Triterpenoid saponins and other glycosides from the stems and bark of *Jaffrea xerocarpa* and their biological activity.

Dima Muhammad[a], Nathalie Lalun[b], Hélène Bobichon[b], Elisabeth Le Magrex Debar [c], Sophie C. Gangloff [c], Mohammed Nour[d] and Laurence Voutquenne-Nazabadioko[a]*

[a] UMR CNRS 7312, Université de Reims Champagne-Ardenne, Bât. 18, Moulin de la Housse, BP 1039, 51687 Reims, Cedex 2, France

[b] CNRS FRE 3481, Université de Reims Champagne Ardenne, 51 rue Cognacq-Jay, 51095 Reims cedex, France

[c] Laboratoire de Microbiologie, EA 4691, UFR de Pharmacie, 1 Rue du Maréchal Juin, 51096 Reims Cedex, France.

[d] Laboratoire Insulaire du Vivant et de l'Environnement (LIVE), EA 4243, Université de la Nouvelle-Calédonie, BP R4, 98851 Nouméa Cedex, Nouvelle-Calédonie

*Correspondance to: L. Voutquenne-Nazabadioko, UMR CNRS 7312, Université de Reims Champagne-Ardenne, Bât. 18, Moulin de la Housse, BP 1039 51687 Reims, Cedex 2, France. Tel.: +33-3-26-91-82-09; Email: laurence.nazabadioko@univ-reims.fr

Supporting Materials

3.6 DPPH radical scavenging assay

The radical scavenging activity of the EtOAc and hydromethanolic extracts of *J. xerocarpa* stems and bark was determined using the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. Briefly, a stock solution of DPPH was prepared at 158 µM in EtOH/H_2O (1:1, v/v). Each sample was dissolved in DMSO (200 µg/mL) and 5 µL were added to the DPPH stock solution (95 µL), in triplicate in 96-well plates. The DPPH$^{\bullet}$ absorbance in each reaction mixture was monitored at λ 515 nm. The EtOAc and hydromethanolic extracts were then tested at 200, 100, 50 and 10 µg/mL to calculate the concentration able to quench 50% of the reaction system (EC_{50}) at 30 min. The EtOH/H_2O (1:1, v/v) solution was used as a blank, the free DPPH solution was used as a negative control and ascorbic acid (5 µg/mL) was used as a positive control. Results were expressed as percentage decrease with respect to control values.

3.7 Tyrosinase inhibitory activity assay

The tyrosinase inhibitory activity of the EtOAc and hydromethanolic extracts of *J. xerocarpa* stems and bark and the four lupane derivatives (**1, 10-12**) was determined against fungi tyrosinase. The assay was performed according to a previously described method using L-DOPA as substrate (Muhammad *et al.*, 2015, 2016). Briefly, the tested compounds were dissolved in DMSO 10% and mixed (1:1) with Na-phosphate buffer (PBS, pH 6.8) to obtain a concentration of 4 mg/mL for the extracts or 1 mg/mL for the compounds. Tyrosinase (100 µL; 135 U/mL) was first pre-incubated with the tested compounds (100 µL) at 25 °C for 10 min, and then 100 µL of L-DOPA (0.5 mM, PBS pH 6.8) was added. The enzyme reaction was monitored by measuring the change in absorbance at λ 475 nm (at 25 °C) after 10 min incubation. These solutions were prepared in triplicate in 96-well plates. Kojic acid (1 mM) was used as positive control. The inhibitory percentage of tyrosinase was calculated as follows: % inhibition = {[(*A* - *B*) - (*C* - *D*)]/(*A* - *B*)} × 100 (*A*: OD at 475 nm without test substance; *B*: OD at 475 nm without test substance and tyrosinase; *C*: OD at 475 nm with test substance; *D*: OD at 475 nm with test substance, but without tyrosinase).

3.8 WST cytotoxicity assay

The cytotoxic activities of compounds **1-3**, **5-6**, **10-12, 22**, and **23** on KB cell lines (ATTC® CCL™-17) were determined by using a colorimetry method based on the cleavage of the WST-1 tetrazolium salt, and using DMEM F12 medium for cells culture (Muhammad *et al.*, 2015). The stock solutions of compounds (1 mg/mL) were prepared in DMSO. Sample dilutions were then performed in medium DMEM F12 (1, 2.5, 5, 7.5 or 10 µg/mL). After removal of pre-incubated culture medium, 200 µL of

DMEM F12 containing various concentrations of samples were added and further incubated for 48 h at 37 °C. Cell viability was determined by adding WST-1 tetrazolium salt and by measuring the absorbance at λ 450 nm after ≈1 h. Each assay was realized in triplicate in 96-well microplates. *α*-hederin was employed as a positive control, which exhibited an IC_{50} value of 5.5 µM under the above conditions (Chwalek *et al.*, 2006).

3.9. Disc diffusion antibacterial assay

Disk diffusion was used to screened antibacterial activity of compounds **1-2**, **5**, **10-12**, **22** and **23** against *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (CIP10907) as gram positive bacteria, and *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) as gram negative bacteria (Acebey-Castellon *et al.*, 2011). 50 µL (of the solution at 10 mg/mL in H_2O) were applied in a sterile atmosphere to 8 mm diameter paper disks corresponding to 500 µg/disk of each compounds**.** After evaporation of the solvent, paper disks were placed in Petri dished of 9 cm diameter containing nutrient Mueller-Hinton agar previously inoculated with 0.2 mL of suspension of bacteria (15 10^7 CFU/mL for *S aureus* and *E. faecalis*; 15 10^6 CFU/mL for *E coli* and *P. aeruginosa*). After 18 hours of incubation at 37°C, the inhibition zone for the active extract was measured (CLSI, 2005). The antimicrobial gentamicin was used as positive control and tested at 50 µg/disk.

Table S1: Antioxidant (DPPH) and anti-tyrosinase activities of *J. xerocarpa*

	antioxidant activity (%) at 200 µg/mL	**antioxidant activity EC_{50} (µg/mL)**	**Tyrosinase inhibitory activity (%)** at 4 mg/mL
AcOEt stem	18.5		5
AcOEt bark	22.6		6
MeOH stem	78.5	73	29.9
MeOH bark	82.5	73	49.6
1	nt	nt	36.5
10	nt	nt	28.4
11	nt	nt	44.8
12	nt	nt	36.6
Ascorbic acid	100		
Kojic acid (1mM)			69.3 ± 1.6

nt : not tested

Table S2: KB cells death (%) induced by compounds **1-3, 5-6, 10-12, 22** and **23** at 10 µg/mL and IC_{50} of compounds **1-2** and **11-12**

	cells death % at 10 µg/mL	**IC_{50} (µg/mL)**	**$IC_{50} \pm \sigma$ (µM)**
1	72.9	3.6	7.9±0.12
2	77.0	3.2	7.0±0.17
3	14.8		
5	9.2		
6	10.3		
10	30.8		
11	79.5	1.2	2.6±0.16
12	79.3	4.0	8.5±0.25
22	13.0		
23	2.5		
***α*-hederin**			5.5 ± 0.11

Table S3: Antimicrobial activities of compounds **1, 2, 5, 10-12, 22** and **23** by disc diffusion and broth diffusion methods

	Inhibition zone (Ø, mm)				**CMI (µg/mL)**	
Compounds (500 µg/disc)	*S. aureus*	*E. faecalis*	*E. coli*	*P. aeruginosa*	*S. aureus*	*E. faecalis*
1	10	12	-	-	4	4
2	-	-	-	-		
5	-	-	-	-		
10	-	-	-	-		
11	16	14	-	-	8	16
12	-	-	-	-		
22	-	-	-	-		
23	-	-	-	-		
Gentamicin	22	22	25	18	5	5

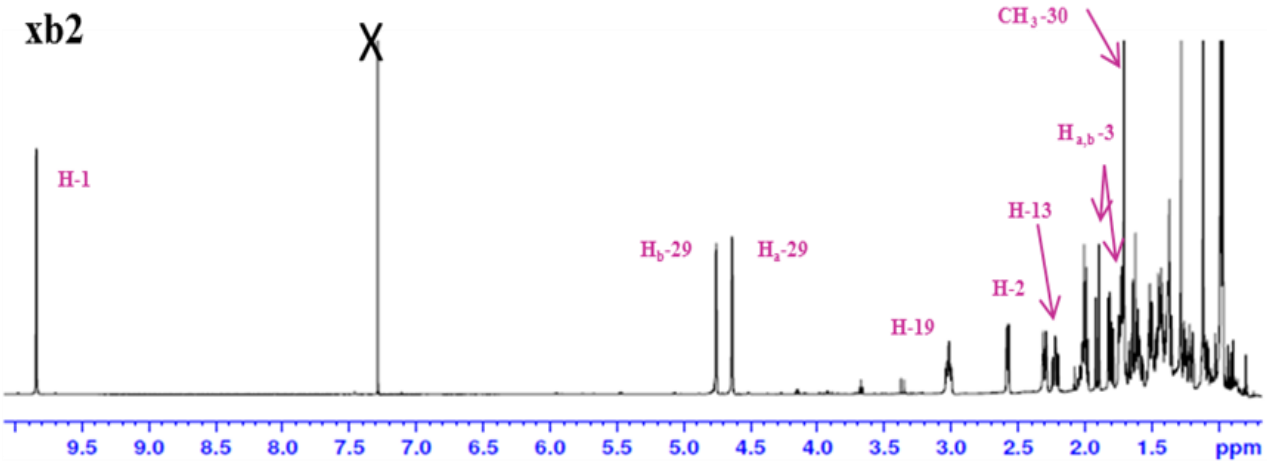

Figure S1: ^{1}H NMR spectrum of compound **1** ($CDCl_3$, 500 MHz)

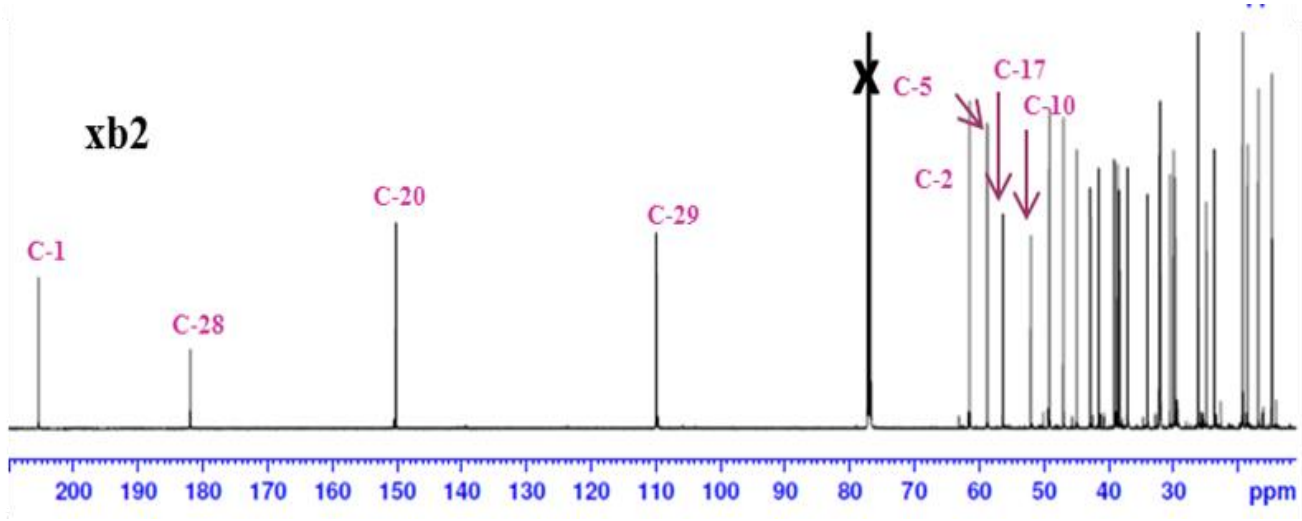

Figure S2: ^{13}C NMR spectrum of compound **1** ($CDCl_3$, 125 MHz)

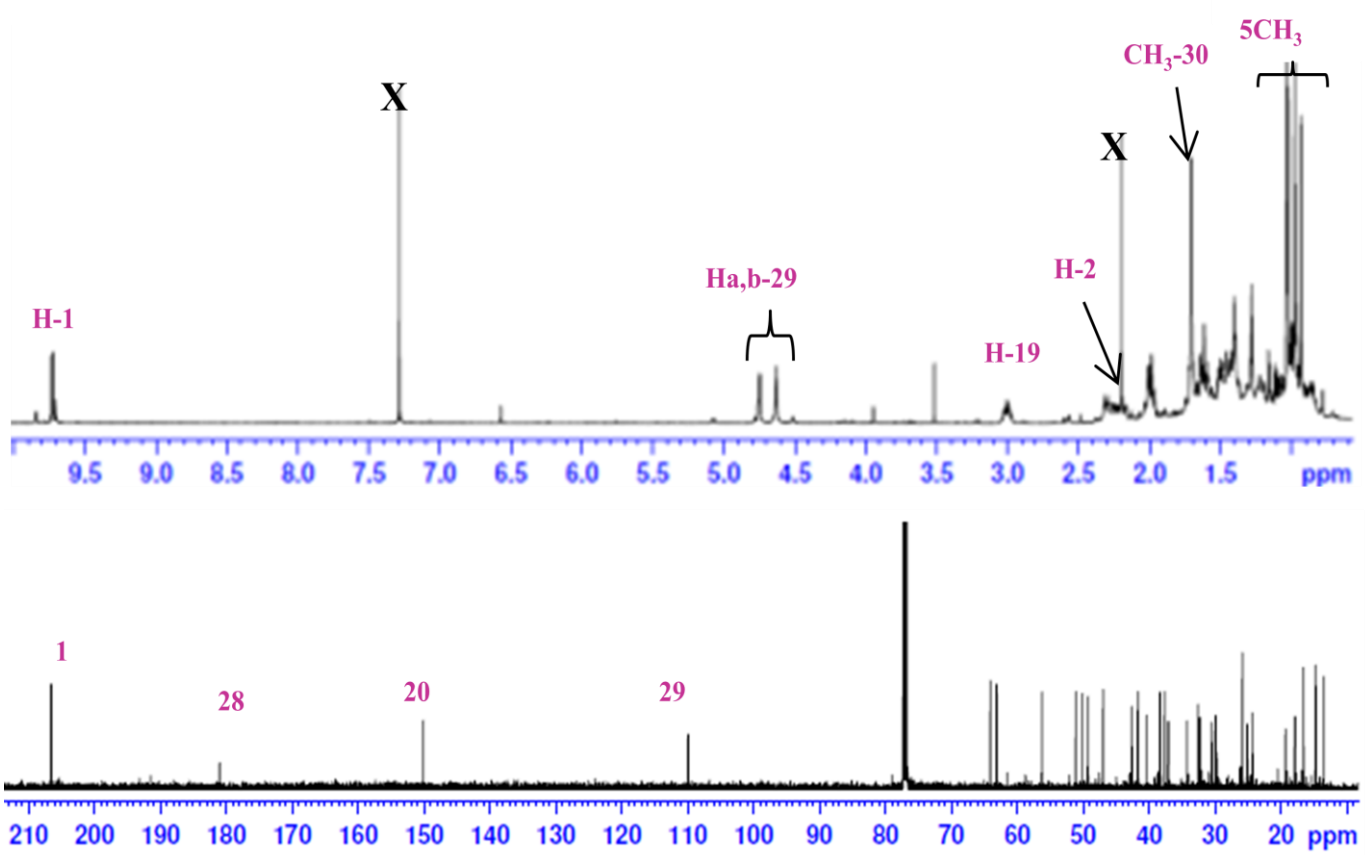

Figure S3 : ^{1}H NMR and ^{13}C NMR spectrum of compound **2** ($CDCl_3$, 600 MHz)

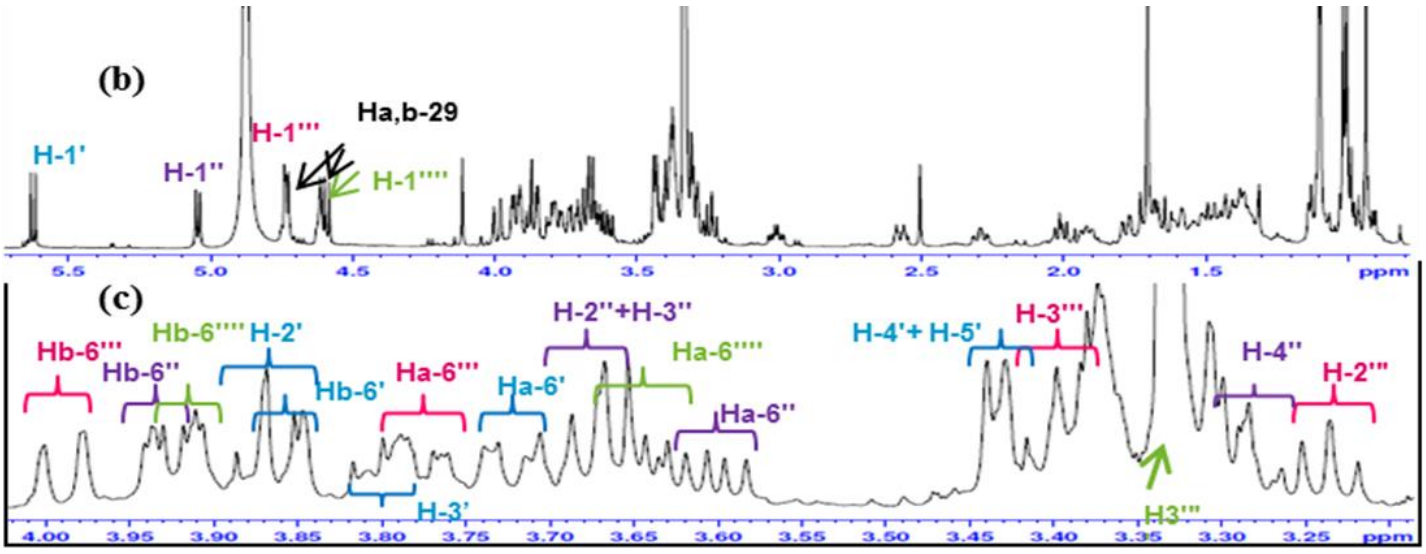

Figure S4: ^{1}H NMR spectrum of compound **3** (CD_3OD, 600 MHz)

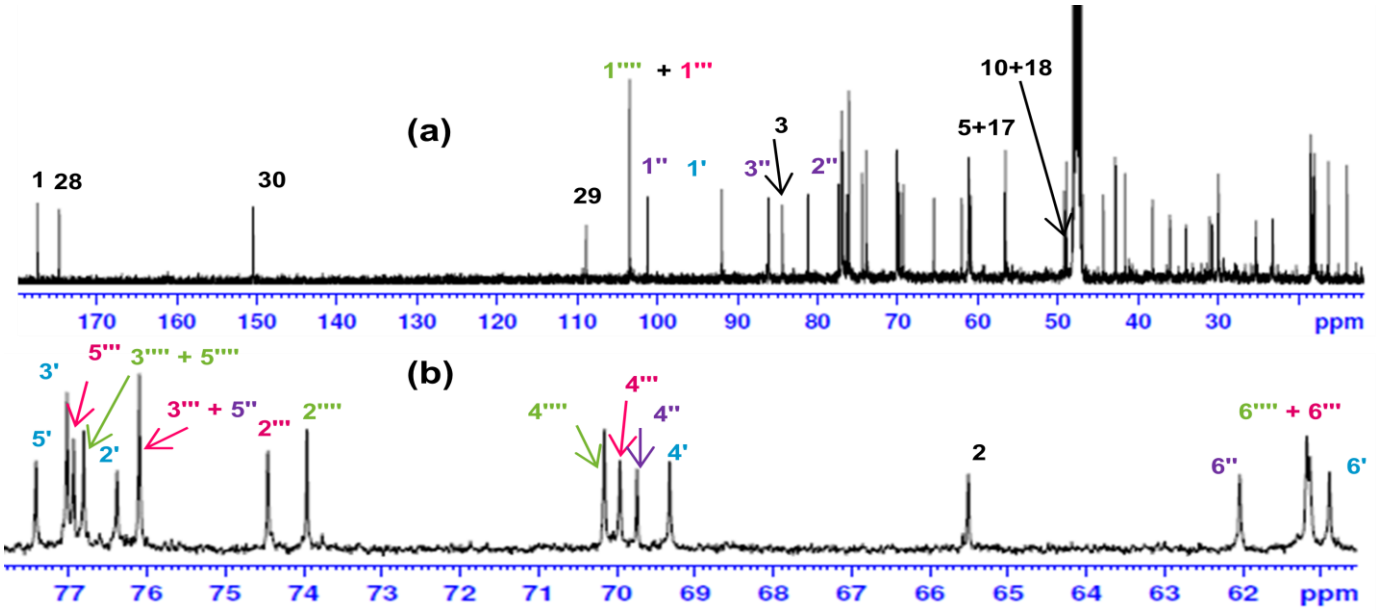

Figure S5: ^{13}C NMR spectrum of compound **3** (CD_3OD, 600 MHz)

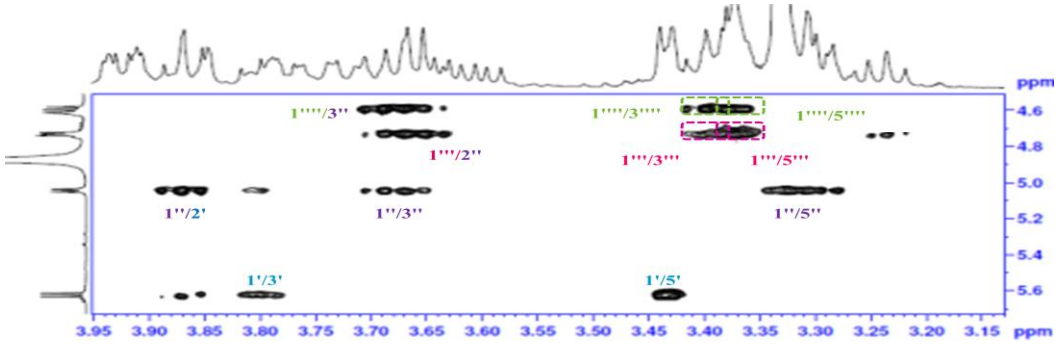

Figure S6: ROESY spectrum of the anomeric zone of compound **3** (CD_3OD)

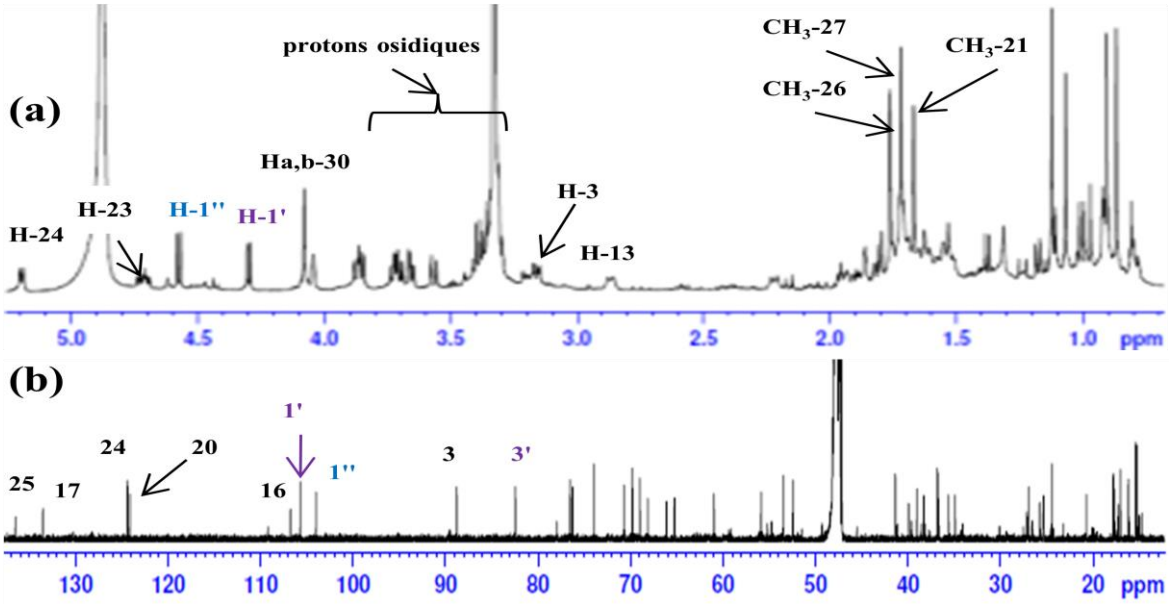

Figure S7: ^{1}H NMR (a) and ^{13}C NMR (b) spectra of compound **4** (CD_3OD, 600 MHz)

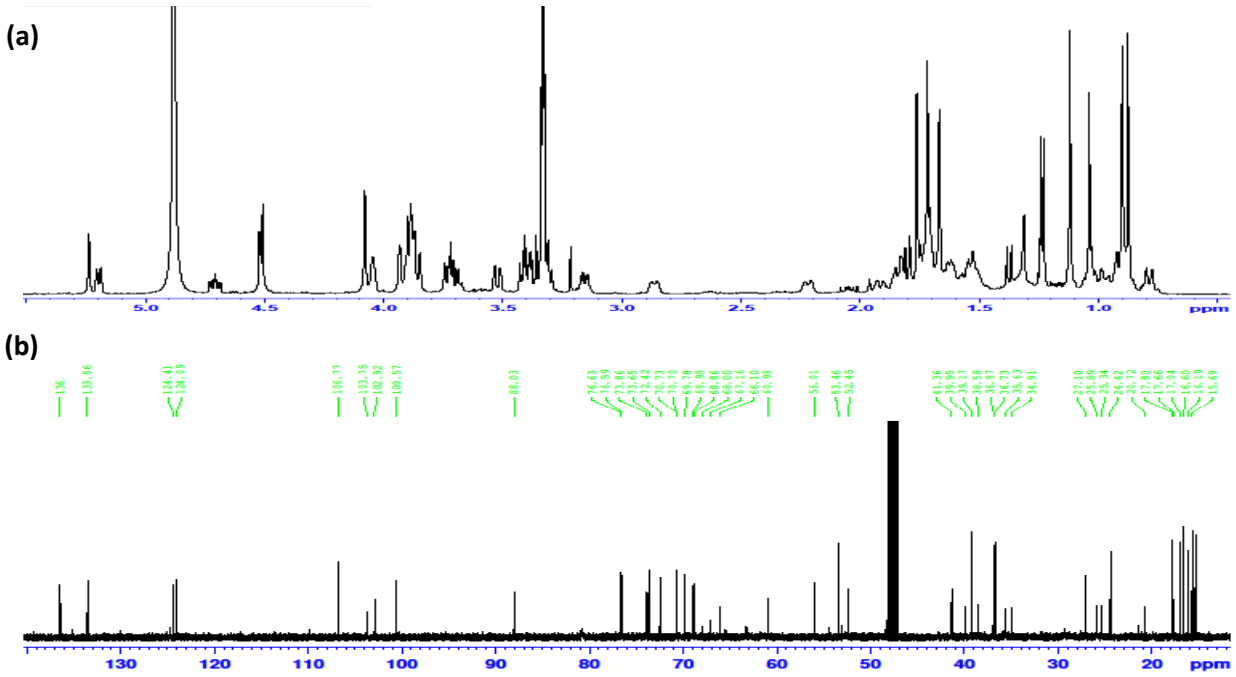

Figure S8: ^{1}H NMR **(a)** and ^{13}C NMR **(b)** spectra of compound **5** (CD_3OD, 600 MHz)

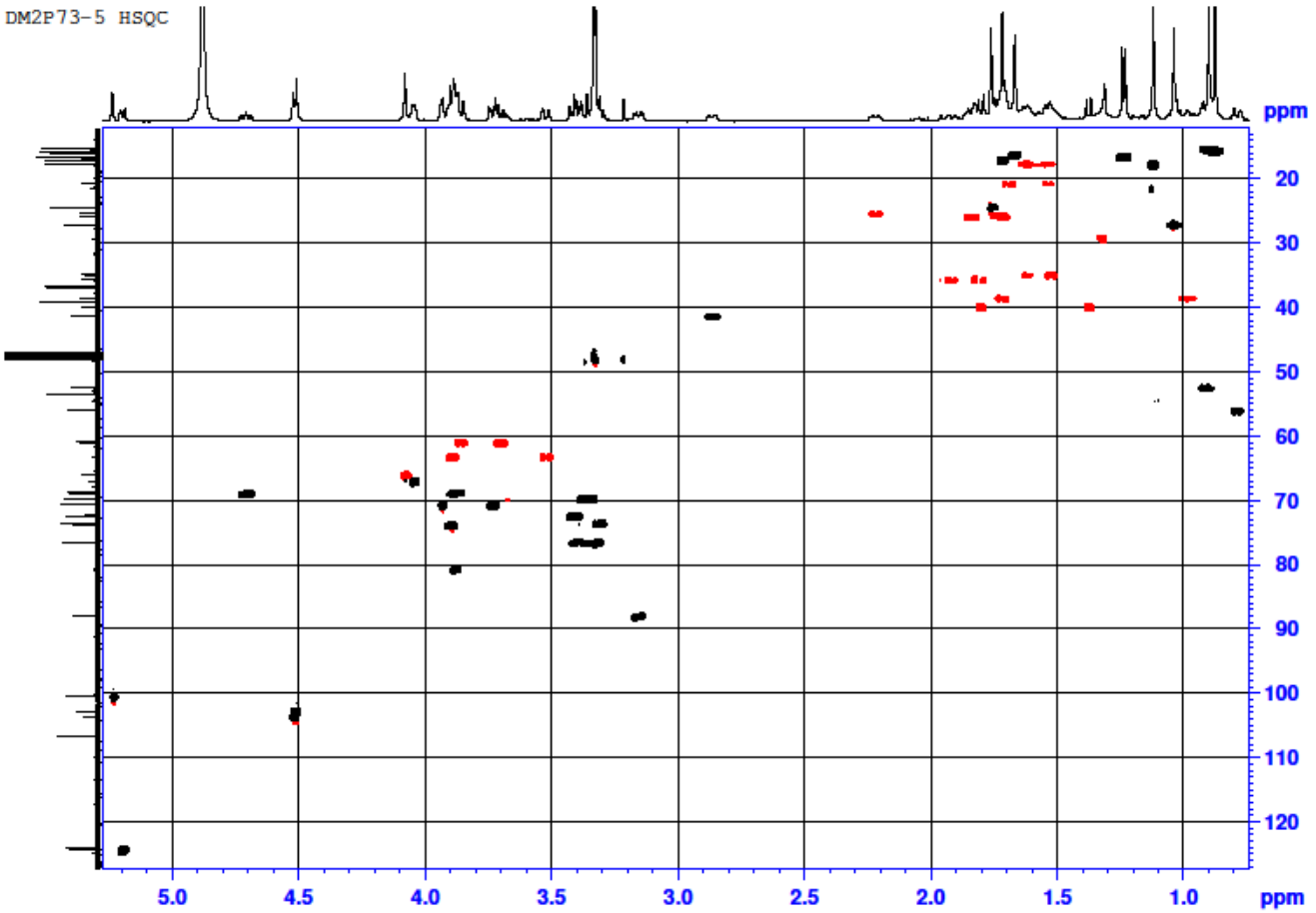

Figure S9: HSQC spectrum of compound **5** (CD_3OD)

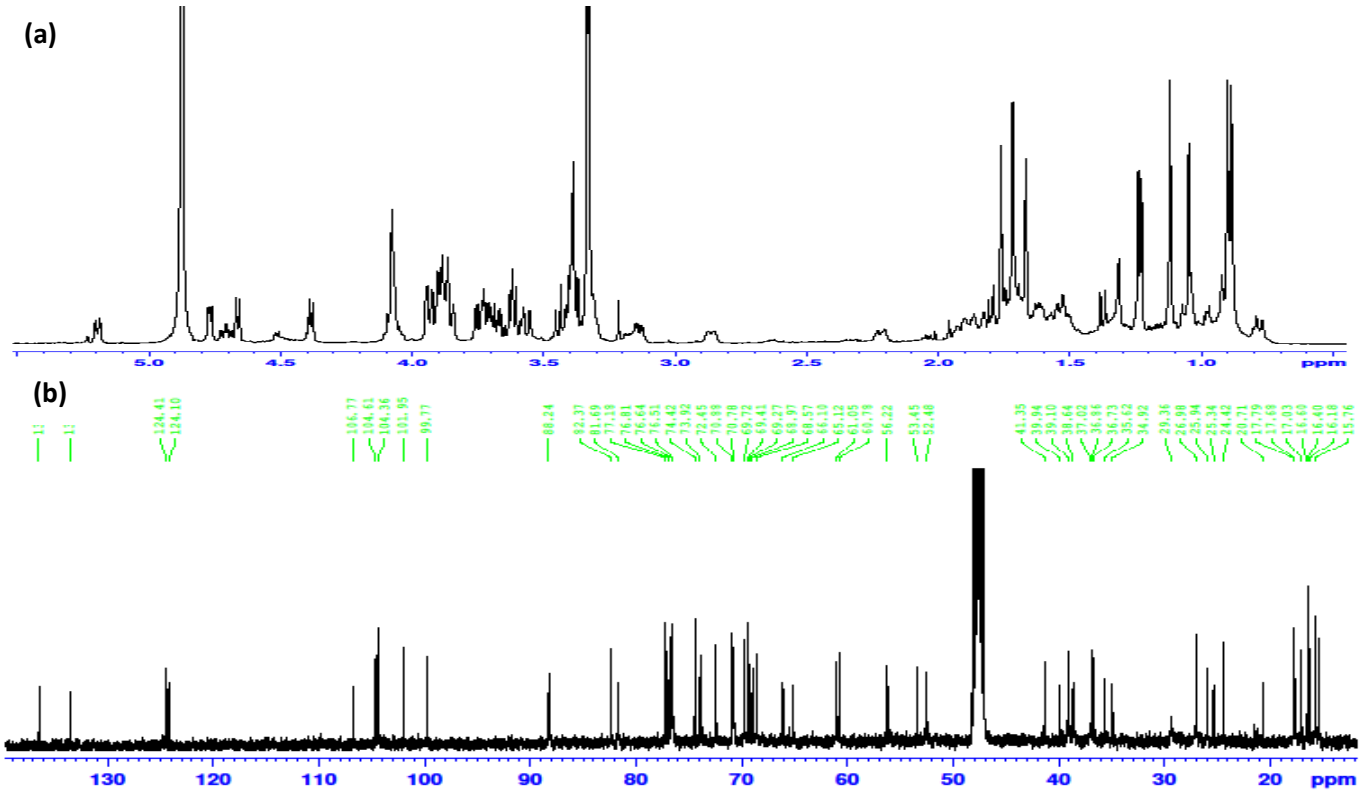

Figure S10: ^{1}H NMR **(a)** and ^{13}C NMR **(b)** spectra of compound **6** (CD_3OD, 600 MHz)

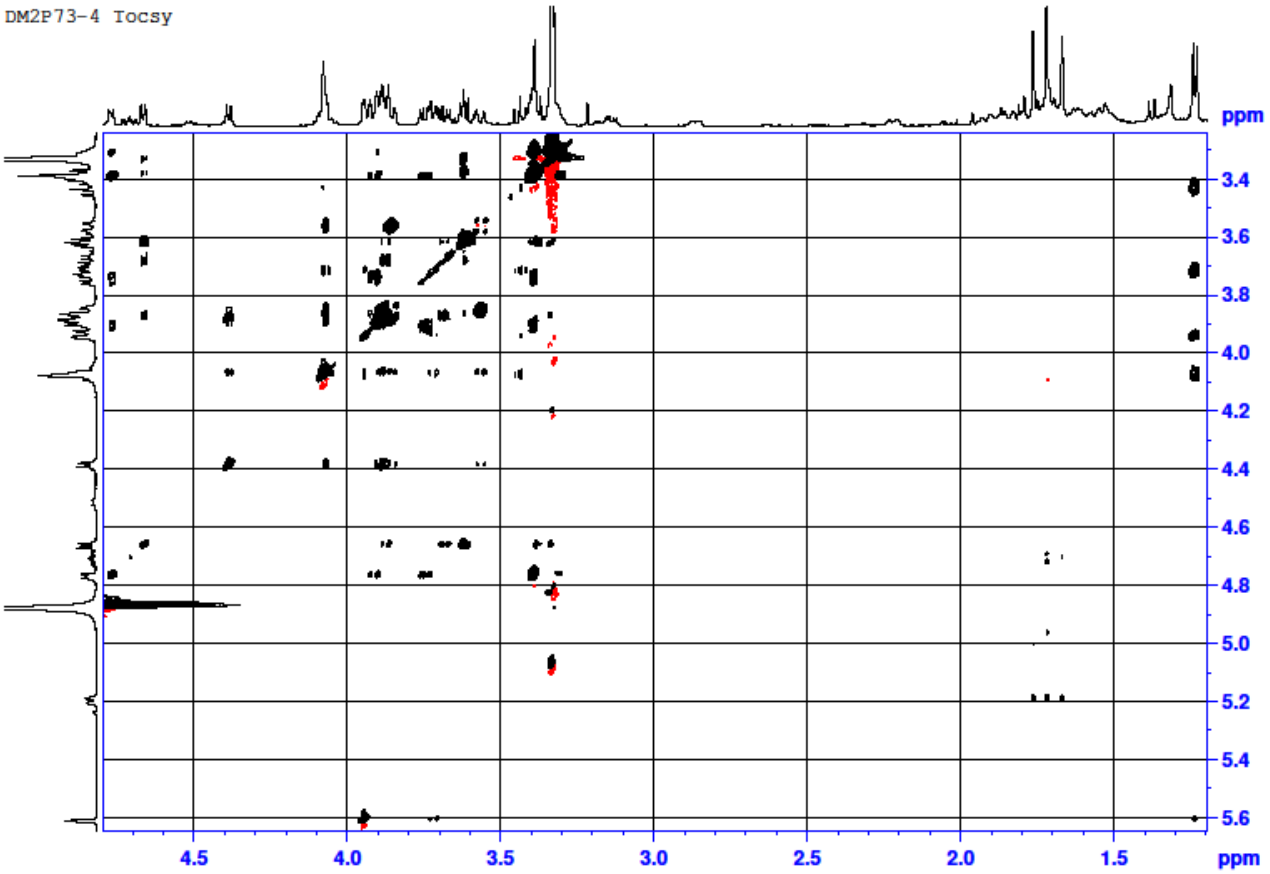

Figure S11: TOCSY spectrum of the osidic part of compound **6** (CD_3OD)

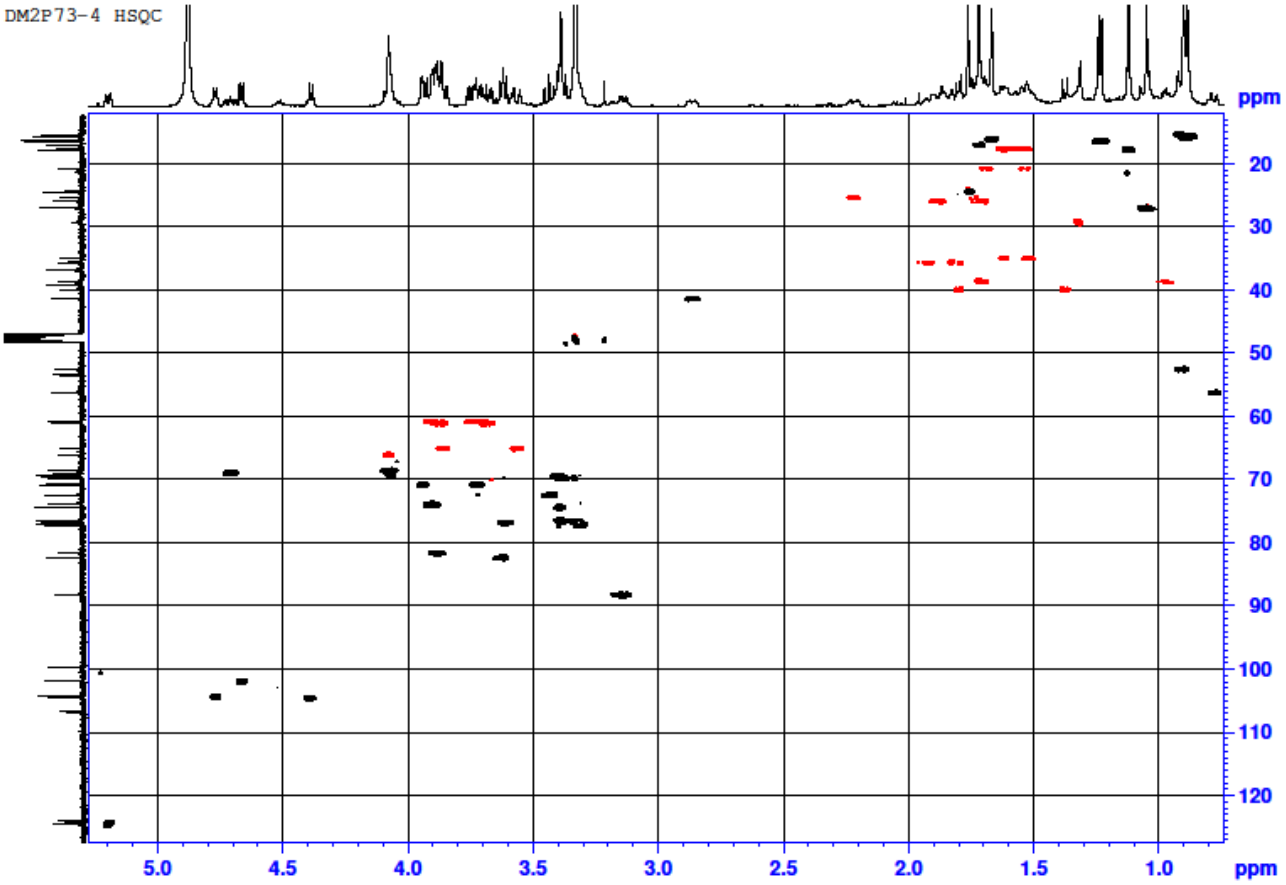

Figure S12: HSQC spectrum of compound **6** (CD_3OD)

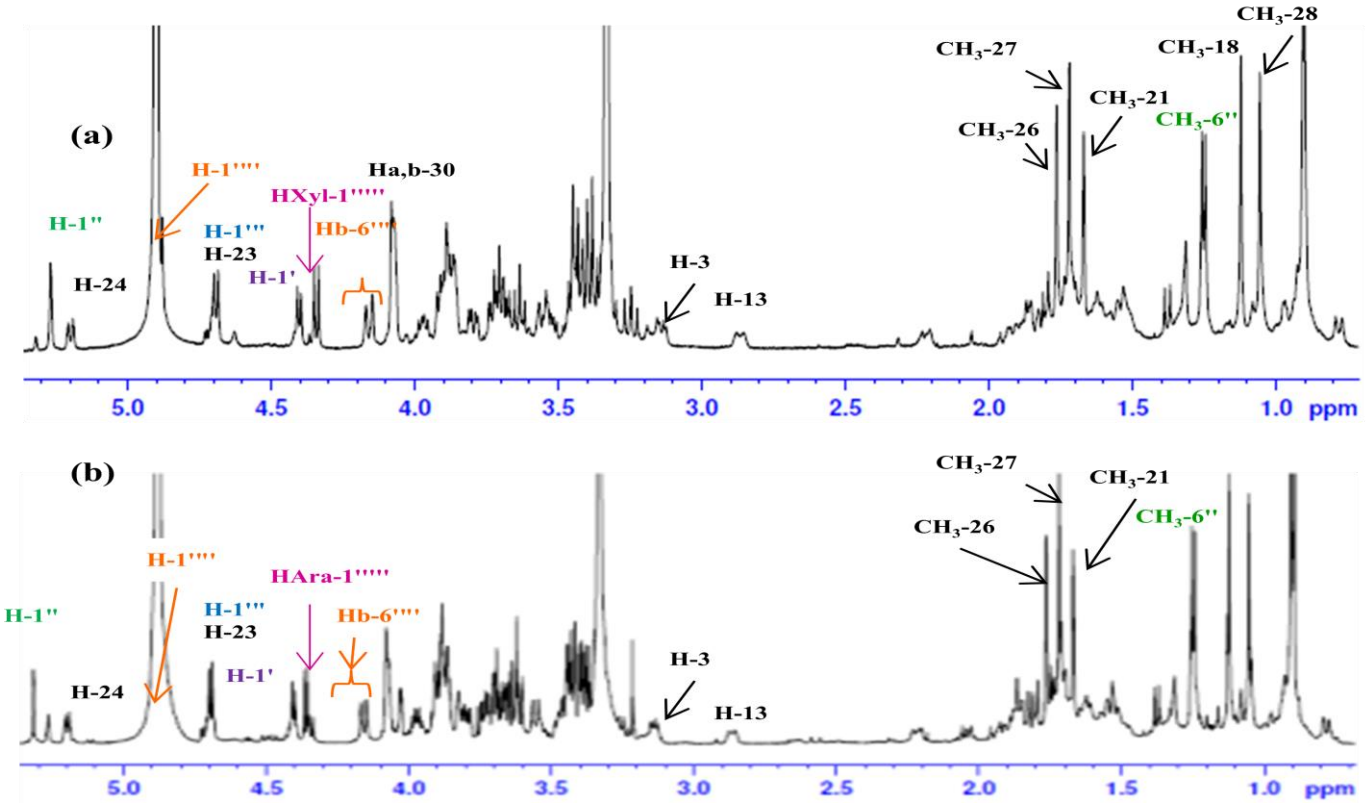

Figure S13: ^{1}H NMR spectra of compounds **7 (a)** and **8 (b)** (CD_3OD, 600 MHz)

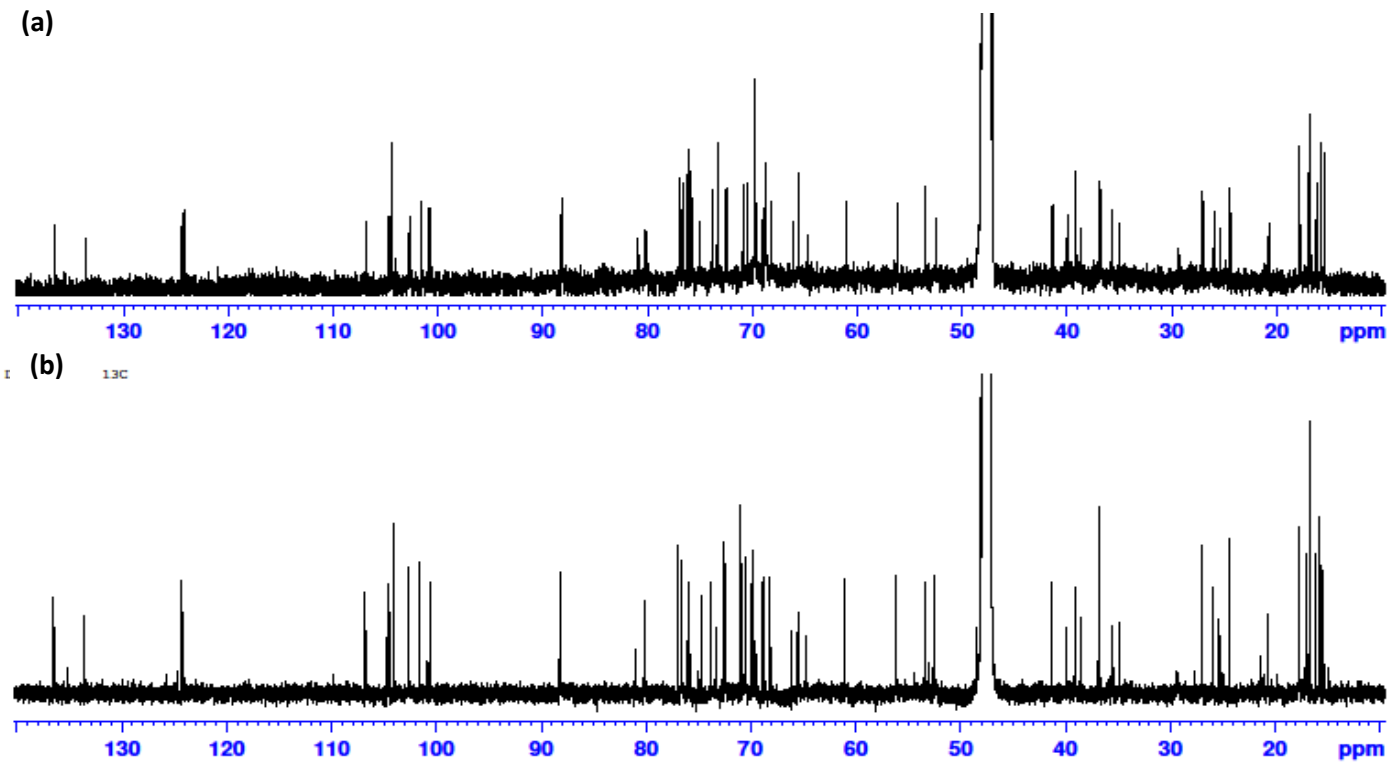

Figure S14: ^{13}C NMR spectra of compounds **7 (a)** and **8 (b)** (CD_3OD, 600 MHz)

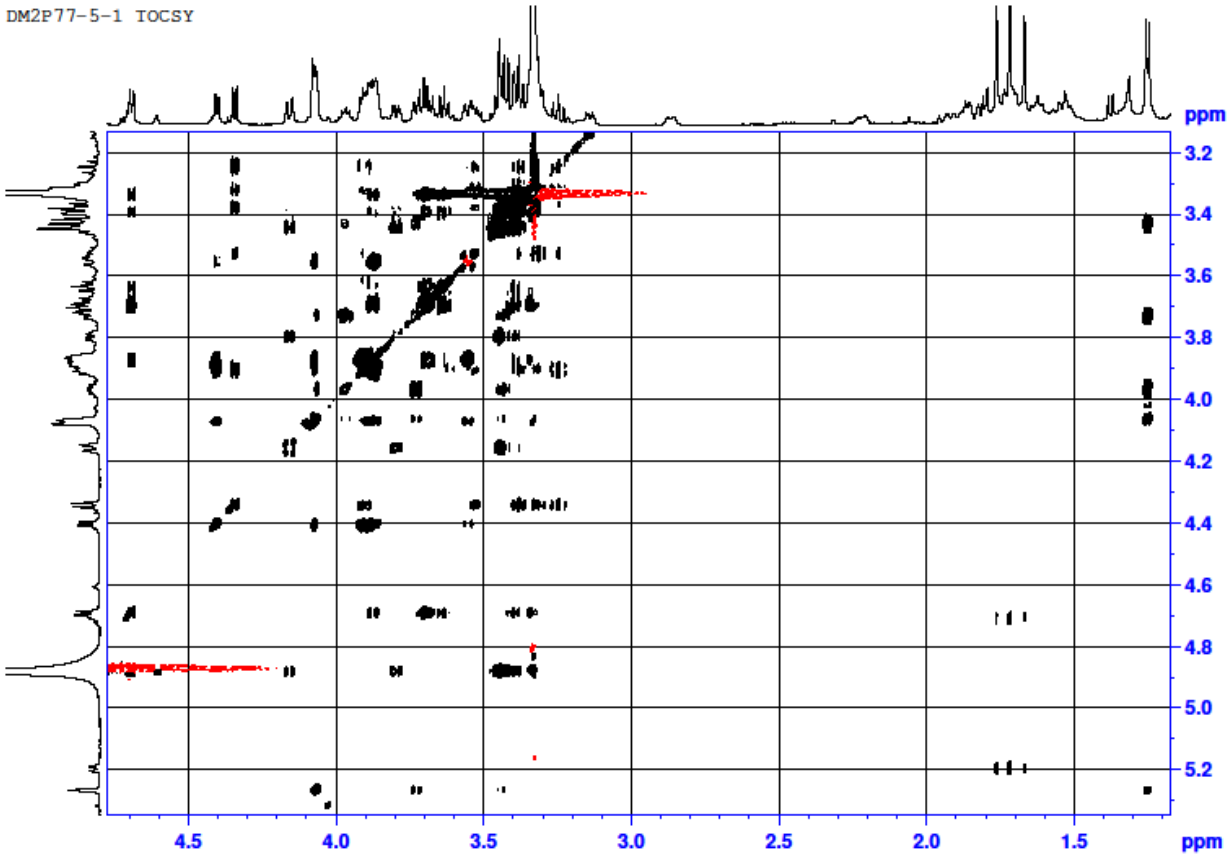

Figure S15: TOCSY spectrum of the osidic part of compound **7** (CD_3OD)

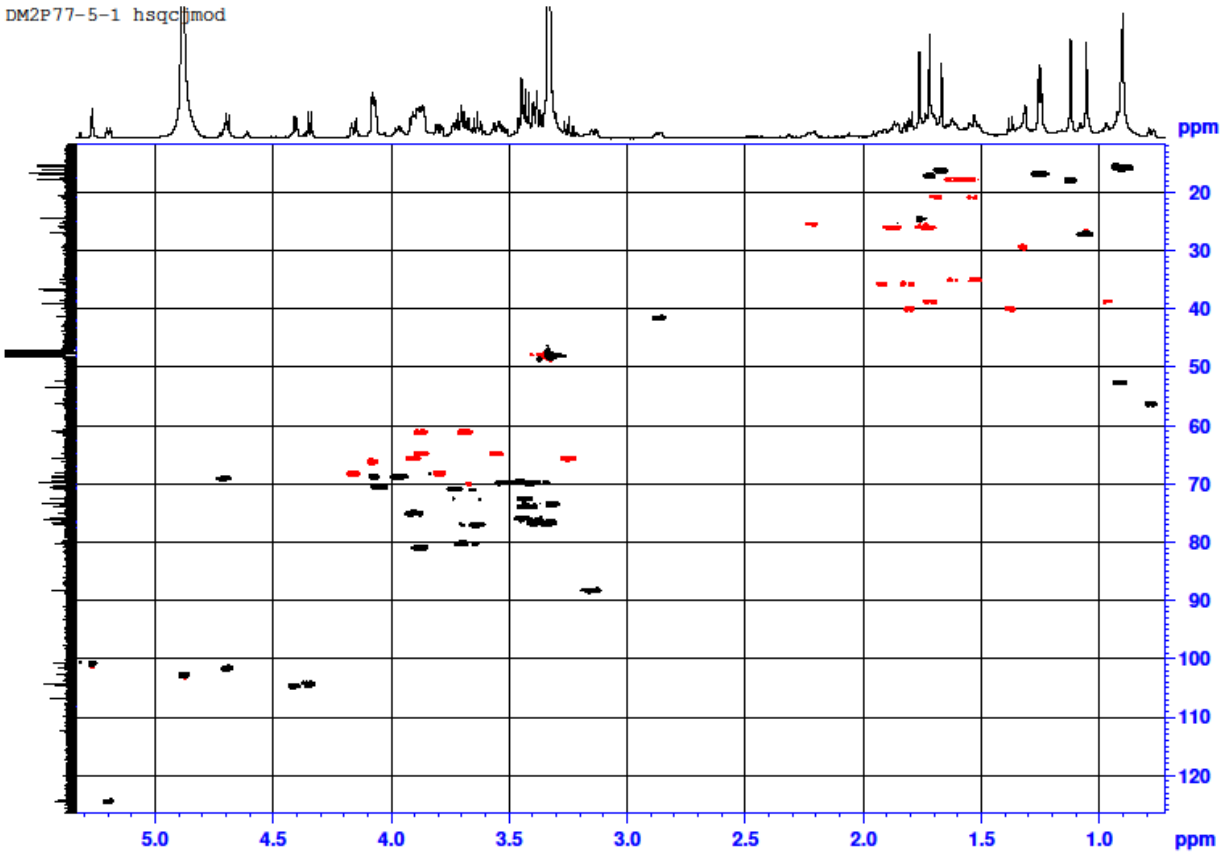

Figure S16: HSQC spectrum of compound **7** (CD_3OD)

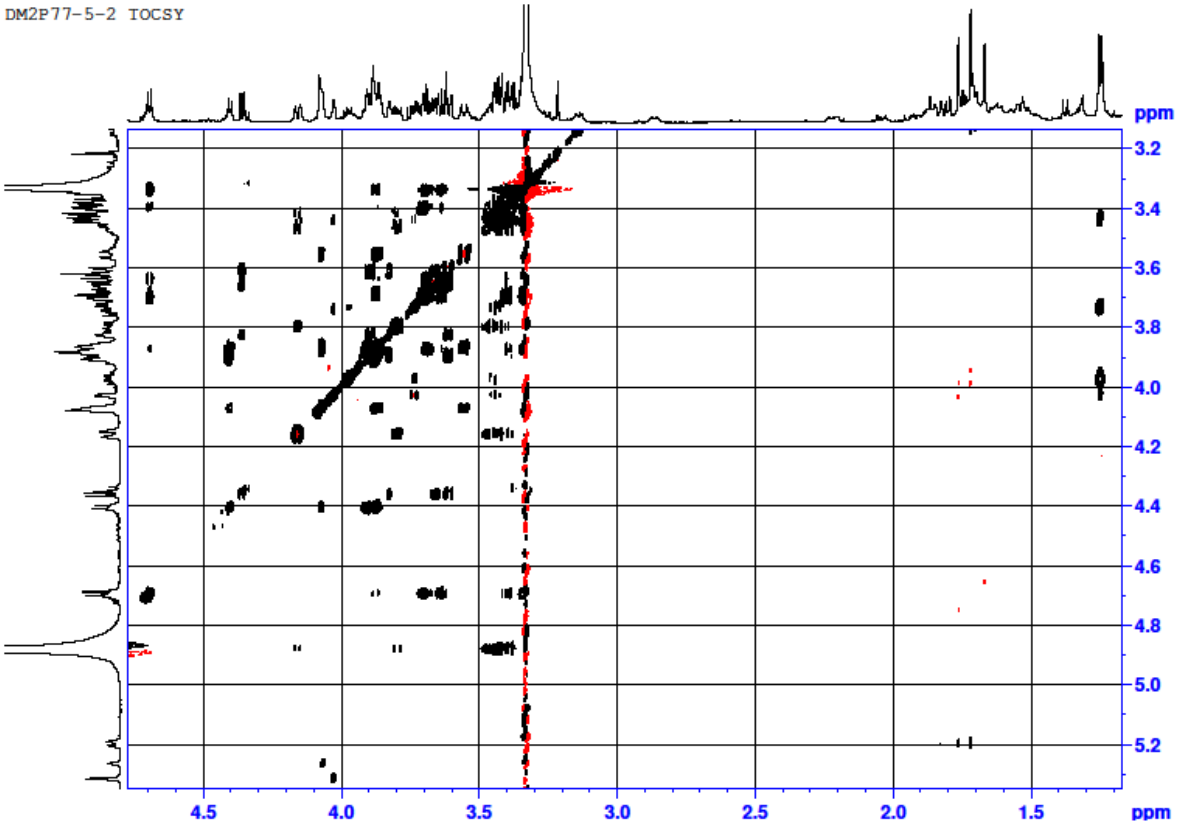

Figure S17: TOCSY spectrum of the osidic part of compound **8** (CD_3OD)

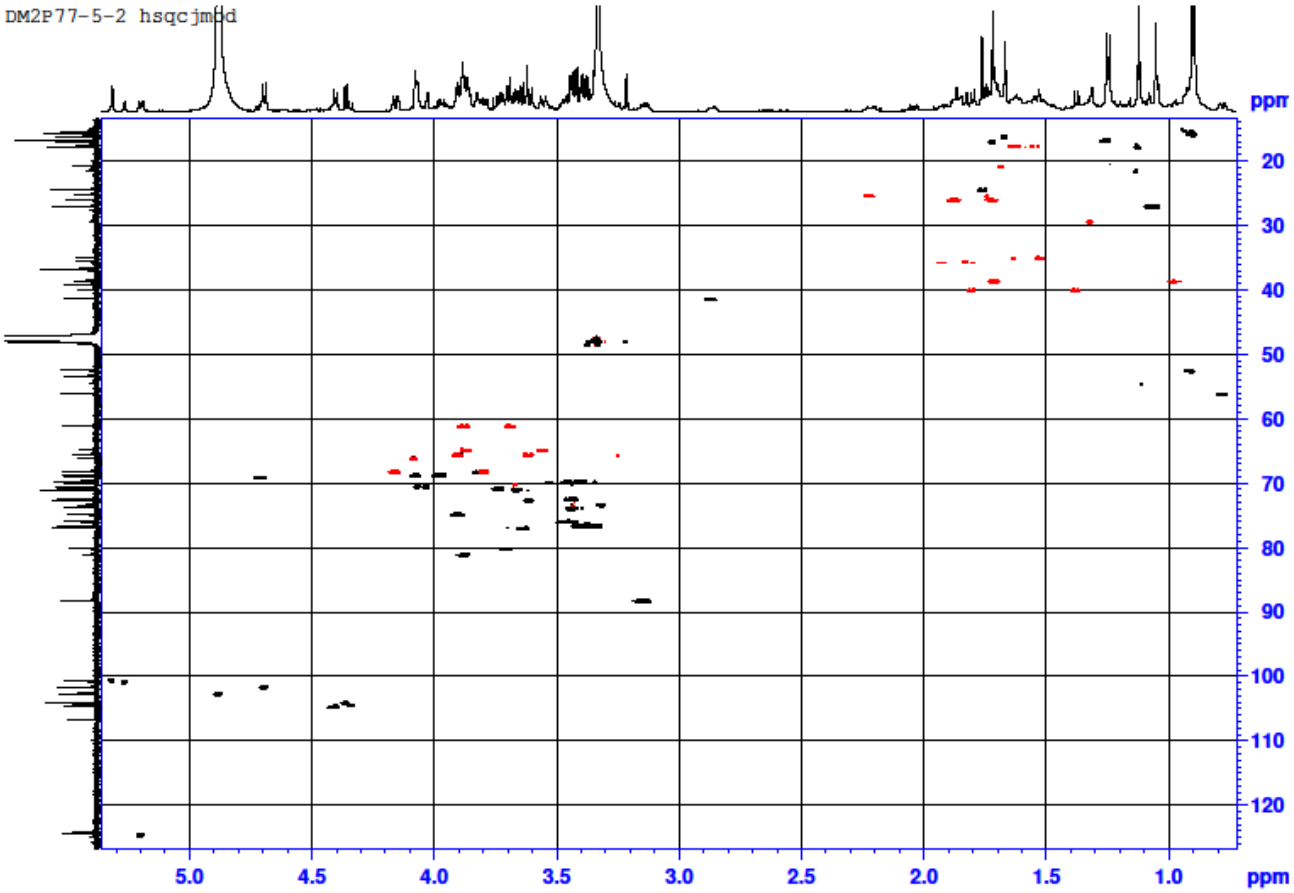

Figure S18: HSQC spectrum of compound **8** (CD_3OD)

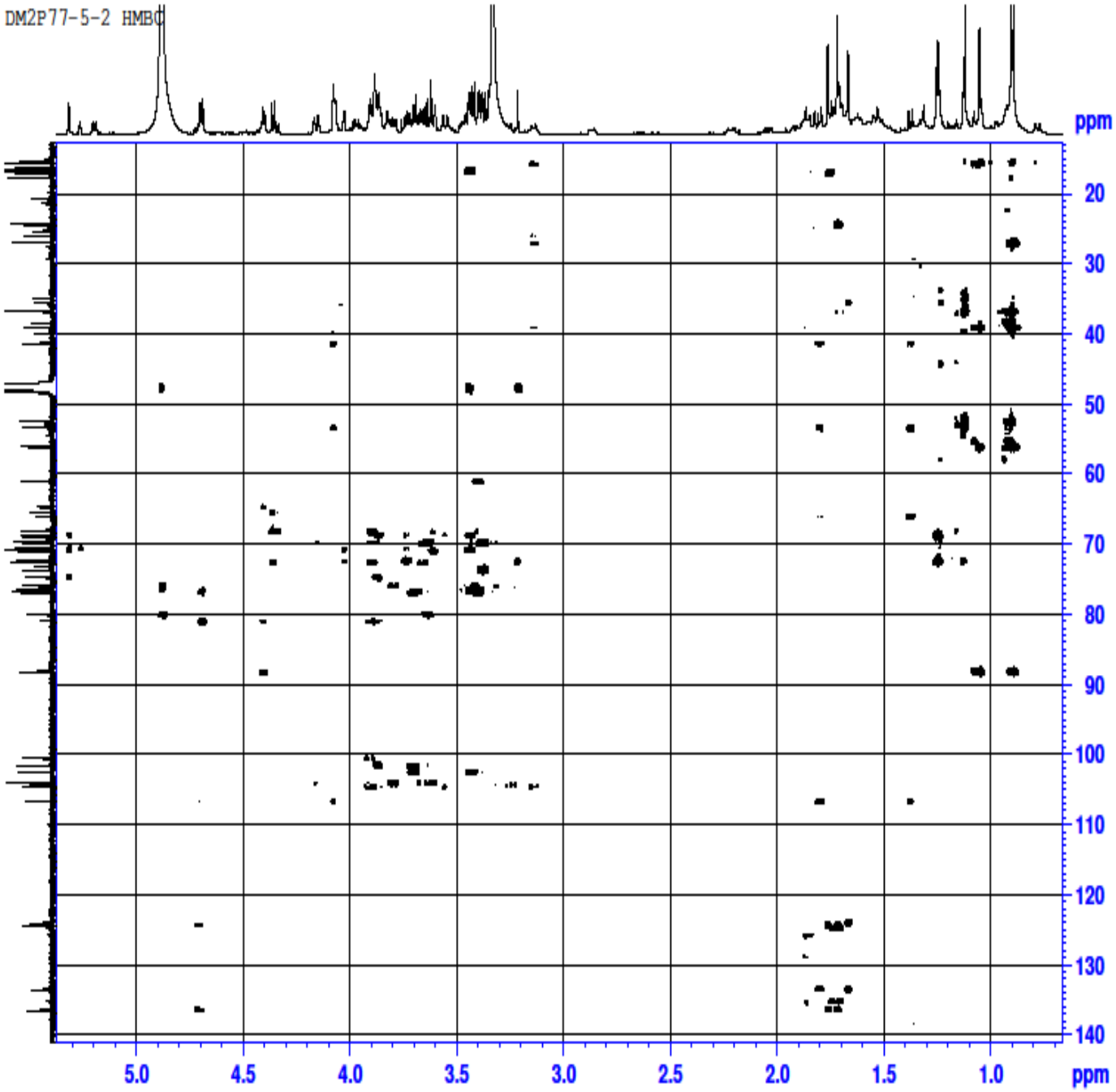

Figure S19: HMBC spectrum of compound **8** (CD_3OD)

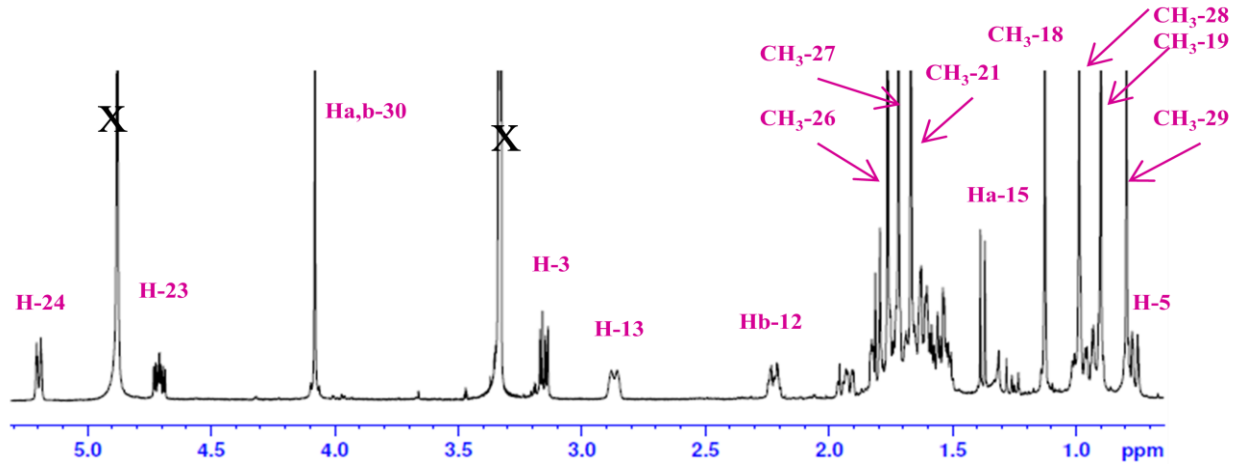

Figure S20: ^{1}H NMR spectrum of compound **9** (CD_3OD, 600 MHz)

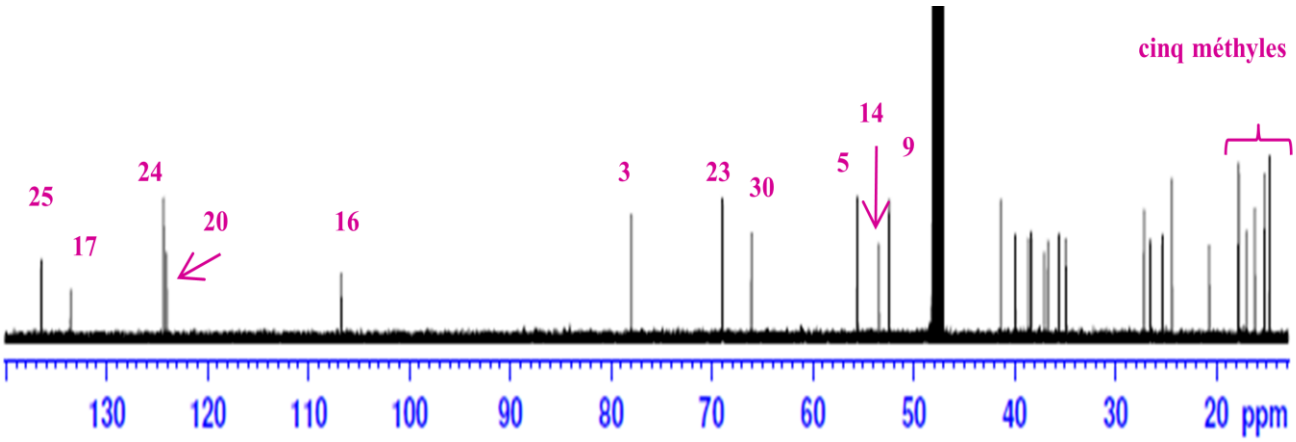

Figure S21: ^{13}C NMR spectrum of compound **9** (CD_3OD, 600 MHz)

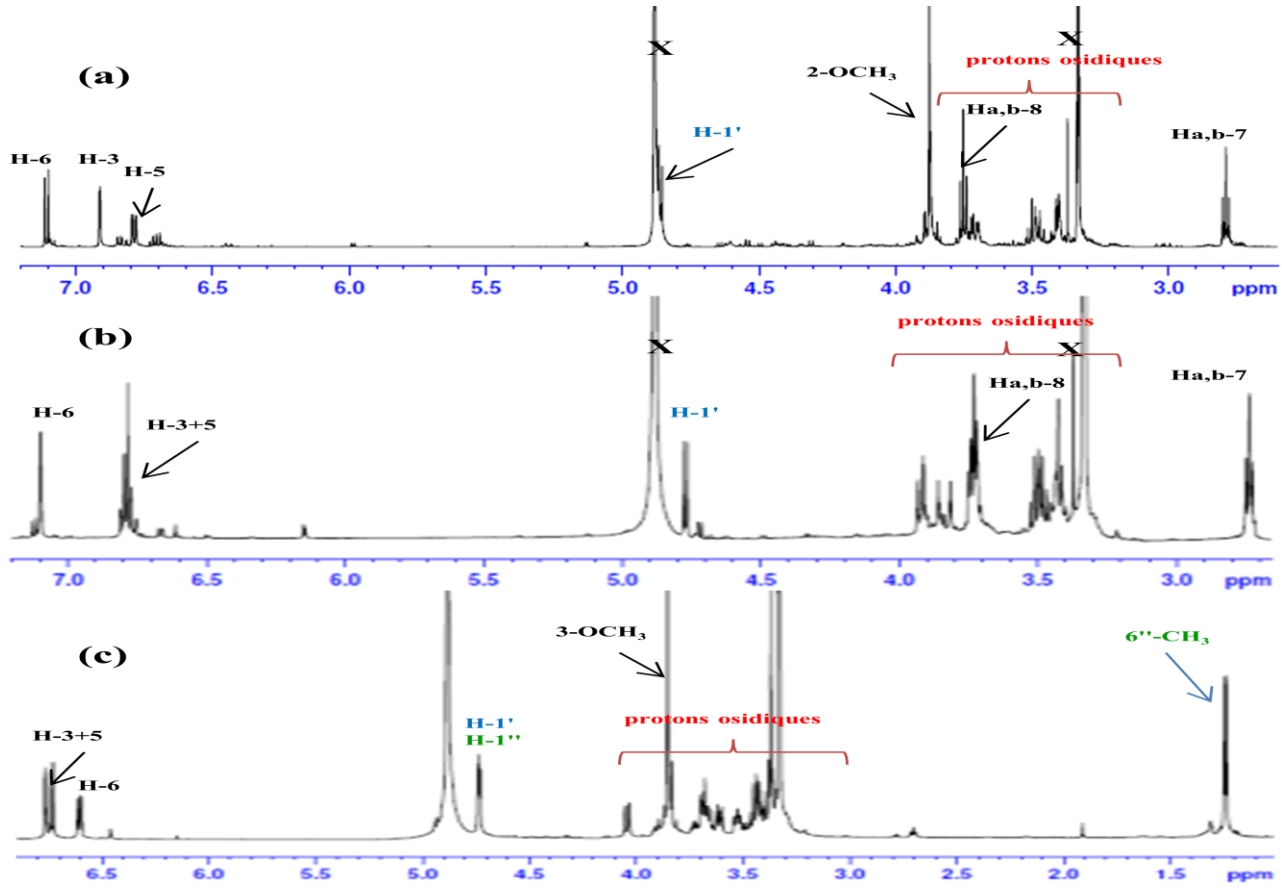

Figure S22 : ^{1}H NMR spectrum of compounds **19 (a) 20 (b)** and **21 (c)** (CD_3OD, 600 MHz)

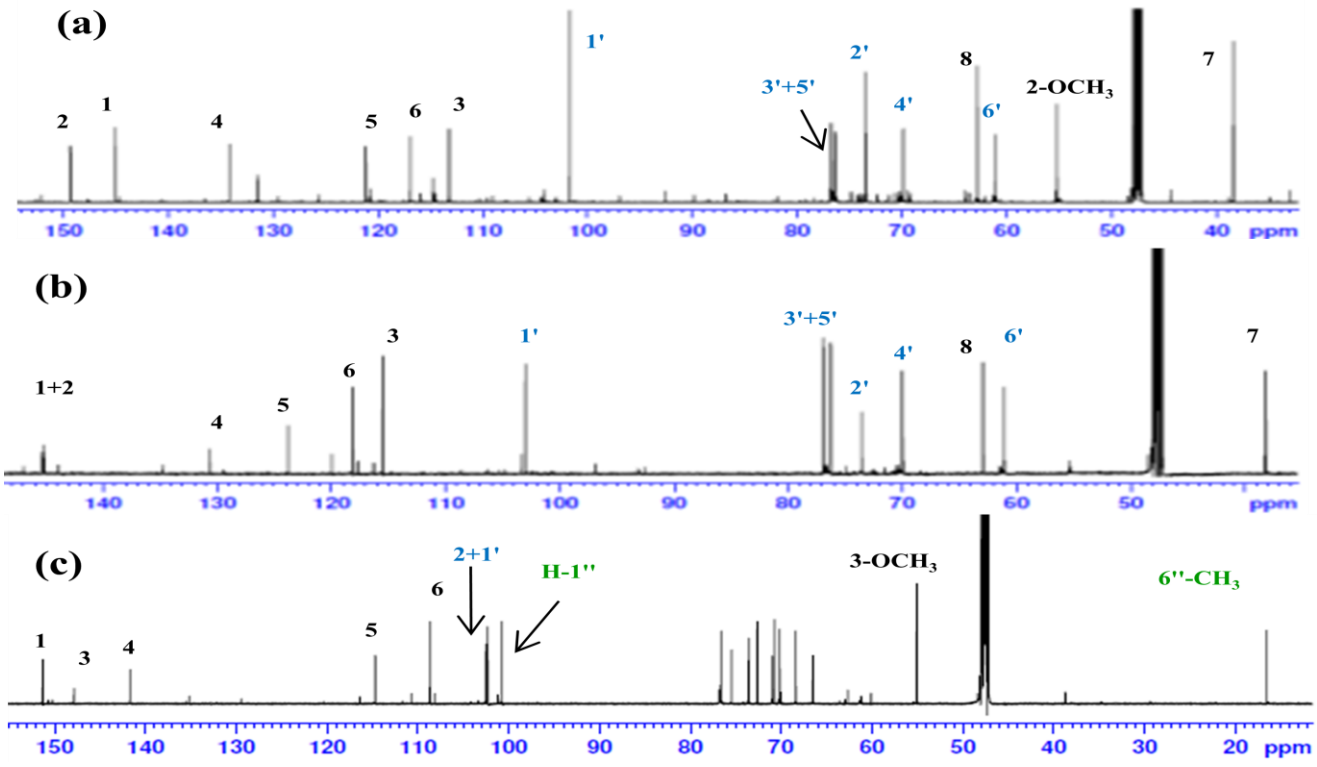

Figure S23 : ^{13}C NMR spectrum of compounds **19 (a) 20 (b)** and **21 (c)** (CD_3OD, 600 MHz)

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