Supporting Information

Rec. Nat. Prod. 17:2 (2023) 318-322

Two New Spirostanol Glycosides from the Roots and Rhizomes of *Helleborus thibetanus* Franch.

Yuze Li[1], Zilong Zhang[1], Wenli Huang[1], Huawei Zhang[1], Yi Jiang[1], Jianli Liu[2], Xiaomei Song[1,*], and Dongdong Zhang[1,*]

[1] School of Pharmacy, Shaanxi University of Chinese Medicine, Xianyang 712046, China

[2] Key Laboratory of Resource Biology and Biotechnology in Western China, Ministry of Education, College of Life Science, Northwest University, Xi'an 710069, China

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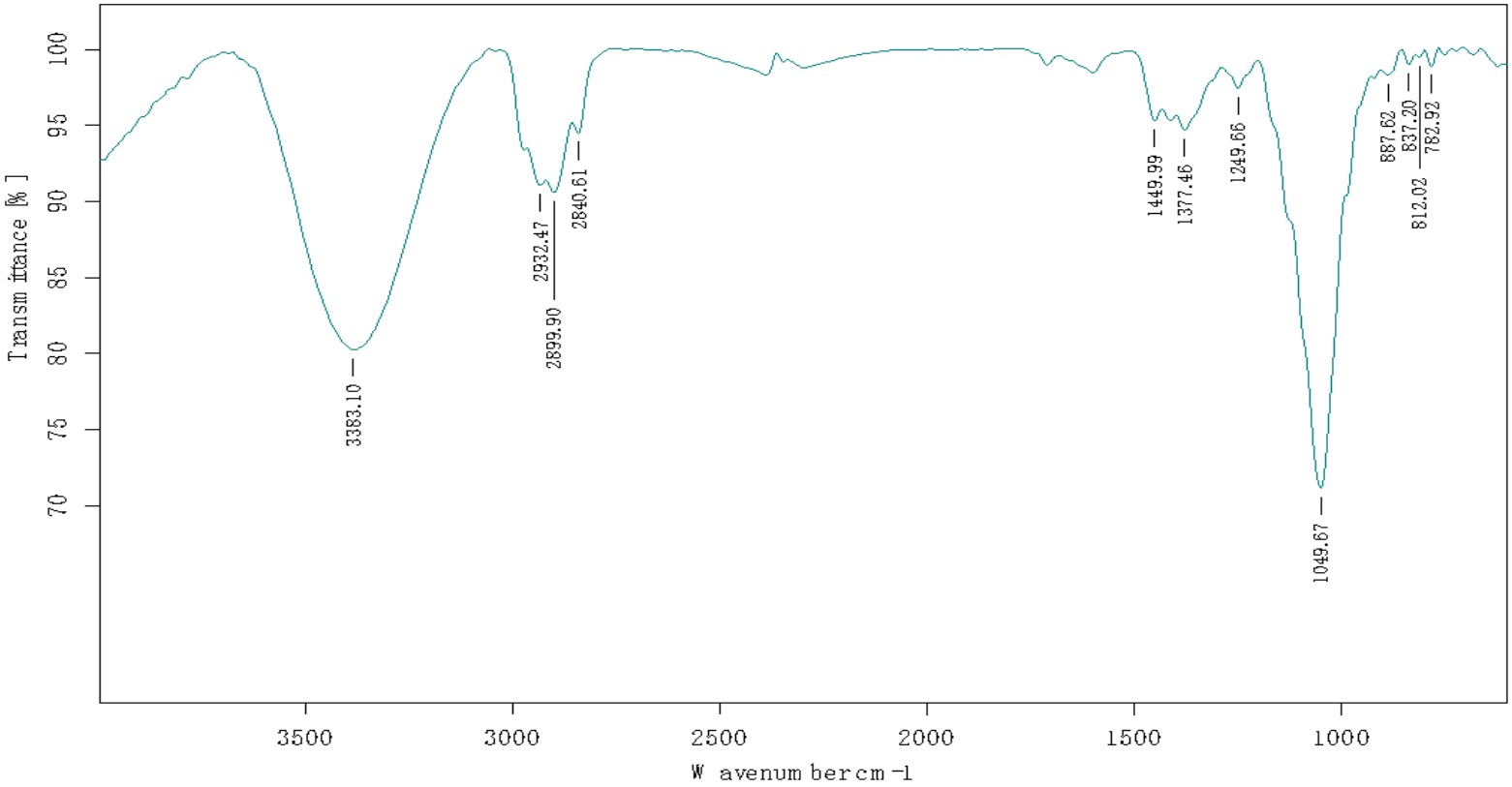

Figure S1: The IR spectrum of **1** (in KBr)

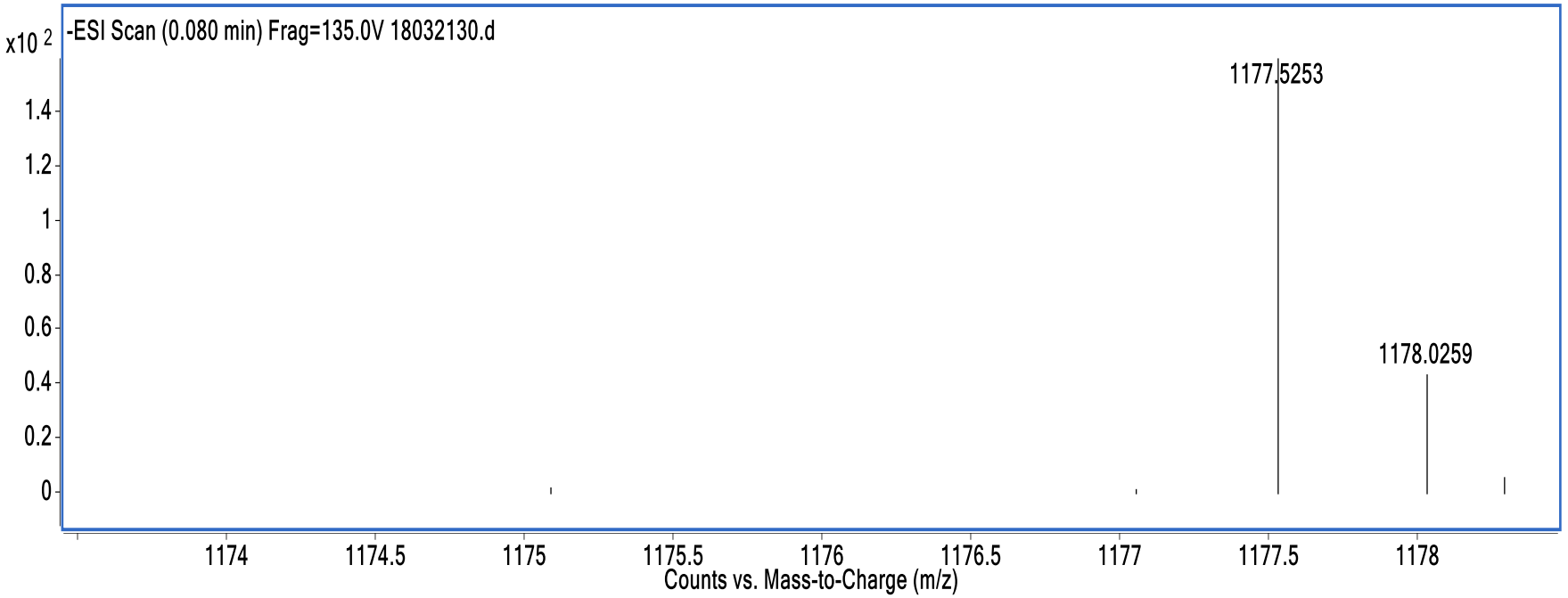

Figure S2: The HR-ESI-MS spectrum of 1 (in MeOH）

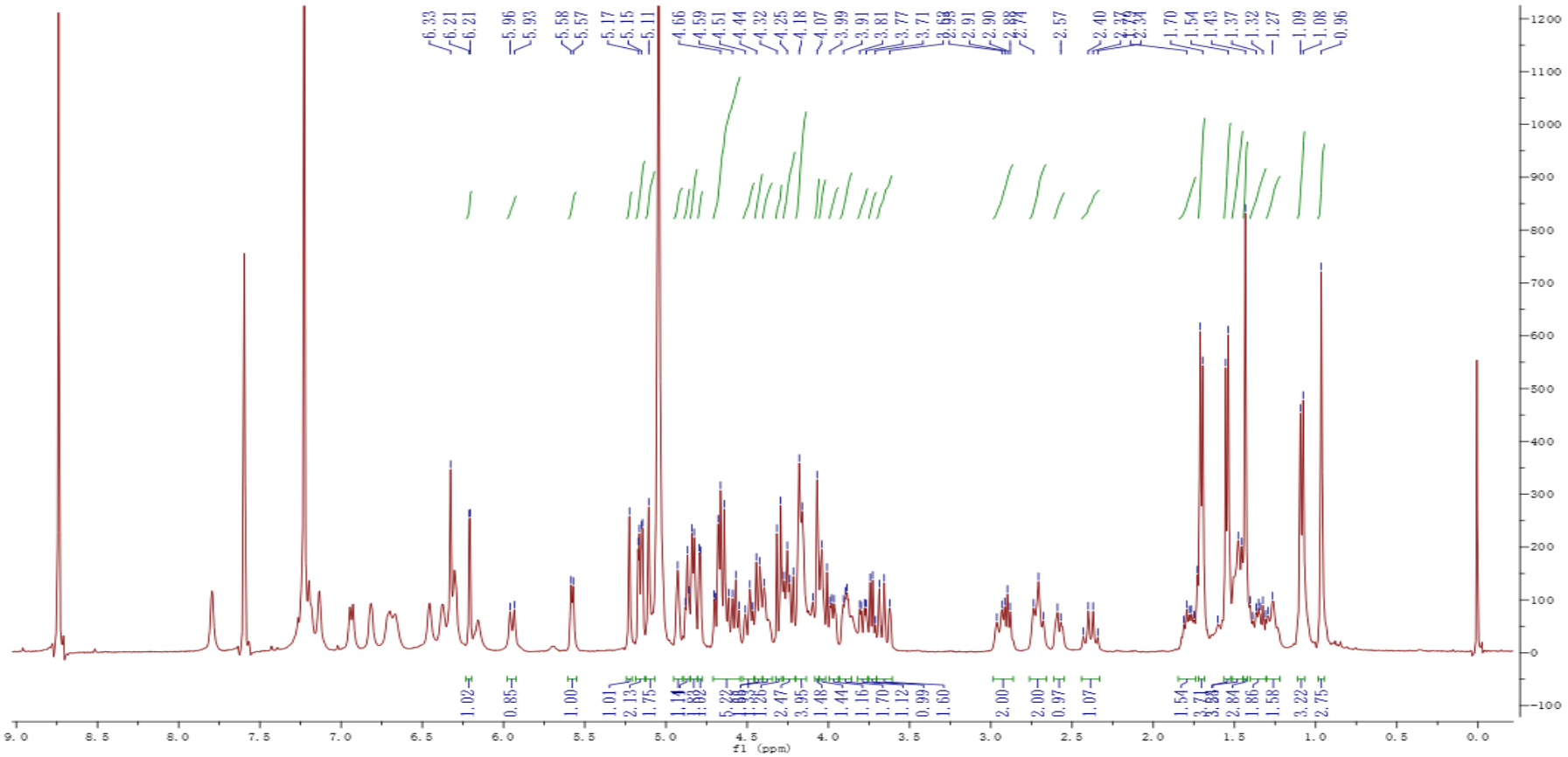

Figure S3: The ^{1}H NMR spectrum of **1** (in C_5D_5N)

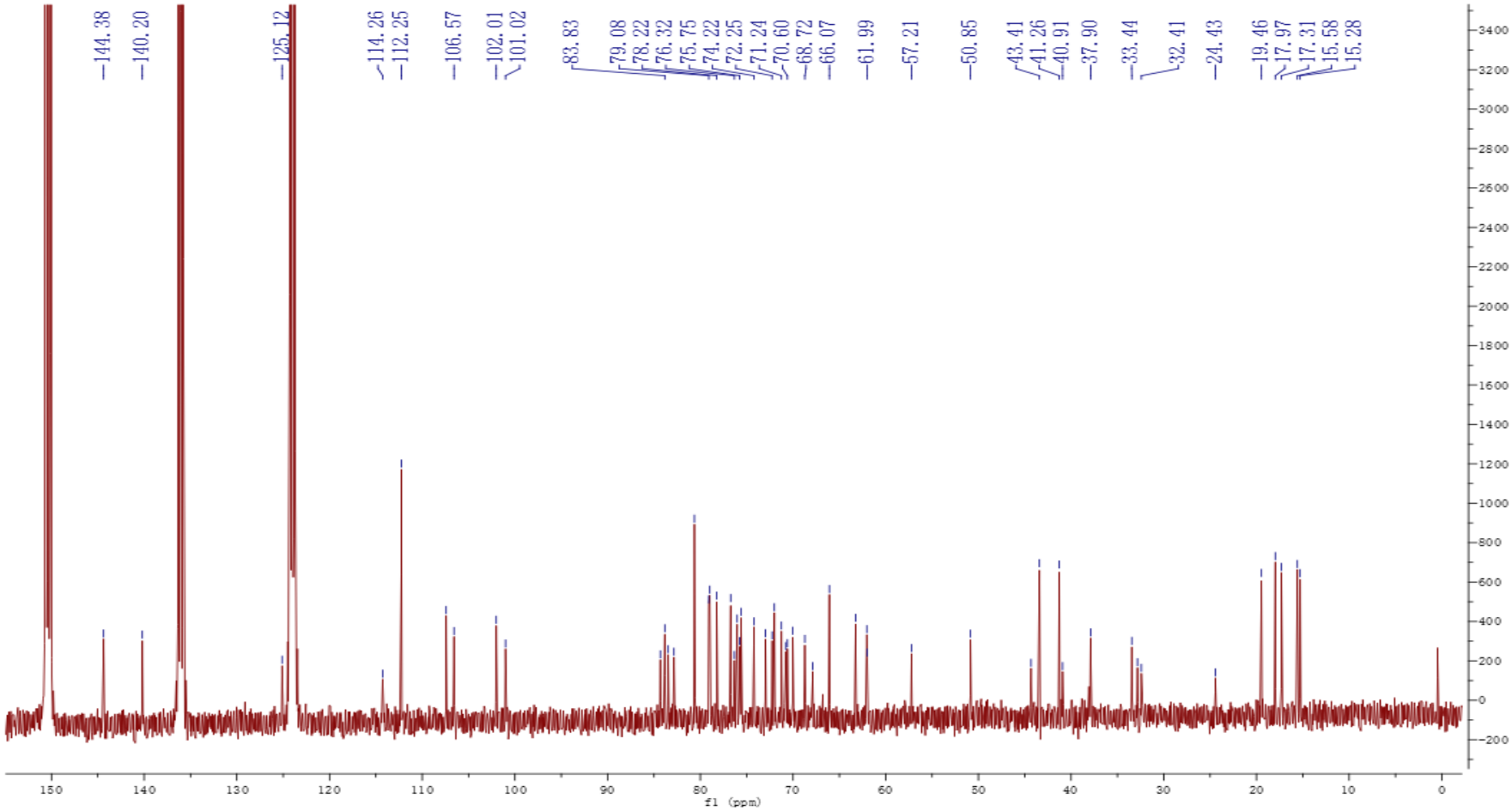

Figure S4: The ^{13}C NMR spectrum of **1** (in C_5D_5N)

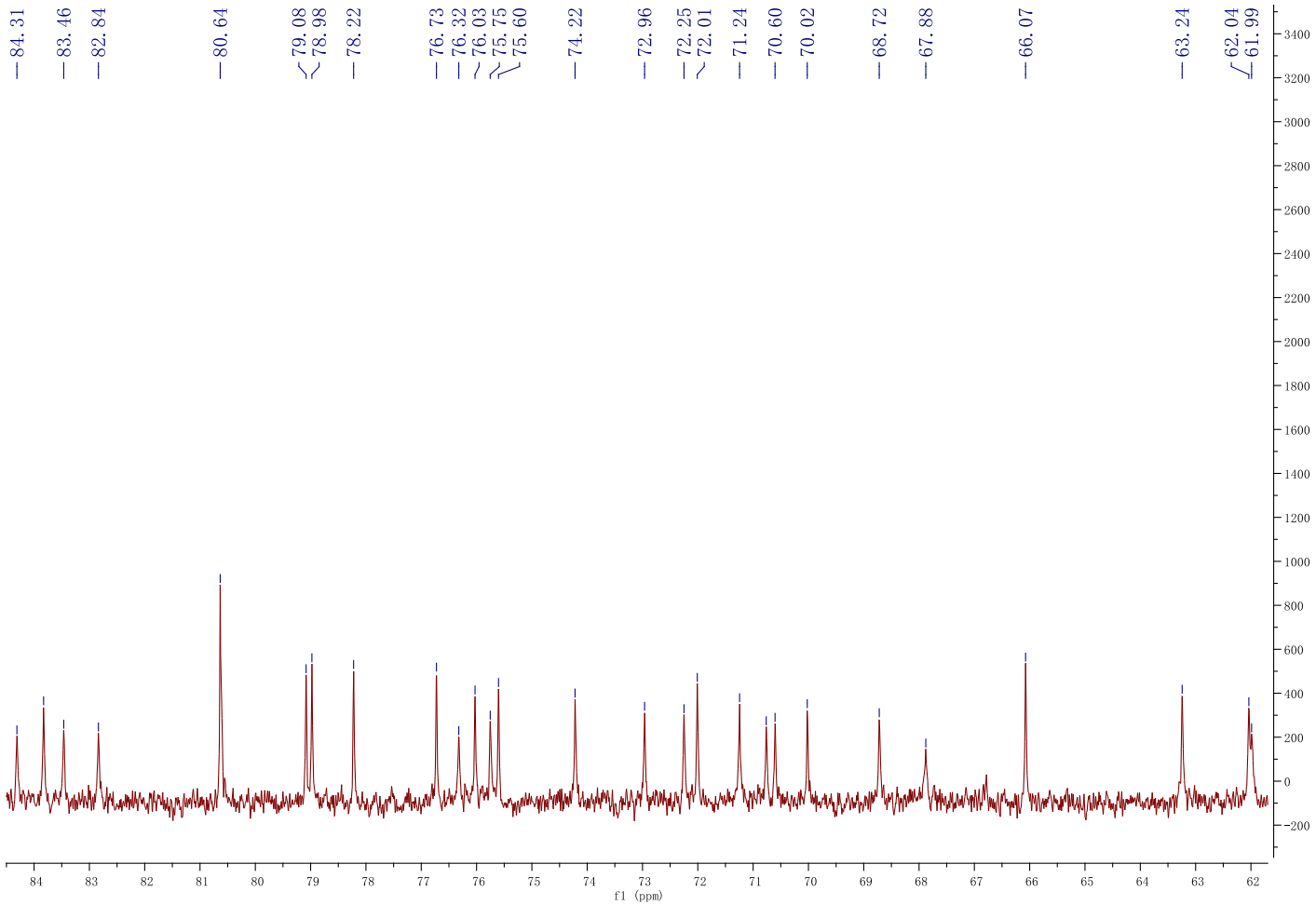

Figure S5: The ^{13}C NMR spectrum of **1** (in C_5D_5N) (From δ_C 60 ppm to δ_C 85 ppm)

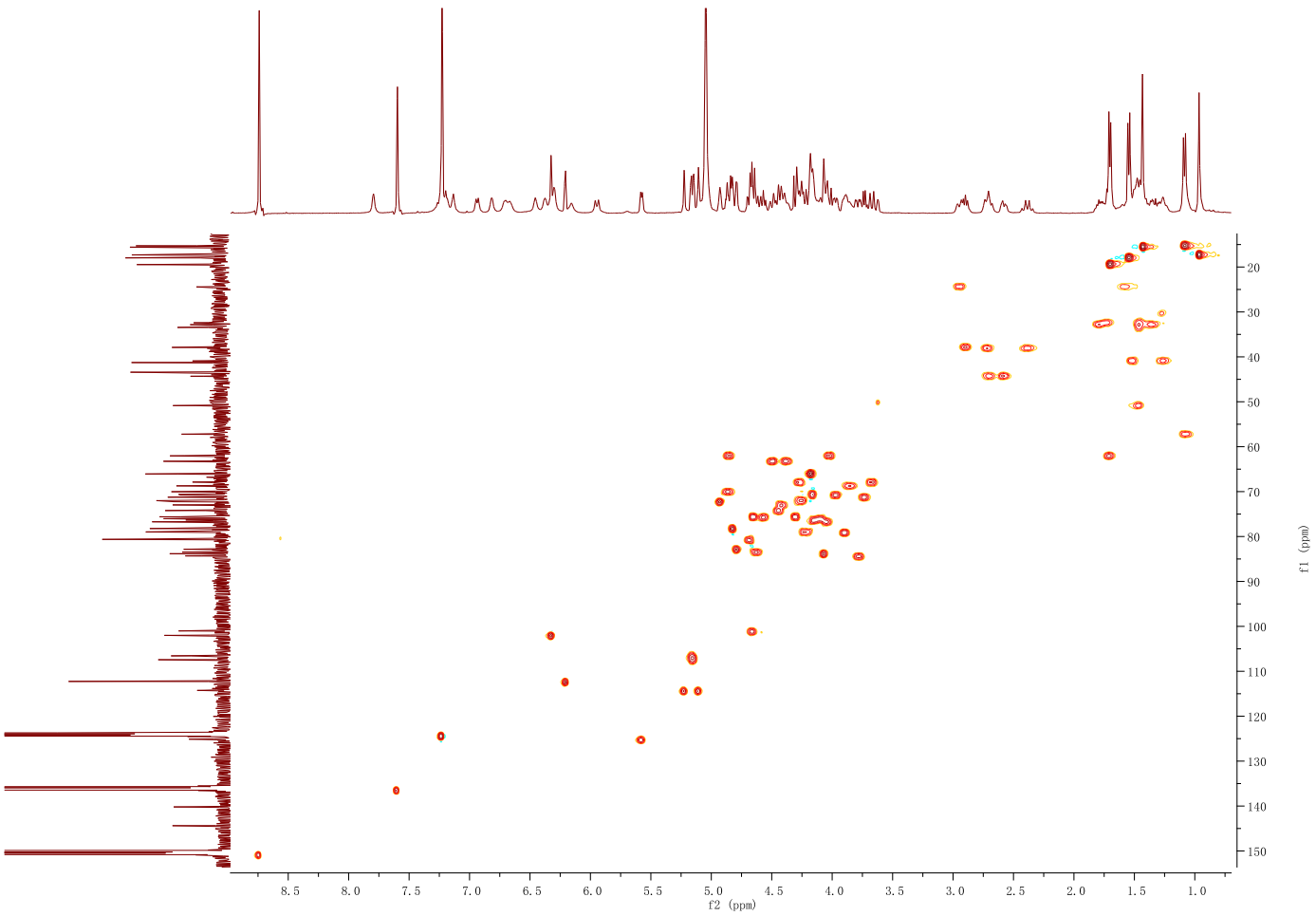

Figure S6: The HSQC spectrum of **1** (in C_5D_5N)

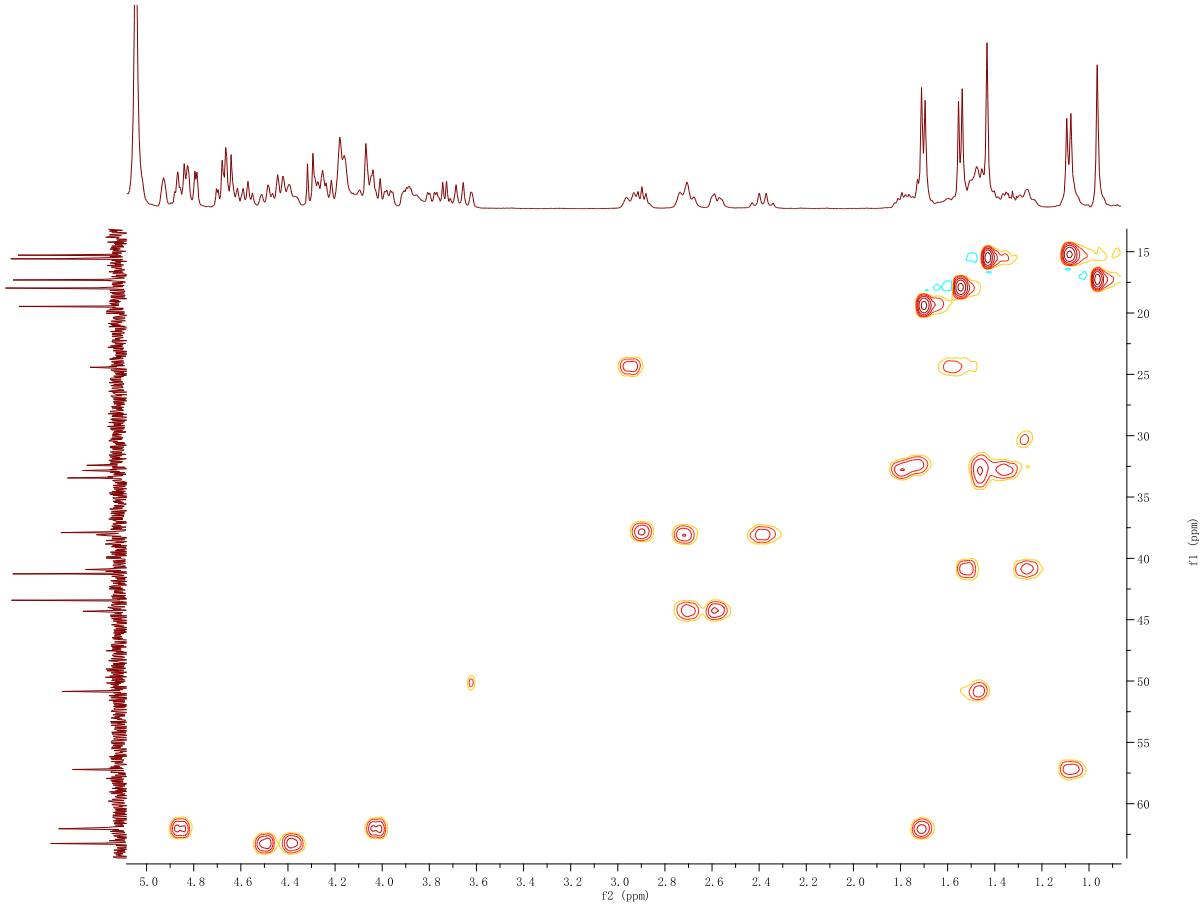

Figure S7: The HSQC spectrum of **1** (in C_5D_5N) (From δ_C 15 ppm to δ_C 65 ppm)

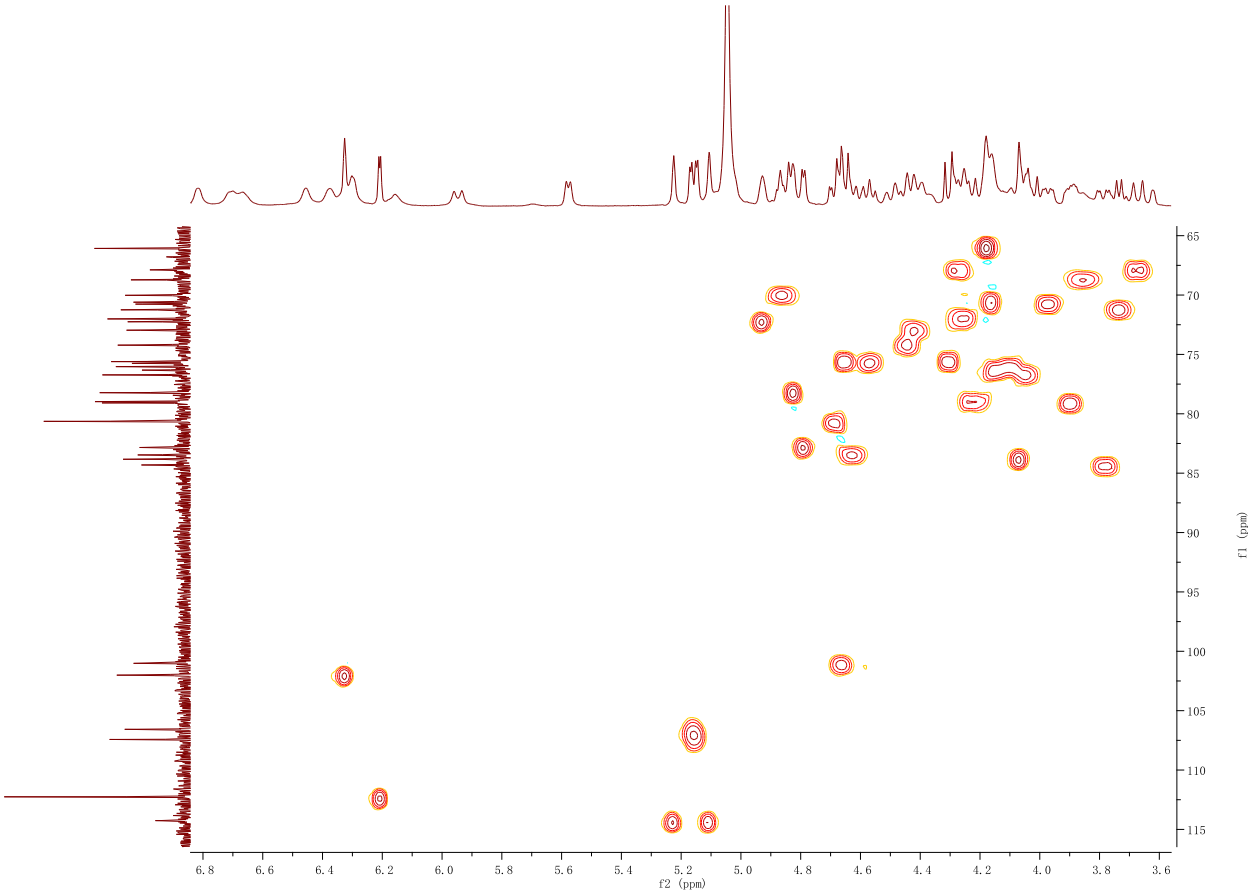

Figure S8: The HSQC spectrum of **1** (in C_5D_5N) (From δ_C 65 ppm to δ_C 115 ppm)

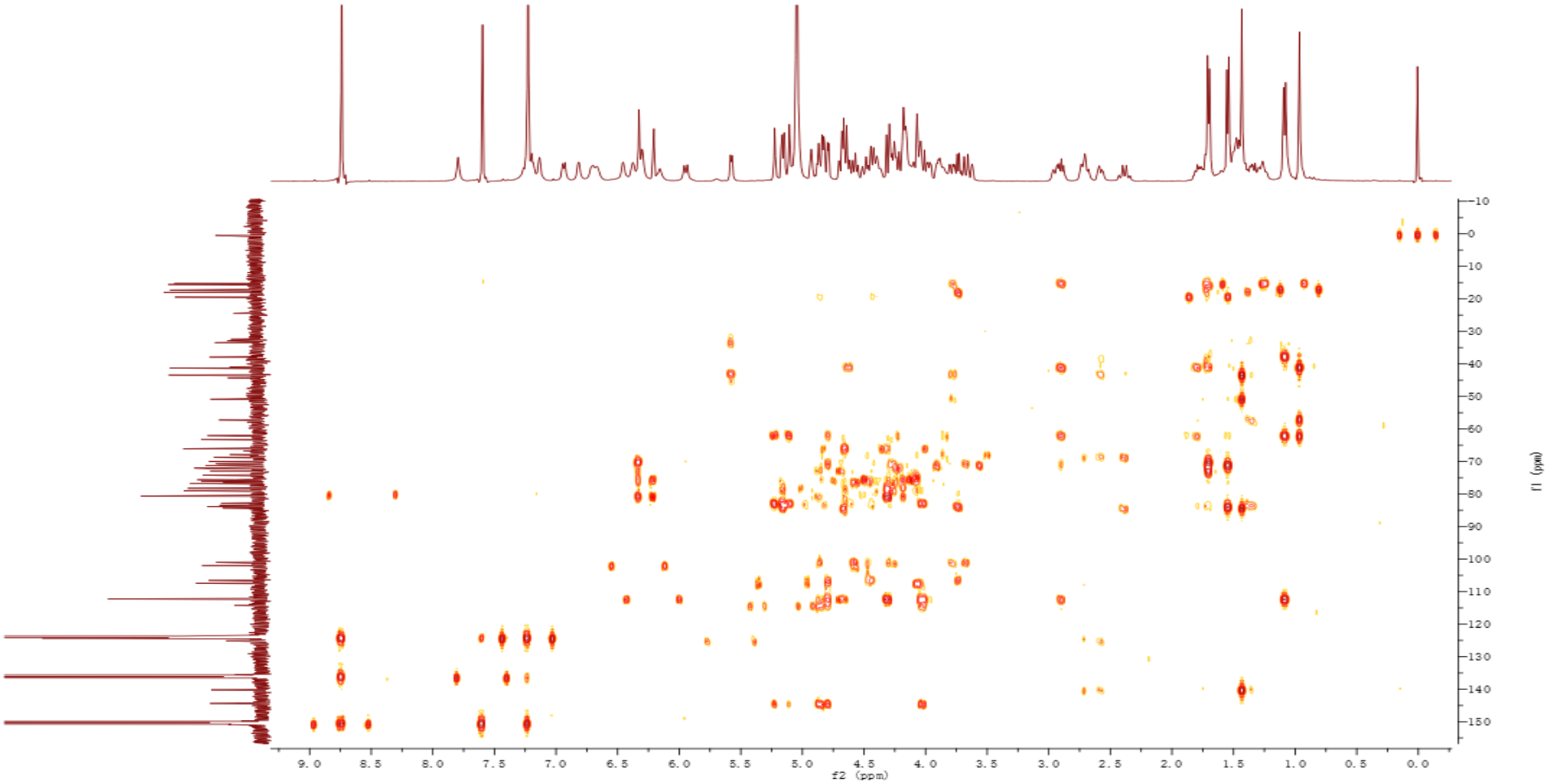

Figure S9: The HMBC spectrum of **1** (in C_5D_5N)

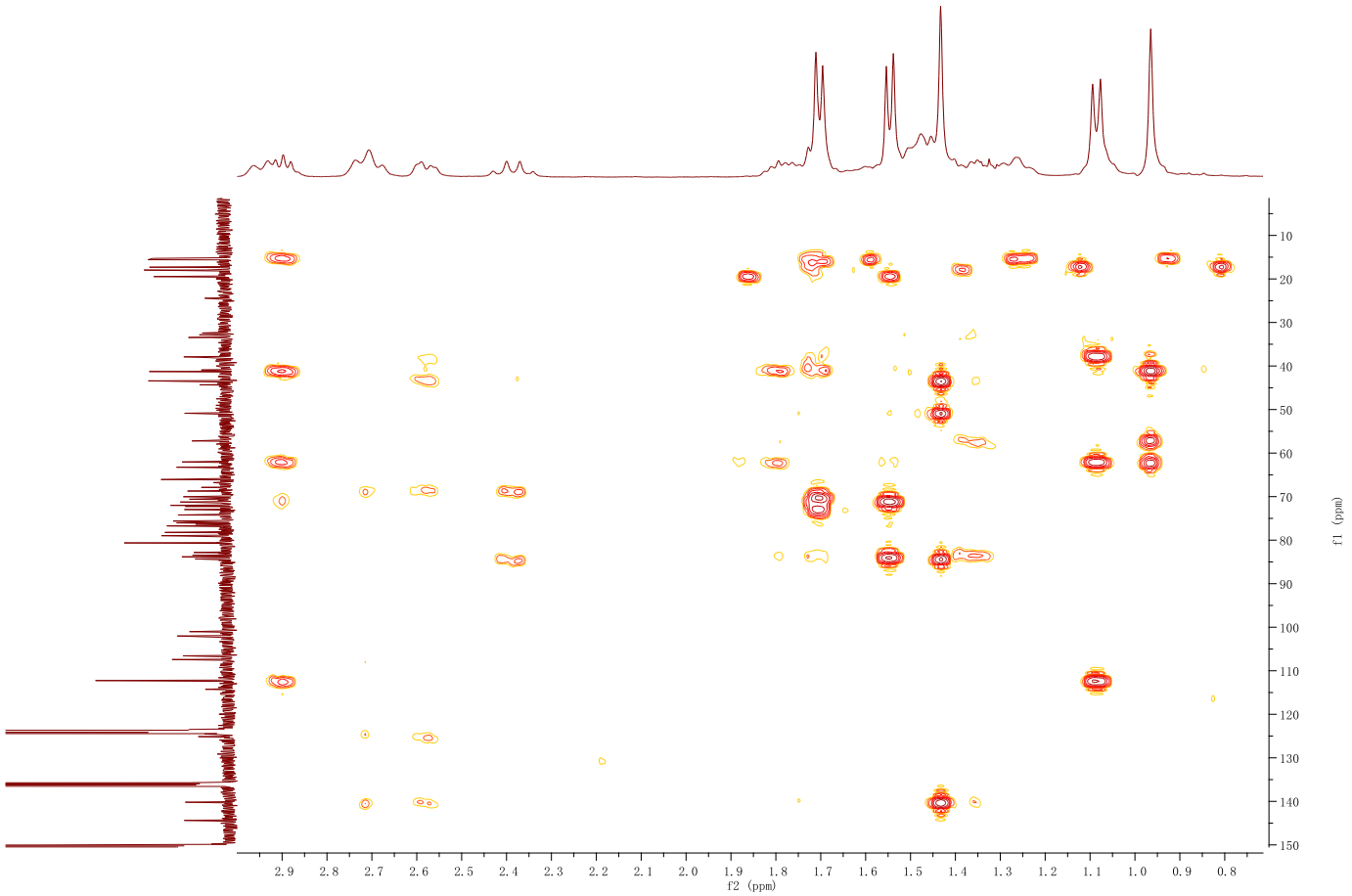

Figure S10: The HMBC spectrum of **1** (in C_5D_5N) (From δ_H 0.8 ppm to δ_H 3.0 ppm)

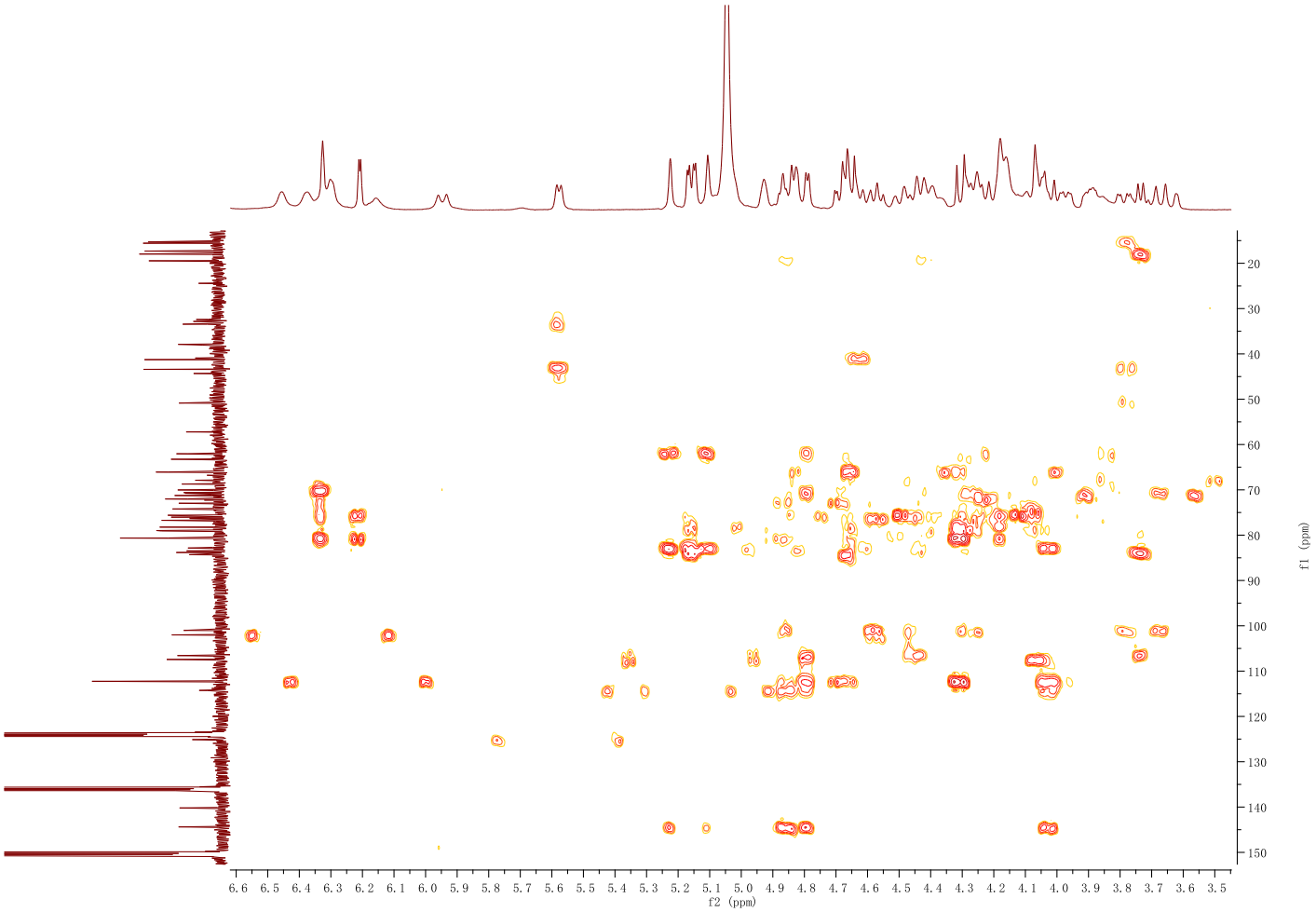

Figure S11: The HMBC spectrum of **1** (in C_5D_5N) (From δ_H 3.3 ppm to δ_H 6.6 ppm)

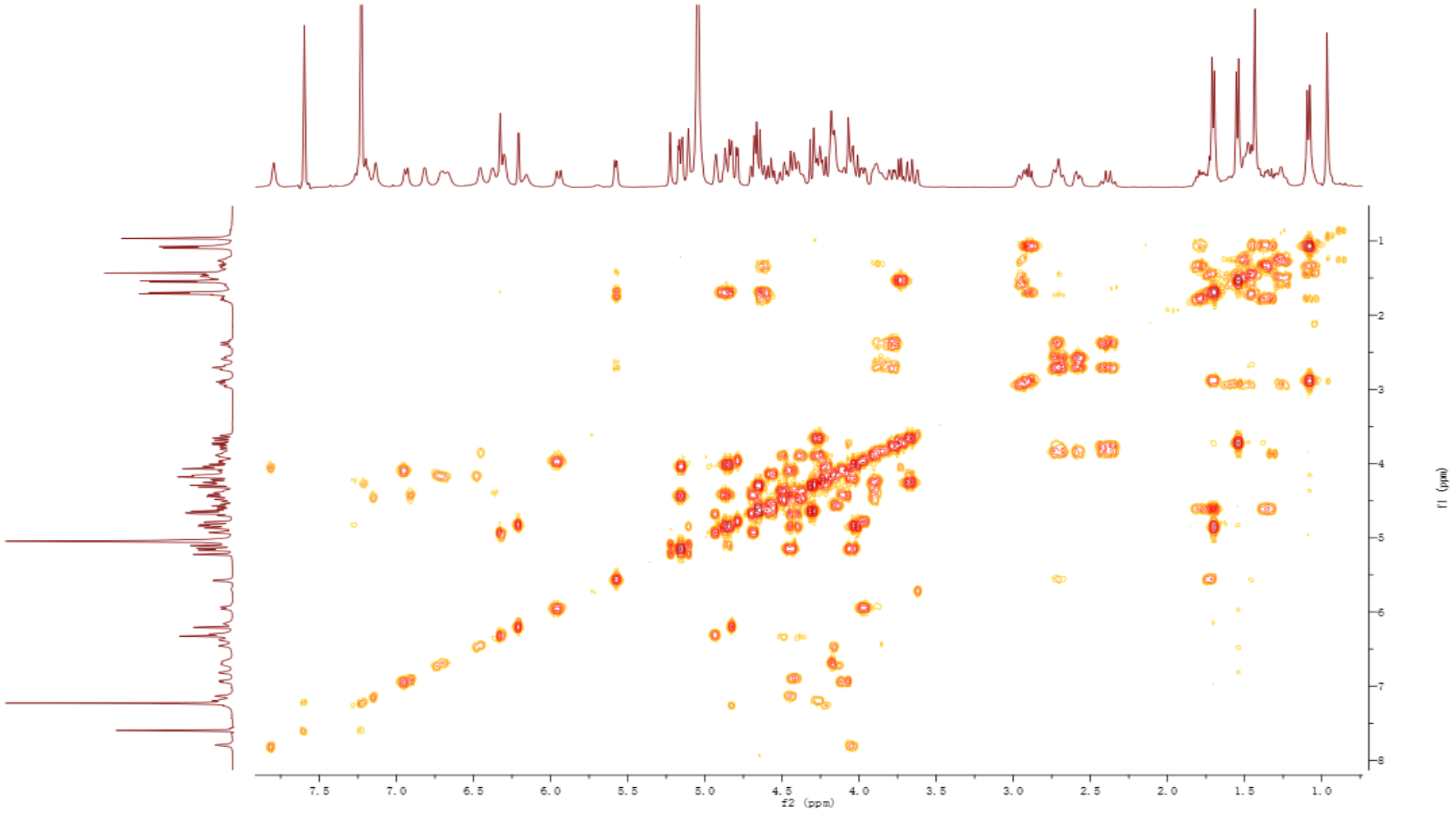

Figure S12: The ^{1}H-^{1}H COSY spectrum of **1** (in C_5D_5N)

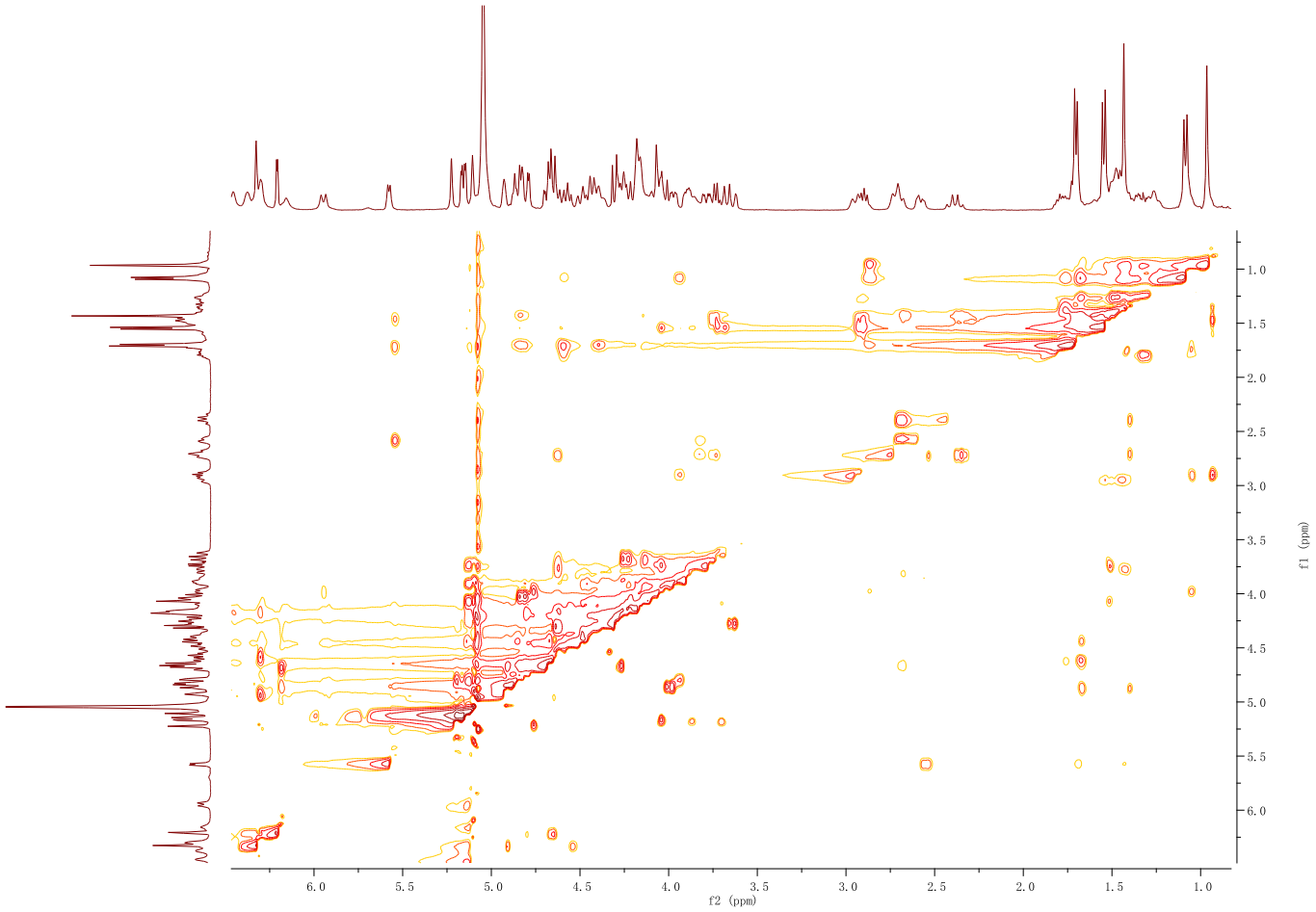

Figure S13: The NOESY spectrum of **1** (in C_5D_5N)

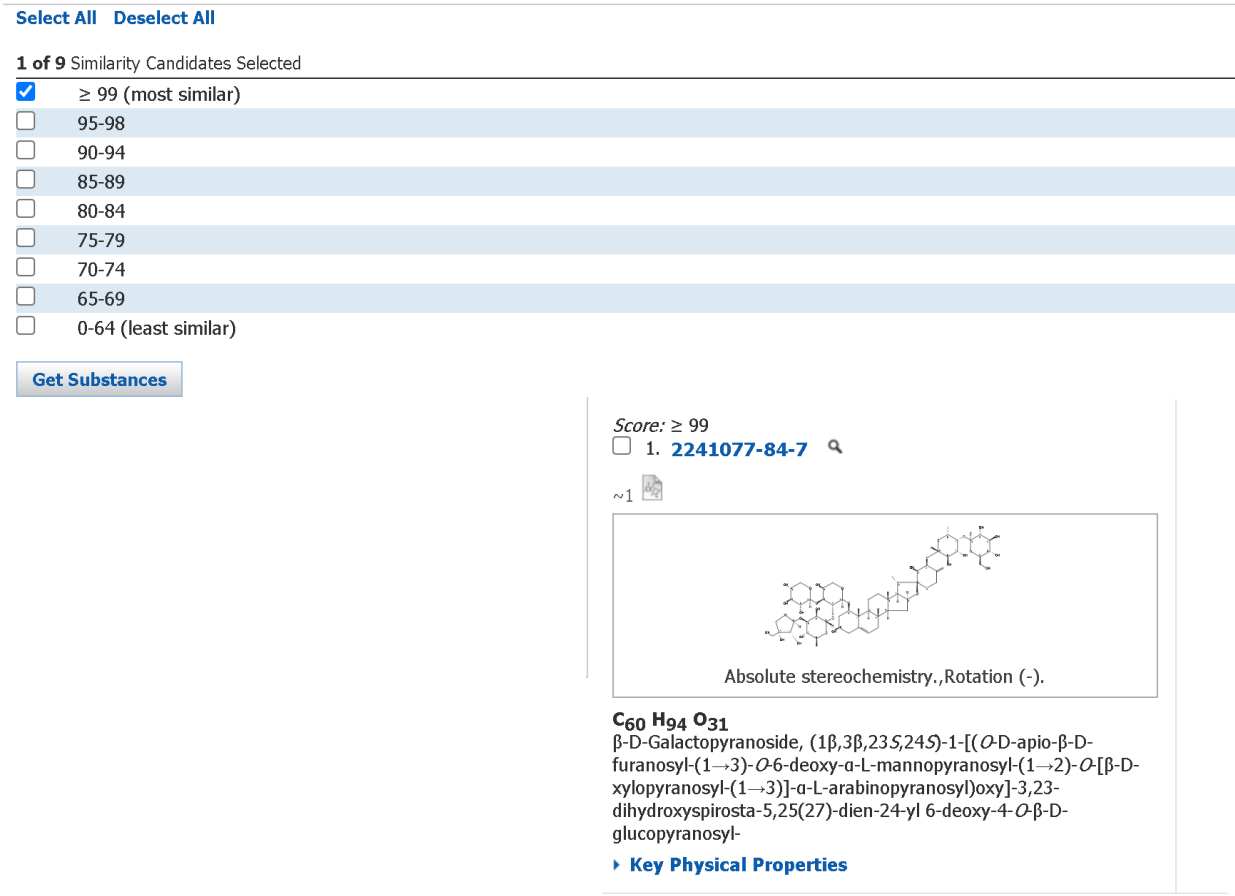

Figure S14: New compound search report of SciFinder

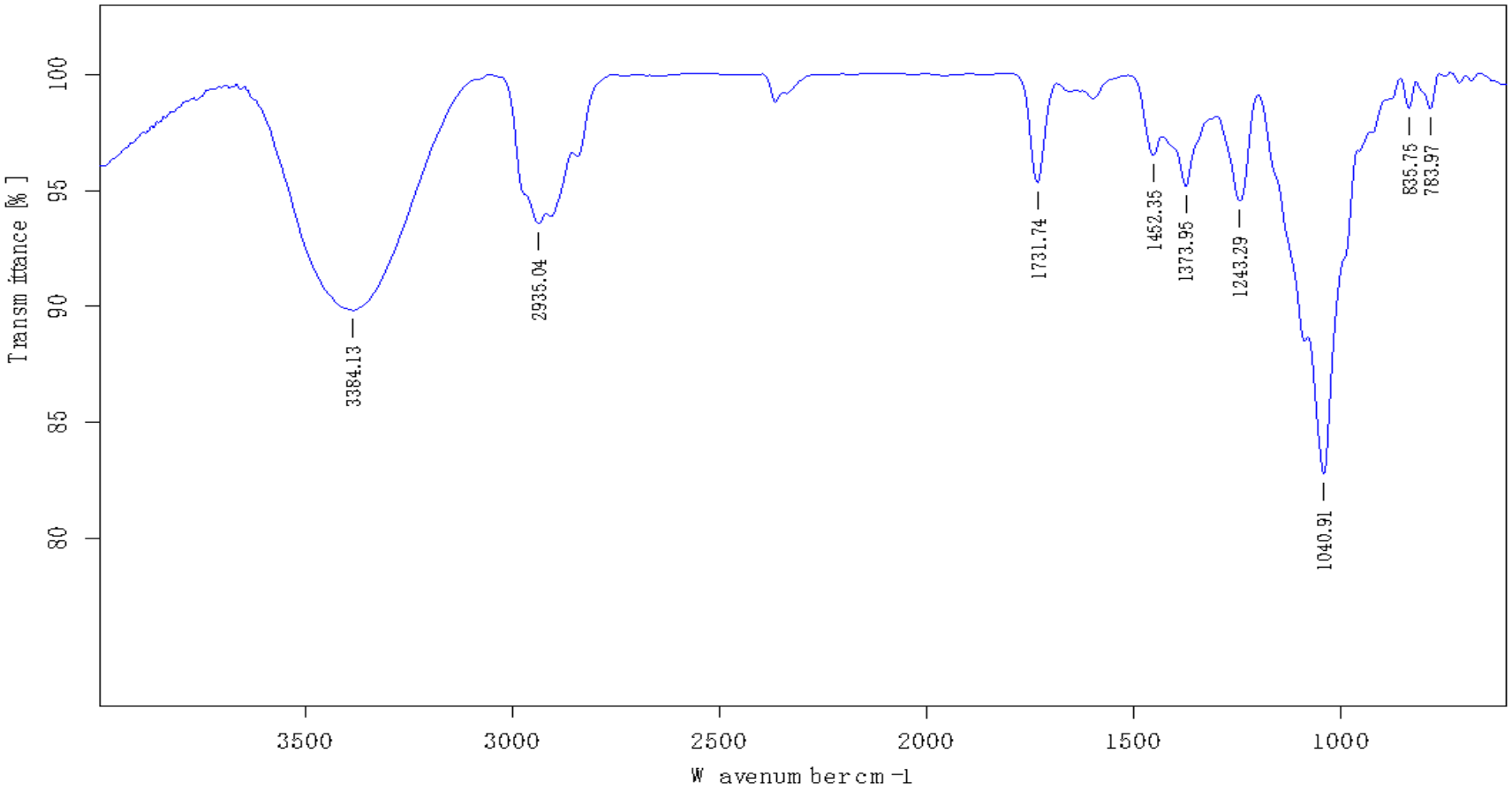

Figure S15: The IR spectrum of **2** (in KBr)

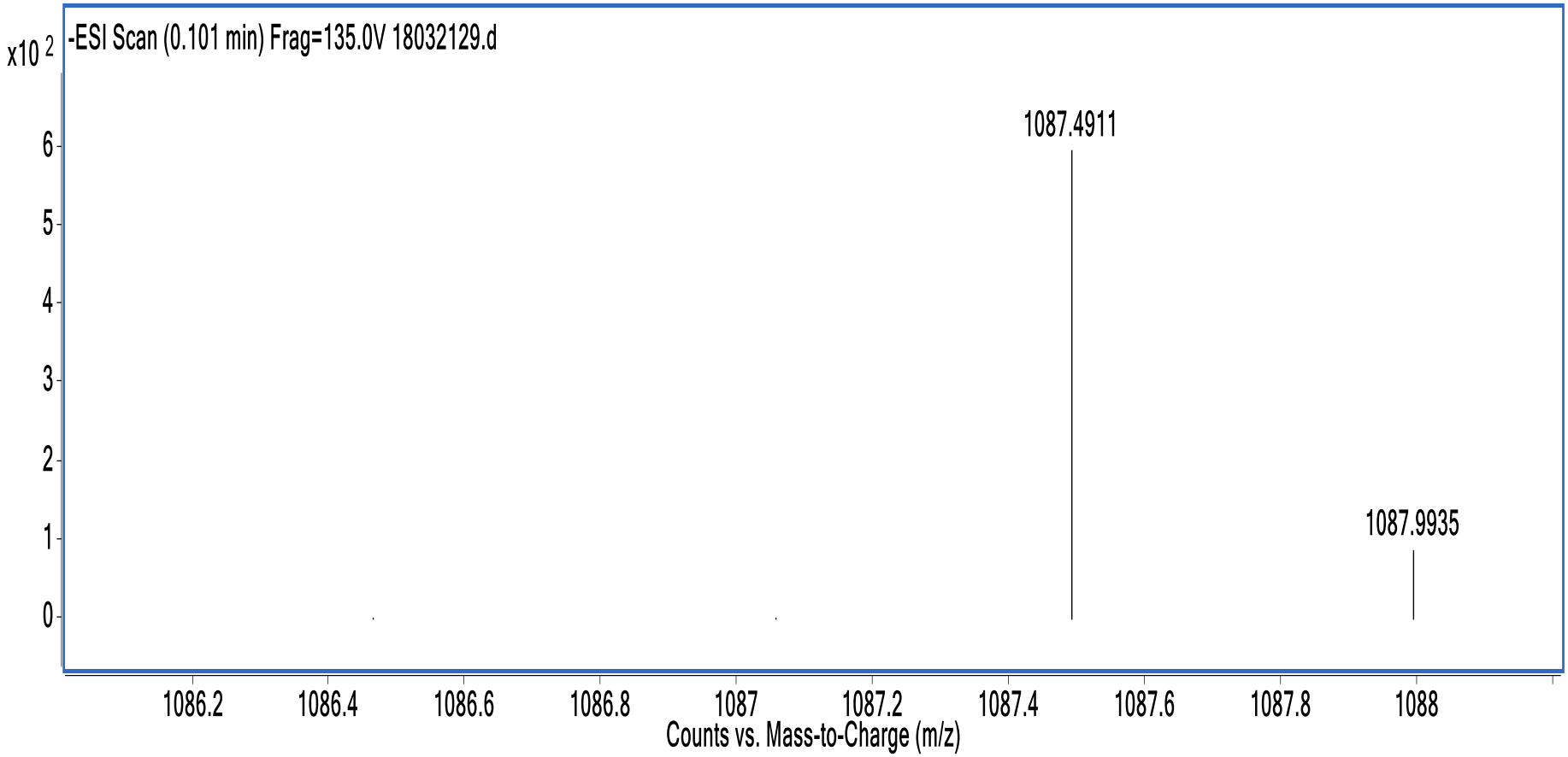

Figure S16: The HR-ESI-MS spectrum of **2** (in MeOH)

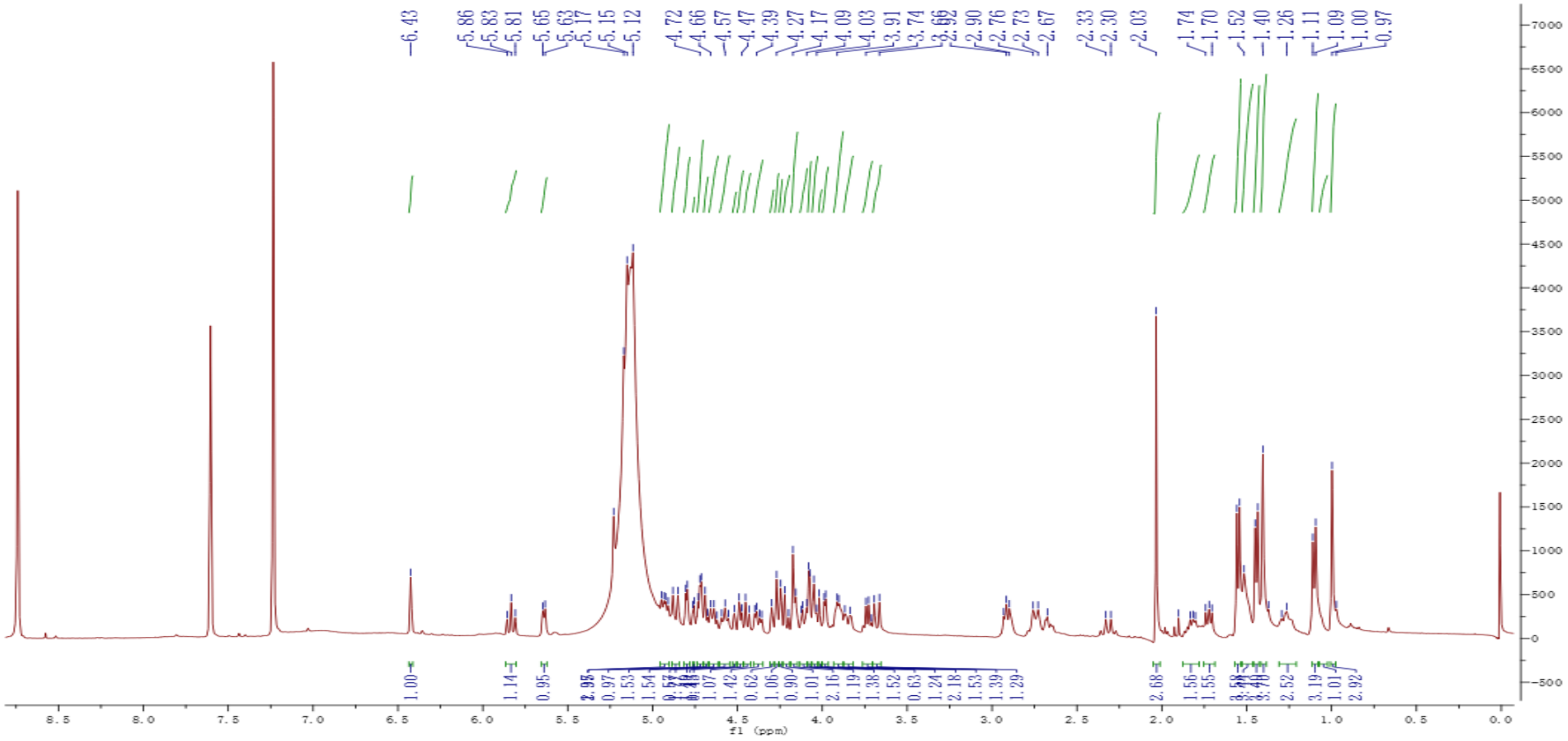

Figure S17: The ^{1}H NMR spectrum of **2** (in C_5D_5N)

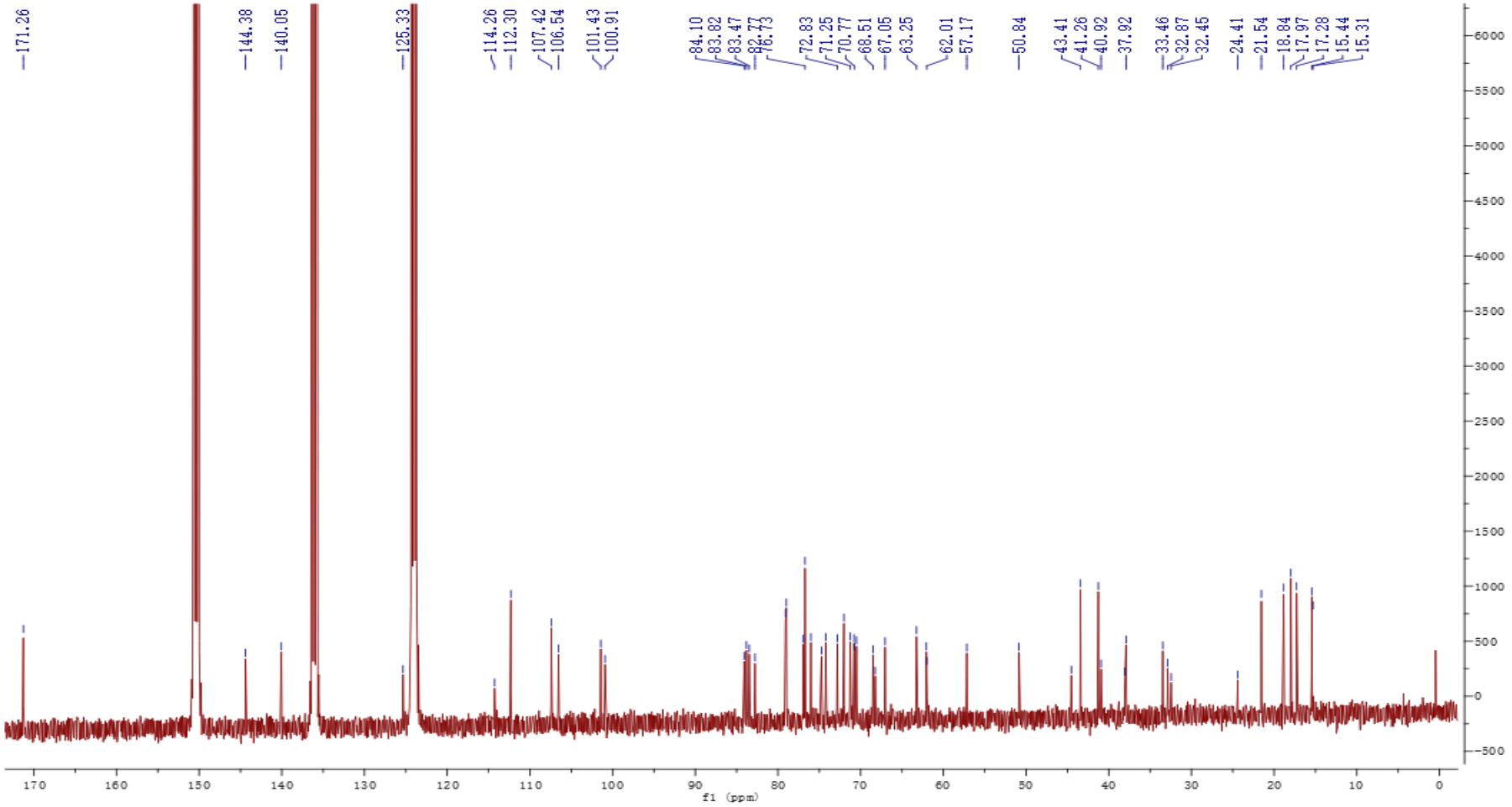

Figure S18: The ^{13}C NMR spectrum of **2** (in C_5D_5N)

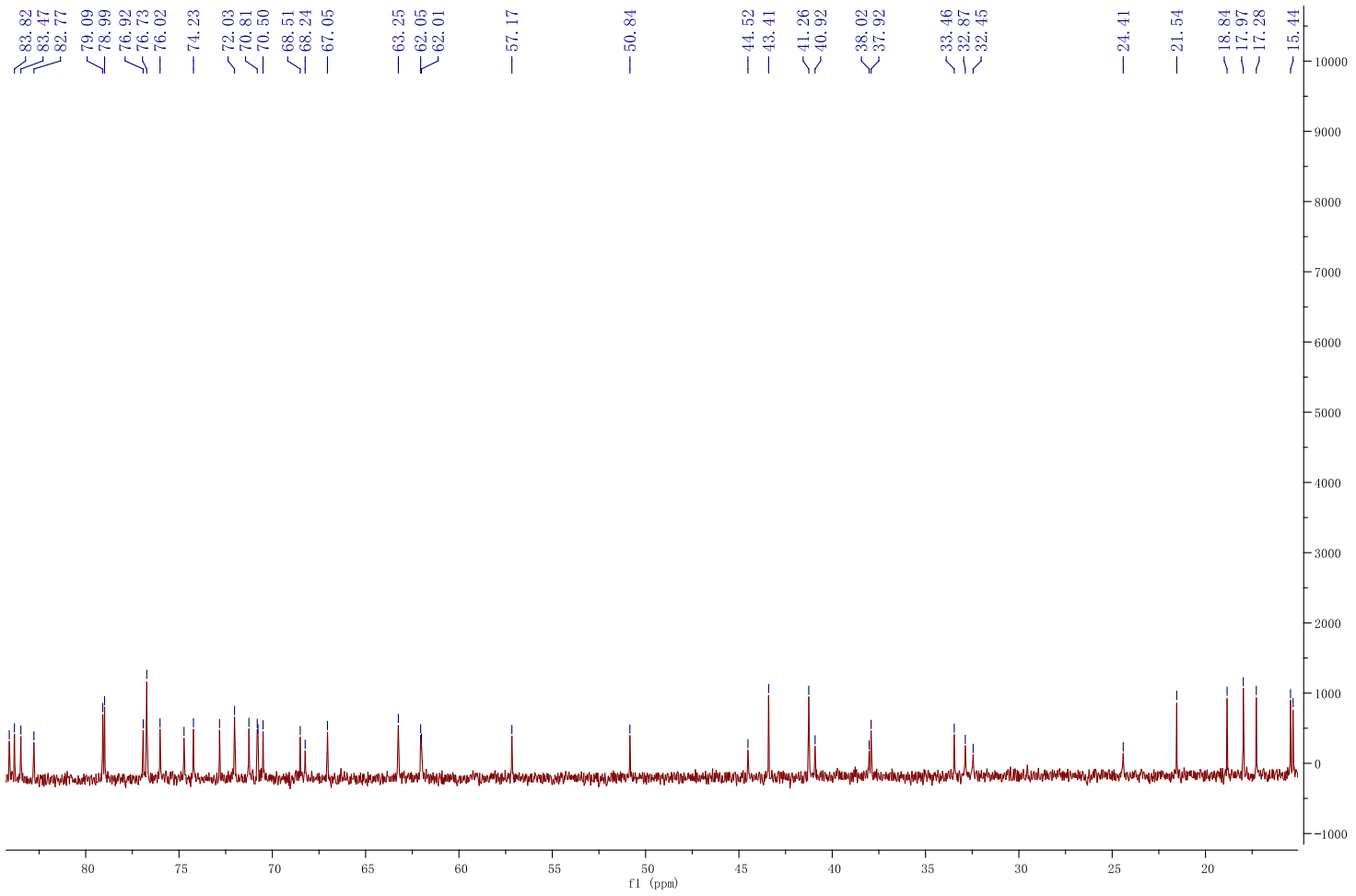

Figure S19: The ^{13}C NMR spectrum of **2** (in C_5D_5N) (From δ_C 15 ppm to δ_C 85 ppm)

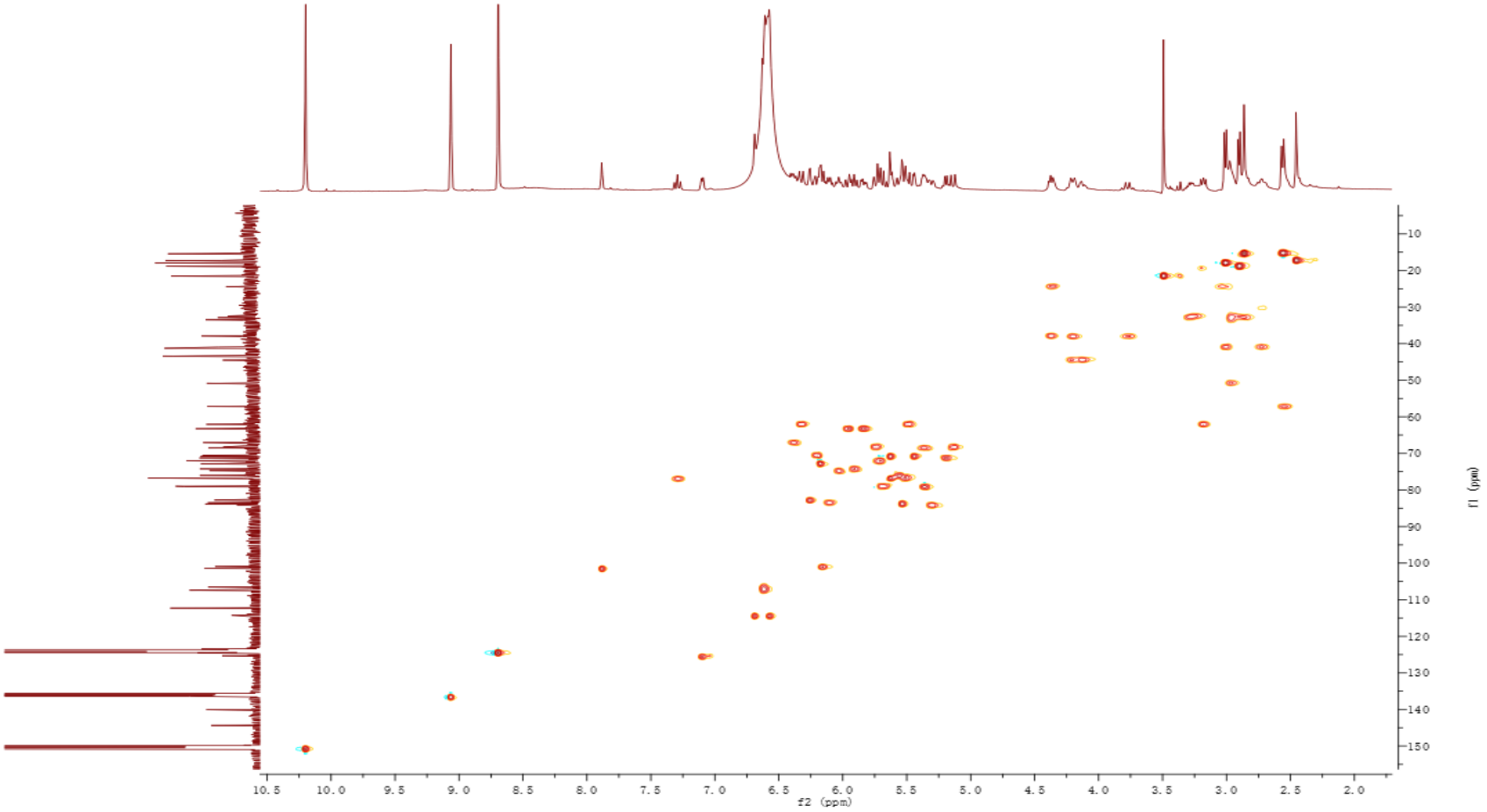

Figure S20: The HSQC spectrum of **2** (in C_5D_5N)

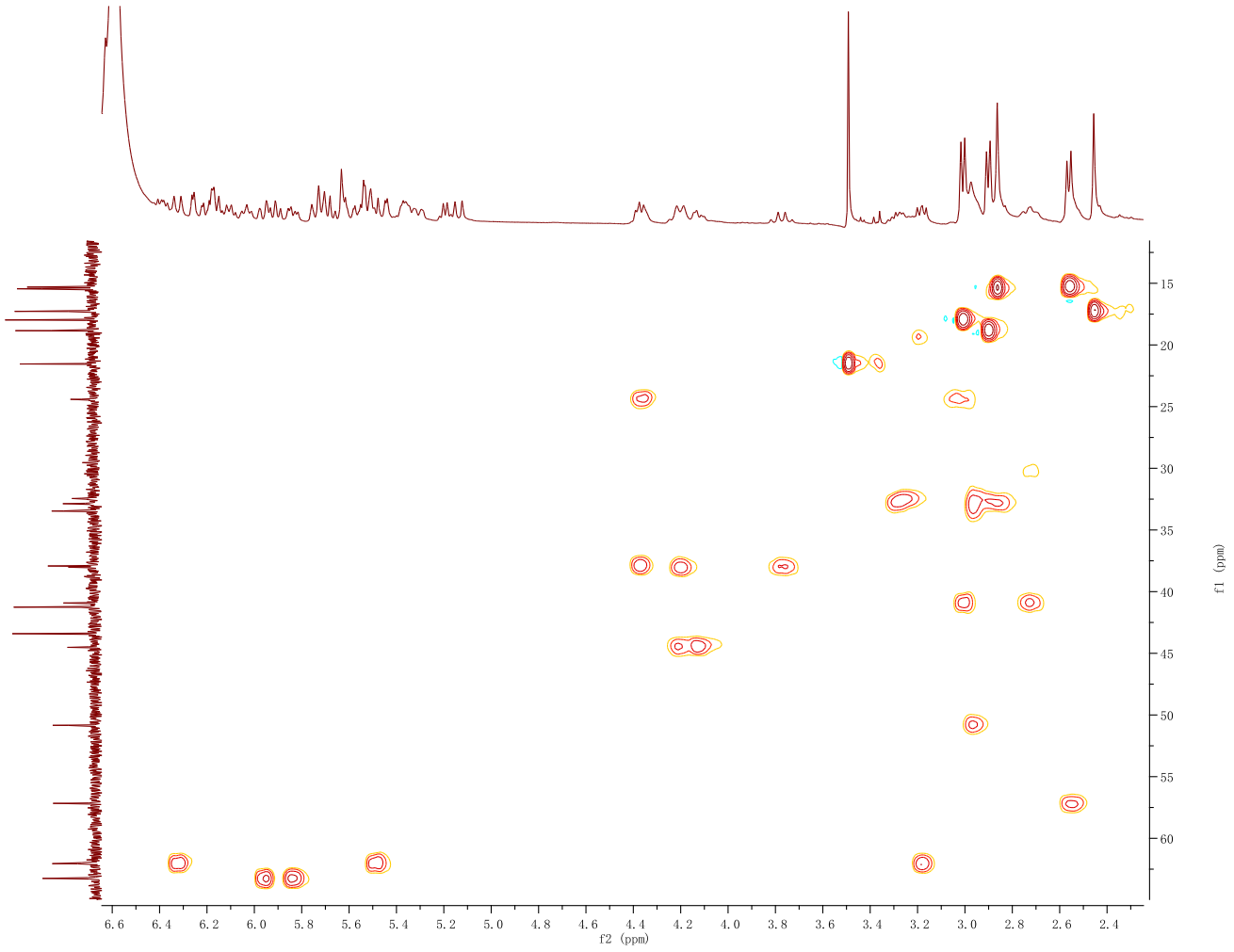

Figure S21: The HSQC spectrum of **2** (in C_5D_5N) (From δ_C 10 ppm to δ_C 65 ppm)

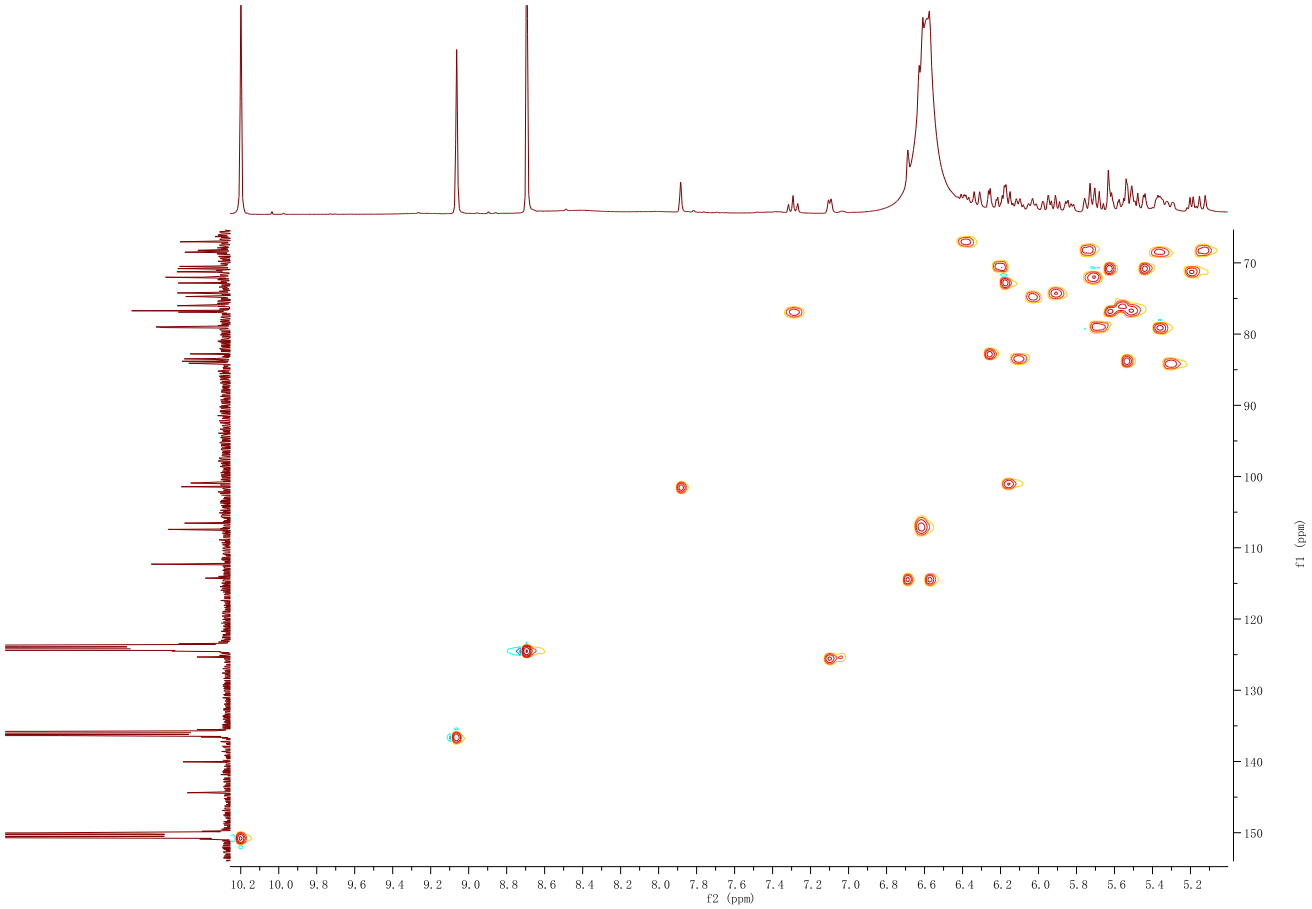

Figure S22: The HSQC spectrum of **2** (in C_5D_5N) (From δ_C 60 ppm to δ_C 125 ppm)

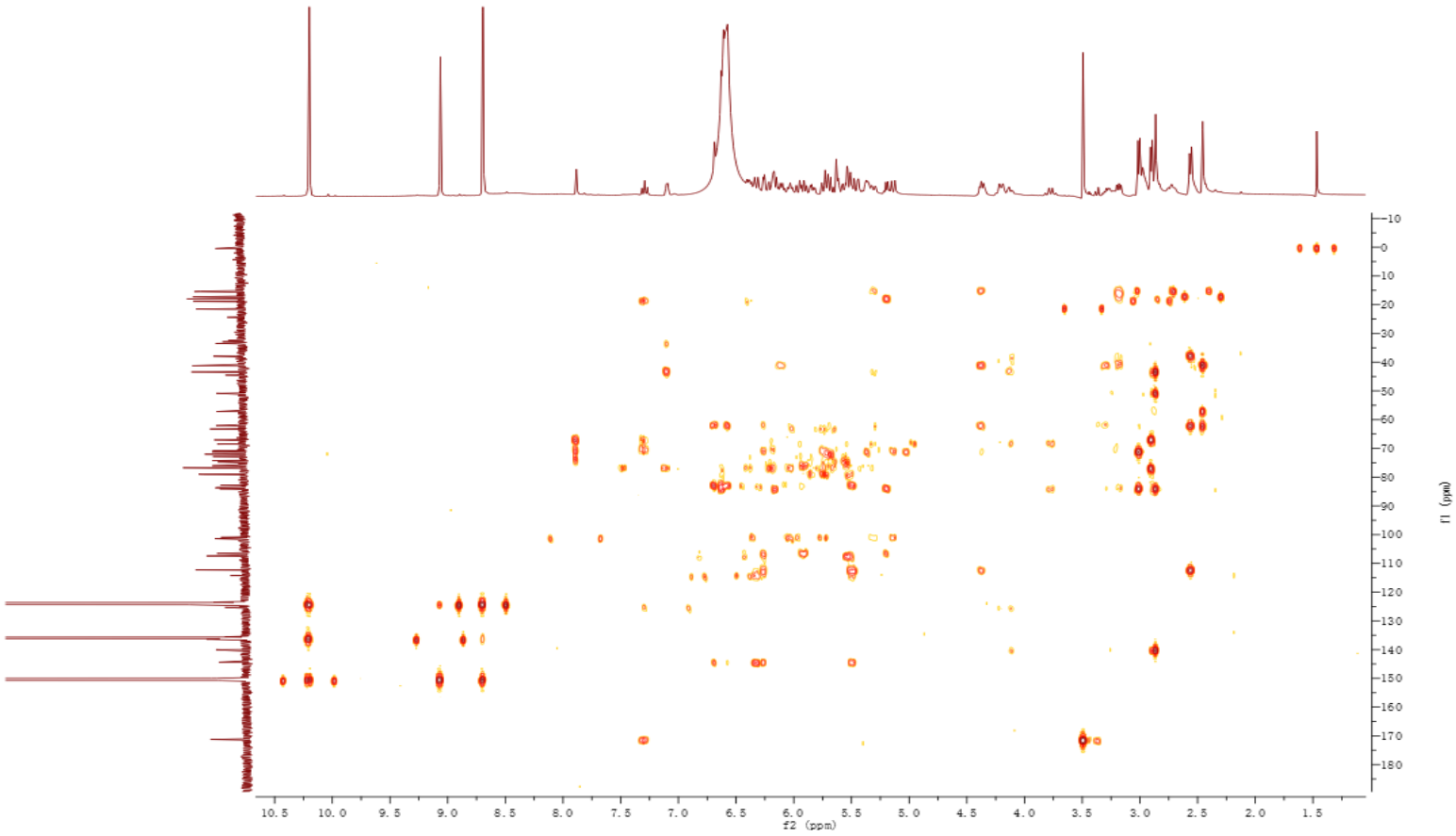

Figure S23: The HMBC spectrum of **2** (in C_5D_5N)

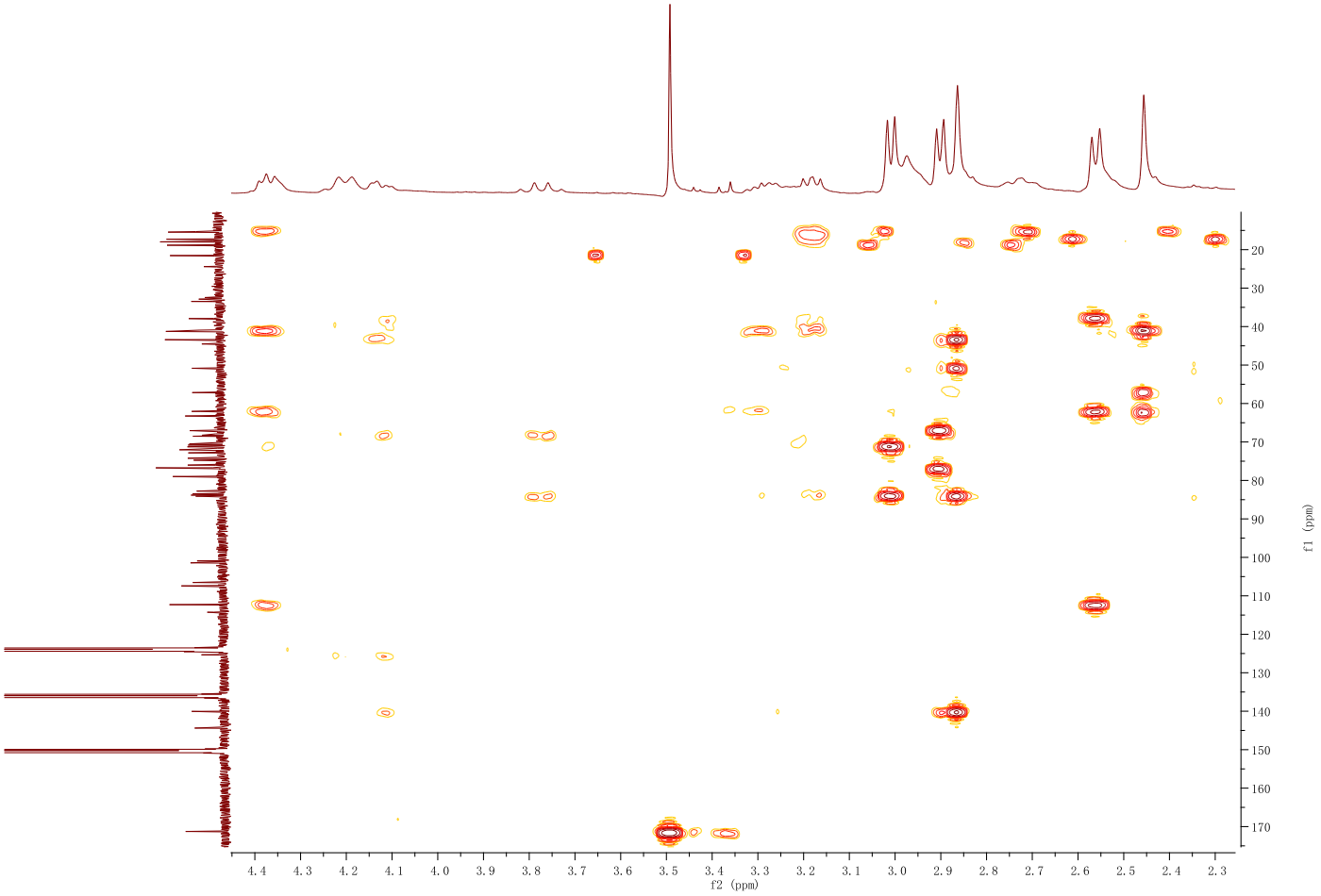

Figure S24: The HMBC spectrum of **2** (in C_5D_5N) (From δ_H 2.2 ppm to δ_H 4.5 ppm)

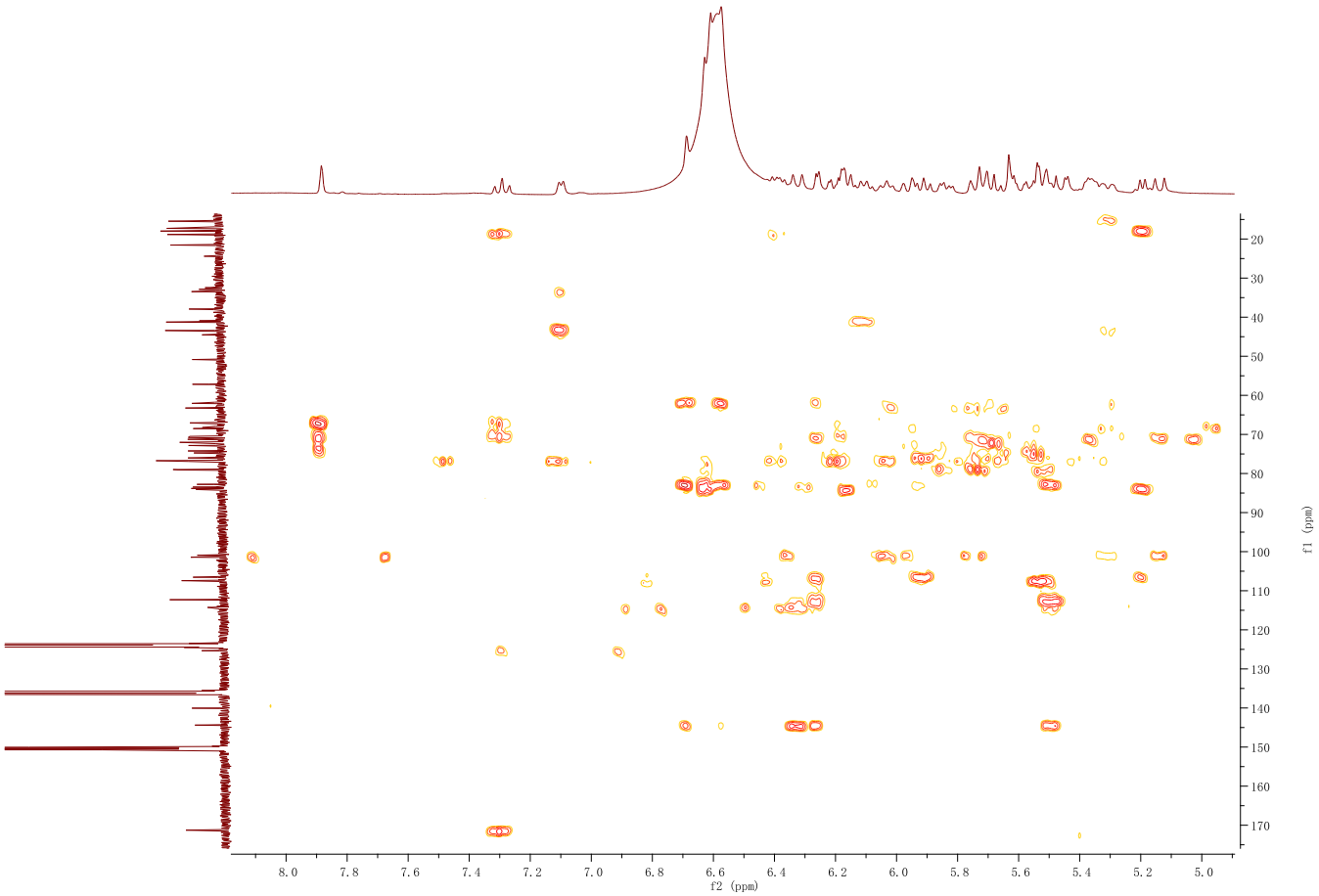

Figure S25: The HMBC spectrum of **2** (in C_5D_5N) (From δ_H 4.6 ppm to δ_H 8.2 ppm)

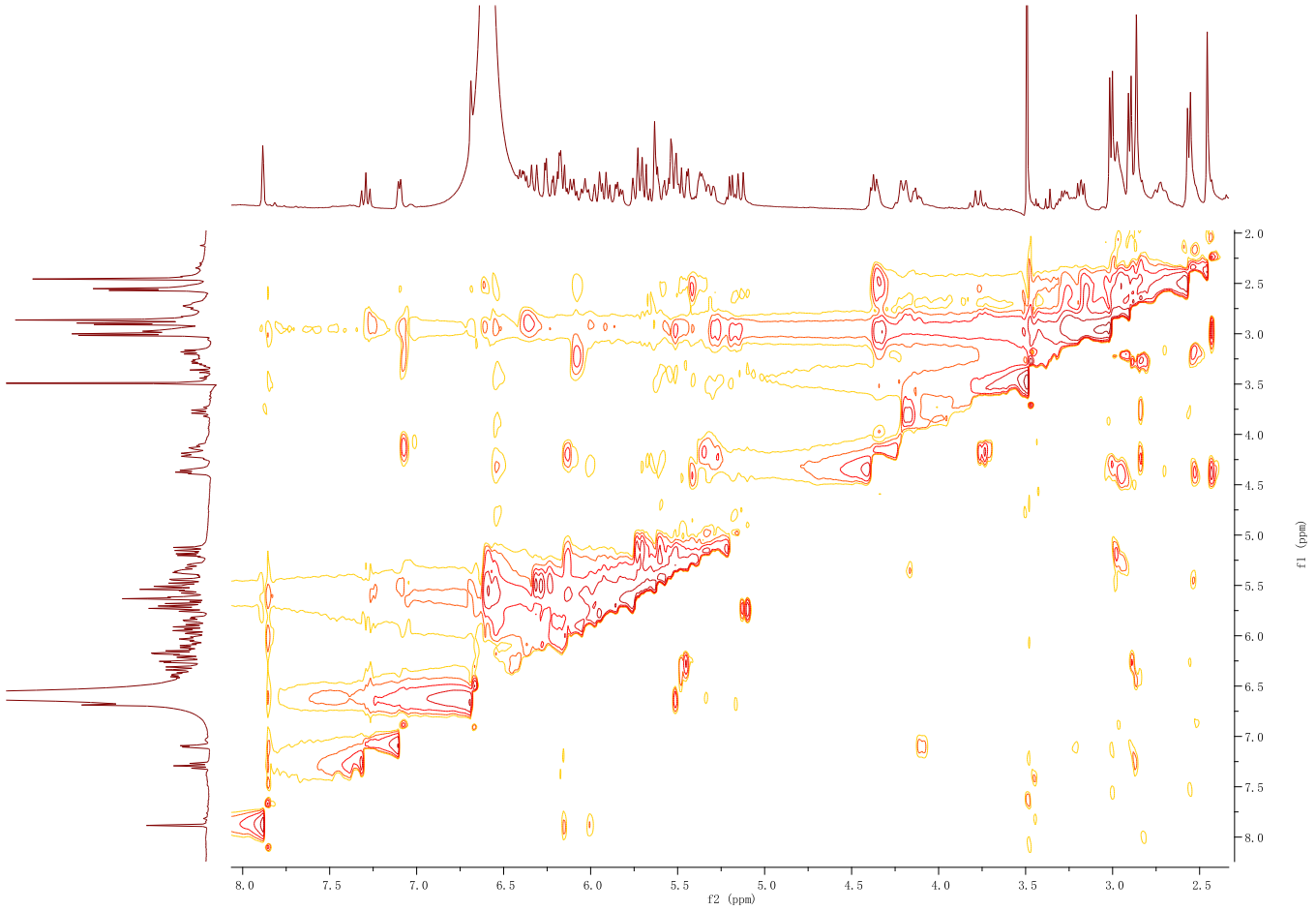

Figure S26: The NOESY spectrum of **2** (in C_5D_5N)

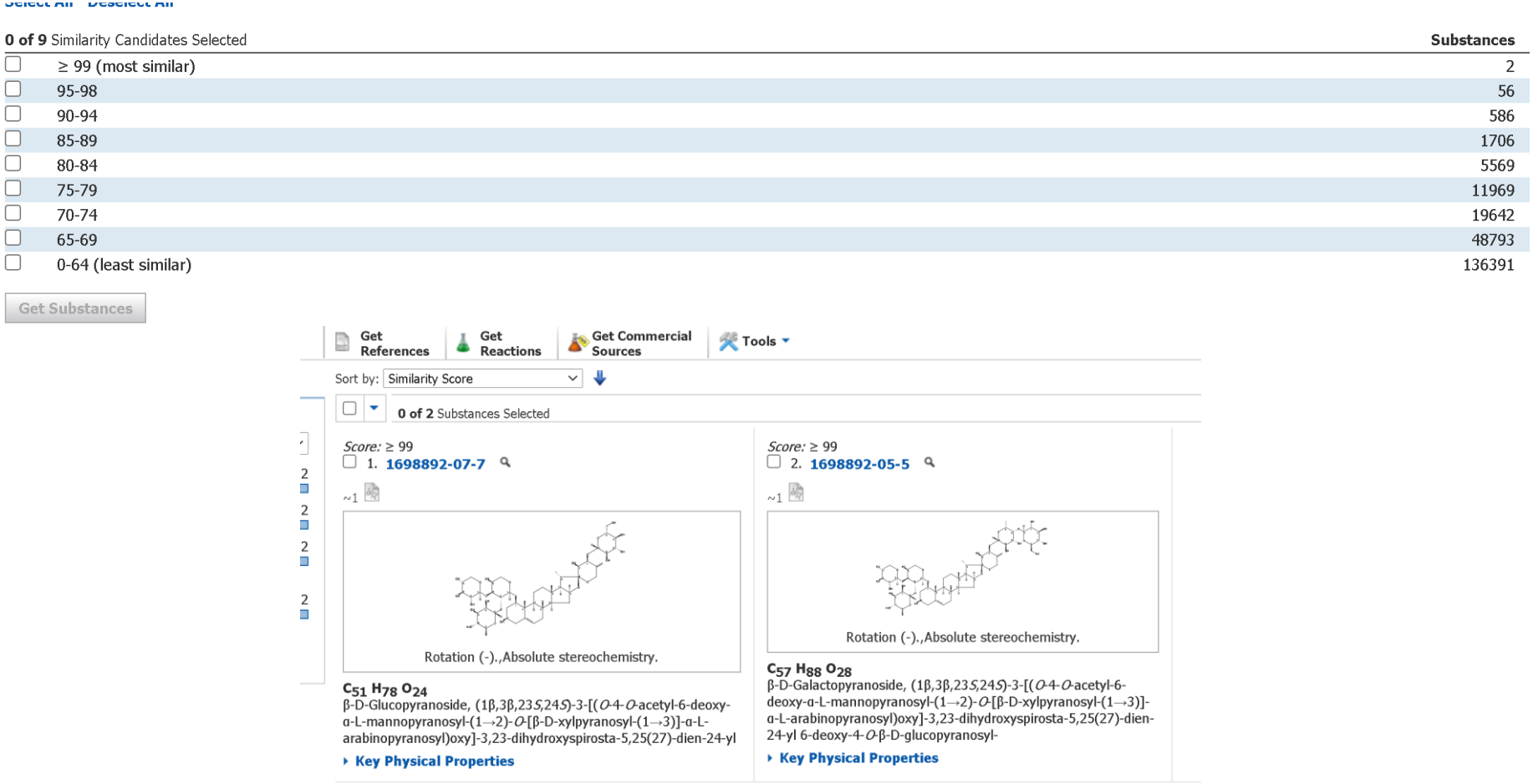

Figure S27: New compound search report of SciFinder

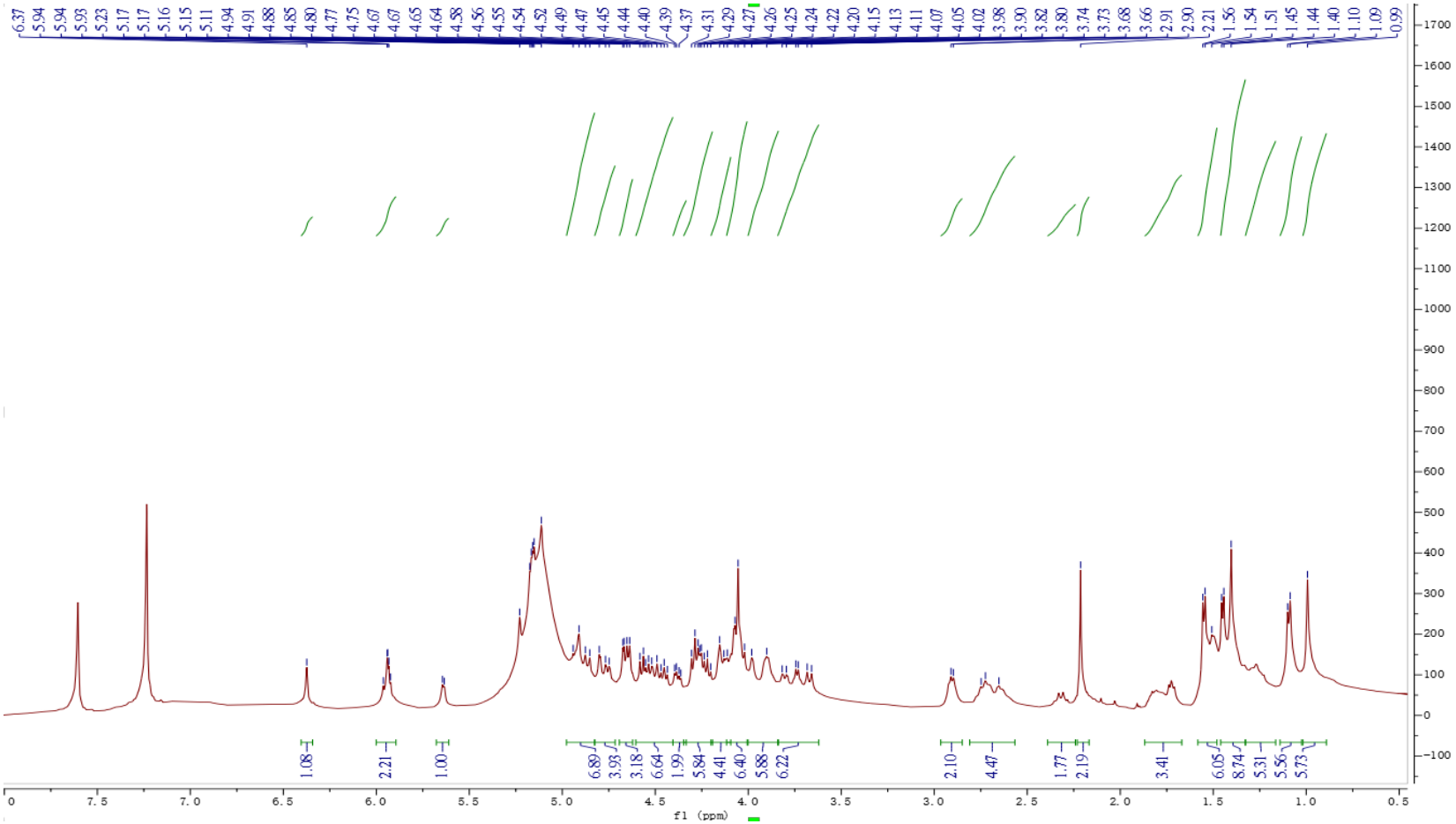

Figure S28: The ^{1}H NMR spectrum of **3** (in pyridine-d_5)

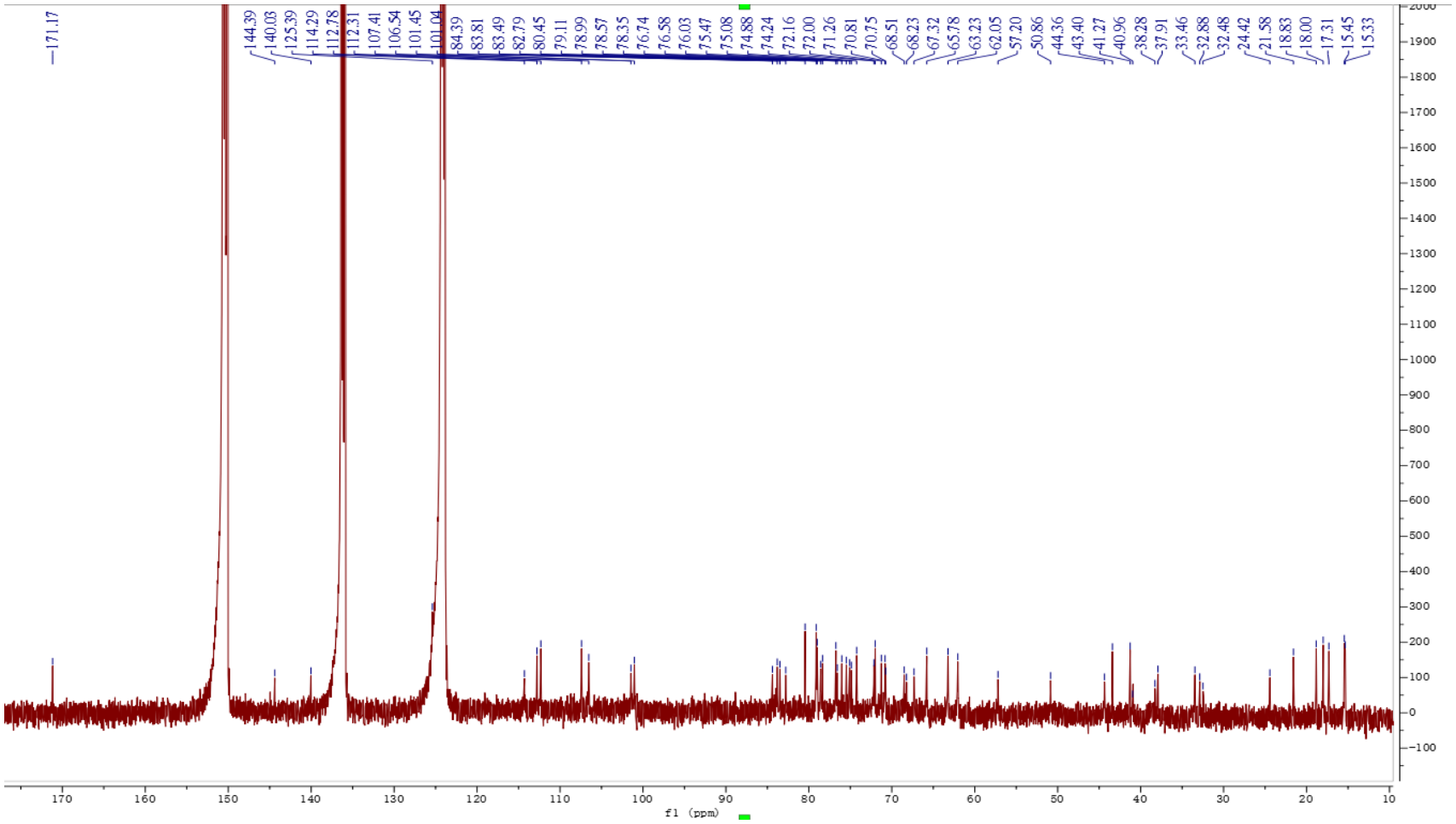

Figure S29: The ^{13}C NMR spectrum of **3** (in pyridine-d_5)

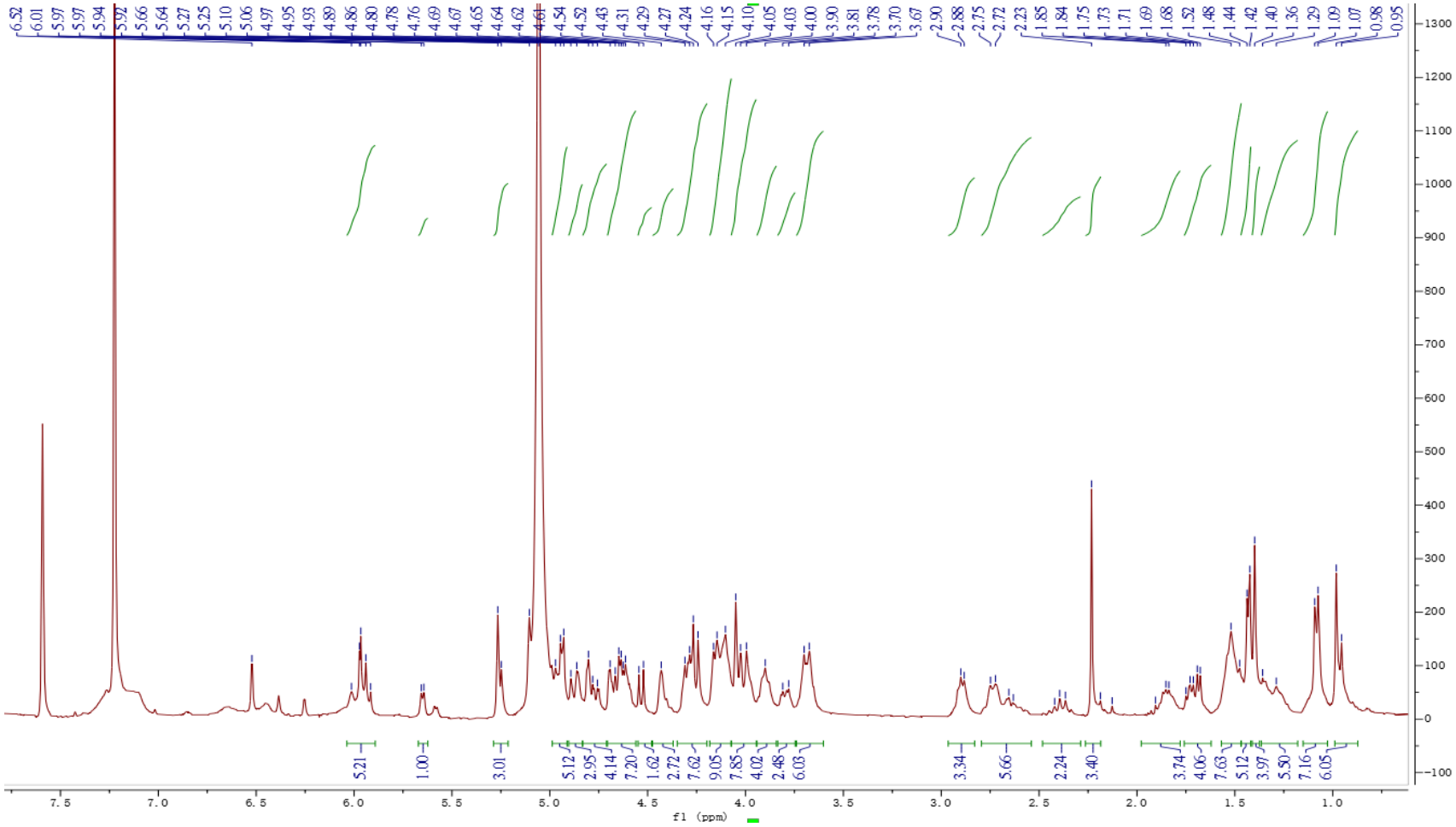

Figure S30: The ^{1}H NMR spectrum of **4** (in pyridine-d_5)

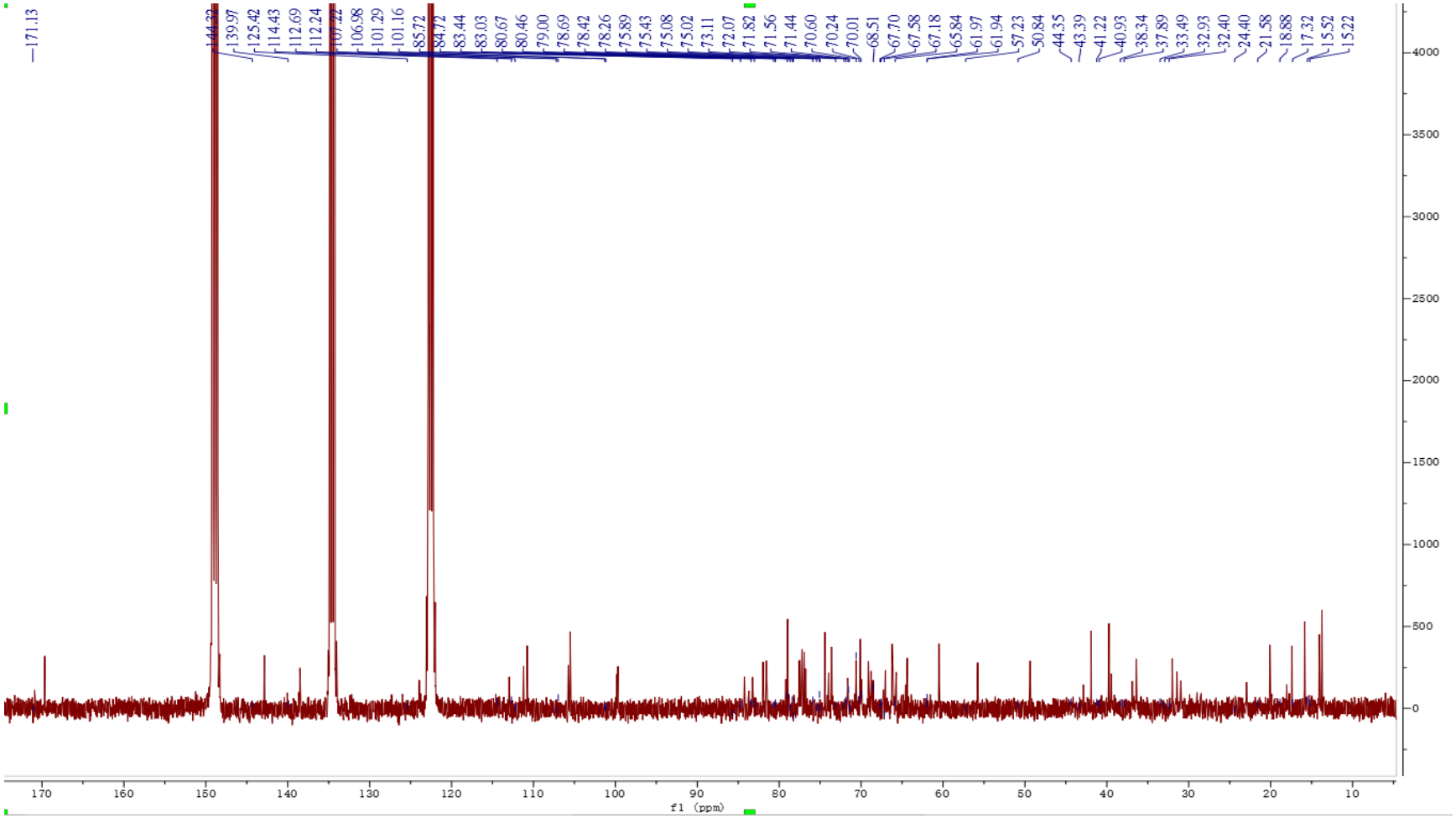

Figure S31: The ^{13}C NMR spectrum of 4 (in pyridine-d_5)

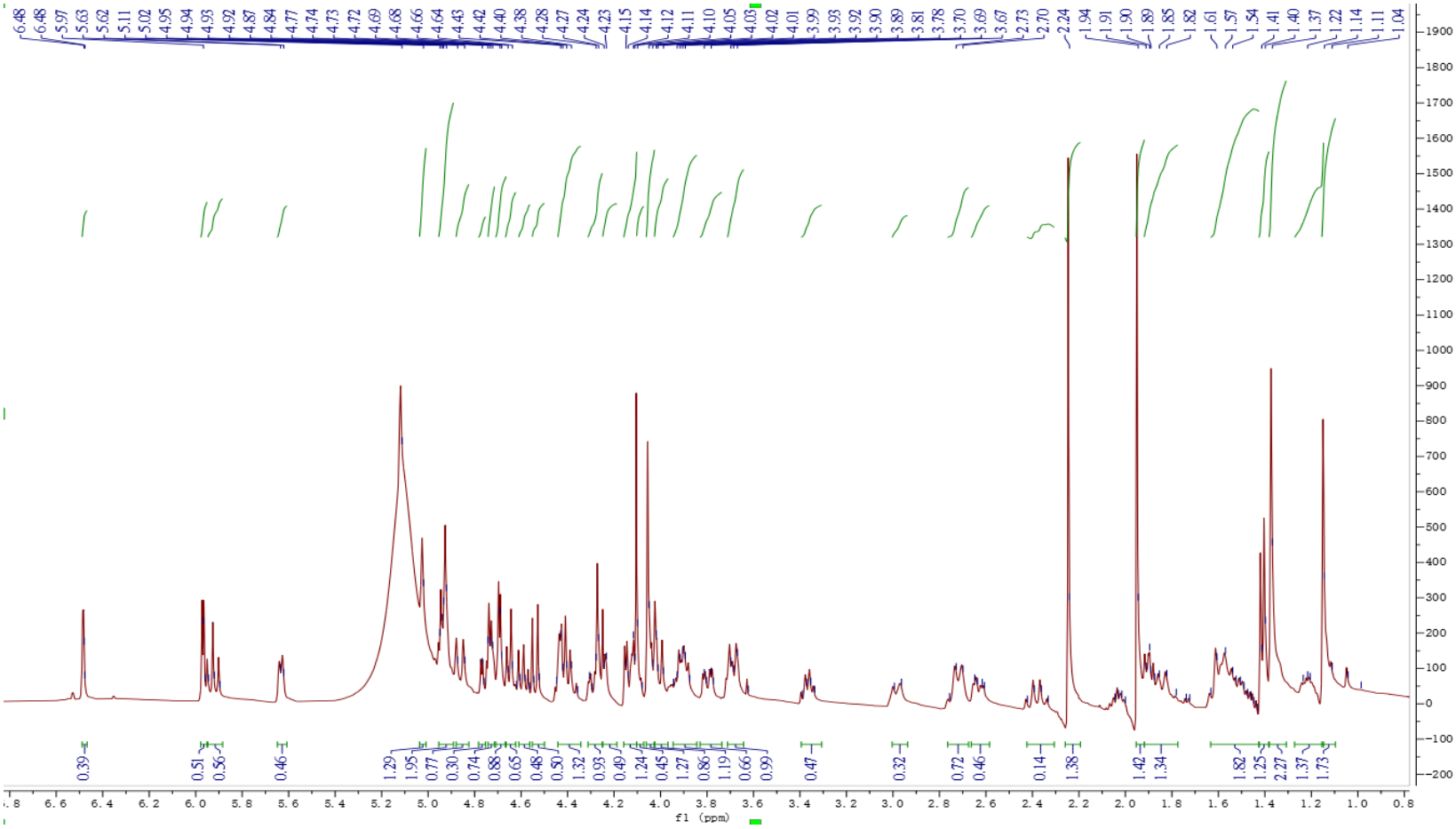

Figure S32: The ^{1}H NMR spectrum of **5** (in pyridine-d_5)

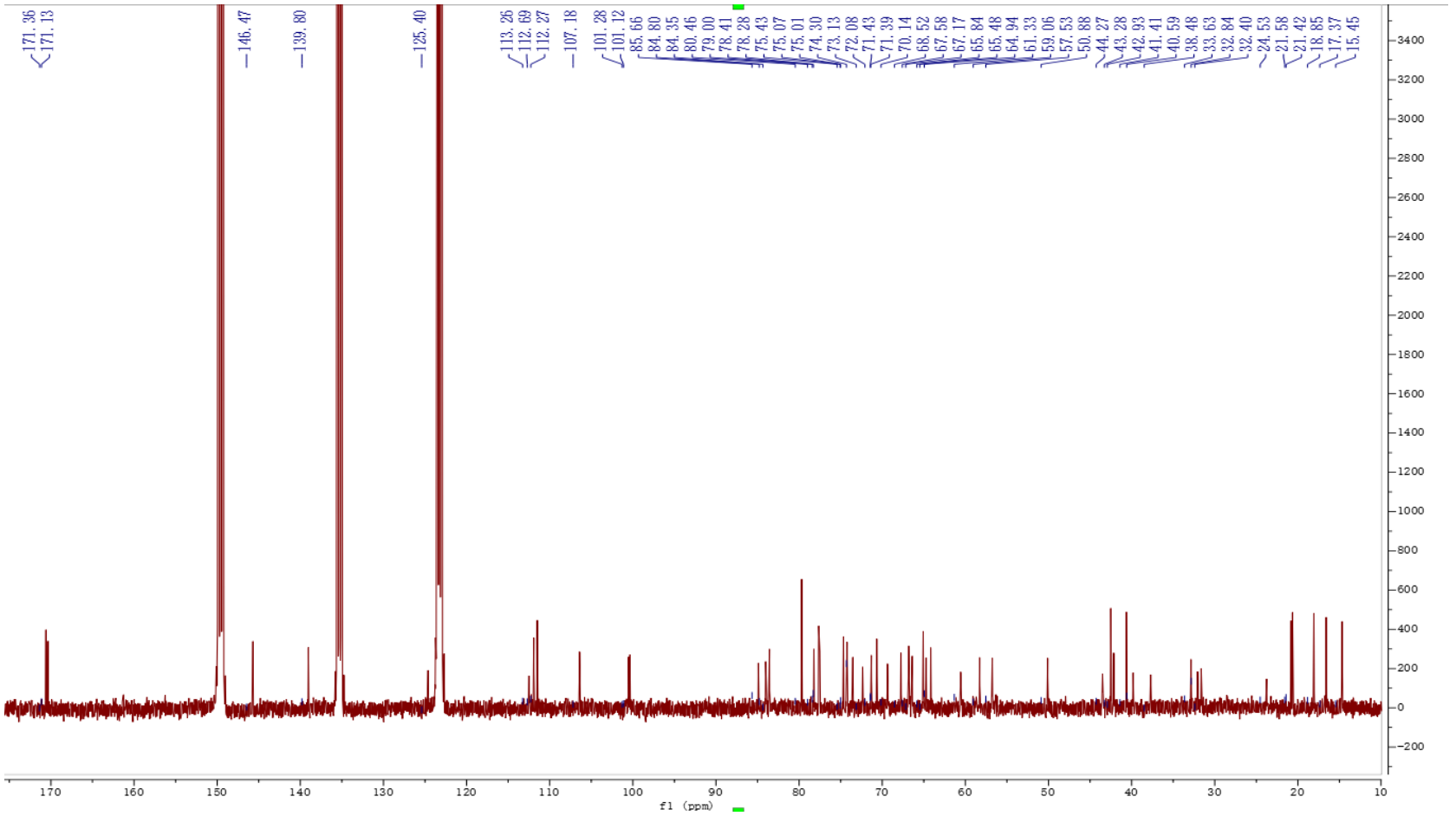

Figure S33: The ^{13}C NMR spectrum of **5** (in pyridine-d_5)

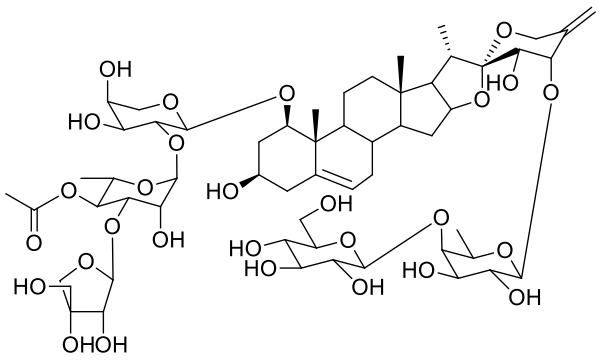

Figure S34: Structure of similar compound (Ref)

(23*S*,24*S*)-24-{[*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-fucopyranosyl]oxy}-3*β*,23-dihydroxyspirosta-5,25(27)-dien-1*β*-yl*O*-*β*-D-apiofuranosyl-(1→3)-*O*-(4-*O*-acetyl-*α*-L-rhamnopyranosyl)-(1→2)-*O*-*α*-L-arabinopyranoside (Ref is similar to compounds **1** and **2**)

Zhang H., Su Y.F., Yang F.Y., et al. Six new steroidal saponins from *Helleborus thibetanus* *Helv. Chim. Acta*. 2014, **97**(**12**), 1652-1665.

Table S1: ^{13}C NMR data for compounds **1-5** and **Ref**.

Position	1	2	3	4	5	Ref.
1	84.3	84.1	83.8	84.7	84.8	83.8
2	37.9	38.0	38.3	38.3	38.5	37.8
3	68.7	68.5	68.5	68.5	67.6	68.0
4	44.3	44.5	44.4	44.4	43.3	43.9
5	140.2	140.1	140.0	140.0	139.8	139.5
6	125.1	125.3	125.4	125.4	125.4	124.8
7	32.4	32.5	32.5	32.4	32.4	32.0
8	33.4	33.5	33.5	33.5	33.6	33.0
9	50.9	50.8	50.9	50.8	50.9	50.4
10	43.4	43.4	43.4	43.4	42.9	42.9
11	24.4	24.4	24.4	24.4	24.5	23.9
12	40.9	40.9	41.0	41.2	40.6	40.4
13	41.3	41.3	41.3	40.9	41.4	40.8
14	57.2	57.2	57.2	57.2	57.5	56.7
15	32.8	32.9	32.9	32.9	32.8	32.4
16	83.5	83.5	83.5	83.0	84.4	83.0
17	62.0	62.1	62.1	62.0	59.1	61.6
18	17.3	17.3	17.3	17.3	17.4	16.8
19	15.6	15.4	15.5	15.5	15.5	15.0
20	37.9	38.0	37.9	37.9	44.3	37.4
21	15.3	15.3	15.3	15.2	64.9	14.8
22	112.3	112.3	112.3	112.2	112.7	111.8
23	70.8	70.8	70.8	70.6	71.4	70.2
24	82.8	82.8	82.8	83.4	75.4	82.3
25	144.4	144.4	144.4	144.3	146.5	143.9
26	62.0	62.0	62.1	62.0	61.3	61.5
27	114.3	114.3	114.3	114.4	113.3	113.7
CO**C**H$_3$	—	—	—	—	21.4	—
COCH$_3$	—	—	—	—	171.1	—
1-*O*-Ara						
1	101.0	100.9	101.0	101.2	101.1	100.5
2	75.8	74.7	74.9	73.1	73.1	74.7
3	76.3	76.7	76.6	85.7	85.7	76.0
4	70.6	70.8	70.8	70.2	70.1	70.3
5	67.9	68.2	67.3	67.2	68.5	67.7
Rha						
1	102.0	101.4	101.5	101.3	101.3	100.9
2	72.3	72.8	71.3	71.6	71.4	71.5
3	80.6	70.5	78.4	78.3	78.3	78.0

4	73.0	76.9	75.1	75.0	75.1	74.4
5	70.0	67.1	68.2	67.6	67.2	66.8
6	19.5	18.8	18.8	18.9	18.9	18.3
CO**C**H$_3$	—	21.5	21.6	21.6	21.6	21.0
COCH$_3$	—	171.3	171.2	171.1	171.4	170.6
Api						
1	112.3	—	112.8	112.7	112.3	112.2
2	78.2	—	78.6	78.4	78.4	77.9
3	80.6	—	80.5	80.5	80.5	79.9
4	75.6	—	75.5	75.1	75.0	75.0
5	66.1	—	65.8	65.8	65.5	65.3
Xyl						
1	—	—	—	107.2	107.2	—
2	—	—	—	75.9	74.3	—
3	—	—	—	79.0	79.0	—
4	—	—	—	72.1	72.1	—
5	—	—	—	67.7	65.8	—
24-*O*-Fuc						
1	106.6	106.5	106.5	—	—	106.0
2	74.2	74.2	74.2	—	—	73.7
3	76.0	76.0	76.0	—	—	76.2
4	83.8	83.8	84.4	—	—	83.3
5	71.2	71.3	72.0	—	—	70.8
6	18.0	18.0	18.0	—	—	17.4
Glc						
1	107.4	107.4	107.4	107.0	—	106.9
2	76.7	76.7	76.7	75.4	—	75.5
3	79.1	79.1	79.0	80.7	—	78.5
4	72.0	72.0	72.2	71.4	—	71.6
5	79.0	79.0	79.1	78.7	—	78.6
6	63.2	63.3	63.2	61.9	—	62.8

Text S1: Detail experiments for Sugar analysis of compounds **1** and **2**

Compounds **1**-**2** (each 2 mg) were individually dissolved with 2 mol/L CF_3COOH (2 mL) at 100°C for 8 h. After dilution with H_2O (15 ml), the reaction mixture was extracted with EtOAc, yielding distinct EtOAc and H_2O phases. The latter was concentrated under reduced pressure by repeated mixing with methanol until the solvent was completely evaporated. The residue was dissolved in pyridine solution (1 mL) of L-cysteine methyl ester hydrochloride (2 mg/L). After heating at 60 °C for 1 h, the solvent was evaporated under N_2 protection. The reaction products were dissolved in the mixed solution of N-(trimethylsilyl)imidazole (0.2 mL) and anhydrous pyridine (2 mL), and the mixture was maintained at 60°C for 1 h, evaporated under a stream of N_2, and dried in a vacuum. The residue was suspended in cyclohexane and water, the cyclohexane layer was the trimethylsilyl ether derivatives of monosaccharide. The mixture was filtered through a 0.45 μm membrane to remove the precipitate and analyzed by GC under the following conditions: HP-5 capillary column (30 m × 0.32 mm × 0.25 μm); flame ionization detector; detector temperature = 280 °C; injection temperature = 250 °C; initial temperature = 100 °C for 2 min, followed by an increase to 280 °C at a rate of 10 °C/min; final temperature = 280 °C for 5 min; and N_2 gas as a carrier. The absolute configurations of sugars isolated from the hydrolysates of compounds **1** - **2** were determined by comparing the retention times (t_R) of their trimethylsilyl-L-cysteine derivatives with those of authentic sugars prepared by a similar procedure. Retention times for authentic sugars after being derivatized were follows: D-glucose, 45.2 min, D-fucose, 35.2 min; D-apiose, 11.2 min; L-arabinose, 12.2 min and L-rhamnopyranose, 14.5 min, respectively.

Text S2: Cytotoxicity assay

Cytotoxic was determined against HCT116, A549 and HepG2 tumor cell lines based on the MTT assay method *in vitro*, and 5-fluorouracil (5-Fu) was used as the positive control. Briefly, 1×10^4 mL^{-1} cells were seeded into 96-well plates and allowed to adhere for 24 h. Compounds **1**-**5** were dissolved in DMSO and diluted with complete medium to six concentration levels (from 0.001 $mmol \cdot L^{-1}$ to 0.3 $mmol \cdot L^{-1}$) for inhibition rate determination. After incubation at 37 °C for 24 h, the supernatant was removed before adding DMSO (100 μL) to each well. The inhibition rate (IR) and IC_{50} were calculated. Values are mean ± SD, $n = 3$, ** $p < 0.01$ *vs.* DMEM control.