Supplemental Material for

A Family of Multicyclic Indolosesquiterpenes from a Bacterial Endophyte

Ling Ding,[a] A. Maier,[b] H.H. Fiebig,[b] Wen-Han Lin,[c] and Christian Hertweck[a,d]*

Experimental Section

General Experimental Procedures. Optical rotation was measured using a 0.5 dm cuvette with a JASCO P-1020 polarimeter at 25°C. UV spectra were recorded on a Varian UV-visible Cary spectrophotometer (Varian, Palo Alto, CA). IR spectra were recorded on a Bruker FT-IR (IFS 55) spectrometer. ^{1}H and ^{13}C NMR, DEPT and 2D NMR spectra were measured on Bruker Avance III 300 MHz and Bruker Avance III 600 MHz instruments. The chemical shifts values (δ) are given in parts per million (ppm), and coupling constants in Hz. ESIMS data were obtained with a triple quadrupole mass spectrometer (Quattro; VG Biotech, Altrincham, Cheshire, UK). Columm chromatography was performed on silica gel 60M (230-400 mesh, Macherey-Nagel, Düren, Germany) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden). TLC analysis was performed on silica gel plates (Sil G/UV_{254} 0.20 mm, Macherey-Nagel).

Plant Material and Strain Isolation. The stems of *K. candel* were collected in Xiamen city of Fujian Province, People's Republic of China, in June 2002 and authenticated by Prof. Peng Lin, Xia Men University, People's Republic of China. A voucher sample of the plant is deposited in the National Research Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing, People's Republic of China (Mangrove 200208082). Bacteria were isolated from the stems of the plant according to the procedure described earlier. *Streptomyces* sp. strain HKI0595 was deposited in the strain collection of the HKI, Jena, Germany.

Fermentation. Liquid organic medium 79 (dextrose 10 g, bacto peptone 10 g, casamino acids 1 g, yeast extract 2 g, NaCl 6 g, H_2O 1 L) (2 × 100 mL/flask) was inoculated with a suspension of mycelium and spores (about 1 × 1cm) of *Streptomyces* sp. (HKI0595) grown on agar slants or agar plates. After incubation for 48 h on a rotary shaker at 28 °C, the culture was transferred to 3200 mL of medium 27 (glucose 20 g, soybean powder 20 g, NaCl 5 g, $CaCO_3$ 3 g, 1 L H_2O, eight 1000 mL-scale Erlenmeyer flasks with 400 mL of medium 27 each) and incubated at 28 °C under shaking conditions for 48 h to yield pre-fermentation culture, which was poured into a 300 L-scale fermenter filled with 200 L of medium 27 and fermented for 5 days.

Extraction and Isolation. The fermentation broth of *Streptomyces* sp. (HKI0595) was separated into culture filtrate and mycelia by centrifugation. The mycelia were extracted by ethyl acetate and the extract was concentrated under

reduced pressure to give 20 g dry crude extract. Separation by flash silica gel chromatography (column 50 × 3 cm, CH_2Cl_2/0-10% CH_3OH gradient), yielding eight fractions 1-8. Fraction 6 was firstly purified by Sephadex LH-20 (CH_2Cl_2/50%CH_3OH) into four fractions 6A-6D. Fraction 6C was further purified by HPLC (RP-18, CH_3OH /H_2O as gradient) to yield two sub-fractions 6C1 and 6C2. Compound **1b** (1.0 mg) and **3** (14.2 mg) was obtained by separation of 6C1 on PTLC (CH_2Cl_2/7% CH_3OH). Compound **1a** (2.2 mg) was obtained by purification of 6C2 on Sephadex LH-20 (MeOH). Fraction 6D was subjected to preparative HPLC to afford **2** (2.5 mg). For HPLC separation, the compounds were eluted with a gradient from 10% methanol in water to 100% methanol in 20 minutes, followed by 100% methanol for 10 minutes using a flow rate of 10 mL min^{-1}.

Anti-microbial assays. Sterile filter paper disks were impregnated with 50 μg of the samples using methanol as the carrier solvent. The impregnated disks were then placed on agar plates previously inoculated with *Bacillus subtilis* ATCC 6633, *Mycobacterium vaccae* IMET 10670, *Pseudomonas aeruginosa* K799/61, *Staphylococcus aureus* SG511, *Staphylococcus aureus* 134/94 R9 (methicillin resistant MRSA), *Enterococcus faecalis* 1528 R10 (vancomycin resistant VRE). Chloramphenicol was used as a positive control against bacteria. Methanol was used as negative control. After the plates were incubated at 37 °C for 24 h, antimicrobial activity was recorded as clear zones (in mm) of inhibition surrounding the disk. The test sample was considered active when the zone of inhibition was greater than 7 mm in diameter.

Antiproliferative and cytotoxic assays. A modified propidium iodide assay was used to determine the cytotoxic activity of compounds **1a**, **2** and **3** against 12 cell lines derived from solid human tumors. The test procedure has been described elsewhere.[17] Cell lines tested were derived from patient tumors engrafted as a subcutaneously growing tumor in NMRI nu/nu mice, or obtained from the American Type Culture Collection, Rockville, MD, USA, National Cancer Institute, Bethesda, MD, USA, or Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig. Inhibitory concentrations are provided as 50% inhibition of cell growth (absolute IC_{50}, determined by two-point-curve-fit after plotting compound concentration versus fluorescence intensity). Compound **2** showed to be more potent (geometric mean IC_{50} = 10.13 µM) compared to compound **1** (geometric mean IC_{50} > 30 µM), with antitumor potency being generally less pronounced.

Xiamycin A (1a): All physicochemical data were in full agreement with those reported.[13]

Xiamyxin B (1b): pale yellow crystal; $[\alpha]_D^{21}$ –75 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) 351 (2.95), 343 (3.12), 329 (3.20), 300 (3.99), 268 (3.60) nm; IR(film) ν_{max} 3355.3, 2922.3, 2852.1, 1734.0, 1594.2, 1464.6, 1375.6, 1254.7, 1121.2, 630.8 cm^{-1}; NMR data, Table 1; ESIMS $[M + NH_4]^+$ 397.1, $[M + Na]^+$ 402.1, $[2M + Na]^+$ 781.3, $[M - H]^-$ 378.2, $[2M - H]^-$ 767.4; HRESIMS *m/z* 378.1756 $[M - H]^-$ (calcd 378.1711 for $C_{23}H_{24}NO_4$).

Indosespene (2): pale yellow crystal; $[\alpha]_D^{27}$ 43.9 (*c* 1.8, MeOH); UV (MeOH) λ_{max} (log ε) 284 (4.14), 225 (4.91), 202 (4.69) nm; IR(film) ν_{max} 3304.0, 2933.6, 1712.6, 1646.7, 1455.6, 1385.8, 1356.5, 1257.3, 1231.8, 1161.6, 1127.8, 1086.3, 1062.6, 1027.6, 1001.9, 979.2, 943.0, 889.9, 809.2, 793.2, 737.5, 703.5, 647.4, 623.2 cm^{-1}; NMR data, Table 1; ESIMS $[M + H]^+$ 368.1, $[2M + Na]^+$ 758.3, $[2M + NH_4]^+$ 752.4, $[M - H]^-$ 366.1, $[2M - H]^-$ 733.4; HRESIMS *m/z* 366.2048 $[M - H]^-$ (calcd 366.2075 for $C_{23}H_{28}NO_3$).

Sespenine (3): pale yellow crystal; $[\alpha]_D^{26}$ –261 (*c* 10.7, MeOH); UV(MeOH) λ_{max} (log ε) 302 (3.57), 245 (3.86), 205 (4.30) nm; IR(film) ν_{max} 3358.7, 2939.0, 1693.8, 1602.9, 1489.2, 1449.6, 1413.1, 1391.1, 1311.2, 1278.1, 1249.7, 1146.9, 1113.3, 1078.7, 1021.9, 1000.9, 978.1, 902.1, 749.2, 672.0, 631.1 cm^{-1}; NMR data, Table 1; ESIMS $[M + H]^+$ 384.2, $[M + Na]^+$ 406.1, $[2M + Na]^+$ 789.4, $[M - H]^-$ 382.2, $[2M - H]^-$ 765.4; HRESIMS *m/z* 384.2191 (calcd 384.2169 for $C_{23}H_{30}NO_4$).

Table 1. NMR Spectroscopic Data (MeOD) for **1b**, **2** and **3**.

position	**1b**		**2**		**3**	
	δ_C [a]	δ_H [b] (*J* in Hz)	δ_C [a]	δ_H [b] (*J* in Hz)	δ_C [c]	δ_H [b] (*J* in Hz)
1	-	-	-	-	-	-
2	136.9	-	123.3	6.89, s	58.7	3.64, brs
3	121.5	-	129.0	-	37.9	-
4	122.9	-	115.6	-	130.5	-
5	119.3	7.92, dd (7.9, 1.0)	119.2	7.53, dd (7.8, 1.0)	126.2	7.28, dd (8.1, 1.1)
6	118.0	7.08, ddd (7.9, 7.0, 1.0)	119.2	6.98, ddd (8.0, 7.0, 1.0)	118.3	6.63, ddd (8.1, 8.1, 1.1)
7	124.7	7.29, ddd (8.1, 7.0, 1.0)	122.0	7.04, ddd (8.0, 7.0, 1.0)	128.5	6.96, ddd (8.1, 8.1, 1.1)
8	110.2	7.38, dd (8.1, 1.0)	112.1	7.28, dd (8.0, 1.0)	115.4	6.55, dd (8.0, 1.1)
9	140.6	-	137.8	-	144.4	-
10	113.8	7.48, s	20.7	2.97, dd (15.3, 1.6) 2.83, dd (15.2, 10.7)	212.0	-
11	147.5	-	57.8	2.27, d (10.3)	37.4	2.27, d (16.2) 2.15, dd (16.2, 8.0)
12	**	-	40.0	-	63.2	1.72, d (8.0)
13	39.3	2.00, m	38.5	2.08, td (13.3, 3.4) 1.50, dt (13.1, 4.2)	37.8	2.39, m 1.54, d (12.9)
14	28.2	1.84, 1.65, m	28.3	1.75, 1.70, m	22.0	1.76, m, 1.29, m
15	74.5	4.14, dd (11.8, 4.4)	76.4	4.05, dd (10.7, 5.6)	51.9	1.75, *
16	54.0	-	54.9	-	54.7	-
17	79.7	-	52.0	1.95, dd (12.6, 2.7)	75.2	4.00, dd (8.4, 8.2)
18	25.1	1.69, dd (12.3, 5.5) 1.53, m	27.4	1.58,1.32, m	27.2	1.78, 1.00, m
19	34.3	2.88, dd (6.1, 5.6)	38.8	2.33, m 1.99, dt (12.9, 4.7)	39.8	1.63, *
20	134.4	-	148.8	-	39.5	-
21	110.1	7.15, s	15.1	1.11, s	33.8	2.70, dd (13.8, 3.6) 1.65 (brd, 13.8)
22	19.9	1.07, s	11.5	0.88, s	11.6	1.11, s
23	9.6	1.05, s	109.0	4.80,d (1.2) 4.71, s	181.4	-
24	179.1	-	181.3	-	17.7	0.94, s

[a] Recorded at 150.94 MHz. [b] Recorded at 600.27 MHz. [c] Recorded at 75.47 MHz.
* Overlapping resonance signal. ** Signal could not be detected.

Table 2. Antiproliferative Activities of **1a**, **2** and **3** Determined in a Panel of 12 Human Tumor Cell Lines.

		IC_{50} [µM]		
Tumor type	**Cell line**	**1a**	**2**	**3**
Colon	HT-29	>30	>10	>10
Stomach	GXF 251L	>30	>10	>10
Lung	LXFA 629L	>30	>10	>10
	LXFL 529L	>30	>10	>10
Breast	MAXF 401NL	>30	>10	>10
Melanoma	MEXF 462NL	>30	>10	>10
Ovary	OVXF 899L	>30	>10	>10
Pancreas	PAXF 1657L	>30	>10	>10
Prostate	22Rv1	>30	>10	>10
Mesothelioma	PXF 1752L	>30	>10	>10
Kidney	RXF 486L	>30	>10	>10
Uterus	UXF 1138L	>30	>10	>10
Mean IC_{50}		**>30**	**>10**	**>10**

Table 3. Antibacterial Activities of **1a**, **2** and **3**. Pa: *Pseudomonas aeruginosa*; Sa: *Staphylococcus aureus*; Bs: *Bacillus subtilis*; Mv: *Mycobacterium vaccae*; MRSA: methicillin-resistant *Staphylococcus aureus*; VRE: vancomycin-resistant *Enterococcus faecalis*. [a] 50 µg/paper disk, d = 7 mm, data in diameter.

Compound	Test strains (mm inhibition zone)[a]					
	Pa	Sa	Bs	Mv	MRSA	VRE
1a	15	15	14	14	14	12
2	0	12	12	10	13	15
3	0	0	10	10	0	0

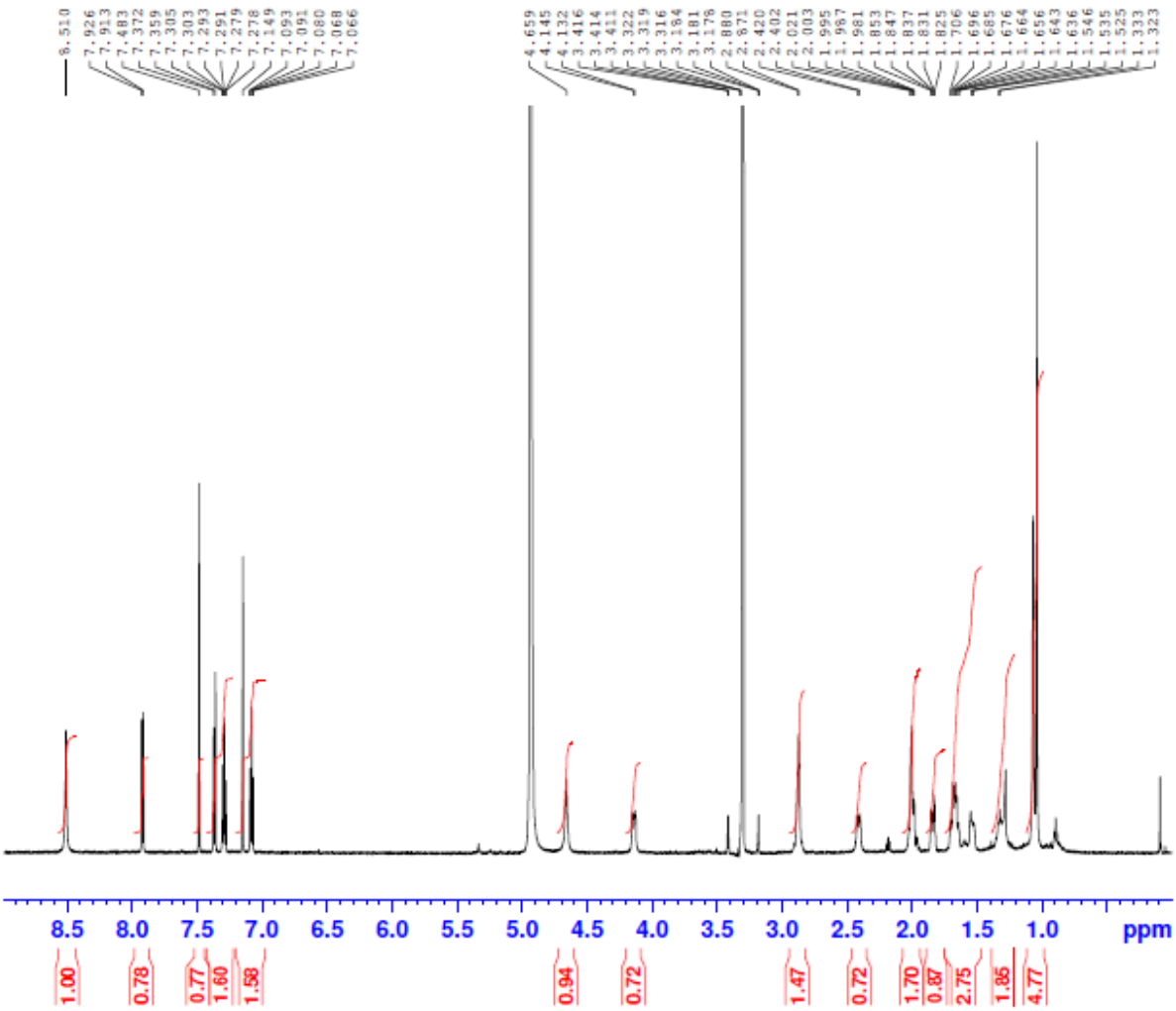

Figure S1. ^{1}H NMR (600 MHz, MeOD) spectrum of compound **1b**.

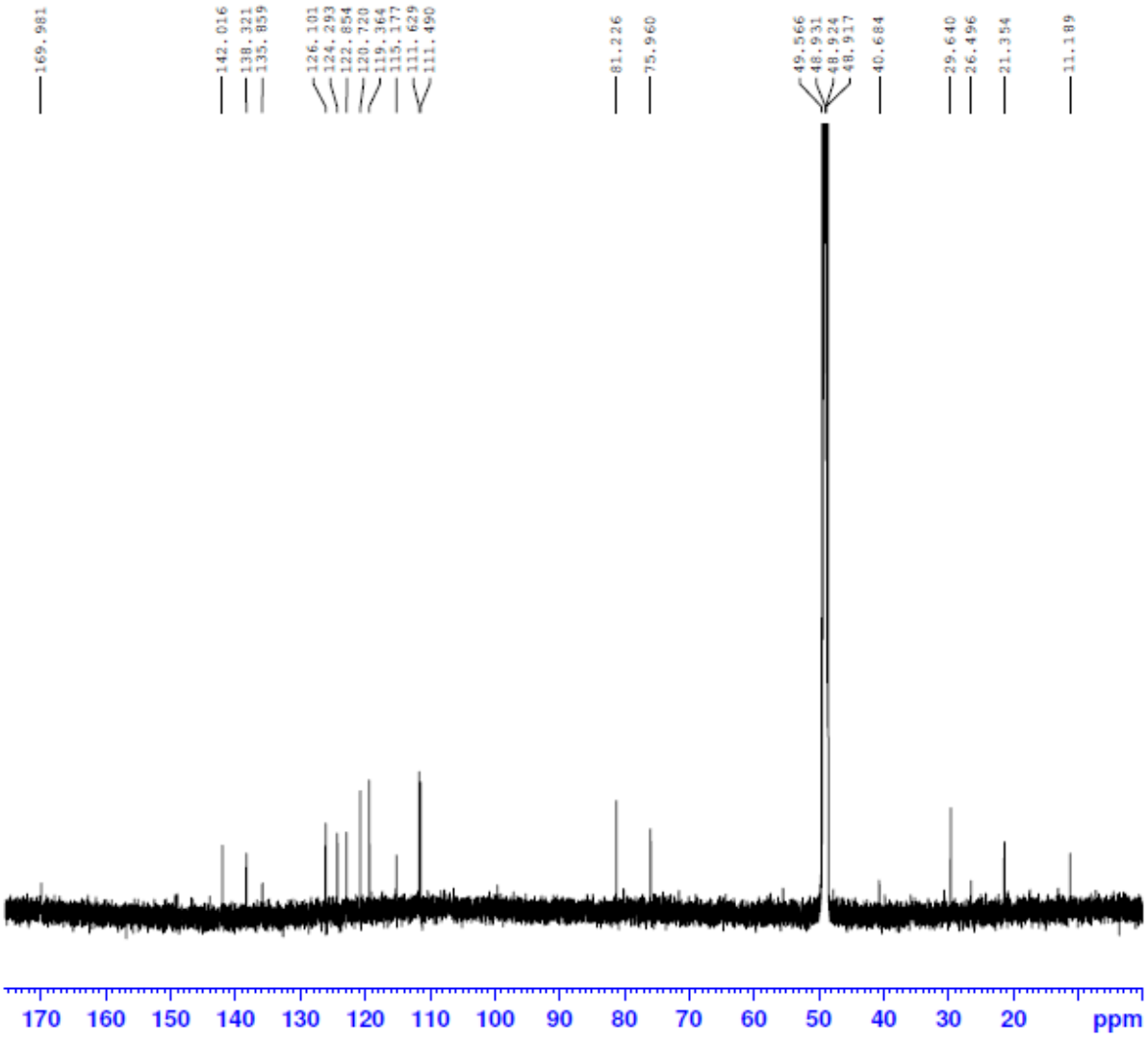

Figure S2. ^{13}C NMR (150 MHz, MeOD) spectrum of compound **1b**.

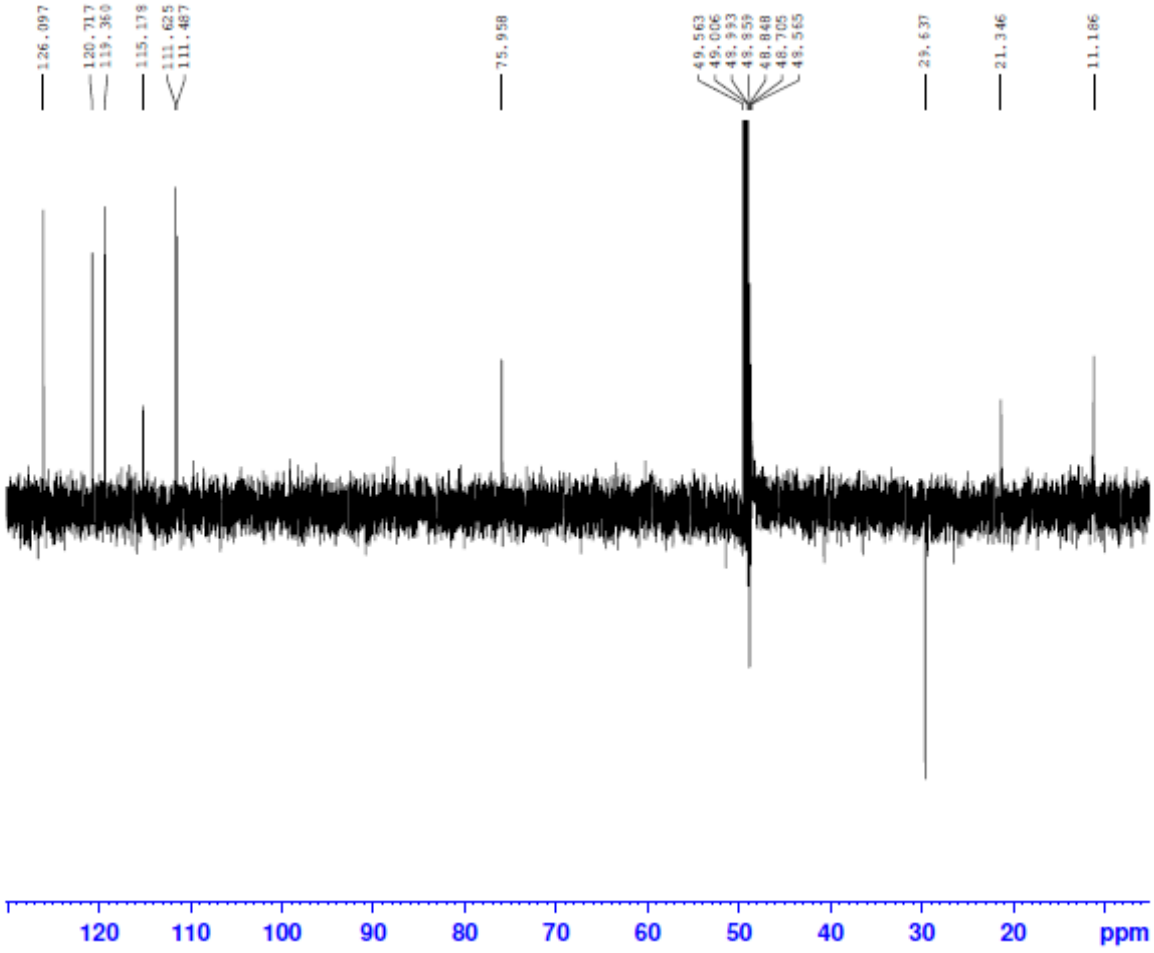

Figure S3. DEPT spectrum (125 MHz, MeOD) of **1b**.

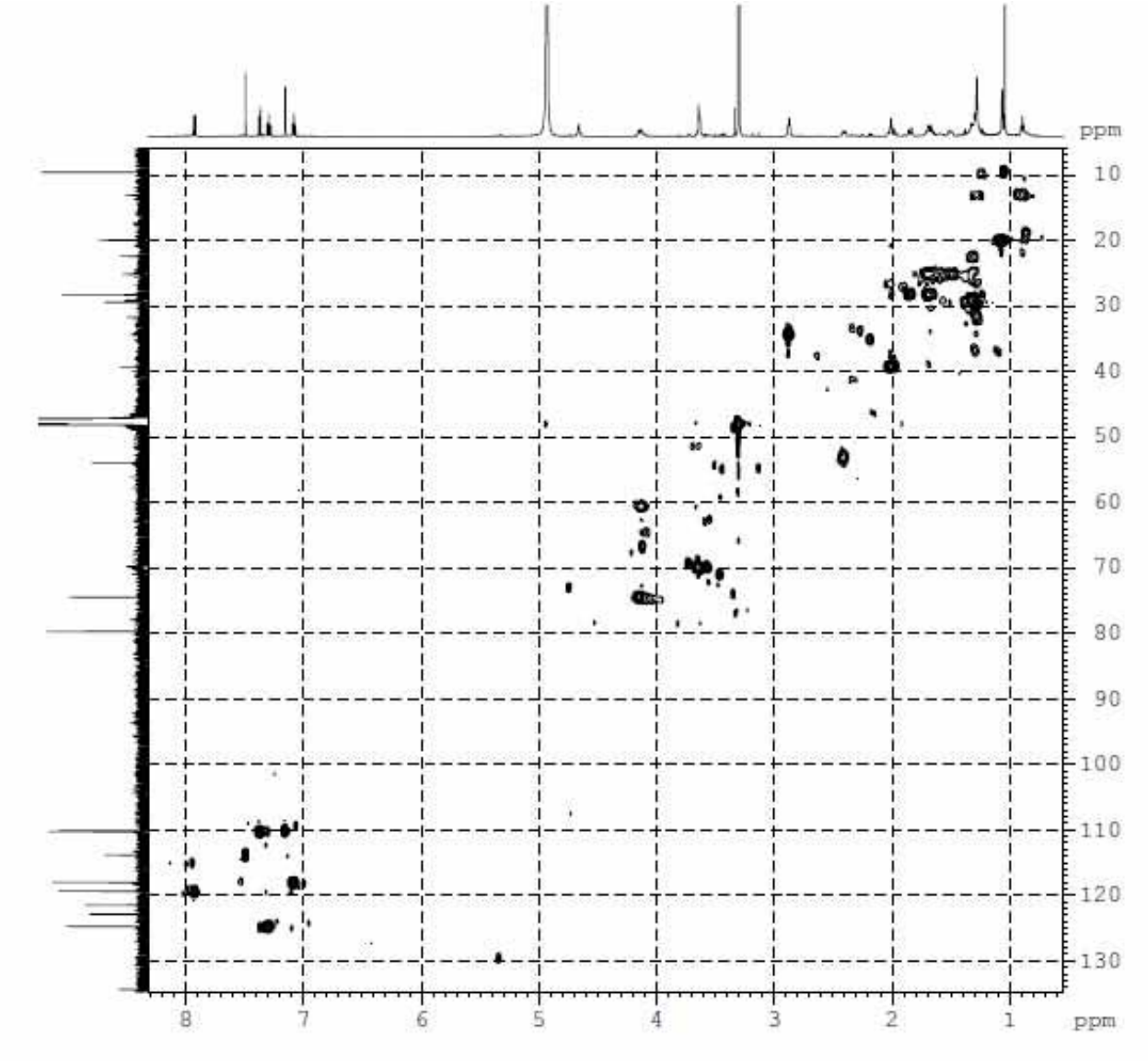

Figure S4. HMQC spectrum (125 MHz, MeOD) of **1b**.

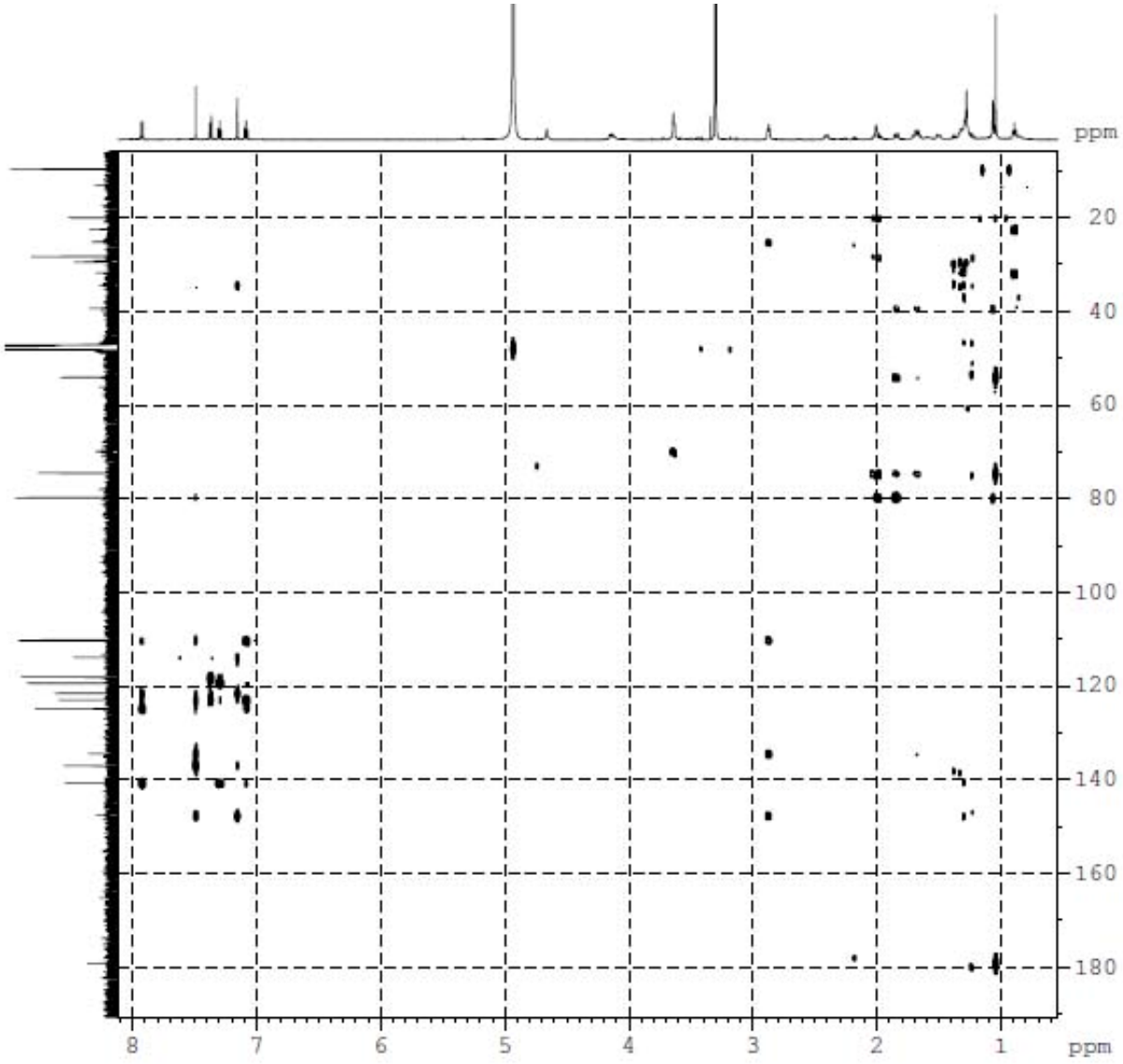

Figure S5. HMBC spectrum (125 MHz, MeOD) of **1b**.

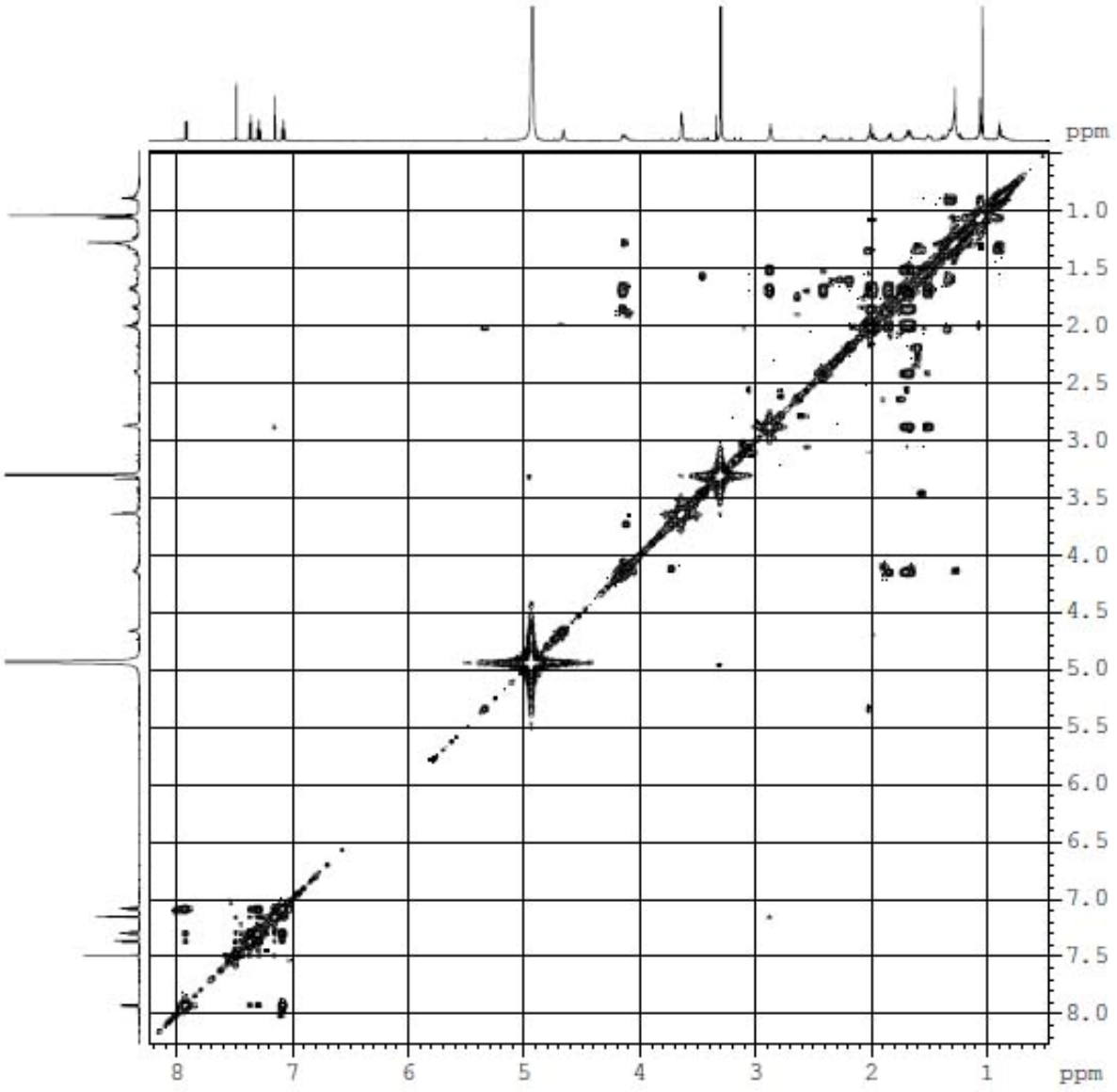

Figure S6. H,H COSY spectrum (500 MHz, MeOD) of **1b**.

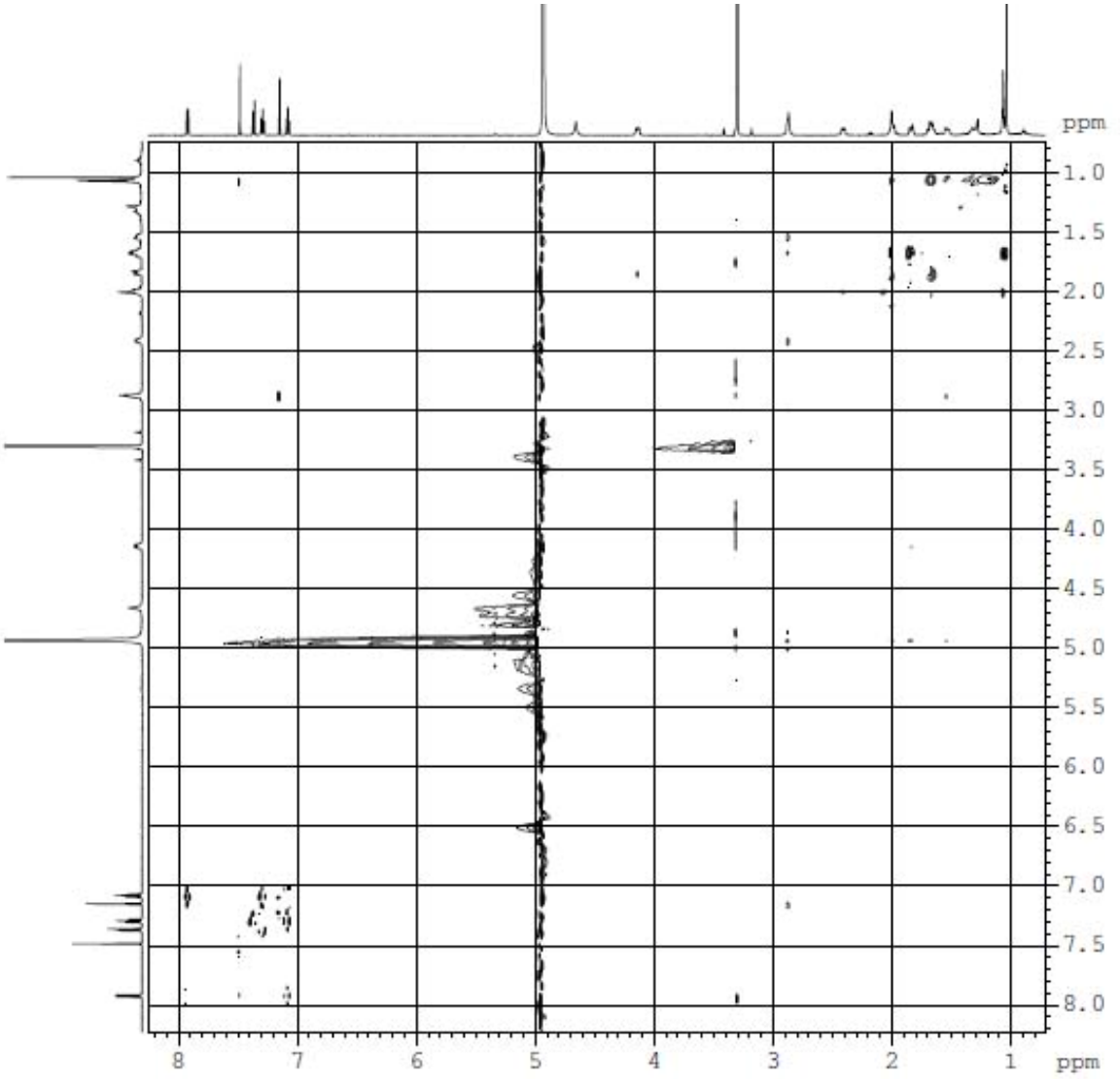

Figure S7. NOESY spectrum (500 MHz, MeOD) of **1b**.

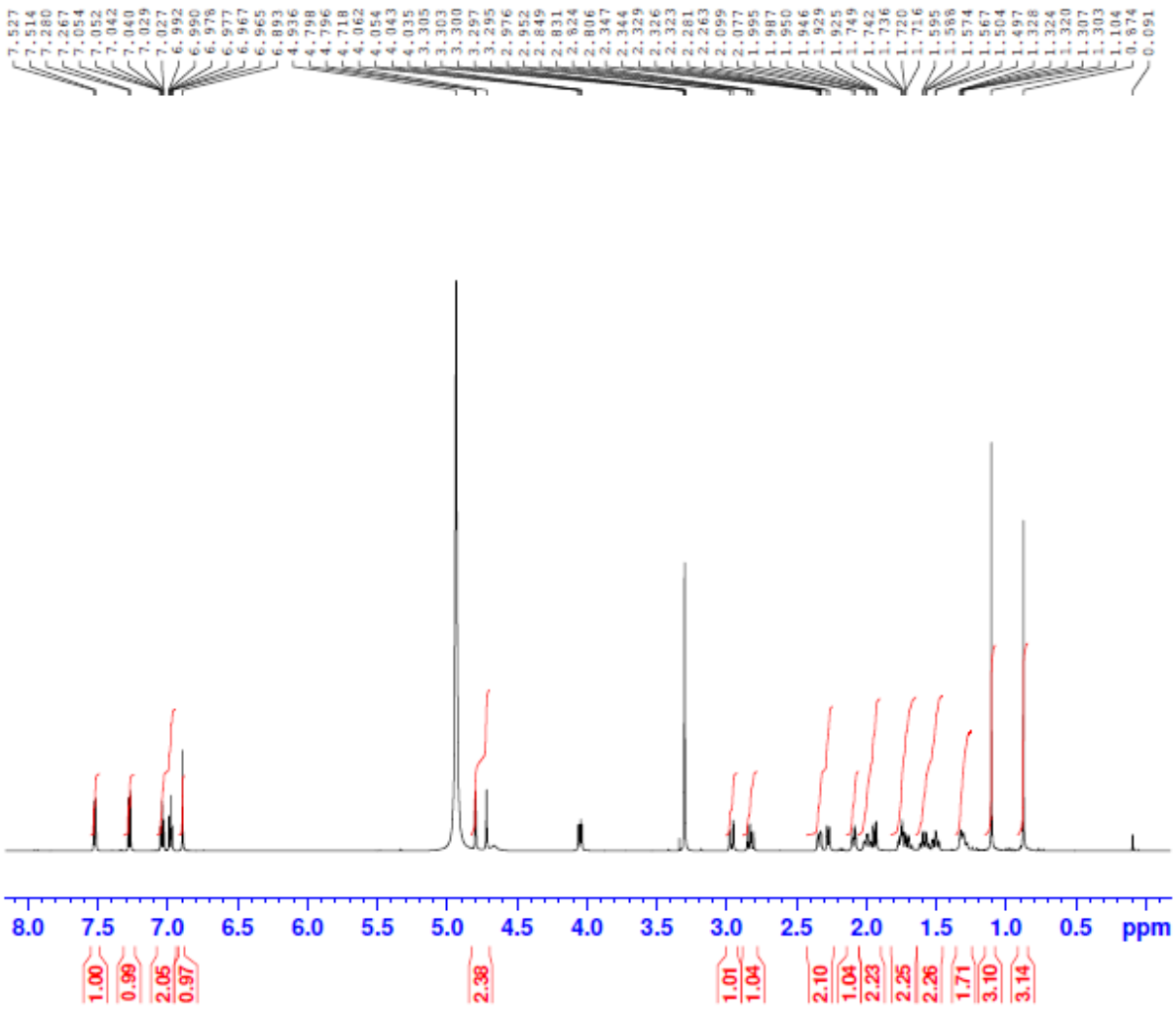

Figure S8. ^{1}H NMR (600 MHz, MeOD) spectrum of compound **2**.

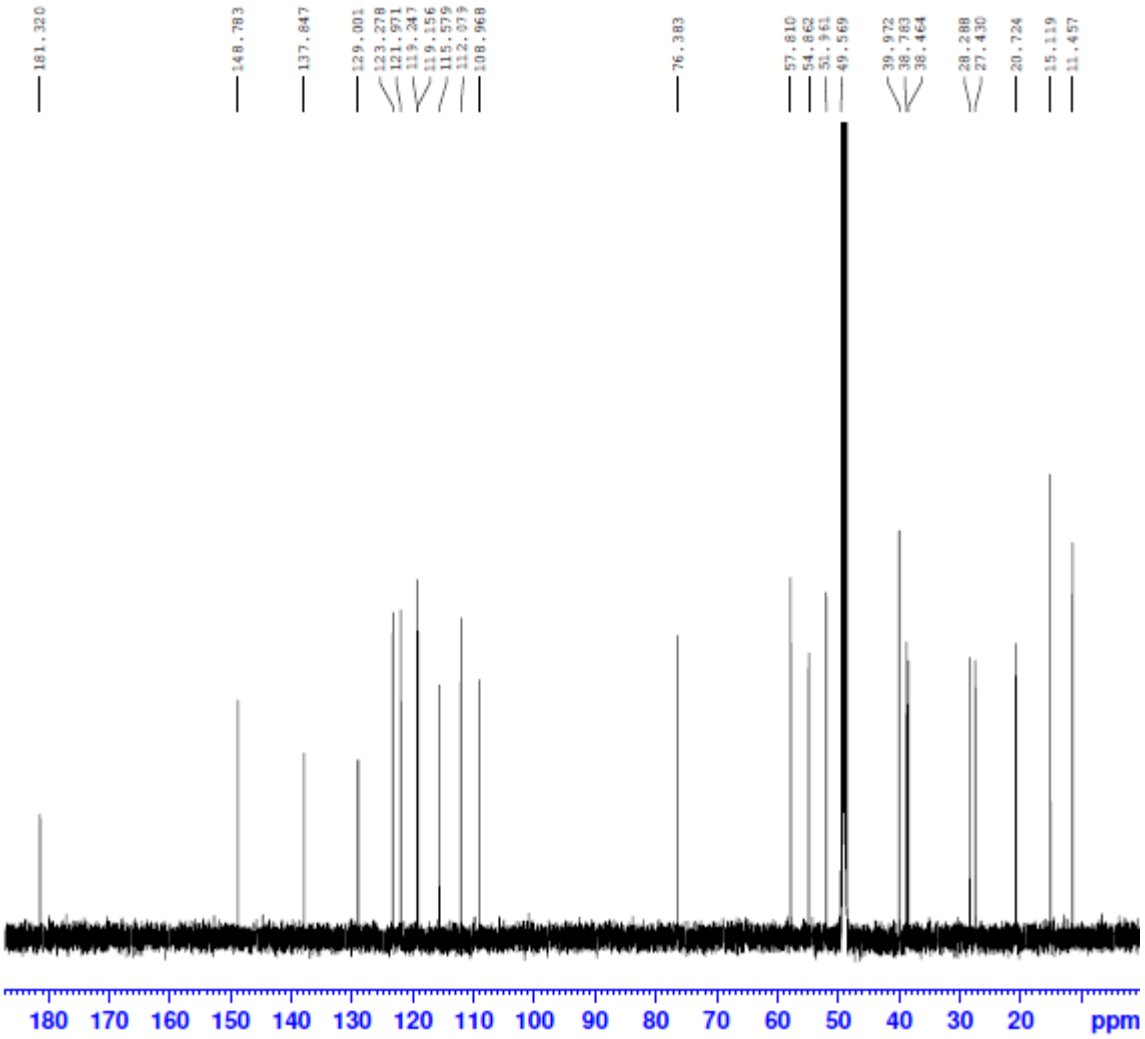

Figure S9. ^{13}C NMR spectrum (150 MHz, MeOD) spectrum of compound **2**.

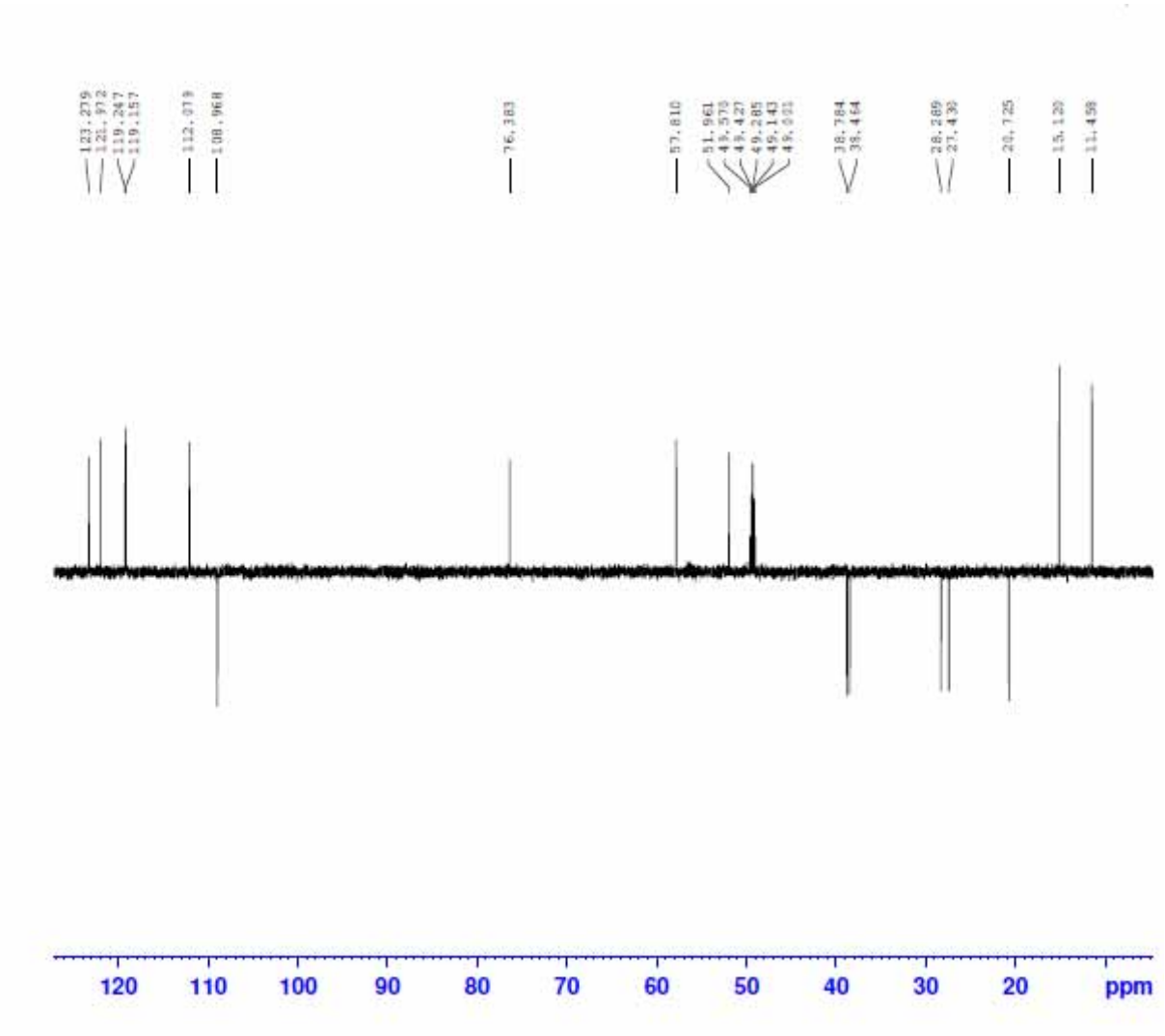

Figure S10. DEPT spectrum (150 MHz, MeOD) of **2**.

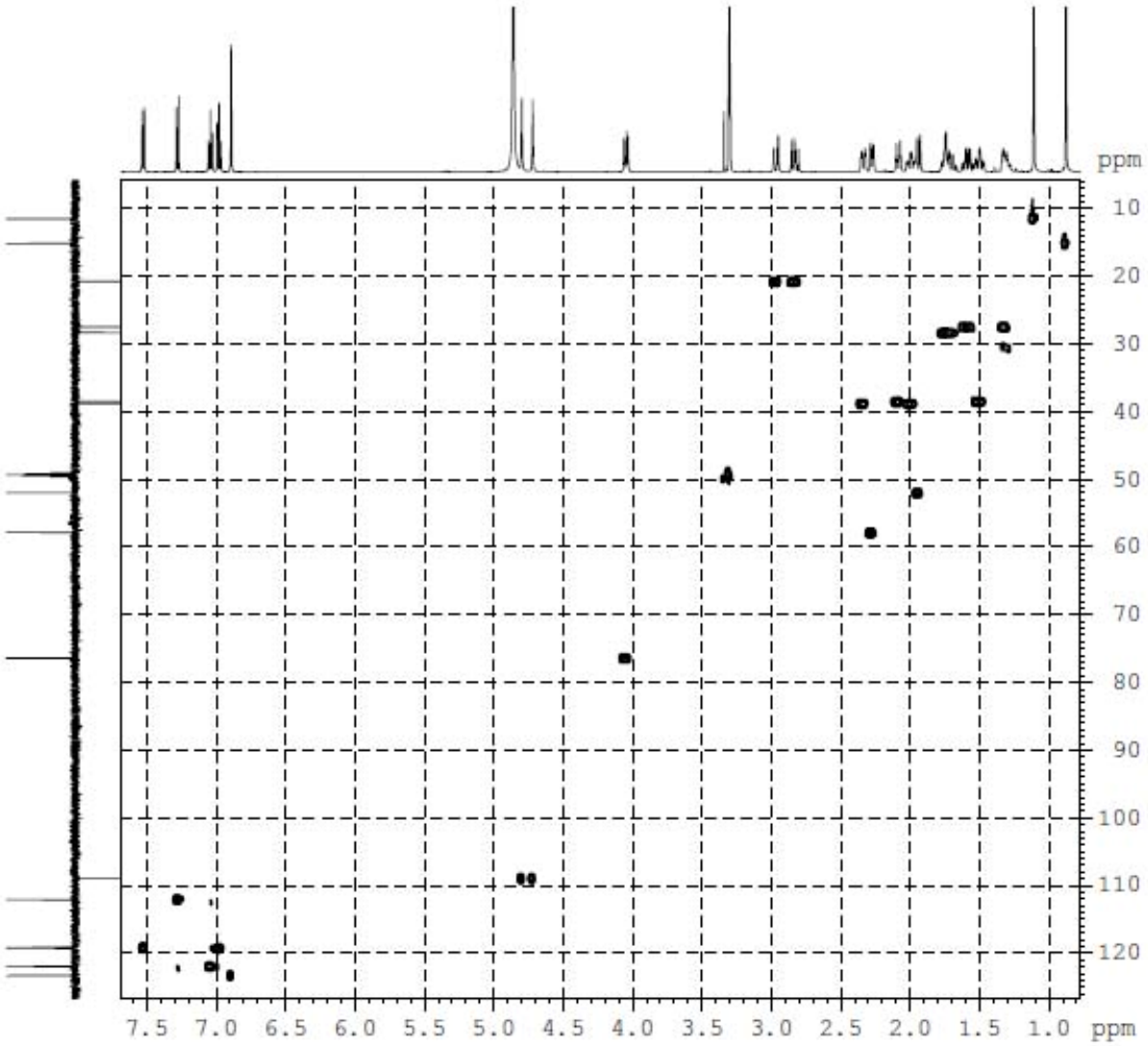

Figure S11. HMQC spectrum (125 MHz, MeOD) of **2**.

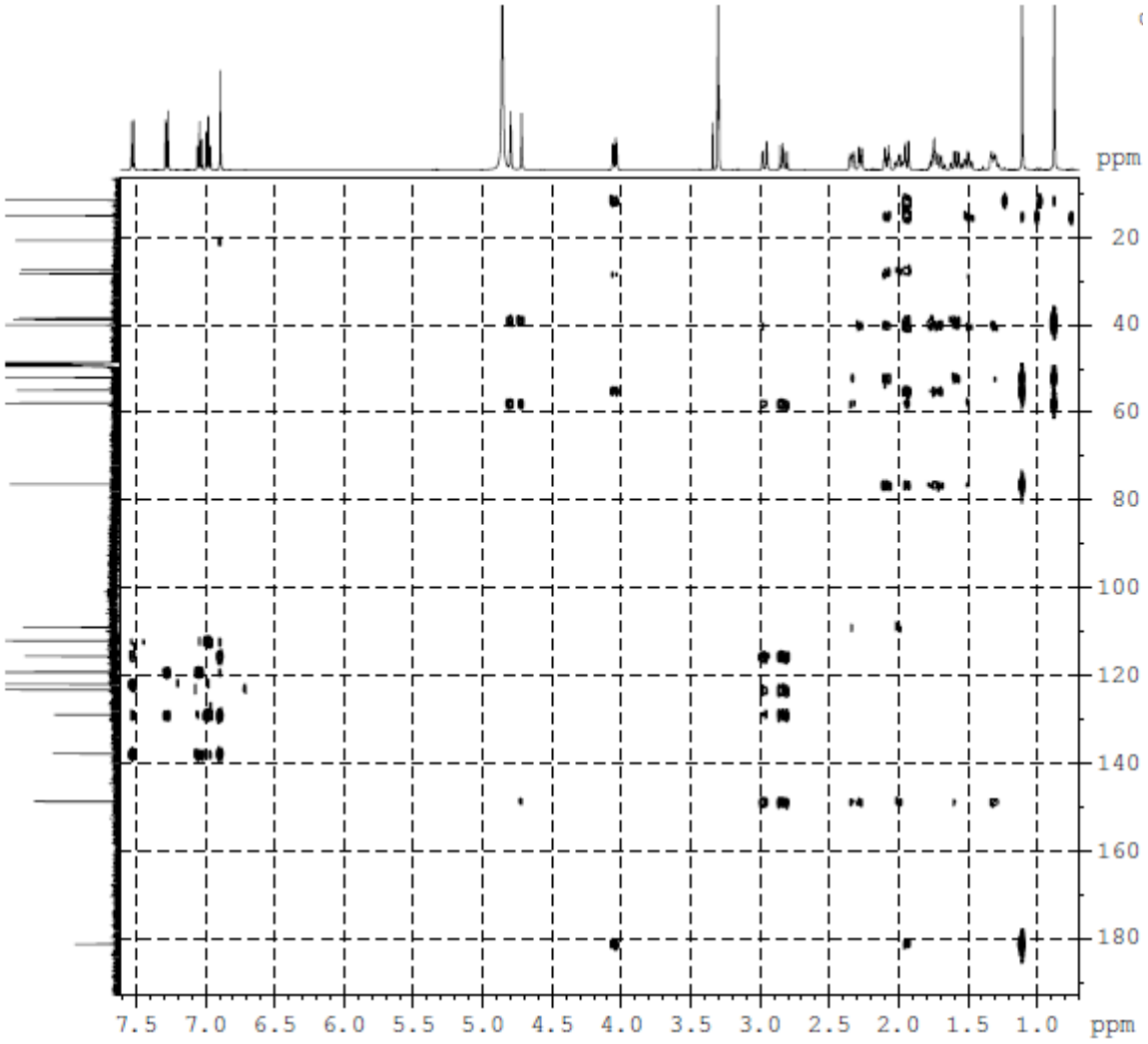

Figure S12. HMBC spectrum (125 MHz, MeOD) of **2**.

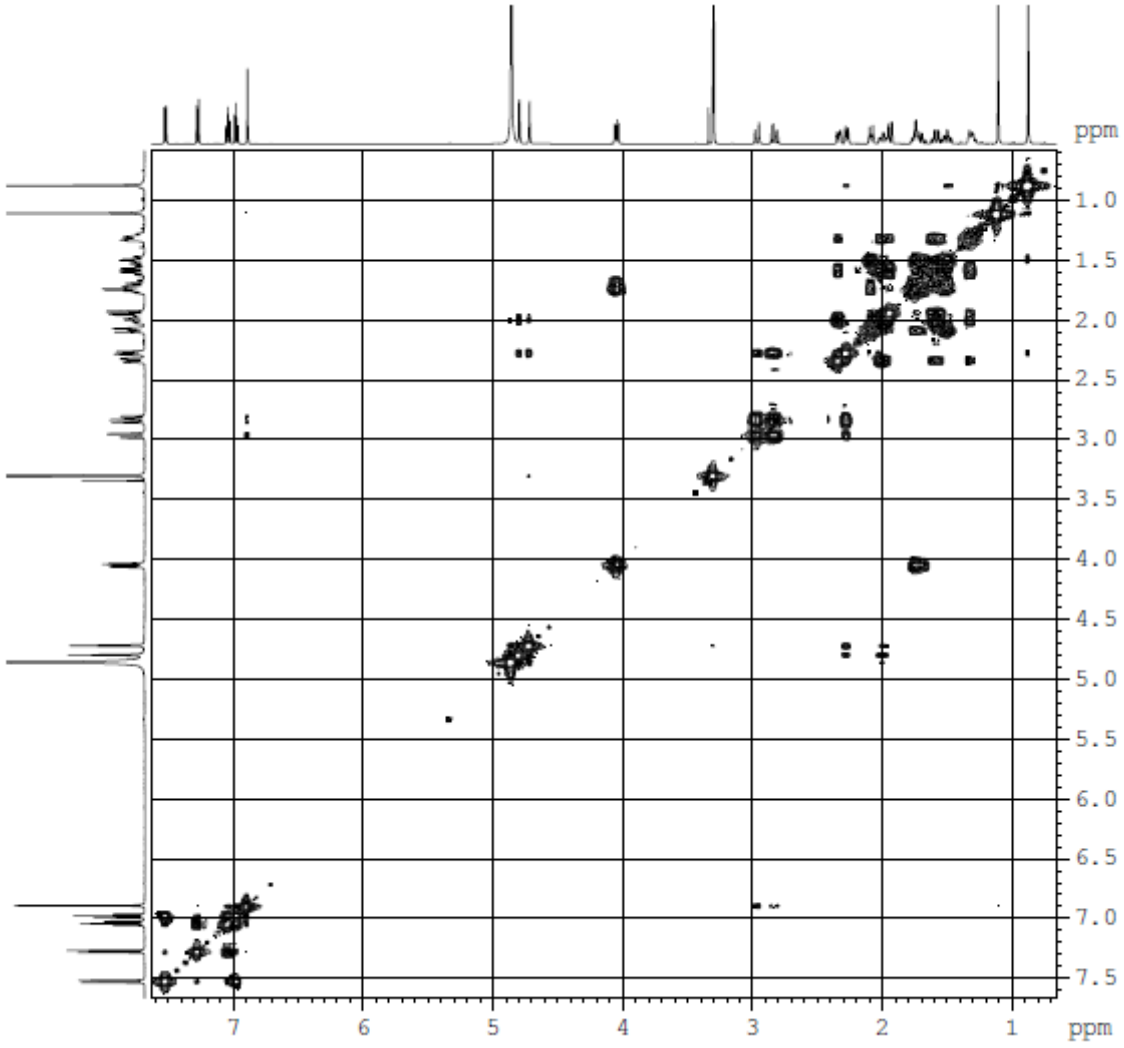

Figure S13. H,H COSY spectrum (500 MHz, MeOD) of **2**.

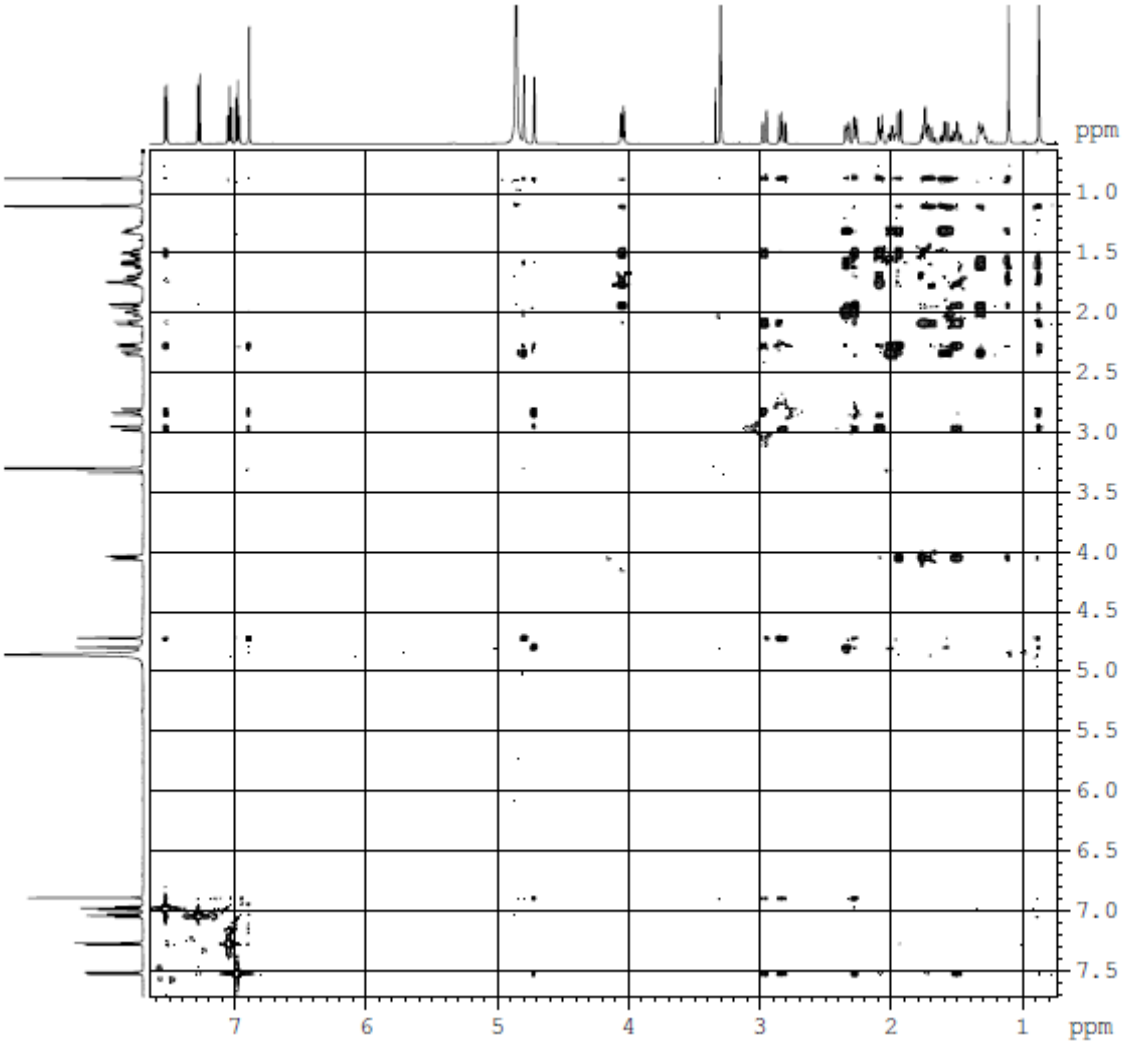

Figure S14. NOESY spectrum (500 MHz, MeOD) of **2**.

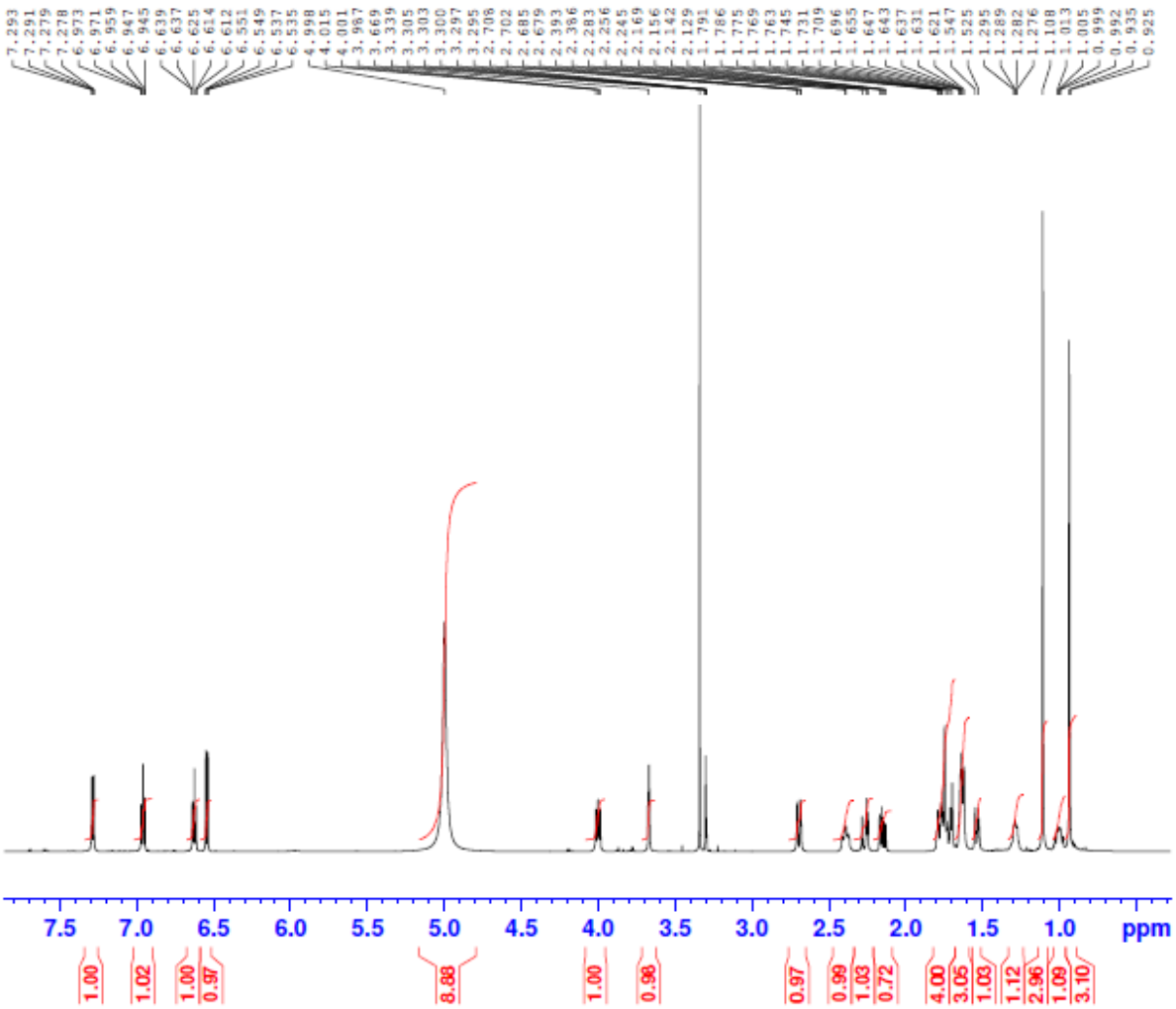

Figure S15. ^{1}H NMR spectrum (600 MHz, MeOD) of **3**.

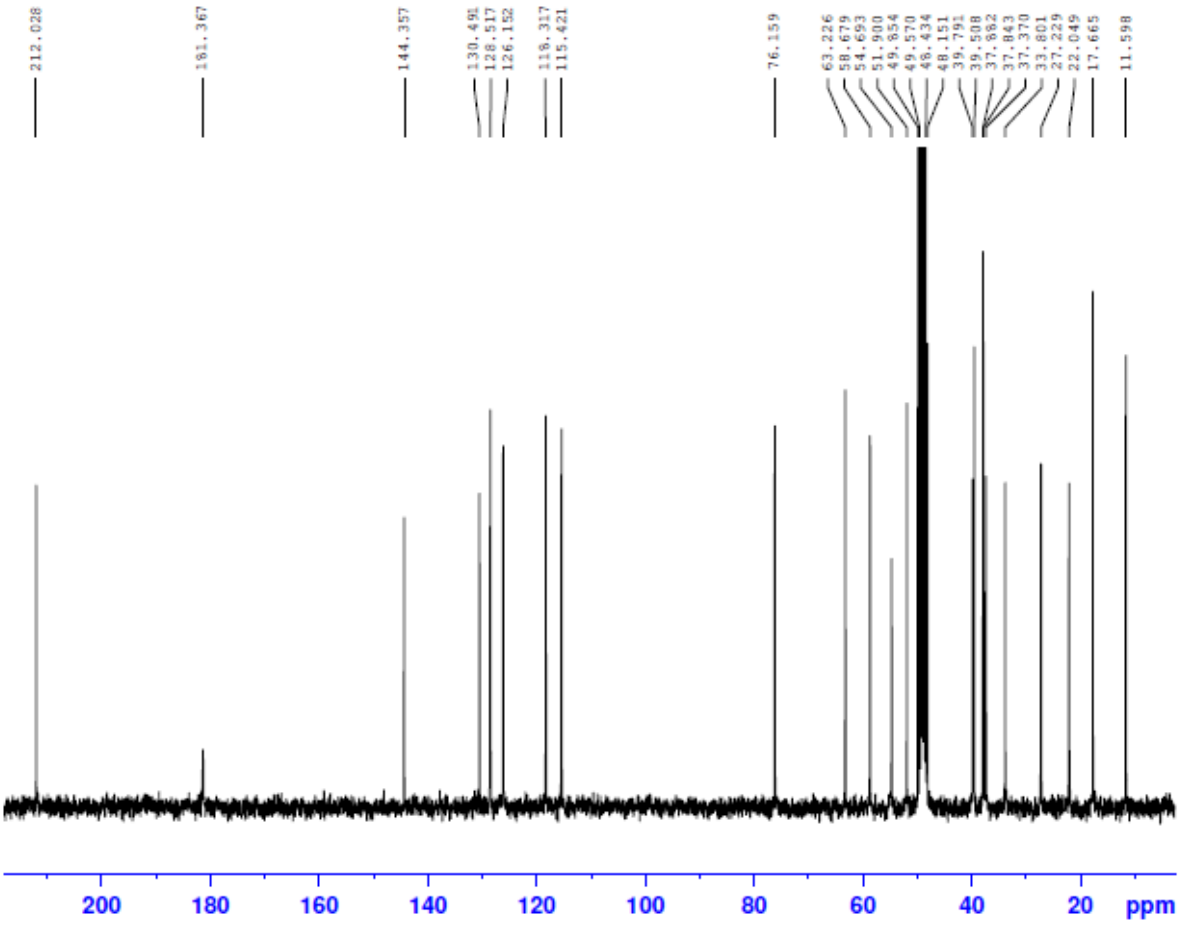

Figure S16. ^{13}C NMR spectrum (75 MHz, MeOD) of **3**.

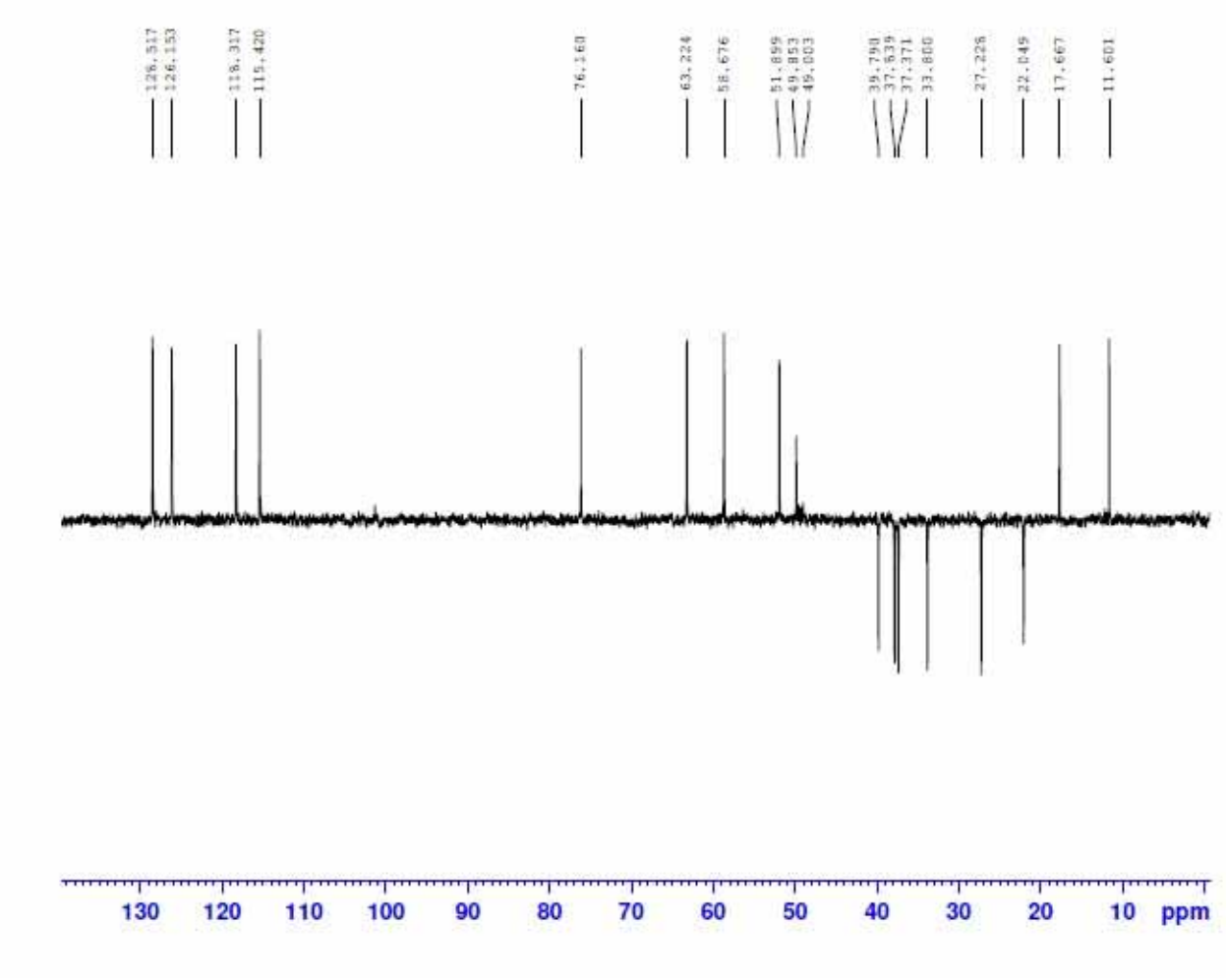

Figure S17. DEPT spectrum (75 MHz, MeOD) of **3**.

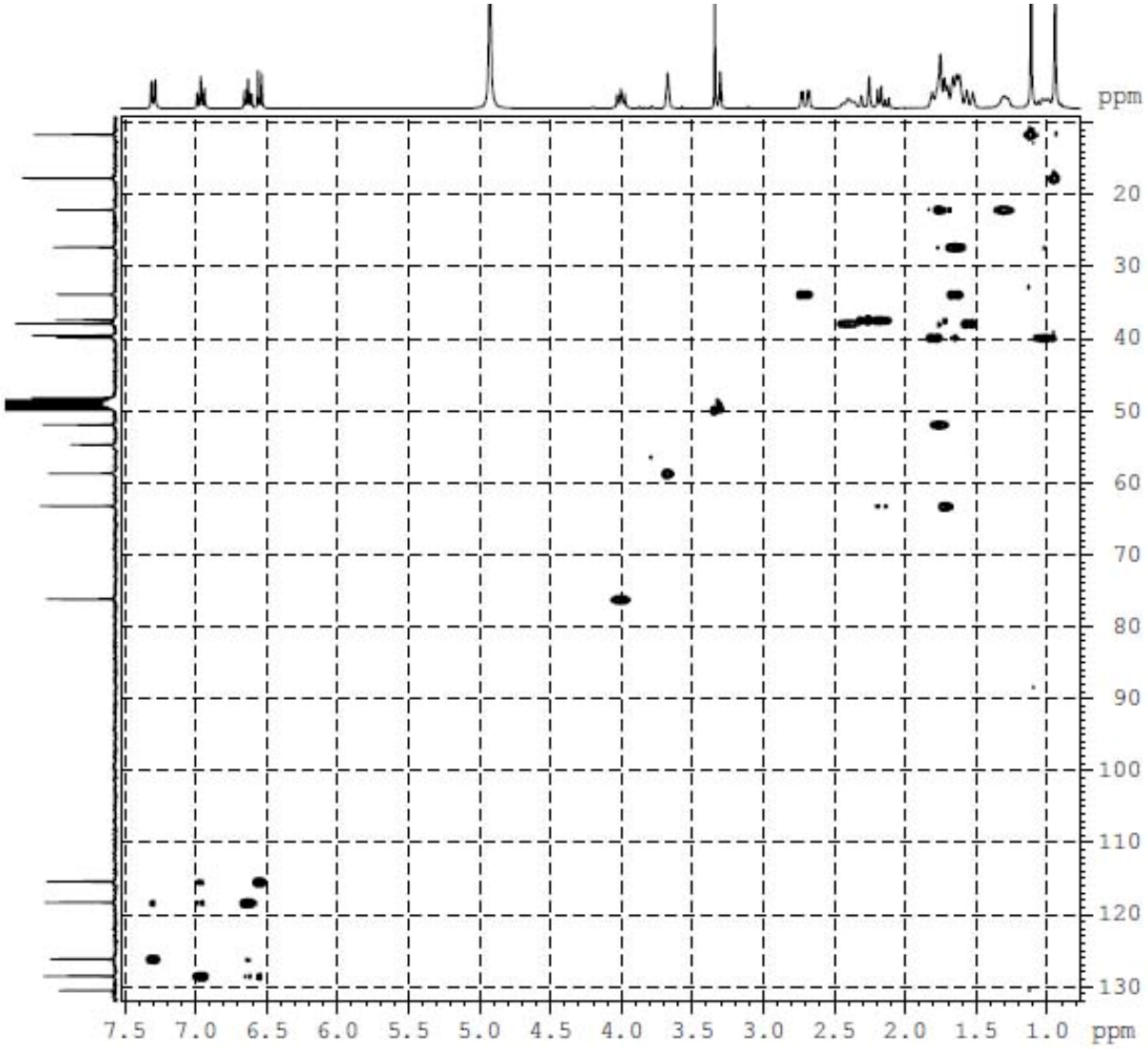

Figure S18. HMQC spectrum (75 MHz, MeOD) of **3**.

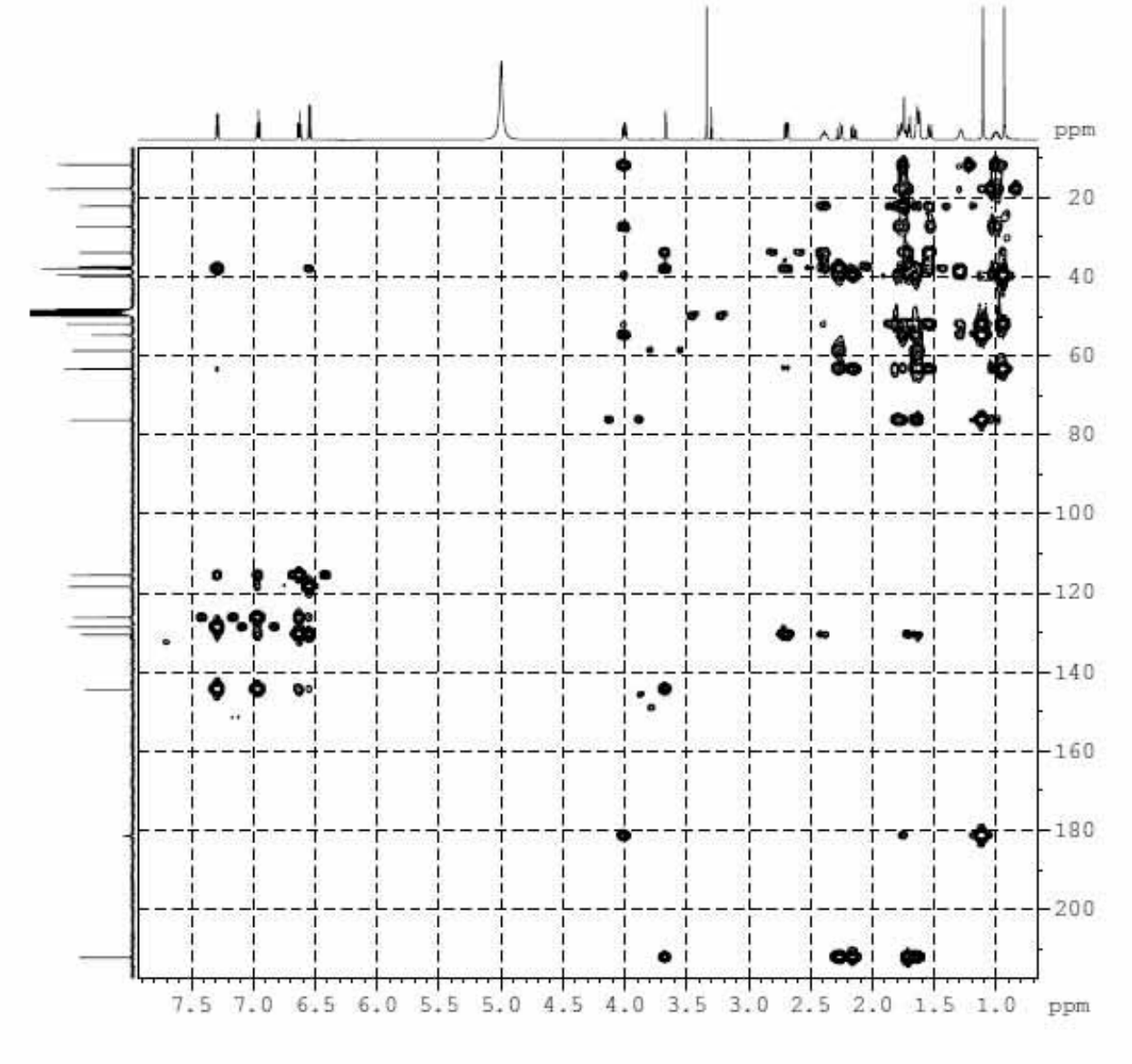

Figure S19. HMBC spectrum (150 MHz, MeOD) of **3**.

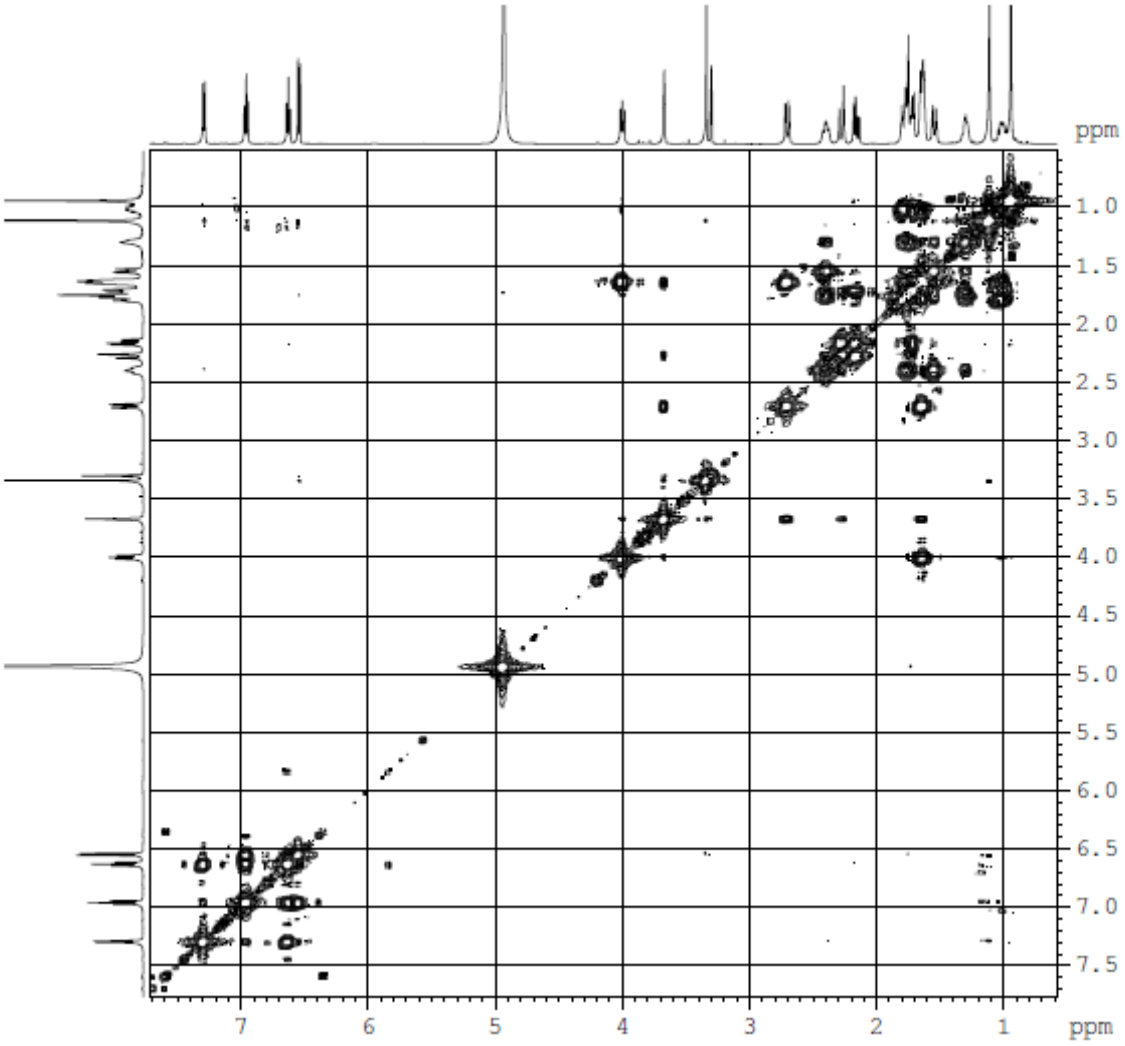

Figure S20. H,H COSY spectrum (500 MHz, MeOD) of **3**.

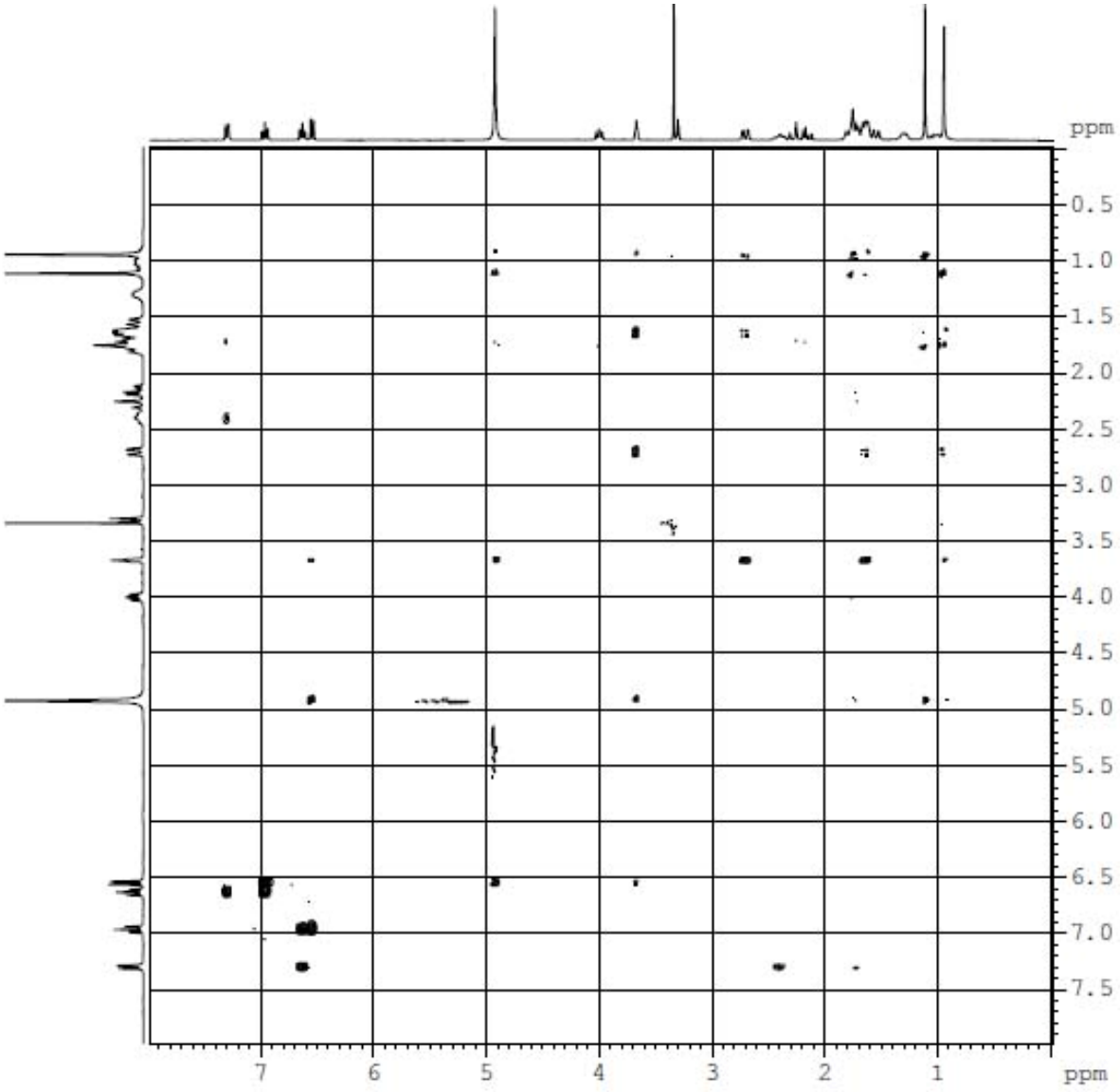

Figure S21. NOESY spectrum (300 MHz, MeOD) of **3**.