Supplementary Material

Lyophilized ash gourd (*Benincasa hispida* (Thunb.) Cogn.) juice alleviates diet-induced prediabetes in rat model

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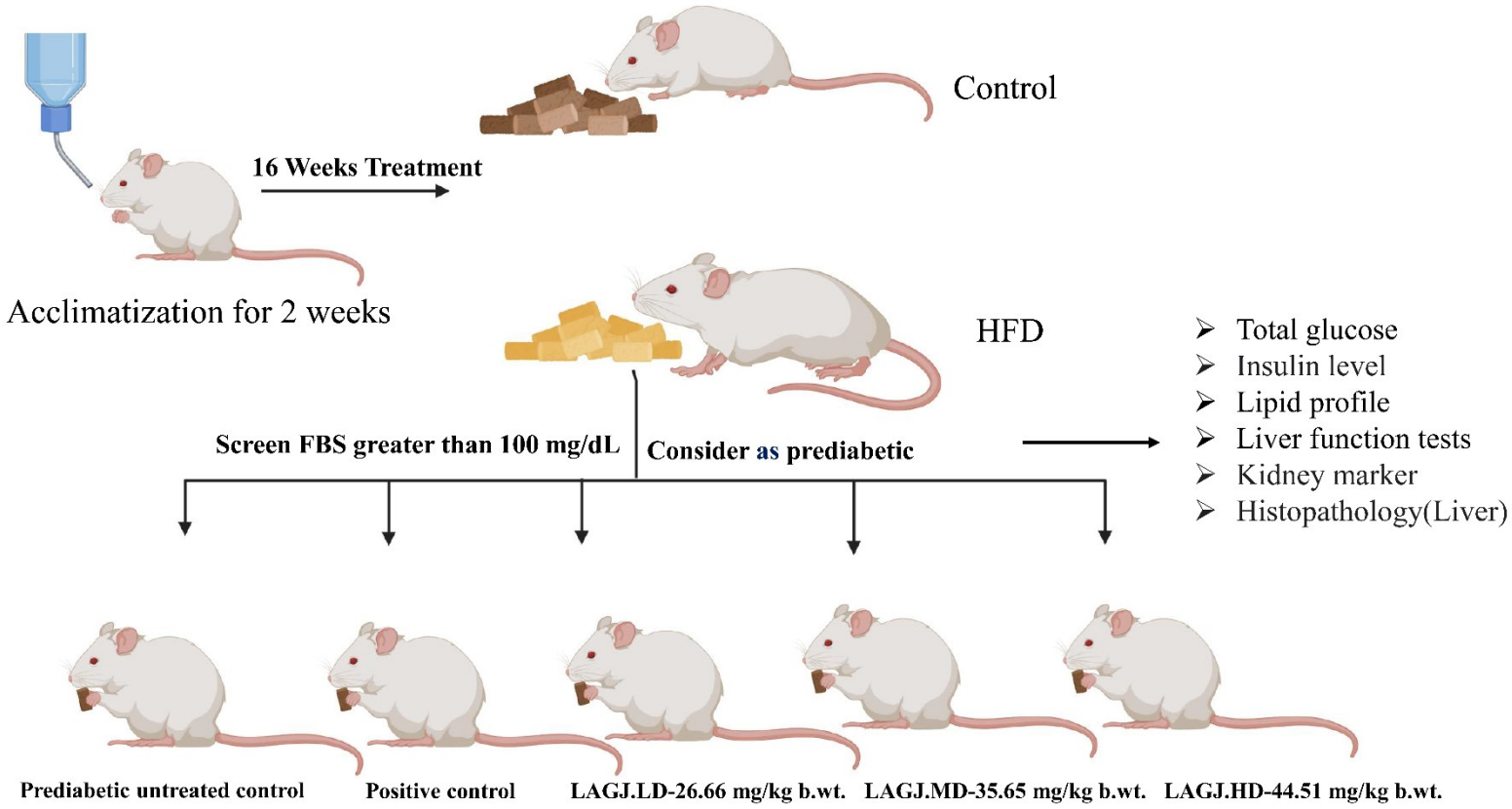

Fig. S1 Experimental design (FBS; Fasting blood sugar, LAGJ; Lyophilized ash gourd juice, LD; Low dose, MD; Medium dose, HD; High dose)

Table S1. Normal rat chow composition

Ingredient	g/100g
Protein	18.0
Corn starch	62.0
Potato Starch	-
Fat	7.0
Vitamin	1.5
Mineral	3.5
Fibre	3.0
Moisture	5.0
Energy	3.8 cal/g

Table S2. Composition of high-fat diet

Ingredient	g/100g
Casein	11.5
Milk powder	25
Fructose	13.8
Potato starch	15
Lard	1
Coconut oil	8.4
Sunflower oil	4
Vitamin mineral mix	9
Deoxycholic acid sodium salt	1
L-cysteine	1
Cellulose	10
Energy	3.8 cal/g

Composition of vitamin and mineral mixture: The mineral mixture contained the following (Nutritional value/kg):vitaminA,700000I.U;vitaminD3,70000I.U;vitaminE,250 mg; cobalt,150mg, copper,1200mg;
iodine,325mg;iron,1500mg;magnesium,6000mg;potassium100mg;sodium,5.9mg;manganese,1500mg; sulphur,0.72%;zinc,9600mg;DL-methionine–1000mg; calcium,25.5%,phosphorus,12.75%.

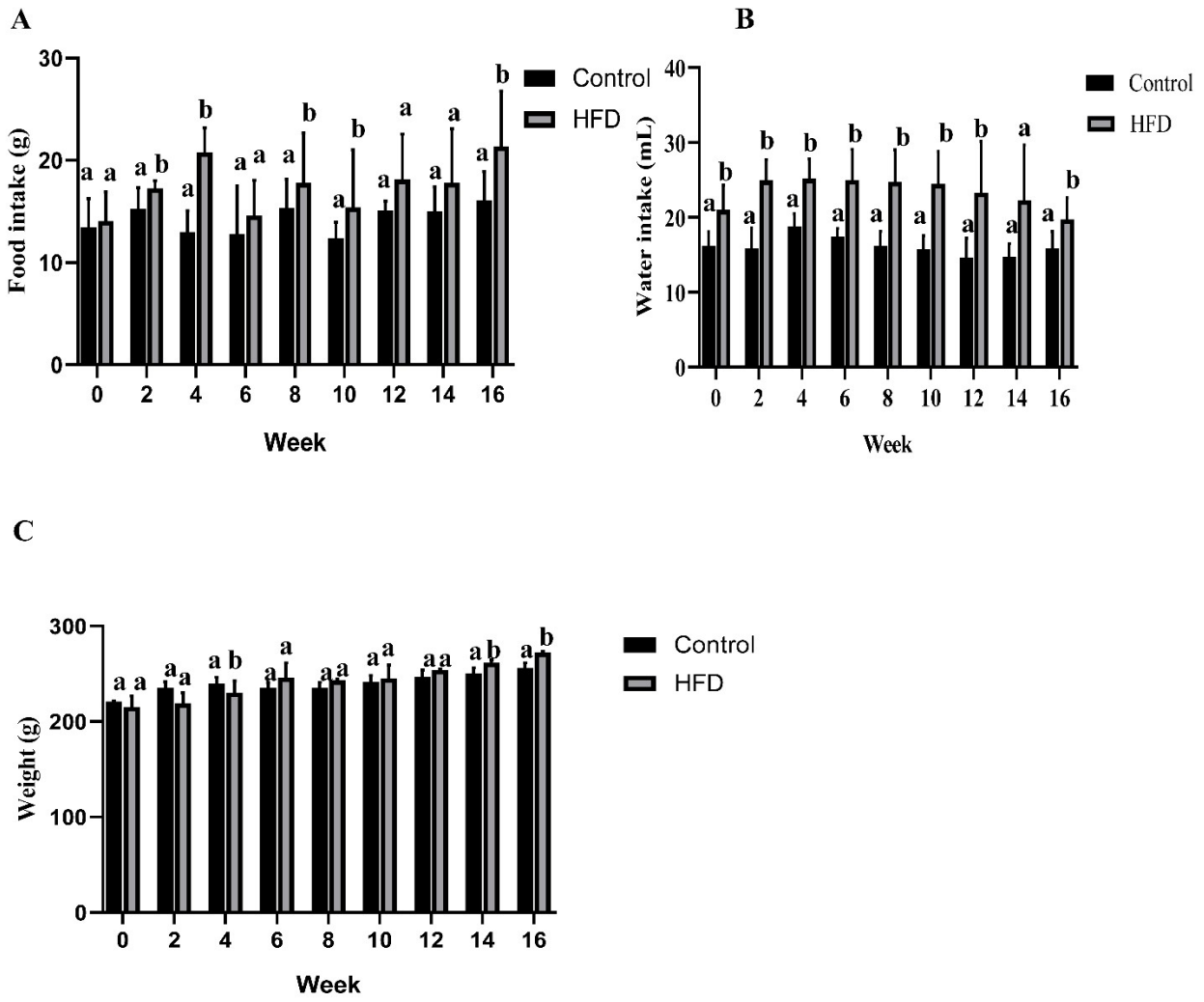

Fig. S2 A. Food intake **B**. water intake and **C.** weight change of control and HFD group over 16 weeks. Values were analyzed by independent sample t-test and expressed as the mean ± SD of 5 rats/group; different letters indicate the significant difference between the samples at $p \leq 0.05$ (HFD; High-fat diet)

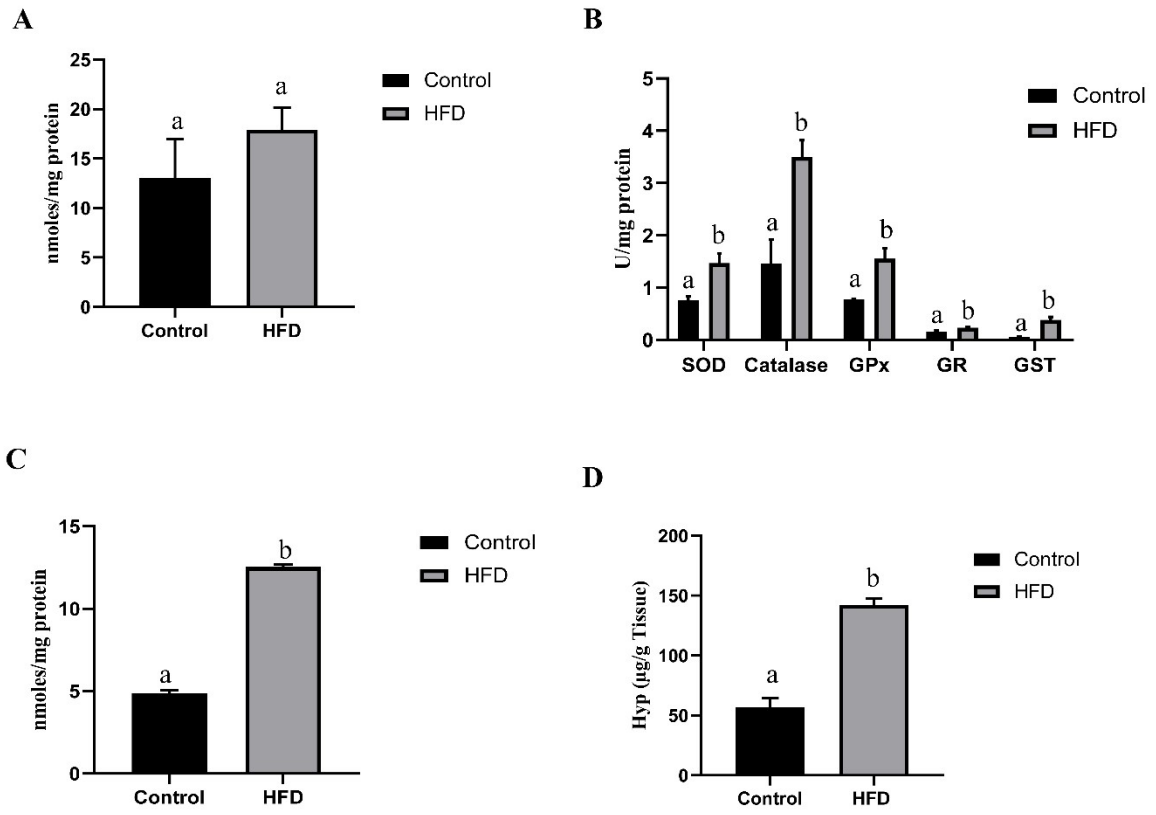

Fig. S3 Comparison of control and HFD groups hepatic redox imbalance as indicated by **A.** GSH **B.** SOD, catalase, GPx, GR and GST **C.** TBARS and **D.** Hyp. Values were analyzed by independent sample t-test and expressed as the mean ± SD of 5 rat/group, different letters indicate the significant difference between the sample at $p \leq 0.05$ (HFD; high-fat diet, GSH; reduced glutathione, SOD; superoxide dismutase, GPx; glutathione peroxidase, GR; glutathione reductase, GST; glutathione S-transferase, TBARS; thiobarbituric acid-reactive substances, Hyp; hydroxyproline)

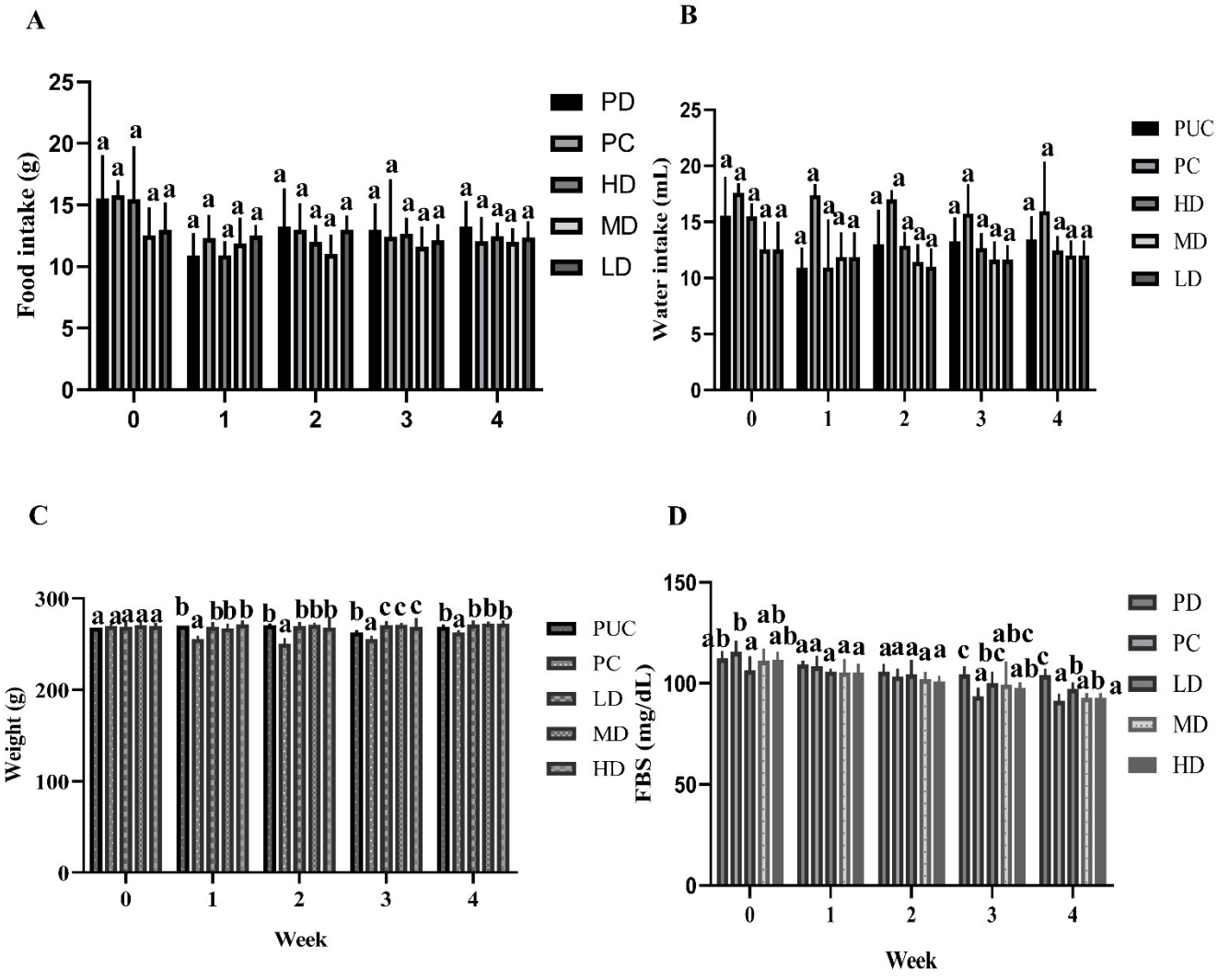

Fig. S4 Effect of LAGJ on **A.** Food intake, **B**. water intake, **C.** Body weight **D**. FBS, of PUC, PC, LD, MD, and HD groups. Values were analyzed by 1-way ANOVA and expressed as the mean±SD of 5 rats/group; different letters indicate the significant difference between the samples at $p \leq 0.05$ (LAGJ; lyophilized ash gourd juice powder, FBS; fasting blood sugar; PUC; prediabetic untreated control, PC; positive control, LD; low dose, MD; medium dose; HD; high dose)

Table S3. Effect of LAGJ on relative organ weight of prediabetic rats

	PUC	PC	LD	MD	HD
Pancreas (%)	0.28±0.08[a]	0.33±0.07[a]	0.38±0.12[a]	0.38±0.13[a]	0.47±0.14[a]
Liver (%)	2.78±0.40[a]	2.94±0.46[a]	2.74±0.27[a]	2.61±0.98[a]	2.68±0.58[a]
Kidney (%)	0.61±0.01[a]	0.66±0.11[a]	0.60±0.09[a]	0.61±0.14[a]	0.60±0.11[a]
Heart (%)	0.34±0.05[a]	0.35±0.03[a]	0.36±0.02[a]	0.31±0.08[a]	0.34±0.70[a]

Values were analyzed by 1-way ANOVA and expressed as the mean ± SD of 5 rats/group, and means with different superscript letters in the same row show significant differences at $p \leq 0.05$ (LAGJ; lyophilized ash gourd juice powder, PUC; prediabetic untreated control, PC; positive control, LD; low dose, MD; medium dose; HD; high dose)

Table S4. Scoring of hepatosteatosis in various groups of experimental rats

Characteristics	Control	HFD	PUC	PC	LD	MD	HD
Macro vesicular steatosis	0	2	0	0	0	0	0
Hepatocellular ballooning	0	1	0	0	0	0	0
Portal tract inflammation	0	2	2	0	0	0	0
Glycogenated nuclei	0	1	0	0	0	0	0
Sinusoidal dilation and nuclear vacuolation	0	0	2	1	1	1	1
Fibrosis and lipogranulomas	0	1	1	1	0	0	0

Scoring indicates 0–3 based on extent of damage in the hepatocytes, where 0 is none; 1 mild; 2 is moderate; 3 is severe (PUC; prediabetic untreated control, PC; positive control, LD; low dose, MD; medium dose; HD; high dose)

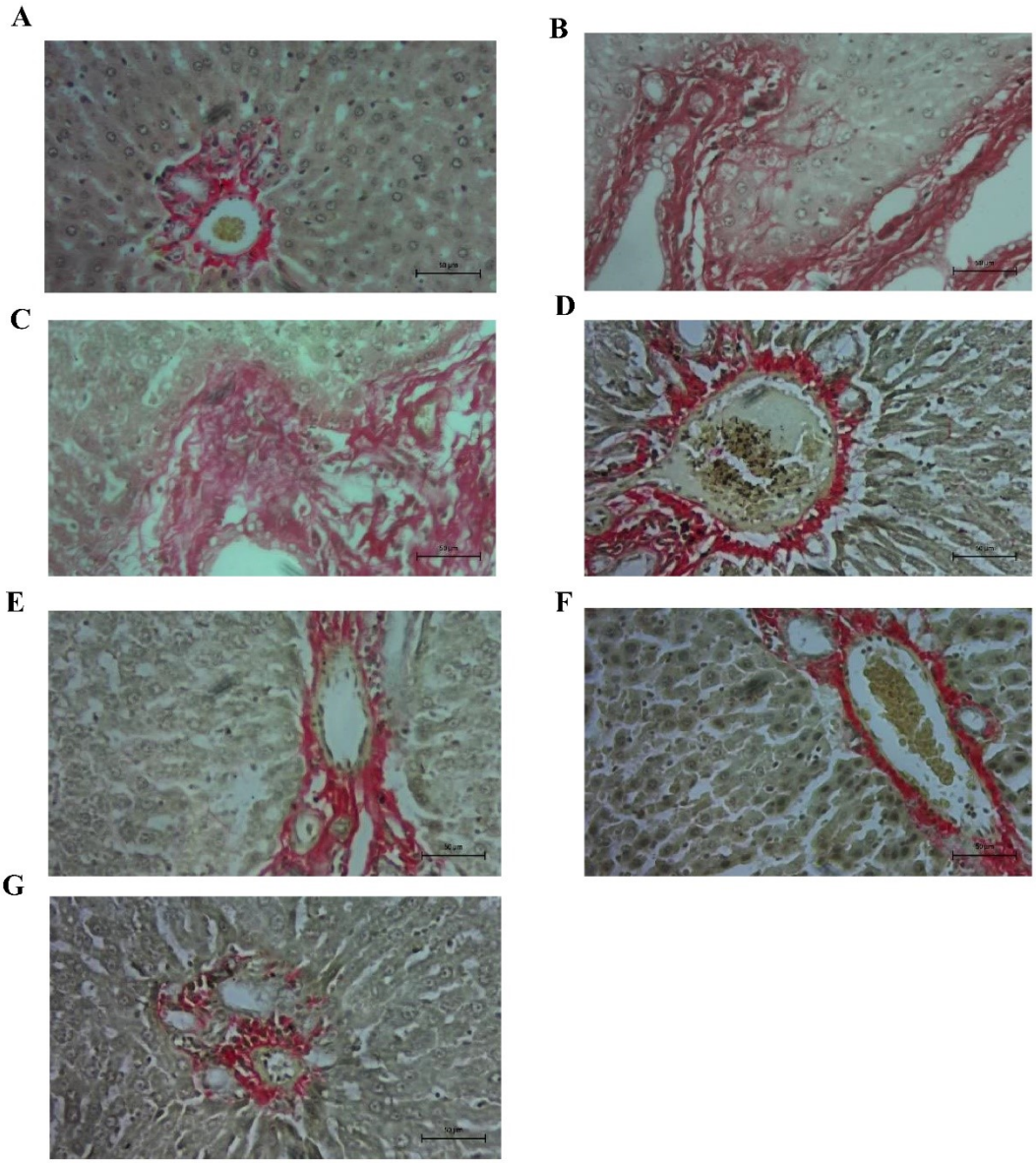

Fig. S5 Picrosirius red staining of hepatic tissues **A**) control, **B**) HFD, **C**) PUC **D**) PC **E**) LD, **F**) MD, and **G**) HD, scale bar = 50 µm (HFD; high-fat diet, PUC; prediabetic untreated control, PC; positive control, LD; low dose, MD; medium dose; HD; high dose)

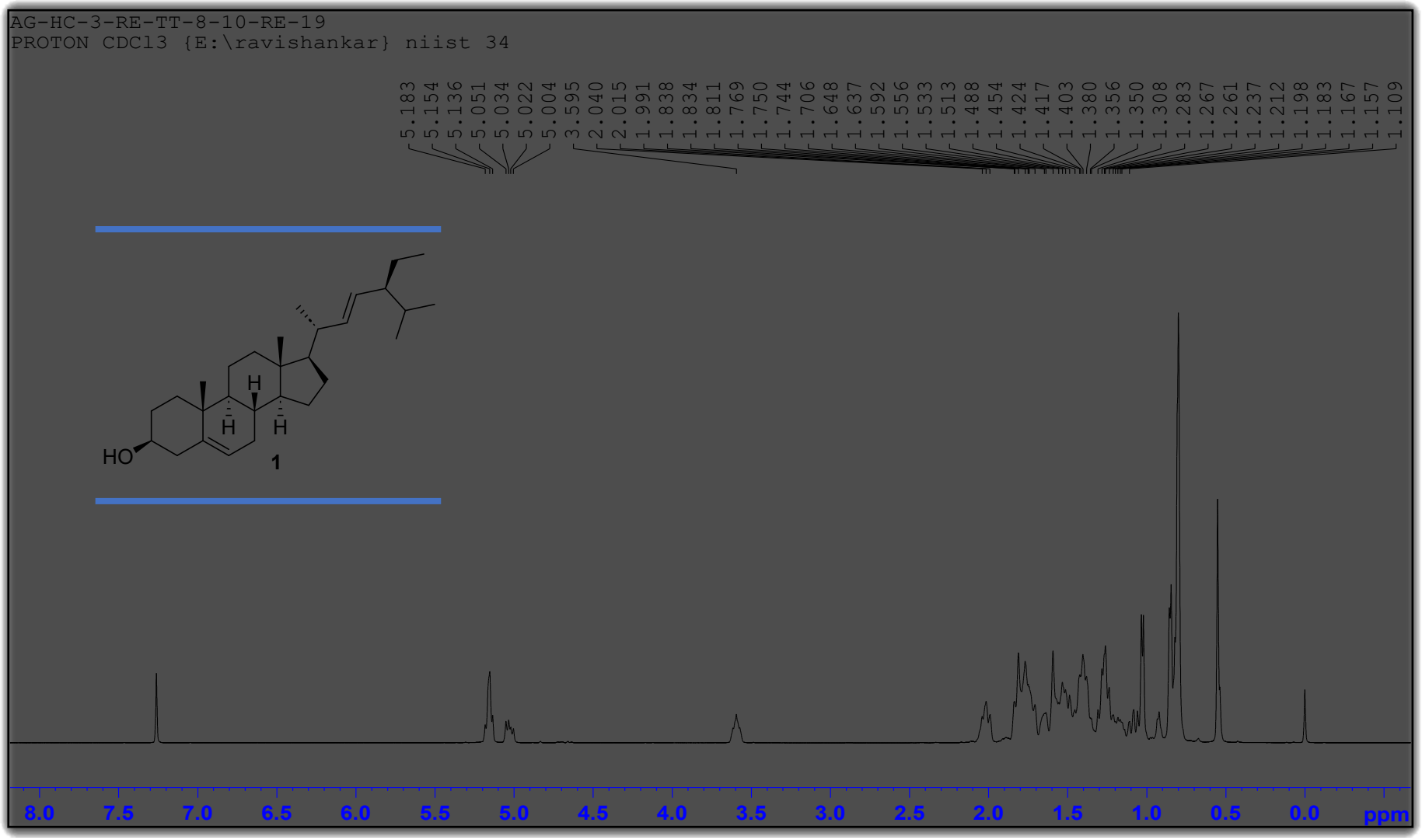

Fig. S6 ^{1}H NMR of compound **1** in $CDCl_3$

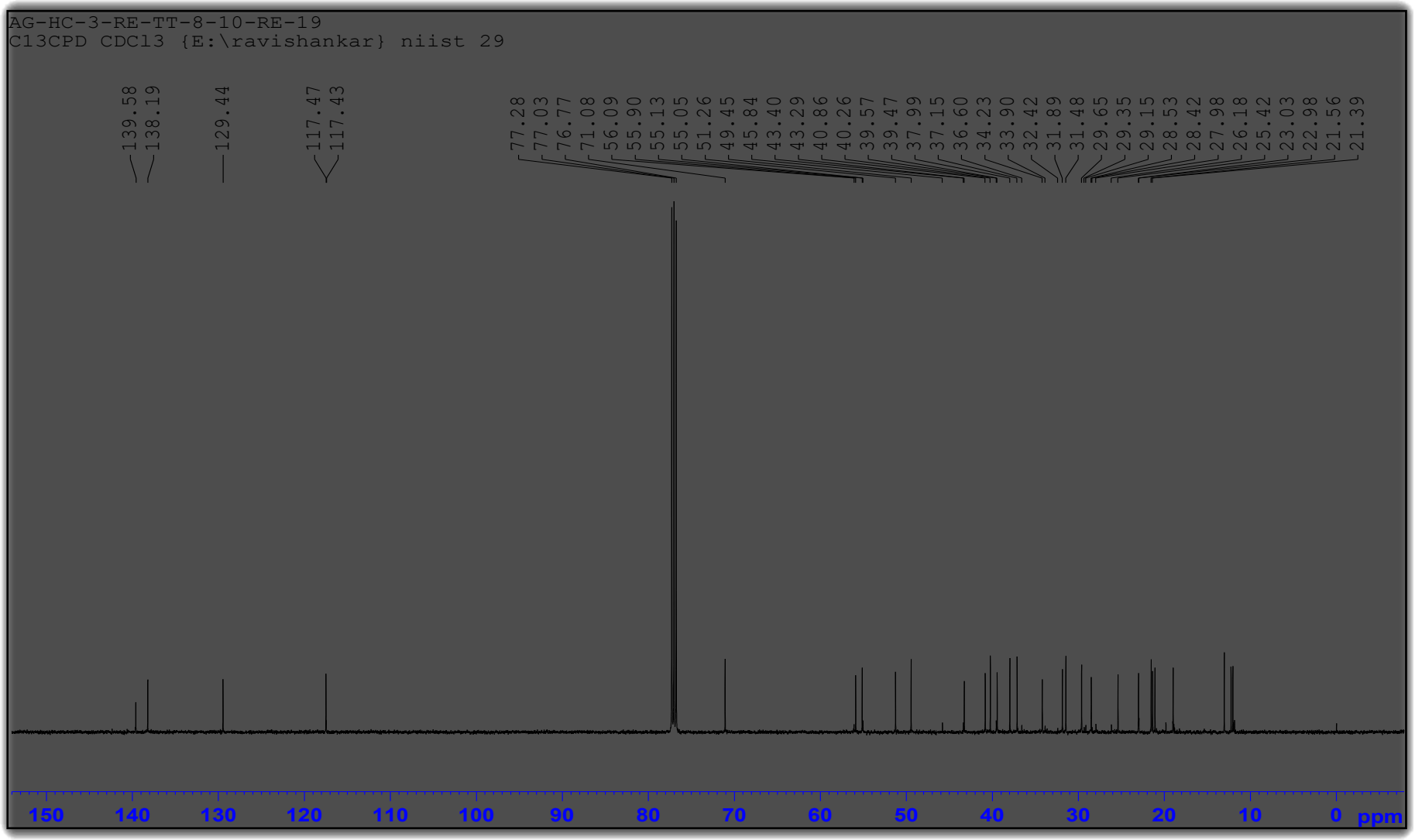

Fig. S7 ^{13}C NMR of compound **1** in $CDCl_3$

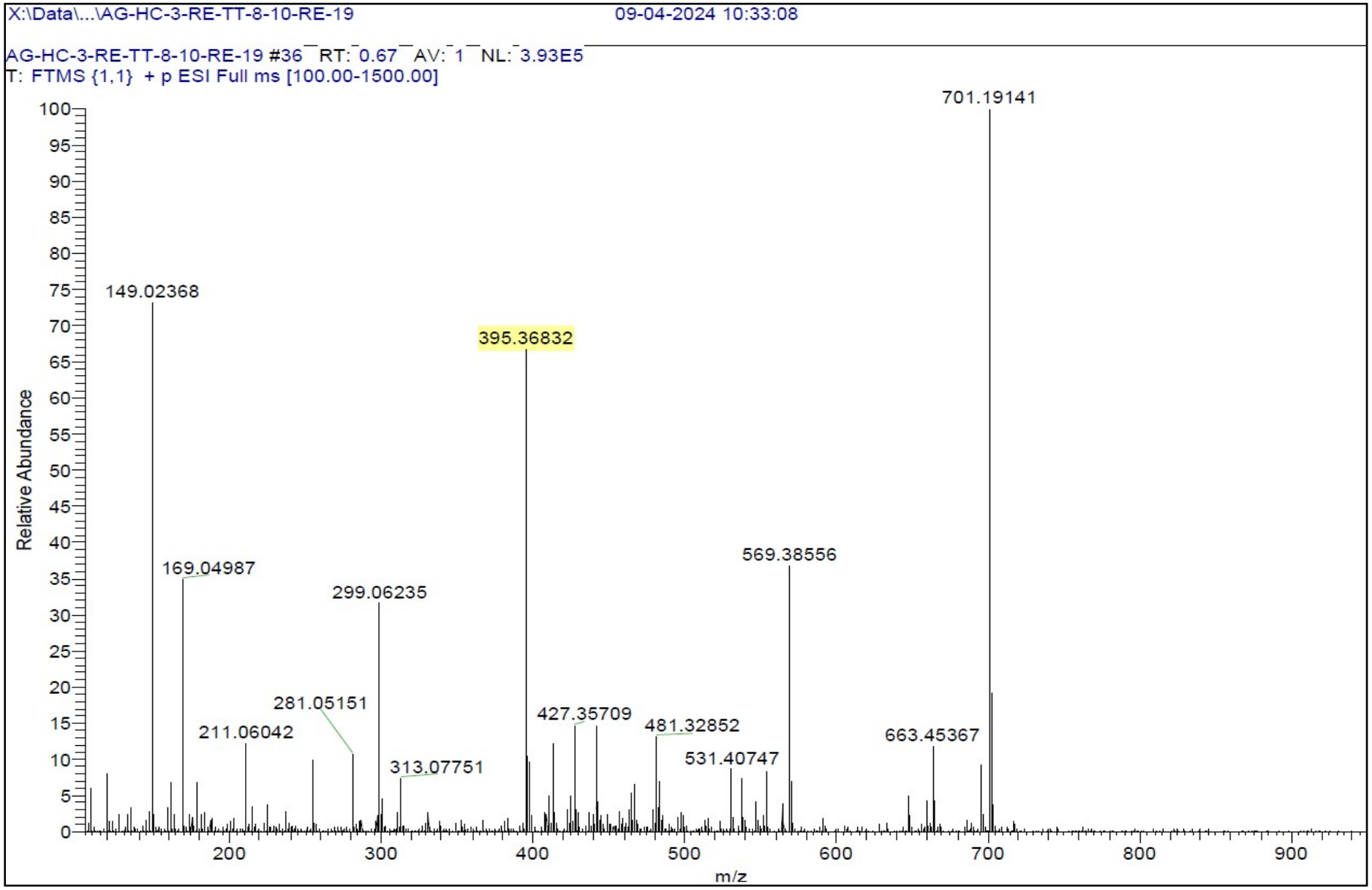

Fig. S8 HR-ESI-MS of compound **1**

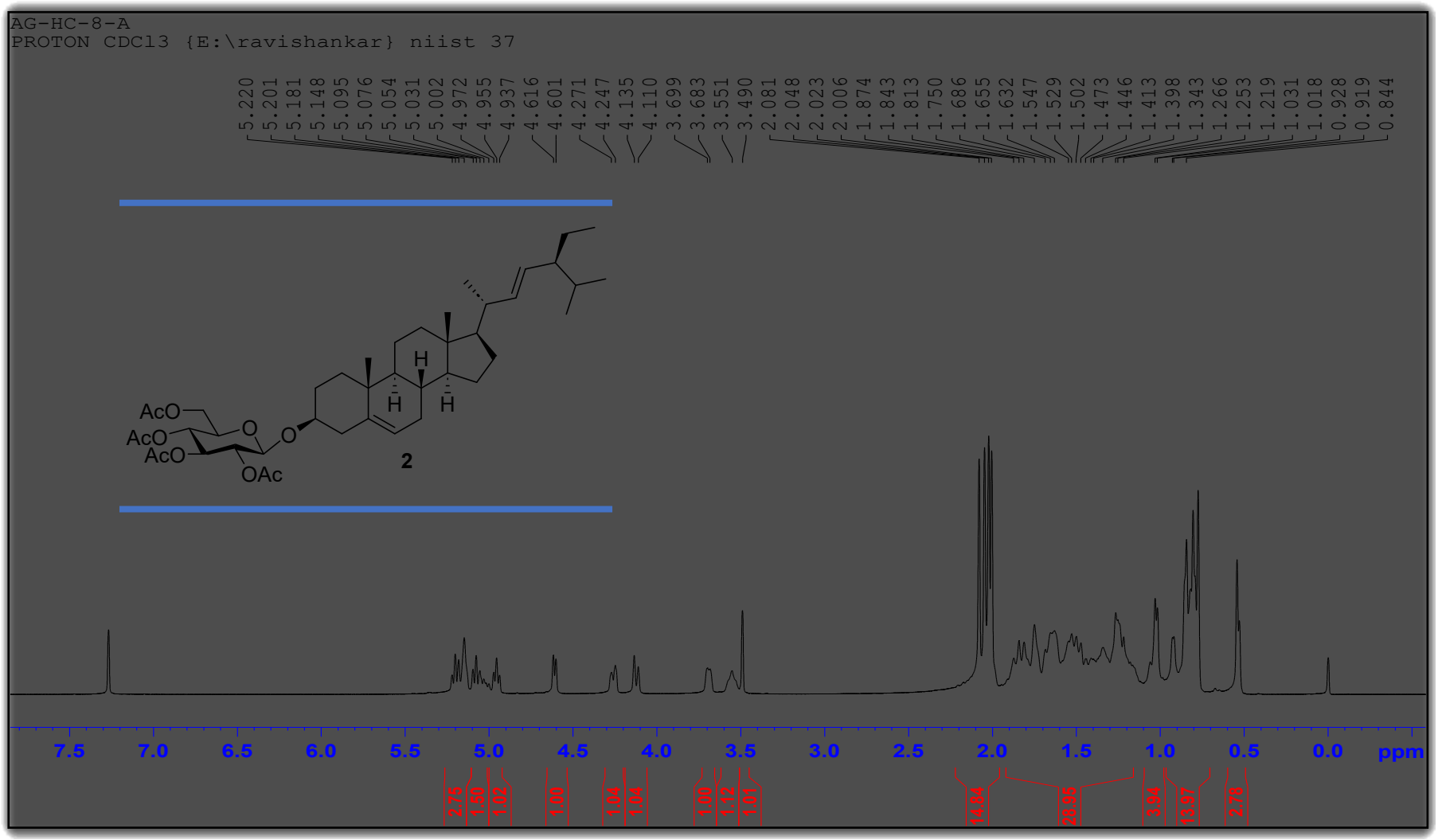

Fig. S9 ^{1}H NMR of peracetylated derivative of compound **2** in $CDCl_3$

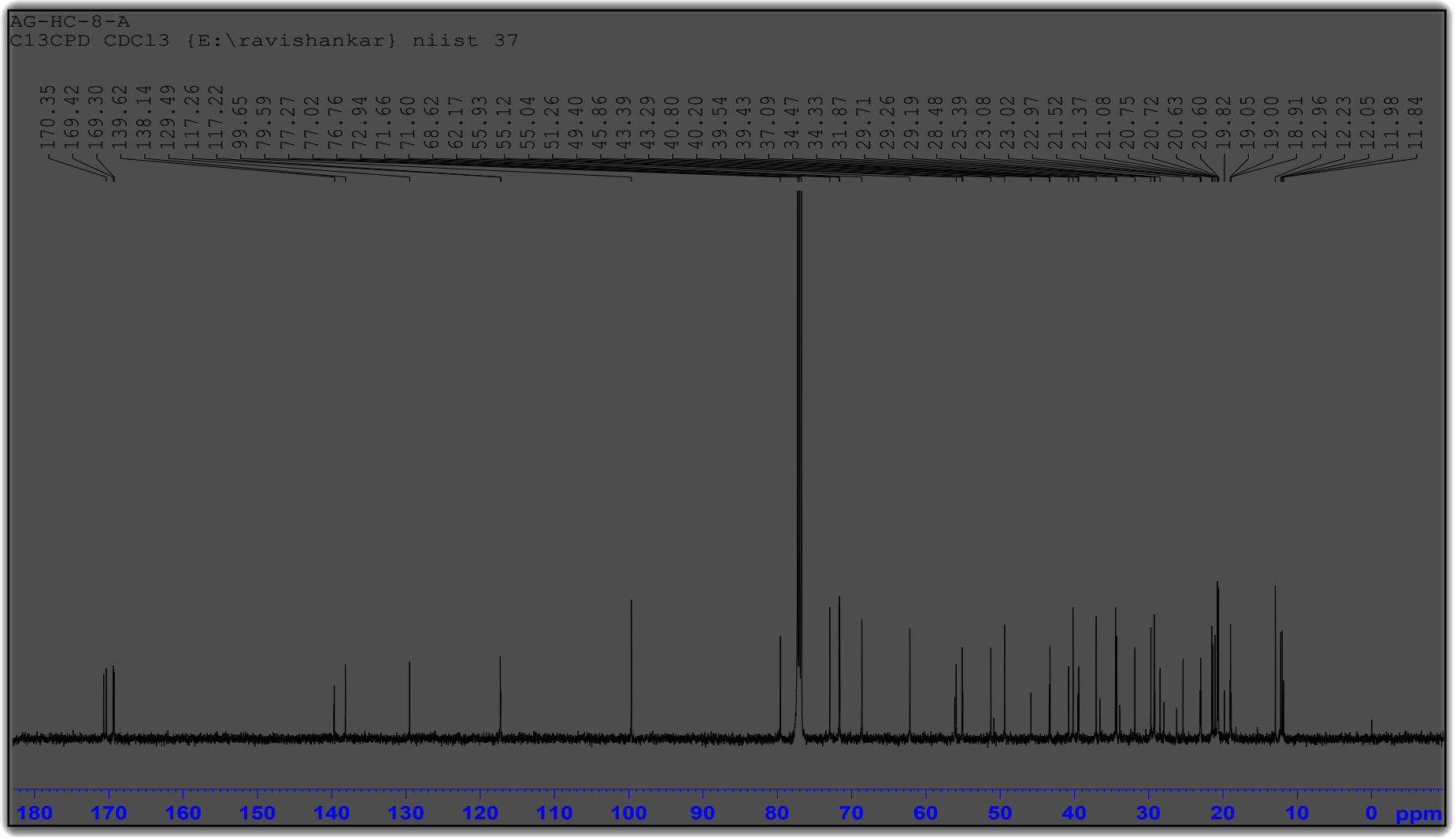

Fig. S10 ^{13}C NMR of peracetylated derivative of compound **2** in $CDCl_3$

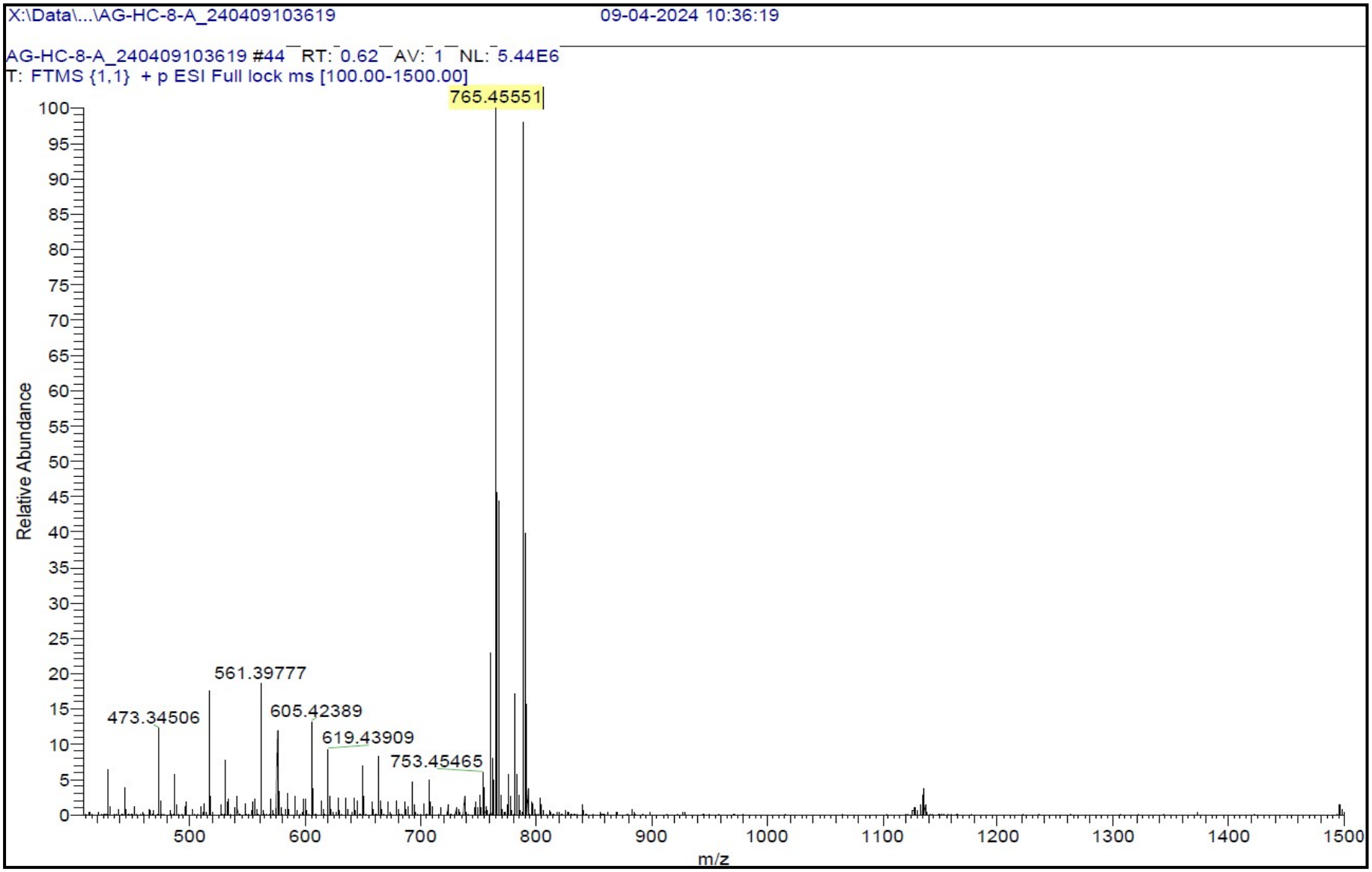

Fig. S11 HR-ESI-MS of peracetylated derivative of compound **2**

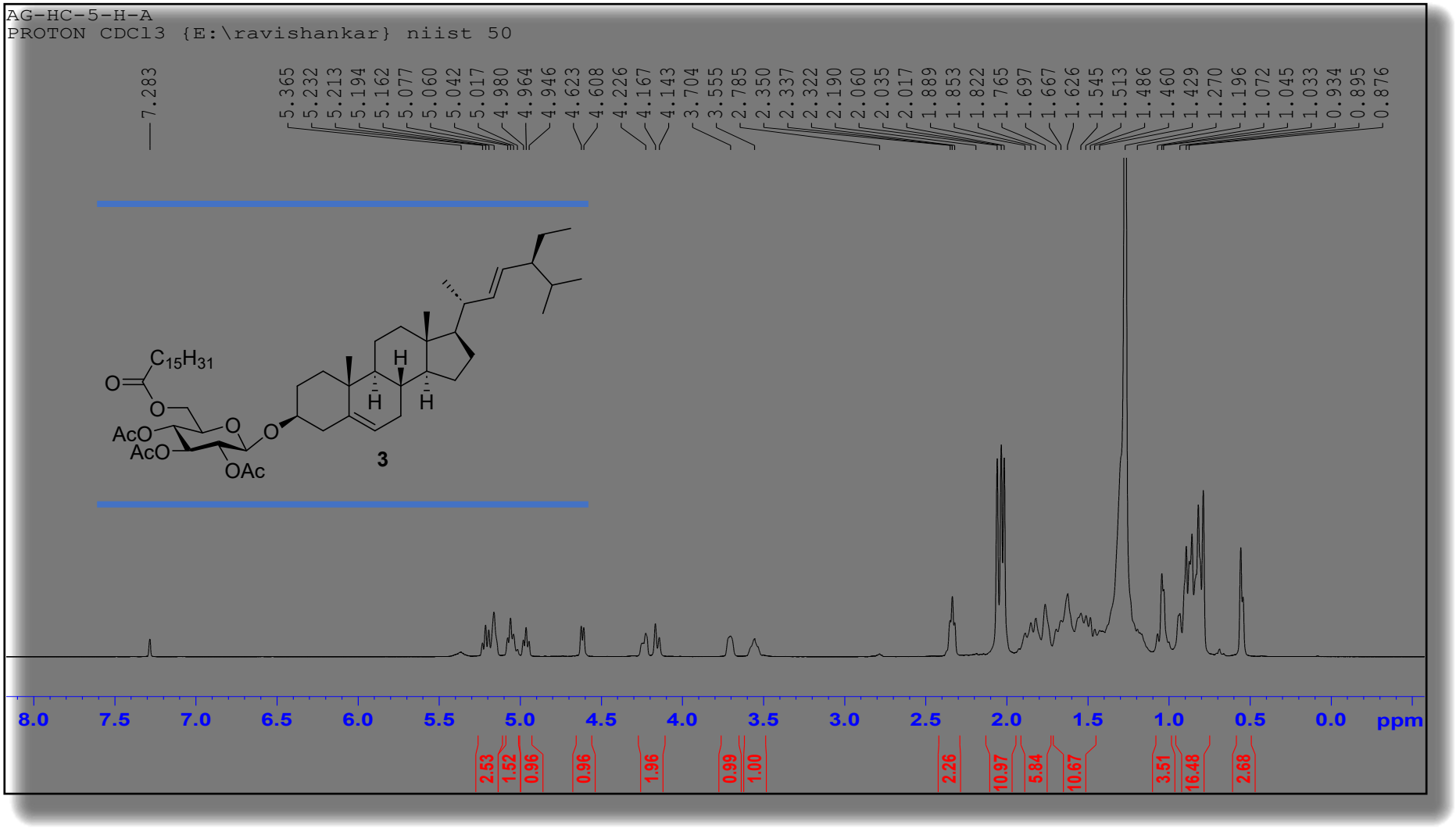

Fig. S12 ^{1}H NMR of peracetylated derivative of compound **3** in $CDCl_3$

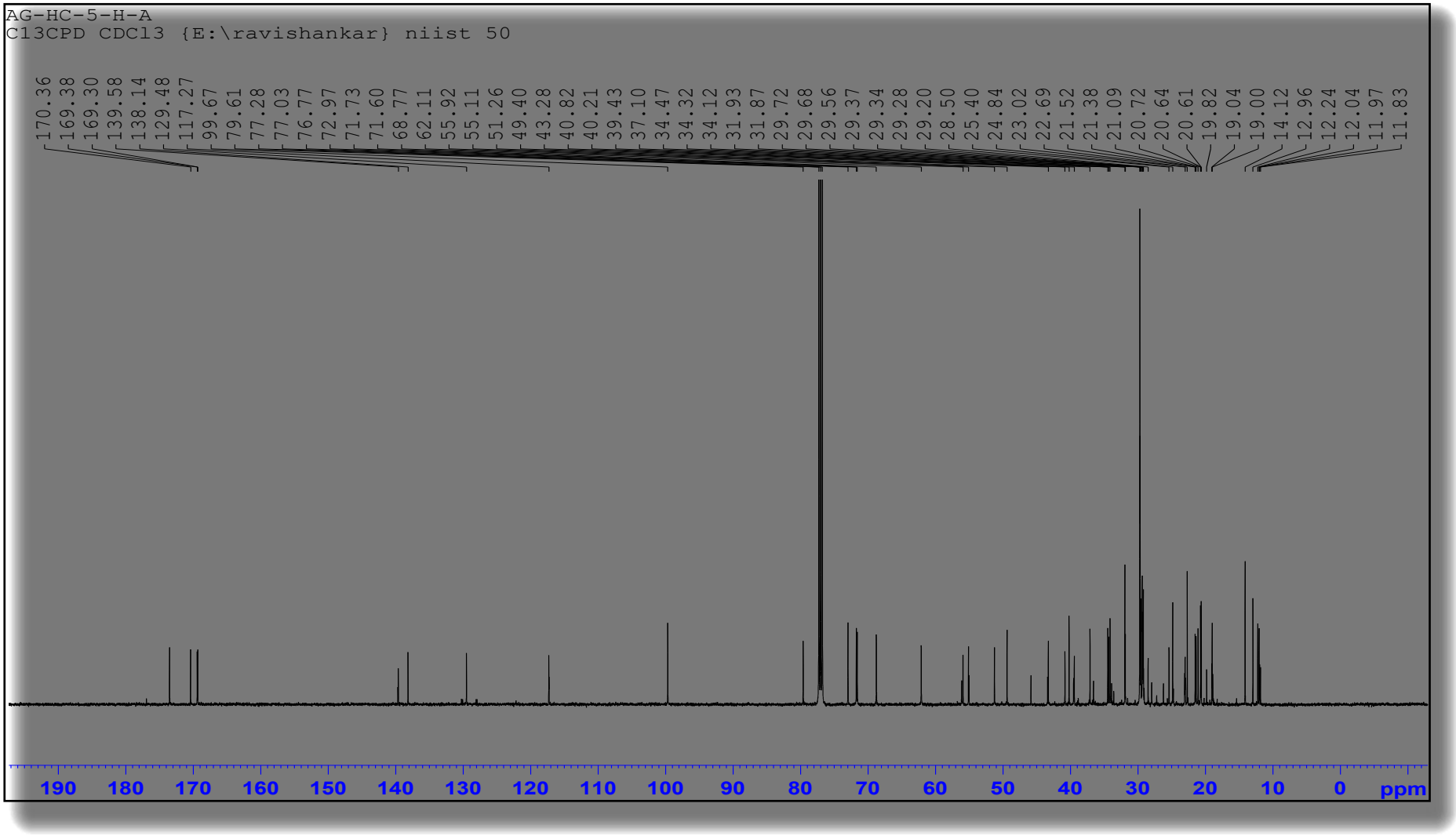

Fig. S13 ^{13}C NMR of peracetylated derivative of compound **3** in $CDCl_3$

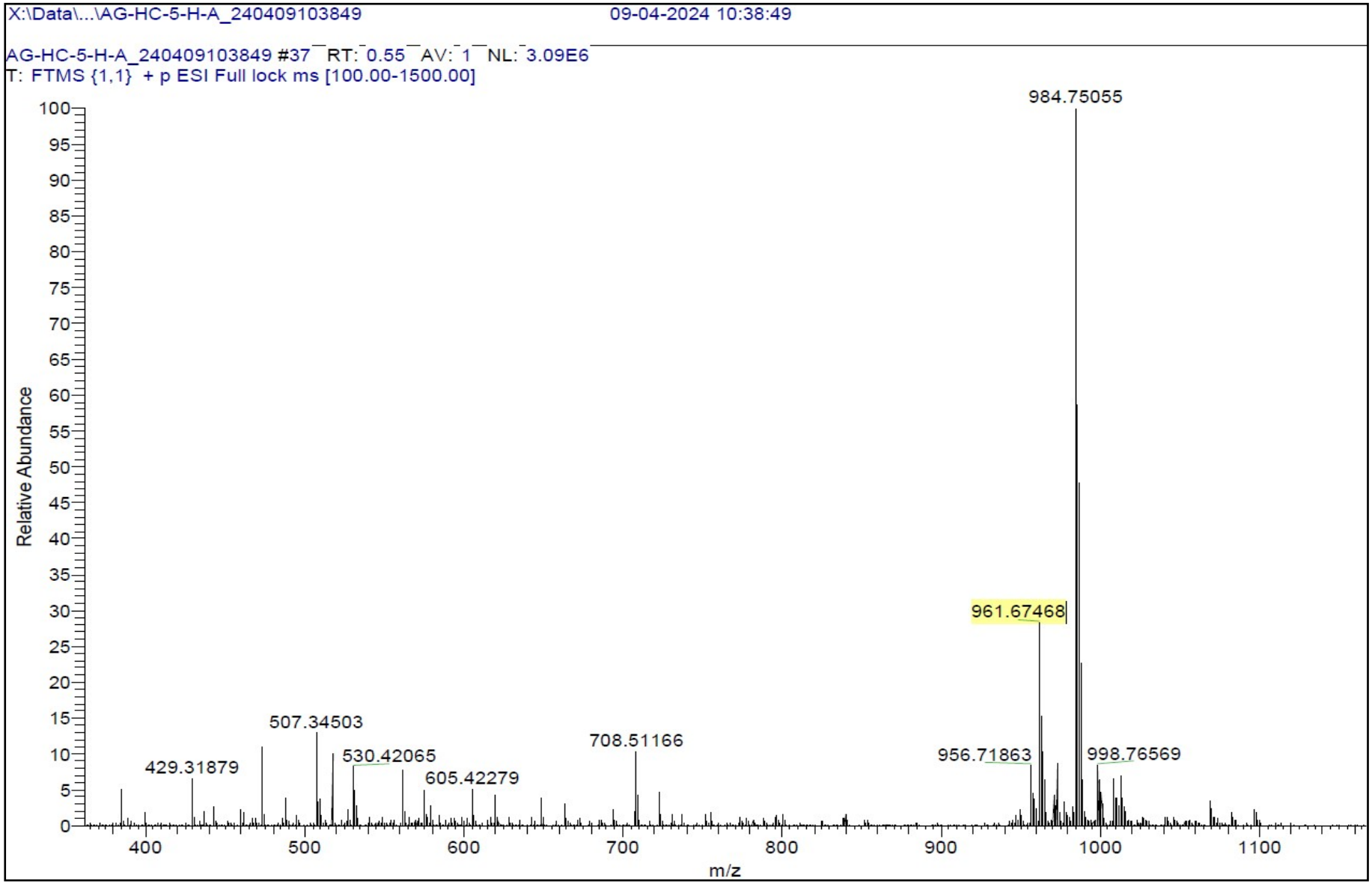

Fig. S14 HR-ESI-MS of peracetylated derivative of compound **3**