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A Teaching Experiment to Elucidate a Cation- π Effect in an Alkyne Cycloaddition Reaction and Illustrate Hypothesis Driven Design of Experiments

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Abstract

An organic chemistry experiment is described that is based on recent research to elucidate a novel cation- π interaction between tetraalkammonium cations and propargyl hydrazines. This non-bonded interaction is a key component of the mechanism of ammonium-catalyzed intramolecular cycloaddition of nitrogen to the terminal carbon of a C-C triple bond of the propargyl substrate. In this teaching experiment, reactions and control experiments are employed to demonstrate the testing of two alternative mechanistic hypotheses. Specifically, cyclization reactions are performed with a soluble base (sodium phenoxide) with and without tetrabutylammonium bromide under homogeneous conditions. Students observe that the added ammonium salt accelerates the reaction. They are then encouraged to develop a testable hypothesis for the role of the ammonium salt in the cyclization mechanism: typical phase transfer or other. IR spectroscopy is then used to directly observe a dose dependent shift of the alkyne stretching mode due to a cation- π interaction. In this experiment, undergraduate “researchers” were able to practice the scientific method on a contemporary system and see how data are generated and interpreted to adjudicate between rival hypotheses in a way that emulates authentic and current research in a lab setting. This experimental design was tested on students enrolled in the introductory undergraduate Organic Chemistry Lab.

Graphical Abstract

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Supporting Information

Detailed student handout of the experiment and instructor notes with IR spectra. This material is available via the Internet at <http://pubs.acs.org>.

Keywords

Second-Year Undergraduate; Organic Chemistry; Inquiry-Based/Discovery Learning; Mechanism of Reactions; IR Spectroscopy; Alkynes

While cation- π interactions have long been known and described in the literature,¹ they are included in relatively few introductory or even advanced textbooks² and laboratory experiments³ of organic chemistry. This is an unfortunate educational omission since cation- π interactions are being used more frequently to explain exciting new phenomena in biology,⁴ materials science,⁵ and chemistry.⁶ Indeed, we have previously invoked this non-bonded interaction to explain cyclization reactions of substituted propargyl hydrazines catalyzed by tetrabutylammonium fluoride (TBAF).⁷ This cyclization was unusual since transition metals are typically required to activate alkynes for nucleophilic attack.⁸ The mechanism of this reaction is believed to involve coordination of a tetrabutylammonium cation with the C-C triple bond of the substrate *via* a cation- π interaction.⁹ The process of investigation in which first indirect and then direct evidence of the cation- π effect were obtained constitute a unique teaching opportunity. Thus, in addition to developing a laboratory experiment to illustrate cation- π interactions, the goal was to train students in the scientific method, formulating hypotheses and designing experiments to test them.

This experiment is divided into two parts. It begins by introducing the student to a hypothetical argument based on indirect evidence¹⁰ for an ammonium-alkyne interaction. It was observed that propargyl hydrazine **1** (Figure 1) is converted to cyclic product **2** in the presence of KF only when tetrabutylammonium bromide (TBAB) is also present. In the introductory lecture, concepts were explained pertaining to two possible competing hypotheses that might account for the catalytic effect of the added ammonium salt. One hypothesis is that the ammonium functions as a phase transfer agent for the insoluble fluoride. The alternative is that the ammonium cation interacts with the alkyne, subtly influencing the cyclization mechanism. Students are prompted to think of an experiment that could distinguish between these two hypotheses and falsify one or the other. What experiment can be designed to demonstrate phase transfer catalysis? The answer is to use a soluble base and perform the reaction as a homogeneous solution, with and without added TBAB, to see whether TBAB enhances the rate of reaction compared to the soluble base alone (Figure 1). If the addition of TBAB to a homogeneous reaction mixture increases its rate, then the phase-transfer catalysis hypothesis would be revealed as an insufficient explanation.

The second part of the lab is based on the recently published spectroscopic evidence for this ammonium-alkyne interaction.⁹ In order to obtain direct evidence¹⁰ of the ammonium-alkyne interaction, we recently reported the use of Raman spectroscopy to observe the effect of TBAB on various terminal alkynes, including the propargyl hydrazine. A shift in frequency of the carbon-carbon triple bond stretching vibrational mode was observed in a direction that is consistent with a cation- π effect. In order to allow students the opportunity to gather novel data in the teaching lab, we adapted this TBAB titration experiment for IR spectroscopy (reported here for the first time), using the spectroscopic equipment available

in the teaching labs. Students performed their own TBAB titration by preparing samples of propargyl hydrazine in solution with increasing amounts of TBAB and drop depositing them on salt crystals. They then analyzed the samples using IR spectroscopy to semi-quantitatively observe the effect of the ammonium ion on the alkyne stretching frequency. Students analyzed the data to determine whether the evidence supports a cation- π interaction.

BACKGROUND

It was reported that an unusual TBAF-mediated intramolecular cycloaddition of a carbamate nitrogen to an unconjugated alkyne formed substituted azaproline derivatives.⁷ Subsequent studies led to the hypothesis that the mechanism involves a cation- π interaction between the ammonium ion (with the positive charge spread between the methylene groups directly attached to the electronegative nitrogen) and the C-C triple bond of the substrate.⁹ The ammonium cation itself was shown to have a catalytic effect, and, critically, experiments revealed that this was due to something other than phase-transfer catalysis. Raman spectroscopy showed that in the presence of ammonium cation, a dose-dependent shift occurred in the wavelength of the carbon-carbon triple bond stretching mode consistent with a cation- π interaction. Critically, other vibrational modes showed no shift in wavenumber in the presence of TBAB.

The propargyl hydrazine **1** was chosen as the substrate for this experiment because it was the compound used to extensively study this mode of organocatalysis in our published work. The product, dehydroazaproline derivative **2**, is representative of a class of compounds that may have interesting applications in peptide design, specifically as beta-turn inducers in protein folding. Therefore, the reaction is of interest beyond its usefulness in studying the organocatalysis and its possible role in this unusual reaction. The goals of this lab were to guide students through the process of forming plausible hypotheses and designing experiments to choose between them, while allowing them to interact with experiments from contemporary research. Unlike most undergraduate organic chemistry experiments, which are drawn from long-established research and classic named reactions, this lab recapitulates the key experiments used in recent research. Students are given the chance to work in small groups in order to recreate the team dynamic of a research group, and they are encouraged to collaborate on hypothetical reasoning and then to come up with a team strategy to carry out the experiments. The two most plausible hypotheses for the organocatalysis reaction under investigation are presented to the students, along with the scientific method rationale for the experiments that will decide between the rival hypotheses using the criterion of falsification¹¹. However, students are reminded that these hypotheses may not exhaust the possibilities. Students participating in this experiment had completed Organic Chemistry 1 and were enrolled in this lab course with a co-requisite of Organic Chemistry 2.

EXPERIMENT

This experiment was carried out by 232 second or third-year undergraduates over two semesters as the capstone learning experience of the organic chemistry lab course. The experiment, including the introductory lecture and the write-up, was completed over four

sessions of two-and-a-half hours each. The class was divided into research teams to more fully replicate the experience of peer interactions in graduate research. Each team of four students was instructed to prepare a division of labor plan such that all members had a role in every stage of the experiments while working together in a coordinated manner. Each team member kept an individual lab notebook for the experiment, and write-ups were completed individually.

In the first part of the experiment, students tested the phase-transfer hypothesis for the role of TBAB in the catalyzed reaction. Each team ran the control reaction combining sodium phenoxide with propargyl hydrazine **1** in ethyl acetate, as well as the same reaction with the addition of TBAB. Each reaction was monitored individually with a sample taken at regular 5-minute intervals for TLC analysis. The TLC plates were developed using KMnO_4 , and the progression of the spot corresponding to **1** to the less polar spot known (from reliable research data shared with the students) to be the azaproline derivative product **2**, was evaluated. By visually comparing the approximate rate at which the spot for compound **1** faded away and the spot for compound **2** appeared and grew stronger, the students were asked to make a qualitative judgement about whether the reaction with added TBAB proceeded to completion faster than the control reaction.

In the second part of the experiment, students carried out a reaction using the same reactants plus TBAB with acetonitrile as a solvent. The reaction was timed and TLC data were collected and evaluated in the same way as before. Students were reminded of the two hypotheses being considered, and were encouraged to discuss the results and their implications with group members throughout the lab period.

IR TITRATION

Students were then prompted to think about how IR spectroscopy can be used to more directly observe an ammonium interaction with the reactant. Perhaps there will be a change in the vibrational frequency of the C-C triple bond? Such changes should be detectable by IR spectroscopy, taking advantage of the fact that the signal appears in a relatively uncluttered region of the spectrum. To obtain this direct observation, students prepared a series of samples of propargyl hydrazine **1** in ethyl acetate and dichloromethane (DCM) (to improve TBAB solubility) with increasing amounts of TBAB. The technique of drop deposition was used: each sample was deposited onto a NaCl crystal, dried, and measured using transmission IR spectroscopy. The alkynyl carbon-carbon stretch was identified at 2120 cm^{-1} , and the spectra were expanded and overlaid to highlight that peak.

For each research group's titration, all spectra were plotted on a single graph with each labeled according to the molar equivalent of TBAB added (see Figure 2 for a representative student example). After obtaining and overlaying the spectra of their titration samples, students printed a copy for each group member and annotated and examined the peaks for evidence of a dose-dependent shift in wavenumber with added TBAB. Discussion of the implications for the competing hypotheses, this time emphasizing possible confirmatory evidence for the cation- π hypothesis, was encouraged between group members (see SI for detailed procedures).

HAZARDS

Proper protective personal equipment (PPE) were used at all times to minimize exposure to all chemicals used. Safety goggles and lab coats were required at all times during this lab. Gloves were required whenever handling chemicals. Ethyl acetate, hexanes, dichloromethane and acetonitrile are flammable and volatile organic solvents, and fume hoods were used to avoid inhalation of vapors. Dichloromethane is also a suspected carcinogen. N-hexane is a neurotoxin. Sodium phenoxide and tetrabutylammonium bromide are corrosive upon skin or eye contact. Potassium permanganate is harmful if swallowed, and a potential environmental toxin that must be disposed of properly. All MSDS information was posted for students and required reading as part of pre-laboratory preparation. Substrate **1** was prepared according to our previously reported procedure⁷ and made available to students as stock solutions. No hazard data is currently available for the experimental propargyl hydrazine **1** or the azaproline derivative **2**. Any potential risks involved in handling them in solution were minimized by working at semi-microscale and by using proper PPE.

RESULTS AND DISCUSSION

In general, a rate increase was qualitatively inferred by TLC from the increasing product intensity and decreasing starting material intensity when TBAB was added to the reaction in ethyl acetate, compared to the uncatalyzed reaction in ethyl acetate. The TBAB catalyzed reaction was fastest in acetonitrile, showing that the rate is solvent dependent. The order of reaction rates, from fastest to slowest, is as follows: TBAB catalyzed in acetonitrile; followed by TBAB catalyzed in ethyl acetate; and, finally, the uncatalyzed reaction in ethyl acetate. (Table 1). The observed relative reaction rates are in agreement with the published data (obtained by more precise NMR experiments) used to establish the catalytic role of the ammonium cation in homogeneous solutions and the importance of the solvent in determining the reaction rate.⁹ However, it is important to note that due to unfamiliarity with the technique of TLC spotting and TLC plate development, not all students were able to produce sufficiently high quality TLC plates to see the difference in rate. The results from the IR titration were uniform across the student groups and showed unambiguously that, in the presence of increasing TBAB, the carbon-carbon triple bond stretching frequency changed noticeably (Figure 2).

Students were asked to consider the original competing hypotheses: catalysis by phase-transfer versus a cation- π interaction. The phase-transfer catalysis hypothesis was falsified by the rate experiments. However, students were guided towards realizing that the falsification of one possibility does not automatically validate the alternative, since there may be other possibilities that were not considered. This is why the IR titration was needed to provide corroborating evidence for the cation- π hypothesis. The IR titration study showed an unambiguous dose dependent shift of the alkynyl carbon-carbon stretching frequency with added TBAB, an observation best explained by the cation- π interaction between the ammonium cation and the alkyne. This result was reproduced in all students' data. Furthermore, the direction of the shift was uniformly towards lower wavenumber with increasing TBAB, just as one would expect given the nature of the hypothetical electrostatic

interaction slightly decreasing the bond order of the alkyne. This evidence was taken to confirm the predictions of the ammonium-alkyne cation- π interaction hypothesis.

For the final portion of the research experience, each student was asked to compose a conclusion in essay format. In this conclusion, they recounted the background information and initial hypotheses given in the introductory lecture. They were expected to take into consideration the potential falsification of the phase-transfer hypothesis by the reaction rate experiments, as well as the potential confirmation of the predictions of the cation- π hypothesis by the IR titration. They were asked to draw some provisional conclusions, keeping in mind that the two hypotheses that were examined may not exhaust the possible explanations. Finally, they were asked to think critically and to come up with a further hypothesis about the reaction and an experiment to test their hypothesis. A qualitative review of student write-ups suggested that students generally comprehended the concepts introduced in this experiment (see Supporting Information for a representative sample).

In conclusion, this lab module adapted a recently published investigation of a novel interaction into an exercise in applying the scientific method to contemporary research. The experiment showed how hypothetical reasoning leads to experimental design. Students found indirect and direct evidence of an intermolecular cation- π interaction. By carrying out experiments from contemporary research and thinking critically about hypothesis formulation and the various ways of obtaining scientific evidence, students had an opportunity to interactively explore the process of scientific discovery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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10. Throughout this laboratory module, we refer to spectroscopic observation for a molecular interaction as ‘direct evidence’ in part because the measurement is occurring in real time. For an example of this usage of ‘direct evidence’, see: Ganguly HK, Majumder B, Chattopadhyay S, Chakrabarti P, Basu G. Direct Evidence for CH $\cdots\pi$ Interaction Mediated Stabilization of Pro-cisPro Bond in Peptides with Pro-Pro-Aromatic Motifs. *J Am Chem Soc*. 2012; 134(10):4661–4669. [PubMed: 22335630]
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Original experimental observations
(described in introduction to experiment)

Rate enhancing effect of TBAB
still observed with soluble base
(PhONa)

Develop hypotheses for role
of TBAB

Figure 1.
Setting up the problem

Figure 2.

Representative student IR titration study of the alkyne C-C stretching region of **1** with added amounts of TBAB (labeled peaks inset)

Table 1

Qualitative rate experiments based on TLC observations

Experiment	Additive	Solvent	Qualitative Rate Ranking
1	TBAB	EtOAc	2 nd
2	No TBAB	EtOAc	3 rd
3	TBAB	MeCN	1 st