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Synthesis of Substituted Stilbene Probes Towards the Investigation of Antagonistic Effects Observed with 3,5-Substituted Paraben Derivatives

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April 29, 2020

Faculty Advisor: Dr. Andrew Yeagley

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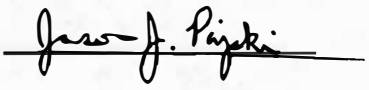
Dr. Jason Pajski (University of Mount Olive) 

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Abstract

In practice, antagonistic xenoestrogen compounds have been used as drugs to treat cancer. Traditional strategies, include preparation of estrogen receptor antagonists exhibiting high affinity for the receptor while preventing rearrangement of the AF-2 binding domain. In a potentially new strategy, substituted parabens have proven to act as antagonists but do not bear the large sidechain associated with this common antagonist strategy. Weak phenolic interactions make determining such paraben binding confirmations and mode of action difficult. To investigate these activities associated with hindered phenolic compounds, substituted bisphenol probes have been synthesized *via* Wittig protocols to produce a series of stilbene derivatives. Herein we present the synthesis of such substituted stilbenes. A model system was used to test the synthesis of many different molecules, with success towards one of the probes. Further work will need to be completed to complete the synthesis of all the probes to allow for testing using TR-FRET and ELISA assays. Antagonism is expected to arise from similar disruption in the role of H12 in the AF-2 ligand binding domain albeit due to alternative binding interactions.

Dedication

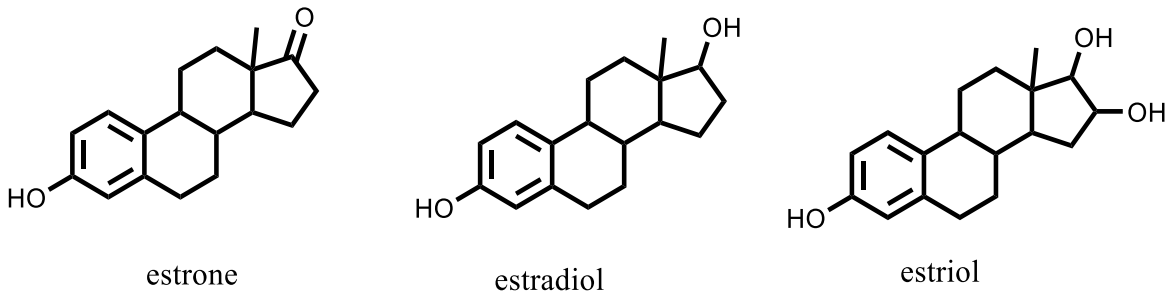
This Senior Honors Research Thesis is dedicated to the chemistry faculty in the Department of Chemistry and Physics at Longwood University, particularly Dr. Andrew Yeagley. Without his guidance, I would not be the scientist that I am today, and with this strong foundation I will be able to expand my knowledge and abilities in the future. Additionally, this document is dedicated to my parents, Michael and Kris Adams, who have fostered an everlasting love of science in me, and without whom this adventure would have been impossible.

Chapter 1

The human body is a very complex system with billions of microscopic moving parts that help the body function. One of these aspects of the body is hormones, small molecules that regulate bodily functions. Many aspects of daily life are regulated by hormones, such as thyroid hormones,[1] which regulate metabolism and heat produced in the body. Insulin works to allow the body to use sugar from carbohydrates in order to supply energy to the body.[2] Hormones work much like drugs in the body, binding receptors and activating them, which prompts the function of the related receptor to activate.

Estrogen functions in this way, binding to an estrogen receptor (ER) and regulating homeostasis in the body. There are two different types of estrogen receptors, ERα and ERβ. They are structurally very similar, but they have differing cellular effects, mostly because of where they are found in the body. ERα is the predominant ER found in reproductive tissues and thus breast cancer cells, while ERβ is ubiquitous and found in many tissues and organs (main receptor found in males). Due to its link to breast cancer, the structure of ERα has been most extensively studied, and for the same reason is the main focus of this work.[3]

Additionally, there are three different types of estrogen shown in Figure 1; they are estrone, estradiol, and estriol, with the diol being the dominant version found in women before menopause.[4] Estrone acts as the precursor to the diol and triol and for this reason is in higher concentration post menopause when in females the production of the latter two are downregulated. Estriol is the estrogen primarily responsible for preparing the body for child birth with its concentration peaking just prior to child birth. All of these estrogen derivatives are produced naturally within the gonads, and are to some extend present in both males and females.



estrone estradiol estriol

Figure 1: Chemical structures of estrone, estradiol, and estriol.

Estrogen regulates many vital systems in the body, including the bones, the cardiovascular system, and the nervous system.[5] The concentration of estrogen in the body is extremely important for this reason. For example, in a review by Grosman-Rinion et al.,[6] the link between estrogen concentration and cardiovascular health was explored, showing that low estrogen levels can lead to low energy and poor cardiovascular health. However, the most important role of estrogen is the one it performs regularly throughout the body. Estrogen controls a variety of cellular responses, including protein synthesis and calcium mobilization. When bound to ERα, the complex is responsible for gene expression, interacting with DNA and controlling complex protein interactions that determine cellular function.[7]

The ligand binding domain (LBD) of ERα consists of two distinguished but cooperating binding clefts. Figure 2 illustrates these clefts which are known as the ligand binding cleft (LBC – shown in blue) and Activation Function-2 (AF-2 – shown in red). In order to activate the receptor, a ligand must come in contact with the LBD. Once the ligand interacts with the LBD, it will then bind within LBC. This binding interaction allows a conformational change to occur within ERα. The solvent exposed helix-12 of AF-2 shifts closer to the other regions shown in red. This generates the active form of the AF-2 binding

site which can then recruit regulatory proteins. Once the regulator proteins have bound to AF-2, the entire complex binds to the estrogen receptor element (ERE) which is the gene in the DNA specific for the ER. This binding initiates gene expression which is the production of related mRNA that will be converted to functioning proteins.[8]

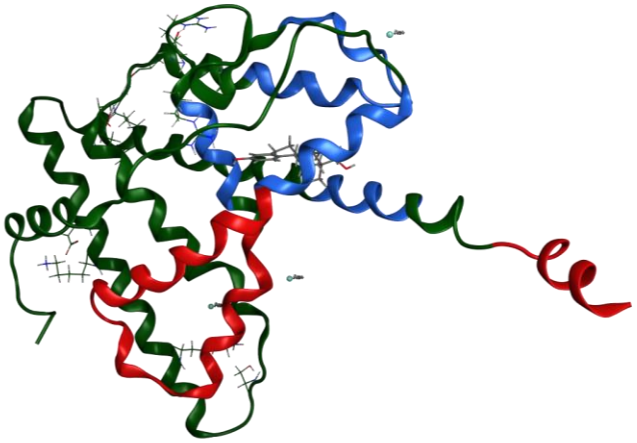


Figure 2: Color coded image of estrogen receptor. Red ribbons denote the AF-2 complex (currently unfolded in image), and the blue ribbons denote the ligand binding cleft.

While many receptors are selective about the substrate they allow to bind, estrogen allows many different molecules to bind and activate ERα. These different molecules that can act as agonists to ERα prompting similar reactions that have slightly different end results. Before binding can happen, estrogen and other molecules must travel through the cell membrane in order to interact with ERα. However, in order for the estrogen or estrogen-mimicking molecule to interact with the receptor, some key requirements need to

be met. The ideal configuration is that of estrogen itself, and it is believed that the important features that allow estrogen to bind with the receptor are the hydroxyl groups, shown in Figure 3.

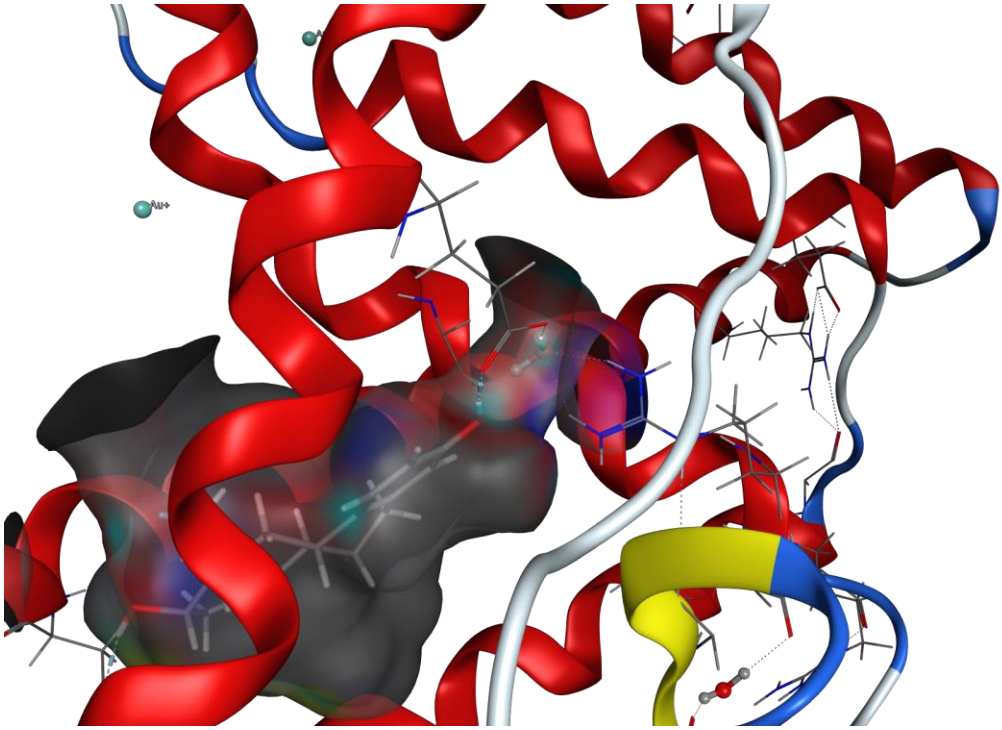


Figure 3: A model highlighting the interactions between estrogen's hydroxyl groups and the binding pocket of ERα, shown in gray.

One of the hydroxyl groups on estrogen is aromatic and called a phenol (interaction in upper righthand corner of binding pocket shown in gray) while the other is aliphatic (interaction in lower lefthand corner). The two hydroxyl groups are approximately 9.6 Å apart with a region of hydrophobicity between them. The best estrogen mimics, or xenoestrogens, are the ones that come close to meeting these standards. For example, bisphenol A is a known xenoestrogen, and it has two aromatic hydroxyl groups that are 9.3 Å apart and have a hydrophobic region between them.[9]

There are two different categories of ligands that can bind ERα; agonists and antagonists. Agonists are molecules that activate ERα after having bound the receptor. Estrogen is an agonist, as well as phenols and their bisphenol derivatives. Agonists each impact ERα activity in different ways. This is due to variations in exposing the AF-2 binding site that can impact its ability to interact with regulatory proteins, and thus impacts the genes that are expressed when the complex binds with ERE.[9]

Molecules that bind the receptor but do not activate it are referred to as antagonists. These molecules inhibit the function of ERα either because of their size or the functional groups. Since hydroxyl groups are so important to the activation of ERα, missing one hydroxyl group or having other large functional groups around it can seriously impede the binding ability of the molecule. Antagonists can work by chemically shutting off the receptor and preventing it from performing its function, or they can have a ligand that is physically too large for the receptor to be activated. One such molecule is raloxifene, a cancer treatment drug with a large ligand that prevents an active AF-2 from forming. Molecules like antagonists that can bind with a receptor are said to have an affinity for the receptor. When a molecule has an affinity for a receptor and can activate it, it is said to also have efficacy. Both of these aspects are important for proper binding and activation of the receptor, leading to proper gene expression.

Chapter 2

The activity of pharmaceuticals is determined by their affinity and efficacy. Binding affinity refers to the ability of a molecule to enter the ligand binding domain (LBD) and bind with the receptor. Molecules that have an affinity for a receptor will be able to enter the binding pocket and interact with the receptor sites, but might not necessarily make the appropriate interactions to activate the receptor. Efficacy refers to the ability of the small molecule to activate the target receptor once it has bound. If a molecule is able to enter the binding pocket and interact with receptor sites in a way that activates the receptor, then the molecule is said to have efficacy for that receptor. As with many protein receptors, when a molecule has efficacy for the ERα, it is due to an invoked conformational change in the receptor. For ERα this change involves the proteins helix 12 (H12) being pulled in towards the LBD and exposes AF-2. If the small molecule does not invoke this conformational change then the ERα has not been activated, and the molecule inside the binding pocket merely has an affinity for the receptor. For this reason, blocking H12 from coming towards the receptor is a common method of preventing molecules from activating ERα.[10]

Raloxifene is a drug used to treat breast cancer[11] by inhibiting ERα.[12] It also functions as a treatment for osteoporosis in post-menopausal women, which indicates that estrogen receptors in different types of tissue can serve different purposes. The structure of raloxifene is shown in Figure 4. The molecule has the two hydroxyl groups that seem to be necessary to bind the ERα (red), but it has a large side chain (blue) that protrudes in between the two hydroxyl groups. The additional bulk of the side chain prevents H12 from interacting with the ligand binding cavity,[10] and therefore inhibits the formation of an

active AF-2, which is essential for recruiting cofactors[9] and thus gene expression.[5] Agonists of the receptor will be able to bind and to varying extents expose AF-2 similarly to estrogen.

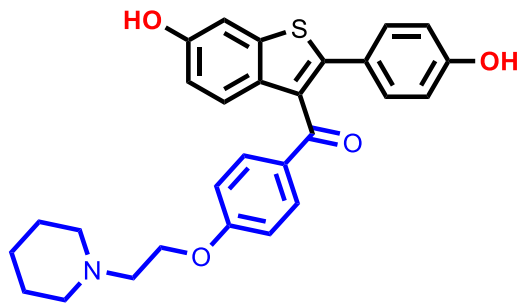
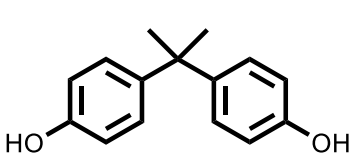
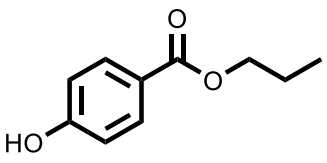


Figure 4: Structure of raloxifene.

Bisphenols are known xenoestrogens that are able to bind and activate ERα.[13] They have two hydroxyl groups on either end of the molecule that makes them full agonists, because their structure allows them to fully mimic the binding of estrogen. Since they are full agonists, they are able to bind and activate ERα, thus leading to the exposure of AF-2 which will lead to gene expression. Parabens only have one phenolic group yet surprisingly activate ERα. However, this feature imparts only partial agonist activity; able to bind and activate ERα, but not activating the receptor to its full potential.[14] A comparison of these structures is shown in Figure 5. Since there is still one hydroxyl group on propyl paraben, these molecules are



Bisphenol A



Propyl paraben

Figure 5: Chemical structures for agonists bisphenol A and propyl paraben.

able to bind but with the lack of a second hydroxyl group the binding is much weaker and the effect on the system is also weaker.

Our lab has made alterations to the standard structure of parabens in order to disrupt their affinity and thus efficacy.[15] By substituting the hydrogens at the 3 and 5 positions on the phenolic ester of the parabens with different halides, it was anticipated that the substituted parabens would block the essential phenolic interaction and prevent binding with ERα. The generic structure of these parabens is shown in Figure 6. However, upon investigation by Bergquist et al.[15], it was determined that the substituted parabens still bound ERα, but they did not activate it. This indicates that the substituted parabens have an affinity for ERα, but do not have any efficacy. Substitution at the 3 and 5 positions was supposed to prevent binding altogether due to sterically blocking phenolic interactions. Since the substituted parabens were still able to bind, there must have been something more complex going on that had not yet been accounted for. This factor remains a mystery, so the exact reason that these substituted parabens are antagonists is unknown.

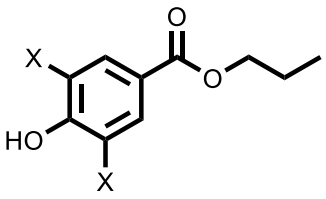


Figure 6: Structure of substituted parabens where X represents different substitutions.

Discerning this activity is important to understanding how substituted parabens work. In order to determine the activity of substituted parabens, there are a variety of experiments that could be conducted. For initial estimates, computational work could be

conducted using molecular modeling software. Using this, substituted parabens could be fit into an estrogen receptor and different binding modes could be explored. The flaw with this method is that parabens are extremely flexible and could fit into the binding pocket in multiple ways. This leads to a multitude of viable interactions with difficulty discerning which is the real binding mode. To account for this, theoretical calculations could be done on bisphenols, molecules that will be similar in size and function to parabens, but are far less flexible. Using different amounts of substitutions, these probes could be used to gain theoretical knowledge of the binding activity of substituted parabens. Since this method is purely theoretical, this would be followed by building the probes and testing their binding, and using x-ray crystallography, to accurately determine their binding activity.

Chapter 3

The affinity that a small molecule has for a receptor is a very important quality to be able to measure. When testing a new drug, it is vital to know if the drug simply binds the receptor, or if it also has efficacy and turns on the receptor as well. In addition to knowing if the drug binds the receptor, it is useful to know how strongly it binds to the receptor. There are two methods to measure the strength of binding; Computational calculations can be run to theoretically determine strength of binding and experimental based calculations can be carried out using terbium based time-resolved fluorescence resonance energy transfer (TR-FRET) to determine the IC_{50}, which is the concentration required to bind 50% of the receptors present in the study. Such binding experiments have been run on a plethora of endogenous and exogenous molecules to determine their affinity for the estrogen receptor, and an experimental design is shown in Figure 7.[16]

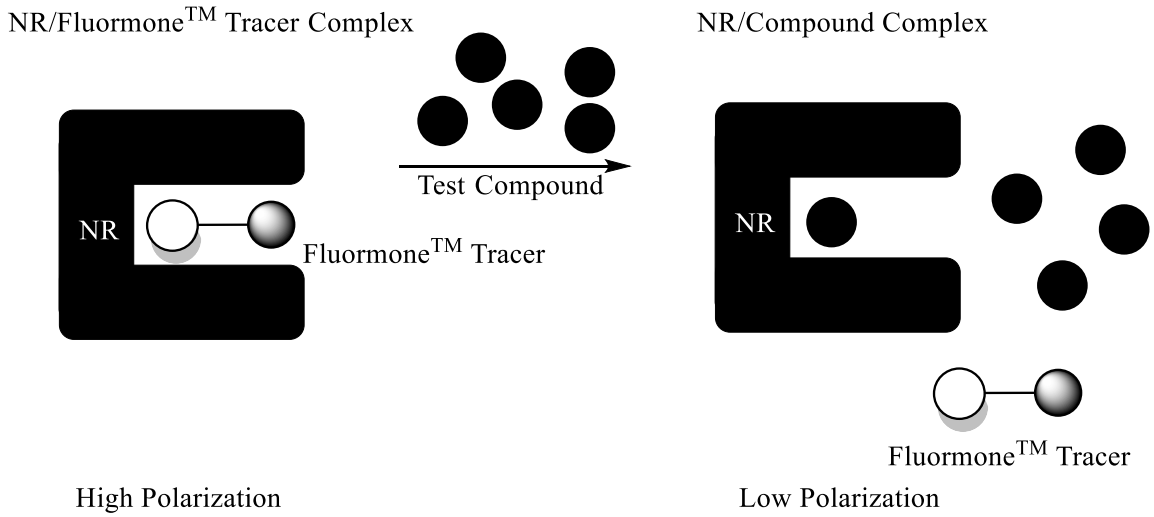


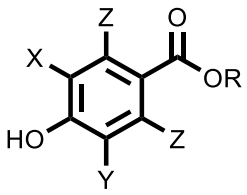
Figure 7: Diagram displaying TR-FRET analysis.

Alternatively, computational work can be used to determine affinity for a receptor. In a study by Byford et al.,[17] theoretical calculations were run on how parabens bind to an

estrogen receptor. A docking study was run on a molecular modeling software, OPTIMOL, that allowed for the group to program two parabens occupying the binding site at the same time. They tested parabens with varying carbon chain lengths, from methylparaben with one carbon, to *n*-decylparaben with ten carbons. The group found that for parabens with chain lengths from one to four, two parabens were able to be contorted into the binding pocket and fill the role of the two necessary hydroxyl groups, theoretically resulting in activation. Unfortunately, since these results are theoretical and not experimental, additional experimentation would need to be conducted to concretely prove that such a dimeric binding is required for activation of the ERα.

There are many xenoestrogens, or estrogen mimics, in nature. For example, halogenated phenols are found in the blood of humans and other animals.[18] The exact nature of their binding to ERα, and whether or not the receptor is activated by the halogenated phenols is an area of significant research. The goal of many groups, such as Olsen et al.,[19] is to test the efficacy of these xenoestrogens. Olsen et al. tested a variety of phenols with varying bromine substitutions that have been found in blood samples in the past. Their results showed that the phenols that were heavily brominated, or had more than two bromines present, were unable to effectively displace estrogen from ERα, indicating that this compound did not have a strong affinity for the receptor. The molecules which had a single bromine or a pair of bromines were able to effectively displace estrogen by binding with the receptor, but had lesser or no efficacy, meaning these compounds were unable to activate the receptor to begin transcription. This may indicate that since the hydroxyl group is important to binding and activating the estrogen receptor, it is necessary to ensure that this group is not sterically blocked from binding with the receptor in order

to activate it. Even when the hydroxyl group is blocked by bromination, binding is still possible because it is impacted to a lesser degree than the efficacy of the molecule. Unfortunately, the binding strength of most mono-phenolic xenoestrogens is not strong enough to stay bound during the recrystallization process, and therefore the binding mode cannot be accurately determined. For this reason, it is impossible to know exactly what is occurring in the binding pocket that allows ERα to bind with so many different substrates.



X, Y, Z	R	IC_{50} (nM)	Efficacy
F, F, F	Butyl	29400	antagonist
Cl, Cl, H	Butyl	55300	antagonist
Br, Br, H	Butyl	39400	antagonist
Br, H, H	Butyl	8210	antagonist
I, I, H	Butyl	3600	antagonist
I, H, H	Butyl	7980	antagonist
CH_3, CH_3, H	Butyl	32500	antagonist
^{t}Bu, ^{t}Bu, H	Butyl	>200000	antagonist
OH, OH, H	Butyl	8970	antagonist
OMe, OMe, H	Butyl	>200000	antagonist
NO_2, NO_2, H	Butyl	>200000	antagonist
I, I, H	Octyl	13200	antagonist
CH_3, CH_3, H	Octyl	16200	antagonist
OH, OH, H	Octyl	8740	antagonist
NO_2, NO_2, H	Octyl	60400	antagonist
H, H, H	Butyl	1420	agonist

Table 1: TR-FRET binding data and ELISA efficacy data from Bergquist et al.[15]

In order to try and understand this binding and exactly how it works, several studies have been conducted. Bergquist et al.[15] synthesized parabens with different substitutions

on the phenol ring in order to prevent binding of ERα. Once these compounds were analyzed with TR-FRET analysis, the results showed that the substituted parabens were still able to bind the receptor. Additional testing using enzyme-linked immunosorbent assay (ELISA) showed that the substituted parabens were unable to activate ERα, thus making them antagonists. These results, shown in Table 1, were highly unexpected, and thus sparked additional research.

Following these results, additional computational work was done, attempting to model how the substituted parabens were binding inside ERα. Using Molecular Operating Environment (MOE), theoretical calculations were run to gain additional understanding of the substituted parabens' binding. Unfortunately, single phenols, such as parabens, are extremely flexible and can contort and bend in many different ways, allowing for a multitude of different binding modes inside the binding site. This means that computational data are very difficult to interpret, and may not be accurate.[20]

In order to obtain clear data, a molecule that is more rigid should be used, but it is important that this new molecule be similar in structure and function to phenols, so that they will be suitable analogs for binding. One proposed molecule would be a bisphenol like stilbene. Stilbenes are composed of two phenols bound together by a short carbon chain. This structure makes them much less flexible, and so allows for clean computational data. Additionally, given the theory that two parabens fit into the binding pocket to bind and activate ERα, a bisphenol probe would be a suitable analog for this conformation. Their rigidity would also make them suitable probes for ERα, and they may be able to stay in the binding site strongly enough for a crystal x-ray to be taken, thus providing the exact binding mode.

Chapter 4

Understanding how parabens bind the ERα is an essential part of knowing how to make them safer. The work of Bergquist et al.[15] showed that parabens substituted at the 3 and 5 positions prevent activation of ERα, but they still bind the receptor. Since the binding of these parabens is not strong enough to allow for a crystal structure to be taken of the bound complex, some other probe must be synthesized. For this study, a set of three stilbene probes has been proposed. These probes are stilbene, disubstituted stilbene, and tetrasubstituted stilbene, shown in Figure 8. These probes are designed to bind more strongly to ERα because they have two hydroxyl groups that can interact with the receptor, but in theory display similar binding characteristics to our own substituted phenols.

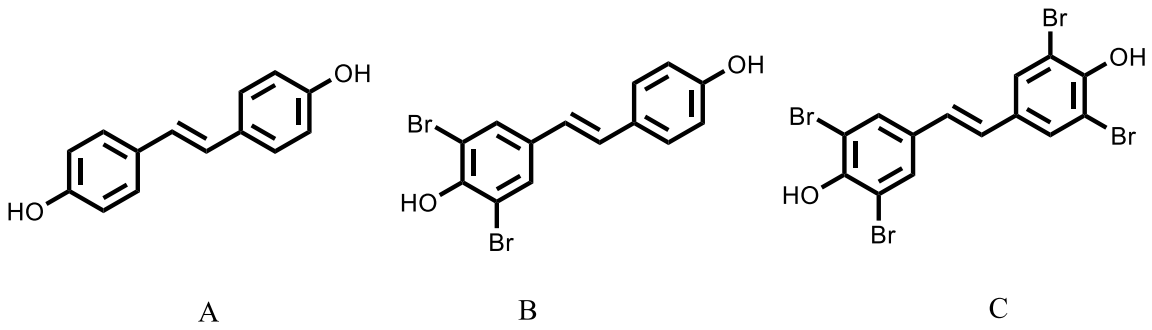


Figure 8: Structures of stilbene probes, where A is unsubstituted stilbene, B is disubstituted stilbene, and C is tetrasubstituted stilbene.

Before synthesis could begin, computational docking was performed using Molecular Operating Environment (MOE)[21] to confirm relative interactions of these probes with the ERα. Each molecule was programmed to bind with ERα with 30 different random conformations and these poses were then energy minimized to find the ones with the lowest energy (best binding). The S-score and energy of refinement were taken into account when

deciding which conformation had the lowest energy. The S-score is a somewhat proprietary score in MOE that accounts for a combination of molecular strains and favorable interaction energies. The energy of refinement represents the strength of the favorable interactions between the receptor and the stilbenes after energy minimizations are complete. The results of this docking study are shown in Figure 9.

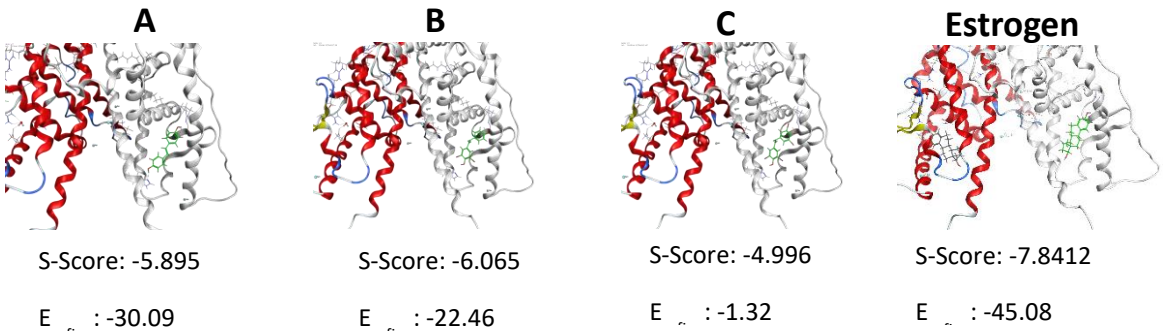
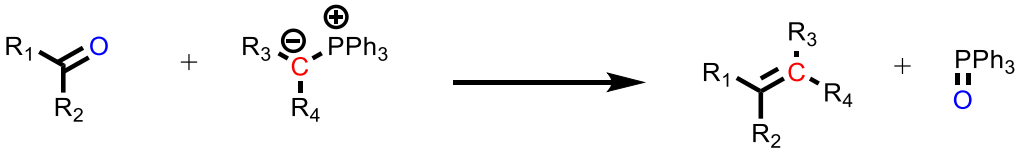


Figure 9: Binding confirmations and computational results from the MOE study. A, B, and C labels taken from Figure 8

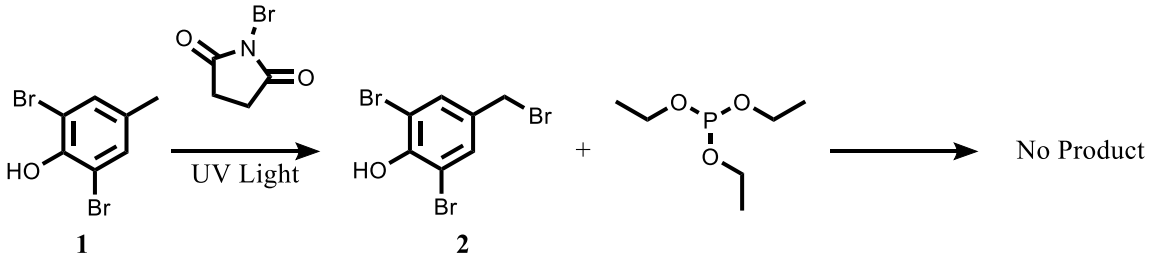
From this data, the unsubstituted stilbene seemed to provide the best fit. It has a low S-score and a low refinement energy, meaning that it binds well with little need for conformation adjustments. The disubstituted stilbene derivative seemed to fall in the middle, with a low S-score but a higher energy of refinement that the unsubstituted version. This version seemed to bind with the hydroxy binding site of ERα. Tetrasubstituted stilbene seemed to be the molecule with the worst binding. It has the highest S-score of the molecules tested, and its refinement energy is quite high, meaning it took a considerable amount of conformational changes to make the molecule bind with the receptor. After these calculations were completed, the expectations for experimental data were set.

In order to synthesize these probes, a Wittig method was used. Wittig reactions are a common technique used in organic chemistry to create elusive carbon-carbon pi-bonds.[22] An example of a simple Wittig synthesis is shown in Scheme 1. Within this reaction an aldehyde or ketone (double bound oxygen) is reacted with a phosphonium. The phosphines desire for oxygen (strength in binding) causes the dehydration of the carbon skeleton to produce the final pi-bond.



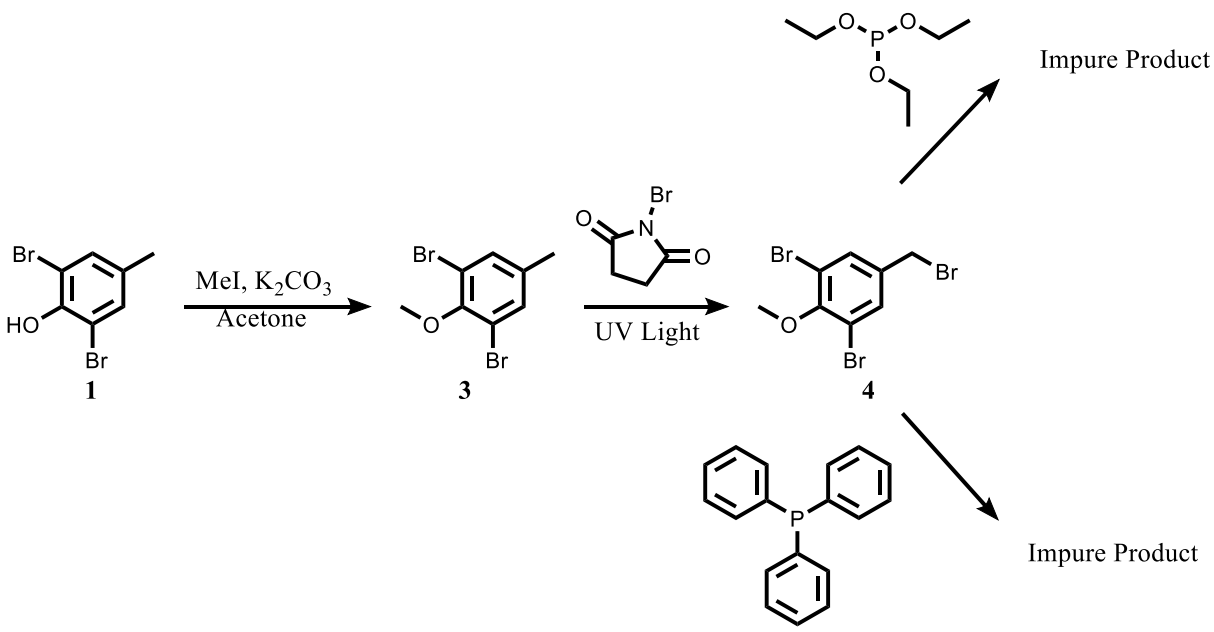
Scheme 1: General scheme for a Wittig reaction.

Initially, synthesis of these probes was attempted using the most direct method, which was attempting to connect two phenolic rings together using a Wittig reaction. First, it was necessary to synthesize the phosphonium (phosphonate in this case) molecule that would undergo the Wittig reaction. This involved the benzylic bromination of the required substituted phenol **1** and attempted displacement with triethylphosphite. The scheme for this reaction is shown in Scheme 2. Unfortunately, several iterations of this method were unsuccessful, and the next step was to use a model system to screen why this system failed.



Scheme 2: Scheme of initial attempt at probe synthesis.

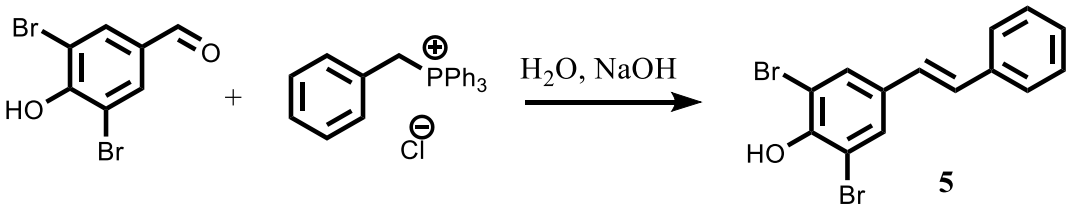
Using a model system is a valuable way to test synthesis with simpler molecules in order to see what is the best fit for the reaction at hand. The first issue we attempted to address was the acidic hydrogen of the phenol. Its presence in the reaction could have been the source of failure for the initial reaction attempt, since the hydrogen may lead to other irreversible substitution products. Therefore, the decision was made to replace the hydrogen with a methyl. This portion of the model system began by taking the initial substituted phenol and using methyl iodide to substitute the hydrogen for a methyl group, creating a substituted anisole **3**. This compound underwent benzylic bromination before two different phosphonium groups were used to try and move towards the final probe. A scheme showing these reactions is shown in Scheme 3.



Scheme 3: Scheme of the initial anisole model system.

Ultimately, this route failed as well, and did not form the desired product. We at this point suspected that we were having difficulty obtaining the unstable benzylic bromide intermediate **4**. Following this, a different approach was taken to attempt the synthesis from

another angle. Instead of generating the substituted phosphonium, a substituted benzaldehyde derivative was used to react with a similar benzyl phosphonium. In addition, we still believed that this approach would be amenable to the presence of the phenolic proton. The scheme for this reaction is shown in Scheme 4.



Scheme 4: Scheme of initial benzaldehyde derivatives.

While this reaction did work, the yield was low, approximately 38%, and was difficult to purify, so other methods were explored to hopefully offer a higher yield. The next round of experiments included the benzaldehyde like the previous run, as well as the same phosphonium group. However, these experiments differed in that once again the hydroxyl group was replaced with a methoxy group. The experiments that were run with this method are detailed in Table 2.

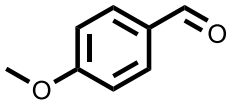
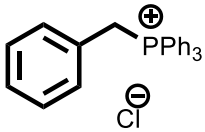
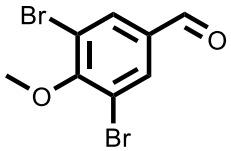
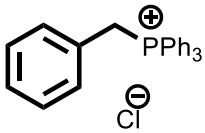
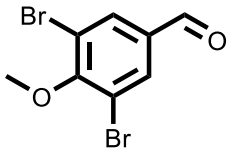
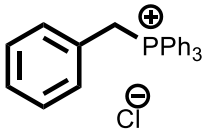
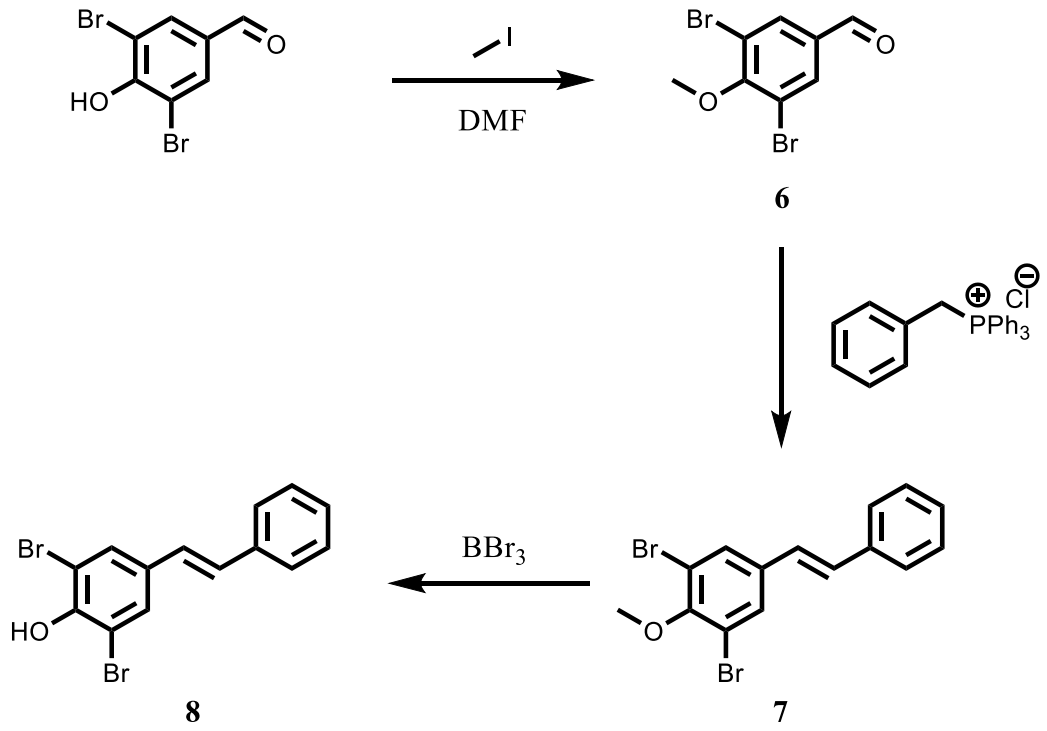
Benzaldehyde	Phosphonium	Solvent	Base
		Water	Sodium hydroxide
		Water	Sodium Hydroxide
		Tetrahydrofuran	Sodium hydride

Table 2: Table detailing the different methyl and benzaldehyde trials.

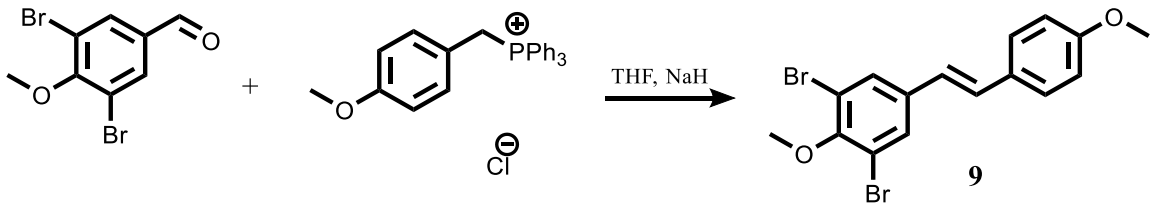
These reactions offered more success than any of the previous trials in the fact that they delivered cleaner products, even if the yield was lower. Of these, the most promising trial is the third reaction in Table 2. Though it had a yield of only 18%, the product was very clean following column chromatography. Following this, the methyl needed to be removed to expose the free phenol, since the desired probe contained a hydroxyl group and not a methoxy group. As this was the most successful trial, its synthesis is detailed from start to finish in Scheme 5.



Scheme 5: Complete synthetic scheme of the most successful trial.

In light of this success, another modification was made to this method. It involved the same benzaldehyde group that was successful in the previous runs, however the phosphonium was altered. It now had a methoxy group on one of the phenyl rings. This alteration was made in hopes to come closer to the monosubstituted stilbene probe, as the

probe has a hydroxyl group on both sides. Production of methylated probe **9** was successfully prepared by the reaction scheme in Scheme 6.



Scheme 6: Reaction scheme for the trial with the methoxylated phosphonium.

This is where our work ends for the moment due to time constraints. The disubstituted probe is well within reach, considering that the only step keeping **9** from the structure of this probe is demethylation. Future members of this lab will continue to synthesize these probes, leading to tests using TR-FRET to determine binding. Once these tests are complete, the data can be used to help determine the binding mode of parabens.

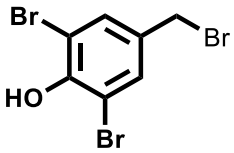
Conclusions

This study was conducted to investigate and understand the binding mode of substituted parabens to ERα. To accomplish this, three different stilbene probes were designed, including unsubstituted, disubstituted, and tetrasubstituted stilbene. Initially, these probes were investigated using computational software in a Molecular Operating Environment (MOE) to determine theoretical binding of ERα. The S-Score and energy of refinement were recorded to be used as a reference for future experimental binding data.

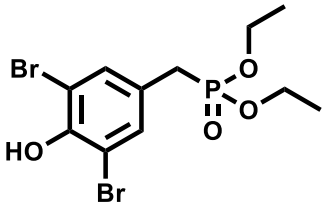
Following these computational results, synthesis began. There was limited success to begin with, but modifying the synthetic route led to near completion of the disubstituted stilbene probe. By taking a less direct route and carefully synthesizing each compound, a derivative of the final product was obtained.

Future work will need to be conducted to truly understand the binding mode of parabens. Following the methods shown in this document, the stilbene probes could be synthesized in order for a docking study to be conducted. With the binding data from that study, and a crystal structure of ERα bound to the probes, more could be known about the way that substituted phenols, such as parabens, bind to ERα.

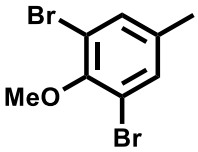
Methods



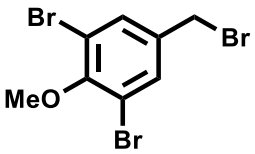
2,6-dibromo-4-(bromomethyl)phenol (2). 3.24 grams of 2,6-dibromo-4-methylphenol and 2.42 grams of N-bromosuccinimide was added to 51 mL of chloroform in a 100 mL round bottom flask, swirling to dissolve solid material. This was placed under ultra-violet light for 3 days while stirring. After 3 days, the reaction was removed from UV diluted with chloroform, then washed with hydrochloric acid. The organic layer was dried using magnesium sulfate, followed by vacuum filtration, and finally evaporation under vacuo.



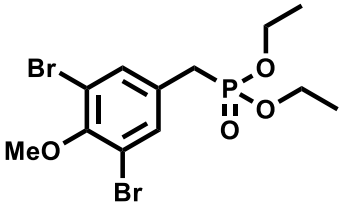
diethyl (3,5-dibromo-4-hydroxybenzyl)phosphonate (2a). 499 mg of 2,6-dibromo-4-(bromomethyl)phenol was added to 2 mL of triethylene phosphite in a 10 mL round bottom flask. The reaction was set to reflux at 150°C while stirring. Thin layer chromatography was performed in 15 minute increments using 15% ethyl acetate in hexanes as an eluent to test the progress of the reaction. TLC failed to identify product material and excess sodium hydride was added to determine phosphonate formation without production of characteristic yellow phosphonium color.



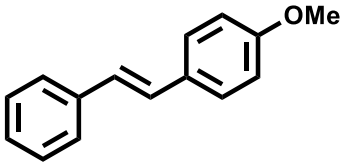
2,6-dibromoanisole (3). 5 g of 2,6-dibromo-4-methylphenol, excess potassium carbonate, and 10 mL of acetone to a 100 mL round bottom flask. Once 2,6-dibromo-4-methylphenol was dissolved in acetone, 2.37 mL of methyl iodide was added to the reaction before it was set to reflux. After 24 hours, the reaction was removed from heat and was vacuum filtered to remove the solid potassium carbonate salt. The product was evaporated under vacuo to yield a cloudy yellow liquid, with a 96.8% yield.



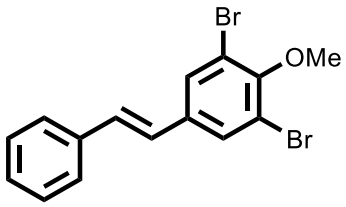
1,3-dibromo-5-(bromomethyl)-2-methoxybenzene (4). 1.10 grams of 2,6-dibromo-4-methylanisole and 0.738 grams of N-bromosuccinimide was added to 17 mL of chloroform in a 100 mL round bottom flask, swirling to dissolve solid material. This was placed under ultra-violet light while stirring. After 3 days, the reaction was removed from UV diluted with chloroform, then washed with hydrochloric acid. The product was dried using magnesium sulfate, followed by vacuum filtration, and finally evaporated under vacuo. The final yield was 1.55 g, or 110%



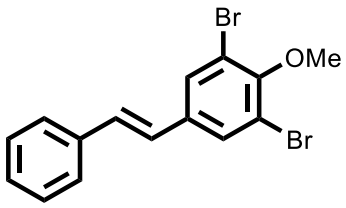
Phosphonium conditions from 4. 499 mg of 2,6-dibromo-4-(bromomethyl)anisole and 2 mL of triethyl phosphite were combined in a 10 mL round bottom flask. The reaction was refluxed at 150°C for two hours before placing the reaction on ice. While cold 0.32 grams of sodium hydride was added. Sodium hydride addition formed the characteristic ylide dark red or brown color, but addition of 3,5-dibromo-4-hydroxy benzoate reverted the color back to a pale yellow. This indicates incomplete formation of the ylide. The product was purified by flash chromatography. Purification began with 10% ethyl acetate in hexanes before increasing to 20% ethyl acetate in hexanes and finally increasing to 50% ethyl acetate in hexanes. The fractions containing product were collected in a 100 mL round bottom flask and underwent evaporation under vacuo to provide 2.711 g of impure product.



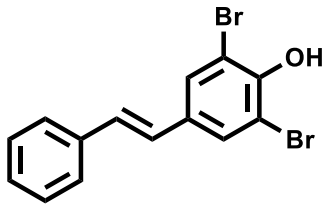
Phosphonium conditions 1, from Table 2. 0.8 mL of water and 0.785 g of tetraphenylphosphonium chloride were added to a 25 mL round bottom flask and allowed to stir for 15 minutes with the slow addition of 0.322 g of sodium hydroxide. After 15 minutes, 0.2 mL of 4-methoxybenzaldehyde was added to the reaction which was then allowed to stir at 70°C. After 3 hours, the reaction was removed from heat and quenched with 15 mL of water. Water was removed from the reaction by vacuum filtration to give off-white crystals. These crystals were purified using column chromatography, with the eluent being 15% ethyl acetate in hexanes. This gave 0.07 g of product, or 20.6%



Phosphonium conditions 2, from Table 2: 0.8 mL of water and 0.790 g of tetraphenylphosphonium chloride were added to a 10 mL round bottom flask and allowed to stir for 15 minutes with the slow addition of .482 g of sodium hydroxide. After 15 minutes, 0.25 g of 3,5-dibromo-4-methoxybenzaldehyde was added to the reaction which was then allowed to stir at 70°C. After 3 hours, the reaction was removed from heat and quenched with 15 mL of water. The product was a sticky yellow solid, which was dissolved with ethyl acetate and purified using flash chromatography, where the eluent began as pure hexanes then transitioning to 5% ethyl acetate in hexanes before increasing to 10% ethyl acetate in hexanes. The final product was 0.226 g, or 72%.

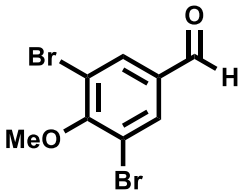


Phosphonium conditions 3, from Table 2. 18 mL of tetrahydrofuran and 7.063 g of tetraphenylphosphonium chloride were added to a 50 mL round bottom flask and allowed to stir for 15 minutes with the slow addition of 1.1 g of sodium hydride. After 15 minutes, 2.02 g of 3,5-dibromo-4-methoxybenzaldehyde was added to the reaction which was then allowed to stir at 70°C. After 3 hours, the reaction was removed from heat and quenched with 15 mL of water. The product was dried using magnesium sulfate and rotary evaporated, yielding a vibrant yellow solid. The product was dissolved with hexanes and purified using flash chromatography, where the eluent was 5% ethyl acetate in hexanes. The yield was 16%.

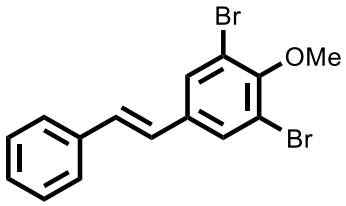


(E)-1,3-dibromo-2-methoxy-5-styrylbenzene (5). 0.8 mL of water and 0.775 g of tetraphenylphosphonium chloride were added to a 10 mL round bottom flask and allowed

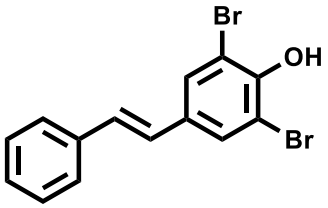
to stir for 15 minutes with the slow addition of 0.419 g of sodium hydroxide. After 15 minutes, 0.239 g of 3,5-dibromo-4-hydroxybenzaldehyde was added to the reaction which was then allowed to stir at 70°C. After 3 hours, the reaction was removed from heat and quenched with 15 mL of water. The product was a sticky orange solid, which was dissolved with isopropyl alcohol and purified using column chromatography, where the eluent began as 5% ethyl acetate in hexanes before increasing to 10% ethyl acetate in hexanes. The yield was 38%.



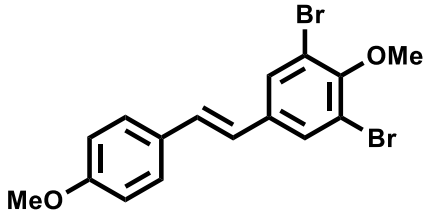
3,5-dibromo-4-methoxybenzaldehyde (6). 5.3 g of 3,5-dibromo-4-hydroxybenzaldehyde was added to a 100 mL round bottom flask containing 75 mL of dimethylformamide and 5.56 g of potassium carbonate. After stirring for a few moments, 2.5 mL of methyl iodide was added to the reaction before the reaction was allowed to stir at 55°C for three hours. After three hours, the reaction was removed from the heat and quenched with 150 mL water, turning the yellow liquid into a white solid. The yield was 98.9%.



(E)-1,3-dibromo-2-methoxy-5-styrylbenzene (7). 18 mL of tetrahydrofuran and 7.063 g of tetraphenylphosphonium chloride were added to a 50 mL round bottom flask and allowed to stir for 15 minutes with the slow addition of 1.1 g of sodium hydride. After 15 minutes, 2.02 g of 3,5-dibromo-4-methoxybenzaldehyde was added to the reaction which was then allowed to stir at 70°C. After 3 hours, the reaction was removed from heat and quenched with 15 mL of water. The product was dried using magnesium sulfate and rotary evaporated, yielding a vibrant yellow solid. The product was dissolved with hexanes and purified using flash chromatography, where the eluent was 5% ethyl acetate in hexanes. The yield was 16%.



(E)-2,6-dibromo-4-(4-hydroxystyryl)phenol (8). A 5 mL round bottom flask was dried and put under nitrogenous conditions. 0.103 g of (E)-1,3-dibromo-2-methoxy-5-styrylbenzene was added to the round bottom flask, followed by 1 mL of boron tribromide. This reaction was allowed to stir overnight. The reaction was quenched with 1 mL of methanol and extracted with ethyl acetate. The extraction was washed with water and salt brine and dried using magnesium sulfate before undergoing rotary evaporation. This product was then purified using column chromatography to obtain a yield of 57%.



(E)-1,3-dibromo-2-methoxy-5-(4-methoxystyryl)benzene (9). 5 mL of tetrahydrofuran and 2 g of (4-methoxybenzyl)triphenylphosphonium chloride were added to a 25 mL round bottom flask and allowed to stir on ice for 10 minutes with the slow addition of 1.1 g of sodium hydride. After 15 minutes, 0.69 g of 3,5-dibromo-4-methoxybenzaldehyde was added to the reaction which was then allowed to stir at 70°C for 30 minutes. After 3 hours, the reaction was removed from heat and quenched with 15 mL of water. The product was washed with hydrochloric acid before being dried using magnesium sulfate and rotary evaporated. The product was dissolved with hexanes and purified using column chromatography, where the eluent was 5% ethyl acetate in hexanes before transitioning to 10% ethyl acetate in hexanes. The yield was 12.6%.

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