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Synthesis and Testing of Second Generation Azatilbenes

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Honors Thesis
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Abstract

Based on previous research, azastilbenes have been reported as effective chemical sensors for organophosphates and organothiophosphates; they are related classes and the active components of agricultural pesticides, environmental pollutants and chemical warfare agents. This project builds on previous work and involves the synthesis of a variety of these azastilbenes and the testing of their respective properties.

Azastilbene sensors are prepared using high-yield organic synthetic methodology developed in Professor Murray's lab.

The next step involves testing the sensors through a measure, by UV-Vis spectroscopy and fluorometry, of an optical change upon the addition of analyte to a solution containing azastilbene sensor.

Introduction

In the modern developing and developed world, environmental pollution remains a common problem to the humans and animals. A major cause of this environmental pollution comes from many aspects of the refinement process and include many products which include synthetic organic compounds such as: “plastics, lubricants, refrigerants, fuels, solvents, preservatives, surfactants, dispersants, and pesticides” (De, C., et al., 2010). Another environmental contaminant previously unmentioned includes pesticides.

Pesticides usage is very prevalent within developed and developing countries. Its usage varies greatly, such as, the personal use of pesticides in private gardens to the use of pesticides for commercial farming which has led to an increased output of produce. However, pesticide usage comes with a high price.

Pesticides, however useful, have a low rate of actually reaching their intended targets. Aside from leakage and spills, “many pesticides are sprayed in large amounts with only 1% reaching the intended target” (De, C., et al., 2010). This nonspecific usage of pesticides have many health related effects that have been reported to highly correlate with increased exposure. These health related problems include: a higher incidence of early pregnancy, cancer, asthma, Attention Deficit Disorder (ADD) and Attention Deficit Hyperactivity Disorder (ADHD), and a wide range of other neurophsycological disorders (De, C., et al., 2010). Increased exposure has also been linked to metabolic defects in lab mice in a pre-diabetic state (Lassiter, T.L., et al. 2008) Many of these problems originate from the cholinesterase properties of the organophosphates (OP’s) and organothiophosphates (OT’s) present within many of these

pesticides hence their usage (Fukoto, 1990). Examples of these OP's and OT's include: parathion, diazinon, propetamphos, phorate, chlorpyrifos and chlorfenvinpho, for which-each substance have a similar general structure. These aforementioned problems arise when many of these OP'a and OT's are "converted within the body into forms which irreversibly inhibit acetylcholinesterase by phosphorylating a serine hydroxyl group in the active site of the enzyme (Mahajan, R., et al. 2006). Thus, the development of a technique to detect the levels of these OP's and OT's within our environment is a very important endeavour.

Currently, the use of bulky instrumentation, complicated procedures, and error prone techniques have been implemented in an attempt to identify toxins within out environment. Some of these include: nuclear magnetic resonance (NMR) spectroscopy, chromatography, mass spectrometry, and other approaches such as enzymatic assays (De, C. et al. 2010). Considering the introduction of false positives within many of the aforesaid techniques, the development of chemical sensors that are able to detect organic toxins within the environment with confidence has been an active endeavor within the community of science as well as the goal of this specific research project.

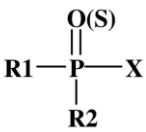
The purpose of this project is to synthesis a chemical molecule facilitating the detection of OP's and OT's within the environment. This project proposes to do so by the use of derivatives of a molecule commonly known as stilbene. The general structure of stilbene is comprised of an ethylene with two phenyl substitutes attached. The derivatives will, however, contain either one or many pyridine rings. The molecular structure of these azastilbenes derivatives gives these chemicals their properties. By exploiting these properties, researchers are

able to use these azastilbenes in a multi-modal manner as a means to detect OP's and OT's within the environment.

Mechanism of action

Before discourse of the mechanism by which azastilbene derivatives sense OP's and OT's, the properties of these azastilbene derivatives must be elucidated. Azastilbene has many attributes because of its unique structure. One such attribute is the property of being colored. The highly conjugated nature of its bonds is the main factor that gives azastilbene its colored characteristic. The conjugation arises from the alternating double bonds within the azastilbene molecule. This conjugation causes an absorption of different wavelengths of light within the visible region of the electromagnetic spectrum, which is typically seen in molecules containing phenyl substituents. Another attribute azastilbene derivatives contain that proves useful their application is the ability to undergo cis/trans isomerism. The double bond interconnecting the two phenyl substituents of stilbene is able to break apart and reform with some amount of energy with the trans form of the molecule being the most favored due to sterics. This is one of the reasons why many azastilbene derivatives fluoresce in solution. Furthermore, azastilbene derivatives have the ability to interact with certain metals and ions. Azastilbenes derivatives such as those that contain pyridine and (1,8)-napthyridine are able to coordinate with metal ions and various molecules (Bera, J., et al. 2009). For example, the pyridine is able to coordinate with certain molecules in a monodentate fashion in which denticity referrers to the number of ligands that are formed upon coordination. Napthyridine containing two nitrogens within its conjoined heterocycles are able to form two ligands. With these attributes, azastilbene derivatives are able to be used in a multi-modal manor reducing the amount of false positives received from tests.

The mechanism by which OP's and OT's bind to azastilbene derivatives begins with a leaving group (X), are able to stabilize its charge, thus leaving the molecule. An example of this is shown in the figure below. With the separation of this group, the molecule takes on a positive charge. With this change in charge, the negative nature of the lone pairs belonging to the nitrogen atoms within heterocycles are able to form ligands with the OP or OT. As a result of this interaction, a change occurs within the properties of the azastilbene sensor. These changes include shifts in the absorption spectrum of the sensor as well as a notable change in fluorescence. By using these changes of properties, shifts in the fluorescence can be used to characterize and analyze toxins within the environment.



- This represents the general structure of organo(thio)phosphates where "X" is a leaving group and the R groups are variable.

- General structure of stilbene

Effects of Denticity

In this experiment, the denticity of the sites is the only dependent variable while other conditions are held constant. However, it is precisely this difference between molecules that account for the differences i9n absorption at various wavelengths and flourecence of the azastilbenes synthesized.

Methodology

Azastilbene Synthesis

Syringe 20 mL of fresh dry DMF into an oven-fried (overnight) 50 mL round bottom flask containing a magnetic stir bar. Atatch a dry, clean condenser with a $CaCL_2$ drying tube to the round bottom flask. Camp the apparatus in place on a magnetic electric stirrer. Measure out and add to the flask in the following order: 15 mL mmol (~0.132 g of) lithium hydride, 2.5 mmol (~0.373g) of 4-dimethylaminobenzaldehyde, 2.5 mmol of methyl azaaromatic which varies according to the proposed product, and 7.5 mmol (~0.8516g) potassium *tert*-butoxide. Note the color change upon the addition of the final reactant. Stir the reaction mixture for 3.5 hours at room temperature. Pour the reaction mixture into a beaker with ice and pour saturated ammonium choride until the mixture has a neutral pH. To ensure this, periodically check the solution through the us of litmus paper. Stir the mixture for 5 minutes. If a solid forms, vacuum filter the reaction mixture us a buchner funnel and air dry the product for one hour. Collect and weigh the product. Calculate the percent yield. Take the NMR of the azastilbene product and compare to NMR of the original methyl azaaromatic and 4-dimethylaminobenzaldehyde. Different azastilbene derivatives are synthesized by using different methyl azaaromatics within the reaction. For example, a monodentate azastilbene may be sythesized using 2-methylpyridine (2-picoline) while a bidentate synthesis is achieved by using a reactant containing two nitrogen atoms within heterocycles as seen in the molecule 2-methyl-1,8-naphthyridine.

Testing of sensors

Visually

To test the effects that OP's and OT's have on the synthesized sensors, stock solutions are made that equate to 100 mL of 5.0 X 10^(-4)M acetonitrile solution of each sensor. Using these stock solutions take 1 mL of each and place that in a vial. It is best to use a syringe to have the utmost accuracy. Next, the stock solution is diluted by adding 5 mL of pure acetonitrile. The OT/OP's are added in 5 μL increments three times and 10 μL one final time. After each incremental increase in the addition of OP or OT, changes of color, color intensity, and florescence are to be noted.

UV-Vis Spectroscopy

Aside from visual changes, changes within the ultraviolet spectrum may be analyzed though the use of instrumentation. A UV-Vis spectrometer is best used in obtaining data. In preparation for spectroscopy, the following steps will be implemented: 1) dilute 1 mL of the original stock solution in 24 mL of acetonitrile and pour 3 mL into a cuvette. 2) Then take 3 mL of pure acetonitrile and pour that amount into a cuvette as well to be used as a reference; 3). Label the λmax. 4) Then take the final concentrations of the solutions constaining OP/OT. 5) Further dilute 1 mL of the solution in 24 mL of acetonitrile. 6) Make note of the shifts in wavelengths in comparison to the stock solution data and to the data of solutions containing other OP's and OT's.

Results and Discussion

Azastilbene Synthesis

The azastilbene synthesis on most occasions resulted in a yield of over fifty percent. This is due impart to the methodology in which the reaction is carried out in one step. This one step processes begins upon the addition of potassium *tert*-butoxide into the reaction mixture. Due to the spontaneity of the reaction at room temperature, a color change is immediately noticed at this step. The mechanism of this reaction involves an acid-base reaction of the methyl group belonging to the methylazaaromatic and the negatively charged oxygen of the *tert*-butoxide. This causes a negative carbanion was then able to subsequently attack the electron deficient carbon within the oxo group of the 4-dimethylaminobenzaldehyde. Following this is a dehydration reaction involving loss of water pushed by the activity of Lithium Hydride on water within the solution.

Nuclear Magnetic Resonance (NMR) analysis

In synthesizing the azastilbene derivatives, conformation of the correct and complete synthesis is best obtained through NMR. An aldehyde peak present

Sensor testing

Visually

Visually, there were some significant changes in the coloration of the compounds tested. Upon addition of OP or OT all solutions of sensor changed a certain color which increased intensity as the concentration of OP/OT increased. On average, the sensor 16A initially appeared red/orange in color which eventually became a deep pink/magenta as the concentration of OP/OT increased. Sensor 17 was a light pink on addition , however, as the concentration of OP and OT increased the color became similar to that of sensor 16. Sensors 17 and 18 were

generally a lighter hue than that of the previous two. Sensor 18 tended to be a lighter pink than the previous two while sensor 19 tended to turn a honey colored yellow as OP/OT was added to solution. One problem that was seen in the data series is the fact that many of these data series are quite similar when the data was obtained by comparing OT or OP to a corresponding OT or OP. However even with the parallel comparison of corresponding OT's or OP's noticeable variability within the response remained. One explanation for this is the similarly in structure of the analytes used. The analytes used include diethylchlorophosphate, diethylcyanophosphonate, and diethylchlorothiophosphate. Each of these analytes only differ from one another in the leaving group each contained with diethylchlorothiophophate which included a thio group instead of the oxo group which was characteristic of the analytes associated with OT's. Thus when the leaving group separates from the molecule, essentially the same structure binds to the analyte producing similar responses. As for florescence, concentration dependent factors played a role in determining whether or not the azastilbene sensors fluoresce in ultraviolet light. It was noted that upon the addition of higher concentrations of OT's and OP's the fluorescence decreased. In addition, only the two solutions for sensors 16 and 17 were florescent visually from the start.

UV-spectroscopy

The results of this test showed variabilities of absorption within the ultraviolet and visible regions of the different samples. Frequently, a shift in the maximum wavelength of absorption * was seen while there also being differences between the spectrums of different trial runs of sensor that contained different analytes were also noted.

Table 1. Wavelengths of Absorption

Table of Wavelengths	
16 Series	Wavelengths (nm)
No analyte	385*, 291, 253
diethylchlorophosphate	621,499-497*,399,379,367,361,210
Diethylcyanophosphonate	498*,499,343,349,334,332
diethylchlorothiophosphate	797,621,505,502,393,391,273,225*
17 Series	
No analyte	408*
diethylchlorophosphate	541*, 378
Diethylcyanophosphonate	539*, 208
diethylchlorothiophosphate	539, 415, 271, 224*
18 Series	
No analyte	266*, 236
diethylchlorophosphate	557*, 336, 281
Diethylcyanophosphonate	540, 272*, 235
diethylchlorothiophosphate	543, 271, 225*
19 Series	
No analyte	371*, 277, 247
diethylchlorophosphate	350.000, 301(λmax)
Diethylcyanophosphonate	370*
diethylchlorothiophosphate	373*, 273, 225

*= λmax-the maximum wavelength of absorption

Conclusion and Future Work

In conclusion, theoretically the synthesis of sensor that may be used in a variety of ways have been a success. Further research would be needed to include the characterization of OT's and OP's based on the results and trends observed. Different defining peaks can be noted in the graphs and in table 1, specifically the characteristic peaks unique to organothiophosphates in the

225 nm region of the UV-Vis spectrum. Further work should be devoted in the development of a technique to measure mechanically the amount of OT's and OP's that bind by the use of a quartz crystal microbalance. The use of these sensing techniques can detect higher, moer accurate levels of toxins within the environment. This may enable changes in the way these chemical compounds are used, and ultimately, may bring about improvements in the health standards nationwide regarding the use of these toxins.

Annotated Bibliography

1. B. Antonijevic, "Unequal Efficacy of Pyridinium Oximes in Acute Organophosphate Poisoning ." *Clinical Medicine & Research* 2007. **5**:71-82
This article is very expansive regarding the history behind organophosphates. It also gives background on the mechanism of toxicity seen in individuals who are exposed to organophosphates and proposes an immediate treatment option for those experiencing acute organophophate poisoning.

2. C. De, et al. "Dual colorimetric and electrochemical sensing of organothiophosphorus pesticides by an azastilbene derivative" *Tetrahedron Letters 51* 2010. **51**:1754-1757
The research mentioned in this paper focuses on many of the same aspects of this paper. In actuality, the research mentioned in the article includes previous research this paper builds on. This paper gives the reasons why the development of a multi-modal sensor is favorable and even gives information about the general structure and some properties of stilbenes in general as well as properties of azastilbene derivatives.

3. F. Kamel, et al. "Neurologic Symptoms in Licensed Private Pesticide Applicators in the Agricultural Health Study." *Environmental Health Perspectives* 2005. **113**:877-82.
This study gives some information what is seen in individuals who undergo acute organophosphate/organothiophosphate exposure. Although some results within this study were non-conclusive, it was noted that there was a general correlation between incidences of persistent neurological symptoms and organophosphate exposure by the pesticide applicators. This article gives readers an idea how testing may be done to access organophosphate poinsoning and how inconclusive some tests are due to extraneous factors within the environment.

4. H. Cataño . et al. "Plasma Cholinesterase Levels and Health Symptoms in Peruvian Farm Workers Exposed to Organophosphate Pesticides. " *Arch Environ Contam Toxicol* 2008. **55**:153–159.
This article involved a study of pesticide applicators employed within Peru. It was found that with higher exposure to pesticides, measured blood acetylcholinesterase activety decreases. In addition, workers that opted not to wear protection equipment tended and those previously poisoned by pesticides also to have lower acetylcholinesterase activity levels. It was found that these levels correlate highly with one's Body Mass Index (BMI). This article reiterates previous work on the subject, but takes into account actual measure blood levels.

5. J. Bera, "1,8-Naphthyridine Revisited: Applications in Dimetal Chemistry." *Eur. J. Inorg. Chem.* 2009: 4023–4038.
This article presents applications of Naphthyridine to be used in chemistry. One of importance to this project is the ability of the naphthyridine to form bidentate ligands

with metal ions. This can be applied to the analytes within the current research. The article gives examples of how these complexes can be made and potential molecules that can be made through the coordination of Naphthyridine to different atoms.

6. K. Roupe, et al. "Pharmacometrics of Stilbenes: Seguing Towards the Clinic." *Current Clinical Pharmacology* 2006. **1**: 81-101.
This article presents information on natural sources of stilbene in nature and the health benefits that come along with them. A complete characterization of the effects of each is listed within the article complete with different biological activites and structure of each stilbene derivative. Information regarding the cis/trans isomerism of stilbenes is also presented.

7. R. Mahajan, et al. "Phorate Exposure and Incidence of Cancer in the Agricultural Health Study." *Environment Health Perspectives* 2006. **114**:1205-1209
This article presents research involving cohort studies focused on the incidence of cancer in association with phorate exposure. The article concludes with presenting evidence that cancer, except for prostate cancer to an extent, doesn't correlate with phorate exposure contrary to previous research. This article presents a contradiction to some of the research done on organophosphates showing evidence of a view not commonly taken.

8. R. Whyatt, et al. "Contemporary-Use Pesticides in Personal Air Samples during Pregnancy and Blood Samples at Delivery amoung Urban Minority Mothers and Newborns." *Environmental Health Perspectives* 2003. **111**:749-756.
The research in particular did not measure the symptoms but the presence of organophosphates and organothiophosphates within the blood of pregnant women belonging to the African American and Dominican demographics. Using their methodology, the researchers were able to deduce the percentage of organophosphates within the blood as well as the percentage of organophosphates in newborns at the time of pregnancy by the use of umbilical cord blood. It was found that all but a few organophosphates where transferred to newborns, and of those that were transferred the same concentration of organophosphate exists in the blood of the newborn in comparison to the mothers. This study shows how organophosphate toxicity theoretically can be passed down from mother to child while at the same time hinting at the dangers that these newborns are exposed to.

9. T.L. Lassiter, et al. "Exposure of Neonatal Rats to Parathion Elicits Sex-Selective Reprogramming of Metabolism and Alters the Response to a High-Fat Diet in Adulthood" *Environmental Health Perspectives* 2008. **116:**1456-1461.
In this work the metabolic activity of rats were scrutinized as different doses of parathion where given neonatally and as adults. Differences within the amount of weight gained or lost differed among sexes and other categories; however, this article points out that in each category a noticeable difference is seen in comparison to a control group. This has

implications not just for disease in regards to organophosphates and organothiophosphates, but also over all function of the body.

10. T.R. Fukuto. "Mechanism of Action of Organophosphorus and Carbamate Insecticids. *Environmental Health Perspectives* 1990. **87**: 245-254.
In his work, Fukuto outlines various aspects of organophophate and carbamate function in pesticides. He notes especially the mechanism whereby organophosphates work to decrease populations of pests. The article mentions the important aspect of organophosphates inhibitory effect on cholinesterase enzymes and the aspect of irreversible binding of organophosphate onto enzymes responsible for regulation of neurotransmitter at the neuromuscular junction in humans and in the central nervous system for many pests. This knowledge of the mechanism helps to further classify and demonstrate health effects that are associated with organophosphates and organothiophosphates within the environment.
11. S. Obare, et al. "Fluorescent Chemosensors for Toxic Organophosphorus Pesticides: A Review" *Sensors* 2010. **10**:7018-7043
This article provides insight into research already done within the area of the current research. It gives specifics on the general properties and formulas of organophosphates and organothiophosphates as well as list many different health hazards of these different compounds. Detailed information regarding structures of organophosphates and organothiophosphates is listed as well as previous procedures done in order to assess organophosphate and organothiophosphate levels with in the environment.