University of North Carolina at Asheville
Journal of Undergraduate Research
Asheville, NC
May 2014

An Approach to Synthesize Phenstatin Analogues from Azidochalcones

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Abstract

Chalcone analogues of established anti-cancer molecules such as Colchicine and Combretastatin A-4 have been shown to be effective inhibitors of microtubule polymerization in rapidly dividing cancer cells. In addition, this group of molecules exhibit high solubility in water, high potency to malignant cells, and are often cheaper to synthesize and less harmful to the non-malignant tissue that surrounds cancerous growths. In an effort to produce chalcone analogues of Combretastatin A-4, various azidochalcones will be thermolized to produce a phenstatin analogue, which belongs to a group of well-known anti-cancer molecules that exhibit similar cytotoxic properties to chalcones. The resultant molecules will be examined for water solubility and cytotoxicity. Past research has shown that, when thermolized, the azidochalcone tends to form a cyanochalcone instead of the desired phenstatin analogue. The aim of this research is to examine the substitution of the rings to see if assorted chemical groups have an effect on desired phenstatin synthesis. In addition, the research will help to determine the reaction mechanism and see what role the substituents play in the mechanistic process. New procedures will be examined to determine if more pure products and greater percent yields can be established by evaluating the various substituents off the main ring of the molecules. Successful bromination of 3'-methoxyacetophenone and 3',4',5'- trimethoxyacetophenone has occurred, as well as a successful azide substitution of 2-bromo-3'-methoxyacetophenone and 2-bromo-3',4',5'-trimethoxyacetophenone.

1. Introduction

1.1 Cancer Inhibition

Colchicine is an anti-inflammatory compound of the meadow saffron plant commonly found in Europe and North Africa (Figure 1). It's commonly used to treat gout, but in recent years, research has shown that colchicine binds to microtubules in rapidly dividing cells and serves as a mitotic poison.[1] This trait has led towards its use as an effective cancer inhibitor.

Tubulin is a G-protein in cells that consists of an alpha and beta dimer. These dimeric subunits come together as a microtubule which is an alternating structure of 13 alpha and beta subunits.[2] Microtubules serve as a support structure for various cellular structures, but more importantly, they come together during the M-phase of mitosis to form the mitotic spindle.[1] Colchicine binds to the beta-tubulin and causes the microtubule structure to depolymerize, virtually halting cell division in cancer cells. Unfortunately, colchicine is expensive to isolate from the meadow saffron, so efforts have been made to synthesize it in the laboratory.

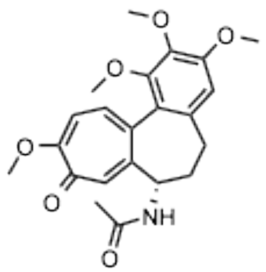


Figure 1. Colchicine

1.2 Colchicine Analogues

It was over 100 years after colchicine's initial discovery before it was finally synthesized by chemists.[3] As a result of colchicine's difficult synthesis, many efforts were made to find analogues of it that would still retain its ability to depolymerize tubulin. Combretastatin A-4 (CA-4), an extract of the South African willow tree, was shown by Pettit et al. to bind to the colchicine binding site on tubulin (Figure 2).[4] It became of immediate interest due to its relatively simple structure, anti-mitotic properties, and lower cytotoxicity than colchicine. CA-4 analogues began to be investigated for various other traits including water solubility.

Chalcones bear a similar structure to CA-4, have a relatively simple structure to synthesize, and can attach to the colchicine binding site of tubulin. They are recognized by two aromatic rings with an alpha-beta unsaturated ketone linking them (Figure 3).[2] The promise of this class of molecules lies in the variation from compound to compound by altering substituent groups on the rings. Different substituents can alter the water solubility or cytotoxicity of a molecule. However, the main objective remains to maintain the cytotoxicity of the mitotic poison, while possibly finding new pathways to an analogue, or making existing pathways more efficient with greater yields.

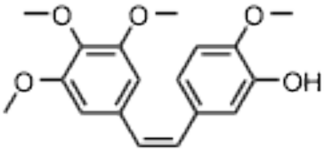


Figure 2. Combretastatin A-4

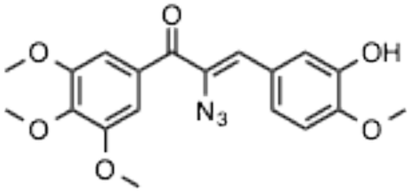


Figure 3. Azidochalcone

Due to the difficult synthesis of colchicine, further research has been undertaken to determine if analogues can serve as immunosuppresants. Dong-Jo et al. derived dozens of colchicine analogues, each with different substituent groups, to determine a molecular structure that could promote the production of macrophages and reduce collateral toxic effects.[5] They found that a colchicine derivative of Cyclosporin A, with an additional nitrate substituent, was cytotoxic enough to be delivered as an oral agent.

1.3 Combretastatin A-4 Analogues

Combretastatin A-4 is widely used as a substitute for colchicine, owing to a simpler synthesis due to its more simple structure. However, its solubility in water and structural instability have led many to synthesize more soluble and more stable analogues. Kerr et al. synthesized various CA-4 analogues including benzothiophenes, benzofurans, indoles and chalcones and discovered some analogues that were not only more stable and potent than CA-4, but were able to attach to the binding site of colchicine.[6] The most potent was a benzofuran analogue of CA-4, which was followed by an indole analogue (Figure 4, 5). Indole analogues are of particular interest to the Holt Research Group.

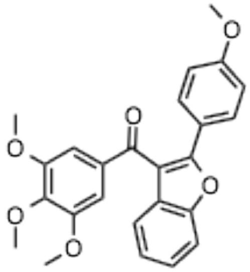


Figure 4. Benzofuran Analogue

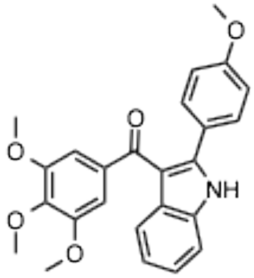


Figure 5. Indole Analogue

1.4 Evaluating Chalcone Derivatives

Ducki et al. has focused their research on the potency of chalcones. They synthesized dozens of chalcones through Claisen-Schmidt and Knoevenagel-like condensations.[7] The most cytotoxic chalcone was a 3',4',5'-trimethoxyacetophenone derivative and member of the alpha-aryl chalcone family (Figure 6). This molecule was shown, through structure-activity relationships, to inhibit two docking sites on tubulin, as compared to the beta-aryl chalcones which could only inhibit one site.[3] This led Ducki et al. to hypothesize that the beta-aryl chalcones should be categorized under a different pharmacore group than CA-4. The attachment of a phosphate group has also been shown to increase the solubility of CA-4 and analogues such as Combretastatin A-4 Phosphate, clinical name Zybrestat, are already undergoing clinical trials.[7]

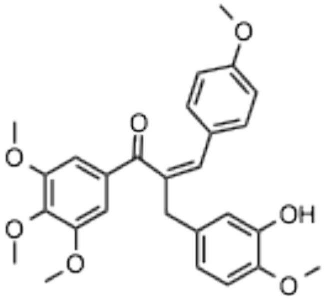


Figure 6. 3',4',5'- trimethoxyacetophenone derivative chalcone

1.5 Indanones

Lawrence et al. have identified a new synthesis pathway for CA-4 indanone analogues, which bear structural similarities to indoles. They took chalcones and created the indanone structure via a Nazarov cyclization (Figure 7).[8] This reaction involves heating a chalcone in the presence of a strong acid for an extended period of time, and irradiating it with microwaves. It was found that the desired indanone was formed at less than ideal yields (< 40%). Also, Lawrence et al. noted that the reaction proceeded best when the beta-aryl ring was electron deficient and the alph-aryl ring was electron rich. This created the best conditions for the indanone structure to form. This indanone procedure is of interest due to the product's structural similarities to the research's target phenstatin analogues, as well as an alternative synthesis scheme to reach the target analogues.

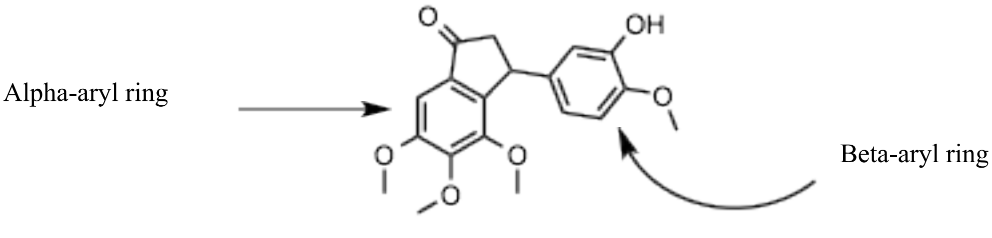


Figure 7. Indanone from Nazarov Cyclization

1.6 Phenstatins

Combretastain A-4 shows great promise in anti-cancer treatments due to its low cytoxicity and high potency as a vascular disrupting agent in malignant tumor cells, however, it also exhibits high levels of cardiovascular toxicity and neurotoxicity.[9] These side effects limit its use which makes it necessary to search for similarly structured compounds that maintain the same binding to cancer cells. Ghinet et. al conducted SAR studies which compared CA-4 to other compounds and found phenstatins to have similar potency against malignant cells. In addition, they found that some of these phenstatins exhibit nearly identical cytotoxicity and potency as CA-4P (Zybrestat). When taking all these factors into account, phenstatins provide a very appealing alternative to CA-4 (Figure 8).

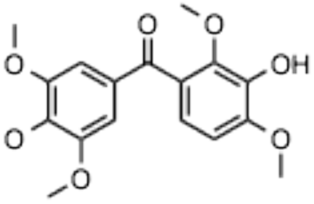


Figure 8. Phenstatin Analogue of Interest

1.7 Research Target

The goal of the research is to take what has been conducted in previous research and "combine" an indole analogue of CA-4 with a phenstatin. This research hopes to synthesize phenstatin analogues from azidochalcones via Hemetsberger-Knittel Thermolysis (Figure 9). Past research has shown that a cyanochalcone forms as opposed to the phenstatin analogue. The intent of this research is to examine the effects of various substituents on the rings to determine what may induce or inhibit phenstatin analogue formation.

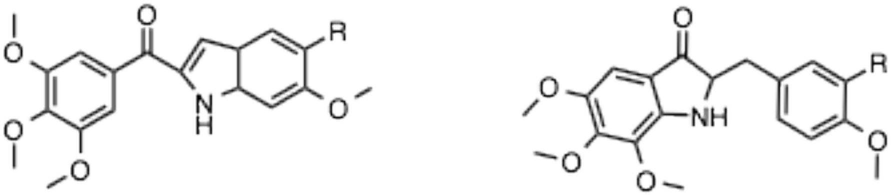
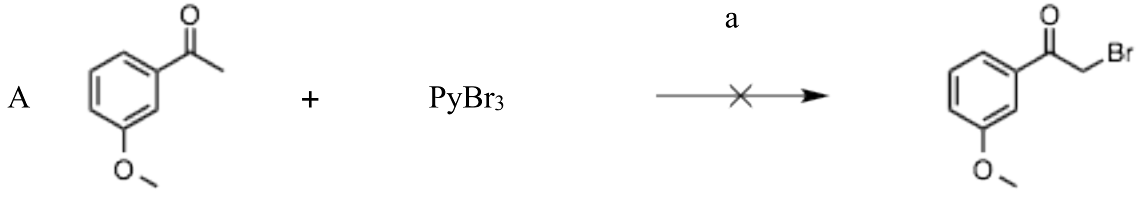
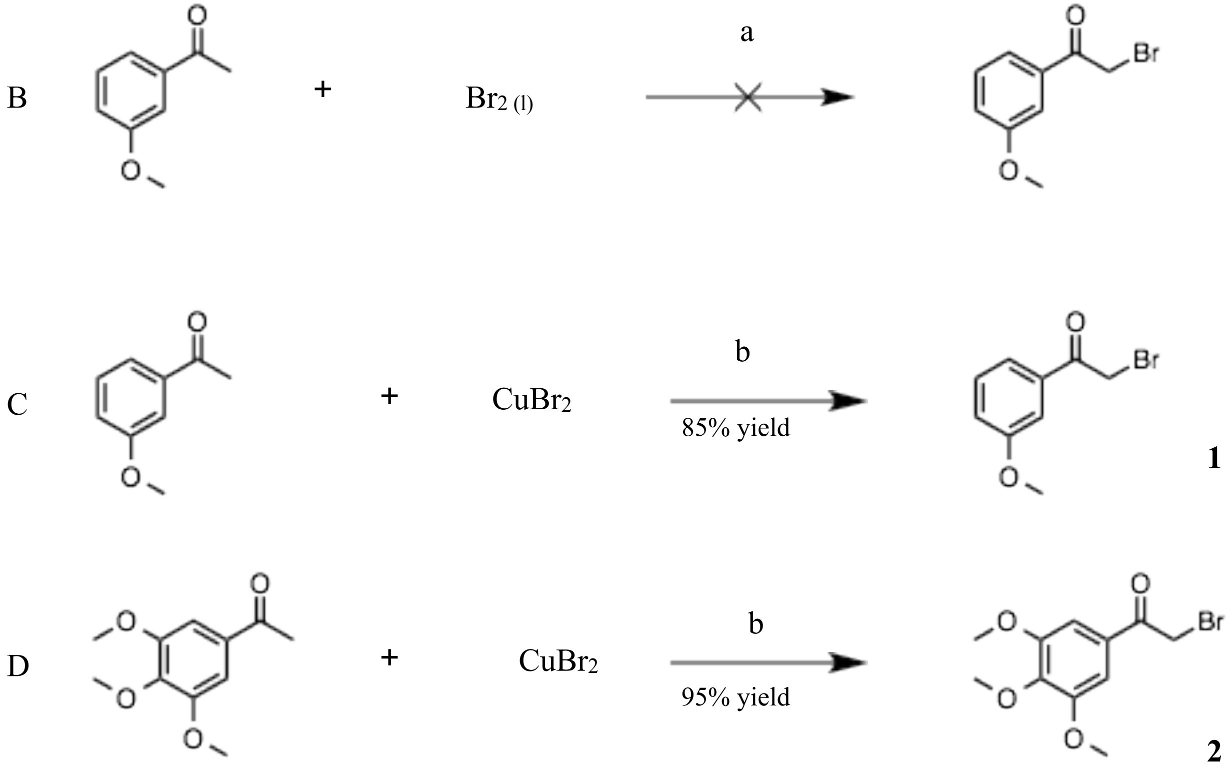


Figure 9. Desired Phenstatin Analogues

2. Synthesis

The first step in phenstatin analogues synthesis was the bromination of 3,4,5-trimethoxyacetophenone. The bromine moiety, when attached to a molecule, serves as a good leaving group and is useful when wanting to perform a substitution reaction on the product molecule from the bromination. (Note: 3-methoxyacetophenone was initially used because it's less expensive than 3,4,5-trimethoxyacetophenone).

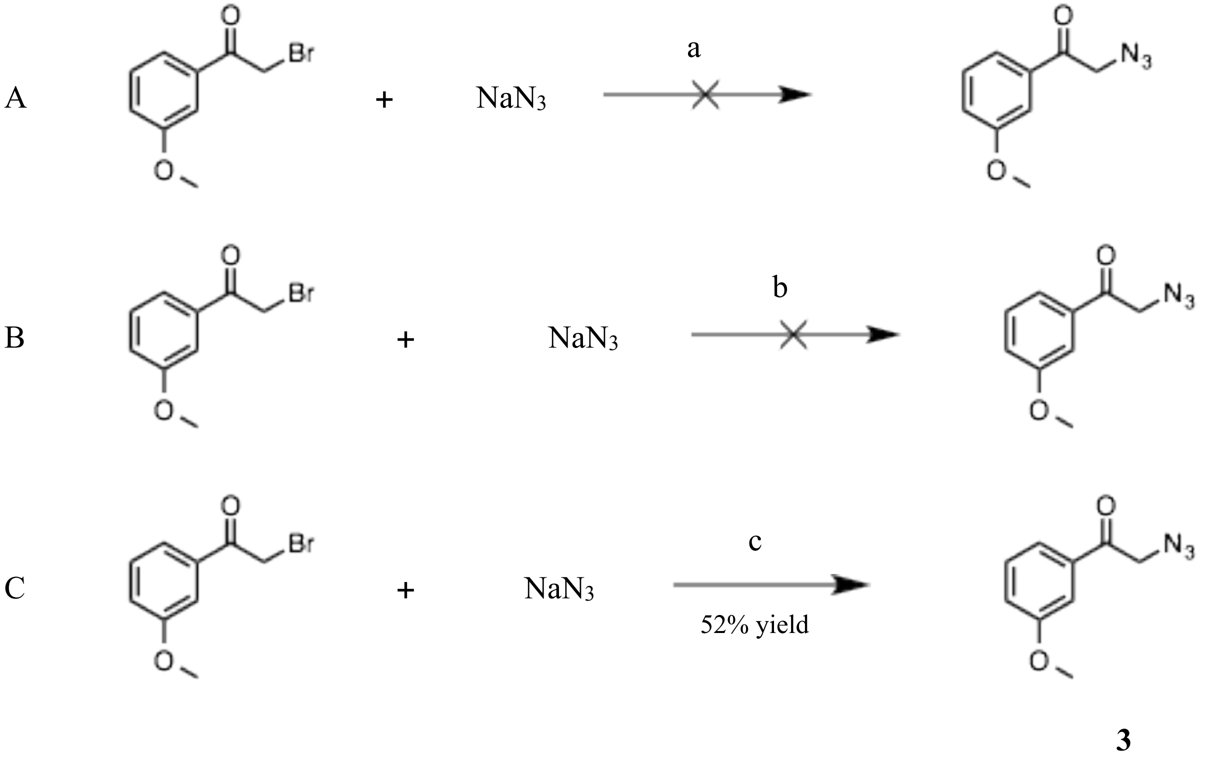


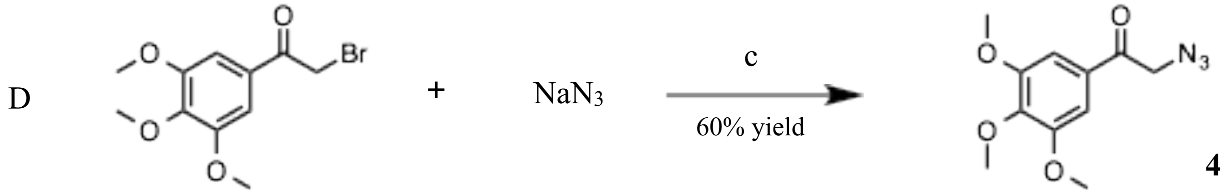


Scheme 1. Bromination of substituted acetophenones.

Reagents and Conditions: a) Acetic Acid, 2 hours; b) EtoAc, 70 ^{o}C, 2 hours. (Note: $PyBr_3$ is the shortened term for pyridinium bromide perbromide)

The products from Scheme 1 were subjected to an azide substitution.





Scheme 2. Azide substitution of bromo-acetophenones.

Reagents and Conditions: a) DMF, 50 ^{o}C, 16 hours; b) MeOH, 50 ^{o}C, 16 hours; c) THF, 50 ^{o}C, 16 hours

3. Results

Exclusive monobromination alpha to the carbonyl of 3-methoxyacetophenone and 3,4,5-trimethoxyacetophenone has been achieved, yielding **1** and **2**. An azide substitution has been performed on **1** and **2**, yielding **3** and **4**.

4. Discussion

4.1 Bromination of Acetophenones

There were multiple difficulties in the synthesis of **1** and **2**. The first obstacle involved a bromination procedure using either liquid Br_2 or pyridinium bromide perbromide, neither of which brominated at the correct position. Instead of exclusively mono-brominating alpha to the carbonyl, these reactants would di-brominate at the alpha position, brominate aromatically (para) to the ketone, or even tri-brominate alpha to the carbonyl. All of these products are useless for this research. An assortment of substituted molecules were tested: 3'-methoxyacetophenone, 3',4'-dimethoxyacetophenone, and 2',4',6'-trimethoxyacetophenone all failed to give the desired results. The lack of fidelity in brominating alpha to the carbonyl necessitated the use of a different bromination procedure.

As a result, a new procedure using copper(II) bromide as the brominating agent was tested and, initially, produced mixed results. Small scale reactions (< 0.2 mol in theoretical product) were implemented. Initially, it appeared that the bromination had not occurred as expected, due to high levels of starting material, however, this was most likely due to a lengthy reflux time (4 hours) and unpredictable exotherms that caused excessive heating. Other factors were changed as well; it was decided that the reaction might proceed better under inert conditions to try and minimize any cooperative binding the copper may have with water in the reaction vessel. Despite these changes, the amount of product being generated was not sufficient.

In the interest of time and materials, it was determined that it may be best to increase the size of the reaction to produce 3 g, 0.013 mol of **1**. In this instance, mono-bromination was achieved with minimal amounts of residual starting material. This can be explained in a couple ways: 1) the size of the reaction vessel increased from 25 mL to 100 mL, which may have given the reactants a larger surface area in which to interact. 2) The molar ratio had accidentally been increased from 1 mol SM : 1.75 mol $CuBr_2$ to 1: 2.3 via a mathematical error. Qualitatively, the reaction proceeded differently. Most noticeably was the formation of two layers following the cessation of reflux: the top layer was a bright green and the bottom a muddy gray. In addition, there were copious amounts of HBr gas produced and it could be inferred that upon cessation of gas production, the reaction came to completion.

The adapted procedure was then used for the synthesis of **2**. Results were similar to the synthesis of **2**, but with a higher percent yield. This could be a result of the presence of the desired product and unwanted starting material. During the reaction, it could be observed that the longer the reaction went, the copper (II) bromide changed from a black powder into a white powder. It can be inferred that this indicates a change in oxidation state to copper (I). It was also observed that once the reaction reached the two hour mark, TLC indicated the presence of a third compound. It was probably best to not let the reflux exceed two hours. With this reaction, dry chloroform was unavailable, which led to a much brighter green layer upon cessation of reflux. Dry solvents are preferred to keep the entire reaction as inert and aprotic as possible to facilitate a substitution reaction.

4.2 Azide Substitution of Brominated Acetophenones.

The azide substitution of **1** and **2** has also proved problematic. To preserve the amount of product from **1** and **2** , 2-bromo 4'-nitroacetophenone was initially used for the azide reaction. DMF was chosen as the solvent for the reaction, but failed to produce the desired product. The reaction was then repeated using methanol, but to the same effect as DMF. THF was then selected and all reactions run in THF showed desired azide substitution alpha to the carbonyl. However, the goal soon became optimizing the reaction. A gentle reflux (50 ^{o}C) was eventually implemented to try and increase the yield. However, the percent yield of the azide substitution appears to hover around 60%.

5. Future

Work still remains to be done in synthesizing the desired phenstatin analogues. Aldol condensations need to be run on **3** and **4**. The subsequent chalcones will then need to be subjected to a Hemetsberger-Knittel Thermolysis to synthesize a phenstatin analogue.

6. Experimental

6.1 2-bromo, 3'-methoxyacetophenone

A 100 mL three neck round-bottomed flask (RBF) was charged with a stir bar, fixed with thermometer and reflux condenser, flame-dried and kept under inert conditions. 6.69 g of $CuBr_2$ was added and stirred in 15-20 mL of dry ethyl acetate. The solution was heated to 70 ^{o}C and upon reaching the desired temperature, a mixture of 1.95 g 3'-methoxyacetophenone and 15-20 mL of dry chloroform added. The reflux was allowed to take place for 1.5-2 hours. The product was filtered and washed with ethyl acetate and dried under sodium sulfate. The product was then condensed under reduced pressure to yield a lime green crystal (2.53 g, 0.011 mol, 85% yield). ^{1}H NMR δ (400 MHz; $CDCl_3$), 3.80 (3H, s, OMe), 4.40 (2H, s, CH_2Br), 7.10 (1H, s, ArH), 7.25 (1H, s, ArH), 7.45 (1H, s, ArH), 7.60 (1H, s, ArH).

6.2 2-bromo, 3',4',5'-trimethoxyacetophenone

A 100 mL three-neck RBF was charged with 7.2 g of $CuBr_2$ and 15-20 mL of dry ethyl acetate. Upon reaching 70 ^{o}C, 2.94 g of 3',4',5'-trimethoxyacetophenone and 15-20 mL of dry chloroform were added and allowed to stir for 1.5-2 hours. The final product was a dark brown crystal (7.64 g, 0.0265 mol, 95% yield). ^{1}H NMR δ (400 MHz; $CDCl_3$), 3.95 (9H, m, OMe), 4.40 (2H, s, CH_2Br), 7.25 (2H, m, ArH)

6.3 2-azido, 3'-trimethoxyacetophenone

A 25 mL three neck RBF was flame dried, and while under inert conditions, was fixed with a thermometer, a reflux condenser, and charged with a stir bar. 0.24 g of 2-bromo-3'-methoxyacetophenone **1**, 0.5 g of sodium azide, and 15-20 mL of dry THF were added. The mixture was heated to 50 ^{o}C and allowed to stir overnight. The reaction mixture was extracted into 3 x 50 mL of diethyl ether and washed with 5 x 80 mL of water. The organic extracts were combined and condensed under reduced pressure to yield a dark yellow oil (0.10 g, 0.000524 mol, 52% yield). ^{1}H NMR δ (400 MHz; $CDCl_3$), 3.95 (3H, s, OMe), 4.55 (2H, s, CH_2N_3), 7.20 (1H, s, ArH), 7.40 (3H, m, ArH)

6.4 2-azido, 3',4',5'- trimethoxyacetophenone

A 100 mL three-neck RBF was flame dried, kept under inert conditions, charged with stir bar, and fitted with thermometer and reflux condenser. 0.3 g of 2-bromo, 3',4',5'-trimethoxyacetophenone **2**, 0.5 g sodium azide, and 15-20 mL dry chloroform were added together. The mixture was heated to 50 ^{o}C and allowed to stir overnight. The reaction mixture was extracted into 3 x 50 mL of diethyl ether and washed with 5 x 80 mL of water. The organic extracts were combined and condensed under reduced pressure to yield a goldenrod colored powder (0.15 g, 0.000595 mol, 60% yield). ^{1}H NMR δ (400 MHz; $CDCl_3$), 3.95 (3H, s, OMe), 4.55 (2H, s, CH_2N_3), 7.15 (2H, s, ArH).

7. Acknowledgements

I would like to thank my advisor, Dr. Herman Holt for his guidance and support over the past couple of years, as well as my labmates Ben and Randy for their assistance in executing and working through some of the experiments. In addition, I would like to thank the UNC Asheville Department of Chemistry for their willingness to teach and inspire. Finally, I would like to express my gratitude to the Undergraduate Research Program for their funding over the course of the research.

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