A Thesis Presented to

The Faculty of Alfred University

# "Synthesizing Blattellaquinone, its Derivatives, and Subsequent Testing as a Means of Interdisciplinary Teaching"

by

Kylee K. Gaier

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Under the Supervision of:

Chair: Dr. John D'Angelo

Committee Members:

Dr. Geoffrey Bowers

Dr. Heather Zimbler-DeLorenzo

**Dedication**

I would like to dedicate this thesis project to my parents, who pushed me to join the Honors Program as a Freshman, and continued to support me throughout the research and writing process.

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## Introduction

The purpose of this research is to show proof of concept for an interdisciplinary, undergraduate laboratory experiment where students in the organic chemistry and animal behavior biology courses collaborate. Currently, in many curricula, courses are separate from one another, making it harder for students to make connections between the various fields of science. However, in real-world applications, the fields are not as distinct as they are made to be in the classroom and traditional academic lab. All fields of science are interconnected in some way. Unfortunately, it can be difficult for students to understand and make those connections, as typical undergraduate courses tend to focus on their subject alone.

Additionally, current undergraduate laboratory exercises tend to be exercise-based as opposed to experiment-based. In these labs, the students all perform the same exercise, and are expected to get the same results. To some extent, this is necessary in order to build technical skills in the laboratory. However, this can make for a boring, uninspiring laboratory experience and may breed an atmosphere of competition that demoralizes some students. Experiments, such as the one demonstrated in this research, allow students to ask different questions of their own and generate the answers to them. Additionally, this kind of project challenges the "right" or "wrong" answer mentality, which has no business in research, where the only failed experiment is the one that the researcher does not learn from. It also

removes the demoralizing sentiment of not getting the yield or result that other students did.

It is our hope that in creating an experiment-based, interdisciplinary lab, students will gain experience making connections between two fields of science. In our study, we have focused on organic chemistry and animal behavior. The kind of relationship this interaction would best model would be between the synthetic lab making pharmaceuticals and the biological lab evaluating them. Such an experience ought to make a student more prepared for graduate school, or the workforce, where diverse topics often inter-rely on one another. Additionally, students may find such labs more exciting and interesting, leading to an overall more impactful lab experience, while also instilling a mindset for research. This was demonstrated by Hecke,[1] where after completion of an interdisciplinary lab course, students filled out questionnaires, with many indicating that the real-world application labs were more interesting. Also, these labs provided "an opportunity to test one's own hypothesis…allow[ing] for creativity and a personal stake in the lab."

In order to try to facilitate the development of such a lab at AU, I spent four semesters working on a research team, lead by Dr. John D'Angelo and Dr. Heather Zimbler-DeLorenzo, to develop a lab that could integrate the animal behavior and organic chemistry courses. I was part of a synthetic team with Emma Robinson and Benjamin Proctor, who worked on the biology component. We first targeted the female German cockroach sex pheromone, blattellaquinone (**1**, Figure 1). Since its

[1] Hecke, Gerald R. Van, Kerry K. Karukstis, Richard C. Haskell, Catherine S. Mcfadden, and F. Sheldon Wettack. "An Integration of Chemistry, Biology, and Physics: The Interdisciplinary Laboratory.". *Journal of Chemical Education* 79.7 (2002): 837.

synthesis was known[2], and the structure contains several easily functionalizable units, we believed it would be amendable to derivatives for future students to explore. We decided to target the ester end of the molecule, because we had hypothesized that the ketone unit, with the cyclohexane, oxygens, and multiple double bonds, may be a key part of what makes the pheromone work, and that changing this could render the molecule ineffective. As there are many ester-containing natural products, it seemed unlikely that this specific ester was entirely responsible for the activity. This, along with the anticipation that the synthesis to change the ester unit was significantly easier, was our rationale behind making it the first part of the molecule we'd alter. Altering this unit would allow us to determine how changes to this portion of the molecule changed its effectiveness at eliciting a reaction from the male cockroaches. Such systematic changing of units in the molecule are very similar to Structure Activity Relationship studies; which are routinely done in the discovery and optimization of pharmaceuticals. Such experience in this topic is uncommon in the undergraduate curriculum. Alternatively, students could decide to alter the ketone structures as well, possibly changing it to an alcohol (although this would introduce some stereochemical issues by creating a stereocenter in a molecule that is right now achiral), alkene, or reducing them altogether to the alkane. Furthermore, after evaluating the natural pheromone, with our positive control in hand, future generations of students could be confident that the protocols they followed were synthetically sound and the

[2] ~Feist, Patty L. "The Synthesis of a Cockroach Pheromone." *Journal of Chemical Education 85.11 (2008): 1548-549.*

testing was capable of producing a positive result for compounds that the cockroaches will respond to. It would also provide them a comparison point for the natural compound and potentially an easy to access family of derivatives.

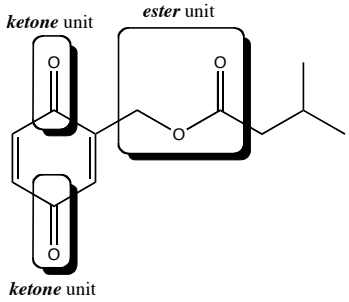
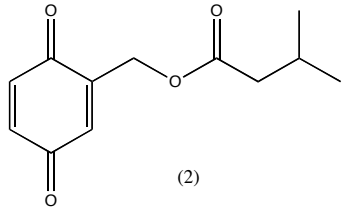


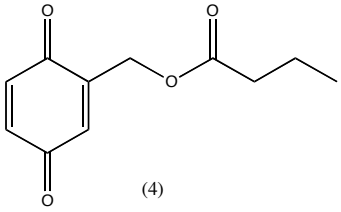

**Figure 1 Structure Functional Groups**

One of our important goals was to optimize the procedure for synthesizing blattellaquinone and its derivatives into the 3-hour lab periods employed at Alfred in the organic lab. After a successful synthesis of crude blattellaquinone (2), we began working on three derivatives: the acetyl chloride derivative (3), butyryl chloride derivative (4) and, valeryl chloride derivative (5). **(**Figure 2).



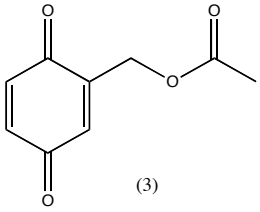
(2)

**Structure of Blattellaquinone**



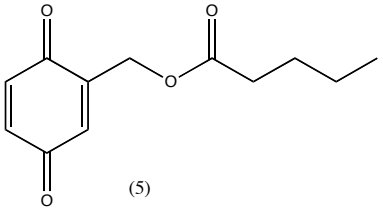
(4)

**Structure of Butyryl Chloride Derivative**



(3)

**Structure of Acetyl Chloride Derivative**



(5)

**Structure of Valeryl Chloride Derivative**

**Figure 2 Blattellaquinone and Derivatives**

While we pursued the synthesis of derivatives, we gave the crude blattellaquinone to Benjamin, who performed experiments to test its effectiveness at eliciting a response from the male cockroaches of the same species.

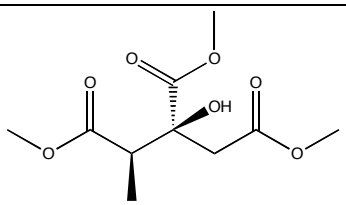
Finally, in an attempt to reduce the process to one lab period while also incorporating modern concepts, we attempted to synthesize the blattellaquinone *via* a one-pot synthesis, in which the entire synthesis is done in one reaction vessel. Recently, the concept of pot economy[3] has been developed as a way to optimize chemical reactions and reduce waste. In some cases, a work-up procedure could take as long to complete as the chemical reaction itself. For a one-pot procedure to work, the solvents, reagents, and by-products of one reaction must not impact the

[3] Hayashi, Yujiro. "Pot Economy and One-pot Synthesis." *Chem. Sci.* 7.2 (2016): 866-80.

subsequent reaction(s). In this one-pot synthesis, rather than separating the procedure so that each chemical step is carried out in its own flask; mandating the use of multiple sets of glassware; and doing multiple work-ups, several steps or in some cases (such as ours) the entire synthesis can be carried out in one round-bottom flask (RBF). This method allows a synthetic chemist to skip certain work up procedures, which are often time consuming to perform and produce large volumes of waste.

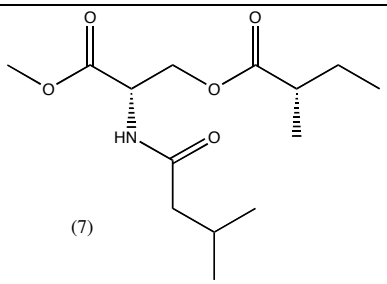
Overall, this research shows that an interdisciplinary lab between the animal behavior biology and organic chemistry like the one described herein has potential. However, there were some challenges that would need to be addressed before experiments of this nature were implemented in the curriculum, particularly if blattellaquinone derivatives are to be further explored. As an alternative, there are other insect sex pheromones students could choose to work with. One example is the female Wasp Spider, *Argiope bruennichi* (6). [4] Another example would be the Australian Red Back Spider, *Lactrodectus hasselti*[5] (7). Both are shown below in Figure 3.

[4] Chinta, S. P.; Goller, S.; Lux, J; Fnke, S.; Uhl, G.; chulz, S. *Angew. Chimie. Inti. Ed.*, **2009,** *49*, 2003-2036
[5] 2. Jerhot, E.; Stoltz, J. A.; Andrade, M. C. B.; Schulz, S. *Angew. Chem. Int. Ed.* **2010,** *49*, 2037-2040




(6)

**Female Wasp Spider Sex Pheromone**




(7)

**Australian Red Back Spider Sex Pheromone**

**Figure 3 Female Wasp Spider and Australian Red Back Spider Sex Pheromones**

Both pheromones have many possible derivatives, like blattellaquinone. Furthermore, the Red Back Spider sex pheromone has been known to illicit a response in low levels (25 μg)[5]. This would not only make it an interesting choice, but a potentially cost-effective one as well if it were to be deployed in insect control. In both cases, the molecules contain difficult to install stereocenters, which would be quite challenging to synthesize with Alfred's current resources, but that should not be a prohibitive factor.

Finally, the decision to use insects rather than other animals is multi-fold. First, the structures we have so far identified are known and relatively simple. Also, use of insects in research does not require approval for animal subject testing. Although for a research project, such approval may not be an issue, as part of a laboratory course, there may be insufficient time to develop a protocol that would earn approval, much less get approval from the committee.

**Prior Work**

There are many different types of labs that can be implemented at the undergraduate level. The most common type is the expository[6], or "cookbook," type labs which we, and countless other institutions now have in place. While this is the easiest kind in terms of student lab assistant training and laboratory preparations, and is to some extent necessary in the acquisition of many essential laboratory skills for the students, it tends to provide little in the way of critical thinking opportunities. Therefore, many researchers in STEM education are developing new, more thought-provoking laboratory exercises.

Other disciplines are taking note of the need for more interdisciplinary laboratory experiences at all levels, particularly in biology. Vale[7] implemented an interdisciplinary course at the Marine Biological Laboratory (MBL) in Woods Hole, Massachusetts. The goal was to improve the education of graduate students taking the physiology course. In 2004, 28 students were selected to take part in the course, with an equal amount of students with cell biology and physical science or computational backgrounds. The physiology course had three major goals:

1. Begin the course with exploration and practice of new techniques.
2. Use the time in the lab to focus on real research questions.
3. Provide an emphasis on collaboration between peers.

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[6] Dunlap, Norma, and Leah J. Martin. "Discovery-Based Labs for Organic Chemistry: Overview and Effectiveness." *ACS Symposium Series Advances in Teaching Organic Chemistry* 1108 (2012): 1-11.
[7] Vale, R. D., J. Derisi, R. Phillips, R. D. Mullins, C. Waterman, and T. J. Mitchison. "Interdisciplinary Graduate Training in Teaching Labs." *Science* 338.6114 (2012): 1542-543

This course, although designed for graduate students, has many of the same benefits we hope to obtain for undergraduate students. The assessment of the project suggests that it helped to more closely mimic a "real world" lab experience, in which the students had the chance to collaborate closely with their peers. The work also helped to develop many skills that the students will need as a professional scientist after graduate school, including: hypothesis/research question development, formulation of methods to collect data, and how to work around problems that develop along the way. The course also attempted to highlight to the students how certain methods they are not familiar with may apply to a problem they are trying to solve. This gives the new (to them) method relevance and helps motivate the student to learn it well. Also, because each "type" of student gets hands-on experience with what the other "type" of student does, they can more easily relate to and understand each other. This makes collaboration easier and the students can more readily see how one field affects the other. For example, the computational and physical science students move away from their typical, "observations plus modeling" approach and experience experimental biology, and the untidiness that comes with it. They learn to develop biological questions, and then develop "controlled experiments to answer [them]." Biological scientists, on the other hand, learn how to use tools more extensively, and how to apply more quantitative thinking to their work. Overall, each student walks away with a better idea of how the other can compliment or inform their work. However, Vale argues that bringing these two fields together at the graduate level does not guarantee success in developing collaboration skills and "novel insights into biological problems", and

that it may be advantageous to implement this type of course earlier, in the undergraduate curriculum.

Other work discusses the need for implementing more authentic research experience in biology undergraduate curricula[8]. Here, the authors compare the effectiveness of "cookbook" lab courses versus research-based lab courses. Specifically it compares the styles of lab regarding the student's attitude towards authentic research practices, the student's confidence in performing lab work, and finally, student's interest in perusing biological research. In order to evaluate this, the study used student surveys, classroom observations, and student interviews in each type of learning environment. They used both pre- and post-course surveys. In order to select participants, students were given the option of taking the normal biology course, or volunteering to be selected to participate in the experimental course. The "comparison group," in the normal course was selected so that their demographics matched those of the experimental group, specifically, gender, GPA, major, class year, and previous research experience. The results were broken down by attitude, confidence and interest.

Students were asked if they "prefer open-ended questions" with no right answer. Students in the experimental lab reported a statistically significant gain in "agree" and "strongly agree" reports in the pre-and post-course surveys. Whereas students in the comparison lab reported a mean that fell between "agree" and "disagree." Their means were statistically lower than the experimental lab's means.

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[8] Brownell, Sara E., Matthew J. Kloser, Tadashi Fukami, and Rich Shavelson. "Undergraduate Biology Lab Courses: Comparing the Impact of Traditionally Based "Cookbook" and Authentic Research-Based Courses on Student Lab Experiences." *Journal of College Science Teaching* 41.4 (2012): 36-45. Web. 9 Apr. 2016.

The students in the experimental lab reported statistically significant boosts in their self-confidence for each part of the lab, including data interpretation, question development, and result presentation. Students in the comparison lab reported smaller boosts in self-confidence overall.

Finally, students in the experimental lab reported being more interested in completing an honors thesis, while students in the comparison lab reported no significant interest. Additionally, students in the experimental lab reported a greater increase in interest in serving as an undergraduate research assistant, whereas the comparison group reported no such rise.

The results of this study show promise, however, replication of the results on a larger sample size is needed. If these results are achieved, then universities will be able to move onto the next challenge: implementing and altering their current biology lab courses. There are potential issues involving financial and logistical burdens, however, if implemented effectively, the study implies that many of the objectives we have for our course can be achieved with experiment-based labs.

Another example is an Argument-Driven Inquiry (ADI)[9] laboratory sequence for undergraduate general chemistry. Using this approach, students go through a seven-step process, beginning with an introduction to the task and research question, and ending with peer review of their reports. Finally, the students can revise their report before turning it in to their professor. The students are responsible for designing their own methods to answer the research question(s)

[9] Walker, Joi Phelps, Victor Sampson, and Carol O. Zimmerman. "Argument-Driven Inquiry: An Introduction to a New Instructional Model for Use in Undergraduate Chemistry Labs." *J. Chem. Educ. Journal of Chemical Education* 88.8 (2011): 1048-1056

they have asked, carrying out the experiments with appropriate record keeping, and finally, interpreting the data and evaluating if the data is sufficient to answer the question(s). Instead of the professor answering specific questions, they respond to questions with more guiding questions, prompting the students to collaborate with each other and think critically about what they are doing, which helps the student-researchers to learn how to think critically *via* a Socratic method. Between the development of their own methods, collaboration, and peer review, this type of lab experience more closely mimics "real world" research. Providing students with the opportunity to participate in more rigorous and authentic laboratory experiments will make for more competent science majors as they end their undergraduate studies.. This will hypothetically allow K-12 teachers to be better trained scientists, and consequently provide younger students with a more thorough science background, allowing them to enter into a college or university better prepared.

Another example is an interdisciplinary course between undergraduate biology and chemistry[10]. In this course, the interdisciplinary nature of brewing is addressed. By teaching a subject that tends to engage college students, professors are given the opportunity to discuss the biology and chemistry behind the brewing process, and thus demonstrate connections between the two sciences. During the course (4-week "semester"), 18/24 h are devoted to the laboratory component, while 6 hours are devoted to lecture. There are three biology-focused labs and five

[10] Hooker, Paul D., William A. Deutschman, and Brian J. Avery. "The Biology and Chemistry of Brewing: An Interdisciplinary Course." *J. Chem. Educ. Journal of Chemical Education* 91.3 (2014): 336-39.

chemistry-focused labs. This shows the interdisciplinary nature of a larger, single topic (brewing). The overall goals of this course were to:

- Expose science majors to instrumental methods and analytical techniques used in modern chemistry and biology.
- Engage students in science by applying the concepts learned in general chemistry and biology to a complex system.
- Share information on a subject in which students are interested, but for the most part not very knowledgeable.
- Promote writing and research skills, especially through good notebook keeping practice.

Finally, another example of an interdisciplinary laboratory involves chemistry, biology and physics[1]. Similar to the work we are doing, this approach strived to create a laboratory experience more centered on research, that is, one that is more experiment-based and less exercise-based. Concepts from at least two disciplines are integrated in each experiment; if concepts from the third discipline cannot reasonably be implemented into the lab itself, they are addressed in the laboratory manual and discussions. In the lab, students take on an investigative approach in which they formulate and test their own hypotheses. The students were selected based on interest in the lab on a questionnaire (only 36 were chosen due to equipment and faculty limitations). There were two methods used to assess the success of the laboratory experience: a post-course exercise and questionnaires (post-course and post-experiment). Evaluations and course reviews were conducted regularly. At the end of each semester, another questionnaire was given

regarding the total semester's experience. At the end of the entire year, a meeting was held between the students and faculty for open discussion of the experience as a whole. The form of the questionnaire involved both numerical scales and free responses and indicated that the students had a "more positive attitude toward the laboratory experience than their peers in the traditional laboratory sequence." This can be considered as a success from the point of view of creating a curriculum that the students are more interested in.

The post-course exercise was administered during the last lab of the Spring semester, and was given to students in the Interdisciplinary (ID) lab as well as the traditional course. A third party (biology faculty member at Pomona College), read and graded the responses without knowing which belonged to the ID student, and which the traditional students. Overall, the results indicated that the ID students were better able to "handle the exercise and seemed to demonstrate 'higher order' thinking." However, these results come after only one year of research, and the students participating in the ID lab were not selected at random, and this could have impacted the results. It is nevertheless promising as it suggests that students who complete such labs are leaving the course better equipped to problem solve. Whether or not the course selected for students who were already stronger appears unknown at this time.

Overall, these various studies had many similar aspects. All focused on implementing more student involvement in the labs. They all addressed how critical it is that students at any level are exposed to the skills that will make them successful scientists. For example: formulating a hypothesis and research

questions; development of their own methods/procedures; collaboration with other students to analyze results; etc. All of the articles agree that these skills are vital to the success of the student and that it is a good idea to implement labs that reinforce these skills early, most likely in during the undergraduate years. The articles that included student surveys found that the majority of students enjoyed these research-based labs, and that their confidence in the laboratory had increased since participating, which is essential for students to get the most out of the lab experience.

## Methods

**Table 1 Blattellaquinone Methods**

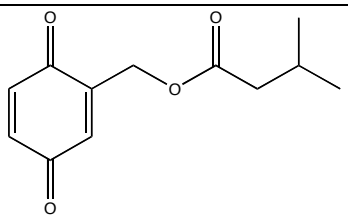
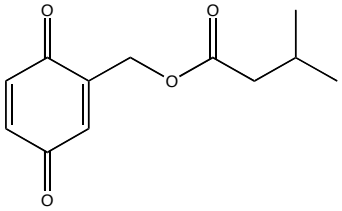
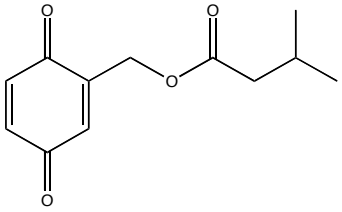
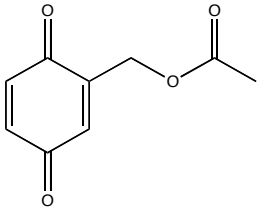
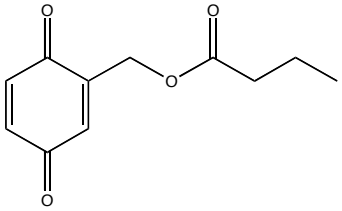
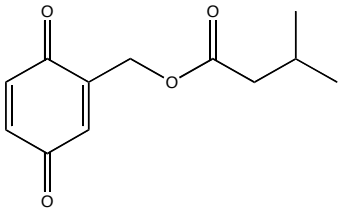
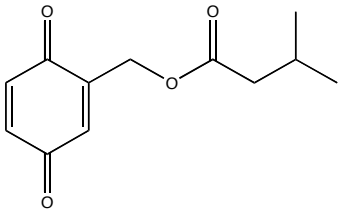
| Blattellaquinone | Method | Problems |
|---|---|---|
|  Trial 1 | We first attempted to synthesize blattellaquinone *via* a two-week procedure. The first week involved steps up to the first washing procedure (intermediate production). The second week involved the second half of the synthesis, and washing of the chloroform layers. Finally, the product was analyzed via GC/MS, vacuum filtrated and recrystallized. The melting point was taken, and found to be 56 ˚C, which is consistent with the literature value[2] of 42-56 ˚C. It was dissolved in dichloromethane and sent to Ben for testing. | • Work-up procedure difficult to understand<br>• Hard to complete in two 3-hour lab periods |
|  Trial 2 | The second trial involved attempting to carry out the synthesis in one research period. The first week, the entire synthesis, including all washing and extraction procedures was performed. The following week, a GC/MS was taken of the product. | • No solid formation<br>• GC/MS results did not look promising. There were multiple peaks, but none of them contained the starting material, or intermediate.<br>• Work-up procedure difficult. |
|  Trial 3 | For the third trial, the entire synthesis was completed in one lab period, except for the final chloroform washing/extracting procedure, which was left for the following week. Additionally, the chloroform layers were placed on the rotavap and saved for the GC/MS, and pentane was added to recrystallize the product. | • No solid formation<br>• Work-up procedure difficult |

**Table 2 Blattellaquinone Derivative Methods**

| **Blattellaquinone Derivative** | **Methods** | **Problems** |
|---|---|---|
| Acetyl Chloride Derivative  Trial 4 | The attempt was made to synthesize a derivative of blattellaquinone using the original two day protocol. The first one selected was the acetyl chloride derivative where isovaleryl chloride was replaced with acetyl chloride. | • Spilled some solution.<br>• Chloroform work-up procedure confusing. |
| Butyryl Chloride Derivative  Trial 5 | Another Derivative was synthesized using the original protocol, but instead of isovaleryl chloride, butyryl chloride was used. | • Work-up procedure confusing. |

Note: The Valeryl Chloride Derivative is not included in the table as it was not a derivative I worked with directly.

**Table 3 Blattellaquinone One-Pot and Half Scale Methods**

| Blattellaquinone (one-pot and half-scale) | Methods | Problems |
|---|---|---|
| One-Pot Blattellaquinone  Trial 6 | The first trial involved a one-pot procedure. However, instead of using dichloromethane as the solvent, acetonitrile was used. | • Solid formed, however GC/MS gave no unusable data.<br>• Suggests one-pot procedure ineffective, or reactant/product degeneration. |
| Half-Scale Blattellaquinone  Trial 7 | The blattellaquinone was synthesized using the regular procedure, but on half the scale. The same synthesis was used as the original trials but the amounts of the reactants were all cut in half. | • No specific issues.<br>• Still failed to isolate solid product.<br>• No product observed by GCMS<br>• Reactant/product degeneration? |

## Benjamin Proctor's Methods

In order to test the success of our compounds, a test bin was obtained (Figure 4). Then, two filter paper discs were added to one end of the bin, with one containing the blattellaquinone and one "blank" as a control. The blattellaquinone was dissolved in dichloromethane and the blank was treated with the same dichloromethane to verify the dichloromethane had no effect. The insects were transfered *via* a BugZooka™ (Figure 5) and transport capsule (Figure 6). In one trial, a very dilute sample of blattellaquinone was used and in another, a highly

concentrated but crude sample was used. The results suggest that although the cockroaches did not respond to the dilute sample nor did they respond to the blank, they did respond to the concentrated crude sample. The lack of response could be due to impurities in the product to the point of near complete product decomposition or the diluted sample being below a detectable threshold for the test subjects. Unfortunately, because this sample is crude, we are unable to complete a quantitative analysis of the response. If an analytically pure sample were obtained, a quantitative analysis could be performed, leading to an understanding of how much pheromone is required to elicit a response. This may interest future students whom are willing to further this study by optimizing the synthesis and purification.


**Figure 4 Test Bins and Filter Paper Samples**

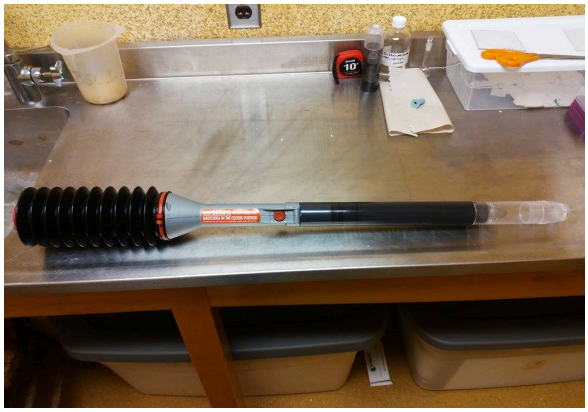


**Figure 5 BugZooka**

**Figure 6 Removable Capture Capsule**

## Results/Discussion

A solid sample of what appeared to be blattellaquinone was prepared and given to Benjamin Proctor, who developed a behavioral assay in order to test the sample's ability to elicit a response from the cockroaches (Figure 4). The cockroaches were observed walking toward the filter paper with the synthesized blattellaquinone while they were not apparently drawn to the control paper that

was only exposed to solvent (dichloromethane). Once arriving at this filter paper they engaged in a display akin to a dance where they extended their wings and moved their legs in a rhythmic pattern of sorts. This dance indicated that not only were they drawn to the sample filter paper, but they responded to it the same way they would a female cockroach signaling it is seeking a mate. This positive response of the cockroaches, where the males appear to be selectively responding to an area that contains female sex pheromone while ignoring an area that does not, suggests that the primary concept for this lab, where one group in the organic chemistry lab prepares a sample and gives it to a second group who tests it, is sound. The result also implies that it possible to synthesize the pheromone with the resources available to Alfred's STEM education, even if only a crude sample.

We also set out to synthesize three derivatives using different acid chloride starting materials: butyryl chloride, acetyl chloride, and valeryl chloride to derivatize the ester position. This position was targeted first because of the commercial availability of starting material acid chlorides, simplifying access to these targets and because of an anticipated ease of chemistry. The first derivative attempted was the acetyl derivative-which produced a small amount of solid. However, it was unable to be analyzed *via* GC/MS, as the instrument was not working at this time. Next was the valeryl derivative, which was completely unsuccessful. Two attempts were made to synthesize the product, and each time a solid failed to form. The final derivative was the butyryl derivative. Similar to the acetyl derivative, a small amount of solid formed, but was unable to be verified.

An assumption is being made, though, that the product is a solid in all cases. Although this is likely a safe assumption due to the fact that the change in the ester unit should not affect physical properties very much, it is possible that some derivatives, unlike blattellaquinone, are liquids. If this is the case we will have to determine another method of purification such as chromatography or distillation, as recrystallization would not work and also may explain some of our difficulties. However, purification by chromatography is infeasible for such a large class, given our resources and also often requires more time than our traditional lab period allows. That being said, if the product were being formed and was a liquid, it should still be identifiable on the GC/MS.

**One-Pot Results**

An attempt was made to evaluate if a one-pot procedure could be used for this experiment. Blattellaquinone was the target, however, the one-pot procedure failed to produce any identifiable product. Because, according to GC/MS this reaction failed to give even a small amount of product, in order to ensure this was a procedure failure and not due to reagent or product degradation, blattellaquinone was synthesized again *via* the two-step method, but on a smaller (half) scale in order to conserve resources. This procedure led to problems as well. It was later learned in a personal communication with the author of the Feist[2] paper that most students had difficulty obtaining product, something that was not indicated in the original report.

The results suggest that there is some issue with product stability, or there are issues with at least one of our reagents. Further, there were peaks observed in

the GC/MS that appeared to be unrelated to our sample, as they were inconsistent with anything in our synthetic protocol. Whether this is due to unexpected chemistry occurring, contaminated samples and/or solvents, or a contaminated GC column or syringe remains unknown. In our experience, this was a recurring problem. If the product was synthesized, and there was nothing in the GC/MS to suggest it was, it was an exceedingly minor product. This sample was discarded for lack of any evidence suggesting a successful reaction.

**Challenges and Future Work**

One issue with the experiment is its scale. Due to the cost of some starting materials, the synthesis must be performed on a very small scale. While not necessarily an issue for experienced chemists, this leaves little room for error in students who have minimal experience. Thus, second semester organic students might not be skilled enough to obtain testable amounts of pure product. It therefore may be more logical to incorporate this kind of laboratory experience into an Advanced Lab course, or a Capstone project. This has an added benefit of timing. Currently, the second semester of organic chemistry runs in the spring, while the animal behavior course runs in the fall. This means that the product would have to sit for at least 10 weeks before it could be tested by the animal behavior class. Advanced lab, however, is offered in the fall. Having the courses run during the same semester would minimize the amount of time the product would have to be stored. This could help resolve some of our concerns regarding the stability of the products.

Another issue is the work-up procedure. My partner and I had significant trouble when it came to understanding the importance of the chloroform layers, and why we needed to collect them. It was quite challenging to grasp exactly what needed to be done during the work-up, and mistakes were made. It wasn't until the third semester of research that the work-up was fully understood. Since two students who have already completed organic chemistry and various other lab courses struggled with it, it is likely that newer students will as well. Extra care will have to be taken on the part of the professor to ensure students understand exactly what they are doing and don't discard valuable product. Therefore, the complete synthesis and analysis may not be able to be completed in two-three hour lab periods with our facilities and instruments, despite what was done by Feist et al[2]. This is also why the one-pot procedure remains an attractive goal since it would resolve most, if not all of these concerns.

Also it may be that students need to work in groups, as there is not only limited time, but also limited equipment, starting materials and access to the instruments. If every student is completing their own synthesis, the lines for the GC/MS or NMR and rotavap will get very long, very quickly, overloading our facilities and creating a bottleneck that will not only frustrate the students but also potentially ruin their product or at the very least, make the results less reproducible. If other analytical methods that are more reliable can be found for future work, it would be very beneficial to preventing the "bottleneck" effect that often occurs in labs. It is for this reason that it may be beneficial to have students work in groups. This would also help solve the issue of limited glassware. However,

this introduces the problem of ensuring each student is actively participating, and getting to study the pheromone/derivative that appeals to their interest.

Instrument failure was another problem throughout this research. The derivative solids synthesized were unable to be analyzed because the GC/MS was down for the majority of our research time. Additionally, we were unable to have any additional samples (derivative or fresh blattellaquinone) tested *via* cockroach testing bin because Ben Proctor graduated before our derivatives were ready. As of now, crude samples have been stored in the refrigerator, but it is unlikely they are stable for months, and probably need to be discarded and resynthesized, particularly with the renovations pending in the lab space.

Despite the challenges, the idea of having an interdisciplinary lab between the two courses is sound and our proof of concept demonstrates it can be done, but needs significant optimization, if it is to fit into 2 or 3, three-hour lab periods. It has been shown that blattellaquinone can be synthesized in a two or three week process, and that the male cockroaches respond positively to it in concentrated, albeit unknown, amounts. However, the analytical and purification procedures may cause the experiment to take closer to 3-5 weeks.

Overall, once optimized, this type of an experiment will give students the chance to not only perform a true experiment as opposed to exercise, but give them ownership over their own education. By allowing them to select their own derivative/pheromone and design the testing protocol, they are responsible for coming up with a hypothesis, collaborating with another lab group to test and perhaps refine their hypothesis, sharing results, and designing their experiment(s).

This type of experiment will more closely resemble a research work environment or graduate program, and give students a better idea of what chemistry/biology is like in the real world. They will have to think more critically about what it is they are doing, such as predicting what changing one functional group on a molecule will do, or deciding how to more effectively test the insect's response to the product. For example, future-testing protocol could involve leading an insect through a maze as oppose to a simple box. It can also entail different pheromones all together. In the end, the students are designing their own experiments and answering their own research questions, making for a more impactful laboratory experience.

**Conclusion**

The overall intent of this research was to show proof of concept of an interdisciplinary lab between the animal behavior and organic chemistry classes. Overall, this lab looks promising, once the issues discussed above have been resolved. If such a lab were to be deployed into the curriculum, care would have to be taken to make sure that all of the reagents and solvents were fresh, that all of the glassware was properly cleaned and dried and that all of the instruments are properly used. Additionally, it may be better implemented as a capstone or Advanced Lab class, as this would allow younger students to gain more experience before embarking on a challenging project. Additionally, the One-Pot procedure should be explored further, as it resolves many of the work-up and time-constraint issues discussed above. If implemented at the appropriate level, and with proper care taken to rectify the issues identified through this research, this lab would offer

students a chance to gain more experience in the “real world” of chemical research, something that would not only enrich their learning experience, but potentially help guide them through determining career paths after college.

**Putative Example Lab-Handout**

Objectives: The objective of this lab is to synthesize insect pheromones as part of an interdisciplinary lab between the animal-behavior and organic chemistry courses. Additionally, students will gain experience in structure-relationship studies, and perform an experiment, as opposed to an exercise. Students could choose to synthesize blattellaquinone (described herein) a derivative (easily adaptable from the procedure described here), or an entirely different, instructor-approved pheromone.

Safety:

Ethyl acetate: Flammable, acute toxicity (oral, dermal, inhalation), skin and eye irritation, specific organ toxicity.

Hexanes: Flammable, acute toxicity (oral, dermal, inhalation), skin and eye irritation, specific organ toxicity, health hazard, respiratory sensitization, germ cell mutagenicity, reproductive toxicity, aspiration hazard, hazardous to aquatic environment.

Pentane: Flammable, acute toxicity (oral, dermal, inhalation), skin and eye irritation, specific organ toxicity, health hazard, respiratory sensitization, germ cell mutagenicity, reproductive toxicity, aspiration hazard, hazardous to aquatic environment.

Dichloromethane: acute toxicity (oral, dermal, inhalation), skin and eye irritation, specific organ toxicity, health hazard, respiratory sensitization, germ cell mutagenicity, reproductive toxicity, aspiration hazard.

Dimethoxy benzyl alcohol: Eye protection, gloves, respirator use suggested.

Pyridine: Flammable, acute toxicity (oral, dermal, inhalation), skin and eye irritation, specific organ toxicity.

Isovaleryl chloride: Flammable, corrosive, acute toxicity (oral, dermal, inhalation).

Acetonitrile: Flammable, acute toxicity (oral, dermal, inhalation), skin and eye irritation, specific organ toxicity.

Ammonium cerium nitrate: Oxidizer, acute toxicity (oral, dermal, inhalation), skin and eye irritation, specific organ toxicity.

Safety Information taken from Sigma-Aldrich

Procedure:

Obtain a clean, dry RBF whose volume is roughly 2x that the total volume of materials to be added. Set it upon a cork-ring on top of a stir plate and obtain a magnetic stir bar and septum. Using a metal clamp, clamp the neck of the RBF to the bars in the back of a hood or ring stand and place the magnetic stir bar into the RBF. Then, obtain the following reagents and add them to the flask in the order provided while stirring magnetically:

1. 15 mL of dichloromethane,
2. 0.5 mL (3.32 mmol) dimethoxy benzyl alcohol, and
3. 0.31 mL (3.83 mmol) of pyridine.
4. Cover the flask fully using the septum and then add 1.53 mL (12.55 mmol) of isovaleryl chloride.
5. Allow the solution to stir at room temperature for approximately 45 minutes.
6. Monitor the progress of the reaction by TLC with 1:1 ethyl acetate and hexanes.

7. Once the reaction has run to completion by TLC (approx. 45 minutes), perform the following workup:
8. Wash the solution 3x with 10 mL HCl,
9. 3x with 10 mL bicarbonate, and finally
10. 1x with 10 mL of brine.
11. During this stage, shaking too strongly may lead to the formation of an emulsion that can be very difficult to separate. Store the organic solution in the refrigerator until the next laboratory period. Next week, remove the dichloromethane using the rotavap.
12. Once this is complete, add a magnetic stirbar, 15 mL of DI water and 15 mL of acetonitrile and leave the reaction to stir magnetically.
13. Remove a small amount (~1 mL) of this solution for a reference TLC
14. Then, obtain 6.0 g (11 mmol) of cerium ammonium nitrate, and add portion-wise over several minutes.
15. As with the previous reaction, monitor the reaction by TLC using the same solvent as before.
16. Once the reaction is complete according to TLC analysis, add 20 mL of brine and 15 mL of chloroform.
17. Extract the bottom (chloroform) layer. Extract the products from the aqueous layer with 20 mL of chloroform, 2x combining all of the chloroform layers.
18. Then, wash the combined chloroform layers 1x with 20 mL of concentrated sodium bicarbonate.

19. Wash 1x 20 mL of DI water.
20. In a clean, dry separatory funnel, wash 1x with 20 mL of brine. Separate the organic layer and store product in the refrigerator until the next laboratory period. The following week, evaporate the dichloromethane using a rotavap. Next, add 10 mL of pentane to recrystallize the product. If crystals form, isolate *via* vacuum filtration and analyze the product *via* GC/MS, IR and NMR to confirm the product.
21. Send product to biology counterparts.

**Questionnaire**

Please rate the following aspects of the course using the following scale:

1-Strongly disagree 2-Disagree 3-Neutral 4-Agree 5-Strongly Agree

1. The lab course helped me make connections between the different fields of science applied?
2. The lab course helped me to gain a better understanding about what science in the "real world" looks like?
3. The lab course was more engaging and interesting that previous lab experiences?
4. The lab course allowed me to gain experience creating my own hypothesis, and implementing my own methods to test said hypothesis.

Please answer the following questions in short-answer format:

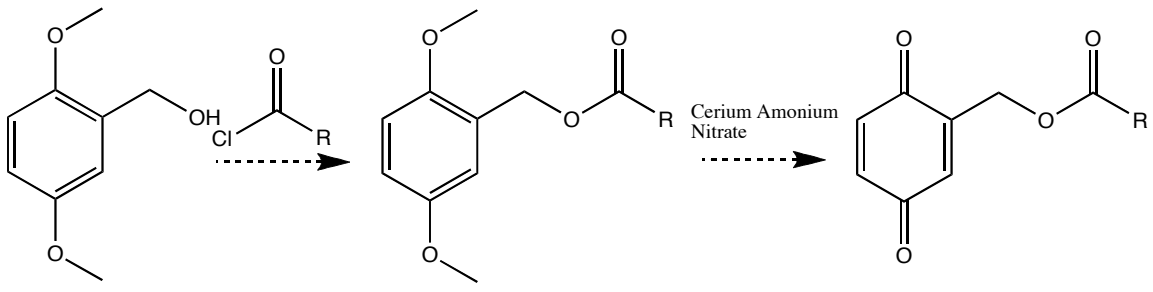
1. What did you like most about the lab course?
2. What did you like least about the lab course?
3. How would you compare this lab course to previous ones?
4. Would you recommend this lab course to future students?
5. If you were constructing the lab course, what are some things you would do differently? What are some things you would keep the same?

**Acknowledgements**

I would like to take this opportunity to thank Alfred University's ARGUS program for providing the funds to supply the materials for this research. I'd also like to thank Dr. D'Angelo, Dr. Zimbler-DeLorenzo and Dr. Bowers, for overseeing the project, providing an opportunity to participate in research group meeting, and serving on the committee. I would also like to thank Benjamin Proctor, for serving as the biology counterpart to Emma and I. Finally, I'd like to extend a huge thank you to my partner, Emma Robinson. The research was a team effort, and without her, there would be significantly less data, and significantly less of my sanity.

**Experimental Protocol**

Shown below is the basic synthesis of blattellaquinone, and its derivatives used for this research (11). Additionally, the mechanism (12) of the first step of the reaction is shown below.




(11)

**Figure 7 Blattellaquinone Synthesis**

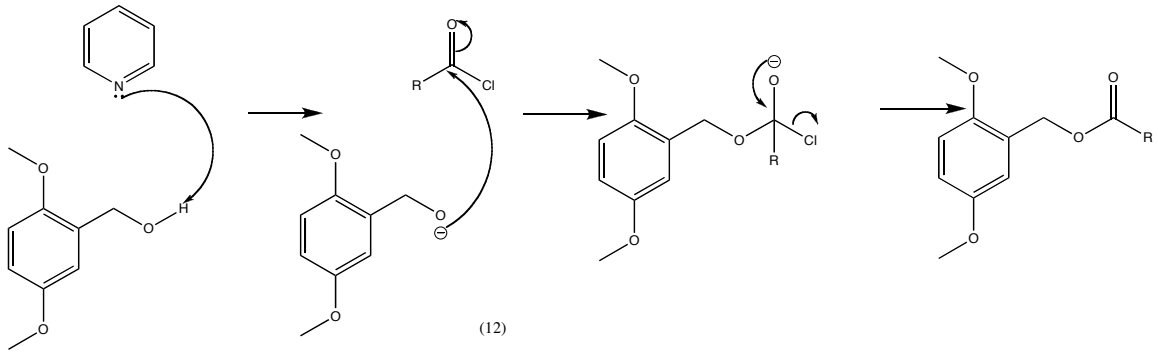



**Figure 8 Step 1 Ester Formation Mechanism**

## Trial 1: Blattellaquinone 2-Week Synthesis

The first experiment (experiment 1) was the original synthesis of blattellaquinone. Dichloromethane (15 mL), dimethoxy benzyl alcohol (0.5 mL), pyridine (0.31 mL), and isovaleryl chloride (1.53 mL) were added to an RBF and condenser apparatus *via* syringe. The apparatus was placed on top of a stir/heat plate and the heat was set to 60 ˚C . A TLC was taken, with a mixture of 1:1 ethyl acetate and hexanes (all TLCs used this same ratio) and was stained with potassium permanganate. After approximately a half-hour, another TLC was taken with the starting material on the left and the partially reacted solution on the right. The partially reacted solution had moved up the TLC, indicating that the reaction had run to completion. The TLC was dipped into potassium permanganate and the reaction was allowed to continue at reflux. After approximately an hour, the heat was turned off and the solution was left to stir as it cooled to room temperature. After cooling, a work-up (which was completed by Dr. D'Angelo because of time constraints) was done in which the solution was washed 3x with HCl (10 mL), 3x

with bicarbonate (10 mL), and finally 1x with brine (10 mL). After standing for one week, a TLC was taken and the dichloromethane was evaporated using a rotavap. This TLC was alarming, because the spot appeared lower on the TLC plate than it had the previous week, matching more closely with the starting material than the reacted solution. Another TLC was taken comparing Emma Robinson's product from the same reaction, the starting material combined with Emma Robinson's product, and the starting material alone. This comparison showed that there might have been product degeneration. Acetonitrile (15 mL) and DI water (15 mL) were added to the solution following the dichloromethane evaporation. A TLC was taken of this solution for comparison purposes during reaction monitoring, and then it was left to stir. Cerium ammonium nitrate (6.04g ) was then added portion-wise. The solution was left to stir at room temperature and another TLC was taken. After 60 minutes, a work-up was done in which the reaction was portioned between brine (20 mL) and chloroform (15 mL). The bottom layer (chloroform layer) was extracted. The aqueous solution was further extracted 2x with chloroform (20 mL), combining the organic layers. The combined organic solutions were washed 1x with concentrated sodium bicarbonate (20 mL), 1x DI water (20 mL) and finally, in a new separatory funnel, 1x with brine (20 mL).

The next week, the product was washed 1x with brine (5 mL) and a TLC was taken, which showed a spot which had moved further up the TLC plate than the starting material. This suggested formation of product. The organic layer was analyzed by GC/MS and suggested formation of the product, as there was a small peak at 222, which is the molecular weight of our target product. The solution was

placed on the rotavap, and then pentane was added in order to recrystallize the product. The mixture was then stored in the refrigerator.

A few weeks later, the product was placed on the rotavap. Then, dichloromethane (15 mL ) was added. The product was washed 3x with sodium bicarbonate (10 mL), and 1x with brine (10 mL). A GC/MS was taken of the product and the dichloromethane was again evaporated. Then, pentane (10 mL) was added to recrystallize it and a solid formed.

A few weeks later, the product was vacuum filtered. The melting point was taken twice. The first time, it melted at 42 °C. However, there was concern that the melt-temp was set to high (level 5) and it was re-done. The melt-temp was set to level 1 and the product began to look "melty" at 51.1 °C. The level was increased to 2.5 and the product fully melted at 56 °C, which agreed with the literature value[2] of 56.5 °C. We were able to isolate 0.043g of crude product. The product was stored in the refrigerator until next semester.

The next semester, the melting point of the solid blattellaquinone was obtained a second time. However, there were issues with the melt-temp selected, as the first trial indicated that the sample would not melt even at temperatures as high as 87 °C (began melting on level 2, increased to level 2.5 and 3). A second trial was completed on a new melt-temp on level 50 and the product melted at 56 °C, suggesting the product was crude blattellaquinone. The product was then dissolved in dichloromethane and given to Benjamin for testing.

**Trial 2: Blattellaquinone 1-Week Synthesis**

Dichloromethane (16.5 mL), isovaleryl chloride (1.55 mL), dimethoxy benzyl alcohol (0.5 mL), and pyridine (0.3 mL) were collected in an RBF. A stir bar was then added and the solution was left to stir for approximately 55 minutes. A workup was then completed in which the solution was washed 3x with HCl (10 mL), 3x with bicarbonate (10 mL) and then finally, 1x with brine (10 mL). The solution was then placed on the rotavap. After the dichloromethane was removed by rotavap, acetonitrile (15 mL) and DI water (15 mL) were added to the RBF. Ammonium cerium nitrate (6.0 g) was added portion-wise. The solution was left to stir. Once the reaction had completed, the solution was washed 1x with brine (20 mL) and chloroform (16 mL). The bottom layer was extracted. The top layer was washed again 2x with chloroform (20 mL). The bottom layers were all collected and combined. Finally, these chloroform layers were washed 3x with sodium bicarbonate (20 mL), 1x with DI water (20 mL), and (in a new separatory funnel) 1x with brine (20 mL).

The next week, the product was analyzed *via* GC/MS. There was no solid formation. At the end of the semester, it was decided that this could maybe be some form of blattellaquinone (labeled kg-10).

The next semester, a TLC was taken of kg-10. The TLC showed one long, messy line that went about ¾ up the plate. Then, pentane (10 mL) was added and an attempt was made to isolate the product *via* vacuum filtration. However, no crystals formed. The RBF was placed in the refrigerator to help promote crystal

formation, and that had no effect. The attempt was made to vacuum filter the solution anyways. No product was able to be isolated and it was discarded.

**Trial 3: Blattellaquinone Synthesis**

The third trial was another attempt to synthesize blattellaquinone. Dichloromethane (15 mL), pyridine (0.31 mL), dimethoxy benzyl alcohol (0.5 mL) and isovaleryl chloride (1.53 mL) were added to an RBF with a stir bar. The reaction was left to stir for approximately 45 minutes. A TLC was taken with dichloromethane at 15 and 45 minutes in, comparing our product, and the starting material. Both TLCs indicated formation of product, as the "unknown" spot had moved further up the TLC plate. Then, a work-up was completed in which the solution was washed 3x with 5%HCl (10 mL), 3x with sodium bicarbonate (10 mL), and finally, 1x with brine (10 mL). The solution was placed on the rotavap, and then acetonitrile (15 mL) and DI water (15 mL) were added. 6.005g of CAN were then added portion-wise and the reaction was left to stir for about 20 minutes. A TLC was taken, which looked similar to the two TLCs taken previously. The product was stored in cupboard 96 until the next week.

The next week, another work-up was performed in which the solution was washed with brine (20 mL) and chloroform (16 mL). The bottom layers were extracted. Then, the top layer was washed again 2x with chloroform (20 mL). Then, all of the bottom layers were collected and washed 3x with concentrated sodium bicarbonate (20 mL), 1x DI water (20 mL), and finally, 1x with brine (20 mL). A TLC was taken of the washed chloroform layers. It looked similar to the previous week's TLCs, however, the spots had not moved as far up the plate. A small vial was saved

for the GC/MS, and the rest was placed on the rotavap. Then, pentane was added in order to recrystallize the product. However, no crystals formed so the product could not be vacuum filtered. It was stored in the refrigerator and no further work was completed on it.

**Trial 4: Acetyl Chloride Derivative**

Dichloromethane (15 mL), acetyl chloride (1.53 mL), dimethoxy benzyl alcohol (0.5 mL), and pyridine (0.31 mL) were collected in an RBF with a stir bar. The solution was placed on a stir plate with the heat (level 3) and stir settings on. However, after approximately 40 minutes, the solution was removed from the heat, and left to stir. A TLC was taken at this time as well. The product was then washed 3x with HCl (10 mL), 3x with bicarbonate (10 mL) and finally, 1x with brine (10 mL). A TLC was taken of the solution, which showed two large spots about half way up the plate. One source of error came from spilling some of the solution under the hood. This could have an affect on the product yield.

The next week, a TLC was taken immediately. It looked similar to the one taken at the end of last week, suggesting the intermediate is table when stored in the refrigerator. The solution was then placed on the rotavap. Then, acetonitrile (15 mL) and DI water (15 mL) were added and the solution was left to stir. of ammonium cerium nitrate (~6.0 g) were added portion-wise. Once the reaction had run to completion, the solution was washed with brine (20 mL) and chloroform (20 mL). The bottom layers were extracted, and the top layer was washed 2x with chloroform (15 mL). The bottom layers were all collected and washed 1x with concentrated sodium bicarbonate (20 mL), and 1x with DI water (20 mL). Finally, in

a new separatory funnel, the solution was washed 1x with brine (20 mL) and stored in the refrigerator.

The next week, the solution was washed with brine (10 mL) and a TLC was taken, with spots that fell at about the same height on the plate as the previous week. Then the solution was placed on the rotavap, leaving behind white crystals. Pentane (10 mL) was added in order to re-crystalize the product and it was stored in the refrigerator.

The next week, the product was vacuum filtered in order to isolate the crystals. The product was an orange, rusty colored solid. The product was stored in a vial labeled: KKG 3/3/15. Then, a melting point was obtained of 91 °C. The product was stored in the refrigerator.

**Trial 5: Butyryl Chloride Synthesis**

Dichloromethane (15 mL), butyryl chloride (1.5 mL), dimethoxy benzyl alcohol (0.5 mL), and pyridine (0.31 mL) were added to an RBF. A stir bar was added and the whole solution was left to stir. About half way through the stirring, a TLC was taken. Two spots were present, a darker one on the line, and a very faint one about 2/3 up the plate. Then, the solution was washed 2x with 5% HCl (10 mL), 3x with saturated sodium bicarbonate (10 mL), and 1x with brine (15 mL). Another TLC was taken and the intermediate was labeled: 3/17/15 KKG and stored in the refrigerator.

The next week, and Rf value was calculated for one of the TLCs. The starting materials had an Rf value of .728 and the intermediate had an Rf value of .847. It was decided to continue on to step two. The product was placed on the rotavap to

evaporate off the dichloromethane. Then, acetonitrile (15 mL) and DI water (15 mL) were added to the RBF. The solution was left to stir and of ammonium cerium nitrate (~6.0 g) were added portion-wise. A TLC was taken about 20 minutes later. Once the reaction had completed, the product was washed 1x with brine (20 mL) and chloroform (20 mL). The bottom layer was extracted and the top layer was washed 2x with chloroform (15 mL). The bottom layers were collected and washed 1x with concentrated sodium bicarbonate (20 mL), 1x with DI water (20 mL) and finally, in a new separatory funnel, 1x with brine (20 mL). A final TLC was taken right before the final wash with brine, and showed a spot at about the same place as the earlier one. The product was then stored in the refrigerator.

The next week, the chloroform layers were washed with brine (20 mL). Then, a TLC was taken, which looked similar to the previous week's. Then the solution was washed with brine (10mL) again. The product was placed on the rotavap and then hexane (10 mL) was added in order to recrystallize the product.

The next week, the product was vacuum filtered and a very small amount of solid, white crystals were obtained. The solid was collected and stored in a vial labeled: 4/7/15 KKG. The filtrate was also saved and stored in the refrigerator in an RBF.

**Trial 6: Blattellaquinone One-Pot Synthesis**

The 6$^{th}$ trial involved a one-pot synthesis of blattellaquinone. Dichloromethane (15 mL), dimethoxy benzyl alcohol (0.5), pyridine (0.31 mL), and isovaleryl chloride (1.53 mL) were added to a 250 mL RBF with a stir bar. The apparatus was covered with a septum and left to stir for ~45 minutes. White the

reaction was stirring, 1 drop of 2,5 dimethoxy benzyl alcohol was dissolved in dichloromethane and a TLC was run side by side with the intermediate. The TLC indicated that the intermediate had formed, as its spot had moved further up the plate. Then, of cerium ammonium nitrate (~6g) was added. The first 3g were added, followed by DI water (10 mL), and then the last 3g. A TLC was taken and then chloroform (60 mL) was added to the solution. Then, it was washed 3x with saturated sodium bicarbonate (10 mL), 3x with 0.1 M HCl (10 mL), 1x with DI water (10 mL) and finally, in a new separatory funnel, 1x with brine (10 mL). Then, another TLC was taken, which looked similar to the TLC taken before the workup. The solution was stored in an Erlenmeyer flask labeled: KG/ER Blattellaq. Int. 9/30/15. The flask was stored in the refrigerator.

The next week, a TLC and GC/MS were taken. The TLC looked similar to the previous week's. The first GC/MS was under the file: KG-26, name: blattellaquinone one pot, (my doc→ D'Angelo research→ Kyma). A second GC/MS was taken because the first one did not match the TLC. The second file was named KG-26 B. Then, the solution was placed on the rotavap in order to evaporate off the dichloromethane. The RBF was stored at room temperature until the next week.

After one week at room temperature, there was solid crystal formation, suggesting that our product was coming off the GC/MS column before it could be read. Dichloromethane was added and a TLC was taken, in which the spot looked like a large smear running almost all of the way up the plate. Then, an HNMR was taken of the sample: File name: PNMRfidERKG. The receiver gain was reduced to 10, and the NMR failed to give useful results. A CNMR was taken also, under the file

name: pnmrfidERKG2. The solution was then placed on the rotavap and pentane was added for recrystallization.

The next week, the solid product was isolated and the melting point was taken and found to be 70-71 ℃. Another GC/MS was taken @ 60 T, hold 2 minutes, solvent delay: 4 minutes. However, the GC/MS was still giving unexplainable results.

**Trial 7: Half-Scale Blattellaquinone Synthesis**

Dichloromethane (7.5 mL), dimethoxy benzyl alcohol (0.25 mL), pyridine (0.15 mL) and isovaleryl chloride (0.75 mL) were added to a 25 mL RBF with a stir bar. A TLC was taken after about 15 minutes, and 25 minutes to check the reaction's progression. Both TLCs gave large smears at the top of the plate, and did not look promising. Then, a work-up was performed in which the solution was washed 3x with HCl (20 mL), 3x with sodium bicarbonate (10 mL) and finally, 1x with brine (10 mL).

The next week, dichloromethane (10 mL) were added to the solution. It was then added to a separatory funnel in order to extract the remaining water. A TLC was then taken twice to ensure results were not caused by beaker contamination. Then, a GC/MS was taken and saved under dangeloresearchkyma, with the name: 11-4-15-Kyma. The GC/MS showed the intermediate and starting material and was stored in cabinet 94.

The next week, the solution was placed on the rotavap. Then DI water (7.5 mL) and acetonitrile (7.5 mL) were added and the solution was left to stir. Then of ammonium cerium nitrate (3.076g) were added portion-wise. Finally, the solution

was washed 1x with chloroform (15 mL) and brine (15 mL). The bottom layers were collected. And then the top layer was washed again 2x with chloroform (15 mL). Then, the bottom layers were collected and washed 1x with sodium bicarbonate (20 mL), 1x with DI water (20 mL), and finally, in a new separatory funnel, 1x with brine (20 mL). The solution was stored in cabinet 94.

The next week, the solution was placed on the rotavap and then pentane (10 mL) was added in order to recrystalize the product. The following week, the product was vacuum filtered with a microkit. The weight of the solid was obtained (0.154g) and then it was stored in the refrigerator.

The following semester, the solid product was dissolved in ~2 pipettes of dichloromethane and a GC/MS was taken under the file names: KG-32 and KG-32b. (D'Angelo Research→ Kyma→ file names: KG-32 and KG-32b→ Sample: blattellaquinone 2 pot). However, both indicated that the product is probably decomposing.

## GC/MS Spectra

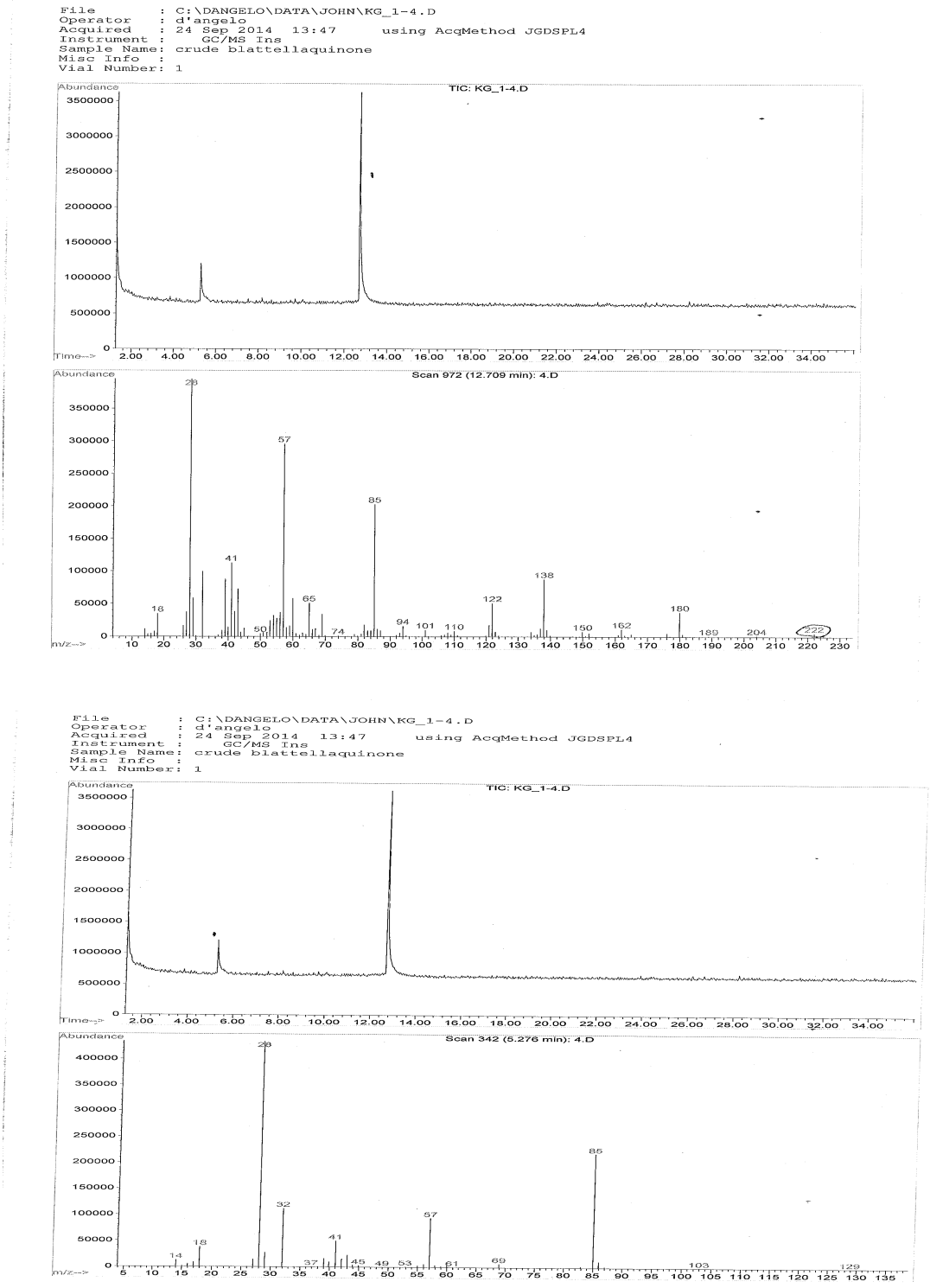
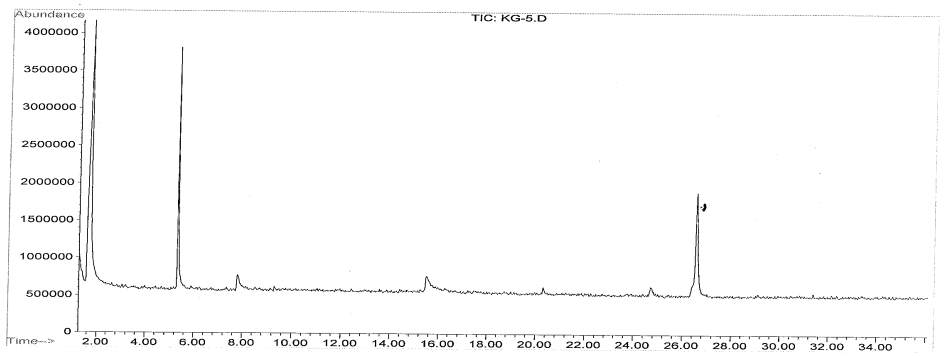


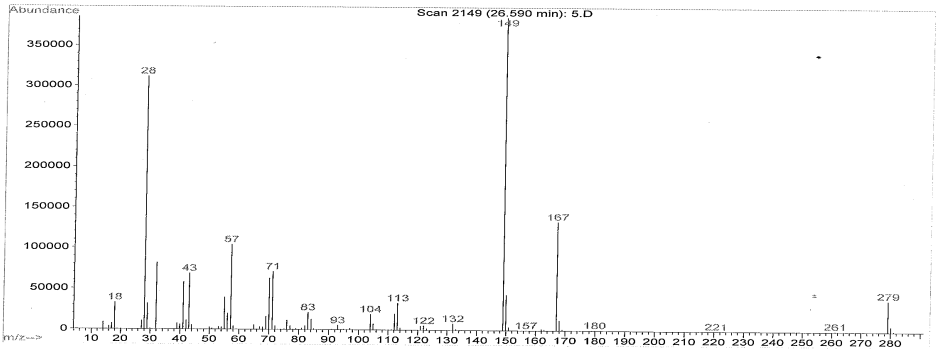




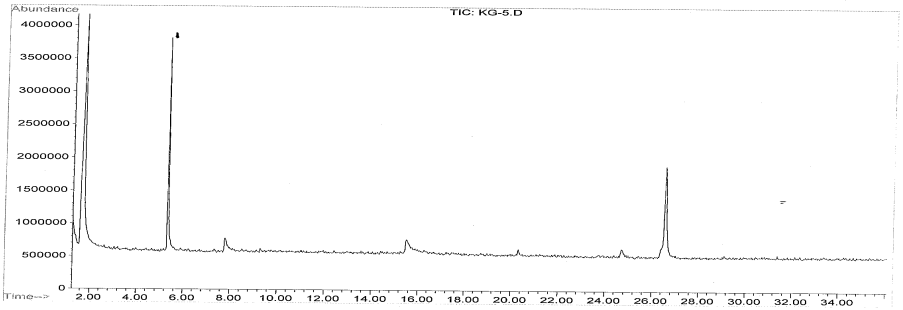
**Figure 9 GC/MS of Successful Blattellaquinone 2-day Synthesis**

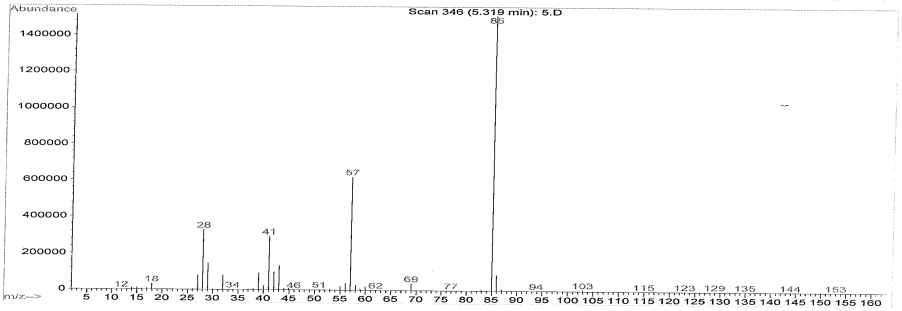
```
File        : C:\DANGELO\DATA\JOHN\KG-5.D
Operator    : dangelo
Acquired    :  8 Oct 2014   13:31      using AcqMethod JGDSPL4
Instrument  :    GC/MS Ins
Sample Name: blattellaquinone attempt 1-day
Misc Info   :
Vial Number: 1
```




```
File        : C:\DANGELO\DATA\JOHN\KG-5.D
Operator    : dangelo
Acquired    :  8 Oct 2014   13:31      using AcqMethod JGDSPL4
Instrument  :    GC/MS Ins
Sample Name: blattellaquinone attempt 1-day
Misc Info   :
Vial Number: 1
```



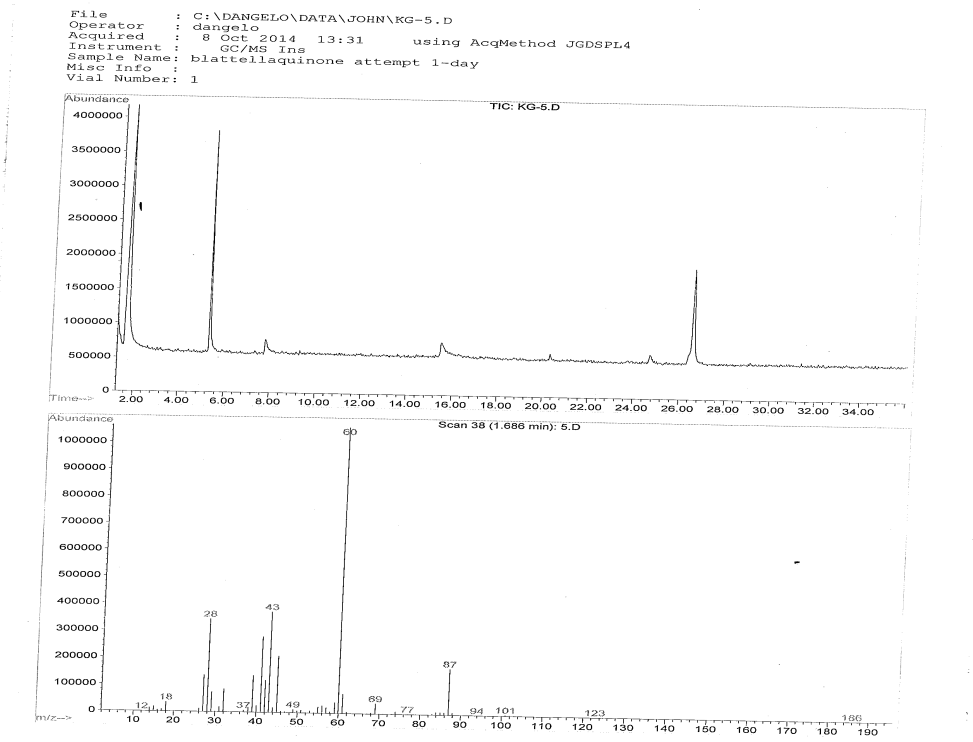
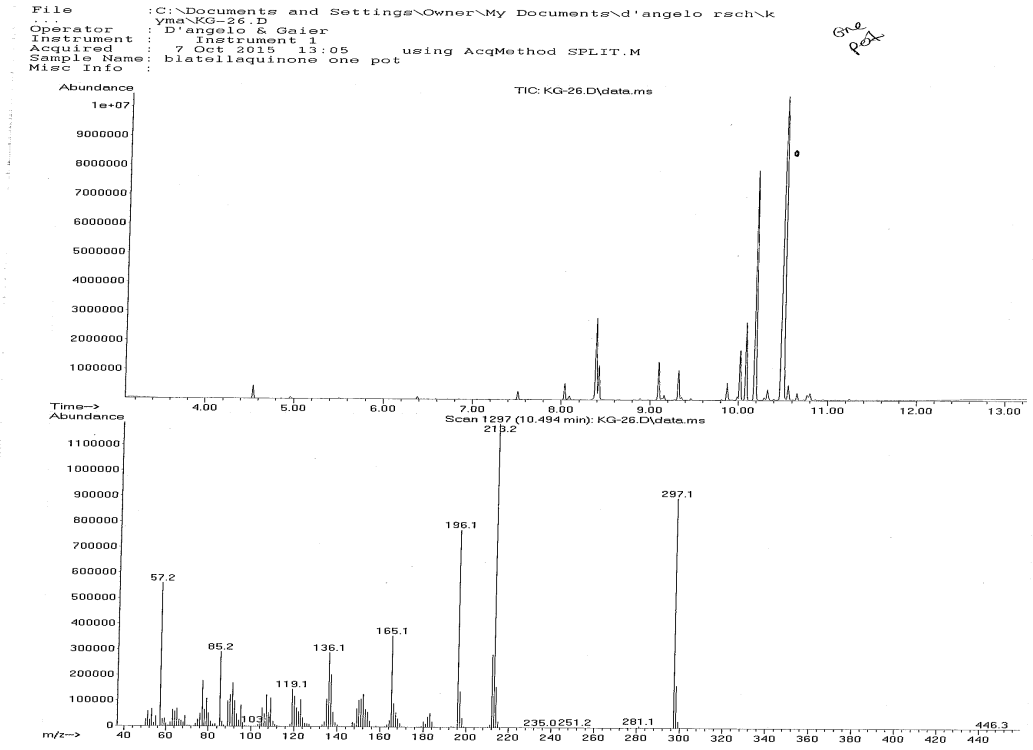
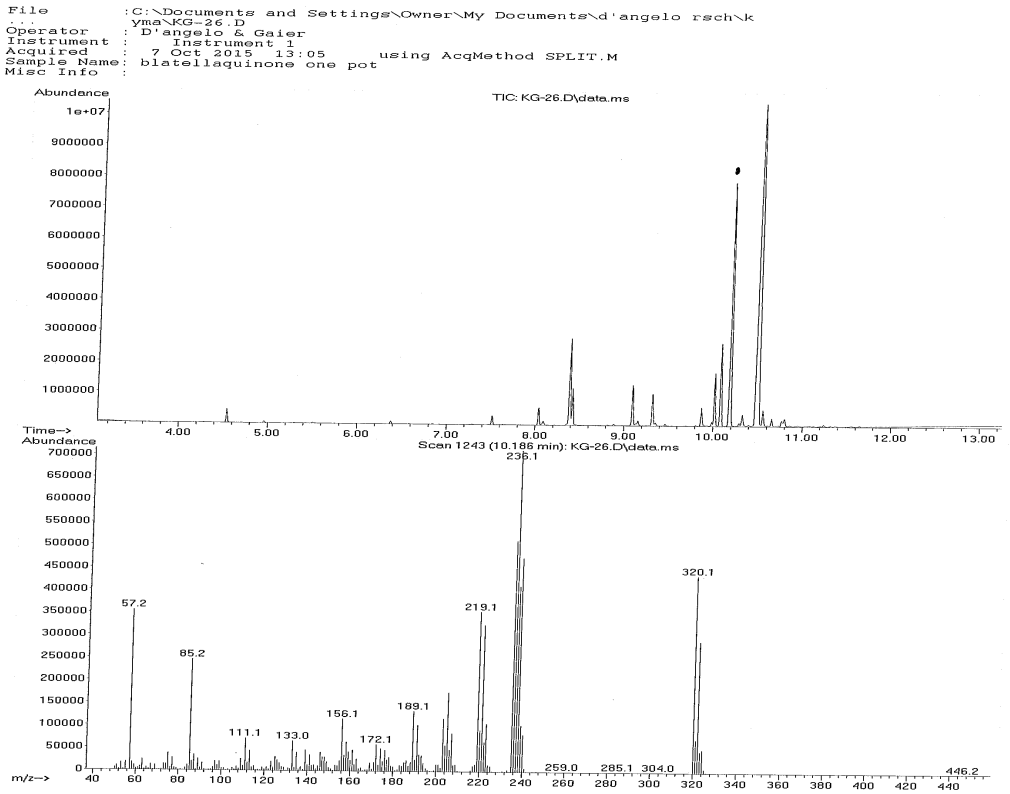
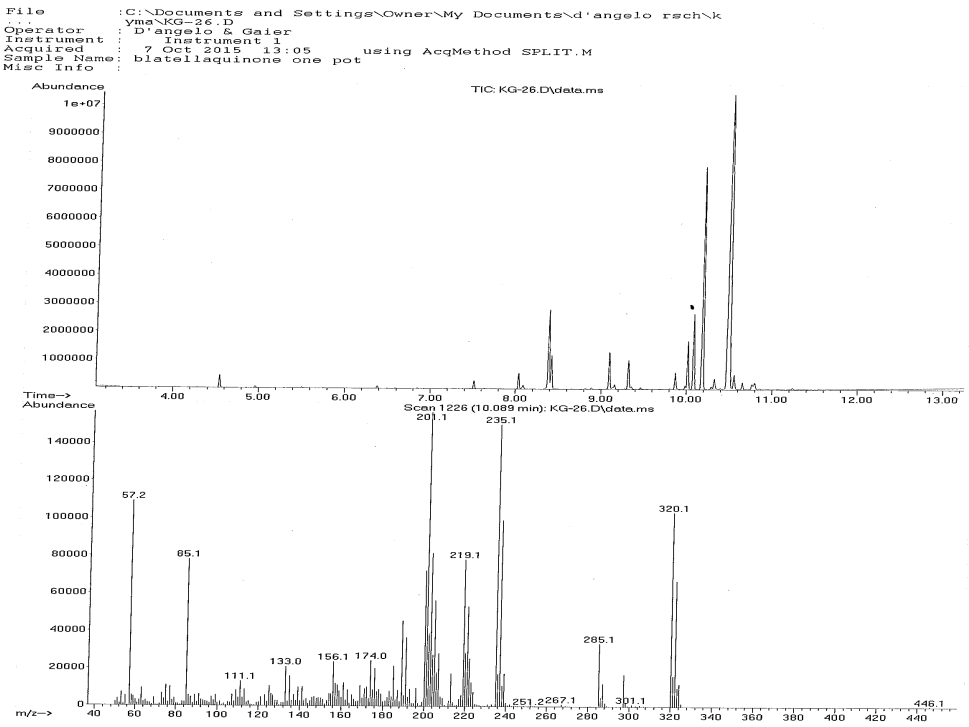


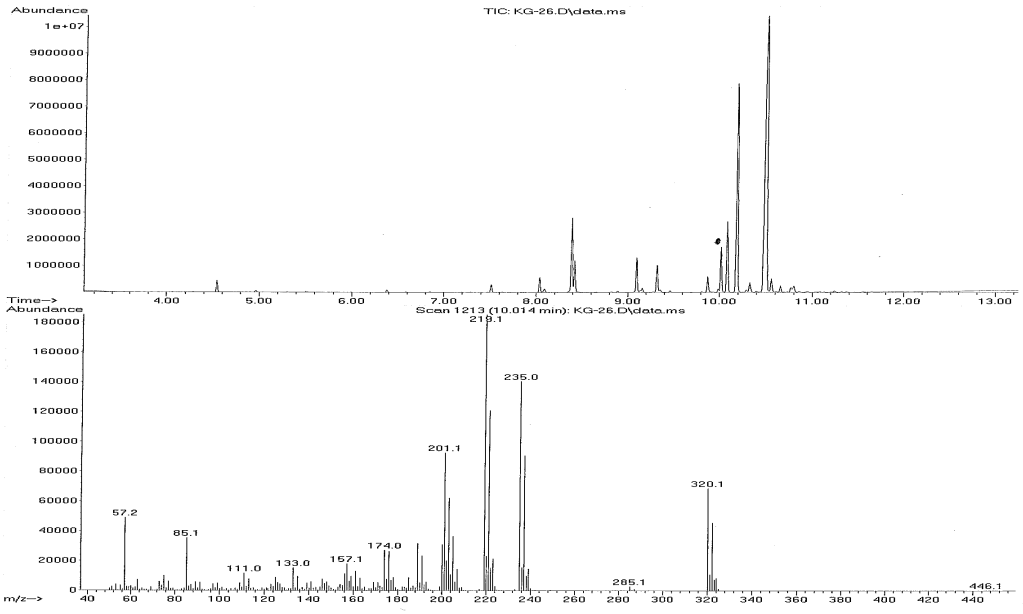

**Figure 10 Unsuccessful 1-Day Synthesis of Blatellaquinone**



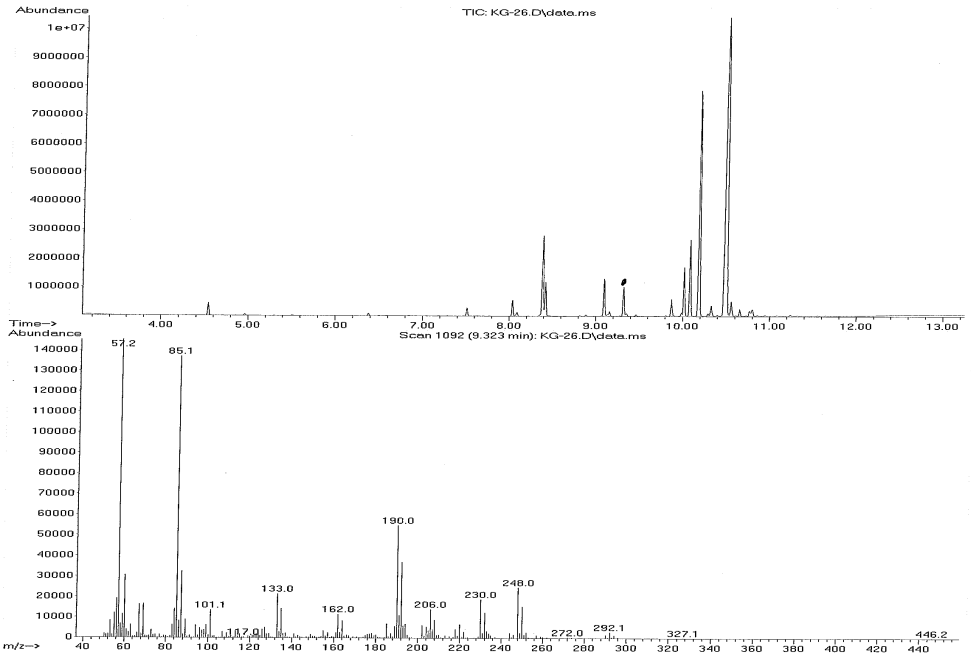

File :C:\Documents and Settings\Owner\My Documents\d'angelo rsch\kyma\KG-26.D
Operator : D'angelo & Gaier
Instrument : Instrument 1
Acquired : 7 Oct 2015 13:05 using AcqMethod SPLIT.M
Sample Name: blatellaquinone one pot
Misc Info :
TIC: KG-26.D\data.ms
Abundance
Time-->
Scan 1243 (10.186 min): KG-26.D\data.ms
m/z-->

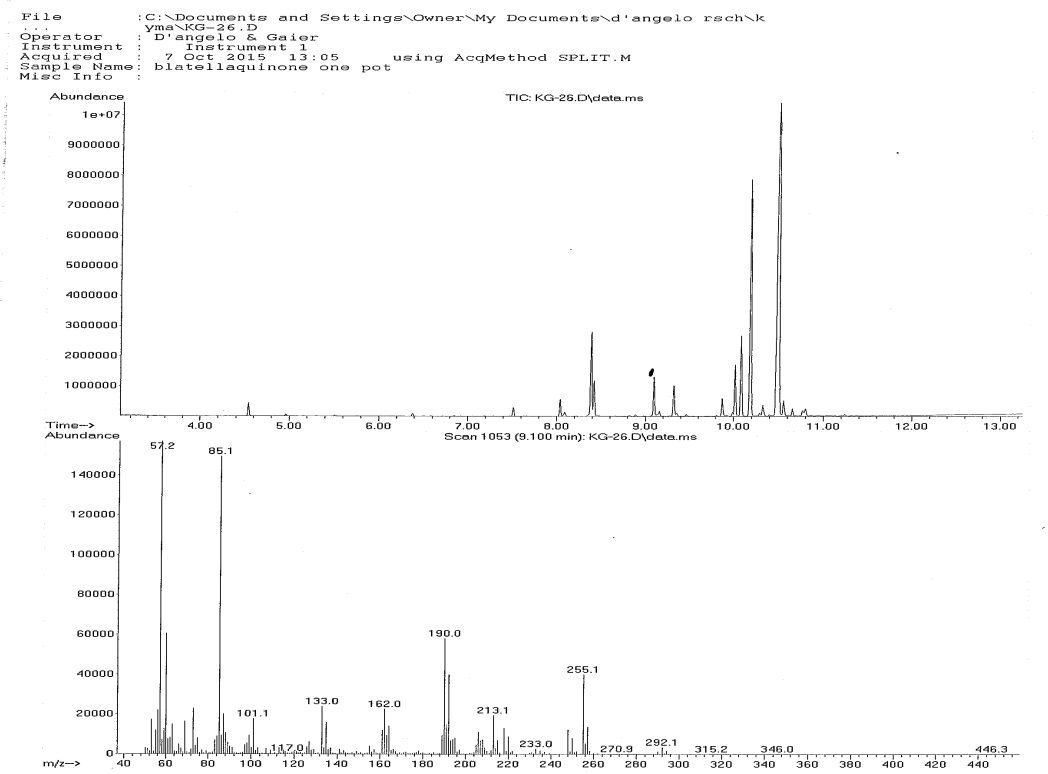

File :C:\Documents and Settings\Owner\My Documents\d'angelo rsch\kyma\KG-26.D
Operator : D'angelo & Gaier
Instrument : Instrument 1
Acquired : 7 Oct 2015 13:05 using AcqMethod SPLIT.M
Sample Name: blatellaquinone one pot
Misc Info :
TIC: KG-26.D\data.ms
Abundance
Time-->
Scan 1226 (10.089 min): KG-26.D\data.ms
m/z-->

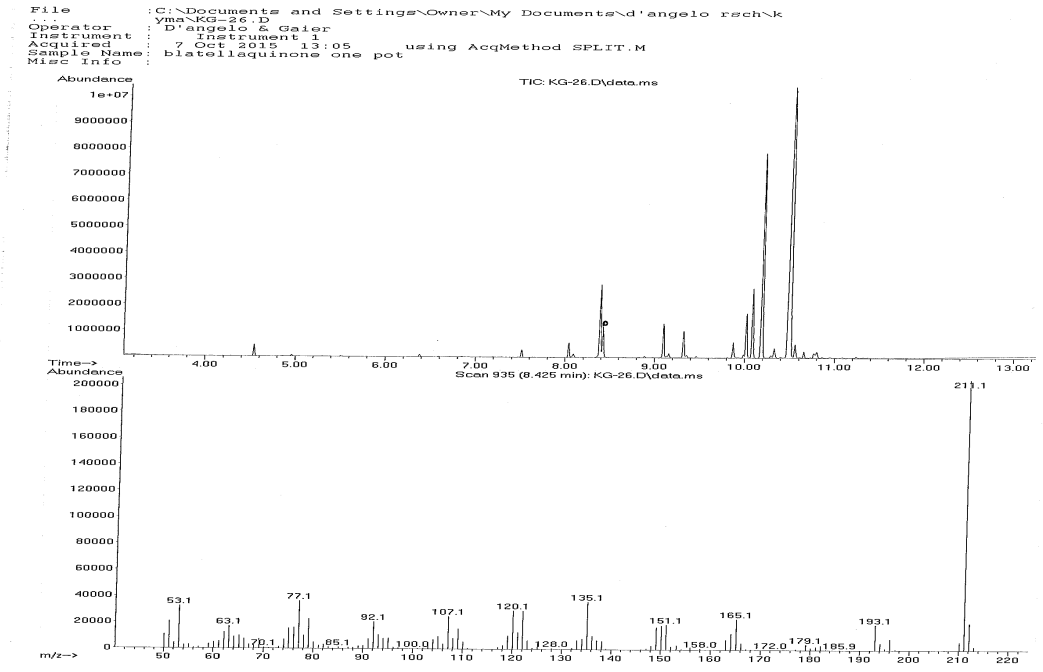
File : C:\Documents and Settings\Owner\My Documents\d'angelo rsch\k
yma\KG-26.D
Operator : D'angelo & Gaier
Instrument : Instrument 1
Acquired : 7 Oct 2015 13:05 using AcqMethod SPLIT.M
Sample Name: blatellaquinone one pot
Misc Info :

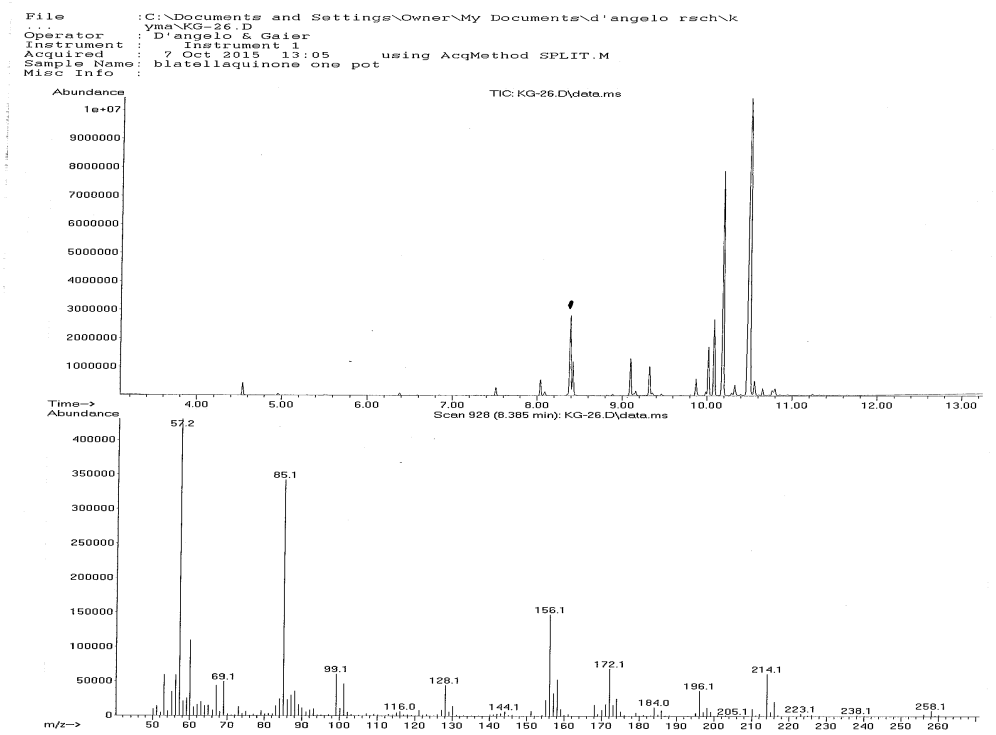



File : C:\Documents and Settings\Owner\My Documents\d'angelo rsch\k
yma\KG-26.D
Operator : D'angelo & Gaier
Instrument : Instrument 1
Acquired : 7 Oct 2015 13:05 using AcqMethod SPLIT.M
Sample Name: blatellaquinone one pot
Misc Info :




File :C:\Documents and Settings\Owner\My Documents\d'angelo rsch\k yma\KG-26.D
Operator : D'angelo & Gaier
Instrument : Instrument 1
Acquired : 7 Oct 2015 13:05 using AcqMethod SPLIT.M
Sample Name: blatellaquinone one pot
Misc Info :
TIC: KG-26.D\data.ms
Abundance
Time-->
Scan 1053 (9.100 min): KG-26.D\data.ms
m/z-->


File :C:\Documents and Settings\Owner\My Documents\d'angelo rsch\k yma\KG-26.D
Operator : D'angelo & Gaier
Instrument : Instrument 1
Acquired : 7 Oct 2015 13:05 using AcqMethod SPLIT.M
Sample Name: blatellaquinone one pot
Misc Info :
TIC: KG-26.D\data.ms
Abundance
Time-->
Scan 935 (8.425 min): KG-26.D\data.ms
m/z-->



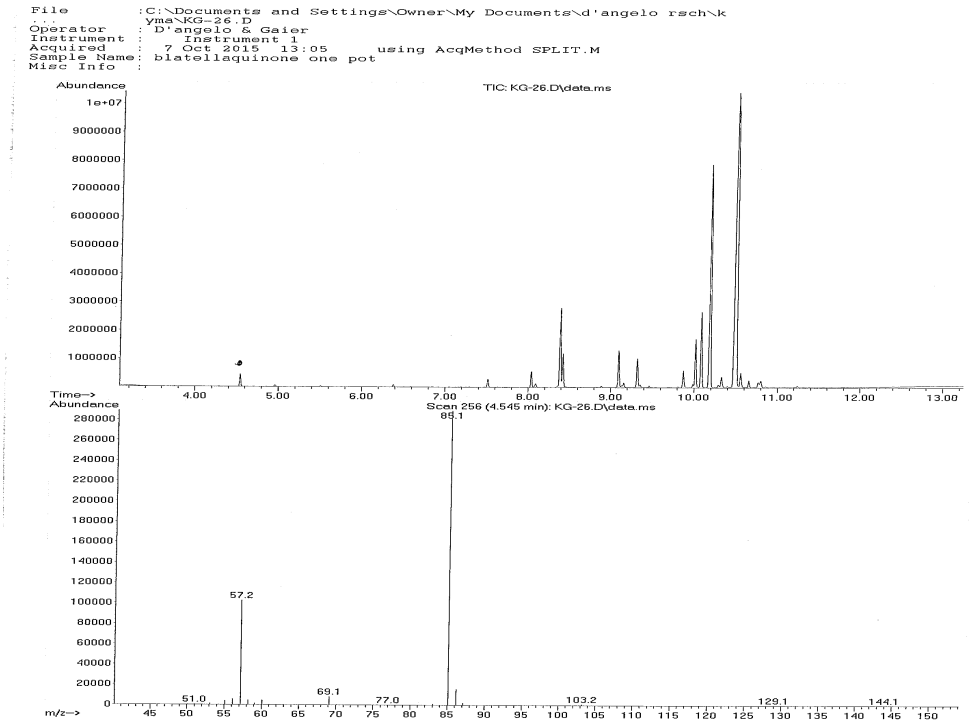
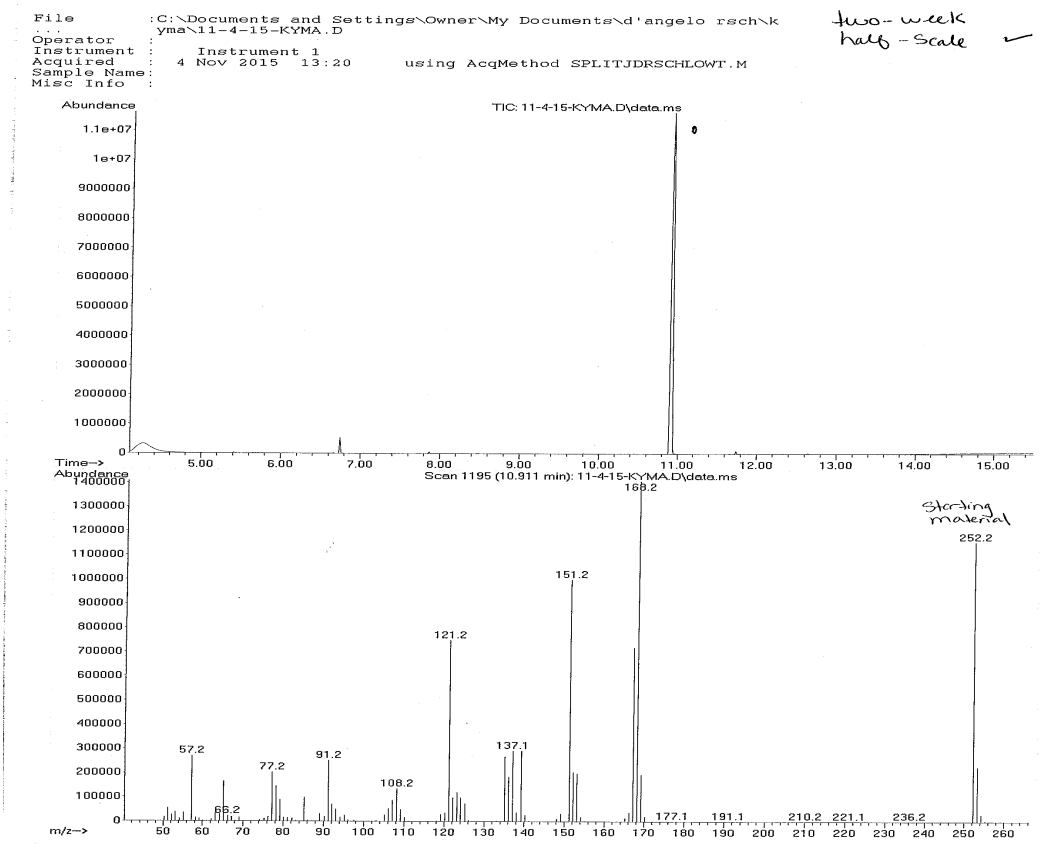




**Figure 11 One-Pot Synthesis of Blattellaquinone**


File :C:\Documents and Settings\Owner\My Documents\d'angelo rsch\kyma\11-4-15-KYMA.D
Operator :
Instrument : Instrument 1
Acquired : 4 Nov 2015 13:20 using AcqMethod SPLITJDRSCHLOWT.M
Sample Name:
Misc Info :
two-week half-scale
Abundance
TIC: 11-4-15-KYMA.D\data.ms
Time->
Abundance
Scan 1195 (10.911 min): 11-4-15-KYMA.D\data.ms
Starting material
168.2
252.2
151.2
121.2
137.1
57.2
77.2
91.2
108.2
65.2
177.1
191.1
210.2
221.1
236.2
m/z->
File :C:\Documents and Settings\Owner\My Documents\d'angelo rsch\kyma\11-4-15-KYMA.D
Operator :
Instrument : Instrument 1
Acquired : 4 Nov 2015 13:20 using AcqMethod SPLITJDRSCHLOWT.M
Sample Name:
Misc Info :
Abundance
TIC: 11-4-15-KYMA.D\data.ms
Time->
Abundance
Scan 466 (6.745 min): 11-4-15-KYMA.D\data.ms
85.1
57.2
50.1
69.1
103.1
129.1
206.9
m/z->

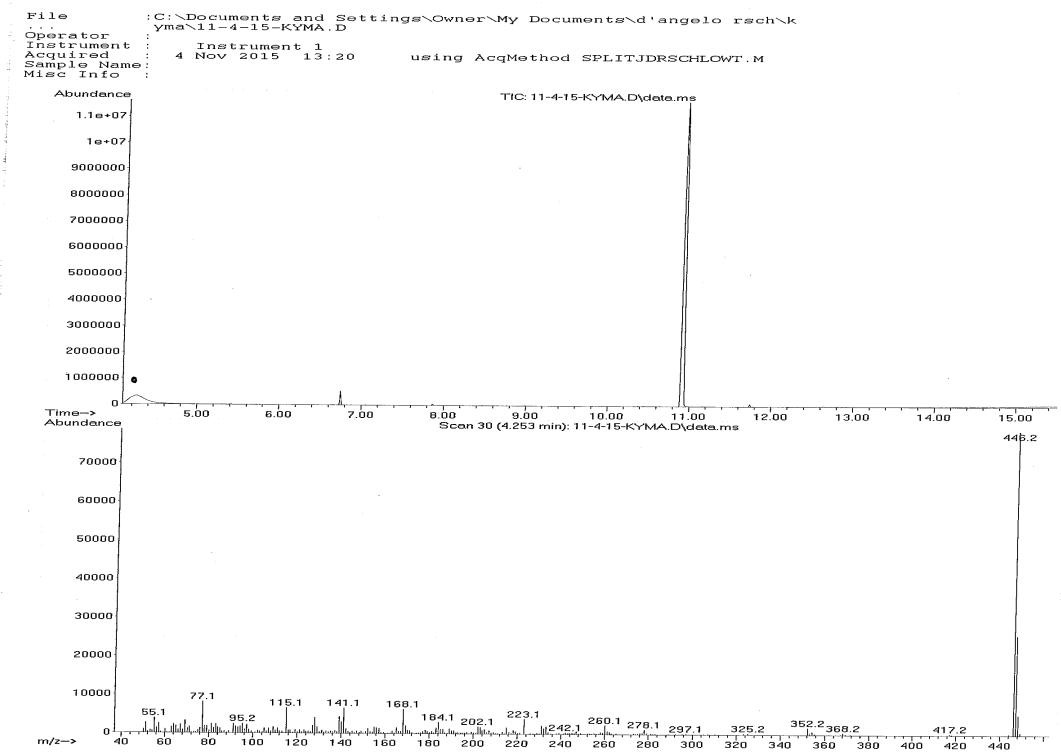



**Figure 12 Two-Week Half-Scale Synthesis of Blattellaquinone**