SUPPLEMENTARY MATERIAL

Sulphated flavones and pregnane-type steroids from *Helicteres viscida*

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ABSTRACT. In our search for anti-inflammatory constituents from Vietnamese plants, the methanolic extract of *Helicteres viscida* was found to exhibit inhibitory effect on LPS-induced NO production in RAW264.7 cells. Phytochemical investigation of this plant led to isolation of four sulphated flavones (**1**–**4**), including one new compound 5,3′,4′-trihydroxy-7-methoxy-8-*O*-sulphate flavone (**1**), and two pregnane-type steroids (**5** and **6**), including one new compound 7-*epi*-heligenin B (**5**). Their structures were elucidated by 1D and 2D NMR as well as HR-QTOF-MS experiments. Among isolated compounds, heligenin B (**6**) exhibited potent inhibitory effect on LPS-induced NO production in RAW264.7 cells with IC_{50} of 1.23 ± 0.05 µM. The activity of **6** was comparable to that of the positive control cardamonin.

Keywords: *Helicteres viscida*, Malvaceae, sulphated flavone, pregnane-type steroid.

CONTENTS

General experimental procedures

Optical rotations were determined on a JASCO P-2000 polarimeter (Tokyo, Japan). The HR-QTOF mass spectra were recorded on an Agilent 6530 Accurate-Mass spectrometer (CA, USA). The ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on an AVANCE III HD 500 (Bruker, Germany) FT-NMR spectrometer with tetramethylsilane (TMS) as an internal standard. Medium pressure liquid chromatography (MPLC) was carried out on a Biotage - Isolera One system (SE-751 03 Uppsala, Sweden). Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70–230 mesh and 230–400 mesh, Merck, Darmstadt, Germany) and YMC*GEL (ODS-A, 12 nm S-150 mm, YMC Co., Ltd., Japan) resins. TLC used pre-coated silica gel 60 F_{254} (Merck) and RP-18 F_{254S} plates (Merck), and compounds were visualized by spraying with aqueous 10% H_2SO_4 and heating for 3–5 min.

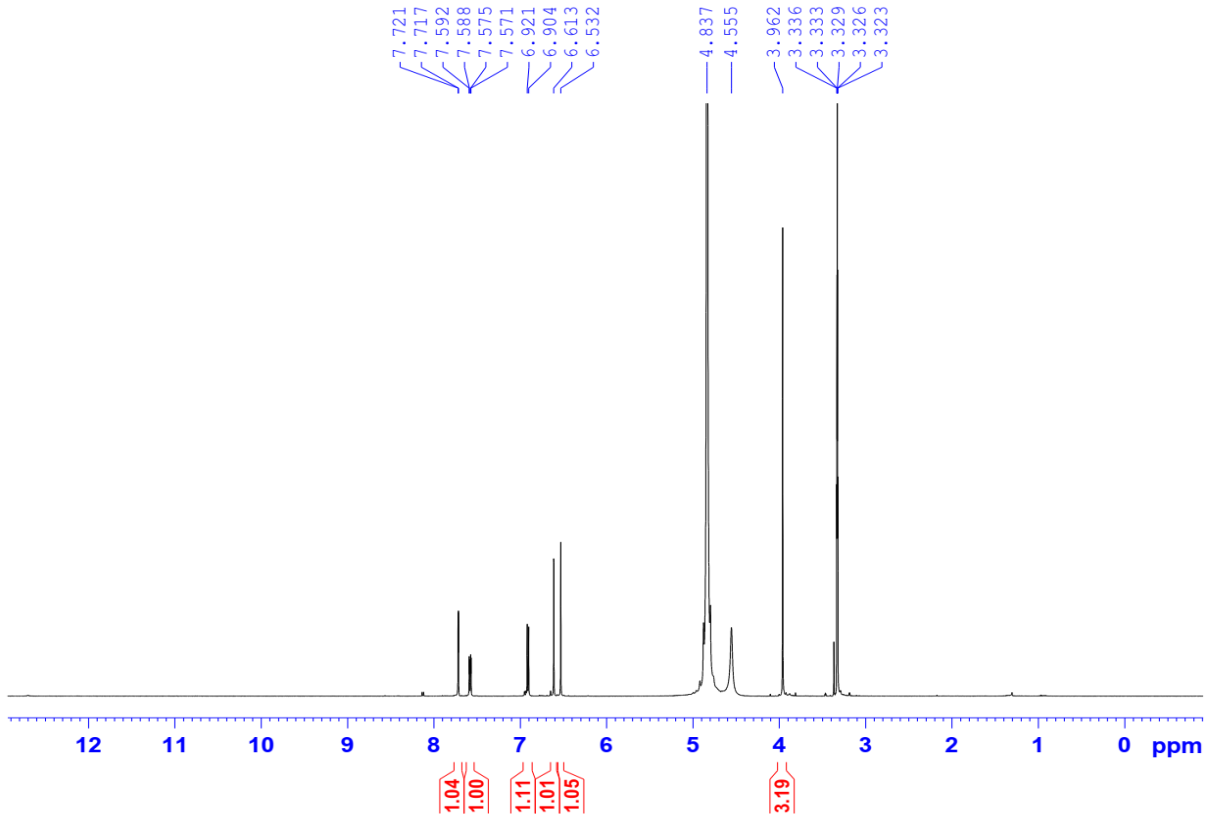

Figure S1. ^{1}H NMR spectrum (CD_3OD, 500 MHz) of compound **1**

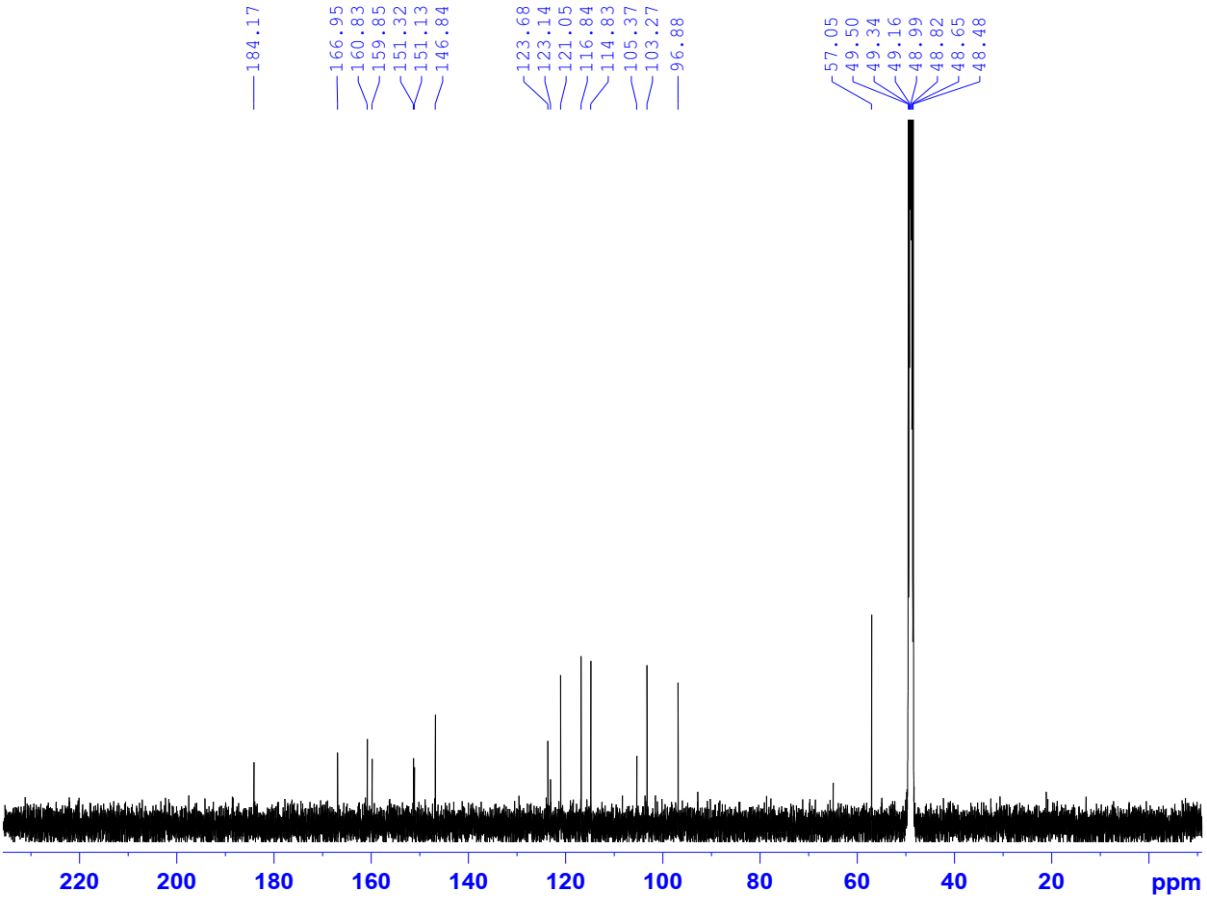

Figure S2. ^{13}C NMR spectrum (CD_3OD, 125 MHz) of compound **1**

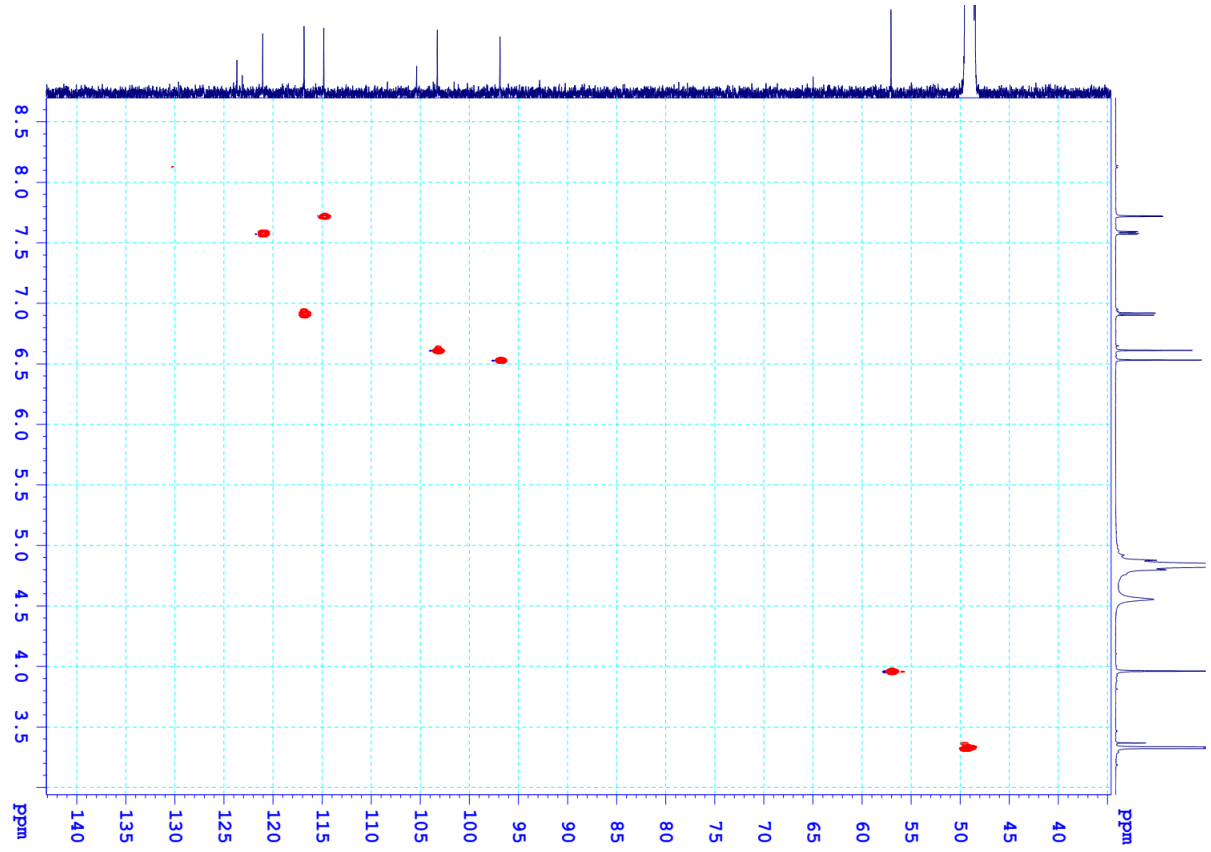

Figure S3. HSQC spectrum (CD_3OD, 500 MHz) of compound **1**

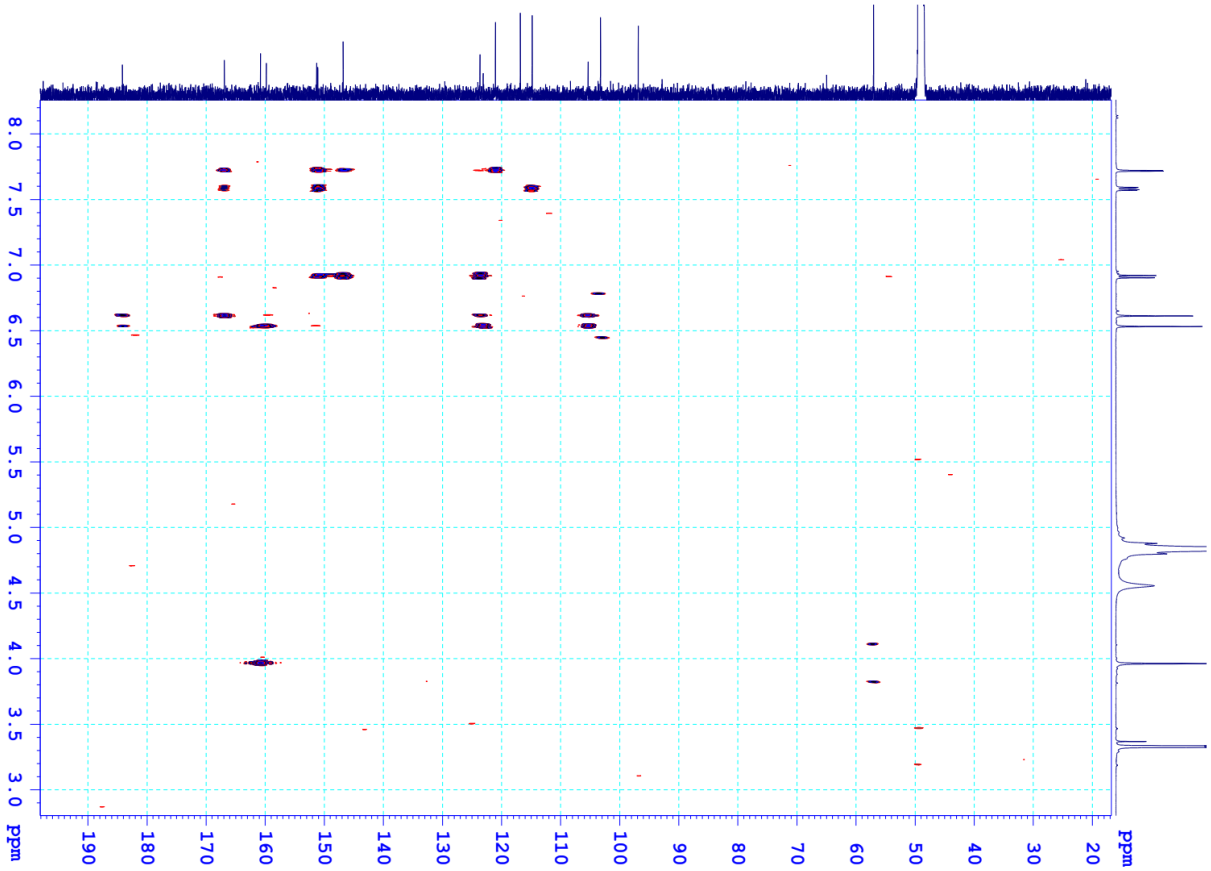

Figure S4. HMBC spectrum (CD_3OD, 500 MHz) of compound **1**

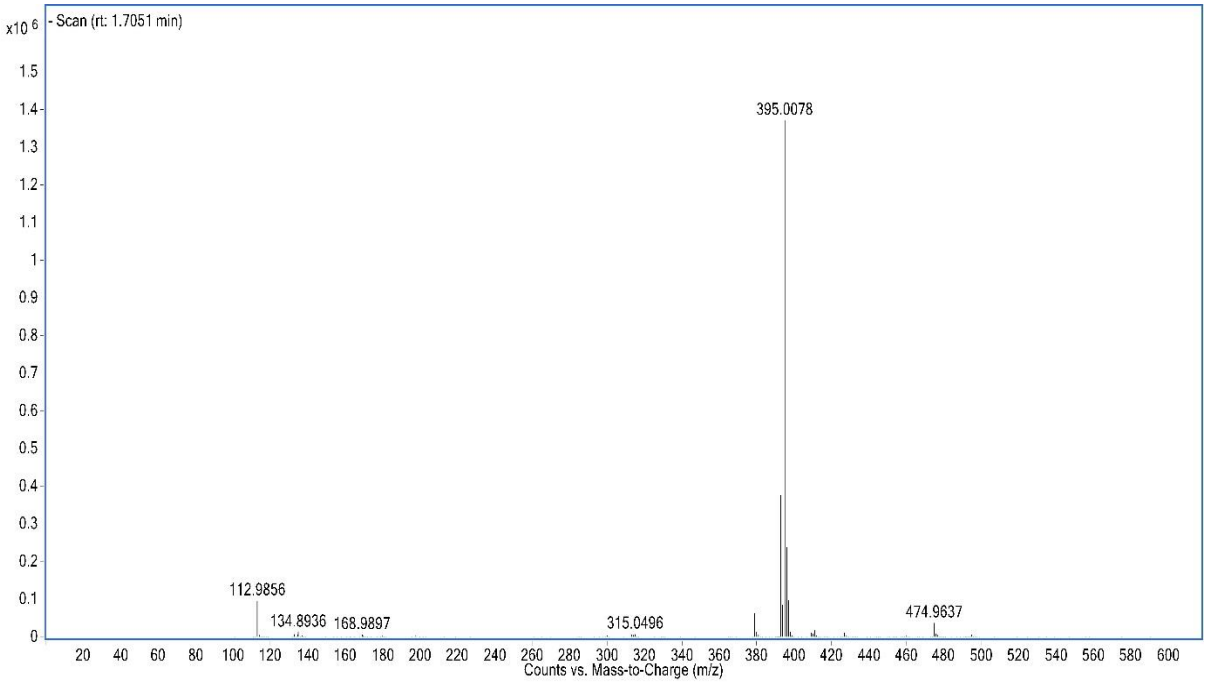

Figure S5. HR-QTOF mass spectrum of compound **1**

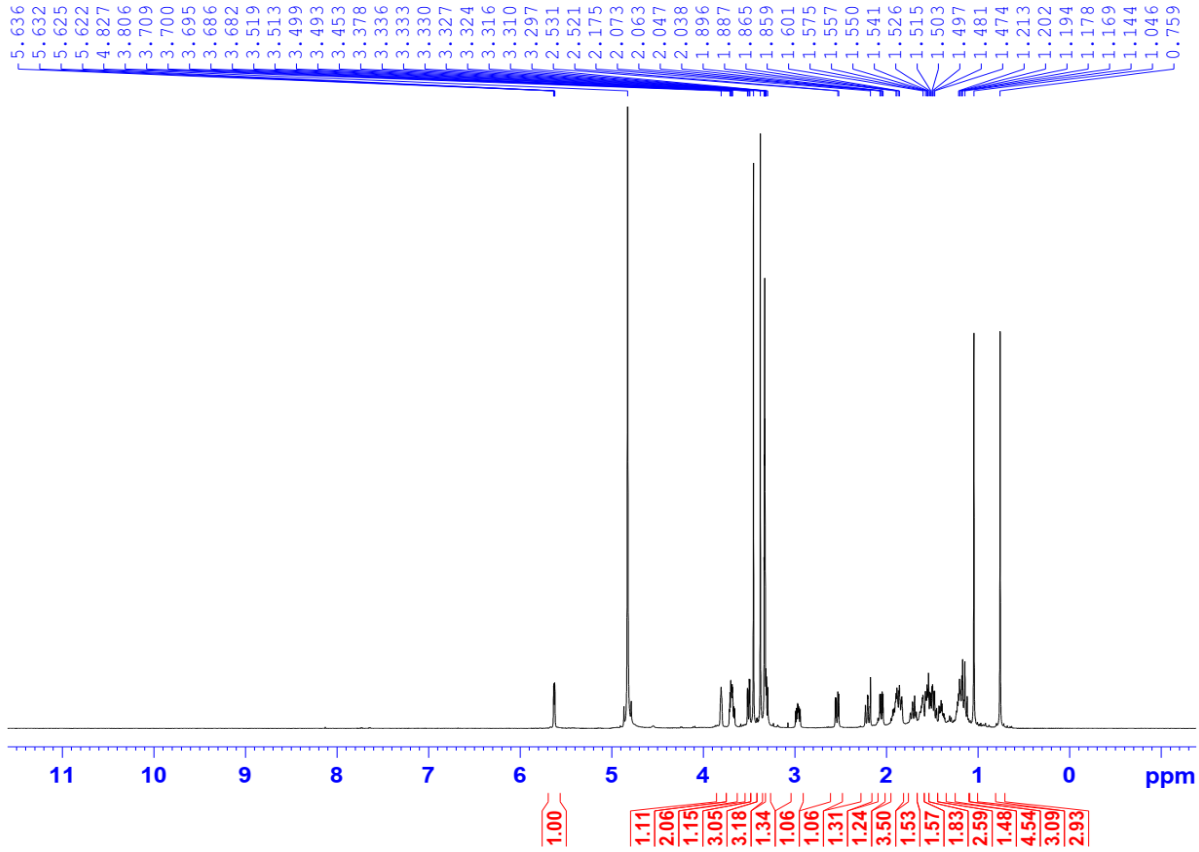

Figure S6. ^{1}H NMR spectrum (CD_3OD, 500 MHz) of compound **5**

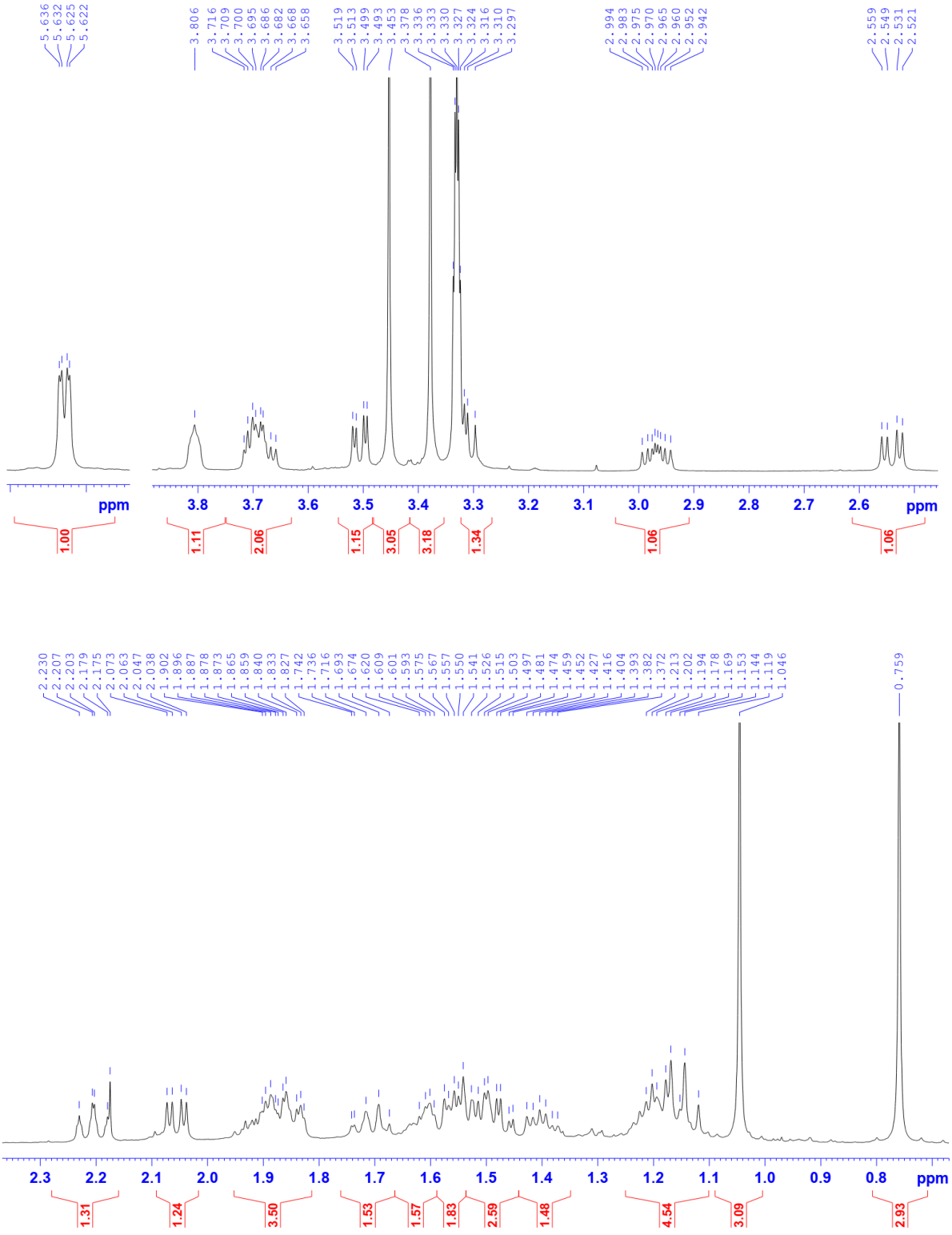

Figure S7. Expaned ^{1}H NMR spectrum (CD_3OD, 500 MHz) of compound **5**

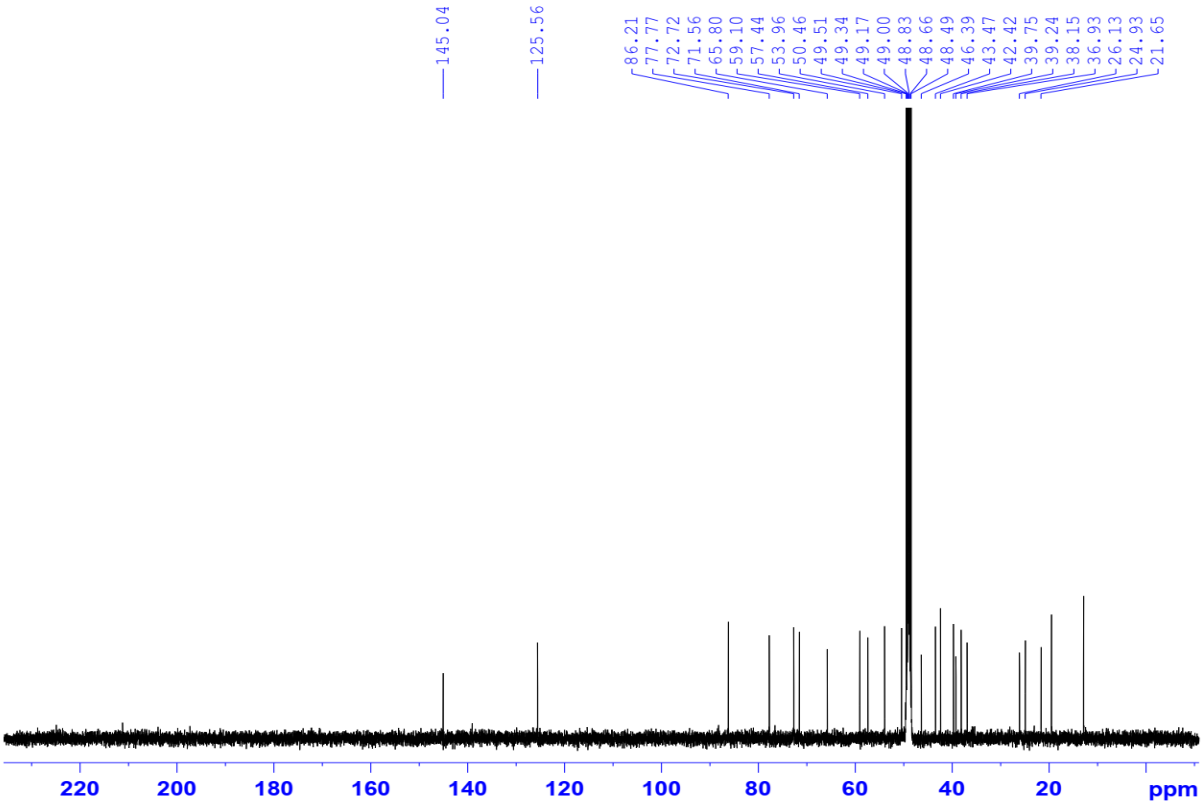

Figure S8. ^{13}C NMR spectrum (CD_3OD, 125 MHz) of compound **5**

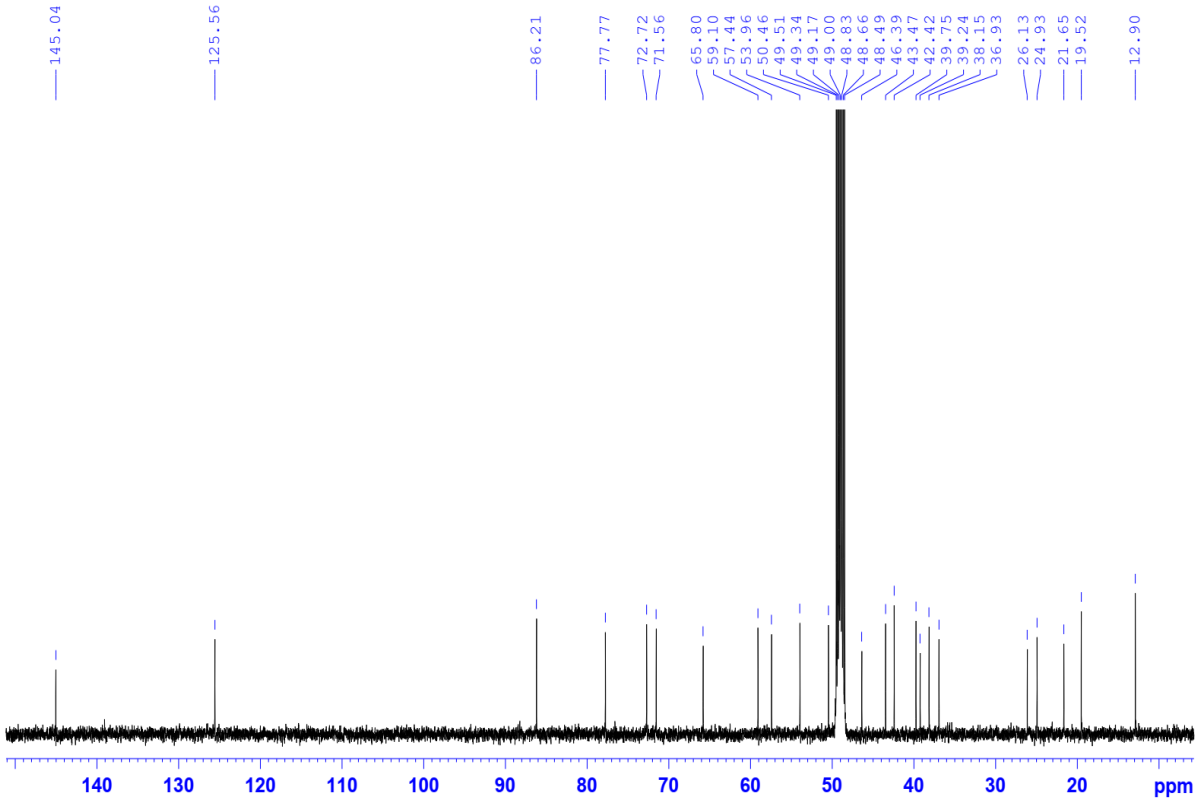

Figure S9. Expaned ^{13}C NMR spectrum (CD_3OD, 125 MHz) of compound **5**

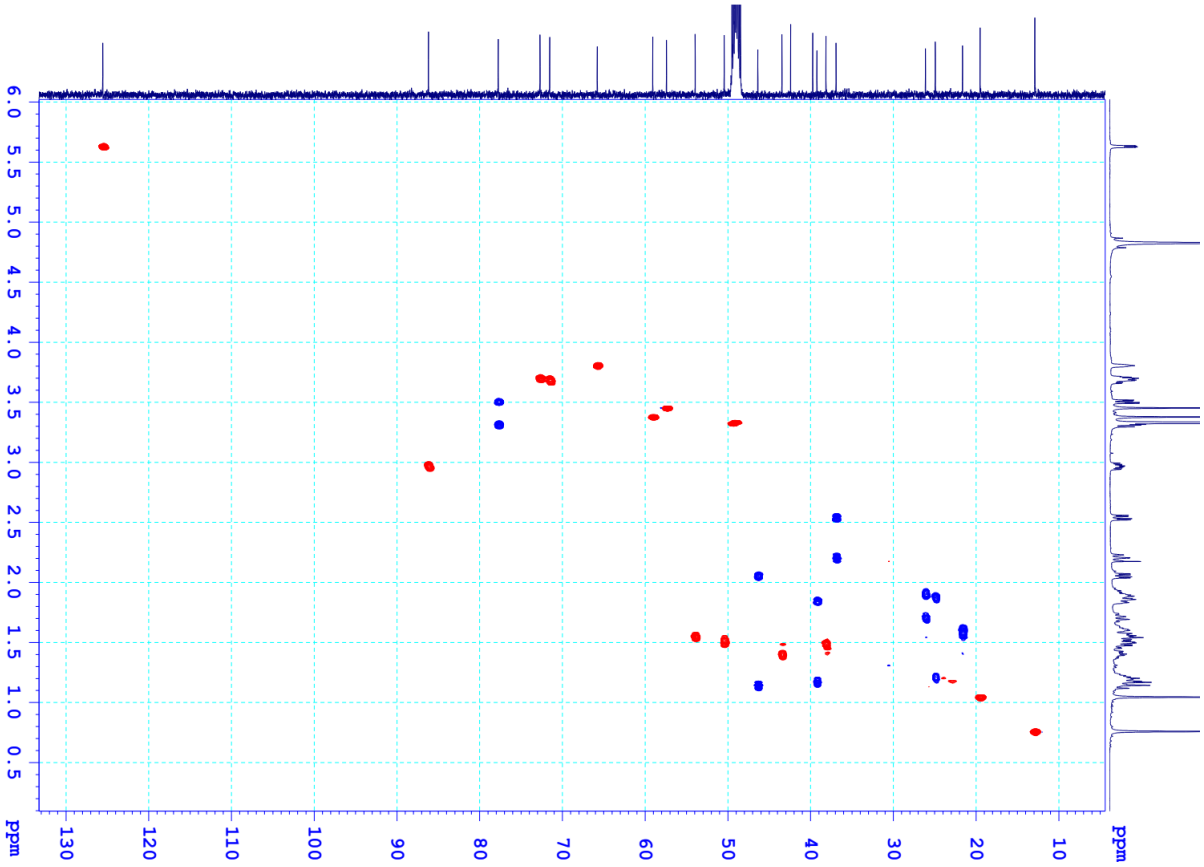

Figure S10. HSQC spectrum (CD_3OD, 500 MHz) of compound **5**

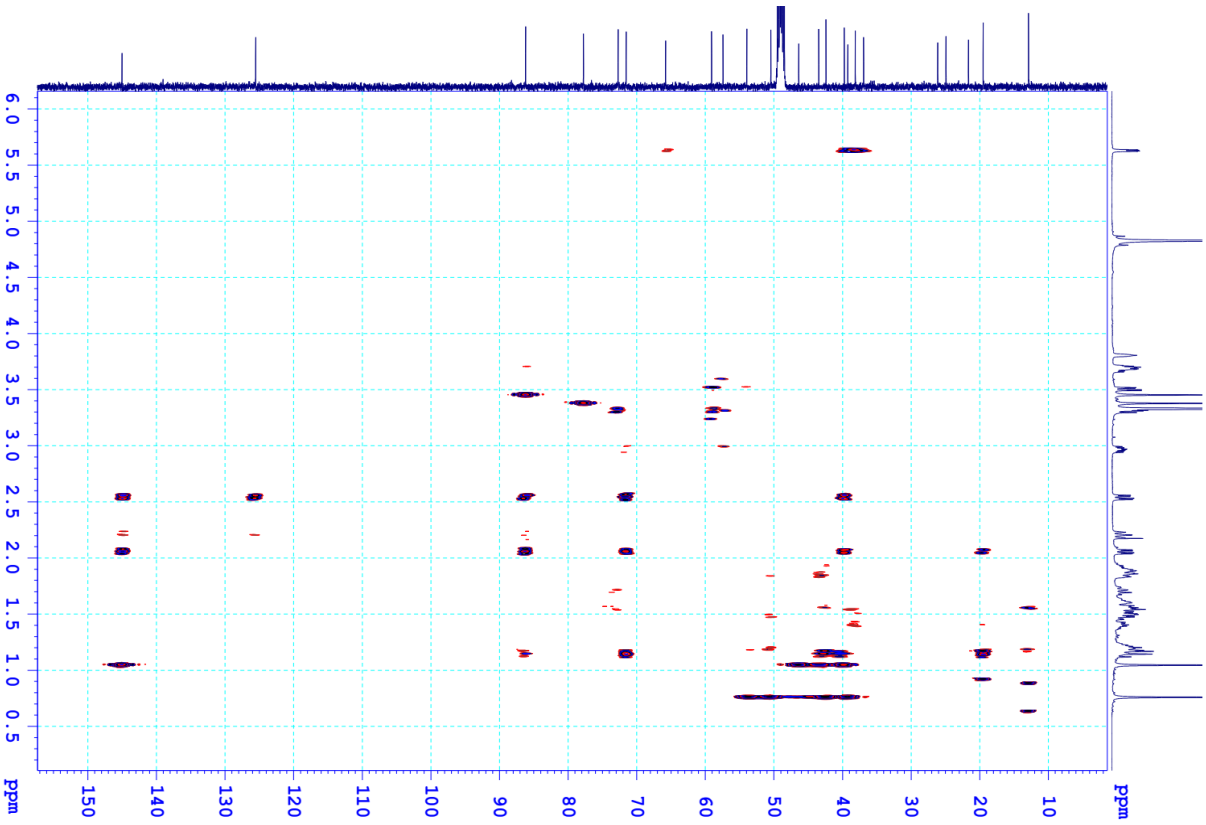

Figure S11. HMBC spectrum (CD_3OD, 500 MHz) of compound **5**

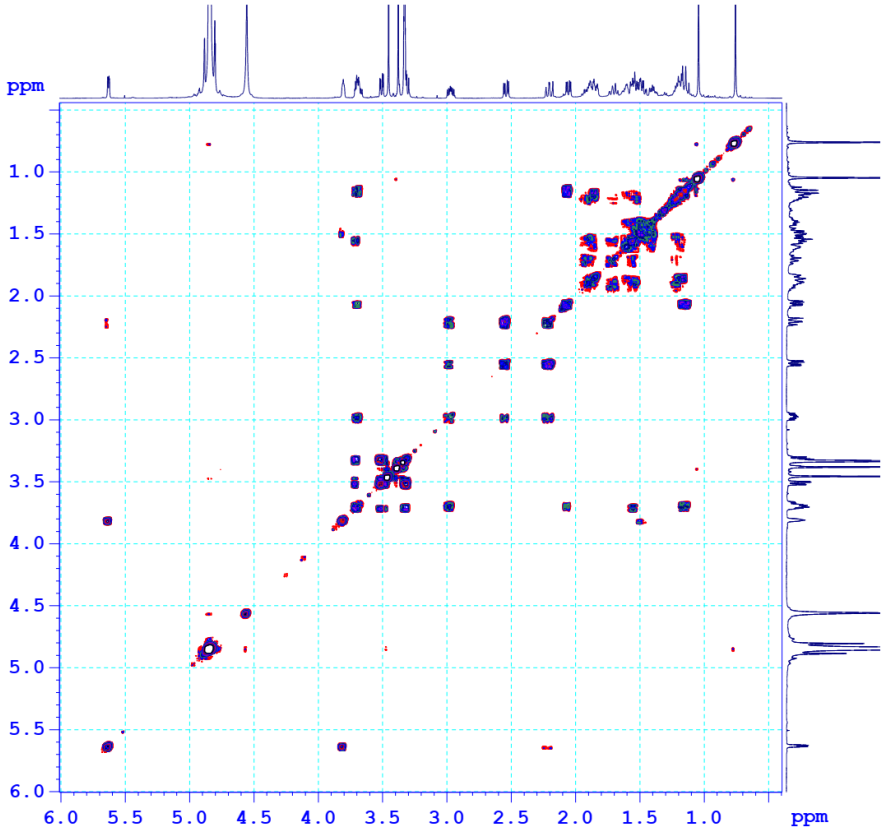

Figure S12. COSY spectrum (CD_3OD, 500 MHz) of compound **5**

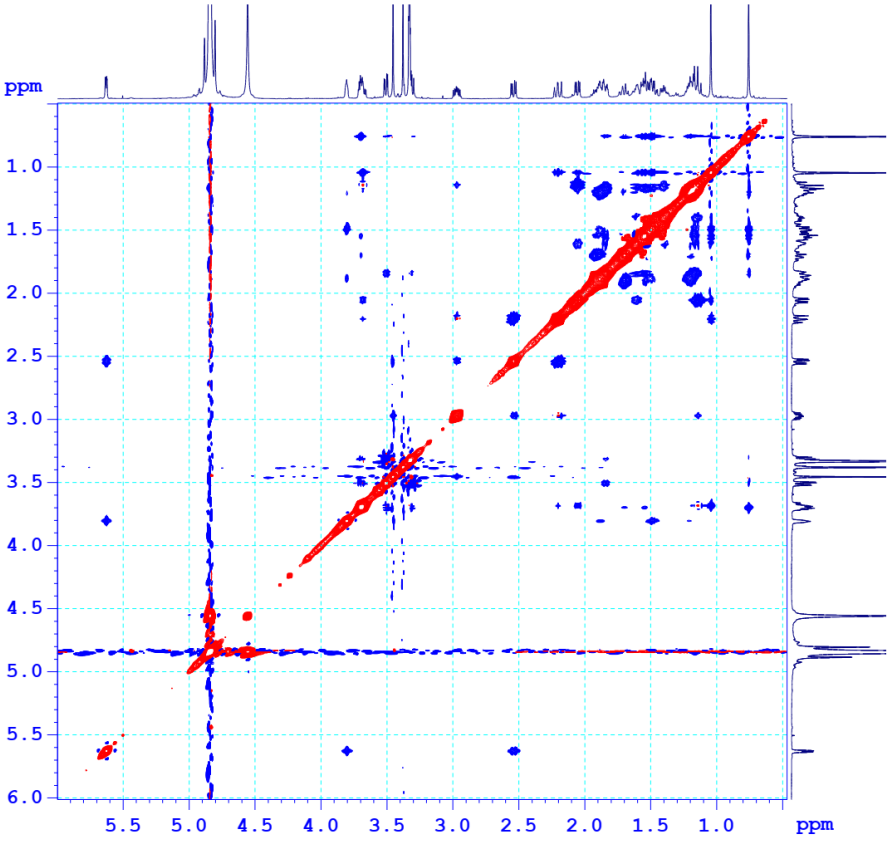

Figure S13. NOESY spectrum (CD_3OD, 500 MHz) of compound **5**

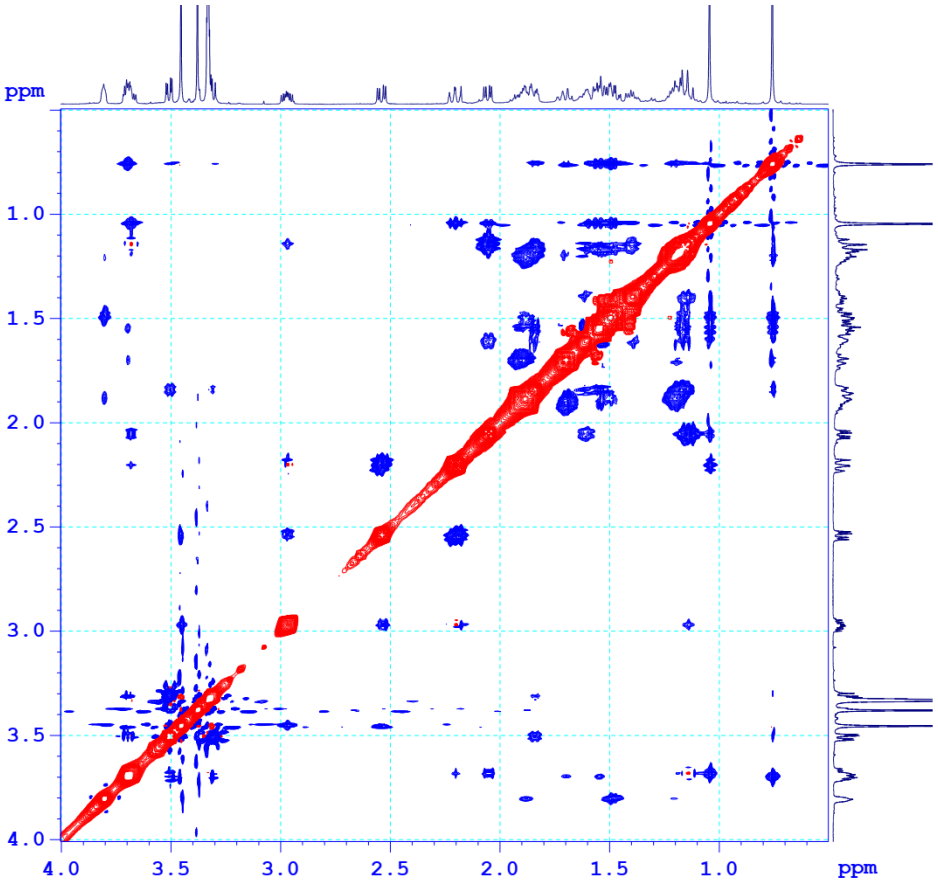

Figure S14. Expaned NOESY spectrum (CD_3OD, 500 MHz) of compound **5**

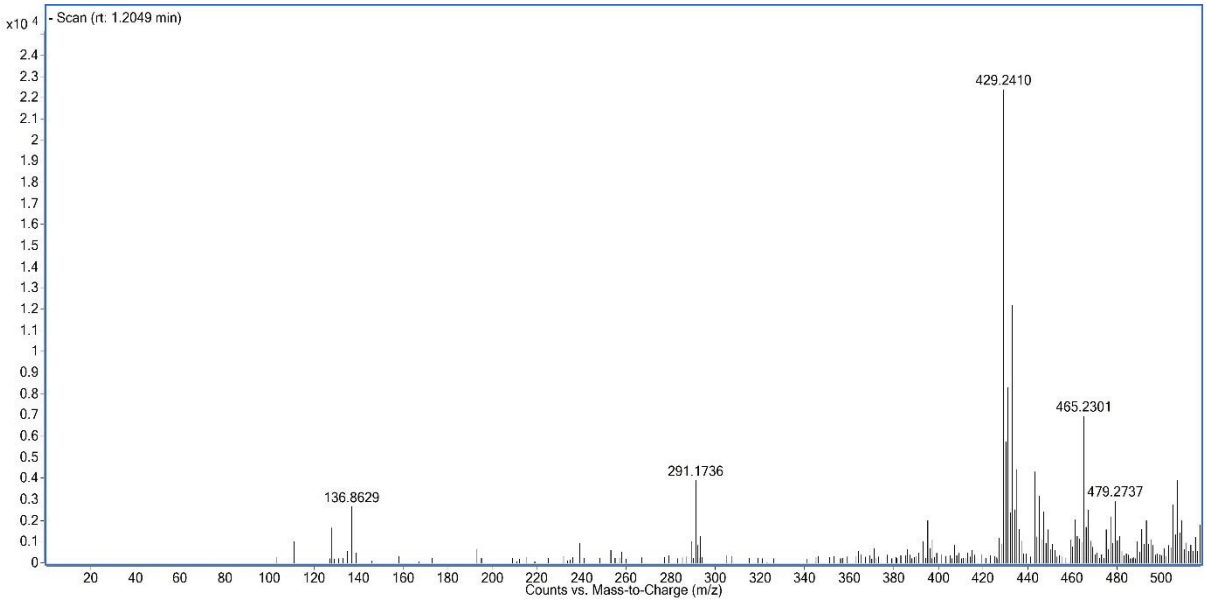

Figure S15. HR-QTOF mass spectrum of compound **5**

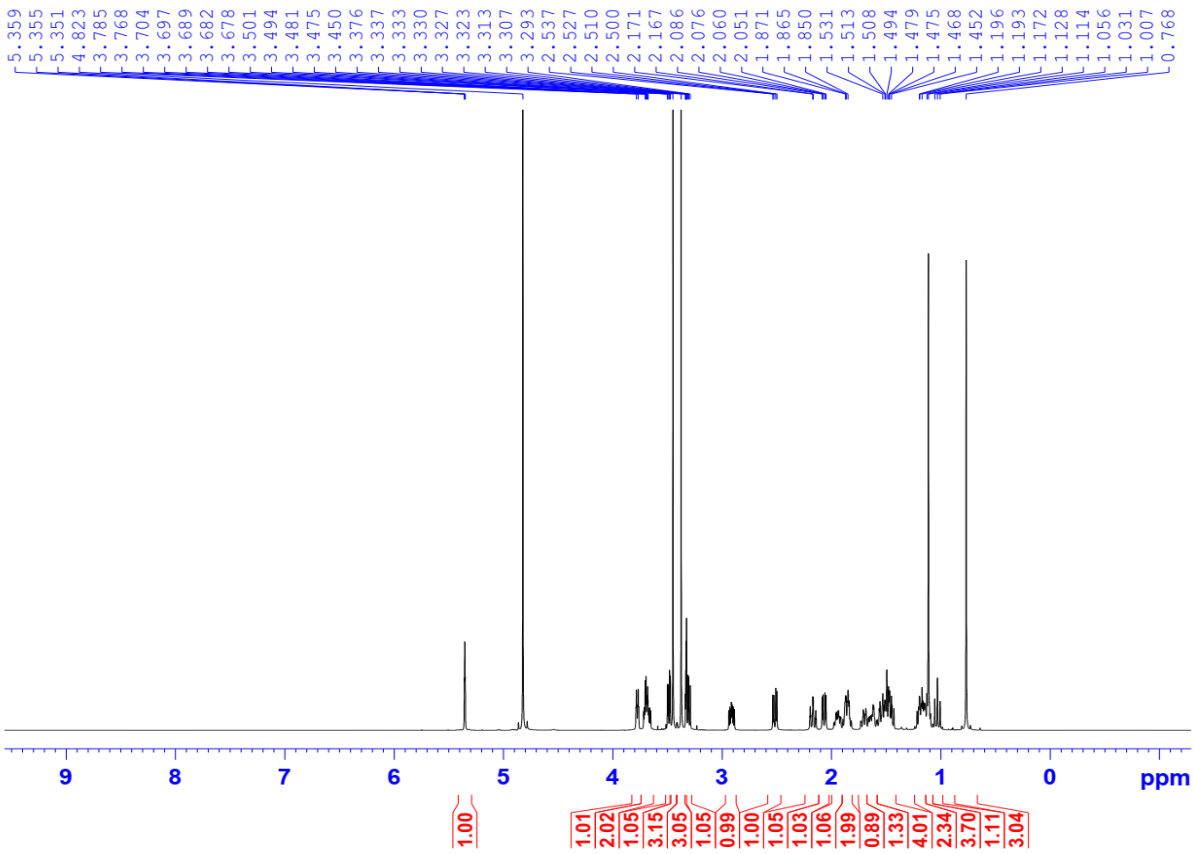

Figure S16. ^{1}H NMR spectrum (CD_3OD, 500 MHz) of compound **6**

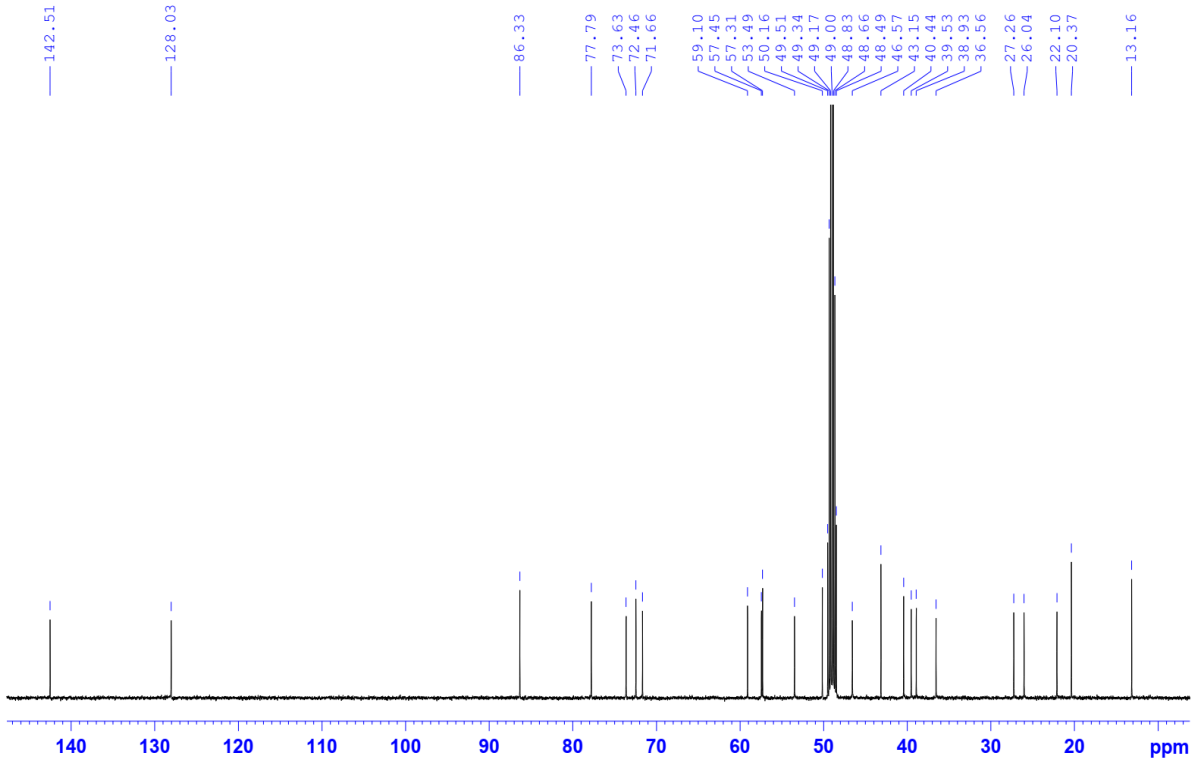

Figure S17. ^{13}C NMR spectrum (CD_3OD, 125 MHz) of compound **6**

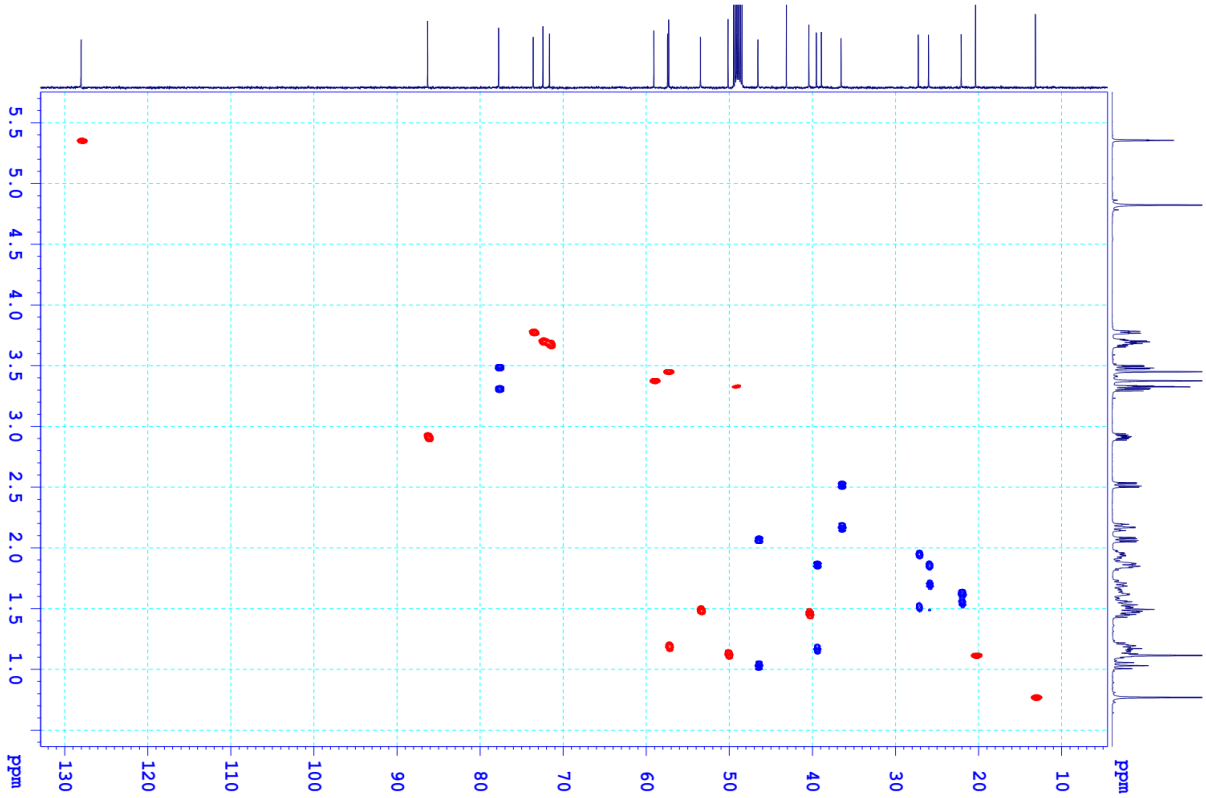

Figure S18. HSQC spectrum (CD_3OD, 500 MHz) of compound **6**

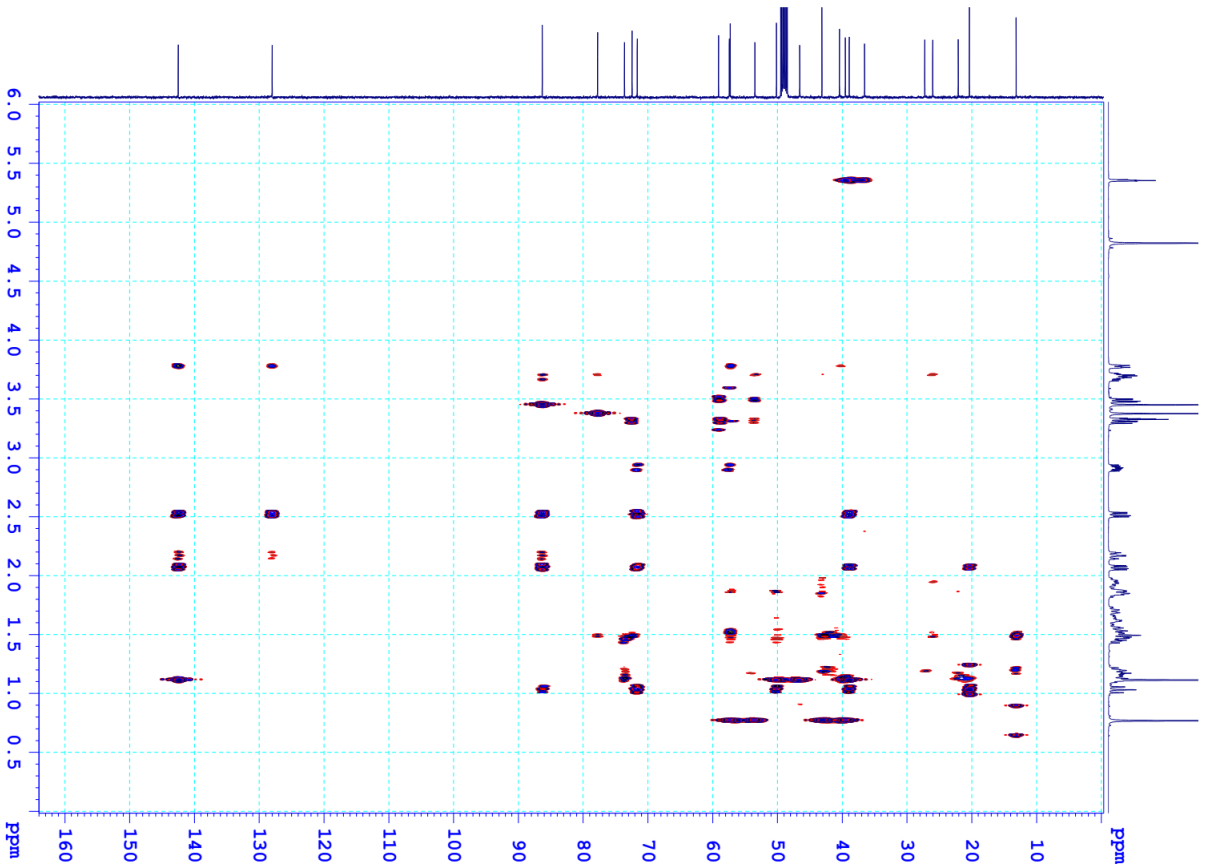

Figure S19. HMBC spectrum (CD_3OD, 500 MHz) of compound **6**

Table S1. ^{13}C NMR (CD_3OD, 125 MHz) spectroscopic data of compounds **1**, **5** and **6**.

C	[a]δ_C	[b]δ_C	δ_C of **1**	C	[c]δ_C	[d]δ_C	[e]δ_C	δ_C of **5**	δ_C of **6**
2	163.4	164.7	166.9	1	37.6	37.0	46.6	46.4	46.6
3	102.9	102.7	103.3	2	32.4	31.4	71.7	71.6	71.6
4	182.3	182.6	184.2	3	71.1	71.4	86.4	86.2	86.3
5	153.0	-	159.8	4	43.4	41.7	36.6	36.9	36.5
6	95.6	95.8	96.9	5	144.9	143.5	142.5	145.0	142.5
7	154.2	159.1	160.8	6	125.5	125.4	128.0	125.6	128.0
8	126.1	123.2	123.1	7	64.8	73.3	73.7	65.8	73.6
9	144.3	148.8	151.3	8	38.4	40.9	40.5	38.1	40.4
10	103.8	104.7	105.4	9	42.7	48.3	50.2	43.5	50.1
1′	122.9	123.6	123.7	10	37.7	36.9	38.9	39.7	38.9
2′	128.4	113.8	114.8	11	21.2	21.1	26.1	21.6	22.1*
3′	114.4	146.8	146.8	12	39.8	39.5	39.5	39.2	39.5
4′	162.3	151.6	151.1	13	42.3	42.0	43.2	42.4	43.1
5′	114.4	111.8	116.8	14	50.2	55.6	57.3	50.5	57.3
6′	128.4	119.8	121.0	15	24.7	26.4	22.1	24.9	26.0*
7-OMe	56.2	56.4	57.0	16	28.7	28.4	27.3	26.1	27.2
4′-OMe	55.5	55.7		17	56.2	55.9	53.5	53.9	53.5
				18	12.0	11.8	13.2	12.9	13.1
				19	18.8	19.1	20.4	19.5	20.4
				20			72.5	72.7	72.4
				21			77.8	77.8	77.8
				3-OMe			57.4	57.4	57.4
				21-OMe			59.1	59.1	59.1

[a]δ_C of 7,4′-di-*O*-methylisoscutellarein in DMSO-d_6 (Hòrie et al. 1998), [b]δ_C of 5,3′-di-hydroxy-7,4′-dimethoxy-8-*O*-sulphate flavone (condadine) in DMSO-d_6 (Fernandes et al. 2018), [c]δ_C of (24*S*)-ergost-5-ene-3β,7α-diol in $CDCl_3$ (Kobayashi et al. 1993), [d]δ_C of (24*S*)-ergost-5-en-3β,7β-diol in $CDCl_3$ (Muralidhar et al. 2005), [e]δ_C of heligenin B in CD_3OD (Wang et al. 2012), *Due to 2D NMR experiments, the reported ^{13}C-NMR chemical shifts at C-11 and C-15 of heligenin B (**6**) (Wang et al. 2012) must be reassigned as shown.

References

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