Synthesis of Sodium Sulfonate Auxin Derivatives

Zachary D. Einwag

Senior Comprehensive Project
Allegheny College
Meadville, PA
4/11/2013

Senior Project Committee Members:

Dr. Shaun Murphree
Dr. Bradley Hersh
Dr. Timothy Chapp

I hereby recognize and pledge to fulfill my responsibilities as defined in the Honor Code, and to maintain the integrity of myself and the college community as a whole.

Pledge: _____

Table of Contents

I. Abstract……..…….……2

II. Introduction

a. Overview of Auxins…….…….……3

b. Auxin Transport …….…….….8

c. Scope of Project……10

III. Results and Discussion……..…..……..……..14

IV. Future Work……..……..……18

V. Experimental……..……..…….…….19

VI. References……..……..…….….21

VII. Spectra……..……..…....25

Abstract

Auxins are a naturally-occurring class of plant growth hormones that are vital to numerous functions within the plant, including root cell differentiation and elongation, phototropism, and gravitropism. Although the physiological effects of these hormones are well documented, there are several gaps in the knowledge surrounding their mechanism of action, as well as their transportation through the plant. The following project describes the synthetic routes to two analogs of known auxins. The proposed routes are similar in nature, first adding a strong leaving group onto the desired carbon, followed by an S_N2 attack by SO_3^{-2}. Both target auxin analogs are designed in such a way as to test various methods of auxin entry into plant cells, or proposed binding characteristics of auxin binding proteins.

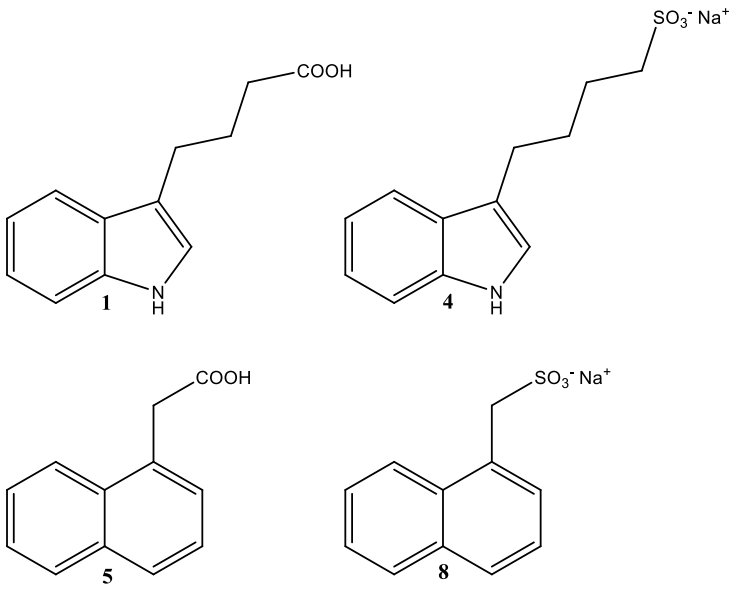


Figure 1: The naturally-occurring Indole-3-butyric acid (IBA) (**1**) and the synthetic 1-Napthaleneacetic acid (NAA) (**3**) besides theirs sulfonate analogs (**4** and **8**).

Introduction

Overview of the auxin

Auxins, one of the first discovered plant hormones, are a class of plant growth hormones responsible for several important physiological responses within plant growth, such as cell elongation and differentiation, phototropism, gravitropism, and organ patterning.[1,2] In particular, the regulation of auxin distribution throughout the plant (read concentration gradient) is vital for dictating a plant's growth in reaction to its environment, and for development of lateral root systems, leaves, and flowers.[3,4] It fulfills these roles by acting as a signaling molecule which enters the cell and binds to specific receptor proteins located throughout the cell. Once bound, a cellular cascade is initiated which then elicits the desired cellular response.

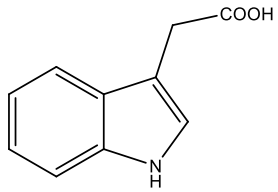


Figure 1: Indole-3-acetic acid, the first isolated auxin

Since the isolation of the first auxin in 1937 by Kenneth V. Thimann, a vast array of synthetic compounds, such as 2,4-dichlorophenoxyacetic acid (2,4-D), α-naphthalene acetic acid (NAA), and 2-methoxy-3,6-dichlorobenzoic acid (dicamba), with similar phenotypic effects have been created in labs around the world.[5] At the current time, the categorization of a compound as an auxin is determined principally by its basic biological functionality,[6] meaning that there is no defining structure against which one can compare a newly discovered molecule. While three of the four naturally-occurring auxins are indolic derivatives with carboxyl functional groups (the fourth, 2-phenylacetic acid, also contains a carboxyl functional group), many synthetic auxins are not bound by this structural constraint. Several studies have noticed this apparent lack of structural limitation, and attempted to define a set of guidelines by which one can structurally

identify a molecule as an auxin.[7,8] As of yet there have been no all-encompassing model found.[5] However, the majority of synthetic auxins can be grouped into five main structural categories (figure 2), all of which contain some aromatic group with an acidic group attached to the ring by an alkyl tether (typically one to four carbons in length).[5,9,10]

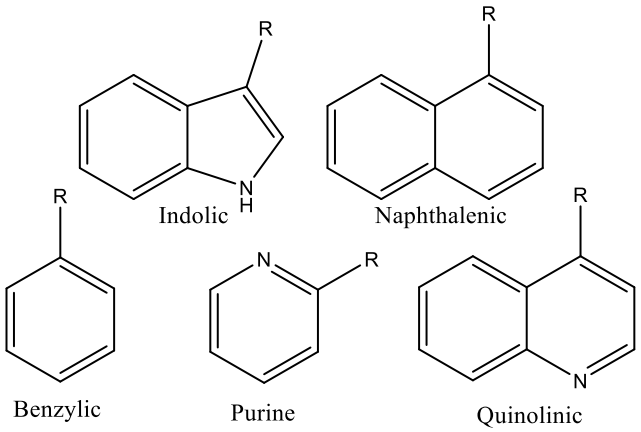


Figure 2: Main structural categories of the auxins (both synthetic and naturally occurring). The R group denotes position for the carboxylic acid containing group.

One way of understanding and defining the structural requirements for auxins is in terms of the binding/receptor sites with which they interact or bind. As with any other hormone, there are specific receptor proteins whose interaction with auxin is vital to producing the desired phenotypic effect.[11] Among the first discovered proteins capable of binding auxin is Auxin Binding Protein$_1$ (ABP1),[12] which is responsible for membrane hyperpolarization, cell expansion, and inhibition of endocytosis.[13] ABP1 also inhibits the efflux of auxin from the cell. However, once auxin is bound to ABP1, it stops inhibiting auxin efflux by preventing the clathrin-mediated endocytosis of PIN protein efflux pumps.[14] This results in more PIN proteins along the cellular membrane, thus resulting in higher efflux of auxin out of the cell.[15] For this strong affinity with auxins, ABP1 is considered to play a crucial role in the overall auxin signaling system.[13]

Another more recently studied auxin receptor is the Transport Inhibitor Response$_1$ (TIR1) protein. TIR1 is an F-box subunit of the SCF complex (a multi-protein ubiquitin ligase complex), meaning it facilitates binding interactions between proteins involved in the ubiquitination process.[16] In the case of TIR1, the SCF complex serves to recognize and bind to AUX/IAA proteins (a system of transcription regulator proteins). This ultimately leads to the degradation of the AUX/IAA protein.[17,18] Through the degradation of the AUX/IAA proteins, the transcription factors responsible for the regulation of auxin responsive genes (Auxin Response Factors or ARFs) are activated.[18]

Both TIR1 and ABP1 are receptor proteins involved in critical roles of the overall auxin binding system, and both bind directly to auxin molecules. By utilizing this knowledge, molecules could be designed in order to 'fit' these specific binding proteins. Because these proteins are known to be relevant in auxin response in plants, designing analogs to fit within their binding sites would likely result in auxin-like physiological response. The crystal structure of each of these proteins has recently been determined, and the binding sites observed.[19,20] From these observations, the interactions of the heterocycle of IAA and the napthyl ring of NAA are found to sit comfortably with both the TIR1 and ABP1 receptor proteins.

As can be noted from Figure 3, a view of ABP1's and TIR1's binding site has been determined through the use of x-ray crystallography.[19] The binding site of ABP1 contains a zinc ion held in place by several amino acid residues, three histidines and a glutamic acid. The carboxylic acid of the auxin can be seen to also interact with zinc ion, adding specificity and stability to the auxin molecule within to the binding pocket.[21] As the carboxylic acid is deprotonated at physiological pH, this interaction would be fairly strong. Figure 3 also shows that the area surrounding the aromatic region of the auxin in composed almost entirely of

hydrophobic amino acids. In addition to the hydrophobic region stabilizing the auxin, three of those hydrophobic amino acids (histidines, phenylalanine, and tryptophan) are aromatic in nature. This may also prove important to the stabilization of the auxin-ABP complex through pi-pi stacking.[21] The location of W151 directly beneath the naphthalene group of NAA (or the indole of IAA) adds support to this theory.

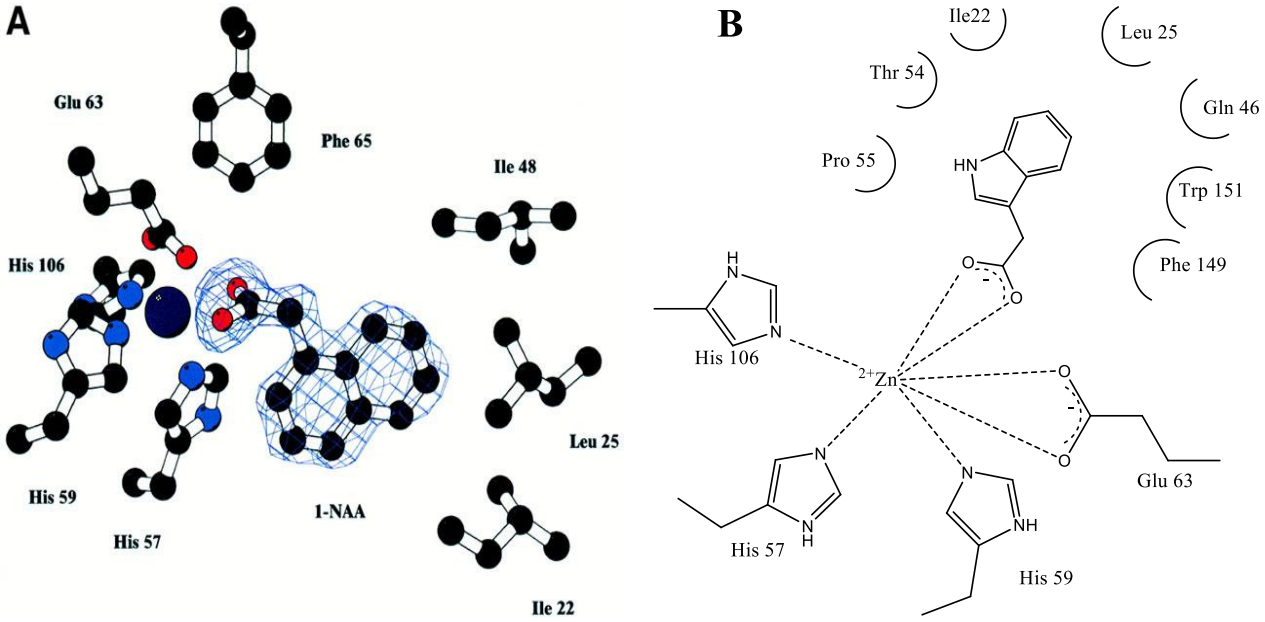


Figure 3: Two views of the active site of ABP1, each with its own auxin within the binding pocket (1-NAA in A and IAA in B). The hydrophobic amino acid residues surrounding the region are clearly visible.[19]

The binding of auxins to TIR1 is slightly similar (figure , in that the binding region contains several aromatic and nonpolar amino acid residues which forms a hydrophobic pocket for the auxin.[18] However, instead of a zinc ion further stabilizing the structure, several basic amino acids containing nitrogen are capable of hydrogen bonding to the carboxyl group of the auxin, and several other charged amino acids (specifically L439 and S438) further stabilize the complex by interacting with the nitrogen of the indole. The binding site is further stabilized by the hydrophobic region around F82 and F380.[20] This particular interaction could explain why most naturally-occurring auxin contains an indolic aromatic structure with the carboxylate functional group.

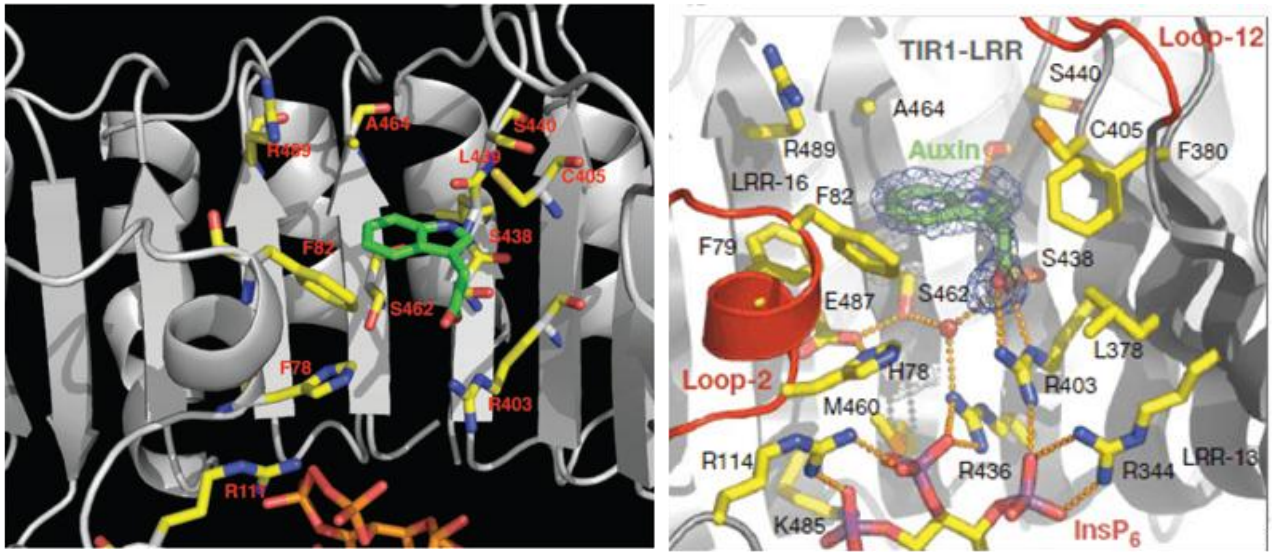


Figure 4: The binding of IAA within the TIR1 protein.[20]

When viewing the receptor proteins, it is clear that the binding sites of each is highly specific to auxins and auxin-like molecules.[22] At the same time, the quantity of molecules capable of binding to ABP1 and TIR1 is quite large, ranging for single ring aromatic systems to indolic and other heterocyclic systems.[5] This variety of auxin-like molecules shows that there is room for modification without losing auxin activity. Understanding the relationship between the structure of each specific auxin and its binding proteins is vital in determining exactly what can be defined as an auxin. With these structural characteristics in mind, this project chose to design analogs of both a naturally occurring auxin similar to IAA, IBA, and a synthetic auxin, 1-NAA. Both of these molecules are known to bind favorably to ABP1 and TIR1.[21] Manipulating the structures of the two active auxins and testing their binding affinity to these proteins should allow for further understanding of the TIR1 and ABP1 receptors in addition to expanding knowledge of auxin structure.

Auxin Transport

Auxin is theorized to be synthesized locally in plants, mainly in younger regions of the plant, such as young leaves, developing seeds, and shoot tips.[24] Altering the location and concentration of auxin results in several changes to the overall structure of the plant, be it by organ development through cell differentiation or simply cell elongation. Therefore, the transport of auxins throughout the plant, along with a plant's ability to create concentration gradients in vital areas, is an essential part of plant development.

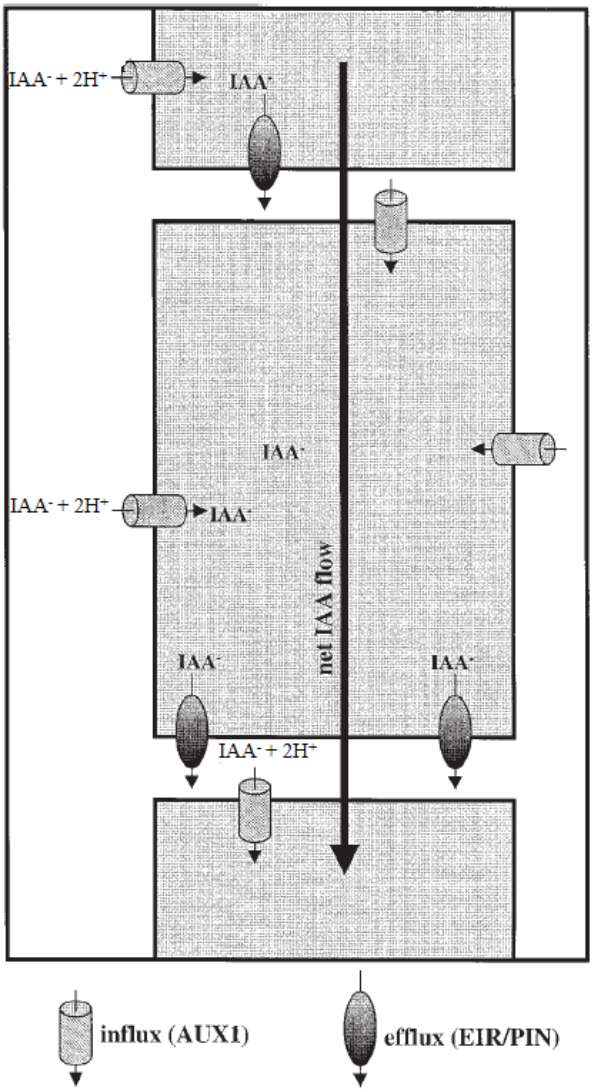


Figure 3*: Model for Polar Auxin Transport through a row of cells

*Figure from M. Estelle's Polar Auxin Transport: New Support for an Old Model

Auxin is transported through the plant in one of two manners. The first is a relatively fast (5-20 cm/h), non-directional transport through the phloem.[24,25] This method moves mostly inactive auxins conjugates. These are formed by the conjugation of IAA to different moieties by esterification to sugars or peptide linkage to amino acids/peptides as a component of auxin homeostasis.[26] As opposed to 'free' auxins (active, unconjugated auxins), these inactive forms make up the majority of auxins in the plant.[27] Because this is a passive transport of auxins, the plant does not expend energy in the movement.

The other method of auxin movement is much slower (5-20 mm/h), specific for free auxins,

and unidirectional.[24] This transport is known as polar auxin transport (PAT). Flow of auxin into the cell begins with Auxin Resistant$_1$ (AUX1), an influx carrier protein which belongs to the Auxin Permease$_1$/LIKE AUX protein family.[28] These proteins co-transport the deprotonated form of the auxin, along with a two protons, via a symport mechanism.[24,28] The activity is dependent upon a proton gradient across the cellular membrane maintained by a proton pumping ATPase.[30] Auxin efflux is controlled by EIR/PIN proteins, and net directional flow is due to the basal localization of said proteins.[31]

This mode of polar transportation is necessary due to the pH of the surrounding environment. When IAA is protonated, it is capable of diffusing across the cell membrane. However, with a pKa of 4.75, the majority of IAA is in its deprotonated form even at the relatively acidic cell membrane, which has a pH between 5 and 5.5.[24] Therefore, the use of an influx-carrier (such as AUX1) is needed to transport auxin into the cell. Once in the cytoplasm of the cell (pH ~7), all IAA is in its deprotonated form, and unable to exit the cell. The efflux facilitating PIN proteins are required to move auxin along the PAT system.[32] Unlike IAA and IBA, 1-NAA (which has a lower pKa than IAA, 4.25) is capable of passively diffusing across the cellular membrane even when deprotonated.[33] The reasoning behind this observation is that the naphthyl ring of NAA is so hydrophobic in nature that the negative charge of the carboxylic acid does not interact with the phospholipid bilayer of the cell membrane. In terms of this project, this factor may come into play. A switch from a carboxyl group to a sulfonate group could result in the 1-NAA analog being unable to passively diffuse into the cell. This possibility will have to be considered if the analog shows evidence of growth regulation in bioassays. It could imply an ability of the analog, and conversely 1-NAA, to use the polar transport system,[34] and thus an ability to bind to AUX1 or other auxin influx proteins.

Scope of Project

Although much is now known about the effects of auxins upon plant growth, the mechanism for their actions, as well as their transport through the plant as a whole, are issues that have not been thoroughly answered. While it is known that ABP1 has a high affinity binding specifically for auxins, and that some of the roles it is responsible for within the cell, the mechanisms for its actions are still unclear. As the ABP1 loss-of-function mutant is fatal, molecules capable of binding to and inhibiting ABP1 are needed to further study this protein.[14] It has also been found that PIN proteins located in the cellular membrane are responsible for the primary direction-determining factors in auxin transport,[35] but the question of how exactly auxin flow is redirected so rapidly is still to be discovered. These two examples are only a minor look into the areas of concern surrounding the transport of auxins within the plant, and movement of auxin molecules into the cell.

In choosing starting points for this project, compounds with similarities or differences to noted auxins are subject to attention. Already, hundreds of synthetic approaches have been taken, most involving some aromatic/π-bond rich system with a carboxylate group, or perhaps a transformation of the carboxyl group into esters, amines, or other functionalities.[5] In multiple studies done by Noel Ferro's group, the biological activity of over 250 different auxin-like molecules which conformed to the above structural requirements were tested using bioactivity assays and computational analysis.[5,36] Because the various physiological responses to auxins are well documented, bioassays are extremely useful in determining compounds that exhibit auxin functionality.[32] The biological activity of each auxin-like molecule was rated on a scale from 0 (inactive) to 3 (most active). From this study, it appears that this approach to designing auxin analogs is relatively successful in reproducing auxin-like behavior.[5] Table 1 includes a brief

sample of some of the molecules tested for auxin activity, including known auxins as well as new synthetic auxin-like molecules which displayed auxin like activity.

Table 1: Reported Activity for Auxin Analogs[5]

Molecule Name	Bio-consensus
4-Cl-indoleacetic acid	3
3-indolebutyric acid	3
2,3,6-trichlorobenzoic acid	3
2-bromo-3-nitrobenzoic acid	2
1-alpha-naphthaleneaceitic acid	3
2-chloro naphthylacetic acid	3

With this results in mind, two known auxins, IBA and 1-NAA, were selected as starting points for creating auxin analogs. As both molecules are known have a high binding affinity to the two major auxin receptor proteins ABP1 and TIR1, they offer a solid base structure from which small portions can be transformed without destroying the basic structure of the auxin. At the same time, the varied structures of the two molecules (the lipophilic napthyl ring of NAA with only a methylene group branching off, compared to the indolic structure of IBA, with a long, rotating alkyl branch on one end) should allow for the diverse analog creation, and thus further areas to expand upon.

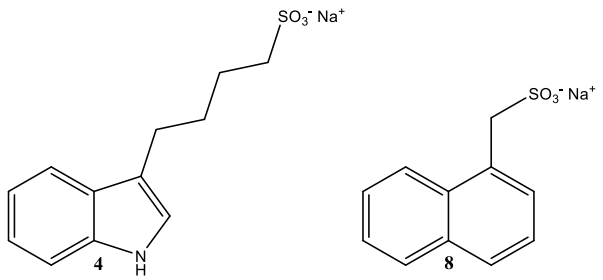


Figure 4: The two target molecules, indole-3-butyl sodium sulfate (IBS) and 1-methylnapthyl sodium sulfonate (NSS)

The use of a sulfonic acid functional group in the biologically active molecule indole-3-methanesulfonic acid (IMS) has already been described by Cohen in 1984.[37] IMS was found to promote growth of isolated soybean hypocotyl sections to roughly the same extent as IAA, although the concentrations necessary to imitate such growth were higher than that of IAA.[37] These finding lend credibility to the use of a sodium sulfonate salt as a possible functional group to be used producing biologically active auxin analogs. The synthesis of a relatively similar indole-3-butyl sodium sulfonate would then test what effects changing the tether length in the auxin has in regards to biological activity. When observing the two naturally occurring auxins, IAA and IBA, the only difference in chemical appearance is the addition of two carbons in the alkyl chain of IBA. Yet it is noted that there are distinctly different physiological responses to each auxin.[38] IBA is noted as promoting earlier and more adventitious root growth in comparison to IAA.[38] It is also more effective when applied exogenously to the plant than IAA, and more resistant to auxin degrading enzyme systems.[38] Because of this observed difference in physiological response based solely on tether length, the further studies where this aspect of the auxin is modified could lead to a more in-depth understanding of auxin binding characteristics. For these reasons, the first molecule of interest is the sulfonated derivative of IBA. As Cohen's work showed, the sulfonic acid is a viable replacement to the carboxylate group.[37] If IMS mimics the behavior of IAA, then it is possible that IBS would mimic the physiological activity of IBA.

While nearly all of IAA is in its deprotonated state outside of the plant cell, there is still a small portion of protonated IAA at the relatively acidic cellular membrane.[24] However, sulphonic acids have particularly low pK_a values, typically far below 0, meaning that these functionalities would be completely deprotonated at physiological pH. The next molecule of

interest, stemming from 1-NAA, would allow for a study of the molecules entry into the cell. Normally, NAA is able to passively diffuse through the cell membrane. The addition of such a large, electronegative group attached would like decrease the molecule's hydrophobicity, and thus force the molecule to use active transport molecules, such as the AUX1 influx protein. It is not known if NAA or its sulfonated analog can interact with such receptors, making the study of its growth activity a matter of interest.

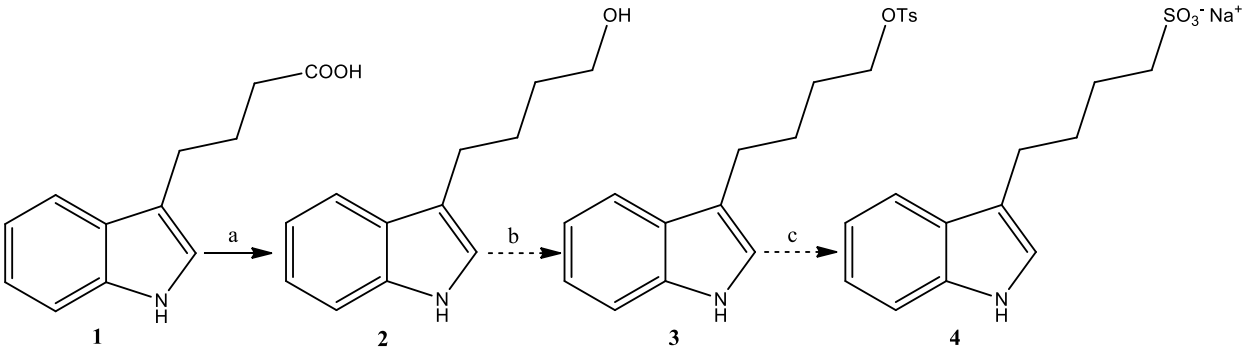
The two target molecules will provide branching points to further analogs capable of stressing and stretching the borders of our knowledge on auxin transport. The use of these analogs in various bioassays will hopefully allow for a more in depth models of endogenous and exogenous auxin transport within plants.

Results and Discussion

The proposed synthetic routes taken towards the two target molecules are straightforward in their manner; both involve attaching a strong leaving group onto the desired carbon followed by a simple S_N2 attack by SO_3^{-2} which is provided by sodium sulfite. In the case of IBA, a reduction of the carboxylic acid was required before a strong leaving group could be placed on end of the alkyl tether, and the method used was documented in the literature.[23] After an initial attempt at tosylation of the alcohol were unsuccessful, the project turned to halogenation as a path to the final product. [39,23]

Indole-3-butyric acid

The initial planned route for attaching the sulfonate functionality was a three step sequence: the reduction of the acetic acid, followed by tosylation of the corresponding alcohol, and finally sulfonylation of **3** to obtain the sulfonate salt analog.



Scheme 1: Proposed route to Indole-3-butyl sodium sulfonate 4: a. LAH, ET_2O; reflux 3hr. b. p-TsOH, silica chloride, CH_2Cl_2; reflux 2 hr. c. Na_2SO_3, 50:50 MeOH/H_2O; reflux 7hr.

The experimental procedure for the reduction of **1** to **2** has been well documented,[34] and in our hands the reaction produced the desired product. A three hour reflux of the starting material in diethyl ether with lithium aluminum hydride as the reducing agent successfully resulted in the corresponding alcohol (90.4% yield). The next stage of the original synthetic route called for the tosylation of **2** using the methodology of the Das group,[39] which had included

the tosylation of primary alcohol 3 carbons away from an aromatic ring. It was believed that the structural similarities between this model system and the actual system would yield a positive result, but our attempts did not result in the desired product. As the only requirement for achieving the final target molecule was a strong leaving group, the decision was made to attempt halogenation of **2** instead of fiddling with unknown methods.

Following the successful reduction of IBA, Benghiat's method of bromination[23] was chosen to convert the alcohol to the halide.[34] The first attempt of Benghiat's method used identical conditions to the original procedures, however the results did not achieve the success of the original paper (18.2% isolated yield). A second reaction was run and crude ^{1}H-NMR spectrum revealed starting material and 3-bromo butyl indole, but and column chromatography was unsuccessful in removing byproducts from the final mixture (36% crude yield). Additional attempts of purification using varied mobile phases and altering column and fraction size have not been successful in removing all contaminants from the desired product.

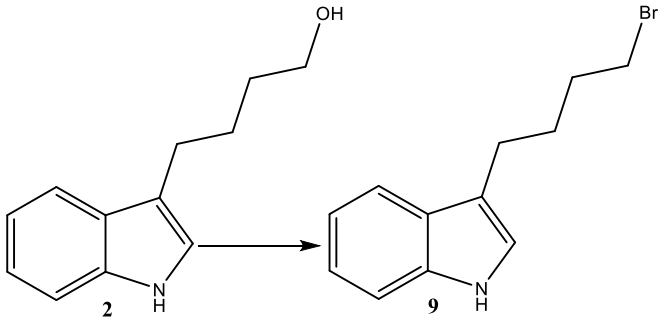


Table 2: Bromination of Indole-3-butanol

Reaction Number	Conditions	Scale	Crude Yield	Isolated Yield
ZDE0122-01	1.2 eq PBr_3, 1 eq indole-3-butanol, Et_2O, RT overnight	1.0 mmol		18.2%
ZDE0123-01	1.7 eq PBr_3, 1 eq indole-3-butanol, Et_2O, RT overnight	1.0 mmol	36%	

In an effort to find a more efficient conversion of **2** to a desirable halide, the next step attempted was chlorination of the hydroxyl functional group. The methodology used by the

Fabrio group[40] was chosen for their substrate's structural similarity (a methyl alcohol off of a benzyl ring) to the products used in this work. However, this method did not result in the anticipated product and further attempts were restricted by time.

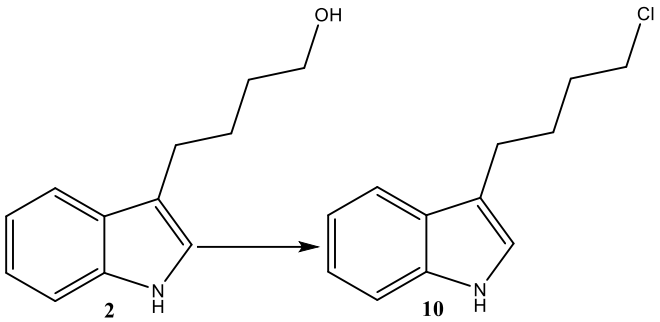
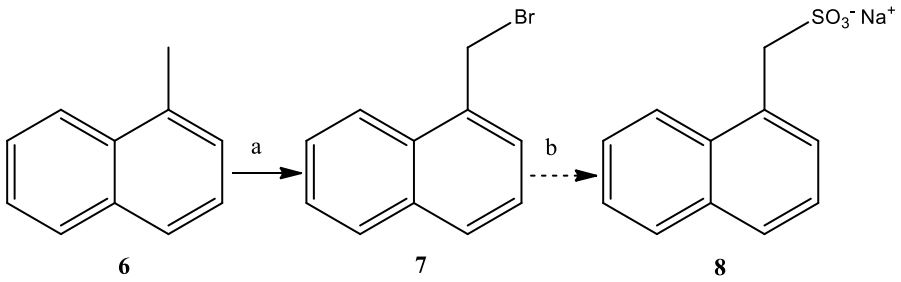


Table 3: Chlorination of Indole-3-butanol

Reaction Number	Conditions	Scale	Result
ZDE0124-01	2.0 eq $SOCl_2$, 1 eq indole-3-butanol, CH_2Cl_2, 2 hr. reflux	1.0 mmol	27.6% starting material recovered

1-methylnapthalene

The proposed route to 1-methylnapthyl sodium sulfonate is a two-step process beginning first with the methylbromination of 1-methylnapthalene followed by sulfonylation of 1-bromomethyl naphthalene.



Scheme 2: Proposed route to 1-methylnapthyl sodium sulfonate: a. NBS, AIBN, CH_2Cl_2; 2min. 100°C (in microwave). b. Na_2SO_3, H_2o/dioxane; reflux 1hr.

Using radical chemistry methodology, **6** was treated with N-bromosuccinimide (NBS) and the radical initiator AIBN. After rapid heating *in micro* for two minutes, **7** was found in satisfying yields. Increasing the scale of the reaction has also proven successful with nearly

quantitative yields. Products were purified using flash chromatography resulting in a clear oil, which then crashed out of solution forming clear, square crystals when left to sit overnight.

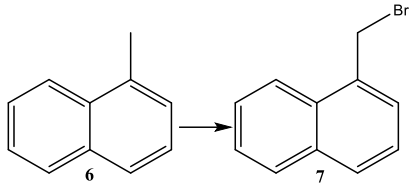


Table 3: Bromination of 1-methylnapthalene

Reaction Number	Conditions	Scale	Crude Yield	Isolated Yield
ZDE0112-01	1.3eq NBS (recrystallized), 1eq 1-methylnapthalene, 0.01eq AIBN, 2 min at 120^0C	2.5 mmol		92.1%

After achieving the purified **7**, the final procedure to the target molecule should have been a straightforward S_N2 reaction. However, although the reaction appears to have occurred as expected, a large quantity of excess sodium sulfite remains in the product. Several attempts at recrystallization of the pure final product were unsuccessful in ridding the solid of excess sodium sulfite.

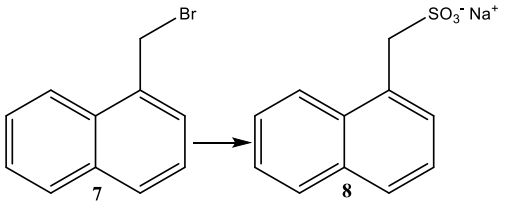


Table 4: Sulfonylation of 1-bromomethyl naphthalene

Reaction Number	Conditions	Comments
ZDE0109-01	5.0eq Na_2SO_3, H_2O, dioxane, reflux 1 hr.	Starting materials recovered, unknown needle-like crystals formed from aqueous layer
ZDE0117-01	5.0eq Na_2SO_3, H_2O, dioxane, reflux 1 hr.	There appear to be peaks associated with the final product in the ^{1}H-NMR, but residual sodium sulfite remains in the crude mixture

Future Work

Continuation of the project will involve complete synthesis of IBA analog, as well as finalizing the purification 1-bromomethylnapthalene. Once the initial target molecules have been reached and purified in reasonable quantities, additional synthesis of sodium sulfonate analogs will continue. The most likely route would begin through the auxin indole-3-propionic acid to the corresponding sodium sulfonate. Indole-3-pentanoic acid could be another starting point to its corresponding sodium sulfonate. The idea behind creating these molecules is that varying the length of the tether on IBA (longer or shorter), would allow for additional insight in determining specific mechanisms for auxin transport/response. Additional research could also be done in alternating the functional group at the end of the alkyl chain. Altering the functional moiety would most likely alter the 'auxins' binding to specific auxin binding proteins and thus allow additional study of plant's abilities to bind these hormones. Synthesis of all of these molecules would most likely follow routes similar to the planned route of indole-3-butylsodium sulfonate. After successful synthesis of the target molecules, the project would then turn towards various biochemical assays, which could be used to test the auxin-like abilities of said molecules. These assays would include applying the compounds to plants exogenously and monitoring growth response, as well as competitive binding assays where the analogs' binding to traditional auxin receptors is compared to its corresponding 'natural' compound.

Experimental Section

Indole-3-butyric acid

Indole-3-butanol

A solution of lithium aluminum hydride (.3795 g, 10. mmol) and anhydrous diethyl ether (30 mL) was brought to reflux. A solution of indole-3-butyric acid and Et_2O (15 mL) was added drop wise by addition funnel. The reaction mixture was stirred at reflux for 3 hours, then allowed to react at room temperature overnight. The reaction was quenched with H_2O (10 mL), then by a 1.0 M HCl(10 mL). The combined mixture was then extracted with Et_2O (30 mL X 2). The ether layers were then combined and dried over sodium sulfate. The crude product was purified via column chromatography (70% EtOAc/hexanes). Solvent was removed *in vacuum*. ^1H NMR ($CDCl_3$) δ: 7.61 (1 H, d, 6Hz), 7.12(3H, m), 6.99 (1H, s), 3.71 (2H, t, 6Hz), 2.88 (2H, t, 6Hz), 2.01 (2H, m); amber oil

3-bromo-butyl indole

In a dried round-bottom flask, indole-3-butanol (0.1892 g, 1.0 mmol) was added to a solution of anhydrous Et_2O (18 mL) and flushed under nitrogen. The stirred solution was cooled to 0°C. A solution of PBr_3 (0.05 ml) and Et_20 (7 mL) was added drop-wise via syringe to the flask. The ice bath was removed and the solution was stirred at room temperature overnight. The reaction mixture was then washed first with H_2O followed by sodium bicarbonate. The ethyl ether layer was collected and dried over $NaSO_4$, and the solvent was removed *in vacuum* Spectra matched reported literature exactly.[23]

^1H NMR ($CDCl_3$) δ: 7.92 (1H, s), 7.61 (1 H, d, 7Hz), 7.36 (1 H, d, 8Hz), 7.19 (1 H, t, 7Hz), 7.13 (1 H, t, 7Hz), 6.99 (1 H, s), 3.45 (2 H, t, 6Hz), 2.81 (2 H, t, 7Hz), 1.86-1.96 (4 H, m); brown solid

1-methylnapthalene

1-bromo-methylnapthalene (**7**)

Using a dry 10 mL microwave vial, the following was added sequentially: recrystallized NBS (.4628 g, 2.6 mmol), CH_2Cl_2 (8 mL), 1-methylnapthalene (.2844 g, 2.0 mmol), and AIBN (3.284 mg, 0.02 mmol). The reaction mixture was heated in a microwave reactor to 120 ^oC for 2 minutes with a 3 second pre-stir. The solution was then extracted with fresh dichloromethane (50 mL) and .1M sodium thiosulfate (40 mL). The organic layer was then extracted again with H_2O (50 mL), then dried over $NaSO_4$. The solvent was removed via rotary evaporation and use of the pump. The product was further purified using column chromatography using 100% hexanes as the mobile phase resulting in a clear oil. Upon allowing the sample sit overnight, clear crystals crashed out of solution. The ^{1}H-NMR spectra matched the reported literature.[41] (Isolated yield: 0.4068 g, 1.84 mmol, 92%).

^{1}H NMR ($CDCl_3$) δ: 8.20 (1 H, d, J = 6 Hz), 7.92 (1 H, d, J = 6 Hz), 7.89 (1 H, d, J = 6 Hz), 7.62 (1H, t, J = 6 Hz), 7.54 (2 H, m), 7.43 (1 H, m), 4.97 (2 H, s). White solid

1-methylnaphthyl-sodium sulfonate (**8**)

Purified **6** (.221g, 1.0mmol) and dioxane (6mL) were added to a 50 mL round bottomed flask equipped with stirbar and flushed with nitrogen. In a separate flask, Na_2SO_3 (.68g, 5.3mmol) was added to H_2O (7ml). This solution was added to the stirred mixture via syringe and stirred at reflux for 1 hour, after which heat was removed. Reaction mixture was allowed to cool to room temperature, then placed in an ice bath. Solid was removed from the mixture using vacuum filtration. The filtrate was extracted with dichloromethane, and the aqueous layer was allowed to sit for several days. Needle-like crystals formed, which were then separated by vacuum filtration.

References

1. Okushima, Y.; Overvoorde, P.J.; Arima, K.; Alonso, J.M.; Chan, A.; Chang, C.; Ecker, J.R.; Hughes, B.; Lui, A.; Nguyen, D.; Onodera, C.; Quach, H.; Smith, A.; Yu, G.; Theologis, A. Multiple AUX/IAA–ARF modules regulate lateral root formation: the role of *Arabidopsis* SHY2/IAA3-mediated auxin signaling. *The Plant Cell*. 2005, *17*, 444-463.

2. Nishimura, T.; Wada, T.; Yamamoto, K.; Okada, K. The Arabidopsis STV1 Protein, Responsible for Translation Reinitiation, is required for Auxin-Mediated Gynoecium Patterning. *The Plant Cell*. 2005, *17*, 2940-2953.

3. Friml, J. Auxin Transport – shaping the plant. *Current Opinion in Plant Biology*. **2000**, *6*, 7-12.

4. Mockaitis, K.; Estelle, M. Auxin Acceptors and Plant Development: A New Signaling Paradigm. *Annu. Rev. Cell Dev. Biol.* **2008**, *24*, 55-88.

5. Ferro, N.; Gallegos, A.; Bultinck, P.; Jacobsen, H.; Carb-Dorca, R.; Reinhard, T. Coulomb and overlap self-similarities: a comparative selectivity analysis of structure-function relationships for auxin-like molecules. *J.Chem. Inf. Model*. **2006**, *46*, 1751-1762.

6. Böttcher, C.; Dennis, E. G.; Booker, G.W.; Polyak, S.W.; Boss, P.K.; Davies, C. A Novel Tool for Studying Auxin-Metabolism: The Inhibition of Grapevine Indole-3-Acetic Acid-Amido Synthetases by a Reaction Intermediate Analogue. *PLoS ONE*. **2012**, *7*, 1-8.

7. Katekar, G. F. Auxins - Nature of the Receptor-Site and Molecular Requirements for Auxin Activity. *Phytochemistry*. **1979**, *18*, 223-233.

8. Kaethner, T. M. Conformational Change Theory for Auxin Structure-Activity-Relationships. *Nature*. **1977**, *267*, 19-23.

9. Bean, T.G. Unpublished Work. Allegheny College. 2011

10. Magnus, V.; Kojic-Prodi, C.B. Auxin structure and activity. *Phyton*. **1999**, *39*, 19-23.

11. Benkova, E.; Michniewicz, M.; Sauer, M.; Teichmann, T.; Seifertova, D.; Jurgens, G.; Friml, J. Local, Efflux-Dependent Auxin Gradients as a Common Module for Plant Organ Formation. *Cell*. **2003**, *115*, 591-602.

12. Hertel R.; Thomson, K.S.; Russo, V. In-vitro auxin binding to particulate cell fractions from corn coleoptiles. *Planta*. **1972**, *107*, 325–340.

13. Sauer, M.; Kleine-Vehn, J. Auxin Binding Protein$_1$: The Outsider. *The Plant Cell*. **2011**, *23*, 2033-2043.

14. Paciorek, T.; Zazimalova, E.; Ruthardt, N.; Petrasek, J.; Stierhof, Y.; Kleine-Vehn, J.; Morris, D.; Emans, N.; Jurgens, G.; Geldner, N.; Friml, J. Auxin inhibits endocytosis and promotes its own efflux from cells. *Nature*. **2005**, *435*, 1251-1256.

15. Robert, S., Kleine-Vehn, J., Barbez, E., Sauer, M., Paciorek,T., Baster,P., Vanneste, S., Zhang, J., Simon, S., Covanova, M., Hayashi, H., Dhonukshe, P., Yang, Z., Bednarek, S., Jones, A., Luschnig, C., Aniento,F., Zazımalova, E., and Friml., J. ABP1 Mediates Auxin Inhibition of Clathrin-Dependent Endocytosis in Arabidopsis. *Cell*. **2010**, *143*, 111-121.

16. Dharmasiri, N.; Dharmasiri, S.; Estelle, M. The F-box protein TIR1 is an auxin receptor. *Nature*. **2005**, *435*, 441-445.

17. Kepinski, S.; Leyser, O. Ubiquitination and auxin signaling: a degrading story. *The Plant Cell*. **2002**, *14*, 81-95.

18. Tan, X.; Calderon-Villalobos, L.; Sharon, M.; Zheng, C.; Robinson, C.; Estelle, M. ; Zheng, N. Mechanism of auxin perception by the TIR1 ubiquitin ligase. *Nature*. **2007**, *446*, 640-645.

19. Woo, E.; Marshall, J.; Bauly, J.; Chen, J.; Venis, M.; Napier, R.; Pickersgill, R. Crystal structure of auxin-binding protein 1 in complex with auxin. *The EMBO J.* **2002**, *21*, 2877-2885.

20. Xu, T.; Luz, I.A.; Villalobos, C.; Sharon, M.; Zheng, C.; Robinson, C.V.; Estelle, M.; Zheng, N. Mechanisms of auxin perception by the TIR1 ubiquitin ligase. *Nature*. **2007**, *446*, 640-645.

21. Bean, T.G. Unpublished Work. Allegheny College. 2011

22. Scherer, G. F. E. Auxin-Binding Protein$_1$, the second auxin receptor: what is the significance of a two-receptor concept in plant signal transduction? *Journal of Experimental Botany*. **2011**, *62*, 3339-3357.

23. Benghiat, E.; Crooks, P.A, Multisubstrate adducts as potential inhibitors of S-adenosylmethionine dependent methylases: inhibition of indole N-methyltransferase by (5'-deoxyadenosyl)[3-(3-indolyl)prop-1-yl]methylsulfonium and (5'-deoxyadenosyl)[4-(3-indolyl)but-1-yl]methylsulfonium salts. *J. Med. Chem.* 1983, *26*, 1470-1477**.**

24. Friml, J.; Palme, K. Polar Auxin Transport – old questions and new concepts. *Plant Molecular Biology*. **2002**, *49*, 273-284.

25. Davies, P.J. Plant Hormones: Physiology, Biochemistry and Molecular Biology. Martinus Nijhoff, Dordrecht, Netherlands. **1995**.

26. Rampey, R.A.; LeClere, S.; Kowalczyk, M.; Ljung, K.; Sandberg, G.; Bartel, B. Family of Auxin-Conjugate Hydrolases that contribute to Free Indole-3-Acetic Acid levels during Arabidopsis Germination. *Plant Physiol.* **2004**, *135*, 978 -988.

27. Tam, Y.Y.; Epstein, E.; Normanly, J. Characterization of Auxin Conjugates in Arabidopsis. Low Steady-State Levels of Indole-3-Acetyl-Aspartate, Indole-3-Acetyl-Glutamate, and Indole-3-Acetyl-Glucose. *Plant Physiol.* **2000**, *123*, 589-596.

28. Marchant, A.; Kargul, J.; May, S.T.; Muller, P.; Delbarre, A.; Perrot-Rechenmann, C.; Bennet, M.J. AUX1 regulates root gravitropism in Arabidopsis by facilitating auxin uptake within root apical tissues. *EMBO J.* **1999**, *18*, 2066-2073.

29. Friml, J.; Palme, K. Polar Auxin Transport – old questions and new concepts. *Plant Molecular Biology*. **2002**, *49*, 273-284.

30. Raven, J.A. Transport of indole-3- acetic acid in plant cells in relation to pH and electrical potential gradients, and its significance for polar IAA transport. *New Phytol*. **1975**, *74*, 163–172.

31. Estelle, M. Polar Auxin Transport: New Support for an Old Model. *The Plant Cell*. **1998**, *10*, 1775-1778.

32. Delker, C.; Raschke, A.; Quint, M. Auxin dynamics: the dazzling complexity of a small molecule's message. *Planta*. **2008**, *227*, 929-941.

33. Ganguly, A.; Lee, S.H.; Cho, M.; Lee, O.R.; Yoo, H.; Cho, H.T. Differential Auxin-Transporting Activities of PIN-FORMED Proteins in Arabidopsis Root Hair Cells. *Plant Physiol*. **2010**, *153*, 1046-1061.

34. Mong, J. and Murphree, S. Unpublished works. Allegheny College. 2010.

35. Wisniewska, J.; Xu, J.; Seinfertova, D.; Brewer, P.B.; Ruzicka, K.; Blilou, I.; Rouquie, D.; Benkova, E.; Scheres, B.; Friml, J. Polar PIN Localization directs Auxin Flow in Plants. *Science.* **2006**, *312*, 883.

36. Ferro, N.; Bultinck, P.; Gallegos, A.; Jacobsen, H.; Carbo-Dorca, R.; Reinard, T. Unrevealed structural requirements for auxin-like molecules by theoretical and experimental evidences. *Phytochemistry*. **2007**, *68*, 237-250.

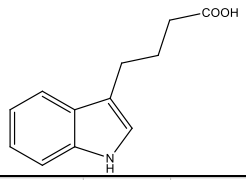
37. Cohen, J.D.; Baldi, B.G.; Bialek, K. Strongly Acidic Auxin Indole-3-methanesulfonic Acid. *Plant Physio.* **1984**, *77*, 195-199

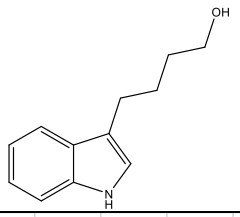
38. Stefanci, M.; Stampar, F.; Osterc, G. Influence of IAA and IBA on root development and the quality of *Prunis* 'GiSelA 5' leafy cuttings. *Hort Science*. **2005**, *40*, 2052-2055.

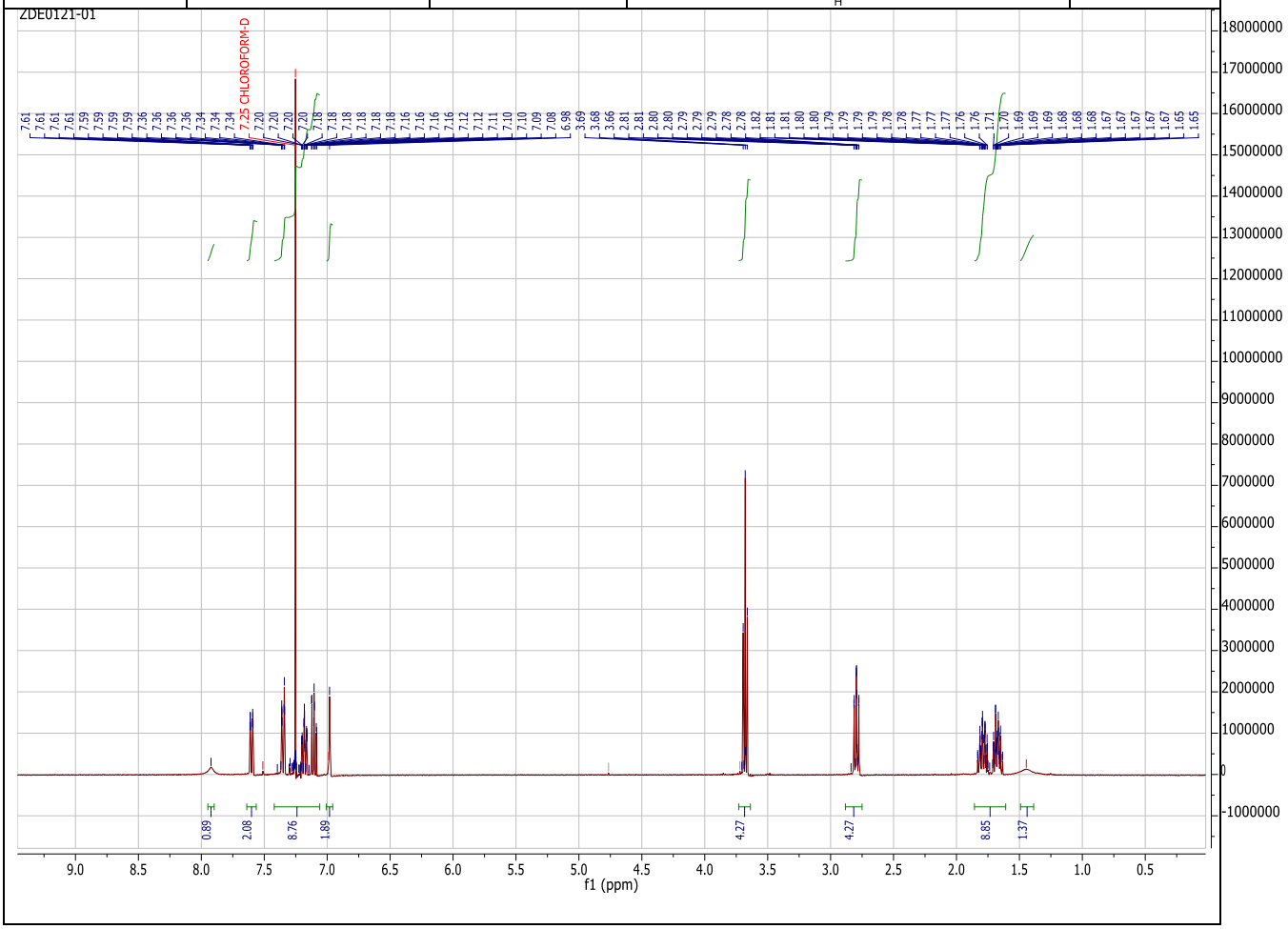
39. Das, B.; Reddy, V.S.; Reddy, M.R. An efficient and selective tosylation of alcohols with p-toluenesulfonic acid. *Tetrahedron Letters*, **2004**, *45*, 6717 - 6719

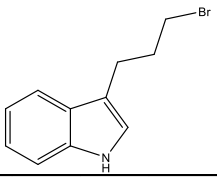
40. Fabrio, R.; Arban, R.; Bernasconi, G.; Braggio, S.; Blaney, F.; Capelli, A.; Castiglioni, E.; Donati, D.; Fazzolari, E.; Ratti, E.; Feriani.; Contini, A.; Gentile, G.; Chirlanda, D.; Sabbatini, F.; Andreotti, D.; Spada, S.; Marchioro, C.; Worby, A.; St. Denis, Y. Dihydropyrrole [2,3-d]pyridine derivatives as novel corticotropin-releasing factor-1 antagonist: mapping of the receptor binding pocket by in silico docking studies. *J. Med. Chem.* **2008** *51*, 7273-7286.

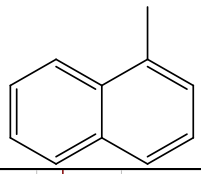
41. Schonleber, M.; Hilgraf, R.; Pfaltz, A. Chiral bis(N-arylamino)phosphine-oxazolines: Synthesis and application in asymmetric catalysis. *Advanced Synthesis and Catalysis*. **2008**, *350*, 2033-2038.

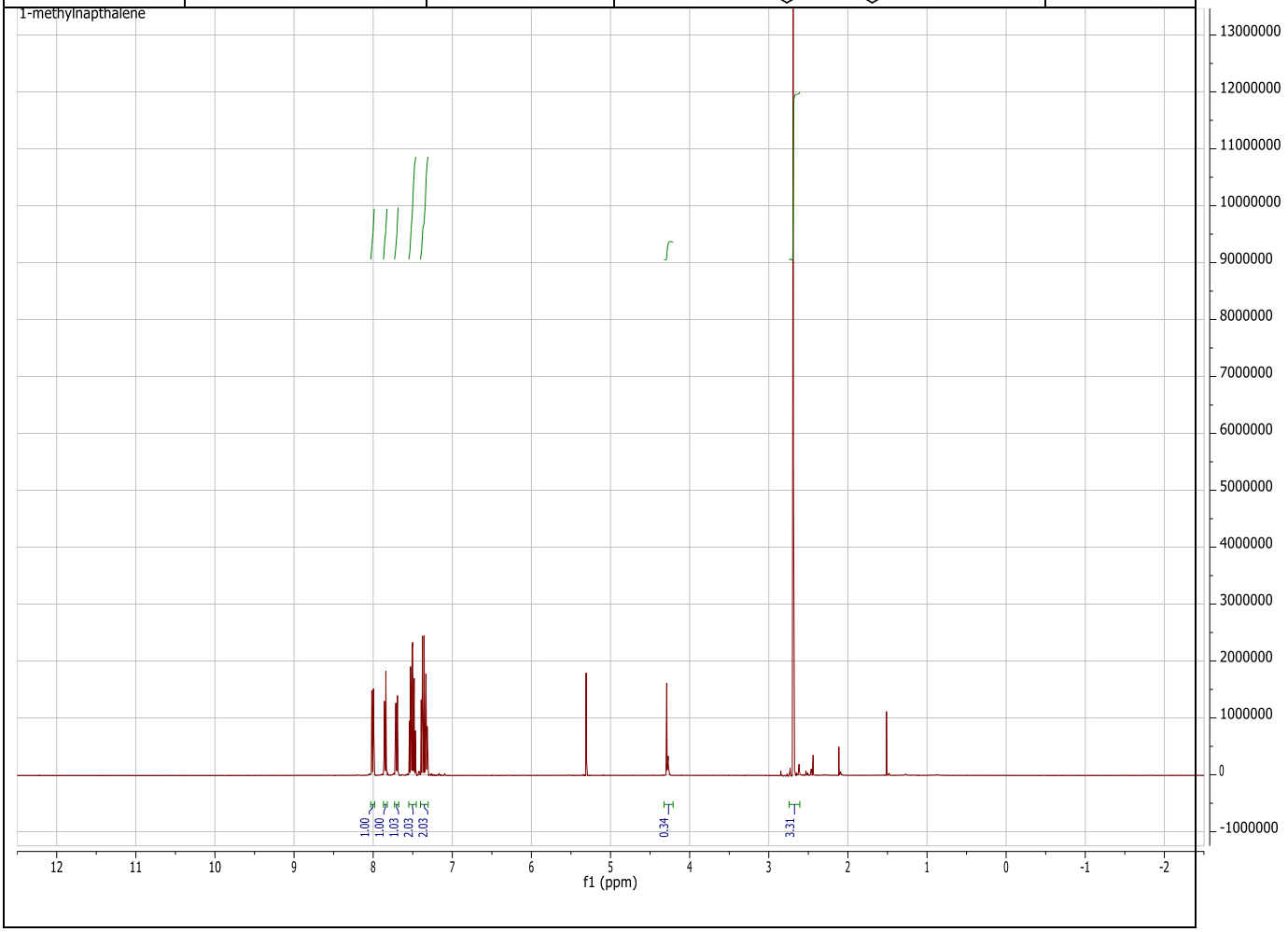
Indole-3-butryic acid				Compd No.
400 MHz	chloroform-d	16 Nov 2012		**1**

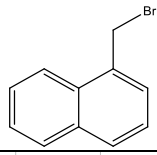
Indole-3-butanol				Compd No.
400 MHz	chloroform-d	29 March 2013		**2**



3-bromo-butyl indole				Compd No.
400 MHz	chloroform-d	31 March 2013		**9**

1-methylnapthalene				Compd No.
400 MHz	chloroform-d	18 Nov 2012		**6**



1-bromo-methylnapthalene				Compd No.
400 MHz	chloroform-d	16 Nov 2012		**7**