JOURNAL OF CHEMICAL EDUCATION

Article

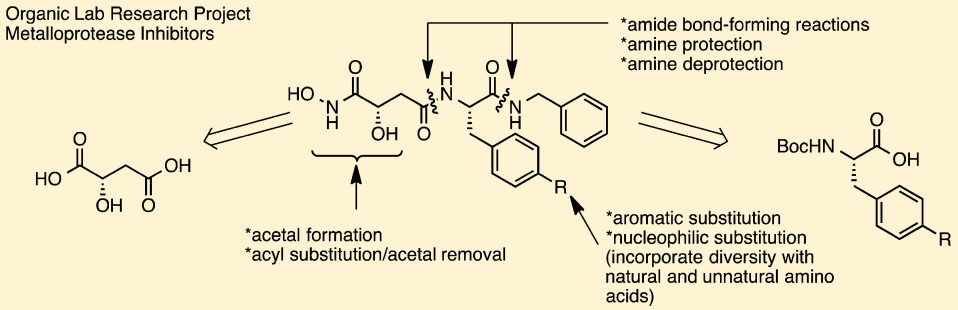
pubs.acs.org/jchemeduc

Metalloprotease Peptide Inhibitors: A Semester-Long Organic Synthetic Research Project for the Introductory Laboratory Course

Jason K. Pontrello*

Department of Chemistry, MS 015, Brandeis University, Waltham, Massachusetts 02454-9110, United States

Supporting Information



ABSTRACT: A semester-long research project to synthesize unique compounds designed after published metalloprotease peptide inhibitors is presented. The research project encompasses a set of nine organic chemistry reactions traditionally taught in the second semester lab course, and the procedures are derived from scientific literature. The two principle goals of the course design are (1) to enhance student interest through the scientific applications of the research project and (2) to introduce students to a synthetic organic chemistry research experience to develop skills needed in this area. In addition to the exploratory synthesis of novel compounds, students read background review articles about metalloprotease inhibitors. The design of the project provides opportunity for collaboration over multiple years, between different courses within the university, and among different schools.

KEYWORDS: *Second-Year Undergraduate, Curriculum, Laboratory Instruction, Organic Chemistry, Inquiry-Based/Discovery Learning, Applications of Chemistry, Medicinal Chemistry*

Over the past several decades, numerous efforts to introduce project-based experiences into undergraduate chemistry laboratory courses have been published, and some also introduce aspects of scientific research through the project design. In preparation of this paper, a large number of published works in this area were reviewed, and these are included as Supporting Information. While some examples exist where additional research-oriented courses or mentoring activities were created, the majority of effort in this area results in the integration of projects into a traditionally offered laboratory course. These efforts can be broadly organized into categories of (1) open-ended questions and (2) an activity chosen by the instructor.[1]

Laboratory projects involving open-ended questions allow students to choose a starting molecule or target to synthesize or something to investigate that they find interesting. For example, students have synthesized molecules like *N*,*N*-diethyl-*m*-toluamide (DEET) or a pharmaceutical.[2] Laboratory projects involving instructor-chosen activities may consist of a target molecule synthesis, following a defined synthetic route, exploring a specific reaction, completion of assigned tasks, and/or development of techniques. For example, one project employed different substrates in a specific multicomponent reaction,[3] while other projects involved a multistep organic synthesis designed toward a single defined target, such as chrysanthemic acid,[4] insect pheromones,[5] or an antithrombotic drug.[6] Alternatively, aspects of scientific research have been incorporated into many multistep projects. For example, structural diversity in target molecules was realized through use of different reactants in the synthesis of fluorous dye molecules,[7] local anesthetics,[8] or catalyst/substrate synthesis and work.[9]

OVERVIEW OF THE LABORATORY COURSE

At Brandeis University, the second semester Organic Chemistry Laboratory course consists of ten, 4 h blocks of time meeting once a week to perform experiments. Overall enrollments typically range from 160 to 240 in any given semester. The class is divided into sections of about 40 students in a room and further divided into small groups of about 10 students supervised by one Teaching Assistant (TA).

Previously, this lab course was designed around use of standardized textbook reactions run on microscale. Students performed reactions following the detailed stepwise directions given in the textbook, completing each experiment within a single lab period. Characterization of compounds consisted of melting point evaluation, and synthesized materials were discarded after each lab.

DOI: 10.1021/ed500193h
J. Chem. Educ. XXXX, XXX, XXX−XXX

Starting in the spring 2010 semester, the course was redesigned and has been iteratively modified with the underlying goals of (1) increasing student interest and engagement through participation in a research project synthesizing novel inhibitors of metalloproteases, and (2) exposing students to an authentic synthetic organic chemistry research environment.

Metalloproteases are an extremely diverse class of enzymes involved in numerous diseases, such as arthritis, cancer, diabetes, HIV, Alzheimer's disease, and Crohn's disease.[10] The area of metalloprotease inhibitor research presents clear challenges and applications, as the discovery of selective and potent inhibitors has been difficult.[10−13] Metalloproteases were selected as a target, anticipating that the applications of these enzymes in biological systems would be of interest to students in the course.

Most of the current metalloprotease inhibitor designs focus on peptide-based structures presenting a hydroxamic acid functional group necessary for binding metalloprotease active-site Zn^{2+} ions.[11] Variation of amino acids within the structure has been observed to affect inhibitor potency and selectivity.[11−13] Since use of unnatural amino acids has not been reported in the literature, an opportunity was apparent to develop a research project for the organic chemistry course.

Based on literature precedent of amino-acid-based inhibitor structures,[11−13] a general, peptide-based target was designed (Figure 1). This target allowed students to synthesize unnatural

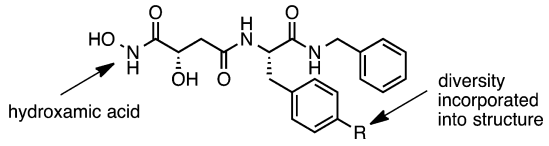


Figure 1. Target molecule is modeled after published peptide-based metalloprotease inhibitors. The hydroxamic acid is important for zinc binding in the metalloprotease targets, while other structural variations can affect inhibitor selectivity and potency.

amino acids in the lab, incorporating structural diversity not previously reported in the literature into the inhibitor structure through syntheses of novel compounds. Using unnatural amino acids also presented the opportunity to develop a multi-year research project that could include possible proposed collaborations with students from other courses and possibly other universities. This type of strategy has been described in detail elsewhere.[14,15]

Over the semester, students complete nine reactions toward the synthesis of novel target metalloprotease inhibitor structures. All students perform the same functional group transformations throughout the course. Diversity results as students carry their own synthesized compounds through the synthetic route and/or employ different commercially available reactants.

Offering this laboratory emulates a long-term research program in that reactions have been optimized in the sequence and stocks of some products from high-yielding reactions have been assembled. Optimizing reactions over the years of the course has allowed utilization of new reactions and reactants, further advancing the research project through incorporation of additional diversity in the target inhibitor structures. At this point, however, the foundation of the overall design is stable.

Additional scaffolding components to the project were added as it became clear which aspects of the program provided the greatest challenges for students. Specifically, an exercise was developed to help students perform calculations, converting information in a literature procedure to serve their own lab needs. Students also struggled to understand the synthetic path followed during the semester, so additional materials were developed to help students put the individual reactions into the larger context of the synthetic route.

Assessment of student performance was done through short laboratory reports for each of nine run reactions (reaction calculations, experimental notebook pages, brief discussion). Two larger final reports (reaction mechanisms, ^{1}H nuclear magnetic resonance spectroscopy (NMR) interpretation, background literature questions) were also assigned in addition to two exams and a formal presentation. Formal course evaluations, including open-ended responses, were used to gain additional insight into the research project.

INSTRUCTIONAL DESIGN FEATURES DRAWN FROM RESEARCH PRACTICES

Organizing instructional design around research practices is an established way to construct a research-based laboratory,[16] and this proved to be a useful organizer. The use of research practices to guide design features of the metalloprotease inhibitor synthetic project is described.

Reading Background Literature

Reviewing the entire area of metalloproteases as therapeutic targets, as well as the drug discovery work that has been accomplished to date, is beyond the scope of an introductory laboratory course. To prepare students to read the background literature for broader relevance rather than becoming overly concerned with small details, they were given specific leading questions from two assigned papers to answer as part of the required final reports (see Supporting Information).

Using Literature-Based Procedures

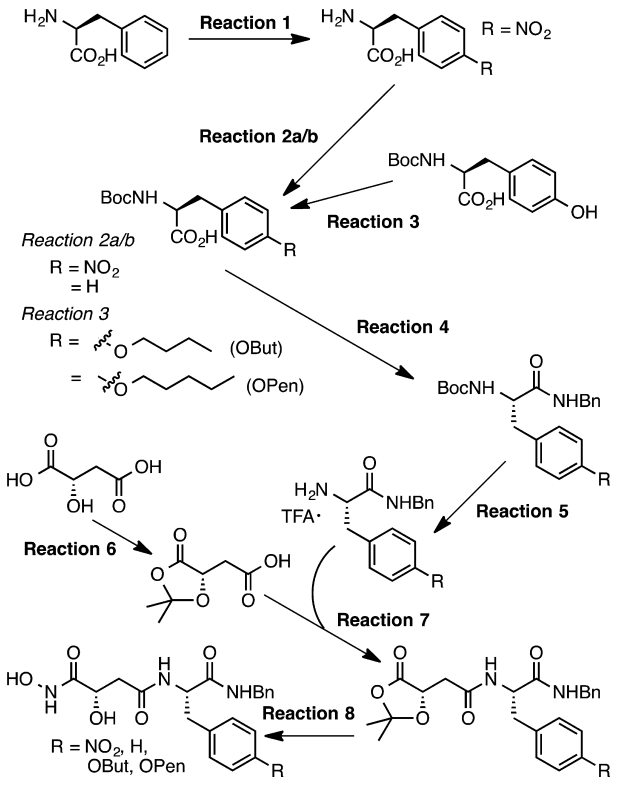
Using experimental procedures from the scientific literature is critical to carrying out a research project. Nine reactions were planned for students to complete throughout the course (Scheme 1), employing experimental procedures from scientific literature (Table 1). Incorporating structural diversity into the target molecule permitted use of a wide variety of procedures from a range of different journals, rather than relying on a single publication reporting an entire synthetic route.

Completion of Reactions 1−3, where Reaction 2 was performed twice using different conditions (a and b) (Scheme 1, Table 1), was planned for the first four lab sessions. Completion of Reactions 4−8 (Scheme 1, Table 1) was planned for the subsequent five lab sessions. Students were given a handout with the target reactions for each lab period and the corresponding literature references (see Supporting Information). Any adaptation to the published procedure was indicated in the handout.

Prior to lab, students' responsibilities included finding the reference indicated for the scheduled reaction, bringing the pages with the reaction and procedure to lab, and completing calculations on the scale of the reaction specified in the handout, using the indicated reactants. The research project consisted of examples where the same published reaction was performed on a different scale (Reactions 1, 2a and b, and 6). Students also performed the same functional group transformation as reported in a published procedure but used different reactant molecules (Reactions 3, 4, 5, 7, and 8). In some cases, all students in the section used the same molecules, comparing data and results. In other cases, different groups of students worked with different reactants, increasing diversity in the target inhibitor structure. All

DOI: 10.1021/ed500193h
J. Chem. Educ. XXXX, XXX, XXX−XXX

Scheme 1[a]



[a]Reactions 1−8 were completed throughout the lab course. Students were given citations to find the reaction procedure and then to use to plan the reaction for the lab.

reactants students used are indicated in the handout (see Supporting Information).

Students performed independent reactions whenever a commercially available reactants could be used (Reactions 1, 2a and b, 3, 4, 5, and 6). In cases where a synthesized reactant was required (Reactions 7 and 8), up to 25% of the class may share the compound or work in pairs when sufficient yields were not obtained in the previous step. Reactions were typically completed within 3−4 h of the scheduled lab time.

Failed Reactions

Regardless of the literature precedent, sometimes reactions simply do not work. This can be especially prevalent in the hands of inexperienced students in an introductory teaching lab course. A failed reaction is a reality of scientific research, and it can be a valuable tool to help students begin to appreciate research challenges. Given the intentional use of novel targets in the project design, it was important to address how failed reactions might affect a student's ability to complete the reaction sequence. In a synthetic research lab, a failed reaction is repeated multiple times until either optimized or abandoned for a different route. In the interest of exposing students to diverse reactions and keeping the class together from week to week, students were not asked to repeat failed reactions. While this is a compromise to the spirit of an all-out research project, it was nonetheless the decision we made to implement this design in our introductory laboratory program.

As shown in Scheme 1, the sequence of reactions contains a linear element (Reactions 1−2−4−5−7−8). However, it is also fed into from several independent reactions (Reactions 3 and 6) not dependent on previous success. Reactions 1, 3, 4, and 6 were designed to utilize commercially available reactants. Reactions 2a and b, 5, 7, and 8 were designed to utilize reactants from a previous synthetic step. For each of these (except Reaction 8), a commercially available reactant could be used in cases where insufficient yield or quality was obtained from the previous step. This allowed students to carry out the planned functional group transformation, incorporating further diversity into the target molecular structure.

Advanced Laboratory Equipment and Characterization

Carrying out synthetic chemistry in a research laboratory can be significantly different from performing traditional classroom laboratory experiments. In a research lab, most reactions are water-sensitive. Large volumes of solvent must be regularly removed. Reaction monitoring and compound characterization routinely utilize thin-layer chromatography (TLC) and ^{1}H NMR spectroscopy. Product purification in a research setting typically employs silica column chromatography.

In order to successfully perform the nine reactions designed to emulate a research experience, several new equipment needs were first addressed (see Supporting Information). Glass manifolds with nitrogen and high vacuum lines were installed in each section of 10 students. The increased reliance on TLC and silica chromatography required that TLC plates and jars of silica be made regularly available. The lab was already using Microscale Apparatus Kits, and the column from these kits was used for silica chromatography. A rotary evaporator was purchased for use by each section of 10 students (a total of four for the lab room of 40 students).

Typically, groups of students working with the same reactants within sections compared TLC data, selected what they believed to be the best sample, and prepared a single NMR sample for analysis. This resulted in four samples per section, run per day, which were collected either with an autosampler or manually by a TA or course instructor. All processed spectra were posted by the course instructor on a course Web site, and spectra for each reaction determined by the instructor to contain product were assigned for all students to interpret as part of the final reports (see Supporting Information).

Using NMR spectra derived from authentic reaction mixtures provided the opportunity to demonstrate principles beyond what might be illustrated by standard textbook spectra. Students identified solvent contaminants from published chemical shift tables,[17] and they became comfortable with identification of diastereotopic groups (Figure 2).

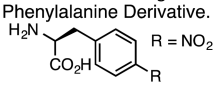
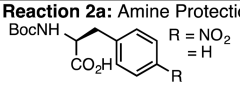
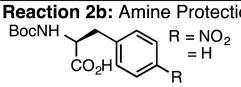
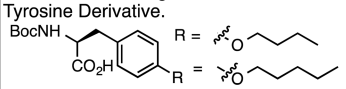
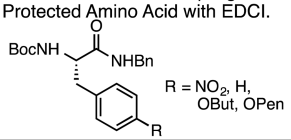
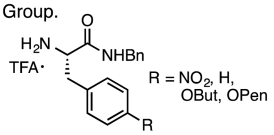
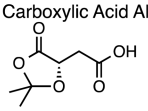
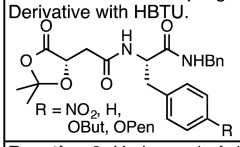
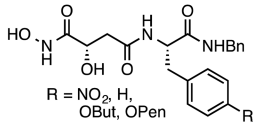
The use of NMR spectra from compounds in a synthetic sequence demonstrated a valuable lesson in interpretation. When carrying a molecule through a synthetic sequence, complexity is often added to the structure in each step, making NMR interpretation difficult toward the end of the sequence. By completing the task of interpreting spectra for this project, students learned that interpretation of spectra of simpler structures early in the synthetic sequence allowed for comparison to spectra of later, more complicated structures.

Formal Presentations of Scientific Literature

As part of the project final reports, students were assigned background articles to read and specific questions to answer. These questions were designed to highlight important aspects of the research project, as well as to guide students through the readings. In the last week of the course, each section of 10 students (four sections per lab day) was given the task of preparing and delivering a formal presentation of one of the assigned background literature articles. While the whole section

DOI: 10.1021/ed500193h
J. Chem. Educ. XXXX, XXX, XXX−XXX

Table 1. Procedures, Compounds Utilized, and Yields for Reactions 1−8

Reaction Product	Literature Procedure	Compounds Used	Yields
Reaction 1: Making an Unnatural Phenylalanine Derivative. 	*Eur. J. Med. Chem.* **2009**, *44*, 3147-3157. *Bioorg. Med. Chem.* **2009**, *17*, 3118-3125.	all students synthesize R=NO_2	yields good (over 50%) *compound stocked*
Reaction 2a: Amine Protection. 	*Heterocycles* **2008**, *75(6)*, 1493-1501.	students select to use R=NO_2 (Reaction 1 product) or R=H (commercial)	yields poor (less than 30%)
Reaction 2b: Amine Protection. 	*Helv. Chim. Acta* **2003**, *86*, 3326-3331.	students select to use R=NO_2 (Reaction 1 product) or R=H (commercial)	yields good (over 50%)
Reaction 3: Making an Unnatural Tyrosine Derivative. 	*J. Org. Chem.* **1983**, *48*, 4127-4129.	half students assigned to make R=OButyl and half R=OPentyl	yields moderate (30-50%) *compound stocked*
Reaction 4: Amine Coupling to a Protected Amino Acid with EDCI. 	*Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101(27)*, 10000-10005.	students select to use R=NO_2 (Reaction 2 product), OBut, OPen (Reaction 3 products), or R=H (commercial)	yields moderate (30-50%)
Reaction 5: Deprotection of the Amino Group. 	*Angew. Chem. Int. Ed.* **2009**, *48*, 1097-1101.	students select to use R=NO_2, OBut, OPen, H (Reaction 4 products)	yields good (over 50%)
Reaction 6: Acetal Protection of a Carboxylic Acid Alcohol. 	*J. Med. Chem.* **1998**, *41*, 199-223.	all students run reaction	yields good (over 50%) *compound stocked*
Reaction 7: Acid Coupling to Amino Acid Derivative with HBTU. 	*Proc. Natl. Acad. Sci. S. A.* **2004**, *101(27)*, 10000-10005.	students select to use R=NO_2, OBut, OPen, H (Reaction 5 products), or benzylamine (commercial)	yields moderate (30-50%)
Reaction 8: Hydroxamic Acid Synthesis. 	*Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101(27)*, 10000-10005.	students select to use R=NO_2, OBut, OPen, H (Reaction 7 products)	yields poor (less than 30%)

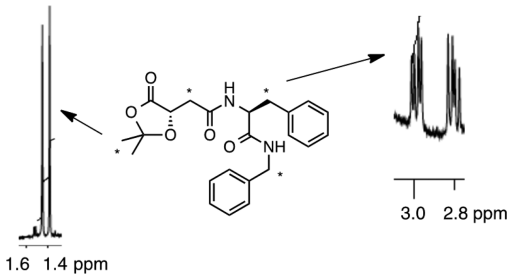


Figure 2. Diastereotopic groups (indicated by *) are observed in multiple compounds in the synthetic reaction scheme. Diastereotopic methylene protons are present in any structure using phenylalanine, tyrosine, or unnatural derivatives of these amino acids. Additionally, protection of malic acid as a dimethyl acetal creates diastereotopic methyl groups, illustrating an extension of this particular NMR theory.

was required to assist in preparation of the presentation, a maximum of three students were allowed to present. This restriction was designed to increase the fluidity of the overall presentation. The audience for the presentation consisted of the course instructor and the lab day's other three sections of students and TAs.

Students received general guidelines for preparing the presentation (see Supporting Information). Students ranked each other's contributions to the presentation, and this was counted toward the lab participation grade. Formal reviews of the presentations from the course instructor and TAs were compiled and given to each group to provide feedback.

■ ADDRESSING CHALLENGES THROUGH COURSE DEVELOPMENTS

Seeing the Big Picture

In the first iteration of this project, it was noted that students had a difficult time drawing the connections between the reactions and seeing how the parts related to the overall sequence. For

DOI: 10.1021/ed500193h
J. Chem. Educ. XXXX, XXX, XXX−XXX

Literature Reference				molar				Lab Reaction
structure	MW,density	quantity	mmoles	equivalents	mmoles	quantity	MW,density	structure

Figure 3. "Planning Reactions" worksheet was designed to help students draw connections between literature procedures and a desired reaction to run in lab. The worksheet consists of multiple rows like the one above, where the values calculated for the literature procedure are then translated into amounts needed for the lab reaction.

Table 2. Comparison of Results from Students' Formal Course Evaluations Prior to and Following Implementation[a]

Evaluation Questions	1) The overall quality of this course was excellent.	2) The course helped me to develop my writing ability.	3) The course helped me to develop my oral communication skills.	4) The course helped me to develop my creative abilities.	5) The course improved my ability to reason and think analytically.	6) The course broadened my perspective.		
Pre-Program (textbook-based) Spring 2009 Enrolled: 111 Avg, SD	3.63, 1.1 N=68	3.34, 1.3 N=32	3.52, 1.3 N=33	3.06, 1.5 N=17	4.06, 1.1 N=66	3.79, 1.1 N=68		
Evaluation Questions	1) The overall quality of this course was excellent.	2) This course improved my writing ability.	3) This course improved my oral communication skills.	4) This course helped me develop my creative abilities.	5) This course improved my quantitative skills.	6) This course helped me to consider alternative perspectives on complex issues.	7) This course helped me to analyze, interpret and synthesize information.	8) This course helped me to reason better and to think more critically about its subject matter.
Pooled (metalloprotease inhibitor synthesis) Spring 2012, 2013, Summer 2012, 2013 Enrolled: 459 Avg, SD	4.18** M, 0.9 N=358	3.98** M, 1.0 N=253	4.03* M, 0.9 N=233	3.93* M, 1.0 N=183	4.26, 0.8 N=306	4.26** M, 0.8 N=272	4.44, 0.7 N=334	4.40, 0.7 N=327

Scale: 1=Strongly Disagree, 2=Disagree, 3=Neutral, 4=Agree, 5=Strongly Agree

Questions 1-4 are the same. Questions 5-6 are variations addressing similar issues.

t-test (2-tailed, unequal variance): *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001
effect size (Cohen d with Hedges' g for pooled standard deviation):
none (d<0.2), small S (0.2≤d<0.5), medium M (0.5≤d<0.8), large L (d≥0.8)

[a]Averages (Avg), standard deviations (SD), and numbers of responses (N) taken from four pooled semesters when the metalloprotease project was implemented and one semester prior to program implementation.

example, students often did not recognize that the product of a previous reaction was intended as a reactant in the next reaction.

In response, students were asked explicitly to complete a flowchart with reactants and products from Reactions 1−8 that would lead to improved recognition of the relationship between individual reactions in assembly of the overall synthetic scheme. Consequently, an open flowchart to complete, structured similarly to Scheme 1, is now a part of each of the final reports (see Supporting Information). An increase in student understanding of the connections between reactions became evident as TAs reported not only significant decreases in student questions about "what to use as a reactant" but also improvement in prelab preparation.

"Planning Reactions" Worksheet

A critical component of the project was to have students find literature procedures for their reactions and then to adapt them to their specific experiment needs. This task involved either simply scaling the published reaction or carrying out the same functional group transformation using different molecules from the published reaction. In the first semester the project was offered, students struggled to complete prelab calculations. Sections spent as much as 40 min at the start of the lab just figuring out the math for the reaction. In response to student confusion about prelab calculations, a "Planning Reactions" worksheet was designed and implemented in subsequent semesters to facilitate completion of the stoichiometric calculations needed to set up the reactions (Figure 3 and Supporting Information). Students brought two copies of the completed "Planning Reactions" worksheet to lab and turned in one at the start of lab to be graded. Students used their second copy to compare and discuss answers with each other in a peer review until a group consensus was reached. The review process took less than 10 min to complete. Refining calculations was valuable so students used the correct amounts when setting up their reaction. In addition, students then reported correct amounts in their lab reports and did not incur additional point deductions.

■ ASSESSMENT

Evaluating Student Performance

Student performance in the course was evaluated using multiple sources

DOI: 10.1021/ed500193h
J. Chem. Educ. XXXX, XXX, XXX−XXX

Weekly Laboratory Participation (10%). TAs evaluated students' abilities to work efficiently and carefully, ask appropriate questions, and become involved with group exercises. Students' abilities to navigate published procedures, perform calculations, and carry out reaction setups and purifications improved over the duration of the course, as indicated in the TAs' evaluations of participation.

Independently Written Laboratory Reports (25%). Reports for each reaction performed included notebook pages, calculations, and discussion of results. Improvement in completion of the "Planning Reactions" worksheet was seen after the first few reactions, as students became more comfortable with calculations.

An Independently Completed Larger Report (20%). The report was submitted in two parts (mid-semester and final). It included NMR analysis for the synthesized compounds, questions to answer about assigned background literature, and reaction mechanisms to propose (see Supporting Information). The larger report grades showed strong improvement with averages typically increasing by about 10% from the mid-semester report to the final report. This increase was attributed to substantial improvements in the interpretation of NMR data.

A Formal Presentation (5%). Each section of 10 students prepared a presentation that addressed the content of an assigned background literature reading. Up to three student speakers delivered the presentation to students and TAs from their lab day and the course instructor. The whole group was responsible for answering questions at the end of the presentation, and evaluations by the four TAs and the course instructor were returned as formal written feedback.

Two Examinations (40%). Examinations included questions about mechanisms, the metalloprotease inhibitor project, specialized equipment use, and NMR interpretation.

Formal Course Evaluations

Evaluation of the change in the course from relying exclusively on standardized textbook reactions to employing the research project was undertaken. To evaluate the program change, results were compared from the University-administered end-of-semester questions for four semesters of its implementation with the single, pre-implementation semester in which the same instructor taught the course. Because there was only one semester for comparison, the differences between it and each of the four semesters of implementation were analyzed separately, as well as against the pooled results from those four semesters. Results from individual years of implementation reflected the same trends, and the data from each year are included in the Supporting Information.

The results of pre-implementation and pooled implementation data are shown in Table 2 derived from *t*-test comparisons of each implementation semester with the pre-implementation semester (two-tailed, unequal variance). To evaluate the effect size,[18] the version of Cohen's *d* was used that incorporates Hedges *g*, for pooled standard deviation, which is more conservative than Glass' Δ and is better at accounting for the difference in population sizes. In 2010, the University changed some of the questions in its end-of-semester surveys, so not all of the questions from 2009 appear today. In Table 2, Q1−4 were conserved, Q5−6 were modified, and since Q7−8 did not previously exist, they cannot be compared.

As seen in Table 2, students have been uniformly positive in their reaction to the new program. The greatest effect observed has been their self-assessment of the course on their creative abilities, which has strong face validity for a course that shifted to more independent research. The second highest effect was on students' assessment about improving their writing skills. The rigors of running reactions based on published procedures rather than established stepwise textbook procedures led to increased thought and independence when recording data in notebooks and discussing data in lab reports. The program also positively affected student comments regarding improved oral communication skills. The course was designed to involve sections of 10 students regularly in optimizing TLC and reaction conditions, as well as delivering a class presentation.

More insight into course structure and design was gained from review of written responses on formal course evaluations (Spring 2010, 2012, 2013, Summer 2012, 2013). Ninety-seven written responses to the question, "Please identify those aspects of the course you found most useful or valuable for learning", have been generally grouped into these three categories:

1. **Project-Based Lab Design and Engaging Format**. This category includes 56 comments, which these quotes exemplify:
   "The novel idea of structuring Orgo lab around a semester long synthesis project is brilliant because it introduces students to the life of a synthetic chemist." [Spring 2010]
   "I like the fact that we did real applicable experiments throughout the semester. Although this created a lot of work at times, it felt more relevant than just running some random reaction, throwing away the product, and starting over next week with a totally unrelated thing. It really showed the relevance of the reactions that we learned in the lecture component." [Spring 2012]
   "I thought that the professor stimulated interest in the subject and put the course into a larger scientific prospective." [Spring 2013]
2. **Scientific Literature Use in Experiments**. This category includes 33 comments, typified by these quotes:
   "I really enjoyed how much freedom we had during labs. It actually felt like we were investigating something, as opposed to simply following a procedure. This improved my skills in decision-making, time management, and cooperation (as well as communication) with others in the lab." [Spring 2010]
   "Though this class was one of the most challenging courses I've taken at Brandeis, I walk away with considerable knowledge of lab techniques and lab analysis methods and a good conceptual understanding of the course material." [Spring 2012]
   "Using real procedures from literature made lab more interesting." [Summer 2013]
3. **Class Presentations**. This category has 8 comments, with this representative quote:
   "I also liked the idea of having students give presentations - I think this an important skill to learn and also enables students to think more critically about the material they are learning. Overall a really terrific course." [Spring 2012]

Written comments indicated that students were strongly supportive of the project-based lab design. Multiple comments addressed enhanced engagement in the course due to the nature of the work. Numerous comments reflected positively about using literature procedures and running "real" reactions.

DOI: 10.1021/ed500193h
J. Chem. Educ. XXXX, XXX, XXX−XXX

Students were able to identify the educational value in completing the project, even if it did require additional "work".

As part of the same formal course evaluations, 100 written responses to the question, "What suggestions would you make to the instructor for improving the course?" have been generally grouped into these four categories:

1. **Project-Based Lab Design and Engaging Format**. This category includes 12 comments, which these quotes exemplify:
   "I still really don't understand what metalloprotease is, or the point of what we synthesized. More extensive background should have been given." [Spring 2010]
   "At first, the purpose of the Metallo Labs was unclear." [Spring 2010]
2. **Scientific Literature Use in Experiments**. This category includes 15 comments, typified by these quotes:
   "Put more lab detail in the handout." [Spring 2012]
   "Also, while the journal articles were helpful, I found that taking the time to find them was often unnecessary." [Spring 2012]
3. **Class Presentations**. This category has 10 comments, with these representative quotes:
   "In our group, it was very difficult trying to get other group members to contribute who weren't presenting. I think a way to remedy this would be to have smaller group sizes (4 or 5 people in a group)." [Spring 2012]
   "Each bay should be broken into two groups for the presentation." [Spring 2013]
4. **Workload and Due Date Timing**. This category includes 63 comments, which these quotes exemplify:
   "Having multiple assignments (and a presentation project!) due just before or during exam periods makes studying considerably more difficult." [Spring 2012]
   "I think the course should either be a full credit class or the workload should be reduced." [Spring 2013]

Written comments expressing a lack of understanding of the project were not observed on evaluations following incorporation of (1) an opening statement in the project handout, (2) the previously described flowchart, and (3) more detail provided in the handout about quantities of reagents to use. Concerns expressed about class presentations were primarily focused around equal contribution by group members. Finally, the majority of concerns involved the credit value of the course. Counting the course as "full" rather than "half" is reasonable and is currently being reviewed.

CONCLUSIONS AND DISCUSSION

Designing and implementing the research project for a second semester introductory organic chemistry lab course revealed several key recurring themes. Students generally appreciated the practical application of laboratory work, expressing enhanced engagement through running "real" reactions with a clear purpose. Although challenging and requiring adaptation over time, most students recognized the value of using literature procedures and were able to adjust to the experiment format.

It was necessary to introduce certain elements of structure to the program to help students at this educational level appreciate the project goals and achieve success in the course. Students did not respond well to receiving only a simple list of reactions to run and corresponding references to read. Incorporating a flowchart assignment helped students to draw connections between reactions as they were performed across the semester. A few pages of introductory materials with project overview and background were necessary to help students appreciate the value of research into metalloprotease inhibitors. Guiding questions in the larger reports focused student attention and led the reading through assigned background literature.

Additional structure was needed to perform reaction calculations and to design a synthetic route that could be followed throughout the course. The designed worksheet resulted in significant improvement in students' abilities to scale reactions and incorporate different reactants from a published procedure. Commercially available reactants at different parts of the synthetic scheme were used for failed or low yielding reactions, a necessary component of scientific research.

The task of interpreting NMR spectra of synthesized compounds provided a substantial challenge for most students. Students had access to thousands of spectra through databases,[19−21] online practice exercises,[22−24] and textbook problems. However, learning to interpret spectra of synthesized compounds containing solvents and byproducts required practice. By the end of the semester, most students were able to master these skills through completion of the synthetic route and interpretation of spectra for each compound generated.

Future Directions—Demonstrating the Value of Collaboration

As in any scientific research project, there are a few clear opportunities upon which to build as the project advances: (1) using materials prepared from previous years, (2) collaboration on the synthesis projects, and (3) collaboration on the biological evaluation. As larger quantities of amino acid building blocks have been successfully synthesized, stocks of these materials have been started. Using these materials will allow time in future semesters for students to develop syntheses of different unnatural amino acids as part of the research effort. We are also currently pursuing collaborations, both with students at other universities to further expand the pool of reactants and also in the biological evaluation of the novel synthesized targets.

ASSOCIATED CONTENT

Supporting Information

A review of published works in the area of project-based chemistry laboratory development is included. Metalloprotease inhibitor project handouts, report forms, NMR data, presentation guidelines, and course evaluation data are included. Finally, the laboratory preparation file contains lists of chemicals, quantities, suppliers, specific hazards, and all equipment used in the course. This material is available via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: pontrell@brandeis.edu.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

I want to thank Brandeis University for allowing the freedom to develop this project. The laboratory supervisor, Gary Koltov, was always helpful in preparing new experiments. I also thank Brian P. Coppola, University of Michigan, for endless support and guidance in general and specifically with assistance and feedback

DOI: 10.1021/ed500193h
J. Chem. Educ. XXXX, XXX, XXX−XXX

on the preparation of this manuscript. Finally, I would like to thank my colleague Suzanne Paradis for asking in a meeting, "Does anyone know anything about metalloprotease inhibitors?"

REFERENCES

(1) Horowitz, G. The State of Organic Teaching Laboratories. *J. Chem. Educ.* **2007**, *84* (2), 346−353.
(2) Ruttledge, T. R. Organic Chemistry Lab as a Research Experience. *J. Chem. Educ.* **1998**, *75* (12), 1575−1577.
(3) Dintzner, M. R.; Maresh, J. J.; Kinzie, C. R.; Arena, A. F.; Speltz, T. A Research-Based Undergraduate Organic Laboratory Project: Investigation of a One-Pot, Multicomponent, Environmentally Friendly Prins−Friedel−Crafts-Type Reaction. *J. Chem. Educ.* **2012**, *89* (2), 265−267.
(4) Schatz, P. F. Synthesis of Chrysanthemic Acid: A Multistep Organic Synthesis for Undergraduate Students. *J. Chem. Educ.* **1978**, *55* (7), 468−470.
(5) Cormier, R. A.; Hoban, J. N. Laboratory Syntheses of Insect Pheromones. *J. Chem. Educ.* **1984**, *61* (10), 927−928.
(6) Nuhrich, A.; Varache-Lembège, M.; Lacan, F.; Devaux, G. Use of Infrared Spectroscopy in Monitoring a New Method for the Preparation of Sulotroban, an Antithrombotic Drug: A Medicinal Chemistry Experiment. *J. Chem. Educ.* **1996**, *73* (12), 1185−1187.
(7) Slade, M. C.; Raker, J. R.; Kobilka, B.; Pohl, N. L. B. A Research Module for the Organic Chemistry Laboratory: Multistep Synthesis of a Fluorous Dye Molecule. *J. Chem. Educ.* **2014**, *91* (1), 126−130.
(8) Demare, P.; Regla, I. Synthesis of Two Local Anesthetics from Toluene: An Organic Multistep Synthesis in a Project-Oriented Laboratory Course. *J. Chem. Educ.* **2012**, *89* (1), 147−149.
(9) Zhang, J.; Phillips, J. A. The Synthesis and Application of Polymer-Supported Hypervalent Iodine Reagent in the Organic Chemistry Laboratory. *J. Chem. Educ.* **2010**, *87* (9), 981−984.
(10) Moss, M. L.; White, J. M.; Lambert, M. H.; Andrews, R. C. TACE and Other ADAM Proteases as Targets for Drug Discovery. *Drug Discovery Today* **2001**, *6* (8), 417−426.
(11) Levy, D. E.; Lapierre, F.; Liang, W.; Ye, W.; Lange, C. W.; Li, X.; Grobelny, D.; Casabonne, M.; Tyrrell, D.; Holme, K.; Nadzan, A.; Galardy, R. E. Matrix Metalloproteinase Inhibitors: A Structure−Activity Study. *J. Med. Chem.* **1998**, *41* (2), 199−223.
(12) Saghatelian, A.; Jessani, N.; Joseph, A.; Humphrey, M.; Cravatt, B. F. Activity-Based Probes for the Proteomic Profiling of Metalloproteases. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101* (27), 10000−10005.
(13) Sieber, S. A.; Niessen, S.; Hoover, H. S.; Cravatt, B. F. Proteomic Profiling of Metalloprotease Activities with Cocktails of Active-Site Probes. *Nat. Chem. Biol.* **2006**, *2* (5), 274−281.
(14) Scott, W. L.; O'Donnell, M. J. Distributed Drug Discovery, Part 1: Linking Academia and Combinatorial Chemistry To Find Drug Leads for Developing World Diseases. *J. Comb. Chem.* **2009**, *11* (1), 3−13.
(15) Scott, W. L.; Alsina, J.; Audu, C. O.; Babaev, E.; Cook, L.; Dage, J. L.; Goodwin, L. A.; Martynow, J. G.; Matosiuk, D.; Royo, M.; Smith, J. G.; Strong, A. T.; Wickizer, K.; Woerly, E. M.; Zhou, Z.; O'Donnell, M. J. Distributed Drug Discovery, Part 2: Global Rehearsal of Alkylating Agents for the Synthesis of Resin-Bound Unnatural Amino Acids and Virtual D3 Catalog Construction. *J. Comb. Chem.* **2009**, *11* (1), 14−33.
(16) Weaver, G. C.; Wink, D. J.; Varma-Nelson, P. V.; Lytle, F. E. Integrating Research into the Curriculum: Using Laboratory Modules To Engage Faculty and Students. In *Broadening Participation in Undergraduate Research: Fostering Excellence and Enhancing the Impact*; Boyd, M. K., Wesemann, J. L., Eds.; Council on Undergraduate Research: Washington, D.C., 2009; pp 109−125.
(17) Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. NMR Chemical Shifts of Common Laboratory Solvents as Trace Impurities. *J. Org. Chem.* **1997**, *62* (21), 7512−7515.
(18) idre UCLA. http://www.ats.ucla.edu/stat/mult_pkg/faq/general/effect_size_power/effect_size_power.htm (accessed Feb 2015).
(19) Sigma-Aldrich. http://www.sigmaaldrich.com/ (accessed Feb 2015).
(20) Spectral Database for Organic Compounds (SDBS). http://sdbs.db.aist.go.jp/sdbs/cgi-bin/cre_index.cgi?lang=eng (accessed Feb 2015).
(21) Database of NMR spectra. http://www.nmrdb.org/ (accessed Feb 2015).
(22) Chem 605—Structure Designation Using Spectroscopic Methods. http://www.chem.wisc.edu/areas/reich/chem605/index.htm (accessed Feb 2015).
(23) WebSpectra—Problems in NMR and IR Spectroscopy. http://www.chem.ucla.edu/~webspectra/ (accessed Feb 2015).
(24) Organic Structure Elucidation, A Workbook of Unknowns. http://www3.nd.edu/~smithgrp/structure/workbook.html (accessed Feb 2015).

DOI: 10.1021/ed500193h
J. Chem. Educ. XXXX, XXX, XXX−XXX