Supporting information

Traversal of the Blood-Brain Barrier by Cleavable L-Lysine Conjugates of Apigenin

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1. General Chemicals.

HPLC and UPLC-MS/MS analysis solvents were LC grade and LCMS grade, respectively. All final products were purified by recrystallization and their purities were confirmed >95% by HPLC prior to *in vivo* use. Thin-layer chromatography (0.25 mm, E. Merck silica gel 60 F_{254}) was used to monitor reaction progress; plates were visualized by UV (λ = 254 nm), or by staining with ninhydrin, and heating. Elution rate and temperature of HPLC condition were set to 1 mL/min and 25 °C, unless otherwise specified.

2. Standard curves of 4, 7, 10 for HPLC analyses

Table S1. UV Absorption Values of 4 under λ = 254 nm

Gly-api			
Conc. (mg/mL)	0.0250	0.0125	0.0063
Area	1877259	908934	786013

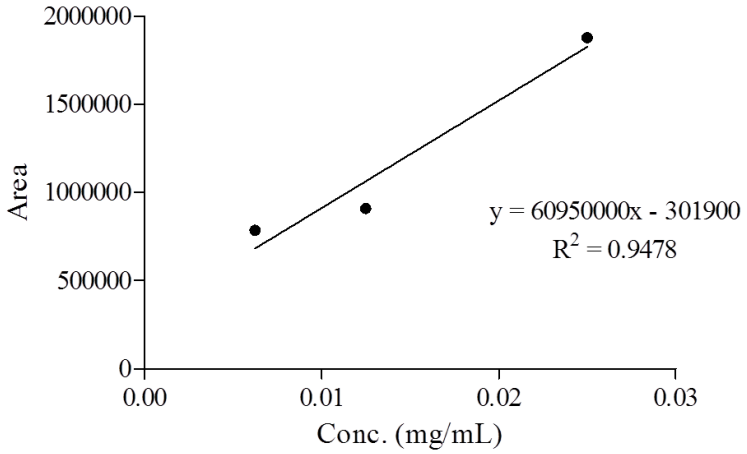


Figure S1. The standard curve of 4 for HPLC analysis.

Table S2. UV Absorption Values of 7 under λ = 254 nm

Phe-api						
Conc. (mg/mL)	5	0.5	0.1	0.05	0.01	0.005
Area	136542838	12688744	2149802	1040573	518985	266527

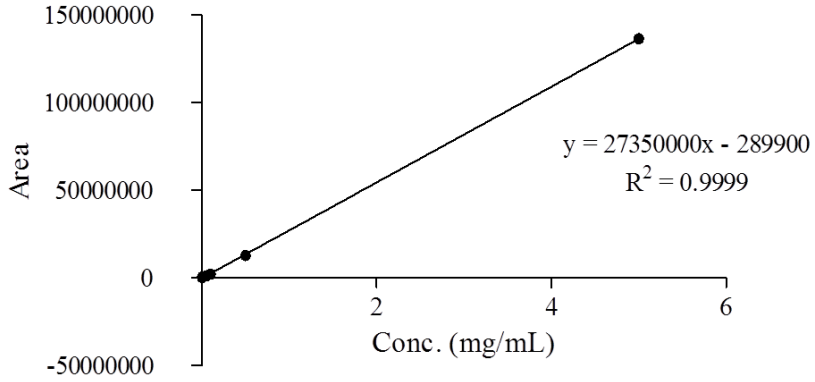


Figure S2. The standard curve of 7 for HPLC analysis.

Table S3. UV Absorption Values of 10 under λ = 254 nm

Lys-api					
Conc. (mg/mL)	0.5	0.1	0.05	0.01	0.005
Area	1029222	243181	121444	52708	25690

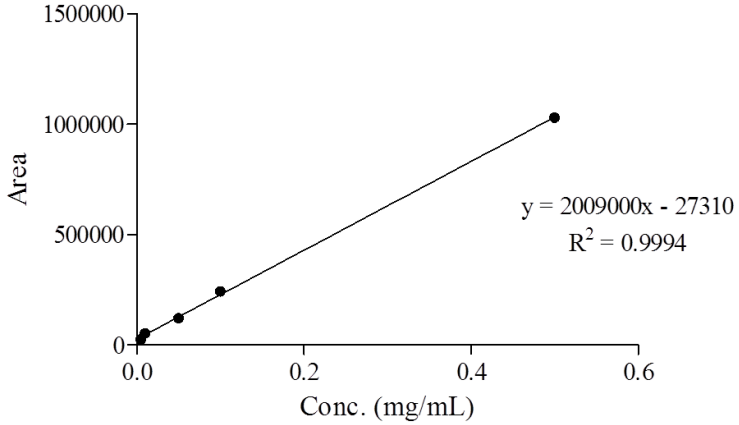


Figure S3. The standard curve of 10 for HPLC analysis.

3. Human S9 Stability Assays

Table S4. UV Absorption Values of 4, 7, and 10 in Human Liver S9 Stability Assays (λ = 254 nm).

	Compounds					
	4		7		10	
	Area	Remaining ratio	Area	Remaining ratio	Area	Remaining ratio
0 h	3624014	100%	971023	100%	178969	100%
6 h	3511848	97%	941963	94%	162695	90%
12 h	3439535	95%	923895	95%	160195	90%
24 h	2820435	78%	901629	93%	138104	77%

4. Log P

Table S5. Retention Times and Literature log P Values of Reference Standards

	$NaNO_2$	Benzyl alcohol	Phenol	Thiophene	Benzene	Toluene
t_0, min	3.09	-	-	-	-	-
t_R, min	-	4.15	5.00	7.13	8.53	12.33
log P (lit.)	-	1.05	1.50	1.81	2.13	2.73

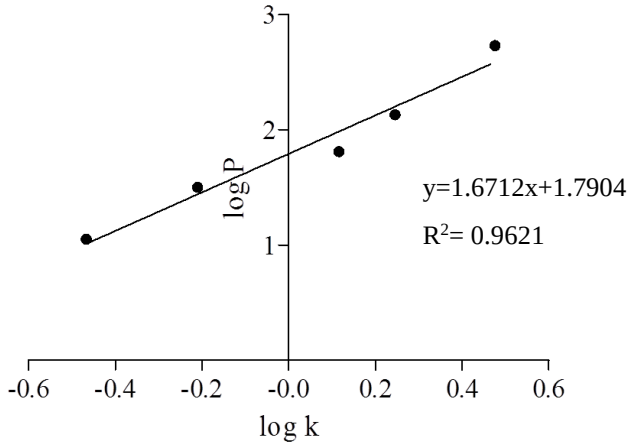


Figure S4. The regression line obtained via log P and log k values of the five compounds.

Table S5. Retention Times and log P Values of 1, 4, 7, and 10

	Apigenin, **1**	Gly-apigenin carbamate, **4**	Phe-apigenin carbamate, **7**	Lys-apigenin carbamate, **10**
t_R, min	5.87	5.20	5.5	4.08
log P	1.71	1.51	1.61	0.96

5. UPLC-MS/MS Analyses

Table S6. Peak Area of m/z Transition of 10 From 443.2 to 271.1

Lys-api									
Conc. (ppm)	10	2	1	0.2	0.1	0.05	0.025	0.0125	0.00625
Area	108462	32800	17201	3495	1726	799	421	252	118

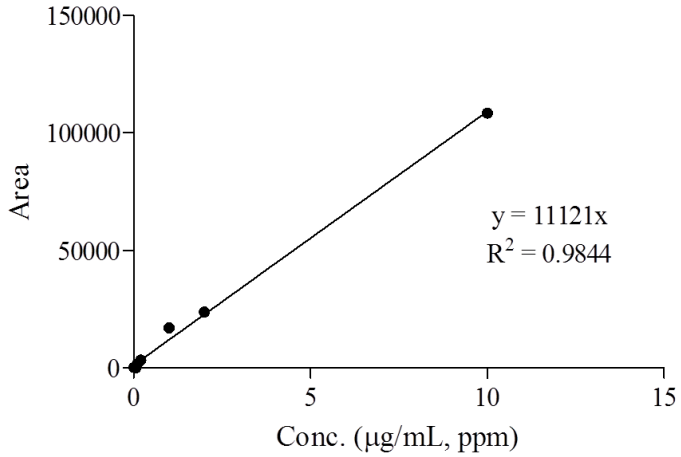


Figure S5. The standard curve of 10 for UPLC-MS/MS analysis.

Table S7. Peak Area of m/z Transition of 1 from 269.0 to 112.0

Apigenin									
Conc. (ppm)	10	2	1	0.2	0.1	0.05	0.025	0.0125	0.00625
Area	38670	16687	6917	1438	632	448	294	136	108

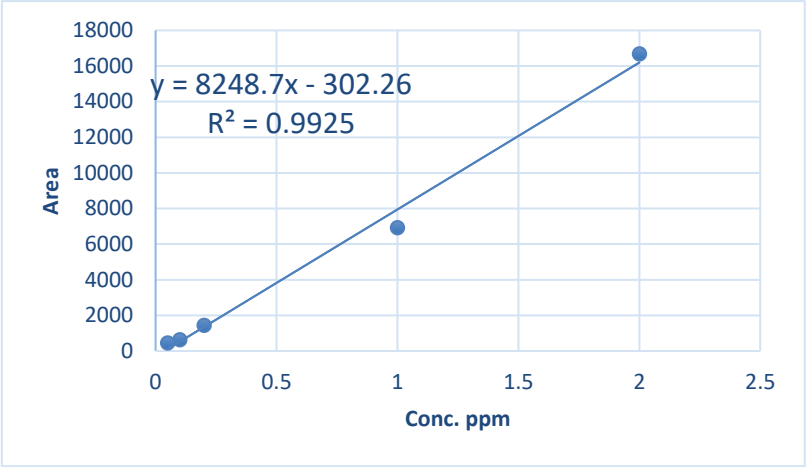


Figure S6. The standard curve of **1** in conc. of 0.05-2 ppm for UPLC-MS/MS analysis

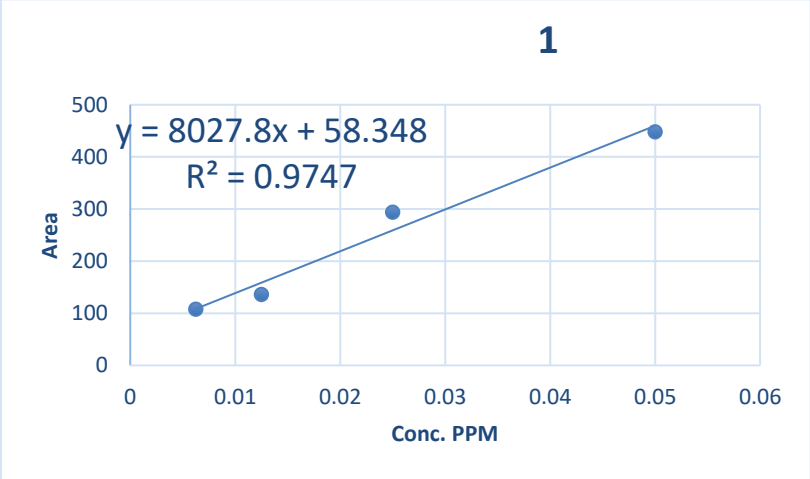


Figure S7. The standard curve of **1** in conc. of 0.00625-0.05 ppm for UPLC-MS/MS analysis

Table S8. Concentrations (PPM) of 10 of Blood Samples after a Single IP Injection of 10. (0.4 mg/g)

Blood samples								
Peak areas of m/z 443.2 → 271.1								
		0.0833 h	0.5 h	1 h	1.5 h	2 h	4 h	6 h	12 h
10		90528	136409	197143	99398	69965	49951	20626	4023
		76810	81631	130387	146237	91413	50060	30085	17624
		74511	123561	99941	206397	100107	52544	20257	7432
Units converted to ppm through the standard curve									
		0.0833 h	0.5 h	1 h	1.5 h	2 h	4 h	6 h	12 h
10	mean	7.2490	10.2389	12.8127	13.5489	7.8376	4.5726	2.1271	0.8716
	stdev	0.7787	2.5759	4.4707	4.8231	1.3950	0.1319	0.5009	0.6363
After multiplied by 3, the dilution factor									
		0.0833 h	0.5 h	1 h	1.5 h	2 h	4 h	6 h	12 h
10	mean	21.75	30.72	38.44	40.65	23.51	13.72	6.38	2.61
	stdev	2.34	7.73	13.41	14.47	4.19	0.40	1.50	1.91
	P value[a]	0.0002	0.023	0.044	0.044	0.011	<0.001	0.02	0.18

[a]Compared to concentration of **1** after IP injection of **10** at the same time point.

Table S9. Concentrations (PPM) of 1 of Blood Samples after a Single IP Injection of 10. (0.4 mg/g)

Blood samples									
Peak areas of m/z 269.0 → 112.0									
		0.0833 h	0.5 h	1 h	1.5 h	2 h	4 h	6 h	12 h
	1	4185	4113	7204	3935	2988	2952	856	622
		6949	6314	6990	7522	3901	2322	1865	1704
		10952	7130	8054	9301	4730	2988	1251	405
Units converted to ppm through the standard curve									
1	mean	0.93	0.75	0.94	0.88	0.51	0.37	0.20	0.15
	stdev	0.41	0.19	0.07	0.33	0.11	0.05	0.06	0.08
After multiplied by 3, the dilution factor									
		0.0833 h	0.5 h	1 h	1.5 h	2 h	4 h	6 h	12 h
1	mean	2.79	2.24	2.81	2.63	1.52	1.11	0.59	0.44
	stdev	1.24	0.57	0.20	0.99	0.32	0.14	0.18	0.25

Table S10. Concentrations (ppm) of 10 of Brain Samples after a Single IP Injection of 10. (0.4 mg/g)

Brain samples (homogenized suspensions of entire intracranial contents in T-PER buffer)									
Peak areas of m/z 443.2 → 271.1									
		0.0833 h	0.5 h	1 h	1.5 h	2 h	4 h	6 h	12 h
10		292	945	948	1604	340	729	252	179
		567	~~1699~~	1275	1224	948	1195	243	209
		549	559	1232	1339	424	298	217	205
Unit converted to ppm through the standard curve									
		0.0833 h	0.5 h	1 h	1.5 h	2 h	4 h	6 h	12 h
10	mean	0.0422	0.0676	0.1036	0.1249	0.0616	0.0666	0.0213	0.0178
	stdev	0.0138	0.0245	0.0160	0.0175	0.0290	0.0403	0.0016	0.0015
After multiplied by 4, the dilution factor									
		0.0833 h	0.5 h	1 h	1.5 h	2 h	4 h	6 h	12 h
10	mean	0.17	0.27	0.41	0.50	0.25	0.27	0.09	0.07
	stdev	0.06	0.10	0.06	0.07	0.11	0.16	0.01	0.01

Table S11. Concentrations (PPM) of 1 of Brain Samples after a Single IP Injection of 10. (0.4 mg/g)

Brain samples (homogenized suspensions of entire intracranial contents in T-PER buffer)									
Peak areas of m/z 269.0 → 112.0									
		0.0833 h	0.5 h	1 h	1.5 h	2 h	4 h	6 h	12 h
	1	N.D.	193	162	N.D.	N.D.	164	N.D.	N.D.
		N.D.	260	299	199	171	292	N.D.	N.D.
		N.D.	143	402	252	N.D.	N.D.	N.D.	N.D.
Unit converted to ppm through the standard curve									
		0.0833 h	0.5 h	1 h	1.5 h	2 h	4 h	6 h	12 h
	1	-	0.0168	0.0129	-	-	0.0132	-	-
		-	0.0251	0.0300	0.0175	0.0140	0.0291	-	-
		-	0.0105	0.0428	0.0241	-	-	-	-
1	mean	-	0.0175	0.0286	0.0208	0.0140	0.0212	-	-
	stdev	-	0.0073	0.0150	-	-	-	-	-
After multiplied by 4, the dilution factor									
		0.0833 h	0.5 h	1 h	1.5 h	2 h	4 h	6 h	12 h
1	mean	-	0.0699	0.1143	0.0832	0.0560	0.0848	-	-
	stdev	-	0.0293	0.0600	-	-	-	-	-

Table S12. Concentrations (ppm) of 1 of Blood Samples after a Single IP Injection of 1. (0.23 mg/g)

Blood samples									
Peak areas of m/z 269.0 → 112.0									
		0.0833 h	0.5 h	1 h	1.5 h	2 h	4 h	6 h	12 h
	1	491	226	N.D.	N.D.	131	N.D.	N.D.	N.D
		1181	207	576	164	207	N.D.	N.D.	N.D
		~~10700~~	176	175	121	224	N.D.	N.D.	N.D.
		0.0833 h	0.5 h	1 h	1.5 h	2 h	4 h	6 h	12 h
1	mean	836.00	203.00	375.50	142.50	187.33	-	-	-
	stdev	487.90	25.24	142.13	30.41	49.52	-	-	-
Units converted to ppm through the standard curve									
		0.0833 h	0.5 h	1 h	1.5 h	2 h	4 h	6 h	12 h
1	mean	0.1170	0.0391	0.0395	0.0105	0.0372	-	-	-
	stdev	0.0742	0.0172	0.0104	-	0.0202	-	-	-
After multiplied by 3, the dilution factor									
		0.0833 h	0.5 h	1 h	1.5 h	2 h	4 h	6 h	12 h
1	mean	0.3510	0.1173	0.1185	0.0420	0.1115	-	-	-
	stdev	0.2225	0.0517	0.0412	-	0.0606	-	-	-

Table S13. Concentrations of 10 in Brain Tissues Calculated via Formula S1.

Time	Suspension (brain+T-PER) vol.	Suspension (brain samples) conc.	Cerebral blood vol.	Blood (blood samples) conc.	Weight of intracranial content	Conc. in brain tissues	P value[a]
hour	mL	μg/mL	mL	μg/mL	g	μg/g	
0.0833	1.8	0.17	0.014	21.75	0.4	0.00	1
0.5	1.8	0.27	0.014	30.72	0.4	0.14	0.1082
1	1.8	0.41	0.014	38.44	0.4	0.50	0.345
1.5	1.8	0.50	0.014	40.65	0.4	0.82	0.0353
2	1.8	0.25	0.014	23.51	0.4	0.30	0.1003
4	1.8	0.27	0.014	13.72	0.4	0.73	0.1155
6	1.8	0.09	0.014	6.38	0.4	0.18	<.0001
12	1.8	0.07	0.014	2.61	0.4	0.22	0.0007

[a]compared to the concentration of **1** at the same time point.

Formula 1.

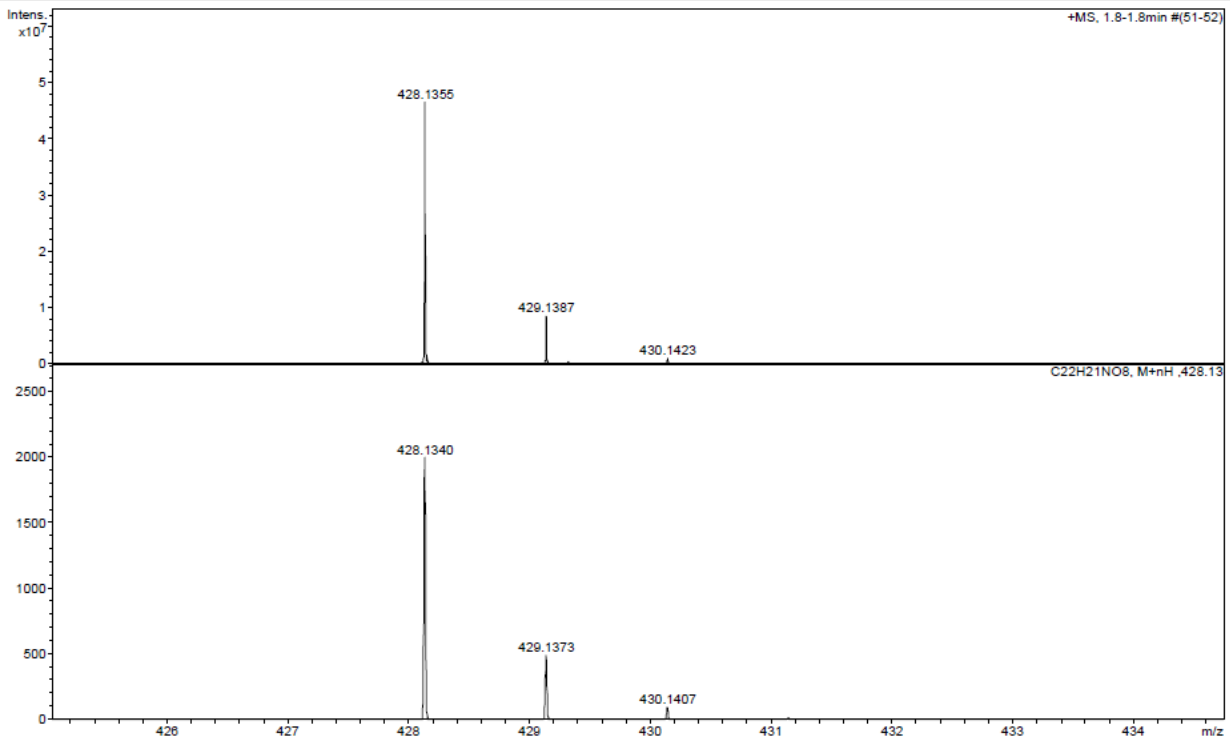
$$\frac{suspension\ volume\ \times suspension\ concentration\ -\ CBV\ \times\ blood\ concentration}{weight\ of\ entire\ intracranial\ contents}$$

Table S14. Concentrations of 1 in Brain Tissues Calculated via Formula 1 after IP injection of 10 (0.4 mg/g).

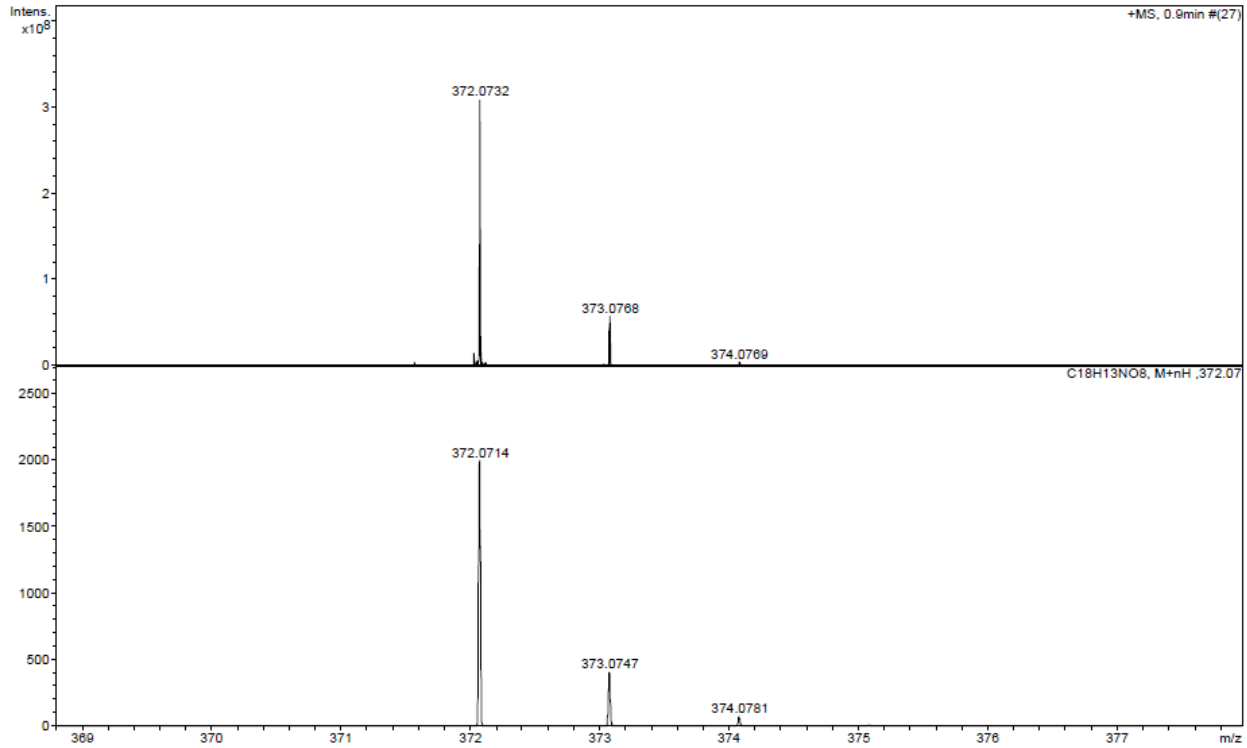
Time	Suspension (brain+T-PER) vol.	Suspension (brain samples) conc.	Cerebral blood vol.	Blood (blood samples) conc.	Weight of intracranial content	Conc. in brain tissues
hour	mL	μg/mL	mL	μg/mL	g	μg/g
0.0833	1.8	-	0.014	2.79	0.4	-
0.5	1.8	0.070	0.014	2.24	0.4	0.236
1	1.8	0.114	0.014	2.81	0.4	0.416
1.5	1.8	0.083	0.014	2.63	0.4	0.282
2	1.8	0.056	0.014	1.52	0.4	0.199
4	1.8	0.085	0.014	1.11	0.4	0.343
6	1.8	-	0.014	0.59	0.4	-
12	1.8	-	0.014	0.44	0.4	-

Formula 1.

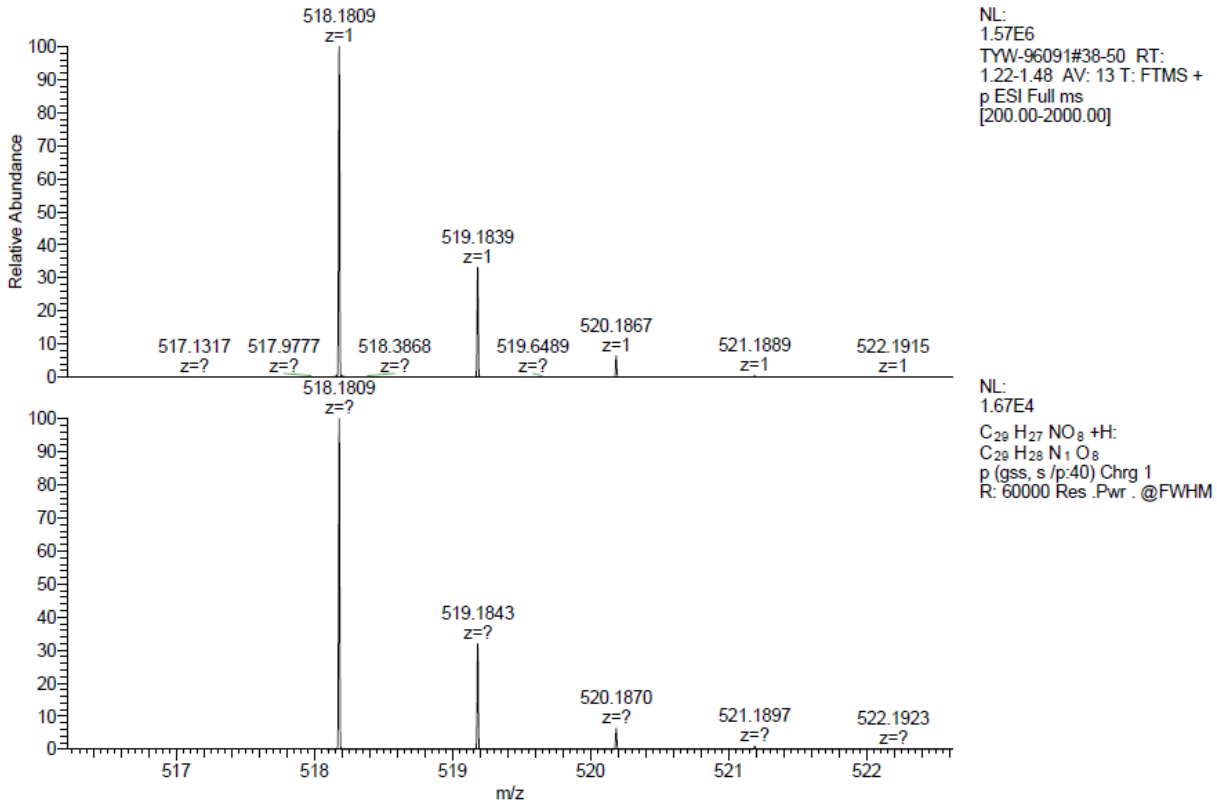
$$\frac{suspension\ volume\ \times suspension\ concentration\ -\ CBV\ \times\ blood\ concentration}{weight\ of\ entire\ intracranial\ contents}$$



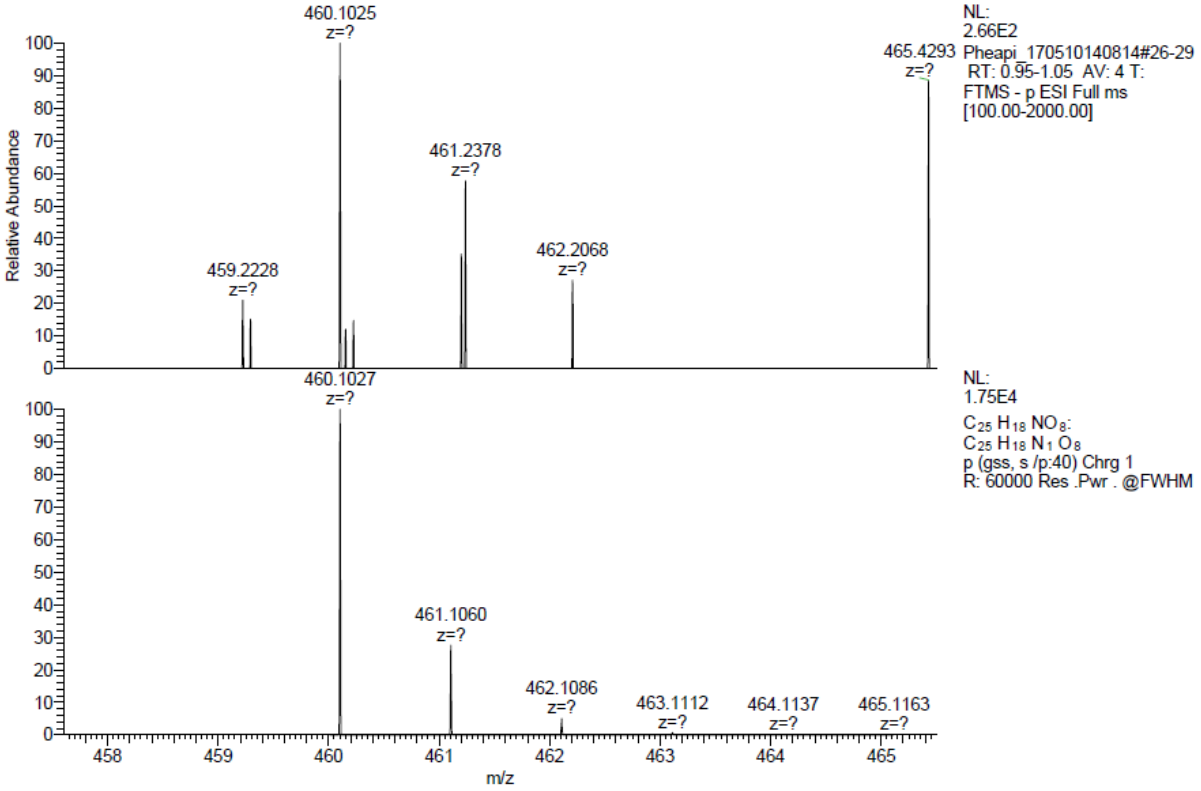
Appendix 1 Mass spectrum of **3**



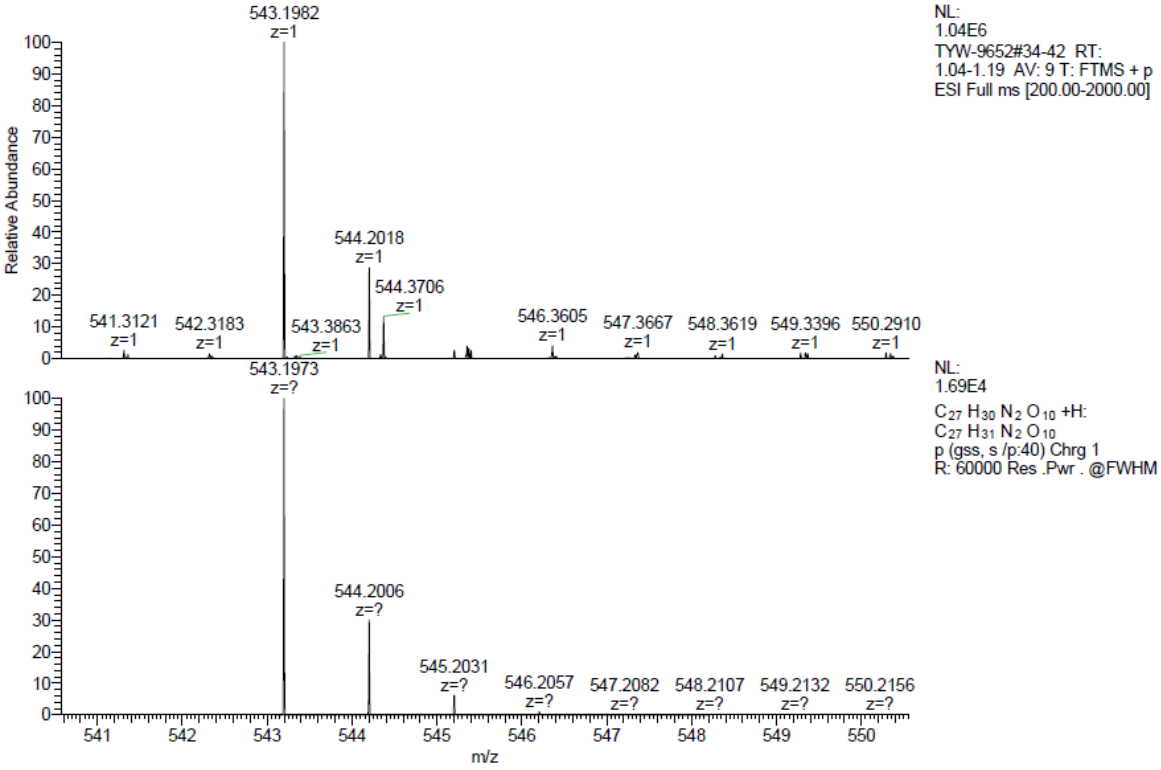
Appendix 2. Mass spectrum of **4**



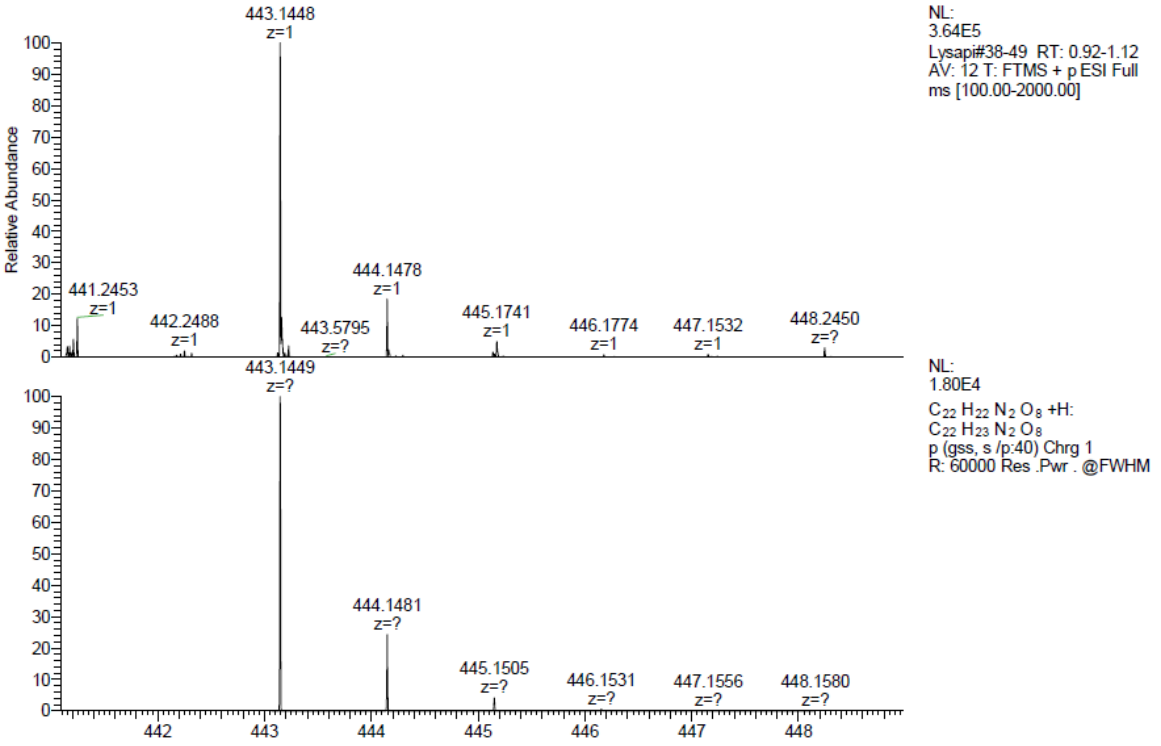
Appendix 3. Mass spectrum of **6**



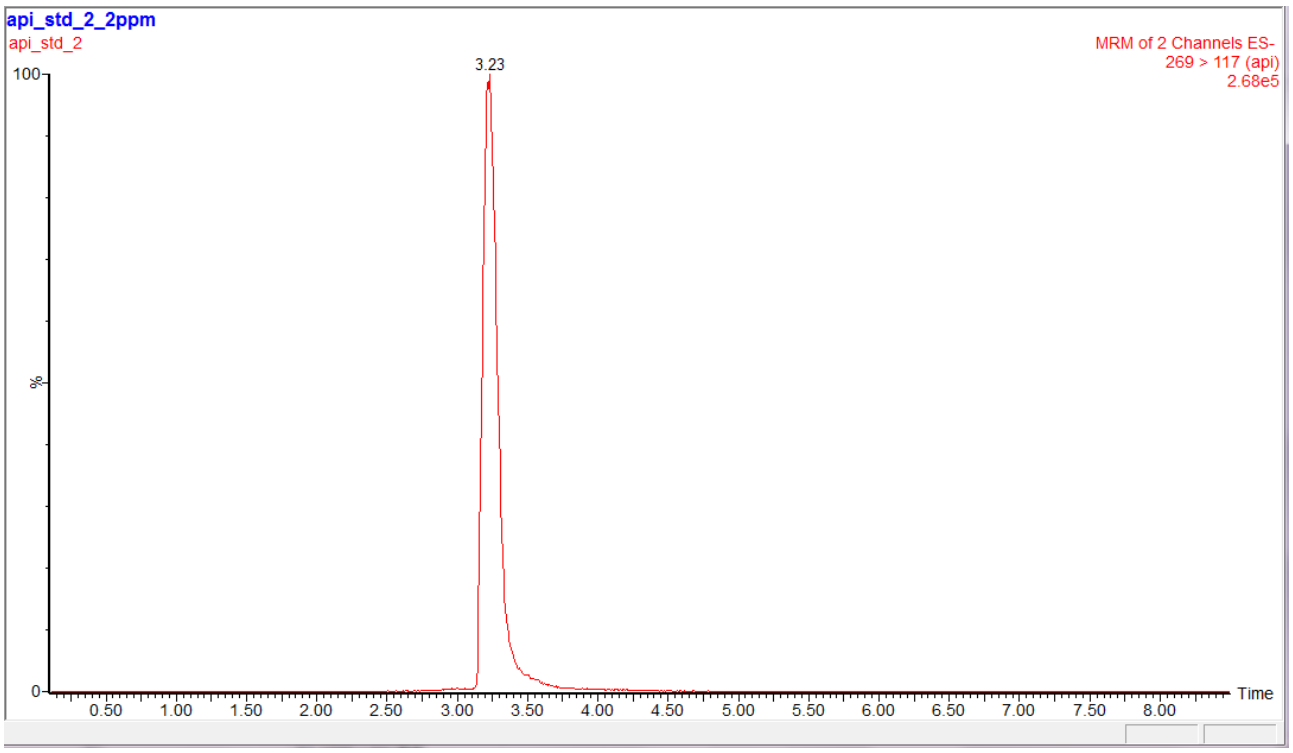
Appendix 4. Mass spectrum of **7**



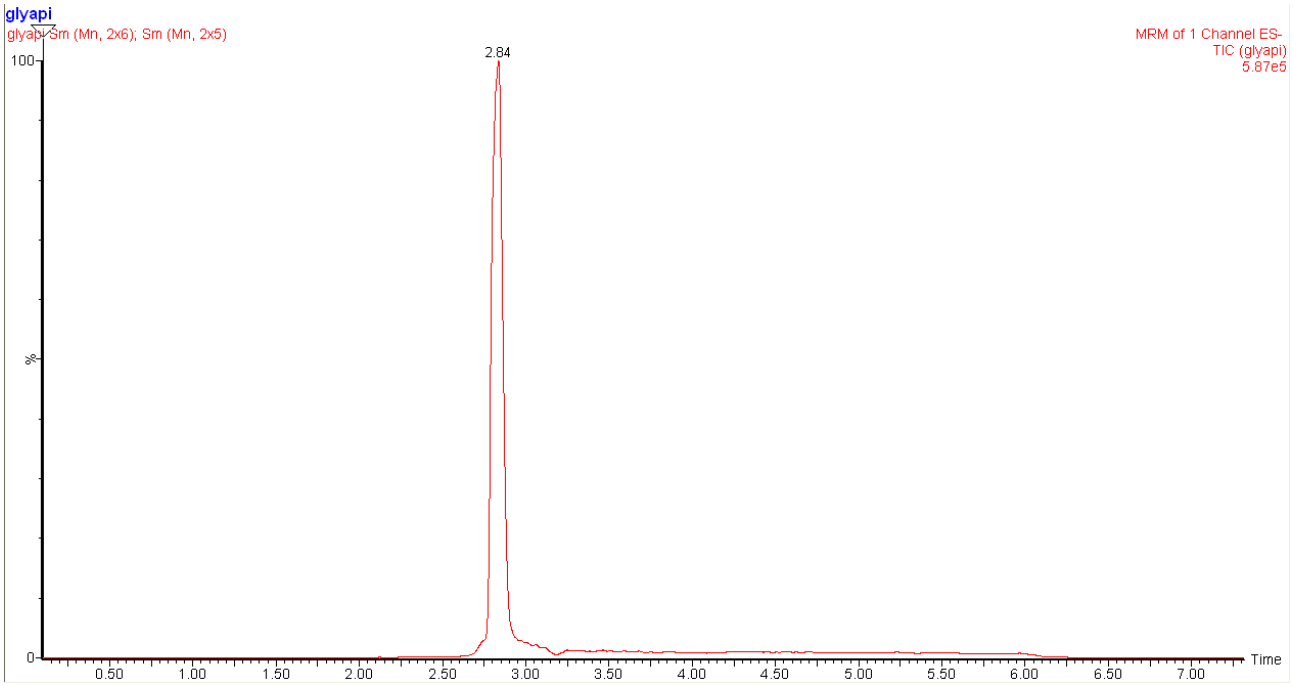
Appendix 5. Mass spectrum of **9**



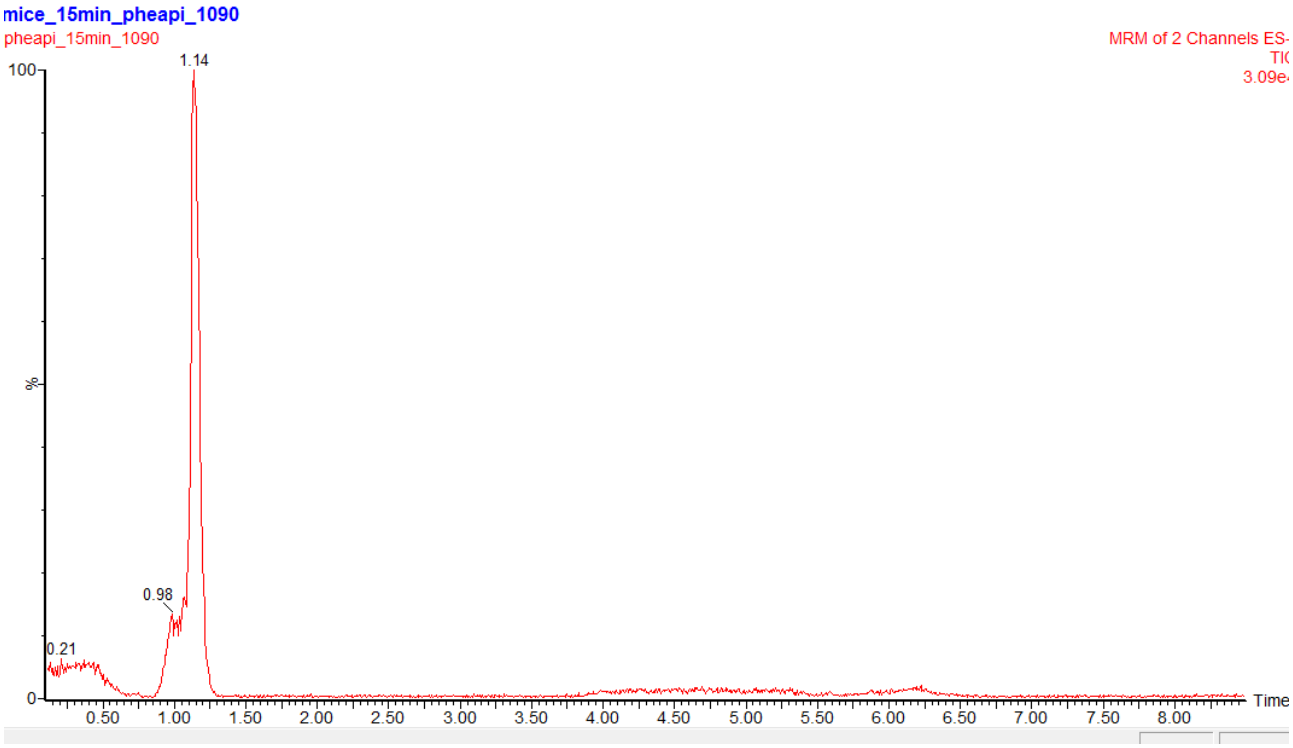
Appendix 6. Mass spectrum of **10**



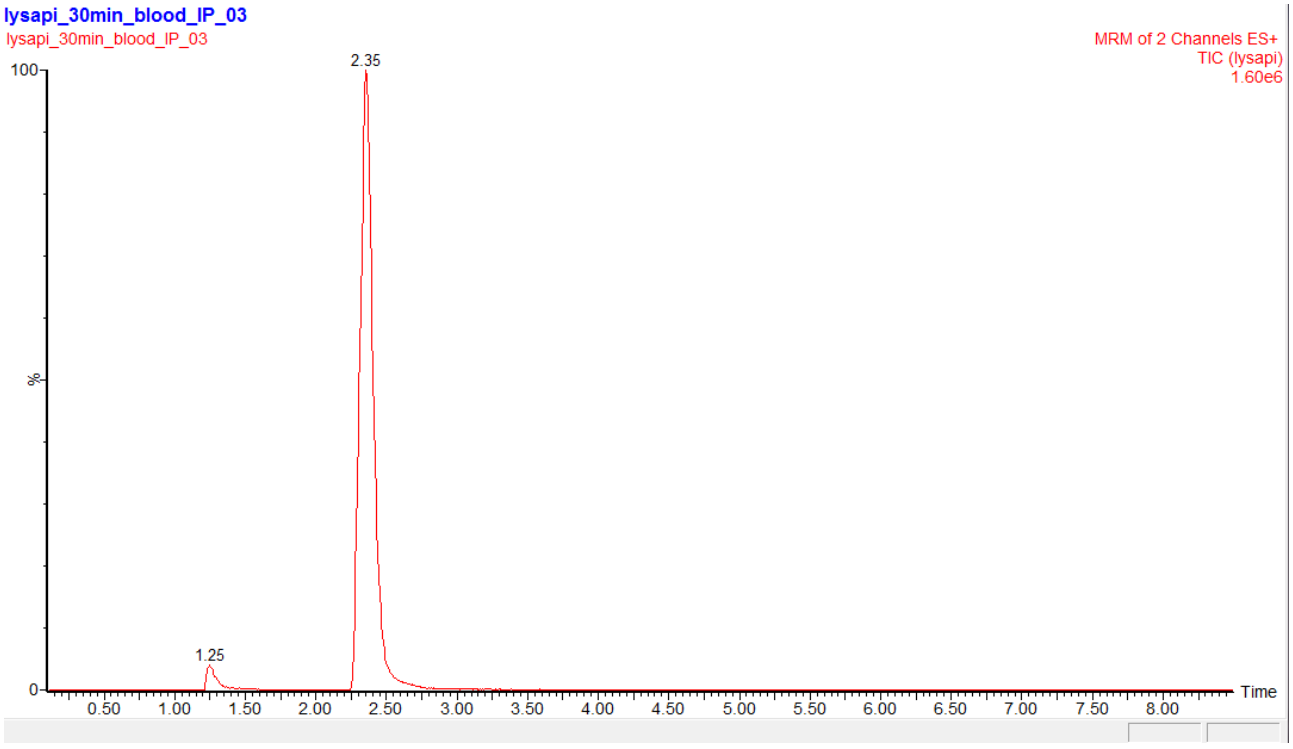
Appendix 7. LC-MS chromatogram of **1**



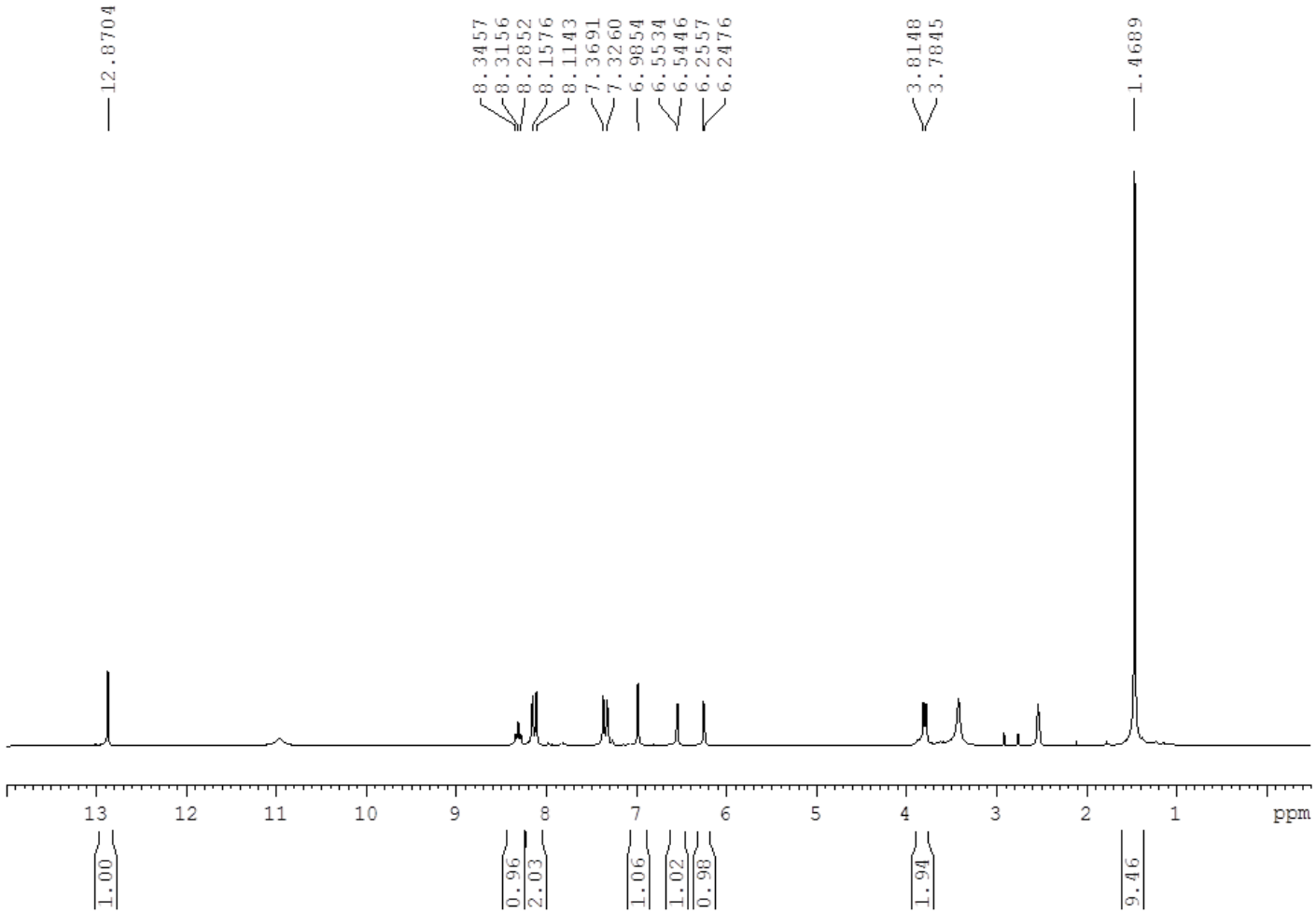
Appendix 8. LC-MS chromatogram of **4**



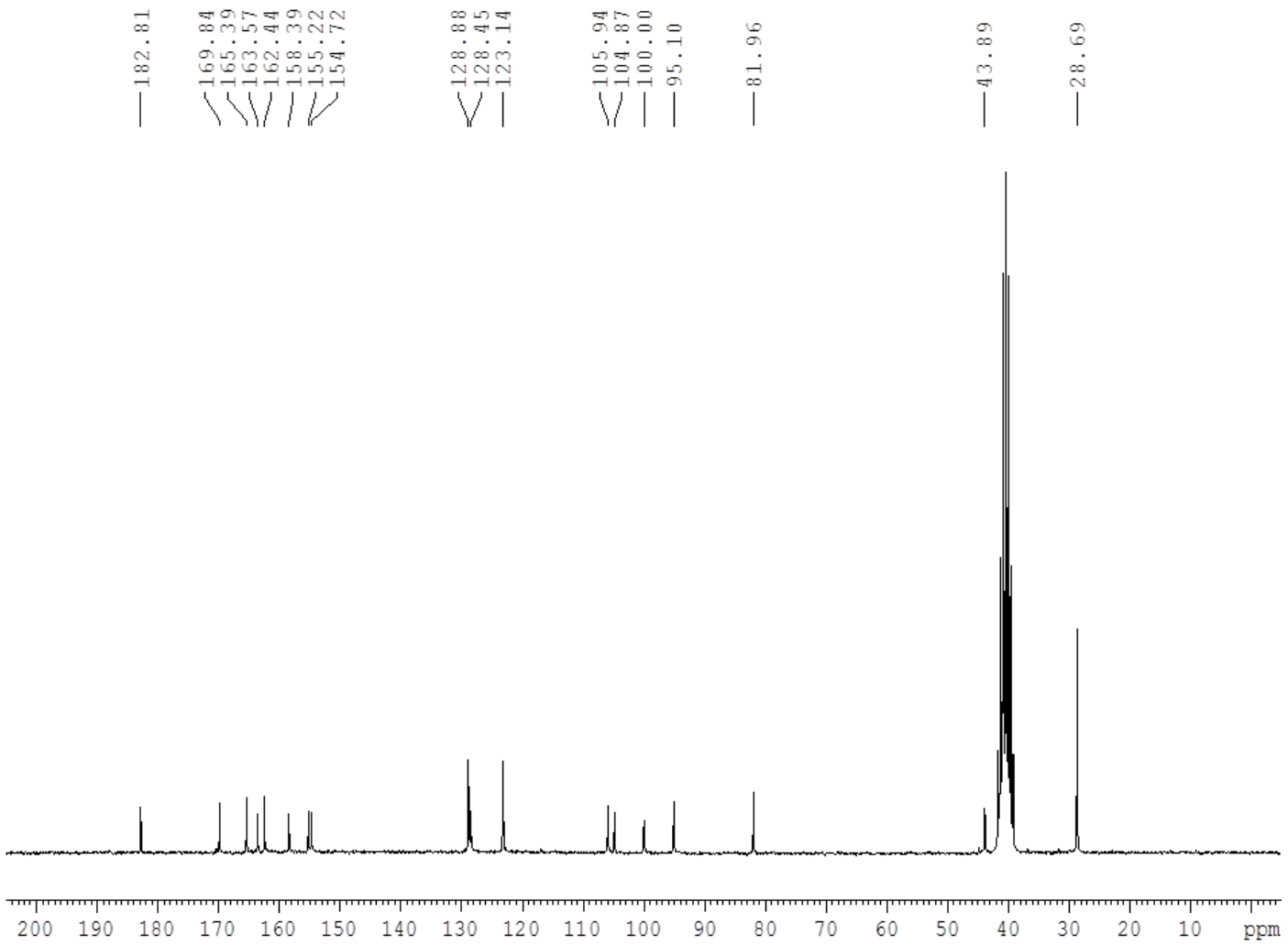
Appendix 9. LC-MS chromatogram of **7**



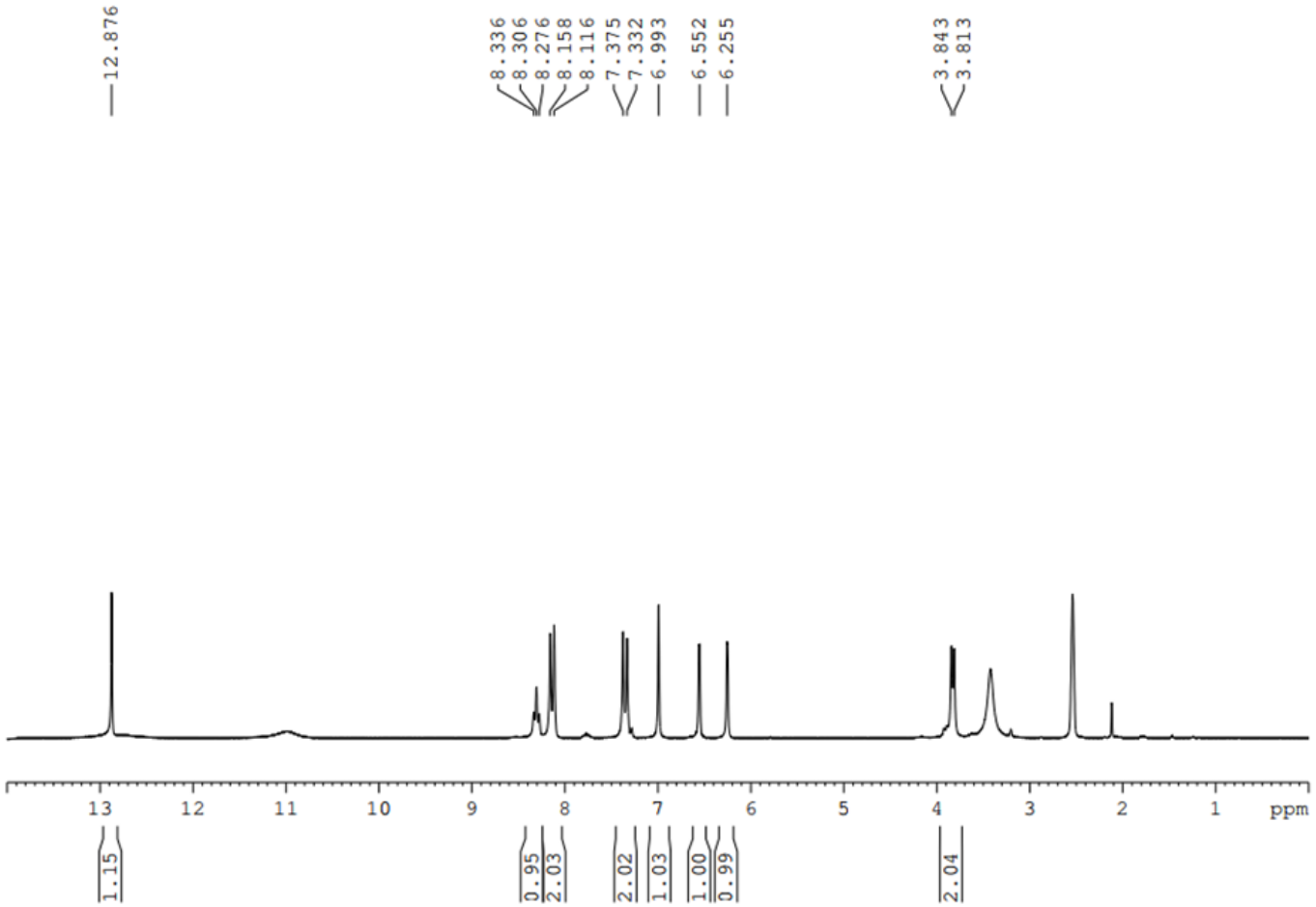
Appendix 10. LC-MS chromatogram of **10**



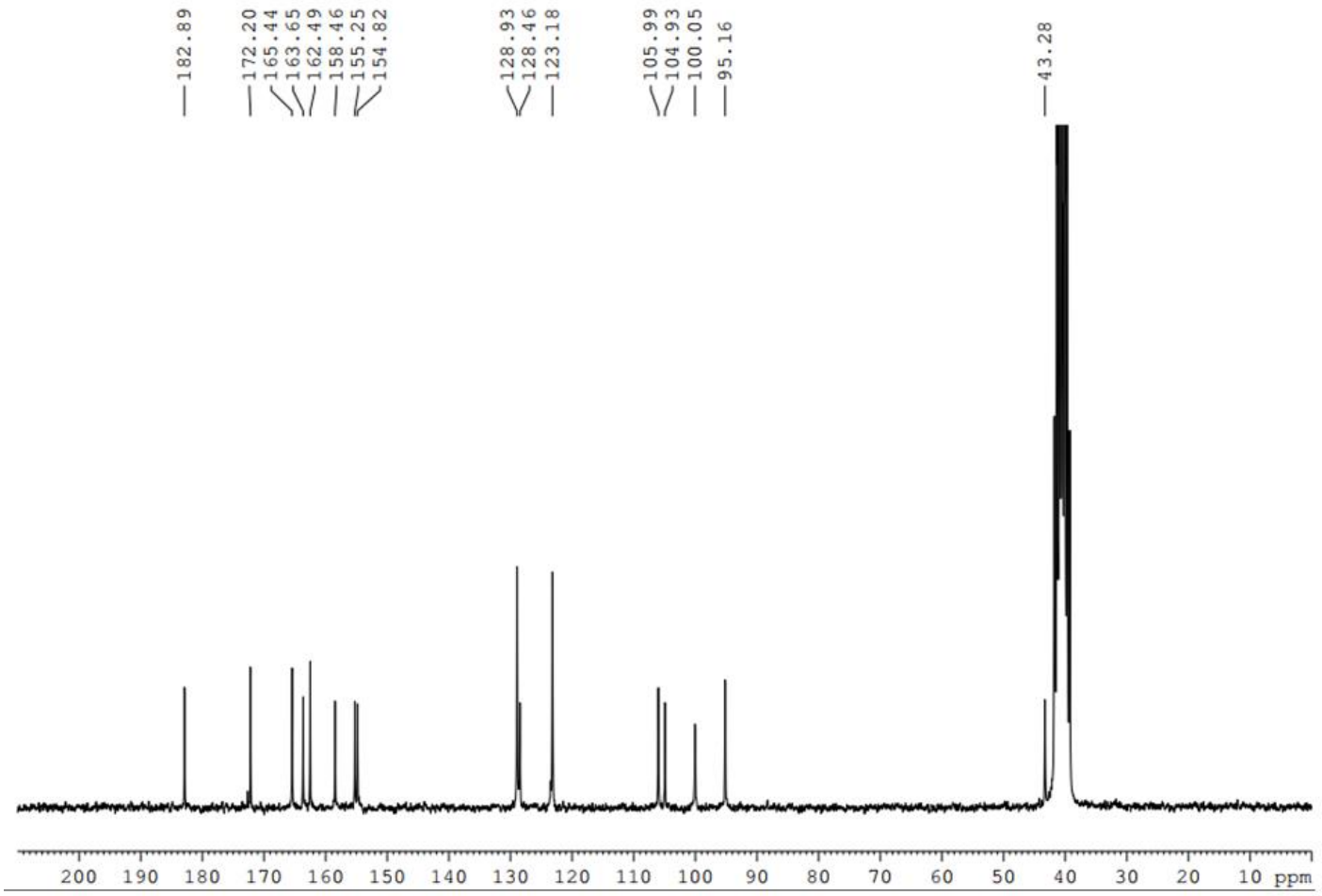
Appendix 11. ^{1}H-NMR spectrum of **3** (d_6-DMSO, 200 MHz)



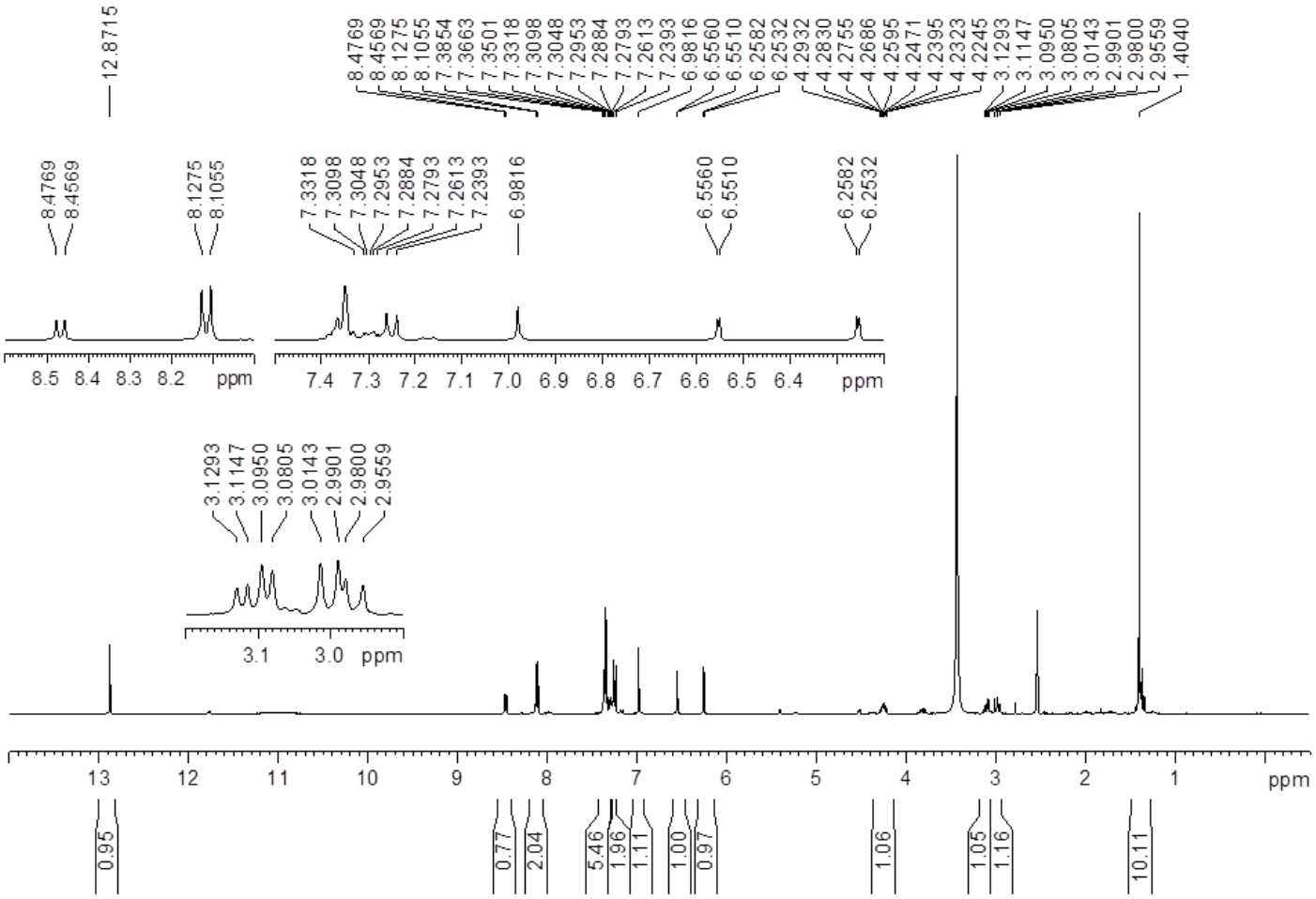
Appendix 12. ^{13}C-NMR spectrum of **3** (d_6-DMSO, 50 MHz)



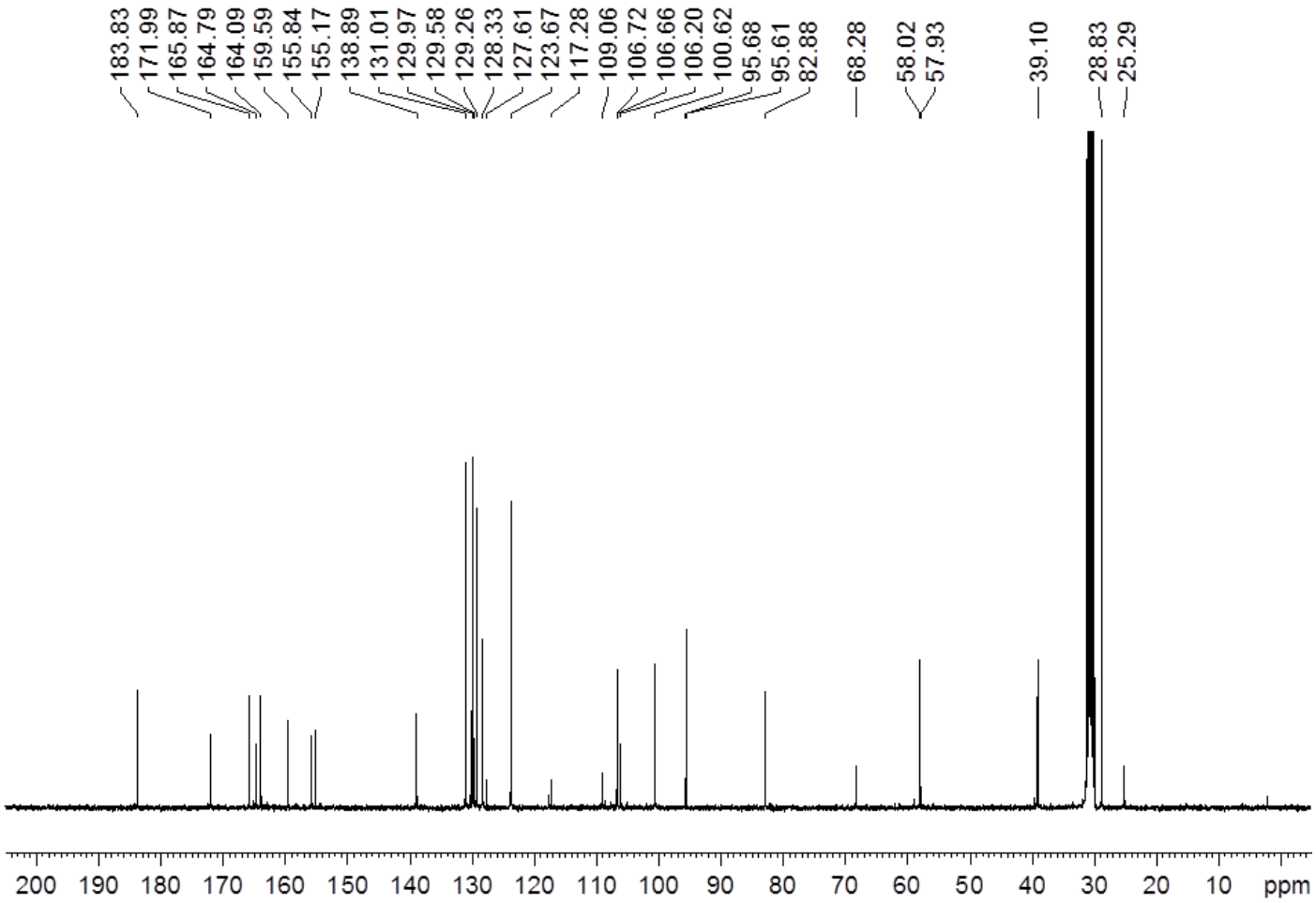
Appendix 13. ^{1}H-NMR spectrum of **4** (d_6-DMSO, 200 MHz)



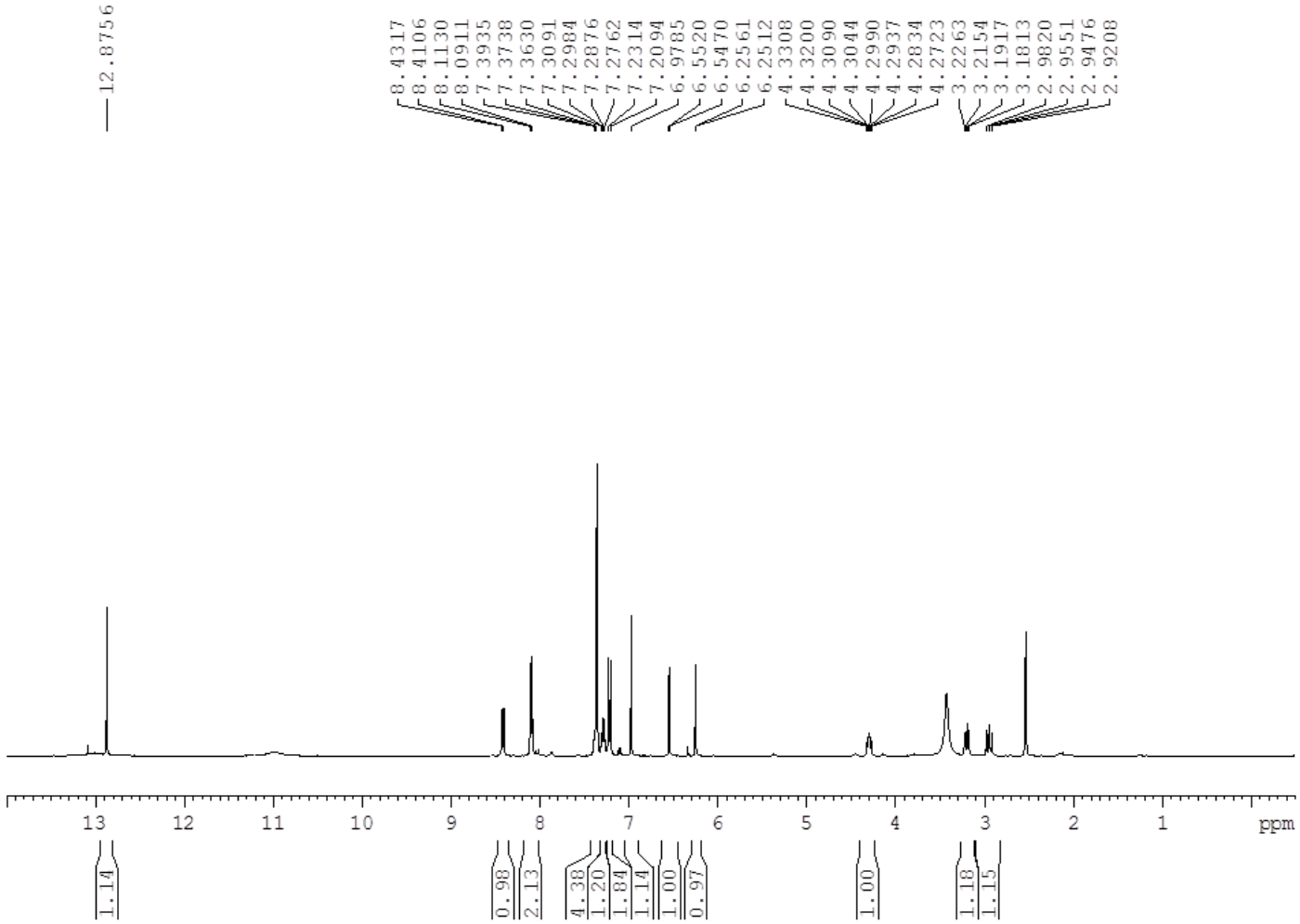
Appendix 14. ^{13}C-NMR spectrum of **4** (d_6-DMSO, 50 MHz)



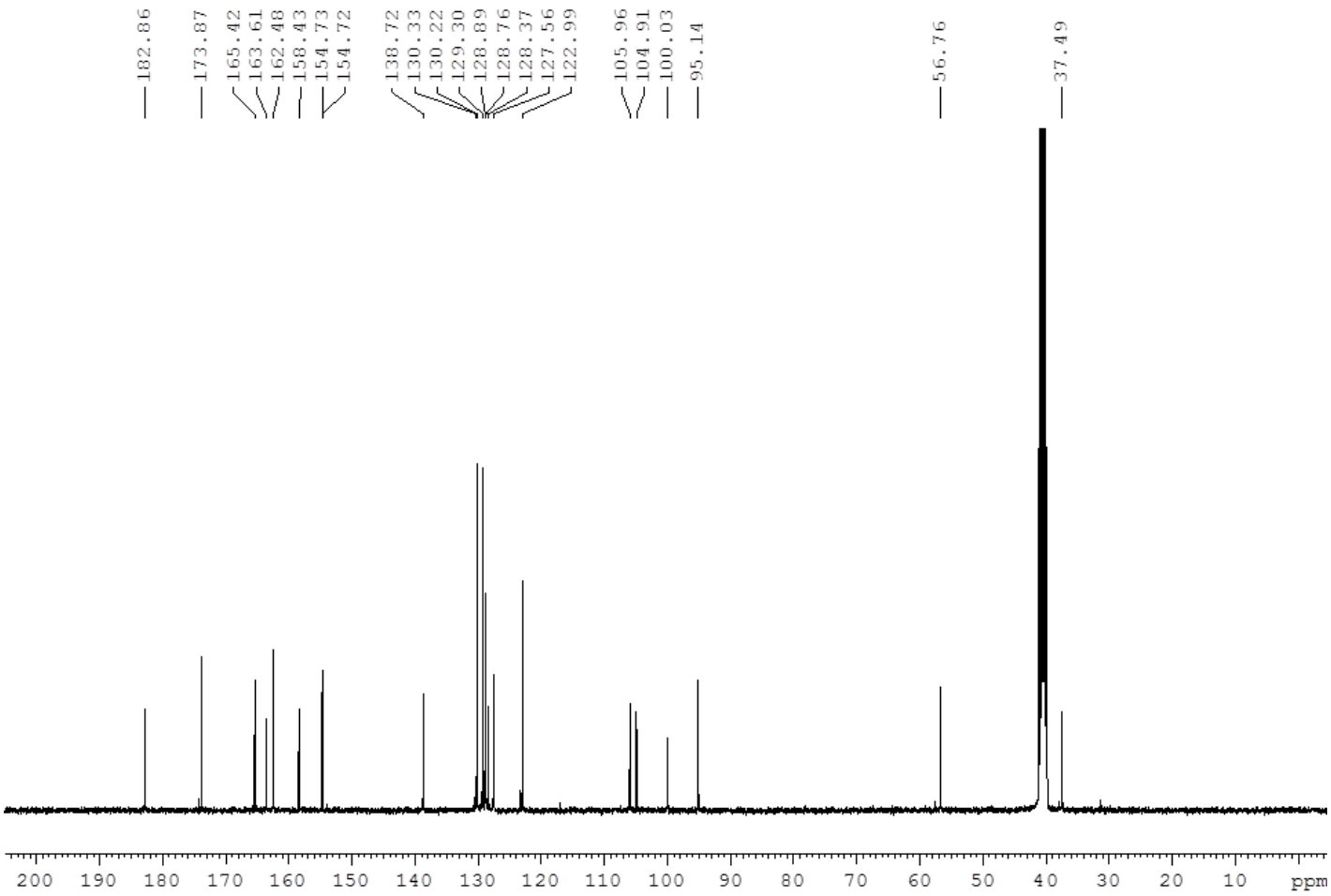
Appendix 15. ^{1}H -NMR spectrum of **6** (d_6-DMSO, 400 MHz)



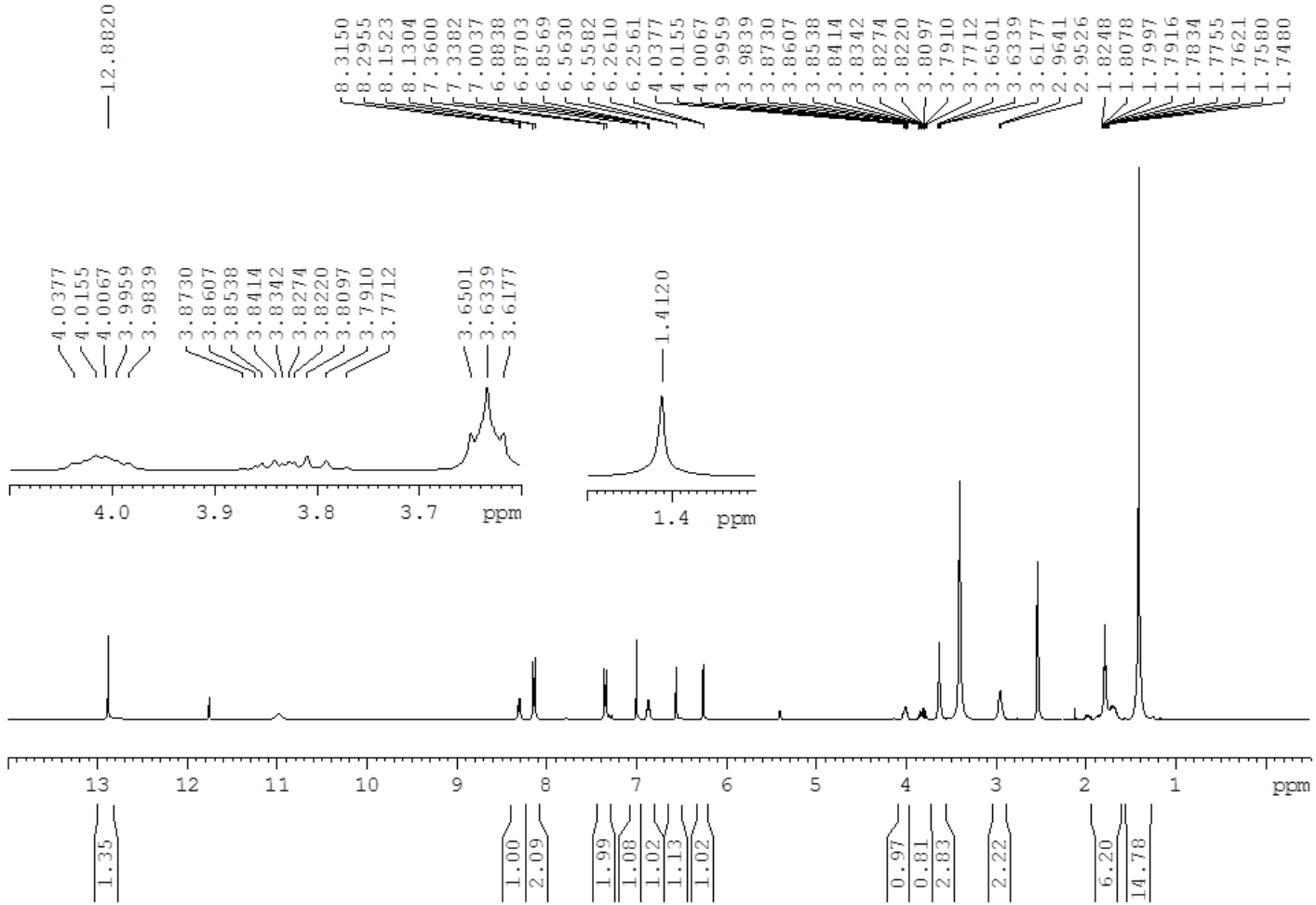
Appendix 16. ^{13}C-NMR spectrum of **6** (d_6-acetone, 100 MHz)



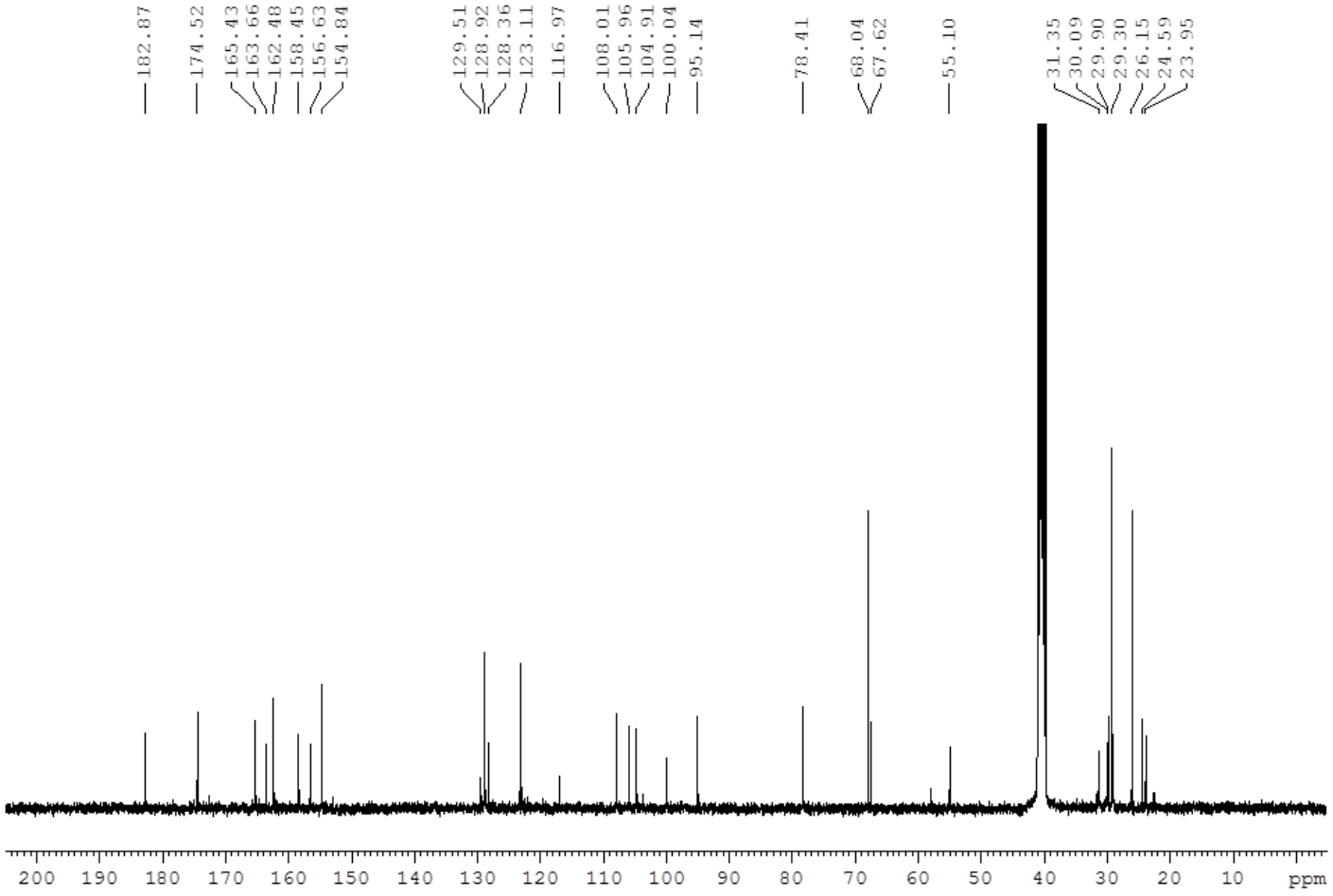
Appendix 17. ^{1}H-NMR spectrum of **7** (d_6-DMSO, 400 MHz)



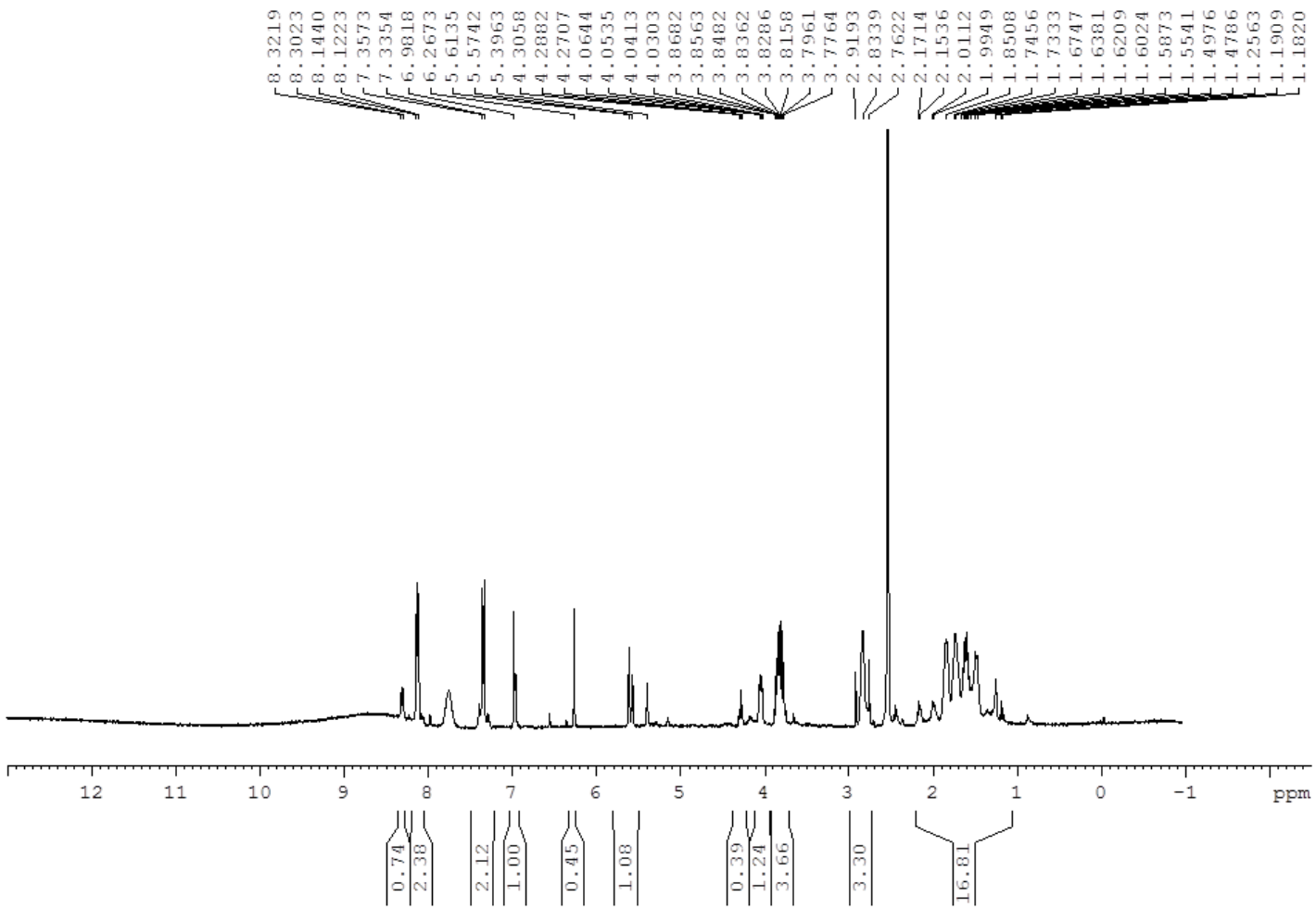
Appendix 18. ^{13}C-NMR spectrum of **7** (d_6-DMSO, 100 MHz)



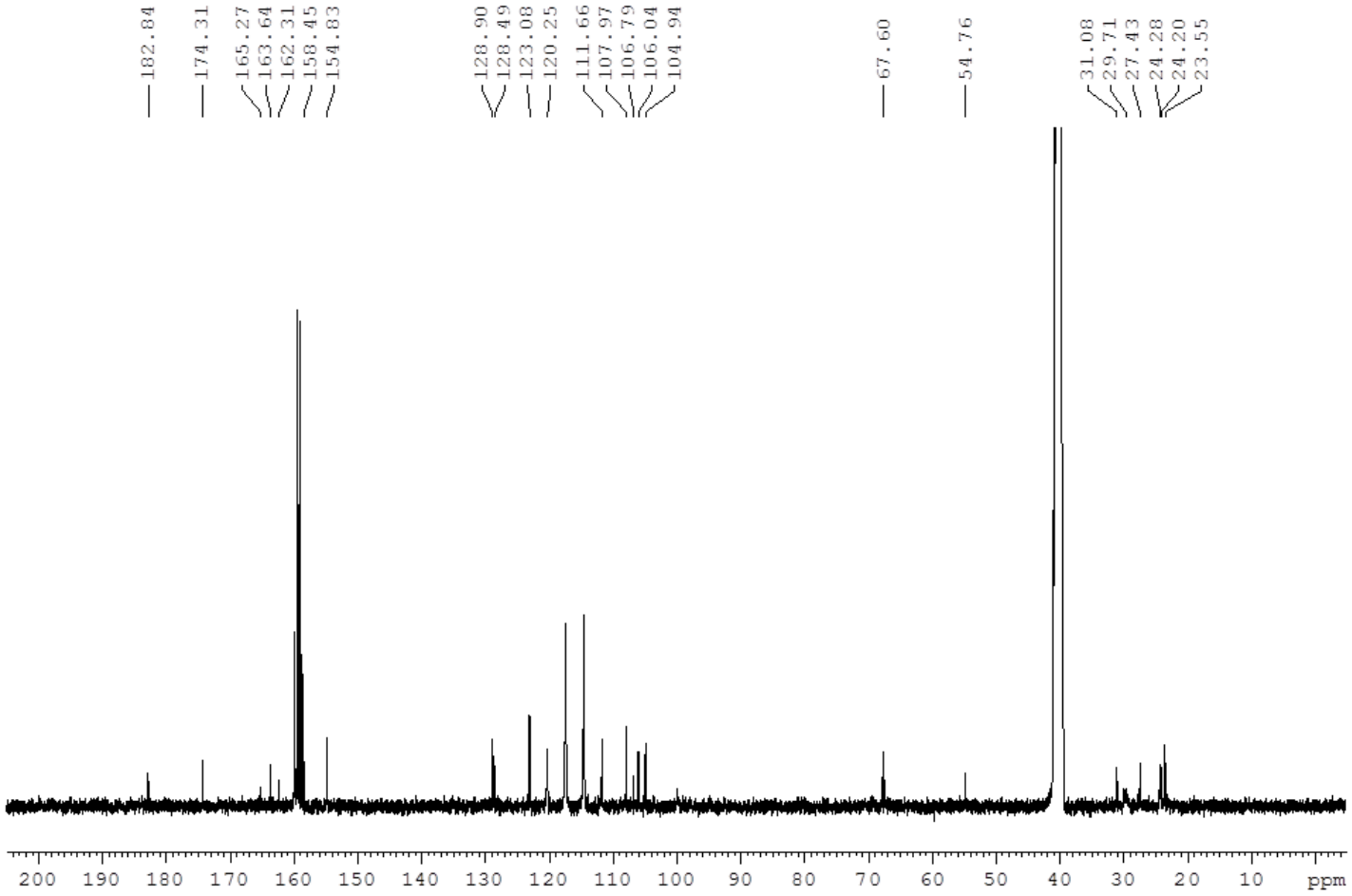
Appendix 19. ^{1}H-NMR spectrum of **9** (d_6-DMSO, 400 MHz)



Appendix 20. ^{13}C-NMR spectrum of **9** (d_6-DMSO, 100 MHz)



Appendix 21. ^{1}H-NMR spectrum of **10** (d_6-DMSO/d-TFA = 10/1, 400 MHz)



Appendix 22. ^{13}C-NMR spectrum of **10** (d_6-DMSO/d-TFA= 10/1, 100 MHz)