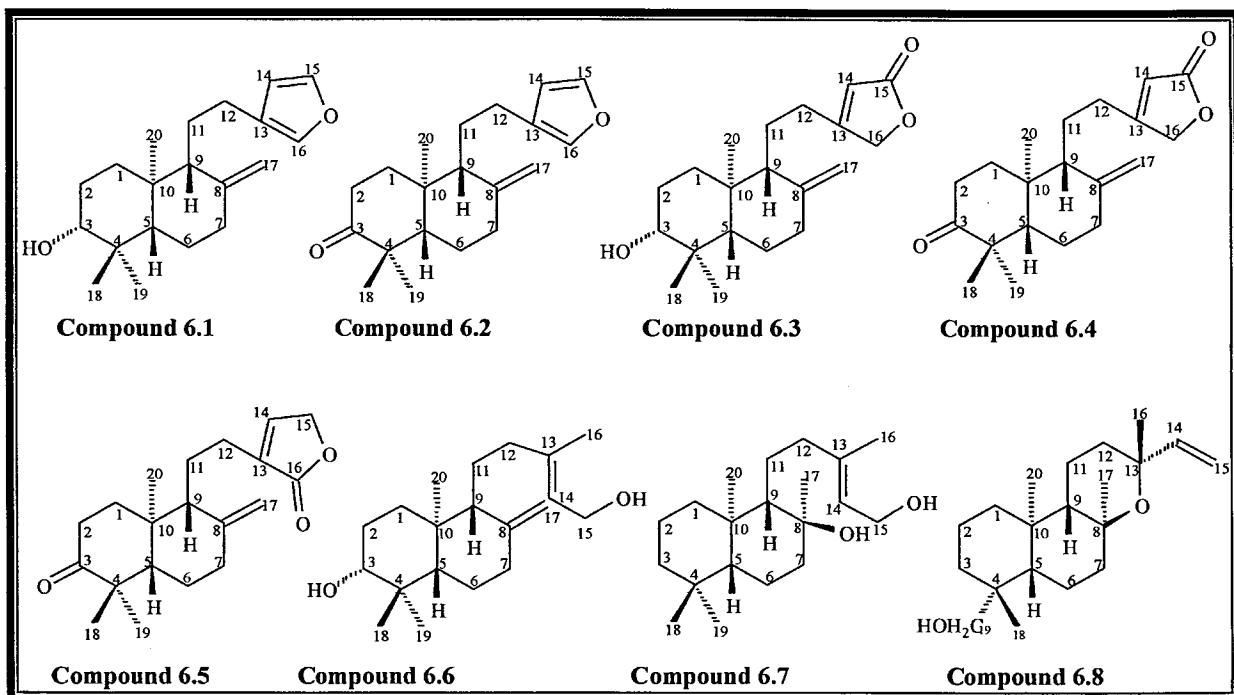


Figure 6.1: *Ent*-labdanes from *M. rakotozafyi*

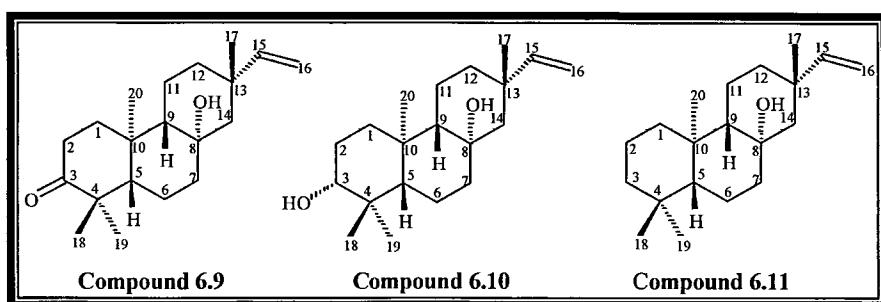
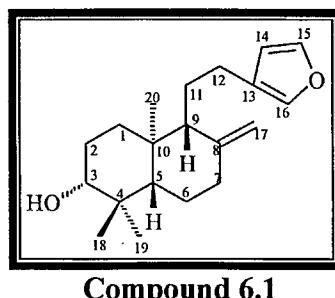


Figure 6.2: *Ent*-pimaranes from *M. rakotozafyi*

**6.1.1: Structural elucidation of compound 6.1: *ent*-15, 16-epoxylabda-8(17), 13 (16), 14-trien-3 $\beta$ -ol.**



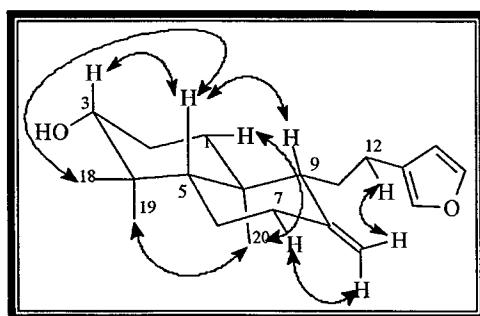
The high resolution mass spectrum of compound **6.1** showed a molecular ion peak at *m/z* 302.2253, which is consistent with the molecular formula of C<sub>20</sub>H<sub>30</sub>O<sub>2</sub>, and indicated that the compound was a diterpenoid. The molecular formula suggested a double bond equivalence of six. The FTIR spectrum showed an absorption band at 3392 cm<sup>-1</sup> typical of an OH group stretch, CH stretching bands at 2855 cm<sup>-1</sup>, a C=C stretching band at 1643 cm<sup>-1</sup> and a furan ring C-O stretching band at 1444 cm<sup>-1</sup>.<sup>1,2</sup>

The <sup>1</sup>H NMR spectrum indicated that a furan ring was present in the molecule. This was indicated by three coupled proton resonances at δ 6.25 (H-14), δ 7.34 (H-15) and δ 7.16 (H-16). Corresponding <sup>13</sup>C NMR resonances occurred at δ 111.1 (C-14), δ 142.9 (C-15) and δ 138.9 (C-16). The resonance at δ 125.7 was assigned to C-13 due to correlations seen in the HMBC spectrum between this resonance and the H-14, H-15 and H-16 resonances, previously assigned. In addition, the C-13 resonance showed a correlation in the HMBC spectrum with the two H-11 methylene proton resonances at δ 1.69 and δ 1.59. The C-14 and C-16 carbon resonances showed correlations with the two H-12 proton resonances at δ 2.23 and δ 2.55. The COSY spectrum showed the two H-12 resonances to be coupled to the two H-11 resonances, which were further coupled to the H-9 proton resonance at δ 1.61. The corresponding C-9 carbon resonance (δ 55.9) showed correlations in the HMBC spectrum to the H-5 methine resonance (δ 1.07), the two non-equivalent H-17 protons of an exocyclic methylene group (δ 4.88, δ 4.58) and the 3H-20 methyl group proton resonance at δ 0.69. The H-5 methine resonance showed coupling in the COSY spectrum with the two H-6 methylene group proton resonances (δ 1.39, δ 1.72) which, in turn, showed further coupling with the two H-7 methylene group proton resonances (δ 2.41, δ 1.96). These resonances showed long range coupling with the 2H-17 resonances, previously assigned.

A resonance at δ 3.25 (dd, *J* = 11.7, *J* = 7.4 Hz) corresponded to a resonance at δ 79.0 in the HSQC spectrum. This resonance was seen to correlate with the previously assigned H-5 methine proton resonance and the 3H-18 and 3H-19 resonances (δ 0.77, δ 0.98) in the HMBC spectrum, and was assigned to C-3. The COSY spectrum showed coupling between the H-3 resonance and

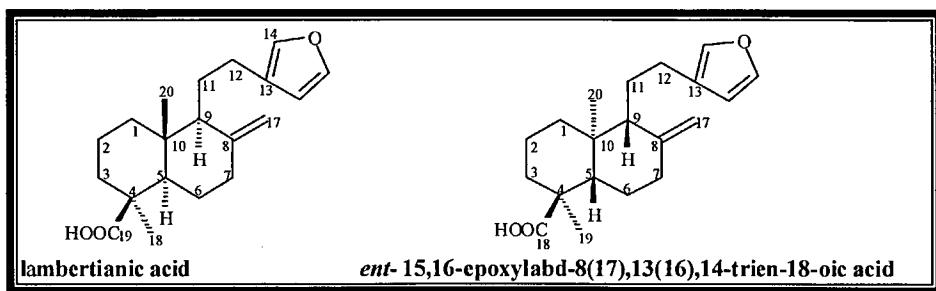
the superimposed H-2 resonances ( $\delta$  1.60), which showed further coupling to the two H-1 resonances ( $\delta$  1.77,  $\delta$  1.12).

The optical rotation of  $-26.98^\circ$  indicated that the compound belonged to the *ent*-labdane series, where the H-5 and H-9 protons are in the  $\beta$ -orientation and the C-20 methyl group in the  $\alpha$ -orientation. The NOESY spectrum indicated a correlation between the  $\beta$ -orientated H-5 resonance and the  $\beta$ -orientated 3H-18 and H-9 methine proton resonance and the H-3 proton resonance. This indicated that H-3 was in the axial or  $\beta$ -orientation and the hydroxyl group was  $\alpha$  or equatorial. The compound was identified as *ent*-15,16-epoxy-labda-8(17),13(16),14-trien-3 $\beta$ -ol. This compound has not been reported previously.



**NOESY correlations for Compound 6.1**

Furanolabdanes have been isolated previously. Lambertianic acid ( $[\alpha]_D = +53.0^\circ$ ) was isolated from *Platycladus orientalis* (Cupressaceae), and was tested for *in vitro* antiplasmodial activity. A weak ( $IC_{50} > 25\mu M$ ) *in vitro* antiplasmodial effect against *Plasmodium falciparum* was noted.<sup>3</sup> *ent*-15,16-epoxylabd-8(17), 13(16),14-trien-18-oic acid ( $[\alpha]_D = -40.9^\circ$ ) was isolated from *Eupatorium buniifolium* (Asteraceae).<sup>4</sup>

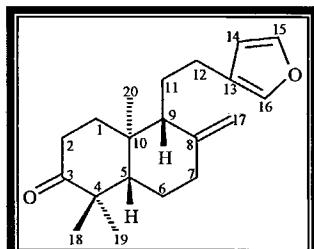


**Table 6.1: NMR data for compound 6.1: *ent*-15,16-epoxy-labda-8(17),13(16),14-trien-3 $\beta$ -ol in CDCl<sub>3</sub>**

No.	<sup>1</sup> H NMR (500 MHz)	<sup>13</sup> C NMR (125 MHz)	HMBC (H → C)	COSY	NOESY
1 $\alpha$	1.77 (1H, m)	37.2 (CH <sub>2</sub> )	C-3, C-4, C-5, C-20	H-1 $\beta$	3H-20
1 $\beta$	1.12 (1H, m)		C-3, C-4, C-5, C-9, C-10, C-20	H-2 $\beta$ , H-2 $\alpha$ , H-1 $\alpha$	3H-2 $\beta$
2 $\alpha$	1.60 (1H, m)	24.4 (CH <sub>2</sub> )	C-2, C-3, C-4, C-5, C-9, C-10, C-20	H-3 $\alpha$ , H-1 $\beta$	3H-20, H-3
2 $\beta$	1.60 (1H, m)		C-2, C-3, C-4, C-5, C-9, C-10, C-20	H-3 $\alpha$	H-1 $\beta$
3	3.22 (1H, dd, J = 11.7, 7.4 Hz)	79.0 (CH)	C-1, C-4, C-5, C-18, C-19	H-2 $\alpha$ , H-2 $\beta$	H-5, H-11A, 3H-18
4	-	39.3 (C)	-	-	-
5	1.07 (1H, m)	54.7 (CH)	C-3, C-9, C-18, C-19, C-20	H-6 $\alpha$ , H-6 $\beta$	H-2 $\beta$ , H-3, H-7 $\beta$ , 3H-19, H-9
6 $\alpha$	1.39 (1H, qd, J=4.00, 12.75 Hz)	24.2(CH <sub>2</sub> )	C-5, C-7	H-5, H-6 $\beta$ , H-7 $\alpha$	3H-20, H-7 $\alpha$
6 $\beta$	1.72 (1H, m)		C-5, C-6	H-5, H-6 $\alpha$ , H-7 $\beta$ , H-7 $\alpha$	3H-18, H-6 $\alpha$ , H-7 $\beta$
7 $\alpha$	2.41 (1H, m)	38.3 (CH <sub>2</sub> )	C-5, C-6, C-8, C-9, C-17	H-7 $\beta$ , H-6 $\beta$	H-17, H-6 $\alpha$
7 $\beta$	1.96 (1H, td, J=5.00, 13 Hz)		C-6, C-8, C-9, C-17	H-7 $\alpha$ , H-6 $\alpha$ , H-6 $\beta$	H-5, H-6 $\beta$
8	-	148.0 (C)	-	-	-
9	1.61 (1H, m)	55.9 (CH)	C-5, C-8, C-12, C-17	H-11A, H-11B	H-5
10	-	39.5 (C)	-	-	-
11A	1.69 (1H, m)*	28.1 (CH <sub>2</sub> )	C-8, C-11, C-12	H-9, H-11B, H-12A, H-12B	H-3, H-12A, 3H-20
11B	1.59 (1H, m)*		C-8, C-10, C-12, C-13, C-17	H-9, H-11A, H-12A, H-12B	
12A	2.55 (1H, m)*	23.7 (CH <sub>2</sub> )	C-9, C-11, C-13, C-14, C-16	H-12B, H-11A, H-11B	H-12B, H-17B, H-11A
12B	2.23 (1H, m)*		C-9, C-13, C-14, C-16	H-12A, H-11A, H-11B	H-12A, H-17B
13	-	125.7 (C)	-	-	-
14	6.25 (1H, s)	111.1 (CH)	C-12, C-13, C-16	H-15	H-15
15	7.34 (1H, s)	142.9 (CH)	C-13, C-14, C-16	H-14	H-14, H-16
16	7.19 (1H, s)	138.9 (CH)	C-12, C-13, C-14, C-15		H-15
17A	4.88 (1H, brs)	106.9 (CH <sub>2</sub> )	C-8, C-7, C-9	H-17B	H-17B, H-7 $\alpha$
17B	4.58 (1H, brs)		C-8, C-7, C-9	H-17A	H-17A, H-12A, H-11A, 3H-20
18	0.98 (3H, s)	28.50 (CH <sub>3</sub> )	C-3, C-4, C-5, C-19	-	H-3, H-6 $\beta$ , H-5
19	0.77 (3H, s)	15.60 (CH <sub>3</sub> )	C-3, C-4, C-5, C-18	-	3H-20
20	0.69 (3H, s)	14.7 (CH <sub>3</sub> )	C-1, C-9, C-10	-	H-1 $\alpha$ , H-2 $\alpha$ , H-7 $\alpha$ , H-17B, H-11A, 3H-19

\* Not assigned as  $\alpha$  or  $\beta$  due to free rotation of the side chain.

**6.1.2: Structural elucidation of compound 6.2: *ent*-15,16-epoxylabda-8(17),13(16),14-trien-3-one.**



Compound 6.2

Compound 6.2 was found to be the 3-keto derivative of compound 6.1. The high resolution mass spectrum of compound 6.2 showed a molecular ion peak at  $m/z$  300.2089, which corresponds to a molecular formula of  $C_{20}H_{28}O_2$ , consistent with a double bond equivalence of seven. The FTIR spectrum showed a carbonyl stretch band at  $1703\text{ cm}^{-1}$ , typical of a ketone absorption band.<sup>1,2</sup> No hydroxyl group stretch was present.

Compound 6.2 was previously reported as a synthetic derivative synthesized from the compound tectograndinol which was isolated from *Tectona grandis* (Verbenaceae).<sup>5</sup> Tectograndinol was oxidized using  $MnO_2$  to form the butenolide shown in Figure 6.3 as the main product. Reaction of this compound with Jones reagent yielded compound 6.2. Only NMR resonances of the furan ring protons of compound 6.2 are reported in the literature.<sup>5,6</sup>

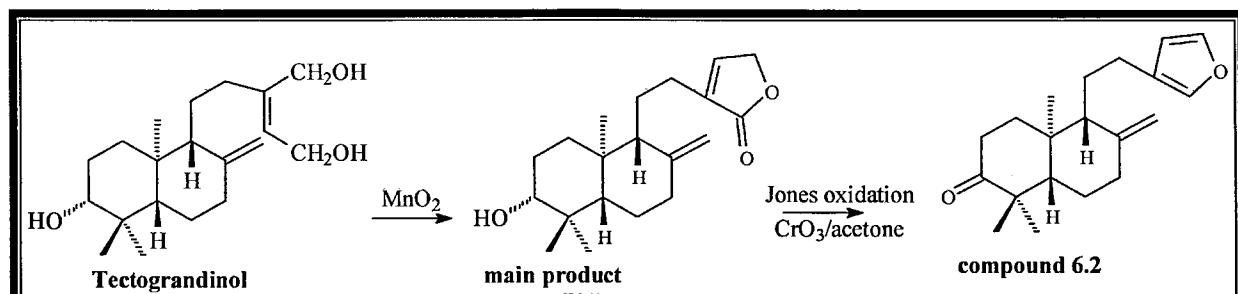


Figure 6.3: Synthesis of *ent*-15,16-epoxylabda-8(17),13(16),14 trien-3-one.

The  $^{13}C$  NMR spectrum of compound 6.2, differed from that of compound 6.1 in that the resonance ascribed to C-3 occurring at  $\delta$  79.0 in compound 6.1 was missing and a ketone carbonyl carbon resonance was present at  $\delta$  217.0 in compound 6.2. This resonance showed

correlations in the HMBC spectrum with the resonances at  $\delta$  1.08 and  $\delta$  1.02 which were ascribed to 3H-18, 3H-19, and the H-5 methine resonance at  $\delta$  1.58.

Assignment of all  $^1\text{H}$  and  $^{13}\text{C}$  NMR resonances was done in a same manner to that of compound 6.1. As with compound 6.2 the negative optical rotation of - 13.7° indicated that compound 6.2 belonged to the *ent*-series of the labdane. Compound 6.2 was found to be *ent*-15, 16-epoxy-labd-8(17), 13(16), 14 - trien-3-one. This is the first report of this compound from a natural source.

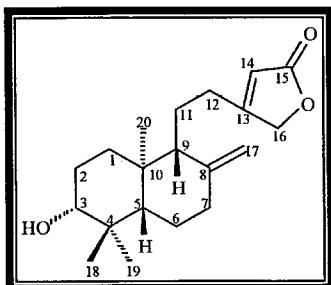
**Table 6.2: NMR data of compound 6.2: *ent*-15,16-epoxylabda-8(17),13(16),14-trien-3-one in  $\text{CDCl}_3$ .**

no	$^1\text{H}$ NMR (500 MHz)	$^{13}\text{C}$ NMR (125 MHz)	HMBC (H $\rightarrow$ C)	COSY	NOESY
1 $\alpha$	2.01 (1H, m)	37.8 (CH <sub>2</sub> )	C-3, C-2, C-9, C-20	H-2 $\alpha$	3H-20, H-2 $\alpha$
1 $\beta$	1.50 (1H, m)		C-2, C-3, C-5, C-9	H-2 $\alpha$	H-2 $\beta$
2 $\alpha$	2.60 (1H, m)	34.9 (CH <sub>2</sub> )	C-1, C-3	H-1 $\beta$ , H-2 $\beta$	H-2 $\beta$ , 3H-20
2 $\beta$	2.38 (1H, m)		C-3	H-2 $\beta$ , H- $\alpha$ , H-1 $\beta$	H-2 $\alpha$
3	-	217.0 (C)	-	-	-
4	-	47.9 (C)	-	-	-
5	1.58 (1H, m)	55.3 (CH)	C-6, C-7, C-9, C-10, C-18, C-19, C-20	H-6 $\beta$	H-6 $\beta$ , H-9, 3H-18
6 $\alpha$	1.51 (1H, m)	25.3 (CH <sub>2</sub> )	C-5, C-10	H-6 $\beta$	
6 $\beta$	1.69 (1H, m)		C-5, C-7, C-8, C-10	H-6 $\alpha$ , H-5	H-5
7 $\alpha$	2.46 (1H, m)	38.1 (CH <sub>2</sub> )	C-6, C-8, C-9, C-17	H-7 $\beta$	H-7 $\beta$
7 $\beta$	1.99 (1H, m)		C-6, C-8, C-9, C-17	H-6 $\alpha$ , H-6 $\beta$ , H-7 $\alpha$	H-7 $\alpha$
8	-	147.3 (C)	-	-	-
9	1.69 (1H, m)	55.1 (CH)	C-1, C-5, C-8, C-10,C-12, C-13, C-17	H-20 (w)	H-5, H-17A, 3H-18
10	-	39.4 (C)	-	-	-
11A	1.68 (1H, m)*	24.8 (CH <sub>2</sub> )	C-8, C-9,C-10, C-12, C-20	H-12A	H-12A, H-12B, 3H-20
11B	1.68 (1H, m)*		C-8, C-9,C-10, C-12, C-20	H-12A, H-12B	H-12A, H-12B, 3H-20
12A	2.58 (1H, m)*	23.7 (CH <sub>2</sub> )	C-8, C-9, C-11, C-13, C-14, C-16	H-12B, H-11B	3H-20
12B	2.26 (1H, m)*		C-8, C-9, C-13, C-14, C-16	H-12A, H-11A	3H-20
13	-	125.4 (C)	-	-	-
14	6.25 (1H, s)	111.1 (CH)	C-11, C-13, C-15, C-16	H-15	
15	7.35 (1H, s)	143.0 (CH)	C-13, C-14, C-16	H-14, H-16 (w)	H-16
16	7.20 (1H, s)	139.0 (CH)	C-13, C-14, C-15	H-15	H-15
17A	4.65 (1H, brs)	107.7 (CH <sub>2</sub> )	C-7, C-8, C-9	H-17B, H-9	H-17B, H-12A
17B	4.95 (1H, brs)		C-7, C-8, C-9	H-17A	H-17A, H-7, H-9
18	1.08 (3H, s)	26.2 (CH <sub>3</sub> )	C-3, C-4, C-5, C-19	-	H-5, H-9
19	1.02 (3H, s)	21.9 (CH <sub>3</sub> )	C-3, C-4, C-5, C-18	-	3H-20, H-18
20	0.87 (3H, s)	14.3 (CH <sub>3</sub> )	C-1, C-9, C-10, C-17	-	3H-19, H-1 $\alpha$ , H-11 $\alpha$ , H-2 $\alpha$

\* Resonances superimposed at  $\delta$  1.68 ppm

\* Not assigned as  $\alpha$  or  $\beta$  due to free rotation of the side chain.

### 6.1.3: Structural elucidation of compound 6.3: *ent*-3 $\beta$ -hydroxylabda-8(17),13-dien-15,16-olide



Compound 6.3

Compound 6.3 was found to differ from compound 6.1 only in the structure of the side chain, which occurred as a butenolide ring instead of the furan ring found in compounds 6.1 and 6.2. The high resolution mass spectrum gave a molecular ion peak at *m/z* 318.2185, indicating a molecular formula of C<sub>20</sub>H<sub>30</sub>O<sub>3</sub>. A double bond equivalence of six was calculated. The IR spectrum showed an absorption band at 3282 cm<sup>-1</sup> consistent with a hydroxyl stretch and a carbonyl stretch at 1736 cm<sup>-1</sup> consistent with an  $\alpha$ ,  $\beta$ -unsaturated butenolide.<sup>1,2</sup>

The <sup>1</sup>H NMR spectrum showed a resonance at  $\delta$  3.25 (dd, *J* = 11.73, *J* = 4.53 Hz) corresponding to the H-3 $\beta$  resonance, as in compound 6.1. The corresponding carbon resonance occurred at  $\delta$  78.8. The C-3 resonance showed correlations with the two methyl group proton resonances at  $\delta$  0.99 and  $\delta$  0.78 which were assigned as 3H-18 and 3H-19 in the HMBC spectrum. A further correlation was observed in the HMBC spectrum between the C-3 resonance and H-5 methine proton resonance at  $\delta$  1.07.

The NMR spectra showed the presence of an  $\alpha$ ,  $\beta$ -unsaturated butenolide ring. An  $\alpha$ ,  $\beta$ -unsaturated carbonyl carbon resonance occurred at  $\delta$  174.6 in the <sup>13</sup>C NMR spectrum and could be ascribed to C-15. A singlet ascribed to H-14 ( $\delta$  5.83) showed long range coupling with the two H-16 oxymethylene proton resonances which occurred as a non-first order pair of doublets at  $\delta$  4.71 and  $\delta$  4.61. The corresponding C-16 methylene carbon resonance at  $\delta$  73.3 showed correlations in the HMBC spectrum with the H-14 resonance and the two H-12 methylene proton resonances ( $\delta$  2.54,  $\delta$  2.25). The two H-12 resonances showed correlations in the HMBC

spectrum with the C-14 ( $\delta$  115.4) carbon resonance and a further alkene carbon resonance at  $\delta$  171.1 which was ascribed to C-13. In addition, they showed a correlation with a methine carbon resonance at  $\delta$  56.1 which was ascribed to C-9. The two H-12 resonances showed coupling in the COSY spectrum to two H-11 methylene proton resonances at  $\delta$  1.74 and  $\delta$  1.65. These resonances were, in turn, seen to be coupled to the H-9 proton resonance at  $\delta$  1.59. The corresponding C-9 carbon resonance showed correlations in the HMBC spectrum with the two non-equivalent H-17 protons of an exocyclic methylene group ( $\delta$  4.89,  $\delta$  4.46).

Compound 6.3 gave a negative optical rotation value of  $-33.17^\circ$ , confirming that it was an *ent*-labdane. The NOESY spectrum showed a correlation between the H- $5\beta$  resonance and the H-3 resonance establishing its stereochemistry as  $\beta$ , and the hydroxy group was assigned as  $\alpha$  or equatorial. Compound 6.3 was identified as *ent*-3 $\alpha$ -hydroxylabd-8(17)-13-dien-15, 16-olide. This compound has not been reported previously.

Butenolide labdanes belonging to the normal series have previously been isolated from other sources. Pinusolidol has been isolated from the leaf extract of *Biota orientalis* (Cupressaceae family) and has a methyl ester at C-18, with an optical rotation value of +58.5. It was found to show platelet-activating factor (PAF) receptor binding antagonist activity.<sup>7</sup> 15-Methoxypinusolidic acid, was isolated from the same source and showed significant protective activity against glutamate neurotoxicity in primary cultures of rat cortical cells.<sup>8</sup>

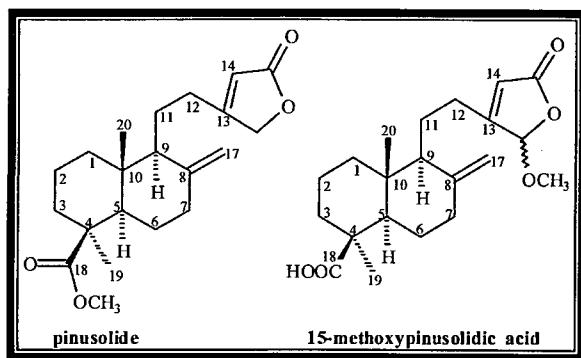


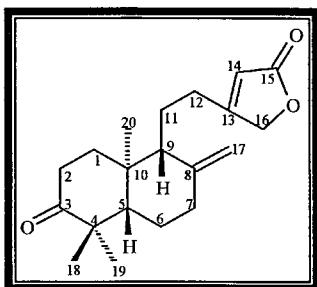
Figure 6.4: Structure of but-13-en-15,16-olide labdanes<sup>7,8</sup>

**Table 6.3: NMR data of compound 6.3: *ent*-3 $\beta$ -hydroxy labd-8(17),13-dien-15,16-olide in CDCl<sub>3</sub>**

No	<sup>1</sup> H NMR (500 MHz)	<sup>13</sup> C NMR (125 MHz)	HMBC (H→C)	COSY	NOESY
1 $\alpha$	1.76 (1H, m)	37.3 (CH <sub>2</sub> )	C-3, C-5, C-9, C-10, C-3, C-2, C-9, C-10, C-20	H-1 $\beta$ , H-1 $\alpha$	3H-20
1 $\beta$	1.14 (1H, m)			H-1	H-3
2 $\alpha$	1.59 (1H, m)	27.9 CH <sub>2</sub> )	C-3, C-10	H-2 $\beta$	3H-19, 3H-20
2 $\beta$	1.71 (1H, m)		C-3, C-9	H-2 $\alpha$	
3	3.25 (dd, <i>J</i> = 11.73, 4.53 Hz)	78.8 (CH)	C-1, C-4, C-18, C-19	2H-2	H-1 $\beta$ , H-5, 3H-18
4	-	39.3 (C)	-	-	-
5	1.07 (1H, m)	54.7 (CH)	C-3, C-4, C-6, C-9, C-18, C-19	H-6 $\beta$	H-3, 3H-18, H-7 $\beta$ , H-6 $\beta$ , H-9
6 $\alpha$	1.38 (1H, m)	24.2 (CH <sub>2</sub> )	C-5, C-7, C-8	H-7 $\beta$	H-6 $\beta$ , 3H-19, 3H-20
6 $\beta$	1.75 (1H, m)		C-5, C-7, C-8	H-7, H-5	H-5, H-6 $\alpha$ , 3H-18
7 $\alpha$	2.42 (1H, m)	38.2 (CH <sub>2</sub> )	C-5, C-6, C-8, C-17	H-17	H-6 $\alpha$ , H-7 $\beta$ , H-17A
7 $\beta$	1.95 (1H, m)		C-5, C-6, C-8, C-17		H-5, H-7 $\alpha$
8	-	147.4 (C)	-	-	-
9	1.59 (1H, m)	56.0 (CH)	C-1, C-4, C-5, C-8, C-10, C-11, C-12, C-17, C-20	2H-11	H-5
10	-	39.7 (C)	-	-	-
11A	1.74 (1H, m)*	21.6 (CH <sub>2</sub> )	C-7, C-8, C-9, C-12, C-13	2H-12, H-9	H-14, H-20
11B	1.65 (1H, m)*		C-8, C-9, C-12, C-13	H-12, H-9	H-17B, 3H-20, H-12B
12A	2.54 (1H, m)*	27.6 (CH <sub>2</sub> )	C-9, C-11, C-13	2H-11A	H-17B
12B	2.25 (1H, m)*		C-9, C-11, C-13, C-14, C-16	2H-11B	H-12A, H-11B
13	-	171.1 (C)	-	-	-
14	5.83 (1H, t, <i>J</i> = 1.55 Hz)	115.4 (CH)	C-12, C-13, C-15, C-16	H-16A	H-16, H-11A
15	-	174.5 (C)	-	-	-
16A	4.71 (1H, d <i>J</i> =17.40 Hz)*	73.3 (CH <sub>2</sub> )	C-13, C-14, C-15	H-14, H-15	H-16B, H-14
16B	4.69 (1H, d, <i>J</i> =17.40)*		C-13, C-14, C-15		H-16A
17A	4.89 (1H, brs)	107.1 (CH <sub>2</sub> )	C-7, C-8, C-9	H-17B	H-17B, H-7 $\alpha$
17B	4.46 (1H, brs)		C-7, C-8, C-9	H-17A	H-17A, H-12 $\alpha$ , H-11A
18	0.99 (3H, s)	28.5 (CH <sub>3</sub> )	C-3, C-4, C-5, C-19	-	H-3, H-5, H-6 $\beta$ , 3H-19
19	0.78 (3H, s)	15.6 (CH <sub>3</sub> )	C-3, C-4, C-18	-	H-6 $\alpha$ , H-2 $\alpha$ , 3H-18, 3H-20,
20	0.69 (3H, s)	14.6 (CH <sub>3</sub> )	C-1, C-5, C-9, C-10	-	H-2 $\alpha$ , H-6 $\alpha$ , 3H-19, H11A

\* Not assigned as  $\alpha$  or  $\beta$  due to free rotation of the side chain.

#### 6.1.4: Structural elucidation of compound 6.4: *ent*-3-oxo-labda-8(17),13-dien-15, 16-oxide



Compound 6.4

The high resolution mass spectrum of compound 6.4, isolated as needle-like crystals, gave a molecular ion peak at  $m/z$  316.2038, which corresponded to a molecular formula of  $C_{20}H_{28}O_3$ . The number of double bond equivalents was found to be seven. Two double bonds, a ketonic carbonyl group and a lactone carbonyl carbon resonance seen in the  $^{13}C$  NMR spectrum, accounted for four double bond equivalents implying the molecule was tricyclic. Compound 6.4 was found to be the 3-keto derivative of compound 6.3, and was previously isolated from representatives of the *Sphaeranthus* group by Zdero *et al.*<sup>9</sup> These authors determined the structure using  $^1H$  NMR, MS and IR techniques, and the compound was assigned as belonging to the *ent*-series based on circular dichroism analysis which gave a negative Cotton effect at 288 nm.<sup>11</sup>

The IR spectrum showed a carbonyl stretch at  $1745\text{ cm}^{-1}$  suggesting the presence of a lactone carbonyl stretch of a butenolide, and a ketone carbonyl group was indicated by the stretching band observed at  $1702\text{ cm}^{-1}$ . No hydroxyl absorption band was present.

The hydroxyl group of compound 6.3 was replaced by a ketone at C-3 in compound 6.4. The keto group was placed at C-3 due to the correlations observed in the HMBC spectrum between the resonance at  $\delta$  216.4 and the 3H-18 and 3H-19 methyl group proton resonances at  $\delta$  1.08 and  $\delta$  1.01, and with the H-5 methine proton resonance at  $\delta$  1.70. The  $^{13}C$  NMR spectrum showed resonances at  $\delta$  115.4,  $\delta$  170.7,  $\delta$  174.2 and  $\delta$  73.2 which were assigned to C-13, C-14, C-15 and C-16 of the butenolide ring. The C-14 resonance showed correlations in the HMBC spectrum with the oxymethylene proton resonances at  $\delta$  4.71 and  $\delta$  4.70, which were ascribed to 2H-16

and with the two H-12 methylene proton resonances at  $\delta$  2.56 and  $\delta$  2.25. Further  $^1\text{H}$ ,  $^{13}\text{C}$  NMR, HMBC and COSY correlations were done as in compound **6.3**. The optical rotation value of -40.59 was determined and confirmed that the compound belonged to the *ent*-series.

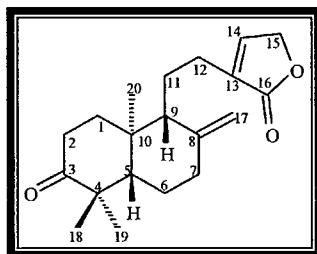
**Table 6.4: NMR data of compound 6.4: *ent*-3-oxolabd-8(17),13-dien-15, 16-olide in  $\text{CDCl}_3$**

No	$^1\text{H}$ NMR (500 MHz)	$^{13}\text{C}$ NMR (125 MHz)	HMBC ( $\text{H} \rightarrow \text{C}$ )	COSY	NOESY	$^1\text{H}$ NMR (400 MHz) <sup>a</sup> in $\text{CDCl}_3$
1 $\alpha$	2.01 (1H, m)	37.9 (CH <sub>2</sub> )	C-3, C-5, C-9, C-10	H-2 $\alpha$ , H-2 $\beta$ , H-1 $\beta$	3H-20, H-2 $\alpha$	2.03 ddd
1 $\beta$	1.54 (1H, m)		C-3, C-5, C-10, C-20	H-2 $\alpha$ , H-2 $\beta$ , H-1 $\alpha$	H-2 $\beta$	1.45 dd
2 $\alpha$	2.64 (1H, m)	34.7 (CH <sub>2</sub> )	C-1, C-3, C-10	H-2 $\beta$ , H-1 $\alpha$ , H-1 $\beta$	3H-20, 3H-19, H-1 $\alpha$	2.65 ddd
2 $\beta$	2.40 (1H, m)		C-3, C-5, C-9	H-2 $\alpha$ , H-1 $\alpha$ , H-1 $\beta$	H-1 $\beta$	2.42 ddd
3	-	216.4 (C)	-	-	-	-
4	-	47.9 (C)	-	-	-	-
5	1.70 (1H, m)	55.4 (CH)	C-9, C-10, C-20	H-6	H-9, 3H-18	1.60 dd
6 $\alpha$	1.50 (1H, m)	25.2 (CH <sub>2</sub> )	C-5, C-7, C-8	H-5, H-6 $\beta$ , H-7 $\alpha$ , H-7 $\beta$	H-7 $\alpha$ , 3H-19, 3H-20	1.51 dq
6 $\beta$	1.68 (1H, m)		C-8, C-10	H-5, H-6 $\alpha$ , H-7 $\alpha$ , H-7 $\beta$	-	1.78 br d
7 $\alpha$	2.47 (1H, m)	37.7 (CH <sub>2</sub> )	C-5, C-8, C-9, C-17	H-7 $\beta$ , H-6 $\alpha$ , H-6 $\beta$	H-17A, H-6 $\alpha$	2.48 ddd
7 $\beta$	2.04 (1H, m)		C-5, C-9, C-17	H-7 $\alpha$ , H-6 $\alpha$ , H-6 $\beta$		2.00 br dt
8	-	146.7 (C)	-	-	-	-
9	1.58 (1H, m)	55.2 (CH)	C-1, C-5, C-10, C-20	H-11 $\alpha$ , H-11 $\beta$	H-5, 3H-18	1.78 m
10	-	39.5 (C)	-	-	-	-
11A	1.72 (1H, m)*	21.9 (CH <sub>2</sub> )	C-8, C-9, C-10, C-12, C-13	H-11B, H-12A, H-12B	H-12A, 3H-20	1.78
11B	1.72 (1H, m)*			H-11B, 12A, 12B	H-12A, 3H-20	1.78
12A	2.56 (1H, m)*	27.6 (CH <sub>2</sub> )	C-9, C-11, C-14	H-11A, H-11B, H-12B	H-11A	2.58 dddd
12B	2.25 (1H, m)*		C-9, C-11, C-13, C-14, C-16	H-11A, H-11B, H-12A	H-11A	2.27 br dt
13	-	170.7 (C)	-	-	-	-
14	5.82 (1H, t, $J = 1.55$ Hz)	115.4 (CH)	C-12, C-13, C-15, C-16	H-14 $\alpha$ , H-14 $\beta$	H-16	5.85 tt
15	-	174.2 (C)	-	-	-	-
16A	4.71 (1H, d, $J=17.40$ Hz)*	73.2 (CH <sub>2</sub> )	C-14, C-15	H-14	H-16B, H-14	4.75 dd
16B	4.70 (1H, d, $J=17.40$ Hz)*		C-14, C-15	H-14	H-16A, H-14	4.69 dd
17A	4.95 (1H, brs)	107.9 (CH <sub>2</sub> )	C-7, C-8, C-9	H-17A	H-17B, H-7A	4.97 dt
17B	4.53 (1H, brs)		C-7, C-8, C-9	H-17B	H-17A, 3H-20, H-11	4.54 br s
18	1.08 (3H, s)	26.2 (CH <sub>3</sub> )	C-3, C-4, C-5, C-19	-	H-5, H-9	1.11 s
19	1.01 (3H, s)	21.9 (CH <sub>3</sub> )	C-3, C-4, C-5, C-18	-	3H-20, H-2, H-6	1.03 s
20	0.87 (3H, s)	14.1 (CH <sub>3</sub> )	C-5, C-9, C-10	-	H-2 $\alpha$ , 3H-19, H-11A	0.90 s

Non-first order \*

\* Not assigned as  $\alpha$  or  $\beta$  due to free rotation of the side chain.

### 6.1.5: Structural elucidation of compound 6.5: *ent*-3-oxo-labd-8(17),13-dien-16, 15-olide



Compound 6.5

The high resolution mass spectrum of compound 6.5 showed a molecular ion peak at  $m/z$  316.2038, which is consistent with a molecular formula of  $C_{20}H_{28}O_3$ . The IR spectrum showed the presence of a carbonyl stretch at  $1750\text{ cm}^{-1}$ , suggesting the presence of a lactone carbonyl group and a typical ketone carbonyl stretch was seen at  $1701\text{ cm}^{-1}$ .<sup>1,2</sup> No hydroxyl group absorption band was present. Compound 6.5 was also isolated previously from the *Sphaeranthus* group, and the structure was determined by using  $^1\text{H}$  NMR, IR, mass spectrometry and circular dichroism measurements.<sup>9</sup>

$^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy showed that rings A and B were the same for compound 6.4 and 6.5, however the two compounds differed in that compound 6.4 had a 15,16-butenolide ring and compound 6.5 had the 16,15-butenolide ring.

The  $^{13}\text{C}$  NMR spectrum showed a carbonyl carbon resonance at  $\delta$  174.5 which was ascribed to C-16, and was seen to correlate in the HMBC spectrum with the methine H-14 proton resonance at  $\delta$  7.10 ( $t, J = 1.35\text{ Hz}$ ), and with the two superimposed H-15 oxymethylene proton resonances at  $\delta$  4.76. The COSY spectrum showed coupling between the H-14 methine proton resonance and the 2H-15 methylene proton resonances, previously assigned. The HMBC spectrum showed correlation between the C-14 and two H-12 methylene proton resonances at  $\delta$  2.48 and  $\delta$  2.13 in the HMBC spectrum.

The negative optical rotation value of  $-5.86^\circ$  confirmed that compound 6.5 belongs to the *ent* series.

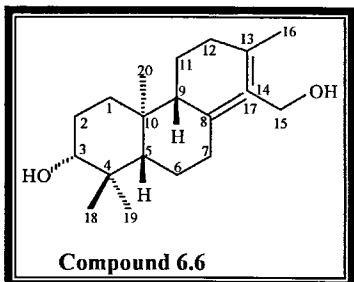
**Table 6.5: NMR data of compound 6.5: *ent*-3-oxo-labd-8(17),13-en-16,15-olide ( $\text{CDCl}_3$ ) and literature values ( $\text{CDCl}_3$ )**

No	$^1\text{H}$ NMR (500 MHz)	$^{13}\text{C}$ NMR (125 MHz)	HMBC (H → C)	COSY	NOESY	$^1\text{H}$ NMR (400 MHz) $\text{CDCl}_3$
1α	2.02 (1H, m)	37.8 ( $\text{CH}_2$ )	C-1, C-3, C-5, C-9, C-10, C-20	H-1β, H-2α, H-2β	H-2α, 3H-20,	2.04 ddd
1β	1.54 (1H, m)		C-2, C-3, C-5, C-9, C-10, C-20	H-1α, H-2α, H-2β	H-2β	1.57 m
2α	2.60 (1H, m)	34.9 ( $\text{CH}_2$ )	C-1, C-3, C-10	H-2β, H-1α, H-1β	H-1α, 3H-19, 3H-20,	2.62 ddd
2β	2.38 (1H, m)		C-1, C-3, C-10	H-2α, H-1α, H-1β	H-1β	2.39 ddd
3	-	216.9 (C)	-	-	-	-
4	-	48.0 (C)	-	-	-	-
5	1.58 (1H, m)	55.3 ( $\text{CH}$ )	C-1, C-3, C-7, C-9, C-10, C-20	H-6α, H-6β	3H-18	1.57 dd
6α	1.50 (1H, m)	25.3 ( $\text{CH}_2$ )	C-4, C-5, C-7, C-8, C-17	H-5, H-6β, H-7α, H-7β	3H-20	1.51 dq
6β	1.68 (1H, m)		C-3, C-5, C-10, C-20	H-5, H-6α, H-7α, H-7β	H-5	1.75 br d
7α	2.45 (1H, m)	38.0 ( $\text{CH}_2$ )	C-5, C-6, C-8, C-9, C-17	H-6α, H-6β, H-7β	H-7β	2.45 ddd
7β	2.00 (1H, m)		C-5, C-8, C-17	H-6α, H-6β, H-7α	H-7α	2.01 br dt
8	-	146.8 (C)	-	-	-	-
9	1.67 (1H, m)	55.6 ( $\text{CH}$ )	C-5, C-8, C-10, C-17, C-20	H-11A, H-11B	3H-18	1.70 m
10	-	39.5 (C)	-	-	-	-
11A	1.74 (1H, m)*	22.4 ( $\text{CH}_2$ )	C-8, C-9, C-13, C-14, C-16	H-9, H-11B, H-12A, H-12B		1.70 m
11B	1.68 (1H, m)*			H-9, H-11A, H-12A, H-12B		1.70 m
12A	2.48 (1H, m)*	24.8 ( $\text{CH}_2$ )	C-8, C-9, C-16, C-17	H-12B, H-11A, H-11B, H-14, H-15	H-12A, 3H-20	1.70 m
12B	2.13 (1H, m)*		C-9, C-16, C-17	H-12A, H-11A, H-11B, H-14, H-15	H-11A	2.15 m
13	-	134.8 (C)	-	-	-	-
14	7.10 ( $t, J = 1.35$ Hz)	144.3 ( $\text{CH}$ )	C-12, C-13, C-15, C-16	H-15A, H-15B, H-15	H-15	7.11 tt
15A	4.76 *	70.3 ( $\text{CH}_2$ )	C-13, C-14, C-16	H-15B,	H-14, H-15B	
15B	4.76 *		C-13, C-14, C-16	H-15A, H-14	H-14, H-15A	4.78 dt
16	-	174.5 (C)	-	-	-	-
17A	4.94 (1H, s)	108.1 ( $\text{CH}_2$ )	C-7, C-8, C-9, 12 (w)	H-17B	H-7A, H-17B	4.96 dt
17B	4.67 (1H, s)		C-7, C-8, C-9, 12 (w)	H-17A	H-17B	4.69 br s
18	1.08 (3H, s)	26.2 ( $\text{CH}_3$ )	C-3, C-4, C-5, C-19	-	H-5, H-9	1.10 s
19	1.00 (3H, s)	21.9 ( $\text{CH}_3$ )	C-3, C-5, C-18	-	H-2α, 3H-20	1.03 s
20	0.85 (3H, s)	14.2 ( $\text{CH}_3$ )	C-1, C-5, C-9, C-10	-	H-2α, H-11A, 3H-19	0.88 s

\*Resonance, superimposed at δ 4.76

\* Not assigned as α or β due to free rotation of the side chain.

### 6.1.6: Structural elucidation of compound 6.6: *ent*-labda 8(17),13*E*-diene-3 $\beta$ ,15-diol



Compound 6.6

The needle-like crystals of compound 6.6 gave a molecular ion peak at  $m/z$  306 from the low resolution mass spectrum, which was consistent with a molecular formula of C<sub>20</sub>H<sub>34</sub>O<sub>2</sub>. A double bond equivalence of four was calculated. The FTIR spectrum showed an OH absorption band at 3450 cm<sup>-1</sup>.

The <sup>1</sup>H NMR spectrum showed proton resonances of an exocyclic double bond at  $\delta$  4.84 and  $\delta$  4.53 which were seen to correspond with the C-17 carbon resonance ( $\delta$  106.7) in the HSQC spectrum. The C-17 carbon resonance showed correlations in the HMBC spectrum with the H-9 methine proton resonance ( $\delta$  1.55) and the two H-7 proton resonances ( $\delta$  2.40,  $\delta$  1.95). The COSY spectrum showed coupling between the 2H-7 proton resonance and two H-6 proton resonances ( $\delta$  1.74, 1.38), which, in turn, showed coupling with the H-5 methine proton resonance at  $\delta$  1.07. The C-5 carbon resonance  $\delta$  54.7 showed correlations in the HMBC spectrum with the 3H-18 and 3H-19 methyl group proton resonances at  $\delta$  0.98 and  $\delta$  0.77, and with the H-3 methine proton resonance (dd,  $J$  = 11.75, 4.35 Hz). The H-3 resonance showed a correlation in the NOESY spectrum with the H-5 resonance. However, compound 6.7 gave an optical rotation of +24.2°, whereas all the previously isolated compounds from this source gave a negative optical rotation. A literature search showed that a compound with the same <sup>13</sup>C NMR data was isolated from *Excoecaria agallocha* Linn (Euphorbiaceae family). An optical rotation of -24.6° and the coupling constant of  $\delta$  3.25 dd ( $J$ =5, 11 Hz) representing the H-3 $\beta$ , was reported and the compound was identified as *ent*-labda-8(17), 13*E*-diene-3 $\alpha$ ,15-diol.<sup>10</sup>

**Table 6.6: NMR data of compound 6.6: *ent*-labda 8(17),13*E*-diene-3 $\beta$ ,15-diol (CDCl<sub>3</sub>) and literature values (CDCl<sub>3</sub>)**

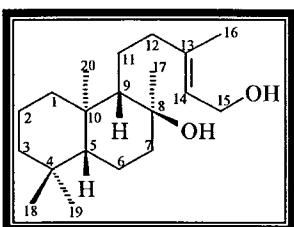
no	<sup>1</sup> H NMR (500 MHz)	<sup>13</sup> C NMR (125 MHz)	HMBC (H $\rightarrow$ C)	COSY	<sup>13</sup> C NMR (CDCl <sub>3</sub> ) <sup>10</sup>
1 $\alpha$	1.79 (1H, m) 1.16 (1H, m)	37.3 (CH <sub>2</sub> ) C-20	C-2, C-5, C-9, C-11 C-20	H-1 $\beta$ H-1 $\alpha$ , H-2 $\beta$ H-2 $\beta$	H-1 $\beta$ , H-2 $\alpha$ , 3H-20, 37.1 (CH <sub>2</sub> )
1 $\beta$	1.59 (1H, m)	28.1 (CH <sub>2</sub> )	C-3, C-4	H-1 $\alpha$ , H-5 H-1 $\alpha$	27.9 (CH <sub>2</sub> )
2 $\alpha$	1.70 (1H, m)		C-3	H-3, H-1 $\beta$ , H-2 $\alpha$	
3	3.25 (dd, <i>J</i> = 11.75, 4.35 Hz)	79.1 (C)	C-2, C-18, C-19	H-2 $\beta$	H-1 $\beta$ , H-5, 3H-18
4	-	39.3 (C)	-	-	78.8 (CH)
5	1.07 (1H, d, <i>J</i> =2.35, 12.40 Hz)	54.8 (CH)	C-2, C-4, C-7, C-9, C-19, C-20	H-6 $\beta$	39.1
6 $\alpha$	1.38 (1H, m)	24.2 (CH <sub>2</sub> )	C-4, C-5, C-7 C-5, C-7	H-7 $\beta$ H-7 $\alpha$ , H-5	H-3, H-9, H-1 $\beta$ 54.7 (CH)
6 $\beta$	1.74 (1H, m)			3H-20 3H-18	24.0 (CH <sub>2</sub> )
7 $\alpha$	2.40 (1H, m)	38.4 (CH <sub>2</sub> )	C-5, C-6, C-9, C-17 C-5, C-6, C-8, C-17	H-6 $\beta$ , H-7 $\beta$ H-6 $\beta$ , H-6 $\alpha$ , H-7 $\beta$	H-7 $\beta$ , H-17B 38.3 (CH <sub>2</sub> )
7 $\beta$	1.95 (1H, m)		-	-	H-7 $\alpha$
8	-	148.1 (C)	-	-	147.0 (C)
9	1.55 (1H, m)	56.2 (CH)	C-1, C-5, C-8, C-11, C-12, C-17, C-20	H-11A	56.1 (CH)
10	-	39.6 (C)	-	-	39.4 (C)
11A	1.58 (1H, m)*	22.1 (CH <sub>2</sub> )	C-8, C-9, C-12, C-17 C-8, C-9, C-12	H-12A, H-12B, H-9 H-12A, H-12B	3H-20 22.0 (CH <sub>2</sub> )
11B	1.46 (1H, m)*			H-17A	
12A	2.15 (1H, m)*	38.6 (CH <sub>2</sub> )	C-11, C-13, C-14 C-8, C-9	H-11A, H-11B, H-12B H-11A, H-11B, H-12A	H-12B H-12A
12B	1.81 (1H, m)*				38.6 (CH <sub>2</sub> )
13	-	140.7 (C)	-	-	140.0 (C)
14	5.37 (1H, t, <i>J</i> =6.02 Hz)	123.3 (CH)	C-12, C-15, C-16	H-15	123.2 (CH)
15A	4.15 (1H, s)	59.6 (CH <sub>2</sub> )	C-12 (w), C-13, C-14 C-12 (w), C-13, C-14	H-14, H-15B H-14, H-15A	H-14, H-15B H-14, H-15A
15B	4.15 (1H, s)		C-11(weak), C-12, C-13, C-14	-	
16	1.66 (3H, s)	16.6 (CH <sub>3</sub> )	C-11 (C-16)	-	16.3 (CH <sub>3</sub> )
17A	4.84 (1H, s)	106.9 (CH <sub>2</sub> )	C-7, C-9	H-17B	H-17B, H-11B
17B	4.53 (1H, s)		C-7, C-8, C-9	H-17A	H-17A, H-7 $\alpha$
18	0.98 (3H, s)	28.5 (CH <sub>3</sub> )	C-3, C-4, C-5, C-19	-	H-3, 3H-19
19	0.77 (3H, s)	15.6 (CH <sub>3</sub> )	C-3, C-4, C-5, C-18	-	3H-18, 3H-20
20	0.68 (3H, s)	14.7 (CH <sub>3</sub> )	C-1, C-9, C-5, C-10	-	H-1 $\alpha$ , H-2 $\alpha$ , H-6 $\alpha$ , H-11A, 3H-19

\* superimposed 4.15

\* Not assigned as  $\alpha$  or  $\beta$  due to free rotation of the side chain.

The optical rotation of +24.2 obtained for compound **6.6** is puzzling. It would appear logical to suppose that a plant would consistently produce either the *ent*- or normal series. There are two possibilities here: either the plant can produce both series (this has been reported previously by Tane *et al.* working on another Meliaceae species *Turraeanthus africanus*<sup>11</sup>) or that impurities have affected the optical rotation. The NMR spectra indicated that the compound is pure but the mass spectrum (Spectrum 21.8) showed some impurities. Thus in accordance with the compounds isolated, compound **6.6** is tentatively assigned as *ent*-labda-8(17),13*E*-3*β*,15-diol.

#### 6.1.7: Structural elucidation of compound **6.7**: *ent*-labd-13-ene-8*α*,15-diol



Compound **6.7**

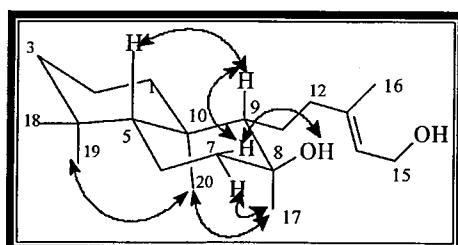
The low resolution mass spectrum of compound **6.7** gave a molecular ion peak at *m/z* 308, which corresponded to a molecular formula of C<sub>20</sub>H<sub>36</sub>O<sub>2</sub>. A double bond equivalence of three was deduced. The FTIR spectrum showed an absorption band at 3360 cm<sup>-1</sup>, characteristic of an OH group.<sup>1,2</sup>

The <sup>1</sup>H NMR spectrum showed the presence of the two H-15 methylene proton resonances at δ 4.14, which corresponded to the C-15 carbon resonance at δ 59.5 in the HSQC spectrum. The 2H-15 proton resonance showed correlations in the HMBC spectrum with the fully substituted carbon resonance C-13 (δ 141.2) and the C-14 methine resonance (δ 123.4). In addition, the C-14 carbon resonance showed a correlation with the 3H-16 methyl group proton resonance (δ 1.69) and with the two equivalent H-12 proton resonances at δ 2.09. The COSY spectrum showed coupling between the 2H-12 and the 2H-11 proton resonances (δ 1.52, δ 1.38) and, in turn, with the H-9 methine proton resonance at δ 1.05. The corresponding H-9 resonance showed correlations in the HMBC spectrum with the C-1 (δ 39.9), C-5 (δ 56.3), C-7 (δ 44.8), C-8 (δ 74.3), C-17 (δ 24.2) and C-20 (15.7) resonances.

The optical rotation of compound **6.7** was found to be  $+17.3^\circ$ . This compound is lacking a chiral centre at C-3 and has an extra one at C-8 so the optical rotation cannot be directly compared with the compounds described previously from this source. . However, based on its co-isolation with compounds **6.1-6.5**, it tentatively assumed that it belongs to the *ent*-series and the stereochemistry was assigned at C-8 based on this assumption.

The  $^1\text{H}$ NMR spectrum in  $\text{CDCl}_3$  did not show the 8-OH proton resonance and had overlapping peaks. Thus, the sample was re-run in *d*-DMSO. This enabled a thorough investigation of the NOESY spectrum to assign the stereochemistry at C-8. The 8-OH proton resonance occurred as a singlet at  $\delta$  3.80. Interestingly, the H-12 proton resonances ( $\delta$  2.09) and the H-15 ( $\delta$  4.14) proton resonances were seen to be superimposed when  $\text{CDCl}_3$  was used. However, when *d*-DMSO was used the H-12 proton resonance were resolved to give two triplets of doublets ( $\delta$  2.07 and 1.89) and the H-15 proton resonances were seen to occur as two triplets at  $\delta$  3.89 ( $J=5.35\text{ Hz}$ ) and  $\delta$  4.36 ( $J=5.35\text{ Hz}$ ).

The NOESY spectrum run in *d*-DMSO showed correlations between the 8-OH proton resonance and the H- $7\beta$  proton resonance, whereas the H- $7\alpha$  resonance was seen to correlate with the 3H-17 methyl group proton resonance, which, in turn, showed correlation with the 3H-20 methyl group proton resonance. Correlations were seen in the NOESY spectrum between the H-5 methine proton resonance and the H-9 methine proton resonance. The H-9 methine proton resonance was seen to correlate with the H- $1\beta$  and H- $7\beta$  proton resonances. Furthermore the H-9 proton resonance showed correlations to both the H- $12\beta$  and H- $12\alpha$  resonances due to the free rotation of the side chain.



**NOESY correlations for compound 6.7**

**Table 6.7: NMR data of compound 6.7: *en*-labd-13(14)-ene-8 $\alpha$ ,15-diol ( $CDCl_3$ ) and aphanamixol ( $CDCl_3$ )<sup>13</sup>**

no	$^1H$ NMR (500 MHz)	$^{13}C$ NMR (125 MHz)	HMBC (H $\rightarrow$ C)	COSY	$^{13}C$ (CDCl <sub>3</sub> ) <sup>13</sup>	NMR	$^1H$ NMR (500 MHz) in DMSO	NOESY in DMSO
1 $\alpha$	1.64 (1H, m)	39.9 (CH <sub>2</sub> )	C-4, C-5, C-20	H-1 $\beta$	39.83 (CH <sub>2</sub> )	H-18	1.55 (1H, m)	
1 $\beta$	0.95 (1H, m)		C-3, C-9, C-18, C-20	H-1 $\alpha$			0.89 (1H, m)	H-9
2 $\alpha$	1.60 (1H, m)	18.1 (CH <sub>2</sub> )	C-3, C-5	H-2 $\beta$ , H-3 $\alpha$	18.48 (CH <sub>2</sub> )		1.53 (1H, m)	
2 $\beta$	1.43 (1H, m)		-	H-2 $\alpha$			1.36 (1H, m)	
3 $\alpha$	1.37 (1H, m)	42.2 (CH <sub>2</sub> )	C-4, C-5, C-19	H-3 $\beta$	42.07 (CH <sub>2</sub> )	H-19	1.29 (1H, m)	
3 $\beta$	1.14 (1H, m)		C-2, C-4, C-18, C-19	H-2 $\alpha$ , H-3 $\alpha$			1.11 (1H, m)	
4	-	33.5 (C)	-		33.3 (C)	-	-	
5	0.92	56.3 (CH)	C-4, C-7, C-9, C-10, C-18, C-20	H-6 $\alpha$ , H-6 $\beta$	56.23 (CH)	H-9	0.81 (1H, m)	H-9
6 $\alpha$	1.64 (1H, m)	20.8 (CH <sub>2</sub> )	C-7, C-17	H-5, H-6 $\beta$ , H-7 $\alpha$	20.61 (CH <sub>2</sub> )		1.52 (1H, m)	
6 $\beta$	1.26 (1H, m)		C-7, C-17	H-5, H-6 $\alpha$			1.19 (1H, m)	
7 $\alpha$	1.86 (1H, m)	44.8 (CH <sub>2</sub> )	C-5, C-6, C-8, C-9, C-17	H-7 $\beta$ , H-6 $\alpha$	43.00 (CH <sub>2</sub> )		1.66 (1H, m)	H-17
7 $\beta$	1.38 (1H, m)			H-7 $\alpha$			1.33 (1H, m)	
8	-	74.3 (C)	-		74.35 (C)	-	-	
9	1.05 (1H, m)	61.4 (CH)	C-1, C-5, C-8, C-10, C-11, C-17, C-20	H-11 $\alpha$	61.31 (CH)	H-5, H-12B	0.96 (1H, m)	H-5, H-1 $\beta$ , 2H-12
10	-	39.6(C)	-		39.34 (C)	-	-	
11A	1.52 (1H, m)*	23.8 (CH <sub>2</sub> )	C-8, C-9, C-12	H-9, H-11B, H-12A	23.60 (CH <sub>2</sub> )		1.49 (1H, m)	H-12
11B	1.38 (1H, m)*		C-8, C-9, C-12	H-11A, H-12B			1.17 (1H, m)	
12A	2.09 (1H, m)*	43.1 (CH <sub>2</sub> )	C-9, C-11, C-13, C-14, C-16	H-11A	44.59 (CH <sub>2</sub> )	H-9, 3H-16	2.07 (1H, td, J=4, 40,	H-9
12B	2.09 (1H, m)*		C-9, C-11, C-13, C-14, C-16	H-11B		H-9, 3H-16	1.89 (1H, td, J=4, 40,	H-9
13	-	141.2 (C)	-		141.23 (C)	-	-	
14	5.43 (1H, t, J=6.00 Hz) <sup>t</sup>	123.4 (CH)	C-12, C-15, C-16	H-15	123.58 (CH)	H-15	5.21 (1H, t, J=6.03 Hz)	
15A	4.14 (1H, m)	59.5 (CH <sub>2</sub> )	C-13, C-14	H-14B	59.38 (CH <sub>2</sub> )	H-1	3.89 (1H, t, J=5.5 Hz)	
15B	4.14 (1H, m)		C-13, C-14	H-14A	59.38 (CH <sub>2</sub> )	H-14, H-16	4.36 (1H, t, J=5.20 Hz)	
16	1.69 (3H, s)	16.7 (CH <sub>3</sub> )	C-12, C-13, C-14	-	16.50 (CH <sub>3</sub> )	H-12, H-15	1.55 (3H, s)	
17	1.13 (3H, s)	24.2 (CH <sub>3</sub> )	C-7, C-8, C-9	-	23.96 (CH <sub>3</sub> )	3H-20	0.96 (3H, s)	3H-20
18	0.87 (3H, s)	33.6 (CH <sub>3</sub> )	C-2, C-3, C-4, C-5, C-19	-	33.48 (CH <sub>3</sub> )		0.81 (3H, s)	
19	0.78 (3H, s)	21.7 (CH <sub>3</sub> )	C-3, C-4, C-5	-	21.56 (CH <sub>3</sub> )		0.73 (3H, s)	3H-20
20	0.79 (3H, s)	15.7 (CH <sub>3</sub> )	C-9, C-11	-	15.52 (CH <sub>3</sub> )	H-11 $\alpha$ ,	0.70 (3H, s)	3H-17, H-7 $\alpha$
							3.800 (1H, s)	H-7 $\beta$

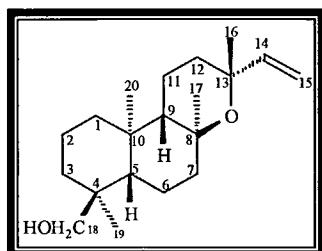
\* resonances superimposed δ 4.14

\* Not assigned as  $\alpha$  or  $\beta$  due to free rotation of the side chain.

A literature survey was undertaken for related compounds. Chandrasekharan and Chakrabortty (1968)<sup>12</sup> reported the isolation of aphanamixol with an optical rotation of -11°, but do not give the stereochemistry at C-8 nor say whether it belongs to the normal or *ent*-series. The <sup>13</sup>C NMR data given<sup>12</sup> agrees well with NMR data for compound 6.7 as shown in Table 6.7. Gonzales *et al.* (1975),<sup>13</sup> draw the structure as *ent* with a 8β-hydroxy group, but they name the compound in their text as *ent*-labd-13-ene-8α,15-diol and report an optical rotation of +0.6°. The <sup>1</sup>H NMR data of Gonzales *et al.* agrees with those obtained for compound 6.7. Caputo *et al.* (1976)<sup>14</sup> report the isolation of *ent*-8β,15-labd-E-13-ene-diol, with an optical rotation of -33 and say they identified it based on comparison against authentic sample and referred to Hugel's paper of 1966, where an optical rotation of -32° was reported.<sup>15</sup> These earlier authors did not have access to 2D NMR analyses, and, in particular, the NOESY experiment. Hugel *et al.* gave the chemical shifts of the methyl groups as occurring at δ 0.83 and δ 0.86 (methyl groups at C-4), δ 0.95 (methyl group at C-10) and δ 1.4 (methyl group at C-8), when determined in CDCl<sub>3</sub>. The corresponding resonances determined for compound 6.7 using the same solvent were δ 0.78 and δ 0.87 (3H-18 and 19 respectively), δ 0.79 (3H-20) and δ 1.13 (3H-17). The difference in shifts indicate that the compounds were probably not the same isomers, and this misassignment of structure was continued by Caputo *et al.*. This compound would be formed by addition of water across the 8,17-double bond, so it is possible that the confusion in the literature could arise because racemic mixtures have been isolated in some cases.

Assuming that in line with the other compounds isolated from the same source that compound 6.7 belonged to the *ent*- series, compound 6.7 was assigned the structure of *ent*-labd-13-ene-8β,15-diol.

#### **6.1.8: Structural elucidation of compound 6.8: *ent*-8,13-epoxy-14-labden-18-ol**



**Compound 6.8**

The low resolution mass spectrum showed a molecular ion peak at *m/z* 306 which was consistent with a molecular formula of C<sub>20</sub>H<sub>34</sub>O<sub>2</sub>, therefore indicating a double bond equivalence of four. The FTIR spectrum showed an absorption band at 3453 cm<sup>-1</sup> consistent with a primary OH group stretch.<sup>1,2</sup> Compound **6.8** was found to be *ent*-8,13-*epoxy*-14-labden-18-ol.

The <sup>1</sup>H NMR spectrum revealed four methyl group proton resonances at δ 1.13, δ 1.22, δ 0.73 and δ 0.76. The fifth methyl group had been oxidized to an oxymethylene group, which was indicated by a pair of doublets at δ 3.41 (d, *J* = 10.85 Hz) and δ 3.15 (d, *J* = 10.85 Hz) which corresponded with a resonance at δ 72.3 in the HSQC spectrum which was assigned to either C-18 or C-19 as it showed a correlation in the HMBC spectrum with the second methyl group at C-4 (δ 0.73) and with the H-5 methine proton resonance at δ 1.25.

The H-5 proton resonance showed correlations with the C-20 (δ 16.4), C-19 (δ 17.3), C-7 (δ 43.0), C-3 (δ 35.6) and C-9 (δ 58.7) resonances. The H-9 resonance (δ 1.25) showed correlations with the C-8 (δ 76.1) and C-12 (δ 35.0) resonances and showed coupling in the COSY spectrum with two H-11 proton resonances (δ 1.54, δ 1.44). The H-12 resonances showed a correlation with the C-13 epoxy carbon resonance at δ 73.5.

An optical rotation value of +7.4° was determined. The amount of sample isolated was small and the NOESY spectrum was weak. A correlation was seen between the 3H-17 and 3H-20 resonances as in compound **6.7**. The H-5 methine proton resonance was seen to correlate with the H-9 methine proton resonance, and the 3H-20 resonance was also seen to correlate with the H-1α resonance. However, no correlations were seen with the C-4 CH<sub>2</sub>OH group methylene protons. The sample was acetylated, but, again, the NOESY spectrum was not useful.

In order to ascertain whether C-18 or C-19 had been oxidised, a literature search was undertaken to look for diterpenoids with one of the methyl groups at C-4 oxidised to an alcohol. San Martin *et al.* isolated a series of *ent*-beyerane diterpenoids.<sup>16</sup> When the -CH<sub>2</sub>OH group is on the same side (β) as H-5, the oxymethylene carbon resonance occurs at δ 72.9 and the C-19 methyl group at δ 17.6. However, when the -CH<sub>2</sub>OH group is on the opposite side (α) of C-5, the oxymethylene carbon resonance occurs at δ 67.0 and the C-18 methyl group at δ 27.4. The <sup>13</sup>C

NMR resonances for C-18 and C-19 for the *ent*-pimarane, (-)-thermanol,<sup>18</sup> which has the C-19 oxymethylene group on the opposite side to H-5 $\beta$ , this is supported, with this resonance occurring at  $\delta$  65.1 and the C-18 methyl group carbon resonance occurring at  $\delta$  27.0. In compound **6.8**, the resonances occur at  $\delta$  72.3 and  $\delta$  17.3, indicating that the oxymethylene group is on the same side as H-5 $\beta$ , or at C-18, and the methyl group is at C-19.

Compound **6.8** was found to be *ent*-8, 13-*epoxy*-14-labden-18-ol.

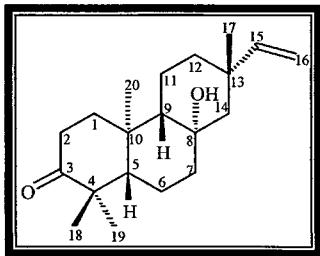
**Table 6.8:** NMR data of compound **6.8**: *ent*-8,13-*epoxy*-14-labden-18-ol in CDCl<sub>3</sub>

no	<sup>1</sup> H NMR (500 MHz)	<sup>13</sup> C NMR (125 MHz)	HMBC (H $\rightarrow$ C)	COSY	NOESY
1 $\alpha$	1.64 (1H, m)	39.1 (CH <sub>2</sub> )	C-2, C-20	H-1 $\beta$	H-20
1 $\beta$	0.90 (1H, m)		C4	H-1 $\alpha$ , H-2 $\alpha$ , H-2 $\beta$	
2 $\alpha$	1.68 (1H, m)	18.2 (CH <sub>2</sub> )	C-4, C-5	H-1 $\beta$	
2 $\beta$	1.60 (1H, m)		C-4, C-9	H-1 $\alpha$ , H-3 $\beta$	
3 $\alpha$	1.41 (1H, m)	35.6 (CH <sub>2</sub> )	C-2, C-4, C-19	H-3 $\beta$	
3 $\beta$	1.25 (1H, m)		C-5	H-3 $\alpha$	
4	-	37.0 (C)	-	-	-
5	1.25 (1H, m)	50.0 (CH)	C-6, C-10		H-9
6 $\alpha$	1.54 (1H, m)	19.9 (CH <sub>2</sub> )	C-5, C-7	H-7 $\alpha$	
6 $\beta$	1.25 (1H, m)				
7 $\alpha$	1.73 (1H, m)	43.0 (CH <sub>2</sub> )	C-5, C-8	H-6 $\alpha$	H-7 $\beta$ , 3H-17
7 $\beta$	1.40 (1H, m)				H-7 $\alpha$
8	-	76.1 (C)	-	-	-
9	1.25 (1H, m)	58.7 (CH)	C-8, C-11, C-12		H-5
10	-	37.9 (C)	-	-	-
11A	1.54 (1H, m)*	16.1 (CH <sub>2</sub> )	C-8, C-9, C-10		
11B	1.44 (1H, m)*				
12A	2.21 (1H, m)*	35.0 (CH <sub>2</sub> )	C-9, C-13	H-12B	
12B	1.41 (1H, m)*			H-12A	
13	-	73.5 (C)	-	-	
14	6.01 (1H, dd, J = 17.9, 10.9 Hz)	147.9 (CH)	No correlation observed	H-14B, H-15	H-15
15A	4.94 (1H, dd, J = 10.9, 0.75 Hz)	109.8 (CH <sub>2</sub> )	C-13, C-14	H-14	H-14
15B	4.90 (1H, dd, J = 17.9, 0.75 Hz)		C-13, C-14		H-14
16	1.11 (1H, s)	32.9 (CH <sub>3</sub> )	C-12, C-13, C-14	-	
17	1.22 (1H, s)	24.1 (CH <sub>3</sub> )	C-7, C-8, C-9	-	3H-20
18A	3.41 (1H, d, J = 10.85)	72.3 (CH <sub>2</sub> )	C-3, C-5	H-18B	H-18B

18B	3.15 (1H, d, $J = 10.85$ )		C-3, C-5	H-18A	H-18A
19	0.73 (1H, s)	17.3 (CH <sub>3</sub> )	C-5, C-10, C-18	-	
20	0.76 (1H, s)	16.4 (CH <sub>3</sub> )	C-5, C-9, C-10	-	H-11 $\alpha$ , 3H-17, H-1 $\alpha$

\* Not assigned as  $\alpha$  or  $\beta$  due to free rotation of the side chain.

### 6.1.9: Structural elucidation of compound 6.9: *ent*-8 $\beta$ -hydroxypimar-15-en-3-one.



Compound 6.9

The high resolution mass spectrum of the needle-like crystals of compound 6.9 gave a molecular ion peak at  $m/z$  304.2393 which was consistent with the molecular formula of C<sub>20</sub>H<sub>32</sub>O<sub>2</sub>. A double bond equivalence of five was deduced. The FTIR spectrum showed a sharp absorption band of a free tertiary OH group at 3456 cm<sup>-1</sup> and a carbonyl stretch at 1685 cm<sup>-1</sup>.<sup>1,2</sup>

The <sup>13</sup>C NMR spectrum showed a ketone carbonyl carbon resonance at  $\delta$  217.9. This resonance showed correlations with the methyl group proton resonances at  $\delta$  1.03 and  $\delta$  1.08 which were ascribed to 3H-18 and 3H-19, and with the H-5 methine proton resonance at  $\delta$  1.39. The H-5 methine resonance showed coupling in the COSY spectrum with the two H-6 proton resonances at  $\delta$  1.75 and  $\delta$  1.40. The H-6 proton resonances, in turn, were seen to be coupled with the H-7 proton resonances at  $\delta$  1.79 and  $\delta$  1.25 in the COSY spectrum.

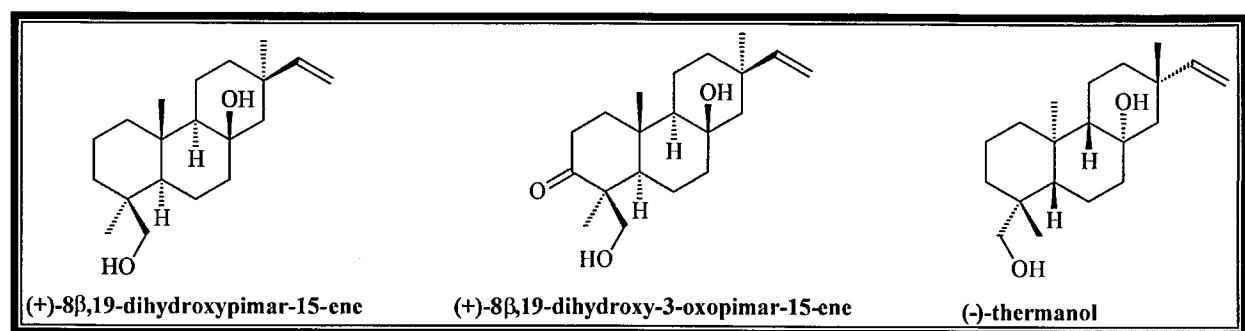
The corresponding C-7 ( $\delta$  41.5) carbon resonance showed correlations in the HMBC spectrum with the H-9 methine proton resonance at  $\delta$  0.91. The H-9 methine proton resonance was seen to be coupled with the superimposed H-11 proton resonances ( $\delta$  1.51), which, in turn, were seen to be coupled with the two H-12 proton resonances at  $\delta$  2.02 and  $\delta$  1.24. The corresponding C-12 carbon resonance showed correlations in the HMBC spectrum with the H-14 proton resonances

at  $\delta$  1.69 and  $\delta$  1.25, and with the equivalent 3H-17 methyl group proton resonance at  $\delta$  0.91. In addition, the C-12 resonance was seen to correlate with the H-15 methine proton resonance at  $\delta$  5.97 (1H,  $J$  = 17.90, 10.90 Hz). The H-15 methine resonance was seen to be coupled with the two H-16 proton resonance at  $\delta$  5.15 ( $J$  = 17.90, 0.90 Hz) and  $\delta$  5.11 ( $J$  = 10.90, 0.90 Hz) in an AMX system. This suggested that compound 6.9 was a pimarane or *ent*-pimarane diterpenoid.

The H-9 and 3H-17 proton resonances were superimposed at  $\delta$  0.91 in the  $^1\text{H}$  NMR spectrum when run in  $\text{CDCl}_3$ , therefore, it was rather difficult to confirm correlations in the NOESY spectrum so the spectra were re-run in *d*-DMSO. The different solvent enabled the spectrum to be resolved and the proton resonances ascribed to H-9 and 3H-17 were found to occur at  $\delta$  0.83 and  $\delta$  0.87 respectively and the 8-OH tertiary hydroxyl group proton resonance was seen at  $\delta$  3.23 enabling the stereochemistry to be assigned using the NOESY experiment.

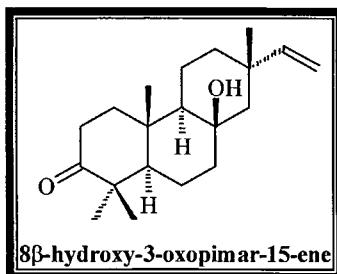
The NOESY spectrum showed no correlations between the 8-OH proton resonance and the 3H-17 and H-9 resonances. However, a correlation between the 8-OH proton resonance and the 3H-20 resonance was evident. The proton of the hydroxyl group showed further correlations with the methine proton resonance assigned to H-15 in the NOESY spectrum and with one of the H-14 resonances at  $\delta$  1.52. This indicates that the 8-OH group, the ethylene group at C-13 and the C-20 methyl group are on the same side of the molecule.

Related pimaranes have been isolated from many plant species of different families.



de Carvalho *et al.* isolated two pimaranes from the aerial parts of *Salzmannia nitida* (Rubiaceae), (+)-8 $\beta$ -,19-dihydroxypimar-15-ene (3-oxo-thermarol) with an optical rotation value

of +22.60° and (+)-8 $\beta$ ,19-dihydroxypimar-15-ene (+8.28°).<sup>17</sup> In 1976, Mastuo *et al.* reported a new *ent*-pimarane-class diterpenoid (-)-thermarol from *Jungermannia thermarum*, with an optical rotation value -17.1°.<sup>18</sup> The NMR data for compound 6.9 and the compounds above are very similar, except for where one would expect changes due to different functional groups.



Pinto *et al.* have isolated 8 $\beta$ -hydroxypimar-15-en-3-one from roots of *Vellozia piresiana* (Velloziaceae), but did not report an optical rotation value.<sup>19</sup> Yang *et al.* isolated the same compound from *Biota orientalis* (Cupressaceae) and reported an optical rotation of +14.7° (in CHCl<sub>3</sub>). This compound was tested and was found to be inactive as a platelet activating factor (PAF) receptor binding antagonist.<sup>8</sup> The NMR resonances for this compound are given in Table 6.10 for comparison purposes.

The optical rotation of compound 6.9 was determined to be -34°. Of the compounds reported above from the literature, the compounds with the 8-OH group, ethylene group at C-13 and the C-20 methyl group on the same side of the molecule are the normal pimaranes, (+)-8 $\beta$ ,19-dihydroxypimar-15-ene (+22.60°) and (+)-8 $\beta$ ,19-dihydroxy-pimar-15-ene (+8.28°), and the *ent*-isopimarane, (-)-thermarol (-17.1°). The optical rotation of compound 6.9 of -34° suggested that it belonged to the *ent*-isopimarane series, with the H-5 and H-9 protons in the  $\beta$ -orientation and the C-20 methyl group in the  $\alpha$ -orientation. This implied that the 8-OH group, the ethylene group at C-13 and C-20 methyl group were all in the  $\alpha$ -orientation. The NOESY spectrum indicated a correlation between the 3H-17 resonance and the H-9 resonance, which in turn showed a correlation with the H-5 resonance which showed a correlation with the 3H-18 proton resonance. Compound 6.9 was thus identified as *ent*-8 $\beta$ -hydroxy-isopimar-15-en-3-one. This compound has not been reported previously.

**Table 6.9: NMR data of compound 6.9: *en*-8 $\beta$ -hydroxyisopimar-15-ene-3-one**

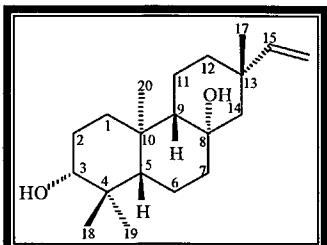
no	$^1\text{H}$ NMR (500 MHz) (CDCl <sub>3</sub> )	$^{13}\text{C}$ NMR (125 MHz) 38.5 (CH <sub>2</sub> )	HMBC (H $\rightarrow$ C)	COSY	NOESY	$^1\text{H}$ NMR (DMSO)	NOESY (DMSO)
1 $\alpha$	1.94 (1H, m)	C-3, C-9	H-2 $\beta$	H-2 $\alpha$ , H-11 $\alpha$ , H-20	1.82 (H, m)	H-2 $\alpha$ , H-20	
1 $\beta$	1.41 (1H, m)	C-10	H-1 $\alpha$	H-2 $\beta$	1.36 (1H, m)		
2 $\alpha$	2.39 (1H, m)	C-1, C-2, C-3, C-10	H-1 $\alpha$	H-1 $\alpha$ , H-20	2.33 (1H, m)	H-1 $\alpha$	
2 $\beta$	2.55 (1H, m)	C-1, C-3	H-1 $\beta$	H-1 $\beta$	2.44 (1H, m)		
3	-	217.9 (C)	-	-	-	-	
4	-	47.7 (C)	-	-	-	-	
5	1.39 (1H, m)	55.6 (CH)	C-3, C-4, C-6 C-10, C-18, C-20	H-9, H-18, H-7 $\beta$ , H-1B	1.39 (1H, m)	H-7 $\beta$ H-9, H-18,	
6 $\alpha$	1.75 (1H, m)	19.1 (CH <sub>2</sub> )	C-7, C-8, C-10	H-5	H-9, H-6 $\beta$ , H-18	1.64 (1H, m)	
6 $\beta$	1.40 (1H, m)	C-8	H-7 $\alpha$	H-5	1.29 (1H, m)	H-18	
7 $\alpha$	1.79 (1H, m)	41.5 (CH <sub>2</sub> )	C-5, C-6, C-9,	H-6 $\alpha$	1.63 (1H, m)		
7 $\beta$	1.25 (1H, m)	C-8, C-9	H-6 $\beta$		1.21 (1H, m)		
8	-	72.3 (C)	-	-	-	-	
9	0.91 (1H, m)	55.5 (CH)	C-5, C-8, C-10, C-20	H-11 $\alpha$ , H-11 $\beta$	H-5	0.83	H-5, H-14 $\beta$
10	-	36.9 (C)	-	-	-	-	
11 $\alpha$	1.51 (1H, m)	18.0 (CH <sub>2</sub> )	C-8, C-9, C-12, C-13	H-9	H-20, H-12 $\alpha$	1.60 (1H, m)	
11 $\beta$	1.51 (1H, m)	C-8, C-9, C-12,	H-12 $\alpha$ , H-12 $\beta$		1.28 (1H, m)		
12 $\alpha$	2.02 (1H, m)	36.2 (CH <sub>2</sub> )	C-8, C-9, C-15	H-11 $\alpha$	H-11 $\alpha$ , H-20	1.81 (1H, m)	H-16B
12 $\beta$	1.24 (1H, m)	C-8, C-15	H-11 $\beta$	H-11	1.16 (1H, m)	H-17	
13	-	36.8 (C)	-	-	-	-	
14 $\alpha$	1.69 (1H, m)	53.2 (CH <sub>2</sub> )	C-7, C-8, C-9,C-12, C-13, C-15	H-14 $\alpha$	H-14 $\beta$	1.52 (1H, m)	8-OH
14 $\beta$	1.25 (1H, m)	C-8, C-15	H-14 $\beta$	H-14 $\alpha$		1.17	H-9
15	5.97 ( $J = 17.9$ Hz, $J = 10.90$ Hz)	C-16	H-16A, H-16B			6.27 (d, $J = 17.9$ Hz, $J = 10.9$ Hz)	8-OH
16A	5.15 ( $J = 17.90$ , $J = 0.90$ Hz)	C-12, C-13, C-14, C-15	H-15	H-16B		4.84 ( $J = 17.90$ , $J = 0.90$ Hz)	H-16B, H-15
16B	5.11 ( $J = 10.90$ , $J = 0.90$ Hz)	C-12, C-13, C-14, C-15	H-15	H-16A, H-15		4.87 ( $J = 10.90$ , $J = 0.90$ Hz)	H-16A, H-15
17	0.91 (3H, s)	32.6 (CH <sub>3</sub> )	C-8, C-15, C-16	H-9		0.87 (3H, s)	
18	1.08 (3H, s)	26.9 (CH <sub>3</sub> )	C-3, C-4, C-5, C-19	H-19.		0.98 (3H, s)	
19	1.04 (3H, s)	21.6 (CH <sub>3</sub> )	C-3, C-4, C-5, C-18	H-18, 3H-20		0.94 (3H, s)	
20	1.03 (3H, s)	15.4 (CH <sub>3</sub> )	C-9, C-1	H-1 $\alpha$ , H-11 $\alpha$ , 3H-19	0.95 (3H, s)	8-OH	
					3.21 (1H, s)	H-14 $\alpha$ , H-15, 3H-20	

\* H-11 proton resonance superimposed at  $\delta$  1.51 and H-9 and 3H-17 superimposed  $\delta$  0.91

**Table 6.10: NMR data of compound 6.9 and related compounds in  $\text{CDCl}_3$**

no	$^1\text{H}$ NMR (500 MHz); compound 6.9	$^{13}\text{C}$ NMR (125 MHz); compound 6.9	$^{13}\text{C}$ NMR (100 MHz) ( $^{+}\text{-}8\beta\text{-},19\text{-dihydroxyiminar-15-en-3-one}^{17}$ )	$^{13}\text{C}$ NMR (50 MHz, $(-\text{hydroxypimar-15-en-3-one})^{17}$ )	$^{13}\text{C}$ NMR (-thermarol); $8\beta\text{-en-19-diol}^{18}$
1 $\alpha$	1.94 (1H, m)	38.5 ( $\text{CH}_2$ )	38.0 ( $\text{CH}_2$ )	37.9 ( $\text{CH}_2$ )	39.5 ( $\text{CH}_2$ )
1 $\beta$	1.41 (1H, m)				
2 $\alpha$	2.39 (1H, m)	34.4 ( $\text{CH}_2$ )	34.5 ( $\text{CH}_2$ )	34.1 ( $\text{CH}_2$ )	18.1 ( $\text{CH}_2$ )
2 $\beta$	2.55 (1H, m)				
3	-	217.9 (C)	220.0 (C)	217.2 (C)	35.6 ( $\text{CH}_2$ )
4	-	47.7 (C)	51.1 (C)	47.4 (C)	38.7 (C)
5	1.39 (1H, m)	55.6 (CH)	56.8 (CH)	55.2 (CH)	56.5 (CH)
6 $\alpha$	1.75 (1H, m)	19.1 ( $\text{CH}_2$ )	18.3 ( $\text{CH}_2$ )	18.8 ( $\text{CH}_2$ )	18.1 ( $\text{CH}_2$ )
6 $\beta$	1.40 (1H, m)				
7 $\alpha$	1.79 (1H, m)	41.5 ( $\text{CH}_2$ )	41.5 ( $\text{CH}_2$ )	42.9 ( $\text{CH}_2$ )	42.3 ( $\text{CH}_2$ )
7 $\beta$	1.25 (1H, m)				
8	-	72.3 (C)	72.0 (C)	72.0 (C)	72.5 (C)
9	0.91 (1H, m)	55.5 (CH)	55.1 (CH)	55.8 (CH)	57.2 (CH)
10	-	36.9 (C)	36.9 (C)	36.7 (C)	36.4 (C)
11 $\alpha$	1.51 (1H, m)	18.0 ( $\text{CH}_2$ )	18.6 ( $\text{CH}_2$ )	17.5 ( $\text{CH}_2$ )	17.4 ( $\text{CH}_2$ )
11 $\beta$	1.51 (1H, m)				
12 $\alpha$	2.02 (1H, m)	36.2 ( $\text{CH}_2$ )	36.3 ( $\text{CH}_2$ )	38.3 ( $\text{CH}_2$ )	36.1 ( $\text{CH}_2$ )
12 $\beta$	1.24 (1H, m)				
13	-	36.8 (C)	36.8 (C)	36.5 (C)	37.1 (C)
14 $\alpha$	1.69 (1H, m)	53.2 ( $\text{CH}_2$ )	53.0 ( $\text{CH}_2$ )	51.5 ( $\text{CH}_2$ )	53.4 ( $\text{CH}_2$ )
14 $\beta$	1.25 (1H, m)				
15	$5.97 (J=17.9 \text{ Hz}, J_{=10.9})$	147.6 (CH)	147.4 (CH)	151.0 (CH)	147.5 (CH)
16A	$5.15 (J=17.90, 0.90$	112.6 ( $\text{CH}_2$ )	112.8 ( $\text{CH}_2$ )	108.6 ( $\text{CH}_2$ )	111.9 ( $\text{CH}_2$ )
16B	$5.11 (J=10.90, 0.90$				
17	0.91 (3H, s)	32.5 ( $\text{CH}_3$ )	32.5 ( $\text{CH}_3$ )	24.3 ( $\text{CH}_3$ )	32.3 ( $\text{CH}_3$ )
18	1.08 (3H, s)	26.9 ( $\text{CH}_3$ )	22.3 ( $\text{CH}_3$ )	26.6 ( $\text{CH}_3$ )	27.0 ( $\text{CH}_3$ )
19	1.04 (3H, s)	21.6 ( $\text{CH}_3$ )	66.0 ( $\text{CH}_3$ )	21.3 ( $\text{CH}_3$ )	65.1 ( $\text{CH}_2$ )
20	1.03 (3H, s)	15.4 ( $\text{CH}_3$ )	16.5 ( $\text{CH}_3$ )	15.4 ( $\text{CH}_3$ )	16.1 ( $\text{CH}_3$ )

### 6.10: Structural elucidation of compound 6.10: *ent*-3 $\beta$ ,8 $\beta$ -dihydroxypimar-15-ene



Compound 6.10

The high resolution mass spectrum gave a molecular ion peak at  $m/z$  306.2553 for compound **6.10**, which is consistent with a molecular formula of  $C_{20}H_{34}O_2$ . A double bond equivalence of four was deduced. The FTIR spectrum showed a sharp absorption band of a free tertiary hydroxyl group at  $3567\text{ cm}^{-1}$  and a secondary OH stretch band at  $3260\text{ cm}^{-1}$ .<sup>1,2</sup> A ketone carbonyl stretch was absent. Compound **6.10** was found to be the  $3\alpha$ -hydroxy derivative of compound **6.9**.

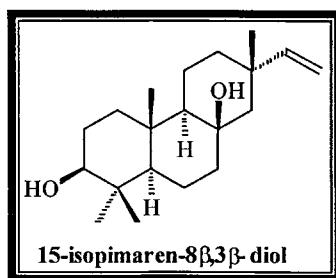
The  $^{13}\text{C}$  NMR spectrum of compound **6.10** showed that the ketone resonance at  $\delta$  217.9 in compound **6.9** was replaced by a resonance at  $\delta$  79.3. This resonance corresponded with a proton resonance at  $\delta$  3.19 (dd,  $J = 10.9\text{ Hz}$ ,  $J = 5.4\text{ Hz}$ ) in the HSQC spectrum that was assigned as C-3, due to correlations seen in the HMBC spectrum with the 3H-18 and 3H-19 methyl group proton resonances at  $\delta$  0.89 and  $\delta$  0.98 and also with the H-5 methine proton resonance at  $\delta$  0.82. The H-3 methine proton resonance was seen to be coupled in the COSY spectrum with the superimposed H-2 resonances ( $\delta$  1.60), which, in turn, were seen to be coupled with the two H-1 proton resonances ( $\delta$  1.70 and  $\delta$  0.98).

The HMBC spectrum showed correlations between the C-1 carbon resonance ( $\delta$  38.0) and the H-9 methine proton resonance at  $\delta$  0.82. This resonance showed coupling in the COSY spectrum with the superimposed H-11 methylene proton resonances at  $\delta$  1.47, which, in turn, showed coupling with the H-12 methylene proton resonances at  $\delta$  2.00 and  $\delta$  1.20. The presence of an AMX system was evident in the  $^1\text{H}$  NMR spectrum of compound **6.10**, as in that of compound **6.9** for the H-15 and 2H-16 resonances.

The NOESY spectrum showed correlations between the H-3, H-5 and 3H-18 resonances. An optical rotation of -22.5°, was determined for this compound.

Meragelman *et al.*<sup>20</sup> isolated the same compound, *ent*-isopimar-15-en-8 $\alpha$ ,3 $\alpha$ -diol from *Gnaphalium gaudichaudianum* and reported an optical rotation of -17.5°. Their NMR data was consistent with that found for compound **6.10**. Koo *et al.*<sup>8</sup> reported to have isolated a compound of the same structure from *Biota orientalis* (Cupressaceae). They reported an optical rotation value of +3°and some different <sup>13</sup>C NMR shifts, especially for C- and H-14, 15 and 16 suggesting that the stereochemistry at C-13 may have been incorrectly assigned. (The paper does not mention how assignment of the stereochemistry at C-13 was undertaken).

The compound 15-isopimaren-8 $\beta$ , 3 $\beta$ -diol, shown below, was isolated by Asili *et al.* from the leaves and branches of *Platycladus orientalis* (Cupressaceae).<sup>3</sup> The NMR spectra of this compound were thoroughly assigned using the NOESY experiment at 600 and 800 MHz. This compound gave the same carbon NMR shifts as the compound reported by Koo *et al.* above. Asili *et al.* reported an optical rotation value of -9°. The compound was tested for *in vitro* antiplasmodial activity and its ability to induce changes of erythrocytes. The results showed that the compound exhibited a ( $IC_{50}>25\mu M$ ) *in vitro* antiplasmodial effects against *Plasmodium falciparum*<sup>3</sup>



The optical rotation of -22.5° found for compound **6.10**, was similar to the -17° found by Meragelman *et al.*<sup>20</sup> for *ent*-isopimar-15-en-8 $\alpha$ ,3 $\alpha$ -diol, for compound **6.10** and the <sup>13</sup>C NMR data are in good agreement, except that the authors appear to have interchanged two of the methyl group carbon assignments.

**Table 6.11: NMR data of compound 6.10: *ent*-3 $\beta$ , 8 $\beta$ -dihydroxy-pimar-15-ene in CDCl<sub>3</sub>**

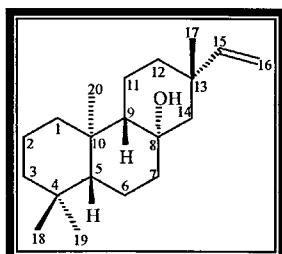
no	<sup>1</sup> H NMR (500 Hz)	<sup>13</sup> C NMR (125 Hz)	HMBC (H→C)	COSY	NOESY
1 $\alpha$	1.70 (1H, m)	38.0 (CH <sub>2</sub> )	C-2, C-3, C-5, C-9	H-2 $\alpha$	3H-20
1 $\beta$	0.97 (1H, m)		C-3, C-5, C-20	H-2 $\beta$	3H-18
2 $\alpha$	1.60 (1H, m)	27.4 (CH <sub>2</sub> )	C-3	H-3	3H-20
2 $\beta$	1.60 (1H, m)		C-3	H-1 $\beta$	
3	3.19 (dd, J= 10.9, 5.4 Hz)	79.3 (CH)	C-4, C-5, C-18, C-19	H-2 $\alpha$	H-5, 3H-18
4	-	39.1 (C)	-	-	-
5	0.82 (1H, m)	55.8 (CH)	C-3, C-4, C-6, C-9, C-18, C-19, 20	H-6 $\alpha$ , H-6 $\beta$	H-3, H-6 $\beta$
6 $\alpha$	1.60 (1H, m)	18.0 (CH <sub>2</sub> )	C-4, C-5, C-8, C-7	H-5	
6 $\beta$	1.48 (1H, m)		C-4, C-5, C-8	H-7 $\alpha$ , H-7 $\beta$	H-5
7 $\alpha$	1.76 (1H, m)	42.2 (CH <sub>2</sub> )	C-5, C-6, C-8, C-10	H-7 $\beta$ , H-7 $\alpha$ , H-6 $\beta$	
7 $\beta$	1.21 (1H, m)		C-5, C-6, C-8		
8	-	72.5 (C)	-	-	-
9	0.82 (1H, m)	56.4 (CH)	C-5, C-7, C-12, C-11, C-20	H-11 $\alpha$	
10	-	37.2 (C)	-	-	-
11 $\alpha$	1.47(1H, m)	17.7 (CH <sub>2</sub> )	C-8, C-9, C-10, C-12, C-	H-9 $\alpha$ , H-12 $\alpha$	H-12 $\alpha$ , 3H-19, 3H-20
11 $\beta$	1.47 (1H, m)		13	H-12 $\beta$	
12 $\alpha$	2.00 (1H, m)	36.2 (CH <sub>2</sub> )	C-11, C-13, C-14, C-17	H-11 $\alpha$	
12 $\beta$	1.20 (1H, m)			H-11 $\beta$	
13	-	36.8 (C)	-	-	-
14 $\alpha$	1.21 (1H, m)	53.6 (CH <sub>2</sub> )	C-7, C-8, C-9, C-12, C-13,	H-14 $\beta$	
14 $\beta$	1.67 (1H, m)		C-15, C-17	H-14 $\alpha$	
15	5.96 (dd, J= 17.90, 10.90 Hz)	147.7 (CH)	C-12, C-14, C-17	H-16 $\beta$ , H-16 $\alpha$	H-16, H-16B
16A	5.13(dd, J=17.90, 0.90 Hz)	112.3 (CH <sub>2</sub> )	C-13, C-15, C-17	H-16B, H-15	
16B	5.01 (dd, J = 10.90 Hz)		C-13, C-15, C-17	H-16A, H-15	H-15
17	0.89 (3H, s)	32.6 (CH <sub>3</sub> )	C-8, C-12, C-13, C-14, C-15	-	H-12 $\beta$ , H-14 $\beta$ ,
18	0.98 (3H, s)	28.6 (CH <sub>3</sub> )	C-3, C-4, C-5, C-19	-	H-3, H-6 $\beta$ , 3H-19, H-5/H-9
19	0.80 (3H, s)	15.7 (CH <sub>3</sub> )	C-3, C-4, C-5,, C-18	-	3H-20, H-2 $\alpha$
20	0.92 (3H, s)	15.7 (CH <sub>3</sub> )	C-1, C-5, C-9	-	3H-19

**Table 6.12: NMR data of compound 6.10 and related compounds**

no	<sup>1</sup> H NMR (500 MHz)	<sup>13</sup> C NMR (125 MHz)	<sup>1</sup> H NMR (600 MHz) CDCl <sub>3</sub> Meragelman <i>et al.</i> <sup>20</sup>	<sup>13</sup> C NMR (50.32 MHz) CDCl <sub>3</sub> <sup>20</sup> Meragelman <i>et al.</i>	<sup>13</sup> C NMR (125 MHz) CDCl <sub>3</sub> Koo <i>et al.</i> <sup>8</sup>	<sup>13</sup> C NMR (125 MHz) CDCl <sub>3</sub> Asili <i>et al.</i> <sup>3</sup>
1 $\alpha$	1.70 (1H, m)	38.0 (CH <sub>2</sub> )	1.71 (dt, 1.31, 1.35 Hz)	37.8 (CH <sub>2</sub> )	37.6 (CH <sub>2</sub> )	37.7 (CH <sub>2</sub> )
1 $\beta$	0.98 (1H, m)		0.98 (1H, m)			
2 $\alpha$	1.60 (1H, m)	27.5 (CH <sub>2</sub> )	1.61 (1H, m)	27.2 (CH <sub>2</sub> )	27.2 (CH <sub>2</sub> )	27.1 (CH <sub>2</sub> )
2 $\beta$	1.60 (1H, m)		1.61 (1H, m)			
3 $\alpha$	3.19 (dd, <i>J</i> = 10.9, 5.4 Hz)	79.3 (CH)	3.21 (dd, <i>J</i> = 11.1 Hz, <i>J</i> = 5.2 Hz)	79.1 (CH)	79.0 (CH)	78.9 (CH)
4	-	39.1 (C)	-	38.9 (C)	38.9 (C)	38.9 (C)
5	0.82 (1H, m)	55.8 (CH)	0.82 (1H, m)	55.6 (CH)	55.1 (CH)	55.5 (CH)
6 $\alpha$	1.60 (1H, m)	18.0 (CH <sub>2</sub> )	1.63 (1H, 13.4, 3.7 Hz)	17.8 (CH <sub>2</sub> )	17.6 (CH <sub>2</sub> )	17.5 (CH <sub>2</sub> )
6 $\beta$	1.48 (1H, m)		1.49 (1H, m)			
7 $\alpha$	1.21 (1H, m)	42.1 (CH <sub>2</sub> )	1.22 (1H, m)	42.0 (CH <sub>2</sub> )	43.6 (CH <sub>2</sub> )	43.0 (CH <sub>2</sub> )
7 $\beta$	1.76 (1H, m)		1.78 (1H, dt, 13.4, 3.2 Hz)			
8	-	72.5 (C)	-	72.3 (C)	72.4 (C)	72.3 (C)
9	0.82 (1H, m)	56.3 (CH)	0.85 (1H, m)	56.2 (CH)	56.8 (CH)	56.7 (CH)
10	-	37.2 (C)	-	37.0 (C)	37.0 (C)	36.9 (C)
11 $\alpha$	1.47 (1H, m)	17.7 (CH <sub>2</sub> )	1.47 (1H, dq, 13.4, 3.1 Hz)	17.4 (CH <sub>2</sub> )	17.6 (CH <sub>2</sub> )	17.1 (CH <sub>2</sub> )
11 $\beta$	1.47 (1H, m)		1.47 (1H, m)			
12 $\alpha$	2.00 (1H, m)	36.2 (CH <sub>2</sub> )	2.01 (1H, dq, 13.7 Hz)	36.1 (CH <sub>2</sub> )	38.1 (CH <sub>2</sub> )	38.0 (CH <sub>2</sub> )
12 $\beta$	1.20 (1H, m)		1.21 (1H, dd, 13.7, 4.4 Hz)			
13	-	36.8 (C)	-	36.5 (C)	36.6 (C)	36.5 (C)
14 $\alpha$	1.21 (1H, m)	53.6 (CH <sub>2</sub> )	1.68 dd (1H, 14.0, 3.1 Hz)	53.6 (CH <sub>2</sub> )	51.5 (CH <sub>2</sub> )	51.4 (CH <sub>2</sub> )
14 $\beta$	1.67 (1H, m)		1.23 (1H, m)			
15	5.96 (dd, <i>J</i> = 17.90, 10.90 Hz)	147.7 (CH)	5.98 (dd, <i>J</i> = 17.90, <i>J</i> = 11.0 Hz)	147.5 (CH)	151.5 (CH)	151.5 (CH)
16A	5.13 (dd, <i>J</i> =17.90, 0.90 Hz)	112.3 (CH <sub>2</sub> )	5.14 (1H, dd, <i>J</i> = 17.9, <i>J</i> =1.2	112.0 (CH <sub>2</sub> )	108.6 (CH <sub>2</sub> )	108.6 (CH <sub>2</sub> )
16B	5.01 (dd, <i>J</i> =10.90, <i>J</i> = 0.90 Hz)		5.09 (11.0, 1.2 Hz)			
17	0.89 (3H, s)	32.6 (CH <sub>3</sub> )	0.91 (3H, s)	28.3 (CH <sub>3</sub> )*	24.3 (CH <sub>3</sub> )	24.2 (CH <sub>3</sub> )
18	0.98 (3H, s)	28.6 (CH <sub>3</sub> )	0.99 (3H, s)	32.4 (CH <sub>3</sub> )*	28.2 (CH <sub>3</sub> )	28.2 (CH <sub>3</sub> )
19	0.80 (3H, s)	15.73 (CH <sub>3</sub> )	0.81 (3H, s)	15.5 (CH <sub>3</sub> )	15.5 (CH <sub>3</sub> )	15.5 (CH <sub>3</sub> )
20	0.92 (3H, s)	15.68 (CH <sub>3</sub> )	0.93 (3H, s)	15.5 (CH <sub>3</sub> )	15.7 (CH <sub>3</sub> )	15.6 (CH <sub>3</sub> )

\*these resonances were incorrectly assigned

### 6.11: Structural elucidation of compound 6.11: *ent*-8 $\beta$ -hydroxypimar-15-ene.

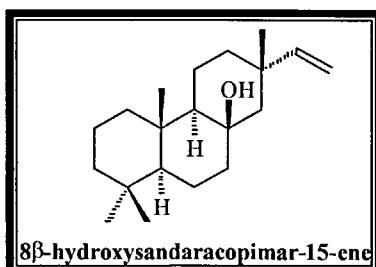


Compound 6.11

Compound 6.11, was found to be related to compounds 6.9 and 6.10 but was not oxygenated at C-3. The high resolution mass spectrum gave a molecular ion peak  $m/z$  290.2604, which is consistent with the molecular formula of  $C_{20}H_{36}O$ . A double bond equivalence of four was deduced. The FTIR spectrum showed a sharp tertiary hydroxyl group at  $3578\text{ cm}^{-1}$ ,<sup>1,2</sup> but did not show a carbonyl stretch, nor secondary hydroxyl band as for compound 6.11.

Evidence of ketone carbonyl and C-3 and hydroxyl group resonances were absent in the  $^{13}\text{C}$  NMR and  $^1\text{H}$  NMR spectra. The  $^1\text{H}$  NMR spectrum revealed four methyl group proton resonances  $\delta$  0.89 (H-17),  $\delta$  0.83 (H-18),  $\delta$  0.86 (H-19) and  $\delta$  0.93 (H-20), which correspond to the carbon resonances at  $\delta$  32.6 (C-17),  $\delta$  33.9 (C-18),  $\delta$  21.9 (C-19) and  $\delta$  15.7 (C-20) in the  $^{13}\text{C}$  NMR spectrum respectively. The C-18 carbon resonance showed correlations with the two H-3 methylene proton resonances ( $\delta$  1.53,  $\delta$  1.45), which, in turn, were seen to be coupled with the H-2 proton resonances ( $\delta$  1.51,  $\delta$  1.44).

The assignments and correlations were similar to those of compounds 6.9 and 6.10. The optical rotation of  $-7.9^\circ$ , indicated this belonged to the *ent*-series, where H-5 and H-9 are in the  $\beta$ -configuration, and 3H-20 in the  $\alpha$ -configuration. Compound 6.11, was identified as *ent*-8 $\beta$ -hydroxy-isopimar-15-ene, and has not been reported previously.



In 1967, Corbett *et al.* isolated  $8\beta$ -hydroxysandaracopimar-15-ene from the volatile oil of *Dacrydium colensoi*. The compound gave an optical rotation value of  $-6.8^\circ$ , but no NMR data was provided, and no reason for the assignment of the stereochemistry given.<sup>21</sup>

**Table 6.13: NMR data of compound 6.11: *ent*- $8\beta$ -hydroxypimar-15-ene in  $\text{CDCl}_3$**

no	$^1\text{H}$ NMR (500 MHz)	$^{13}\text{C}$ NMR (125 MHz)	HMBC (H→C)	COSY	NOESY
1 $\alpha$	1.66 (1H, m)	39.8 (CH <sub>2</sub> )	C-2	H-1 $\beta$	H-3, H-20
1 $\beta$	0.81 (1H, m)		C-20	H-1 $\alpha$ , H-2 $\alpha$ , H-2 $\beta$	
2 $\alpha$	1.51 (1H, m)	17.5 (CH <sub>2</sub> )	C-5	H-1 $\alpha$	
2 $\beta$	1.44 (1H, m)		C-9	H-1 $\alpha$	
3 $\alpha$	1.53 (1H, m)	18.3 (CH)	C-4, C-5	H-3 $\beta$ , H-2 $\beta$	
3 $\beta$	1.45 (1H, m)		C-4, C-5	H-3 $\alpha$	
4	-	37.5 (C)	-	-	
5	0.87 (1H, m)	56.7 (CH)	C-7, C-9, C-18, C-20	H-6 $\beta$	H-9
6 $\alpha$	1.61 (1H, m)	18.7 (CH <sub>2</sub> )		H-6 $\beta$ , H-7 $\beta$	
6 $\beta$	1.38 (1H, m)		C-4, C-5, C-18	H-5, H-6 $\alpha$	
7 $\alpha$	1.38 (1H, m)	42.4 (CH <sub>2</sub> )	C-5, C-6, C-8	H-7 $\beta$	
7 $\beta$	1.12 (1H, m)		C-8	H-6 $\alpha$ , H-7 $\alpha$	3H-18
8	-	72.8 (C)	-	-	
9	0.82 (1H, m)	56.9 (CH)	C-5, C-10, C-11, C-20	H-11 $\alpha$	H-5, H-14 $\beta$
10	-	33.5 (C)	-	-	
11 $\alpha$	1.74 (1H, m)	42.2 (CH <sub>2</sub> )	C-8, C-9	H-9, H-11 $\beta$	
11 $\beta$	1.21 (1H, m)			H-12 $\alpha$ , H-12 $\beta$ , H-11 $\alpha$	
12 $\alpha$	2.00 (1H, m)	36.3 (CH <sub>2</sub> )	C-8, C-9, C-11, C-13, C-15, C-16, C-17	H-12 $\beta$ , H-11 $\beta$	
12 $\beta$	1.21 (1H, m)			H-12 $\alpha$ , H-11 $\beta$	
13	-	36.7 (C)	-	-	
14 $\alpha$	1.66 (1H, m)	53.8 (CH <sub>2</sub> )	C-7, C-8, C-9, C-12, C-15, C-17	H-14 $\beta$	3H-19
14 $\beta$	1.23 (1H, m)			H-14 $\alpha$	H-9
15	6.02 (1H, dd, $J=17.90, 10.90$ Hz)	147.9 (CH)	C-12, C-14, C-17	H-16A, H-16B	H-14, H-16A, H-16B
16A	5.09 (dd, $J=17.90, 0.90$ )	112.0 (CH <sub>2</sub> )	C-13, C-15, C-17 (l.r)	H-15, H-16B	
16B	5.09 (dd, $J=10.90, 0.90$ Hz)			H-15, H-16A	
17	0.89 (3H, s)	32.6 (CH <sub>3</sub> )	C-12, C-14, C-15	-	

18	0.86 (3H, s)	33.9 (CH <sub>3</sub> )	C-3, C-5, C-18	-	H-5, H-9, H-7 $\beta$
19	0.83 (3H, s)	21.9 (CH <sub>3</sub> )	C-3,C-5, C-19	-	3H-20, H-14 $\alpha$
20	0.93 (3H, s)	15.7 (CH <sub>3</sub> )	C-1, C-5, C-9	-	H-1 $\alpha$ , 3H-19

### 6.1.3: Conclusion

The combined hexane and dichloromethane extracts of the stem bark and the leaves of *Malleastrum rakotozafayi* yielded nine *ent*-labdanes and three *ent*-pimaranes diterpenoids. Compounds **6.1**-**6.11** were isolated from the combined hexane and dichloromethane extracts of the leaves. Compounds **6.1**, **6.2**, **6.4**, **6.5**, **6.10** and **6.11** were isolated from the hexane extract of the stem bark. The structures of the *ent*-labdanes and the *ent*-pimaranes isolated and the optical rotations obtained are shown below.

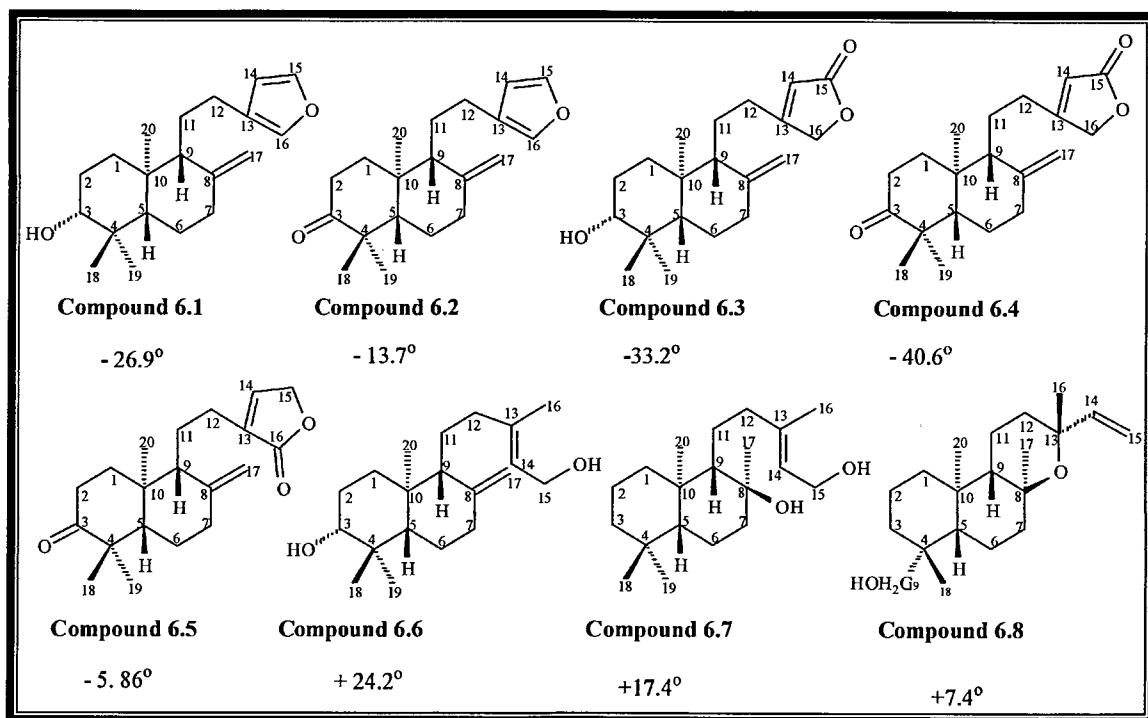


Figure 6.1: *Ent*-labdanes from *M. rakotozafayi* and optical rotations found

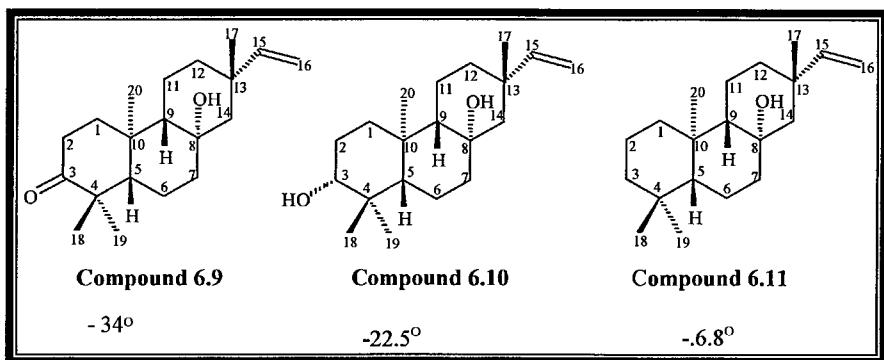
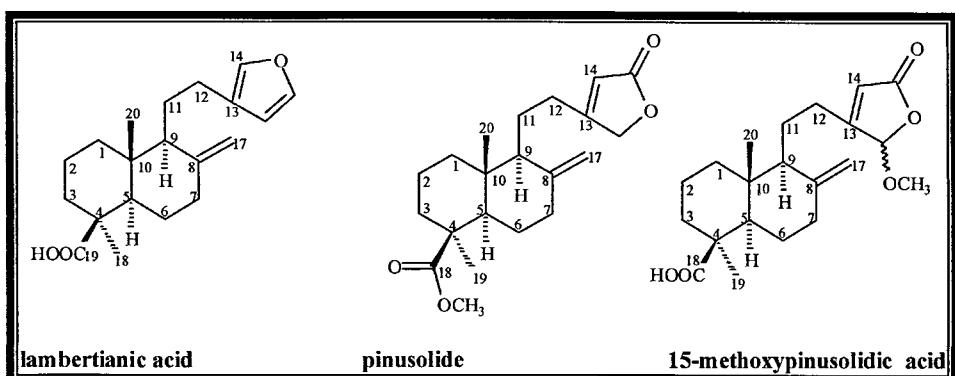


Figure 6.2: *Ent*-pimaranes from *M. rakotozafyi* and optical rotations found

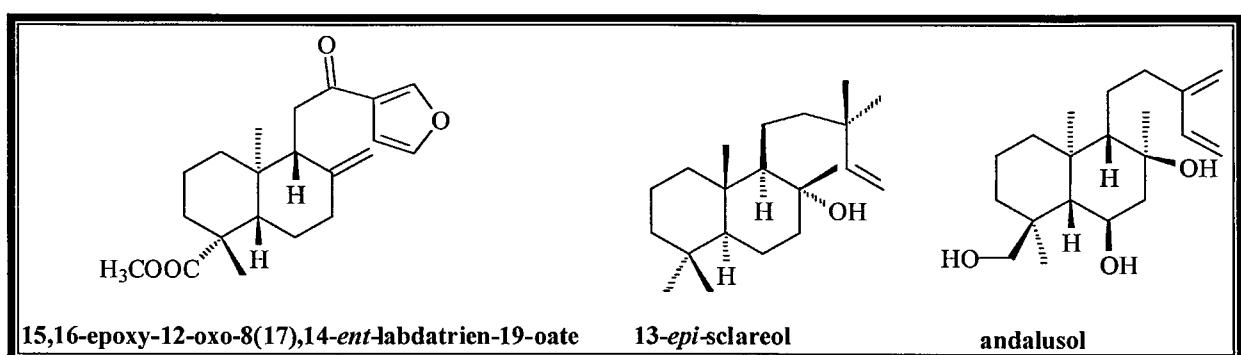
This plant has yielded diterpenoids of the *ent*-labdane type: *ent*-15,16-epoxylabda-8(17), 13 (16), 14-trien-3 $\beta$ -ol **6.1**, *ent*-15,16-epoxylabda-8(17),13(16),14-trien-3-one **6.2**, *ent*-3 $\beta$ -hydroxylabda-8(17),13-dien-15,16-oxide **6.3**, *ent*-3-oxo-labd-8(17),13-dien-15,16-oxide **6.4**, *ent*-3-oxo-labd-8(17),13-dien-16,15-oxide **6.5**, *ent*-labda-8(17),13E-diene-3 $\beta$ ,15-diol **6.6**, *ent*-labd-13(14)-ene-8 $\alpha$ ,15-diol **6.7**, 8,13-*epoxy*-14-labden-18-ol **6.8** and three *ent*-pimaranes, *ent*-0.8 $\beta$ -hydroxypimar-15-en-3-one **6.9**, *ent*-3 $\beta$ ,8 $\beta$ -dihydroxypimar-15-ene **6.10** and *ent*-8 $\beta$ -hydroxypimar-15-ene **6.11**. The combined hexane and dichloromethane extract of the stem bark yielded compounds **6.1**, **6.2**, **6.4**, **6.5**, **6.10** and **6.11**. The optical rotations found for the compounds isolated are given in the figures above. Assigning the stereochemistry as *ent*- for compounds **6.1** -**6.5** was straightforward by comparison of optical rotation values against reliable literature sources. The assignment of compounds **6.6**-**6.8** as *ent* is tentative, and based on their co-occurrence with the other compounds of the *ent*-series. However, the relative stereochemistry at C-8 for compound **6.7** could be determined by running the NOESY experiment in *d*-DMSO. The stereochemistry at C-4 for compound **6.8** could be assigned by comparison against literature values. Compounds **6.9**- **6.11** could be assigned as *ent*-pimaranes based on their optical rotations and correlations seen in the NOESY spectra.

Labdane diterpenoids are known to exhibit a range of biological activities, such as antibacterial, antifungal, anti-inflammatory, anti-leishmanial and cytotoxic activities. Related furanolabdanes such as lambertianic acid from *Platycladus orientalis* (Cupressaceae) was tested for *in vitro* antiplasmodial activity. A weak ( $IC_{50} > 25\mu M$ ) *in vitro* antiplasmodial effect against *Plasmodium falciparum* was noted.<sup>3</sup>

Pinusolide has been isolated from the leaf extract of *Biota orientalis* (Cupressaceae family) and has a methyl ester at C-18. It was found to show platelet-activating factor (PAF) receptor binding antagonist activity.<sup>7</sup> 15-Methoxypinusolidic acid, isolated from the same source, showed significant protective activity against glutamate neurotoxicity in primary cultures of rat cortical cells.<sup>8</sup>



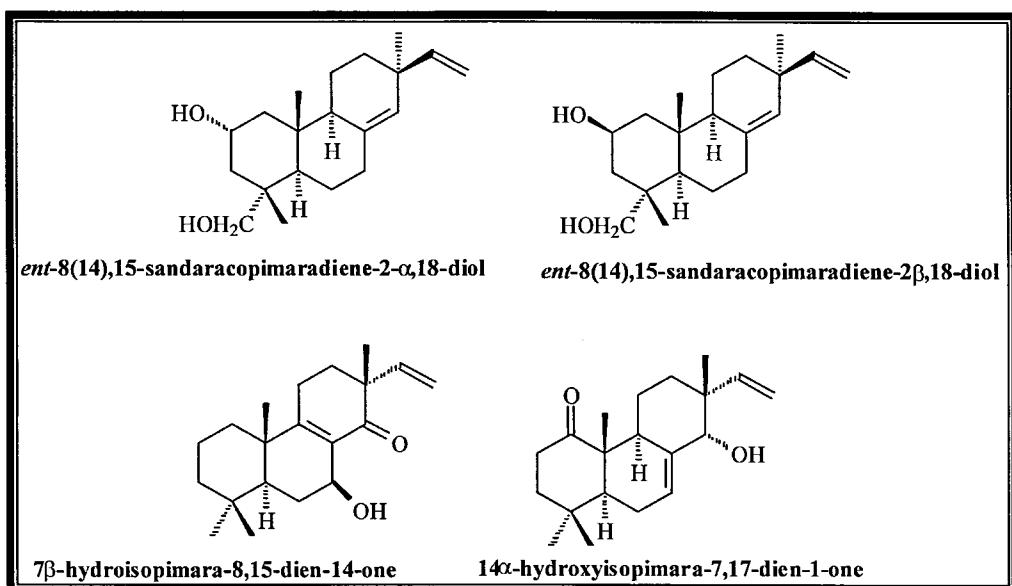
The furano-*ent*-labdane *ent*-14,15,16-epoxy-12-oxo-8(17)-labdatrien-19-oate, isolated from *Potamogeton pectinatus*, exhibited strong algicidal activity against *Raphidocelis subcapitata*.<sup>22</sup> The labdane diterpenoid, 13-*epi*-sclareol, isolated from the root bark of *Coleus forskohlii*, was found to exhibit antiproliferative activity.<sup>23</sup> An *ent*-labdane, andanusol, isolated from *Sideritis foetens* (Lamiaceae), exhibited *in vivo* anti-inflammatory activity.<sup>24</sup>



Pimarane and *ent*-pimaranes, iso and *ent*-isopimaranes have been isolated before from the Meliaceae family and other plant sources. Two diterpenoids were isolated from *Guarea rhophalocarpa* and identified as *ent*-8(14),15-sandaracopimaradiene-2 $\alpha$ -,18-diol and *ent*-

8(14),15-sandaracopimaradiene-2 $\beta$ ,18-diol. The two diterpenoids were found to have antileishmanial activity, however, *ent*-8(14),15-sandaracopimaradiene-2 $\beta$ ,18-diol was 3.3 times more active than the first against *L. donovani* promastigotes, but was found inactive against *Trypanosoma brucei brucei* blood stream trypomastigotes.<sup>25</sup>

The pimaranes 7 $\beta$ -hydroxyisopimara-8,15-dien-14-one and 14 $\alpha$ -hydroxyisopimara-7,15-dien-1-one which were isolated from *Hypoestes serpens*, showed antifungal activity against *Cladiosponium cucumerinum* and the yeast, *Candida albicans*.<sup>26</sup>



The *ent*-labdanes and *ent*-isopimaranes isolated from *Malleastrum rakotozafyi* are now available to be tested for anti-inflammatory, antiplamodial, anti-fungal and other activities.

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## **CHAPTER 7: EXPERIMENTAL**

### **7.1: Experimental techniques**

#### **7.1.1: Nuclear Magnetic Resonance spectroscopy (NMR)**

Nuclear Magnetic Resonance spectroscopy was performed on a 400 MHz Varian UNITY-INOVA NMR spectrometer at the University of KwaZulu-Natal, Durban, South Africa and a 500 MHz Bruker AVANCE NMR spectrometer at the University of Surrey, Guildford, UK. The spectra were recorded in deuteriochloroform ( $\text{CDCl}_3$ ) and deuterio- dimethyl sulfoxide ( $d\text{-DMSO}$ ). The chemical shifts were all recorded in ppm (parts per million) relative to the solvents. The deuteriochloroform was referenced according to the central line for both  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR as  $\delta$  7.260 and 877.23.

#### **7.1.2: Fourier Transform Infrared Spectroscopy (FTIR)**

The infrared spectra were recorded using a Perkin-Elmer System (2000 FTIR) spectrometer. The samples were dissolved in dichloromethane and analyzed using NaCl plates.

#### **7.1.3: Mass Spectrometry (M.S)**

High-resolution mass spectra were recorded at Oxford University by Mr Colin Sparrow. The EI/FI (Electron Impact/Field Ionisation) samples were run on a Waters GCT Time of Flight Mass Spectrometer using a Temperature Programmed Solids Probe to introduce the samples into the instrument source. The Electrospray samples were run on a Bruker MicroToF Mass Spectrometer using an Agilent 1100 HPLC to introduce the samples.

Low resolution mass spectra acquired using a Hewlett Packard G1800A GCD system in the Chemistry Division, at the University of Surrey.

#### **7.1.3: Optical Rotation**

Optical rotations were measured at room temperature in chloroform using a JASCO P-1020 Polarimeter.

#### **7.1.4: Melting Points**

Melting points were carried out using a STUART melting point SMP10 apparatus.

### **7.2: Chromatographic Techniques.**

#### **7.2.1: General Chromatography**

The isolation process employed column and thin layer chromatographic techniques. In column chromatography, different sized columns were used ranging from 2-6 cm in diameter depending on the amount of sample available and the purification stage. Separation of the crude extracts was generally carried out on a column using Merck Art. 9385 silica gel. Final purification was carried using a 1 cm or 0.75 cm diameter Pasteur pipette, which was also packed with Merck Art 9385 silica gel. All separations were carried out under gravity. Both the column and thin layer chromatography techniques made use of varying ratios of hexane/methylene chloride, hexane/ethyl acetate and dichloromethane and methanol. Thin layer chromatography was carried out on 0.2 mm silica-gel, aluminium-backed plates (Merck Art. 5554). The plates were developed using anisaldehyde: conc. H<sub>2</sub>SO<sub>4</sub>: methanol [1:2:97] spray reagent.

#### **7.2.2: Dry Packing of Material**

This method was employed for crude extracts that did not dissolve in a relatively non-polar solvent. Generally these were the methanol extracts. This was carried out by dissolving the extract by heating in a minimal amount of methanol. The extract was mixed with silica gel until all the extract was adsorbed onto the silica gel. The result was a fine, dry, powdery material which was left to dry overnight and then loaded onto a column.

#### **7.2.3: Acetylation**

Pyridine (1ml) and acetic anhydride (1ml) were added to the sample (15 mg) in a round bottomed flask. The sample was left to stand for 48 hours. MeOH (5 ml) was then added to the sample to remove the excess acetic anhydride and toluene (4x10 ml) was added successively to remove the pyridine. After each addition, the solvent was evaporated off on the Rotavapor. Thereafter, MeOH (5x10 ml) was added to remove the toluene. The sample was then spotted on

a t.l.c. plate to see whether the reaction had gone to completion or needed to be separated from the starting material.

#### 7.2.4: Procedure for extraction of plant material.

The plant materials were collected by Professor N. Crouch (South African National Biodiversity Institute, South Africa) and Dr M. Randrianarivelojosia (Pasteur Institute, Madagascar).

##### 7.2.4.1: Extractives from *Phyllanthus cedrelifolius* I. Verd (Phyllanthaceae)

Plant material was collected by Professor N. Crouch of the South African National Biodiversity Institute (SANBI) from a cultivated specimen in Kloof, South Africa. A voucher specimen was retained at the Natal Herbarium (N.Crouch 1012, NH).

The milled stem bark (754 g) and leaves (350 g) of *Phyllanthus cedrelifolius* were extracted using a Soxhlet apparatus (hot extraction) for 48 hours with hexane, dichloromethane, ethyl acetate and methanol. The solvent was then removed under pressure using a Rotary evaporator, to yield an extract which was subjected to column chromatography. Extracts of *Phyllanthus cedrelifolius* were investigated in this study and weighed masses of the eight extracts of both the stem bark and leaves are shown below.

Masses of the extracts of *Phyllanthus cedrelifolius*

Plant material	hexane	dichloromethane	ethyl acetate	methanol
Leaves	17.21 g	2.60 g	5.65 g	114.7 g
Stem bark	18.234 g	7.75 g	1.65 g	88.45 g

Silica gel (Merck 9385) was used as the stationary phase and a solvent step gradient of hexane:dichloromethane/ethyl acetate was used. Fractions of 40 ml each were collected.

##### Chromatography of the hexane extract of the stem:

Fractions 30-40 (40% dichloromethane: 60% hexane): compound **2.4** (phyllanthone). (This was rechromatographed using a 30% dichloromethane: 70% hexane solvent mixture).

Fractions 44-52 (40% dichloromethane: 60% hexane): compound **2.5** ( $\delta$ -amyrin acetate). (This was recrystallised from chloroform).

Fractions 66-71 (50% dichloromethane: 50% hexane): compound **2.6** (lupenone). (This was rechromatographed using a 40% dichloromethane: 60% hexane solvent mixture).

Fractions 80-98 (80% dichloromethane: 20% hexane): compound **2.3** (phyllanthol).

#### **Chromatography of the dichloromethane extract of the stem:**

Fractions 170-180 (100% dichloromethane): compound **2.1** ((20*S*)-3 $\beta$ -acetoxy-24-methylidenedammaran-20-ol). (This was rechromatographed using a 100% dichloromethane as solvent).

#### **Chromatography of the ethyl acetate extract of the stem:**

Fractions 150-166 (1% MeOH, 99% dichloromethane): compound **2.1** ((20*S*)-3 $\beta$ -acetoxy-24-methylidenedammaran-20-ol). (This was rechromatographed using a 100% dichloromethane as solvent).

#### **Chromatography of the combined dichloromethane and hexane extracts of the leaves:**

Fractions 190-209 (100% dichloromethane): compound **2.2** ((20*S*)-3 $\alpha$ -acetoxy-24-methylidenedammaran-20-ol). (This was rechromatographed using a 100% dichloromethane as solvent).

The methanol extract contained sugars and no compounds of interest were isolated.

#### **Physical data for compound **2.1****

Name: (20*S*)- 3 $\beta$ -acetoxy-24-methylidenedammaran-20-ol

Yield: 6.2 mg

Physical description: amorphous solid

Mass spectrum: [M+H]<sup>+</sup> ion peak at *m/z* 501.4303, (calculated for C<sub>33</sub>H<sub>57</sub>O<sub>3</sub> 501.4308)

Infrared spectrum: 3298 cm<sup>-1</sup> (O-H stretching), 1726 cm<sup>-1</sup> (C=O stretching), 2923, 2858 cm<sup>-1</sup>, C-H stretching,

[ $\alpha$ ]<sub>D</sub><sup>24</sup> = +7.6 (c= 4.06x10<sup>-3</sup>, CHCl<sub>3</sub>)

Mp: 201-203°

$^1\text{H}$  and  $^{13}\text{C}$  NMR data are presented in Table 2.1.

#### Physical data for compound 2.2

Name: **(20S)-3 $\alpha$ -acetoxy-24-methylidenedammaran-20-ol**

Yield: 7.7 mg

Physical description: pale green solid

Mass spectrum:  $[\text{M}+\text{H}]^+$  ion peak at  $m/z$  501.4308, (calculated for  $\text{C}_{33}\text{H}_{57}\text{O}_3$  501.4308)

Infrared spectrum:  $1726 \text{ cm}^{-1}$  (C=O stretch),  $2933, 2851 \text{ cm}^{-1}$  (CH stretches)

$[\alpha]_D^{24} = +9.3^\circ$  ( $c = 6.98 \times 10^{-3}$ ,  $\text{CHCl}_3$ )

Mp: 189-191°

$^1\text{H}$  and  $^{13}\text{C}$  NMR data are presented in Table 2.3

#### Physical data for compound 2.3

Name: **13, 27-cycloursan-3 $\beta$ -ol (phyllanthol)**

Yield: white crystalline powder

Physical description: 15 mg

Mass spectrum:  $\text{M}^+$  at  $m/z$  426.3849 (calculated for  $\text{C}_{30}\text{H}_{50}\text{O}$ : 426.3862)

Infrared spectrum:  $3235 \text{ cm}^{-1}$  (O-H stretch),  $2924 \text{ cm}^{-1}$  (CH stretch), a cyclopropane ring stretch at  $1449 \text{ cm}^{-1}$

$[\alpha]_D^{24} : +49^\circ$  ( $c = 4.16 \times 10^{-3}$  in  $\text{CHCl}_3$ ) (literature  $+43^\circ$ ,  $c = 1.23$ ,  $\text{CHCl}_3$ )<sup>1</sup>

Mp: 233-234°, (literature 233-234°)<sup>1</sup>

$^1\text{H}$  and  $^{13}\text{C}$  NMR data are presented in Table 2.4

Name: **phyllanthol acetate**

Yield: 8.7 mg

Physical description: needle-like crystals

Mass spectrum:  $\text{M}^+$  at  $m/z$  468 ( $\text{C}_{32}\text{H}_{52}\text{O}_2$ ), 408, 453, 393, 281, 207

Infrared spectrum:  $1731 \text{ cm}^{-1}$  (C=O),  $3439 \text{ cm}^{-1}$  (water peak),  $2926, 2849 \text{ cm}^{-1}$  (C-H stretch).

$[\alpha]_D^{24} = +43.3^\circ$  ( $c = 5.76 \times 10^{-3}$  in  $\text{CHCl}_3$ ), (literature  $+50^\circ$ ,  $c = 1.54$ )<sup>2</sup>

Mp: 197-199 ° (literature 271°)<sup>1</sup>

$^1\text{H}$  and  $^{13}\text{C}$  NMR data are presented in Table 2.4

### **Physical data for compound 2.4**

Name: **13, 27-cycloursan-3-one (phyllanthone)**

Yield: 10.6 mg

Physical description: needle-like crystals

Mass spectrum: M<sup>+</sup> at *m/z* 424.3707 (calculated for C<sub>30</sub>H<sub>48</sub>O, 424.3705)

Infrared spectrum: 1708 cm<sup>-1</sup> (C=O stretch), 2920, 2858 cm<sup>-1</sup> (C-H stretches)

[α]<sub>D</sub><sup>24</sup> = + 55.8° (c = 6.2 × 10<sup>-3</sup>) (literature +52°, c = 1.54, CHCl<sub>3</sub>)<sup>2</sup>

Mp: 157-159°, (literature 164-165°)<sup>2</sup>

<sup>1</sup>H and <sup>13</sup>C NMR data are presented in Table 2.5

### **Physical data for compound 2.5**

Name: **3β-acetoxyolean-13(18)-ene; (δ-amyrin acetate)**

Yield: 11.8 mg

Physical description: crystalline solid

Mass spectrum: M<sup>+</sup> at *m/z* 468 (C<sub>32</sub>H<sub>52</sub>O<sub>3</sub>), 453, 408, 393 (base peak)

Infrared spectrum: 1726 cm<sup>-1</sup> (C=O stretch), 2934, 2844 cm<sup>-1</sup> (C-H stretch)

[α]<sub>D</sub><sup>24</sup> = -41 (c = 7.17 × 10<sup>-3</sup>, CHCl<sub>3</sub>), (literature -36.5°, c = 0.87, CHCl<sub>3</sub>)<sup>3,4</sup>

Mp: 210-211° (literature 207-209°)<sup>3,4</sup>

<sup>1</sup>H and <sup>13</sup>C NMR data are presented in Table 2.6

### **Physical data for compound 2.6**

Name: **lupenone (3-oxolup-20(29)-ene)**

Yield: 18 mg

Physical description: amorphous solid

Mass spectrum: M<sup>+</sup> at *m/z* 424 (C<sub>30</sub>H<sub>40</sub>O), 409, 205, 95 (100)

Infrared: 1707 cm<sup>-1</sup> (C=O stretch), 2922, 2858 cm<sup>-1</sup> (C-H stretch)

[α]<sub>D</sub><sup>24</sup> = 60.6° (c = 2.14 × 10<sup>-3</sup> in CHCl<sub>3</sub>), (literature 62.8°, c = 1, CHCl<sub>3</sub>)

Mp: 174-176° (literature 170-171°)<sup>3</sup>

<sup>1</sup>H and <sup>13</sup>C NMR data are presented in Table 2.7.

#### **7.2.4.2: Extractives from *Phyllanthus reticulatus* Poir var. (Phyllanthaceae)**

Plant material was collected from a cultivated specimen from Kloof, South Africa and a voucher specimen was retained (N.Crouch 1013, NH).

The leaves (331 g) and stem bark (746 g) of *Phyllanthus reticulatus* were extracted in the same manner as *Phyllanthus cedrelifolius* using a Soxhlet apparatus. The same step gradient procedure was used for the plant *P. reticulatus*.

Masses of the extracts of *Phyllanthus reticulatus*:

Plant material	hexane	dichloromethane	ethyl acetate	methanol
Leaves	8.76 g	4.55 g	1.36 g	123.7 g
Stem bark	2.33 g	3.36 g	2.01 g	116.65 g

#### **Chromatography of the combined hexane and dichloromethane extracts of the stem bark:**

Fractions 10-30 (20% dichloromethane: 80% hexane): compound **2.10** (*n*-tetraeicosanyl-*trans*-4-hydroxy-3-methoxycinnamate). (This compound was rechromatographed using a 20% dichloromethane: 80% hexane solvent mixture).

Fractions 45-60 (40% dichloromethane: 60% hexane): compounds **2.7** (friedelin) and **2.8** (glochidone). (These were separated by means of recrystallization from chloroform).

Fractions 61-80 (60% dichloromethane: 30% hexane): compound **2.9** (lupeol) (This compound was rechromatographed using a 40% dichloromethane: 60% hexane solvent mixture).

#### **Chromatography of the combined hexane and dichloromethane extracts of the leaves:**

Fractions 10-29 (20% dichloromethane: 80% hexane): compound **2.10** (*n*-tetraeicosanyl-*trans*-4-hydroxy-3-methoxycinnamate). (This compound was rechromatographed using a 20% dichloromethane: 80% hexane solvent mixture).

Fractions 40-70 (50% dichloromethane: 50% hexane): stigmasterol.

Fractions 7-76 (50% dichloromethane: 50% hexane): compound **2.9** (lupeol). (Rechromatographed using a 50% dichloromethane: 50% hexane solvent mixture).

#### **Physical data for compound 2.7**

Name: **friedelan-3-one (friedelin)**

Yield: 16.7 mg

Physical description: needle-like crystals

Mass spectrum: M<sup>+</sup> at *m/z* 426 (C<sub>30</sub>H<sub>46</sub>O), 411, 341, 302 (100)

Infrared spectrum: 1709 cm<sup>-1</sup> (C=O stretch), 2922, 2858 cm<sup>-1</sup> (CH stretches), 3277 cm<sup>-1</sup> (water peak)

[α]<sub>D</sub><sup>24°</sup> = -24.9 ° (c=3.76 x10<sup>-3</sup> CHCl<sub>3</sub>), (literature -22.55°, CHCl<sub>3</sub>)<sup>5</sup>

Mp: 257-259° (literature 260-263°)<sup>5</sup>

<sup>1</sup>H and <sup>13</sup>C NMR data are presented in Table 2.8

Physical data for compound 2.8

Name: **3-oxolup-1:20 (29)-diene (glochidone), lupadienone**

Yield: 4.4 mg

Physical description: amorphous solid

Mass spectrum: M<sup>+</sup> at *m/z* 424 (C<sub>30</sub>H<sub>46</sub>O), 409, 281, 207 (100), 123

Infrared spectrum: 1671 cm<sup>-1</sup> carbonyl (C=O stretch), 2926, 2851 cm<sup>-1</sup> (C-H stretch)

[α]<sub>D</sub><sup>24°</sup> = +76.6°, c=0.87x10<sup>-3</sup>, CHCl<sub>3</sub> (+70.2°, c=1.00, CHCl<sub>3</sub>)<sup>3</sup>

Mp: 165-168 ° (literature 163-164°)<sup>3</sup>

<sup>1</sup>H and <sup>13</sup>C NMR data are presented in Table 2.9.

Physical data for compound 2.9

Name: **3β-hydroxylup-20(29)-ene (lupeol)**

Yield: 18.2 mg

Physical description: white solid

Mass spectrum: M<sup>+</sup> at *m/z* 426 (C<sub>30</sub>H<sub>50</sub>O), 393, 365, 229, 207 (100)

Infrared: 3438 cm<sup>-1</sup> (O-H stretch), 3050, 2928 cm<sup>-1</sup> (C-H stretch), 3438 cm<sup>-1</sup> (water peak)

[α]<sub>D</sub><sup>24°</sup> = +33.5° (c=3.8x10<sup>-3</sup>, CHCl<sub>3</sub>) (literature +26°, c=0.80, CHCl<sub>3</sub>)<sup>3</sup>

Mp: 199-201° (literature 210 °)<sup>3</sup>

<sup>1</sup>H and <sup>13</sup>C NMR data are presented in Table 2.10

Physical data for compound 2.10

Name: ***n*-Tetraicosanyl-*trans*-4-hydroxy-3-methoxycinnamate**

Yield: 8.7

Physical description: amorphous solid

Mass spectrum: M<sup>+</sup> at *m/z* 530 (C<sub>34</sub>H<sub>58</sub>O<sub>4</sub>), 529 (100%), 207.

Infrared spectrum: 1709 cm<sup>-1</sup> (C=O carbonyl stretch) 3296 cm<sup>-1</sup> (O-H stretch), 2916, 2849 cm<sup>-1</sup> (C-H stretch)

<sup>1</sup>H and <sup>13</sup>C NMR data are presented in Table 2.1.

#### **Extractives from *Heywoodia lucens* Sim (Phyllanthaceae)**

The stem bark of *Heywoodia lucens* (786 g) was collected from a cultivated specimen in the garden of the Natal Herbarium, Durban, South Africa. A voucher specimen was retained (N.Crouch 1079, NH). Plant material was extracted using a shaker for 48 hours (cold extraction method) with hexane, dichloromethane and methanol.

Masses of the extracts of *Heywoodia lucens*:

Plant material	hexane	dichloromethane	methanol
Stem bark	4.6 g	5.1 g	44.6 g

Three compounds were isolated from the combined hexane and dichloromethane extracts of the stem bark of *Heywoodia lucens*.

Fractions 18-40 (20% dichloromethane/ 80 % hexane): Compound **2.10** (*n*-tetraicosanyl-*trans*-4-hydroxy-3-methoxycinnamate) and compound **2.9** (lupeol). These compounds were separated by further column chromatography.

Fractions 60-80 (80% dichloromethane/ 20 % hexane): Compound **2.11** ( $\alpha$ -glutinol). This compound was recrystallised from MeOH.

#### **Physical data for compound 2.11**

Name: ( $\alpha$ -glutinol), 3 $\alpha$ -hydroxy-D:B-friedoolean-5-ene

Yield: 5.5 mg

Physical description: white solid

$[\alpha]$ <sup>24</sup> = +77° (c=6.4x10<sup>-4</sup>, CHCl<sub>3</sub>) (literature +96°, c=1.0 CHCl<sub>3</sub>)<sup>3</sup>

Mp:197-198° (literature 204-206°)<sup>3</sup>

Mass spectrum: M<sup>+</sup> at m/z 426 (C<sub>30</sub>H<sub>50</sub>O), 411, 259 (100), 205

Infrared: 3451 cm<sup>-1</sup> (O-H stretch), 2925, 2867 cm<sup>-1</sup>(C-H stretches)

<sup>1</sup>H and <sup>13</sup>C NMR data are presented in Table 2.11.

#### 7.2.4.3: Extractives from *Sapium integrerrimum* (Hochst) J.Leonard (Euphorbiaceae).

Plant material was collected from a cultivated specimen in the Natal Herbarium Garden, Durban, South Africa. A voucher specimen was retained (N.Crouch 1063, NH). The leaves and stem bark of *Sapium integrerrimum* were extracted in the same manner as *Phyllanthus cedrelifolius* and *Phyllanthus reticulatus* using a Soxhlet apparatus. The same step gradient procedure was used for this extract. *S. integrerrimum* yielded eight compounds.

Chromatography of combined hexane and dichloromethane extracts of the stem bark:

Fractions 51-62 (60 % hexane: 40% dichloromethane): compound 2.6, lupenone (this was further rechromatographed using a 50 % hexane: 50% dichloromethane solvent mixture).

Fractions 63-65 (50 % hexane: 50% dichloromethane ): compound 2.7, friedelin (this was further rechromatographed using a 60 % hexane: 40% dichloromethane solvent mixture).

Fractions 70-92 (20 % hexane: 80% dichloromethane ): compound 4.2, betulone (this was further rechromatographed using a 35 % hexane: 65% dichloromethane solvent mixture).

Fractions 160-169 (100% dichloromethane ): compound 4.1, 17 $\alpha$ -hydroxy pimara-8(14),15-dien-3-one (this was further rechromatographed using a 10% ethyl acetate: 90% hexane solvent mixture)

Chromatography of the ethyl acetate extract of the stem bark:

Fractions 40-61 (50 % dichloromethane, 50% hexane) compound 2.6, lupenone.

Fractions 100-102 (100 % dichloromethane) compound 4.2, betulone.

Chromatography of combined hexane and dichloromethane extracts of the leaves:

Fractions 10-30 (10 % dichloromethane, 90 % hexane) compound 4.4,  $\alpha$ -tocopherol (this was further rechromatographed using the same solvent system).

Fractions 66-68 (60 % dichloromethane, 40 % hexane) compound 4.3 (*trans*-phytol).

Fractions 70-75 (60 % dichloromethane, 40 % hexane) stigmasterol.

**Physical data for compound 4.1:**

Name: **pimara-8(14),15-dien-17-hydroxy-3-one**

Yield: 4.6 mg

Physical description: amorphous solid

Mass spectrum: M<sup>+</sup> at *m/z* 302.2298 (calculated for C<sub>20</sub>H<sub>30</sub>O<sub>2</sub>: 302.2246), 271.1658 (100), 236, 0707

Infrared: 3446 cm<sup>-1</sup> (OH stretch), 1702 cm<sup>-1</sup> (C=O carbonyl stretch), 2943, 2870 cm<sup>-1</sup> (C-H stretch)

[α]<sub>D</sub><sup>24°</sup> = +19.2° (c = 1.34x10<sup>-3</sup>, CHCl<sub>3</sub>)

Mp: 128-130°

<sup>1</sup>H and <sup>13</sup>C NMR data are presented in Table 4.1.

**Physical data for compound 4.2**

Name: **betulone**

Yield: 8.8 mg

Physical description: amorphous solid

Mass spectrum: M<sup>+</sup> at *m/z* 440 (C<sub>30</sub>H<sub>48</sub>O<sub>2</sub>), 409 (100), 383, 281, 245

Infrared spectrum: 3446 cm<sup>-1</sup> (O-H stretch), 3446 cm<sup>-1</sup> water peak, 1702 (C=O carbonyl stretch)

[α]<sub>D</sub><sup>24°</sup> = +22.7° (c = 1.34x10<sup>-3</sup>, CHCl<sub>3</sub>), (literature +16.4°, c = 0.2, CHCl<sub>3</sub>)<sup>6</sup>

Mp: 181-183° (literature 175-176°)<sup>6</sup>

<sup>1</sup>H and <sup>13</sup>C NMR data are presented in Table 4.2

**Physical data for compound 4.3**

Name: ***trans*-phytol**

Yield: 12.6

Physical description: amorphous solid

Mass spectrum: M<sup>+</sup> at *m/z* 296 (C<sub>20</sub>H<sub>40</sub>O), 280, 279

Infrared spectrum: 3298 cm<sup>-1</sup> (OH stretch), 1726 cm<sup>-1</sup> (C-O stretch)

<sup>1</sup>H and <sup>13</sup>C NMR data are presented in Table 4.3

#### Physical data for compound 4.4

Name:  $\alpha$ -tocopherol

Yield: 39.32 mg

Physical description: amorphous solid: 20.6 mg

Mass spectrum: M<sup>+</sup> at m/z 430 (C<sub>29</sub>H<sub>50</sub>O<sub>2</sub>), 387, 281, 205, 165 (100)

Infrared spectrum: 2925, 2854 cm<sup>-1</sup> (C-H stretch)

[ $\alpha$ ]<sup>24</sup> = +46.9° (c=2.56x10-3, CHCl<sub>3</sub>)

<sup>1</sup>H and <sup>13</sup>C NMR data are presented in Table 4.4

#### 7.2.4.4: Extraction from *Malleastrum rakotozafayi* (Meliaceae)

The leaves (346 g) and stem bark (1133 g) of *M. rakotozafayi* were collected from the Angavo Forest of Madagascar. A voucher specimen (004-nJ/nDul) was deposited at the Department of Botany of the University of Antananarivo. The dried, milled leaves were extracted successively using a Soxhlet apparatus with hexane, dichloromethane, ethyl acetate, and methanol for 48 h each. The extracts were separated using column chromatography over silica gel (Merck 9385) and a mixture of hexane and dichloromethane were used as the mobile phase.

Masses of the extracts of *Malleastrum rakotozafayi*:

Plant material	hexane	dichloromethane	Ethyl acetate	methanol
Leaves	9.21 g	8.23 g	4.43 g	152.76 g.
Stem bark	12.32 g	17.34 g	7.25 g	180.65 g

#### Chromatography of the combined hexane and dichloromethane extracts of the leaves:

Fractions 21-23 (20% dichloromethane: 80% hexane): compound 6.11 (*ent*-8 $\beta$ -hydroxy-pimar-15-ene). (This was rechromatographed using a 20% dichloromethane: 80% hexane solvent mixture).

Fractions 54-75 (40% dichloromethane: 60% hexane): compound 6.1 (*ent*-15,16-epoxy-labda-8(17),13(16), 14-trien-3 $\beta$ -ol.). (This was rechromatographed using a 50% dichloromethane: 50% hexane solvent mixture).

Fractions 88-97 (80% dichloromethane: 20% hexane): compound **6.2** (*ent*-15,16-epoxy-labd-8(17),13(16),14-trien-3-one). (This was rechromatographed using a 15% ethyl acetate: 75% hexane solvent mixture).

Fractions 120-130 (4% ethyl acetate: 96% hexane): compound **6.9** (*ent*- 8 $\beta$ -hydroxyisopimar-15-ene-3-one). (This was recrystallised from chloroform).

Fractions 170-180 (20% ethyl acetate: 80% hexane): compound **6.10** (*ent*-3 $\beta$ ,8 $\beta$ -dihydroxy pimar-15-ene).

Fractions 212-219 (30% ethyl acetate: 70% hexane): compound **6.4** (*ent*-3-oxo-labd-8(17),13-dien-15,16-oxide. (This was recrystallised from chloroform).

Fractions 220-259 (30% ethyl acetate: 70% hexane): compound **6.5** (*ent*-3-oxolabd-8(17),13-dien-15, 16-oxide). (This was recrystallised from chloroform).

Fractions 263-264 (40% ethyl acetate: 60% hexane): compound **6.3** (*ent*-3 $\beta$ -hydroxylabd-8(17),13-dien-15,16-oxide). (This was rechromatographed using a 30% ethyl acetate: 70% hexane solvent mixture).

Fractions 266-269 (50% ethyl acetate: 50% hexane): compound **6.6** (*ent*-labda-8(17),13E-diene-3 $\beta$ ,15-diol) and compound **6.7** (*ent*-labd-13(14)-ene-8 $\beta$ ,15-diol). (This mixture was separated using a 30% ethyl acetate: 70% hexane solvent mixture).

#### **Chromatography of the combined hexane and dichloromethane extracts of the leaves:**

Fractions 10-20 (20% dichloromethane: 80% hexane): compound **6.11** (*ent*-8 $\beta$ -hydroxypimar-15-ene). (This was rechromatographed using a 20% dichloromethane: 80% hexane solvent mixture).

Fractions 40-60 (40% dichloromethane: 60% hexane): compound **6.1** (*ent*-15,16-epoxylabd-8(17),13(16),14-trien-3 $\beta$ -ol.). (This was rechromatographed using a 50% dichloromethane: 50% hexane solvent mixture).

Fractions 70-81 (80% dichloromethane: 20% hexane): compound **6.2** (*ent*-15,16-epoxylabd-8(17),13(16),14-trien-3-one). (This was rechromatographed using a 15% ethyl acetate: 75% hexane solvent mixture).

Fractions 110-118 (20% ethyl acetate: 80% hexane): compound **6.10** (*ent*-3 $\beta$ ,8 $\beta$ -dihydroxy pimar-15-ene).

Fractions 190-199 (30% ethyl acetate: 70% hexane): compound **6.4** (*ent*-3-oxolabd-8(17), 13-dien-15,16-oxide) and compound **6.5** (*ent*-3-oxolabd-8(17), 13-dien-15,16-oxide). (This was rechromatographed using a 30% ethyl acetate: 70% hexane solvent mixture).

#### Physical data for compound **6.1**

Name: ***ent*-15, 16-epoxy-labda-8(17),13(16),14-trien-3 $\beta$ -ol.**

Yield: 6 mg

Physical description: amorphous solid

Mass spectrum: M<sup>+</sup> at *m/z* 302.2253, (calculated for C<sub>20</sub>H<sub>30</sub>O<sub>2</sub> M<sup>+</sup> at *m/z* 302.2246), 256.220

Infrared: 3392 cm<sup>-1</sup> (O-H stretch), 2930, 2855 cm<sup>-1</sup> (C-H stretch), 1444 cm<sup>-1</sup>, C-O stretch of furan ring

[ $\alpha$ ]<sub>D</sub><sup>24°</sup> = -26.9 ° (c=8.58x10<sup>-3</sup>, CHCl<sub>3</sub>)

Mp= 147-149°

<sup>1</sup>H and <sup>13</sup>C NMR data are presented in Table 6.1.

#### Physical data for compound **6.2**

Name: ***ent*-15, 16-epoxy-labda-8(17),13(16),14-trien-3-one**

Yield: 11.6 mg

Physical description: yellowish oil

Mass spectrum: M<sup>+</sup> at *m/z* 300.2089, (calculated for C<sub>20</sub>H<sub>28</sub>O<sub>2</sub> M<sup>+</sup> at *m/z* 300.2089)

Infrared spectrum: 3446 cm<sup>-1</sup> (O-H stretch), 2939, 2851 cm<sup>-1</sup> (C-H stretch), 1457 cm<sup>-1</sup>, C-O of furan ring.

[ $\alpha$ ]<sub>D</sub><sup>24°</sup> = -13.7 ° (c=1.24x10<sup>-3</sup>, CHCl<sub>3</sub>)

<sup>1</sup>H and <sup>13</sup>C NMR data are presented in Table 6.2

#### Physical data for compound **6.3**

Name: ***ent*-3 $\beta$ -hydroxy labd-8(17),13-dien-15,16-oxide**

Yield: 12.8 mg

Physical description: white crystals

Mass spectrum: M<sup>+</sup> at *m/z* 318.2185, 316.1768, 301.2186, 300.2355, (calculated for C<sub>20</sub>H<sub>30</sub>O<sub>3</sub> 318.2195)

Infrared spectrum: 3282 cm<sup>-1</sup> (O-H stretch), 1736 (butenolide), 2921, 2857 cm<sup>-1</sup>

$[\alpha]_D^{24} = -33.2^\circ$  ( $c=4.28 \times 10^{-3}$ , CHCl<sub>3</sub>)

Mp: 128-129°

<sup>1</sup>H and <sup>13</sup>C NMR data are presented in Table 6.3

#### Physical data for compound 6.4

Name: *ent*-3-oxolabda-8(17),13-dien-15,16-oxide

Yield: 50.5 mg

Physical description: white crystals

Mass spectrum: M<sup>+</sup> at *m/z* 316.2038] (calculated for C<sub>20</sub>H<sub>28</sub>O<sub>3</sub> *m/z* 316.2038)

Infrared spectrum: 1702 (C=O carbonyl stretch), 1745 (butenolide), 2936, 2855 (CH stretch)

$[\alpha]_D^{24} = -40.6^\circ$  ( $c=2.524 \times 10^{-3}$ , CHCl<sub>3</sub>)

Mp: 121-127°, (literature 122°)<sup>7</sup>

<sup>1</sup>H and <sup>13</sup>C NMR data are presented in Table 6.4.

#### Physical data for compound 6.5

Name: *ent*-3-oxolabda-8(17),13-dien-15,16-oxide

Yield: 19.7 mg

Physical description: white solid

Mass spectrum: M<sup>+</sup> at *m/z* 316.2038, (calculated for C<sub>20</sub>H<sub>28</sub>O<sub>3</sub> at *m/z* 316.2038)

Infrared spectrum: 1701 (C=O carbonyl stretch), 1750 (lactone), 2940, 2863 (C-H stretch)

$[\alpha]_D^{24} = -5.9^\circ$  ( $c=4.06 \times 10^{-3}$  CHCl<sub>3</sub>)

Mp: 125-126°, (literature 103°)<sup>7</sup>

<sup>1</sup>H and <sup>13</sup>C NMR data are presented in Table 6.5

#### Physical data for compound 6.6

Name: *ent*-labda-8(17),13E-diene-3 $\beta$ ,15-diol

Yield: 4.3 mg

Physical description: brownish crystals

Mass spectrum: M<sup>+</sup> at *m/z* 306 (C<sub>20</sub>H<sub>34</sub>O<sub>2</sub>)

Infrared: 3310 (O-H stretch), 2923, 2859 (C-H stretch)

$[\alpha]_D^{24} = +24.0^\circ$  ( $c=5.9 \times 10^{-3}$ ,  $\text{CHCl}_3$ )

Mp: 117-118°

$^1\text{H}$  and  $^{13}\text{C}$  NMR data are presented in Table 6.5

#### Physical data for compound 6.7

Name: *ent-labd-13(14)-ene-8 $\alpha$ ,15-diol*

Yield: 12.5 mg

Physical description: white crystals

Mass spectrum:  $M^+$  at  $m/z$  308 ( $\text{C}_{20}\text{H}_{36}\text{O}_2$ )

Infrared: 3310 (O-H stretch), 2923, 2867 (C-H stretch)

$[\alpha]^{24} = +17.3^\circ$  ( $c=2.08 \times 10^{-3}$   $\text{CHCl}_3$ )

Mp: 125-127°

$^1\text{H}$  and  $^{13}\text{C}$  NMR data are presented in Table 6.5

#### Physical data for compound 6.8

Name: *ent-8,13-epoxy-14-labden-18-ol*

Yield: 4.7 mg

Physical description: brownish crystals

Mass spectrum:  $M^+$  at  $m/z$  306 ( $\text{C}_{20}\text{H}_{34}\text{O}_2$ ), 273, 255, 177

Infrared: 3453 (O-H stretch), 2944 (C-H stretch)

$[\alpha]^{24} = +7.4^\circ$  ( $c=1.76 \times 10^{-3}$   $\text{CHCl}_3$ )

Mp: 131-133°

$^1\text{H}$  and  $^{13}\text{C}$  NMR data are presented in Table 6.5

#### Physical data for compound 6.9

Name: *ent- 8 $\beta$ -hydroxypimar-15-en-3-one*

Yield: 44 mg

Physical description: white needles

Mass spectrum:  $M^+$  at  $m/z$  304.2393, 286.2228, 244.2537, 205.6488, (calculated for  $\text{C}_{20}\text{H}_{32}\text{O}_2$  304.2402)

Infrared spectrum: 3456 (tertiary O-H stretch), 1685 (C=O carbonyl stretch), 2944, 2857 (C-H stretch)

$[\alpha]_D = -35.7^\circ$  ( $c=9 \times 24 \times 10^{-3}$  CHCl<sub>3</sub>)

Mp: 147-149°

<sup>1</sup>H and <sup>13</sup>C NMR data are presented in Table 6.9

#### Physical data for compound 6.10

Name: *ent*-3β, 8β-dihydroxyisopimar-15-ene

Yield: 14.4 mg

Physical description: white powder

Mass spectrum: M<sup>+</sup> at *m/z* 306.2553, (calculated for C<sub>20</sub>H<sub>34</sub>O<sub>2</sub> 306.2559)

Infrared: 3567 (tertiary O-H stretch), 3260 (primary O-H stretch), 2859, 2962 (CH stretch)

$[\alpha]_D^{24} = -22.3^\circ$  ( $c=1.484 \times 10^{-3}$  CHCl<sub>3</sub>)

Mp: 132-133°

<sup>1</sup>H and <sup>13</sup>C NMR data are presented in Table 6.10.

#### Physical data for compound 6.11

Name: *ent*-8β-hydroxyisopimar-15-ene.

Yield: 6.6 mg

Physical description: clear oil

Mass spectrum: M<sup>+</sup> at *m/z* 290.2604, (calculated for C<sub>20</sub>H<sub>36</sub>O 290.2609)

$[\alpha]_D^{24} = +7.6^\circ$  ( $c=1.425 \times 10^{-3}$ , CHCl<sub>3</sub>)

Infrared spectrum: 3578 (tertiary O-H stretch), 2918, 2844 (CH stretch)

<sup>1</sup>H and <sup>13</sup>C NMR data are presented in Table 6.11.

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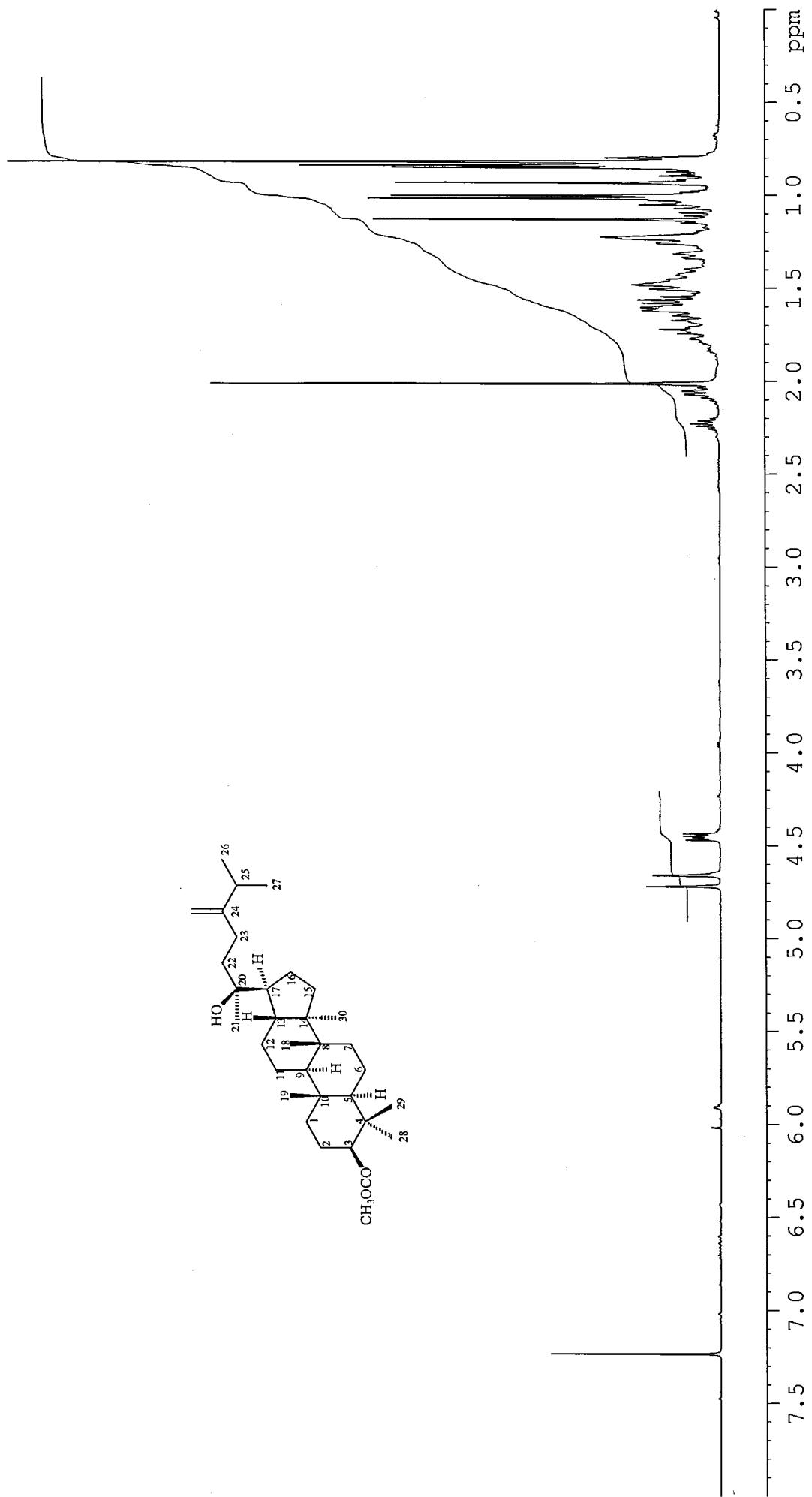
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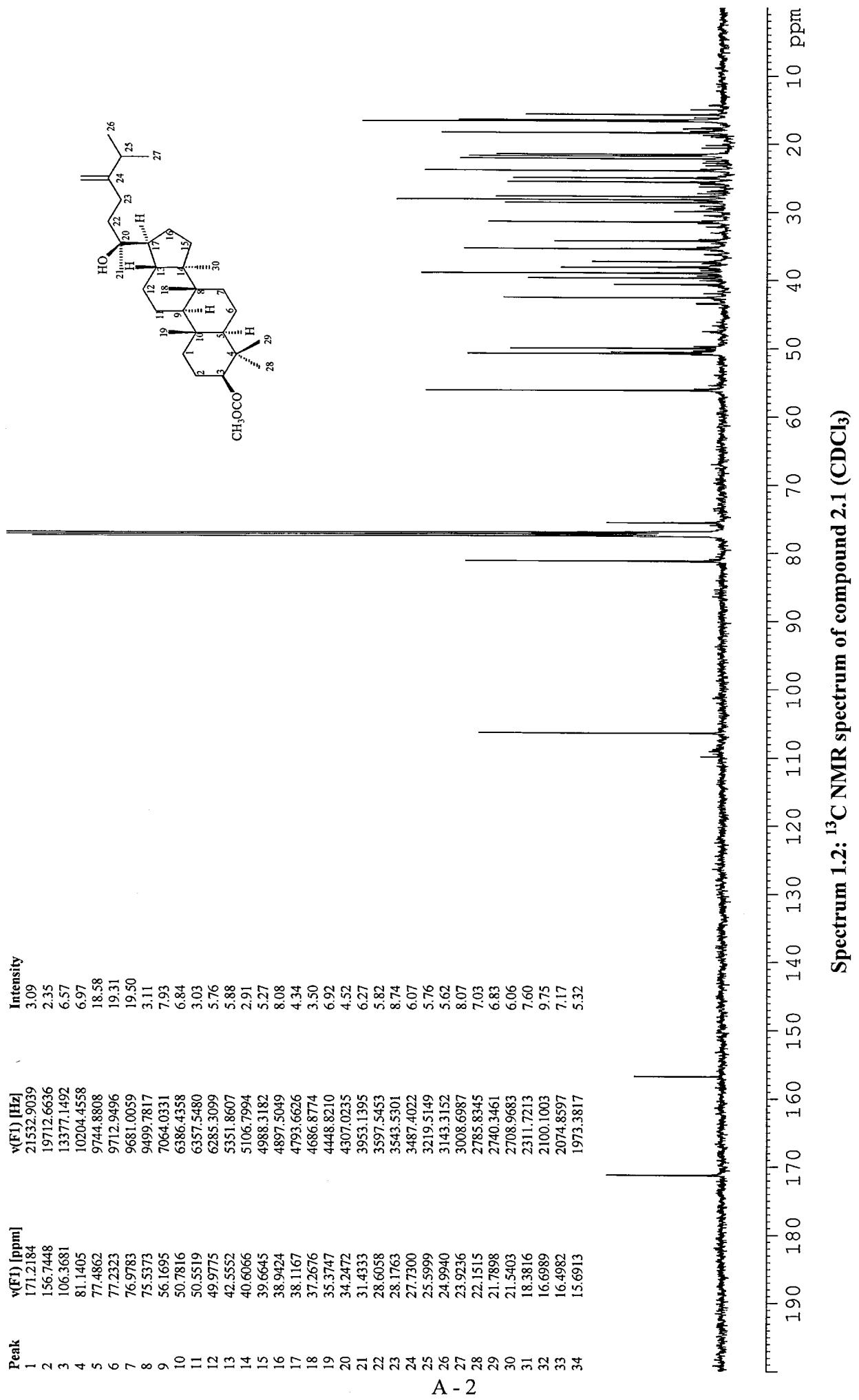
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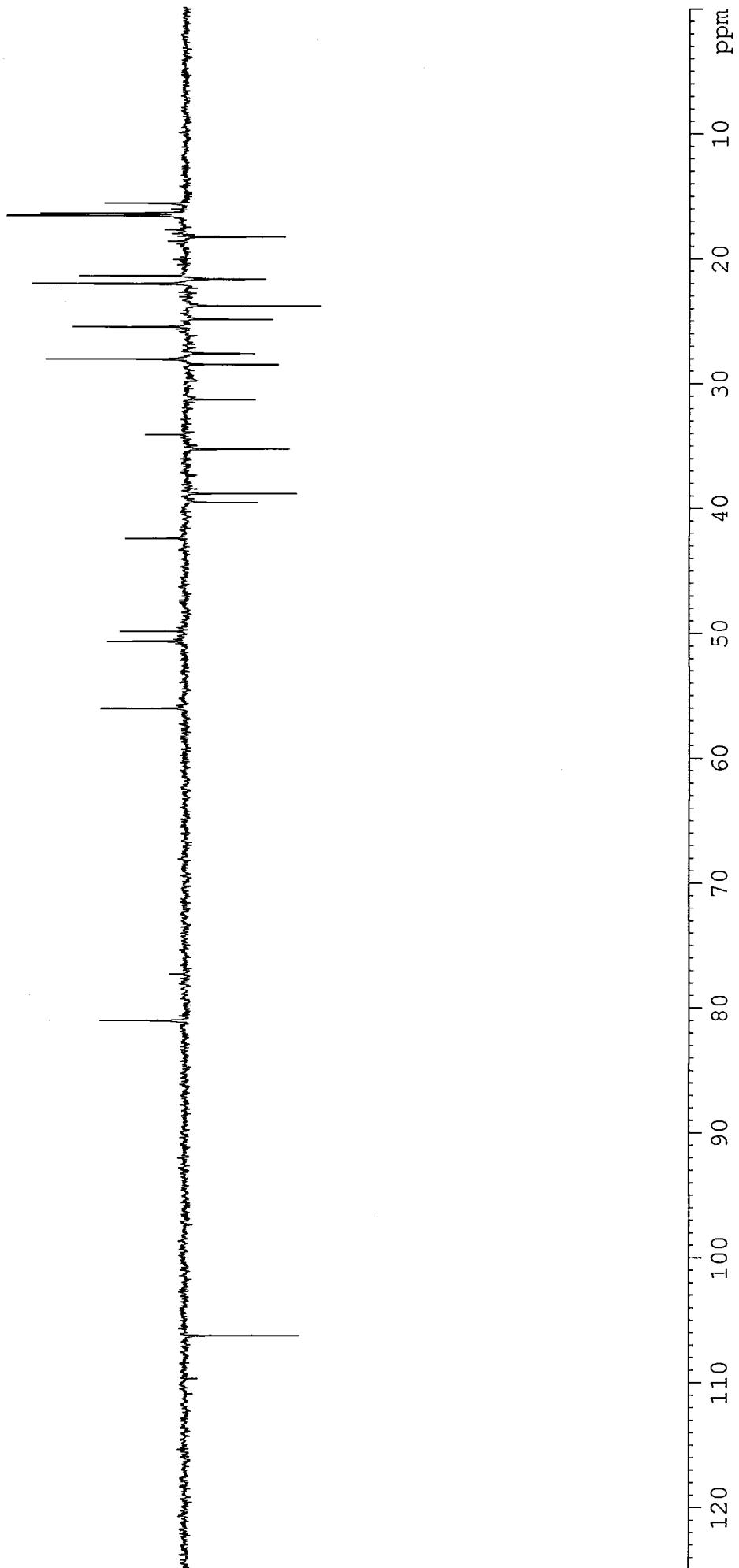


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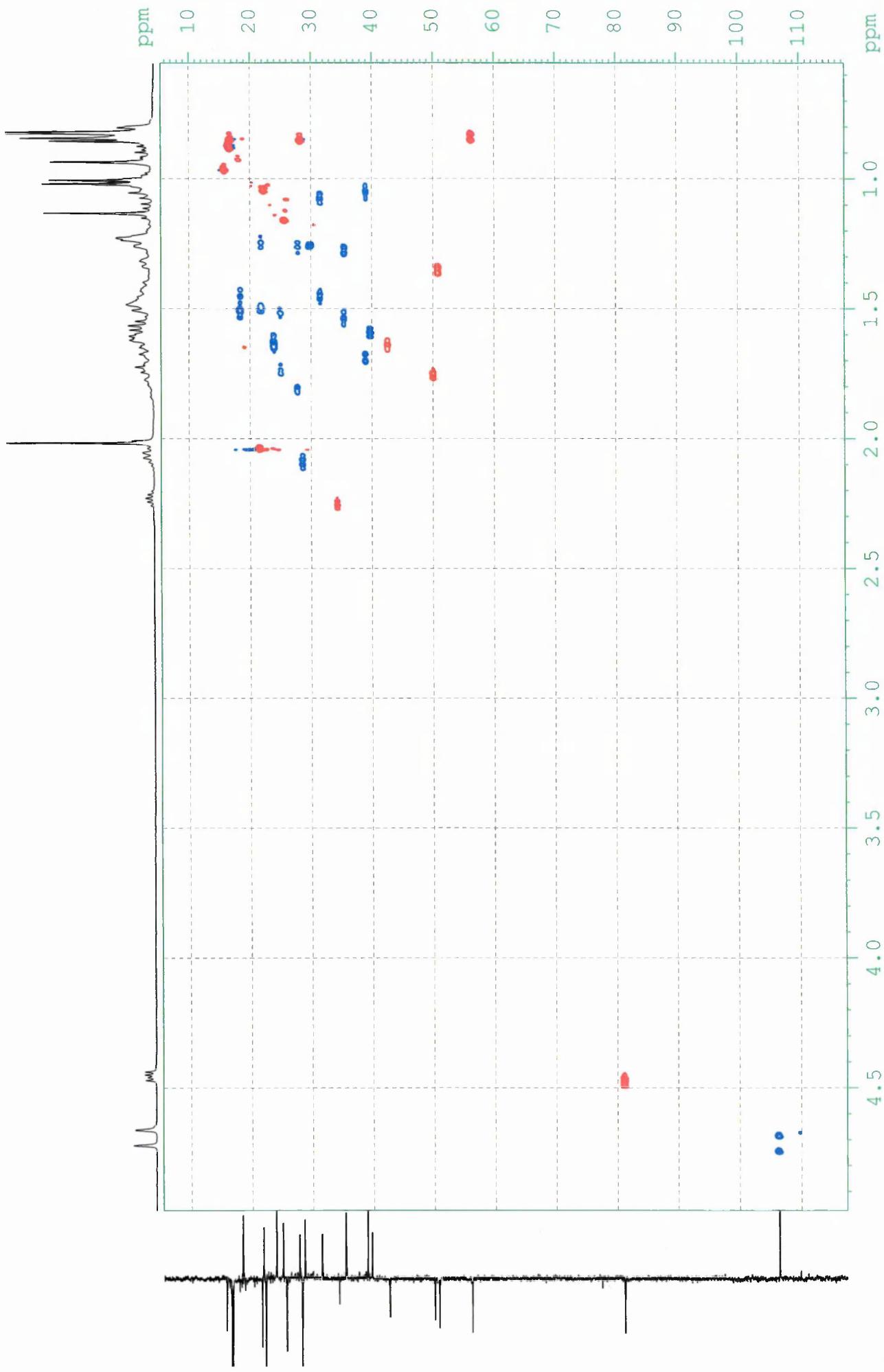
Spectrum 1.1:  $^1\text{H}$  NMR spectrum of compound 2.1 ( $\text{CDCl}_3$ )



Spectrum 1.2:  $^{13}\text{C}$  NMR spectrum of compound 2.1 ( $\text{CDCl}_3$ )



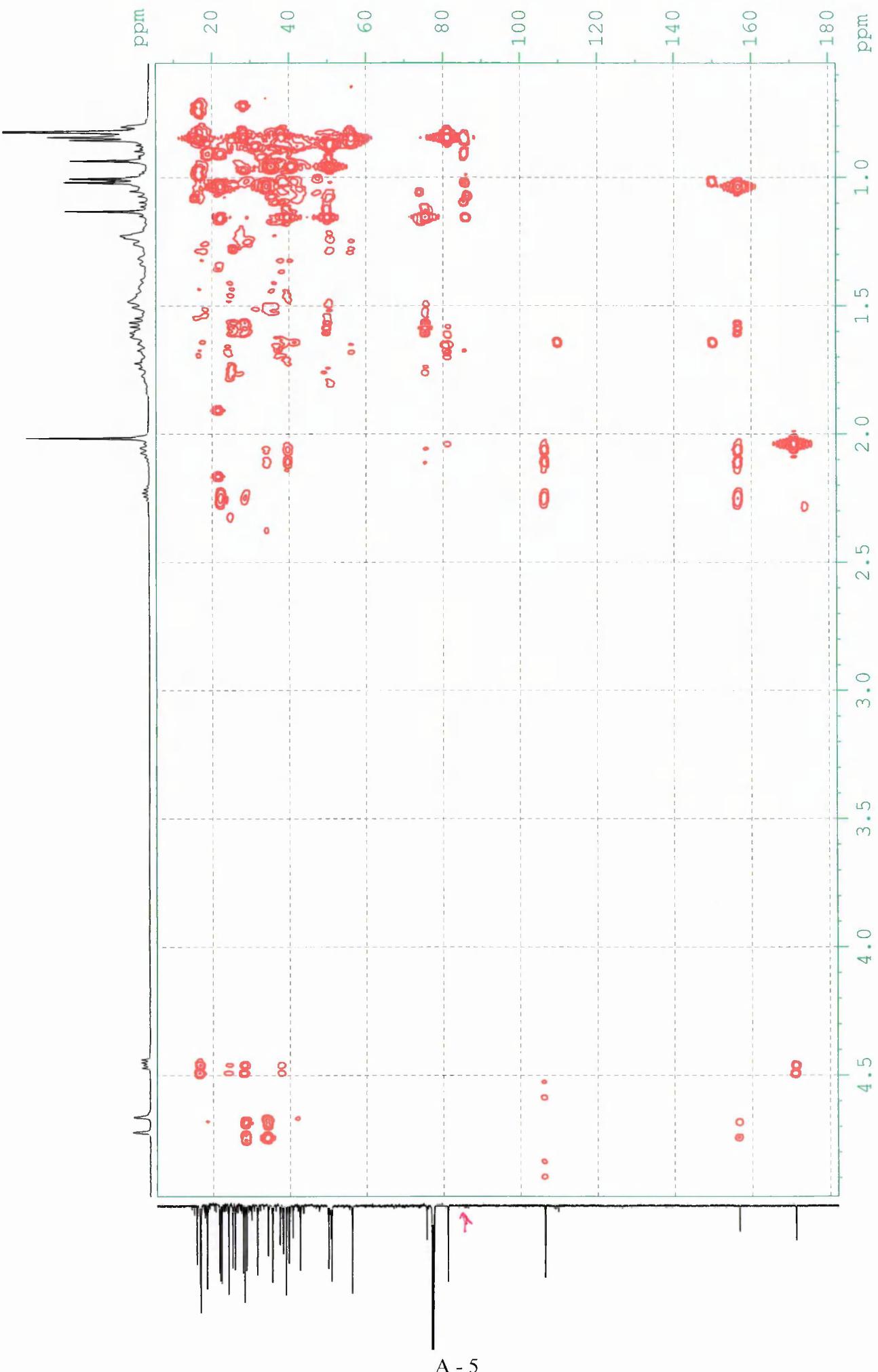
Spectrum 1.3: DEPT spectrum of compound 2.1 ( $\text{CDCl}_3$ )



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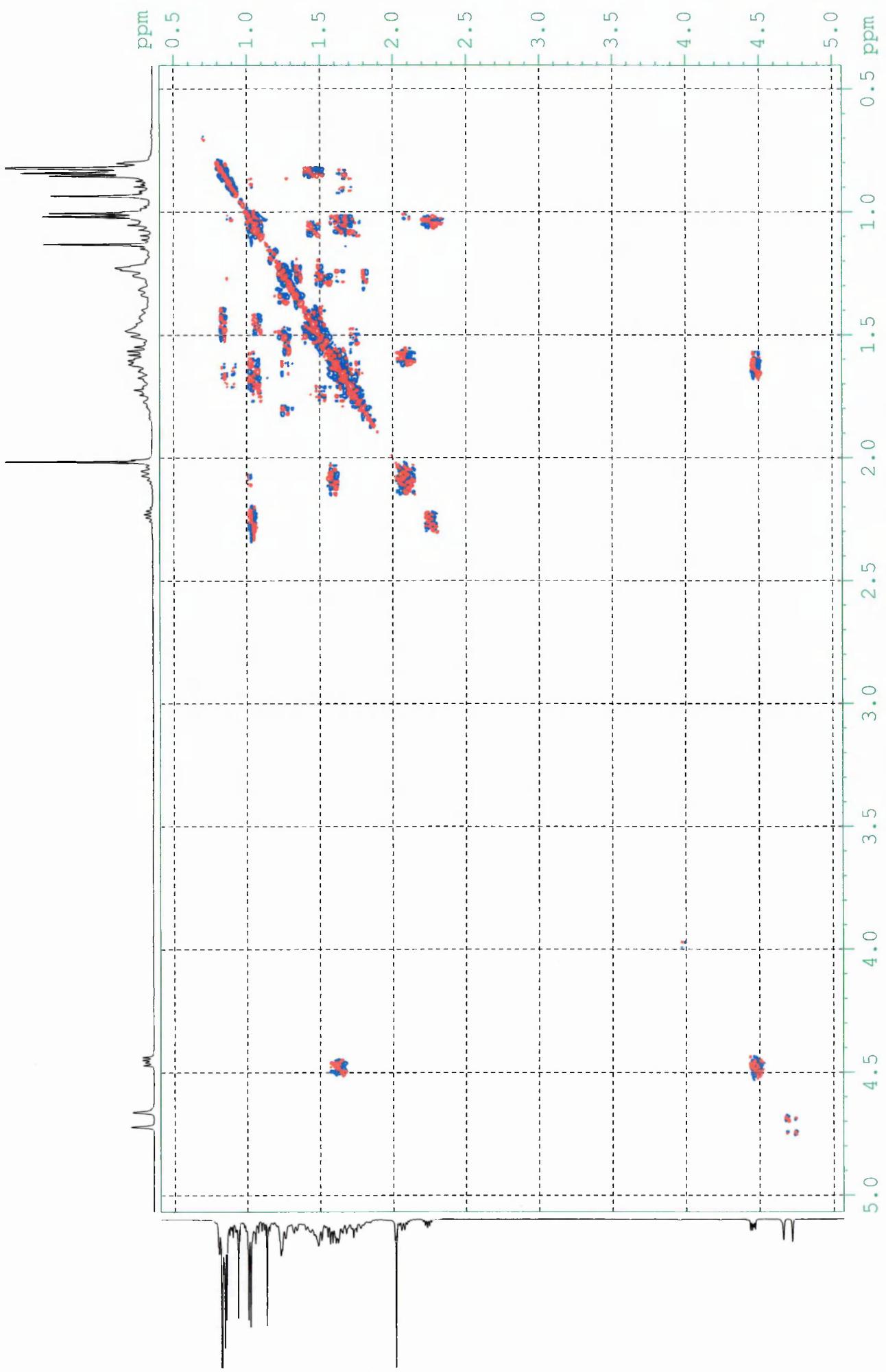
Spectrum 1.4: HSQCDEPT spectrum of compound 2.1 ( $\text{CDCl}_3$ )

**Spectrum 1.5: HMBCLP spectrum of compound 2.1 ( $\text{CDCl}_3$ )**

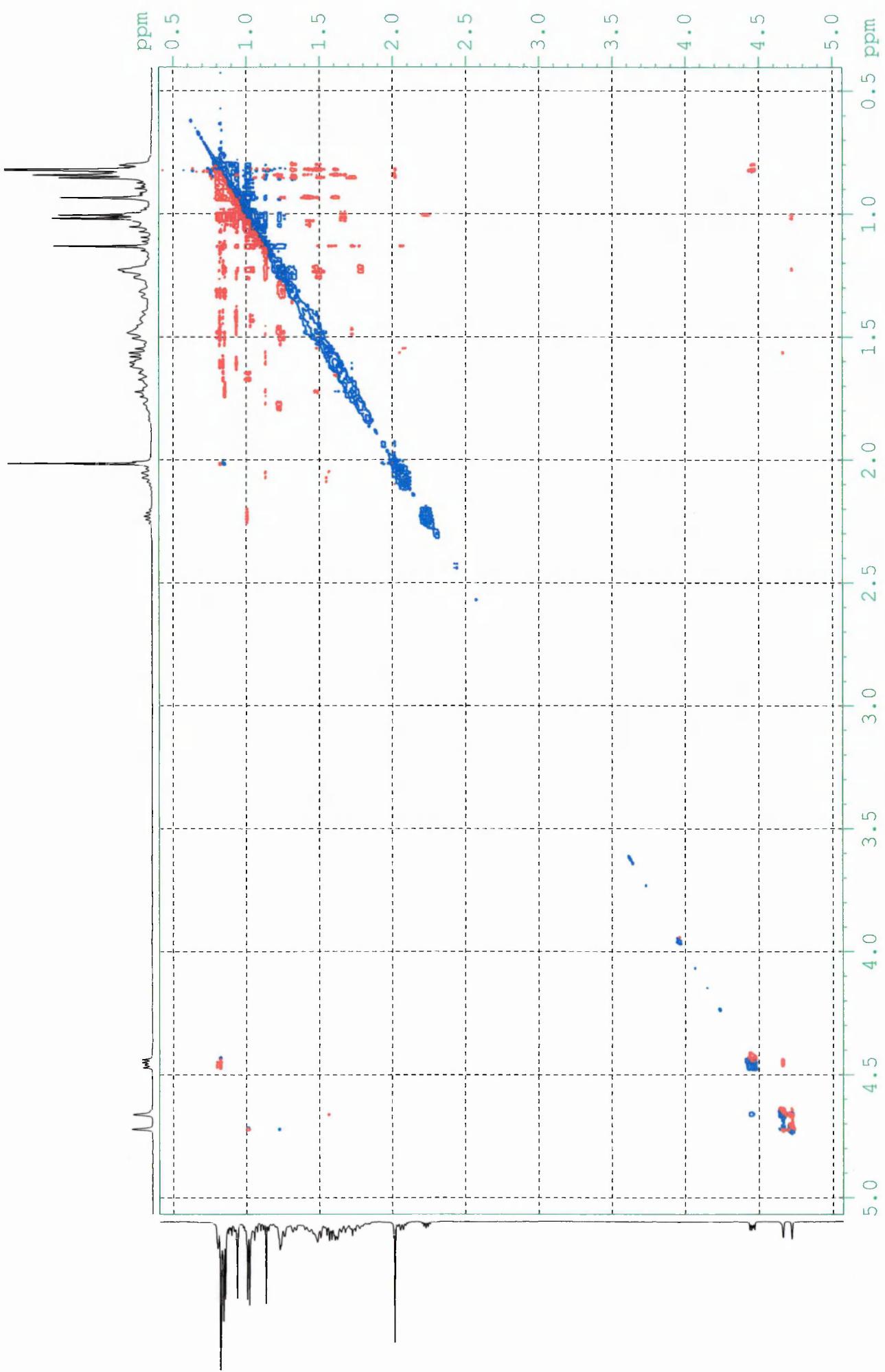


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Spectrum 1.6: COSYPH spectrum of compound 2.1 ( $\text{CDCl}_3$ )



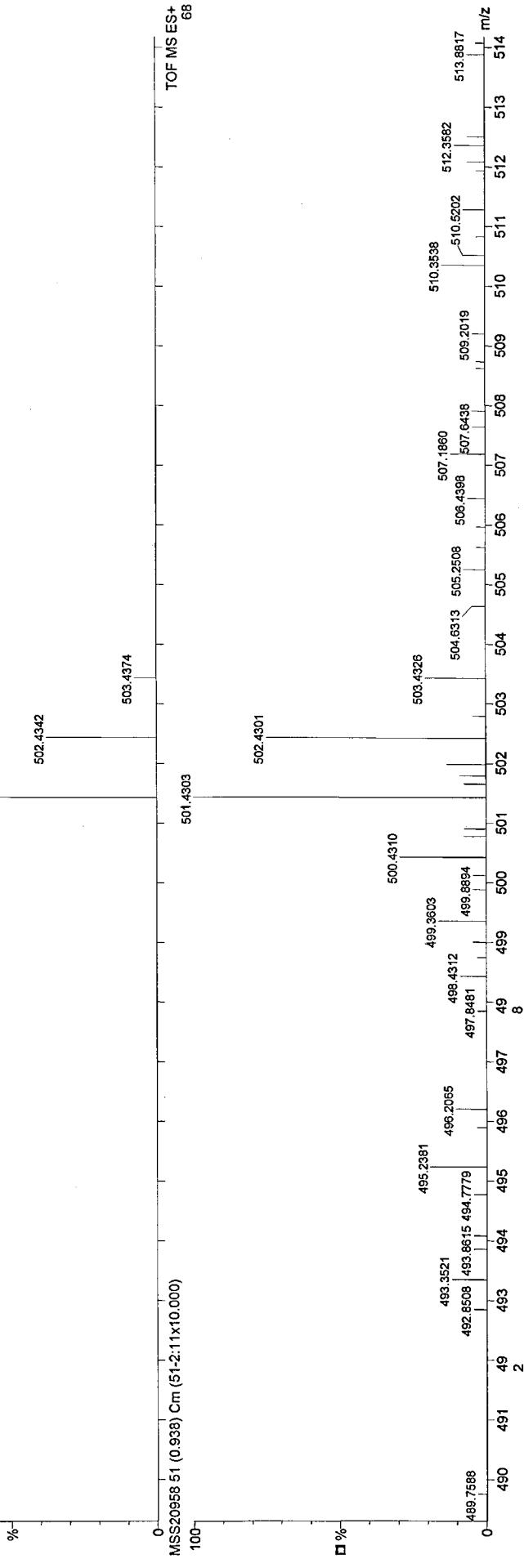
Spectrum 1.7: NOESY spectrum of compound 2.1 ( $\text{CDCl}_3$ )



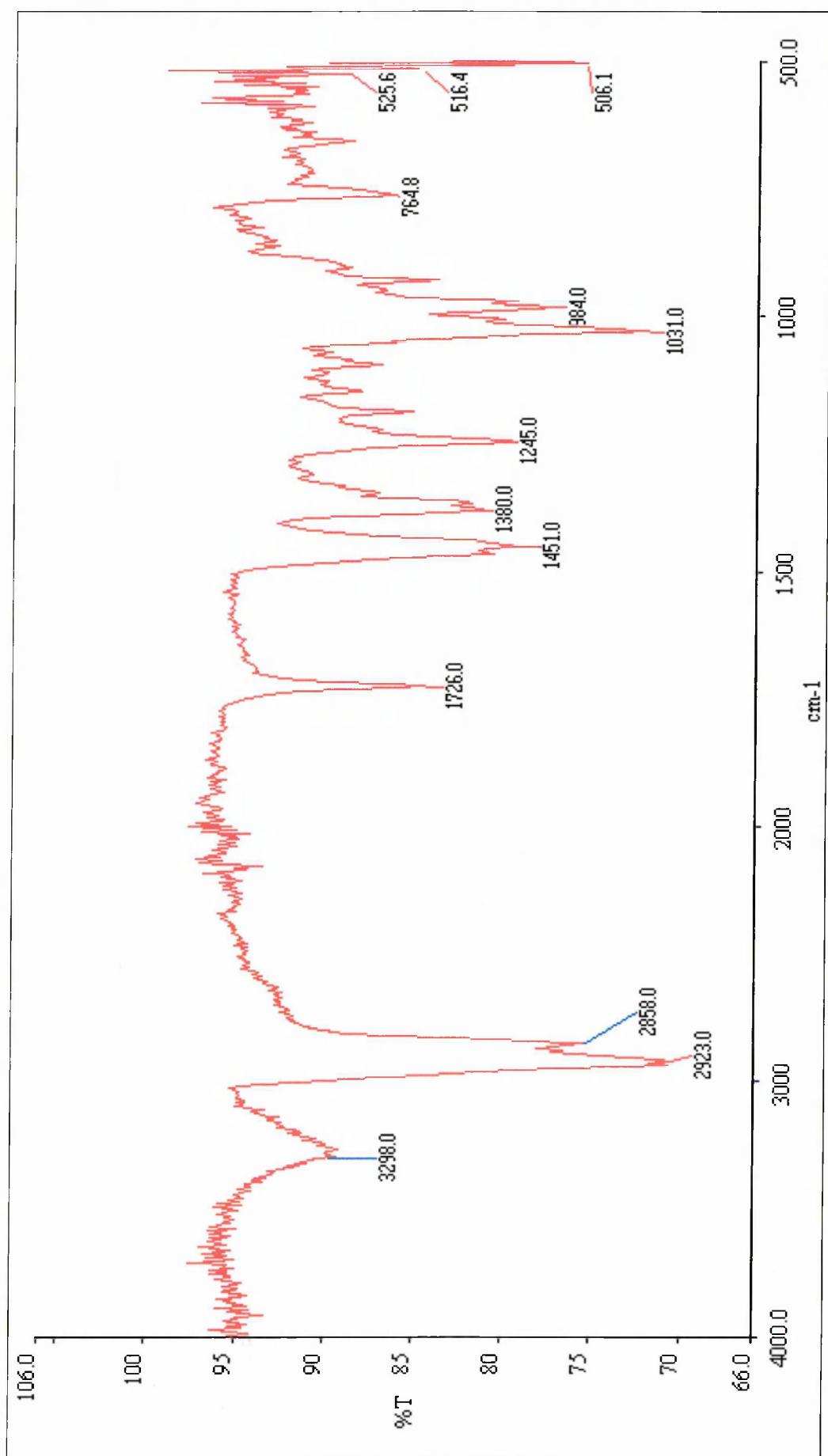
**MSS 20958 SAMPLE 1 [C<sub>33</sub>H<sub>56</sub>O<sub>3</sub>]**  
MSS20958 (0.021) ls (1.00,1.00) C<sub>33</sub>H<sub>57</sub>O<sub>3</sub>

Electrospray  
501.4308

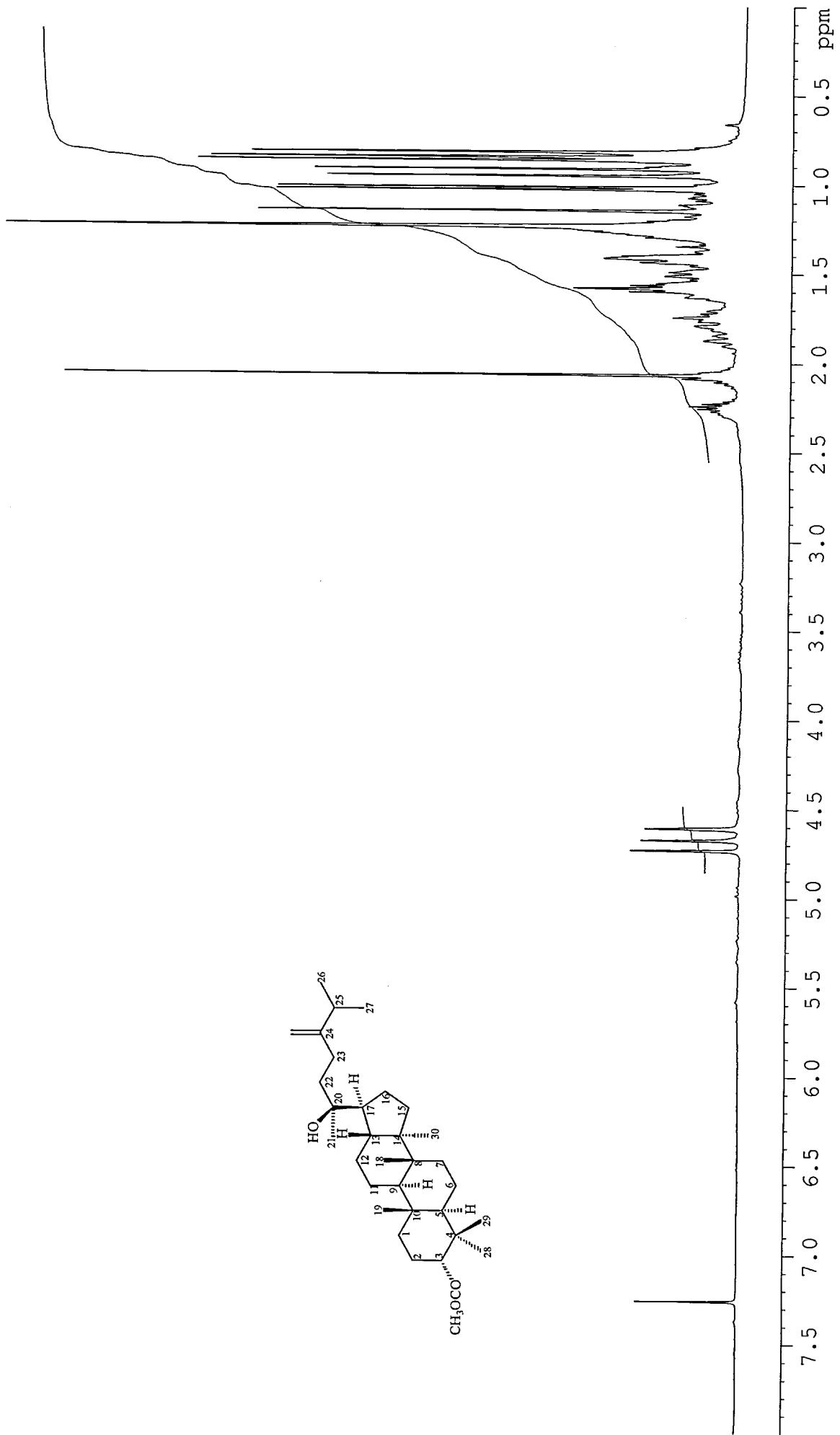
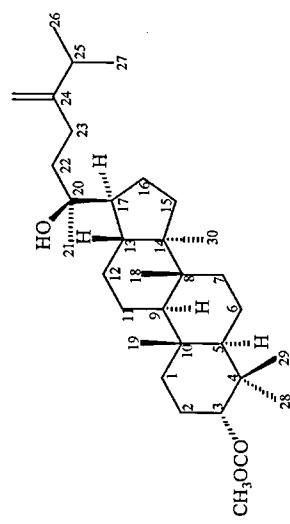
ISOTOPE MODEL [C<sub>33</sub>H<sub>57</sub>O<sub>3</sub>]  
M+1 ION



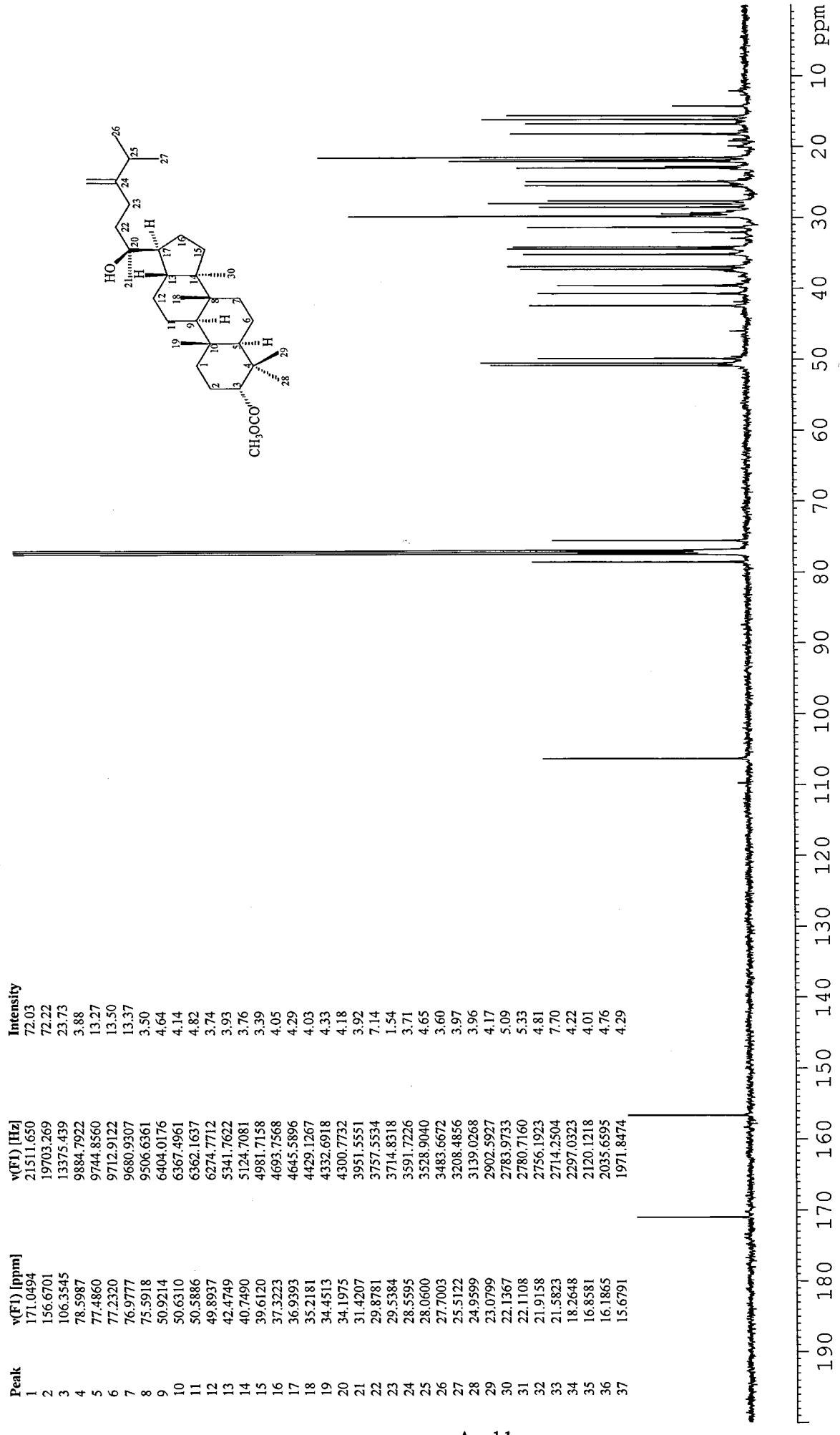
**Spectrum 1.8: Mass spectrum of compound 2.1**



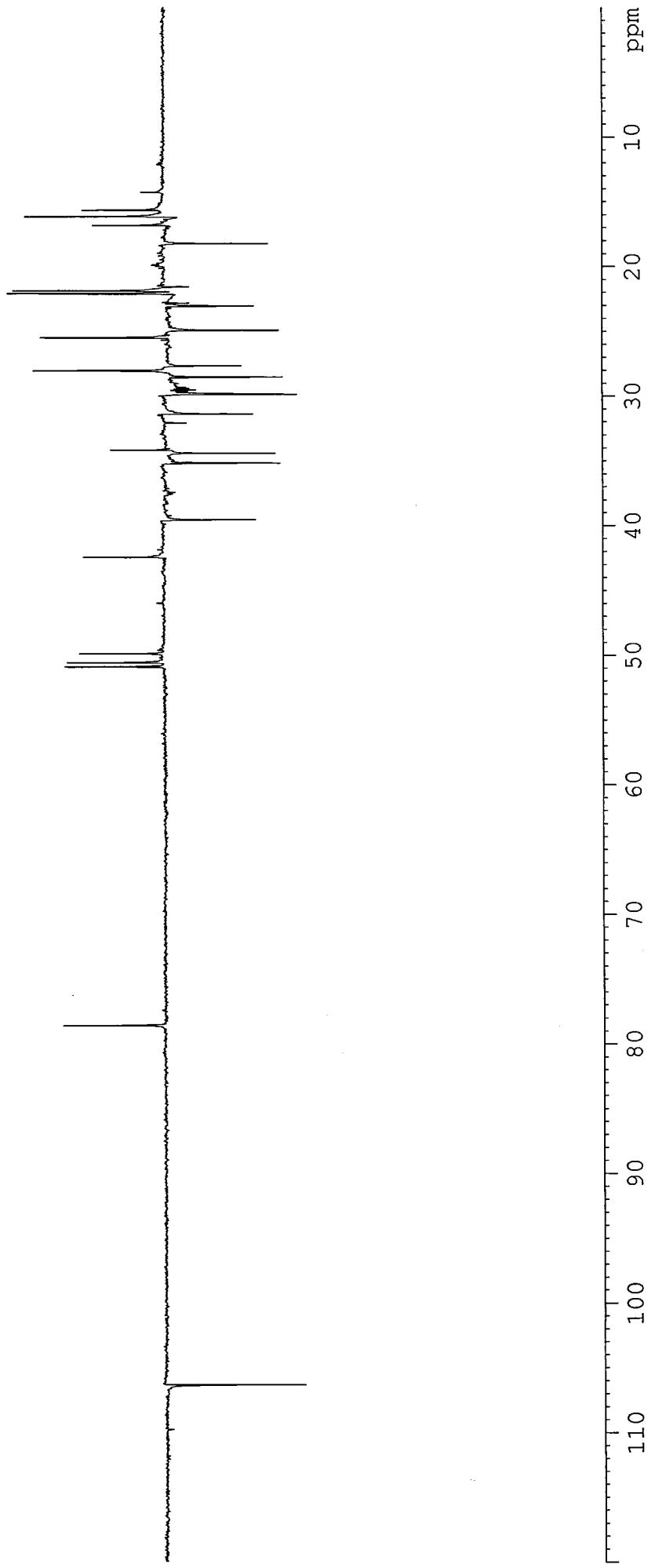
Spectrum 1.9: FTIR spectrum of compound 2.1 ( $\text{CDCl}_3$ )



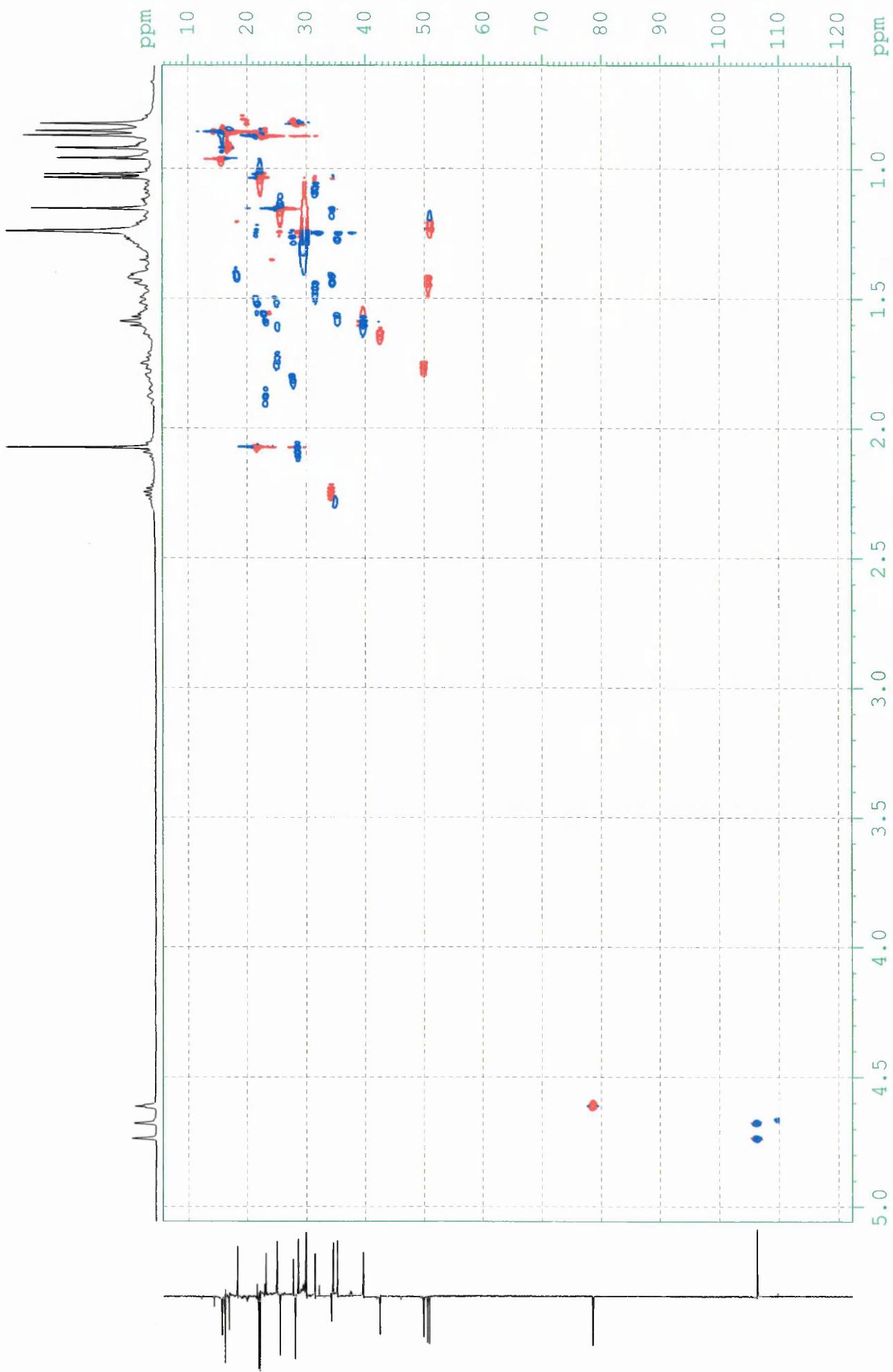
Spectrum 2.1: <sup>1</sup>H NMR spectrum of compound 2.2 (CDCl<sub>3</sub>)



Spectrum 2.2:  $^{13}\text{C}$  NMR spectrum of compound 2.2 ( $\text{CDCl}_3$ )



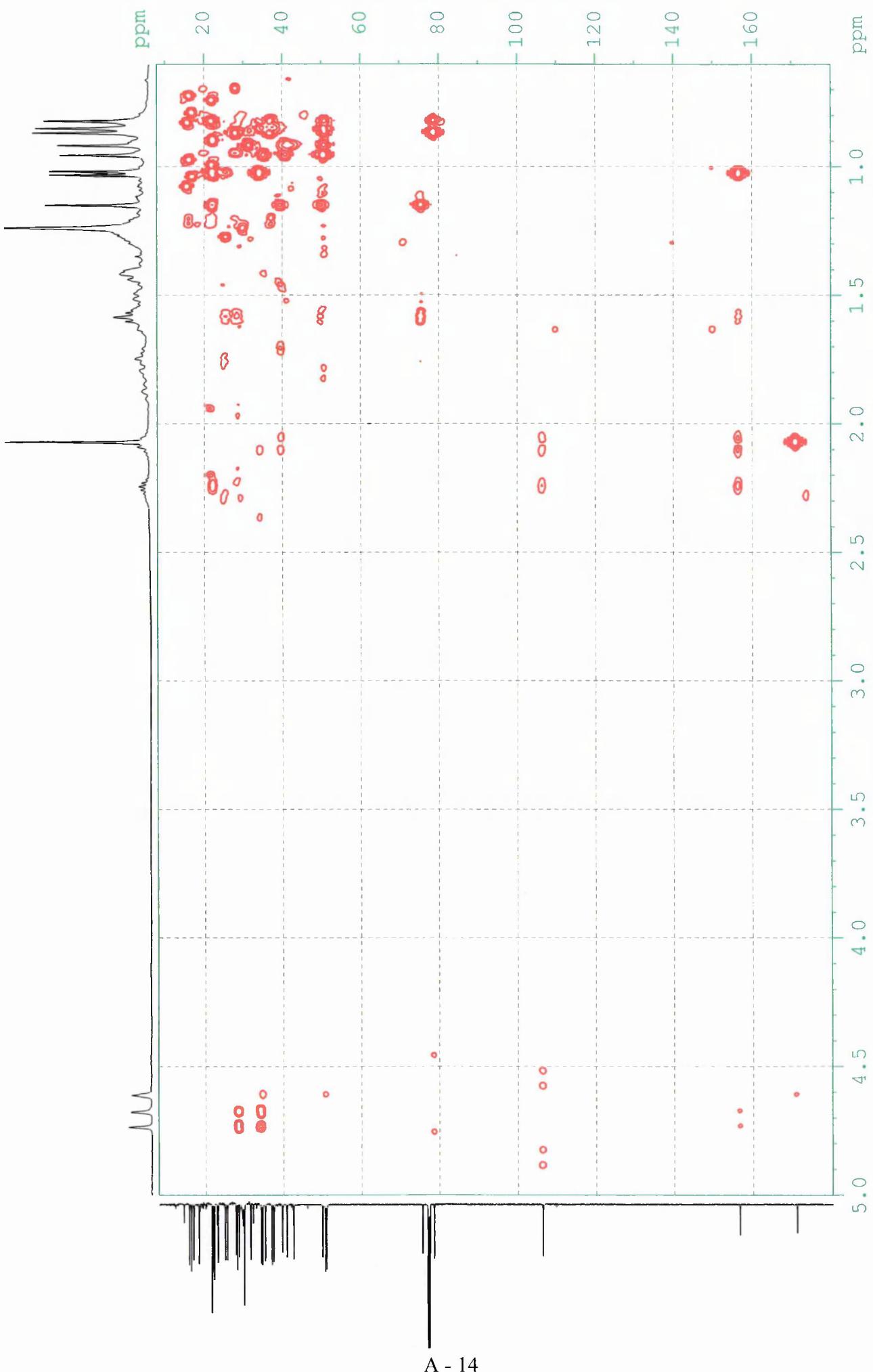
Spectrum 2.3: DEPT spectrum of compound 2.2 ( $\text{CDCl}_3$ )

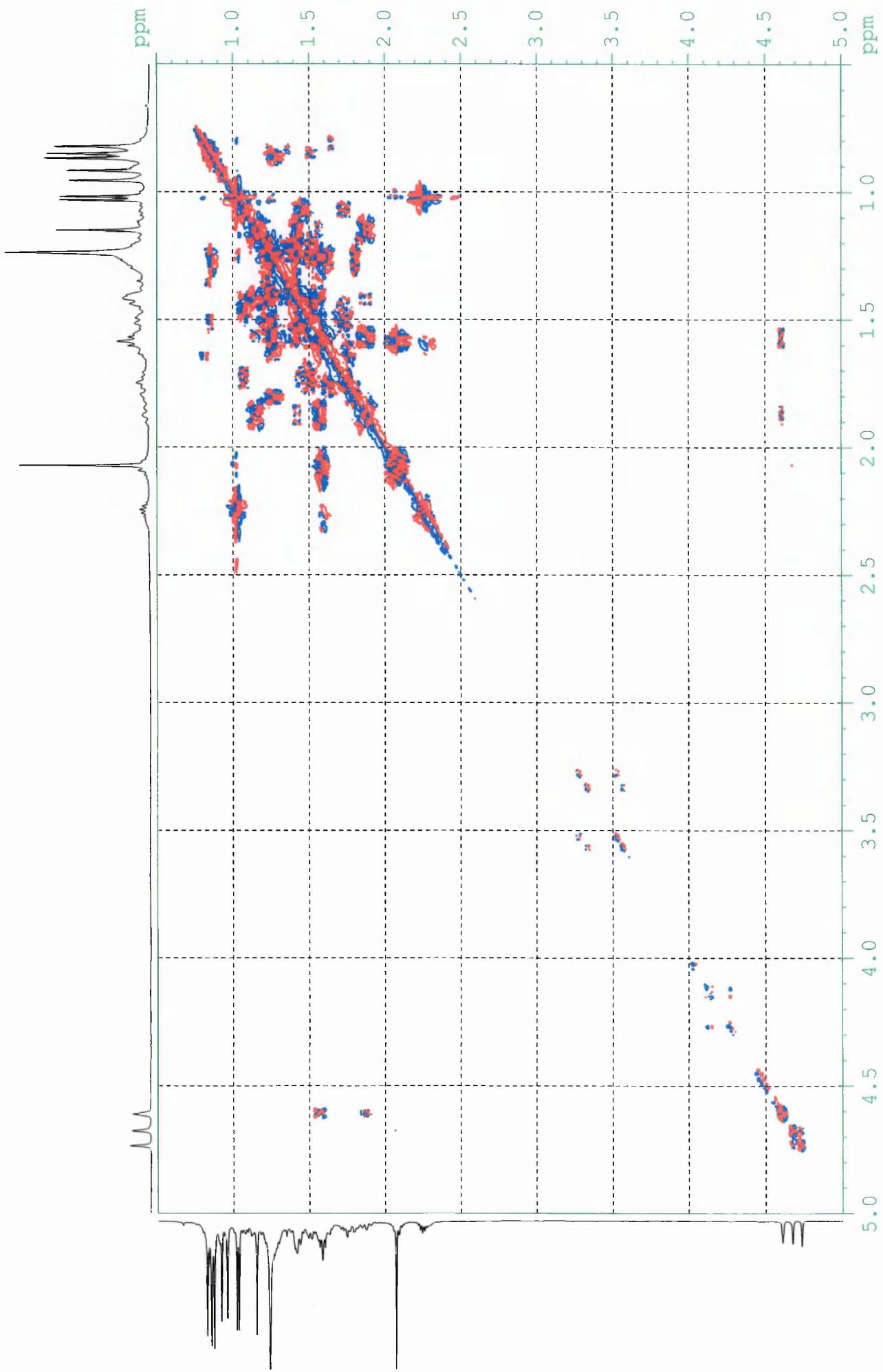


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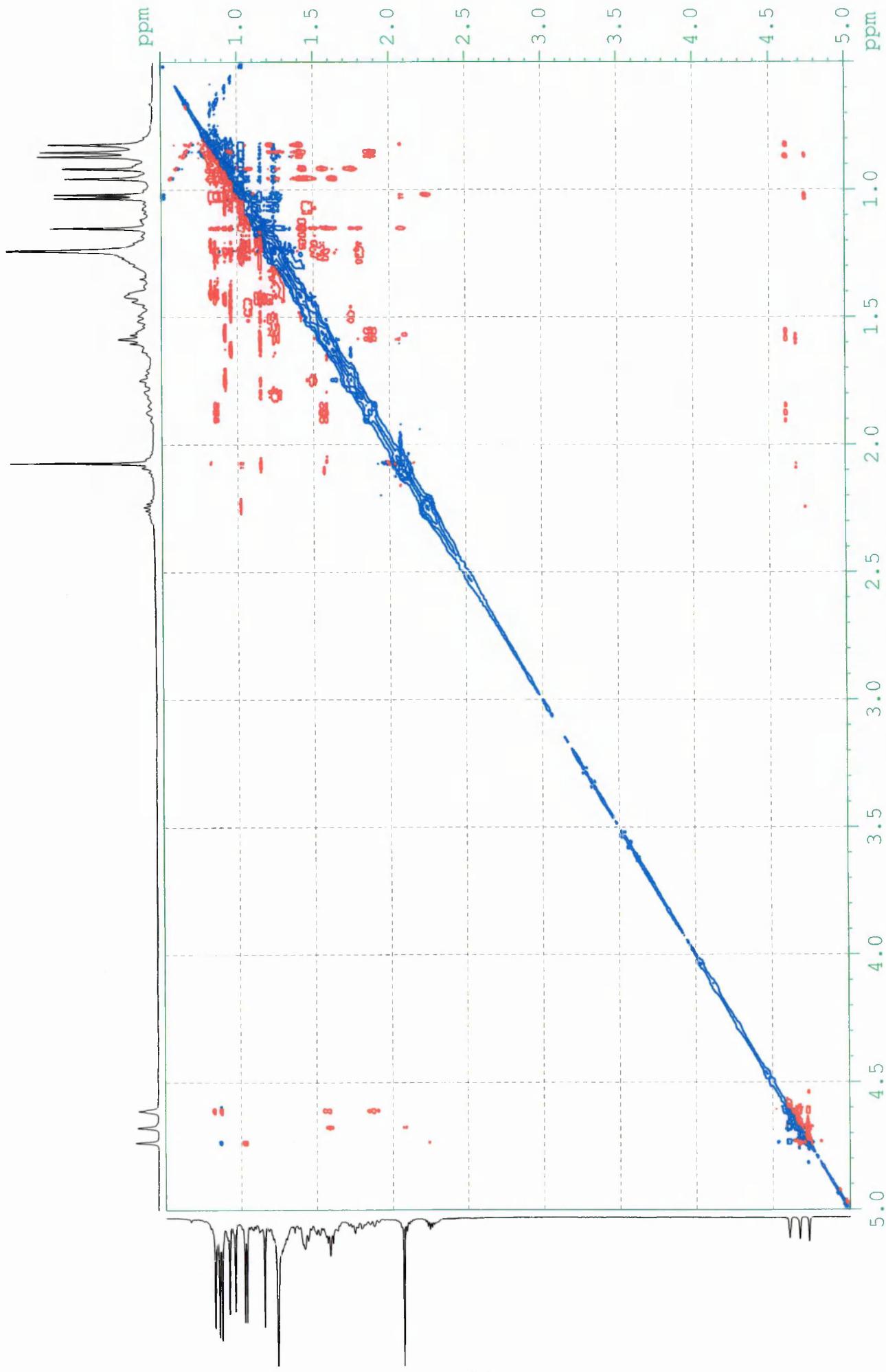
Spectrum 2.4: HSQCDEPT spectrum of compound 2.2 ( $\text{CDCl}_3$ )

**Spectrum 2.5: HMBCLP spectrum of compound 2.2 ( $\text{CDCl}_3$ )**





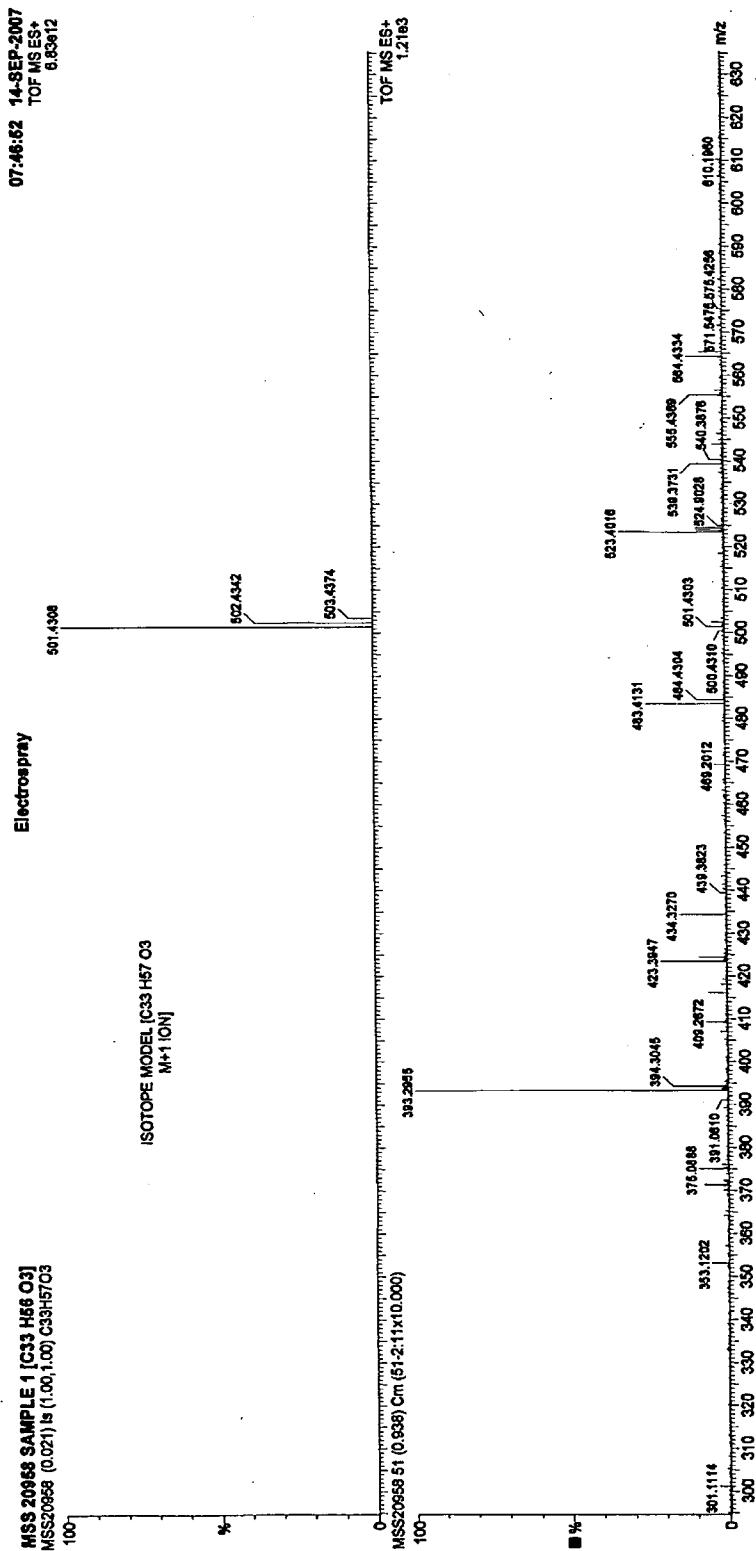
Spectrum 2.6: COSYPH spectrum of compound 2.2 ( $\text{CDCl}_3$ )

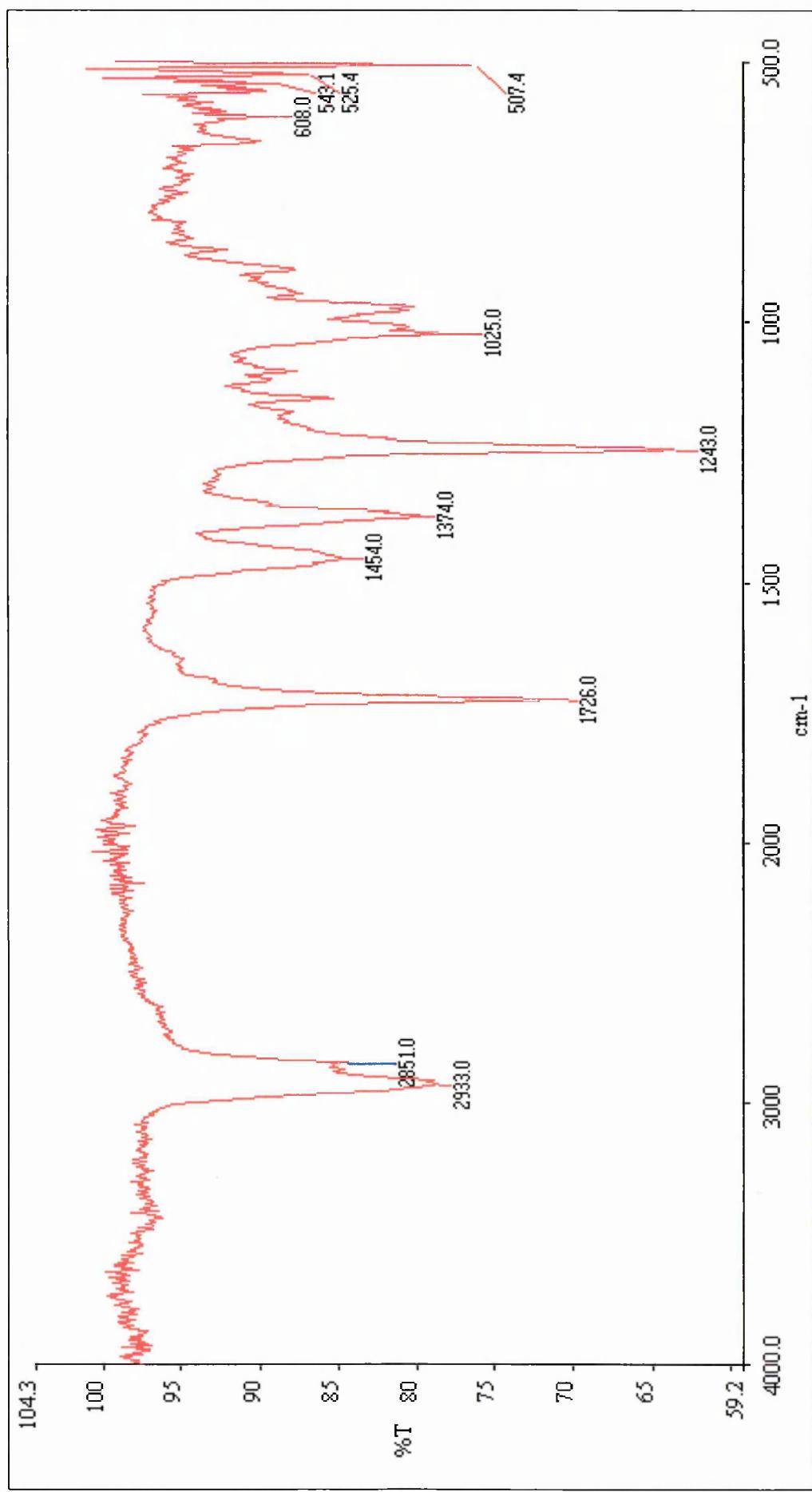


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Spectrum 2.7: NOESY spectrum of compound 2.2 ( $\text{CDCl}_3$ )

Spectrum 2.8: Mass spectrum of compound 2.2



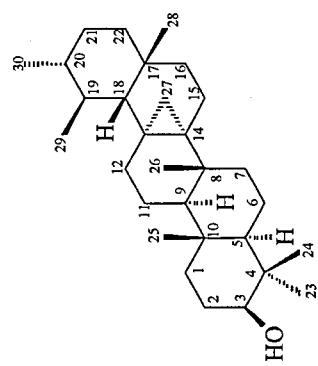


Spectrum 2.9: FTIR spectrum of compound 2.2 ( $\text{CDCl}_3$ )

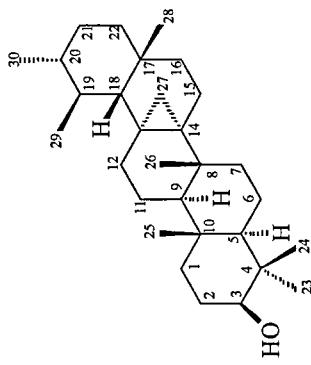
ppm

0  
1  
2  
3  
4  
5  
6  
7

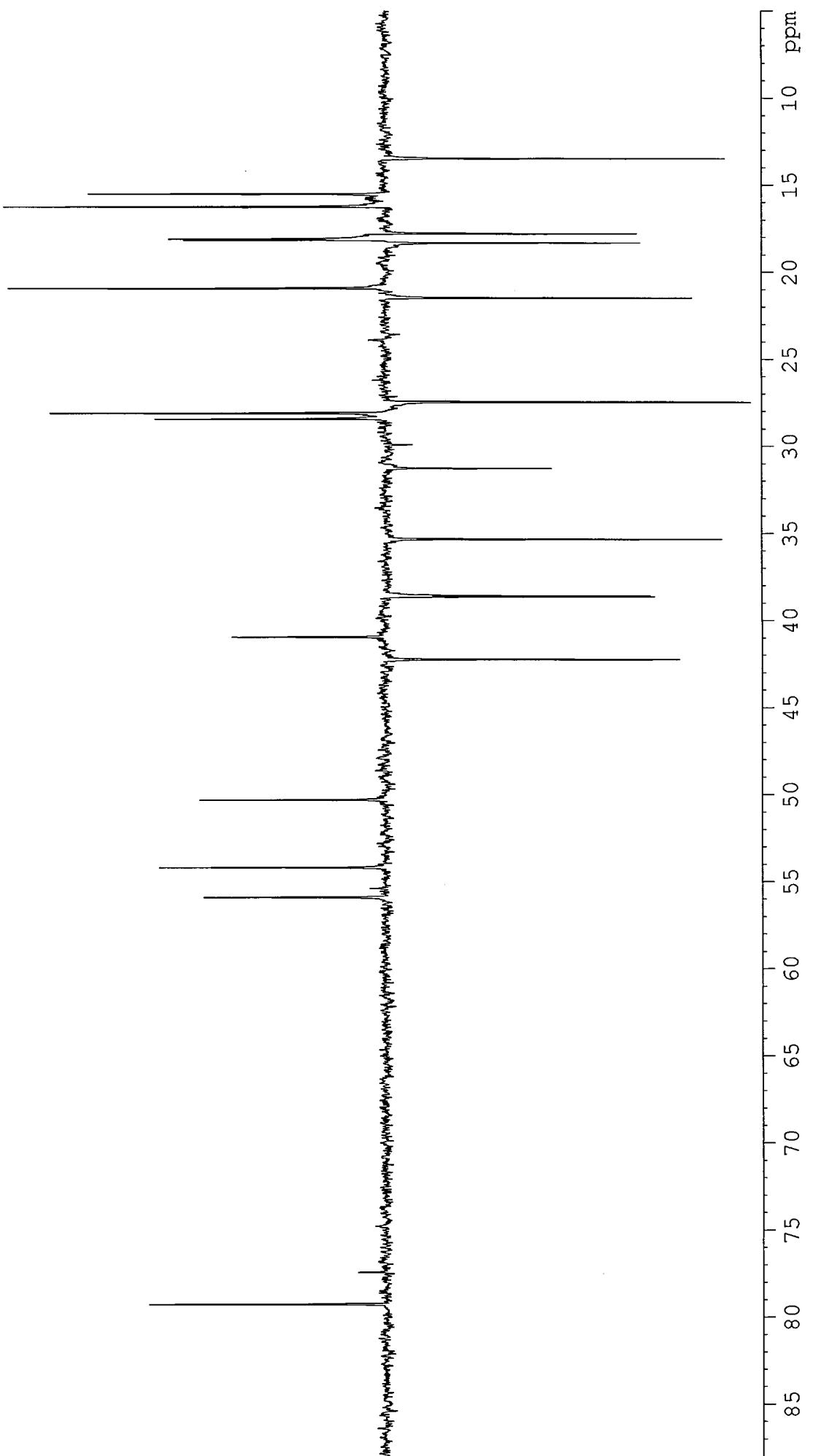
Spectrum 3.1:  $^1\text{H}$  NMR spectrum of compound 2.3 ( $\text{CDCl}_3$ )



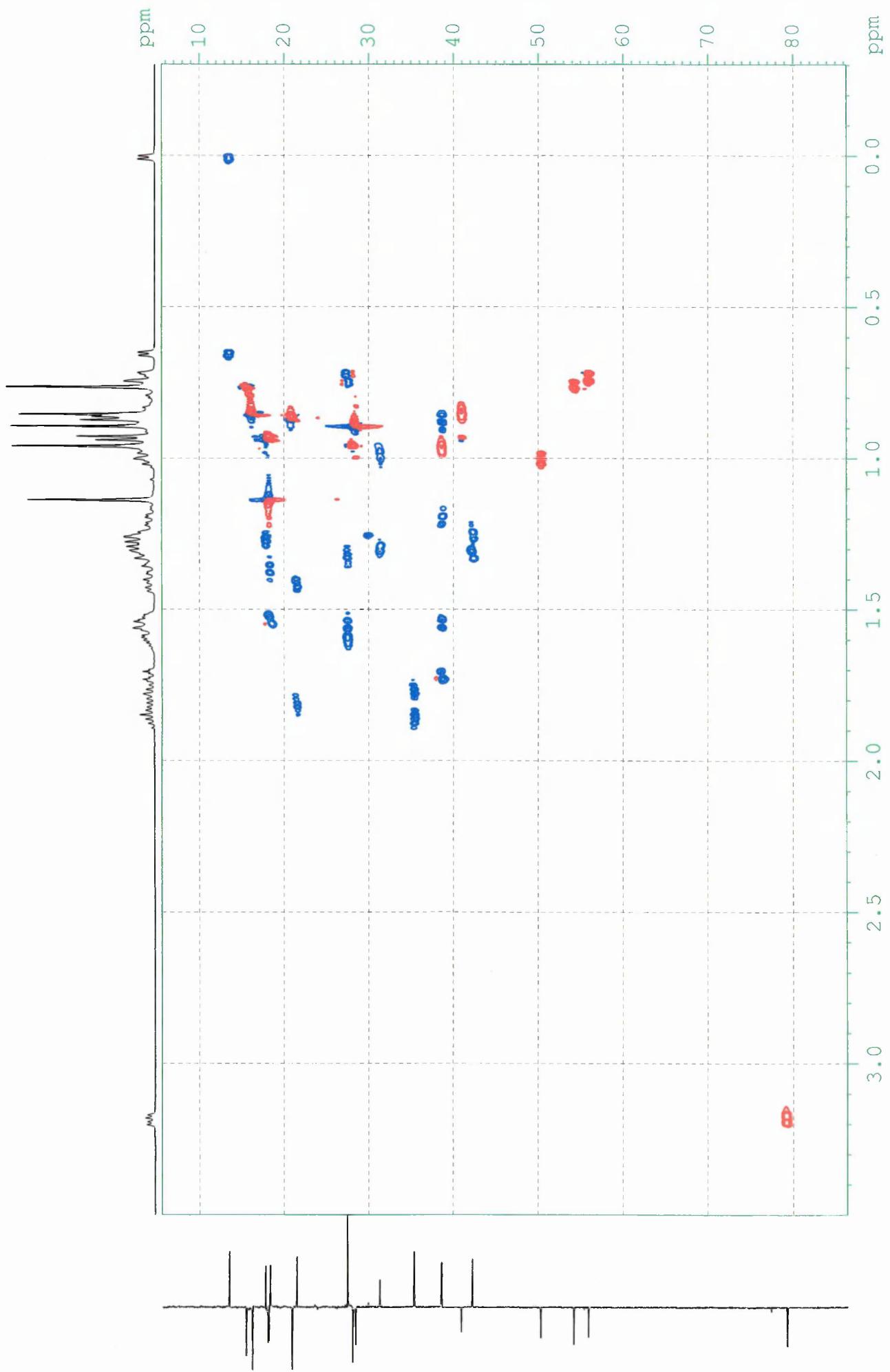
Peak	$\nu(F1)$ [ppm]	$\nu(F1)$ [Hz]	Intensity
1	79.2726	9969.5434	8.58
2	77.4864	9744.9059	18.78
3	77.2324	9712.9622	19.42
4	76.9784	9681.0184	19.50
5	55.9190	7032.5295	8.34
6	54.2114	6817.7770	8.17
7	50.3362	6330.4210	8.37
8	42.2678	5315.7165	8.97
9	40.9737	5152.9669	8.46
10	39.0649	4912.9108	3.15
11	38.6582	4861.7631	10.89
12	38.6194	4856.8835	16.66
13	37.4986	4715.9286	3.08
14	37.1653	4674.0119	2.66
15	35.3641	4447.4879	8.36
16	32.4190	4077.1039	2.42
17	32.1141	4038.7588	3.40
18	31.2950	3935.7465	8.98
19	29.9294	3764.0049	1.20
20	28.4385	3576.5051	9.09
21	28.1035	3534.3746	9.24
22	27.4749	3455.3201	18.37
23	26.7538	3364.6326	2.72
24	21.5009	2704.0132	8.84
25	20.9356	2632.9195	8.95
26	18.3387	2306.3261	9.12
27	18.1821	2285.6316	7.53
28	18.0968	2275.9041	9.47
29	17.8112	2239.9862	8.88
30	16.2578	2044.6263	8.60
31	15.5156	1951.2852	8.42
32	13.5060	1698.5523	8.54



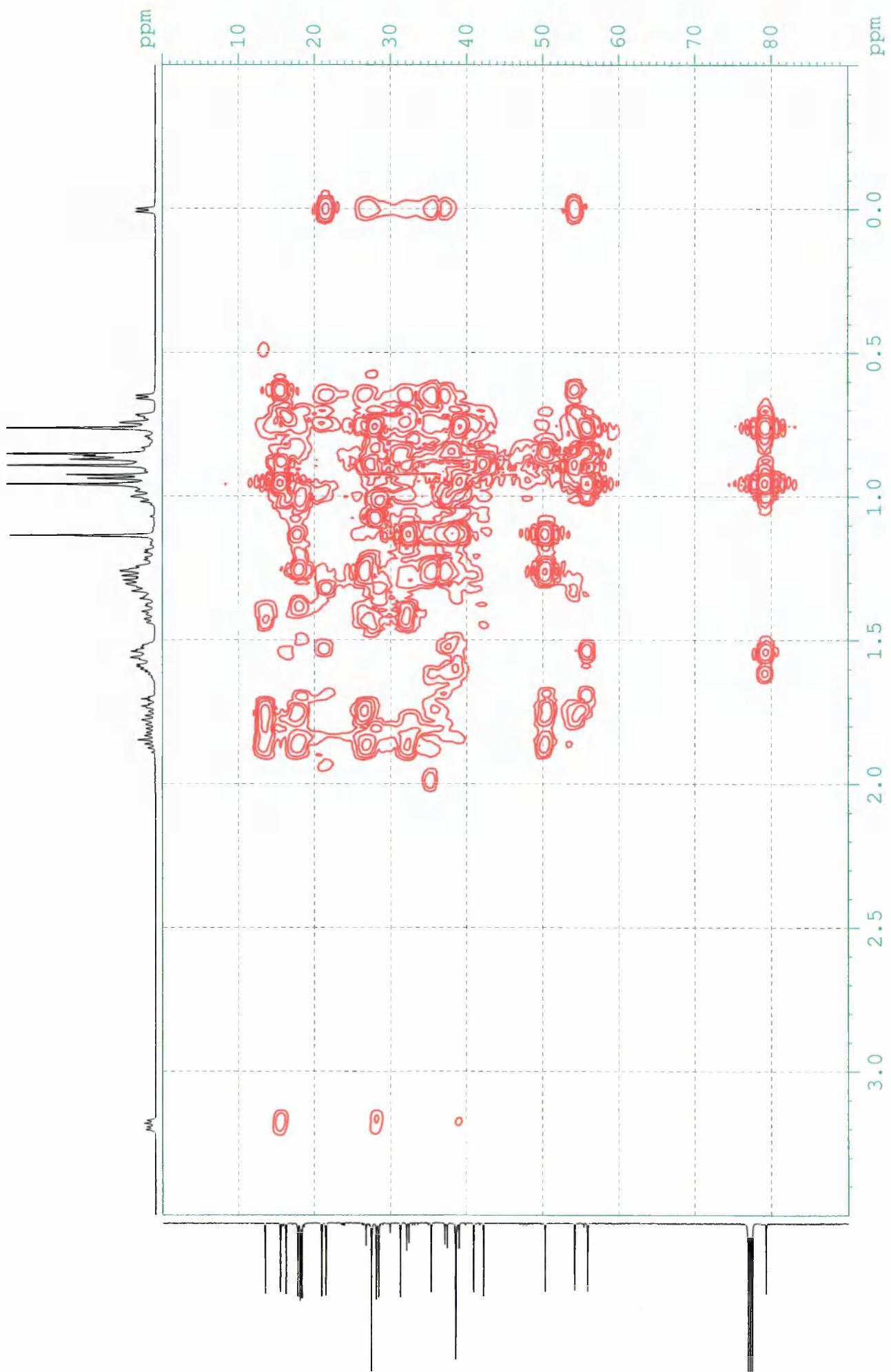
Spectrum 3.2:  $^{13}\text{C}$  NMR spectrum of compound 2.3 ( $\text{CDCl}_3$ )



Spectrum 3.3: DEPT spectrum of compound 2.3 ( $\text{CDCl}_3$ )

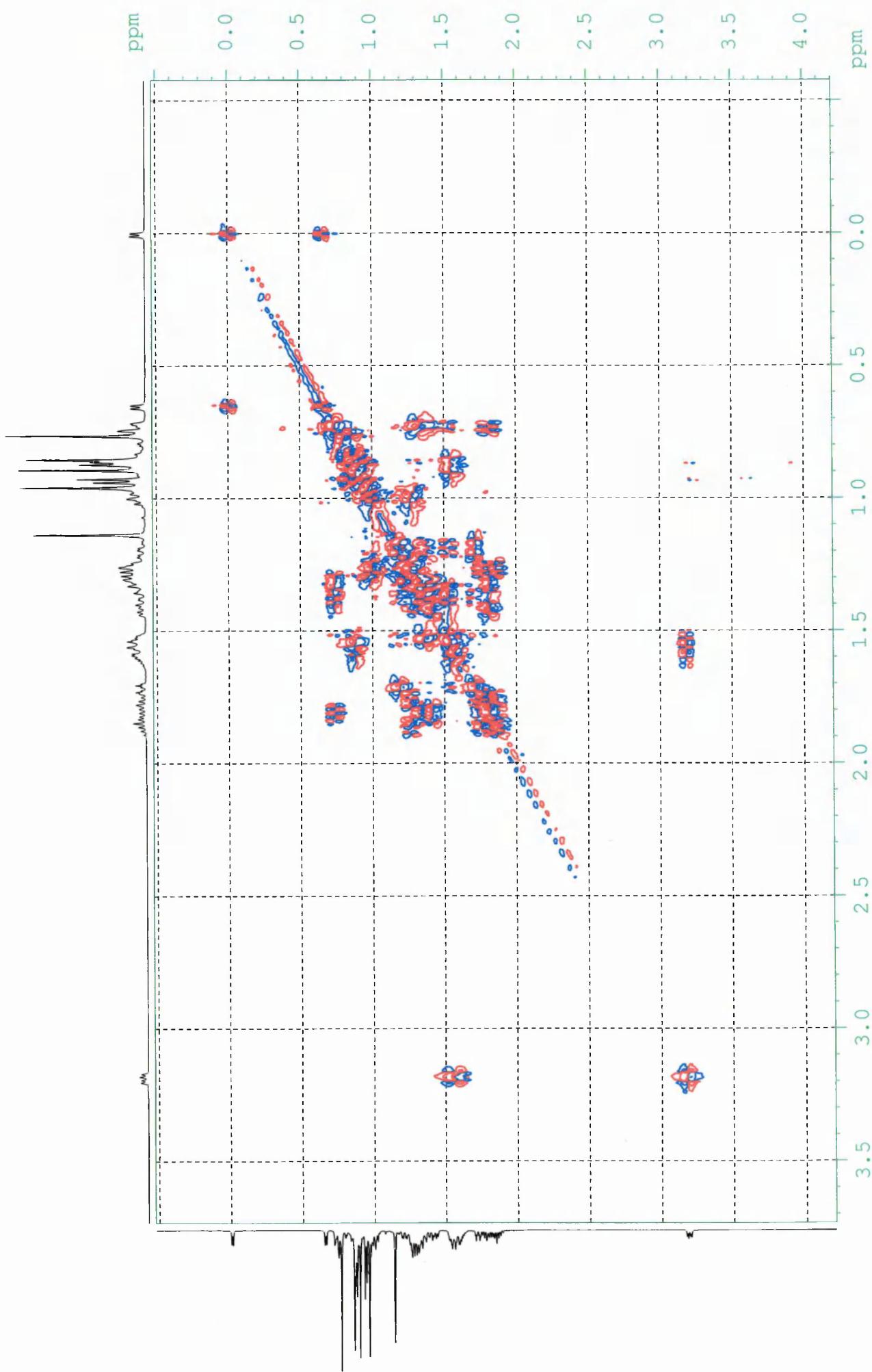


Spectrum 3.4: HSQCDEPT spectrum of compound 2.3 ( $\text{CDCl}_3$ )

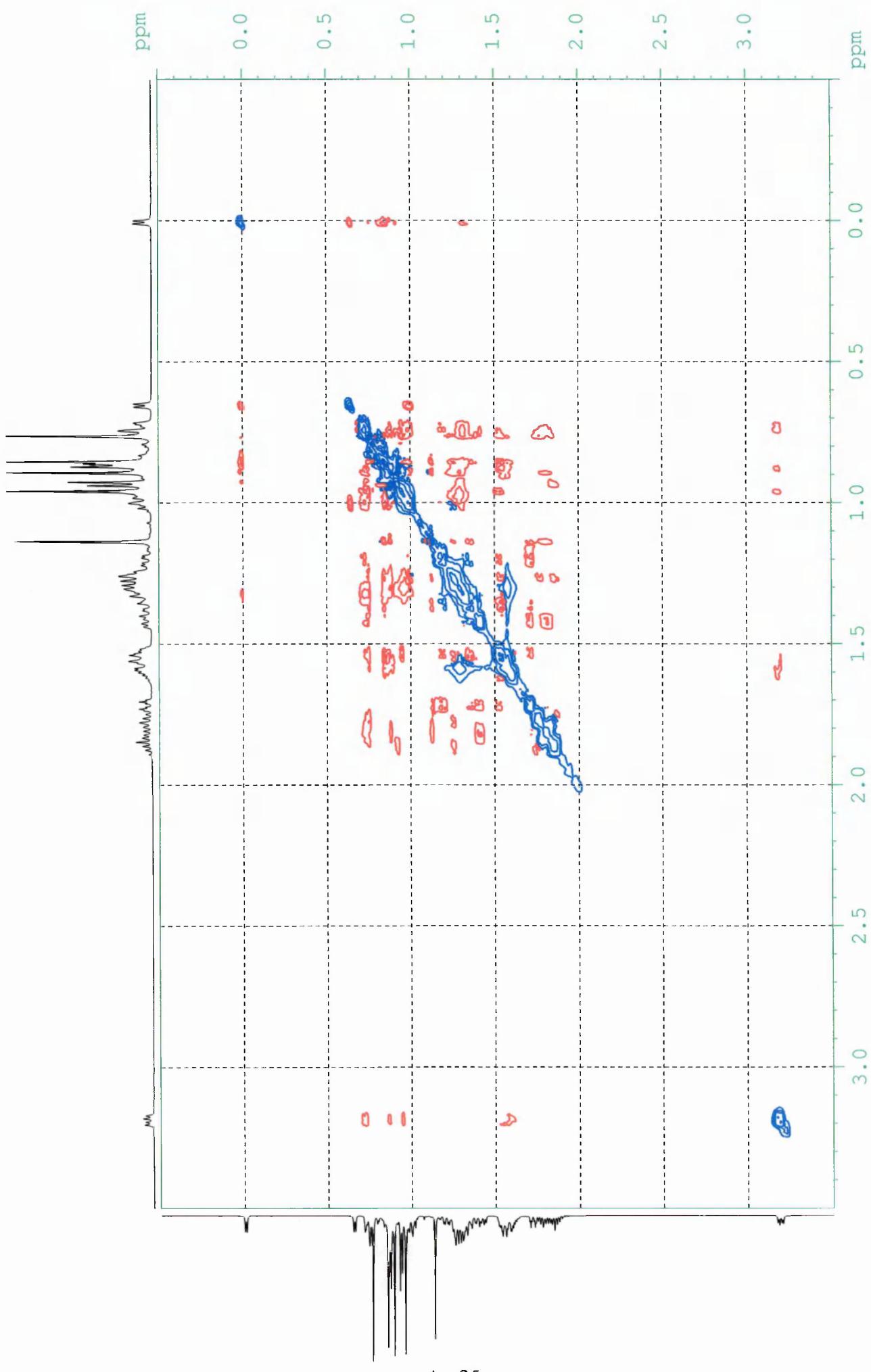


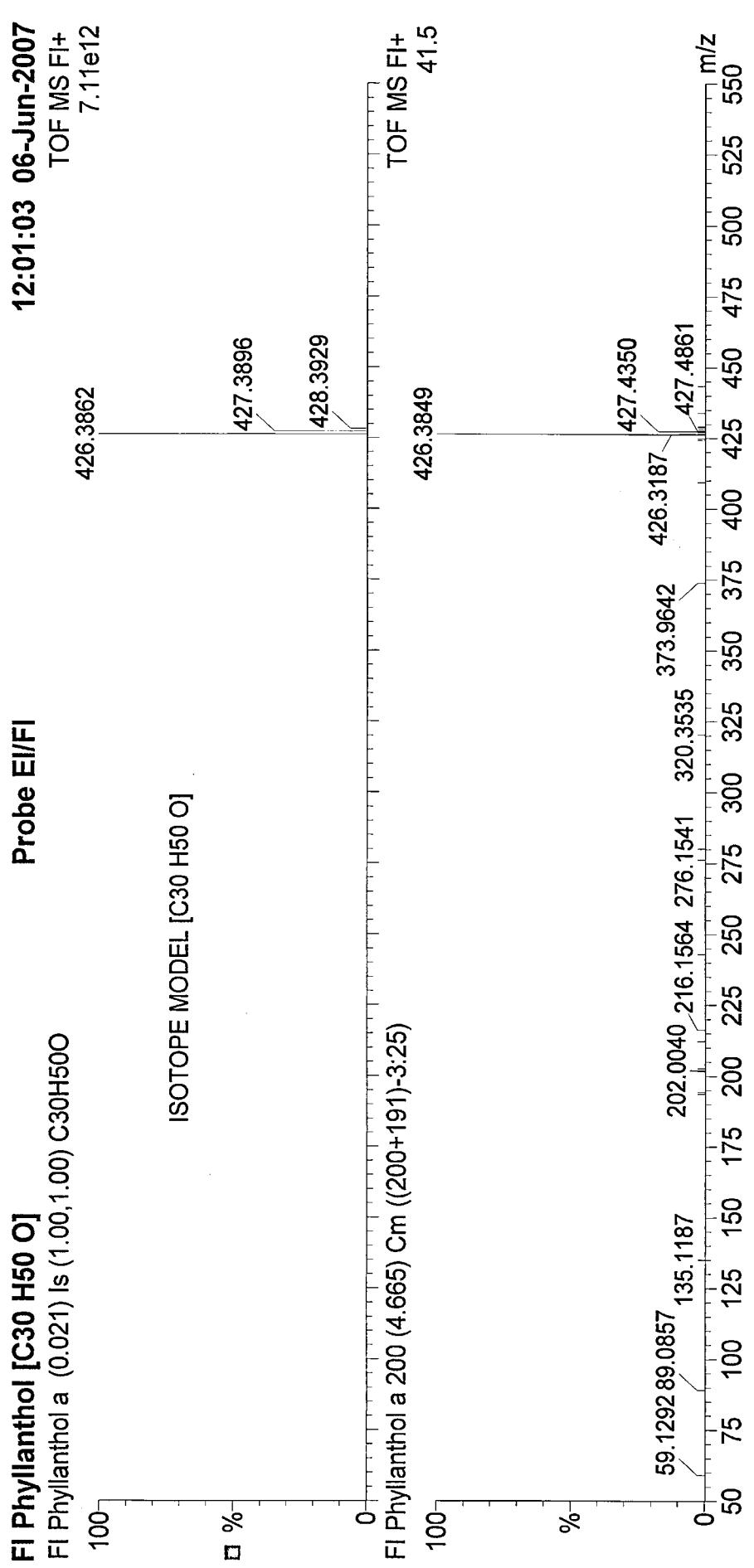
Spectrum 3.5: HMBCCLP spectrum of compound 2.3 ( $\text{CDCl}_3$ )

Spectrum 3.6: COSYPH spectrum of compound 2.3 ( $\text{CDCl}_3$ )

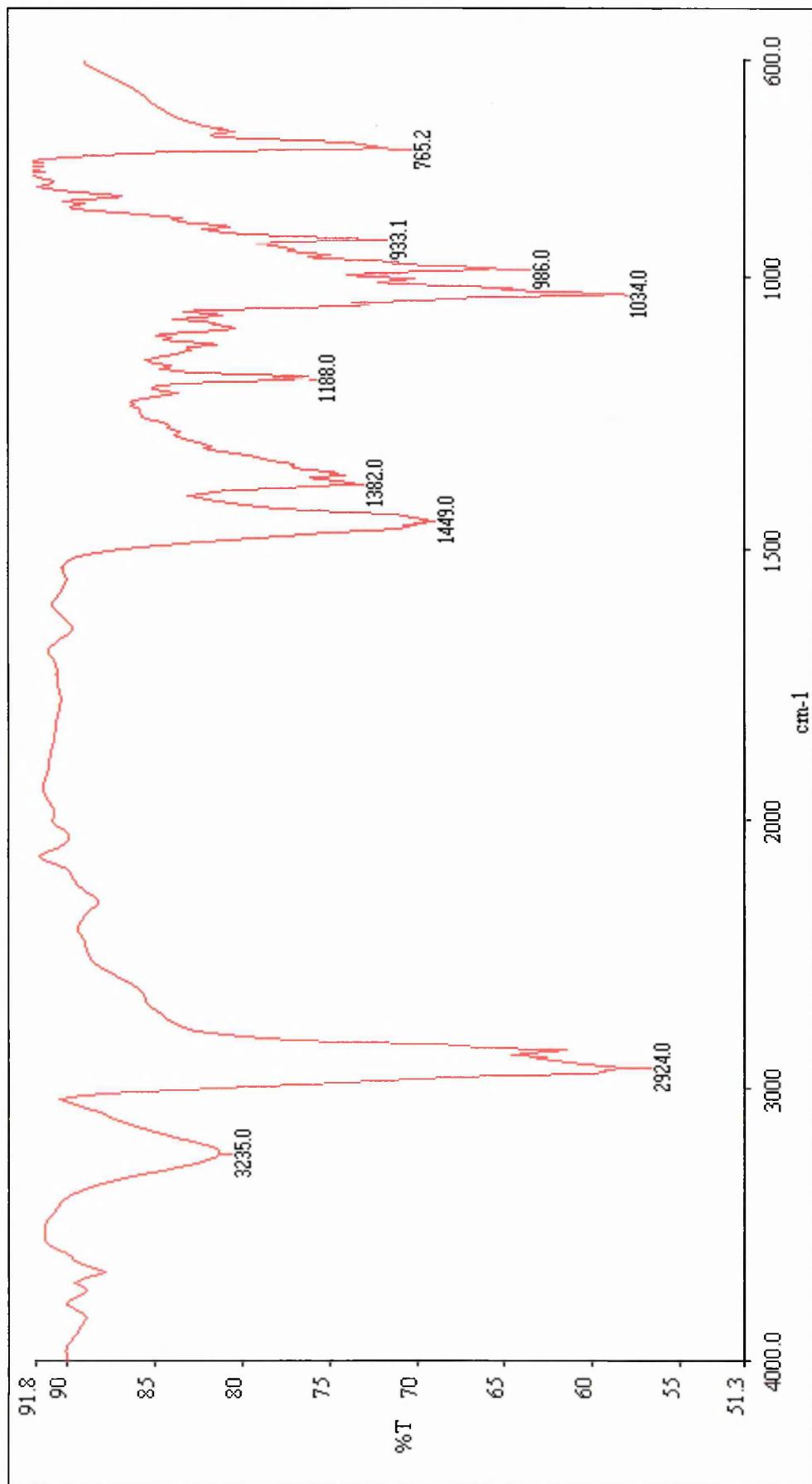


Spectrum 3.7: NOESY spectrum of compound 2.3 ( $\text{CDCl}_3$ )

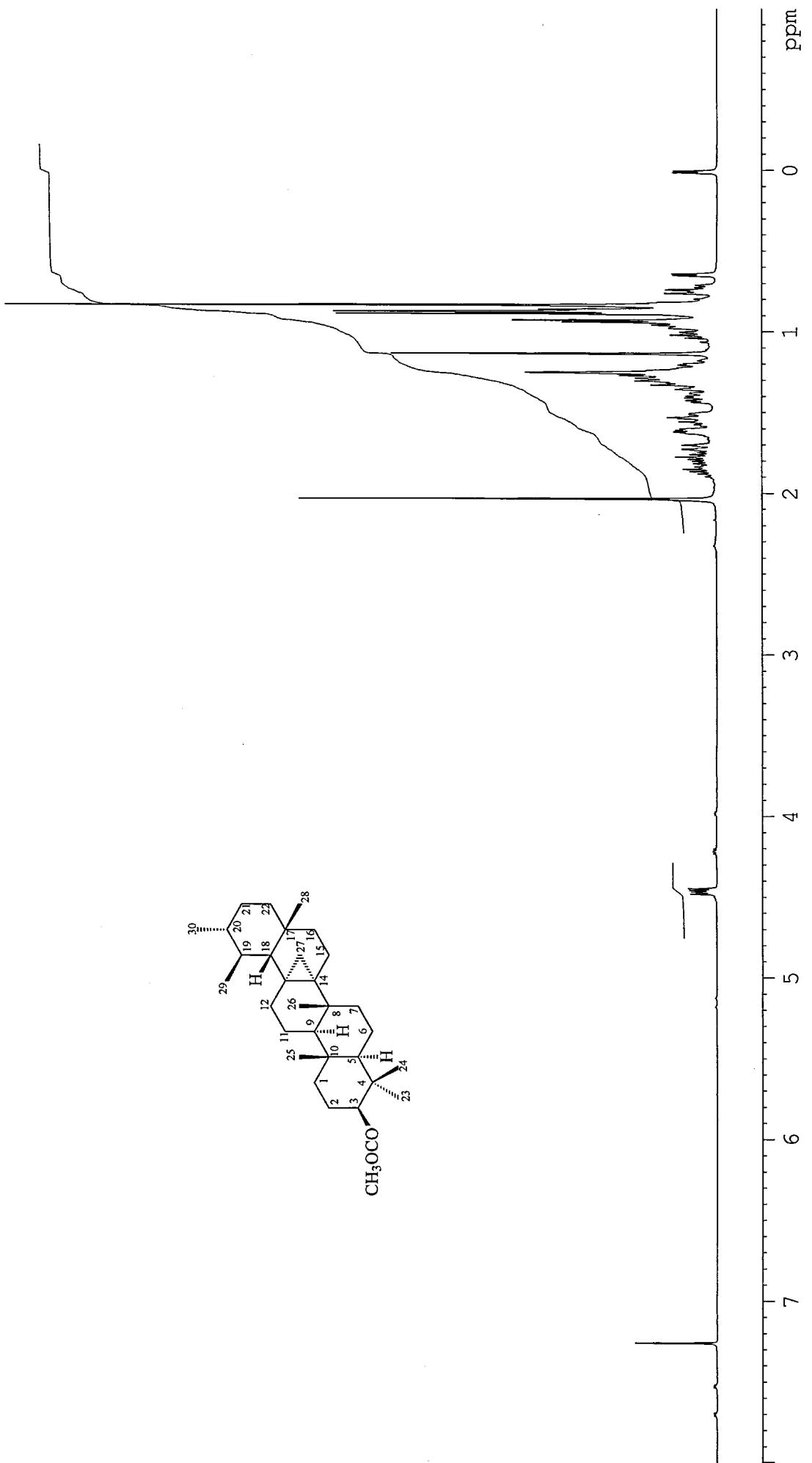




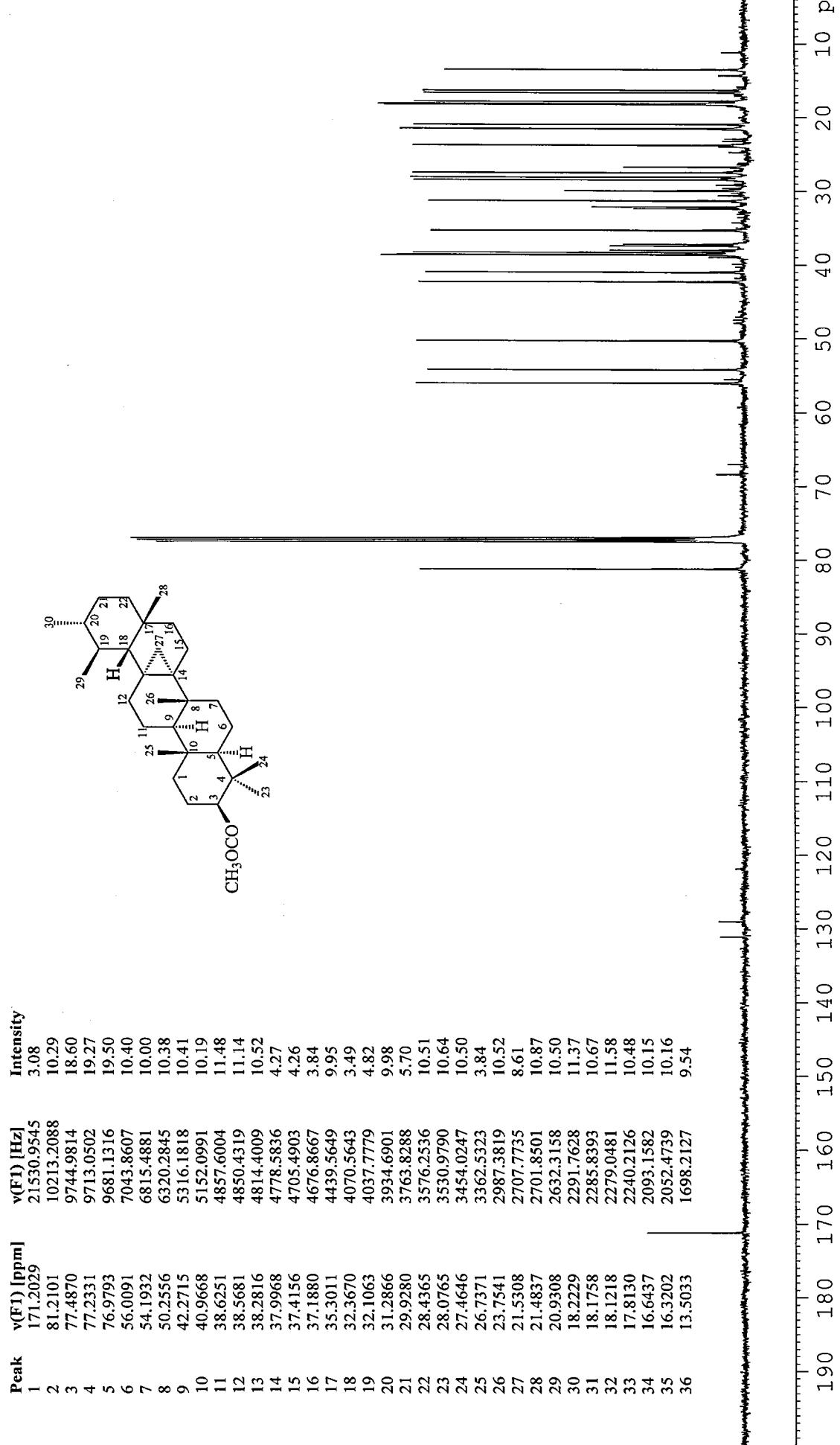
Spectrum 3.8: Mass spectrum of compound 2.3



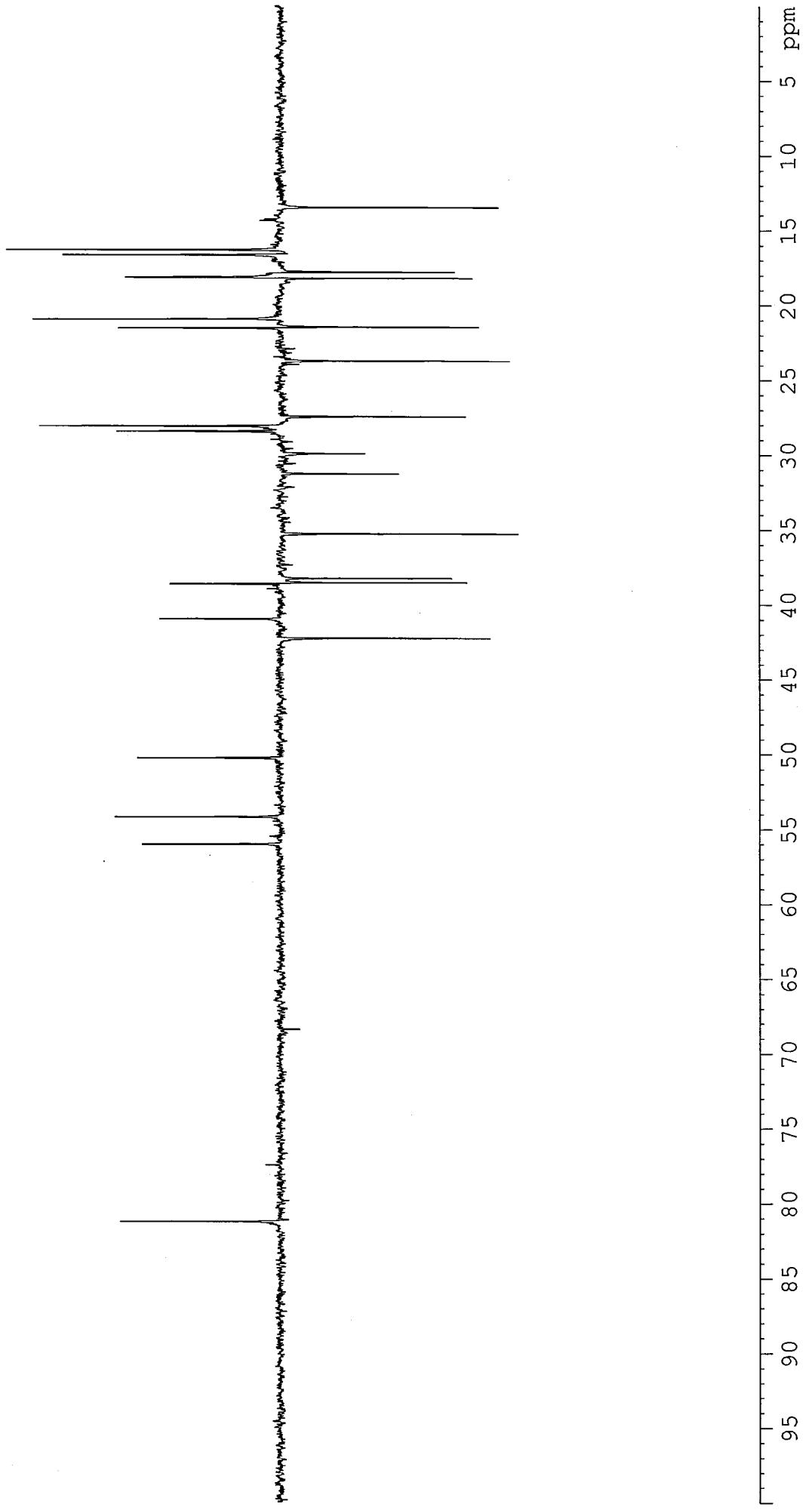
Spectrum 3.9: FTIR spectrum of compound 2.3 ( $\text{CDCl}_3$ )



Spectrum 3.1a:  $^1\text{H}$  NMR spectrum of acetylated compound 2.3 ( $\text{CDCl}_3$ )



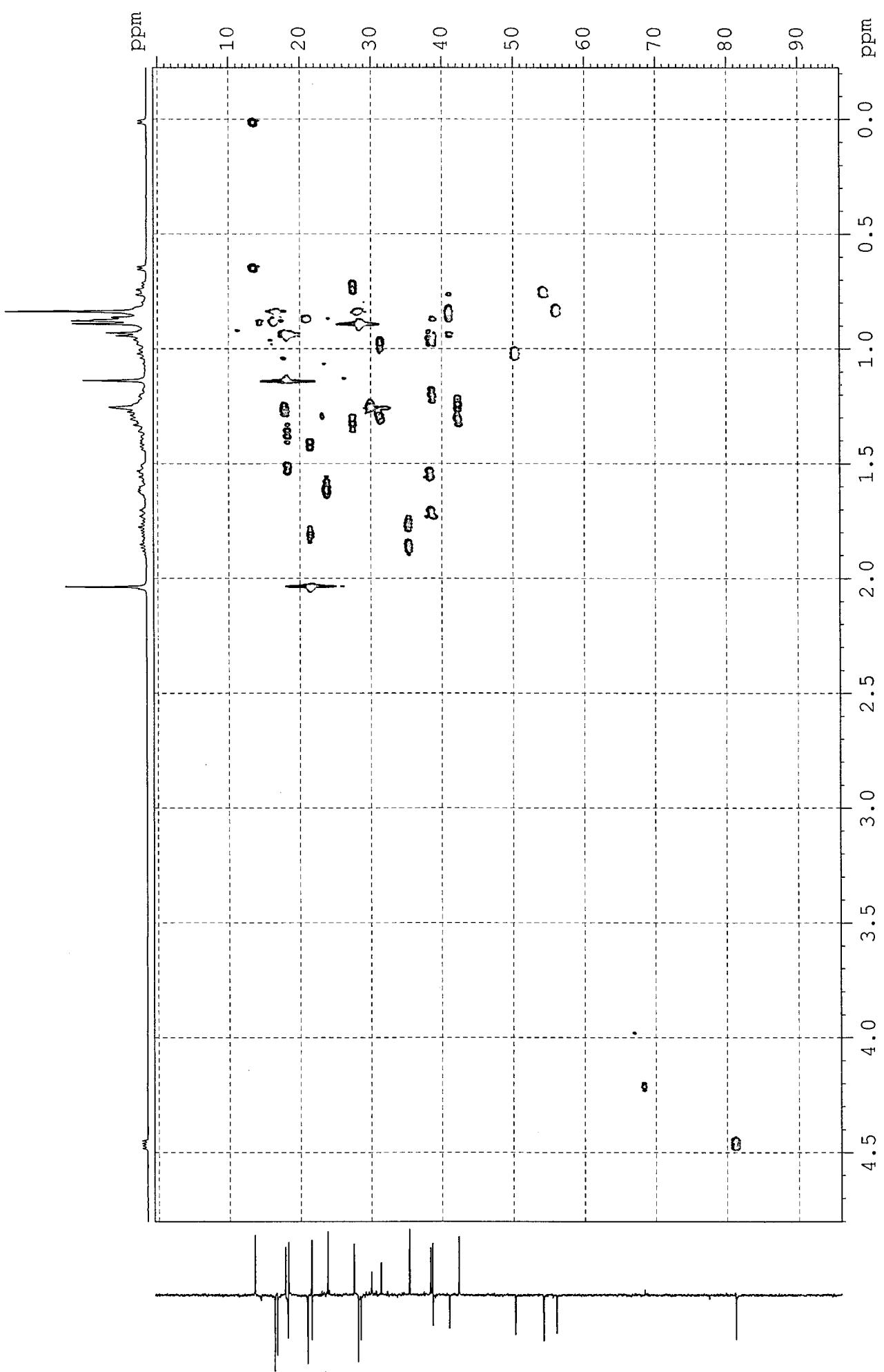
Spectrum 3.2b:  $^{13}\text{C}$  NMR spectrum of acetylated compound 2.3 ( $\text{CDCl}_3$ )



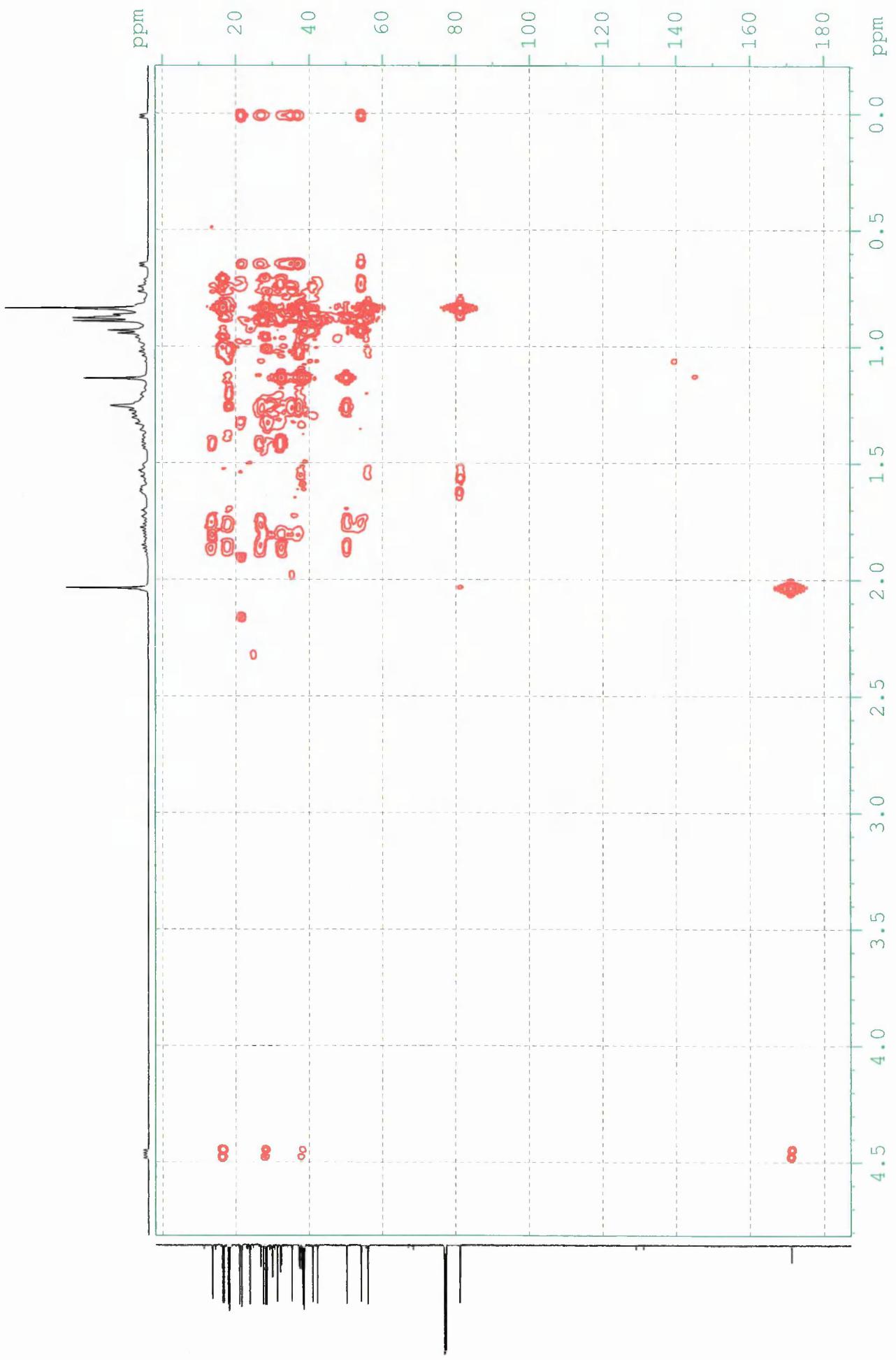
A - 30

Spectrum 3.3c: DEPT spectrum of acetylated compound 2.3 ( $\text{CDCl}_3$ )

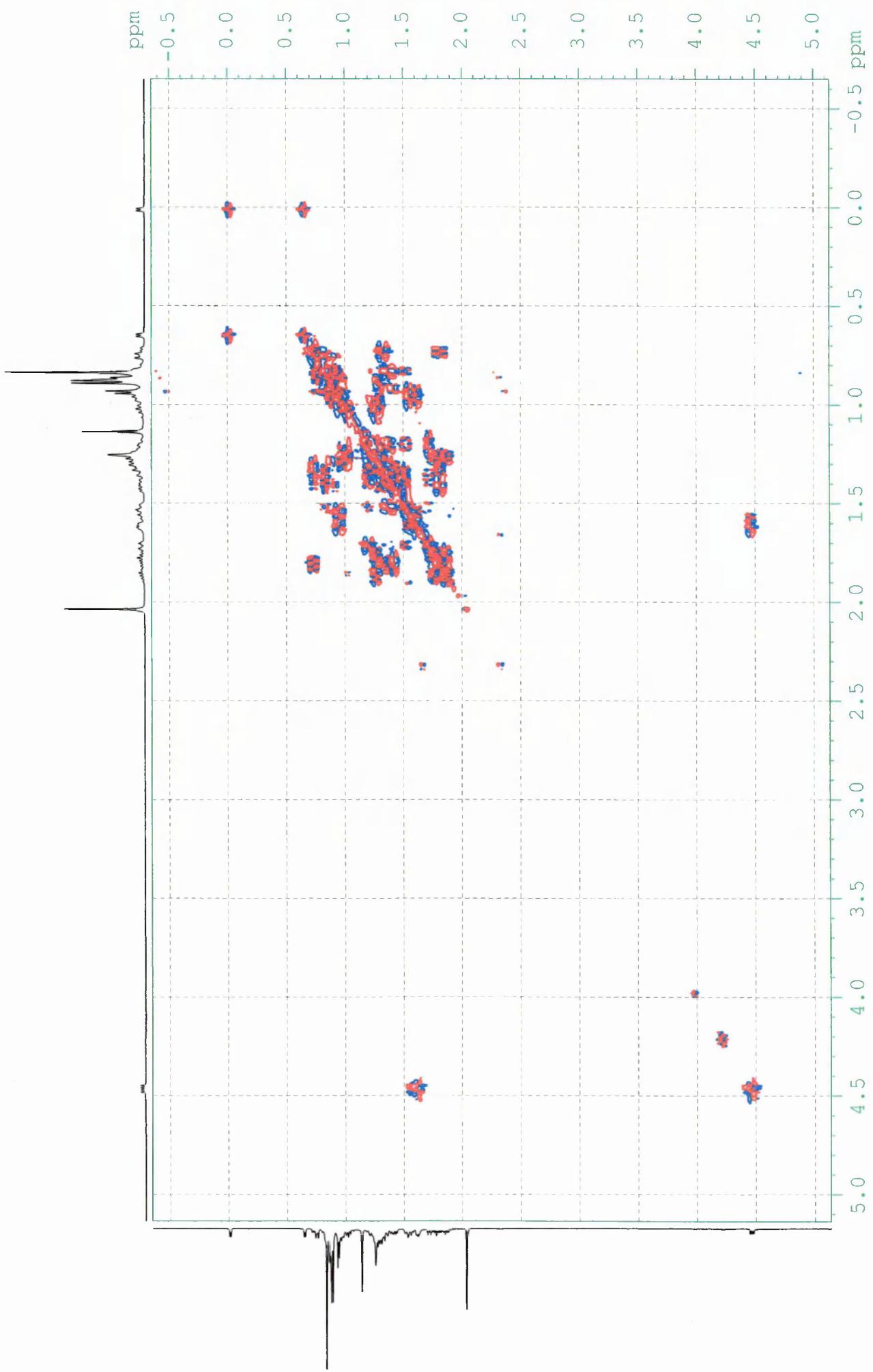
Spectrum 3.4d: HSQCDEPT spectrum of acetylated compound 2.3 ( $\text{CDCl}_3$ )



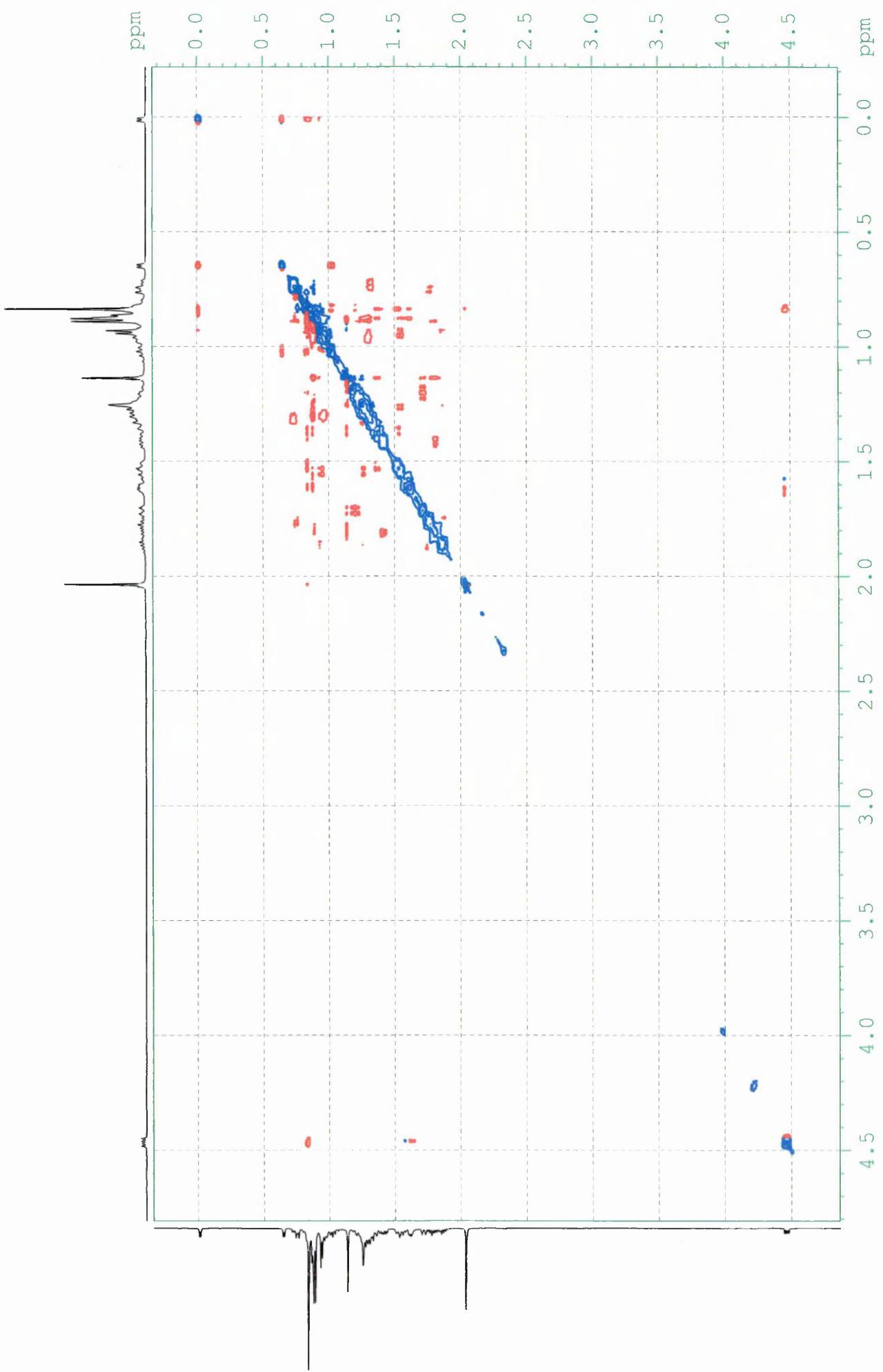
**Spectrum 3.5e:** HMBCLP spectrum of acetylated compound 2.3 ( $\text{CDCl}_3$ )



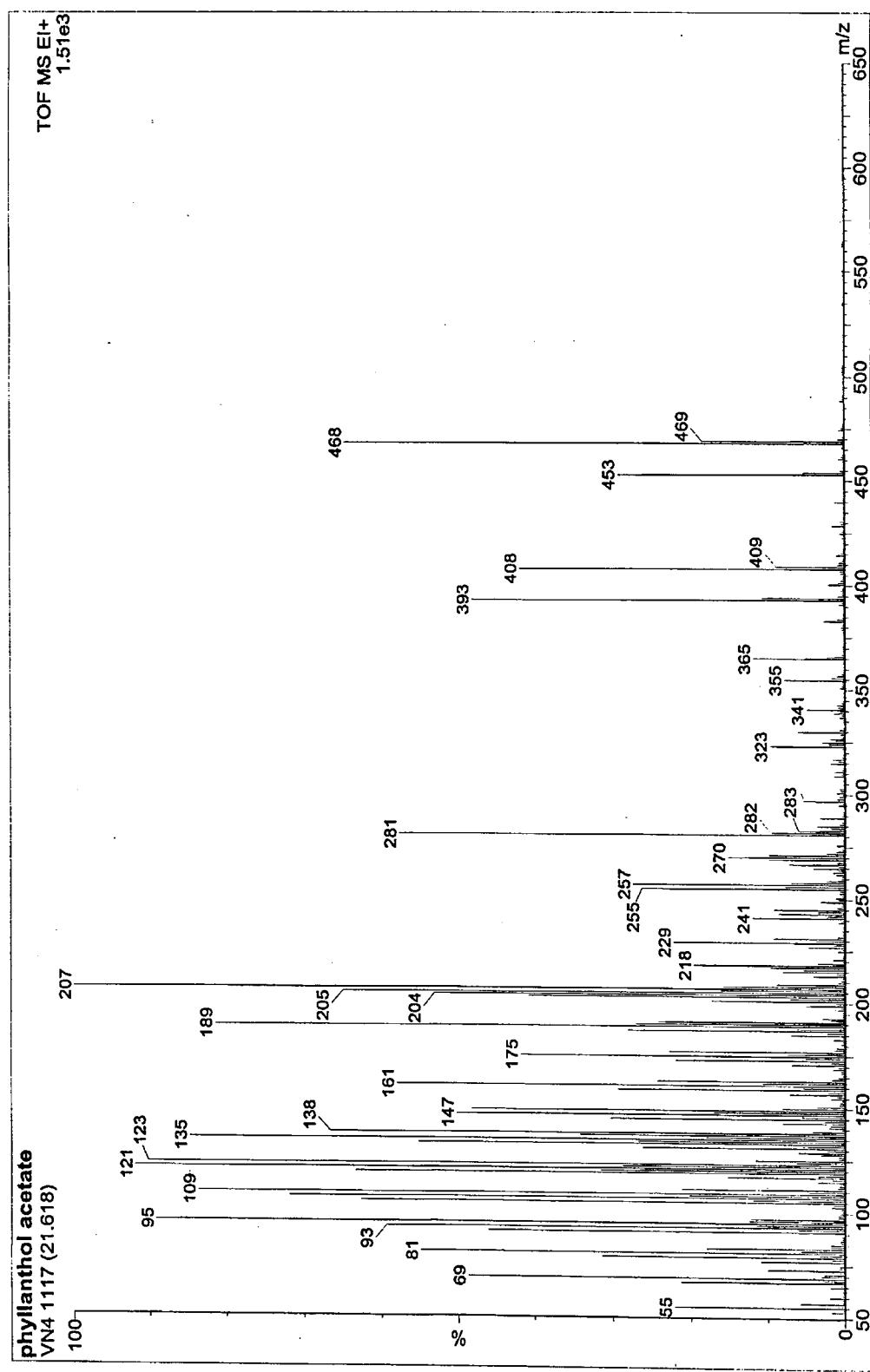
**Spectrum 3.6f:** COSYPH spectrum of acetylated compound 2.3 ( $\text{CDCl}_3$ )

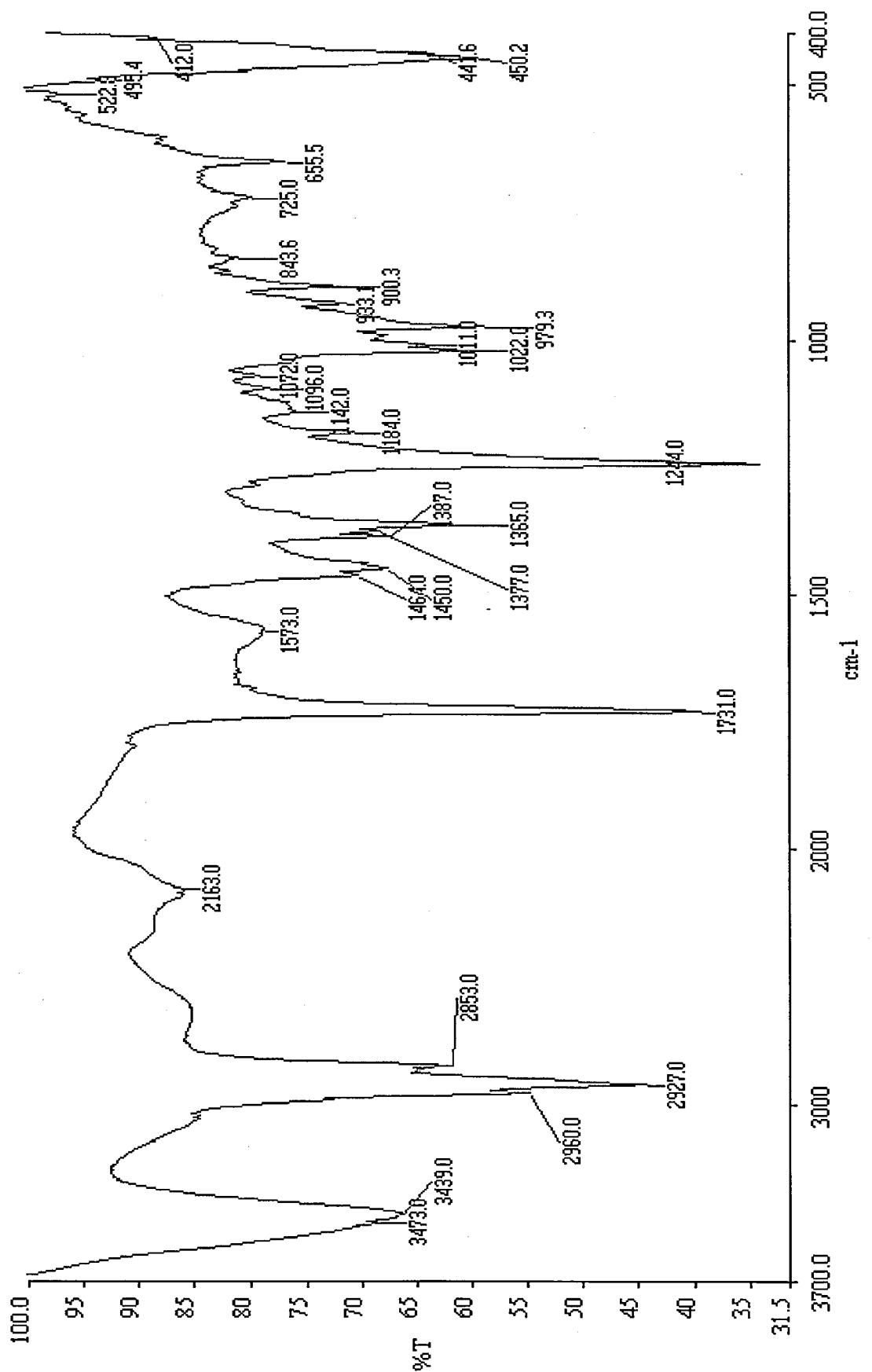


Spectrum 3.7g: NOESY spectrum of acetylated compound 2.3 ( $\text{CDCl}_3$ )



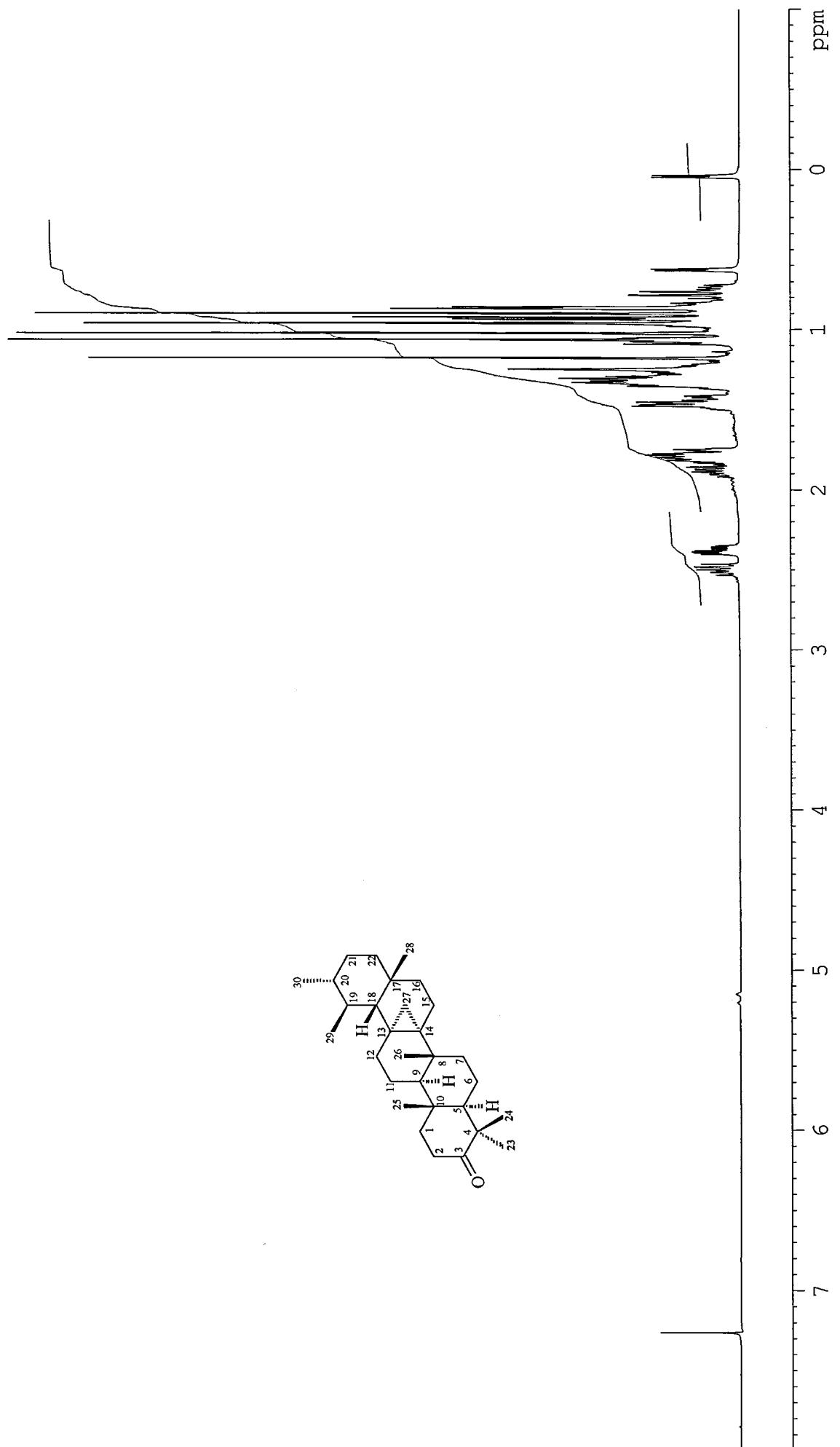
Spectrum 3.8: Mass spectrum of acetylated compound 2.3

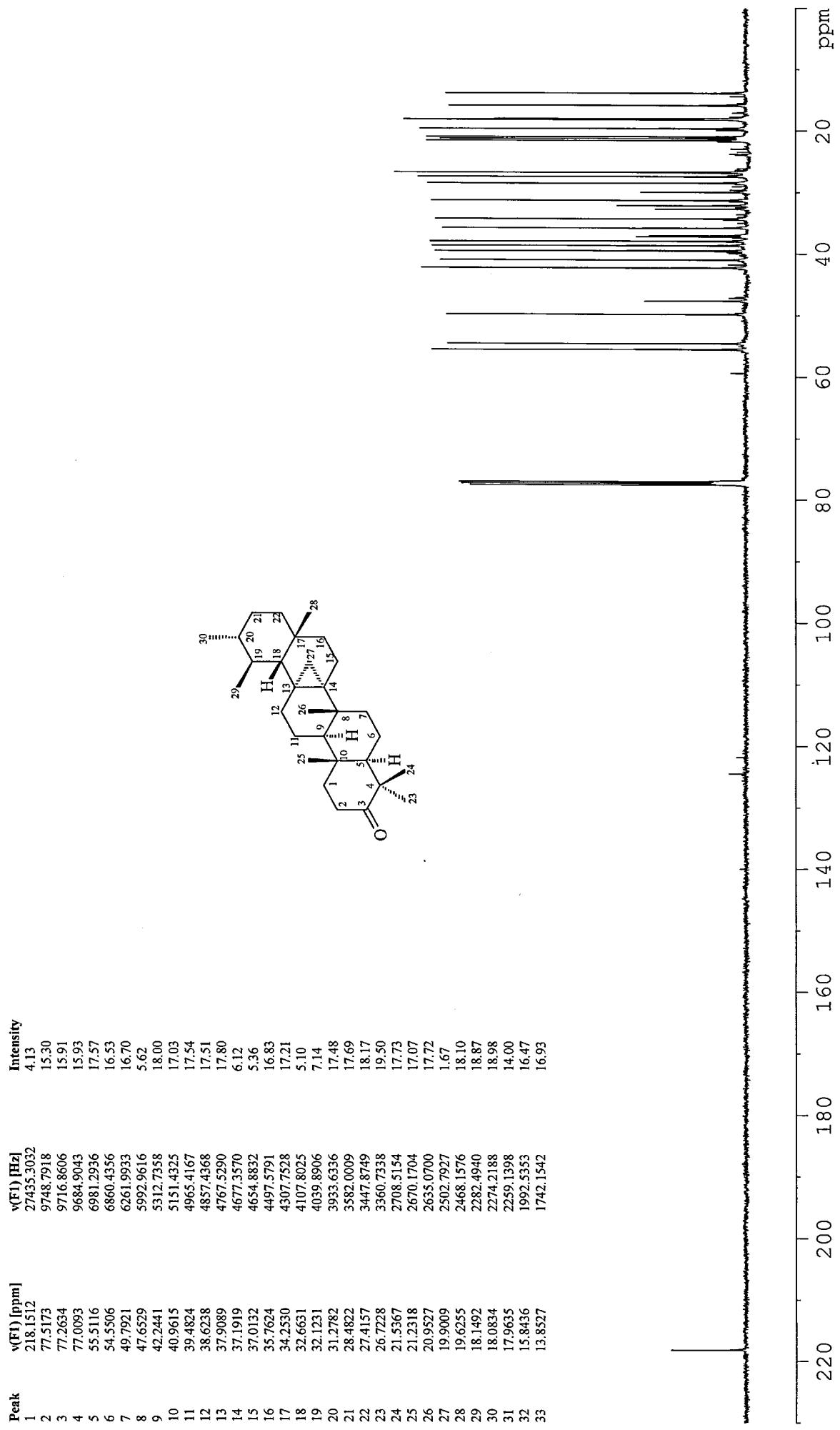




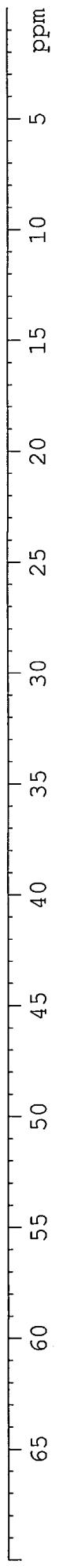
Spectrum 3.9: FTIR spectrum of acetylated compound 2.3

Spectrum 4.1:  $^1\text{H}$  NMR spectrum of compound 2.4 ( $\text{CDCl}_3$ )

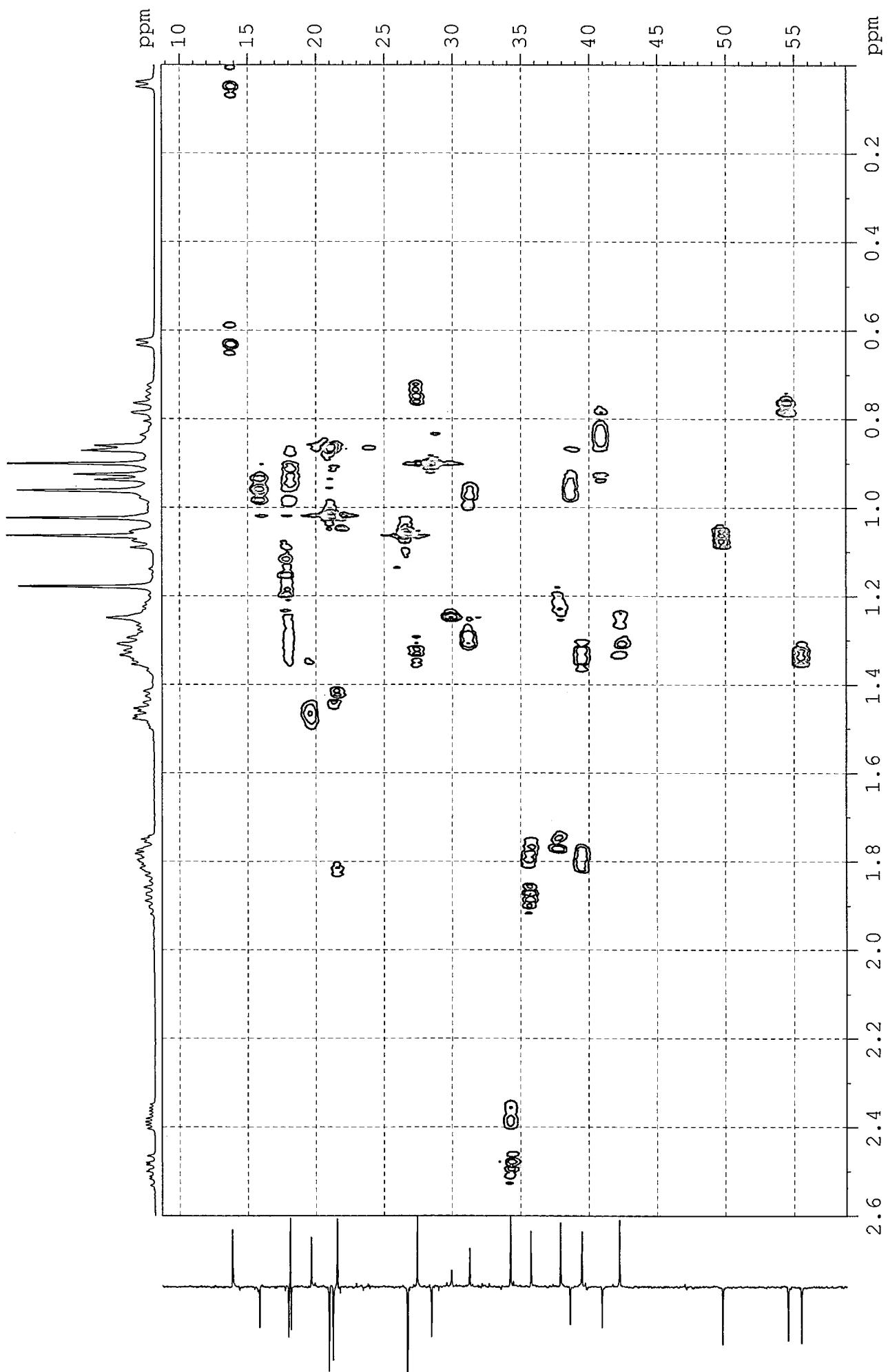




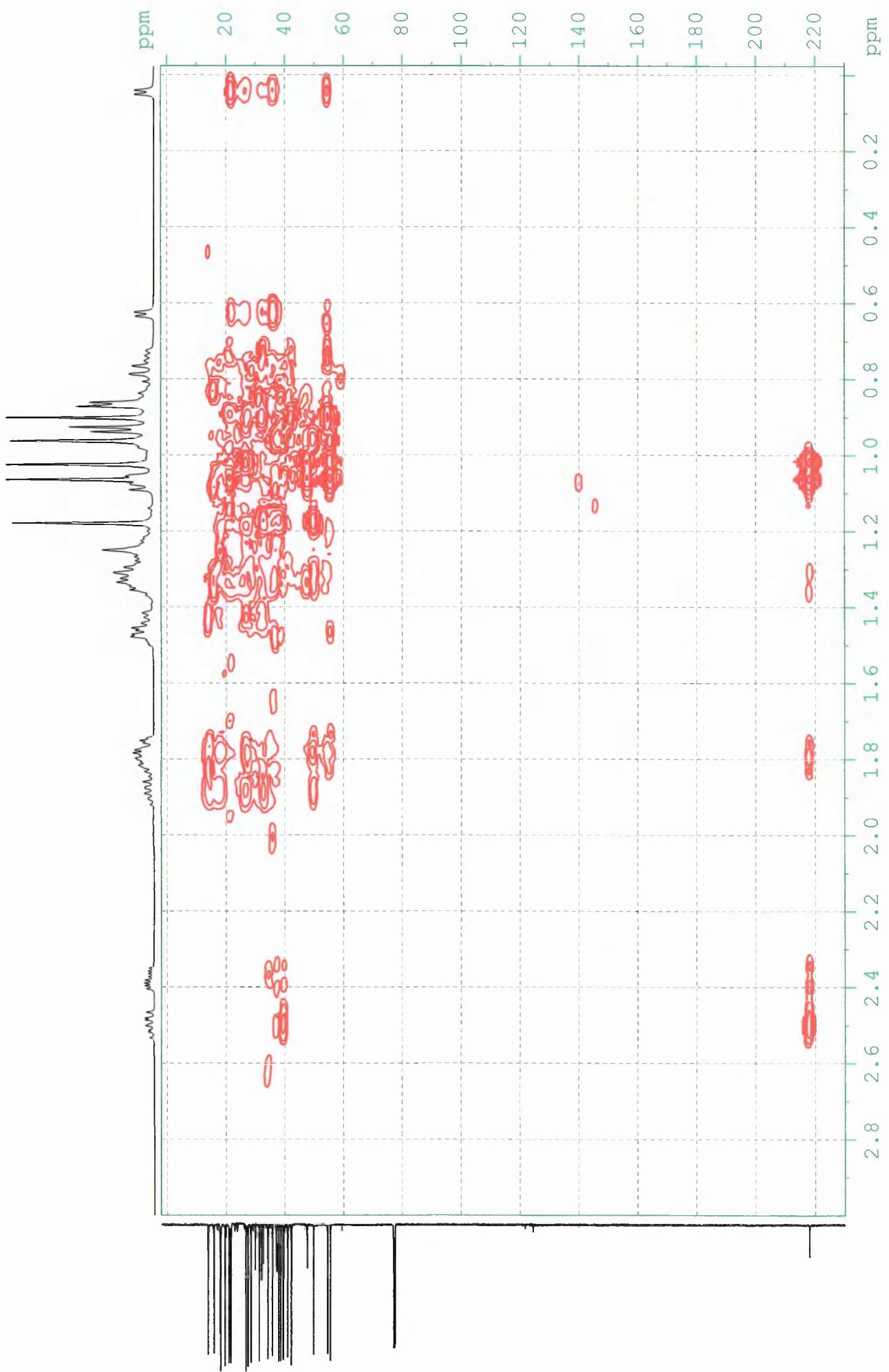
Spectrum 4.2:  $^{13}\text{C}$  NMR spectrum of compound 2.4 ( $\text{CDCl}_3$ )



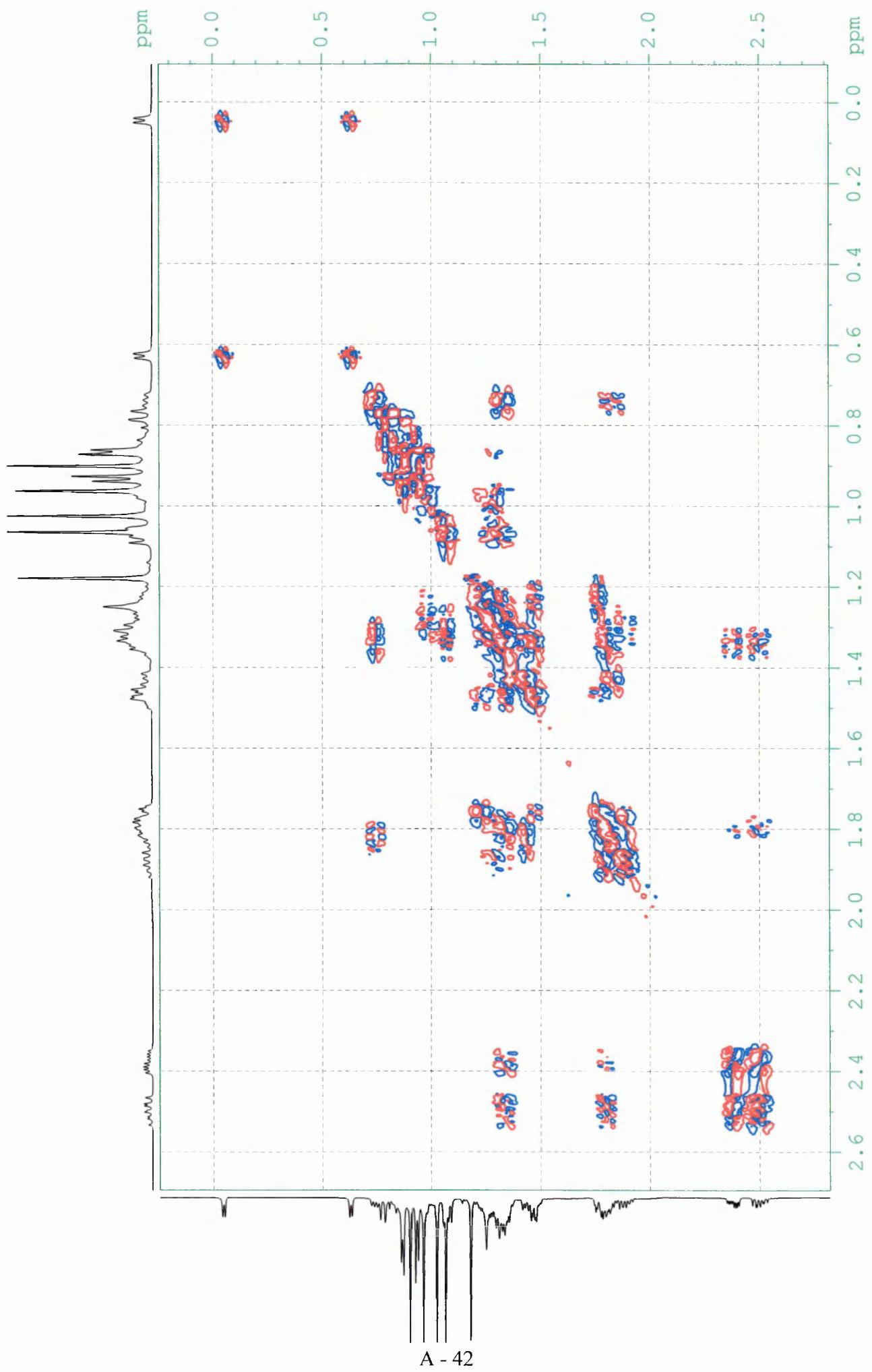
Spectrum 4.3: DEPT spectrum of compound 2.4 ( $\text{CDCl}_3$ )



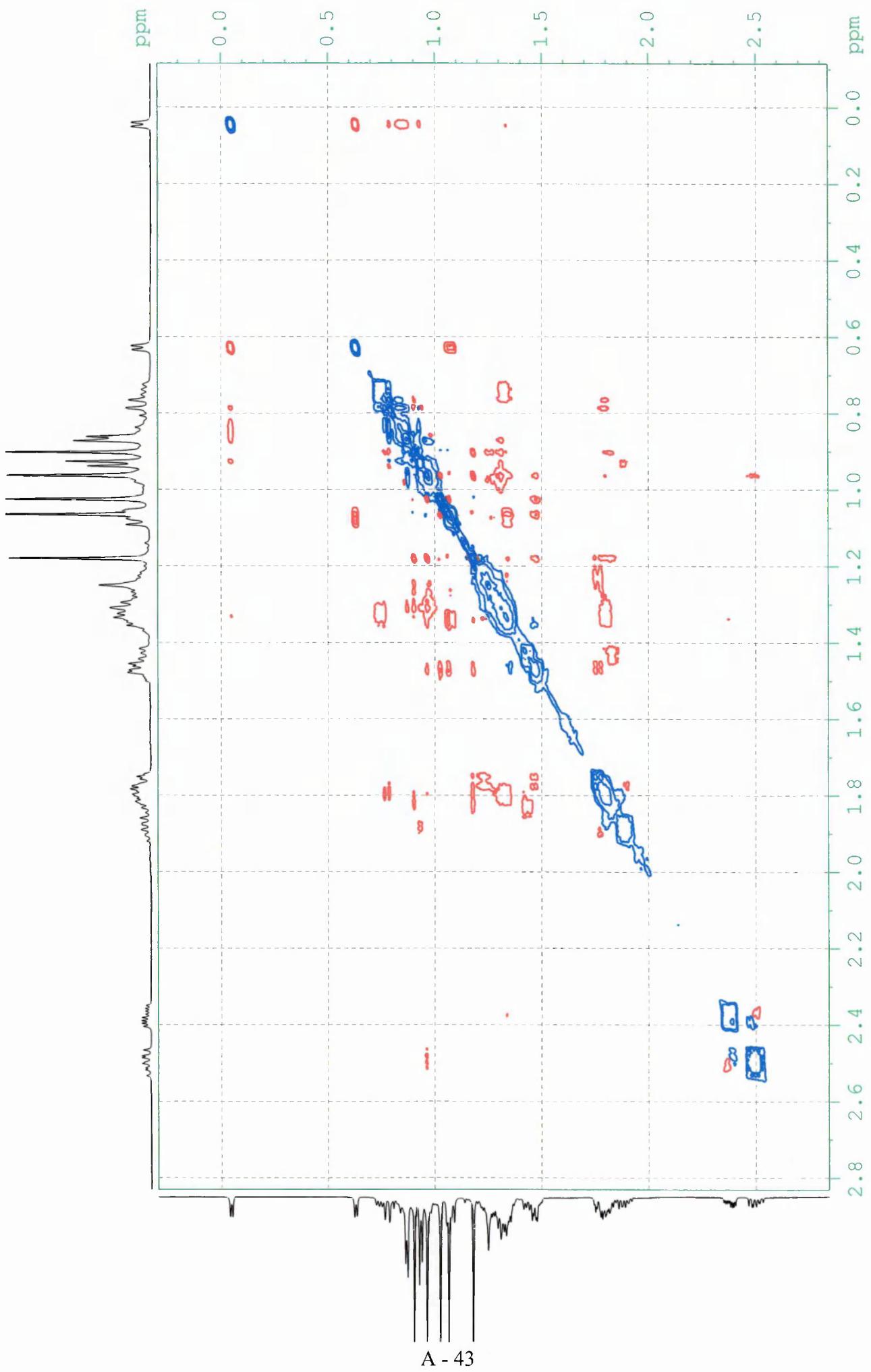
Spectrum 4.4: HSQCDEPT spectrum of compound 2.4 ( $\text{CDCl}_3$ )



Spectrum 4.5: HMBCLP spectrum of compound 2.4 ( $\text{CDCl}_3$ )



Spectrum 4.6: COSYPH spectrum of compound 2.4 ( $\text{CDCl}_3$ )



Spectrum 4.7: NOESY spectrum of compound 2.4 ( $\text{CDCl}_3$ )

**FI Phyllanthone [C<sub>30</sub>H<sub>48</sub>O]**

FI Phyllanthone (0.023) ls (1.00,1.00) C<sub>30</sub>H<sub>48</sub>O

100

□ %

ISOTOPE MODEL [C<sub>30</sub>H<sub>48</sub>O]

**Probe EI/Fl**

11:33:45 06-Jun-2007  
TOF MS Fl+  
7.11e12

424.3705

425.3739

426.3773

FI Phyllanthone 192 (4.479) Cm (192-3:14)

100

A - 44

TOF MS Fl+  
344

425.3766

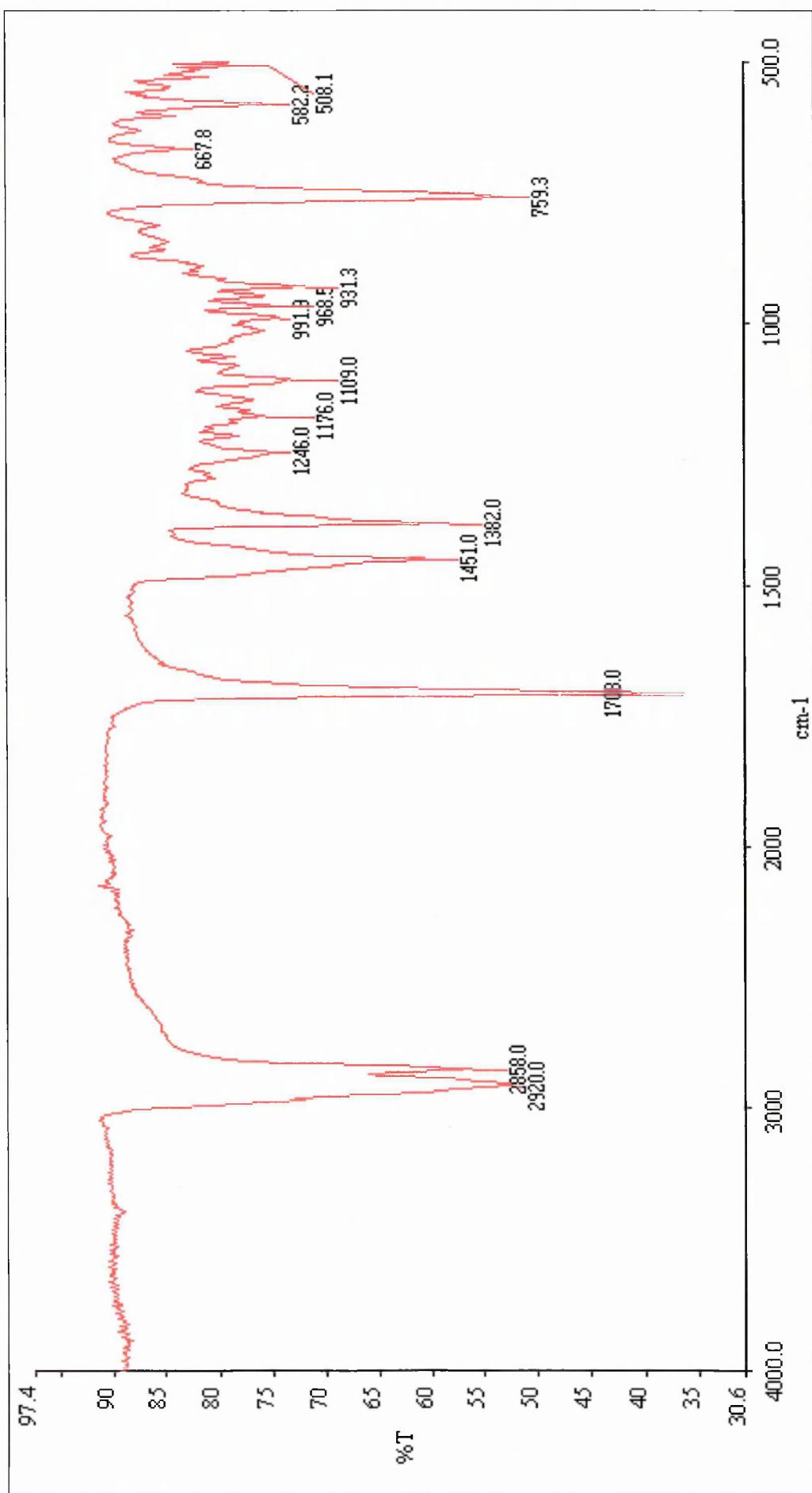
426.3750

TOF MS Fl+  
550

100

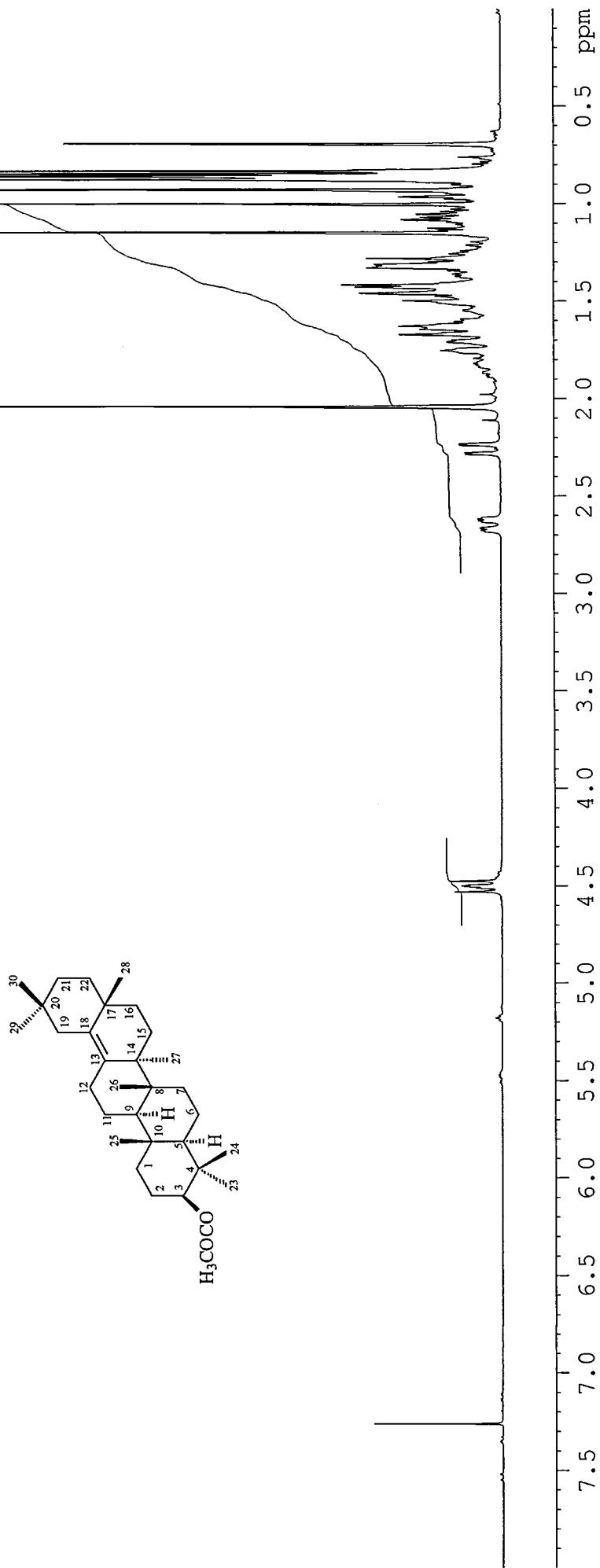
m/z

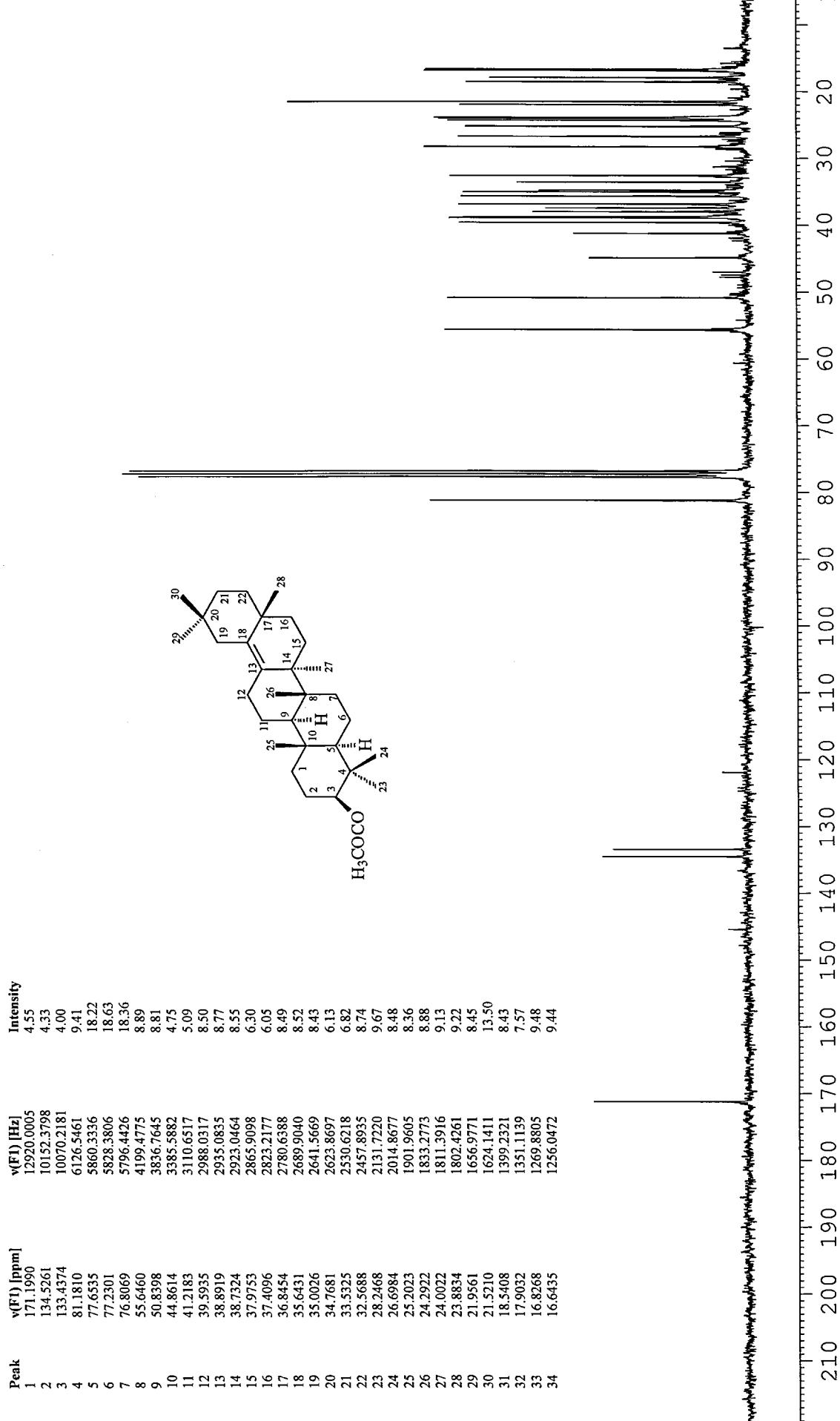
Spectrum 4.8: Mass spectrum of compound 2.4



Spectrum 4.9: FTIR spectrum of compound 2.4 ( $\text{CDCl}_3$ )

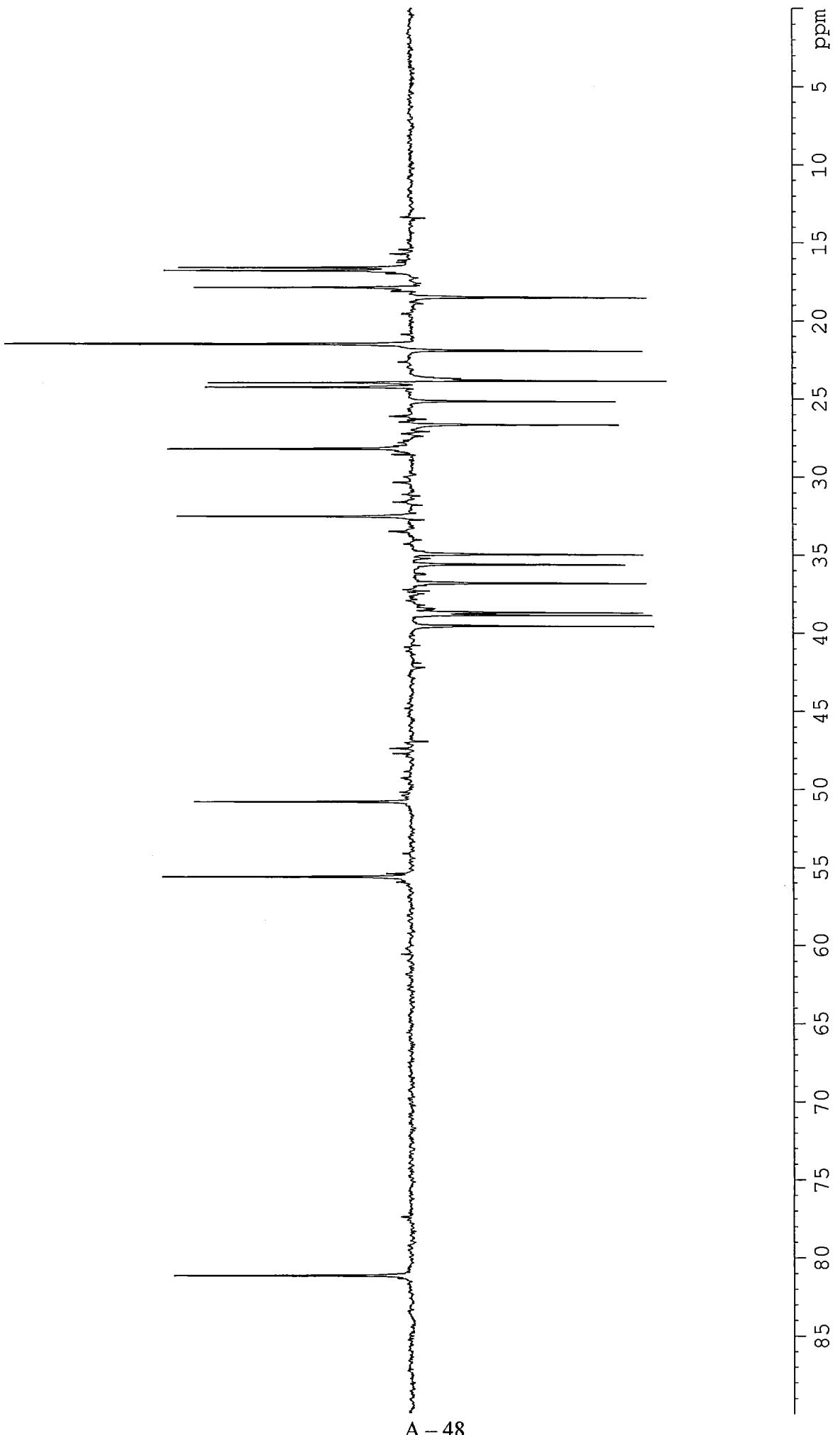
Spectrum 5.1:  $^1\text{H}$  NMR spectrum of compound 2.5 ( $\text{CDCl}_3$ )



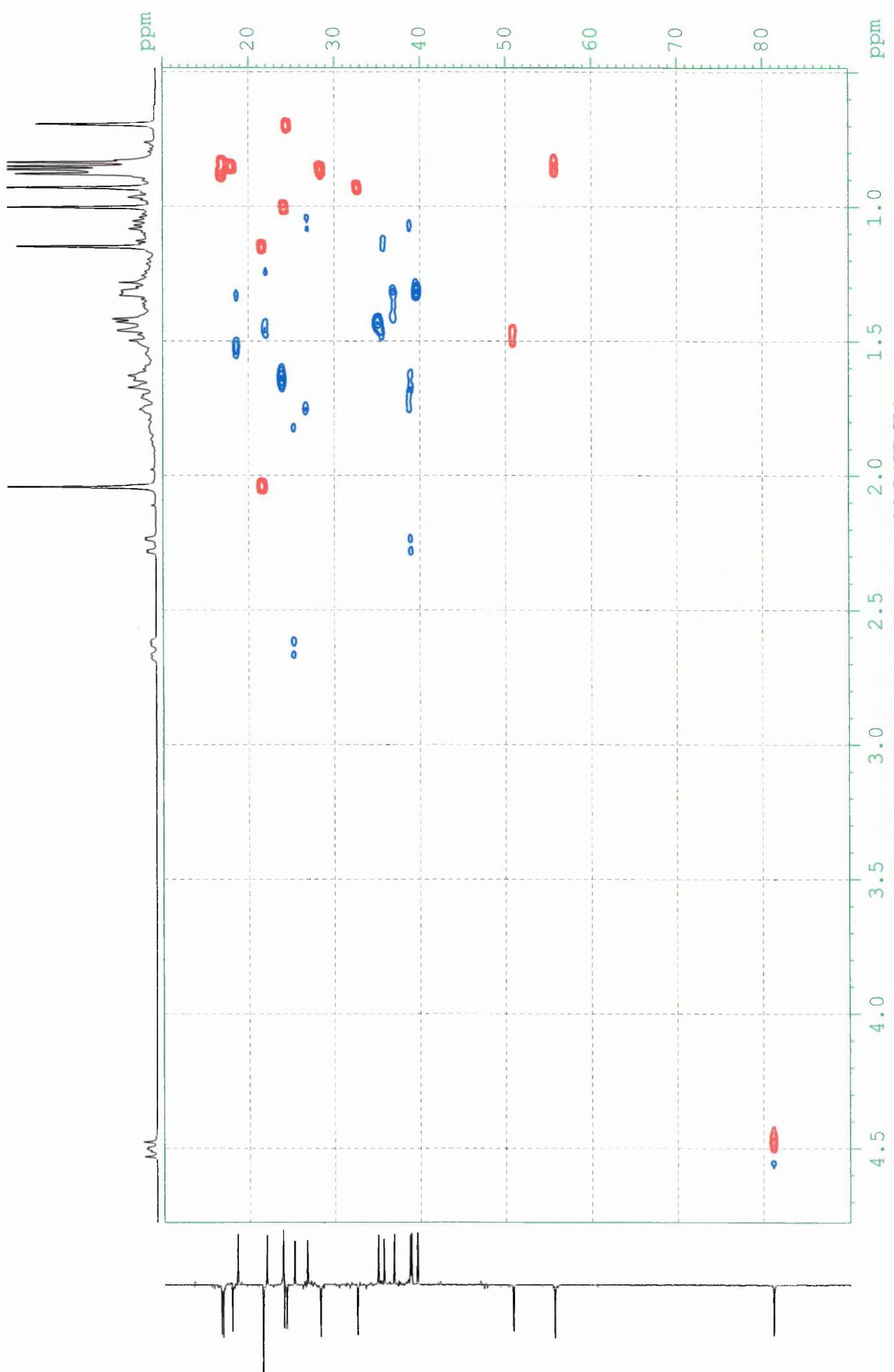


Spectrum 5.2:  $^{13}\text{C}$  NMR spectrum of compound 2.5 ( $\text{CDCl}_3$ )

Spectrum 5.3: DEPT spectrum of compound 2.5 ( $\text{CDCl}_3$ )



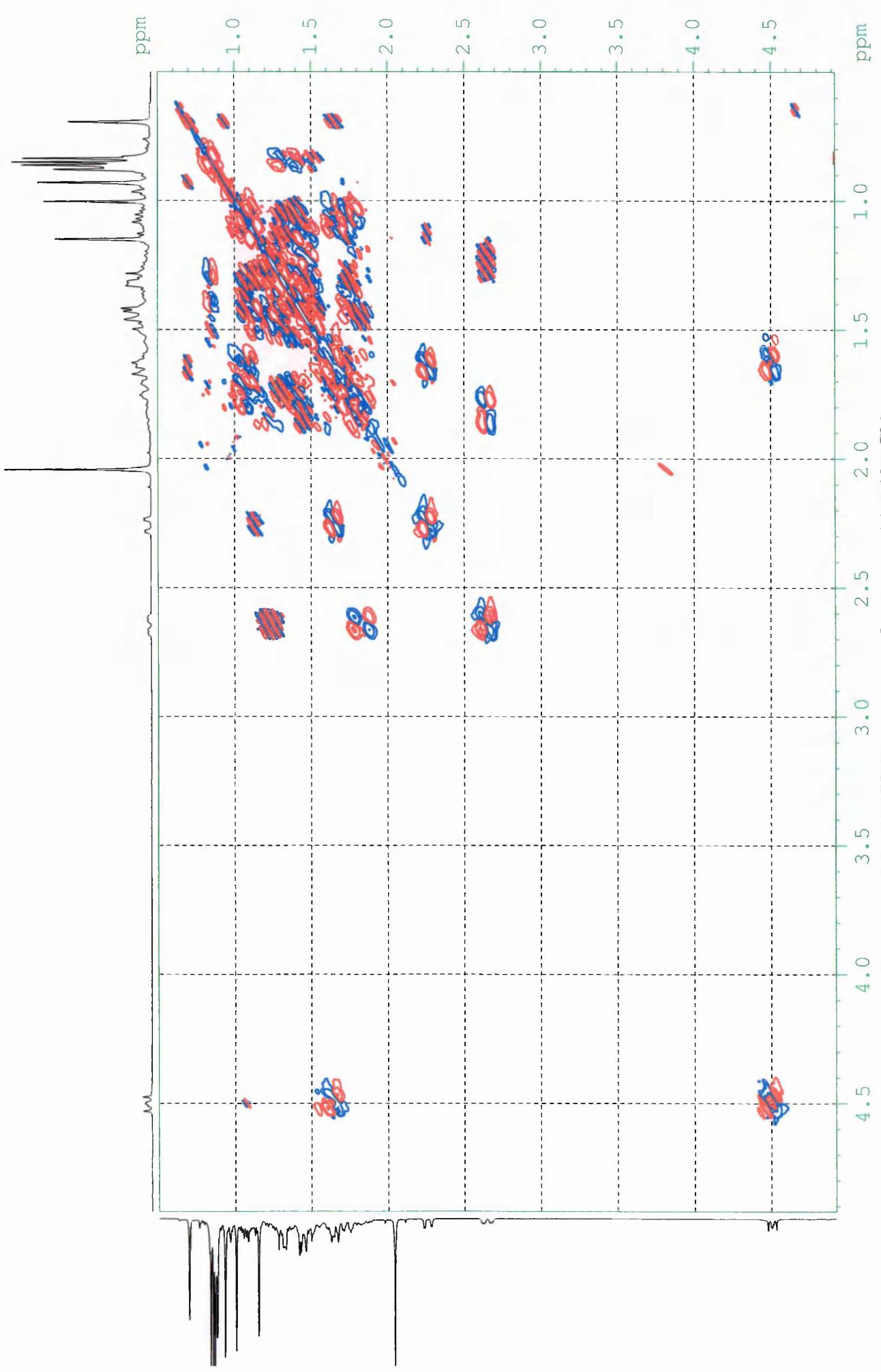
**Spectrum 5.4: HSQCDEPT spectrum of compound 2.5( $\text{CDCl}_3$ )**



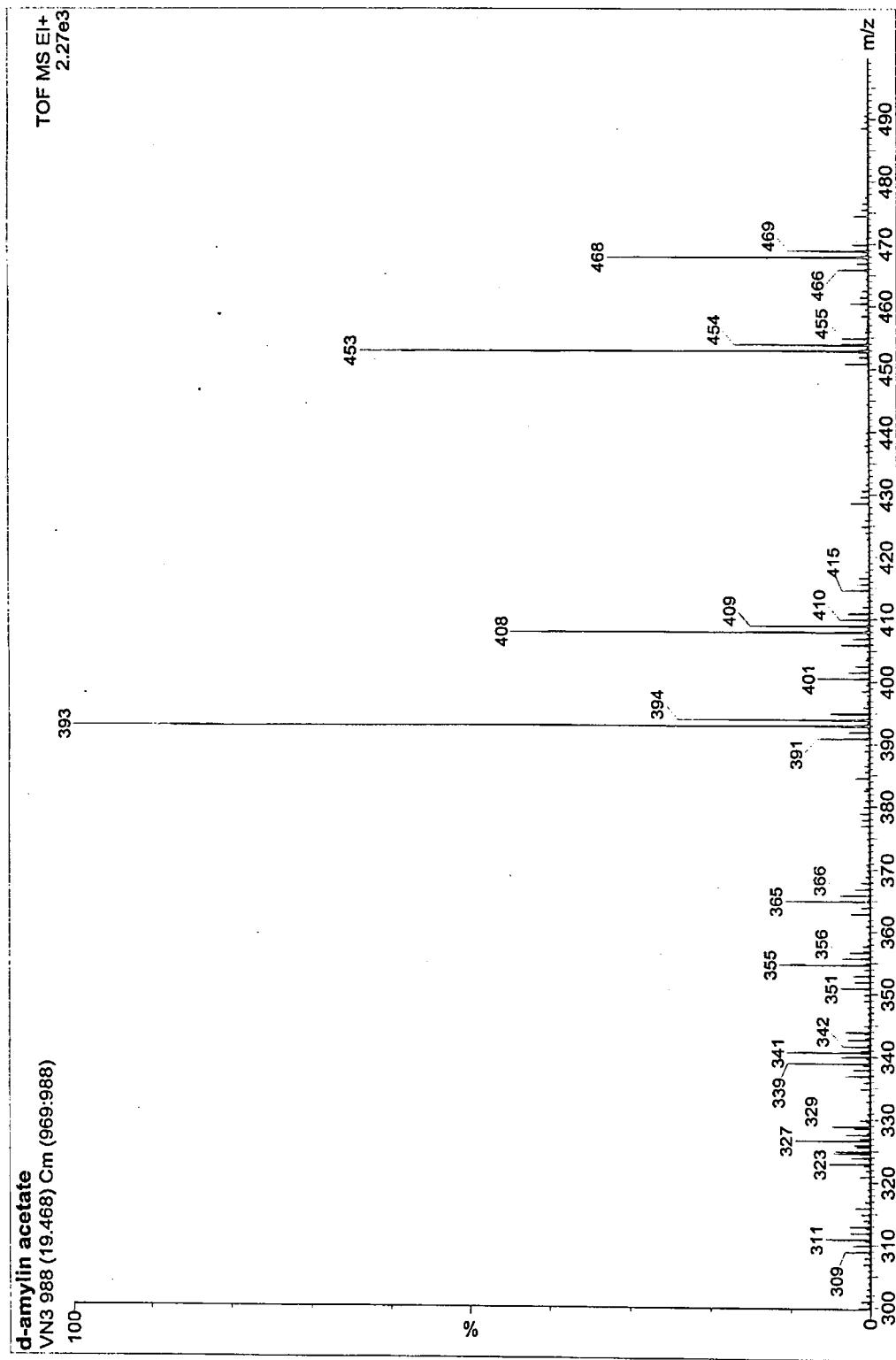
**Spectrum 5.5: HMBCCP spectrum of compound 2.5 ( $\text{CDCl}_3$ )**

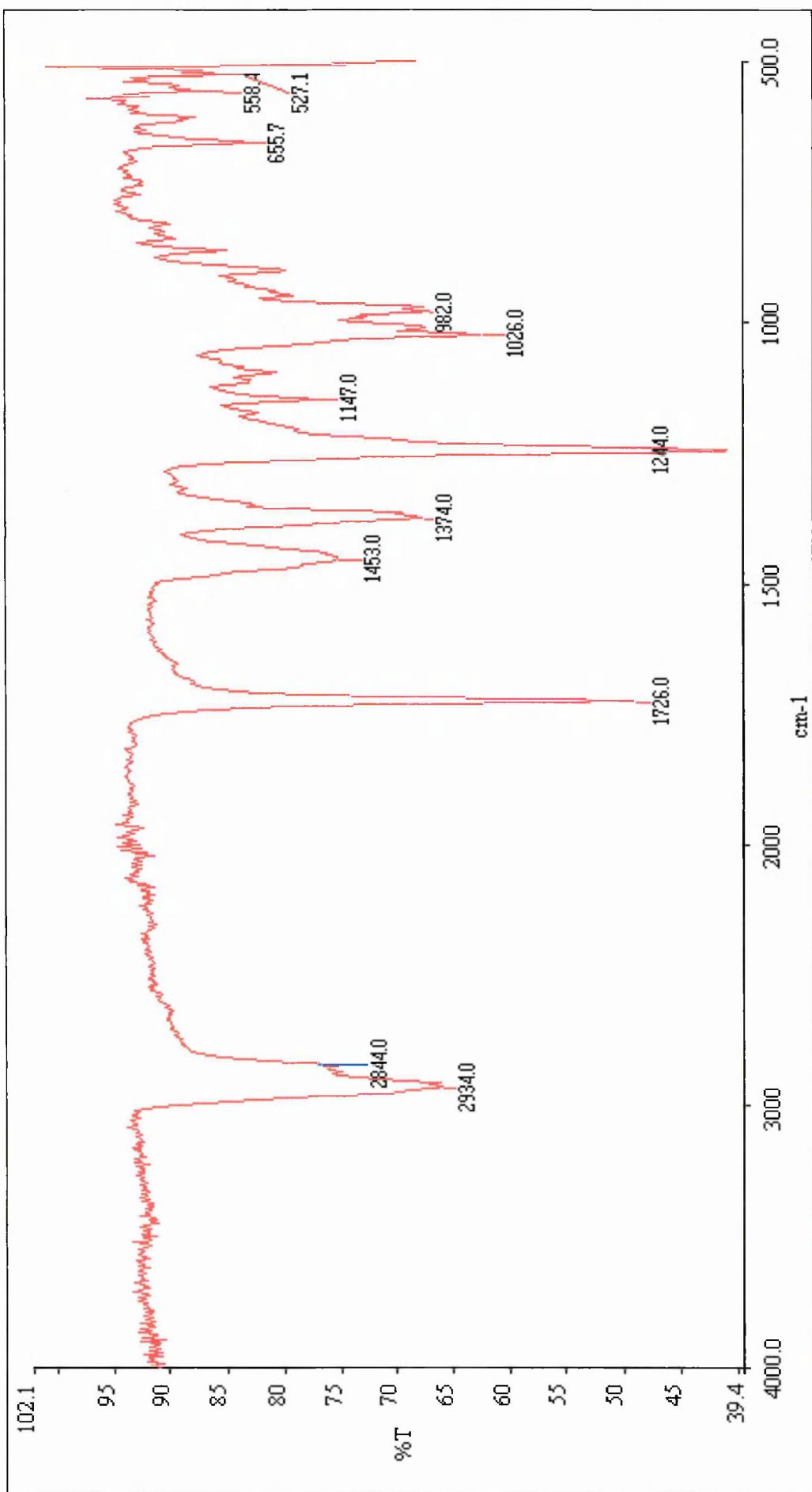


**Spectrum 5.6: COSYPH spectrum of compound 2.5 ( $\text{CDCl}_3$ )**



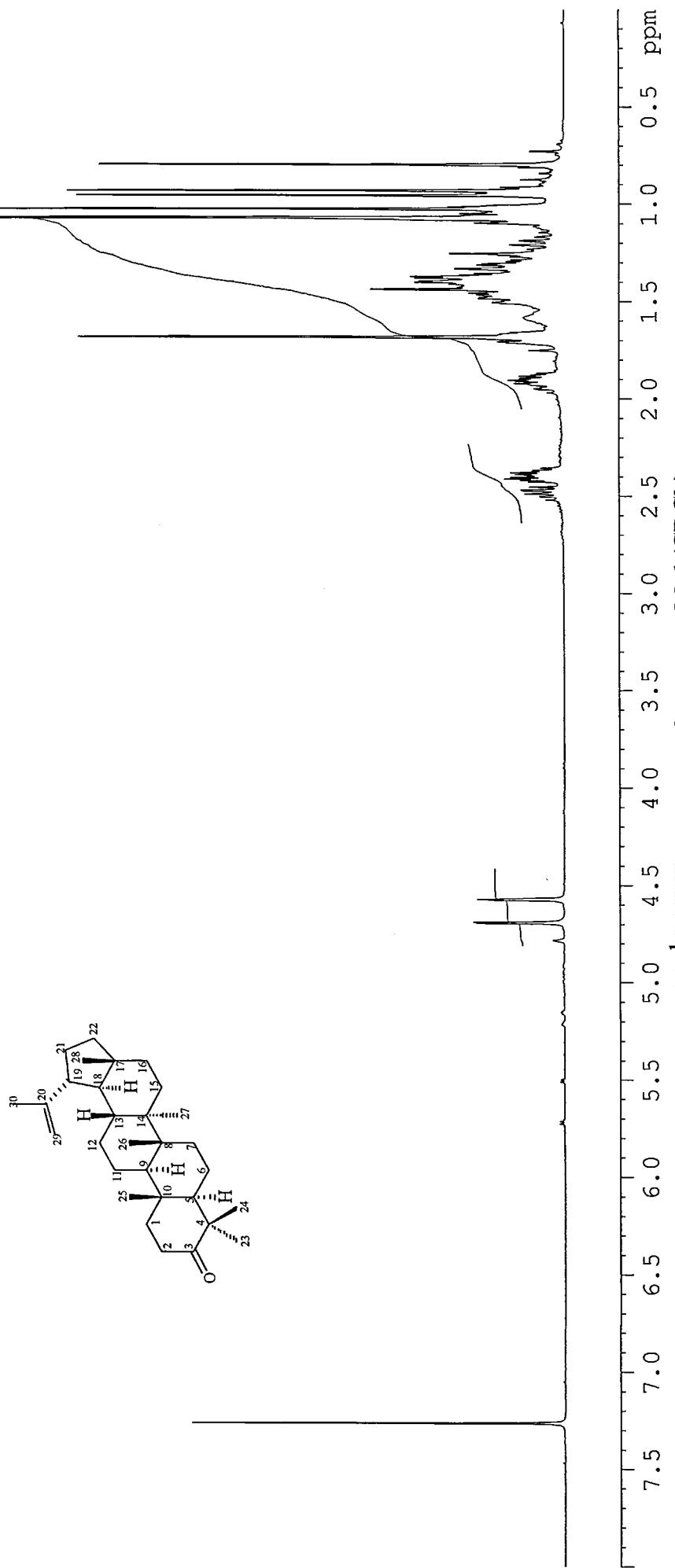
Spectrum 5.7: Mass spectrum of compound 2.5



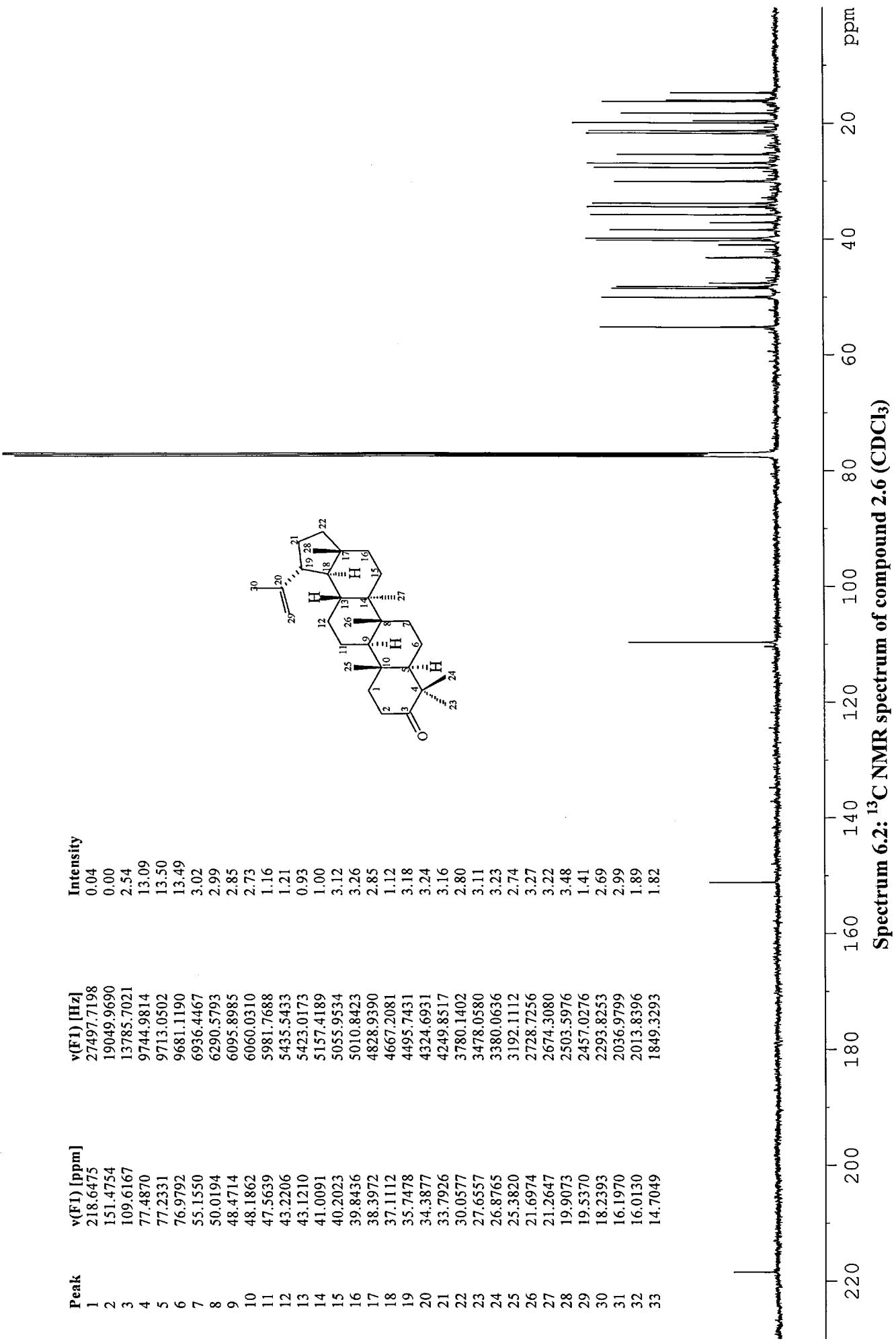
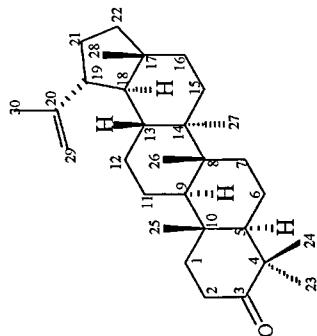


Spectrum 5.8: FTIR spectrum of compound 2,5 ( $\text{CDCl}_3$ )

Spectrum 6.1:  $^1\text{H}$  NMR spectrum of compound 2.6 ( $\text{CDCl}_3$ )

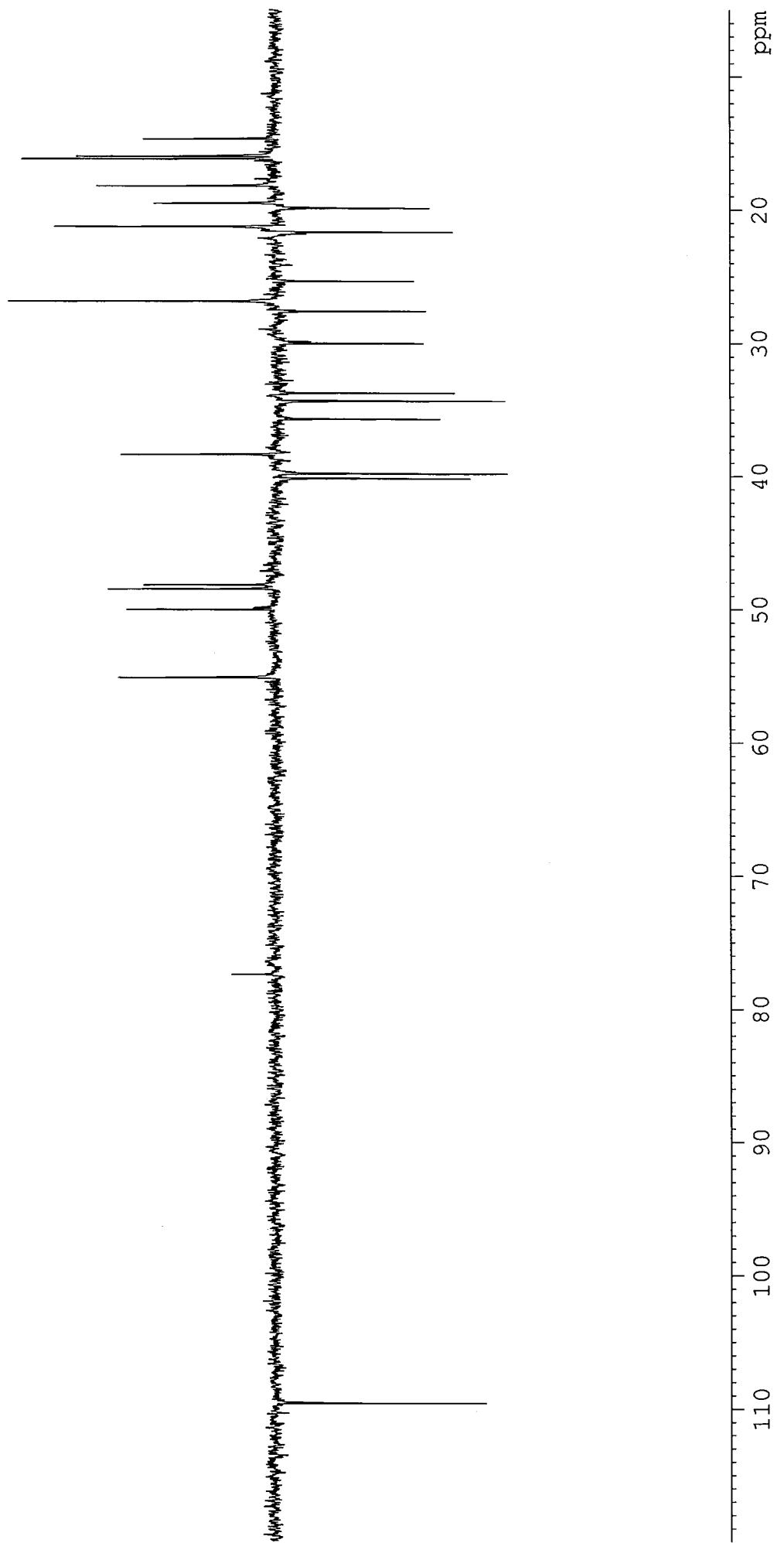


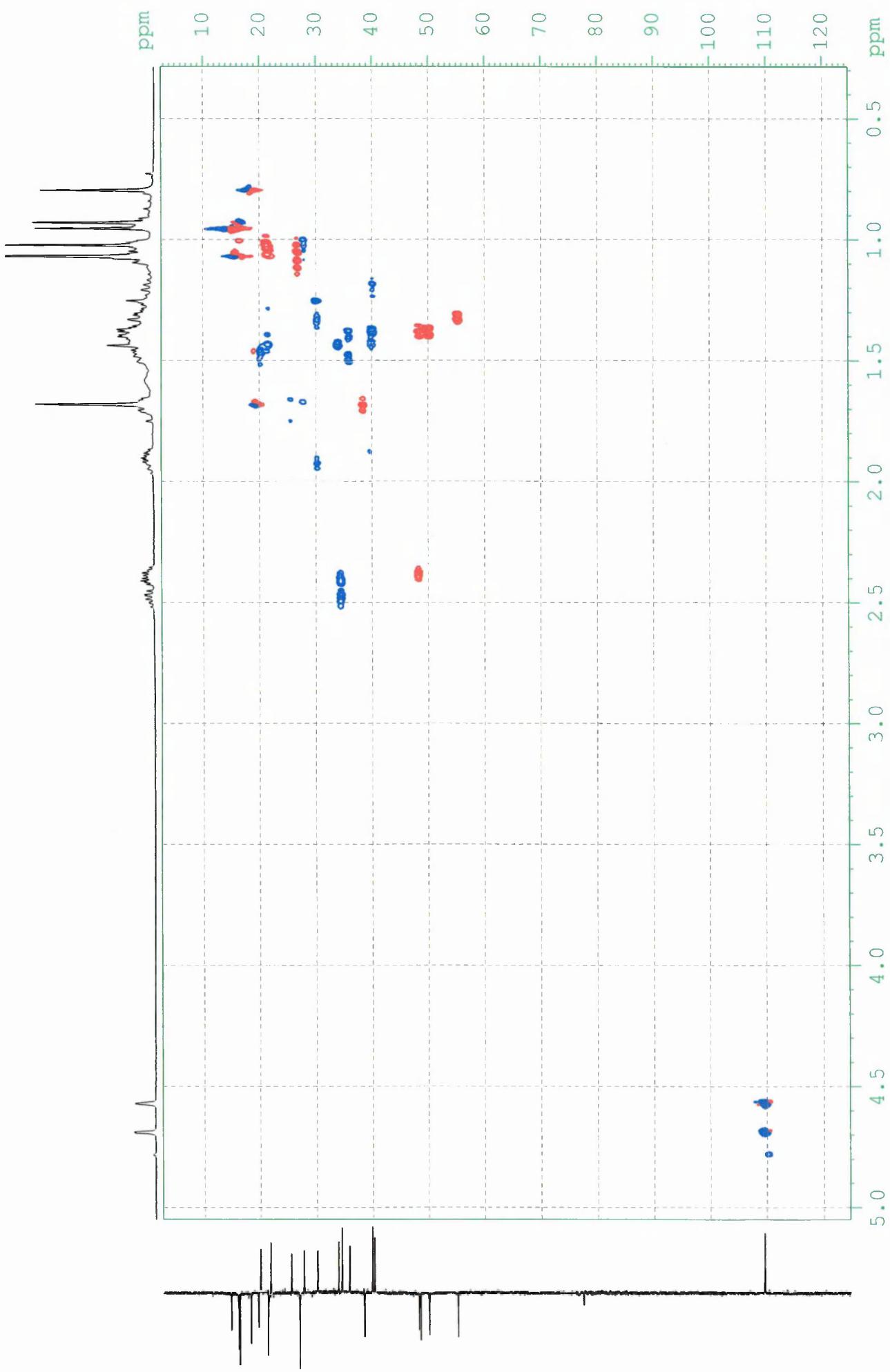
Peak	$\nu(F1)$ [ppm]	$\nu(F1)$ [Hz]	Intensity
1	218.6475	27497.7198	0.04
2	151.4754	19049.9690	0.00
3	109.6167	13785.7021	2.54
4	77.4870	9744.9814	13.09
5	77.2331	9713.0502	13.50
6	76.9792	9681.1190	13.49
7	55.1550	6936.4467	3.02
8	50.0194	6290.5793	2.99
9	48.4714	6095.8985	2.85
10	48.1862	6060.0310	2.73
11	47.5639	5981.7688	1.16
12	43.2206	5435.5433	1.21
13	43.1210	5423.0173	0.93
14	41.0091	5157.4189	1.00
15	40.2023	5055.9534	3.12
16	39.8436	5010.8423	3.26
17	38.3972	4828.9390	2.85
18	37.1112	4667.2081	1.12
19	35.7478	4495.7431	3.18
20	34.3877	4324.6931	3.24
21	33.7926	4249.8517	3.16
22	30.0577	3780.1402	2.80
23	27.6557	3478.0580	3.11
24	26.8765	3380.0636	3.23
25	25.3820	3192.1112	2.74
26	21.6974	2728.7256	3.27
27	21.2647	2674.3080	3.22
28	19.9073	2503.5976	3.48
29	19.5370	2457.0276	1.41
30	18.2393	2293.8253	2.69
31	16.1970	2036.9799	2.99
32	16.0130	2013.8396	1.89
33	14.7049	1849.3293	1.82



Spectrum 6.2: <sup>13</sup>C NMR spectrum of compound 2.6 ( $\text{CDCl}_3$ )

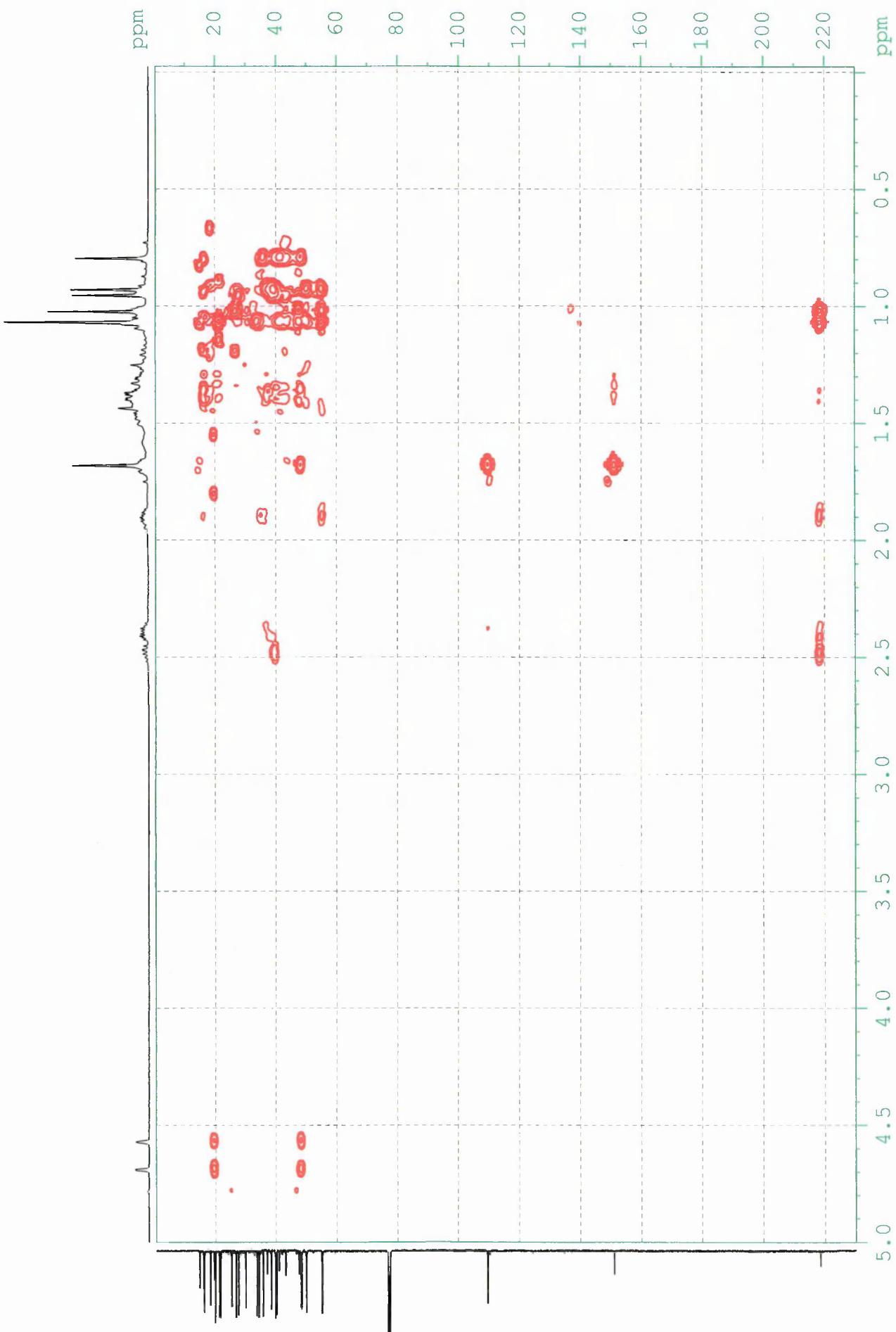
Spectrum 6.3: DEPT spectrum of compound 2.6 ( $\text{CDCl}_3$ )



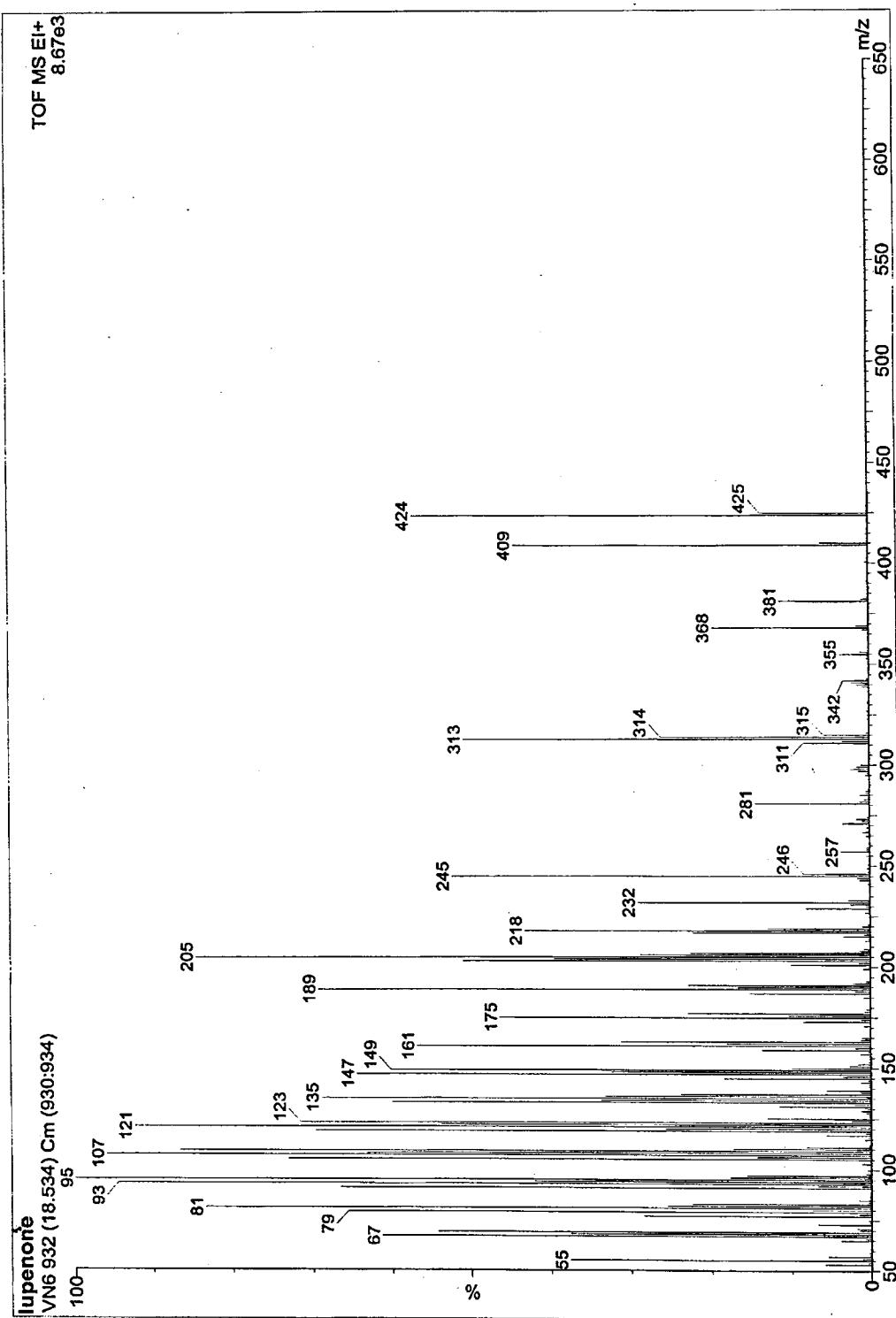


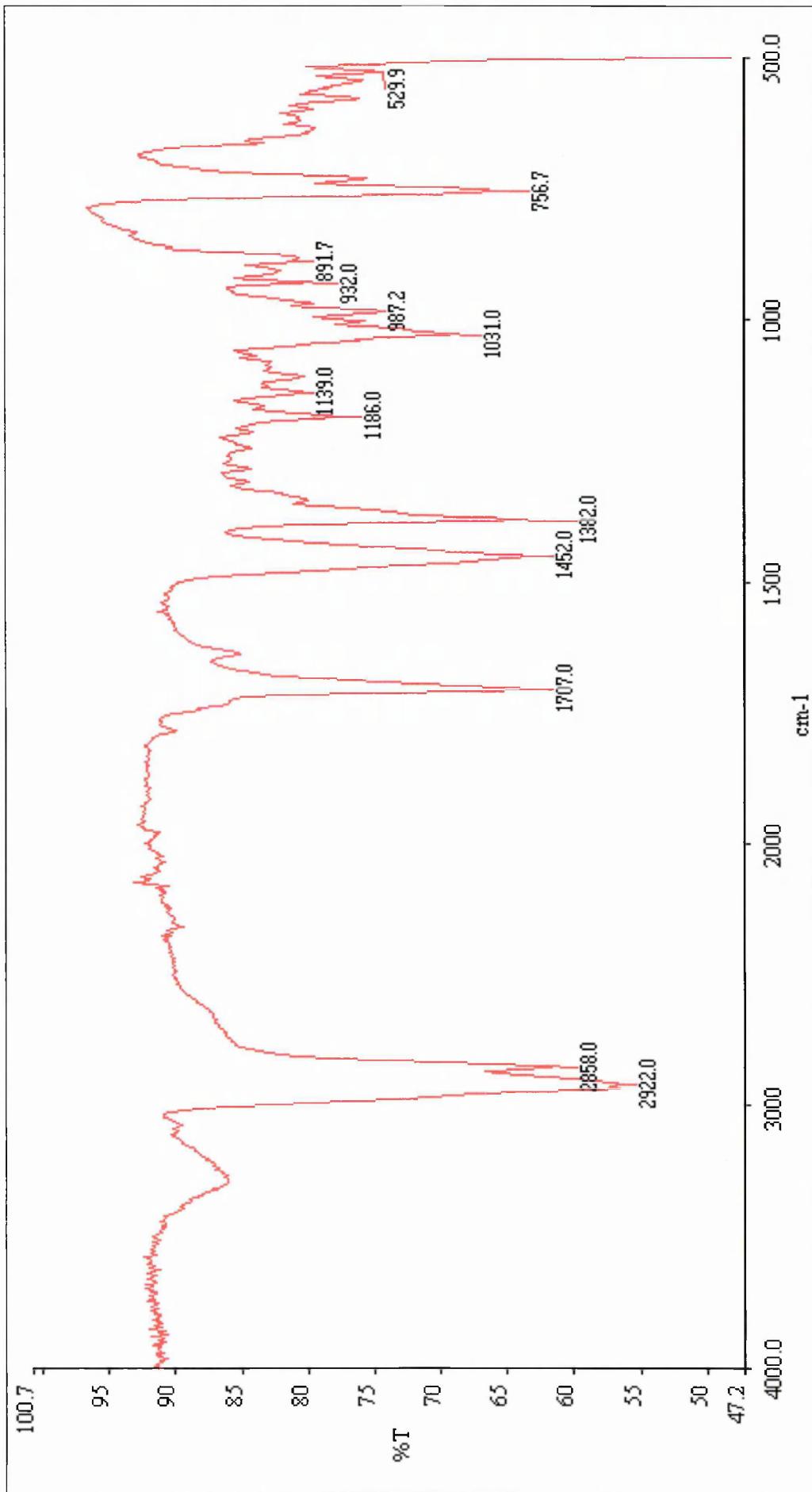
Spectrum 6.4: HSQCDEPT spectrum of compound 2.6 ( $\text{CDCl}_3$ )

Spectrum 6.5: HMBCCP spectrum of compound 2.6 ( $\text{CDCl}_3$ )



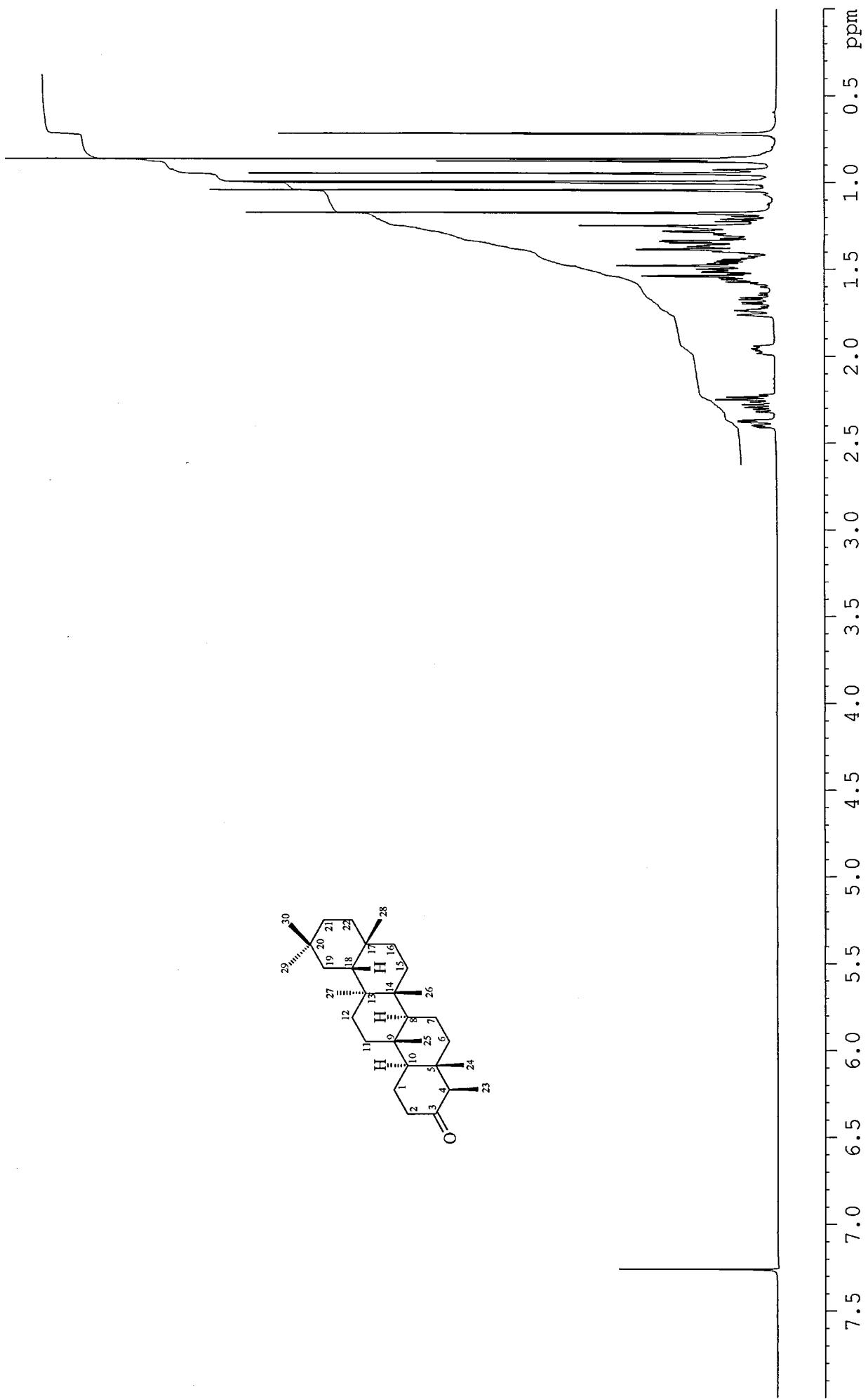
Spectrum 6.6: Mass spectrum of compound 2.6



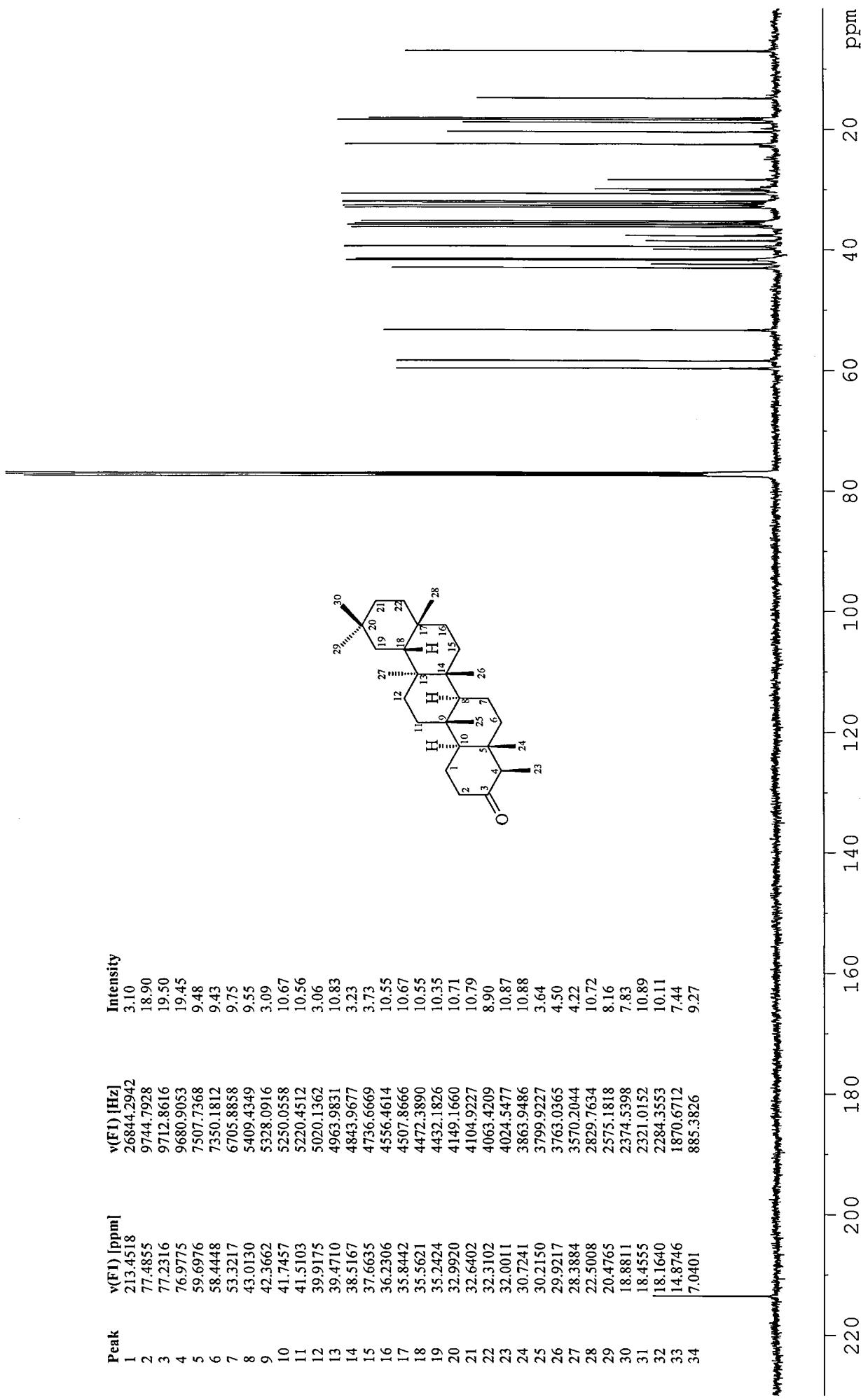
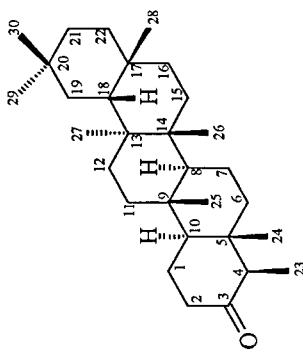


Spectrum 6.7: FTIR spectrum of compound 2,6 ( $\text{CDCl}_3$ )

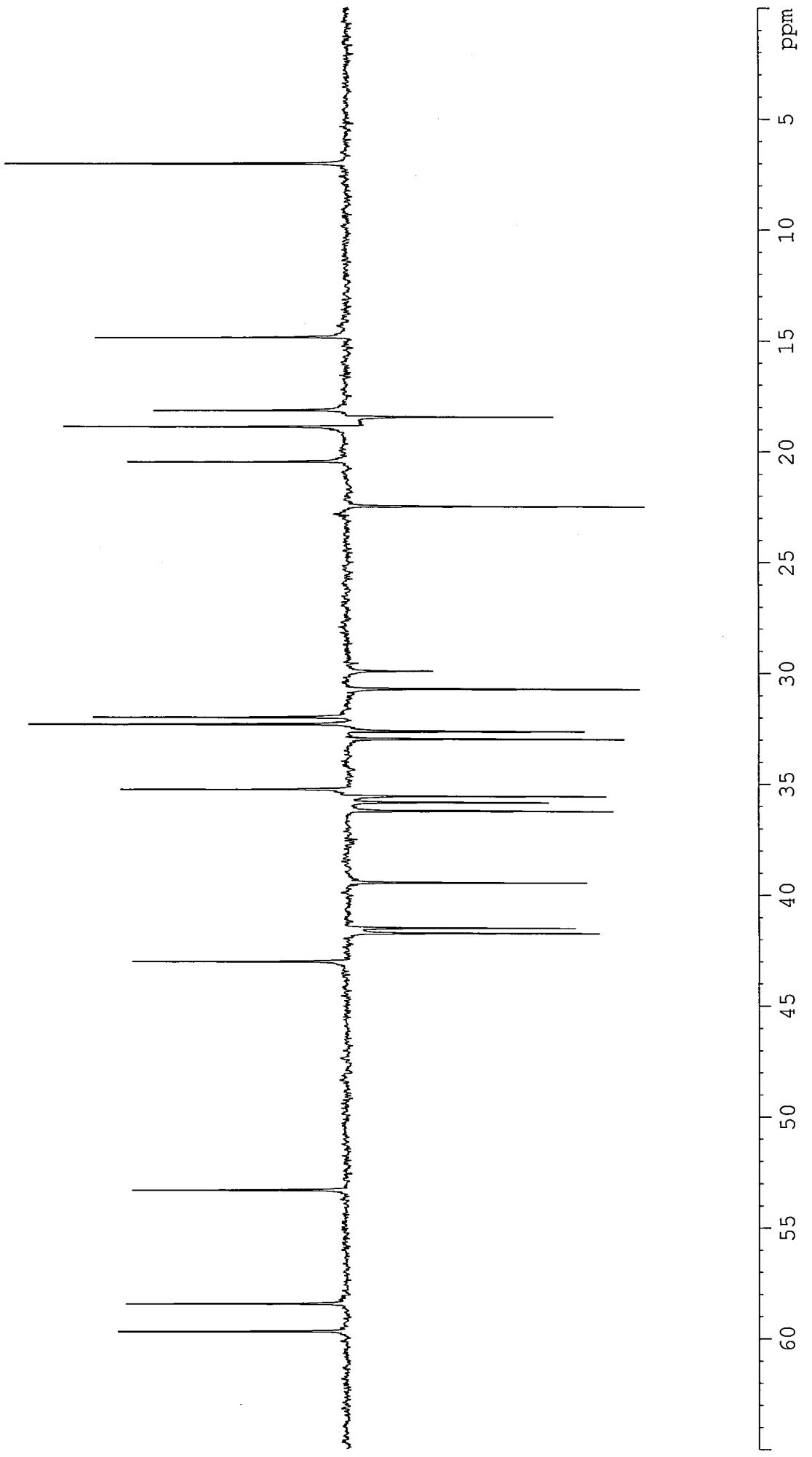
Spectrum 7.1:  $^1\text{H}$  NMR spectrum of compound 2.7 ( $\text{CDCl}_3$ )



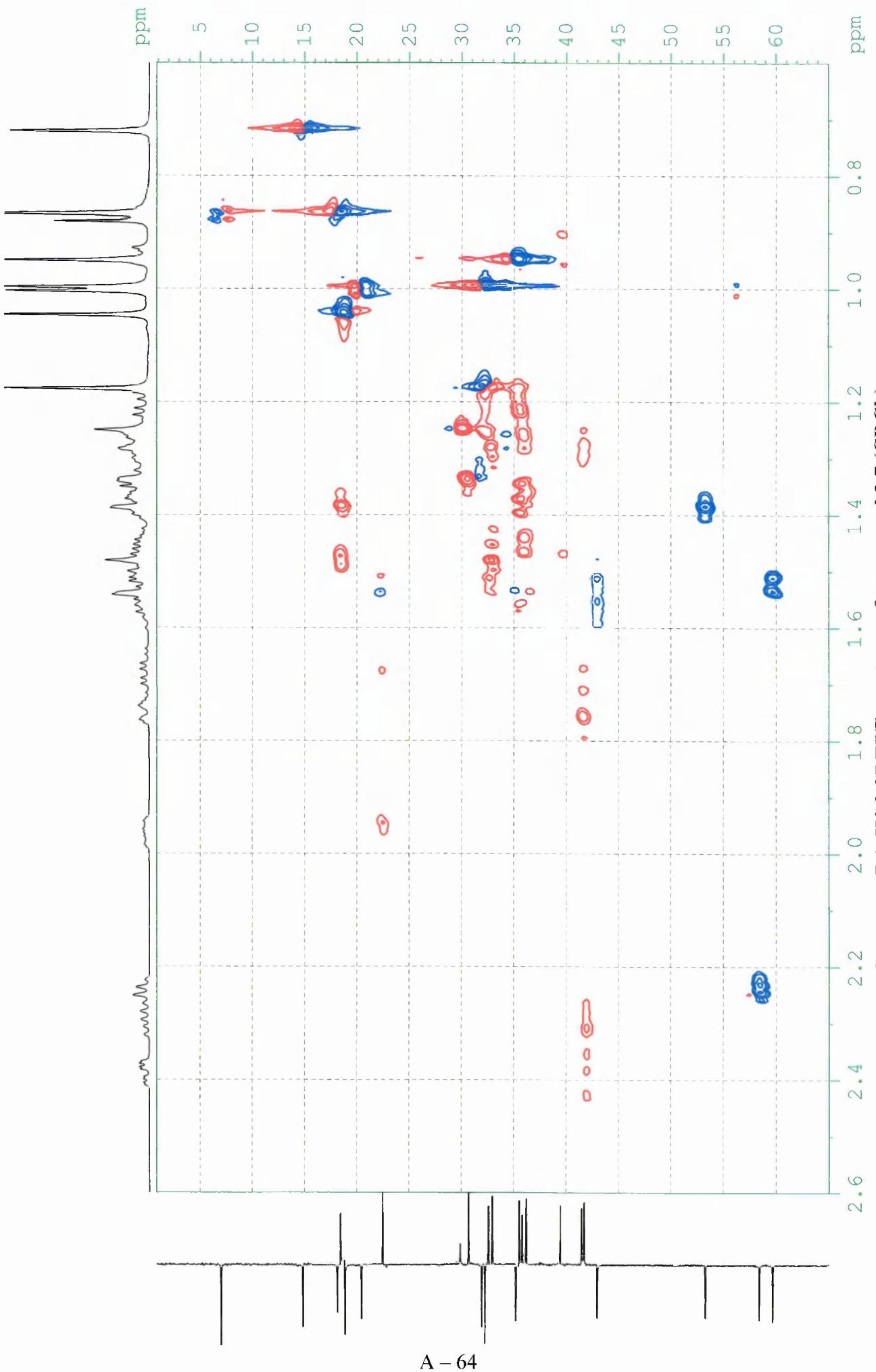
Peak	$\nu(F1)$ [ppm]	$\nu(F1)$ [Hz]	Intensity
1	213.4518	26844.2942	3.10
2	77.4855	9744.7928	18.90
3	77.2316	9712.8616	19.50
4	76.9775	9680.9053	19.45
5	59.6976	7507.7368	9.48
6	58.4448	7350.1812	9.43
7	53.3217	6705.8858	9.75
8	43.0130	5409.4349	9.55
9	42.3662	5328.0916	3.09
10	41.7457	5250.0558	10.67
11	41.5103	5220.4512	10.56
12	39.9175	5020.1362	3.06
13	39.4710	4963.9831	10.83
14	38.5167	4843.9677	3.23
15	37.6635	4736.6669	3.73
16	36.2306	4556.4614	10.55
17	35.8442	4507.8666	10.67
18	35.5621	4472.3890	10.55
19	35.2424	4432.1826	10.35
20	32.9920	4149.1660	10.71
21	32.6402	4104.9227	10.79
22	32.3102	4063.4209	8.90
23	32.0011	4024.5477	10.87
24	30.7241	3863.9486	10.88
25	30.2150	3799.9227	3.64
26	29.9217	3763.0365	4.50
27	28.3884	3570.2044	4.22
28	22.5008	2829.7634	10.72
29	20.4765	2575.1818	8.16
30	18.8811	2374.5398	7.83
31	18.4555	2321.0152	10.89
32	18.1640	2284.3553	10.11
33	14.8746	1870.6712	7.44
34	7.0401	885.3826	9.27



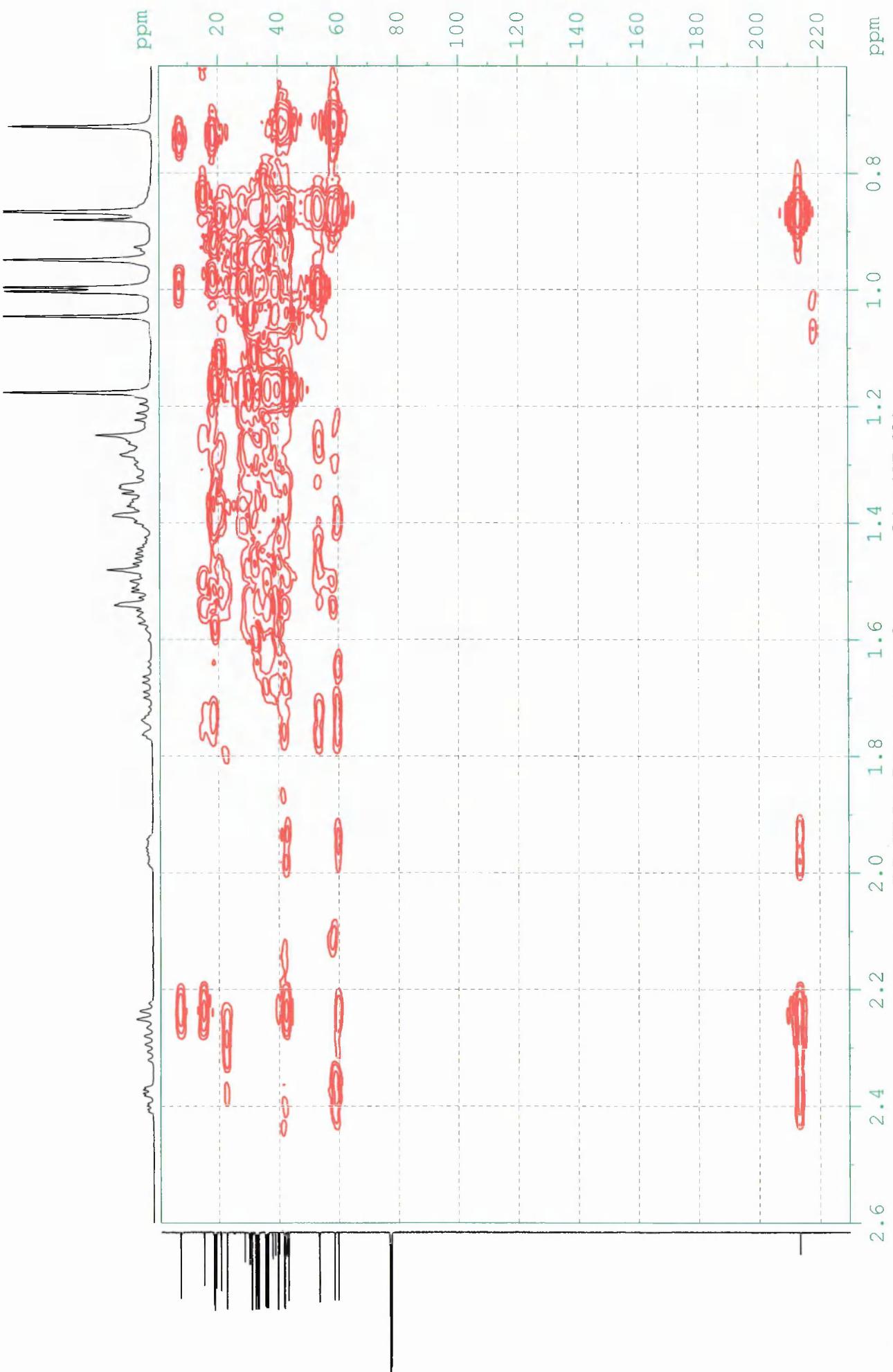
Spectrum 7.2:  $^{13}\text{C}$  NMR spectrum of compound 2.7 ( $\text{CDCl}_3$ )

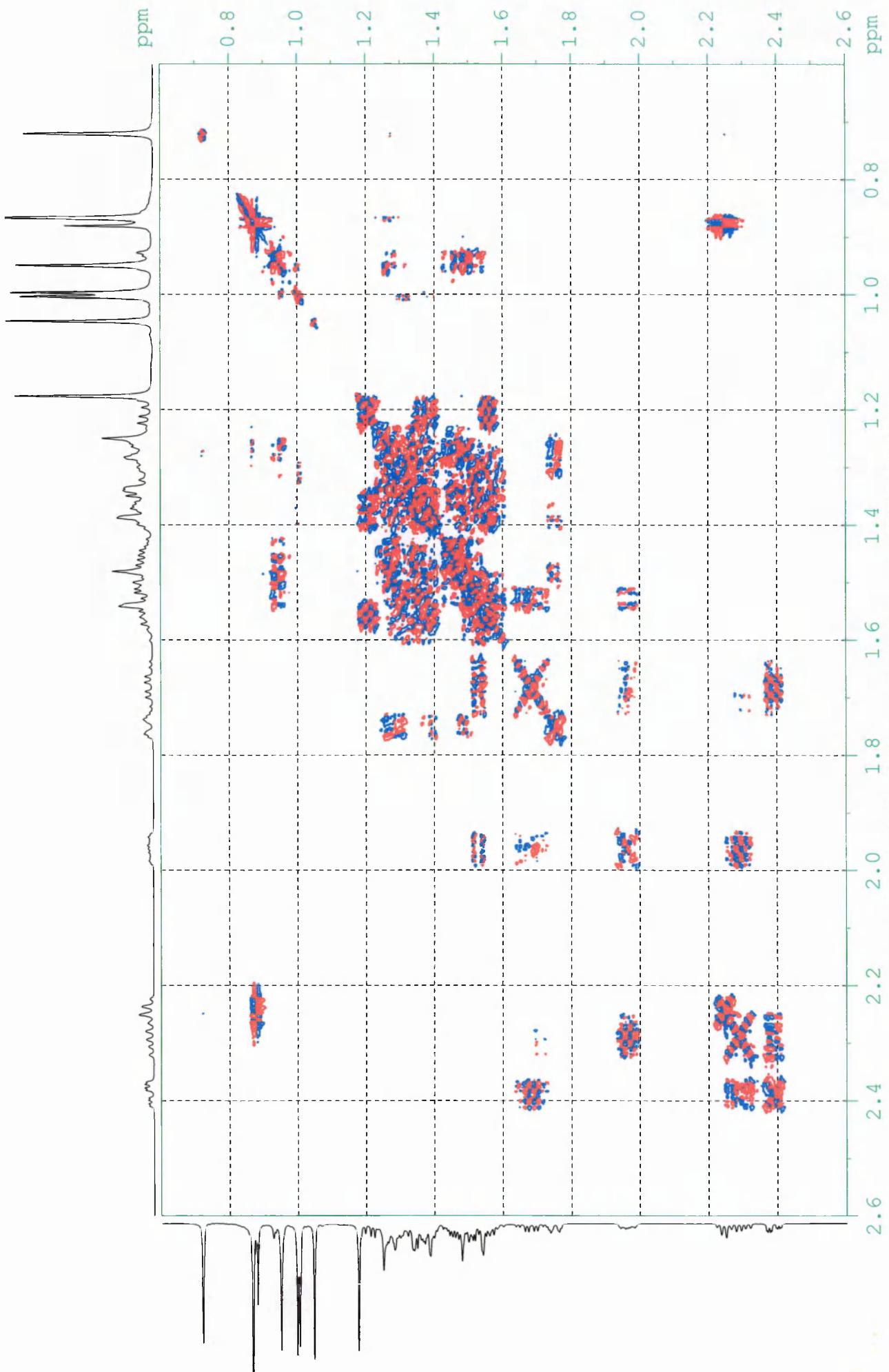


Spectrum 7.3: DEPT spectrum of compound 2.7 ( $\text{CDCl}_3$ )



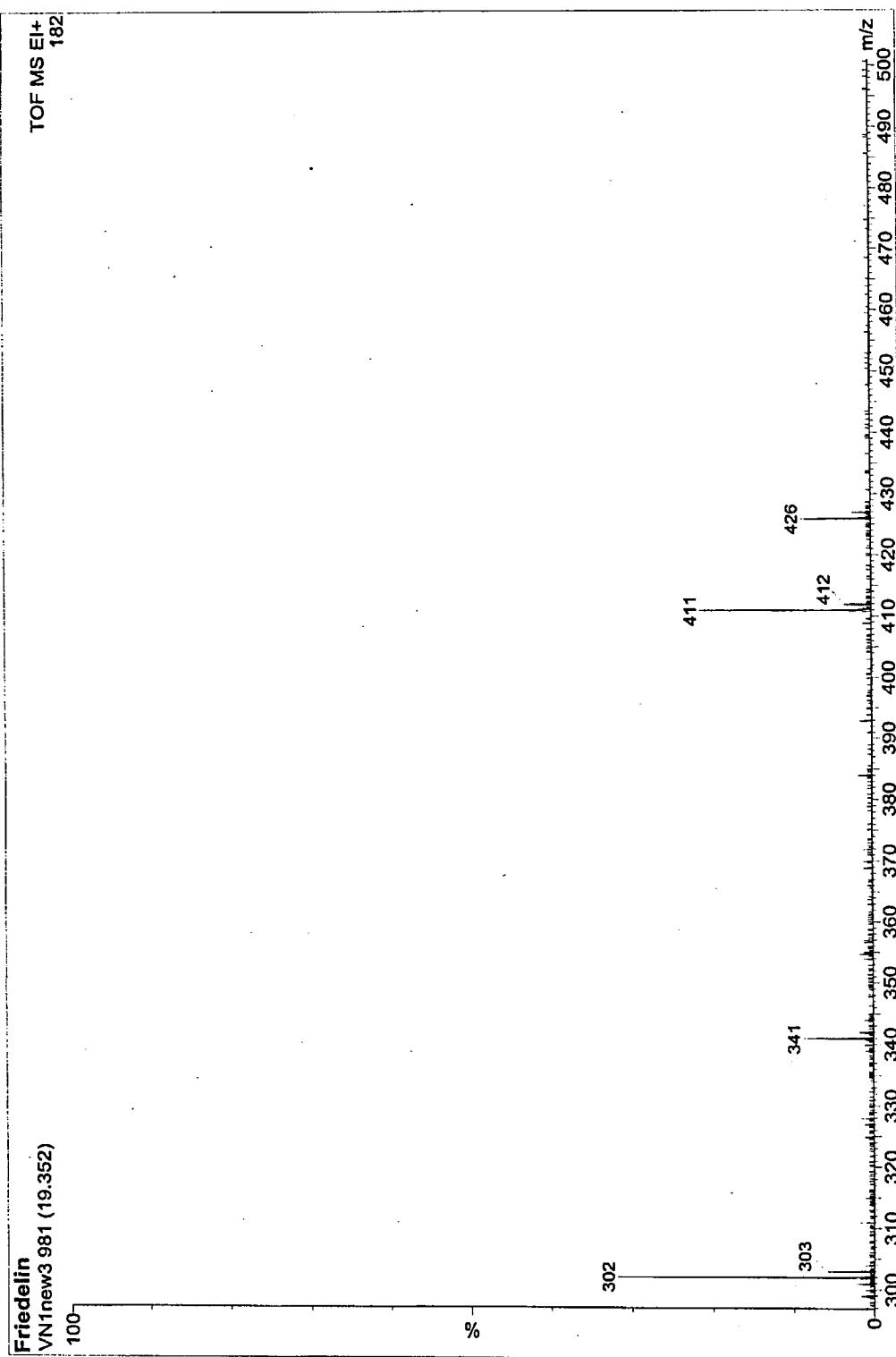
Spectrum 7.4: HSQCDEPT spectrum of compound 2.7 ( $\text{CDCl}_3$ )

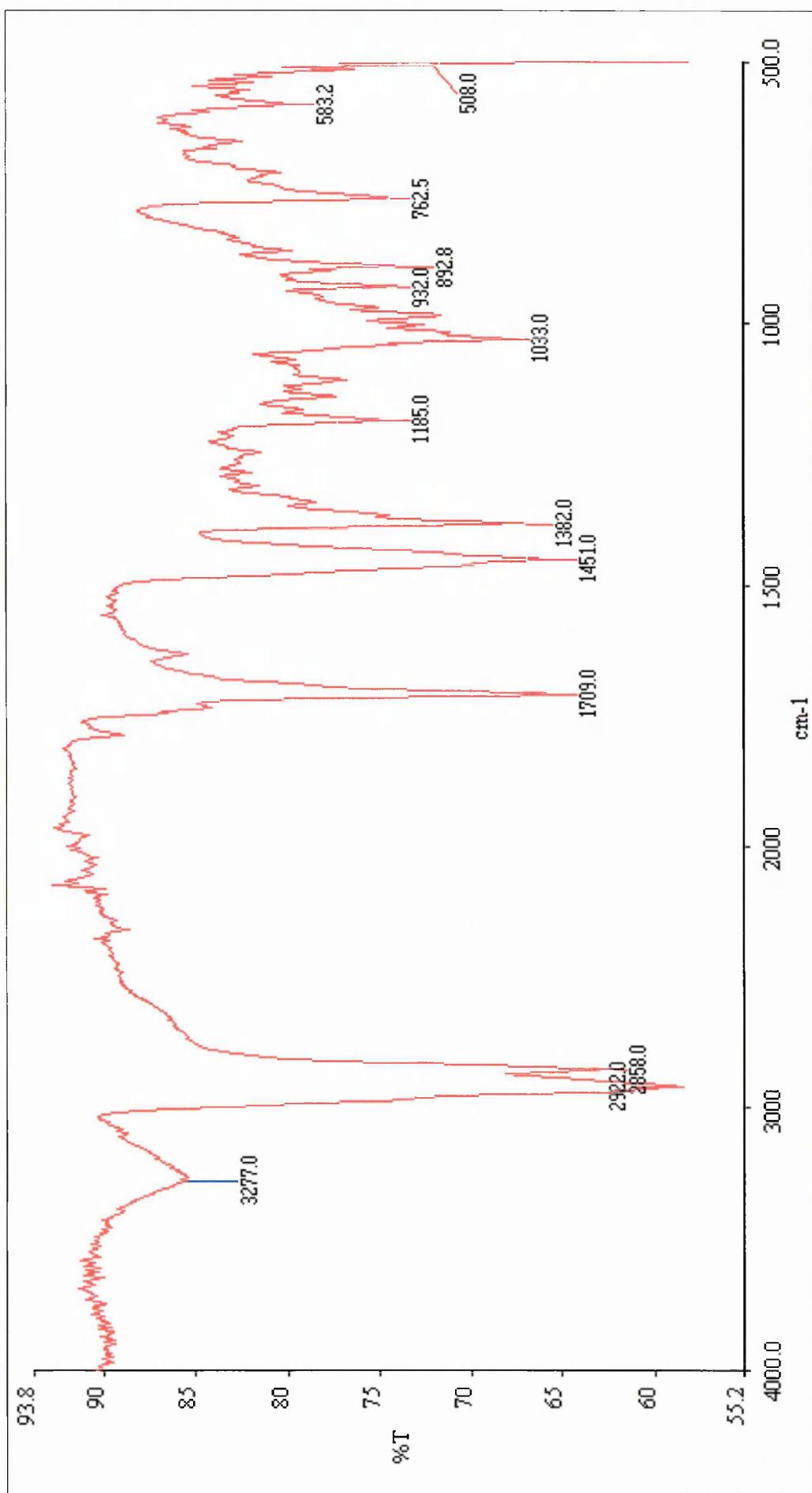




Spectrum 7.6: COSYPH spectrum of compound 2.7 ( $\text{CDCl}_3$ )

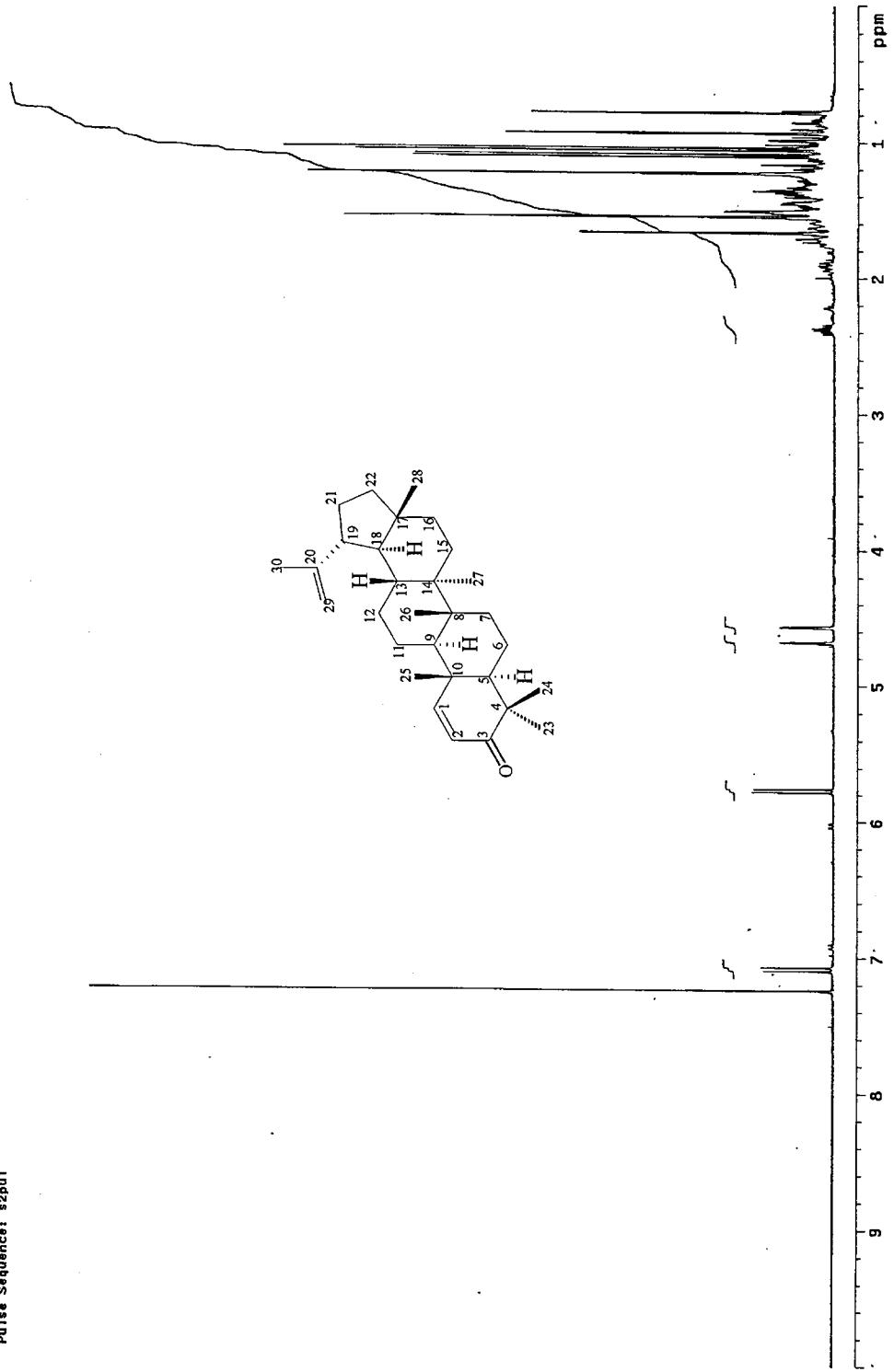
Spectrum 7.7: Mass spectrum of compound 2.7





Spectrum 7.8: FTIR spectrum of compound 2.7 ( $\text{CDCl}_3$ )

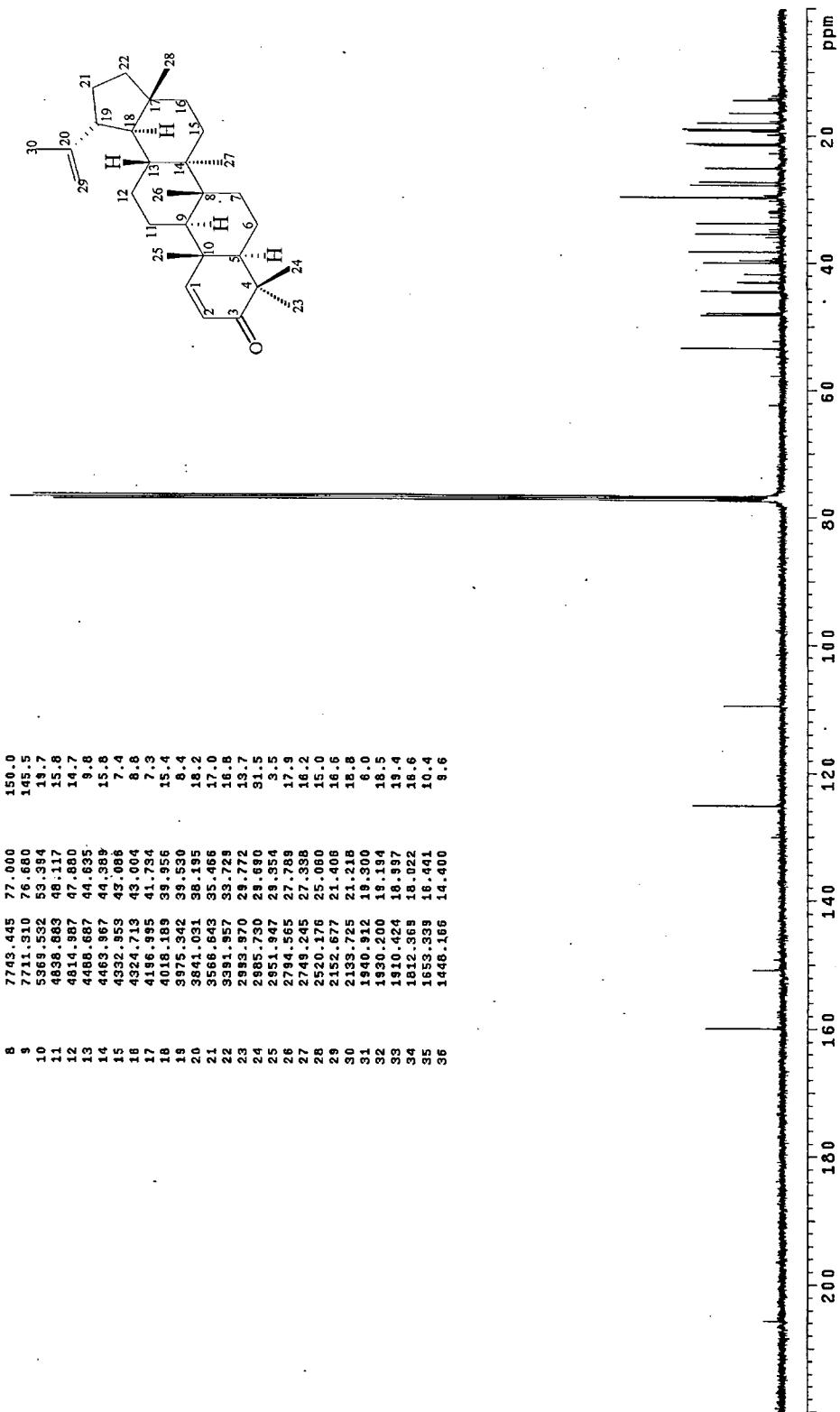
hpx13-Phyretch 72.13 in cdcl3  
pulse sequence: s2pu1



Spectrum 8.1:  $^1\text{H}$  NMR spectrum of compound 2.8 ( $\text{CDCl}_3$ )

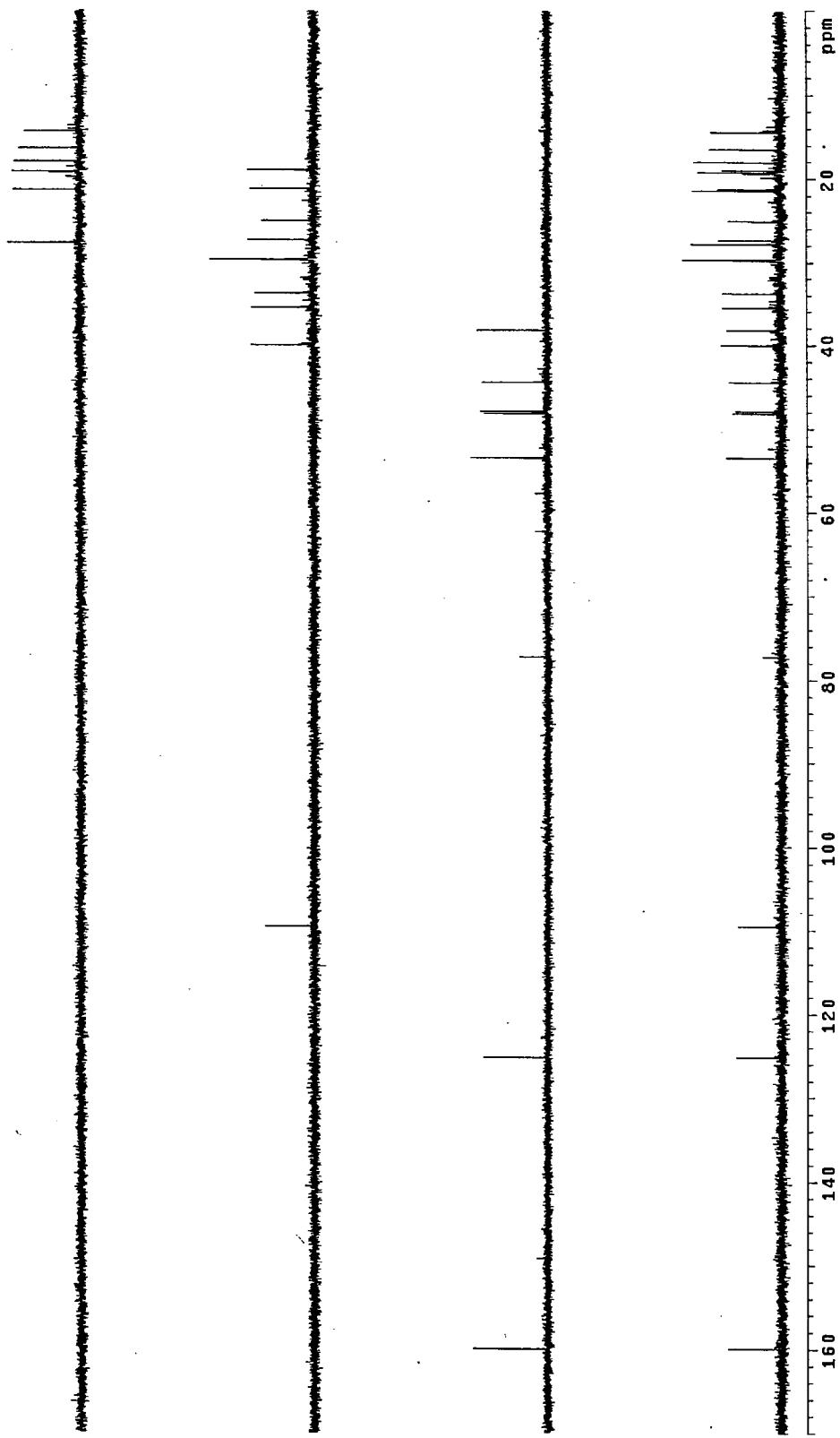
cpv13-phrsth 72.13 in cdcl<sub>3</sub>  
probe=5mmaw  
Pulse Sequence: s2pu1

INDEX	FREQUENCY	PPM	HEIGHT
1	269.6 .784	6.008	3.6
2	16092.219	159.920	15.0
3	15162.648	150.776	5.8
4	12583.559	125.130	17.5
5	11009.740	109.480	11.4
6	7775.581	77.320	141.6
7	7784.045	77.205	9.5
8	7713.445	77.000	157.0
9	7711.310	76.680	145.5
10	5869.532	58.384	18.7
11	4838.383	48.117	15.8
12	4814.387	47.880	14.7
13	4888.687	48.635	9.8
14	4663.967	44.388	15.8
15	4532.353	43.088	7.4
16	4324.713	43.004	8.8
17	4186.995	41.734	7.3
18	4018.189	39.955	15.4
19	3975.342	39.530	8.4
20	3841.031	39.195	18.2
21	3666.643	31.465	17.0
22	3581.557	33.725	16.8
23	2893.970	28.272	13.7
24	2985.730	28.690	31.5
25	2951.947	28.354	3.5
26	2794.565	27.785	17.9
27	2749.245	27.385	16.2
28	2520.476	25.080	15.0
29	2452.677	21.408	16.6
30	2433.725	21.218	18.8
31	1940.912	18.300	6.0
32	1930.200	18.184	18.5
33	1810.424	18.397	19.4
34	1812.368	18.022	18.6
35	1853.339	18.441	10.4
36	1446.166	14.400	9.6

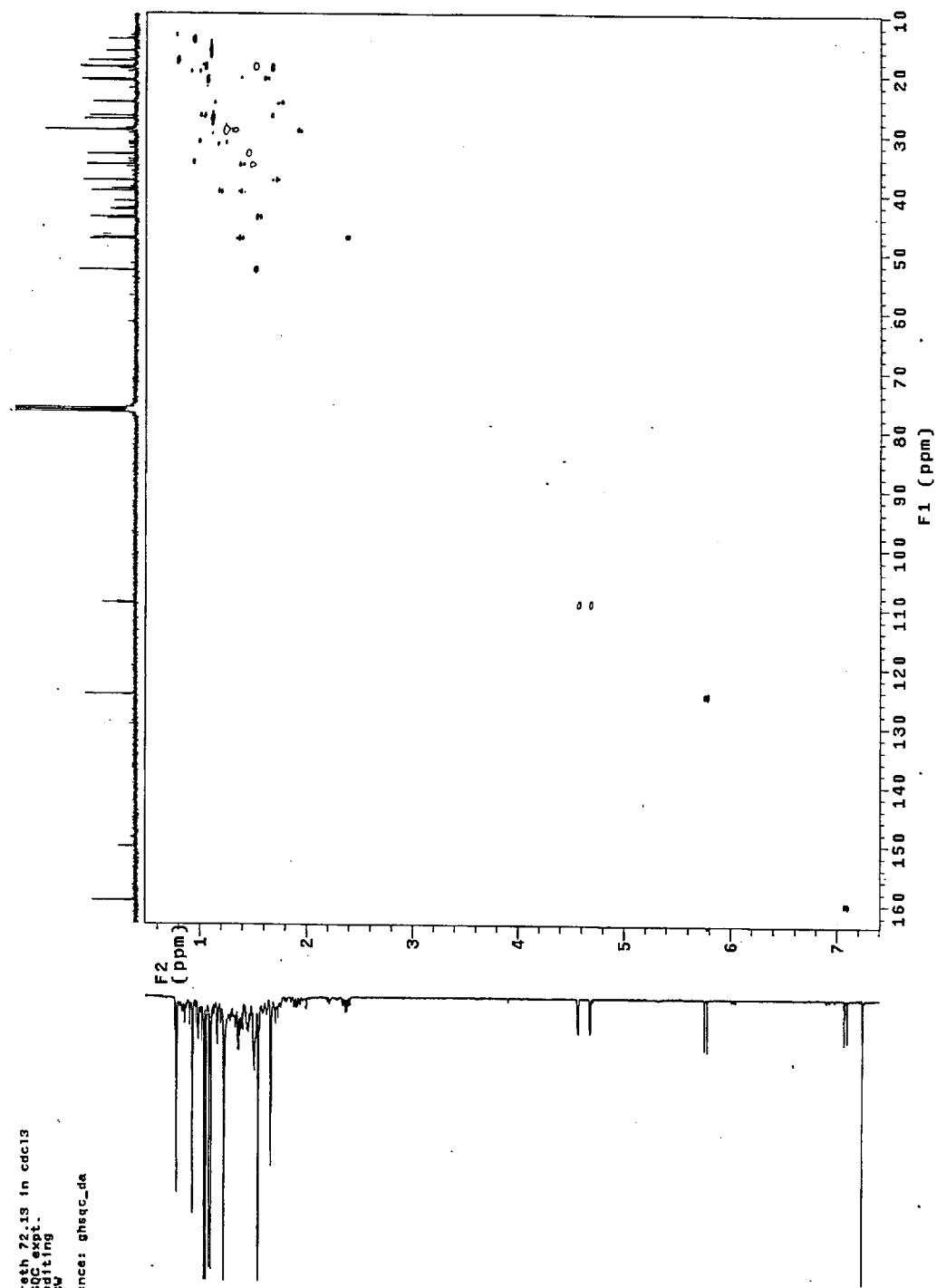


Spectrum 8.2: <sup>13</sup>C NMR spectrum of compound 2.8 (CDCl<sub>3</sub>)

Spectrum 8.3: DEPT NMR spectrum of compound 2.8 ( $\text{CDCl}_3$ )

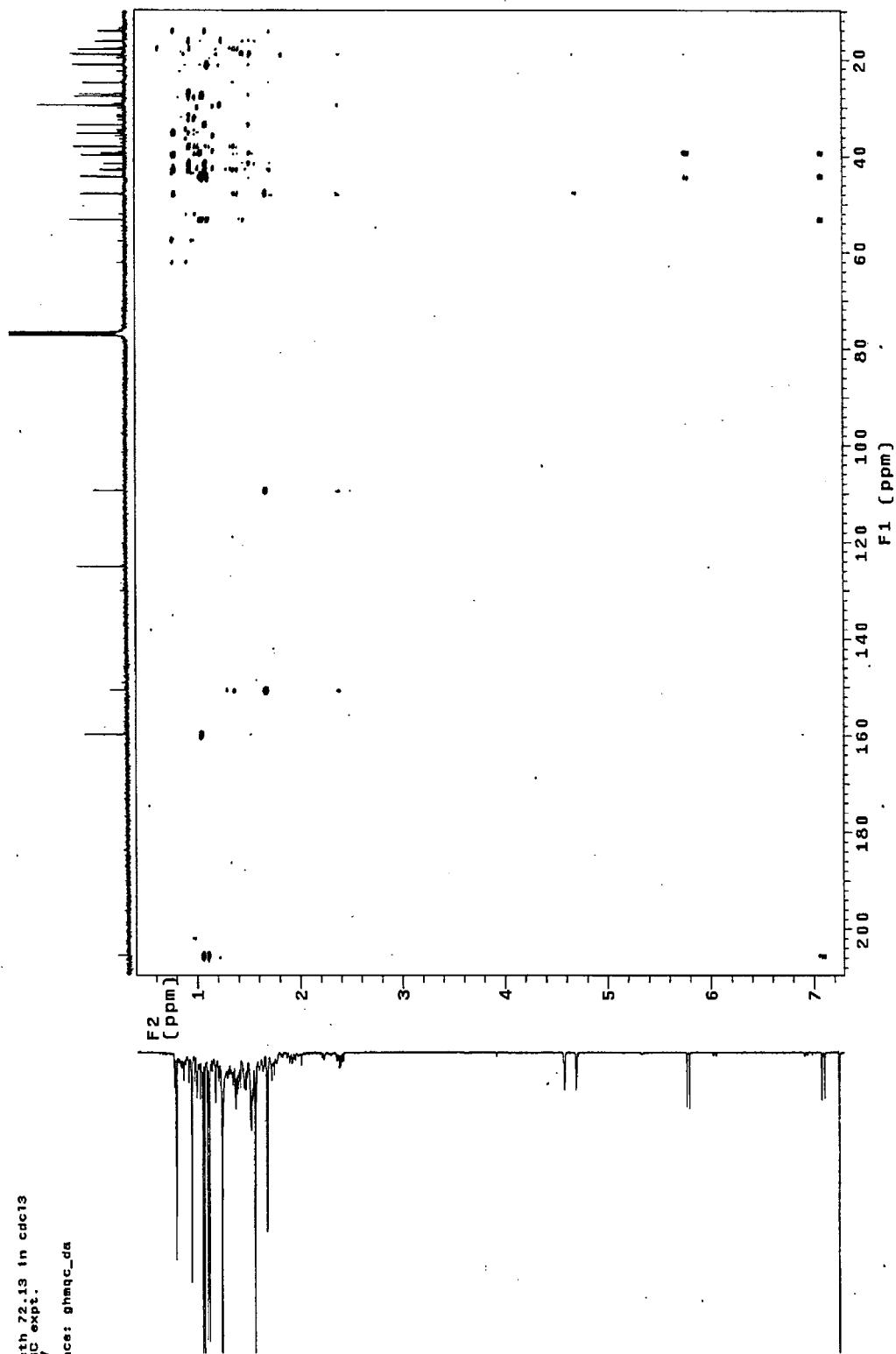


HQPYIS-Phyreth 72.13 in cdc13  
Gradient HSQC expt.  
with mult-editing  
probe-Sma3g  
Pulse Sequence: ghsqc-de



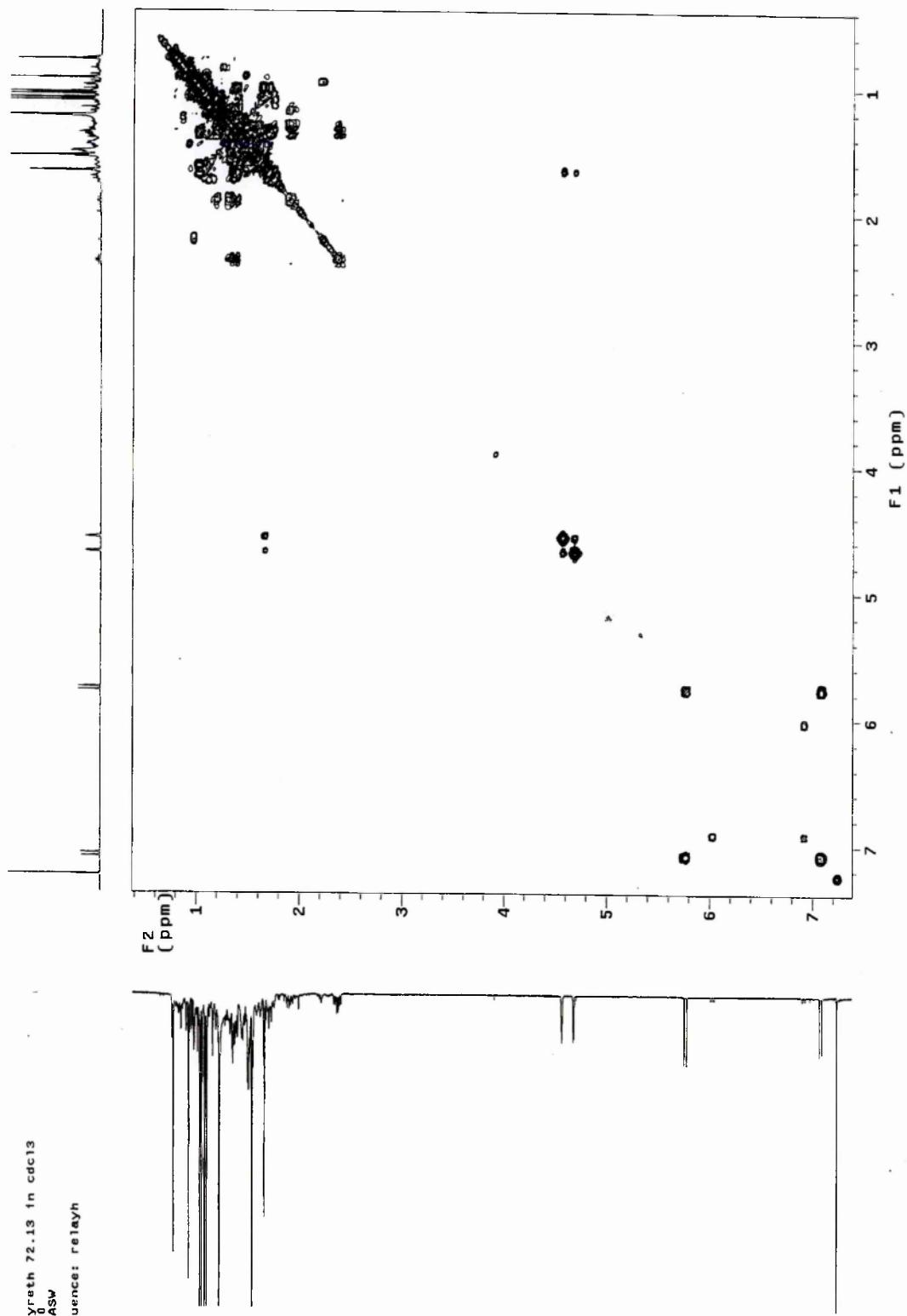
Spectrum 8.4: HSQC spectrum of compound 2.8 ( $\text{CDCl}_3$ )

HBPY13.phyreth 72.13 in cdc13  
Gradient HMBC expt.  
probe:gammaSW  
Pulse Sequence: ghsqc\_dq



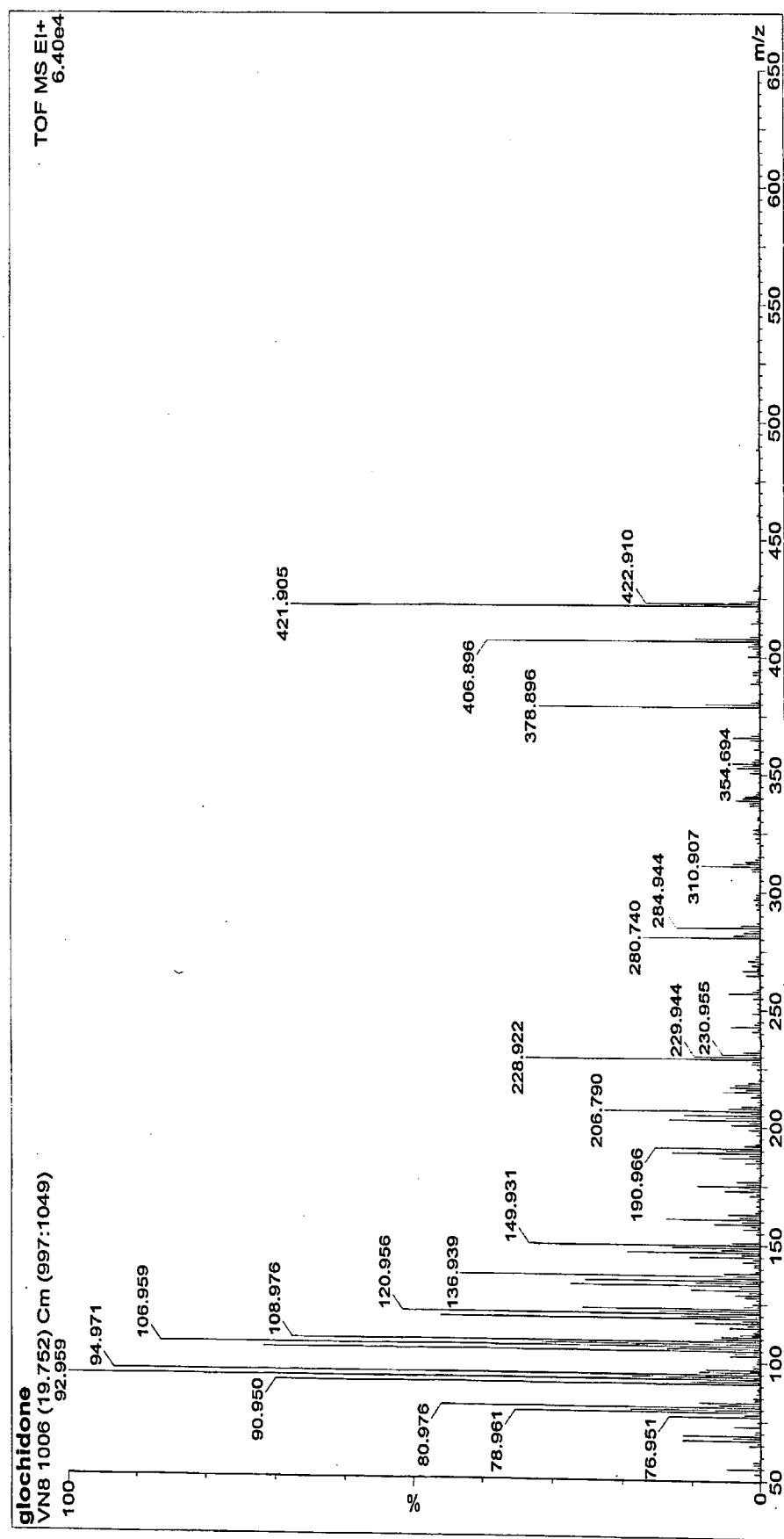
Spectrum 8.5: HMBC spectrum of compound 2.8 ( $\text{CDCl}_3$ )

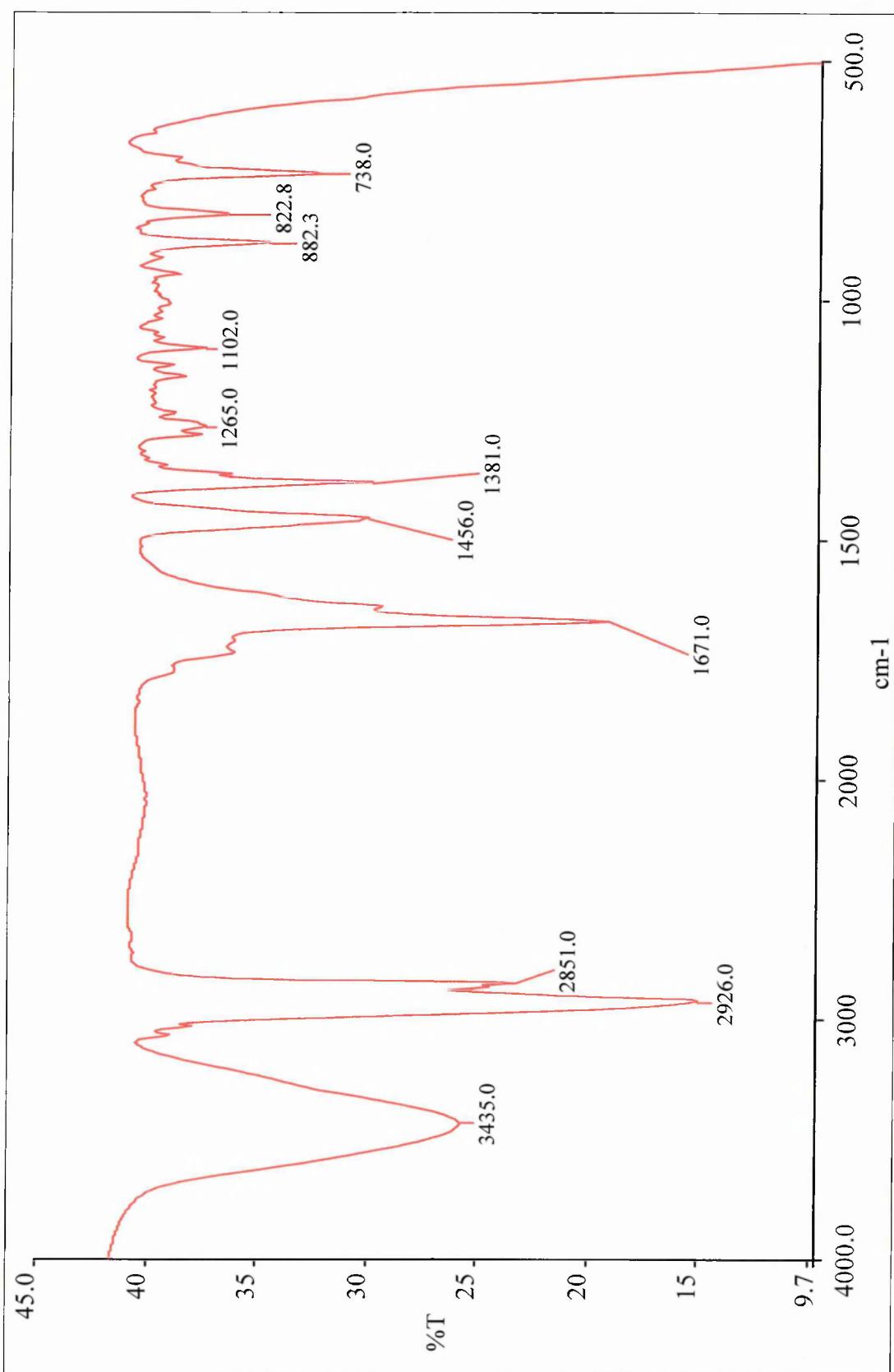
CYPY13.phy  
1H COSY-102  
probe:5mmASW  
Pulse Sequence: relayh



Spectrum 8.6: COSY spectrum of compound 2.8 ( $\text{CDCl}_3$ )

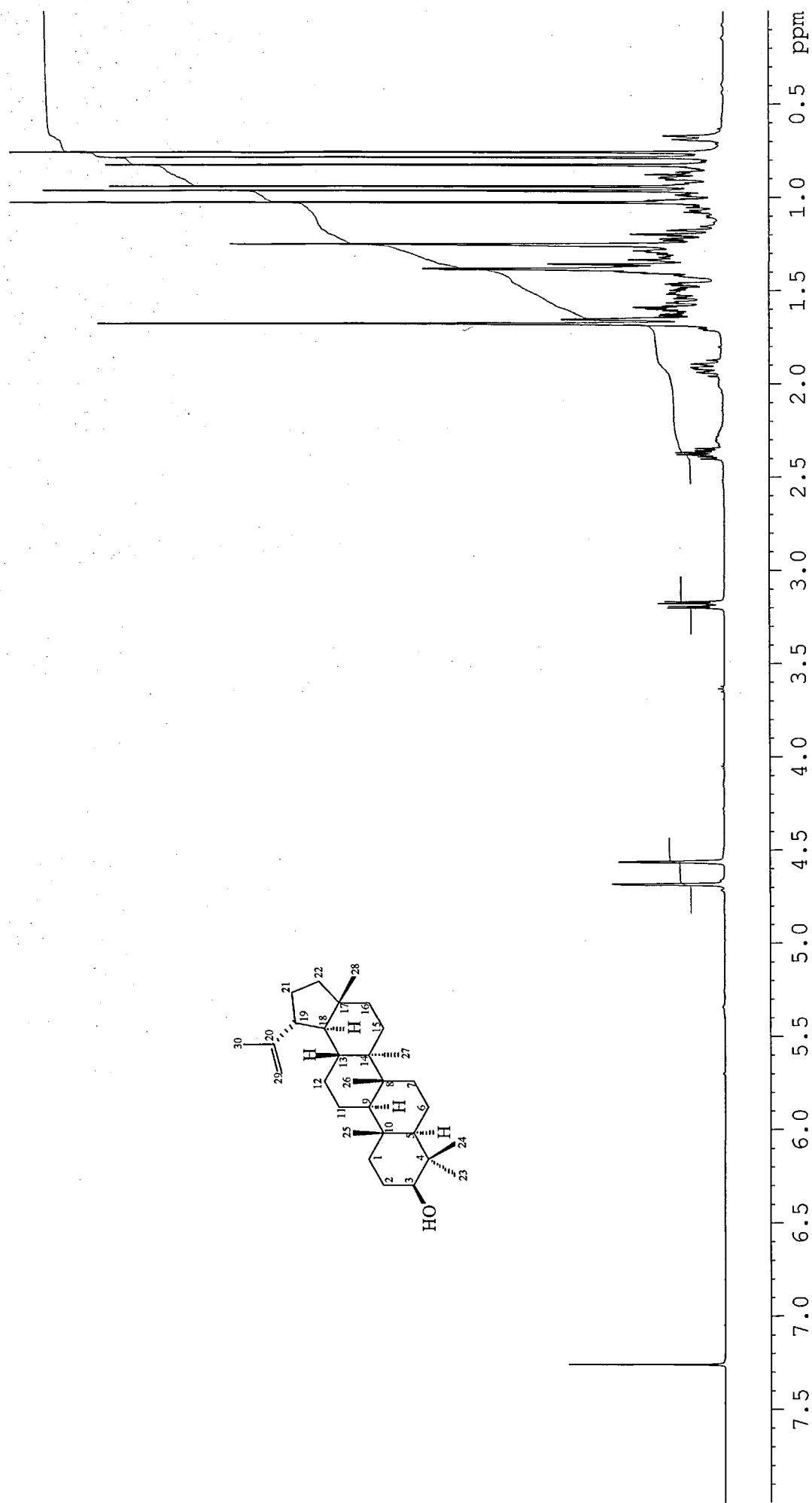
Spectrum 8.7: Mass spectrum of compound 2.8

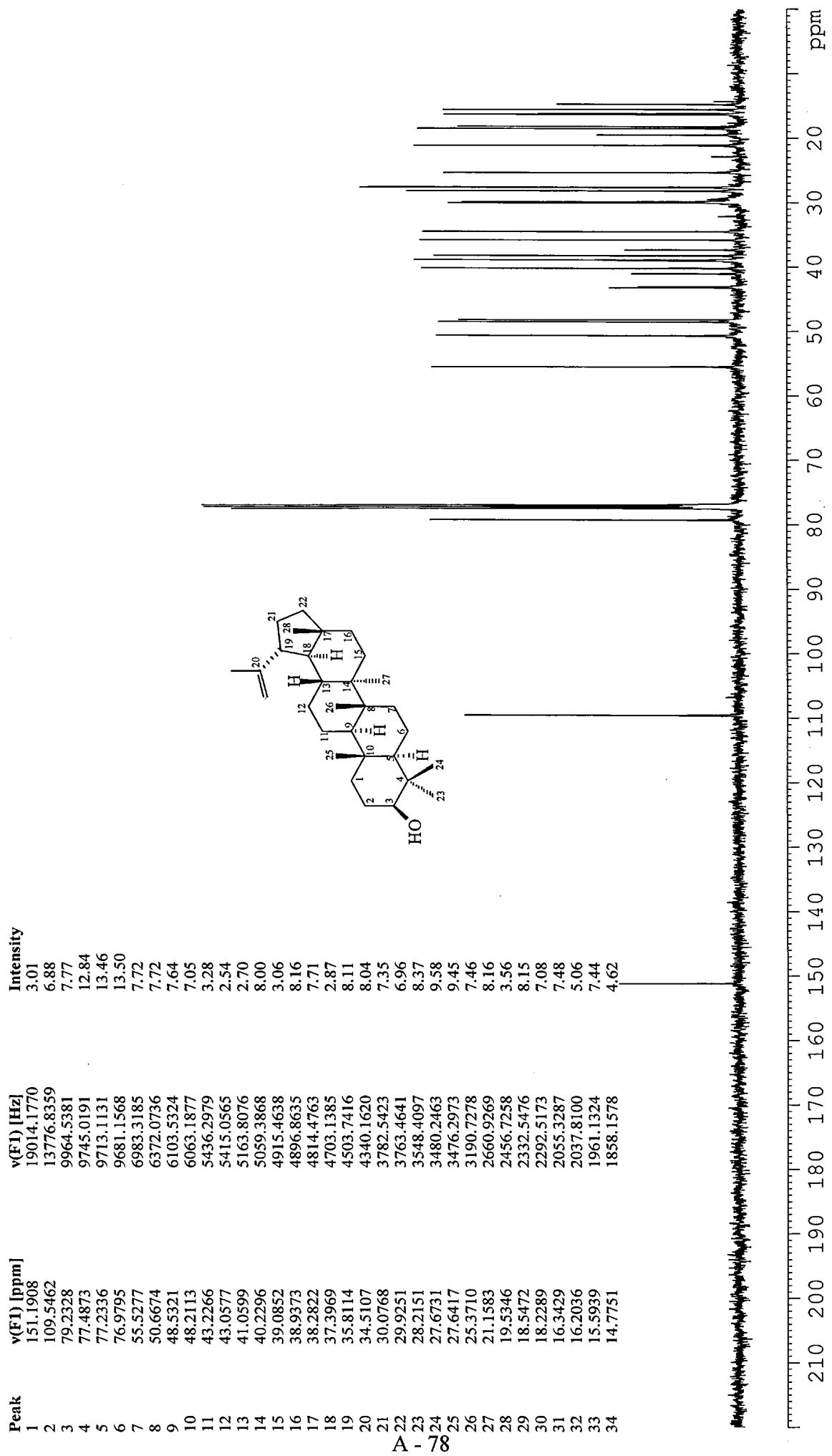




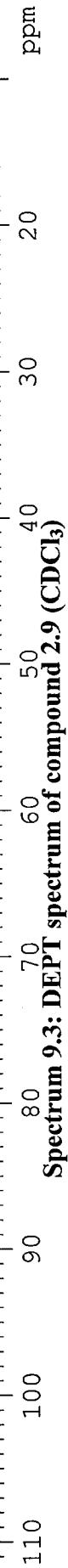
Spectrum 8.8: FTIR spectrum of compound 2.8

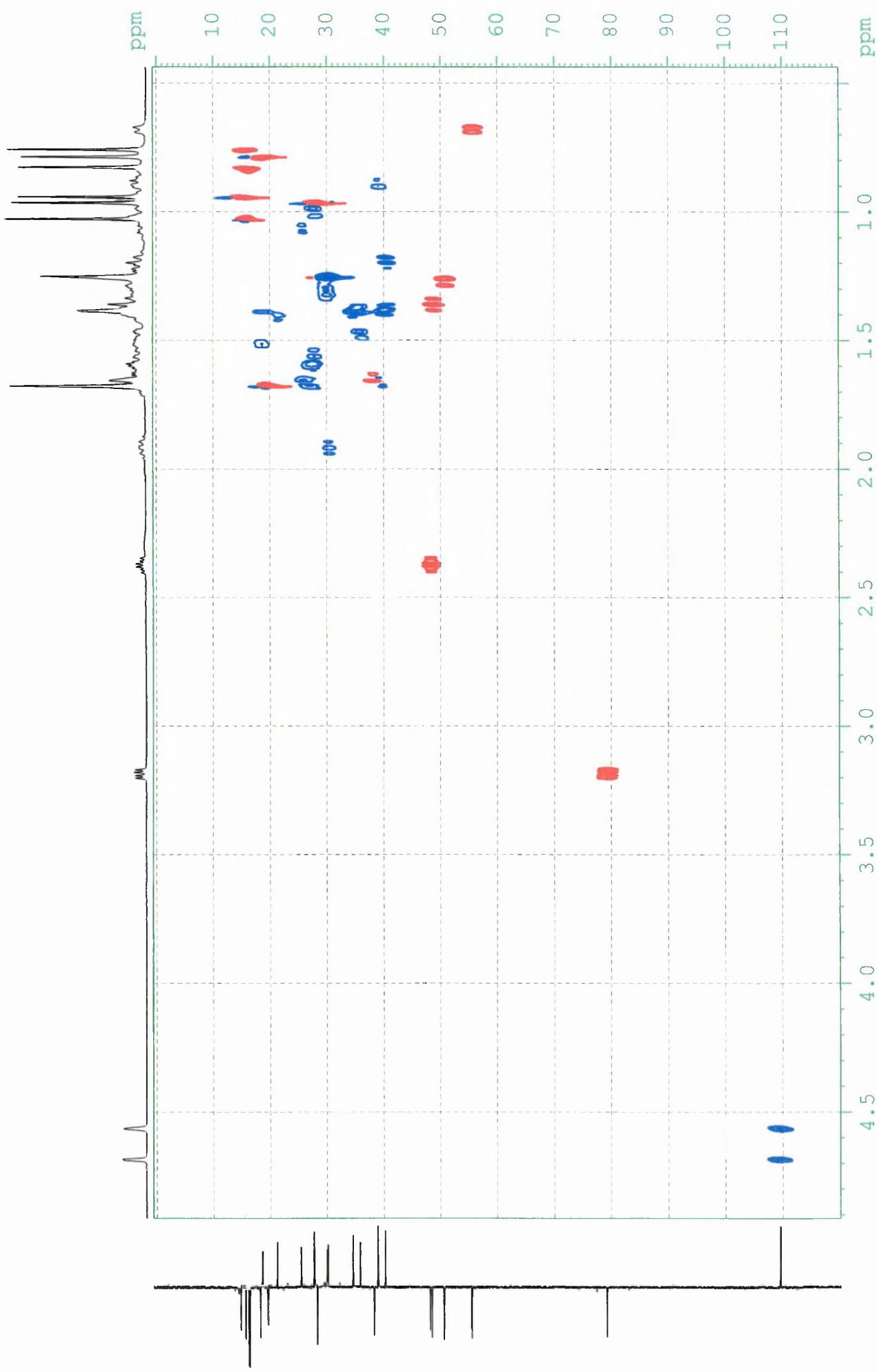
Spectrum 9.1:  $^1\text{H}$  NMR spectrum of compound 2.9 ( $\text{CDCl}_3$ )





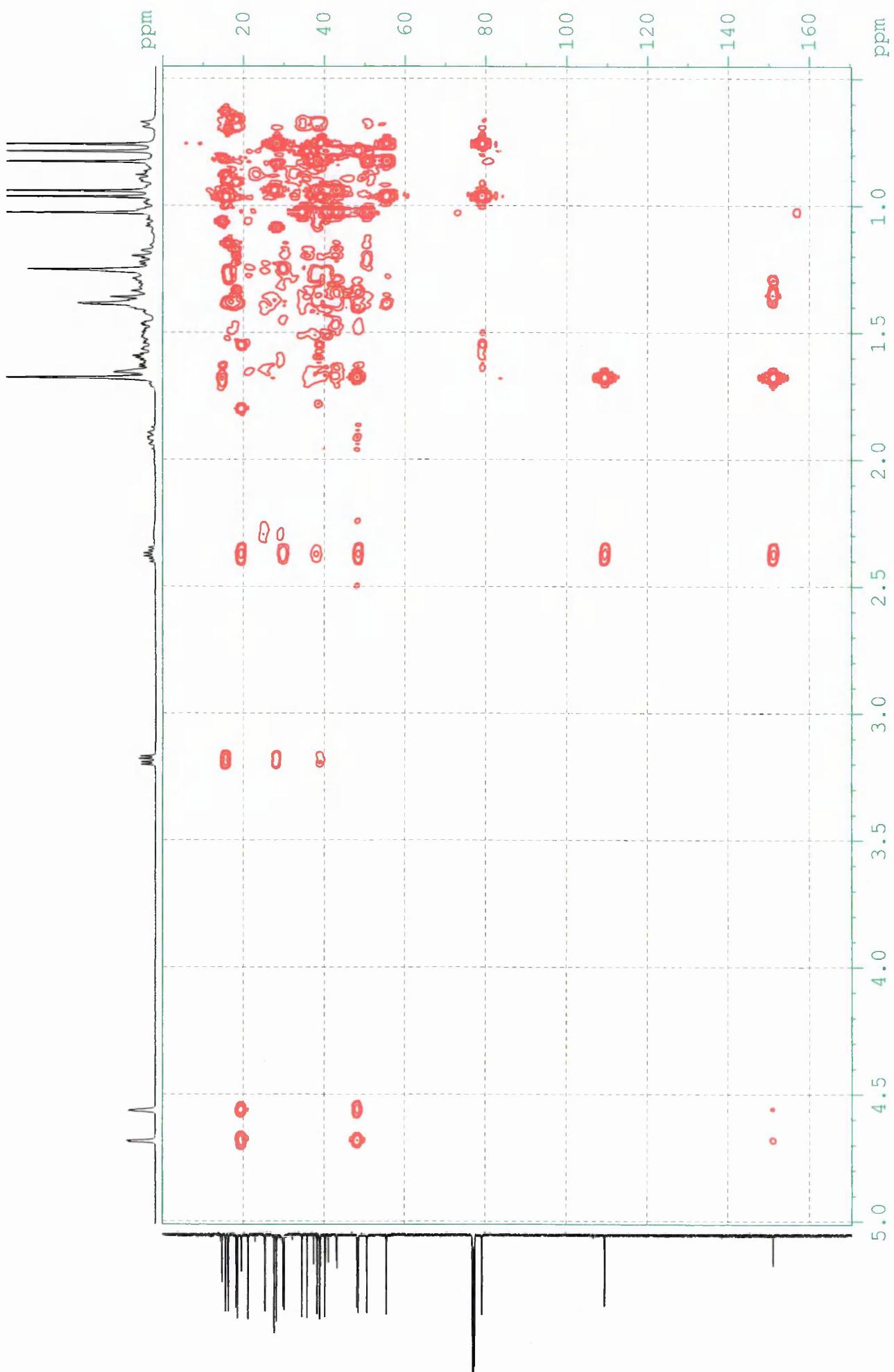
Spectrum 9.2:  $^{13}\text{C}$  spectrum of compound 2.9 ( $\text{CDCl}_3$ )



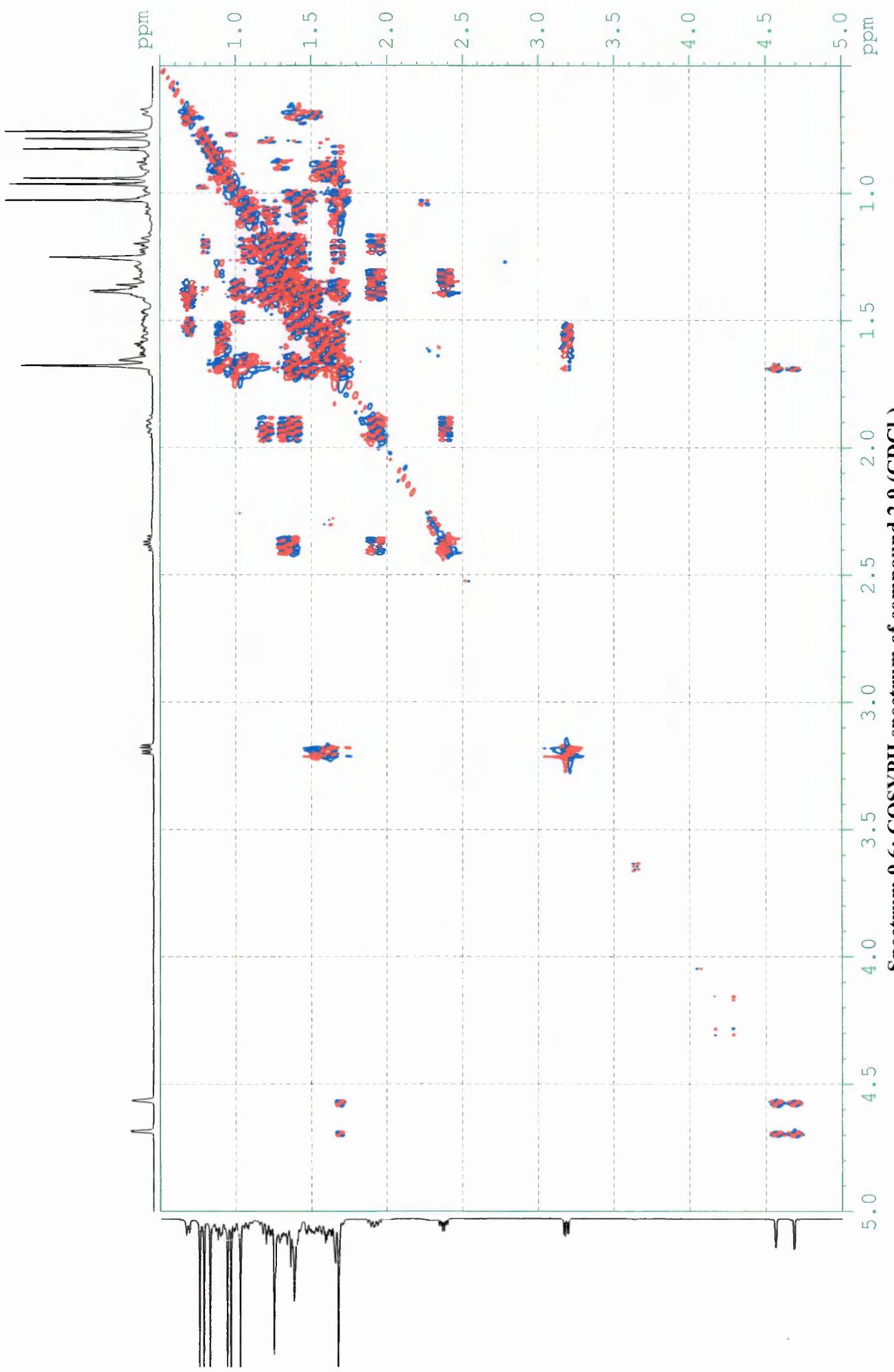


A - 80

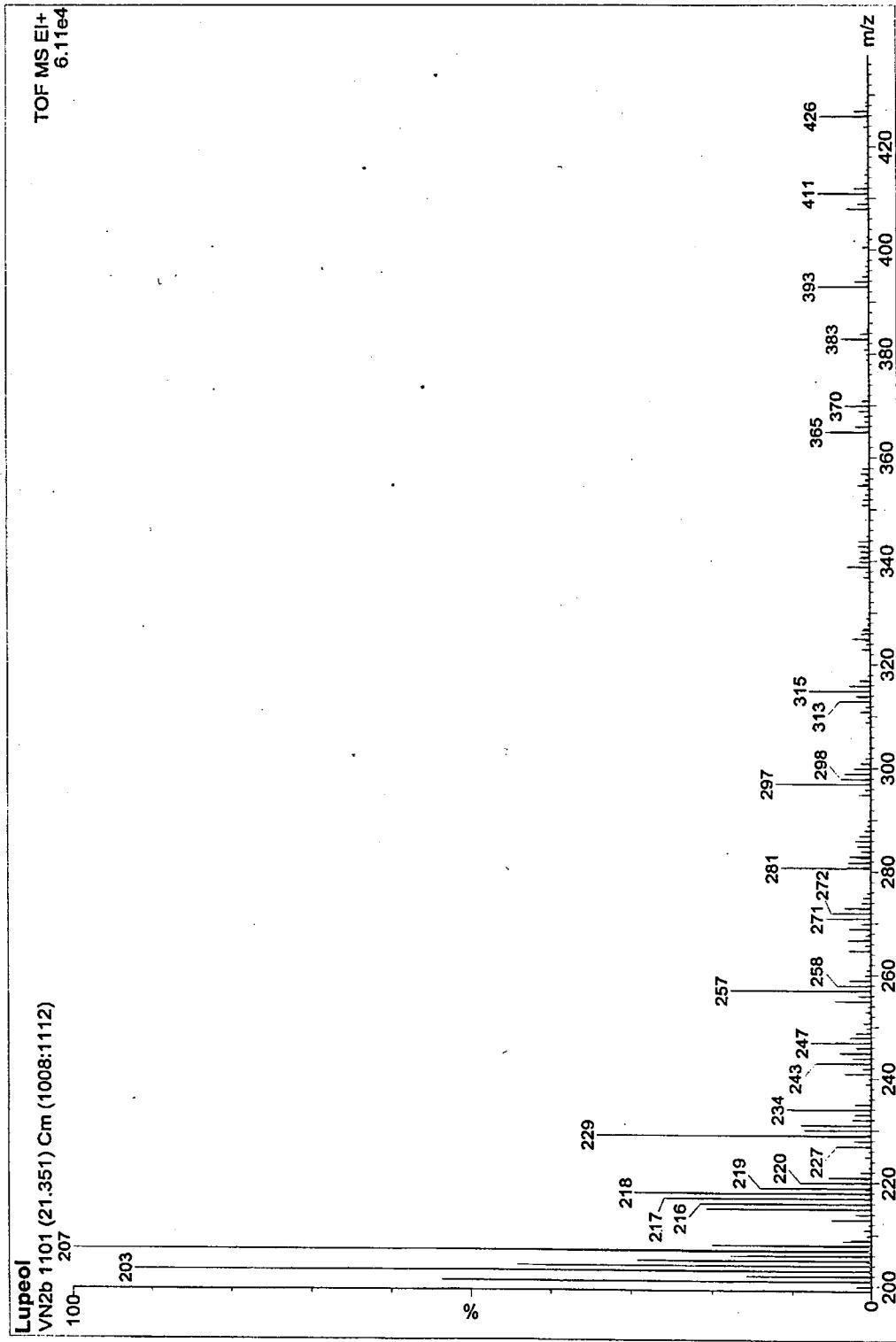
Spectrum 9.4: HSQCDEPT spectrum of compound 2.9 ( $\text{CDCl}_3$ )

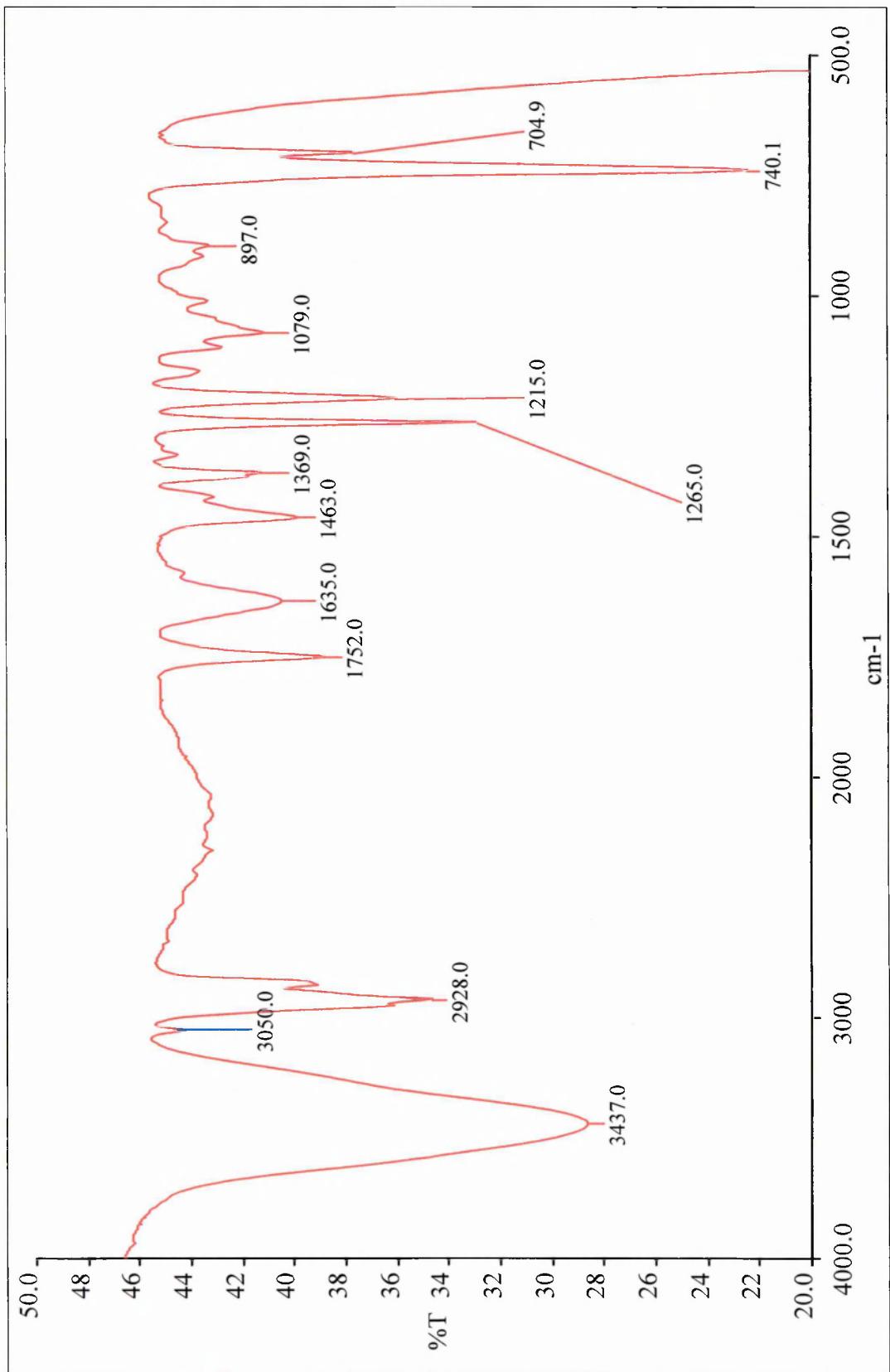


Spectrum 9.5: HMBCCLP spectrum of compound 2.9 ( $\text{CDCl}_3$ )



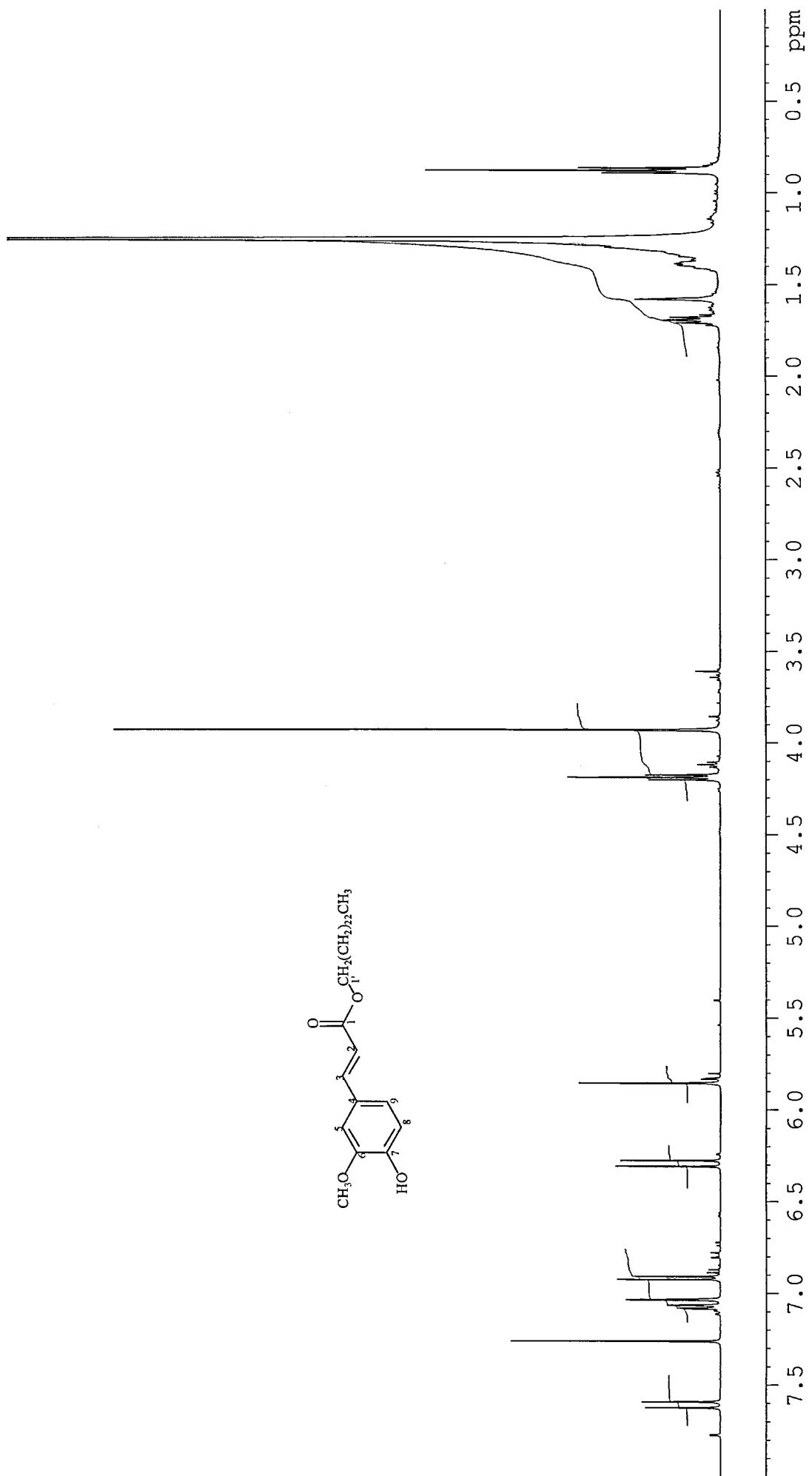
Spectrum 9.7: Mass spectrum of compound 2.9





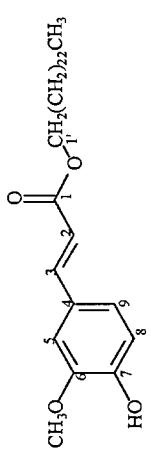
Spectrum 9.8: FTIR spectrum of compound 2.9

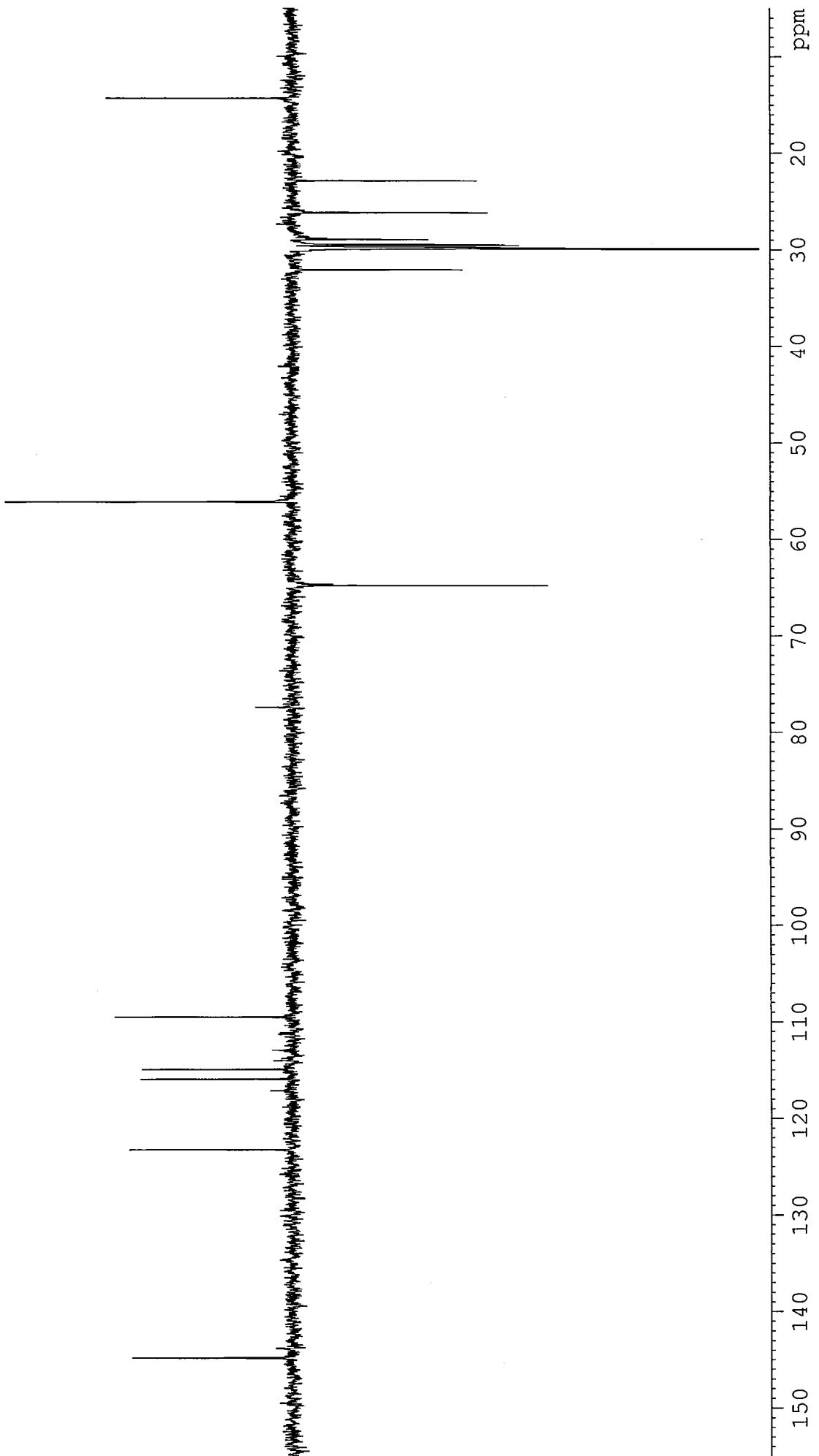
Spectrum 10.1:  $^1\text{H}$  NMR spectrum of compound 2.10 ( $\text{CDCl}_3$ )



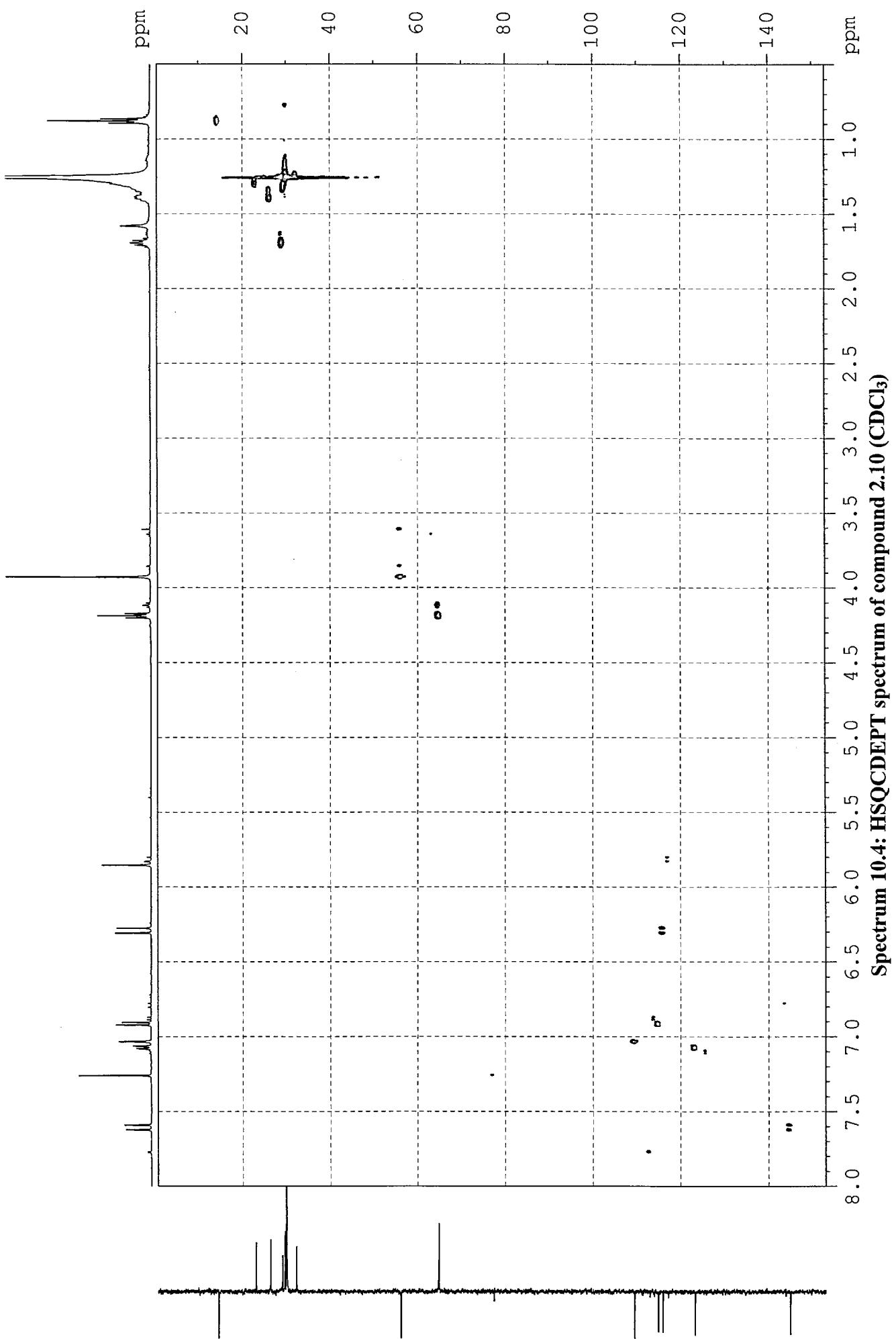
Spectrum 10.2:  $^{13}\text{C}$  NMR spectrum of compound 2.10 ( $\text{CDCl}_3$ )

Peak	$\nu(\text{F1})$ [ppm]	$\nu(\text{F1})$ [Hz]	Intensity
1	167.6512	21084.2828	0.02
2	148.2160	18640.0578	0.00
3	146.9446	18480.1630	0.04
4	143.8568	18091.8326	0.02
5	144.8366	18215.0550	0.75
6	127.2995	16009.5404	0.24
7	125.8133	15822.6317	0.07
8	123.2628	15501.8737	0.79
9	117.3377	14756.7166	0.00
10	113.8866	14322.6966	0.01
11	112.7968	14185.6403	0.00
12	115.9306	14579.7558	0.74
13	114.9167	14452.2449	0.78
14	109.5110	13772.4090	0.74
15	77.4885	9745.1700	2.47
16	77.2345	9713.2263	2.57
17	76.9807	9681.3077	2.58
18	64.8426	8154.7863	0.87
19	56.1599	7062.8257	0.72
20	32.1538	4043.7516	0.84
21	29.7789	3745.0776	1.37
22	29.5878	3721.0443	1.15
23	29.5369	3714.6430	1.10
24	29.0073	3648.0390	0.91
25	26.2292	3298.6574	0.89
26	22.9174	2882.1562	0.78
27	14.3409	1803.5516	0.73

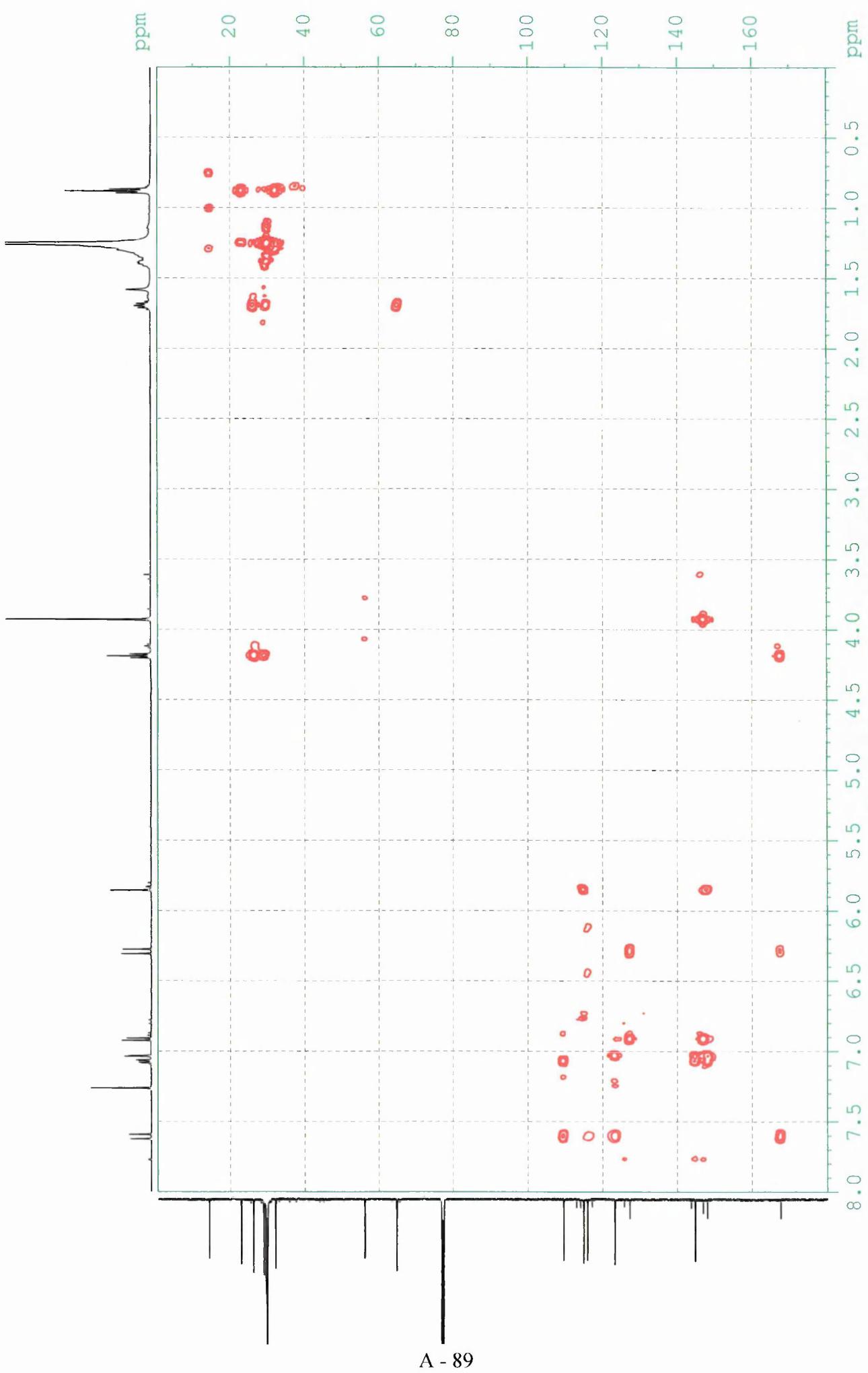


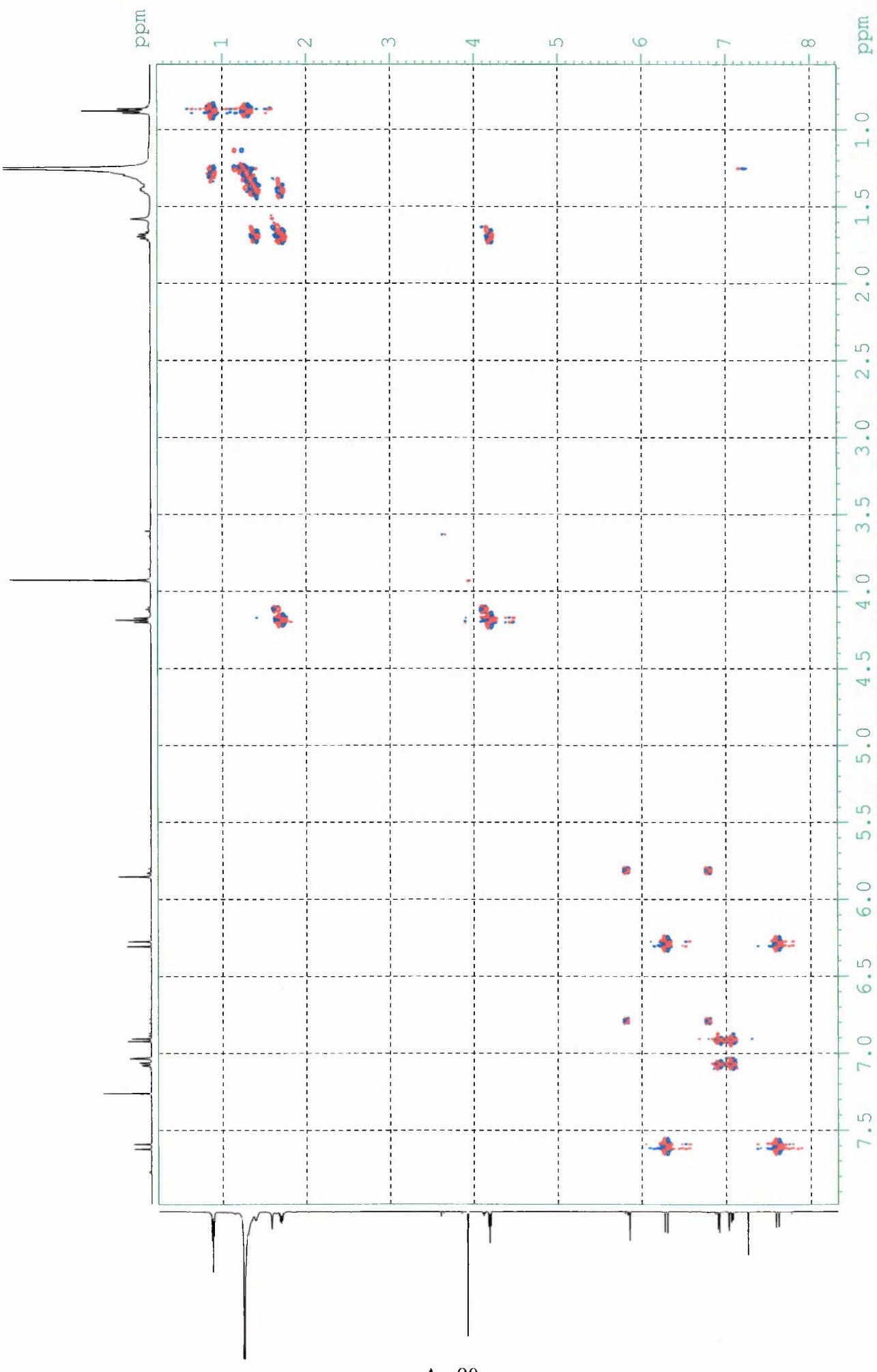


Spectrum 10.3: DEPT NMR spectrum of compound 2.10 ( $\text{CDCl}_3$ )



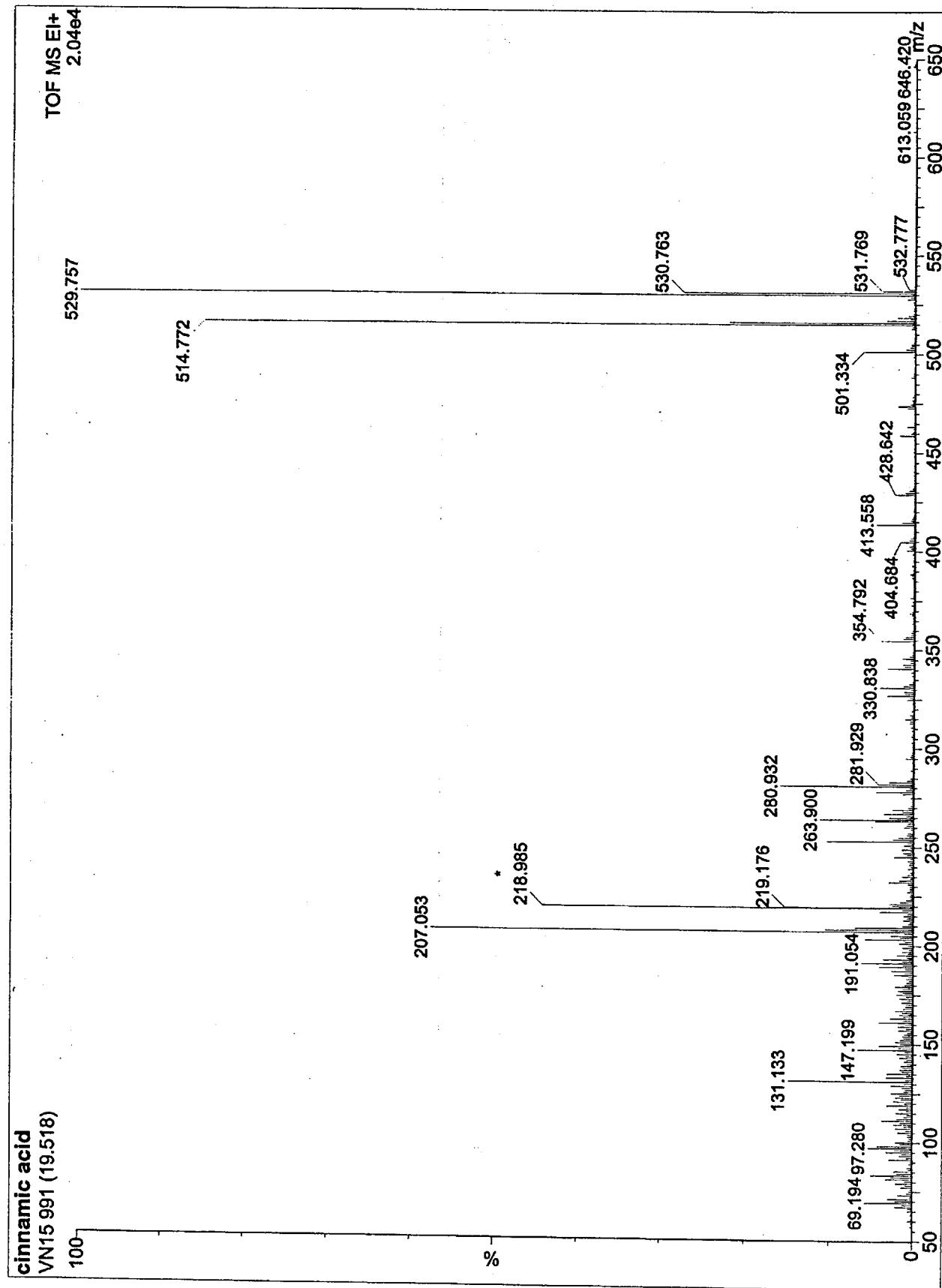
**Spectrum 10.5: HMBCLP spectrum of compound 2.10 ( $\text{CDCl}_3$ )**



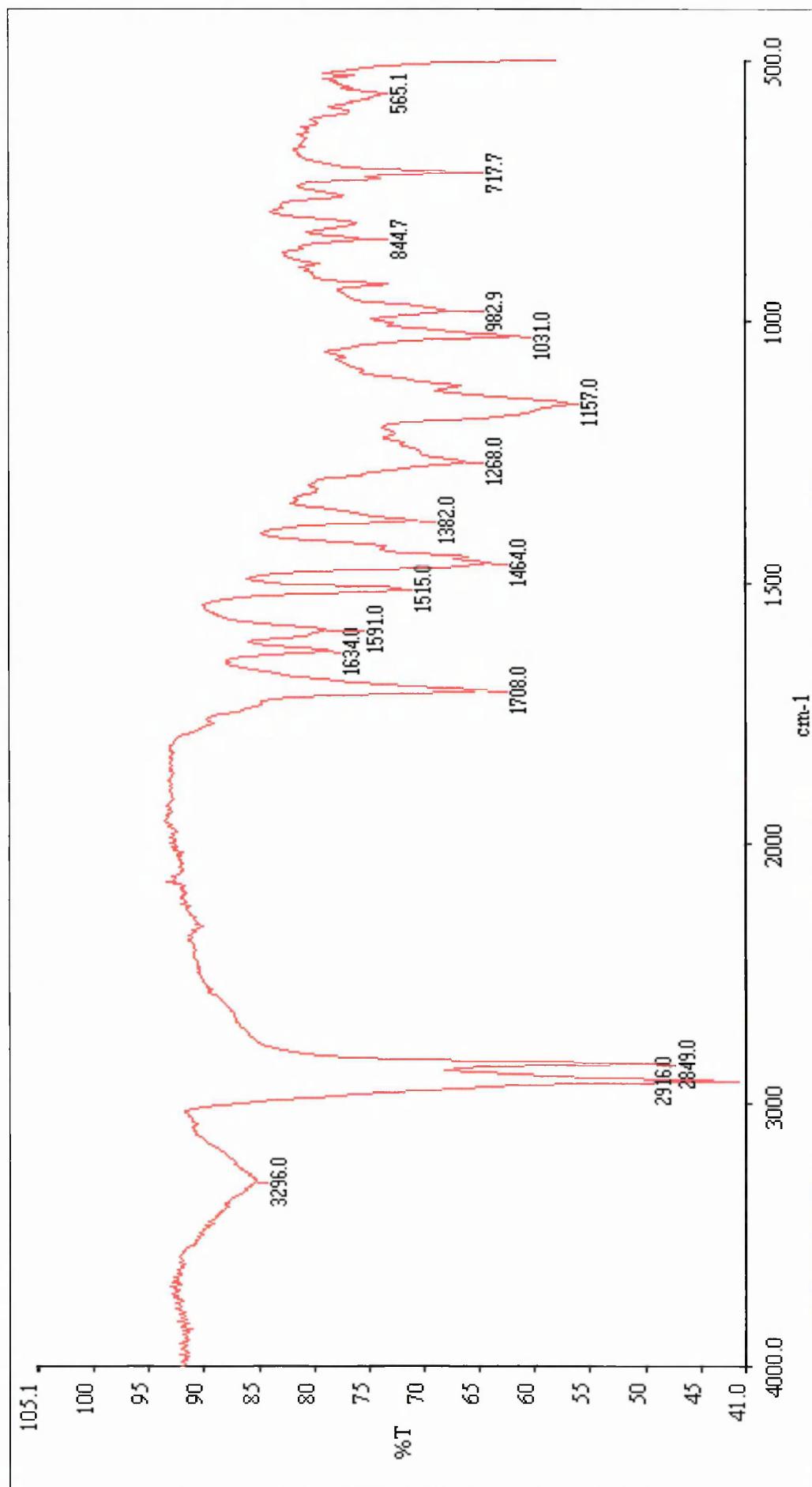


Spectrum 10.6: COSYPH spectrum of compound 2.10 ( $\text{CDCl}_3$ )

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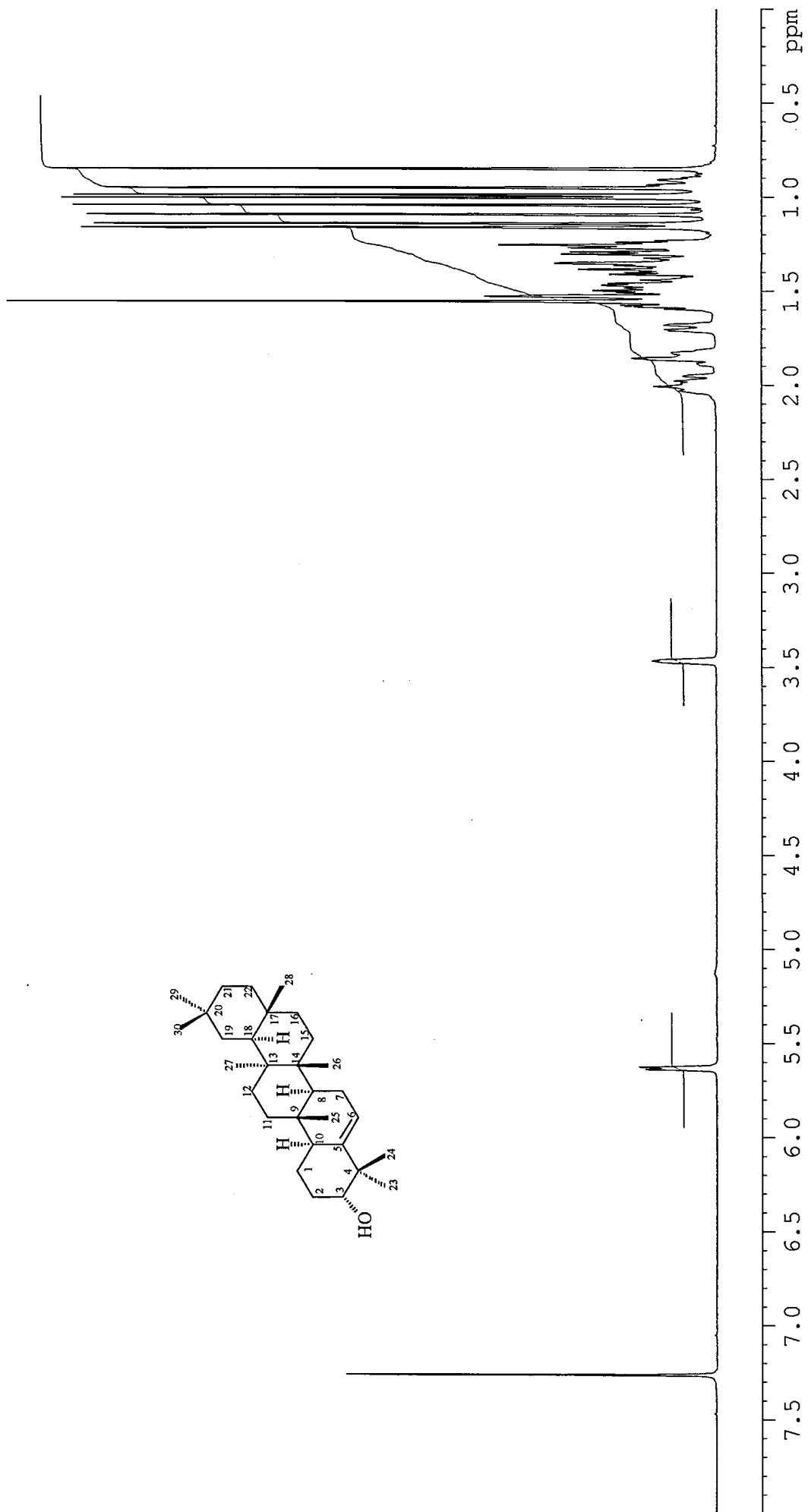


Spectrum 10.7: Mass spectrum of compound 2.10

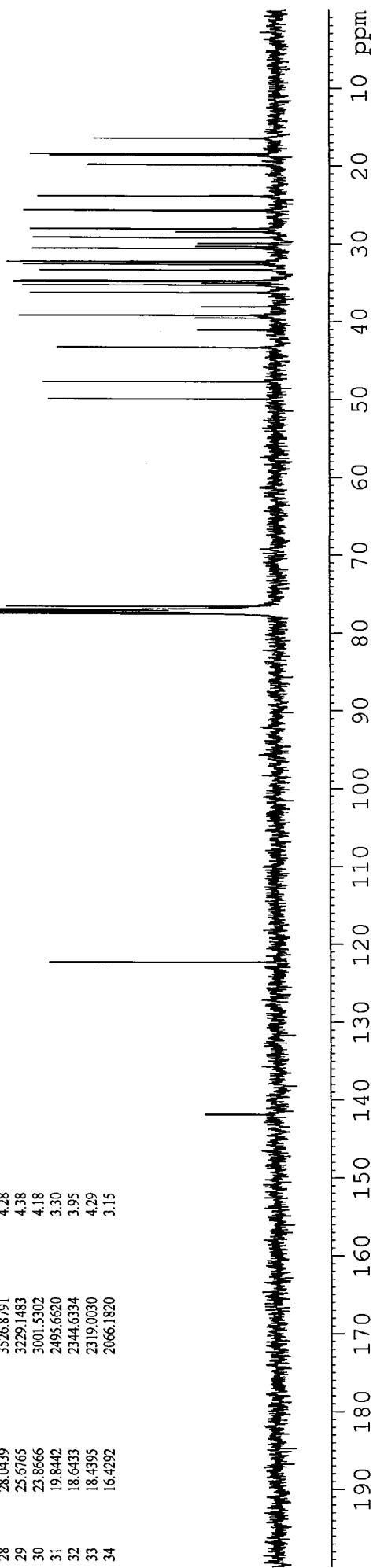
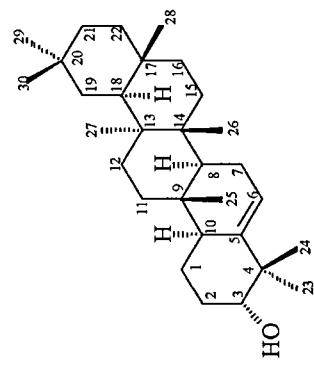


Spectrum 10.8: FTIR spectrum of compound 2.10 ( $\text{CDCl}_3$ )

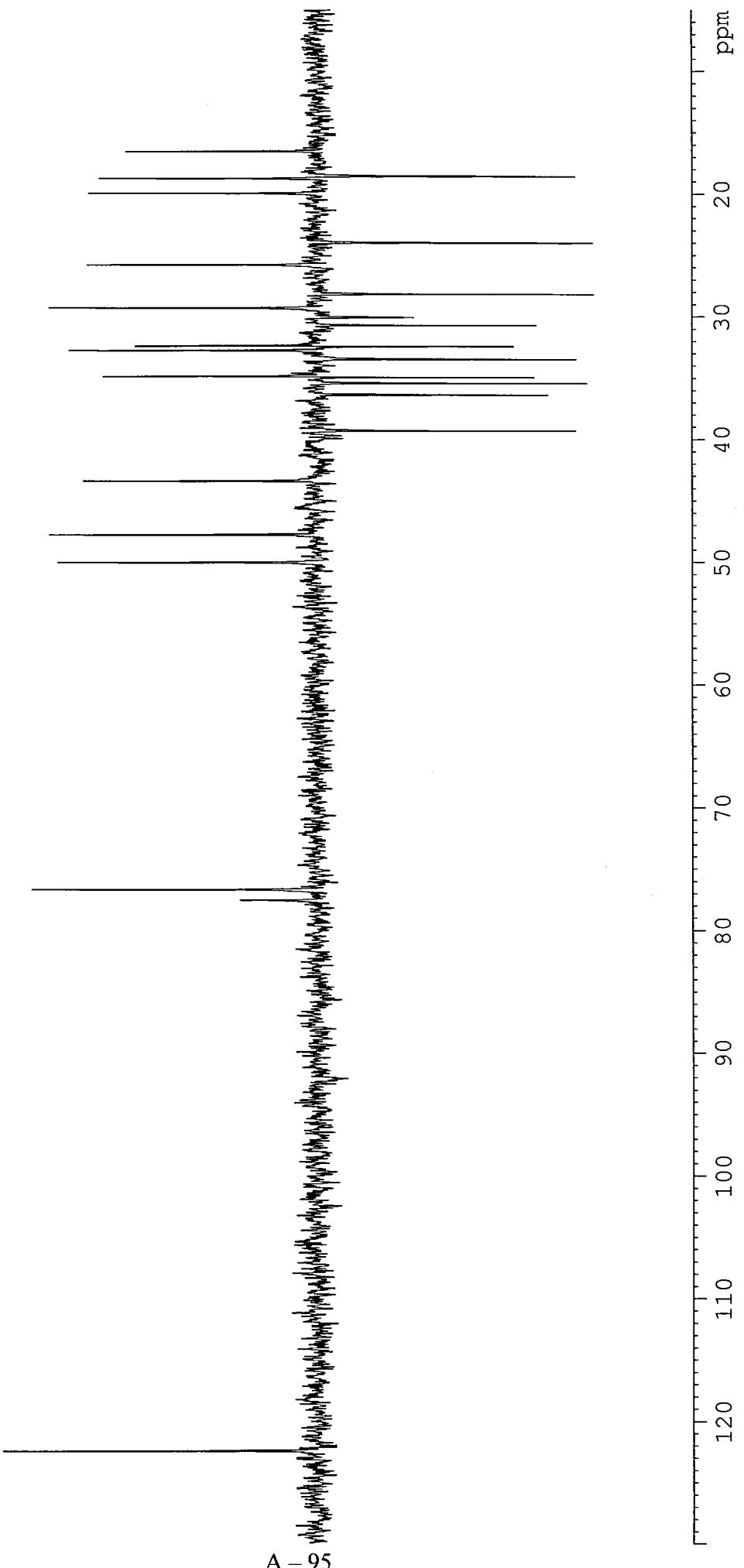
Spectrum 11.1:  $^1\text{H}$  NMR spectrum of compound 2.11 ( $\text{CDCl}_3$ )



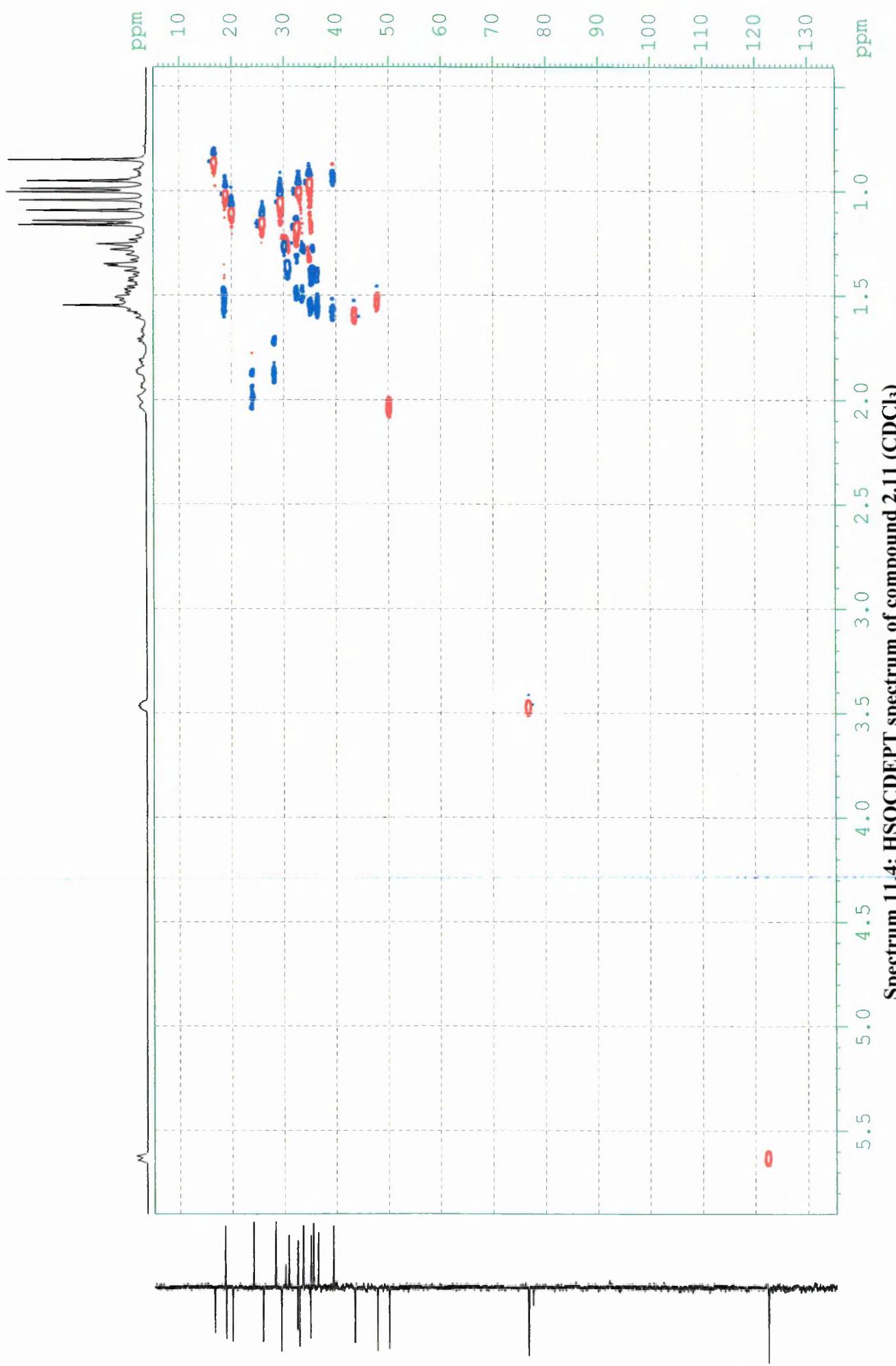
Peak	$\delta$ (F1) [ppm]	$\nu$ (F1) [Hz]	Intensity
1	141.8362	17837.7164	1.27
2	122.2971	15380.4246	3.93
3	77.4849	9744.7173	18.46
4	77.2311	9712.7987	19.43
5	76.9771	9680.0549	19.50
6	76.5680	9629.4054	4.74
7	49.9167	6277.635	4.00
8	47.6608	5993.5552	4.03
9	43.2876	5443.5694	3.81
10	41.0523	5162.518	1.37
11	39.5388	4971.2522	1.41
12	39.1823	4927.6754	4.46
13	38.0633	4786.5468	1.30
14	36.2457	4558.5604	4.31
15	35.3053	4440.0931	4.40
16	35.0695	4410.3282	1.31
17	34.8330	4380.6953	4.57
18	34.7487	4370.0935	4.43
19	33.3407	4193.0195	4.16
20	32.6178	4102.1056	4.38
21	32.3057	4062.5550	4.71
22	32.2615	4057.2963	4.41
23	30.5799	3845.8136	4.24
24	30.3174	3812.8008	1.41
25	29.9258	3763.3521	1.37
26	29.1722	3668.7773	4.25
27	28.4718	3580.6930	1.74
28	28.0439	3526.6791	4.28
29	25.6765	3229.1483	4.38
30	23.8866	3001.5302	4.18
31	19.8442	2495.6620	3.30
32	18.6433	2344.6334	3.95
33	18.4395	2319.0030	4.29
34	16.4292	2066.1820	3.15

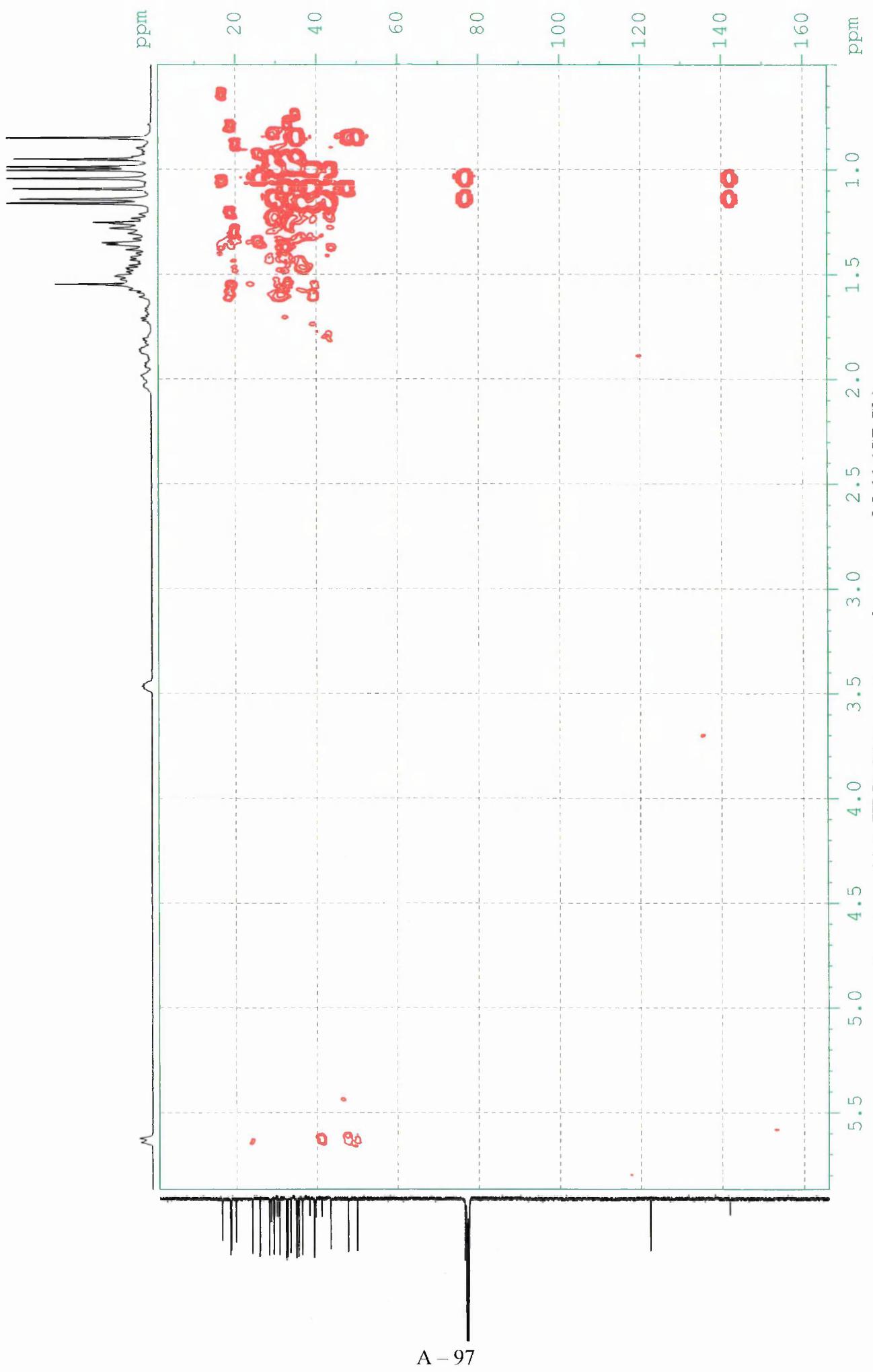


Spectrum 11.2:  $^{13}\text{C}$  NMR spectrum of compound 2.11 ( $\text{CDCl}_3$ )

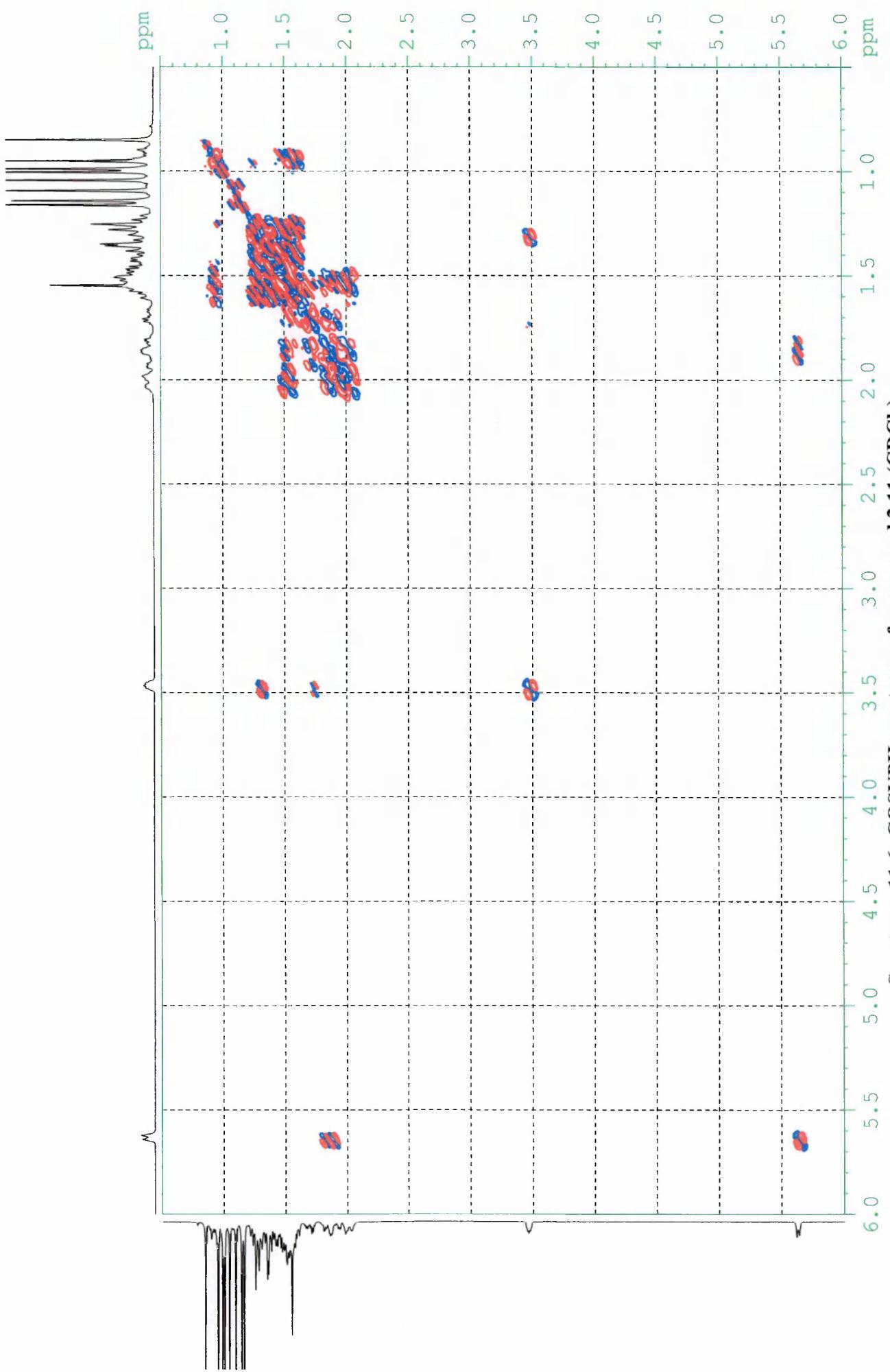


Spectrum 11.3: DEPT spectrum of compound 2.11 ( $\text{CDCl}_3$ )





Spectrum 11.5: HMQCCLP spectrum of compound 2.11 ( $\text{CDCl}_3$ )

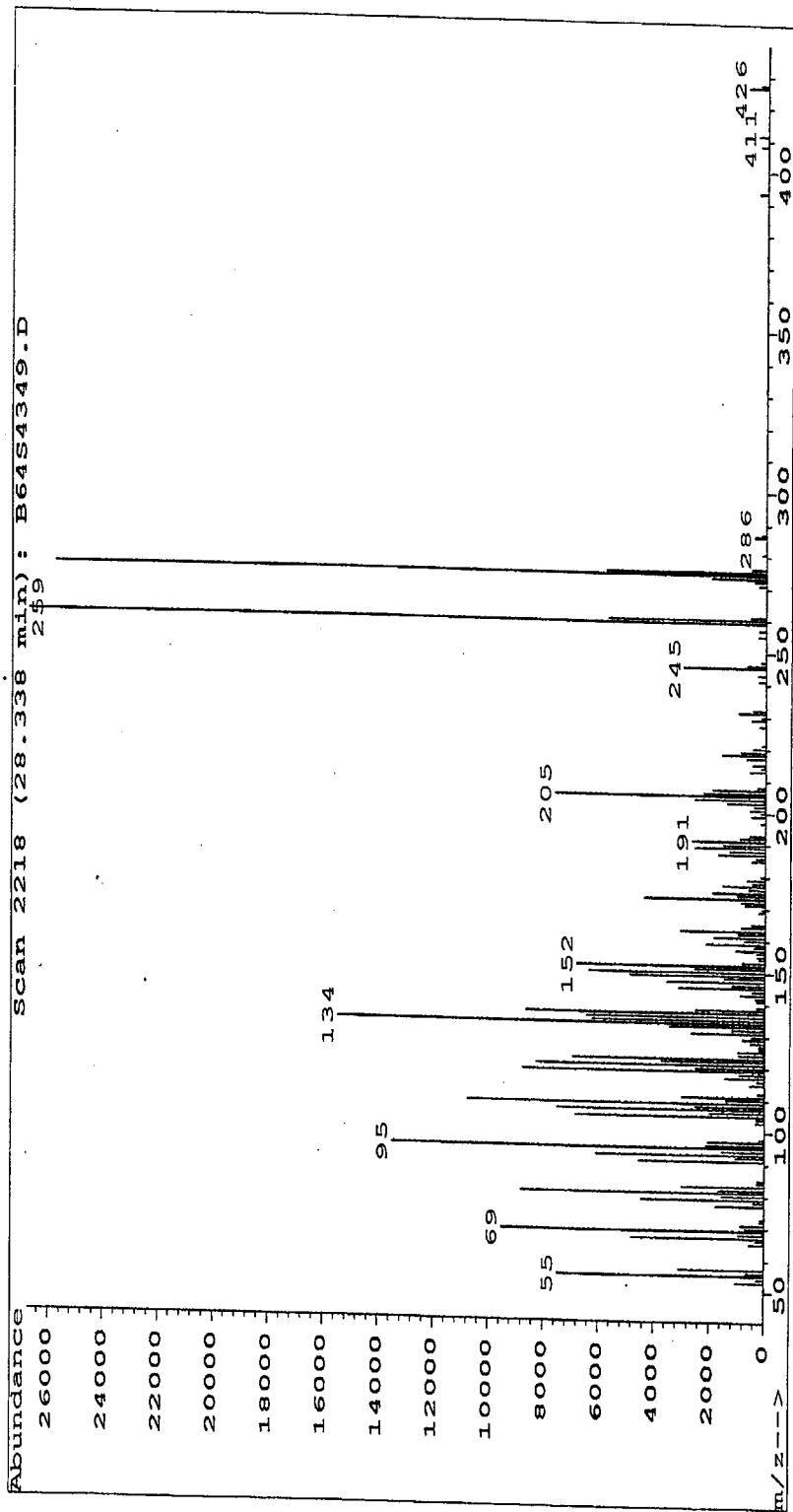


Spectrum 11.6: COSYPH spectrum of compound 2.11 ( $\text{CDCl}_3$ )

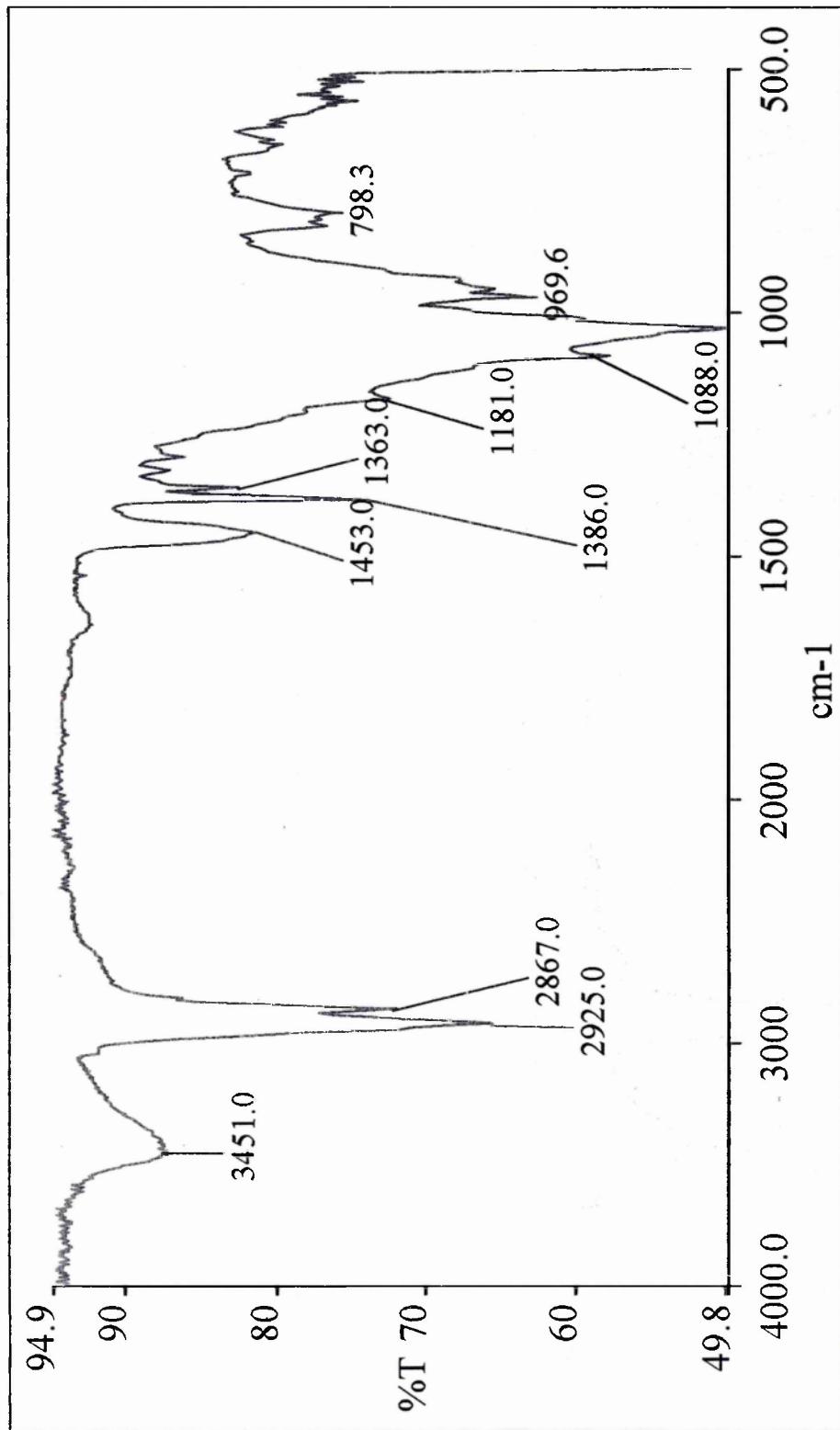
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File : C:\HPCHEM\1\DATA\STUDENTS\M_LANGAT\B64S4349.D
Operator : 16 May 106
Acquired : 4:26 pm using AcqMethod TARIQ2
Instrument : 5971 - In
Sample Name : b64s4349
Misc Info :
Vial Number : 2

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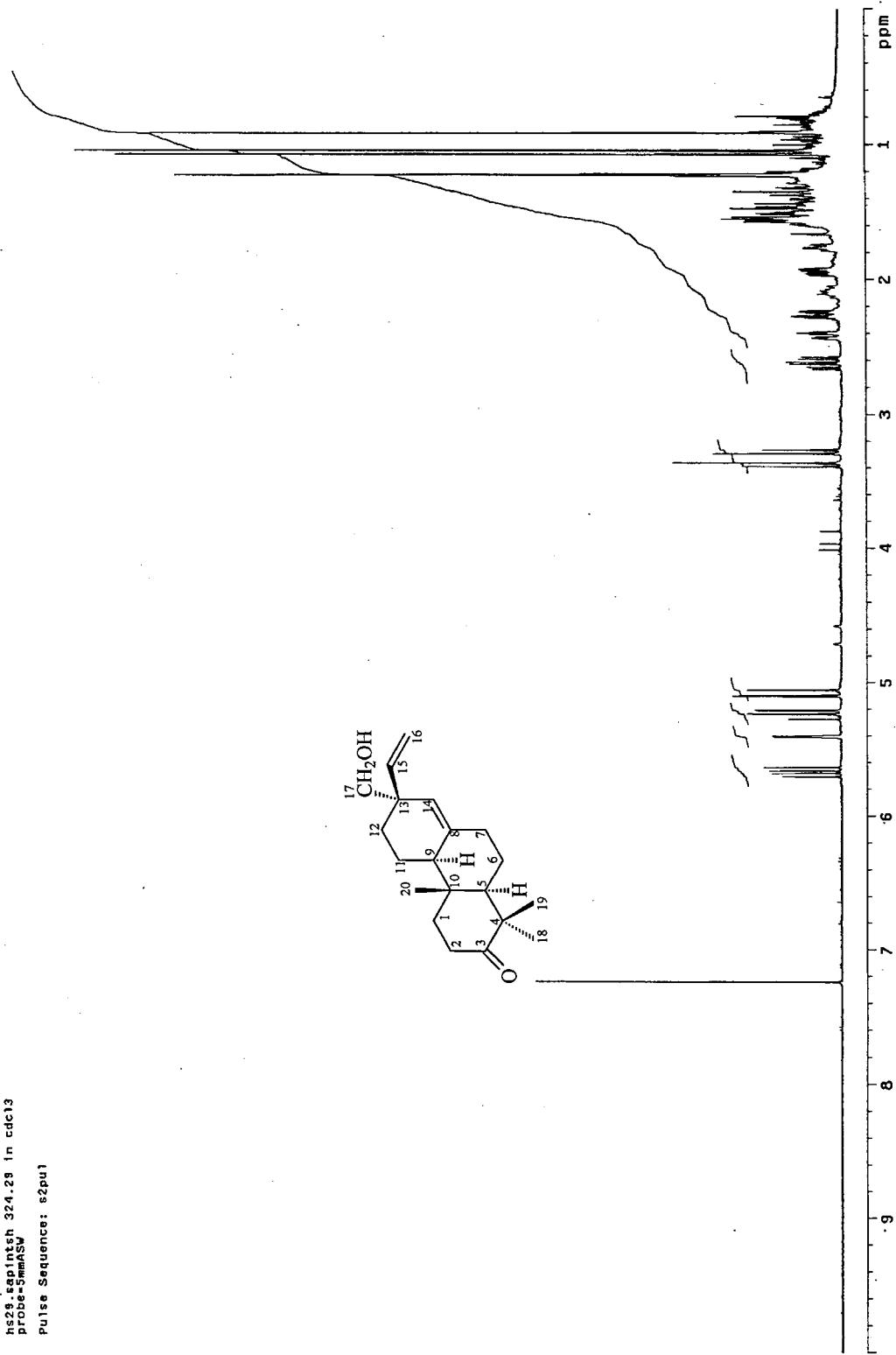


Spectrum 11.7: Mass spectrum of compound 2.11



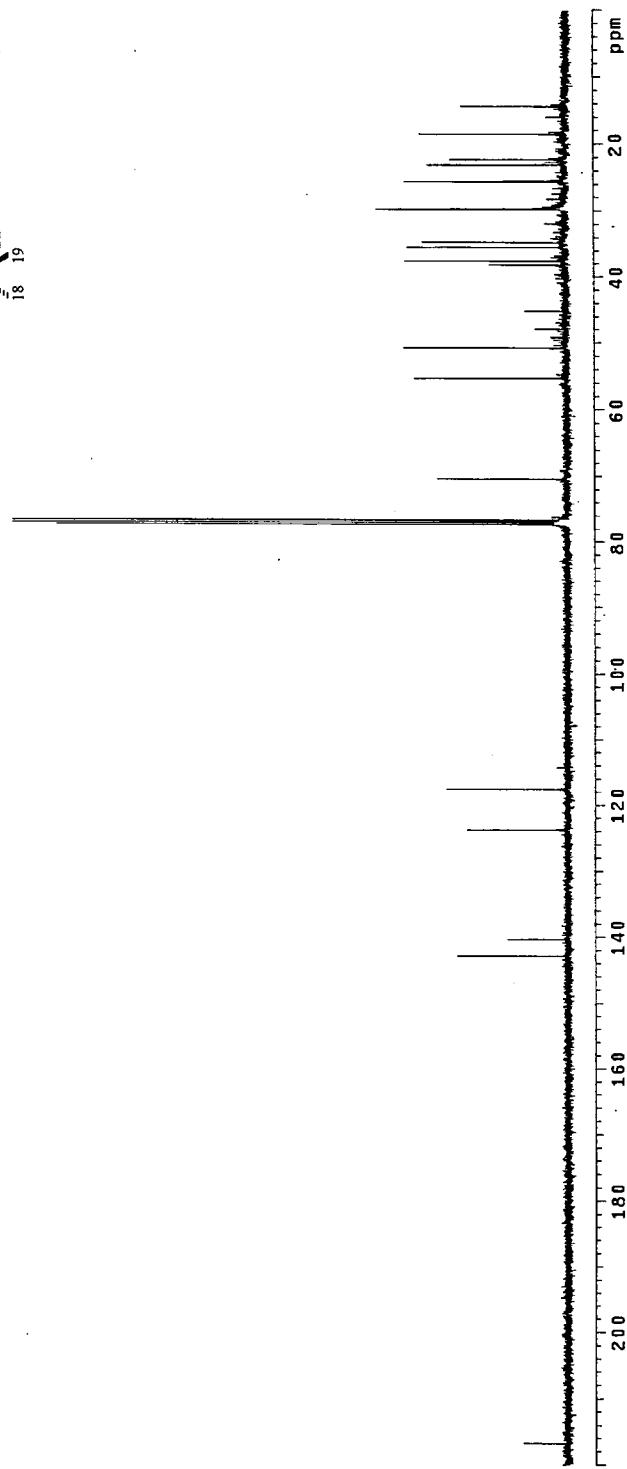
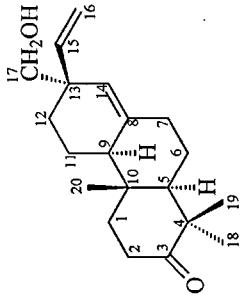
Spectrum 11.8: FTIR spectrum of compound 2.11

hs2s\_rap1tsh 324.2s in cdc13  
probe=5mmASW  
pulse Sequence: s2pu1



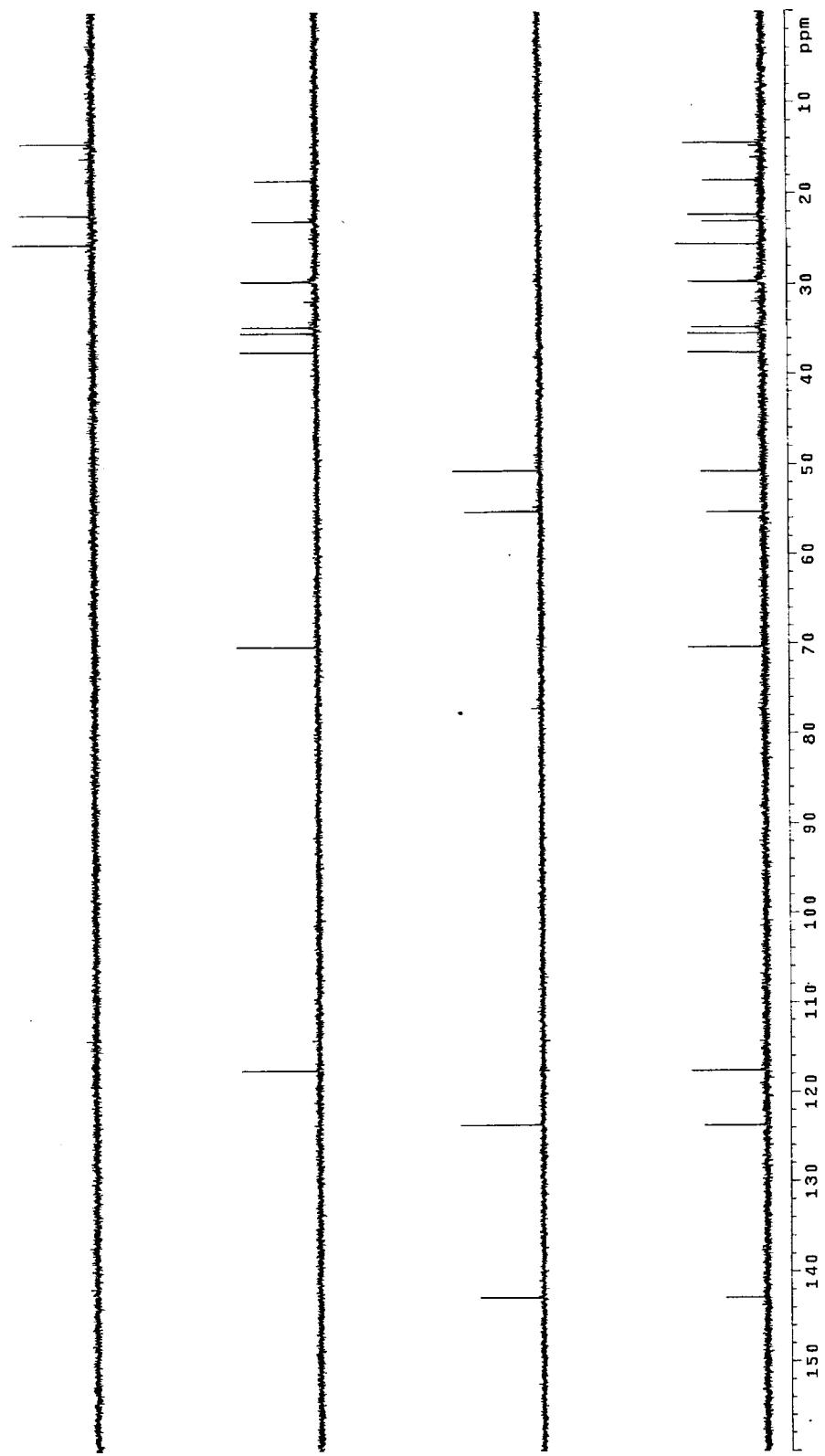
Spectrum 12.1:  $^1\text{H}$  NMR spectrum of compound 4.1 ( $\text{CDCl}_3$ )

C59-sapintsh 324.29 in cdc13			
	FREQUENCY	PPM	HEIGHT
1	2179.07	216.775	8.2
2	1439.170	142.885	19.3
3	14115.381	140.361	10.9
4	12401.859	123.720	18.2
5	11825.514	117.591	21.5
6	7775.406	77.320	91.9
7	7743.471	77.000	100.0
8	7711.335	76.680	95.9
9	7078.511	70.388	23.4
10	5559.075	55.273	27.4
11	5106.-04	50.760	29.4
12	4542.272	45.168	7.6
13	3334.465	38.128	14.0
14	3779.257	37.580	29.1
15	3553.372	35.434	28.7
16	3494.157	34.745	25.3
17	2989.875	29.731	26.6
18	2884.932	29.682	34.3
19	2572.937	25.585	28.2
20	2318.324	23.053	25.1
21	2246.637	22.340	21.0
22	1865.954	18.555	26.5
23	1446.543	14.384	19.0



Spectrum 12.2:  $^{13}\text{C}$  NMR spectrum of compound 4.1 ( $\text{CDCl}_3$ )

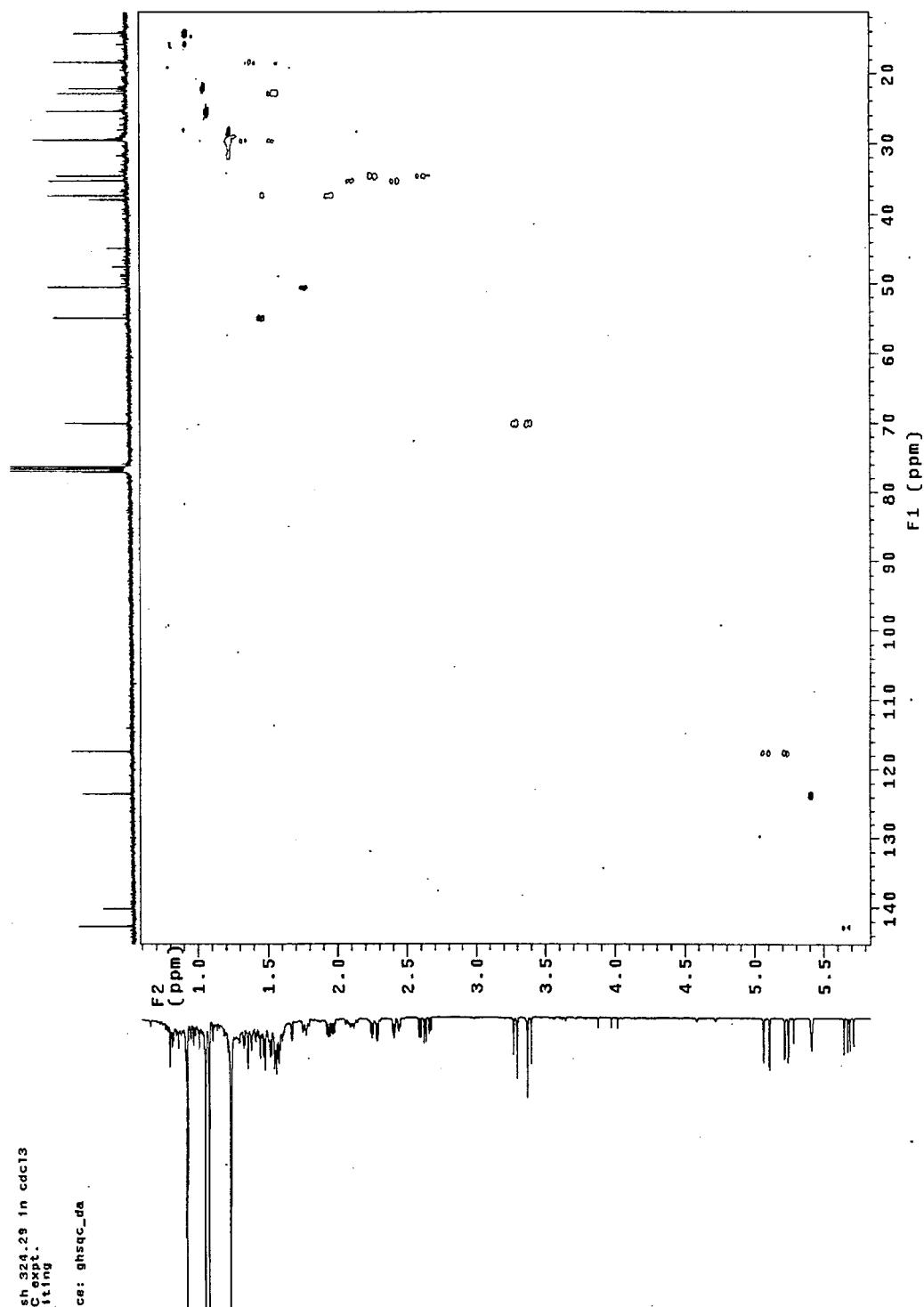
d529-sapintsh 324.29 in cdc13  
probe=5mmASW  
Pulse Sequence: dept



Spectrum 12.3: DEPT spectrum of compound 4.1 (CDCl<sub>3</sub>)

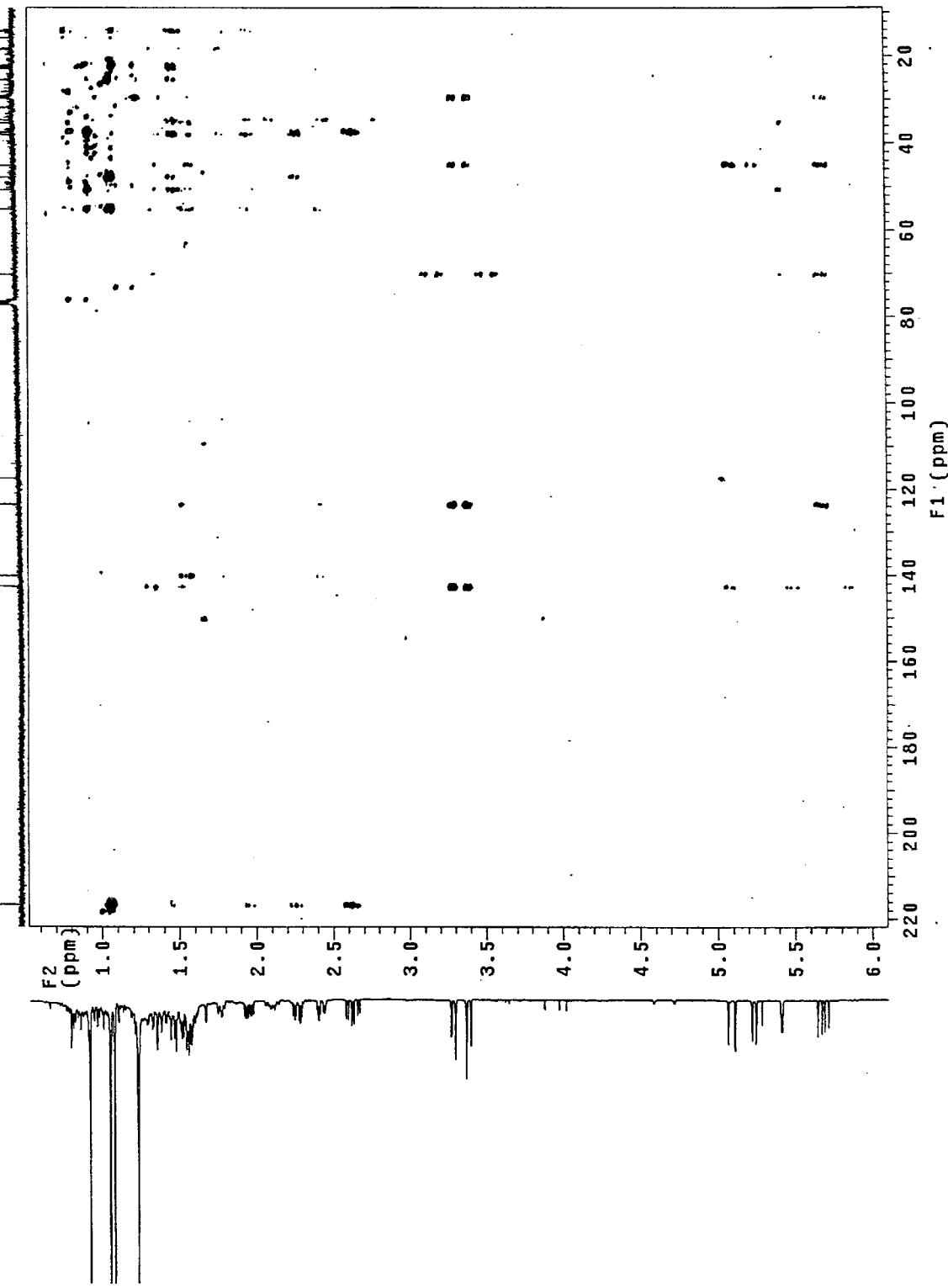
H0529.sep11ish\_324.29 in cdc13  
Grid 1.0 ant HSCC opt.  
with m1.2d setting  
Probe 3mm ASY

Pulse Sequence: ghsqc\_da



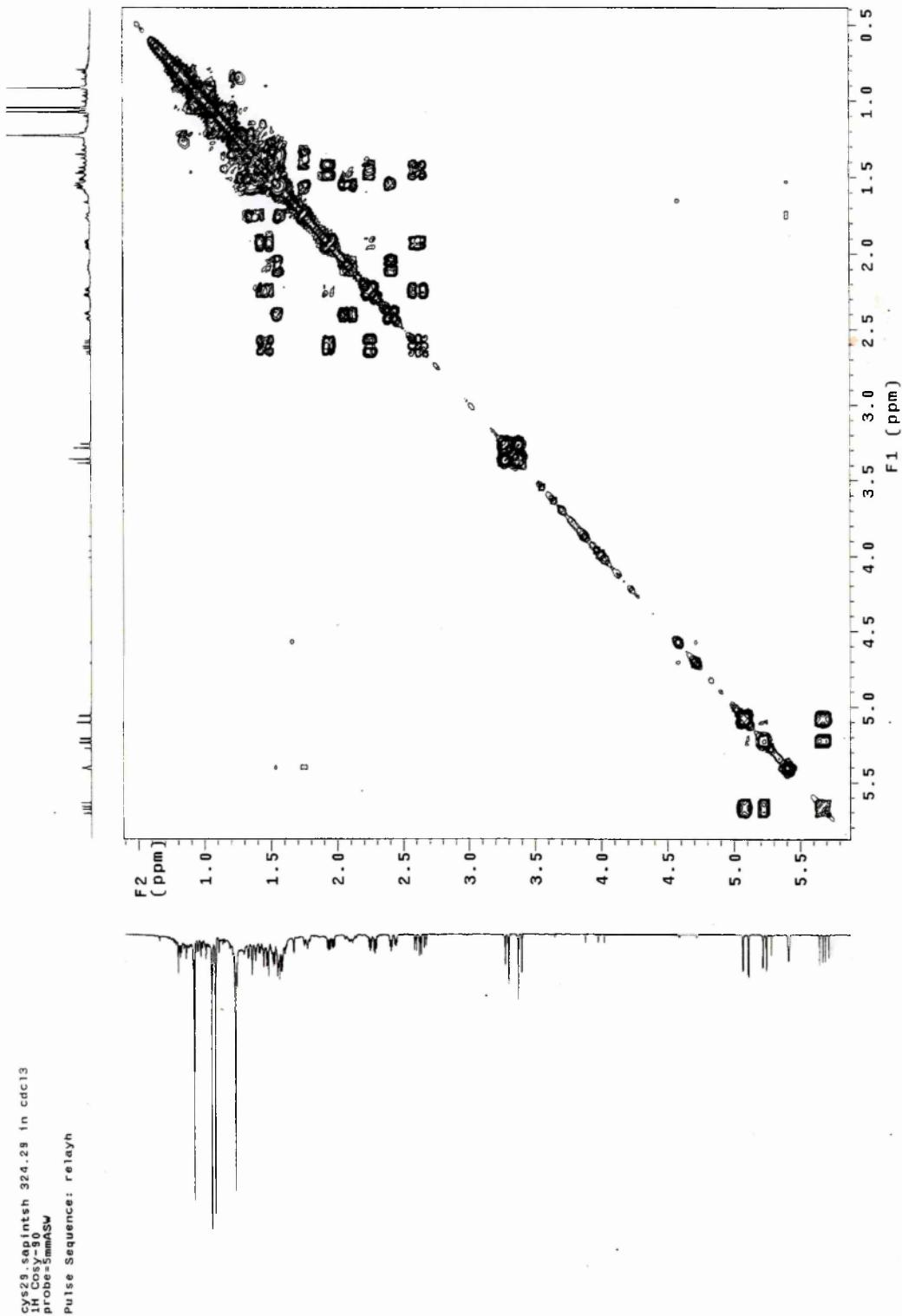
Spectrum 12.4: HSQC spectrum of compound 4.1 ( $\text{CDCl}_3$ )

HBS29.saplntsh 324.29 in cdc13  
Gradient HMBC expt.  
probe:5mmASW  
Pulse Sequence: ghmqc\_da

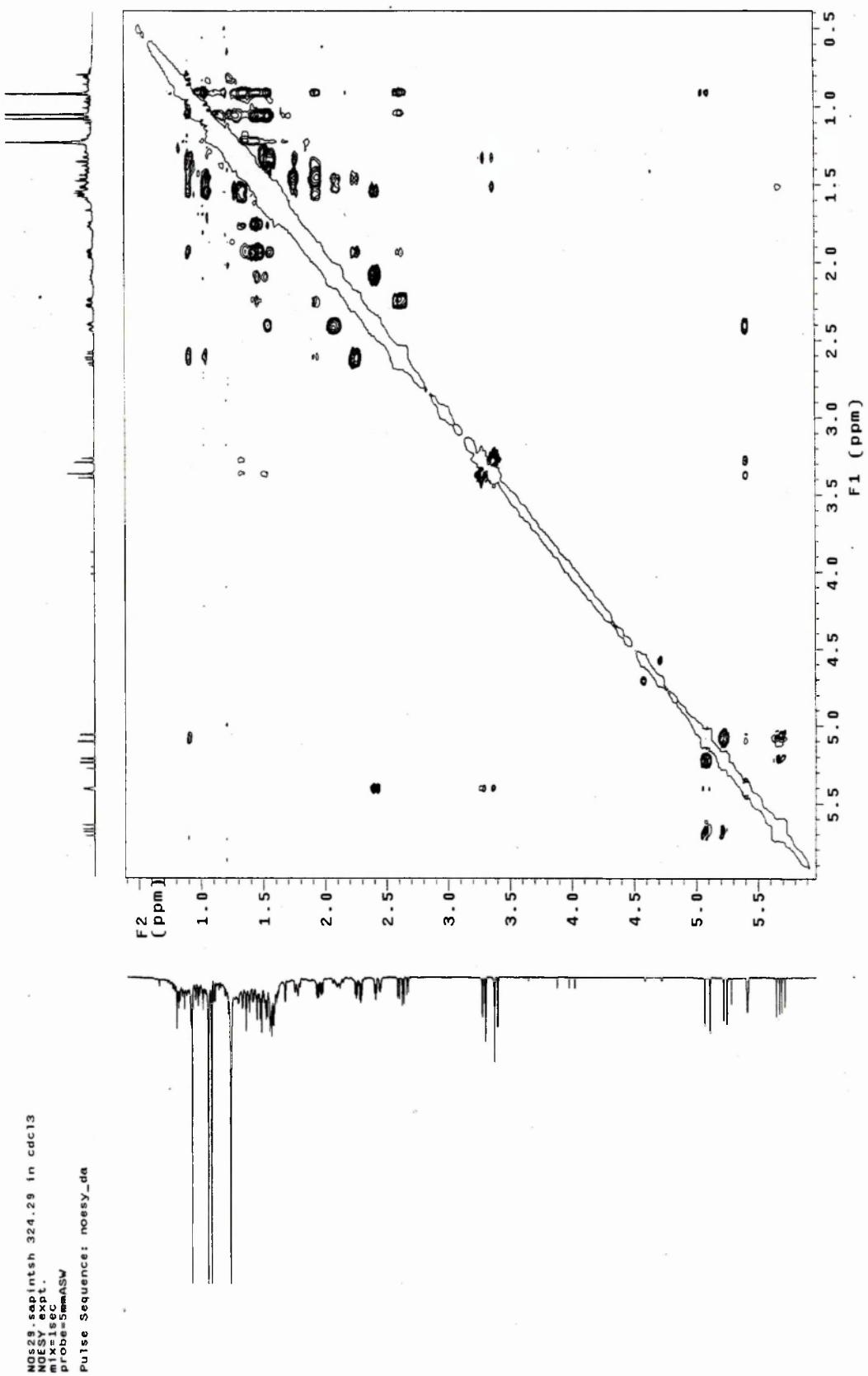


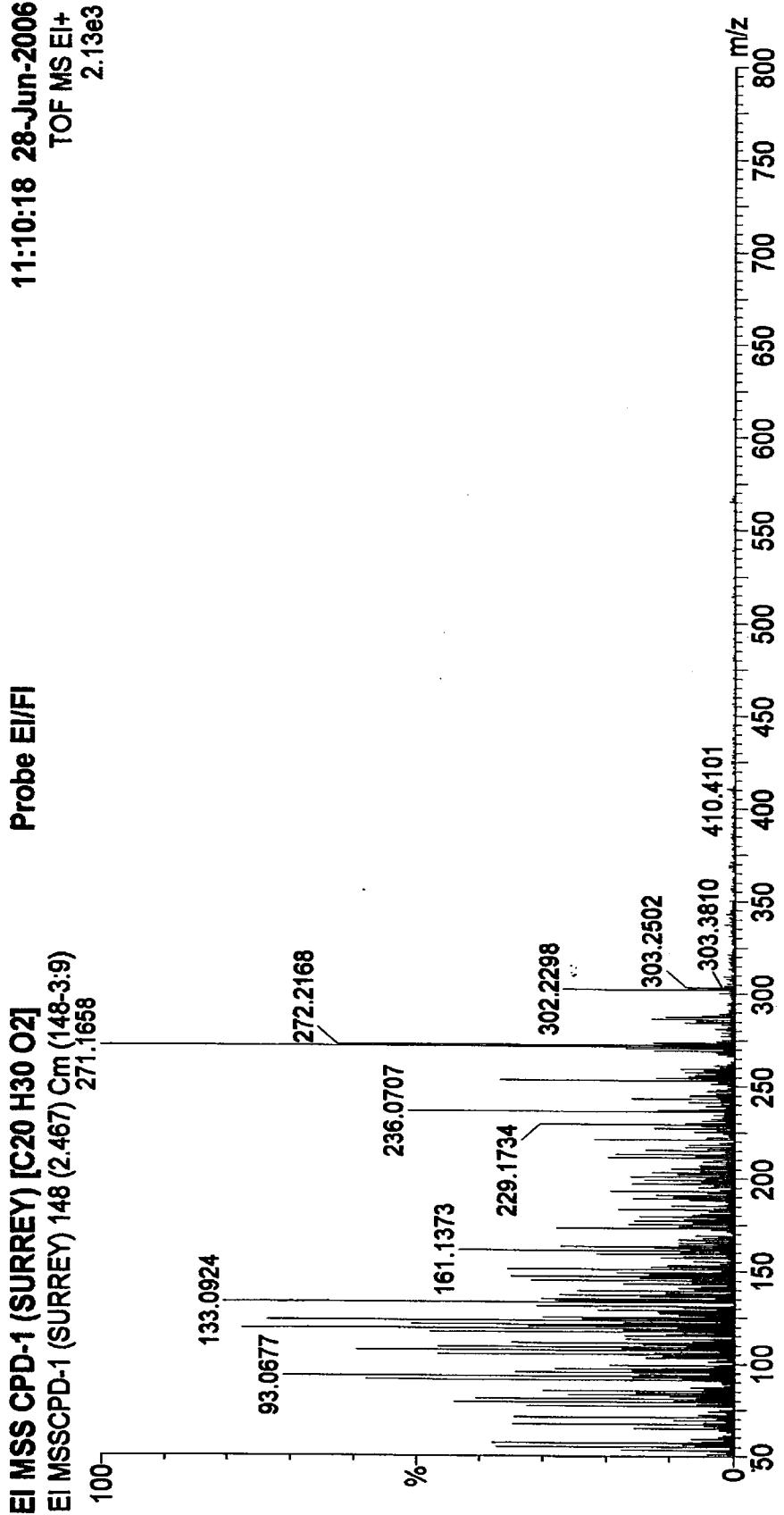
Spectrum 1.8: HMBC spectrum of compound 4.1  $\text{CDCl}_3$

Spectrum 12.6: COSY spectrum of compound 4.1 ( $\text{CDCl}_3$ )

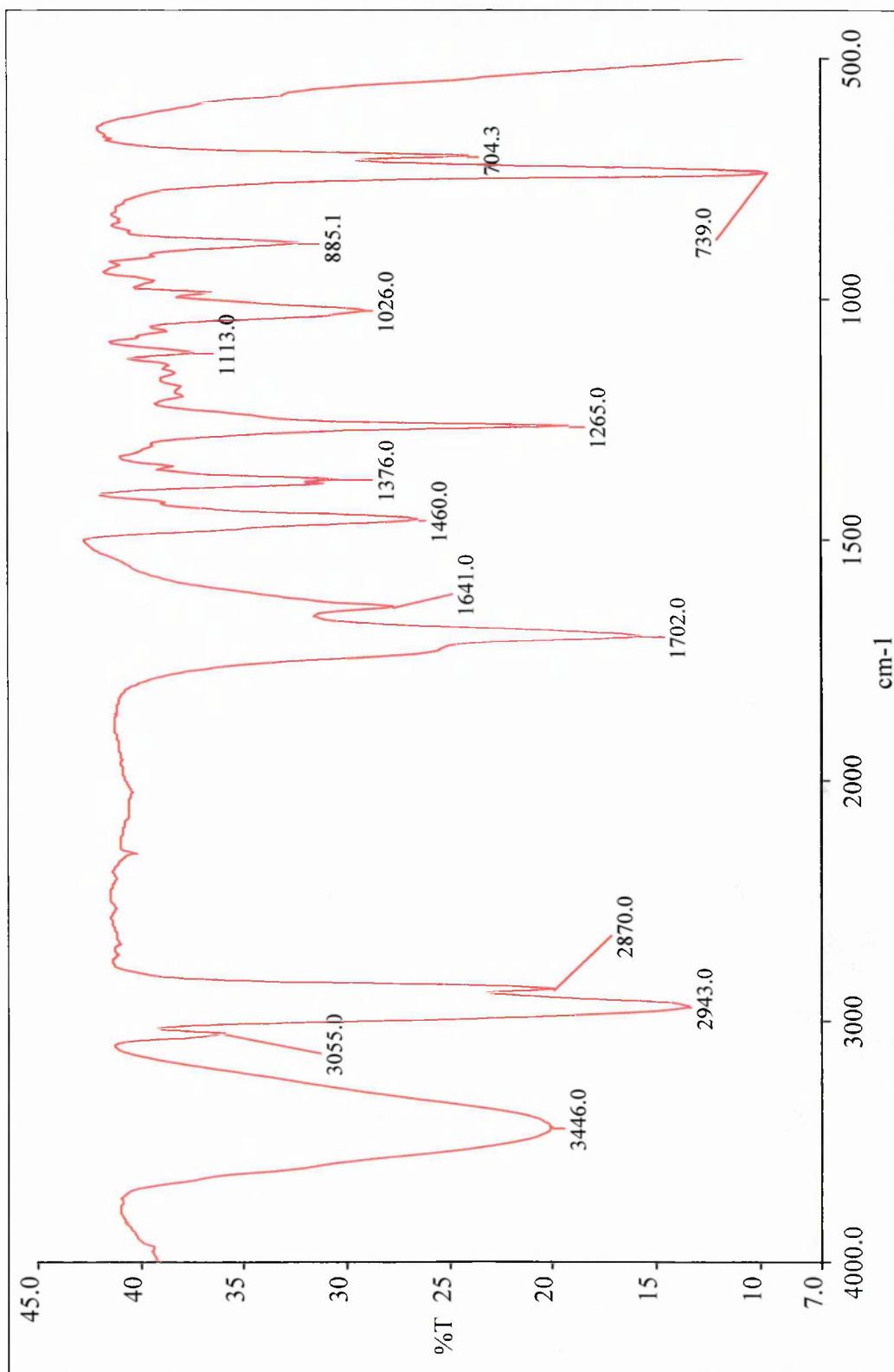


Spectrum 12.7: NOESY of compound 4.1 ( $\text{CDCl}_3$ )

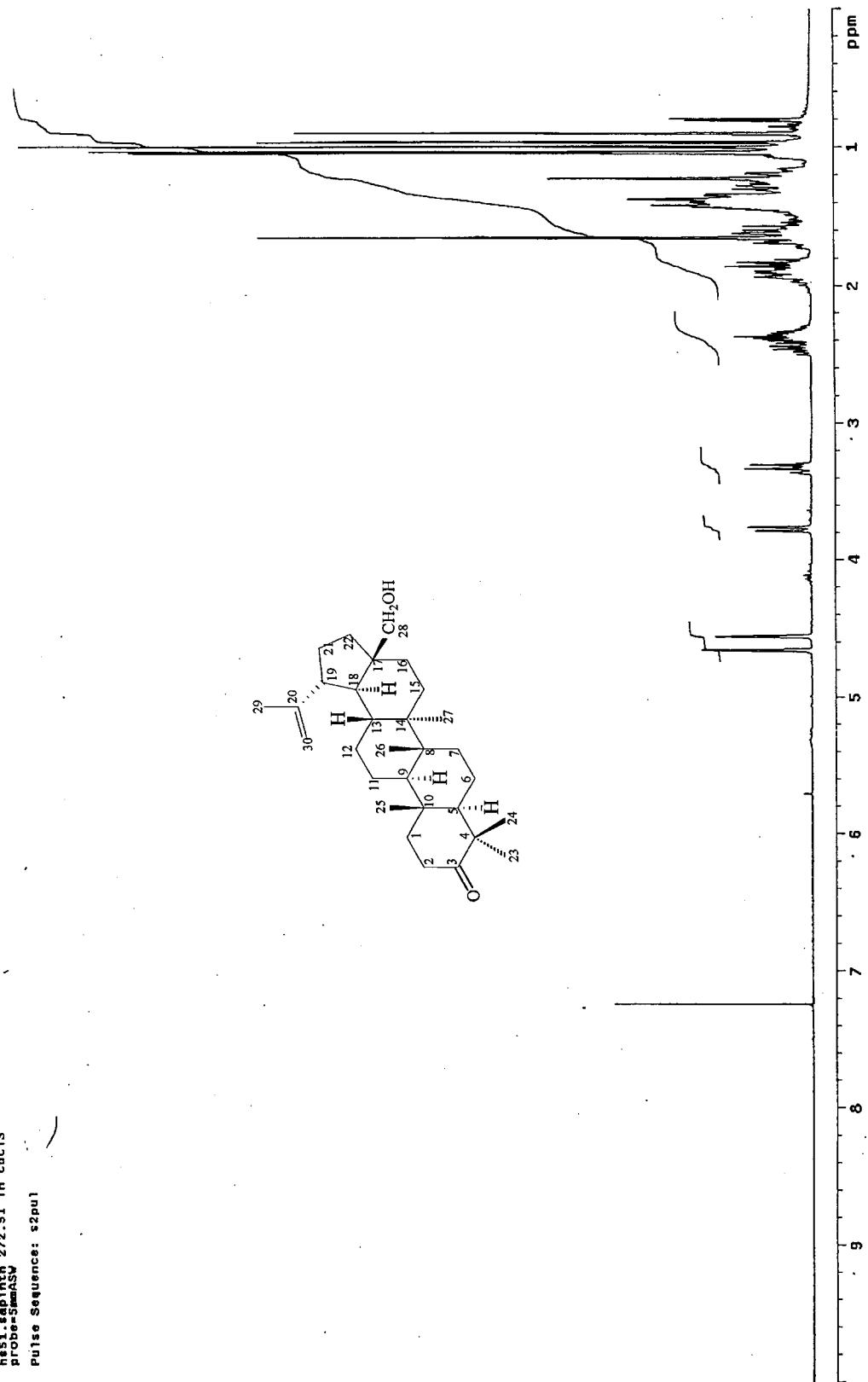




Spectrum 12.8: Mass spectrum of compound 4.1

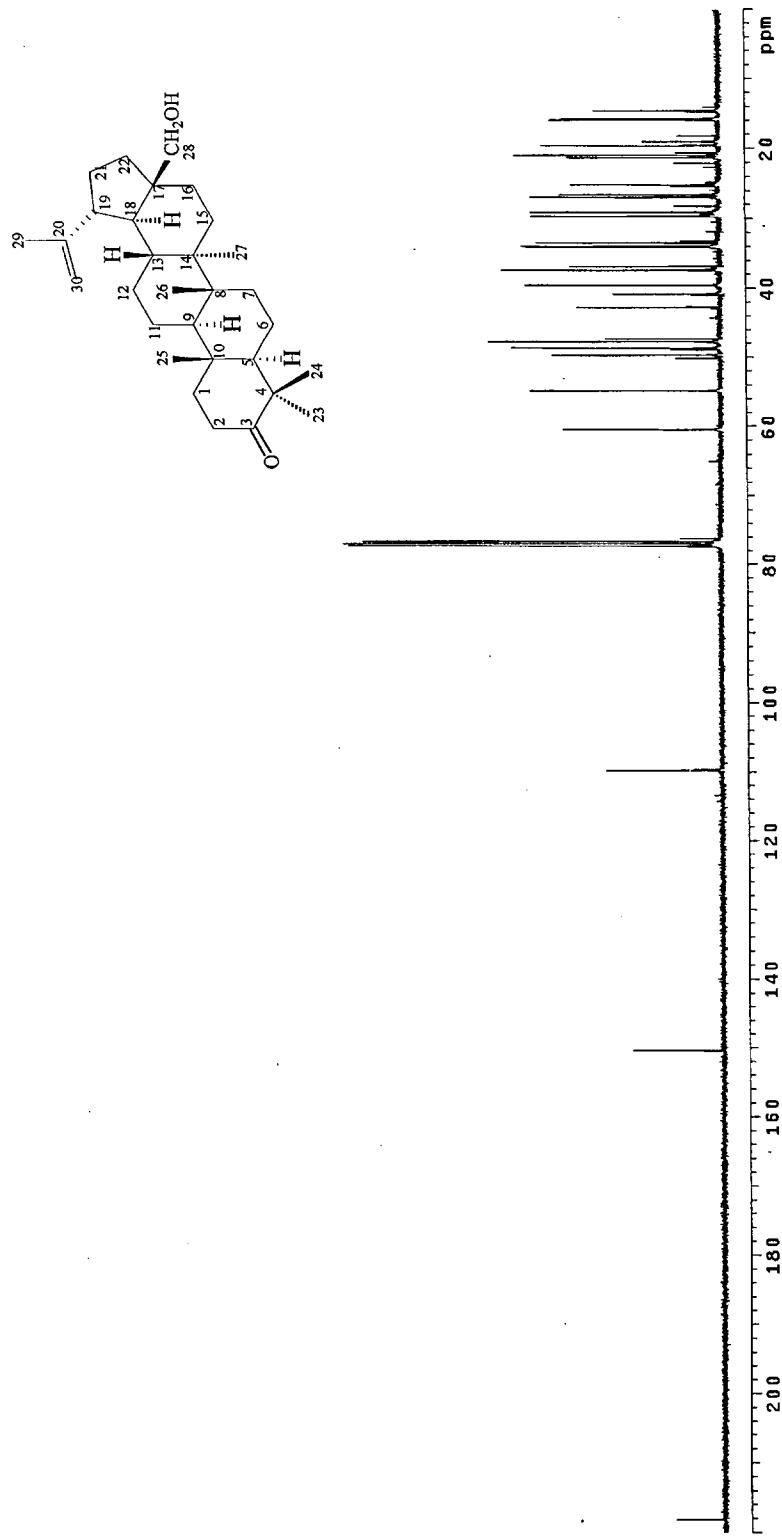


he51-sabInth 272.51 in cdc13  
probe 5mmASY  
Pulse Sequence: s2pul



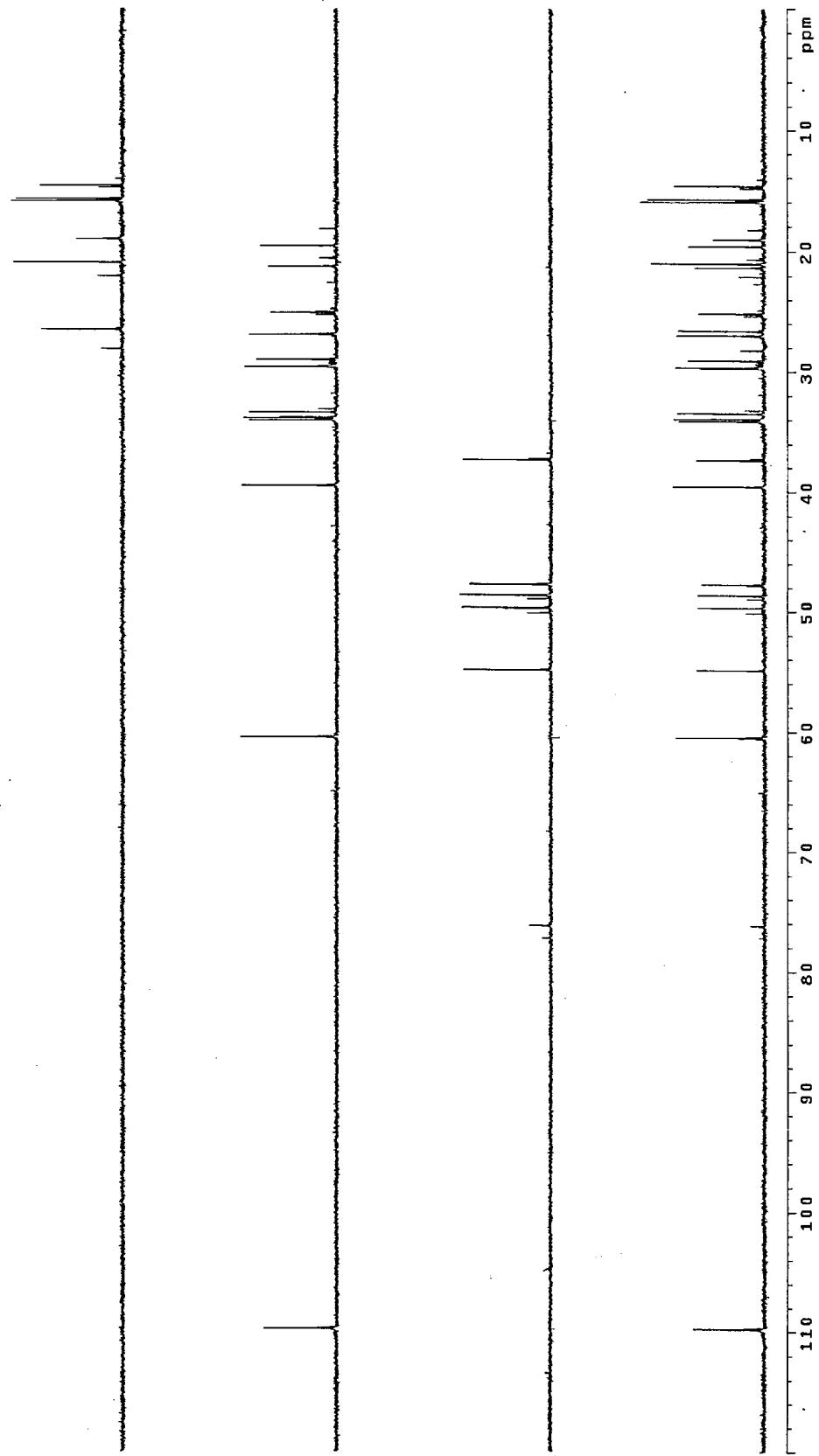
Spectrum 13.1:  $^1\text{H}$  NMR spectrum of compound 4.2 ( $\text{CDCl}_3$ )

ciss1-saplnth 272.51 in cdc13  
probe:SMA5W  
Pulse Sequence: zgpu1



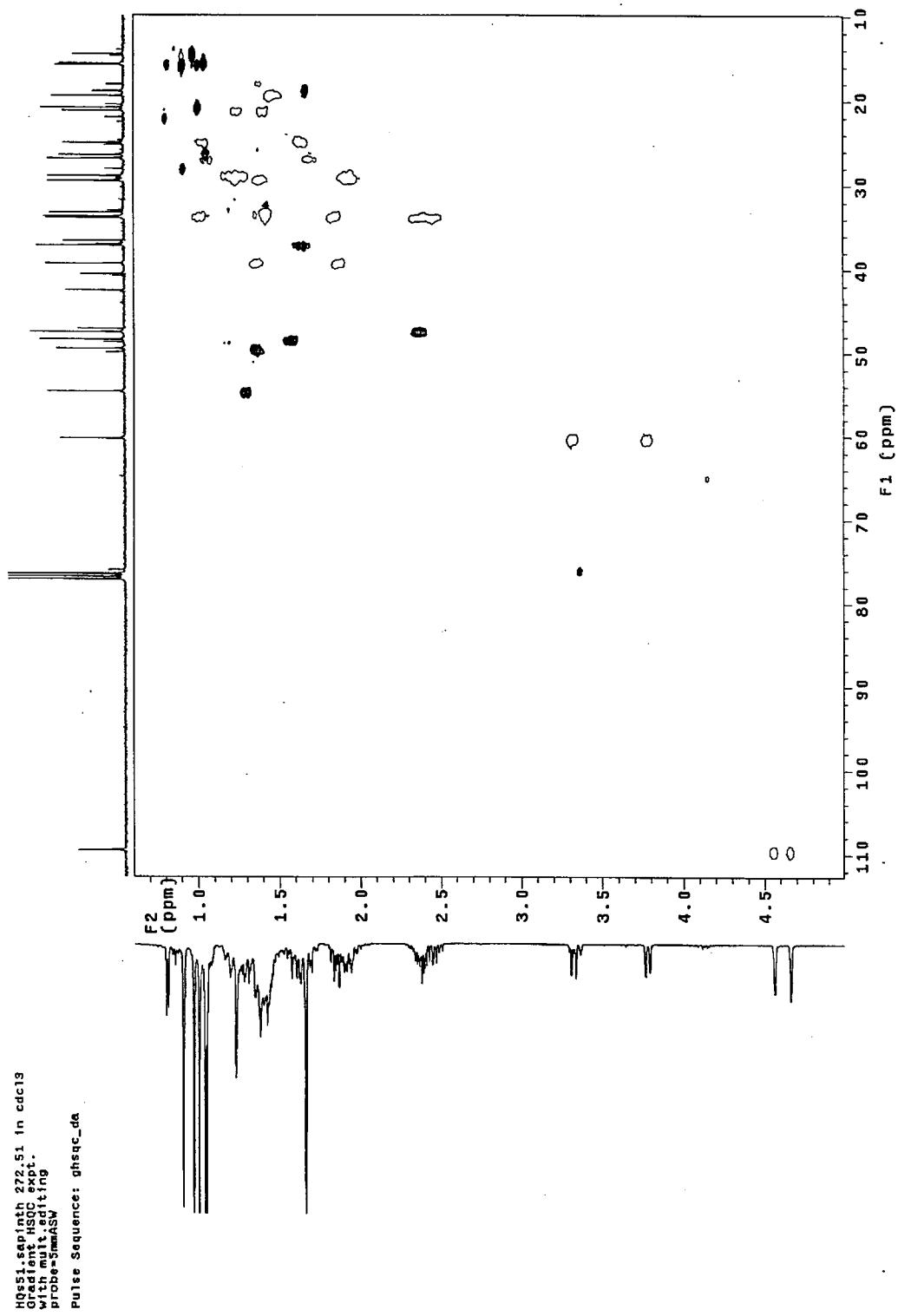
Spectrum 13.2:  $^{13}\text{C}$  NMR spectrum of compound 4.2 ( $\text{CDCl}_3$ )

ds51.sqplnth 272.51 in cdc13  
probe: mmSW  
Pulse Sequence: dept

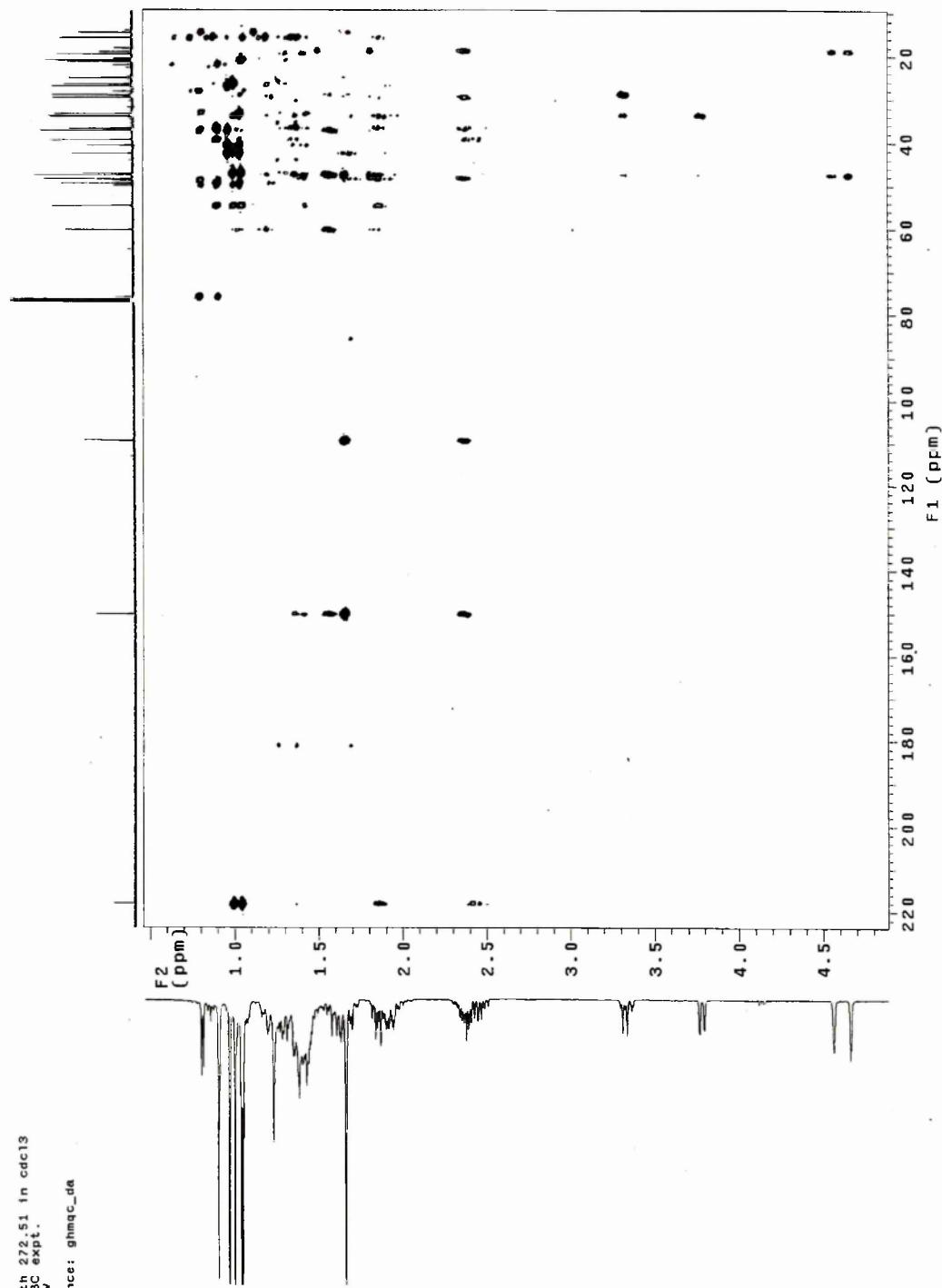


Spectrum 13.3: DEPT spectrum of compound 4.2 ( $\text{CDCl}_3$ )

Spectrum 13.4: HSQC spectrum of compound 4.2 ( $\text{CDCl}_3$ )

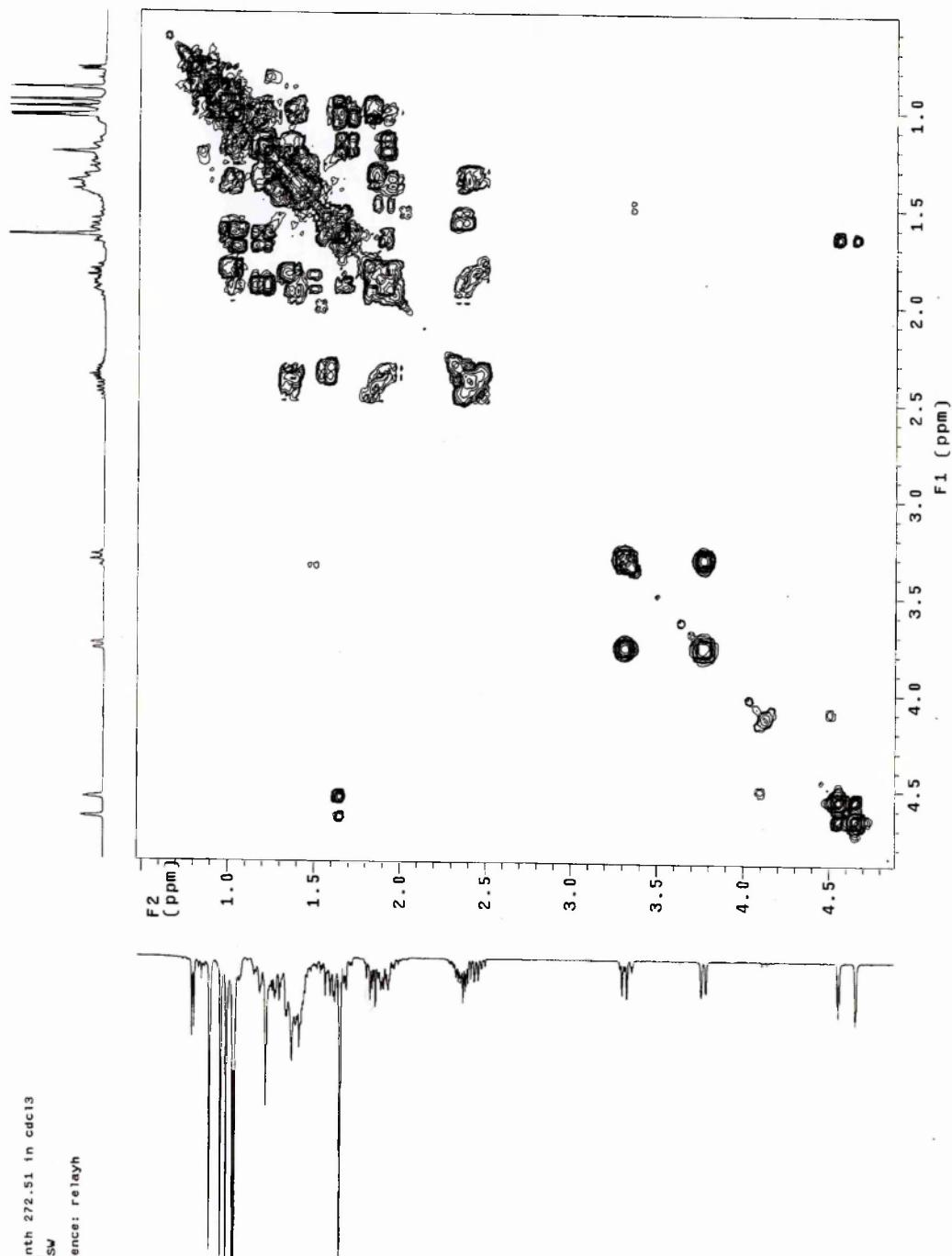


HBS51.sap in th 272.51 in cdc13  
Gradient HMBC expt.  
Probe:5mmASW  
Pulse Sequence: ghmqc\_da



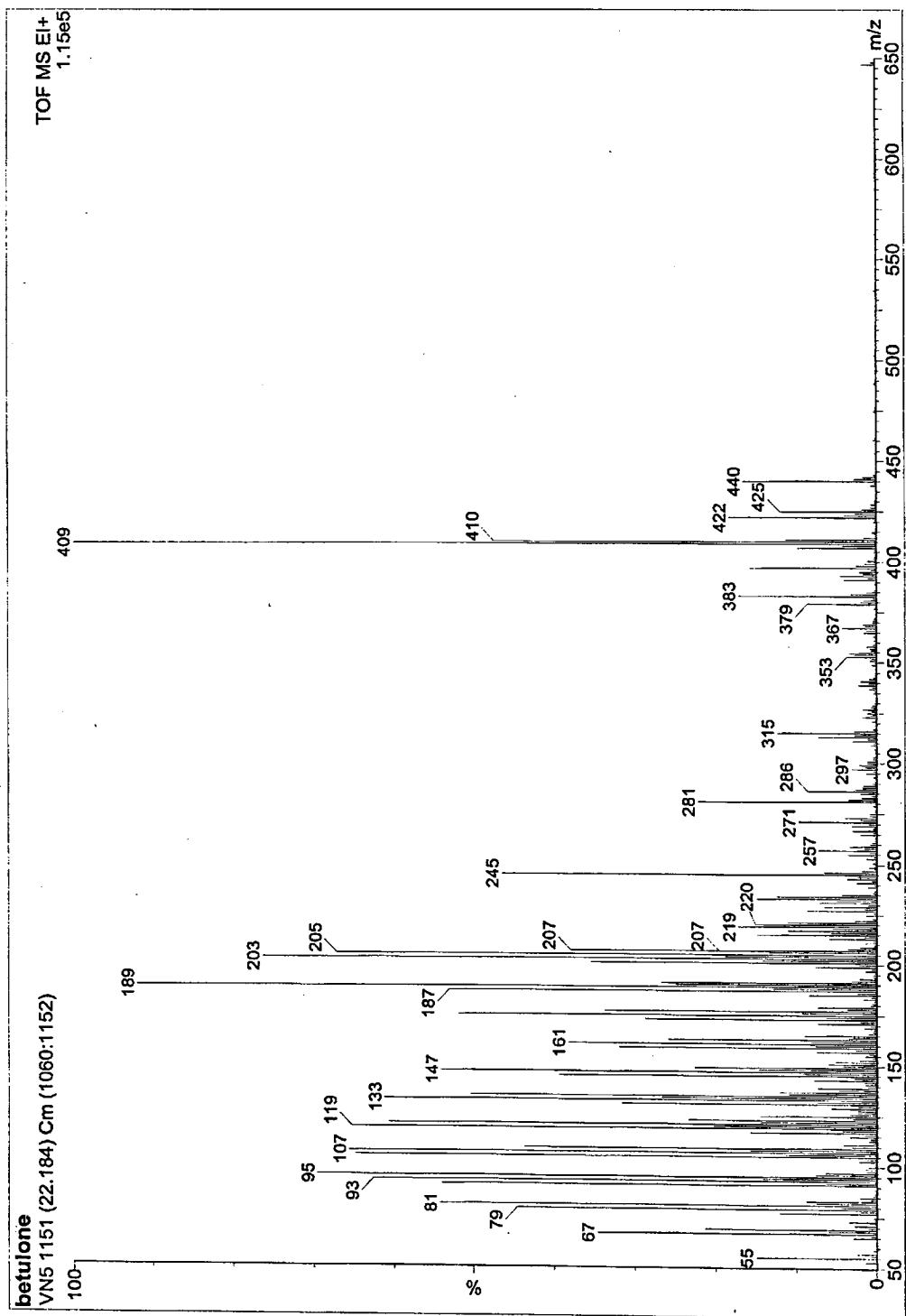
Spectrum 13.5: HMBC spectrum of compound 4.2 ( $\text{CDCl}_3$ )

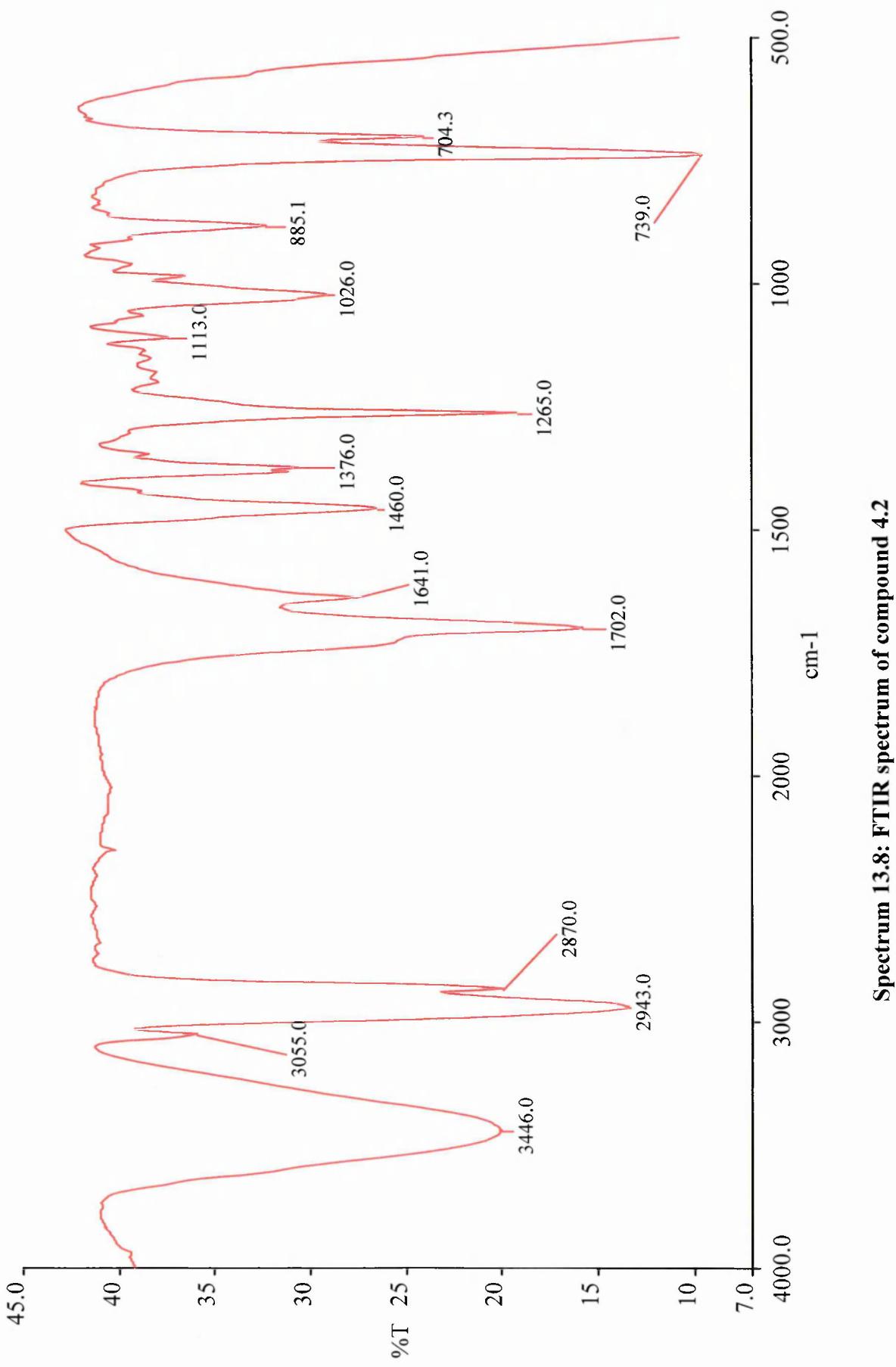
Cysteineapatin 272.51 in  $\text{CDCl}_3$   
1H COSY-90  
probe=5mmSW  
Pulse Sequence: relayh



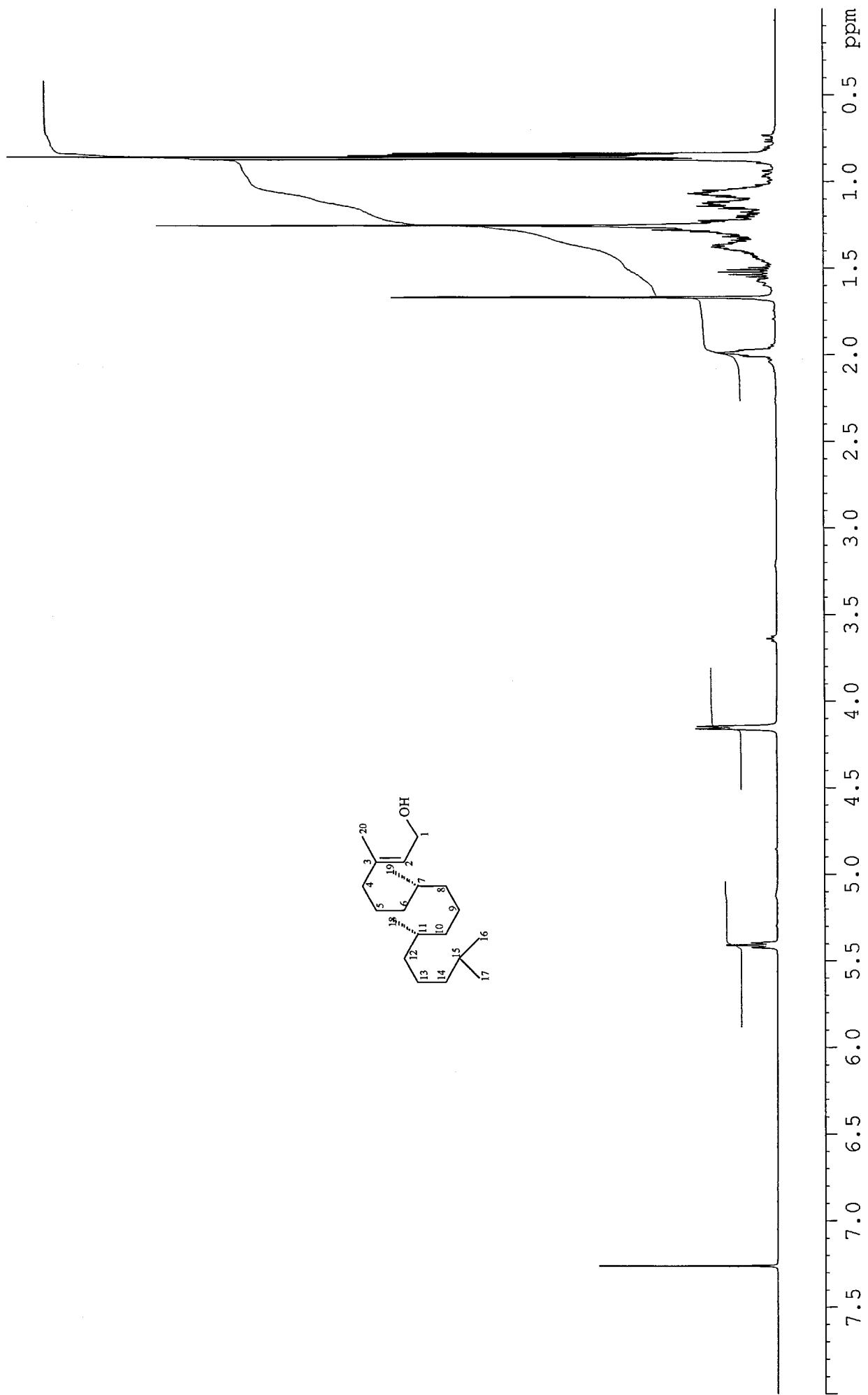
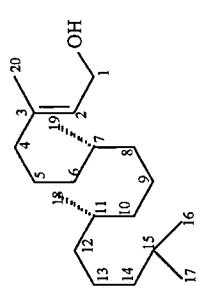
Spectrum 13.6: COSY spectrum of compound 4.2 ( $\text{CDCl}_3$ )

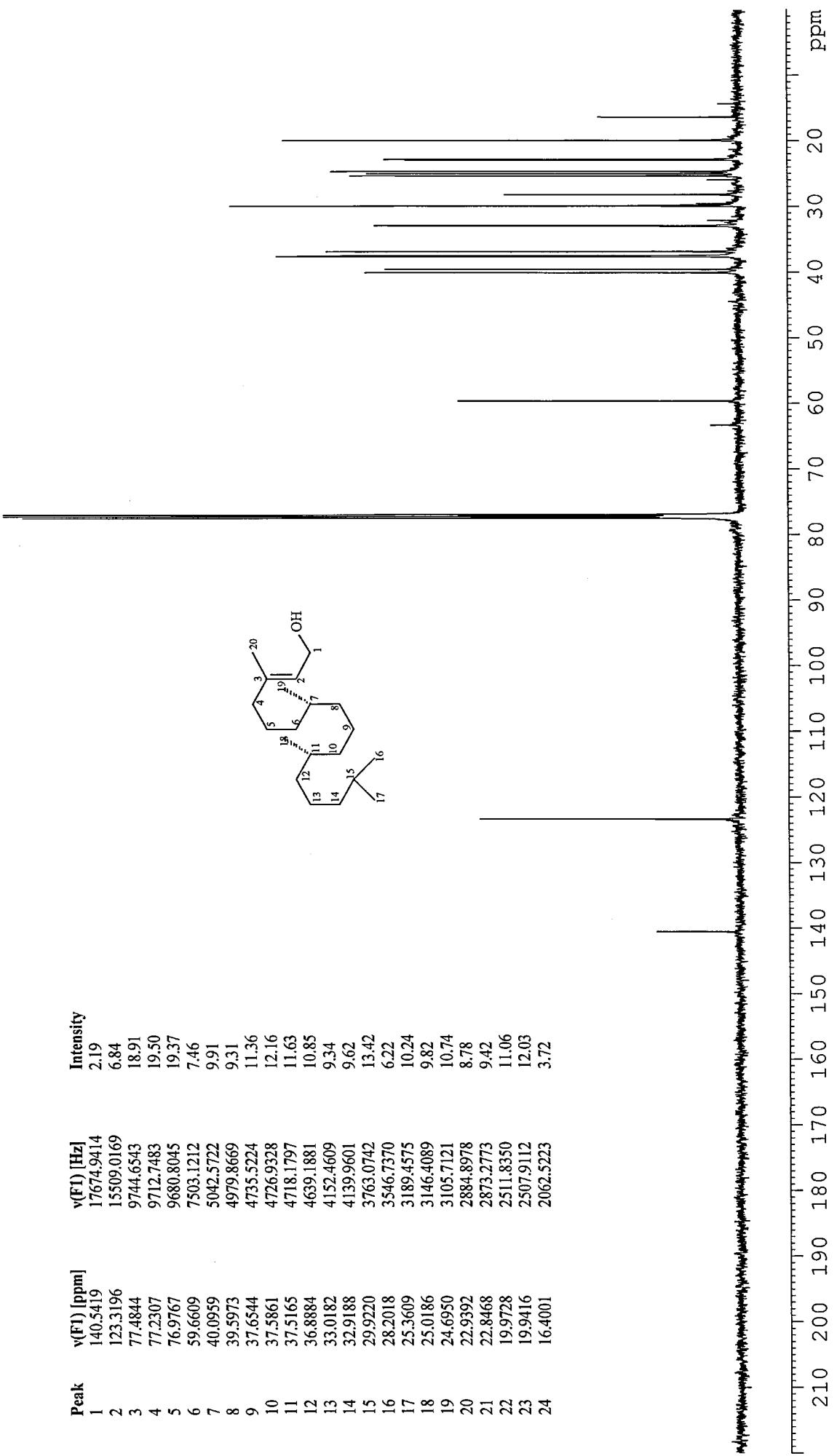
Spectrum 13.7: Mass spectrum of compound 4.2



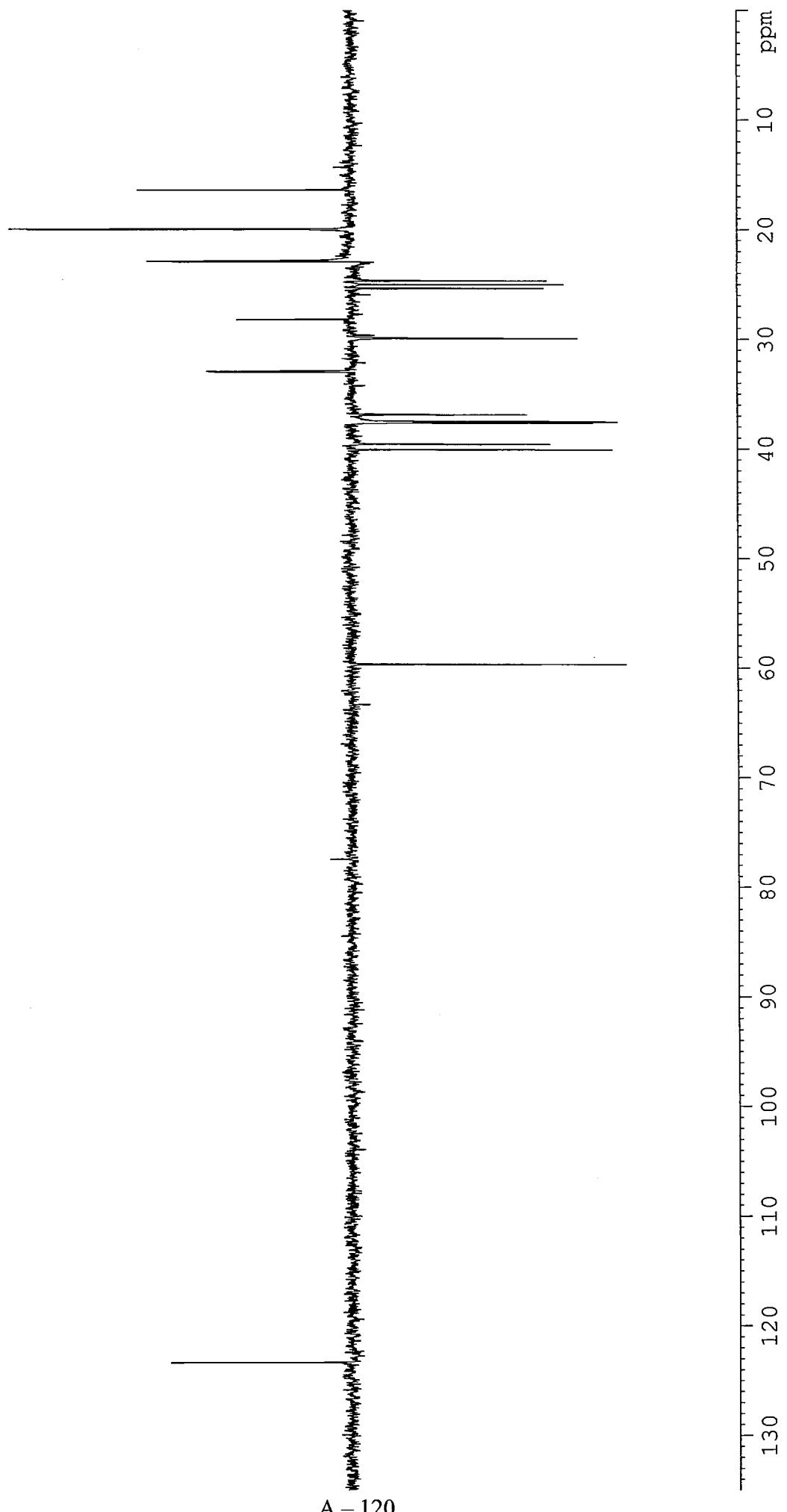


Spectrum 14.1:  $^1\text{H}$  spectrum of compound 4.3 ( $\text{CDCl}_3$ )

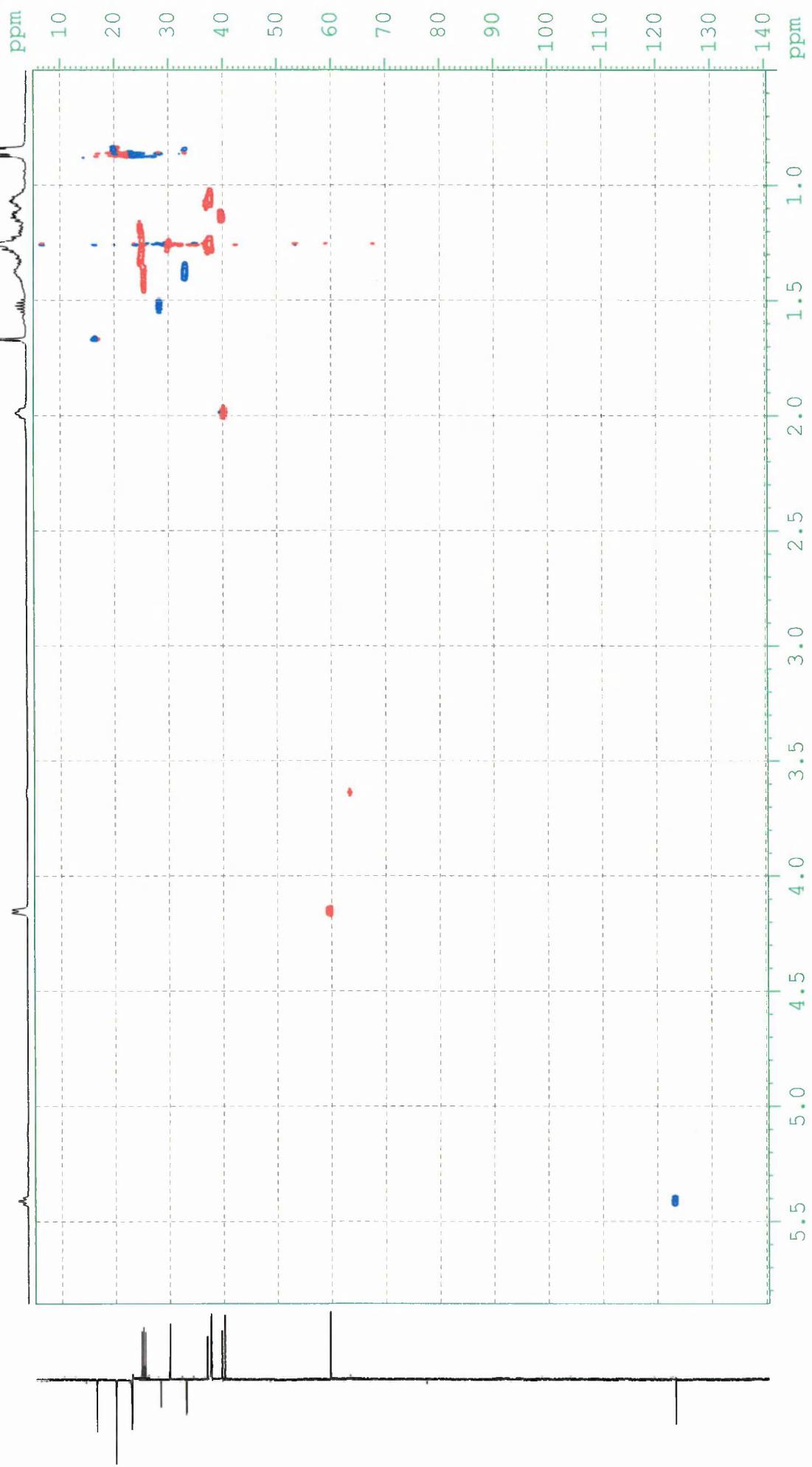




Spectrum 14.2:  $^{13}\text{C}$  spectrum of compound 4.3 ( $\text{CDCl}_3$ )

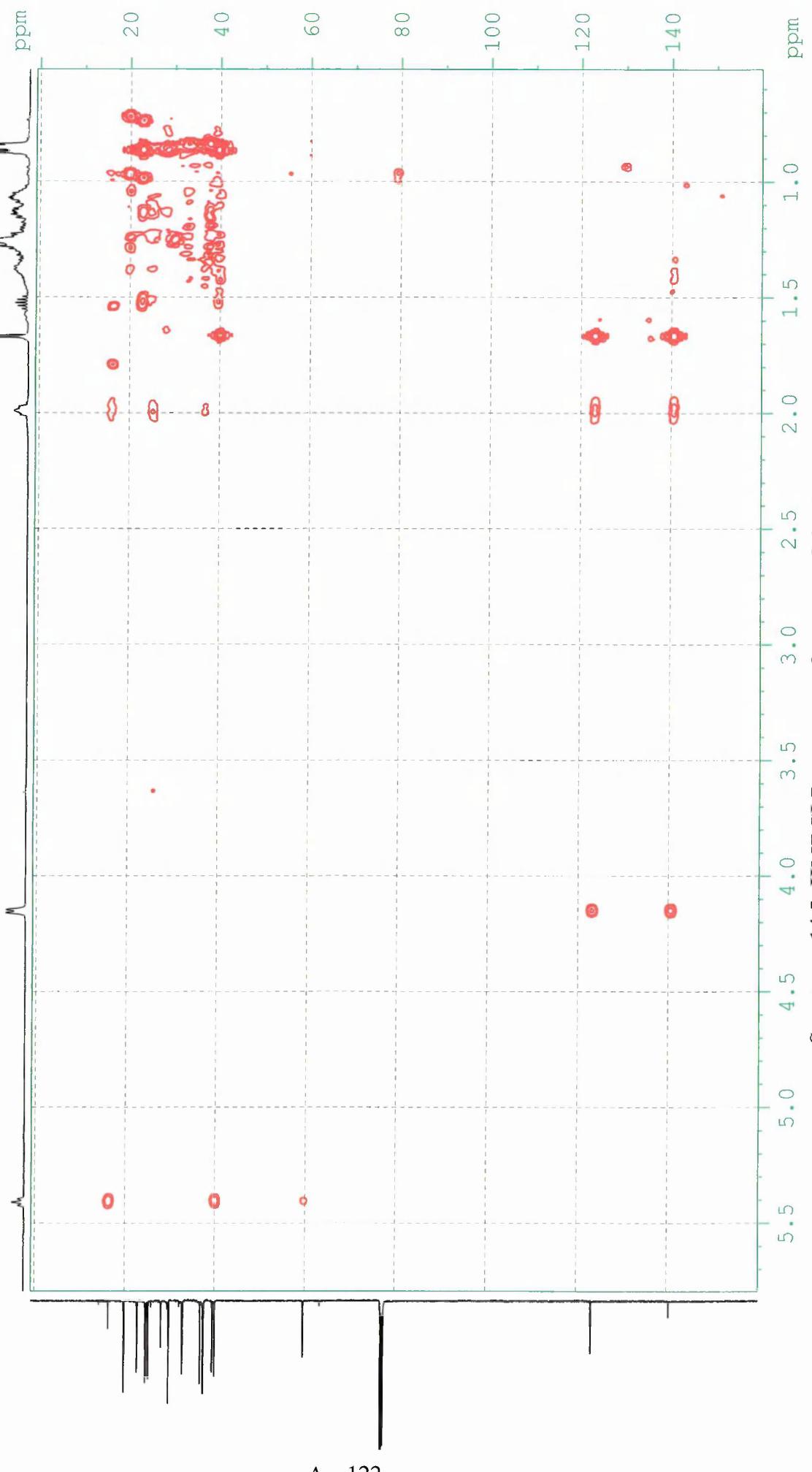


Spectrum 14.3: DEPT spectrum of compound 4.3 ( $\text{CDCl}_3$ )



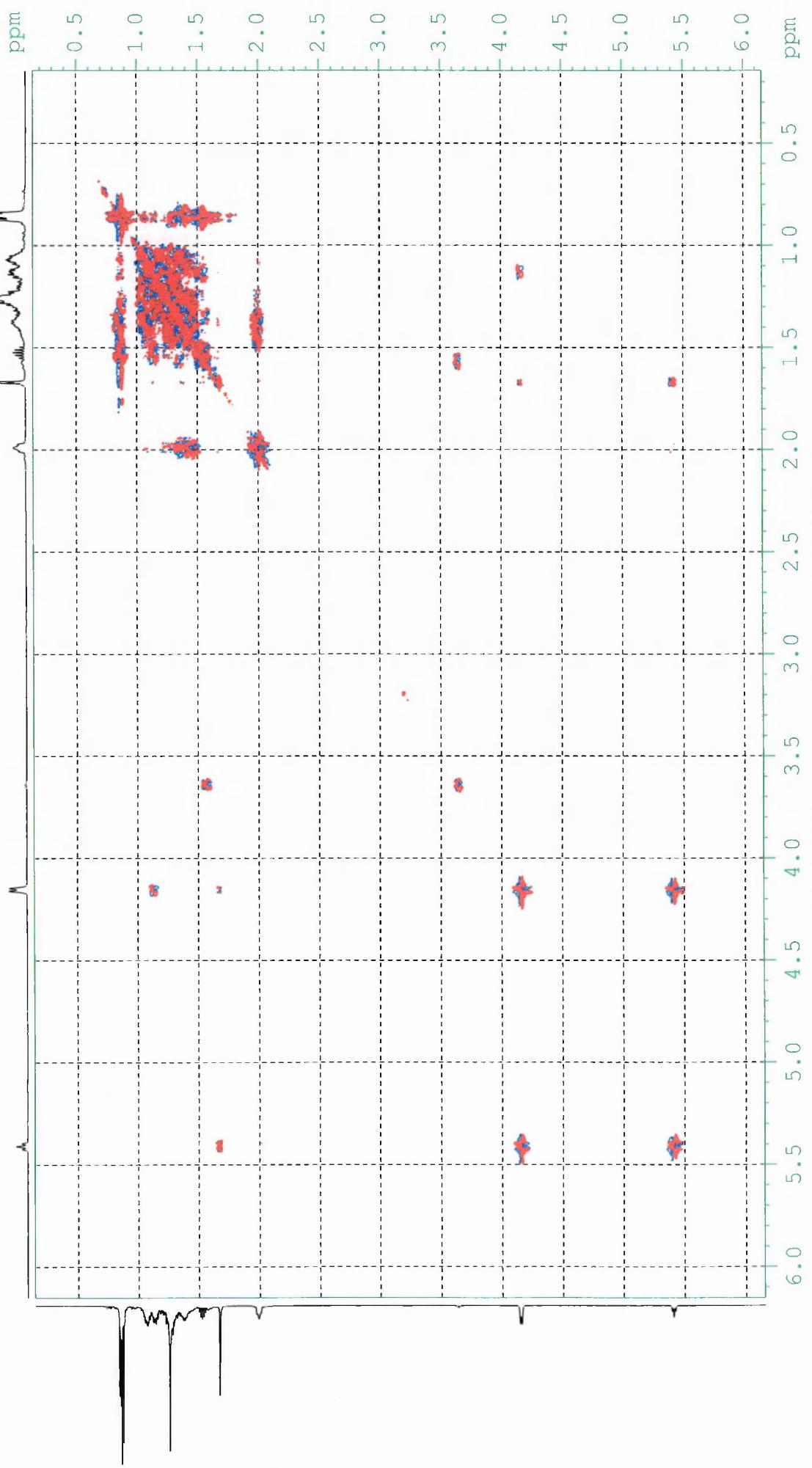
Spectrum 14.4: HSQCDEPT spectrum of compound 4.3 ( $\text{CDCl}_3$ )

**Spectrum 14.5: HMBCCLP spectrum of compound 4.3 ( $\text{CDCl}_3$ )**

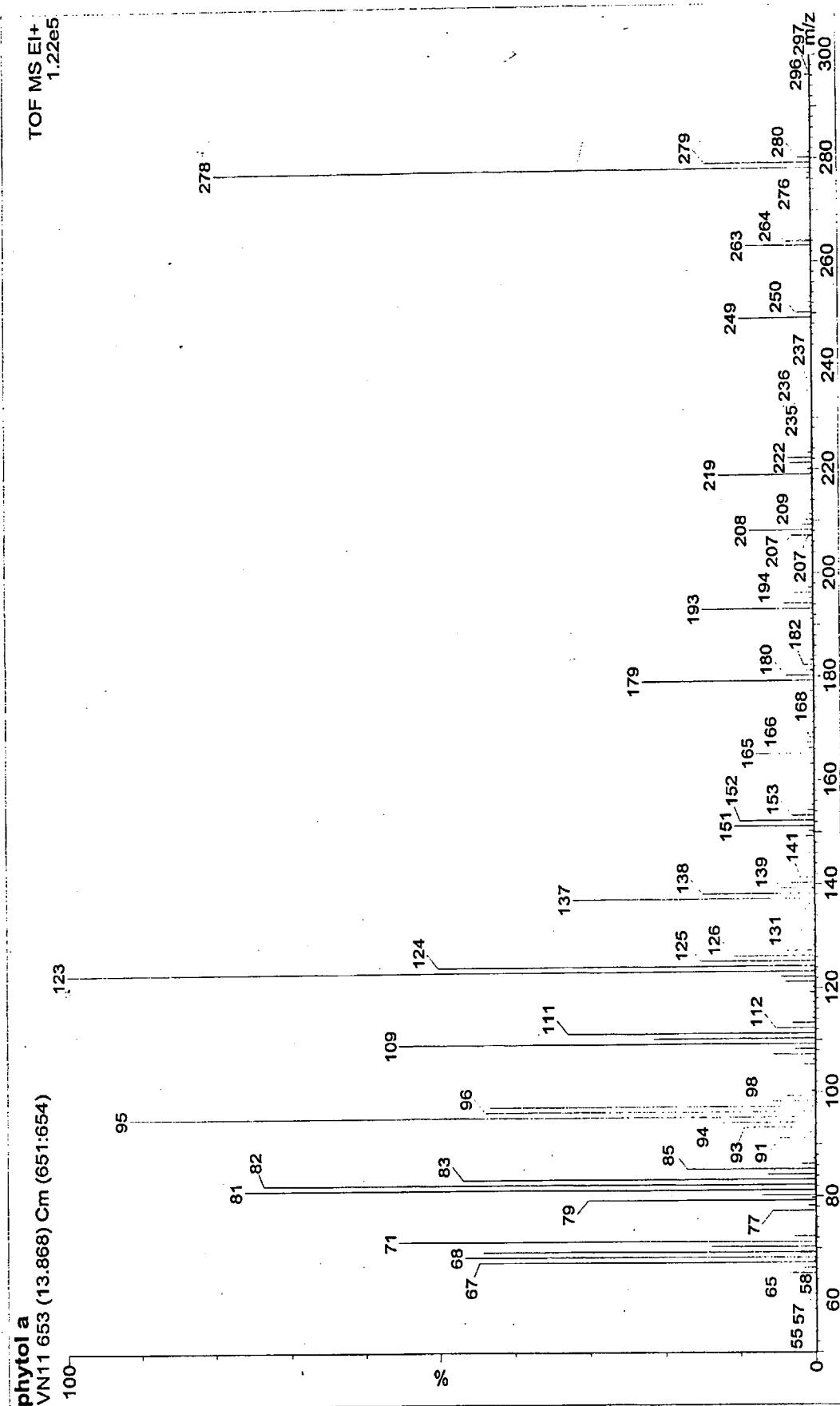


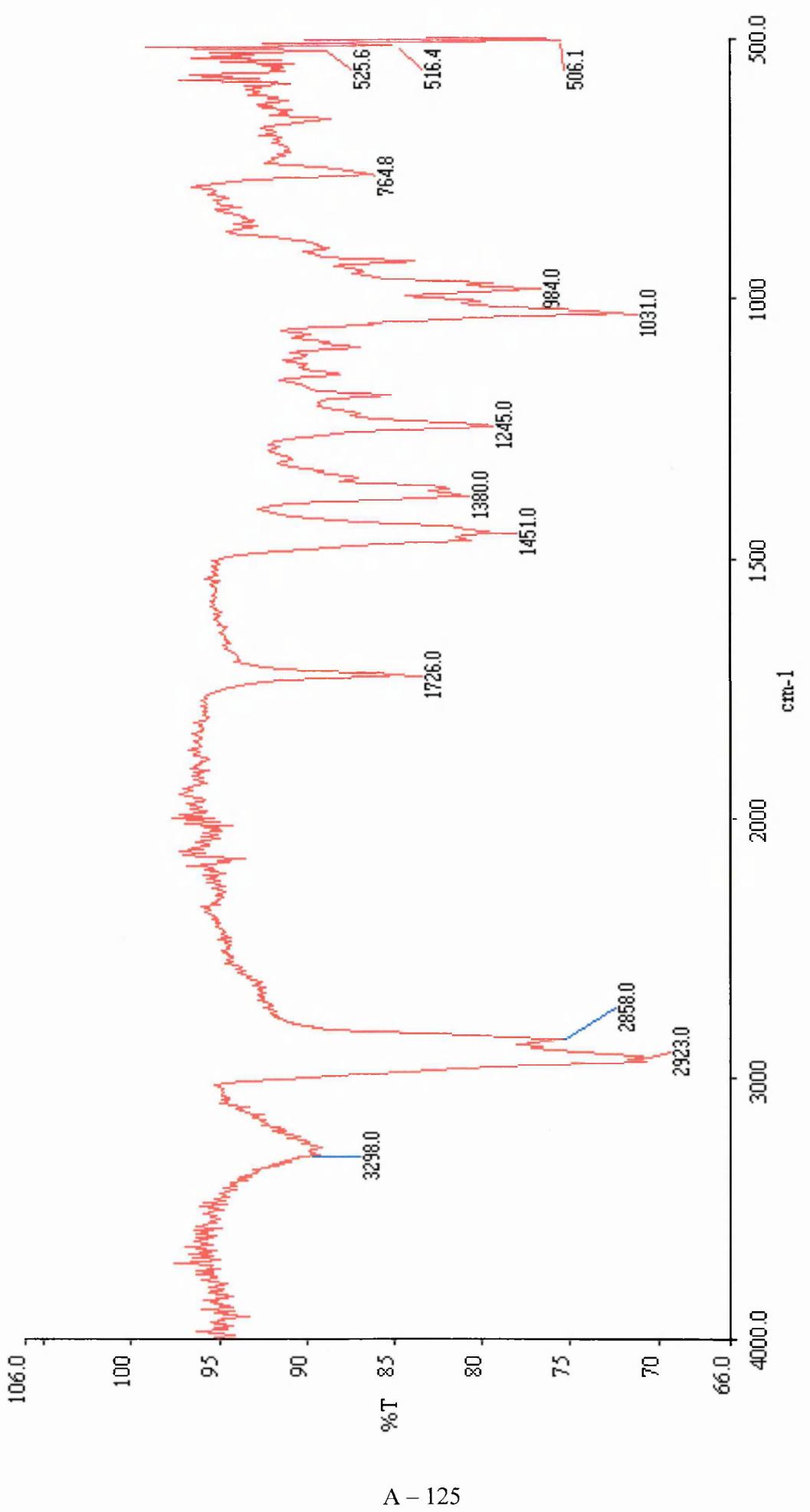
A - 122

**Spectrum 14.6: COSY spectrum of compound 4.3 ( $\text{CDCl}_3$ )**



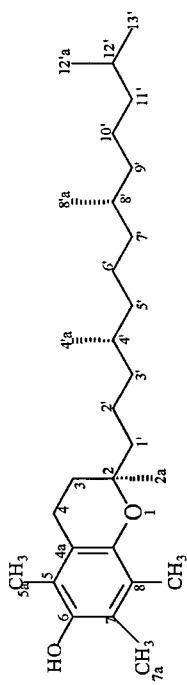
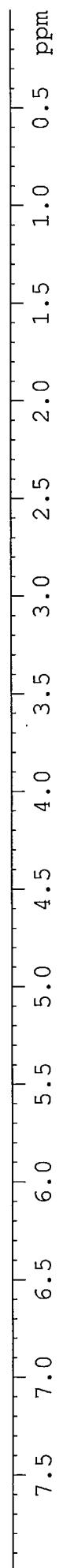
Spectrum 14.7: Mass spectrum of compound 4.3



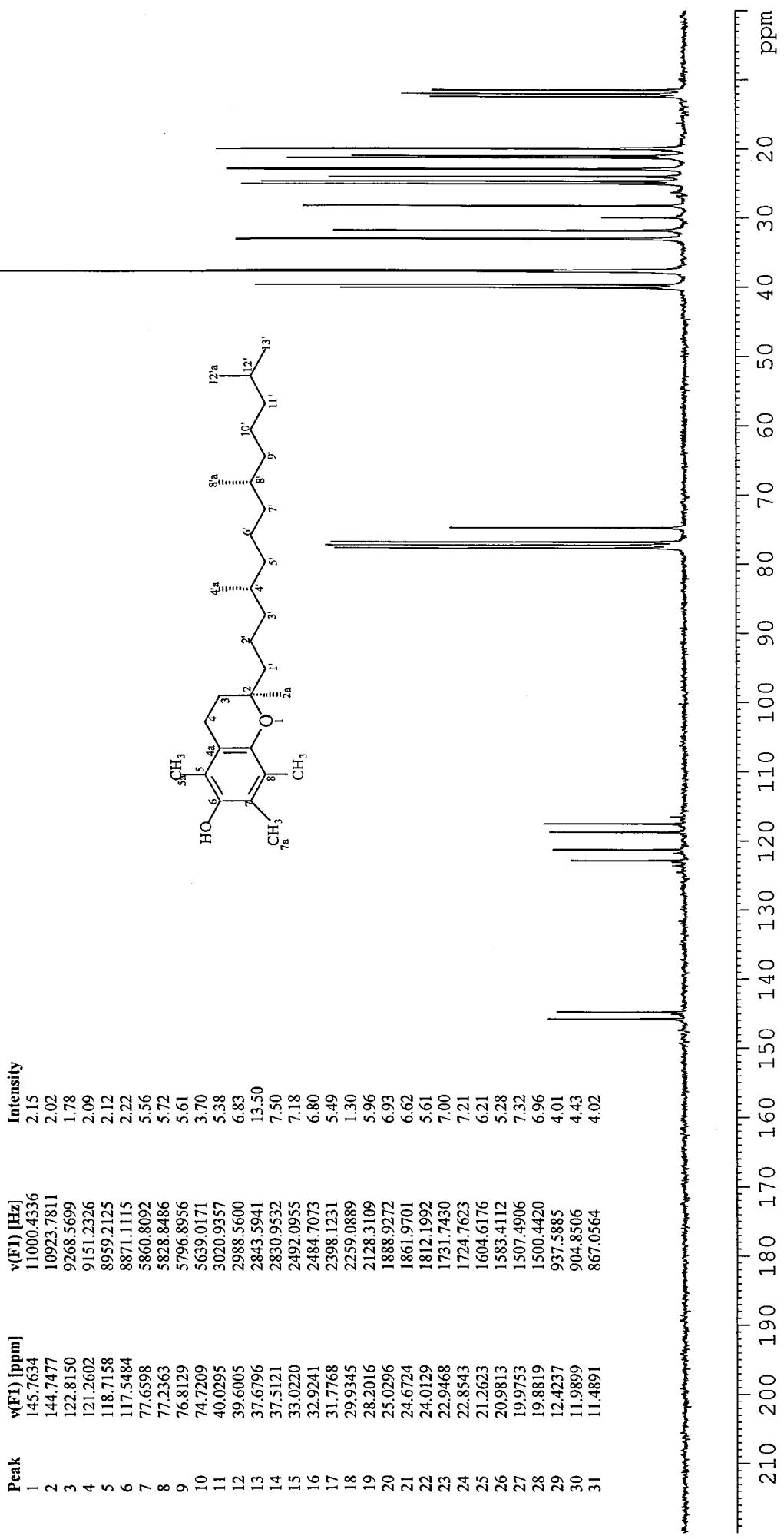


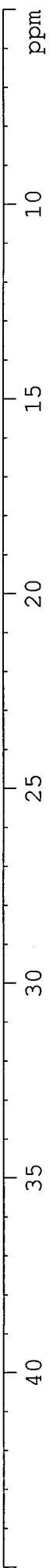
Spectrum 14.8: FTIR spectrum of compound 4.3

Spectrum 15.1:  $^1\text{H}$  NMR spectrum of compound 4.4 ( $\text{CDCl}_3$ )



Spectrum 15.2:  $^{13}\text{C}$  NMR spectrum of compound 4.4 ( $\text{CDCl}_3$ )

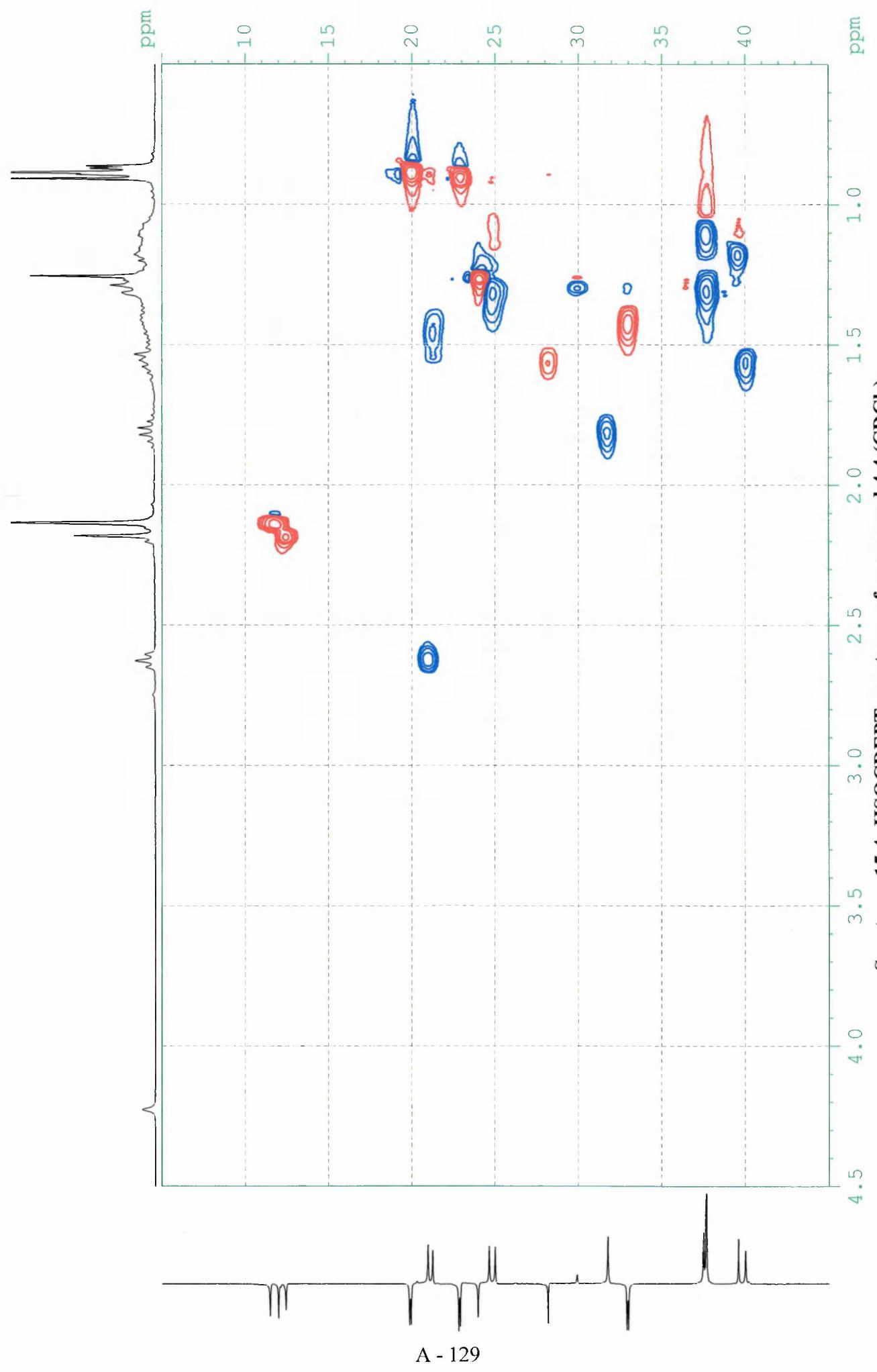




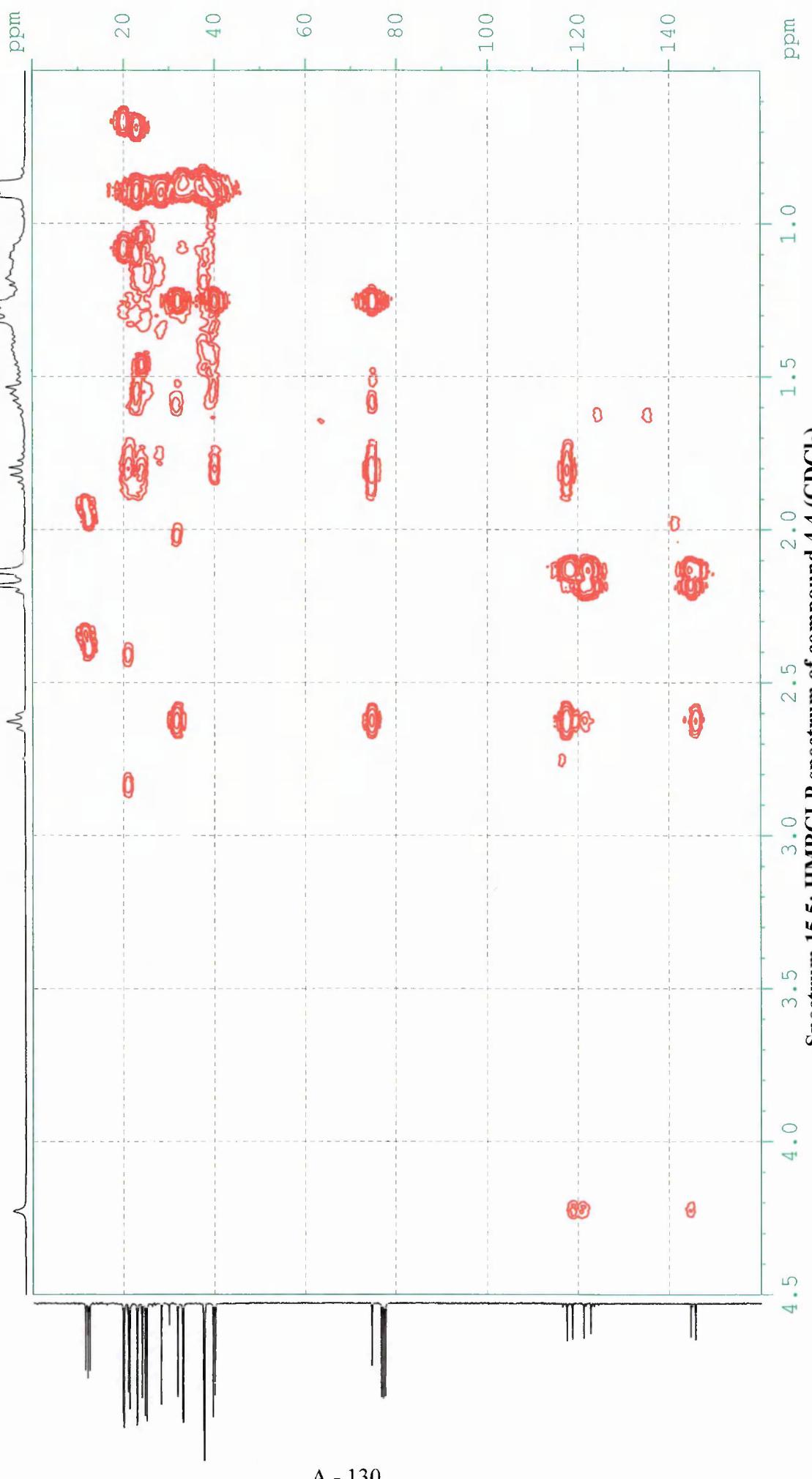
Spectrum 15.3: DEPT spectrum of compound 4.4 ( $\text{CDCl}_3$ )

A - 128

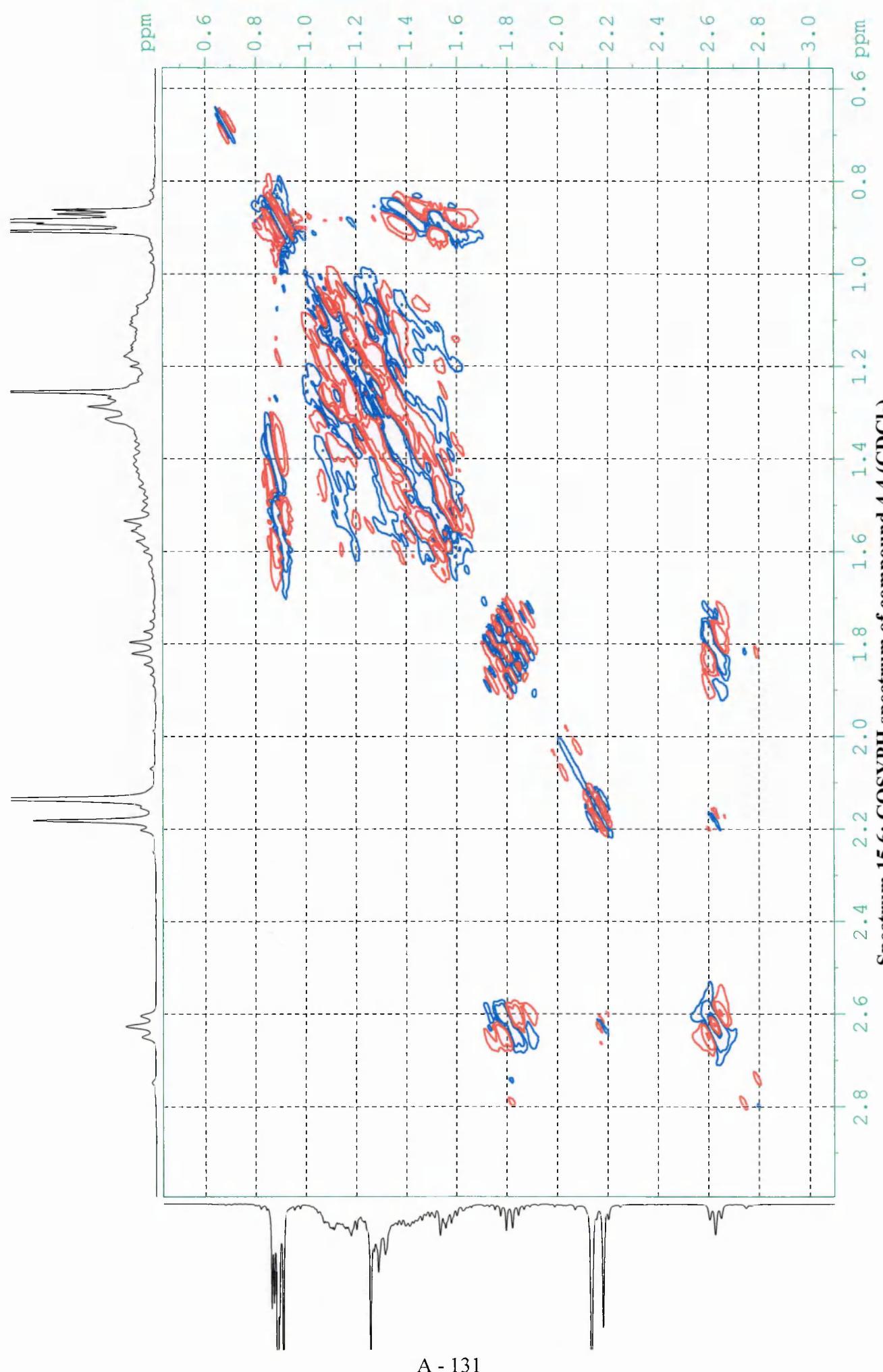
**Spectrum 15.4: HSQCDEPT spectrum of compound 4.4 ( $\text{CDCl}_3$ )**



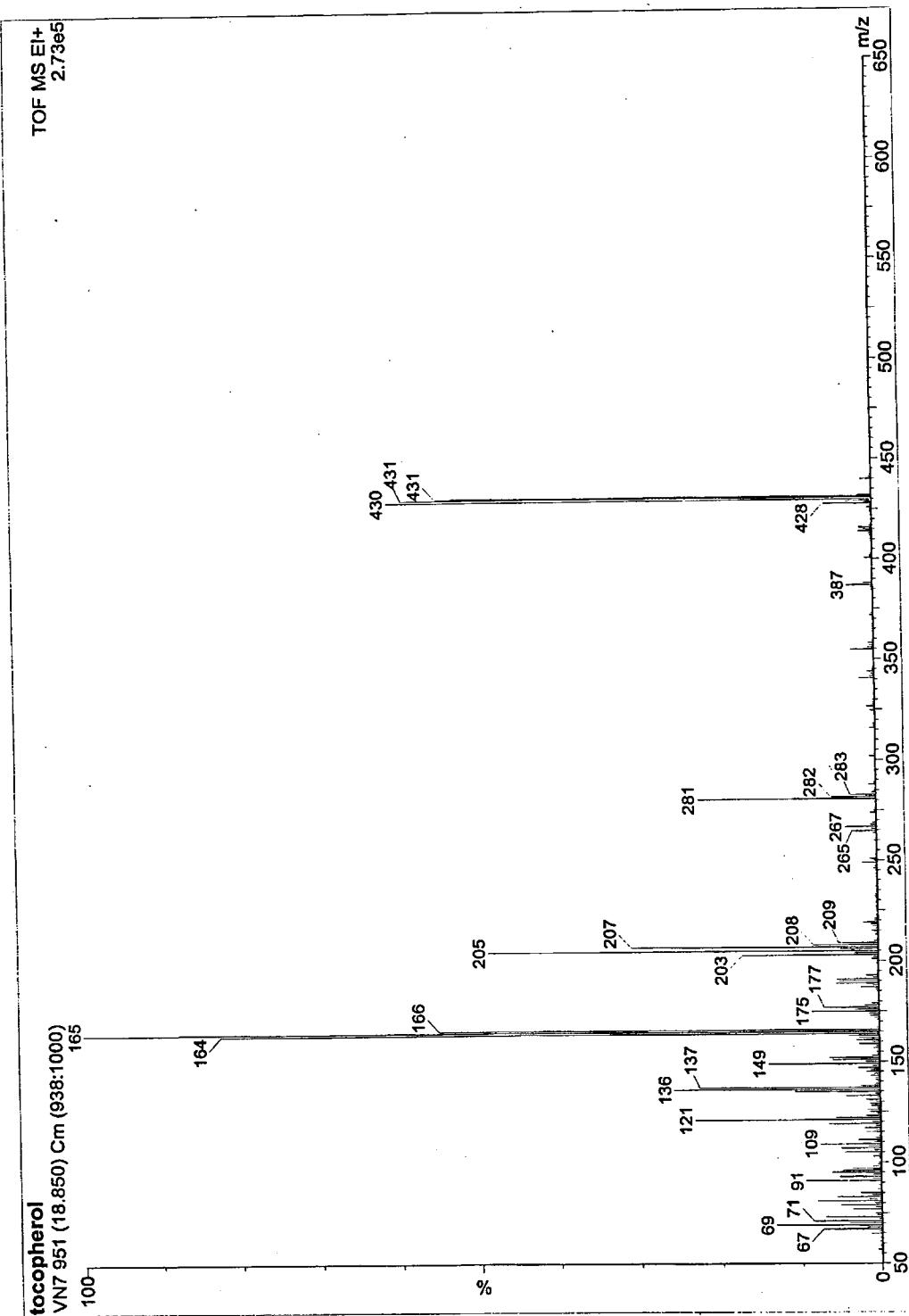
**Spectrum 15.5: HMBCCP spectrum of compound 4.4 ( $\text{CDCl}_3$ )**

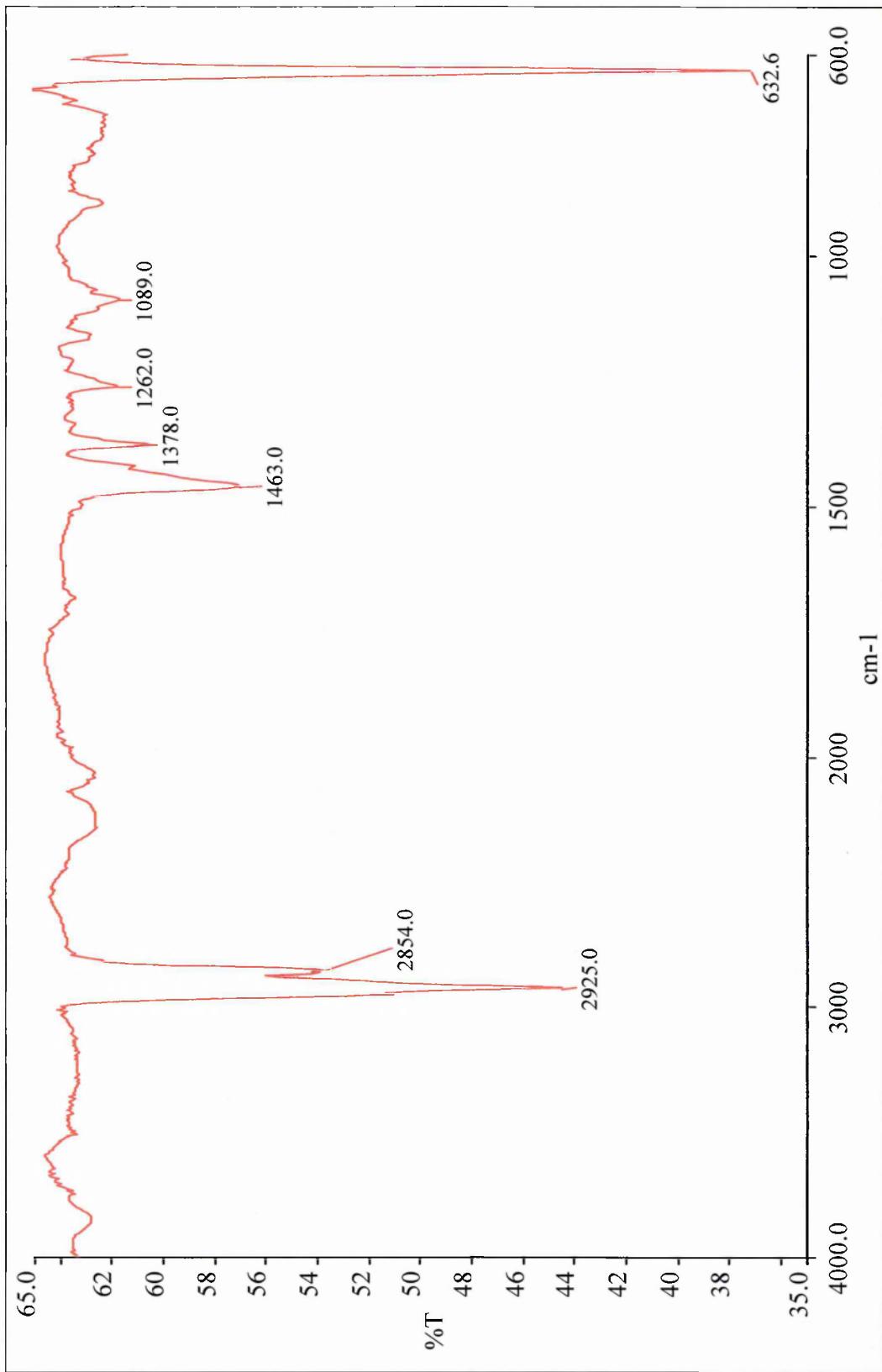


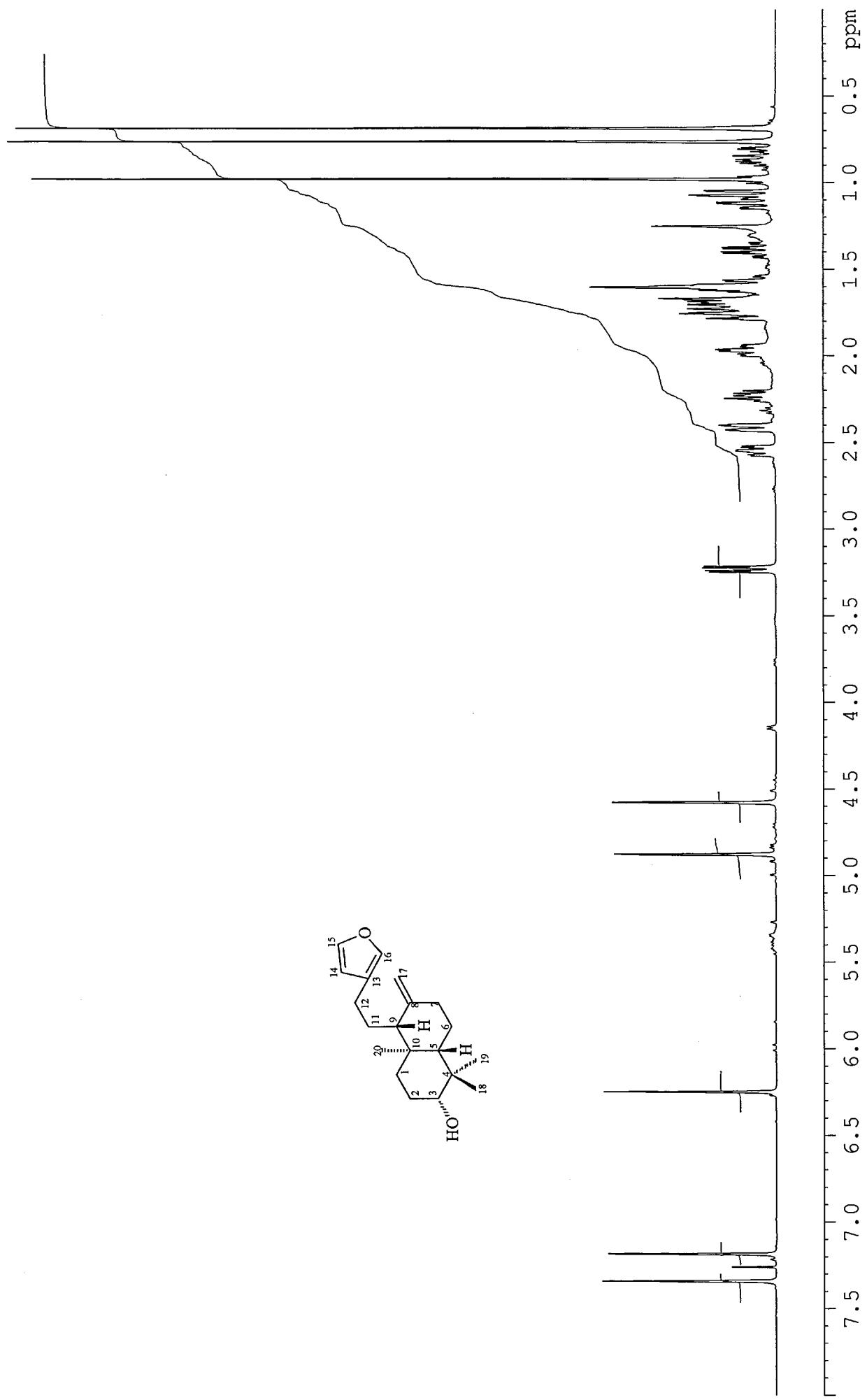
Spectrum 15.6: COSYPH spectrum of compound 4.4 ( $\text{CDCl}_3$ )



Spectrum 15.7: Mass spectrum of compound 4.4.

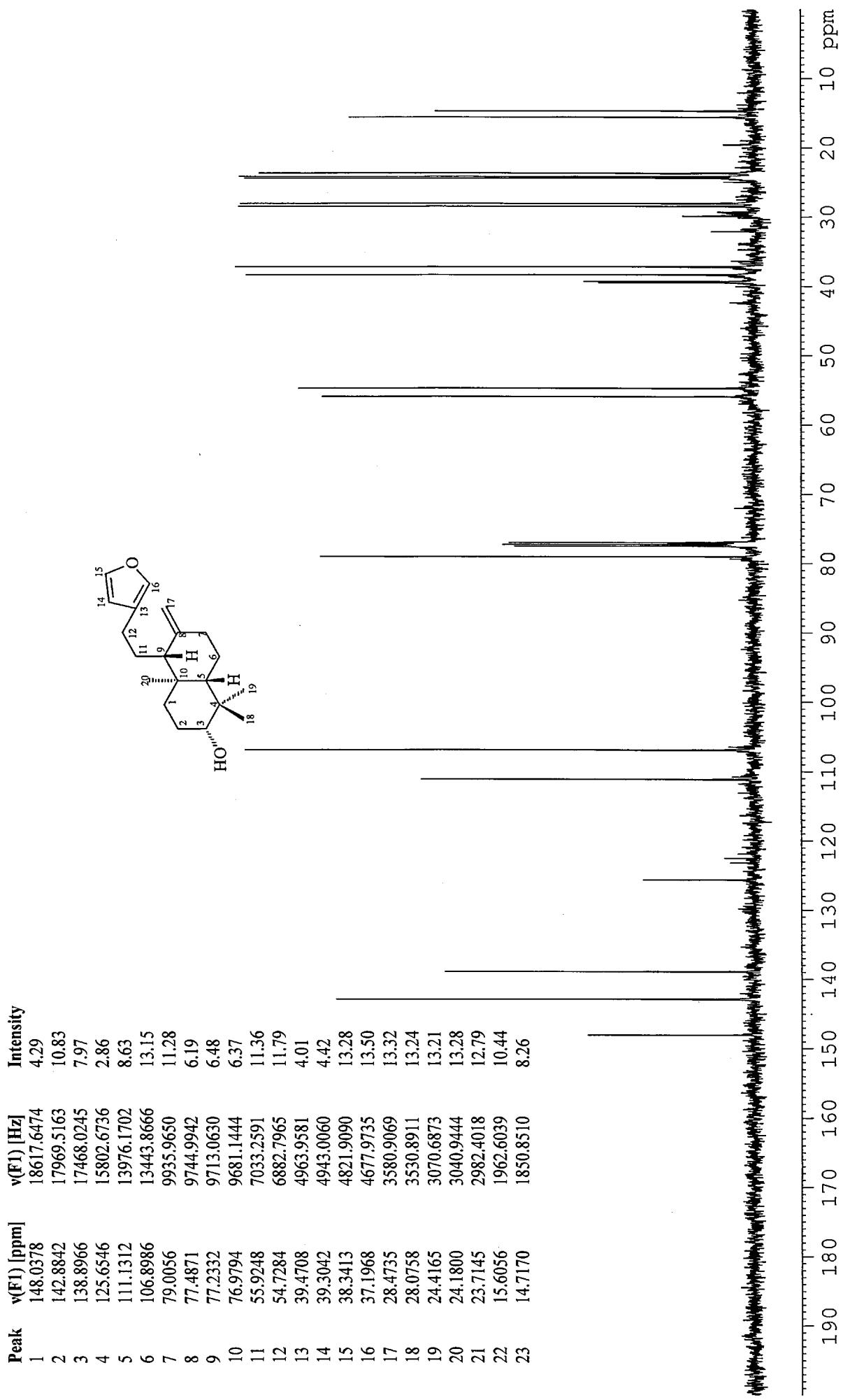


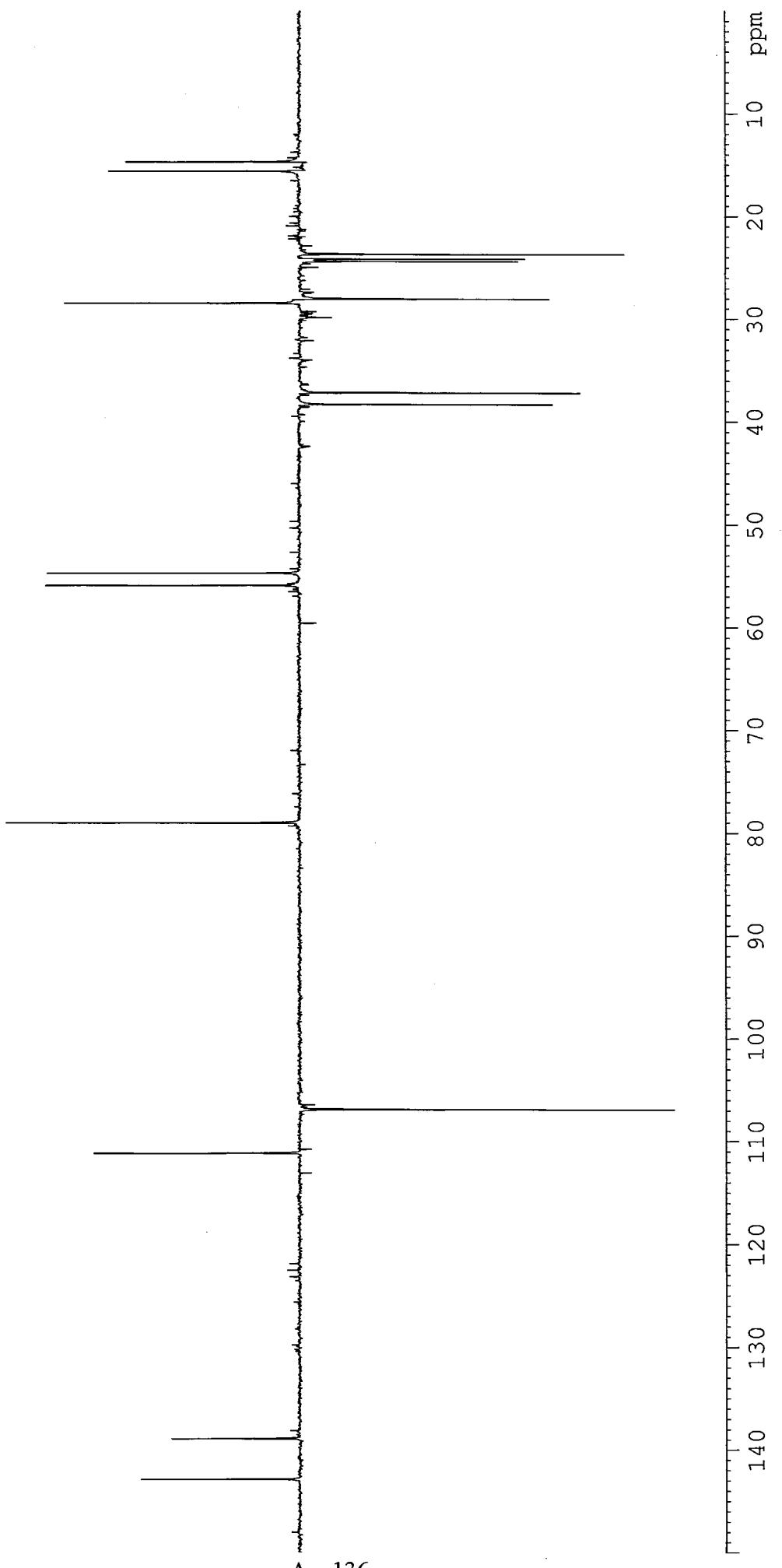




Spectrum 16.1:  $^1\text{H}$  NMR spectrum of compound 6.1 ( $\text{CDCl}_3$ )

Spectrum 16.2:  $^{13}\text{C}$  NMR spectrum of compound 6.1 ( $\text{CDCl}_3$ )

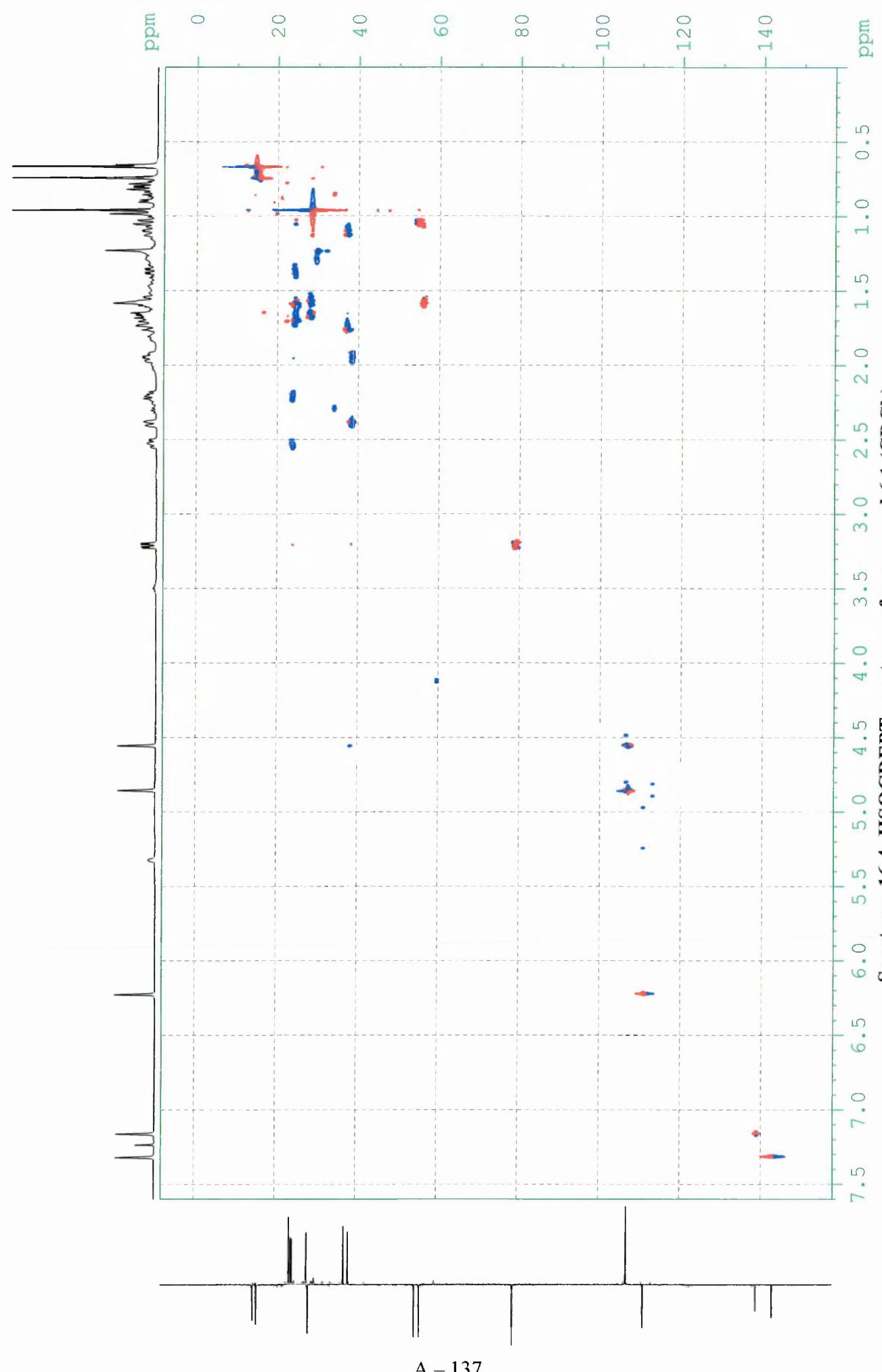




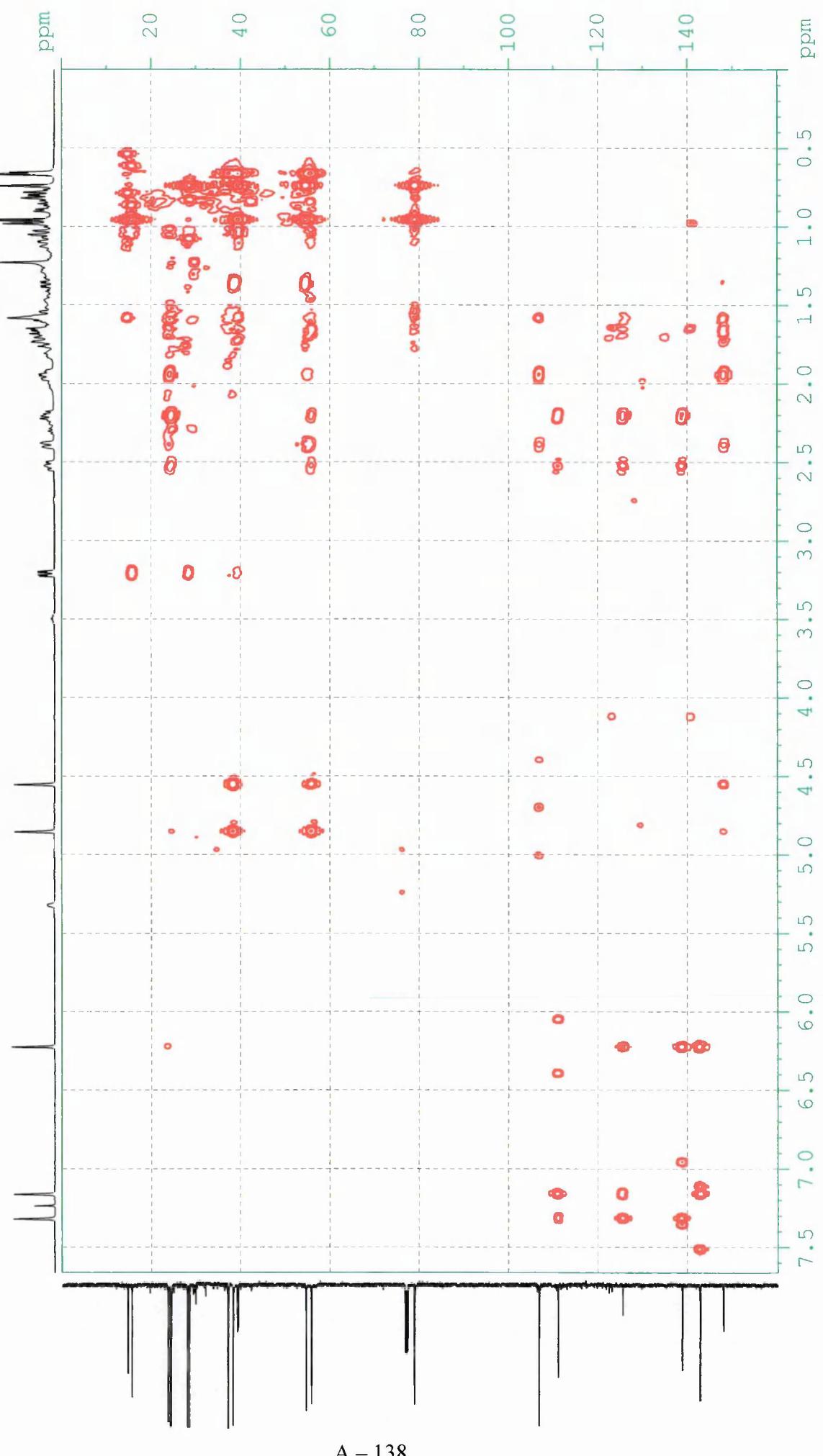
Spectrum 16.3: DEPT spectrum of compound 6.1 ( $\text{CDCl}_3$ )

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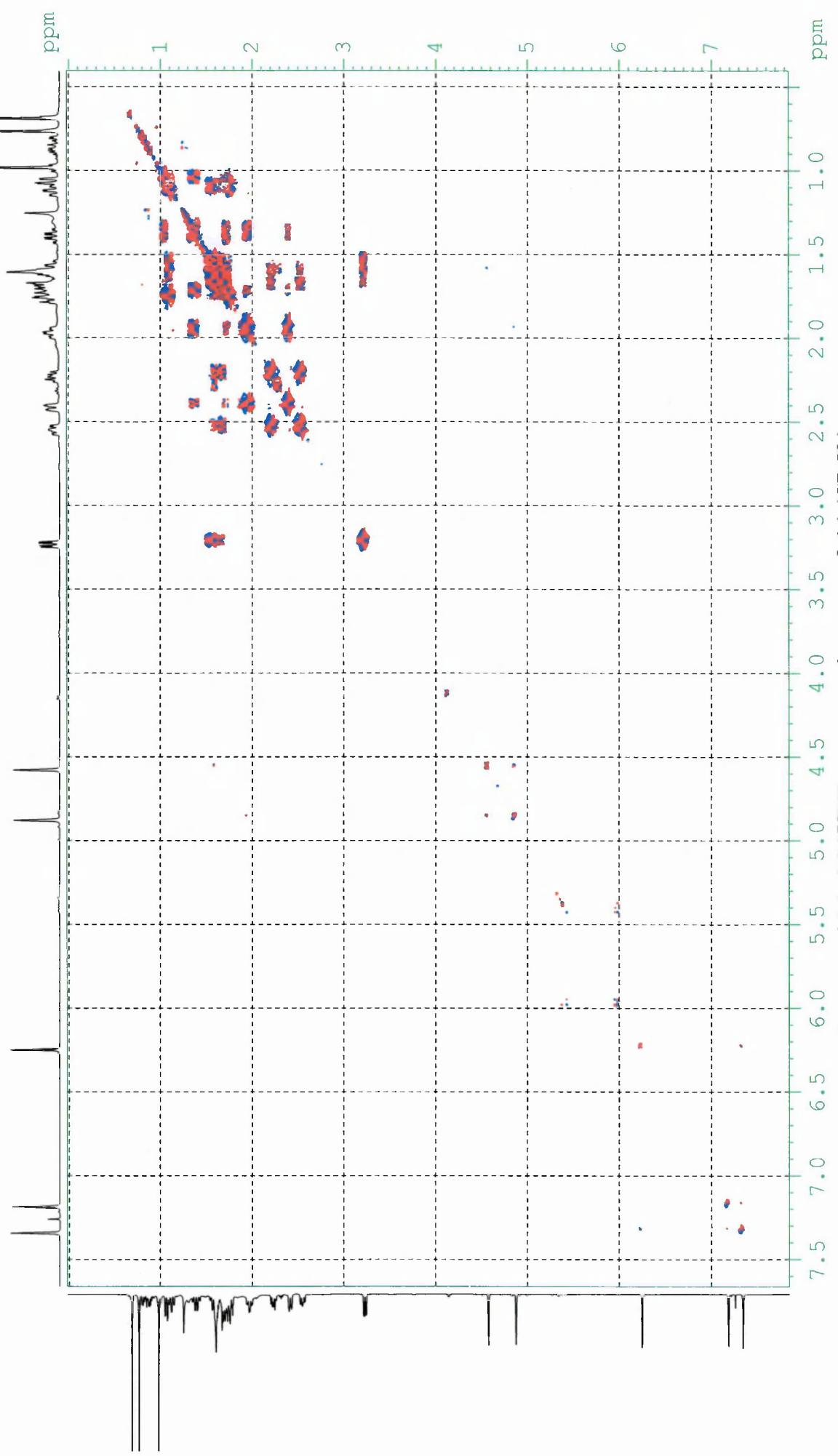
**Spectrum 16.4: HSQCDEPT spectrum of compound 6.1 ( $\text{CDCl}_3$ )**

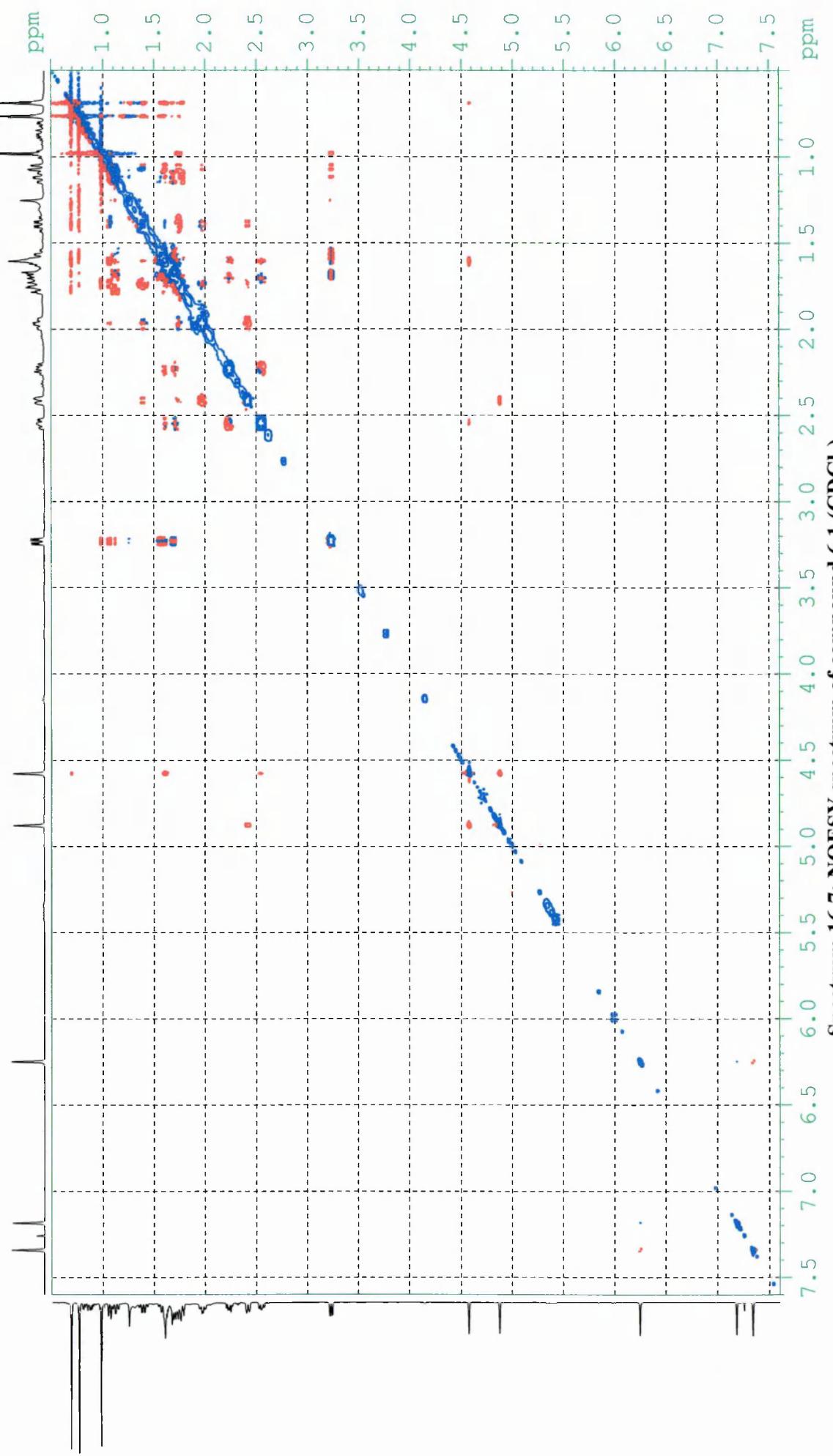


**Spectrum 16.5: HMBCCLP spectrum of compound 6.1 ( $\text{CDCl}_3$ )**

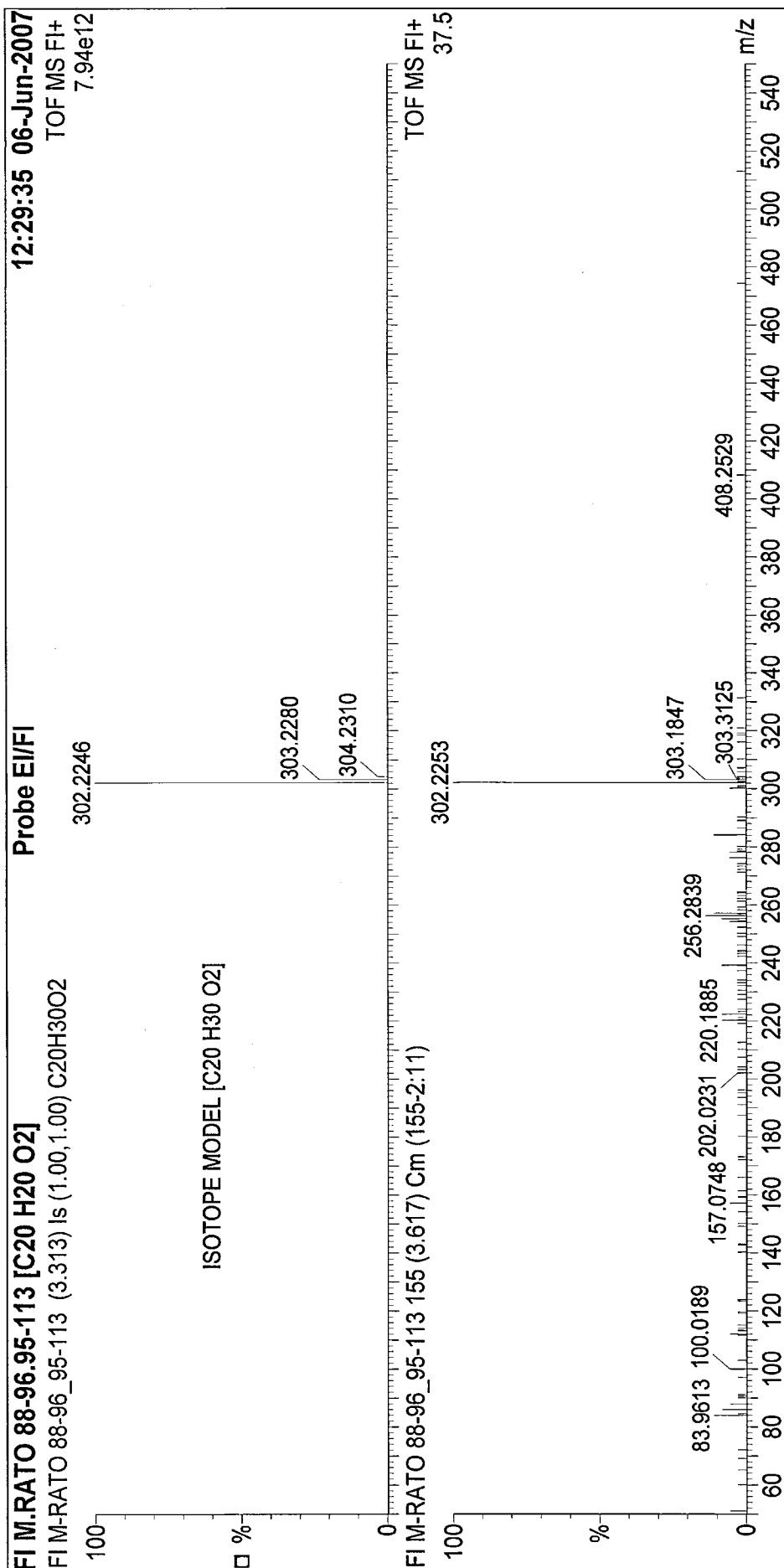


**Spectrum 16.6: COSYPH spectrum of compound 6.1 ( $\text{CDCl}_3$ )**

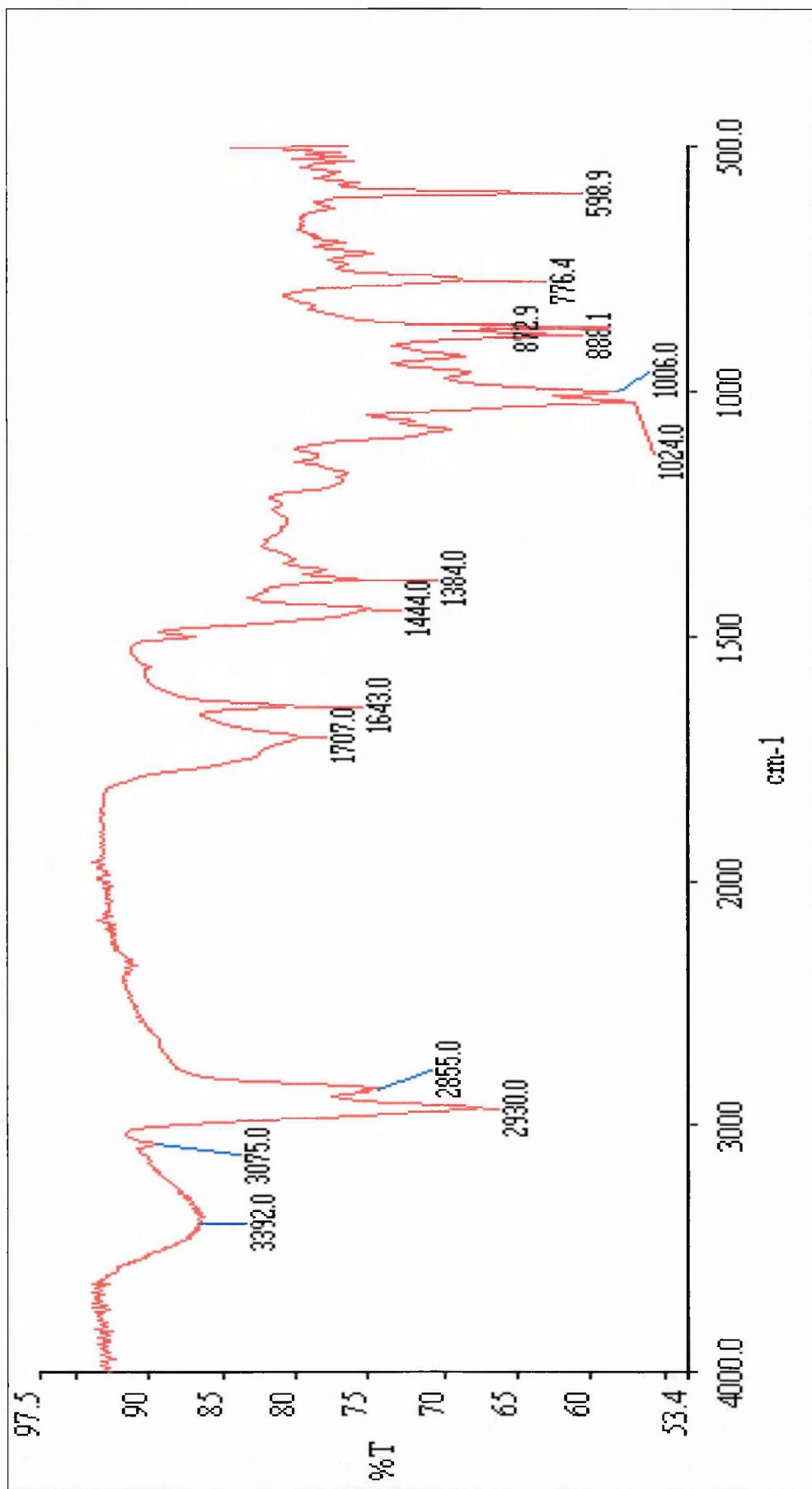




**Spectrum 16.7: NOESY spectrum of compound 6.1 ( $\text{CDCl}_3$ )**

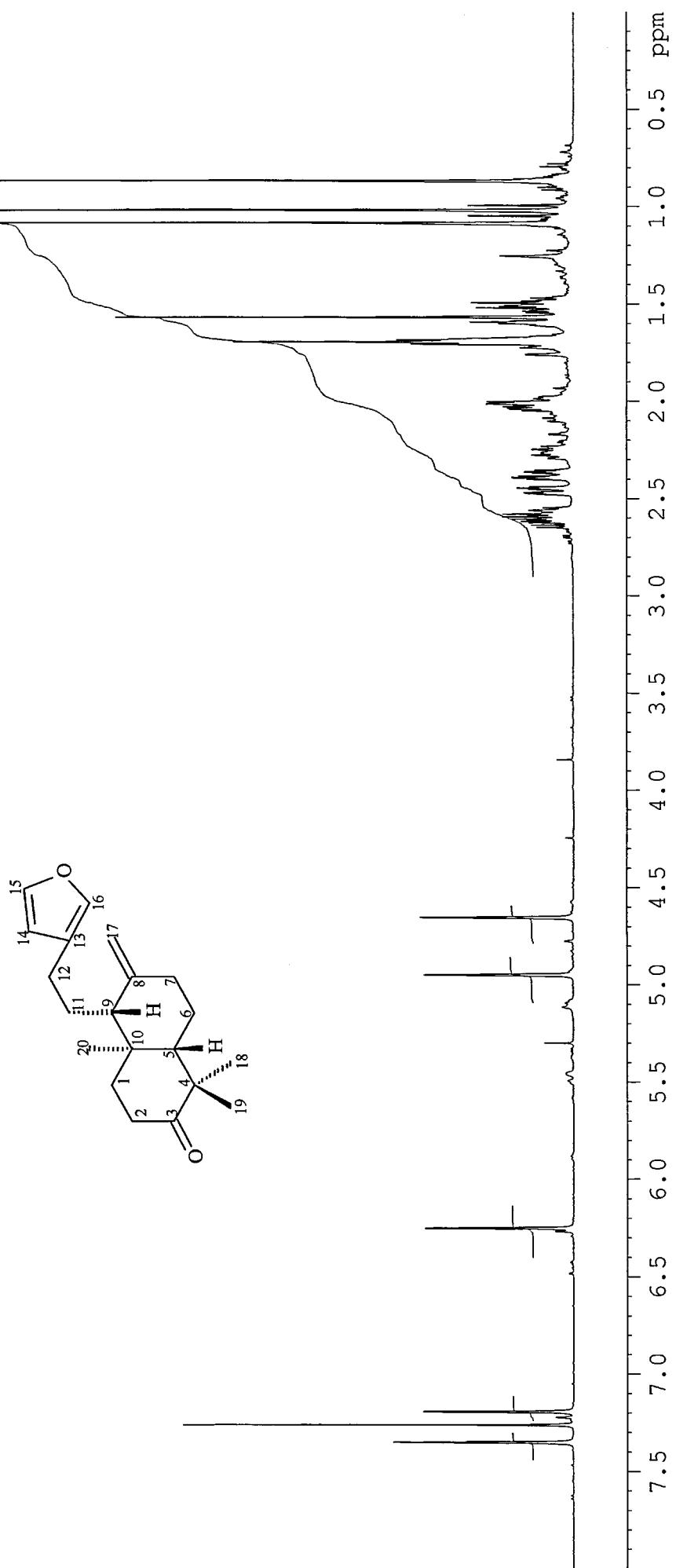


Spectrum 16.8: Mass spectrum of compound 6.1

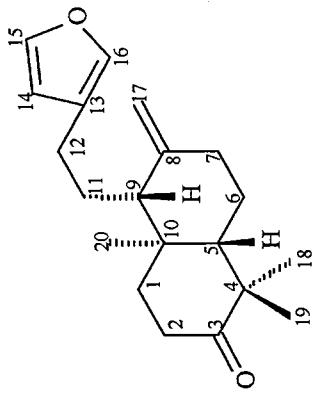


Spectrum 16.9: FTIR spectrum of compound 6.1

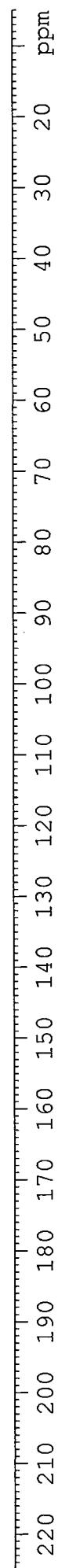
**Spectrum 17.1:**  $^1\text{H}$  NMR spectrum of compound 6.2 ( $\text{CDCl}_3$ )

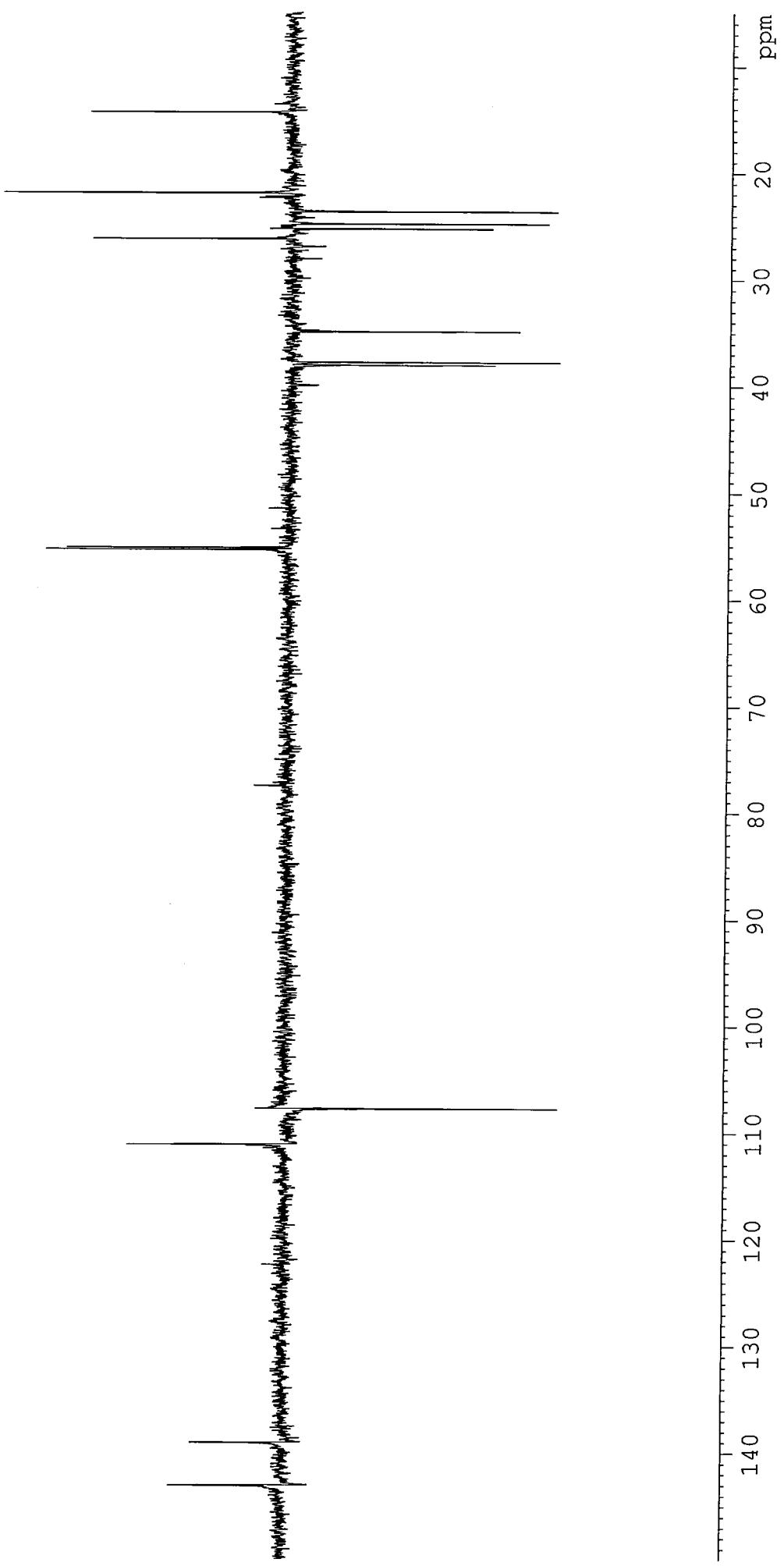


Peak	$\nu(\text{F1})$ [ppm]	$\nu(\text{F1})$ [Hz]	Intensity
1	217.0295	27294.2356	1.43
2	147.2695	18521.0233	2.00
3	143.0317	17988.0658	5.20
4	138.9667	17480.6129	4.03
5	125.4019	15770.8929	1.13
6	111.0520	13966.2095	4.17
7	107.7475	13550.6263	6.58
8	77.4870	9744.9814	18.64
9	77.2331	9713.0502	19.40
10	76.9792	9681.1190	19.50
11	55.3053	6955.3489	5.75
12	55.1251	6932.6684	5.54
13	47.9764	6033.6460	1.63
14	39.3833	4952.9537	1.71
15	38.0500	4785.2742	6.59
16	37.7842	4751.8464	6.63
17	34.9122	4390.6557	6.26
18	34.7683	4372.5584	0.83
19	26.9197	3385.4966	0.83
20	26.2048	3295.5888	6.54
21	25.2832	3179.6658	6.75
22	24.8205	3121.4954	6.30
23	23.6688	2976.6543	6.27
24	21.8956	2753.6518	5.88
25	14.3188	1800.7723	4.92



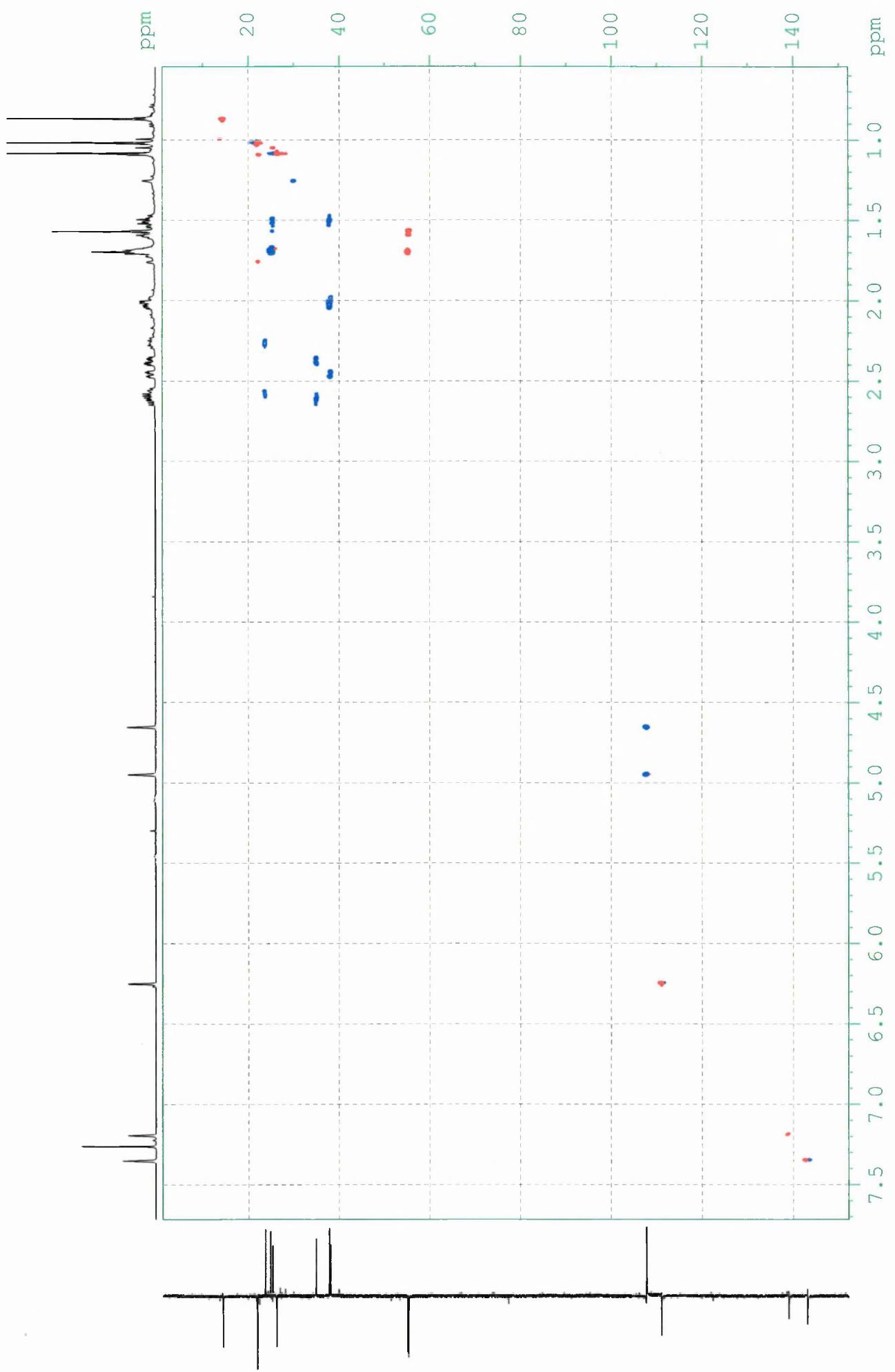
Spectrum 17.2:  $^{13}\text{C}$  NMR spectrum of compound 6.2 ( $\text{CDCl}_3$ )



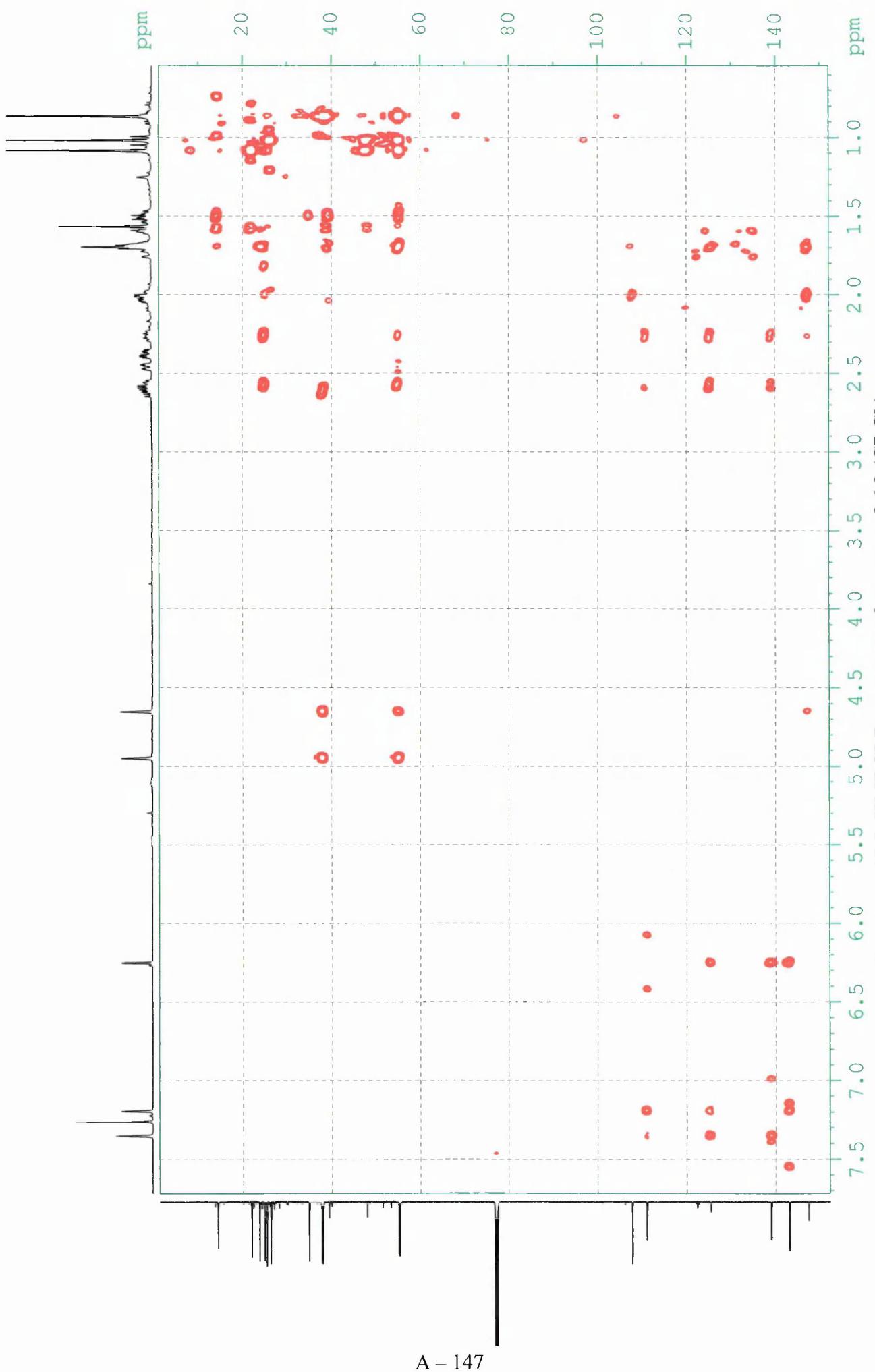


Spectrum 17.3: DEPT spectrum of compound 6.2 ( $\text{CDCl}_3$ )

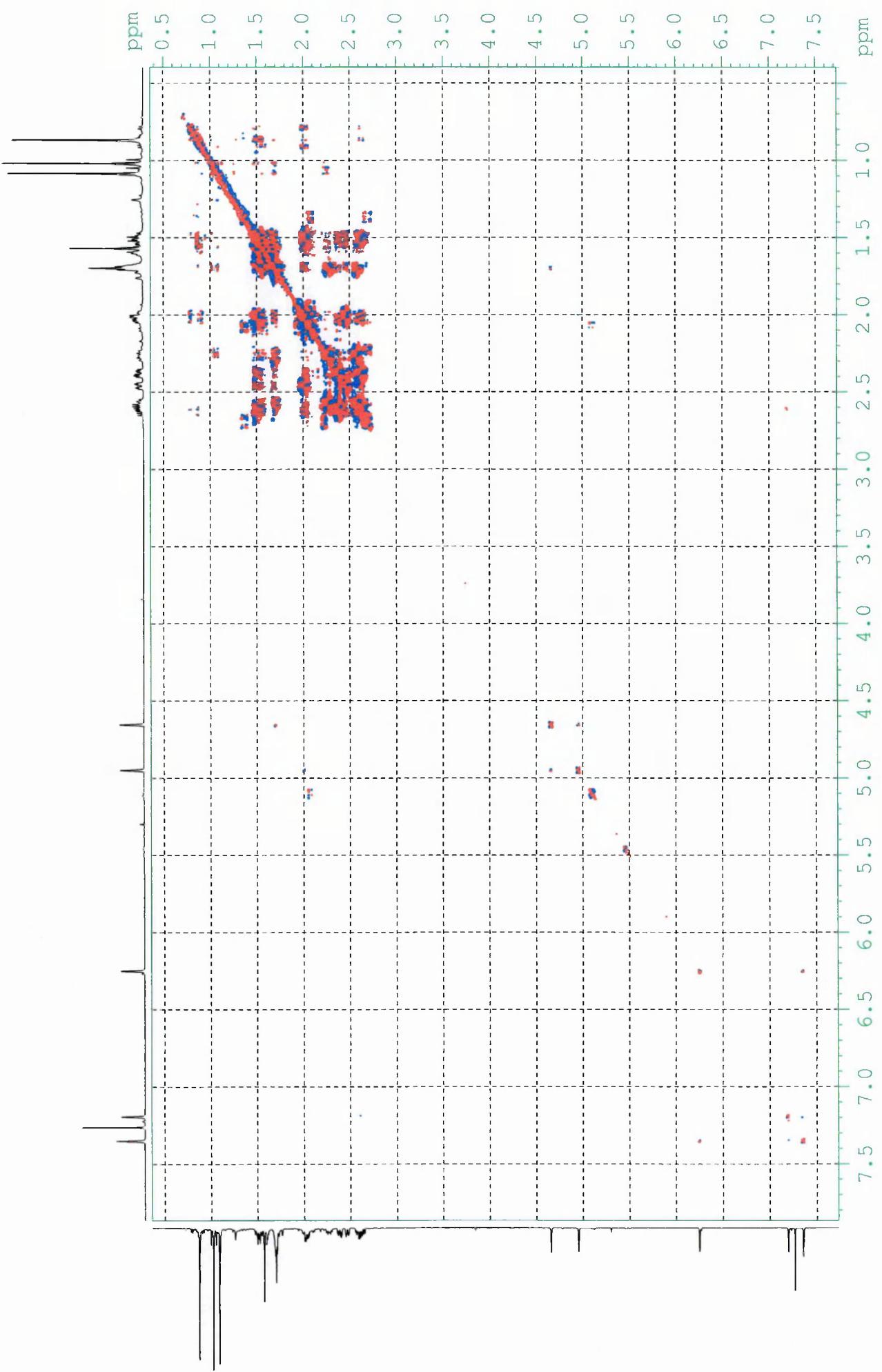
**Spectrum 17.4: HSQCDEPT spectrum of compound 6.2 ( $\text{CDCl}_3$ )**



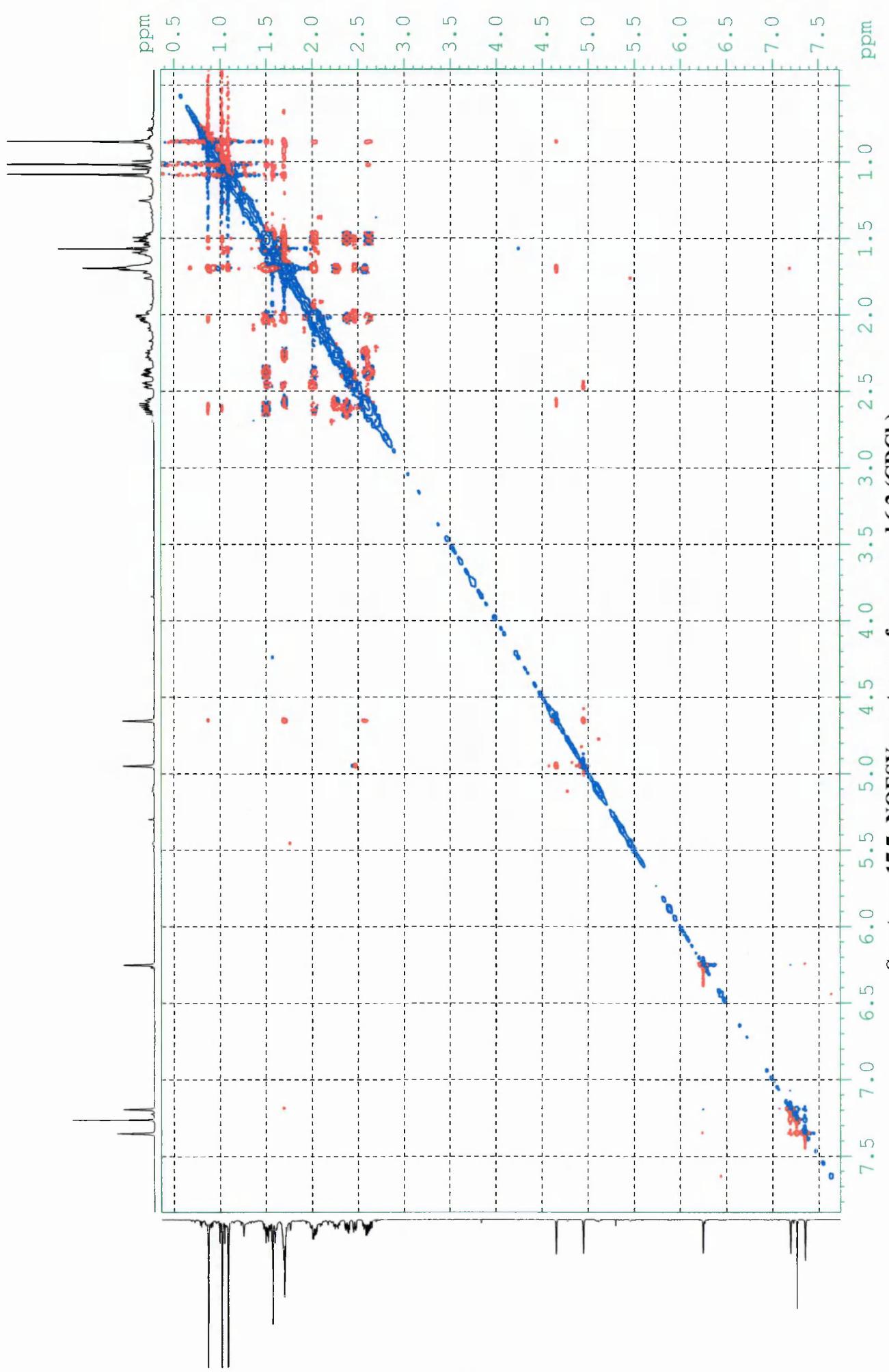
**Spectrum 17.5: HMBCCP spectrum of compound 6.2 ( $\text{CDCl}_3$ )**



**Spectrum 17.6: COSYPH spectrum of compound 6.2 ( $\text{CDCl}_3$ )**



**Spectrum 17.7: NOESY spectrum of compound 6.2 ( $\text{CDCl}_3$ )**



## Elemental Composition Report

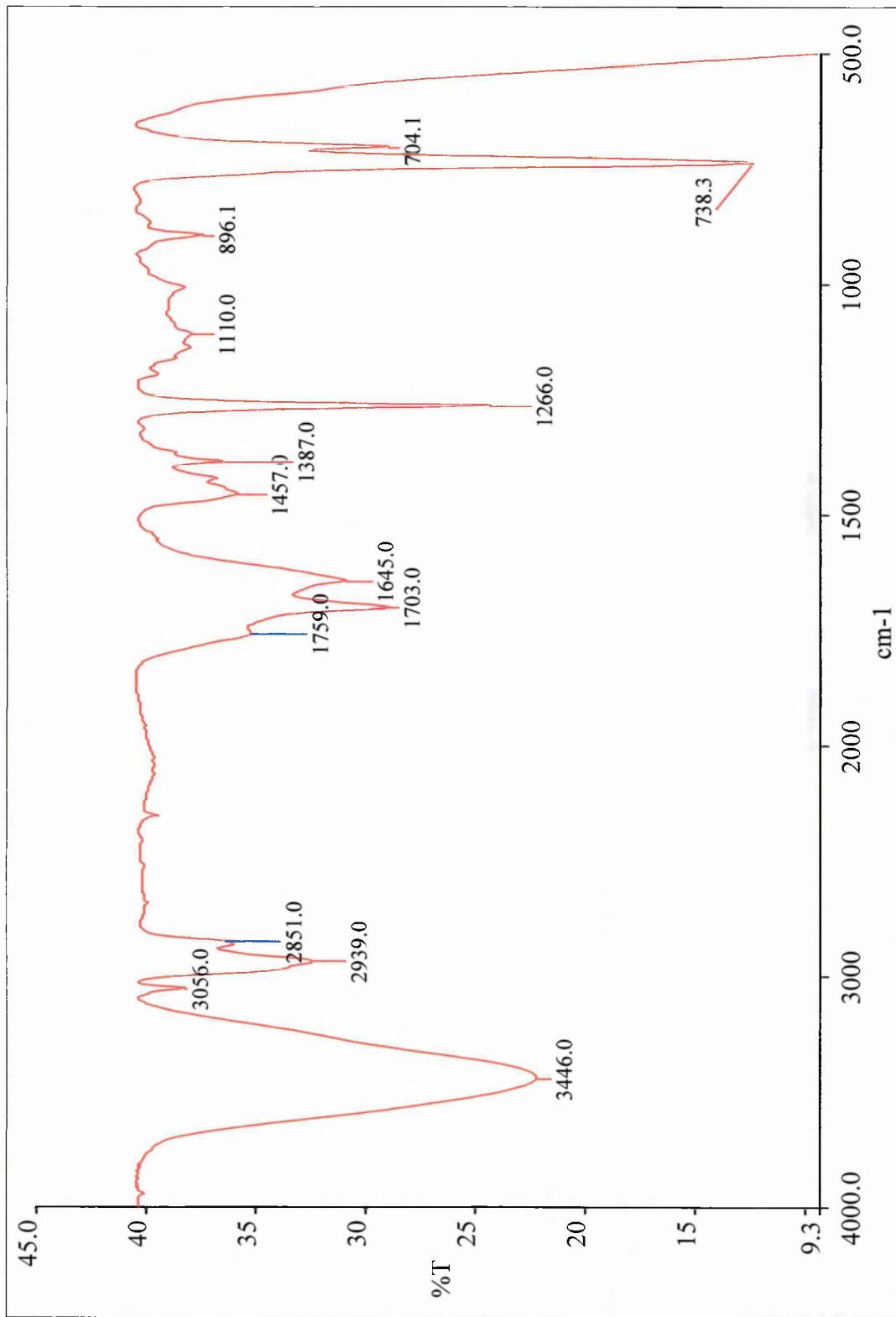
### Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -0.5, max = 50.0

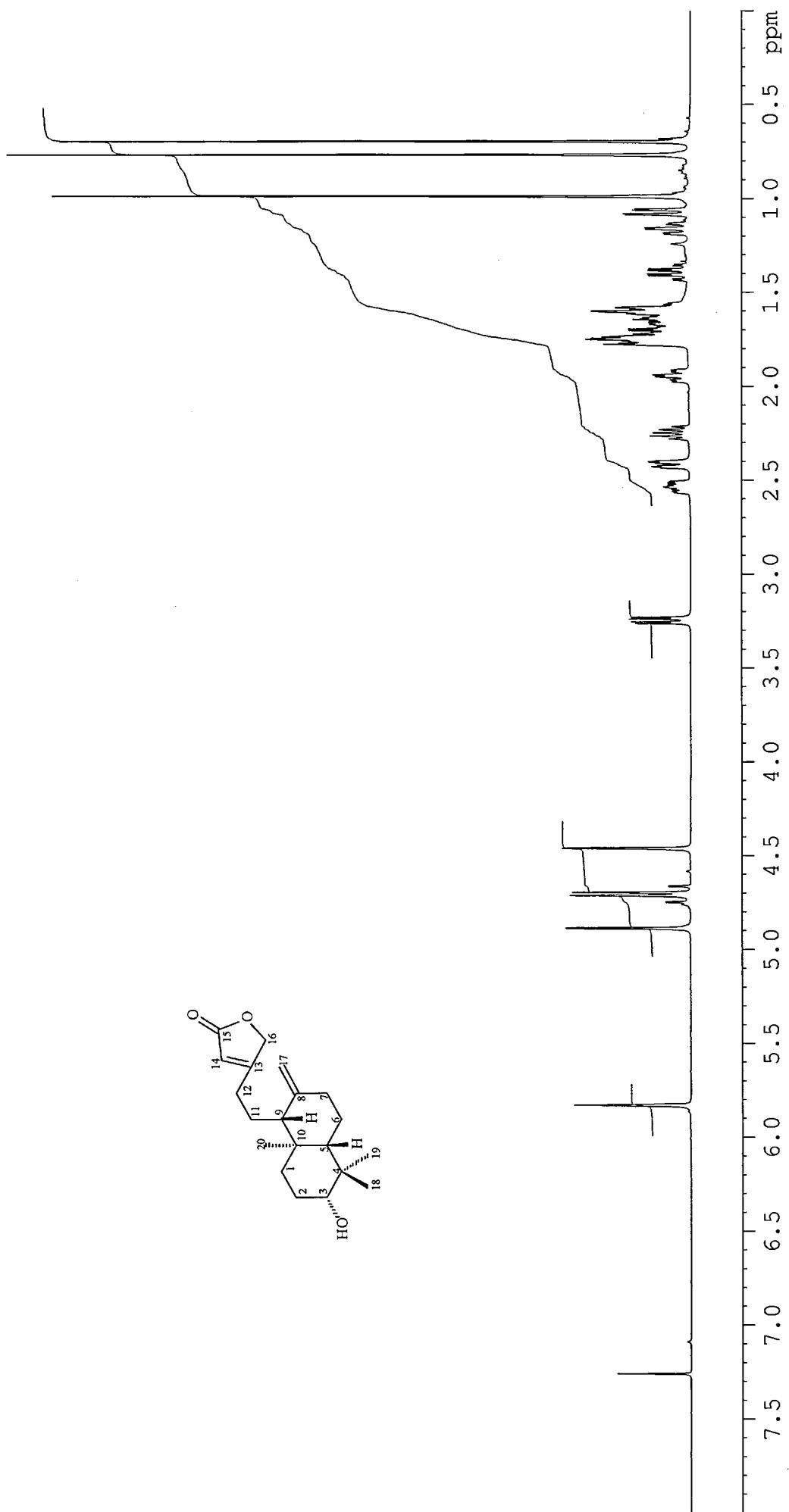
Monoisotopic Mass, Odd and Even Electron Ions  
13 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Mass	Calc. Mass	mDa	PPM	DBE	Formula	
301.2167	301.2168	-0.1	-0.2	6.5	C20 H29 O2	( <i>actual</i> $H^+$ )

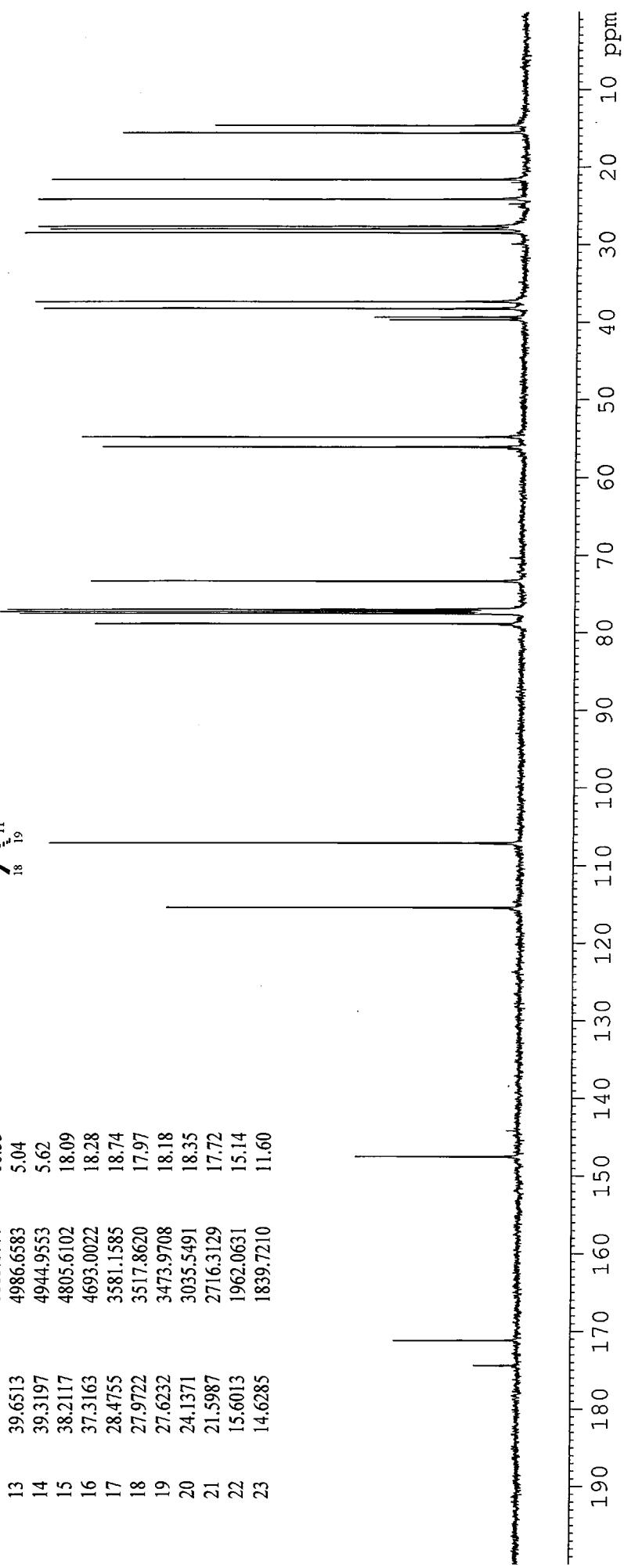
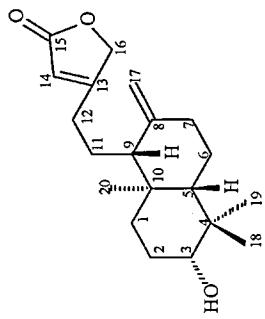
Spectrum 17.9: FTIR spectrum of compound 6.2



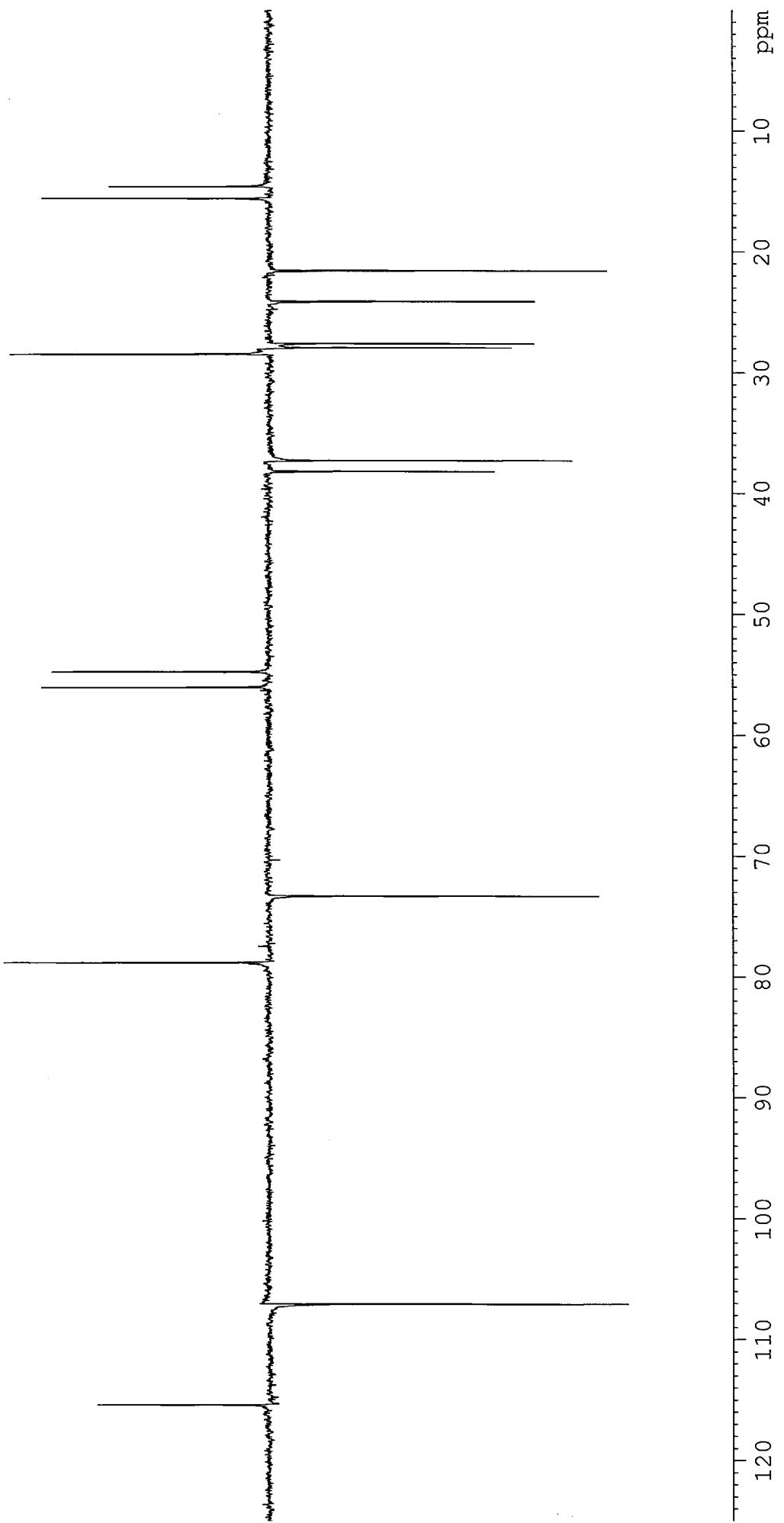
**Spectrum 18.1:**  $^1\text{H}$  NMR spectrum of compound 6.3 ( $\text{CDCl}_3$ )



Peak	$\nu(F)$ [ppm]	$\nu(F)$ [Hz]	Intensity
1	174.4672	21941.4826	0.00
2	171.1082	21519.0454	4.62
2	147.4435	18542.9066	6.08
4	115.3787	14510.3477	13.24
5	107.0710	13465.5482	17.65
6	78.7928	9909.2027	16.02
7	77.4860	9744.8559	18.85
8	77.2319	9712.8996	19.50
9	76.9778	9680.9432	19.30
10	73.3083	9219.4567	16.17
11	56.0456	7048.4513	15.74
12	54.7529	6885.8777	16.55
13	39.6513	4986.6583	5.04
14	39.3197	4944.9553	5.62
15	38.2117	4805.6102	18.09
16	37.3163	4693.0022	18.28
17	28.4755	3581.1585	18.74
18	27.9722	3517.8620	17.97
19	27.6232	3473.9708	18.18
20	24.1371	3035.5491	18.35
21	21.5987	2716.3129	17.72
22	15.6013	1962.0631	15.14
23	14.6285	1839.7210	11.60



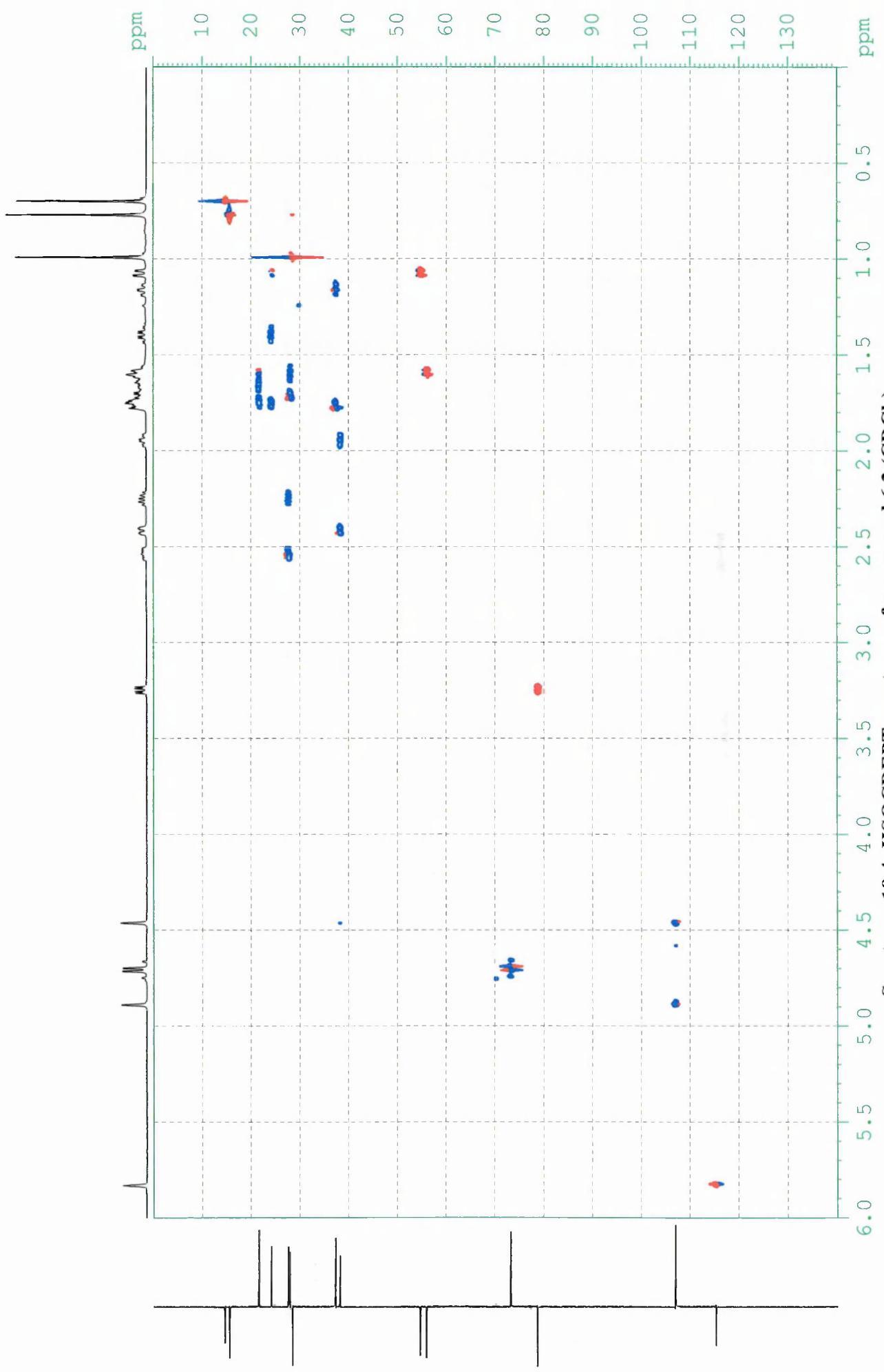
Spectrum 18.2:  $^{13}\text{C}$  NMR spectrum of compound 6.3 ( $\text{CDCl}_3$ )



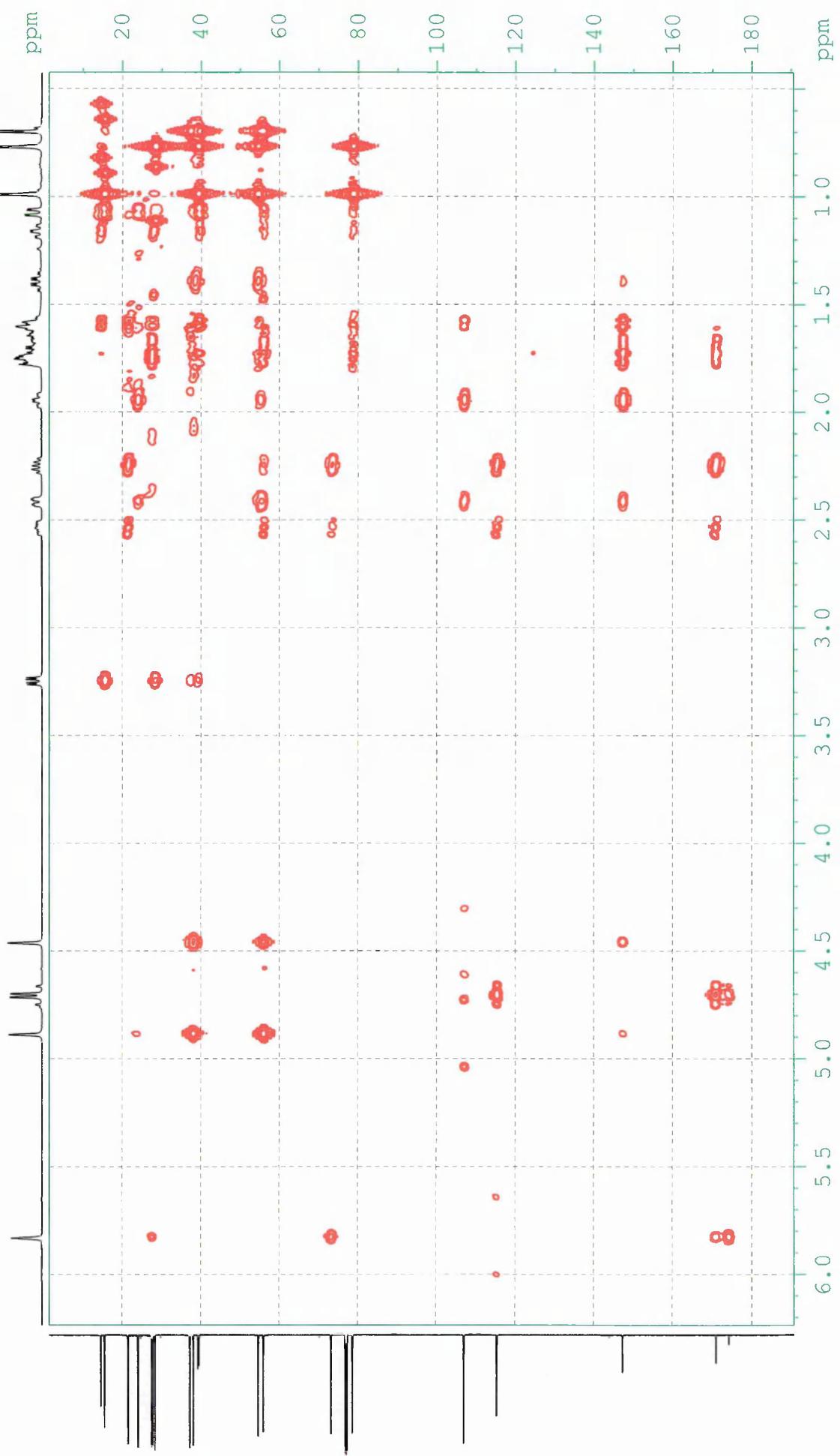
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**Spectrum 18.3:** DEPT NMR spectrum of compound 6.3 ( $\text{CDCl}_3$ )

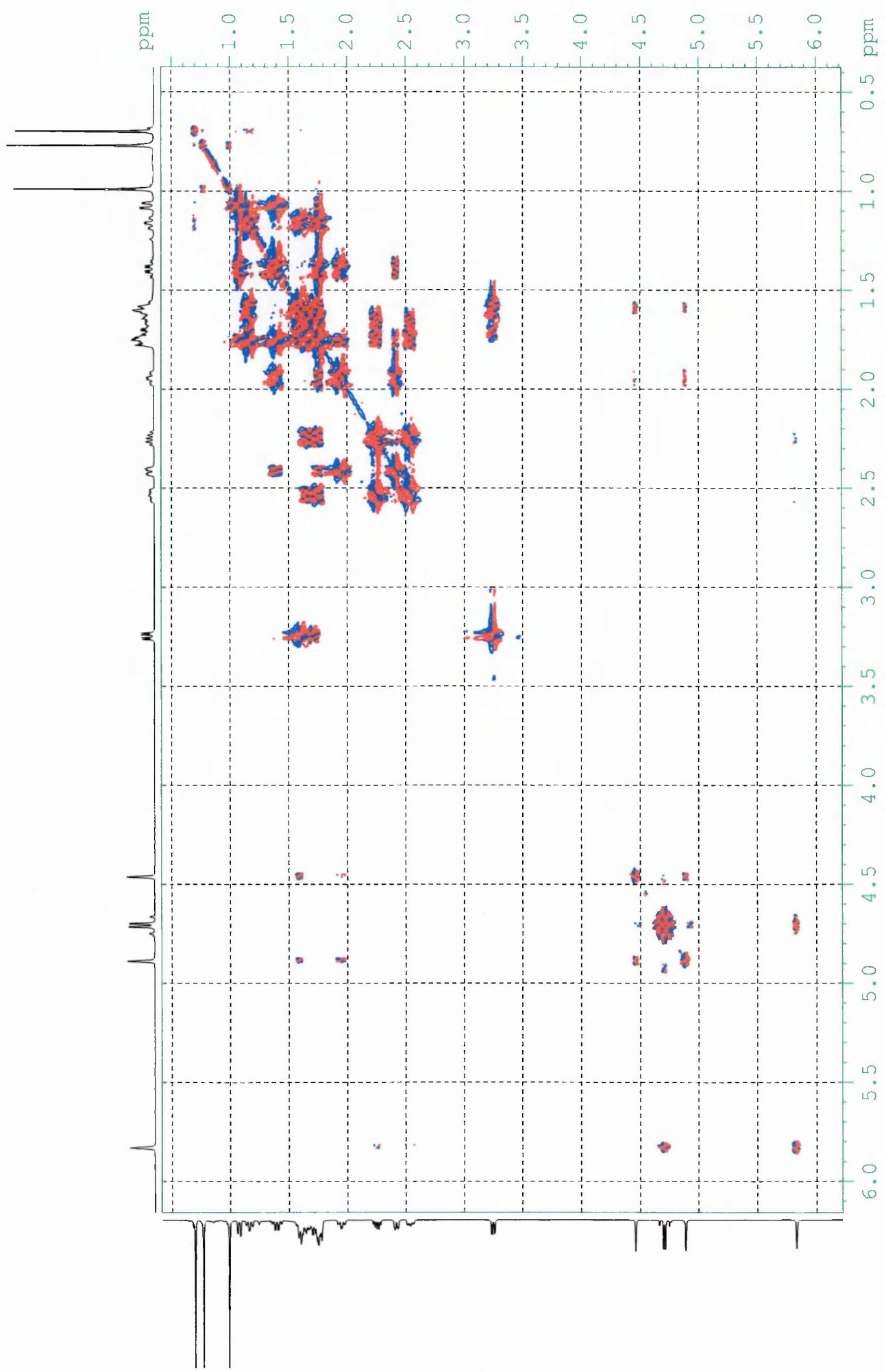
**Spectrum 18.4: HSQCDEPT spectrum of compound 6.3 ( $\text{CDCl}_3$ )**

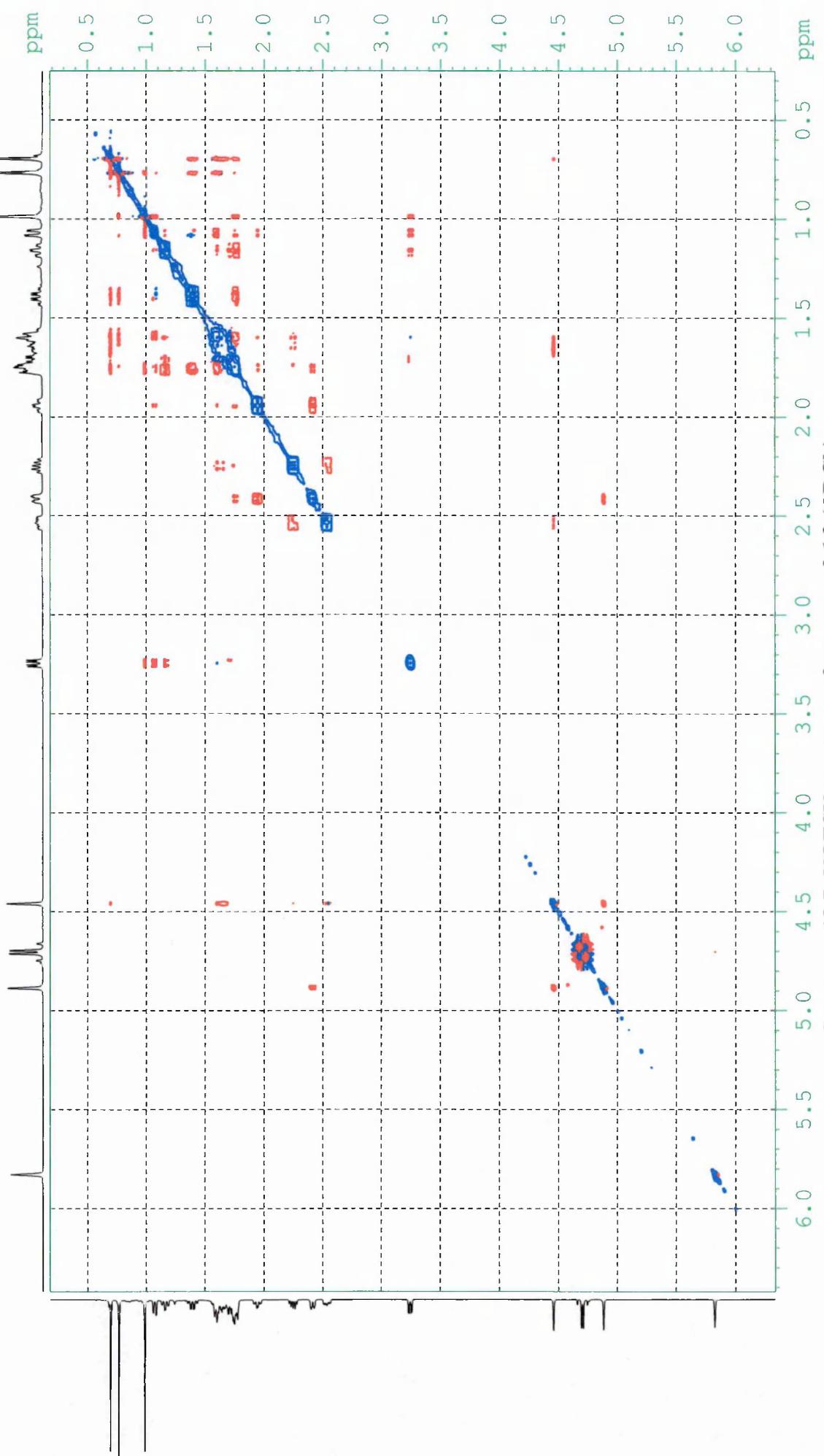


**Spectrum 18.5: HMBCCLP spectrum of compound 6.3 ( $\text{CDCl}_3$ )**



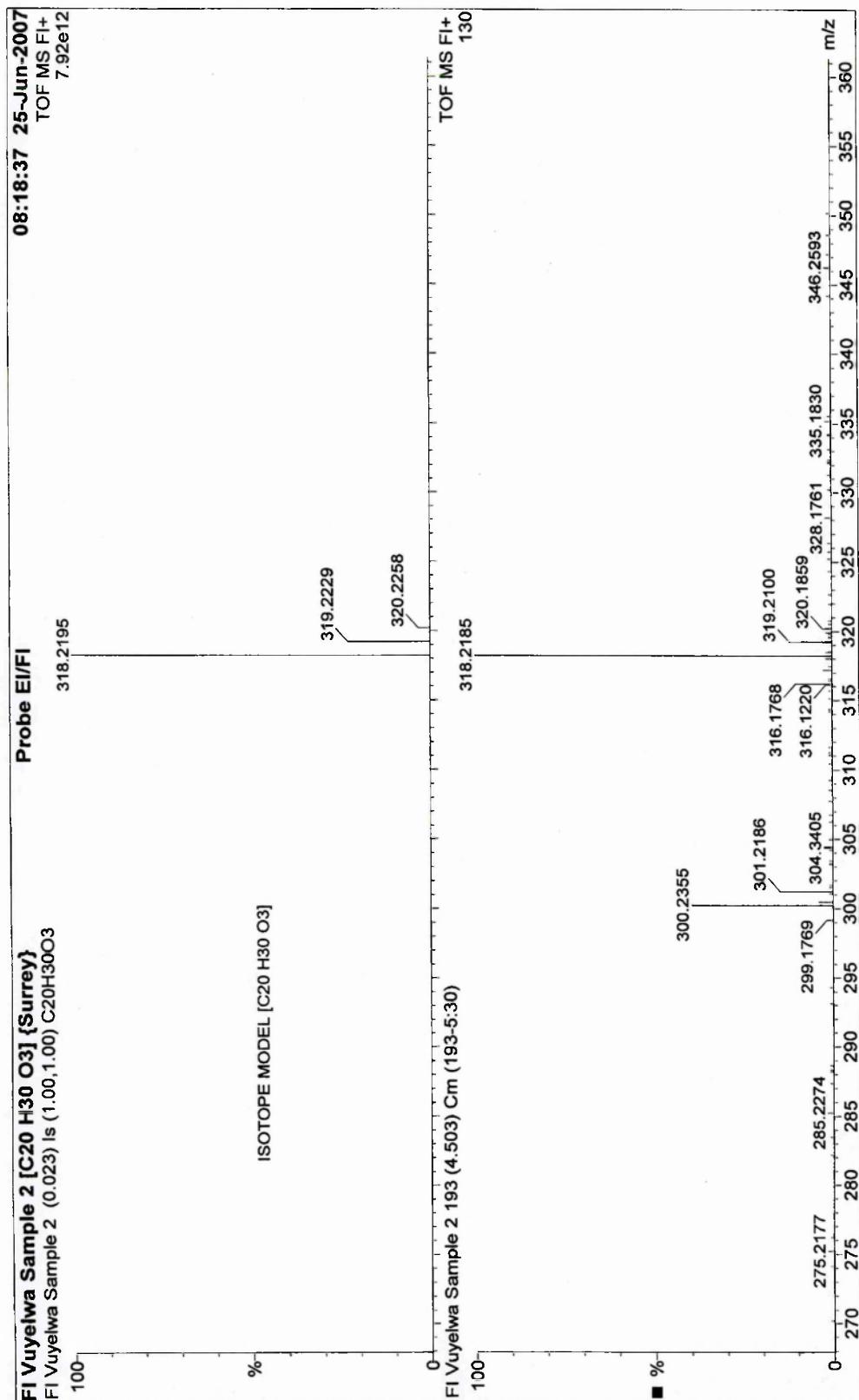
**Spectrum 18.6: COSYPH spectrum of compound 6.3 ( $\text{CDCl}_3$ )**

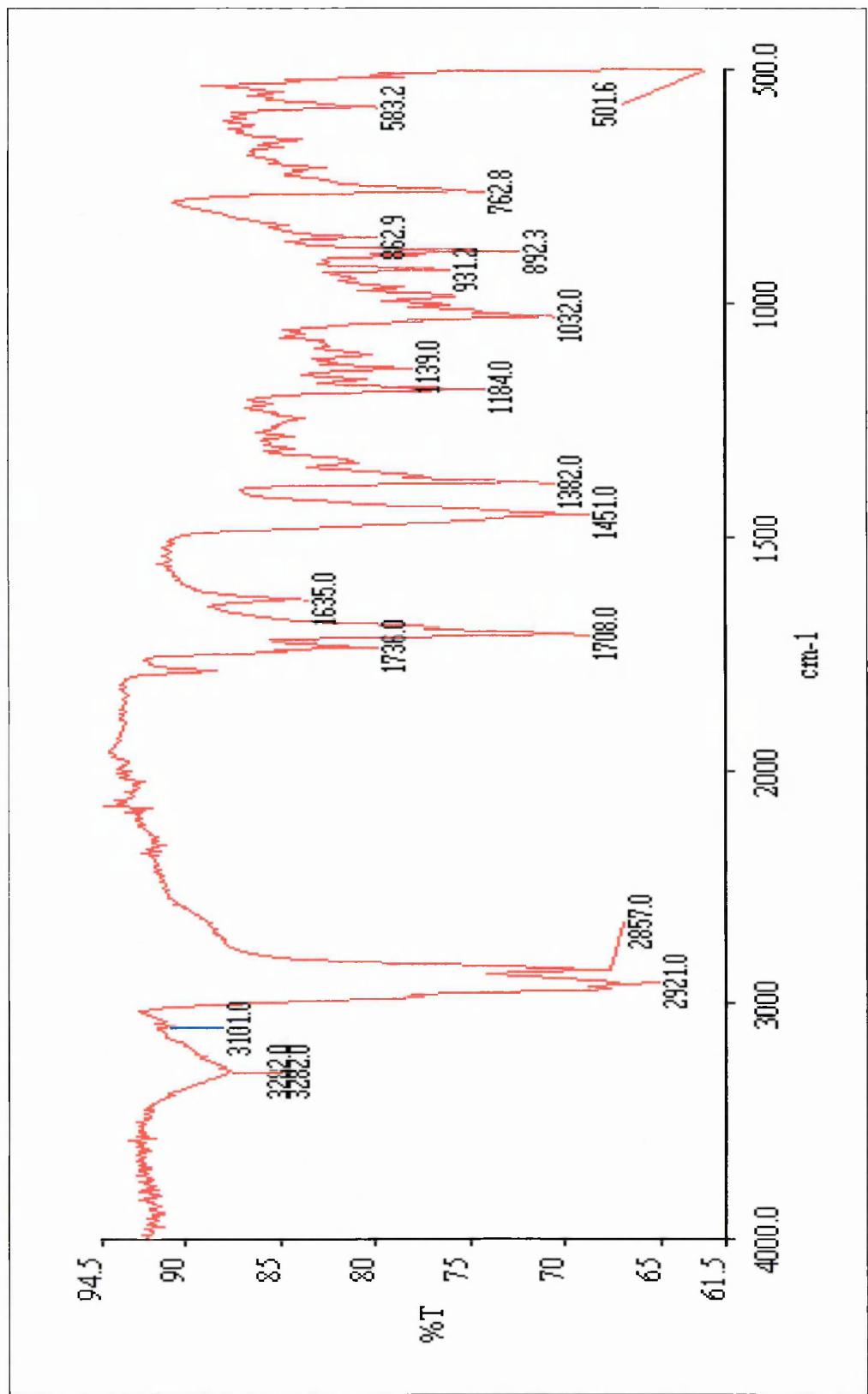




Spectrum 18.7: NOESY spectrum of compound 6.3 ( $\text{CDCl}_3$ )

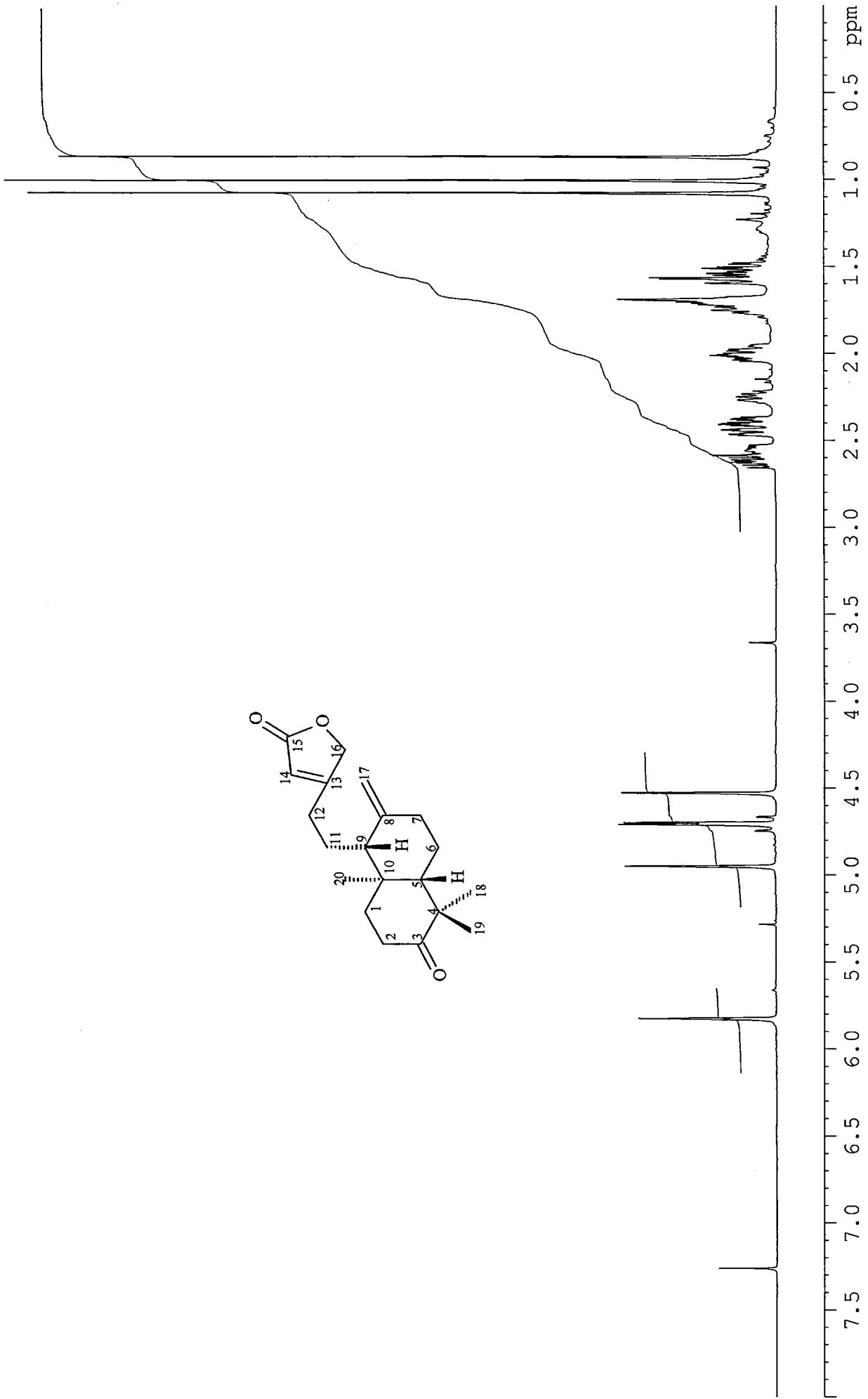
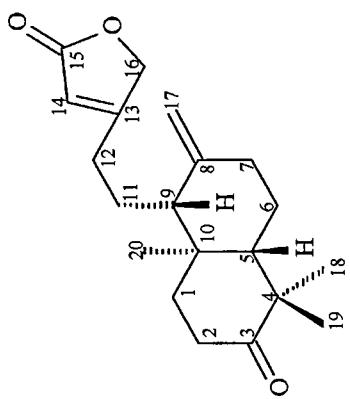
Spectrum 18.8: Mass spectrum of compound 6.3



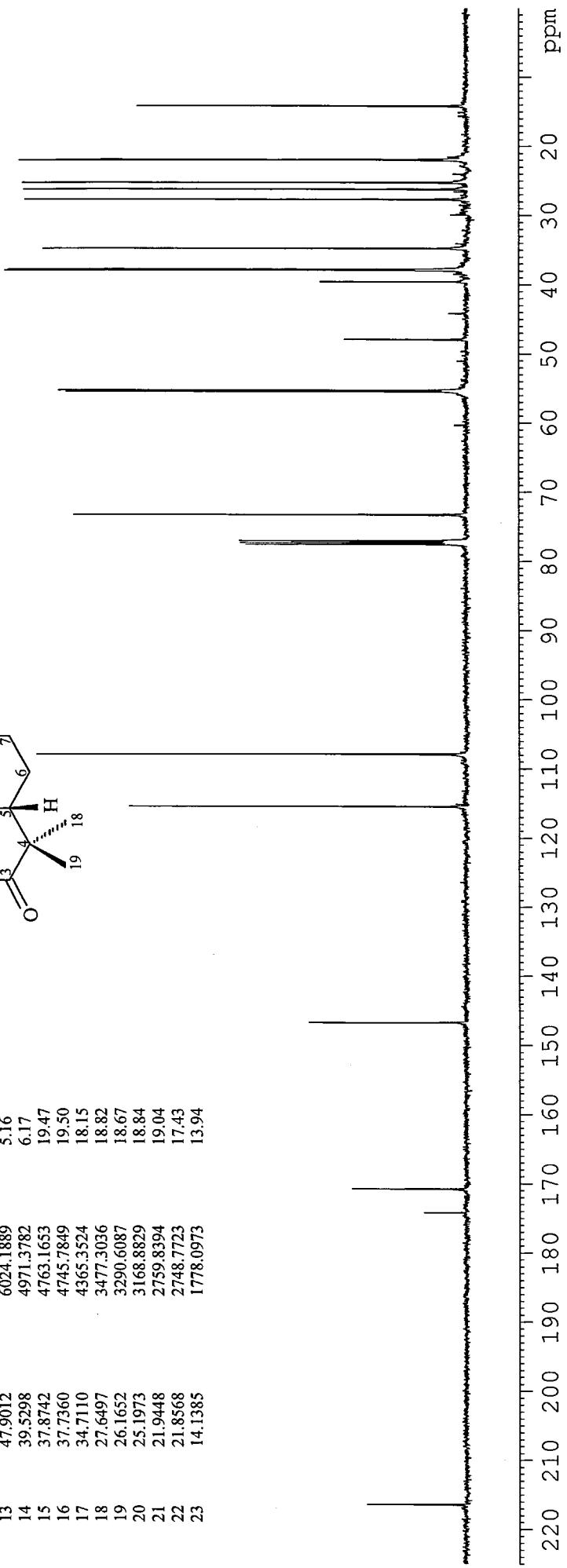
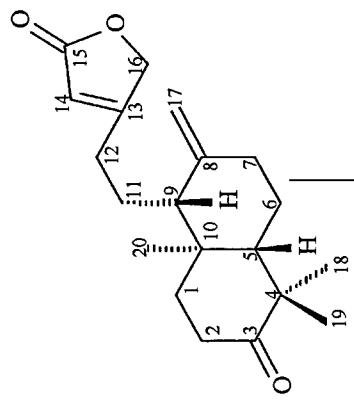


Spectrum 18.9: FTIR spectrum of compound 6.3

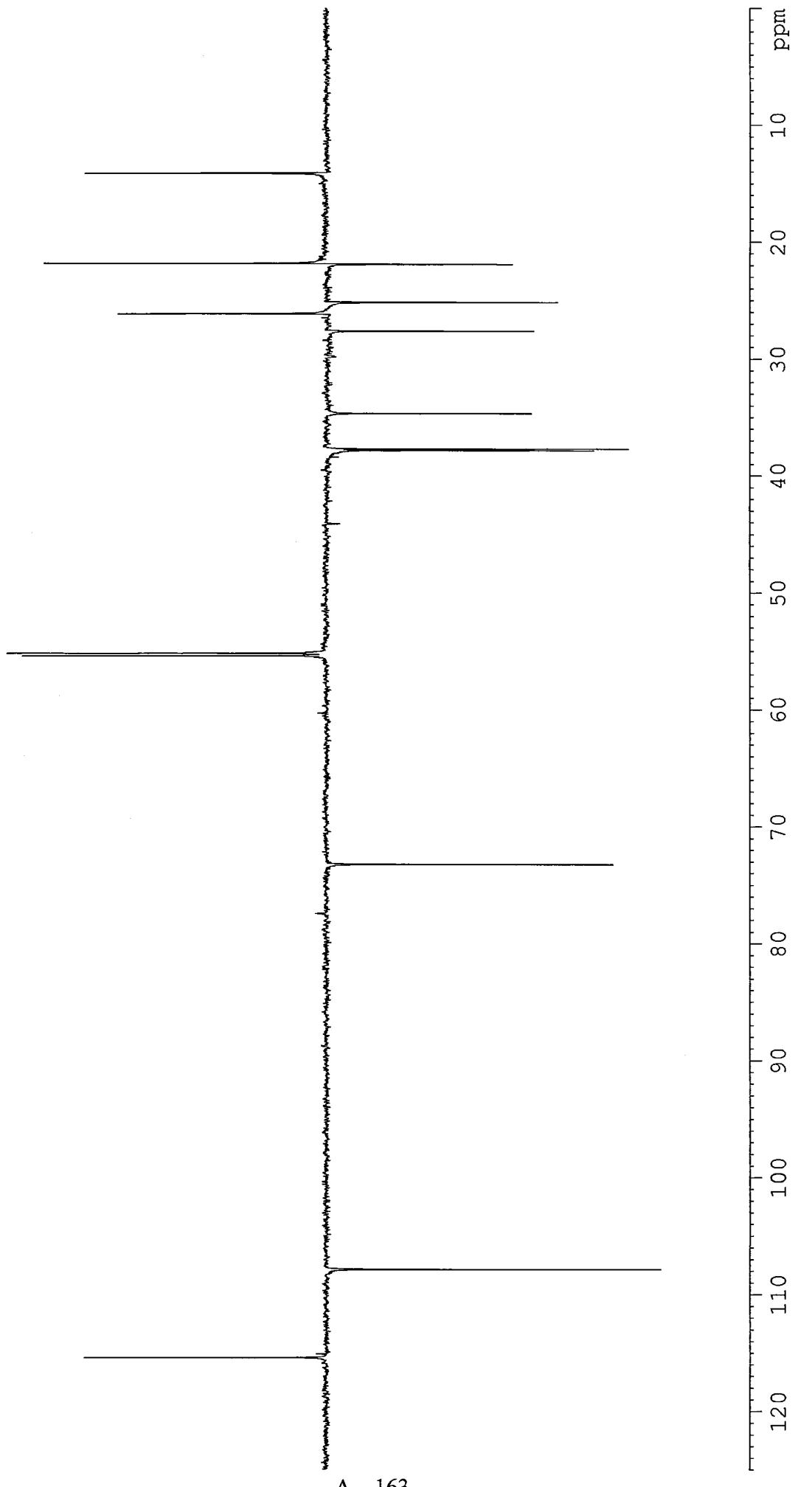
**Spectrum 19.1:**  $^1\text{H}$  NMR spectrum of compound 6.4 ( $\text{CDCl}_3$ )



Peak	$\nu(\text{F1})$ [ppm]	Intensity
1	216.3678	4.22
2	174.1548	1.79
3	170.7394	4.83
4	146.6701	6.64
5	115.4016	14.25
6	107.8706	18.21
7	77.4900	9.32
8	77.2358	9.63
9	76.9815	9.61
10	73.2348	16.69
11	55.4230	17.02
12	55.2180	17.40
13	47.9012	5.16
14	39.5298	6.17
15	37.8742	19.47
16	37.7360	19.50
17	34.7110	4365.3524
18	27.6497	18.15
19	26.1652	18.82
20	25.1973	3290.6087
21	21.9448	18.67
22	21.8568	3168.8829
23	14.1385	18.84
		2759.8394
		2748.7723
		19.04
		17.43
		13.94
		1778.0973



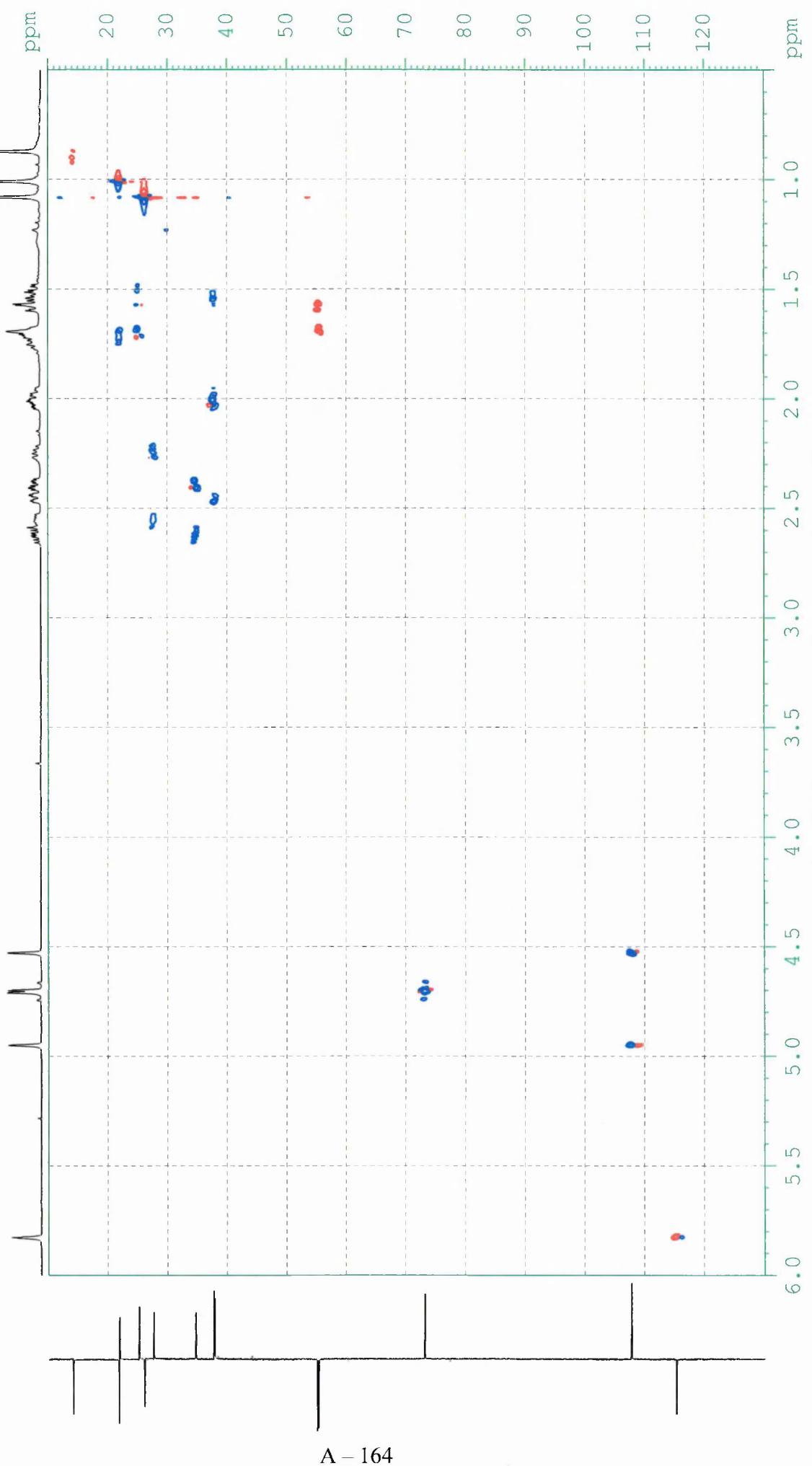
Spectrum 19.2:  $^{13}\text{C}$  NMR spectrum of compound 6.4 ( $\text{CDCl}_3$ )



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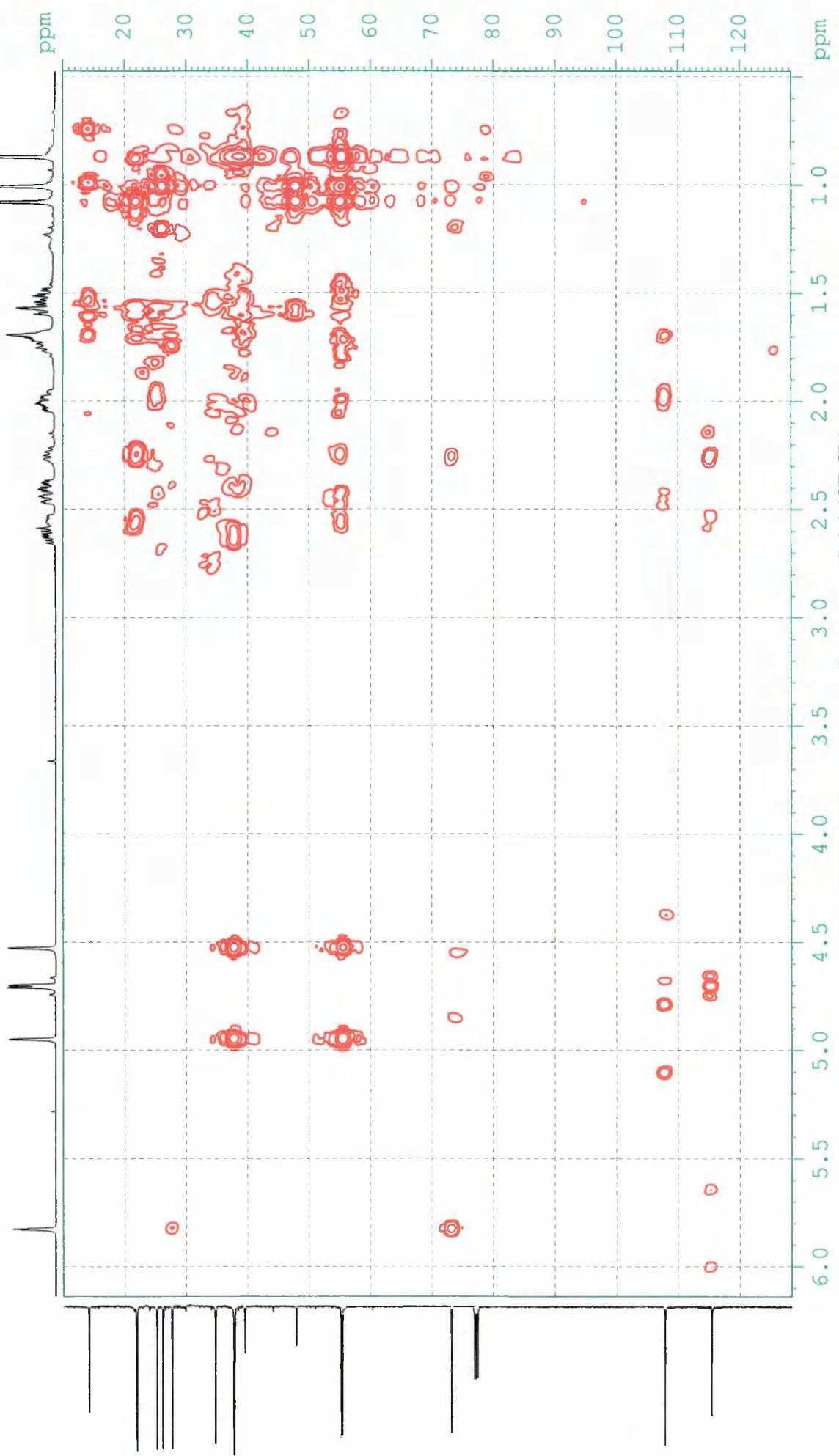
Spectrum 19.3: DEPT spectrum of compound 6.4 ( $\text{CDCl}_3$ )

**Spectrum 19.4: HSQCDEPT spectrum of compound 6.4 ( $\text{CDCl}_3$ )**

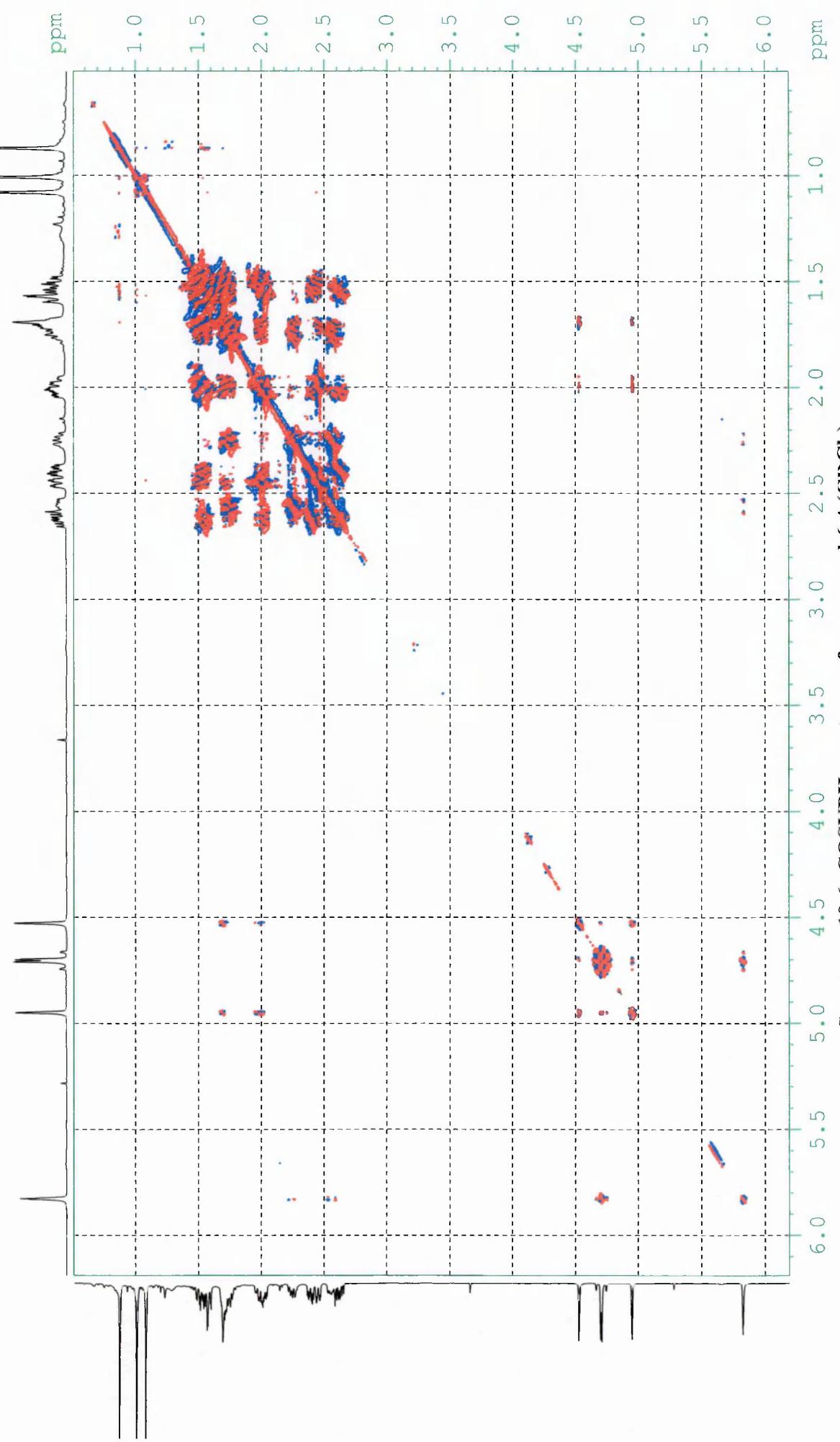


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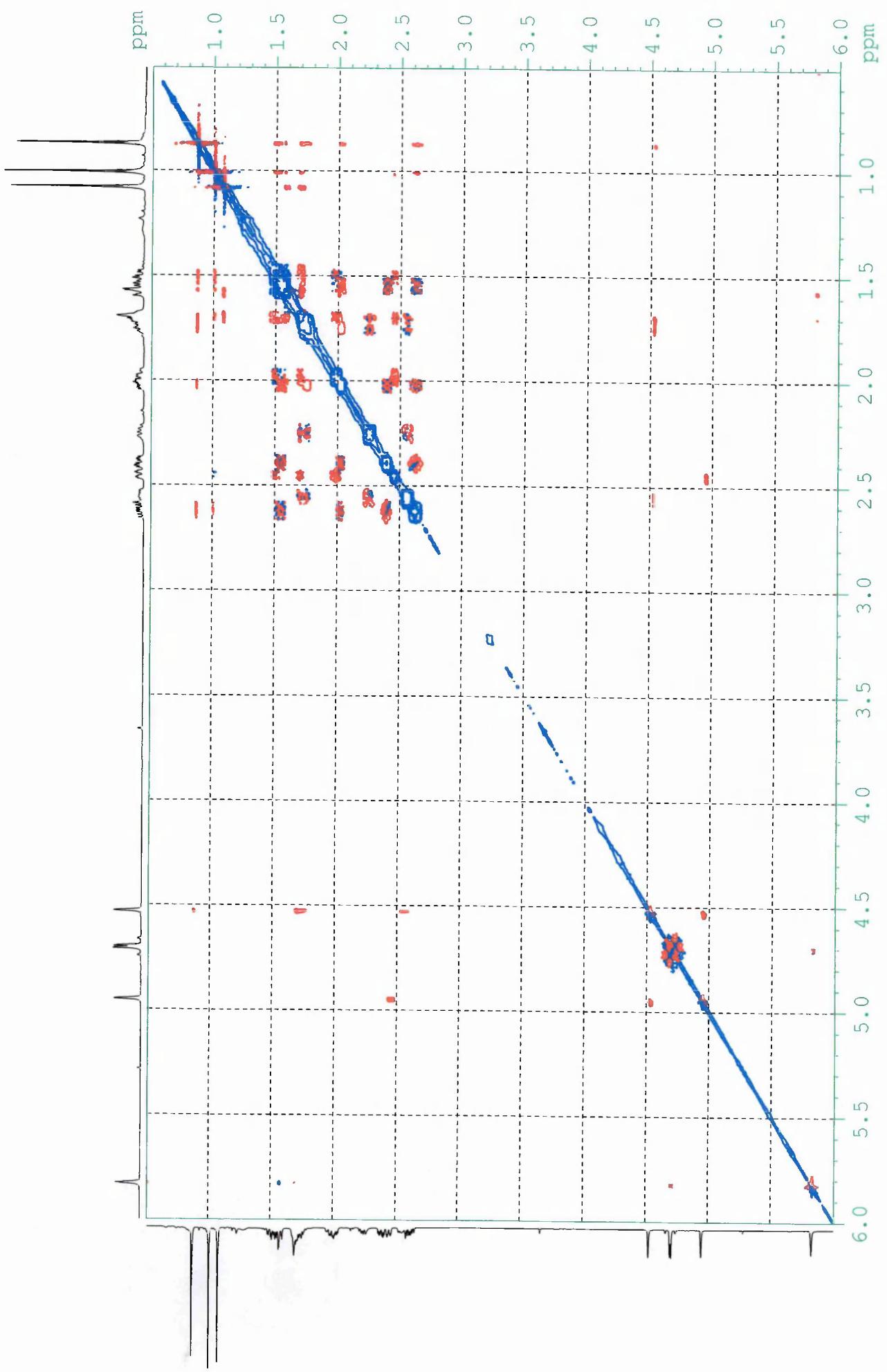
**Spectrum 19.5: HMBCCLP spectrum of compound 6.4 ( $\text{CDCl}_3$ )**



Spectrum 19.6: COSYPH spectrum of compound 6.4 ( $\text{CDCl}_3$ )



Spectrum 19.7: NOESY spectrum of compound 6.4 ( $\text{CDCl}_3$ )



## **Elemental Composition Report**

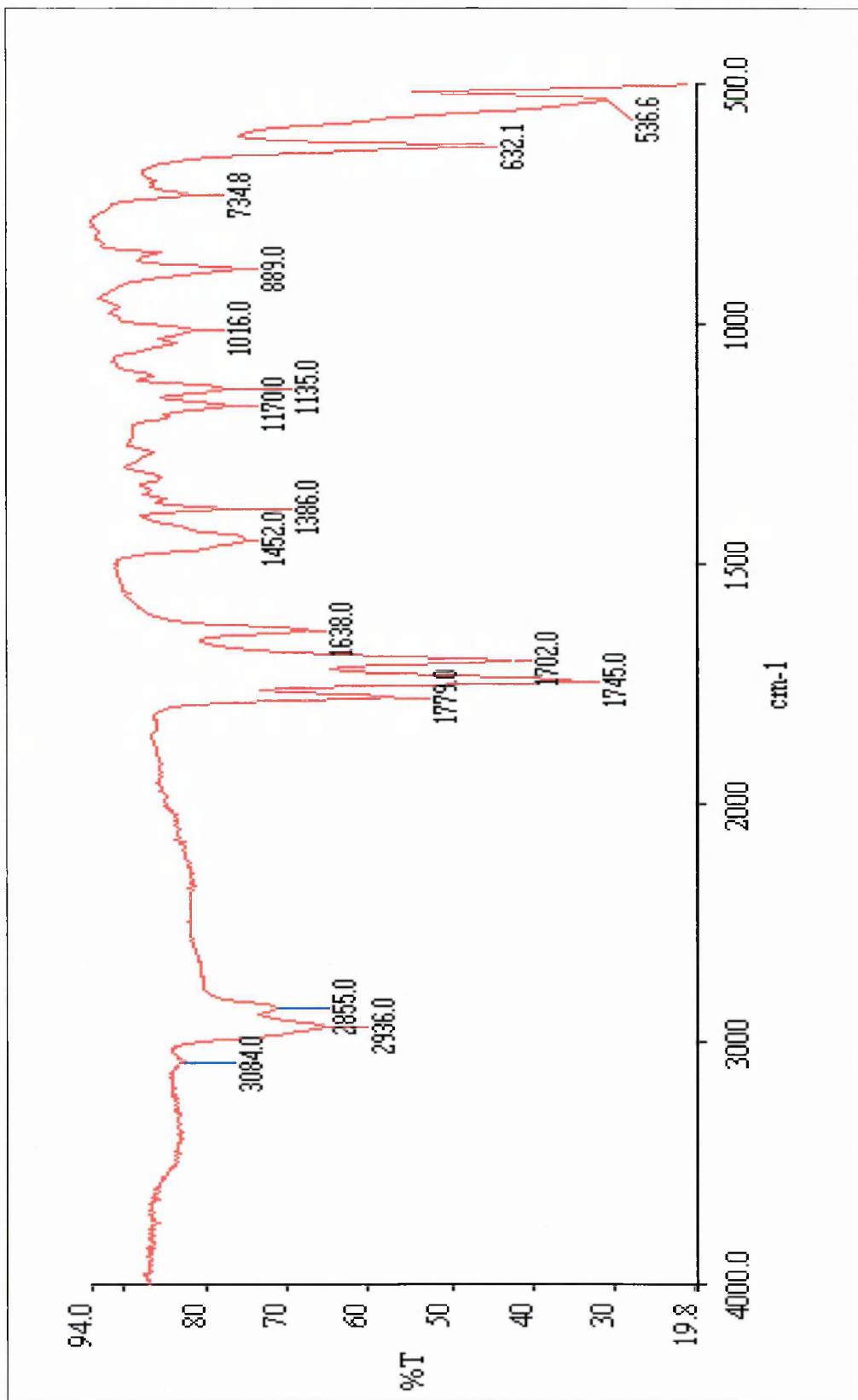
### **Single Mass Analysis**

Tolerance = 10.0 PPM / DBE: min = -0.5, max = 50.0

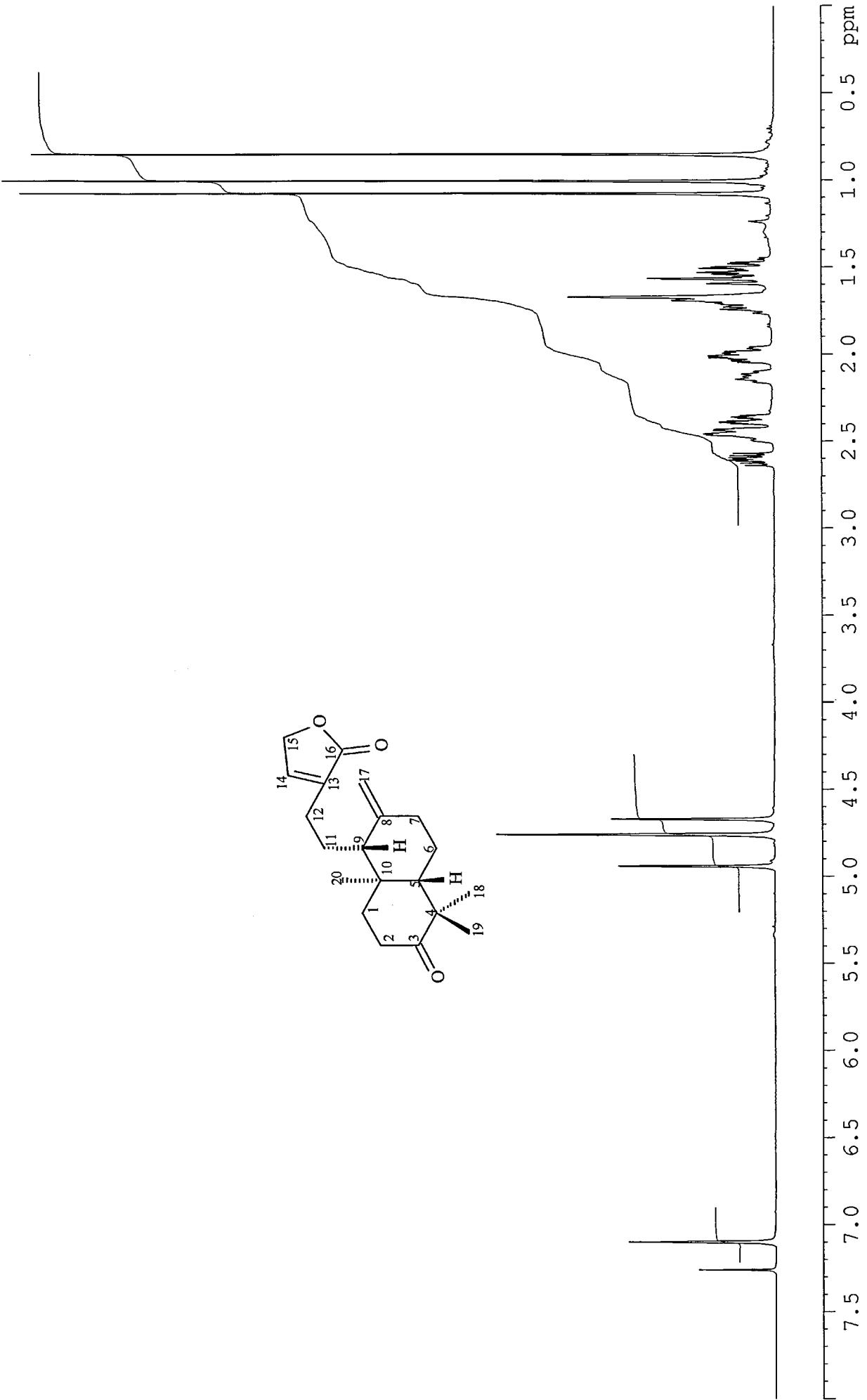
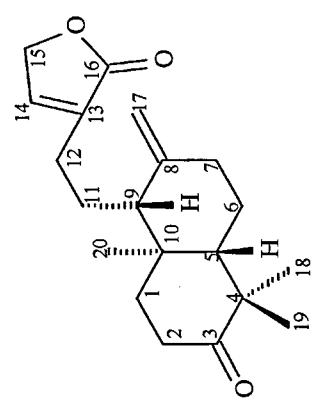
Monoisotopic Mass, Odd and Even Electron Ions  
6 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Mass	Calc. Mass mDa	PPM	DBE	Formula
334.2391	334.2382	0.9	2.6	5.5 C20 H32 N O3

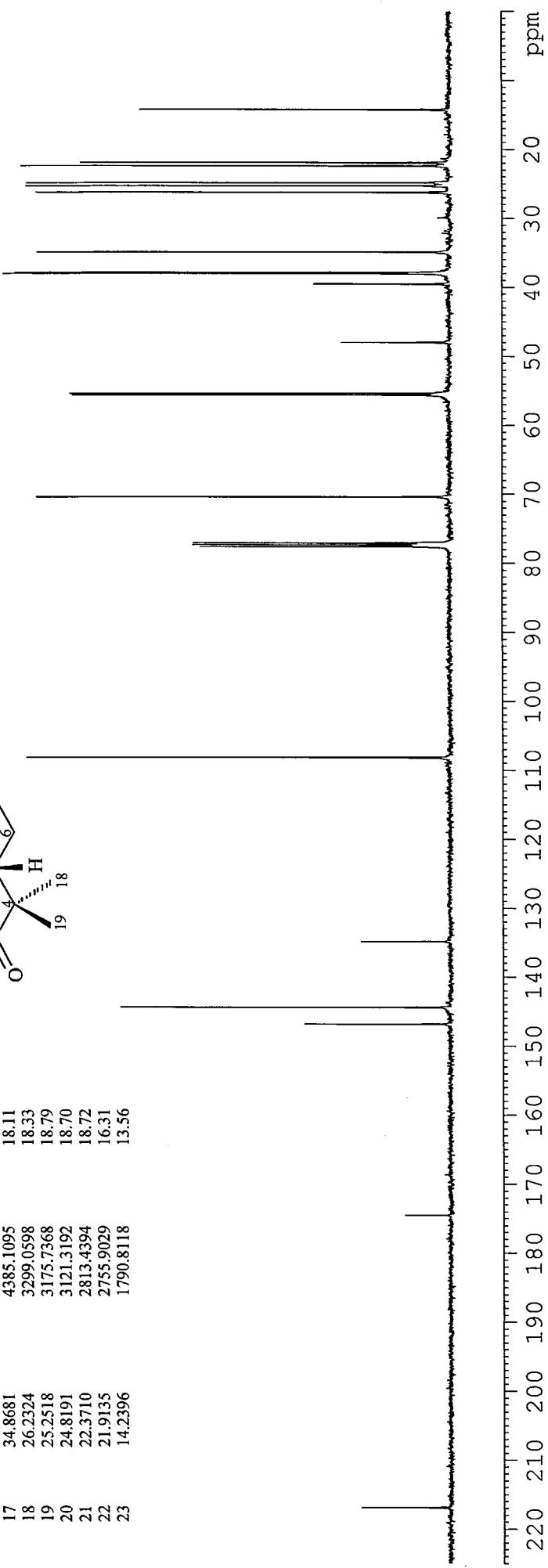
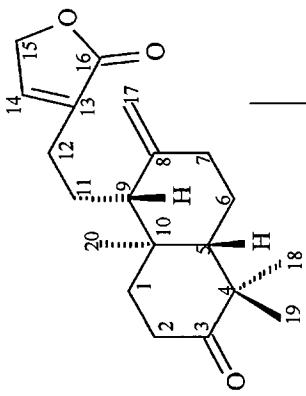
Spectrum 19.9: FTIR spectrum of compound 6.4



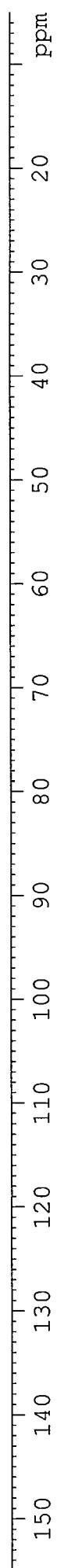
Spectrum 20.1:  $^1\text{H}$  NMR spectrum of compound 6.5 ( $\text{CDCl}_3$ )



Peak	$\nu(F)$ [ppm]	$\nu(F)$ [Hz]	Intensity
1	216.8639	27273.4090	3.90
2	174.4797	21943.0538	2.00
3	146.7720	18458.4561	6.38
4	144.3400	18152.6010	14.39
5	134.7953	16932.2329	3.95
6	108.1244	13598.0262	18.57
7	77.5328	9750.7412	10.99
8	77.2789	9718.8100	11.27
9	77.0247	9686.8411	11.21
10	70.3413	8846.3181	18.13
11	55.6013	6992.5746	16.56
12	55.3481	6960.7314	16.72
13	47.9832	6034.5011	4.73
14	39.4952	4967.0265	5.93
15	37.9851	4777.1121	19.50
16	37.8317	4757.8201	19.26
17	34.8681	4385.1095	18.11
18	26.2324	3299.0598	18.33
19	25.2518	3175.7368	18.79
20	24.8191	3121.3192	18.70
21	22.3710	2813.4394	18.72
22	21.9135	2755.9029	16.31
23	14.2396	1790.8118	13.56

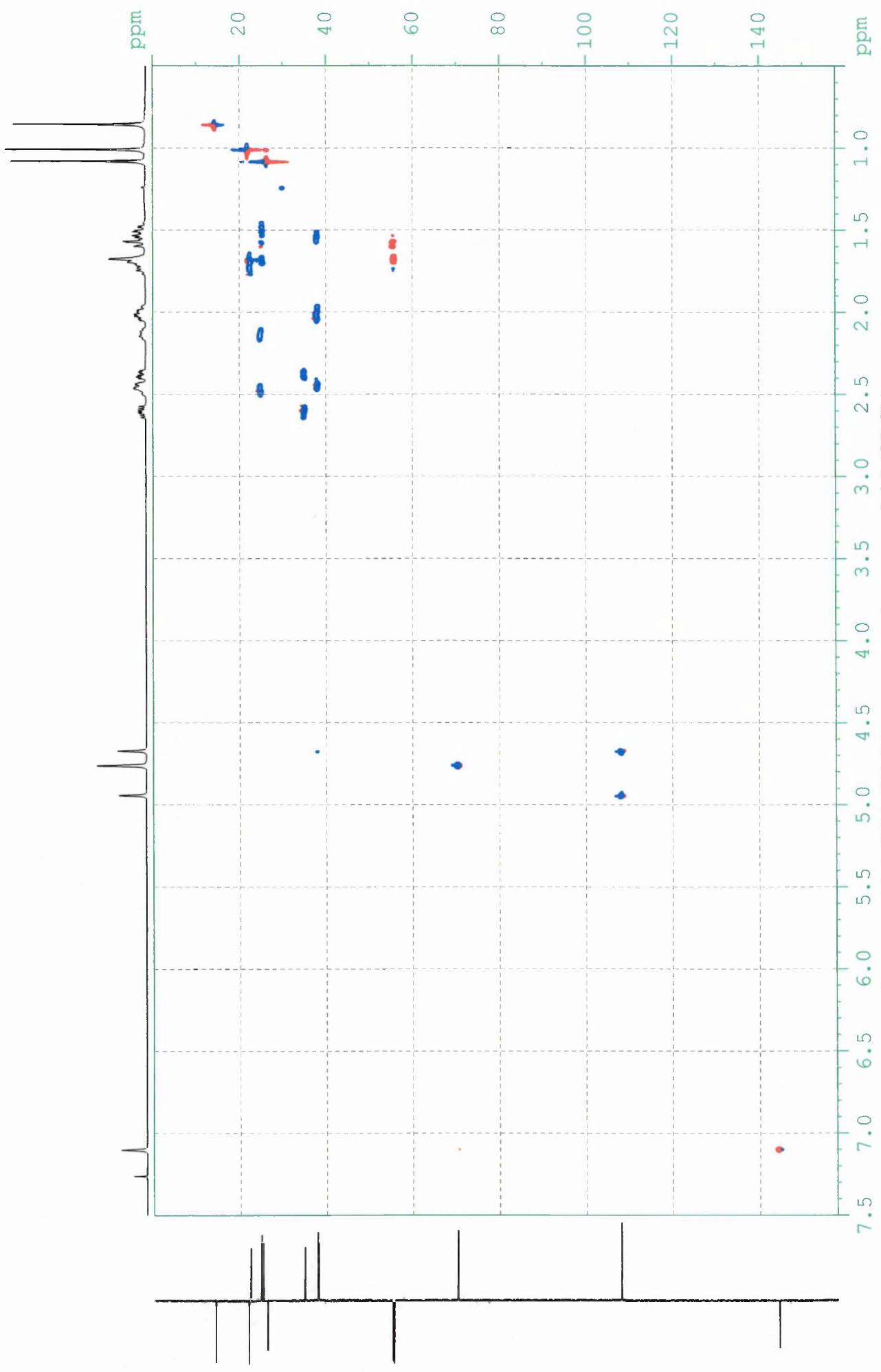


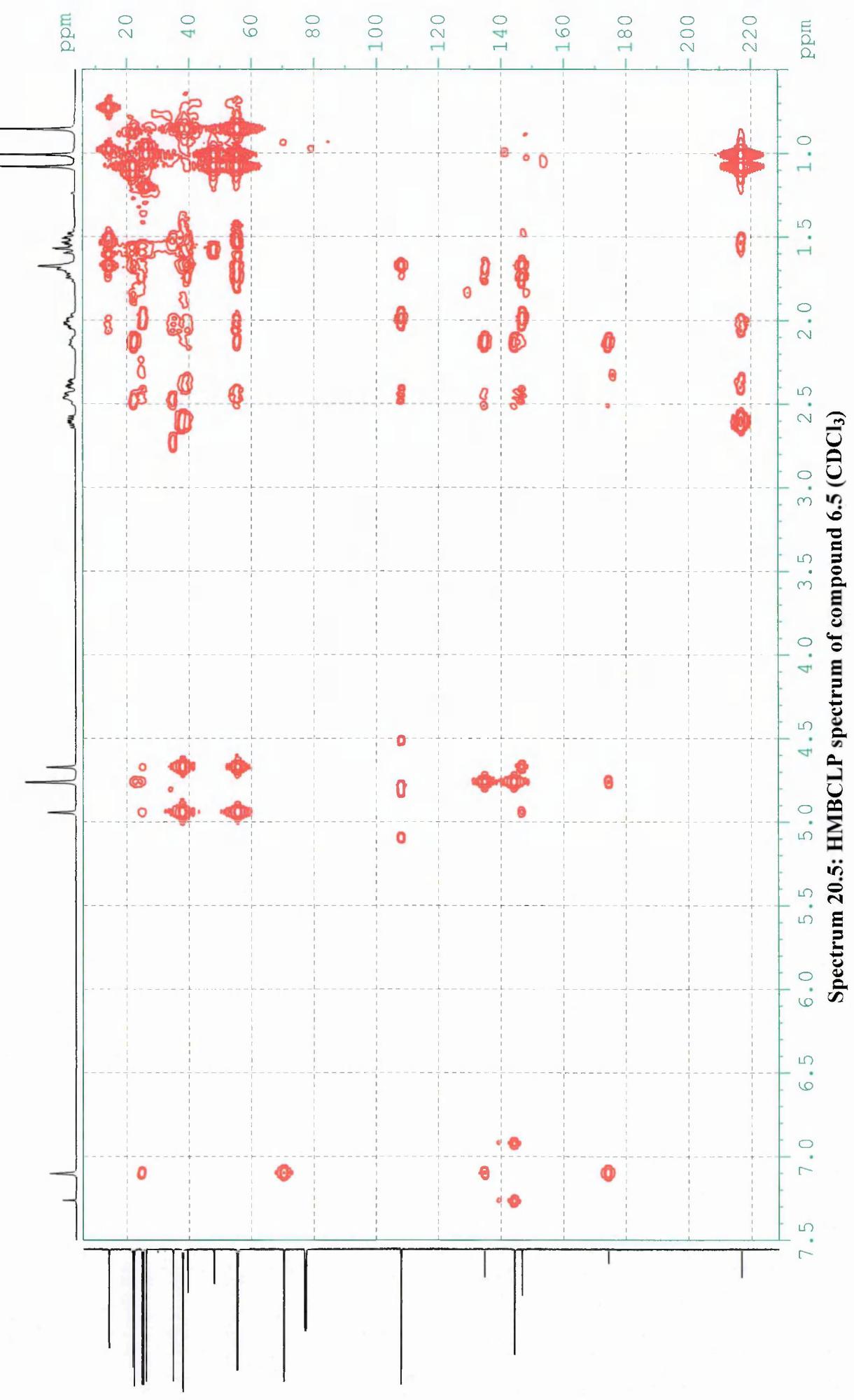
Spectrum 20.2:  $^{13}\text{C}$  NMR spectrum of compound 6.5 ( $\text{CDCl}_3$ )



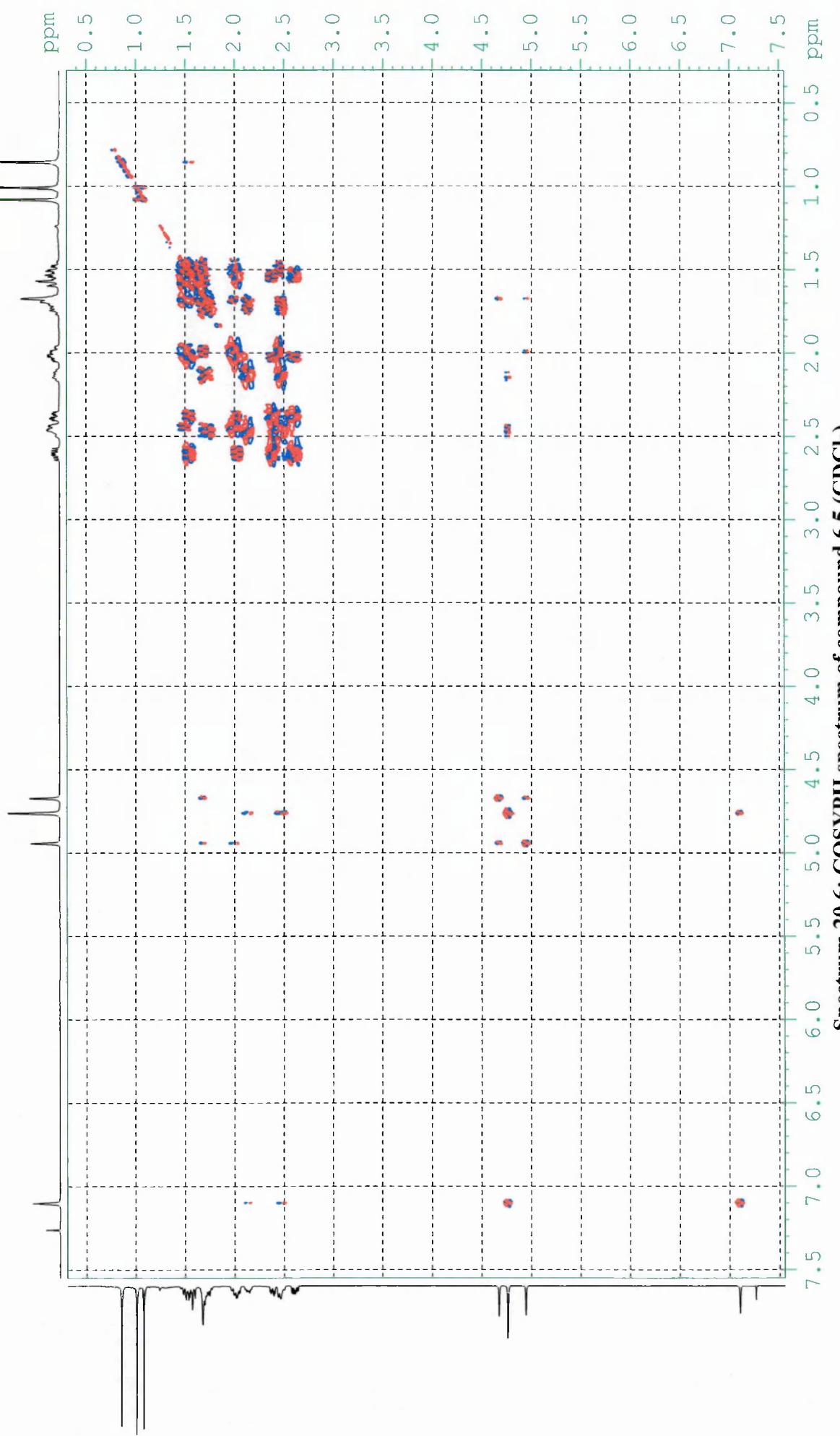
Spectrum 20.3: DEPT spectrum of compound 6.5 ( $\text{CDCl}_3$ )

**Spectrum 204: HSQCDEPT spectrum of compound 6.5 ( $\text{CDCl}_3$ )**



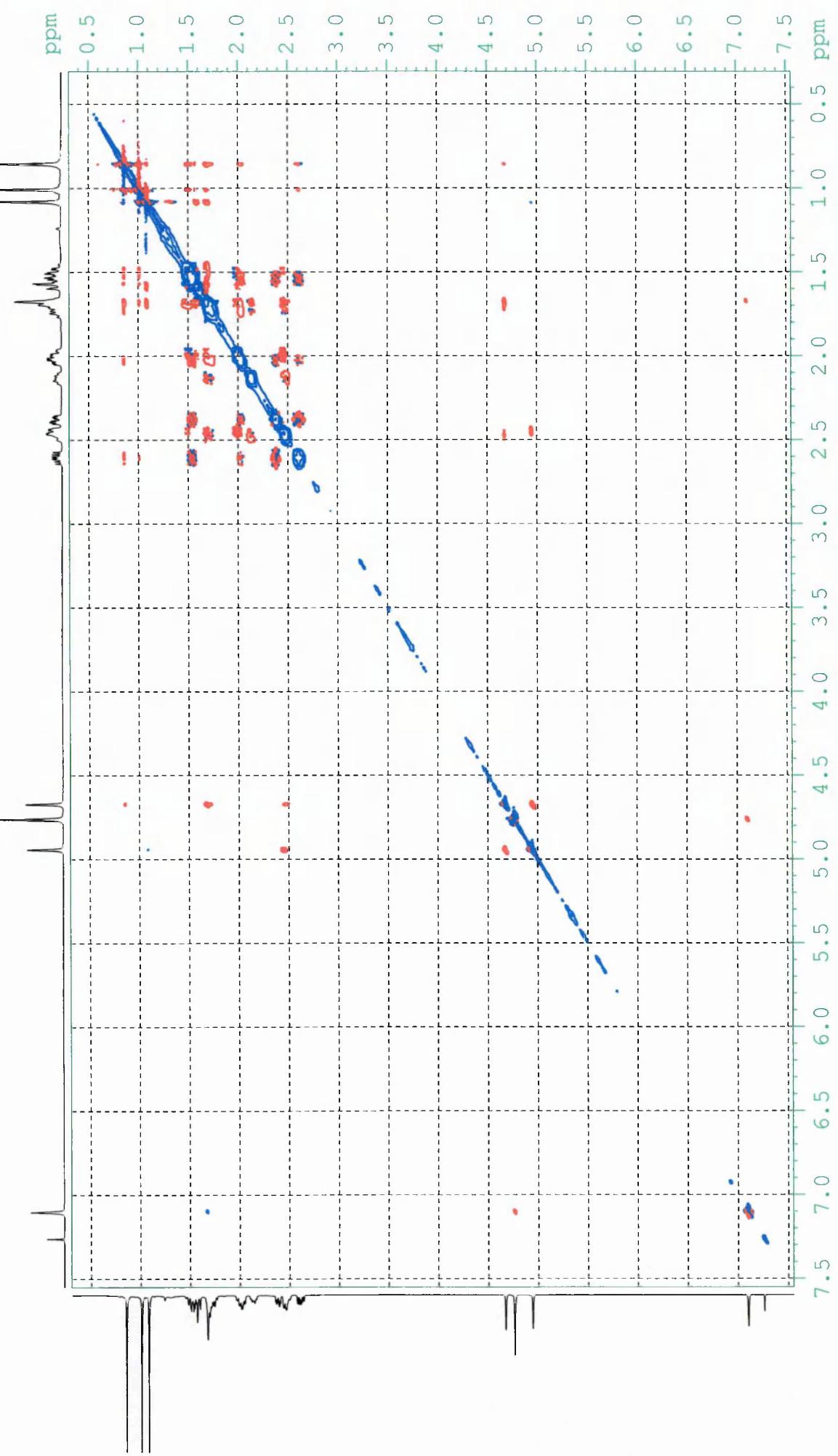


Spectrum 20.5: HMQC spectrum of compound 6.5 ( $\text{CDCl}_3$ )



Spectrum 20.6: COSYPH spectrum of compound 6.5 ( $\text{CDCl}_3$ )

**Spectrum 20.7: NOESY spectrum of compound 6.5 ( $\text{CDCl}_3$ )**



## Elemental Composition Report

### Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -0.5, max = 50.0

Monoisotopic Mass, Odd and Even Electron Ions  
6 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Minimum:

200.0

10.0

-0.5

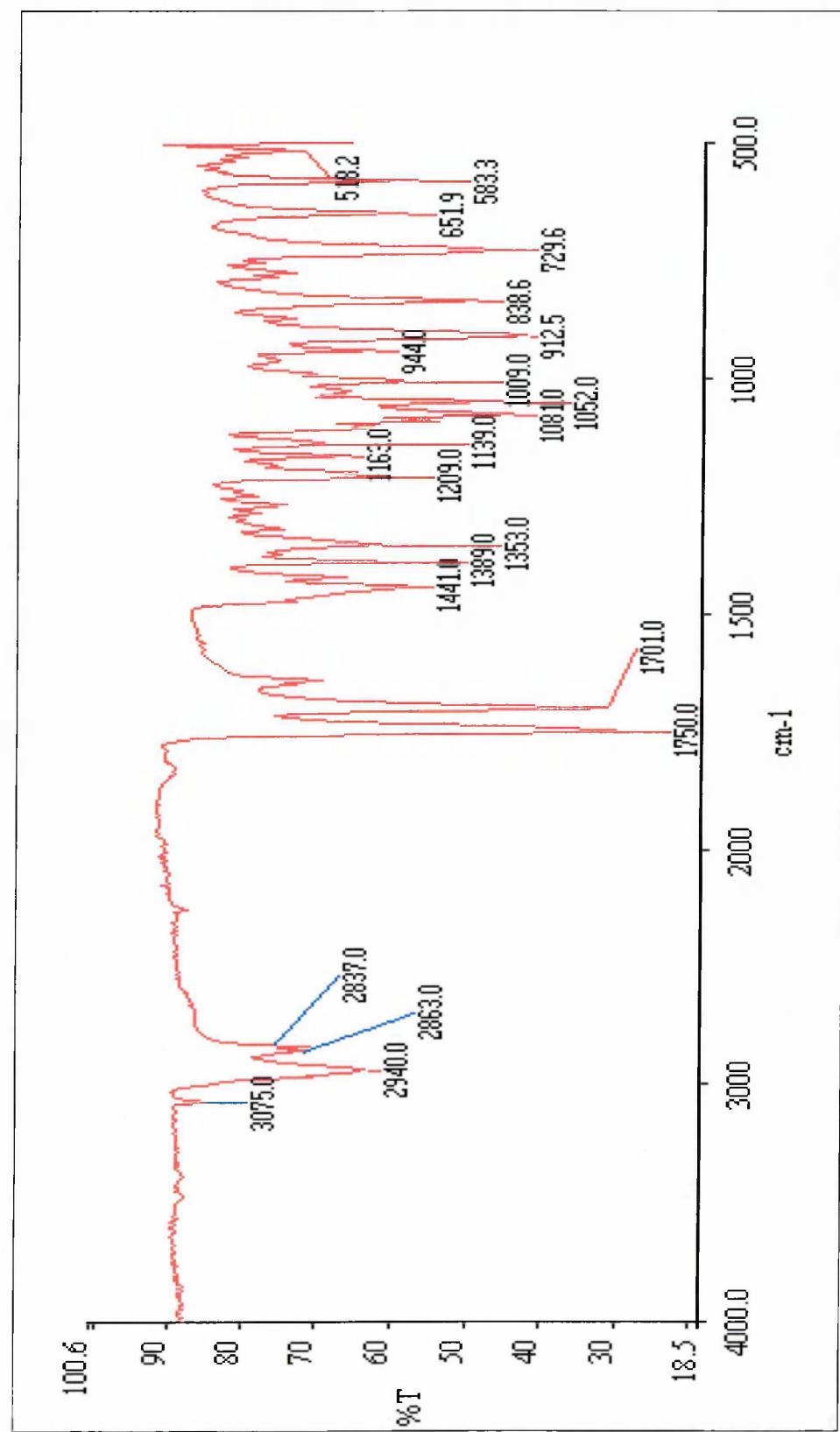
Maximum:

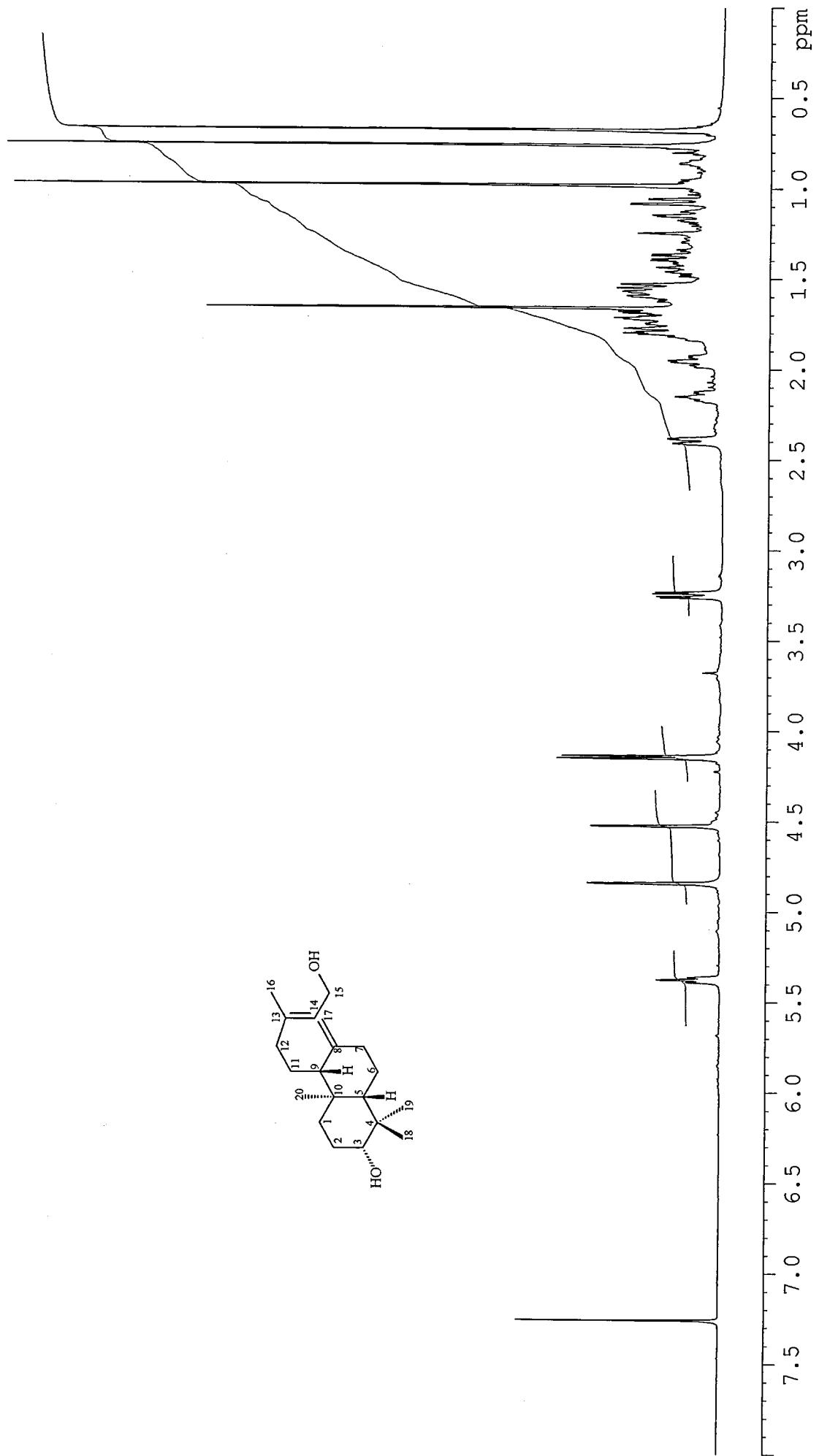
50.0

50.0

-0.5

Mass	Calc.	Mass	mDa	PPM	DBE	Formula
334.2382	334.2382	-0.1	-0.2	5.5	C20 H32 N O3	

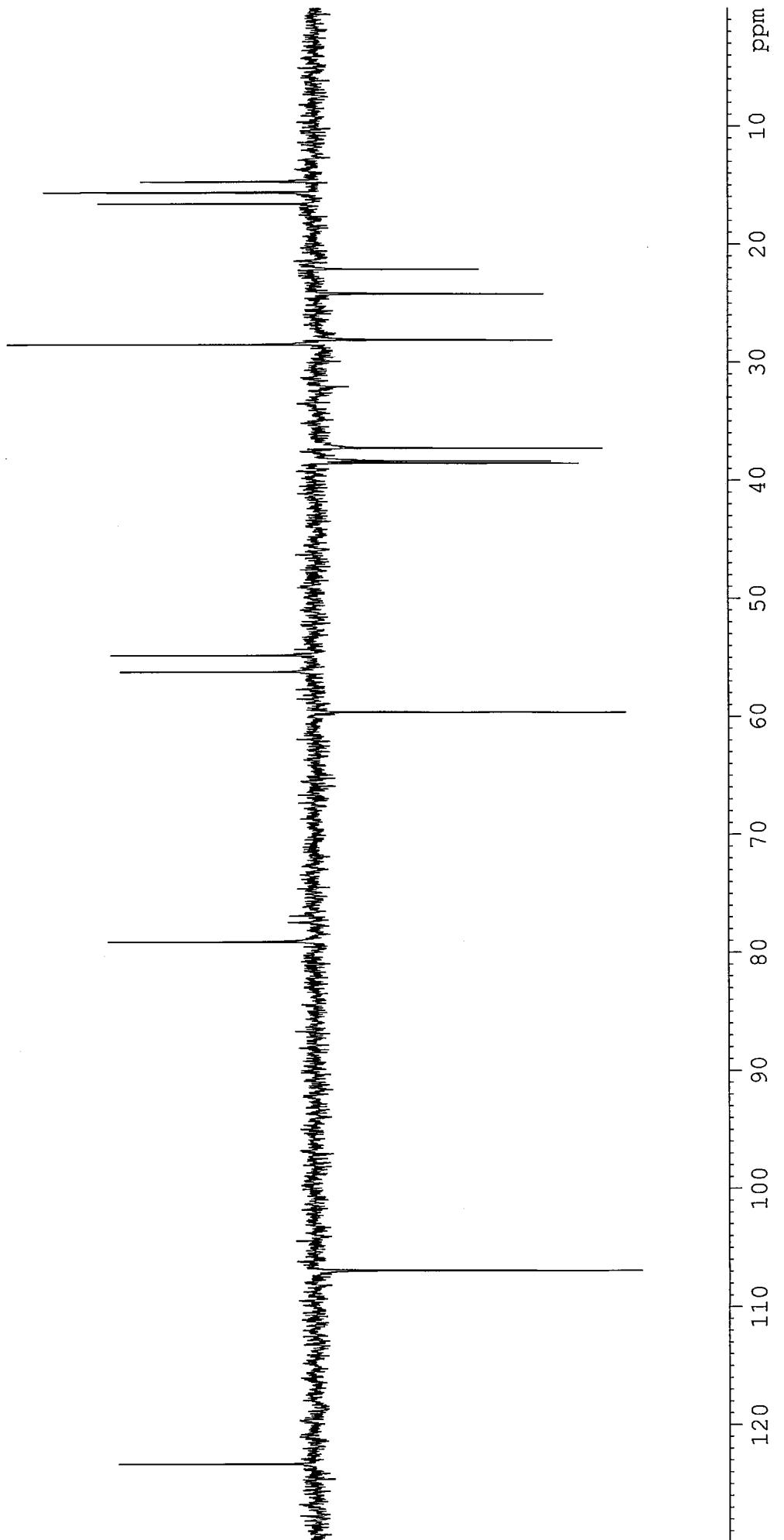




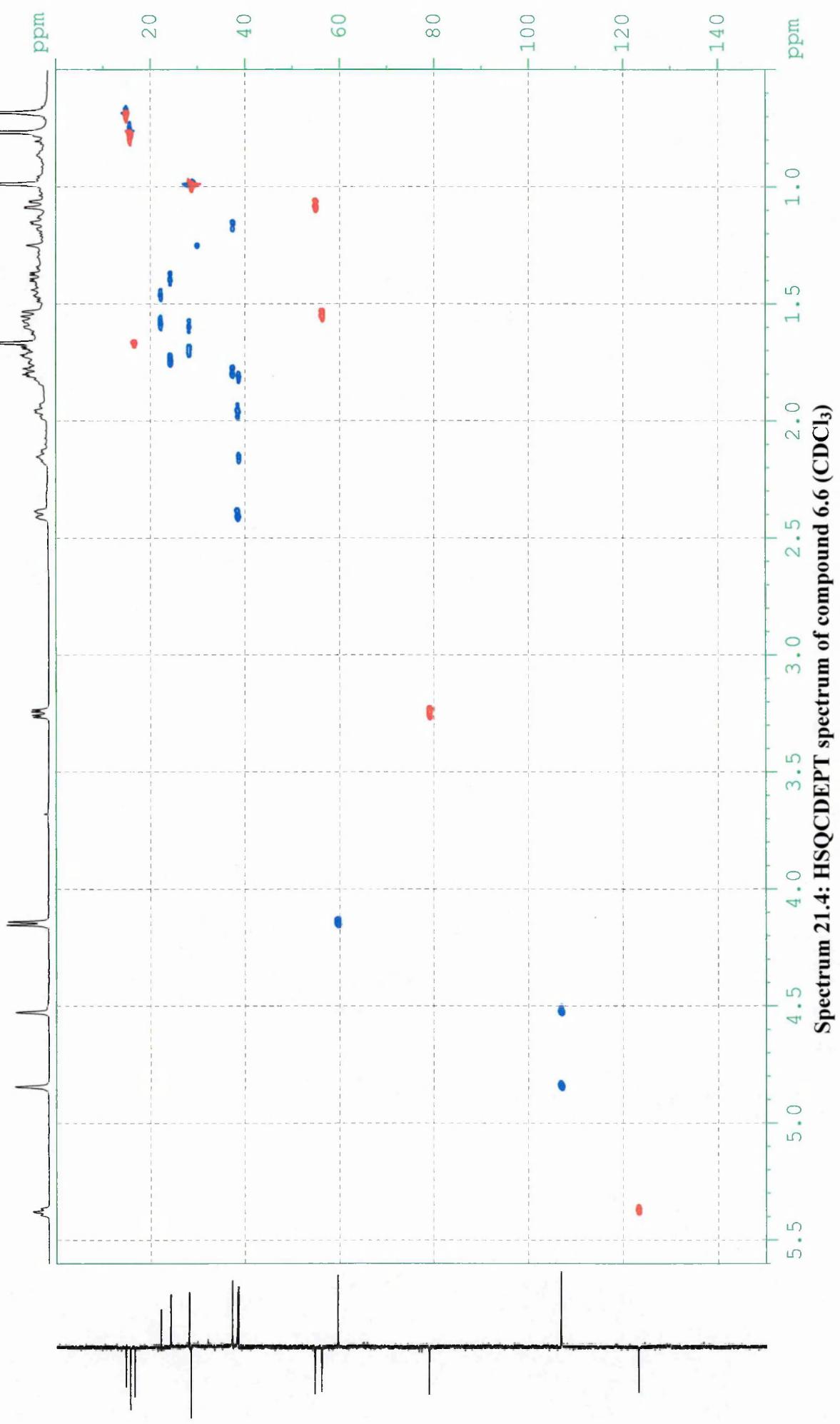
Spectrum 21.1:  $^1\text{H}$  NMR spectrum of compound 6.6 ( $\text{CDCl}_3$ )

Peak	$\nu(F1)$ [ppm]	$\nu(F1)$ [Hz]	Intensity
1	148.0943	18624.7527	0.19
2	140.6557	17689.2536	1.11
3	123.2907	15505.3827	3.27
4	106.9173	13446.2182	4.61
5	79.0790	9945.1958	4.20
6	77.4871	9744.9940	13.05
7	77.2330	9713.0377	13.50
8	76.9789	9681.0814	13.37
9	59.6283	7499.0215	3.64
10	56.2263	7071.1765	4.03
11	54.8146	6893.6371	4.60
12	39.5654	4975.8352	1.40
13	39.3348	4946.8543	1.72
14	38.5530	4848.5329	4.60
15	38.3768	4826.3735	4.82
16	37.2865	4689.2343	5.11
17	28.5015	3584.4282	5.89
18	28.1146	3535.7706	4.85
19	24.2001	3043.4721	5.02
20	22.1350	2783.7594	4.44
21	16.5734	2084.3171	1.68
22	15.6149	1963.7734	4.57
23	14.7224	1851.5501	3.12

Spectrum 21.2:  $^{13}\text{C}$  NMR spectrum of compound 6.6 ( $\text{CDCl}_3$ )



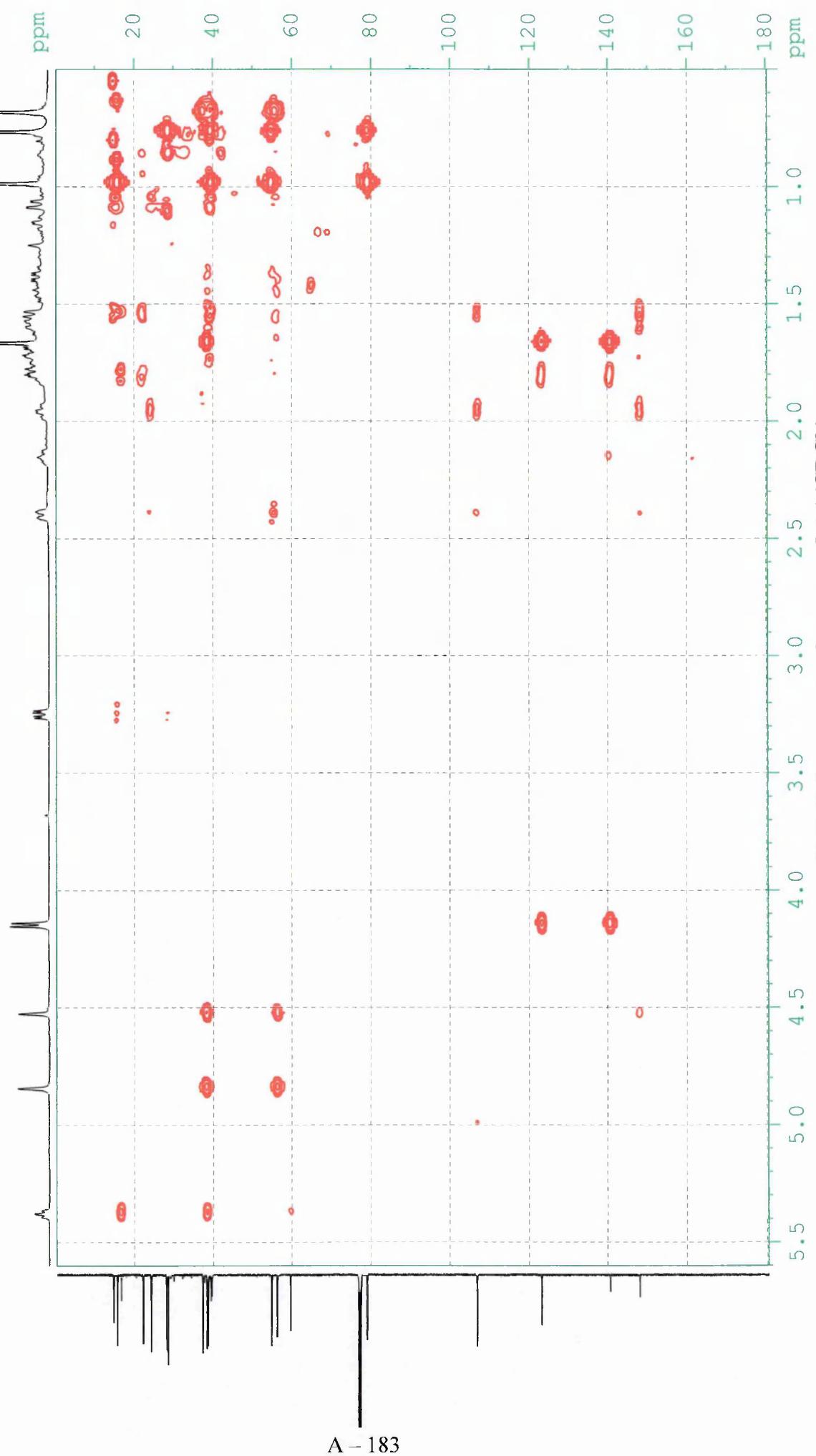
Spectrum 21.3: DEPT spectrum of compound 6.6 ( $\text{CDCl}_3$ )



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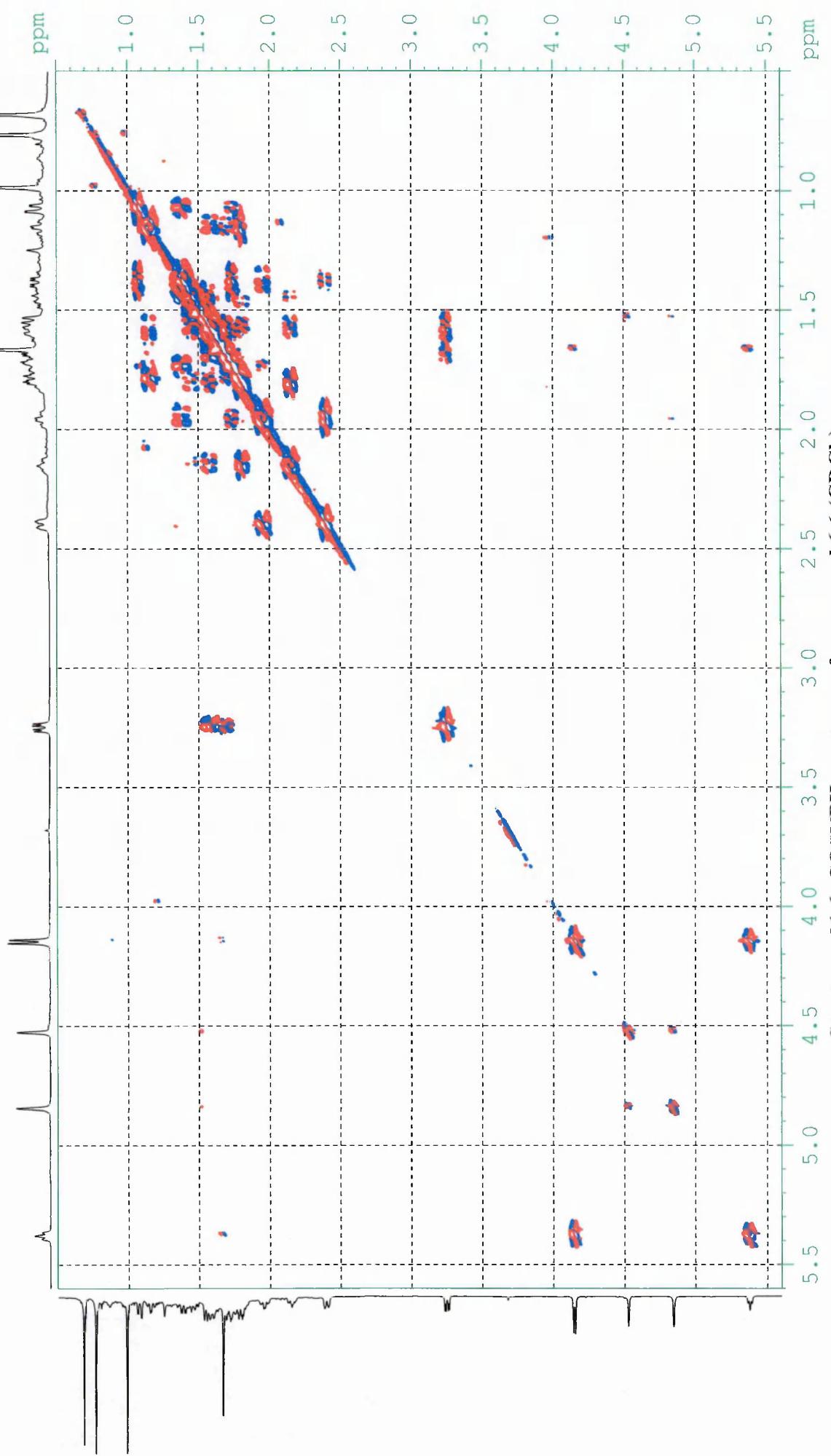
Spectrum 21.4: HSQCDEPT spectrum of compound 6.6 ( $\text{CDCl}_3$ )

**Spectrum 21.5: HMBCLP spectrum of compound 6.6 ( $\text{CDCl}_3$ )**

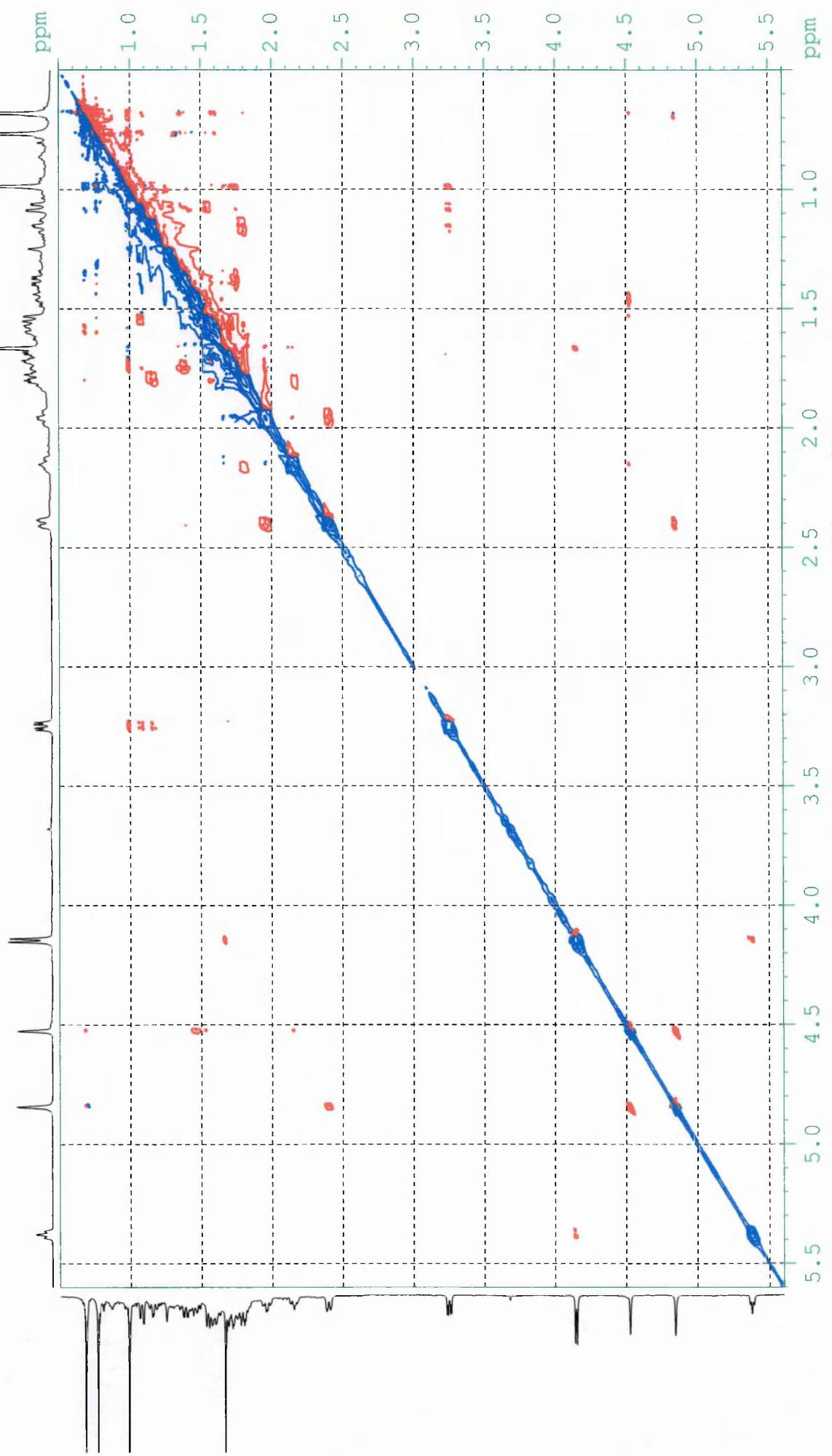


A - 183

**Spectrum 21.6: COSYPH spectrum of compound 6.6 ( $\text{CDCl}_3$ )**

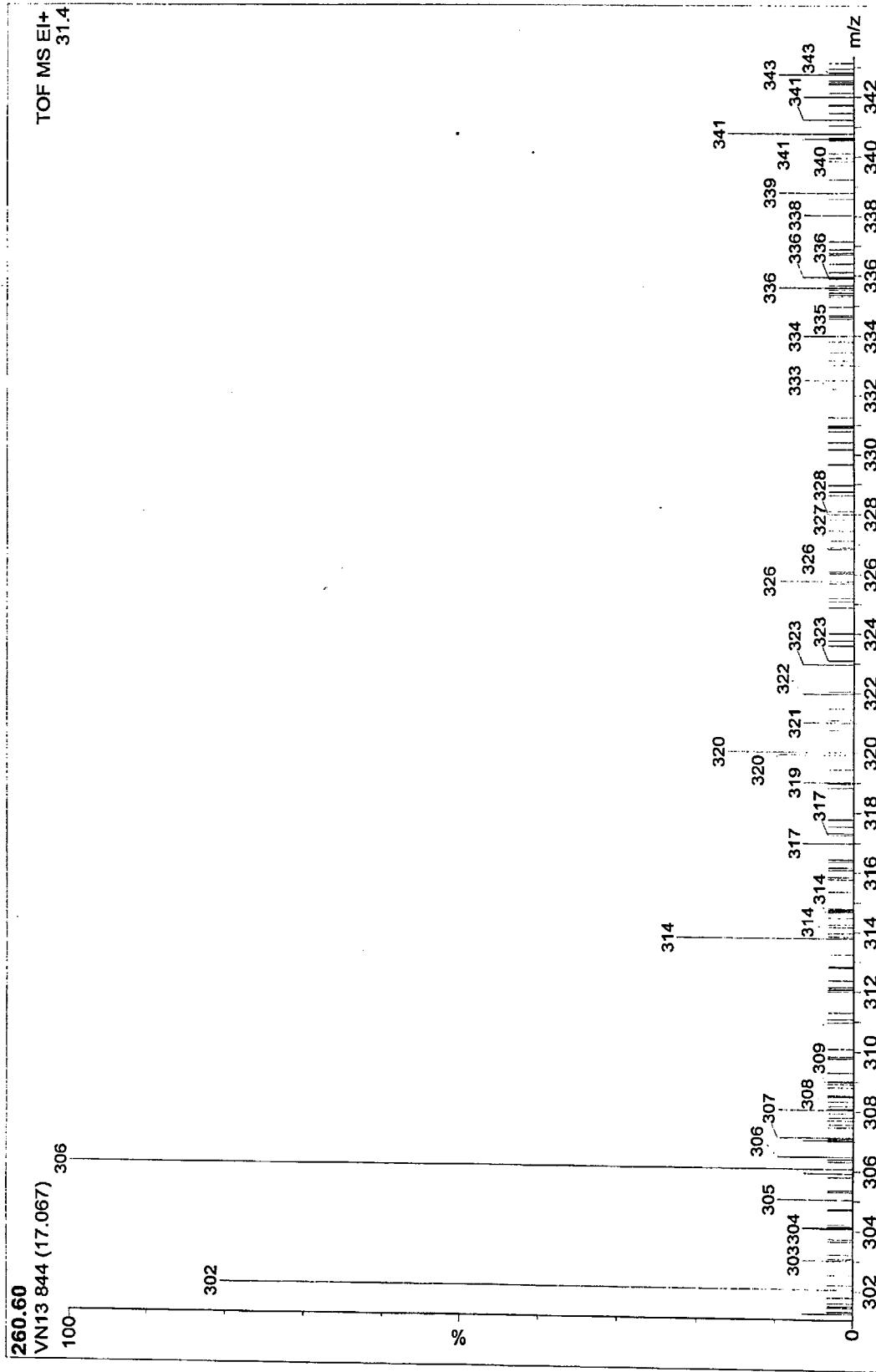


**Spectrum 21.7: NOESY spectrum of compound 6.6 ( $\text{CDCl}_3$ )**



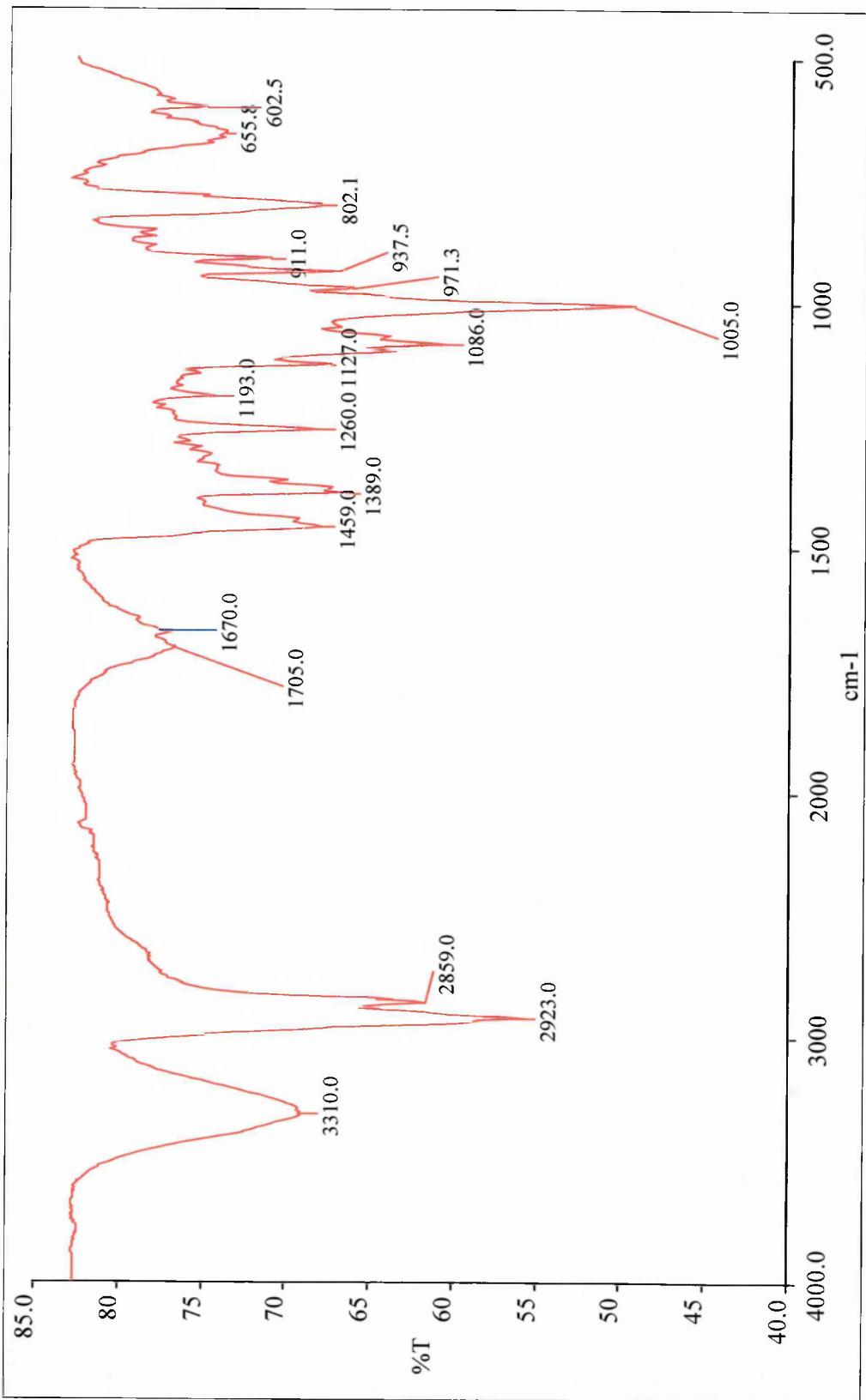
**260.60**  
VN13 844 (17.067) 306  
100-

TOF MS EI+  
31.4

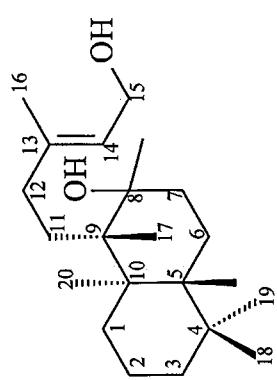
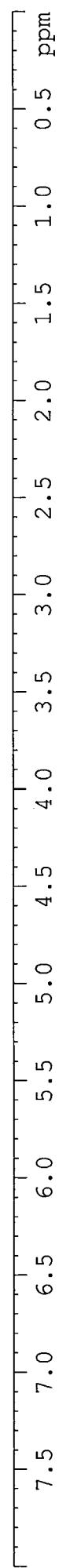


Spectrum 21.8: Mass spectrum of compound 6.6

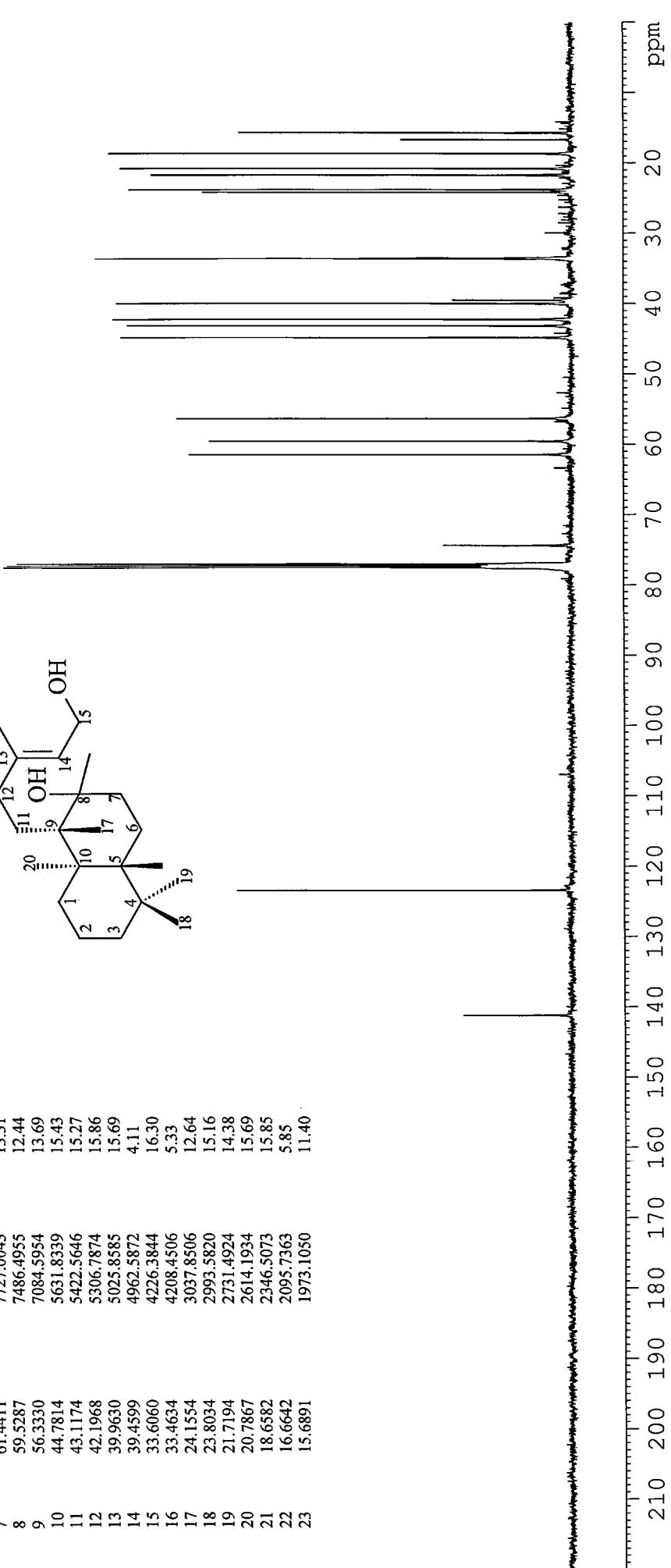
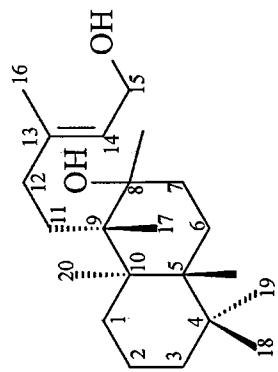
Spectrum 21.9: FTIR spectrum of compound 6.6



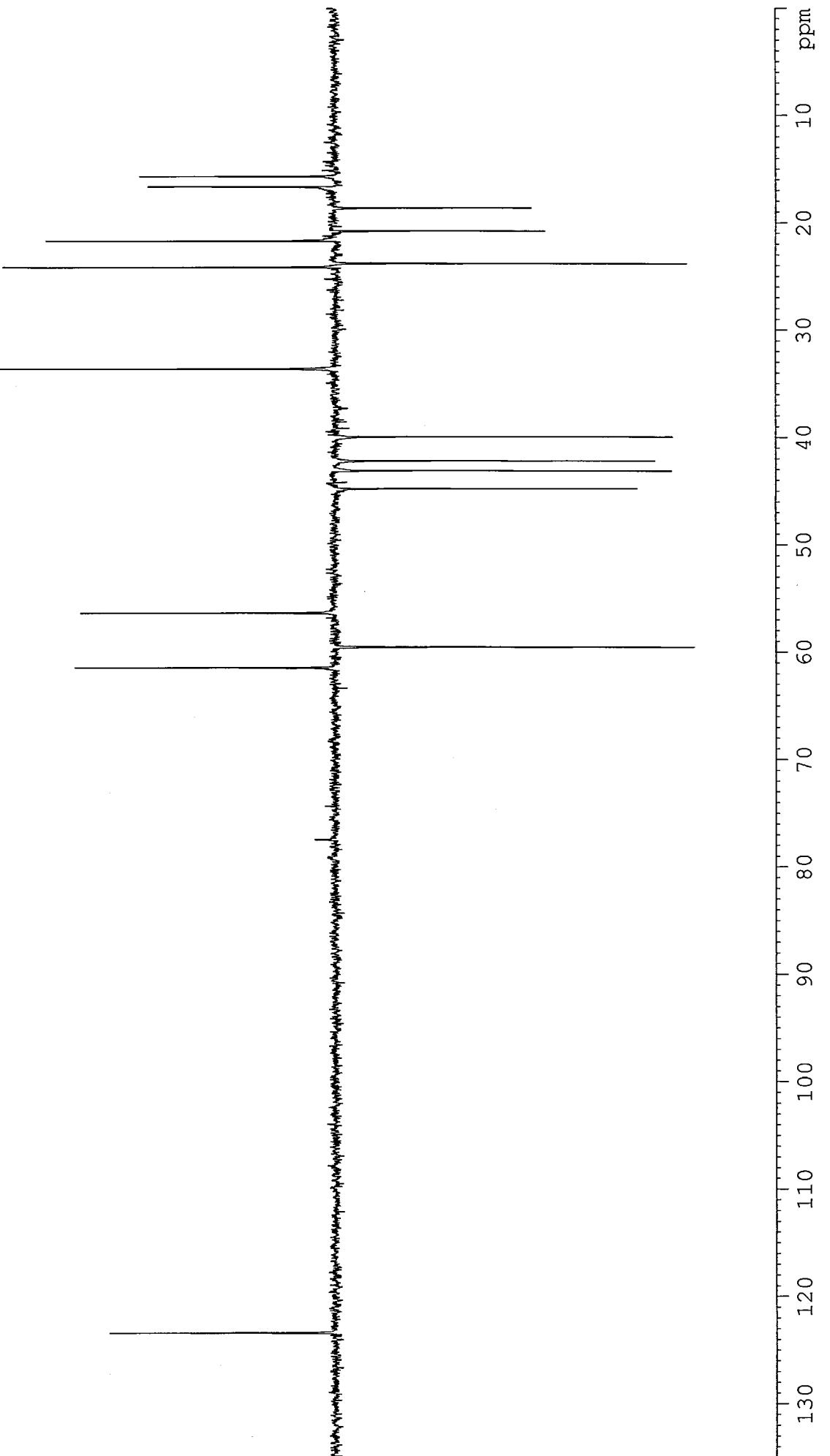
Spectrum 22.1:  $^1\text{H}$  NMR spectrum of compound 6.7 ( $\text{CDCl}_3$ )



Peak	$\delta$ (F1) [ppm]	$\nu$ (F1) [Hz]
1	141.1912	17756.5995
2	123.3880	15517.6194
3	77.4845	9744.6671
4	77.2305	9712.7233
5	76.9766	9680.7922
6	74.3443	9349.7468
7	61.4411	7727.0043
8	59.5287	7486.4955
9	56.3330	7084.5954
10	44.7814	5631.8339
11	43.1174	5422.5646
12	42.1968	5306.7874
13	39.9630	5025.8585
14	39.4599	4962.3872
15	33.6060	4226.3844
16	33.4634	4208.4506
17	24.1554	3037.8506
18	23.8034	2993.5820
19	21.7194	2731.4924
20	20.7867	2614.1934
21	18.6582	2346.5073
22	16.6642	2095.7363
23	15.6891	1973.1050

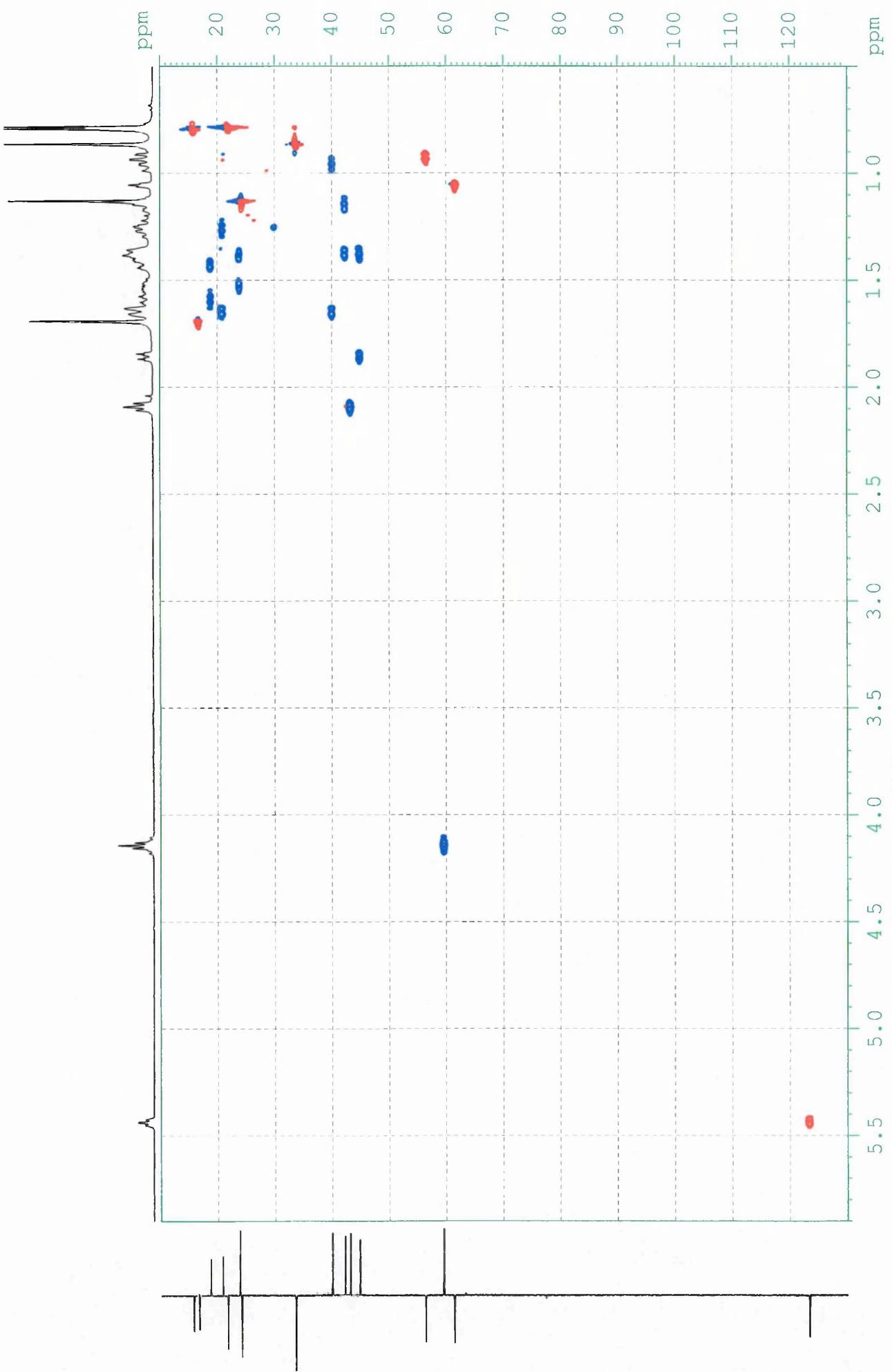


Spectrum 22.2:  $^{13}\text{C}$  NMR spectrum of compound 6.7 ( $\text{CDCl}_3$ )



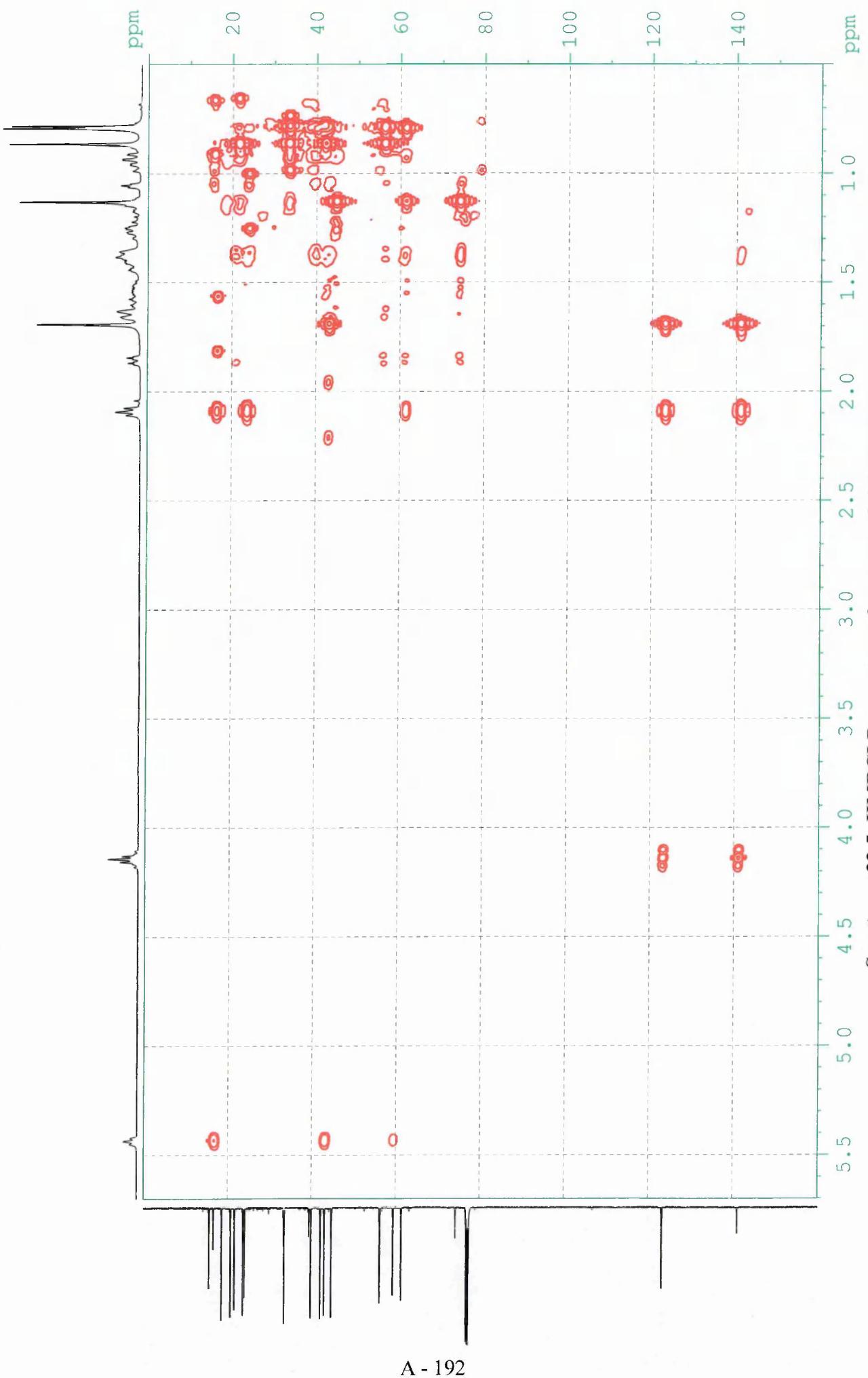
A - 190

Spectrum 22.3: DEPT spectrum of compound 6.7 ( $\text{CDCl}_3$ )



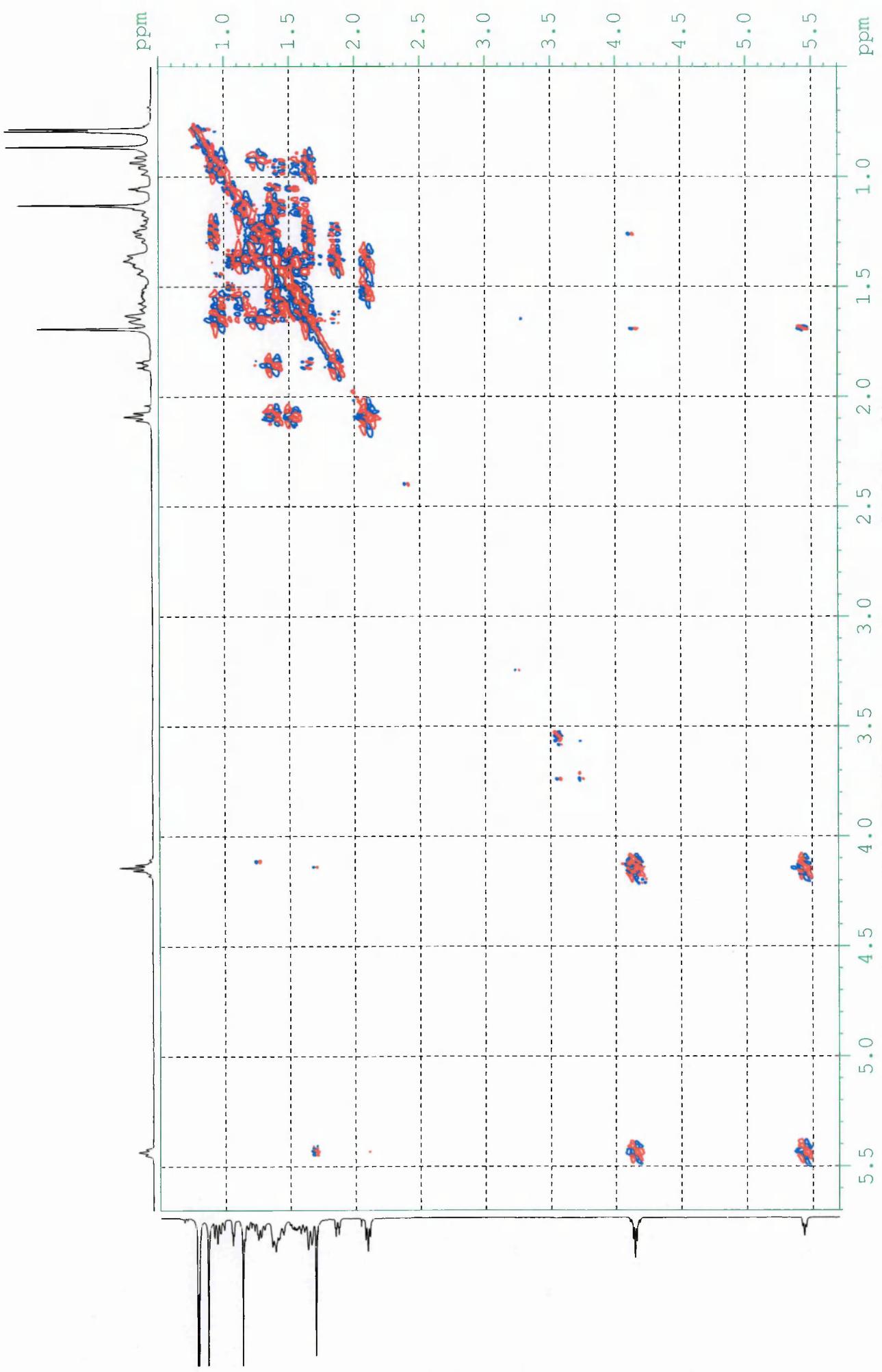
Spectrum 22.4: HSQCDEPT spectrum of compound 6.7 ( $\text{CDCl}_3$ )

**Spectrum 22.5: HMBCCP spectrum of compound 6.7 ( $\text{CDCl}_3$ )**

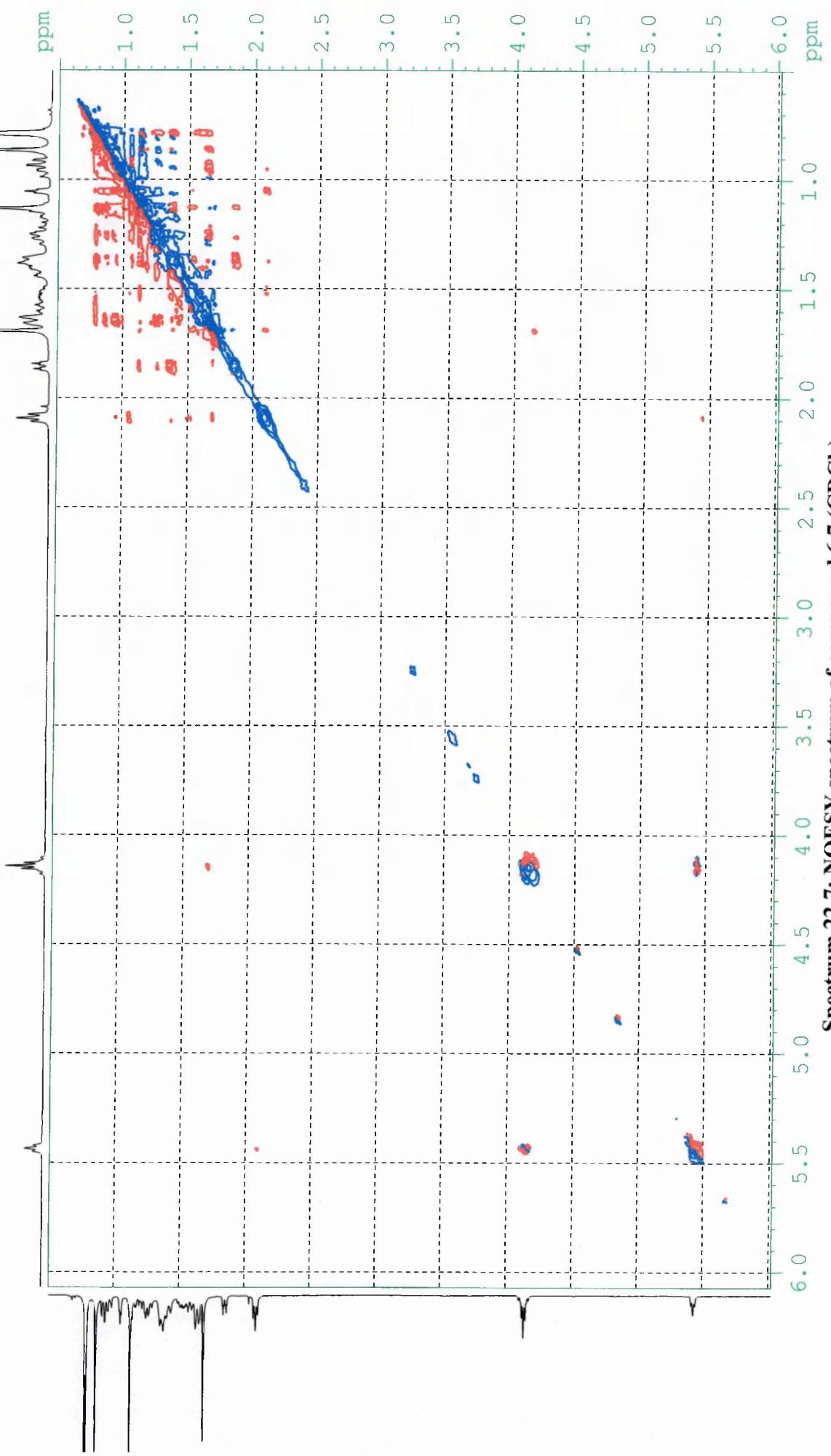


A - 192

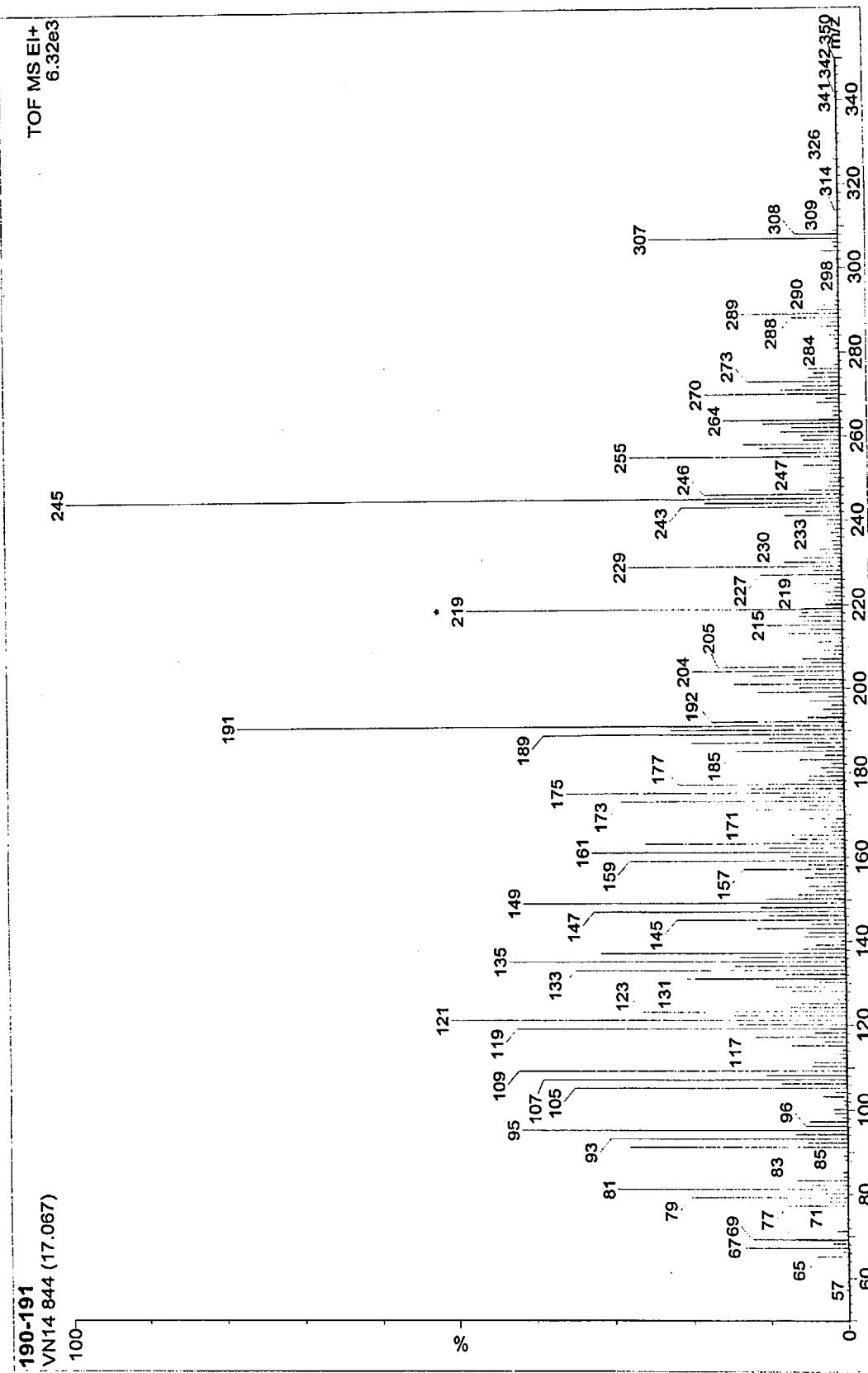
Spectrum 22.6: COSYPH spectrum of compound 6.7 ( $\text{CDCl}_3$ )



**Spectrum 22.7: NOESY spectrum of compound 6.7 ( $\text{CDCl}_3$ )**

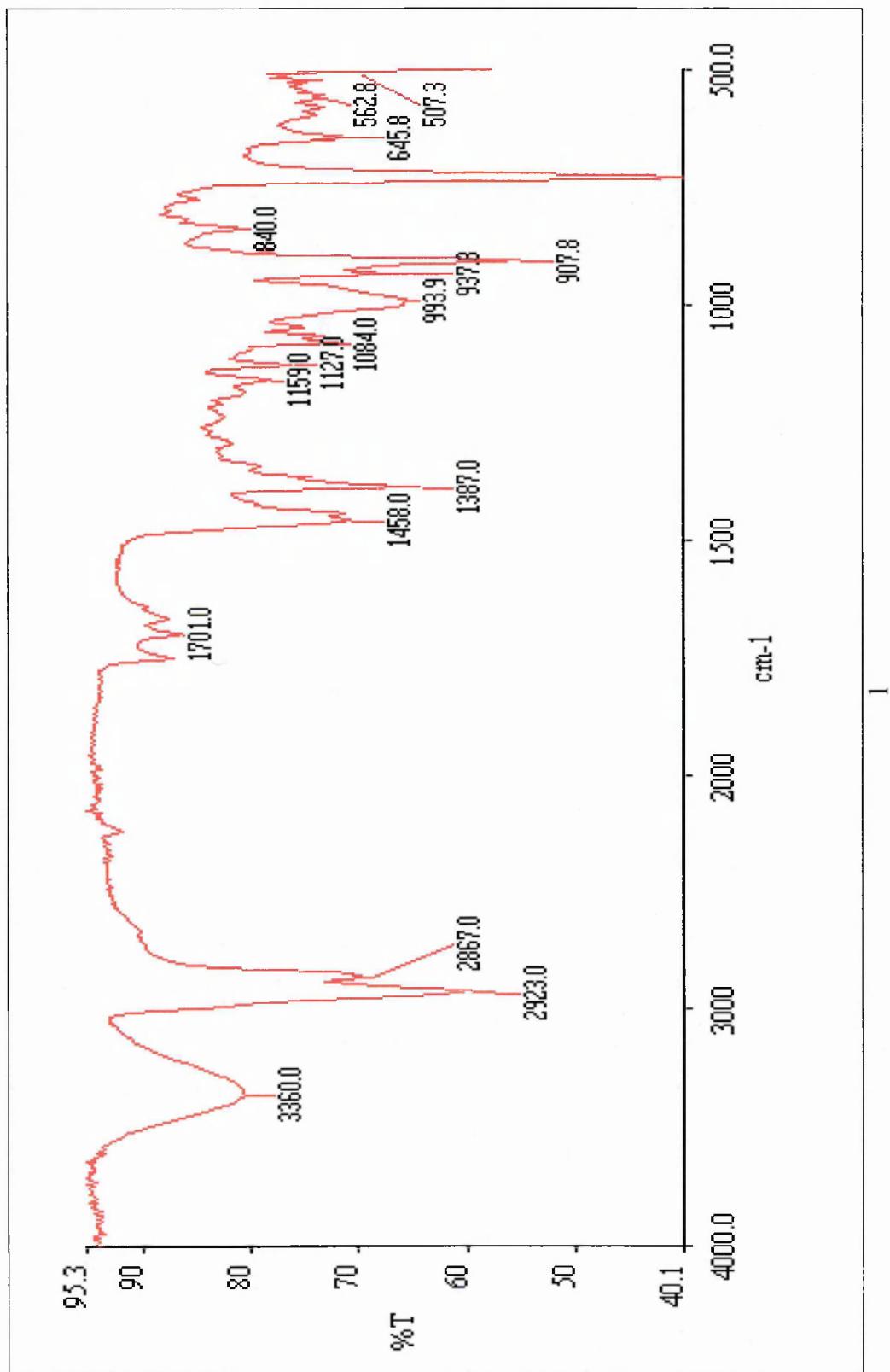


Spectrum 22.8: Mass spectrum of compound 6.7

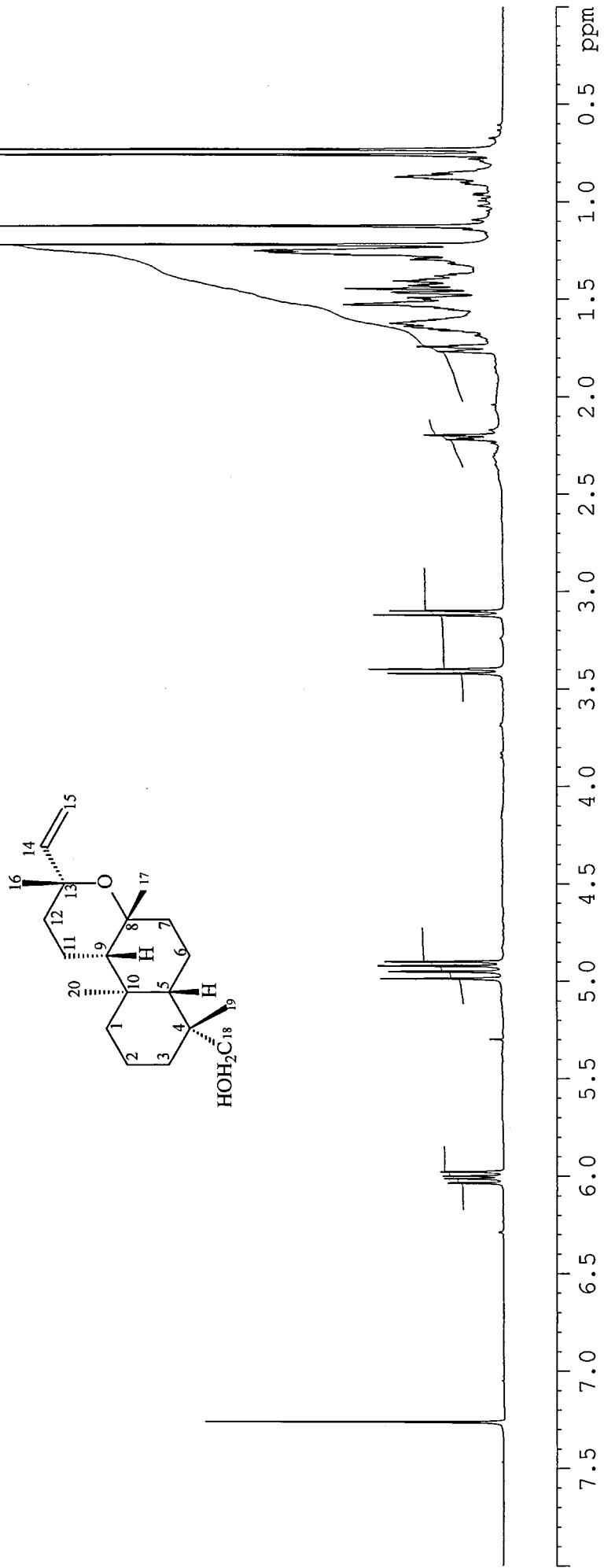


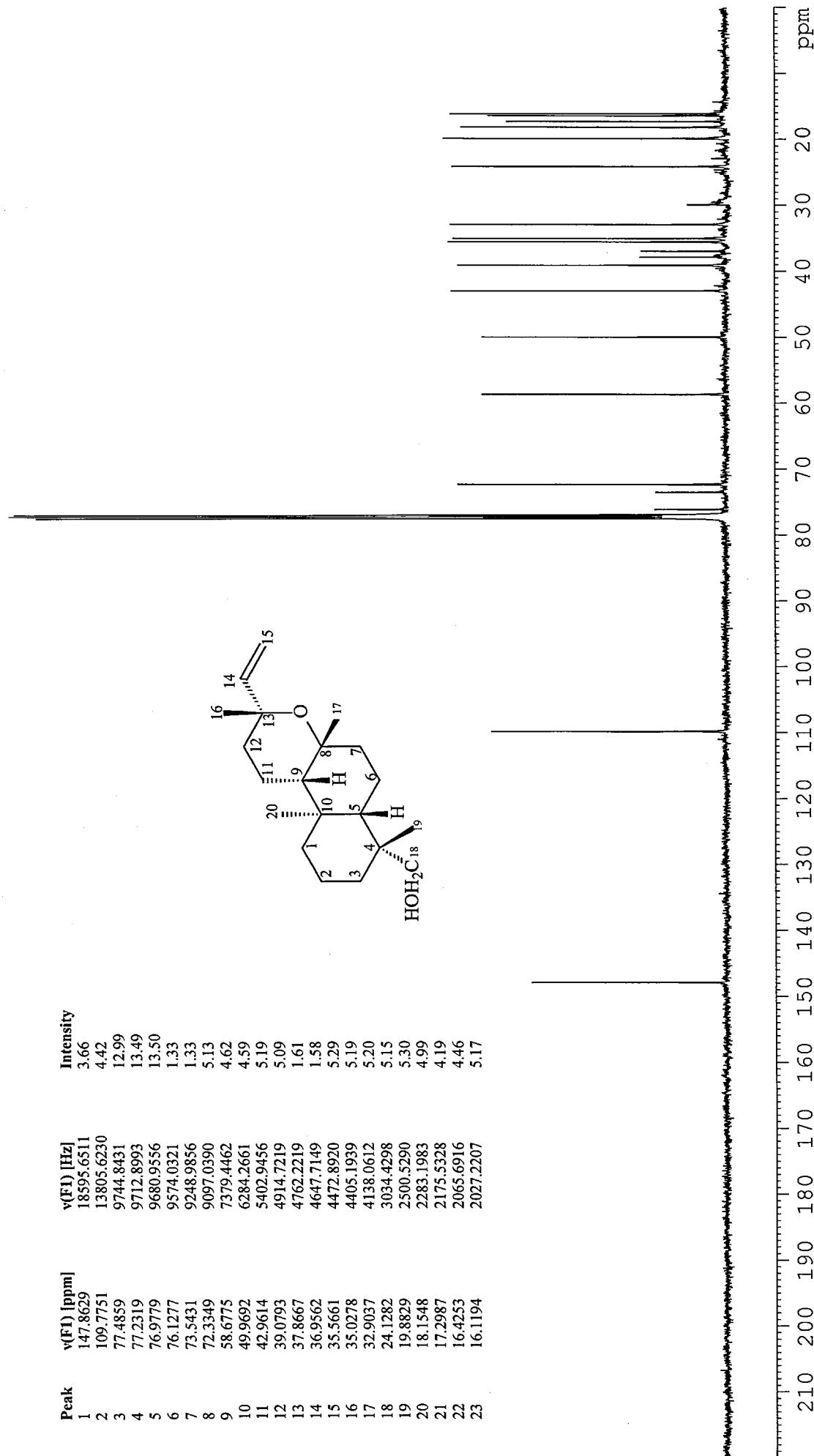
A - 195

Spectrum 22.9: FTIR spectrum of compound 6.7

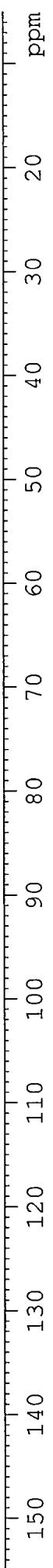


Spectrum 23.1:  $^1\text{H}$  NMR spectrum of compound 6.8 ( $\text{CDCl}_3$ )





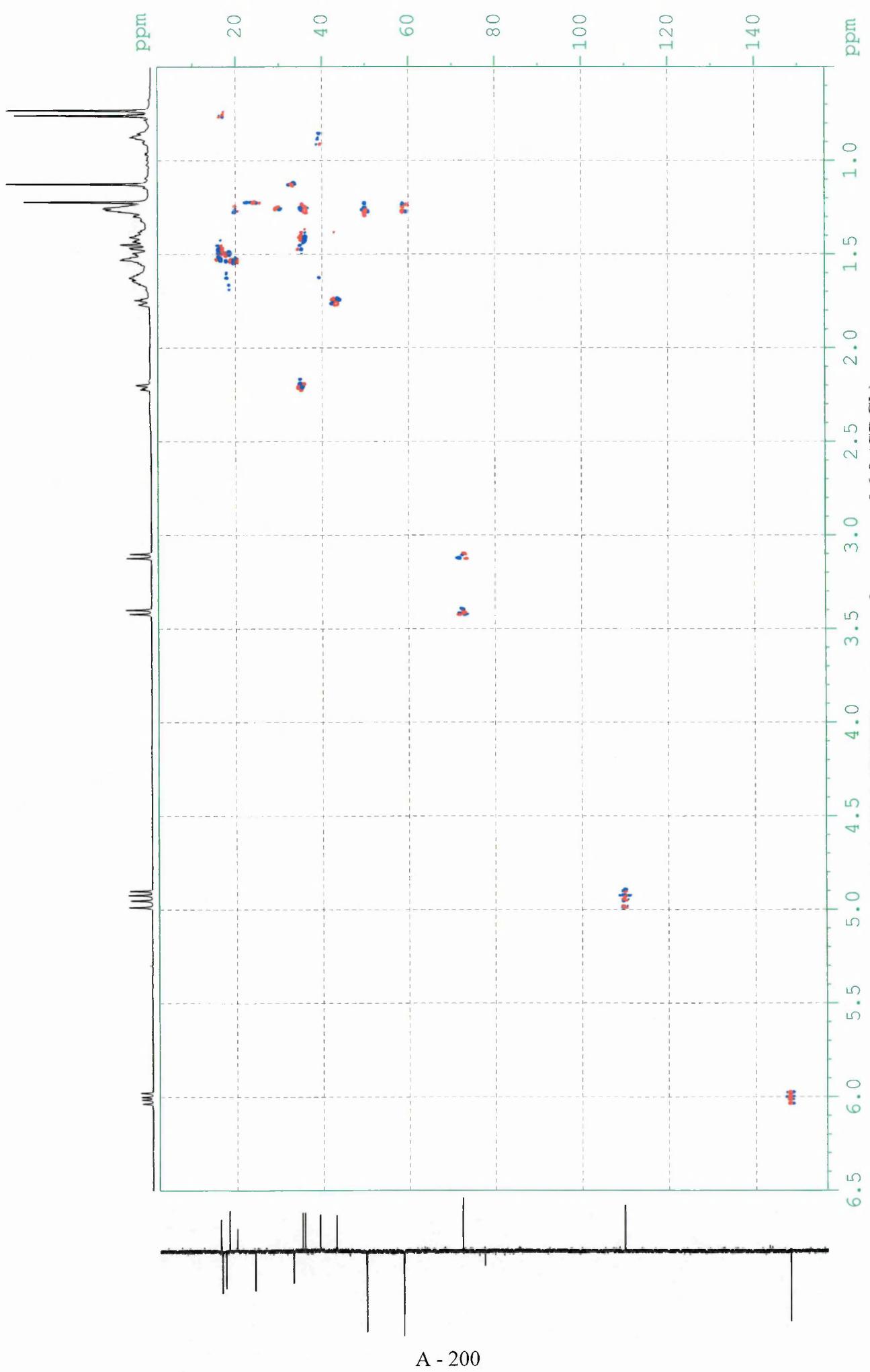
Spectrum 23.2:  $^{13}\text{C}$  NMR spectrum of compound 6.8 ( $\text{CDCl}_3$ )



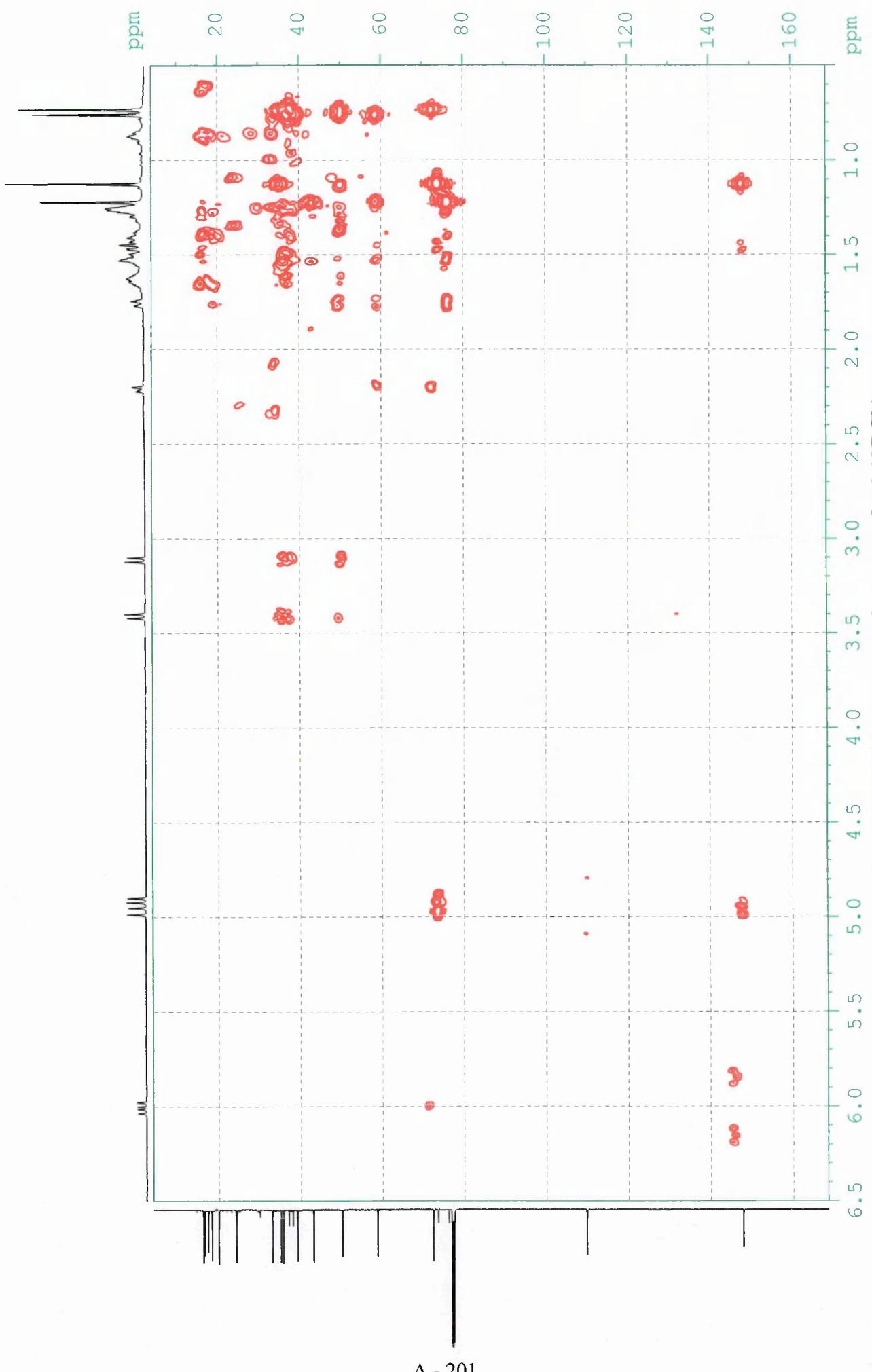
Spectrum 23.3: DEPT spectrum of compound 6.8 ( $\text{CDCl}_3$ )

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**Spectrum 23.4: HSQCDEPT spectrum of compound 6.8 ( $\text{CDCl}_3$ )**

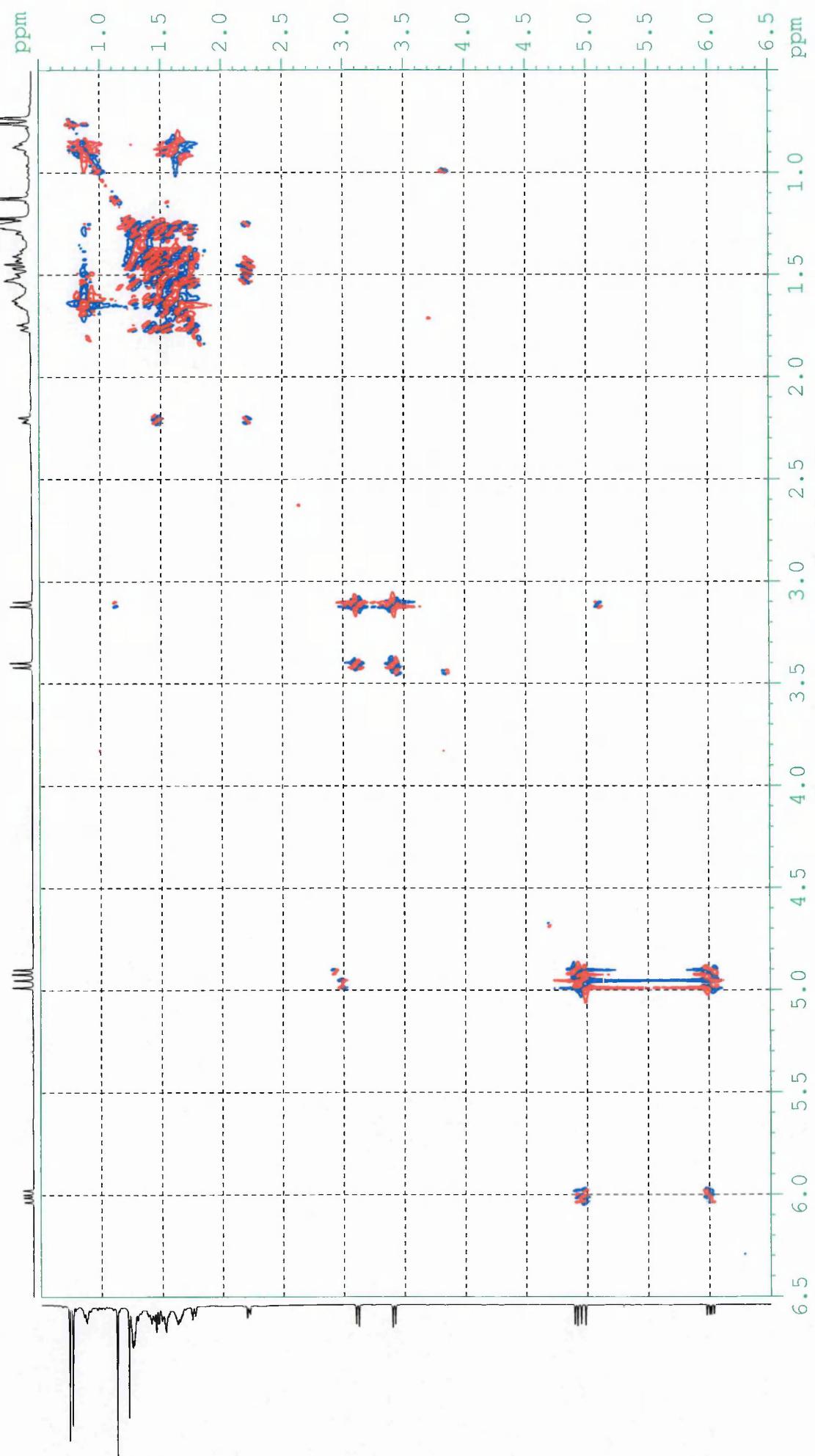


**Spectrum 23.5: HMQC spectrum of compound 6.8 ( $\text{CDCl}_3$ )**

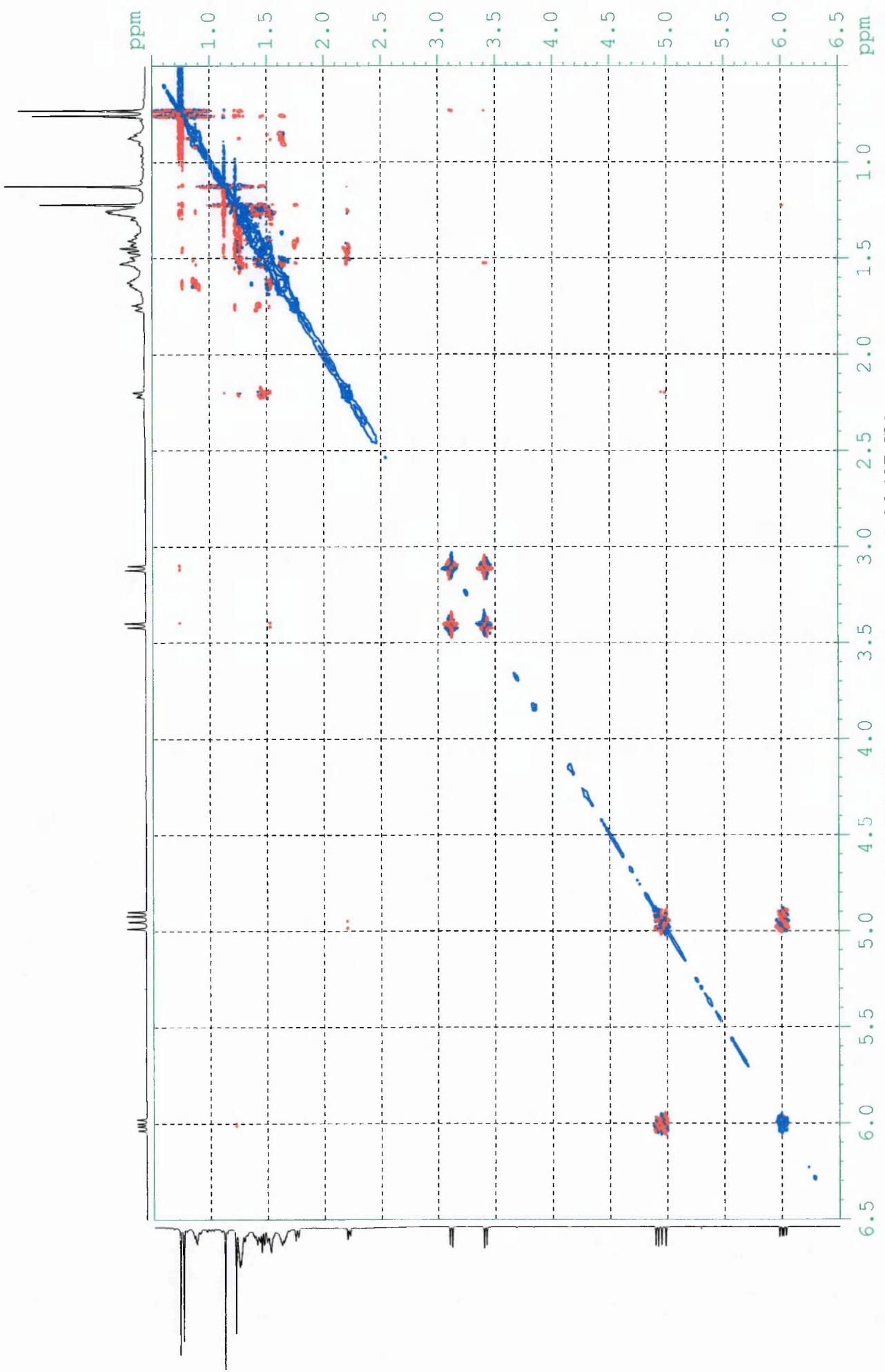


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Spectrum 23.6: COSYPH spectrum of compound 6.8 ( $\text{CDCl}_3$ )



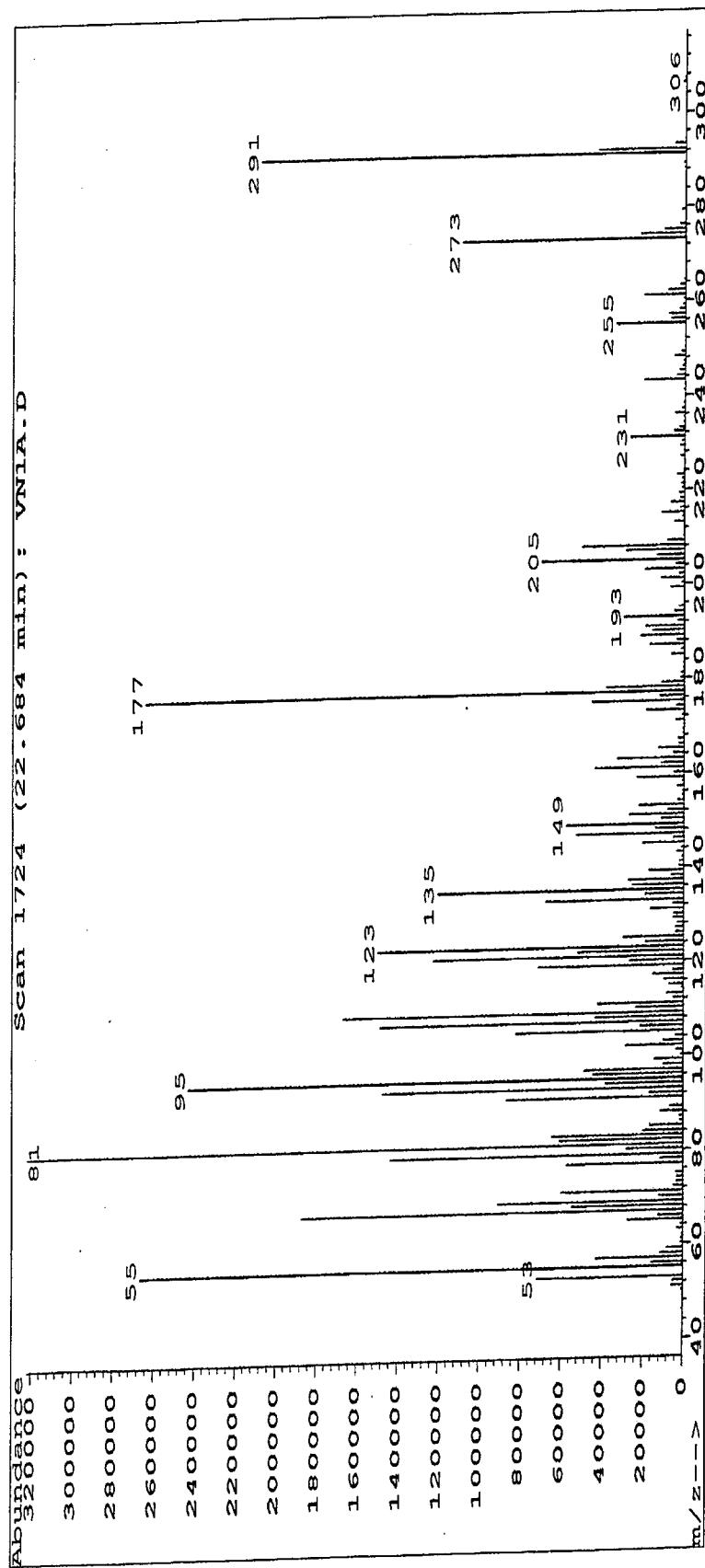
**Spectrum 23.7: NOESY spectrum of compound 6.8 ( $\text{CDCl}_3$ )**



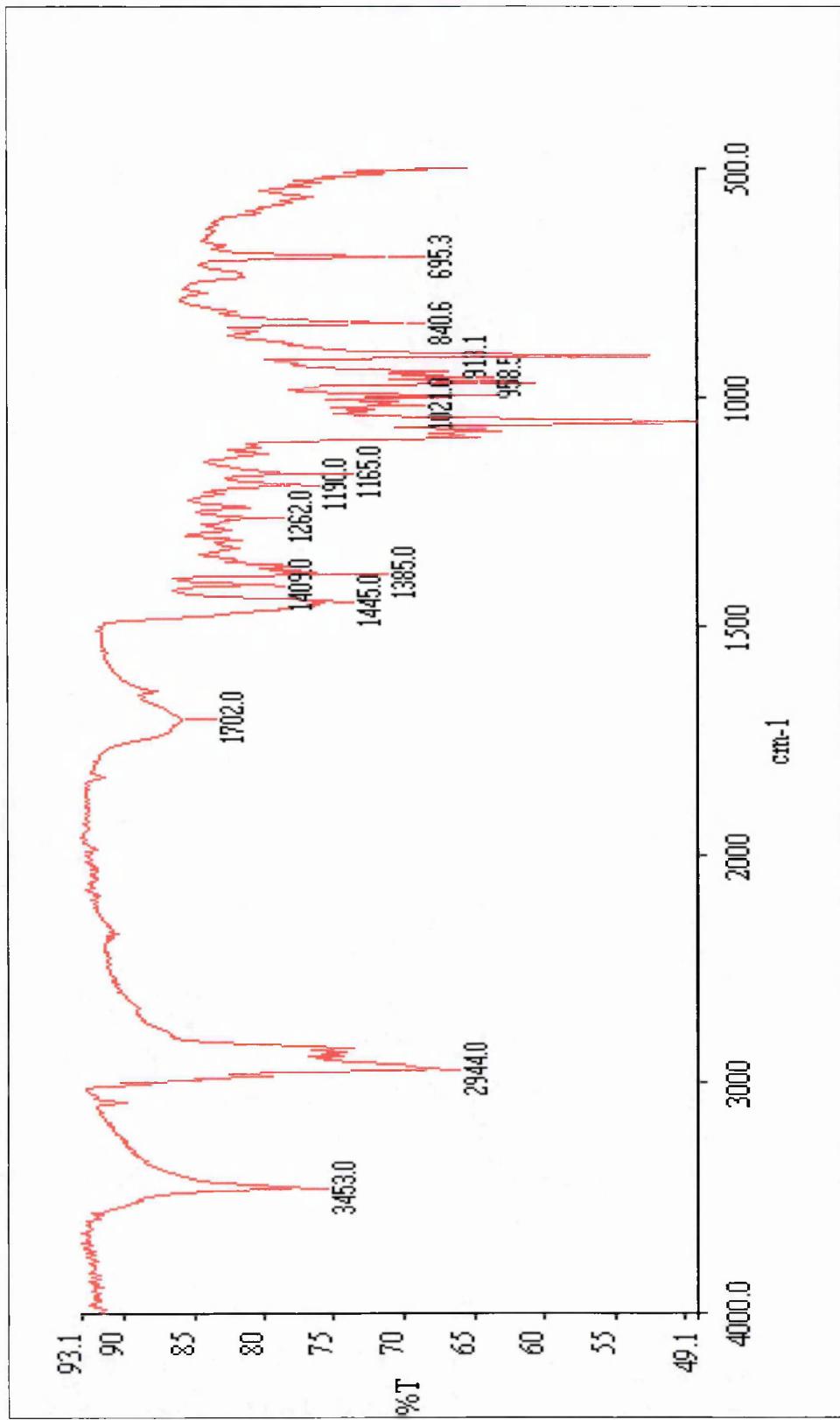
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File : C:\HPCHEM\1\DATA\STUDENTS\V_NDLEBE\VNLAD.D
Operator : 
Acquired : 10 OCT 106 11:28 am using ACQMETHOD STANDARD
Instrument : 5971 IN
Sample Name : 263 32 3
Misc Info : 
Vial Number: 10

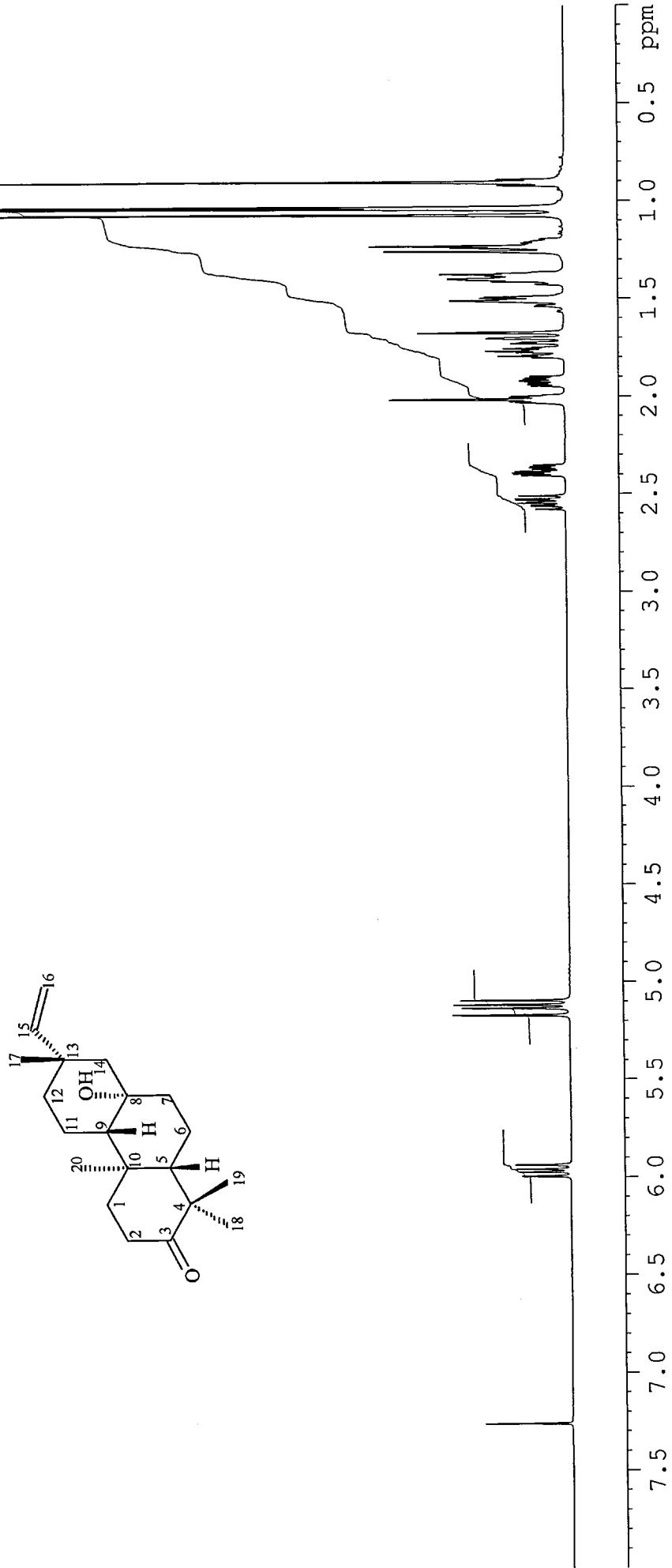
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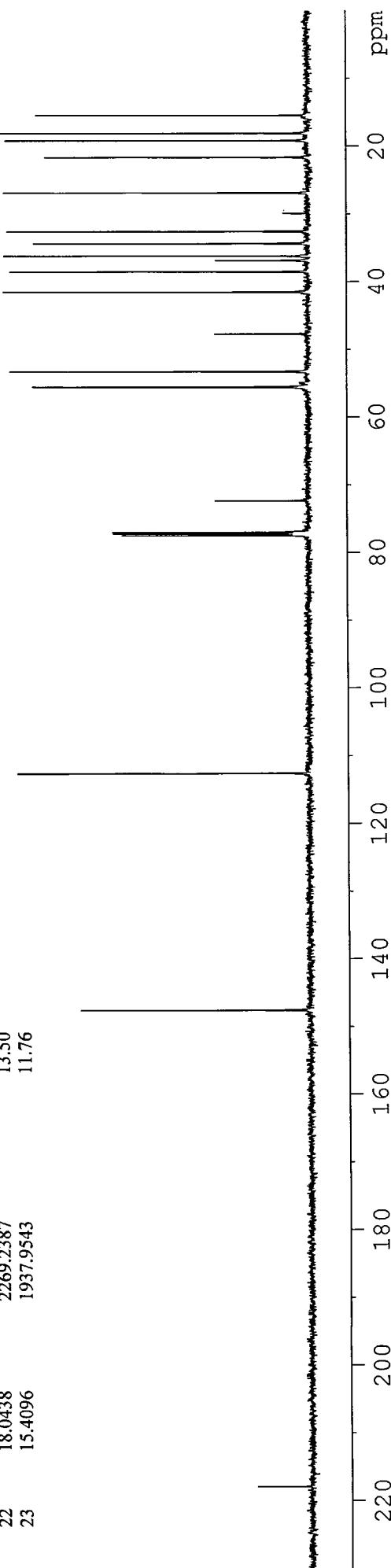
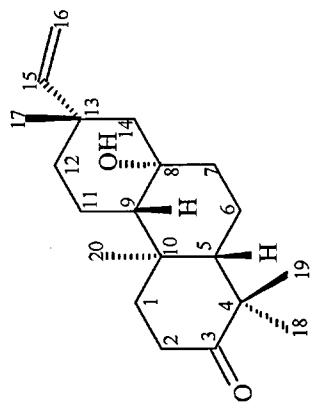
Spectrum 23.9: FTIR spectrum of compound 6.8



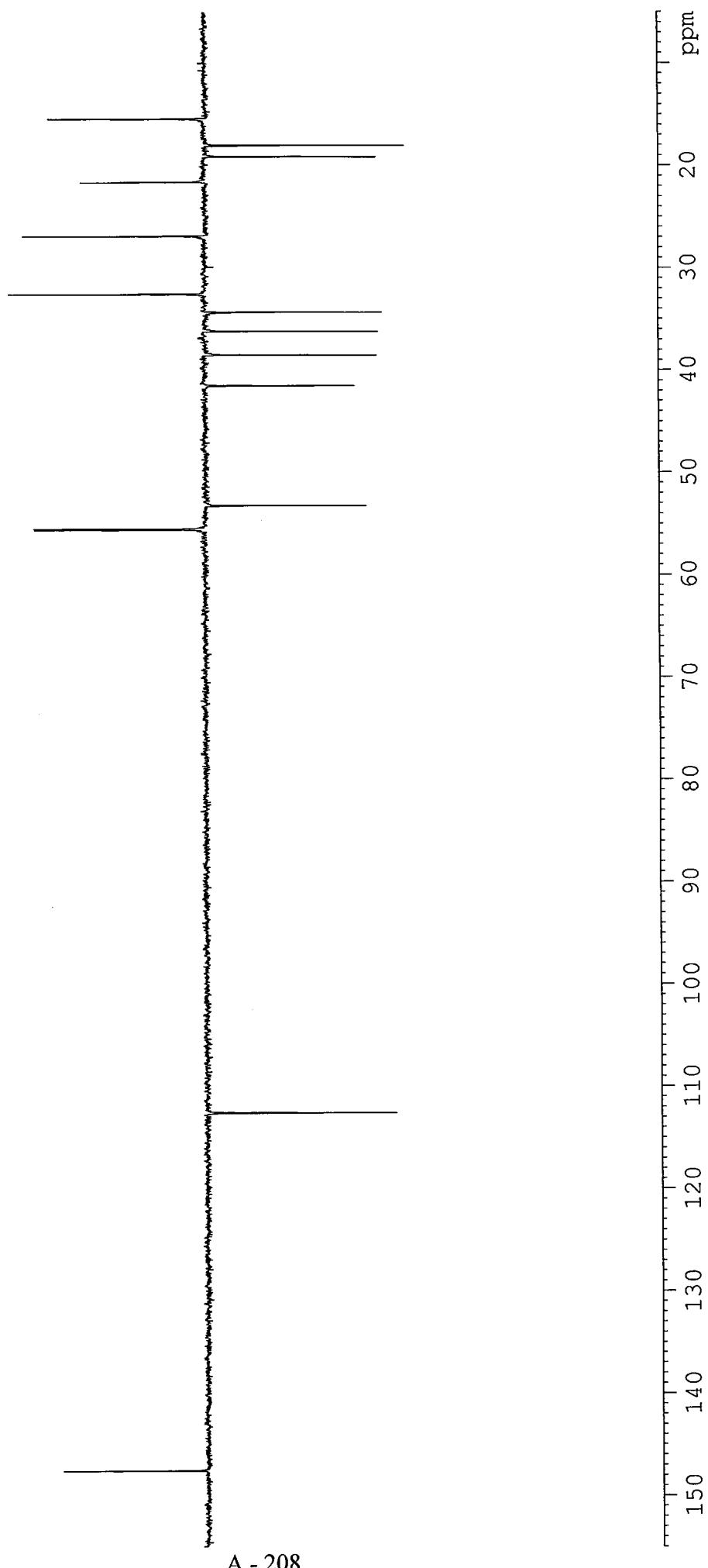
**Spectrum 24.1:**  $^1\text{H}$  NMR spectrum of compound 6.9 ( $\text{CDCl}_3$ )



Peak	$\nu(\text{F1})$ [ppm]	$\nu(\text{F1})$ [Hz]	Intensity
1	217.9302	27407.5107	2.37
2	147.5573	18557.2182	9.91
3	112.5954	14160.3120	12.59
4	77.4851	9744.7426	8.14
5	77.2310	9712.7863	8.44
6	76.9769	9094.4358	4.02
8	55.5660	6988.1354	11.84
9	55.4927	6978.9170	11.89
10	53.2426	6695.9381	13.00
11	47.6914	5997.8037	4.07
12	41.5018	5219.3823	13.33
13	38.5121	4843.3893	12.87
14	36.8834	4638.5594	3.43
15	36.8123	4629.6177	4.05
16	36.2046	4553.1916	13.19
17	34.3362	4318.2164	11.96
18	32.5501	4093.5915	12.98
19	26.8803	3380.5416	13.10
20	21.6223	2719.2808	11.40
21	19.1366	2406.6723	13.15
22	18.0438	2269.2387	13.50
23	15.4096	1937.9543	11.76



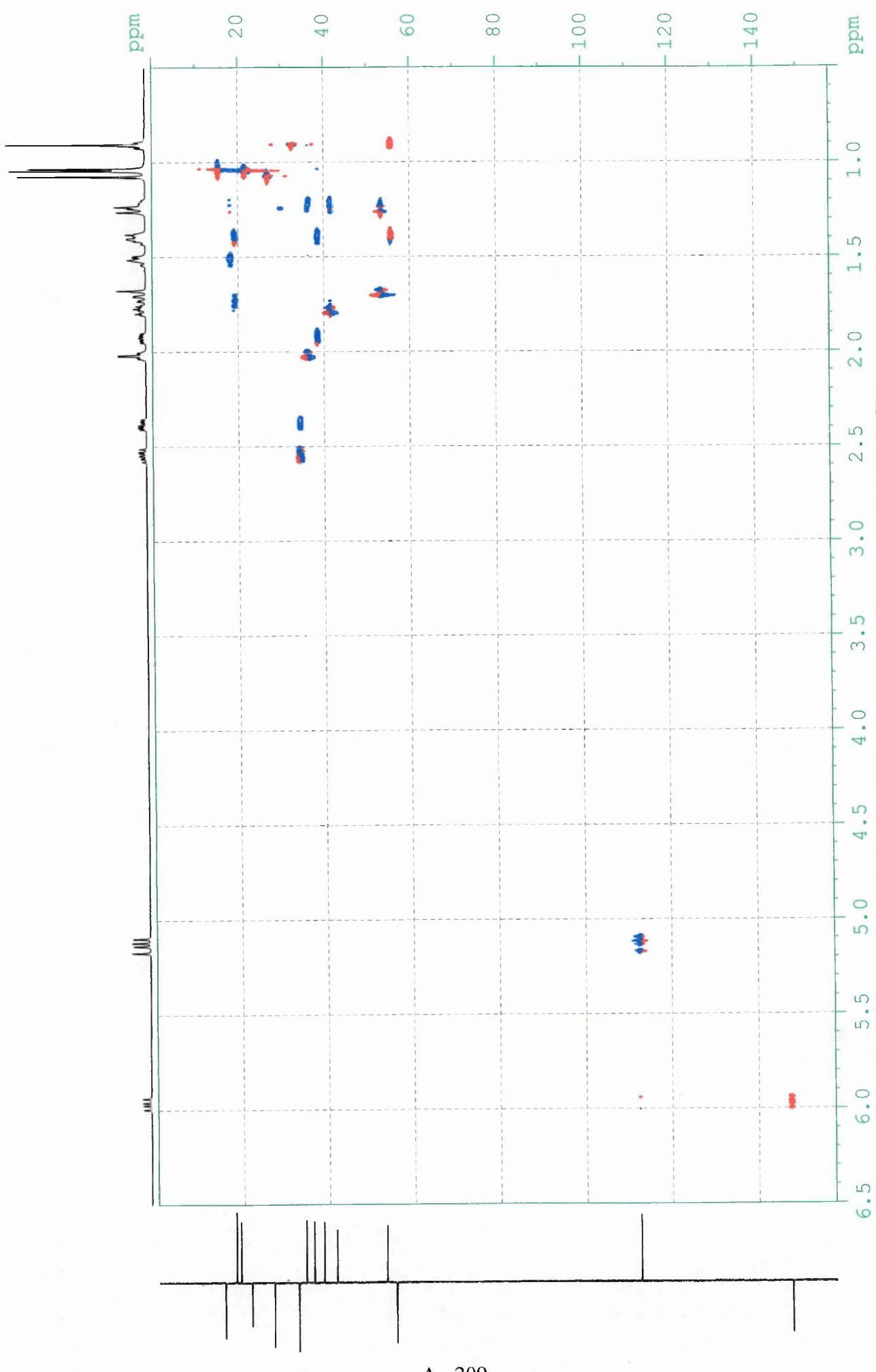
Spectrum 24.2:  $^{13}\text{C}$  NMR spectrum of compound 6.9 ( $\text{CDCl}_3$ )



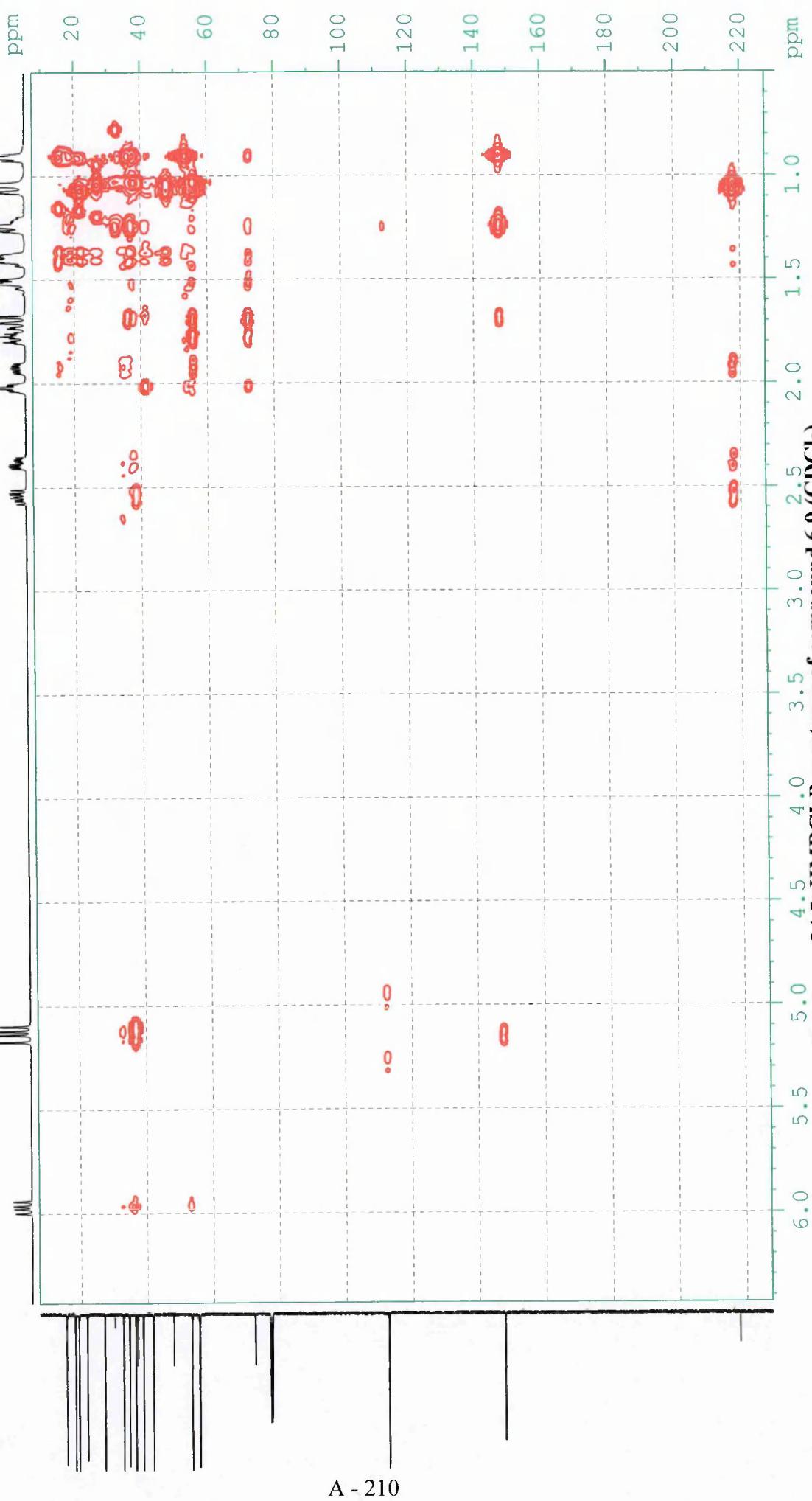
A - 208

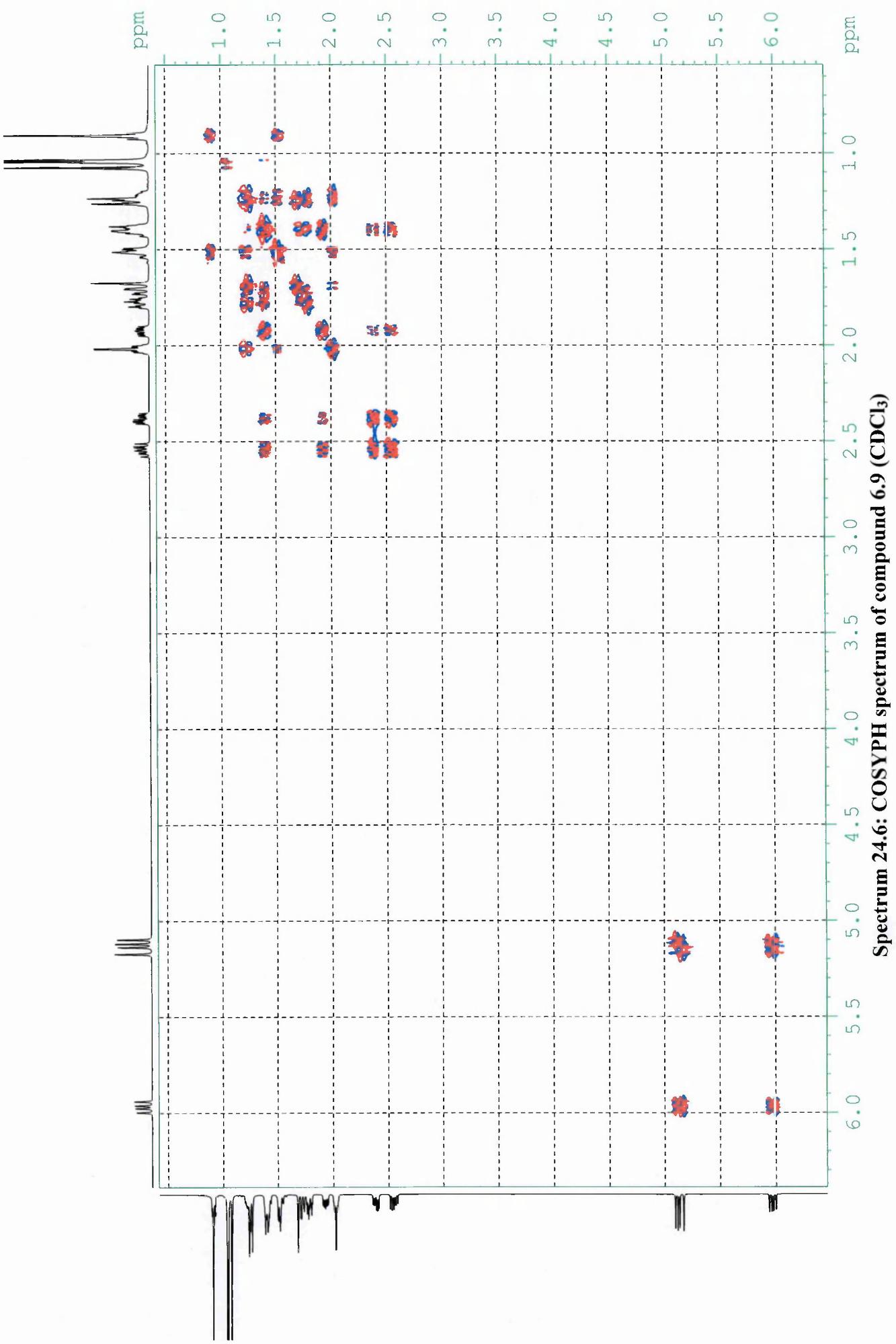
**Spectrum 24.3:** DEPT spectrum of compound 6.9 ( $\text{CDCl}_3$ )

**Spectrum 24.4: HSQCDEPT spectrum of compound 6.9 ( $\text{CDCl}_3$ )**

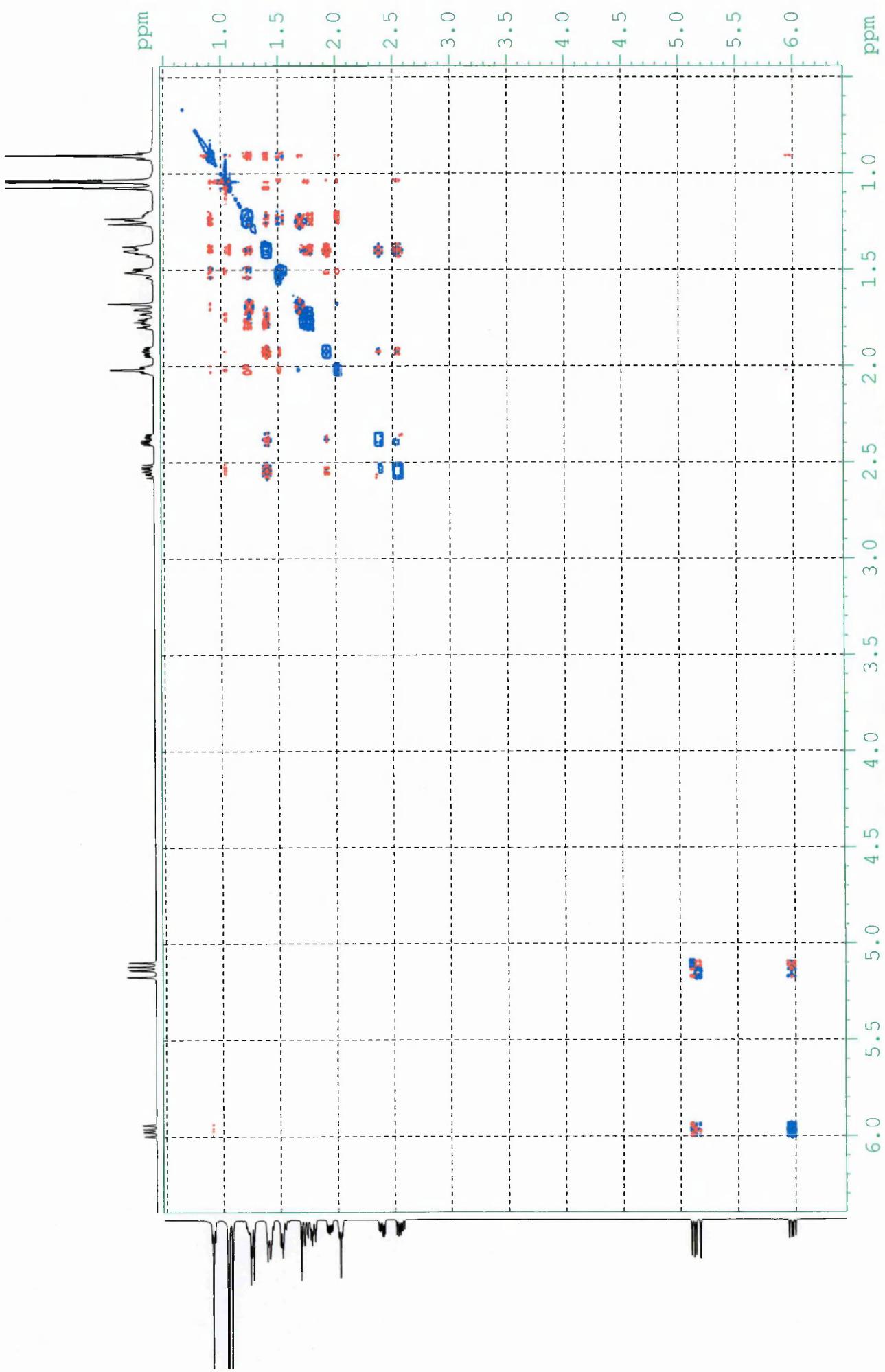


**Spectrum 24:5: HMBCLP spectrum of compound 6.9 ( $\text{CDCl}_3$ )**



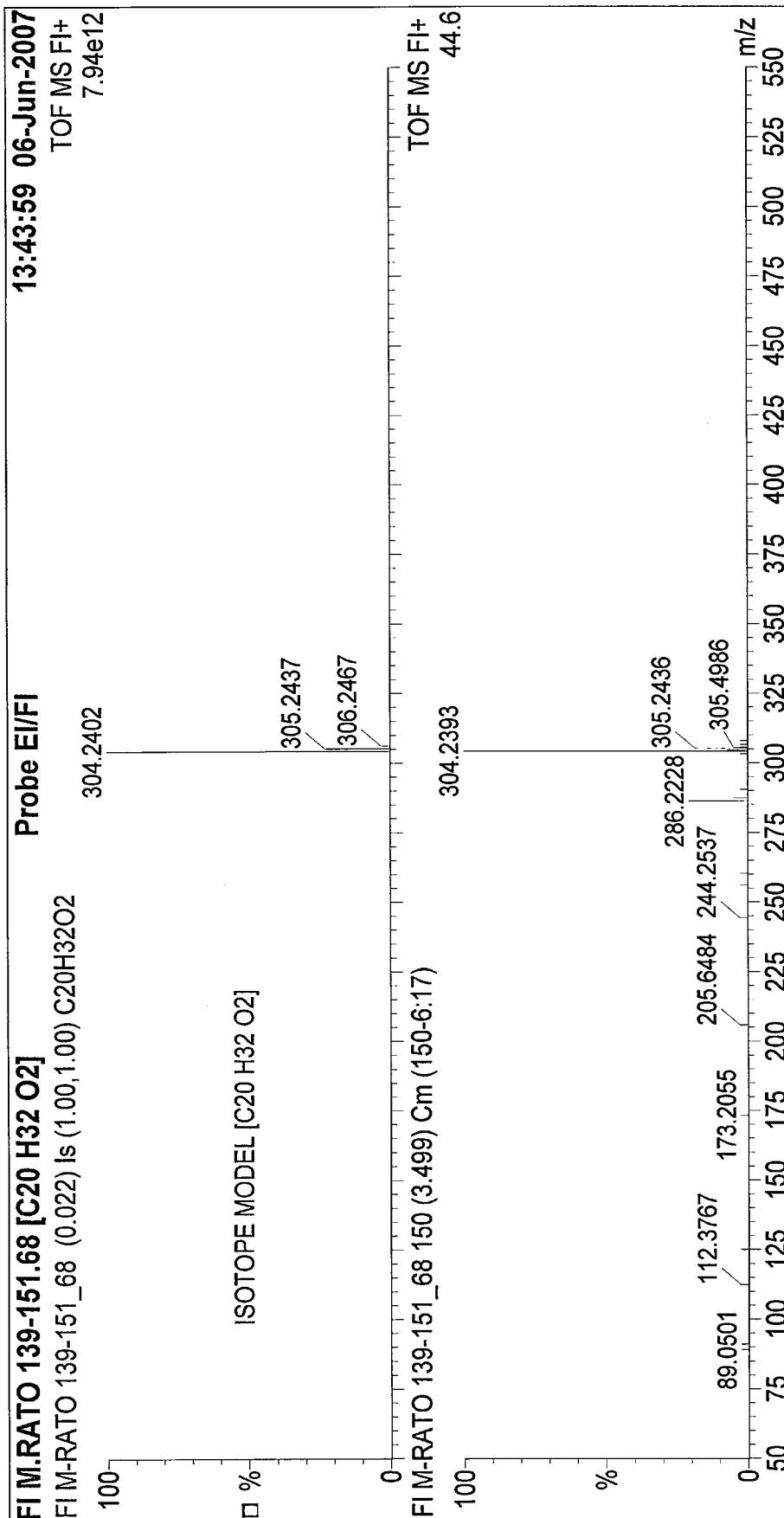


Spectrum 24.6: COSYPH spectrum of compound 6.9 ( $\text{CDCl}_3$ )



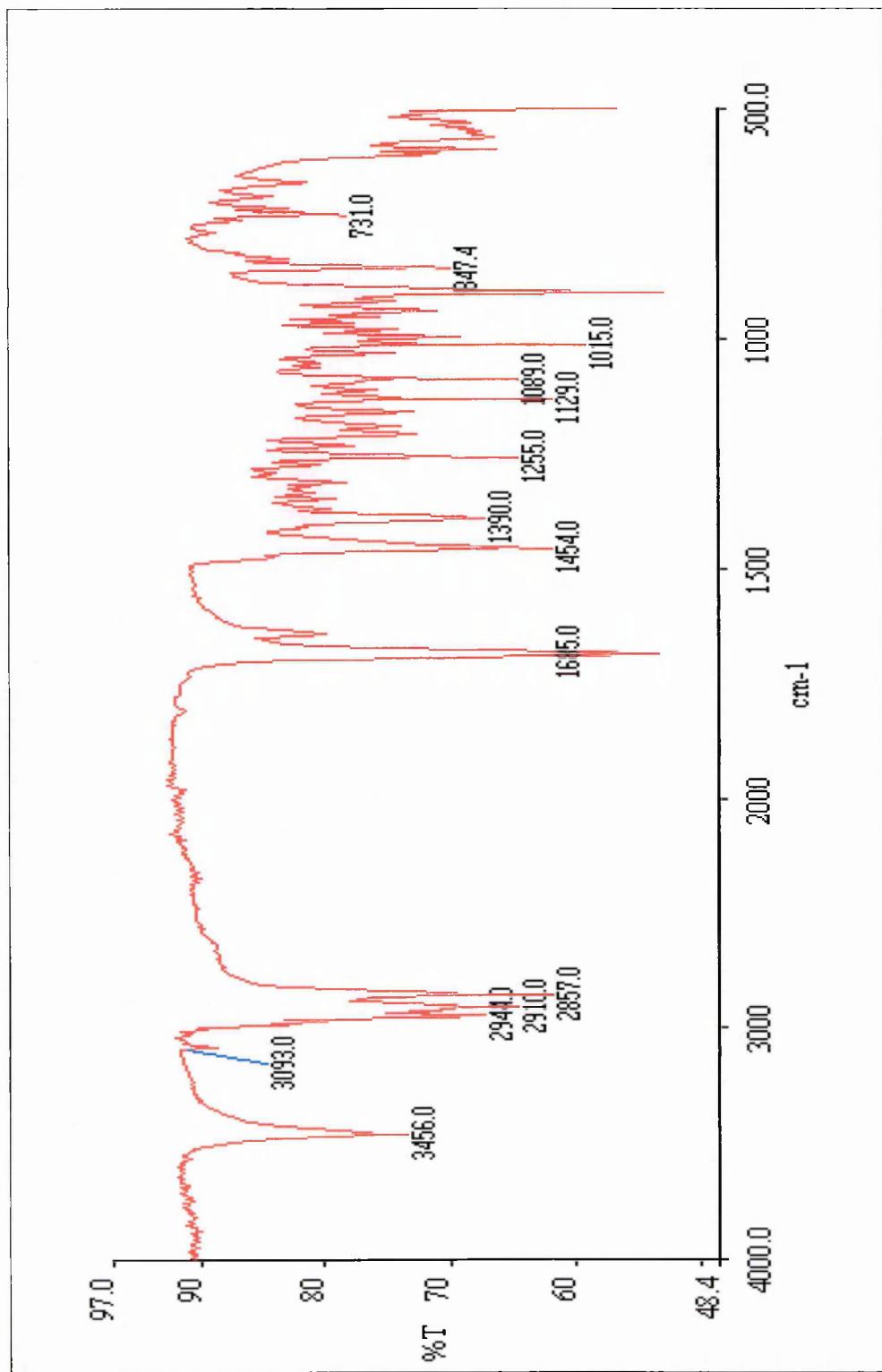
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Spectrum 24.7: NOESY spectrum of compound 6.9 ( $\text{CDCl}_3$ )

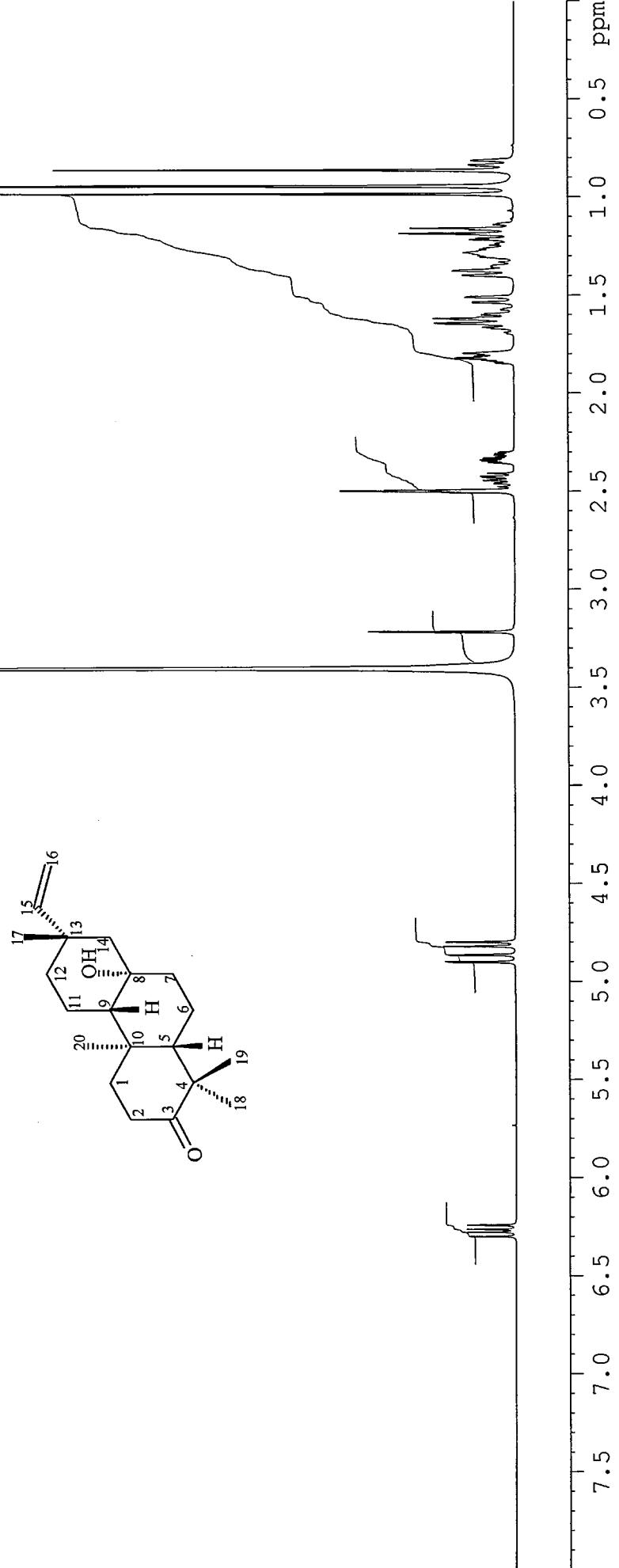


Spectrum 24.8: Mass spectrum of compound 6.9

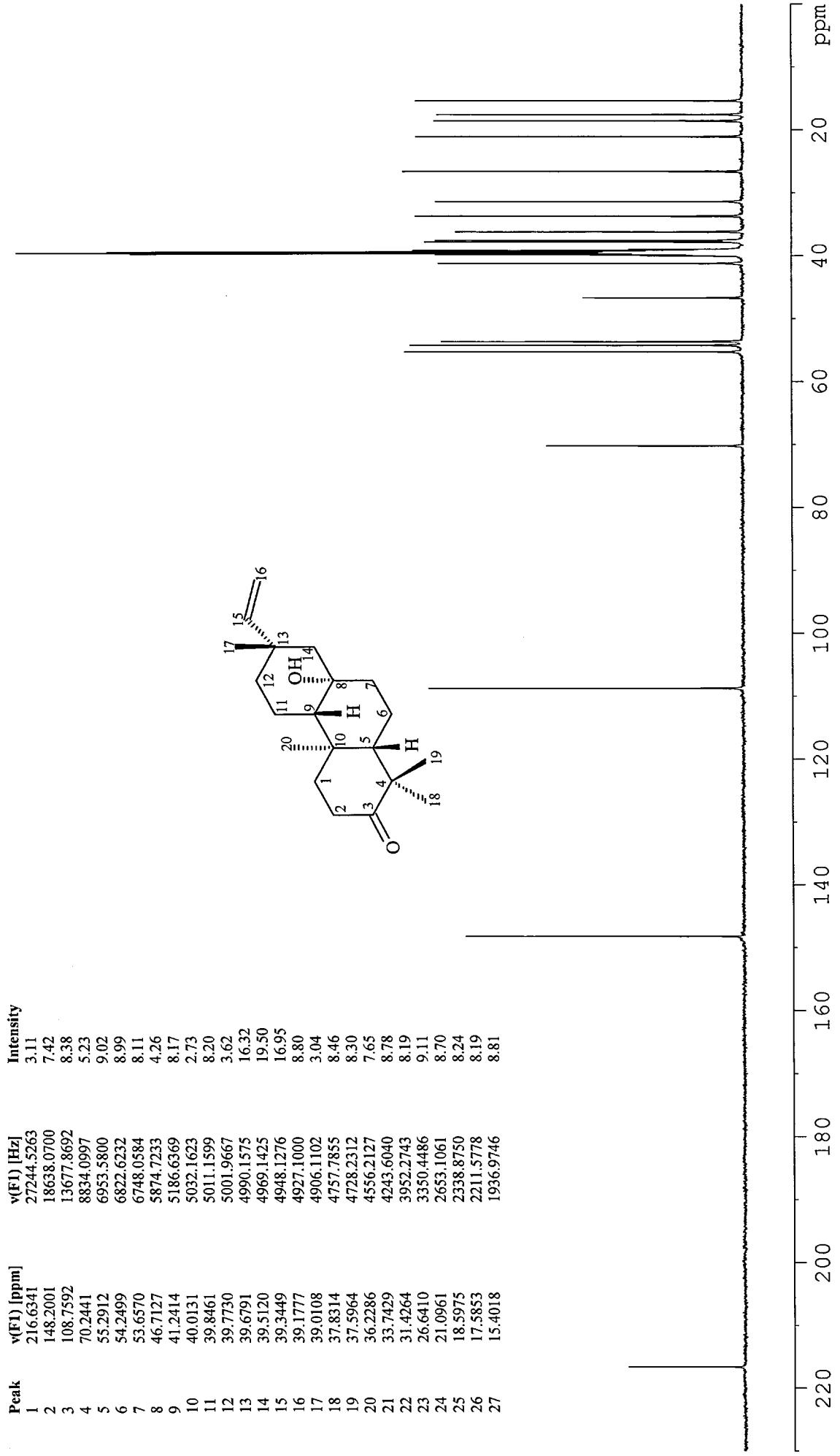
Spectrum 24.9: FTIR spectrum of compound 6.9

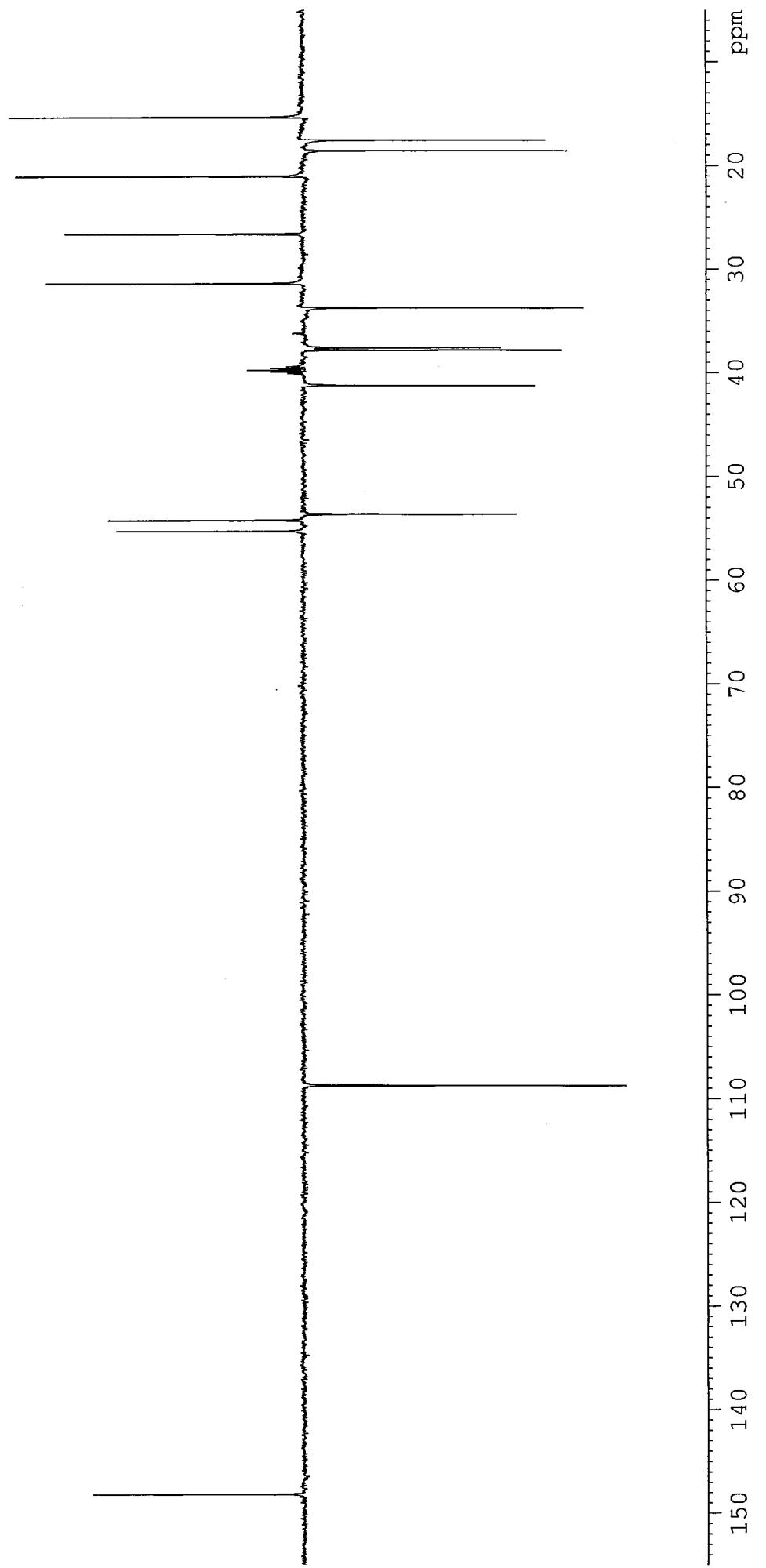


**Spectrum 25.1:**  $^1\text{H}$  NMR spectrum of compound 6,9 (DMSO)

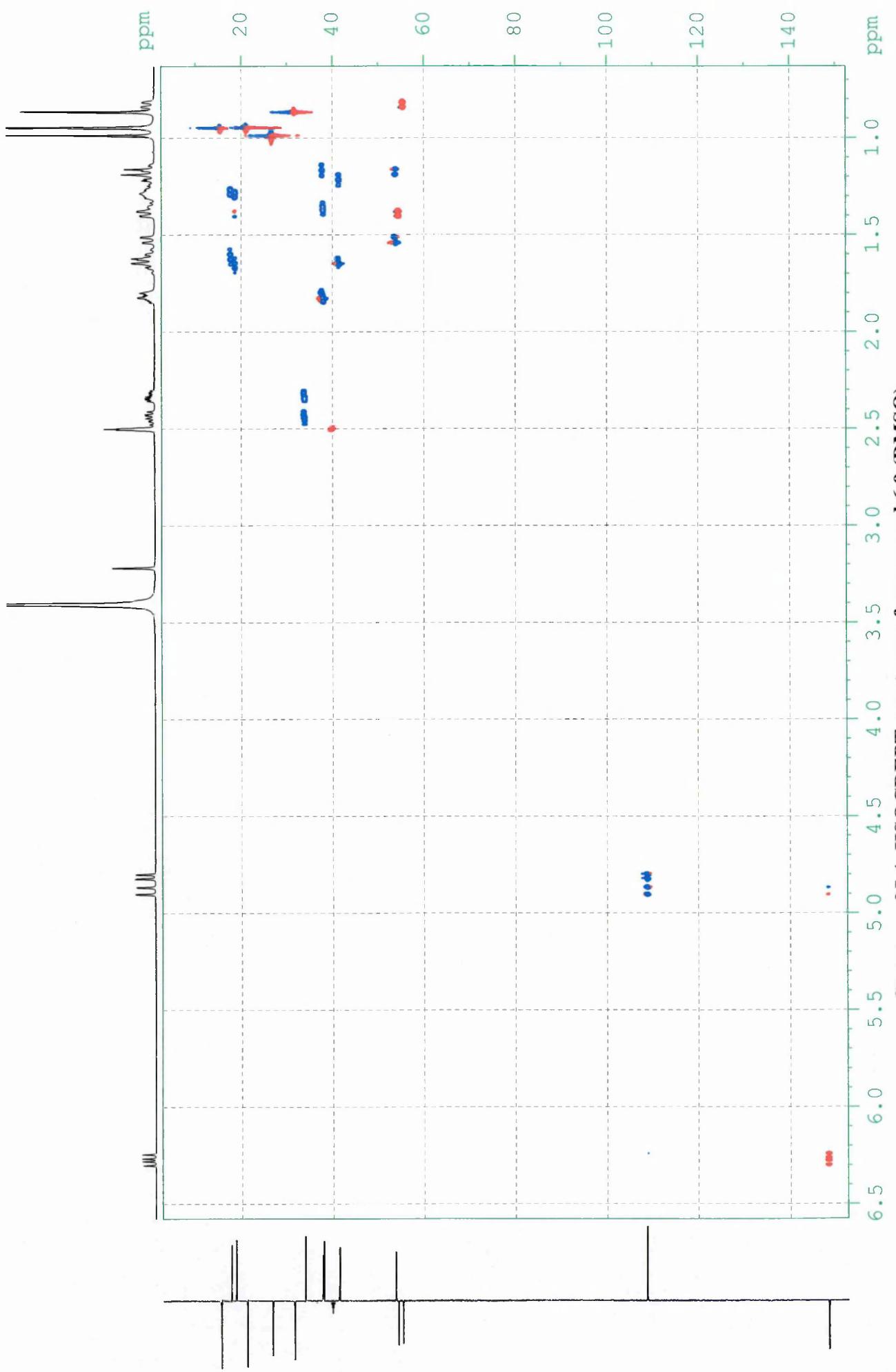


**Spectrum 25.2:**  $^{13}\text{C}$  NMR spectrum of compound 6.9 (DMSO)





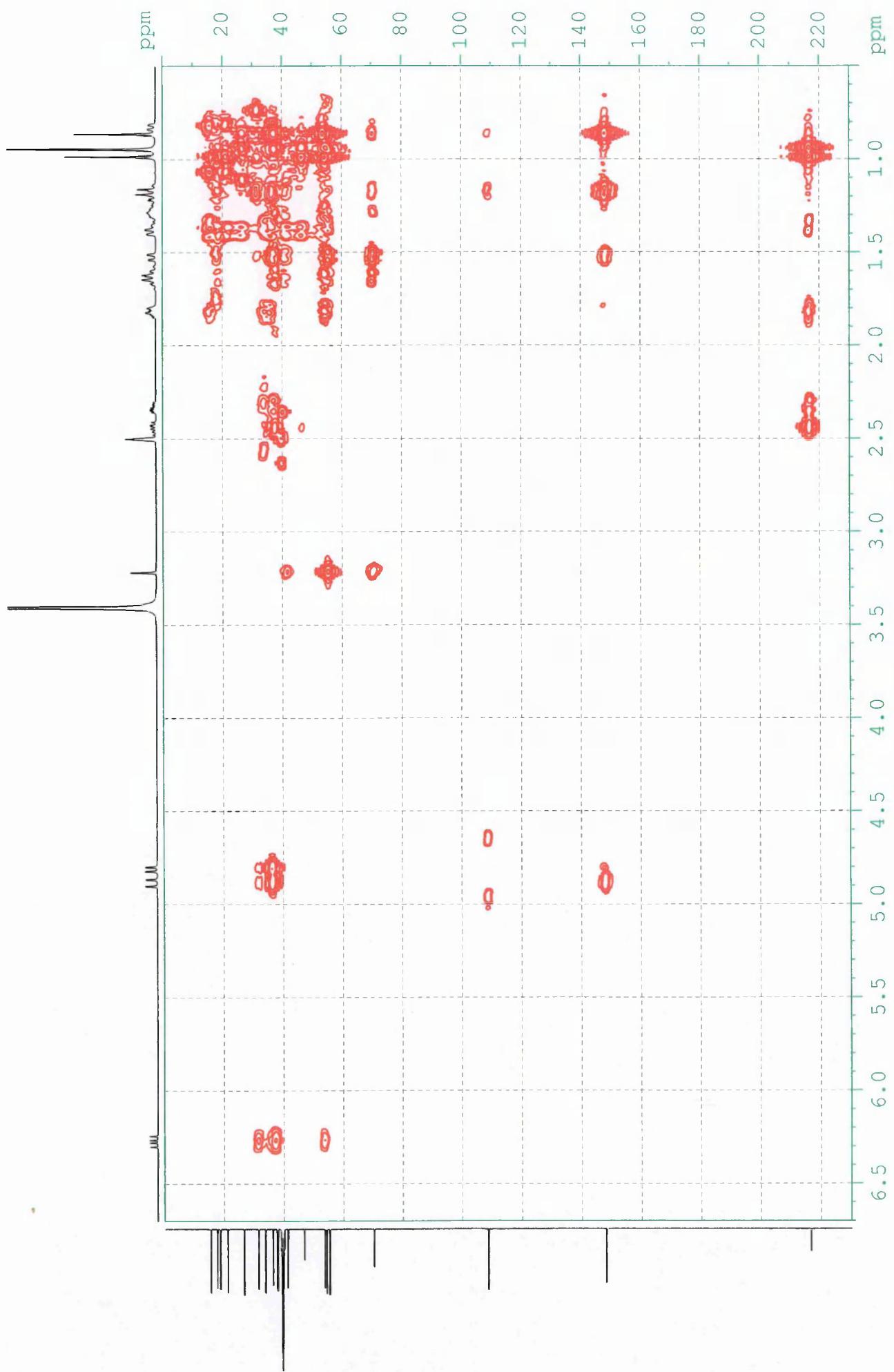
Spectrum 25.2: DEPT spectrum of compound 6.9 (DMSO)



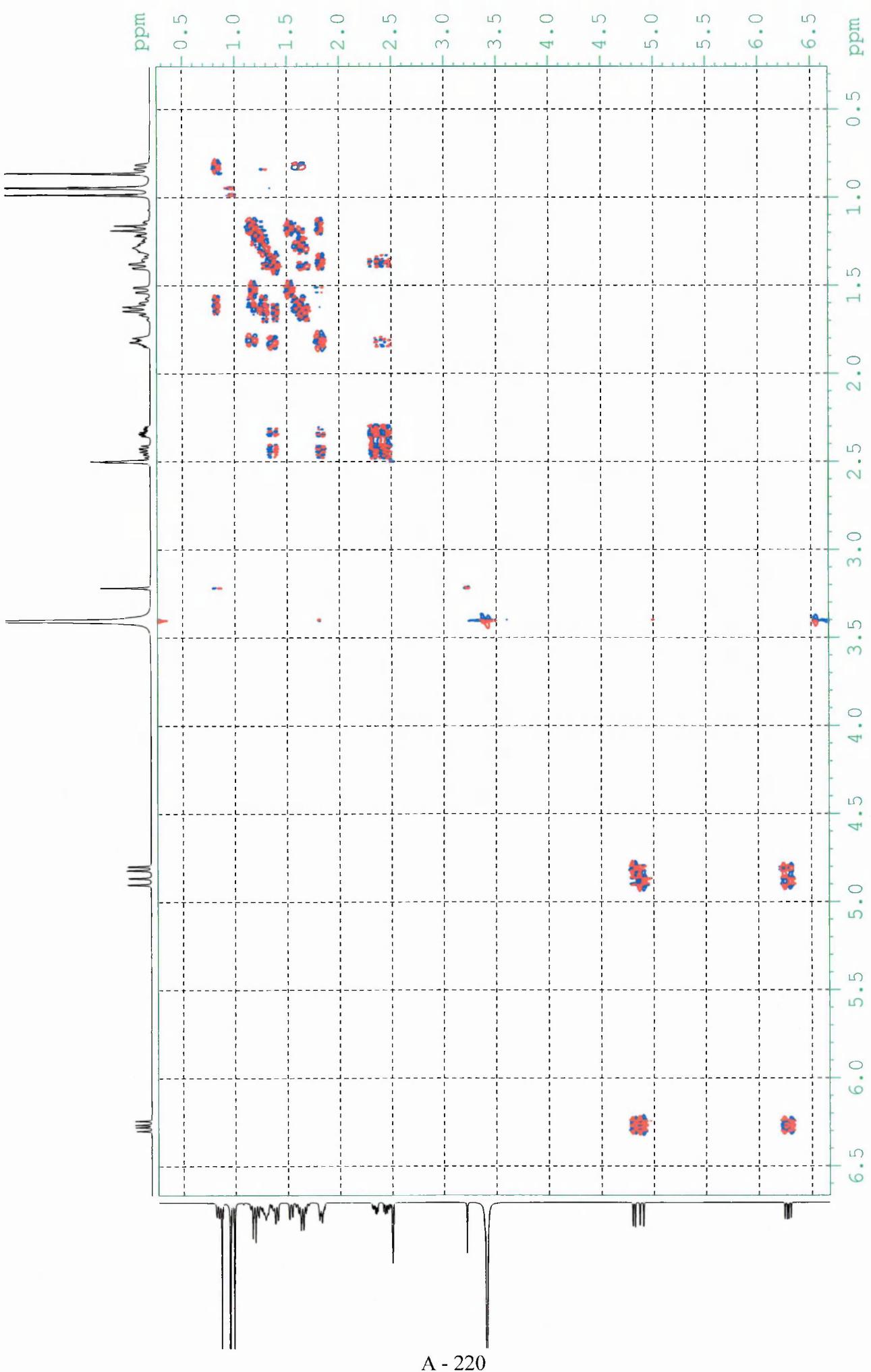
A - 218

Spectrum 25.4: HSQCDEPT spectrum of compound 6.9 (DMSO)

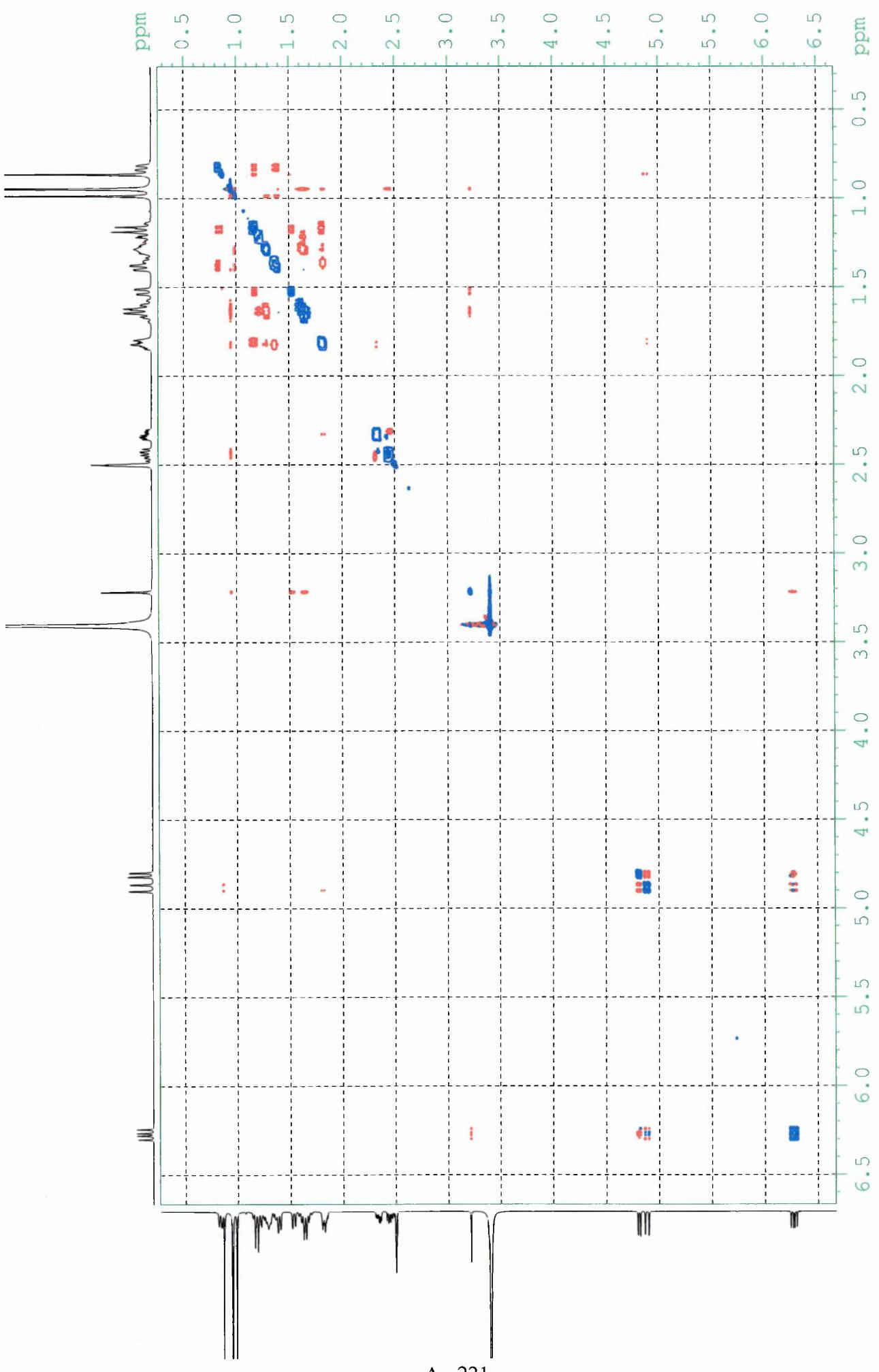
Spectrum 25.5: HMBCLP spectrum of compound 6.9 (DMSO)



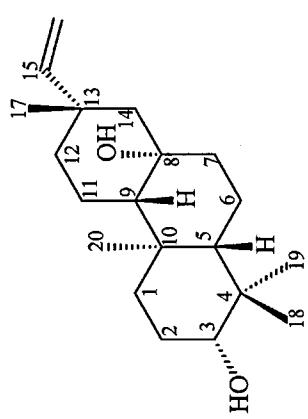
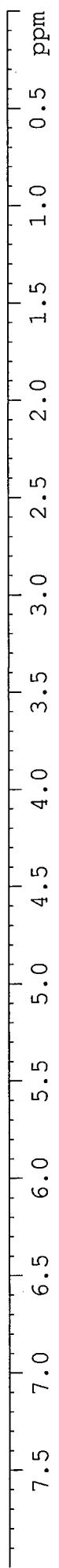
**Spectrum 25.6: COSYPH spectrum of compound 6.9 (DMSO)**



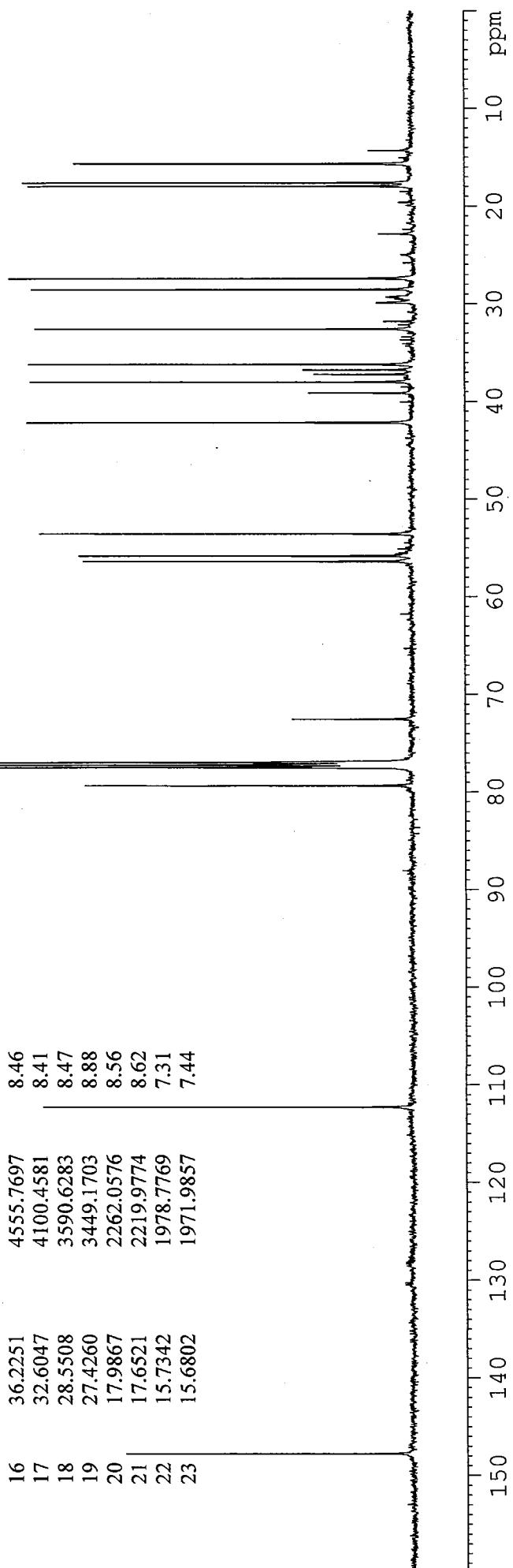
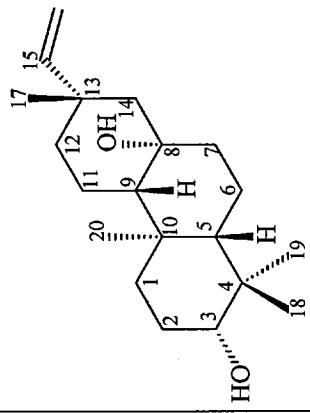
**Spectrum 25.7: NOESY spectrum of compound 6.9 (DMSO)**



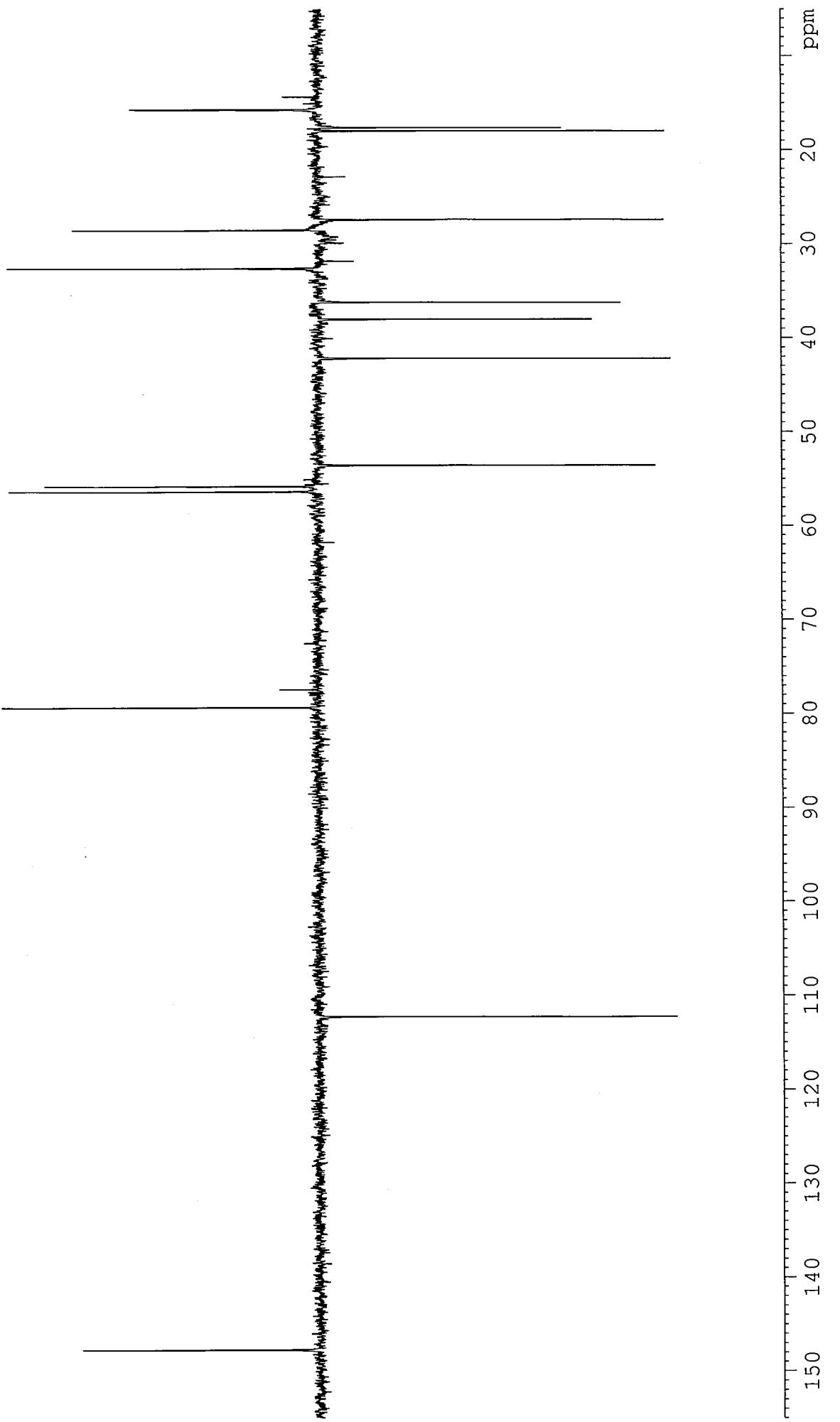
Spectrum 26.1:  $^1\text{H}$  NMR spectrum of compound 6.10 ( $\text{CDCl}_3$ )



Peak	$\nu(F1)$ [ppm]	$\nu(F1)$ [Hz]	Intensity
1	147.7256	18578.3838	6.34
2	112.2586	14117.9549	8.30
3	79.3476	9978.9756	7.24
4	77.4861	9744.8682	18.59
5	77.2322	9712.9370	19.31
6	76.9782	9680.9933	19.50
7	72.5406	9122.9083	2.65
8	56.3822	7090.7828	7.31
9	55.8152	7019.4753	7.34
10	53.5782	6738.1440	8.32
11	42.1746	5303.9954	8.64
12	39.1546	4924.1918	2.30
13	38.0192	4781.4007	8.56
14	37.2257	4681.6079	2.20
15	36.7662	4623.8199	2.41
16	36.2251	4555.7697	8.46
17	32.6047	4100.4581	8.41
18	28.5508	3590.6283	8.47
19	27.4260	3449.1703	8.88
20	17.9867	2262.0576	8.56
21	17.6521	2219.9774	8.62
22	15.7342	1978.7769	7.31
23	15.6802	1971.9857	7.44

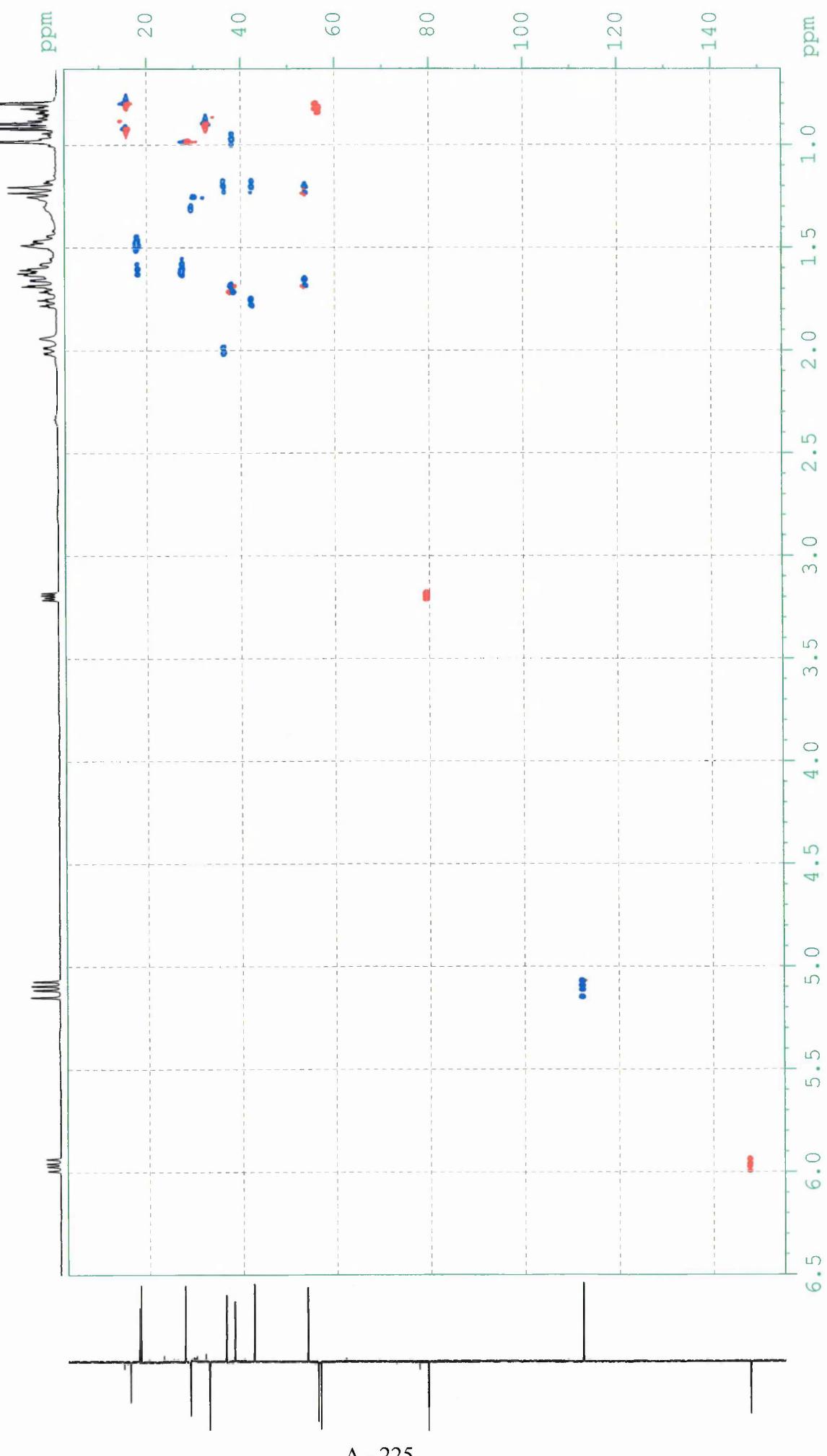


Spectrum 26.2:  $^{13}\text{C}$  NMR spectrum of compound 6.10 ( $\text{CDCl}_3$ )

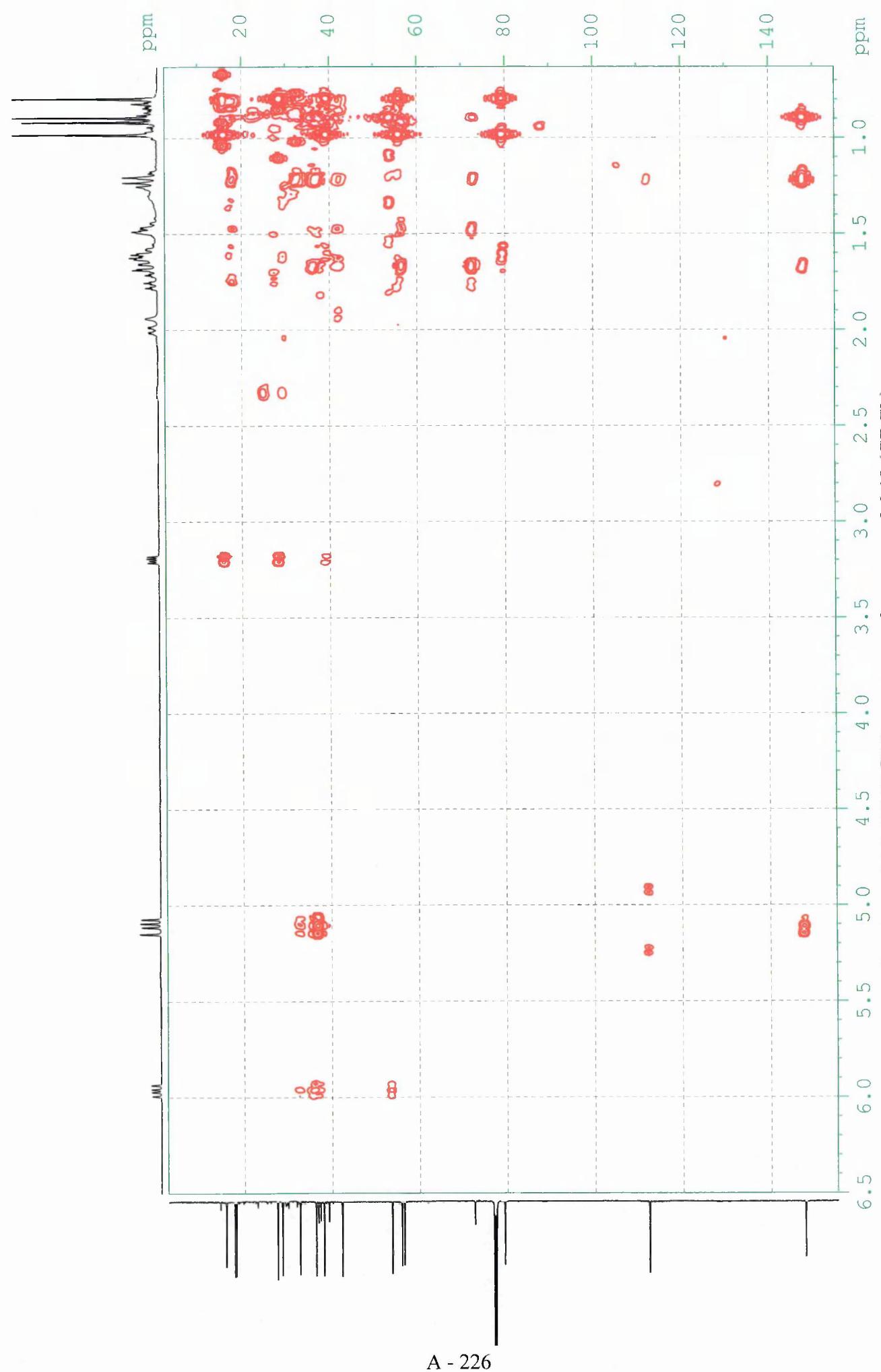


Spectrum 26.3: DEPT spectrum of compound 6.10 ( $\text{CDCl}_3$ )

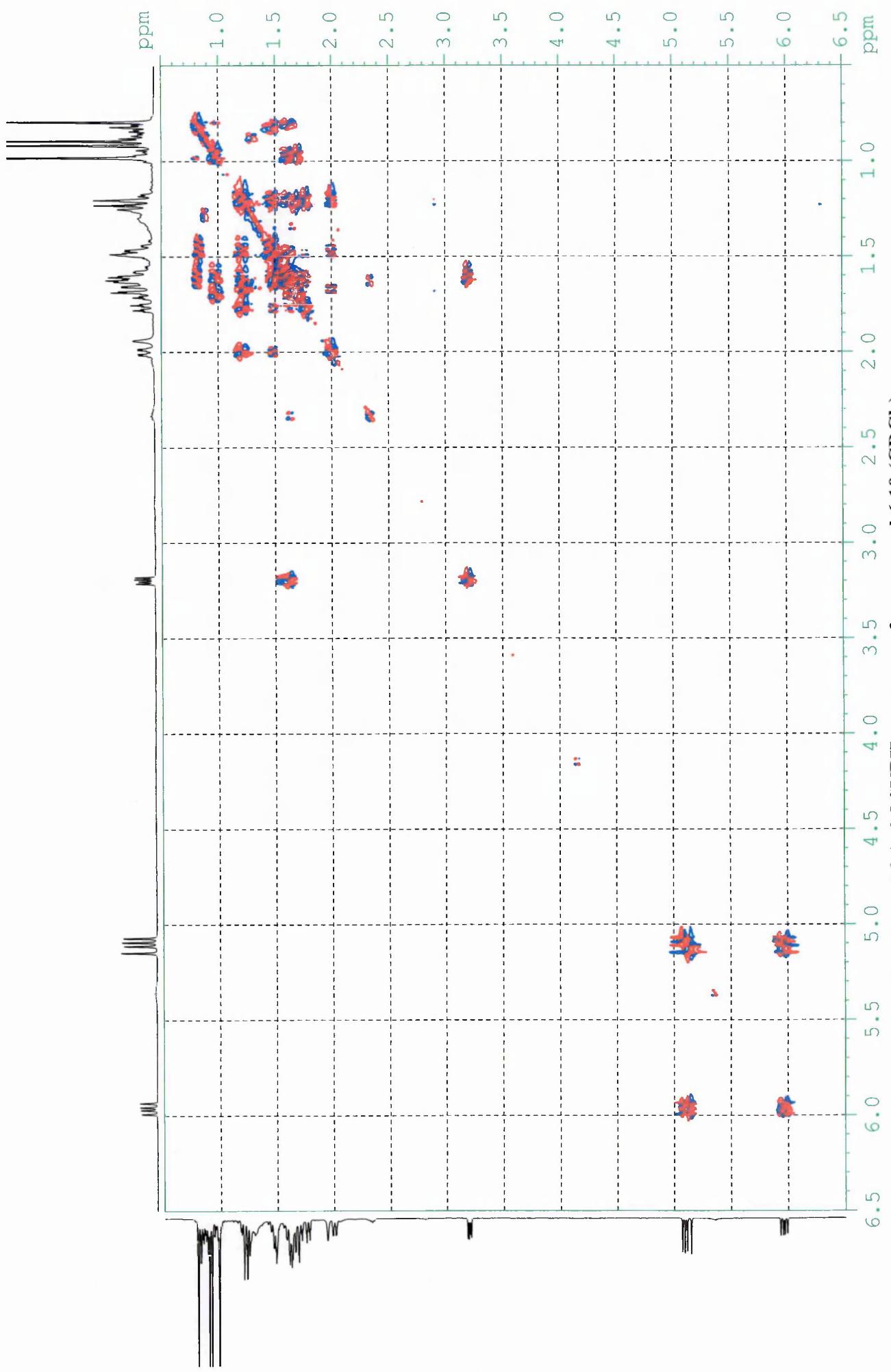
**Spectrum 26.4: HSQCDEPT spectrum of compound 6.10 ( $\text{CDCl}_3$ )**



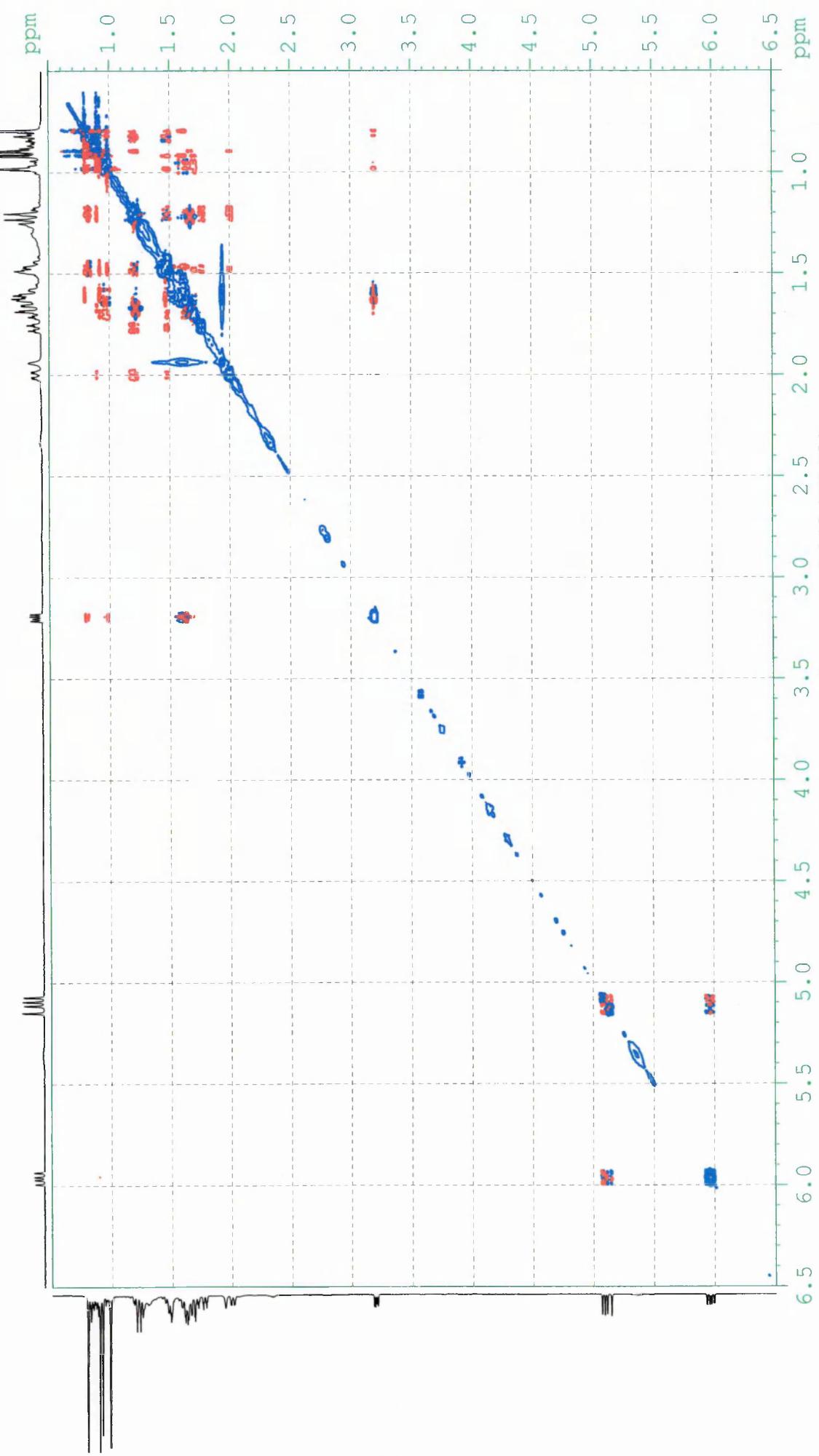
**Spectrum 26.5: HMBCLP spectrum of compound 6.10 ( $\text{CDCl}_3$ )**

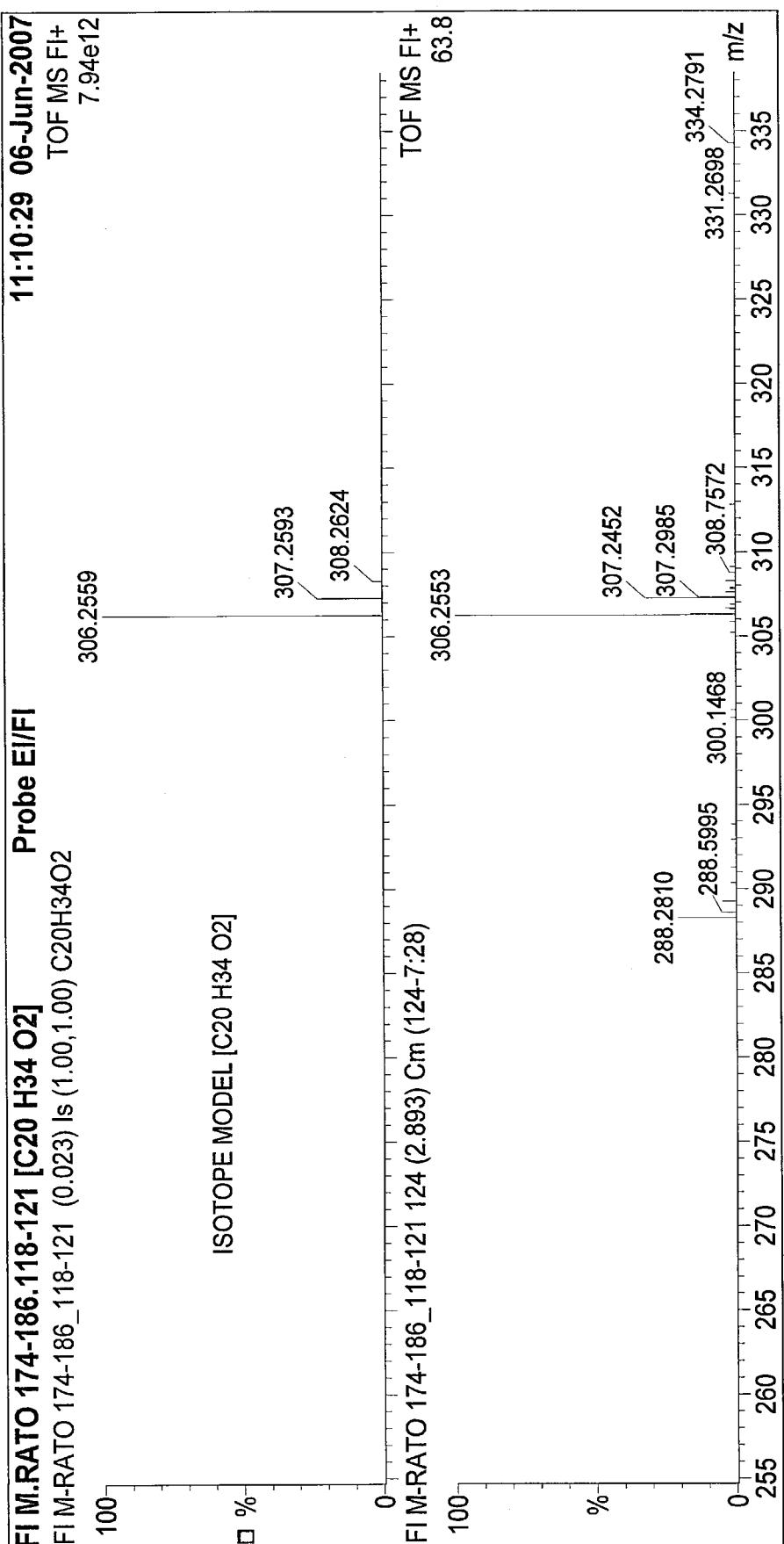


**Spectrum 26.6: COSYPH spectrum of compound 6.10 ( $\text{CDCl}_3$ )**

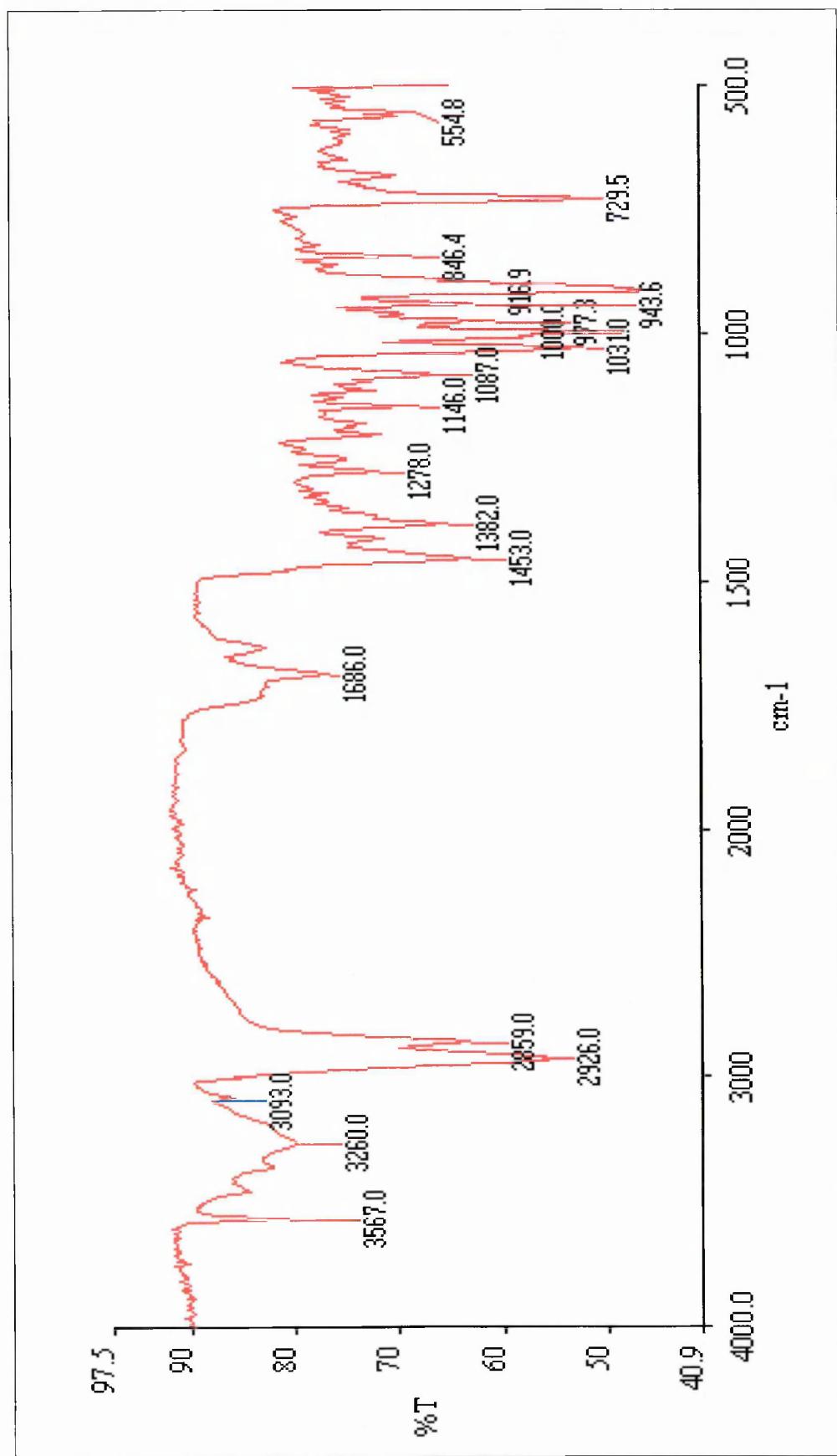


**Spectrum 26.7: NOESY spectrum of compound 6.10 ( $\text{CDCl}_3$ )**

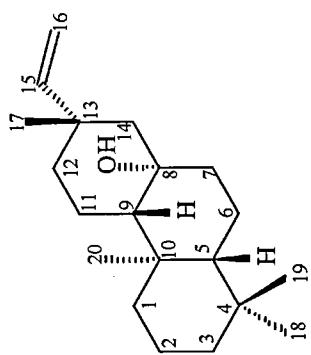
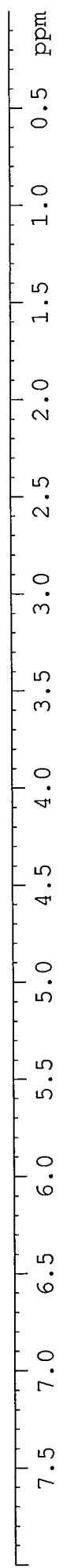




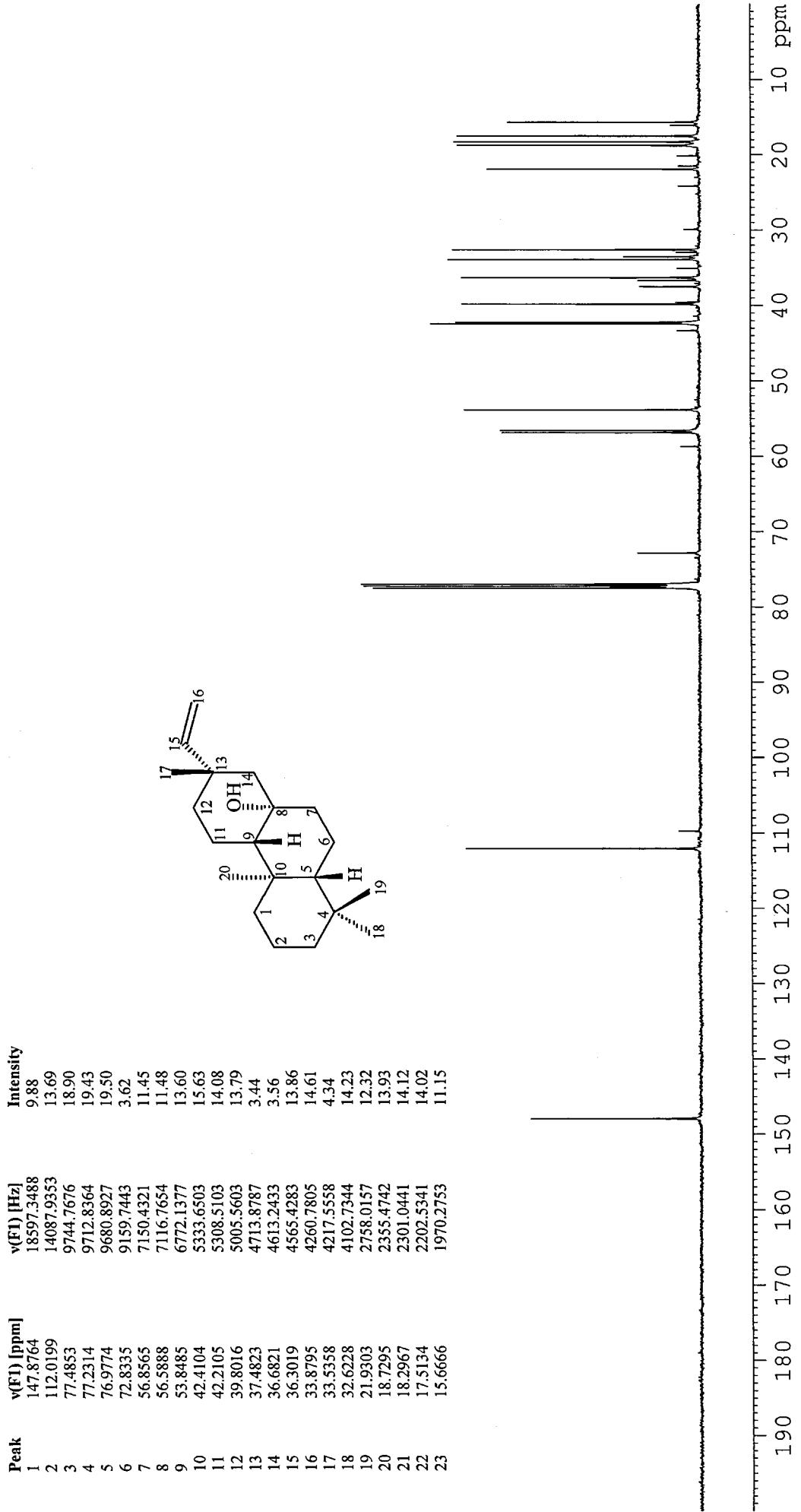
Spectrum 26.9: FTIR spectrum of compound 6.10

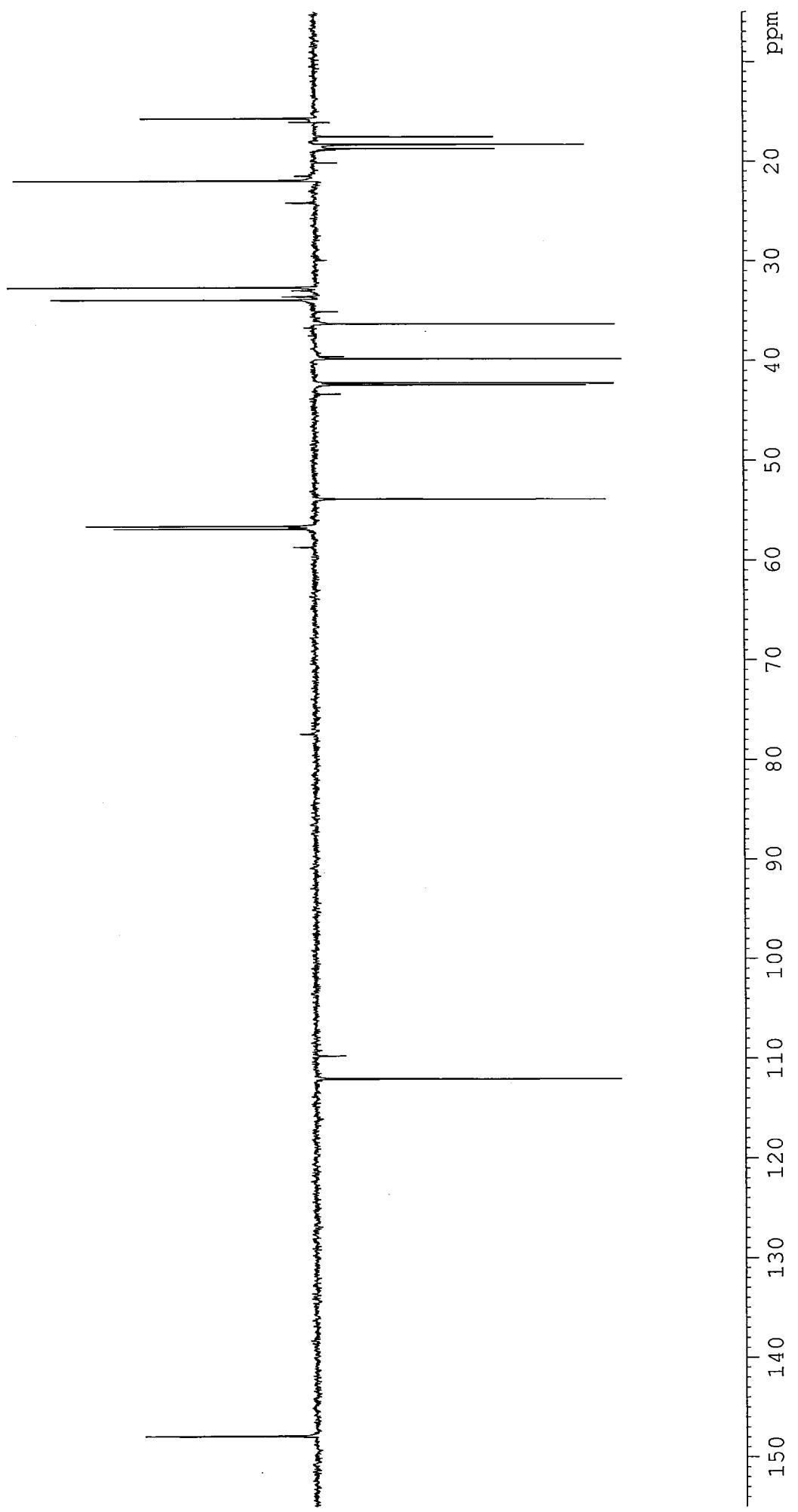


Spectrum 27.1:  $^1\text{H}$  NMR spectrum of compound 6.11 ( $\text{CDCl}_3$ )



**Spectrum 27.2:**  $^{13}\text{C}$  NMR spectrum of compound 6.11 ( $\text{CDCl}_3$ )

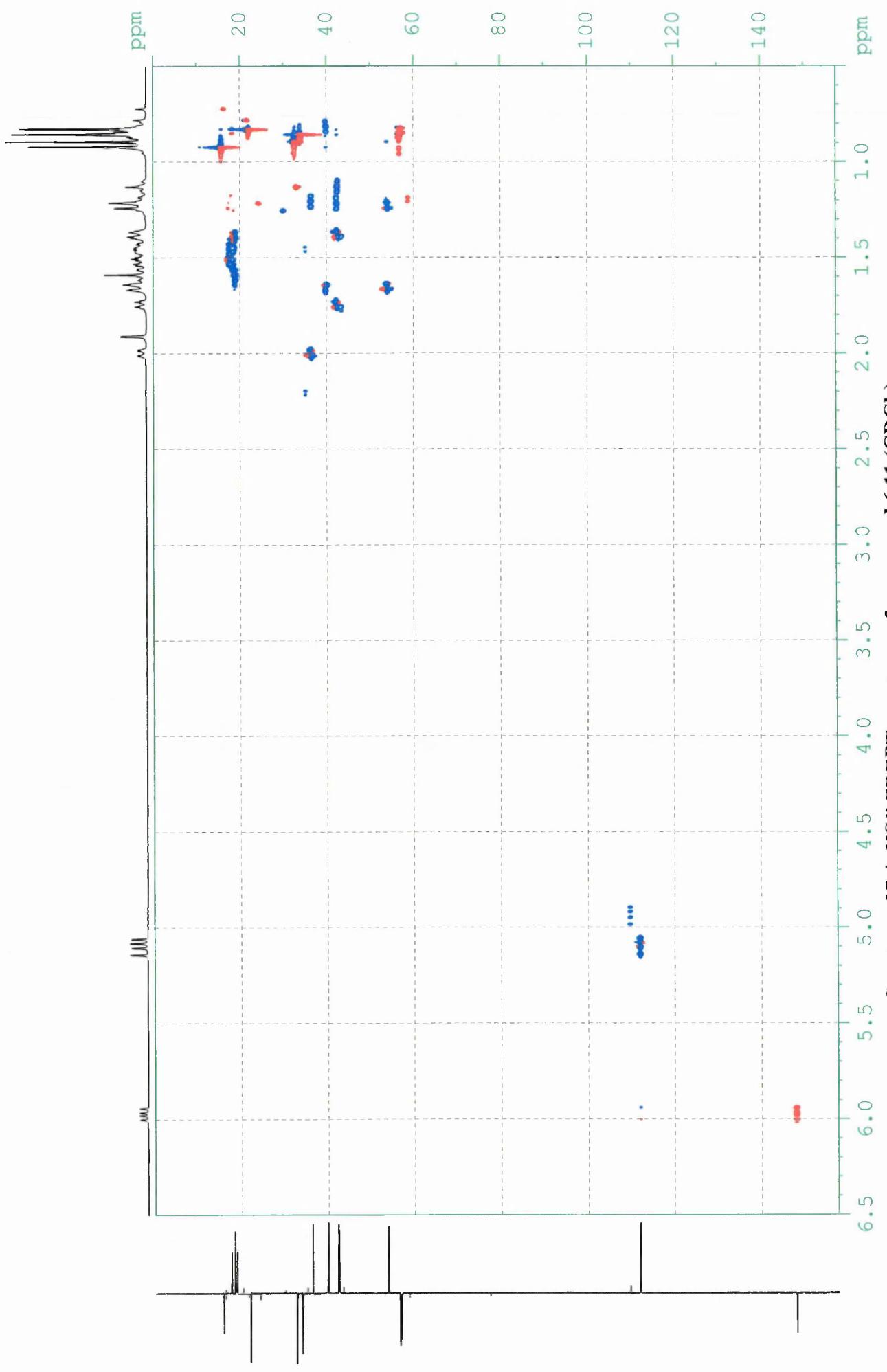




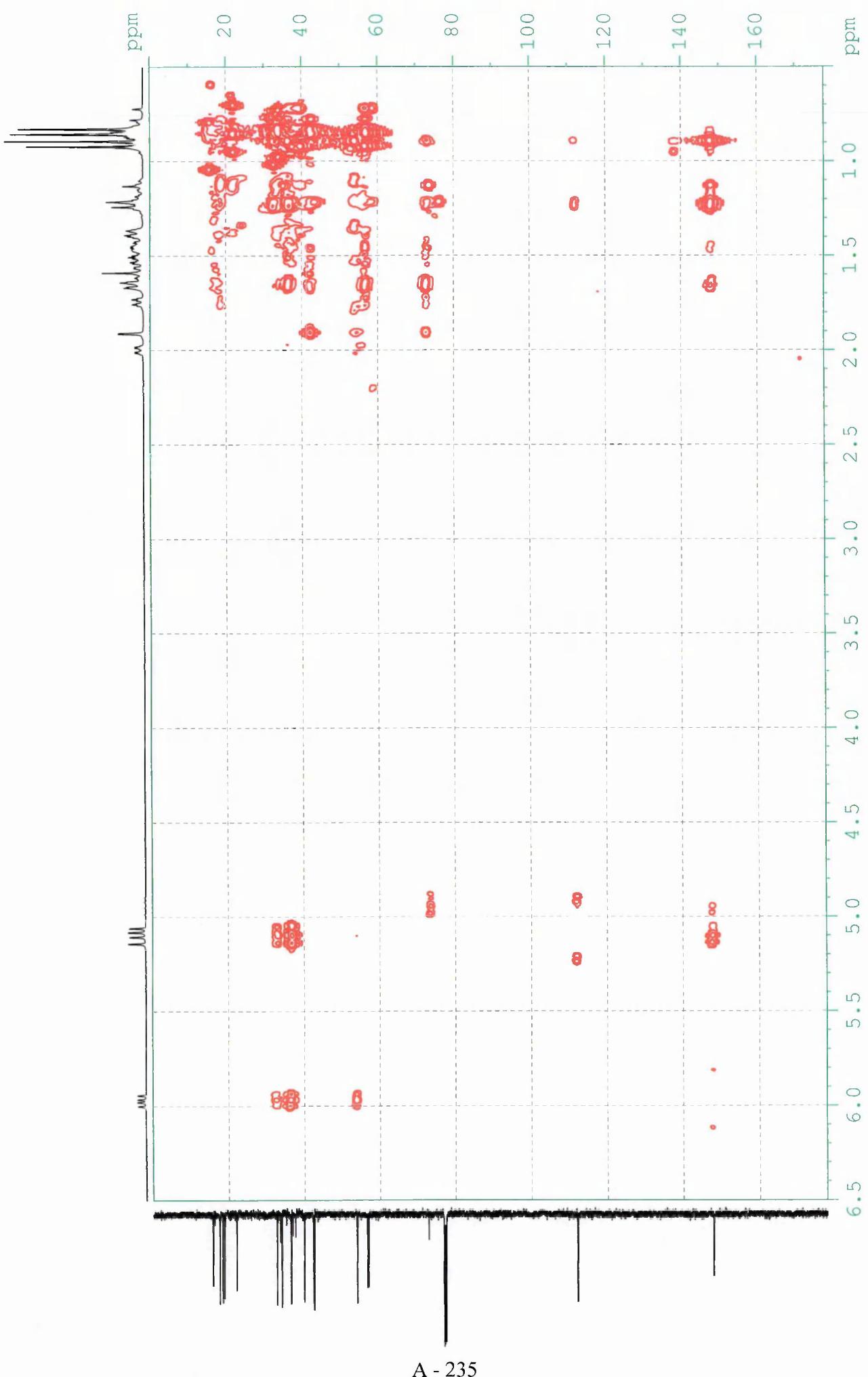
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Spectrum 27.3: DEPT spectrum of compound 6.11 ( $\text{CDCl}_3$ )

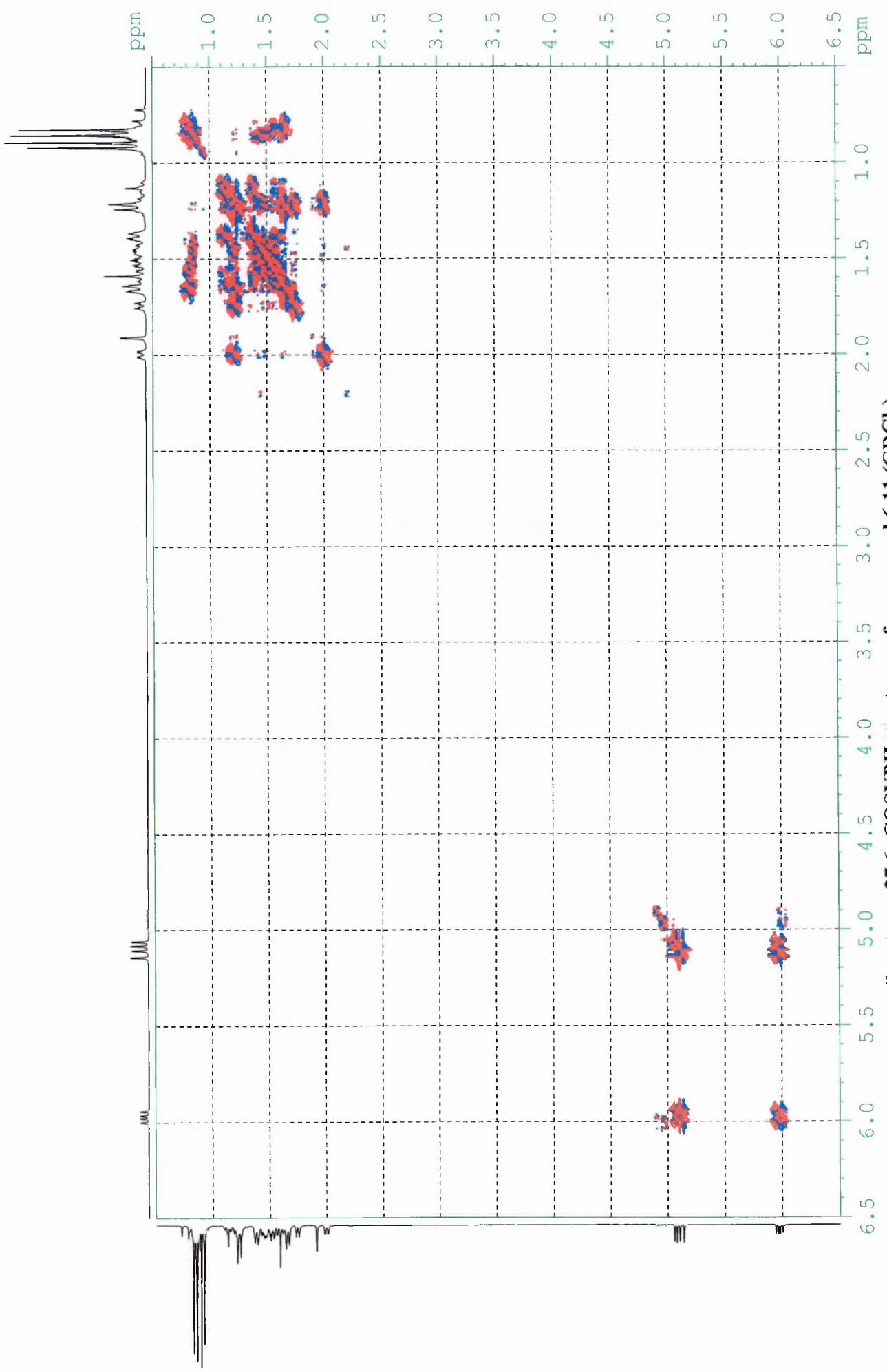
**Spectrum 27.4: HSQCDEPT spectrum of compound 6.11 ( $\text{CDCl}_3$ )**



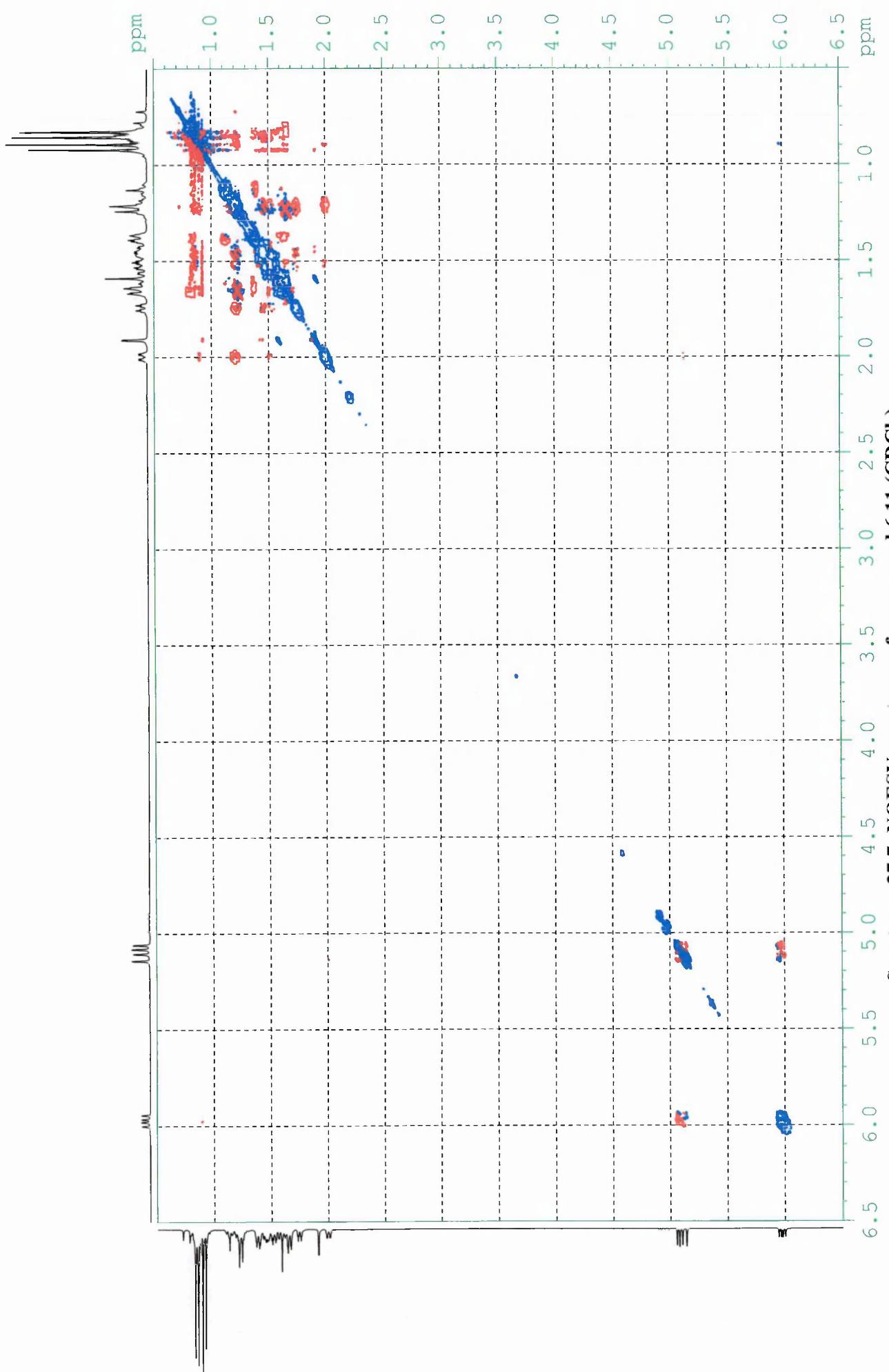
**Spectrum 27.5: HMBCCP spectrum of compound 6.11 ( $\text{CDCl}_3$ )**



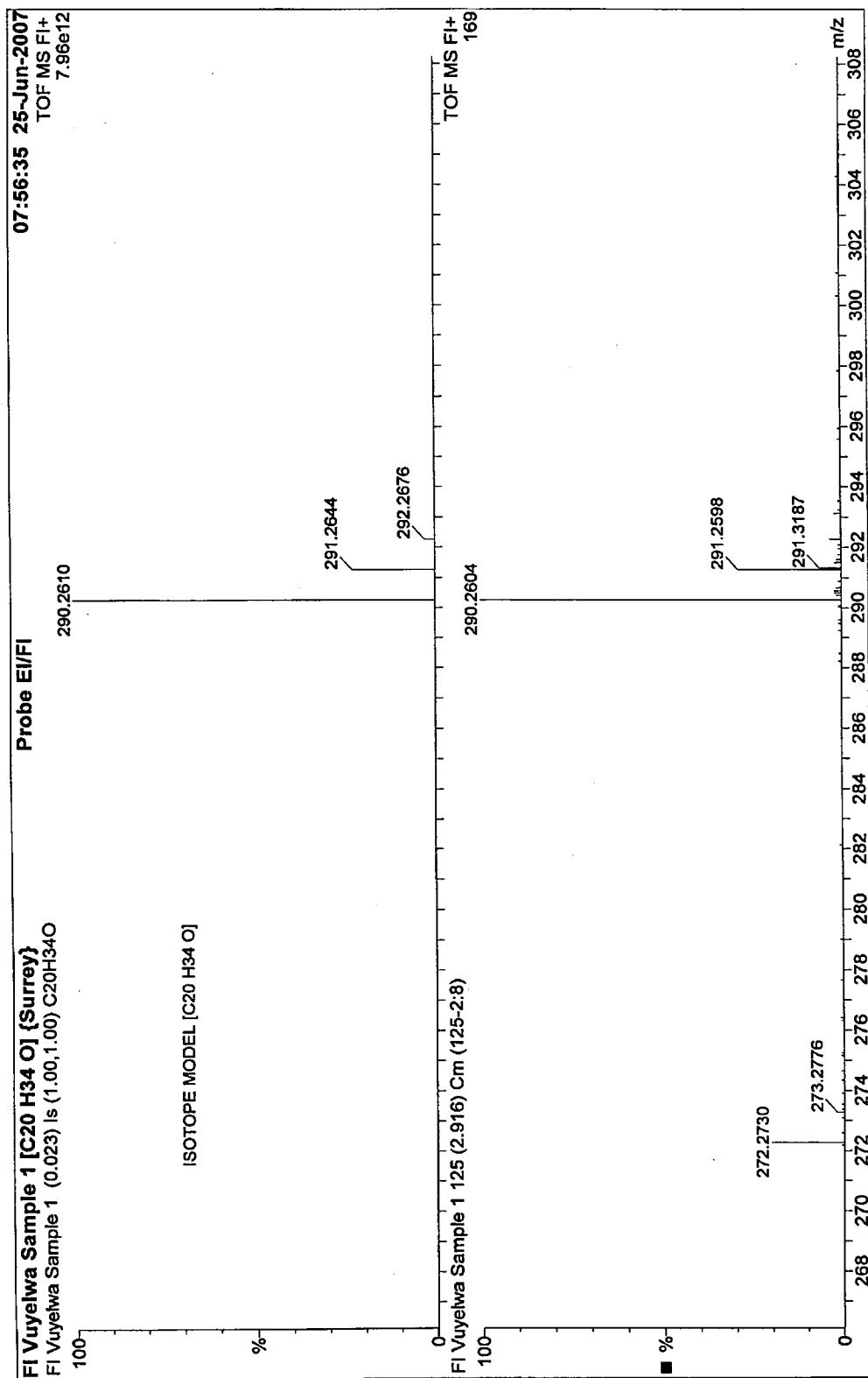
**Spectrum 27.6: COSYPH spectrum of compound 6.11 ( $\text{CDCl}_3$ )**



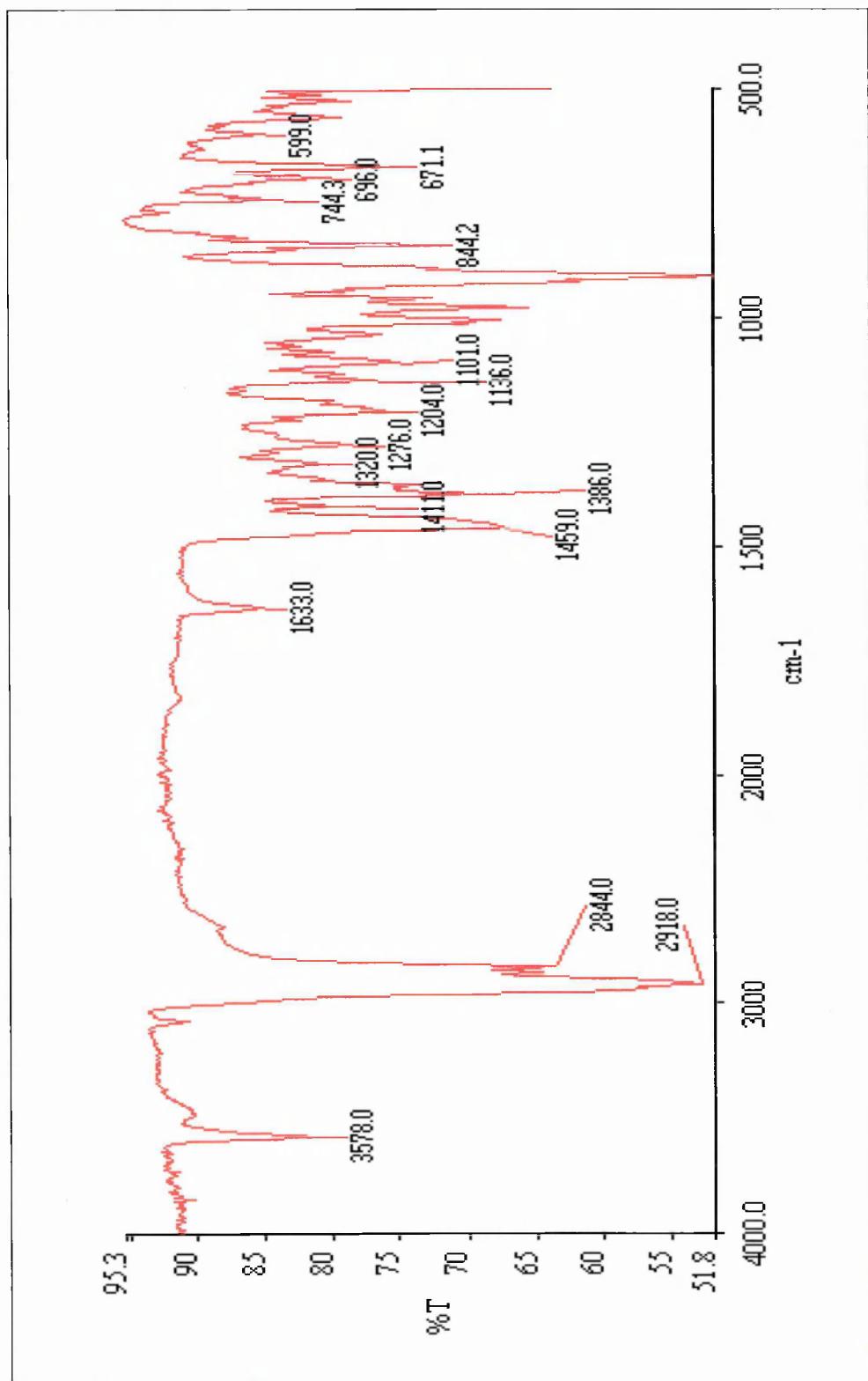
**Spectrum 27.7: NOESY spectrum of compound 6.11 ( $\text{CDCl}_3$ )**



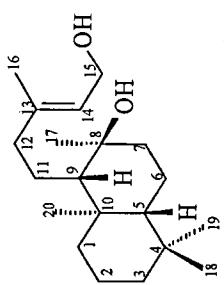
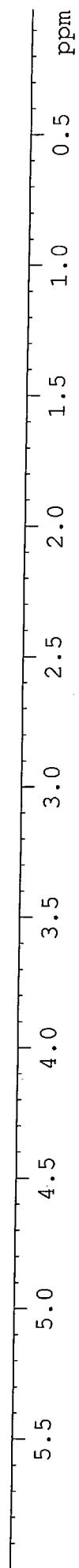
Spectrum 27.8: Mass spectrum of compound 6.11



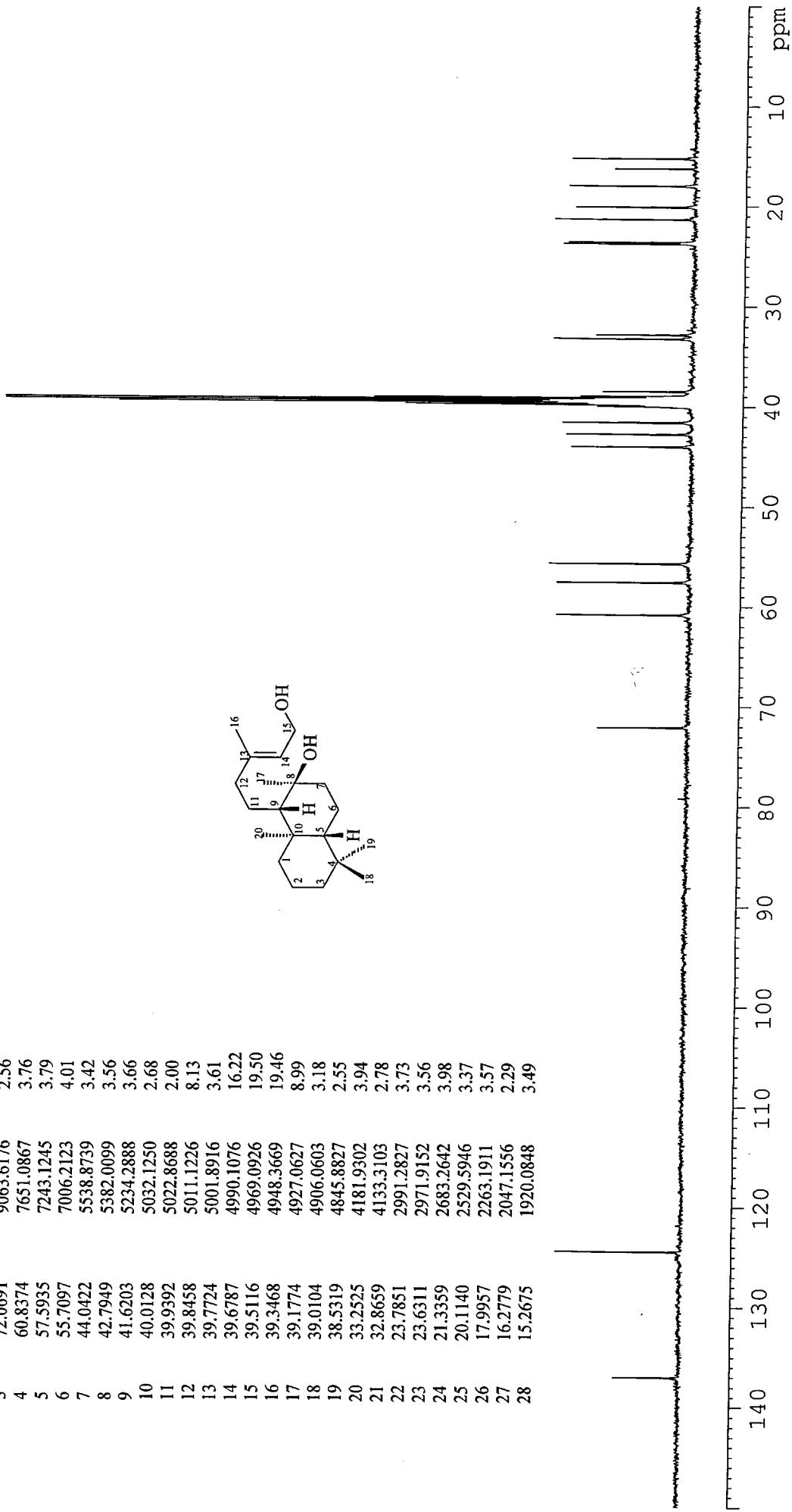
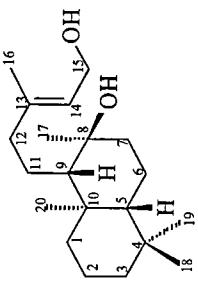
Spectrum 27.9: FTIR spectrum of compound 6.11



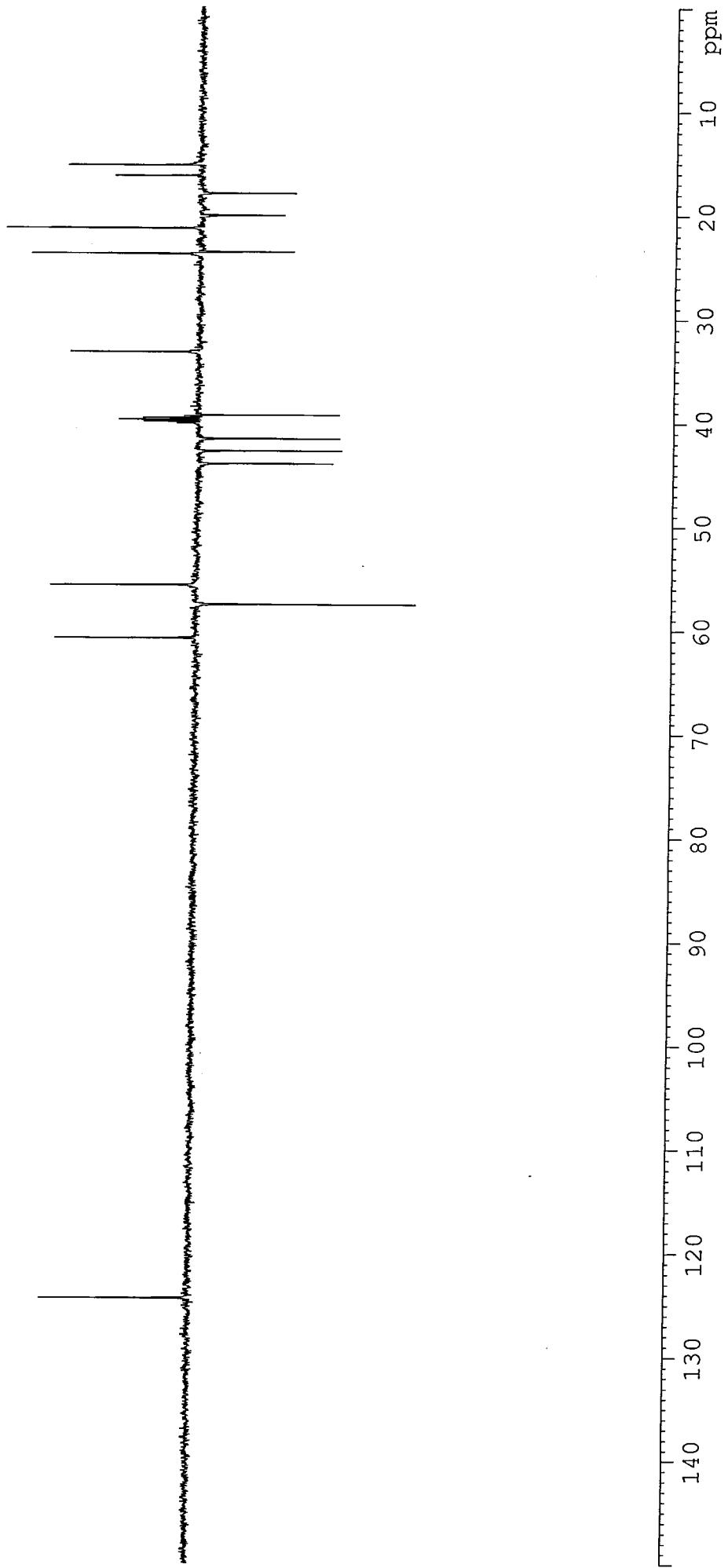
Spectrum 28.1:  $^1\text{H}$  NMR spectrum of compound 6.7 (DMSO)



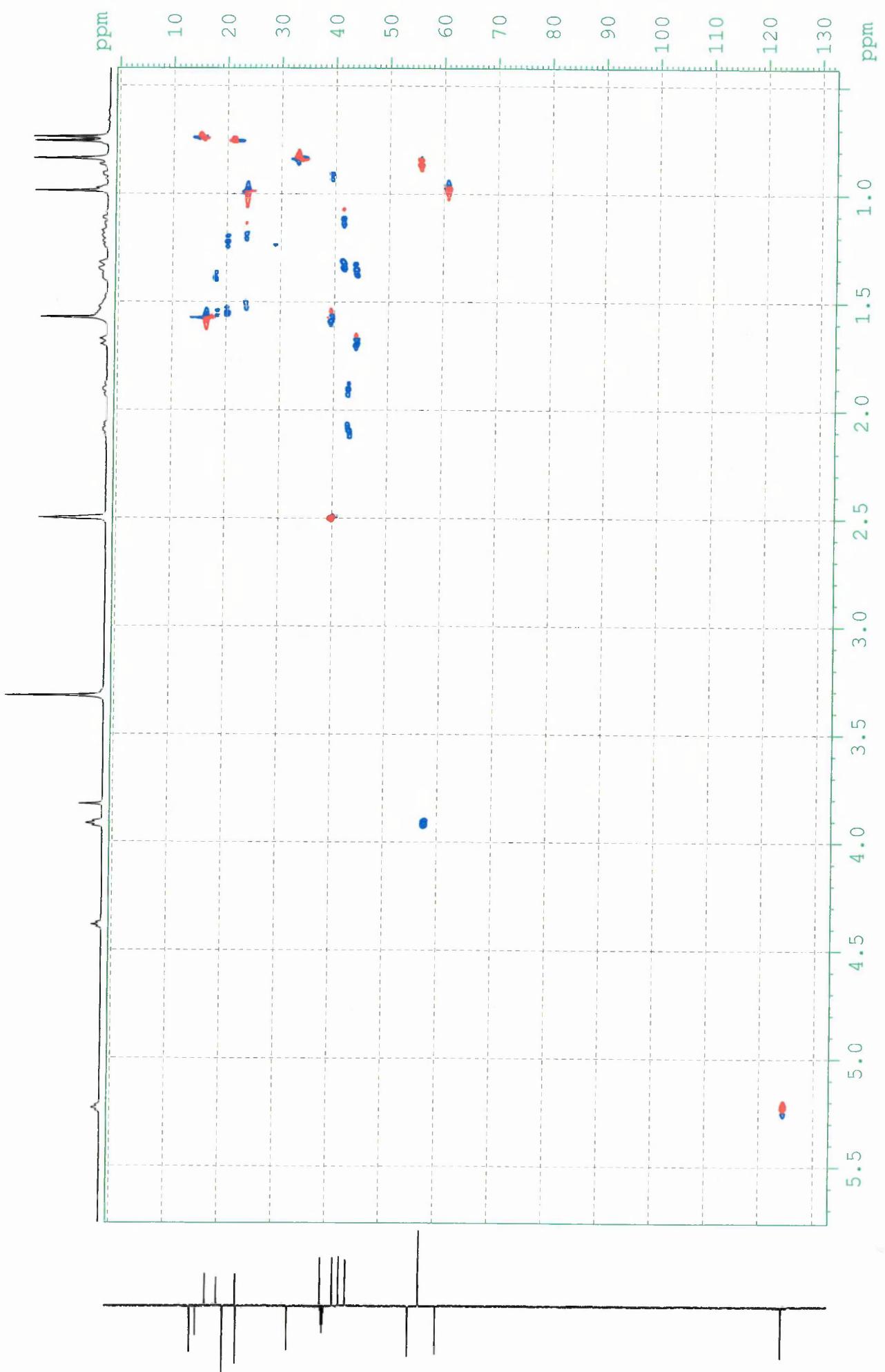
Peak	$\nu(F)$ [ppm]	$\nu(F1)$ [Hz]	Intensity
1	136.9947	17228.8481	1.87
2	124.4387	15649.7694	3.57
3	72.0691	9063.6176	2.56
4	60.8374	7651.0867	3.76
5	57.5935	7243.1245	3.79
6	55.7097	7006.2123	4.01
7	44.0422	5538.8739	3.42
8	42.7949	5382.0099	3.56
9	41.6203	5234.2888	3.66
10	40.0128	5032.1250	2.68
11	39.9392	5022.8688	2.00
12	39.8458	5011.1226	8.13
13	39.7724	5001.8916	3.61
14	39.6787	4990.1076	16.22
15	39.5116	4969.0926	19.50
16	39.3468	4948.3669	19.46
17	39.1774	4927.0627	8.99
18	39.0104	4906.0603	3.18
19	38.5319	4845.8827	2.55
20	33.2525	4181.9302	3.94
21	32.8659	4133.3103	2.78
22	23.7851	2991.2827	3.73
23	23.6311	2971.9152	3.56
24	21.3359	2683.2642	3.98
25	20.1140	2529.5946	3.37
26	17.9957	2263.1911	3.57
27	16.2279	2047.1556	2.29
28	15.2675	1920.0848	3.49



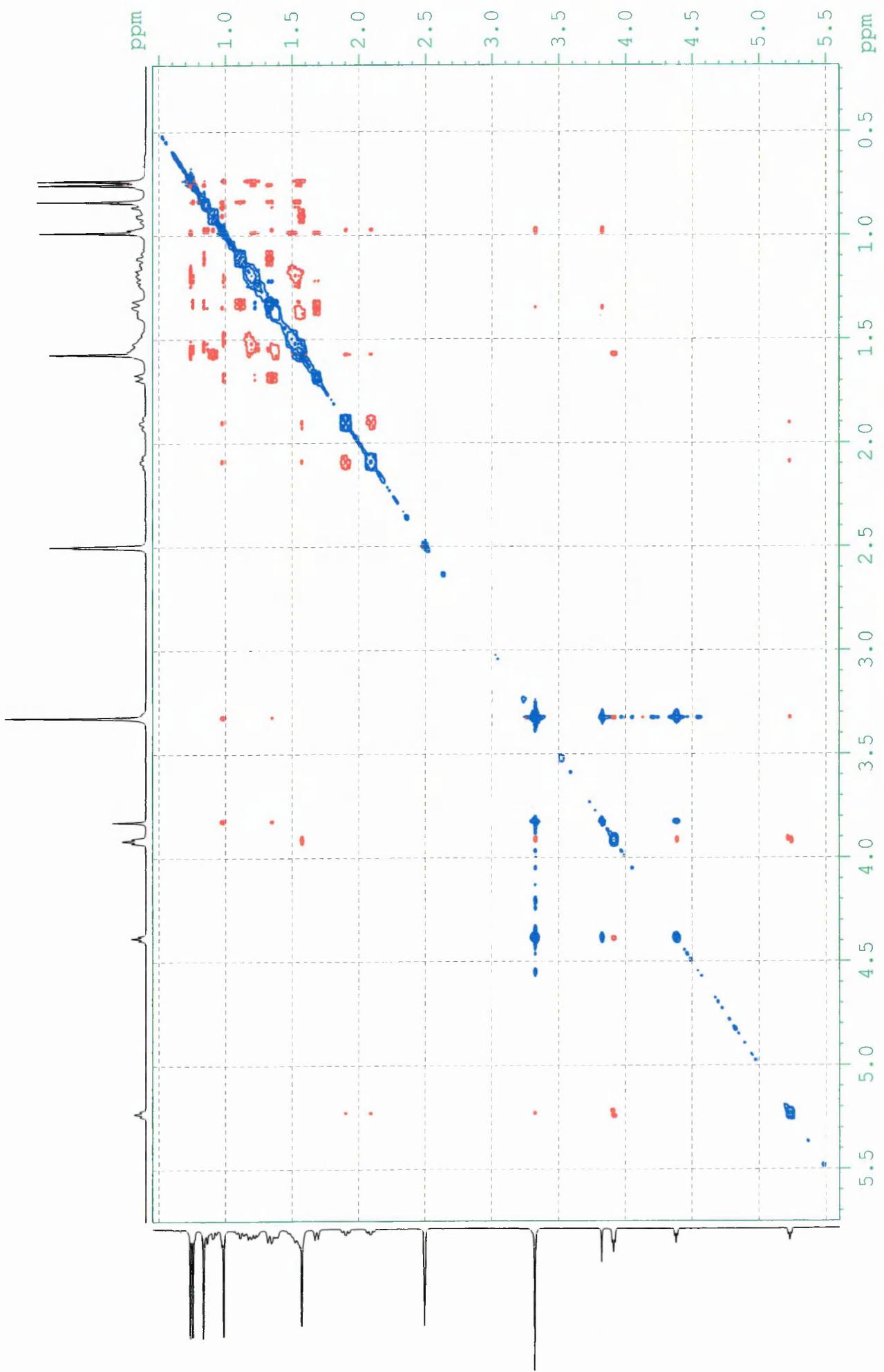
Spectrum 28.2: <sup>13</sup>C NMR spectrum of compound 6.7 (DMSO)



Spectrum 28.3: DEPT spectrum of compound 6.7 (DMSO)



Spectrum 28.4: HSQCDEPT spectrum of compound 6.7 (DMSO)



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Spectrum 28.5: NOESY spectrum of compound 6.7 (DMSO)