Synthesis and Biological Evaluation of Prodigiosene Analogues

by

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The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled "Synthesis and Biological Evaluation of Prodigiosene Analogues" by Cassandra Hawco in partial fulfilment of the requirements for the degree of Master of Science.

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DEDICATION PAGE

I dedicate this work to my grandfather, who remains a constant influence in my life and my inspiration for completing this program.

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ABSTRACT

Prodigiosenes are a family of tripyrrolic 4-methoxypyrrolyl-dipyromethane natural products based on the parent compound, prodigiosin. Prodigiosin exhibits significant anti-cancer activity including some ability to specifically target breast cancer cells. By appending targeting moieties to these compounds with known selectivity for carcinomas, the prodigiosene selectivity should be enhanced and the corresponding residual toxicities lowered. Targeting groups chosen for use in this study include estrogens and anti-estrogens, porphyrins and glucose. Following known synthetic strategies, prodigiosenes with appended targeting moieties were synthesized, via an ester or amide linker. MTT assays using human breast cancer cell lines MCF-7 and MDA-MB231 were performed for each of the conjugates and the results demonstrate promise for the targeting strategy. A cell viability screen was also performed on a prodigiosene-estrone conjugate against the NCI60 cell line and demonstrated differential activity for the compound.

LIST OF ABBREVIATIONS USED

4-OHT	4-hydroxy Tamoxifen
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DMAP	Dimethylamino pyridine
DME	Dimethylethane
DMSO	Dimethyl sulfoxide
dsDNA	Double strand DNA
DTP	Developmental Therapeutics Program
E2	Estradiol
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
eq	equivalent
ER	Estrogen receptor
ESI	Electrospray ionization
EtOAc	Ethyl acetate
FDA	Food and Drug Administration
GI50	50% inhibition of cell growth
HBTU	*o*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*″-tetramethyluronium hexafluorophosphate
HR-MS	High resolution mass spectrometry
HRT	Hormone replacement therapy
Hz	Hertz
LC50	Lethal concentration for 50% cells
LR-MS	Low resolution mass spectrometry
M	Molar
MAPK	Mitogn-activated protein kinase
MDR	Multidrug resistance
MHz	MegaHertz
mol	Mole
MS	Mass spectrometry
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NCI	National Cancer Institute
NMR	Nuclear magnetic resonance
PDT	Photodynamic therapy
SAR	Structure activity relationships
SERM	Selective estrogen receptor modulator
TAM	Tamoxifen
TGI50	Total growth inhibition 50%
THF	Tetrahydrofuran

TLC	Thin layer chromatography
TEOF	Triethyl orthoformate
TMOF	Trimethyl orthoformate
TMS	Trimethylsilyl
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
TNF	Tumor necrosis factor
TPP	5,10,15,20-tetraphenyl-21*H*,23*H*-porphyrin
V-ATPase	Vacuolar-type H^+-ATPase

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CHAPTER 1: INTRODUCTION

1.1 PRODIGIOSENES

Prodigiosin is the parent member of a class of tripyrrolic natural compounds based on a common 4-methoxypyrrolyldipyrrin core unit, known as prodigiosenes, which exhibits a characteristic bright red color.[1] The name prodigiosin, was derived due to the association of prodigiosin with various historical events.[2] Colonies of the prodigiosin-producing bacteria were thought to resemble droplets of blood due to the bright red color of the pigment.[2] There are many reports throughout history of the "bleeding" of bread and other foods. A very early example of this dates back to 322 B.C. during Alexander the Great's siege of Tyre, when Macedonian soldiers noticed what appeared to be blood inside a piece of bread. The royal seers took this as an omen and as a way to justify the real bloodshed which the soldiers inflicted upon the inhabitants of Tyre.[1] The most famous historical example of the presence of prodigiosins is referred to the Miracle of Bolsena, which occurred in 1263 in the Church of Saint Christina near Bolsena, a small town north of Rome. A priest, struggling to maintain his faith, discovered "blood" dripping from the host while celebrating mass during a pilgrimage to Rome. Pope Urban IV approved this miracle and this is now commemorated as the festival of Corpus Christi, an integral part of the Christian calendar and still a holiday in many Western European countries.[1] The linking of these and other historical events viewed by many to be miraculous (prodigious) with the presence of the red pigment, lead to the pigment being named prodigiosin.[2] However, it was not until the 1960s that the correct chemical constitution of prodigiosin was established.[1]

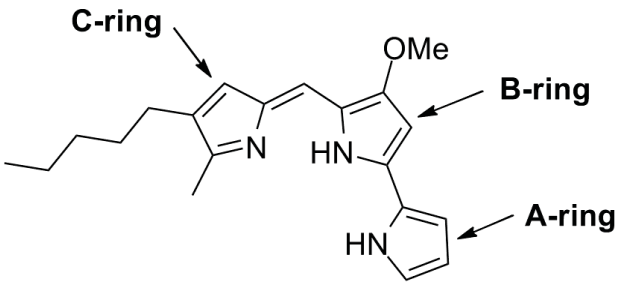


Figure 1. Prodigiosin

While the "miraculous" events are now explained due to advances in microbiology and chemistry, prodigosenes are still of interest due to their demonstrated immunosuppressive, antimicrobial, antifungal, antiprotozoal, antimalarial and anticancer activities.[1-3] Coley's toxins, synthesized by Dr. William B. Coley in 1888 by combining cultures of *Streptococcus* sp. and *Bacillus prodigiosus*, (S. *marcercens*), and then sterilizing them by either heat or filtration. This mixture was then used to treat tumors of mesodermal origin with fascinating results.[4] While the biologically active substance in Coley's toxin is described to be tumor necrosis factor (TNF), a cytokine, it is suspected that prodigion is also present and plays a role.[4] Coley's toxin was used in the clinic until being banned by the FDA in 1963. Due to systemic toxicity, prodigiosin is not a suitable target for clinical development. However, its derivatives (prodigiosenes) have shown many desirable qualities leading to increased interest in the study and the development of new analogues.

Due to their clinical significance, the immunosuppressive and anti-cancer properties of prodigiosenes are the most widely studied. Prodigiosenes have been shown to be a T cell specific immunosuppressant using *in vitro* studies on mouse and human lymphocytes,

while significant immunosuppressive activity has been demonstrated *in vivo* in models of various transplantations and autoimmune disorders. JAK3, a tyrosine kinase, has been shown to be the main molecular target for the immunosuppressive activity.[2]

Due to prodigiosenes' fluorescence, investigations into the location of prodigiosenes within cancer cells have been performed. Due to their hydrophobicity, prodigiosenes are able to associate with the cellular membrane and enter cells. The mechanism of action and the location of the compounds within the cell vary by cell type, which may in part lead to the selectivity of various prodigiosenes for certain cancer cell types. It has been shown that they typically accumulate in the cytoplasm as well as often in the mitochondria. Unfortunately, currently utilized diagnostic techniques are not sensitive enough to detect the presence of prodigiosenes within the nucleus.[2] Further studies are needed to further understand the localization of prodigiosenes within cells.

A singular mechanism of action by which prodigiosenes induce apoptosis has not been discovered, rather a diverse range of activities have been noted which are thought to accumulate to account for the cytotoxic effects. Prodigiosenes have demonstrated the ability to inflict copper-mediated double strand DNA (dsDNA) breakage and can effectively intercalate DNA.[1-2, 4-5] The planar prodigiosene core is capable of dsDNA intercalation while the methoxy substituent and amine groups provide locations for hydrogen bonding to occur.[4] The cationic nature of prodigiosenes at neutral pH leads to electrostatic interactions with the negative phosphate group of DNA.[4] Oxidation of the electron rich prodigiosene facilitates copper mediated oxidative dsDNA breakage by

producing the reductive Cu(II) species.[4] One of the most studied actions of prodigiosenes is the uncoupling of V-ATPase through promotion of H^+/Cl^- symport, leading to the neutralization of acidic cellular compartments and acidification of the cytoplasm which leads to apoptosis.[2, 4-5] The tripyrrolic core has been determined to be essential to this symport mechanism as protonation of the basic site allows for the formation of a tight ion pair that allows transmembrane transport of the H+/Cl- ion pair.[6] It is uncertain if the altering of pH significantly contributes to the cytotoxicity of the prodigiosene, however it likely plays another function in the overall action of prodigiosenes.[2] Control of the cell cycle has also been discovered in cells treated with prodigiosenes.[4-5] Prodigiosenes are capable of mediating apoptosis via the mitochondria as well.[5b, c] Other potential mechanisms of action for prodigiosenes include influencing transcription factors and the AKT/PI3K pathway[2], regulating mitogen-activated protein kinase (MAPK)[4] and triggering the reorganization of the active cytoskeleton[5a]

Multidrug resistance (MDR), a resistance to a range of structurally unrelated compounds, may develop in tumor cells following drug exposure. This may significantly limit the effectiveness of chemotherapeutic treatments. Prodigiosenes have gained a lot of interest due to their potential to combat MDR for two main reasons; the number of potential targets it affects and its inability to be transported by the MDR transporter.[2] The larger the range of potential ways by which a compound can exhibit its anti-neoplastic effects, the less likely it is for MDR to develop. It has also been shown that prodigiosenes are not a substrate for the MDR transporter BCRP.[2]

Structure activity relationships (SAR) using natural and synthetic prodigiosene analogues have been performed to further understand the variety of mechanisms of action.[2] The presence of the C-6 methoxy substituent and the A-pyrrole ring unit have been shown to be essential for the cytotoxicity of prodigiosnes. Replacing the methoxy substituent with a larger alkoxy group was associated with a decrease in cytotoxicity but conversely with an increase in selectivity. The A-pyrrole ring unit was shown to be essential for copper nuclease activity and overall cytotoxicity; this group may also be present as an indole.[2]

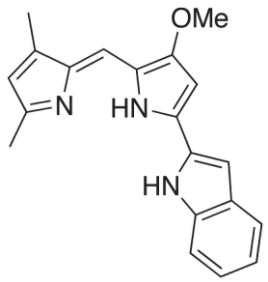


Figure 2. Obatoclax (GX15-070)

Obatoclax (GX15-070), shown in Figure 2, is an A-ring indole prodigiosene that functions as a Bcl-2 inhibitor to induce apotosis. Obatoclax was developed by Gemin X and recently acquired by Cephalon; it and is currently in Phase 2b clinical trials against extensive stage small cell lung cancer. It is the primary example of a prodigiosene showing promising results in clinical studies for use as an antineoplastic agent.[7]

Thompson and coworkers[8] have previously synthesized a collection of prodigiosene anologues possessing pendant ester moieties on the C-ring (Figure 3). This functionality has been shown to be beneficial for two reasons; the adjacent carbonyl group facilitates isolation and purification due to an increase in stability and the ester group provides a site

for further derivitization. These analogues also maintain the biological activity of prodigiosin.[8]

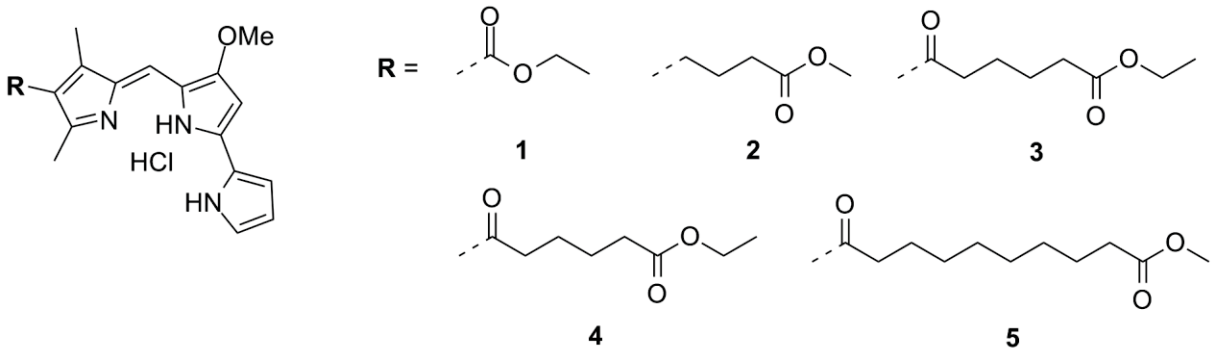


Figure 3. C-ring modified prodigiosenes.

These synthetic C-ring modified prodigiosenes exhibited significant H+/Cl- transmembrane transport. However, the introduction of the carbonyl group adjacent to the pyrrolic skeleton was shown to decrease transport efficiency relative to prodigiosin itself. The prodigiosenes were also shown to maintain the oxidative, copper-mediated DNA cleavage ability of the parent prodigiosin, although at a slightly slower rate.[8]

A compound synthesized as part of the study by Thompson and coworkers, prodigiosene **12**[8] (Figure 4), was chosen as the base prodigiosene skeleton for this project as it possesses both the conjugated carbonyl group for stabilization and an ester group to be used for derivitization. Other members of the Thompson research group are synthesizing various prodigiosene analogues containing various chain lengths on the C-ring.

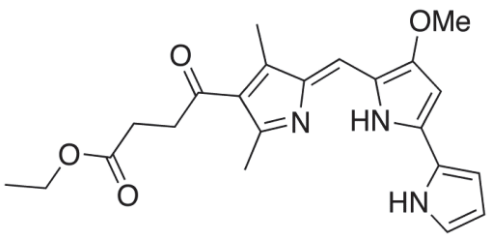


Figure 4 Prodigiosene **12**

1.2 Targeted Drug Design

Chemotherapy remains the primary mode of treatment for disseminated or metastatic cancers.[9] One of the problems with currently used chemotherapeutics is that most drugs used in the treatment of cancer are actually anti-proliferative, rather than anti-cancer.[10] This leads to the increased uptake of drugs into all proliferative cells such as hair, stomach lining and cancer cells.[9] This causes side-effects such as hair loss and mouth sores which are often experienced with many types of chemotherapy drugs.[10] A strategy that researchers have been investigating to limit these side-effects is targeted drug design. Targeted drug design (represented as a schematic in Figure 5) involves attaching a compound known to accumulate in cancer cells, to an anti-cancer compound which will in turn increase its uptake into the cancerous cell compared to healthy cells.[10]

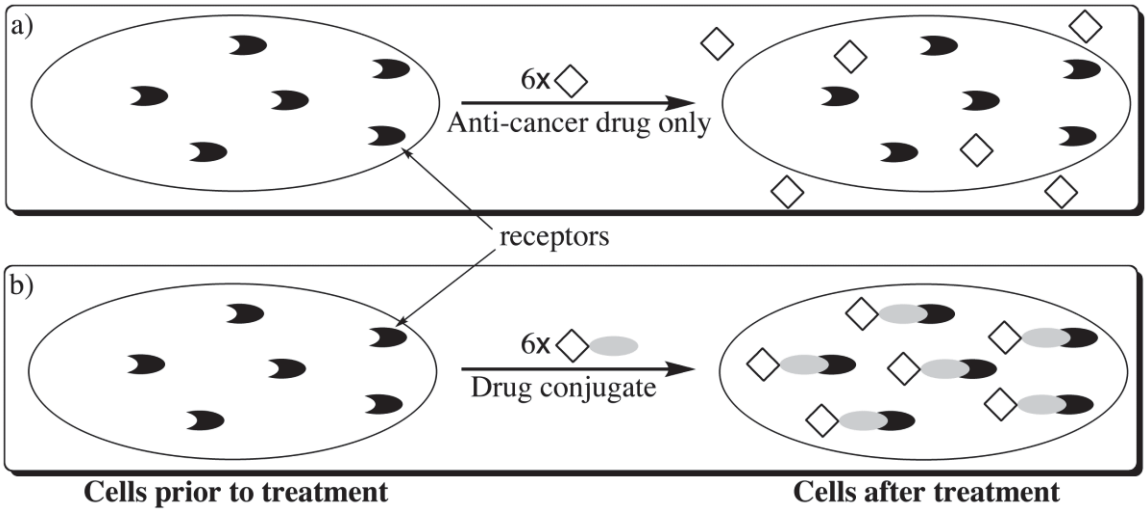


Figure 5. Targeted drug design.

Researchers theorize that the toxicity of anticancer drugs could be decreased or even eliminated if the drug was targeted to selectively uptake within cancerous cells.[9] This strategy would also permit more aggressive treatment schedules, due to the lower accumulation in healthy cells, therefore improving the therapeutic outcome as such schedules limit the amount of time available for cells to recover.[9] Further research into tumor specific drug delivery is crucial for the future effective treatment of breast cancer.[9] Biological pathways and other cullular targets are being identified to aid in the development of cell-specific therapies.[11]

Examples of "targeting agents" are hormones (or their mimics) and vitamins which are known to have an overabundance of receptors in the cancer cells.[9, 12] A further potential benefit to using this strategy is that the targeting agent itself could be an anti-cancer compound, therefore increasing the ability of the compound as a whole to exhibit anti-cancer effects.

Drug conjugates are formed by chemically tethering an anti-cancer compound to the targeting moiety using ester, amide or ether bonds for example. An example of targeted drug design from the literature can be seen in Figure 6.[13] In this example, estradiol is bound to a porphyrin. The estradiol is utilized to target estrogen-receptor positive breast cancer cells, and porphyrins are a class of compound currently used in photodynamic therapy for the treatment of cancer.[13] The conjugate must stay intact until it enters the cell, and may remain intact or cleave within the cell depending on the mechanism of action of the drug. The type of bond chosen for a targeted compound is chosen based upon the biological stability and synthetic ease of each type of bond. Ethers and amides are typically more robust than esters and are therefore more often used.[14]

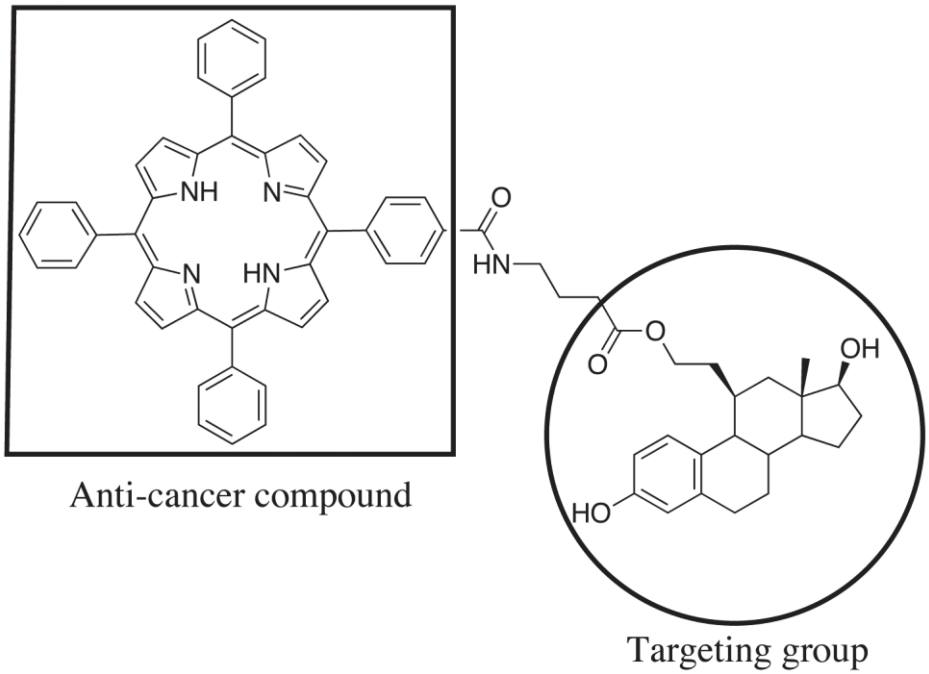


Figure 6. Example of targeted drug design.

1.2.1 Estrogen Receptor

The first targeting group chosen for use in this study was estrogen. Estrogens are endocrine regulators of the vertebrate reproductive system, which also exhibit profound effects on the growth, differentiation and function of a variety of other non-reproductive tissues including bone, liver, the cardiovascular system and the central nervous system.[15] Estrogens also play an important role in pathological processes associated with breast, uterine and other cancers.[16] Estrogen-based pharmaceuticals have been investigated for the prevention and control of hormone-responsive breast cancer, hormonal treatment of symptoms caused by menopause and regulation of fertility.[15a]

Estrogens interact with the intracellular estrogen receptor (ER), a member of the steroid/thyroid super-family of related proteins which also includes the vitamin D receptor and retinoic receptor.[15b, 17] The ER can be divided into two different subtypes; ERα and ERβ.[15-17] ERα was first discovered in 1967[16] and was first cloned from the human breast cancer cell line MCF7 in the 1980's.[17] ERβ was later discovered in 1996[16] and cloned from a rat's prostate.[17] The levels and proportions of the receptor types vary based on cell and tissue type and function. For example, ERα is associated with malignant breast cancer while ERβ can be associated with benign breast cancer[16] and is present in colon cancer cells and the prostate while ERα is not.[17]

The ER is well known as a nuclear receptor but has also recently been discovered to exist as a G-protein coupled cell membrane receptor.[16] The nuclear receptor action is tripartite involving the receptor, ligand and coregulated proteins,[15b] which together act as a transcription factor which binds specifically to estrogen-responsive areas in the promoter region of estrogen related genes.[15a] ER ligands include natural estrogens, the simplest steroid hormones which are distinguished via a phenolic A-ring and include estradiol (E2) and estrone as well as synthetic estrogens which also typically contain a phenolic ring.[15a] Examples of natural and synthetic estrogens are shown in Figure 7.

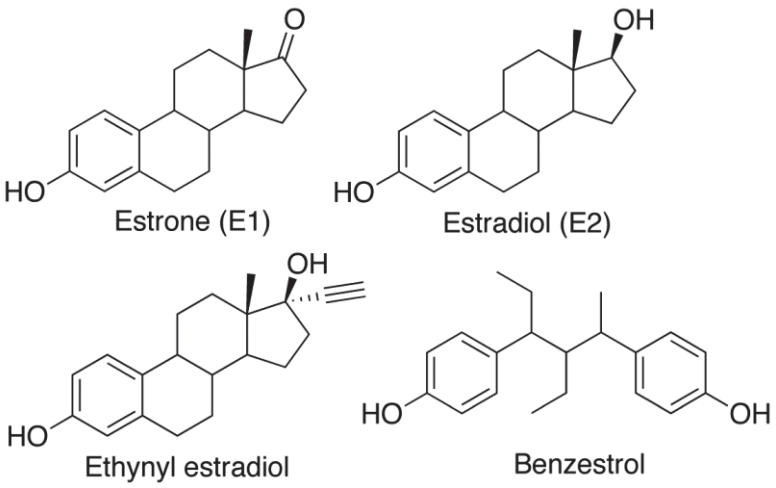


Figure 7. Synthetic and natural estrogens.

An increase in knowledge of the biological action of estrogens and other sexual steroid hormones resulted in the development of hormone replacement therapy (HRT) for the treatment of menopause.[15d] This lead to the interesting discovery that while HRT could increase the likelihood of the development of breast and uterine tumors, it decreased the chances of the patient developing colorectal cancer.[15d] This result suggests a protective nature of ERβ, the predominant ER present in colon cancer. However the mechanism of action of this protection is not fully understood.[15d] While ERα and ERβ are structurally

quite similar, studies have demonstrated the potentially drastic differences in their actions, particularly in relation to cancer.[15b]

As estrogen is known to promote the growth and development of breast tumors, the ER was identified as an effective target for the treatment of breast cancer.[17] Selective estrogen receptor modulators (SERMs) (e.g. Tamoxifen and Raloxifene) act as competitive inhibitors of the natural estrogen E2 at the estrogen receptor which can act acts as either an agonist or antagonist depending on cell type or as pure anti-estrogens (ie. Fulvestrant).[17] Examples of anti-estrogens and SERMs are shown in Figure 8. Each of these types of SERM have demonstrated effectiveness in the treatment of breast cancer.[17] Tamoxifen (TAM) is a non-steroidal triphenolic anti-cancer agent widely used in the treatment of estrogen-receptor positive breast cancer.[15b] Tamoxifen and its active metabolite 4-hydroxy Tamoxifen (4-OHT) are type I antagonists, possessing mixed antagonist and agonist activity depending on cell type and the promoters present.[15b]

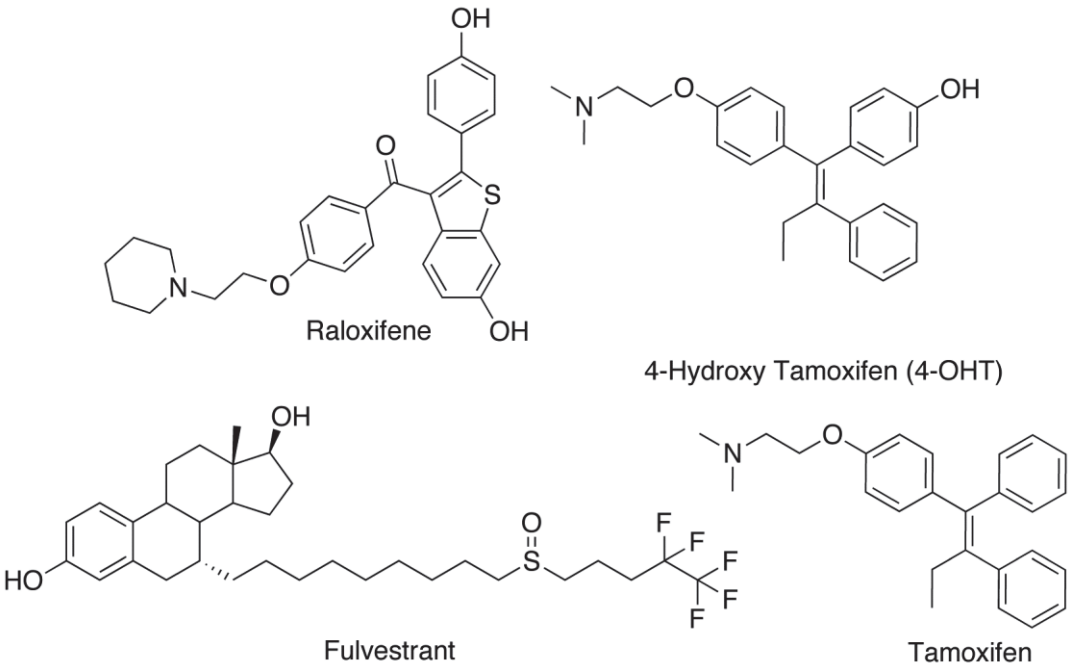


Figure 8. Anti-estrogens and SERMs.

While approximately 50% of breast cancers possess an initial response to anti-estrogen therapy, many develop a resistance to the treatment.[16] It is thought that by combining an anti-estrogen compound with prodigiosene the drug resistance could be over came/avoided.

ER-targeting has previously been exploited extensively by coupling estrogen with cytotoxins such as nitrogen mustards, genotoxins and geldaanamycin.[13, 18] The ER-targeting moieties chosen in this study are; E2, estrone and 4-OHT (shown in Figure 9), which differ in substrate preference and binding affinity. E2 is capable of binding to both ERα and ERβ while estrone exclusively binds ERα. Furthermore, 4-OHT demonstrated a three-times increased binding affinity for ER as compared to E2[15b] and the ER dissociation constant for estrone is also significantly greater than that for E2.[19]

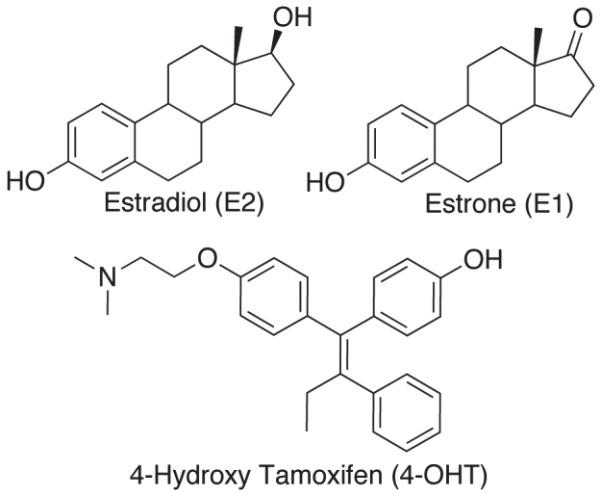


Figure 9. ER-targeting molecules used in this study.

1.2.2 GLUCOSE

Another type of targeting agent chosen for this study is glucose. In order to promote their rapid growth and invasion of surrounding tissues, cancer cells have a higher metabolic rate than normal, healthy cells.[20] This is particularly the case for glucose metabolism.[20-21] The first biochemical hallmark noted for cancer cells was the shift from typical oxidative phosphorylation to anaerobic glycolysis[20-21] which provides cancerous cells with a metabolic advantage allowing the maintenance of a constant supply of energy even in the absence of oxygen.[21c] Therapeutic strategies have been developed to target this increase in metabolism: it is hypothesized that this would provide a selective mechanism for drug delivery and action.[21a, b] For this research study, glucose (shown in Figure 10) was chosen to be attached to a prodigiosene in order to potentially increase the cancer cell specific delivery of the prodigiosene.

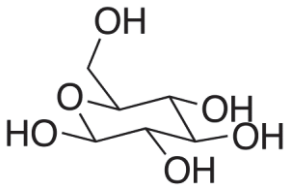


Figure 10. Glucose utilized in this study.

1.2.3 PORPHYRINS

The final targeting moieties chosen were porphyrins. Porphyrins are aromatic macrocycles that consist of four "pyrrole-type" rings which are joined by four methine carbons. A general porphyrin structure is shown in Figure 11.

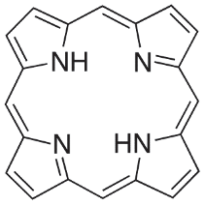


Figure 11. Porphyrin skeleton.

Porphyrins have demonstrated an ability to accumulate in cancer cells as compared to normal cells.[13, 18, 22] This ability of naturally occurring hematoporphyrin (Figure 12) and other synthetic derivates to accumulate in tumor cells has been demonstrated via fluorescent imaging.[22b] Porphyrins are known to localize in the subcellular structures including lysosomes, endoplasmic reticulum, mitochondria and Golgi apparatus.[22a]

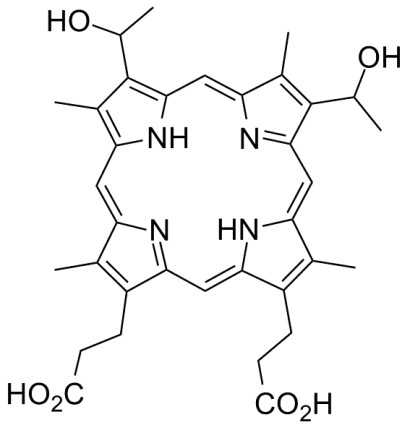


Figure 12. Hematoporphyrin.

Presently, porphyrins are used in medicine as photosensitizers (PS) which, when irradiated by visual light, produce the highly reactive singlet oxygen species which induces apoptosis.[18] Photodynamic therapy (PDT) is employed in a variety of aspects of health care including the treatment of early stage malignant tumors, palliative care patients and in sterilization of the tumor bed following surgery to ensure all cancerous cells were removed.[18] Porfimer sodium, also known as Photofrin, was the first approved PS, approved in Canada in 1993.[23] Photofrin consists of purified hematoporphyrin derivatives as well as a mixture of other porphyrins and is used in the treatment of a variety of cancers.[23] Foscan (*m*-tetrahydroxyphenylchlorin) is a second generation photosensitizer which is the most effective to date used in the treatment of head and neck cancers.[24] Visudyne, is another example of a porphyrin-based photosensitizer which is used in the treatment of age-related macular generation.[18, 24] The structures of these photosensitizers are shown below in Figure 13.

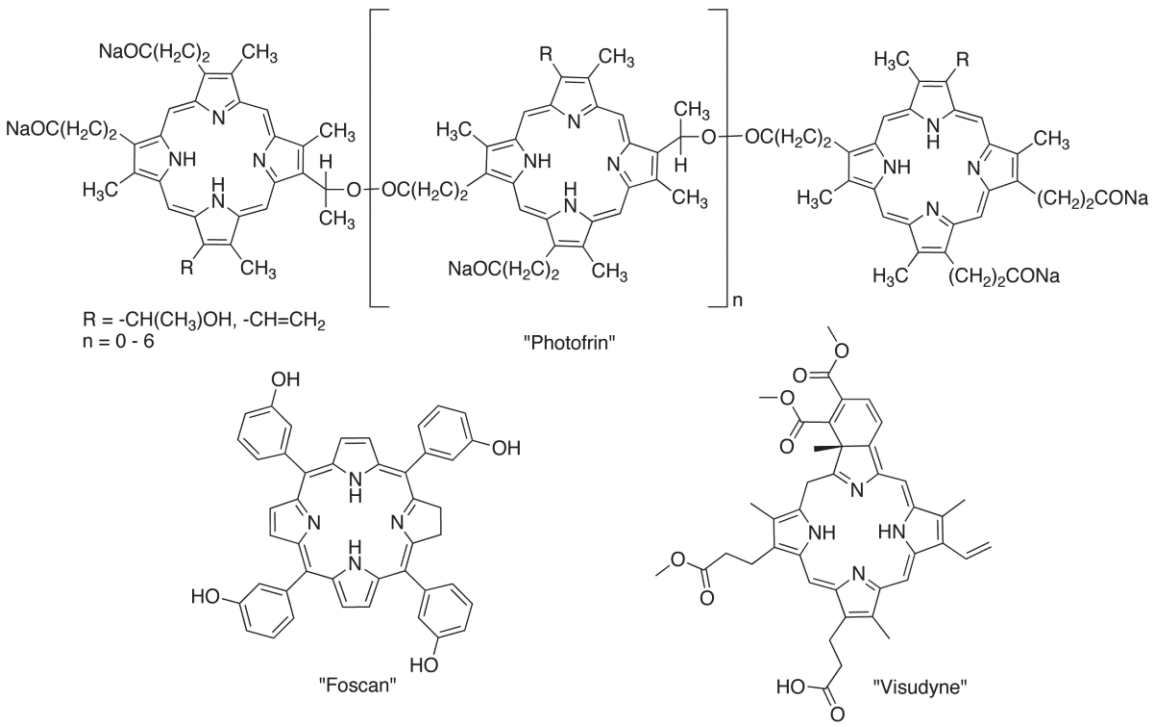


Figure 13. Photosensitizers presently used therapeutically.

PDT has been investigated for the palliative treatment of cutaneous reoccurrence of breast cancer.[18] Delay of tumor growth in murine orthotropic breast tumor model has shown the potential for use of photodynamic therapy in breast cancer.[18] The attachment of a lipophilic and cationic moiety to porphyrins causes an increase in membrane targeting ability and mitochondrial accumulation.[25] Prodigiosene is both lipophilic and cationic at physiological pH, providing the possibility for this accumulation. This could also benefit the apoptotic action of prodigiosenes as prodigiosenes are thought to exert some effect within the mitochondria.

CHAPTER 2: RESULTS AND DISCUSSION

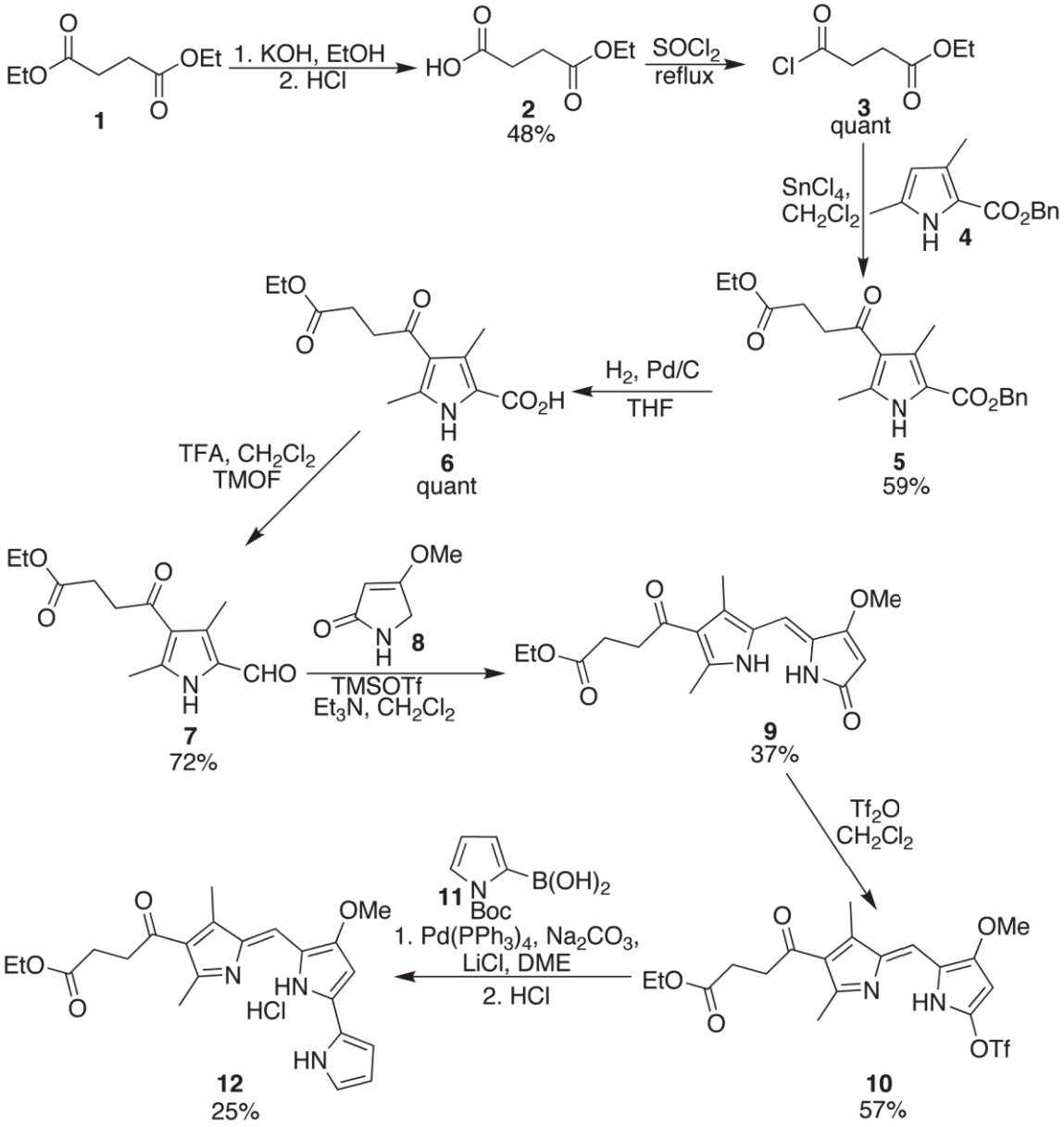
2.1 SYNTHESIS OF PROIGIOSENE FOR DERIVITIZATION

The aim of this research project was the synthesis of prodigiosenes coupled with compounds known to selectively accumulate in cancer cells with the goal of lowering system toxicity by increasing the delivery of the prodigiosene to the cancerous cells as compared to healthy cells. Estrogens, estrogen mimics and porphyrins containing alcohol or amine functional groups were chosen as targeting moieties for this study, as discussed in Chapter 1. The first stage in the production of these conjugates required synthesizing the prodigiosene core skeleton following previously reported synthetic procedures from the Thompson group.[8, 26] Ethyl 4-[5-(4-methoxy-1*H*,1'*H*-[2,2']bipyrrolyl-5-ylmethylene)-2,4-dimethyl-5*H*-pyrrol-3-yl]-oxobutanoate (prodigiosene **12**) was chosen to be the base prodigiosene for the conjugates synthesized in this study, as previous biological screens on this compound yielded promising results. The synthesis of this prodigiosene is depicted in Reaction Scheme 1.[8, 26] This synthesis was repeated multiple times over the duration of the program in order to obtain a significant quantity of prodigiosene **12**.

The first stage of the synthetic route towards prodigiosene **12** involved the production of a functionalized pyrrole via Friedel-Crafts acylation of benzyl 3,5-dimethyl-pyrrole-2-carboxylate (**4**),[27] with the intention that this heterocycle would become the C-ring in the final product.[27] The incorporation of a conjugated β-carbonyl group allows for more facile isolation and purification of the corresponding prodigiosenes,[8] while the pendant

ester moiety offers the option for further derivatization and attachment of targeting groups. The required chlorocarbonyl ester was obtained via monosaponification of diethyl succinate (**1**) using potassium hydroxide in ethanol followed by chlorination with thionyl chloride at reflux temperature. Yields for the monosaponification and isolation of **2** tended to be low (48%), due to the sequential production of the disaponified product. This result had been previously reported in the literature.[8, 28] Monosaponification was optimized, at the expense of the disaponification, by controlling the equivalents of potassium hydroxide. In order to ensure complete reaction of diethyl succinate, some of the corresponding di-acid was formed, and was successfully removed using hot filtration. The desired monosaponified product **2** was soluble in ethanol at reflux temperature while the disaponified product was not. Chlorination of butanedioic acid monoethyl ester (**2**) subsequently proceeded in a quantitative yield. Friedel-Crafts acylation of benzyl 3,5-dimethyl-pyrrole-2-carboxylate (**4**)[27] utilizing chlorocarbonyl **3** yielded the desired compound **5** in a 59% yield.

Following hydrogenolysis of the benzyl ester group of 4-(3-ethoxycarbonyl-propanoyl)-3,5-dimethyl-1*H*-pyrrole-2-carboxylic acid benzyl ester (**5**), formyl pyrrole **7** was synthesized via formylation of pyrrole carboxylic acid **6** using trimethyl orthoformate and trifluoroacetic acid.[8] This method was chosen over Vilsmeier-Haack formylation, which was previously used in prodigiosene synthesis by Thomspon and coworkers,[8, 26] as it does not require the isolation of the unstable α-free pyrrole as was required in the original synthesis. The yield obtained via the trifluoroacetic acid/trimethyl orthoformate method (72%) was comparable to a previously reported yield by Thompson and coworkers using Vilsmier-Haack conditions for the same compound (68%).[8]



Reaction Scheme 1. Synthesis of ethyl 4-[5-(4-methoxy-1*H*,1'*H*-[2,2']bipyrrolyl-5-ylmethylene)-2,4-dimethyl-5*H*-pyrrol-3-yl]-oxobutanoate.

Thompsonand coworkers have previously reported the synthesis of dipyrrinones via condensation of a formyl pyrrole with a pyrrolinone using potassium hydroxide in a mixed water/tetrahydrofuran solution, followed by re-esterification.[8] The need for two

separate reactions to form the dipyrrinone along with the need for elevated temperatures over two days lead to the desire to use another method for the synthesis of dipyrrinone **9**. The condensation was performed in this study using trimethylsilyl trifluoromethanesulfonate and triethylamine to couple formyl pyrrole **7** and pyrrolinone **8**.[26, 29] Yields for this reaction however were often quite low (37%), which can be largely attributed to the poor solubility of dipyrrinone **9** in organic solvents which leads to difficulty in work-up and purification. Following extraction of the reaction mixture using dichloromethane, solvent was removed under reduced pressure and the resulting yellow solid was washed using water and hexanes. Dichloromethane was chosen for the extraction as dipyrrinone **9** possesses the greatest solubility in it as compared to other organic solvents. However, the dipyrrinone is not fully soluble which leads to the need to use extremely large amounts of solvent in the extraction, approximately 1 L of solvent for each gram of dipyrrinone **9** produced. It is predicted that a significant amount of the product remained in the reaction mixture as it was not completely extracted as it could still be observed in the aqueous layer using TLC.

In order to facilitate the cross-coupling reaction required in the last step of the synthesis to form prodigiosene **12**, dipyrrinone **9** was triflated. Triflate (**10**) was unstable and was found to decompose during purification via column chromatography and was required to be used immediately in the next step of the reaction or stored under N_2 in the freezer before use inside 72 hours. The final step involving palladium-catalyzed coupling of triflate **10** and 1-*N*-Boc-pyrrole-2-boronic acid **11** successfully yielded prodigiosene as its free base. Following purification of the prodigiosene using column chromatography over

alumina, the free-base prodigiosene was converted to the corresponding hydrochloric salt by treatment with an HCl/methanol solution. This facilitated further purification and isolation of the final product prodigiosene **12**, via filtration and washing with water and diethyl ether, in a 25% yield.

One major problem associated with the synthesis of prodigiosenes arises at the stage of dipyrrinone formation: a mixture of *E*/*Z* isomers may be formed (Figure 14). The presence of both isomers has been previously noted by members of the Thompson research group in the synthesis of a variety of prodigiosenes.[26]

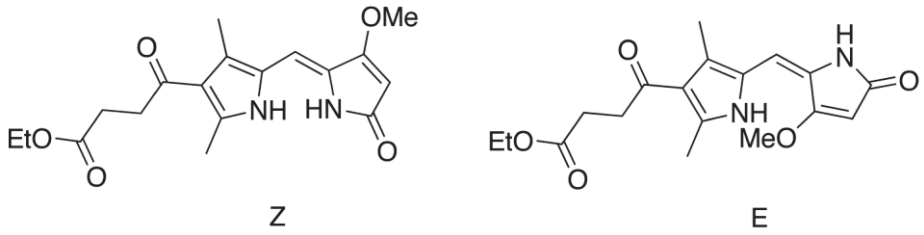
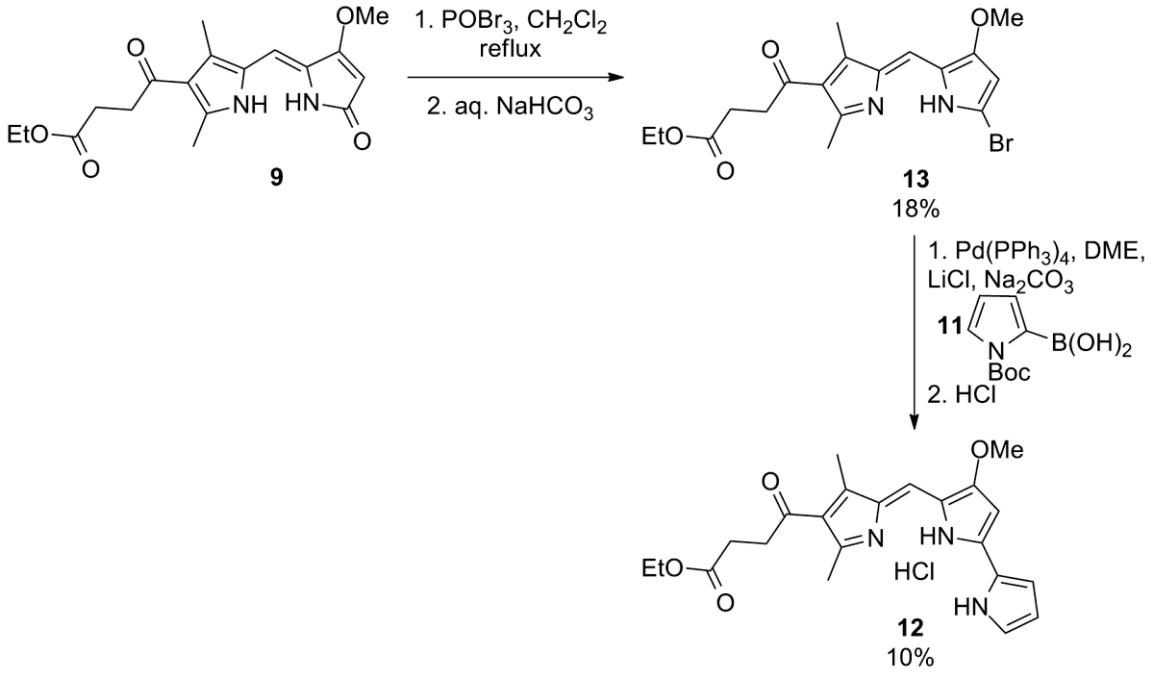


Figure 14. *E*/*Z* isomers of dipyrrinone **9**.

The isomers can be separated using column chromatography; however, it has been found that reaction conditions for the bromination of dipyrrinones using phosphorous oxybromide converts the undesired *E*-isomer to the desired *Z*-isomer for a variety of dipyrrinones.[26] This would increase the overall amount of compound available to continue on in the synthesis. The brominated compounds are often significantly more stable than the corresponding triflates[26] and can be stored on the bench-top, facilitating their use. The synthesis of prodigiosene **12** using the developed bromination method is shown in Reaction Scheme 2.[26] The bromination method was not desirable for future production of prodigiosene **12** for several reasons. Firstly, the obtained yields of bromodipyrrin **13** were lower (18% and 10%) than the yields obtained using the triflation

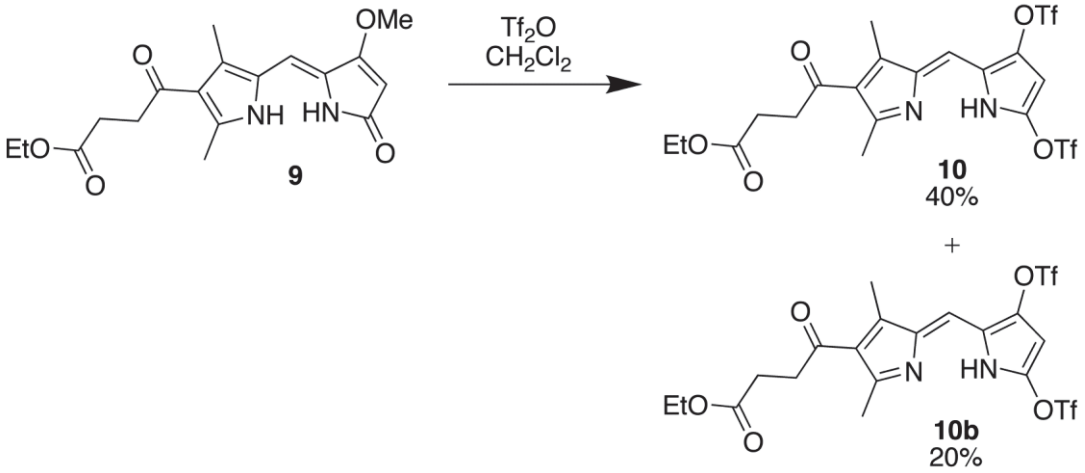
method (57% and 25%). Secondly, the brominated compound (**13**) contained a significant amount of the unwanted *E*-isomer necessitating purification using the Biotage flash purification system over alumina before continuing on with the synthesis. Finally, the overall length of synthesis for the bromination method was significantly longer than that of the triflation route as the bromination took 48 hours compared to the 4 hours required for triflation, and the cross-coupling using the brominated compound took 120 hours compared to 32 hours for the corresonding triflate. For these reasons, the triflation method was chosen as the route for further prodigiosene syntheses in this project.



Reaction Scheme 2. Bromination method for the synthesis of ethyl 4-[5-(4-methoxy-1*H*,1'*H*-[2,2']bipyrrolyl-5-ylmethylene)-2,4-dimethyl-5*H*-pyrrol-3-yl]-oxobutanoate.

The triflation reaction resulting in the formation of triflate **10** took a significantly longer amount of time and produced a lower yield as compared to the same reaction on dipyrromethanes previously synthesized by members of the Thompson group.[8] For this reason, optimization of the reaction conditions for my particular substrate (**9**) was attempted. It was a concern that the longer reaction time required for this compound (**9**)

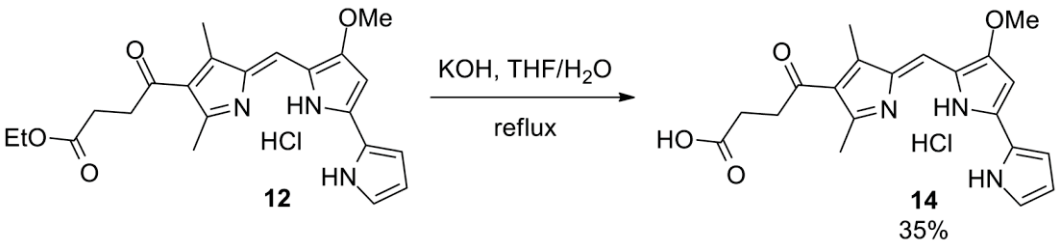
was leading to product decomposition as it is unstable. The amount of trifluoromethane sulfonic anhydride used in the reaction was investigated, whereby increasing from 2.8 eq. to 5 eq. (shown in Reaction Scheme 3) resulted in the reaction going to completion in 3 hours as compared to 4 hours for the previously utilized method. The yield however decreased to 40% when using the 5 eq. method, compared to 57% for the usual 2.8 eq. method. Another bright yellow compound was isolated during purification using column chromatography in 20% yield from the 5 eq. method that was not previously noted. Using ^{1}H, ^{13}C and ^{19}F NMR spectroscopy and mass spectrometry, the product was determined to be bistriflate **10b**. This discovery is of interest as it provides a possible method to alter the substitution on the B-ring at the end of the prodigioesne synthesis rather than having to synthesize pyrrolinones with various substitutions. Further investigations into this reaction are needed to determine the viability of this strategy.



Reaction Scheme 3. Synthesis of bistriflate **10b**.

Hydrolysis of the ethyl ester functionality of prodigiosene **12** was then performed, thus producing a carboxylic acid ready for coupling to targeting moieties via ester and amide linkages. Prodigiosene **12** was hydrolyzed, using potassium hydroxide in a mixed

tetrahydrofuran/water solution at reflux temperature,[30] and converted to the hydrochloride salt (Reaction Scheme 4). Unfortunately, these reaction conditions also lead to decomposition of the prodigiosene which, along with difficulties in purification due to poor solubility, lead to a low isolated yield of the final product (35%).



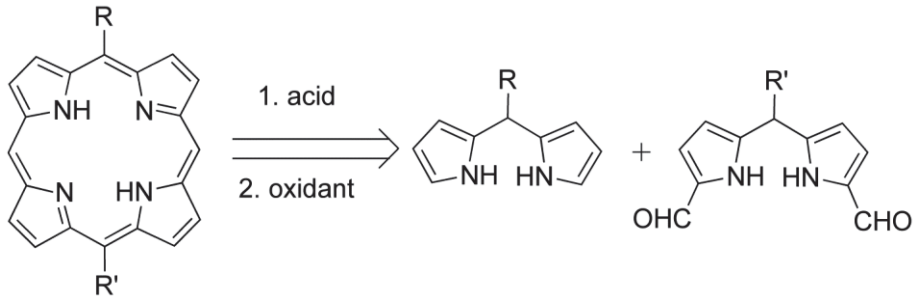
Reaction Scheme 4. Prodigiosene acid formation.

2.2 Porphyrin Synthesis

In order to perform coupling studies, the targeting moieties needed to be synthesized. The targeting group to be synthesized as part of this project is a porphyrin containing an alcohol or amine group to enable coupling with prodigiosene **14**. The remaining functionality of the porphyrin was not viewed as important in this initial stage of research as the goal was to show the ability of a porphyrin to be coupled to a prodigiosene and to obtain initial biological data. There are many examples of porphyrin synthesis in the literature which follow very distinct paths; condensation of an α-free dipyrromethane with an α-formyl dipyrromethane,[31] self-condensation of an α-free dipyrromethane using functionalized aldehydes[32] and coupling pyrrole using functionalized aldehydes.[32-33]

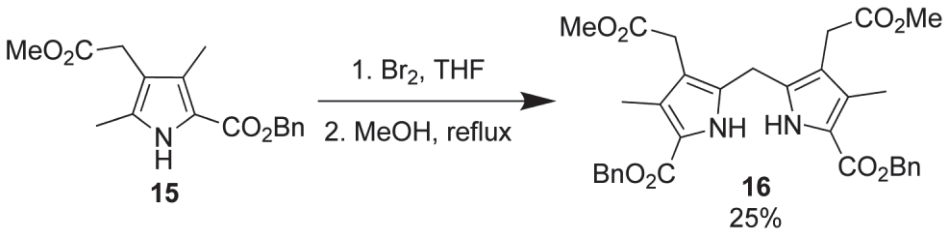
The initial route for the synthesis of a suitable porphyrin investigated followed methods previously employed by Dr. Rosa Saez Diaz, a former member of the Thompson group.

This route involved the condensation of an α-free dipyrromethane with an α-formyl dipyrromethane (shown in Reaction Scheme 5), one of which contained a functional group to facilitate coupling to prodigiosene **14**. The functional groups of interest presently are either a hydroxyl or amine group situated on a *meso*-phenyl substituent of either dipyrromethane.



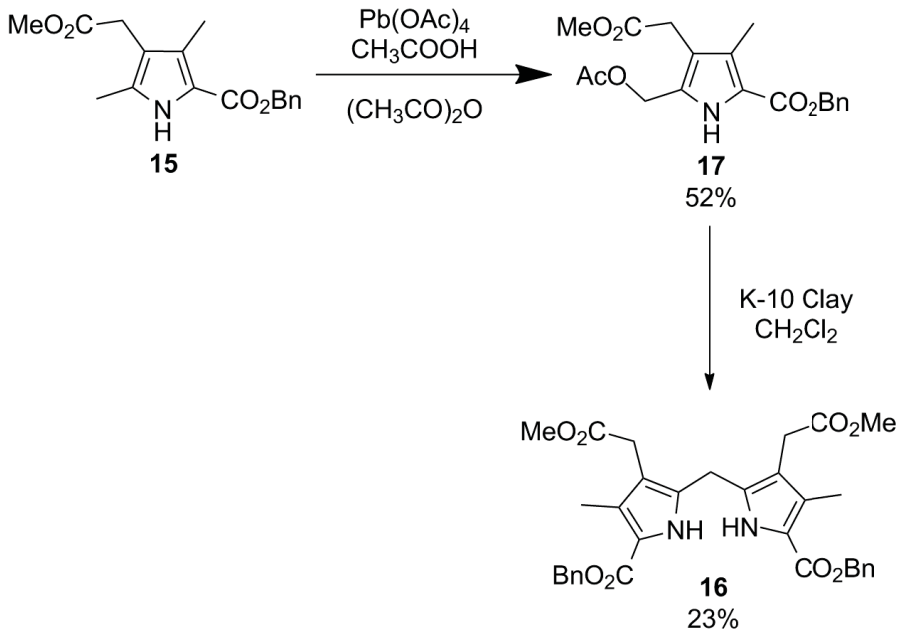
Reaction Scheme 5. General porphyrin synthesis utilizing an α-free dipyrromethane and an α-formyl dipyrromethane.

The first step required in this synthetic route was the synthesis of the α-formyl dipyrromethane, which can be formed via hydrogenolysis followed by formylation of a 2,2'-dibenzyl ester dipyrromethane.[34] The first attempt made to synthesize such a dibenzyl ester dipyrromethane was bromination of benzyl ester pyrrole **15**[35] using molecular bromine in tetrahydrofuran followed by heating to reflux temperature in methanol (Reaction Scheme 6).[31a] After drying overnight under vacuum, an oil remained following the bromination, rather than a solid as previously reported for this compound.[31a] The oil was then dissolved in methanol and heated to reflux temperature and allowed to crystallize overnight in the freezer, producing the desired product (**16**) in a low yield of 25%.



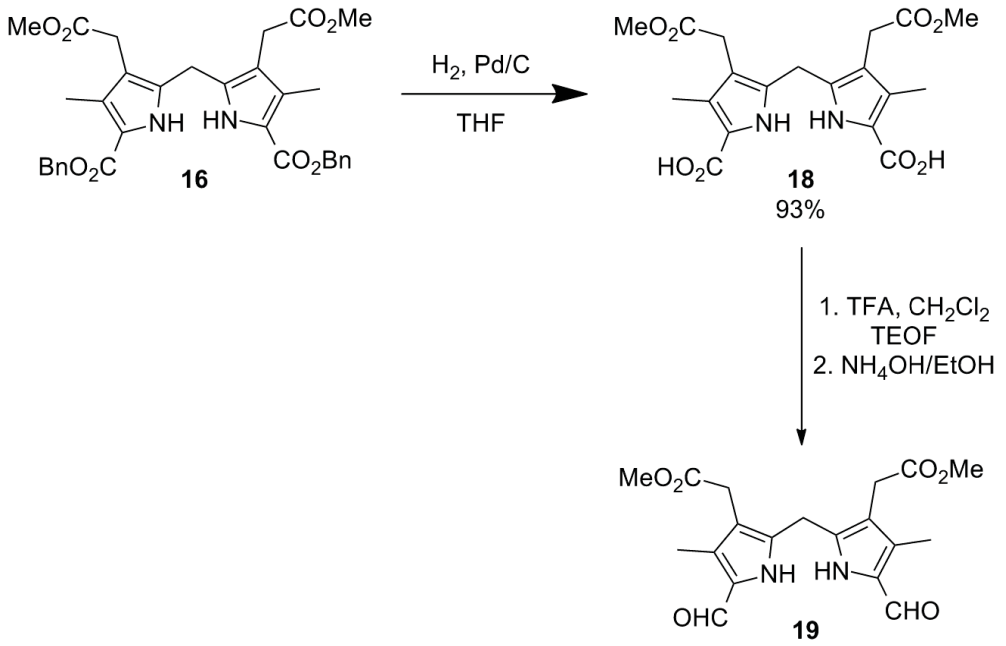
Reaction Scheme 6. Dipyrromethane formation *via* bromination method.

Further attempts to repeat the synthesis in order to increase the yield by changing the time for the bromination stage and not placing the brominated intermediate under vacuum overnight were unsuccessful; therefore an alternative method (shown in Reaction Scheme 7) was utilized.[31] This alternative method also involved mono-oxidation of the α-methyl group of pyrrole **15**, but rather than bromination, instead involved acetylation. The fully substituted pyrrole (**15**) was acetylated at the 5-position using lead(IV)acetate, in a mixed acetic acid/acetic anhydride solvent system, to give 4-acetic acid methyl ester-5-[acetyloxy)methyl]-1*H*-pyrrole-2-carboxylic acid benzyl ester (**17**) in a 52% yield, which is comparable to literature yields.[31b] Self-condensation of pyrrole **17** using montmorillonite (K-10) clay as catalyst, and dichloromethane as the solvent, afforded dipyrromethane **16** in a 23% yield which is quite low compared to literature yields for this compound (88%[31b]). This low yield is suspected to be caused by difficulty rinsing product from the clay, therefore further attempts of the synthesis involved washing the clay with larger amounts of dichloromethane to increase the yield. In comparing the two investigated strategies for synthesizing the dipyrromethane, it can be noted that while neither reaction afforded the desired product in high yield, the second route involving acetylation and self-condensation is more synthetically straight forward and the products obtained were easier to purify via recrystallization.



Reaction Scheme 7. Dipyrromethane formation *via* acetylation method.

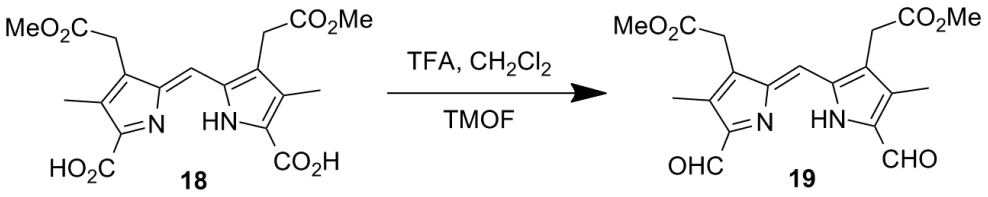
Hydrogenolysis of the 2,2'-benzyl ester dipyrromethane **16** to form the α-carboxylic acid dipyrromethane **18** followed. The hydrogenolysis was successful (93% yield) yielding the desired α-carboxylic acid which was shown to be highly insoluble in most organic solvents , therefore ^{13}C NMR data was not obtained. Formylation to yield the α-formyl dipyrromethane **19** was the final step in synthesizing the precursors needed for this porphyrin synthesis. Formylation was first attempted utilizing a method previously used by Dr. Rosa Saez Diaz on a similar compound for porphyrin synthesis which is shown in Reaction Scheme 8.



Reaction Scheme 8. Hydrogenolysis and attempted formylation of dipyrromethane **16**.

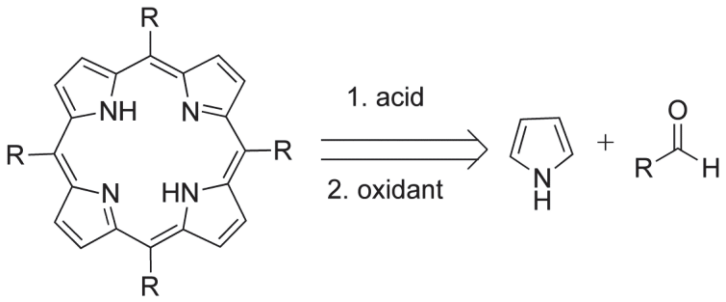
The reaction produced a sticky dark green product which was shown using ^{1}H NMR spectroscopy to be decomposition products as characteristic pyrrolic peaks were not visible. It was thought that the conditions were perhaps too harsh, leading to product decomposition. Compound **18** was instead exposed to the formylation conditions (shown in Reaction Scheme 9) that had previously been successful for the formylation of 4-(3-ethoxycarbonyl-propanoyl)-3,5-dimethyl-1*H*-pyrrole-2-carboxylic acid (**6**). As such, compound **18** was first dissolved in dichloromethane, on ice, prior to the addition of trifluoroacetic acid and subsequent treatment with trimethyl orthoformate. The di-α-carboxylic acid dipyrromethane starting material (**18**) did not appear to fully dissolve under these conditions; therefore more trifluoroacetic acid was added in an attempt to complete the dissolution. Following an aqueous work-up and extraction using dichloromethane, the solvent was removed under vacuum. No product was isolated. Attempts to isolate either the desired product **19** or starting material **18** from the aqueous

fractions were also unsuccessful. The poor solubility of dipyromethane **18** appeared to prevent successful formylation, therefore it was decided that other methods for the synthesis of a porphyrin were to be investigated.



Reaction Scheme 9. Attempted formylation of dipyrromethane **18**.

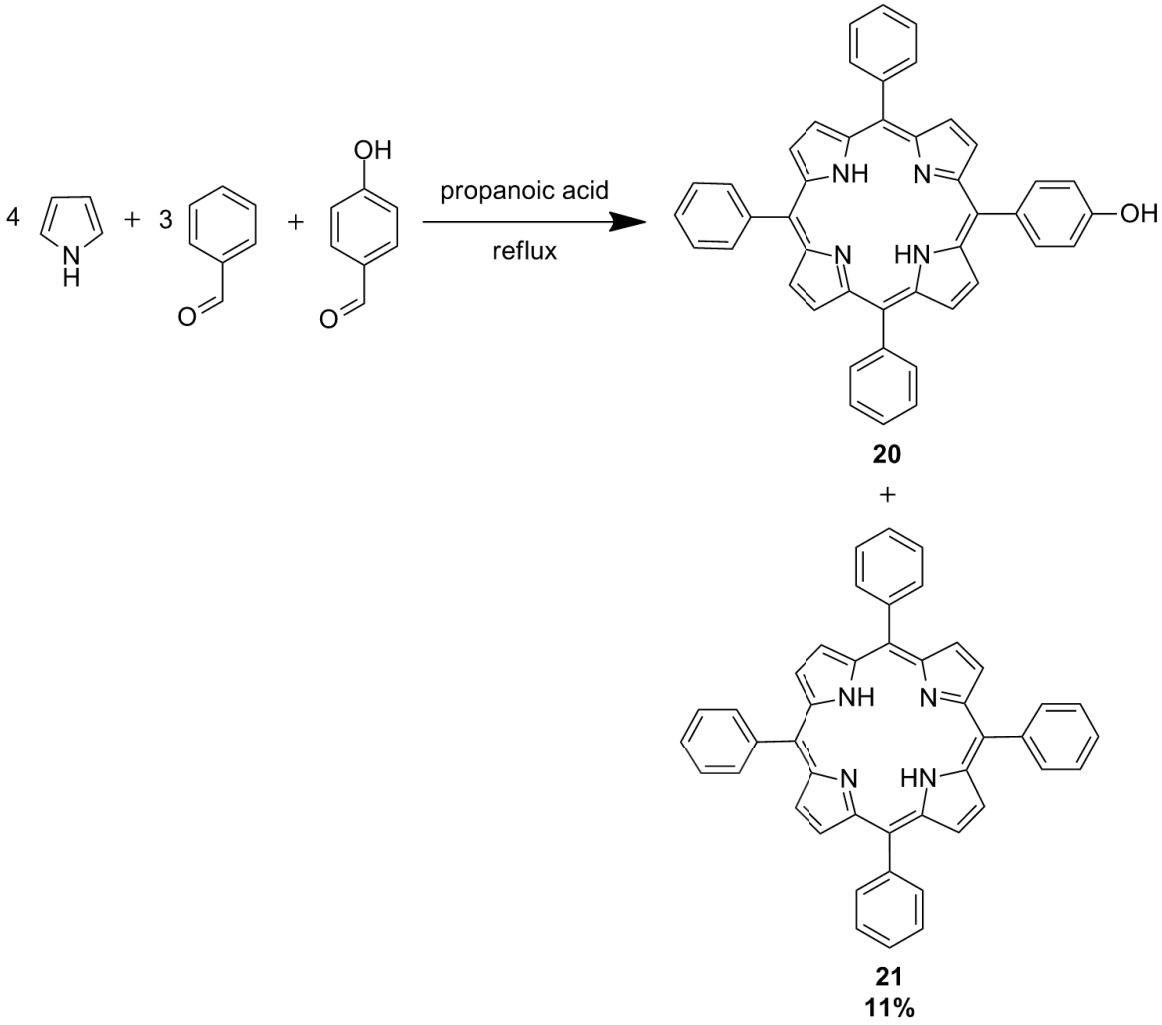
Due to the number of steps involved in porphyrin synthesis using the above-mentioned route, one-step syntheses using materials already available in the lab were investigated. Porphyrins can be formed by condensing pyrrole with an aldehyde in the presence of acid (Reaction Scheme 10). This strategy has been investigated by numerous research groups using a variety of aldehydes and acids to promote the reaction.[23, 33]



Reaction Scheme 10. General porphyrin synthesis utilizing pyrrole and a functionalized aldehyde.

The first one-step synthesis investigated in this research study is shown in Reaction Scheme 11,[33] whereby pyrrole, benzaldehyde and 4-hydroxybenzaldehyde were reacted in propanoic acid at reflux temperature overnight in hopes of obtaining porphyrin **20** with only one alcohol moiety. A mixture of products was to be expected using these reaction conditions; however it was predicted that porphyrin **20** would be isolated in significant

quantities as reported in the literature.[33] Two purifications using column chromatography with dichloromethane and a dichloromethane:hexanes gradient as eluents afforded 5,10,15,20-Tetraphenyl-21*H*,23*H*-porphyrin (TPP) (**21**) as the only product in an 11% yield. This result can be attributed to benzaldehyde being more reactive than 4-hydroxybenzaldehyde, which makes the desired product **20** less likely to be formed than TPP (**21**).

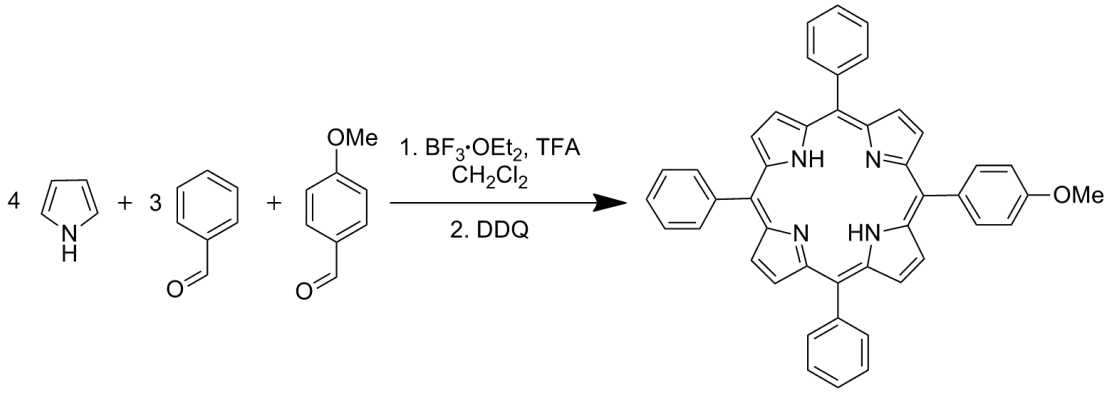


Reaction Scheme 11. One-pot porphyrin synthesis.

The synthesis of mono-hydroxy porphyrin **20** could pontentially be achieved by first synthesizing the mono-anisole porphyrin **22**, followed by conversion of the methoxy

group to an alcohol. It was hoped that the different activity of *p*-anisaldehyde from *p*-hydroxy benzaldehyde would increase the yield of the desired porphyrin **22** compared to TPP. The use of boron trifluoride diethyl ethereate and tifluoroacetic acid as acids to catalyze the condensation was also investigated in hopes of increasing the ease of purification as compared to the previously attempted synthesis which required the removal of significant amounts of propanoic acid. The synthesis of mono-anisole porphyrin **22** was thus attempted (shown in Reaction Scheme 12)[23] by reacting pyrrole, benzaldehyde, *p*-anisaldehyde, boron trifluoride diethyl etherate and trifluoroacetic acid in dichloromethane at room temperature, followed by oxidation using DDQ. Attempted purification of the isolated product was unsuccessful as all of the material stuck to the silica during column chromatography using a mixture of dichloromethane and methanol as eluent. It was decided that all further purifications using column chromatography of porphyrins would be carried out using neutral alumina, regardless of what stationary phases were suggested in literature reports. The porphyrins appeared to react with the acidic silica, changing from a deep purple color to green and this product would not elute from the column. As a precaution, before each purification using chromatography on alumina the reaction mixture was washed with a saturated aqueous sodium bicarbonate solution to ensure that the product mixture was basic and less likely to react with the alumina. This significantly increased the ease by which prophyrins could be purified using column chromatography for the remainder of the compounds synthesized in this

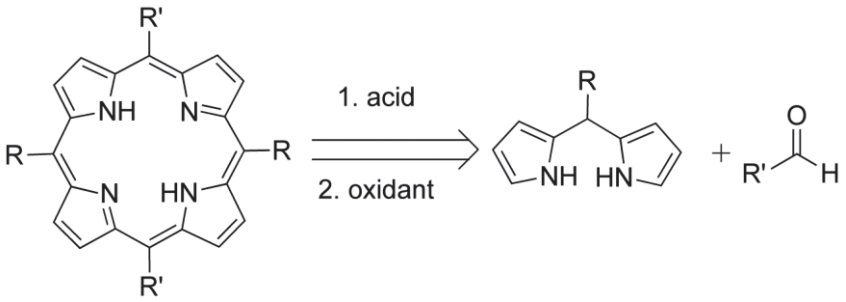
project.



22

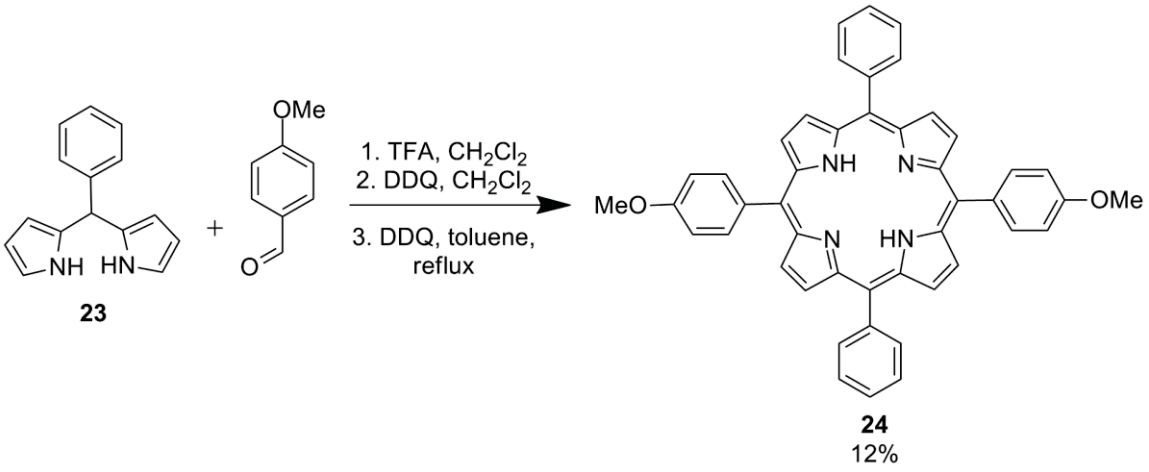
Reaction Scheme 12. One-pot porphyrin synthesis using BF_3-OEt_2 and TFA.

Literature results,[23, 32a, 33, 36] along with the results obtained in this study, highlighted the difficulties associated with porphyrin synthesis following this "one-step method". The conditions promote the formation of oligomers as well as the desired porphyrin, which causes low yields and difficult purifications due to the number of products present in the reaction mixture. For this reason, another "one-step synthesis" for functionalized porphyrins was investigated which involves the self-condensation of an α-free dipyrromethane with an aldehyde as is shown in Reaction Scheme 13.[32] Either the α-free dipyrromethane or the aldehyde could contain the desired functionalization. This synthesis would lead to fewer potential products being formed, and therefore would increase the yield obtained as well as improve the ease of purification.



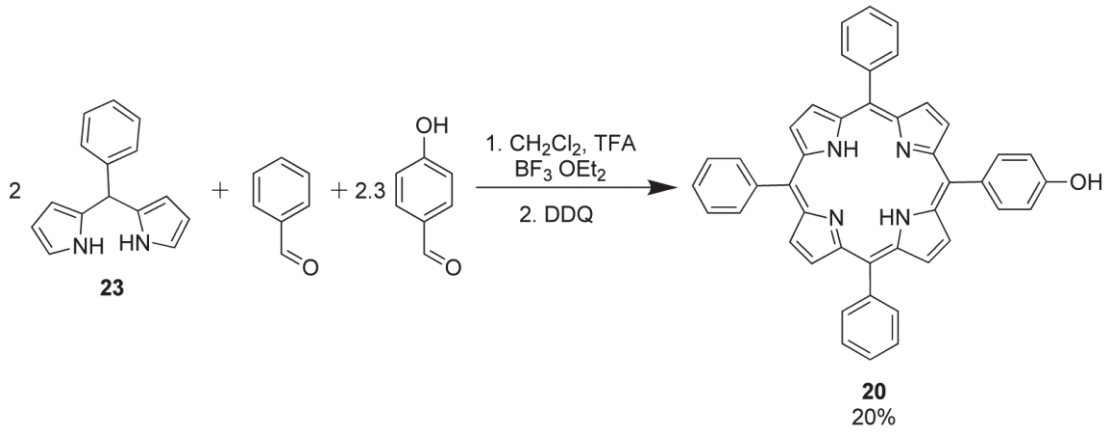
Reaction Scheme 13. General porphyrin synthesis utilizing an α-free dipyrromethane and aldehyde.

The first reaction attempted using this strategy aimed to synthesize di-methoxy containing porphyrin **24** as shown in Reaction Scheme 14. The mono-deprotection of one methoxy group would yield a compound containing only one hydroxyl group available for coupling with prodigiosene **14**. Dipyrromethane **23**,[35] which was available in the lab, and *p*-anisaldehyde were reacted in dichloromethane in the presence of trifluoroacetic acid at room temperature followed by oxidation using DDQ. Purification using column chromatography on alumina yielded porphyrin **24** as a dark purple solid in a 12% isolated yield. Deprotection of only one methoxy group of this compound (**24**) would yield a porphyrin with only one position available for coupling with prodigiosene.



Reaction Scheme 14. Synthesis of porphyrin **24**.

At the same time as porphyrin **24** was being synthesized, the synthesis of porphyrin **20** was attempted once again following a different procedure (shown in Reaction Scheme 15).[23] This porphyrin would be immediately ready for coupling, unlike porphyrin **24** which would require further transformation to establish the hydroxyl group required for coupling. Dipyrromethane **23** was reacted with benzaldehyde and 4-hydroxybenzaldehyde in the presence of catalytic amounts of both trifluoroacetic acid and boron trifluoride diethyl etherate overnight, before oxidation using DDQ. Following purification using column chromatography on alumina, the desired compound (**20**) was isolated in 20% yield. This reaction yielded a sufficient amount of porphyrin to be used in coupling trials with prodigiosenes by myself and other members of the Thompson group. Each time a poprhyrin was synthesized, a significant amount of TPP was also isolated. This is to be expected due to the wide range of possible products that could form under the reaction conditions.



Reaction Scheme 15. Synthesis of porphyrin **20**.

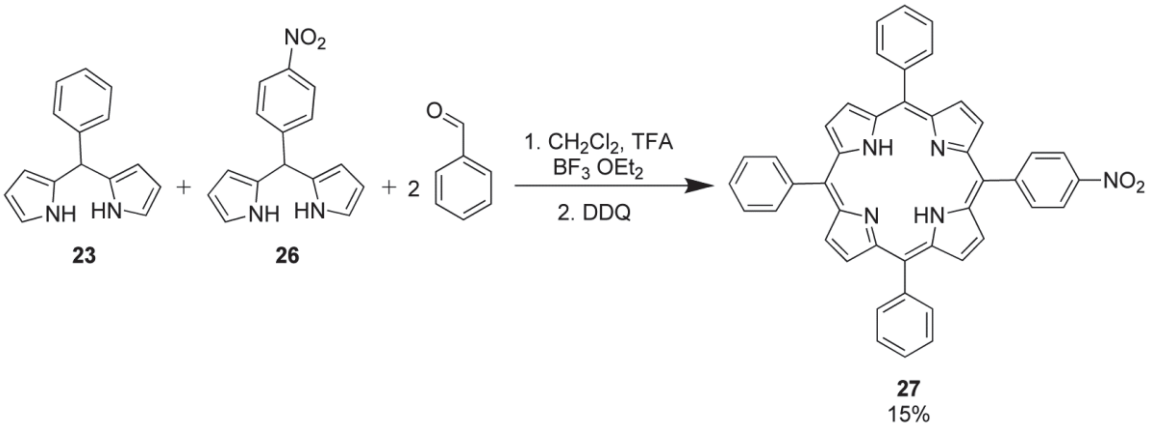
Due to the greater stability of an amide bond as compared to an ester, a porphyrin containing an amino group was synthesized. The first method investigated used TPP which was previously isolated from the synthesis of other porphyrins in this research

study. The synthetic route towards an amine-containing porphyrin is shown in Reaction Scheme 16.[37] The first stage of the synthesis involved nitration of one of the phenyl rings of TPP using sodium nitrate and trifluoroacetic acid. The reaction time was very short (5 minutes) in an attempt to prevent multiple nitrations from occurring. Following removal of the trifluoroacetic acid under reduced pressure, reduction of the nitro functional group to an amine using tin(II) chloride and concentrated hydrochloric acid was attempted. Following an aqueous work-up and removal of solvent under reduced pressure, a very small amount of a brown film was isolated. This film was shown to be a mixture of products and decomposition products using ^{1}H NMR spectroscopy.

21 **25**

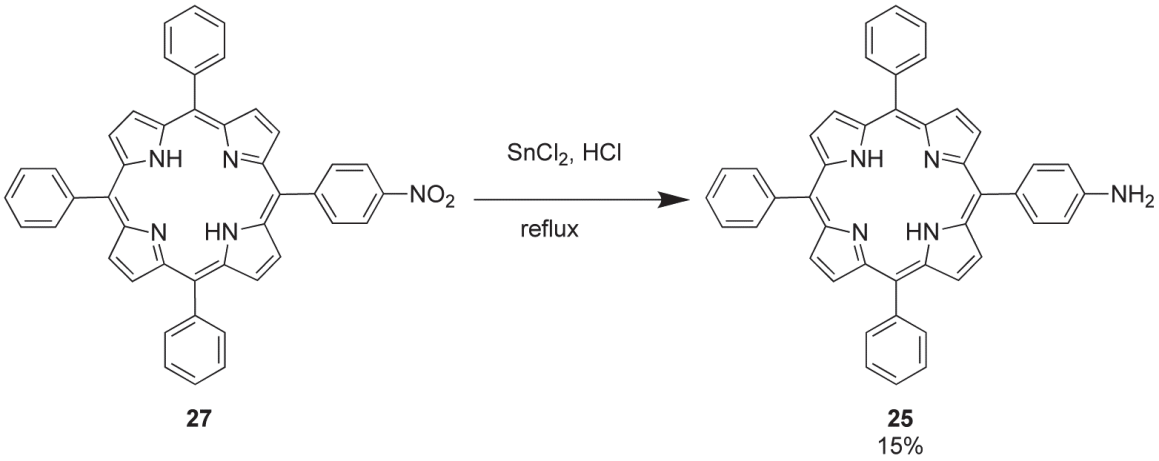
Reaction Scheme 16. One-pot nitration and reduction to form porphyrin **25**.

This synthesis was not repeated due to the wide variety of possible products formed during the nitration. A second method was chosen to be investigated involving first synthesizing the nitro-containing porphyrin **27** from two different α-unsubstituted dipyrromethanes and benzaldehyde in a one-pot procedure as had been previously used in the synthesis of compound **20** (Reaction Scheme 17).[23] This was followed by reduction of the nitro group to form the amine-containing porphyrin **25**, shown in Reaction Scheme 18.[38]



Reaction Scheme 17. One-pot syntheis of nitro-containing porphyrin **27**.

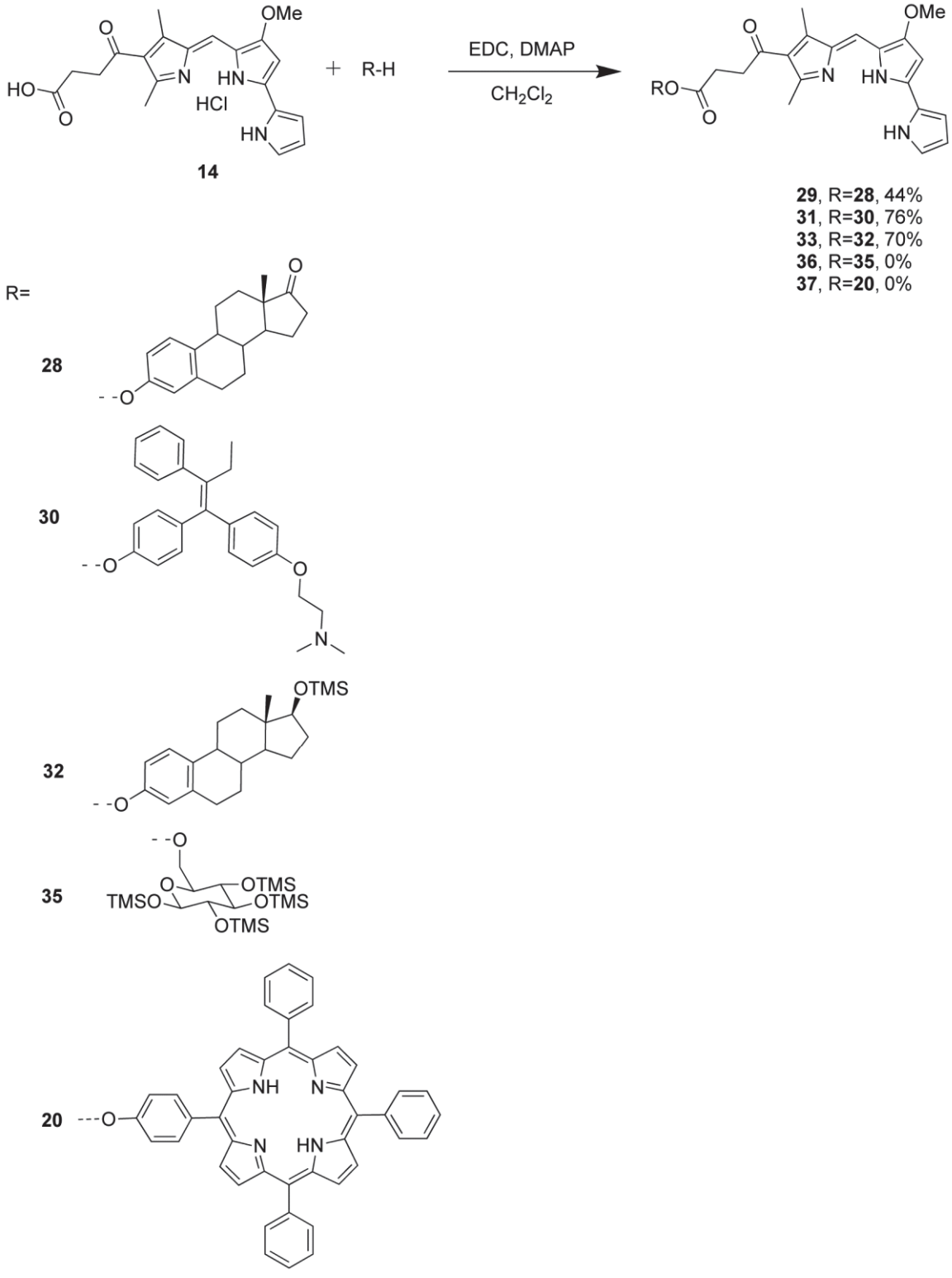
Following purification using column chromatography on alumina, the desired mono-nitro porphyrin **27** was isolated in a 15% yield as well as TPP (8%) and the di-nitro porphyrin (6%). Porphyrin **27** was then subjected to reduction conditions to yield the desired amino-porphyrin **25** in an 86% yield following purification using column chromatography on alumina. This reaction successfully yielded enough amine-containing porphyrin for coupling reactions to be investigated with prodigiosenes.



Reaction Scheme 18. Reduction of amino-containing porphyin **25**.

2.3 Prodigiosene Coupling Reactions

When a sufficient amount of each starting material required for coupling reactions had been synthesized, trials of the coupling reactions were carried out following procedures previously attempted by Dr. Estelle Marchal on a prodigiosene with an adipate side chain rather than the succinate shown in this work.[39] An sterification utilizing 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and dimethylamino pyridine (DMAP) was chosen for the coupling of prodigiosenes with targets containing a hydroxyl group as shown below in Reaction Scheme 19. EDC was chosen for the reaction rather than the traditional *N*,*N*'-dicyclohexylcarbodiimide (DCC) as both the EDC and its urea byproduct are water-soluble and can be removed from the reaction mixture using an aqueous work-up, while DCC is not water soluble. Estrone **28** was commercially available while *Z*-hydroxy Tamoxifen **30,** TMS-protected estradiol **34** and TMS-protected glucose **35** were synthesized by Dr. Estelle Marchal and Dr. Imam Uddin.

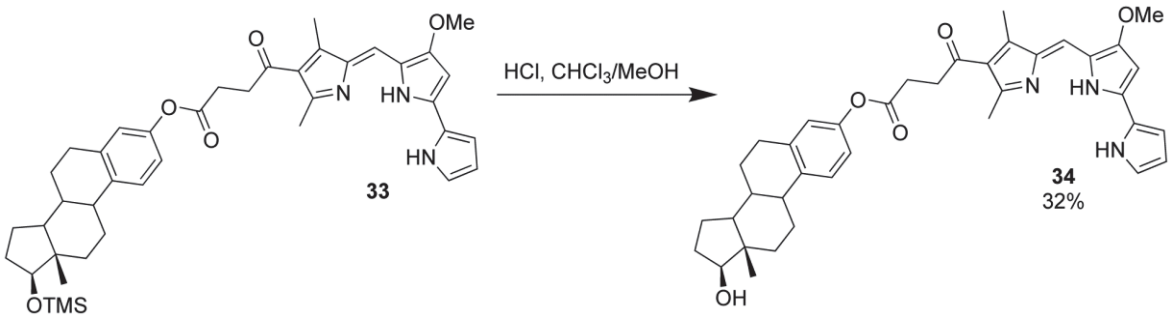


Reaction Scheme 19. Prodigiosene conjugate coupling reactions.

Prodigiosene conjugates with estrone (**29**), Tamoxifen (**31**) and TMS-protected estradiol (**33**) were successfully synthesized and purified to yield the desired products in 44, 76 and 70% yields, respectively. The prodigiosene conjugate with TMS-protected glucose (**36**) was successfully synthesized as shown *via* ^{1}H NMR and mass spectra of the crude reaction mixture. However, the product was not successfully purified despite multiple attempts using column chromatography with varying solvent systems. Starting material, TMS-glucose (**35)** remained with the product and was not successfully removed, therefore, an accurate yield was not calculated and the compound was not deprotected and used in biological trials. The prodigiosene-hydroxy porphyrin conjugate **37** was also shown using ^{1}H NMR spectroscopy and mass spectrometry to have been synthesized from the coupling. However the compound was not successfully purified of reaction byproducts and starting materials so it was also not suitable for biological testing. The difficulty in purifying both of these conjugates was also noted by Dr. Estelle Marchal.[39a] The stability of the ester bond in the conjugate is unknown and it is possible the conjugate could decompose during purification leading to the variety of products isolated following column chromatography.

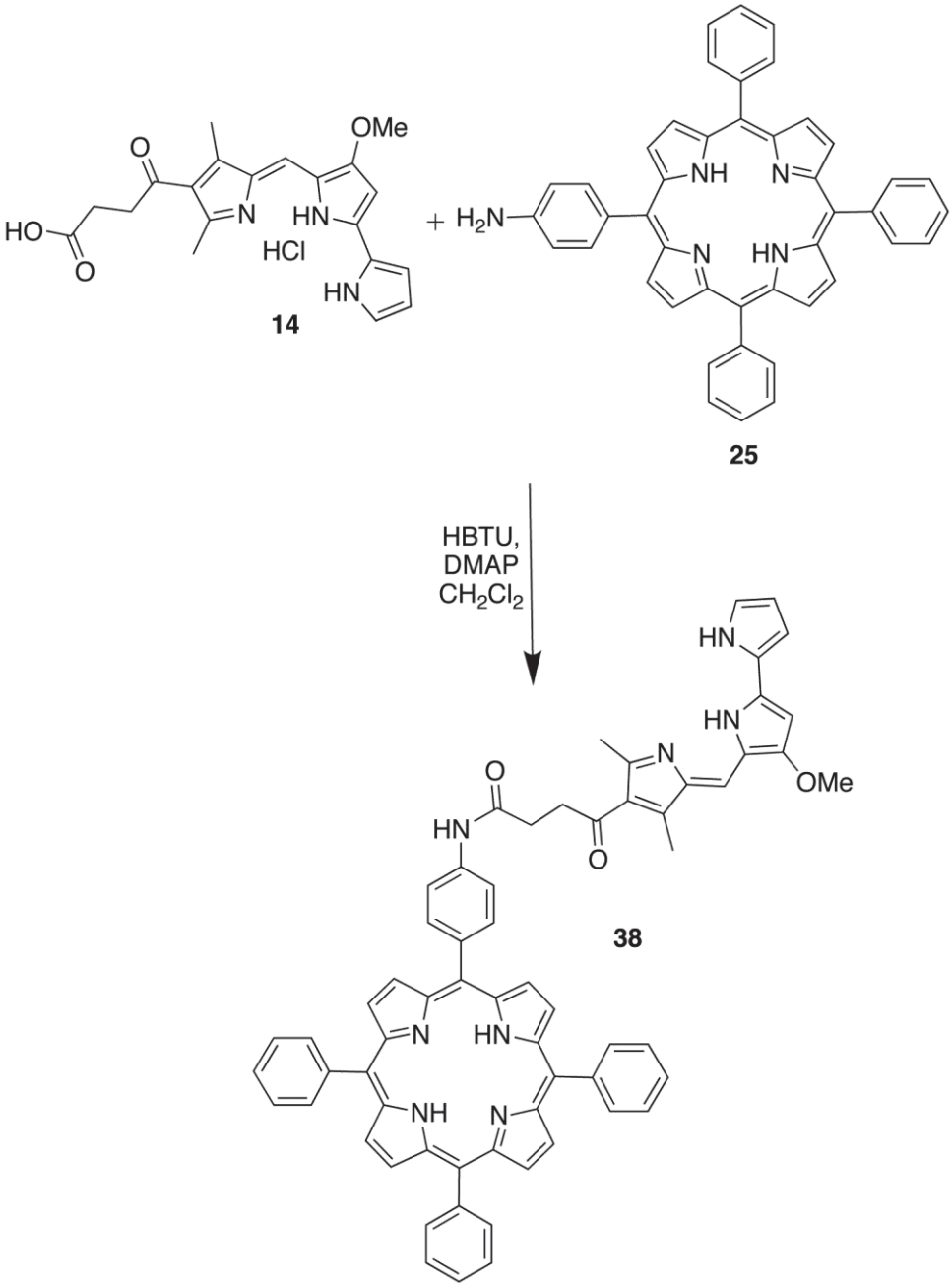
Conjugate **33** required removal of the TMS protecting group in order for this construct to be used for biological testing (shown in Reaction Scheme 20). The deprotection was performed using an acidic methanol solution followed by filtration to yield the desired compound **35** as a dark pink solid (32%). The isolated yield for this reaction was quite low, even though TLC of the reaction mixture showed no starting material remained at the end of the reaction time. It is thought that this is due to the solubility of the product.

The product was washed with methanol and diethyl ether as was previously done for both the adipate and sebacate conjugates. However it appeared that **34** was partially soluble in these solvents leading to the loss of product.



Reaction Scheme 20. Deprotection of TMS estrone-prodigiosene conjugate **34**.

The final conjugate synthesis attempted involved the creation of an amide bond which we believed would be more stable biologically and during purification. Peptide coupling of prodigiosene acid **14** and amino-porphyrin **25** was attempted using *o*-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HBTU) and DMAP as shown below in Reaction Scheme 21. Once again the presence of the product was shown using ^{1}H NMR spectroscopy and mass spectrometry. However complete purification of the product using column chromatography was not successful.



Reaction Scheme 21. Attempted synthesis of prodigiosene-amino porphyrin conjugate **38**.

2.4 Biological Testing

2.4.1 MTT Testing

In order to examine the potential cytotoxic effects of the compounds synthesized in this study, as well as several compounds synthesized by other members of the Thompson group, a series of colorimetric assays was performed. This research was carried out in the Department of Pathology at Dalhousie University under the supervision of Dr. Graham Dellaire. The assay chosen uses 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Viable cells have the ability to convert the water-soluble yellow MTT into an insoluble formazan precipitate which is deep purple in color.[40] The formazan crystals can be dissolved in a variety of organic solvents and the optical density of the resulting solution is measured using a multiwell spectrophotometer. Optimized conditions were utilized, whereby cells that had been treated with the selected compound for 72-hours were incubated with an MTT solution in a phosphate buffer solution for 4 hours and the resulting formazan precipitate was dissolved in DMSO for measurement.[40] The MTT cell viability assay was chosen as it is extremely rapid and provides reliable results.[40] Another reason the MTT assay was chosen is that prodigiosenes are highly colored and the MTT assay provided a way to prevent false results due to absorbance of the prodigiosenes themselves. Wells containing the prodigiosene drug and no cells were used to provide a reference for each concentration of compound tested to provide an accurate zero-reading. There were four replicates for each compound being tested in each run, and each compound was tested two separate times to account for human error, cell pass effects and to ensure the reliability of the results. The standard deviations for these

readings are shown on the cell viability graphs as error bars. Cell viability is presented as a ratio of the absorbance of the treated cells to the absorbance of wells containing cells which had not been treated with a compound. Therefore, a cell viability value of one corresponds to no growth inhibition or cytotoxicity. Both the MCF-7 and MDA-MB-231 breast cancer cell lines were chosen to be used in this study. These cell lines are classic examples of estrogen receptor positive (MCF-7) and estrogen receptor negative (MDA-MB-231) breast cancer cell lines. This provides the possibility to observe differential activity based on the presence of the estrogen receptor.

Testing of ethyl 4-[5-(4-methoxy-1*H*,1'*H*-[2,2']bipyrrolyl-5-ylmethylene)-2,4-dimethyl-5*H*-pyrrol-3-yl]-oxobutanoate **12** and prodgiosene acid **14** (shown in Figure 15) to determine the activity of the prodigiosene without appended targeting moieties on MCF-7 breast cancer cells was first carried out. The dose-response graphs for these compounds are shown in Figure 16. The results show that the ethyl ester prodigiosene **12** possesses significantly greater activity than the prodigiosene acid **14**. This result can be attributed to the presence of the carboxylic acid moiety. Carboxylic acids are known to have difficulty entering cells and could also be interacting with proteins in the cell medium.[41]

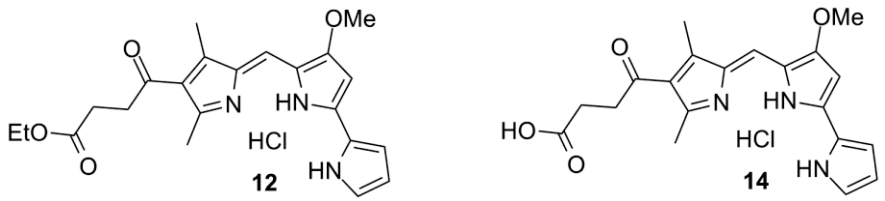


Figure 15. Prodigiosene **12** and prodigiosene acid **14**.

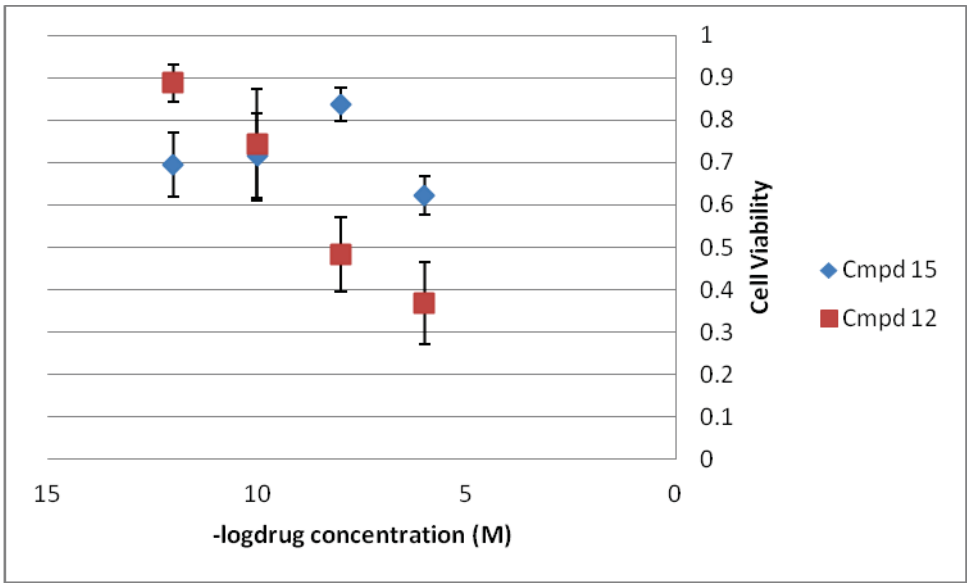


Figure 16. Dose response curves for prodigiosenes **12** and **14**.

Each prodigiosene conjugate (Figure 17) was then tested on the MCF-7 cell line to examine the effect of attaching an estrogen receptor-targeting moiety to the prodigiosene on its activity in an estrogen receptor enriched cell line.

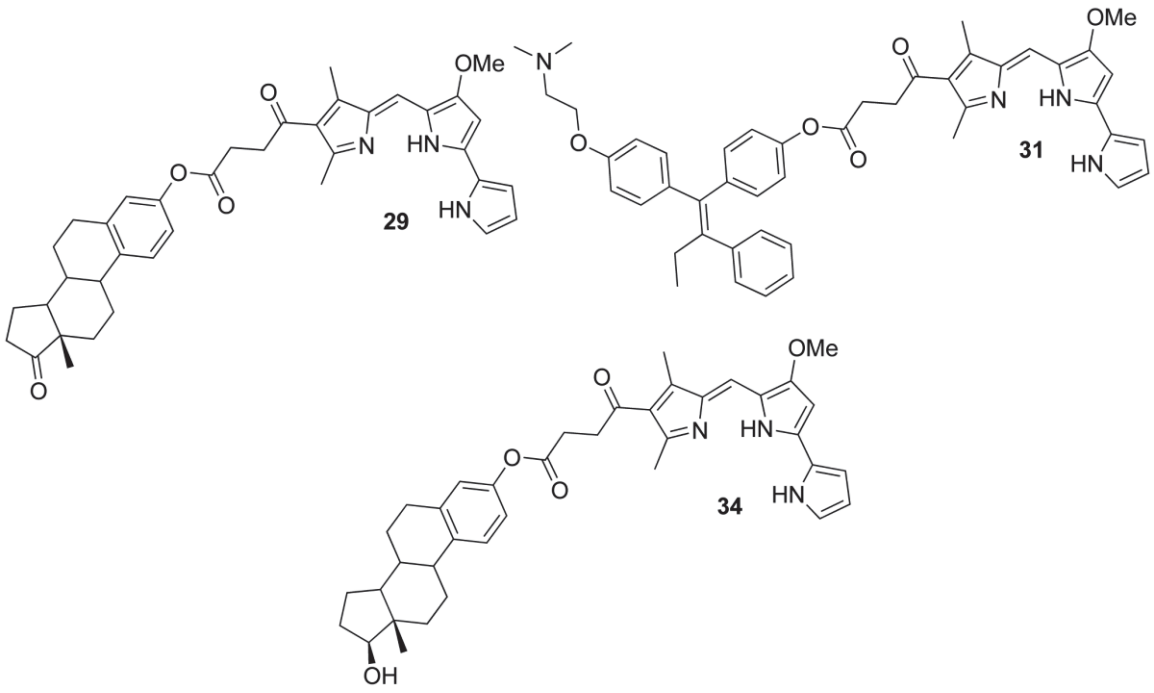


Figure 17. C-2 prodigiosene conjugates.

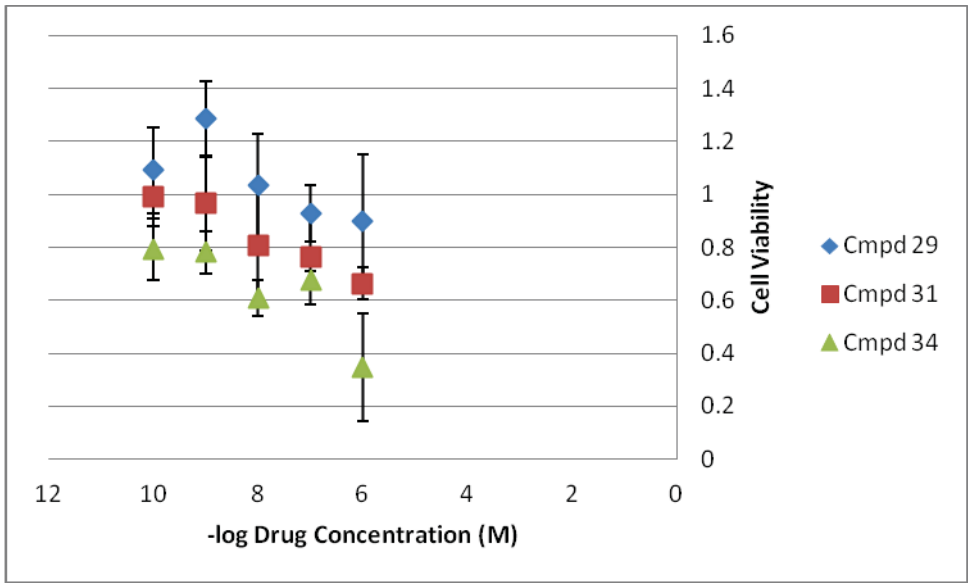


Figure 18. Dose response graphs for ethyl 4-[5-(4-methoxy-1*H*,1'*H*-[2,2']bipyrrolyl-5-ylmethylene)-2,4-dimethyl-5*H*-pyrrol-3-yl]-oxobutanoate conjugates.

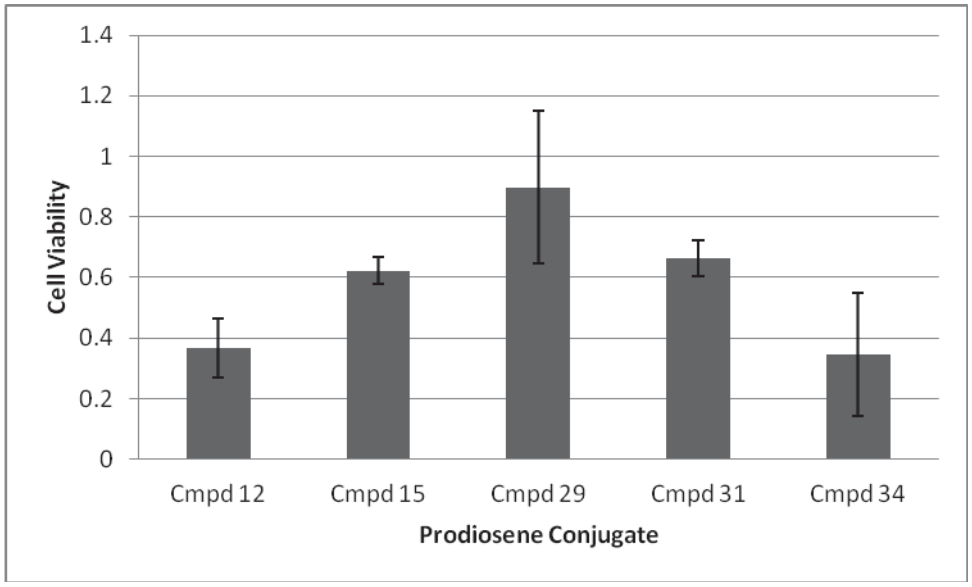
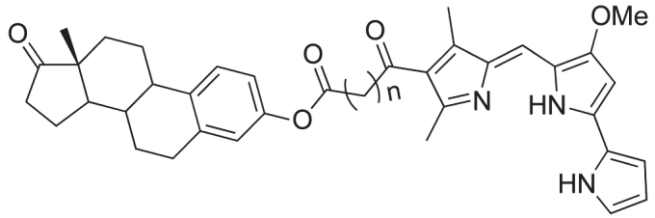


Figure 19. Cell viability following treatment of MCF-7 cells with various ethyl 4-[5-(4-methoxy-1*H*,1'*H*-[2,2']bipyrrolyl-5-ylmethylene)-2,4-dimethyl-5*H*-pyrrol-3-yl]-oxobutanoate at 10^{-6} M.

Dose-response graphs for each conjugate can be seen in Figure 18 while the measured cell viability for cells treated with 10^{-6} M of each prodigiosene conjugate are shown in Figure 19. It can be seen that the estradiol-prodigiosene conjugate **34** (0.347) has an activity similar to the unaltered ethyl 4-[5-(4-methoxy-1*H*,1'*H*-[2,2']bipyrrolyl-5-

ylmethylene)-2,4-dimethyl-5*H*-pyrrol-3-yl]-oxobutanoate **12** (0.367) while the Tamoxifen-prodigiosene conjugate **31** (0.663) has an activity similar to the prodigiosene acid **14** (0.623). The estrone-prodigiosene conjugate **29** (0.898) is shown to have the least activity of all the conjugates. This lower activity of compound **29** may be due to the lower apparent dissociation constant for estradiol compared to estrone.[19] This suggests that while the conjugate is bound to the estrogen receptor it is unable to successfully carry out its apoptosis-promoting activities. While the estrone is bound to the receptor it promotes growth of the cell line, while the prodigiosene is counteracting that by promoting apoptosis. These two counter-active activities are in competition as long as the conjugate remains bound to the receptor.

MTT assays were also performed on the MCF-7 cell line using conjugates of estrone, estradiol and Tamoxifen with prodigiosenes of different chain lengths with hopes to notice a difference in activity which could be attributed to chain length. The prodigiosene conjugates with adipate and sebacate side chains were synthesized by Dr. Estelle Marchal and Dr. Imam Uddin of the Thompson research group.



Compound **29**, **n**=2
Compound **39**, **n**=4
Compound **40**, **n**=8

Figure 20. Estrone-prodigiosene conjugates of varying chain lengths.

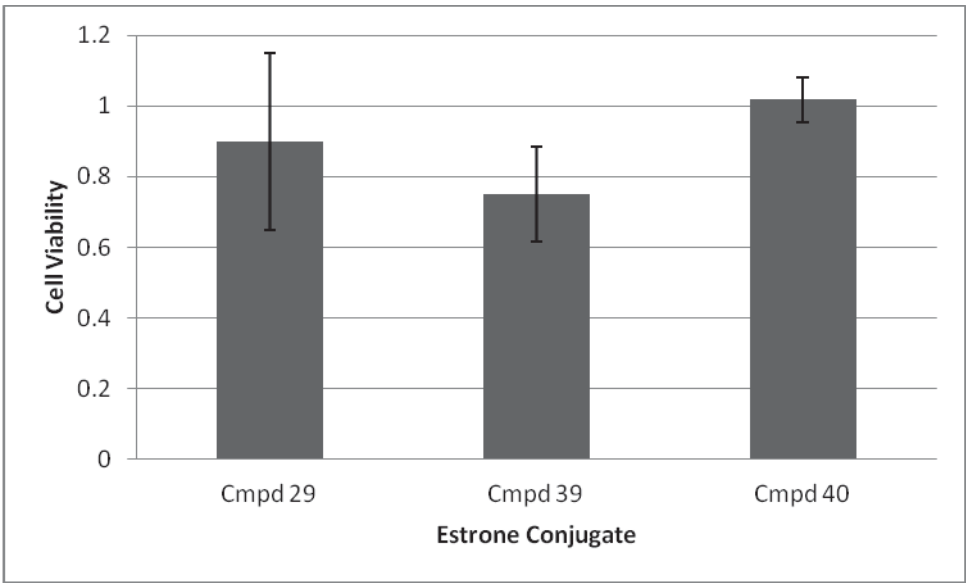
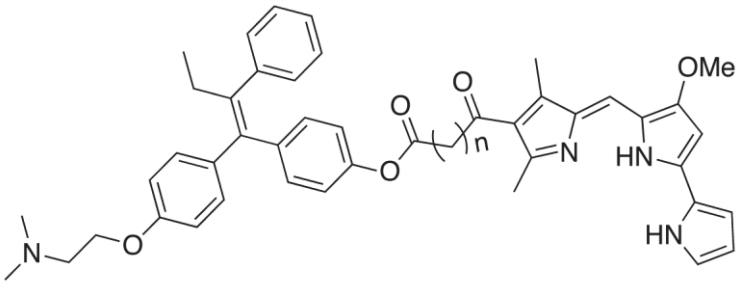


Figure 21. Cell viability following treatment of MCF-7 cell line with 10^{-6} M prodigiosene-estrone conjugates.

The estrone-prodgiosene conjugates (Figure 20) all demonstrated poor activity against the MCF-7 cell line, shown in Figure 21. There was no statistically significant difference between the cell viability of succinate and adipate conjugates **29** and C-4 conjugate **39** ($t(11)=1.387$, $p>0.05$). Both compound **29** and **39** exhibited a statistically significant increase in activity as compared to sebacate conjugate **40**, $t(11)=1.97$, $p<0.05$ and $t(14)=2.198$, $p<0.05$, respectively.



Compound **31**, **n**=2
Compound **41**, **n**=4
Compound **42**, **n**=8

Figure 22. Tamoxifen-prodigiosene conjugates of varying chain lengths.

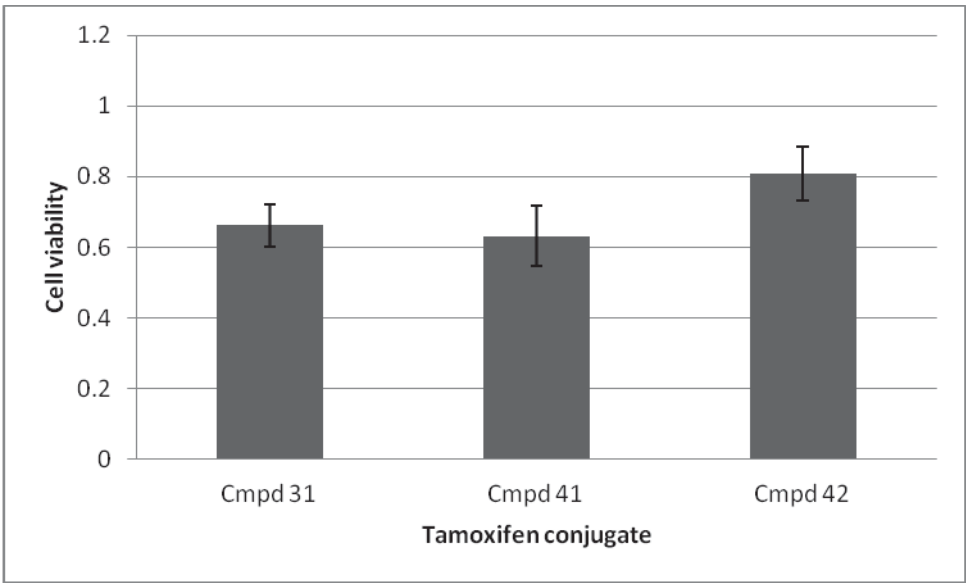
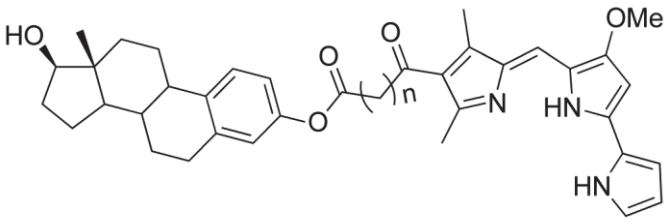


Figure 23. Cell viability following treatment of MCF-7 cell line with 10^{-6} M prodigiosene-Tamoxifen conjugates.

The observed activity of Tamoxifen-prodigiosene conjugates (Figure 22) against the MCF-7 cell line is shown in Figure 23. There was no statistically significant difference between the cell viability of succinate conjugate **31** and adipate conjugate **41** ($t(10)=0.771$, $p>0.05$). Both compound **31** and **41** exhibited enhanced activity as compared to sebacate conjugate **42**, $t(10)=1.823$, $p<0.05$ and $t(6)=3.088$, $p<0.05$, respectively.



Compound **34**, **n**=2
Compound **43**, **n**=4
Compound **44**, **n**=8

Figure 24. Estradiol-prodigiosene conjugates of varying chain lengths.

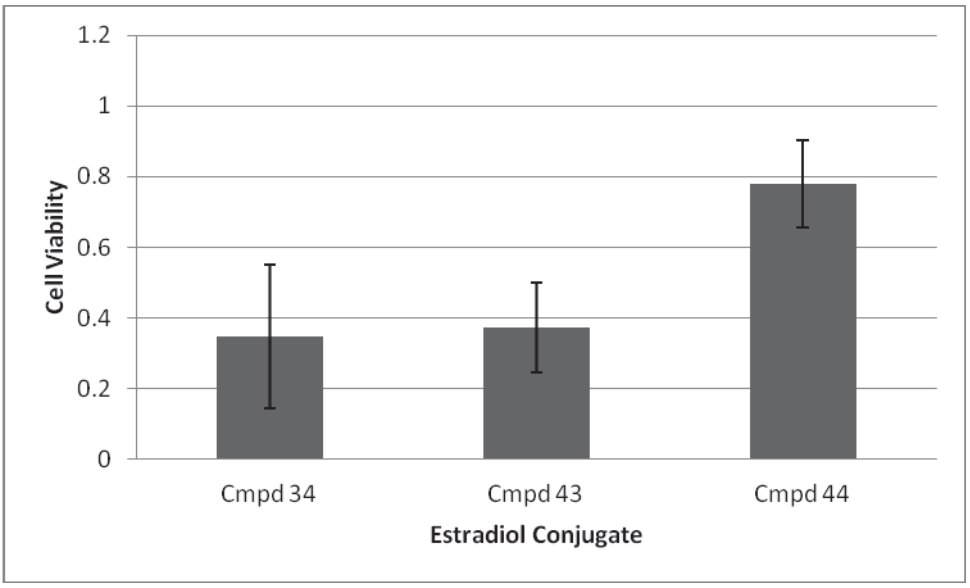


Figure 25. Cell viability following treatment of MCF-7 cell line with 10^{-6} M prodigiosene-estradiol conjugates.

Estradiol-prodgiosene conjugates (Figure 24) exhibited the greatest activity against the MCF-7 cell line (Figure 25).There was no statistically significant difference between the cell viability of succinate conjugate **34** and adipate conjugate **43** ($t(9)=0.219$, $p>0.05$). Both compound **34** and **43** exhibited enhanced activity as compared to sebacate conjugate **44**, $t(9)=3.810$, $p<0.05$ and $t(6)=4.625$, $p<0.05$, respectively. It was shown that for each of the conjugates, the sebacate chain linker performed poorly as compared to the other chain lengths.

To determine any possible effect caused by the presence of estrogen receptors, the differential activity of each of the succinate conjugates and ethyl 4-[5-(4-methoxy-1*H*,1'*H*-[2,2']bipyrrolyl-5-ylmethylene)-2,4-dimethyl-5*H*-pyrrol-3-yl]-oxobutanoate was then investigated using the MDA-MB-231 cell line. Following the same protocol for the MCF-7 cell line, the biological activity of each compound was determined using MTT

assays. The results from these studies, compared to results from the MCF-7 cell line are shown in Figure 26.

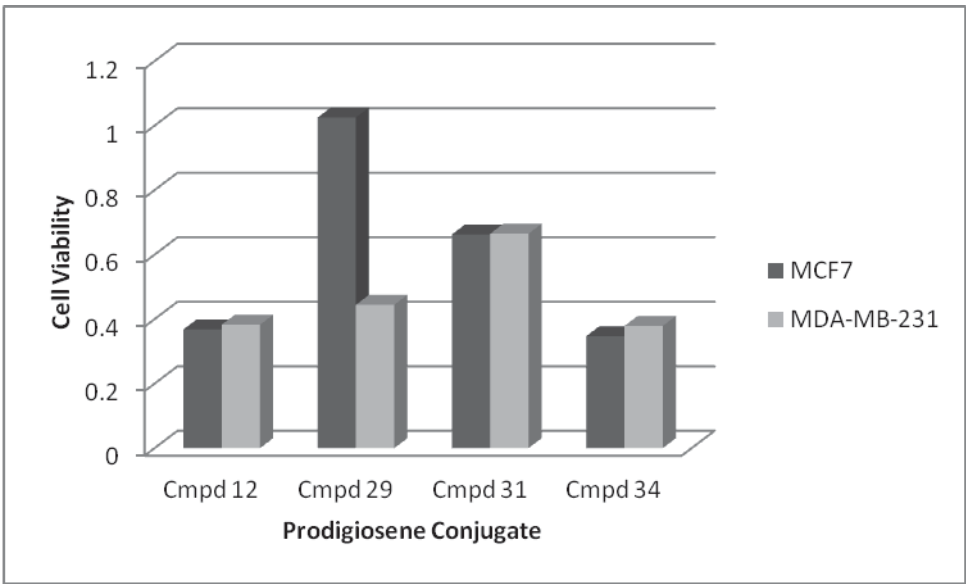


Figure 26. Cell viability following treatment of MCF-7 and MDA-MB-231 breast cancer cell lines with C-2 ethyl ester prodigiosene conjugates at 10^{-6} M.

There was no notable difference in activity between cell lines for compounds **12**, **31** and **34**. This suggests that these compounds, while possessing estrogen targeting moieties, possess the same cytotoxic effect on estrogen receptor positive and negative cell lines. This result has been seen before in cell viability studies using MTT assays on prodigiosin.[42] The natural product prodigiosin, the parent compound of the prodigiosenes tested in this study, was shown not to exhibit differential activity between MCF-7 and MDA-MB-231 cell lines.[42] The estrone-prodigiosene conjugate **29**, however, did exhibit differential activity in the two studied cell lines. There was a significant difference in cell viability following treatment with 10^{-6} M concentration of the compound ($t(8) = 3.813$,

$p<0.005$). The cell viability assays were repeated three times to confirm this differential activity.

2.4.2 NCI60 Cell Line Testing

Estrone-prodigiosene conjugate **29** was sent for further analysis against the NCI60 panel of human tumor cell lines. The Developmental Therapeutics Program (DTP) of the National Cancer Institute (NCI) employs the NCI60 cell line screen as an early stage of drug discovery and development. The NCI60 cell line screen consists of 60 human tumor cell lines, each chosen for their ability to perform consistently and provided appropriate representation of a variety of tumor types.[43] Each cell line used has been extensively characterized by various research groups in the world.[43] The drug screen involves treatment of each cell line with compounds over a 5-log mol/L concentration range for 2 days.[43-44] The cells are then fixed and stained with sulphorhodamine B and optical densities are measured.[43-44] Growth inhibition is calculated relative to cells at the time zero control and those without drug treatment.[43-44] Selected results for the NCI60 cell line screen are depicted in Table 1 and GI50 values are shown in Figure 27. The results for the entire cell line screen are presented in Table 2 and, found in Appendix A.

Table 1. Activity of estrone-conjugate **29** against selected human tumor cell lines.

Cell Line	Tumor Type	GI50	TGI	LC50
MCF-7	Breast Cancer	1.87E-06	2.43E-05	>1.00E-04
MDA-MB-231	Breast Cancer	2.91E-07	9.79E-07	1.90E-05
HCT-116	Colon Cancer	5.77E-07	>1.00E-04	>1.00E-04
HT29	Colon Cancer	6.37E-07	>1.00E-04	>1.00E-04
SW-620	Colon Cancer	7.39E-07	>1.00E-04	>1.00E-04
CCRF-CEM	Leukemia	6.46E-07	>1.00E-04	>1.00E-04
SR	Leukemia	3.85E-07	>1.00E-04	>1.00E-04
LOX IMVI	Melanoma	5.63E-07	2.37E-06	8.06E-06
DU-145	Prostate Cancer	8.03E-07	>1.00E-04	>1.00E-04

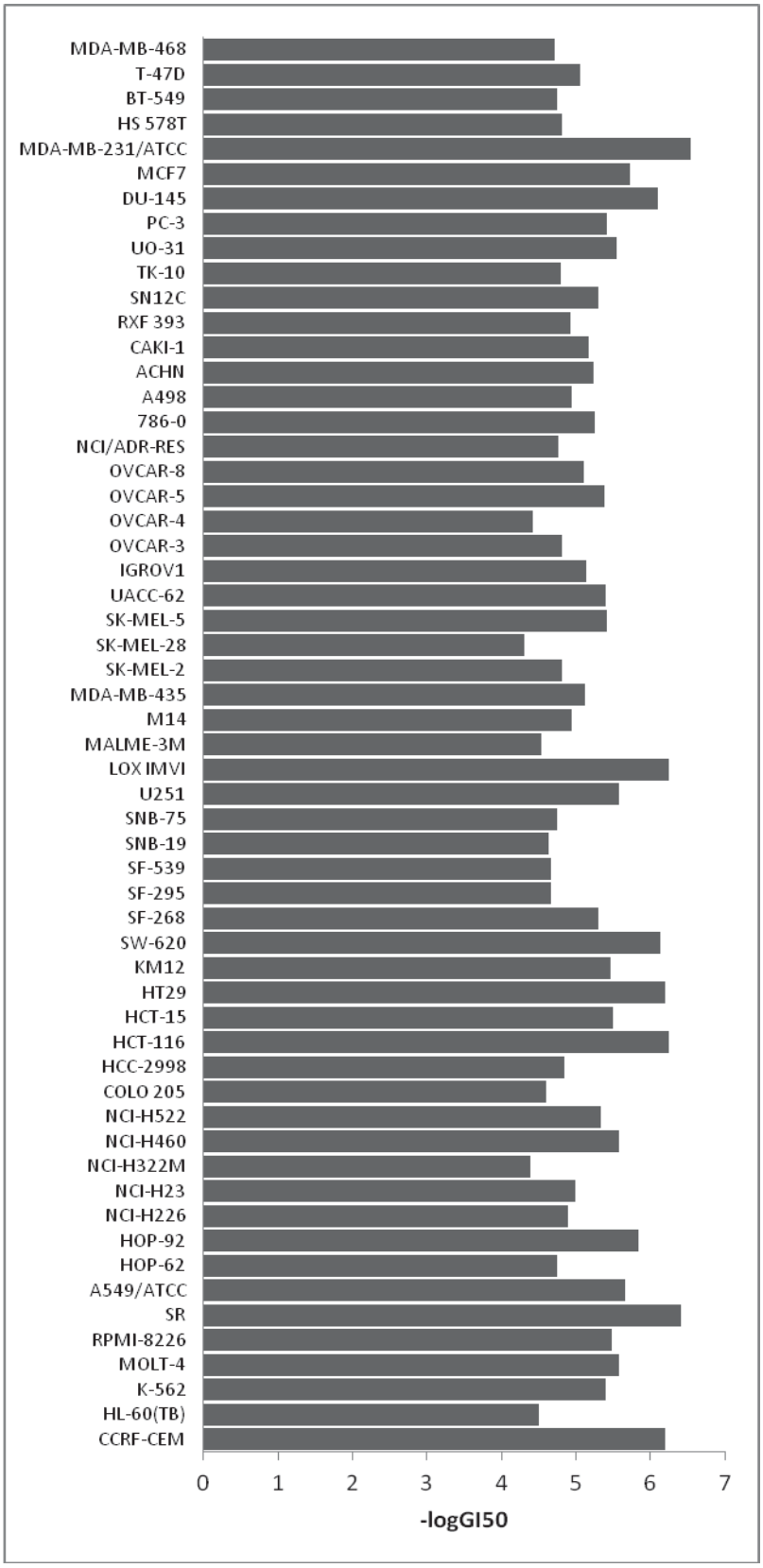


Figure 27. NCI60 cell line screen results for compound **29.**

The results obtained are quite interesting. As seen in the results from the MTT assays, there is a significant difference in activity between the estrogen receptor-positive MCF-7 cell line and the estrogen receptor-negative cell line MDA-MB-231 cell line as can be seen by the differing GI50 values for these cell lines. While numerical results may vary between the types of cell viability assays used, the appearance of the same trend in the NCI60 cell line screen supports the previous results obtained from the MTT assays. Compound **29** was shown to be active against other cell lines that were not related to breast cancer, most notably selected colon cancer, leukemia, melanoma and prostate cancer cell lines. This is interesting as none of these cell types would be enriched with ER α as is the MCF-7 cell line, while the different types of breast cancer and ovarian cancer for example would. However, previous research studies have noted the presence of either estrogen metabolites or the estrogen receptor β in each of the cell lines for which an increase in activity was noted for compound **29**.[45] Estrone possesses an affinity for estrogen receptor α which is present in the MCF-7 cell line. These results suggest that the activity of the targeted compound may not necessarily be attributed to the presence of the receptor but instead to the metabolism of the cell. Cancer cells are rapidly dividing and therefore have higher metabolic rates than healthy cells, with an increased rate of production for various products including cholesterol. It is possible that the targeted compound enters the cell as the cancer cell has an increased uptake of estrogens in order to scavenge starting materials for cholesterol synthesis.[46] Previous studies have also noted that prodigiosin has the ability to trigger apoptosis in hematopoietic cancer cell lines as well as colon and gastric cell lines.[5a] This provides another potential explanation for the activity seen of compound **29** against the leukemia and colon cancer cell lines.

Also notable is the difference between the GI50 and TGI50 values for the cell lines treated with compound **29**. This is the first time such a large difference between these two values has been seen in compounds tested by the Thompson lab.[8] This shows that the compound can inhibit growth without completely stopping the growth at low concentrations. The GI50 values obtained across the 60-cell line were overall higher than some previously determined values for other prodigiosenes from the Thompson group, however there was still excellent activity determined for certain cell lines (shown in Table 1). The LC50 values for compound **29** were significantly lower than those previously obtained for prodigiosene analogues from the Thompson lab, meaning that it is less cytotoxic than those previously synthesized and tested. The large difference between GI50 and LC50 demonstrates the ability of compound **29** to stop the growth of the cells without killing them. These results are promising and reinforce the need for further exploration into the targeting of prodigiosenes and further studies on conjugates already synthesized.

The prodigiosene-estrone conjugate was submitted for further testing by the NIH against the NCI60 cell line. Differential activity was also demonstrated; corresponding to the results obtained *via* the MTT assays. It is predicted that the uptake of the prodigiosene-estrone conjugate into cancer cells is not related to the presence of the ER, but rather estrogen metabolism. Further studies are required to further understand the biological activity of the conjugates.

CHAPTER 3: CONCLUSIONS AND FUTURE DIRECTION

The goal of this project was to synthesize and evaluate the biological activity of a small series of prodigiosenes. The prodigiosenes synthesized were based on an ethyl 4-[5-(4-methoxy-1*H*,1'*H*-[2,2']bipyrrolyl-5-ylmethylene)-2,4-dimethyl-5*H*-pyrrol-3-yl]-oxobutanoate core skeleton conjugated to a series of tumor-targeting moieties. The synthesis of porphyrins for use in conjugation with prodigiosene was also completed as part of the study.

Conjugates of prodigiosene with estradiol (E2), Tamoxifen and estrone were successfully synthesized. The protocols to produce and purify conjugates with glucose, 5,10,15-triphenyl-20-(4-aminophenyl)-21*H*,23*H*-porphyrin and 5,10,15-triphenyl-20-(4-hydroxyphenyl)-21*H*,23*H*-porphyrin did not yield the desired compounds. Further research into the synthesis and purification of these conjugates is required.

Results for MTT assays demonstrated promising initial results for the conjugates which were tested. Two trends in activity were observed for the conjugates against MCF-7 cells; the succinate and adipate linker conjugates possessed increased activity as compared to the sebacate linker, and the conjugates ranged in activity with estradiol conjugates as the most active followed by Tamoxifen conjugates and estrone conjugates being least active. The succinate prodigiosene-estrone conjugate **29** was shown to possess differential activity between MCF-7 and MDA-MB231 cells. This result is of interest as a conjugate

with an ER-targeting group had an enhanced activity against cells which were not ER enriched as compared to cells enriched with ER.

In conclusion, the results of this study demonstrate promise for targeted drug design as a way to enhance uptake of anti-neoplastic compounds into cancer cells. Further studies to examine the biological activity of the prodigiosene conjugates are required including their activity against healthy, non-cancerous cells compared to cancerous cells. The synthesis of further conjugates to broaden the series available to be tested will also enhance the understanding the utility of this strategy.

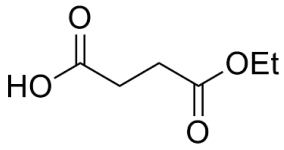
CHAPTER 4: EXPERIMENTAL

4.1 General Experimental

All ^{1}H and ^{13}C NMR experiments performed using either a Bruker 250 MHz or AVANCE 500 MHz spectrometer. Chemical shifts are reported in ppm using the solvent signal $CDCl_3$ (^{1}H NMR 7.26 ppm, ^{13}C NMR 77.2 ppm), CD_2Cl_2 (^{1}H NMR 5.32 ppm, ^{13}C NMR 54.0 ppm) or DMSO-d_6 (^{1}H NMR 2.50 ppm, ^{13}C NMR 39.6 ppm) as internal reference. All coupling constants (*J*) are reported in Hertz (Hz). Column chromatography was performed using 230-400 mesh ultra pure silica or 150 mesh, Brockman III, activated, neutral or basic alumina oxide, as indicated. Preparative thin-layer chromatography plates were made in-lab using 230-400 mesh ultra pure silica. With the exclusion of solvents, which were obtained crude and purified *via* distillation under air and at atm pressure, chemicals were used as received unless otherwise stated. HR and LR mass spectra were obtained using TOF and LCQ Duo ion trap instruments operating in ESI+ mode.

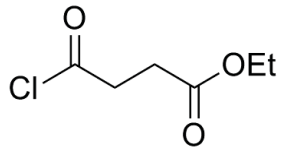
Human breast cancer cell lines MCF-7 and MDA-MB-231 were maintained in DMEM (Sigma Aldrich) medium supplemented with 10% fetal bovine serum (Sigma Aldrich) as well as 1% penicillin and streptomycin (Sigma Aldich). The cells were incubated at 37 ºC and 5% CO_2. MTT analyses followed standard protocol,[40] using a treatment time of 72 h.

4.2 Butanedioic Acid Monoethyl Ester (2)[8]



Following a previously published synthesis,[8] KOH (32.5 g, 0.58 mol) in ethanol (300 mL) was added slowly to a solution of diethyl succinate **1** (100 mL, 0.58 mol) in ethanol (300 mL) at 0 °C. The reaction mixture was stirred for 18 h at room temperature. The reaction mixture was then heated to reflux temperature and hot-filtered to remove the insoluble dipotassium salt. The solvent was removed under reduced pressure, yielding a white solid which was then dissolved in water (90 mL) and extracted with diethyl ether (2x100 mL). The aqueous layer was treated with hydrochloric acid (12 M, 50 mL) at 0˚C and stirred for 1.5 h. Following extraction with dichloromethane (3x100 mL) and drying with anhydrous sodium sulfate, the solvent was removed under reduced pressure giving the title product as a colourless oil (41.0 g, 48 %); δ_H (500 MHz, $CDCl_3$) 11.51 (1H, bs), 4.12 (2H, q, *J* 7.1), 2.64 (2H, t, *J* 6.7), 2.58 (2H, t, *J* 6.7), 1.22 (3H, t, *J* 7.1). The ^{1}H NMR data corresponds with literature values.[8]

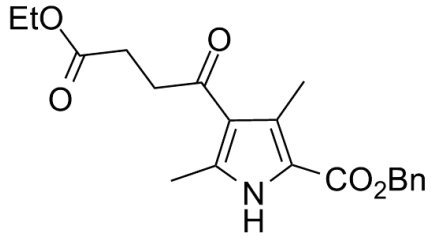
4.3 3-Chlorocarbonyl-propanoic Acid Ethyl Ester (3)[47]



Following a literature method,[47] mono-acid **2** (8.0 g, 54 mmol) was reacted with $SOCl_2$ (4.9 mL, 68 mmol) at reflux temperature for 4 h. Excess $SOCl_2$ was removed under reduced pressure to yield the title product as a yellow oil (8.9 g, quant.); δ_H (250 MHz,

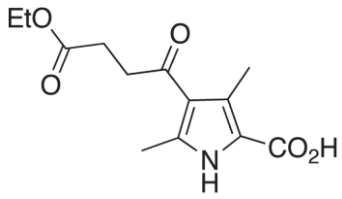
$CDCl_3$) 4.04 (2H, q, *J* 7.1), 3.10 (2H, t, *J* 6.5), 2.55 (2H, t, *J* 6.5), 1.14 (3H, t, *J* 7.1). The ^{1}H NMR data corresponds with literature values.[8]

4.4 4-(3-Ethoxycarbonyl-propanoyl)-3,5-dimethyl-1*H*-pyrrole-2-carboxylic Acid Benzyl Ester (5)[8]



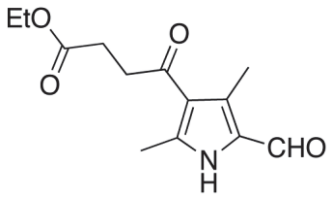
Following a literature procedure,[8] benzyl-3,5-dimethyl-pyrole-2-carboxylate **4**[27] (12.4 g, 54 mmol) was dissolved in anhydrous DCM (100 mL) and the solution was stirred at 0 °C for 10 min under N_2. $SnCl_4$ (6.3 mL, 65 mmol) was added drop-wise and the mixture was stirred at 0°C for an additional 10 min, after which a solution of chlorocarbonyl ester **3** (8.9 g, 54 mmol) in anhydrous DCM (30 mL) was added slowly and the reaction mixture was then stirred at 0˚C for 3.5 h. The solution was then poured into aqueous HCl (1 M, 250 mL) and stirred for 10 min. The reaction mixture was extracted with DCM (3x50 mL), washed with brine (50 mL) and the combined organics were then dried using anhydrous sodium sulfate and concentrated under reduced pressure. The crude product was crystallized from hot methanol to give the title compound as a white solid (9.8 g, 59%); δ_H (500 MHz, $CDCl_3$) 9.49 (1H, s), 7.41-7.33 (5H, m), 5.31 (2H, s), 4.14 (2H, q, *J* 7.1), 3.03 (2H, t, *J* 6.5), 2.68 (2H, t, *J* 6.5), 2.61 (3H, s), 2.48 (3H, s), 1.25 (3H, t, *J* 7.1). The ^{1}H NMR data corresponds with literature values.[8]

4.5 4-(3-Ethoxycarbonyl-propanoyl)-3,5-dimethyl-1*H*-pyrrole-2-carboxylic Acid (6)[8]



As described in the literature,[8] a solution of pyrrole **5** (9.8 g, 28 mmol) and 10% Pd/C (1.0 g) in THF (100 mL) was stirred under H_2 for 23 h. The reaction mixture was filtered through celite, which was then rinsed with methanol (50 mL). The filtrate solvent was removed under reduced pressure to give the title compound as an off-white solid (7.5 g, quant.); δ_H (500 MHz, $CDCl_3$) 9.49 (1H, s), 4.16 (2H, q, *J* 6.9), 3.49 (2H, s), 3.07 (2H, t, *J* 6.1), 2.71 (2H, t, *J* 6.1), 2.66 (3H, s), 2.54 (3H, s), 1.32 (3H, t, *J* 6.9). The ^{1}H NMR data corresponds with literature values.[8]

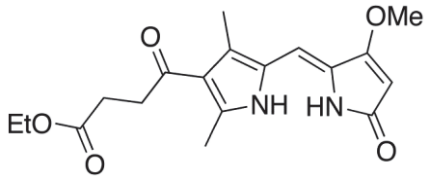
4.6 Ethyl 6-(5-Formyl-2,4-dimethyl-1*H*-pyrrol-3-yl)-6-oxohexanoate (7)[8]



A solution of the acid **6** (4.6 g, 17 mmol) in anhydrous DCM (200 mL) was cooled to 0°C under N_2 and trifluoroacetic acid (36.0 mL, 464 mmol) was added slowly. The reaction mixture was stirred for 3.5 h before addition of trimethyl orthoformate (25.0 mL, 224 mmol) and stirring for a further 20 min. The reaction mixture was quenched using

sat. $NaHCO_3$ (aq), and extracted with DCM (3x50 mL). The combined organic fractions were washed with brine (100 mL) and then dried using anhydrous magnesium sulfate. The crude material was purified using crystallization from hot methanol to yield the desired compound as a white solid (3.1 g, 72% yield); δ_H (500 MHz, $CDCl_3$) 9.66 (1H, s), 9.49 (1H, s), 4.17 (2H, q, *J* 7.1), 3.06 (2H, t, *J* 6.5), 2.72 (2H, t, *J* 6.5), 2.59 (3H, s), 2.57 (3H, s), 1.27 (3H, t, *J* 7.1). The ^1H NMR data corresponds with literature values.[8]

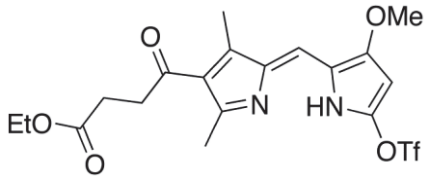
4.7 Ethyl 4-[5-(3-Methoxy-5-oxo-1,5-dihydro-pyrrol-2-ylidene-methyl)-2,4-dimethyl-1*H*-pyrrol-3-yl]-4-oxobutanoate (9)[26]



Following a literature procedure,[26] a solution of the aldehyde **7** in anhydrous DCM (200 mL) was added slowly to a solution of 4-methoxy-3-pyrrolin-2-one (1.5 g, 13 mmol), TMSOTf (3.2 mL, 18 mmol) and triethylamine (5.0 mL, 36 mmol) in anhydrous DCM (200 mL) at 0 °C under N_2 and the reaction mixture was stirred for 3 h. A phosphate buffer solution (250 mL) was added, followed by extraction with DCM (3x100 mL). The combined organic fractions were washed with brine (200 mL), dried with anhydrous magnesium sulfate and the solvent was then removed under reduced pressure. The resulting dark brown solid was dissolved in THF (400 mL), acidified to pH 2 using conc. HCl and the mixture was then stirred for 20 min before the addition of water (200 mL), extracton with DCM (3x200 mL), drying of the combined organics with $MgSO_4$ and removal of the solvent under reduced pressure. The resulting yellow solid was collected

using suction filtration and washed with water and hexanes to yield the title compound (0.761 g, 37%); δ_H (500 MHz, $CDCl_3$) 10.98 (1H, s), 10.57 (1H, s), 6.41 (1H, s), 5.14 (1H, s), 4.17 (2H, q, *J* 7.1), 3.93 (3H, s), 3.08 (2H, t, *J* 6.5), 2.72 (2H, t, *J* 6.5), 2.70 (3H, s), 2.41 (3H, s), 1.28 (3H, t, *J* 7.1). The ^{1}H NMR data corresponds with literature values.[8]

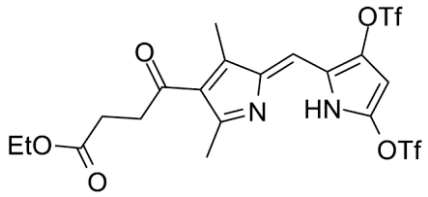
4.8 Ethyl 4-[5-(3-methoxy-5-trifluoromethanesulfonyloxy-1*H*-pyrrol-2-ylidenemethyl)-2,4-dimethyl-5*H*-pyrrol-3-yl]-4-oxobutanoate (10)[8]



As described in the literature,[8] trifluormethanesulfonic anhydride (0.40 mL, 4.3 mmol) was added slowly to a solution of dipyrrinone **9** (530 mg, 1.5 mmol) in anhydrous DCM (200 mL) at 0 °C under N_2 and the reaction mixture was stirred for 4 h. The reaction mixture was poured into 5% $NaHCO_3$ (aq.) (50 mL), extracted with DCM (3x25 mL) and the combined organic fractions were then washed with brine (50 mL). The combined extracts were dried using anhydrous sodium sulfate and the solvent was removed under reduced pressure. The crude material was purified using column chromatography (SiO_2, EtOAc/hexane 0/10-3/7) to yield the desired compound as a yellow solid (350 mg, 57% yield); δ_H (500 MHz, $CDCl_3$) 11.07 (1H, s), 7.20 (1H, s), 5.51 (1H, s), 4.16 (2H, q, *J* 7.1), 3.93 (3H, s), 3.07 (2H, t, *J* 6.5), 2.72 (2H, t, *J* 6.5), 2.65 (3H, s), 2.48 (3H, s), 1.27 (3H, t, *J* 7.1); δ_C (500 MHz, $CDCl_3$) 194.7, 173.3, 162.1, 143.9, 133.9, 133.3, 126.1,

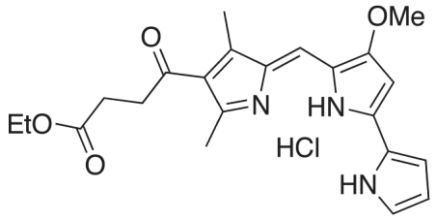
123.1, 122.6, 120.0, 118.8, 117.4, 114.9, 87.7, 59.0, 37.5, 16.2, 12.6. 1 C unaccounted for. HR-MS **(ESI):** $[M+Na]^+$ calcd. for $C_{19}H_{21}F_3N_2NaO_7S_1$: 501.0919; found 501.0914.

4.9 Ethyl 4-[5-(3, 5-trifluoromethanesulfonyloxy-1*H*-pyrrol-2-ylidenemethyl)-2,4-dimethyl-5*H*-pyrrol-3-yl]-4-oxobutanoate (10b)[8]



Modifying a literature procedure,[8] trifluormethanesulfonic anhydride (0.70 mL, 7.5 mmol) was added slowly to a solution of dipyrrinone **9** (530 mg, 1.5 mmol) in anhydrous DCM (200 mL) at 0 °C under N_2 and the reaction mixture was stirred for 4 h. The reaction mixture was poured into 5% $NaHCO_3$ (aq.) (50 mL), extracted with DCM (3x25 mL) and the combined organic fractions were then washed with brine (50 mL). The combined extracts were dried using anhydrous sodium sulfate and the solvent was removed under reduced pressure. The crude material was purified using column chromatography (SiO_2, EtOAc/hexane 0/10-3/7) to yield the bistriflate **10b** as a yellow solid (186 mg, 20%); δ_H (500 MHz, $CDCl_3$) 11.43 (1H, s), 7.24 (1H, s), 6.24 (1H, s), 4.17 (2H, q, *J* 7.2), 3.08 (2H, t, *J* 6.3), 2.73 (2H, t, *J* 6.3), 2.68 (3H, s), 2.55 (3H, s), 1.28 (3H, t, *J* 7.2); δ_C (500 MHz, $CDCl_3$) 194.6, 173.0, 150.1, 127.6, 125.3, 123.5, 120.0, 119.9, 117.4, 117.3, 100.1, 75.9, 60.8, 37.6, 31.6, 28.1, 16.4, 12.8. 1 C unaccounted for. HR-MS (ESI): $[M+Na]^+$ calcd. for $C_{19}H_{18}F_6N_2NaO_9S_2$: 619.0256; found 619.0250.

4.10 Ethyl 4-[5-(4-methoxy-1*H*,1'*H*-[2,2']bipyrrolyl-5-ylmethylene)-2,4-dimethyl-5*H*-pyrrol-3-yl]-4-oxobutanoate Hydrochloride Salt (12)[8, 26]



Trial 1:

LiCl (76 mg, 1.8 mmol), dipyrrin triflate **10** (250 mg, 0.6 mmol) and boc-pyrrole boronic acid **11** (155 mg, 0.7 mmol) were dissolved in DME (20 mL) and the solution was purged with N_2 for 10 min. $Pd(PPh_3)_4$ (70 mg, 0.1 mmol) was added and the reaction mixture was stirred for a further 10 min before the addition of 2 M Na_2CO_3 (aq.) (1.2 mL). The reaction mixture was heated at 85 °C for 32 h prior to cooling to room temperature and being poured into water (50 mL), and extracted using ethyl acetate (3x15 mL) and the combined organic fractions were then washed with brine (15 mL). Following drying of the organic fraction using anhydrous sodium sulfate and removal of solvent under reduced pressure a crude, dark brown solid was isolated. Purification using column chromatography (Al_2O_3, type III, EtOAc/hexane 0/10-3/7) yielded the free-base prodigiosene as a magenta solid. The product was dissolved in methanol and treated with a methanolic HCl solution (1 M, 2.6 mL) for 20 min. Following collection of the resulting dark pink solid using vacuum filtration and washing with water and diethyl ether the desired product was obtained as a dark pink powder (63 mg, 25%); δ_H (500

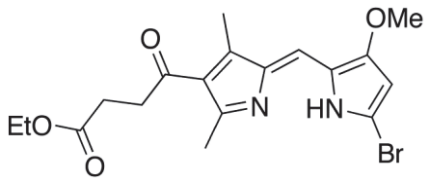
MHz, $CDCl_3$) 13.02 (1H, bs), 12.75 (1H, bs), 12.69 (1H, bs), 7.32 (1H, s), 7.14 (1H, s), 7.03 (1H, s), 6.41 (1H, s), 6.12 (1H, s), 4.17 (2H, q, *J* 7.1), 4.07 (3H, s), 3.09 (2H, t, *J* 6.5), 2.88 (3H, s), 2.73 (2H, t, *J* 6.5), 2.53 (3H, s), 1.28 (3H, t, *J* 7.1). The ^{1}H NMR data corresponds with literature values.[8]

Trial 2:

Following a published procedure,[26] LiCl (93 mg, 2.2 mmol), bromo dipyrrin **13** (300 mg, 0.7 mmol) and boc-pyrrole boronic acid **11** (177 mg, 0.80 mmol) were dissolved in DME (25 mL) and the solution was purged with N_2 for 10 min. $Pd(PPh_3)_4$ (81 mg, 0.07 mmol) was added and the reaction mixture was stirred for a further 10 min before the addition of 2 M Na_2CO_3 (aq.) solution (1.4 mL). The reaction mixture was heated at 85 °C for 120 h prior to cooling to room temperature and being poured into water (100 mL), then extracted using ethyl acetate (3x20 mL). The combined organics were then washed with brine (20 mL). Following drying of the organic fraction using anhydrous sodium sulfate and removal of solvent under reduced pressure a crude, dark brown solid was isolated. Purification using column chromatography (Al_2O_3, type III, EtOAc/hexane 0/10-3/7) yielded the free-base prodigiosene as a magenta solid. The product was dissolved in methanol and treated with a methanolic HCl solution (1 M, 2.6 mL) for 20 min. Following collection of the resulting dark pink solid using vacuum filtration and washing with water and diethyl ether the desired product was obtained (31 mg, 10% yield); δ_H (500 MHz, $CDCl_3$) 13.02 (1H, s), 12.75 (1H, s), 12.69 (1H, s), 7.32 (1H, s), 7.14 (1H, s), 7.03 (1H, s), 6.41 (1H, m), 6.12 (1H, d, *J* 1.7), 4.17 (2H, q, *J* 7.1), 4.07 (3H, s), 3.09 (2H,

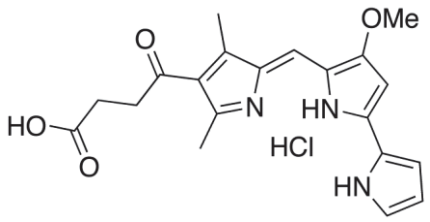
t, *J* 6.5), 2.88 (3H, s), 2.73 (2H, t, *J* 6.5), 2.53 (3H, s), 1.28 (3H, t, *J* 7.1). The ^{1}H NMR data corresponds with literature values.[8]

4.11 Ethyl 4-[5-(5-bromo-3-methoxy-1*H*-pyrrol-2-ylidenemethyl)-2,4-dimethyl-5*H*-pyrrol-3-yl]-4-oxobutanoate (13)[26]



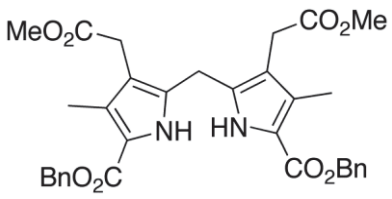
Following a literature procedure,[26] $POBr_3$ (2.33 g, 8.20 mmol) was added to a suspension of dipyrrinone **9** (1.41 g, 4.10 mmol) in anhydrous DCM (125 mL) and the reaction mixture was heated at reflux temperature for 48 h. Upon cooling, the reaction mixture was poured into sat. $NaHCO_3$ (aq) (250 mL) on ice. The organic layer was separated and washed with brine (100 mL) and water (100 mL), then dried using anhydrous sodium sulfate. The solvent was then removed under reduced pressure. The resulting crude material was dissolved in ethyl acetate (150 mL) and the solution was passed through a pad of silica (12 g), eluting using ethyl acetate:hexanes (80:20). The solvent was then removed in vacuo. The resulting crude material was purified using a Biotage purification system (Al_2O_3, type III, EtOAc/hexane 0/10-4/6) to yield the title compound as a yellow-brown solid (300 mg, 33%); δ_H (500 MHz, $CDCl_3$) 11.3 (1H, s), 6.94 (1H, s), 5.59 (1H, s), 4.16 (2H, q, *J* 7.1), 3.85 (3H, s), 3.06 (2H, t, *J* 6.6), 2.71 (2H, t, *J* 6.6), 2.62 (3H, s), 2.43 (3H, s), 1.27 (3H, t, *J* 7.1). The product was used immediately in the subsequent step of the synthesis, therefore further characterization was not performed.

4.13 4-[5-(4-methoxy-1*H*,1′*H*-[2,2′]bipyrrolyl-5-ylmethylene)-2,4-dimethyl-5*H*-pyrrol-3-yl]-4-oxobutanoic acid Hydrochloride Salt (14)[30]



Following a literature procedure,[30] KOH (7.0 g, 123 mmol) was dissolved in water (25 mL) and the solution was degassed before the addition of this aqueous solution to a solution of prodigiosene **12** (113 mg, 0.26 mmol) in THF (25 mL) under N_2. The reaction mixture was heated at reflux temperature for 24 h before removal of THF under reduced pressure. The resulting suspension was acidified to pH 2 using 12 M HCl and the mixture was then stirred for 10 minutes before the resulting dark pink solid was collected using vacuum filtration. Washing with water (10 mL) and acetone (5 mL), and then drying of the solid in a vacuum oven for 5 h gave the desired compound as a dark pink solid (40 mg, 35% yield); δ_H (500 MHz, DMSO-d_6) 12.95 (1H, s), 12.82 (1H, s), 12.53 (1H, s), 12.02 (1H, s), 7.64 (1H, s), 7.51 (1H, s), 7.05 (1H, s), 6.83 (1H, s), 6.43 (1H, s), 4.02 (3H, s), 3.03 (2H, t, *J* 6.5), 2.95 (2H, s), 2.67 (3H, s), 2.47 (2H, t, *J* 6.5). Due to poor solubility, no ^{13}C NMR spectroscopy or MS data were obtained.

4.14 Dibenzyl 3,3′-dimethyl-4,4′-bis(methoxycarbonylmethyl)-pyrromethane-5,5′-dicarboxyate (16)[31b]



Method 1:

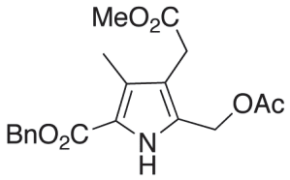
Following a literature procedure,[31a] pyrrole **15**[35] (1.5 g, 5.0 mmol) was dissolved in THF (5 mL) and a solution of bromine (0.3 mL, 5.8 mmol) in THF (3.2 mL) was added drop-wise to the solution. The reaction mixture was stirred for 3 h and the solvent was then removed under reduced pressure. The resulting reddish brown oil was dissolved in methanol (1.5 mL) and the resulting solution was heated to reflux temperature for 3 h before the cooled solution was placed in the freezer overnight. The desired product was then collected by vacuum filtration as a dark purple solid (720 mg, 25%); δ_H (500 MHz, $CDCl_3$) 10.05 (2H, s), 7.42-7.26 (10H, m), 5.25 (4H, s), 3.85 (2H, s), 3.61 (6H, s), 3.49 (4H, s), 2.29 (6H, s). The ^{1}H NMR data corresponds with literature values.[31b]

Method 2:

Following a literature procedure,[31a] pyrrole **17** (483 mg, 1.39 mmol) was dissolved in DCM (35 mL) prior to the addition of Montmorillonite (K-10) clay (2.8 g) and the reaction mixture was then stirred for 2 h. The reaction mixture was filtered to remove the clay and rinsed with DCM. The solvent was removed under reduced pressure yielding the desired dipyrrin as a beige solid (180 mg, 23%); δ_H (500 MHz, $CDCl_3$) 10.04 (2H, s),

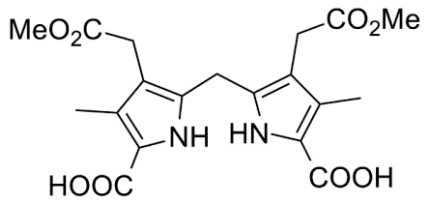
7.42-7.26 (10 H, m), 5.25 (4H, s), 3.85 (2H, s), 3.61 (6H, s), 3.49 (4H, s), 2.29 (6H, s). The observed ^{1}H NMR data corresponds to literature values.[31b]

4.15 4-Acetic acid methyl ester-5-[(acetyloxy)methyl]-1*H*-pyrrole-2 carboxylic Acid Benzyl Ester (17)[36]



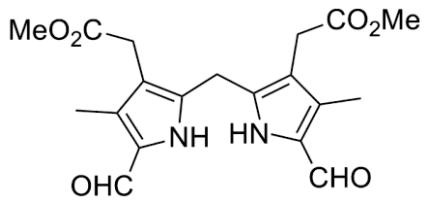
Following a literature procedure,[36] lead (IV) acetate (1.33 g, 3.0 mmol) was added over a period of 20 minutes to a solution of pyrrole **15** (680 mg, 2.6 mmol) in glacial acetic acid (15 mL) and acetic anhydride (2.5 mL) and the reaction mixture was stirred overnight. The white-yellow suspension was poured into ice-cold water (25 mL) and extracted using DCM (2x25 mL). The combined organic fractions were washed with water (15 mL) and sat. $NaHCO_3$ (aq) (15 mL) and then dried using anhydrous sodium sulfate and the solvent was then removed under reduced pressure. Following crystallization from hot methanol the desired compound was isolated as a light brown solid (483 mg, 52%); δ_H (500 MHz, $CDCl_3$) 9.09 (1H, s), 7.42-7.33 (5H, m), 5.05 (2H, s), 3.67 (3H, s), 3.49 (2H, s), 2.29 (3H, s), 2.06 (2H, s). The ^{1}H NMR data corresponds with literature values.[36]

4.17 3,3′-Dimethyl-4,4′-bis(methoxycarbonylmethyl)-pyrromethane-5,5′-dicarboxyate (18)



Dipyrromethane **16** (462 mg, 0.79 mmol) was dissolved in THF (10 mL) and the solution was then degassed with N_2. Following the addition of 10% Pd/C (50 mg), the reaction mixture was stirred overnight under H_2. Following filtration, the residue and catalyst were collected and then treated with NaOH (7.5 mg, 1.9 mmol) in methanol (5 mL) and the mixture was stirred for 2 min. The mixture was filtered to remove the catalyst and the filtrate was acidified to pH 2 and filtered once again to collect the title product as a white precipitate (300 mg, 93%); δ_H (500 MHz, DMSO) 12.13 (2H, s), 11.13 (2H, s), 3.78 (2H, s), 3.38 (6H, s), 3.34 (4H, s), 2.09 (6H, s). Due to poor solubility, no ^{13}C NMR or MS data were obtained.

4.18 3,3′-Dimethyl-4,4′-bis(methoxycarbonylmethyl)-5,5′-diformyl Pyrromethane (19)



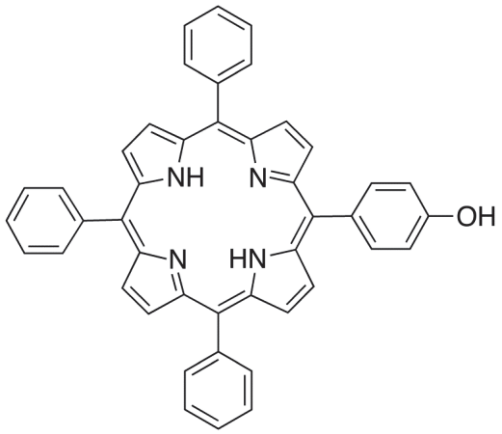
Method 1:

Dipyrromethane **18** (300 mg, 0.74 mmol) was combined with trifluoroacetic acid (1.7 mL) and the mixture was stirred at room temperature until the solid dissolved. The solution was then cooled to 0 °C and triethyl orthoformate (0.4 mL, 2.4 mmol) was added drop-wise and stirring was continued for a further 15 min. The reaction mixture was poured into water (10 mL) and the resulting precipitate was collected using suction filtration before being stirred in ethanol (2.2 mL) and 1 M NH_4OH (4.4 mL). The reaction mixture was then filtered to afford a sticky dark green product which was shown by ^1H NMR to be decomposition products.

Method 2:

Dipyrromethane **18** (345 mg, 0.85 mmol) was combined with anhydrous DCM under N_2 and the solution was cooled to 0 °C. Trifluoroacetic acid (0.18 mL, 2.3 mmol) was added slowly to the reaction mixture and the mixture was then stirred for 2 h. The starting material did not appear to dissolve during the reaction but the reaction was continued by adding trimethyl orthoformate (1.20 mL, 11.1 mmol) and stirring for a further 30 min. The reaction mixture was quenched using sat. $NaHCO_3$ (aq.) and it was then extracted with DCM (3x20 mL). The combined organic fractions were then washed with brine (50 mL) and dried using anhydrous magnesium sulfate. No product was isolated following removal of the organic solvent under reduced pressure and attempts to recover starting material and/or product from the aqueous phase were unsuccessful.

4.20 5,10,15-Triphenyl-20-(4-hydroxyphenyl)-21*H*,23*H*-porphyrin (20)[23, 33]



Method 1:

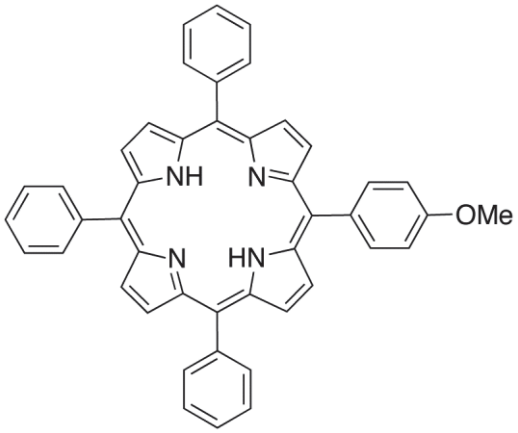
Following a literature procedure,[33] 4-hydroxybenzaldehyde (0.75 g, 6.0 mmol), freshly distilled benzaldehyde (1.9 mL, 18 mmol) and freshly distilled pyrrole (1.7 mL, 24 mmol) were dissolved in propanoic acid (50 mL) and heated to reflux temperature overnight. Purification using column chromatography (SiO_2, DCM, 100%), followed by a second column chromatography (Al_2O_3, Type III, basic, DCM, 100%) yielded TPP (**21**) as a deep purple solid (50 mg, 11%); δ_H (500 MHz, $CDCl_3$) 8.87 (8H, s), 8.24-8.25 (8H, m), 7.75-7.79 (12H, m), -2.75 (2H, s). LR-MS *m/z* ESI^+ 615.3. The desired compound was not isolated from this synthesis.

Method 2:

Modifying a literature procedure,[23] dipyrromethane **23**[35] (500 mg, 2.2 mmol), benzaldehyde (0.11 mL, 1.1 mmol) and 4-hydroxy benzaldehyde (305 mg, 2.5 mmol) were stirred in DCM (400 mL) for 15 minutes before the addition of boron trifluoride

diethyl etherate (0.4 μL, 0.003 mmol) and trifluoroacetic acid (0.1 mL, 1.5 mmol). The solution was stirred overnight and then DDQ (535 mg, 2.4 mmol) was added and the reaction mixture was stirred for a further 2 days. The reaction mixture was washed with sat. $NaHCO_3$(aq) (2x250 mL) and brine (400 mL) before being dried using anhydrous sodium sulfate and removal of solvent under reduced pressure. The crude mixture was purified using column chromatography (Al_2O_3, neutral, type III, DCM/methanol 100/0-95/5) to provide the desired compound as a dark purple solid (278 mg, 20%); δ_H (500 MHz, $CDCl_3$) 8.87 (3H, s), 8.85-8.84 (5H, m), 8.22-8.21 (6H, m), 8.08-8.06 (2H, m), 7.77-7.75 (12 H, m), -2.78 (2H, s).

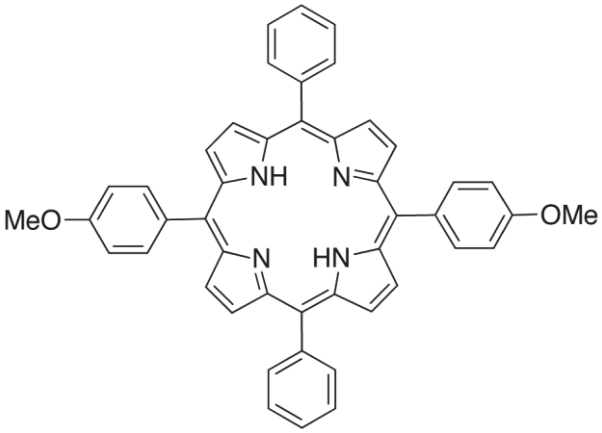
4.21 5,10,15-Triphenyl-20-(4-methoxyphenyl)-21*H*,23*H*-porphyrin (22)[23]



Following a published procedure,[23] $BF_3 \cdot OEt_2$ (12 μL, $1.2x10^{-2}$ mmol) and trifluoroacetic acid (0.35 mL, 4.5 mol) were added to a solution of freshly distilled pyrrole (0.35 mL, 5.0 mmol), *p*-anisaldehyde (0.15 mL, 1.25 mmol) and benzaldehyde (0.38 mL, 3.75 mmol) in DCM (500 mL) and the reaction mixture was stirred for 4 h. DDQ (570 mg, 2.5 mmol) was added and the reaction mixture was stirred for a further 2 h. The solvent was

removed under reduced pressure to yield a dark green solid. Purification was attempted using column chromatography (SiO_2, DCM/hexane 6/4). No product was isolated from the column.

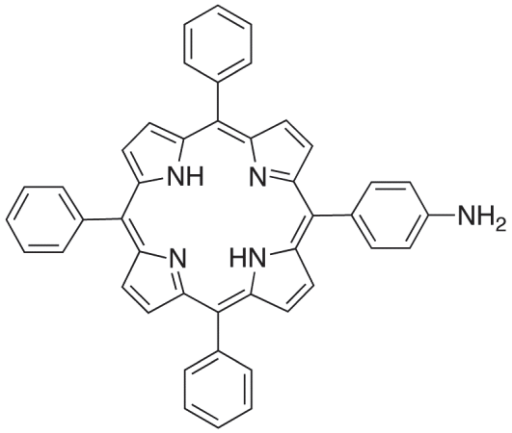
4.22 5,15-Diphenyl-10,20-(4-methoxyphenyl)-21*H*,23*H*-porphyrin (24)[32]



Following published procedures,[32] trifluoroacetic acid (0.15 mL, 2.0 mmol) was slowly added to a solution of meso-phenyl dipyrromethane **23**[35] (111 mg, 0.5 mmol) and *p*-anisaldehyde (68 mg, 0.5 mmol) in DCM (50 mL). After stirring for 40 min, DDQ (114 mg, 0.5 mmol) was added and the reaction mixture was stirred for 1 h. Following filtration through neutral alumina the solvent was removed under reduced pressure to yield a dark red solid which was then dissolved in toluene (20 mL) and the solution was then heated at reflux temperature in the presence of DDQ (114 mg, 0.5 mmol) for 1 h. Once cooled, the reaction mixture was passed through a pad of neutral alumina and eluted with DCM before purification using column chromatography (Al_2O_3, neutral, type III, DCM/hexane 4/6) to yield the desired compound as a dark purple solid (40 mg, 12%);

δ_H (500 MHz, $CDCl_3$) 8.87 (8H, d, *J* 7.6), 8.23 (4H, d, *J* 7.6), 8.14 (4H, d, *J* 8.6), 7.74-7.79 (6 H, m), 7.29 (4H, d, *J* 8.6), 4.10 (6H, s), -2.74 (2H, s). LR-MS m/z ESI^+ 675.3.

4.24 5,10,15-Triphenyl-20-(4-aminophenyl)-21*H*,23*H*-porphyrin (25)[37-38]



Method 1:

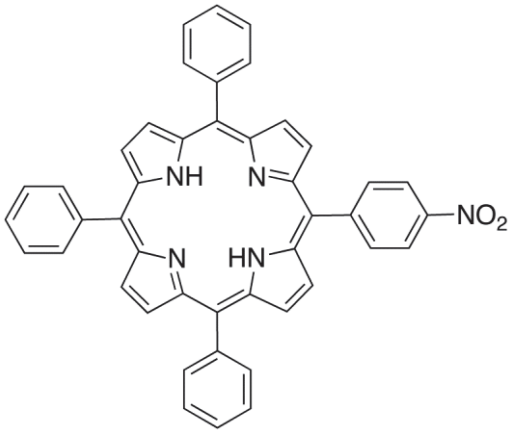
Following a literature procedure,[37] sodium nitrate (28 mg, 0.40 mmol) was added to a solution of tetraphenylporphyrin **21** (188 mg, 0.30 mmol) in trifluoroacetic acid (8 mL). The reaction mixture was stirred for 5 min and the solvent was then removed under reduced pressure. The crude product was dissolved in anhydrous DCM (6 mL) and conc. HCl (25 mL) and the resulting solution was stirred overnight at 60 °C under N_2 in the presence of tin(II) chloride dihydrate (690 mg, 3.0 mmol). The reaction mixture was neutralized by slowly adding KOH pellets to the reaction mixture at 0℃, then extracted with DCM (3x20 mL), washed with brine (50 mL) and dried using anhydrous sodium sulfate before removal of the solvent under reduced pressure. The reaction yielded

several milligrams of a brown film which was shown by ^{1}H NMR to be an intractable mixture of unidentifiable products.

Method 2:

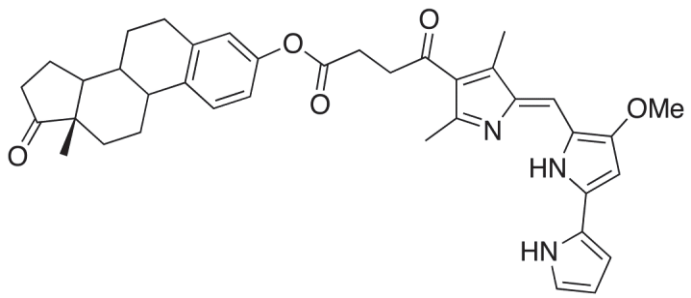
Following a published procedure,[38] conc. HCl (40 mL) was degassed with N_2 prior to addition of porphyrin **27** (220 mg, 0.33 mmol) and tin(II) chloride dihydrate. The reaction mixture was heated at 60℃ overnight under N_2. The reaction was poured into water (50 mL) and slowly quenched with KOH pellets at 0℃ before being extracted with DCM (3x30 mL). The combined organics were then washed with brine (100 mL) and dried using anhydrous sodium sulfate. The crude material was purified using column chromatography (Al_2O_3, neutral, type III, DCM/hexane 75/25) to yield the desired compound as a dark purple solid (178 mg, 86%); δ_H (500 MHz, $CDCl_3$) 8.97-8.96 (2H, m), 8.85 (6H, s), 8.23 (6H, d, *J* 7.5), 8.01 (2H, d, *J* 8.3), 7.76 (9H, d, *J* 7.5), 7.06 (2H, d, *J* 8.3), 4.01 (2H, s), -2.74 (2H, s). The ^{1}H NMR data corresponds with literature values.[38]

4.25 5,10,15-Triphenyl-20-(4-nitrophenyl)-21*H*,23*H*-porphyrin (27)[23]



Following a modified literature procedure,[23] dipyrromethane **23**[35] (500 mg, 2.20 mmol), dipyrromethane **26**[35] (601 mg, 2.20 mmol) and benzaldehyde (0.45 mL, 4.40 mmol) were stirred in DCM (800 mL) for 15 min before the addition of $BF_3 \cdot OEt_2$ (0.70 μL, 0.006 mmol) and trifluoroacetic acid (0.22 mL, 2.9 mmol). The solution was stirred overnight and then DDQ (1.05 g, 4.62 mmol) was added and the reaction mixture was stirred for a further 2 d. The reaction mixture was washed with sat. $NaHCO_3$ (aq.) (3x250 mL) and brine (400 mL). The combined organic fractions were dried using anhydrous sodium sulfate and the solvent was then removed under reduced pressure. The crude mixture was purified using column chromatography (Al_2O_3, neutral, type III, DCM/hexane 5/5) to provide the desired compound as a dark purple solid (220mg, 15%); δ_H (500 MHz, $CDCl_3$) 8.89 (2H, d, *J* 4.8), 8.86 (3H, s), 8.74 (2H, d, *J* 4.8), 8.64 (2H, d, *J* 8.6), 8.41 (2H, d, *J* 8.6), 8.22-8.21 (6H, m), 7.81-7.75 (10H, m), -2.79 (2H, s). The ^1H NMR data corresponds with literature values.[38]

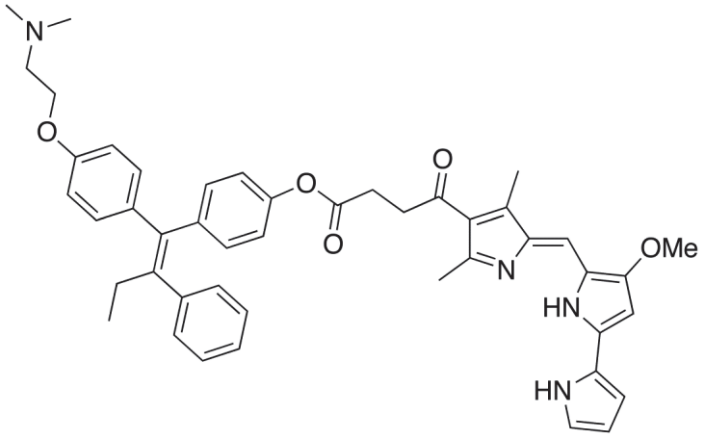
4.27 Prodigiosene-estrone Conjugate (29)



Acid **14** (100 mg, 0.25 mmol), estrone **28** (100 mg, 0.38 mmol), DMAP (33 mg, 0.27 mmol) and EDC (42 mg, 0.27 mmol) were dissolved in anhydrous DCM (25 mL) under nitrogen. After stirring at room temperature for 3 d, water (25 mL) was added and the mixture was extracted with DCM (4x25 mL). The combined organic fractions were

washed with brine (100 mL), then dried using anhydrous sodium sulfate. Following removal of the solvent under vacuum the crude solid was purified using column chromatography (Al_2O_3, neutral, type III, EtOAc/hexane 5/5) to yield the title compound as a dark red solid (68 mg, 44%). δ_H (500 MHz, $CDCl_3$) 7.27 (1H, s), 6.91 (1H, s), 6.88-6.87 (1H, m), 6.87-6.86 (1 H, m), 6.85-6.84 (1H, m), 6.73 (1H, d, *J* 3.0), 6.29 (1H, t, *J* 3.0), 5.99 (1H, s), 3.95 (3H, s), 3.16-3.13 (2H, m), 2.92 (2H, t, *J* 6.5), 2.89 (2H, t, *J* 6.5), 2.48 (3H, s), 2.45 (3H, s), 2.42-2.38 (2H, m), 2.28-2.27 (1H, m), 2.14-2.12 (1H, m), 2.08-1.94 (3H, m), 1.65-1.53 (4H, m), 1.48-1.42 (2H, m), 0.90 (3H, s); δ_C (500 MHz, $CDCl_3$) 194.8, 172.4, 168.8, 158.0, 152.0, 148.8, 142.5, 138.0, 137.3, 126.4, 122.8, 121.7, 118.9, 113.8, 112.0, 111.0, 95.9, 95.8, 58.7, 50.6, 48.1, 44.3, 38.1, 37.5, 36.4, 36.0, 31.7, 29.5, 28.7, 26.5, 25.9, 21.7, 15.1, 14.0, 12.6. 3 C unaccounted for. HR-MS (**ESI**): $[M+H]^+$ calcd. for $C_{38}H_{42}N_3O_5$: 620.3124; found 620.3119.

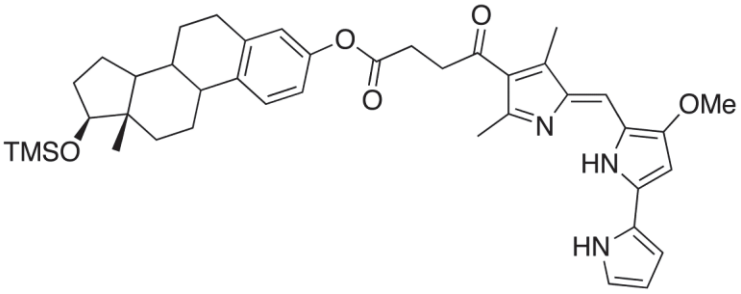
4.28 PRODIGIOSENE-TAMOXIFEN CONJUGATE (31)



Acid **14** (32 mg, 0.08 mmol), *Z*-hydroxy Tamoxifen **30**[48] (34 mg, 0.08 mmol), DMAP (11 mg, 0.09 mmol) and EDC (14 mg, 0.09 mmol) were dissolved in anhydrous DCM (10

mL) under nitrogen. After stirring at room temperature for 2 d, water (10 mL) was added and the mixture was extracted with DCM (4x20 mL). The combined organic factions were washed with brine (50 mL), then dried using anhydrous sodium sulfate. Following removal of the solvent under vacuum the crude solid was purified using column chromatography (Al_2O_3,neutral, type III, methanol/DCM 5/100) to yield the title compound as a dark red solid (45 mg, 76%) ; δ_H (500 MHz, CD_2Cl_2) 7.24-7.21 (2H, m), 7.19-7.16 (2H, m), 7.13-7.11 (3H, m), 7.09-7.06 (2H, m), 6.93 (1H, s), 6.87 (1H, s), 6.78 (1H, t, *J* 2.6), 6.77-6.75 (2H, m), 6.56-6.53 (2H, m), 6.26 (1H, dd, *J* 4.8, 2.6), 6.05 (1H, s), 3.95 (3H, s), 3.89 (2H, t, *J* 4.8), 3.13 (2H, t, *J* 6.4), 2.87 (2H, t, *J* 6.4), 2.58 (2H, t, *J* 5.8), 2.44 (4H, t, *J* 3.7), 2.22 (6H, s), 1.25 (4H, s), 0.91-0.88 (3H, m); δ_C (500 MHz, CD_2Cl_2) 194.8, 172.2, 157.3, 149.9, 142.7, 142.2, 141.6, 137.8, 135.7, 132.1, 130.6, 130.0, 128.4, 128.1, 126.4, 123.2, 121.6, 116.6, 113.9, 113.6, 112.1, 111.1, 96.0, 66.4, 66.3, 66.2, 58.9, 58.5, 53.4, 45.9, 37.8, 30.1, 30.0, 29.3, 29.0, 15.1, 13.6, 12.6. 1 C unaccounted for. HR-MS (**ESI**): $[M+H]^+$ calcd. for $C_{46}H_{49}N_4O_5$: 737.3703; found 737.3697

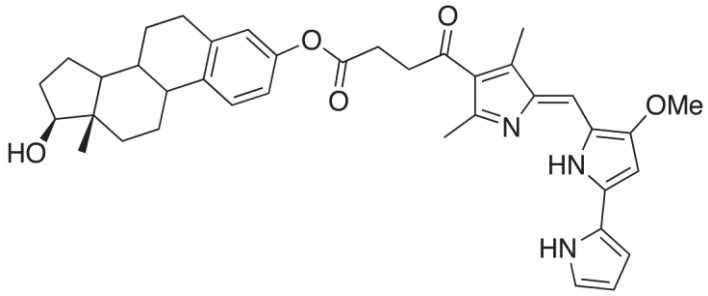
4.29 TMS-PROTECTED PRODIGIOSENE-ESTRADIOL CONJUGATE (33)



Acid **14** (100 mg, 0.25 mmol), estradiol **32** (102 mg, 0.30 mmol), DMAP (37 mg, 0.30 mmol) and EDC (47 mg, 0.30 mmol) were dissolved in anhydrous DCM (25 mL) under

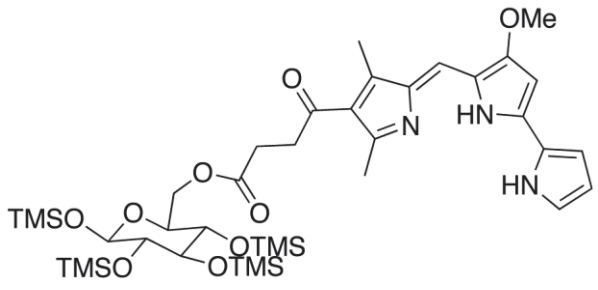
nitrogen. After stirring at room temperature for 2 d, water (25 mL) was added and the mixture was extracted with DCM (4x25 mL). The combined organic fractions were washed with brine (100 mL), then dried using anhydrous sodium sulfate. Following removal of the solvent under vacuum the crude solid was purified using column chromatography (Al_2O_3, neutral, type III, EtOAc/hexane 5/5) to yield the title compound as a dark red solid (122 mg, 70%); δ_H (500 MHz, $CDCl_3$) 7.27 (1H, s), 6.94 (1H, s), 6.86-6.84 (1H, m), 6.80-6.81 (1H, m), 6.78-6.66 (1H, s), 6.75-6.74 (1H, m), 6.23-6.22 (1H, m), 6.06-6.05 (1H, m), 3.99 (3H, s), 3.65-3.63 (1H, m), 3.10 (2H, t, *J* 6.6), 2.90 (2H, t, *J* 6.6), 2.86-2.83 (2H, m), 2.44 (3H, s), 2.26 (3H, s), 2.19 (1H, s), 1.96-1.92 (1H, m), 1.90-1.86 (2H, m), 1.53-1.41 (4H, m), 1.37-1.27 (4H, m), 1.18-1.17 (1H, m), 0.75-0.74 (3H, m), 0.11 (9H, s); δ_C (500 MHz, $CDCl_3$) 194.9, 172.4, 169.0, 160.9, 150.0, 148.6, 142.5, 138.3, 138.1, 128.2, 126.5, 126.4, 123.3, 122.8, 121.6, 118.6, 113.9, 112.1, 110.9, 81.8, 58.7, 49.9, 44.4, 43.4, 38.6, 37.5, 37.1, 31.0, 29.7, 28.7, 27.2, 26.4, 24.8, 23.4, 12.6, 11.4, 0.4. 1 C unaccounted for. HR-MS (**ESI**): $[M+H]^+$ calcd. for $C_{41}H_{52}N_3O_5Si$: 694.3676; found 694.3671.

4.30 PRODIGIOSENE-ESTRADIOL CONJUGATE (34)



Compound **33** was dissolved in $CHCl_3$ (5 mL) and treated with 0.06 M HCl in methanol (3 mL). After stirring for 5 min, the solvent was removed under vacuum and the product was then collected using suction filtration, before being washed with diethyl ether to yield the title compound as a deep red solid (12 mg, 32%); δ_H (500 MHz, $CDCl_3$) 12.97 (1H, s), 12.73 (1H, s), 12.67 (1H, s), 7.32-7.30 (1H, m), 7.27-7.26 (1H, m), 7.11 (1H, s), 7.02 (1H, s), 6.87-6.86 (1H, m), 6.83-6.81 (1H, m), 6.41-6.40 (1H, m), 6.11-6.10 (1H, m), 4.05 (3H, s), 3.73-3.72 (1H, m), 3.17 (2H, t, *J* 6.4), 2.95 (2H, t, *J* 6.4), 2.88 (3H, s), 2.86-2.84 (2H, m), 2.53 (3H, s), 2.33-2.29 (1H, m), 2.21-2.18 (1H, m), 2.15-2.08 (1H, m), 1.97-1.93 (1H, m), 1.89-1.85 (1H, m), 1.73-1.66 (1H, m), 1.59 (1H, s), 1.50-1.25 (7H, m), 0.77 (3H, s); δ_C (500 MHz, $CDCl_3$) 172.1, 166.9, 161.2, 160.7, 158.4, 150.6, 148.9, 148.6. 138.7, 138.3, 138.0, 129.0, 126.8, 126.5, 126.1, 125.6, 124.5, 123.5, 123.2, 122.1, 121.6, 121.5, 119.6, 118.7, 112.8, 93.6, 82.0, 59.2, 50.2, 44.3, 43.3, 38.6, 37.8, 36.8, 30.7, 29.7, 28.6, 27.2, 26.3, 23.3, 15.9, 12.8, 11.2. HR-MS (ESI): $[M+H]^+$ calcd. for $C_{41}H_{44}N_3O_5$: 622.3281; found 622.3275

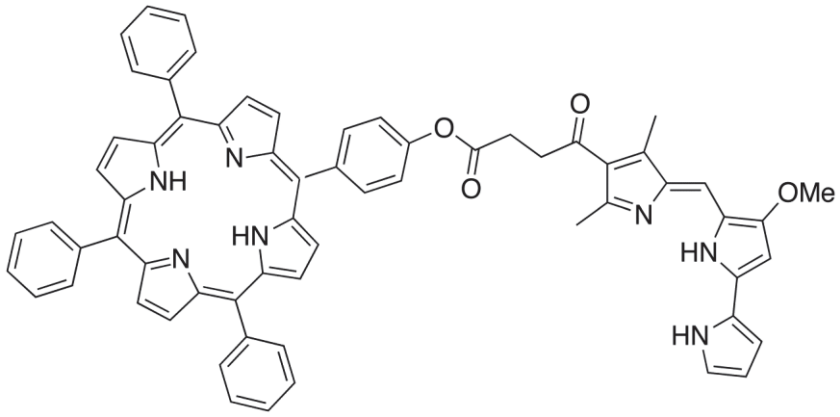
4.31 PRODIGIOSENE-GLUCOSE CONJUGATE (36)



Acid **14** (32 mg, 0.08 mmol), glucose derivative **35**[49] (56 mg, 0.12 mmol), DMAP (15 mg, 0.12 mmol) and EDC (14 mg, 0.09 mmol) were dissolved in anhydrous DCM (10 mL) under nitrogen. After stirring at room temperature for 3 d, water (10 mL) was added

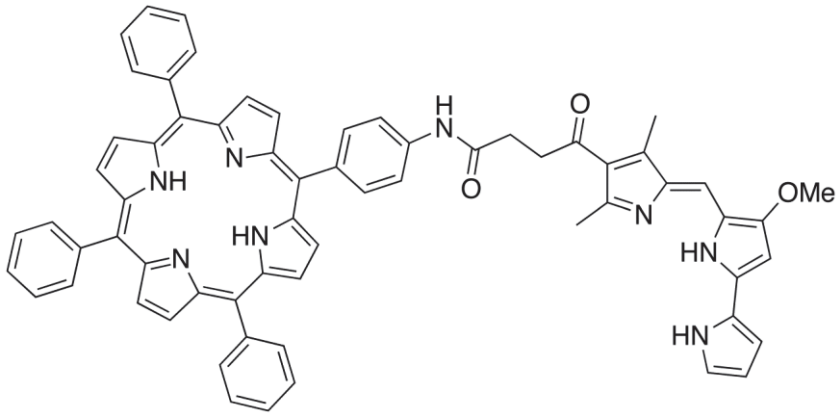
and the mixture was extracted with DCM (4x15 mL). The combined organic fractions were washed with brine (50 mL), then dried with anhydrous sodium sulfate. Following removal of the solvent under vacuum the crude solid was purified using column chromatography (Al_2O_3, neutral, type III, EtOAc/hexane 3/7) to yield a dark red solid (13 mg) which was shown to contain the desired product and some unknown impurities. Further purification attempts were unsuccessful.

4.32 Prodigiosene-hydroxyporphyrin Conjugate (37)



Acid **14** (24 mg, 0.06 mmol), porphyrin **20** (40 mg, 0.06 mmol), DMAP (9 mg, 0.08 mmol) and EDC (12 mg, 0.08 mmol) were dissolved in anhydrous DCM (12 mL) under nitrogen. After stirring at room temperature for 3 d, water (20 mL) was added and the mixture was extracted with DCM (4x20 mL). The combined organic fractions were washed with brine (50 mL), then dried with anhydrous sodium sulfate. Following removal of the solvent under vacuum the crude solid was purified using column chromatography (Al_2O_3, neutral, type III, EtOAc/hexane 5/5) to yield a dark red solid. The obtained solid was shown by mass spectrometry and NMR spectroscopy to contain the desired product, however further purification attempts were unsuccessful.

4.33 Prodigiosene-aminoporphyrin Conjugate (38)



Acid **14** (24 mg, 0.06 mmol), porphyrin **25** (40 mg, 0.06 mmol), DMAP (9 mg, 0.08 mmol) and HBTU (27 mg, 0.08 mmol) were dissolved in anhydrous DCM (12 mL) under nitrogen. After stirring at room temperature for 3 d, water (20 mL) was added and the mixture was extracted with DCM (4x20 mL). The combined organic fractions were washed with brine (50 mL), then dried with anhydrous sodium sulfate. Following removal of the solvent under vacuum the crude solid was purified using column chromatography (Al_2O_3, neutral, type III, EtOAc/hexane 5/5 – methanol/DCM 1/100) to yield a dark red solid. The obtained solid was shown by mass spectrometry and NMR spectroscopy to contain the desired product, however further purification attempts were unsuccessful.

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APPENDIX A LIST OF REACTION SCHEMES

Reaction Scheme 1. Synthesis of ethyl 4-[5-(4-methoxy-1*H*,1'*H*-[2,2']bipyrrolyl-5-ylmethylene)-2,4-dimethyl-5*H*-pyrrol-3-yl]-oxobutanoate. 20

Reaction Scheme 2. Bromination method for the synthesis of ethyl 4-[5-(4-methoxy-1*H*,1'*H*-[2,2']bipyrrolyl-5-ylmethylene)-2,4-dimethyl-5*H*-pyrrol-3-yl]-oxobutanoate. 23

Reaction Scheme 3. Synthesis of bistriflate **10b**..... 24

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Reaction Scheme 12. One-pot porphyrin synthesis using BF_3-OEt_2 and TFA. 33

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Reaction Scheme 14. Synthesis of porphyrin **24**. 34

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APPENDIX B NCI DTP IN VITRO TESTING RESULTS

Table 2. Results obtained from in vitro cell viability assays performed by the NCI DTP.

Panel/Cell Line	GI50	TGI	LC50
Leukemia			
CCRF-CEM	6.46E-07	>1.00E-04	>1.00E-04
HL-60(TB)	3.16E-05	>1.00E-04	>1.00E-04
K-562	4.07E-06	>1.00E-04	>1.00E-04
MOLT-4	2.62E-06	>1.00E-04	>1.00E-04
RPMI-8226	3.29E-06	>1.00E-04	>1.00E-04
SR	3.85E-07	>1.00E-04	>1.00E-04
Non-Small Cell Lung Cancer			
A549/ATCC	2.23E-06	>1.00E-04	>1.00E-04
HOP-62	1.80E-05	6.53E-05	>1.00E-04
HOP-92	1.45E-06	9.16E-06	>1.00E-04
NCI-H226	1.26E-05	>1.00E-04	>1.00E-04
NCI-H23	1.04E-05	>1.00E-04	>1.00E-04
NCI-H322M	4.09E-05	>1.00E-04	>1.00E-04
NCI-H460	2.65E-06	>1.00E-04	>1.00E-04
NCI-H522	4.71E-06	6.01E-05	>1.00E-04
Colon Cancer			
COLO 205	2.48E-05	>1.00E-04	>1.00E-04
HCC-2998	1.45E-05	>1.00E-04	>1.00E-04
HCT-116	5.77E-07	>1.00E-04	>1.00E-04
HCT-15	3.22E-06	3.82E-05	>1.00E-04
HT29	6.37E-07	>1.00E-04	>1.00E-04
KM12	3.47E-06	>1.00E-04	>1.00E-04
SW-620	7.39E-07	>1.00E-04	>1.00E-04
CNS Cancer			
SF-268	5.09E-06	>1.00E-04	>1.00E-04
SF-295	2.14E-05	8.54E-05	>1.00E-04
SF-539	2.13E-05	>1.00E-04	>1.00E-04
SNB-19	2.34E-05	>1.00E-04	>1.00E-04
SNB-75	1.82E-05	9.33E-05	>1.00E-04
U251	2.61E-06	>1.00E-04	>1.00E-04
Melanoma			
LOX IMVI	5.63E-07	2.37E-06	8.06E-06
MALME-3M	2.98E-05	>1.00E-04	>1.00E-04
M14	1.14E-05	3.33E-05	9.77E-05
MDA-MB-435	7.63E-06	>1.00E-04	>1.00E-04

Panel/Cell Line	GI50	TGI	LC50
SK-MEL-2	1.54E-05	5.53E-05	>1.00E-04
SK-MEL-28	5.03E-05	>1.00E-04	>1.00E-04
SK-MEL-5	3.89E-06	1.96E-05	5.50E-05
UACC-257	>1.00E-04	>1.00E-04	>1.00E-04
UACC-62	4.00E-06	1.77E-05	4.77E-05
Ovarian Cancer			
IGROV1	7.21E-06	>1.00E-04	>1.00E-04
OVCAR-3	1.55E-05	>1.00E-04	>1.00E-04
OVCAR-4	3.75E-05	>1.00E-04	>1.00E-04
OVCAR-5	4.08E-06	6.66E-05	>1.00E-04
OVCAR-8	7.98E-06	>1.00E-04	>1.00E-04
NCI/ADR-RES	1.71E-05	>1.00E-04	>1.00E-04
SK-OV-3	>1.00E-04	>1.00E-04	>1.00E-04
Renal Cancer			
786-0	5.55E-06	4.87E-05	>1.00E-04
A498	1.15E-05	7.34E-05	>1.00E-04
ACHN	5.73E-06	>1.00E-04	>1.00E-04
CAKI-1	6.68E-06	>1.00E-04	>1.00E-04
RXF 393	1.18E-05	>1.00E-04	>1.00E-04
SN12C	5.06E-06	>1.00E-04	>1.00E-04
TK-10	1.62E-05	8.47E-05	>1.00E-04
UO-31	2.85E-06	2.35E-05	>1.00E-04
Prostate Cancer			
PC-3	3.85E-06	>1.00E-04	>1.00E-04
DU-145	8.03E-07	>1.00E-04	>1.00E-04
Breast Cancer			
MCF7	1.87E-06	2.43E-05	>1.00E-04
MDA-MB-231/ATCC	2.91E-07	9.79E-07	1.90E-05
HS 578T	1.56E-05	>1.00E-04	>1.00E-04
BT-549	1.83E-05	>1.00E-04	>1.00E-04
T-47D	8.75E-06	>1.00E-04	>1.00E-04
MDA-MB-468	1.96E-05	>1.00E-04	>1.00E-04