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Short Report

Maytensifolone, a New Triterpene from *Maytenus distichophylla* Mart. ex Reissek

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O estudo fitoquímico das folhas de *Maytenus distichophylla* Mart. ex Reissek levou ao isolamento de um novo triterpeno 3,16,21-trioxo-6β,12α-dihidroxi-1-en-friedelano, nomeado maytensifolona, juntamente com os triterpenos conhecidos, 3-oxofriedelano, 3,12-dioxofriedelano, 3β-hidroxifriedelano, 3-oxo-29-hidroxifriedelano, 3-oxo-12α-hidroxifriedelano e 3-oxo-30-hidroxifriedelano. A identificação estrutural foi baseada em métodos espectroscópicos e comparação com dados da literatura

Phytochemical study of the leaves of *Maytenus distichophylla* Mart. ex Reissek led to the isolation of the new triterpene 3,16,21-trioxo-6β,12α-dihydroxy-1-en-friedelane, named maytensifolone, along with the known triterpenes 3-oxofriedelane, 3,12-dioxofriedelane, 3β-hydroxyfriedelane, 3-oxo-29-hydroxyfriedelane, 3-oxo-12α-hydroxyfriedelane and 3-oxo-30-hydroxyfriedelane. Their structural identification was based on spectroscopic methods and comparison with literature data.

Keywords: Celastraceae, *Maytenus distichophylla*, maytensifolone, friedelane triterpene

Introduction

The family Celastraceae is composed of 90 genera and approximately 1300 species.[1] The genus *Maytenus* comprises about 80 species distributed throughout Brazil.[2] They are chemically characterized mainly by the presence of flavonoids and pentacyclic triterpenes, which are considered taxonomic markers for this genus.[3-5] Various pharmacological activities have been reported for triterpenes isolated from *Maytenus*, such as antiulcerogenic and antifungal.[6,7]

In previous works, we reported the isolation and structural characterization of triterpenes and flavonoids from *M. obtusifolia*,[3] as well as their toxicity and antiulcerogenic activity.[8] In continuing our work on *Maytenus sp.*, we conducted a phytochemical study of *M. distichophylla* Mart. ex Reissek, a species that was not previously subjected to chemical or pharmacological studies. Accordingly, seven triterpenes of the friedelane group were isolated and characterized (Figure 1), including the new 3,16,21-trioxo-6β,12α-dihydroxy-1-en-friedelane, here named as maytensifolone (**1**), and known triterpenes 3-oxofriedelane (**2**),[9] 3,12-dioxo-friedelane (**3**),[10] 3β-hydroxyfriedelane (**4**),[11] 3-oxo-29-hydroxyfriedelane (**5**),[12] 3-oxo-12α-hydroxyfriedelane (**6**)[13] and 3-oxo-30-hydroxyfriedelane (**7**)[14] which are being reported for the first time in this species.

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Results and Discussion

Compound **1** was isolated in the form of a white amorphous solid, mp 310-312 ºC, $[\alpha]_D^{25}$ +1.4, (*c.* 0.001 in $CHCl_3$). The high resolution mass spectrum utilizing the ESI^+ ionization mode showed a quasi-molecular peak at *m/z* 485.3306 $[M + H]^+$, compatible with molecular formula $C_{30}H_{44}O_5$ (calc. 485.3261). The infrared (IR) spectrum showed absorptions in the region of 3504 cm^{-1} (hydroxyl group), 1681 cm^{-1} (ketone carbonyl) and 1662 cm^{-1}

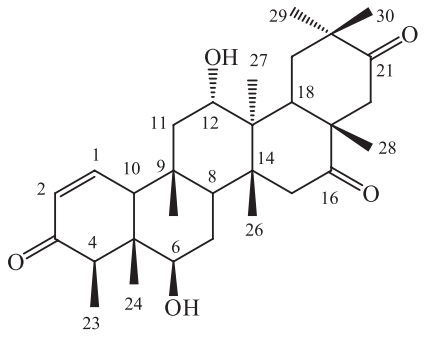

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Figure 1. Triterpene isolated from *M. distichophylla*.

(α,β unsaturated carbonyl). The ^{13}C APT (attached proton test) NMR spectrum (125 MHz, $CDCl_3 + C_5D_5N$) showed 29 signals corresponding to 30 carbon atoms: eight methyl, five methylene, eight methine and nine non-hydrogenated, in line with a triterpene skeleton of the friedelane type.[4] The signals at δ_C 200.87, 146.28 and 130.14 were attributed to C-3, C-1 and C-2, respectively, consistent with an α,β unsaturated carbonyl system of friedelane triterpenes.[9] The signals at δ_C 77.25 and 69.32 were attributed to C-6 and C-12, respectively.[4,15] The location of the hydroxyl groups at C-6 and C-12 was corroborated by the chemical shift at 8.69, corresponding to the methyl groups CH_3-24 and CH_3-27, both subject to a γ effect. Also, this spectrum displayed signals at δ_C 214.24 and 218.13 attributed to carbons C-16 and C-21.[15] The ^1H NMR spectrum showed signals at δ_H 0.74 (s), 0.84 (s), 0.89 (s), 0.91 (s), 1.04 (s), 1.09 (s), 1.11 (s), and 1.17 (d, 1H, *J* 6.5 Hz), attributed to eight methyl groups. In agreement with literature, the doublet at δ_H 1.17 is consistent with CH_3-23 of Δ^1 friedelanes.[16] In addition, signals were observed at δ_H 6.65 (d, 1H, *J* 10.5 Hz, H-1), 5.92 (dd, 1H, *J* 10.5, 3.0 Hz, H-2), 3.63 (dd, 1H, *J* 9.5, 5.0 Hz, H-6ax) and 3.94 (dd, 1H, *J* 11.0, 4.0 Hz, H-12ax), thereby inferring the equatorial orientation of the hydroxyl groups bound to C-6 and C-12. Observed HMQC (heteronuclear multiple quantum coherence) correlations are shown in Table 1. In the long-range (HMBC) ^1H-^{13}C NMR correlation spectrum, the following correlations were observed: δ_H 2.23 (H-4) with carbons at δ_C 200.87, 49.10, 60.35, 8.69 and 9.80 confirming the attributions of C-3, C-5, C-10, CH_3-24 and CH_3-23, respectively; δ_H 0.74 (CH_3-24) with carbons at δ_C 77.25 and 57.72, corresponding to C-6 and C-4, respectively; δ_H 2.08 (H-10) with the carbon at δ_C 19.57 (CH_3-25) and δ_H 0.89 (CH_3-25) with carbons at δ_C 48.19, 36.66 and 45.37, which were attributed to C-8, C-9 and C-11, respectively; δ_H 1.40 (H-8) with carbons at δ_C 19.93 and 49.56 (CH_3-26 and C-15), respectively; δ_H 1.82/1.17 (2H-11) with the carbon at δ_C 69.32 (C-12); and δ_H 0.84 (CH_3-27) with carbons at δ_C 44.66 (C-18) and 69.32 (C-12). The other correlations are given in Table 1. The relative stereochemistry was determined by NOESY (nuclear Overhauser effect spectroscopy) and is shown in Figure 2, confirming the equatorial orientation of the hydroxyls at C-6 and C-12. The above findings support the structure of **1** as 3,16,21-trioxo-6β,12α-dihydroxy-1-en-friedelane, a new natural product named as maytensifolone.

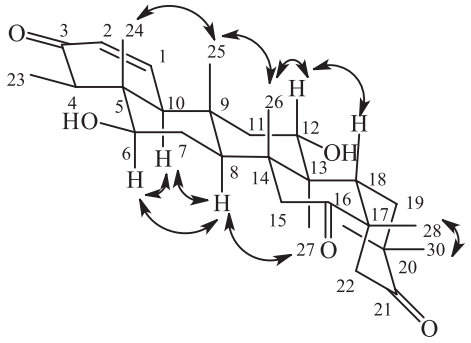

Figure 2. NOESY correlations of **1**.

The other compounds were identified by comparison of their spectroscopic data with those described in literature, as 3-oxofriedelane (**2**),[9] 3,12-dioxofriedelane (**3**),[10] 3β-hydroxyfriedelane (**4**),[11] 3-oxo-29-hydroxyfriedelane (**5**),[12] 3-oxo-12α-hydroxyfriedelane (**6**)[13] and 3-oxo-30-hydroxyfriedelane (**7**),[14] and are reported here for the first time in *M. distichophylla.*

Experimental

General experimental procedures

Melting points were determined with a digital apparatus model MQAPF-302 from Microchemical and were not corrected. IR spectra were recorded on a BOMEM-MB 100 spectrophotometer. One-dimensional (^1H and ^{13}C) and two-dimensional (gHMQC, gHMBC, gCOSY and gNOESY) NMR analyses were performed on a Varian-System spectrometer operating at 500 MHz (^1H) and 125 MHz (^{13}C). $CDCl_3$ and C_5D_5N were used as solvents with TMS (tetramethylsilane) as an internal standard. HRESIMS (high resolution electrospray ionization mass spectrometry) was obtained using a micrOTOF-II system from Bruker. Silica gel 60 (0.063-0.20 and 0.04-0.063 mm; Merck), was used in column chromatography (CC), whereas silica gel TLC (thin layer chromatography) plates PF254 7749 (Merck) stained with iodine vapor and viewed under UV light (254/366 nm) were used to monitor chromatographic purification procedures.

Table 1. NMR data for **1**[a]

	HMQC		HMBC	
	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$
		C		
3	200.87	–	H-4	3H-23
5	49.10	–	H-4; 3H-24	2H-7; 3H-25
9	36.66	–	H-8; 2H-11; 3H-25	2H-7
13	39.97	–	3H-27	H-8; 2H-15; 3H-26
14	45.56	–	3H-26	3H-27
16	214.24	–	2H-15	3H-28
17	47.12	–	3H-28	
20	42.29	–	2H-19; 3H-29; 3H-30	
21	218.13	–		2H-19; 3H-29; 3H-30
		CH		
1	146.28	6.65 (d, 10.5)		
2	130.14	5.92 (dd, 10.5, 3.0)		
4	57.72	2.23 (q)	3H-23	3H-24
6	77.25	3.63 (dd, 9.5, 5.0)	2H-7	3H-24
8	48.19	1.40 (m)		3H-25
10	60.35	2.08 (br s)		H-4; 3H-24; 3H-25
12	69.32	3.94 (dd, 11.0, 4.0)	2H-11	3H-27
18	44.66	2.46 (m)	2H-19	H-11a; 3H-27; 3H-28
		CH_2		
7	28.67	1.49 (m), 1.46 (m)		
11	45.37	1.82 (m), 1.17 (m)		3H-25
15	49.56	2.24 (d, 19.0) 2.06 (d, 19.0)		3H-26; H-8
19	39.58	2.03 (m), 1.82 (m)		3H-29; 3H-30
22	47.16	2.46 (m)		3H-28
		CH_3		
23	9.80	1.17 (d, 6.5)	H-4	
24	8.69	0.74 (s)		H-4
25	19.57	0.89 (s)		H-8; H-11b; H-10
26	19.93	1.11 (s)		H-8; 2H-15
27	8.69	0.84 (s)		H-18
8	29.07	1.09 (s)		2H-22
29	28.39	0.91 (s)		H-19b; 3H-30
30	24.40	1.04 (s)		2H-19; 3H-29

[a]Data obtained at 500 MHz in $CDCl_3$ + C_5D_5N (δ in ppm, J in Hz).

Plant material

The botanical material utilized was collected in the Matureia city (Paraíba, Brazil) in June 2009, and identified by Prof. Dra. Maria de Fatima Agra. A dried specimen is deposited in the Herbarium Professor Lauro Pires Xavier at Universidade Federal da Paraíba (Paraíba, Brazil) under No. 7448.

Extraction and isolation

The leaves of *M. distichophylla* (3.5 kg), dried and pulverized, were extracted with 95% ethanol at room temperature for 3 days. The obtained extract was concentrated in a rotary evaporator under reduced pressure at 40 °C, yielding 685.0 g of ethanolic extract. A portion (100.0 g) was suspended in MeOH:H_2O (7:3) and partitioned successively with *n*-hexane, $CHCl_3$ and EtOAc to obtain the *n*-hexane (2.5 g), chloroform (5.4 g) and ethyl acetate (6.5 g) fractions. The ethyl acetate fraction (5.4 g) was separated by CC, on silica gel 60 (0.063-0.200 mm), eluted with *n*-hexane, EtOAc and MeOH, pure or in binary mixtures, in increasing order of polarity, resulting in 110 fractions of 100 mL each, which were submitted to analytical NMR. Fraction 14 eluted with *n*-hexane:EtOAc (7:3) yielded compound **2** (50.3 mg). Fractions 1-2 (168.3

mg) were submitted to another CC utilizing similar conditions as before, providing 25 subfractions of 10 mL each. Subfractions 10-15 eluted with *n*-hexane:EtOAc (8:2) gave the triterpene friedelane **1** (13.4 mg). Fractions 27-35 (99.3 mg) were rechromatographed as before, resulting in 55 subfractions of 10 mL each. Subfractions 33-37 eluted with *n*-hexane:EtOAc (6:4) yielded **3** (26.2 mg).

The chloroform fraction (5.0 g) was submitted to CC, on silica gel 60 (0.063-0.200 mm), eluted with hexane, EtOAc and MeOH, pure or in binary mixtures and with increasing order of polarity, resulting in 110 fractions of 100 mL each, which were concentrated in a rotary evaporator and submitted to analytical NMR. Fractions 42-45 eluted with *n*-hexane:EtOAc (8:2) provided compound **4** (22.7 mg). Fractions 67-81 (675.3 mg) were submitted to another CC, utilizing a column packed with silica gel 60 (0.04-0.063 mm) and the eluents *n*-hexane and EtOAc and MeOH, resulting in 53 subfractions of 100 mL each, which were submitted to analytical NMR and combined into 10 groups. Subfractions 2-3 eluted with *n*-hexane:EtOAc (85:15) yielded compounds **5** (8.5 mg), 6-7 eluted with *n*-hexane:EtOAc (6:4) yielded compound **6** (5.0 mg) and 9-10 eluted with *n*-hexane:EtOAc (1:1) yielded compound **7** (9.0 mg).

3,16,21-Trioxo-6β,12α-dihydroxy-1-en-friedelane (**1**)

White amorphous powder; mp 310-312 ºC; IR (KBr) ν_{max}/cm^{-1} at 3504, 1681, 1662; HRESIMS *m/z* 485.3306 $[M + H]^+$, (calc. for $C_{30}H_{44}O_5$, 485.3261); ^1H NMR (500 MHz, $CDCl_3$ + C_5D_5N) and ^{13}C NMR (125 MHz, $CDCl_3$ + C_5D_5N), see Table 1.

Supplementary Information

Supplementary data associated with this paper are available free of charge at http://jbcs.sbq.org.br as a PDF file.

Acknowledgments

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References

1. Simmons, M. P.; Savolainen, V.; Clevinger, C. C; Archer, R. H.; Davis, J. I.; *Mol. Phylogenet. Evol.* **2001**, *19*, 353.
2. Oliveira, D. M.; Silva, G. D. de F.; Duarte, L. P.; Vieira Filho, S. A.; *Biochem. Syst. Ecol*. **2006**, *34*, 661.
3. Silva, M. S.; Sousa, D. P.; de Medeiros, V. M.; Folly, M. A. B.; Tavares, J. F.; Barbosa-Filho, J. M.; *Biochem. Syst. Ecol.* **2008**, *36*, 500.
4. Silva, F. C.; Duarte, L. P.; Silva, G. D. F.; Vieira-Filho, S. A; Lula, I. S.; Takahashi, J. A.; Sallum, W. S. T.; *J. Braz. Chem. Soc.* **2011**, *22*, 943.
5. Niero, R.; Andrade, S. F.; Cechinel-Filho, V.; *Curr. Pharm. Des*. **2011**, *17*, 1851.
6. Andrade, S. F.; Comunello, E.; Noldin, V. F.; Monache, F. D.; Filho, V. C.; Niero, R.; *Arch. Pharmacal Res*. **2008**, *31*, 41.
7. Orabi, K. Y.; Al-Qasoumi, S. I.; El-Olemy, M.; Mossa, J. S.; Muhammad, I.; *Phytochemistry* **2001**, *58*, 475.
8. Mota, K. S. L.; Pita, J. C. L. R.; Estevam, E. C.; Medeiros, V. M.; Tavares, J. F.; Agra, M. F.; Diniz, M. F. F. M.; Silva, M. S.; Batista, L. M.; *Rev. Bras. Farmacogn*. **2008**, *18*, 442.
9. Sousa, G. F.; Duarte, L. P.; Alcântara, A. F. C.; Silva, G. D. F.; Vieira-Filho, S. A,; Silva, R. R.; Oliveira, D. M.; Takahashi, J. A.; *Molecules* **2012**, *17*, 13439.
10. Ming-Xiang, C.; Ding-Yong, W.; Jiao, G.; *J. Chem. Res*. **2010**, *34*, 114.
11. Salazar, G. C. M.; Silva, G. D. F.; Duarte, L. P.; Vieira-Filho, S. A.; Lula, I. S.; *Magn. Reson. Chem*. **2000**, *38*, 977.
12. Alves, J. S.; Castro, J. C. M.; Freire, M. O.; Cunha, E. V. L.; Barbosa-Filho, J. M.; Silva, M. S.; *Magn. Reson. Chem*. **2000**, *38*, 201.
13. Oliveira, M. L. G.; Duarte, L. P.; Silva, G. D. F.; Vieira-Filho, S. A.; Knupp, V. F.; Alves, F. G. P.; *Magn. Reson. Chem*. **2007**, *45*, 895.
14. Magalhães, C. G.; Ferrari, F. C.; Guimarães, D. A. S.; Silva, G. D. F.; Duarte, L. P.; Figueiredo R. C.; Vieira-Filho, S. A.; *Rev. Bras. Farmacogn*. **2011**, *21*, 415.
15. Nozaki, H.; Matsuura, Y.; Hirono, S.; Kasai, R.; Tada, T.; Nakayama, M.; Lee, K. H.; *Phytochemistry* **1991**, *30*, 3819.
16. Itokawa, H.; Shirota, O.; Ikuta, H.; Morita, H.; Takeya, K.; Iitaka, Y.; *Phytochemistry* **1991**, *30*, 3713.

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Supplementary Information

Maytensifolone, a New Triterpene from *Maytenus distichophylla* Mart. ex Reissek

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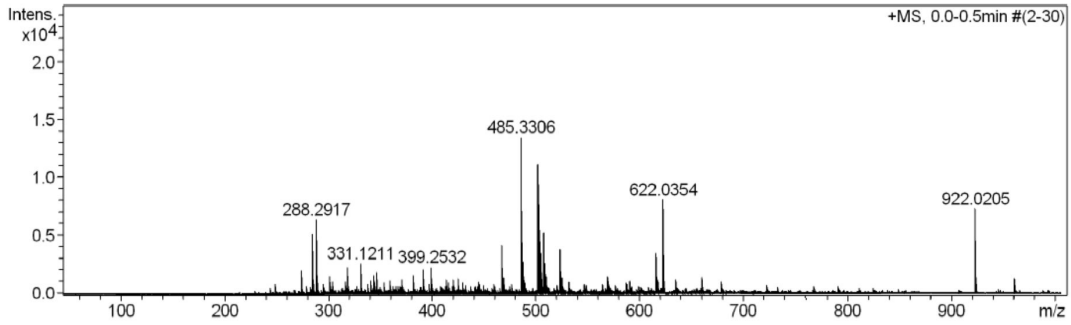

Figure S1. HRESIMS spectrum of compound **1**.

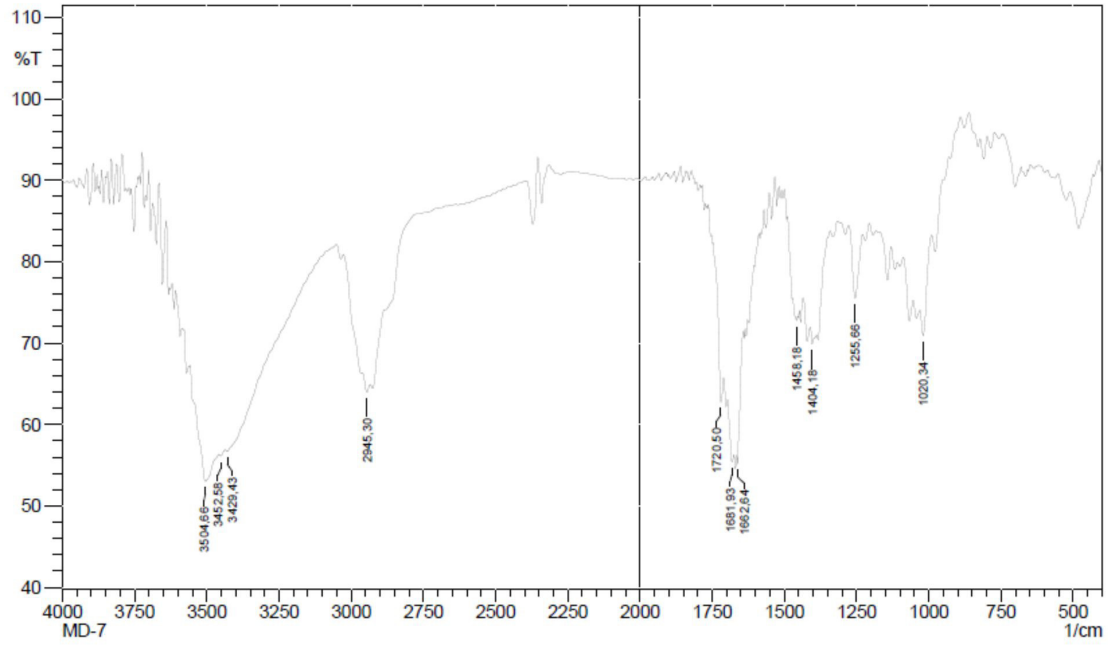

Figure S2. IR spectrum (KBr) of compound **1**.

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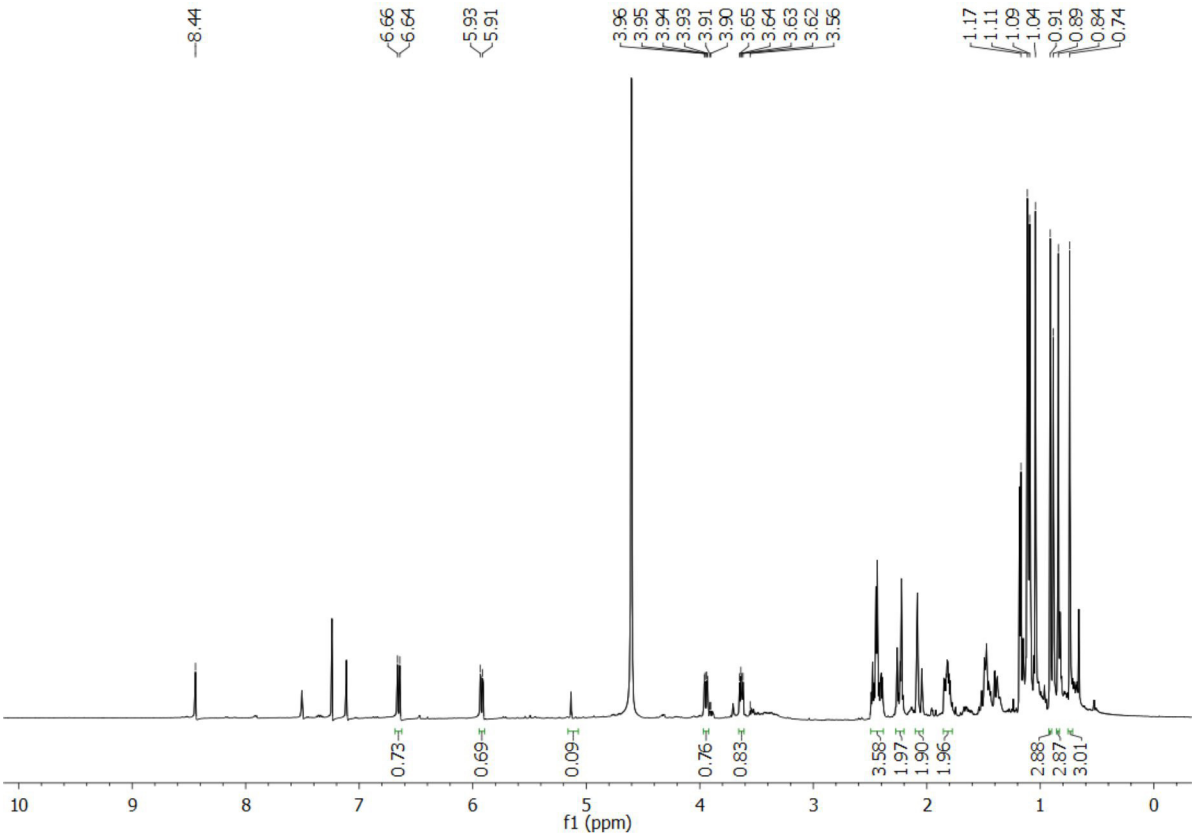

Figure S3. ^{1}H NMR spectrum (500 MHz, $CDCl_3$ + C_5D_5N) of compound **1**.

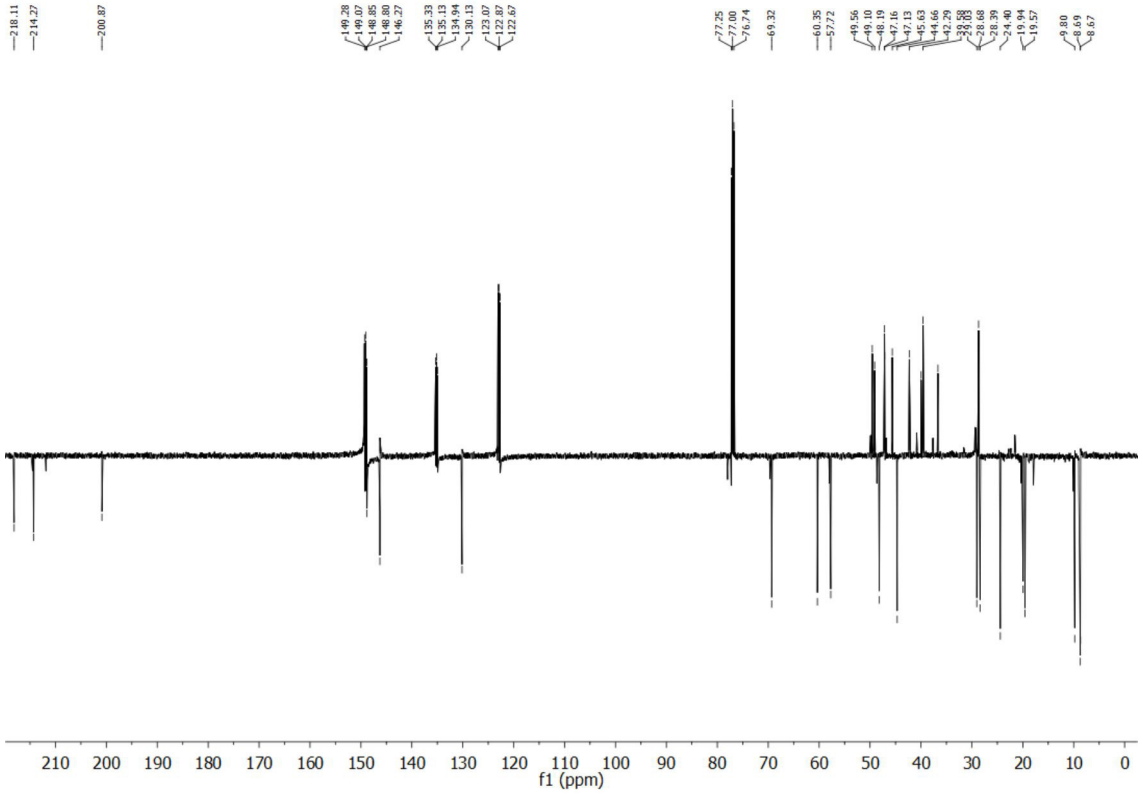

Figure S4. ^{13}C APT NMRspectrum (125 MHz, $CDCl_3$ + C_5D_5N) of compound **1**.

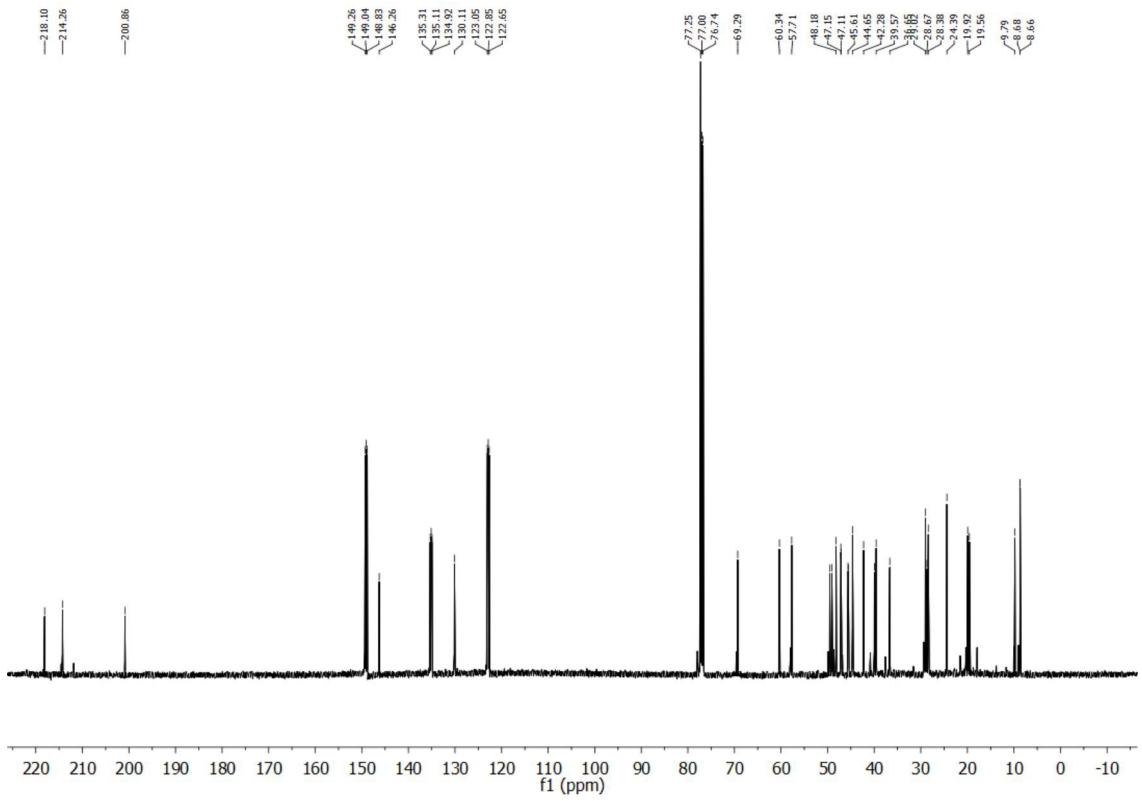

Figure S5. ^{13}C BB NMR spectrum (125 MHz, $CDCl_3$ + C_5D_5N) of compound **1**.

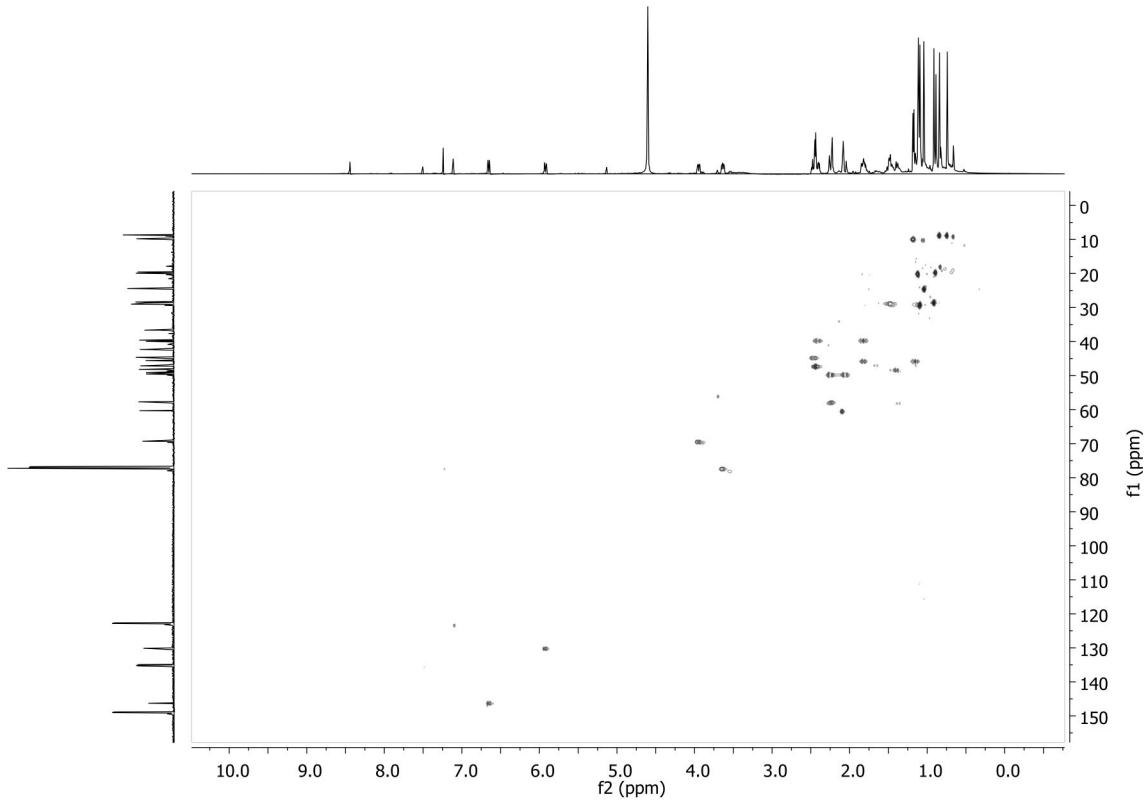

Figure S6. HMQC NMR spectrum (500 MHz, $CDCl_3$ + C_5D_5N) of compound **1**.

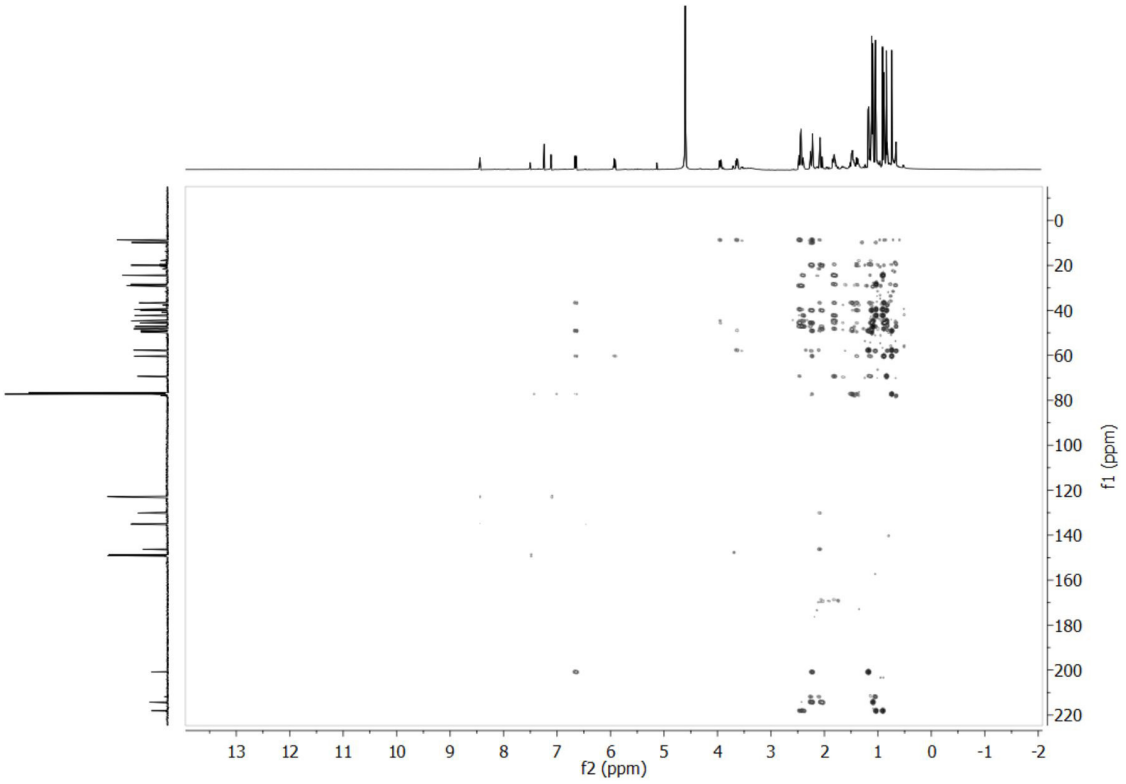

Figure S7. HMBC NMR spectrum (500 MHz, $CDCl_3$ + C_5D_5N) of compound **1**.

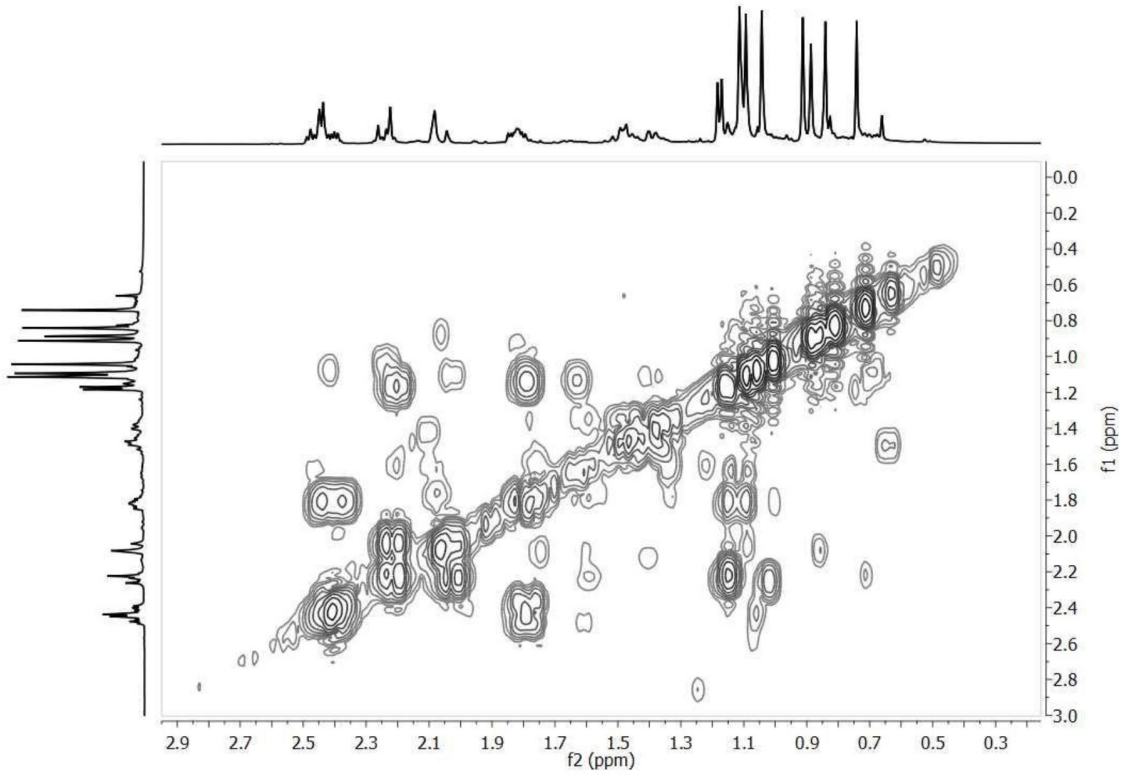

Figure S8. COSY NMR experiment (500 MHz, $CDCl_3$ + C_5D_5N, δ_H 2.9-0.2) of compound **1**.

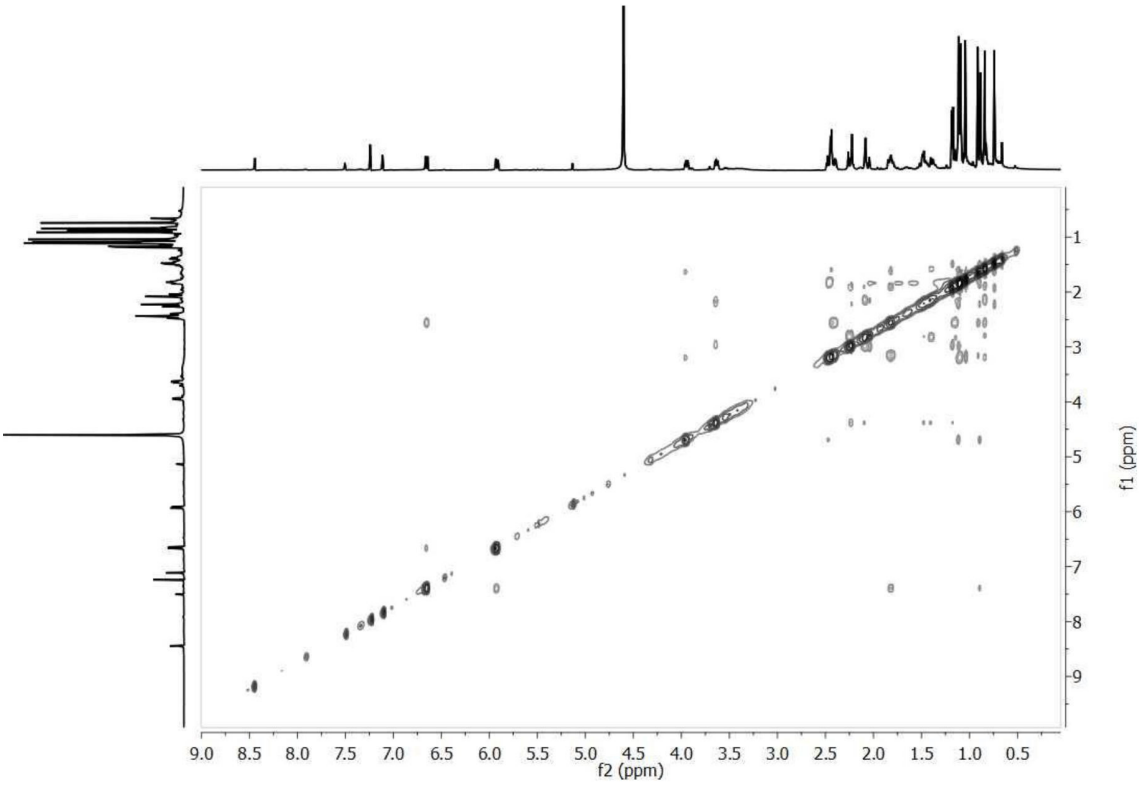

Figure S9. NOESY NMR experiment (500 MHz, $CDCl_3$ + C_5D_5N, δ_H 9.0-0.0) of compound **1**.