

Preparation of a Cobalt(II) Cage: An Undergraduate Laboratory Experiment That Produces a ParaSHIFT Agent for Magnetic Resonance Spectroscopy

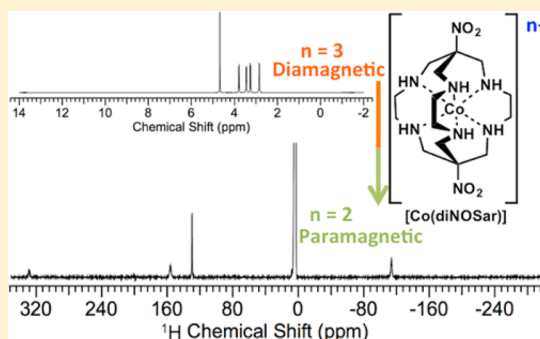
Patrick J. Burns, Pavel B. Tsitovich, and Janet R. Morrow*

Department of Chemistry, University at Buffalo, the State University of New York, Amherst, New York 14260, United States

S Supporting Information

ABSTRACT: Laboratory experiments that demonstrate the effect of paramagnetic complexes on chemical shifts and relaxation times of protons are a useful way to introduce magnetic resonance spectroscopy (MRS) probes or magnetic resonance imaging (MRI) contrast agents. In this undergraduate inorganic chemistry experiment, a paramagnetic Co(II) cage complex is prepared by reduction of a classical Co(III) cage complex. The Co(II) cage behaves as a paramagnetic shift agent (paraSHIFT) and produces relatively sharp and highly dispersed proton resonances. Comparison of the proton NMR spectra of the diamagnetic and paramagnetic cage complexes provides opportunities for students to consider coordination complex symmetry and the effect of unpaired electrons on the chemical shifts of proton resonances. Oxidation and spin state changes of transition metal complexes, Co(III)/Co(II), are also illustrated in this experiment.

KEYWORDS: Aqueous Solution Chemistry, Bioinorganic Chemistry, Coordination Compounds, Inorganic Chemistry, Magnetic Properties, Metals, NMR Spectroscopy, Oxidation/Reduction, Qualitative Analysis, Upper-Division Undergraduate



The application of magnetic resonance imaging (MRI) contrast agents in biomedical diagnostics is familiar to most students. Many students, however, may not be aware that these contrast agents contain paramagnetic metal ions. A search of this *Journal* for examples of MRI contrast agents yields a single article on Gd(III) complexes and measurements of T_1 relaxivity.¹ The lack of examples is surprising given the emphasis on paramagnetic states of metal ions in undergraduate inorganic chemistry courses. Notably, there are several articles in this *Journal* on magnetic susceptibility measurements^{2–5} and EPR spectroscopy⁶ that lay the foundation for paramagnetic metal ion interactions with ligands. Further, paramagnetic cobalt complexes have been studied before in this *Journal*.⁷ Paramagnetic shifts are reported, as part of the characterization of a complex or a metalloenzyme, but not with an emphasis on imaging.^{6,7} The undergraduate laboratory experiment described here is designed to give students an appreciation for paramagnetic metal ions and their effect on ^1H NMR resonances including changes in shift and relaxation time constants. The experiment focuses on the synthesis of a paramagnetic metal ion complex that produces highly shifted proton resonances. NMR spectra of the paramagnetic complex may be taken with short (millisecond) delay times to demonstrate the rapid collection of data for chemical shift imaging applications.⁸

Undergraduate students may be under the impression that paramagnetic metal ion complexes do not produce useful ^1H NMR spectra. In fact, certain paramagnetic metal ions give

beautifully sharp and highly shifted ^1H NMR spectra.^{9,10} Metal ions with rapid electron spin relaxation rate constants (10^{11} to 10^{12} s^{-1}) do not efficiently relax neighboring protons so that resonances are not severely broadened. For example, paramagnetic NMR spectra with proton resonances spread out over the range of several hundred parts per million (ppm) with line widths as narrow as 50–200 Hz for macrocyclic ethylene protons have been reported in our laboratory for complexes of Fe(II), Co(II), and Ni(II).^{8,11–13} These large changes in chemical shifts of protons induced by the paramagnetic center are known as hyperfine shifts.¹⁴ There are two primary components of the paramagnetic effect, including (1) the through bond or contact shift which corresponds to the amount of unpaired electron spin density at the nucleus and, (2) the dipolar or pseudocontact contribution which involves a through-space interaction between paramagnetic metal ion center and proton nucleus which is related to the geometry of the complex.¹⁵ Further reading on this topic is summarized in two early articles in this *Journal*^{14,16} and is given in a brief description in the student handout as [Supporting Information](#).

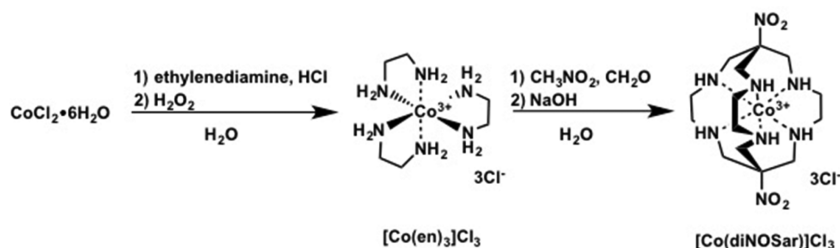
Co(II) complexes are studied here to capitalize on their excellent properties as NMR shift agents. The useful paramagnetic properties of this ion are demonstrated in Co(II)

Received: October 9, 2015

Revised: March 5, 2016

Published: March 25, 2016

Scheme 1. Synthesis of Co(III) Cage Complex



substituted metalloenzymes.^{9,17} Co(II) complexes have also been used as paraCEST (paramagnetic chemical exchange saturation transfer) MRI contrast agents.^{12,18} Co(II) paraCEST agents produce negative MRI contrast that can be switched on or off via a presaturation pulse.¹² More recently, paramagnetic lanthanide(III), Fe(II), and Co(II) complexes have been developed as paramagnetic probes for chemical shift imaging (paraSHIFT).^{8,19,20} In particular, the narrow proton resonances of the Co(II) complexes are shifted far outside of the range of diamagnetic compounds in tissue, making these complexes useful for magnetic resonance spectroscopy (MRS) as shown in this experiment.⁸ The large temperature dependence of the hyperfine-shifted proton resonances of paraSHIFT agents leads to their applications in monitoring tissue temperature.^{20,21}

The procedure detailed here demonstrates the synthesis and subsequent reduction of a diamagnetic Co(III) cage complex, 1,8-dinitro-3,6,10,13,16,19-hexaazabicyclo-(6,6,6)icosane (also called dinitrosarcophagine or “diNOSar”)²² to form a Co(II) cage complex. The Co(III) center of the $[\text{Co}(\text{diNOSar})]^{3+}$ cage²³ (Scheme 1) is reduced by sodium dithionite, while the nitro groups can be reduced under more stringent conditions involving excess dithionite.²⁴ The experiment here takes advantage of this early work and uses less than an equivalent of dithionite to produce the paramagnetically shifted proton resonances of the Co(II) complex. Cage complexes are chosen here to capitalize on the bicyclic scaffold, which encapsulates either Co(III) or Co(II) cation and, thus, imparts a large degree of kinetic inertness toward competing ligands and subsequent release of the metal ion.²⁵ Preparation of cage complexes proceeds by using a template synthesis based on a metal complex as one of the reagents. The metal ion is encapsulated by reaction with formaldehyde in the presence of base, which forms the connecting carbon fragment of the cage through a condensation reaction (Scheme 1).²² Previously reported undergraduate experiments on diamagnetic Co(III) cage complexes feature characterization of the complexes by using electronic spectroscopy, cyclic voltammetry, and NMR spectroscopy.^{22,26,27} Notably, the cage is prepared from relatively common and inexpensive chemicals.

In addition to paramagnetic ^1H NMR spectroscopy, this experiment gives hands on experience in important concepts in inorganic coordination chemistry including redox and properties of high spin/low spin transition metal ion complexes. Students gain experience in the characterization of coordination complexes by several spectroscopic techniques, including ^1H NMR and ^{13}C NMR spectroscopy, and mass spectrometry (Figures S1–S4).

LEARNING GOALS

In this laboratory experiment students learn that

1. Transition metal ion complexes with different oxidation states have distinct magnetic and spectroscopic properties as well as reactivity. This experiment has two redox steps including the preparation of Co(III) complexes from Co(II) precursors and the reduction of the diamagnetic Co(III) cage to the paramagnetic Co(II) cage.
2. The number of observed ^1H and ^{13}C NMR resonances corresponds to the symmetry of the complex. The simple ^1H NMR spectra of both diamagnetic and paramagnetic cobalt cages are a result of the high symmetry of the complexes.
3. The ^1H NMR spectra of certain paramagnetic transition metal complexes are readily observed. Paramagnetically shifted proton resonances can be used in magnetic resonance spectroscopy.

EXPERIMENTAL OVERVIEW

Synthesis of the individual compounds in the lab takes approximately 1 day each (3 h period), and the analysis of the paramagnetic cage is done on the final day. In total, the experiment is scheduled for three afternoons. The first two steps in the synthetic procedure are shown in Scheme 1. The first day the students prepare a Co(III) tris(ethylenediamine) complex from CoCl_2 and ethylenediamine also using HCl and hydrogen peroxide.²⁸ The yellow to yellow-orange product is isolated by chilling the solution in an ice bath or by letting the solution stand overnight in a freezer. The next day in the laboratory is used to convert the tris(ethylenediamine)Co(III) chloride ($[\text{Co}(\text{en})_3]\text{Cl}_3$) to the Co(III) cage complex ($[\text{Co}(\text{diNOSar})]\text{Cl}_3$) by using nitromethane, formaldehyde and base.²⁹ An indication that the synthesis will be successful is the change of the solution to a deep violet color when the base is added. If the color does not change, more formaldehyde and base may be added as described in the Supporting Information, instructor notes. After isolating the complex as a solid, the material is confirmed by electrospray ionization mass spectrometry (ESI MS, Figure S1) prior to preparing NMR samples (Figure S2). Students further analyze their product by ^1H NMR spectroscopy and are typically given the ^{13}C NMR spectrum (Figure S3). This two-step procedure is fairly straightforward and students are generally successful in obtaining product in two afternoons.

The final day in the laboratory is used to prepare the paramagnetic Co(II) cage complex. This step involves the reduction of the diamagnetic cage by sodium dithionite under inert atmosphere using argon-purged solutions (Figure 1). Students prepare the sample and the ^1H NMR spectrum of the Co(II) complex is taken within an hour of preparation to prevent reversible oxidation to Co(III) cage. Tips for taking and phasing paramagnetic ^1H NMR spectra are given in the

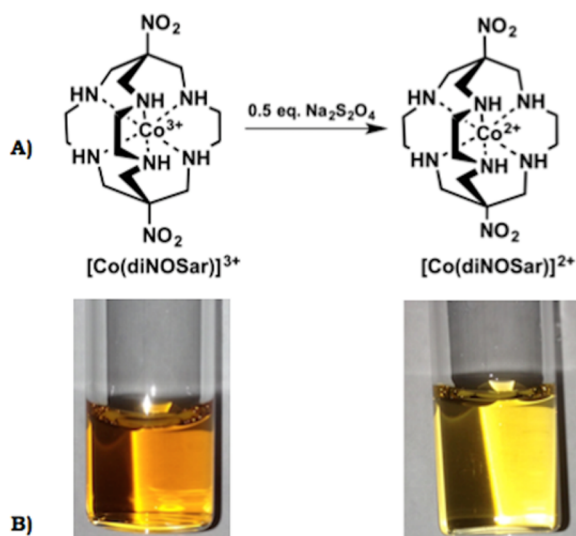


Figure 1. (A) Reduction of cage complex. (B) Aqueous solutions of Co(diNOSar) before (orange) and after (yellow) addition of 0.5 equiv of sodium dithionite solution. Solution of Co(II) cage complex is purged and stored under argon.

Supporting Information (Figure S4). It is most efficient if ^1H NMR spectra of the paramagnetic cages are collected with short pulse delay times. This allows for rapid collection of many transients over a period of 5–10 min depending on concentration. The experimental delay times are limited by longitudinal relaxation times (T_1) of individual proton resonances of paramagnetic Co(II) complex as listed in **Figure S5**. Notably, the resolution of the paramagnetically shifted proton resonances will be adequate regardless of magnetic field strength, whereas the proton resonances of the diamagnetic Co(III) cage complex may be poorly resolved at magnetic fields which are lower than those used here.

HAZARDS

These reactions involve the use of hydrochloric acid, a strong acid that should be handled with care. The synthesis of the ethylenediamine precursor requires hydrogen peroxide, a strong oxidant. Synthesis of the cage complex requires formaldehyde and nitromethane, both volatile organic reagents, and sodium hydroxide, a strong base. It is important for the students to know that these materials are all irritants and should be properly washed off if they get any on their skin. The reduction of the complex uses sodium dithionite, a powder that is poisonous if ingested and must be handled with care. The two Co(III) complexes that are synthesized are inert solids with unknown toxicity. The Co(II) cage is formed in aqueous solution and is not isolated or handled, but is disposed of in a standard glass waste container after characterization by NMR spectroscopy. Compressed argon should be used with care and an appropriate gas manifold should be used. Further information is given in **Table S1**.

DISCUSSION

The formation of the Co(III) cage complex was monitored by ESI mass spectrometry to illustrate methods that are commonly used to monitor the synthesis of coordination complexes (**Figure S1**).²⁹ The visible color of the Co(III) cage product was also an important indicator of whether the complex was successfully prepared as described in notes to the instructor.

Both the diamagnetic and paramagnetic cobalt cages are characterized by using NMR spectroscopy.

The ^1H NMR and ^{13}C NMR spectra of the Co(III) cage complex are remarkably simple due to the high degree of symmetry of the complex (**Figures S2 and S3**). The ^{13}C NMR of $[\text{Co}(\text{diNOSar})]^{3+}$ shows three resonances, one for six equivalent ethylene carbons, one for six equivalent methylene carbons and a 3-fold lower intensity resonance for two equivalent cap carbon atoms. The ^1H NMR spectrum shows ^1H – ^1H coupling in the ethylene and methylene groups for the diamagnetic Co(III) cage complex as analyzed previously.²⁹ Interactive exercises for identification of magnetically inequivalent protons in the diamagnetic cage complex have recently been published.²⁷

The ^1H NMR of the paramagnetic Co(II) cage shows four proton resonances that range from 350 to -120 ppm. The ^1H – ^1H coupling is no longer detected due to broadening of the resonances. While the proton resonances cannot be assigned to specific protons, the pattern corresponds to the high symmetry of the cage and the large chemical shifts demonstrate that the unpaired electrons of the Co(II) influence proton chemical shifts (**Figure 2**). Each of the four sets of proton resonances

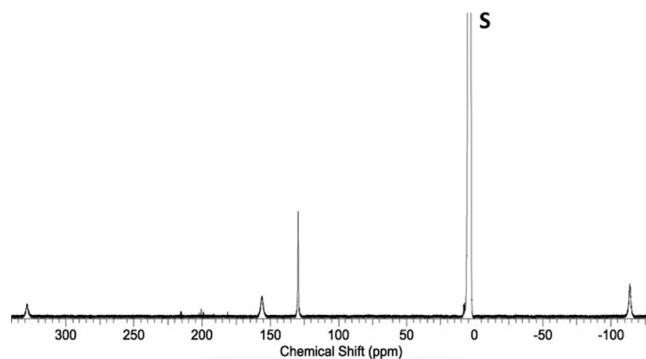


Figure 2. ^1H NMR spectrum of $[\text{Co}(\text{diNOSar})]^{2+}$ showing four paramagnetically shifted proton resonances (reference is the solvent peak labeled “S” in the diamagnetic region).

have similar integrated intensity consistent with the D_3 -symmetry of the Co(II) cage. Two sets of distinct protons for the methylene groups and two for the ethylene groups are expected as described in the **Supporting Information**. The more rapid relaxation of the proton in the presence of the paramagnetic Co(II) center (decreasing the T_1 time constant) is utilized by collecting data with short delay times to increase the number of transients collected and decrease the time required for data collection. In general, it is best to use delay times that are no shorter than the $4\times$ of the longest T_1 value (**Figure S5**). Short (200–400 ms) delay times give more pronounced paramagnetic spectra in the presence of diamagnetic species such as unreduced cage complex and water (solvent). An example of the ^1H NMR spectrum of the Co(II) cage in the presence of albumin is given in **Figure S6**. This shows that the paramagnetic cage proton resonances are clearly not obscured by the presence of a biological macromolecule. An example of variable temperature proton NMR studies is given in the **Supporting Information** to demonstrate how paraSHIFT agents may be used to measure temperature by using calibration lines for the temperature dependences of individual proton resonances (**Figure S7**).

These latter two experiments are presented to the students in lecture.

The theory of paramagnetic induced proton shifts and their temperature dependence may be discussed if there is a lecture that accompanies the laboratory, or alternatively, the students may be directed to the discussion presented in Drago's articles in this *Journal*.^{14,16} A brief discussion is also given in the [Supporting Information](#) in the student handout. Discussions in class may also cover the effect of paramagnetic centers on the proton relaxivity for common MRI agents such as Gd(III) complexes that act through increased relaxation of neighboring water protons. Other topics that may be included for discussion are crystal field splitting for Co(II) versus Co(III), and the corresponding formation of high-spin or low-spin complexes. Suggested postlaboratory questions for students are given in the student handout as [Supporting Information](#).

SUMMARY

This experiment was first carried out by second and third-year undergraduate research students several times over two semesters, and then implemented in an undergraduate laboratory/lecture course which is offered as a second semester of inorganic chemistry. An average of 26 third and fourth-year students take the course each semester, and the experiment has been incorporated into the laboratory for two semesters. Each undergraduate student prepared and characterized their own complexes. Typically, three 3-h laboratory periods are required; however, four periods were used by students who had to repeat the second step of the cage synthesis.

Students' answers to the questions in their lab writeup and on a course final exam showed that the majority of them (>75%) could describe certain aspects of the interaction of unpaired electrons with ligand protons. For example, they could describe ways in which unpaired electrons on the metal complex produce a shift in proton resonances, including through space and through bond interactions. Nearly all students (>85%) appreciated how differences in oxidation state affected the magnitude of the crystal field. However, most of the students (>60%) had difficulty in giving a rationale for the requirement of Co(II) in the synthesis of the cage. This demonstrated a lack of appreciation of the differences in reaction kinetics of Co(II) versus Co(III) complexes. Students were able to rationalize the number of observed proton resonances in diamagnetic and paramagnetic cage complexes.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the [ACS Publications website](#) at DOI: [10.1021/acs.jchemed.5b00818](https://doi.org/10.1021/acs.jchemed.5b00818).

Instructor handout, mass spectra, ¹H NMR, and ¹³C NMR spectra as well as discussion on theory and processing of paramagnetic ¹H NMR data ([PDF](#), [DOCX](#))

Student handout ([PDF](#), [DOCX](#))

AUTHOR INFORMATION

Corresponding Author

*E-mail: jmorrow@buffalo.edu.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We gratefully acknowledge the NSF (CHE-1310374) for support. This laboratory experiment was used in the advanced inorganic chemistry laboratory at the University at Buffalo. We thank the professor of the course, David Watson, as well as graduate students Zachary Schmidt and Christopher Bond, undergraduate students Alexandra Van Hall and Gregory Sokolow, and the CHE 322 undergraduate students who participated in the development of this experiment.

REFERENCES

- (1) Dawsey, A. C.; Hathaway, K. L.; Kim, S.; Williams, T. J. Introductory Chemistry: A Molar Relaxivity Experiment in the High School Classroom. *J. Chem. Educ.* **2013**, *90* (7), 922–925.
- (2) Schubert, E. M. Utilizing the Evans Method with a Superconducting NMR Spectrometer in the Undergraduate Laboratory. *J. Chem. Educ.* **1992**, *69* (1), 62.
- (3) Ostfeld, D.; Cohen, I. A. A Cautionary Note on the Use of the Evans Method for Magnetic Moments. *J. Chem. Educ.* **1972**, *49* (12), 829.
- (4) Deutsch, J. L.; Poling, S. M. The Determination of Paramagnetic Susceptibility by NMR: A Physical Chemistry Experiment. *J. Chem. Educ.* **1969**, *46* (3), 167–168.
- (5) Piguet, C. Paramagnetic Susceptibility by NMR: The "Solvent Correction" Removed for Large Paramagnetic Molecules. *J. Chem. Educ.* **1997**, *74* (7), 815–816.
- (6) Linenberger, K.; Bretz, S. L.; Crowder, M. W.; McCarrick, R.; Lorigan, G. A.; Tierney, D. L. What Is the True Color of Fresh Meat? A Biophysical Undergraduate Laboratory Experiment Investigating the Effects of Ligand Binding on Myoglobin Using Optical, EPR, and NMR Spectroscopy. *J. Chem. Educ.* **2011**, *88* (2), 223–225.
- (7) Uffelman, E. S.; Doherty, J. R.; Schulze, C.; Burke, A. L.; Bonnema, K. R.; Watson, T. T.; Lee, D. W. Microscale Syntheses, Reactions, and ¹H NMR Spectroscopic Investigations of Square Planar Macrocyclic Tetraamido-N Co(III) Complexes Relevant to Green Chemistry. *J. Chem. Educ.* **2004**, *81* (3), 325–329.
- (8) Tsitovich, P. B.; Cox, J. M.; Benedict, J. B.; Morrow, J. R. Six-Coordinate Iron(II) and Cobalt(II) ParaSHIFT Agents for Measuring Temperature by Magnetic Resonance Spectroscopy. *Inorg. Chem.* **2016**, *55* (2), 700–716.
- (9) Bertini, I.; Turano, P.; Vila, A. J. Nuclear Magnetic Resonance of Paramagnetic Metalloproteins. *Chem. Rev.* **1993**, *93* (8), 2833–2932.
- (10) Tsitovich, P. B.; Morrow, J. R. Macrocyclic ligands for Fe(II) ParaCEST and Chemical Shift MRI Contrast Agents. *Inorg. Chim. Acta* **2012**, *393*, 3–11.
- (11) Dorazio, S. J.; Tsitovich, P. B.; Sifers, K. E.; Sperryak, J. A.; Morrow, J. R. Iron(II) PARACEST MRI Contrast Agents. *J. Am. Chem. Soc.* **2011**, *133* (36), 14154–14156.
- (12) Dorazio, S. J.; Olatunde, A. O.; Sperryak, J. A.; Morrow, J. R. CoCEST: Cobalt(II) Amide-Appended ParaCEST MRI Contrast Agents. *Chem. Commun.* **2013**, *49* (85), 10025–10027.
- (13) Olatunde, A. O.; Dorazio, S. J.; Sperryak, J. A.; Morrow, J. R. The NiCEST Approach: Nickel(II) ParaCEST MRI Contrast Agents. *J. Am. Chem. Soc.* **2012**, *134* (45), 18503–18505.
- (14) Drago, R. S.; Zink, J. I.; Richman, R. M.; Perry, W. D. Theory of Isotropic Shifts in the NMR of Paramagnetic Materials. *J. Chem. Educ.* **1974**, *51* (6), 371–376.
- (15) Viswanathan, S.; Kovacs, Z.; Green, K. N.; Ratnakar, S.; Sherry, A. D. Alternatives to Gadolinium-Based Metal Chelates for Magnetic Resonance Imaging. *Chem. Rev.* **2010**, *110* (5), 2960–3018.
- (16) Drago, R. S.; Zink, J. I.; Richman, R. M.; Perry, W. D. Theory of Isotropic Shifts in the NMR of Paramagnetic Materials: Part II. *J. Chem. Educ.* **1974**, *51* (7), 464–467.
- (17) Pladziewicz, J. R.; Accola, M. A.; Osvath, P.; Sargeson, A. M. Metalloprotein-Cobalt Cage Electron Transfer and the Stereoselective Reduction of Spinach Plastocyanin by Λ - and Δ -[Co((N(CH₃)₃)₂-sar)⁴⁺]. *Inorg. Chem.* **1993**, *32* (11), 2525–2533.

(18) Tsitovich, P. B.; Sperryak, J. A.; Morrow, J. R. A Redox-Activated MRI Contrast Agent that Switches Between Paramagnetic and Diamagnetic States. *Angew. Chem. Int. Ed.* **2013**, *52* (52), 13997–14000.

(19) Harvey, P.; Blamire, A. M.; Wilson, J. I.; Finney, K.-L. N. A.; Funk, A. M.; Senanayake, P. K.; Parker, D. Moving the Goal Posts: Enhancing the Sensitivity of PARASHIFT Proton Magnetic Resonance Imaging and Spectroscopy. *Chem. Sci.* **2013**, *4* (11), 4251–4258.

(20) Coman, D.; Trubel, H. K.; Rycyna, R. E.; Hyder, F. Brain temperature and pH measured by ^1H chemical shift imaging of a thulium agent. *NMR Biomed.* **2009**, *22* (2), 229–239.

(21) Hekmatyar, S. K.; Hopewell, P.; Pakin, S. K.; Babsky, A.; Bansal, N. Noninvasive MR thermometry using paramagnetic lanthanide complexes of 1,4,7,10-tetraazacyclododecane- $\alpha,\alpha',\alpha'',\alpha'''$ -tetramethyl-1,4,7,10-tetraacetic acid (DOTMA⁺). *Magn. Reson. Med.* **2005**, *53* (2), 294–303.

(22) Harrowfield, J. M.; Lawrance, G. A.; Sargeson, A. M. Facile Synthesis of a Macrobicyclic Hexamine Cobalt(III) Complex Based on Tris(Ethylenediamine)Cobalt(III) - An Advanced Undergraduate Inorganic Laboratory Experiment. *J. Chem. Educ.* **1985**, *62* (9), 804–806.

(23) Harrowfield, J. M.; Herlt, A. J.; Lay, P. A.; Sargeson, A. M.; Bond, A. M.; Mulac, W. A.; Sullivan, J. C. Synthesis and Properties of Macrobicyclic Amine Complexes of Rhodium(III) and Iridium(III). *J. Am. Chem. Soc.* **1983**, *105* (16), 5503–5505.

(24) Balahura, R. J.; Ferguson, G.; Ruhl, B. L.; Wilkins, R. G. Kinetics of the Reduction of Nitro to Hydroxylamine Groups by Dithionite in a Cobalt(II) Cryptand Complex. X-ray Analysis of [1,8-Bis-(hydroxylamino)-3,6,10,13,16,19-hexaazabicyclo[6.6.6]eicosane]-cobalt(III). *Inorg. Chem.* **1983**, *22* (26), 3990–3992.

(25) Gahan, L. R.; Harrowfield, J. M. Sepulchrate: Four Decades on. *Polyhedron* **2015**, *94*, 1–51.

(26) Gahan, L. R.; Healy, P. C.; Patch, G. J. Synthesis of Cobalt(III) "Cage" Complexes - a Twist on an Old Theme in the Inorganic Laboratory. *J. Chem. Educ.* **1989**, *66* (5), 445–446.

(27) McClellan, M. J.; Cass, M. E. Improved Syntheses and Expanded Analyses of the Enantiomerically Enriched Chiral Cobalt Complexes $\text{Co}(\text{en})_3\text{I}_3$ and $\text{Co}(\text{diNOsar})\text{Br}_3$. *J. Chem. Educ.* **2015**, *92* (10), 1766–1770.

(28) Krause, R. A.; Megargle, E. A. Student Synthesis of Tris(ethylenediamine)cobalt(III) Chloride. *J. Chem. Educ.* **1976**, *53* (10), 667.

(29) Cai, H.; Fissekis, J.; Conti, P. S. Synthesis of a Novel Bifunctional Chelator AmBaSar based on Sarcophagine for Peptide Conjugation and ^{64}Cu Radiolabelling. *Dalton Trans.* **2009**, *27*, 5395–5400.