

Summer Scholar Report

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

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Introduction

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. Paul Anastas and John Warner developed the 12 Principles of Green Chemistry¹, Figure 1, to provide the framework for a sustainable future in the design of more efficient technologies to produce consumer products that are better, safer and cheaper.

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	Whenever practicable, synthetic methods should be designed to use and generate substances that possess 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 auxiliary 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 or feedstock should be renewable rather than depleting whenever technically and economically practicable.
8. Reduce Derivatives	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 stoichiometric 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, in-process 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 1. *The Twelve Principles of Green*

3-Bromooxindole-3-acetic acid (BOAA) is an important intermediate in our group's work on the synthesis of small-molecule mechanism-based enzyme inhibitors. BOAA is readily transformed into a variety of oxindole derivatives designed to inhibit specific enzymes known to be therapeutic targets, Figure 2.

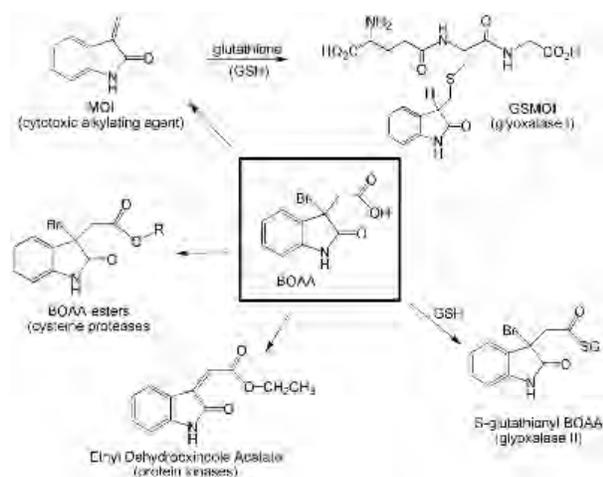


Figure 2. Potential mechanism-based inhibitors based on oxindole derivatives.

The traditional method to synthesize BOAA from indole-3-acetic acid (IAA) is illustrated in Figure 3.2 N-bromosuccinimide (NBS) serves as brominating agent, and tertbutanol is 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 including HBr and succinimide (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 literature precedent,^{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.

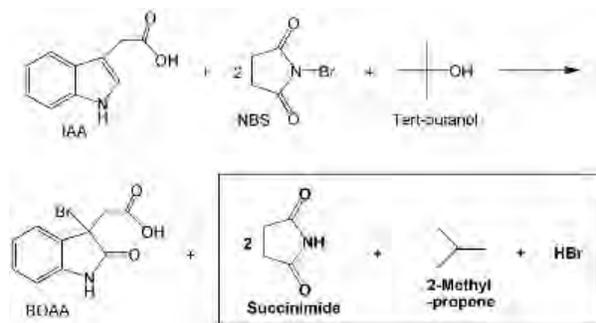


Figure 3. Traditional method to synthesize BOAA from IAA.

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 1H 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 is helping 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)⁵ 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.⁶

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 reaction 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 was worked up by evaporation of tert-butanol solvent on a high vac, and then suspending the residue in diethyl ether. The succinimide byproduct was removed by pipette filtration into a 10 mL round bottom flask and solvent again evaporated under reduced pressure. Acetone-d₆ 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-d₆ was added as the internal qNMR reference standard. This screening method allowed us to run and analyze up to five reactions per day. This general procedure allowed us to test the effect of various reaction parameters on the product distribution, including the IAA:NBS stoichiometry, and addition of potential catalysts.

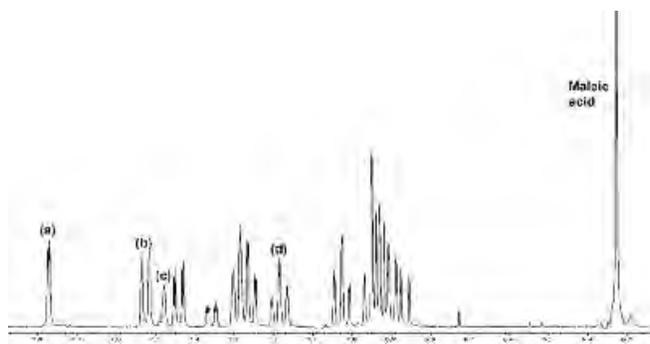


Figure 4. *¹H NMR (400 MHz) of IAA reaction with two equivalents of NBS.*

Results and Discussion

The traditional reaction mixture with two equivalents of NBS was worked up to remove residual solvent and succinimide byproduct, and the residue analyzed by ¹H NMR, Figure 4. By focusing exclusively on signals in the aromatic region, we were able to identify four major products from

IAA bromination, Figure 5.

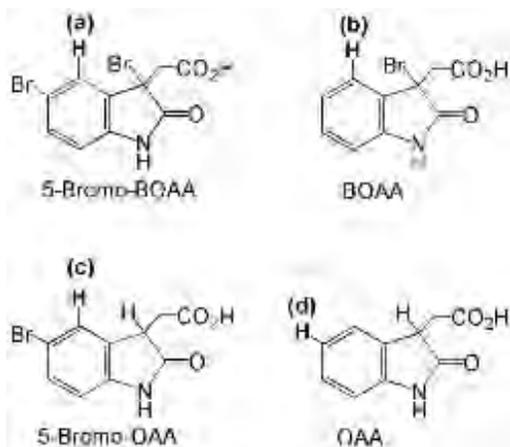


Figure 5. Oxindole byproducts of complete IAA-NBS reaction: (a) 3,5-dibromooxindole-3-acetic acid (5-Bromo-BOAA), (b) 3-bromooxindole-3-acetic acid (BOAA), (c) 5-bromooxindole-3-acetic acid (5-Bromo-OAA), (d) oxindole-3-acetic acid (OAA).

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 equivalent of electrophilic bromine adds rapidly (within seconds) to the C-2,3 double bond (possibly with assistance from N-1), 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 addition of a second equivalent of bromine to C-5 of OAA (consistent with literature precedent⁷). Reaction #2 is slow addition of the second 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.^{3,4,7} 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). The authors from 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 ¹H NMR analysis.

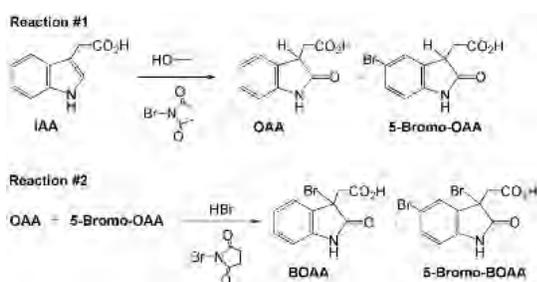


Figure 6. Proposed reaction scheme for IAA bromination.

To test this idea 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 equivalent gives the typical product distribution shown in Figure 5.

As our preliminary data suggested that addition of the first equivalent of electrophilic bromine to IAA was the critical step, in that multiple oxindole byproducts are produced and that this poor regioselectivity was ultimately responsible for the poor efficiency of this reaction. As we could now successfully run the BOAA synthesis as two separate reactions (Figure 6), this allowed us to focus exclusively on Reaction #1, and conduct preliminary experiments to improve the poor regioselectivity.

It has been reported that the selectivity 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.⁸⁻¹¹ Furthermore, in the traditional synthesis we suspected that HBr generated during the course of Reaction #1 catalyzes C-3 halogenation of OAA through an enol intermediate. We subsequently surveyed a variety of Lewis base, and acid catalysts for improving the overall efficiency of BOAA synthesis. Representative data are presented in Table I.

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

Table I. %Composition of oxindole products as a function of reaction conditions

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 as well as 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

Acknowledgements

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