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Bioactive Compounds and Insecticidal Activity of *Hysterionica pinifolia*, a Native South American Plant

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Abstract

Phytochemical investigation of the purified fractions of the active dichloromethane extract of *Hysterionica pinifolia* (Poir.) Baker led to the identification of five compounds. New acetylenic alcohol (E)-undec-3-en-5,7-diyne-1-ol **(1)** and three other compounds **(3)**, **(4)**, and **(5)** were reported for the first time in this species. Furthermore, forty-six components from the volatile fraction of *H. pinifolia* were identified. These compounds were elucidated using 1D and 2D NMR spectroscopy as well as MS-ESI and GC-FID-MS experiments. The dichloromethane extract, its fractions, and the methanolic extract were tested for insecticidal activity against *Tribolium castaneum* under laboratory conditions. The dichloromethane extract and the fraction F2 were found to be active, showing high larval mortality. The dichloromethane extract was also active against *T. castaneum* adults. The results have shown that *H. pinifolia* could be considered, in a near future, as a potential source for the development of a botanical insecticide for pest control.

Keywords

Hysterionica pinifolia, Bioactive Compounds, Polyacetylene Compounds, (E)-undec-3-en-5,7-diyne-1-ol, Insecticidal Activity

1. Introduction

Hysterionica pinifolia (Poir.) Baker (Asteraceae) is a perennial subshrub, native to the Argentine flora. It grows in hilly areas of the province of Buenos Aires,

between 0 and 800 meters above sea level, and in other South American countries such as Brazil and Uruguay [1]. Previous investigations on this plant have led to the identification of acetylenic compounds [2] but no reports of biological activities were found for this species.

At the moment, there is growing concerned about the negative effects on human health, and the environment due to the widespread and indiscriminate use of conventional insecticides [3] [4]. For that reason, obtaining and characterizing new molecules of plant origin with insecticidal activity is, currently, one of the main research focuses for pest control [5] [6] [7].

In this work, we report the identification of a new acetylenic alcohol and four other compounds from the active CH_2Cl_2 extract of *H. pinifolia*. The insecticidal activity of the CH_2Cl_2 extract, its fractions, and the CH_3OH extract were assayed against *Tribolium castaneum* Herbst, the red flour beetle, a stored grain pest.

2. Materials and Methods

2.1. Plant Material

Aerial parts of *Hysterionica pinifolia* were collected in Tandil, Buenos Aires province (west 59.1369, south 37.3286), Argentina, in March 2016 and were authenticated by Dr. Gustavo Giberti. A voucher specimen (BAF 858) was deposited in the Pharmacobotanical Museum of the Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Argentina. The material was dried at room temperature and finely powdered.

2.2. Extraction and Bioguided Fractionation

The powered aerial parts (100 g) were extracted at room temperature by maceration using CH_2Cl_2 (1000 ml) for 1 h under continuous shaking. This procedure was repeated 5 times changing the solvent each time. These extracts were gathered. The plant residue was then extracted using the same volume of CH_3OH. After evaporation of the solvent, 4.6 and 8.1 g of each extract were obtained respectively.

A portion of the active CH_2Cl_2 extract (2.0 g) was fractionated by a preparative chromatography column (60 cm × 5 cm) using silica gel 60 MN (70 - 230 mesh, Macherey-Nagel, Germany), eluted with a gradient mixture of hexane (Hx)/ethyl acetate (EtOAc) (100:0 to 50:50, v/v), then EtOAc/CH_3OH (100:0 to 0:100, v/v). The chromatographic analyses were made by Thin Layer Chromatography (TLC) on silica gel F254 (MERCK-Germany), developed by different solvent systems and the spots were visualized under UV light at 254 and 366 nm. Four fractions (F1 to F4) were collected based on their TLC profiles. The insecticidal activity was found to be concentrated only in F2 (**Table 4**). Then, F2 (313 mg) was rechromatographed on an analytical column (45 cm × 3 cm) using silica gel 60 MN (70 - 230 mesh, Macherey-Nagel, Germany) and was eluted with gradient mixtures of Hx/ethyl ether (Et_2O) (100:0 to 50:50, v/v), then Et_2O/CH_3OH (100:0 to 0:100, v/v). Fifteen subfractions (F2-A to F2-O) were obtained. After the TLC

analysis, the cromatographic profile of the subfractions F2-C and F2-E showed the main compounds present in the active fraction F2. For this reason, the subfractions F2-E and F2-C were selected for the identification of their bioactive compounds.

2.3. Volatile Fraction Obtention

Volatile fraction was obtained by hydrodistillation of naturally air dried aerial parts during 3 h in a Clevenger-type trap [8]. The resulting volatile fraction was dried over anhydrous sodium sulfate and stored at 2˚C prior to GC-FID-MS analysis

2.4. Compounds Identification

The identification of the compounds in the subfractions F2-E and F2-C was conducted by GC-FID-MS, ^{1}H and ^{13}C NMR 1D and 2D and MS-ESI. The identification of unknown compounds in complex mixtures using the combination of 1D and 2D NMR and MS, without the need for prior isolation, allows rapid identification [9] [10].

2.4.1. NMR and MS Analysis

^{1}H and ^{13}C-NMR spectra and homo- and heteronuclear correlation spectroscopy experiments were recorded in chloroform-d_3 ($CDCl_3$) on an Avance II 500 Bruker at 500 and 125 MHz. Chemical shifts (δ) are reported as ppm based on the tetramethylsilane signal.

Mass spectra were measured on a Bruker micrOTOF-Q II spectrometer, ionization was performed by electrospray (ESI) in positive mode.

2.4.2. GC-FID-MS Analysis

GC-FID-MS analysis was performed using a GC-FID-MS Agilent 7890A/5975C equipped with one injector (split ratio 1:100) connected by a flow splitter to two capillary columns (HPWAX and HP-1, both 60 m × 0.25 mm with 0.25 microns of fixed phase). The polar column was connected to a FID, whereas the non-polar column was connected to a quadrupolar mass detector (HP 5975C) (70 eV). Helium was used as gas carrier, at 1.8 mL/min. The injector temperature was set at 250˚C. The column temperature was programmed according to the following gradient: 100˚C, increasing at 2˚C/min to 240˚C and kept constant for 15 min. FID temperature was 260˚C, and temperatures for the transference line and the ionic source were set at 280 and 230˚C, respectively. Mass range (m/z) was 40 - 500 Da. Data acquisition, processing and instrument control was performed using the Agilent Chem Station software.

2.4.3. GC-FID-MS Identification

The identification of the compounds was achieved by analyzing the retention indexes (relative to C8-C24 n-alkanes) obtained in both columns and compared with those of reference compounds, compounds identified in chemically well-known essential oils and from bibliography [11] [12]. Additionally, each mass spectra

obtained was compared to those from the literature libraries [11] [13] [14] and mass spectra obtained from reference compounds.

Relative percentage contribution of the compounds was calculated from the FID responses by a computerized integration assuming all of the responses factors were 1.

2.5. Insecticidal Activity Bioassay

2.5.1. Insect Rearing

T. castaneum Herbst. (Coleoptera: Tenebrionidae) larvae from an established laboratory colony in the Organic Plant Production Laboratory, Faculty of Agronomy, University of Buenos Aires, Argentina, were employed. *T. castaneum* larvae were reared with an artificial diet (wheat flour, beer yeast, cornstarch 10:1, 5:10) and environmental standard conditions (25˚C ± 1˚C, 60% ± 5% RH, in darkness). This laboratory strain is susceptible to all insecticides [15].

2.5.2. Larvicidal Activity Ingestion Bioassay

The insecticidal activity of the CH_2Cl_2 extract, its fractions (F1 to F4) and the CH_3OH extract were investigated.

The concentrations employed for the determination of larvicidal activity were: CH_2Cl_2 and CH_3OH extracts: 5.0 to 25.0 mg/ml and the fractions (F1 to F4): 25.0 mg/ml. Two grams of the artificial diet were mixed with 1.0 ml of each acetonic solution of *H. pinifolia* extracts and fractions. The solvent was evaporated from the diet at room temperature during 24 h. Then, ten neonate larvae of *T. castaneum* were placed in each glass vessel containing a treated artificial diet. All tests were performed in quadruplicate with acetone as a negative control. The bioassay was conducted for nine weeks under standardized conditions (25˚C ± 1˚C and 60% ± 5% RH). The mortality at each stage of the life cycle of the red flour beetle as well as the overall mortality was weekly assessed. The sublethal effects expressed as delays in the development of the insect produced by the extracts and fractions were also evaluated [15].

2.5.3. Study of the Survival of *T. castaneum* Adults

Ten adults of *T. castaneum* were added to each glass vessel containing two grams of artificial diet previously mixed with an acetonic solution of *H. pinifolia* CH_2Cl_2 extract. The concentrations employed for the study of the survival of *T. castaneum* adults were: 25.0 and 50.0 mg/ml. The number of surviving insects was observed and recorded for 35 days or until the death of all the individuals. Insects were considered dead when there were no responses to tactile stimuli. All tests were performed in quadruplicate with acetone as a negative control [16].

2.5.4. Statistical Analysis

Statistical differences in mortality ($p \leq 0.05$) were calculated with ANOVA and Tukey's multiple range tests [17]. Sublethal effects and EC_{50} values were calculated by Probit analysis using a computer program [18].

3. Results and Discussion

3.1. Phytochemical Analysis

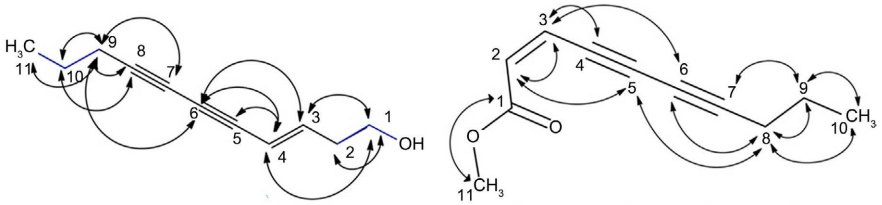
In order to identify the bioactive compounds, the subfractions F2-E and F2-C from the active CH_2Cl_2 extract of *H. pinifolia* were analyzed. The analysis was conducted by GC-FID-MS, ^1H and ^{13}C NMR 1D and 2D experiments (COSY, TOCSY, HSQC, HMBC) and MS-ESI. The compounds were identified in the subfractions using the combination of 1D and 2D NMR and MS, without the need of prior isolation, wich allowed to a rapid identification [9] [10].

A new acetylenic alcohol was identified in subfraction F2-E. The molecular formula was assigned as $C_{11}H_{14}O_1$ on the basis of its MS-ESI analysis, which showed a protonated molecular ion peak at m/z 145.10 $[M + H]^+$ (calcd for $C_{11}H_{14}O_1$, 162.23) (**Figure S1**). This molecular ion was obtained by dehydration of the alcohol functional group under electrospray ionization conditions [19]. In the ^{13}C-NMR spectrum the signal of a carbon linked to a hydroxyl group at δ_C 72.11 ppm, the signals of alkenes carbons at δ_C 148.47 and 108.83 ppm and the signals of alkyl carbons at δ_C 75.02, 72.99, 65.19 and 84.42 ppm were observed. Aliphatic carbon signals δ_C 36.87, 21.52, 21.75 and 13.41 ppm were also observed (**Figure S2**). The ^1H-NMR spectrum showed signals of protons from alkenes with *trans* isomerism at δ_H 6.28 and 6.19 ppm (J = 15.9 Hz) and signals from protons bound to aliphatic carbons at δ_H 4.19, 2.32, 1.60, 1.56 and 1.02 ppm (**Figure S3**). The ^1H and ^{13}C NMR spectra showed the signals corresponding to an acetylene skeleton. These compounds are characterized by the presence of two or more triple bonds [20]. With the aid of ^1H-^1H COSY, TOCSY, HSQC and HMBC spectroscopic data, all protons and carbons were fully assigned (**Table 1**, **Figures S4-S7**). Analysis of the ^1H-^1H COSY and TOCSY spectrum allowed to determine the connection between the protons and led to the establishment of two spin systems (H-1 to H2/H3/H4 and H9 to H10/H11) (**Figure 1**). Furthermore, the HMBC correlations between C1/H3 and H4; C3/H1; C4/H1; C5/H4 and H9; C6/H3, H4 and H9; C7/H9; C8/H9 and H10; C9/H10 and H11; C10/H9 and H11 and C11/H9 and H10, confirm the structure of this new polyacetylene alcohol as (E)-undec-3-en-5,7-diyne-1-ol **(1)**. This compound is reported for the first time in *H. pinifolia* and no reports have been found in other species.

The major compound, *cis* lachnophyllum methyl ester **(2)**, was identified in subfraction F2-C by GC-FID-MS (63.0%, rt: 45.45 min) and ^1H and ^{13}C NMR 1D and 2D (**Table 2**, **Figures S8-S11**). In the ^{13}C-NMR spectrum, signals of a carbonyl carbon at δ_C 166.8 ppm and of a methyl carbon at δ_C 51.6 ppm were observed, indicating the presence of an ester group. The alkene carbon signals at δ_C 130.7 and 122.5 ppm and the alkyl carbon signals δ_C70.8, 86.6, 65.2 and 90.1 ppm indicated the presence of one double bond and two triple bonds in the molecule. Signals of aliphatic carbons δ_C 21.60, 21.79 and 13.5 ppm were also observed (**Figure S8**). The ^1H-NMR spectrum showed signals of protons of alkenes with *cis* isomerism at δ_H 6.23 and 6.19 ppm (J = 11.4 Hz), a singlet at δ_H 3.80 ppm corresponding to the methyl protons of the ester group and the signals of

the protons linked to aliphatic carbons at δ_H 2.37, 1.61 and 1.03 ppm (Figure S9). Like in compound 1 the ^1H and ^{13}C NMR spectra showed the signals corresponding to an acetylene skeleton. Additionally, the heteronuclear correlation spectra HSQC (Figure S10) and HMBC allowed to fully assign the protons and carbons in the molecule (Table 2, Figure 1). The HMBC correlations between C1/H2, H3 and H11; C3/H2; C4/H2, H3 and H8; C5/H2 and H8; C6/H2, H3 and H8; C7/H8 and H9; C8/H9 and H10; C9/H8 and H10 and C10/H8 and H9 (Figure S11) confirm the structure of this polyacetylene ester as *cis* lachnophyllum methyl ester. The results of NMR analysis agreed with the data described in the literature [21]. This compound was previously described by Bohlmann *et al.* [2] for this species.

The compounds eucalyptol **(3),** benzoic aldehyde **(4)** and benzyl alcohol **(5)** were identified in fractions F2-E and F2-C by GC-FID-MS. Eucalyptol (rt: 6.51 min) 3.50% in F2-C and 4.90% in F2-E, benzoic aldehyde (rt: 14.2 min) 4.90% in F2-C and 30.0% in F2-E and benzyl alcohol (rt: 28.69 min) 2.50% in F2-C and 13.5 % in F2-E. These compounds are reported for first time in *H. pinifolia.*



(E)-undec 3-en-5,7-dyin-1-ol (2) *cis* lachnophyllum methyl ester (2)

Figure 1. Key H-^1H COSY (blue line) and HMBC (arrows) correlations for compounds 1 and 2.

Table 1. ^{13}C and ^1H NMR data and 2D correlations of compound **1** in $CDCl_3$.

Position	(E)-undec-3-en-5,7-diyne-1-ol			
	δ_C[a], type	δ_H[b] (*J* in Hz)	COSY	HMBC
1	72.11, CH_2OH	4.19 m	2, 3	3
2	36.87, CH_2	1.56 m	1, 3	-
3	148.47, CH	6.28 dd, *J* = 15.9; 5.9	4, 2	1
4	108.83, CH	5.75 d, *J* = 15.9	3	1
5	75.02, C	-	-	4
6	72.99, C	-	-	3, 4, 9
7	65.19, C	-	-	9
8	84.42, C	-	-	9, 10
9	21.52, CH_2	2.32 t, *J* = 7.5	10	10, 11
10	21.75, CH_2	1.60 m	9, 11	9
11	13.41, CH_3	1.02 t, *J* = 7.3	10	9

[a] Data recorded at 125 MHz. [b]Data recorded at 600 MHz.

Table 2. ^{13}C and ^{1}H NMR data and 2D correlations of compound **2** in $CDCl_3$.

Position	*cis* lachnophyllum methyl ester		
	δ_C[a], type	δ_H[b] (*J* in Hz)	HMBC
1	164.8, CO	-	11
2	122.5, CH	6.19 d, *J* = 11.4	-
3	130.7, CH	6.23 d, *J* = 11.4	2
4	70.8, C	-	3
5	86.6, C	-	2, 8
6	65.2, C	-	3, 8
7	90.1, C	-	9
8	21.60, CH_2	2.37 t, *J* = 7.0	9, 10
9	21.79, CH_2	1.61 m	8, 10
10	13.5, CH_3	1.03 t, *J* = 7.4	8, 9
11	51.6, OCH_3	3.80 s	-

[a] Data recorded at 125 MHz. [b]Data recorded at 600 MHz.

Acetylenic compounds have also been found in essential oils [22]. For this reason, the volatile fraction of *H. pinifolia* was obtained and analyzed. Forty-six components were identified by GC-FID-MS and these constituted the 84.2% of the volatile fraction.

The principal compounds identified were *cis* lachnophyllum methyl ester (38.4%) as the major constituent, *trans*-*β*-ocimene (17.5%), *γ*-curcumene (12.5%), ar-curmumene (4.5%), *β*-caryophyllene (1.9%) and geranylacetone (1.3%) (Table 3).

3.2. Insecticidal Activity Results

The insecticidal activity was evaluated on *T. castaneum* larvae from a laboratory strain susceptible to all insecticides. The CH_2Cl_2 extract exhibited only larval mortality with EC_{50} value of 15.7 mg/ml (CI = 14.9 - 16.6 mg/ml) and no sublethal effects were observed (Table 4). These promising results led to a bioguided fractionation in order to identify the active compounds present in the CH_2Cl_2 extract. For that reason, the activity of the four fractions (F1 to F4) of the CH_2Cl_2 extract was evaluated. Only the fraction F2 showed lethal effects with 95% of larval mortality at 25.0 mg/ml and no sublethal effects were observed (Table 4). The mortality observed in F2 fraction was comparable to that obtained with the crude CH_2Cl_2 extract (92.5%) at the same concentration of 25.0 mg/mL (Table 4). These results showed that the insecticidal activity of the crude CH_2Cl_2 extract would be concentrated in the F2 fraction. The CH_3OH extract did not present lethal or sublethal effects (Table 4).

The high larval mortality obtained with the CH_2Cl_2 extract and the fraction F2 was very interesting due to the extensive losses in stored grains caused by the voracity of *T. castaneum* larvae in their different instars [23]. In addition, adults

of *T. castaneum* also cause an important damage. Therefore, the insecticidal activity of the CH_2Cl_2 extract on *T. castaneum* adults was evaluated. Mortality and immobility were initially observed and, at the end of the trial, 100% mortality only at 50.0 mg/ml was obtained. In the control group, no mobility alteration or mortality was exhibited.

Table 3. Relative percentage of the main constituents of the volatile fraction of *H. pinifolia.*

LRI[a]	LRI[b]	Compound	%
634	917	Isovaleraldehyde	0.1
738		Dimethyldisulfide	T
800		2-Methyl-4-heptene	T
900	900	Nonane	0.1
929	1037	Tricyclene	0.3
935	1043	*α*-Pinene	0.2
951	1100	Camphene	T
968	1138	Sabinene	0.2
979	1133	*β*-Pinene	1.0
1005	1191	*α*-Phellandrene	0.2
1012	1206	*α*-Terpinene	T
1018	1286	*p*-Cimene	T
1020		Cosmene	0.1
1021	1216	Sylvestrene	0.3
1032	1260	***trans β* Ocimene**	**17.5**
1047	1264	*γ*-Terpinene	0.1
1047	1264	*β*-Phellandrene	0.1
1073	1452	*p*-Cymene	T
1077	1409	*γ*-Clausenane	T
1081	1549	Linalool	T
1082	1305	Terpinolene	T
1103	1313	4.8-*trans*-Dimethyl-1.3.7-Nonatriene	0.6
1151	1513	Pyrazine. 2-Methoxy 3-sec Butyl	T
1155	1787	*p*-Methylacetophenone	T
1164	1614	Terpinen-4-ol	0.2
1169	1789	MethylSalicylate	T
1172	1705	*α*-Terpineol	0.1
1304	1675	*cis*-3-Hexenyl tiglate	T
1387	1603	*β*-Elemene	0.1
1404		7-epi-*α* -Cedrene	T
1405	1545	Italicene	T
1417	1614	***β*-caryophyllene**	**1.9**

Continued

1428	1858	**Geranylacetone**	**1.3**
1448	1668	*trans*-*β*-farnesene	1.0
1449	1683	*α*-Humulene	0.6
1470	1783	***α*-Curcumene**	**4.5**
1472	1696	***γ*-Curcumene**	**12.5**
1477	2266	***cis* Lachnophyllummethylester**	**38.4**
1479		Matricaria methylester	T
1489	1742	Bicyclogermacrene	0.2
1503	1744	*β*-Curcumene	0.4
1512	1763	*δ*-Cadinene	0.1
1521		Italiceneether	0.5
1540	2117	*cis*-3-hexenyl benzoate	0.2
1546	2042	*trans* Nerolidol	0.2
1554	2207	*α*-Turmerol	0.5
1565	1989	Oxycaryophyllene	0.8
		TOTAL	**84.2**

T: trace, less than 0.05%. [a]Linear retention index on non polar column, experimentally determined using homologous series of C8-C24 alkanes. [b]Linear retention index on polar column experimentally determined using homologous series of C8-C24 alkanes.

Table 4. Insecticidal activity of the CH_2Cl_2 extract, its fractions (F1 to F4) and the CH_3OH extract against *T. castaneum* larvae.

Extract or fraction	Concentration (mg/mL)	Mortality (%)
CH_2Cl_2 extract	25.0	92.5 c
	18.0	61.1 c
	15.0	42.5 b
	12.0	10.0 a
	10.0	10.0 a
	5.00	10.0 a
F 1	25.0	5 a
F 2		95.0 c
F 3		2.50 a
F 4		0 a
CH_3OH extract	25.0	0 a
	18.0	0 a
	15.0	0 a
	12.0	0 a
	10.0	0 a
	5.00	0 a
Control	-	0 a

Different letters indicate significant differences ($p < 0.05$).

The insecticidal activity showed with the CH_2Cl_2 extract and its F2 fraction could be attributed to the identified compounds. The major compound *cis* lachnophyllum methyl ester has reported larvicidal, nematicidal, and antifeedant activities on various pests [24] [25] [26] as well as other acetylene alcohols [27]. Additionally, the compounds eucalyptol, benzoic aldehyde, and benzyl alcohol have also reported insecticidal activity [28] [29] [30]. The *cis* lachnophyllum methyl ester was also the major compound in the volatile fraction. Therefore it is expected that this volatile fraction presents insecticidal activity such as the CH_2Cl_2 extract and the F2 fraction.

4. Conclusion

In the present study, the acetylenic alcohol (E)-undec-3-en-5,7-diyne-1-ol **(1)** was reported for the first time in *H. pinifolia* and no reports have been found in other species. The compounds eucalyptol **(3),** benzoic aldehyde **(4)**, and benzyl alcohol **(5)** were also reported for the first time in this species. The volatile fraction was studied and forty-six components were identified. *cis* lachnophyllum methyl ester **(2)** was the major compound identified in the fraction F2-C and in the volatile fraction. Our results showed that the CH_2Cl_2 extract of *H. pinifolia* and its fraction F2 have significant insecticidal activity in *T. castaneum* neonate larvae. The CH_2Cl_2 extract was also active on *T. castaneum* adults. This is the first report of bioactivity for this species. These results allow considering *H. pinifolia* as a potential source of extracts, purified fractions, and eventually secondary metabolites with insecticidal activity for the development, in a near future, of a botanical insecticide for controlling pests.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Appendix

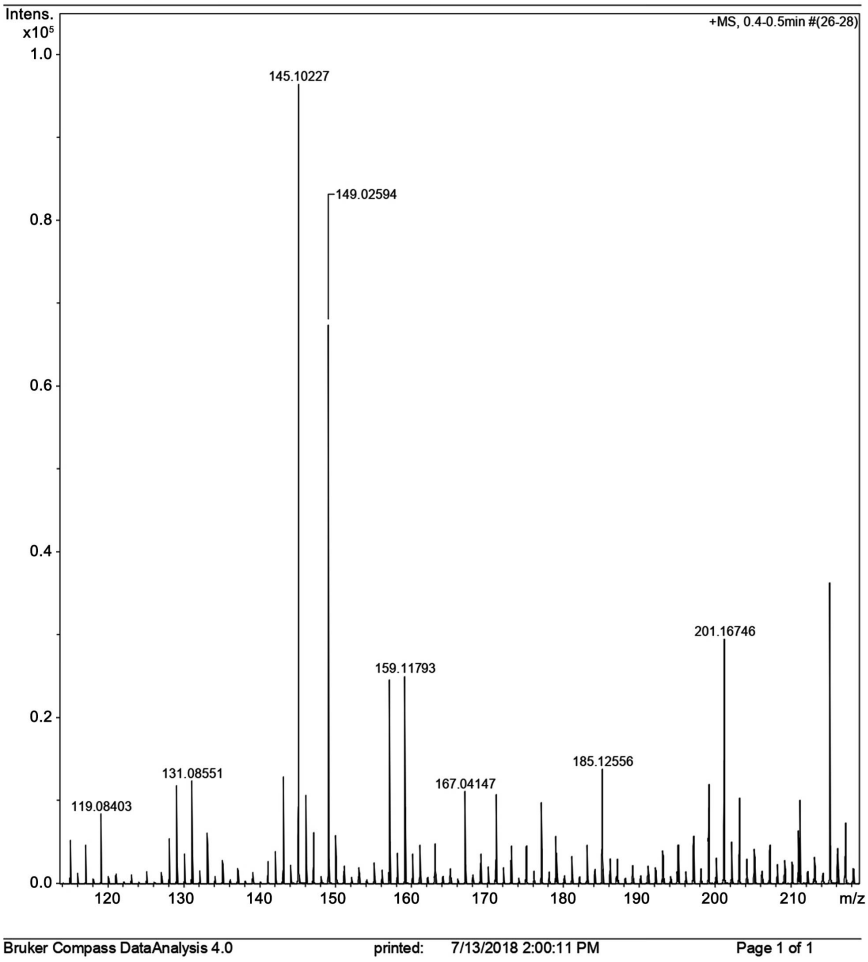

Figure S1. Massspectrumof fraction F2-E**(1)**.

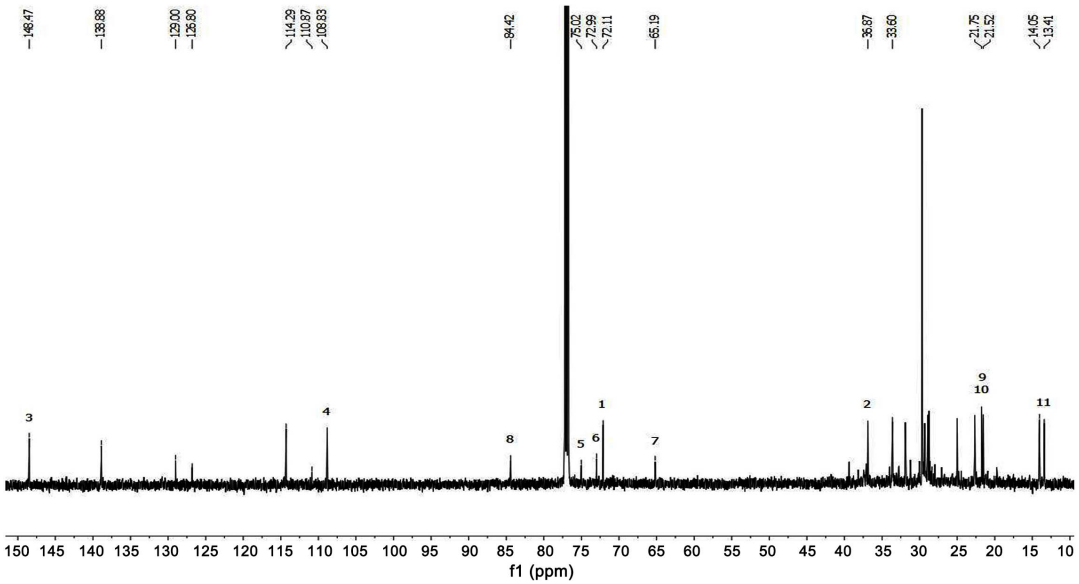

Figure S2. ^{13}CNMR spectrum of fraction F2-E**(1)** in $CDCl_3$.

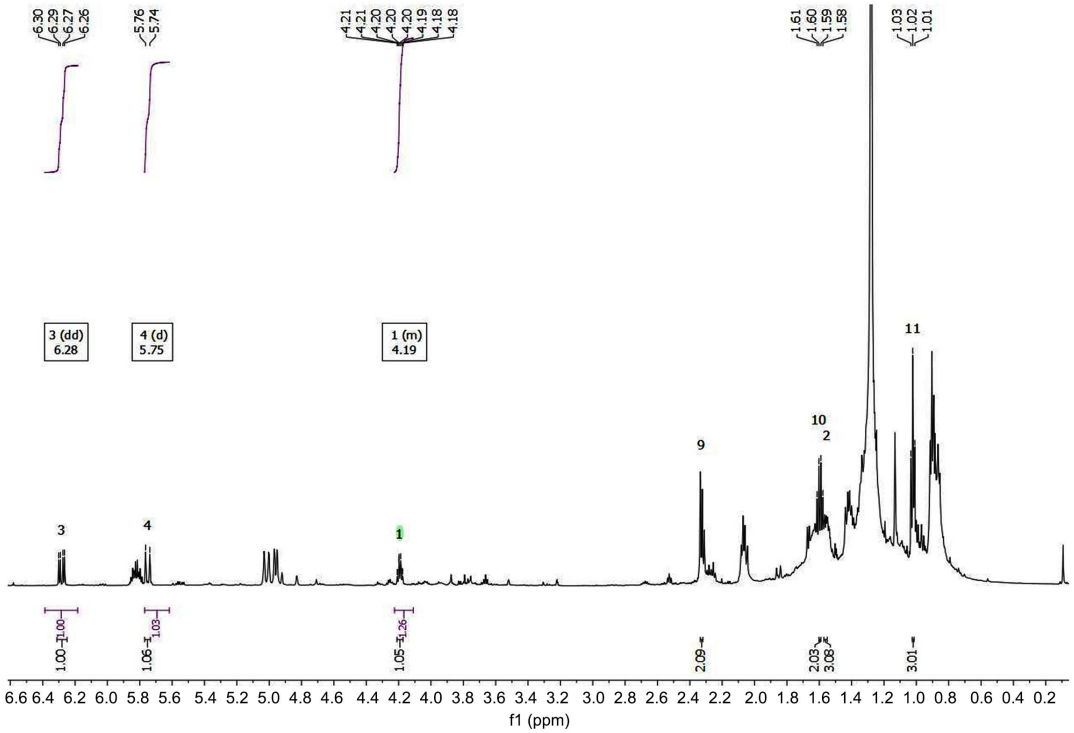

Figure S3. ^{1}H-NMR spectrum of fraction F2-E**(1)** in $CDCl_3$.

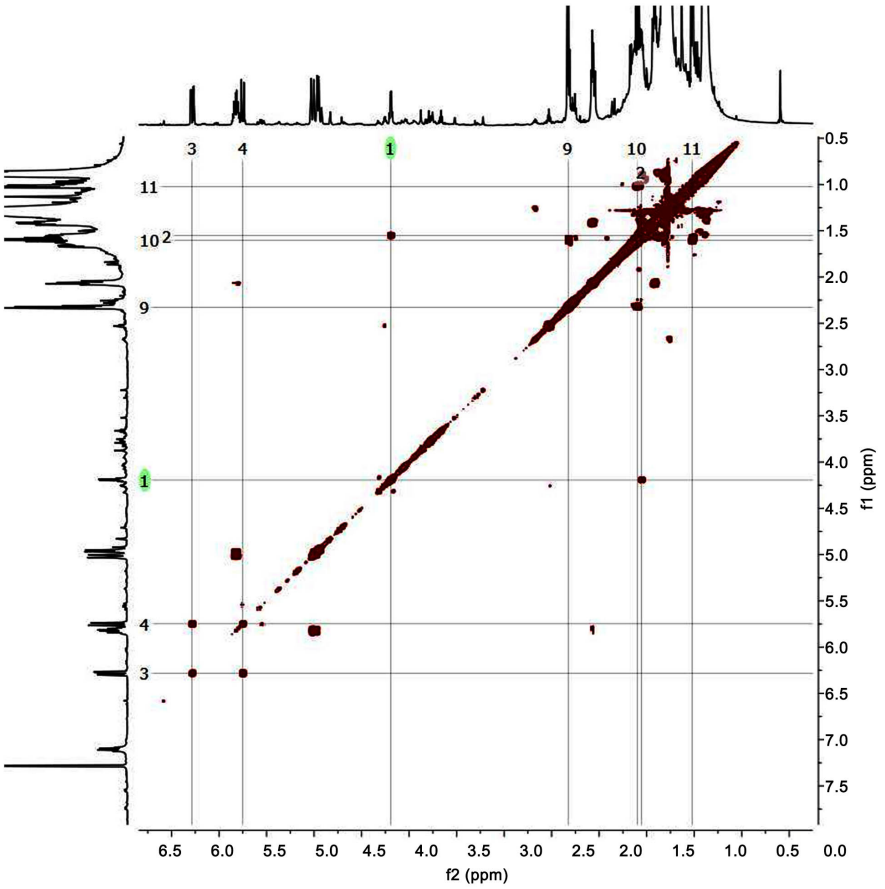

Figure S4. COSY spectrumof fraction F2-E**(1)** in $CDCl_3$.

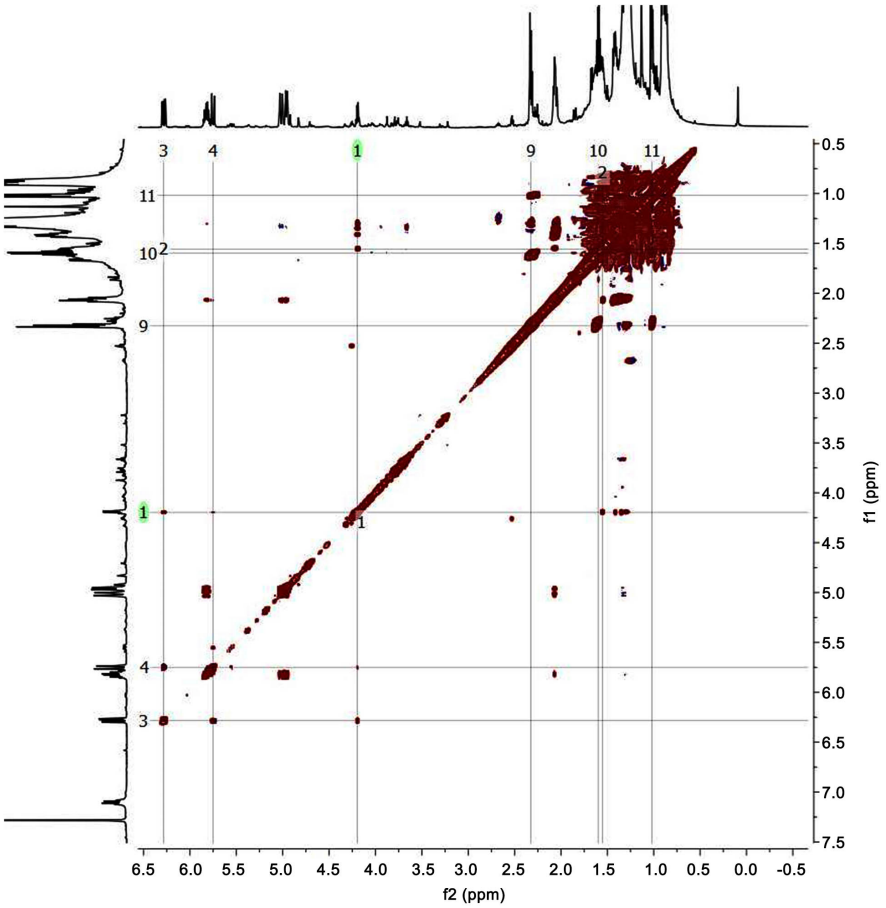

Figure S5. TOCSY spectrumof fraction F2-E**(1)** in $CDCl_3$.

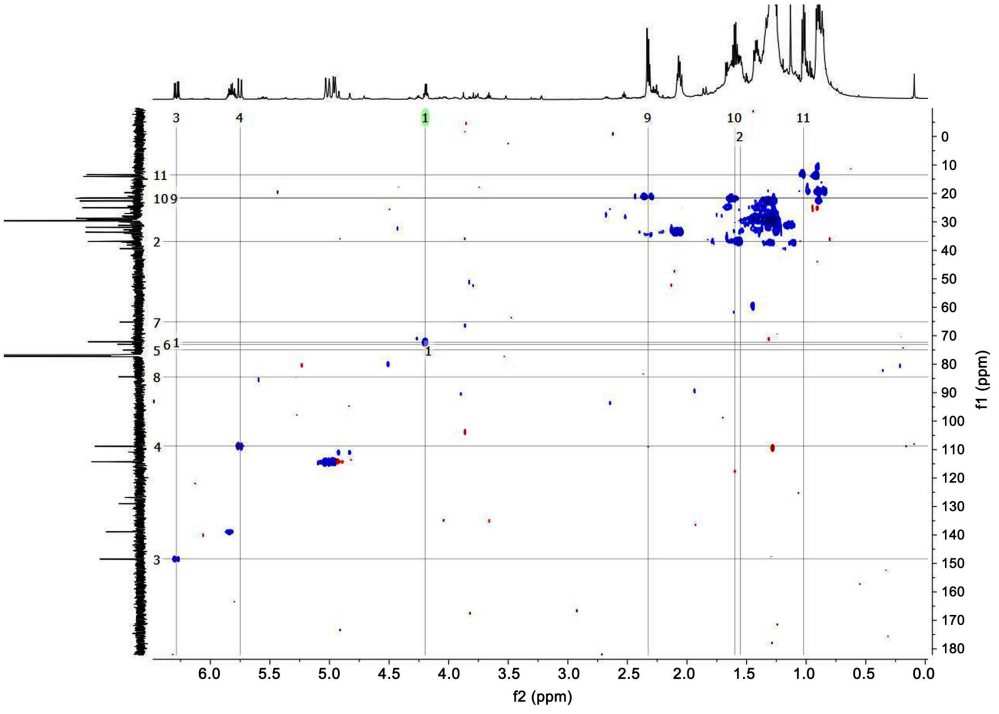

Figure S6. HSQC spectrumof fraction F2-E**(1)** in $CDCl_3$.

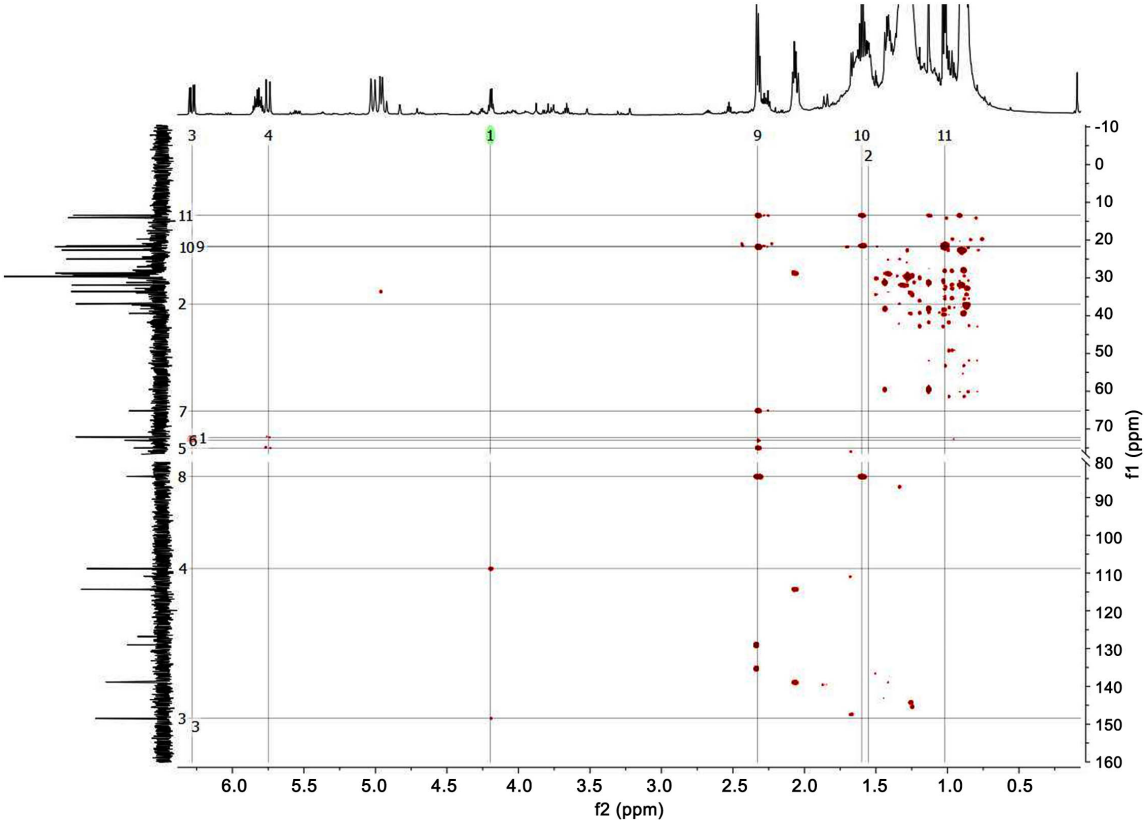

Figure S7. HMBC spectrumof fraction F2-E**(1)** in $CDCl_3$.

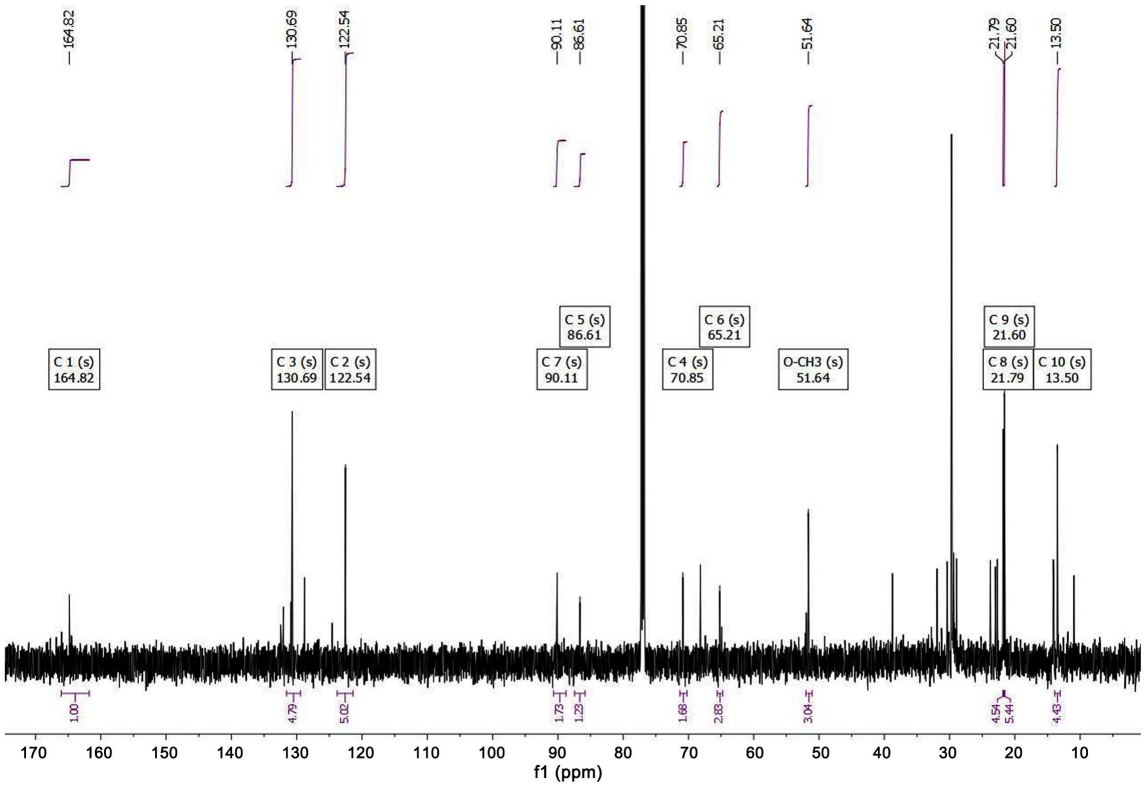

Figure S8. ^{13}C-NMR spectrum of fraction F2-C**(2)** in $CDCl_3$.

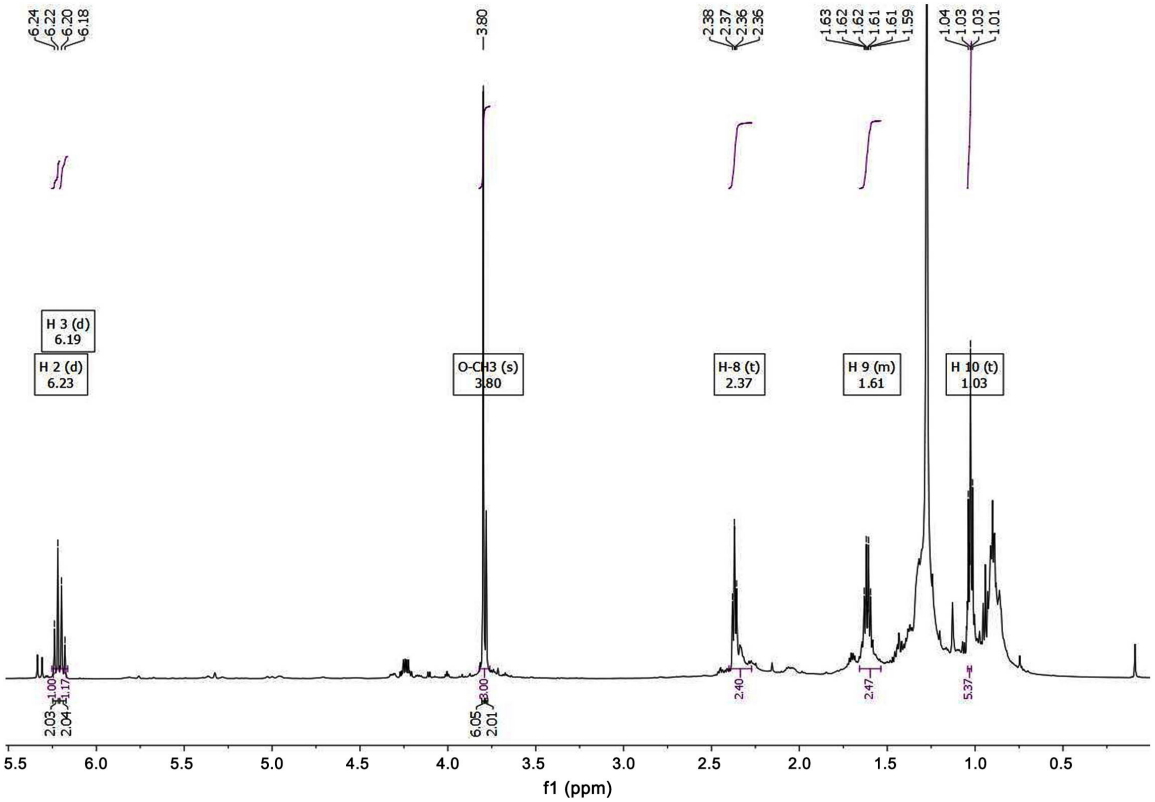

Figure S9. ^{1}H-NMR spectrum of fraction F2-C**(2)** in $CDCl_3$.

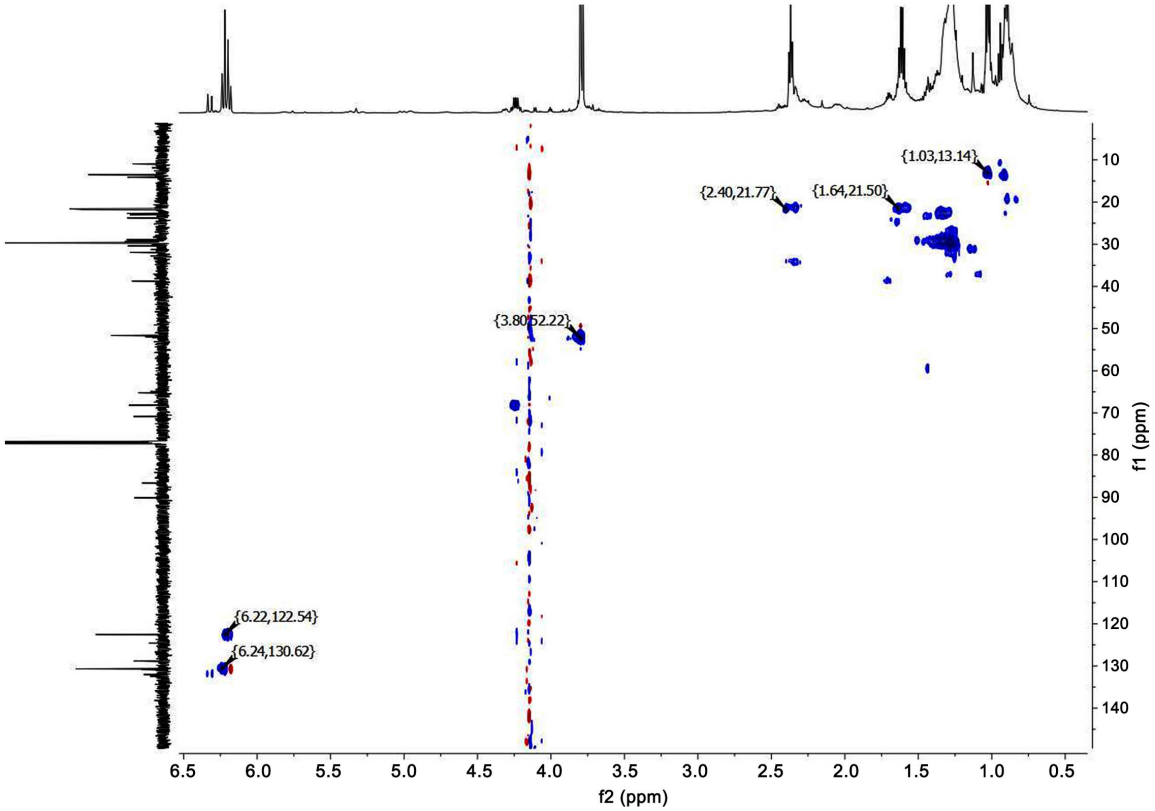

Figure S10. HSQC spectrum of fraction F2-C**(2)** in $CDCl_3$.

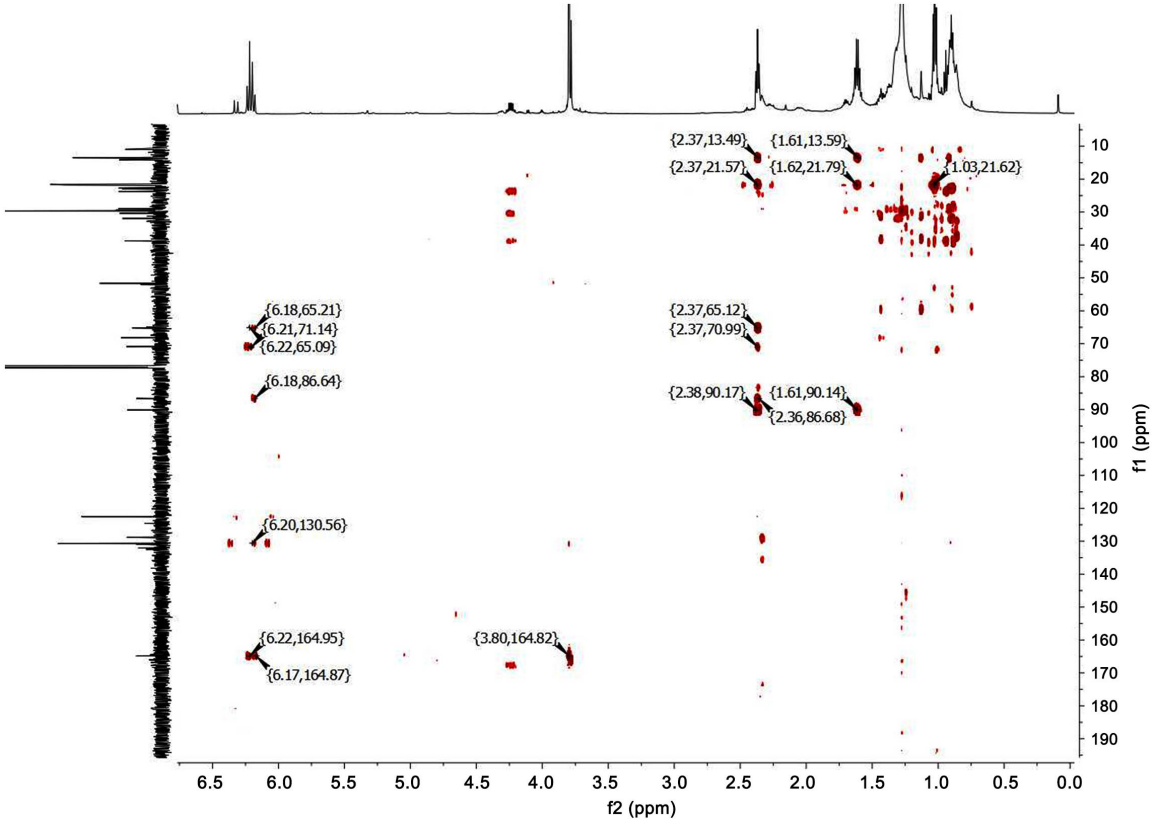

Figure S11. HMBC spectrum of fraction F2-C**(2)** in $CDCl_3$.