SUPPORTING INFORMATION

Computational and experimental investigation of mono-septanoside binding by Concanavalin A: Correlation of ligand stereochemistry to enthalpies of binding

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Computational Approach

Modeling of all protein-ligand complexes was based on the X-ray structure of Concanavalin A (ConA) with **9** (PDB access code: 1GIC, chain A).[1] Since the co-crystal contains one of the ligand studied, we constructed all other six-member ring ligands by *in situ* modification of **9**. For the septanosides we constructed the ligands in the gas phase and aligned common stereocenters to those of **9** inside the protein. After *in situ* modification with the program Maestro[2] and removal of all crystallographic waters except Wat335, each ligand (including **9**) was re-docked with the program Glide,[3] always maintaining the structure of the protein frozen. Wat335 was kept in the system following the studied by Kadirvelraj *et al*[4], in which they show that Wat335 is a highly conserved molecule. Hydrogen atoms were added to this molecule and their orientation optimized to form hydrogen bonds to Asn14, Arg228, and Asp16.

Docking computations were followed by QM/MM energy minimizations using Qsite,[5] where the ligand and Asp208 were treated at the quantum mechanical (QM) level and the rest of the protein at the MM level. In one set of calculations, the MM region was kept frozen in all QM/MM calculations, while in another set the relevant residues Asn14, Leu99, Tyr100, Asp208, Arg228, and ligand were relaxed during the QM/MM minimization. Since the Docking procedure generates a manifold of poses, we considered no just the one with highest score, but others that presented qualitative differences in the conformation of the ligand. The final energies reported in Table 2 are those corresponding to the lowest QM/MM energy found. For the QM/MM calculations, cuts between the QM and MM region were treated with the frozen-orbital method as implemented in Qsite. The MM region was treated with the OPLSAA force field,[6] and the QM region with Density Functional Theory (DFT). The functional B3LYP and basis set 6-31g* were used in all QM calculations. Gas phase calculations were carried out with Jaguar at

the same level of theory specified above.[7] As explained in the main manuscript, the starting geometry for the ligand in the gas phase was that obtained by the highest scored conformation using Glide. However, we considered other conformations with different OH rotamers and ring conformations. Selection of these rotamers was guided by a previous study in which a rigorous conformational analysis of septanosides **5** and **6** was done by Monte Carlo (MC) simulations.[8] In fact, our minimum energy structures of **5** and **6** were the same as those obtained in these previous MC simulations. From the study of DeMatteo *et al*[8] it was concluded that the septanosides studied were rigid enough to prefer one conformation at room temperature. Thus, the sole use of the lowest energy configurations, found in both the Glide/QM/MM and gas phase QM calculations, to correlate binding energies with experimental enthalpies appears to be a reasonable assumption.

Convergence of results with respect to larger QM regions

As explained in the computational methods section, we performed all QM/MM calculations with a minimal QM region (i.e. Asp208 and ligand). This selection was based on several factors: 1) A large number of calculation was required to test different conformers for a same ligand. We typically tested about 5 conformers per ligand. Conformers were selected from the manifold of poses generated by Glide and also by the possible conformers a ligand can adopt in the gas phase. 2) We noticed from the preliminary Docking calculations that Asp208 was the only residue that conserved the number of hydrogen bonds with all ligands studied. 3) Of the two charged residues (208 and 228), Asp208 is the only residue that can substantially polarize the electronic structure of the ligand (Arg228's side chain is actually not in close contact with the ligands and it is not involved in hydrogen bonds).

To further test that this minimal QM region was appropriate, we recomputed the binding energy using a larger QM region: Asn14, Leu99, Tyr100, Asp208, and Arg228. We considered some of the ligands studied as a benchmark. As shown in Table S1, the difference in using the proposed minimal QM region and a larger QM region, spanning the relevant residues inside the cavity, will not alter the interpretation proposed in the manuscript regarding the differential binding among the ligands. Both models agree well with experiment.

Table S1. Comparison between the calculated binding energy with the experimental binding enthalpies considering a minimal QM region (Asp208 + ligand) and a larger QM region (Asn14, Leu99, Tyr100, Asp208, Arg228 + ligand).

Ligand	ΔE_b (minimal QM region (kcal/mol)	ΔE_b (minimal QM region (kcal/mol)	$\Delta\Delta H$ (exp.) (kcal/mol)
6	5.2	5.3	4.67/2.8
7	0.0	0.0	0.0
8	16.9	21.2	no binding
9	1.7	1.8	2.0

Analysis of Methyl 2-*O*-methyl β-septanoside (15)

In the main text of the manuscript we gave a rational on the reason of the difference in the enthalpy of binding between **7** and **6** (both septanosides). We showed that the main difference between them comes from the reorientation of the ring hydroxyl groups in going from the free to the bound state (Figure 8 of the main text). One way to describe these changes was to count the number of electrostatic OH···O interactions before and after binding. In methyl "manno" β-septanoside **7**, this number changes from three to two (see arrows in Figure 8), while in methyl

"gluco" β-septanoside **6** it changes from five to three, accounting for most of the difference in binding energy between these two ligands. A similar analysis can be invoked for ligand **15** (Fig. S1), which has the same stereochemistry as **6**, but contains a methyl ether rather than a hydroxyl group at C2. We can also see that for **15** the number of electrostatic interactions changes from four to two (i.e, changes by two as in **6**), as shown in Fig. S2, which accounts for the similar magnitudes in $\Delta\Delta H$ in **15** than in **6**.

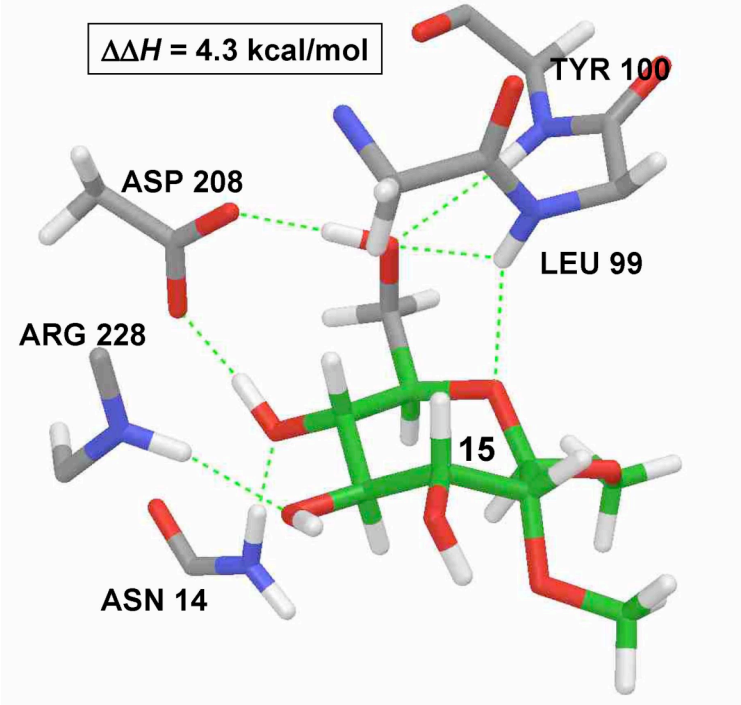


Figure S1

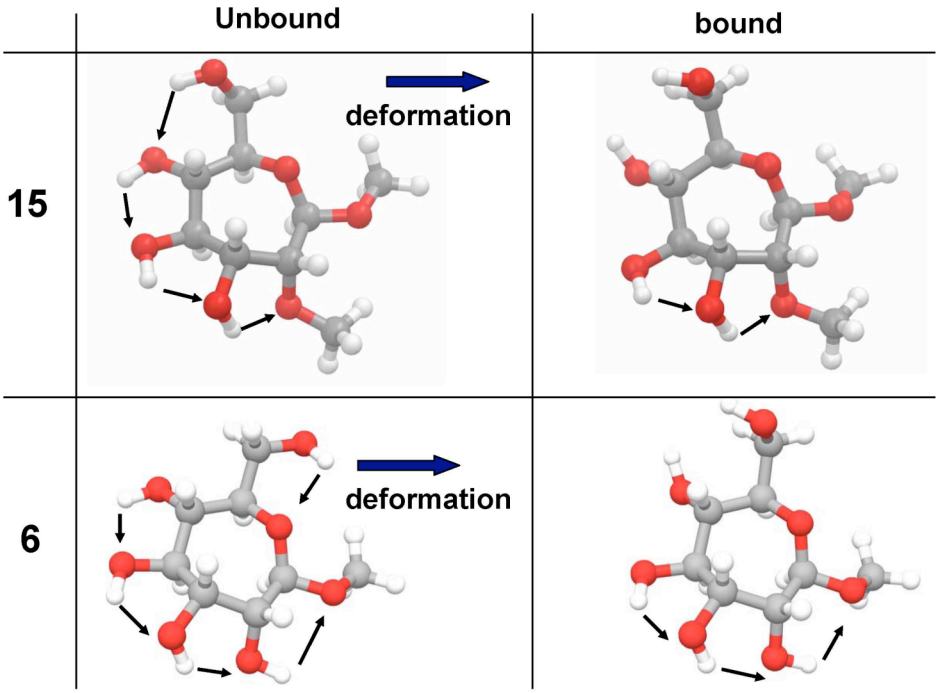


Figure S2

Analysis of Methyl "gluco" α-septanoside (5)

The large enthalpy of binding of the ConA•**5** complex relative to ConA•**7** (8.8 kcal/mol) correlates well with no-binding events seen in ITC. The QM/MM structure of this complex reveals that no hydrogen bond is lost with respect to **7** (Figure S3). Thus all its destabilization with respect to **7** comes from deformation energy upon binding. While in **7** one OH···O contact is lost, in **5** two of these contacts are lost (Figure S4).

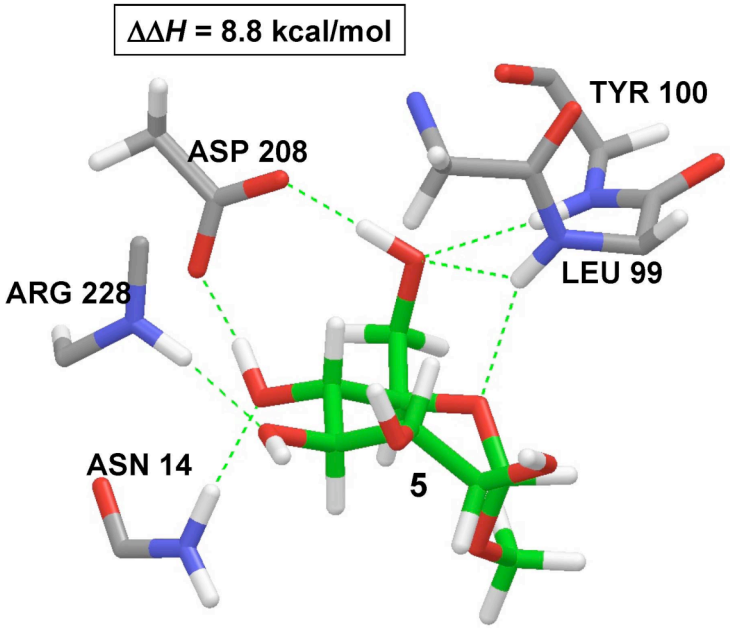


Figure S3

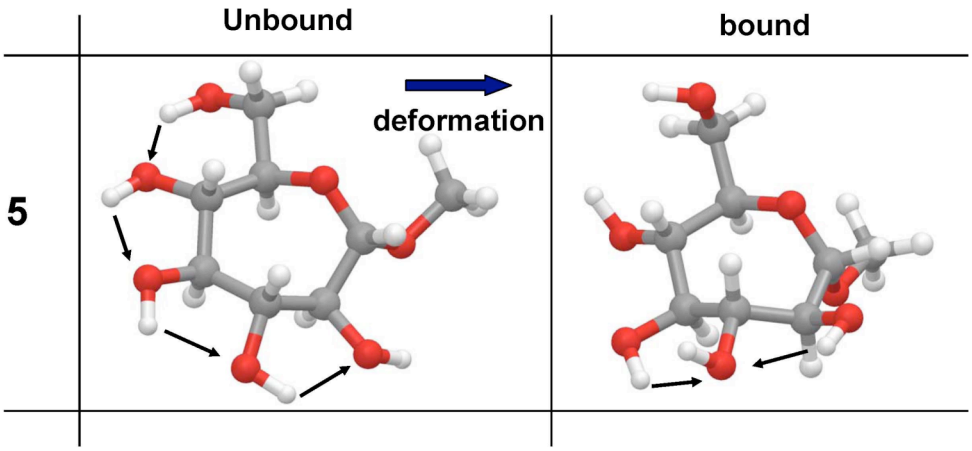


Figure S4

Comparison of Scoring functions with experimental binding affinities

Table S2 presents scoring results of the best pose for all ligands studied. Notice that the ranking of affinities according to the Docking calculations only marginally correlate with the ranking given by the experimental free energy of binding. The reason for this is twofold: 1) Although the scoring function has in it some sort of binding energy incorporated (via Molecular Mechanics terms), the docking algorithm misses the reorientation of the OH bonds that must necessary take

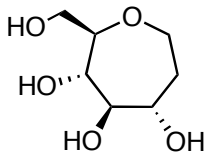
place upon binding. 2) Also, it has been shown before by DeMatteo et al (ref 8) that the relative energies of the various conformers according to a molecular mechanics force field did not coincide with the QM relative energies, not even qualitatively.

Table S2. Comparison between the calculated binding energy with the experimental binding enthalpies. Values in parenthesis correspond to a QM/MM calculation in which residues Asn14, Leu99, Tyr100, Asp208, and Arg228 are relaxed. Also shown are the scoring function according to docking calculations and their comparison with experimental binding affinities. Ranks are presented based on the scoring function and free energy of binding.

Ligand	ΔE_b (kcal/mol)	$\Delta\Delta H$ (exp.) (kcal/mol)	Scoring Function	Scoring Rank	$\Delta\Delta G$ (exp.) (kcal/mol)	Experimental Rank
5	8.8 (**9.0**)	no binding	-6.58	3	no binding	
6	5.2 (**5.5**)	4.67/2.8	-5.45	7	-3.7/3.6	4
7	0.0 (**0.0**)	0.0	-6.04	4	-4.0	3
8	16.9 (**15.4**)	no binding	-5.84	5	no binding	
9	1.7 (**1.1**)	2.0	-6.72	1	-4.5	2
10	1.0 (**1.0**)	1.0	-6.70	2	-5.5	1
15	4.3 (**4.7**)	5.37/4.1	-5.62	6	-3.6/3.1	5

References

1. S. J. Harrop, J. R. Helliwell, T. C. M. Wan, A. J. Kalb, L. Tong and J. Yariv, *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, 1996, **52**, 143-155.
2. *Maestro, version 9.0*, Schrodinger LLC, New York, NY.
3. *Glide, version 502076*, Schrodinger LLC, New York, NY.
4. R. Kadirvelraj, B. L. Foley, J. D. Dyekjaer and R. J. Woods, *J. Am. Chem. Soc.*, 2008, **130**, 16933-16942.
5. *Qsite, version 7.5*, Schrodinger LLC, New York, NY.
6. W. L. Jorgensen, D. S. Maxwell and J. TiradoRives, *J. Am. Chem. Soc.*, 1996, **118**, 11225-11236.
7. *Jaguar, version 7.5*, Schrodinger LLC, New York, NY.
8. M. P. DeMatteo, N. L. Snyder, M. Morton, D. M. Baldisseri, C. M. Hadad and M. W. Peczuh, *J. Org. Chem.*, 2005, **70**, 24-38.



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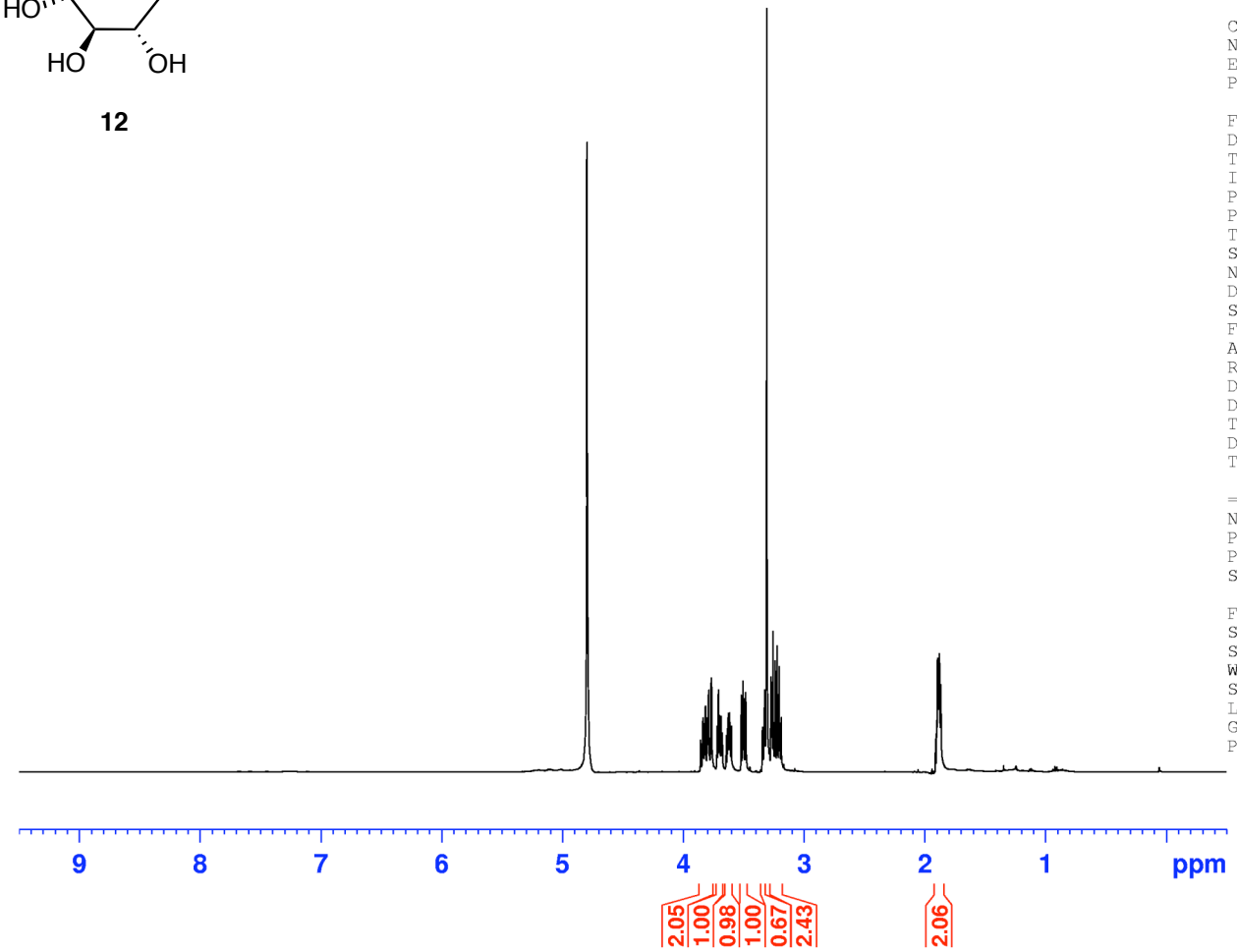


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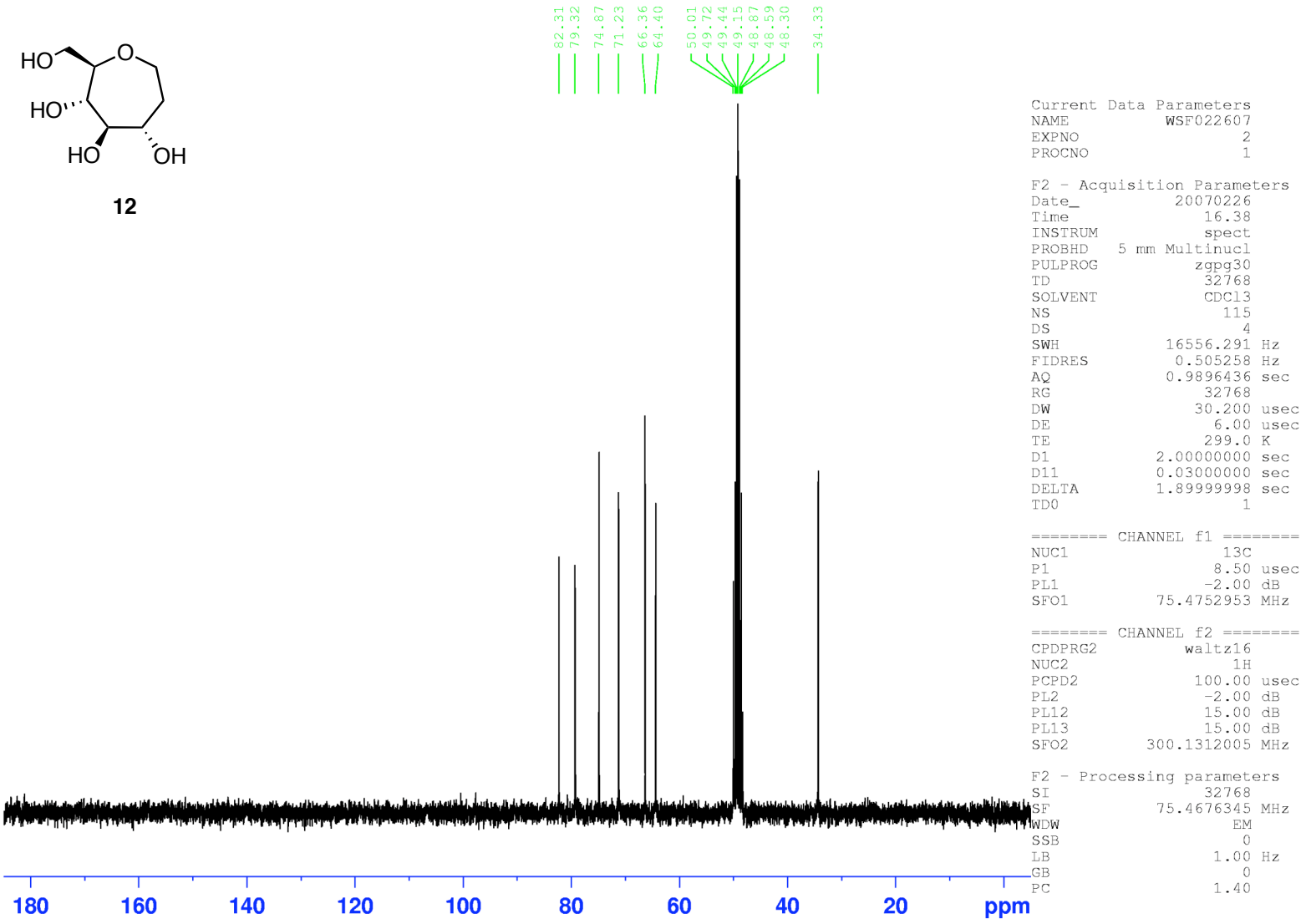
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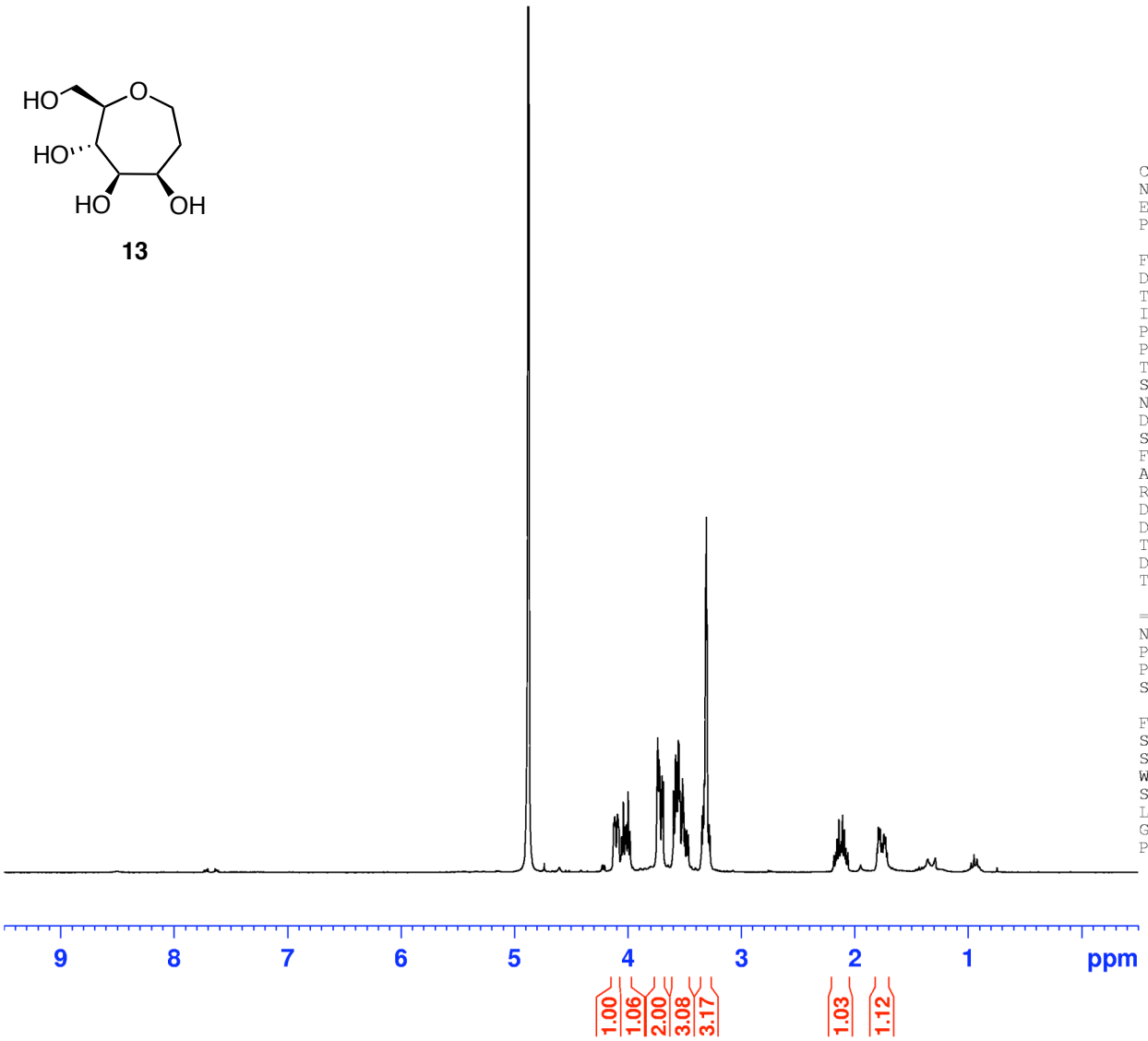


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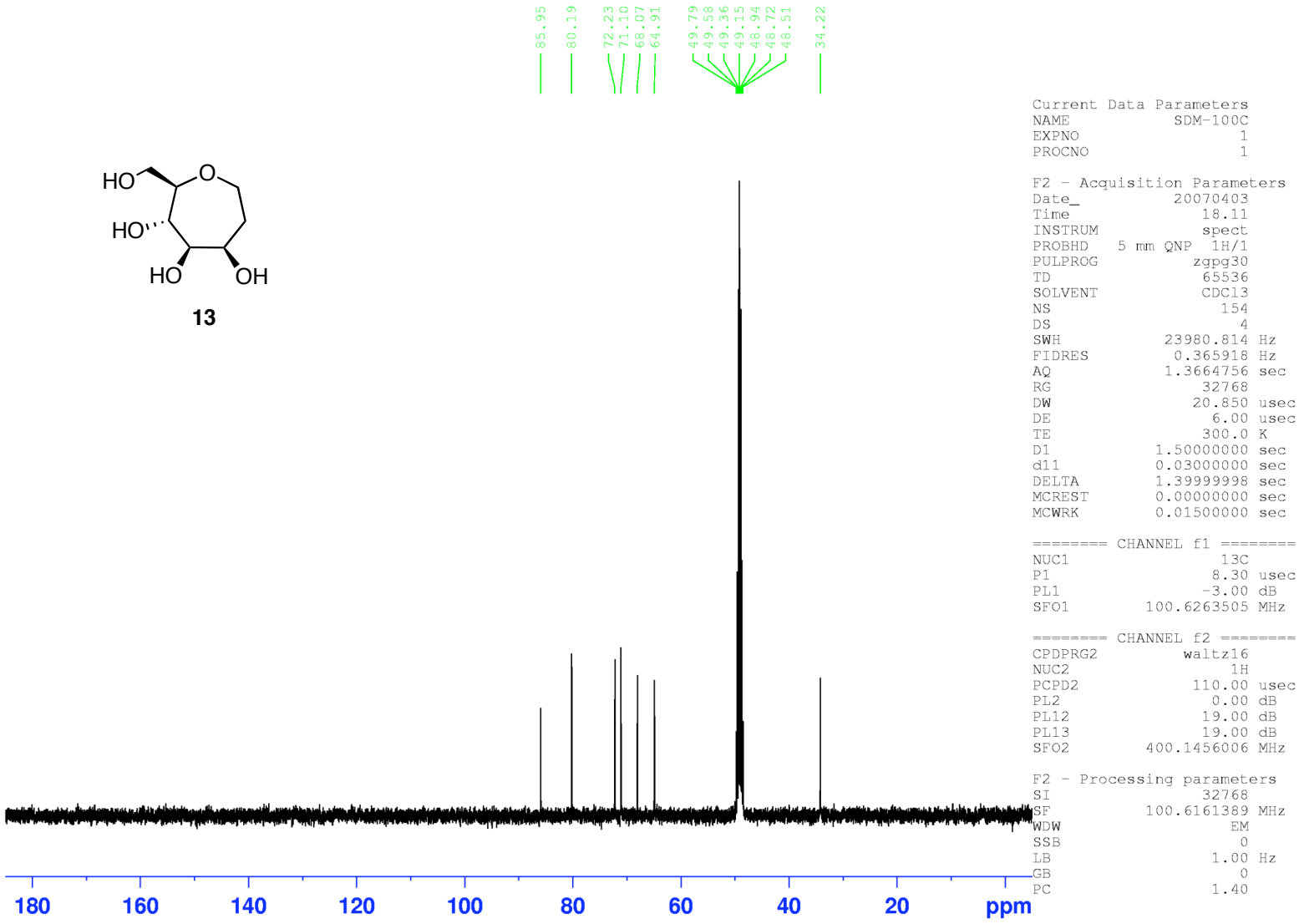
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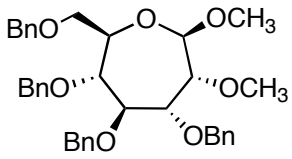

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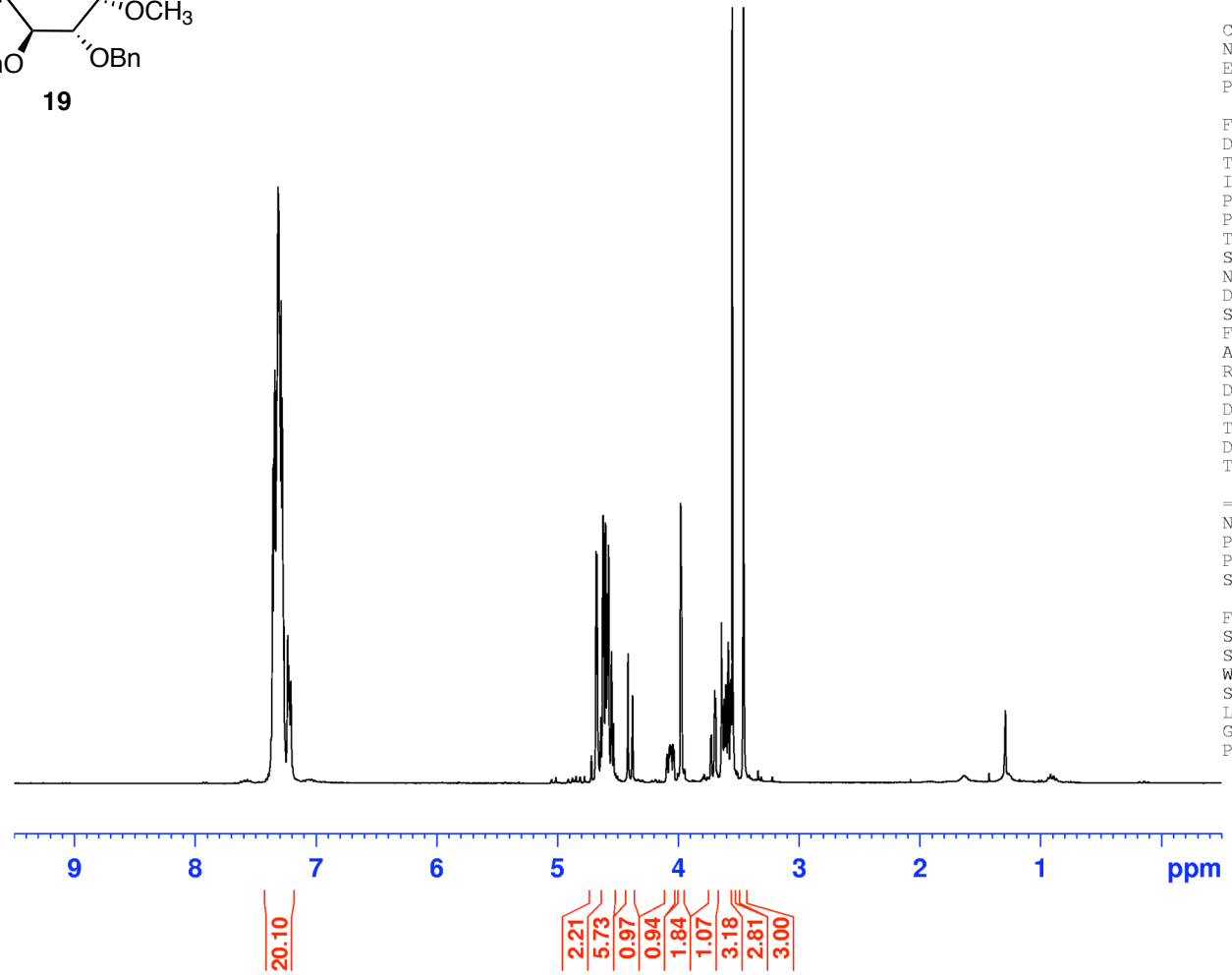


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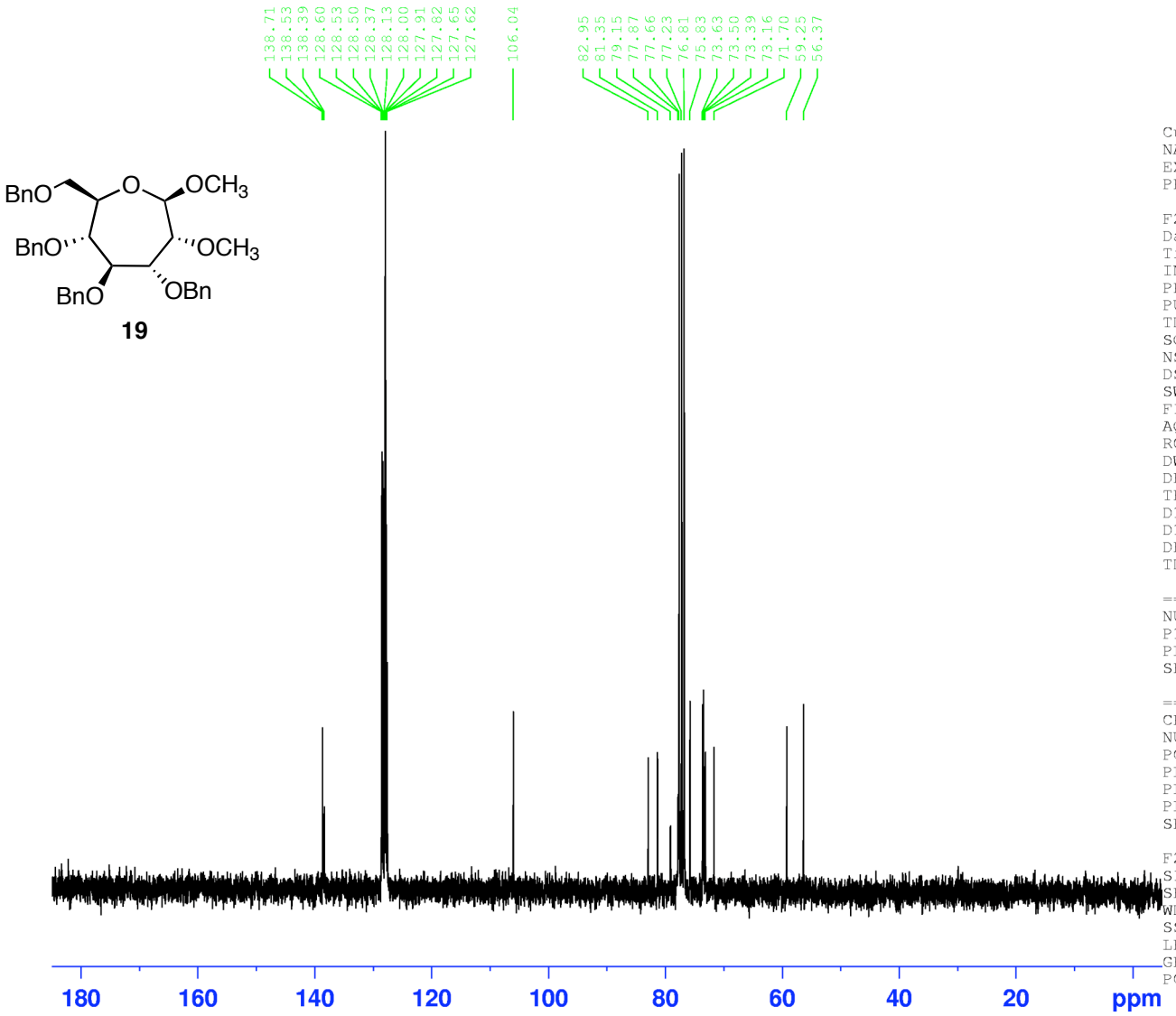


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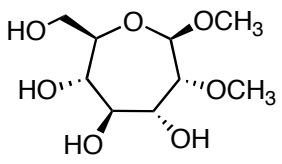

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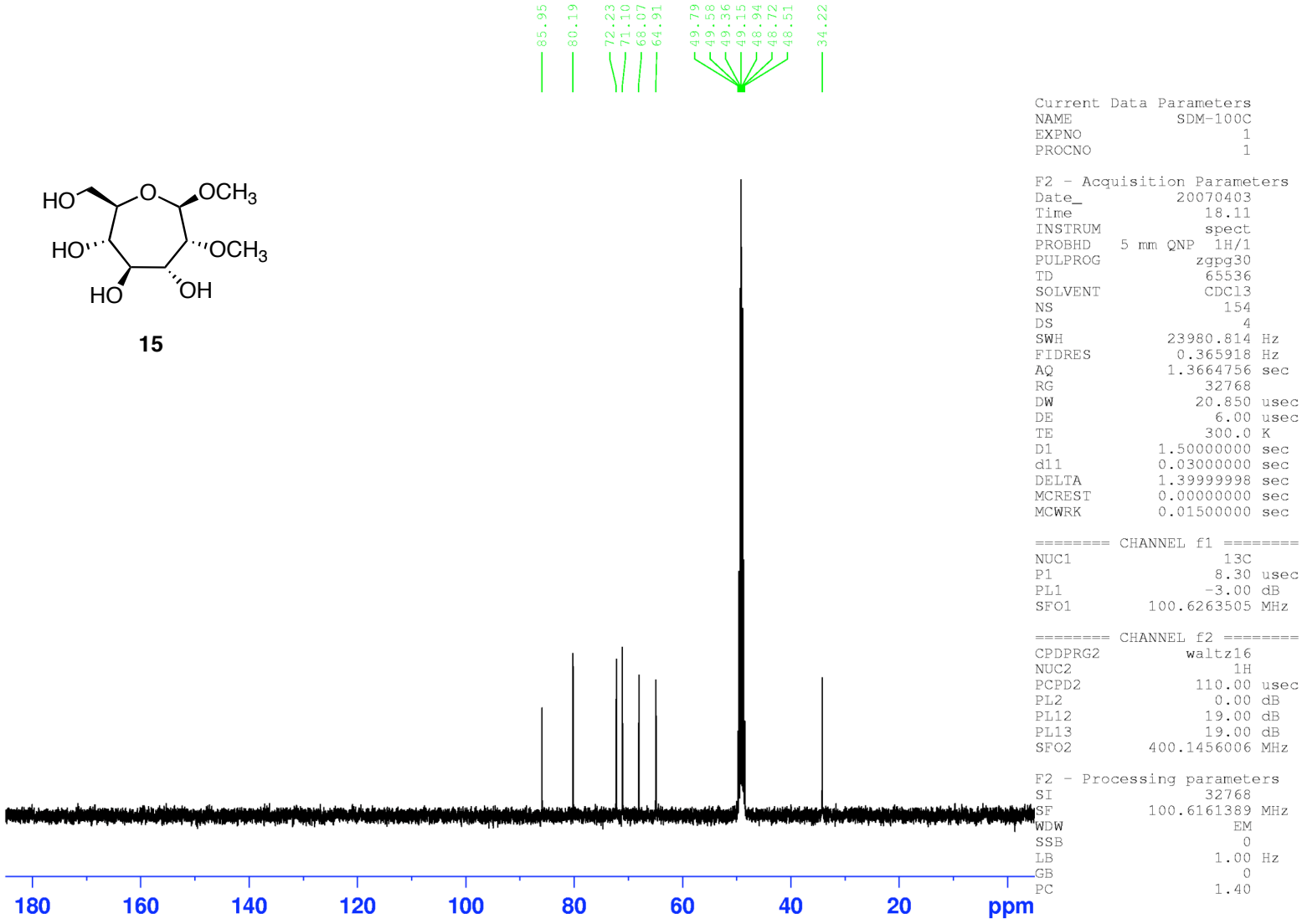
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