

## Two New Compounds from *Allii Macrostemonis Bulbus* and Their In Vitro Antioxidant Activities

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**Abstract:** Two new compounds named 4,4'-bis( $\beta$ -D-glucopyranosyloxy)biphenyl (**1**) and spirostane-25(27)-en-2 $\alpha$ ,3 $\beta$ -diol-3-O- $\beta$ -D-xylopyranosyl(1 $\rightarrow$ 3)- $\beta$ -D-glucopyranosyl(1 $\rightarrow$ 4)- $\beta$ -D-galactopyranoside (**2**) were isolated from n-butanol extraction part of 80% ethanol extract of *Allii Macrostemonis Bulbus*. Alongside these, ten known compounds (**3–12**) were also identified, including a flavonoid glycoside (**3**), seven steroids (**4–10**), a nucleoside (**11**), and a phenylpropanoid glycoside (**12**) were found. Notably, compounds **3–6** were isolated from this plant for the first time. The structures of all compounds were confirmed using high-resolution electrospray ionization mass spectrometry (HR-ESI-MS), 1D, and 2D NMR spectroscopy. Some of these compounds showed strong antioxidant activity, and compound **1** demonstrated the most potent reduction of ferric ions ( $\text{Fe}^{3+}$ ) with an  $\text{IC}_{50}$  value of  $0.59 \pm 0.18$  mg/mL. Compounds **2** and **3** exhibited the highest scavenging activity against superoxide anion radicals ( $\text{O}_2^{\cdot-}$ ) with an  $\text{IC}_{50}$  value of  $0.02 \pm 0.01$  mg/mL. Additionally, compound **3** displayed substantial scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) with  $\text{IC}_{50}$  values of  $0.21 \pm 0.17$  mg/mL and  $0.02 \pm 0.01$  mg/mL, respectively. The discovery of these two new compounds is a reference for identifying *Allii Macrostemonis Bulbus* quality markers. Moreover, their exceptional antioxidant activity offers a promising avenue for uncovering novel natural antioxidants.

**Keywords:** *Allii Macrostemonis Bulbus*; biphenyl glycoside; steroidal saponin; antioxidant

## CONTENTS

<b>Table S1.</b> The assignment of carbon signals of compound <b>3-12</b>	<b>3</b>
<b>Figure S1.</b> HR-ESI-MS spectrum of compound <b>1</b>	<b>6</b>
<b>Figure S2.</b> $^1\text{H}$ -NMR spectrum of compound <b>1</b> (in Methanol- $d_4$ , 600 MHz)	<b>6</b>
<b>Figure S3.</b> $^{13}\text{C}$ -NMR-APT spectrum of compound <b>1</b> (in Methanol- $d_4$ , 150 MHz)	<b>7</b>
<b>Figure S4.</b> HMQC spectrum of compound <b>1</b>	<b>7</b>
<b>Figure S5.</b> HMBC spectrum of compound <b>1</b>	<b>8</b>
<b>Figure S6.</b> COSY spectrum of compound <b>1</b>	<b>8</b>
<b>Figure S7.</b> NOESY spectrum of compound <b>1</b>	<b>9</b>
<b>Figure S8.</b> HR-ESI-MS spectrum of compound <b>2</b>	<b>9</b>
<b>Figure S9.</b> $^1\text{H}$ -NMR spectrum of compound <b>2</b> (in Pyridine- $d_5$ , 600 MHz)	<b>10</b>
<b>Figure S10.</b> $^{13}\text{C}$ -NMR-APT spectrum of compound <b>2</b> (in Pyridine- $d_5$ , 150 MHz)	<b>10</b>
<b>Figure S11.</b> HMQC spectrum of compound <b>2</b>	<b>11</b>
<b>Figure S12.</b> HMBC spectrum of compound <b>2</b>	<b>11</b>
<b>Figure S13.</b> COSY spectrum of compound <b>2</b>	<b>12</b>
<b>Figure S14.</b> NOESY spectrum of compound <b>2</b>	<b>12</b>

**Table S1.** The assignment of carbon signals of compound **3-12**

Position	$\delta_c(\text{ppm})$									
	<b>3<sup>a</sup></b>	<b>4<sup>b</sup></b>	<b>5<sup>b</sup></b>	<b>6<sup>c</sup></b>	<b>7<sup>b</sup></b>	<b>8<sup>b</sup></b>	<b>9<sup>d</sup></b>	<b>10<sup>d</sup></b>	<b>11<sup>b</sup></b>	<b>12<sup>c</sup></b>
1	-	37.61	37.41	36.54	36.54	37.95	37.37	37.37	-	133.78
2	158.54	30.35	30.15	29.04	29.05	30.73	31.77	31.78	153.77	152.74
3	136.22	78.33	78.13	76.35	75.94	79.11	71.94	71.95	-	104.42
4	179.65	35.24	35.04	34.01	34.02	39.81	42.33	42.44	150.43	132.62
5	163.24	45.04	44.85	44.04	44.04	141.36	140.87	140.87	121.96	104.42
6	99.81	29.35	29.16	28.32	28.32	122.43	121.86	121.88	158.19	152.74
7	165.95	32.83	32.63	31.89	31.89	32.65	31.77	34.04	-	128.46
8	94.70	35.66	35.46	34.66	34.66	32.51	32.02	32.01	141.04	130.21
9	159.32	54.78	54.59	53.68	53.68	50.79	50.25	50.23	91.29(Rib-C-1')	61.50
10	105.87	36.24	36.04	35.33	35.33	37.40	36.64	36.63	76.02(C-2')	102.53(Glc-C-1')
11	122.84(C-1')	21.70	21.50	20.60	20.60	21.76	21.19	19.98	72.89(C-3')	74.19(C-2')
12	116.89(C-2')	40.56	40.37	40.02	39.44	40.41	39.79	39.88	88.28(C-4')	77.27(C-3')
13	146.44(C-3')	41.21	41.01	40.12	40.12	42.95	42.42	42.42	63.50(C-5')	69.93(C-4')
14	149.82(C-4')	56.85	56.66	55.63	55.63	57.28	56.04	56.15		76.57(C-5')
15	116.36(C-5')	32.58	32.38	31.43	31.42	24.99	24.50	23.17		60.89(C-6')
16	122.94(C-6')	81.59	81.39	79.33	79.41	29.04	29.08	26.12		56.36(OCH <sub>3</sub> ×2)
17	103.55(Man-C-1'')	63.45	63.25	61.90	61.90	56.69	56.98	56.88		
18	72.04(C-2'')	17.08	16.88	16.25	16.25	12.45	12.18	12.12		
19	72.08(C-3'')	12.74	12.55	12.12	12.12	19.91	19.11	19.55		
20	73.22(C-4'')	42.42	42.23	40.05	41.12	36.88	40.67	36.28		
21	71.90(C-5'')	15.52	15.32	14.70	14.70	19.49	21.26	18.91		
22	17.66(C-6'')	109.69	109.49	108.42	108.42	34.66	138.48	28.39		
23		32.25	32.05	30.91	30.91	26.79	129.37	24.44		
24		29.71	29.52	28.50	28.50	46.49	51.37	45.93		
25		31.06	30.86	29.84	29.84	29.89	32.02	29.22		
26		67.30	67.11	67.57	65.92	19.67	21.36	19.55		

27	17.80	17.61	17.14	17.14	20.48	19.55	19.15
28	-		-	-	23.85	25.56	21.21
29	-		-	-	12.64	12.42	12.00
Gla-1	102.83	102.63	100.90	100.90	-		
2	75.76	75.80	73.82	71.52	-		
3	75.99	75.84	75.89	73.80	-		
4	81.97	81.36	80.21	79.28	-		
5	77.73	76.47	74.91	74.30	-		
6	61.05	60.85	68.93	59.47	-		
Glc-1	105.02	104.83	102.48	103.37	103.04		
2	80.76	81.77	74.29	80.20	75.84		
3	88.93	88.74	77.03	86.90	79.03		
4	71.29	71.82	69.96	69.62	72.14		
5	79.13	80.57	76.83	76.06	78.53		
6	62.71	62.51	63.27	60.81	63.29		
Glc-1	105.41	105.21	-	102.95			
2	76.04	77.54	-	73.54			
3	80.72	80.52	-	76.36			
4	73.68	73.48	-	69.15			
5	78.03	78.93	-	75.87			
6	63.51	63.32	-	60.97			
Glc-1	-	-	-	102.50			
2	-	-	-	73.68			
3	-	-	-	76.99			
4	-	-	-	70.11			
5	-	-	-	76.52			
6	-	-	-	61.41			
Xyl-1	105.60	105.40	-				
2	76.67	77.83	-				
3	79.17	78.98	-				

4	72.02	75.57	-
5	67.30	67.11	-
Ara-1			103.33
2			71.48
3			73.54
4			69.61
5			65.91

<sup>1</sup>H-NMR and <sup>13</sup>C-NMR were measured at 150 MHz and 600 MHz, respectively, using deuterated <sup>a)</sup> Methanol-d<sub>4</sub>, <sup>b)</sup> Pyridine-d<sub>5</sub>, <sup>c)</sup> DMSO-d<sub>6</sub>, <sup>d)</sup> Chloroform-d as the deuterated solvent.

WJF-2-4-H\_230717145944 #1 RT: 0.00 AV: 1 NL: 1.28E7  
T: FTMS + p ESI Full ms [200.00-2000.00]

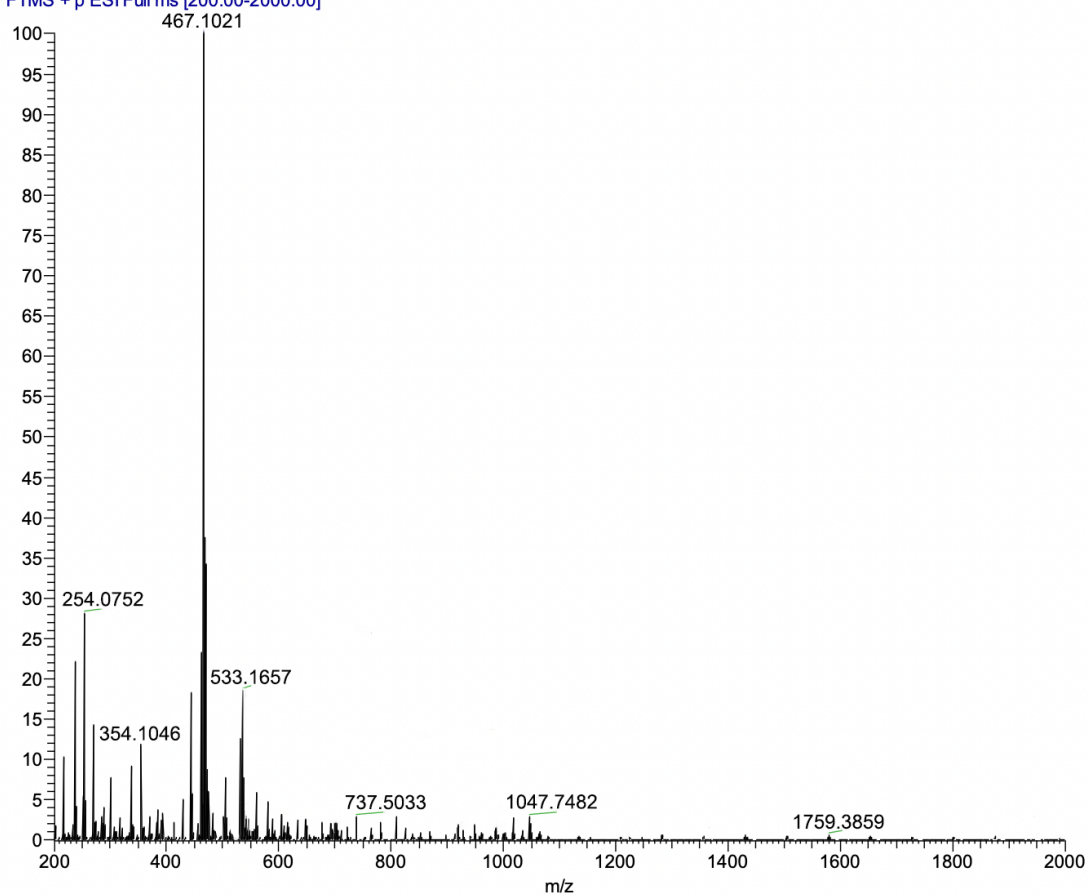


Figure S1. HR-ESI-MS spectrum of compound 1

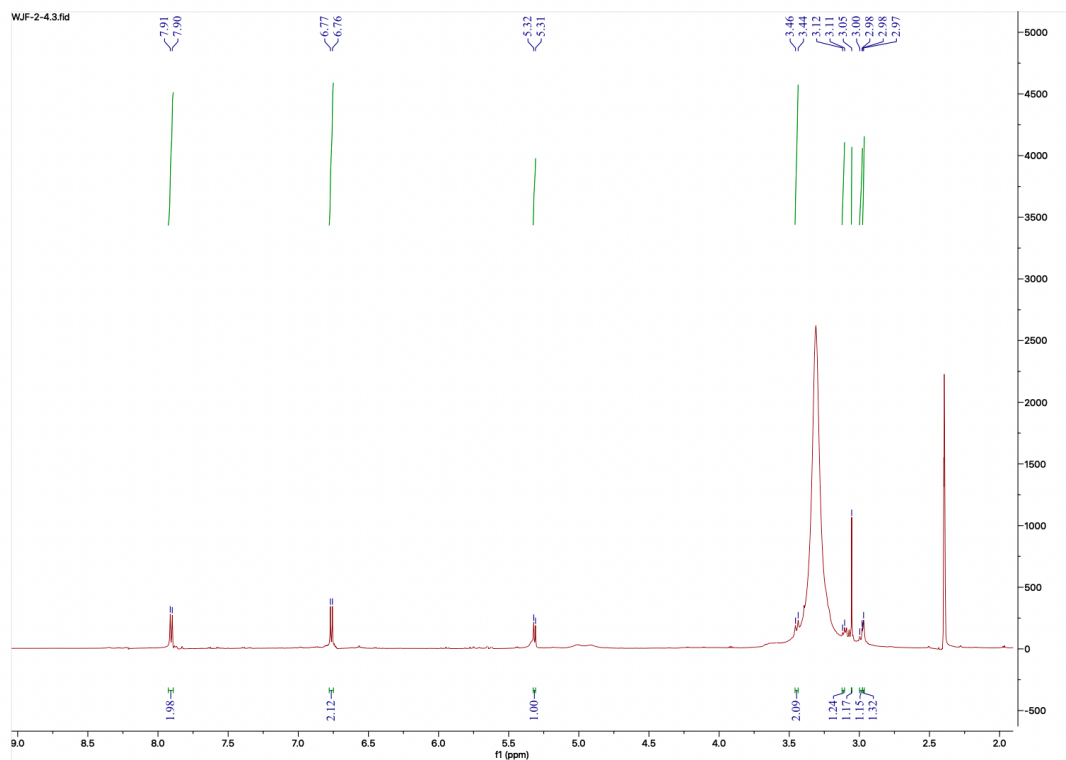
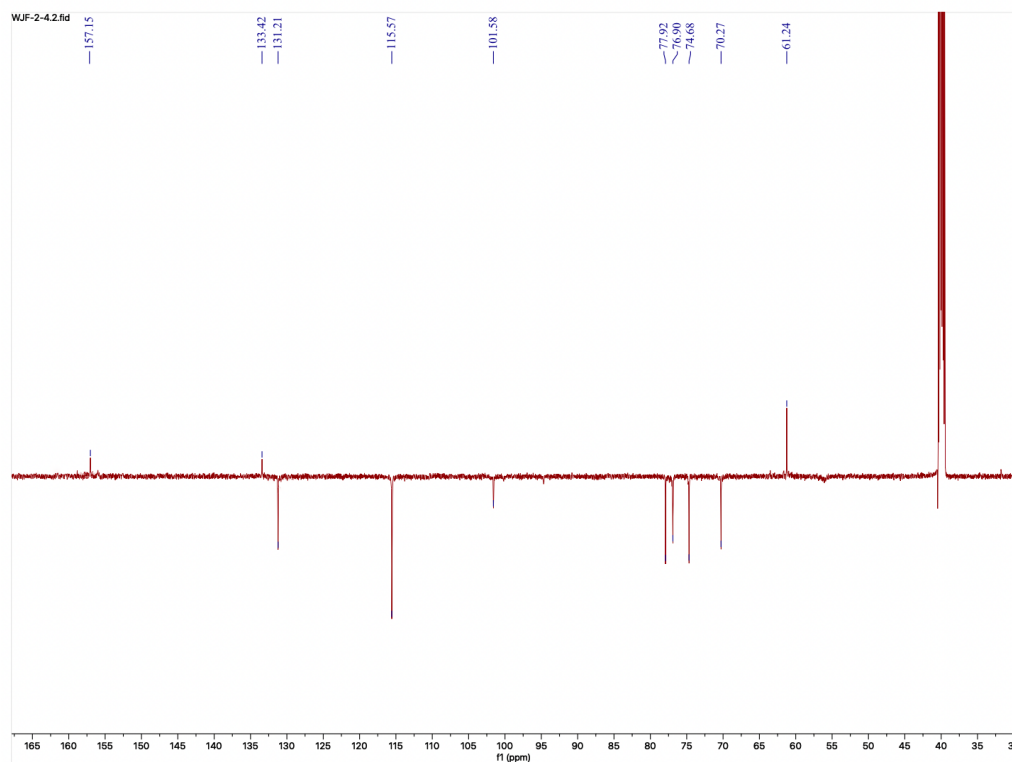
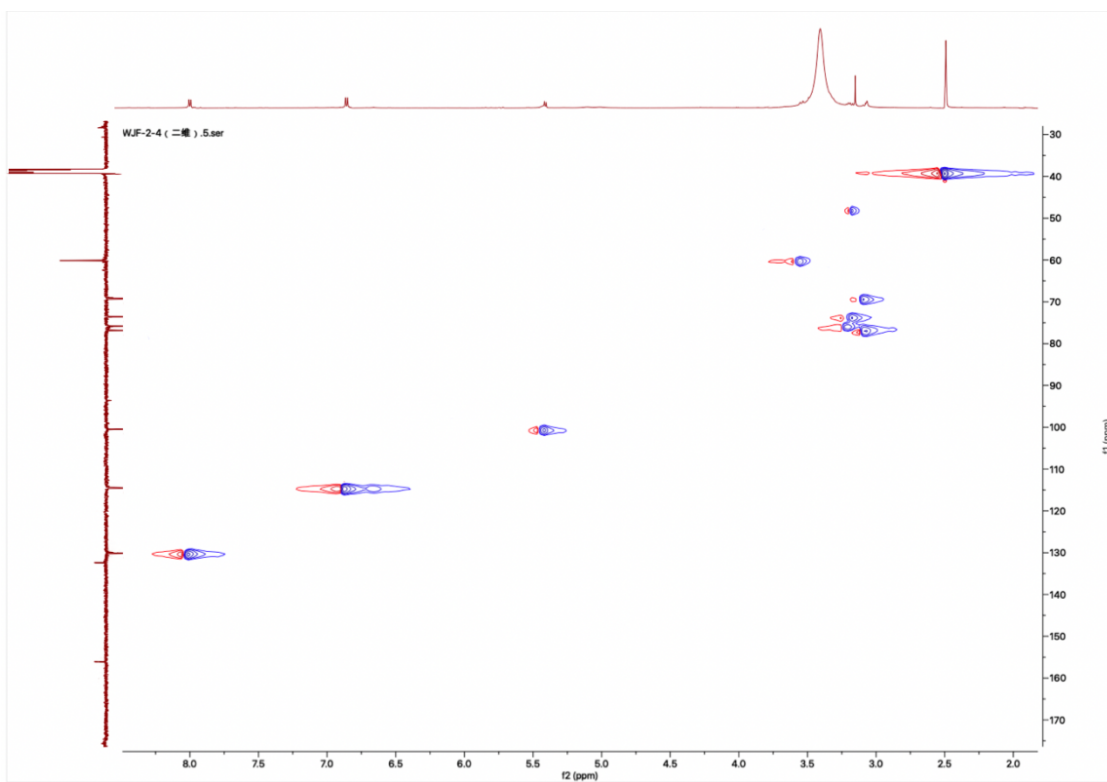


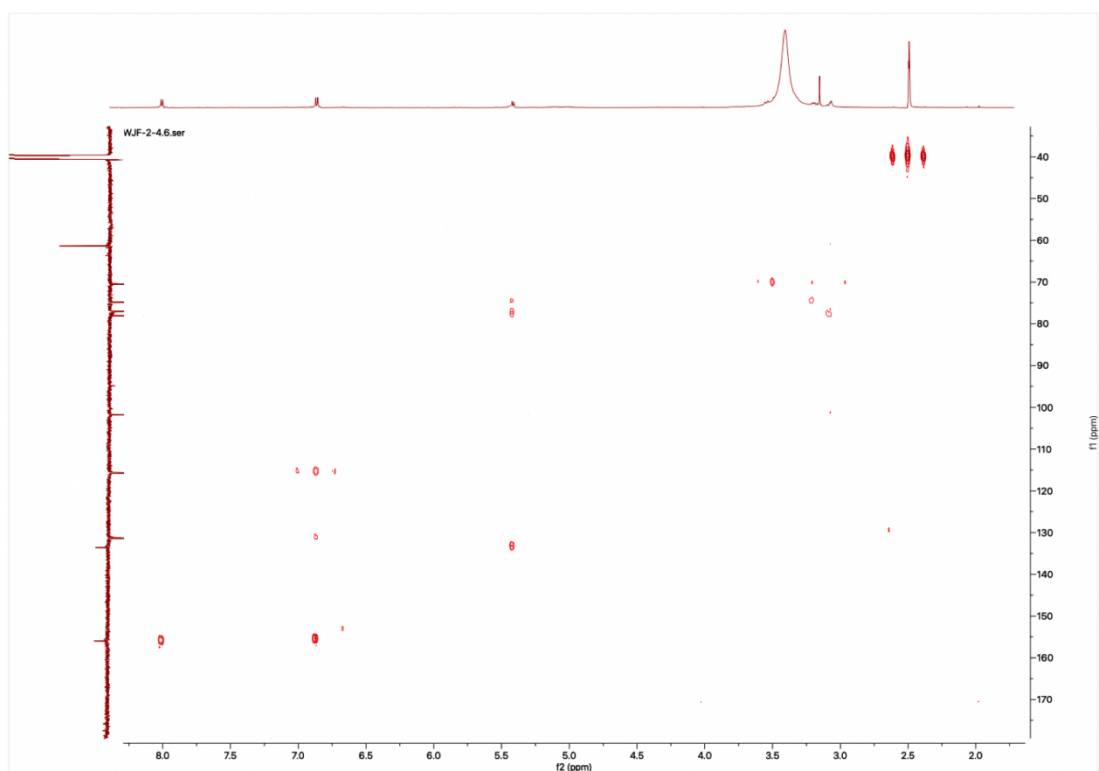
Figure S2. <sup>1</sup>H-NMR spectrum of compound 1 (in Methanol-*d*<sub>4</sub>, 600 MHz)



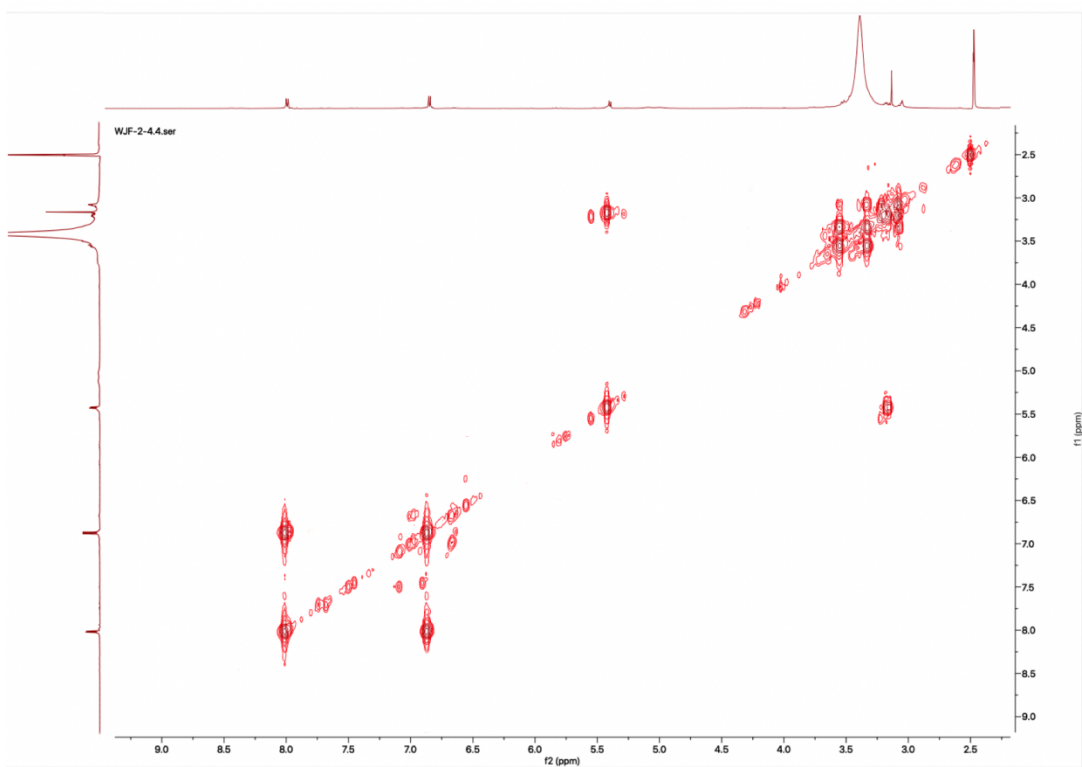
**Figure S3.**  $^{13}\text{C}$ -NMR-APT spectrum of compound **1** (in Methanol- $d_4$ , 150 MHz)



**Figure S4.** HMQC spectrum of compound **1**

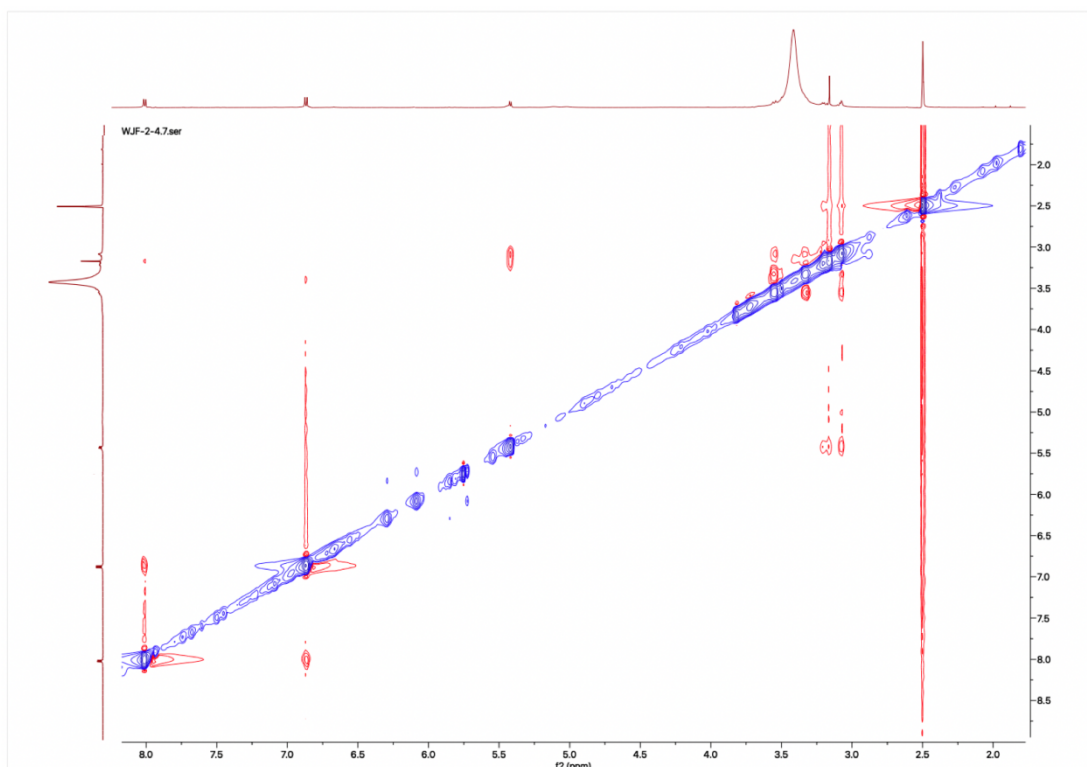


**Figure S5.** HMBC spectrum of compound **1**



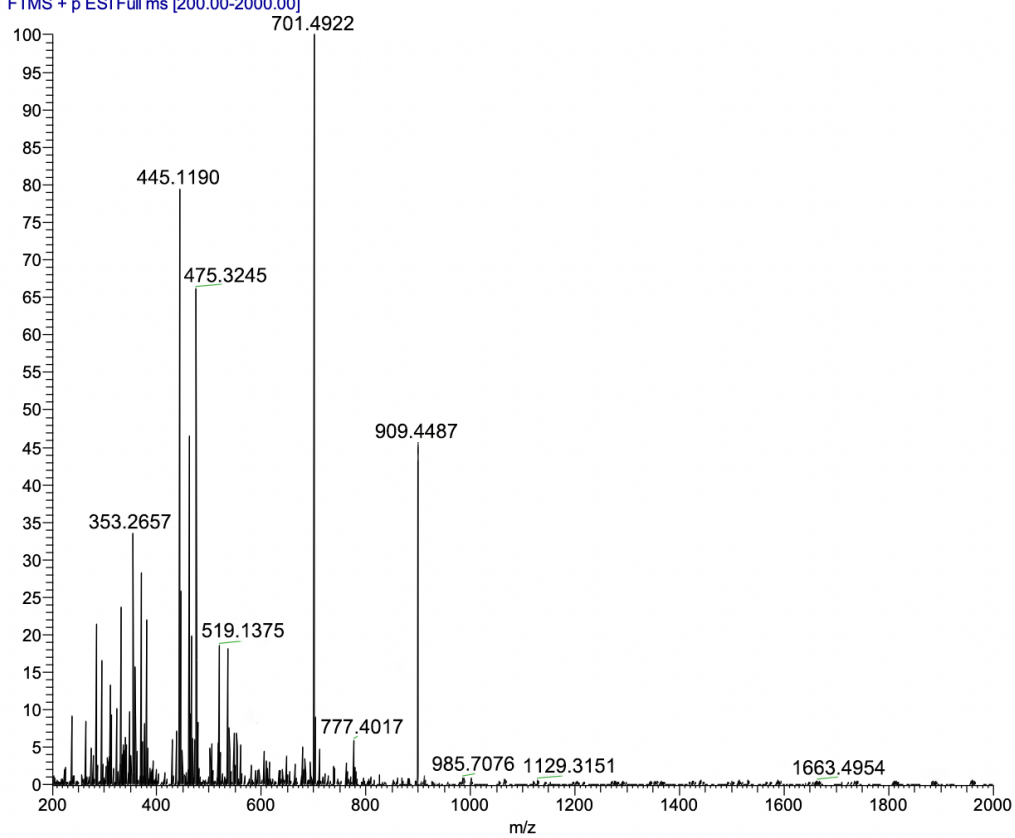
**Figure S6.** COSY spectrum of compound **1**





**Figure S7.** NOESY spectrum of compound **1**

WJF-2-23-H\_230717145944 #1 RT: 0.00 AV: 1 NL: 1.23E7  
T: FTMS + p ESI Full ms [200.00-2000.00]



**Figure S8.** HR-ESI-MS spectrum of compound **2**

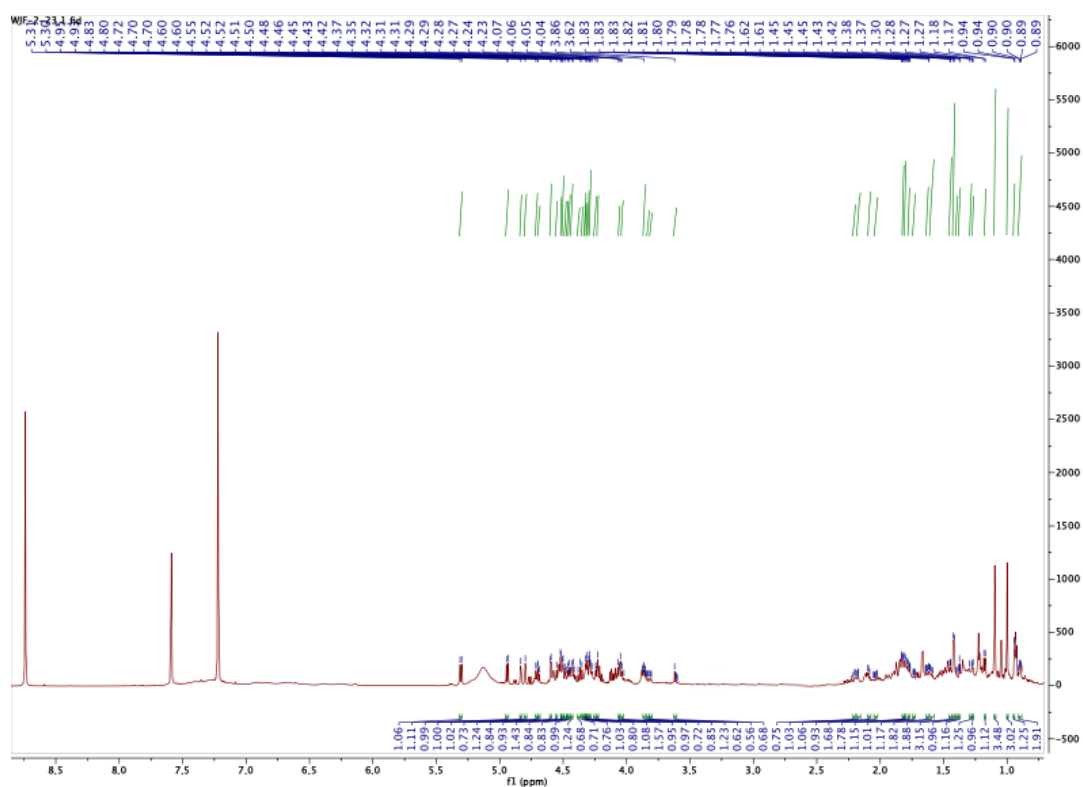


Figure S9.  $^1\text{H}$ -NMR spectrum of compound **2** (in Pyridine- $d_5$ , 600 MHz)

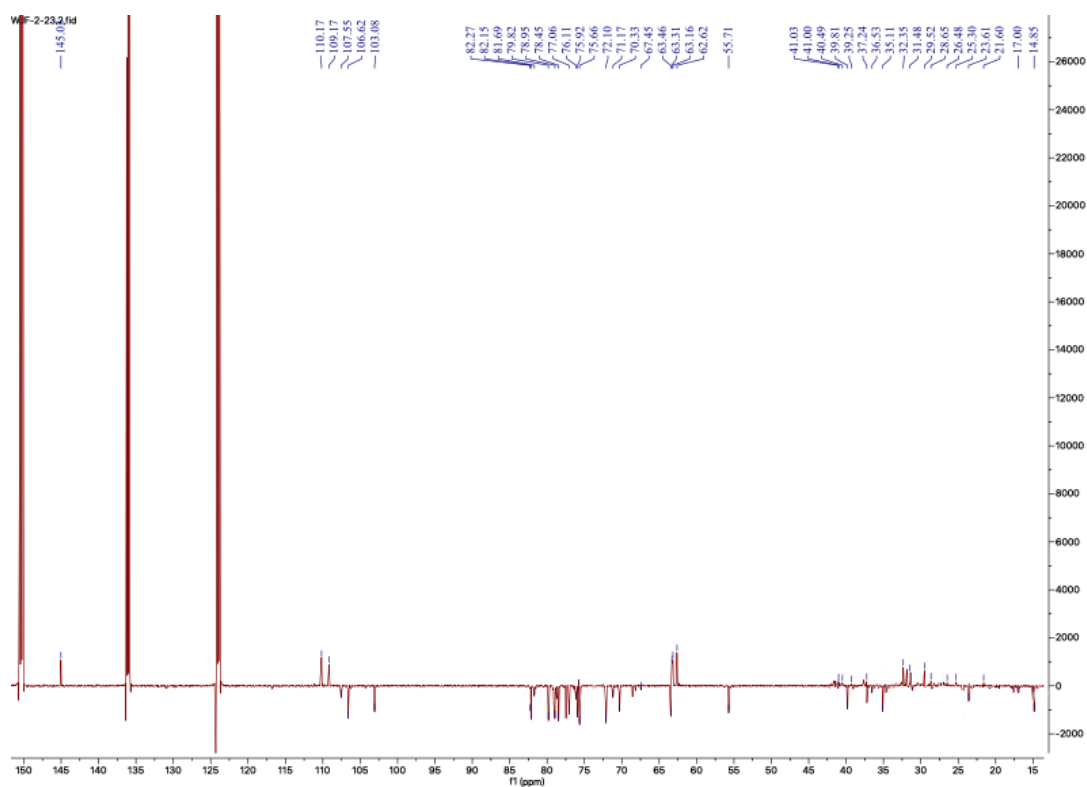


Figure S10.  $^{13}\text{C}$ -NMR-APT spectrum of compound **2** (in Pyridine- $d_5$ , 150 MHz)

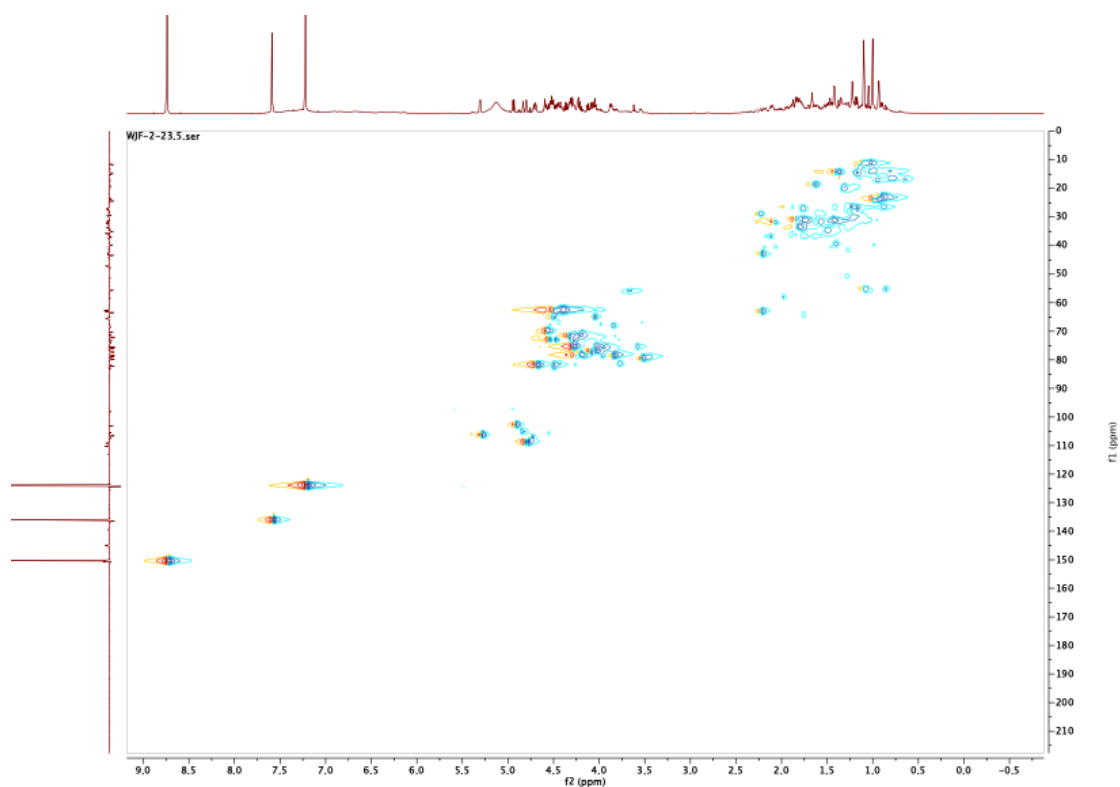


Figure S11. HMQC spectrum of compound 2

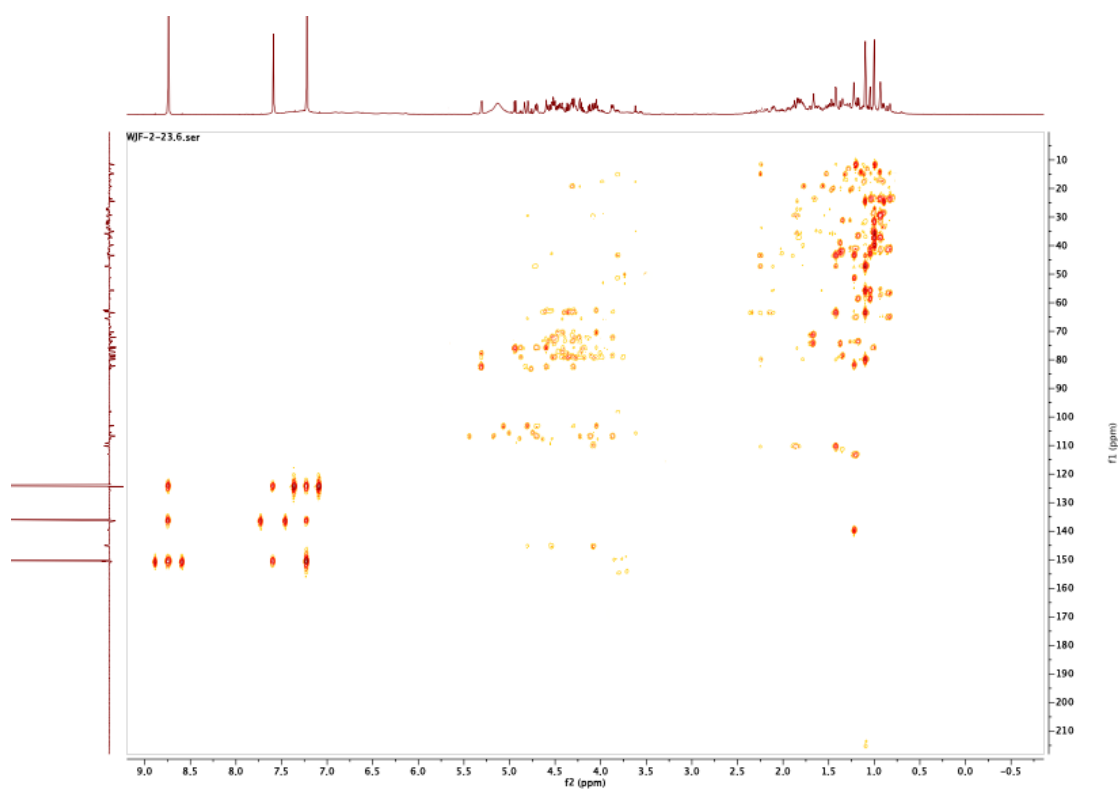


Figure S12. HMBC spectrum of compound 2

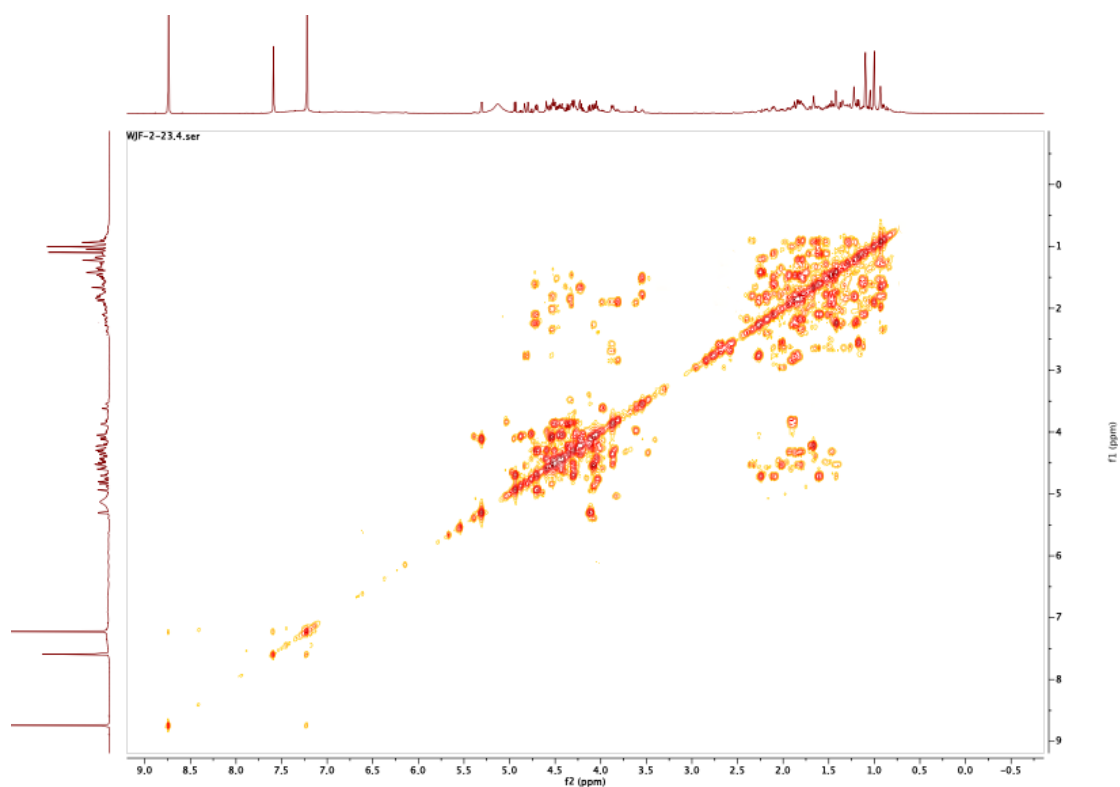


Figure S13. COSY spectrum of compound 2

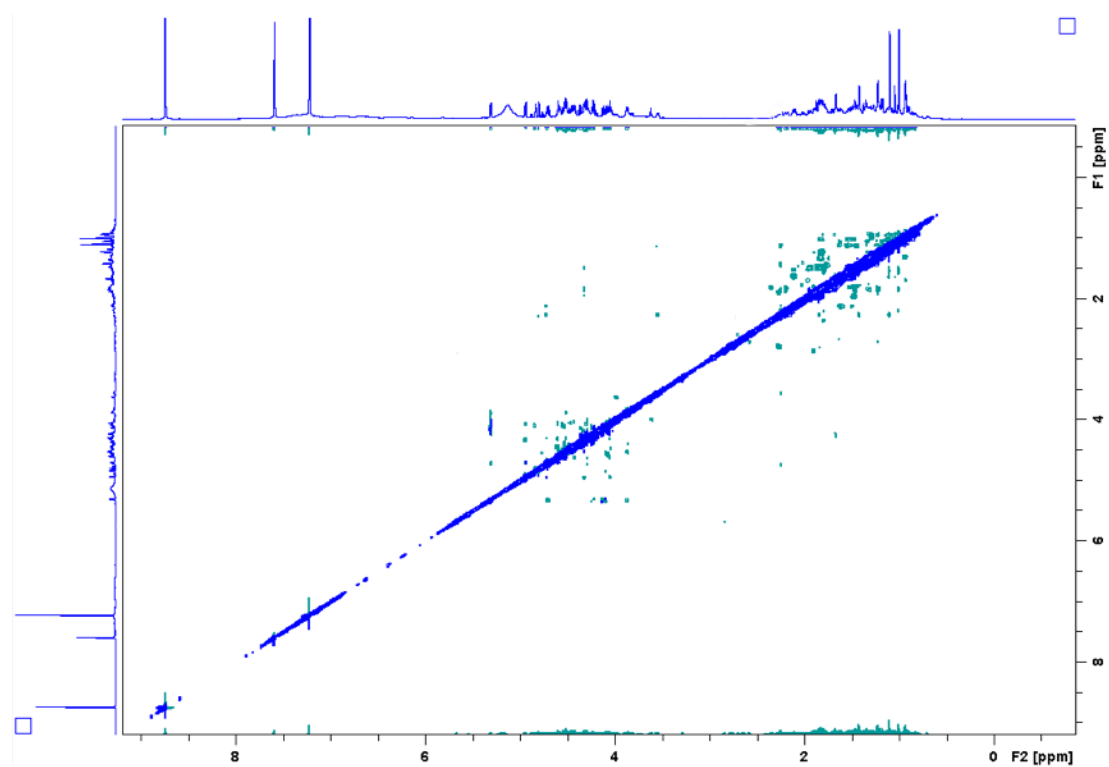


Figure S14. NOESY spectrum of compound 2