SYNTHESIS OF COMPOUNDS CAPABLE OF PRODUCING CYTOTOXIC N3-METHYLADENINE DNA ADDUCTS IN ESTROGEN RECEPTOR POSITIVE CELLS

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A Thesis Submitted to the
University of North Carolina Wilmington in Partial Fulfillment
Of the Requirements for the Degree of
Master of Science

Department of Chemistry and Biochemistry

University of North Carolina Wilmington

2007

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This thesis has been prepared in the style and format consistent with the journal

Journal of Organic Chemistry

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ABSTRACT

This project describes the design and synthesis of new compounds that are capable of targeting cells that express the estrogen receptor and producing cytotoxic N3-methyladenine DNA adducts in those cells. These compounds contain a reactive methylsulfonate group connected to a unit that binds to DNA in the minor groove at adenine-thymine rich regions, and therefore, selectively methylates adenines in these regions at the N3 position. This component is connected by a linking unit to estradiol, which binds to the estrogen receptor, and therefore, is expected to selectively target these compounds to cells that express the estrogen receptor. The linking unit is the only variable component in the design, and can be altered in order to optimize the DNA and estrogen receptor binding properties of the compound, and to modulate the water solubility of the compounds. The synthesis of three compounds varying in linker length by one, two, and three methylene units is described.

ACKNOWLEDGEMENTS

I would first like to thank Dr. Sridhar Varadarajan. His support as an undergraduate and a graduate student has proved invaluable and I've learned more chemistry from him in four years of research than I have in six years of chemistry coursework. He always pushed me to achieve what he always knew I was capable of, even when I didn't believe in myself. He has supported my ideas and provided me with an experience that I will carry with me for a lifetime. I would also like to thank Dr. Pamela Seaton for being on my committee, for advising me to see Dr. Varadarajan, and for guiding me thorough experimentation. I would also like to thank Dr. John Tyrell, for being on my committee and providing such a memorable experience in organic chemistry. Lastly, I would like to acknowledge my family for their continued support. I want to thank my mother, Mary Swartz, and my sister Candace Lewis, for always being there for me when I needed them the most and for listening to chemistry when they had no idea what I was talking about. We've always had each other, and we always will. I never would have achieved so much in my life without their continued encouragement. I would also like to thank my father Michael Lewis, he has always encouraged me to do whatever my heart desired, he never held me back from any ideas I had, and always encouraged my creativity. I would finally like to thank my husband Christopher Perry, if it weren't for his continued love and support, I'm not so sure that I wouldn't be where I am today.

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

DNA in all cells is subject to many chemical alterations. Some agents that damage DNA include ultraviolet light, oxygen radicals produced during normal cellular respiration, chemicals in the environment (poly aromatic hydrocarbons), and drugs used for chemotherapy.[1] If the genetic information encoded in the DNA is to remain uncorrupted, any chemical changes that occur must be corrected. A failure to repair DNA can produce cell death and/or mutations.[2] Cell death resulting from DNA-damage is put to beneficial use in the treatment of diseases like cancer.

The hallmark of all cancers is continuous cell division.[3] Each cell division requires both the replication of DNA and the transcription and translation of many genes needed for continued growth. Many drugs used for cancer therapy function by targeting the rapidly dividing cancer cells and damaging their DNA.[3] Certain classes of anti cancer drugs that specifically target DNA such as, melphalan, bizelesin and cyclophosphamide, which are DNA alkylating agents.[4] These drugs damage the DNA of both normal and cancerous cells. Normal cells upon DNA-damage go into cell-cycle arrest, and repair enzymes are triggered, which are able to repair the damage before the cell can progress to cell division. This DNA-damage is not repaired in cancerous cells before cell division starts because these rapidly- dividing cells do not enter cell cycle arrest, and also because the repair systems are not usually functioning well in these cells. When replication of damaged (unrepaired) DNA starts in these cancer cells during cell division, recognition of the damage triggers a cascade of events resulting in the death of the cell.

Since the cytotoxicity of these alkylating drugs results from the existence of damaged DNA during cell division, many other cell types that are also proliferating rapidly, such as the cells of the intestinal lining, bone marrow and hair follicles are also destroyed, because their DNA repair enzymes are unable to repair the damage before initiation of cell division. This

undesired damage in these normal cells is what results in many of the unpleasant side effects of chemotherapy such as gastric irritation, fatigue and hair loss.[3]

A more disturbing aspect of chemotherapy with DNA alkylating drugs is that it poses a significant risk of causing a new or secondary cancer (often leukemia) that is a direct result of the extensive DNA-damage caused by the drugs used to treat the initial or primary cancer. This secondary cancer is often untreatable with the drugs that were used to treat the primary cancer.

These undesired side-effects of cancer chemotherapy can be minimized or eliminated by using drugs that damage DNA in a particular manner, such that it causes only cell death and does not result in mutations, and by making this drug specific to the targeted cell. For example, when DNA-methylation occurs at the N3 position of adenine, cytotoxic non-mutagenic N3-methyladenine adducts are formed. This specific DNA-damage can be caused by Me-Lex (Figure 1.1) a compound that has been shown to methylate the N3 position of adenines in the minor groove of DNA at A/T rich regions. Over 95% of the DNA-adducts formed by Me-Lex is N3-methyladenine. Additionally, the DNA-damage caused by Me-Lex has been shown to be exclusively cytotoxic and non-mutagenic. Therefore, if Me-lex could be targeted to specific cells, it may be possible to achieve cell-selective cytotoxicity.

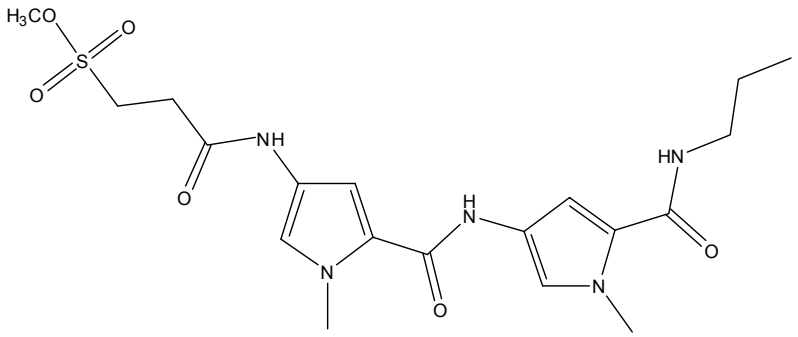


Figure 1.1: Structure of Me-Lex

There are many examples of targeting ligands used to send chemical agents to specific cells. One example is glucoseamine, which is a naturally occurring ligand that is selectively transported into the pancreatic β-cells that produce insulin via the Glut-2 transporter.[5]

Estradiol is another ligand that has been used for targeting specific cell types, in particular, cells that express the estrogen receptor (ER), like early stage breast cancer cells. Other alkylating agents have been selectively targeted to ER positive cells by tethering them to estradiol.[23]

This thesis describes the synthetic methodology used to make novel compounds that combined the ability of estradiol to target cell that express the ER, with the ability of Me-Lex to produce cytotoxic N3-methyladenine adducts. Such new compounds can be used to test the hypothesis that N3 methyl adenine adducts can be generated in specific cells by tethering Me-Lex to a ligand that specifically targets those cells.

CHAPTER 2: BACKGROUND AND SIGNIFICANCE

2.1. Background:

The research described in this thesis involves the development of an efficient synthetic methodology for making new compounds that are capable of delivering cytotoxic damage to DNA in estrogen receptor positive cells. The strategy that was employed to design these compounds was to chemically combine a compound that binds selectively in a sequence specific manner and forms exclusively cytotoxic adducts with a compound that targets estrogen receptors using suitable linkers. In order to be successful in the design (and synthesis) of such compounds, it is necessary to understand the structural features of the compounds that influence site and sequence specific DNA binding and methylation, and those that affect binding to the ER. An understanding of these factors will influence the site and modes of connection of the two functional units of the desired target compounds.

2.1.1. DNA Structure:

DNA (deoxyribose nucleic acid) is a double-stranded molecule twisted into a double helix. Each strand is comprised of a sugar-phosphate backbone and attached bases, which is connected to a complementary strand by non-covalent hydrogen bonding between the paired bases. The bases are adenine (A), thymine (T), cytosine (C) and guanine (G), where A and T are paired together by two hydrogen bonds and G and C are paired together by three hydrogen bonds. The twisting of the double helix results in the DNA having two distinct grooves, the major and minor grooves (Figure 2.1). The major groove is shallow and wide and this is where many proteins and enzymes interact with DNA. The minor groove is the deep and narrower groove.

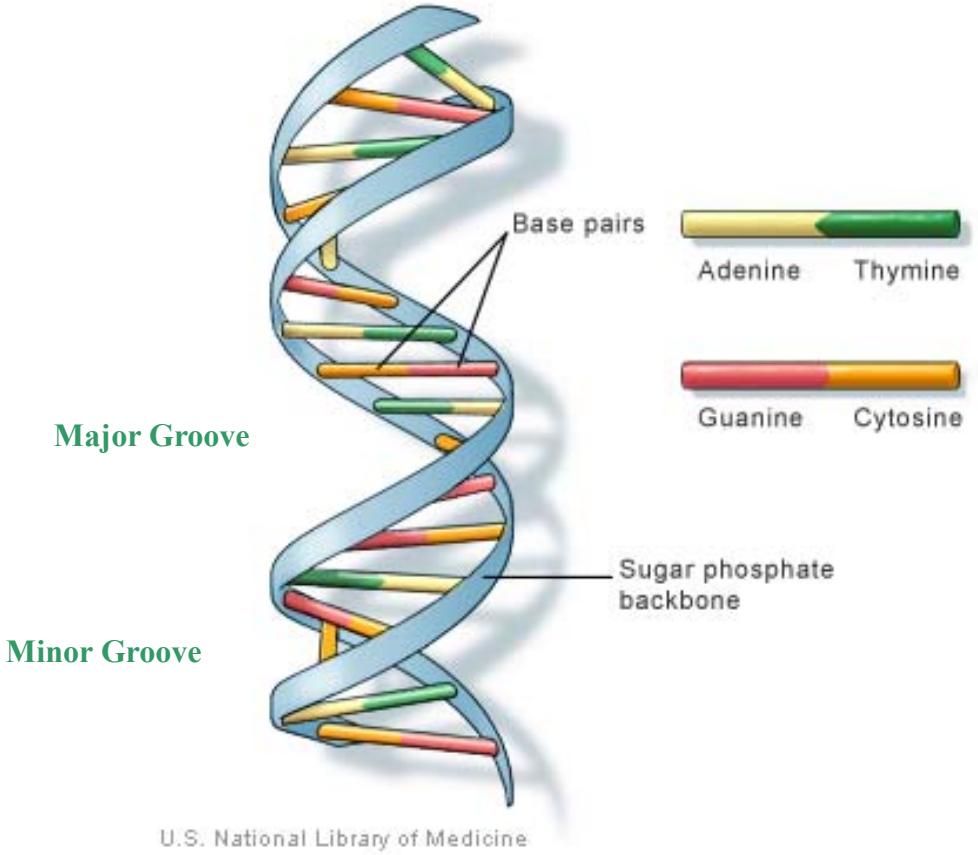


Figure 2.1: DNA base pairing (A with T and G with C), and the major and minor grooves

Figure 2.2 illustrates a top view of DNA if it were sliced perpendicular to the axis of the helix at the A/T and G/C base pairs, and also shows the hydrogen bonding between each of the base pairs which bring the two strands together forming the major and minor grooves. As a result, certain sites on the base pairs face the major groove and others face the minor groove. Sites in the major groove are more easily accessible and include the O^4 of thymine, the N7 and N^6 of adenine, N7 of guanine, N^4 of cytosine, and the O^6 of guanine. The sites that lie in the minor groove are more difficult to access and include the O^2 of thymine, the N3 of adenine, the O^2 of cytosine and the N^2 and N3 of guanine.

2.1.2. Reactivity of DNA Towards Alkylating Agents:

Numerous nucleophilic sites on DNA are susceptible to alkylation by alkylating agents, these sites are indicted by arrows in Figure 2.2. The extent of alkylation is a function of accessibility and nucleophilicity of the particular site. The sites on the G/C base pair, in the major groove, that are susceptible to alkylation include the N^4 of cytosine, the O^6 of guanine and the N7 of guanine. In fact, the N7 of guanine is the site on DNA most commonly alkylated since it is highly nucleophilic and most easily accessible, by alkylating agents. The G/C base pair also includes sites in the minor groove that are vulnerable to alkylation, examples of these are the O^2 of cytosine, and the N^2 and N3 of guanine. Since these sites lie within the minor groove, and are consequently less accessible, they are less commonly alkylated as compared to the N7 of guanine. On the A/T base pair the sites susceptible to alkylation include the O^4 of thymine, and the N7 and N^6 of adenine in the major groove and the O^2 of thymine and N3 position of adenine. In addition, the phosphate on the backbone of DNA can also be alkylated.

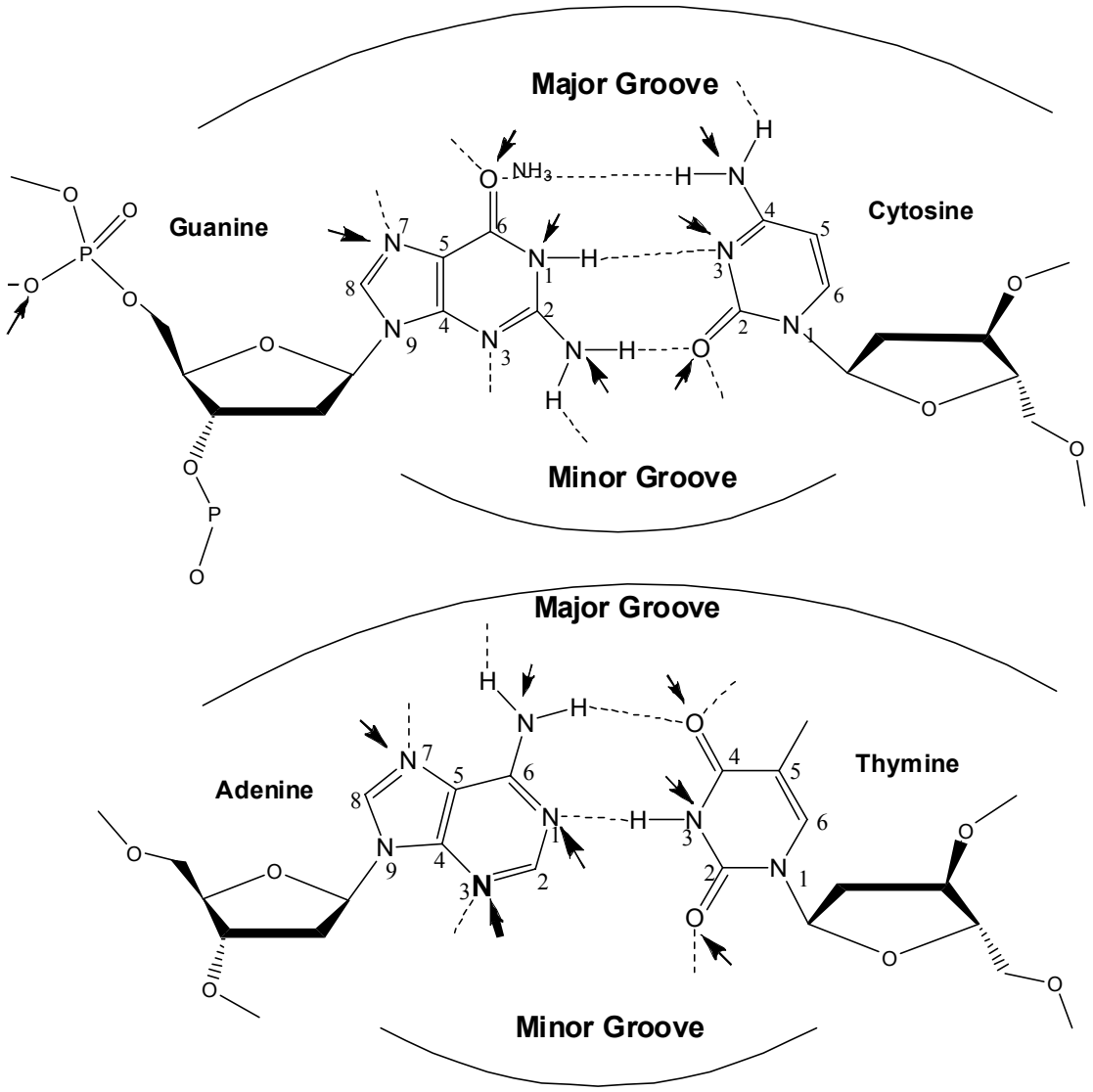


Figure 2.2: Structure and pairing of DNA bases indicating major and minor groove sites. Each arrow indicates a site which can be alkylated.

2.1.3. Biological Consequences of DNA Methylation:

Methylation of DNA can result in different biological consequences depending on what site has been methylated. The N7 position of guanine in the major groove is the most commonly methylated site on DNA. Methylation at this site appears to have no biological outcome and leads to neither cytotoxic nor mutangenic consequences.[7-10] The O^6 site, which too lies in the major groove, and is also involved in the base-pairing hydrogen bonding interactions, is another site that is commonly methylated by methylating agents. Formation of O^6-methylguanine adducts is known to lead to both mutations and cell-toxicity. [7-11]

Methylation at other sites such as the N^2 of guanine, the O^2 and O^4 of thymine and O^2 of cytosine has been implicated in mutagenicity.[7-11] Methylation at the N3 site of adenine, which lies in the minor groove, has been shown to result in cytotoxicity, but does not lead to mutations.[7, 9,11-14] Thus, compounds that can generate exclusively N3-methyladenine adducts can be used to destroy cells without any risk of mutagenicity.

2.1.4. Structure and Properties of Me-Lex:

Me-Lex (shown in Figure 2.3) is a neutral compound that exclusively produces N3-methyladenine adducts in the minor groove of DNA at A/T rich regions. These N3-methyladenine adducts produced by Me-Lex have been shown to be highly cytotoxic and non-mutagenic.

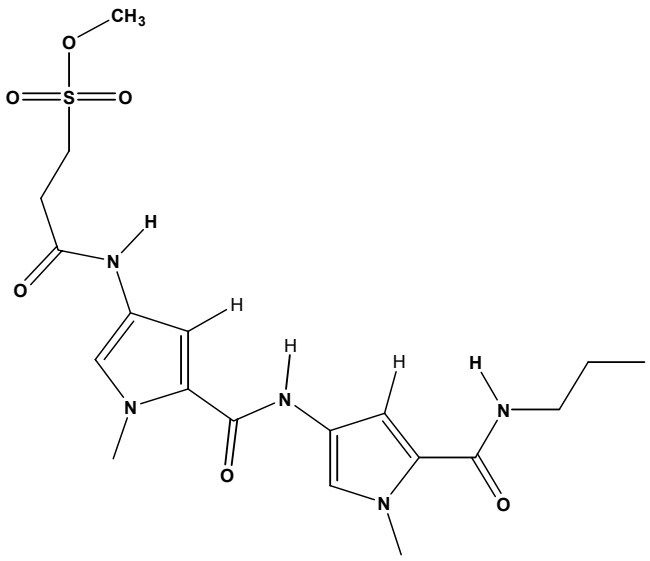


Figure 2.3: Structure of Me-Lex

Me-Lex has a natural crescent shape, which enables it to fit well in the minor groove of DNA (Figure 2.4). It binds at A/T rich regions due to the favorable hydrogen bonding and Van der Waals interactions between the pyrrole units of Me-Lex and adenine thymine base pair (Figure 2.5, a, b). Me-lex does not bind to guanines because of the unfavorable steric interactions between the pyrrole units of Me-lex and the exocyclic amine present on guanine (Figure 2.5 c). When Me-Lex binds to the minor groove of DNA at A/T rich regions, it reacts with the most nucleophilic site in those regions, which is the N3 position of adenine. Once the methyl group is transferred to the adenine, the resultant sulfonate anion leaves the DNA due to electrostatic repulsion from the poly anionic DNA backbone.[15]

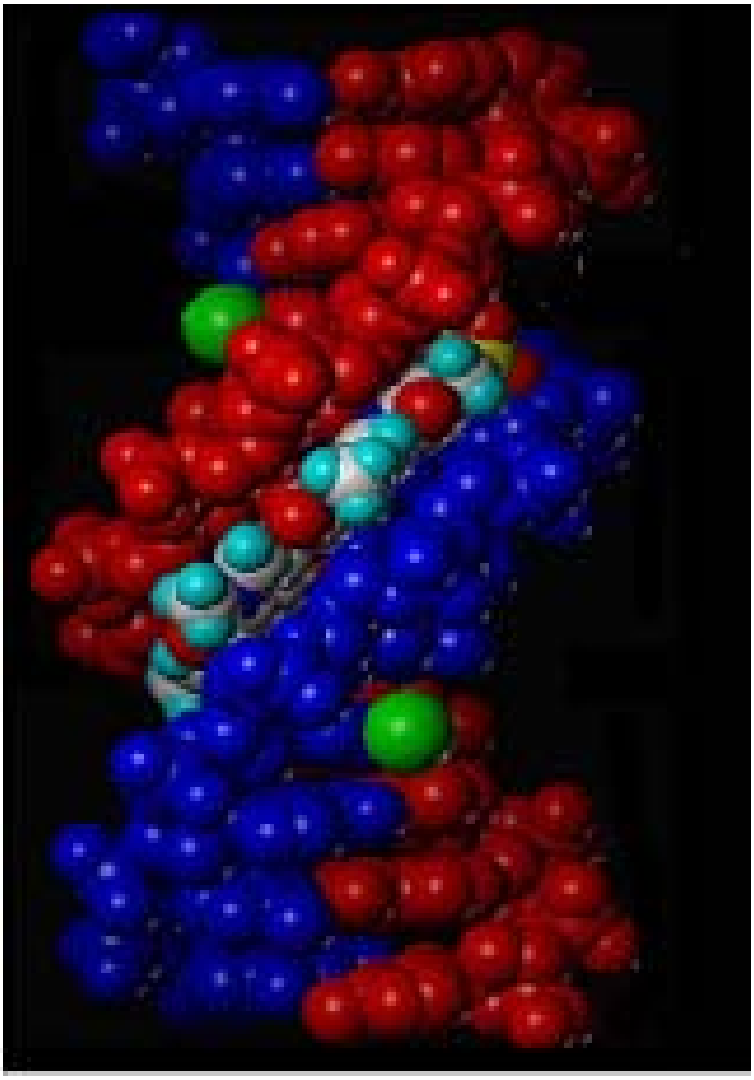


Figure 2.4: Molecular model illustrating Me-lex bound within the minor groove of DNA.

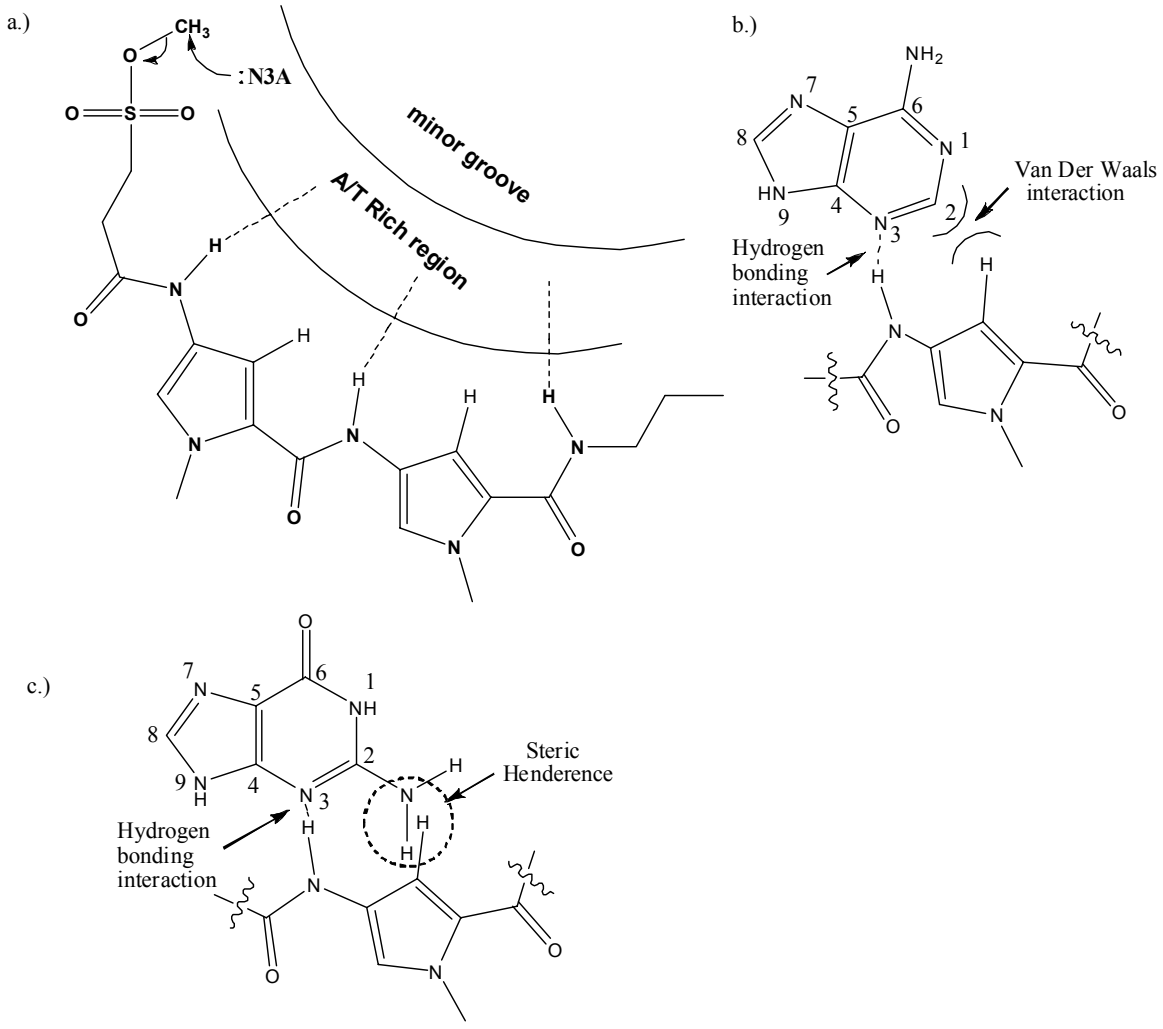


Figure 2.5: a.) Interaction of Me-Lex at A-T rich regions of DNA, in the minor groove, resulting in methylation of N3-adenine sites

b.) Favorable H-bonding and van der Waals interactions between adenine and the pyrrole amide units of Me-Lex, which result in its selective binding at A/T rich regions.

c.) Unfavorable steric conflicts between guanine and the pyrrole amide units of Me-Lex preventing it from binding to the minor groove of DNA at G/C regions.

2.1.5. Structure and Properties of the Estrogen Receptor and its Naturally Occurring Ligand, β-Estradiol:

The ER is a member of the nuclear hormone receptor family found in the cytoplasm of cells.[16, 22] It functions as a ligand activated transcription regulator when it is bound with its natural ligand, estradiol.[16] The ER is found in various tissues and cell types including the brain, bones, arteries and the human ovaries and breast cells. The structure of the ER has a canonical alpha-helical sandwich shape that is made up of 12 alpha helices that are arranged into three anti-parallel layers as shown in Figure 2.6.[17,18] This arrangement allows for a large buried binding pocket where estradiol is sequestered after entering the receptor channel. The ER, when inactive, resides mainly in the nucleus of cells, to become activated it moves into the cytosol of the cell where it then binds estradiol.[18] The binding of estradiol to the ER allows for a conformational change of the receptor that places a "lid" over the steroid binding pocket, exposing transcriptional residues that are necessary for DNA binding.[17-20]

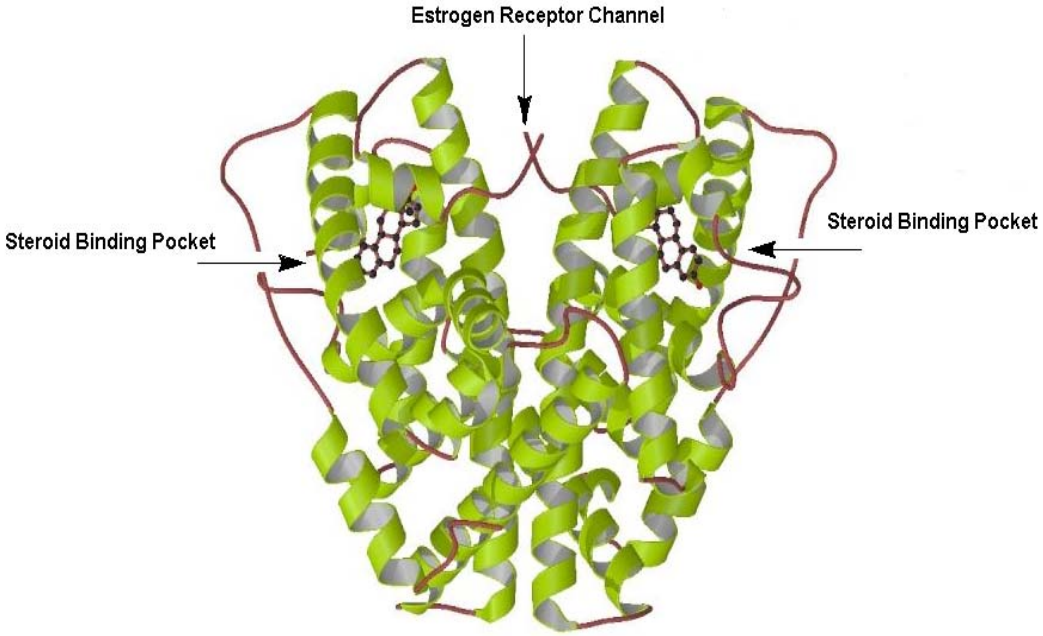


Figure 2.6: Structure of the Human Estrogen Receptor, with Estradiol bound within the hormone binding pockets.[6]

It has been shown that the ER is over-expressed in early stage breast cancer cells, which require estradiol for their continued growth.[21, 22] Therefore, there are several reports in literature of therapeutic agents being attached to estradiol in order to selectively deliver them to breast cancer cells. The site and mode of connection of agents to estradiol must be such that it does not interfere with the binding of estradiol to the ER, It has been shown, in literature that certain modifications at the 7α, 11α, 16α, and 17β sites (Figure 2.7) still allow estradiol to bind to the estrogen receptor, whereas modifications at other sites drastically diminish ER binding. [19, 23, 24] Also, since estradiol binds within a long narrow channel of the ER, it is necessary that the tether used be long enough to extend out of this channel. There is evidence to show that tethers have to be 6 carbons in length or longer to allow for estradiol to bind within the ER.

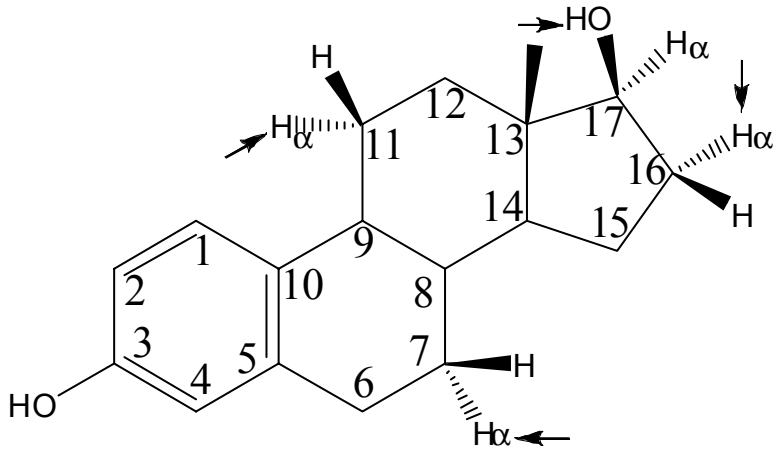


Figure 2.7: Structure of estradiol. The sites to which tethers can be attached, without significantly interfering with estradiol's binding to the ER, are 7α, 11α, 16α, and 17β (indicated by arrows).

The compound shown in Figure 2.8 is an example in which a DNA alkylating nitrogen mustard has been attached to estradiol in order to selectively destroy breast cancer cells. This compound was reported to be successfully transported to the nucleus of estrogen receptor positive cells, and resulted in alkylation at the N7 site of guanines.

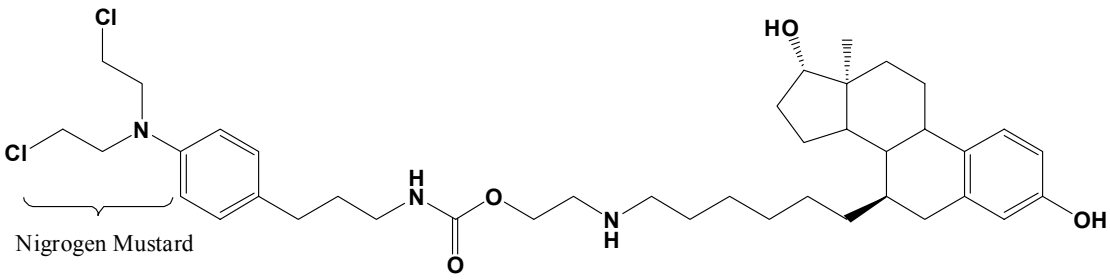


Figure 2.8: Structure of an alkylating compound (nitrogen mustard) attached to the 7α position of estradiol, which has been shown to alkylate DNA in ER-positive breast cancer cells.

2.2. Design:

The goal of this research was to synthesize compounds that can produce cytotoxic N3-methyladenine adducts in ER-positive cells. The molecules designed for the project are shown in Figure 2.9. The estradiol and DNA-damaging components were connected by a linking unit, which is the only variable component in the design, and can be altered in order to optimize the DNA and estrogen receptor binding properties of the compound, and to modulate the water solubility of the compounds.

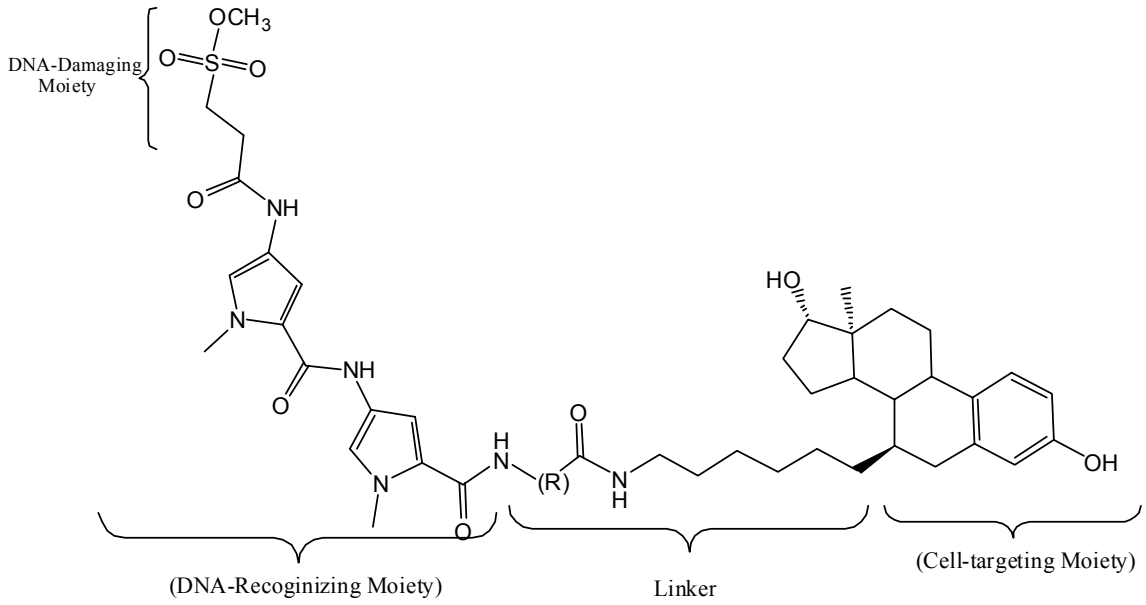


Figure 2.9: Design of molecules synthesized in this project.

2.3 Significance:

The successful preparation of the target compounds will enable us to test whether cytotoxic N3-methyladenine adducts can be selectively produced in ells that express the ER by using this strategy. The success of the approach described here will also demonstrate the feasibility of using this strategy to target different kinds of cells that express a unique protein/receptor, ad generate cytotoxic, non-mutagenic, N3-methyladenine adducts in these cells by attaching Me-Lex to an appropriate ligand. The ability to successfully target and generate only cytotoxic, non-mutagenic N3-methyladenine adducts in specific tumor cells can lead to the direct destruction of these cells while minimizing the common chemotherapy side-effects (such as hair loss and gastric irritation). Since the specific DNA-damage caused by these new compounds do not cause mutations (mutations resulting from the extensive DNA-damage caused by common chemotherapy drugs are believed to be responsible for the occurrence of secondary cancers in patients who survive the primary cancer), such new drugs can also eliminate the incidence of secondary cancers that are reported for several chemotherapy drugs.

CHAPTER 3: SYNTHESIS OF THE CELL TARGETING LIGAND

Estradiol is being used as a cell-targeting ligand, which is to have Me-Lex attached at the 7α position to maintain sufficient binding to the ER. This compound must be synthesized in such a way that it can easily be attached to Me-Lex. The synthesis of this compound starts with commercially obtained β-estradiol. Amine functionality is added to the end of the 6-carbon tether, this would make it possible to form an amide linkage between Me-Lex and estradiol. Therefore, the unit that was synthesized is shown in Figure 3.1. The synthesis has been described in literature, with minor modifications.[25-29] The outline of the procedures adopted is shown in Scheme 3.1.

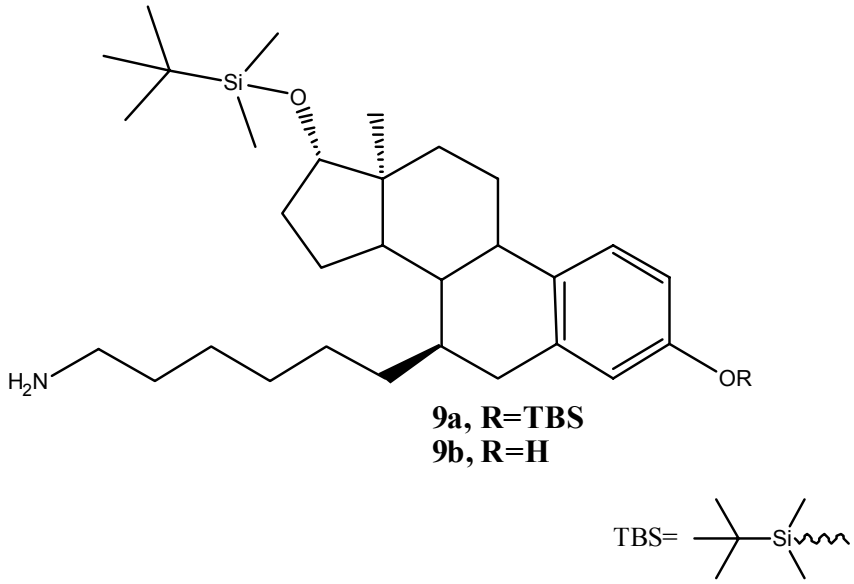
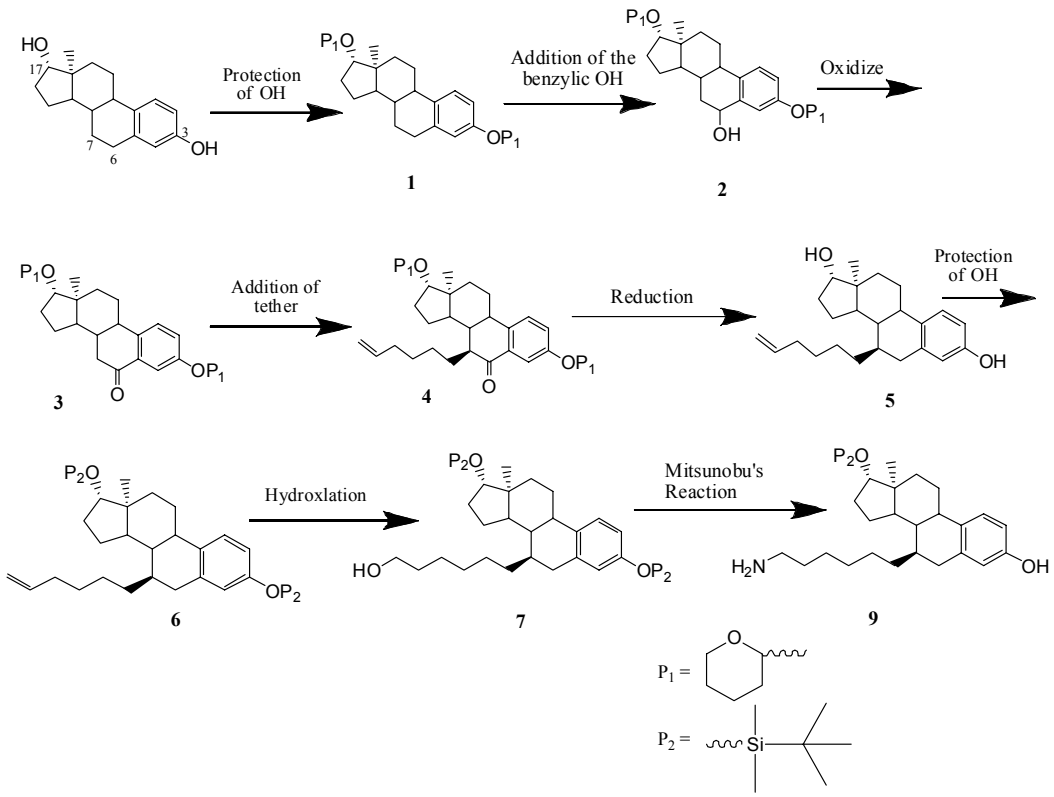


Figure 3.1. Structure of the cell-targeting segment that will be synthesized.

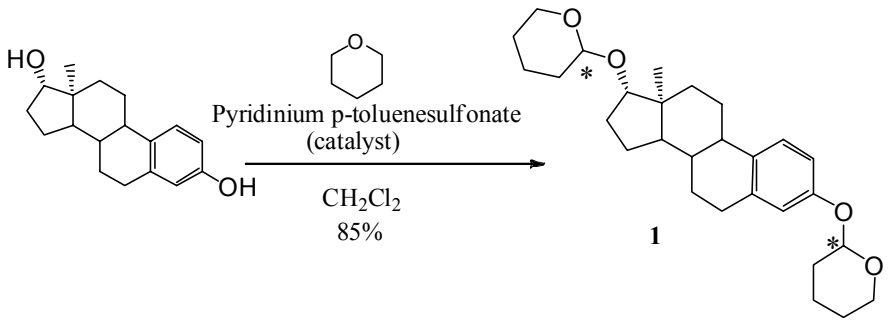


Scheme 3.1

The alkyl group was attached to the 7α position, however the 7α position was not reactive and it must first be made reactive by the conversion the 6-carbon into a ketone, which then rendered the 7α position acidic. Base was then used to make the carbanion, followed by alkylation. To prevent interference from the OH groups, they were first protected. Details of the synthesis are described below.

The synthesis of **9** began with commercially obtained β-estradiol, where the 2 OH groups were protected using dihydropyran (Scheme 3.2) as described in literature.[25-29] This reaction of protecting the OH groups proceeded well, however since the site of reaction produced chiral centers, different stereoisomers were formed. In this case 4 diasteromers were produced due to the two chiral centers indicated by an (*) in Scheme 3.2. This lead to a complicated NMR

spectrum, however, the formation of the isomers was of no consequence because these protecting groups were removed later in the synthesis.

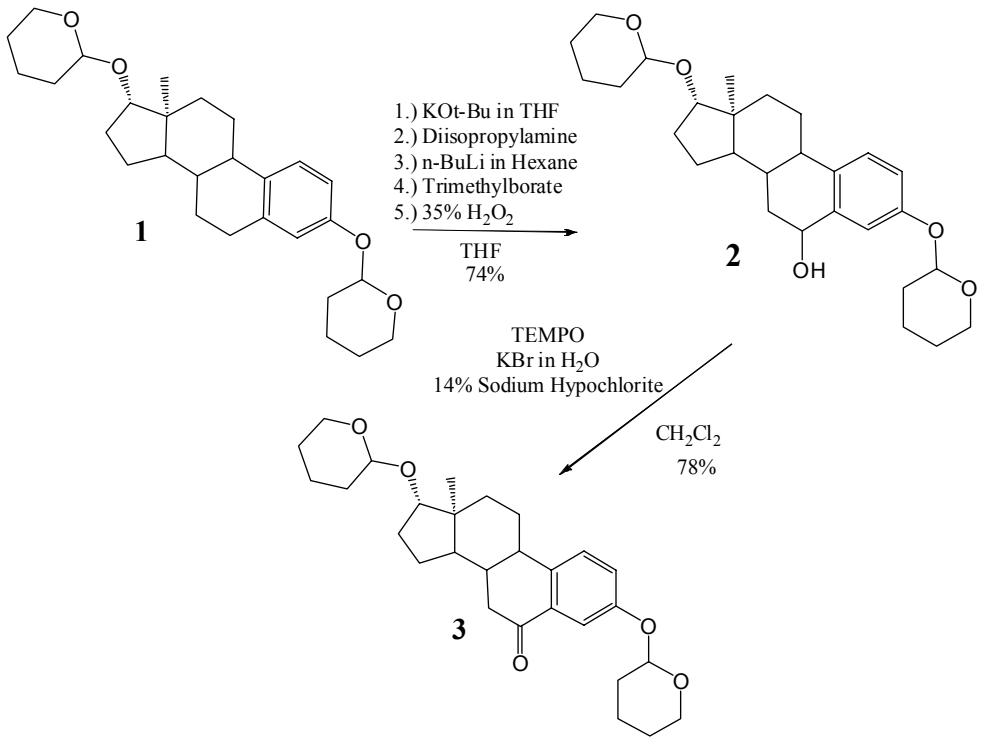


Scheme 3.2

Compound **1** was isolated as a viscous oil upon removal of the solvent. However, complete removal was not achieved even under high vacuum for prolonged time. Therefore, determination of the quantity was difficult because part of it was solvent. This made calculations in the subsequent step more difficult due to the fact that the calculations would not be accurate considering solvent was still present. However, the product could be crystallized using a 9:1 EtOH/H_2O mixture. To obtain the compound as a white solid, it was first slowly heated in a warm water bath to 50°C for 30 minutes and cooled slowly to precipitate the product. All previous reports described compound **1** as a viscous oil.[25] This time, compound **1** was isolated as a powdery white solid in an 85% yield.

The next step of the synthesis was the introduction of an alcoholic group in the 6-postion to form **2** followed by the conversion of the alcohol to a ketone to form **3** (Scheme 3.3). The addition of the alcohol was achieved using potassium tert butoxide, diisopropylamine and n-BuLi to produce the lithium salt. This was followed by the addition of trimethylborate to trap the compound as a borate salt. Compound **2** was obtained as a white foamy solid in a 74% yield.

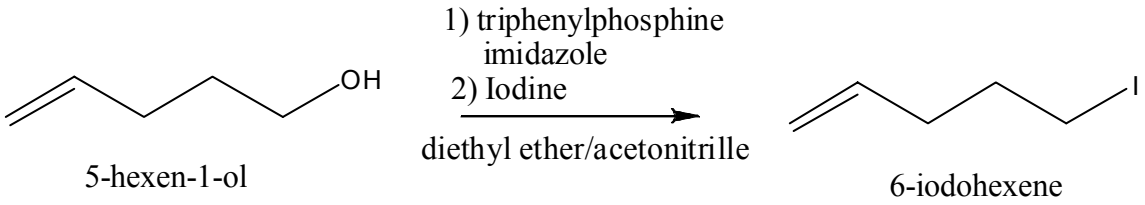
Compound **2** was formed as two stereoisomers; no further attempt was made to separate the isomers because the alcohol was subsequently oxidized into the ketone.



Scheme 3.3

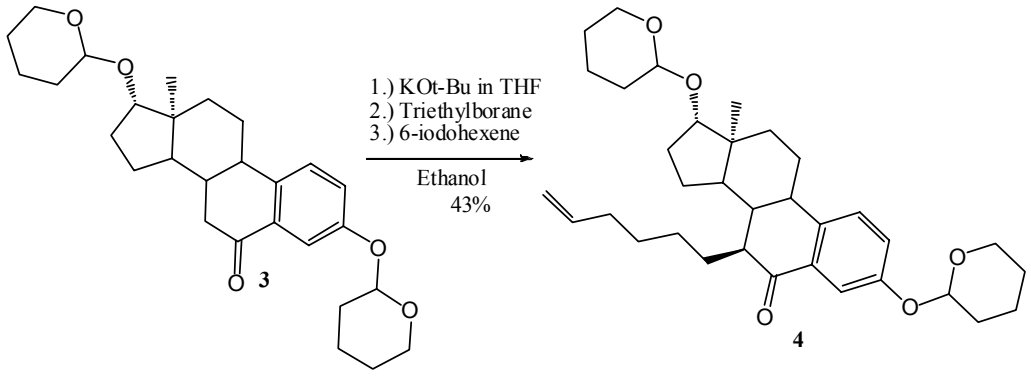
The introduction of the ketone rendered the hydrogen in the 7α position acidic. This conversion involved the oxidation of the benzylic alcohol **3** into a ketone. The oxidation was accomplished using TEMPO (2,2,6,6-Tetramethylpiperidine 1-oxy) [25] as a catalyst and was achieved in a reasonable yield of 78% as a yellow foamy solid. Good yields were obtained for this reaction when the temperature was held lower between 0-5°C. Higher temperatures above 5°C resulted in lower yields, while temperatures any cooler than 0°C drastically slowed the reaction.

After the synthesis of the ketone the 7α-position could be alkylated with 6-iodohexene, which was synthesized from 5-hexen-1-ol (Scheme 3.4). 5-Hexen-1-ol was dissolved in a mixture of diethyl ether/acetonitrile, and triphenylphosphine, imidazole, and iodine was added. This product was purified by column chromatography using hexanes as the elutant, but removal was difficult due the product being volatile. Therefore, it could not be placed under high vacuum. Because of this, the product was used in excess to account for the presence of some remaining solvent.



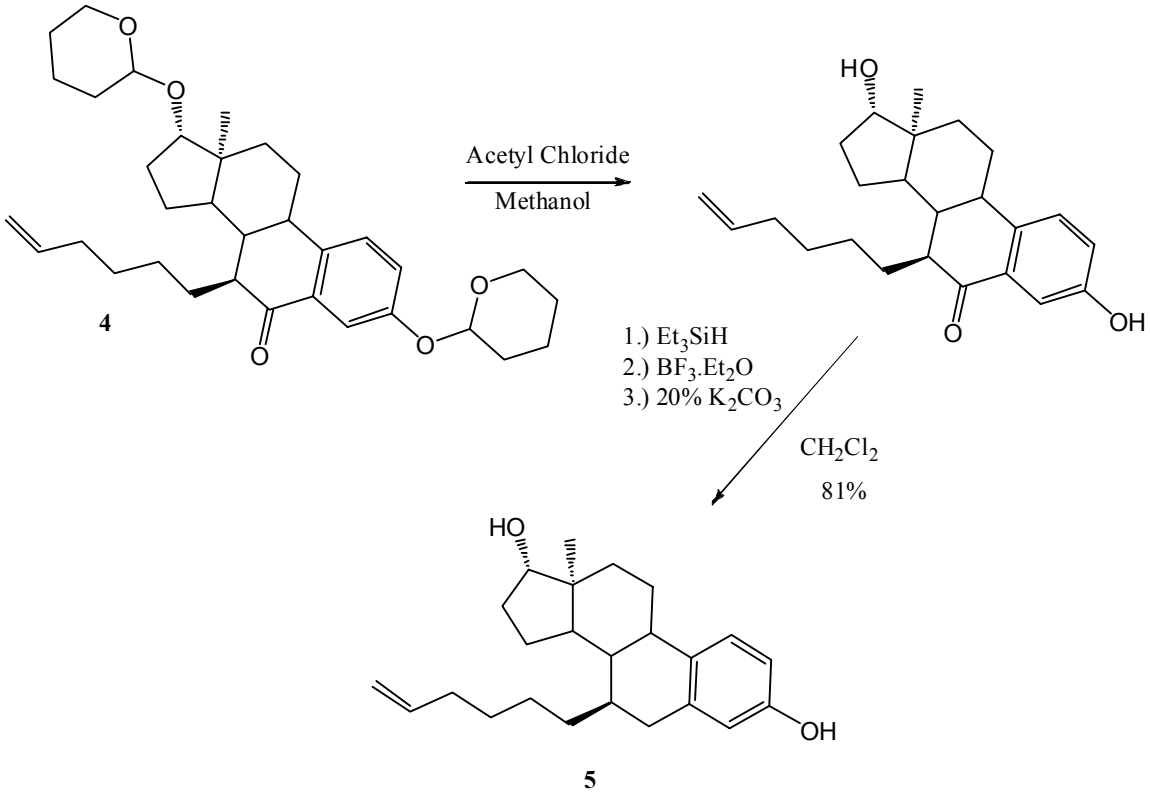
Scheme 3.4

Once the alkylating agent, 6-iodohexene, was synthesized it was then reacted with **3**. Introduction of the alkyl group at the 7α position of estradiol was achieved first by generating the enolate with potassium tertbutoxide, then trapping the enolate with triethyl borate. This prevented O-alkylation and also ensured that the 6-iodohexene was added to the 7α position and not the 7β position. This was then followed by the addition of 6-iodohexene. This resulted in the introduction of the alkyl group at the α-position, (Scheme 3.5) in a 43% yield. No attempt was made to verify the addition at the 7α position, however all reports indicate that the addition of the alkyl group was present at the 7α position, and the NMR spectra is an exact match to the NMR of the reported compounds.[25-27]



Scheme 3.5

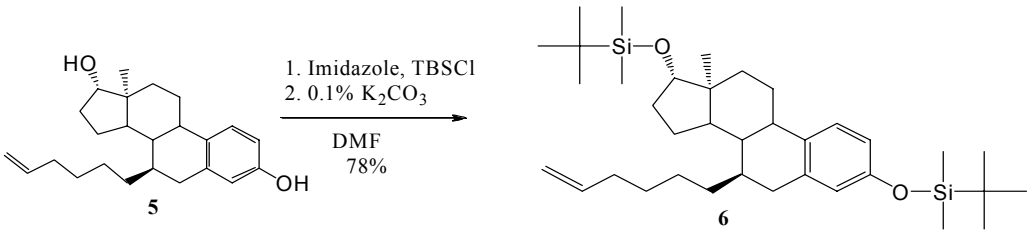
After the addition of the alkyl group to the 7α position, the deprotection of the 3-phenolic and 17-hydroxyl OH groups was done, followed by the reduction of the ketone (Scheme 3.6). The process of reducing the ketone ultimately resulted in the deprotection of the OH groups. Published reports indicate that it was necessary to first deprotect the OH groups before reducing the ketone to avoid low yields.[28] Therefore, removing the protecting groups first, followed by the reduction of the ketone resulted in higher yields, which resulted in obtaining compound **5** as a foamy white solid in an 81% yield.



4 **5**

Scheme 3.6

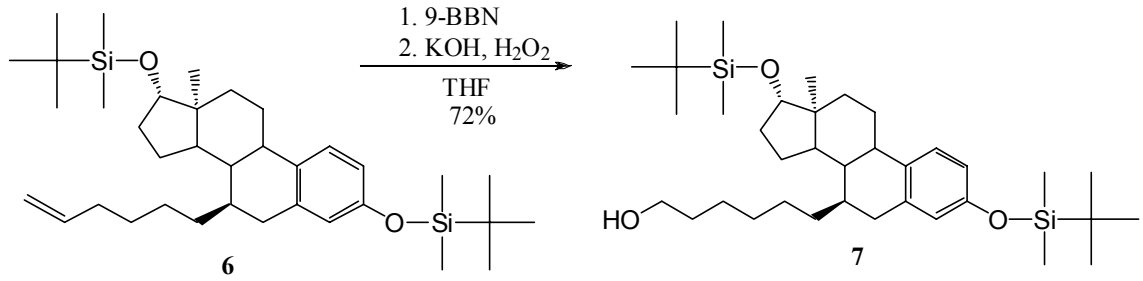
Once the ketone was reduced, the OH groups were reprotected using TBSCl. This protecting group affords only 1 isomer, making the interpretation of NMR relatively simple. This is illustrated in Scheme 3.7 below. TBSCl was activated by imidazole to form a complex that is then reacted with **5**, which resulted in the reprotected compound **6** in a 78% yield.



5 **6**

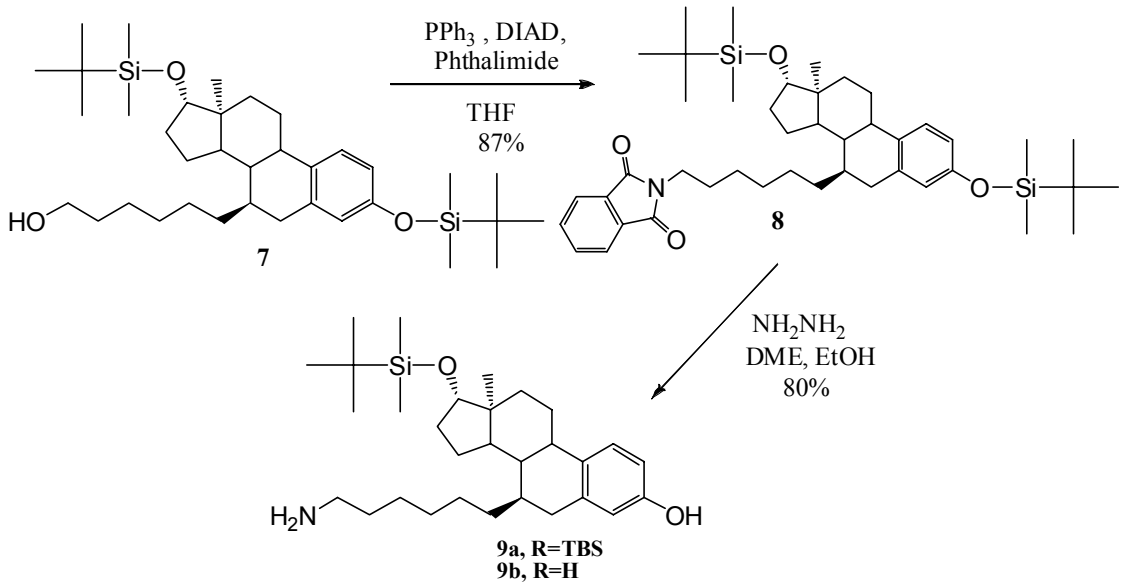
Scheme 3.7

Once the alcoholic groups were reprotected, the terminal alkene was converted to the alcohol via anti-markovnikov addition of water across a double bond using 9-BBN (9-borabicyclo[3.3.1]nonane), KOH, and hydrogen peroxide to form the alcohol **7** as a white solid in a 72% yield (Scheme 3.8).



Scheme 3.8

This alcohol was converted to the amine (Scheme 3.9), by the addition of phthalamide by using Mitsunobo conditions and subsequently treating the phthalamide with hydrazine to form the amine in an 80% yield.



Scheme 3.9

The introduction of the hexyl amino was a laborious 9-step synthesis with an overall yield of 6%. Because of this, some of the compound was obtained commercially from Quality Chemical Laboratories for subsequent reactions. This compound **9 a** or **b** could then be coupled with the DNA methylating unit that was suitably functionalized as a carboxylic acid.

CHAPTER 4: SYNTHESIS OF THE DNA METHYLATING UNIT

There are several key features of the target molecules. The first is DNA recognition, which is achieved by the bis-pyrrole units. The hydrogen bonding interactions with the minor groove is just the same as with Me-Lex. The second is the reactive methyl group that will be transferred to the N3 position of adenine. The reactive methyl sulfonate that transfers the methyl group was introduced late in the synthesis, in the very last step, because of its reactivity. We were trying to make these compounds with linker variations that would be introduced at the C-terminus of the molecule. They were composed of $(CH_2)_n$, where n is either 1, 2, or 3. These linkers are esters that were ultimately hydrolyzed to carboxylic acids, which allowed for the addition of **9**, forming an amide linkage.

A synthesis was attempted that would allow for the easy addition of the linker at a later stage in the synthesis, this would be followed by the addition of the cell-targeting component, then functionalized to the reactive methyl sulfonate, this is illustrated in Figure 4.1

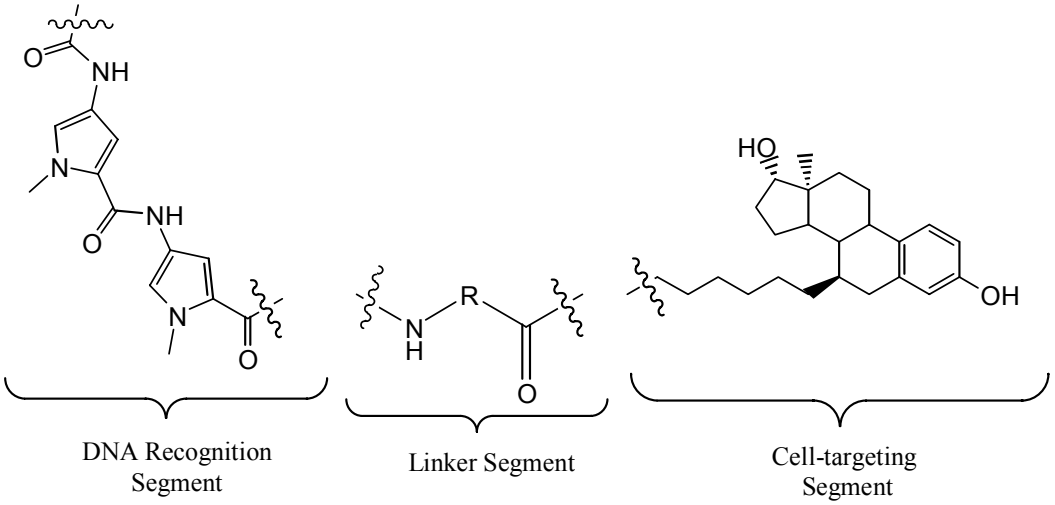


Figure 4.1: Attempted strategy to prepare compounds to target ER positive cells, and methylate N3-adenine in those cells.

The synthesis used for the DNA-recognition unit was done following published procedures, with some modifications.[13, 30] The overall strategy adopted for the synthesis of the minor groove recognition unit is such that it could be easily combined with the cell-targeting unit estradiol, and also so the reactive methyl sulfonate would be added last in the overall synthesis. The synthesis of **9** was expensive in time and resources, so it was desirable to minimize the number of manipulations after the addition of **9**. Which means it was necessary to functionalize the bis-pyrrole unit as much as possible before the addition of **9**. The first attempt was to synthesize the compound shown below in Figure 4.2.

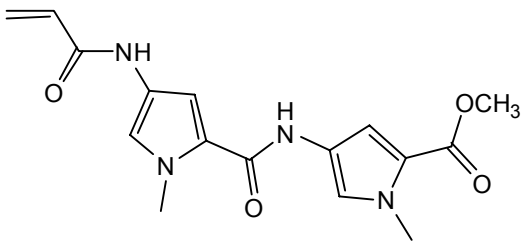
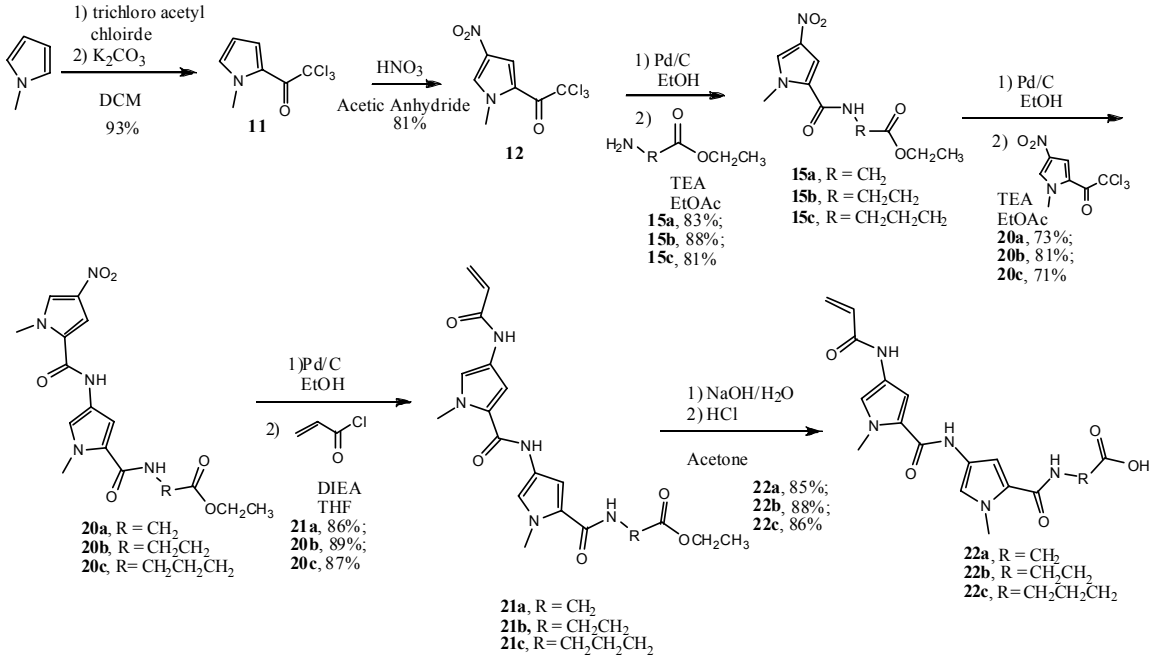


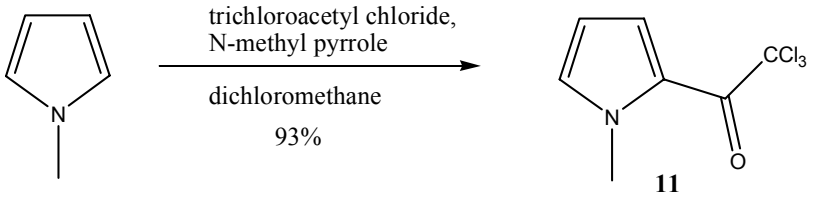
Figure 4.2: Bis-pyrrole unit to allow for the easy addition of linkers at a later stage of the Synthesis.

All attempts to hydrolyze the ester without affecting the alkene were unsuccessful. This is due to the aromatic ester requiring harsher conditions for hydrolysis. This complication made it necessary to add the linkers earlier, so the synthesis adopted is shown below in Scheme 4.1. This modification was of no consequence considering the addition of the linkers and the steps thereafter were produced in high yields.



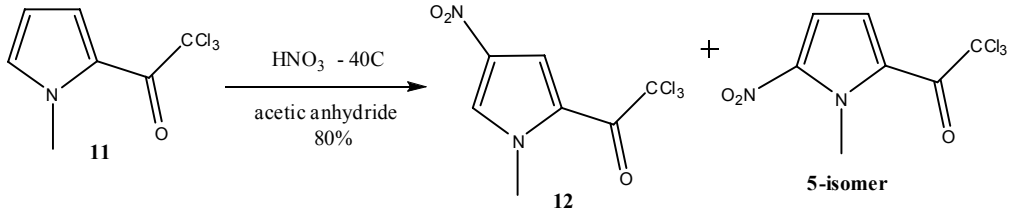
Scheme 4.1

The synthesis of the DNA recognizing dipyrrole unit, **22** started with the trichloroacetylation of N-methyl pyrrole. Compound **11** was synthesized following scheme 4.2 by reacting trichloroacetyl chloride with N-methyl pyrrole, this afforded compound **11** in a 93 % yield.



Scheme 4.2

Compound **11** was nitrated using fuming nitric acid as the nitrating reagent (Scheme 4.3). Upon nitration, the formation of the desired 4-nitro product, along with a small amount of the undesired 5-nitro isomer was obtained. When complete, the addition of water precipitated the product, which has a lower solubility due to the hydrolysis of the acetic anhydride to acetic acid.



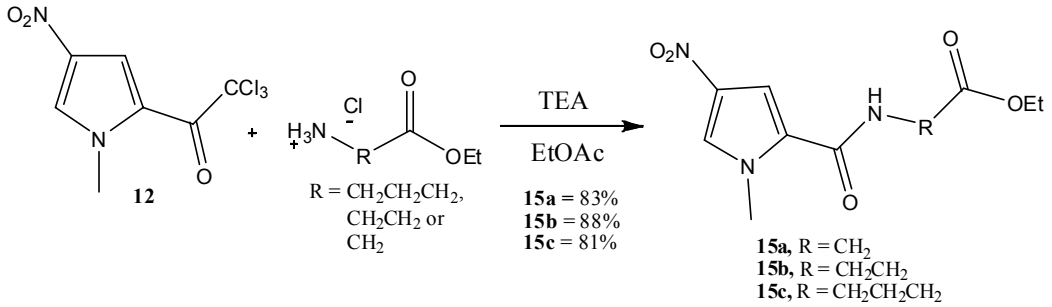
Scheme 4.3

It was found that by stirring the precipitate with hexane overnight eliminated all of the undesired isomer. It was then possible to obtain **12** as a pure isomer in good yields (80%).

Compound **12** is stable to aqueous hydrolysis. Usually, trichloro acetyl groups are very reactive with nucleophiles. With this compound, the electronic configuration is just right so that water is too weak of a nucleophile to react, making **12** stable to aqueous hydrolysis. However, amines are stronger nucleophiles, displacing chloroform as a by-product, which was then easily removed by rotary evaporation.

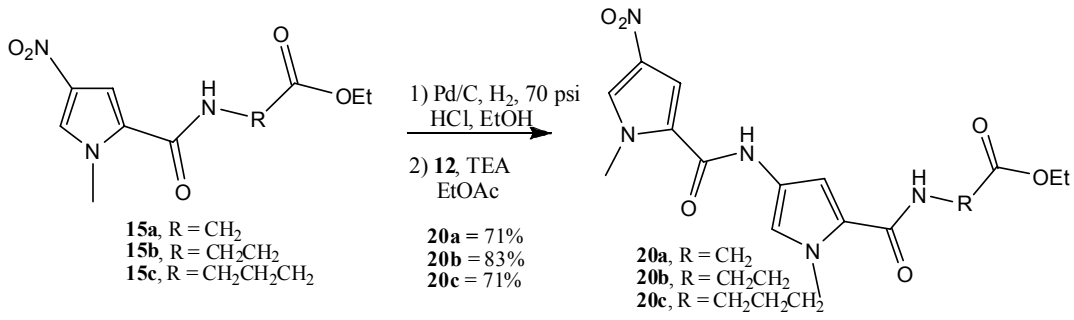
At this stage of the synthesis, various linking units were attached. They were obtained as an amino ester, which was commercially available. The three linkers chosen are shown in Scheme 4.4, where R = CH_2, CH_2CH_2, or $CH_2CH_2CH_2$. These amines were stirred with **12** at

room temperature in EtOAc, which resulted in the formation of the product, without the necessity of dry conditions. CCl_3 is eliminated as $CHCl_3$ and aqueous HCl eliminated any unreacted amine, since the linkers were used in excess to ensure the complete reaction of **12.** These compounds were obtained in yields greater than 80%. These compounds now contain the component to be attached to **9**.



Scheme 4.4

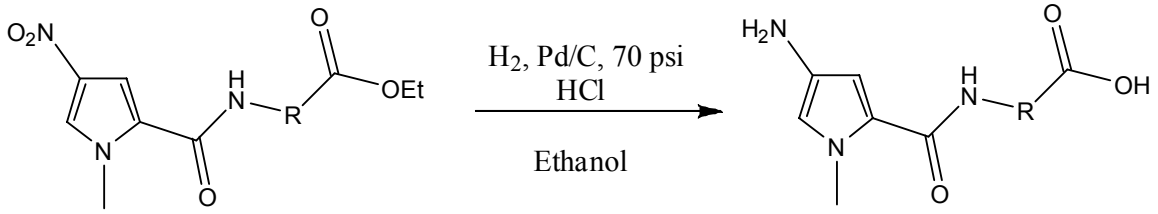
The nitro group in compound **15** was reduced with H_2 gas under pressure in the presence of Pd/C catalyst and subsequently reacted with another unit of **12** to give the bis-pyrrole unit **20** (Scheme 4.5) in yields greater than 70%.



Scheme 4.5

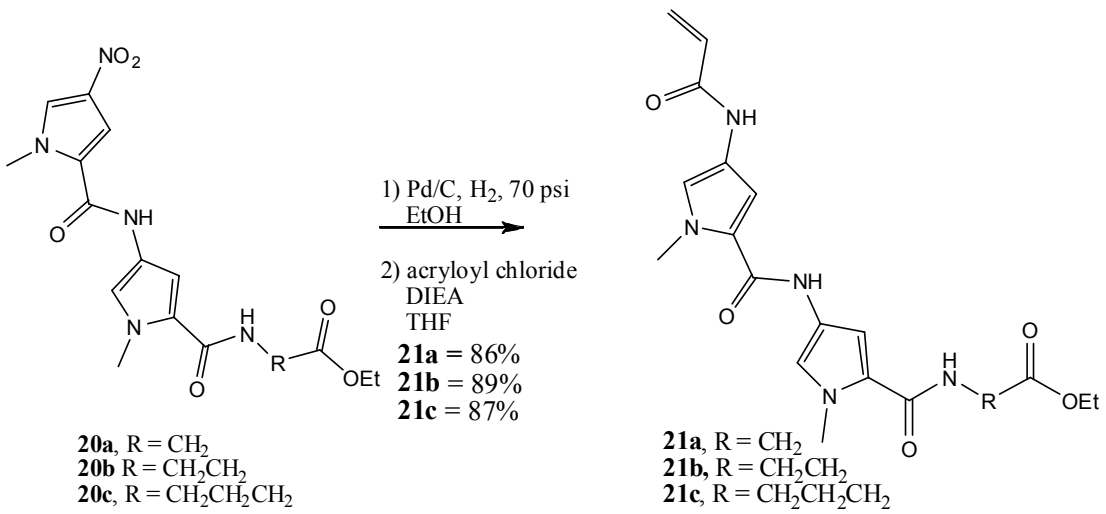
Initially compound **15** was reduced and not characterized. The amine produced is aromatic, so the reaction with **12** took several days. Low yields were obtained due to

decomposition and air oxidation of the amine. To overcome this problem, producing the amine salt in the hydrogenator could minimize the decomposition. To test this, HCl was added directly to the hydrogenator during the reduction of the **15**. The HCl salt of the amine was formed, but the ester was also hydrolyzed (Scheme 4.6). To avoid acid hydrolysis of the ester, the HCl was added to the parr jar after the complete reduction of the amine, which formed only the HCl salt of the amine leaving the ester intact. The free amine could then be formed in situ by the slow addition of TEA, which resulted in yields greater than 70% for compound **20**.



Scheme 4.6

These nitro bis-pyrrole compounds were reduced under high pressure hydrogenation, then subsequently reacted with acroloyl chloride to produce the olefins (Scheme 4.7). In this reaction, DIEA was used as the scavenger of the HCl that was formed upon the reaction of the amine and the acryloyl chloride. The reaction was performed at low temperatures and protected from light to prevent polymerization. Compound **21** was produced in high yields (>85%).



Scheme 4.7

The hydrolysis of the ethyl ester **21** produced the carboxylic acid that could then be condensed with compound **9**. Initially, the hydrolysis was performed as reported in the literature, by first dissolving the compound in ethanol, adding the NaOH dissolved in H_2O and reacting under reflux. Performing the reaction in this manner hydrolyzed the ester but also resulted in the addition of an OH across a double bond via Michael addition, the proposed product is shown in Figure 4.3.

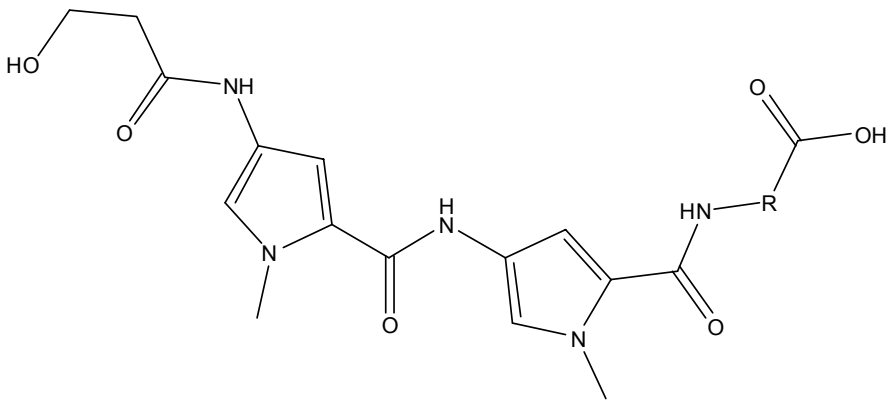
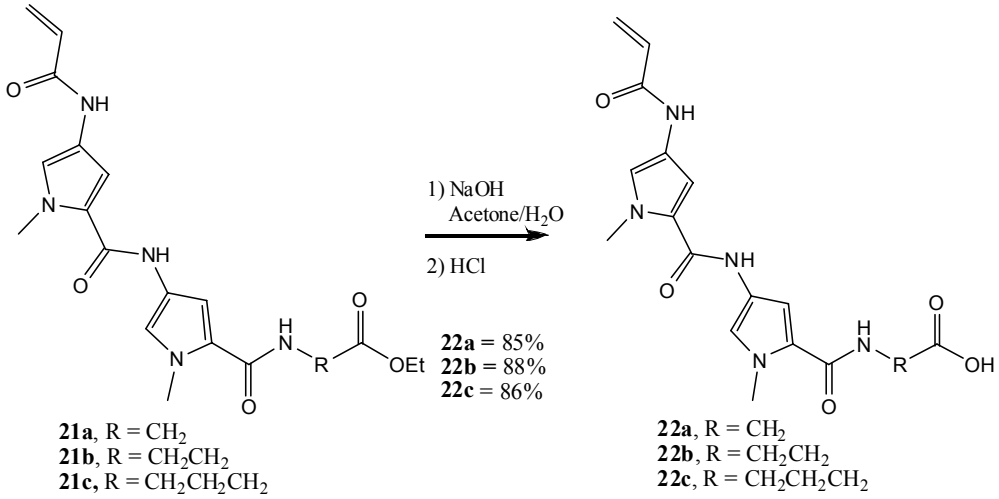


Figure 4.3: Proposed Michael Addition product from the base hydrolysis of the Ester.

The reaction was then performed at room temperature and methanol was used instead of ethanol as the solvent. If HCl was added with methanol present, the carboxylic acid was esterified to the methyl ester by trans-esterification as indicated by NMR. When the reaction was carried out in the polar aprotic solvent acetone, the addition of HCl did not give rise to complications, which resulted in high yields for compound **22** (Scheme 4.8).



Scheme 4.8

Using base in acetone produced the enolate of acetone, which may have resulted in side products, derived from acetone, being formed. However, they were easily washed away with a 50/50 hexane/ether mixture, in which the desired products are not soluble.

Compound **22** was synthesized from N-methyl pyrrole with overall yields greater than 30%. These compounds can now be condensed with **9** to form the bifunctional molecule that has the ability to bind to the A/T rich regions of the minor groove of DNA and to be specific to ER positive cells.

CHAPTER 5: SYNTHESIS OF TARGET COMPOUNDS AFTER ASSEMBLING THE CELL-TARGETING AND DNA-RECOGNIZING COMPONENTS

The DNA sequence recognition compound capable of recognizing A/T rich regions in the minor groove of DNA and the cell-targeting component capable of binding to the ER, have been obtained as compounds **22** and **9** and are shown in Figure 5.1a) and b). The DNA recognizing unit **22** is present as an acroloyl imide that has to be derivatized as the methyl sulfonate capable of methylating the N3 position of adenine. The other terminus is a carboxylic acid, which was combined with the amine of **9** to form an amide linkage. The cell-targeting compound **9** is estradiol, with a 6-carbon tether functionalized as an amine at the 7α position, and the OH groups protected. Occasionally with the synthesis of **9,** the protecting group of the 17-hydroxyl OH was removed as shown in Figure 5.1b, however it was found that both **9a** and **9b** participated equally in subsequent reactions, therefore **9b** was not reprotected to **9a**.

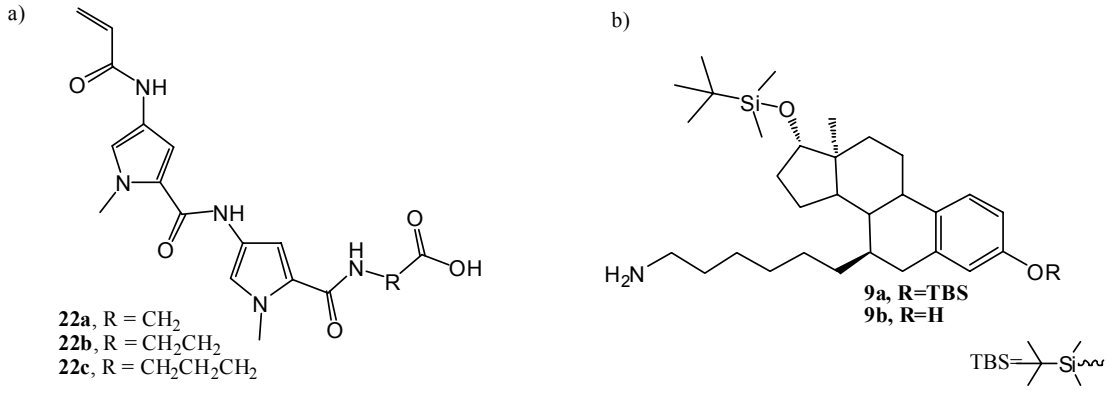
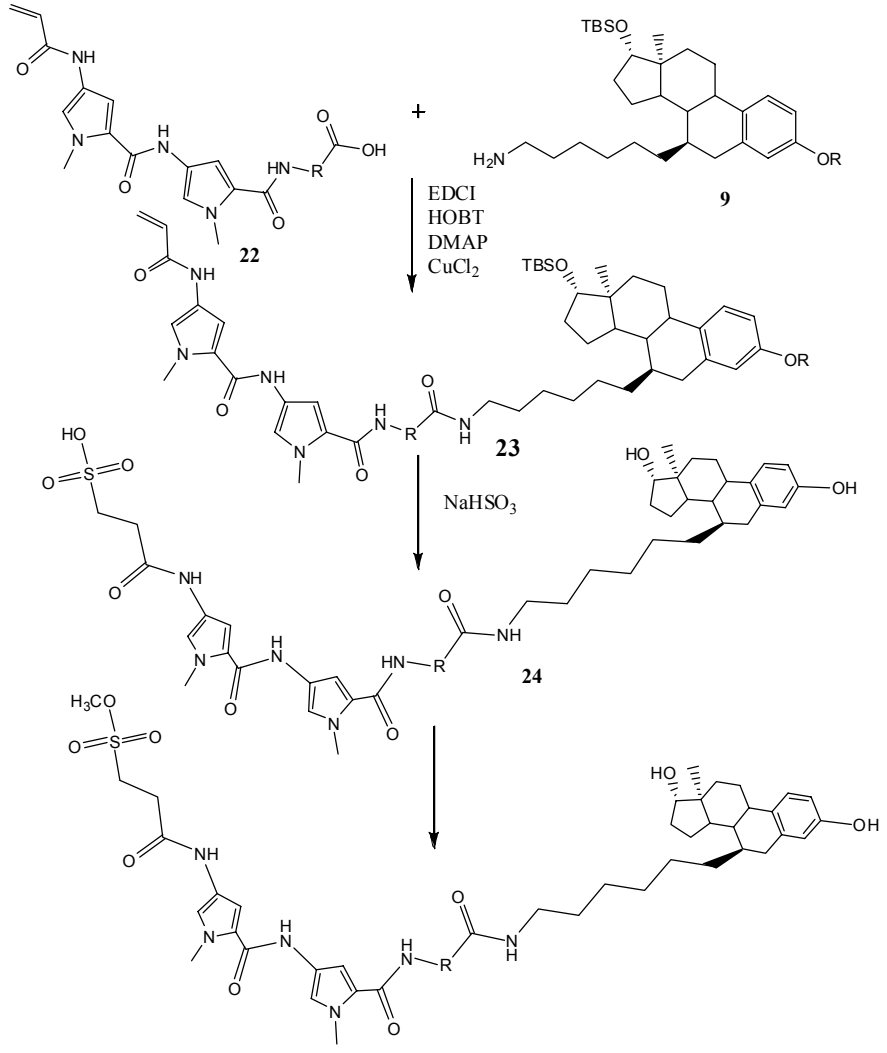


Figure 5.1: a) DNA sequence recognizing compound **22** functionalized as a carboxylic acid

b) Cell-targeting compound **9** synthesized with variance in 17-hydroxyl OH protection.

5.1: Combination of the two functional units and derivitazation to final desired compounds:

Scheme 5.1 below outlines the proposed synthesis for the assembling the functional units **22** and **9** and derivatizing further to the reactive methyl sulfonate. These compounds contained both the DNA recognition and cell-targeting units. The carboxylic acid of **22** and the amine of **9** were first coupled, followed by the conversion of the alkene to the sulfonic acid, which also resulted in the removal of the protecting groups on estradiol. Finally, the reactive methyl group could be introduced to the molecule.



Scheme 5.1

The condensation of **22** and **9** was achieved using EDCI and HOBT as coupling agents. Initial reactions using these reagents resulted in low yields of the products. The mechanism of how these reagents function is shown in Figure 5.2. EDCI is a water-soluble carbodiimide that first reacted with the carboxylic acid, which was then immediately trapped by HOBT forming the O-acylisourea intermediate. This intermediate was then able to react with the desired amine, which formed the product and regenerated HOBT. Typically, HOBT reacts quickly to form the O-acylisourea, but, it is possible for EDCI to form an unreactive N-acylurea intermediate before HOBT is able to form the desired intermediate.[39] It has been reported that $CuCl_2$ and DMAP lowered the incidence of the undesired N-acylurea intermediate and increased product yields.[39, 40] Compound **23** was obtained in yields greater than 70% using this mixture of reagents, (Scheme 5.2).

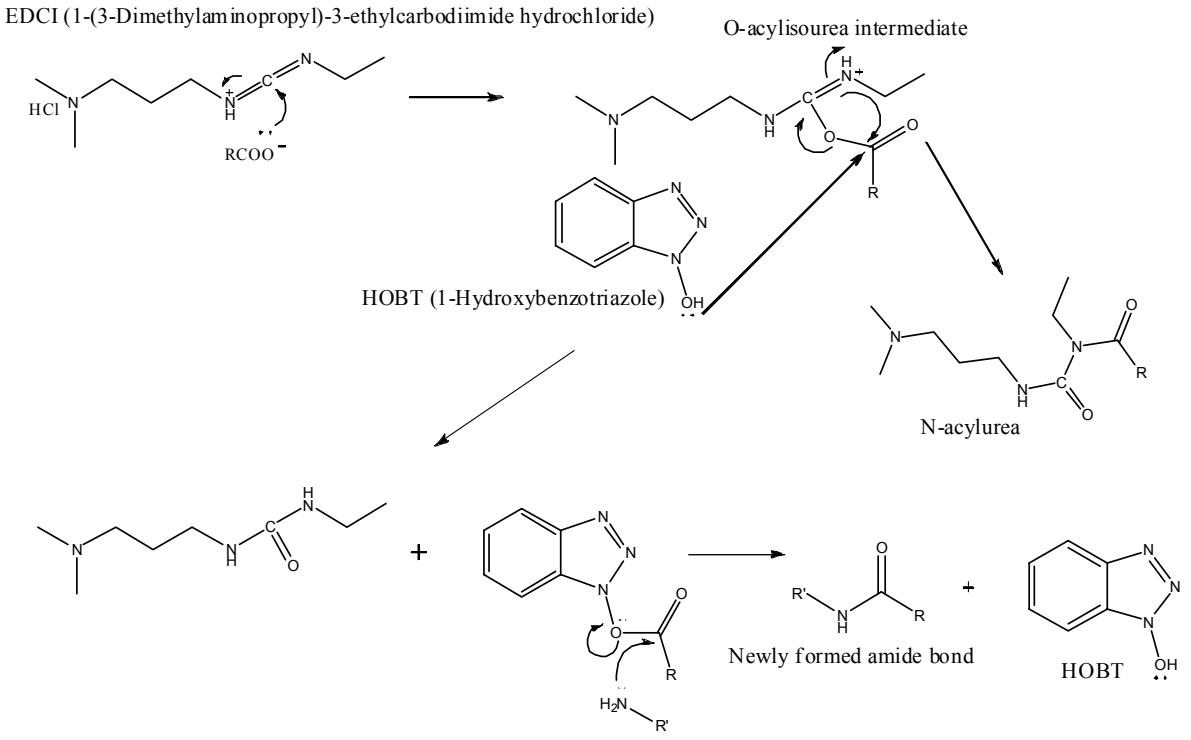
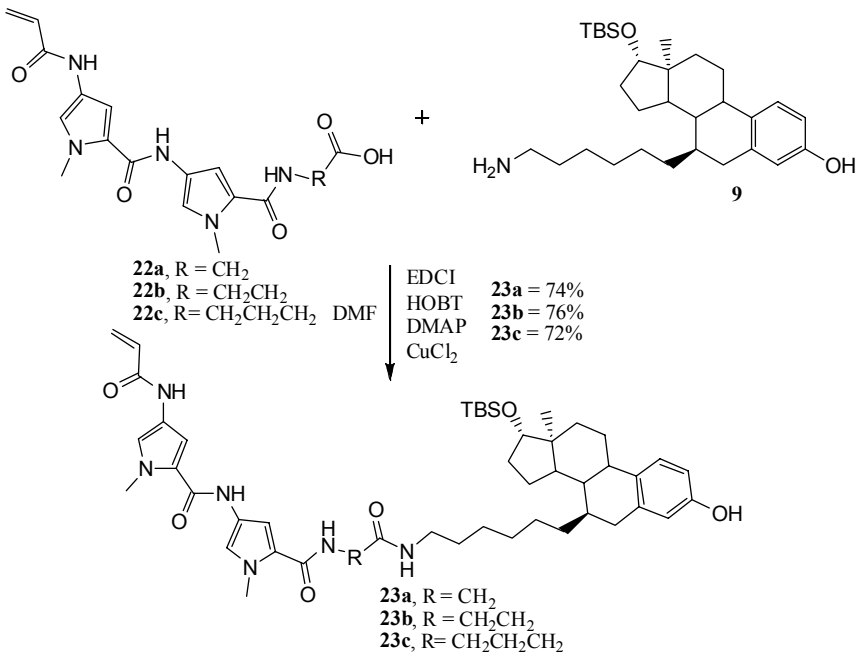
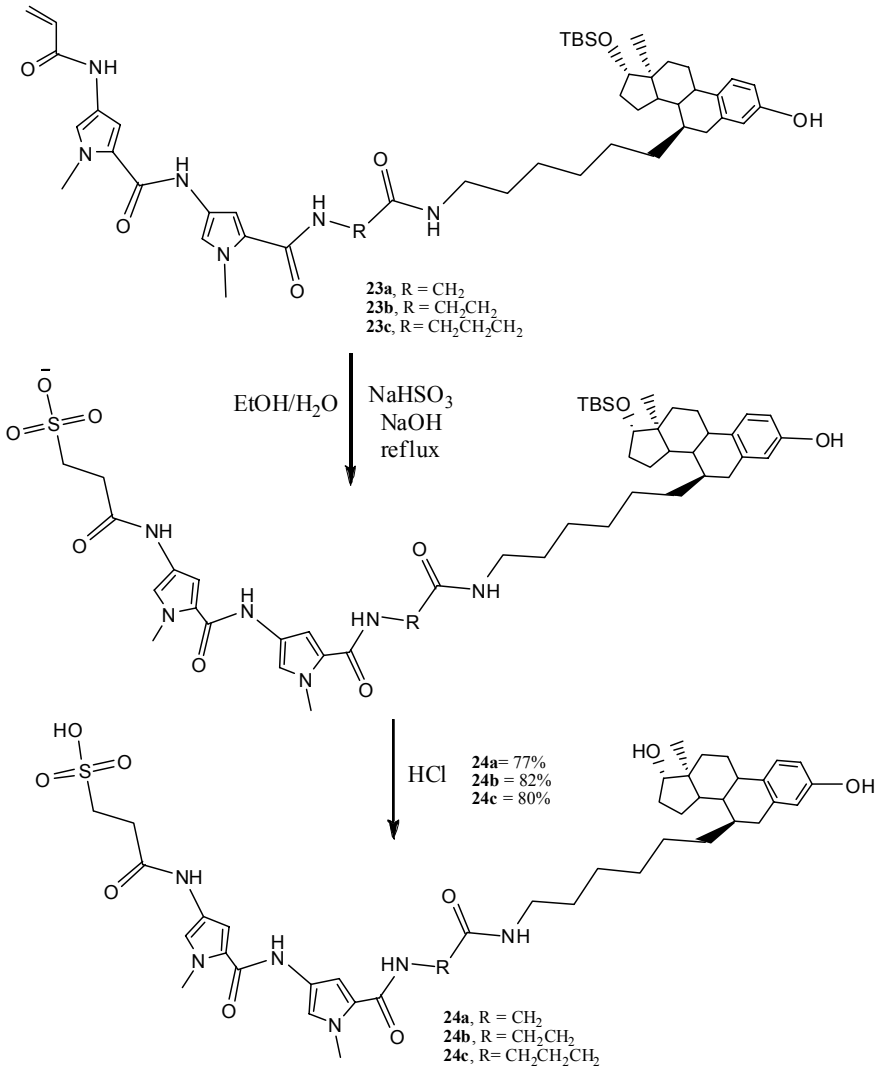


Figure 5.2. Mechanism by which EDCI and HOBT form an amide bond.



Scheme 5.2

The next step was the conversion of the alkene to the sulfonic acid (Scheme 5.3). This reaction was accomplished by using NaOH and $NaHSO_3$ under reflux as described in literature.[44] The acidification using HCl during the workup of the reaction resulted in the protonation of the sulfonate salt to form the sulfonic acid, as well as the removal of the protecting groups present on estradiol. Compound **24** was obtained in yields greater than 70%.



Scheme 5.3

The proposed mechanism for the addition of the sulfonic acid is shown below in Figure 5.3. This reaction proceeds via Michael addition, where the sulfur of sodium bisulfite ($NaHSO_3$) acted as the Michael donor, and the alkene of **23** acted as the Michael acceptor. The reaction was initiated by the addition of base, which acted as a catalyst removing the hydrogen from sodium bisulfite. This formed an intermediate that subsequently reacted with the alkene to form the desired Michael product that existed in equilibrium between the enol and the ketone, favoring the ketone tautomer.[4]

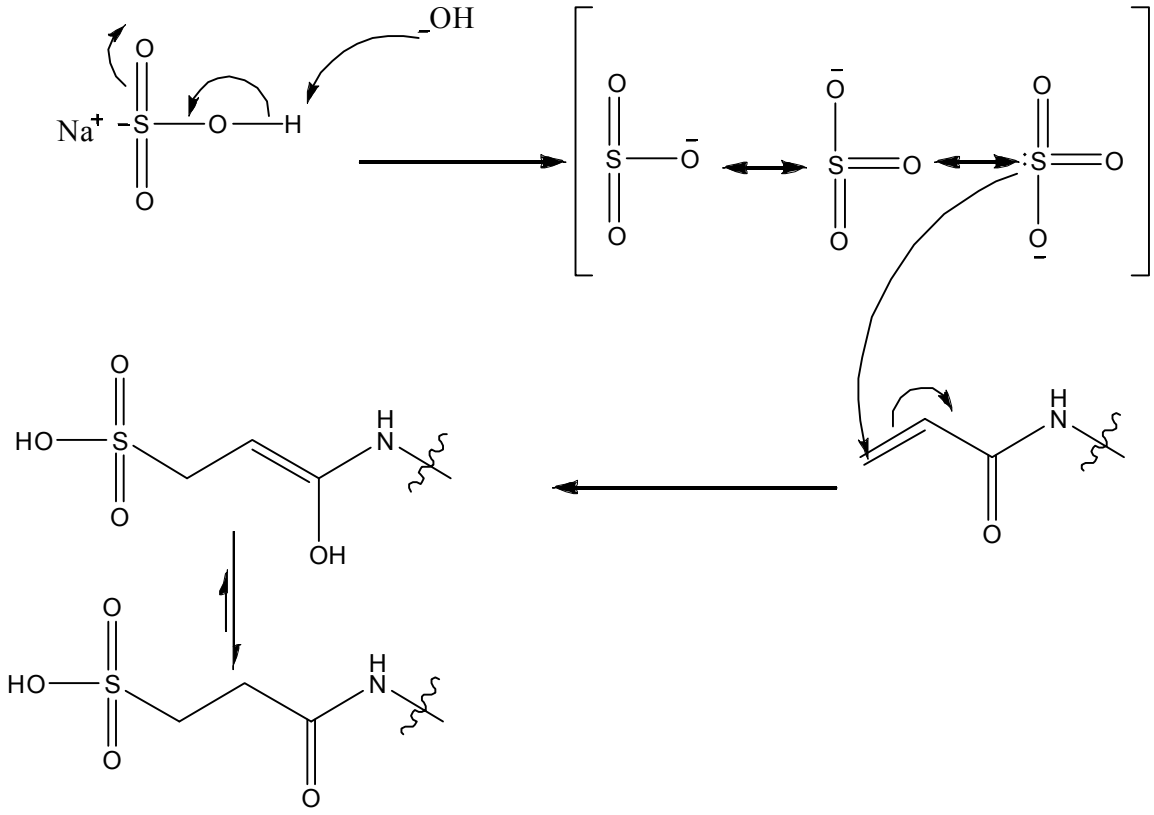


Figure 5.3. The mechanism for the Michael addition of sodium bisulfite across an alkene.

5.2: Attempted Methylation of the Sulfonic Acids:

The final step of the synthesis was the conversion of the sulfonic acid **24** to the methyl sulfonate. There are several methylating agents available to methylate sulfonic acids. The agent used to methylate Me-lex is 3-methyl-p-tolyltriazene; this is a methylating agent that requires an acidic hydrogen for methylation. Figure 5.4 illustrates the mechanism where the acidic hydrogen of the sulfonic acid first protonates 3-methyl-p-tolyltriazene, which then methylates the sulfonate ion, forming the desired methyl sulfonate, nitrogen gas, and p-toluidine as side products. This reaction was carried out in dioxane under reflux conditions for 5 hours.[13, 30]

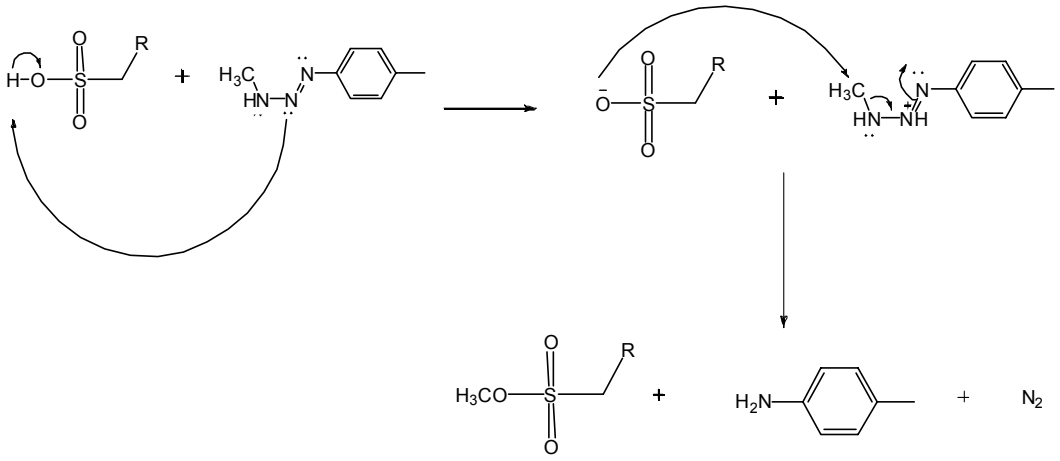
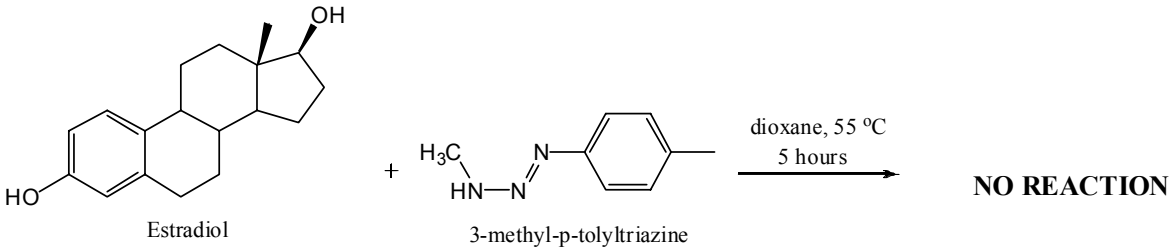


Figure 5.4: Mechanism for the methylation of a sulfonic acid using 3-methyl-p-tolyltriazene.

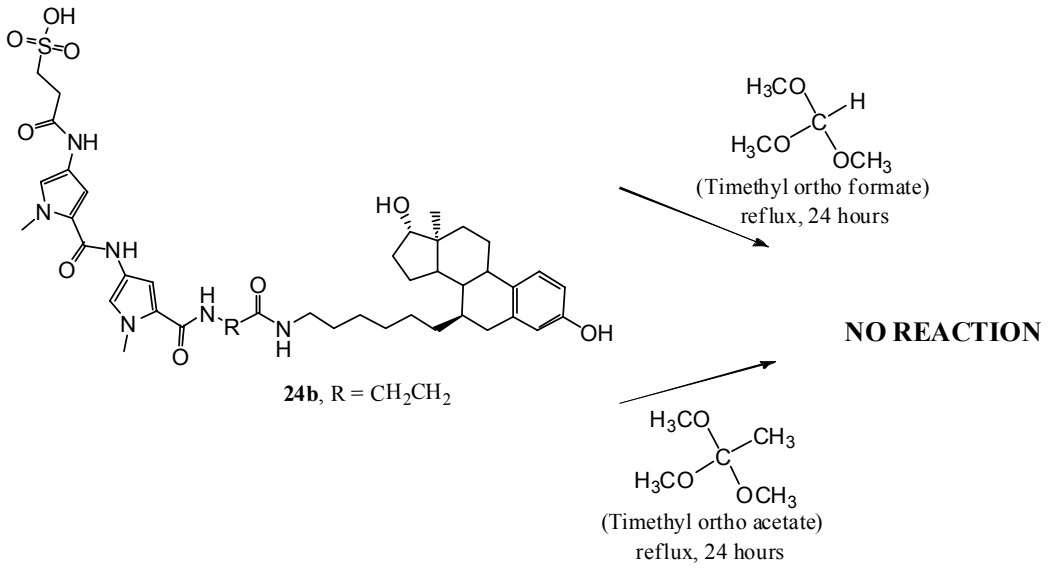
3-Methyl-p-tolyltriazene is an effective methylating agent for sulfonic acids, but it must react such that methylation does not occur on the 3-phenolic and 17-hydroxyl OH of estradiol. To test that these groups of estradiol will not get methylated, estradiol was reacted under the conditions mentioned above (Scheme 5.4). No methylation of the OH groups was observed, indicating that this would be a suitable reagent for the methylation of compound **24**.



Scheme 5.4

However, methylation of compound **24b** was not achieved with the above reaction conditions. No methylation was observed, which may be due to the compound not having suitable solubility in dioxane. Therefore, the reaction was subjected to prolonged heating under reflux conditions, still with no methylation achieved.

Alkylation with two other methylating agents was attempted. Trimethylorthoformate (TMOF) and trimethylorthoacetate (TMOA) have both been reported to convert sulfonic acids to methyl sulfonates, where the methylating agents act as both the solvent and reagent.[31-33] Compound **24b** was taken up in these reagents separately. While compound **24b** was soluble in both, no methylation was observed, even under reflux conditions for 24 hours (Scheme 5.5). 100% of starting material was recovered in both cases.



Scheme 5.5

Since the attempts to convert the sulfonic acid **24b** using the reagents mentioned were unsuccessful, other reported literature showed that iodomethane has been used to convert silver salts of sulfonic acids to their respective methyl sulfonates.[34] However, when attempted with compound **24b**, the synthesis was also unsuccessful, presumably because of complications with solubility.

Other reagents and methods have to be attempted to convert these sulfonic acids to their respective methyl sulfonates. For example, alternative solvents could be used for the reaction with 3-methyl-p-tolyltriazene. Other methylating agents should also be explored, such as diazomethane, which is well known and very reactive.

CHAPTER 6: SYNTHESIS OF MODEL COMPOUNDS

When all three components are assembled, the DNA recognition unit, the cell-targeting unit, and the reactive methyl sulfonate, they can directly be tested for their ability to methylate the N3 position of adenine by reacting these compounds with genomic DNA and observing adducts formed. Also, these compounds can be tested in cells that express the ER compared to cells without the ER, for their toxicity. If the compounds are not selectively cytotoxic for ER positive cells, there are several factors to account for. The compounds may not be taken up into cell, due to inefficient binding of the compounds to the ER, or they are getting into the cell, but not binding to DNA to allow for methylation. Also, the methyl group of these compounds might not be reactive which could be a result from the methyl group being hydrolyzed within the cytoplasm before reaching the DNA. One has to verify that each of the individual components are functioning as envisioned. To do this, the DNA-recognizing component must be assessed for its ability to bind to the A/T rich regions of the minor groove of DNA, separately from the cell-targeting component, which must be assessed for its binding affinity to the ER.

Crystal structures of compounds similar to the final compounds, but lacking the cell-targeting component, have been developed, and computational studies on these compounds revealed their binding affinity to DNA. Figure 6.1 illustrates the information gathered from similar computational studies that were performed on one of the target molecules, and have revealed that the bis-pyrrole compound **27a** is capable of achieving binding at the A/T rich regions of the minor groove of DNA, without major steric interference from the cell-targeting component. These computational studies can be further verified by using model compounds.

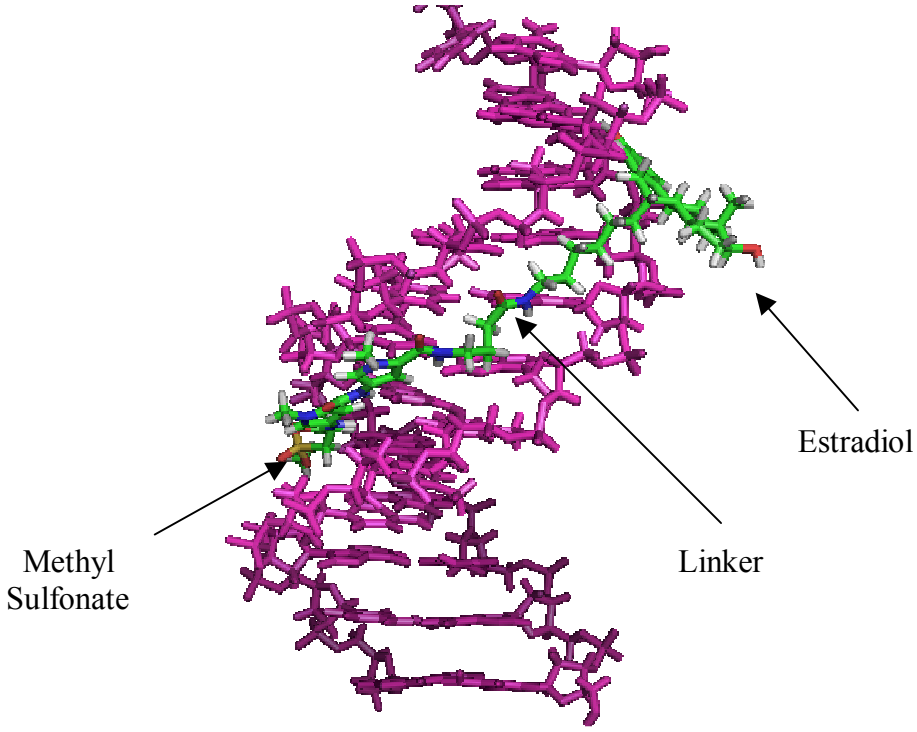


Figure 6.1: Computational model of compound **27a** bound into the minor groove of DNA

In order to test compounds for their binding, unreactive model compounds must be made. If the model compound were reactive, binding would be unable to be measured since a reaction would be taking place within the molecule. Therefore, model compounds that lack the reactive methyl sulfonate must be used to determine first, if DNA methylation would occur within the minor groove of DNA at A/T rich regions when estradiol attached as the cell-targeting ligand, and second if estradiol would bind to the ER with the DNA recognition component attached. Figure 6.2 illustrates the one difference between the target methylating compounds and the unreactive model compounds, which is that the target molecules possess a reactive methyl sulfonate, and the model compounds contain an unreactive methyl sulfone.

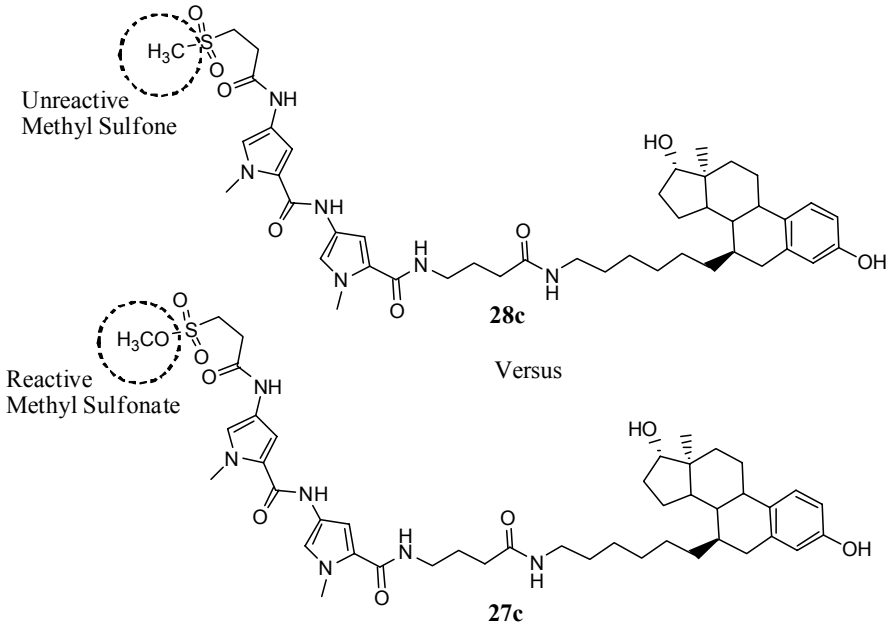
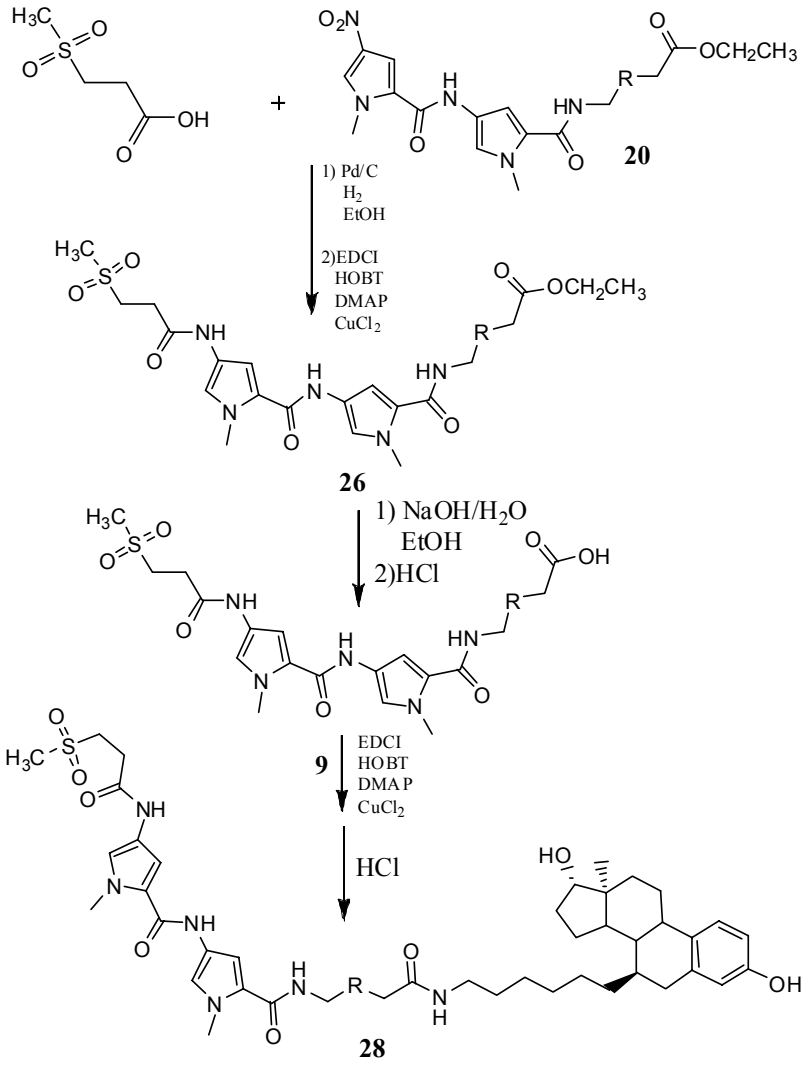


Figure 6.2: The unreactive model compound containing an unreactive methyl sulfone, compared to the reactive DNA methylating compound, containing a reactive methyl sulfonate.

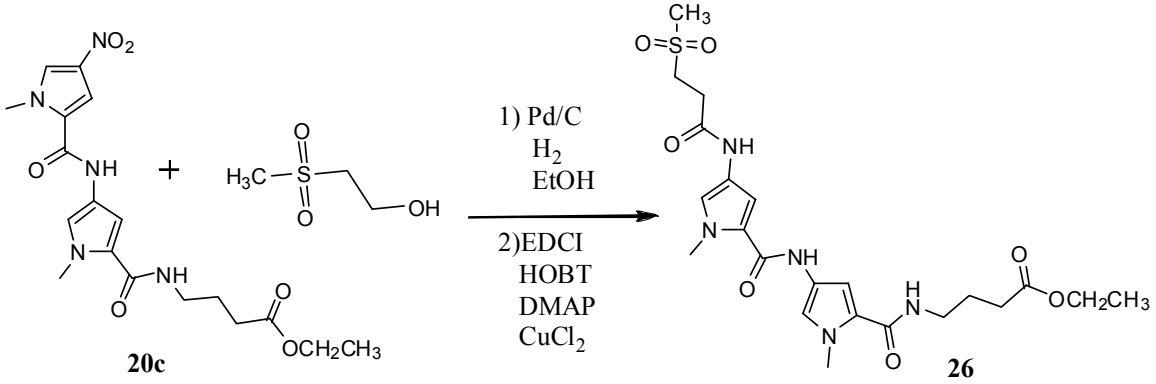
The binding ability of these model compounds will be compared to the binding of known compounds. For example, the binding of the model compounds to the A/T rich regions of the minor groove of DNA will be compared to the binding of the known compound Me-Lex, also, the model compounds will be tested for their binding affinity for the ER, which will be compared to the binding affinity of unmodified estradiol. If these results are similar, it is likely that the target reactive molecules will behave in a similar fashion.

The model compounds with varying lengths of R will be tested to see how this variation in the molecule will affect the binding to the minor groove of DNA, and to the ER. The planned synthesis of these molecules is outlined in Scheme 6.1 below. Compound **20** was first be coupled to 3-(methylsulfonyl) propanoic acid, which was previously synthesized from the oxidation of the methyl sulfide, forming an amide linkage. The ester of **26** could be subsequently hydrolyzed and coupled at the amine terminus of **9** forming an amide bond. This could then be followed by the deprotection of the OH groups, yielding the final model compounds.


1) Pd/C
H2
EtOH
2)EDCI
HOBT
DMAP
CuCl2
20
26
1) NaOH/H2O
EtOH
2)HCl
9
EDCI
HOBT
DMAP
CuCl2
HCl
28

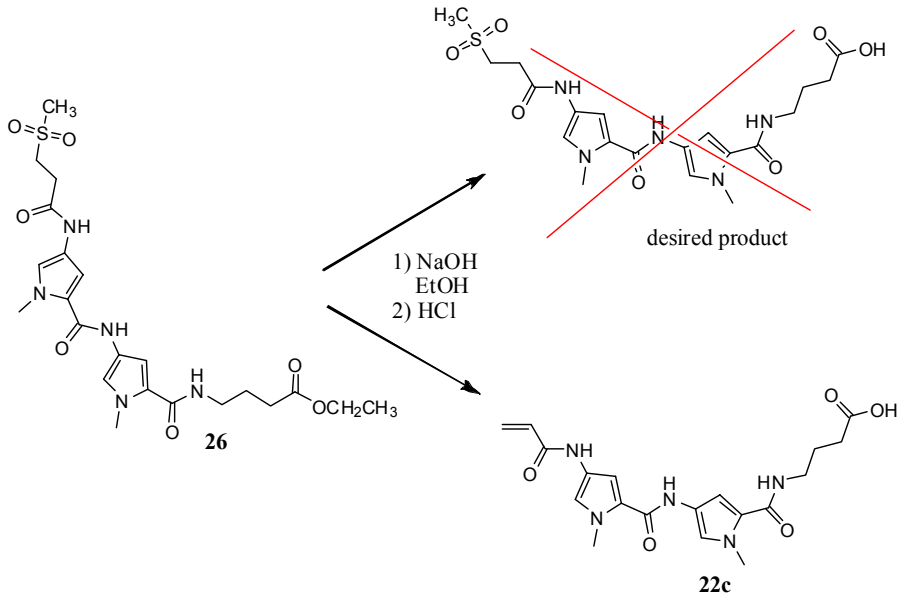
Scheme 6.1

The coupling of 3-(methylsulfonyl) propanoic acid to **20c** was accomplished using EDCI and HOBT (Scheme 6.2), resulting in a 74% yield of **26**.



Scheme 6.2

Scheme 6.3 illustrates that when compound **26** was subjected to standard hydrolysis, none of the desired compound was formed; the entire product was obtained as compound **22c.** The proposed mechanism of the removal of the methyl sulfone is illustrated in Figure 6.3 below, where base extracts the acidic hydrogen forming the double bond, and elimination of the methyl sulfonate.[41-42]



Scheme 6.3

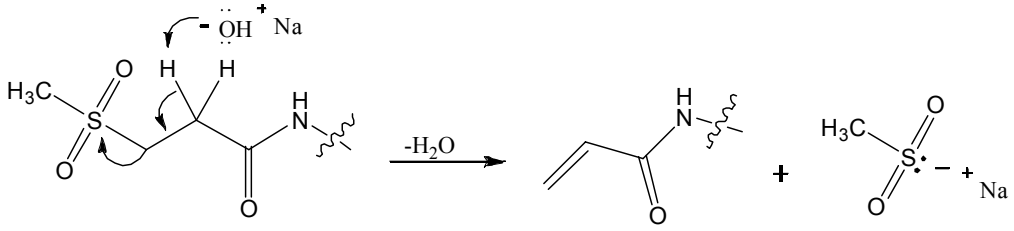
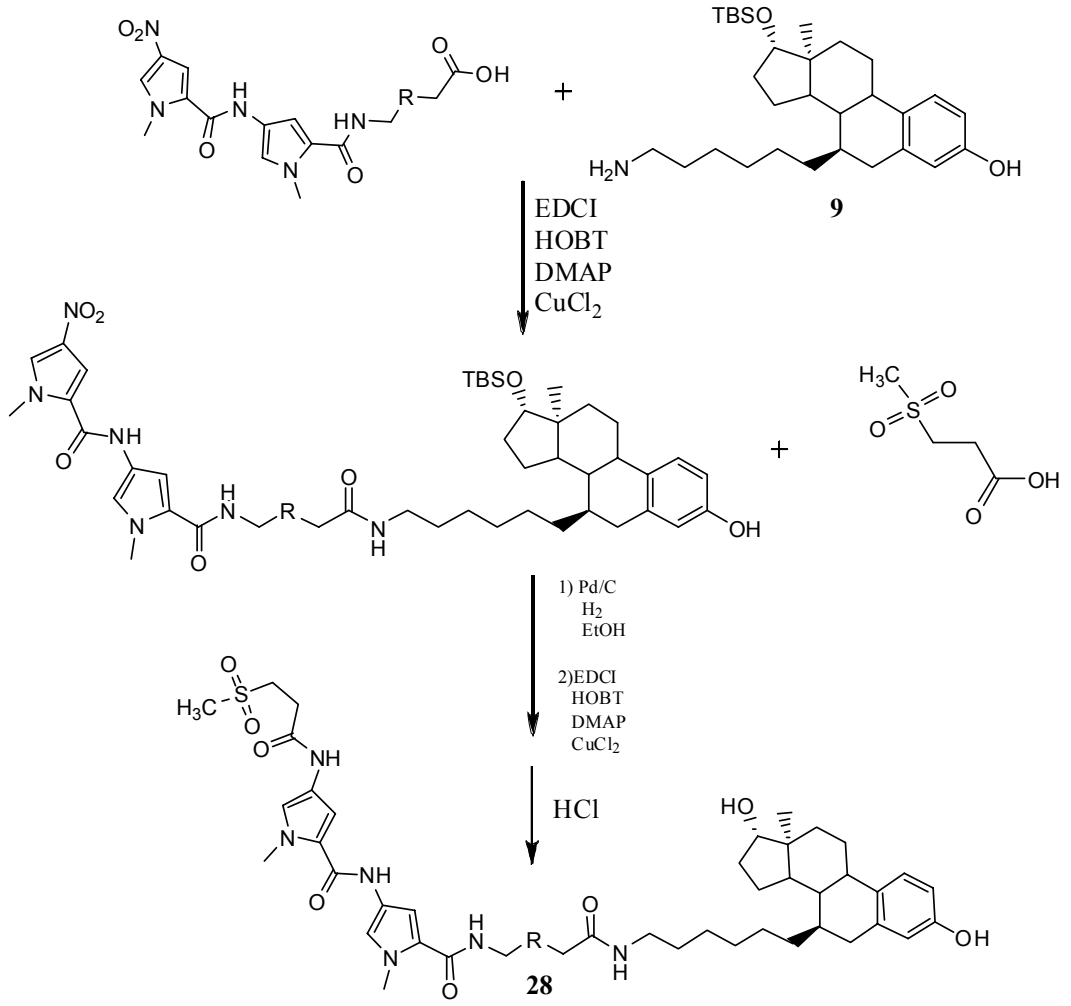


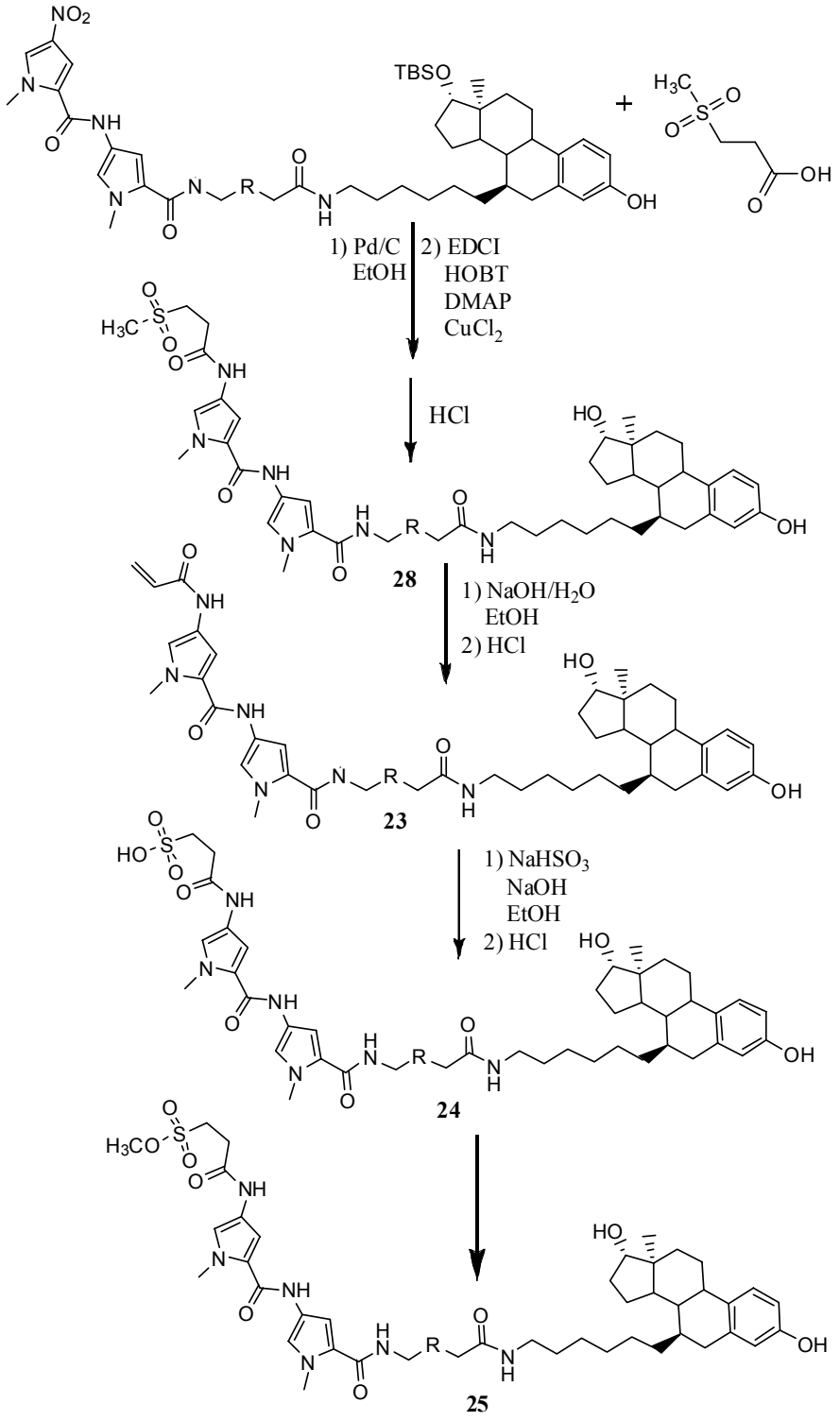
Figure 6.3: Mechanism for the removal of the methyl sulfone during base hydrolysis

Therefore, the ester with the methyl sulfone, **26** cannot be used for the synthesis of the model compounds. This pathway was abandoned and an alternate route was devised. In Scheme 6.4, a nitro bis-pyrrole unit containing a linker functionalized as a carboxylic acid would be condensed with **9** forming an amide linkage. The coupling would be followed by first the reduction of the nitro to an amine, followed by the coupling of 3-(methylsulfonyl) propanoic acid to form another amide linkage. The final step would be the removal of the OH protecting group with strong acid, yielding the final unreactive model compounds. Once these compounds are synthesized they can be used to test their ability to bind to the minor groove of DNA and to the ER.



Scheme 6.4

The unexpected advantage of forming compound **22c** during the hydrolysis of **26** implies that **28** could be synthesized and used directly as a model compound for binding studies, and as Scheme 6.5 illustrates, by using conditions for base hydrolysis, **28** could easily be converted to **23**, which would then be followed by the addition of the sulfonic acid to for **24** and finally, the compound could be methylated forming the reactive compound **25.** By using this method, time and resources could be saved.


1) Pd/C
EtOH
2) EDCI
HOBT
DMAP
CuCl2
HCl
28
1) NaOH/H2O
EtOH
2) HCl
23
1) NaHSO3
NaOH
EtOH
2) HCl
24
25

Scheme 6.5

CHAPTER 7: EXPERIMENTAL

7.1. General:

All solvents and reagents were purchased from VWR International (West Chester, Pennsylvania) or Sigma-Aldrich (Atlanta, Georgia) and were of the highest grade available unless otherwise noted. Flash chromatography was performed with silica gel (230/400 mesh, Life Force Inc.). TLC was performed on glass plates coated with silica gel (Whatman International, Maidstone, England, 150 A) that had a fluorescence indicator and were detected by UV visualization. All rotary evaporations were carried out using a Buchi R-3000 or a Buchi R-114 rotary evaporator equipped with a Brinkmann model B-16 vacuum aspirator. Hydrogenations were performed using a Parr Hydrogenation Apparatus in a 500 mL Parr jar. Melting points were determined using a Mel-Temp II.

All anhydrous reactions were carried out under positive pressure argon. Glassware used for anhydrous reactions were dried overnight in an oven at 140 °C or dried over a flame, assembled while still hot, and cooled to room temperature under argon. Solvents and reagents (liquids) for anhydrous reactions were obtained in bottles with sure-seal caps and transferred by using oven-dried needles and glass syringes.

All ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a Bruker 400MHz NMR spectrometer, using deuterated DMSO or deuterated chloroform as the solvent. The deuterated DMSO was obtained in sealed ampoules from Sigma-Aldrich. The deuterated chloroform was obtained from Alfa Aesar. The spectra are reported in ppm and referenced to deuterated DMSO (2.49 ppm for ^{1}H, 39.5 ppm for ^{13}C) or referenced to deuterated chloroform (7.26 ppm for ^{1}H, 77 ppm for ^{13}C). The samples were contained in 5 mm Pyrex glass NMR tubes obtained from Wilmad-LabGlass, Buena, New Jersey.

7.2. Synthesis:

(3,17β-Bis(2-tetrahydropyranyloxy)estra-1,3,5(10)triene) (1). To a solution of β-estradiol (30 g, 110.2 mmol) in CH_2Cl_2 (375 mL) was added dihydropyran (101 mL) and then pyridinium ρ-toluenesulfonate (20 mg). The reaction mixture was fitted prepared with a reflux condenser and was allowed to reflux for 3 hours while monitoring by TLC. The solvent was then removed by rotary evaporation to yield clear oil. Ethanol was added to the clear oil, the flask was then spun in a warm water bath until the oil dissolved and a white precipitate formed. The flask was allowed to cool to room temperature upon which a white solid further fell out of solution, then the flask was placed into the refrigerator overnight, followed by placement in the freezer for another 24 hours, upon which a white solid precipitated. The precipitate was filtered to yield 48.51 g (85%) of **1** as a white solid. mp = 77-82°C, R_f = 0.43 (5:1, hexane/EtOAc), ^{1}H NMR ($CDCl_3$): Identical to that published in literature.[25-27]. ^{13}C NMR ($CDCl_3$): Identical to that published in literature.[25-27]

(3,17β-Bis(2-tetrahydropyranyloxy)estra-1,3,5(10)-triene-6-ol) (2). To a cooled (-78 °C) solution of **1** (18.5 g, 42 mmol) in THF (105 mL) was added 1.0 M potassium tert-butoxide (KOt-Bu) in THF (85 mL), diisopropylamine (1.5 mL), and 1.6 M n-BuLi in hexanes (48 mL). Upon addition of n-BuLi the reaction mixture turned dark red. After 15 minutes trimethyl borate (11.3 mL) was added and the reaction mixture turned pale yellow. After 5 minutes 35% H_2O_2 (52 mL) was added to the reaction, and a white solid formed. After stirring, the solid dissolved to yield a clear pale yellow liquid. The reaction mixture then stirred at room temperature for 1 hour. The organic phase was partitioned between toluene and diluted with 10% HCl. The organic layer was dried over $MgSO_4$ and filtered. The solvent was removed by rotary evaporation and the product was purified by flash chromatography (2:1, hexane/EtOAc) to

give 14.3 g (74%) of **2** as a white solid. mp = 74-79°C, R_f =0.32 (2:1, hexane/EtOAc), ^{1}H NMR ($CDCl_3$): Identical to that published in literature.[25-27]. ^{13}C NMR ($CDCl_3$): Identical to that published in literature.[25-27]

(3,17β-Bis(2-tetrahydropyranyloxy)estra-1,3,5(10)-triene-6-one) (3). To a cooled (0-5 °C) solution of **2** (12 g, 26.3 mmol) in CH_2Cl_2 was added 2,2,6,6-Tetramethylpiperidine 1-oxy (TEMPO) (0.220 g) and KBr (0.330 g in 11 mL of DI H_2O). 14% Sodium hypochlorite (pH was adjusted to 8.5 by adding saturated $NaHCO_3$) was added until a less polar compound was formed. The organic phase was then washed with 10% sodium thiosulfate and deionized H_2O. The organic layer was dried over $MgSO_4$ and gravity filtered. The solvent was removed by rotary evaporation and the product was then purified by flash chromatography (3:1, hexane/EtOAc) to give 9.3 g (78%) of **3** as a white solid. mp = 52-57°C, R_f = 0.70 (3:1, hexane/EtOAc), ^{1}H NMR ($CDCl_3$): Identical to that published in literature.[25-27]. ^{13}C NMR ($CDCl_3$): Identical to that published in literature.[25-27]

(3,17β-Bis(2-tetrahydropyranyloxy)-7α-(5-hexen-1-yl)-estra-1,3,5(10)-triene-6-one) (4). To a solution of **3** (2.0 g, 4.40 mmol) in ethyl ether (21 mL) was added 1.0 M KOt-Bu in THF (4.8 mL). After 10 minutes 1.0 M triethylborane (5.5 mL) was added and the reaction mixture was stirred for 1 hour. 6-iodohexene (1.1g) in ethyl ether was canulated into the reaction flask mixture. After 30 minutes of stirring an equivalent amount of 1.0 M KOt-Bu in THF (4.8 mL) was added and the reaction mixture was stirred overnight. The reaction was then quenched with DI water and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and gravity filtered. The solvent was removed by rotary evaporation and the product was purified by flash chromatography (5.5:0.5, hexane/EtOAc) to give 1.02 g (43%) of **4** as a white solid. mp = 39-

46°C, R_f = 0.19 (5.5:0.5, hexane/EtOAc), ^{1}H NMR ($CDCl_3$): Identical to that published in literature.[25-27]. ^{13}C NMR ($CDCl_3$): Identical to that published in literature.[25-27]

(6-iodo-1-hexene). To a solution of 5-hexen-1-ol (3.0g, 30 mmol) in a mixture of diethyl ether/acetonitrile (145/37 ml) was added triphenylphosphine (15.8g) and imidazole (4.1g). Iodine (15.3 g) was then added slowly to the reaction and the reaction was allowed to stir at room temperature until only one spot was observed by TLC. Diethyl ether (100ml) was then added and the reaction mixture was then filtered through celite. The filtrate was then washed with saturated sodium bicarbonate and saturated NaCl, followed by a water wash. The organic layer was then dried over Mg SO_4 and gravity filtered. The solvent was then removed by rotary evaporation and the product was purified by flash chromatography (hexanes) to give 5.9 (94%) of 6-iodo-1-hexene as a clear liquid. ^{1}H NMR ($CDCl_3$): δ 1.51 (m, 2H), 1.84 (m, 2H), 2.08 (m, 2H), 3.19, (t, 2H, J = 7 Hz), 4.99 (m, 2H), 5.78 (m, 1H).

(7α-(5-hexen-1-yl)-estra-1,3,5(10)-triene-3,17β-diol) (5). To a cooled (0 °C) solution of **4** (0.550 g, 1.0 mmol) in MeOH (24 mL) was added acetyl chloride (2.4 mL) in 0.4 mL aliquots. The ice water bath was removed and the reaction was allowed to stir at room temperature for 30 minutes. The solvent was removed by rotary evaporation to give a yellow oil. To a cooled (0 °C) solution of the crude oil in CH_2Cl_2 (12 mL) was added triethylsilane (12.1 mL) and boron trifluoride diethyl etherate (38.5 mL). The ice water bath was removed and the reaction solution was allowed to stir overnight. 20% K_2CO_3 (120 mL) was added resulting in a biphasic mixture. The biphasic mixture was then filtered through silica and to the filtrate was added CH_2Cl_2. The organic layer was then dried over Mg SO_4 and gravity filtered. The solvent was then removed by rotary evaporation and the product was purified by flash chromatography (2:1, hexane/EtOAc) to give 0.286g (81%) of **5** as a white solid. mp = 108-113°C, R_f = 0.26 (2:1,

hexane/EtOAc), ^{1}H NMR ($CDCl_3$): Identical to that published in literature.[25-27]. ^{13}C NMR ($CDCl_3$): Identical to that published in literature.[25-27]

(3,17β-Bis(τ-butyldimethylsilanyloxy)-7α-(5-hexen-1-yl)-estra-1,3,5(10)-triene) (6). To a cooled (0 °C) solution of imidazole (4.29 g) in DMF (22 mL) was added tertbutyl dimethylsilyl chloride (4.27 g). The reaction was then stirred for 30 minutes and then a solution of **5** (2.2 g, 6.2 mmol) in DMF (4 mL) was added all at once. The reaction was then stirred overnight and then was hydrolyzed with 0.1% K_2CO_3. The reaction mixture was then extracted with CH_2Cl_2 and washed with deionized H_2O. The organic layer was dried over $MgSO_4$ and gravity filtered. The product was then purified by flash chromatography (2:1, hexane/EtOAc) to give 2.8 g (78%) of **6** as an oil, which was converted to a white solid under high pressure vacuum. mp = 54-57 °C, R_f = 0.82 (2:1, hexane/EtOAc), ^{1}H NMR ($CDCl_3$): Identical to that published in literature.[25-27]. ^{13}C NMR ($CDCl_3$): Identical to that published in literature.[25-27]

(3,17β-Bis(τ-butyldimethylsilanyloxy)-7α-(6-hydroxy-hexen-1-yl)-estra-1,3,5(10)-triene) (7) . To a solution of **6** (0.170 g, 0.292 mmol) in THF (10 mL) was added 0.5 M 9-borabicyclo[3.3.1]nonane in THF (1.5mL). The reaction was then stirred overnight and hydrolyzed with 3 M KOH (1.3 mL). After 5 minutes 30% H_2O_2 (1.3 mL) was added and the reaction was stirred for 3 hours. Saturated $NaHCO_3$ (12 mL) was added and then extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and gravity filtered. The solvent was removed by rotary evaporation and the product was then purified by flash chromatography (20%, EtOAc/hexane) to give 0.128 g (74%) of **7** as a white solid. mp = 53-55°C, R_f = 0.58 (20%, EtOAc/hexane), ^{1}H NMR ($CDCl_3$): Identical to that published in literature.[25-27]. ^{13}C NMR ($CDCl_3$): Identical to that published in literature.[25-27]

(3,17β-Bis(τ-butyldimethylsilanyloxy)-7α-(6-phthalimido-hexen-1-yl)-estra-1,3,5(10)-triene) (8). To a solution of triphenylphosphine (0.086 g) in THF (1.5 mL) was added diisopropyl azodicarboxylate (0.065 mL) dropwise. The reaction was allowed to stir for 40 minutes and a white precipitate formed. The phthalimide (0.048 g) was then added followed by **7** (0.100 g, 0.165 mmol). The reaction was stirred at 0 °C for 1 hour and then at room temperature overnight. The solvent was removed by rotary evaporation and the product was then purified by flash chromatography (5% hexane/EtOAc) to give 0.105 g (87%) of **8** as a yellow solid. mp = 55-58°C, R_f = 0.83 (5% hexane/EtOAc), ^{1}H NMR ($CDCl_3$): Identical to that published in literature.[25-27]. ^{13}C NMR ($CDCl_3$): Identical to that published in literature.[25-27]

(3,17β-Bis(τ-butyldimethylsilanyloxy)-7α-(6-phthalimido-hexen-1-yl)-estra-1,3,5(10)-triene) (9a and 9b). To a solution of **8** (0.084 g, 0.114 mmol) in ethyl ether/EtOH (1.11 mL/1.11 mL) was added anhydrous hydrazine (555 μL) at room temperature in one installment. The reaction mixture was then prepared with a reflux condenser and refluxed for 2 hours. During this time a white solid formed on the walls and the solution turned a pale green/yellow. 5% NaOH was added after the reaction cooled to dissolve the white solid. After 30 minutes DI H_2O was added and then was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and gravity filtered. The solvent was then removed to give 0.055 g (80%) of **9** as a white solid. mp = 69-72 °C, R_f = 0.67 (6:1 $CHCl_3$/MeOH), ^{1}H NMR ($CDCl_3$): Identical to that published in literature.[25-27]. ^{13}C NMR ($CDCl_3$): Identical to that published in literature.[25-27]

3-Methoxy-17β-hydroxy-1, 3, 5(10)-estratriene (10). β-estradiol (0.200 g, 0.734 mmol) and potassium carbonate (0.203 g, 1.47 mmol) were added to a 25 mL round bottom flask and dissolved in 7 mL of acetonitrile. Iodomethane (0.91 mL, 1.47 mmol) was then added all at once. The flask was then equipped with a reflux condenser and allowed to reflux overnight. The

solution was concentrated by rotary evaporation until dry. This solid was dissolved in dichloromethane ad extracted with 1M HCl (1 x 100 mL) and DI water (1 x 100mL). The organic layers were dried over $MgSO_4$. The solution was concentrated by rotary evaporation. The was placed under vacuum until dry which yielded a white foam **10** (0.185 g, 89%): mp 49-52 °C. TLC (EtOAc) $R_f = 0.74$. ^{1}H NMR data ($CDCl_3$): δ 7.17 – 7.21 (m, 1H) 6.83 (d, 1H, J = 8 Hz), 6.77 (d, 1H, J = 2 Hz), 3.78 (s, 3H), 3.72 (m, 1H) 2.82 (d, J = 4 Hz, 2H), 0.77 (s, 3H). ^{13}C NMR data ($CDCl_3$): δ 157.43, 138.300, 132.66, 126.36, 113.82, 111.48, 81.95, 55.23, 50.03, 43.96, 43.28, 38.86, 36.72, 30.59, 29.83, 27.26, 26.34, 23.14, 11.08.

2,2,2-trichloro-1-(1-methyl-1*H*-Pyrrol-2-yl)ethanone (11). Trichloro acetyl chloride (50 mL, 0.170 mol) was added to 250 mL of dry dichloromethane in a 1000 mL flask flushed with argon. N-methyl pyrrole (42 mL, 0.569 mol) was then added to 100 mL of dry dichloromethane in a dropping funnel. This N-methyl pyrrole mixture was added drop wise to the trichloro acetyl chloride and stirred overnight. A solution of potassium carbonate (32 g in 250mL of water) was then added to the empty dropping funnel and dropped into the reaction mixture over a period of 2 hours to quench the reaction. This mixture was extracted with DI water (2 x 300 mL) and dichloromethane (1x 300 mL). The organic layers were dried over $MgSO_4$. The solution was concentrated by rotary evaporation until dry. This was placed under vacuum until dry, which yielded a dark brown solid **11** (93.1 g, 93%): mp 53-57 °C. TLC (1:1 EtOAc/Hexane) $R_f = 0.81$. ^{1}H NMR data: δ 7.44 (d, J = 3.6 Hz, 2H), 6.29 (t, J =3.4 Hz, 1H), 3.93 (s, 3H). ^{13}C NMR data: δ 172.30, 135.73, 124.17,. 121.17, 109.54, 96.56, 38.44.

2,2,2-trichloro-1-(1-methyl-4-nitro-1*H*-pyrrol-2-yl)ethanone (12). Pyrrole **11** (25.00 g, 0.11 mol) was dissolved in acetic anhydride (175 mL) in a 500 mL round bottom flask and cooled to -78 °C in a dry ice/acetone bath. This temperature was maintained for 10 minutes

when fuming nitric acid (12.3 mL) was added drop wise, using a drop funne,l over a period of 45 minutes with constant stirring, ensuring that the temperature did not warm above -40 °C. This mixture stirred for one hour at -40 °C. The solution was then allowed to warm to room temperature and stirred for an additional two hours. The solution was then cooled in an ice bath and cold DI water (101 mL) was added slowly in installments over a period of 45 minutes. This mixture was stirred overnight, during which a yellow precipitate formed and was filtered under vacuum and dried to give **12** (23.48 g, 80%): mp 114-121 °C. TLC (1:1 EtOAc/Hexane) R_f = 0.56. ^{1}H NMR data: δ 8.56 (d, J = 1.73 Hz, 1H), 7.78 (d, J = 1.71 Hz, 1H), 3.98 (s, 3H). ^{13}C NMR data: δ 1.73.30, 134.73, 133.09, 121.09, 116.82, 95.02, 79.44.

(5-carboxy-1-methyl-1*H*-pyrrol-3-yl)(hydroxy)oxoammonium (13). The nitro pyrrole **12** (0.500 g, 0.0018 mol) was dissolved in ethanol (15 mL) in a 50 mL round bottom flask and a solution of NaOH (0.368 g, 5 eq.) in H_2O (2 mL) was added. This was allowed to reflux until the disappearance of **12** was indicated by TLC (EtOAc). The solution was concentrated by rotary evaporation to remove the majority of the EtOH. The aqueous solution was cooled in an ice bath and then acidified with concentrated HCl until the final pH was approximately 1 when a precipitate fell out of solution. The mixture was allowed to cool in the freezer for one more hour and the dark yellow solid precipitate as filtered out and rinsed with ice-cold water and air-dried to give pure **13** (0.250 g, 82%): mp 177-181 °C. TLC (5:2 $CHCl_3$/MeOH) R_f = 0.38. ^{1}H NMR data: δ 13.15 (s, 1H), 8.23 (d, J = 2.0 Hz , 1H), 7.26 (d, J = 2.0 Hz , 1H), 3.92 (s, 1H). ^{13}C NMR data: δ 204.39, 161.45, 134.47, 129.67, 124.26, 111.86, 37.96.

[5-(ethoxycarbonyl)-1-methyl-1*H*-pyrrol-3-yl](hydroxy)oxoammonium (14). Absolute ethanol (250 mL) was added to a flask flushed with argon. This flask was then placed

in an ice bath. To the flask was added solid sodium (0.635g, 0.0276 mol) in small increments. Once all the sodium was dissolved, pyrrole **12** (5.00g, 0.0184 mol) was added to the reaction flask all at once. The reaction proceeded overnight and was indicated complete by TLC (1:1 EtOAc/Hexane) to give **14** (2.98 g, 80%): mp 290-294 °C. TLC (1:1 EtOAc/Hexane) R_f = 0.80. ^{1}H NMR data: δ 8.30 (s, 1H), 7.31 (s, 1H), 4.26 (q, J = 6.0, 1.2 Hz, 2H), 3.93 (s, 3H), 1.29 (t, J = 6.0 Hz, 3H). ^{13}C NMR data: δ 159.88, 134.59, 129.94, 123.35, 111.98, 61.06, 37.98, 14.53.

ethyl *N*-({4-[hydroxy(oxo)ammonio]-1-methyl-1*H*-pyrrol-2- yl}carbonyl)glycinate (15a). EtOAc dried over sieves (100 mL) was added to a flask flushed with argon. The nitro pyrrole **12** (5.00g, 19 mmol) was added to the reaction flask followed by glycine ethyl ester hydrochloride (2.58 g, 22 mmol). TEA (7.8 mL, 56 mmol) dissolved in EtOAc dried over sieves (20 mL) was added to a dropping funnel equipped to the reaction flask. Argon was bubbled through this solution, and this mixture was added drop wise over a period of 15 hours under argon. Once all of the TEA mixture was added the reaction was allowed to stir for an additional 48 hours under argon. The white precipitate formed was filtered off and the filtrate was extracted with 1M HCl (2 x 100 mL) and DI water (1x 100 mL). The organic layers were dried over $MgSO_4$. The solution was concentrated by rotary evaporation until dry and was placed under vacuum until a yellow solid formed to give **15a** (3.72 g, 83%): mp 96-99 °C. TLC (1:1 EtOAc/Hexane) R_f = 0.54. ^{1}H NMR data: δ 8.89 (t, J= 1.2Hz, 1H), 8.16 (d, J = 2.4 Hz, 1H), 7.51 (d, J = 1.6 Hz, 1H), 4.14 (q, J = 6.0, 1.2 Hz, 2H), 3.96 (d, J = 6.0 Hz, 2H), 3.92 (s, 3H), 1.21 (t, J = 7.0 Hz, 3H). ^{13}C NMR data: δ 170.12, 160.71, 134.35, 128.61, 126.11, 108.32, 60.97, 41.15, 37.87, 14.51.

Ethyl *N*-[(1-methyl-4-nitro-1*H*-pyrrol-2-yl)carbonyl]-β-alaninate (15b). Compound **15b** was synthesized by a procedure similar to the one described above for **15a** using

(5.00 g, 19 mmol) of **12** and (3.40 g, 22 mmol) of β-alanine ethyl ester hydrochloride to obtain **15b** (3.97g, 88%): mp 127-129 °C. TLC (1:1 EtOAc/Hexane) $R_f = 0.33$. ^{1}H NMR data: δ 8.49 (t, J = 5.2 Hz, 1H), 8.12 (d, J = 2.0 Hz, 1H), 7.41 (d, J = 2.0 Hz, 1H), 4.01 (q, J = 6.8, 7.2 Hz, 2H), 3.91 (s, 3H), 3.42 (q, J = 5.6, 2.1 Hz, 2H), 2.55 (t, J = 7.0 Hz, 2H), 1.18 (t, 6.8 Hz, 3H). ^{13}C NMR data: δ 171.63, 160.34, 134.23, 128.32, 126.71, 107.86, 60.42, 37.78, 35.41, 34.11, 14.51.

Ethyl 4-{[(1-methyl-4-nitro-1*H*-pyrrol-2-yl)carbonyl]amino}butanoate (15c). Compound **17** was synthesized by a procedure similar to the one described above for **15a** using (5.00 g, 19 mmol) of **12** and (3.71 g, 22 mmol) of aminobutyrate hydrochloride to obtain **15c** (4.08 g, 81%): mp 54-58 °C. TLC (1:1 EtOAc/Hexane) $R_f = 0.25$. ^{1}H NMR data: δ 8.38 (t, 5.3 Hz, 1H), 8.11 (d, J = 1.6 Hz, 1H), 7.43 (d, J = 2.0 Hz, 1H), 4.05 (q, J = 6.8, 7.2 Hz, 2H), 3.91 (s, 3H), 3.22 (q, J = 6.0, 7.6 Hz, 2H), 2.35 (t, J = 7.4 Hz, 2H), 1.75 (p, 7.2, 7.1, 7.2 Hz, 2H), 1.18 (t, 7.0 Hz, 3H). ^{13}C NMR data: δ 173.05, 160.31, 134.24, 128.21, 126.91, 107.72, 60.21, 38.41, 37.77, 31.46, 34.84, 14.54.

(5-{[(3-carboxypropyl)amino]carbonyl}-1-methyl-1*H*-pyrrol-3-yl)(hydroxy)oxoammonium (16). The nitro pyrrole **15c** (0.730 g, 2.6 mmol) was dissolved in ethanol (15 mL) in a 50 mL round bottom flask and a solution of NaOH (0.515 g, 13 mmol) in H_2O (2 mL) was added. This was allowed to reflux until the disappearance of **15c** was indicated by TLC (EtOAc). The solution was concentrated by rotary evaporation to remove the majority of the EtOH. The aqueous solution was cooled in an ice bath and then acidified with concentrated HCl until the final pH was approximately 1 when a precipitate fell out of solution. The mixture was allowed to cool in the freezer for one more hour and the yellow solid precipitate was filtered out and rinsed with ice-cold water and air-dried to give pure **16** (0.660 g, 98%): mp 248-250 °C. TLC ($CHCl_3$/MeOH) $R_f = 0.60$. ^{1}H NMR data: δ 8.45 (t, J = 5.3 Hz, 1H), 8.11 (d, J

= 2.0 Hz, 1H), 7.45 (d, 2.0 Hz), 3.91 (s, 3H), 3.22 (q, J = 6.0, 7.6 Hz, 2H), 2.35 (t, J = 7.4 Hz, 2H), 1.75 (p, 7.2, 7.1, 7.2 Hz, 2H). ^{13}C NMR data: δ 174.67, 160.31, 134.22, 128.21, 126.91, 107.74, 38.51, 37.79, 31.56, 24.86.

Methyl 1-methyl-4-{[(1-methyl-4-nitro-1*H*-pyrrol-2-yl)carbonyl]amino}-1H-pyrrole-2-carboxylate (17). Argon was bubbled through 50 mL EtOAc containing 2.5 mL TEA. Methyl 4-amino-1-methyl-1*H*-pyrrole-2-carboxylate, HCl (1.62 g, 0.0085 mol) was then added to the solution followed by the addition of the nitro compound **12** (2.34 g, 0.0085 mol) and the solution was allowed to stir under Ar for 24 hours. The pale yellow solid that was formed was filtered out, washed with DI H_2O, and dried under vacuum to yield the pale yellow solid **17** (2.41 g, 92%): mp 232-237 °C. TLC (2:1 EtOAc/Hexane) R_f = 0.34. ^{1}H NMR data: δ 10.26 (s, 1H), 8.18 (d, J = 1.4 Hz, 1H), 7.56 (d, J = 2.0 Hz, 1H), 7.46 (d, J = 2.0 Hz, 1H), 6.89 (d, J = 2.0 Hz, 1H), 3.96 (s, 3H), 3.85 (s, 3H), 3.75 (s, 3H). ^{13}C NMR data: δ 161.18, 157.39, 134.28, 128.72, 126.57, 122.62, 121.29, 119.33, 108.78, 108.09, 51.46, 37.88, 36.69.

1-methyl-4-{[(1-methyl-4-nitro-1*H*-pyrrol-2-yl)carbonyl]amino}-1*H*-pyrrole-2-carboxylic acid (18). The nitro ester **17** (7.00 g, 23 mmol) was suspended in EtOH (115 mL), and a solution of NaOH (2.8 g, 3 eq.) in H_2O (15 mL) was added. This suspension was allowed to reflux until the disappearance of **17** was indicated by TLC (EtOAc). The solution was concentrated by rotary evaporation to remove all the EtOH. The aqueous solution was cooled in an ice bath and then acidified with concentrated HCl until the final pH was approximately 1, which is when a precipitate fell out of solution. The mixture was allowed to cool in the refrigerator overnight, and the brown solid precipitate was filtered out and rinsed with ice-cold water and air dried to yield pure **18** (6.01g, 89%): mp 199-203 °C. TLC (EtOAc) R_f = 0.54. ^{1}H NMR data: δ 10.39 (s, 1H), 8.17 (d, J = 1.6 Hz, 1H), 7.66 (d, J = 1.6 Hz, 1H), 7.44 (d, J = 2.0 Hz,

1H), 6.90 (d, J = 1.6 Hz, 1H), 3.97 (s, 3H), 3.84 (s, 3H). ^{13}C NMR data: δ 162.31, 157.39, 134.29, 128.64, 126.65, 122.40, 120.88, 120.32, 108.93, 108.23, 37.89, 36.63.

[5-({[5-(ethoxycarbonyl)-1-methyl-1*H*-pyrrol-3-yl]amino}carbonyl)-1-methyl-1*H*-pyrrol-3-yl](hydroxy)oxoammonium (19). The nitro pyrrole ester **14** (2.0 g, 0.010 mol) was dissolved in ethanol (30 mL) in a 500 mL parr jar and Pd/C (0.500 g) was added to it. The mixture was shaken in a hydrogenator under pressurized hydrogen (75 psi) until the reaction was complete as indicated by TLC (EtOAc in 6 hours.). The mixture was then acidified with concentrated HCl (1 mL). The Pd/C was filtered out through celite, and the filtrate was concentrated by rotary evaporation and kept under vacuum overnight to yield yellow solid. Ethyl acetate dried over sieves (20 mL) was added to this solid in a 250 mL round bottom flask and the nitro pyrrole **12** (2.74 g, 0.010 mol) was added to the mixture. TEA (4.2 mL, 0.030 mol) dissolved in EtOAc (5 mL) was then added drop wise using a dropping funnel. This was allowed to stir for 48 hours during which time a yellow solid fell out of solution. After the reaction was complete, as indicated by the disappearance of the starting material by TLC (EtOAc), the yellow solid was vacuum filtered to yield pure **19** (2.2g, 68%): mp 163-167 °C. TLC (1:1 EtOAc/Hexane) R_f = 0.58. ^{1}H NMR data: δ 10.57 (s, 1H), 8.18 (d, J = 1.6 Hz, 1H), 7.75 (d, J = 2.0 Hz, 1H), 7.46 (d, J = 2.0 Hz, 1H), 7.01 (d, J = 1.6 Hz, 1H), 4.22 (q, J = 6.6, 7.0 Hz, 2H), 3.96 (s, 3H), 3.85 (s, 3H), 1.3 (t, J = 7.0 Hz, 3H). ^{13}C NMR data: 157.44, 134.29, 128.67, 126.57, 122.72, 121.25, 119.50, 109.02, 108.45, 59.87, 45.72, 37.93, 36.66, 14.74.

ethyl *N*-({4-[({4-[hydroxy(oxo)ammonio]-1-methyl-1*H*-pyrrol-2-yl}carbonyl)amino]-1-methyl-1*H*-pyrrol-2-yl}carbonyl)glycinate (20a). Compound **20a** was synthesized following a procedure similar to the one described above for **19** using 3.17g (0.012 mol) of pyrrole **15a** and 3.26 g (0.012 mol) of **12** to give **20a** (3.4 g, 73%): mp 170-172 °C. TLC

(1:1 EtOAc/Hexane) R_f = 0.28. ^{1}H NMR data: 10.29 (s, 1H), 8.48 (t, J = 1.8 Hz, 1H), 8.18 (d, J = 2.0 Hz, 1H), 7.59 (d, J = 1.6 Hz, 1H), 7.28 (d, J = 2.0 Hz, 1H), 6.93 (d, J = 2.0 Hz, 1H), 4.14 (q, J = 6.8, 7.2 Hz, 2H), 3.98 (s, 3H), 3.91 (d, J = 2.0 Hz, 2H), 3.82 (s, 3H), 1.21 (t, 6.8, 7.2 Hz, 3H). ^{13}C NMR data: δ 170.65, 161.84, 157.36, 134.24, 128.70, 126.72, 122.79, 121.97, 119.06, 108.07, 104.95, 60.82, 41.15, 37.94, 36.60, 14.58.

Ethyl *N*-[(1-methyl-4-{[(1-methyl-4-nitro-1*H*-pyrrol-2-yl)carbonyl]amino}-1*H*-pyrrol-2-yl)carbonyl]-β-alaninate (20b). Compound **20b** was synthesized following a procedure similar to the one described above for **20a** using 4.0 g (0.015 mol) of pyrrole **15b** and 3.60 g (0.015 mol) of **12** to give **20b** (4.73 g, 81%): mp 199-202 °C. TLC (EtOAc) R_f = 0.64. ^{1}H NMR data: δ 10.26 (s, 1H), 8.18 (d, J = 1.2 Hz, 1H), 8.06, (t, J = 5.6 Hz, 1H), 7.59 (d, J = 1.2 Hz, 1H), 7.23 (d, J = 1.6 Hz, 1H), 6.85 (d, J = 1.2 Hz, 1H), 4.10 (q, J = 6.8, 7.2 Hz, 2H), 3.96 (s, 3H), 3.82 (s, 3H), 3.42 (q, J = 7.2, 6.8 Hz, 2H), 2.56 (t, J = 7.0 Hz, 2H), 1.21 (t, J = 7.2 Hz, 3H). ^{13}C NMR data: δ 204.39, 171.83, 161.63, 157.30, 134.23, 128.66, 126.73, 123.41, 121.83, 118.56, 60.35, 37.93, 36.49, 35.28, 34.45, 14.53.

Ethyl 4-{[(1-methyl-4-{[(1-methyl-4-nitro-1*H*-pyrrol-2-yl)carbonyl]amino}-1*H*-pyrrol-2-yl)carbonyl}amino}butanoate (20c). Compound **20c** was synthesized following a procedure similar to the one described above for **20a** using 4.0 g (0.014 mol) of pyrrole **15c** and 3.8g (0.014 mol) of **12** to give **20c** (4.01 g, 71 %): mp 139-144 °C. TLC (EtOAc) R_f = 0.69. ^{1}H NMR data: δ 10.25 (s, 1H), 8.17 (d, J = 1.6 Hz, 1H), 8.09 (t, 1.8 Hz, 1H), 7.58 (d, J = 2.0 Hz, 1H), 7.21 (d, J = 1.6 Hz, 1H), 6.86 (d, J = 1.4 Hz, 1H), 4.07 (q, J = 6.4, 7.2 Hz, 2H), 3.95 (s, 3H), 3.81 (s, 3H), 3.20 (t, J = 1.8 Hz, 2H), 2.32 (t, J = 7.4 Hz, 2H), 1.76 (p, J = 6.8, 7.6, 7.2 Hz, 2H), 1.19 (t, J = 7.0 Hz, 3H). ^{13}C NMR data: δ 173.18, 161.64, 157.30, 134.23, 128.67, 126.75, 123.61, 121.78, 118.44, 107.99, 104.48, 60.22, 38.17, 37.93, 36.48, 31.49, 25.14, 14.56.

ethyl *N*-{[4-({[4-(acryloylamino)-1-methyl-1*H*-pyrrol-2-yl]carbonyl}amino)-1-methyl-1*H*-pyrrol-2-yl]carbonyl}glycinate (21a). Compound **20a** (2.0 g, 5.2 mmol) was dissolved in 15 mL absolute ethanol and 250 mg Pd/C was added in a Parr jar. The mixture was shaken under pressurized hydrogen (75 psi) until **20a** had disappeared as indicated by TLC (EtOAc). The Pd/C was filtered through celite, and the solution was rotary evaporated and the residue was kept under vacuum overnight. The flask containing the residue was flushed with Ar and the residue was dissolved in anhydrous THF (60 mL) and DIEA (2.02 mL, 12.2 mmol) was added. The solution was bubbled with argon and cooled to -40°C. Acryloyl chloride (0.421 mL, 5.2 mmol) was then added to the cooled solution drop wise. The reaction temperature was regulated between –40°C to –20 °C and protected from light. The reaction continued until it was complete, as indicated by TLC (6:1, $CHCl_3$: MeOH). The solution was concentrated by rotary evaporation re-dissolved in EtOAc and extracted with DI water (2x 250 mL). The organic layers were dried over MgSO4. The solution was concentrated by rotary evaporation until dry. This was placed under vacuum to yield the product as yellow foam **21a** (1.78 g, 86 %): mp 100-105°C. TLC (6:1 $CHCl_3$/MeOH) $R_f = 0.70$. ^{1}H NMR data: δ 10.12 (s, 1H), 9.95 (s, 1H), 8.42 (t, J = 5.5 Hz, 1H), 7.26 (d, J = 2.0 Hz, 1H), 7.25 (d, J = 2.0 Hz, 1H), 6.92 (d, J = 2.0 Hz, 1H), 6.91 (d, J = 1.6 Hz, 1H), 6.37 (m, 1H), 6.19 (dd, J = 2.0, 16.8 Hz, 1H), 5.67 (dd, J = 2.0, 8.0 Hz, 1H), 4.12 (q, J = 6.8, 7.2 Hz, 2H), 3.88 (d, J = 6.0 Hz, 2H), 3.83 (s, 3H), 3.78 (s, 3H), 1.21 (t, J = 6.8 Hz, 3H). ^{13}C NMR data: δ 170.68, 162.09, 161.93,158.79, 131.95, 123.44, 122.64, 122.52, 122.15, 118.92, 105.12, 104.48, 60.80, 41.14, 36.63, 36.51, 14.59.

ethyl *N*-{[4-({[4-(acryloylamino)-1-methyl-1*H*-pyrrol-2-yl]carbonyl}amino)-1-methyl-1*H*-pyrrol-2-yl]carbonyl}-β-alaninate (21b). Compound **21b** was synthesized following a procedure similar to the one described above for **21a** using 2.0 g (5.03 mmol) of **20b**,

0.407 mL (5.03 mmol) of acryloyl chloride and 1.88 mL (11.73 mmol) of DIEA to yield **21b** as a yellow foam (1.85 g, 89 %): mp 87-92 °C. TLC (6:1 $CHCl_3$/MeOH) $R_f = 0.69$. ^1H NMR data: δ 10.11 (s, 1H), 9.91 (s, 1H), 8.06 (t, J = 5.2, 5.6 Hz, 1H), 7.27 (d, J = 1.6 Hz, 1H), 7.19 (d, J = 2.0 Hz, 1H), 6.91 (d, J = 1.6 Hz, 1H), 6.84 (d, J = 1.6 Hz, 1H), 6.41 (m, 1H), 6.21 (dd, J = 2.0, 14.8 Hz, 1H), 5.68 (dd, J = 2.0, 8.0 Hz, 1H), 4.07 (q, J = 6.8, 7.2, 7.2 Hz, 2H), 3.84 (s, 3H), 3.79 (s, 3H), 3.42 (q, J = 6.2, 7.2, 6.8 Hz, 2H), 2.52 (t, J = 6.8, 7.2 Hz, 2H), 1.20 (t, J = 6.8, 7.2 Hz, 3H). ^{13}C NMR data: δ173.46, 172.32, 171.85, 162.10, 161.72, 158.75, 131.96, 126.02, 123.46, 123.17, 123.12, 122.49, 122.15, 118.90, 118.42, 104.72, 104.44, 36.64, 35.26, 34.27.

ethyl 4-({[4-({[4-(acryloylamino)-1-methyl-1*H*-pyrrol-2-yl]carbonyl}amino)-1-methyl-1*H*-pyrrol-2-yl]carbonyl}amino)butanoate (21c). Compound **21c** was synthesized following a procedure similar to the one described above for **21a** using 2.0 g (4.9 mmol) of **20a**, 0.396 mL (4.9 mmol) of acryloyl chloride and 1.9 mL (11.33 mmol) of DIEA to yield **21c** as a yellow foam (1.81 g, 87 %): mp 71-77 °C. TLC (6:1 $CHCl_3$/MeOH) $R_f = 0.68$. ^1H NMR data: δ 10.11 (s, 1H), 9.98 (s, 1H), 8.03 (t, J = 5.3 Hz, 1H), 7.26 (d, J = 2.0 Hz, 1H), 7.17 (d, J = 1.6 Hz, 1H), 6.91 (d, J = 2.0 Hz, 1H), 6.85 (d, J = 1.6 Hz, 1H), 6.35 (m, 1H), 6.19 (dd, J = 2.4, 17.2 Hz, 1H), 5.67 (dd, J = 2.0, 8.0 Hz, 1H), 4.05 (q, J = 7.2, 6.8 Hz, 2H), 3.83 (s, 3H), 3.78 (s, 3H), 3.17 (t, J = 1.8 Hz, 2H), 2.31 (t, J = 7.0 Hz, 2H), 1.72 (p, J = 6.8, 7.6, 7.2 Hz, 2H), 1.18 (t, J = 7.0 Hz, 3H). ^{13}C NMR data: δ 173.18, 162.08, 161.74, 158.74, 131.95, 126.05, 123.47, 123.33, 122.45, 122.14, 118.89, 118.31, 104.63, 104.40, 60.22, 38.16, 36.65, 36.42, 31.50, 25.17, 14.58.

***N*-{[4-({[4-(acryloylamino)-1-methyl-1*H*-pyrrol-2-yl]carbonyl}amino)-1-methyl-1*H*-pyrrol-2-yl]carbonyl}glycine (22a).** Compound **21a** (0.300 g, 0.747 mmol) was dissolved in acetone (4 mL). NaOH (0.120 g, 2.99 mmol) was dissolved in 1 mL of DI H_2O and added all at once to the reaction flask. After 6 hours no starting material was left as indicated by TLC (6:1

$CHCl_3$:MeOH). To the reaction flask was added concentrated HCl until a pH of 1. This material was stirred for 10 min. during which time a salt fell out of solution. The solvents were then evaporated, followed by the addition of cold DI (1 mL) to the reaction flask. Additional impurities were removed by stirring overnight in 50/50 mix of hexane/ether (50 mL/50 mL) to yield a yellow powder **22a,** which was obtained by vacuum filtration (0.231 g, 85%): mp 130-133 °C. TLC (5:2 $CHCl_3$/MeOH) R_f = 0.31. ^{1}H NMR data: δ 10.15 (s, 1H), 9.96 (s, 1H), 8.45 (t, J = 5.3 Hz, 1H), 7.28 (d, J = 2.0 Hz, 1H), 7.26 (d, J = 1.6 Hz, 1H), 6.94 (d, J = 2.2 Hz, 1H), 6.92 (d, J = 2.0 Hz, 1H), 6.42 (m, 1H), 6.21 (dd, J = 2.0, 16.8 Hz, 1H), 5.68 (dd, J = 2.2, 10.8 Hz, 1H), 3.92 (d, J = 6.0 Hz, 2H), 3.85 (s, 3H), 3.80 (s, 3H). ^{13}C NMR data: δ 172.15, 171.19, 162.12, 158.80, 131.95, 126.05, 123.44, 122.65, 122.46, 122.15, 118.94, 118.82, 105.19, 104.50, 52.12, 41.03, 36.63

***N*-{[4-({[4-(acryloylamino)-1-methyl-1*H*-pyrrol-2-yl]carbonyl}amino)-1-methyl-1*H*-pyrrol-2-yl]carbonyl}-β-alanine (22b).** Compound **22b** was synthesized similar to the one described above for **22a** using 0.500 g (1.2 mmol) of **21b**, 6.42 mL of Acetone, 0.192 g (4.8 mmol) NaOH, and 1.6 mL of DI water over a period of 30 min. to yield **22b** as a yellow solid (0.410 g, 88%): mp 108-111 °C. TLC (5:2 $CHCl_3$/MeOH) R_f = 0.33. ^{1}H NMR data: δ 10.15 (s, 1H), 9.19 (s, 1H), 8.04 (t, J = 5.4 Hz, 1H), 7.27 (d, J = 1.2 Hz, 1H), 7.19 (d, J = 2.2 Hz, 1H), 6.92 (d, J = 1.2 Hz, 1H), 6.84 (d, J = 2.0 Hz, 1H), 6.42 (m, 1H), 6.21 (dd, J = 2.0, 15.2 Hz, 1H), 5.68 (dd, J = 2.0, 8.0 Hz, 1H), 3.84 (s, 3H), 3.80 (s, 3H), 3.38 (q, J = 6.2, 7.2Hz, 2H), 2.47(t, J = 7.0 Hz, 2H). ^{13}C NMR data: δ 173.47, 162.10, 161.72, 158.75, 131.96, 126.03, 123.47, 123.17, 122.48, 122.15, 118.90, 118.43, 104.72, 104.44, 36.64, 36.44, 35.31, 34.49.

4-({[4-({[4-(acryloylamino)-1-methyl-1*H*-pyrrol-2-yl]carbonyl}amino)-1-methyl-1*H*-pyrrol-2-yl]carbonyl}amino)butanoic acid (22c). Compound **22c** was synthesized similar to

the one described above for **22a** using 0.400 g (0.932 mmol) of **21c**, 4.98 mL of Acetone, 0.149 g (3.7 mmol) NaOH, and 1.24 mL of DI water over a period of 90 minutes to yield **22c** as a yellow solid (0.308 g, 82 %): mp 71-76 °C. TLC (5:2 $CHCl_3$/MeOH) R_f = 0.36. ^{1}H NMR data: δ 10.24 (s, 1H), 9.93 (s, 1H), 8.05 (t, J = 5.5 Hz, 1H), 7.27 (d, J = 2.0 Hz, 1H), 7.18 (d, J = 1.6 Hz, 1H), 6.93 (d, J = 1.6 Hz, 1H), 6.85 (d, J = 1.6 Hz, 1H), 6.44 (m, 1H), 6.18 (dd, J = 2.0, 14.8 Hz, 1H), 5.65 (dd, J = 2.0, 8.0 Hz, 1H), 3.83 (s, 3H), 3.78 (s, 3H), 3.17 (t, J = 5.8 Hz, 2H), 2.25 (p, J = 3.6, 3.5, 3.8 Hz, 2H), 1.71 (t, J = 7.0, 2H). ^{13}C NMR data: δ 174.76, 162.09, 161.74, 158.74, 131.96, 126.02, 123.47, 123.38, 122.44, 122.14, 118.89, 118.30, 104.62, 104.42, 38.27, 36.64, 36.40, 31.61, 30.06, 25.21, 15.64.

4-(acryloylamino)-*N*-[5-({[2-({6-[(7ξ,8ξ,9ξ,14ξ,17ξ)-17-{[*tert*-butyl(dimethyl)silyl]oxy}-3-hydroxyestra-1,3,5(10)-trien-7-yl]hexyl}amino)-2-oxoethyl]amino}carbonyl)-1-methyl-1*H*-pyrrol-3-yl]-1-methyl-1*H*-pyrrole-2-carboxamide (23a). In a flask flushed with argon, **22a** (0.200 g, 0.536 mmol) was dissolved in 5 mL anhydrous DMF along with, EDCI (0.132 g, 1.5 eq.) DMAP (0.136 g, 2.5 eq.), HOBT (0.204 g, 3 eq.), $CuCl_2$ (0.006 g, 0.1 eq.). After stirring for 30 min. the amine **9** (0.216 g, 0..446 mmol) was added and the solution was allowed to stir at room temperature over a period of two days until the disappearance of starting material as indicated by TLC (6:1 $CHCl_3$/MeOH). The solution was diluted with 100 mL DCM and the organic solution was extracted with H_2O (2x, 100mL), saturated $NaHCO_3$ (2x, 150 mL), and 1 M HCl (2x, 150 mL). The organic layer was then dried over $MgSO_4$. The resulting solution was rotary evaporated until dry to yield the product, **23a** as dark yellow foam (0.278 g, 74 %): mp 152-158 °C. TLC (6:1 $CHCl_3$/MeOH) R_f = 0.64. ^{1}H NMR data: δ 10.13 (s, 1H), 9.94 (s, 1H), 8.99 (s, 1H) 8.15 (t, J = 1.8 Hz, 1H), 7.77 (t, J = 1.8 Hz, 1H), 7.25 (d, J = 1.6 Hz, 1H), 7.22 (d, J = 2.0 Hz, 1H), 7.03 (d, J = 2.0 Hz, 1H), 6.91

(d, J = 1.6 Hz, 1H), 6.87 (d, J = 2.0 Hz, 1H), 6.49 (d, J = 2.0 Hz, 1H), 6.40 (s, 1H), 6.37 (m, 1H), 6.18 (dd, J = 2.0, 15.2 Hz, 1H), 5.66 (dd, J = 2.0, 8.0 Hz, 1H), 3.83 (s, 3H), 3.78 (s, 3H). ^{13}C NMR data: 169.43, 162.77, 162.09, 161.81, 158.77, 155.45, 136.44, 131.98, 129.96, 127.14, 125.99, 123.45, 122.93, 122.58, 122.15, 118.89, 116.25, 113.32, 104.93, 104.49, 81.66, 45.86, 43.71, 42.18, 38.96, 38.21, 36.64, 36.51, 36.25, 34.57, 33.21, 31.23, 30.98, 29.65, 28.05, 27.43, 26.95, 26.21, 25.58, 22.48, 18.24, 11.80, -4.04.

4-(acryloylamino)-*N*-[5-({[3-({6-[(7ξ,8ξ,9ξ,14ξ,17ξ)-17-{[*tert*-butyl(dimethyl)silyl]oxy}-3-hydroxyestra-1,3,5(10)-trien-7-yl]hexyl}amino)-3-oxopropyl]amino}carbonyl)-1-methyl-1*H*-pyrrol-3-yl]-1-methyl-1*H*-pyrrole-2-carboxamide (23b). Compound **23b** was synthesized similar to the procedure described above for **23a** using 0.250 g (0.645 mmol) of **22b,** 5 mL anhydrous DMF along with, EDCI (0.191 g, 1.5 eq.) DMAP (0.197 g, 2.5 eq.), HOBT (0.296 g, 3 eq.), $CuCl_2$ (0.009 g, 0.1 eq.) and 0.314 g (0.645 mmol) of **9** to yield **23b** as yellow foam (0.421 g, 76 %): mp 139-144 °C. TLC (6:1 $CHCl_3$/MeOH) R_f = 0.62. ^{1}H NMR data: δ 10.10 (s, 1H), 9.89 (s, 1H), 8.97 (s, 1H), 7.98 (t, J = 1.8 Hz, 1H), 7.83 (t, J = 1.8 Hz, 1H), 7.26 (d, J = 2.0 Hz, 1H), 7.25 (d, J = 1.6 Hz, 1H), 7.18 (d, J = 1.6 Hz, 1H), 6.90 (d, J = 2.0 Hz, 1H), 6.79 (d, J = 2.0 Hz, 1H), 6.49 (d, J = 2.2 Hz, 1H), 6.40 (s, 1H), 6.39 (m, 1H), 6.19 (dd, J = 2.0, 15.2 Hz, 1H), 5.67 (dd, J = 2.0, 8.0 Hz, 1H), 3.82 (s, 3H), 3.78 (s, 3H). ^{13}C NMR data: δ 170.70, 162.07, 161.64, 158.71, 155.42, 136.46, 131.95, 129.99, 127.15, 126.01, 123.46, 123.23, 122.50, 122.14, 118.87, 118.36, 116.23, 113.30, 104.52, 104.41, 81.66, 45.87, 43.70, 42.16, 38.86, 38.19, 37.21, 36.64, 36.43, 35.97, 33.18, 30.97, 29.65, 29.61, 28.01, 27.44, 27.01, 26.21, 25.57, 22.77.

4-(acryloylamino)-*N*-[5-({[4-({6-[(7ξ,8ξ,9ξ,14ξ,17ξ)-17-{[*tert*-butyl(dimethyl)silyl]oxy}-3-hydroxyestra-1,3,5(10)-trien-7-yl]hexyl}amino)-4-

oxobutyl]amino}carbonyl)-1-methyl-1*H*-pyrrol-3-yl]-1-methyl-1*H*-pyrrole-2-carboxamide (23c). Compound **23c** was synthesized similar to the procedure described above for **23a** using 0.500 g (0.0012 mol) of **22c,** 5 mL anhydrous DMF along with, EDCI (0.332 g, 1.5 eq.) DMAP (0.342 g, 2.5 eq.), HOBT (0.513 g, 3 eq.), $CuCl_2$ (0.015 g, 0.1 eq.) and 0.504 g (0.645 mmol) of **9** to yield **23c** as yellow foam (0.421 g, 72 %): mp 122-126 °C. TLC (6:1 $CHCl_3$/MeOH) R_f = 0.60. ^{1}H NMR data: δ 10.10 (s, 1H), 9.88 (s, 1H), 8.97 (s, 1H), 8.10 (t, J = 1.8 Hz, 1H), 7.83 (t, J = 1.8 Hz, 1H), 7.25 (d, J = 1.2 Hz, 1H), 7.16 (d, J = 1.2 Hz, 1H), 7.08 (d, J = 1.6 Hz, 1H), 6.89 (d, J = 1.2 Hz, 1H), 6.83 (d, J = 1.2 Hz, 1H), 6.49 (d, J = 2.2 Hz, 1H), 6.40 (s, 1H), 6.41 (m, 1H), 6.23 (dd, J = 2.0, 15.2 Hz, 1H), 5.71 (dd, J = 2.0, 8.0 Hz, 1H), 3.81 (s, 3H), 3.77 (s, 3H). ^{13}C NMR data: δ 172.08, 170.82, 162.77, 162.09, 161.69, 158.74, 155.44, 136.45, 131.97, 129.98, 127.15, 126.02, 123.48, 123.40, 122.45, 122.15, 118.88, 118.28, 116.25, 113.32, 104.58, 104.43, 81.67, 45.88, 43.71, 42.18, 38.88, 38.67, 38.20, 37.22, 36.64, 36.42, 36.25, 34.57, 33.56, 33.20, 31.54, 31.23, 30.97, 29.64, 28.02, 27.44, 27.00, 26.22, 26.12.

3-{[5-({[5-({[2-({6-[(7ξ,8ξ,9ξ,14ξ,17ξ)-3,17-dihydroxyestra-1,3,5(10)-trien-7-yl]hexyl}amino)-2-oxoethyl]amino}carbonyl)-1-methyl-1*H*-pyrrol-3-yl]amino}carbonyl)-1-methyl-1*H*-pyrrol-3-yl]amino}-3-oxopropane-1-sulfonic acid (24a). To a solution of alkene **23a** (0.240 g, 0.285 mmol) dissolved in 16 mL of EtOH, $NaHSO_3$ (0.178 g, 1.7 mmol) dissolved in 4 mL DI H_2O was added all at once. The pH was adjusted to about 8 with 5 % NaOH and the mixture was refluxed until **23a** had disappeared and a spot appeared on the baseline on TLC (6:1 $CHCl_3$/MeOH). The solution was cooled in an ice bath and concentrated HCl was added until the pH was about 1, the solution was cooled further in the refrigerator overnight. A pale yellow precipitate fell out of solution, which was vacuum filtered to yield **24a** as a pale yellow solid (0.110 g, 77 %): mp 210-215 °C. TLC (5:2 $CHCl_3$/MeOH) R_f = 0.25. ^{1}H NMR data: δ 9.96 (s,

1H), 9.89 (s, 1H), 8.14 (t, J = 6.2 Hz, 1H), 7.77 (t, J = 6.2 Hz, 1H), 7.22 (d, J = 1.2 Hz, 1H), 7.13 (d, J = 1.2 Hz, 1H), 7.04 (d, J = 2.0 Hz, 1H), 6.87 (d, J = 1.2 Hz, 1H), 6.85 (d, J = 1.2 Hz, 1H), 6.49 (d, J = 2.0 Hz, 1H), 6.40 (s, 1H), 3.81 (s, 3H), 3.78 (s, 3H), 0.65 (s, 3H). ^{13}C NMR data: δ 169.46, 168.83, 161.83, 158.84, 155.42, 136.46, 130.09, 127.125, 123.07, 122.89, 122.65, 122.51, 118.59, 116.23, 113.32, 104.91, 104.39, 80.54, 48.00, 46.43, 43.42, 42.51, 42.20, 38.96, 38.23, 37.22, 36.54, 36.50, 34.59, 33.23, 32.82, 30.32, 29.68, 29.64, 28.05, 27.52, 26.96, 25.62, 22.74, 11.77.

3-{[5-({[5-({[3-({6-[(7ξ,8ξ,9ξ,14ξ,17ξ)-3,17-dihydroxyestra-1,3,5(10)-trien-7-yl]hexyl}amino)-3-oxopropyl]amino}carbonyl)-1-methyl-1*H*-pyrrol-3-yl]amino}carbonyl)-1-methyl-1*H*-pyrrol-3-yl]amino}-3-oxopropane-1-sulfonic acid (24b). Compound **24b** was synthesized similar to the procedure described above for **24a** using 0.300 g (0.350 mmol) of **23b** dissolved in 8 mL of EtOH, and 0.146 g (1.4 mmol) of $NaHSO_3$ in 2 mL H_2O. To yield a pale yellow precipitate that fell out of solution, which was vacuum filtered to yield **24b** as a pale yellow solid (0.238 g, 82 %): mp 208.212 °C. TLC (5:2 $CHCl_3$/MeOH) R_f = 0.23. ^{1}H NMR data: δ 9.96 (s, 1H), 9.84 (s, 1H), 7.97 (t, J = 6.4 Hz, 1H), 7.83 (t, J = 6.4 Hz, 1H), 7.18 (d, J = 1.2 Hz, 1H), 7.13 (d, J = 1.2 Hz, 1H), 7.07 (d, J = 2.2 Hz, 1H), 6.83 (d, J= 1.2 Hz, 1H), 6.78 (d, J = 1.2 Hz, 1H), 6.49 (d, J = 2.2 Hz, 1H), 6.40 (s, 1H), 3.79 (s, 3H), 3.77 (s, 3H), 0.64 (s, 3H). ^{13}C NMR data: δ 170.71, 168.90, 161.67, 158.80, 155.41, 136.49, 130.11, 127.13, 123.20, 123.08, 122.53, 118.55, 118.32, 116.24, 113.31, 104.34, 80.55, 48.03, 46.43, 43.42, 42.20, 38.87, 38.22, 37.22, 36.53, 36.41, 35.97, 34.60, 33.20, 32.91, 30.33, 29.60, 28.02, 27.53, 27.02, 25.59, 22.74.

3-{[5-({[5-({[4-({6-[(7ξ,8ξ,9ξ,14ξ,17ξ)-3,17-dihydroxyestra-1,3,5(10)-trien-7-yl]hexyl}amino)-4-oxobutyl]amino}carbonyl)-1-methyl-1*H*-pyrrol-3-yl]amino}carbonyl)-1-methyl-1*H*-pyrrol-3-yl]amino}-3-oxopropane-1-sulfonic acid (24c). Compound **24c** was

synthesized similar to the procedure described above for **24a** using 0.750 g (0.862 mmol) of **23c** dissolved in 24 mL of EtOH, and 0.146 g (5.1 mmol) of $NaHSO_3$ in 6 mL H_2O. To yield a pale yellow precipitate that fell out of solution, which was vacuum filtered to yield **24c** as a pale yellow solid (0.350 g, 80 %): mp 197-202 °C. TLC (5:2 $CHCl_3$/MeOH) R_f = 0.21. ^{1}H NMR data: δ 9.97 (s, 1H), 9.85 (s, 1H), 8.00 (t, J = 6.4 Hz, 1H), 7.80 (t, J = 6.4 Hz, 1H), 7.16 (d, J = 1.2 Hz, 1H), 7.13 (d, J = 1.2 Hz, 1H), 7.03 (d, J = 2.0 Hz, 1H), 6.83 (d, J = 1.2 Hz, 2H), 6.49 (d, J = 2.0 Hz, 1H), 6.40 (s, 1H), 3.79 (s, 3H), 3.77 (s, 3H), 0.64 (s, 3H). ^{13}C NMR data: δ 172.13, 168.74, 161.71, 158.81, 155.42, 136.45, 130.08, 127.10, 123.35, 123.10, 122.50, 118.55, 188.23, 116.23, 113.31, 104.58, 104.33, 80.55, 49.05, 47.99, 46.43, 43.41, 42.20, 40.99, 38.90, 38.65, 38.22, 37.21, 36.54, 36.40, 34.59, 33.53, 33.20, 32.73, 30.32, 29.62, 28.02, 27.52, 27.01, 26.12, 25.58, 22.74, 11.76.

1-methyl-4-{[(1-methyl-4-nitro-1*H*-pyrrol-2-yl)carbonyl]amino}-*N*-propyl-1*H*-pyrrole-2-carboxamide (25). To a solution of proplyamine (0.01 mL, 0.137 mmol), EDCI (0.041 g, 1.5 eq), DMAP (0.033 g, 2.5 eq), HOBt (0.63 g, 3 eq.), and $CuCl_2$ (0.002 g, 0.1 eq.) in dry DMF (5 mL), the carboxylic acid (**18**) (0.050 g, 0.137 mmol) was added. The solution was allowed to sir under Ar until **18** had disappeared as indicated by TLC (EtOAc). The solution was diluted with 60 mL EtOAc and washed with H_2O (2x, 20 mL), saturated $NaHCO_3$(2x, 50 mL), and 1M HCl (2x, 50 mL). The organic layer was dried over $MgSO_4$ and the resulting yellow solution was concentrated by rotary evaporation to give pure **25** (0.36 g, 65 %): mp 220-225 °C. TLC (EtOAc) R_f = 0.41. ^{1}H NMR data: δ 10.23 (s, 1H), 8.18 (d, J = 2.0 Hz, 1H), 8.05 (t, J = 5.0 Hz, 1H), 7.58 (d, J = 2.0 Hz, 1H), 7.20 (d, J = 1.6 Hz, 1H), 6.84 (d, J = 2.0 Hz, 1H), 3.95 (s, 3H), 3.80 (s, 3H), 3.13 (q, J = 7.2, 5.8 Hz, 2H), 1.50 (p, J = 7.8, 6.2, 5.2 Hz, 2H), 0.88 (t, J = 7.4 Hz,

3H). ^{13}C NMR data: δ 161.54, 157.28, 134.23, 128.67, 126.76, 123.81, 121.74, 118.31, 107.99, 104.35, 37.94, 36.46, 23.03, 11.90.

ethyl 4-({[4-({[4-(methylsulfonyl)-1-methyl-1*H*-pyrrol-2-yl]carbonyl}amino)-1-methyl-1*H*-pyrrol-2-yl]carbonyl}amino)butanoate (26) Compound **20c** (0.100 g, 0.266 mmol) was dissolved in 15 mL absolute ethanol and 250 mg Pd/C was added in a parr jar. The mixture was shaken under pressurized hydrogen (75 psi) until **20c** had disappeared as indicated by TLC (EtOAc). The Pd/C was filtered through celite, and the solution was rotary evaporated and the residue was kept under vacuum overnight. The 3-(methylsulfonyl) propanoic acid (0.048 g, 0.319 mmol) was dissolved in 10 mL anhydrous DMF along with, EDCI (0.078 g, 1.5 eq.) DMAP (0.081 g, 2.5 eq.), HOBT (0.121 g, 3 eq.), $CuCl_2$ (0.004 g, 0.1 eq.), After stirring for 30 min. the reduced compound **20c** was then added, and the solution was allowed to stir at room temperature over a period of three days until the disappearance of starting material as indicated by TLC (6:1 $CHCl_3$/MeOH). The solution was diluted with 100 mL DCM and the organic solution was extracted with H_2O (2x, 100mL), saturated $NaHCO_3$ (2x, 150 mL), and 1 M HCl (2x, 150 mL). The organic layer was then dried over $MgSO_4$. The resulting solution was rotary evaporated until dry to yield the product, **26** as an orange solid (0.101 g, 74%): mp °C. TLC (6:1 $CHCl_3$/MeOH) R_f = 0.64. ^{1}H NMR data: δ 10.06 (s, 1H), 9.87 (s, 1H), 8.03 (t, J = 6.2 Hz, 1H), 7.17 (d, J = 2.0, 1H), 7.16 (d, J = 1.6 Hz, 1H), 6.86 (d, J = 2.0 Hz, 1H), 6.85 (d, J = 2.0 Hz, 1H), 4.07 (q, J = 7.2, 6.8 Hz, 2H), 3.82 (s, 3H), 3.79 (s, 3H), 3.42 (t, J = 7.2 Hz, 2H), 3.18 (q, J = 7.2, 6.4 Hz, 2H), 3.01 (s, 3H), 2.51 (t, J = 7.0 Hz, 2H), 1.17 (t, J = 7.0 Hz, 2H), 1.19 (t, J = 7.0 Hz, 3H). 13C NMR data: δ 173.17, 166.53, 161.74, 158.74, 123.32, 123.28, 122.45, 122.13, 118.61, 118.30, 104.63, 104.27, 60.21, 50.20, 38.15, 36.61, 36.40, 31.50, 28.65, 25.16, 14.58.

CHAPTER 8: RESULTS AND DISCUSSION

This thesis describes the efforts to synthesize the bifunctional compounds shown below in Figure 8.1. The main goal was to synthesize compounds that could potentially produce N3-methyladenine adducts in estrogen receptor positive cells. The design of these compounds utilized the properties of both portions of the molecule; the ability to bind to the ER, and to produce N3-methyladenine adducts.

Figure 8.2 illustrates the synthetic strategy that was developed in order to synthesize these compounds in a modular fashion. This is achieved by synthesizing the cell-targeting ligand and the DNA-recognizing ligand separately. The two would then be combined by a suitable linker.

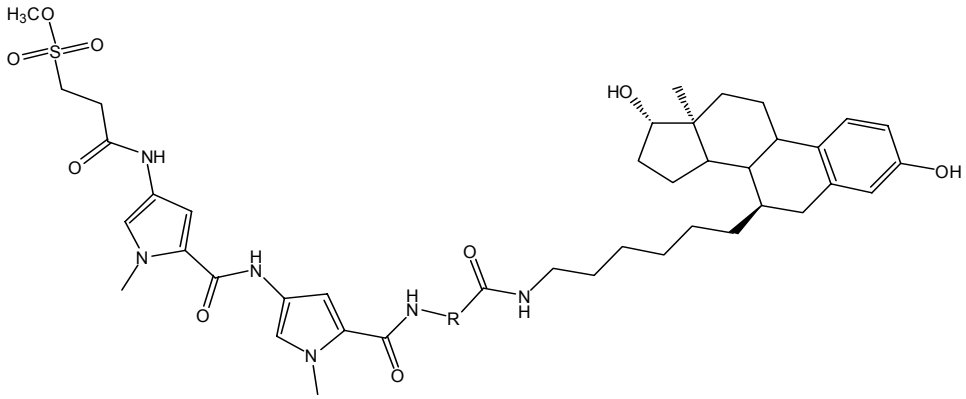


Figure 8.1: Structure of bifunctional compounds capable of producing N3-methyladenine adducts in ER positive cells.

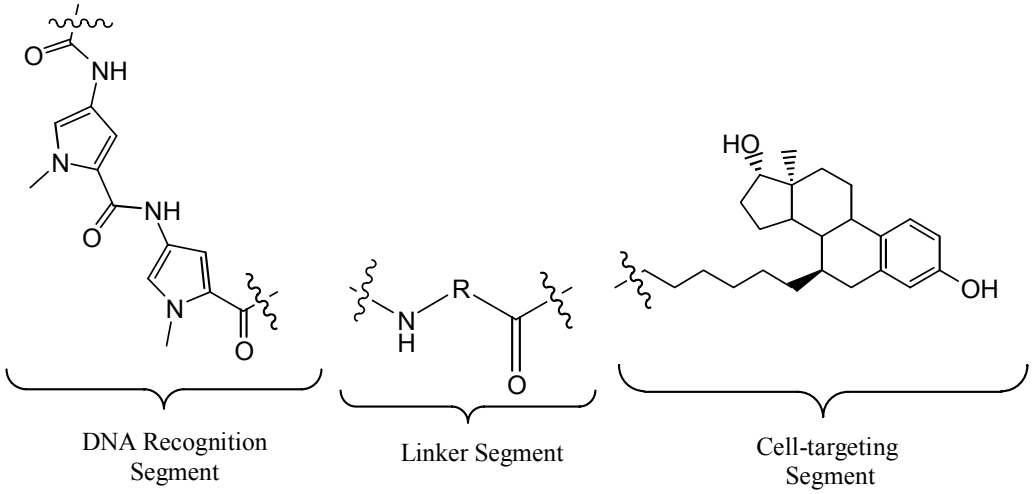


Figure 8.2: Synthetic strategy used to prepare compounds that can target estrogen receptor positive cells causing N3-methyladenine adducts in those cells

The linker that connects these two segments must be modified in such a way that estradiol would still be able to bind to the ER, and the DNA-methylating portion would still be able to produce N3-methyladenine adducts. Three compounds containing the DNA-recognizing functionality and the cell-targeting functionality were synthesized with varying linker lengths, methyl, ethyl or propyl. Having synthesized compound **24,** the final step that remains is the addition of the reactive methyl sulfonate to form **27**.

The cell-targeting unit was synthesized following published procedures, many of which were air and moisture sensitive. Compound **9** was synthesized in a 6% yield, with low reported yields. Occasionally, during the workup of the final step of this sequence, the protecting group was removed from the phenolic OH. It was determined that this was of no consequence to subsequent reactions. Due to time and resource expense, compound **9** was commercially obtained from Quality Chemical Laboratories.

The synthesis of the DNA-methylating unit was synthesized from N-methyl pyrrole following published procedures with minor modifications. Compounds **22a, 22b,** and **22c** were synthesized in 32%, 43%, and 32% yields respectively.

Initially, attempts were made to synthesize the compound shown below in Figure 8.3, which would allow for the addition of linkers at a later stage of the synthesis. However, as Scheme 8.1 illustrates, it was proposed that Michael addition across the alkene had occurred in addition to hydrolysis of the linker ester, so this methodology was abandoned.

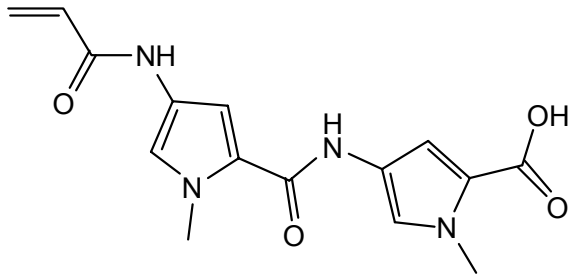
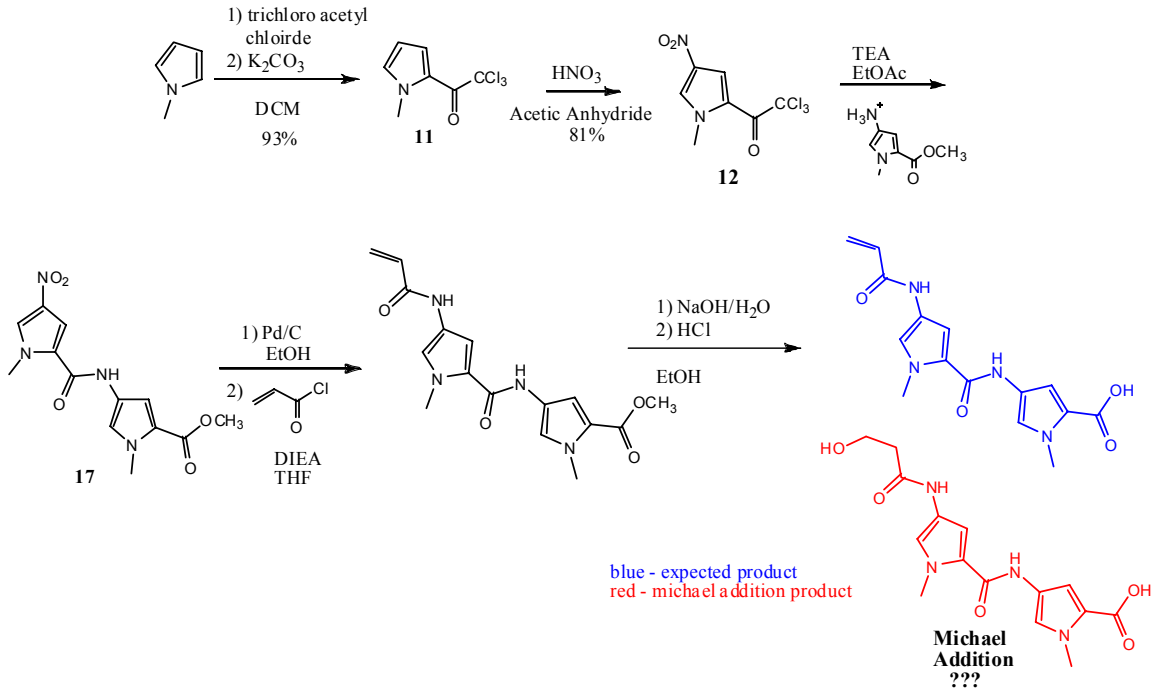
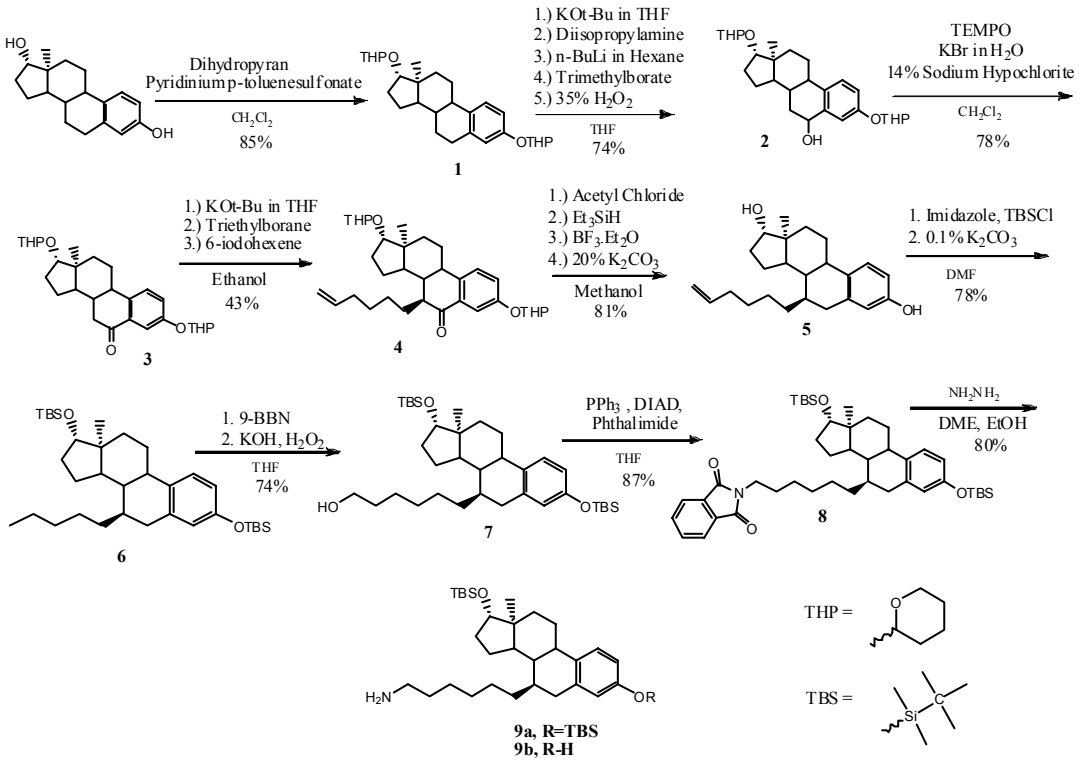


Figure 8.3: compound to be synthesized for easy addition of linkers

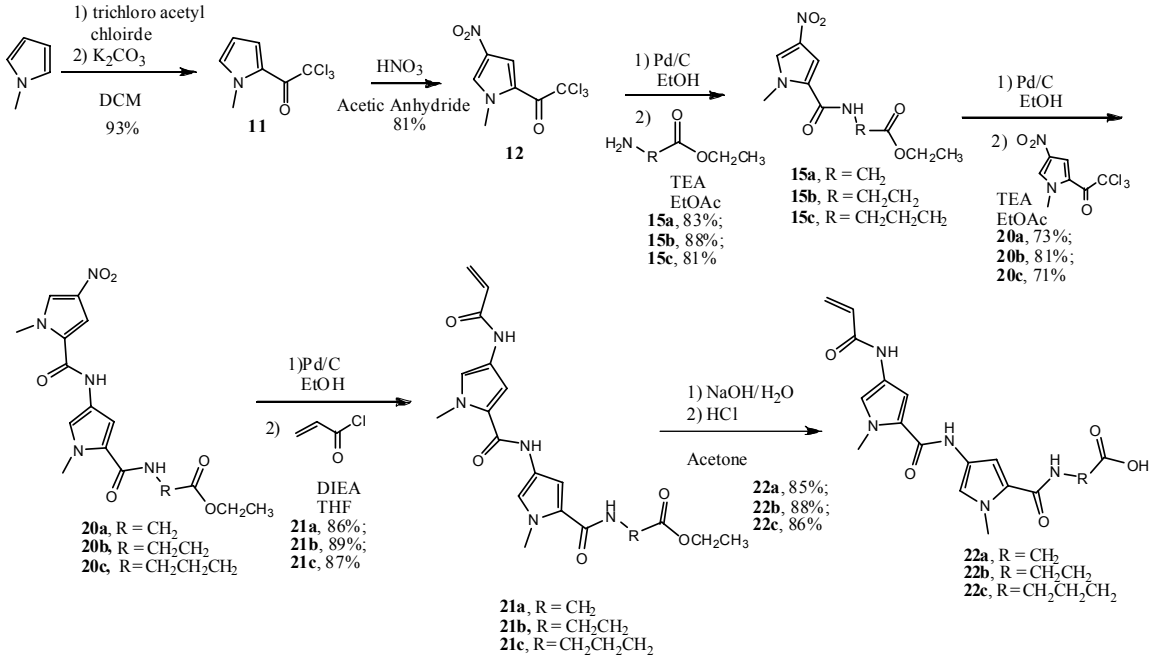


Scheme 8.1

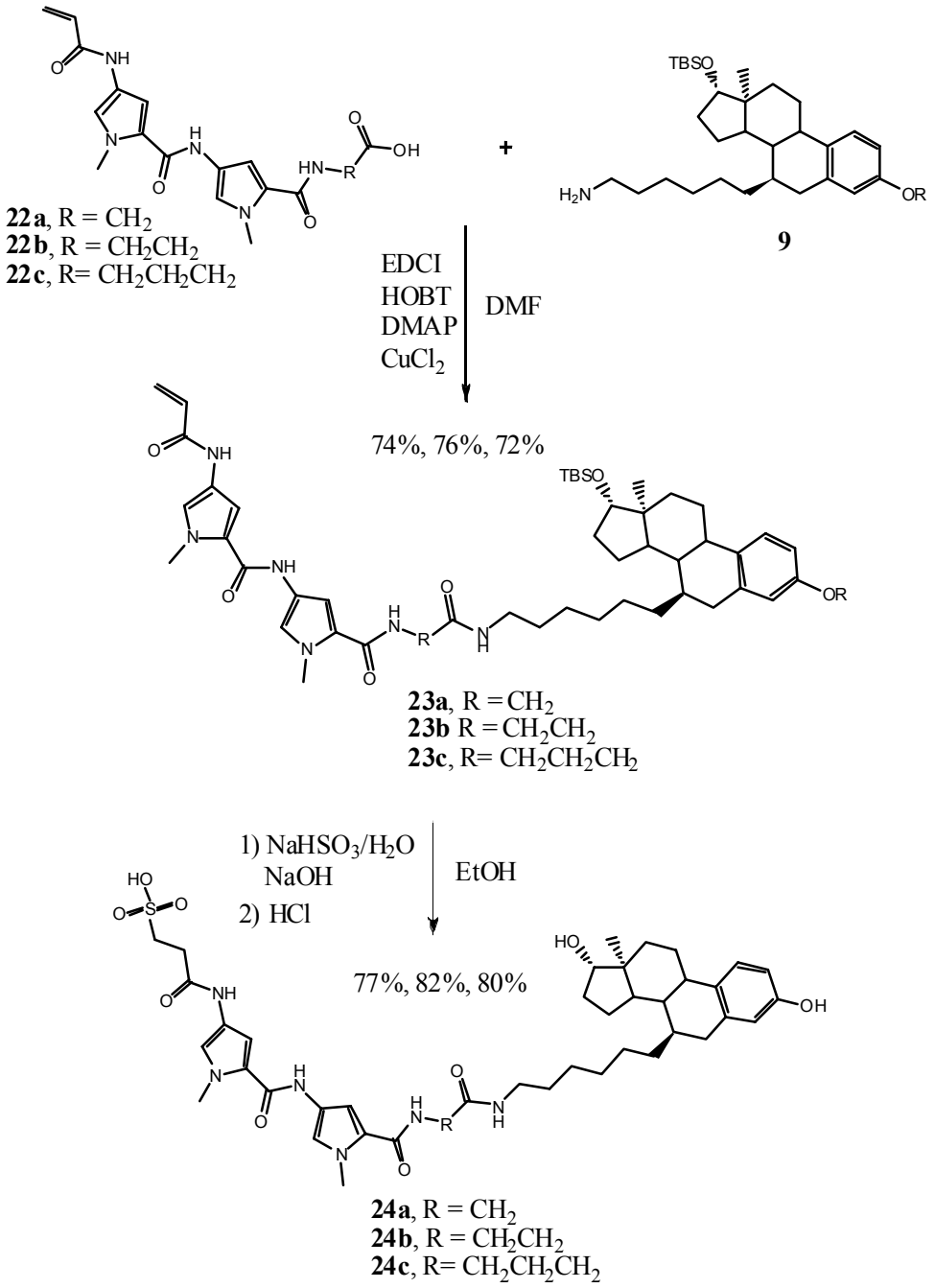
The new methodology employed utilized the addition of the linker earlier in the synthesis; this did not pose a problem since the addition of the linker and reactions thereafter result in high yields. The overall synthesis employed for these compounds is illustrated below in Scheme 8.2a, 8.2b, and 8.2c.


Dihydropyran
Pyridinium p-toluenesulfonate
CH2Cl2
85%
1.) KOt-Bu in THF
2.) Diisopropylamine
3.) n-BuLi in Hexane
4.) Trimethylborate
5.) 35% H2O2
THF
74%
TEMPO
KBr in H2O
14% Sodium Hypochlorite
CH2Cl2
78%
1.) KOt-Bu in THF
2.) Triethylborane
3.) 6-iodohexene
Ethanol
43%
1.) Acetyl Chloride
2.) Et3SiH
3.) BF3.Et2O
4.) 20% K2CO3
Methanol
81%
1. Imidazole, TBSCl
2. 0.1% K2CO3
DMF
78%
1. 9-BBN
2. KOH, H2O2
THF
74%
PPh3 , DIAD,
Phthalimide
THF
87%
NH2NH2
DME, EtOH
80%
THP =
TBS =
9a, R=TBS
9b, R-H

Scheme 8.2a

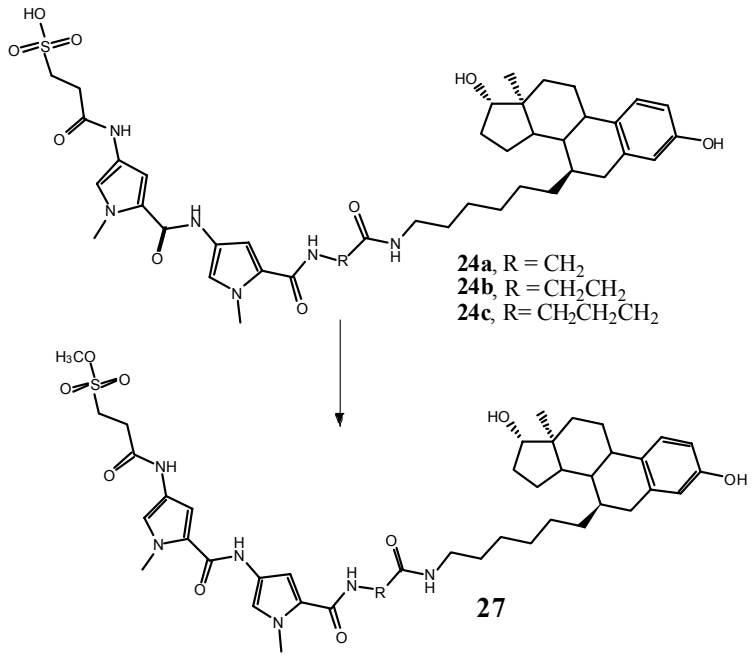

1) trichloro acetyl chloride
2) K2CO3
DCM
93%
11
HNO3
Acetic Anhydride
81%
12
1) Pd/C
EtOH
2)
TEA
EtOAc
15a, 83%;
15b, 88%;
15c, 81%
15a, R = CH2
15b, R = CH2CH2
15c, R = CH2CH2CH2
1) Pd/C
EtOH
2)
TEA
EtOAc
20a, 73%;
20b, 81%;
20c, 71%
20a, R = CH2
20b, R = CH2CH2
20c, R = CH2CH2CH2
1)Pd/C
EtOH
2)
DIEA
THF
21a, 86%;
21b, 89%;
21c, 87%
21a, R = CH2
21b, R = CH2CH2
21c, R = CH2CH2CH2
1) NaOH/H2O
2) HCl
Acetone
22a, 85%;
22b, 88%;
22c, 86%
22a, R = CH2
22b, R = CH2CH2
22c, R = CH2CH2CH2

Scheme 8.2b


22a, R = CH2
22b, R = CH2CH2
22c, R= CH2CH2CH2
9
EDCI
HOBT
DMAP
CuCl2
DMF
74%, 76%, 72%
23a, R = CH2
23b R = CH2CH2
23c, R= CH2CH2CH2
1) NaHSO3/H2O
NaOH
2) HCl
EtOH
77%, 82%, 80%
24a, R = CH2
24b, R = CH2CH2
24c, R= CH2CH2CH2

Scheme 8.2c

Scheme 8.3 illustrates the conversion of the sulfonic acid to the final methyl sulfonate. Several attempts to synthesized **27** were done using different methylating agents such as, 3-methyl-p-tolyltriazene, trimethylorthoformate, trimethylorthoacetate, and iodomethane.



Scheme 8.3

While these attempts were unsuccessful, other options exist. For example, diazomethane, which has been reported in literature for methylating sulfonic acids, could be employed. A potential complication with this method is the possibility of methylating the OH groups of estradiol. Literature reports suggest that in order for phenolic or alcoholic OH groups to react under diazomethane conditions, a catalyst must be present.[36-38] Therefore; this method should not result in the methylation of the OH groups of estradiol. Another potential complication with this method involves the solubility of the molecules. Diazomethane is prepared in either ether or DCM, both of which are non-polar, **24** is a polar compound that is readily soluble in methanol, not ether or DCM. Methanol could be used, except that the desired methyl sulfonate is reactive,

and will methylate the methanol to form dimethyl ether. This would eliminate methanol as a potential solvent. A way to avoid this is to use trifluoro methanol, the fluorine renders the oxygen of methanol less nucleophillic, slowing the reaction of the methyl sulfonate with methanol.

Once the final conversion to the methyl sulfonate is achieved, these compounds can be reacted with genomic DNA to see if N3-methyladenine is formed, and whether the length of the tether affects the compounds ability to methylate DNA. Also, by obtaining the ER commercially, these compounds can be tested for their ability to bind to the ER. Once compounds are identified that posses both of these qualities the compounds will be tested in ER positive and ER negative cells to see if there is selective cytotoxicity for cells over-expressing the estrogen receptor.

.

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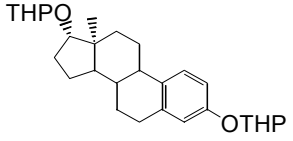
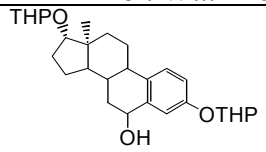
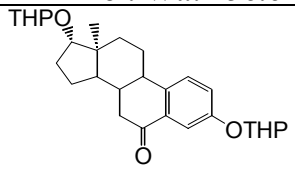
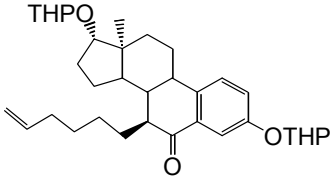
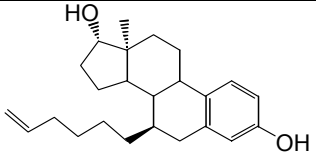
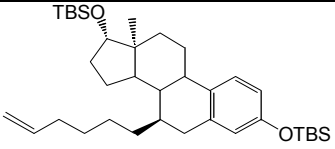
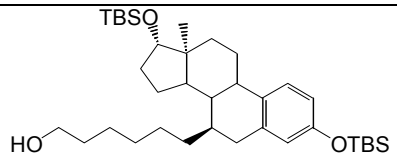
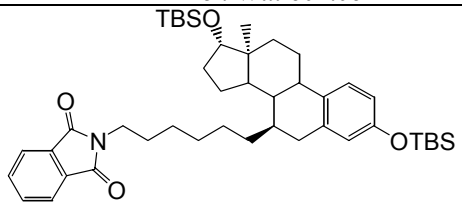
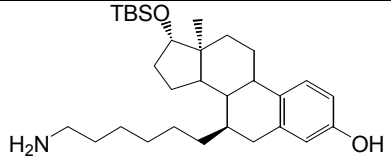
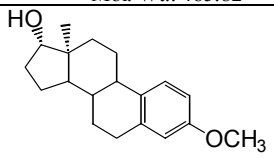
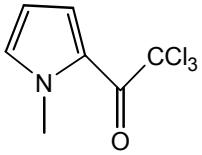
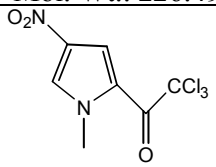
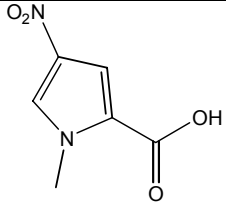
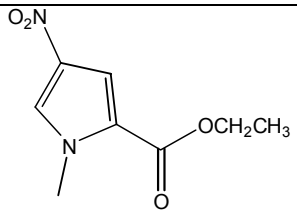
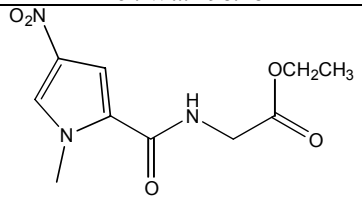
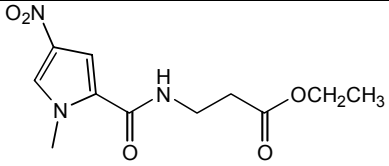
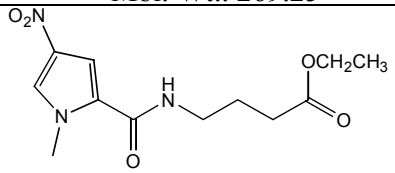
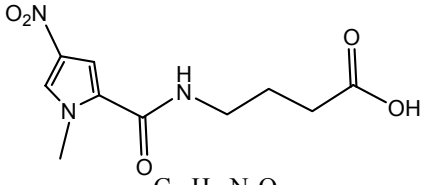
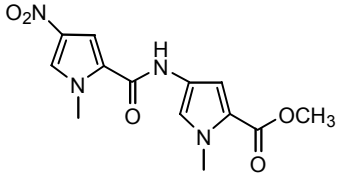
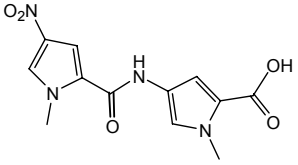
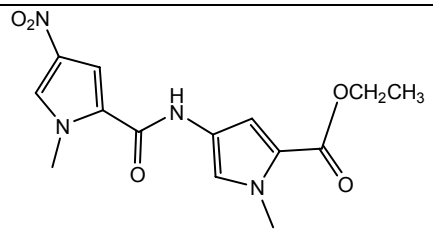
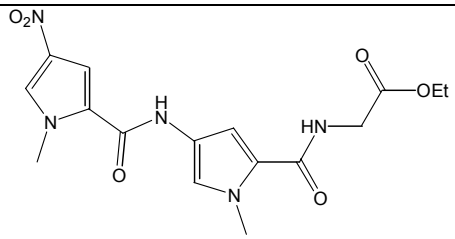
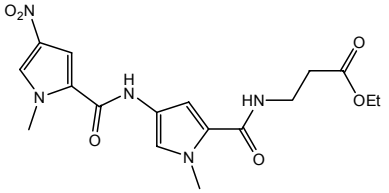
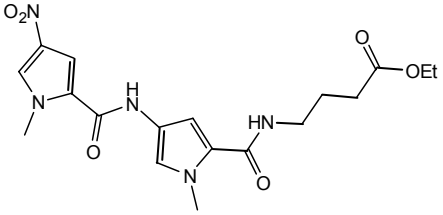
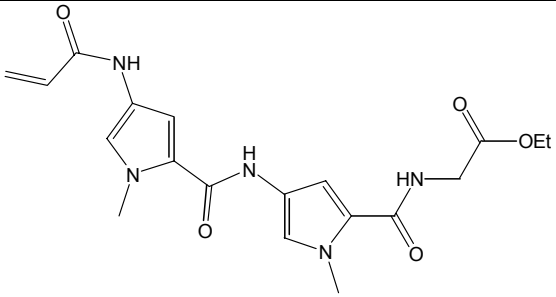
APPENDIX

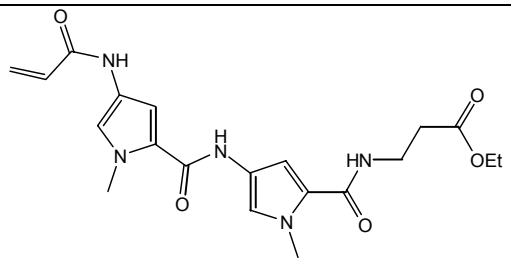
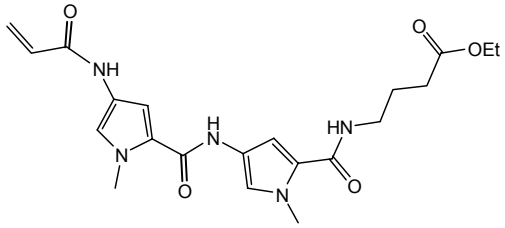
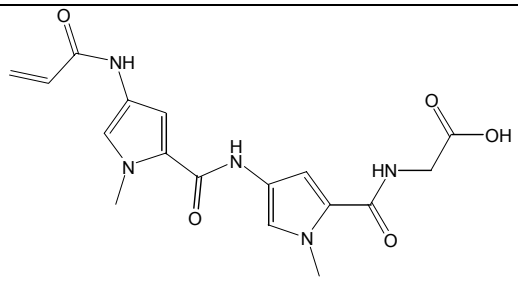
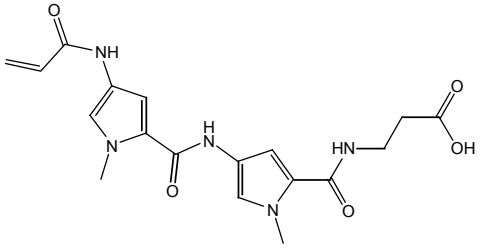
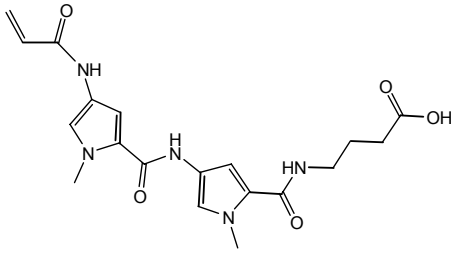
Appendix A. Number, structure and yields of synthesized compounds.

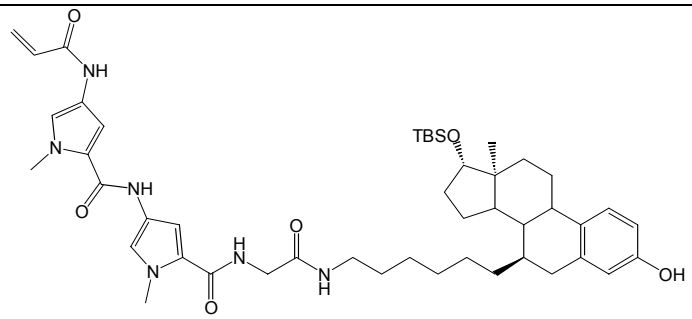
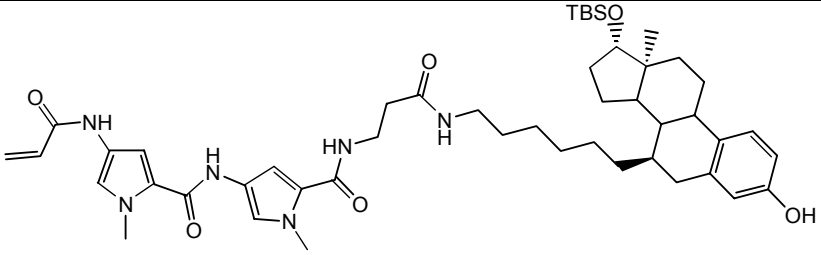
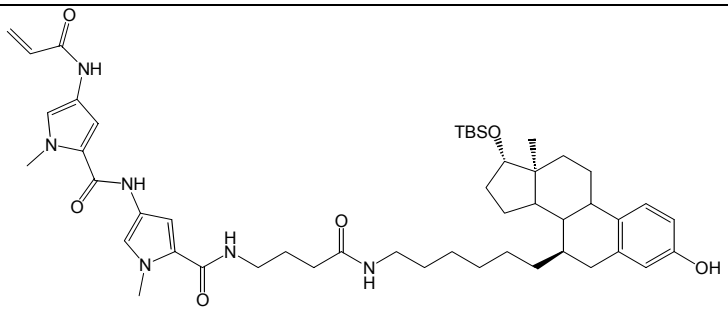
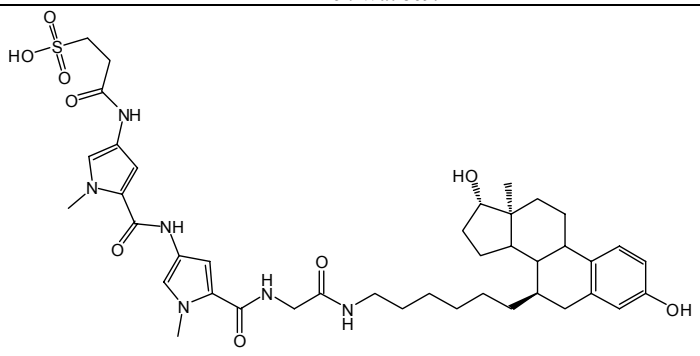
Number	Compound	Yield
1	 $C_{28}H_{40}O_4$ Mol. Wt.: 440.61	85%
2	 $C_{28}H_{40}O_5$ Mol. Wt.: 456.61	74%
3	 $C_{28}H_{38}O_5$ Mol. Wt.: 454.60	78%
4	 $C_{34}H_{48}O_5$ Mol. Wt.: 536.74	43%
5	 $C_{24}H_{34}O_2$ Mol. Wt.: 354.53	81%
6	 $C_{36}H_{62}O_2Si_2$ Mol. Wt.: 583.05	78%

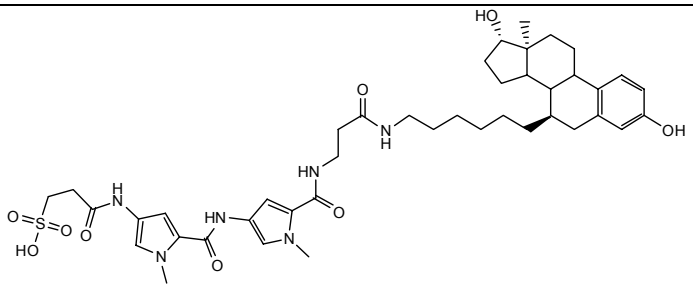
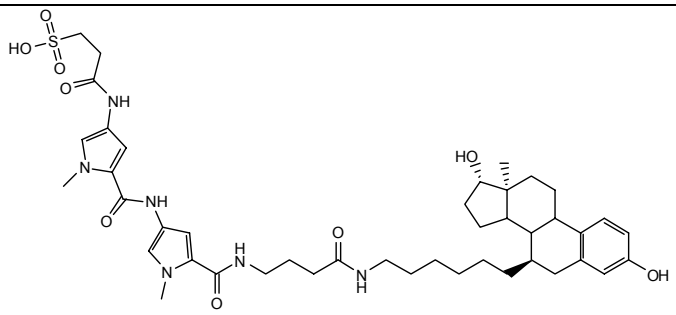
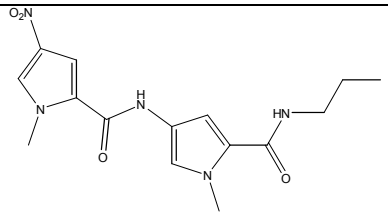
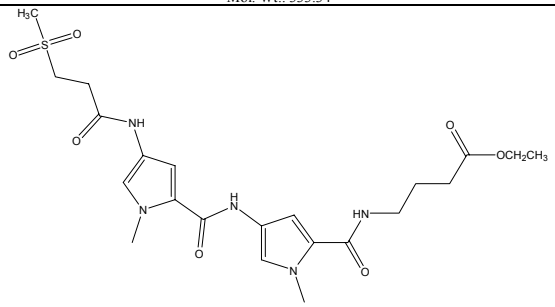
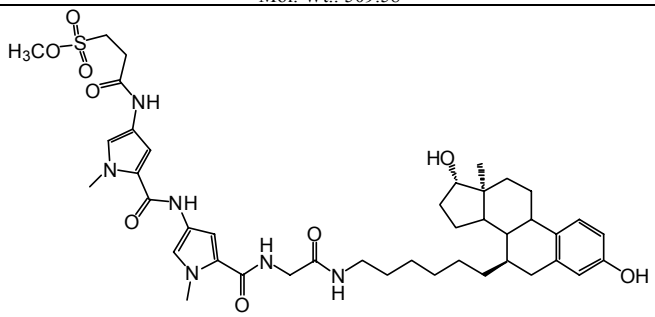
7	 $C_{36}H_{64}O_3Si_2$ Mol. Wt.: 601.06	74%
8	 $C_{44}H_{67}NO_4Si_2$ Mol. Wt.: 730.18	87%
9	 $C_{30}H_{51}NO_2Si$ Mol. Wt.: 485.82	80%
10	 $C_{19}H_{26}O_2$ Mol. Wt.: 286.41	89%
11	 $C_7H_6Cl_3NO$ Mol. Wt.: 226.49	93%
12	 $C_7H_5Cl_3N_2O_3$ Mol. Wt.: 271.48	81%
13	 $C_6H_6N_2O_4$ Mol. Wt.: 170.12	98%

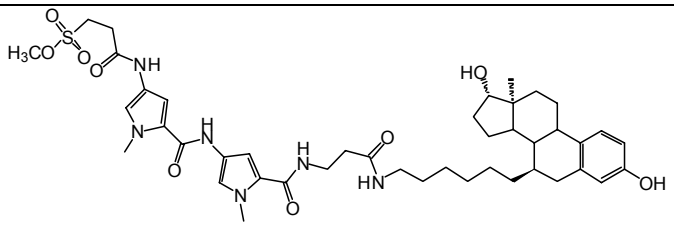
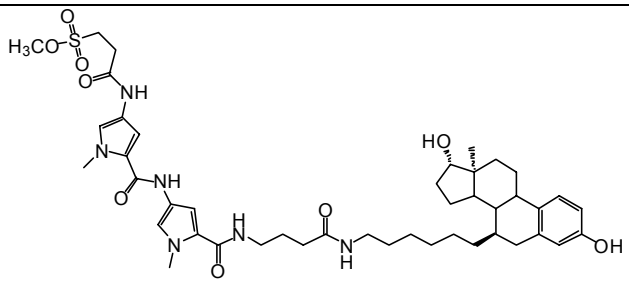
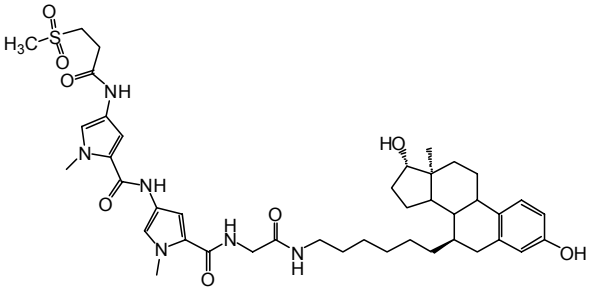
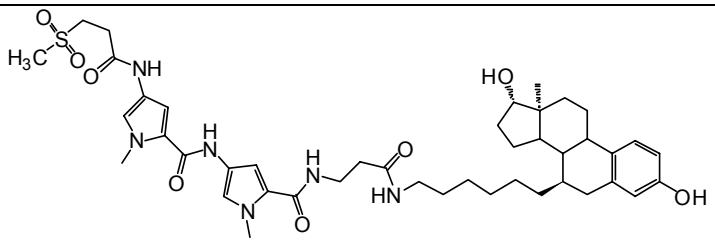
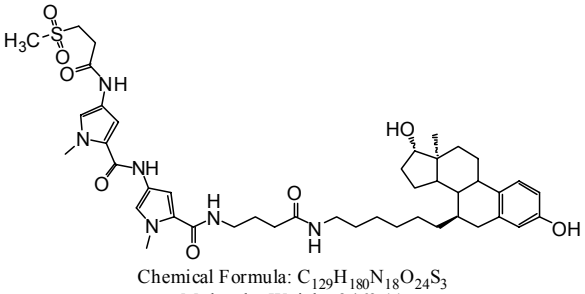
14	 $C_8H_{10}N_2O_4$ Mol. Wt.: 198.18	80%
15a	 $C_{10}H_{13}N_3O_5$ Mol. Wt.: 255.23	83%
15b	 $C_{11}H_{15}N_3O_5$ Mol. Wt.: 269.25	88%
15c	 $C_{12}H_{17}N_3O_5$ Mol. Wt.: 283.28	81%
16	 $C_{10}H_{13}N_3O_5$ Mol. Wt.: 255.23	98%
17	 $C_{13}H_{14}N_4O_5$ Mol. Wt.: 306.27	92%
18	 $C_{12}H_{12}N_4O_5$ Mol. Wt.: 292.25	99%

19	 $C_{14}H_{16}N_4O_5$ Mol. Wt.: 320.3	68%
20a	 $C_{16}H_{19}N_5O_6$ Mol. Wt.: 377.35	73%
20b	 $C_{17}H_{21}N_5O_6$ Mol. Wt.: 391.38	81%
20c	 $C_{18}H_{23}N_5O_6$ Mol. Wt.: 405.41	71%
21a	 $C_{19}H_{23}N_5O_5$ Mol. Wt.: 401.42	86%

21b	 $C_{20}H_{25}N_5O_5$ Mol. Wt.: 415.44	89%
21c	 $C_{21}H_{27}N_5O_5$ Mol. Wt.: 429.47	87%
22a	 $C_{17}H_{19}N_5O_5$ Mol. Wt.: 373.36	85%
22b	 $C_{18}H_{21}N_5O_5$ Mol. Wt.: 387.39	88%
22c	 $C_{19}H_{23}N_5O_5$ Mol. Wt.: 401.42	86%

23a	 $C_{47}H_{68}N_6O_6Si^{\bullet}$ Mol. Wt.: 841.17	74%
23b	 $C_{48}H_{70}N_6O_6Si^{\bullet}$ Mol. Wt.: 855.19	76%
23c	 $C_{49}H_{72}N_6O_6Si^{\bullet}$ Mol. Wt.: 869.22	72%
24a	 $C_{41}H_{56}N_6O_9S$ Mol. Wt.: 808.98	77%

24b	 $C_{42}H_{58}N_6O_9S$ Mol. Wt.: 823.01	82%
24c	 $C_{43}H_{60}N_6O_9S$ Mol. Wt.: 837.04	80%
25	 $C_{15}H_{19}N_5O_4$ Mol. Wt.: 333.34	65 %
26	 $C_{22}H_{31}N_5O_7S$ Mol. Wt.: 509.58	74%
27a	 Chemical Formula: $C_{42}H_{58}N_6O_9S$ Molecular Weight: 823.01	Not yet synthesized

27b	 Chemical Formula: $C_{43}H_{60}N_6O_9S$ Molecular Weight: 837.04	Not yet synthesized
27c	 Chemical Formula: $C_{129}H_{180}N_{18}O_{25}S_3$ Molecular Weight: 2479.11	Not yet synthesized
28a	 Chemical Formula: $C_{42}H_{58}N_6O_8S$ Molecular Weight: 807.01	Not yet synthesized
28b	 Chemical Formula: $C_{43}H_{60}N_6O_8S$ Molecular Weight: 821.04	Not yet synthesized
28c	 Chemical Formula: $C_{129}H_{180}N_{18}O_{24}S_3$ Molecular Weight: 2463.11	Not yet synthesized

Appendix B. List of Abbreviations

3-MeA – 3-methyladenine

3-MeG – 3-methylguanine

6-MeG – 6-methylguanine

A – adenine

A/T – adenine-thymine

C-cytosine

$CDCl_3$ - Deuterated Chloroform

DCM – dichloromethane

DIEA – diisopropylethylamine

DNA – deoxyribose nucleic acid

DMAP – 4-dimethylaminopyridine

DMF – dimethylformamide

DMSO – methyl sulfoxide

EDCI – 1-(3-dimethylaminopropyl)-3-ethylcarbodiimidehydrochloride

ER – estrogen receptor

EtOAc – ethyl acetate

EtOH – ethanol

G – guanine

HCl – hydrochloric acid

HOBT – hydroxybenotriazole

KOt-Bu – potassium tert-butoxide

Me-Lex – methyl 3-(1-methyl-5-(1-methyl-5-(propylcarbmoyl)-1H-pyrrol-3-ylcarbamoyl)-1H-pyrrol-3-ylamino)-3-oxopropane-1-sulfonate

MeOH – methanol

mp – melting point

NaCl – sodium chloride

NaOH – sodium hydroxide

NMR – muclear magnetic resonance

Pd/C – palladium on carbon

R_f – retention factor

TBS – tertbutyl dimethylsilyl

TEA – triethylamine

THF – tetrahydrofuran

THP - tetrahydropyranyl

TLC – thin layer chromatography

T – thymine

UV – ultra violet