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The Synthesis and Characterization of Novel Imino Stilbenes

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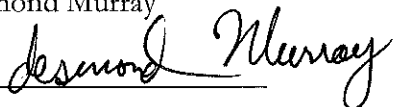
Honors Thesis

The Synthesis and Characterization of Novel Imino Stilbenes

Shieun Jang

April 1 2011

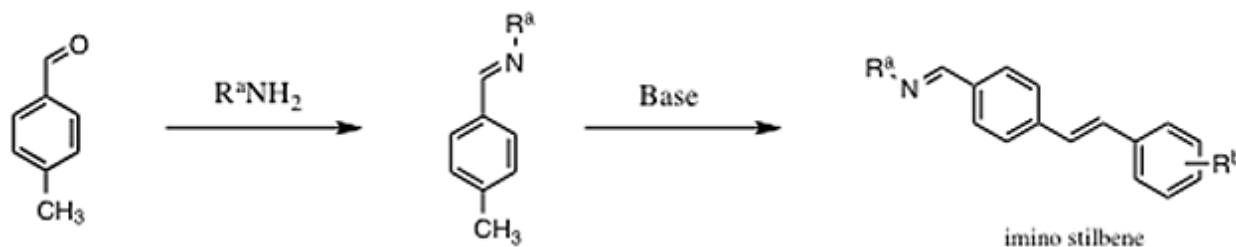
Advisor: Dr. Desmond Murray

Primary Advisor Signature: 

Department: Andrews University Department of Chemistry and Biochemistry

Abstract

We are in the process of developing a two-step synthesis of imino stilbenes as potential molecular switches, which are important in biology, electronics and other applications especially at nanoscopic scale.



While both imines and stilbenes are well known, we have not uncovered any prior research and documentation on our proposed imino stilbenes in the scientific literature. In pursuit of our goal we have developed a new procedure for synthesis of *t*-butyl imines derived from aromatic aldehydes and ketones. We are also currently evaluating four different stilbene synthesis methods. Future work will systematically investigate the effect of electron withdrawing and donating imino (R^a) groups on the stilbene synthesis process.

Background

The objective of this research is to synthesize and characterize imino stilbenes, novel compounds that have not been well-characterized. Below is the general molecular structure. We believe that imino stilbenes have the potential to produce molecular switches or molecular sensors. The demand for smaller electronics and nanotechnology incite the interest of researchers in molecular switches that are small and programmable. Molecular sensors that fluoresce are of particular interest due to its sensitivity, many factors that can be used as analytic data, and other aspects unique to it in the field of spectroscopy. We believe that imino stilbenes will have the properties to make molecular switches and sensors because of the characteristics that imines and stilbenes possess.

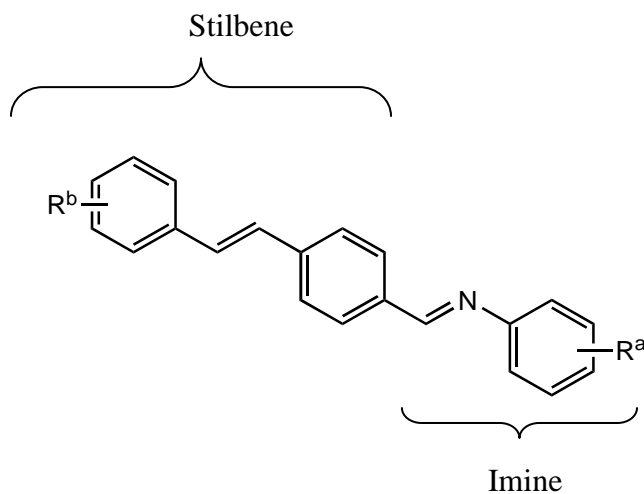


Figure 1 General Structure of an Iminostilbene

Stilbenes, flavanoids, isoflavonoids, and lignans are all derived from phenolic compounds in plants. As shown in the diagram below, biosynthesis of both stilbenoids and flavonoids derive from the same compound, cinnamoyl-CoA. Stilbenoid compounds are found in the woody sections of

plants but the phenylpropanoid pathway that produces stilbenes is activated in response to environmental stressors. Such stilbenes, classified as phytoalexins, protect the plants against viral or microbial attacks, UV exposure, and disease (Roupe *et al.* 81). The general structure of stilbenes consist of double bond with phenyl groups on the carbons. They occur naturally in two isomers, (E)-stilbene or *trans*-stilbene and (Z)-stilbene or *cis*-stilbene. Different conformation incurs different characteristics, exemplified in stilbenes by the dramatic difference in melting point: the sterically hindered, and hence less stable, *cis*-stilbene has a melting point of 6 °C while the more stable *trans*-stilbene has a melting point of about 125 °C (Likhtenshtein 1).

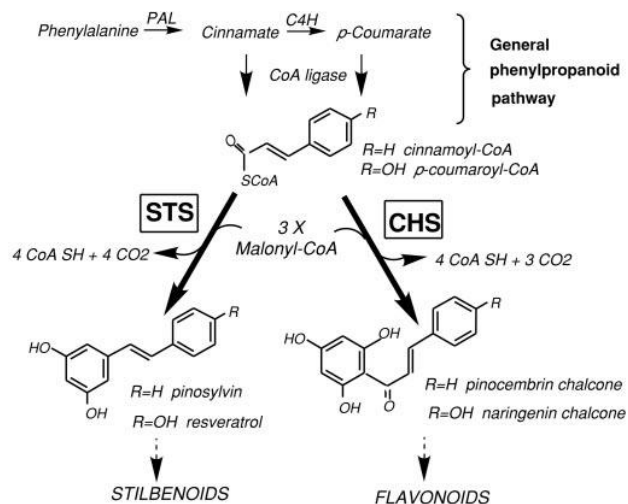


Figure 2 Pathways for stilbenoid and flavonoid biosyntheses. Stilbene synthase (STS) and chalcone synthase (CHS), respectively, lead to stilbenoid and flavonoid biosynthesis from a cinnamoyl-CoA_p-coumaroyl-CoA with three malonyl-CoAs. PAL, phenylalanine ammonia-lyase; C4H, cinnamate 4-hydroxylase.

Figure and caption from

Atsushi Kodan, Hiroyuki Kuroda, and Fukumi Sakai. "A stilbene synthase from Japanese red pine (*Pinus densiflora*): Implications for phytoalexin accumulation and down-regulation of flavonoid biosynthesis" *PNAS* (2002): 99 (5) 3335-3339

Imines, a chemical compound containing nitrogen double-bonded to a carbon and single-bonded to a hydrogen or organic group are common ligands and also present in the deamination of amino acids. Imines are valued for their applications as electrophiles in many organic reactions including reduction, addition, and condensation (Kwon *et al.* 2877). A Schiff base is an imine but the nitrogen is connected to an aryl or alkyl group but no hydrogen. C-N double bonds have a wide range of photochemical reactions including, electronic absorption phenomenon, luminescence, nonradioactive decay, energy transfer, and chemical reactions.

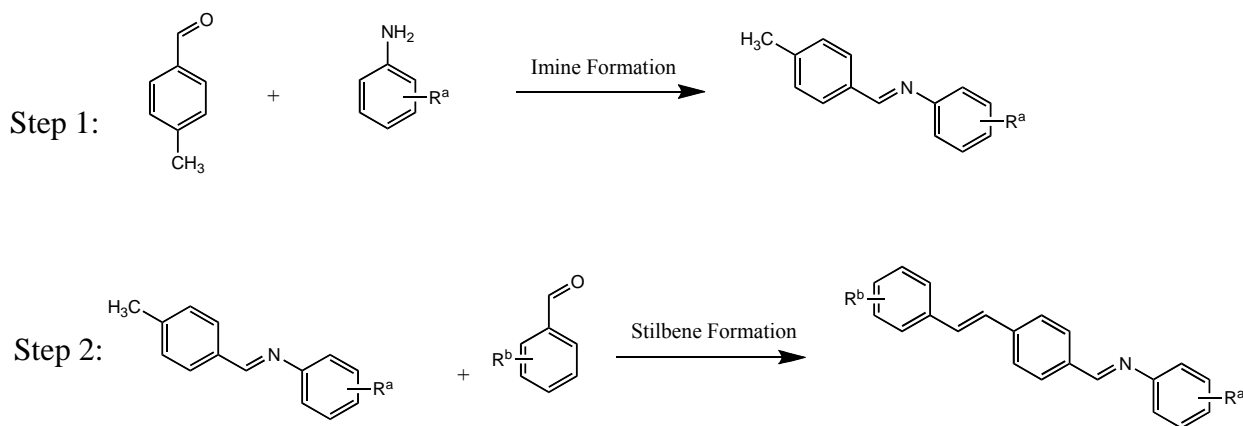


Figure 3 Steps of Iminostilbene formation: Step 1 makes Imine, Step 3 makes a stilbene from that imine.

In the above diagram, the general synthesis of an imino stilbene is shown. The synthesis is divided into the formation of the imine, then the formation of stilbene using the prepared imine. The procedure for imine synthesis is reported in literature as a simple warming of the two starting reagents of aromatic aldehyde and amine, in an alcohol solvent and a trace of acid to aid the reaction. However, past researchers with Dr. Murray had some difficulty with producing a variety of imines with this procedure so a better synthesis for imines was developed, using a secondary amine to further the imine synthesis. The procedure to make the stilbene is an aldol-type condensation where the produced imine is reacted with a substituted benzaldehyde under dry conditions. Literature reports that this method while simple has low yields. Water is the primary side-product in both reactions.

Other established methods for stilbene production are: the Siegrist method, Wittig reaction, Heck reaction, Negishi-Stille coupling, Barton-Kellogg-Staudinger reaction, McMurry reaction, and Perkin reaction. In the future, another method may be considered to use for the synthesis of stilbenes. Currently, the aldol-type condensation reaction fits best within our plans to synthesize a

variety of novel imino stilbenes as both steps are modular, meaning that we have complete control over the molecular structure based on a diversity of building blocks of aromatic amines and aromatic aldehydes.

Although we have little information on imino stilbenes, according to the general structure containing stable benzene rings and multiple double bonds capable of switching conformation, we have certain hopes for imino stilbenes to be used in molecular switches and possibly molecular motors as well, especially in the use of nano-sized devices.

Molecular switches are molecules with the capability of reversibly changing between two or more stable forms. The change in molecular structure can be due

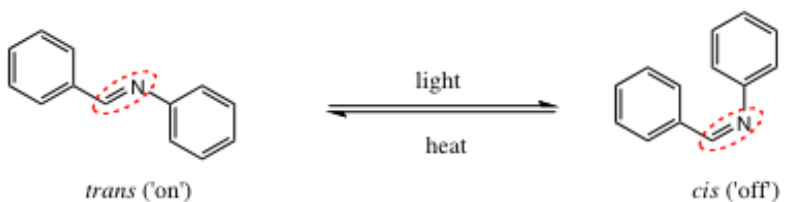


Figure 4 *trans* and *cis* conformations of stilbene - conformation change occurs by excitation through light or losing.

to a number of factors including change in pH, light, temperature, an electrical current, or the presence of a ligand (an ion or molecule bound to a central metal atom). Vision contains a molecular switch, opsin, which turns “on” and “off” photoreceptor cells in response to light. Light photoisomerizes the structure from *cis*- to *trans*-opsin, turning “on” our photoreceptors. Due to the highly conjugated (alternating double and single bond molecular structure) nature of an imino stilbene, it is hoped that a more complex molecular switch can be created. A complex switch is favorable in nano-sized devices where one switch that is capable of producing multiple responses would have an advantage over a single function switch.

Molecular sensors are molecules that interact with another compound that produces a visible change in the sensor; a form of molecular recognition with observable results. Receptors can be

sensitive to a specific molecule or a certain molecular group. Stilbenes have been known to fluoresce while Schiff base fluorescence in the presence of certain metal cations has been recently recognized. The expansion in knowledge of fluorescent sensors is necessary because of the challenges of specificity and molecular recognition.

Methodology

Synthesis is divided into two parts: an imine synthesis from an aromatic aldehyde and an aromatic amine then a synthesis of the stilbene using an imine and another aromatic benzaldehyde that can have another functional group substituted on the ring. The stilbene synthesis uses the previously created imine with another benzaldehyde in a room temperature reaction with dehydrating reagents to create the stilbene half of the desired molecule. Below are the

Imine Synthesis Experimental Procedure

In a 50 mL round bottom flask containing a magnetic stir bar 30 mL of methanol is added. Equivalent moles of aromatic aldehyde and aromatic amine are measured out and added to the reaction flask. A few (5) drops of glacial acetic acid and 1 g of magnesium sulphate was added. A water-cooled reflux condenser was attached to the round bottom flask and the reaction mixture is refluxed, with stirring, for 24 hours. The reflux is stopped by pouring the warm reaction mixture with vigorous stirring into a beaker containing 20 g of ice. To collect the solid the reaction mixture is vacuum filtered. While on the vacuum

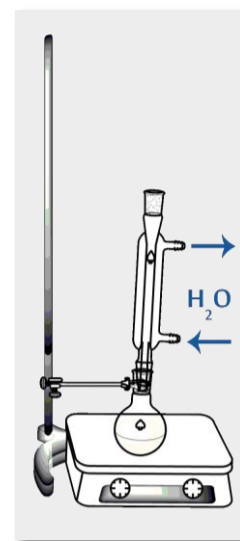


Figure 5 Setup for a Reflux

line, the solid product was washed with at least 100mL of cold distilled water. The product was left on the vacuum for at least an additional 30 minutes to air dry the product. The product is weighed and percent yield is determined. Digital photos are taken of the final product.

Newly Developed Imine Synthesis Experimental Procedure

In a 100 mL round bottom flask containing a magnetic stir bar 30 mL of chloroform is added. Equivalent moles of aromatic aldehyde and aromatic amines (except t-butylamine where an excess of x9 molar equivalent is used) are measured out and added to the reaction flask. About 1 mL (or 10 drops) of glacial acetic acid, 1 mL (or 10 drops) of piperidine and 1 g of magnesium sulphate was added. A water-cooled reflux condenser was attached to the round bottom flask and the reaction mixture is refluxed, with stirring, for 24 hours. The reflux is stopped by pouring the warm reaction mixture with vigorous stirring into a beaker containing 20 g of ice. If there is solid product visible in the beaker (not MgSO_4) proceed to step a, or if there is no visible product proceed to step b: a) *To collect the solid the reaction mixture was vacuum filtered. While on the vacuum line, the solid product was washed with at least 100 mL of cold distilled water. The product was left on the vacuum for at least an additional 30 minutes to air dry the product.* b) *Prepare to extract the organic layer from reaction with chloroform. In a properly secured 250 mL separatory funnel add 30 mL of sat. NaCl and check for any leaks. Then add the reaction mixture to the sep. funnel along with 30 mL of chloroform. Shake thoroughly the layers in the separatory funnel, making sure to release gas build-up in the sep. funnel occasionally and more frequently in the beginning. Let rest and wait till separation of layers is clear. Extract bottom, organic layer into a clean beaker. Wash the inorganic layer with about 25 mL of chloroform three times, collecting the organic layer each time. Dry the collected organic layer with anhydrous sodium sulphate (Na_2SO_4). Through gravity filtration, transfer the organic layer into a round bottom flask that is appropriately sized before. The product is weighed and percent yield is determined. Digital photos are taken of the final product.*

Imine Analysis

Infrared (IR) spectroscopy and proton nuclear magnetic resonance (NMR) spectroscopy were taken of both starting reagents and final products. The disappearance of aldehyde peaks and the appearance of a C-N double-bond signal should be seen in the spectroscopy of the final product. In IR, the disappearance of peaks in the 1740-1720 cm^{-1} range (indicating the C=O double-bond) and 2830-2695 cm^{-1} range (indicating the C-H bond of an aldehyde) would help indicate the success of making the imine. The appearance of peaks in the 1690-1640 cm^{-1} range would indicate the C-N double-bond of the imine. In NMR, the aldehyde peak is usually a distinct singlet in the 9-10 ppm region. Aromatic protons should be present in the 6.5-8.5 ppm range.

Imino Stilbene Synthesis Experimental Procedure

In an oven-dried (20 minutes) 25 mL round bottom flask containing a magnetic stir bar, 15 mL of fresh dry DMF was syringed. A dry, clean condenser with a CaCl_2 drying tube was attached to the round bottom flask. The apparatus was clamped in place on a hot plate stirrer. These reagents were measured out and added to the flask in the following order: (1) 12.5 mmol LiH, (2) 6.25 mmol KTB, (3) 2.5 mmol aryl aldehyde and (4) 2.5 mmol imine. The reaction mixture was stirred for 4 hours at room temperature. The mixture was poured into a beaker with ice and 20mL of phosphoric acid. To ensure that neutralization had taken place, the pH was taken with a litmus paper. The mixture was stirred for 10 minutes. The mixture was vacuum filtered to collect the solid and air dried for 6 – 12 hours. Afterwards the product was Collected and weighed. The percent yield was calculated and digital photos taken throughout the procedure. IR and NMR of the imino stilbene product were taken and compared with the IR and NMR of the organic starting materials.

Imino Stilbene Analysis

Comparing the IR and NMR of the starting reagents and the product, the conjunctive appearance of the starting materials with the exception of an aldehyde peak is expected for an imino stilbene. The same aldehyde peaks mentioned in the imine synthesis analysis should not be present in the IR and NMR results. If a product is suspected to have fluorescent characteristics, it is exposed to high frequency light by an ultraviolet lamp. Then the material is observed for fluorescence in the dark.

*Note that these reactions can be scaled up.

Results and Discussion

Imine p-tolualdehyde + amine

A researcher before me had successfully made imines using the imine synthesis (not containing now supplemental secondary amines). The first imine made was with the aldehyde p-tolualdehyde and with the amine p-anisidine. The NMR done on this first imine synthesis showed that the imine had formed and was quite pure as well with 61.22-66.64 %yield. The product was a very light brown powder. The bulk synthesis (scaled up by 20) had 86.87 %yield and the color and consistency was slightly different, being a very fine light powder and the color a light golden-brown.

Following the synthesis of the imine from p-tolualdehyde and p-anisidine different amines were tried with p-tolualdehyde. The amines were: o-anisidine, 2-aminobenzothiazole, and benzenesulfonamide. The *ortho* substitution of the anisidine gave surprising results as there was no product at all, perhaps due to steric interference at the amine sight. The 2-aminobenzothiazole and benzenesulfonamide also produced disappointing results having only 24.77 %yield and 11.74 %yield, respectively.

The existing imine synthesis method worked very well for making imines from p-tolualdehyde and p-anisidine having a high percent yield. However other amines, even o-anisidine which is structurally only different by the placement of one substituent, did not give nearly such good results. The next section details how we overcame this challenge by developing a new general synthesis method for imines.

Development of Imine Synthesis (specifically for p-tolualdehyde + t-butylamine)

After the failure to make a variety of imines from the pre-existing imine synthesis method, we began to tweak and change the method to optimize the purity of the imine product from p-tolualdehyde and t-butylamine. Purity was determined by the introduction of the imine peak and mostly on the reduction of the aldehyde peak in the NMR readings of the products. Below is a table illustrating the development of the new synthesis method.

Exp. #	Ratio of aldehyde to amine	Solvent	Changes to Exp. Procedure	Reduction of NMR aldehyde peak
1	1:1	methanol	None	87.50%
2	1:1	methanol	Replace glacial acetic acid with 20% molar equivalent L-proline	91.25%
3	1:1	DMSO		15.00%
4	1:1	chloroform		97.50%
5	1:1.5	chloroform	Increased amine to excess.	97.50%
6	1:1.5	chloroform	Add 20% molar equivalent piperidine	95.00%
7	1:1.5	"	Add 20% molar equivalent piperidine and replace acetic acid with boric acid	94.38%
8	1:1.5	"	Replace acetic acid with L-proline (repeat of exp. 5)	97.50%
9	1:3	"	Add 20% molar equivalent piperidine	95.00%
10	1:4.5	"	"	97.50%
11	1:9	"	"	98.75%
12	1:13.5	"	"	98.75%

Figure 6 Table mapping development of the new imine synthesis method

The first time we prepared the imine using the same method that had worked well for p-tolualdehyde and p-anisidine, however when the reflux was stopped and the reaction mixture quenched with ice-water the separation of two different layers and no solid product was observed. Therefore, instead of vacuum filtration, we proceeded to extract the organic layer with ethyl ether using separation techniques. After extraction, drying, and rotovapping the amount of product was minimal for the purposes of NMR testing. The spectroscopy results showed moderate purity and a significant 87.50% reduction of the aldehyde peak, however its presence meant that the reaction had not proceeded to completion.

We quickly found that chloroform was the better solvent for this reaction. Also, the addition of L-proline, a secondary amine, greatly improved the results, as can be seen in experiments 2 and 4 (not so with 3 as the DMSO seems to have reacted adversely to the imine production). The secondary amine seems to help push the formation of the imine. However, L-proline is commonly used in the synthesizing chiral products. Since chirality was not an issue for us, we chose piperidine, another secondary amine to replace L-proline. These two changes were giving us aldehyde peak reductions of over 90%, but the final change that completed the method was increasing the amine content by nine times. We increased the amount of t-butylamine because this reagent's boiling point is only 44 °C while the solvent chloroform is 61 °C and in a 24 hour reflux we suspected the amine was boiling out.

The newly developed imine synthesis method seems to work well with a variety of aldehyde and amine combinations. Using the aldehyde t-butylamine and amines p-anisaldehyde and p-nitrobenzaldehyde produced clean NMR results. The new synthesis method was also used with p-anisaldehyde with the amines 4-aminobenzonitrile and 4-bromoaniline. In these, the NMRs showed the return of the aldehyde peaks, however the NMRs show imine peaks as well. For the 4-aminobenzonitrile the imine peak is roughly 50% the height of the aldehyde peak while 4-

bromoaniline has aldehyde peak roughly 33% the height of the imine peak. This indicates that slight modification of the existing (new) imine synthesis method can yield pure results. Interestingly, while the p-anisaldehyde imine is an aqueous product, as most of the successfully synthesized imines are, the p-nitrobenzaldehyde crystallized at room temperature. Likewise, the 4-aminobenzonitrile remained an aqueous product while the 4-bromoaniline imine crystallized while still in the rotovap. Why some crystallize while other imines do not is unknown and more experiments would have to be conducted to find a pattern. Currently we are working on a series of imine formations with t-butylamine and a variety of aldehydes which include: *ortho*-, *para*-, and *meta*-anisaldehyde, in order to see if this imine synthesis method will prove better than the old method in creating imines when only the substituent position has changed slightly. Another set of amines are for the purpose of making intermediates in chalcone synthesis. For the chalcones, the amines used are: 2-acetylfuran, 2-acetyl pyridine, 2-hydroxyacetophenone, and 2-acetylphenylboronic acid. Unfortunately the results for this set of data are unavailable currently as the NMR machine is under repair.

Iminostilbene Synthesis

The successfully synthesized imine using p-tolualdehyde and p-anisidine (Imine 1a) was used for several iminostilbene syntheses. The aldehydes chosen for iminostilbene syntheses were chosen to determine if there is a connection between the effects of the electron donation of a substituent to the efficacy of iminostilbene synthesis. Those with electron donating groups: 4-dimethylaminobenzaldehyde, p-anisaldehyde, 4-(methylthio)benzaldehydes; and those with electron withdrawing groups: 4-cyanobenzaldehyde, 4-nitrobenzaldehyde, and 4-carboxybenzaldehyde. The results so far are inconclusive and remain in study. Due to the generally low yield and impurities in the NMRs, a modified or new method of iminostilbene synthesis is being sought. To the existing method we have tried using a stronger base, potassium bis(trimethylsilyl)amide, which increased the

percent yield of the 4-cyanobenzaldehyde stilbene from 51.60% to 62.48% and the p-nitrobenzaldehyde stilbene from 32.21% to 67.29%.

With the successfully synthesized imine using p-tolualdehyde and t-butylamine (Imine 17) the stilbene synthesis procedure that worked for making Imine 1a's iminostilbenes failed to work with Imine 17. So, we attempted synthesizing stilbenes through some reflux methods, of which I cannot reveal the details of as the methods are Dr. Murray's unpublished methods for a different paper. If further information needs to be requested, please inquire with Dr. Desmond Murray, Assistant Professor of Chemistry at Andrews University.

Future Work

Above I have already mentioned some future work. In iminostilbene synthesis there may be a trend between the electron withdrawing or donating character of the aldehyde substituent and the success of stilbene synthesis. Further experimentation is needed to confirm this hypothesis. With the second imine we have not had comparable success in stilbene synthesis as with the first imine. So, we are trying four different methods of stilbene synthesis and currently analyzing results.

Furthermore, the new imine synthesis method seems to be a good general synthesis method for imines. We are still testing the general scope of the new synthesis method, to see what varieties of imines can be synthesized. Also, if the chalcone intermediates are successful this will open another branch of future research.

Annotated Bibliography

1. Atsushi Kodan, Hiroyuki Kuroda, and Fukumi Sakai. "A stilbene synthase from Japanese red pine (*Pinus densiflora*): Implications for phytoalexin accumulation and down-regulation of flavonoid biosynthesis". PNAS (2002): 99 (5) 3335-3339

This article focuses on the stilbene and chalcone synthases specific to plants and their key roles in stilbenoid and flavonoid biosyntheses. The researchers isolated the cDNA of various stilbene and chalcone synthases and expressed them in *Escherichia coli* and characterized the synthases. One of the stilbene synthase seems to regulate stilbenoid and flavonoid biosynthetic pathways in pine trees. We were mostly interested in the biosynthesis of stilbenes which an illustrative diagram provided.

2. Hu Y, Li QQ, Li H, Guo QN, Lu YG, Li ZY. "A novel class of Cd(ii), Hg(ii) turn-on and Cu(ii), Zn(ii) turn-off Schiff base fluorescent probes." Dalton Trans. (2010): Nov 1

This article describes the novel use of Schiff base fluorescent probes as molecular sensors for metal cations. The researchers found that the Schiff base probes responded in an elegant lamp-like behavior. It would turn on in the presence of Cd(II) or Zn(II) and when Cu(II) or Hg(II) is added the lamp switches off. It is an example of imines being used as molecular sensors in current research.

3. Likhtenshtein, Gertz. "Stilbenes, Applications in Chemistry, Life Sciences and Materials Science." Verlag GmbH & Co. KGaA, Weinheim (2010): 1-41.

The author of several scientific textbooks, Gertz Likhtenshtein is the professor emeritus at Ben Gurion University. This book goes in depth on the synthesis, reaction, and characteristics known about stilbenes as well as their many applications. The excerpt on the preparation and analysis of stilbenes was particularly helpful in our research as the author talks about various methods to prepare stilbenes and reviews each method's limits and attractions.

4. Min Serk Kwon, Sungjin Kim, Sungho Park, William Bosco, Ravi Kumar Chidrala and Jaiwook Park. "One-Pot Synthesis of Imines and Secondary Amines by Pd-Catalyzed Coupling of Benzyl Alcohols and Primary Amines." J. Org. Chem. (2009): 74 (7), 2877-2879.

This research group synthesized imines and secondary amines selectively by Pd-catalyzed one-pot reactions of alcohols with primary amines. This type of reaction did not require any additional reagents and worked for a variety of alcohols and amines. Although we did not use one-pot methods, this research group also used a Diels-Alder reaction to synthesize imines.

5. Padwa, Albert. "Photochemistry of the Carbon-Nitrogen Double Bond." Chemical Reviews (1977): 77 (1), 37-68.

Albert Padwa is the William Patterson Timmie Professor of Organic Chemistry at Emory University. This article gives an overview on the characteristics, especially the photochemical aspects, of a carbon-nitrogen double bond, which imines are categorized under.

6. Roupe, Kathryn A., Remsber, Connie M., Yanez, Jaime A., Davies, Neal M. "Pharmacometrics of Stilbenes: Seguing Towards the Clinic." *Cur. Clin. Pharma.* 2006. (1) 81-101.

The investigators introduce stilbenes and known stilbene derivatives. One of the most well-known stilbene derivative, resveratrol, has been found to have positive therapeutic effects. The purpose of the review is to describe four compounds structurally similar to resveratrol:

piceatannol, pinosylvin, phapontigenin, and pterostilbene and to review their applications in pharmaceutical and agricultural research. The introductory information and natural uses of stilbenes is particularly relevant.

7. Singleton, F. G., Pollard, C. B. "Reactions of Aldehydes with Amines. I. With o-Aminophenol" *Journal of the American Chemical Society* (1940): 62 (9), 2288-2289

Unfortunately this article is quite short and does not relay much information on any procedures. However, it is important for its correction of erroneous data in the chemical database of Beilstein regarding the reaction of aldehydes with amines.

8. Sprung, Murray A. "A Summary of the Reactions of Aldehydes with Amines" *Chemical Reviews* (1940): 26 (3), 297-338

This review gives a summary of aldehyde and amine reactions, including those of aromatic aldehyde and aromatic amines. This review is important as it goes into detail about the reaction and how it takes place as well as linking to many other resources where a more complete procedure can be found.

9. Wolfbeis, Otto. "Materials for fluorescence-based optical chemical sensors". *J. Mater. Chem.* (2005): 15, 2657-2669.

This article reviews current research on fluorescent sensors. Much of the article focuses on different classifications of sensors. It contains information on the uses and challenges in designing molecular sensors. One of the key problems that has slowed down the utilization in the many possible applications (chemical industry, biotechnology, medicine) is the lack of the right materials. We consider that stilbenes are a possible solution to this problem, as some possess fluorescent characteristics.