CHEMICALEDUCATION

An Advanced Organometallic Lab Experiment with Biological Implications: Synthesis and Characterization of $Fe_2(\mu-S_2)(CO)_6$

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Supporting Information

ABSTRACT: The organometallic complex $Fe_2(\mu-S_2)(CO)_6$ has interesting biological implications. The concepts of bio-organometallic chemistry are rarely discussed at the undergraduate level, but this experiment can start such a conversation and, in addition, teach valuable synthetic techniques. The lab experiment takes a total of five, 3-h lab periods and includes synthesis, purification, and characterization of the title molecule. The synthesis is done using standard Schlenk line techniques, but the product is air stable, which allows for standard benchtop purification. The compound is characterized using UV–vis spectroscopy, Fourier transform infrared spectroscopy, ¹³C NMR spectroscopy, and mass spectrometry.



KEYWORDS: Inorganic Chemistry, Upper-Division Undergraduate, Bioinorganic Chemistry, Coordination Compounds, IR Spectroscopy, Organometallics, UV—vis Spectroscopy, Hands-On Learning/Manipulatives, Synthesis, Laboratory Instruction

INTRODUCTION

Hydrogenase enzymes catalyze the reversible oxidation of molecular hydrogen and are found in bacteria that activate or fix small molecules or perform hydrogenation reactions.¹ There are NiFe, Fe-only, and nonmetal hydrogenase enzymes, and all are capable of hydrogen production and activation. The active sites of the metal hydrogenase enzymes are organometallic and have either a metal carbonyl or a metal cyano bond. There exist numerous reports of structurally similar small inorganic molecules throughout the chemical literature.¹⁻³ For example, the synthesis and characterization of an iron-iron hydrogenase model compound have been published previously in this Journal starting with $Fe_3(CO)_{12}$ and an appropriate thiol.² Although the compound discussed in this paper is structurally similar to the propane-bridged dimer, their synthetic routes are vastly different. Despite the large number of scholarly publications regarding $Fe_2(\mu-S_2)(CO)_{6}^4$ there are no accounts of it in the educational literature. $Fe_2(\mu-S_2)(CO)_6$ has been suggested in the literature to be the evolutionary precursor to the biological active site so important in hydrogen utilization.⁴ The synthesis of $Fe_2(\mu-S_2)(CO)_6$ was written in the German language in the 1950s⁵ and later reported in English in Inorganic Syntheses.⁶

The synthesis and purification of $Fe_2(\mu-S_2)(CO)_6$ are described along with characterization by UV–vis spectroscopy, Fourier transform infrared (FT-IR) spectroscopy, ¹³C NMR spectroscopy, and mass spectrometry. The approach presented here breaks down the experiment to allow students to accomplish the synthesis and purification of $Fe_2(\mu-S_2)(CO)_6$ in a teaching lab of 3 h or less time blocks and with the option to eliminate the use of HCl and, therefore, the generation of $\rm H_2S$ gas. The experiment could be used as a stand-alone experiment in a typical inorganic lab course or as a semester-long project. If desired, it would be possible to expand this experiment into a research project. For example, addition of a reducing agent, such as $\rm LiBH_{4^{\rm J}}$ followed by an alkyl halide to the titled molecule can provide a synthetic route to new and interesting thiol-bridged molecules.⁴ In addition, this approach can be used as a complement for iron–sulfur carbonyl clusters reported previously and allow for added synthetic flexibility.²

Pedagogical goals for this laboratory experiment include:

- (1) Demonstration of the biological relevance of inorganic chemistry
- (2) Demonstration that many synthetic techniques learned in the organic chemistry laboratory are also relevant in inorganic synthesis
- (3) Practice of new laboratory techniques for handling airsensitive compounds
- (4) Product characterization by spectroscopy

EXPERIMENTAL SECTION

Synthesis and Purification

Details of the experiment are included in the Supporting Information. Students work individually. Students use the first lab period to organize glassware and purge all of the solvents with either nitrogen or argon. During the second lab period, a degassed, basic methanol solution of $Fe(CO)_5$ is mixed with elemental sulfur at 0 °C and allowed to stir for about 30



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Journal of Chemical Education

min.^{5–7} Excess sulfur can be removed as H_2S by addition of acid or, optionally, left in the reaction mixture.⁷ If excess sulfur is removed with acid, this is done in a hood, and acid is added slowly to avoid a vigorous reaction; the reaction flask is well vented. This step will increase time in the lab by an additional hour. The acid can also be added during the following lab period. During the third lab period, $Fe_2(\mu-S_2)(CO)_6$ is extracted with either pentane or hexanes.^{6,7} During the fourth period, $Fe_2(\mu-S_2)(CO)_6$ is purified either by silica gel chromatography or by sublimation to remove other iron carbonyl clusters. Students require a full, 3-h lab period to pack and run a column. Alternatively, students purify by sublimation, which will also take at least one lab period. During the fifth lab period, students characterize the complex.

HAZARDS

Organic solvents are flammable and should be handled with both care and caution. Iron pentacarbonyl is a liquid at room temperature, is toxic and harmful if inhaled or swallowed, and could be a skin irritant; handling of this chemical should be done in a fume hood using a glass syringe. Hydrogen sulfide is a gas, considered toxic if inhaled, and is very odorant. Acidic addition to sulfur should be done in a fume hood. Hexanes (a known neurotoxin) should be handled in a hood under inert conditions. Pentane is a good substitute for hexanes and will work for the extractions. The final product, a metal carbonyl, should be treated with care, although toxicity issues are not known. Gloves and safety glasses are worn during the preparation and purification stages, and safety glasses are worn at all times including collection of the spectroscopic data. In addition, the use of a lab coat is suggested during synthesis. The ¹³C NMR was taken in CDCl₃, which is harmful if swallowed and causes skin and eye irritations. Gloves and safety glasses should be worn during NMR sample preparation.

RESULTS

Four upper-division undergraduate students successfully completed this experiment in five 3-h lab periods (see Table 1); all data shown are from students in an advanced synthesis

Table 1. Experimental Timeline and Topics

Lab Period	Activity	Topics for Discussion
1	Deaerate all solutions	Solvent purification and different methods of degassing
2	Synthesis of $Fe_2(\mu$ -S $_2)(CO)_6$	Schlenk line techniques, gas evolution
3	Extraction	Solubility
4	Purification	Sublimation and/or chromatography, recrystallization
5	Characterization	IR, UV–vis, ¹³ C NMR spectroscopy and mass spectrometry

course. Student yields varied with a range of 11–23% and seemed to depend on the synthetic skill of the student and rigorous degassing of solvents. The highest yield obtained from the four students was 23% and was in good comparison to literature values.⁷ These molecules are light sensitive, and care should be taken to avoid prolonged light exposure. Students generally purged with nitrogen, and degassing under reduced pressure and backfilling with nitrogen as well as freeze–pump– thaw techniques were discussed. Results for students who used HCl versus results for students who did not use HCl in the workup were similar. However, the reaction mixture was more tar-like when HCl was not added to react with excess sulfur.

Two students purified using silica gel chromatography, and two students used sublimation. The most abundant impurity was $Fe_3S_2(CO)_9$, which is an interesting molecule in its own right. The two compounds ($Fe_3S_2(CO)_9$ and $Fe_2(\mu-S_2)(CO)_6$) separated well using silica gel chromatography with either pentane or hexanes as an elutent. The desired product elutes first as an orange band and $Fe_3S_2(CO)_9$ second as a magenta band (Figure 1). By using sublimation as the purification



Figure 1. A photograph of the reaction mixture in a silica gel column eluting with pentane. The bottom band is $Fe_2(\mu-S_2)(CO)_{6}$, and the top band is $Fe_3S_2(CO)_9$.

technique, the desired product collected on a coldfinger when the solid was heated to 40 °C in a water bath, while $Fe_3S_2(CO)_9$ remained in the flask. Both purification techniques gave pure product and are viable options.

At least one whole lab period was required for students to collect the spectra of their compounds as suggested in Table 1. Information in the literature was given to students for comparison. The pure product was characterized by UV-vis spectroscopy (Figure 2) and FT-IR spectroscopy (Figure 3). The UV-vis spectrum compared very nicely with the literature spectrum, with a peak at 355 nm assigned to a $\sigma
ightarrow \sigma^*$ transition.⁸ The FT-IR data showed three strong peaks (2085, 2044, and 2008 cm⁻¹) for the CO stretching region and, again, was in agreement with literature values.⁶ The mass spectrum (Figure 4) and the ¹³C NMR spectrum (Figure 5) were collected. The mass spectrum was collected using time-of-flight (TOF), which is a soft ionization technique to avoid fragmentation and capture the molecular ion at 343.73 m/zratio, which agrees with theoretical calculations. Two other students also used a quadrupole mass spectrometer (data not shown) but only observed the fragments and did not obtain a peak for the parent compound. Lastly, the ¹³C NMR spectrum showed one peak at 210 ppm for both the apical and basal COs, which suggests that these carbons are equivalent. However, an alternative explanation for this apparent equivalency is the inherent fluxionality of iron carbonyl complexes, which allows



Figure 2. UV–vis spectrum of $Fe_2(\mu-S_2)(CO)_6$ in dichloromethane.



Figure 3. FT-IR spectrum of $Fe_2(\mu - S_2)(CO)_6$ in dichloromethane.



Figure 4. TOF mass spectrum of $Fe_2(\mu-S_2)(CO)_6$ showing a large molecular ion peak at 343.73 m/z.

for rapid exchange between COs. This rapid exchange makes distinguishing these carbons difficult on the relatively slow NMR time scale. Berry Pseudorotation, a behavior of fluxional molecules, is demonstrated in many organometallic complexes and discussed in some inorganic textbooks.⁹ Interestingly, these textbook discussions focus on 5-coordinate mononuclear complexes as examples of CO scrambling to explain these types of NMR spectra. The single ¹³CO resonance observed for $Fe_2(\mu-S_2)(CO)_6$ may represent the average of the fluxional behavior in solution at room temperature. When a solution of $Fe_2(\mu-S_2)(CO)_6$ is cooled to -73 °C, the carbonyl carbons show two resonances (210 and 204 ppm, basal and apical) in a 2:1 ratio because the exchange of the apical and basal COs is

Laboratory Experiment



Figure 5. ¹³C NMR spectrum of $Fe_2(\mu-S_2)(CO)_6$ in CDCl₃ with added tetramethylsilane (TMS) as an internal standard obtained using a Varian 400 MHz (¹H) 100 MHz (¹³C) spectrometer.

slow on the NMR time scale.¹⁰ However, the exact nature in which the $Fe_2(\mu-S_2)(CO)_6$ rearranges may be complicated by the rigid structure compared to 5-coordinate mononuclear metal carbonyls.

DISCUSSION

Iron sulfur clusters are well-known in biochemistry but are rarely the site of substrate binding and activation. The title compound, $Fe_2(\mu-S_2)(CO)_{6i}$ has been suggested to have formed from the primordial soup and incorporated into enzyme active sites.⁴ It has been the subject of many journal articles,³ but none of them have been educational or have targeted undergraduate students. Incorporation of inorganic synthesis of compounds with biological relevance into an undergraduate curriculum has inspired many students at our university to advance their graduate studies in bioinorganic chemistry.

Two students used this experiment as a traditional lab experiment in an advanced synthesis course, and two others as part of an introduction to research course in which they explored inorganic synthesis but later developed a modification of the target compound. These are upper-division classes with a small student to faculty ratio, which allows for close supervision. The pedagogical goals of the two courses are different, but synthesis and characterization of compounds and comparing spectra to literature results are important research skills that do overlap in these courses. Of the four students who completed this experiment, two did so in the advanced synthesis course. One of those two had considerable trouble with this experiment, which resulted in a lower than expected yield; however, this student was still able to obtain spectra that demonstrated a successful synthesis. Learning goals for the introduction to research and advanced synthesis courses were evaluated by presentations where students connected their results to the primary literature and as written reports in which students presented data and a discussion of that data in relation to the successful synthesis.

The pedagogical goals listed in the Introduction were assessed in several ways. First, all students had to write a detailed proposal and orally present their proposal to faculty teaching the course, and during the course of the semester, each student gave at least two oral presentations to the class, which reported on the background of the project, how they would

Journal of Chemical Education

complete the project, and last progress or results of the project. During these assignments, the link between inorganic chemistry and biochemistry was made not only to the students working directly on the project, but also to the whole class. The faculty assessed this subjectively and concluded that students gained an increased understanding of bioinorganic chemistry. The three learning objectives dealing directly with development of lab skills underwent formative assessment by faculty working directly with students in the lab, carefully observing their synthetic techniques and giving direct feedback. Progress in synthetic technique was assessed by whether or not students were able to successfully make the desired compound. Lastly, product characterization by spectroscopy was assessed when students discussed these results both in the class presentation and in their written report. The direct formative assessment during the presentations resulted in students developing a deeper understanding of spectroscopy and the applications for molecular characterization. Discussions of the results were presented in final reports, which allowed summative assessments of students' understanding of spectroscopy. Results of this assessment led to the conclusion that students were very good at using spectroscopic data to determine successful synthesis and developed a better understanding of synthetic adaptation from the literature during the course.

In conclusion, if students work in 3-h time blocks, the experiment should take five lab periods to complete. Students were able to generate the desired product with a range of 11-23% yield. Although yields were poor when students were not careful about degassing solvents, each student was able to obtain spectra of the desired product.

ASSOCIATED CONTENT

Supporting Information

Complete experimental details, student handouts, sample spectra. 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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