

Megastimanes and Ergostane Type Steroid from Leaves *Cratylia mollis* (Leguminosae)

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Do extrato metanólico das folhas de *Cratylia mollis*, foram isolados através de técnicas cromatográficas, (3*S*,5*S*,6*S*,9*R*)-3,6-di-hidróxi-5,6-diidro-β-ionol (**1**) e um novo *nor*-isoprenoide identificado como (4*S**, 6*S**)-4-but-1*E*-enil-4,6-diidroxi-3,5,5-trimetil-ciclo-hex-2-enona (**2**) além do 5α,8α-epidioxyergosta-6,22-dien-3-β-ol. As estruturas foram elucidadas por meio da análise dos dados de EM, IV, RMN ¹H e ¹³C.

From the methanolic extract of the leaves of *Cratylia mollis* were obtained by chromatographic techniques (3*S*,5*S*,6*S*,9*R*)-3,6-dihydroxy-5,6-dihydro-β-ionol (**1**), and a new bis-*nor*isoprenoid named (4*S**, 6*S**)-4-but-1*E*-enyl-4,6-dihydroxy-3,5,5-trimethyl-cyclohex-2-enone (**2**) as well as 5α,8α-epidioxyergosta-6,22-dien-3-β-ol. The structures of the pure compounds were elucidated based on MS, ¹H and ¹³C NMR spectroscopic data analyses.

Keywords: megastimanes, Leguminosae, *Cratylia mollis*

Introduction

Cratylia is one of 670 genera belonging to Leguminosae family,¹ it is included in the Phaseolae tribe (subtribe Diocleinae)² and this position is maintained until the most recent classifications.³ This genus comprises only five species, *C. argentea* (Desvaux) O. Kuntze, *C. bahiensis* L. P. de Queiroz, *C. hypargyrea* Martius ex Benth., *C. intermedia* (Hassler) L. P. de Queiroz and *C. mollis* Martius ex Benth. From chemical point of view the subtribe genera can be characterized by the presence of the non-proteic aminoacids, especially canavanine.⁴ However, there are no data of the occurrence of this aminoacid in *Cratylia* species. On the other hand, other chemical characteristic of *Cratylia* is the presence of lectins in their seeds which shows great similarity with the lectins isolated from seeds of other species of same tribe.⁵

Cratylia mollis is a legume shrub native to the Northeast semi-arid region of Brazil, especially in “caatinga”. This species is popularly known as “camaratuba” or “camaratu”

and is highly resistant to desiccation. The leaves have been an alternative source of nutrition for cattle, being recommended to be employed by locals as forage to improve cattle’s nutrition, especially during the dry seasons, contributing to regional development of the semi-arid.⁶ However, in spite of studies the about of the biological activities of this and related species⁷ to date there are no phytochemical studies regarding *C. mollis*.

In the present work it is described the phytochemical study of leaves of *Cratylia mollis* led to isolation of two bis-*nor*isoprenoids (**1** and **2**) besides the 5α,8α-epidioxyergosta-6,22-dien-3-β-ol (**3**).

Results and Discussion

The C₁₃-*nor*isoprenoid (**1**) is known as 3,6-dihydroxy-5,6-dihydro-β-ionol. It was identified by analysis of ESIMS, IR, optical rotation, ¹H and ¹³C NMR and comparison with data previously published in the literature.⁸ Moreover, HMQC, HMBC and COSY spectra permitted to attribute unequivocally all the NMR signals. The ¹³C NMR data of **1** are compatible with that previously described for

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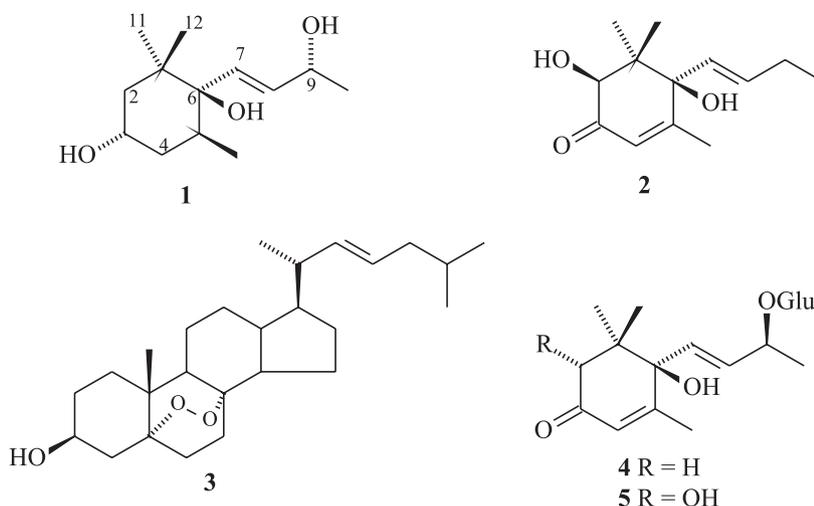


Figure 1. Isolates of leaves of *Cratylia mollis* and other megastimanes.

(3*S*,5*R*,6*S*,9*R*)-3,6-dihydroxy-5,6-dihydro- β -ionol obtained from *Apollonias barbujana* (Lauraceae)⁸ except for C-1. However, the HMQC and HMBC of **1** permitted to attribute the peak at δ 16.4 to C-13 by the observed correlations of one doublet at δ 0.79 and this carbon in the HMQC and C-4 (δ 39.9) and C-6 (δ 78.1) observed in the HMBC experiment. This findings permitted to determinate an axial position of CH₃-13 instead of equatorial as previously proposed and, concluded compound **1** is (3*S*,5*S*,6*S*,9*R*)-3,6-dihydroxy-5,6-dihydro- β -ionol. Thus, it is a must a revision of ¹³C NMR data and structure of the megastimane isolated from *A. barbujana*.

The ¹³C NMR spectra (including DEPT experiments) of **2** showed signals were assigned to four methyls, four methines, one methylene and four non-hydrogenated carbons. These findings besides the protonated molecular ion [M-H]⁺ observed at *m/z* 223.1330 in the negative

HRESIMS permitted to propose molecular formula C₁₃H₂₀O₃ (requires 223.1334) which was indicative of a C₁₃-norisoprenoid derivative. The signals at δ 198.5, 126.7 and 163.4 observed in the ¹³C NMR spectra, and the singlet at δ 5.95 in the ¹H NMR spectra were indicative of presence of a α,β -unsaturated ketone group. These findings together with the additional chemical shifts indicated the structural similarity of **2** and the known megastigmane glycosides **4** and **5**.⁹ The HMQC spectrum was elucidative once it permitted to identify the correlations of closer hydrogen signals with respective carbon resonances. However, the analysis of HMBC spectral data was conclusive to confirm the structure of compound **2**. Briefly, the correlations of H-4 (δ 5.95), H-7 (δ 5.79) and the C-6 (δ 78.9) and, H-10 (δ 1.22) and C-9 (δ 49.6) and C-8 (δ 135.6) were indicative the *E*-butenyl group is attached at C-6 (Table 1).

Table 1. ¹H and ¹³C NMR assignments for the compounds 1 and 2 (500/300 and 125/75 MHz)

Position	1 ^a		2 ^b		HMBC(H → C)
	δ_H	δ_C	δ_H	δ_C	
1	-	40.5	-	41.1	-
2	1.65 (m) 1.40 (m)	45.9	4.38(m)	68.0	-
3	3.78 (m)	67.4	-	198.5	-
4	1.65 (m) 1.40(m)	39.9	5.95 (s)	126.7	C-6
5	1.95(m)	35.4	-	163.4	-
6	-	78.1	-	78.9	-
7	5.52 (d, 15.6)	135.4	5.79 (d,15.6)	129.0	C-6, C-13
8	5.69 (dd, 15.6, 6.3)	133.8	5.81 (ddd, 15.6, indt, indt)	135.6	-
9	4.40 (m)	69.1	2.43 (ddt, 8.1, indt, indt) 2.37 (ddt, 8.1, 6.6)	49.6	-
10	1.23(d, 6.3)	24.1	1.22 (t, 6.6)	23.6	C-8, C-9
11	0.97 (s)	25.3	0.92 (s)	23.9	C-2
12	0.83 (s)	25.2	0.95 (s)	22.8	C-2
13	0.79 (d, 6.9)	16.4	1.90(s)	19.1	C-2, C-3, C-4

^aRecorded in CD₃OD; ^bCDCl₃; multiplicities obtained using DEPT experiments.

The relative configuration of compound **2** was determinate by phase-sensitive NOESY, once the spatial interactions of H-7 and H-2 were indicative the butenyl group and H-2 were in same face (Figure 2). The proposed relative stereochemistry was also confirmed by analysis of coupling constant of derivative **2a**, obtained as main product of reduction of **2** by NaBH₄/MeOH. The ¹H NMR spectrum of **2a** showed the peak of H-3 as a double doublet at δ 3.4 (*J* 12.9 6.5 Hz) revealing *pseudodiaxial* coupling of H-3 and H-2, which allowed to confirm the relative configuration of compound **2**.

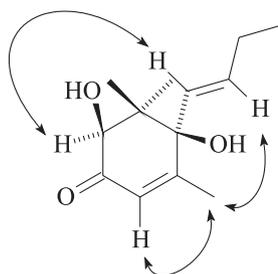


Figure 2. The NOESY correlations of compound **2**.

The APCI-MS, ¹H NMR and ¹³C NMR spectra of compound **3** and comparison with data previously describe in literature¹⁰ permitted to identify this steroid. However correlations observed in COSY, HMQC and HMBC experiments indicated the values of ¹³C NMR data previously established for C-6 and C-7 must be changed.

This is the first occurrence of the megastimane 4-but-1-enyl-4,6-dihydroxy-3,5,5-trimethyl-cyclohex-2-enone (**2**). Compound **1** was previously isolated from *Apollonia barbujana* (Lauraceae)⁸ but this is the first time it is being reported in Leguminosae family. Compound **3** was previously isolated from fungus *Lactarium volemus*, *Schinopsis brasiliensis* and *Typha latifolia*.¹⁰ However the detailed analysis of correlations observed in HMQC and HMBC experiments permitted to attribute unequivocally the C-13 NMR data for this steroid.

Experimental

General procedures

¹H (300 MHz); ¹³C NMR and DEPT (75 MHz) experiments were carried out in a Varian mod. Gemini 2000. HMQC, NOESY and HMBC were run on a Varian INOVA 500: chemical shifts were recorded in δ (ppm) from the solvent peak relative to TMS; APCI and ESIMS were obtained on Shimadzu LCMS-2010; HRESIMS was recorded on Bruker micrOTOF II, IR spectra were taken on a Varian mod. 640-IR spectrophotometer and optical

rotations were measured with a Perkin Elmer polarimeter mod. 341.

Column chromatography was carried out on silica gel 60 (Akros 0.04-0.073 mm) and, silica gel TLC plates were used to monitor the chromatographic fractionment employing iodine fumes, Libermann-Bouchard spray reagent, and UV light (254/366 nm).

Plant material

Botanical material of *C. mollis* was collected at Jacobina, Bahia State, a region where “caatinga” vegetation is prevalent. A voucher is deposited at Herbarium of Universidade Estadual de Feira de Santana under number LP5119.

Extraction and isolation

The powdered leaves (4.1 Kg) were repeatedly extracted with MeOH at room temperature. The leaf crude extract was immediately partitioned with CHCl₃/MeOH:H₂O (6:4), and after the evaporation of CHCl₃ under vacuum, the extract (73.2 g) obtained was partitioned with hexane/MeOH:H₂O (9:1). The hydromethanolic partition phase (28.89 g) was submitted to CC using Silica gel as adsorbent and eluted with mixtures CHCl₃:MeOH with gradient of polarity (95:5 →3:2). The fractions (1.88 g) eluted with CHCl₃:MeOH (9:1) were jointed and submitted to another CC on silica gel which was eluted with mixtures of CHCl₃:MeOH (95:5 and 9:1). The fractions (88.8 mg) eluted with the system CHCl₃:MeOH (95:5) were subjected to PTLC and developed with a mixture of CHCl₃:MeOH:HOAc (90:9:1). This procedure permitted to obtain compound **1** (15.0 mg). The fractions (175 mg) eluted with CHCl₃:MeOH (9:1) were rechromatographed on Sephadex LH-20 column with Hex:CH₂Cl₂ (2:8) as eluent to yield compound **2** (25.0 mg).

The hexane partition phase (42.9 g) was submitted to a CC on Si gel 60 with mixtures of Hex:EtOAc. The fractions (4.5 g) eluted with Hex:EtOAc (95:5) were further submitted to a flash CC on silica gel eluted with CHCl₃:MeOH (98:2) affording compound **3** (7.2 mg).

(1*S*,4*S*,6*S*)-1-(3-Hydroxy-but-1*E*-enyl)-2,2,6-trimethyl-cyclohexane-1,4-diol or (3*S*,5*S*,6*S*,9*R*)-3,6-Dihydroxy-5,6-dihydro-β-ionol (**1**)

Colorless syrup. [α]_D²⁰ -7.0° (*c* 0.48, MeOH), ¹H and ¹³C NMR: Table 1.

(4*S**, 6*S**)-4-But-1*E*-enyl-4,6-dihydroxy-3,5,5-trimethyl-cyclohex-2-enone (**2**)

Oil. [α]_D²⁰ + 56.0° (*c* 1.47, MeOH) ESIMS (*m/z*) 223

[M-H]⁻, and 255 [M-H+MeOH], IR ν_{\max} /cm⁻¹: 3200-3600 (OH), 1713 (C=O), 1656 (C=C), ¹H and ¹³C NMR: Table 1.

Reaction of reduction of compound 2

Compound **2** (5 mg) was dissolved in MeOH (2.0 mL) and added a suspension containing NaBH₄ in MeOH. The mixture was stirred at room temperature during 30 min. After the solvent was evaporated the residue was dissolved in CHCl₃ and **2a** (3.5 mg) was obtained.

Acknowledgments

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil), IMSEAR (CNPq/MCT), FAPESB and Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for fellowship support and grants.

Supplementary Information

Supplementary information for compounds **1-3** is available free of charge at <http://jbcs.sbq.org.br>, as a PDF file.

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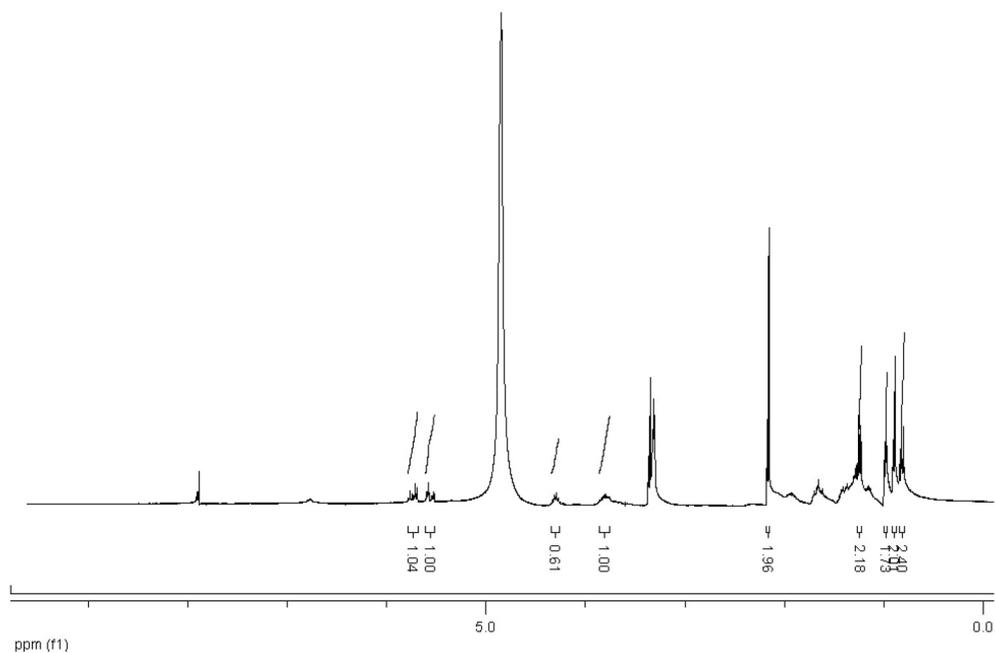


Figure S1. ¹H NMR spectrum of compound 1 (CD₃OD, 300 MHz).

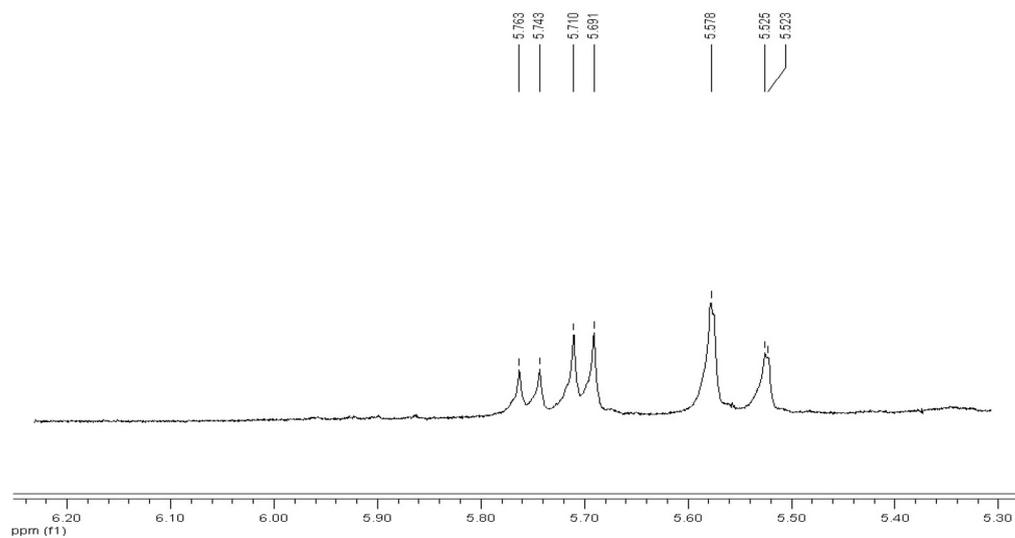


Figure S2. Expansion of ^1H NMR spectrum of compound **1** (CD_3OD , 300 MHz).

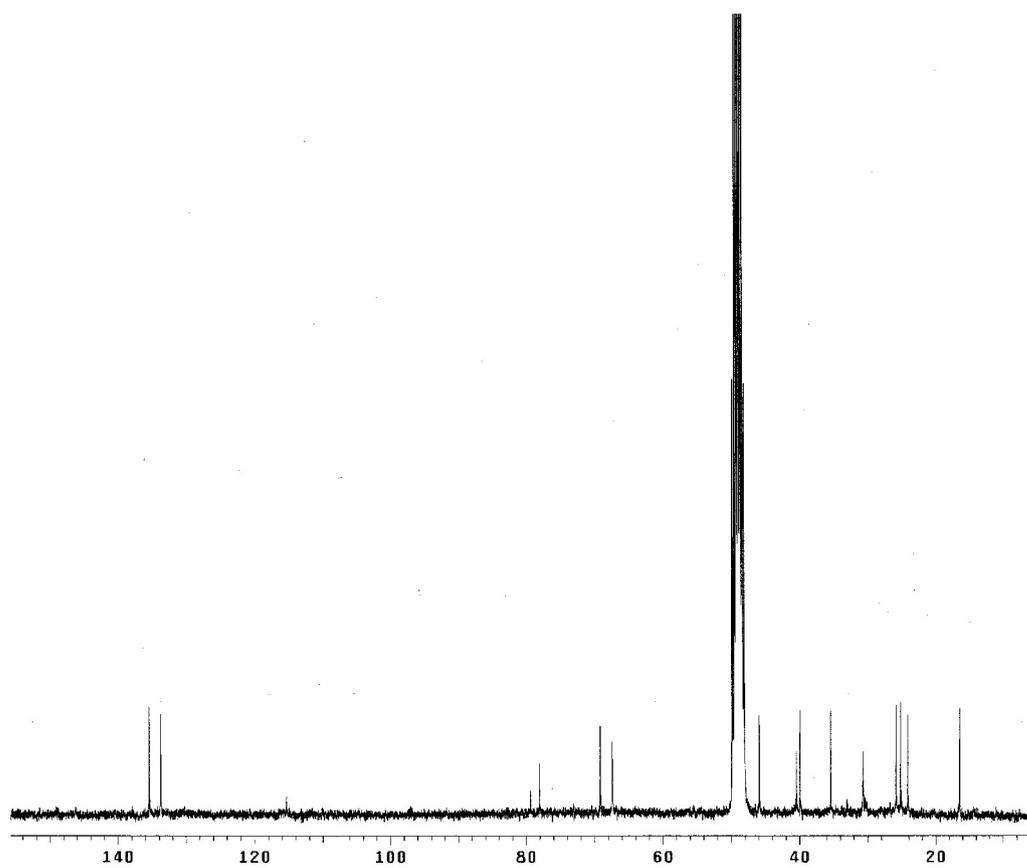


Figure S3. ^{13}C spectrum of compound **1** (CD_3OD , 75 MHz).

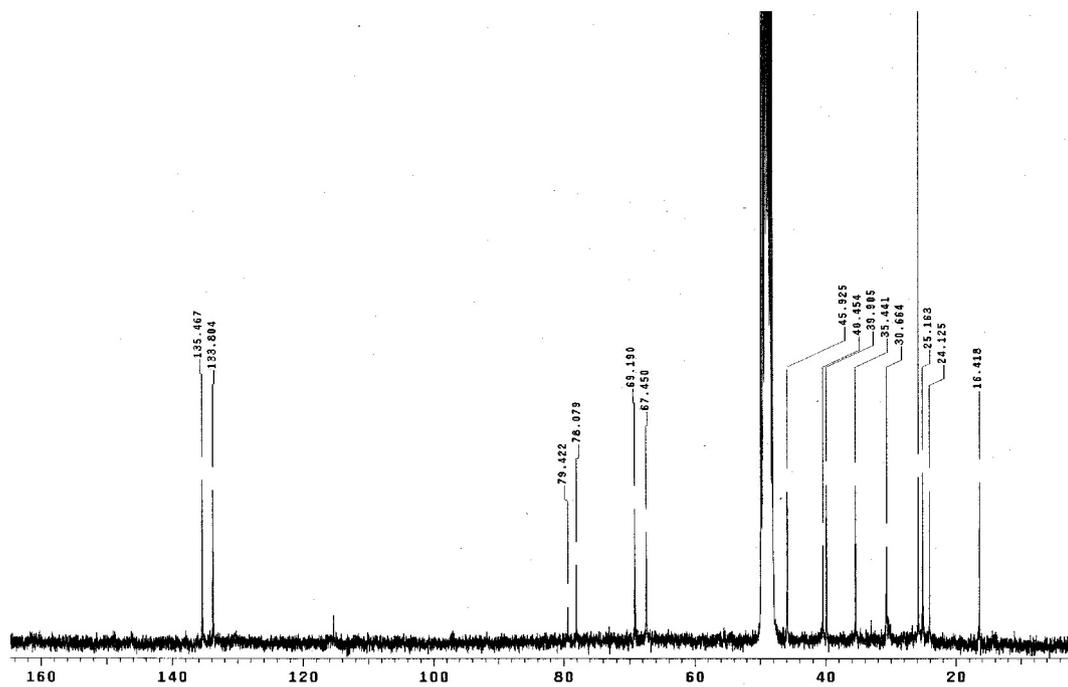


Figure S4. Expansion of ^{13}C NMR spectrum of compound 1 (CD_3OD , 75 MHz).

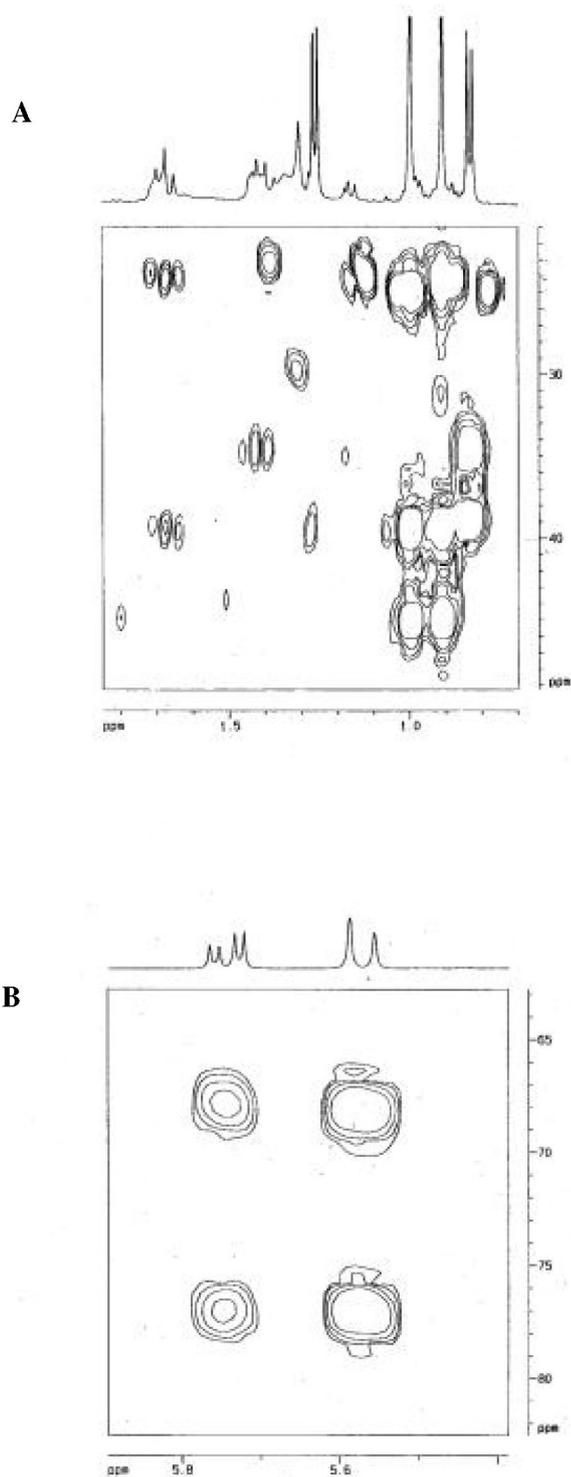


Figure S5. Expansions of the gHMBC spectrum of compound **1** (CD_3OD , 500 MHz).

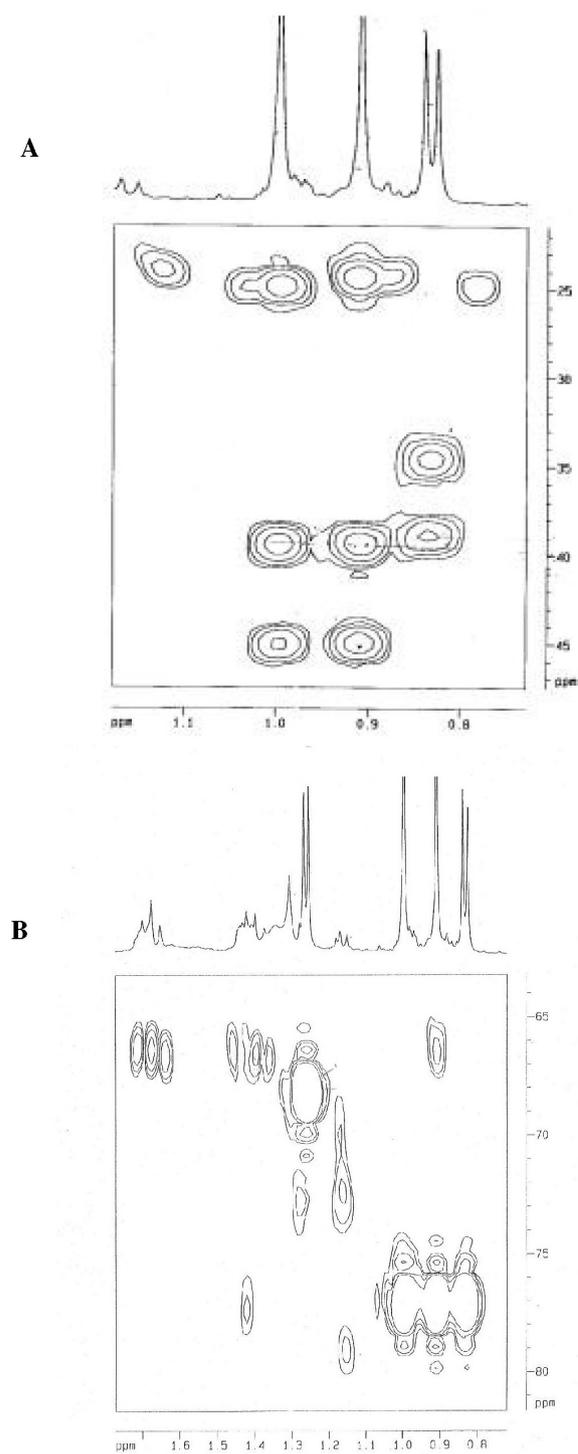


Figure S6. Expansion of the HMBGCS spectrum of compound **1** (CD_3OD , 500 MHz).

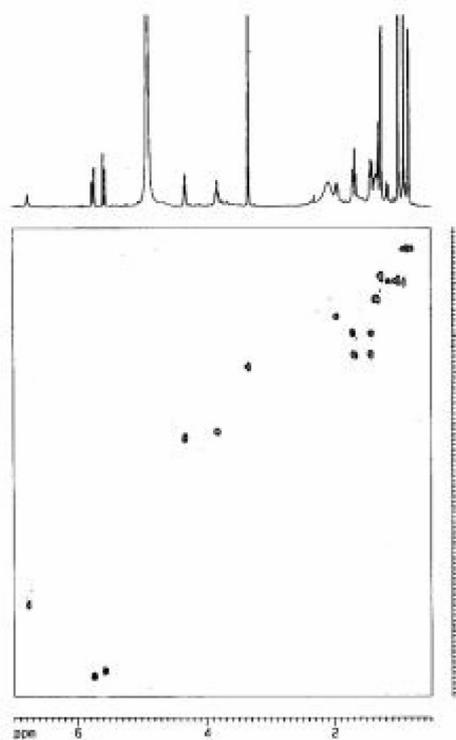


Figure S7. gHMBC spectrum of compound **1** (CD₃OD, 500 MHz).

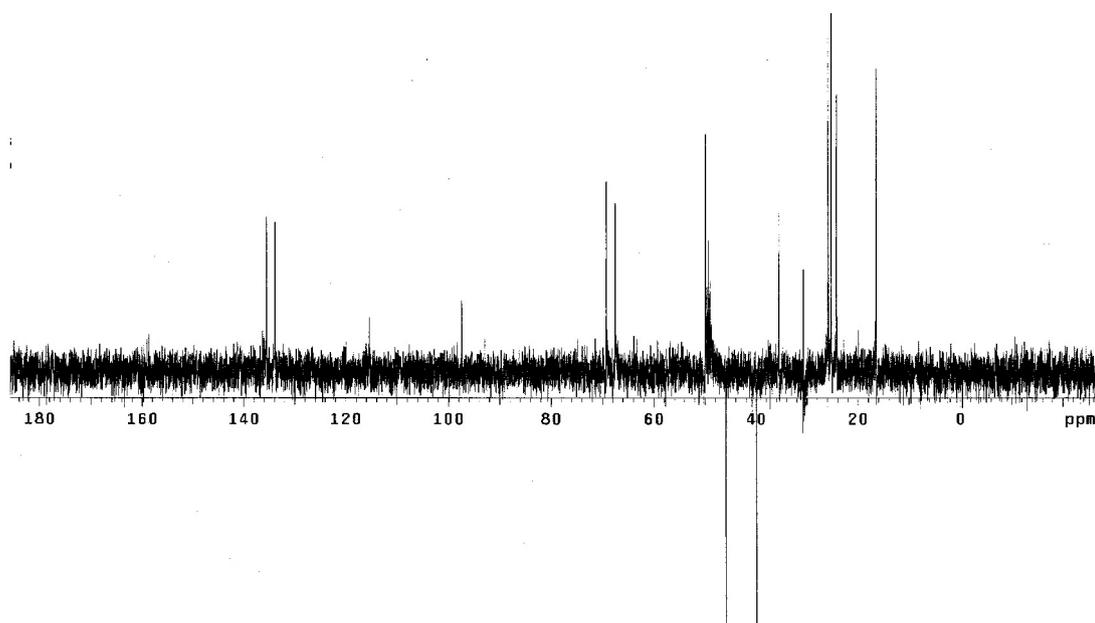


Figure S8. DEPT 135° spectrum of compound **1** (CD₃OD, 75 MHz).

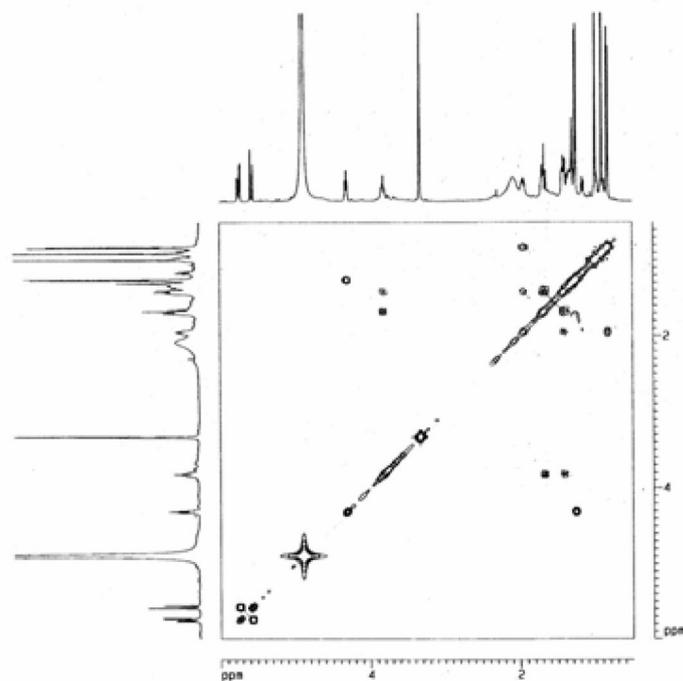


Figure S9. COSYGS spectrum of compound 1 (CD_3OD , 500 MHz).

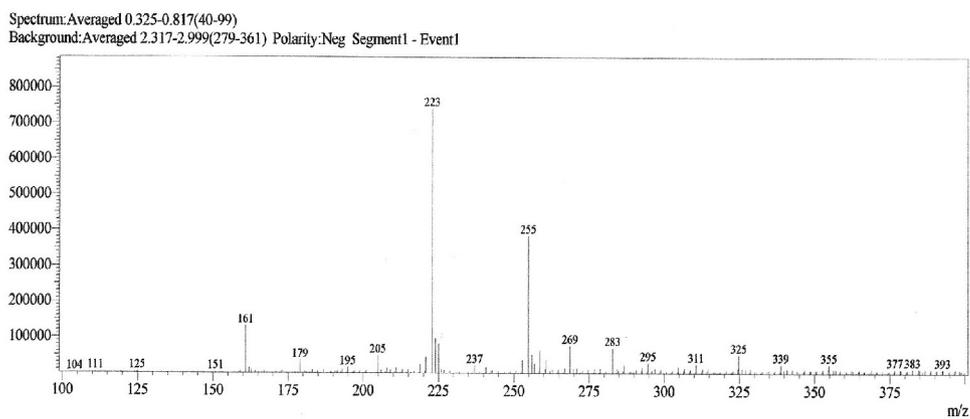


Figure S10. Low resolution negative APCIMS of compound 2.

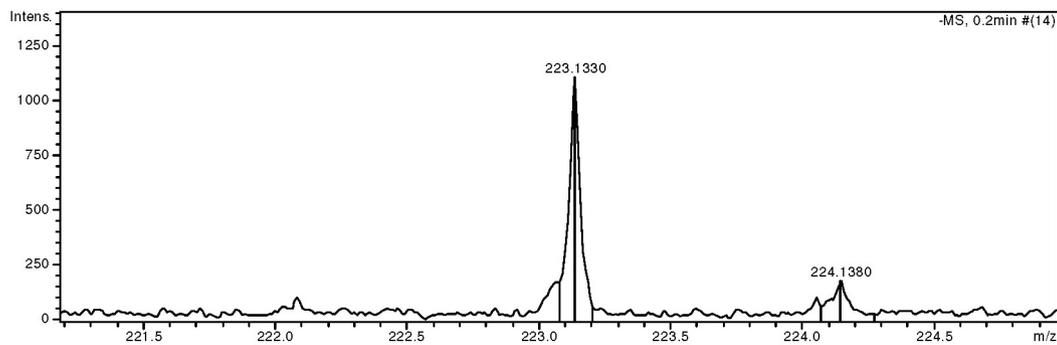


Figure S11. Negative HRESIMS of compound 2.

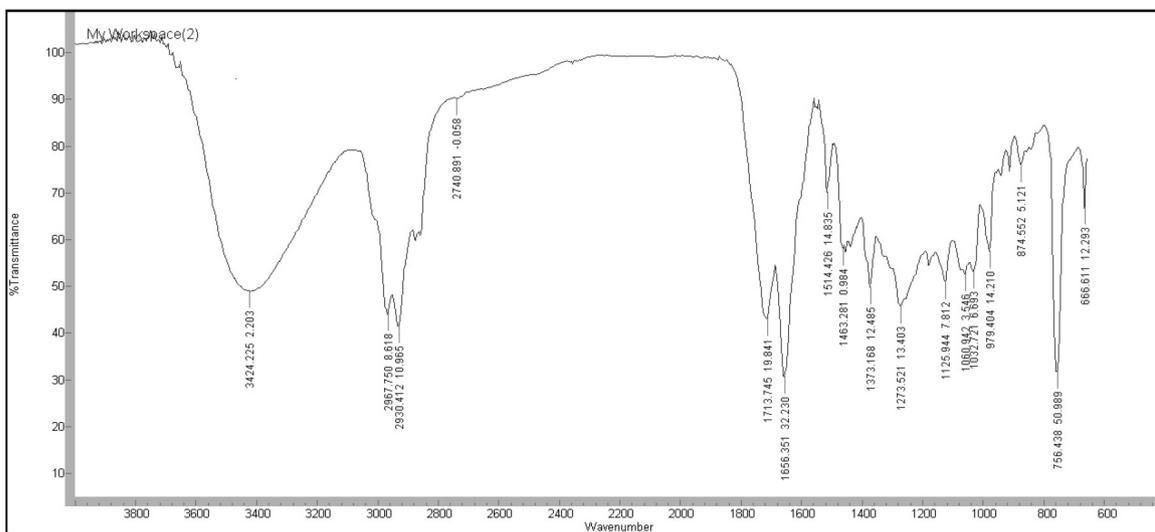


Figure S12. IR spectrum of compound 2 (film).

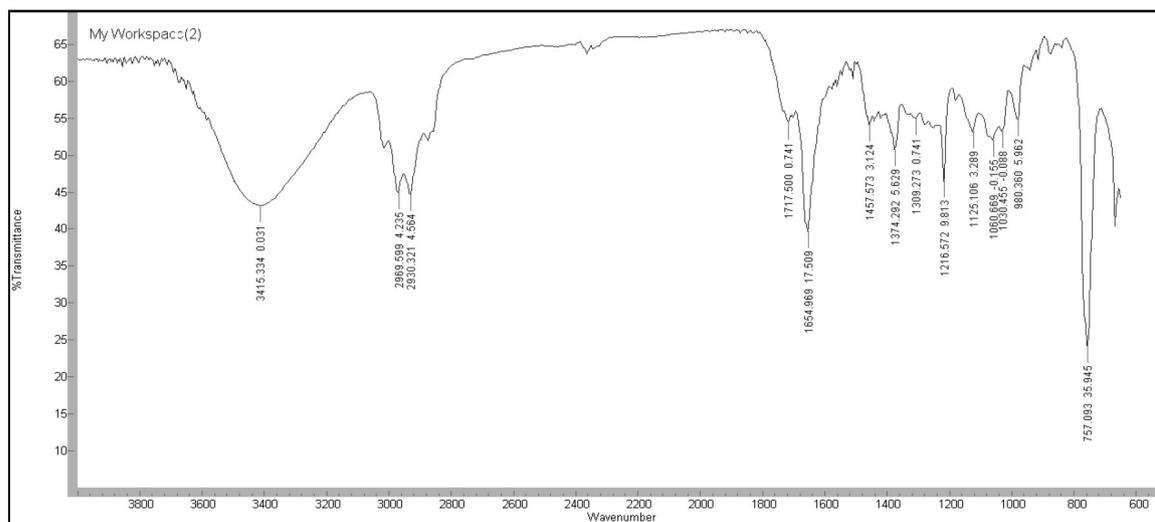


Figure S13. IR spectrum of product reduction of compound 2 (film).

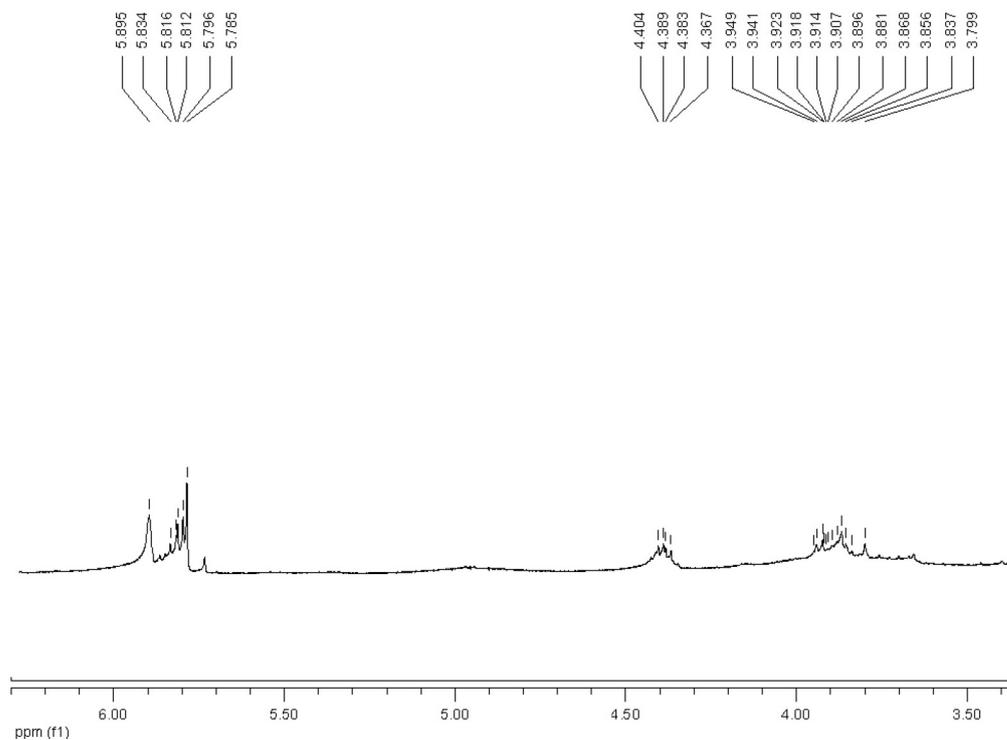


Figure S14. Expansion of ¹H NMR spectrum of compound 2 (CDCl₃, 300 MHz).

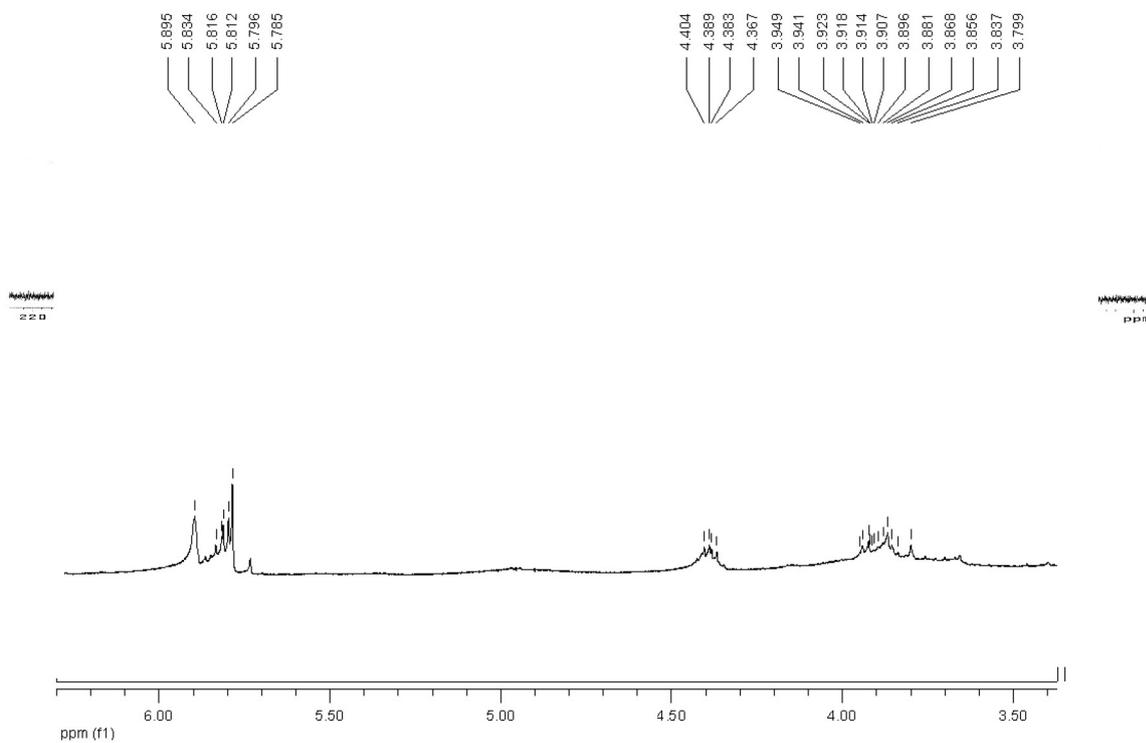


Figure S15. ¹³C NMR spectrum of compound 2 (CDCl₃, 75 MHz).

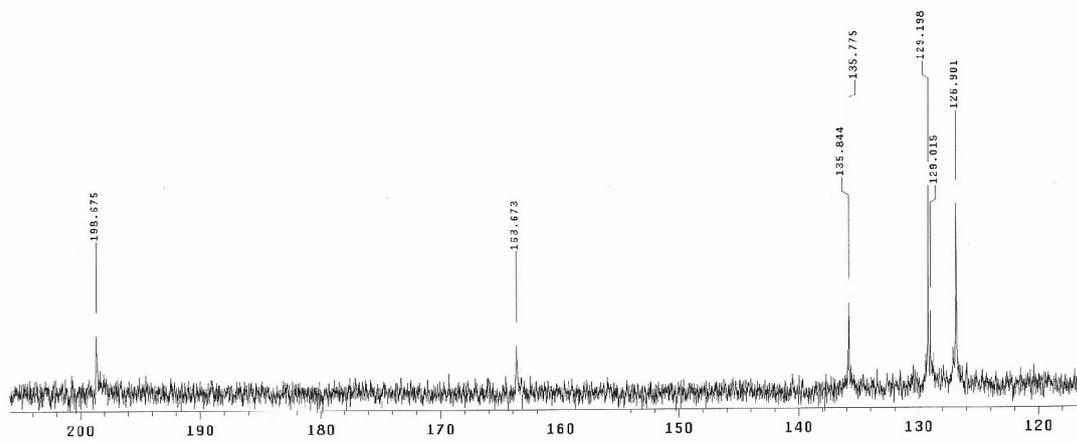
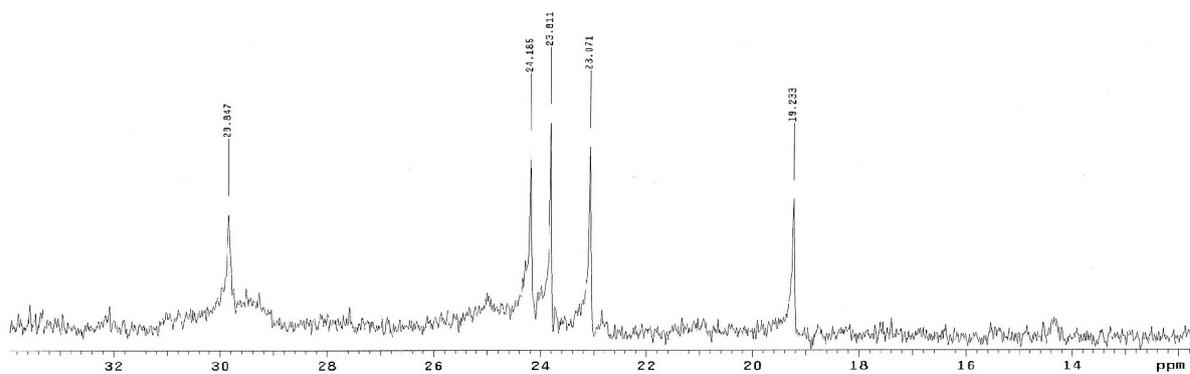


Figure S16. Expansion of ^{13}C NMR spectrum of compound 2 (CDCl_3 , 75 MHz).

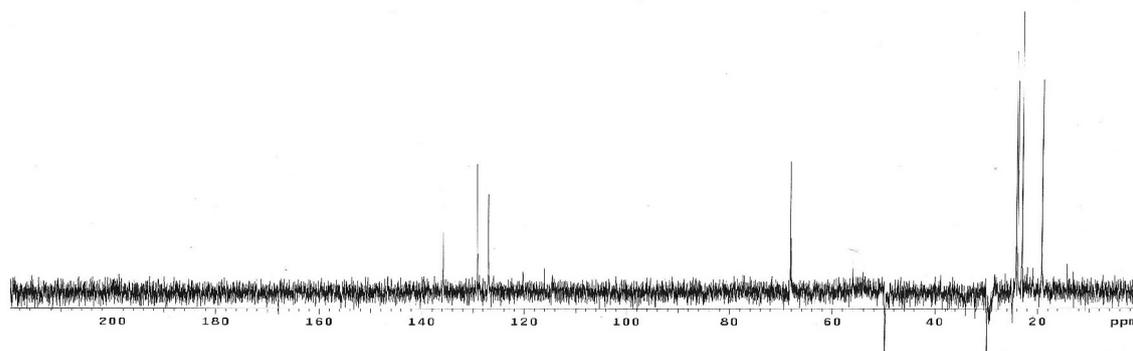


Figure S17. DEPT 135° spectrum of compound 2 (CDCl_3 , 75 MHz).

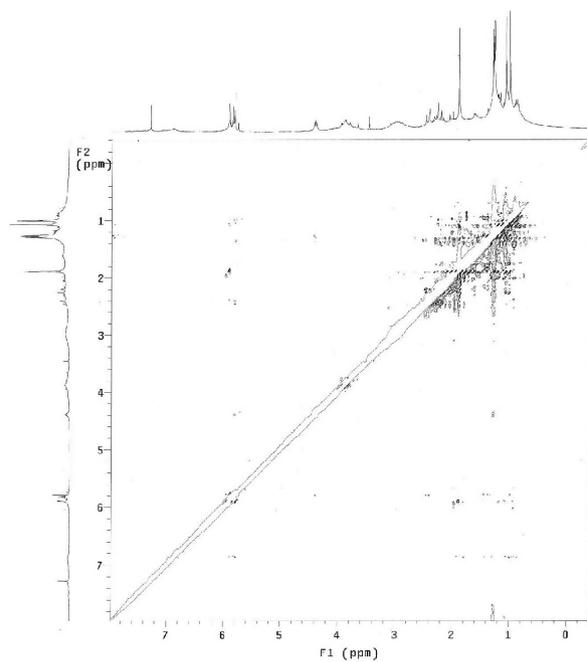
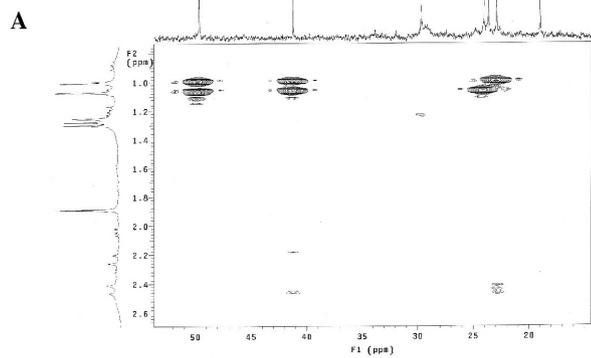


Figure S19. NOESY spectrum compound 2.

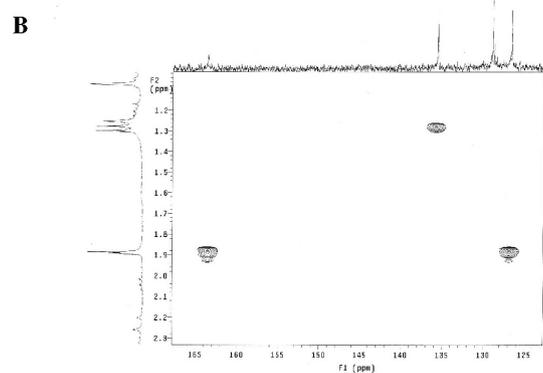


Figure S18. Expansion of the gHMBC spectrum compound 2.

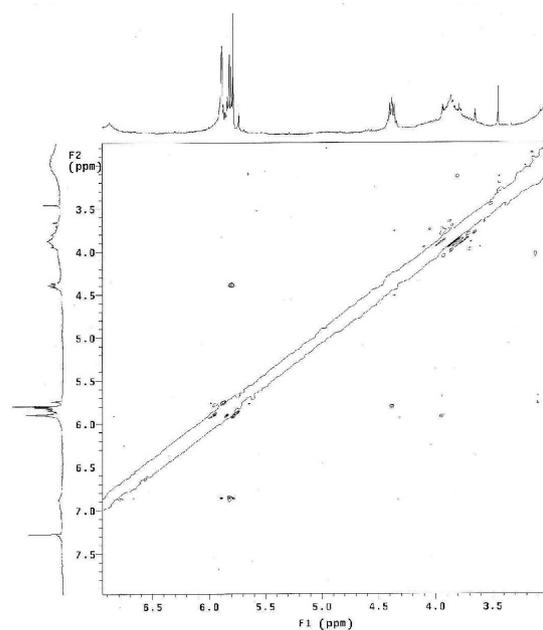


Figure S20. Expansion of the NOESY spectrum compound 2.

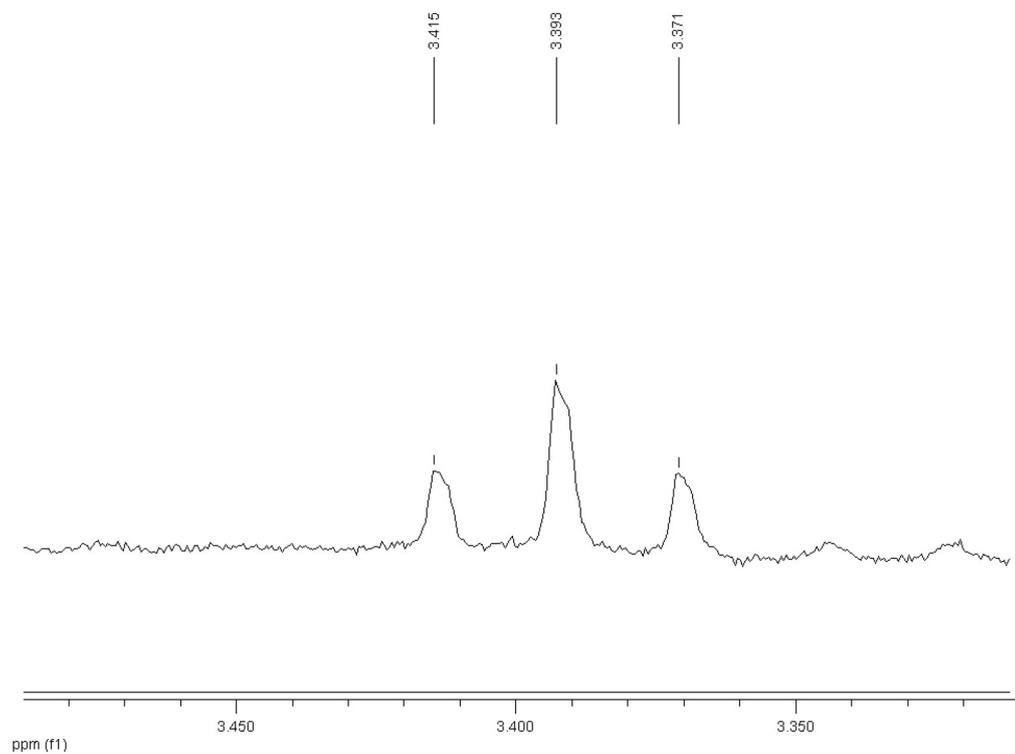


Figure S21. Expansion of ¹H NMR spectrum of product reduction of compound 2 (C₅D₄N, 300 MHz).

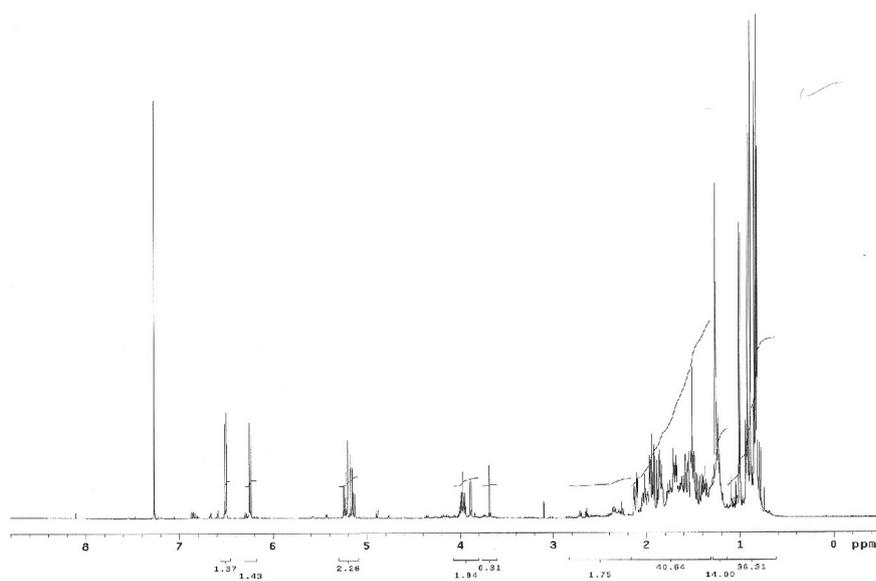


Figure S22. ¹H NMR spectrum of compound 3 (CDCl₃, 500 MHz).

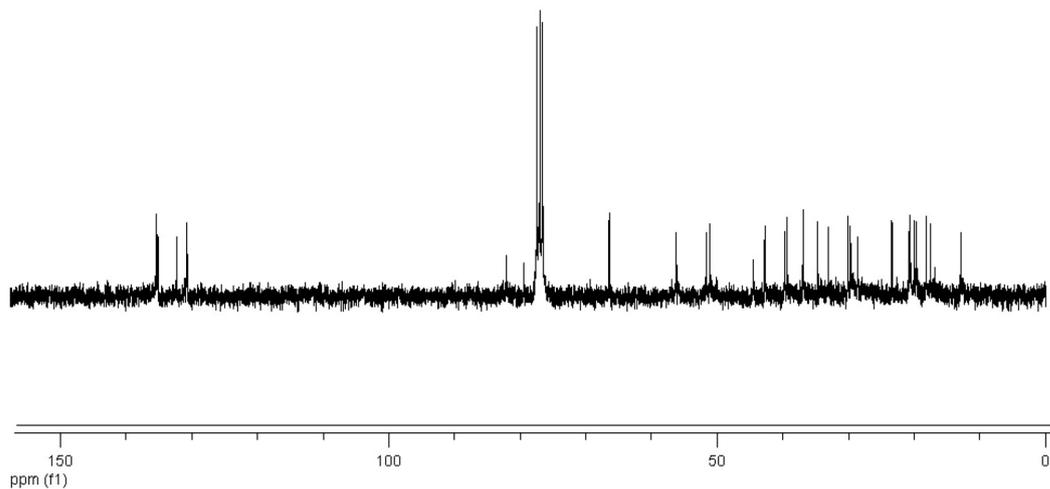


Figure S23. ^{13}C NMR spectrum of compound 3 (CDCl_3 , 75 MHz).

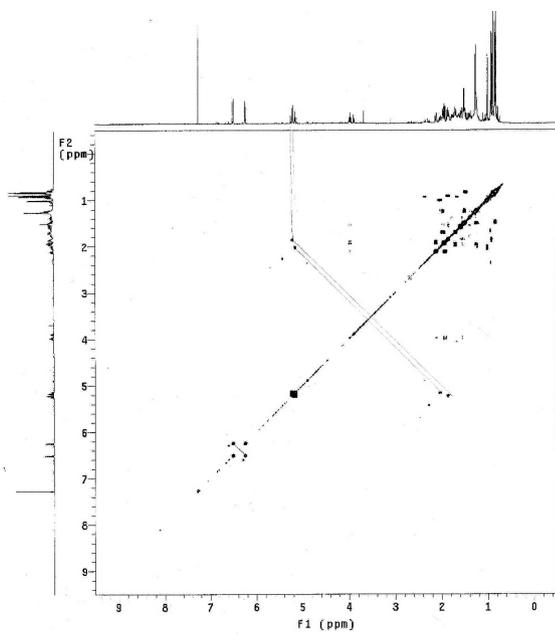


Figure S24. COSY of compound 3 (CDCl_3 , 500 MHz).

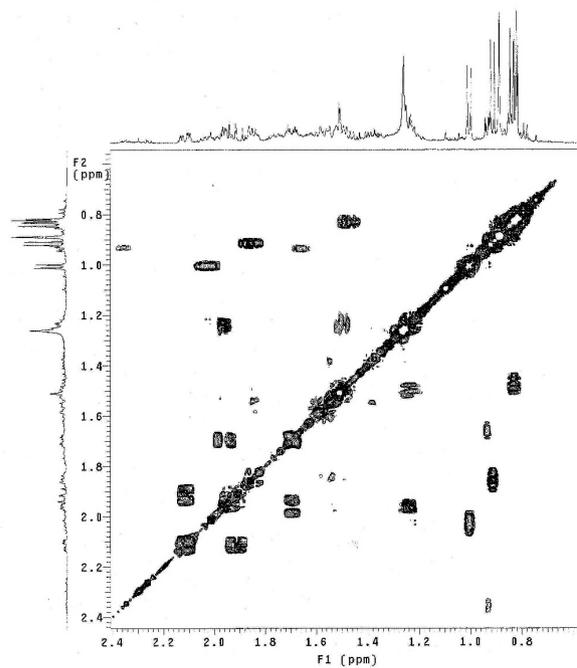


Figure S25. gHSQC of compound 3 (CDCl_3).

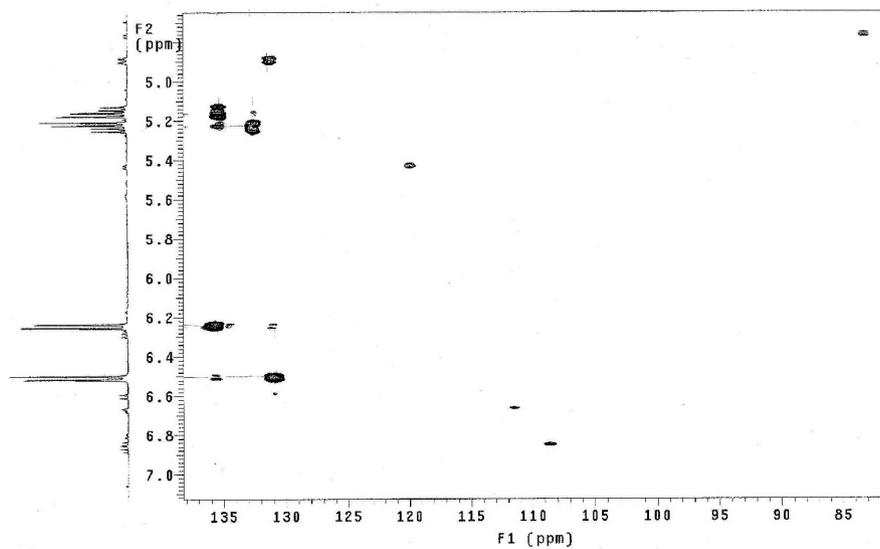


Figure S26. Expansion of gHSQC of compound 3 (CDCl₃).

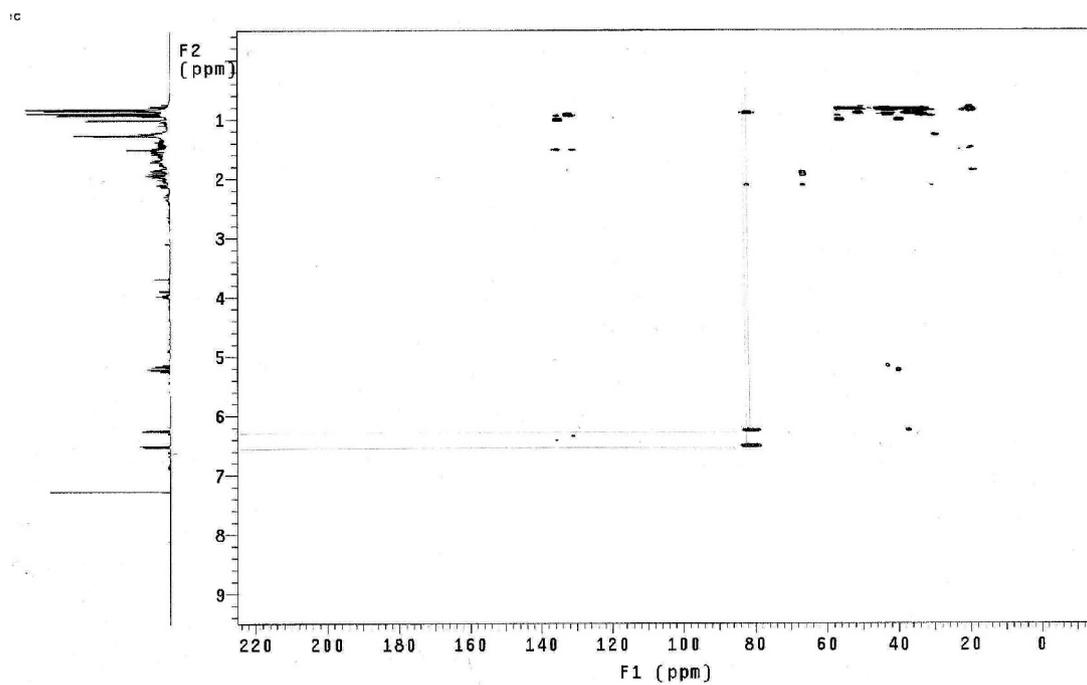


Figure S27. gHMBC of compound 3 (CDCl₃).

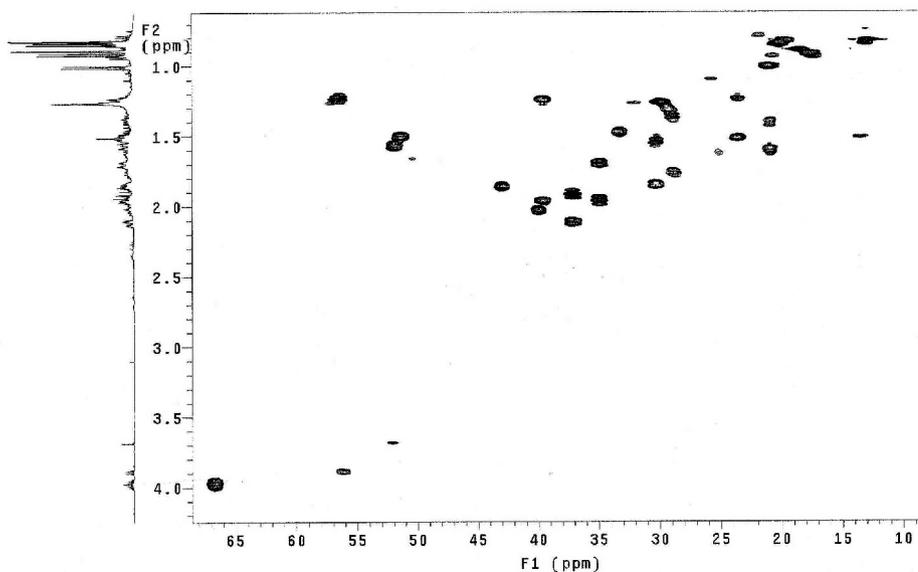
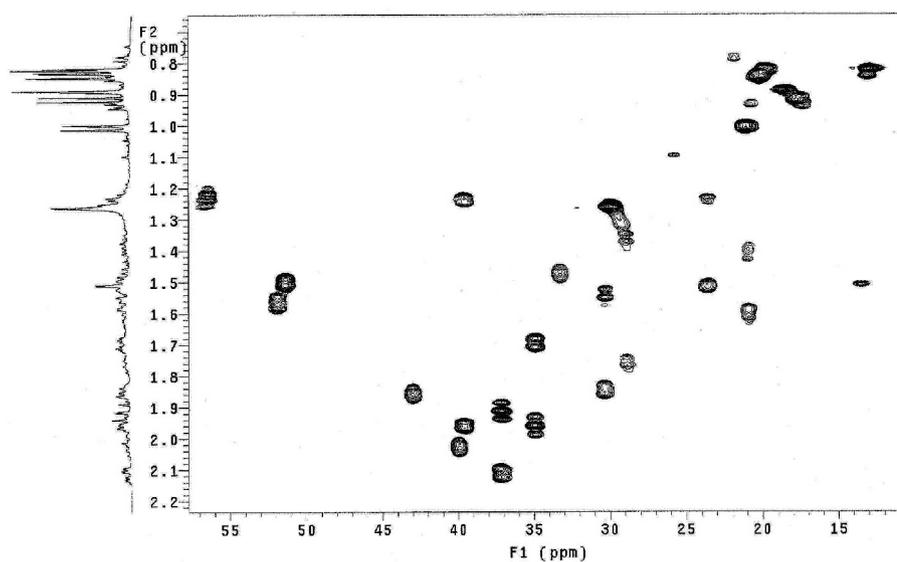
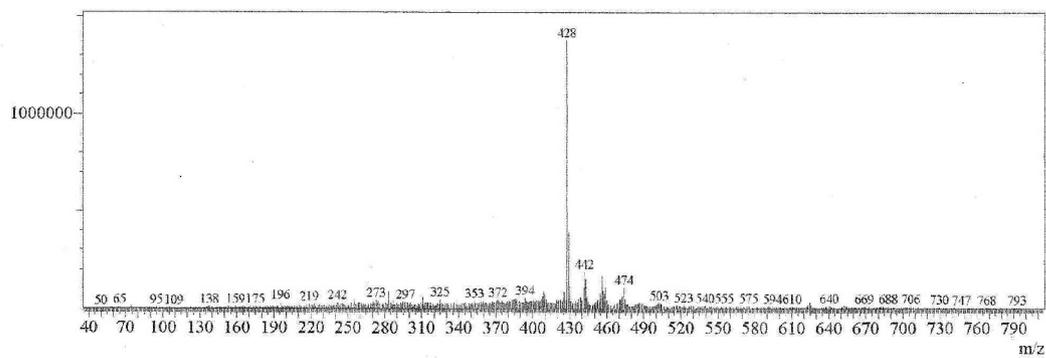
Figure S28. gHSQC of compound 3 (CDCl₃).Figure S29. Expansion of gHSQC of compound 3 (CDCl₃).

Figure S30. Negative ESIMS of compound 3.