BEILSTEIN JOURNAL OF ORGANIC CHEMISTRY

Supporting Information

for

Designed whole-cell-catalysis-assisted synthesis of 9,11-secosterols

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General material and methods for the construction of the biocatalyst as well as NMR spectra of synthesized compounds

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Supporting material and methods

Polyacrylamide gel electrophoresis (SDS-PAGE)

E. coli BL21 (DE3) cells carrying pAJ30 plasmid for expression of *kshA5* and *kshB* were pregrown overnight at 37 °C. Next morning, 200 mL of LB medium in a 2 L baffled flask was inoculated with 1 mL of a preculture. The cultures were grown until OD600 = 0.1 at 37 °C with continuous shaking at 220 rpm, and then the expression was induced by adding 1 mM IPTG. Thereafter, the temperature of the cultures was decreased to 30 °C, and the growth was continued with continuous shaking at 220 rpm overnight. 1 mL of culture samples were collected prior to induction of kshA5 and kshB synthesis and 2, 4 and 20 hours postinduction. The cells from these 1 mL samples were harvested by centrifugation at 11,000 × g and resuspended in 1x SDS sample buffer to final concentration of 5 OD600 units/mL (cells from 1 mL of culture at optical density OD600 = 1 is defined as 1 OD600 units/mL of sample), followed by heating the samples at 97 °C for 10 minutes to lyse the cells and denature the proteins. Either 0.03 or 0.003 OD600 units of each sample was loaded to a 12% polyacrylamide gel for protein staining or western blotting, respectively, and separated by gel electrophoresis. The proteins in the gel were stained using InstsantBlue Coomassie Protein Stain (Abcam, ab119211).

Western blotting

Protein lysates separated by SDS-PAGE were transferred to a PVDF membrane (Trans-Blot Turbo Mini 0.2 µm PVDF Transfer Packs, BioRad #1704156) using Trans-Blot Turbo Transfer System (BioRad) according to recommended protocol by the manufacture. HisProbe™-HRP Conjugate (ThermoFisherScientific, 15165) and SuperSignal™ West Pico PLUS Chemiluminescent Substrate (ThermoFisherScientific, 34579) was used to illuminate 6His-tagged KSH proteins and recorded in LI-COR Fc imaging system.

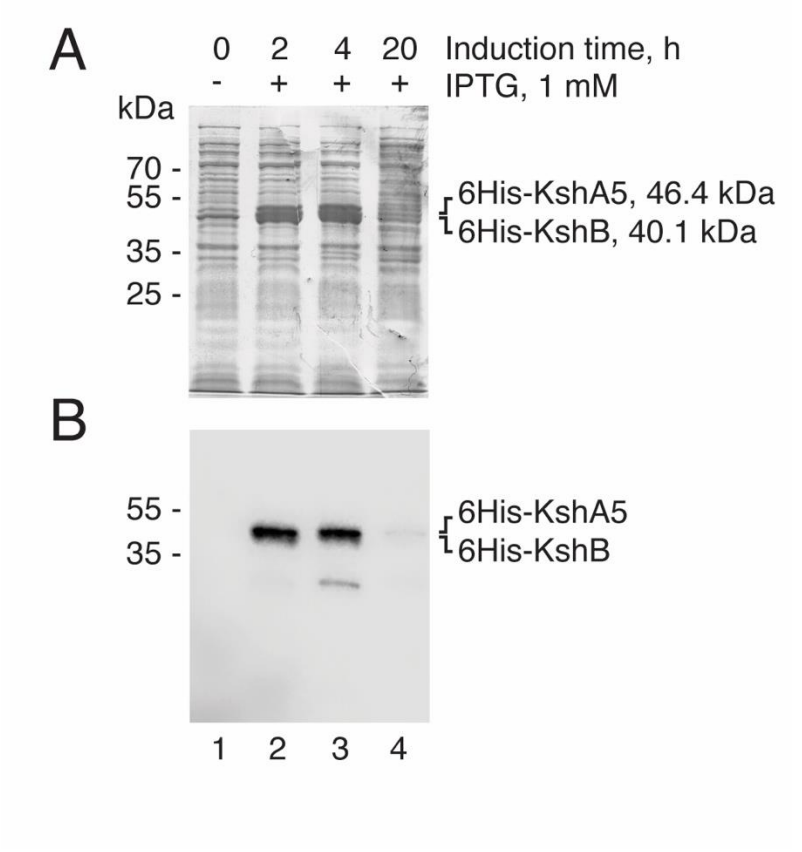


Figure S1: Expression analysis of KSH proteins. The expression of 6His-tagged kshA5 and kshB was monitored in *E. coli* BL21 (DE3) cells carrying pAJ30 plasmid. The culture was grown in LB medium and at OD600 = 0.1. Protein synthesis was induced with 1 mM IPTG (final concentration). 1 mL samples were collected at indicated time points after induction of the protein synthesis and subjected to SDS polyacrylamide gel electrophoresis (A) and western blotting (B).

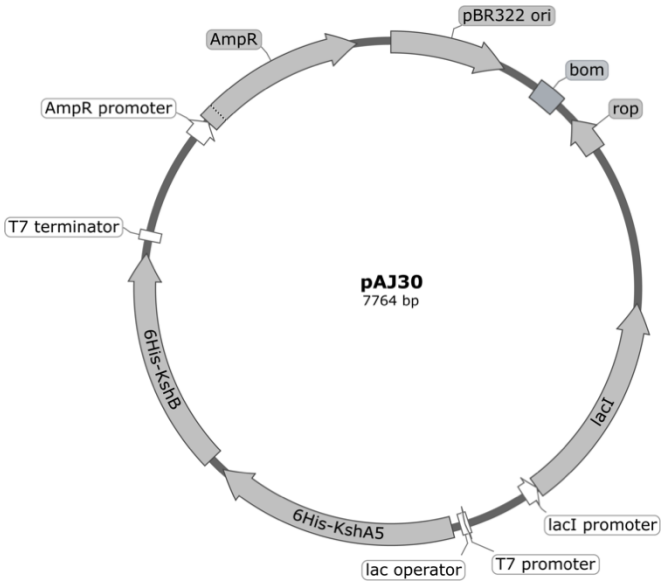
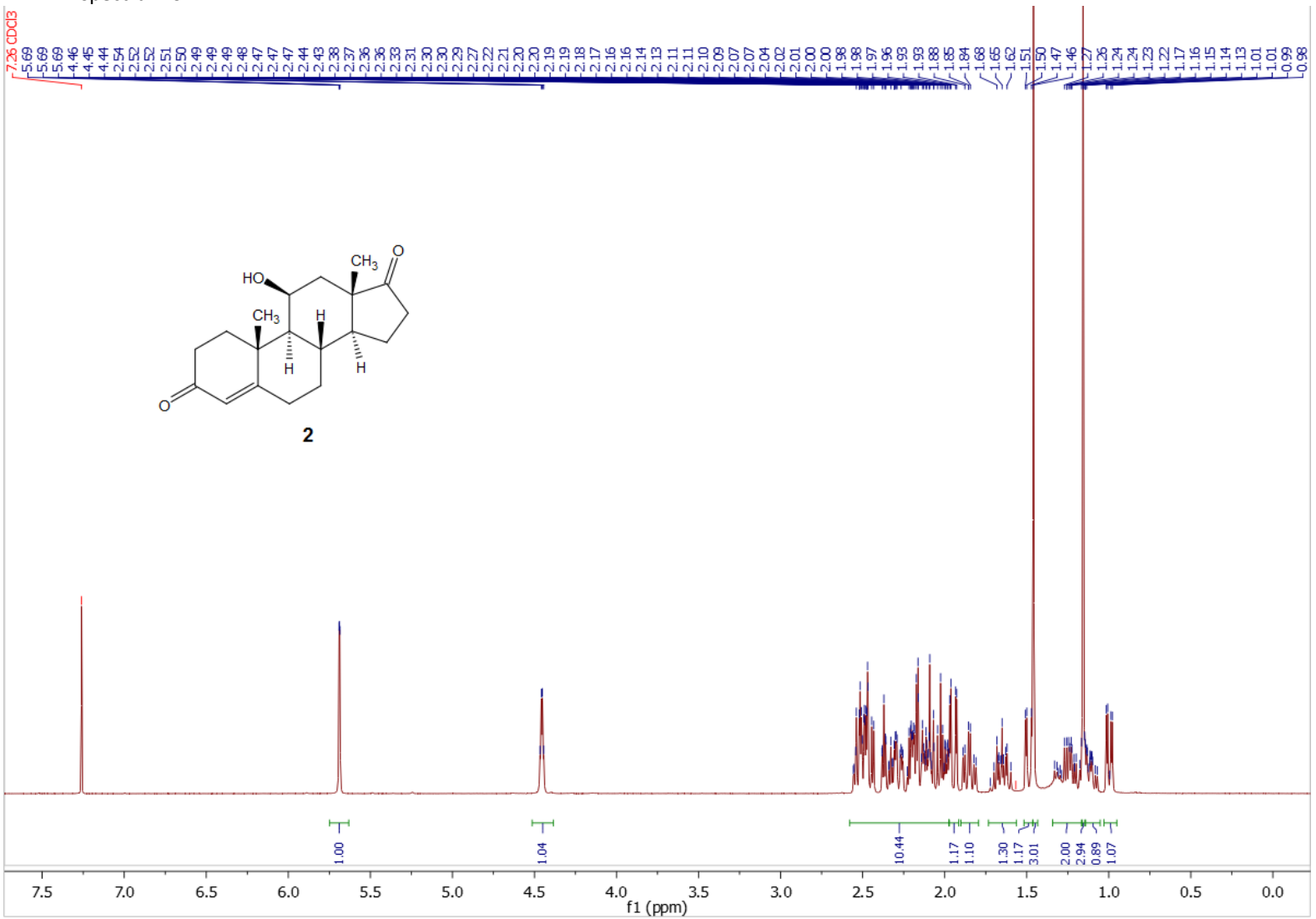


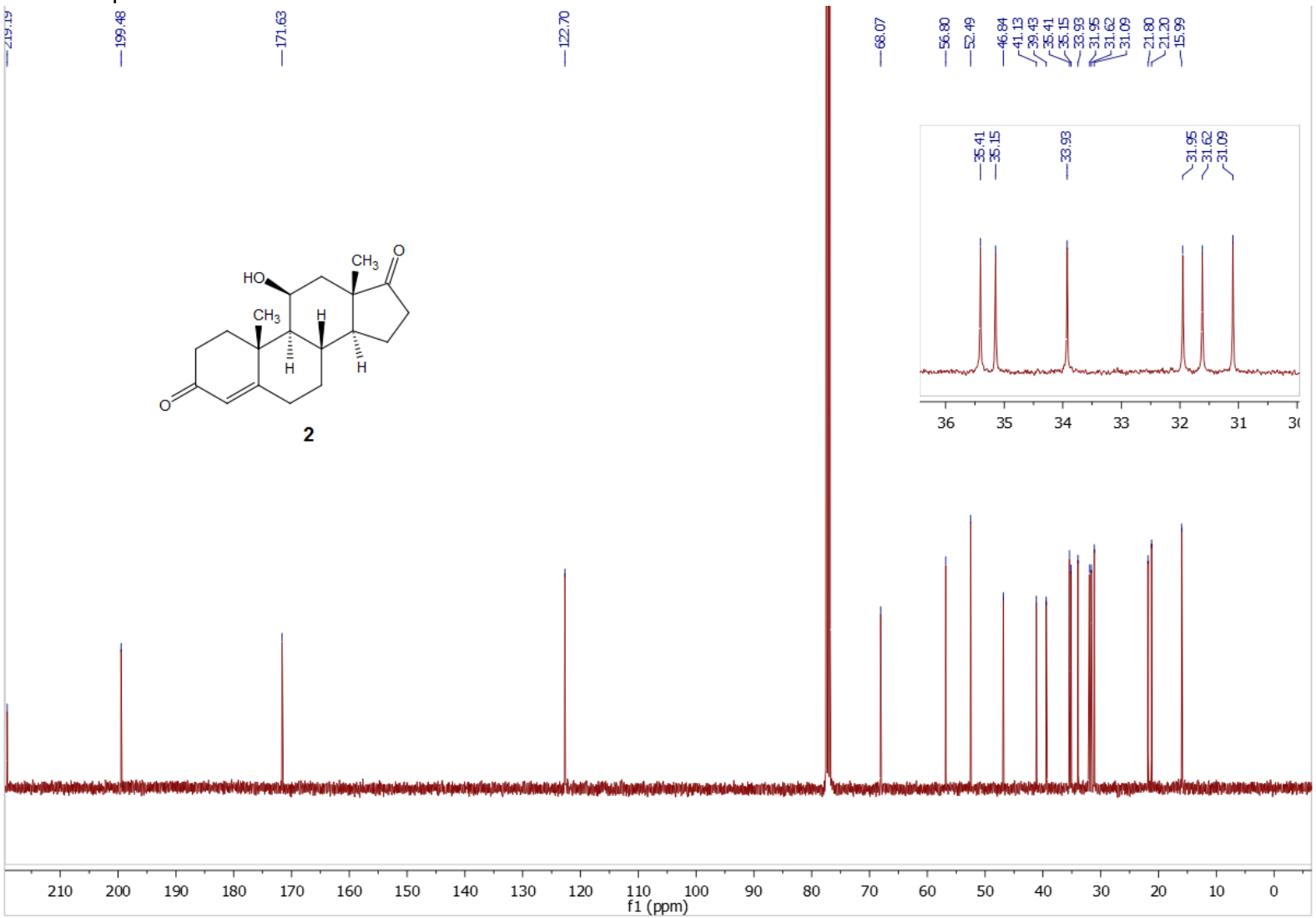
Figure S2: Map of kshA5 and kshB expression vector pAJ30. Codon-optimized *kshA5* and *kshB* with N-terminal 6His-tags were placed under the control of T7 promoter as a single operon in pET21a plasmid. The map was created using SnapGene software. The sequence of the plasmid is available as GenBank file (Supplementary File 2).

NMR spectra

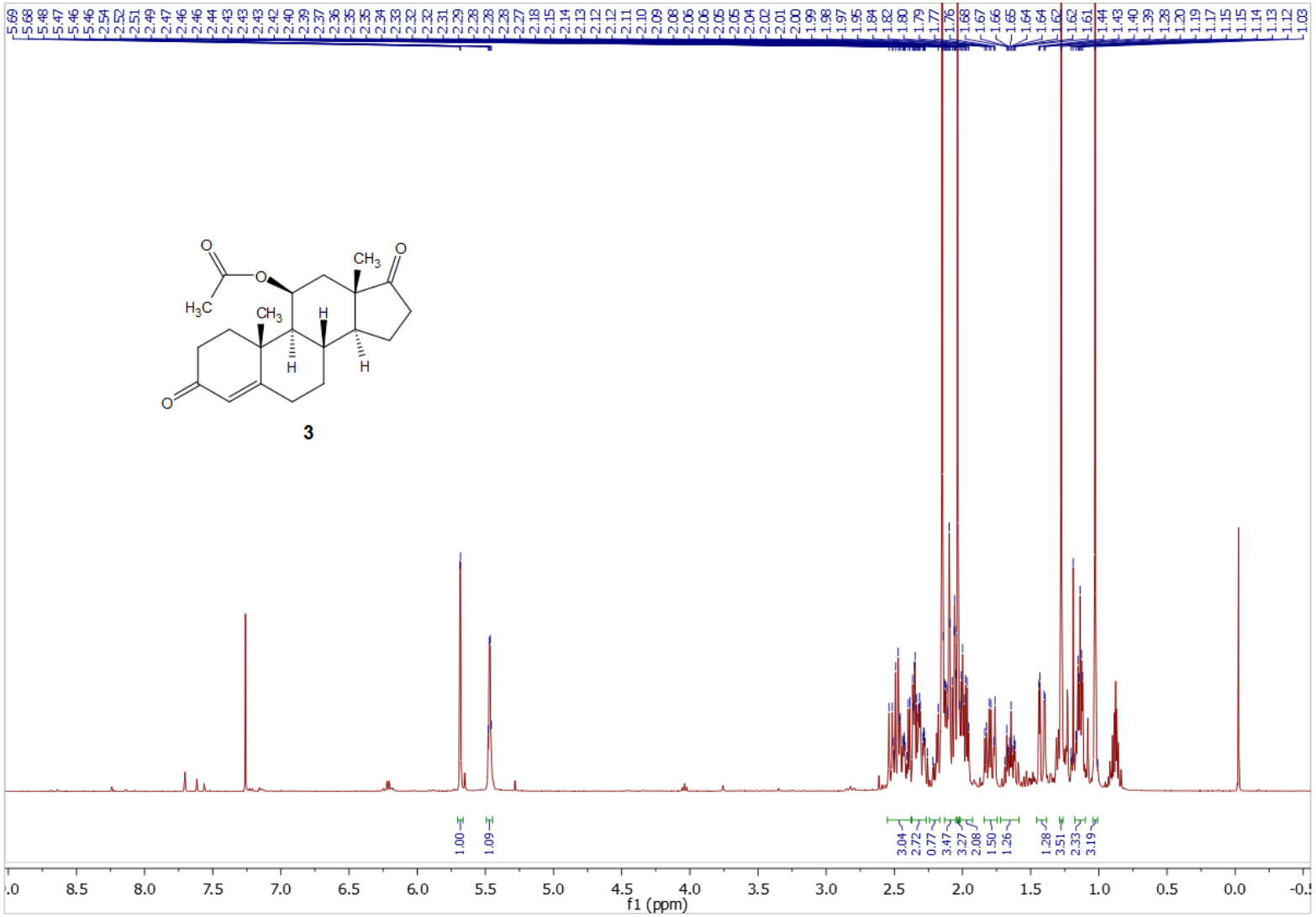
^{1}H NMR spectrum of **2**



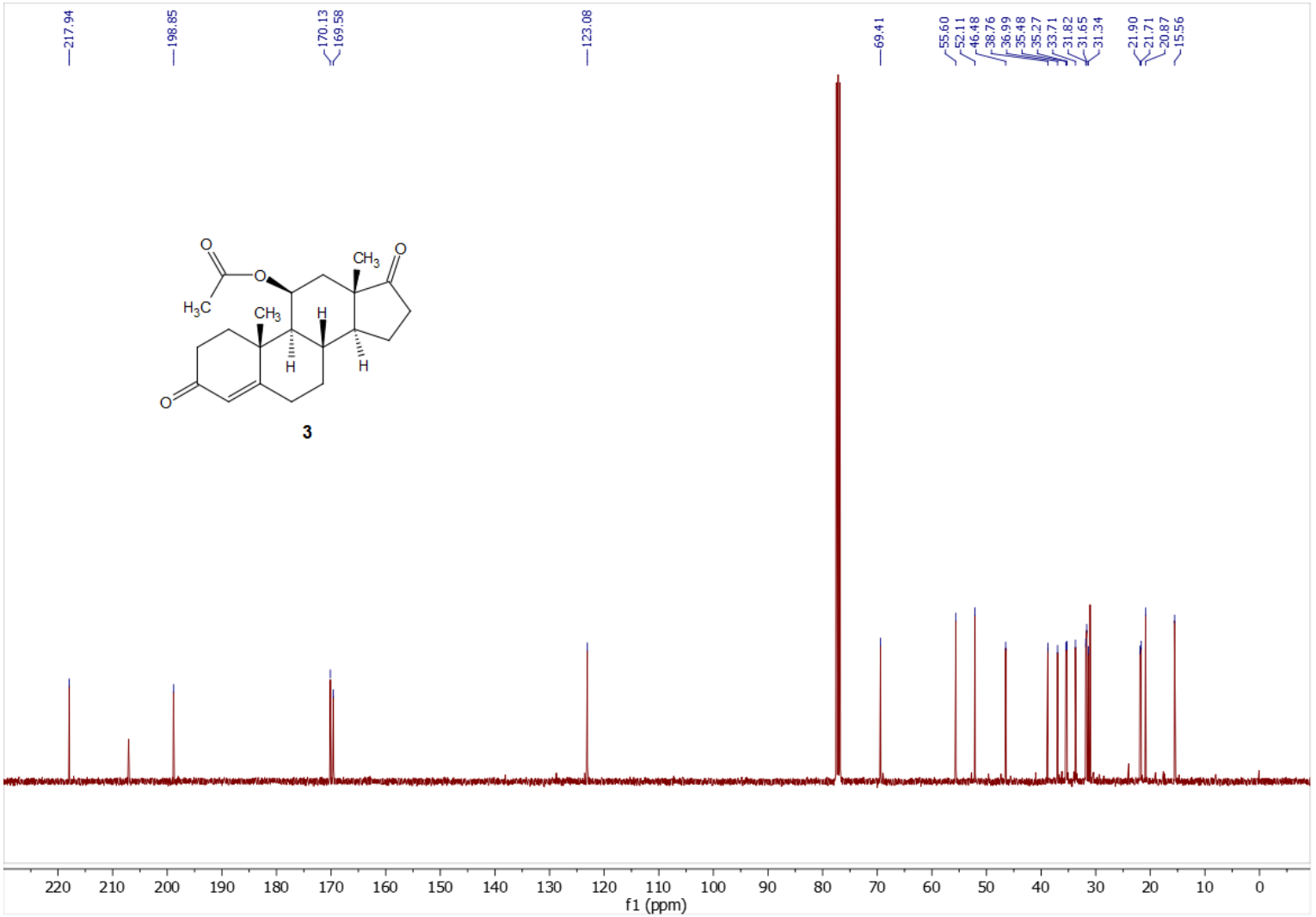
^{13}C NMR spectrum of **2**



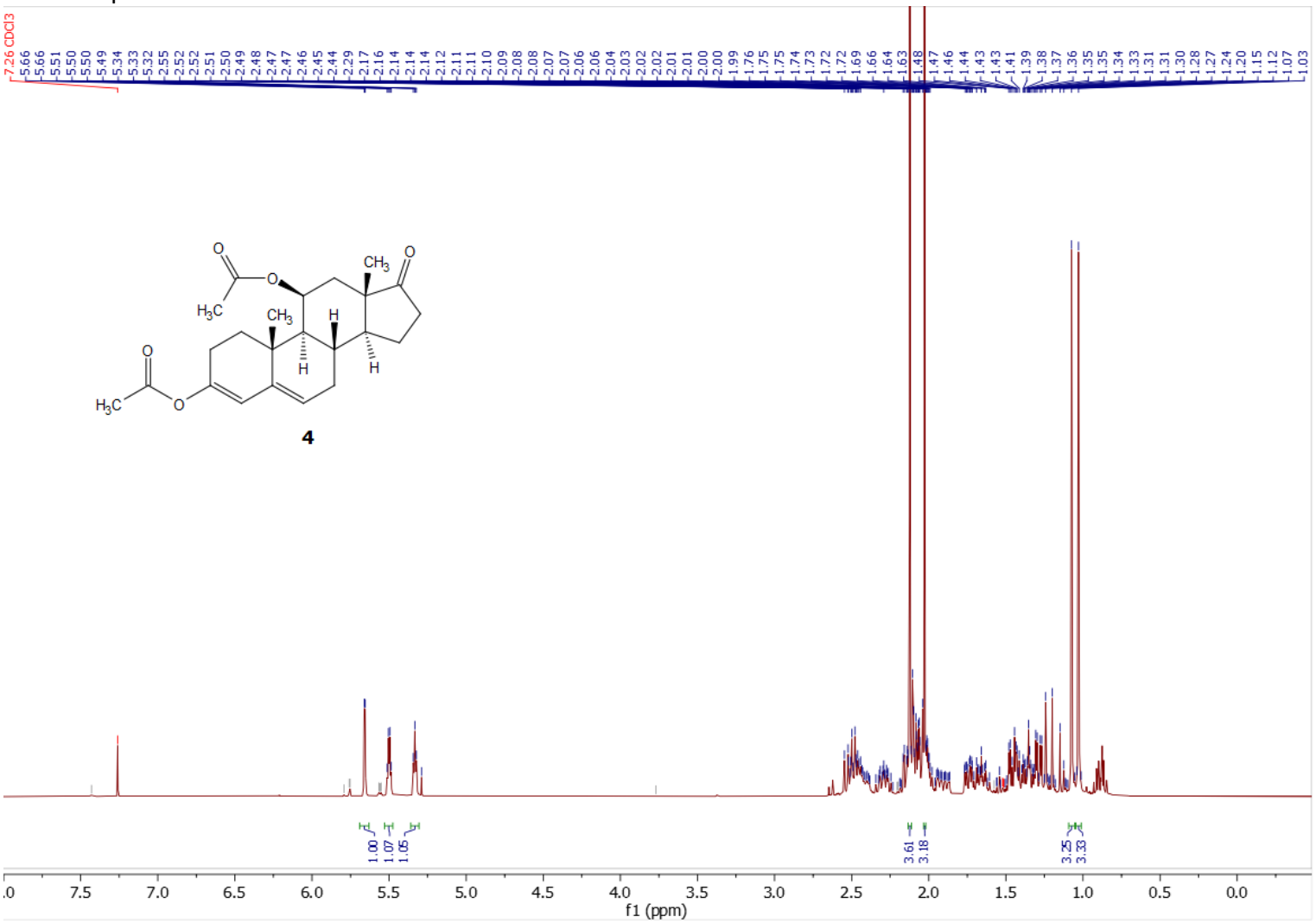
^{1}H NMR spectrum of **3**



^{13}C NMR spectrum of **3**



^{1}H NMR spectrum of **4**



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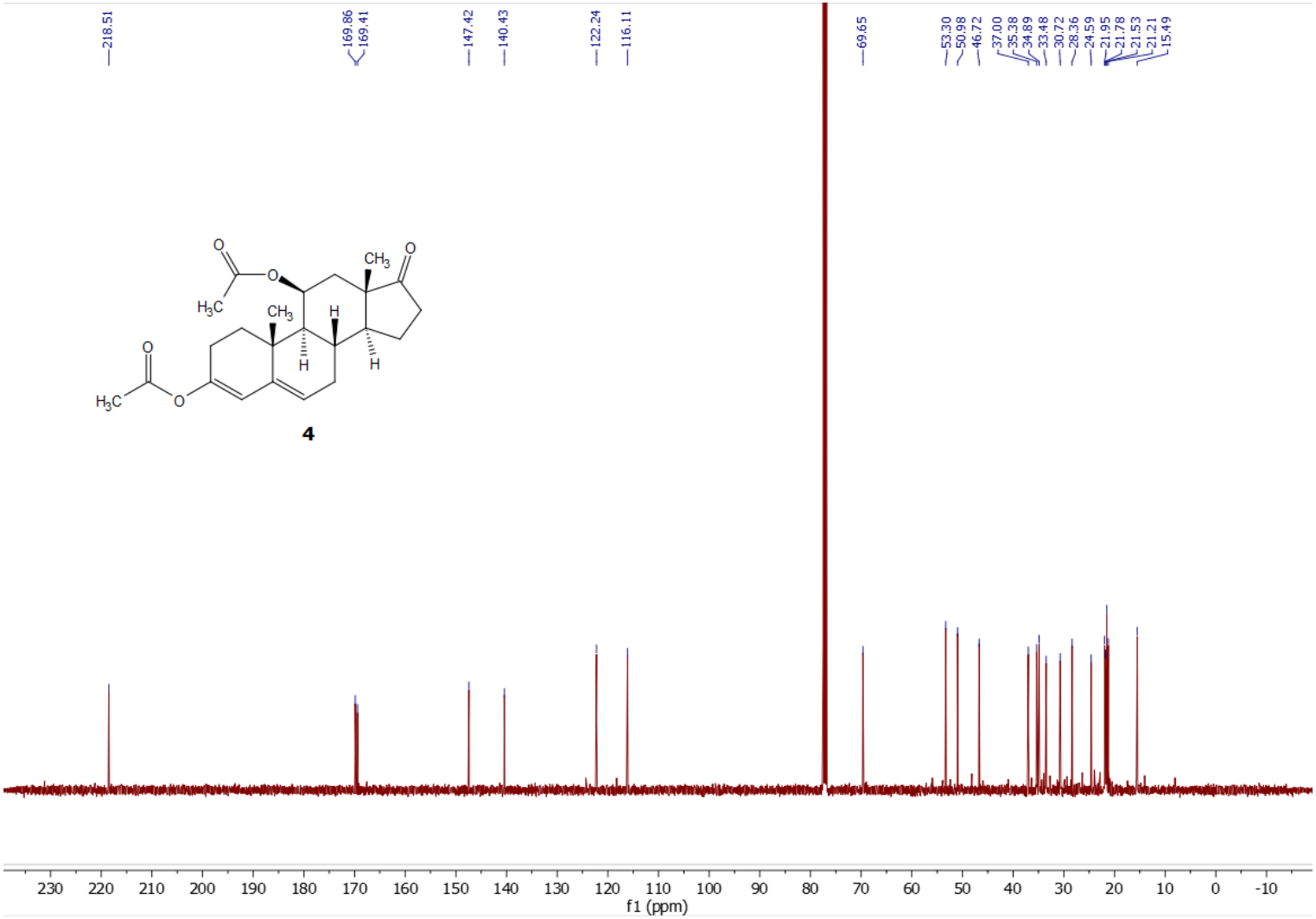
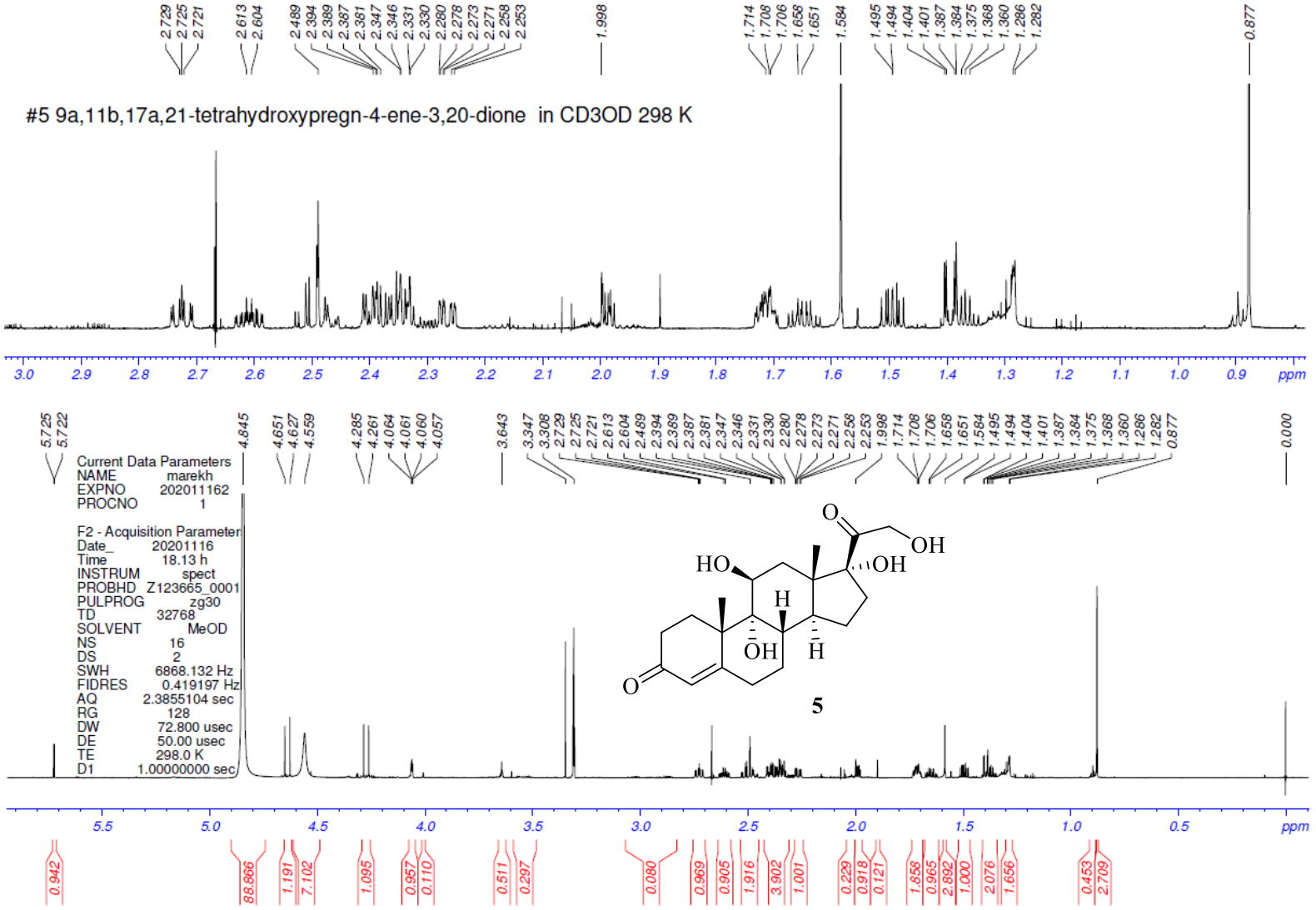
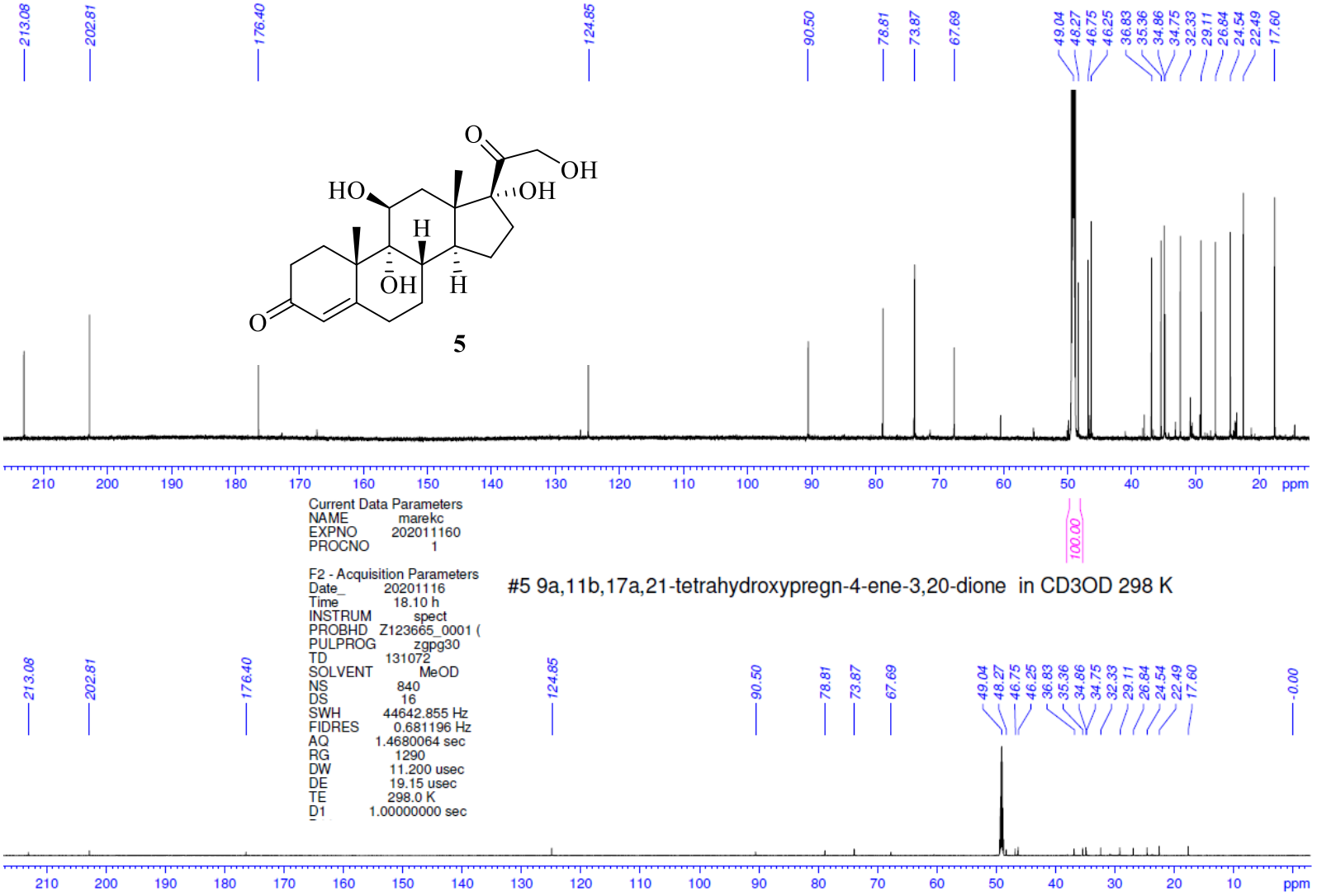


4

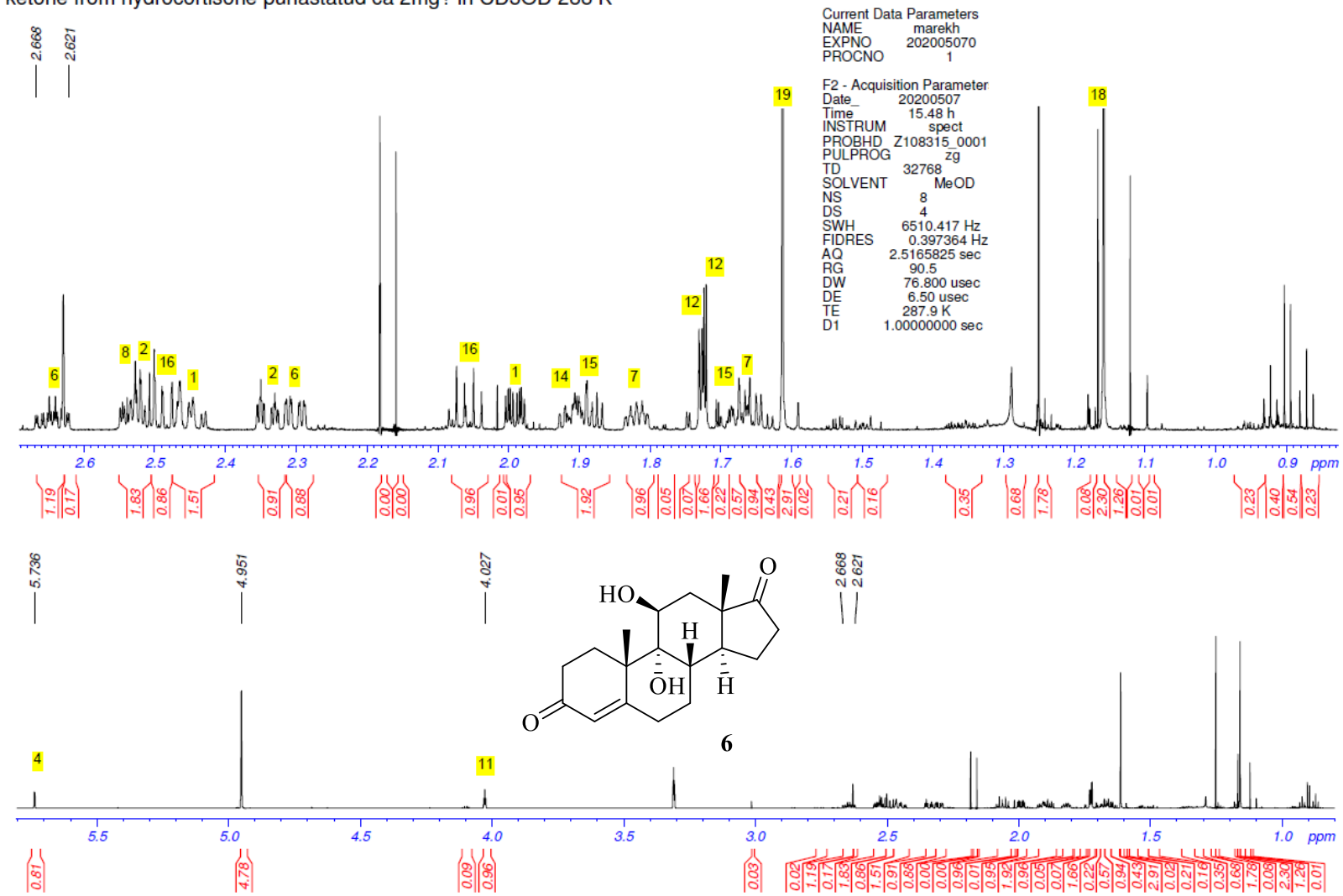
^{1}H NMR spectrum of **5**



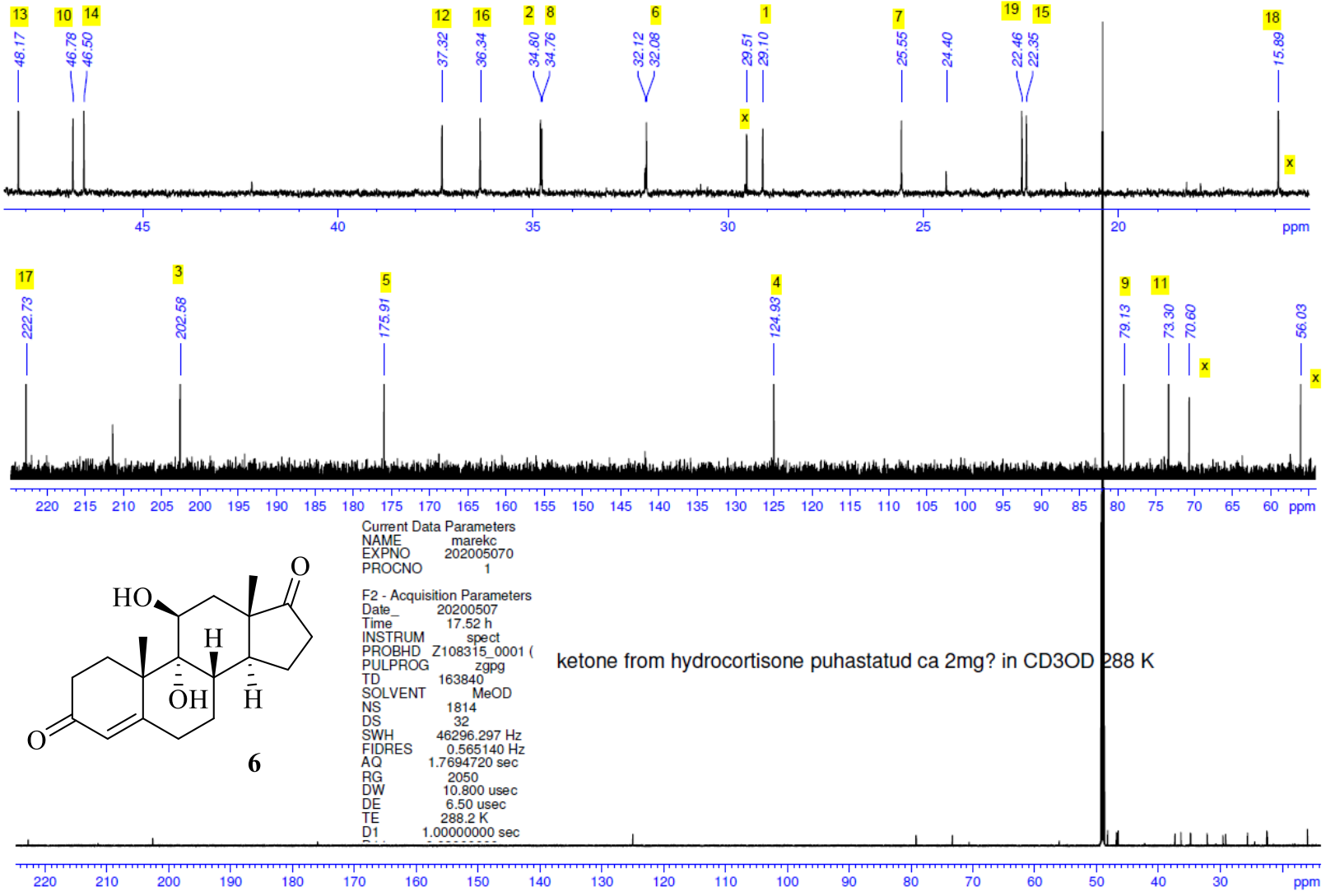
^{13}C NMR spectrum of **5**



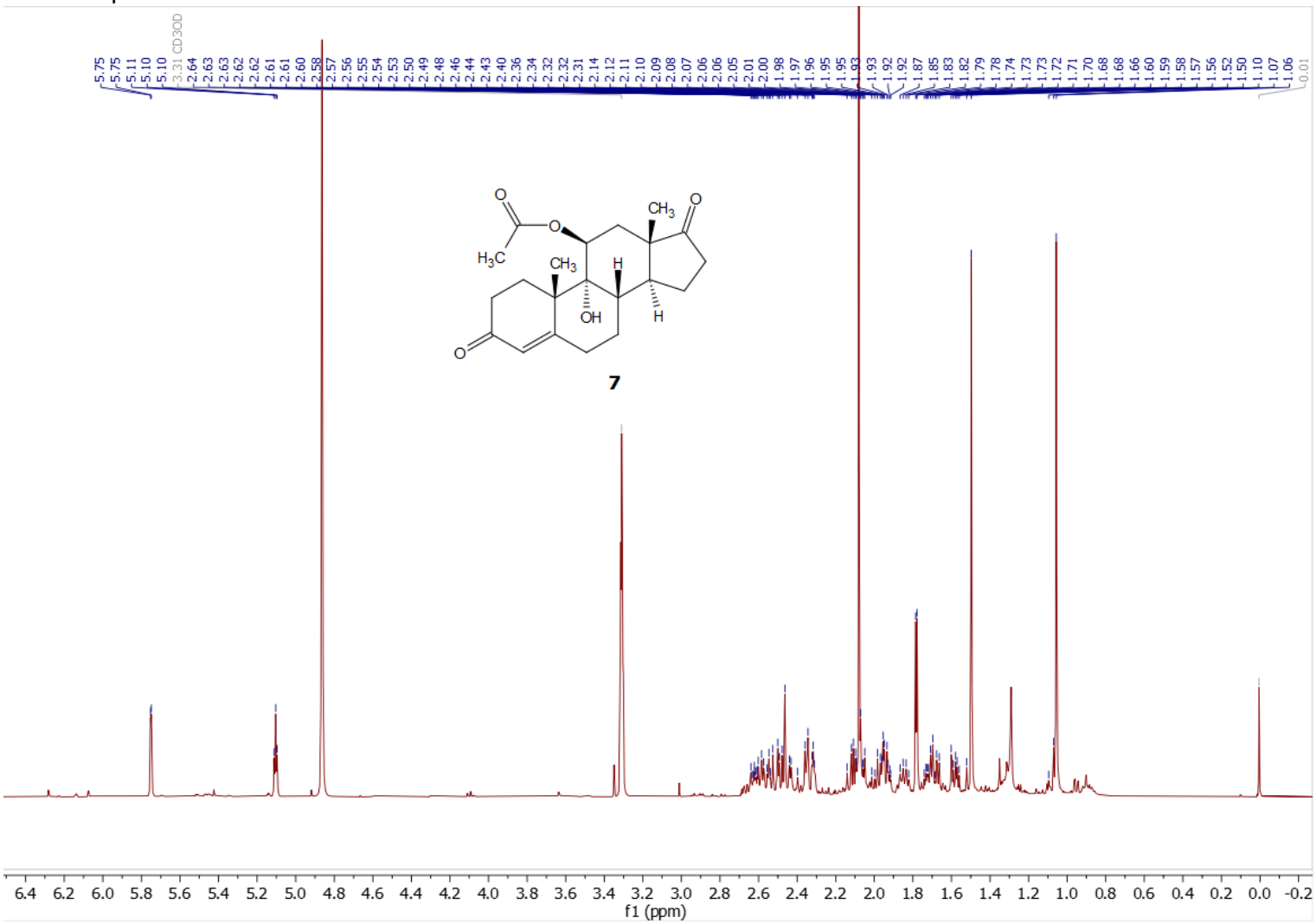
^{1}H NMR spectrum of **6**



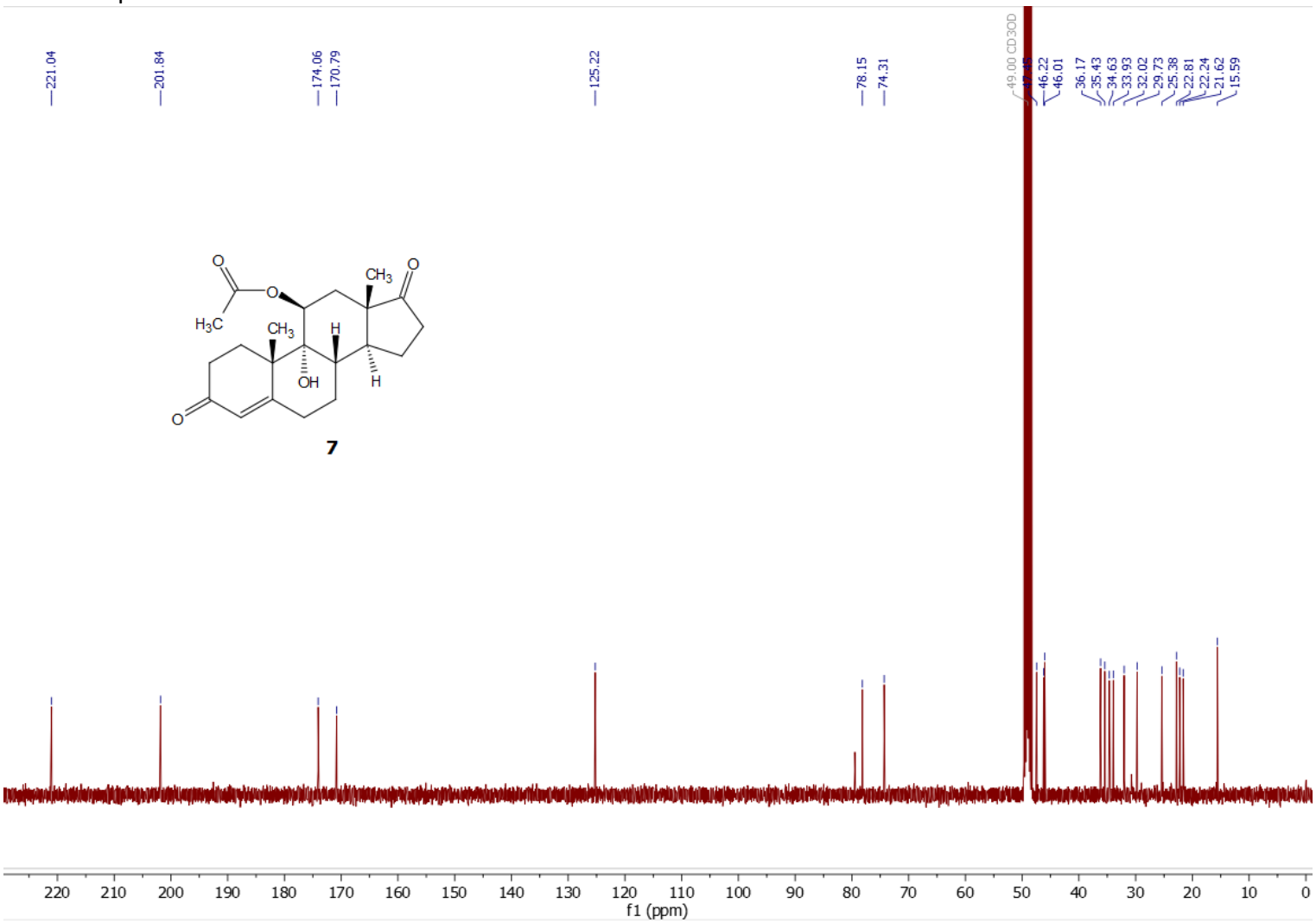
^{13}C NMR spectrum of **6**



^{1}H NMR spectrum of **7**



^{13}C NMR spectrum of **7**



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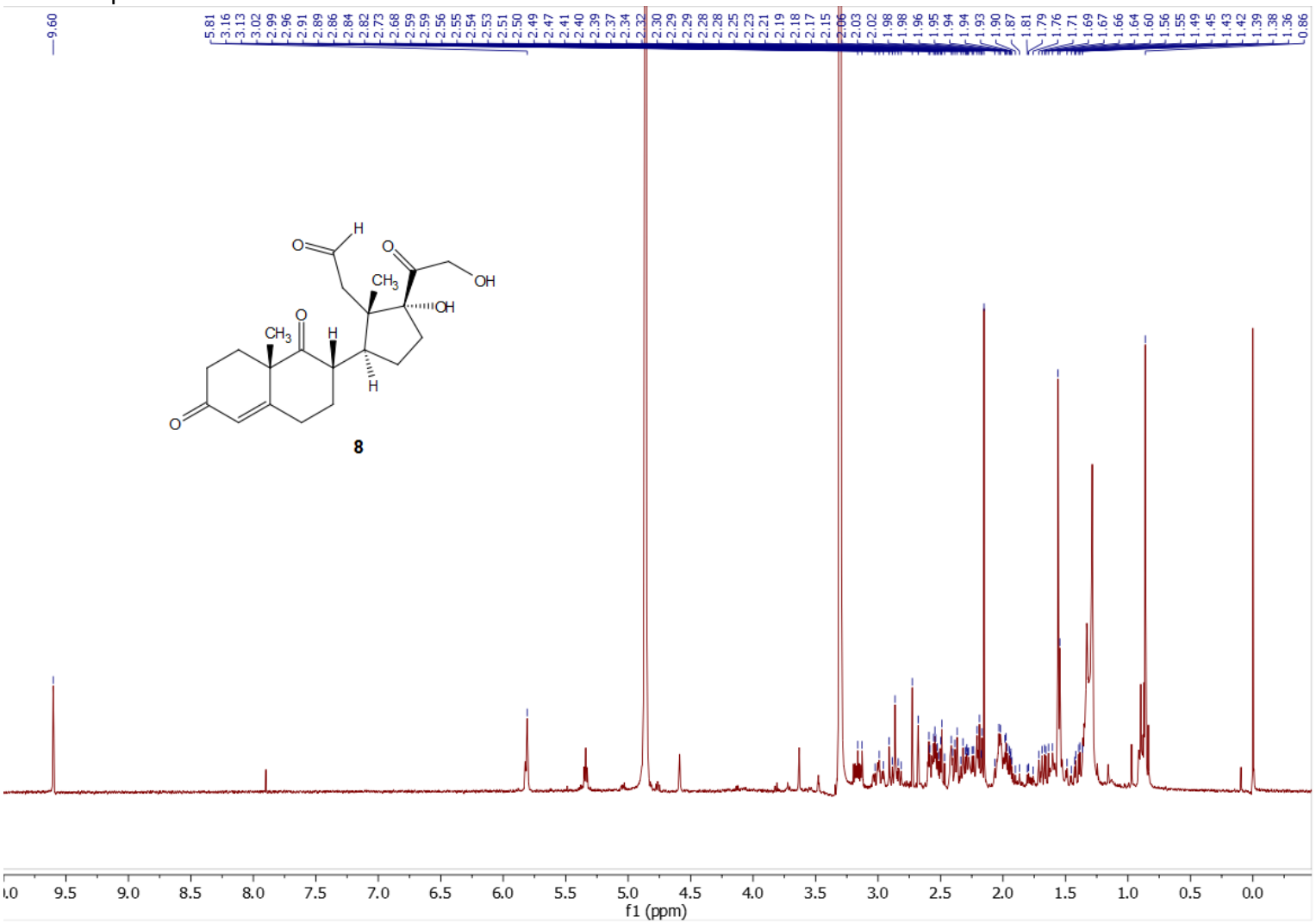
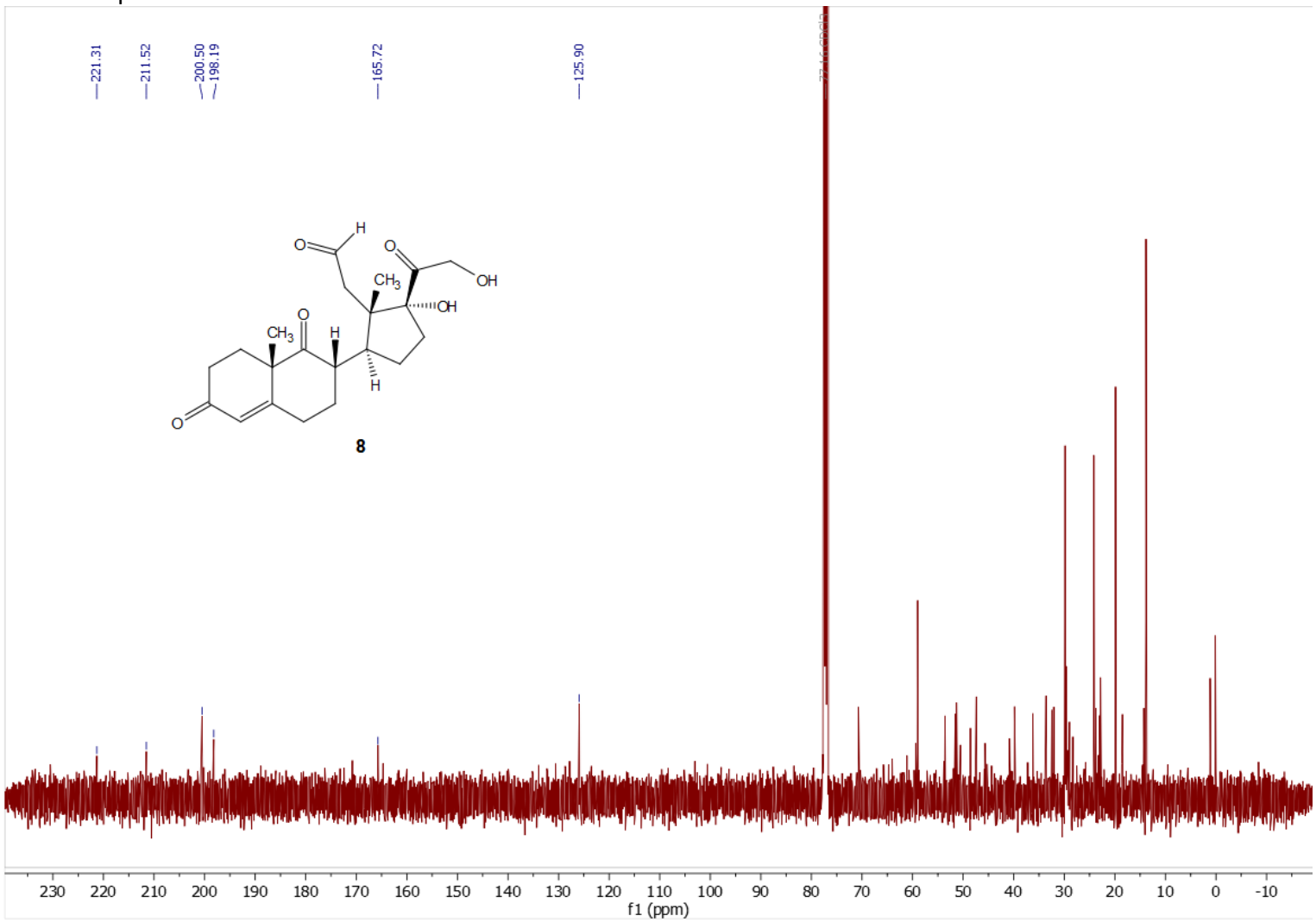
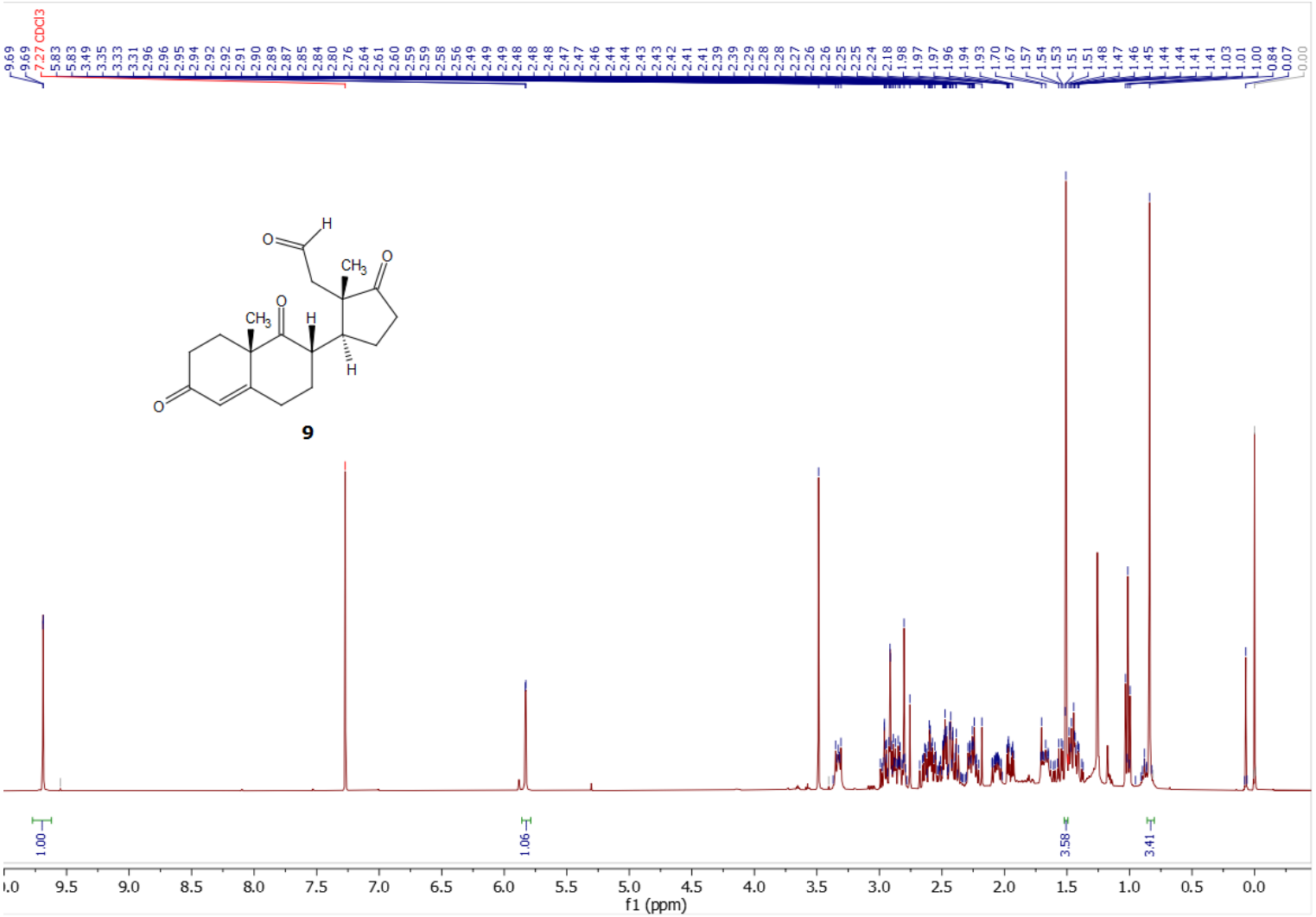


^{13}C NMR spectrum of **8**



^{1}H NMR spectrum of **9**



^{13}C NMR spectrum of **9**