Supporting data

Isolation, crystal structure and *in silico* aromatase inhibition activity of ergosta-5,22-dien-3β-ol from the fungus *Gyromitra esculenta*

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S.1. Experimental

S.1.1. Isolation of 1

The raw material of *G. esculenta* was collected in summer in the vicinity of the city of Karkaraly, Karaganda region, Kazakhstan. *G.a esculenta* was extracted *via* adding 300 mL of MeOH to 207.5 g of a semi-dried powder and sonicated at 40-50 ˚C for 3 hrs. The procedure was repeated 3 times per day. The obtained extracts were combined and evaporated under reduced pressure. Total weight of the obtained extract – 62.9 g.

The total extract was subjected to SiO_2 column (400 g) using hexane – EtOAc and CH_2Cl_2 – MeOH as mobile phases in a manner of increasing polarity. Pure white colored crystal of **1** was obtained from fraction 54 (hexane – EtOAc 1: 10).

S.1.2. X-ray analysis

X-ray intensity data for the compound $C_{28}H_{46}O \cdot H_2O$ were collected at 100 K, on a Rigaku Oxford Diffraction Supernova Dual Source (Cu at zero) diffractometer equipped with an Atlas CCD detector using ω scans and CuKα (λ = 1.54184 Å) radiation. The images were interpreted and integrated with the program CrysAlisPro [62]. Using Olex2 [63], the structure was solved by direct methods using the ShelXT structure solution program and refined by full-matrix least-squares on F^2 using the ShelXL program package [64, 65]. Non-hydrogen atoms were anisotropically refined and the hydrogen atoms in the riding mode with isotropic temperature factors fixed at 1.2 times U(eq) of the parent atoms (1.5 times for methyl and hydroxyl groups). The absolute configuration was established showing a refined Flack parameter of 0.0(2).

CCDC 2060747 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/structures.

Details of the X-ray crystal structure data collection and refinement are given in Table 7.

Table 7. Crystal data and details of the data collection and structure refinement for hydrate brassicasterol.

Parameters	hydrate brassicasterol
Empirical formula	$C_{28}H_{46}O \cdot H_2O$
Formula weight (g/mol	416.66
Temperature (K)	100(1)
Crystal system	monoclinic
Space group	$P2_1$
a (Å)	9.8621(2)

b (Å)	7.5289(2)
c (Å)	34.4423(5)
β (°)	93.926(1)
Volume (Å^3)	2551.37(9)
Z	4
ρ_{calc} (g/cm^3)	1.085
μ (mm^{-1})	0.495
F(000)	928.0
Crystal size (mm^3)	0.351 × 0.129 × 0.072
Radiation	CuK_α (λ = 1.54184)
2Θ range for data collection (°)	7.718 to 150.484
T_{min}, T_{max}	0.760, 1.000
Index ranges	-12 ≤ h ≤ 12, -8 ≤ k ≤ 9, -40 ≤ l ≤ 43
Reflections collected	47210
Independent reflections	9920 [R_{int} = 0.0542, R_{sigma} = 0.0442]
Data/restraints/parameters	9920/1/561
Goodness-of-fit on F^2	1.037
Final R indexes [I≥2σ (I)]	R_1 = 0.0738, wR_2 = 0.1944
Final R indexes [all data]	R_1 = 0.0773, wR_2 = 0.1994
Largest diff. peak/hole (e·Å^{-3})	0.54/-0.34
Flack parameter	0.0(2)

S.1.3. Molecular Docking studies

Crystal structure of aromatase [PDB ID: 3S7S, resolution: 3.21 Å] was obtained from Protein Data Bank. The docking investigation was accomplished using MOE2014 software. At first, the crystal structure of aromatase was prepared by removing water molecules. Only one chain was retained besides the co-crystallized ligand (EXM). Then, the selected chain was protonated and subjected to a minimization of the energy process. Next, the active site of the target protein was defined.

Structures of ergosta-5,22-dien-3β-oland EXM were drawn using ChemBioDraw Ultra 14.0 and saved in MDL-SD format. Such a file was opened using MOE to display the 3D structures which were protonated and subjected to energy minimization. Formerly, flexible alignment was performed using the alignment protocol of MOE. Then, validation of the docking process was performed by docking the

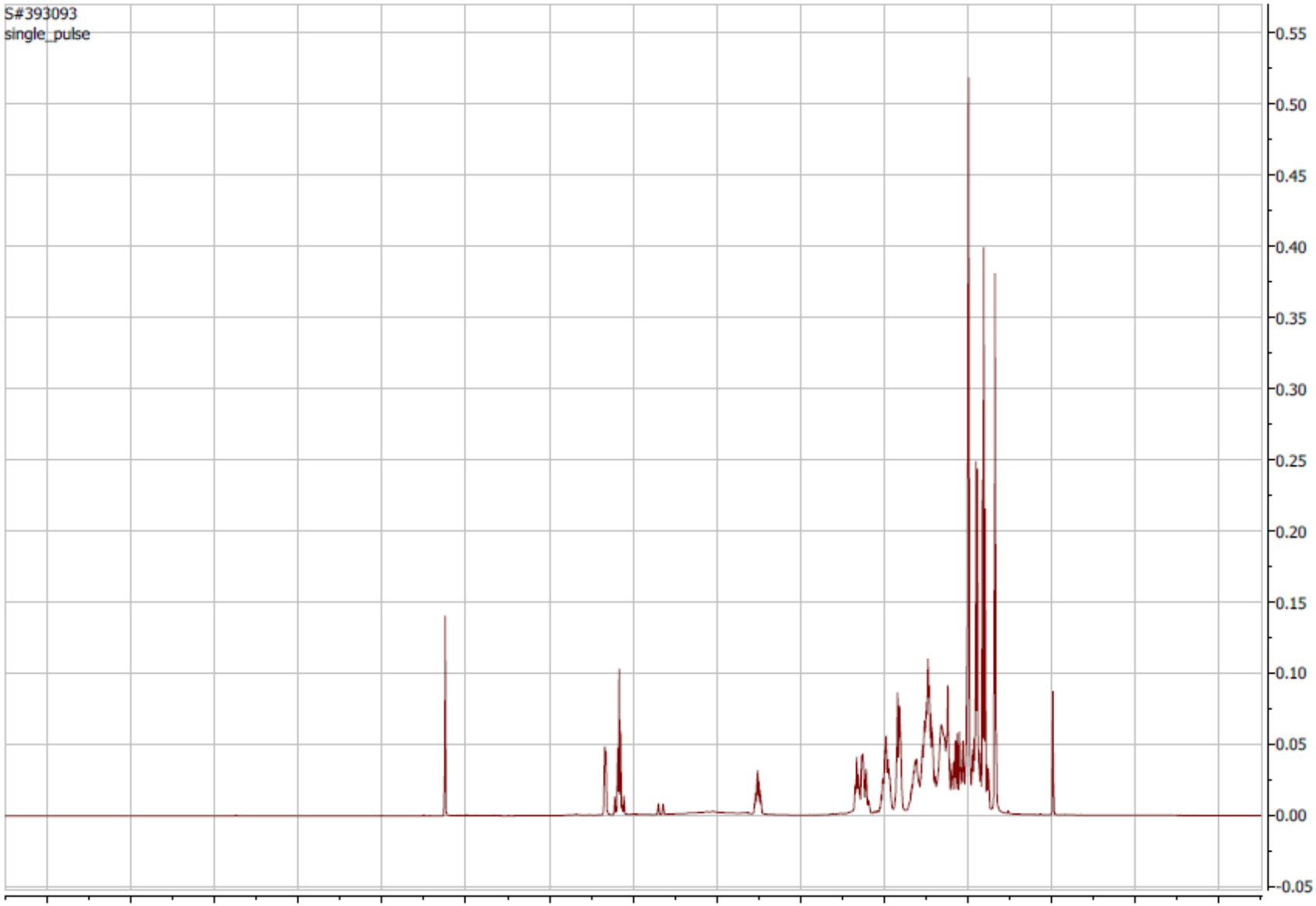
co-crystallized ligand against the isolated pocket of the active site. The produced RMSD value indicated the validity of the process. Finally, docking of the tested compounds was done through the dock option inserted in compute window. For each docked molecule, 30 docked poses were produced using ASE for scoring function and force field for refinement. The results of the docking process were then visualized using Discovery Studio 4.0 software [66-72].

S.1.4. *In silico* ADMET studies

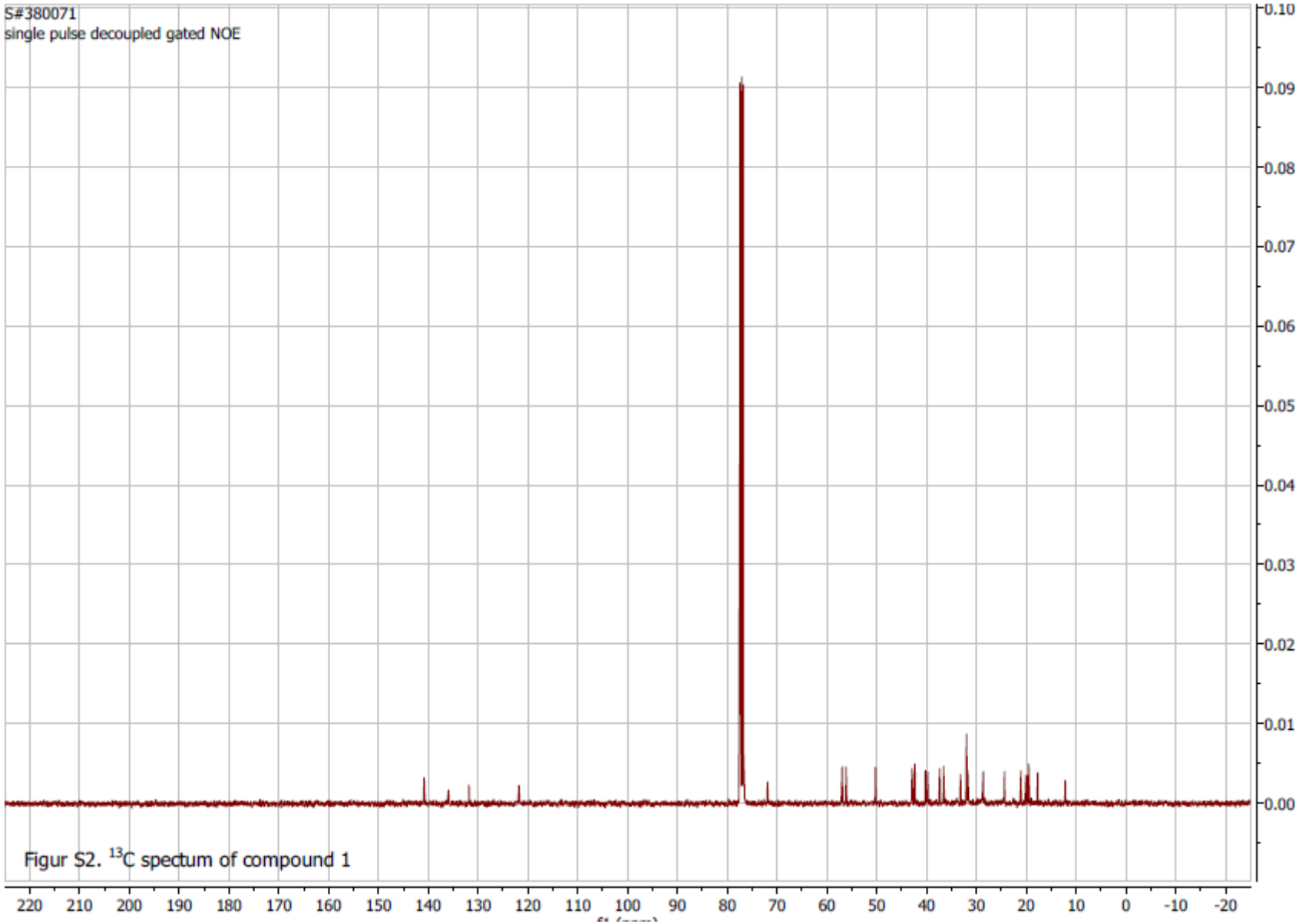
ADMET descriptors (absorption, distribution, metabolism, excretion and toxicity) of ergosta-5,22-dien-3β-ol and the co-crystallized ligand were determined using Discovery studio 4.0. At first, the CHARMM force field was applied then the compounds were prepared and minimized according to the preparation of small molecule protocol. Then ADMET descriptors protocol was applied to carry out these studies [66, 67].

S.1.5. *In silico* Toxicity studies

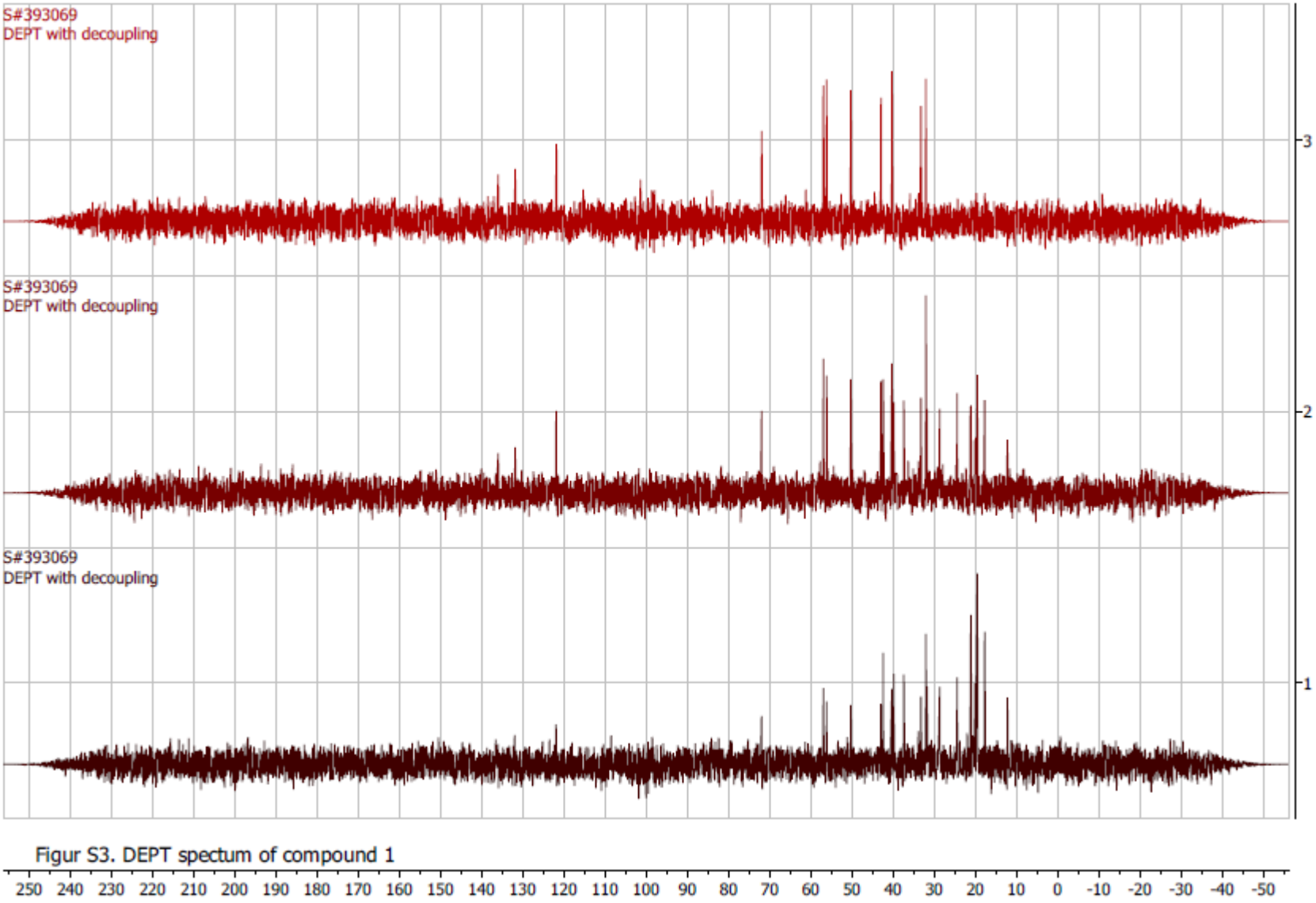
The toxicity parameters of ergosta-5,22-dien-3β-oland the Co-crystallized ligand were calculated using Discovery studio 4.0. At first, the CHARMM force field was applied then the compounds were prepared and minimized according to the preparation of small molecule protocol. Then different parameters were calculated from the toxicity prediction (extensible) protocol.



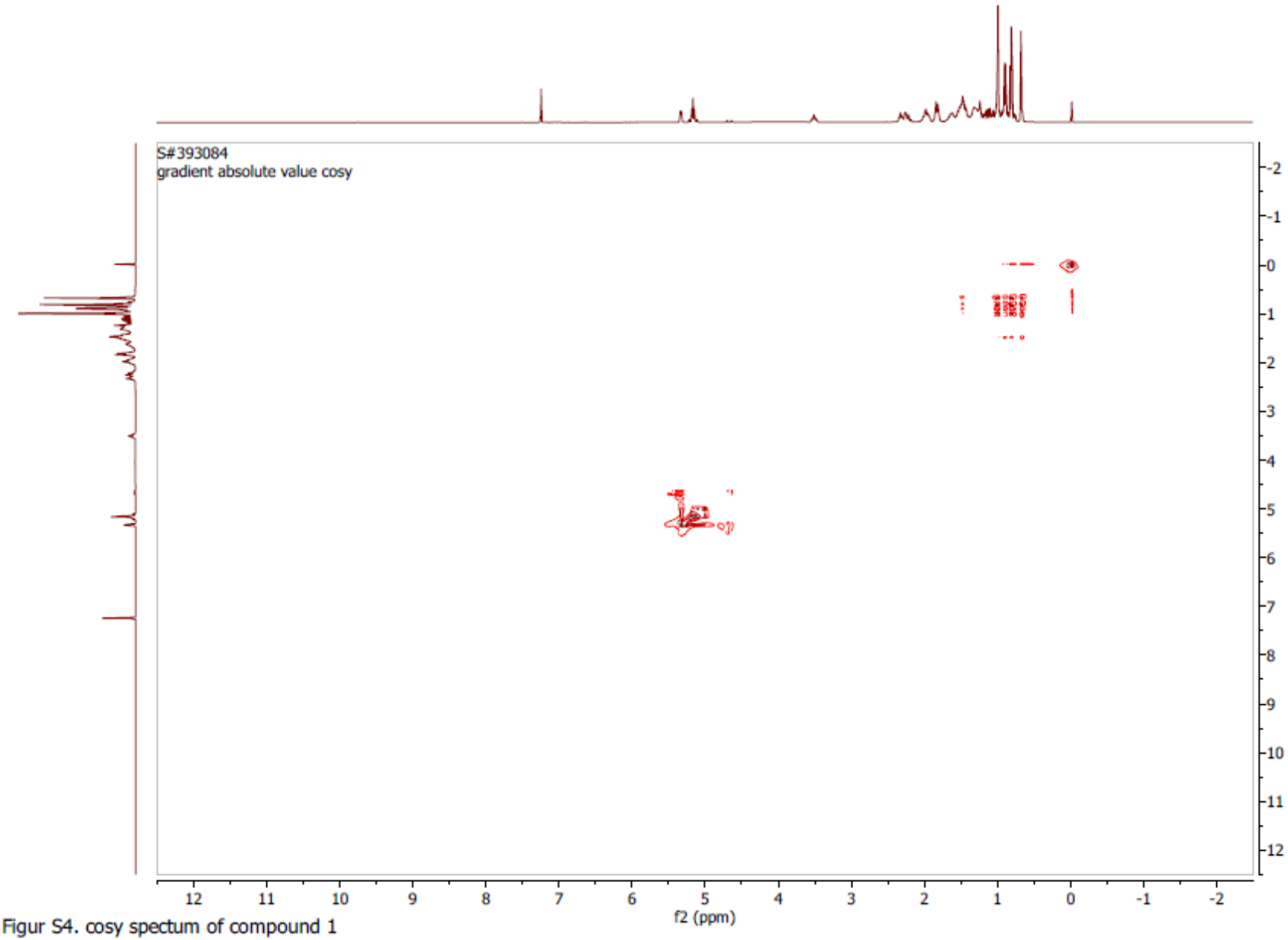
Figur S1. ^{1}H spectum of compound **1**



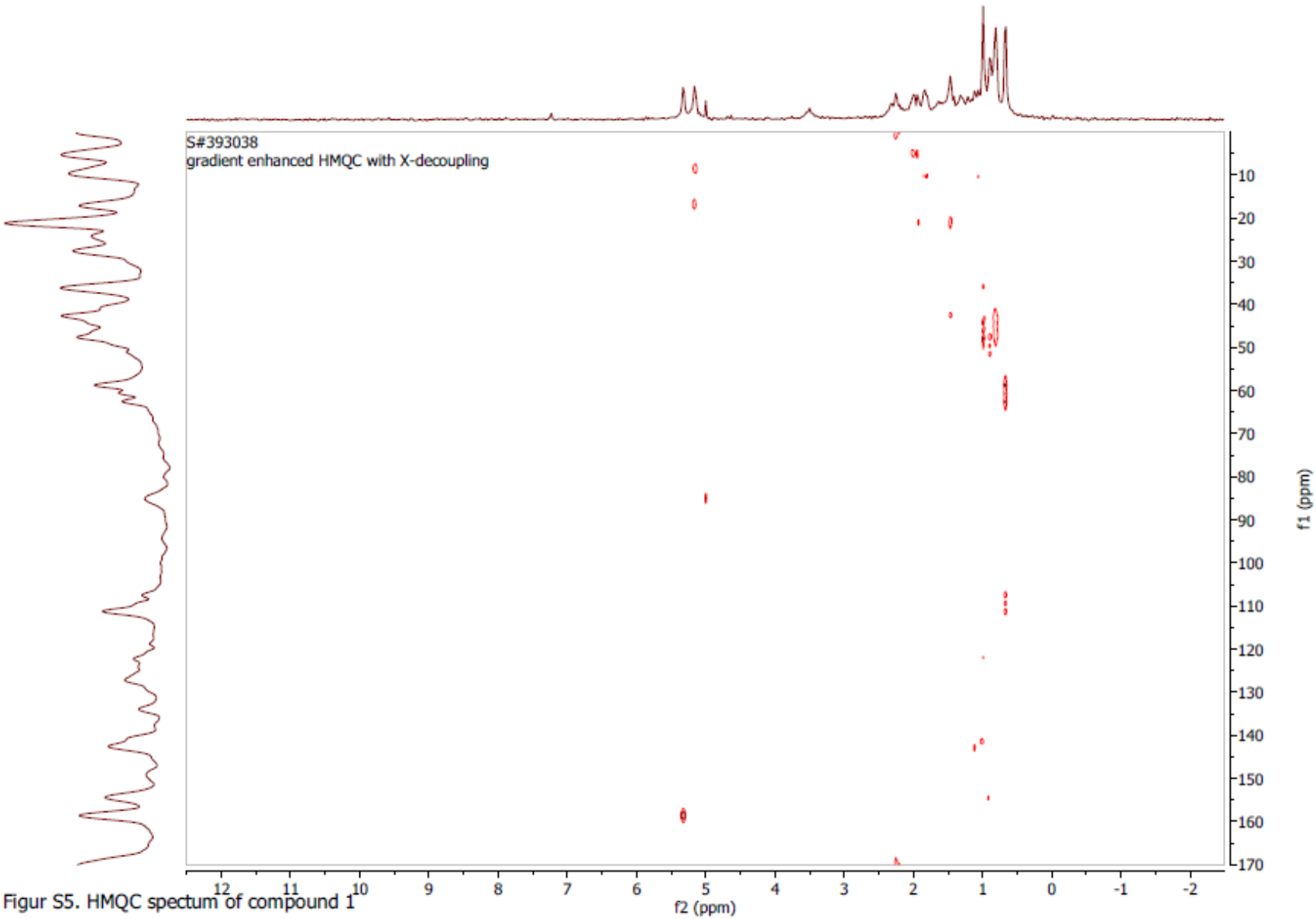
Figur S2. ^{13}C spectum of compound 1



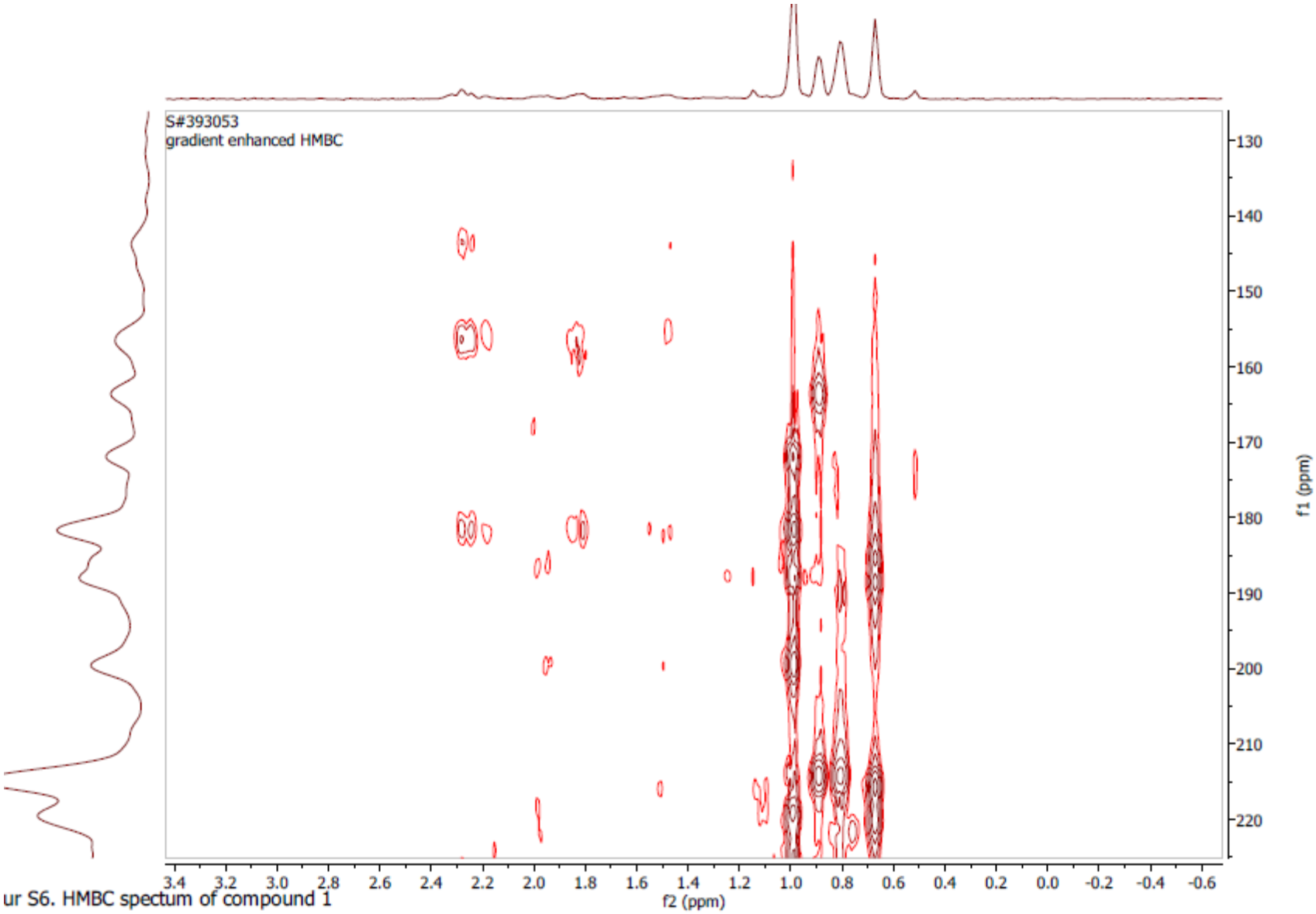
Figur S3. DEPT spectum of compound 1



Figur S4. cosy spectum of compound 1



Figur S5. HMQC spectum of compound 1



ur S6. HMBC spectum of compound 1