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Synthesis and Isolation of 5,6,7-Trimethoxy Indoles for Binding Diverse Functional Groups at the 3-Position of the Indole to Make Novel Combretastatin Analogs

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Abstract

Combretastatin A4 (CA4) is an effective chemotherapeutic (anticancer) drug that decreases cell proliferation and selectively kills tumors by deforming the tumor's vasculature, or the pathway through which tumors obtain nutrients, and thus starves the tumor cells. CA4 works by targeting and inhibiting the formation of tubulin, a key protein component in cell division and structure. CA4 has been used as a model for the production of many new drugs because of how effective it has proven to be against cancer cell lines. While CA4 is an effective chemotherapeutic drug, it is not the miracle cure the world needs, thus necessitating the development of new drugs. Indoles are prominent in pharmaceuticals because of their high bioactivity and they are commonly found in nature bonded at the 3-position (an example being the amino acid tryptophan). This combination encourages the utilization of indoles with substituents at the 3-position in new chemotherapeutic compounds. Synthesis of new potential drugs based on CA4 have been extensively investigated but an indole substituted analog bonded at the 3-position has yet to be studied in the present literature. Trimethoxy indole, due to its high cost, will be synthesized. This will be done through the use of Hemetsberger-Knittel indole methodology in order to create an indole ester. The indole ester will then undergo saponification, subsequent decarboxylation and then will be halogenated at the 3-position. A coupling reaction will then be performed between the halogen substituted indole and an aromatic alkyne (3'-hydroxy-4'-methoxy phenylacetylene). The coupled product must then undergo selective hydrogenation in order to produce the desired indole CA4 analog. Should this CA4 analog exhibit good drug properties (as will be determined by an outside group), the outlined synthetic pathway will then provide potential for the generation of a new library of CA4 analogs. This suggested pathway may also be applied to the functionalization of other chemotherapeutic drugs, such as chalcones and estrogen modulators which are being studied by fellow group members. Presently 5,6,7-trimethoxyindole-2-carboxylic acid has been made with 72% yield and its decarboxylation is being pursued through the use of a well known procedure while other potential methods are being considered.

Keywords: Cancer, Combretastatin A4, Indole

1. Background

Since the 1990s cancer has been a leading cause of death in the United States with a report in 2010 stating that 50% of men and 33% of women will be diagnosed with cancer in his or her lifetime.[1,2,3] Cancer, as defined by the American Cancer Society, is many diseases that cause uncontrolled cell growth.[3] Research into how these diseases work has resulted in the procurement of a variety of treatments including surgery, radiation and chemotherapy, hormonal therapy as well as combinations of these treatment methods.[1]

Chemotherapy is the use of a drug to treat a disease and is most often associated with the treatment of cancer.[3] A potential chemotherapeutic drug, combretastatin A4 (CA4), was isolated from the plant *combretum caffrum* in 1988.[4] CA4 was found to possess a similar structure to that of another molecule, colchicine, which had previously been evaluated for its viability as a chemotherapeutic drug but was found to be too toxic towards healthy tissue for commercial use. Possessing a similar structure, CA4 is able to bind at the same binding site as colchicine on tubulin thus inhibiting microtubule formation (see Figure 1). Inhibition of microtubule formation affects both cell shape and division which leads to cell death and the disruption of tumor vasculature.[5] Combretastatin A4 is less toxic than colchicine, is effective against multi-drug resistant cancer cell lines, induces selective tumor vascular disruption at relatively low concentrations and does not cause bone marrow toxicity or hair loss, which are common side effects of other chemotherapeutic drugs.[6,7,8,9] Some notable problems with CA4 are its low polarity, requiring it to be administered as a pro drug, and that the ring of cells where the tumor attaches to the normal tissue is not affected.[7,10]

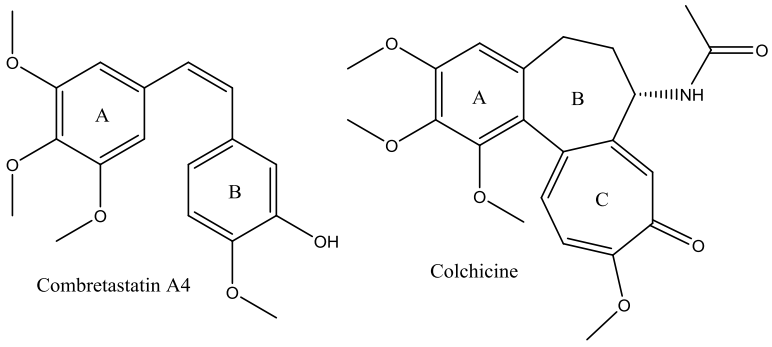


Figure 1. Molecular structure of combretastatin A4 and colchicine

Analogs of CA4 have been studied extensively with alterations to the A-ring, the B-ring and the *cis*-alkene connecting the two rings. Modifications of both the B-ring and the *cis*-alkene have shown retention of activity while alterations to the A ring, such as removal or relocation of the methoxy functional groups, have deactivated past analogs. The tendency for CA4 analogs to deactivate upon modification of the A-ring's methoxy groups has resulted in a relatively meager amount of research on modifying the A-ring. This lack of research creates a need for further investigations into the A-ring, such as replacing the trimethoxy benzene with a trimethoxy indole, so long as the trimethoxy moiety is maintained.[10,11] Most tubulin inhibiting indole analogs of CA4 enhance tubulin inhibition, but contain alterations that do not directly interact with the main binding site.[4,12,13] Recent work by Zhao *et al.* and Arthuis *et al.*, utilizing trimethoxy indoles in place of phenstatin's (a CA4 analog) A-ring, showed promising results thus urging forward the research of novel indole CA4 analogues with modified A-rings.[14,15] Generation of CA4 analogues with modified A-rings will further the field's knowledge of what modifications may be conducted in the formation of CA4 analogs that enable retention of bioactivity. In addition, these analogues may procure new effects when introduced to the colchicine binding pocket, thus further characterizing the site. Indoles are found within many pharmaceuticals such as anti-depressive, anti-inflammatory, anti-fungicidal, anti-tuberculostatic and tubulin inhibiting drugs which makes their use in new analogs appealing.[16]

Past group researchers, McDonald and Graham, investigated the synthesis of a novel CA4 analog replacing CA4's A-ring with a 5,6,7-trimethoxy indole bonded at the 2-position (see Figure 2).[17,18] Due to the prominence of indoles in nature bonded at the 3-position, such as the amino acid tryptophan, a novel CA4 analog will be synthesized replacing CA4's A-ring with a 5,6,7-trimethoxy indole bonded at the 3-position (see Figure 2). Unfortunately, due to the high cost of 5,6,7-trimethoxy-1*H*-indole (over $1500 per gram upon last price check), it is necessary to synthesize the indole A-ring. In the present study, the desired indole A-ring will be synthesized for its use in future work towards the synthesis of a novel CA4 analog.

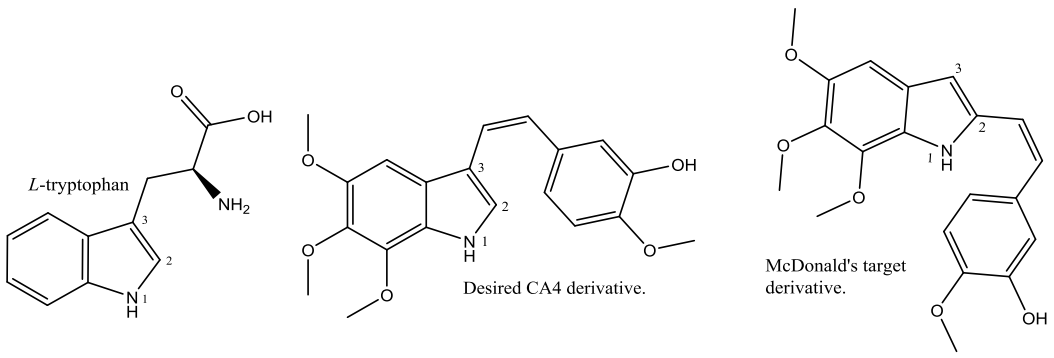
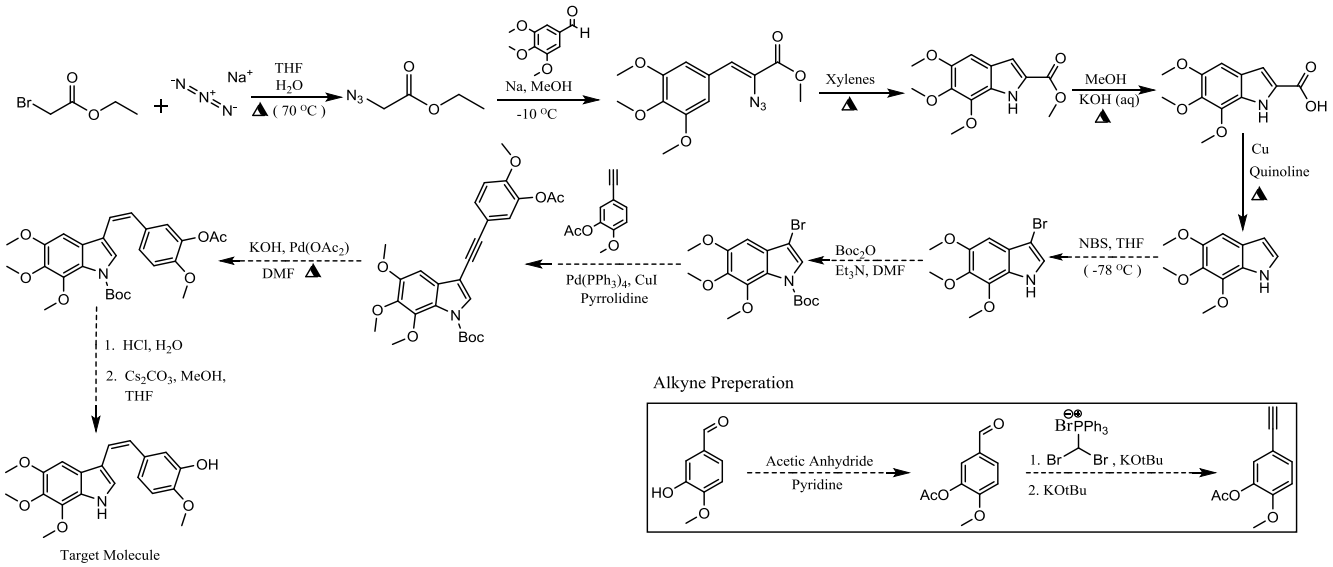


Figure 2. L-Tryptophan and CA4 analogs with an indole substituted at the 2- and 3-positions.

A synthetic scheme was developed utilizing McDonald and Graham's synthetic schemes synthesizing an indole ester through Hemetsberger-Knittel methodology, an alkyl benzene from a Wittig reaction, and a *cis*-alkene bridge through a combination of Sonogashira coupling and selective hydrogenation with palladium acetate. This will be done in conjunction with a saponification and decarboxylation method from Fukuda *et al.* and a halogenation method from Yang *et al.* in order to synthesize the desired indole A-ring and then later complete the CA4 analog (see Scheme 1).[18,19,19,20,21,22,23,24,25,26] Products will be identified through utilization of nuclear magnetic resonance (NMR) Spectroscopy.

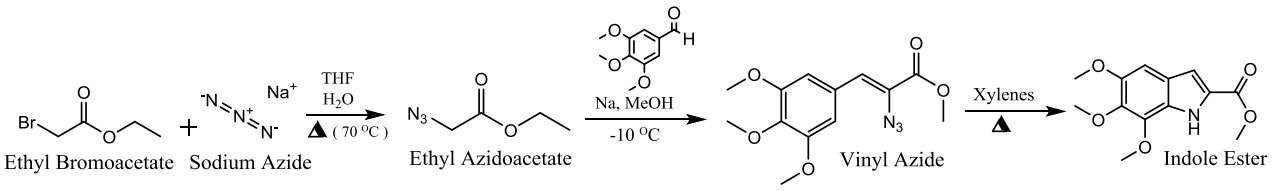
Scheme 1. Proposed synthetic scheme for synthesis of the target CA4 analogue.



3. Results and Discussion

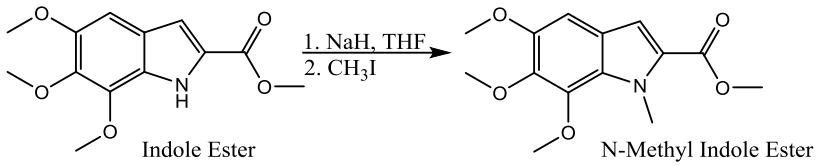
Hemetsberger-Knittel methodology was utilized for the synthesis of methyl 5,6,7-trimethoxy-1*H*-indole-2-carboxylate (indole ester) (see Scheme 2).

Scheme 2. Indole ester synthesis using Hemetsberger-Knittel methodology.



An SN_2 reaction formed the ethyl azidoacetate from ethyl bromoacetate and sodium azide with the highest yield being 93%. The experiment by McDonald utilized a polar protic solvent, MeOH. Upon obtaining lower yields, another group member, Long, used a polar aprotic solvent, THF, which more strongly favors SN_2 reactions.[26] An aldol condensation was then used to attach 5,6,7-trimethoxy benzaldehyde to the carbonyl carbon of ethyl azidoacetate in order to produce methyl (Z)-2-azido-3-(3,4,5-trimethoxyphenyl)acrylate (vinyl azide) with the highest yield being 86%. It should be noted that sodium is very reactive and should not be exposed to air for long periods of time. The sodium used for this reaction was stored in an oil and excess amounts of the required equivalents were used in an effort to counter the mass of the oil coating the pieces of sodium that were used. Refluxing the resulting vinyl azide in xylenes induced ring closure and produced methyl 5,6,7-trimethoxy-1*H*-indole-2-carboxylate (indole ester) with the highest yield being 96%. McDonald used hexanes to recrystallize the product but after a failed attempt, the method by Bridges, using warm MeOH, was adopted.[27] The ^{1}H-NMR spectrum showed extra signals which looked indicative of an isomer but due to the symmetry of the trimethoxy phenyl ring, creation of an isomer was not thought to be possible. It was hypothesized that the hydrogen attached to the nitrogen was spatially interacting with the methoxies to create the extra signals.[24] To test this hypothesis, a methylation of the indole nitrogen was performed (see Scheme 3). The resulting methyl 5,6,7-trimethoxy-1-methyl-1*H*-indole-2-carboxylate (methyl indole ester) was produced with a highest yield of 79% and subsequent analysis with ^{1}H-NMR revealed a loss of the previous peaks, which had indicated an isomer, thus supporting the proposed hypothesis.

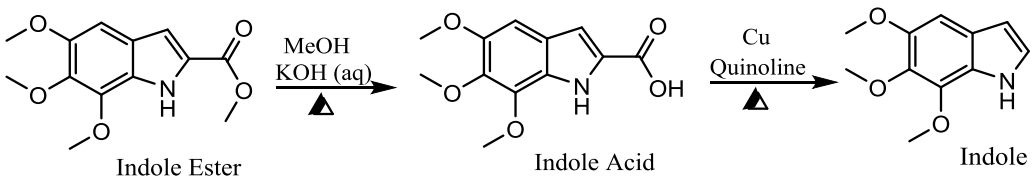
Scheme 3. Methylation of indole ester.



For the decarboxylation, the indole ester was first converted into an indole acid (see Scheme 4) through utilization of a saponification reaction producing 5,6,7-trimethoxy-1*H*-indole-2-carboxylic acid (indole acid) with a maximum yield of 85%. The experimental by Fukuda *et al.* reports testing for reaction completion through TLC using a 5:4:1 ratio of toluene, HCO_2Et and HCO_2H, respectively, but an alternative method was utilized in which a partial work-up of the product was done in a vial using 1 *M* HCl and ethyl acetate.[24] The proceeding workup listed in Fukuda *et al.*'s was unspecific on the amount of 1*M* HCl needed for the workup but a conversation with Dr. Wolfe revealed that the product should crash out upon acidification of the solution containing it.[25] This proved to be accurate as pure product precipitated upon acidification. Next, a successful decarboxylation was performed yielding 5,6,7-trimethoxy-1*H*-indole (indole) with a maximum yield of 23% (see Scheme 4). Successful decarboxylation was determined through analysis of ^{1}H NMR (see Figure 3) in which an extra signal was seen between 6 and 7ppm, indicating that a third hydrogen is directly attached to the aromatic indole ring. This method of decarboxylation is the most common with aromatic rings but the work-up proved to be difficult and inefficient, and produced low yields.[27] According to literature, yields may improve through either increasing the reaction time or by using a copper salt instead of copper metal.[28] In addition, quinoline is reactive with light and therefore shielding the reaction from light may decrease the difficulty of the work up. It has been further proposed to filter the crude reaction materials through a plug of silica gel

to make the work up less difficult.[24] These methods will be pursued in future work in order to make the desired indole in a more efficient manner. Other future work towards the desired CA4 analogue will follow the synthetic scheme outlined in Scheme 1.

Scheme 4. Saponification and subsequent decarboxylation of indole ester to provide the indole.



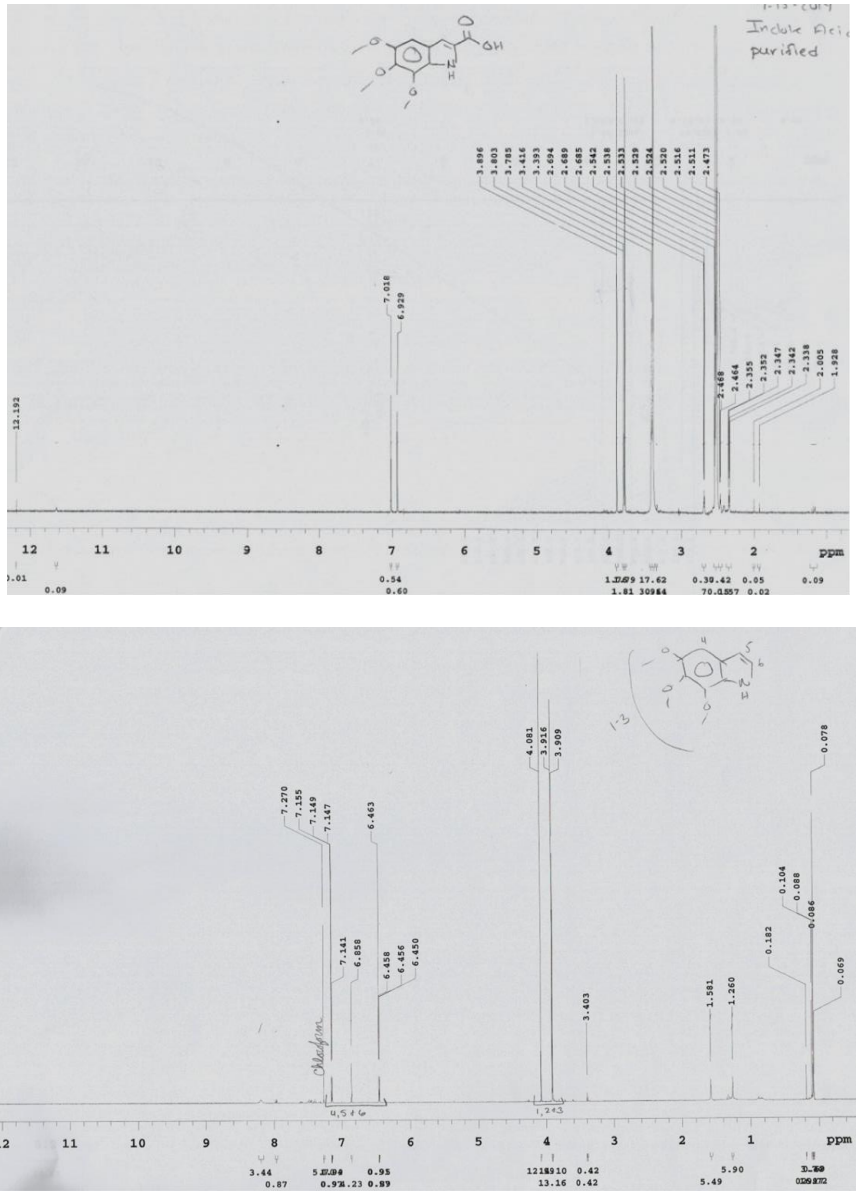


Figure 3. [1]H-NMR spectral data of indole acid (top) and indole (bottom).

4. Conclusion

Trimethoxy indole was successfully synthesized but with modest yields. Because of these modest yields, methods are currently being pursued to increase the efficiency of the reaction. Upon increased reaction efficiency future work will be done to synthesize a novel indole substituted CA4 analog. The bioactivity of this analog may then be assessed and, should it possess good activity, a new library of CA4 analogs may be explored.

5. Experimental

5.1 General Considerations

Proton NMR spectra were recorded using a Varian Unity Inova 400 MHz nuclear magnetic resonance spectrometer. IR spectra for the indole acid were recorded using a Nicolet Thermo Scientific iS10 IR.

5.2 Ethyl Azidoacetate

A 100 mL, 3-neck round bottom flask (RBF) was fitted with a stir bar, condenser, addition funnel, thermometer adapter and thermometer. The system was then placed under nitrogen. Using a funnel, 14.7 g NaN_3 (0.227 mol, 1.1 eq) was poured into the flask and rinsed with a small amount of water. Fifteen milliliters of water was then added, dissolving the solid NaN_3, making a tan solution. Using a syringe, 22.8 mL of ethyl bromoacetate (0.206mol, 1 eq) was transferred into the addition funnel. Using a graduated cylinder, 30.0 mL of THF was added to the addition funnel. The contents of the addition funnel were then added to the RBF at a fast drip over 30 minutes at which point a red-tan solution was observed. The flask was warmed to 70℃ and maintained at this temperature for 4.5 hours.

The solution was reduced through rotary evaporation. The resulting oil was treated with 20.0 mL of water and then extracted with diethyl ether (3x15mL). The combined organic layers were washed with 25.0 mL of brine and dried with $MgSO_4$. The organic solution was filtered and concentrated to yield 13.8 g of a yellow oil (0.107 mol, 52% yield). Identification of the desired product was performed using proton nuclear magnetic resonance (^{1}H NMR) spectroscopy. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.31 (t, 3H), 3.84 (s, 2H), 4.23 (q, 2H).

5.3 Vinyl Azide

A 100 mL 2-neck RBF was fitted with a stir bar, addition funnel and an air inlet adapter, closing the system under N_2 gas. A 50 mL 1-neck RBF was closed with a septum and placed under nitrogen using a nitrogen balloon. Using a syringe, 41.0 mL of dry MeOH was added to the 2-neck RBF. The 2-neck RBF was then placed into a dewer of dry ice and wet MeOH that was kept at -10℃. One and 83 hundreths grams of Na(s) (0.0794 mol, 3.7 eq) was then added piecewise, stirring so that it dissolved in the MeOH. Into the 1-neck RBF, 4.19 g of 3,4,5-trimethoxybenzaldehyde (0.0213 mol, 1 eq), 6.4 mL of ethyl azidoacetate (0.0559 mol, 2.6 eq) and 16.0 mL of dry MeOH were added. The contents were then swirled until all solids had dissolved, making a clear, yellow solution. The contents of the 1-neck RBF were then transferred into the addition funnel using a syringe. This solution was added dropwise via an addition funnel to the sodium/methanol mixture over a period of 56 min. Once the contents of the addition funnel had been added to the 2-neck RBF, the system was covered in foil and allowed to warm to room temperature and react overnight. The resulting solution was a light brown.

Thin-layer chromatography (TLC) was performed on the light brown solution using 80/20 Hex/EA to confirm product formation. A 250 mL beaker was filled to the 100 mL mark with crushed ice and saturated NH_4Cl. The reaction solution was then poured into the saturated NH_4Cl and ice mixture and stirred for ~1.5 hr at which point a precipitate formed. The precipitate was collected through vacuum filtration producing 5.37 g (0.0183 mol, 86 % yield). Identification of the precipitate as the desired product was indicated through ^{1}H NMR spectroscopy. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.90 (s, 3H), 3.90 (s, 6H), 3.92 (s, 3H), 6.86 (s, 1H), 7.11 (s, 2H).

5.4 Indole Ester

A 500 mL 3-neck RBF was fitted with a stir bar, air inlet adapter, condenser, thermometer adapter, thermometer and addition funnel. The system was closed and placed under N_2(g). A 100 mL 1-neck RBF was closed using a septum and placed under N_2(g) through use of a nitrogen balloon. To the 3-neck RBF 106.0 mL of dry xylenes was added and brought to reflux. An additional 40.0 mL of dry xylenes was added to 2.20 g (0.00751mol, 1 eq) of vinyl azide in the 1-neck RBF while swirling. Heat was applied to the 1-neck flask to help dissolve any remaining solid. Once the xylenes in the 3-neck RBF started refluxing, the vinyl azide solution was transferred to the addition funnel and then added to the 3-neck flask dropwise. This was allowed to reflux overnight. TLC was then performed using 80/20 Hex/EA.

The solution was cooled to 80℃ and then transferred to a 500 mL 1-neck RBF. Through rotary evaporation, the solution was concentrated. Warm MeOH was then added to the concentrated solution. A crystalline solid formed and then the flask was placed in the freezer overnight for further recrystallization. The resulting 1.04 g (0.00392 mol, 52 % yield) of yellow crystals were collected via vacuum filtration. The crystals were attached to the high-vac to remove any remaining solvent and the desired product was confirmed through ^{1}H NMR spectroscopy. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.91 (s, 3H), 3.93 (s, 3H), 3.94 (s, 3H), 4.08 (s, 3H), 6.83 (s, 1H), 7.11 (s, 1H), 8.88 (s, 1H).

5.5 Methyl Indole Ester

A 100 mL 2-neck RBF was fitted with an air inlet adapter and stir bar and the system was closed under N_2(g). A 50 mL 1-neck RBF was closed with a septum and placed under N_2(g) with a N_2(g) balloon. To the 2-neck RBF, 0.019 g (0.79 mmol, 2.1 eq) of NaH in mineral oil and 4.0 mL of dry THF was added and the solution was stirred. In the 1-neck RBF, 0.100 g of indole ester (0.377 mmol, 1 eq) was solvated in 6.0 mL of dry THF and then added at a fast drip to the sodium hydride solution. After mixing for an hour, 0.4 mL of CH_3I (6.4 mmol, 1 eq) was added drop- wise. (Caution: CH_3I is an irritant and permeator, a second set of gloves is advised.) The reaction was allowed to occur overnight and then TLC was performed using 70/30 Hex/EA. Upon indication of starting material, additional equivalents were added of CH_3I and NaH every couple of hours until no more starting material was indicated by TLC (4 eq CH_3I and 2.2 eq NaH).

MeOH and water were added to quench the NaH. The solution was then extracted with ethyl acetate and washed with brine. The organic layer was dried with anhydrous sodium sulfate, filtered and then concentrated yielding 0.076 g of product (0.272 mmol, 72 %yield). Identification of the desired product was confirmed through ^{1}H NMR spectroscopy. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.89 (s, 3H), 3.90 (s, 3H), 3.93 (s, 3H), 4.00 (s, 3H), 4.30 (s, 3H), 6.81 (s, 1H), 7.15 (s, 1H).

5.6 Indole Acid

A 2-neck, 50 mL RBF was fitted with a condenser, stir bar, thermometer adapter and thermometer. The system was then placed under N_2(g). To the 2-neck RBF 13.0 mL MeOH and 0.500 g (1.88 mmol, 1 eq) of indole ester were added and stirred to form a yellow solution. Five milliliters of 3M KOH was added to the flask. Upon addition of the KOH, condensation formed on the sides of the condenser indicating an exothermic reaction. Heat was applied and the solution refluxed (~69℃) for 1 hour. A small amount of the solution in the flask was worked up in a vial by adding ~8 drops of 1M HCl and 8 drops of EA. The top layer of this mixture was then used for TLC (70/30 Hex/EA).

The solution was concentrated through rotary evaporation and 1M HCl was added drop wise until the pH reached 2, forming a precipitate. The precipitate was then collected through vacuum filtration yielding 0.401g (85% yield) of product. Identification of desired product was confirmed through infrared (IR) spectroscopy and ^{1}H NMR spectroscopy. IR ν_{max} 3350-2630, 3278, 1652, 1261 cm^{-1}; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 3.78 (s, 3H), 3.80 (s, 3H), 3.90 (s, 3H), 6.93 (s, 1H), 7.02 (s, 1H), 11.52 (s, 1H).

5.7 Trimethoxy Indole

A 2-neck, 50 mL RBF was fitted with a condenser, stir bar, thermometer adapter and thermometer. The system was then placed under N_2(g). The flask was charged with 5.0 mL of quinoline and then 0.100 g (0.398 mmol, 1 eq) of indole acid was added. Next, 0.034 g (0.535 mmol, 1.3 eq) of copper powder was added and heat was applied to the

mixture. The mixture was allowed to reflux for 3 hours. TLC (70/30 Hex/EA) was used to compare indole acid that had been solvated in DCM to the product.

Vacuum filtration was performed using P5 filter paper in a Buchner funnel to remove the copper powder. A second filtration was performed utilizing Q4 filter paper. To the collection flask, 50.0 mL of ice was added and then HCl was added dropwise until a pH of 4 was reached. The solution was then extracted with DCM (3x10 mL) and washed with 2M HCl (3x13 mL), saturated $NaHCO_3$ (3x14 mL) and brine (2x15 mL). The solution was then dried over anhydrous Na_2SO_4 and then gravity filtered into a 1-neck RBF. A column was then run on the resulting material to give a purified yield of 23%. ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 3.91 (s, 3H), 3.92 (s, 3H), 4.08 (s, 3H), 6.46 (q, 1H), 7.14 (q, 1H), 7.27 (s, 1H), 8.25 (s, 1H).

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