SYNTHETIC EFFORTS TOWARDS THE LAGUNAMIDE AND CHALCONE BASED NATURAL PRODUCTS.

by

DOUGLAS ALEXANDER FARLEIGH

Chemistry Major, Biology Minor

A FINAL HONORS PROJECT

submitted in accordance with the University Honors Program requirements

Department of Chemistry
College of Arts and Sciences

KANSAS STATE UNIVERSITY
Manhattan, Kansas

Graduation: May 2021

Approved by:

Dr. Ryan J. Rafferty

I. ABSTRACT

The Lagunamides are a series of secondary metabolites produced by marine cyanobacteria that possess almost identical polypeptide and polyketide moieties and display potency against various cancer cell lines. They are differentiated by the side chain on the polyketide moiety. Chalcones are a class of molecules characterized by an α,β-unsaturated ketone moiety and adjacent phenyl groups; some species that fall under this classification have displayed a similar efficacy when tested against various cancer cell lines. Synthetic procedures on a 100 mg scale and their associated mechanisms regarding the construction of Crimmins' auxiliaries (yield = 72%), diazotization of D-alloisoleucine (yield = 40%/35% for 300 mg scale), and the esterification (yield = 72%), Boc deprotection (yield = 84%), and first peptide coupling (yield = N/A) in the formation of the polyketide moiety of Lagunamides are discussed. Furthermore, the synthetic procedure and mechanism for the acylation (yield = 24%) and subsequent methylation (yield = N/A) of phloroglucinol are provided. For the unsuccessful peptide coupling reaction, the most likely point of failure was the lack of carboxylic acid activation by EDC. For the unsuccessful acylation of phloroglucinol, consideration of the kinetics of aromatic substitutions and the NMR spectrum of the product indicated that diacylation had occurred (the desired product was monoacylated).

II. INTRODUCTION

Herein this senior thesis is the efforts towards to family of natural products: the lagunamides and the chalcones. To be discussed are their isolation, cytotoxicities, and current and completed synthetic efforts to gain access to these natural products.

Lagunamides: The cytotoxic depsipeptides lagunamide A (LagA) and lagunamide B (LagB) were first isolated by Tripathi et al. in 2010 (Figure 1). The objective was to identify new compounds with antimicrobial, antimalarial, cytotoxic, and neurotoxic properties of secondary metabolites produced by marine cytobacterial strains of the *Lyngbya* genus.[1] LagA and LagB possess identical polypeptide moieties (highlighted in green) while differing in their polyketide moieties (highlighted in black). Initial interest in the cytotoxicity of LagA and LagB was taken to the compounds' structural similarities to a series of potent cytotoxic marine cyanobacterial compounds, such as the aurilides (the classification under which LagA and LagB fall), kulokehalide-2, and pulau'amide.[1, 2] LagA and LagB displayed potent cytotoxicity against the P388 cancer cell line with IC_{50} values of 6.4 nM and 20.5 nM, respectively.

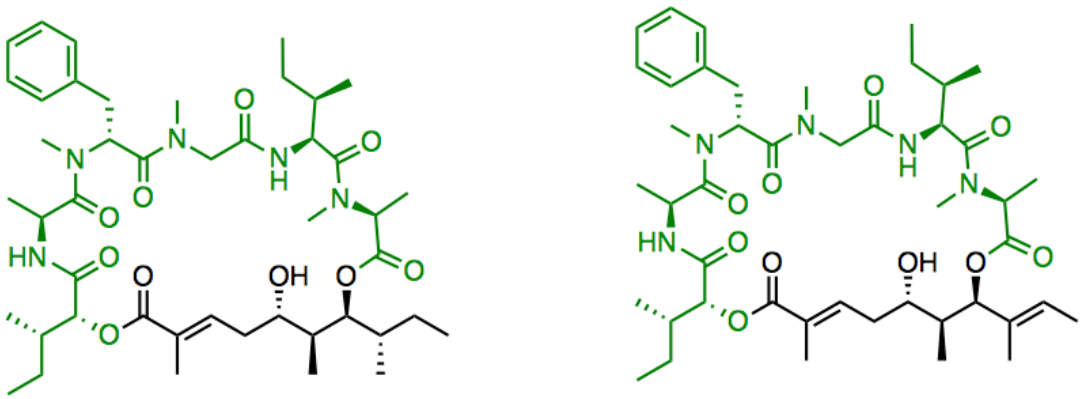


Figure 1. Structures of LagA (left) and LagB (right).

The cytotoxic depsipeptide lagunamide C (LagC, Figure 2) was isolated in 2011 by Tripathi et al. from the marine cyanobacterium *Lyngbya majuscula*. In a manner similar to LagA

and LagB, LagC differs primarily in its polyketide moiety. LagC is related to the aurilide class of molecules, and its distinguishing feature is the presence of an additional methylene carbon in the polyketide moiety.[3] This results in the macrocyclic structure consisting of a 27-membered ring instead of a 26-membered ring system.[3] In addition to the enlarged macrocyclic ring, two stereocenters within the polyketide moiety. LagC exhibited potent cytotoxicity against a range of cancer cell lines, such as the p388, A549, PC3, HCT8, and SK-OV3 lines, with IC_{50} values ranging from 2.1 nM to 24.4 nM.[3] Furthermore, LagC displayed substantial anti-malarial activity when tested against *Plasmodium falciparum* with an IC_{50} value of 0.29 μM and weak anti-swarming activity against the Gram-negative *Pseudomonas aeruginosa* when tested at 100 ppm.[3] The presence of the extra methylene carbon, potent biological activity, and ambiguity of the two stereocenters in the polyketide moiety drew the attention of the Rafferty lab group to the total synthesis of LagC as its proposed structure.[2]

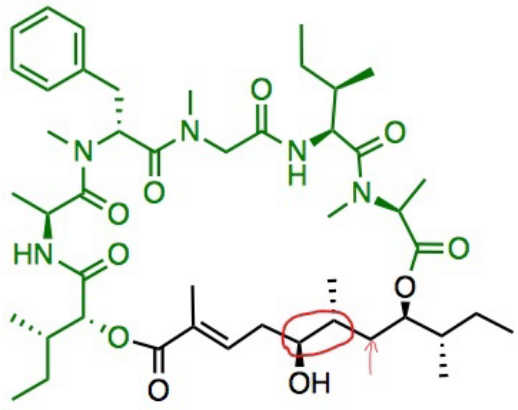


Figure 2. Structure of LagC. The two stereocenters are circled and the extra methylene carbon is marked by an arrow, both in red.

Lagunamide D (LagD) was isolated in 2018 by Luo et al. from a collection of marine cyanobacteria that included *Lyngbya majuscula*. Although the molecule has only been known for approximately two years, it was discovered to possess antiproliferative properties against A549 human lung adenocarcinoma cells in concentrations as low as 7.1 nM.[4] Lagunamide E differs

from Lagunamide D in the presence of an additional methyl group (circled in red); isolation and retrosynthetic efforts are currently being conducted by the Rafferty Laboratory.

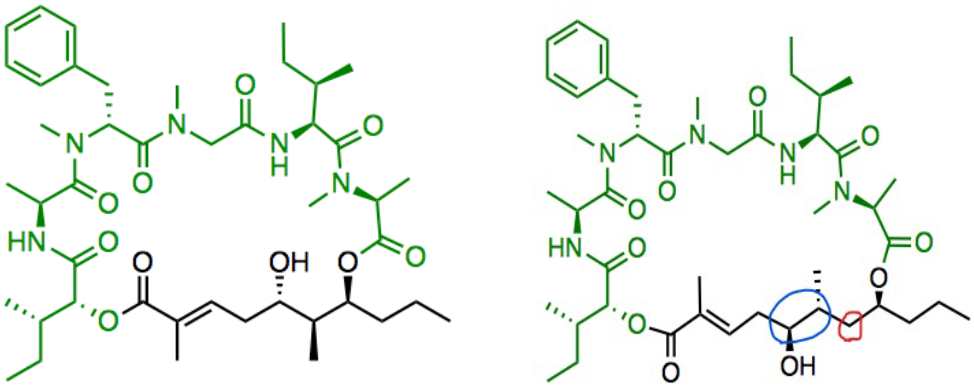


Figure 3. Structures of LagD (left) and LagE (right). The structural difference is circled in red on LagE. Note that LagE possesses the same two ambiguous stereocenters as LagE, circled in blue.

The Chalcones: The natural product families of the chalcones, defined by their α,β-unsaturated ketone moiety and adjacent phenyl groups, are a central component to numerous natural products.[5] They are classified as flavonoids and possess a wide range of applications as pharmacophores. The aforementioned α,β-unsaturated ketone moiety is involved in the cytotoxicity and apoptotic activity as therapeutic agents in cancer treatments.[2] They have been observed to possess a considerable range of biological properties, such as anticancer, anti-malarial, antileishmanial, antituberculotic, antimitotic, antibacterial, and antifungal properties. It has been shown that many chalcone-containing natural products and synthetic compounds achieve biological effect via addition of an enone group. It was found that the *o*-hydroxybenzyl group present in the uvaretin family of chalcone-containing natural products possessed cytotoxic properties. Additionally, chalcone hybrids possessing both a cytotoxic enone group and a small molecule potentiating reduced enone group have been developed.[5]

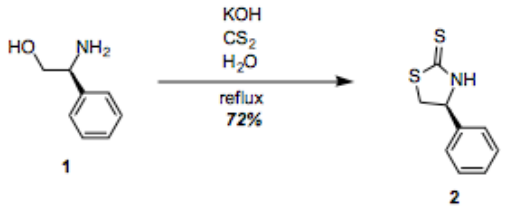
Herein, efforts toward the total synthesis of LagC and a member of the uvaretin family of chalcone-based natural products are discussed.

III. EXPERIMENTAL

All NMR spectra were taken with a 400 MHz Bruker NMR spectrometer and analyzed with MestReNova. All reactions were carried out with 100 mg of the starting material unless otherwise stated. The sources of compounds were stated in parentheses after their names. Each reaction was carried out multiple times, and the best non-erroneous yields were reported in the scheme for each reaction sequence.

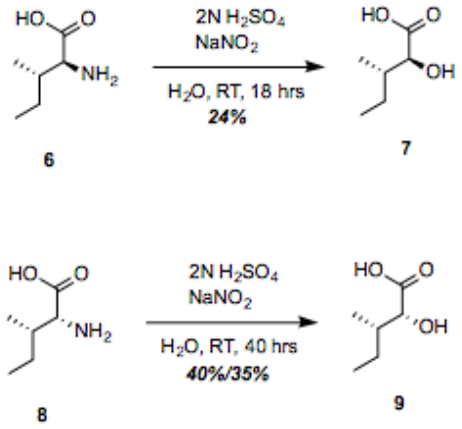
In collaboration with Shashika Perera, a 3rd year graduate student, the following efforts towards the key starting materials of the lagunamide project are delineated below.

Synthesis of the Crimmin's Auxillary



1.32 mL of water were added to a round-bottom flask containing 0.223 g of KOH (VWR) and stirred until all of the solid had dissolved. The solution was cooled to room temperature, and (**1**) (AK Scientific) was added, followed by 0.200 mL of CS_2 (Fisher). The system was purged with inert gas, and the reaction mixture was refluxed at 103 °C for two hours (the inert stream was maintained during reflux). Once refluxed, the reaction mixture was extracted with DCM, dried over anhydrous Na_2SO_4, and subjected to rotary evaporation. The presence of the product (**2**) was confirmed by TLC and, later, ^{1}H NMR.

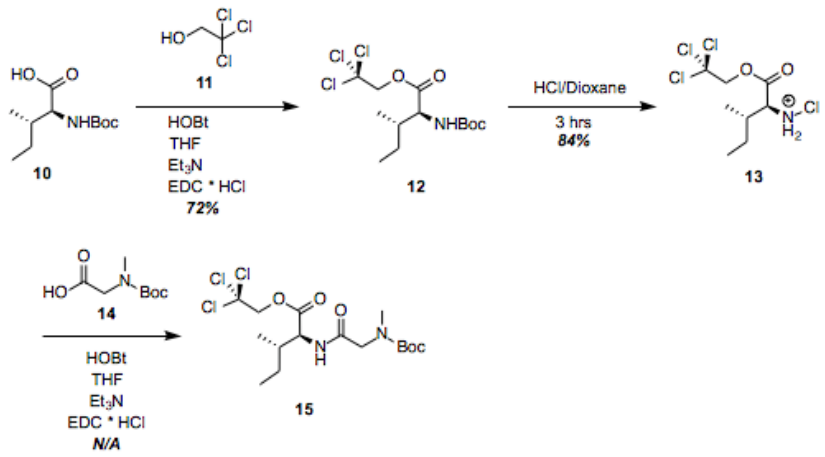
Diazotization of Isoleucine



1.0 M H_2SO_4 and 3.0 M $NaNO_2$ were prepared prior to the beginning of the procedure. It was imperative that the $NaNO_2$ solution was utilized immediately after preparation. For the model system, (**6**) (AK Scientific) was added to a round-bottom flask and dissolved in 2.286 mL of 1.0 M H_2SO_4. Once the solid was completely dissolved, the flask was cooled to 0 ℃ and 2.54 mL of 3.0 M $NaNO_2$ were added dropwise over a period of three minutes. The ice bath was removed and the solution was stirred for 40 hours. Once the reaction was complete, the solution was saturated with NaCl and extracted with EtOAc. The combined organic layers were washed twice by distilled water, then twice more by a brine solution before being dried over anhydrous Na_2SO_4 and subjected to rotary evaporation. The presence of the product (**8**) was confirmed by TLC and, later, ^{1}H NMR.

For the actual system, a scale of 300 mg can be utilized to facilitate identification by TLC and NMR data. (**7**) was provided by Combi Blocks, and the volumes of 1.0 H_2SO_4 and 3.0 M $NaNO_2$ to be used are 6.86 mL and 7.63 mL, respectively. Otherwise, the reaction procedure and workup to obtain (**9**) can be carried out in the same fashion as for the model system.

Synthesis of the Dipeptide Subunit of the Pentapeptide

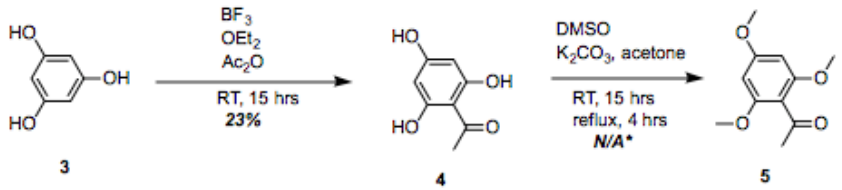


(**10**) (AK Scientific) and 0.070 g of HOBt hydrate (AK Scientific) were added to a flame-dried round-bottom flask. Under inert gas, 4.32 mL of dry THF (such that the molarity of (**10**) was 0.1 M) was added, and once the solid was fully dissolved, 0.041 mL of (**11**) (Sigma-Aldrich) was added. The system was cooled to 0 °C and stirred for fifteen minutes. Afterward, 0.211 mL of Et_3N (Oakwood Chemicals) were added under inert gas, and the system was stirred for fifteen minutes. 0.0870 g of EDC · HCl (AK Scientific) was added under inert gas after fifteen minutes had passed, and the reaction was allowed to run overnight (if preferred, the workup may be carried out immediately). TLC was performed at this point to determine the presence of a product. The reaction mixture was neutralized with two equivalents by volume of distilled water and extracted with DCM (minimum of four extractions). The combined organic layers were washed with $NaHCO_3$ and brine (separate solutions), dried over anhydrous Na_2SO_4, and subjected to rotary evaporation. Utilization of column chromatography was required to purify the product (**12**).

To the container of (**12**), a sufficient volume of a 4.0 M solution of HCl in dioxane (Oakwood Chemicals) was added so that the concentration of (**12**) in the solution was 0.25 M.

The reaction was stirred for 3 hours at room temperature, and the presence of (**13**) was confirmed by TLC and ^{1}H NMR.

1 molar equivalent of (**14**) and 1.05 molar equivalents of HOBt hydrate were added to the vial containing (**13**) and placed under vacuum. A sufficient volume of dry THF was added to the vial under inert gas such that the molarity of (**13**) was 0.1 M. Once all of the solid dissolved, the reaction system was cooled to 0 °C and allowed to stir for fifteen minutes. After the allotted time had passed, 3.5 molar equivalents of Et_3N were added under inert gas and the reaction mixture was allowed to stir for fifteen minutes. Afterward, 1.05 molar equivalents of EDC · HCl were added under inert gas and the reaction was allowed to run overnight (if preferred, the workup may be carried out immediately). TLC was performed at this point to determine the presence of a product. The reaction mixture was neutralized with two equivalents by volume of distilled water and extracted with DCM (minimum of four extractions). The combined organic layers were washed with $NaHCO_3$ and brine (separate solutions), dried over anhydrous Na_2SO_4, and subjected to rotary evaporation. Utilization of column chromatography was required to purify the product (**15**).Synthetic efforts towards the numerous chalcone based natural products commenced with the acetylation of phloroglucinol, followed by the trimethylation of the phenolic groups. Below is a delineation of the synthetic efforts.



Two round-bottom flasks were flame-dried and placed under vacuum until they cooled to room temperature. Phloroglucinol (**3**) (Sigma-Aldrich) was added to one of the flasks and placed under vacuum for ten minutes. In the other round-bottom flask, 0.294 mL of $BF_3 \cdot OEt_2$ (Sigma-

Aldrich) and 0.075 mL of Ac_2O (Sigma-Aldrich) was added and allowed to stir under inert gas for thirty minutes. The solution in the latter flask was transferred dropwise to the flask containing phloroglucinol over a period of thirty minutes. The resulting mixture was stirred under a stream of inert gas for 15 hours. Afterward, 2.8 mL of 10% NaOAc were added to the flask, and the reaction mixture was stirred overnight (the inert gas was not needed). Once complete, the solid precipitate was isolated via vacuum filtration and allowed to air-dry. A third round-bottom flask was flame dried and allowed to cool to room temperature, and 40.0 mg of (**4**) were added. The system was placed under vacuum for thirty minutes. 0.80 mL of acetone were dried by molecular sieves and added in conjunction with 0.226 mL of DMSO (Sigma-Aldrich) to the flask, followed by 164.4 mg of K_2CO_3 (VWR). The reaction mixture was stirred at room temperature under inert gas for 15 hours. After the allotted time had passed, the mixture was refluxed for 4 hours. The resulting mixture was quenched with saturated NH_4Cl until the pH was approximately 7.0, then extracted via liquid-liquid extraction with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and subjected to rotary evaporation. The presence of the product (**5**) was confirmed by TLC and, later, ^1H NMR.

IV. RESULTS AND DISCUSSION

The NMR spectra for each reaction outlined in the Experimental section are shown below.

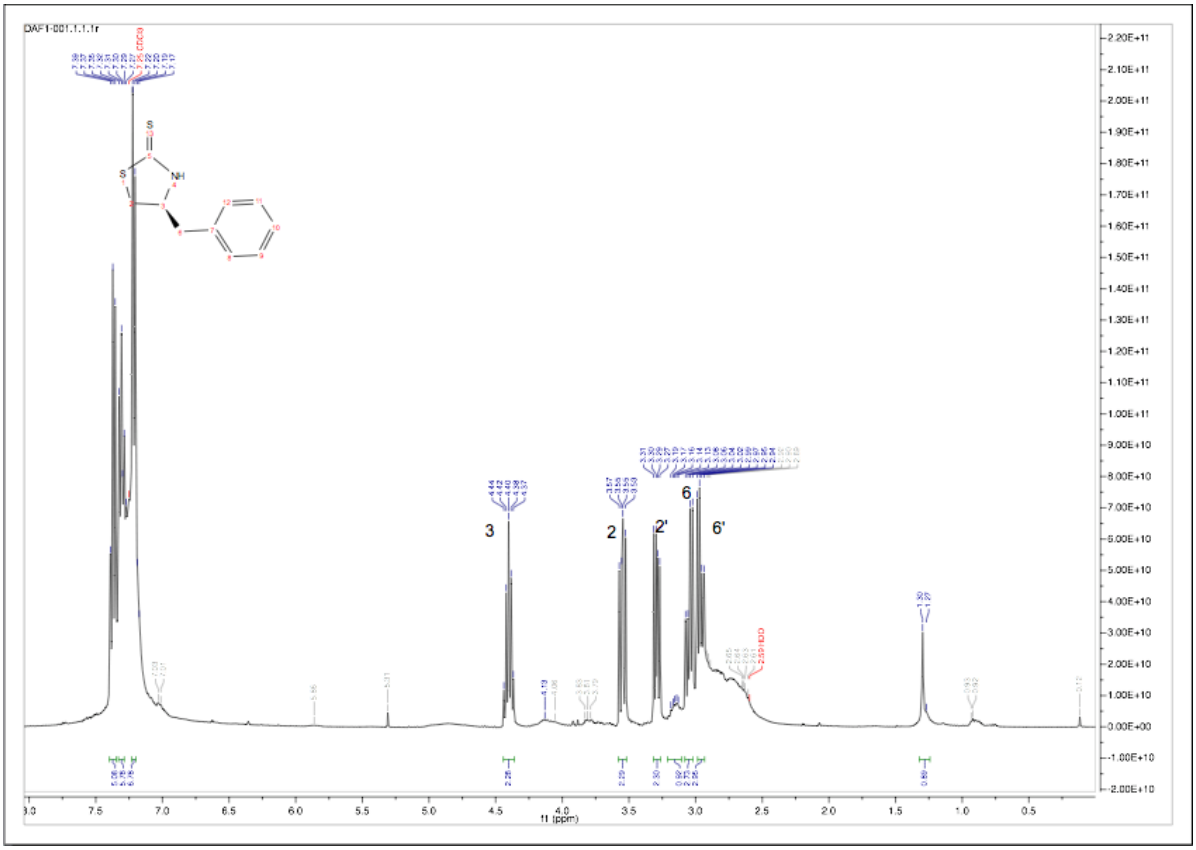


Figure 4. NMR Spectrum of the Crimmins Auxiliary Reaction product. Coupling Constants: 7.37 (t, J = 7.3 Hz, 2H), 7.30 (dd, J = 9.2, 6.0 Hz, 3H), 7.21 (d, J = 7.5 Hz, 3H), 4.40 (p, J = 7.2 Hz, 1H), 3.55 (dd, J = 11.2, 7.7 Hz, 1H), 3.29 (dd, J = 11.2, 6.6 Hz, 1H), 3.05 (dd, J = 13.6, 7.5 Hz, 1H), 2.99 ? 2.93 (m, 1H).

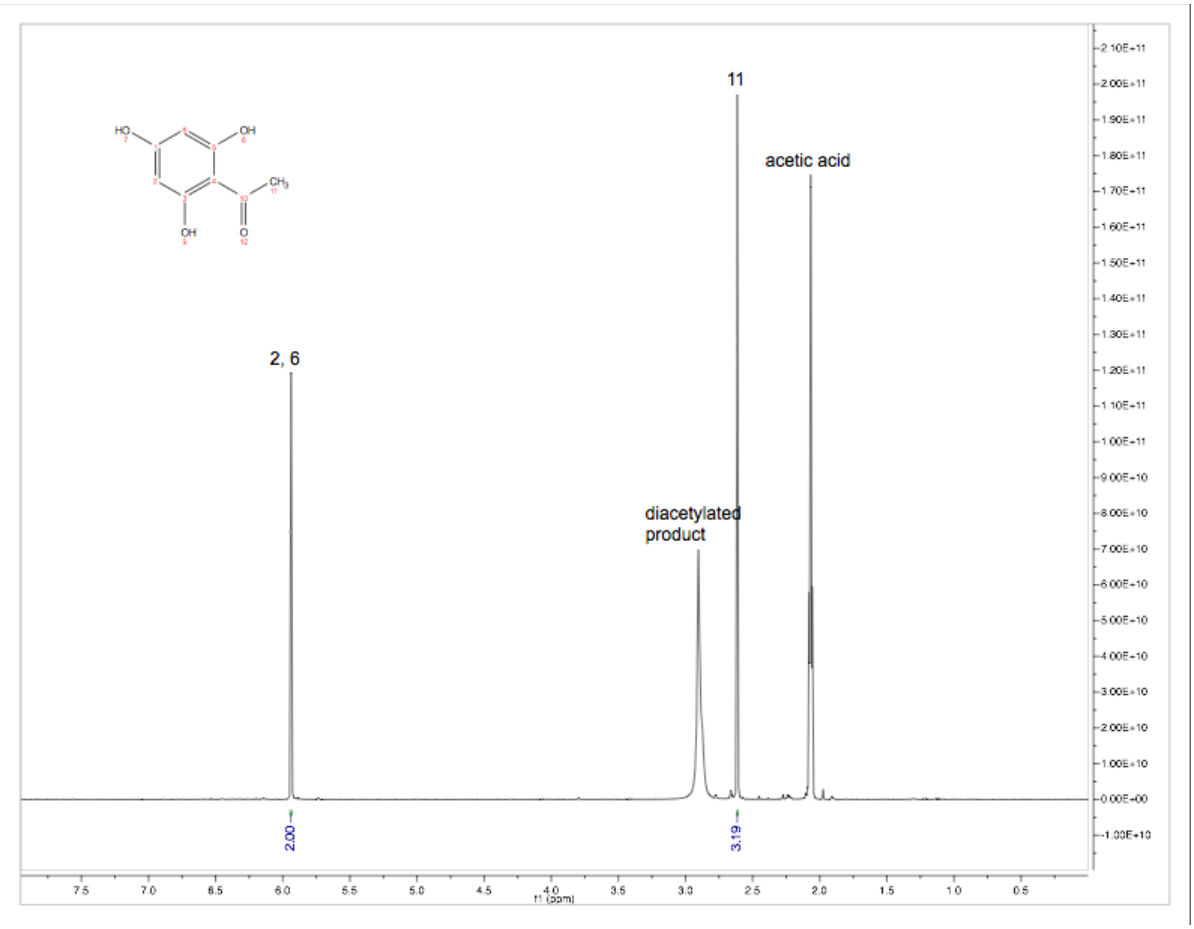


Figure 5. NMR spectrum of monoacetylated phloroglucinol. Coupling Constants: 5.94 (s, 2H), 2.61 (s, 3H).

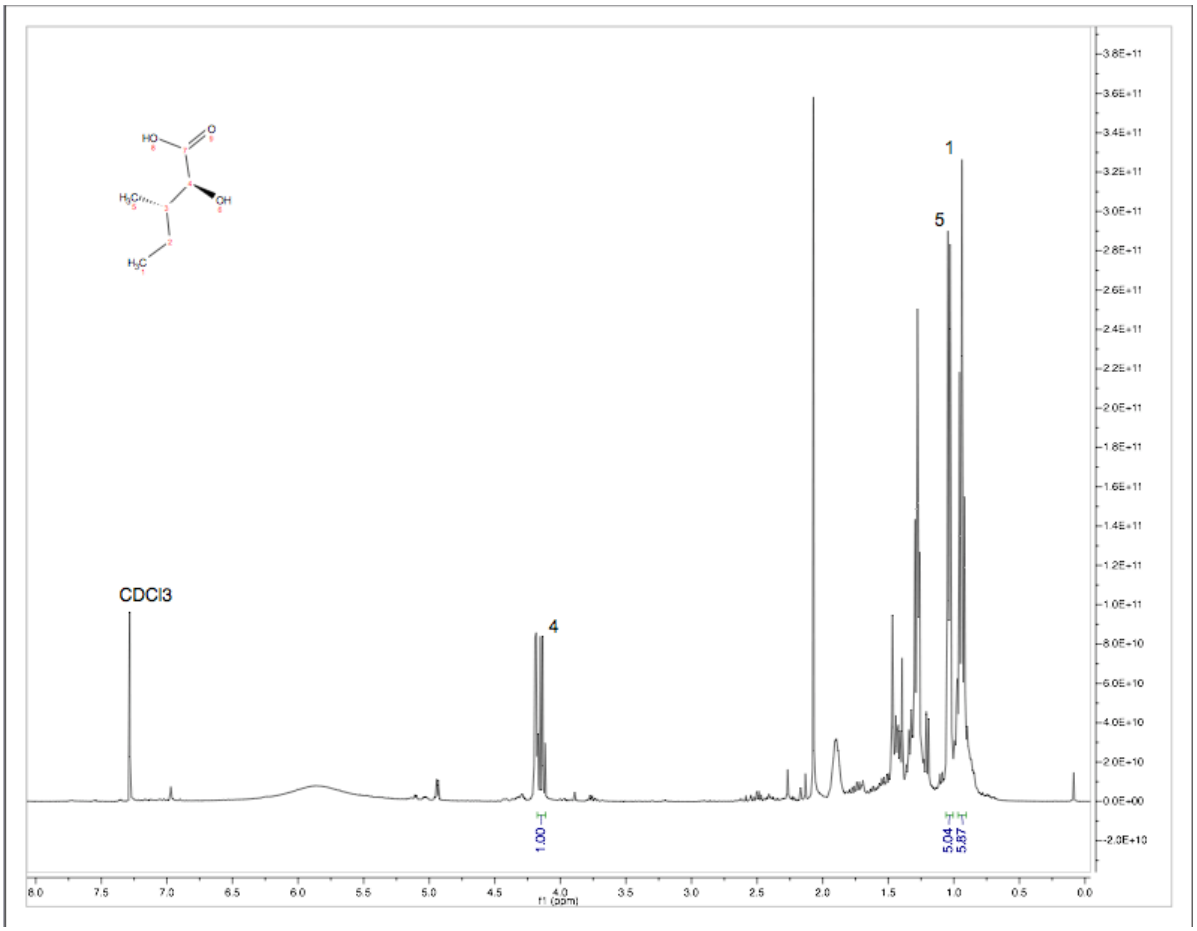


Figure 6. NMR spectrum of model Diazotization product. Coupling Constants: 4.14 (q, J = 7.1 Hz, 1H), 1.04 (d, J = 6.9 Hz, 5H), 0.94 (t, J = 7.5 Hz, 6H).

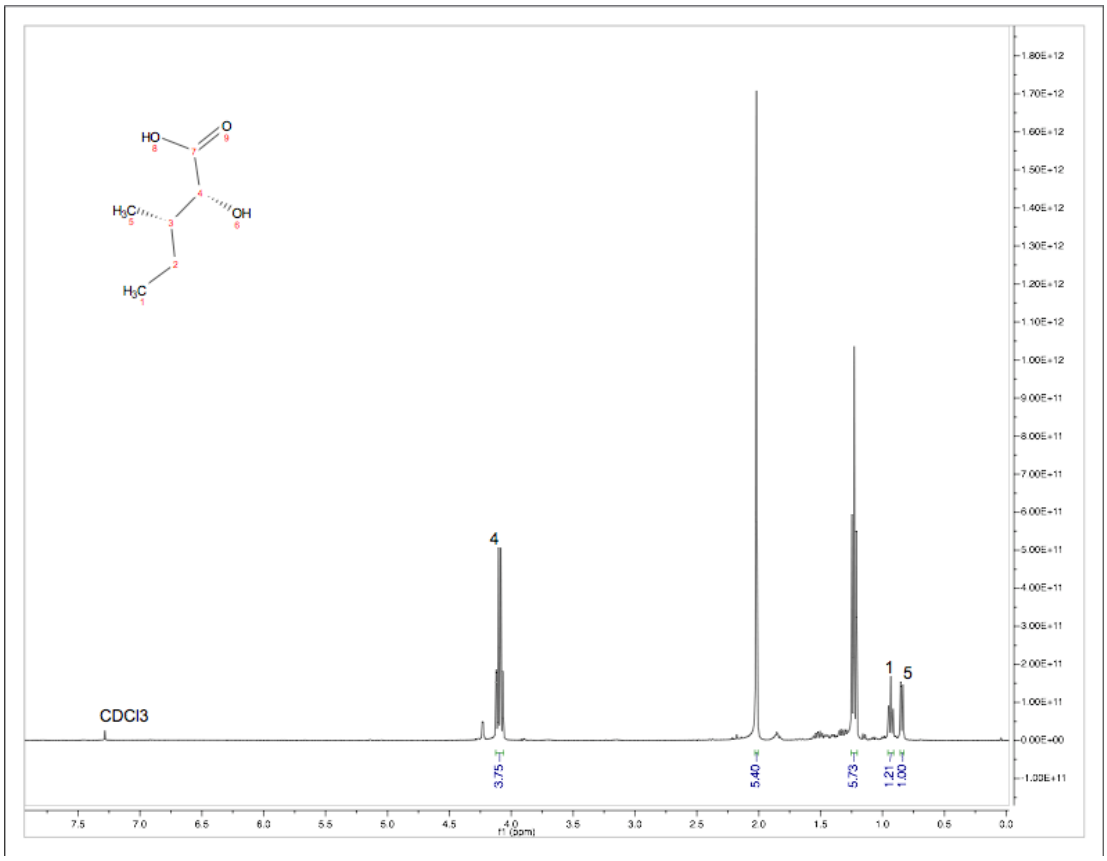


Figure 7. NMR spectrum of Diazotization product carried out on the real system. Coupling Constants: 4.09 (q, J = 7.1 Hz, 4H), 2.02 (s, 5H), 1.23 (t, J = 7.2 Hz, 6H), 0.93 (t, J = 7.3 Hz, 1H), 0.84 (d, J = 6.9 Hz, 1H).

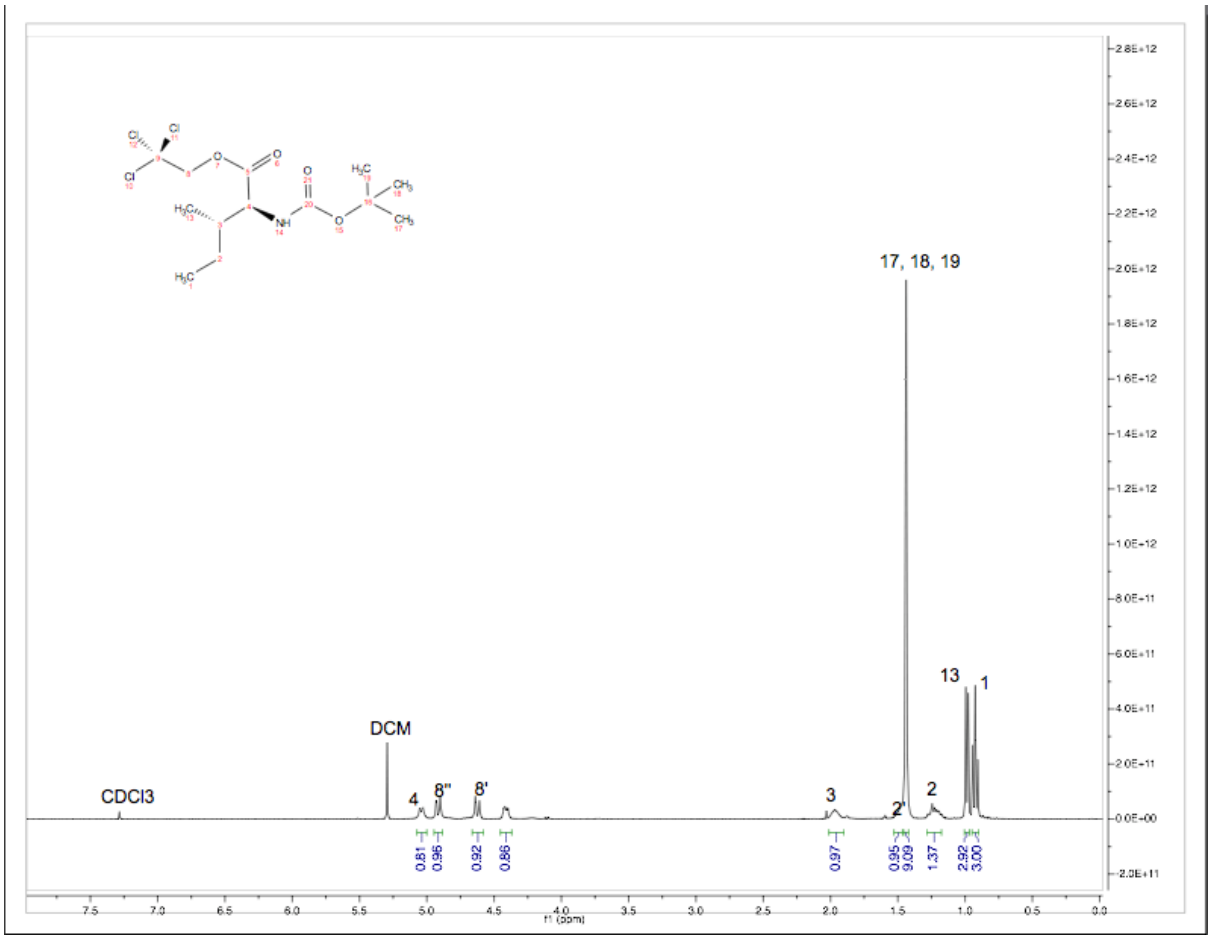


Figure 8. NMR spectrum of Fischer Esterification product for use in peptide coupling. Coupling Constants: 5.04 (d, J = 9.1 Hz, 1H), 4.92 (d, J = 12.0 Hz, 1H), 4.62 (d, J = 12.0 Hz, 1H), 4.41 (dd, J = 9.1, 4.5 Hz, 1H), 2.01 ? 1.90 (m, 1H), 1.53 ? 1.46 (m, 1H), 1.44 (s, 9H), 1.28 ? 1.18 (m, 1H), 0.99 (d, J = 6.9 Hz, 3H), 0.92 (t, J = 7.4 Hz, 3H).

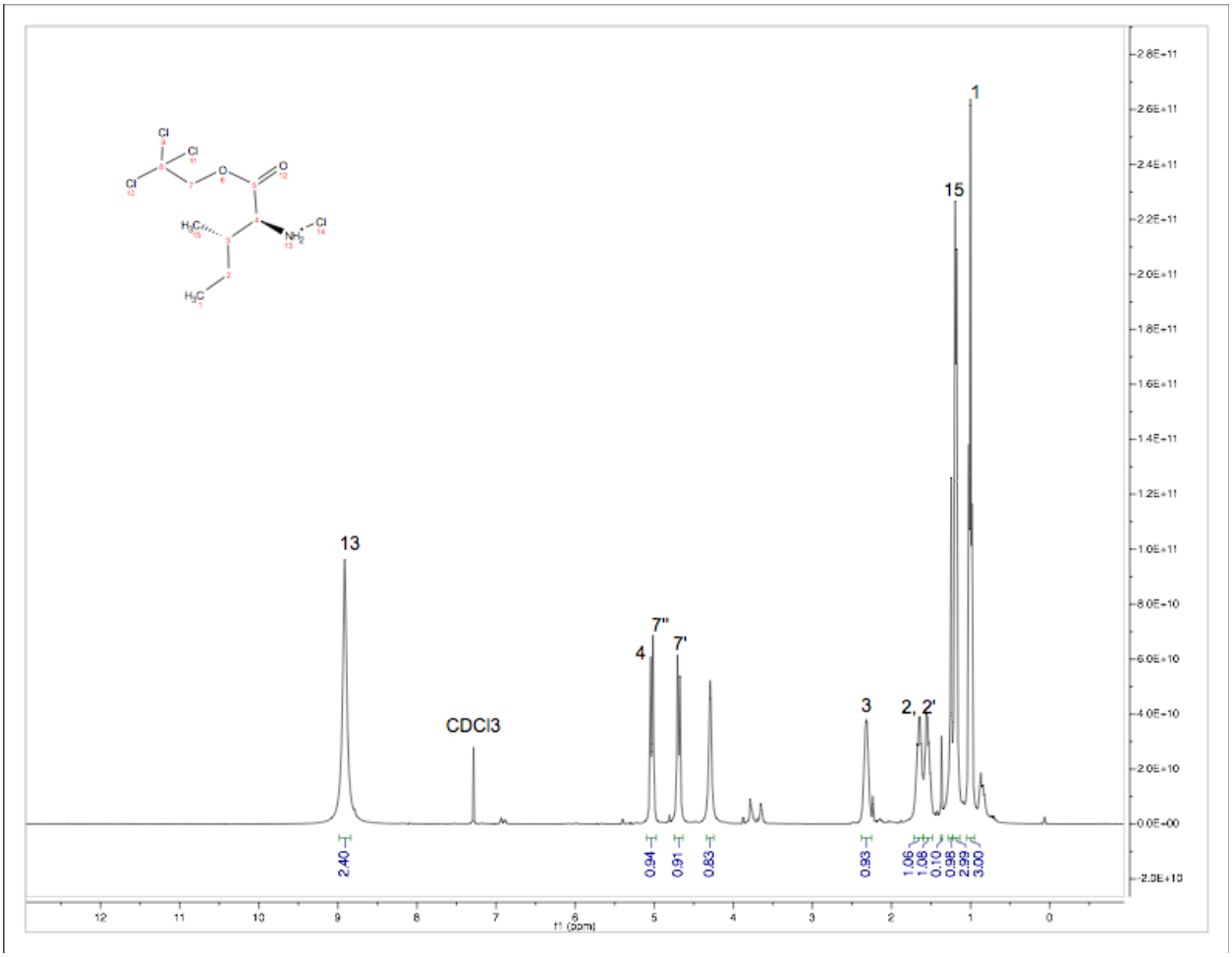
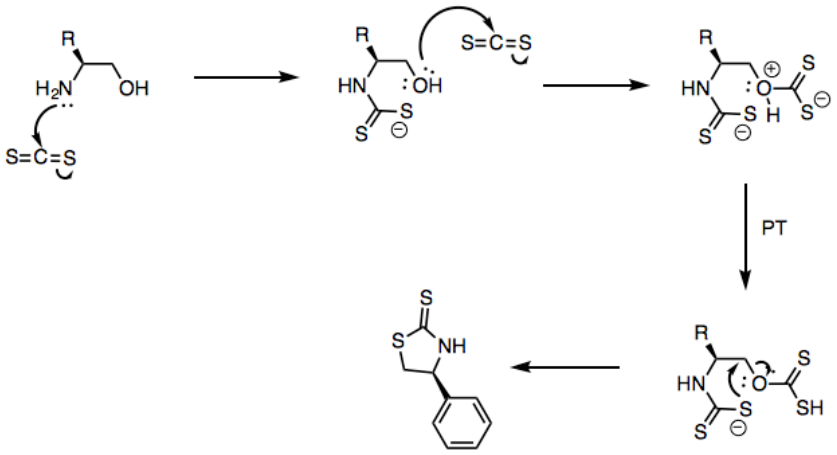


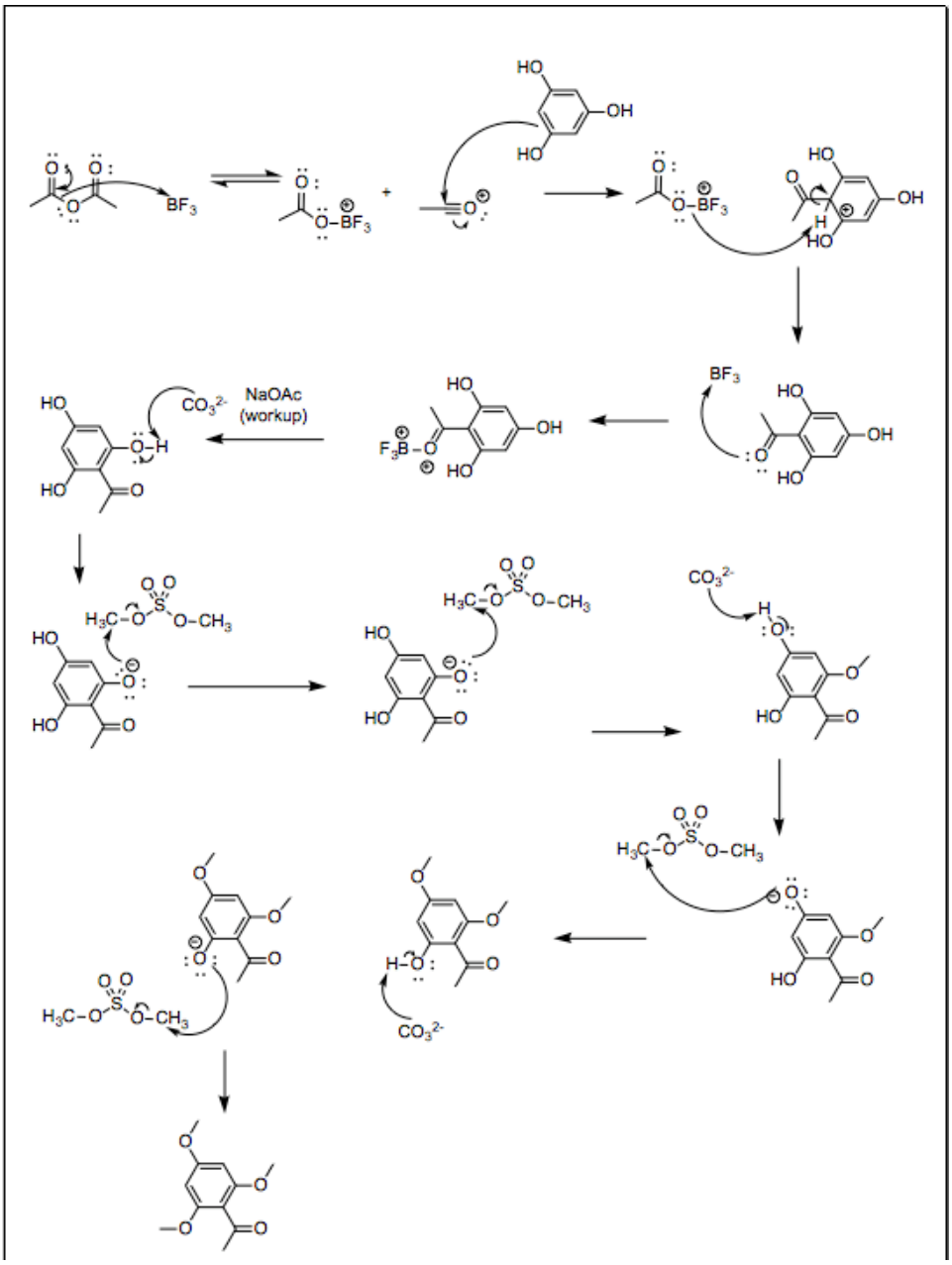
Figure 9. NMR spectrum of Boc-deprotected product for use in peptide coupling. Coupling Constants: 8.91 (s, 2H), 5.03 (d, J = 11.6 Hz, 1H), 4.69 (d, J = 11.7 Hz, 1H), 4.29 (s, 1H), 2.32 (d, J = 8.1 Hz, 1H), 1.65 (dp, J = 14.4, 7.8, 7.3 Hz, 1H), 1.54 (dt, J = 14.4, 7.5 Hz, 1H), 1.18 (s, 3H), 1.00 (t, J = 7.2 Hz, 3H).

The mechanisms by which these reactions occur warrant some discussion.



Scheme 1. Mechanism of Crimmins Auxiliary synthesis (first step).

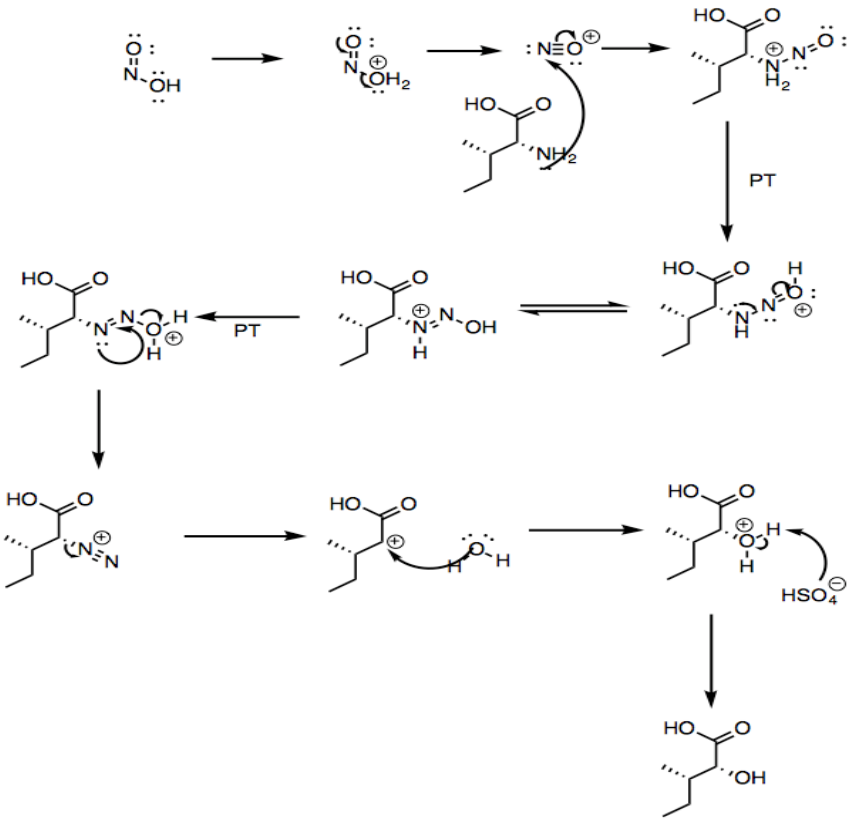
The synthesis of Crimmins-type auxiliaries for use in chiral synthetic applications was found to be more feasible for the synthesis of Lagunamide C than its Evans-type analog due to titanium (from later steps, sourced from $TiCl_4$) preferentially coordinates to the bidentate amine in the product as opposed to the thiocarbonyl sulfur.[6] The proton transfer is carried out by the excess of potassium hydroxide added to the reaction solution prior to adding (**1**). The procedure being discussed involved refluxing an aqueous solution of a β-amino alcohol in the presence of five equivalents of carbon disulfide and an excess of potassium hydroxide, but it should be noted that the refluxing of an aqueous ethanol solution of (**1**) in the presence of moderate excesses of carbon disulfide and potassium hydroxide provides similar yields and may be logistically advantageous.[7,8]



Scheme 2. Mechanism of acylation and subsequent methylation of phloroglucinol.

Although acid chlorides are more commonly employed as acylating reagents in Friedel-Crafts reactions, acid anhydrides are also viable.[9] The reaction kinetics of the acylation are such that monoacylation of phloroglucinol is viable if the acetic anhydride is added dropwise. Di- and triacylation is possible if enough acetic anhydride is added, but is relatively "slow" due to decreasing availability of reactive sites and the m-deactivating inductive effects of ketone groups

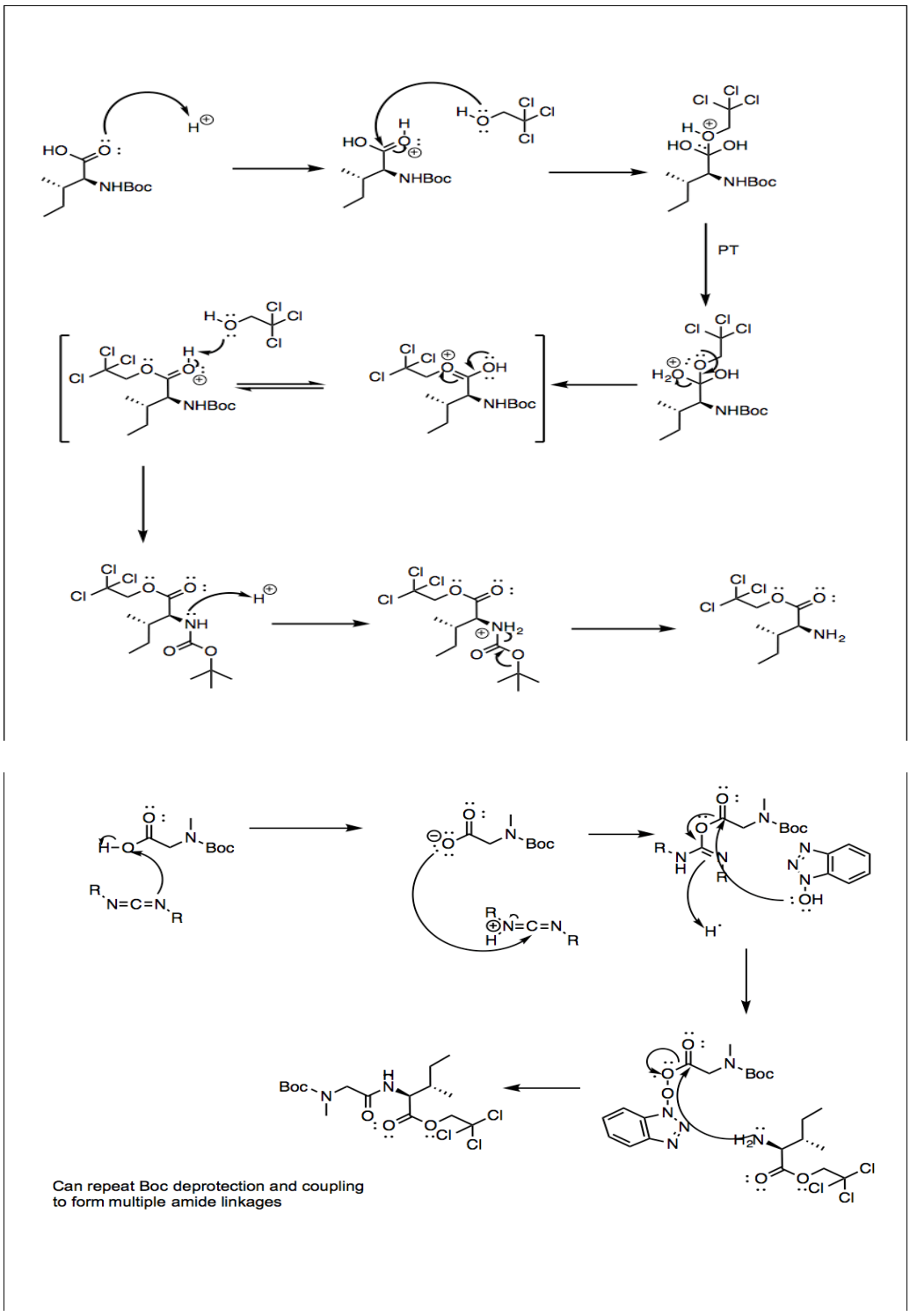
bonded to benzene rings. Following the orthodox Friedel-Crafts acylation, dimethyl sulfate was employed as a methylating agent. The reaction mechanism was written with the assumption that the three methylation events occur in sequence. Dimethyl carbonate has been known to function as a methylating agent if dimethyl sulfate is unavailable.[10]



Scheme 3. Mechanism of the diazotization of D-alloisoleucine.

It is worth noting that diazotization mechanisms are first presented in the context of aromatic substitution reactions, which may imply that aromatics are the only species on which the reactions can be carried out. However, they have been successfully employed on linear, non-aromatic species as well. They are most commonly employed as a means of converting the amino groups of amino acids into alcohols for the purpose of peptide coupling.[11] Retention of

stereochemistry is observed in these reactions, which may imply that the nucleophilic attack by water and the leaving of N_2 occur simultaneously instead of sequentially.[11]



Scheme 4. Mechanism of activation and subsequent coupling of L-isoleucine to Boc-L-sarcosine.

A Fischer esterification is carried out in order to protect the reactive carboxylic acid moiety of Boc-L-isoleucine, ensuring that the hydrochloric acid (source of H^+ in the mechanism) selectively reacts with and cleaves the Boc-protected amine.[12] Once deprotected, the highly reactive primary amine is kept until HOBt reacts with the EDC-Boc-L-sarcosine complex. As noted in Scheme 4, the latter two phases of the overall mechanism (selective Boc deprotection and the coupling reaction itself) can be repeated until the desired polypeptide is obtained.[13]

V. CONCLUSION

The syntheses outlined in this report were examined with the intent to acquire a greater understanding of the underlying mechanism the govern such reactions. With the exception of the acylation and methylation of phloroglucinol and the peptide coupling, the outlined reactions were carried out successfully. A kinetic rationale was developed for the failed phloroglucinol reactions, while a theoretical explanation was postulated for the failed peptide coupling. The NMR spectra provided confirmed the presence of the desired product, the presence of an undesired product, and/or the absence of any product. The additional reactions necessary for the complete formation of the polyketide and polypeptide subunits of the lagunamides have yet to be performed. Once those reactions are completed, analysis of the mechanisms can be carried out. Similarly, the mechanisms governing the reactions through which an extensive chalcone library can be developed can be analyzed once the reactions are carried out in a laboratory setting.

REFERENCES

1) Tripathi, A.; Puddick, J.; Prinsep Michèle R.; Rottmann, M.; Tan, L. T. Lagunamides A and B: Cytotoxic and Antimalarial Cyclodepsipeptides from the Marine Cyanobacterium Lyngbya Majuscula. *J. Nat. Prod.* **2010**, *73* (11), 1810–1814.

2) Perera, S. Natural Product Synthesis and Methodology Development in Exploring New Bioactive Agents. **2020**, 1–21.

3) Tripathi, A.; Puddick, J.; Prinsep, M. R.; Rottmann, M.; Chan, K. P.; Chen, D. Y.-K.; Tan, L. T. Lagunamide C, a Cytotoxic Cyclodepsipeptide from the Marine Cyanobacterium Lyngbya Majuscula. *Phytochem.* **2011**, *72* (18), 2369–2375.

4) Luo, D.; Putra, M.Y.; Ye, T.; Paul, V.J.; Luesch, H. Isolation, Structure Elucidation and Biological Evaluation of Lagunamide D: A New Cytotoxic Macrocyclic Depsipeptide from Marine Cyanobacteria. *Mar. Drugs* **2019**, *17*, 83.

5) Perera, S.; Fernando, A.; Dallman, J.; Weeramange, C.; Lansakara, A.; Nguyen, T.; Rafferty, R. J. Construction and Biological Evaluation of Small Libraries Based of the Intermediates within the Total Synthesis of Uvaretin. *Chem. Med. Chem.* **2021**.

6) Fatino, A.; Weese, C.; Valdez, S.; Jiménez-Somarribas, A.; Rafferty, R. J. Synthetic Studies towards Lagunamide C: Polyketide Assembly Investigations. *Tet. Let.* **2018**, *59* (7), 624–627.

7) Nagao, Y.; Hagiwara, Y.; Kumagai, T.; Ochiai, M.; Inoue, T.; Hashimoto, K.; Fujita, E. New C-4-Chiral 1,3-Thiazolidine-2-Thiones: Excellent Chiral Auxiliaries for Highly Diastereo-Controlled Aldol-Type Reactions of Acetic Acid and α,β-Unsaturated Aldehydes. *J. Org. Chem.* **1986**, *51* (12), 2391–2393.

8) Delaunay, D.; Toupet, L.; Le Corre, M. Reactivity of β-Amino Alcohols with Carbon Disulfide Study on the Synthesis of 2-Oxazolidinethiones and 2-Thiazolidinethiones. *The Journal of Organic Chemistry* **1995**, *60* (20), 6604–6607.

9) Somerville, L. F.; Allen, C. F. H. β-Benzoylpropionic Acid. http://orgsyn.org/demo.aspx?prep=CV2P0081 (accessed May 2, 2021).

10) Kabra, S. K.; Huuhtanen, M.; Keiski, R. L.; Yadav, G. D. Selectivity Engineering of O-Methylation of Hydroxybenzenes with Dimethyl Carbonate Using Ionic Liquid as Catalyst. *React. Chem. Eng.* **2016**, *1* (3), 330–339.

11) Pettit, G. R.; Hu, S.; Knight, J. C.; Chapuis, J.-C. Antineoplastic Agents. 571. Total Synthesis of Bacillistatin 2. *J. Nat. Prod.* **2009**, *72* (3), 372–379.

12) Han, G.; Tamaki, M.; Hruby, V. J. Fast, Efficient and Selective Deprotection of the Tert-Butoxycarbonyl (Boc) Group Using HCl/Dioxane (4 m). *J. Peptide Res.* **2001**, *58* (4), 338–341.

13) Coupling Reagents. https://www.peptide.com/resources/solid-phase-peptide-synthesis/coupling-reagents/ (accessed May 2, 2021).