

SUPPLEMENTARY MATERIAL

Two new 14, 15-secopregnane-type steroidal glycosides from the roots of *Cynanchum limprichtii*

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Abstract

Two new steroidal glycosides **1** and **2**, along with three known ones (**3-5**), were isolated from the 95% ethanol extract of the roots of *Cynanchum limprichtii* Schltr. The structure of the new compounds was elucidated as 3-O- α -L-diginopyranosyl-(1 \rightarrow 4)- β -D-digitoxopyranosyl-(1 \rightarrow 4)- β -D-cymaropyranosyl-(1 \rightarrow 4)- β -D-thevetopyranosyl-14, 16:15, 20: 18, 20-triepoxy-14, 15-secopregn-4, 6, 8 (14)-triene (**1**) and 3-O- α -L-cymaropyranosyl-(1 \rightarrow 4)- β -D-digitoxopyranosyl-(1 \rightarrow 4)- β -D-3-demethyl-2-deoxythevetopyranosyl-14, 16: 15, 20: 18, 20-triepoxy-14, 15- secopregn-5, 8 (14)-diene (**2**) on the basis of spectroscopic analysis together with acidic hydrolysis. All compounds showed cytotoxic activity against the human cancer cell line HL60, with IC₅₀ values of 55.36, 65.41, 17.88, 17.68, and 33.5 μ M, respectively. While only compound **3** showed cytotoxicity against the Caco-2 cell line, with an IC₅₀ value of 67.47 μ M.

Keywords: *Cynanchum limprichtii*; steroidal glycoside; cytotoxicity

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Table 1S. ^1H and ^{13}C NMR spectral data of 1 and 2 (400 MHz for ^1H and 100 MHz for ^{13}C , $\text{C}_5\text{D}_5\text{N}$)

Mass Spectrum Molecular Formula Report

Analysis Info	D:\Data\20160506ceyang\K2-9-8-7-5_1-B_4_01_7114.d	Acquisition Date 5/6/2016 11:12:59 AM
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Method	K2-9-8-7-5	Instrument / Ser# microTOF-Q 125
Sample Name		
Comment		

Acquisition Parameter		
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Focus	Active	Set Capillary 4500 V
Scan Begin	50 m/z	Set End Plate Offset -500 V
Scan End	3000 m/z	Set Collision Cell RF 400.0 Vpp
		Set Nebulizer 1.2 Bar
		Set Dry Heater 180 °C
		Set Dry Gas 8.0 l/min
		Set Divert Valve Source

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Formula, max.			
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		Charge	1
		Maximum	0
		Maximum	3

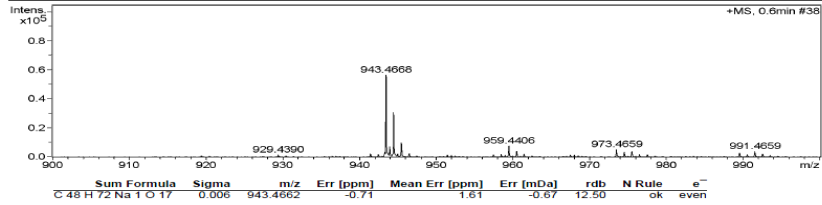


Figure 1S. HR-ESI-MS spectrum of 1

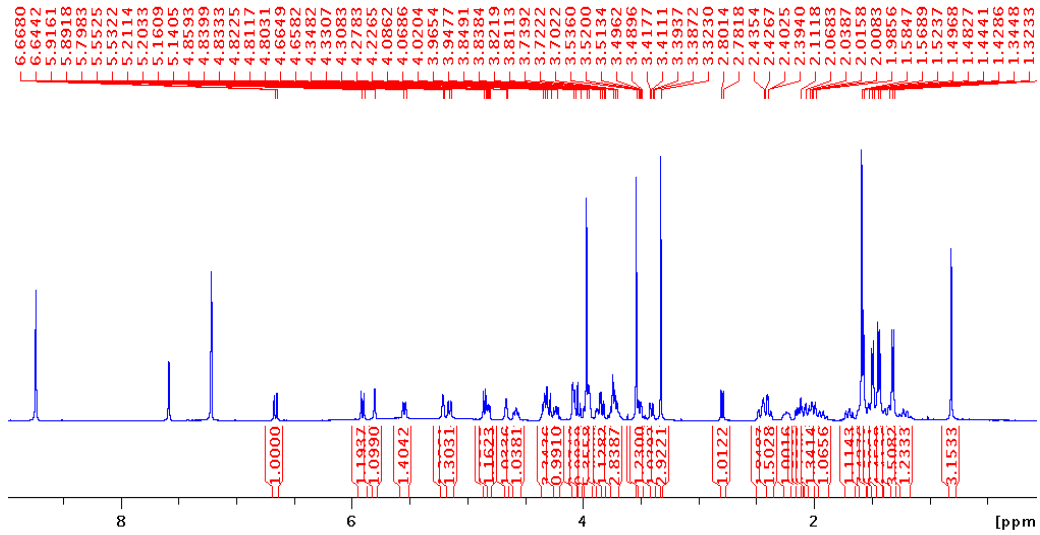


Figure 2S. The ¹H NMR (C₅D₅N, 400 MHz) data of 1

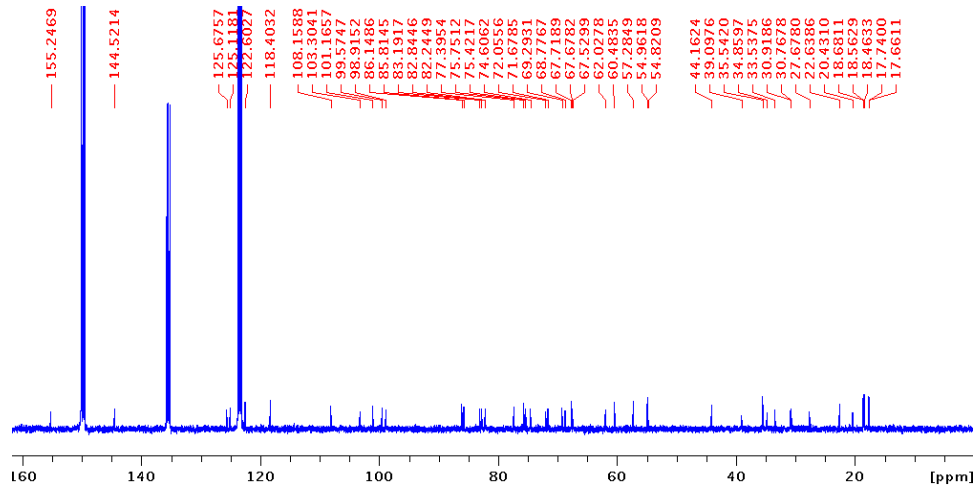


Figure 3S. The ¹³C NMR (C₅D₅N, 100 MHz) data of 1

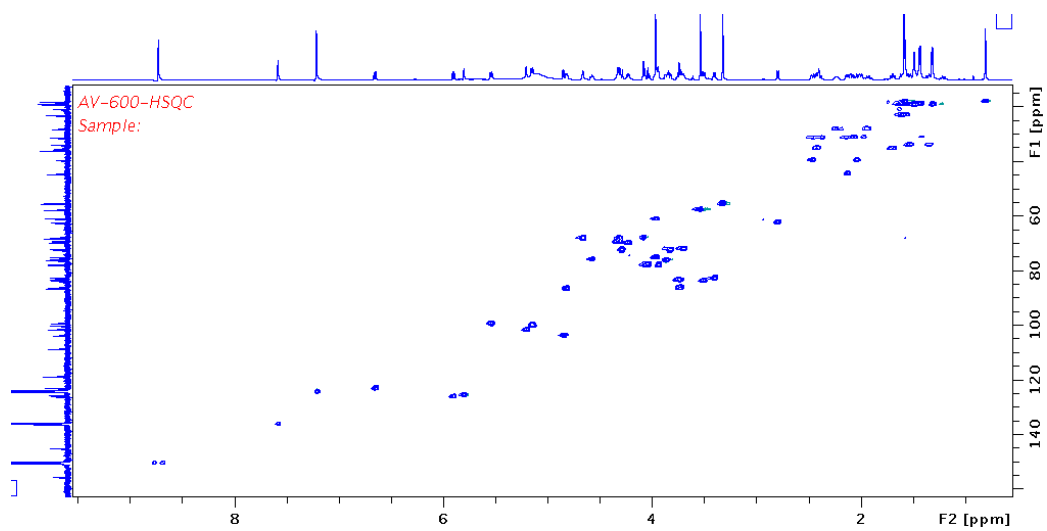


Figure 4S. HSQC data of 1

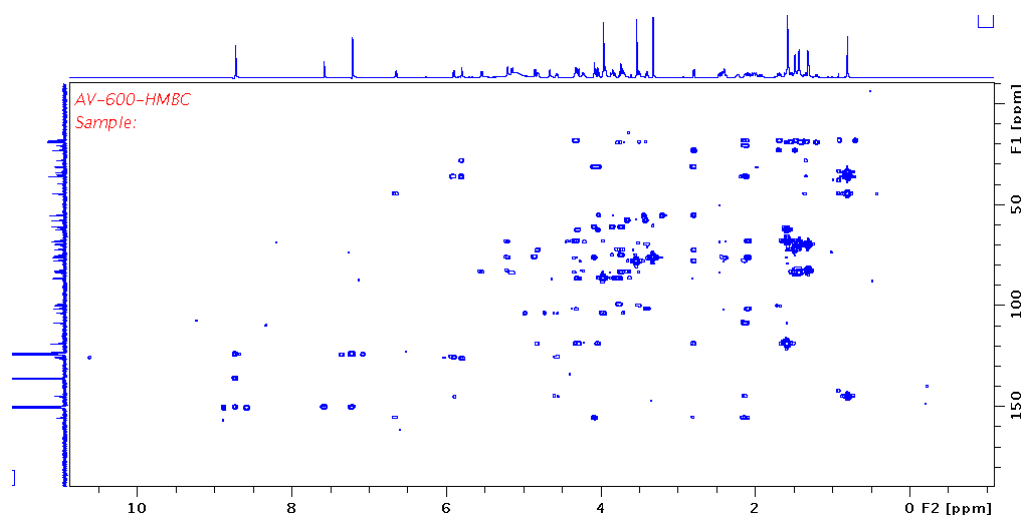


Figure 5S. HMBC data of 1

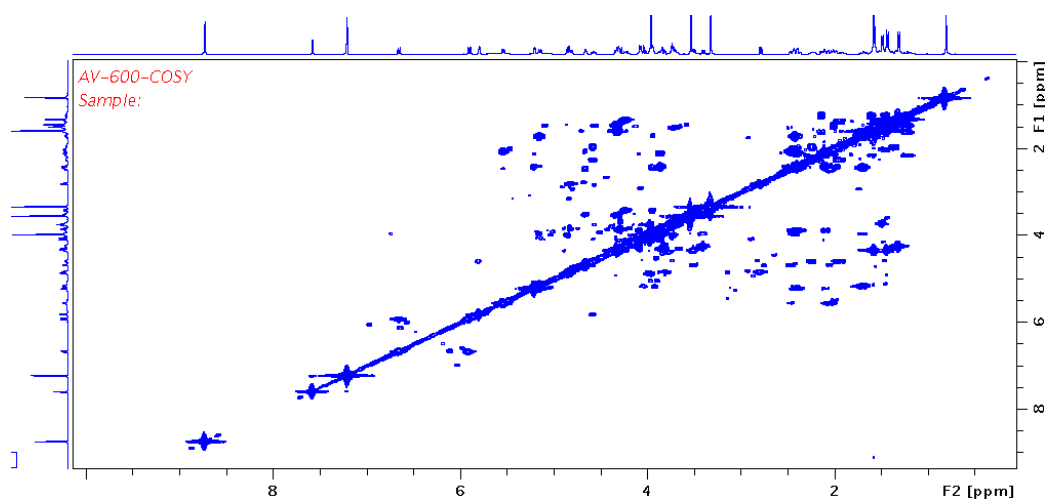


Figure 6S. ^1H - ^1H COSY data of 1

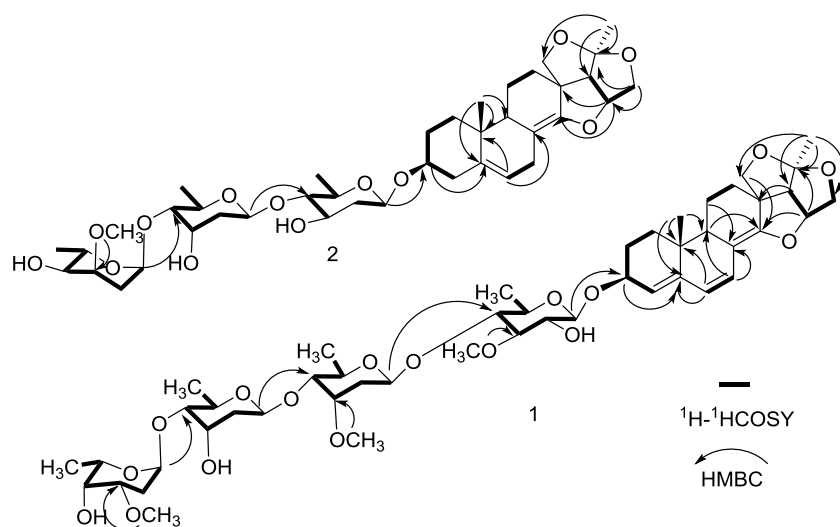


Figure 7 S. Key ¹H-¹HCOSY and HMBC correlations of compounds 1 and 2

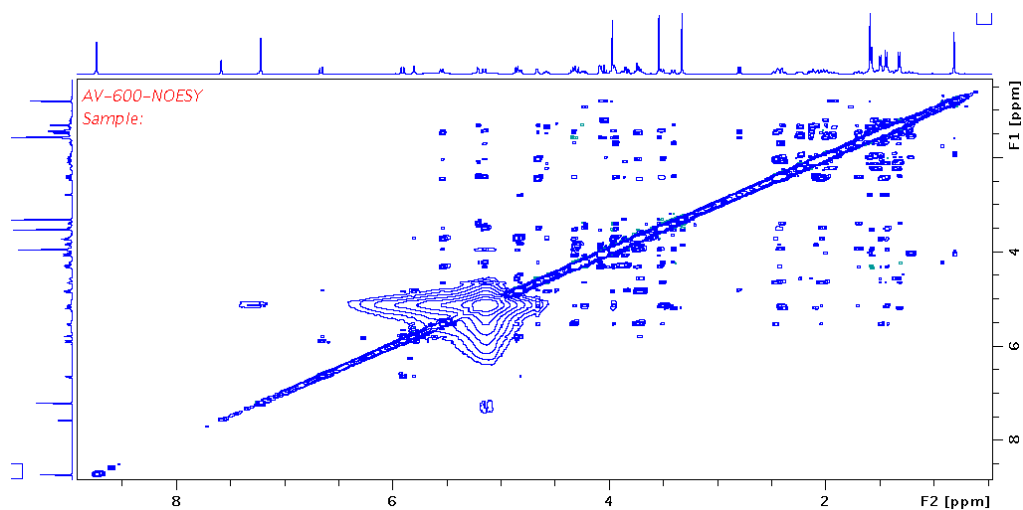


Figure 8 S. NOESY data of 1

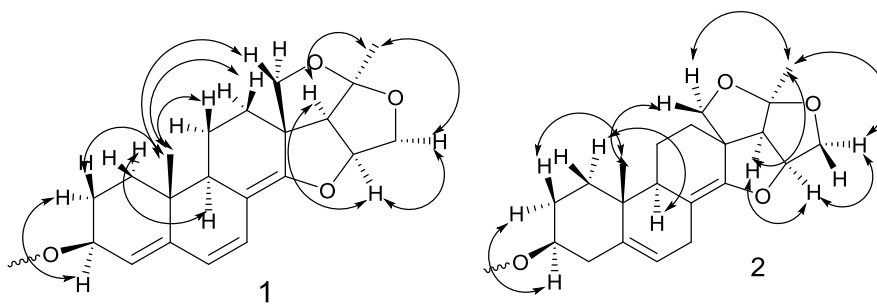


Figure 9 S. Key NOESY correlations of compounds 1 and 2

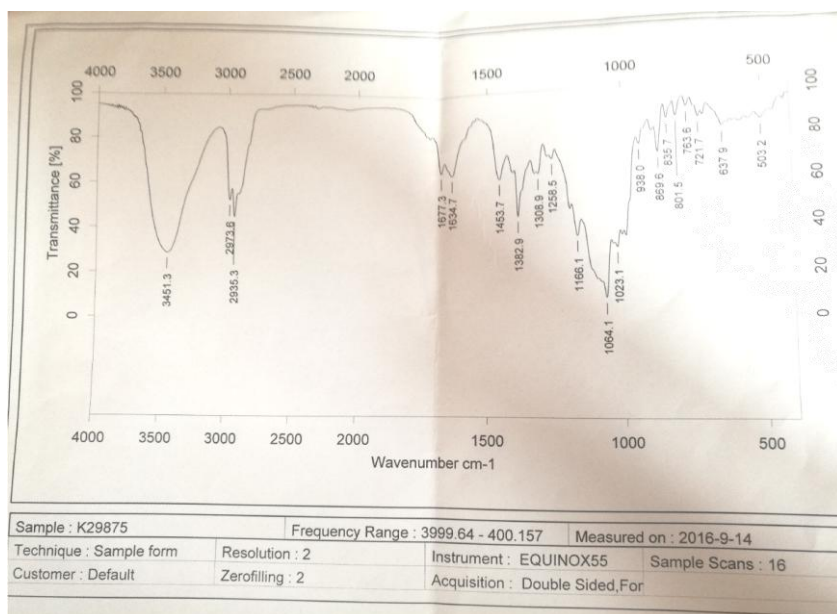


Figure 10S. the IR spectra of 1

Mass Spectrum Molecular Formula Report

Analysis Info					
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Sample Name	K2-9-6-1-5-a	Instrument / Ser#	microTOF-Q 125		
Comment					
Acquisition Parameter					
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Scan End	3000 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Source
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Formula, max.					
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Nitrogen Rule	no	Electron Configuration	both		
Filter H/C Ratio	no	Minimum	0	Maximum	3
Estimate Carbon	yes				

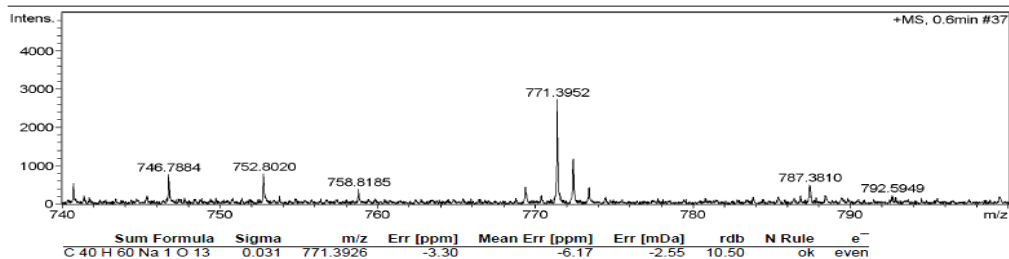


Figure 11S. HR-ESIMS spectrum of 2

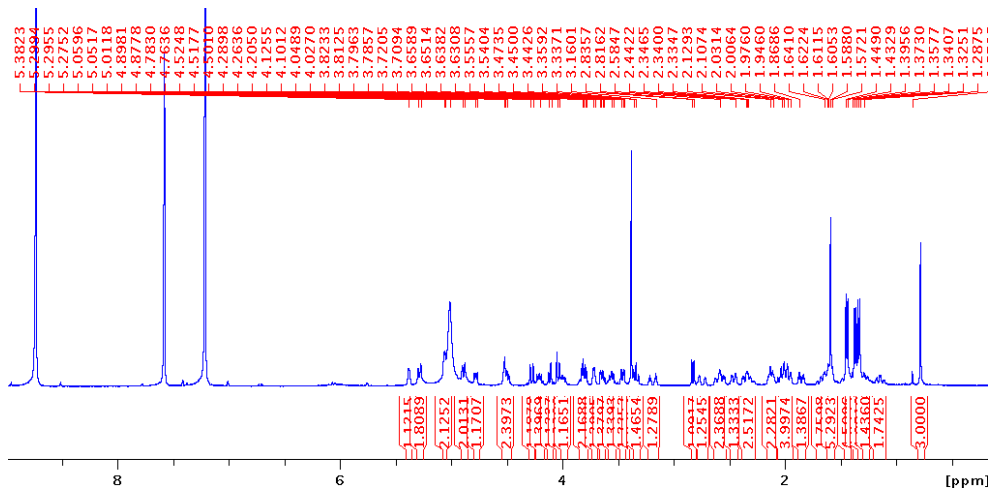


Figure 12S. The ^1H NMR ($\text{C}_5\text{D}_5\text{N}$, 400 MHz) data of 2

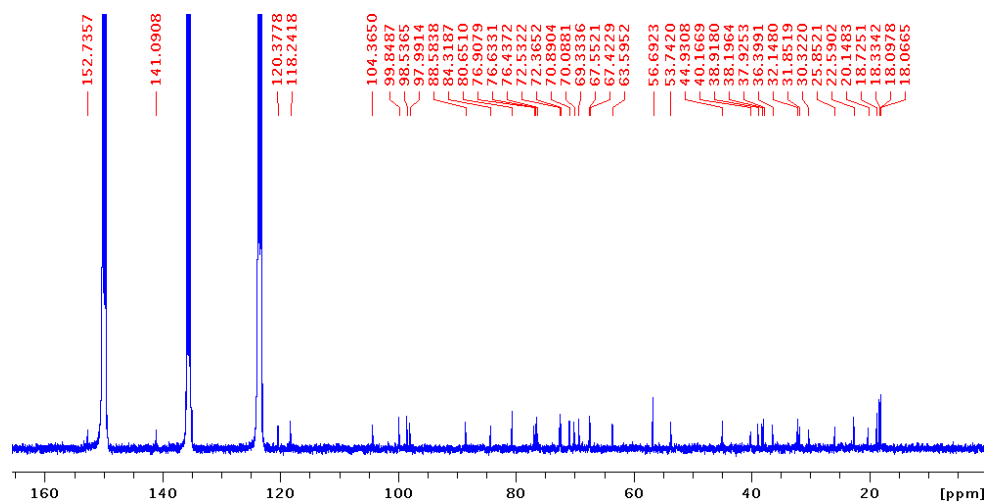


Figure 13S. The ^{13}C NMR ($\text{C}_5\text{D}_5\text{N}$, 100 MHz) data of 2

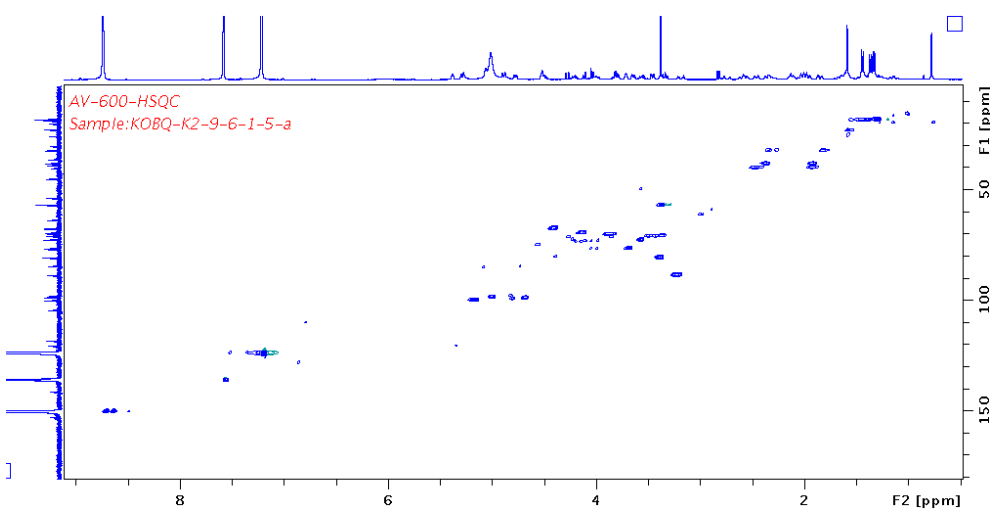


Figure 14S. HSQC data of 2

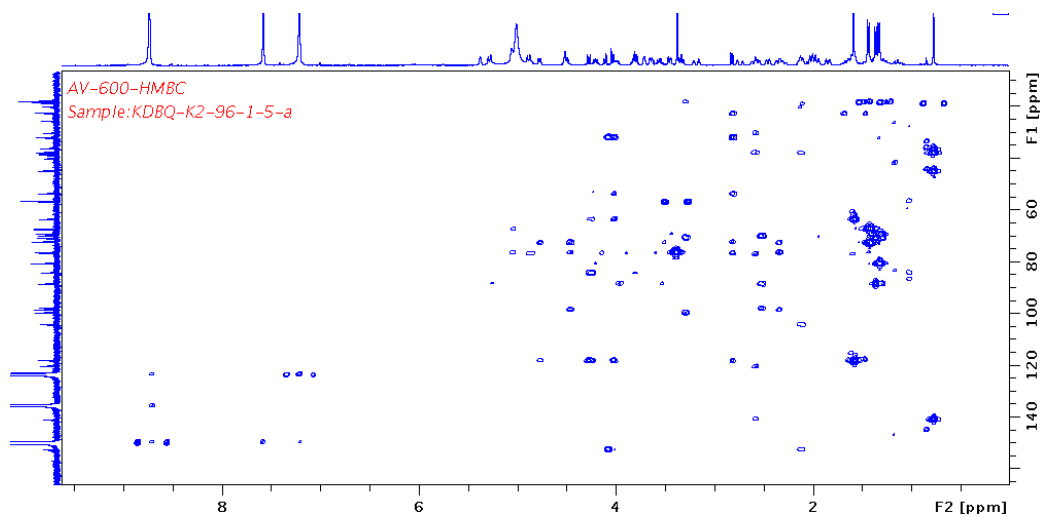


Figure 15S. HMBC data of **2**

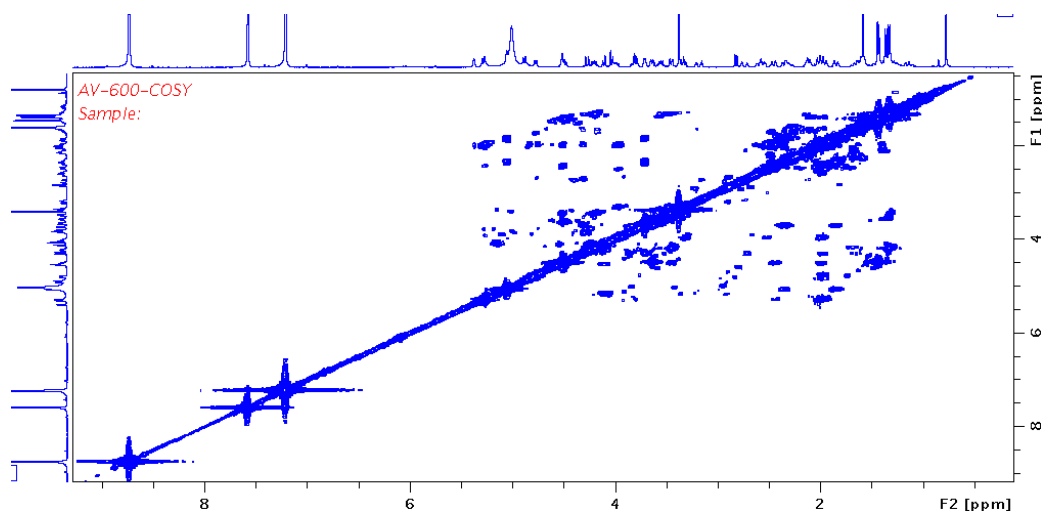


Figure 16S. ^1H - ^1H COSY data of **2**

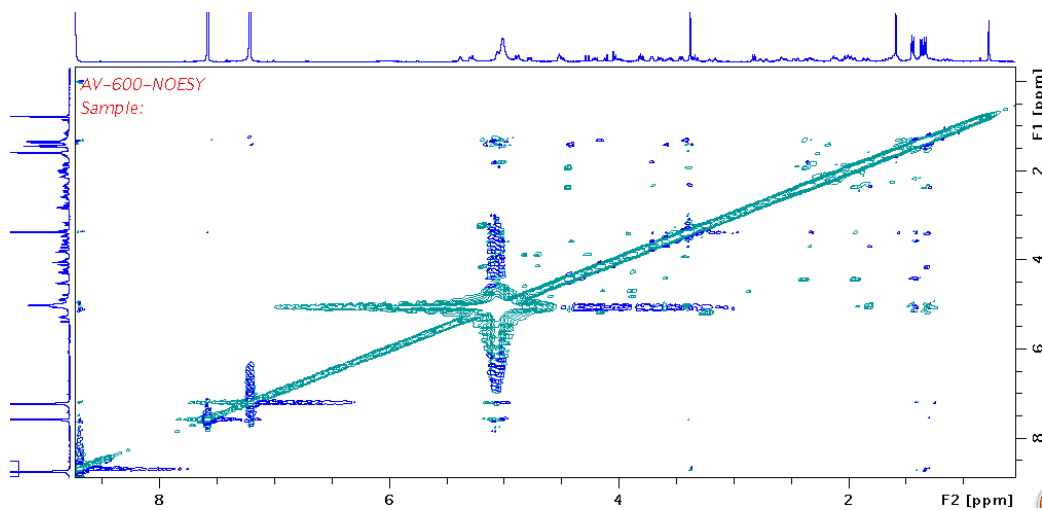


Figure 17S. NOESY data of **2**

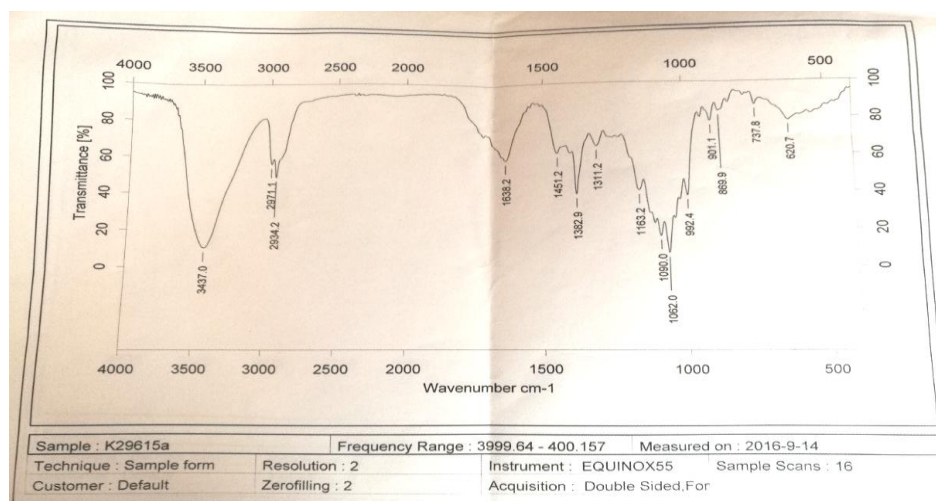


Figure 18S. the IR spectra of 2

Table 1S. ^1H and ^{13}C NMR spectral data of **1** and **2** (400 MHz for ^1H and 100 MHz for ^{13}C , $\text{C}_5\text{D}_5\text{N}$)

No.	1		2	
	δ_{C}	δ_{H} (J Hz)	δ_{C}	δ_{H} (J Hz)
1	33.5	1.34(m), 1.54(m)	37.9	2.02(m)
2	27.2	2.24(m), 1.94(m)	31.8	2.32(m), 1.83(dt, 14.2, 3.7)
3	75.4	4.56(brt, 7.8)	76.9	3.71(m)
4	125.1	5.80(brs)	38.9	2.56(m), 2.30(m)
5	144.5		141.1	
6	125.7	5.90(d, 9.7)	120.4	5.38(brs)
7	122.6	6.65(d, 9.7)	25.8	1.62(m), 1.59(m)
8	108.2		104.4	
9	44.2	2.13(dd, 11.2, 5.8)	44.9	2.12(m)
10	35.5		36.4	
11	20.4	1.62(o), 1.21(dd, 13.7, 11.2)	20.1	1.29(m), 1.15(m)
12	30.8	1.99(m), 1.45(m)	30.3	2.11(m)
13	54.8		53.7	
14	155.2		152.7	
15	72.1	4.28(d, 10.8), 3.82(dd, 4.3, 10.8)	72.4	4.28(d, 10.5), 3.64(dd, 8.3, 3.4)
16	86.1	4.82(dd, 7.8, 4.3)	84.3	4.77(dd, 7.6, 3.4)
17	62.0	2.80(d, 7.8)	63.6	2.80(d, 7.8)
18	77.3	4.02(d, 8.8), 4.07(d, 8.8)	76.6	4.12(d, 9.7), 4.03(d, 9.7)
19	17.6	0.81(s)	18.7	0.77(s)
20	118.4		118.3	
21	22.6	1.59(s)	22.6	1.59(s)
	β -D-the	β -D-the	β -D-3-demeth	β -D-3-demeth
1'	103.3	4.85(d, 7.8)	98.0	4.87(dd, 9.8, 1.7)
2'	74.6	3.95(m)	40.2	2.45(m), 2.01(m)

3'	85.8	3.73(m)	69.3	4.17(m)
4'	82.8	3.74(m)	88.6	3.35(m)
5'	71.7	3.70(m)	70.9	3.56(m)
6'	18.7	1.49(d, 5.6)	18.1	1.33(d, 6.2)
3-OCH ₃	60.5	3.96(s)		
	<i>β</i> -D- cym	<i>β</i> -D-cym	<i>β</i> -D-digt	<i>β</i> -D-digt
1''	99.6	5.15(d, 8.1)	99.8	5.29(dd, 9.7, 1.6)
2''	34.9	2.41(m), 1.68(m)	38.2	2.47(m), 1.95(m)
3''	77.4	3.94(m)	67.5	4.52(m)
4''	82.2	3.40(m)	80.4	3.45(m)
5''	69.3	4.22(m)	70.9	4.18(m)
6''	18.6	1.31(d, 6.2)	18.07	1.36(d, 6.1)
3-OCH ₃	57.3	3.54(s)		
	<i>β</i> -D-digt	<i>β</i> -D-digt	<i>α</i> -L-cym	<i>α</i> -L-cym
1'''	98.9	5.54(d, 8.2)	98.5	5.02(d, 3.2)
2'''	39.1	2.46(m), 2.04(m)	32.1	2.36(m), 1.84(m)
3'''	67.68	4.66(dd, 9.9, 3.2)	76.4	3.80(m)
4'''	83.2	3.50(dd, 9.9, 2.6)	72.5	3.71(m)
5'''	68.8	4.32(m)	67.4	4.48(m)
6'''	18.5	1.42(6.2)	18.3	1.43(d, 6.4)
3-OCH ₃			56.7	3.38(s)
	<i>α</i> -L-dign	<i>α</i> -L-dign		
1''''	101.2	5.21(d, 3.2)		
2''''	30.9	2.41(m), 2.11(m)		
3''''	75.8	3.86(m)		
4''''	67.5	4.08(m)		
5''''	67.72	4.31(m)		
6''''	17.74	1.57(d, 6.3)		
3-OCH ₃	55.0	3.32(s)		