SUPPLEMENTARY MATERIAL

Two new alkaloids from the roots of *Cocculus hirsutus* (L.) W. Theob.

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Abstract

Two undescribed alkaloids, 15-carboxydihydroerysotrine (**1**) and (14*R*)-4-methoxy-13,14-dihydrooxypalmatine (**2**), along with six known compounds, 1,6-didehydro-3,15,16-trimethoxy-9-methylerythrinanium (**3**), 8-oxytetrahydropalmatine (**4**), 20-hydroxyecdysone (**5**), makisterone A (**6**) turkesterone (**7**) and magnoflorine (**8**) were isolated from the root part of *Cocculus hirsutus* (L.) W. Theob. Their structures were established based on detailed analysis of NMR, UV-Vis, HRESIMS, and single-crystal XRD spectroscopic experiments. Compounds **3, 4** and **7** were reported for the first time from the genus *Cocculus*. All the compounds were analysed *in silico* to investigate their human acetylcholinesterase inhibition potential. This analysis revealed that compounds **1** and **8** interacted well with the selected protein, which suggested their further exploration as acetylcholinesterase inhibitors *via in vitro* and *in vivo* investigation.

Keywords: *Cocculus hirsutus*, erythrinan-type alkaloids, protoberberine alkaloids, acetylcholinesterase, molecular docking.

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List of supplementary material

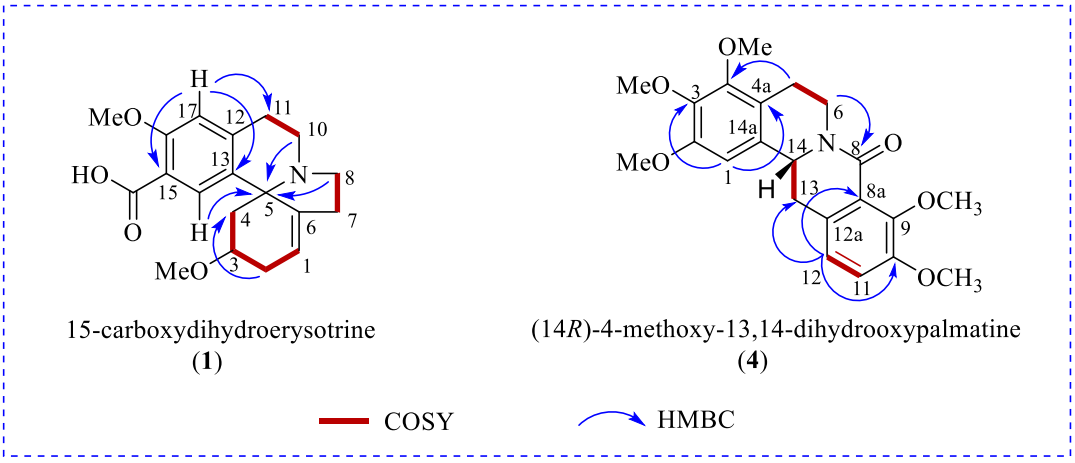


Fig. S1: (^{1}H-^{1}H) COSY and (^{1}H→^{13}C) HMBC correlations of compound **1** and **2.**

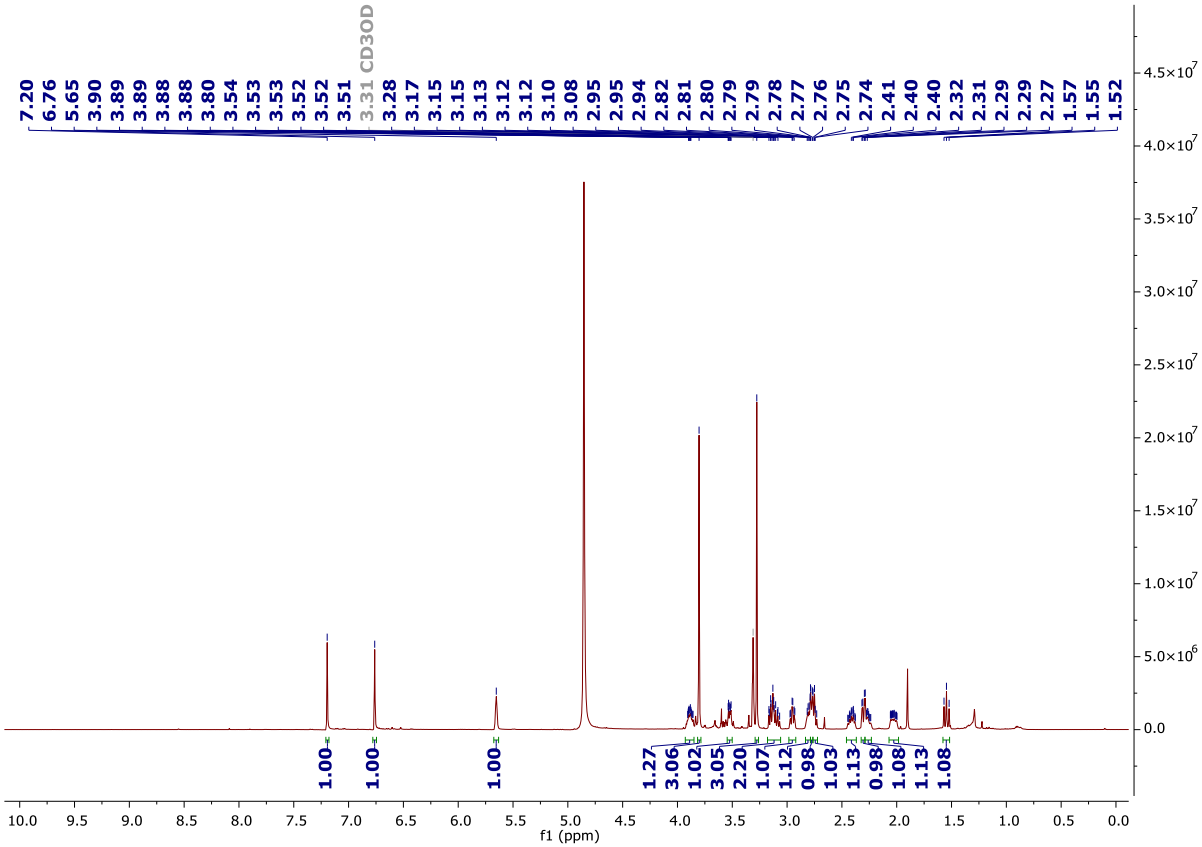


Fig. S2 (a) ^{1}H-NMR spectrum of compound **1**

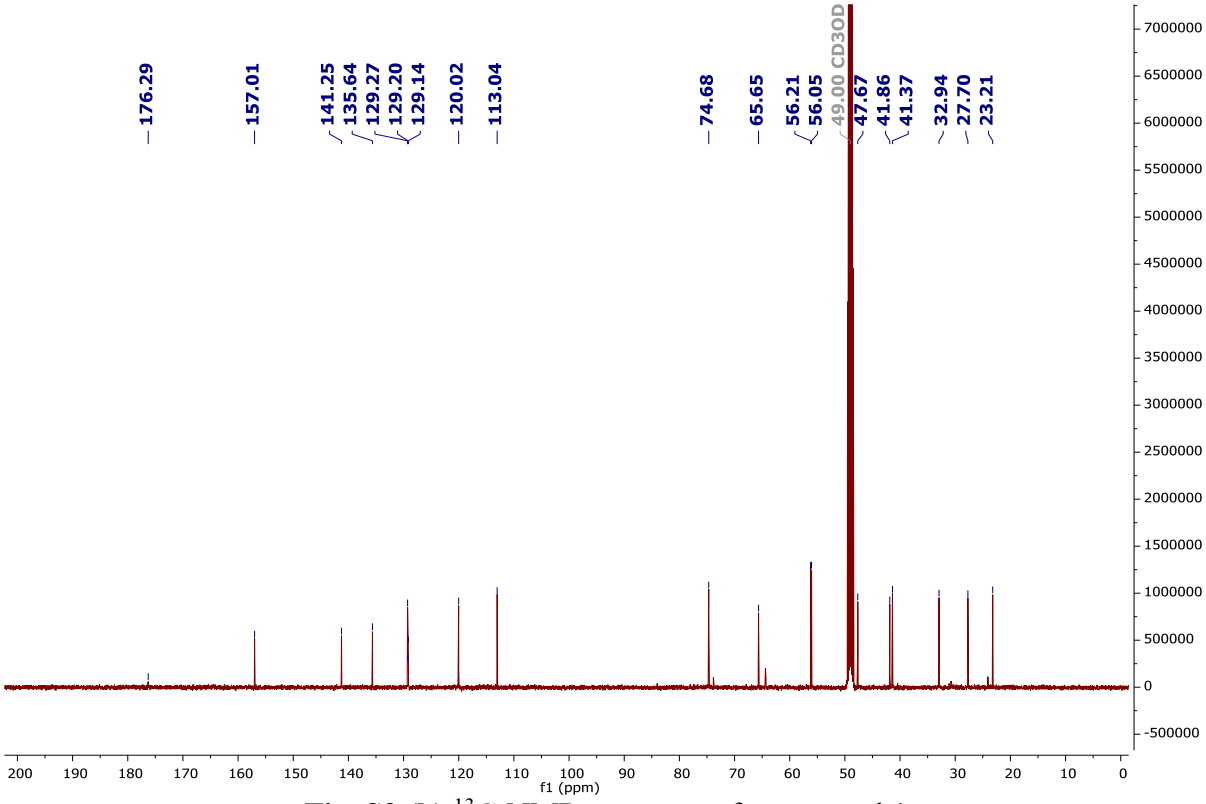


Fig. S2 (b) ^{13}C-NMR spectrum of compound **1**

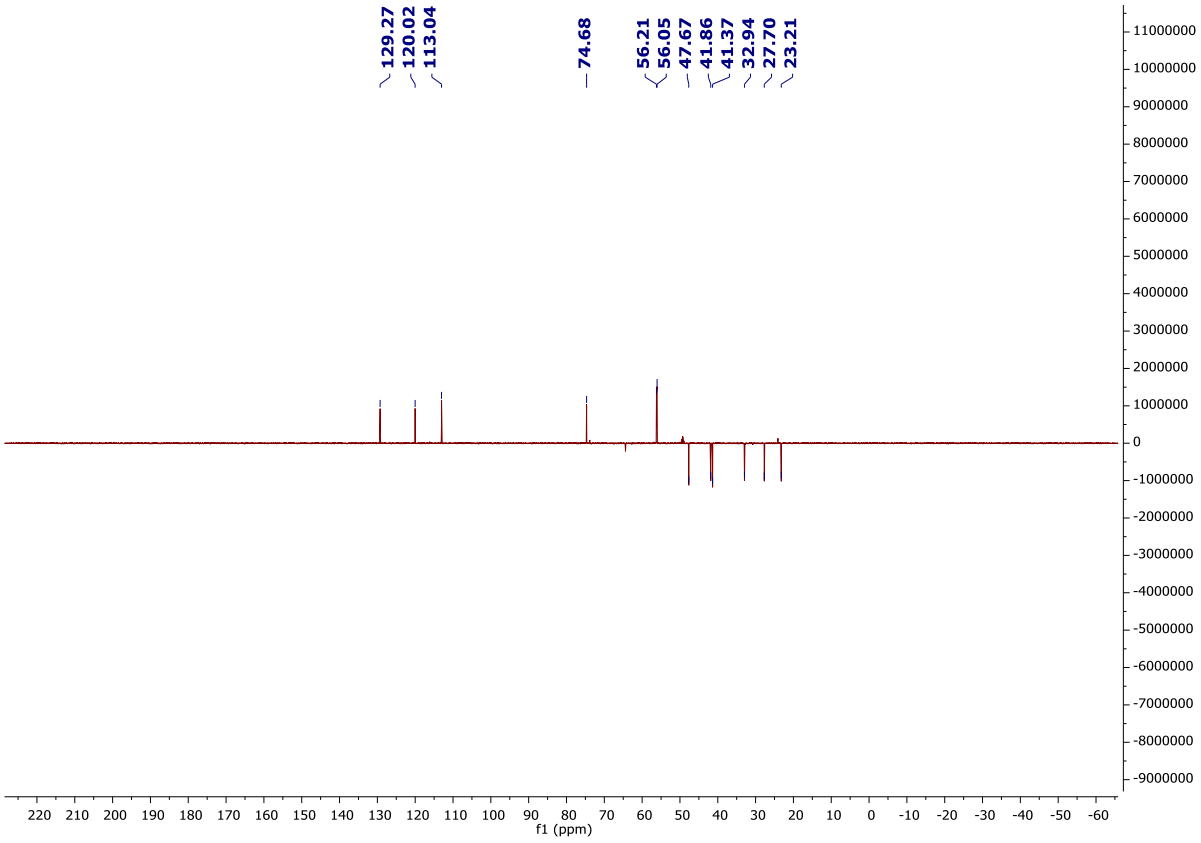


Fig. S2 (c) DEPT-135 NMR spectrum of compound **1**

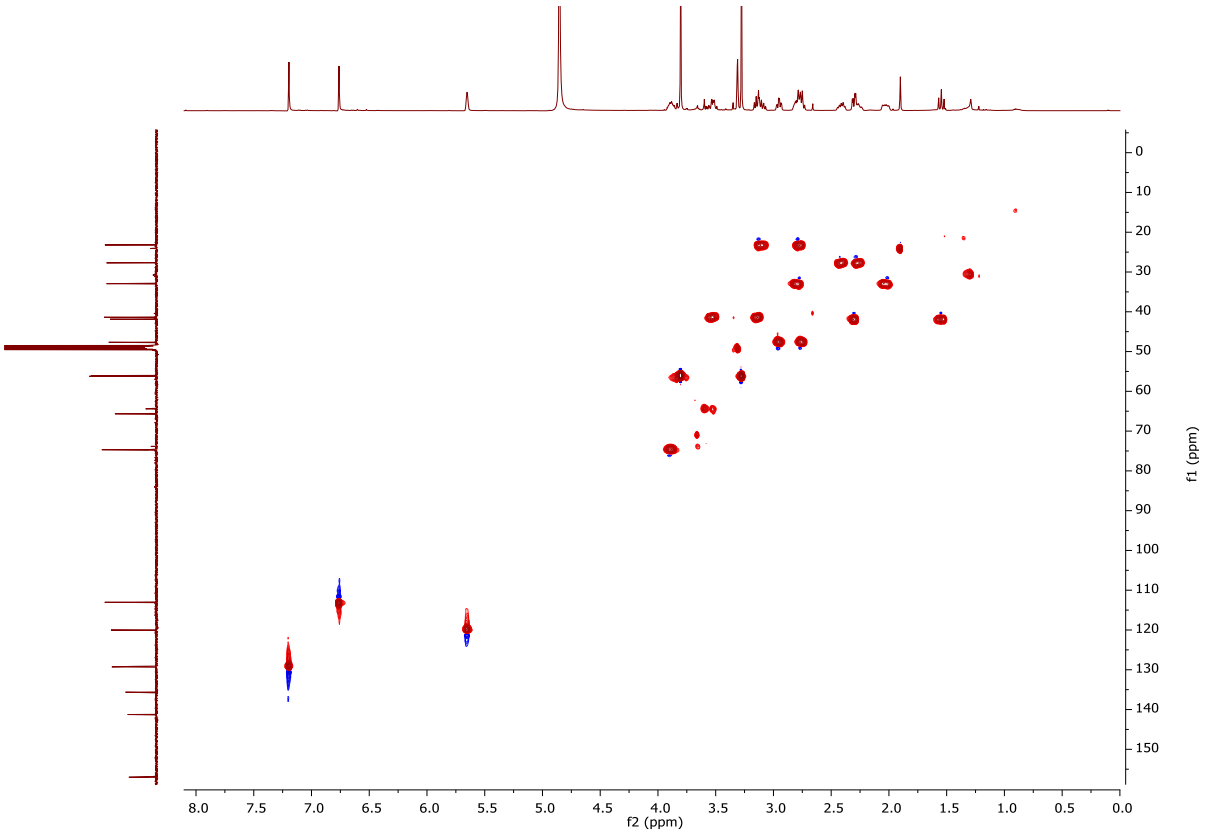


Fig. S2 (d) HSQC spectrum of compound **1**

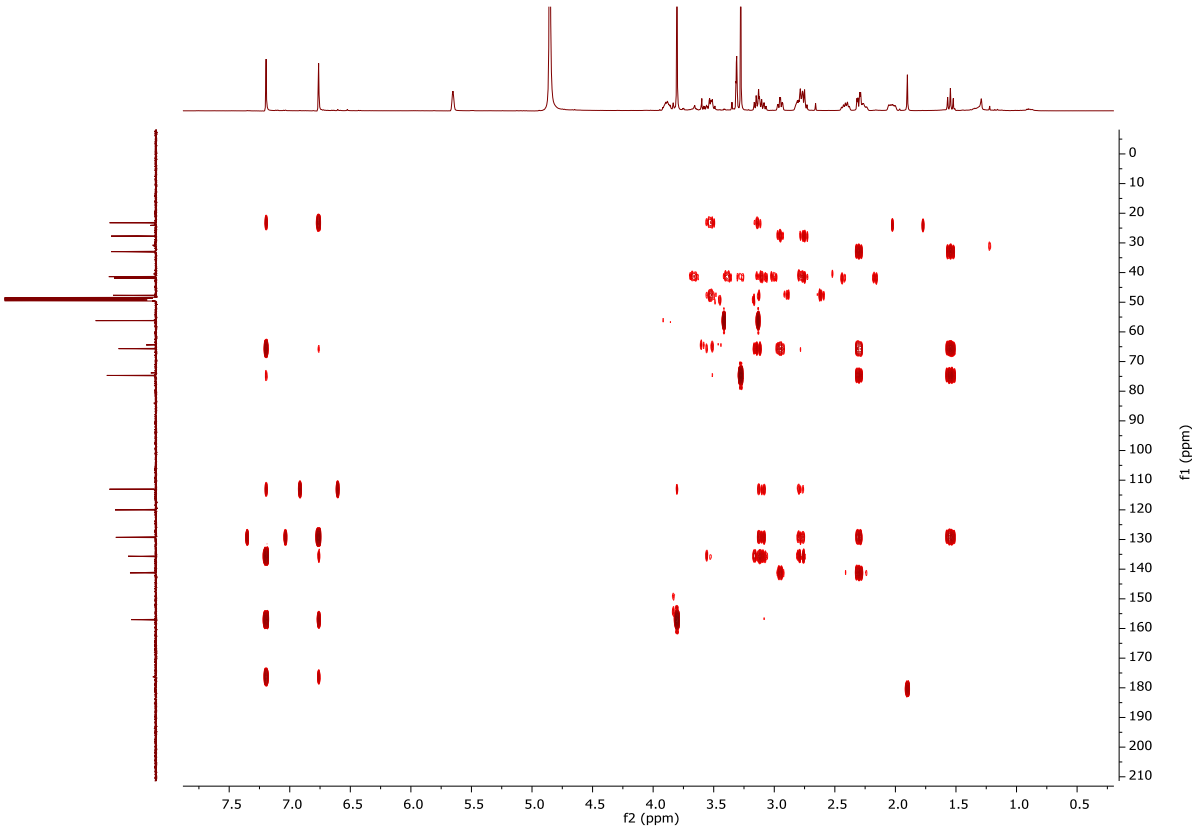


Fig. S2 (e) HMBC spectrum of compound **1**

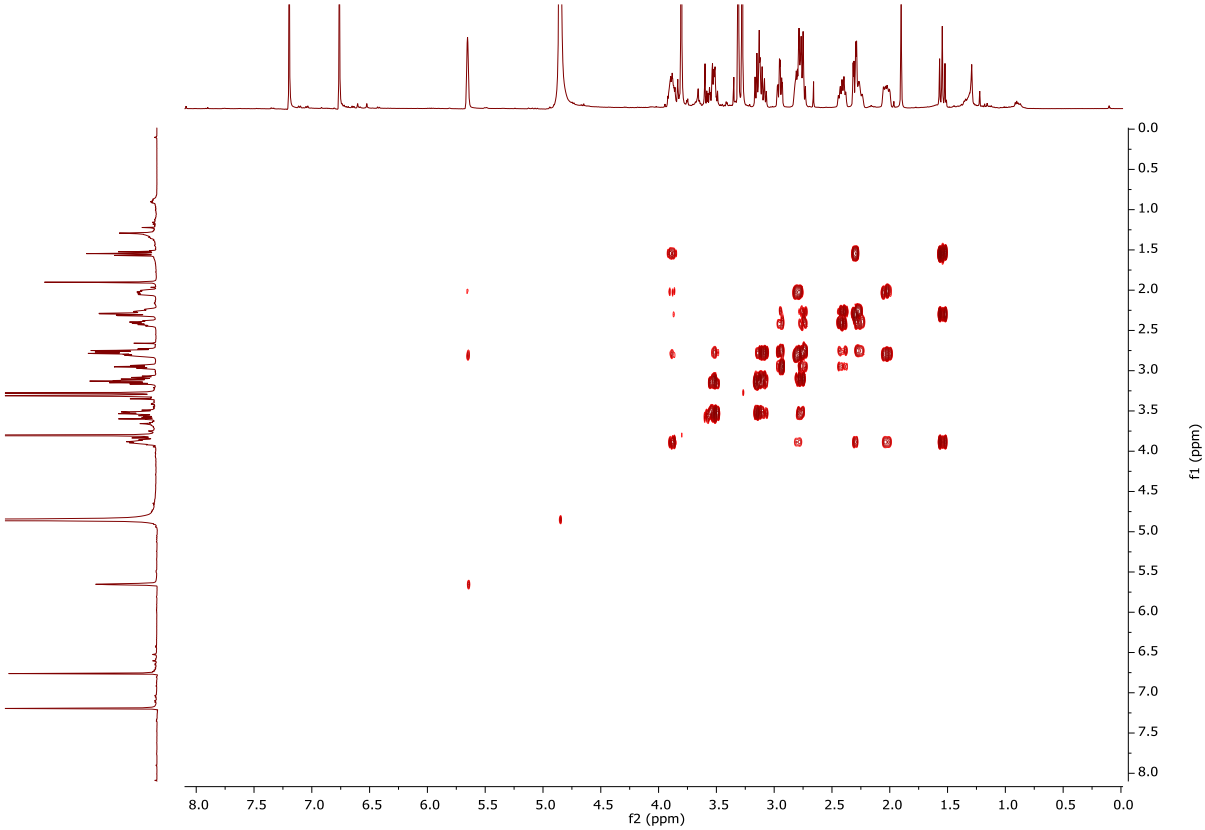


Fig. S2 (f) COSY spectrum of compound **1**

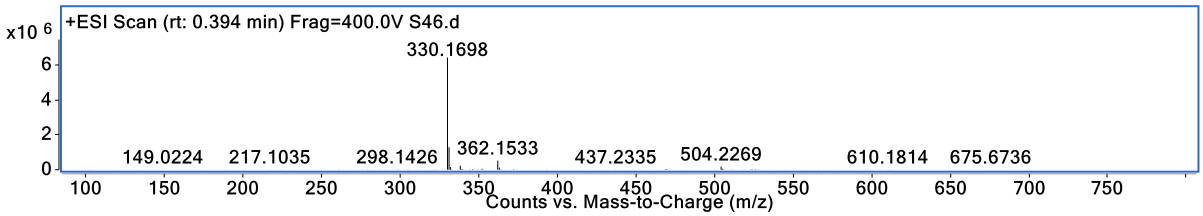
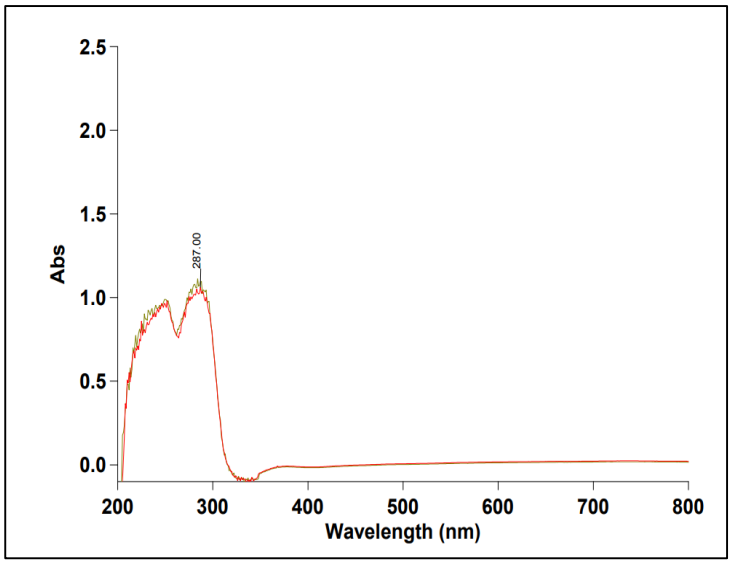


Fig. S3 HR-ESI-MS spectrum of compound **1**



Wavelength	Absorbance
287.0	1.062
252.0	0.981

Fig. S4 UV-Vis spectrum of compound **1**

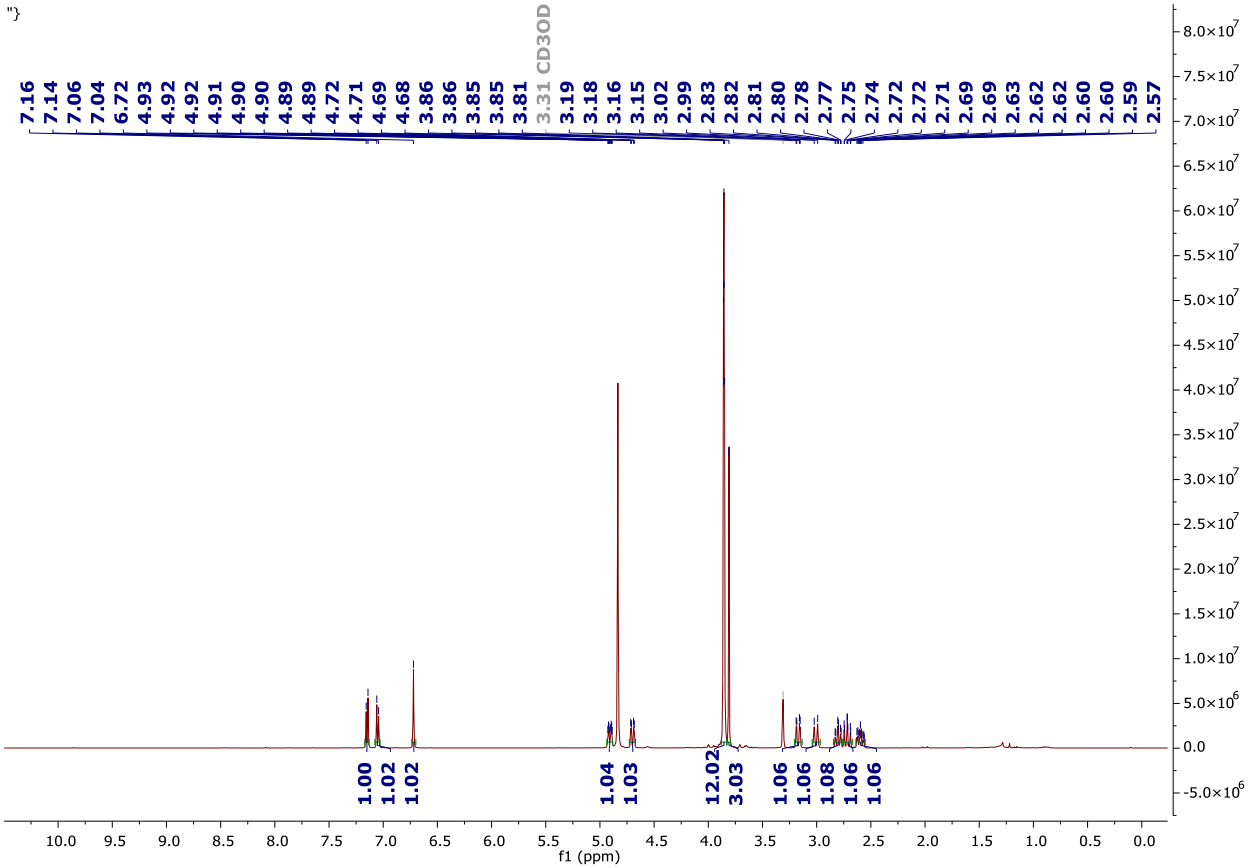


Fig. S5 (a) ^{1}H-NMR spectrum of compound **2**

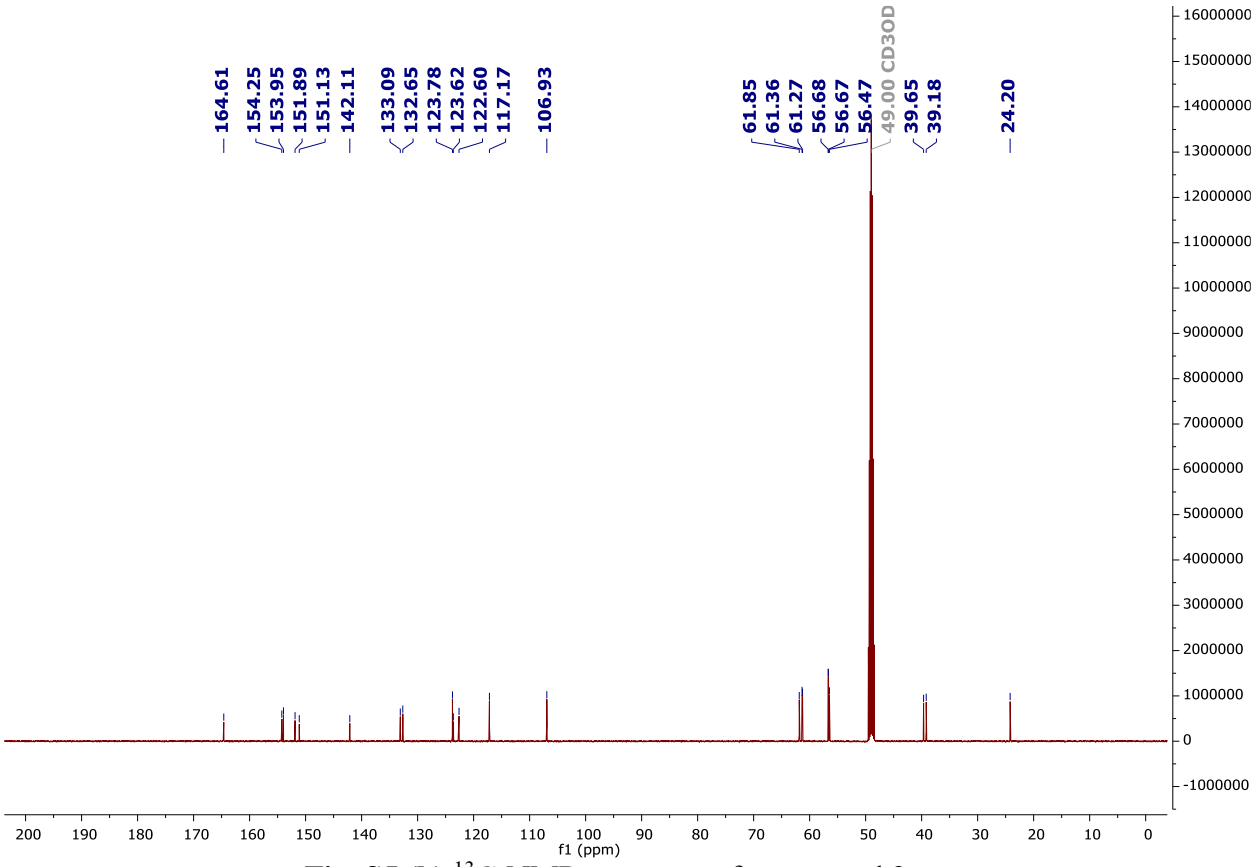


Fig. S5 (b) ^{13}C-NMR spectrum of compound **2**

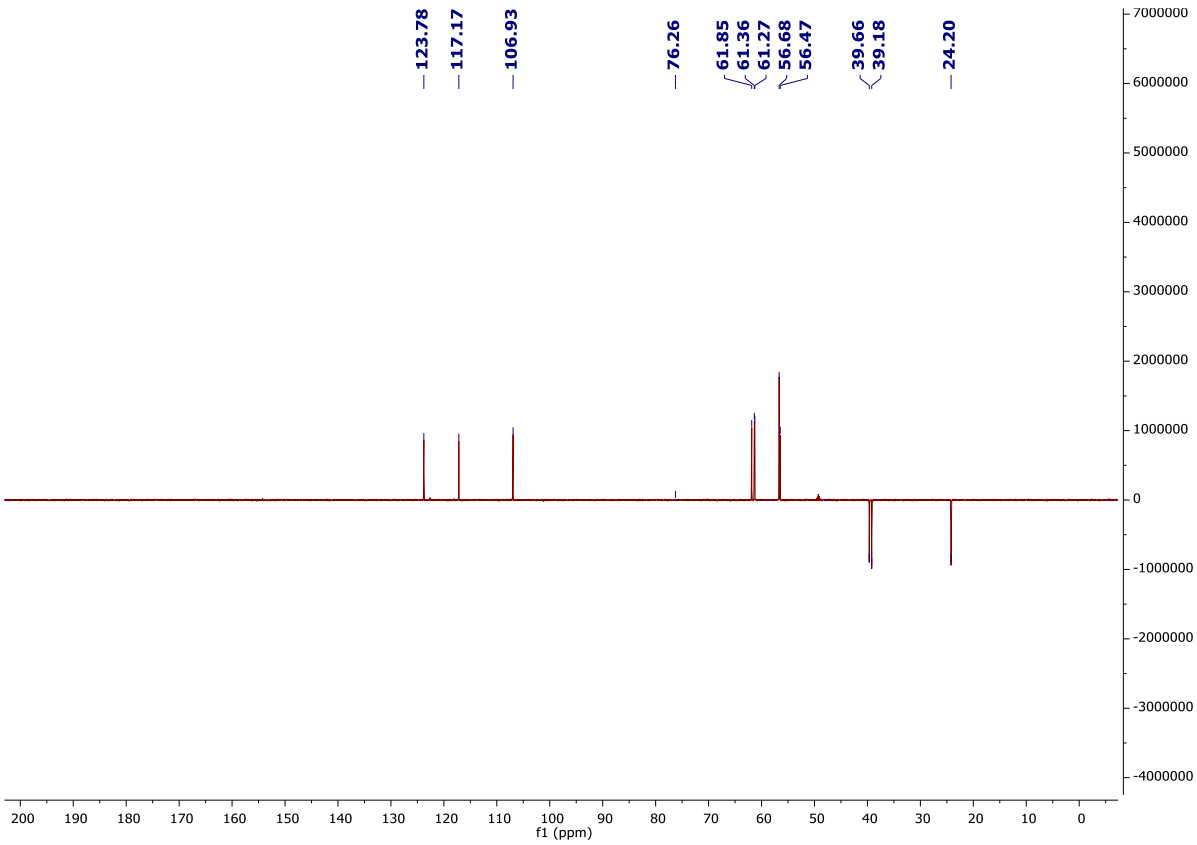


Fig. S5 (c) DEPT-135 NMR spectrum of compound **2**

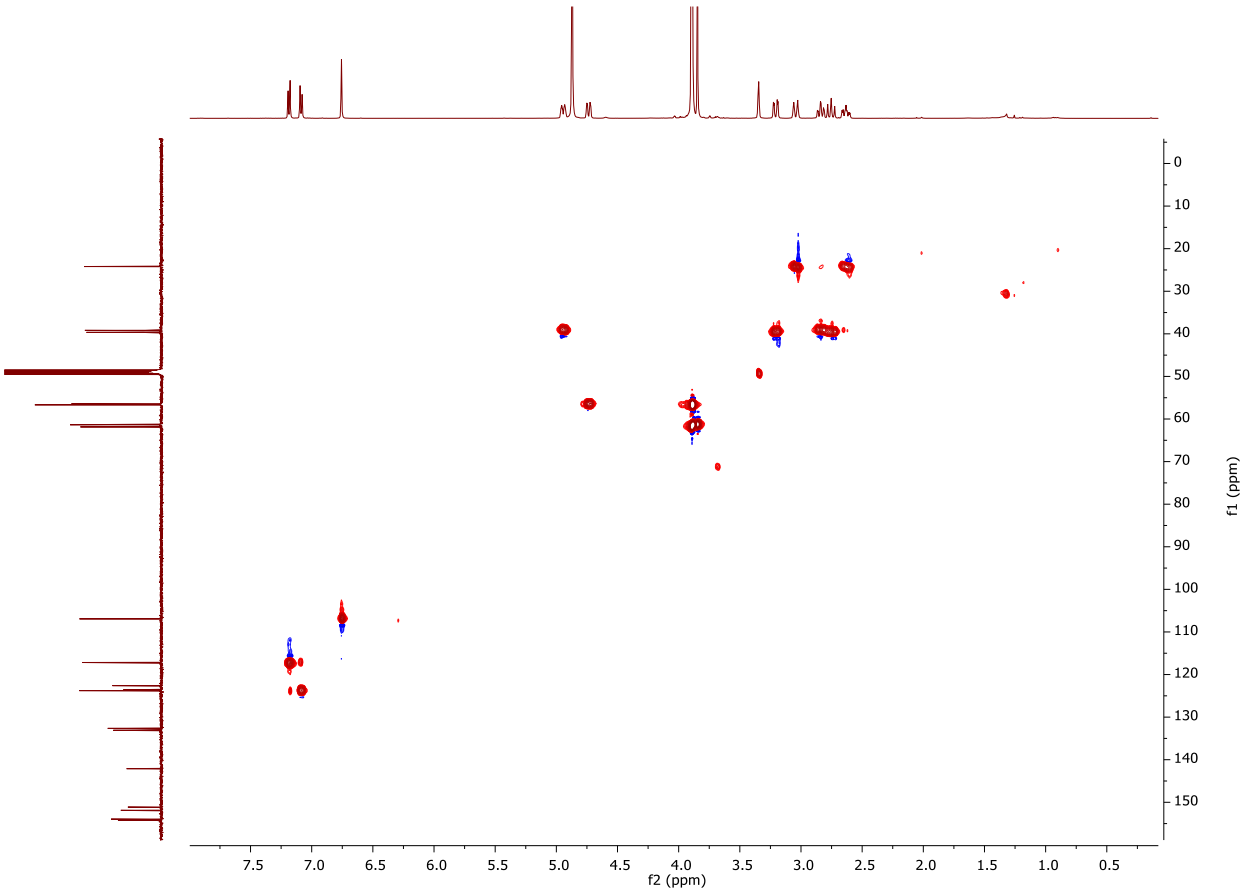


Fig. S5 (d) HSQC spectrum of compound **2**

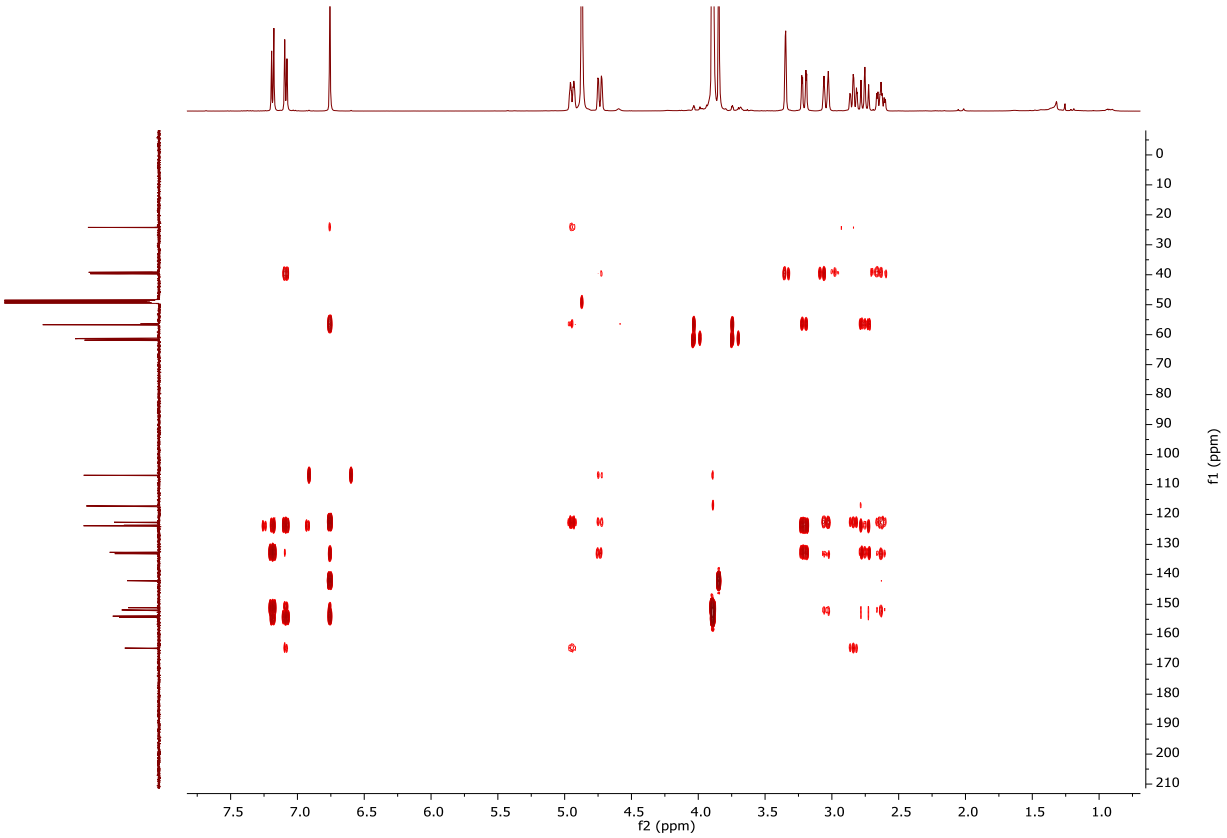


Fig. S5 (e) HMBC spectrum of compound **2**

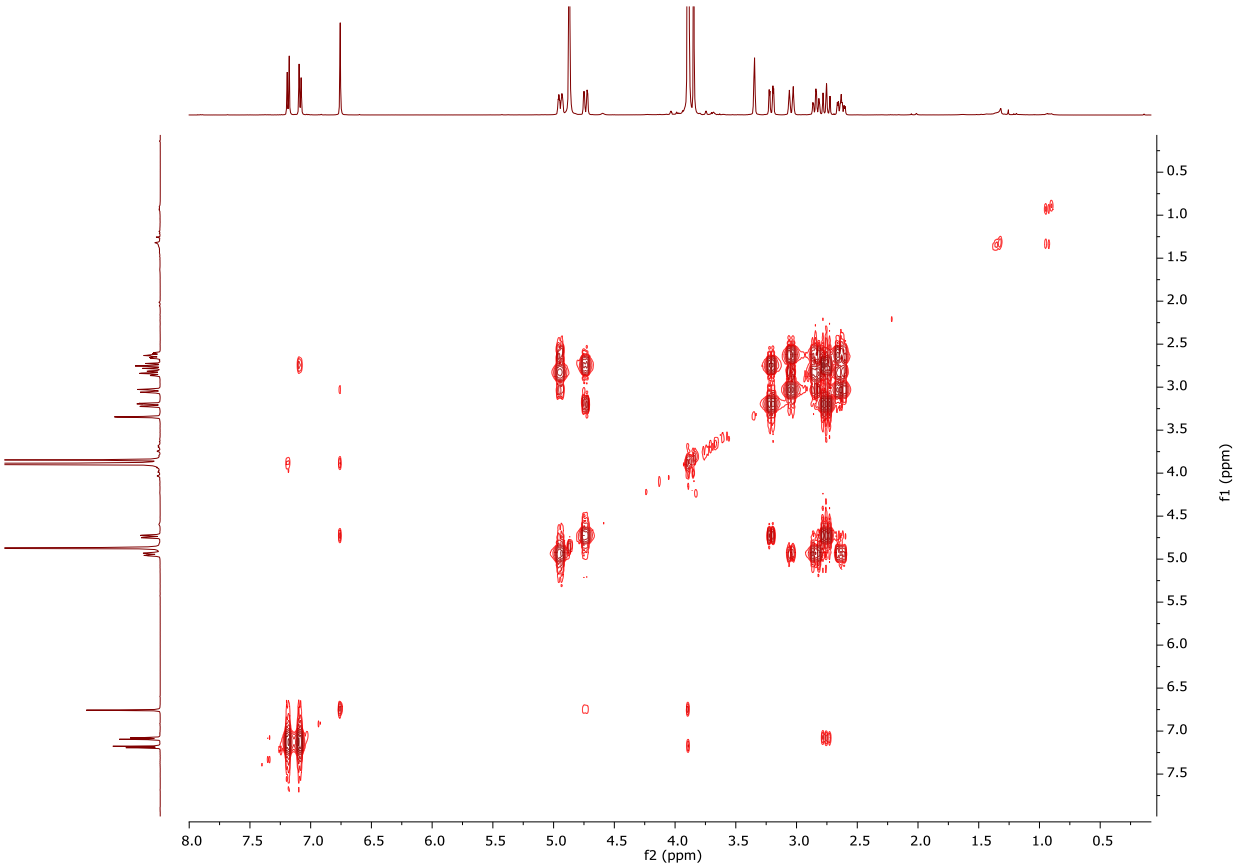


Fig. S5 (f) COSY spectrum of compound **2**

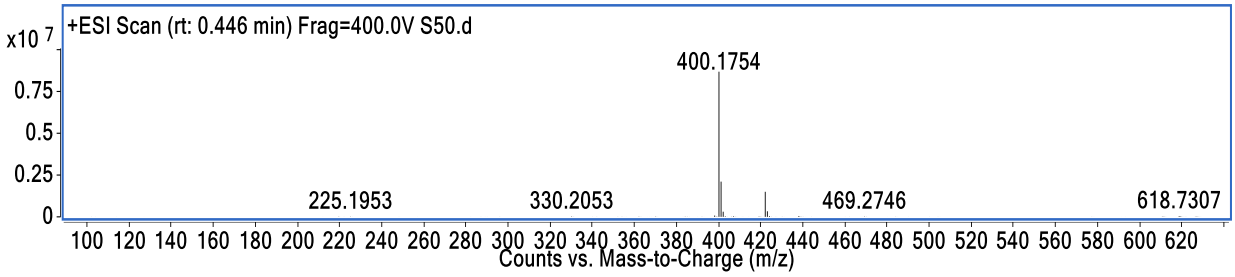
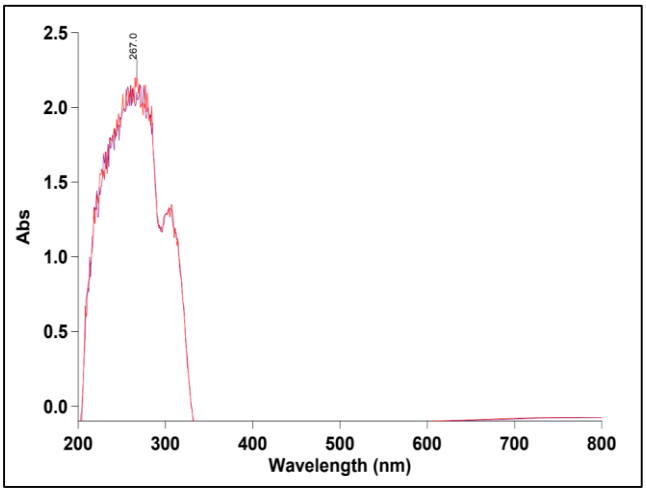


Fig. S6 HR-ESI-MS spectrum of compound **2**



Wavelength	Absorbance
307.0	1.351
267.0	2.208

Fig. S7 UV-Vis. spectrum of compound **2**

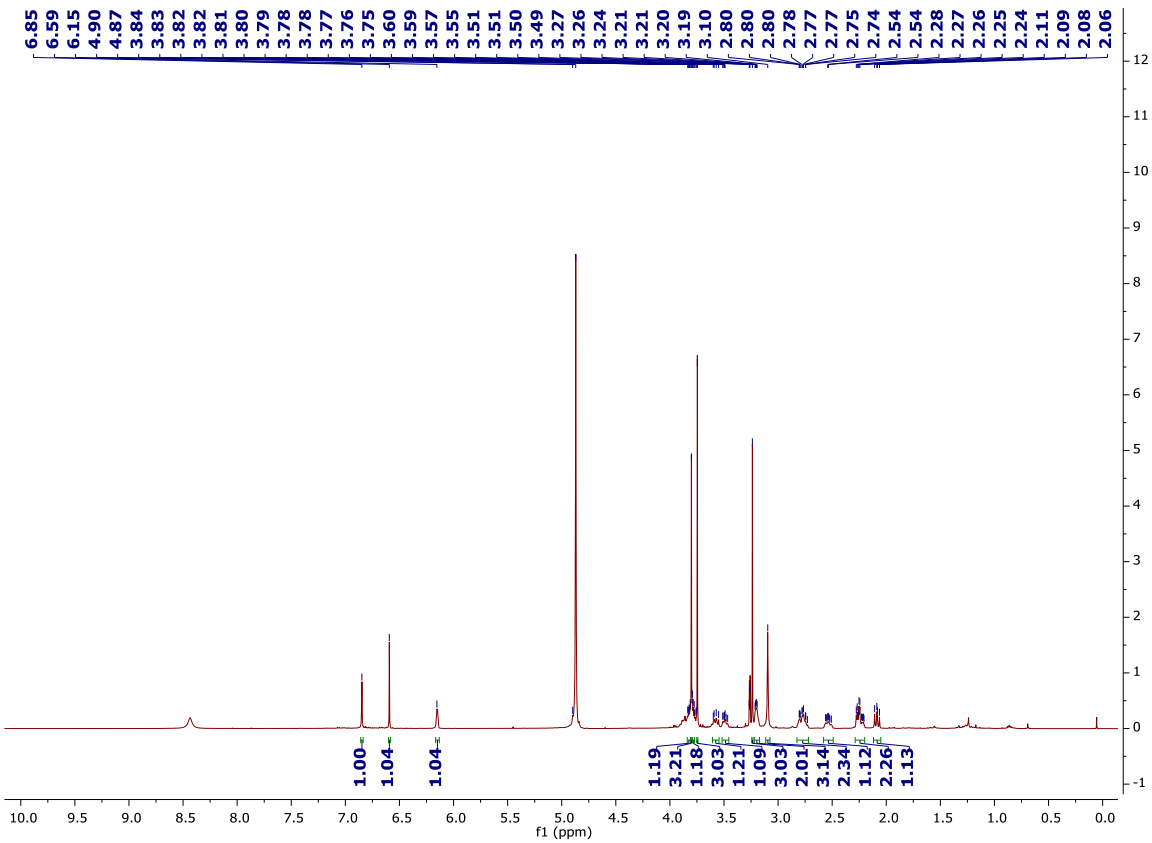


Fig. S8 (a) ^{1}H-NMR spectrum of compound **3**

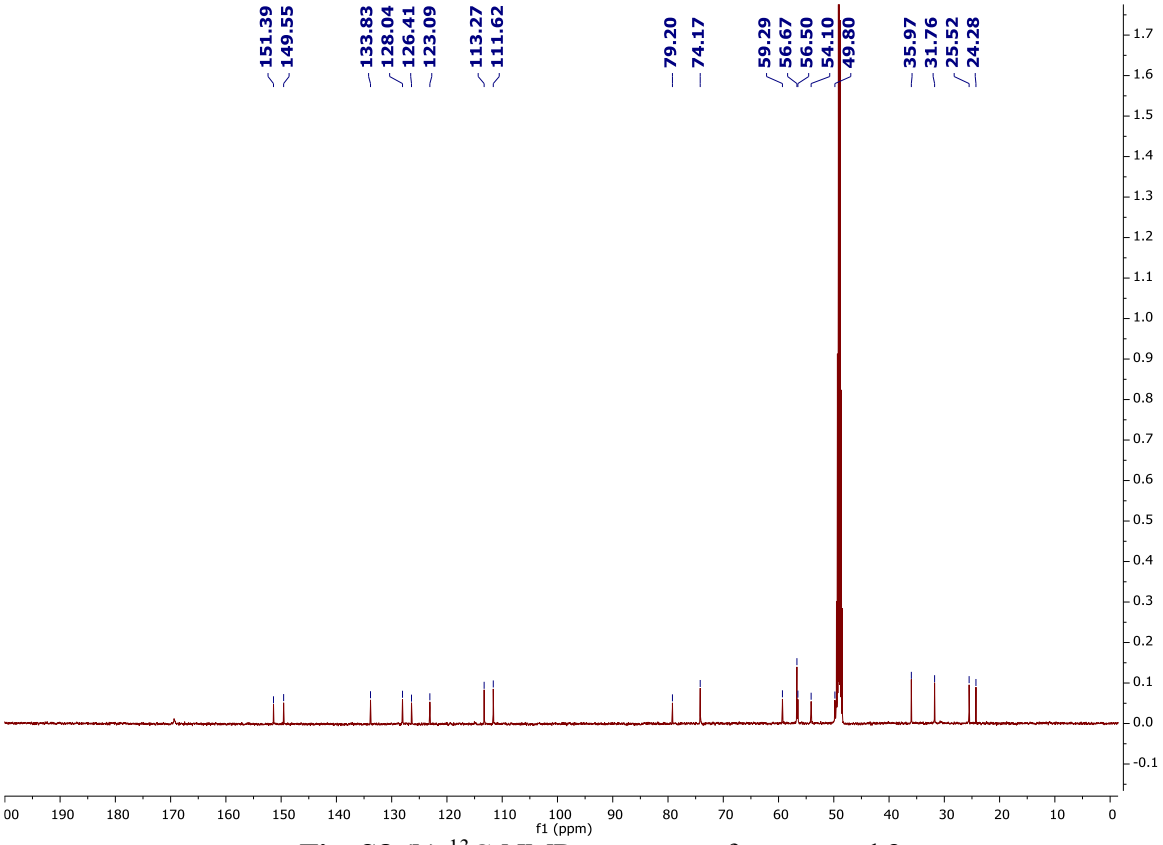


Fig. S8 (b) ^{13}C-NMR spectrum of compound **3**

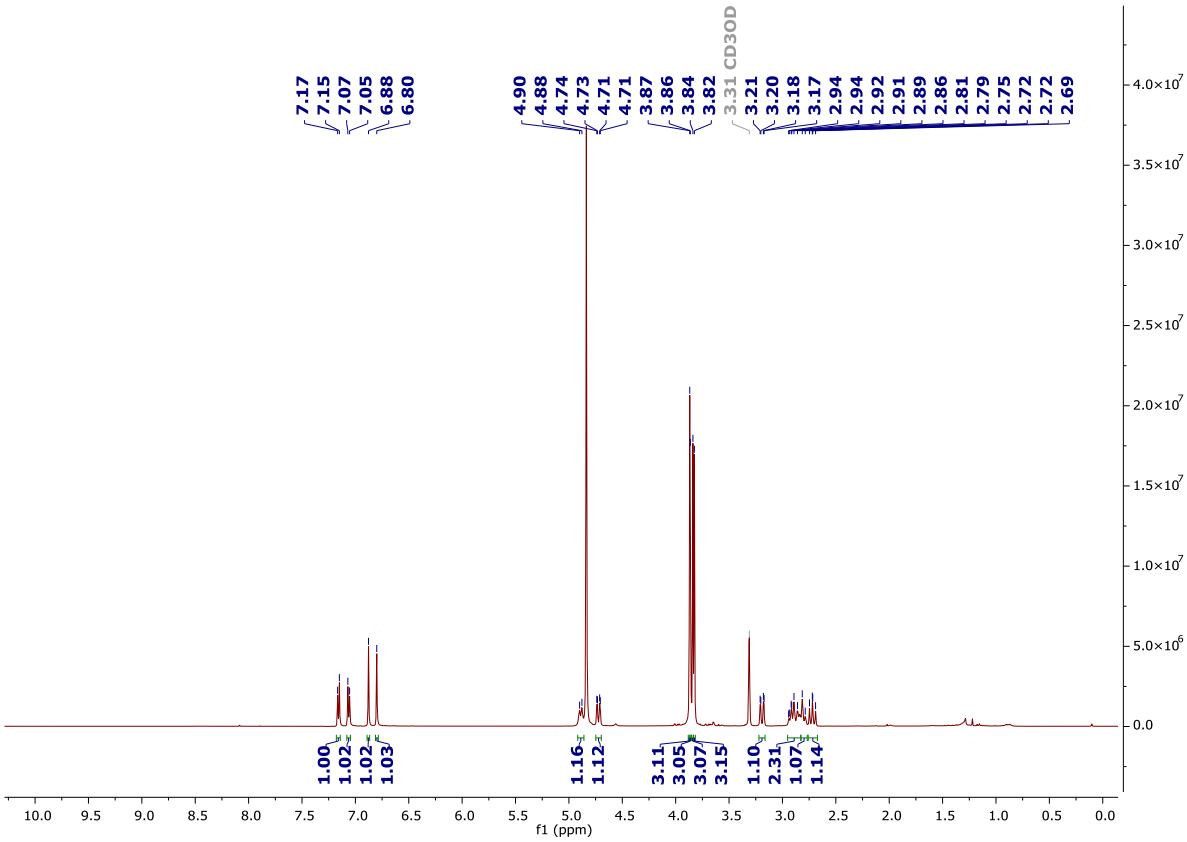


Fig. S9 (a) ^{1}H-NMR spectrum of compound **4**

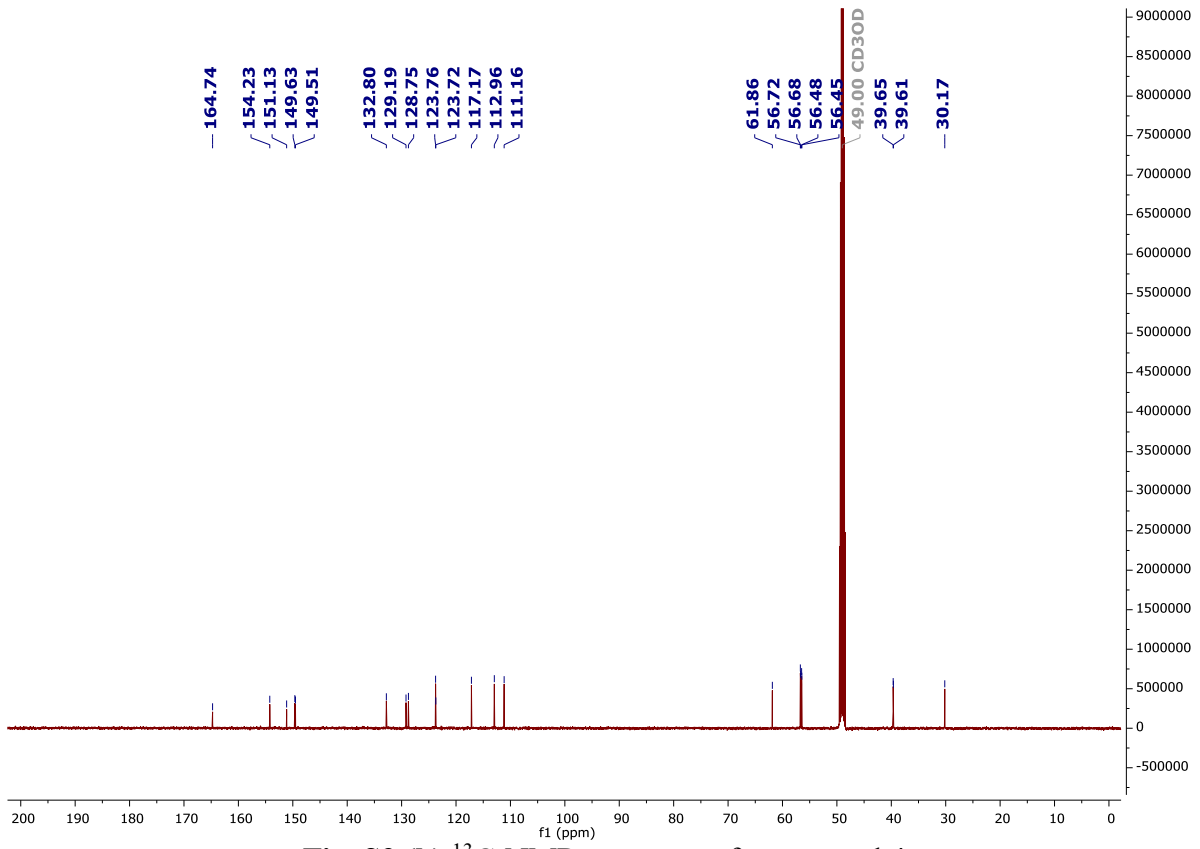


Fig. S9 (b) ^{13}C-NMR spectrum of compound **4**

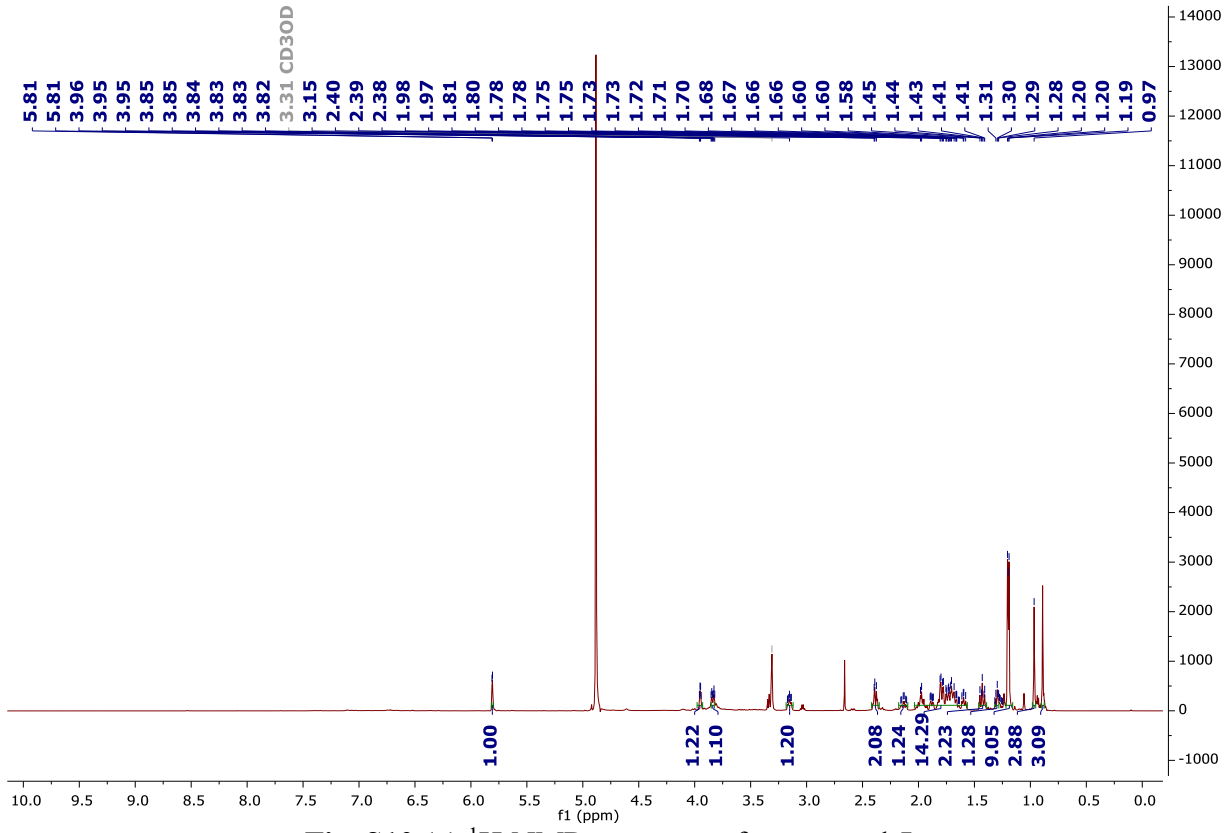


Fig. S10 (a) ^{1}H-NMR spectrum of compound **5**

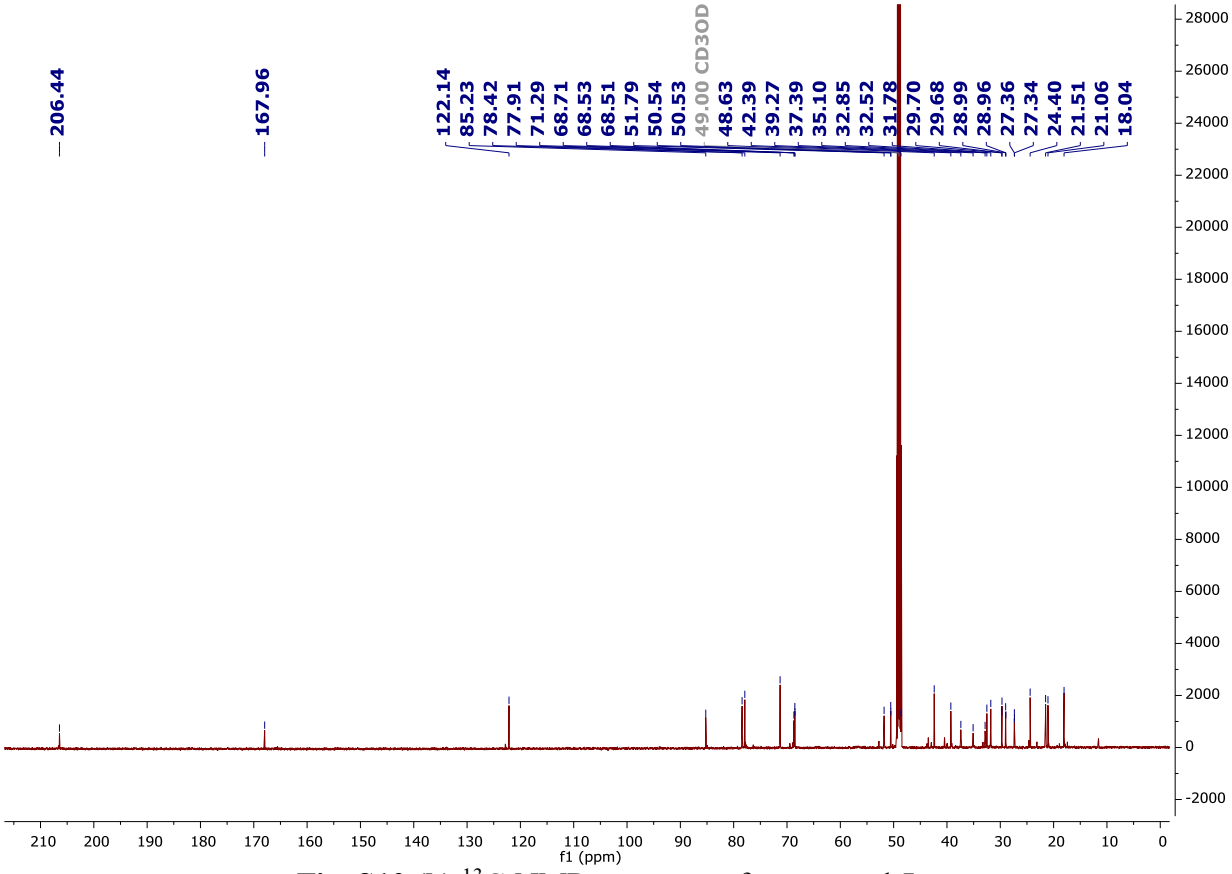


Fig. S10 (b) ^{13}C-NMR spectrum of compound **5**

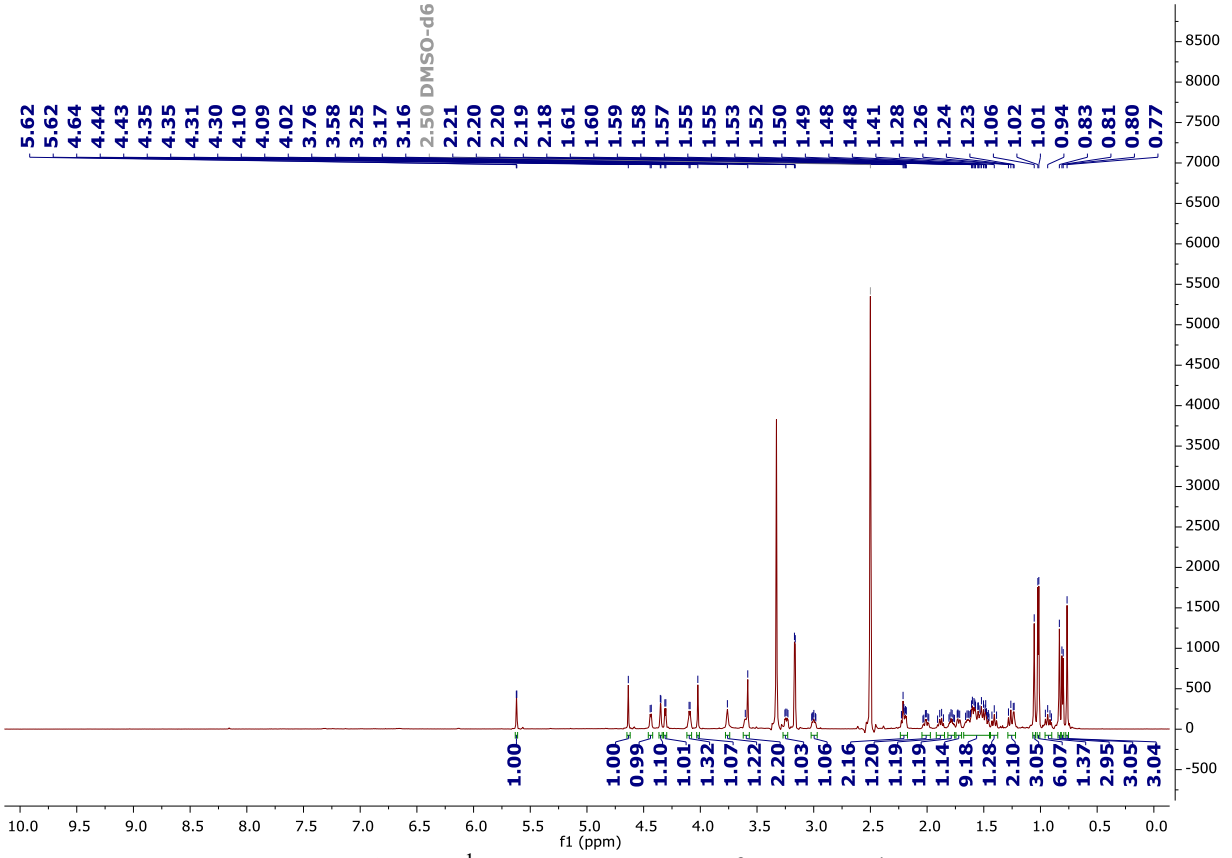


Fig. S11 (a) ^{1}H-NMR spectrum of compound **6**

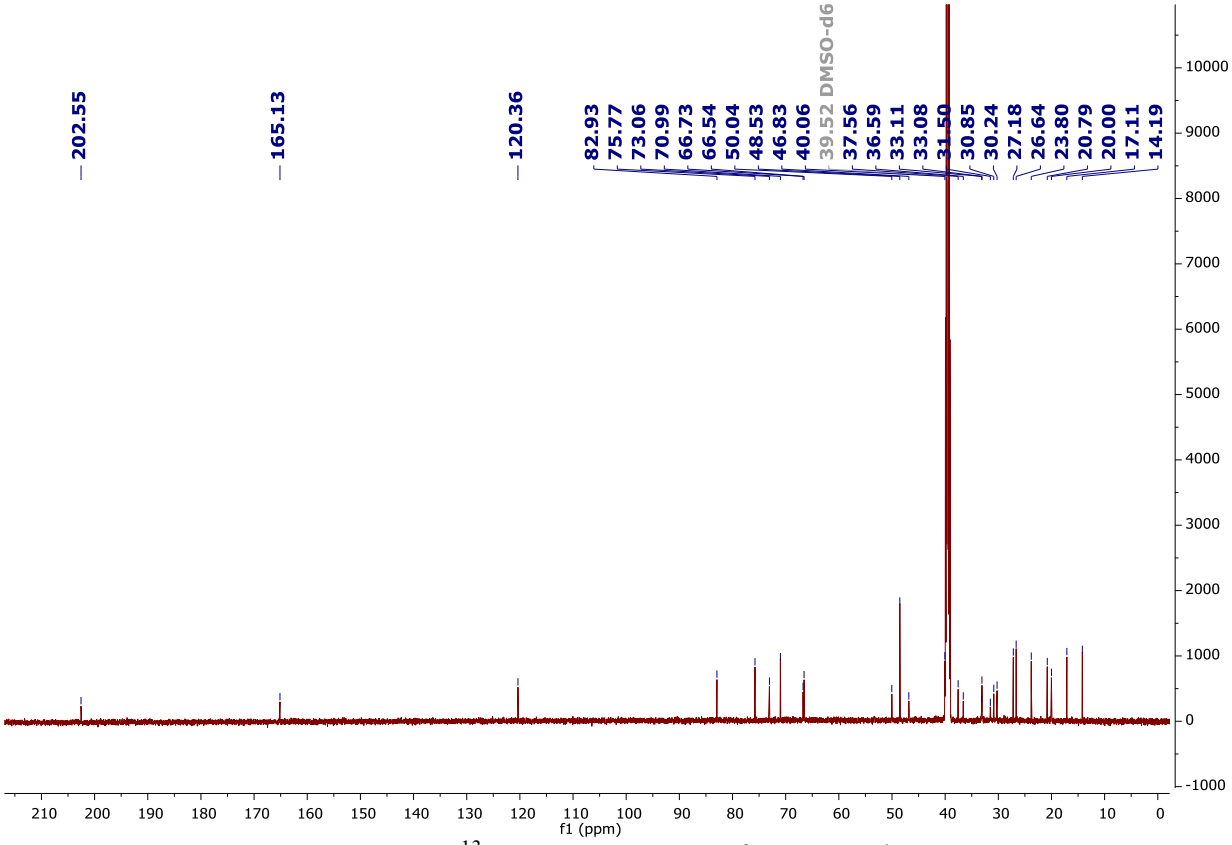


Fig. S11 (b) ^{13}C-NMR spectrum of compound **6**

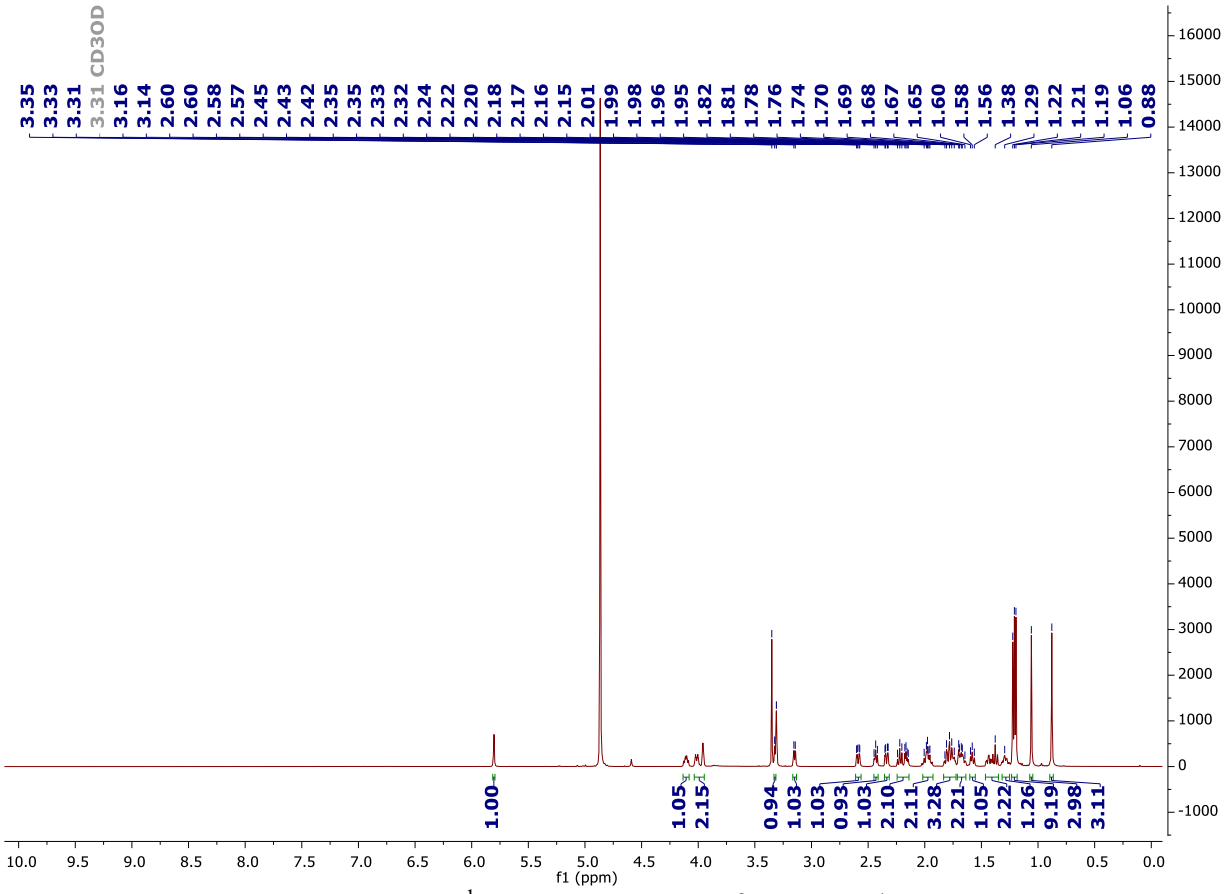


Fig. S12 (a) ^{1}H-NMR spectrum of compound **7**

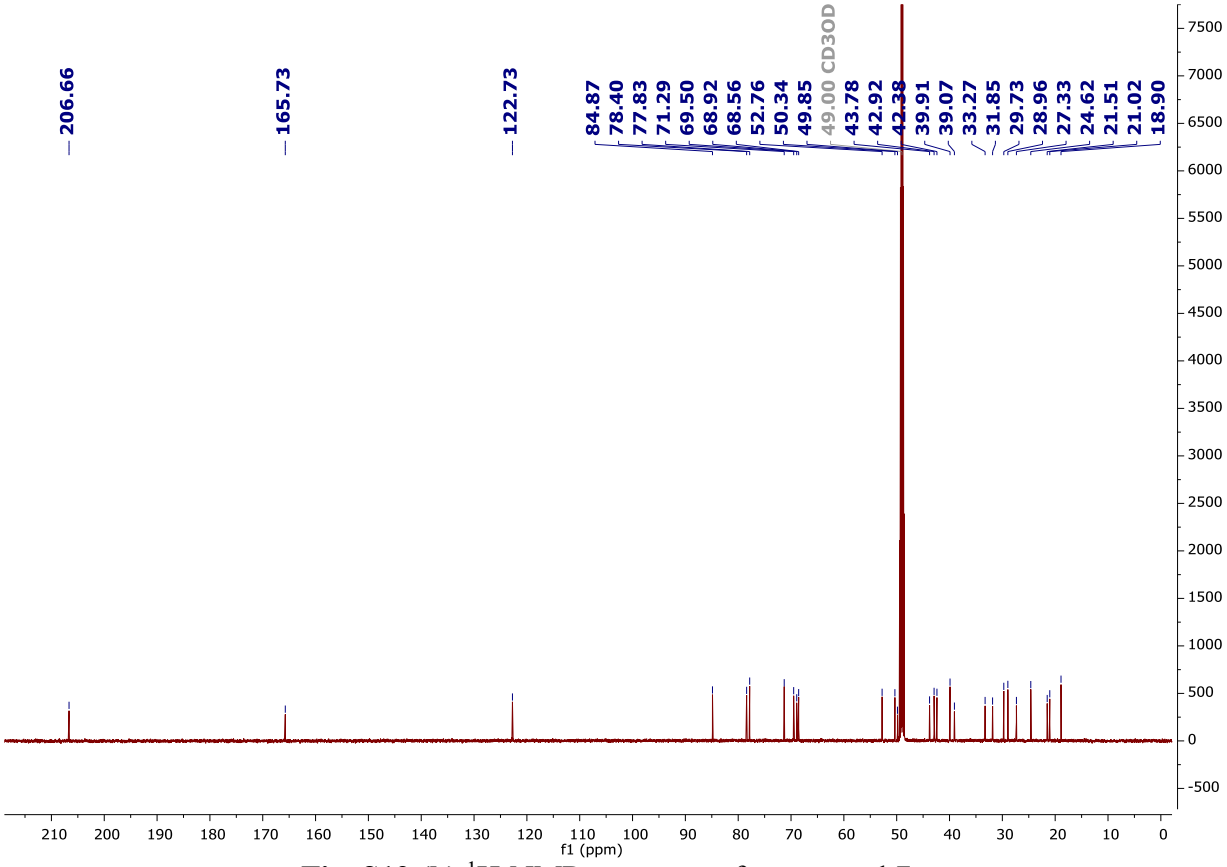


Fig. S12 (b) ^{1}H-NMR spectrum of compound **7**

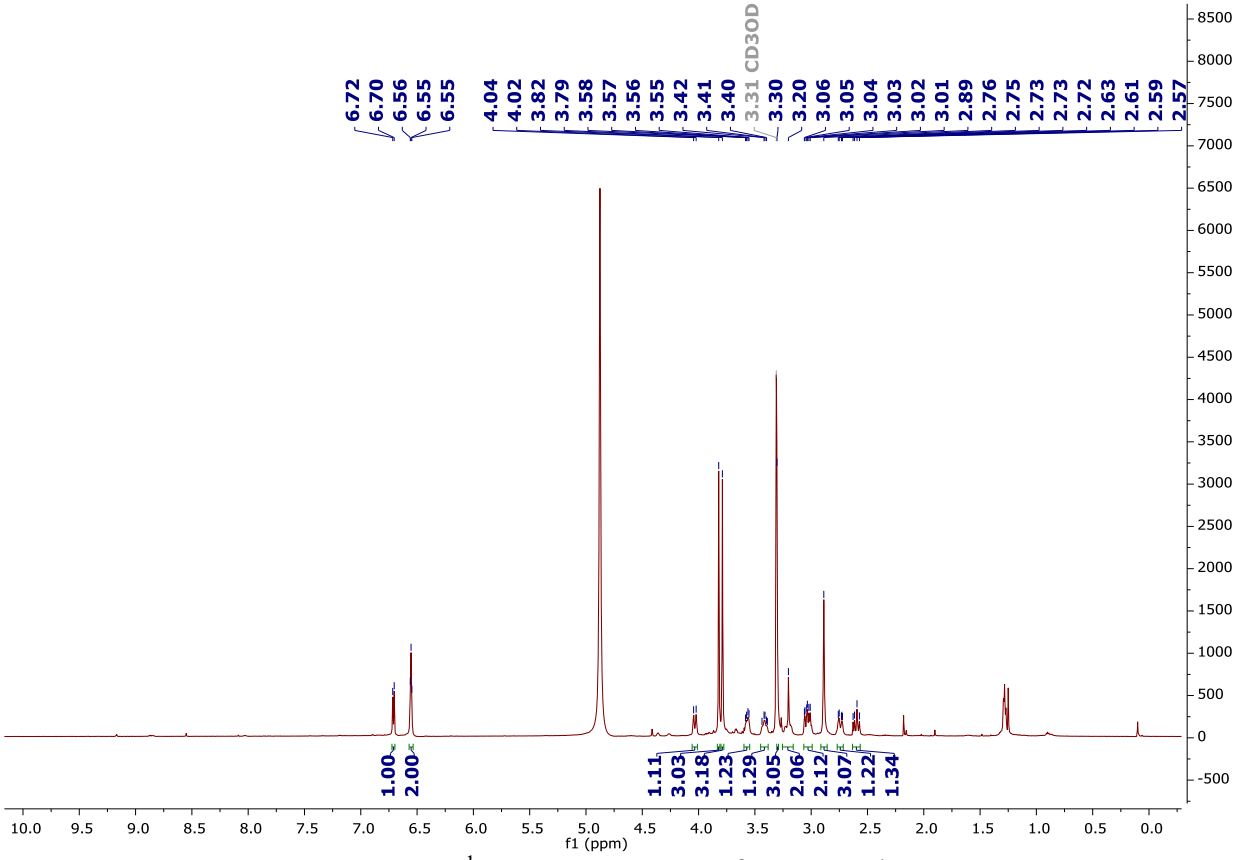


Fig. S13 (a) ^{1}H-NMR spectrum of compound **8**

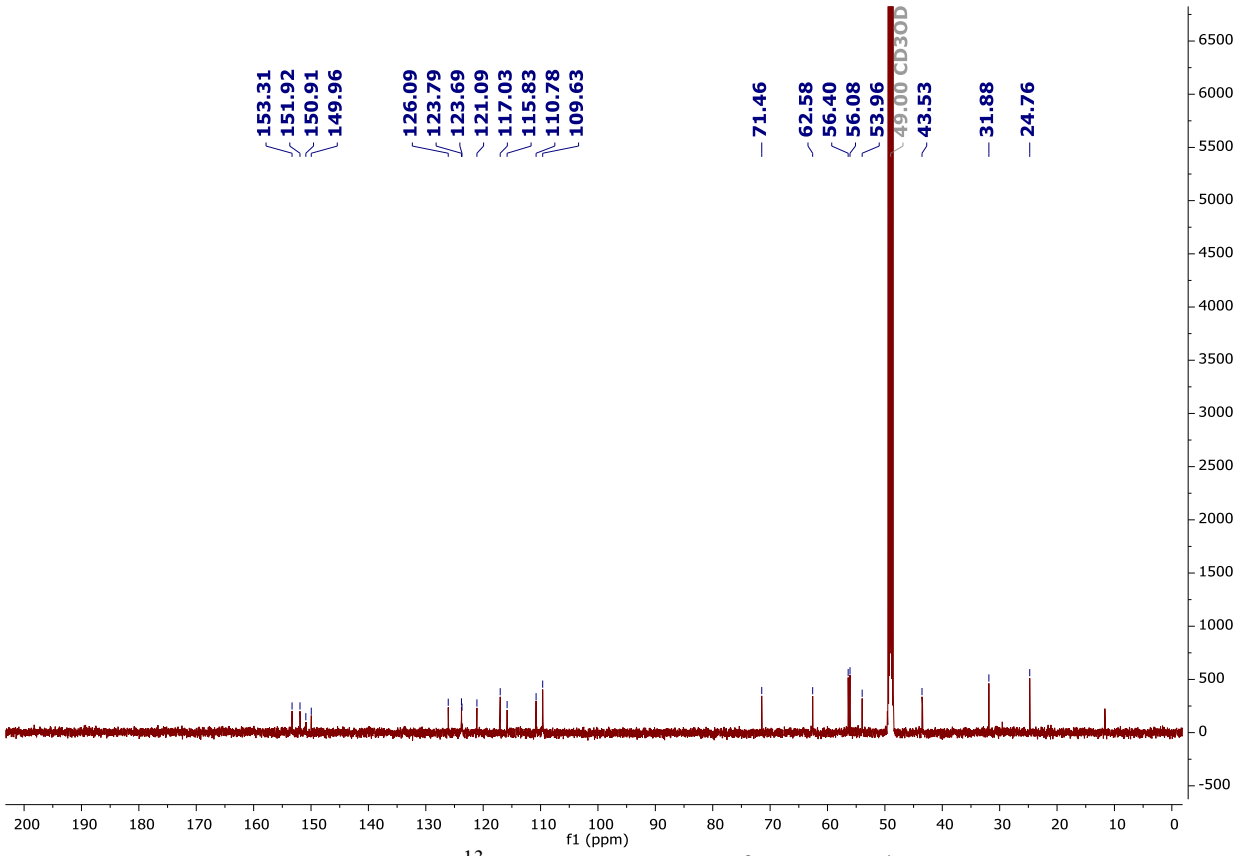


Fig. S13 (b) ^{13}C-NMR spectrum of compound **8**

Table S1. ^{1}H NMR (500 MHz) and ^{13}C-NMR (125 MHz) data of compound **1** in CD_3OD

Positions	**δ_C (ppm)**	**δ_H (ppm)**
1	120.0	5.65 (s)
2	32.9	2.00-2.06, (m); 2.79-2.82 (overlapped)
3	74.6	3.86-3.91 (m)
4	41.8	2.29-2.32 (m[a]); 1.52-1.57 (m)
5	65.6	-
6	141.2	-
7	27.7	2.38-2.45 (m); 2.24-2.29 (m[a])
8	47.6	2.93-2.97 (m); 2.73-2.76 (m[a])
10	41.3	3.51-3.54 (m); 3.13-3.17 (m)
11	23.2	2.76-2.79 (m[a]); 3.07-3.12 (m)
12	129.20	-
13	135.6	-
14	129.27	7.20 (s)
15	129.1	-
16	157.0	-
17	113.0	6.76 (s)
3(-OCH_3)	56.2	3.28 (s)
16(-OCH_3)	56.0	3.80 (s)
15(-COOH)	176.2	-

a = overlapped

Table S2. ^{1}H NMR (500 MHz) and ^{13}C-NMR (125 MHz) data of compound **2** in CD_3OD

Positions	δ_C, **Type (ppm)**	δ_H **(ppm)**
1	106.9	6.72 (s)
2	153.9	-
3	142.1	-
4	151.8	-
4a	133.0	-
5	24.2	2.56-2.63 (m); 2.99-3.02 (m)
6	39.1	2.77-2.83 (m); 4.89-4.93 (m)
8	164.6	-
8a	123.6	-
9	151.1	-
10	154.2	-
11	117.1	7.14 (d, *J* = 8.5 Hz)
12	123.7	7.06 (d, *J* = 8.5 Hz)
12a	132.6	-
13	39.6	2.69-2.75 (m); 3.19 (dd, *J* = 15.5, 3.0 Hz)
14	56.4	4.68 (dd, *J* = 13.0, 3.5 Hz)
14a	122.6	-
2(-OCH_3)	61.8	3.86 (s*)
3(-OCH_3)	61.2	3.81 (s)
4(-OCH_3)	61.3	3.86 (s*)
9(-OCH_3)	56.68	3.86 (s*)
10(-OCH_3)	56.67	3.86 (s*)

* = overlapped

Table S3. Crystal data and structure refinement for compound **2**

Identification code	US_2_Rt_Cu
Empirical formula	$C_{22}H_{25}NO_6$
Formula weight	399.43
Temperature/K	293(2)
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a/Å	7.9434(4)
b/Å	11.4579(7)
c/Å	22.5173(13)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	2049.4(2)
Z	4
ρ_{calc}g/cm^3	1.295
μ/mm^{-1}	0.779
F(000)	848.0
Crystal size/mm^3	0.243 × 0.16 × 0.117
Radiation	Cu Kα (λ = 1.54184)
2Θ range for data collection/°	7.852 to 139.07
Index ranges	$-9 \leq h \leq 4$, $-13 \leq k \leq 13$, $-27 \leq l \leq 21$
Reflections collected	3706
Independent reflections	2838 [R_{int} = 0.0231, R_{sigma} = 0.0353]
Data/restraints/parameters	2838/0/268
Goodness-of-fit on F^2	1.045
Final R indexes [I>=2σ (I)]	R_1 = 0.0436, wR_2 = 0.1129
Final R indexes [all data]	R_1 = 0.0529, wR_2 = 0.1230
Largest diff. peak/hole / e Å^{-3}	0.17/-0.15
Flack parameter	0.8(2)

Table S4. Bond Lengths for compound **2**

Atom	Atom	Length/Å		Atom	Atom	Length/Å
O2	C4	1.374(3)		C5	C4	1.400(4)
O2	C11	1.419(5)		C7	C19	1.514(4)
O3	C6	1.232(3)		C7	C8	1.511(4)
O1	C3	1.365(4)		C9	C1	1.370(5)
O1	C10	1.421(4)		C9	C8	1.503(5)
O5	C16	1.385(4)		C3	C4	1.398(4)
O5	C21	1.418(5)		C3	C2	1.387(4)
N1	C6	1.356(4)		C19	C18	1.394(4)
N1	C7	1.474(4)		C19	C14	1.394(4)
N1	C12	1.455(4)		C18	C17	1.383(5)
O6	C17	1.372(4)		C1	C2	1.383(5)
O6	C22	1.413(5)		C14	C15	1.407(5)
O4	C15	1.378(4)		C14	C13	1.499(5)
O4	C20	1.403(5)		C16	C17	1.393(5)
C6	C5	1.490(4)		C16	C15	1.367(5)
C5	C9	1.406(4)		C12	C13	1.507(6)

Table S5. Bond Angles for compound **2**

Atom	Atom	Atom	Angle/°		Atom	Atom	Atom	Angle/°
C4	O2	C11	113.9(3)		C14	C19	C7	122.0(3)
C3	O1	C10	117.9(3)		C14	C19	C18	120.5(3)
C16	O5	C21	114.1(3)		O2	C4	C5	121.7(3)
C6	N1	C7	121.7(3)		O2	C4	C3	117.3(3)
C6	N1	C12	119.0(3)		C3	C4	C5	120.9(3)
C12	N1	C7	118.1(3)		C17	C18	C19	120.3(3)
C17	O6	C22	118.3(3)		C9	C1	C2	121.4(3)
C15	O4	C20	117.9(3)		C19	C14	C15	117.9(3)
O3	C6	N1	120.7(3)		C19	C14	C13	120.4(3)
O3	C6	C5	121.9(3)		C15	C14	C13	121.6(3)
N1	C6	C5	117.2(2)		C1	C2	C3	120.1(3)
C9	C5	C6	119.9(3)		O5	C16	C17	118.6(3)
C4	C5	C6	121.5(3)		C15	C16	O5	121.6(3)
C4	C5	C9	118.6(3)		C15	C16	C17	119.8(3)
N1	C7	C19	112.1(3)		C9	C8	C7	112.4(3)
N1	C7	C8	108.4(2)		O6	C17	C18	124.7(3)
C8	C7	C19	110.4(3)		O6	C17	C16	115.6(3)
C5	C9	C8	118.7(3)		C18	C17	C16	119.8(3)
C1	C9	C5	119.9(3)		O4	C15	C14	117.0(3)
C1	C9	C8	121.3(3)		C16	C15	O4	121.2(3)
O1	C3	C4	115.9(3)		C16	C15	C14	121.7(3)
O1	C3	C2	125.1(3)		N1	C12	C13	109.5(3)
C2	C3	C4	119.0(3)		C14	C13	C12	109.6(3)
C18	C19	C7	117.5(3)					

Table S6. ^{1}H NMR (500 MHz) and ^{13}C-NMR (125 MHz) data of compound **3** in CD_3OD

Positions	**δ_C (ppm)**	**δ_H (ppm)**
1	128.0	6.15 (s)
2	74.1	3.78-3.79 (m)
3	35.9	2.06-2.11, (m); 2.26-2.28 (m)
4	31.7	2.21-2.24 (m); 2.77-2.80 (m)
5	79.2	-
6	133.8	-
7	24.2	3.19-3.21 (m)
8	59.2	3.55-3.60, (m); 3.47-3.51, (m)
9-(*N*-CH_3)	49.8	3.10 (s)
10	54.1	3.77-3.80 (m); 3.81-3.84 (m)
11	25.5	2.73-2.77 (m); 2.51-2.56 (m)
12	123.0	-
13	126.4	-
14	111.6	6.59 (s)
15	149.5	-
16	151.3	-
17	113.2	6.85 (s)
2-(OCH_3)	56.5	3.24 (s)
15-(OCH_3)	56.6	3.75 (s)
16-(OCH_3)	56.6	3.80 (s)

Table S7. ^{1}H NMR (500 MHz) and ^{13}C-NMR (125 MHz) data of compound **4** in CD_3OD

Positions	**δ_C (ppm)**	**δ_H (ppm)**
1	111.1	6.88 (s)
2	149.5	-
3	149.6	-
4	112.9	6.80 (s)
4a	128.7	-
5	30.1	2.79-2.81 (m); 2.86-2.89 (m)
6	39.6	3.17-3.21 (dd, *J*=15.3, 3.5 Hz); 4.88-4.90 (d, *J* = 10.8 Hz)
8	164.7	-
8a	123.7	-
9	151.1	-
10	154.2	-
11	117.1	7.17-7.15 (d, *J* = 8.2 Hz)
12	123.7	7.05-7.07 (d, *J* = 8.2 Hz)
12a	132.8	-
13	39.6	2.69-2.75 (m); 2.91-2.94 (m)
14	56.4	4.71-4.74, (dd, *J* = 13.3, 3.1 Hz)
14a	129.1	-
2-(OCH_3)	56.7	3.82 (s)
3-(OCH_3)	56.4	3.84 (s)
9-(OCH_3)	61.8	3.86 (ovd)
10-(OCH_3)	56.6	3.86 (s)

Table S8. ^{1}H NMR (600 MHz) and ^{13}C-NMR (150 MHz) data of compound **5** in CD_3OD

Positions	**δ_C (ppm)**	**δ_H (ppm)**	**Positions**	**δ_C (ppm)**	**δ_H (ppm)**
1	37.3	1.77-1.79 (m[a]); 1.41-1.43 (m[a])	15	31.7	1.97-2.00 (m[a]); 1.57-1.62 (m[a])
2	68.7	3.82-3.85 (m)	16	21.5	1.95-1.97 (m[a]); 1.98-2.02 (m[a])
3	68.5	3.95 (br s)	17	50.5	2.38-2.40 (m[a])
4	32.8	1.72-1.73 (m[a]), 1.70-1.71 (m[a])	18	18.0	0.89 (s)
5	51.7	2.37-2.38 (m)	19	24.4	0.97 (s)
6	206.4	-	20	77.9	-
7	122.1	5.81 (s)	21	21.0	1.20 (s[a])
8	167.9	-	22	78.4	3.33 -3.35 (m)
9	35.1	3.14-3.17 (m)	23	27.3	1.65-1.68 (m[a]); 1.27-1.32 (m)
10	39.2	-	24	42.3	1.41-1.45 (m[a]); 1.80 (m[a])
11	21.5	1.82-1.84 (m[a]); 1.75-1.77 (m[a])	25	71.2	-
12	32.5	2.11-2.16 (m); 1.86 -1.90 (m[a])	26	28.9	1.19 (s[a])
13	48.6	-	27	29.6	1.20 (s[a])
14	85.2	-			

a = overlapped signal

Table S9. ^{1}H (600 MHz) and ^{13}C-NMR (150 MHz) data of compound **6** in DMSO

Positions	**δ_C (ppm)**	**δ_H (ppm)**	**Positions**	**δ_C (ppm)**	**δ_H (ppm)**
1	36.5	1.23-1.24 (m[a]); 1.26-1.28 (m)	15	31.5	1.50 (m[a]); 1.63-1.66 (m[a])
2	66.7	3.60-3.61 (m[a])	16	20.0	1.86-1.91 (m); 1.76-1.81 (m[a])
3	66.5	3.76 (br s)	17	48.5	2.21-2.23 (m)
4	30.2	1.48-1.49 (m[a]); 1.55 (m[a])	18	17.1	0.76 (m)
5	50.0	2.18-2.20 (m); 1.57-1.58 (m[a])	19	23.8	0.83 (s)
6	202.5	-	20	75.7	-
7	120.3	5.62 (s)	21	20.7	1.06 (s)
8	165.13	-	22	73.0	3.24 (dd, *J* = 11.1, 5.0 Hz)
9	33.1	2.98-3.02 (m)	23	33.0	1.39-1.43 (m[a]); 0.90-0.96 (m[a])
10	37.5	-	24	40.0	1.61-1.62 (m[a])
11	20.0	1.52 (m[a]); 1.59-1.61 (m[a])	25	70.9	-
12	30.8	1.99-2.04 (m[a]); 1.71-1.73 (m[a])	26	26.6	1.01 (s)
13	46.8	-	27	27.1	1.02 (s)
14	82.9	-	28	14.1	0.80 (s)

a = overlapped signal

Table S10. ^{1}H NMR (600 MHz) and ^{13}C-NMR (150 MHz) data of compound **7** in CD_3OD

Positions	**δ_C (ppm)**	**δ_H (ppm)**	**Positions**	**δ_C (ppm)**	**δ_H (ppm)**
1	39.0	1.36-1.40 (t, *J* = 12.4 Hz,), 2.58 (dd, *J* = 12.8, 3.9 Hz)	15	31.8	1.56-1.60 (t, *J* = 10.2 Hz), 1.99-2.02 (m[a])
2	68.9	4.00-4.02 (m)	16	21.5	1.74-1.76 (m[a]); 1.93-1.98 (m[a])
3	68.5	3.96 (br s)	17	50.3	2.42-2.45 (m)
4	33.2	1.65-1.68 (m[a]); 1.77-1.78 (m[a])	18	18.9	0.88 (s)
5	52.7	2.34 (dd, *J* = 13.2, 3.5 Hz)	19	24.6	1.06 (s)
6	206.6	-	20	77.8	-
7	122.7	5.80 (s)	21	21.0	1.22 (s)
8	165.7	-	22	78.4	-
9	42.9	3.15 (d, *J* = 7.2 Hz)	23	27.3	1.26-1.31 (m)
10	39.9	-	24	42.3	1.81-1.82 (m[a]); 1.41-1.46 (m[a])
11	69.5	4.09-4.13, (m)	25	71.2	-
12	43.7	2.20-2.24 (t, *J* = 11.4 Hz); 2.16, (dd, *J* =12.1, 6.0 Hz)	26	28.9	1.19 (s)
13	48.5	-	27	29.7	1.21 (s)
14	84.8	-			

a = overlapped signal

Table S11. ^{1}H NMR (600 MHz) and ^{13}C-NMR (150 MHz) data of compound **8** in CD_3OD

Positions	**δ_C (ppm)**	**δ_H (ppm)**
1	150.9	-
2	153.3	-
3	109.6	6.55 (s)
3a	123.6	-
3b	121.0	-
4	24.7	2.72-2.76 (m); 3.17-3.20 (m)
5	62.5	3.39-3.44 (m); 3.55-3.58 (m)
6 -(*N*-CH_3)	43.5	2.89 (s)
6 -(*N*-CH_3)	53.9	3.30 (s)
6a	71.4	4.04 (d, *J*=13.6 Hz)
7	31.8	2.57-2.63 (m); 3.03-3.06 (m)
7a	126.0	-
8	117.0	6.56 (d, *J* = 7.9 Hz)
9	110.7	6.71 (d, *J* = 7.9 Hz)
10	151.9	-
11-(OH)	149.9	-
11a	123.7	-
11b	115.8	-
2- (OCH_3)	56.0	3.79 (s)
10- (OCH_3)	56.4	3.82 (s)

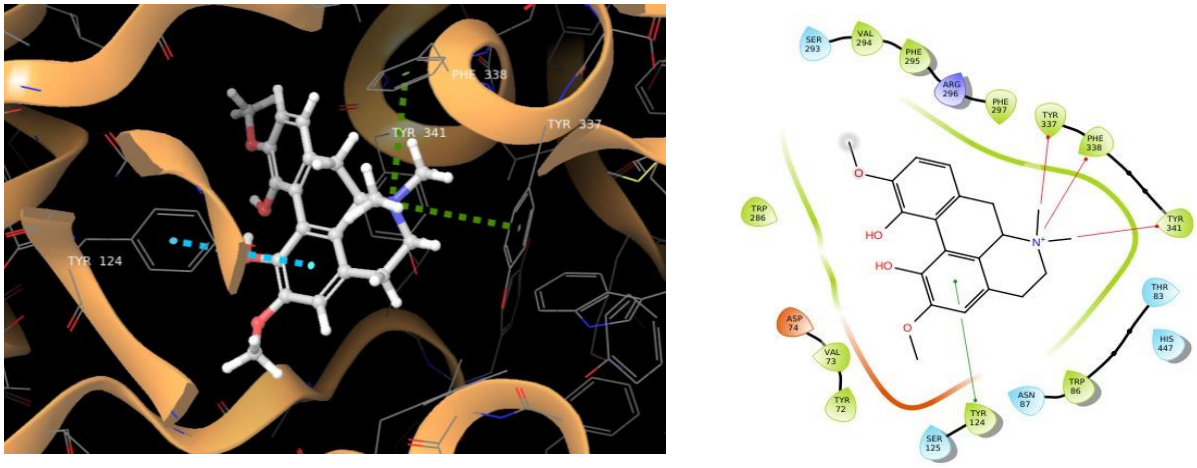


Fig. S14 (a) Binding modes of compound **8** with human AChE

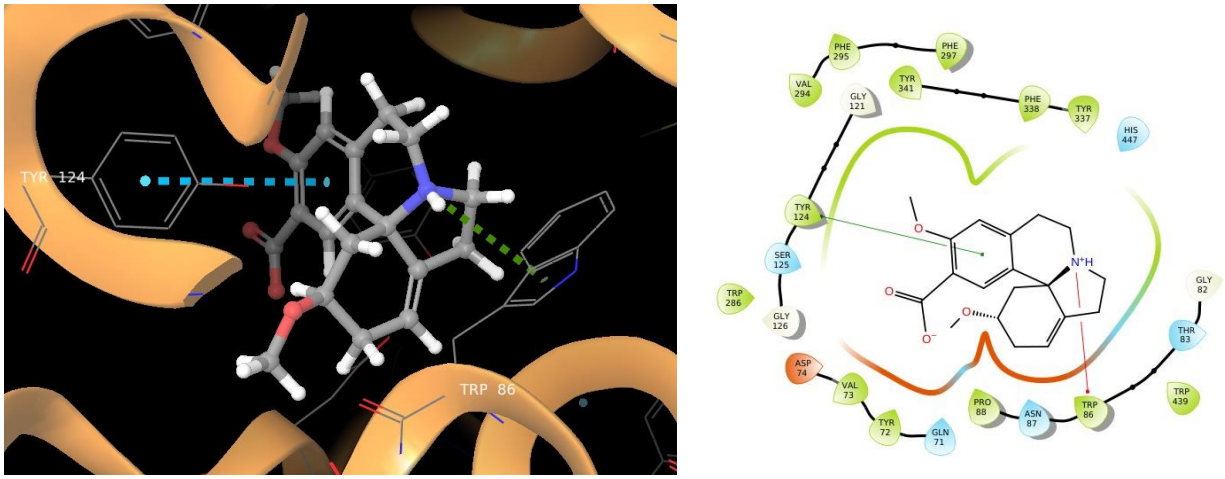


Fig. S14 (b) Binding modes of compound **1** with human AChE

Table S12. Docking score and Glide energy score in Kcal/mol of isolated molecules from *C. hirsutus* against human AChE *in silico* screened by the Schrodinger.

Compounds	Docking score	Glide energy (kcal/mol)	Interactions with residues
1	-11.49	-24.12	Trp86, Tyr124
2	-8.36	-42.88	Phe295
3	-9.36	-31.17	Trp86, Tyr124
4	-7.43	-41.30	Phe295, His447
5	-4.30	-30.44	Tyr72, Tyr341, Ser293
6	-9.14	-31.08	Tyr124, Tyr341, Ser293
7	-6.5	-34.33	Tyr124, Phe295
8	-12.96	-41.97	Tyr337, Tyr341, Phe338, Tyr124