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Applying Green Chemistry Principles in the Electrophilic Bromination of Indole-3-Acetic Acid

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Kyle Murphy is a graduating senior majoring in Chemistry with concentrations in Professional Chemistry as well as Environmental Chemistry. This research began in the spring of 2011 under the mentorship of Dr. Edward Brush (Chemistry) and was funded by an Adrian Tinsley Program (ATP) Summer Research Grant. It was continued with funding from the Center for Sustainability in the summer of 2012. It was further supported by the Norris-Richards Undergraduate Summer Research Scholarship from the Northeastern Section of the American Chemical Society, as well as BSU's Center for Sustainability, in the summer of 2013. Kyle is thankful for the opportunities and support provided to him by the Office of Undergraduate Research, the Center for Sustainability, the Chemistry Department faculty, and Dr. Brush. In the fall of 2014 Kyle will begin a Ph.D. program in organic chemistry.

The goals of green chemistry are to reduce or eliminate the use of hazardous reagents, prevent the synthesis of toxic products and byproducts, and improve the overall efficiency of chemical reactions. Green chemistry is incredibly important today as chemical products are produced and used around the world, resulting in the use and generation of hazardous chemicals, and unintended consequences to human health and the environment. Figure 1 shows the 12 Principles of Green Chemistry[1], developed by Paul Anastas and John Warner, which provide the framework for a sustainable future in the design of more efficient technologies to produce consumer products that are better, safer and cheaper. As the research in our group is focused on improving the efficiency of chemical reactions, Principles 1, 2, 3, 5 and 8 were applied to this project.

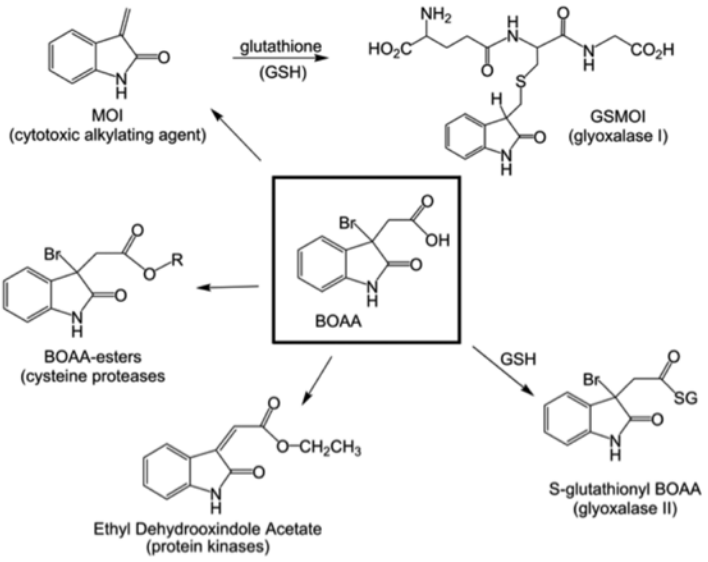
3-Bromooxindole-3-acetic acid (BOAA) is an important intermediate in our group's work on the design and synthesis of small-molecule mechanism-based enzyme inhibitors. Also referred to as "suicide substrates," this unique class of inhibitors that initially unreactive molecules are disguised to "trick" enzymes into acting on them as normal substrates. Once chemically activated the inhibitor molecule then rapidly, specifically and irreversibly attacks and shuts down the target enzyme. As shown in Figure 2, BOAA is readily transformed into a variety of oxindole derivatives that have the ability to inhibit anticancer therapeutic target enzymes such as glyoxalases I&II and the cysteine proteases.

The traditional method[2] to synthesize BOAA from indole-3-acetic acid (IAA) is illustrated in Figure 3 where N-bromosuccinimide (NBS) serves as brominating agent and tert-butanol acts as both solvent and reactant, providing the C-2 oxindole oxygen. We have found major complications with this synthesis, including the use and generation of hazardous chemicals, poor atom economy (30%), and low percent yield (25%). NBS is a major source of the waste byproducts that include succinimide, and hydrobromic acid (HBr), which is a strong acid (see box in Figure 3). Note that of the 24 atoms in two mole equivalents of NBS, only one bromine atom is incorporated in the final BOAA product, contributing to the low atom economy. Based on previous studies[3,4] we suspected that the low percent yield was primarily due to poor regioselectivity in the bromination of IAA, resulting in the formation of isomeric brominated products. It has been reported that the selectivity

Figure 1. The Twelve Principles of Green Chemistry

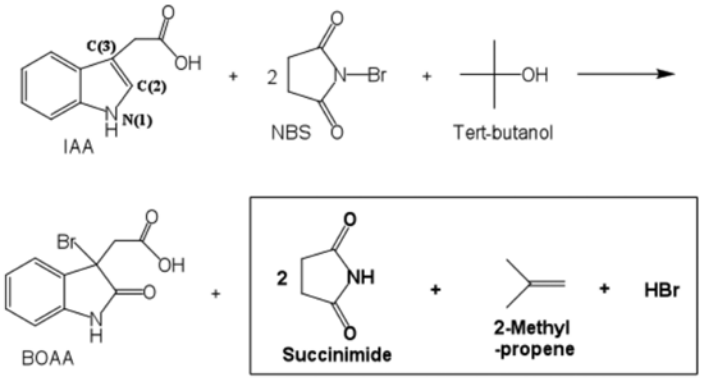
1. **Prevention**
   It is better to prevent waste than to treat or clean up waste after it has been created.
2. **Atom Economy**
   Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.
3. **Less Hazardous Chemical Syntheses**
   Wherever practicable, synthetic methods should be designed to use and generate substances that process little or no toxicity to human health and the environment.
4. **Designing Safer Chemicals**
   Chemical products should be designed to affect their desired function while minimizing their toxicity.
5. **Safer Solvents and Auxiliaries**
   The use of auxilary substances (e.g., solvents, separation agents, etc.) should be made unnecessary wherever possible and innocuous when used.
6. **Design for Energy Efficiency**
   Energy requirements of chemical processes should be recognized for their environmental and economic impacts and should be minimized. If possible, synthetic methods should be conducted at ambient temperature and pressure.
7. **Use of Renewable Feedstocks**
   A raw material of feedstock should be renewable rather than depleting whenever technically and economically practicable.
8. **Reduce Derivates**
   Unnecessary derivatization (use of blocking groups, protection/deprotection, temporary modification of physical/chemical processes) should be minimized or avoided if possible, because such steps require additional reagents and can generate waste.
9. **Catalysis**
   Catalytic reagents (as selective as possible) are superior to stochiometric reagents.
10. **Design for Degradation**
    Chemical products should be designed so that at the end of their function they break down into innocuous degradation products and do not persist in the environment.
11. **Real-time Analysis for Pollution Prevention**
    Analytical methodologies need to be further developed to allow for real-time, inprocess monitoring and control prior to the formation of hazardous substances.
12. **Inherently Safer Chemistry for Accident Prevention**
    Substances and the form of a substance used in a chemical process should be chosen to minimize the potential for chemical accidents, including releases, explosions, and fires.

Figure 2. Potential Mechanism-Based Inhibitors Based on Oxindole Derivatives.



of NBS-mediated brominations can be controlled with amides and amidines that act as Lewis base catalysts by either facilitating the regioselective transfer of bromine from NBS to the acceptor, or stabilizing the bromonium ion intermediate.[5-8]

Figure 3. Traditional Method of Synthesizing BOAA from IAA.



The goal of this research project was to obtain a better understanding of the overall BOAA synthetic process and apply the Principles of Green Chemistry to improve reaction efficiency. Utilizing ^{1}H NMR we have: identified four major products from IAA bromination, determined that the reaction process occurs in two discrete steps, and obtained key mechanistic insight that will help us improve on the overall efficiency of this reaction.

Experimental

All reagents were purchased from Sigma-Aldrich or Fisher Scientific and used without further purification. Tert-butanol was stored over 3Å molecular sieves. Nuclear Magnetic Resonance (NMR) spectra were obtained on a JEOL ECX-400 MHz instrument.

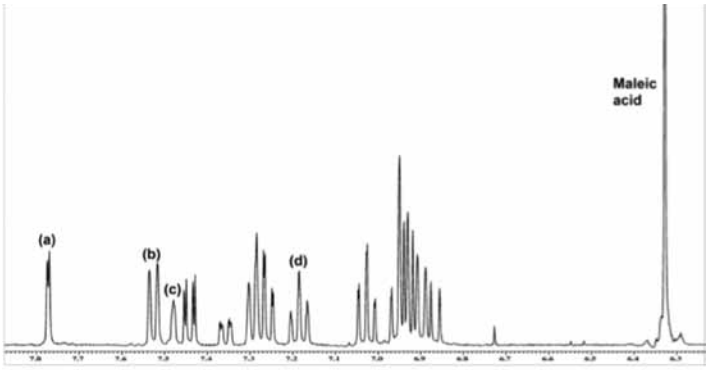
Evaluating different reaction parameters for the synthesis of BOAA would require substantial amounts of solvent and reagents, produce liters of hazardous waste, and an entire day would be needed to run and analyze a single reaction. To optimize our "green chemistry" approach, we developed a quick, reproducible and efficient reaction screening method that required milliliters of solvent, milligrams of reagents, and minimized waste. The screening method used quantitative NMR (qNMR)[9] to determine % yield and recovery. In qNMR the quantity of a particular analyte could be determined by comparing the integrated value of an analyte signal of known number of H's to the integrated value of the vinyl protons (2H, 6.33 ppm) of the maleic acid internal NMR reference standard.[10]

The standard screening reaction used 200 µL of a 0.174 M solution of IAA in tert-butanol (6.11 mg, 34.9 µmole) added to a 10 mL reaction vial with an additional 800 µL of tert-butanol. The solution was stirred at room temperature and the reaction was initiated by the addition of two mole equivalents (12.4 mg, 69.8 µmole) of NBS. After stirring for an additional 10 minutes, the reaction mixture was concentrated by evaporation of tert-butanol solvent on a high-vac followed by suspension of the residue in diethyl ether. The succinimide precipitate was removed by filtration into a 10 mL round bottom flask and the diethyl ether evaporated under reduced pressure. Acetone-d_6 containing 0.05% TMS (1.0 mL) was added to dissolve the residue, then 2.00 mg (0.0172 mol) of maleic acid dissolved in 25 µL of dimethylsulfoxide-d_6 was added as the internal qNMR reference standard. This screening method allowed us to run and analyze up to five reactions per day. We employed this screening method to survey a variety of Lewis base and acid catalysts for improving the overall efficiency of BOAA synthesis.

Results and Discussion

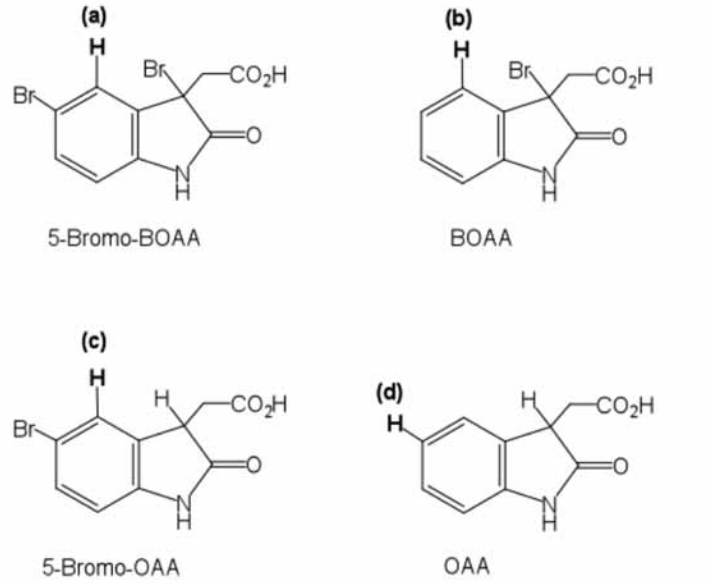
The ^{1}H-NMR spectrum of the residue obtained from the traditional reaction mixture with two equivalents of NBS, is shown in Figure 4. By focusing exclusively on signals in the aromatic region between 6.5 and 8 ppm, we were able to identify four major IAA bromination products as shown in Figure 5.

Figure 4. ^{1}HNMR (400 MHz) of IAA Reaction with Two Equivalents of NBS.



We propose that the bromination of IAA with two equivalents of NBS occurs in two reaction steps as shown in Figure 6. In Reaction #1 the initial mole equivalent of electrophilic bromine (NBS) adds rapidly (within seconds) to the C-2,3 double bond (possibly with assistance from N-1 of IAA), followed by addition of tert-butanol to C-2. Subsequent elimination steps (mechanism not shown) produce oxindole-3-acetic acid (OAA). We also observe 5-bromo-OAA most likely from the

Figure 5. Oxindole Byproducts of Complete IAA-NBS Reaction.

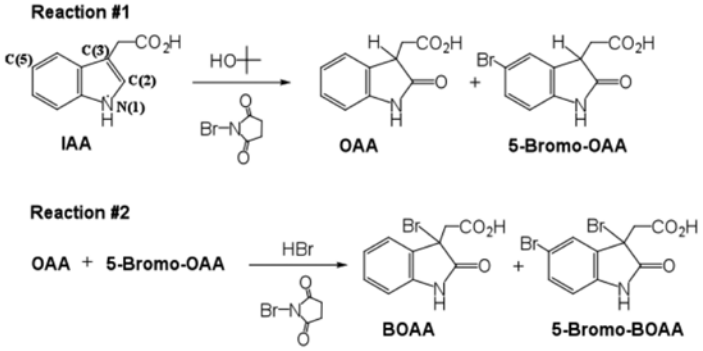


(a) 3,5-dibromooxindole-3-acetic acid (5-Bromo-BOAA), (b) 3-bromooxindole-3-acetic acid (BOAA), (c) 5-bromooxindole-3-acetic acid (5-Bromo-OAA), (D) oxindole-3-acetic acid (OAA).

addition of a second equivalent of bromine to C-5 of OAA (consistent with literature precedent[11]). Reaction #2 is slow addition of the second mole equivalent of NBS to C-3, producing BOAA and 5-bromo-BOAA. Our proposal is consistent

with the observation of C-3 and C-5 brominated products from indoles.[3,4,11] The C-3 position of indoles is known to undergo bromination very rapidly, while C-5 bromination was based on the known activating effects of the N-1 nitrogen (C-7 brominated product was not observed). These original studies employed indirect methods for product analysis based on chemical transformation to known compounds, followed by melting point determination. Our work is the first to conclusively identify the IAA bromination products using ^{1}H NMR analysis.

Figure 6. Proposed Reaction Scheme for IAA Bromination.



To test the two-step reaction scheme proposed in Figure 6, we ran the traditional BOAA synthesis from IAA by adding NBS in two separate steps. The results of these reactions (data not shown) clearly show that addition of one mole equivalent of NBS produces OAA and 5-bromo-OAA. Subsequent addition of a second NBS mole equivalent gives the typical product distribution shown in Figure 5.

Our preliminary data suggested that addition of the first equivalent of electrophilic bromine to IAA was critical for the efficient production of brominated product. As shown in Step 1 in Figure 6, multiple oxindole byproducts are produced and that this poor regioselectivity was ultimately responsible for the poor overall efficiency of this reaction. Our ability to successfully run the BOAA synthesis as two separate reactions (Figure 6) allowed us to examine the effect of changing reaction parameters on the overall reaction efficiency. We conducted preliminary experiments to examine the effect of amides and amidines as Lewis base catalysts in controlling the selectivity of NBS-mediated IAA bromination. Furthermore, we suspected that the HBr generated during the course of Reaction #1 (Figure 6) catalyzes C-3 halogenation of OAA through an enol intermediate. The effect of using various Lewis base and acid catalysts on the overall synthesis is shown in Table 1.

Table 1. %Composition of Oxindole Products as a Function of Reaction Conditions

Compound	NMR Signal (ppm)/ (#protons)	% Composition of oxindole products as a function of reaction conditions			
		Control (Standard Reaction)	DMF	Acetic acid	Triethyl-amine
Maleic Acid	6.33(2)	------	------	------	------
BOAA	7.52(1)	47.5%	46.8%	42.9%	trace
OAA	7.18(1)	27.8%	34.4%	45.8%	71.8%
5-Br-OAA	7.48(1)	6.1%	2.8%	3.1%	trace
5-Br-BOAA	7.77(1)	18.1%	15.8%	7.8%	trace

When dimethylformamide (DMF) was added to Reaction #1 there was modest improvement in the regioselectivity by promoting BOAA production as compared to the oxindole byproducts. Similar results were obtained when acetic acid was added to Reaction #2, consistent with acid catalyzed halogenation. Furthermore, addition of triethylamine inhibited production of BOAA and other oxindole byproducts, possibly by neutralizing any HBr produced in Reaction #1.

Conclusions and Future Work

We have applied Green Chemistry principles and NMR analysis to better understand the synthetic reactions in the preparation of BOAA from IAA. We have identified four oxindole products from this reaction and determined that IAA bromination occurs in two discrete steps. These preliminary results have provided key mechanistic insight that will help us improve the low yield and overall efficiency of this reaction. We are currently evaluating additional Lewis base catalysts to improve the regioselectivity in Reaction #1, and the effect of acid catalysts for Reaction #2. We are also very interested in developing an efficient gram-scale synthesis of oxindole-3-acetic acid (OAA) in Reaction #1, as this compound has recently been identified in the regulation of auxin homeostasis and response mechanisms in plants[12], suggesting that OAA may play a role in controlling plant growth and regulation.

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References

[1]Anastas, P. T.; Warner, J. C. *Green chemistry: theory and practice;* Oxford University Press: Oxford [England]; New York, 1998.

[2]Hinman, R.L and Bauman, C.P. (1964), "Reactions of N-Bromosuccinimide and Indoles. A simple synthesis of 3-bromooxindoles," *J. Org. Chem*., 29, 1206-1215.

[3]Lawson, W.B., Patchornaink, A., and Witkop, B. (1960), "Substitution, oxidation and group participation in the bromination of indoles," *J. Amer. Chem. Soc*., 82: 5918-23.

[4]Condie, G.C., Channon, M.F., Ivory, A.J., Kumar, N. and Black, D.St.C. (2005), "Regioselective reactivity of some 5,7-dimethoxyindoles," *Tetrahedron*, 61(21): 4989-5004.

[5]Denmark, S.E. and Burk, M.T. (2010), "Lewis base catalysis of bromo- and iodolactonization, and cycloetherification," *Proc. Natl. Acad. Sci. USA*., 107(48), 20655-20660.

[6]Pathak, T. P. and Miller, S. J. (2012), "Site-selective bromination of vancomycin," *J. Amer. Chem. Soc.,* 134, 6120-6123.

[7]Ahmad, S. M., Braddock, D. C., Cansell, G., Hermitage, S. A., Redmond, J. M., and White, A. J. P. (2007), "Amidines as potent nucleophilic organocatalysts for the transfer of electrophilic bromine from N-bromosuccinimide to alkenes," *Tetrahedron Letters*, 48, 5948-5952.

[8]Ahmad, S. M., Braddock, D. C., Cansell, G. and Hermitage, S. A. (2007), "Dimethylformamide, dimethylacetamide and tetramethylguanidine as nucleophilic organocatalysts for the transfer of electrophilic bromine from N-bromosuccinimide to alkenes," *Tetrahedron Letters*, 48, 915-918.

[9]Peterson, J. (1992), "[1]H NMR Analysis of Mixtures Using Internal Standards," *J. Chem. Educ.,* 69 (10): 843-5.

[10]JEOL Resonance Application Note NM090009E (2011), "What is qNMR (quantitative NMR)?", www.jeol.co.jp (accessed October 2013).

[11]Sumpter, W.C., Miller, M., and Hendrick, L.N. (1945), "A study of certain brominated derivatives of oxindole", *J. Amer. Chem. Soc.,* 67(10): 1656-8.

[12]Pencik, A., Simonovik, B., Petersson, S.V., Henykova, E., Simon, S., Greenham, K., Zhang, Y., Kowalczyk, M., Estelle, M., Zazimalova, E., Novak, O., Sandberg, G., and Ljung, K. (2013), "Regulation of auxin homeostasis and gradients in *Arabidopsis* roots through the formation of the indole-3-acetic acid catabolite 2-oxindole-3-acetic acid," *Plant Cell*, October 25 Epub ahead of print, http://www.plantcell.org/content/early/2013/10/23/tpc.113.114421.full.pdf (checked November 2013).