

**Aryldihydronaphthalene-type lignans from *Bursera fagaroides* var. *fagaroides* and their antimitotic mechanism of action**

Mayra Y. Antúnez Mojica<sup>a</sup>, Alejandra León<sup>a</sup>, Andrés M. Rojas-Sepúlveda<sup>b</sup>, Silvia Marquina<sup>a</sup>, Mario A. Mendieta-Serrano<sup>c</sup>, Enrique Salas-Vidal<sup>c,\*</sup>, María Luisa Villarreal<sup>d</sup>, Laura Alvarez<sup>a,\*</sup>

<sup>a</sup>Centro de Investigaciones Químicas-IICBA, and <sup>d</sup>Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos, Avenida Universidad 1001, Chamilpa, Cuernavaca, Morelos 62209, México. <sup>b</sup>Facultad de Ciencias, Universidad Antonio Nariño, Armenia, Quindío, Colombia. <sup>c</sup>Departamento de Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México, A.P. 510-3, Cuernavaca, Morelos 62271, México.

Corresponding authors

Laura Alvarez

Centro de Investigaciones Químicas-IICBA, Universidad Autónoma del Estado de Morelos.  
Av. Universidad 1001, Col. Chamilpa, Cuernavaca, Morelos 62209, México

[lalvarez@uaem.mx](mailto:lalvarez@uaem.mx)

Phone: +52 777 3297997; +52 777 3079200

Enrique Salas Vidal

Departamento de Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México, A.P. 510-3, Cuernavaca, Morelos 62271, México.

[esalas@ibt.unam.mx](mailto:esalas@ibt.unam.mx)

Phone: +52 7773291663; Fax: +52 777 3172388

## List of Figures

**Figure S.1.1**  $^1\text{H}$  NMR spectrum of compound **1** (400 MHz,  $\text{CDCl}_3$ )

**Figure S.1.2.**  $^{13}\text{C}$  NMR spectrum of compound **1** (100 MHz,  $\text{CDCl}_3$ )

**Figure S.1.3.** HSQC spectrum of compound **1** ( $\text{CDCl}_3$ )

**Figure S.1.4.** HMBC spectrum of compound **1**( $\text{CDCl}_3$ )

**Figure S.1.5.** Mass spectrum of compound **1**

**Figure S.1.6.** IR spectrum of compound **1**

**Figure S.1.7.** UV spectrum of compound **1**

**Figure S.1.8.** CD spectrum of compound **1**

**Figure S.2.1.**  $^1\text{H}$  NMR spectrum of compound **2** (400 MHz,  $\text{CDCl}_3$ )

**Figure S.2.2.**  $^{13}\text{C}$  NMR spectrum of compound **2** (100 MHz,  $\text{CDCl}_3$ )

**Figure S.2.3.** HSQC spectrum of compound **2** ( $\text{CDCl}_3$ )

**Figure S.2.4.** HMBC spectrum of compound **2**( $\text{CDCl}_3$ )

**Figure S.2.5.** Mass spectrum of compound **2**

**Figure S.2.6.** IR spectrum of compound **2**

**Figure S.2.7.** UV spectrum of compound **2**

**Figure S.2.8.** CD spectrum of compound **2**

**Figure S.3.1.**  $^1\text{H}$  NMR spectrum of compound **3** (400 MHz,  $\text{CDCl}_3$ )

**Figure S.3.2.**  $^{13}\text{C}$  NMR spectrum of compound **3** (100 MHz,  $\text{CDCl}_3$ )

**Figure S.3.3.** HSQC spectrum of compound **3** ( $\text{CDCl}_3$ )

**Figure S.3.4.** HMBC spectrum of compound **3**( $\text{CDCl}_3$ )

**Figure S.3.5.** Mass spectrum of compound **3**

**Figure S.3.6.** IR spectrum of compound **3**

**Figure S.3.7.** UV spectrum of compound **3**

**Figure S.3.8.** CD spectrum of compound **3**

## Tables

**Table S.1.**

**Table S. 2.**

Alvarez id  
Sample: BF 4.15x2  
Sample ID: s\_20120222\_03  
File: 0703.fid  
Pulse Sequence: s2pul  
Solvent: cdc13  
Temp. 25.0 C / 298.1 K  
Sample #7, Operator: gaby  
File: 0703  
Mercury-400BB "mercury400"  
Relax. delay 1.000 sec  
Pulse 45.0 degrees  
Acq. time 1.998 sec  
Width 3856.5 Hz  
8 repetitions  
OBSERVE H1, 399.6281478 MHz  
DATA PROCESSING  
FT size 16384  
Total time 0 min, 25 sec

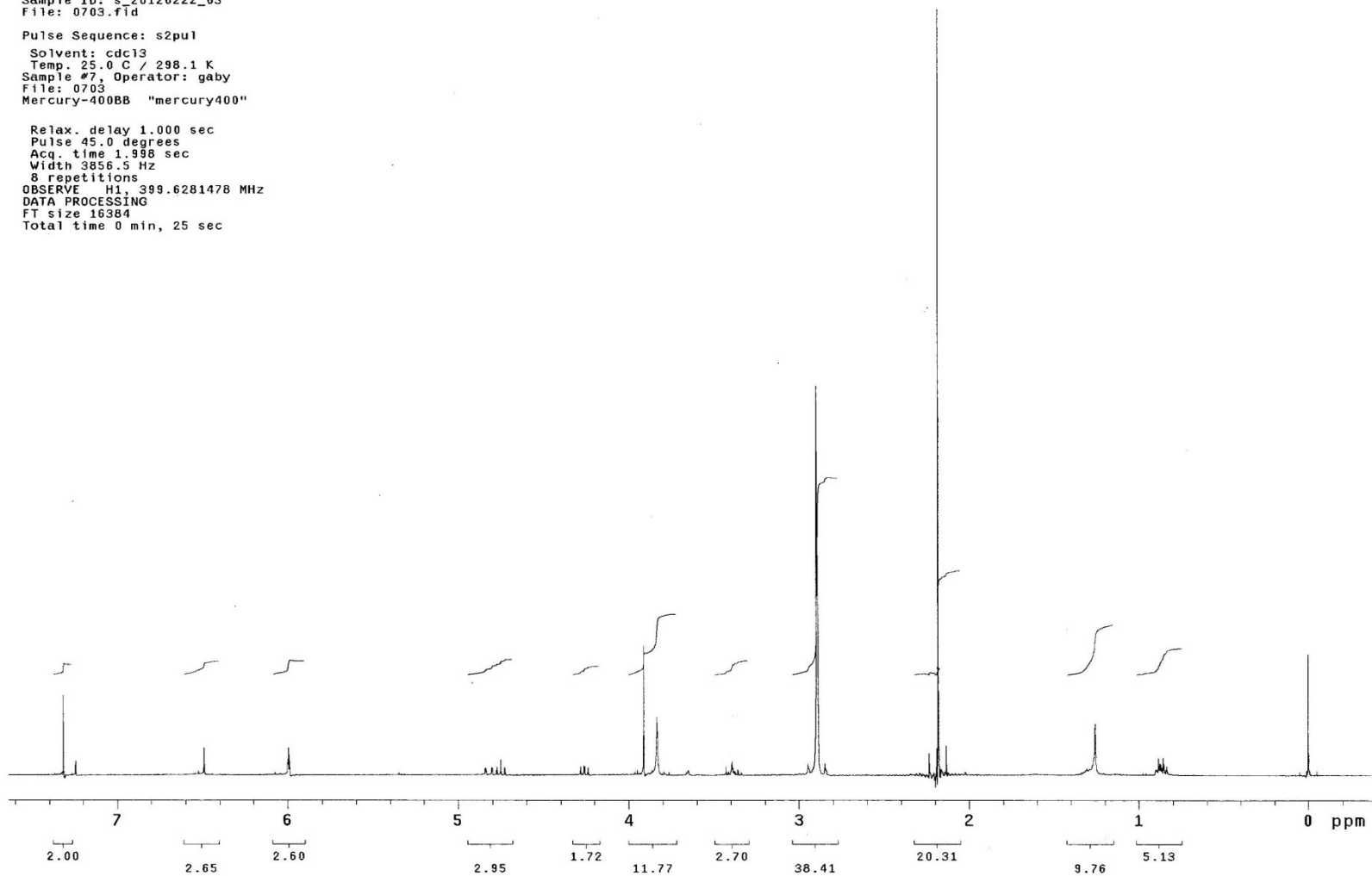
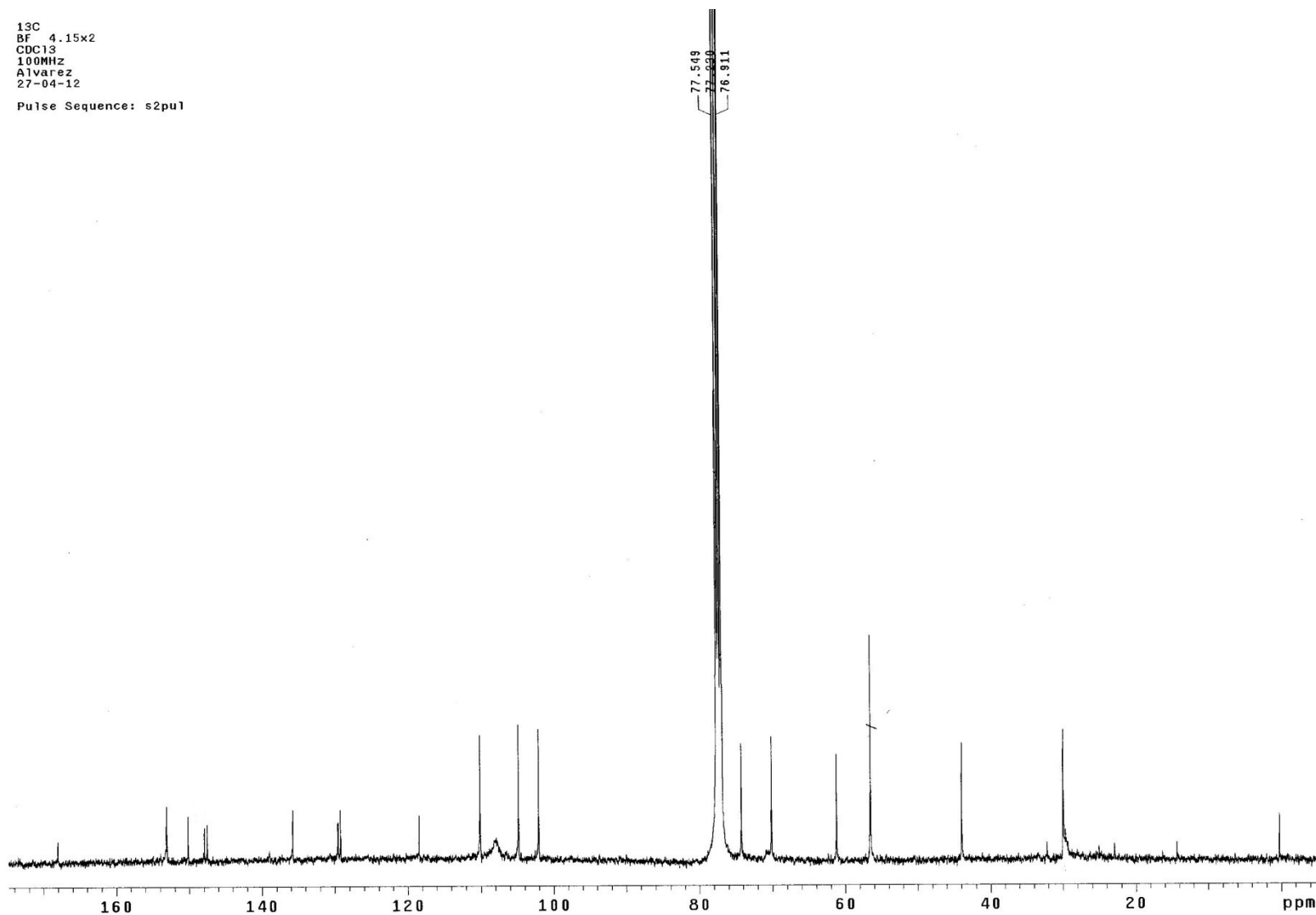


Figure S.1.1 <sup>1</sup>H NMR spectrum of compound 1 (400 MHz, CDCl<sub>3</sub>)

<sup>13</sup>C  
BF 4.15x2  
CDC13  
100MHz  
Alvarez  
27-04-12  
Pulse Sequence: s2pu1



**Figure S.1.2.** <sup>13</sup>C NMR spectrum of compound **1** (100 MHz, CDCl<sub>3</sub>)

Alvarez id

Sample: BF 4.15x2  
Sample ID: s\_20120222\_03  
File: 0702.Fid

Pulse Sequence: gHSQC

Solvent: cdc13  
Temp. 25.0 C / 298.1 K  
Sample #7, Operator: gaby  
File: 0702  
Mercury-400BB "mercury400"

Relax. delay 1.301 sec  
Acq. time 0.199 sec  
Width 3856.5 Hz  
2D Width 17083.1 Hz  
32 repetitions  
2 x 200 increments  
OBSERVE H1, 399.6281421 MHz  
DECOUPLE C13, 100.4941204 MHz  
Power 45 dB  
on during acquisition  
off during delay  
GARP-1 modulated  
DATA PROCESSING  
Gauss apodization 0.029 sec  
F1 DATA PROCESSING  
Gauss apodization 0.005 sec  
FT size 1096 x 2048  
Total time 5 hr, 39 min, 38 sec

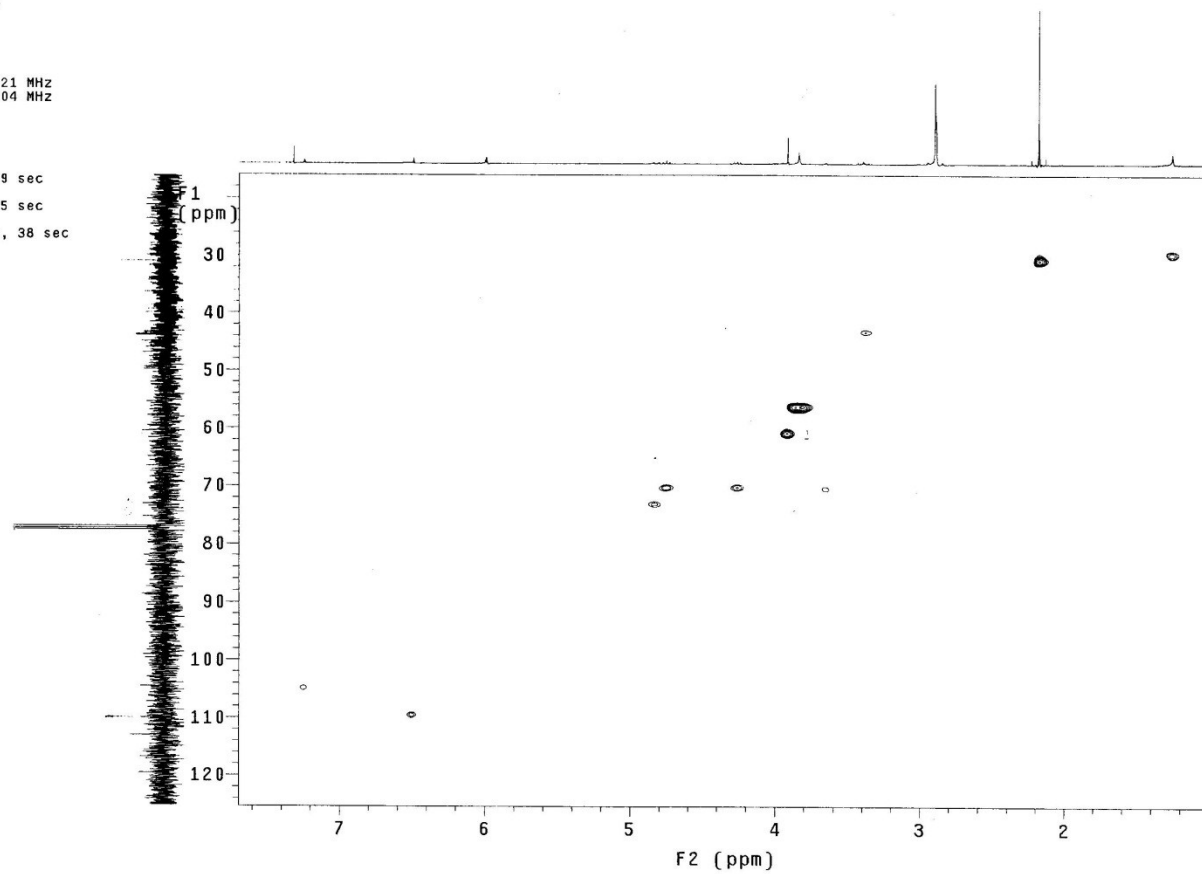


Figure S.1.3. HSQC spectrum of compound 1 ( $\text{CDCl}_3$ )

Alvarez id

Sample: BF 4.15x2  
Sample ID: s\_20120222\_03  
File: 0706.ftd

Pulse Sequence: gHMBC

Solvent: cdcl3  
Temp. 25.0 C / 298.1 K  
Sample #7, Operator: gaby  
File: 0706  
Mercury-400BB "mercury400"

Relax. delay 1.500 sec  
Mixing 0.080 sec  
Acq. time 0.128 sec  
Width 3856.5 Hz  
2D Width 24118.2 Hz  
64 repetitions  
200 increments  
OBSERVE H1, 399.6281436 MHz  
DATA PROCESSING  
Sine bell 0.055 sec  
F1 DATA PROCESSING  
Sine bell 0.005 sec  
FT size 2048 x 2048  
Total time 6 hr, 13 min, 23 sec

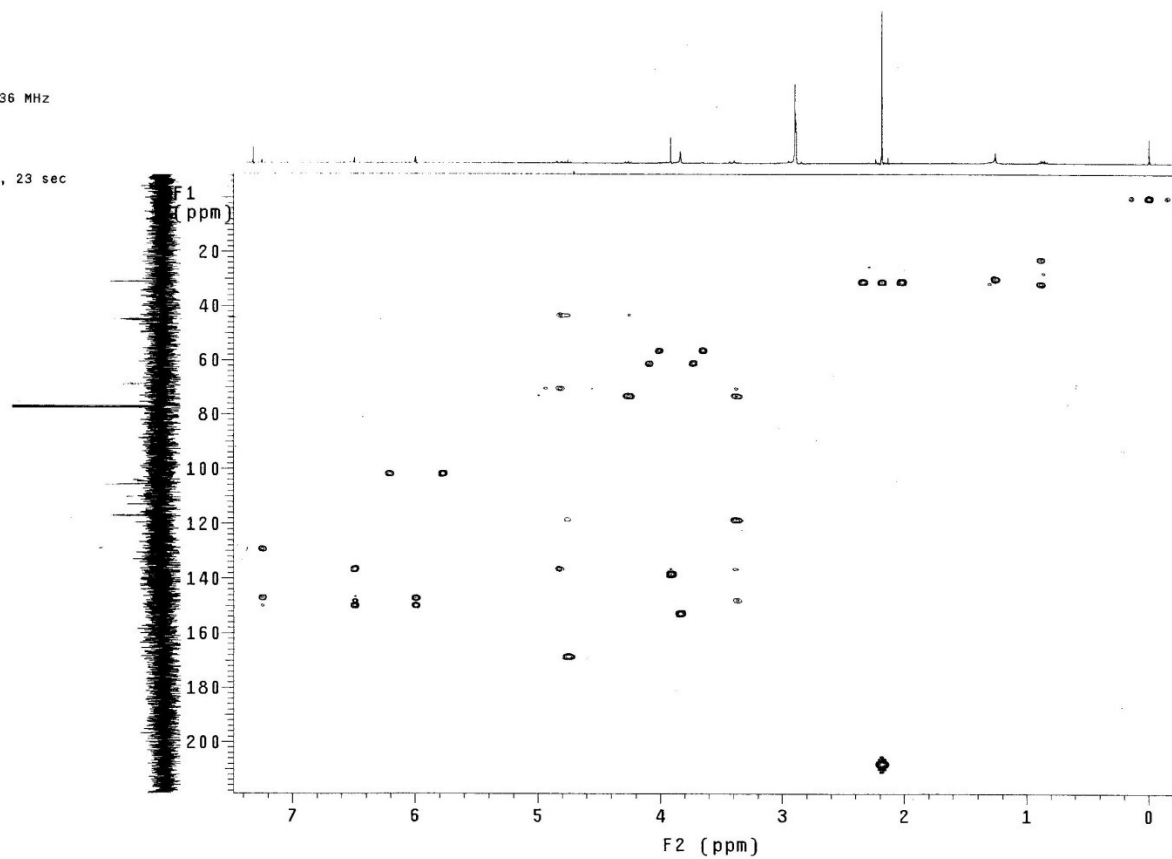


Figure S.1.4. HMBC spectrum of compound 1(CDCl<sub>3</sub>).



[ Mass Spectrum ]  
Data : LAB008 Date : 07-Mar-2012 01:38  
Sample: BF 4.5 Operator name Ing.Victoria Labastida G.  
Note : Dra.Laura Alvarez/Andres Centro de Investigaciones Quimicas UAEM  
Inlet : Direct Ion Mode : FAB+  
Spectrum Type : Normal Ion [MF-Linear]  
RT : 0.13 min Scan# : (2,3)  
BP : m/z 69.0000 Int. : 2.49  
Output m/z range : 40.0000 to 800.0000 Cut Level : 0.00 %

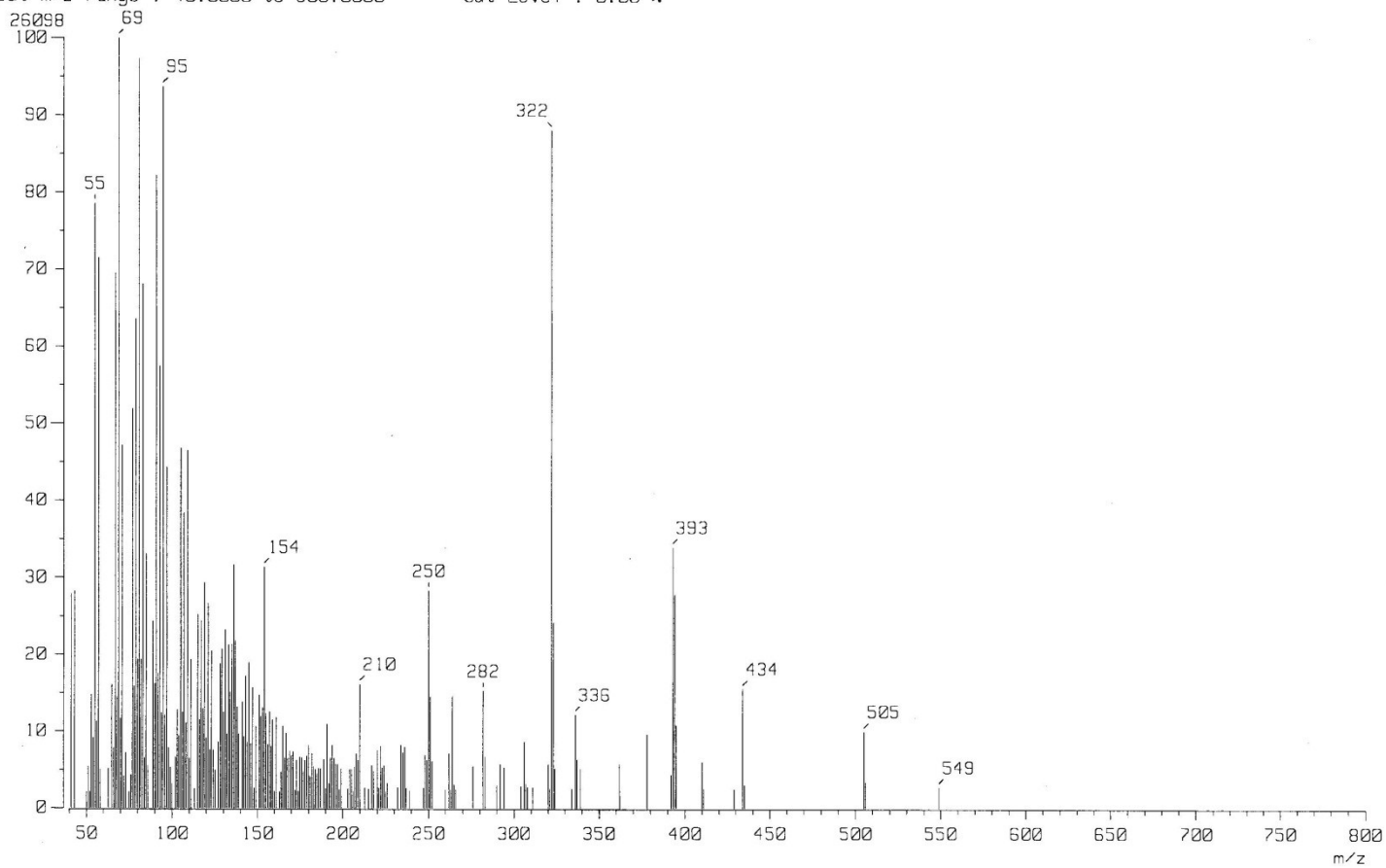


Figure S.1.5. Mass spectrum of compound 1.

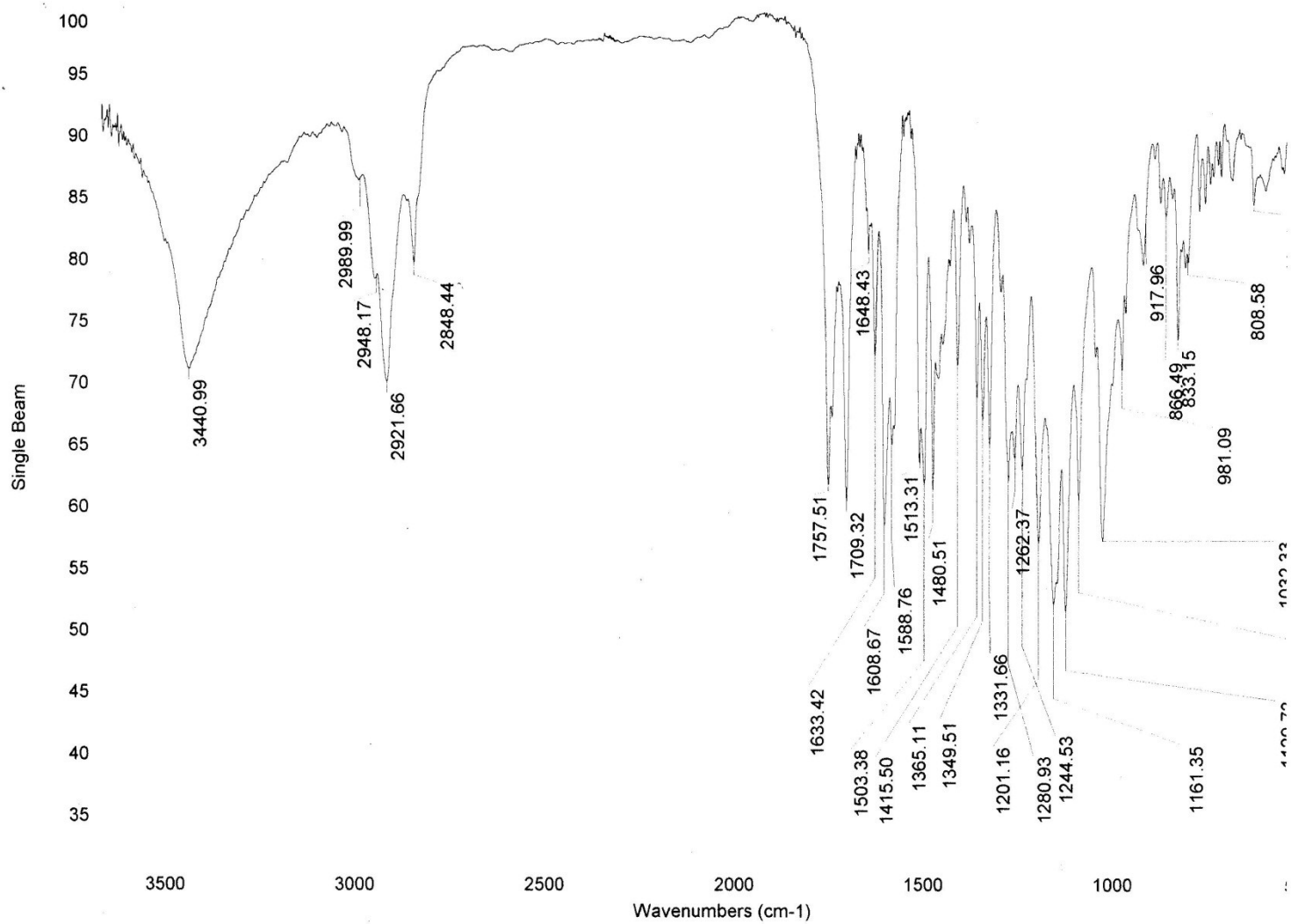
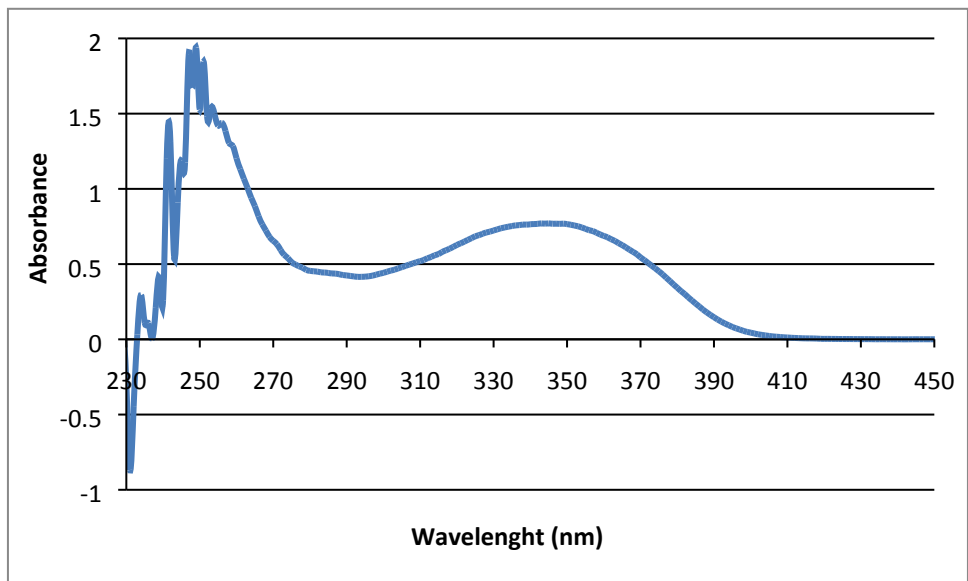
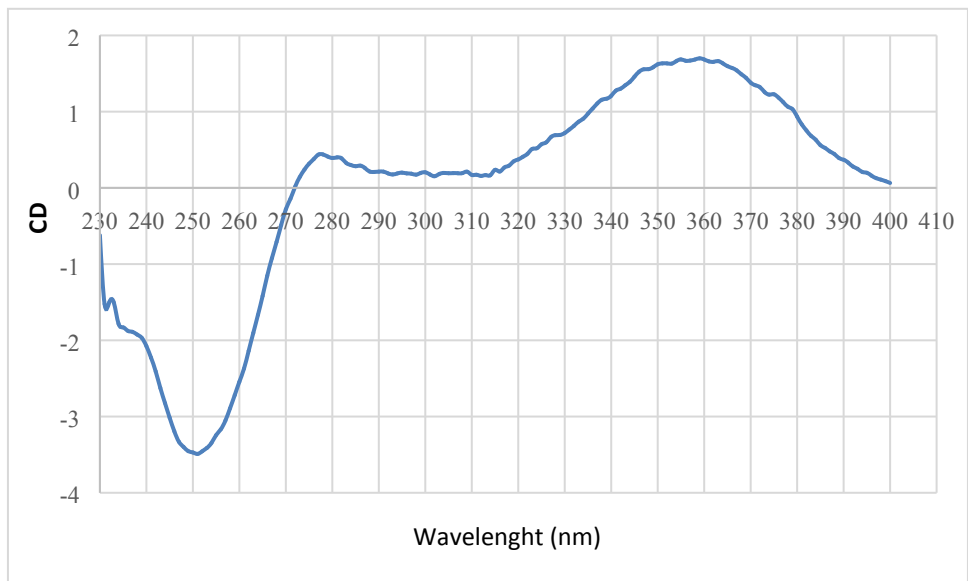


Figure S.1.6. IR spectrum of compound 1.



**Figure S.1.7.** UV spectrum of compound **1**



**Figure S.1.8.** CD spectrum of compound **1**

AcPdx  
1H-RMN  
400MHz-1d  
CDC13-DMS0d6  
Alvarez  
27-02-14  
MARC

File: /home/walkup/vnmrsys/data/ene-feb-14/Alvarez/AcPdx-1H.fid  
Pulse Sequence: s2pu1

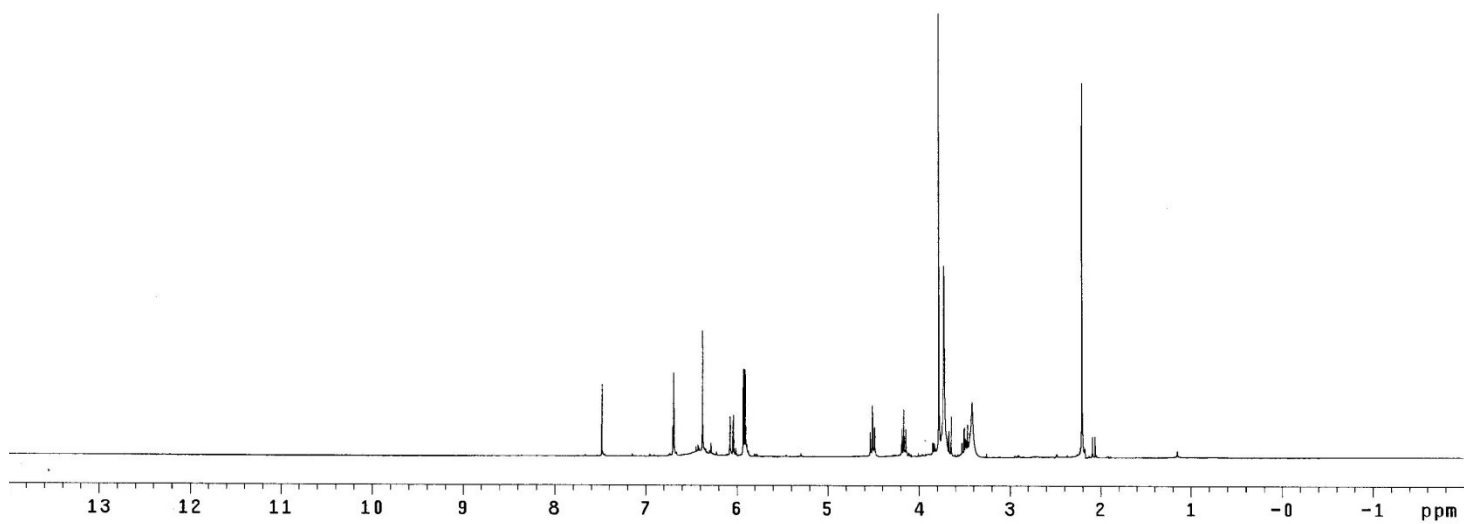
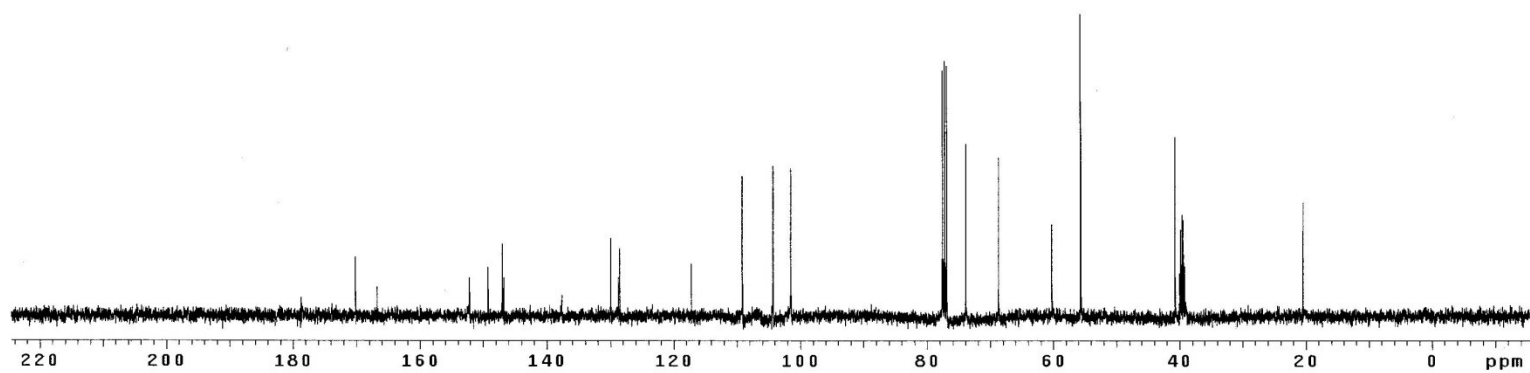


Figure S.2.1. <sup>1</sup>H NMR spectrum of compound 2 (400 MHz, CDCl<sub>3</sub>)

AcPdx  
13C-RMN  
100MHZ-1d  
CDC13-DMSOd6  
Alvarez  
27-02-14  
MARC

File: exp  
Pulse Sequence: s2pu1



**Figure S.2.2.** <sup>13</sup>C NMR spectrum of compound 2 (100 MHz, CDCl<sub>3</sub>).

AcPdx  
1H-RMN  
400MHZ-1d  
CDC13-DMSOd6  
Alvarez  
27-02-14  
MARC

File: exp

Pulse Sequence: gHSQC

Solvent: dmsd  
Ambient temperature  
Operator: walkup  
INOVA-400 "localhost.localdomain"

Relax. delay 1.000 sec  
Acq. time 0.199 sec  
Width 3800.7 Hz  
2D Width 17101.3 Hz  
32 repetitions  
2 x 128 increments  
OBSERVE H1, 400.0691404 MHz  
DECOUPLE C13, 100.6050102 MHz  
Power 41 dB  
on during acquisition  
off during delay  
GARP-1 modulated  
DATA PROCESSING  
Gauss apodization 0.092 sec  
F1 DATA PROCESSING  
Gauss apodization 0.007 sec  
F1 size 4096 x 2048  
Total time 2 hr, 51 min, 31 sec

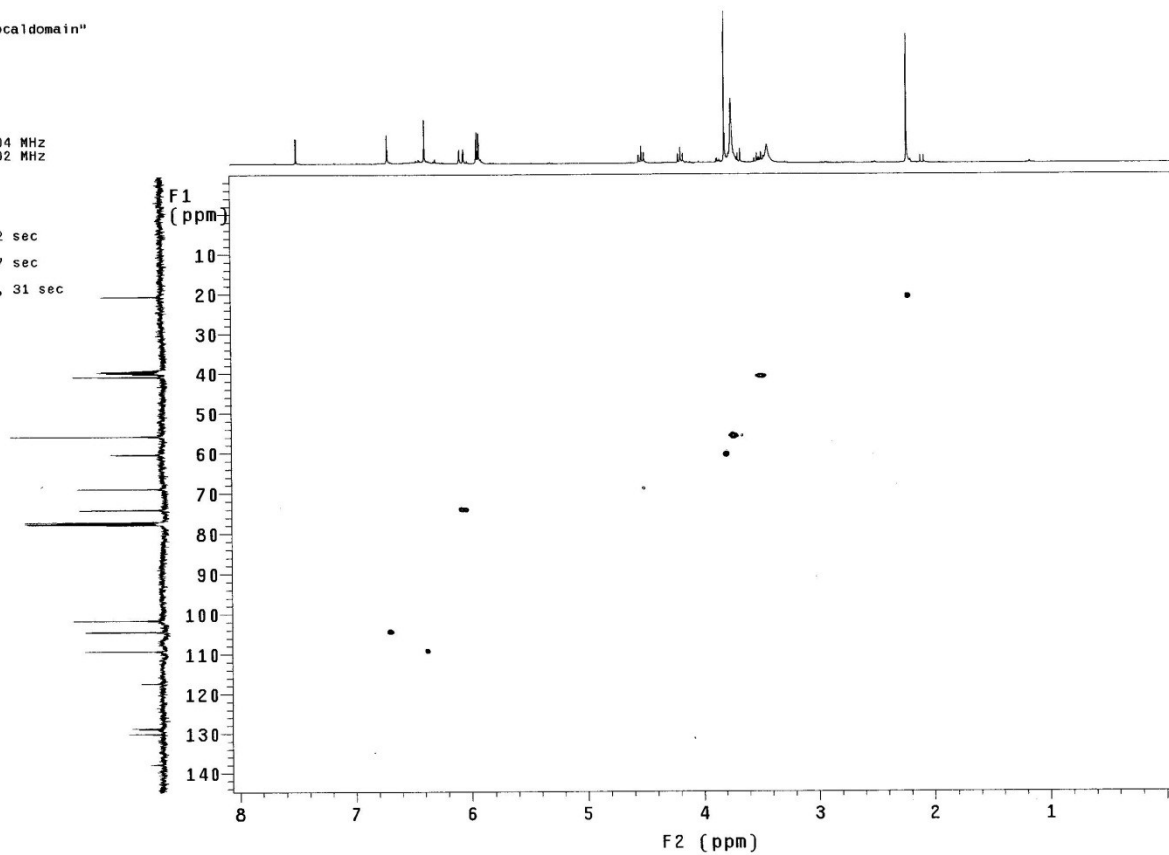


Figure S.2.3. HSQC spectrum of compound 2 ( $\text{CDCl}_3$ ).

AcPdx  
1H-RMN  
400MHZ-id  
CDC13-DMSOd6  
Alvarez  
04-03-14  
MARC

File: exp

Pulse Sequence: gHMBC

Solvent: dmsd  
Ambient temperature  
Operator: walkup  
INOVA-400 "localhost.localdomain"

Relax. delay 1.000 sec  
Mixing 0.080 sec  
Acq. time 0.128 sec  
Width 3000.5 Hz  
2D Width 24140.0 Hz  
64 repetitions  
200 increments  
OBSERVE H1, 400.0691354 MHz  
DATA PROCESSING  
Sine bell 0.064 sec  
F1 DATA PROCESSING  
Sine bell 0.008 sec  
FT size 2048 x 2048  
Total time 4 hr, 20 min, 29 sec

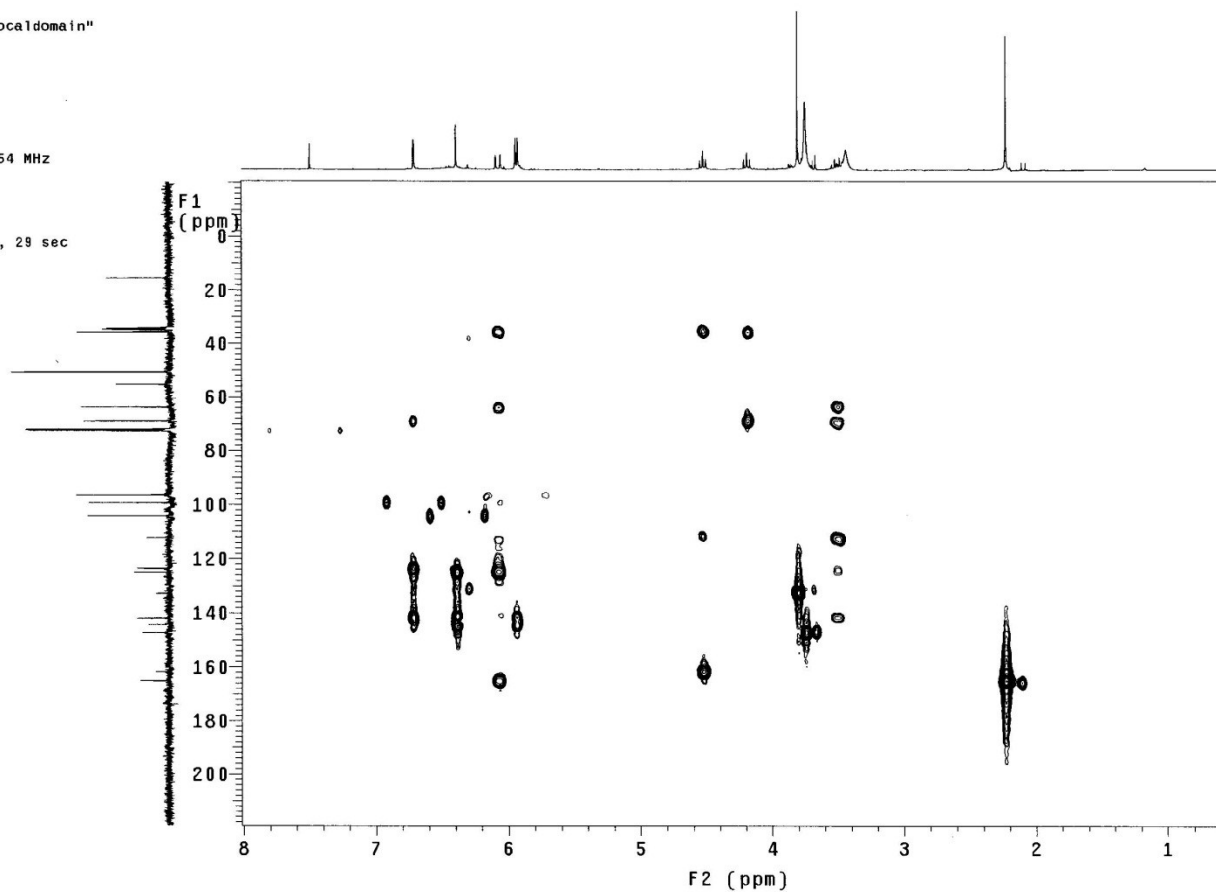


Figure S.2.4. HMBC spectrum of compound 2(CDCl<sub>3</sub>)



[ Mass Spectrum ]  
Data : LFB029 Date : 11-Dec-2015 11:58  
Sample: Comp A(2) Operator Gabriela Vargas Instrument: MSStation JMS-7  
Note : Dra.Laura Alvarez/Mayra Centro de Investigaciones Quimicas UREM  
Inlet : Direct Ion Mode : FHB+  
Spectrum Type : Normal Ion DMF-Linear1  
RT : 0.31 min Scan# : 2  
BP : m/z 55.0000 Int. : 4.68  
Output m/z range : 0.0000 to 692.1958 Cut Level : 0.00 %

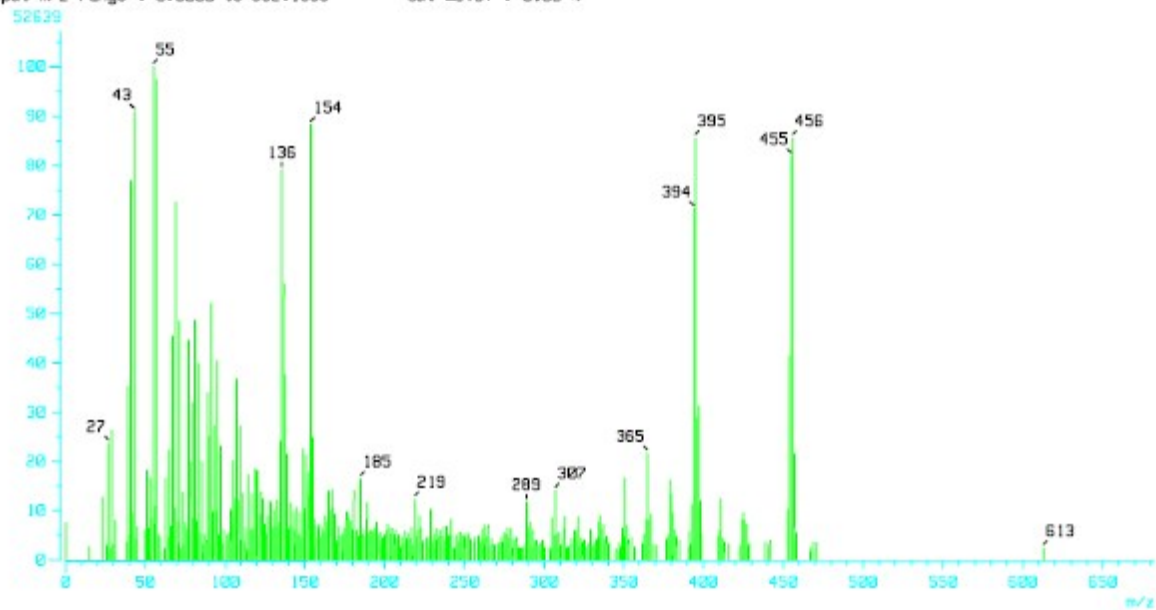


Figure S.2.5. Mass spectrum of compound 2.

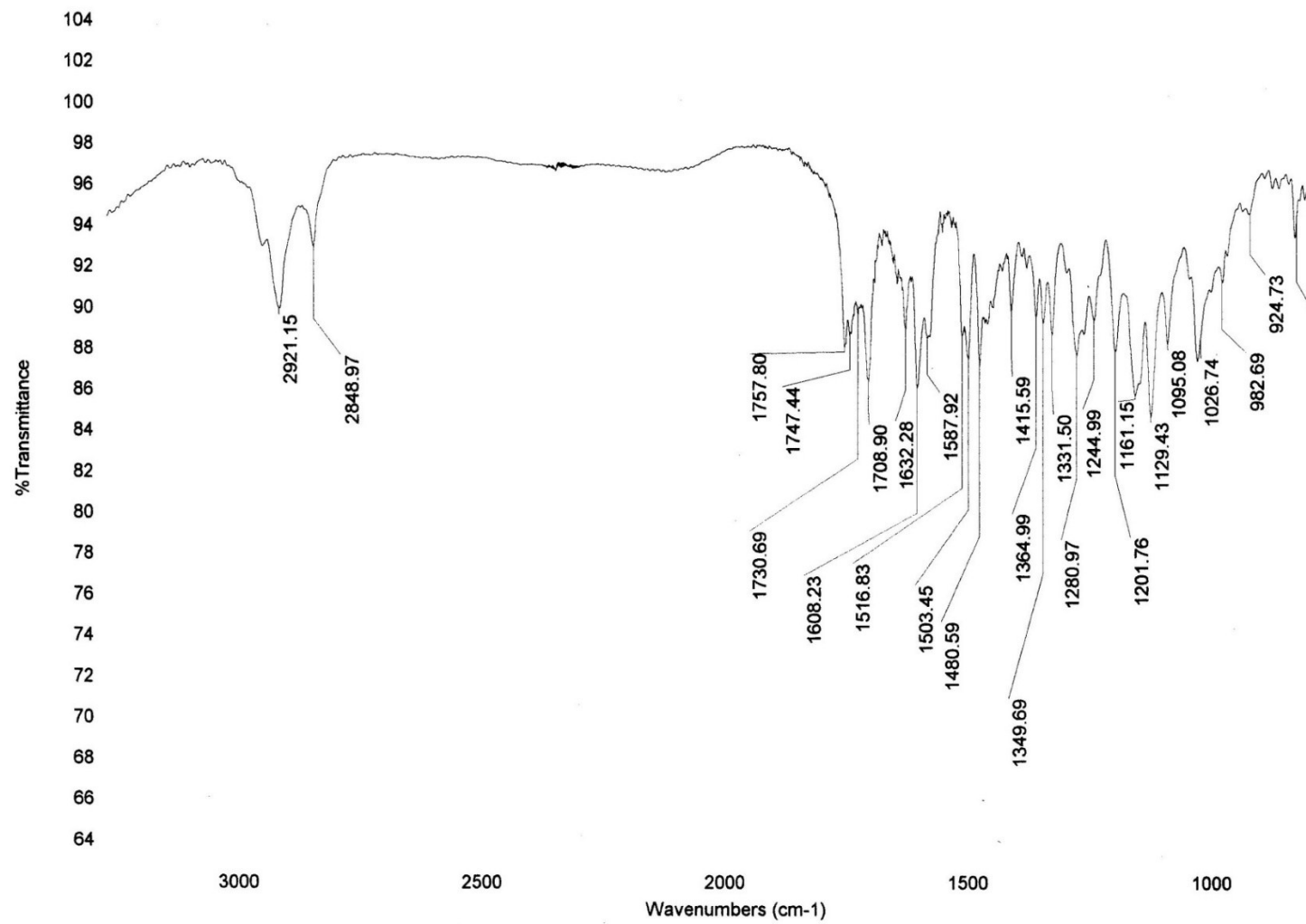
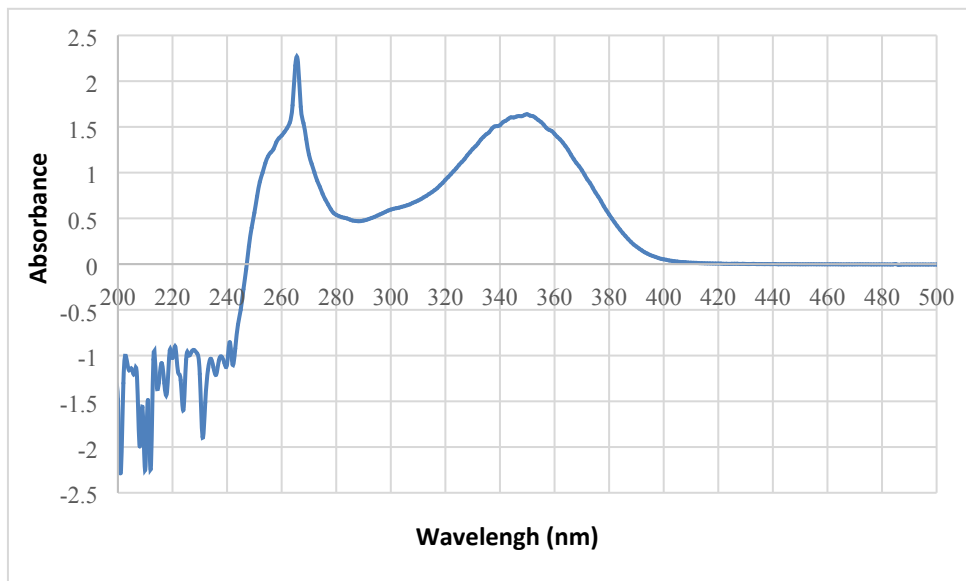
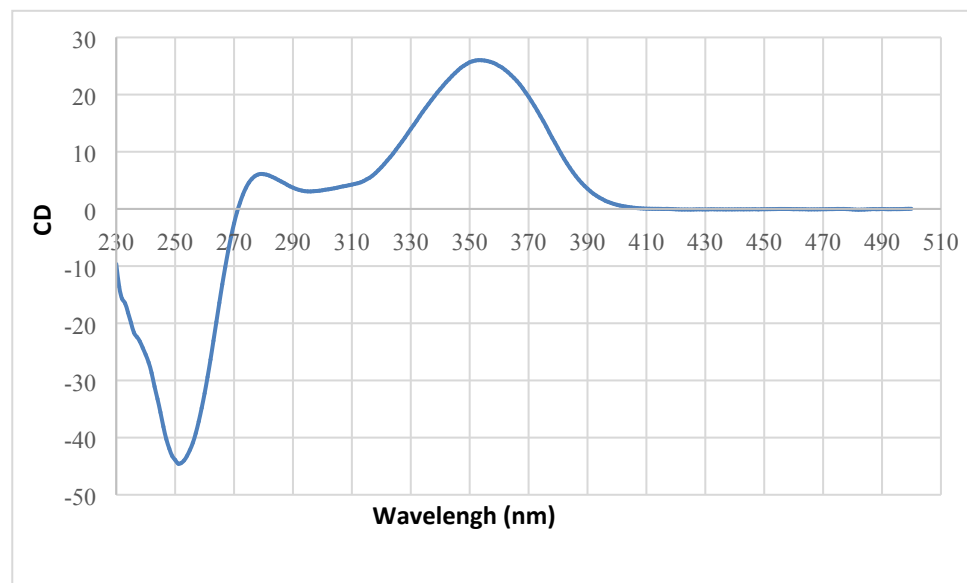


Figure S.2.6. IR spectrum of compound 2.



**Figure S.2.7.** UV spectrum of compound 2.



**Figure S.2.8.** CD spectrum of compound **2**.

<sup>1</sup>H  
BF1.4  
CDC13  
400MHz  
Alvarez  
17-02-12  
Pulse Sequence: s2pu1

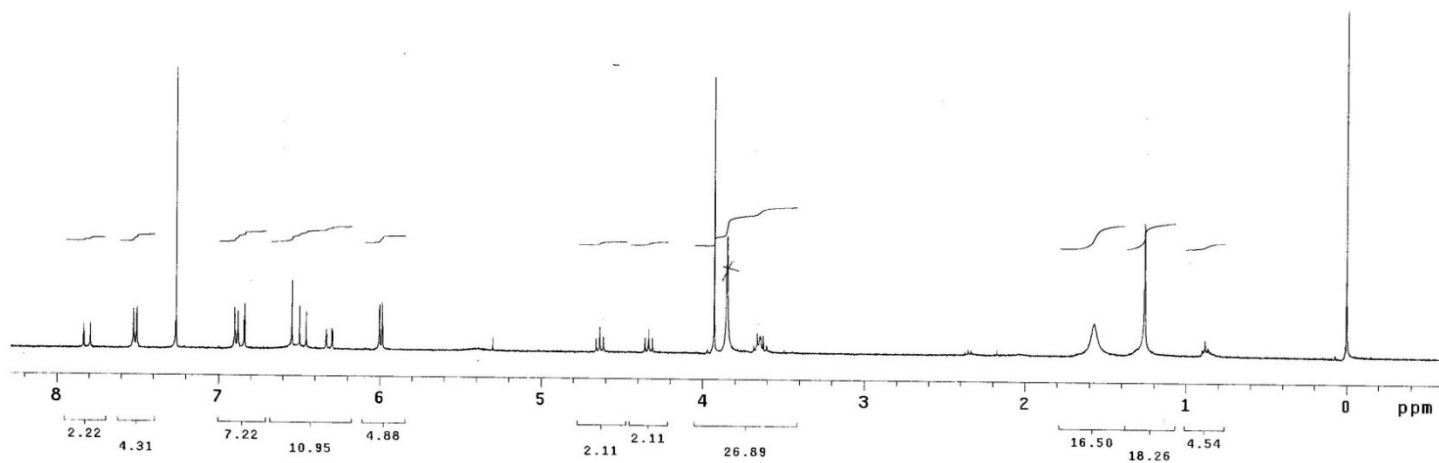
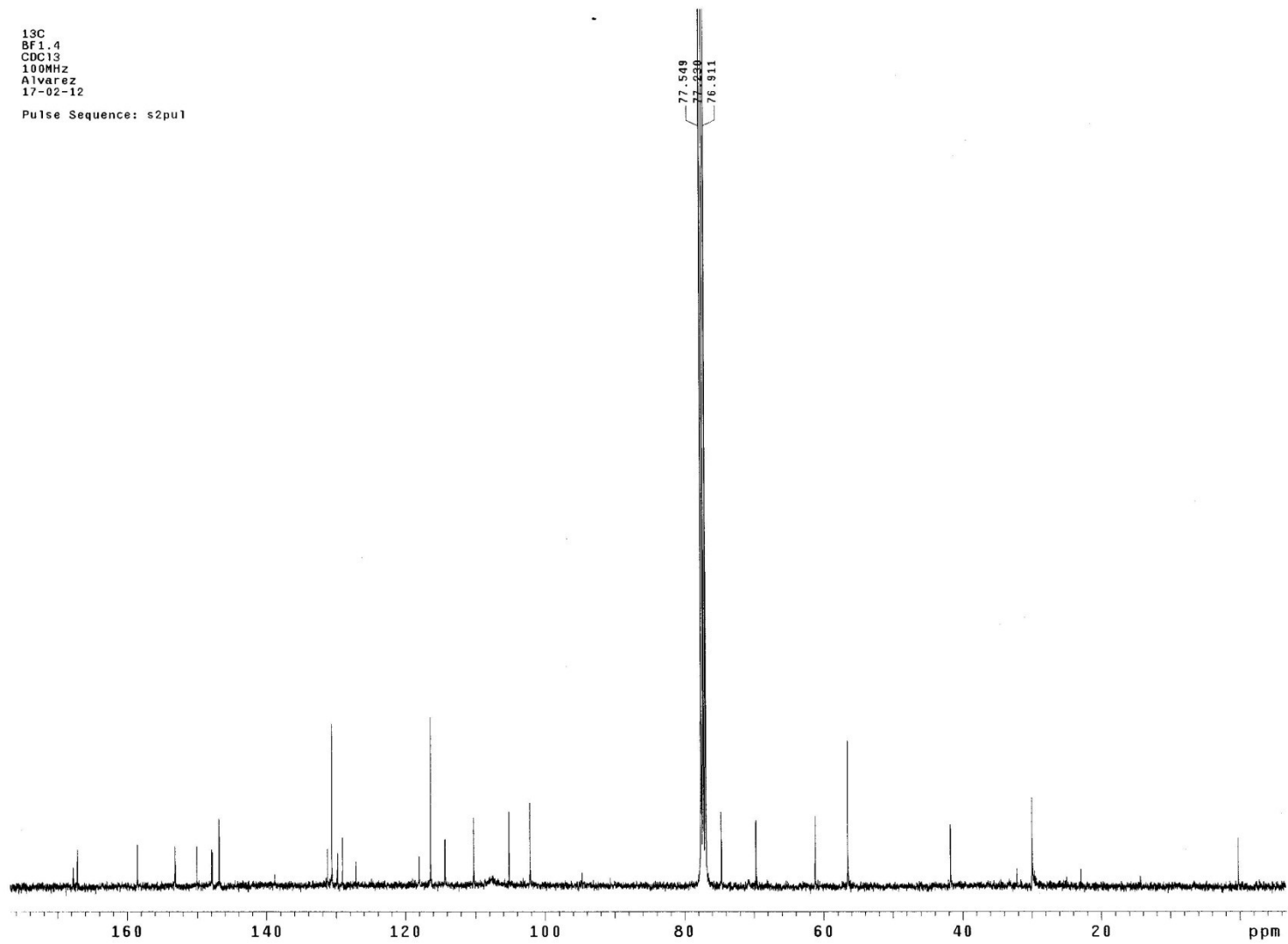


Figure S.3.1. <sup>1</sup>H NMR spectrum of compound **3** (400 MHz, CDCl<sub>3</sub>)

<sup>13</sup>C  
BF1.4  
CDC13  
100MHz  
Alvarez  
17-02-12  
Pulse Sequence: s2pu1



**Figure S.3.2.** <sup>13</sup>C NMR spectrum of compound **3** (100 MHz, CDCl<sub>3</sub>)

Alvarez id

Sample: BF 1.4  
Sample ID: s\_20120220\_08  
File: 0510.fid

Pulse Sequence: gHSQC

Solvent: cdcl3  
Temp. 25.0 C / 298.1 K  
Sample #5, Operator: gaby  
File: 0510  
Mercury-400BB "mercury400"

Relax. delay 1.301 sec  
Acq. time 0.199 sec  
Width 4040.4 Hz  
2D Width 17083.1 Hz  
32 repetitions  
2 x 200 increments  
OBSERVE H1, 399.6281725 MHz  
DECOUPLE C13, 100.4941204 MHz  
Power 45 dB  
on during acquisition  
off during delay  
GARP-1 modulated  
DATA PROCESSING  
Gauss apodization 0.039 sec  
F1 DATA PROCESSING  
Gauss apodization 0.005 sec  
FT size 4096 x 4096  
Total time 5 hr, 39 min, 36 sec

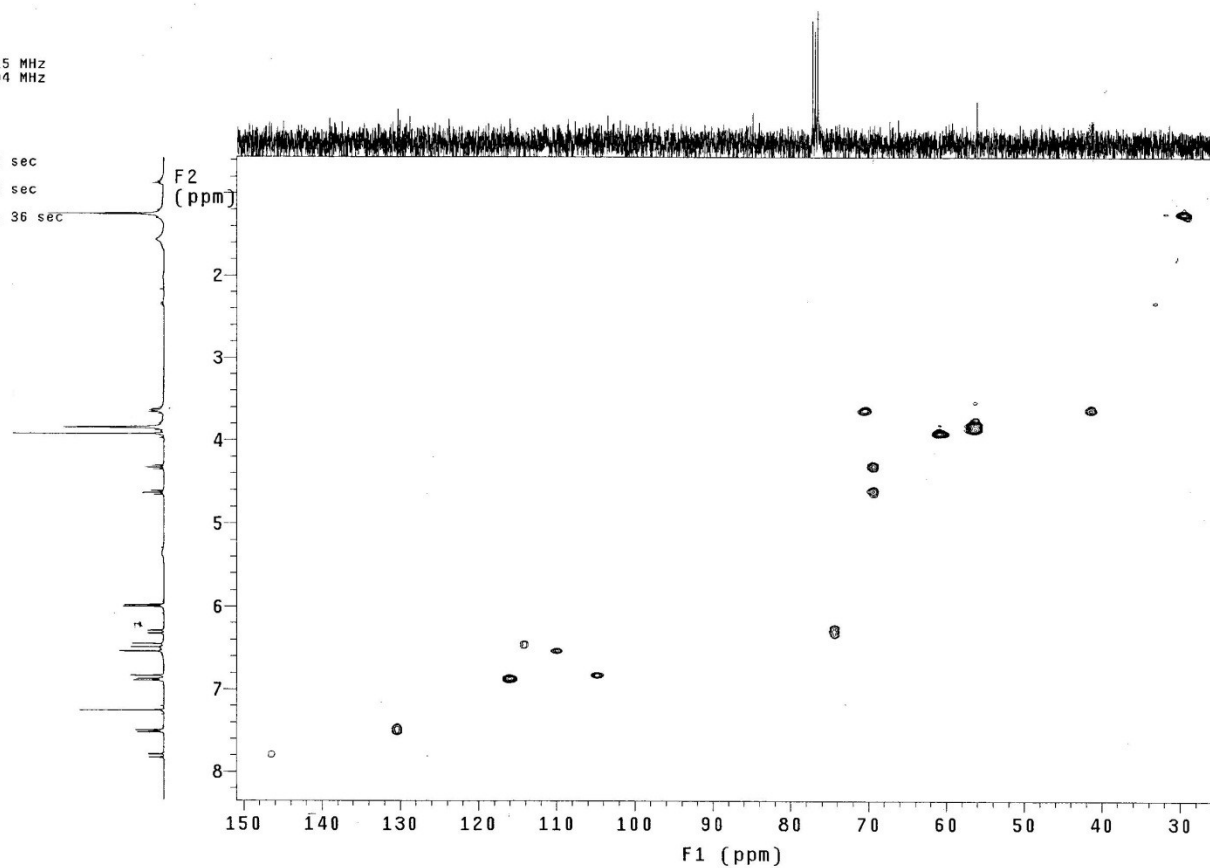


Figure S.3.3. HSQC spectrum of compound **3** (CDCl<sub>3</sub>).

Alvarez id

Sample: BF 1.4  
Sample ID: s\_20120220\_08  
File: 0511.fid

Pulse Sequence: gHMBC  
Solvent: cdc13  
Temp. 25.0 C / 298.1 K  
Sample #5, Operator: gaby  
File: 0511  
Mercury-400BB "mercury400"

Relax. delay 1.500 sec  
Mixing 0.080 sec  
Acq. time 0.128 sec  
Width 4040.4 Hz  
2D Width 24118.2 Hz  
64 repetitions  
200 increments  
OBSERVE H1, 399.6281725 MHz  
DATA PROCESSING  
Sine bell 0.050 sec  
F1 DATA PROCESSING  
Sine bell 0.005 sec  
FT size 2048 x 2048  
Total time 6 hr, 13 min, 34 sec

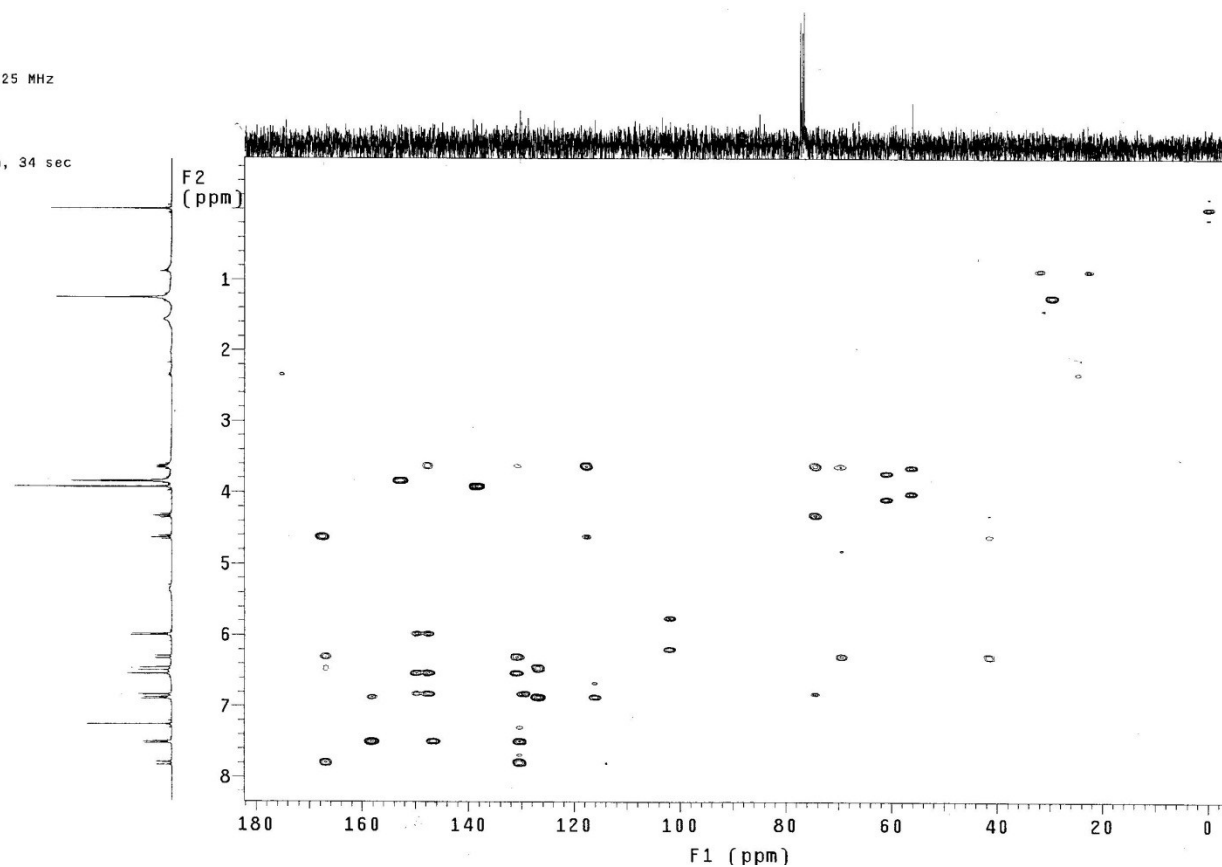


Figure S.3.4. HMBC spectrum of compound 3(CDCl<sub>3</sub>)



[ Mass Spectrum ]  
Data : 1.03009 Date : 07-Mar-2012 01:45  
Sample: BF 1.4 Operator name Ing.Victoria Labastida G.  
Note : Dra.Laura Alvarez/Andres Centro de Investigaciones Quimicas UAEM  
Inlet : Direct Ion Mode : FAB+  
Spectrum Type : Normal Ion [MF-Linear]  
RT : 0.00 min Scan# : (1,2)  
BP : m/z 55.0000 Int. : 8.22  
Output m/z range : 40.0000 to 800.0000 Cut Level : 0.00 %

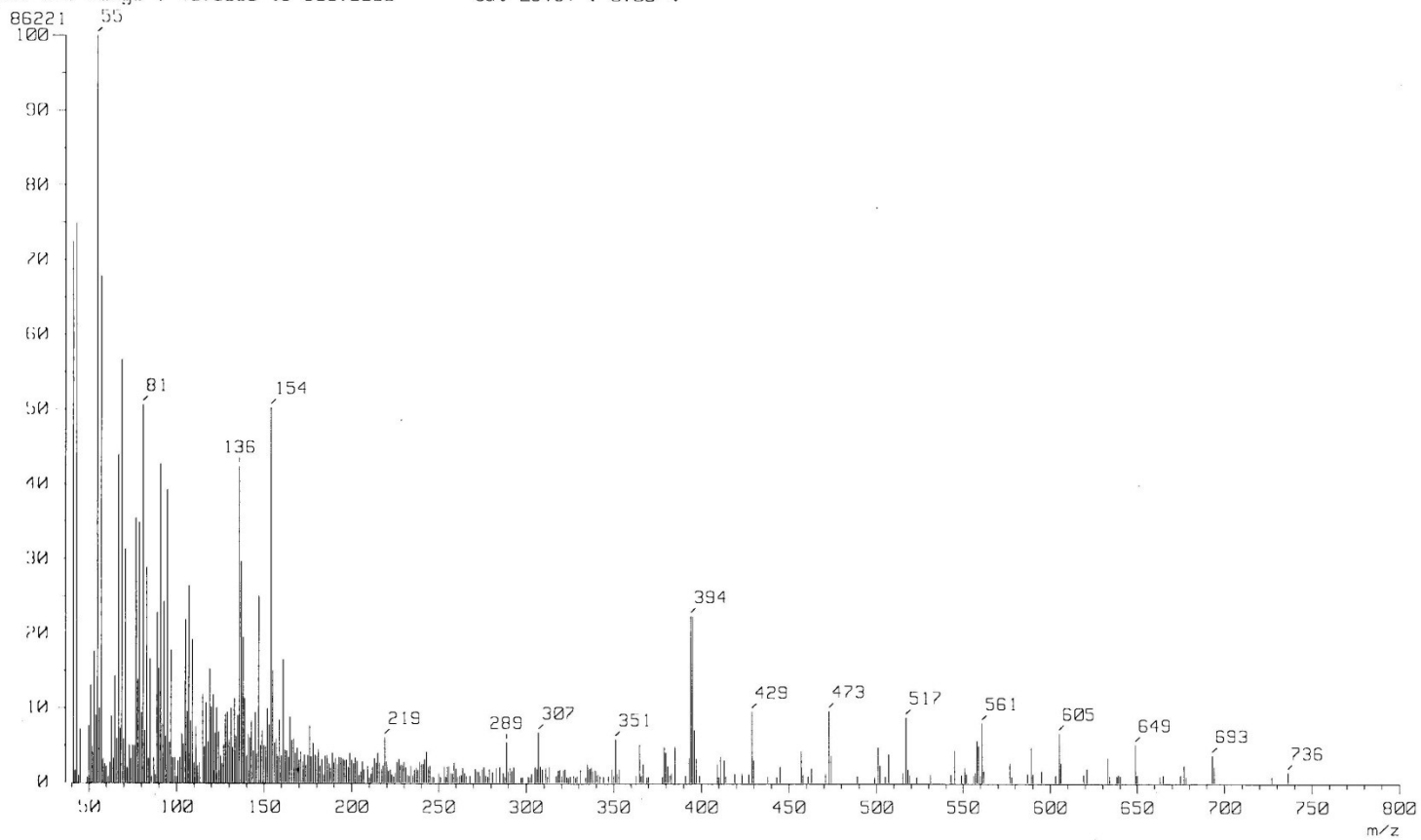


Figure S.3.5. Mass spectrum of compound 3

Table S.1. Quantification of the effect of *B. fagaroides* lignans **1-3** on the cell cycle in zebrafish embryos.

Treatment	Cell cycle activity	H3S10ph fold change	$p < 0.001$	n	N	Student's t-test
DMSO		1.00 ± 0.13		10	1	
Aphidicolin	-	0.23 ± 0.05	*	9	1	7.53E-12
Nocodazole	+	1.80 ± 0.20	*	9	1	9.81E-09
1	+	1.92 ± 0.23	*	10	1	2.41E-09
2	+	2.41 ± 0.19	*	10	1	1.73E-13
3	+	2.57 ± 0.26	*	10	1	1.88E-12

Cell cycle activity; -, denotes decrease, +, increase.

Table S.2. Quantification of the effect of *B. fagaroides* lignans **1-3** on the morphology of zebrafish embryos.

Treatment	Effect on morphology	Circularity	$p < 0.001$	n	N	Student's t-test
DMSO		0.42 ± 0.02		10	1	
Aphidicolin	=	0.38 ± 0.05		9	1	4.90E-02
Nocodazole	+	0.76 ± 0.06	*	9	1	4.80E-12
1	+	0.78 ± 0.07	*	10	1	3.10E-12
2	+	0.73 ± 0.06	*	10	1	2.56E-11
3	+	0.77 ± 0.04	*	10	1	1.68E-15

Circularity, measured circularity. Morphological effect; +, increase in circularity, = without change. n, total number of embryos analyzed. Student's t-test was used to determine the p-value. **1** (7',8'-dehydropodophyllotoxin). **2**, (7',8'-dehydro acetyl podophyllotoxin). **3** (7',8'-dehydro-*trans*-p-coumaroyl podophyllotoxin).