

Introducing 2D NMR Spectroscopy to Second-Year Undergraduate Chemistry Majors Using a Building-Up Approach

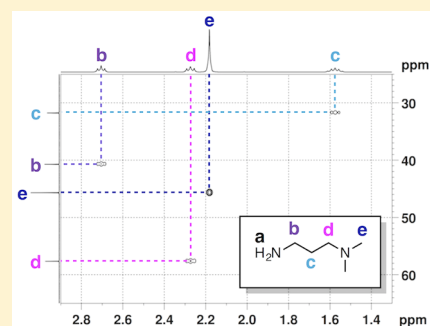
Amelia M. Anderson-Wile*

Department of Chemistry and Biochemistry, Ohio Northern University, 525 South Main Street, Ada, Ohio 45810, United States

S Supporting Information

ABSTRACT: 2D NMR spectroscopy techniques are introduced to second-year undergraduate organic chemistry students using a 3 h classroom activity. By first studying simple organic molecules, students are able to build up to solve more complex structures. This classroom activity first utilizes an instructor-guided problem-solving session focused on simple organic molecules (*N,N'*-dimethylethylene diamine, *N,N*-dimethylethylene diamine, and 3-(dimethylamino)-1-propylamine). Students then work together in teams to assign a more structurally complex molecule (2,4-dimethylphenol or 2,4-di-*tert*-butylphenol) using both 1D (^1H , ^{13}C , and DEPT-135) and 2D (COSY, HSQC, and HMBC) NMR spectra. By teaching second-year undergraduate students 2D NMR spectroscopy, the techniques are easily revisited in advanced undergraduate courses.

KEYWORDS: Second-Year Undergraduate, Organic Chemistry, Hands-On Learning/Manipulatives, Amines/Ammonium Compounds, NMR Spectroscopy, Phenols, Problem Solving/Decision Making



With the increasing availability of high-field NMR spectrometers at undergraduate institutions, it is useful to develop approachable activities that allow undergraduate students to explore advanced techniques such as 2D NMR spectroscopy.¹ The activity reported herein focuses on introducing 2D NMR spectroscopy to second-year undergraduate students nearing the end of their second semester in organic chemistry. The exercise starts by presenting students with NMR spectra for simple organic molecules; ultimately, they build up to solving more complex NMR spectra on their own.² Many articles published in this *Journal* have incorporated 2D NMR spectroscopy into laboratory experiments;³ however, few have focused on how to introduce the topic to undergraduate students using guided classroom activities.⁴ Due to the increased complexity of 2D NMR spectroscopy, there is not a clear consensus on when this concept should be introduced to undergraduate students.^{4a} We have found that second-year students with a prior knowledge of basic 1D NMR spectroscopy can begin solving 2D NMR spectra. Exposing students to a simple 2D NMR spectroscopy exercise incorporating ^1H - ^1H correlation spectroscopy (COSY), ^1H - ^{13}C heteronuclear single quantum coherence spectroscopy (HSQC), and ^1H - ^{13}C heteronuclear multiple bond coherence spectroscopy (HMBC) during their second year allows the concept to be easily revisited and utilized in advanced courses.

ORGANIZATION

The 2D NMR classroom exercise described herein is carried out over one 3 h period; however, the activity could be modified easily to fit into three 1 h lecture periods. The exercise was designed to complement an interdisciplinary laboratory experiment that focuses on the synthesis of an amine-

(bisphenolate) ligand using a Mannich reaction during second-year organic chemistry. The ligand is then metalated the following year in an introductory inorganic course.⁵ This 2D NMR activity was developed as a stand-alone exercise, and may easily be carried out without the laboratory experiment. The presentation given by an instructor and the NMR analysis done by students focus on complete NMR characterization of the Mannich reaction starting materials (Figure 1). This forces

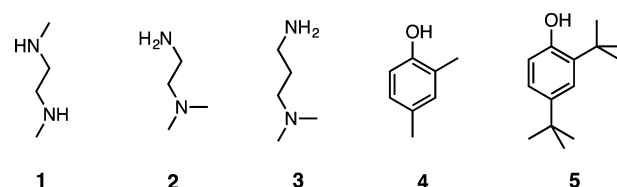


Figure 1. Compounds analyzed using 1D and 2D NMR spectroscopy.

students to analyze and interpret NMR spectra of their starting materials prior to carrying out a laboratory experiment, as well as providing them with simple molecules to begin their interpretation of 2D NMR spectra.

The starting materials analyzed in this exercise are ideal for an introductory discussion of 2D NMR spectroscopy. The amine compounds (1–3) presented by an instructor exhibit simple ^1H and ^{13}C NMR spectra that are easy to interpret for any student still mastering organic chemistry, which makes them an ideal starting point for the discussion of 2D NMR

Received: September 10, 2015

Revised: January 12, 2016

Published: January 29, 2016

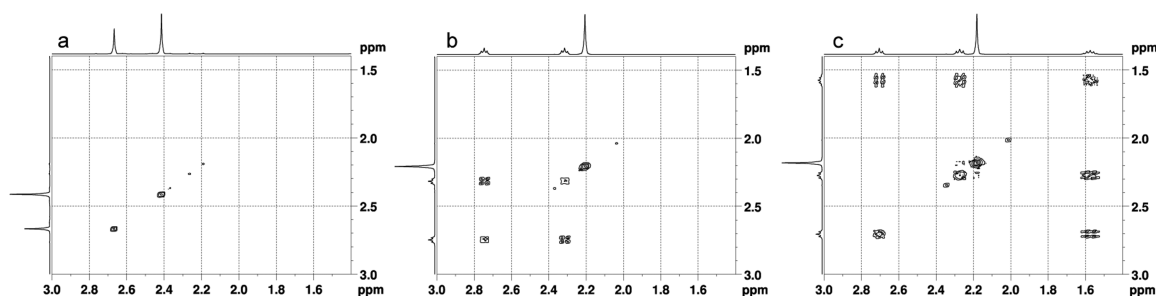


Figure 2. COSY NMR spectra of (a) **1**, *N,N'*-dimethylethylene diamine; (b) **2**, *N,N*-dimethylethylene diamine; and (c) **3**, 3-(dimethylamino)-1-propylamine.

spectroscopy. Since **1–3** are relatively simple molecules, students can focus on the structural features that result in the signals observed in the 2D NMR spectra rather than getting confused in the process of assigning complicated ^1H and ^{13}C NMR spectra. Once a background in 2D NMR spectroscopy is established, students move on to analyze both the 1D and 2D NMR spectra for one of two phenol molecules (**4**, **5**). It is not possible to assign the ^1H and ^{13}C NMR spectra of these phenols fully without the use of 2D NMR spectroscopy, which highlights the utility of 2D NMR techniques.

The students who participated in this activity were chemistry majors in the second semester of a year-long organic chemistry course. The students were all enrolled in a laboratory section designed specifically for chemistry majors that meets for a total of 6 h a week. The students are introduced to ^1H and ^{13}C NMR spectroscopy during their first semester of organic chemistry. By the time the students participate in this exercise, they have been working with NMR spectra for three to four months. The students already have a background in determining splitting patterns, calculating coupling constants, assigning ^1H NMR spectra, utilizing DEPT spectra, and assigning ^{13}C NMR spectra. Therefore, this activity focuses on introducing the students to 2D NMR spectroscopy.

MATERIALS AND METHODS

The exercise has been carried out two times with groups of 12 and 15 students. Compounds **1–5** and CDCl_3 were purchased from Sigma-Aldrich and used without further purification. The compounds were characterized by ^1H and $^{13}\text{C}\{^1\text{H}\}$ NMR spectroscopy using CDCl_3 as the solvent, with peak assignments assisted by the use of DEPT-135, ^1H – ^1H COSY, ^1H – ^{13}C HSQC, and ^1H – ^{13}C HMBC experiments. NMR spectra were recorded on a 400 MHz NMR spectrometer operating at 400.1325 MHz (^1H) or 100.6208 MHz (^{13}C). Chemical shifts are reported in ppm downfield of SiMe_4 and referenced using the solvent residual peak. NMR samples were prepared using one to three drops of liquid reagents or ~ 10 to 20 mg of solid reagents in 0.7 mL of CDCl_3 . All NMR spectra are included in the accompanying [Supporting Information](#).

HAZARDS

Gloves and goggles should be worn at all times during the preparation of NMR samples. All organic amines (*N,N'*-dimethylethylene diamine, CAS 110-70-3; *N,N*-dimethylethylene diamine, CAS 108-00-9; and 3-(dimethylamino)-1-propylamine), CAS 109-55-7), phenols (2,4-dimethylphenol, CAS 105-67-9; and 2,4-di-*tert*-butylphenol, CAS 96-76-4), and CDCl_3 (CAS 865-49-6) should be treated as flammable and harmful if inhaled, swallowed, or absorbed through the skin.

CDCl_3 is a carcinogen and should only be handled in a ventilated fume hood.

INTRODUCTORY 2D NMR SPECTROSCOPY DISCUSSION

The exercise begins with a 45 min presentation by an instructor to explain 2D NMR techniques, including COSY, HSQC, and HMBC. The lecture first focuses on the COSY experiment, which is a method to observe ^1H – ^1H correlations. Initially, students view a 1D ^1H NMR spectrum of **1** ([Supporting Information](#)) that is easy to assign using chemical shift and integration values. A COSY spectrum of **1** is presented, and students observe only peaks on the diagonal ([Figure 2a](#)), which provides an opportunity to discuss the nature of the diagonal peaks in the absence of ^1H – ^1H coupling. Subsequently, **2** is presented and the increased complexity in both the ^1H and COSY spectra is observed. After discussing the ^1H NMR spectrum of **2**, the COSY spectrum ([Figure 2b](#)) is presented, and students observe off-diagonal peaks consistent with coupling between the methylene hydrogens. Finally, the ^1H and COSY spectra of **3** ([Figure 2c](#)) are presented, and students learn how multiple off-diagonal correlations may be observed when a proton is coupling to more than one type of neighboring hydrogen atom. Starting from the simple COSY of **1**, students build up to the more complex COSY spectra of **2** and **3**, making it possible for them to see the relationship between the diagonal and off-diagonal peaks.

Next, the HSQC experiment, which is a type of ^1H – ^{13}C correlation spectroscopy resulting from one-bond couplings, is presented. Using the ^1H NMR assignments obtained above, students are shown how the ^{13}C NMR spectra may be assigned using the ^1H – ^{13}C correlations from the HSQC spectra. Using **2** as an example, the indicated hydrogen ([Figure 3](#)) has a resonance at 2.32 ppm in the ^1H NMR spectrum and shows a correlation to the carbon directly bound to it, which exhibits a ^{13}C NMR resonance at 62.8 ppm. It should be noted for molecules **2** and **3** that the ^1H NMR spectra cannot be unambiguously assigned until the HMBC spectra are analyzed. Therefore, students are given all of the ^1H NMR assignments obtained from the HMBC spectra to assist in the interpretation of the HSQC NMR spectra. Using this approach allows the more complicated HMBC spectra to be solved last. Students typically find both the COSY and HSQC experiments to be very approachable following this introductory analysis.

Finally, students are introduced to the HMBC experiment, which is a type of ^1H – ^{13}C correlation spectroscopy that detects two-bond and three-bond couplings. For both the HSQC and HMBC experiments, the lowest complexity molecule (**1**) is presented first and the material builds up to the molecule of

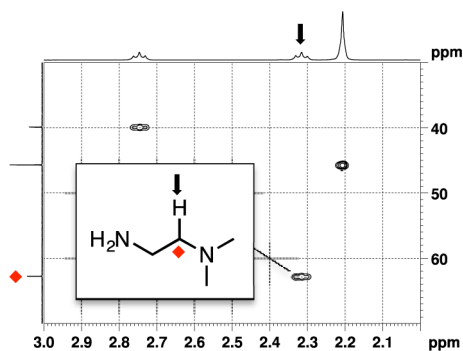


Figure 3. HSQC NMR spectrum of **2** in CDCl_3 . The one-bond correlation between the ^1H NMR resonance at 2.32 ppm (black arrow) and ^{13}C NMR resonance at 62.8 ppm (red diamond) is highlighted.

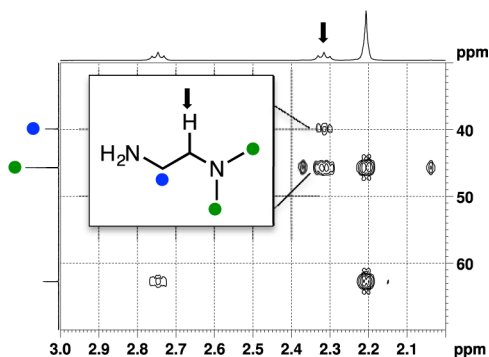


Figure 4. HMBC NMR spectrum of **2** in CDCl_3 . The two-bond (blue circles) and three-bond correlations (green circles) between the ^1H NMR resonance at 2.32 ppm (black arrow) and ^{13}C NMR resonances at 39.9 ppm (blue circle) and 45.7 ppm (green circles) are highlighted. Two ^{13}C satellite peaks are visible at (2.36 ppm, 45.7 ppm and 2.04 ppm, 45.7 ppm).

highest complexity (**3**). Using **2** as an example, the indicated hydrogen (Figure 4) has a resonance in the ^1H NMR spectrum at 2.32 ppm and exhibits correlations to carbons that are two (^{13}C NMR = 39.9 ppm) and three bonds (^{13}C NMR = 45.7 ppm) away. This advanced 2D NMR technique may be a little challenging for second-year organic students to comprehend; however, working together in groups, second-year undergraduate students have been able to solve the HMBC spectra of **4** and **5** with minimal assistance.

STUDENT EXPLORATION OF 2D NMR SPECTROSCOPY

Following the introductory lecture, students assign the ^1H , ^{13}C , DEPT-135, COSY, and HSQC NMR spectra for one of two phenol starting materials. Due to the moderate increase in

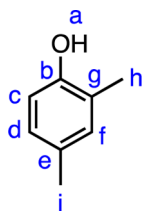


Figure 5. ^1H and ^{13}C NMR reference labels for all C and H atoms in 2,4-dimethylphenol (**4**).

difficulty level associated with assigning the spectra of the phenols, students work together in groups of three or four to complete the exercise. With the exception of the HMBC spectra, the students are given all of the spectral data at the start of the exercise, and it typically takes students about an hour to assign the spectra. Students begin by analyzing the ^1H NMR spectrum of their assigned phenol. The analysis of 2,4-dimethylphenol (**4**) is discussed; however, a very similar analysis procedure would be employed for 2,4-di-*tert*-butylphenol (**5**). The labeled structure of compound **4** is given in Figure 5, and the ^1H NMR assignments that are possible using either ^1H , COSY, or HSQC NMR techniques are listed in Table 1. The meta coupling between hydrogens f

Table 1. Hydrogen Assignments for Compound **4** using 1D and 2D NMR Spectroscopy

ppm	^1H NMR	COSY	HMBC
6.94	f	f	f
6.88	c/d	d	d
6.67	c/d	c	c
4.55	a	a	a
2.26	h/i	h/i	i
2.22	h/i	h/i	h

and d (Figure 5) is not observed in the ^1H NMR spectrum, making it difficult to assign the aromatic region completely. To address this, students analyze the COSY spectrum and observe that hydrogen d correlates with both hydrogens f and c, making it possible to assign all of the aromatic hydrogens. At this point, students cannot distinguish the methyl groups (h and i) since no coupling is observed in either the ^1H NMR or COSY spectrum. Using only the ^1H NMR spectrum, students assign two of the six types of hydrogens, whereas using the COSY spectrum will allow them to assign four of the six types of hydrogens (Table 1).

Students then move on to analyze the ^{13}C and DEPT-135 NMR spectra (Table 2), which allows them to identify the

Table 2. Carbon Assignments for Compound **4** using 1D and 2D NMR Spectroscopy

ppm	^{13}C and DEPT	HSQC	HMBC
151.6	b/g/e	b/g/e	b
131.8	c/d/f	f	f
130.0	b/g/e	b/g/e	e
127.6	c/d/f	d	d
123.5	b/g/e	b/g/e	g
114.9	c/d/f	c	c
20.6	h/i	h/i	i
15.8	h/i	h/i	h

aromatic methine (c, d, f), quaternary aromatic (b, e, g), and methyl (h, i) carbons. Since no distinction between carbons of a similar class can be made, students cannot definitively assign the eight carbons present in the molecule. To clarify the assignments using only ^{13}C and DEPT-135 NMR spectra, students would have to base predictions on proximity to the electron withdrawing group, which is not always a reliable method, or use an NMR prediction software package. Here is another point where the utility of 2D NMR spectroscopy may be highlighted. Using the partially assigned ^1H NMR spectrum from the analysis above and the HSQC spectrum, students

assign the aromatic methine carbons based on experimental data. When the HSQC experiment is utilized, students definitively assign three of the eight carbon resonances (Table 2).

At this point, students will have realized that, using the experiments listed above, they cannot unambiguously assign the quaternary carbons (b, e, g), methyl carbons (h, i), and methyl hydrogens (h, i) present in the molecule. This incomplete assignment has been a factor to motivate students to tackle the tougher HMBC spectra. Instructors not wishing to discuss HMBC correlations would still be able to have students assign a significant number of peaks in the ^1H and ^{13}C NMR spectra, as well as illustrate the utility of 2D NMR spectroscopy. Handing out the HMBC spectra after completion of the other NMR exercises allows the instructor to check the accuracy of the assignments before the students to move on to the more challenging HMBC activity. The HMBC portion of the exercise typically takes about an hour to complete and is ideally carried out with an instructor present to field questions and to guide students in the appropriate direction. Using the HMBC spectra, second-year undergraduate students have fully assigned all of the resonances in both the ^1H and ^{13}C NMR spectra for 4 and 5.

Students have generally been very successful at this exercise with all students receiving grades of 80–100% on the assignment. The most common stumbling point in the exercise is the assignment of the aromatic methine protons using the COSY spectrum. This is easily addressed by the instructor circulating during the activity to answer questions and check assignments. Students typically quickly grasp the HSQC exercise, and there are usually few questions for the instructor. The HMBC activity can be challenging for some students. This may be addressed by the instructor guiding the students to useful starting resonances for their analysis. Assisting the students in solving one of the resonances typically points them in the right direction for the rest of the analysis. The Supporting Information provides a detailed assessment of the resonances for each phenol molecule as well as more information about common stumbling points. In general, working in groups has allowed students to completely assign the ^1H and ^{13}C NMR spectra of the phenol molecules.

Since the students are largely successful carrying out this exercise when the instructor is present to field questions, we wanted to learn more about their ability to utilize 2D NMR techniques on their own. During our second year running this exercise, we taught the students how to acquire their own 2D NMR spectra and started requiring the use of 2D NMR spectroscopy in the characterization of an amine(bisphenolate) ligand⁵ and in the structure determination of an unknown organic molecule. For the structure determination assignment, the students are assigned an unknown organic molecule and they must obtain all of the necessary spectral data to prove the structure of their molecule. Each student is required to carry out COSY and HSQC experiments on their unknown organic molecule. Since we view HMBC as a more advanced 2D NMR technique, we have not required this characterization method. We found that 11 of 14 students successfully acquired and analyzed both the HSQC and COSY spectra for their unknown organic molecules. From this we concluded that our introductory exercise successfully taught the students basic 2D NMR spectroscopy.

CONCLUSION

The exercise utilized simple organic molecules to teach second-year undergraduate students about 2D NMR spectroscopy techniques. Students who participated in this exercise used these techniques in an organic chemistry lab for the characterization of an amine(bisphenolate) ligand, as well as in the determination of unknown organic molecules. By building up their NMR assignments from the simpler 1D NMR spectra to the more complicated 2D NMR spectra, students were able to assign the structure of small organic molecules definitively, as well as appreciate the utility of 2D NMR spectroscopy in structure determination.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.5b00741.

Student handouts, instructor notes, and NMR spectra (^1H , ^{13}C , COSY, HSQC, and HMBC) (ZIP)

AUTHOR INFORMATION

Corresponding Author

*E-mail: a-anderson.4@onu.edu.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The author acknowledges Ohio Northern University for funding and support in the development of this activity. The author thanks Tevye Celius and Bradley Wile for assistance with planning and implementation.

REFERENCES

- (1) (a) Chou, S.-C.; Mercier, J. E.; Wilson, K. A.; Beck, J. J. Complete Proton and Carbon Assignment of (+)-Catechin via One- and Two-Dimensional Nuclear Magnetic Resonance (NMR) Analysis: A Hands-on Learning Experiment for Upper-Division Undergraduate Chemistry Students. *Chem. Educ.* **2006**, *11* (1), 15–22. (b) Wilson, K. A.; Beck, J. J. Complete Proton and Carbon Assignment of Triclosan via One- and Two-Dimensional Nuclear Magnetic Resonance Analysis. *Chem. Educ.* **2007**, *12* (5), 338–342.
- (2) Sims, P. A. An “Aufbau” Approach to Understanding How the King-Altman Method of Deriving Rate Equations for Enzyme-Catalyzed Reactions Works. *J. Chem. Educ.* **2009**, *86* (3), 385–389.
- (3) (a) French, L. G. Isolation and Structure Elucidation of the Terpene β -Thujone from Cedar Leaf Oil. *J. Chem. Educ.* **2011**, *88* (6), 829–831. (b) Wagner, C. E.; Marshall, P. A.; Cahill, T. M.; Mohamed, Z. Visually Following the Hydrogenation of Curcumin to Tetrahydrocurcumin in a Natural Product Experiment That Enhances Student Understanding of NMR Spectroscopy. *J. Chem. Educ.* **2013**, *90* (7), 930–933. (c) Mohd Nazri, M.; Samat, F. D.; Kavanagh, P. V.; Walsh, J. J. Nature’s Cholesterol-Lowering Drug: Isolation and Structure Elucidation of Lovastatin from Red Yeast Rice-Containing Dietary Supplements. *J. Chem. Educ.* **2012**, *89* (1), 138–140. (d) Clausen, T. P. Combining a Standard Fischer Esterification Experiment with Stereochemical and Molecular-Modeling Concepts. *J. Chem. Educ.* **2011**, *88* (7), 1007–1009. (e) Fernández, I.; Fernández Sánchez, J. F. Diamagnetic Anisotropy: Two Iron Complexes as Laboratory Examples. *J. Chem. Educ.* **2010**, *87* (3), 320–322. (f) Lefebvre, B.; Corrin, T.; Simpson, A. J.; Shirzadi, A.; Burrow, T. E.; Dicks, A. P. Use of NMR and NMR Prediction Software To Identify Components in Red Bull Energy Drinks. *J. Chem. Educ.* **2009**, *86* (3), 360–362. (g) Ball, D. B. Diastereoselectivity in the Reduction of α -

Hydroxyketones. An Experiment for the Chemistry Major Organic Laboratory. *J. Chem. Educ.* **2006**, 83 (1), 101–105. (h) Shaw, R.; Severin, A.; Balfour, M.; Nettles, C. Diels–Alder Cycloadditions: A MORE Experiment in the Organic Laboratory Including A Diene Identification Exercise Involving NMR Spectroscopy and Molecular Modeling. *J. Chem. Educ.* **2005**, 82 (4), 625–629. (i) Mitra, A.; Assarpour, A.; Seaton, P. J.; Williamson, R. T. Synthesis of Quinolines and Their Characterization by 2-D NMR Spectroscopy. *J. Chem. Educ.* **2002**, 79 (1), 106–110. (j) Al-Ajlouni, A. M.; Bose, R. N.; Volckova, E. Linkage Isomerization by Two-Dimensional ^{31}P Nuclear Magnetic Resonance Spectroscopy. An Undergraduate Inorganic Laboratory Experiment. *J. Chem. Educ.* **2001**, 78 (1), 83–87. (k) Mills, N. S. Complete Assignment of Proton Chemical Shifts in Terpenes: An Experiment Combining 2D NMR Spectroscopy with Molecular Modeling. *J. Chem. Educ.* **1996**, 73 (12), 1190–1192. (l) Branz, S. E.; Miele, R. G.; Okuda, R. K.; Straus, D. A. Double Unknown” Microscale Preparation and COSY Analysis of an Unknown Ester: An Introductory 2D-NMR Experiment. *J. Chem. Educ.* **1995**, 72 (7), 659–661. (m) Gonzalez, E.; Dolino, D.; Schwartzburg, D.; Steiger, M. A. Dipeptide Structural Analysis Using Two-Dimensional NMR for the Undergraduate Advanced Laboratory. *J. Chem. Educ.* **2015**, 92 (3), 557–560. (n) Augé, J.; Lubin-Germain, N. Hetero Diels-Alder Reaction with Aqueous Glyoxylic Acid: An Experiment in Organic Synthesis and 2-D NMR Analysis for Advanced Undergraduate Students. *J. Chem. Educ.* **1998**, 75 (10), 1285–1287. (o) LeFevre, J. W. Isolating trans-Anethole from Anise Seeds and Elucidating Its Structure: A Project Utilizing One- and Two-Dimensional NMR Spectrometry. *J. Chem. Educ.* **2000**, 77 (3), 361–363. (p) Stark, R. E.; Gaede, H. C. NMR of a Phospholipid: Modules for Advanced Laboratory Courses. *J. Chem. Educ.* **2001**, 78 (9), 1248–1250. (q) Dwyer, T. J.; Norman, J. E.; Jasien, P. G. Rotational Barriers in Push-Pull Ethylenes: An Advanced Physical-Organic Project Including 2D EXSY and Computational Chemistry. *J. Chem. Educ.* **1998**, 75 (12), 1635–1640. (r) Caes, B.; Jensen, D., Jr. Synthesis and Characterization of 9-Hydroxyphenalenone Using 2D NMR Techniques. *J. Chem. Educ.* **2008**, 85 (3), 413–415. (s) Zelisko, P. M.; Amarne, H. Y.; Bain, A. D.; Neumann, K. Extensions of a Basic Laboratory Experiment: [4 + 2] and [2 + 2] Cycloadditions. *J. Chem. Educ.* **2008**, 85 (1), 104–106. (t) Freyer, A.; Freyer, A.; DiMeglio, C. Synthesis and Characterization of (σ^2 -terpyridyl)Re(CO) $_3$ Cl with Determination of Chemical Ring Exchange Constant. *J. Chem. Educ.* **2006**, 83 (5), 788–790. (u) Timmers, F. J.; Wacholtz, W. F. An Advanced Inorganic Laboratory Experiment Using Synthesis and Reactivity of a Cycloheptatriene Molybdenum Complex. *J. Chem. Educ.* **1994**, 71 (11), 987–990.

(4) (a) Alonso, D. E.; Warren, S. E. NMR Analysis of Unknowns: An Introduction to 2D NMR Spectroscopy. *J. Chem. Educ.* **2005**, 82 (9), 1385–1386. (b) Helms, E.; Arpaia, N.; Widener, M. Assigning the NMR Spectrum of Glycidol: An Advanced Organic Chemistry Exercise. *J. Chem. Educ.* **2007**, 84 (8), 1328–1330. (c) Alty, L. T. Monoterpene Unknowns Identified Using IR, ^1H -NMR, ^{13}C -NMR, DEPT, COSY, and HETCOR. *J. Chem. Educ.* **2005**, 82 (9), 1387–1389.

(5) Kasting, B. J.; Bowser, A. K.; Anderson-Wile, A. M.; Wile, B. M. Synthesis and Metalation of a Ligand: An Interdisciplinary Laboratory Experiment for Second-Year Organic and Introductory Inorganic Chemistry Students. *J. Chem. Educ.* **2015**, 92 (6), 1103–1109.