The Synthesis of Cribrostatin 6 Analogs

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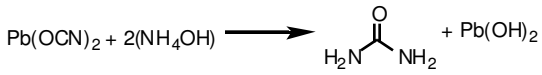
ABSTRACT

The compound cribrostatin 6, isolated in 2003 from the blue colored marine sponge Cribrochalina sp., has been found to exhibit both potent antimicrobial activity, as well as antineoplastic activity, making it of interest for its biological functionalities, as well as its unique tricyclic imidazo[5,1-a]isoquinolinedione structure. Using recently established methodology by Knueppel and Martin, known to be the shortest reported sequence of steps to synthesize this compound, analogs of cribrostatin 6 can be designed and synthesized to potentially find more biologically effective analogues, which can also be tested for antimicrobial and antineoplastic activity. Currently efforts are underway on the synthesis of additional analogs of cribrostatin 6.

Introduction

In a note written in 1828 from the German chemist Friedrich Wöhler to his mentor Jöns Jacob Berzelius, Wöhler proclaimed "I must tell you that I can make urea without the use of kidneys, either man or dog. Ammonium cyanate is urea," and thus from his discovery, the field of natural product [organic] synthesis was born.[1] Up until the mid-18th century, scientists believed mostly in a widespread belief called "vitalism" which maintained that organic compounds could be modified by chemistry but could only be produced through the agency of a vital force present in living plans and animals. The term "organic" had not been used even until 1807 when Berzelius suggested its use to distinguish between compounds devised from living (organic) and nonliving (inorganic) matter. When Wöhler synthesized urea, an organic compound, from the inorganic substances, lead cyanate and ammonium hydroxide (Scheme 1), scientists began to realize that organic compounds could arise from sources other than live plants and animals.

Scheme 1: Synthesis of Urea: lead cyanate + ammonium hydroxide → urea + lead hydroxide



The theory of vitalism eroded as synthetic chemists began producing organic compounds in the lab over the next two decades that followed. Hermann Kolbe's synthesis of acetic acid in 1844 and Marcellin Berthelot's prolific work, which produced many organic compounds, such as methane and acetylene in 1850, marked the end of the vitalism theory and ever since then, organic chemists have not looked back.

Of the many significant awards that have been bestowed upon chemists, The Nobel Prize in Chemistry and the Wolf Prize in Chemistry both have been awarded to many chemists for their work in the field of or contributions to natural product synthesis. Bookended currently by E. Fischer's Nobel Prize in 1902 for his work on sugar and purine synthesis as the earliest synthetic award and R. Grubbs's Nobel Prize in 2005 for the development of the metathesis method in organic synthesis, the field continues to grow, even as we approach the 200-year anniversary of the birth of the field.

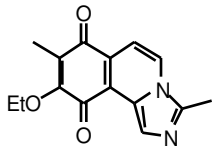
Chemists continue to pursue the field of natural product synthesis for the many challenges and rewards it offers. K.C. Nicolaou aptly commented that "There are many reasons why natural product synthesis withstood the test of time as an enabling and rewarding science and technology, not to mention its attractiveness as an intellectual and creative endeavor offering opportunities for discovery and invention."[2] These reasons include taking on the challenge presented by certain molecular architectures in order to discover and invent new synthetic strategies and methods, finding a route to synthesizing a natural product in larger quantities for exploration of its biological activity and applications, applying a unique set of strategies and methods to alter the structure of a natural product in an effort to enhance its selectivity and biological potency, confirming and characterizing natural products in the literature, and attempting to synthesize monumental natural products simply for the sake of synthesis.

My thesis work ties together a couple of these reasons in a project that looks to utilize the intriguing molecular architecture of cribrostatin 6 and explore the biological activity of analogs of this compound with respect to anticancer and antibacterial activity.

Background

As antibiotic-resistant Gram-positive bacteria becomes a bigger threat to society because of their increasing resistance to multiple drugs, the need for new structural classes of antibiotics continues to increase. Cribrostatin 6 (Figure 1) is one of a number of natural product compounds isolated from the blue marine sponge *Cribrochalina* sp.

Figure 1: Cribrostatin 6



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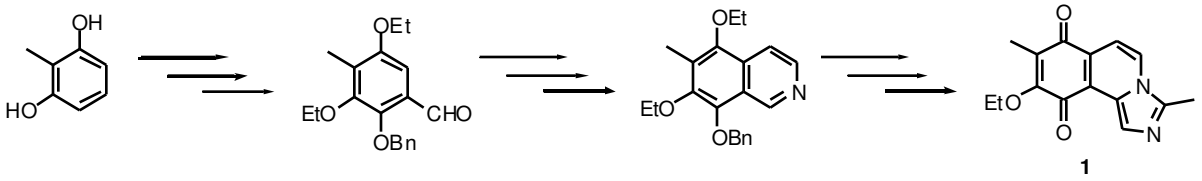
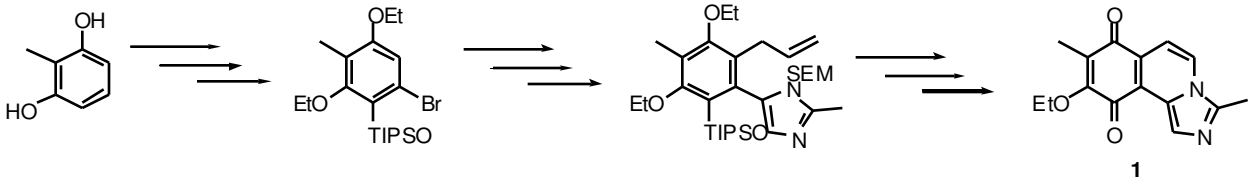
Isolated in 2003 by Pettit et al.[3], cribrostatin 6 (**1**) is a dark blue cancer cell growth inhibitor of both murine P388 lymphocytic leukemia and human cancer cell lines. In addition to this broad anticancer activity, cribrostatin 6 also exhibits antimicrobial potency against a number of antibiotic-resistant Gram-positive bacteria and pathogenic fungi, with the most efficacy against *Streptococcus pneumoniae*, the most common bacterial cause of bacterial meningitis.

There has been interest in synthesizing cribrostatin 6, due in part to its interesting biological activity, but also because of its unique tricyclic imidazo[5,1-a]isoquinolinedione structure. Both the Nakahara[4] (Scheme 2) and Kelly[5] (Scheme 3) groups have devised syntheses of cribrostatin 6 before, but the novel total synthesis by Knueppel and Martin[6] (Scheme 4) is less than half as along as the shorter of the two previous syntheses, with a much higher total yield.

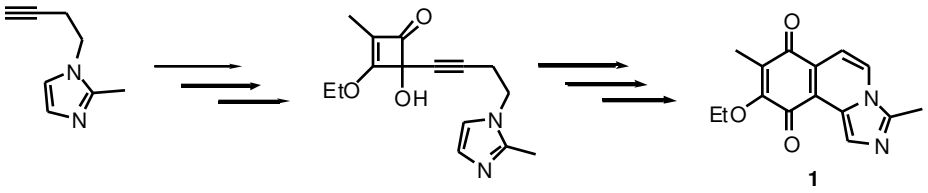
Scheme 2: Nakahara's synthesis (18 linear/total steps, 0.79% yield)



Scheme 3: Kelly's synthesis (13 linear steps, 15 total steps, 3.10% yield)



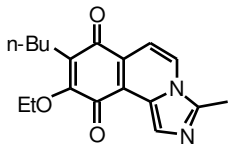
Scheme 4: Knueppel and Martin synthesis (4 linear steps, 5 total steps, 14.1% yield)



Results and Discussion

The goal of this project was to synthesize new analogs of cribrostatin 6 for biological testing using the synthetic route pioneered by Daniel Knueppel as a template for assembling together the starting materials. From an ease of accessibility standpoint, the n-butyl/ethoxy analog (Figure 2) was the first compound pursued because of the high similarity between the synthetic approach to it and cribrostatin 6 (**1**).

Figure 2: n-butyl/ethoxy cribrostatin 6 analog



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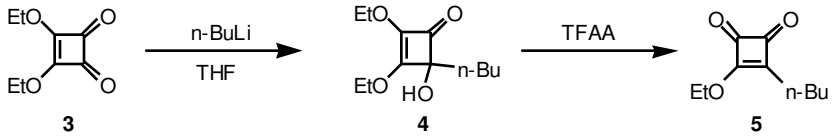
An n-butyl/ethoxy squarate derivative (**5**) was prepared as shown in Scheme 5, using the same approach that was used to obtain the methyl/ethoxy squarate used in Daniel's natural product synthesis.

Scheme 5: n-butyl/ethoxy squarate synthesis

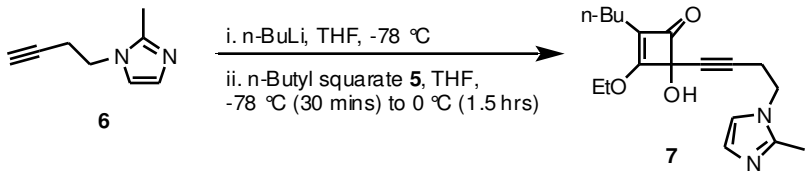


3 **4** **5**

The starting material for this reaction was synthesized following a literature procedure calling for squaric acid to be converted into diethyl squarate (**3**)[7], and then the addition of n-butyl lithium to the **3**, gave the alkoxide (**4**), which was then treated with TFAA to obtain the desired n-butyl/ethoxy squarate (**5**).

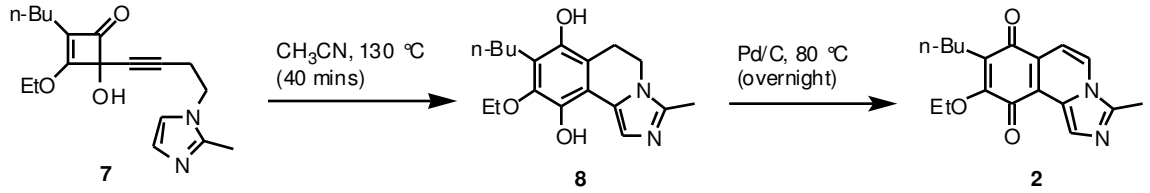
The linear forward synthesis used was the same used for cribrostatin 6; upon bringing up enough of the necessary alkyne XX, the key intermediate XX was generated by coupling the alkyne XX with the n-butyl/ethoxy squarate derivative used in place of the methyl/ethoxy squarate like in the cribrostatin 6 synthesis, as shown in Scheme 6.

Scheme 6: Synthesis of key intermediate 7



Multiple attempts to generate the key intermediate (**7**) were necessary; azeotroping the alkyne (**5**) with benzene helped eliminate all water from the reaction and was perhaps the best approach to obtaining the key intermediate. Despite the low 33% yield of key intermediate (**7**), 123mg was enough to push forward to obtain analog **2**.

Scheme 7: Completion of the synthesis of the n-butyl/ethoxy cribrostatin 6 analog



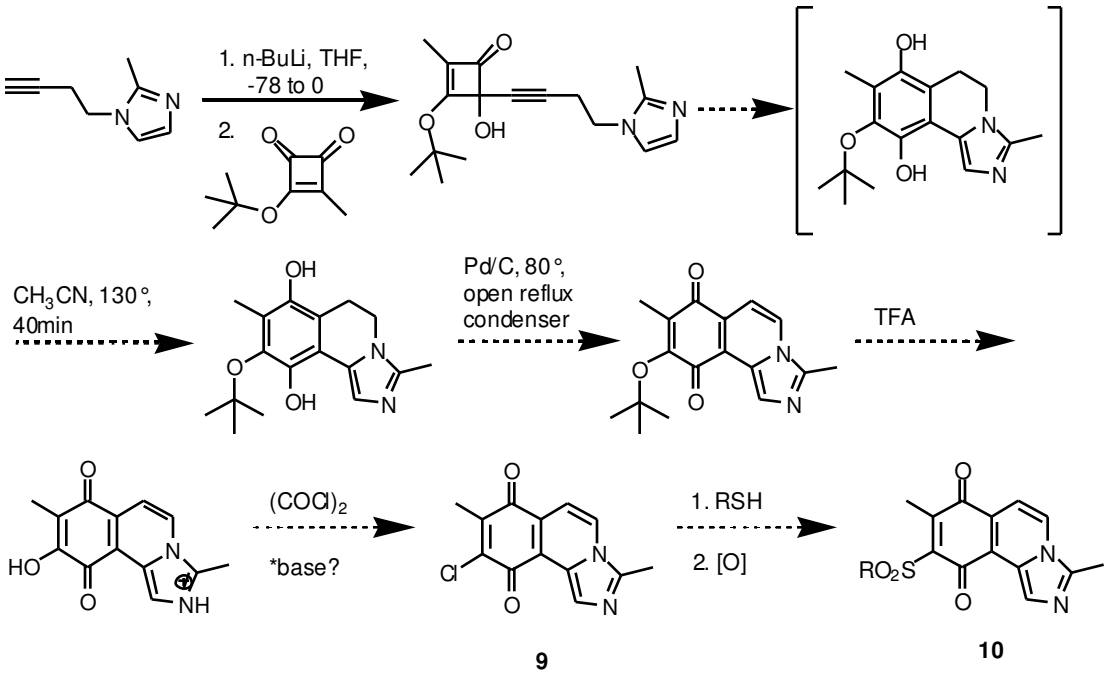
Using the one-pot procedure designed for the cribrostatin 6 synthesis, the key intermediate **7** was heated alone in acetonitrile under argon for 40 minutes to form the hydroquinone **8** (Scheme 7), as indicated by a color change from peach to dark brown/amber. A majority of the solvent was then evaporated before Pd/C was added to the hydroquinone, and the reaction was left to stir overnight with a reflux condenser while open to air, to aid in oxidation, to yield the n-butyl/ethoxy cribrostatin 6 analog (**2**).

Upon workup, the cribrostatin 6 analog **2** was obtained as a dark navy blue solid in 11.4% yield from **7**. On the TLC plate the analog was easily identified by a blue spot, a trademark

similar to that of cribrostatin 6. Analog **2** was characterized and then sent to the Hergenrother group for biological activity testing.

Results obtained from the Hergenrother group revealed that analog **2** was not anywhere near being significantly more effective than cribrostatin 6 against the two cell lines tested (HL-60, leukemia; U-937, lymphoma). Based on the currently known information about the cribrostatin 6 mode of action, a suggested focus/idea for the synthesis of more effective compounds was to focus on synthesizing cribrostatin 6 analogs that would be more easily bioreduced, most likely by cytochrome p450s, in the cell. Because bioreduction correlates with electrochemical reduction potentials, the focus was shifted toward designing compounds with higher [more positive] electron reduction potentials. This led to the current pursuit of analogs **9** and **10**, as shown in Scheme 8.

Scheme 8: Current progress to cribrostatin 6 analogs **9** and **10**



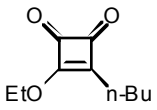
Conclusion

In summary, using the synthetic route devised by Knueppel and Martin as a template, a new cribrostatin 6 analog was produced and submitted for biological activity testing. Based upon preliminary results obtained, new analogs were identified as being potentially more effective candidates and work is ongoing to synthesize those analogs in the lab.

Ideally, future work on this project would be centered around synthesizing analogs **9** and **10** with higher reduction potentials and testing them for biological activity while also measuring their chemical reduction potentials in order to find whether or not a correlation of cytotoxicity and reduction potential exists.

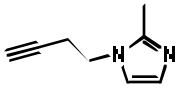
General Methods

General Methods. Solvents and reagents were reagent grade and used without purification unless otherwise specified. THF was passed through two columns of neutral alumina. CH_3CN was passed through two columns of molecular sieves. Reactions involving air- or moisture-sensitive reagents or intermediates were performed under an inert atmosphere of argon or nitrogen in glassware that had been oven dried. ^1H and NMR spectra were obtained as solutions in $CDCl_3$, and chemical shifts are reported in parts per million (ppm) in reference to $CDCl_3$ (7.24 ppm). Coupling constants are reported in hertz (Hz). Spectral splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; app, apparent; br, broad; m, multiplet; comp, complex multiplet. All products that were used without further purification were >95% pure by ^1H NMR spectroscopy.



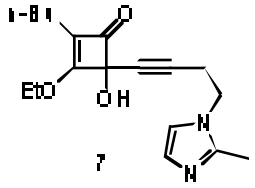
5

3-butyl-4-ethoxycyclobut-3-ene-1,2-dione (5) (1vc41). A solution of *n*-BuLi (3.34 mL, 2.6 M, 8.82 mmol) in hexanes was added dropwise to a solution diethyl squarate (1.5 g, 8.82 mmol) in THF (20 mL) at –78 °C. After 10 minutes, the solution was quenched with trifluoroacetic anhydride (1.47 mL, 10.58 mmol) and 15 mL of 10% aqueous NH_4Cl, whereupon it was cooled to room temperature. The mixture was extracted with Et_2O/5% aqueous $NaHCO_3$ (1:1) (3 × 10 mL each). The aqueous layers were washed with Et_2O (2 x 5 mL). The combined organic phases were dried (Na_2SO_4), and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography eluting with EtOAc/CH_3Cl (1:3) to give 0.91 g (57%) of **5** as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.78 (m, 2 H), 2.59 (m, 2 H), 1.68 (m, 2 H), 1.44 (m, 3 H), 1.38 (m, 2 H), 0.95 (m, 3 H).



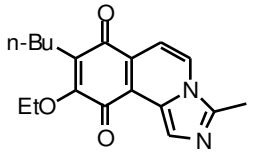
6

1-(but-3-ynyl)-2-methyl-1H-imidazole (6) (1vc25). 2-Methylimidazole (4.58 g, 55.74 mmol) was added to a solution of but-3-ynyl 4-methylbenzenesulfonate (2.5 g, 11.15 mmol) in CH_3CN (15.5 mL). The reaction was heated for 18 h at 70 °C, whereupon it was cooled to room temperature, and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography eluting with $CHCl_3$/MeOH (10:1) to give 0.81 g (54%) of **34** as a clear oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.85 (d, J = 1.2 Hz, 1 H), 6.82 (d, J = 1.2 Hz, 1 H), 3.95 (t, J = 7.0 Hz, 2 H), 2.53 (dt, J = 7.0 Hz, 2 H), 2.35 (s, 3 H), 1.99 (t, J = 2.6 Hz, 1 H).



7

2-butyl-3-ethoxy-4-hydroxy-4-(4-(2-methyl-1H-imidazol-1-yl)but-1-ynyl)cyclobut-2-enone (7) (1vc44). A solution of *n*-BuLi (0.55 mL, 2.6 M, 1.42 mmol) in hexanes was added dropwise to a solution of **6** (158 mg, 1.18 mmol) in THF (5.9 mL) at –78 °C. After 35 min at –78 °C, a solution of 3-butyl-4-ethoxycyclobut-3-ene-1,2-dione (**5**) (323 mg, 1.77 mmol) in THF (5 mL) at –78 °C was added dropwise via cannula. After 15 min at –78 °C, stirring was continued for 1.5 h at 0 °C. Saturated aqueous NH_4Cl (5 mL) and brine (5 mL) were added, and the mixture was extracted with EtOAc (3 × 10 mL). The combined organic phases were dried (Na_2SO_4), and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography eluting with $CHCl_3$/MeOH (10:1) to give 124 mg (33%) of **7** as an amber oil.



2

8-butyl-9-ethoxy-3-methylimidazo[5,1-a]isoquinoline-7,10-dione (2) (1vc45). A solution of **7** (124 mg, 0.40 mmol) in CH_3CN (300 mL) was heated at reflux for 40 min in a preheated oil bath (130 °C). After cooling to room temperature, the solution was concentrated to approximately 5 mL by evaporation under reduced pressure. Pd/C (17 mg, 10 wt. % loading) was added along with 12 mL CH_3CN and approximately 1 mL MeOH, and the reaction was heated for 18 h at 80 °C. After cooling to room temperature, the solvent was removed under reduced pressure, and the

residue was purified by flash column chromatography eluting with $CHCl_3$/MeOH (10:1) to give 13.9 mg of **2** (11%) as a blue solid.

References

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Vicki Chang was born in Richardson, Texas on September 30th, 1987 and grew up there for a few years before moving with her family to Sugar Land, Texas in 1990. She enrolled in the Plan II Honors Program at The University of Texas at Austin in 2006 and studied chemistry in the Dean's Scholars Program as well. During the summer after her junior year, she studied abroad with the American Institute of Roman Culture. During her time in college, she was a member of the American Chemical Society, Camp Texas, the Natural Sciences Council, Orange Jackets, the Plan II Students' Association, and thoroughly enjoyed the opportunity to mentor younger students in any fashion possible. She graduated Phi Beta Kappa in 2010 and plans to attend The Scripps Research Institute this fall to pursue her PhD in chemistry.