Electronic Supplementary Material (ESI) for RSC Advances.

Biologically guided isolation and ADMET profile of new Factor Xa inhibitors from *Glycyrrhiza glabra* roots using *in-vitro* and *in-silico* approaches

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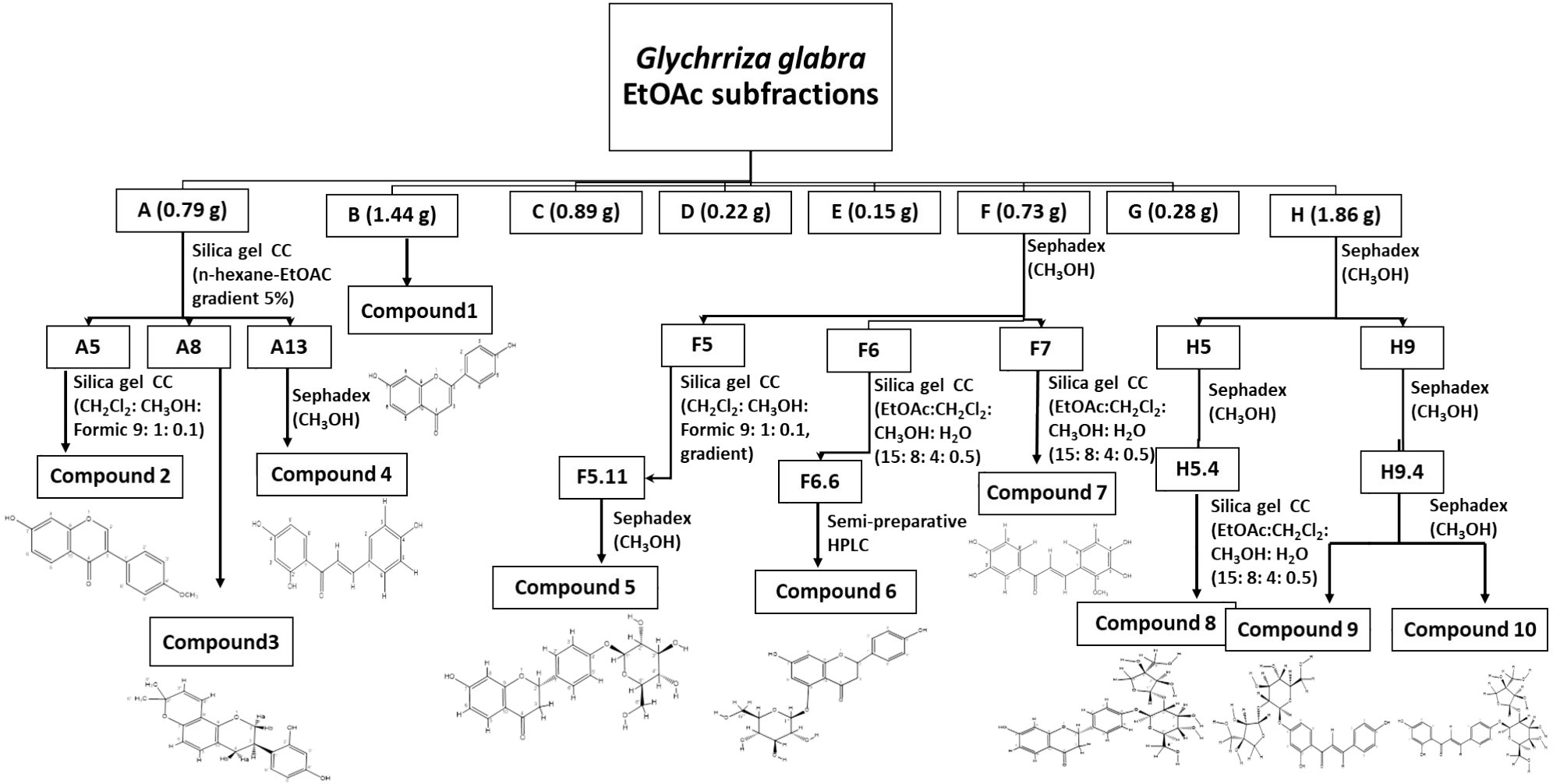

Fig. S1 Scheme for the isolation of 10 compounds from EtOAc subfractions of the roots of *G. glabra*

Table S1. % inhibition of different fractions of *G. glabra* roots extract on FXa*

Fraction	Concentration (mg/ml)	% Inhibition (%)
Light petroleum	1.5	1.55±0.056
	3	0.69±0.083
CH_2Cl_2	1.5	62.31±0.097
	3	71.94±0.034
EtOAc	1.5	87.26±0.034
	3	**97.93±0.019**
n-BuOH	1.5	13.08±0.095
	3	21.51±0.04
Rivaroxaban (positive control)	100 nM	88.64±0.76

***** Inhibition rate was expressed as mean±SD, n=2

Table S2. *In-vitro* % inhibition of EtOAc subfractions of *G. glabra* on FXa enzyme

Subfraction[#] (1 mg/ml)	% inhibition
(A)	60.94±1.18
(B)	61.03±1.99
(C)	63.85±0.18
(D)	65.64±0.45
(E)	77.66±0.66
(F)	84.94±0.82
(G)	76±0.87
(H)	77.39±0.46

[#] Subfractions are arranged according to polarities from the least polar; A to the most polar; H

***** Inhibition rate was expressed as mean±SD, n=2

Table S3. ^{1}H-NMR spectral data of compounds (1- 10), δ values in ppm, J values in Hz

δ H (J in Hz)											
Position	**(1)**	**(2)**	**(3)**	**(5)**	**(6)**	**(8)**	**Position**	**(4)**	**(7)**	**(9)**	**(10)**
2	-	8.15, s	Ha (S): 3.96, t (10.1) Hb (R): 4.31, dd (10.35, 1.1)	5.45, dd (12.76, 3.02)	5.27, dd (13.1, 3.4)	5.43, dd (12.94, 2.87)	C- α	7.61, d (15.36)	7.61, d (15.62)	7.59, d (15.29)	7.68, d (15.35, 9.57)
3	6.70, s	-	3.44, m	Ha: 2.73, dd (16.92, 3.04) Hb: 3.04, dd (16.94, 12.80)	Ha: 2.63, dd (16.4, 3.1) Hb: 2.93, dd (17.3, 13.0)	Ha: 2.72, dd (16.91, 2.92) Hb: 3.03, dd (16.84, 12.96)	C- β	7.79, d (15.4)	7.96, d (15.71)	7.78, d (15.35, 9.57)	7.80, dd (15.34, 10.04)
4	-	-	Ha (S): 2.79, dd (15.7, 4.2) Hb (R): 2.90, dd (15.7, 10.7)	-	-	-	C=O	-	-	-	-
5	7.86, d (8.70)	8.07, d (8.7)	6.77, d (8.3)	7.73, d (8.72)	-	7.72, d (8.73)	1	-	-	-	-
6	6.89, dd (7.51, 2.23)	7.00, dd, (9.3, 1.65)	6.36, d (8.2)	6.5, dd (8.73, 2.27)	5.99, s	6.5, dd (8.70, 2.29)	2	7.63, d (8.64)	-	7.61, d (8.52)	7.69, d (8.79)
7	-	-	-	-	-	-	3	6.85, d (8.61)	-	6.84, d (8.41)	7.12, d (8.77)
8	6.95, d (2.22)	6.92, s	-	6.37, d (2.25)	6.33, d (17.5)	6.36, d (2.30)	4	-	-	-	-
9	-	-	-	-	-	-	5	6.85, d (8.61)	6.65, d (8.52)	6.84, d (8.41)	7.12, d (8.77)
10	-	-	-	-	-	-	6	7.63, d (8.64)	7.20, d (8.52)	7.61, d, (8.52)	7.69, d (8.79)
1'	-	-	-	-	-	-	1'	-	-	-	-
2'	7.91, d (8.80)	7.56, d (8.7)	-	7.44, d (8.70)	7.30, dd (8.7, 2.8)	7.43, d (8.69)	2'	-	7.51, d (2.10)	-	-
3'	6.91, d (8.84)	6.99, d (8.7)	6.24, d (2.0)	7.14, d (8.68)	6.80, dd (8.6, 2.3)	7.11, d (8.68)	3'	6.28, d (2.4)	-	6.57, d (2.39)	6.30, d (2.36)
4'	-	-	-	-	-	-	4'	-	-	-	-
5'	6.91, d (8.84)	6.99, d (8.7)	6.32, dd (8.3, 2.1)	7.14, d (8.68)	6.80, dd (8.6, 2.3)	7.11, d (8.68)	5'	6.41, dd (8.86, 2.42)	6.88, d (8.28)	6.64, dd (8.95, 2.48)	6.42, dd (8.85, 2.34)
6'	7.91, d (8.80)	7.56, d (8.7)	6.86, d (8.4)	7.44, d (8.70)	7.30, dd (8.7, 2.8)	7.43, d (8.69)	6'	7.96, d (8.92)	7.54, dd (8.28, 2.08)	8.02, d (9.01)	7.97, d (8.93)
OCH3-4'	-	3.83, s	-	-	-	-	OCH3-2	-	3.85, s	-	-
2"	-	-	-	-	-	-	-	-	-	-	-
3"	-	-	5.53, d (9.9)	-	-	-	-	-	-	-	-
4"	-	-	6.61, d (9.9)	-	-	-	-	-	-	-	-
5"	-	-	1.40, s	-	-	-	-	-	-	-	-
6"	-	-	1.40, s	-	-	-	-	-	-	-	-

Table S3. continued

δ H (*J* in Hz)											
Position	**(1)**	**(2)**	**(3)**	**(5)**	**(6)**	**(8)**	**Position**	**(4)**	**(7)**	**(9)**	**(10)**
- β -Glu moiety							**- β -Glu moiety**				
1"	-	-	-	4.94, d (7.94)	4.75, d (7.2)	5.00, d (7.47)	1"	-	-	5.09, d (6.1)	5.05, d (7.33)
2"	-	-	-	3.43- 3.47, m	3.53, m	3.66, m	2"	-	-	3.67, m	3.67, m
3"	-	-	-	3.43- 3.47, m	3.44, m	3.66, m	3"	-	-	3.49, m	3.49, m
4"	-	-	-	3.43- 3.47, m	3.65, s	3.40- 3.44, m	4"	-	-	3.43, m	3.42, m
5"	-	-	-	3.43- 3.47, m	3.49	3.40- 3.44, m	5"	-	-	3.98, d (1.58)	3.97, d (1.62)
6"	-	-	-	Ha: 3.70, dd (12.05, 5.44) Hb: 3.90\, d (11.90, 2.07)	Ha: 3.67, t (12) Hb: 3.92, t (11)	Ha: 3.70, m (12.13) Hb: 3.89, dd (12.17, 2.17)	6"	-	-	Ha: 3.73, m Hb: 3.91, d (12.23)	Ha: 3.71, m Hb: 3.91, d (12.18)
- β -Api moiety							**- β -Api moiety**				
1'"	-	-	-	-	-	5.48, d (1.47)	1'"	-	-	5.48, dd (5.23, 1.62)	5.47, dd (5.66, 1.62)
2'"	-	-	-	-	-	3.96, d (1.52)	2'"	-	-	3.67, m	3.67, m
3'"	-	-	-	-	-	-	3'"	-	-	-	-
4'"	-	-	-	-	-	Ha: 3.80, d (9.53) Hb: 4.06, d (9.60)	4'"	-	-	Ha: 3.82, d (9.56) Hb: 4.05, t (10.15)	Ha: 3.81, d (9.59) Hb: 4.05, dd (11.76, 9.61)
5'"	-	-	-	-	-	3.55, d (2.50)	5'"	-	-	3.55, d (2.17)	3.55, d (2.17)

Table S4. ^{13}C-NMR spectral data of compounds (1- 10), δ values in ppm

δ C (ppm)											
Position	**(1)**	**(2)**	**(3)**	**(5)**	**(6)**	**(8)**	**Position**	**(4)**	**(7)**	**(9)**	**(10)**
2	162.70	153.45	69.98	80.85	79.98	80.89	C- α	118.54	120.77	117.94	120.17
3	104.49	124.86	31.46	45.11	46.47	45.06	C- β	145.72	141.09	146.52	144.94
4	176.39	175.77	30.38	193.35	192.59	193.36	C=O	193.74	191.65	194.02	193.48
5	126.50	128.53	129.09	130.01	162.94	130.02	1	128.01	121.63	127.82	130.61
6	114.89	115.75	108.78	112.04	102.06	112.00	2, 6	131.96	150.07	132.15	131.57
7	162.53	163.34	151.53	167.11	164.97	166.99	3, 5	117.10	139.84	117.09	117.93
8	102.51	103.21	109.94	104.01	100.96	103.99	4	161.68	150.90	161.81	161.02
9	157.44	158.77	149.58	165.55	166.84	165.53	5	117.10	112.90	117.09	117.93
10	116.09	118.59	114.65	115.08	103.90	115.10	6	131.96	120.55	132.15	131.57
1'	121.82	125.63	120.04	134.58	131.58	134.48	1'	114.71	131.98	116.67	114.81
2'	128.16	131.08	154.51	128.92	129.06	128.98	2'	166.48	116.53	166.92	165.00
3'	115.96	114.48	103.16	117.94	116.42	117.74	3'	104.06	146.72	105.03	103.98
4'	160.74	160.41	154.88	159.36	159.01	159.21	4'	166.48	152.27	164.96	167.69
5'	115.96	114.48	107.83	117.94	116.42	117.74	5'	109.55	116.10	110.95	109.38
6'	128.16	131.08	128.29	128.92	129.06	128.98	6'	133.51	123.57	133.14	133.61
OCH3-4’	-	55.63	-	-	-	-	OCH3-2	-	61.90	-	-
2"	-	-	75.86	-	-	-	-	-	-	-	-
3"	-	-	129.24	-	-	-	-	-	-	-	-
4"	-	-	116.83	-	-	-	-	-	-	-	-
5"	-	-	27.61	-	-	-	-	-	-	-	-
6"	-	-	27.41	-	-	-	-	-	-	-	-
- β -Glu moiety							**- β -Glu moiety**				
1"	-	-	-	102.32	105.45	100.92	1"	-	-	100.03	100.51
2"	-	-	-	75.04	74.90	78.73	2"	-	-	78.63	78.79
3"	-	-	-	78.11	77.36	78.75	3"	-	-	78.25	78.30
4"	-	-	-	71.50	71.34	71.52	4"	-	-	71.31	71.51
5"	-	-	-	78.31	78.74	78.23	5"	-	-	78.74	78.71
6"	-	-	-	62.64	62.61	62.61	6"	-	-	62.47	62.64
- β -Api moiety							**- β -Api moiety**				
1'"	-	-	-	-	-	110.92	1'"	-	-	109.45	110.93
2'"	-	-	-	-	-	78.16	2'"	-	-	78.65	78.79
3'"	-	-	-	-	-	80.82	3'"	-	-	80.89	80.88
4'"	-	-	-	-	-	75.60	4'"	-	-	75.59	75.69
5'"	-	-	-	-	-	66.18	5'"	-	-	66.11	66.19

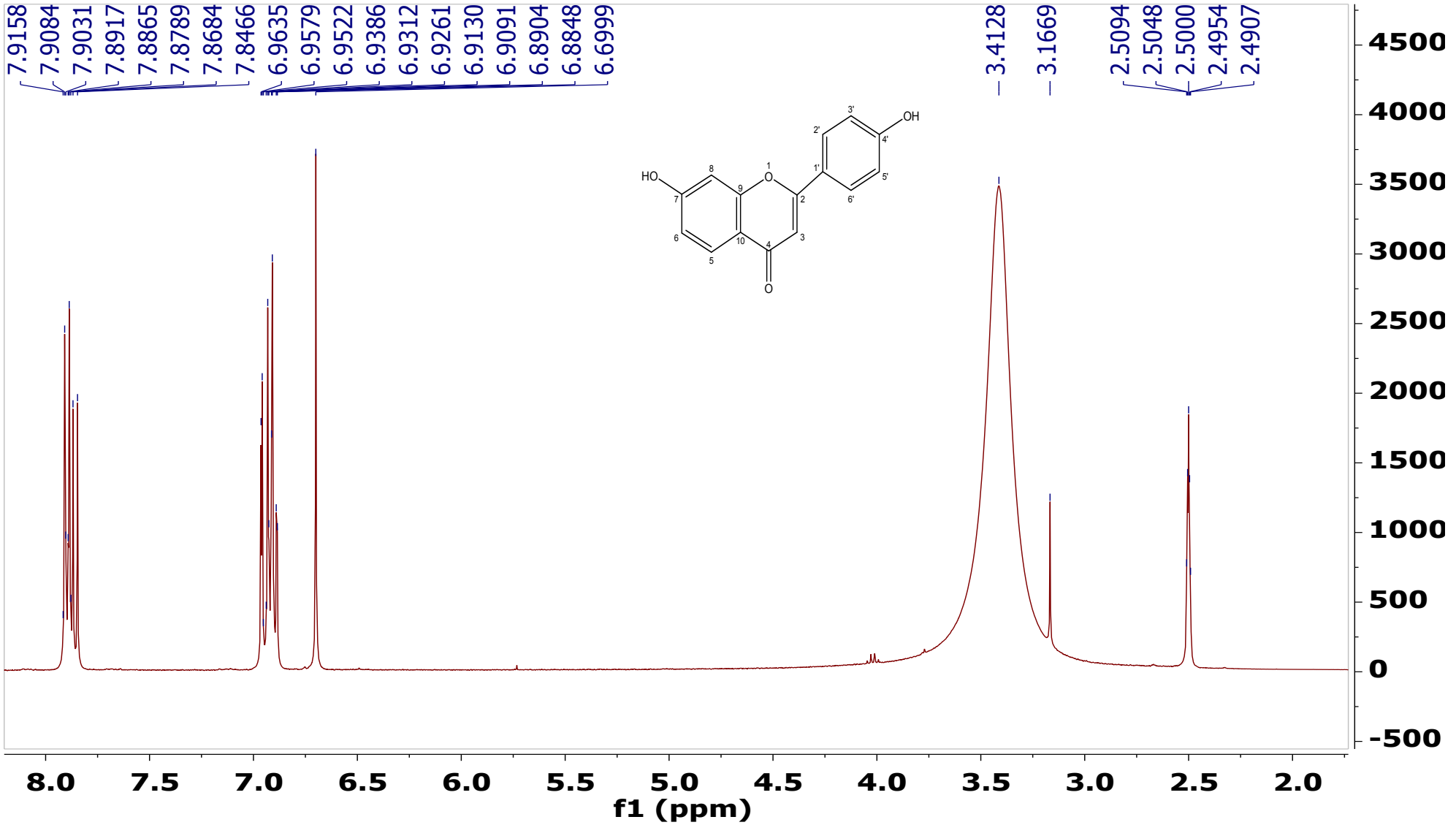

Fig. S2 ^{1}H-NMR spectrum of 7, 4'-dihydroxyflavone (1) in DMSO, d6 at 400 MHz

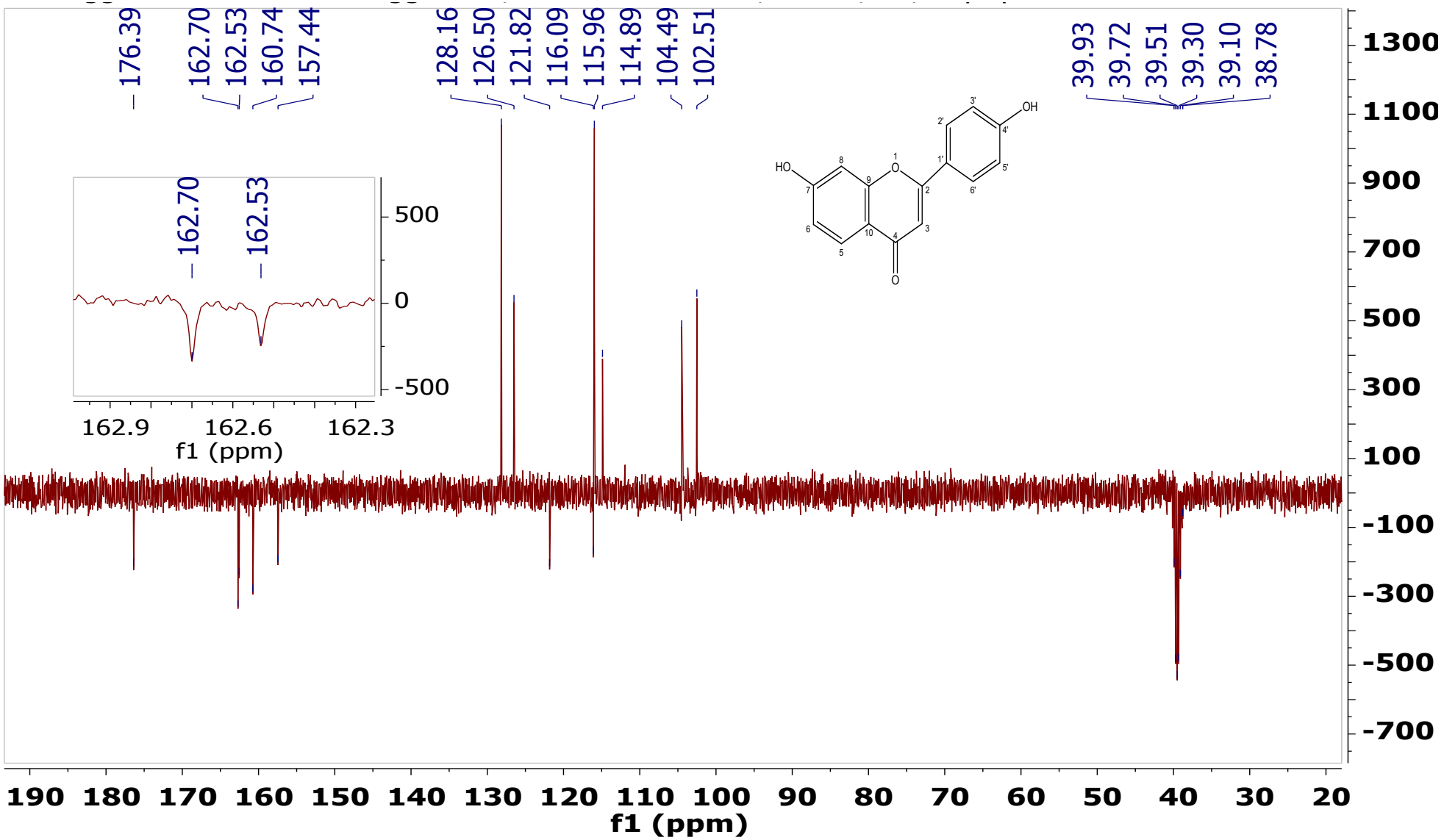

Fig. S3 ^{13}C-DEPTQ spectrum of 7, 4'-dihydroxyflavone (1) in DMSO, d6 at 100 MHz (with expansion at 162.3-162.9 ppm)

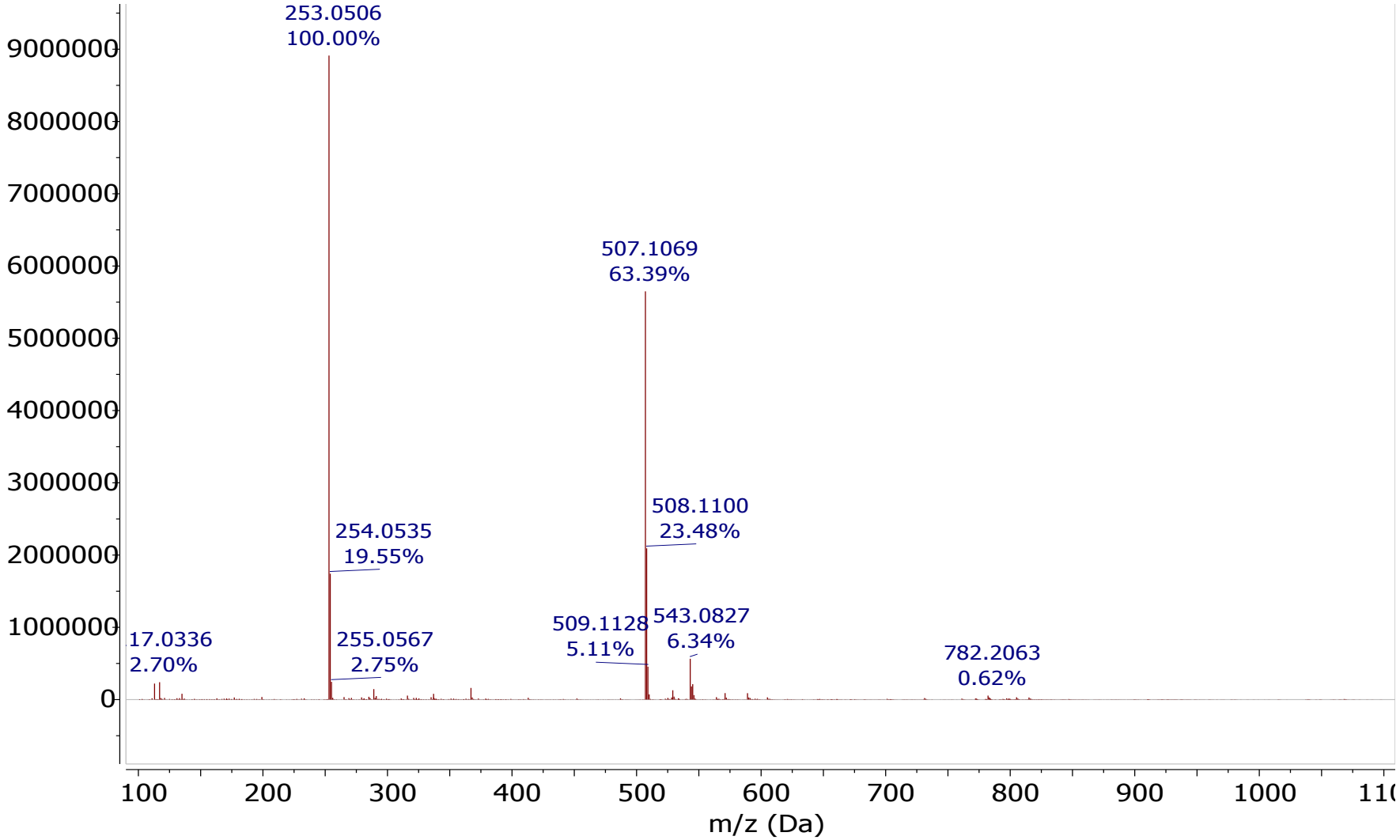

Fig. S4 HRESIMS spectrum of 7, 4'-dihydroxyflavone (1) in negative mode

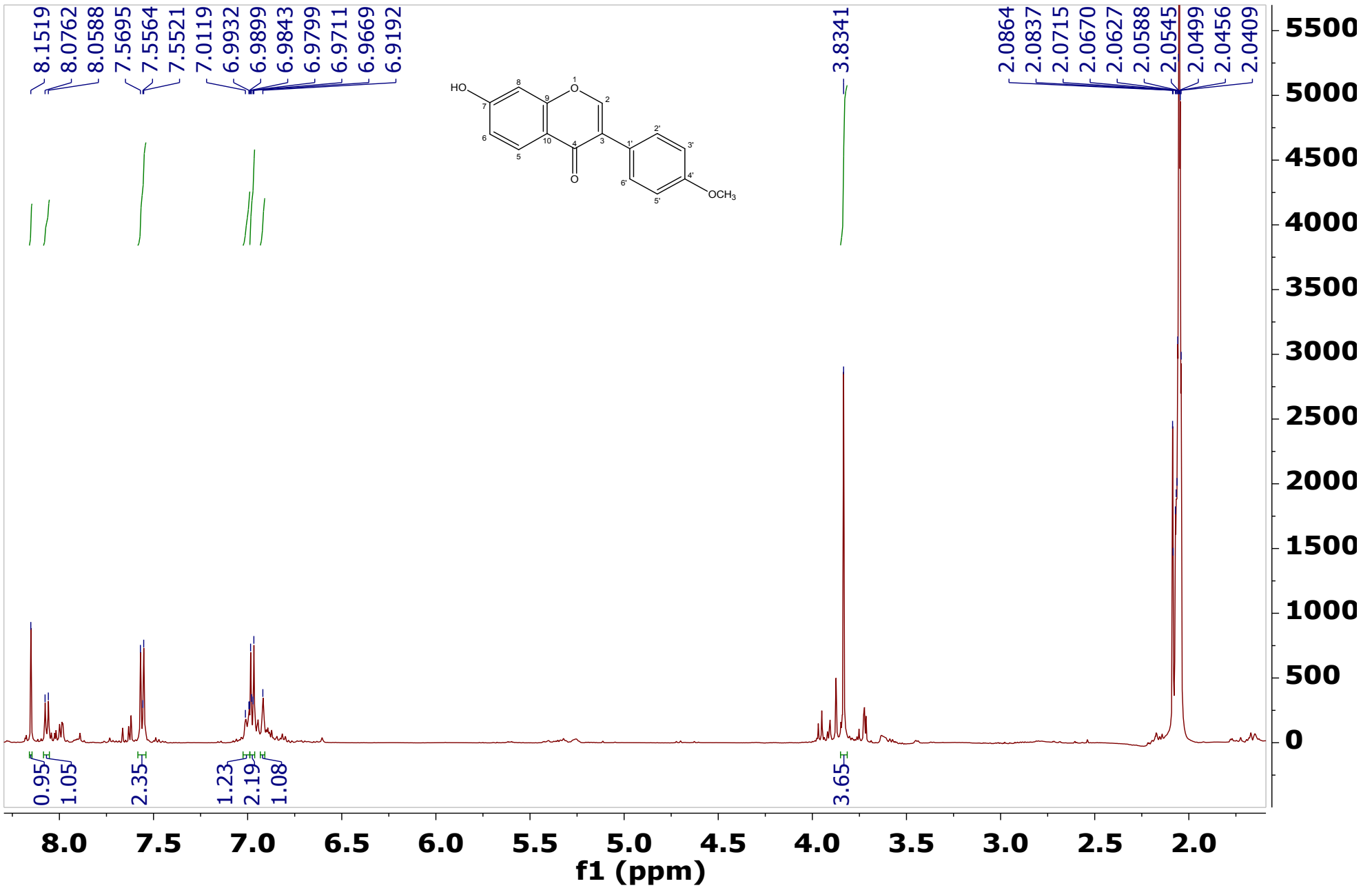

Fig. S5 ^{1}H-NMR spectrum of 7-hydroxy-4'-methoxyisoflavone (2) in Acetone, d6 at 500 MHz

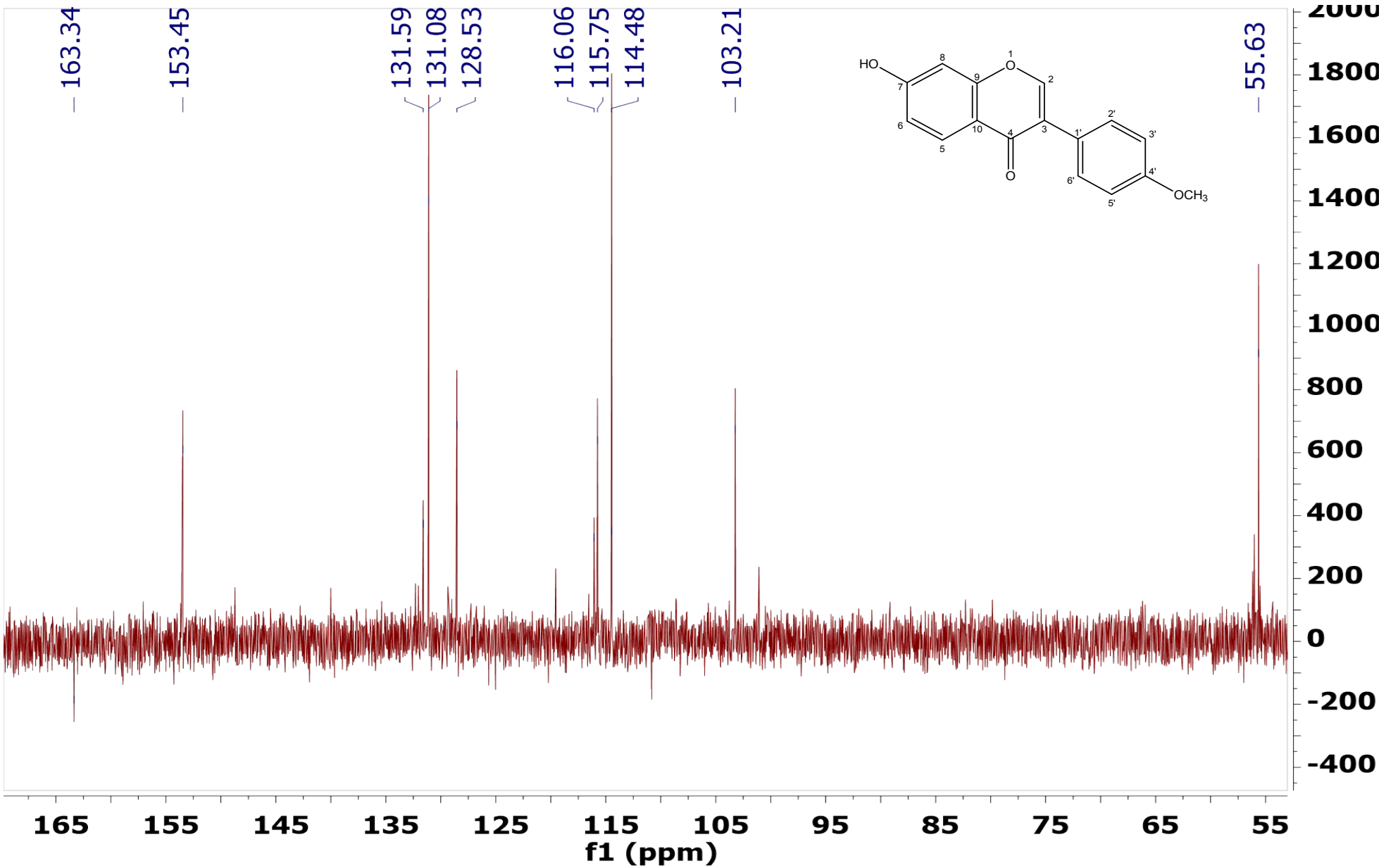

Fig. S6 ^{13}C-DEPTQ spectrum of 7-hydroxy-4'-methoxyisoflavone (2) in Acetone, d6 at 100 MHz

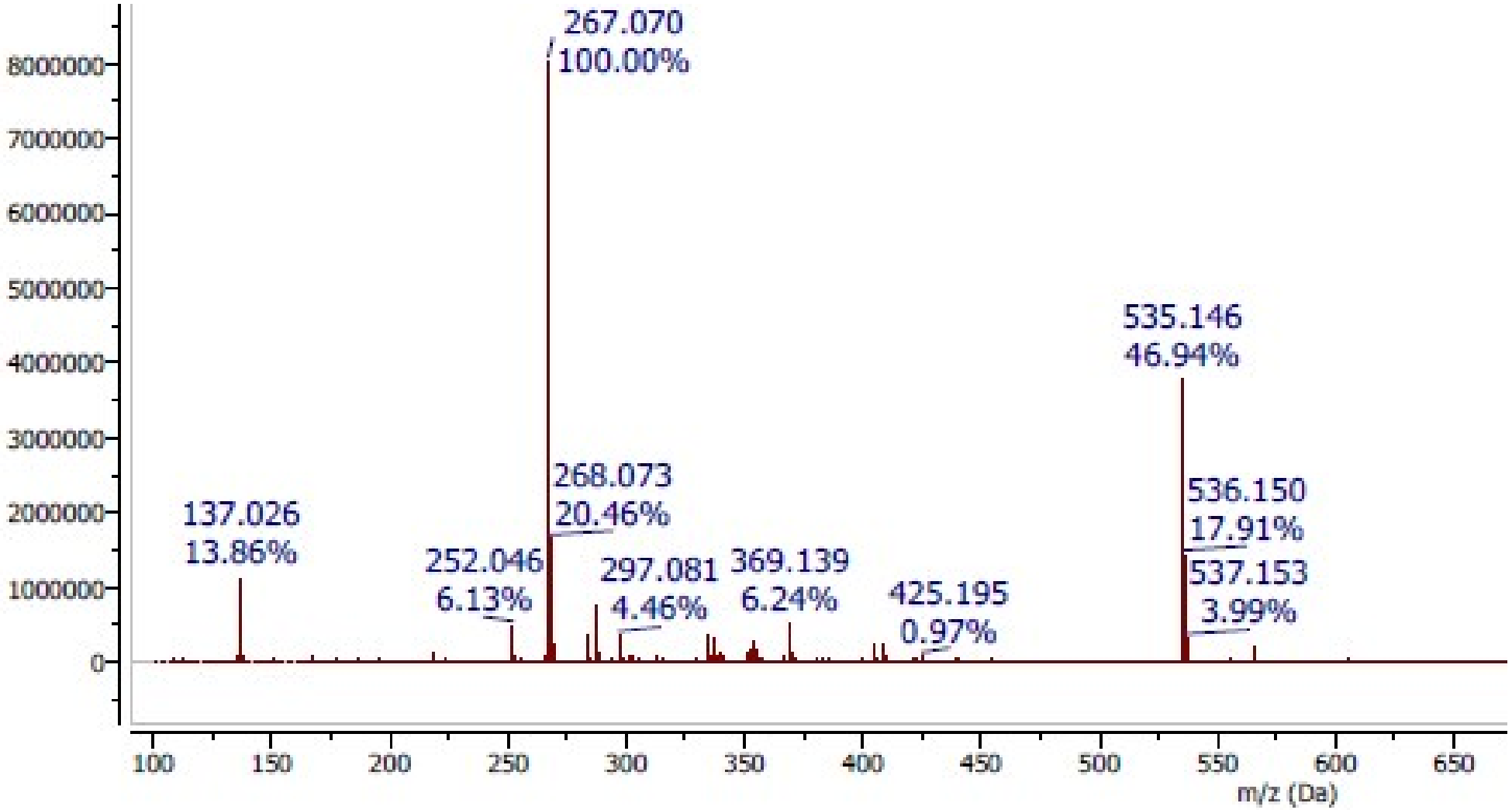

Fig. S7 HRESIMS spectrum of 7-hydroxy-4'-methoxyisoflavone (2) in negative mode

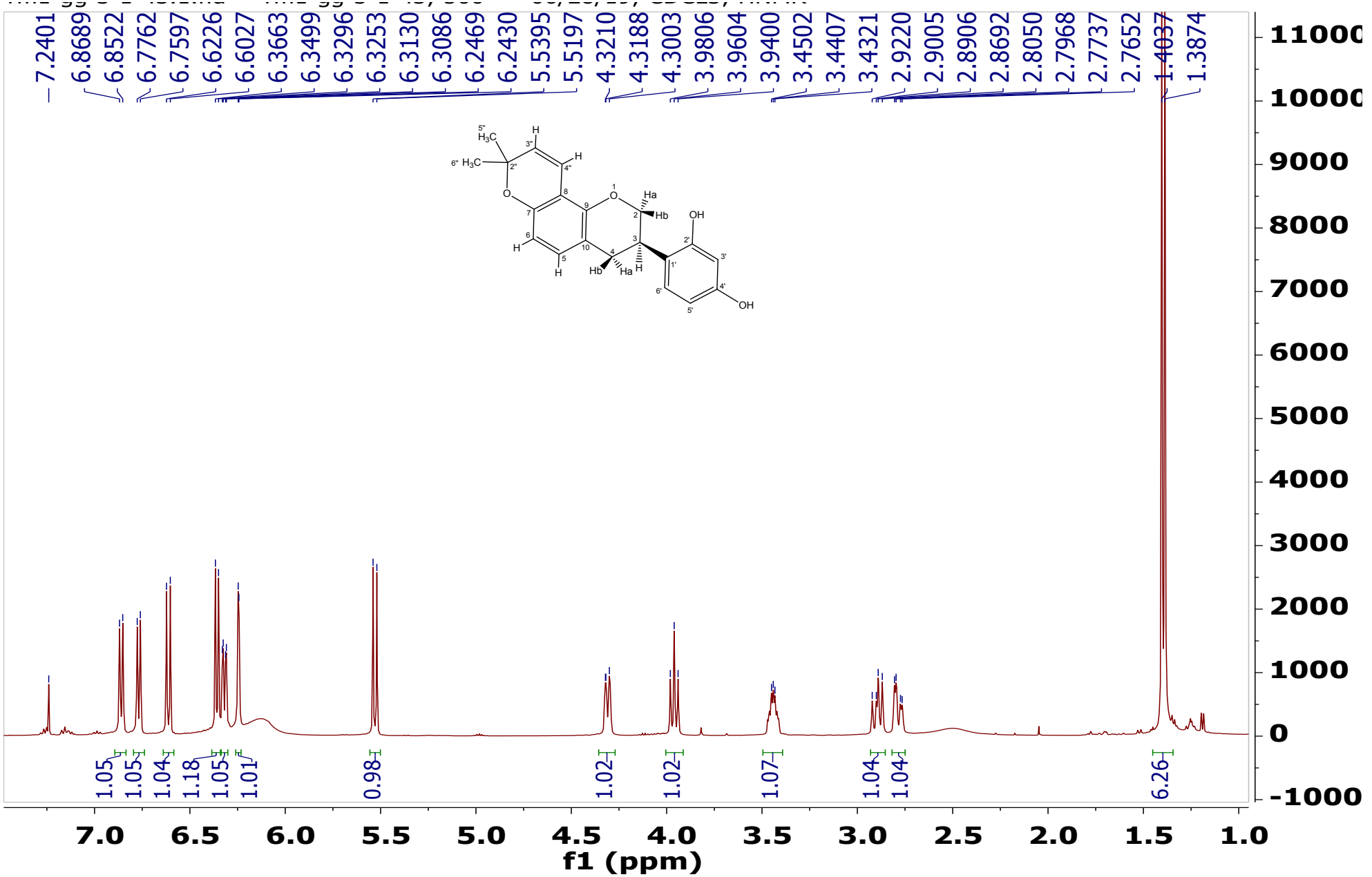

Fig. S8 ^{1}H-NMR spectrum of 3-R-glabridin (3) in $CDCl_3$ at 500 MHz

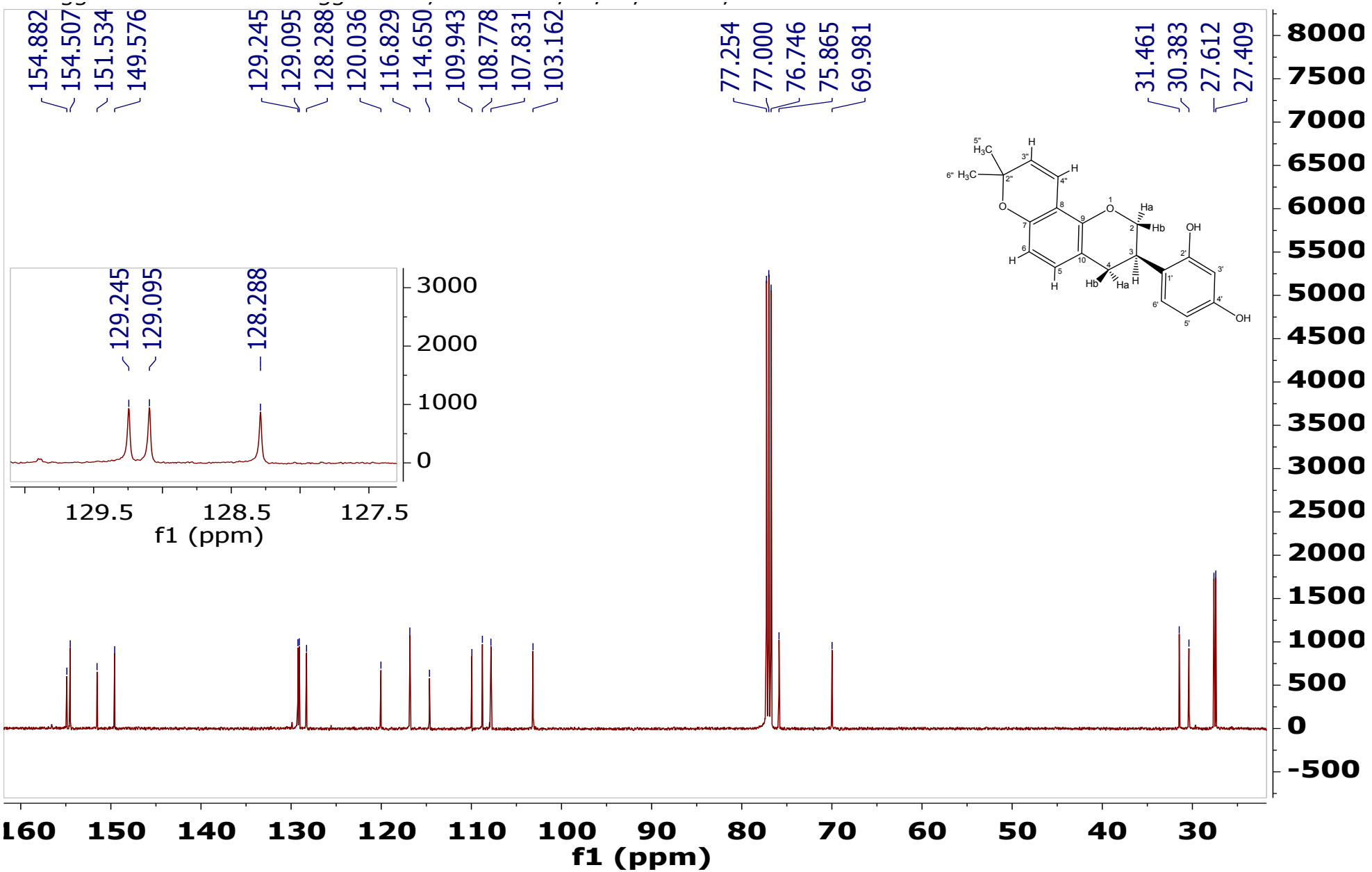

Fig. S9 ^{13}C-NMR spectrum of 3-R-glabridin (3) in $CDCl_3$ at 125 MHz with expansion at (127.5- 129.5 ppm)

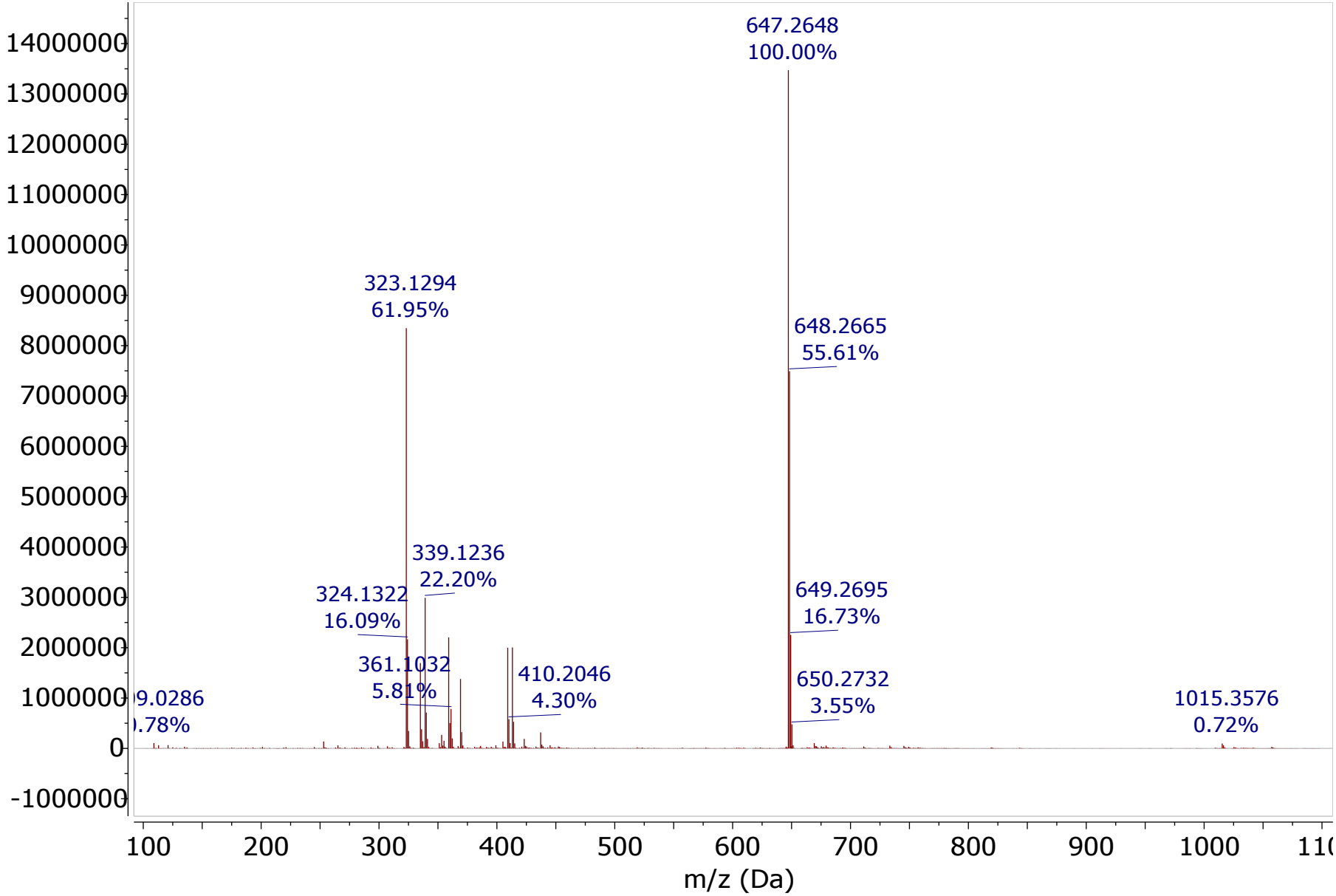

Fig. S10 HRESIMS spectrum of 3-R-glabridin (3) in negative mode

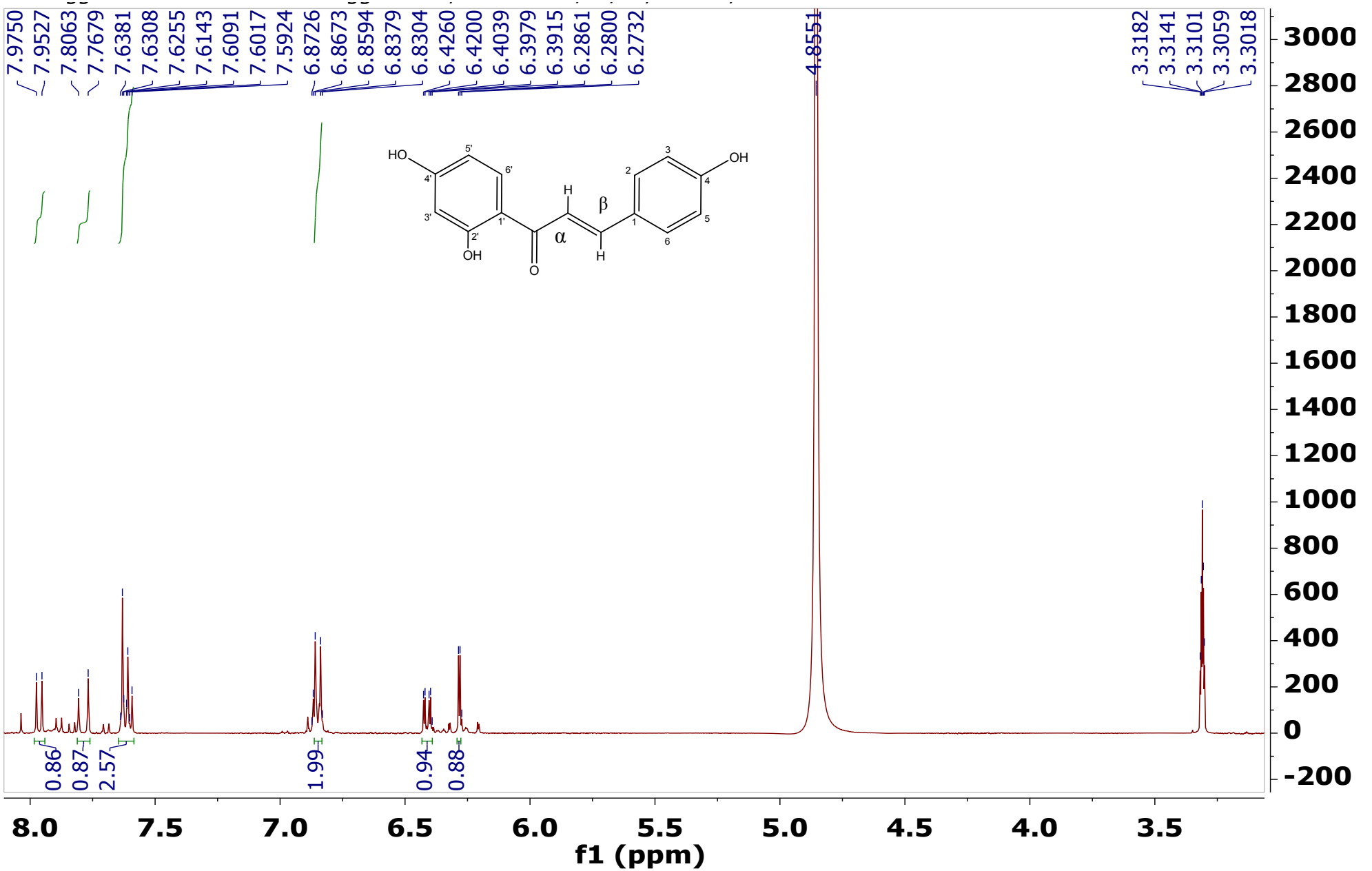

Fig. S11 ^{1}H-NMR spectrum of isoliquirtigenin (4) in CD_3OD at 400 MHz

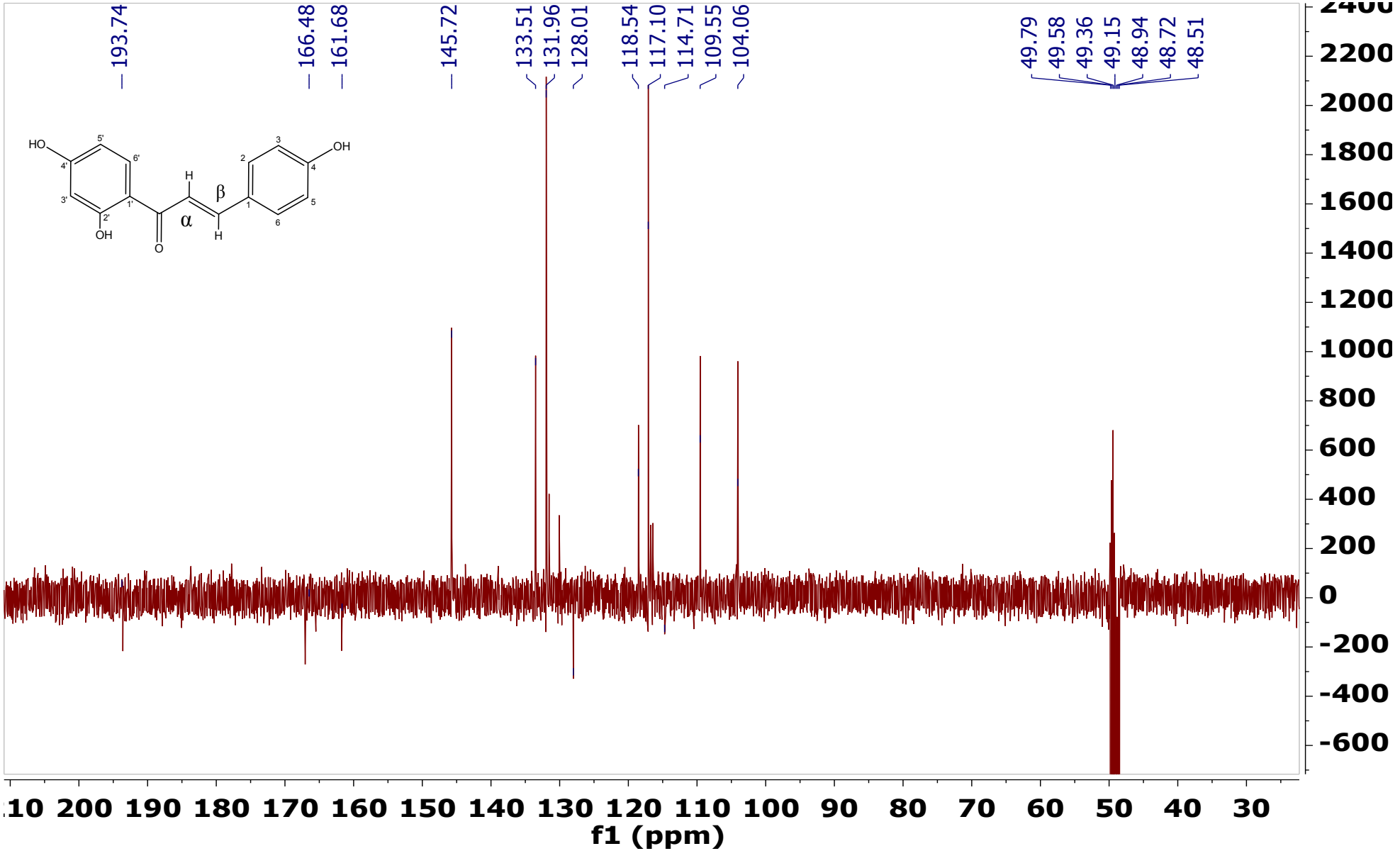

Fig. S12 ^{13}C-DEPTQ spectrum of isoliquirtigenin (4) in CD_3OD at 100 MHz

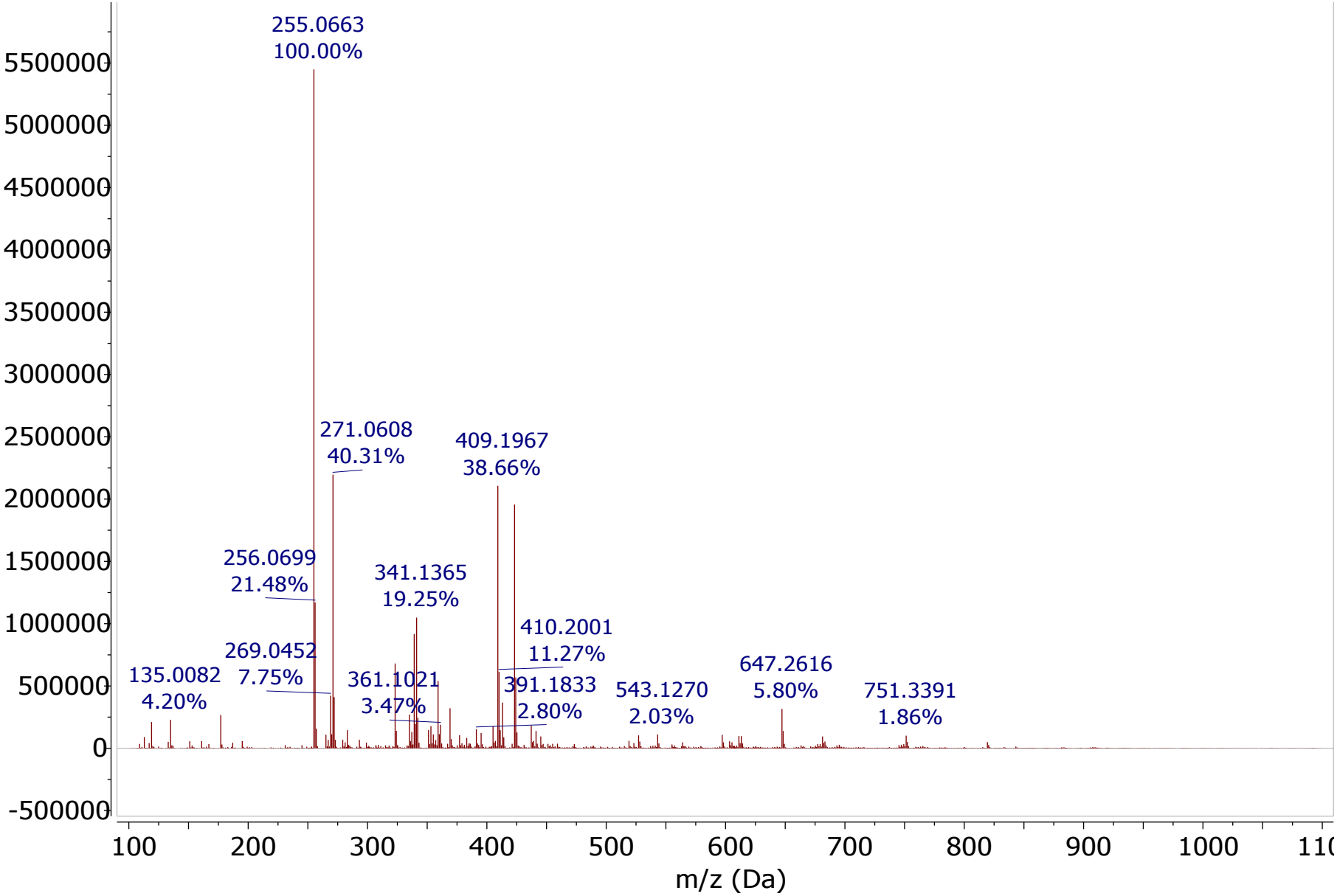

Fig. S13 HRESIMS spectrum of isoliquirtigenin (4) in negative mode

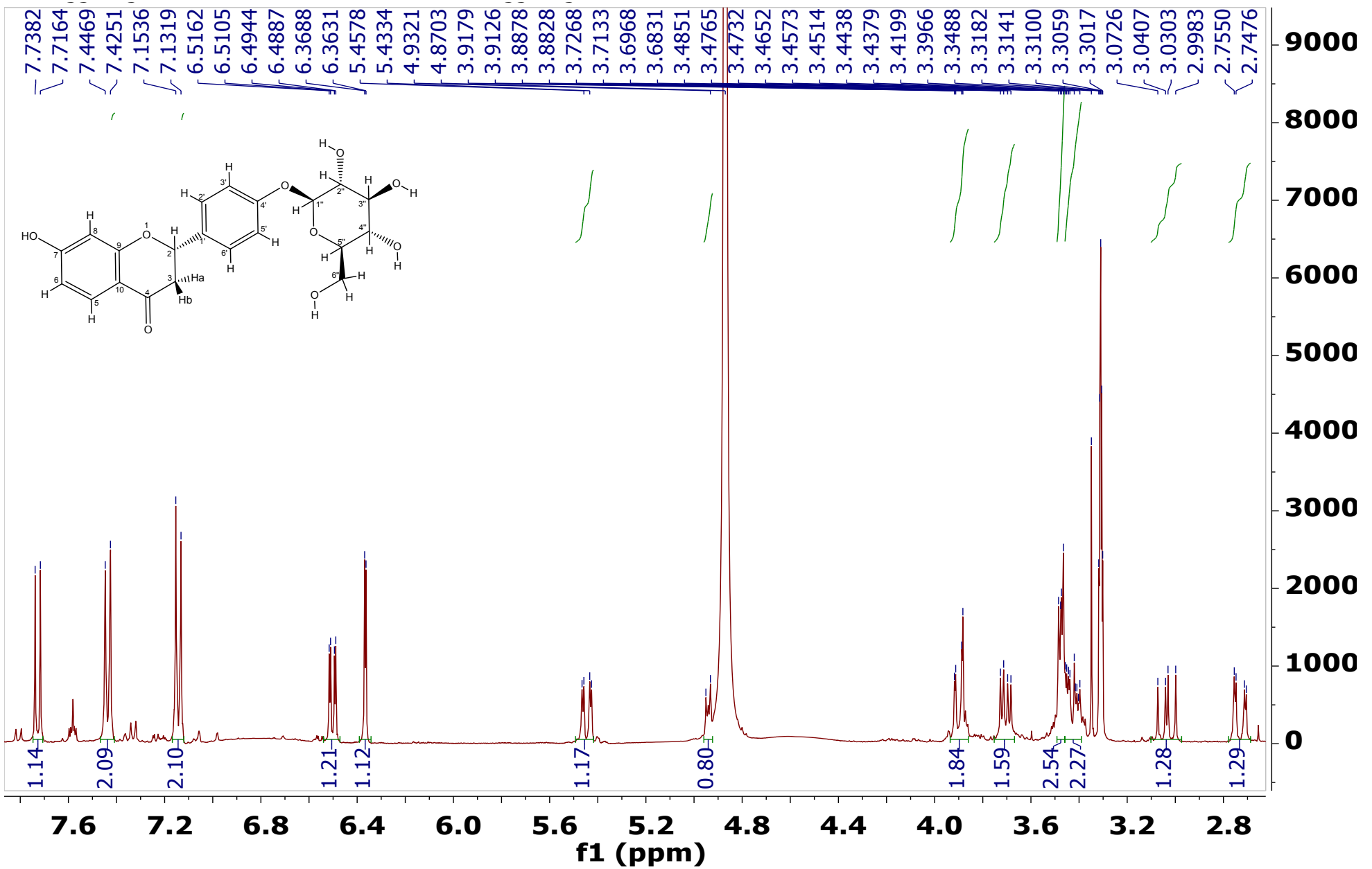

Fig. S14 ^{1}H-NMR spectrum of liquirtin (5) in CD_3OD at 400 MHz

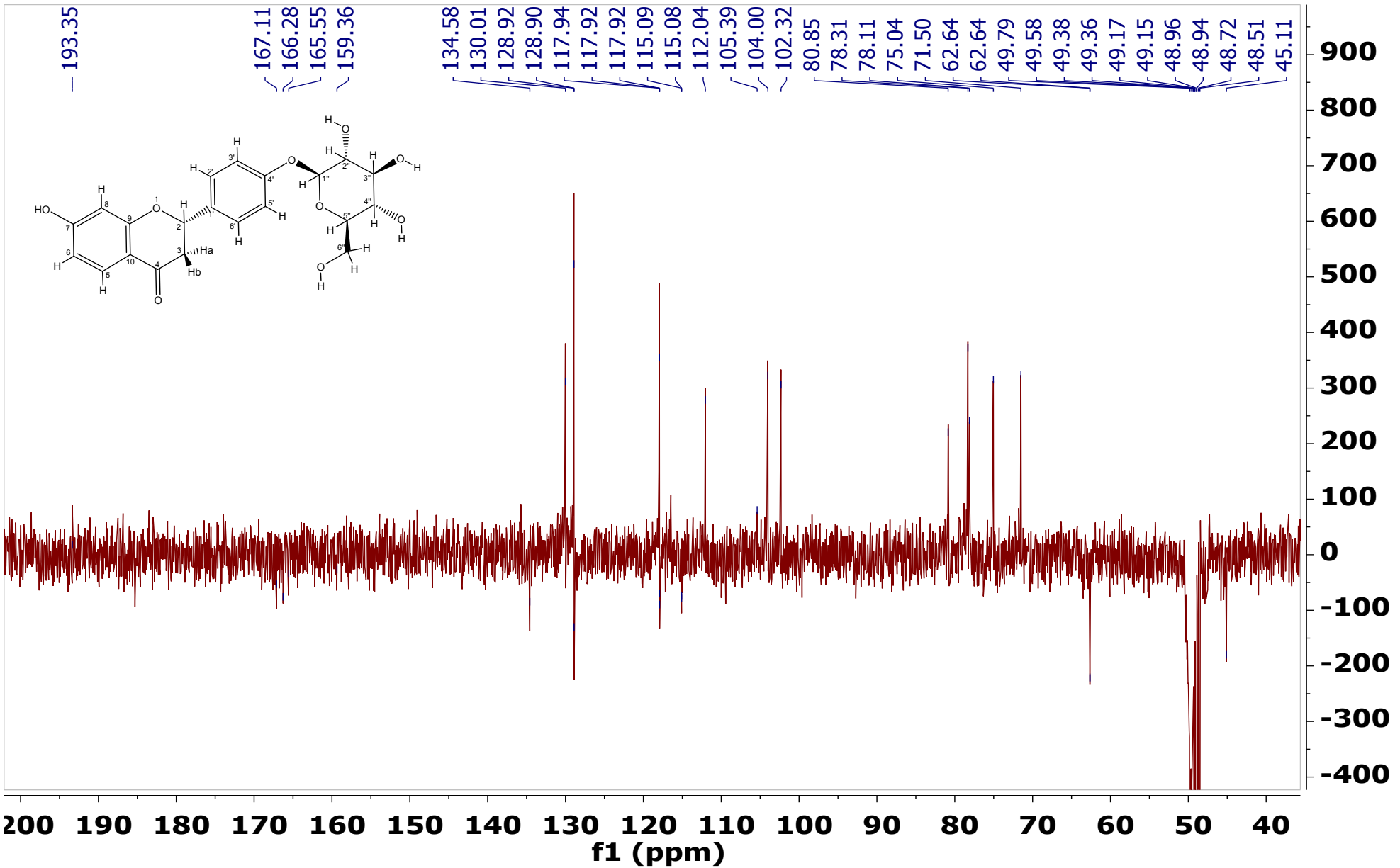

Fig. S15 ^{13}C-DEPTQ spectrum of liquirtin (5) in CD_3OD at 100 MHz

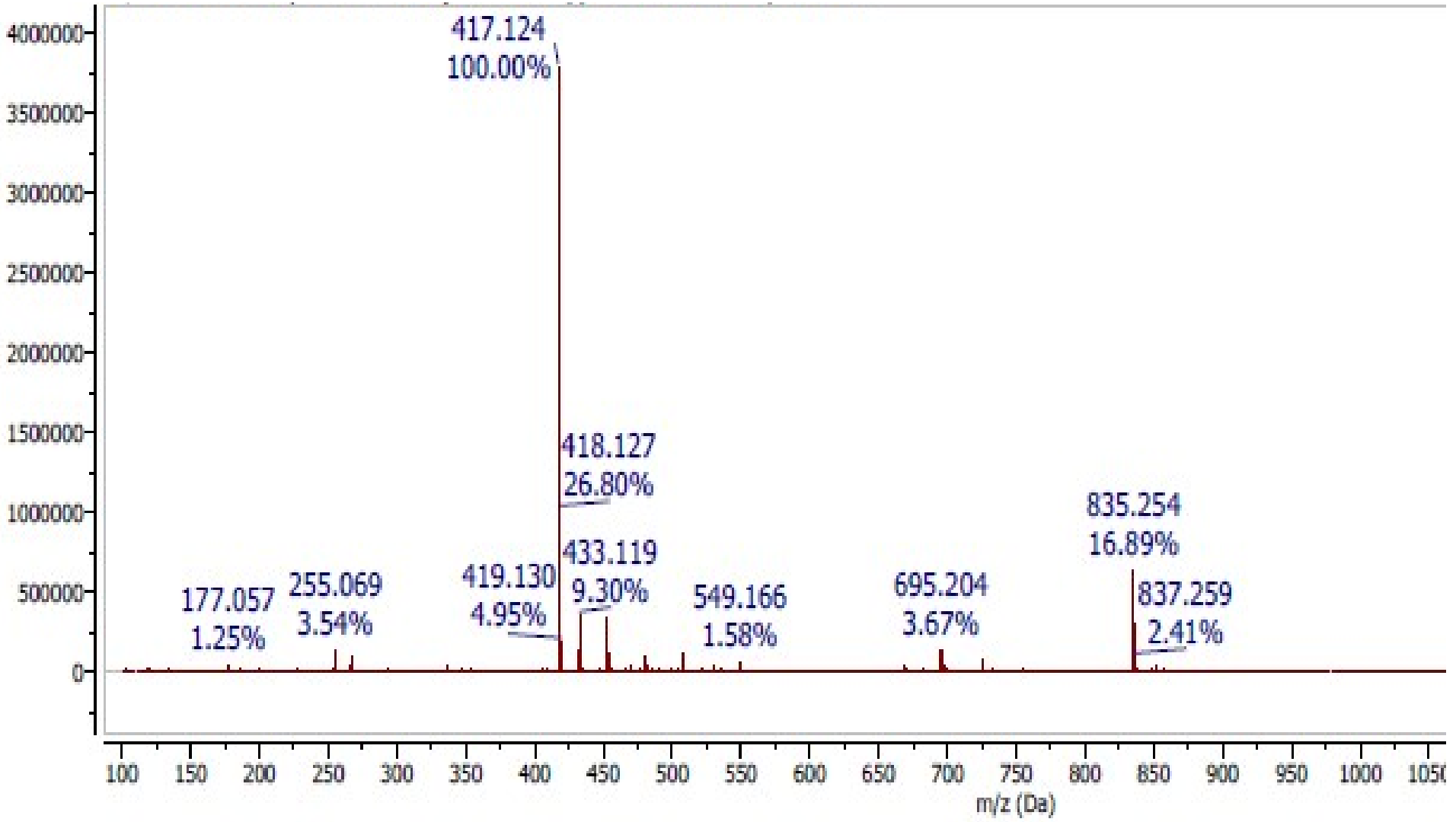

Fig. S16 HRESIMS spectrum of liquirtin (5) in negative mode

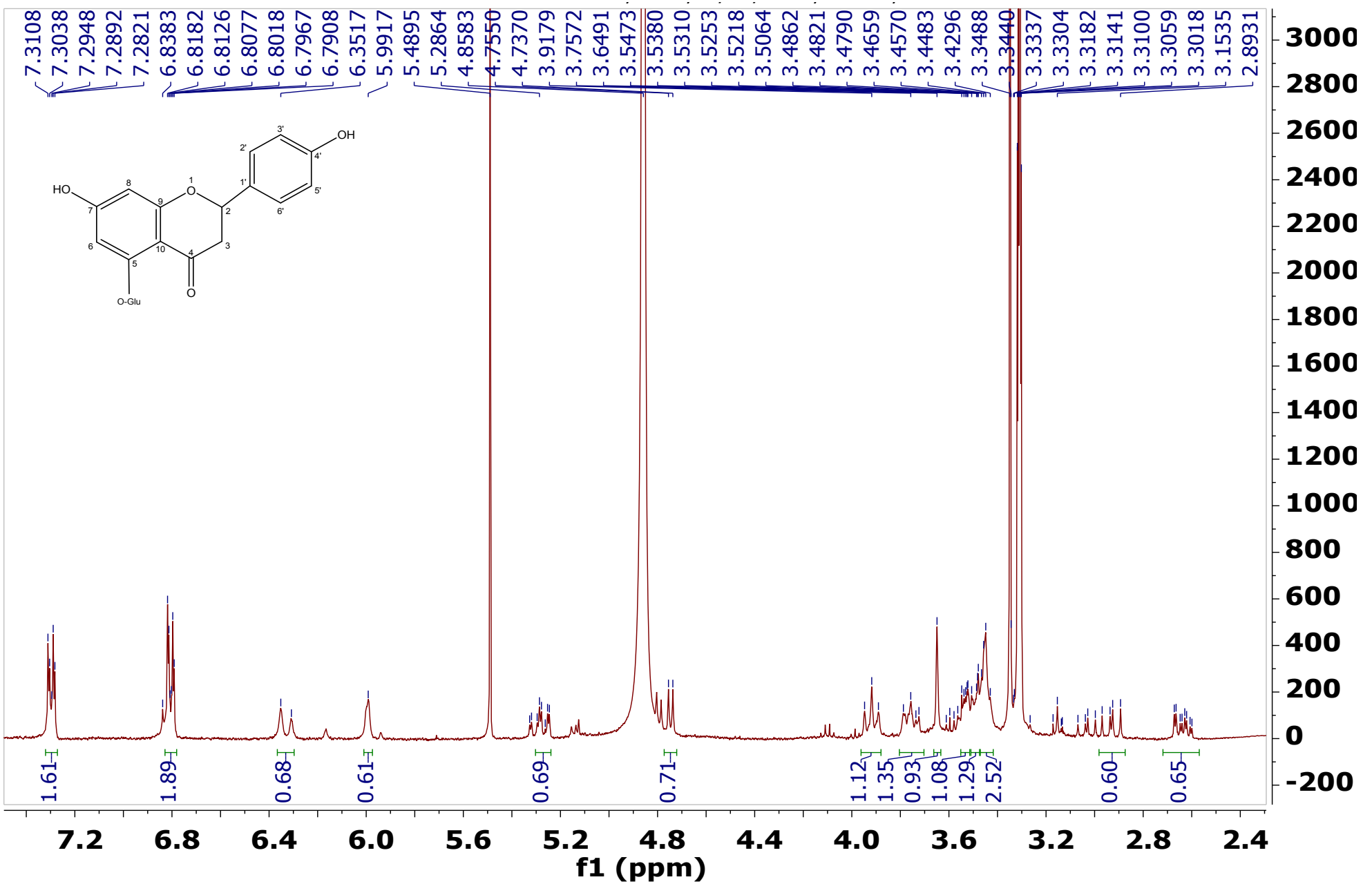

Fig. S17 ^{1}H-NMR spectrum of naringenin 5-O-glucoside (6) in CD_3OD at 400 MHz

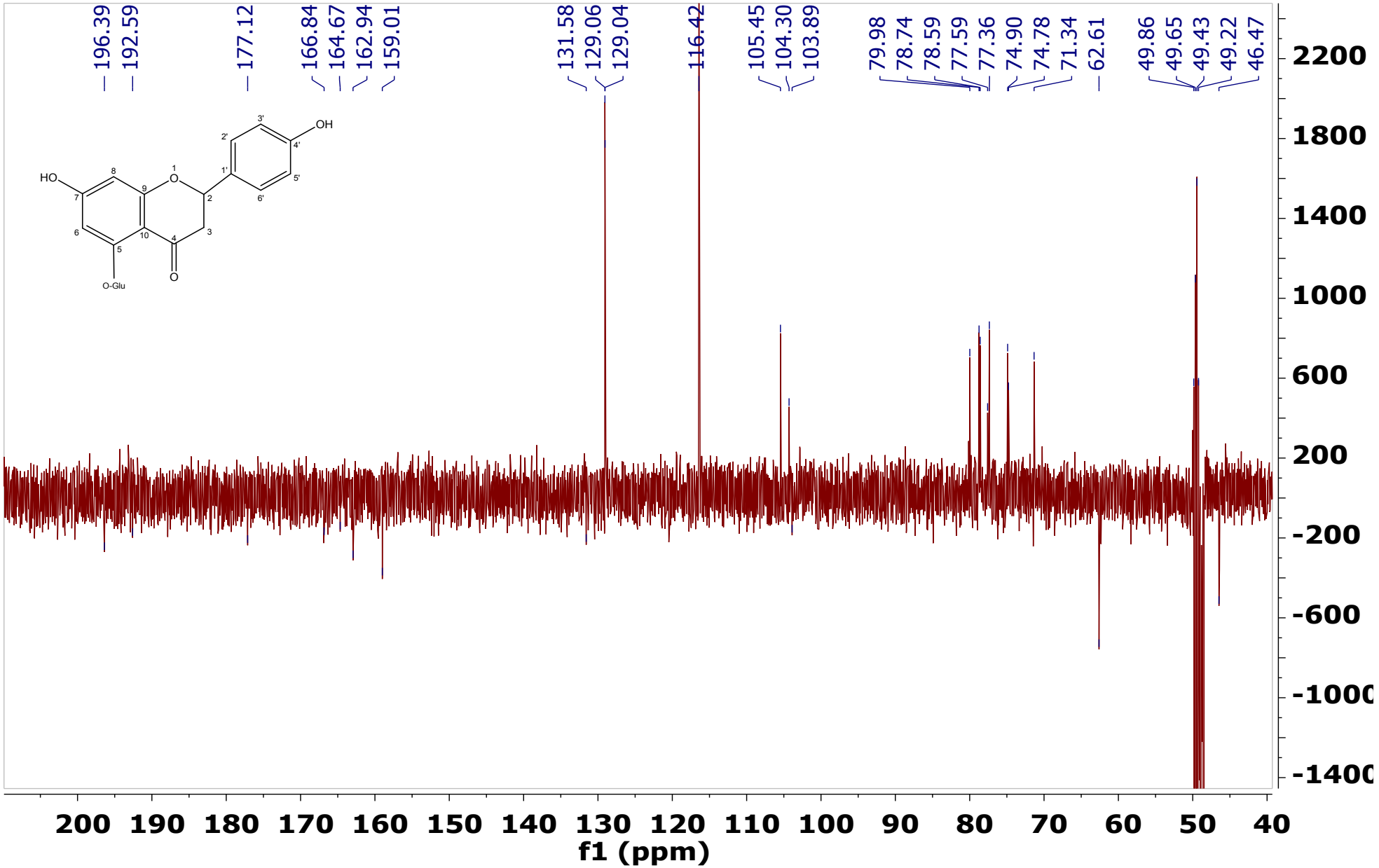

Fig. S18 ^{13}C-DEPTQ spectrum of naringenin 5-O-glucoside (6) in CD_3OD at 100 MHz

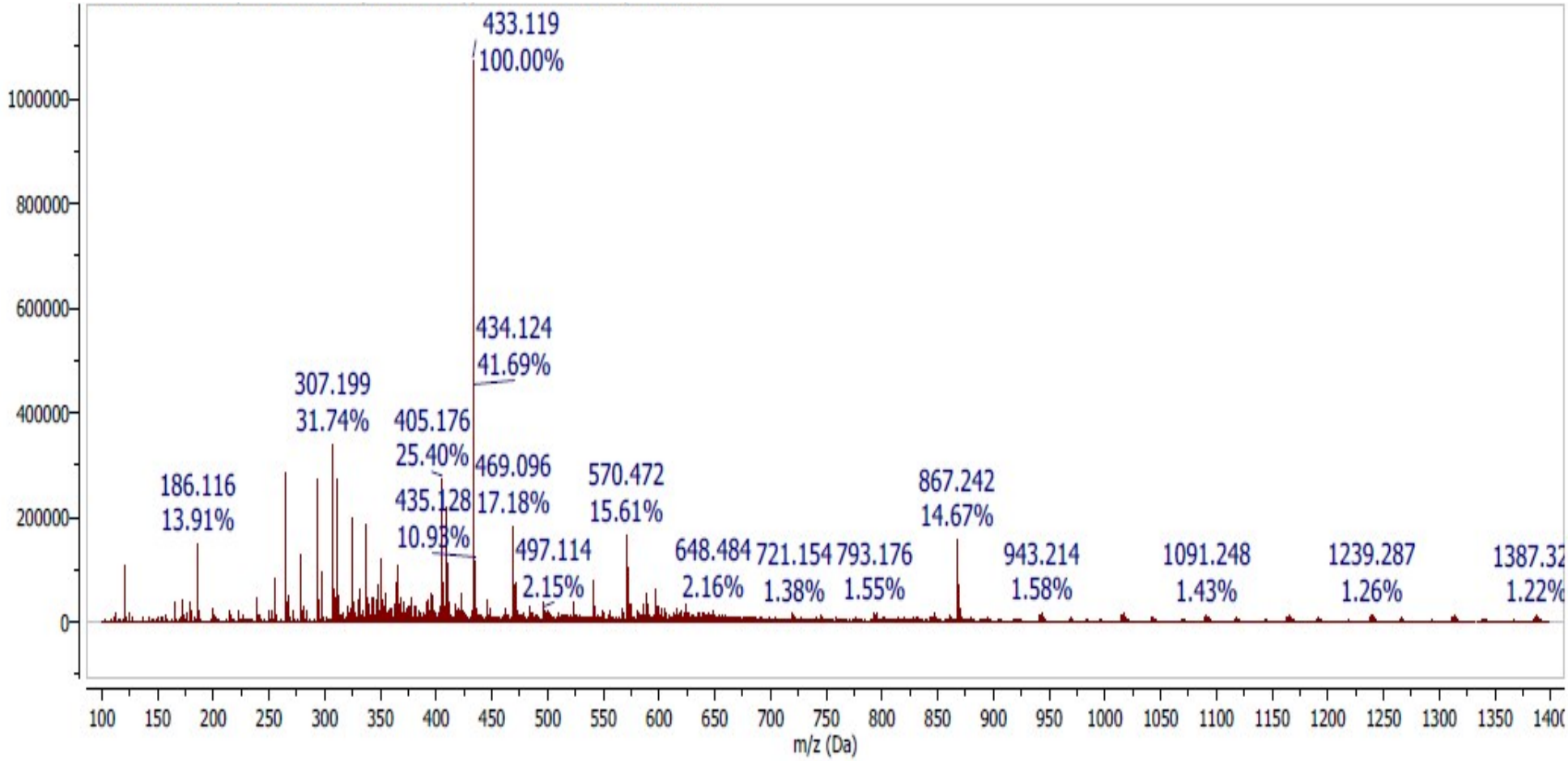

Fig. S19 HRESIMS spectrum of naringenin 5-O-glucoside (6) in negative mode

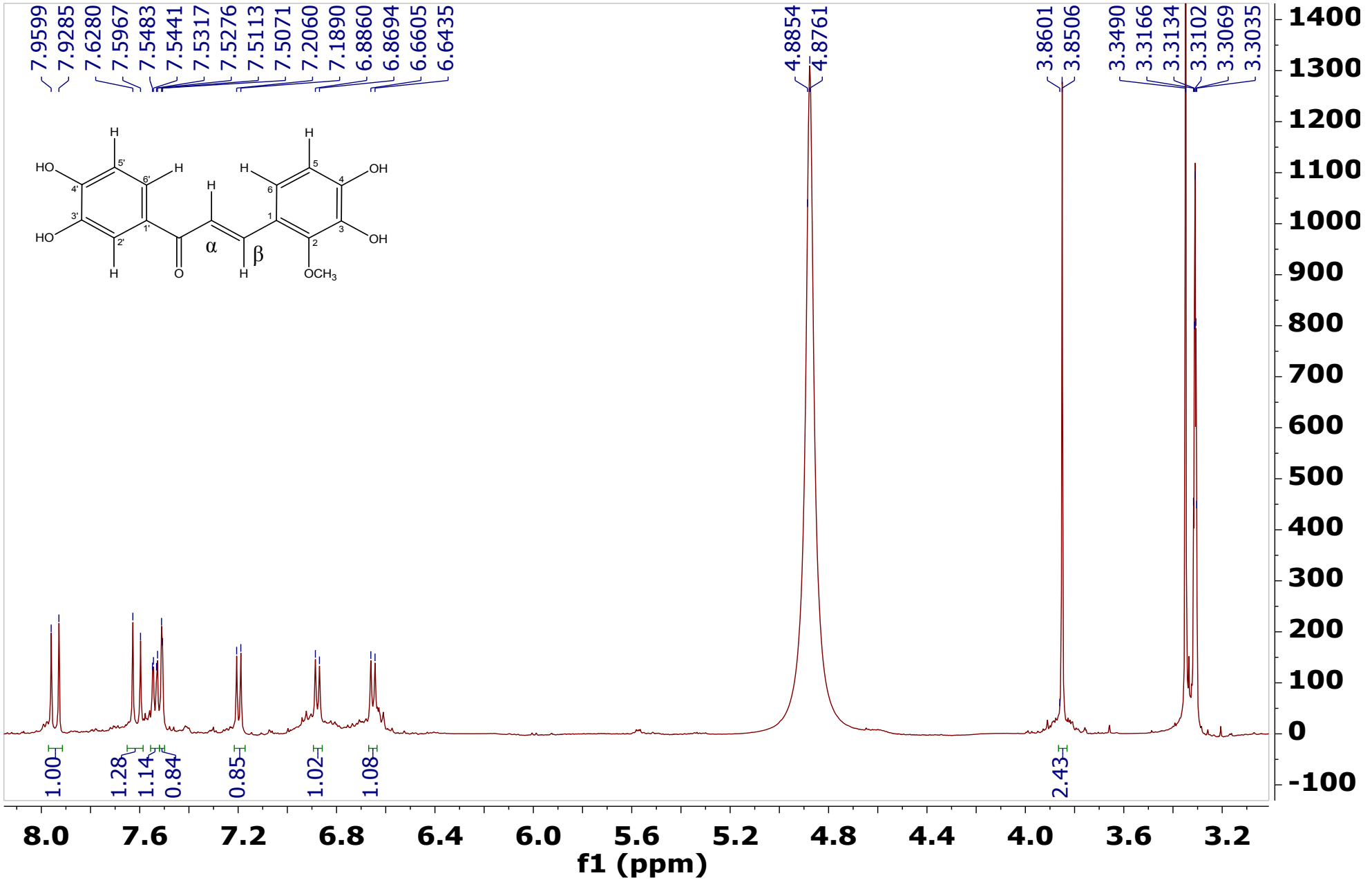

Fig. S20 ^{1}H-NMR spectrum of 3,3',4,4'-tetrahydroxy-2-methoxychalcone (7) in CD_3OD at 400 MHz

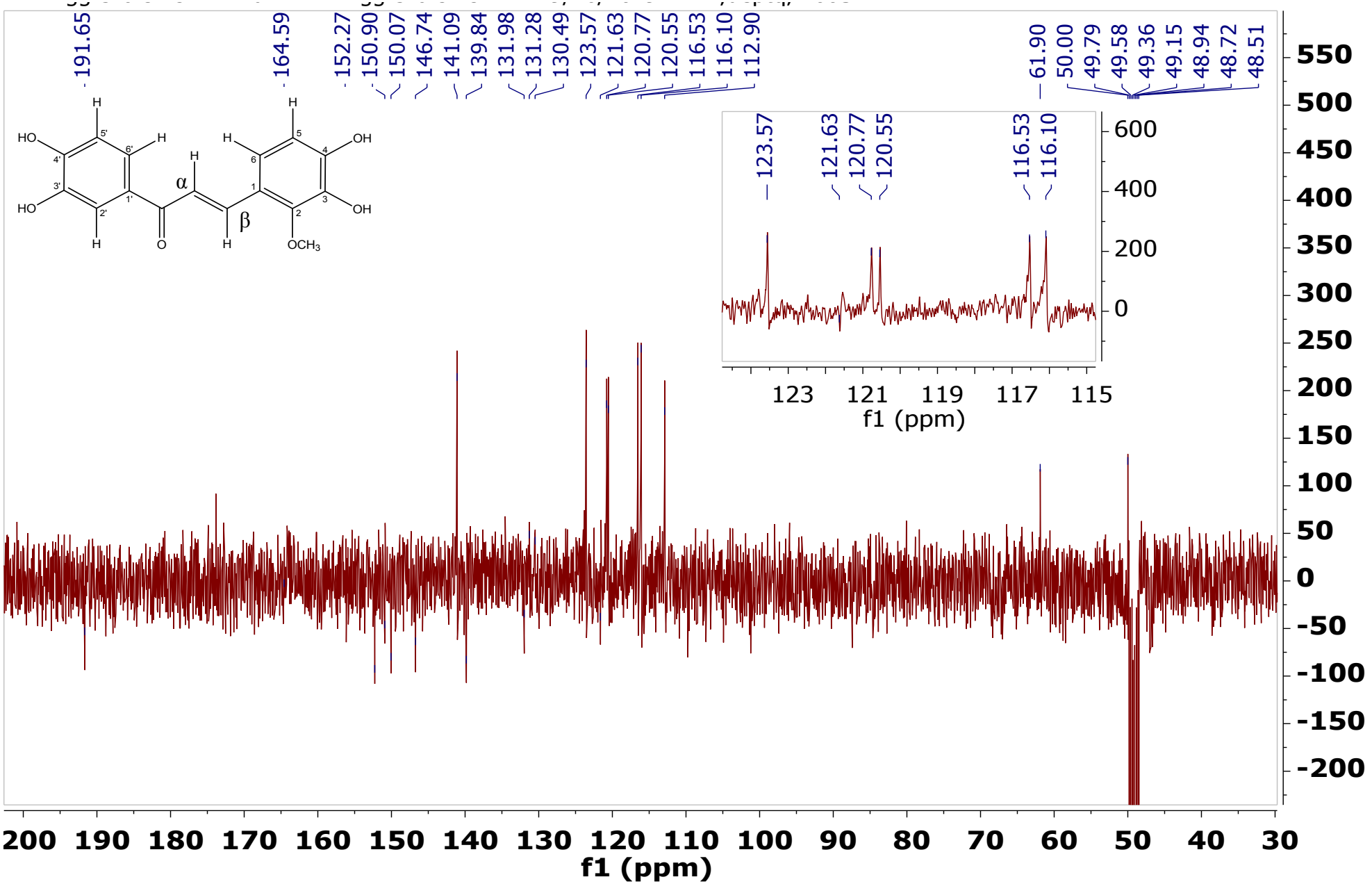

Fig. S21 ^{13}C-DEPTQ spectrum of 3,3',4,4'-tetrahydroxy-2-methoxychalcone (7) in CD_3OD at 100 MHz with expansion at (115- 123 ppm)

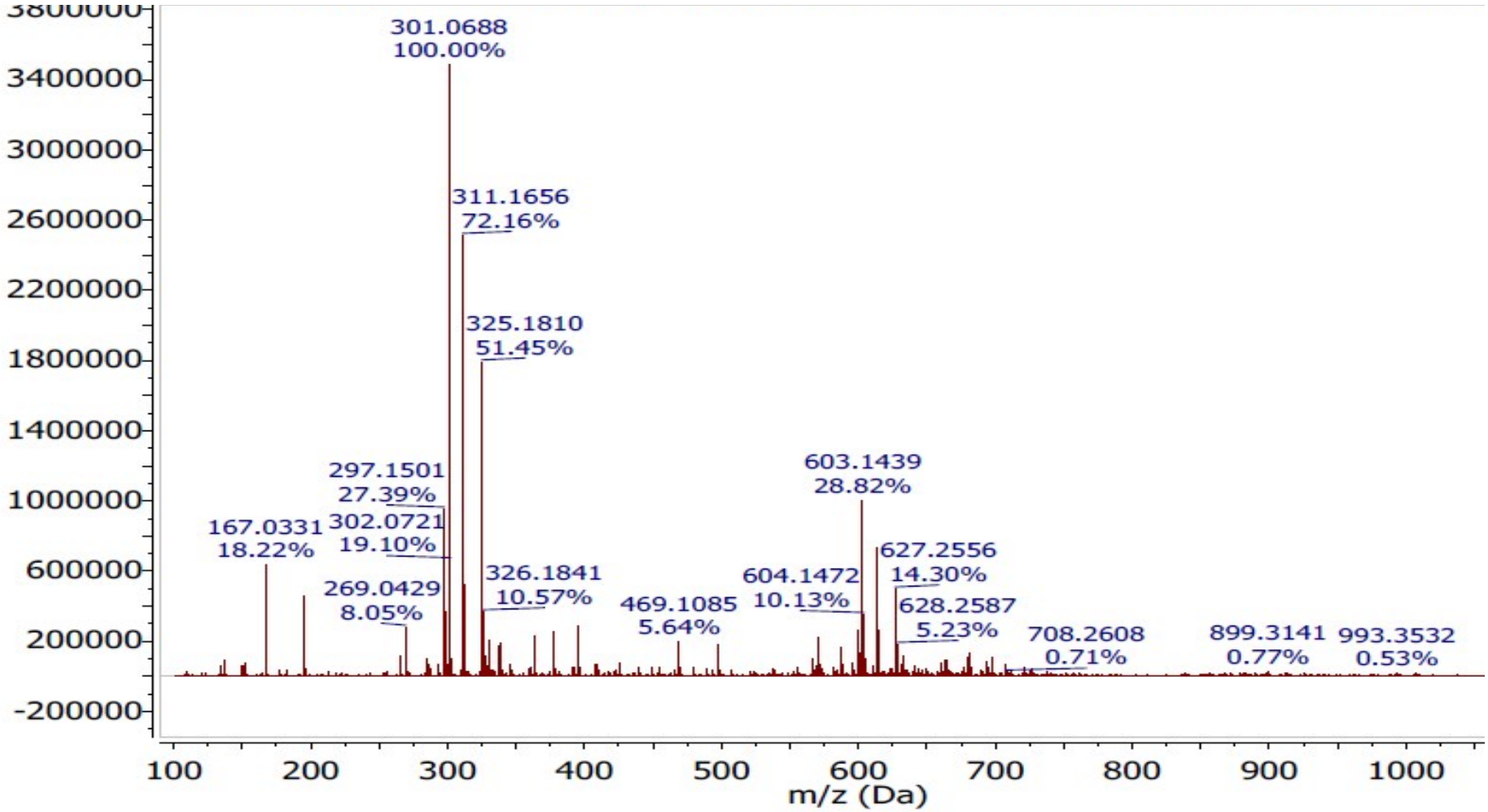

Fig. S22 HRESIMS spectrum of 3,3',4,4'-tetrahydroxy-2-methoxychalcone (7) in negative mode

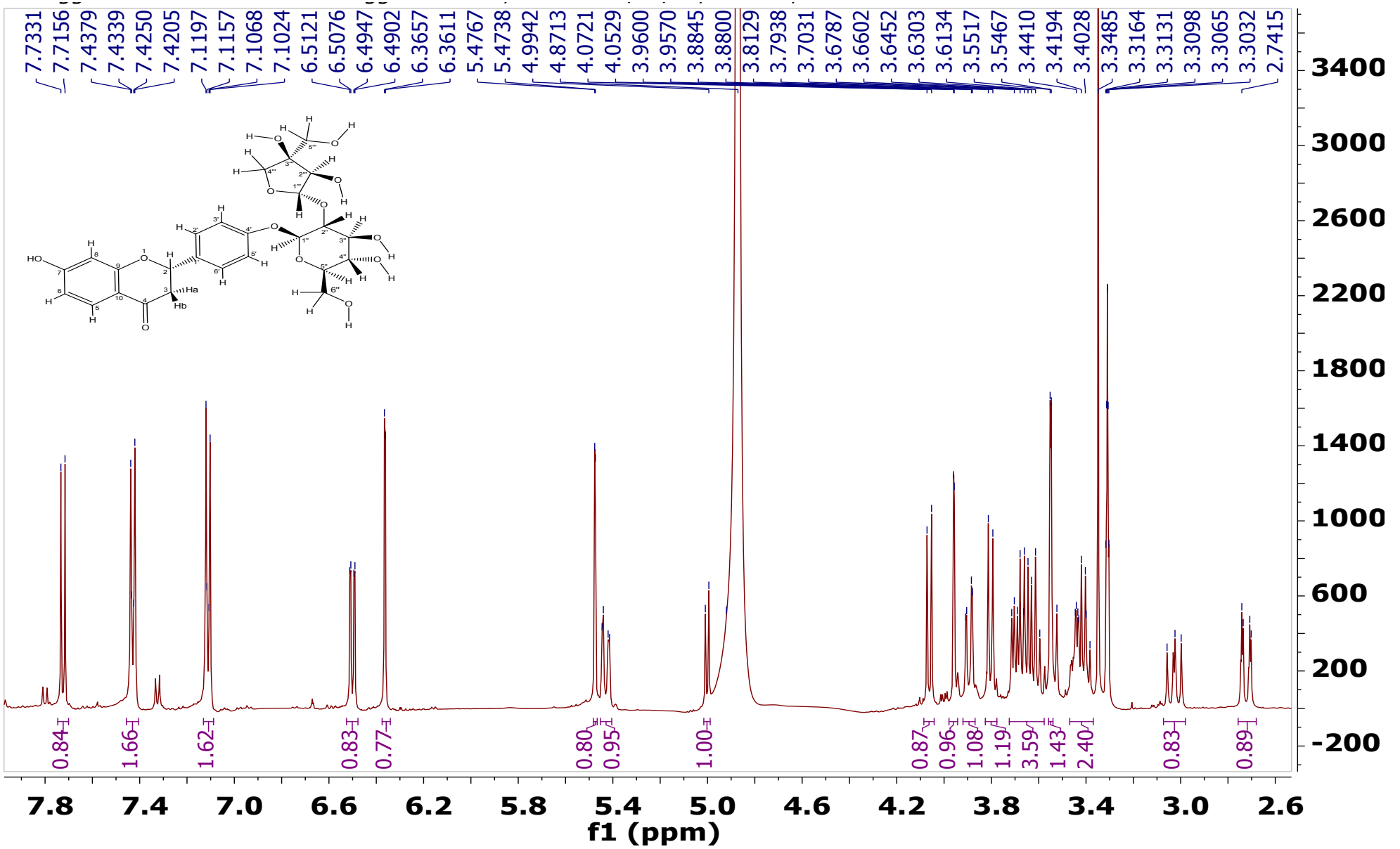

Fig. S23 ^{1}H-NMR spectrum of liquiritinapioside (8) in CD_3OD at 500 MHz

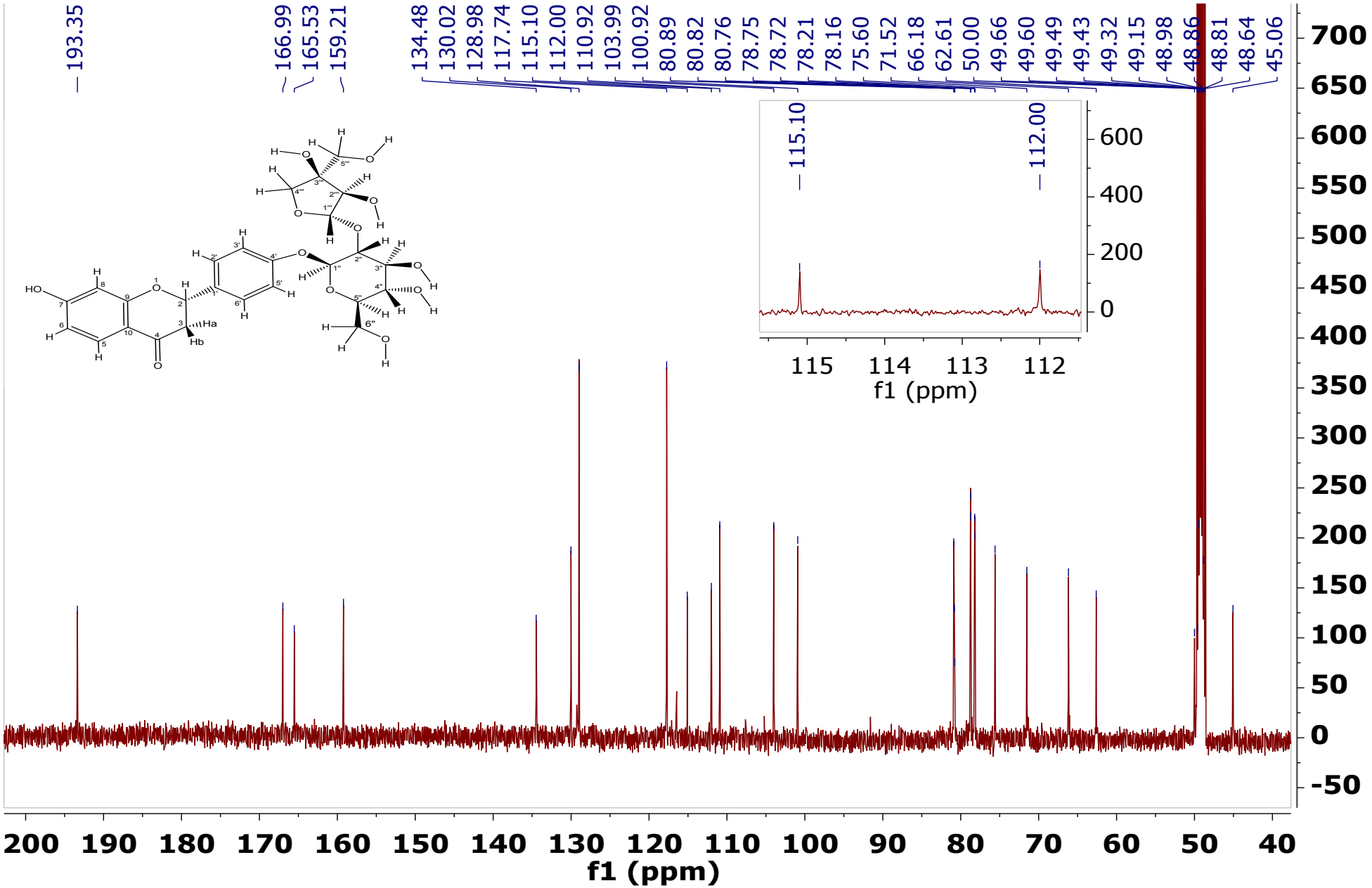

Fig. S24 ^{13}C-NMR spectrum of liquiritinapioside (8) in CD_3OD at 125 MHz with expansion at (112-115 ppm)

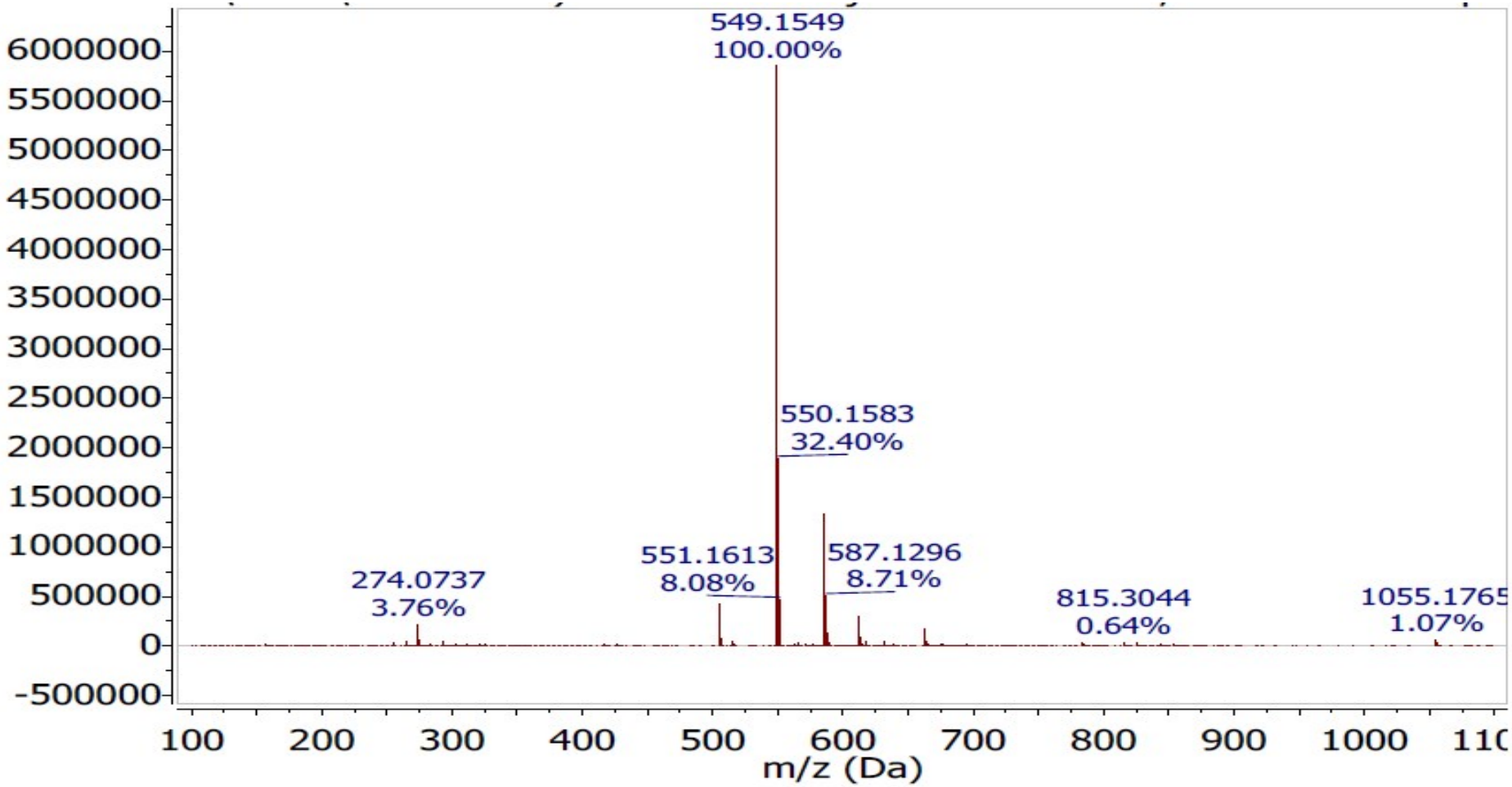

Fig. S25 HRESIMS spectrum of liquiritinapioside (8) in negative mode

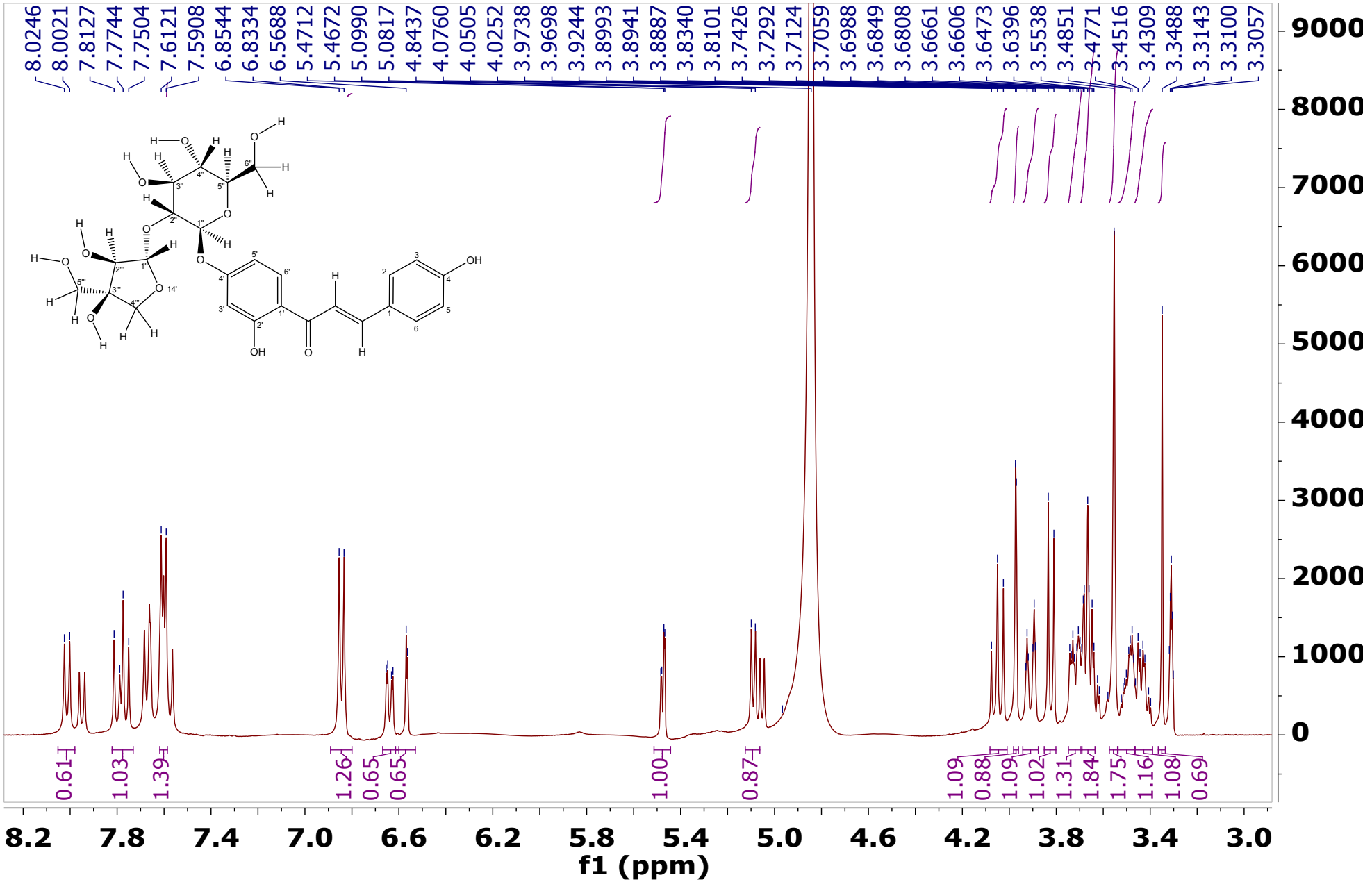

Fig. S26 ^{1}H-NMR spectrum of isoliquiritigenin-4'- O- β-D-apiosylglucoside (9) in CD_3OD at 400 MHz

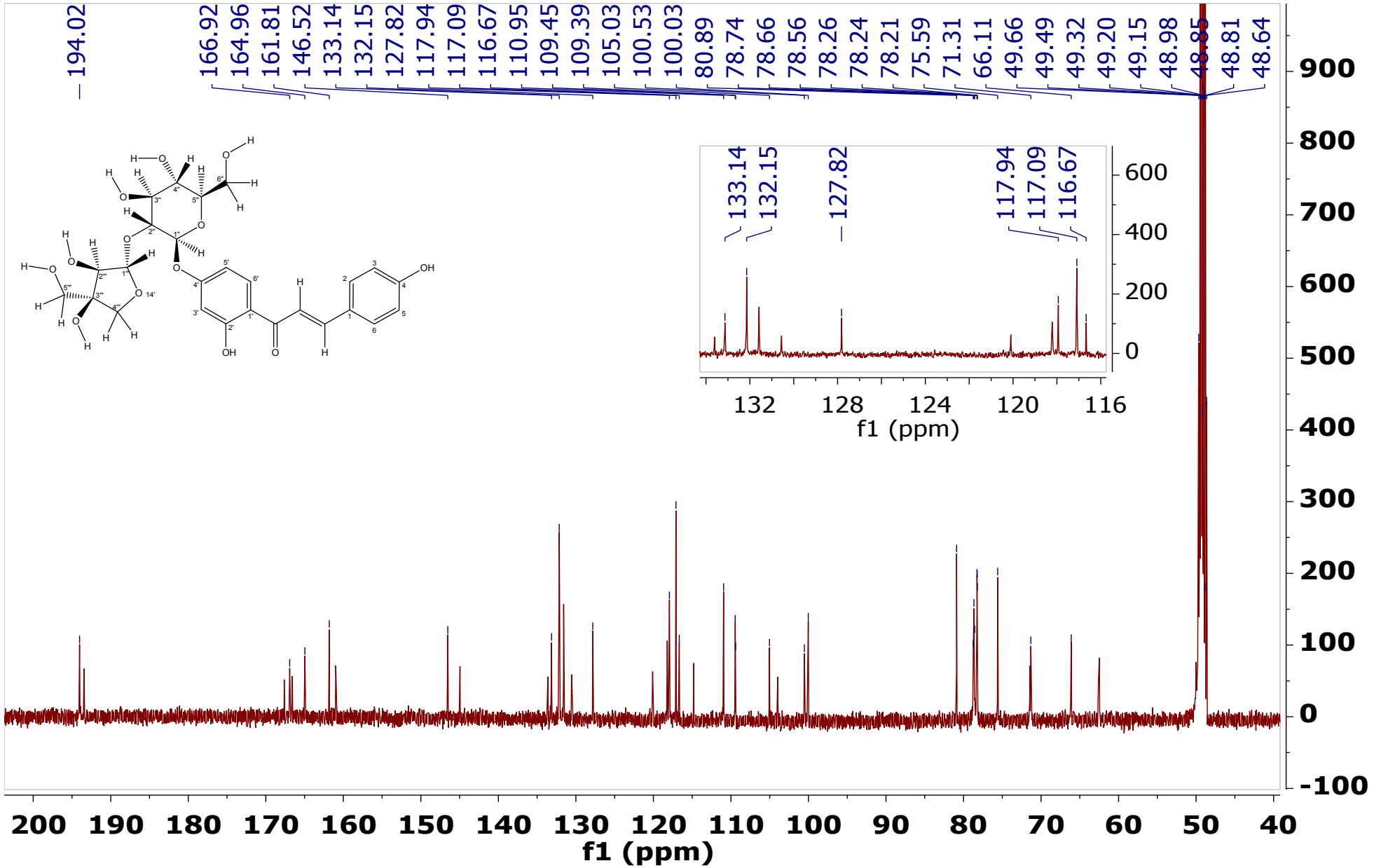

Fig. S27 ^{13}C-NMR spectrum of isoliquiritigenin-4'- O- β-D-apiosylglucoside (9) in CD_3OD at 125 MHz with expansion at (115- 130 ppm)

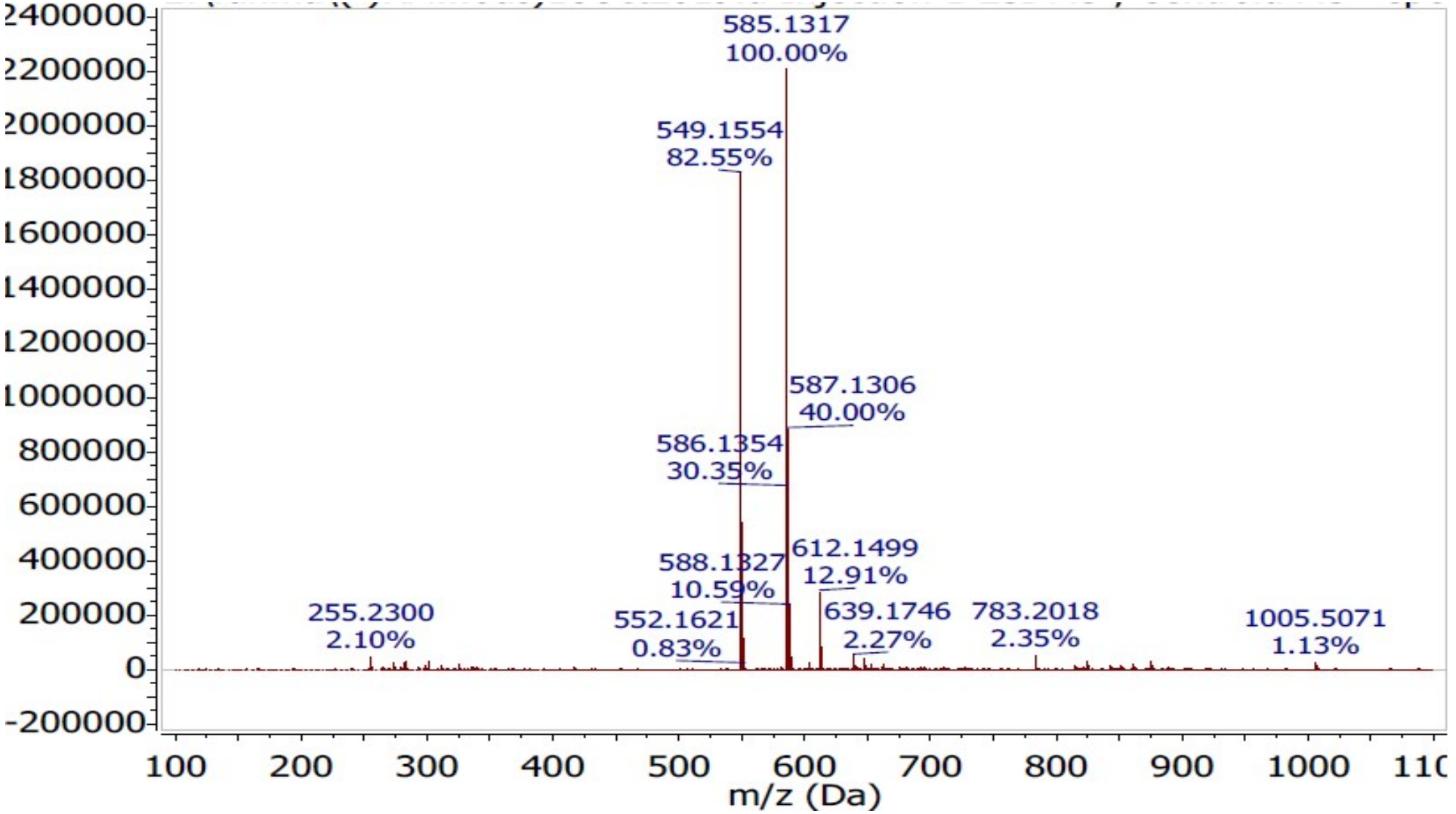

Fig. S28 HRESIMS spectrum of isoliquiritigenin-4'- O- β-D-apiosylglucoside (9) in negative mode

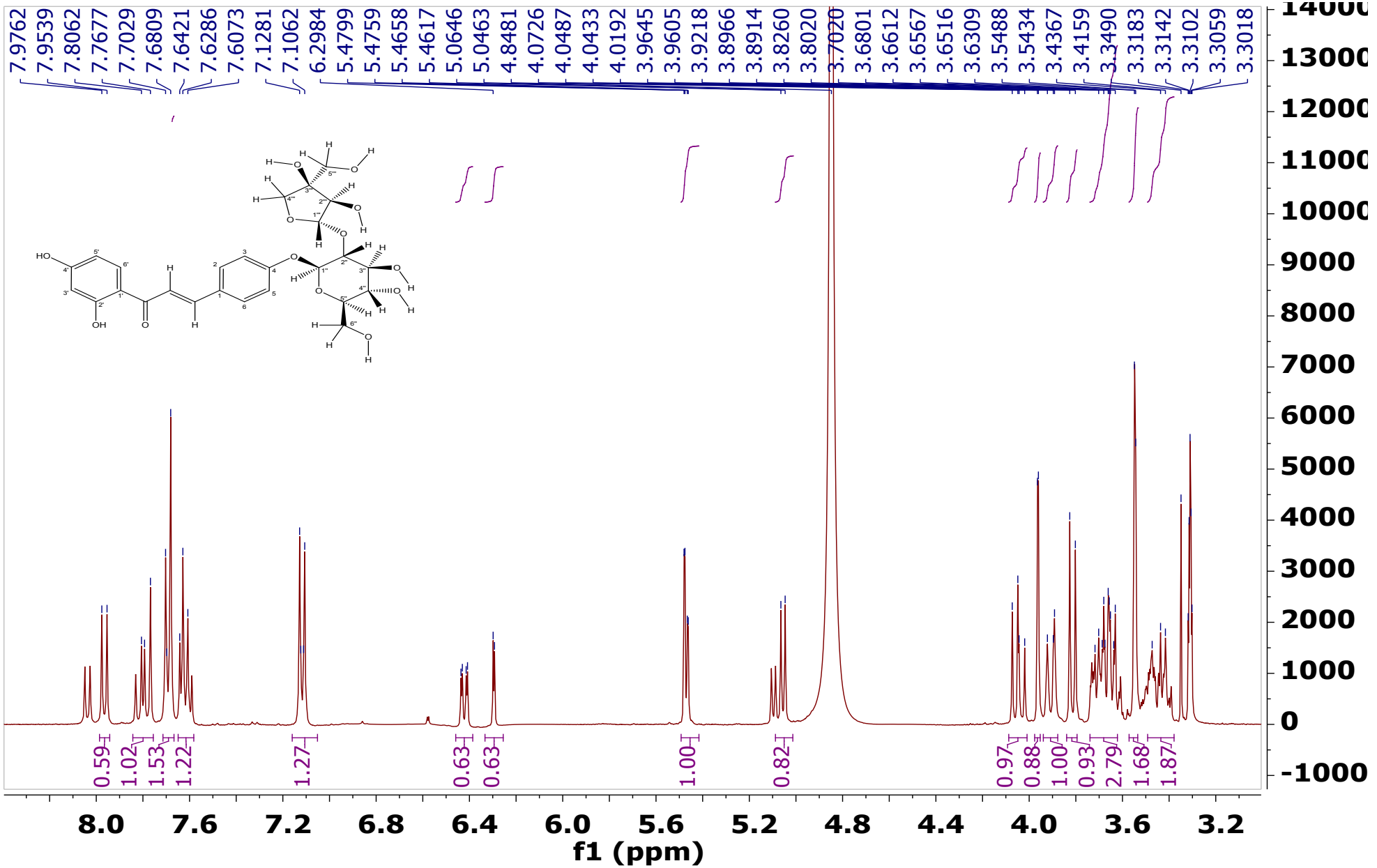

Fig. S29 ^{1}H-NMR spectrum of isoliquiritigenin-4-O-β-D-apiosylglucoside (10) in CD_3OD at 400 MHz

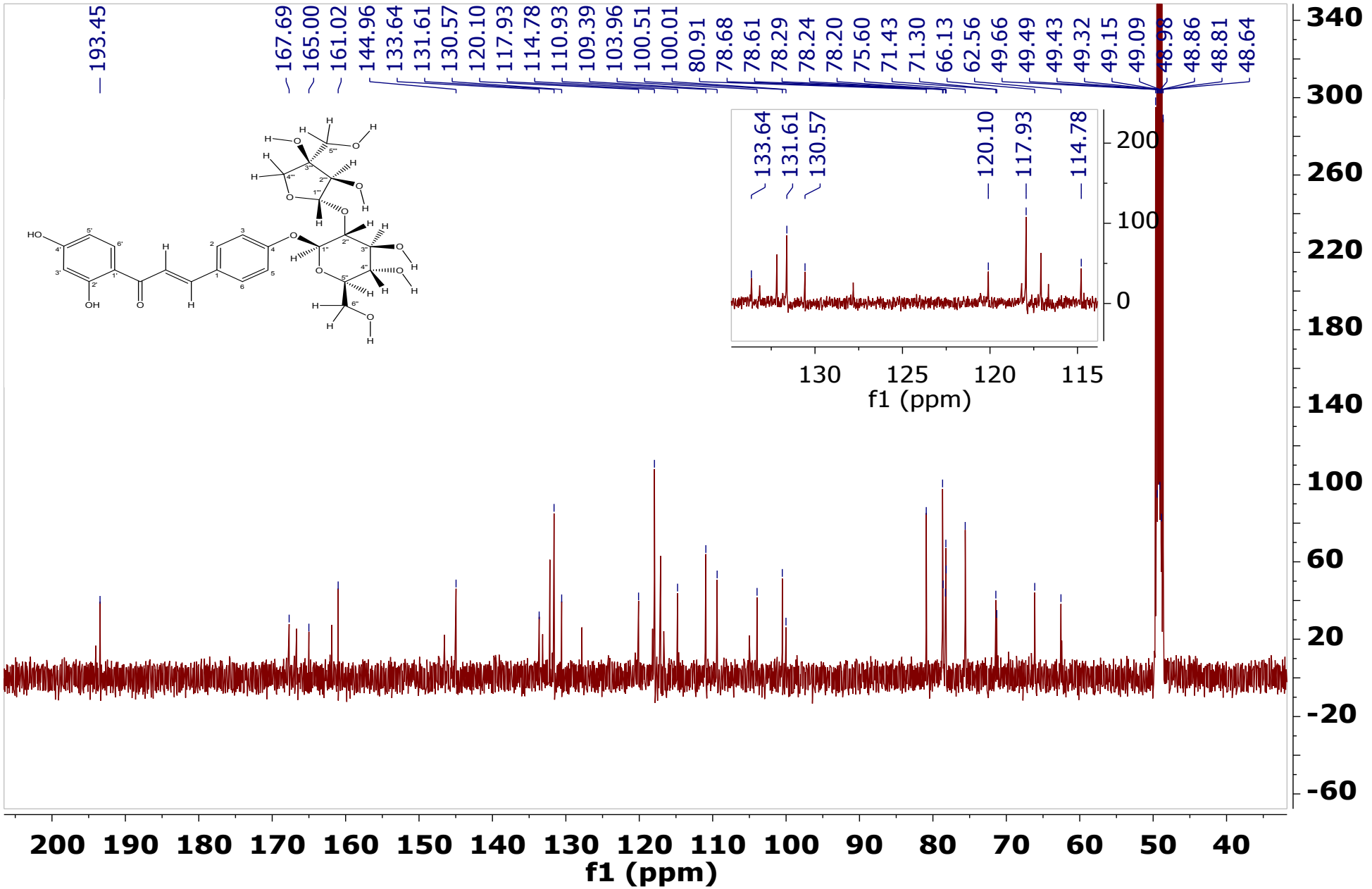

Fig. S30 ^{13}C-NMR spectrum of isoliquiritigenin-4-O-β-D-apiosylglucoside (10) in CD_3OD at 125 MHz with expansion at (115- 130 ppm)

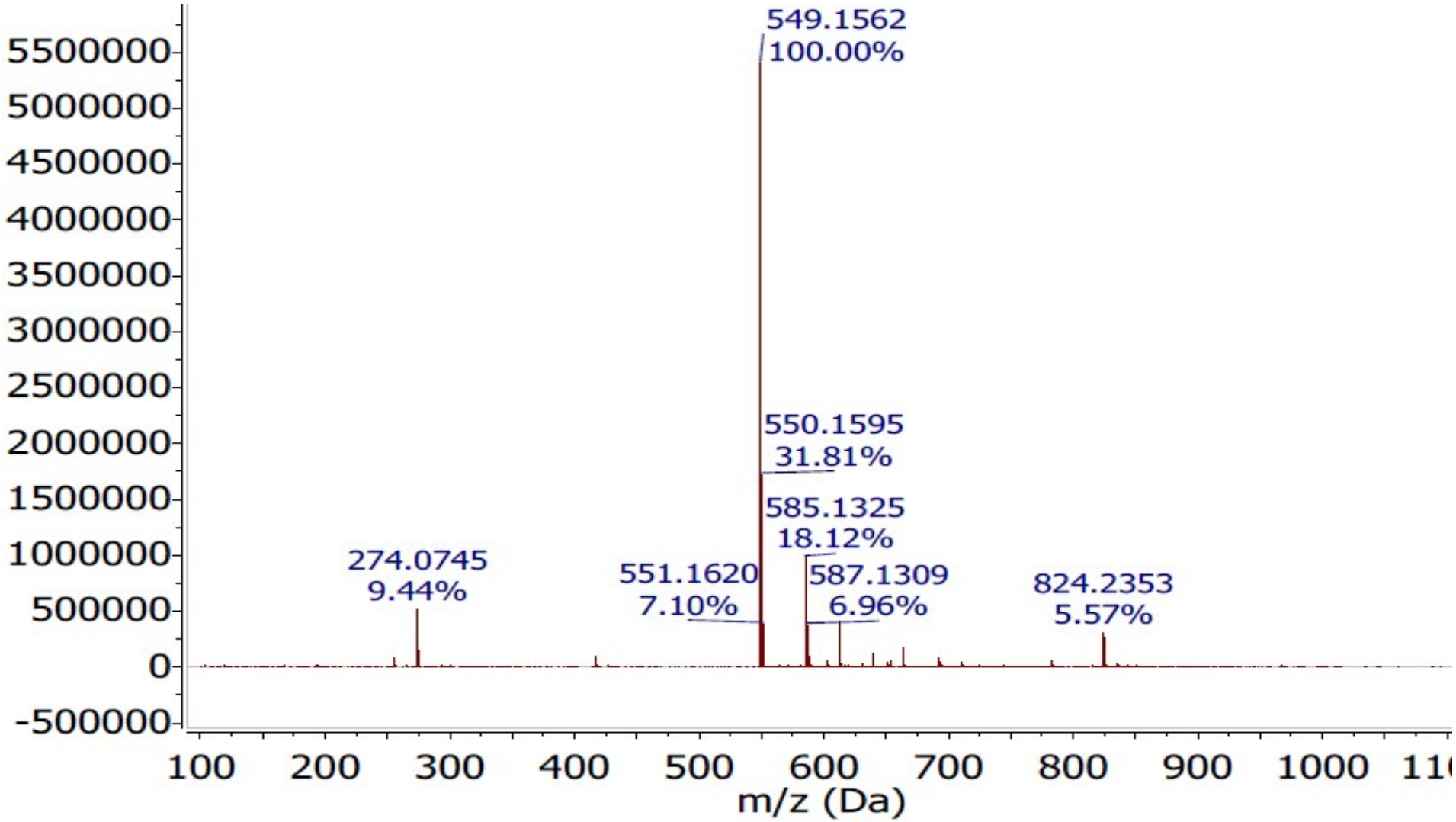

Fig. S31 HRESIMS spectrum of isoliquiritigenin-4-O-β-D-apiosylglucoside (10) in negative mode

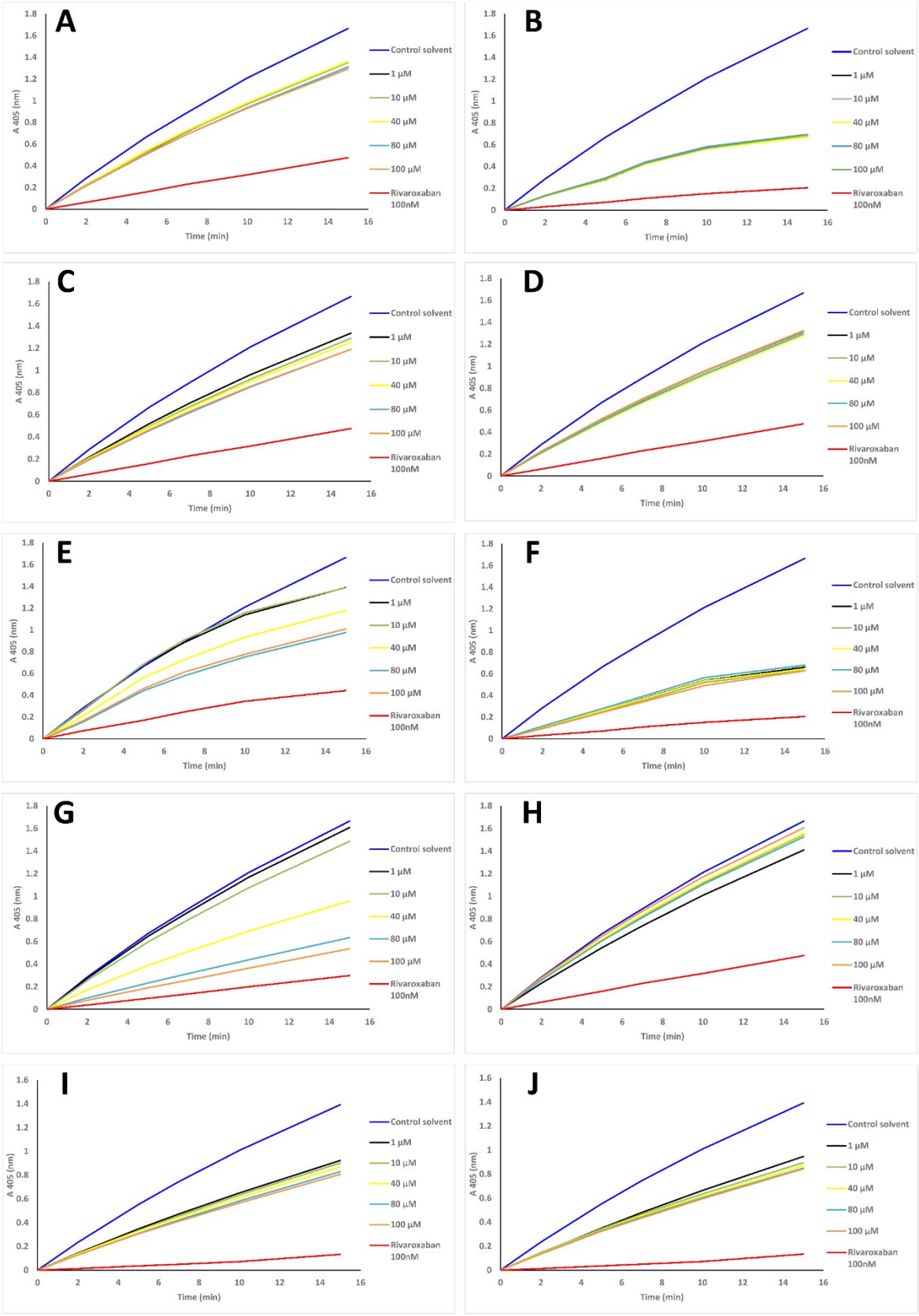

Fig. S32 Absorbance-time curves of FXa mediated hydrolysis of chromogenic substrate using different concentrations of **A**. 7, 4'-dihydroxyflavone, **B**. 7-hydroxy-4'-methoxyisoflavone, **C**. 3-R-glabridin, **D**. isoliquirtigenin, **E**. liquirtin, **F**. naringenin 5-O-glucoside, **G**. 3,3',4,4'-tetrahydroxy-2 methoxychalcone, **H**. liquirtinapioside, **I**. licraside and **J.** isoliquirtinapioside

Table S5. ADMET profile of compounds isolated from licorice compared to synthetic FXa inhibitors

No	Compound	#stars[a]	MW[b]	HBD[c]	HBA[d]	QP logPo/w[e]	QP logS[f]	QPP Caco[g]	CNS[h]	QP logBB[i]	QPP MDCK[j]	% Human Oral Absorption[k]	QP Log Khsa[L]	#metab[m]	QP Log HERG[n]	Rule Of 5[o]	Rule Of 3[p]
A	**Rivaroxaban***	0	435.881	1	10.7	2.245	-5.128	425.979	-1	-0.87	837.713	87.151	-0.291	3	-5.884	0	0
B	**Apixaban***	0	459.504	2	10.25	2.719	-6.394	171.933	-2	-1.608	73.767	82.875	0.314	4	-6.326	0	1
C	**Edoxaban***	0	548.058	1.5	13.5	1.782	-4.603	27.918	-2	-1.347	59.787	37.343	-0.324	6	-5.585	2	0
D	**Betrixaban***	0	451.911	2	7.25	4.048	-5.424	503.108	-2	-1.112	512.497	100	0.424	2	-5.84	0	0
1	7, 4'-Dihydroxyflavone	0	254.242	2	4	1.817	-3.271	269.304	-2	-1.031	119.815	**81.084**	-0.073	2	-5.247	0	0
2	7-hydroxy-4'-methoxyisoflavone	0	268.268	1	4	2.585	-3.422	**1274.915**	0	-0.448	643.225	**100**	0.019	2	-5.133	0	0
3	3-R- Glabridin	0	380.483	2	3	5.096	-6.465	**1891.682**	0	-0.269	985.349	**100**	1.133	3	-5.129	1	1
4	Isoliquiritigenin	0	256.257	2	3.25	2.074	-3.078	174.841	-2	-1.562	75.117	**79.226**	-0.084	3	-5.389	0	0
5	Liquiritin	0	418.399	5	12.75	-0.31	-3.232	21.721	-2	-2.737	7.883	49.06	-0.711	7	-5.672	0	2
6	Naringenin 5-O-glucoside	0	434.399	6	13.5	-0.7	-2.996	17.242	-2	-2.898	6.142	32.022	-0.823	8	-5.587	1	2
7	3,3',4,4'-Tetrahydroxy-2 methoxychalcone	0	302.283	4	5.75	0.949	-2.657	58.255	-2	-2.183	22.899	**64.096**	-0.419	5	-5.105	0	0
8	Liquiritin apioside	6	550.515	7	18.6	-1.293	-2.999	7.111	-2	-3.791	2.358	0	-1.166	9	-6.073	3	2
9	Licraside	7	550.515	7	17.6	-1.054	-2.294	4.771	-2	-4.231	1.532	0	-1.238	8	-6.056	3	2
10	Isoliquirtin apioside	7	550.515	7	17.6	-1.036	-2.318	4.621	-2	-4.246	1.48	0	-1.228	8	-6.043	3	2

***Synthetic FXa inhibitors**

a) ADME-compliance score – druglikeness parameter (range 0 to 5). b) Molecular Weight of the molecule (range: 130.0 to725.0)
c) Hydrogen Bond Donor (range: 0.0 to 6.0). d) Hydrogen Bond Acceptor (range: 2.0 to 20.0)
e) Predicted octanol/water partition coefficient (range: -2.0 to 6.5). f) Predicted aqueous solubility (range: -6.5 to 0.5)
g) Predicted Caco cell permeability (range: < 25 is poor and >500 is great). h) Predicted central nervous system activity (-2 (inactive) to +2 (active)).
i) Predicted brain/blood partition coefficient (range: -3.0 to 1.2). j) Predicted apparent MDCK cell permeability (range: < 25 is poor and >500 is great).
k) Percentage of human oral absorption (range: <25 is poor and >80% is high). L) Prediction of binding to human serum albumin (range: –1.5 to 1.5).
m) Number of likely metabolic reactions (range: 1 to 8). n) Predicted IC_{50} value for blockage of HERG K+ channels (concern < –5).
o) Number of violations of Lipinski's rule of five (maximum is 4). p) Number of violations of Jorgensen's rule of three (maximum is 3).