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Laboratory Experiment

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Reassigning the Structures of Natural Products Using NMR Chemical Shifts Computed with Quantum Mechanics: A Laboratory Exercise

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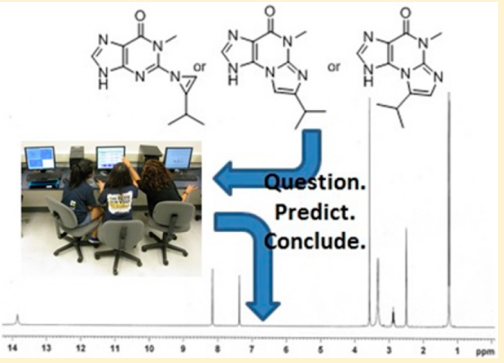
Supporting Information

ABSTRACT: An applied computational chemistry laboratory exercise is described in which students use modern quantum chemical calculations of chemical shifts to assign the structure of a recently isolated natural product. A pre/post assessment was used to measure student learning gains and verify that students demonstrated proficiency of key learning objectives.



KEYWORDS: *Computational Chemistry, Upper-Division Undergraduate, Computer-Based Learning, NMR Spectroscopy, Organic Chemistry, Laboratory Instruction, Laboratory Computing/Interfacing*

INTRODUCTION

The elucidation of the structures (connectivity, relative stereochemistry, and configuration) of complex natural products is a challenging endeavor despite huge leaps in spectroscopic technology over the past century. Among the most useful tools for structure elucidation is nuclear magnetic resonance (NMR) spectroscopy. Recently, Breton and Reynolds presented an excellent review of the various tools natural products chemists utilize in assigning structures, with an emphasis on how the use of NMR spectroscopy has evolved over the years.[1] Previous work has demonstrated the utility of NMR computations, in conjunction with traditional analyses, in the assignment and reassignment of natural product structures.[2]

Upper-division undergraduate chemistry courses generally do not expose students to this important aspect of NMR spectroscopy. Herein, a computer-based laboratory experiment is described that highlights this application of NMR spectroscopy, as well as the utility of quantum chemical calculations, through a brief project that models a state-of-the-art research project in the field of structural elucidation. This exercise is appropriate for students who have been introduced to the basic concepts of NMR spectroscopy and quantum mechanics; advanced knowledge of neither is required. Consequently, this lab exercise could be adapted for an undergraduate or graduate organic, natural products, quantum chemistry, or applied theoretical chemistry course (and perhaps others).

Key features of the approach include the following:

1. The exercise described is derived from recent reports on current research problems in the natural products and applied theoretical chemistry fields.
2. The exercise is designed to encourage hypothesis generation and testing, experimental design, discovery, active learning,[3] and data analysis by utilizing many activities and questions that involve higher order cognitive processes.[4]
3. The exercise provides a basic framework for applying NMR calculations to structure elucidation that is suitable for a 1−2 h laboratory period, but possible extensions appropriate for different courses, cohorts of students, and instructors are provided in the Supporting Information.

Previous Laboratory Exercises Involving Natural Product Isolation and/or Derivatization

Various undergraduate laboratory exercises that focus on the isolation and/or derivatization of natural products have been described. Five noteworthy examples are highlighted here.

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Greenberg presented a laboratory exercise in which students isolate orange oil directly from an orange peel and analyze the components via gas chromatography, IR spectroscopy, elemental analysis, and optical rotation.[5] Wagner and co-workers described an exercise in which a mixture of capsacinoids (**1** and **1**′, Figure 1) is isolated from dried chili

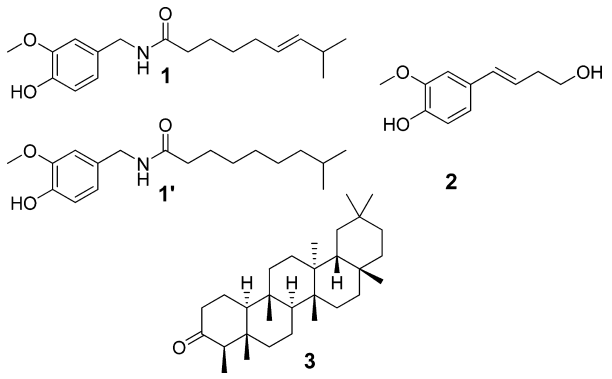

Figure 1. Various natural products and derivatives that have been studied in the context of undergraduate chemistry laboratories.[6,7,9]

peppers and characterized using NMR spectroscopy (^{1}H and ^{13}C NMR, along with proton−proton correlation spectroscopy [COSY] and proton−carbon correlation spectroscopy [HSQC]).[6] Taber and Frankowski presented an activity in which eugenol (a natural product that can be extracted from various spices) is subjected to cross-metathesis using Grubb's catalyst and *cis*-2-butene-1,4-diol to produce **2** (Figure 1).[7] This natural product derivative is characterized with a variety of spectroscopic techniques including NMR spectroscopy. O'Shea and co-workers published an exercise wherein various essential oils are isolated from spices, and high performance liquid chromatography (HPLC) and gas chromatography−mass spectrometry (GC−MS) are then used to deduce the spice associated with each oil.[8] Finally, LeFevre and co-workers described a laboratory experiment in which friedelin (**3**, Figure 1) is isolated from cork and reduced to the corresponding alcohol.[9] To determine which diastereomer is formed in this reaction, force field minimizations are used to determine optimal dihedral angles for each diastereomer, followed by application of the Karplus equation[10] to calculate the corresponding coupling constants expected for each diastereomer. Comparing these coupling constants to the experimental data allows students to identify which diastereomer was formed preferentially.

Previous Laboratory Exercises Utilizing Chemical Shift Calculations

Several laboratory exercises that make use of chemical shift predictions have been described.[11] For example, Wang described an activity in which chemical shifts predicted using *ChemDraw*[12] were correlated to pK_a's for a series of benzoic acids.[13] These chemical shifts are not predicted using quantum chemistry, however, but rather by an empirical scheme that makes use of known experimental data for molecules with similar functional groups; such approaches have obvious limitations.[2b] Simpson and co-workers described an activity in which a related empirical approach from *Advanced Chemistry Development* (*ACD*)[14] was utilized to analyze the organic molecules present in energy drinks.[15] Van Arnum utilized both of these NMR predictors in a laboratory exercise in which the utility of these methods in identifying products (including relative stereochemistry) of two sequential synthetic steps is evaluated through comparisons with experimental chemical shifts.[16] Recently, Pritchard et al. described a computational laboratory for upper-division undergraduates in which students compute the chemical shifts of paramagnetic inorganic complexes from first principles.[17] Baias et al. recently documented the use of powder NMR crystallography prediction as a novel means of using computational methods (verified by experiment) to predict the absolute structure of AZD8329 (a novel type I diabetes treatment) accurately (although this was not used for the development of a lab manual).[18]

THE PROBLEM STUDENTS TACKLE

Acremolin, originally isolated by Julianti and co-workers from the marine fungus *Acremonium strictum*, was first assigned as **6** (Figure 2).[19] Interestingly, this structure contains an azirine

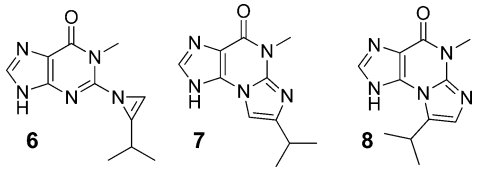

Figure 2. Originally proposed structure (**6**)[19] for acremolin and suggested alternatives (**7** and **8**).[21−23]

moiety, which would suffer the consequences of antiaromaticity (to the extent that distortion to remove cyclic conjugation is very difficult in 3-membered rings).[20] Soon after this structure was reported, Banert published a proposed reassignment in which two structures, each containing an imidazole moiety (**7** and **8**, Figure 2), were deemed much more likely; structure **7** was favored on the basis of a detailed analysis of the experimental chemical shift data.[21] It was also emphasized that azirines are exceedingly unstable and have only been detected at low temperatures.[22] Recently, Januar and Molinksi confirmed that the correct structure of acremolin is indeed **7** by synthesizing **7** from guanosine.[23] The exercise described herein challenges students to use NMR chemical shifts computed with quantum chemical methods to decide which possible acremolin structure, **6**−**8**, best matches the experimental chemical shift data, highlighting the fact that the correct structure can be chosen on the basis of this computed data alone.

THE METHODS STUDENTS USE

Quantum chemical predictions of NMR chemical shifts now play an important role in many studies aimed at elucidating the structures of complex organic molecules.[2b] Through the activities described herein, students gain firsthand experience with this useful technique.

The structures of compounds **6**−**8** were optimized[24] at various levels of theory to determine which theoretical methods provided meaningful results in a time frame appropriate for a laboratory exercise. Frequency calculations were used to ensure that each structure was a minimum on the potential energy surface (a minimum has no imaginary frequencies, while a transition state structure has one). ^{1}H and ^{13}C chemical shift data were generated by computing isotropic shielding constants for each atom in **6**−**8**.

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To account for the effects of solvent, here DMSO, the multistandard (MSTD) approach was utilized.[2b,25] In this approach, isotropic shielding constants for several model compounds (Figure 3) with known shifts in DMSO are computed (after structural optimization).[26]

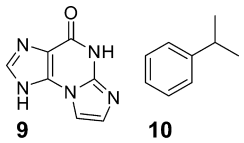

Figure 3. Guanine and cumene, chosen as model compounds due to their structural similarity to the compounds of interest and the availability of experimental NMR data in DMSO-d_6.[27,28]

These compounds are chosen to reflect structural features present in the compounds of interest (e.g., **6**–**8**). Guanine derivative **9** (Figure 3)[27] was used to correct ^{1}H and ^{13}C chemical shifts for the heterocycles in **6**–**8**, while isopropylbenzene (cumene, **10**, Figure 3)[28] was used to correct ^{1}H and ^{13}C chemical shifts for their isopropyl groups.

The calculated and experimental data for the model compounds are then used to convert the computed isotropic shielding constants for **6**–**8** into chemical shifts in DMSO using eq 1.

$$\delta_{COI} = \sigma_{MC} - \sigma_{COI} + \delta_{MC} \quad (1)$$

δ_{COI} is the predicted chemical shift for the compound of interest (COI) in a prescribed solvent, σ_{MC} is the computed isotropic shielding value in the model compound (MC), σ_{COI} is the computed isotropic shielding value in the COI, and δ_{MC} is the known experimental chemical shift for the MC in the solvent of interest. A flowchart illustrating the process students undertake is shown in Figure 4.

Results from four different combinations of methods for geometry optimization and chemical shift calculations are described here. As shown in Table 1, depending on the model chemistry chosen and the size of basis set used, these calculations can take minutes to hours. Different computers, software, and numbers of processors used will affect the results, but the data shown are intended to give readers a feeling for the time blocks generally necessary to complete these sorts of calculations at a chosen level of theory. Note that since NMR chemical shift calculations are generally faster than geometry optimizations (and associated frequency calculations), they can be computed using higher levels of theory than the corresponding geometry optimization and still be feasible within laboratory time constraints.

Representative computed isotropic shielding constants computed with HF/3-21G//HF/3-21G (NMR single point//geometry optimization) and experimental chemical shifts for model compound **9** are shown in Table 2. The data in this table are used to generate predicted chemical shifts for compounds **6**, **7**, and **8** using eq 1. Students generate (or are provided) such tables for whichever theoretical methods are to be used for the lab exercise.

Using the process outlined above (eq 1 and Figure 4), students generate predicted chemical shifts such as those shown in Figure 5. The data shown here were derived from HF/3-21G//HF/3-21G calculations, which are generated rapidly and are accurate enough to solve the structural problem posed herein.[26]

DATA ANALYSIS

Students use their predicted chemical shifts to decide which structure (**6**–**8**) is the best match for the experimental data available for acremolin. An effective means of performing such analysis involves the construction of bar graphs showing the difference between each predicted chemical shift and the corresponding experimental chemical shifts for acremolin (e.g., Figure 6, a "$\Delta\delta$" graph). Visual inspection of these graphs immediately rules out structure **6** as the correct structure of acremolin. Structures **7** and **8** both appear reasonable, although structure **7** appears to be a closer match upon further inspection.

It is useful to calculate mean absolute deviations (MAD) for compounds **6**–**8**. These values are obtained by determining the absolute difference of the δ_{COI} relative to the experimental data available for each nucleus in the COI, and taking an average. In general, if a structure is correct, it will have a MAD of less than 0.5 ppm for ^{1}H and less than 5 ppm for ^{13}C when a good level of theory and/or empirical scaling is employed.[2b] Compounds **6**, **7**, and **8** have MADs of 9.95, 4.39, and 5.17, respectively, for ^{13}C and 1.51, 1.48, and 1.46, respectively, for ^{1}H. These values

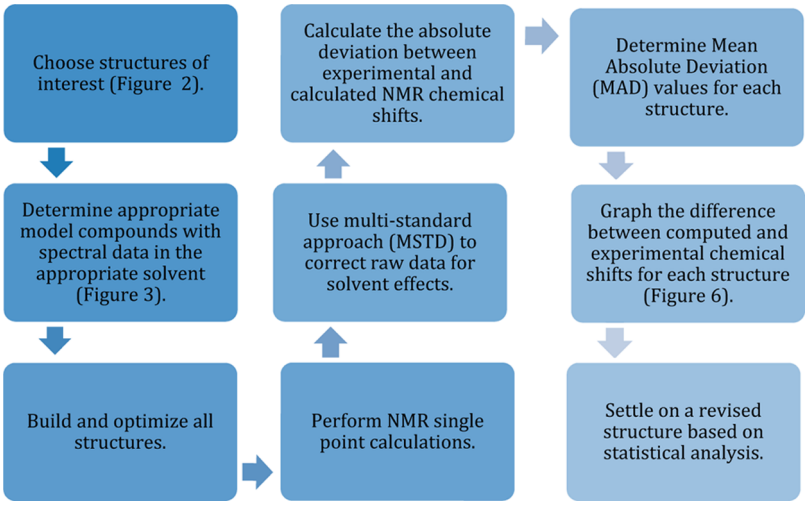

Figure 4. Process overview of the multistandard approach (MSTD) to computational NMR.

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Table 1. Timing Data for Selected Calculations on 9[26]

Geometry optimization	HF/3-21G [3 min, 1 processing core]	B3LYP/3-21G [15 min, 1 processing core]	B3LYP/6-31+G(d) [2 h 8 min, 1 processing core]	M06-2X/6-31+G(d) [1 h, 4 processing cores]
NMR calculation	HF/3-21G [2 min, 1 processing core]	B3LYP/6-31G(d) [15 min, 1 processing core]	B3LYP/6-31+G(d,p) [47 min, 1 processing core]	M06-2X/6-31+G(d,p) [22 min, 4 processing core]

Table 2. Computed (HF/3-21G//HF/3-21G) Isotropic Shielding Constants and Experimental Chemical Shifts (in DMSO, ppm) for Model Compound 9

^{13}C Type	Computed (σ_{MC})	Experimental (δ_{MC})	^{1}H Type	Computed (σ_{MC})	Experimental (δ_{MC})
Tertiary aromatic sp^2	108.2	109.9	Amide NH	27.1	12.3
Secondary aromatic sp^2	106.2	107.7	Secondary aromatic sp^2, 2 nitrogen adjacent	25.3	8.1
Amide carbonyl	63.9	154.4	Secondary aromatic sp^2, 1 nitrogen adjacent	25.6	7.6
Tertiary aromatic sp^2, 3 nitrogen adjacent	77.2	142.7	NH aromatic	25.1	13.8
Secondary aromatic sp^2, 2 nitrogen adjacent	85.2	140.0	Tertiary sp^3	31.2	1.2
Tertiary sp^3	184.0	23.9	Primary sp^3	32.4	2.9
Primary sp^3	192.6	33.4			

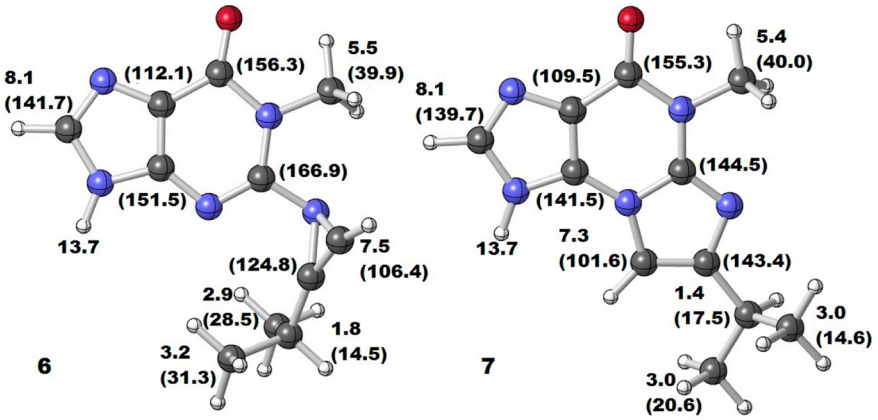

Figure 5. Ball-and-stick structures of **6** and **7** with predicted ^{1}H and (^{13}C) chemical shifts in DMSO computed with HF/3-21G//HF/3-21G.

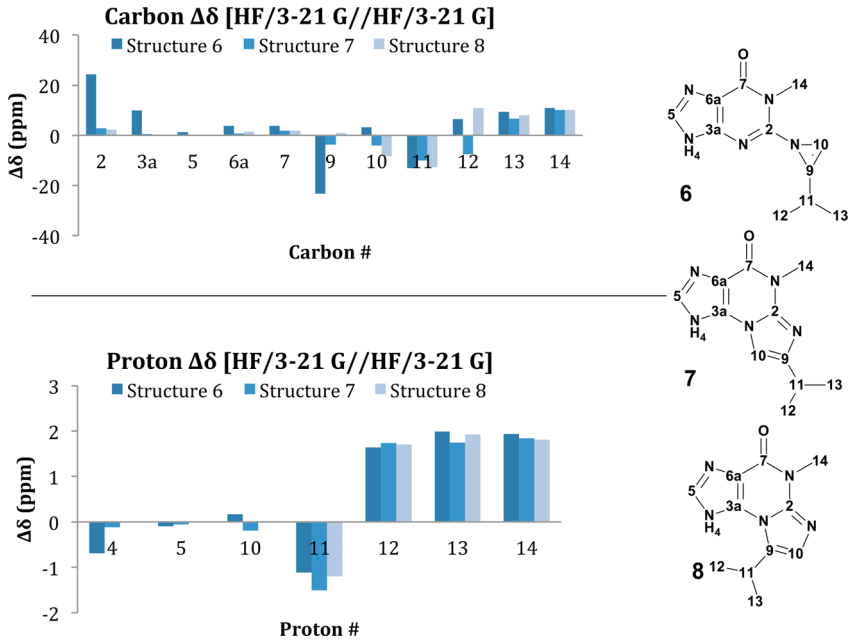

Figure 6. Carbon and proton "Δδ" graphs for data obtained with HF/3-21G//HF/3-21G. Bars indicate differences between computed and experimental chemical shifts.[26]

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do not include data for exchangeable protons as they are notoriously difficult to model in hydrogen bonding solvents.[2b] Also, it is noteworthy that chemical shifts for ^{13}C nuclei are often more accurately predicted than those for ^{1}H nuclei, since they are less subject to solvent effects (particularly in polar solvents such as DMSO).[2] Compound 7, the structure determined to be correct through synthesis,[23] is the closest match to the available ^{13}C experimental data. Note that, for the structural problem chosen, even the low HF/3-21G//HF/3-21G level is suitable for choosing the correct structure, despite the large magnitude of its MAD for ^{1}H shifts.[26]

HAZARDS

There are no physical hazards associated with this laboratory session.

IMPLEMENTATION

This laboratory exercise was implemented in an upper-division pharmaceutical chemistry computational lab course in a single, 3 h lab period in which students completed the prelab and the entirety of the lab as described (the students were provided with a template for the spreadsheet they were required to complete). A short response quiz was designed to assess each of the main learning objectives for this laboratory exercise (see Supporting Information). This assessment was given to students before the lab (the pre-assessment) and then in a subsequent lab (the post-assessment). The pre/post results (92 students total) are shown in Figure 7. The mean score

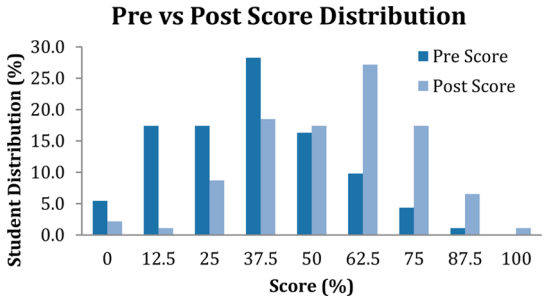

Figure 7. Pre vs post score assessment score distribution.

increased from 37.5% to 62.5%, demonstrating a substantial learning gain. A *t* test run on the 92 students' pre/post results demonstrated that this increase in quiz performance was statistically significant at the $\alpha = 0.05$ level (see Supporting Information). This pre/post assessment data demonstrated that this laboratory exercise was an effective instructional activity.

SUMMARY AND OUTLOOK

A theoretical chemistry laboratory exercise for upper-division undergraduates was developed and implemented; a complete laboratory manual is provided as Supporting Information.[26] The learning outcomes of this exercise were as follows: (1) students understood that natural product structures are sometimes misassigned[29] and gained an appreciation for why misassignments occur; (2) students learned how to build and optimize structures of complex organic molecules, and predicted their NMR chemical shifts, using quantum chemistry; and (3) students learned how NMR chemical shifts predicted using quantum chemistry can be employed to distinguish between several possible structures for an organic molecule. Students also gained experience with managing and analyzing multiple sets of data. Not only did this exercise provide students with practical tools they can use in future research projects, it also reinforced core concepts of NMR spectroscopy and molecular structure elucidation, all through a real world example selected from the recent primary research literature. Assessment data indicated a statistically significant knowledge gain associated with this laboratory exercise.

ASSOCIATED CONTENT

Supporting Information

Laboratory manuals, computational details, and sample student work. This material is available via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Breton, R. C.; Reynolds, W. F. Using NMR to Identify and Characterize Natural Products. *Nat. Prod. Rep.* **2013**, *30* (4), 501−24.
(2) (a) Tantillo, D. J. Walking in the Woods with Quantum Chemistry—Applications of Quantum Chemical Calculations in Natural Products Research. *Nat. Prod. Rep.* **2013**, *30* (8), 1079−86. (b) Lodewyk, M. W.; Siebert, M. R.; Tantillo, D. J. Computational Prediction of ^{1}H and ^{13}C Chemical Shifts: A Useful Tool for Natural Product, Mechanistic, and Synthetic Organic Chemistry. *Chem. Rev.* **2012**, *112* (3), 1839−62. (c) Willoughby, P. H.; Jansma, M. J.; Hoye, T. R. A Guide to Small-Molecule Structure Assignment through Computation of (^{1}H and ^{13}C) NMR Chemical Shifts. *Nat. Protoc.* **2014**, *9* (3), 643−60. (d) Toukach, F. V.; Ananikov, V. P. Recent Advances in Computational Predictions of NMR Parameters for the Structure Elucidation of Carbohydrates: Methods and Limitations. *Chem. Soc. Rev.* **2013**, *42*, 8376−415.
(3) Handelsman, J.; Miller, S.; Pfund, C. *Scientific Teaching*, 1st ed.; W. H. Freeman: New York, NY, 2006.
(4) Bloom, B. S. Reflections on the development and use of the taxonomy. In *Bloom's Taxonomy: A Forty-Year Retrospective*, 1st ed.; Anderson, L. W., Sosniak, L. A., Eds.; The National Society for the Study of Education: Chicago, IL, 1994.
(5) Greenberg, F. H. Natural Products Isolation—Orange Oil: An Undergraduate Organic Experiment. *J. Chem. Educ.* **1968**, *45* (8), 537−8.
(6) Wagner, C. E.; Cahill, T. M.; Marshall, P. A. Extraction, Purification, and Spectroscopic Characterization of a Mixture of Capsaicinoids. *J. Chem. Educ.* **2011**, *88* (11), 1574−9.
(7) Taber, D. F.; Frankowski, K. J. Grubbs's Cross Metathesis of Eugenol with cis-2-Butene-1,4-diol To Make a Natural Product. *J. Chem. Educ.* **2006**, *83* (2), 283−4.
(8) O'Shea, S. K.; Von Riesen, D. D.; Rossi, L. L. Isolation and Analysis of Essential Oils from Spices. *J. Chem. Educ.* **2012**, *89* (5), 665−8.
(9) LeFevre, J. W.; McNeill, K. I.; Moore, J. L. Isolating Friedelin from Cork and Reducing It to Friedelinol and Epifriedelinol - A

Project Involving NMR Spectrometry and Molecular Modeling. *J. Chem. Educ.* **2001**, *78* (4), 535−8.
(10) Minch, M. J. Orientational dependence of vicinal proton−proton NMR coupling constants: The Karplus relationship. *Concepts Magn. Reson.* **1994**, *6* (1), 41−56.
(11) In addition, several activities using quantum calculations of molecular geometries and electronic structures to rationalize experimental chemical shifts and coupling constants are described in the following: Hehre, W. J.; Shusterman, A. J.; Nelson, J. E. *The Molecular Modeling Workbook for Organic Chemistry*; Wavefunction, Inc.: Irvine, CA, 1998; Chapter 19.
(12) *ChemBioDraw Ultra, 12.0*; CambridgeSoft:Waltham, MA, 2009.
(13) Wang, H. Application of ChemDraw NMR Tool: Correlation of Program-Generated ^{13}C Chemical Shifts and pKa Values of para-Substituted Benzoic Acids. *J. Chem. Educ.* **2005**, *82* (9), 1340−1.
(14) *Advanced Chemistry Development, 12.0*; Advanced Chemistry Development, Inc.: Toronto, Ontario, Canada, 2012.
(15) Simpson, A. J.; Shirzadi, A.; Burrow, T. E.; Dicks, A. P.; Lefebvre, B.; Corrin, T. Use of NMR and NMR Prediction Software To Identify Components in Red Bull Energy Drinks. *J. Chem. Educ.* **2009**, *86* (3), 360−2.
(16) Van Arnum, S. D. A Laboratory Assignment in ^{1}H NMR Spectroscopy: A Comparative Analysis of Calculated and Experimental ^{1}H NMR Chemical Shifts. *J. Chem. Educ.* **2006**, *83* (3), 429−31.
(17) Pritchard, B. P.; Simpson, S.; Zurek, E.; Autschbach, J. Computation of Chemical Shifts for Paramagnetic Molecules: A Laboratory Experiment for the Undergraduate Curriculum. *J. Chem. Educ.* **2014**, *91* (7), 1058−63.
(18) Baias, M.; Dumez, J. M.; Svenson, P. H.; Schantz, S.; Day, G. M.; Emsley, L. De Novo Determination of the Crystal Structure of a Large Drug Molecule by Crystal Structure Prediction-Based Powder NMR Crystallography. *J. Am. Chem. Soc.* **2013**, *135* (46), 17501−7.
(19) Julianti, E.; Oh, H.; Lee, H. S.; Oh, D. C.; Oh, K. B.; Shin, J. Acremolin, a New 1H-azirine Metabolite from the Marine-Derived Fungus *Acremonium strictum*. *Tetrahedron Lett.* **2012**, *53* (23), 2885−6.
(20) (a) Wiberg, K. B. Antiaromaticity in Monocyclic Conjugated Carbon Rings. *Chem. Rev.* **2001**, *101* (5), 1317−32. (b) Breslow, R. Antiaromaticity. *Acc. Chem. Res.* **1973**, *6* (12), 393−8.
(21) Banert, K. Acremolin, a Stable Natural Product with an Antiaromatic 1H-Azirine Moiety? A Structural Reorientation. *Tetrahedron Lett.* **2012**, *53* (47), 6443−5.
(22) Banert, K.; Hagedorn, M.; Peisker, H. Synthesis of Stable 1*H*-Azirines Reinvestigated: A Structural Corrigendum. *Syn. Lett.* **2012**, *23* (20), 2943−6.
(23) Januar, L. A.; Molinski, T. F. Acremolin from *Acremonium strictum* is N^2,3-Etheno-20-isopropyl-1-methylguanine, Not a 1H-Azirine. Synthesis and Structural Revision. *Org. Lett.* **2013**, *15* (10), 2370−3.
(24) Frisch, M. J. *Gaussian 09, Revision B.01*; Gaussian, Inc.: Wallingford, CT, **2010**. Other quantum chemical software could, of course, be used. No-cost access to Gaussian is available via the National Science Foundation's XSEDE program, for example: https://portal.xsede.org/ (accessed Nov 2014).
(25) (a) Sarotti, A. M.; Pellegrinet, S. C. Application of the Multi-standard Methodology for Calculating ^{1}H NMR Chemical Shifts. *J. Org. Chem.* **2012**, *77* (14), 6059−65. (b) Sarotti, A. M.; Pellegrinet, S. C. A Multi-standard Approach for GIAO ^{13}C NMR Calculations. *J. Org. Chem.* **2009**, *74* (19), 7254−60. (c) Watts, H. D.; Mohamed, M. N. A.; Kubicki, J. D. Comparison of Multistandard and TMS-Standard Calculated NMR Shifts for Coniferyl Alcohol and Application of the Multistandard Method to Lignin Dimers. *J. Phys. Chem. B* **2011**, *115* (9), 1958−70.
(26) See Supporting Information for "Going Further" exercises for classes with more time.
(27) (a) Fedtke, N.; Boucheron, J. A.; Turner, M. J., Jr.; Swenberg, J. A. Vinyl Chloride Induced DNA Adducts. I: Quantitative Determination of N^2,3-Ethenoguanine Based on Electrophore Labeling. *Carcinogenesis* **1990**, *11* (8), 1279−85. (b) Lee, S. H.; Oe, T.; Blair, I. A. 4,5-Epoxy-(*E*)-2-Decenal-Induced Formation of 1,N^6-Etheno-2′-deoxyadenosine and 1,N^2-Etheno-2′-Deoxyguanosine Adducts. *Chem. Res. Toxicol.* **2002**, *15* (3), 300−4.
(28) Jones, I. C.; Sharman, G. J.; Pidgeon, J. Spectral Assignments and Reference Data. *Magn. Reson. Chem.* **2005**, *43* (6), 497−509.
(29) Nicolaou, K. C.; Snyder, S. A. Chasing Molecules That Were Never There: Misassigned Natural Products and the Role of Chemical Synthesis in Modern Structure Elucidation. *Angew. Chem., Int. Ed.* **2005**, *44* (7), 1012−44.