Assigning NMR Spectra of Bacillithiol Derivatives Using Various Computational Methods

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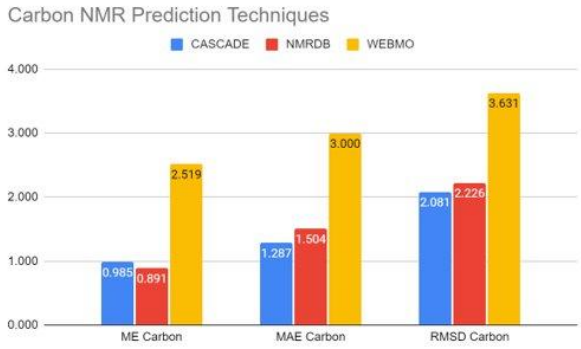
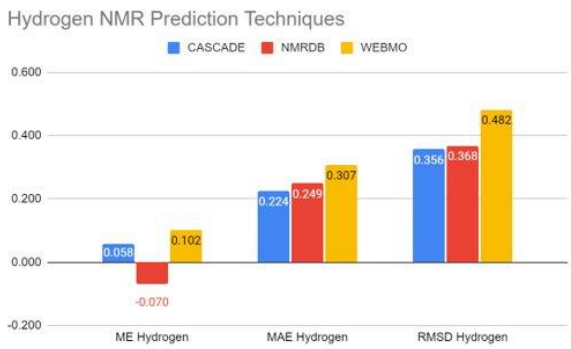
By 2050, antibiotic resistance could cause 10 million deaths annually, making it deadlier than cancer unless researchers can develop new ways to combat antibiotic resistance.[1] For example, methicillin-resistant Staphylococcus aureus (MRSA) synthesize bacillithiol, which interferes with the antibiotic fosfomycin, the treatment for acute simple cystitis.[2] Current research is working towards effectively inhibiting bacillithiol by interfering with its production, therefore shutting down the mechanism that MRSA use to become antibiotic resistant. To understand how bacillithiol confers antibiotic resistance and combat it, bacillithiol derivatives have been synthesized and analyzed.

Nuclear Magnetic Resonance (NMR) is a crucial technique in identifying derivatives from this synthesis. The NMR chemical shift data can be used to assign or reassign experimental spectra, confirm regio- or diastereoselectivity of a reaction, or verify the structure of complex products[3]. The goal of research this summer was to utilize computational chemistry to accurately assign NMR shifts for all derivatives of the synthesis pathway in order to aid in future research efforts.

Before working directly with the bacillithiol derivatives, three NMR prediction techniques were compared: NMRdb, CASCADE, and WebMO (Tantillo). Each method follows a different process to output chemical shift values, and the accuracy of each was measured by comparing data to known spectra values. Based on the test set, which contained 6 molecules for a total of 62 H and 45 C, it was found that CASCADE was the best model for assignments and determining the most stable conformer of molecules, meaning it had the lowest ME and MAE.





Charts 1 and 2. These charts display the Mean Error (ME), Mean Average Error (MAE), and RMSD comparisons for Carbon and Hydrogen base on each assignment method.

After becoming comfortable with the methods, the bacillithiol derivatives were assigned. Each molecule was run through all three computational models which output the C and H NMR assignments. The C NMR data was averaged and matched to the C NMR peaks from the Woodward Spectra[2] fairly easily. The H NMR data was a bit more complex as the chemical shifts, multiplicity, integration, and coupling constants all had to be taken into account when matching the averages to that of the spectra. However, all 6 molecules were fully assigned including differentiating between the Alpha and Beta conformers of Molecule 1.

Both Coupled and Deallylated n-methyl-bacillithiol were also tested which included a trip to Wooster to use their high field NMR instrument to gather data. The raw spectras of C NMR, H NMR, COSY, HSQC, and HMBC were analyzed for both molecules meaning that all

assignments had to be worked through and matched up with the computational predictions. Unfortunately, deallylated was inconclusive as the molecule seemed to be impure or only half-deallylated, but coupled assignments were a success! By the end of the summer, an additional 175 C and 206 H had been assigned.

Image 1. High Field NMR Instrument at Wooster

In addition to the assignment of the bacillithiol derivatives, an app was coded in order to integrate all three computational models in one place. The purpose of this was to help simplify the process of assigning molecules and make it more cohesive. This included displaying pictures of the various conformers with options for visualization, the relative weight and energy of each conformer, and the chemical shift data using each method. The finished website can be found here: https://js.munano.org/nmr/

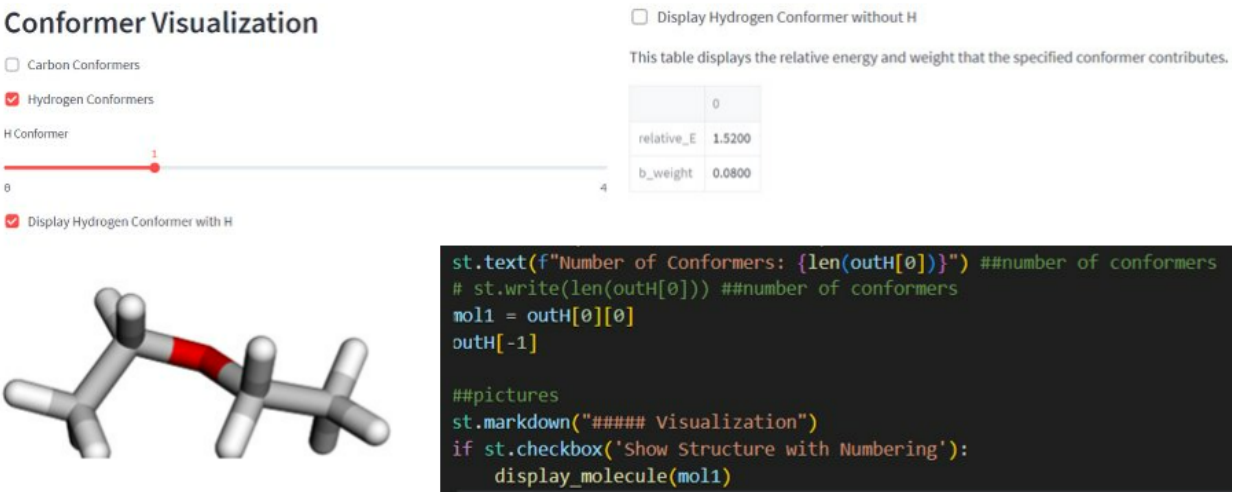


Image 2. Screenshot of a Section of NMR App Conformer Visualization and Coding

I am very fortunate and appreciative to have had the opportunity to conduct this research over the summer! I was able to learn a multitude of new techniques and methods that I had not previously been exposed to and gain hands-on experience. Having taken Organic Chemistry I and II last year, I was familiar with C and H NMR spectra and assignments, but had never utilized 3D spectra like COSY, HSQC, or HMBC to aid in predictions. I also was unaware that computational resources like NMRdb, CASCADE, and WebMO (Tantillo) were available until research began, so using these sites was very interesting! Additionally, I had never before attempted to code even the simplest of projects, so that was a new endeavor that ended up

paying off with the app! The opportunity to dive into this facet of organic and computational chemistry and gain familiarity with all of the various aspects has been a great educational experience and has afforded me with numerous new skills and abilities that I will carry with me in my future schooling and career!

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