Supporting information for:

Doing the methylene shuffle – further insights into the inhibition of mitotic kinesin Eg5 with *S*-trityl L-cysteine

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Classical Molecular Dynamics methodology

Two minimisation cycles were applied as follows: first, the solute was restrained, while water molecules and ions were minimised over 500 steps each of steepest descents (SD) and conjugate gradients (CG). Solute restraints were then removed and 1000 steps of SD and 1500 steps of CG applied. MD simulations initially involved the NVT ensemble, with the system being heated from 0 to 300 K over 20 ps, during which the solutes were constrained with a force constant of 10.0 Kcal/mol/Å^2. Solute restraints were then removed and NVT was replaced by the NPT ensemble at a constant temperature and pressure of 300 K and 1 atm for the production phase [1], with bonds involving hydrogen atoms constrained using the SHAKE algorithm [2] and a 2 fs time step employed throughout all simulations (15 ns for all systems). Periodic boundary conditions with a 12 Å cutoff for non-bonded interactions were used, with the particle mesh Ewald (PME) method [3] applied to account for the long-range electrostatic interactions.

Free energy calculations

The total binding free energies ($\Delta G_{binding}$) of the complexes were calculated using the MM/PBSA method based on Equation 1.

$$\Delta G_{binding} = \langle G_{complex} \rangle - \left(\langle G_{receptor} \rangle + \langle G_{ligand} \rangle \right) \qquad \text{(Equation 1)}$$

The composite terms of the binding free energies for the complex, receptor (in this case Eg5) and the ligand(s) were calculated from Equation 2, which contains the enthalpic (ΔH), entropic (ΔS) and solvation free energy ($\Delta G_{solvation}$) contributions. ΔH is the summation of the standard bonded and non-bonded terms obtained from the force field equation and represents the molecular mechanics energy. ΔS is determined from translational, rotational and vibrational contributions based on classical statistical thermodynamics [4, 5]. The solvation free energies ($\Delta G_{solvation}$) consist of polar (ΔG_{polar}) and non-polar ($\Delta G_{non\text{-}polar}$) contributions (Equation 3), with the former being calculated by Poisson-Boltzmann (PB) calculations [6] and the latter by a function of the solvent-accessible surface area (SASA) [7]. To calculate ΔG_{polar}, grid spacing was set at 0.5 Å with evaluation of all pairwise interactions using an internal and external dielectric constant of 1.0 and 80, respectively. The SASA term was based on Equation 4, where γ and b are 0.0054 Kcal/mol.Å^2 and 0.92 Kcal/mol, respectively. The binding free

energies of Eg5 in a complex with STLC and/or ADP were averaged from 200 snapshots collected from the last 5 ns of the MD simulations.

$$\Delta G_{binding} = \Delta H - T\Delta S + \Delta G_{solvation} \quad \text{(Equation 2)}$$

$$\Delta G_{solvation} = \Delta G_{polar} + \Delta G_{non-polar} \quad \text{(Equation 3)}$$

$$\Delta G_{non-polar} = \gamma \cdot SASA + b \quad \text{(Equation 4)}$$

Solute entropic contributions were estimated from the sampled structures based on mass-weighted covariance matrix analysis (quasiharmonic analysis) using the *ptraj* modules in AMBER [8]. This method can calculate the entropy, heat capacity, and internal energy from the structure of a molecule. The masses and the moments of inertia of the complexes are calculated for the translational and the rotational degrees of freedom [1]. The vibrational contribution to the entropy of the complexes is calculated using quasiharmonic analysis from the mass-weight covariance matrix. Due to demanding computational times, the first, 50th, and 100th, 150th, and 200th configurations of the last 5 ns of the trajectory were selected.

The MM-PBSA approach calculates free energies (G) based on Equation S1. We used 200 snaphots of the solute sampled regularly from the last ns of the MD trajectories, with the water and counterions stripped away. This method combines the enthalpic or molecular mechanics energies (E_{MM}) that represent the internal energies (bond, angle and dihedral; E_{BADH}) along with van der Waals (E_{vdW}) and electrostatic interactions (E_{elec}), with the solvation free energies (G_{sol}) calculated by the finite difference Poisson-Boltzmann (PB) model for polar solvation (G_{PB} or G_{polar}) [9] and the non-polar contribution ($G_{non\text{-}polar}$) as a function of the solvent-accessible surface area (SASA). All terms were computed from the MM-PBSA module in AMBER. Solute entropic contributions were estimated from the sampled structures based on mass-weighted covariance matrix analysis (Quasiharmonic analysis) using the ptraj modules in AMBER [8].

$$G = \langle E_{MM} \rangle + \langle G_{sol} \rangle - T\langle S \rangle \quad \text{(Eq. S1)}$$

Figure S1 – ^{13}C NMR spectrum for 8c: full spectrum.

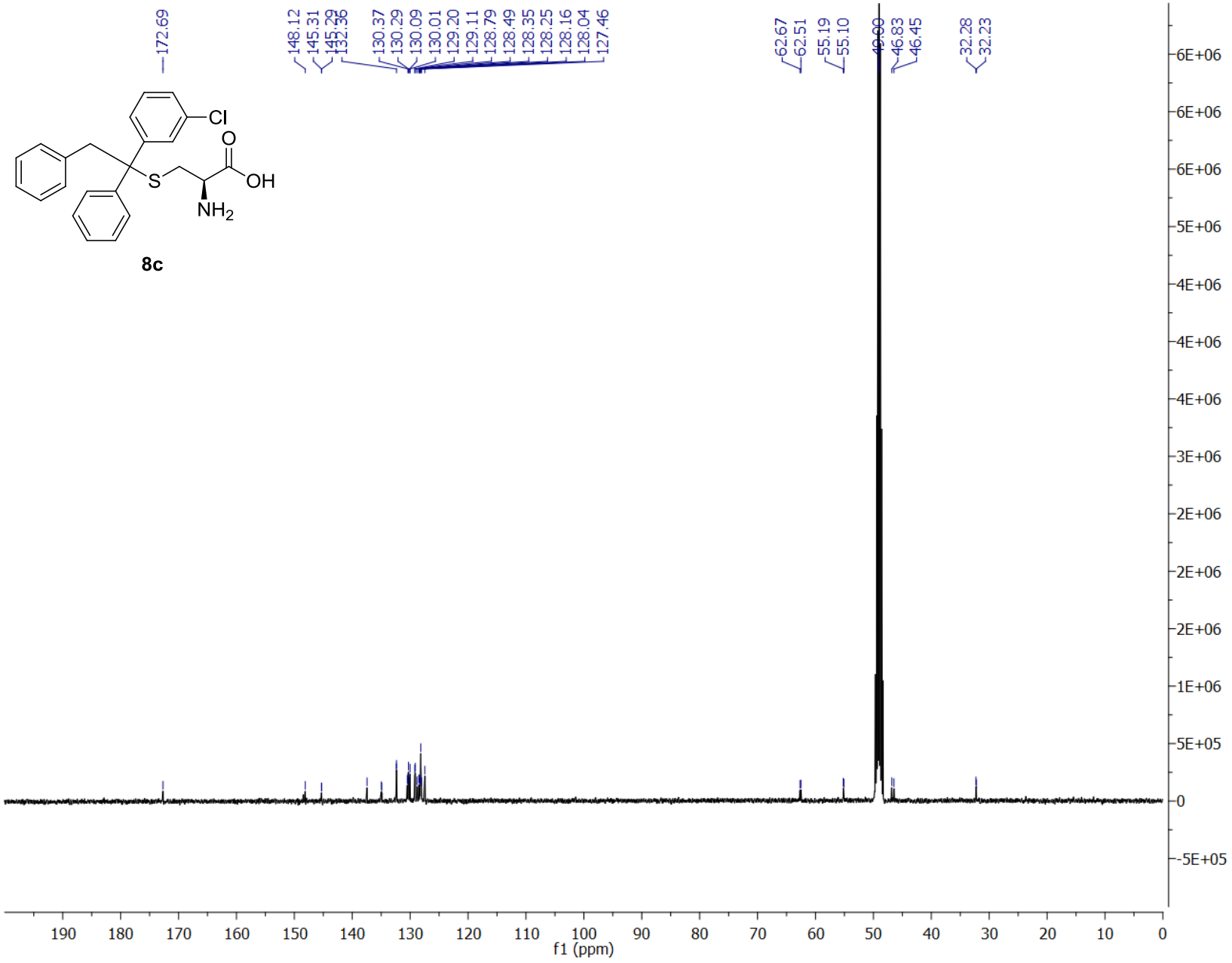


Figure S2 – ^{13}C NMR spectrum for 8c: expansion 1.

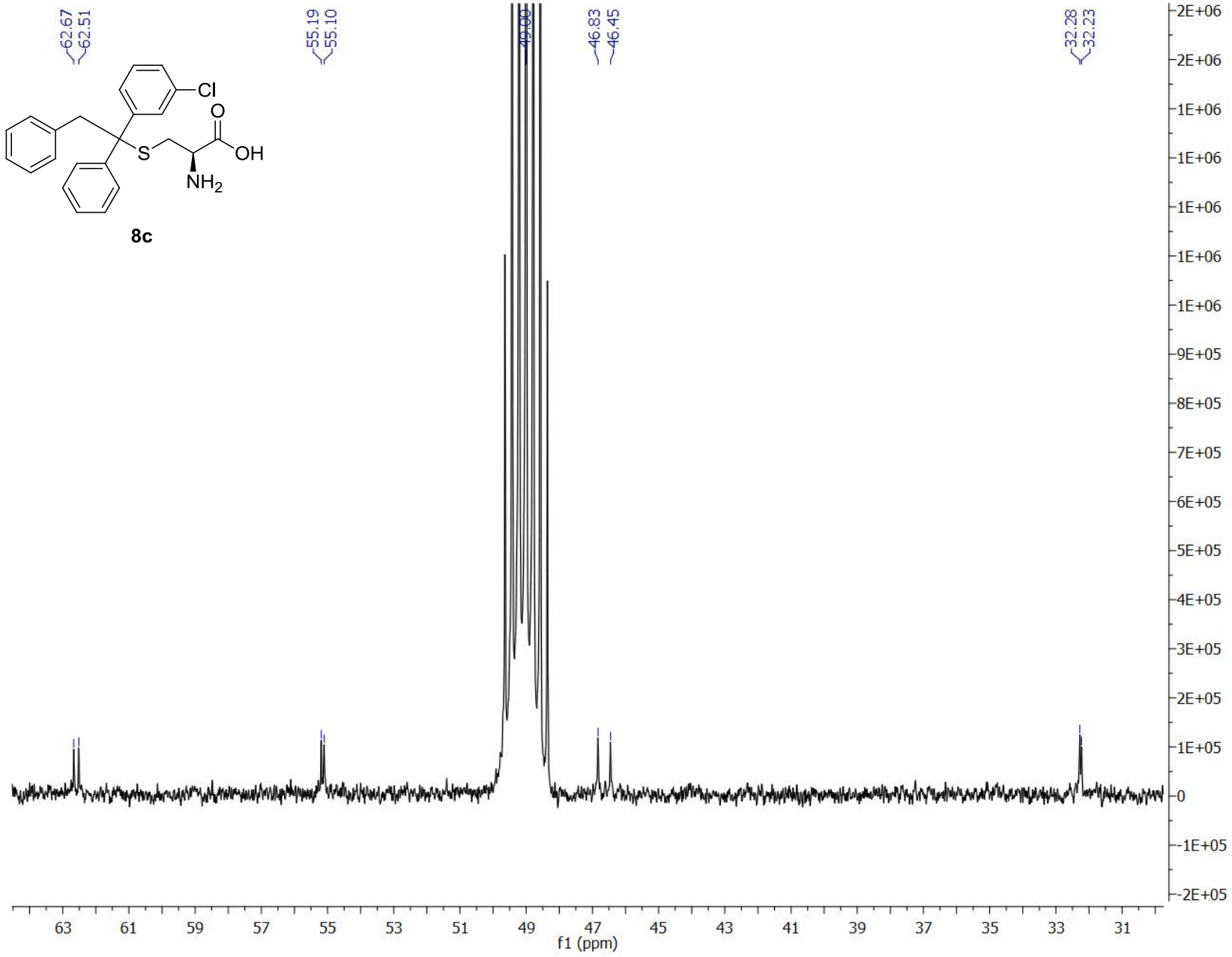


Figure S3 – ^{13}C NMR spectrum for 8c: expansion 2.

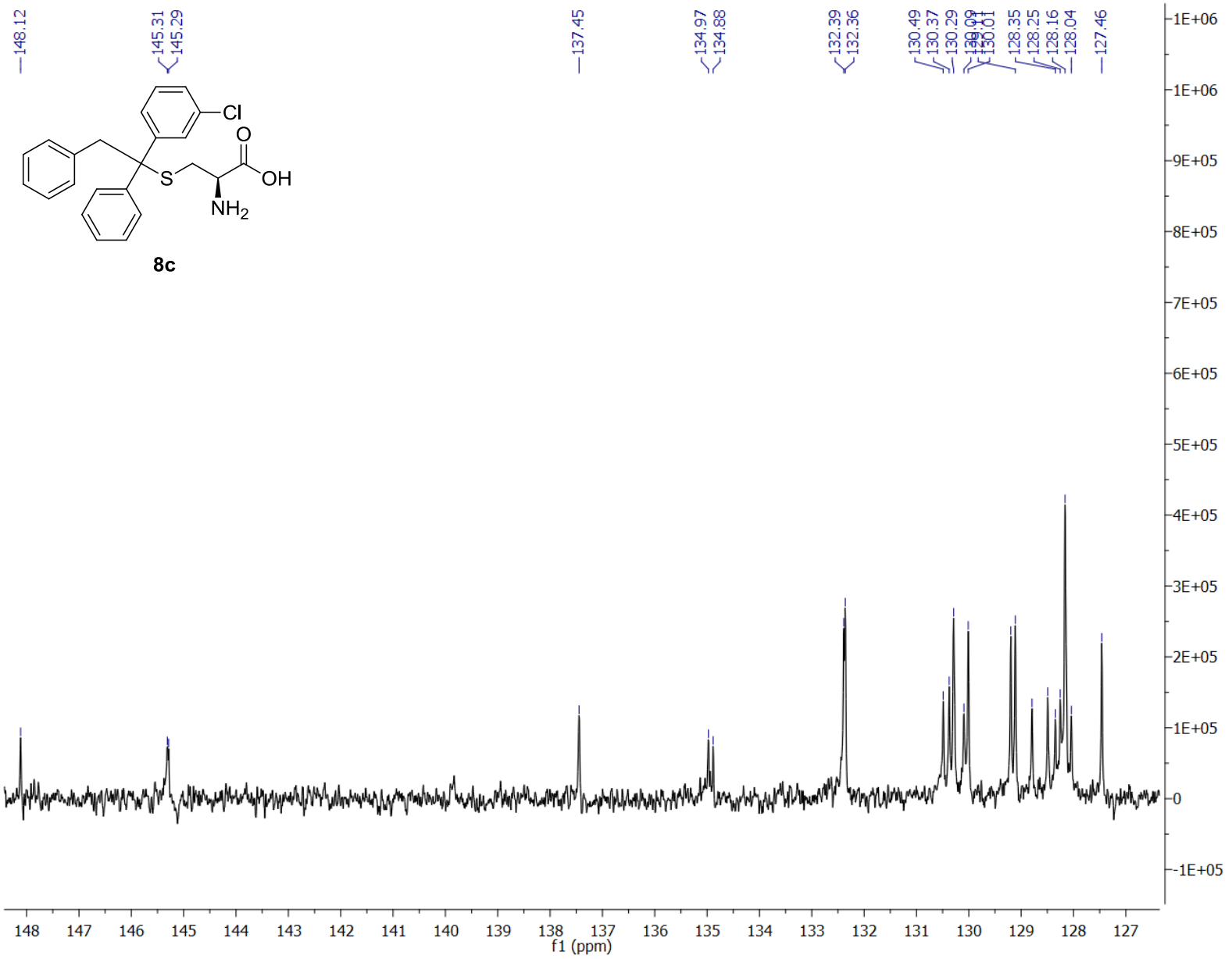


Figure S4 – ^{13}C NMR spectrum for 8d: full spectrum.

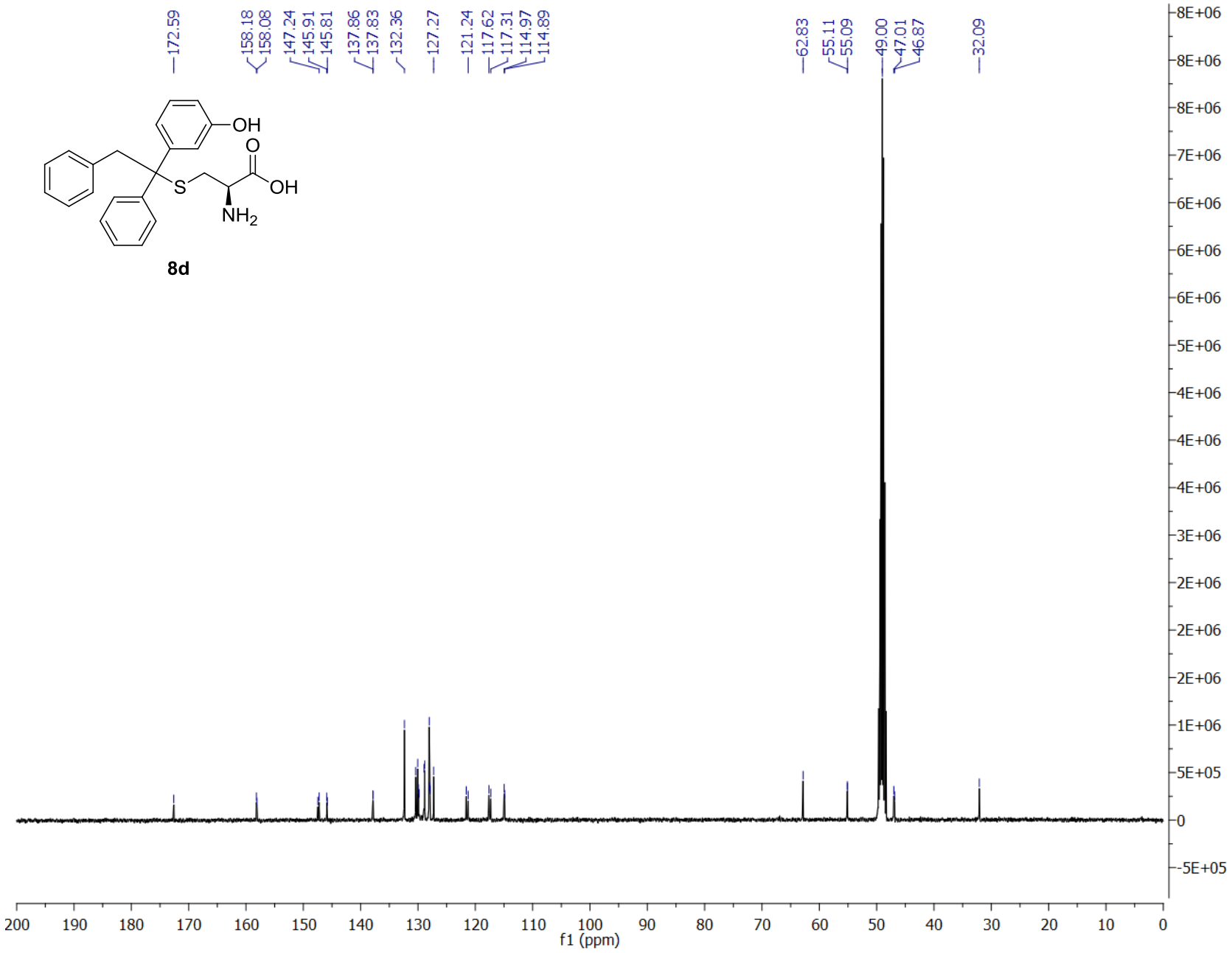


Figure S5 – ^{13}C NMR spectrum for 8d: expansion 1.

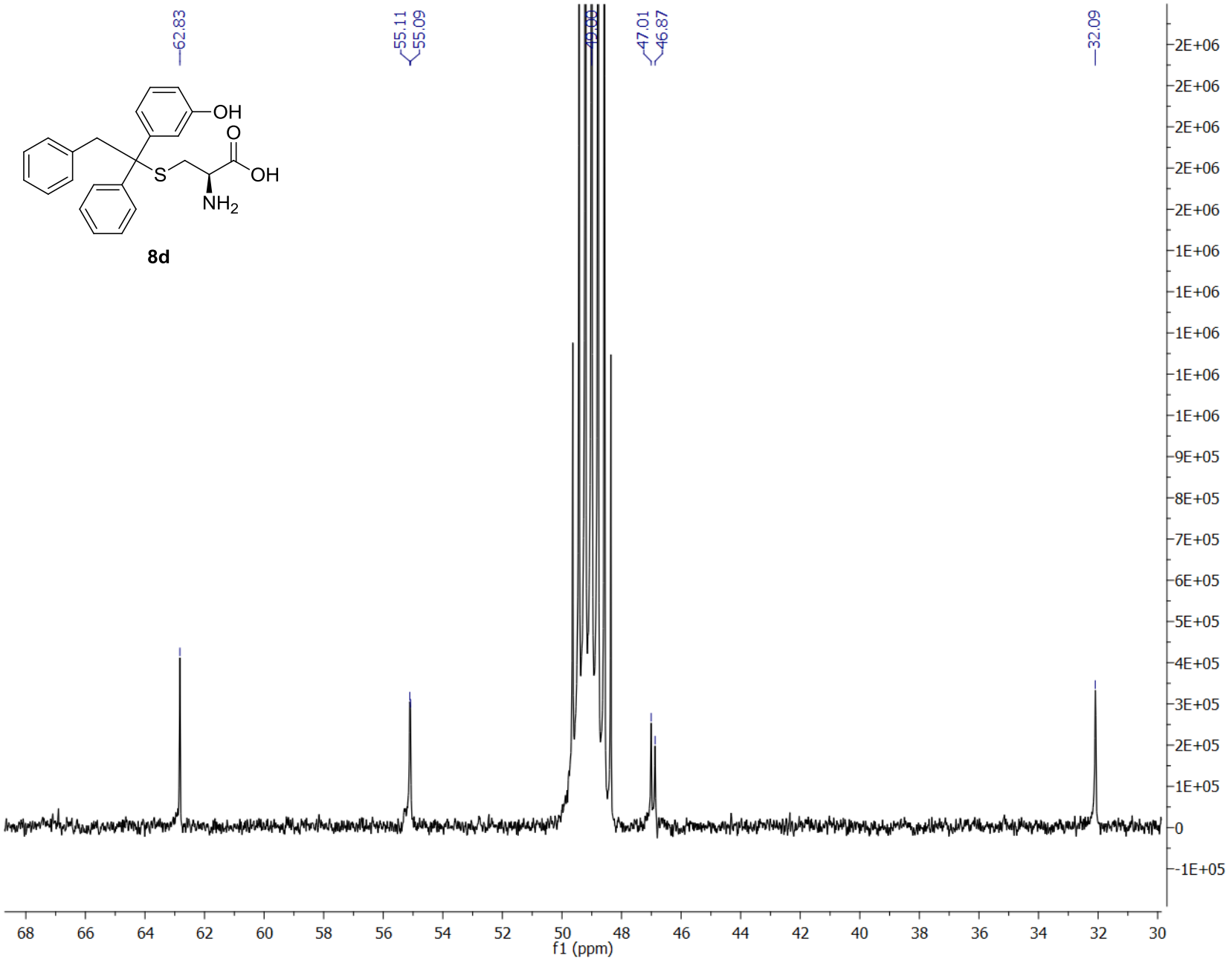


Figure S6 – ^{13}C NMR spectrum for 8c: expansion 2.

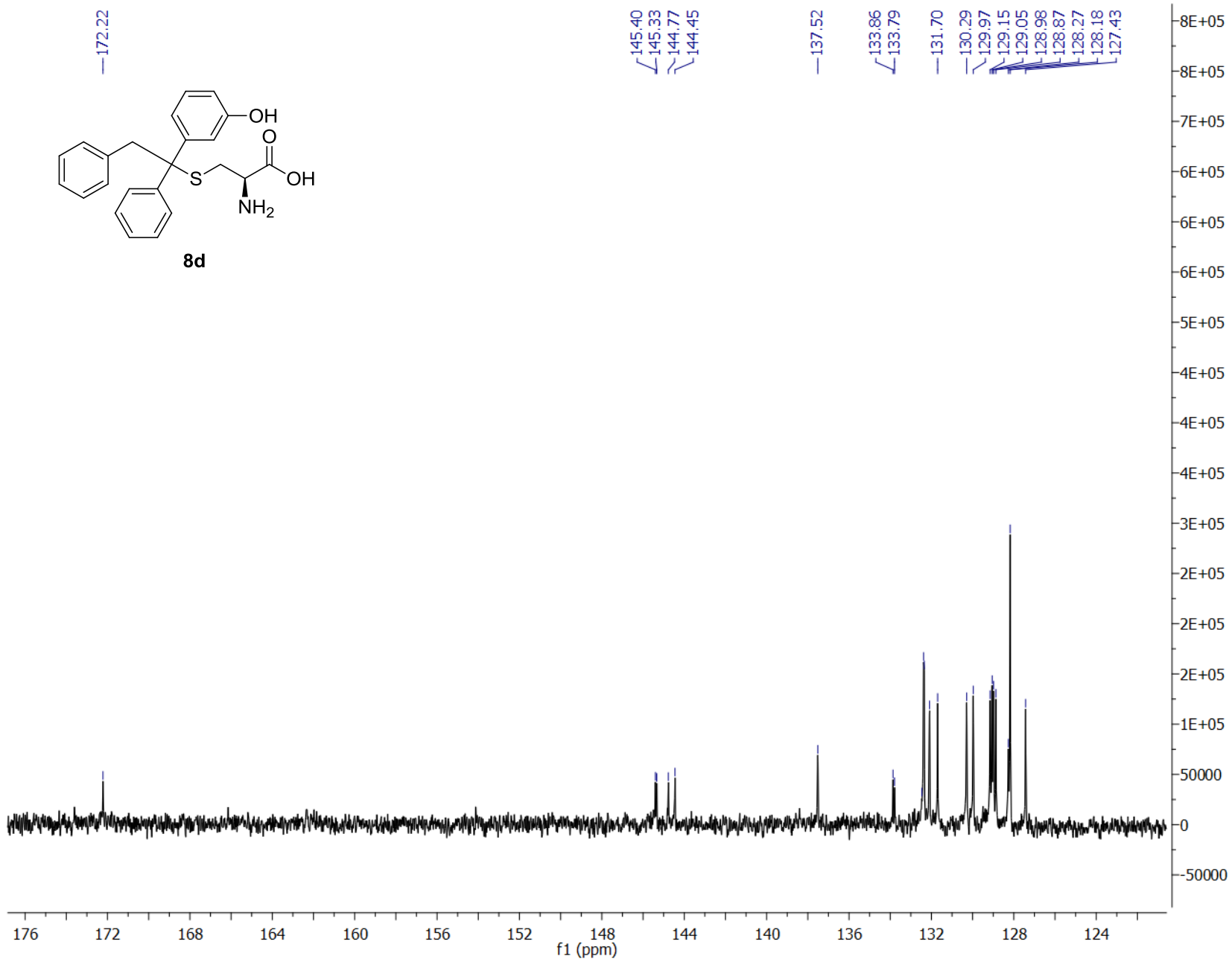


Figure S7 – ^{13}C NMR spectrum for 8f: full spectrum.

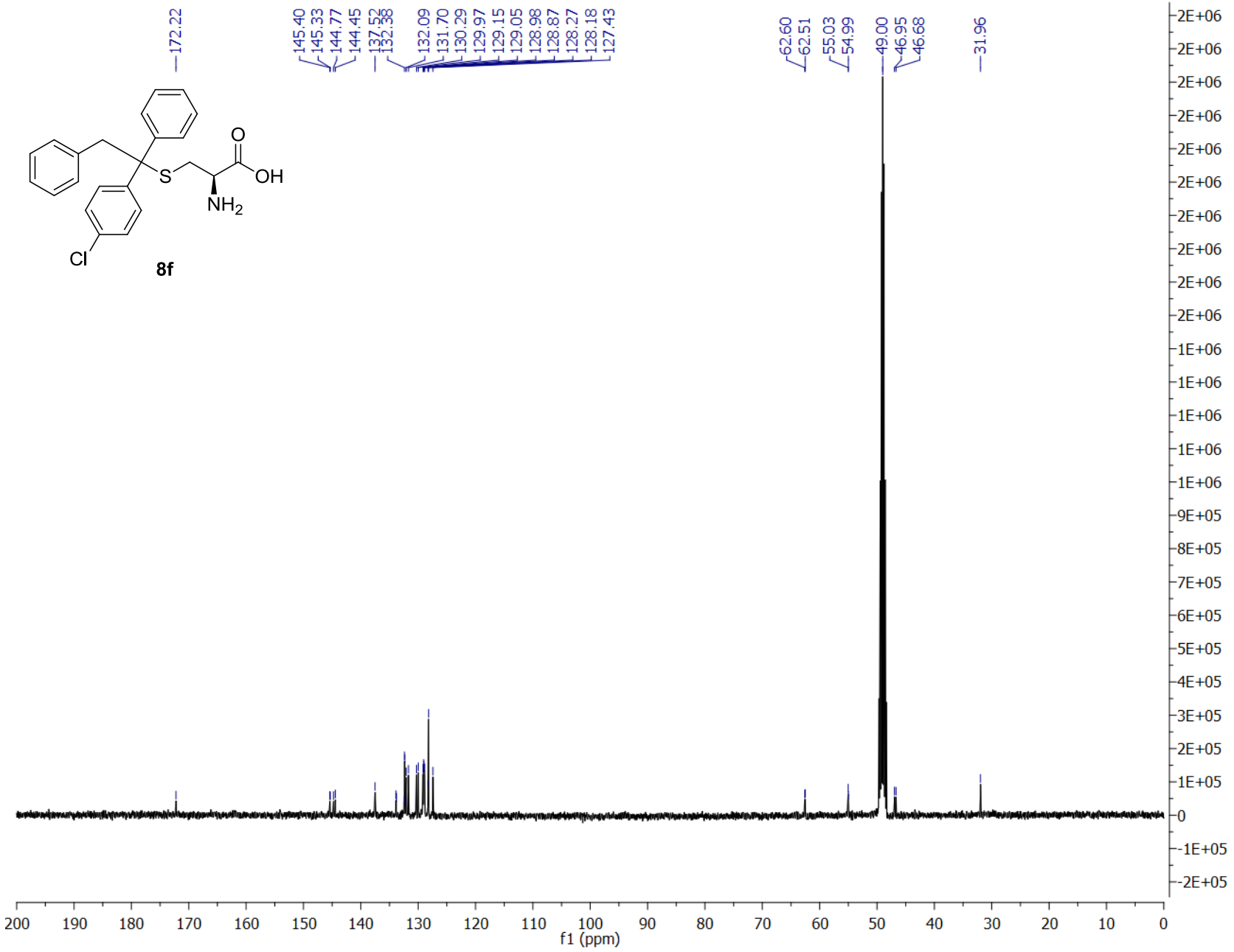


Figure S8 – ^{13}C NMR spectrum for 8f: expansion 1.

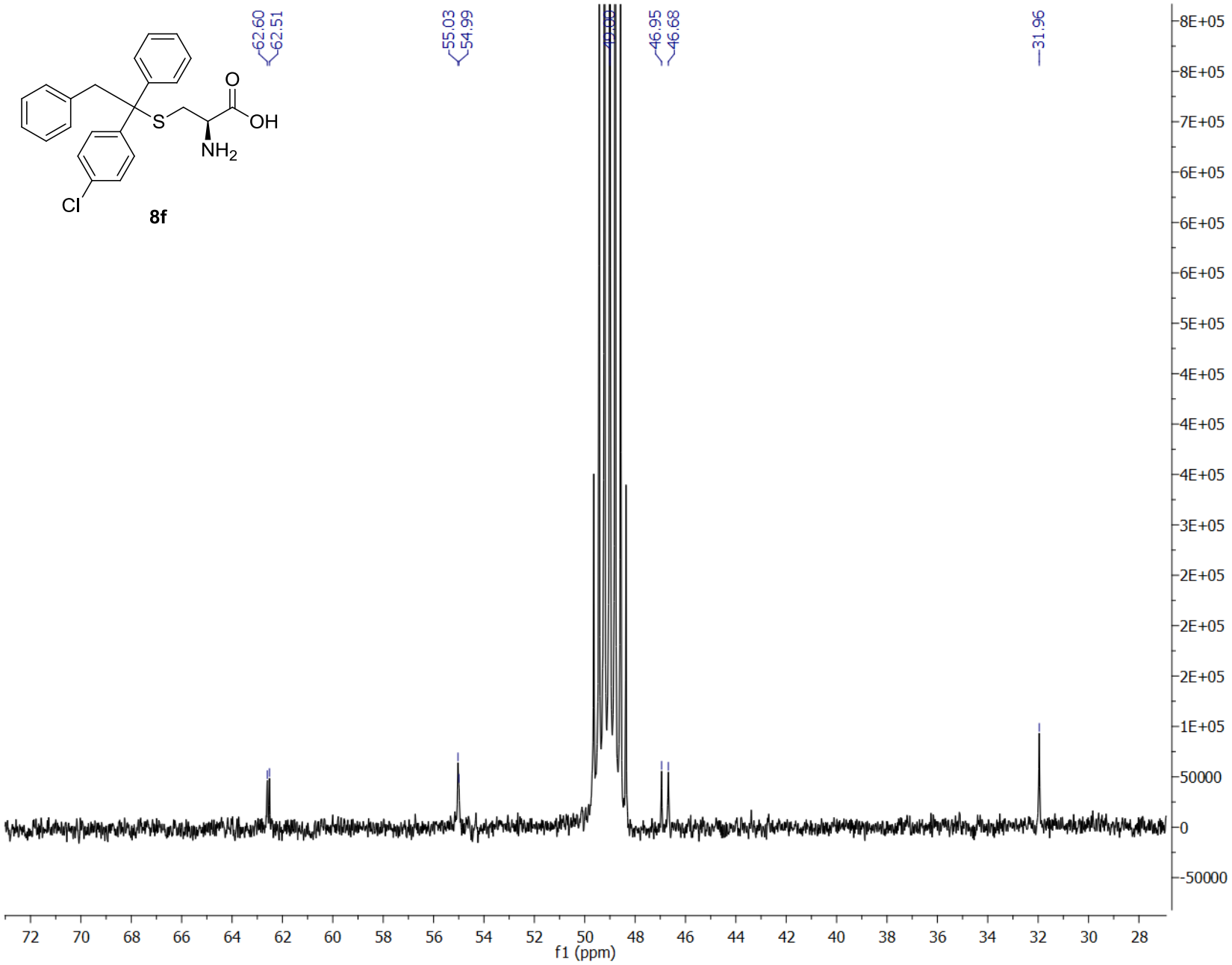
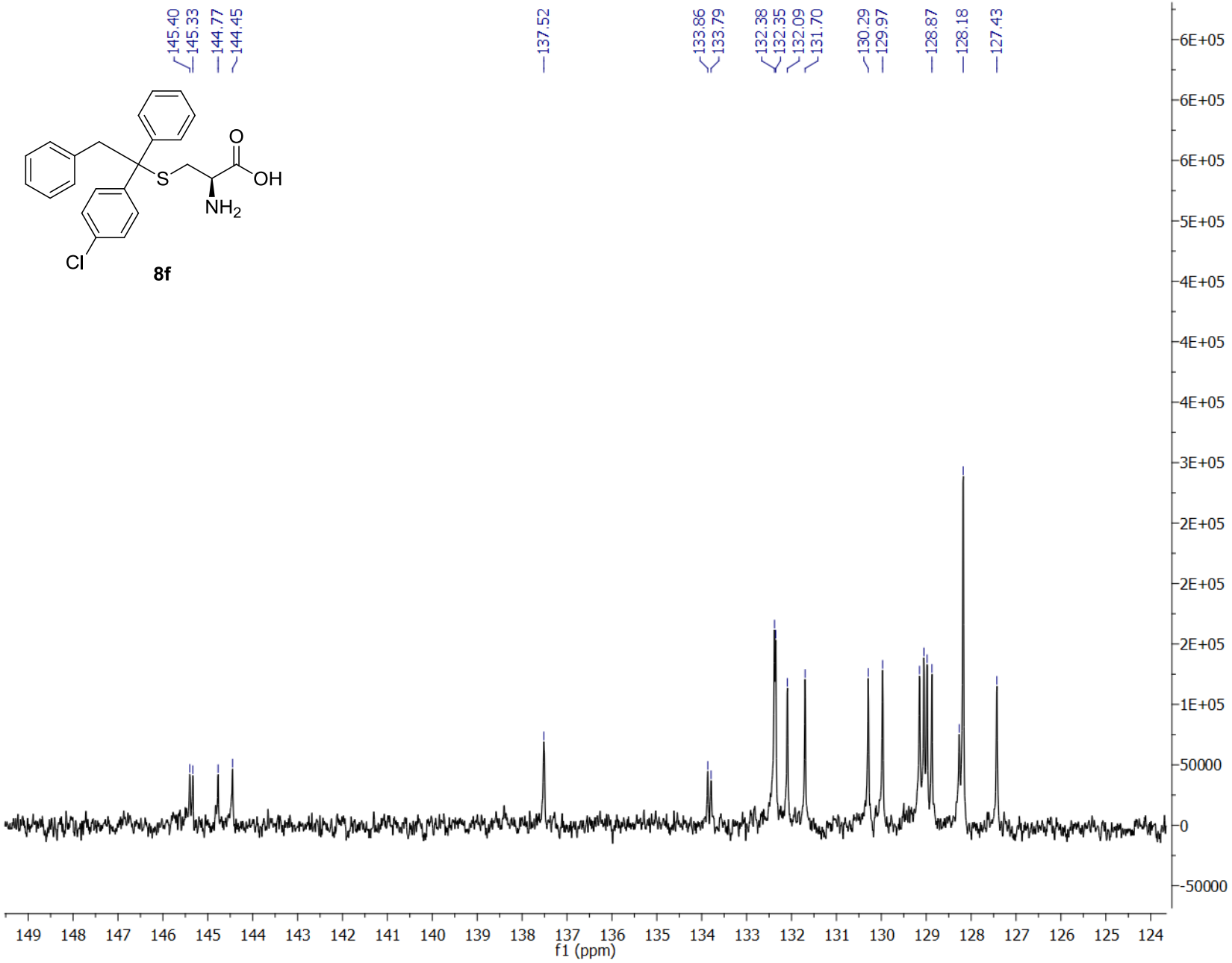


Figure S9 – ^{13}C NMR spectrum for 8f: expansion 2.



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