

## Supplementary Material for

### One-pot synthesis of Vinca Alkaloids-Phomopsin Hybrids

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## Molecular dynamics simulations for the two possible epimers of compound **22**

The charge distribution for both epimers at C-8' of compound **22** were obtained by fitting the quantum mechanically calculated (HF/6-31G\*//HF/3-21G\*) molecular electrostatic potential (MEP), as implemented in Gaussian 09<sup>1</sup> to a restrained electrostatic potential (RESP) point-charge model.<sup>2</sup> The general AMBER force field (leaprc.ff10) was used to assign bonded and nonbonded parameters (parm10) to all atoms. (8*R*)-**22** and (8*S*)-**22** plus two chloride ions to achieve electroneutrality were immersed in a box of 3233 TIP3P water molecules<sup>3</sup> and subjected to molecular dynamics (MD) simulations under periodic boundary conditions for 30 ns at 300 K making use of the pmemd module in the AMBER 12 suite of programs.<sup>4</sup> Firstly, solvent molecules and chloride ions were positionally optimized by energy minimization and then allowed to redistribute around the positionally restrained solute (25 kcal mol<sup>-1</sup> Å<sup>-2</sup>) during 50 ps of MD at constant temperature (300 K) and pressure (1 atm). These initial harmonic restraints were gradually removed so as to sample the conformational space of the free ligands during 30 ns. Electrostatic interactions were treated using the smooth particle mesh Ewald method<sup>5</sup> with a grid spacing of 10 Å. The SHAKE algorithm<sup>6</sup> was applied to all bonds and an integration step of 2.0 fs was used throughout. The program ptraj distributed within AmberTools 13.0 was used to analyze the intramolecular distances along the MD simulations. The molecular graphics program PyMOL (v. 0.99rc6, DeLano Scientific, LLC, Palo Alto, CA) was employed for molecular editing, visualization and representation. A clustering

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analysis of the MD trajectories was performed and the geometry of a representative structure of each most populated epimer was relaxed by subjecting it to a cooling process over 60 ps during which the temperature was reduced from 300 to 273 K.

**Docking experiments.** Compounds **20** and **21** were model-built using vinblastine as a template, essentially as reported earlier for other vinca derivatives.<sup>7</sup> Point charges for the energy-minimized geometries were assigned by fitting the quantum mechanically calculated (RHF/6-31G\*\*/RHF/3-21G\*) molecular electrostatic potential (MEP) using Gaussian 03 (Gaussian, Inc., Wallingford, CT). Consistent bonded and non-bonded AMBER parameters for these ligands were assigned by analogy or through interpolation from those already present in the AMBER database for protein atoms (ff03). **20** and **21** were manually docked at the longitudinal interface between two tubulin heterodimers by superimposing them onto the vinblastine structure in the tubulin-vinblastine complex.<sup>3</sup> The resulting complexes were first energy-minimized *in vacuo* to remove any steric clashes within the binding site and then immersed in a truncated octahedron containing ~32,300 TIP3P water molecules and 23 Na<sup>+</sup> ions. The *sander* and *pmemd* modules from the AMBER12 suite of programs (<http://ambermd.org/>) were used for the restrained and unrestrained MD simulations, respectively. Periodic boundary conditions were applied and electrostatic interactions were treated using the smooth particle mesh Ewald method with a grid spacing of 1 Å. The cutoff distance for the non-bonded interactions was 9 Å, the SHAKE algorithm was applied to all bonds, and an integration step of 2.0 fs was used throughout. After an initial energy minimization of the water molecules and counterions, the system was heated to 300 K in 25 ps after which the solvent was allowed to redistribute around the positionally restrained solute for 220 ps. After this time, the restraints were removed and the system was further simulated for 20 ns. Snapshots from each 10-ns MD trajectory were collected every 20 ps for structural and energetic analyses. The *ptraj* module was used to assess the stability of the complexes by calculating the root-mean-square deviations (RMSD) from the initial geometries and for calculating a representative average structure for each complex from the unrestrained MD simulations after removal of the water molecules for visualization purposes. The molecular

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graphics program PyMOL version 0.99 (DeLano Scientific, LLC, Palo Alto, CA) was employed for visualization and model building.

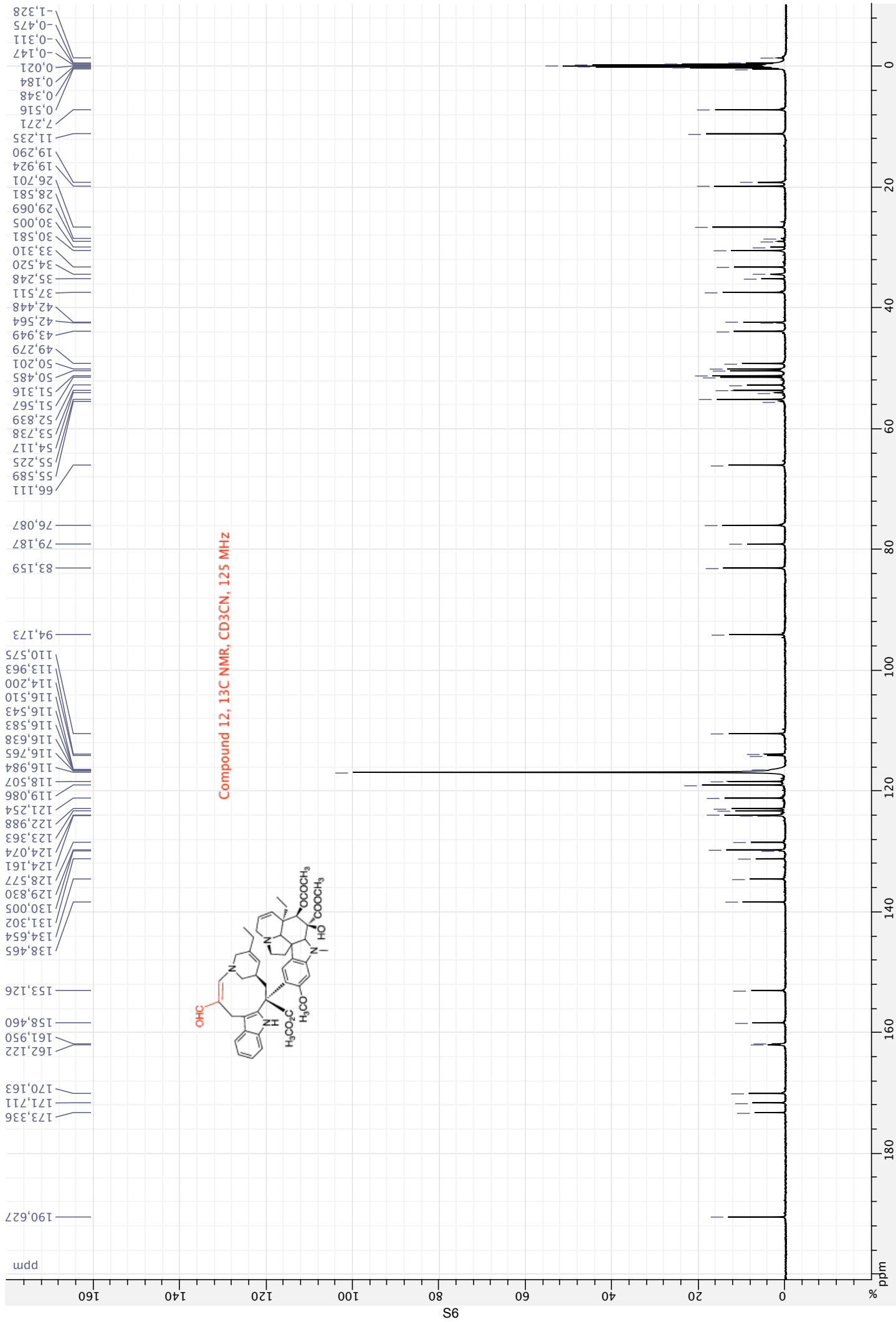
**Inhibition of Tubulin Assembly.** The drug, dissolved in DMSO at different concentrations, was added to a solution of free tubulin (obtained from sheep brain and prepared according to a published procedure<sup>8</sup>) at 0 °C. Then the solution was placed in a temperature controlled cell at 37 °C (micro-tubule assembly), and the increase of the optical density was monitored in a UV spectrophotometer at 350 nm (the maximum was reached in about 1 min). The maximum rate of assembly was recorded and compared to a drug-free sample.

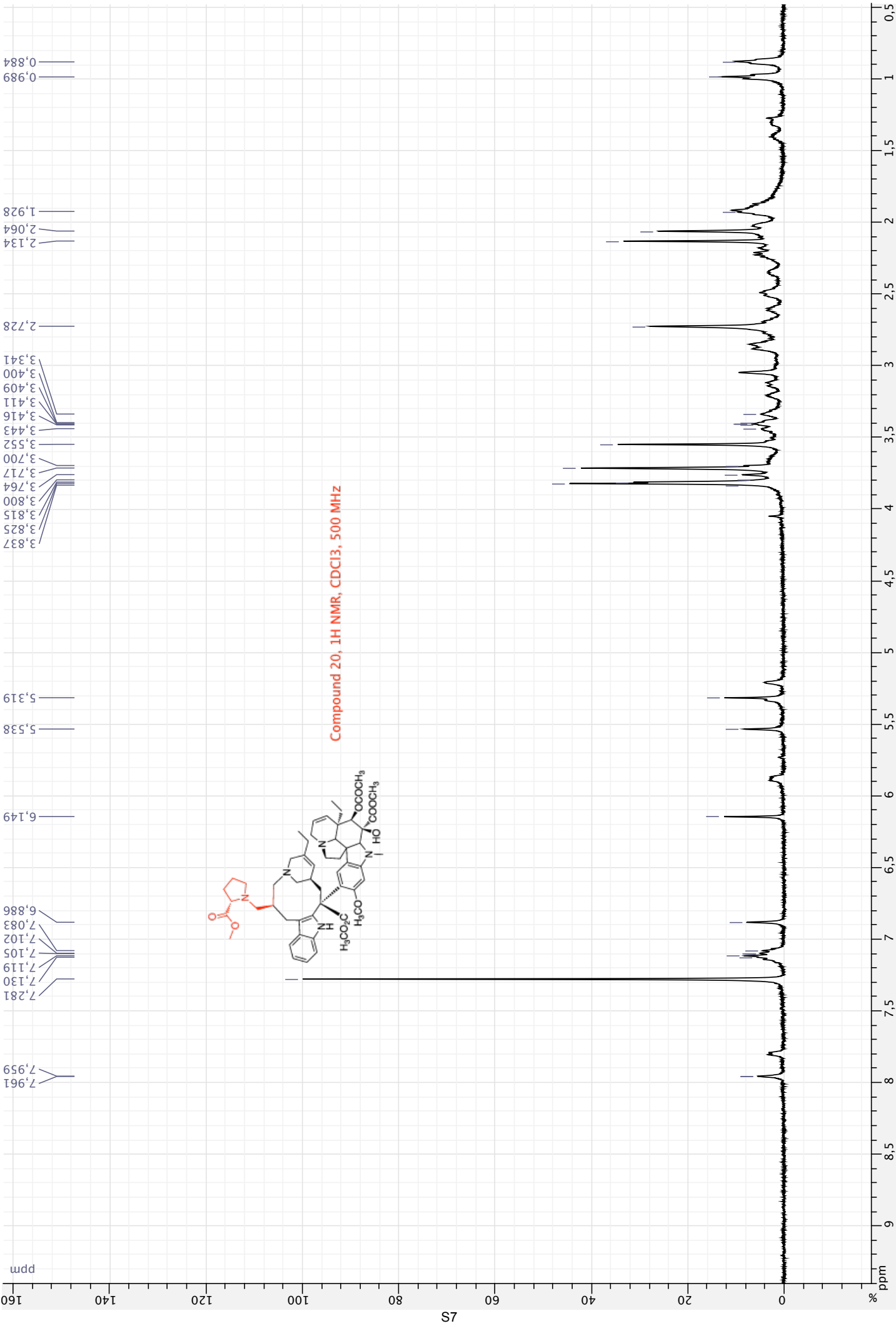
**Cell Culture and Proliferation Assay.** Cancer cell lines were obtained from the American Type Culture Collection (Rockville, MD, USA) and were cultured according to the supplier's instructions. Human HCT116 colorectal carcinoma cells, MCF7 breast cancer cells and K562 leukemia cells were grown in RPMI 1640 supplemented with 10% fetal calf serum (FCS) and 1% glutamine. U87-MG human glioblastoma cells were grown in Dulbecco's Minimal Essential Medium (DMEM) containing 10% FCS and L-glutamine. Cell lines were maintained at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub>. Cell viability was assessed using Promega CellTiter-Blue reagent (Promega, Madison, WI, USA) according to the manufacturer's instructions. Briefly, the cells were seeded in 96-well plates (2.5 × 10<sup>3</sup> cells/well) containing 50 µL of growth medium. After 24 h of culture, the cells were supplemented with 50 µL of medium containing different concentrations of the tested compound dissolved in DMSO (less than 0.1% in each preparation). After 72 h of incubation, 20 µL of resazurin was added for 1.5 h before recording fluorescence ( $\lambda_{\text{ex}} = 560 \text{ nm}$ ,  $\lambda_{\text{em}} = 590 \text{ nm}$ ) using a micro-titer plate fluorimeter. The IC<sub>50</sub> corresponds to the concentration of compound that induced a 50% decrease in fluorescence of drug-treated cells compared with untreated cells. Experiments were performed in triplicate.

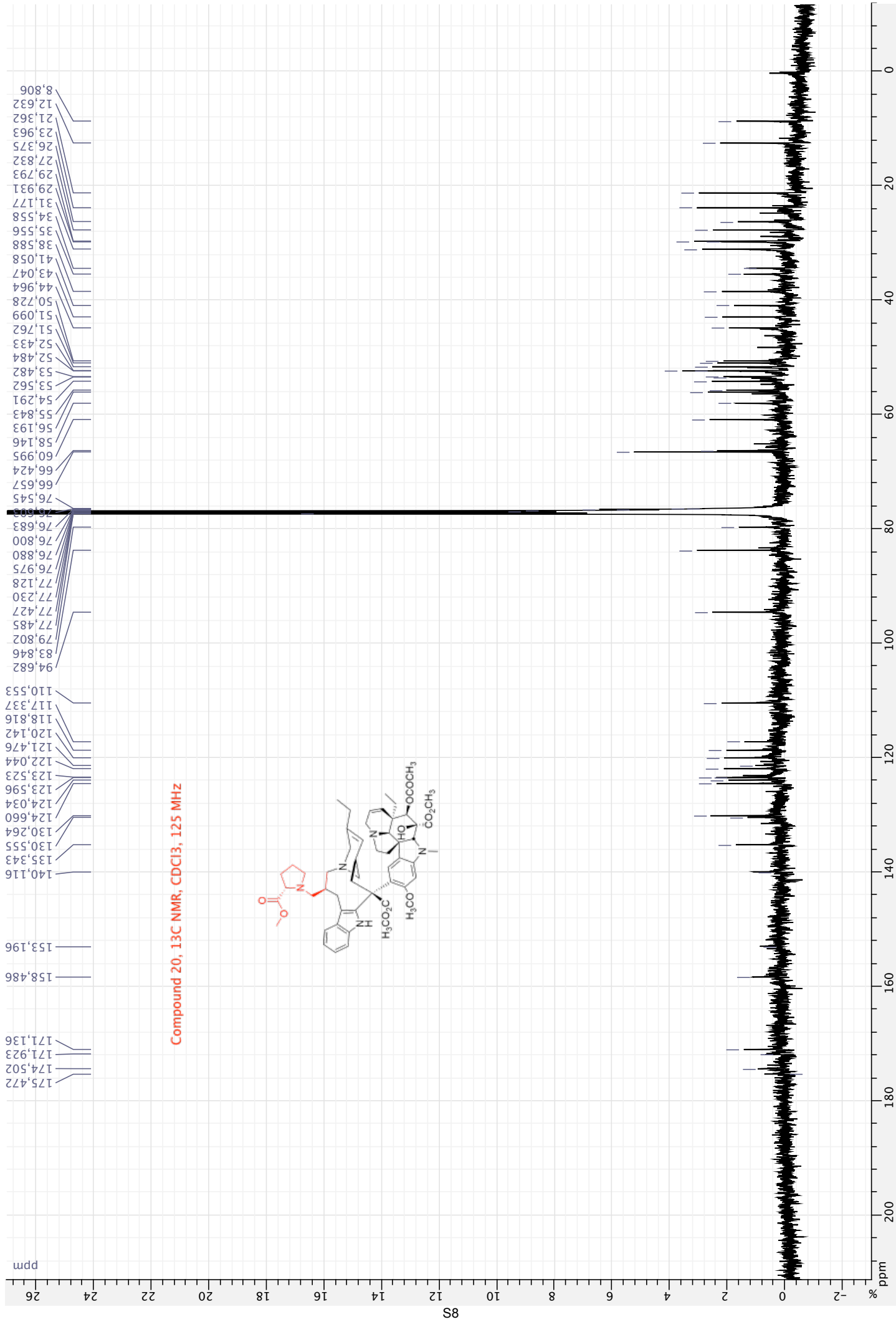
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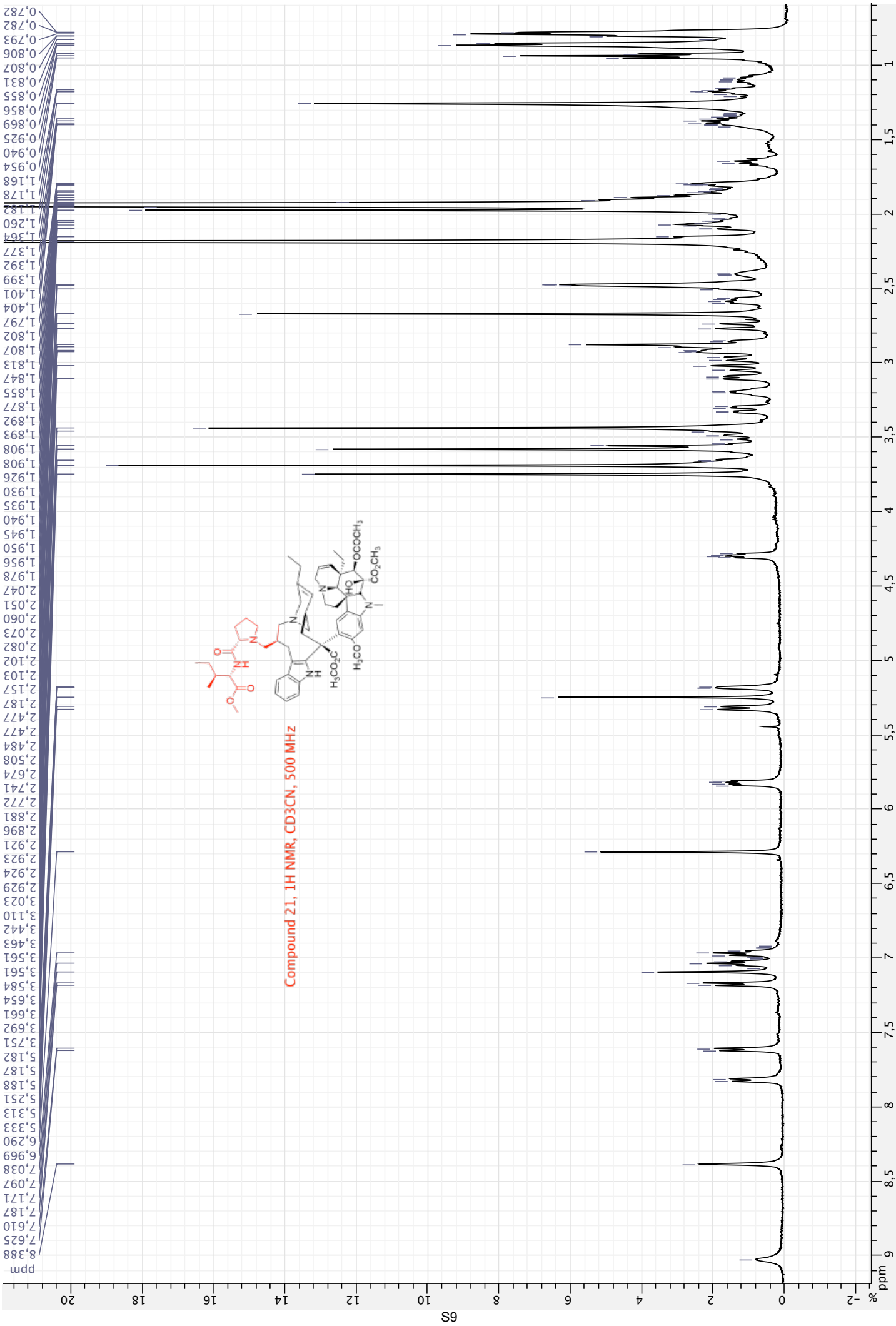


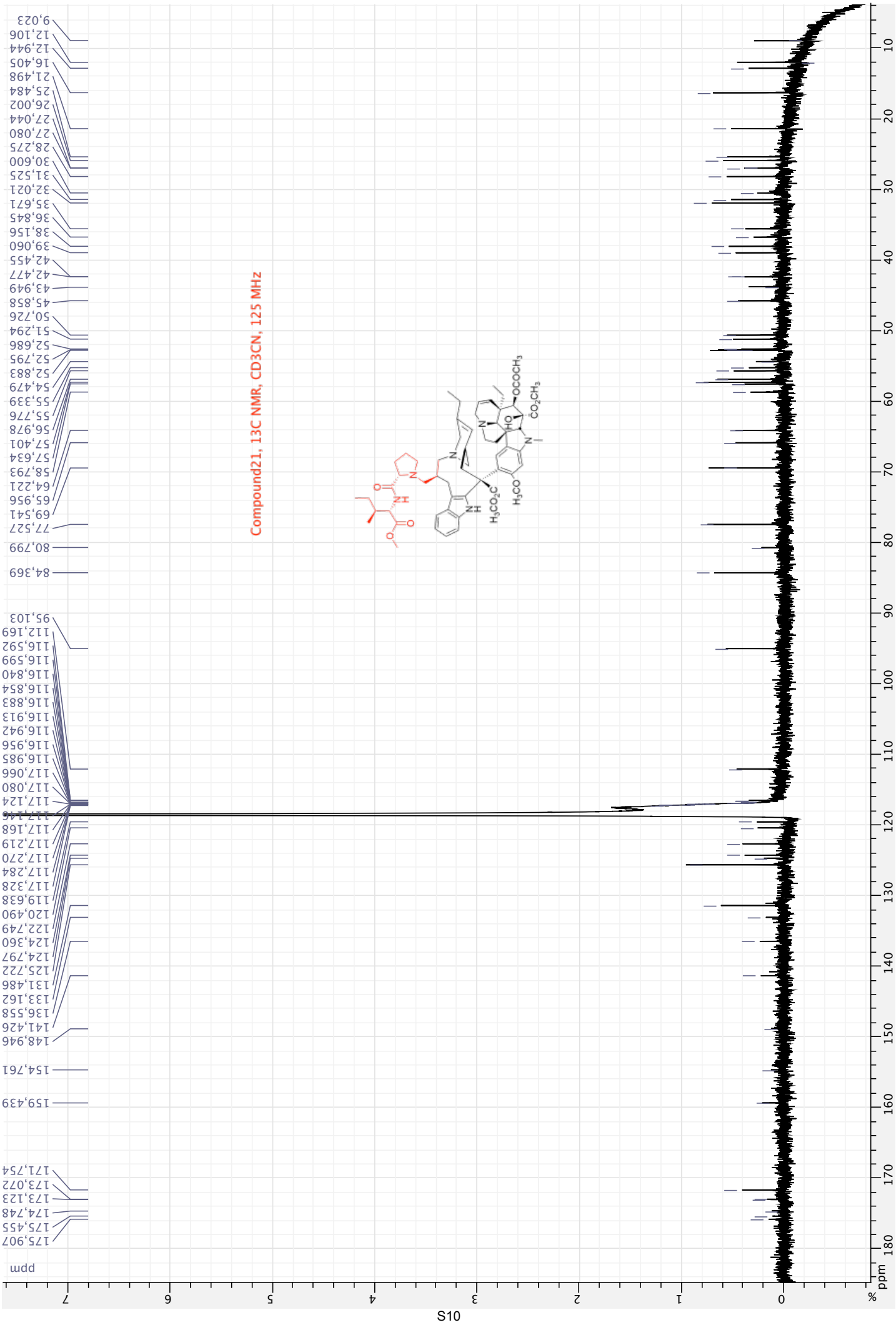


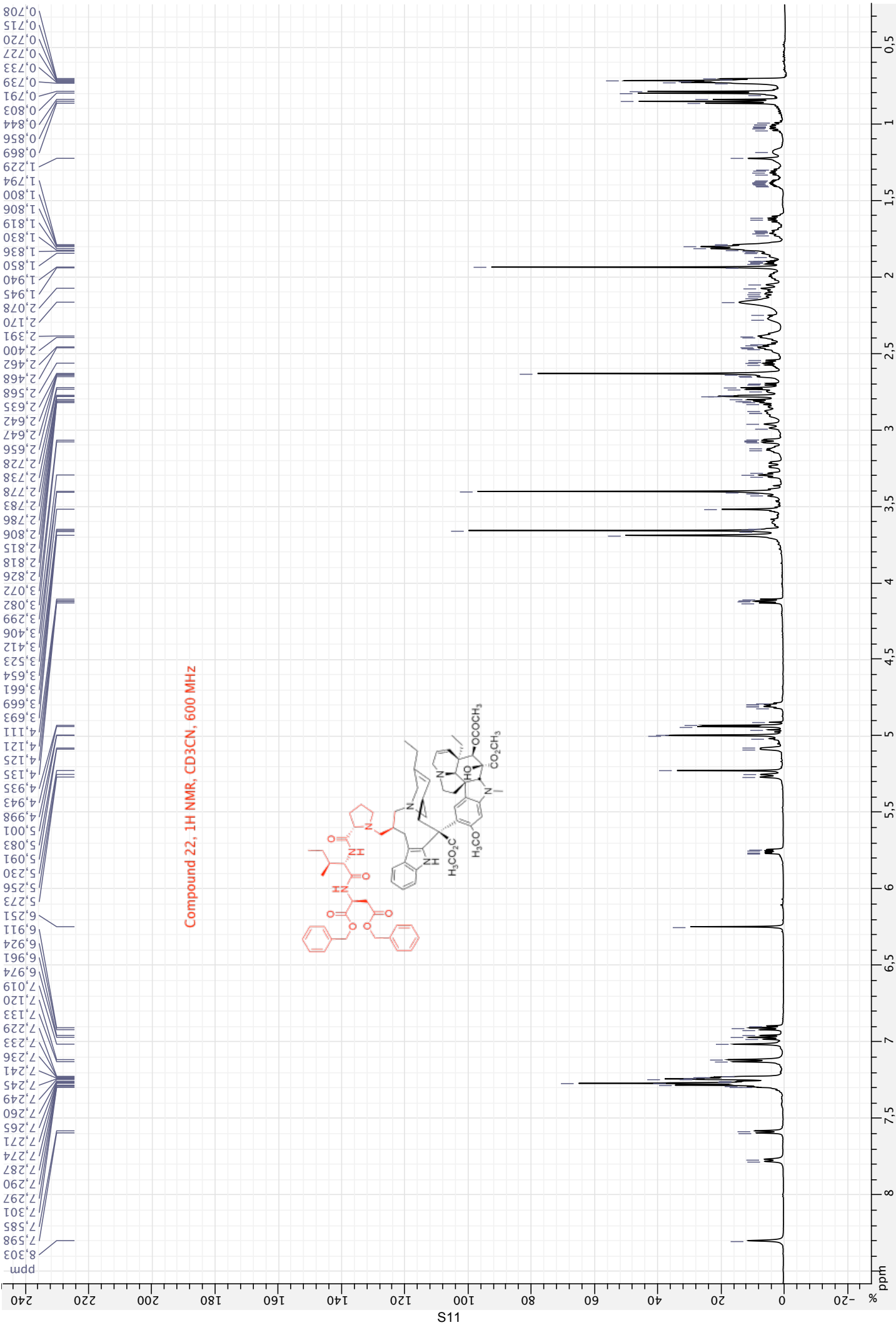


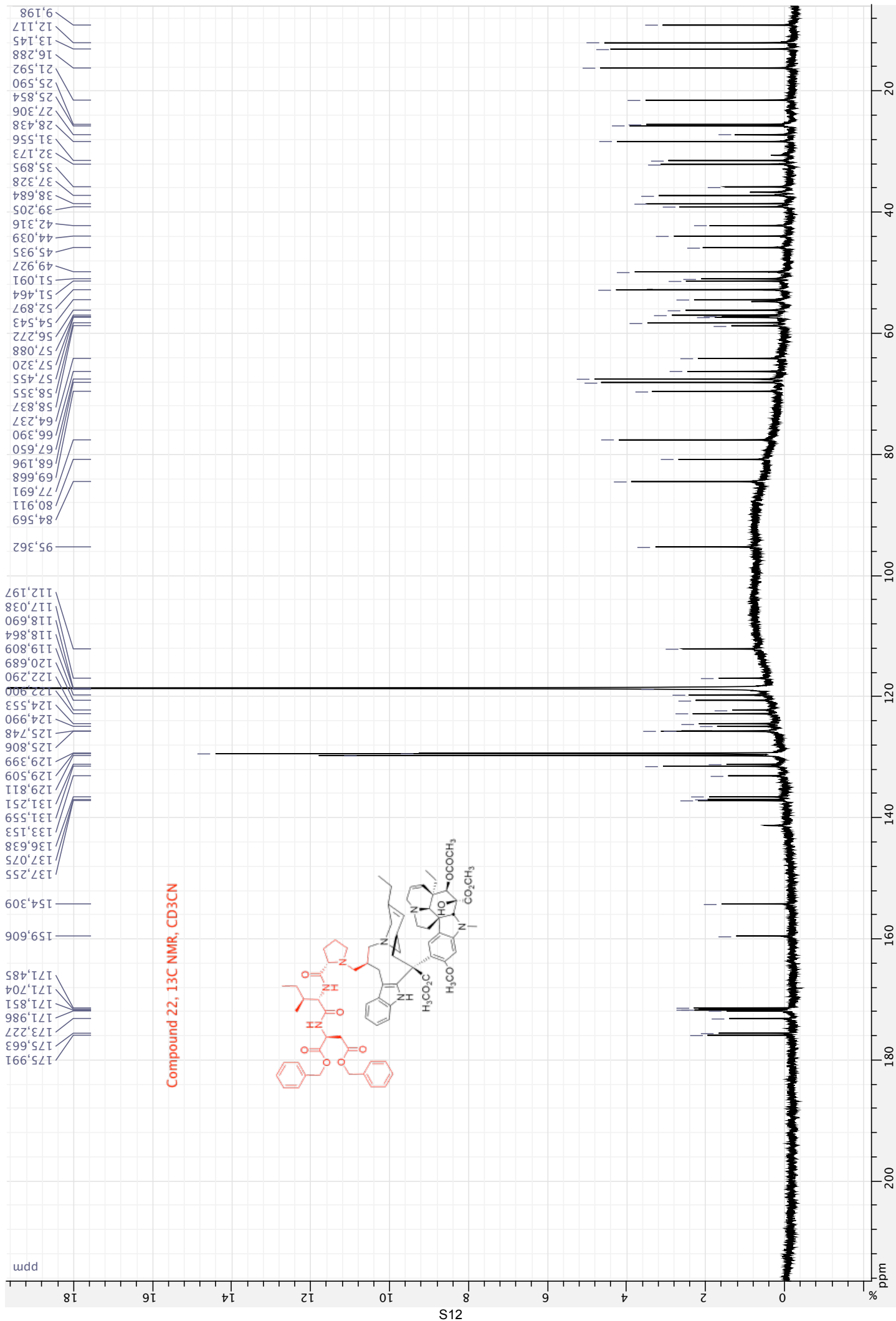


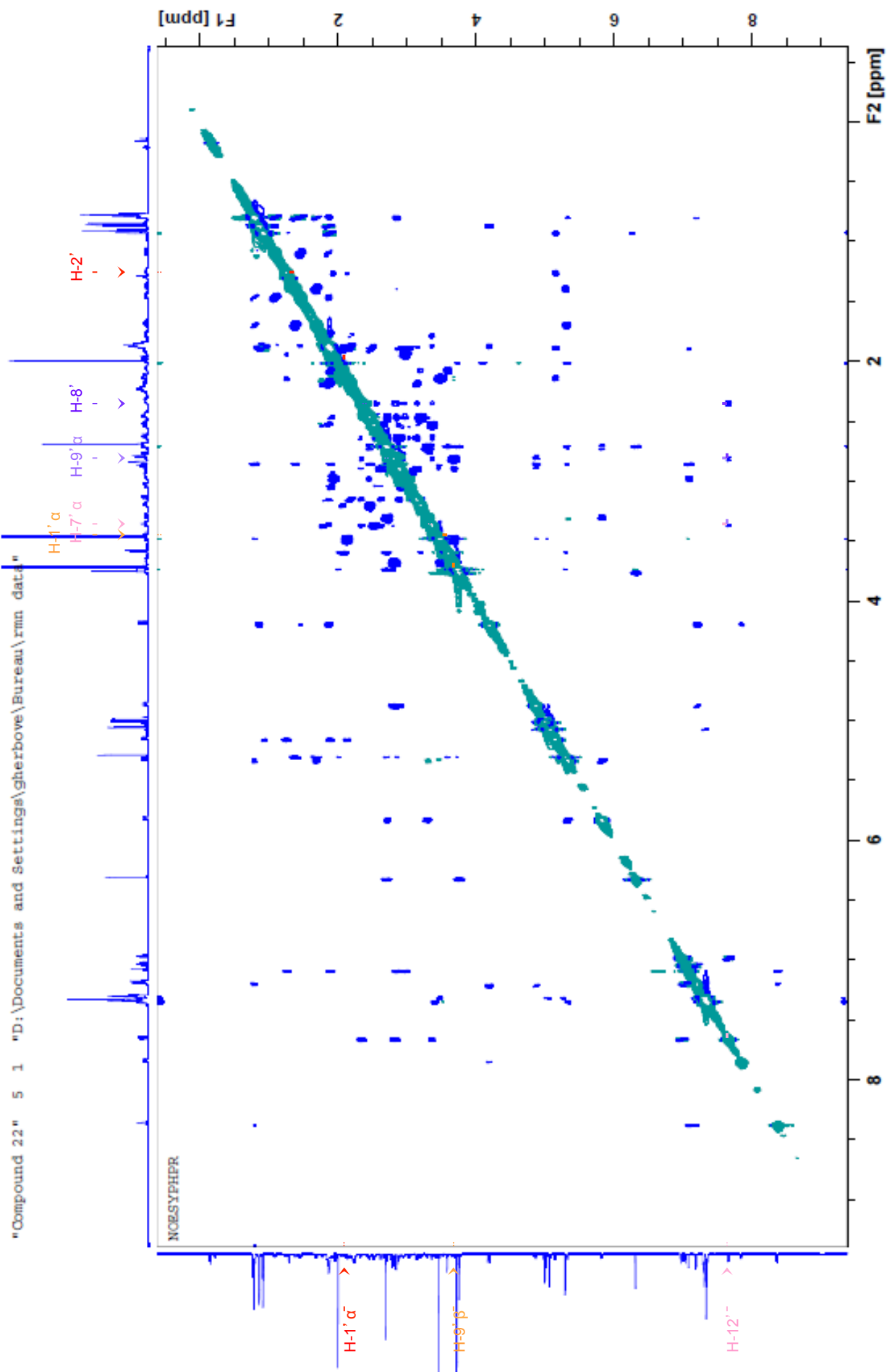




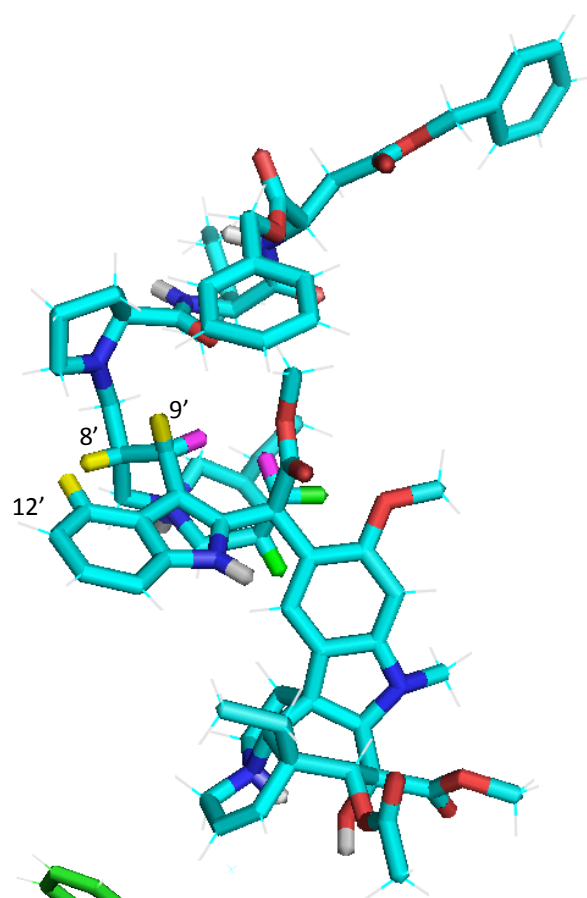




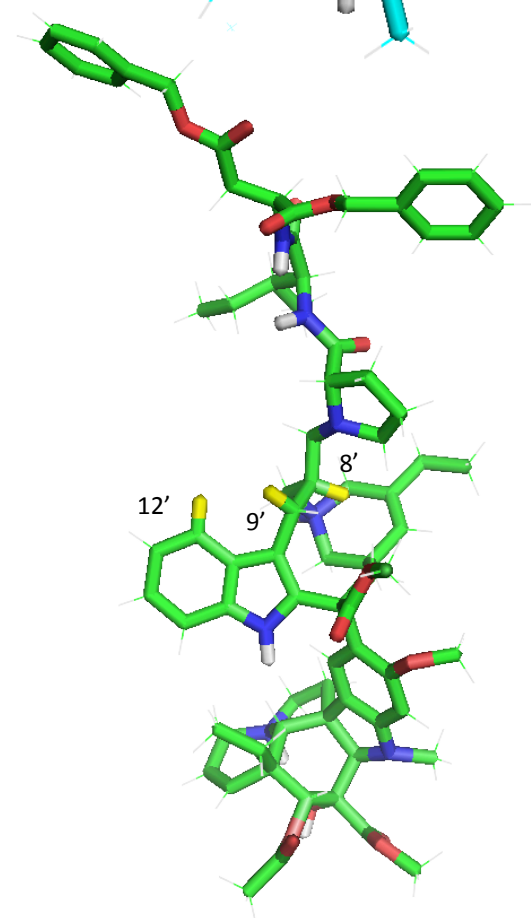




Minimized structures of the two epimers of compound **22**



**8'(*R*)-22**



**8'(*S*)-22**