

PRENYLATED FLAVONOIDS FROM *Maclura tinctoria* FRUITS

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A phytochemical investigation of *Maclura tinctoria* fruits yielded five flavonoids, including one prenylated flavonol (licoflavonol) and four prenylated isoflavones (wighteone, derrone, alpinum isoflavone, and 6-(2,2-dimethyl-2H-chromen-6-yl)-2-(1-hydroxy-1-methylethyl)-2,3-dihydro-5H-furo[3,2-g]chromen-5-one). The structures of the isolates were established by analyzing their spectroscopic data. Compound 6-(2,2-dimethyl-2H-chromen-6-yl)-2-(1-hydroxy-1-methylethyl)-2,3-dihydro-5H-furo[3,2-g]chromen-5-one is new and the other isolates are described for the first time in this species.

Keywords: Moraceae; *Maclura tinctoria*; prenylated flavonoids.

INTRODUCTION

Maclura tinctoria (L.) D. Don ex Steudel (*Chlorophora tinctoria* Gaud.) according to Barroso,¹ popularly known as “amoreira,” “amora-do-mato” or “taíuva,” is a tree having a height of 10–20 m and a diameter of 40–60 cm, belonging to the family Moraceae and growing in humid and dry tropical forests of Central and South America.^{1,2} Its wood has been used in carpentry, furniture manufacturing, and civil construction, as well as for extraction of morin, a yellowish pigment employed for spot testing of certain metal ions.^{3,4} *M. tinctoria* bark infusions have been reported to be used against toothache by the Kaiowá and Guarani indigenous populations living in the Caarapó Reserve in Mato Grosso do Sul state, Brazil.⁵ The resin of the plant is used for the same purpose in the Amazonian indigenous community of Porvenir, Bajo Paragua Indian Reservation, Bolivia.⁶ The species also furnishes diuretic and anti-venereal agents and is used in Latin America to treat urinary infections, cough, gout, pharyngitis, rheumatism, sore throat, and syphilis.⁷

Chemical studies of the *Maclura* genus have revealed prenylated flavonoids as main constituents. In *M. pomifera*, the most studied species of the genus, several flavonoids have been described, including isoflavones from the fruits, root, bark, and heartwood of the plant.⁸⁻¹³ Five prenylated flavones and three prenylated isoflavones have been isolated from chloroform extracts of stems and leaves.¹¹

M. tinctoria is relatively well studied from a phytochemical viewpoint; however, the studies reported have used only the bark and leaves of the plant. Five prenylated flavonoids, four prenylated chalcones and one diprenylisoflavone, were isolated from an ethanol extract of *M. tinctoria* leaves and identified as 2',4',4,2'-tetrahydroxy-3'-(3'-methylbut-3'-enyl)-chalcone, isobavachalcone, bakuchalcone, bavachromanol, and 5,7,3:4'-tetrahydroxy-6,8-diprenylisoflavone (6,8-diprenylorobol).¹⁴ Bioassay-guided fractionation of the bark extract by tracking of HIV-inhibitory activity afforded seven prenylated phenolic derivatives from the active fractions, including the prenylated xanthenes macluraxanthone B, macluraxanthone C, gartanin, and desoxygartanin, along with the prenylated flavones cudraflavone C, dihydrocudraflavone B, and isocyclomulberrin.¹⁵

The flavonoids steppogenin, steppogenin 4'-O-β-D-glucoside, orobol, orobol 5,3'-di-O-methyl-8-C-glucoside, orobol 7-O-β-D-glucoside, aromadendrin, and dihydromorin were isolated from the *M. tinctoria* bark extract.¹⁶ Antioxidant chalcone glycosides and flavanones were also reported from the stem bark of this plant. The isolated compounds were characterized as eriodictyol 7-O-α-D-glucopyranoside, naringenin, naringenin 4'-O-α-D-glucopyranoside, 3'-(3-methyl-2-butenyl)-4'-O-α-D-glucopyranosyl-4,2'-dihydroxychalcone, 4'-O-α-D-(2"-p-coumaroyl)glucopyranosyl-4,2',3'-trihydroxychalcone, 4'-O-α-D-(2"-p-coumaroyl-6"-acetyl)glucopyranosyl-4,2',3'-trihydroxychalcone, and 4'-O-α-D-(2"-acetyl-6"-cinnamoyl)glucopyranosyl-4,2',3'-trihydroxychalcone.¹⁷

In this study, we report the isolation and structural elucidation of a new compound, identified as 6-(2,2-dimethyl-2H-chromen-6-yl)-2-(1-hydroxy-1-methylethyl)-2,3-dihydro-5H-furo[3,2-g]chromen-5-one, along with four known prenylated flavonoids from the fruits of *M. tinctoria*.

EXPERIMENTAL

General experimental

¹H and ¹³C NMR spectra were recorded on a Varian Mercury Plus spectrometer at 300 and 75.5 MHz, respectively, using (CD₃)₂CO as solvent and TMS as reference. High resolution mass spectra were obtained in a LTQ-Orbitrap hybrid mass spectrometer (Thermo Fischer Scientific, Bremen, Germany). EI-MS spectra were recorded on a Thermolectron Corporation Focus-DSQ II spectrometer at 70 eV. Column chromatography (CC) was performed using Sephadex LH-20 or silica gel 60 Merck (70–30 mesh ASTM). TLC was performed on precoated silica gel 60G or 60GF₂₅₄ Merck. All solvents were redistilled before use.

Plant material

Fruits of *M. tinctoria* were collected in December 2008 at the Bosque dos Pinheiros, a remnant forest in an urban area in Maringá-PR, Brazil. The plant was identified by Dr. Sergio Romaniuc Neto (Instituto de Botânica, São Paulo, Brazil). A voucher specimen

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has been deposited in the herbarium of the Biology Department, Universidade Estadual de Maringá (HUEM 16499).

Extraction and isolation

Dried fruits with seeds (670 g) of *M. tinctoria* were powdered and exhaustively extracted with methanol at room temperature. Vacuum concentration yielded the crude methanol extract (123 g), which was suspended in MeOH:H₂O (1:1) and partitioned into CH₂Cl₂, EtOAc, and BuOH. The solvents were evaporated to give CH₂Cl₂ (24.7 g), EtOAc (9.60 g), BuOH (32.4 g), and aqueous (57.1 g) fractions. An aliquot of the CH₂Cl₂ fraction (3 g) was passed through a Sephadex LH-20 chromatographic column using MeOH as eluent, yielding 18 fractions of 15 mL each. This procedure was repeated four times, and the fractions were grouped on the basis of TLC similarity (A1 to A15). Fraction A4 (543 mg) was purified using CC on silica gel with a mixture of *n*-hexane, CH₂Cl₂, and EtOAc in increasing polarity as eluent, followed by preparative TLC (CH₂Cl₂:EtOAc; 7:3) of the subfraction eluted from CC with CH₂Cl₂:EtOAc (6:4), to yield pure compound **1** (8 mg). Fraction A9 (376 mg) was subjected to CC on silica gel, using *n*-hexane containing increasing amounts of EtOAc (up to 100%) as eluent, to give 108 fractions of 20 mL each (A9.1 to A9.108). Subfraction A9.20 (72 mg) was subjected to preparative TLC with CH₂Cl₂ (100%) elution. The fraction with *R_f* = 0.28 was dissolved in CHCl₃ and subjected to precipitation with hexane. The precipitate (10 mg) was identified as pure compound **2**, and the supernatant yielded a mixture of compounds **2** and **3** (12 mg). Fraction A15 (84 mg) was subjected to preparative TLC with hexane:CH₂Cl₂ (2:8) elution. The fraction with *R_f* = 0.20 was subjected to subsequent preparative TLC using hexane:CH₂Cl₂ (2:8) as eluent, to yield pure compounds **4** (9 mg) and **5** (11 mg).

RESULTS AND DISCUSSION

Fractionation of the CH₂Cl₂ fraction by gel permeation chromatography using a Sephadex LH-20 column, followed by CC on silica gel and preparative TLC of subfractions, afforded five prenylated flavonoids (**1–5**) (Figure 1). The structures of the isolated compounds were determined by analysis of their spectral data (MS and 1D and 2D NMR).

The new prenylated isoflavone (**1**), 6-(2,2-dimethyl-2*H*-chromen-6-yl)-2-(1-hydroxy-1-methylethyl)-2,3-dihydro-5*H*-furo[3,2-*g*]chromen-5-one, was obtained as an amorphous yellow powder. The HR-ESIMS of **1**, in the positive-ion mode, exhibited a molecular ion peak [M + H]⁺ at *m/z* 435.1800, consistent with the molecular formula C₂₆H₂₆O₆. The ¹³C NMR spectra (Table 1) of compound **1** revealed characteristic signals for C-2, C-3, and C-4 of an isoflavone at δ 151.5, 122.7, and 175.1, respectively. The additional signals for twelve aromatic carbons, eight non-hydrogenated, and four methine sp²-carbons, evidenced an isoflavone skeleton with di- and tri-substituted aromatic rings. This was confirmed by the signals of one aromatic hydrogen as singlet at 6.49 (s, H-8) and of the coupled aromatic hydrogens at δ 7.19 (d, *J* = 2.1 Hz, H-2'), 6.72 (d, *J* = 8.4 Hz, H-5') and δ 7.25 (dd, *J* = 8.4 and 2.1 Hz, H-6'), in the ¹H NMR spectra. The signals at δ 3.29 (m, 2H, H-3''') and δ 4.74 (t, *J* = 7.20 Hz, 1H, H-2''') and of two methyl groups at δ 1.24 (s, 3H) and δ 1.21 (s, 3H) evidenced the presence of the 2-(1-hydroxy-1-methylethyl)-2,3-dihydrofuran system, which was confirmed by the corresponding ¹³C NMR signals at δ 28.0 (C-3'''), 92.0 (C-2'''), and δ 71.4 (C-4'''). The linkage of this group at C-6 and C-7 of ring A was established by the correlation between H-3''' (δ 3.29) and C-6 (δ 118.4) in the HMBC experiment (Table

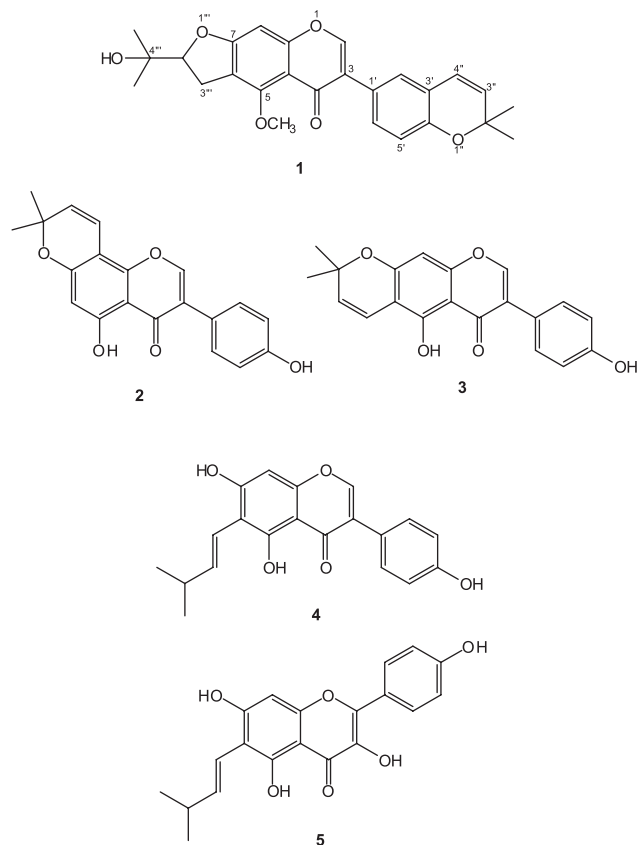


Figure 1. Prenylated flavonoids isolated from the fruits of *Maclura tinctoria*

1). In addition, the ¹H and ¹³C NMR spectra indicated the presence of a dimethylchromene system at δ 7.19 (d, 2.1 Hz, H-2'), 6.72 (d, 8.4 Hz, H-5'), 7.25 (dd, 8.4 Hz and 2.1 Hz, H-6'), 6.37 (d, 9.9 Hz, H-4''), and 5.68 (d, 9.9 Hz, H-5''').

The assignment of all signals observed in ¹H and ¹³C NMR spectra of **1** was corroborated by HMQC and HMBC experiments (Table 1). These data revealed that **1** is closely related to ulexin D, isolated from *Ulex aereus*,¹⁸ differing by the presence of a 5-OCH₃ instead of a 5-OH group in ulexin D. The linkage of the methoxyl group (δ_H 3.87, s, 3H) at C-5 was confirmed by the downfield (approximately 6–9 ppm) shift observed for C-6 and C-10 of compound **1** owing to the methylation effect, compared with the corresponding carbons of ulexin D. This evidence was also corroborated by the carbonyl signal at δ 175.1 in the ¹³C NMR spectrum of **1**, instead of at δ 181.7 as seen in ulexin D, in agreement with the lack of a free 5-OH group.

The known prenylated isoflavones derrone (**2**),¹⁹ alpinum isoflavone (**3**),^{19,20} and wightone (**4**),¹⁸ were identified by comparing their NMR data with those reported in the literature. Derrone (**2**) and alpinum isoflavone (**3**) are isomers, possessing isoprenyl groups linked at C-8 and C-6, respectively. In the ¹H and ¹³C NMR spectra of derrone (**2**), the H-6 and C-6 appears at δ 6.19 (s, 1H) and δ 101.9, respectively, whereas in alpinum isoflavone (**3**) the signals for H-8 and C-8 are observed at δ 6.36 (s, 1H) and δ 95.5, respectively.

Derrone (**2**) and alpinum isoflavone (**3**) along with other prenylated isoflavones have been isolated from *Ficus nymphaefolia*, a species of Moraceae.²¹ Wightone (**4**) was previously isolated from *M. pomifera* leaves¹¹ and *Erythrina indica* stem bark. Its potential cytotoxicity to human KB cells has also been reported.²²

The prenylated flavonol **5** was first isolated from *Glycyrrhiza glabra* roots²³ and identified as licoflavonol by comparing its data with those previously reported.^{23,24}

Table 1. ¹H and ¹³C NMR data of compound **1** in (CD₃)₂CO

Position	¹³ C	¹ H (mult., integ., J in Hz)	HMBC H→C
1			
2	151.5	7.95 (s, 1H)	4, 9, 1'
3	122.7		-
4	175.1		-
5	156.7		-
6	118.4		-
7	165.6		-
8	93.9	6.49 (s, 1H)	6, 7, 9, 10
9	160.0		-
10	113.0		-
1'	125.5		-
2'	127.9	7.19 (d, 1H, 2.1)	3, 4', 6'
3'	121.6	-	-
4'	153.3	-	-
5'	116.4	6.72 (d, 1H, 8.4)	1'
6'	130.5	7.25 (dd, 1H, 8.4; 2.1)	2', 4'
2''	76.9	-	-
3''	131.6	5.68 (d, 1H, 9.9)	3', 2''
4''	125.5	6.37 (d, 1H, 9.9)	4', 2''
CH ₃ -2''	28.1	1.40 (s, 6H)	2'', 3''
2'''	92.0	4.74 (t, 1H, 7.2)	-
3'''	28.0	3.29 (m, 2H)	6
4'''	71.4	-	-
CH ₃ a-4'''	25.1	1.24 (s, 3H)	CH ₃ b-4''', 4'''
CH ₃ b-4'''	25.8	1.21 (s, 3H)	CH ₃ a-4''', 2''', 4'''
OCH ₃	60.8	3.87 (s, 3H)	5

CONCLUSION

Excepting wighetone (**4**), all isolated compounds are being described for the first time from *Maclura* genus. However, several prenylated isoflavones have been reported to occur in *M. pomifera*⁸⁻¹² and other species of the Moraceae family.^{21,25,26} The isolation of prenylated isoflavones from *M. tinctoria* may contribute to further chemotaxonomic studies on the genus *Maclura* and family Moraceae.

SUPPLEMENTARY MATERIAL

The RMN and mass spectra for compounds **1–5** are available at <http://quimicanova.s bq.org.br> as a free-access PDF file.

ACKNOWLEDGMENTS

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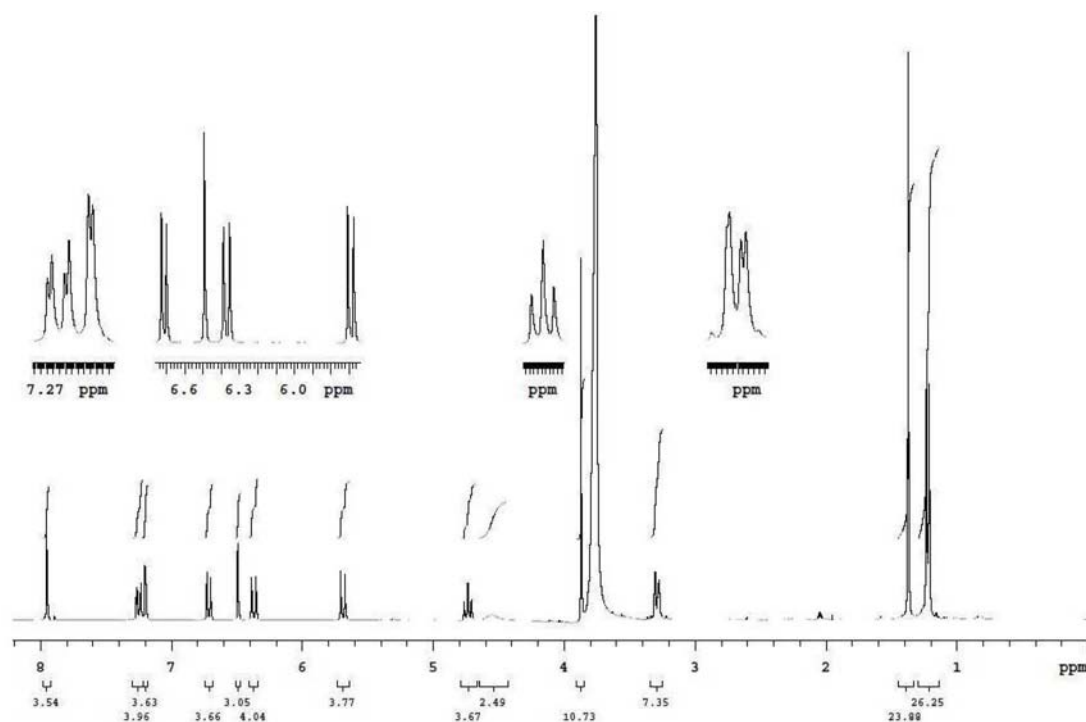


Figure 1S. ¹H NMR spectrum (300 MHz, (CD₃)₂CO) of compound 1

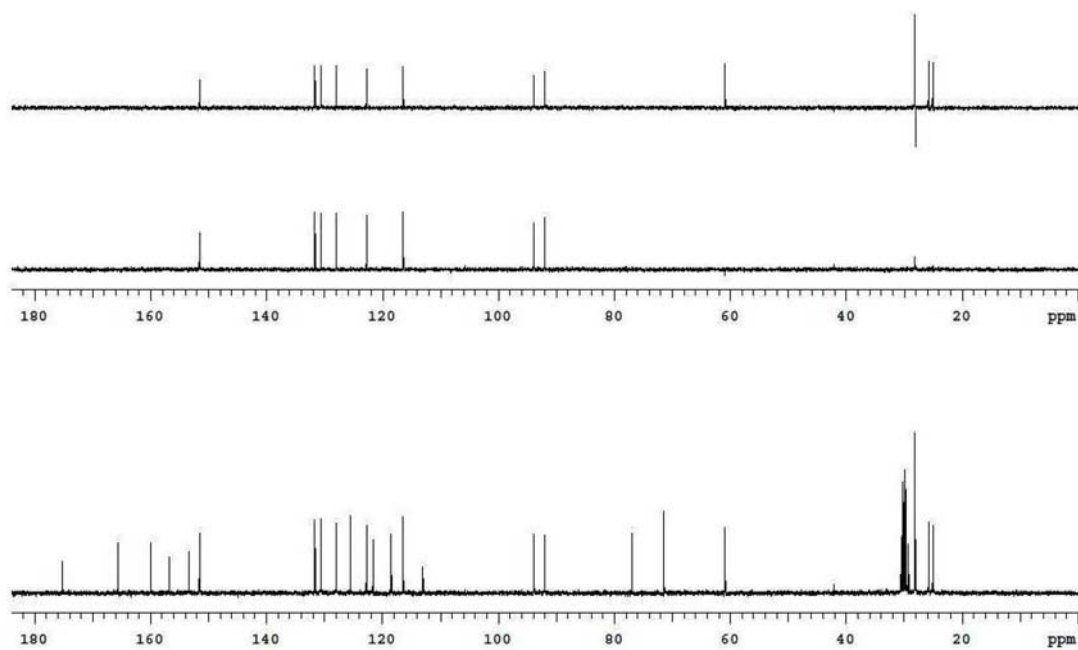


Figure 2S. ^{13}C and DEPT 90 spectrum (75 MHz, $(\text{CD}_3)_2\text{CO}$) of compound 1

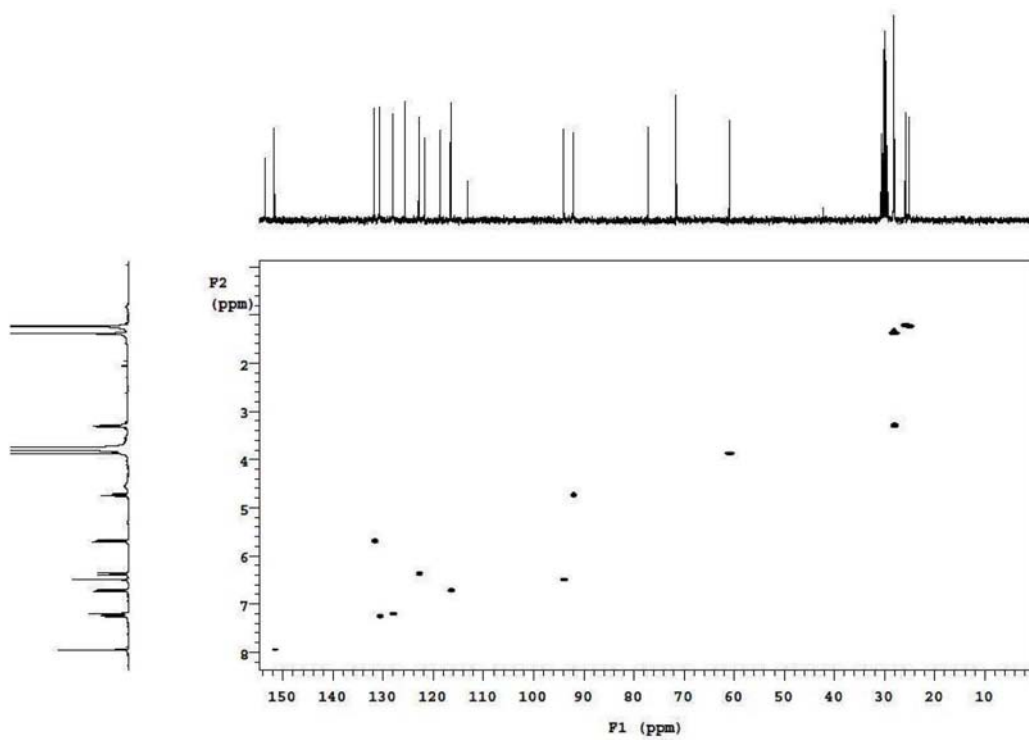


Figure 3S. HSQC spectrum (300/75 MHz, $(\text{CD}_3)_2\text{CO}$) of compound 1

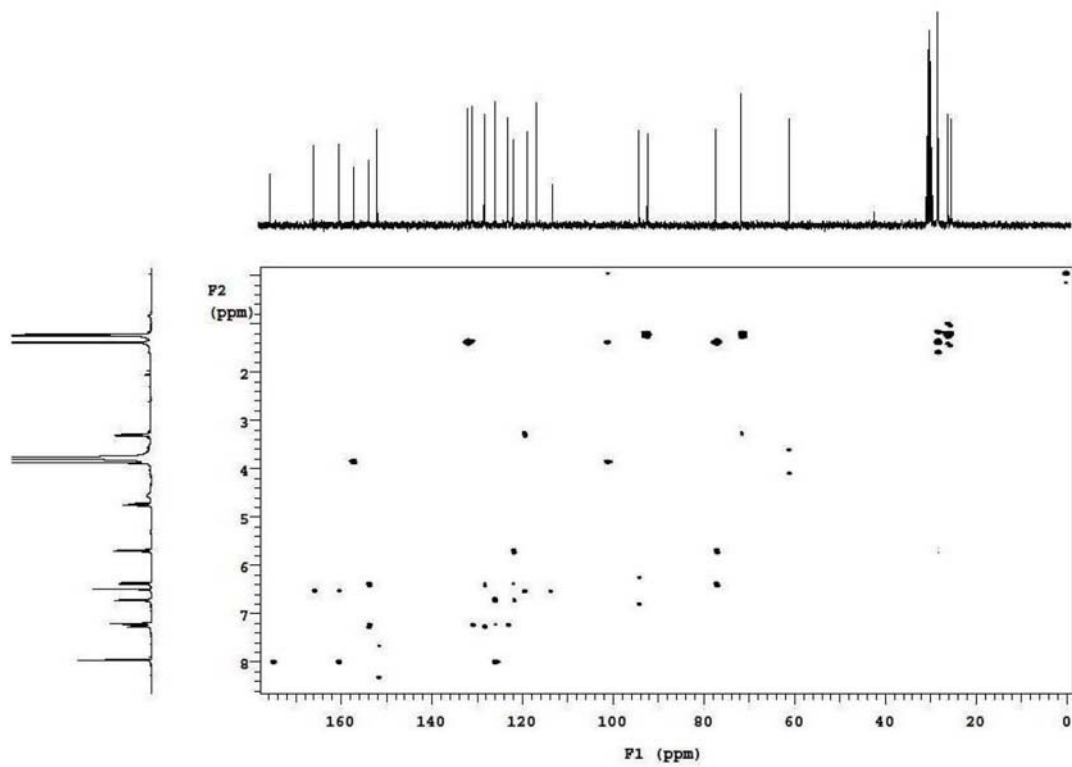


Figure 4S. HMBC spectrum (300/75 MHz, $(CD_3)_2CO$) of compound 1

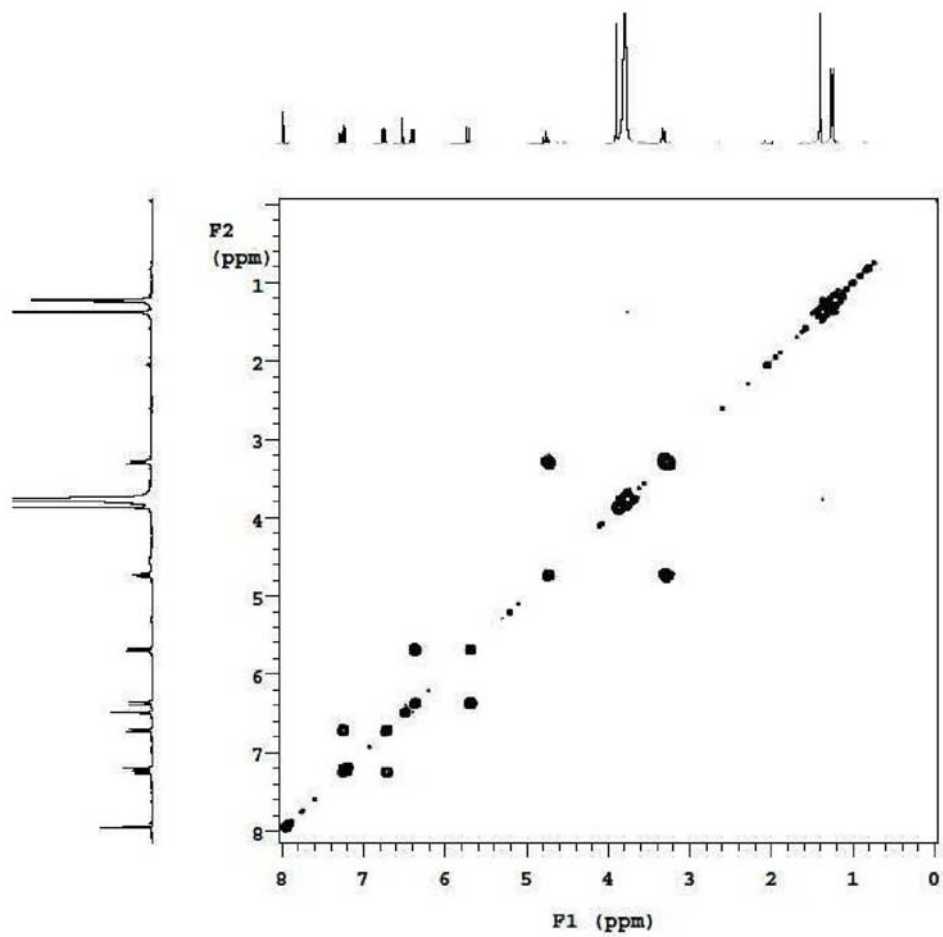


Figure 5S. COSY spectrum (300 MHz $(CD_3)_2CO$) of compound 1

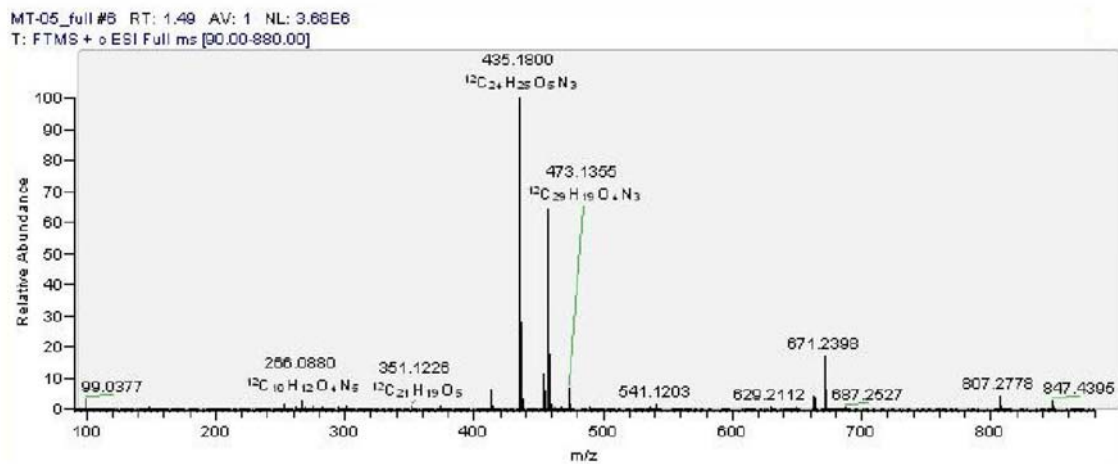


Figure 6S. HR-ESIMS positive mode of compound 1

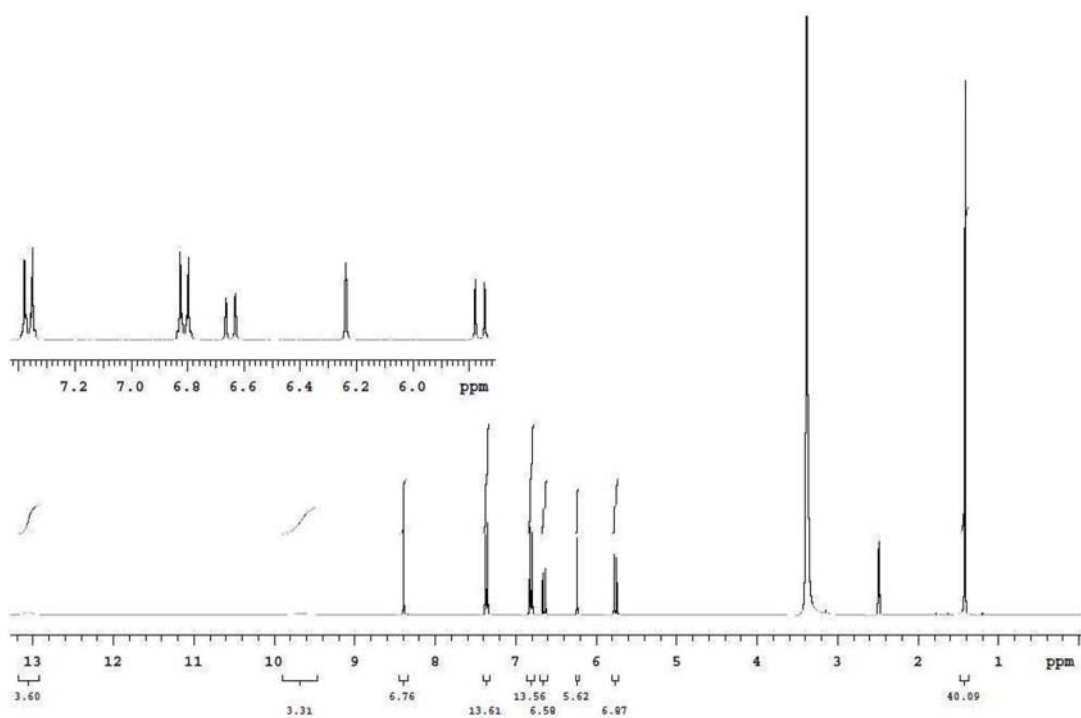


Figure 7S. ^1H NMR spectrum (300 MHz, $(\text{CD}_3)_2\text{CO}$) of compound 2

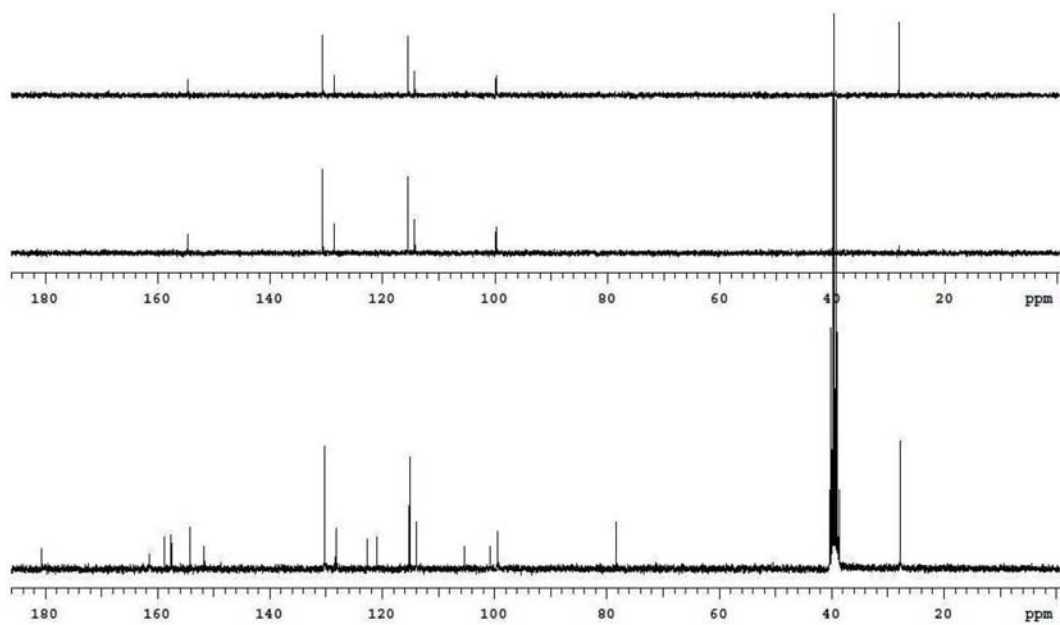


Figure 8S. ¹³C and DEPT 90 spectrum (75 MHz, (CD₃)₂CO) of compound 2

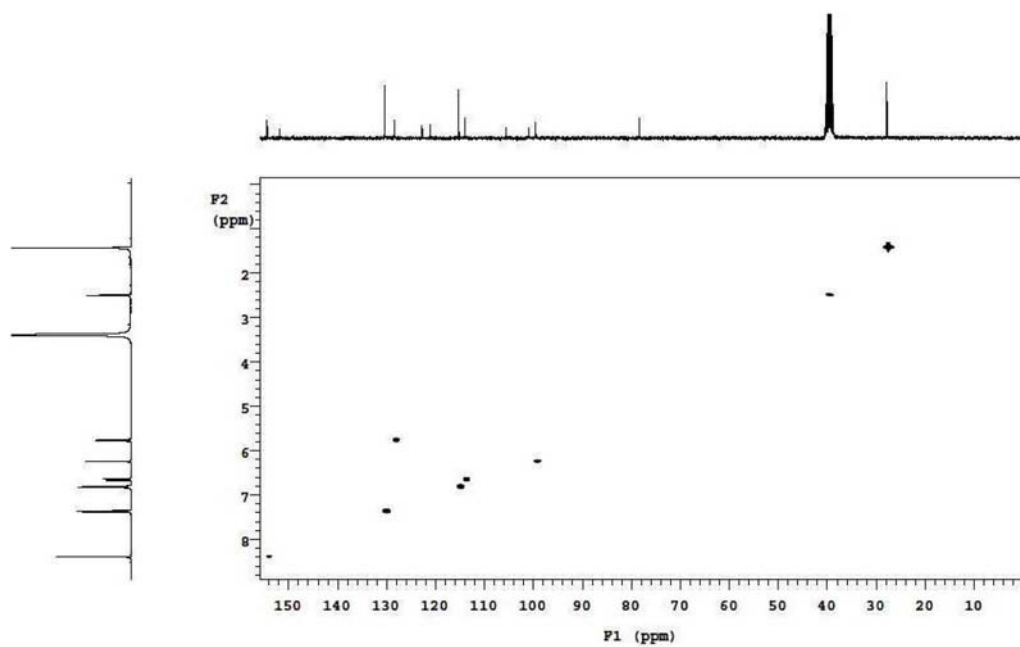


Figure 9S. HSQC spectrum (300/75 MHz, (CD₃)₂CO) of compound 2

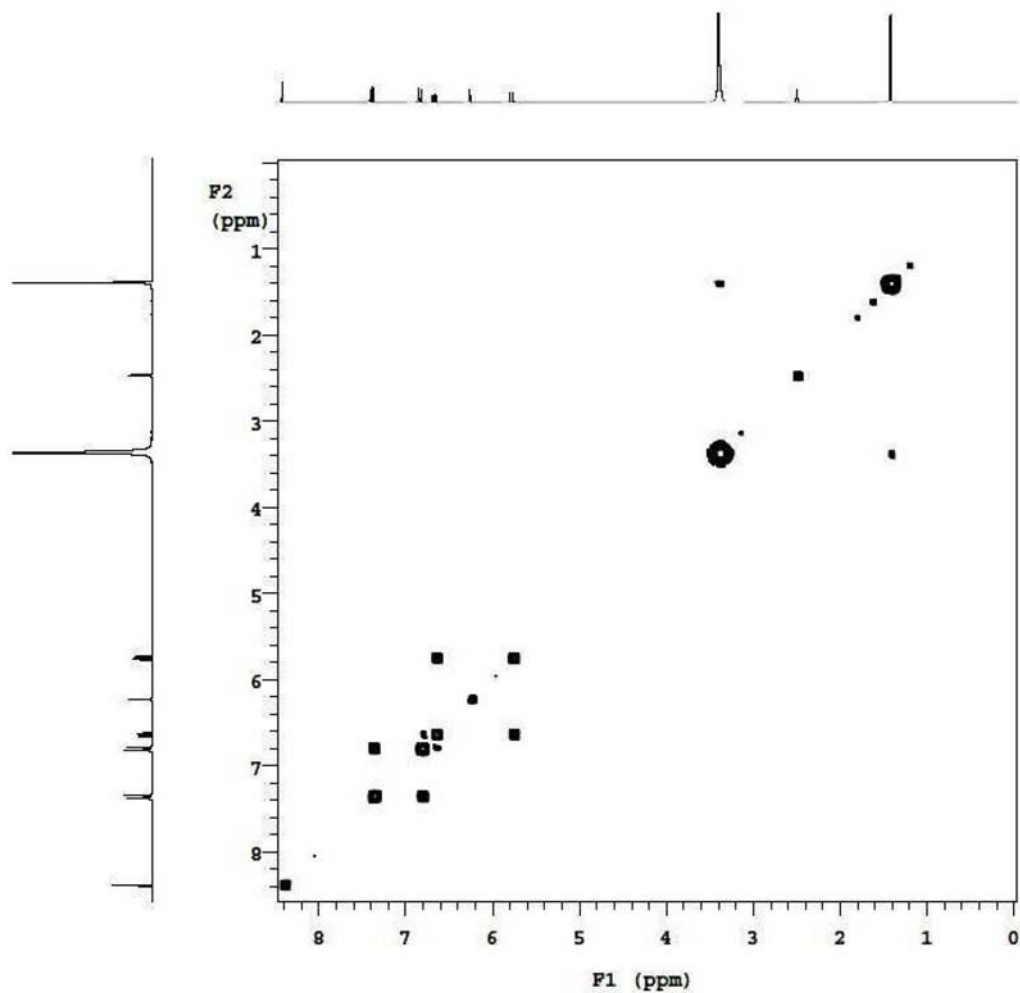


Figure 10S. COSY spectrum (300 MHz $(CD_3)_2CO$) of compound 2

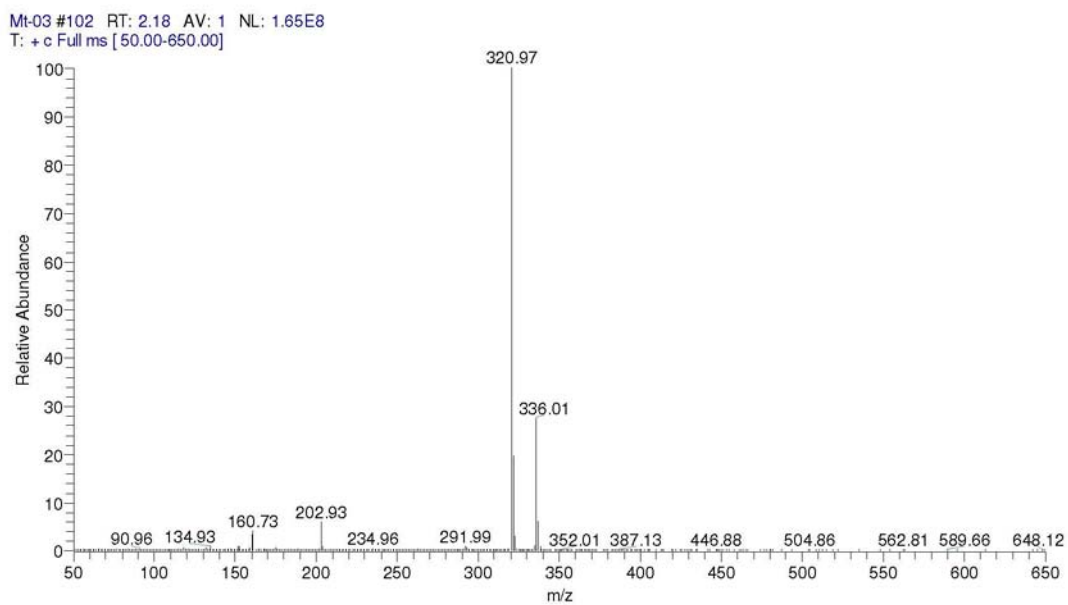


Figure 11S. EIMS (70 eV) of compound 2

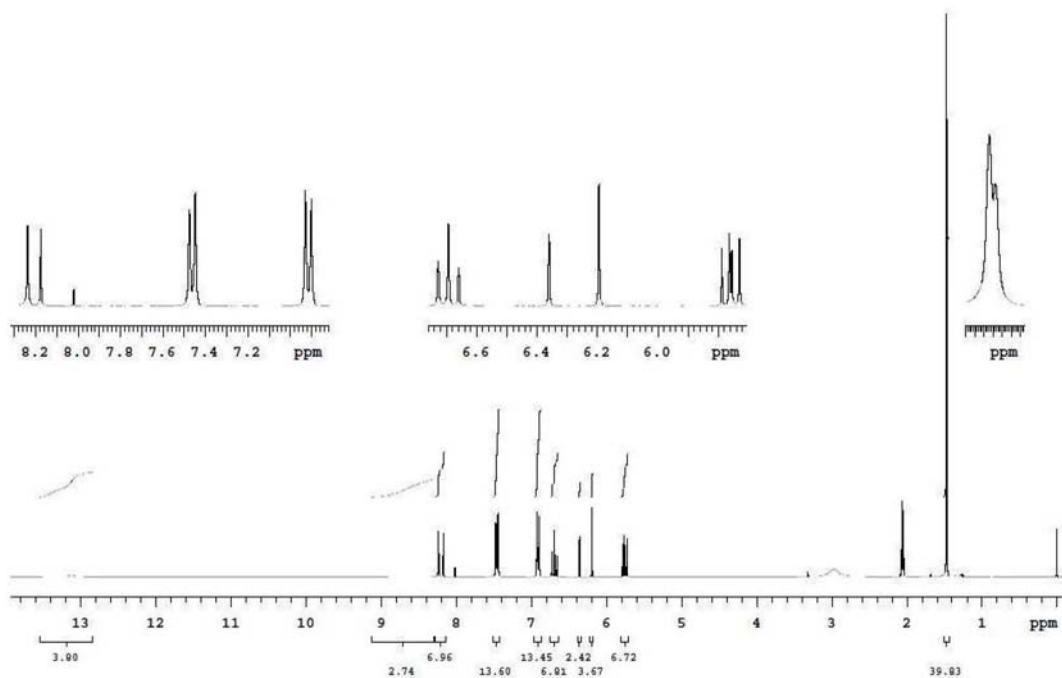


Figure 12S. ^1H NMR spectrum (300 MHz, $(\text{CD}_3)_2\text{CO}$) of compound 3

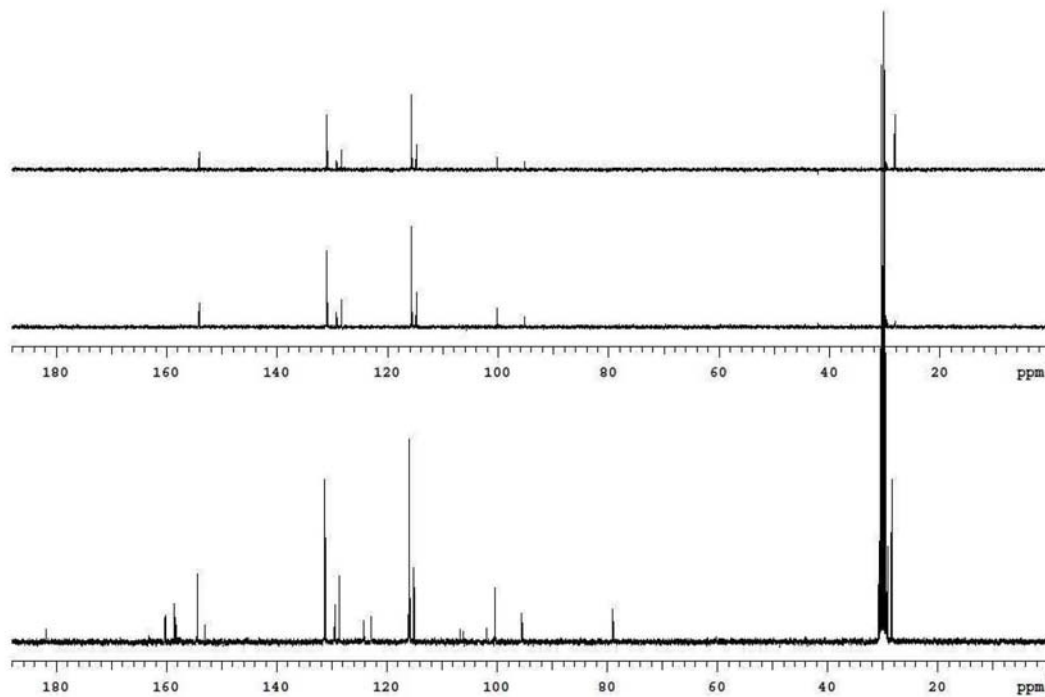


Figure 13S. ^{13}C and DEPT 90 spectrum (75 MHz, $(\text{CD}_3)_2\text{CO}$) of compound 3

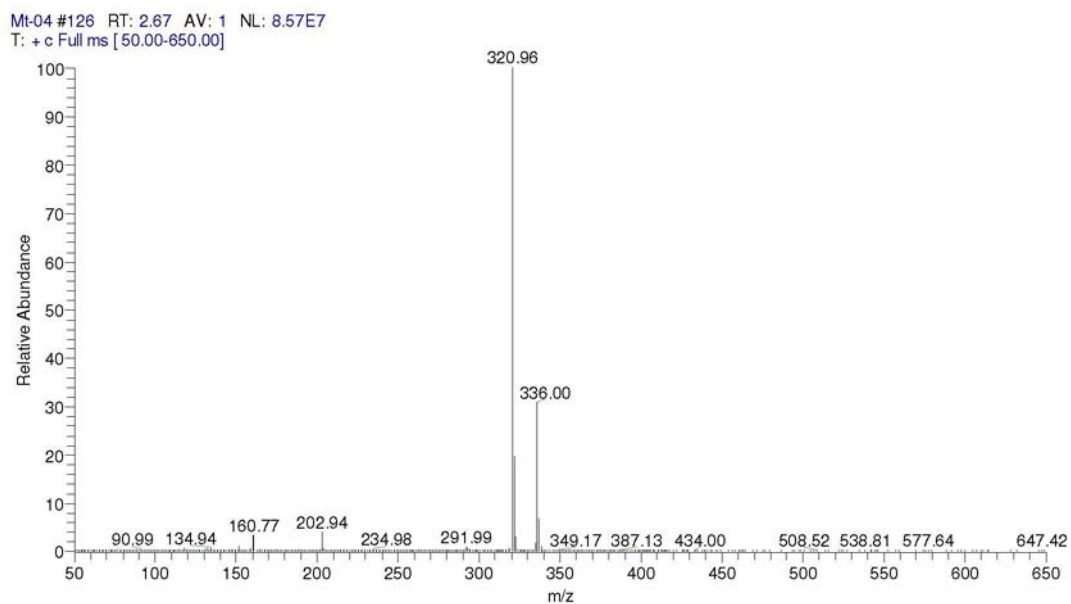


Figure 14S. EIMS (70 eV) of compound 3

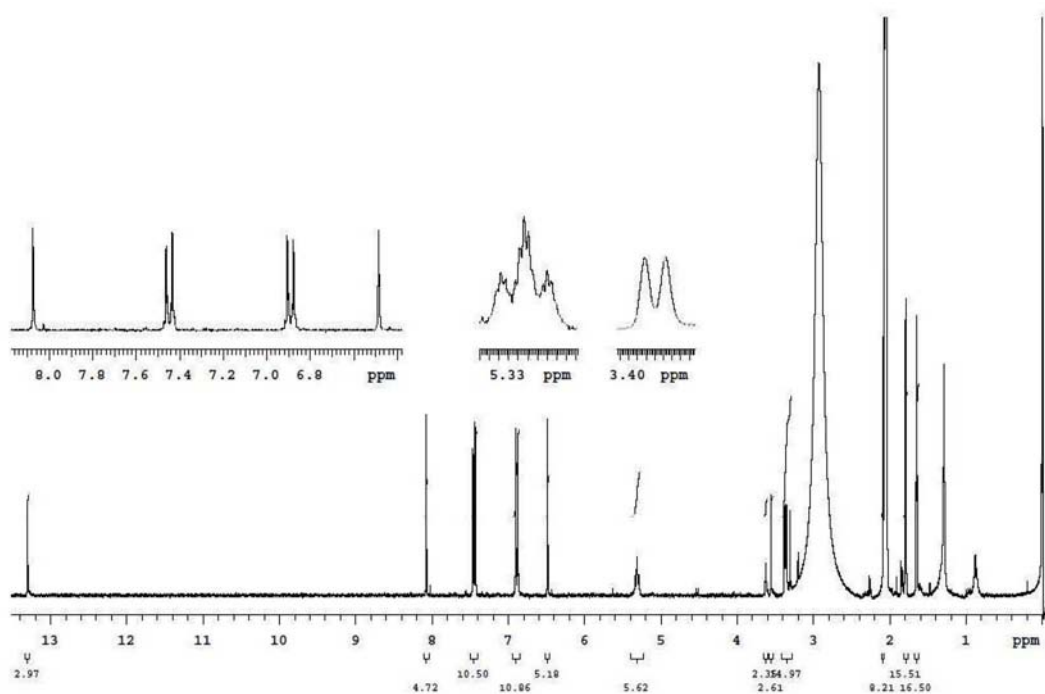


Figure 15S. ^1H NMR spectrum (300 MHz, $(\text{CD}_3)_2\text{CO}$) of compound 4

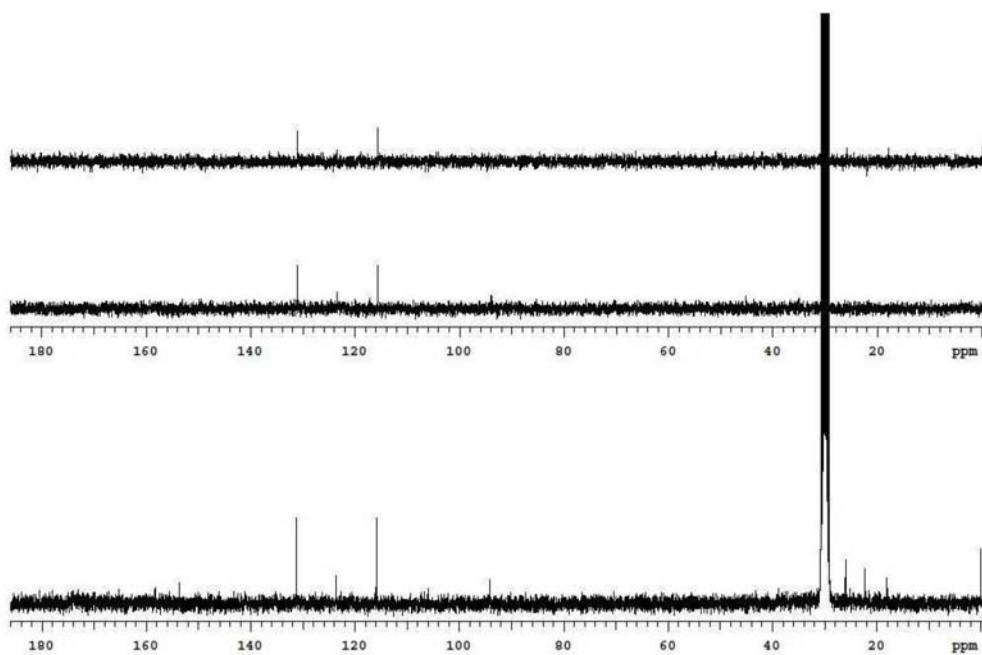


Figure 16S. ^{13}C and DEPT 90 spectrum (75 MHz, $(\text{CD}_3)_2\text{CO}$) of compound 4

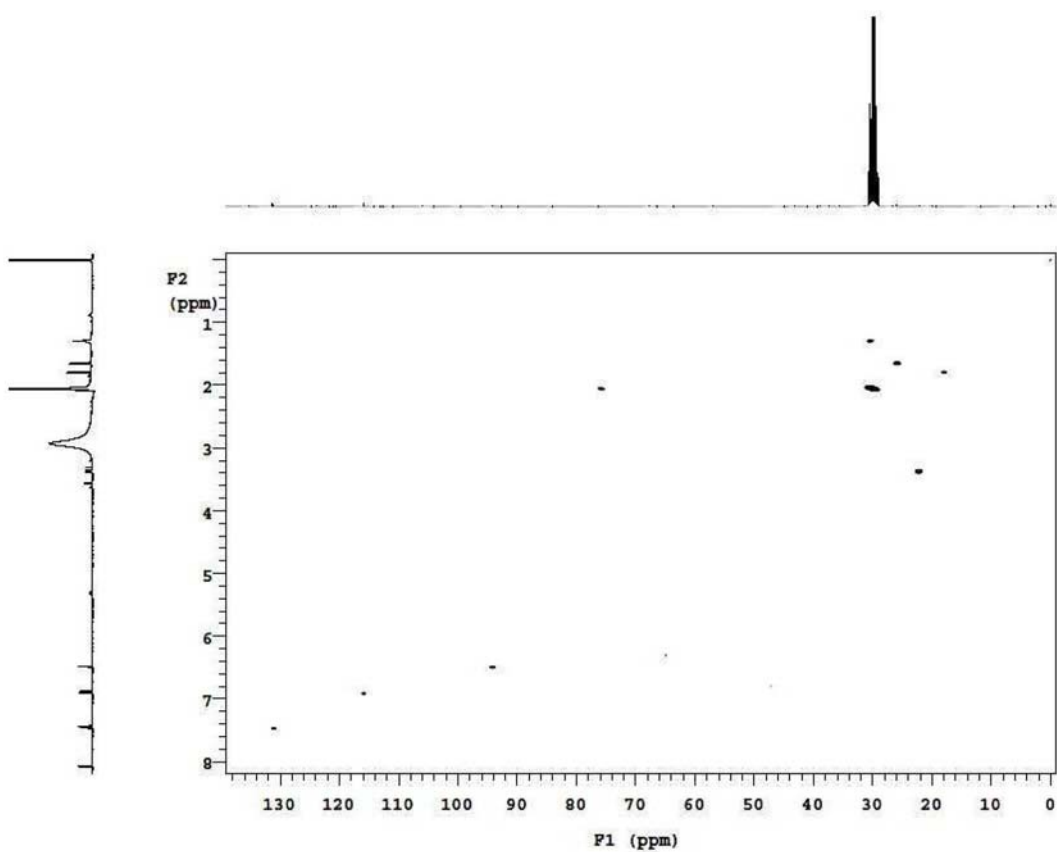


Figure 17S. HSQC spectrum (300/75 MHz, $(\text{CD}_3)_2\text{CO}$) of compound 4

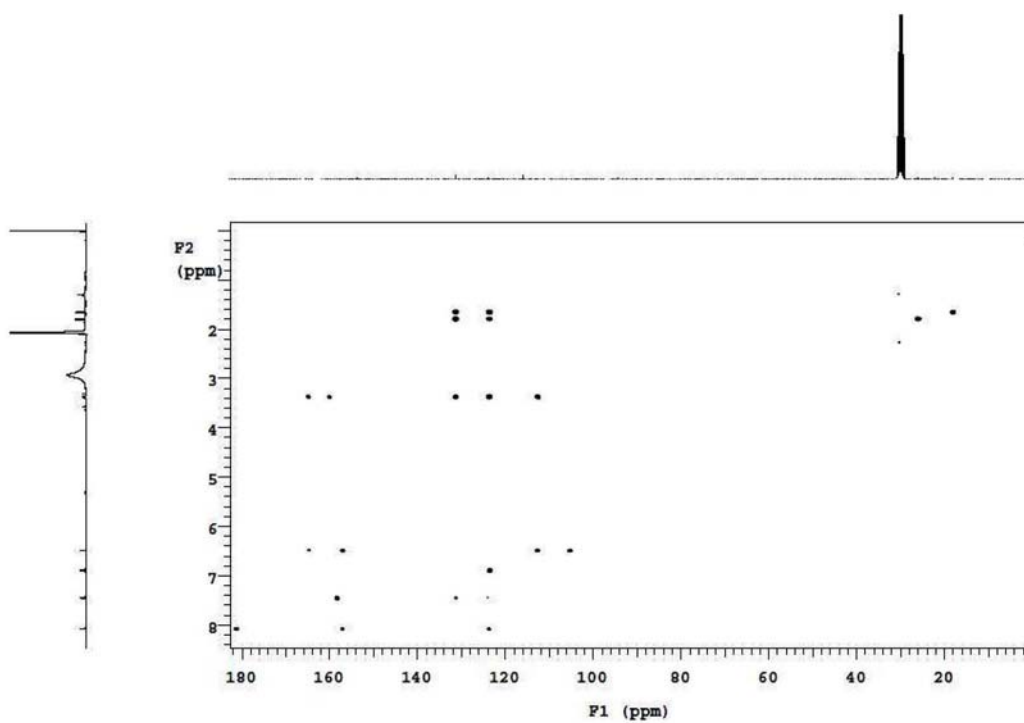


Figure 18S. HMBC spectrum (300/75 MHz, $(CD_3)_2CO$) of compound 4

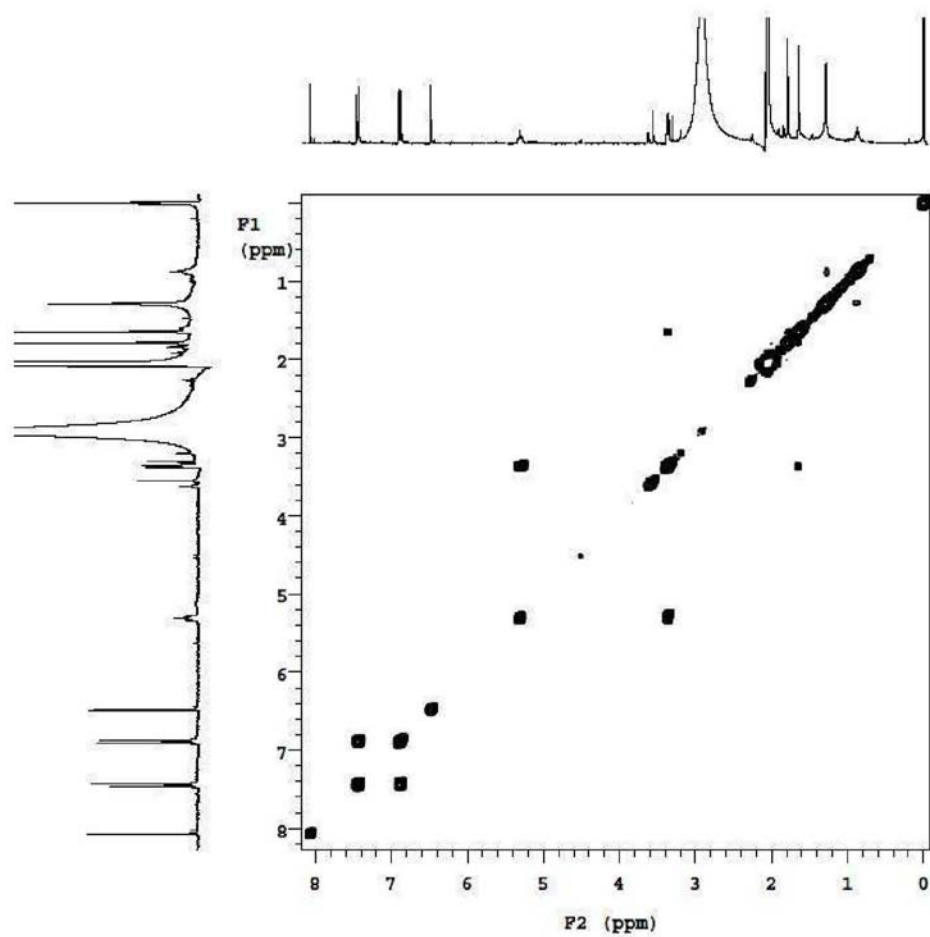


Figure 19S. COSY spectrum (300 MHz $(CD_3)_2CO$) of compound 4

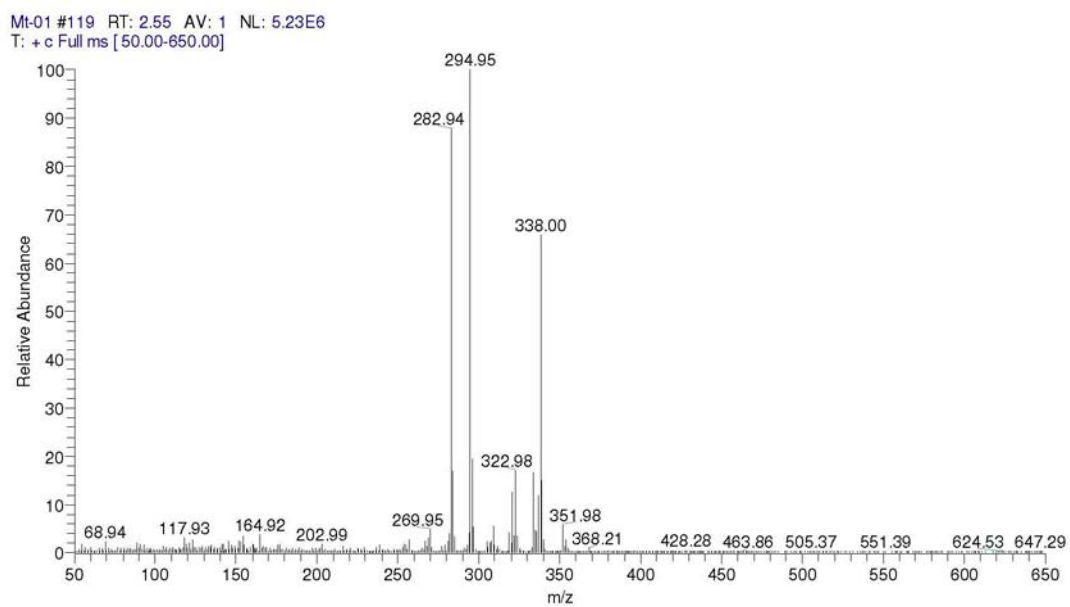


Figure 20S. EIMS (70 eV) of compound 4

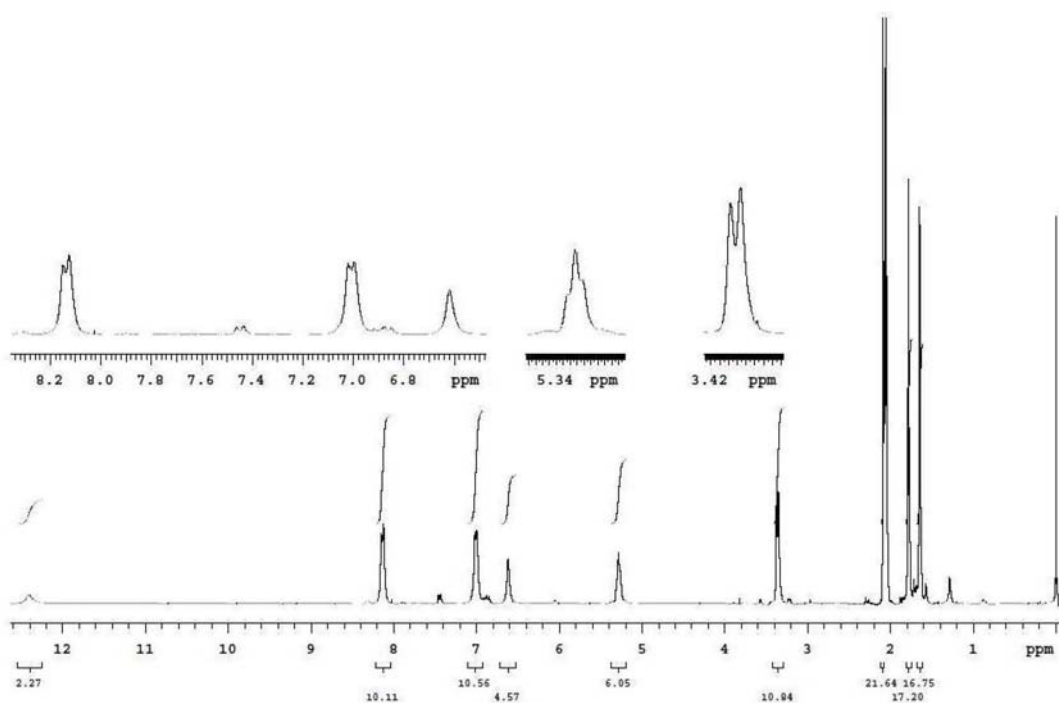


Figure 21S. ^1H NMR spectrum (300 MHz, $(\text{CD}_3)_2\text{CO}$) of compound 5

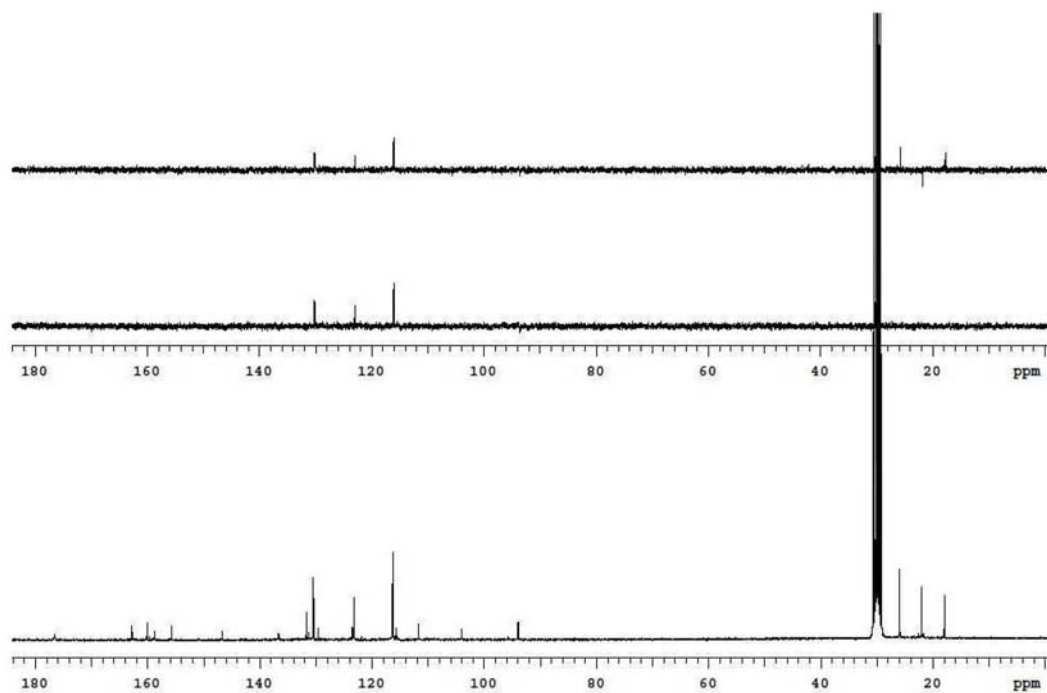


Figure 22S. ^{13}C and DEPT 90 spectrum (75 MHz, $(\text{CD}_3)_2\text{CO}$) of compound 5

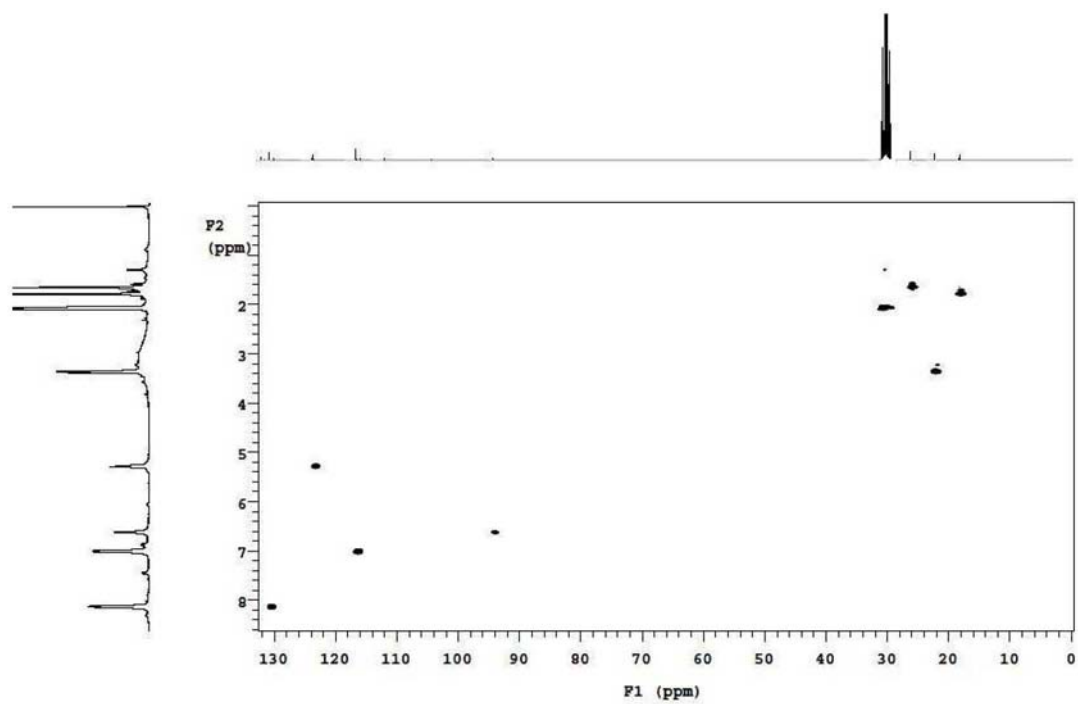


Figure 23S. HSQC spectrum (300/75 MHz, $(\text{CD}_3)_2\text{CO}$) of compound 5

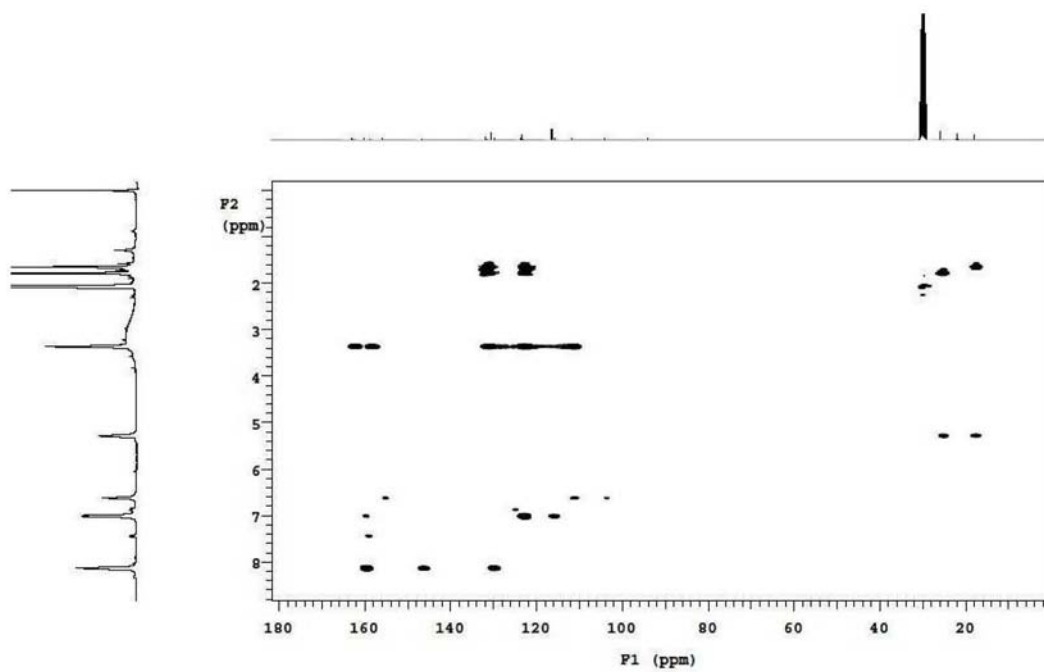


Figure 24S. HMBC spectrum (300/75 MHz, $(CD_3)_2CO$) of compound 5

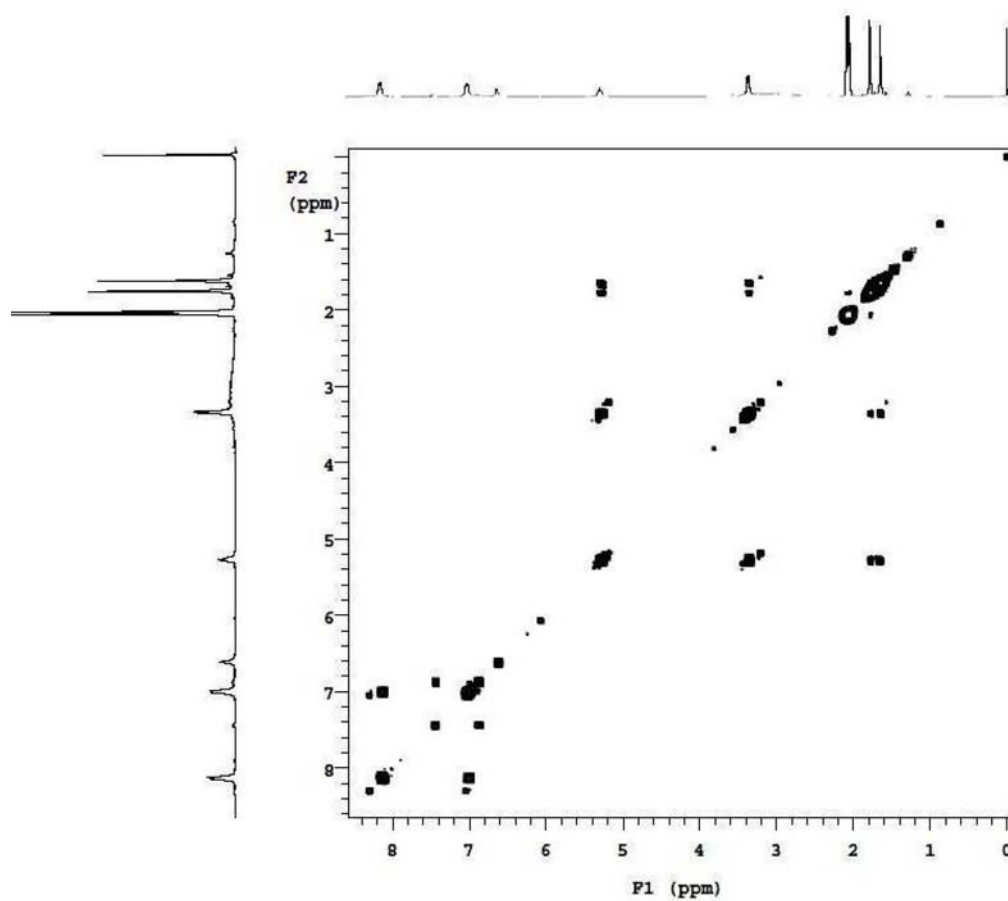


Figure 25S. COSY spectrum (300 MHz $(CD_3)_2CO$) of compound 5

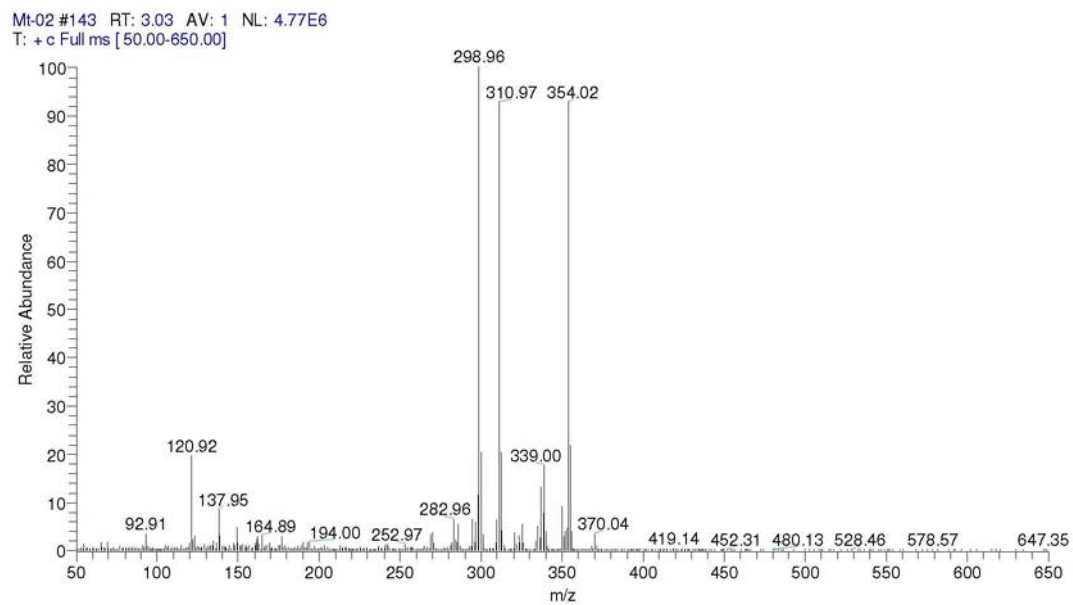


Figure 26S. EIMS (70 eV) of compound 5