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Orotidine-5'-monophosphate decarboxylase (ODCase) is an enzyme that catalyzes the final step during the *de novo* synthesis of uridine-5'-monophosphate (UMP). Several UMP derivatives modified at the C6 position, including 6-iodouridine-5'-monophosphate, have exhibited potent inhibition of ODCase catalytic activity. As part of this thesis, various analogs of UMP were synthesized by attaching small heterocycle moieties at the C6 position through a C-C bond. This was achieved by exploring Stille-coupling and Click chemistry, to prepare four novel C6 derivatives of UMP. These derivatives are 6-(tetrazol-4-yl)-UMP, 6-(pyrazol-4-yl)-UMP, 6-(thiophen-2-yl)-UMP and 6-(thiophen-3-yl)-UMP, and the corresponding nucleosides. The nucleotide derivatives are under investigation as inhibitors of ODCase, and the nucleoside derivatives are investigated as potential antimalarial and anticancer agents. Synthetic strategies, the design of inhibitors, and the accomplishments to isolate C6-heterocycle substituted uridine derivates in the context of therapeutic potential are discussed.

SYNTHESIS OF C6-SUBSTITUTED URIDINE-5'-MONOPHOSPHATE DERIVATIVES AS POTENTIAL INHIBITORS OF OROTIDINE-5'-MONOPHOSPHATE DECARBOXYLASE

by

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APPROVAL PAGE

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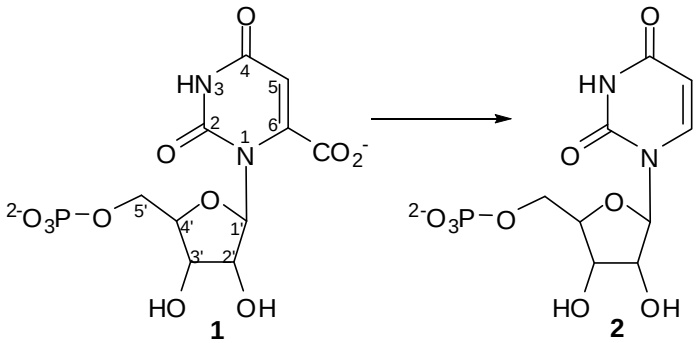
CHAPTER I

INTRODUCTION AND BACKGROUND

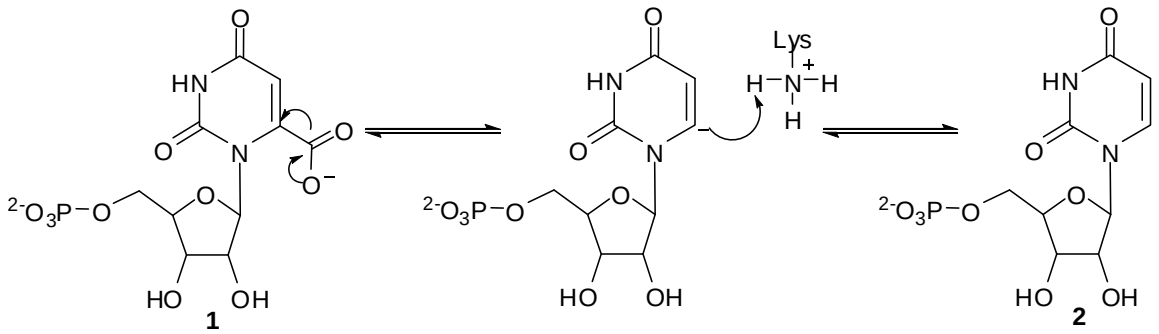
I.a. Orotidine-5'-Monophosphate Decarboxylase (ODCase)

Orotidine-5'-monophosphate decarboxylase (ODCase), is an essential enzyme that catalyses the last of six steps of the uridine-5'-monophosphate (UMP) synthesis, which converts orotidine-5'-monophosphate (OMP) (compound **1**) to UMP (compound **2**) (Scheme 1) (Appleby, 2000; Langely, 2008). The final product of this pyrimidine synthesis is UMP, which is an essential building block for other pyrimidine nucleotides (Meza-Avina, 2008). These nucleotides (including UMP) are vital for a number of cellular processes, making them indispensable for the survival of the cell (Meza-Avina, 2008).



Scheme 1. Final step in the *de novo* synthesis of UMP (**2**) from OMP (**1**) catalyzed by ODCase.

The mechanism of reaction for ODCase is the center of a wide debate, as it is difficult to account for the high rate of efficiency with any of the favored reactions (Vleet, 2008). However, the most widely accepted mechanism involves the direct removal of the C6 carboxylic acid group (Meza-Avina, 2008; Hu, 2008). This generates an anion at the C6 position, which is supposedly stabilized by Lys93 (based on yeast numbering) (Vleet, 2008). This anion then picks up a proton to form the final product of uridine (Vleet, 2008; Meza-Avina, 2008). This suggested mechanism can be seen below in Scheme 2.



Scheme 2. Proposed mechanism of decarboxylation by ODCase.

While the mechanism of this reaction is still being debated, crystal structures of the enzyme with bound UMP have been obtained (Figure 1) and show the C6 position buried in the active site and the residues around the area (Appleby, 2000). The enzyme is active as a dimer, with both monomers having an active site containing the majority of the residues from the monomer it is in and a few from the adjoining monomer (Langely, 2008). Aside from its importance as a potential anti-malarial target, ODCase has also garnered attention through the fact that it is one of the most proficient enzymes known,

increasing the rate of decarboxylation by a factor of 10^{17} without the use of any cofactors (Langely, 2008; Vleet, 2008).

Although the catalytic mechanism of decarboxylation by ODCase is not completely understood, the crystal structures of known inhibitors with ODCase have shown the residues around the C6 and C5 positions, opening doors for the design of inhibitors of this enzyme (Appleby, 2000; Bello, 2008). Some C6 derivatives of UMP already exist that have been shown to inhibit malarial ODCase, including 6-iodo-UMP (Bello, 2007; Poduch, 2006). Another basis for design of inhibitors is on the substrate, orotidine-5'-monophosphate (OMP). From these two starting points, several ideas for inhibitors were designed based on substitutions at the C6 position of uridine-5'-monophosphate (UMP).

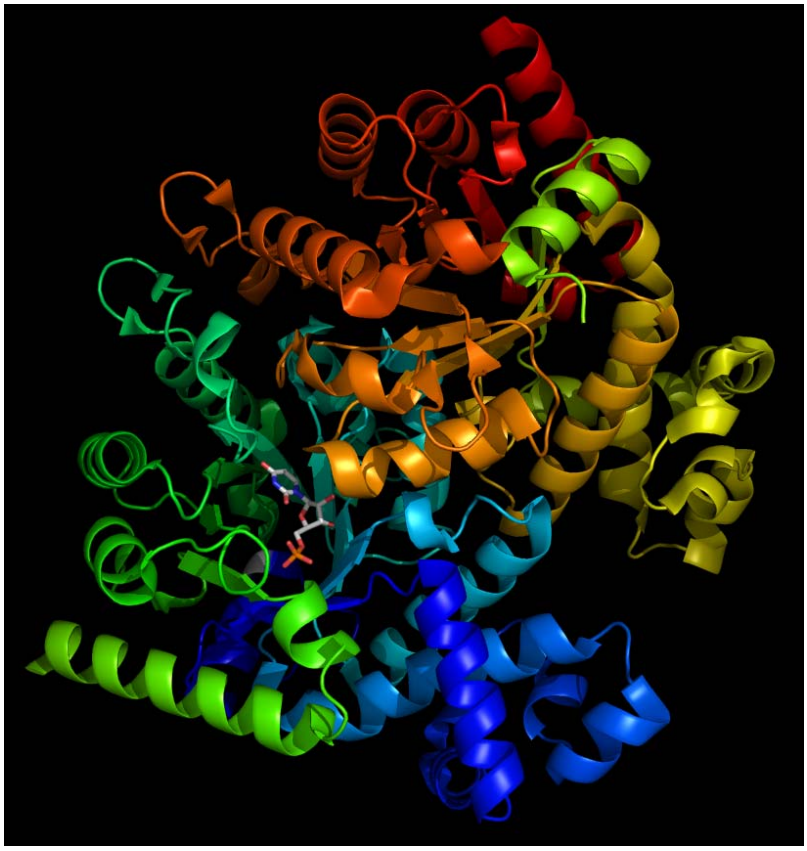


Figure 1. Crystal structure of *Pf* ODCase monomer with bound UMP (Bello, 2008).

I.b. Therapeutic Application

Malaria is a parasitic disease caused by the *Plasmodia* genus, which consists of *P. falciparum*, *P. vivax*, *P. ovale* and *P malariae* (Meza-Avina, 2008; Vera, 2009). The life cycle of this parasite consists of three stages. Initially, the infected mosquito injects the sporozoites into the lymph which puts them in circulation. Once these sporozoites reach the liver, they develop into merozoites which then exit from the liver and invade red blood cells (Good, 2009). Here, they either continue to develop more merozoites and

eventually rupture the cell, or develop into gametocytes which are then passed on to a new mosquito to spread the parasite. The life cycle of the malaria parasite can be seen in Figure 2 (Good, 2009).

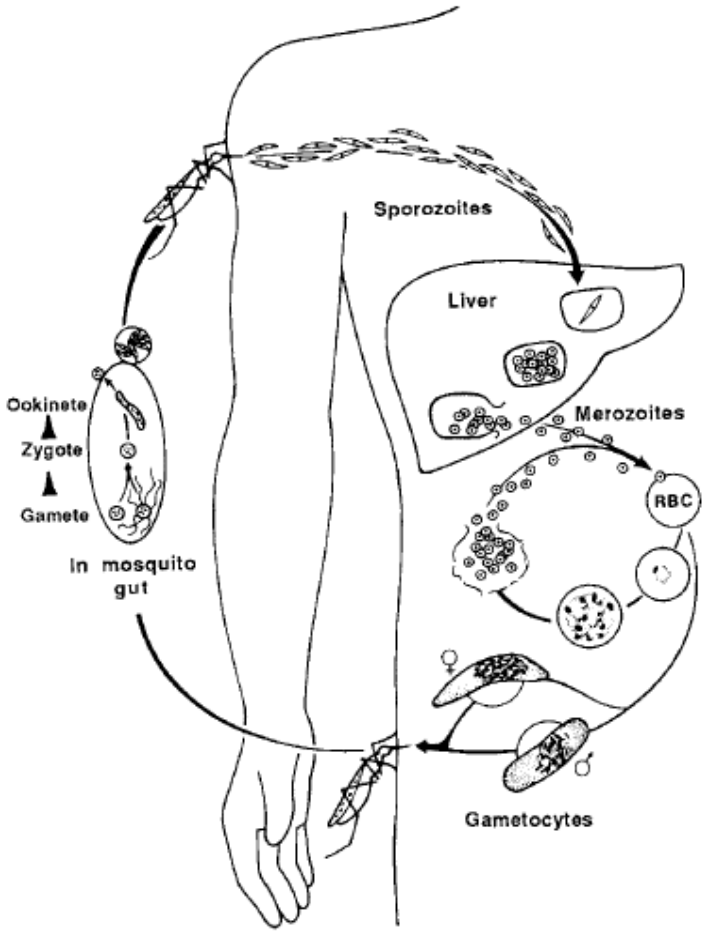


Figure 2. Schematic of the life cycle of the malaria parasite (Good, 2009).

Malaria poses a great threat to developing countries, predominantly in Africa. It is estimated that there were 247 million cases of malaria in 2006, resulting in approximately

881,000 deaths (World Health Organization, 2008). Of these, they report that 91% of these deaths were in Africa, while 4% occurred in both the South-East Asia and Eastern Mediterranean regions. Even though it is most prevalent in African countries it is still present around the world, with the WMR 2008 reporting that there were 109 countries and territories with malaria present. Malaria's spread specifically throughout African countries has led the WHO to label the disease as an endemic.

Malaria is starting to spread outside of its once shrinking areas, predominantly in developing countries, mainly due to newly emerging drug-resistant strains (Verra, 2009; Choi, 2008). This increase has caused a widespread interest in the finding of vaccines and new drugs, as well as combination therapy with existing drugs (Verra, 2009; Good, 2009). Special emphasis is placed on making these new products affordable and easily available, since most of the people of these countries are unable to access preventative measures, such as treated mosquito nets, and the current drugs and combination therapies necessary once they are infected (Verra, 2009). Of the *Plasmodia* genus, *P. falciparum*(*Pf*) is the most widespread in humans, as well as having the most severe symptoms and highest instance of resulting deaths, followed by *P. vivax* (Bello, 2008).

Plasmodial ODCase presents itself as a good antimalarial target for multiple reasons. In *Plasmodia*, the *de novo* pathway is the only source of pyrimidine nucleotides, as these parasites have neither pyrimidine transporters or a salvage pathway. Whereas in human, both a salvage pathway and a *de novo* pathway are operational, providing an opportunity to design ODCase inhibitors that would be nontoxic (Bello, 2008). So if ODCase is knocked out by inhibition, even in the human, the human can still generate the

necessary nucleic bases while the parasite will be unable to reproduce and function normally, leading to it's death. While the focus of therapeutic application is on antimalarials, the target of inhibition of these compounds also leads to potential anticancer effects (Meza-Avina, 2008). The final product of the pyrimidine pathway, UMP, is essential to both DNA and RNA (UMP is an essential building block of the RNA and a precursor to both thymidine and cytidine nucleotides). Thus the inhibitors of ODCase could exhibit potential inhibition of cell proliferation, leading to anticancer effects. With this in mind, all synthesized compounds are also tested against cancer cell lines.

I.c. Methods of Malarial Inhibition

One of the main targets of the malaria causing *Plasmodia* species is the *de novo* synthesis of pyrimidines (Choi, 2008). This 6 step synthesis has many potential targets. Of these, two enzymes are the focus of most antimalarial research for this pathway. Those are dihydroorotate dehydrogenase (DHODH) which catalyzes the fourth step of the synthesis, and orotidine 5'-monophosphate decarboxylase (ODCase), which catalyzes the sixth and final step of the synthesis of uridine (Heikkila, 2007; Hurt, 2006). ODCase is the target chosen for the present study.

Also being researched for potential antimalarial treatments are enzymes of the purine salvage pathway and of the folate synthesis pathway (antifolates) (Kouni, 2003; Rodenko, 2006; Nzila, 2006). The purine synthesis pathway is under some scrutiny for similar reasons as the pyrimidine pathway, except in the purines case the parasites have

only a salvage pathway (Kouni, 2003; Rodenko, 2006). Some of the major enzymes in this pathway are potential targets for combination therapy inhibition, although this is still a developing field (Kouni, 2003). Finally, antifolates are used to target enzymes, mainly dihydrofolate reductase (DHFR), of the folate synthesis pathway (Yuthavong, 2005; Nzila, 2006). Multiple groups are studying new antifolates, as resistance is growing to the ones presently on the market, although no new antifolates as antimalarials have made it to the market besides the originals in the 1940s (Hunt, 2005; Vilaivan, 2003; Muregi, 2009).

I.d. Rationale

Based on the crystal structures of ODCase, it could be seen that there is a hydrophobic pocket situated adjacent to the C5 position when 6-iodo-UMP was bound (Bello, 2008). This pocket suggested the possibility of placing hydrophobic groups at the C5 or C6 position of UMP to promote strong binding of the proposed inhibitor to the enzyme. In this case, the focus was placed on derivatives at the C6 position. This reasoning led to the decision to try to place thiophene and furan heterocycles at this desired position, specifically through a carbon-carbon bond. Another hydrophobic possibility looked at for a C6 derivative of UMP was a tributyltin group.

When basing the design of the derivatives on the substrate, two strategies were adopted based on the discoveries thus far in Kotra laboratory: First was to identify methods to synthesize a nitro group at the C6 position. It was conceived by Profs. Kotra and Pai that the similarities between the carboxylic acid group (at the C6 position of OMP) and a nitro group are isoelectronic, with both the charge and the size of the two

groups being similar. However, the nitro group is perceived to be resistant to removal by ODCase from the C6 position and such compounds will aid in the elucidation of mechanism of decarboxylation of OMP, as well as potentially could be an inhibitor of this enzyme.

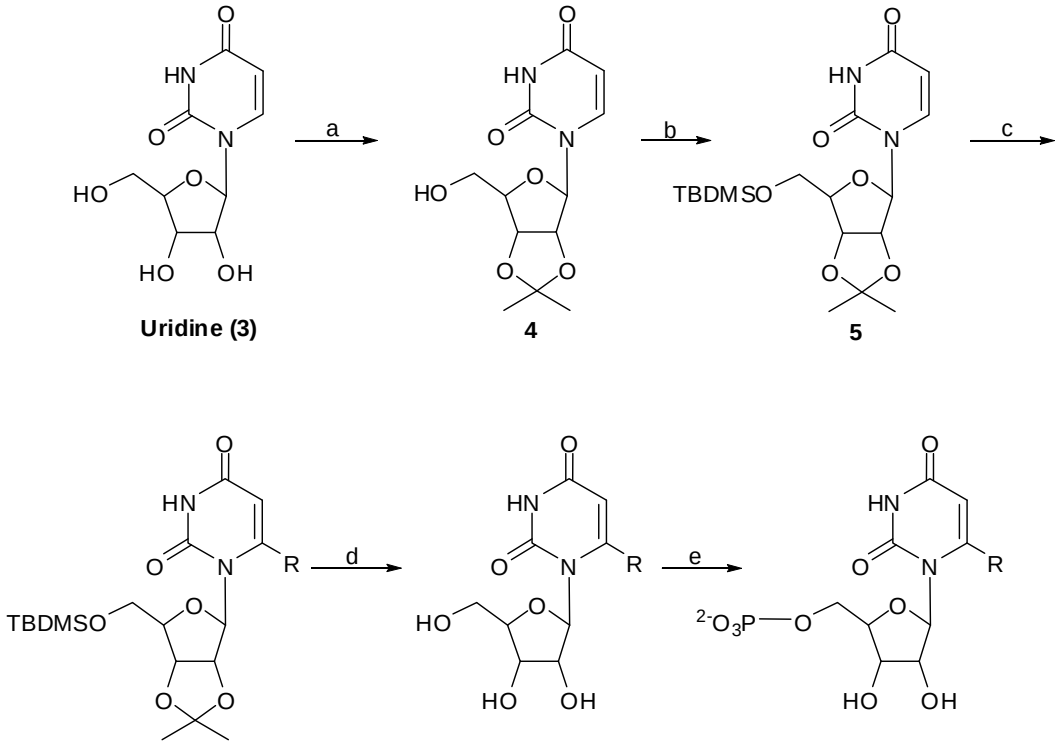
The other strategy is to replace the carboxylic acid group with a tetrazole through a C-C bond. Tetrazole rings have been used multiple times in past medicinal chemistry to replace carboxylic acids, as they are bioisosteres of each other, meaning they have similar physicochemical properties, and so will act similarly under biological conditions (Herr, 2002). Because of the free N-H bond present on tetrazole, they are acidic molecules and have a similar pK_a to carboxylic acid (Herr, 2002). Other similarities include the planar structure, and the fact that tetrazoles are ionized at physiological pH (Herr, 2002). Very importantly, unlike carboxylic acids, tetrazoles have shown resistance to many biological metabolic degradation pathways (Herr, 2002).

I.e. Retrosynthetic Analysis

After deciding on the compounds to be synthesized, retrosynthetic analysis was used to design a synthesis pathway for the desired compounds. Although the desired compound is based on UMP (**1**), the monophosphate group is very reactive and easily lost, so uridine (**3**) was used as a starting material. The reactivity of the free hydroxyl groups on the sugar ring required their protection before further reactions were carried out. This protection was performed by placing a *tert*-butyldimethyl silyl (TBDMS) moiety at the 5'

position and an isopropylidene group at the 2' and 3' positions using known methods (Bello, 2007).

The resulting fully protected uridine was then subjected to different procedures, based on the desired product, to be modified at the C6 position. These coupling procedures include Stille coupling, Click chemistry and conventional nucleophiles to generate the C-C coupled products. The focus for these modifications was primarily on heterocycles connected to C6 through C-C bonds. Finally, the protecting groups were removed and the monophosphate was added to the 5' hydroxyl moiety (Bello, 2007). This general synthesis can be seen in Scheme 3.



Scheme 3. General scheme for synthesis of C6 derivatives of UMP. Reaction conditions: a) acetone, H_2SO_4 b) CH_2Cl_2, imidazole, TBDMSCl, 0 °C→RT c) Specific to derivative d) 50% trifluoroacetic acid in H_2O, 0 °C→RT e) H_2O, $POCl_3$, acetonitrile, pyridine, 0 °C, NH_4OH.

This method of design resulted in the successful synthesis of eight novel compounds that can be seen in Figure 3.

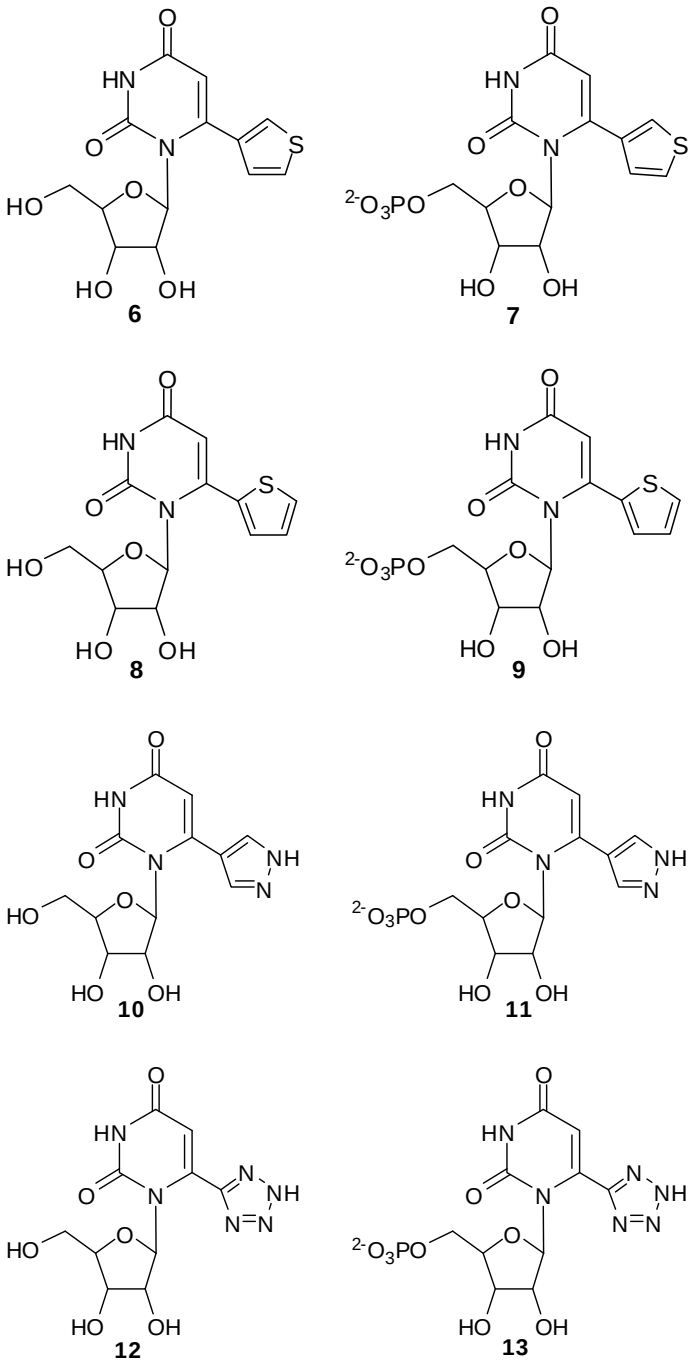


Figure 3. Final successfully synthesized novel compounds. 6-(thiophen-3-yl) uridine (**6**), 6-(thiophen-2-yl) uridine (**8**), 6-(pyrazol-4-yl) uridine (**10**) and 6-(tetrazol-4-yl) uridine (**12**), along with the corresponding nucleotides, 6-(thiophen-3-yl) uridine-5'-monophosphate (**7**), 6-(thiophen-2-yl) uridine-5'-monophosphate (**9**), 6-(pyrazol-4-yl) uridine-5'-monophosphate (**11**) (impure) and 6-(tetrazol-4-yl) uridine-5'-monophosphate (**13**) (impure).

CHAPTER II

MATERIALS AND METHODS

II.a. General Materials

Uridine and all reagents and solvents were obtained from commercially available sources. Argon was used for all reactions that were carried out under anhydrous conditions. Thin layer chromatography plates (TLC) silica gel 60 F_{254} were used to monitor reactions. Charring of TLCs was done using $KMnO_4$. For column chromatography purification on manual and Biotage™ separation system columns, 63-200 μm, 60Å silica gel was used. For purification using reverse phase column chromatography, C18 silica gel was used. Biotage™ column sizes used were 10 g, 25 g and 60 g for regular phase, and 12 g and 60 g for reverse phase C18. For purification and analytical analysis using HPLC, Atlantis T3 preparatory and analytical C18 columns were used on a Varian ProStar. All ^{1}H, ^{13}C and ^{31}P NMRs were obtained using a 500 MHz JEOL NMR instrument. ^{31}P NMRs were referenced to 0 ppm using H_3PO_4. UV and epsilon values were obtained using a Varian.

II.b. Synthesis

2',3'-O-isopropylidene uridine (4). A stirred suspension of anhydrous acetone (100 mL) and uridine 1 (2.0 g, 8.2 mmol) was brought to 0 °C and sulfuric acid (1.0 mL) was added dropwise. After 5 minutes, ice was removed and mixture was allowed to stir at room

temperature for 1.5 hours. Et_3N was then added to neutralize and reaction mixture was filtered and concentrated. The crude mixture was then purified using silica gel column chromatography (4-8% MeOH: CH_3Cl) to obtain the product as a white solid (1.43 g, 61%). ^1H NMR ($CDCL_3$) δ 1.31 (s, 3H, CH_3), 1.53 (s, 3H, CH_3), 3.74 (dd, 1H, H-5''), 3.83 (dd, 1H, H-5'), 4.24 (m, 1H, H-4'), 4.88 (dd, 1H, H-3'), 4.92 (dd, 1H, H-2'), 5.62 (d, 1H, H-1'), 5.70 (d, 1H, H-5), 7.46 (d, 1H, H-6).

5'-O-tert-Butyldimethylsilyl-2',3'-O-isopropylidene uridine (5). A solution was made of 2',3'-*O*-isopropylidene uridine (**4**) (1.2 g, 4.2 mmol) and anhydrous CH_2Cl_2 (18 mL) at 0 °C. Imidazole (0.77 g, 11.3 mmol) and *tert*-butyldimethylsilyl chloride (0.83 g, 5.4 mmol) were added and mixture was allowed to return to room temperature and stirred for 2 hours. Once reaction was complete, the crude mixture was evaporated and the solid dissolved in ethyl acetate (30 mL), washed with water (15 mL) and brine (15 mL) and dried with Na_2SO_4 and concentrated. The crude solid was purified using silica gel column chromatography (0-5% MeOH:$CHCL_3$). Pure product was concentrated to 1.21 g (72%) as a white solid. ^1H NMR ($CDCL_3$) δ 0.07 (s, 6H, CH_3), 0.88 (s, 9H, CH_3), 1.34 (s, 3H, CH_3), 1.57 (s, 3H, CH_3), 3.77 (dd, 1H, H-5''), 3.89 (dd, 1H, H-5'), 4.31 (m, 1H, H-4'), 4.66 (dd, 1H, H-3'), 4.73 (dd, 1H, H-2'), 5.65 (d, 1H, H-5), 5.96 (d, 1H, H-1'), 7.68 (d, 1H, H-6), 8.49 (brs, 1H, -NH).

5'-O-tert-Butyldimethylsilyl-6-iodo-2',3'-O-isopropylidene uridine (22). A solution was made of 5'-*O*-*tert*-butyldimethylsilyl-2',3'-*O*-isopropylidene uridine 5 (2.0 g, 5.0 mmol)

in anhydrous THF (10 mL) at -78 °C. LDA (6.52 mL, 13.0 mmol, 2 M solution in THF) was added dropwise and mixture was allowed to stir for 1 hour at -78 °C. After 1 hour, iodine (1.53 g, 6.0 mmol) in anhydrous THF (5 mL) was added and mixture was stirred for 2.5 hours at -78 °C. Reaction was then quenched with 4.2 mL water and dissolved in ethyl acetate (40 mL), washed with brine (25 mL) and dried with Na_2SO_4. Crude mixture was then concentrated and purified using silica gel column chromatography (0-30% ethyl acetate:hexanes) and 1.0 g (38%) pure compound was obtained as a dark yellow solid. UV ($CHCl_3$): λ_{max}=268 nm. ^{1}H NMR ($CDCL_3$) δ 0.08 (s, 6H, CH_3), 0.90 (s, 9H, CH_3), 1.30 (s, 3H, CH_3), 1.51 (s, 3H, CH_3), 3.75 (m, 2H, H-5', H-5''), 4.13 (m, 1H, H-4'), 4.77 (dd, 1H, H-3'), 5.14 (dd, 1H, H-2'), 6.04 (d, 1H, H-1'), 6.40 (s, 1H, H-5), 9.43 (brs, 1H, -NH).

5'-O-tert-Butyldimethylsilyl-2',3'-O-isopropylidene-6-tributyltin uridine (14). Used same method as above, replacing iodine with tributyltin chloride (10.0 mmol, 2 equivalents). Crude mixture was purified using silica gel column chromatography (0-20% ethyl acetate:hexanes), giving 2.66 g (77%) of pure product as a yellow oil. UV ($CHCl_3$): λ_{max}=269 nm; ^{1}H NMR ($CDCL_3$) δ 0.01 (s, 6H, CH_3), 0.67-1.65 (m, 42H), 3.78 (m, 2H, H-5', H-5''), 4.08 (m, 1H, H-4'), 4.76 (dd, 1H, H-3'), 5.17 (dd, 1H, H-2'), 5.26 (d, 1H, H-1'), 5.67 (s, 1H, H-5), 8.05 (brs, 1H, -NH).

5'-O-tert-Butyldimethylsilyl-6-(furan-2-yl)-2',3'-O-isopropylidene uridine.

Used same method as above, replacing iodine with 3-bromofuran (5.5 mmol, 1.1 equivalents). Additional quenching methods tried on separate reaction attempts included saturated NH_4Cl solution, and a 1:1 saturated NH_4Cl:ethyl acetate solution, both followed by work-up in above procedure. Clean product not obtained.

5'-O-tert-Butyldimethylsilyl -2',3'-O-isopropylidene-6-(thiophene-3-yl) uridine (17). A solution of 5'-*O-tert*-butyldimethylsilyl-2',3'-*O*-isopropylidene-6-tributyltin uridine (**14**) (0.20 g, 0.29 mmol) in DMF (5 mL) was added to a flask with CuI (0.0114 g, 0.060 mmol), $Pd(TPP)_4$ (0.035 g, 0.030 mmol) and 3-iodothiophene (0.04 mL, 0.39 mmol). The reaction mixture was stirred at 80 °C for 1.5 hours. The reaction mixture was allowed to return to room temperature, and was treated with saturated NH_4Cl (15 mL), then extracted twice with ethyl acetate (20 mL). Organic layers were combined and washed with saturated $KF{\cdot}H_2O$ (20 mL), twice with water (20 mL) and brine (20 mL). Reaction mixture was then dried with $MgSO_4$ and concentrated. Crude product was then purified using silica gel column chromatography (0-30% ethyl acetate:hexanes). Pure product obtained as white solid with 91% yield (0.13 g). UV ($CHCl_3$) λ_{max}=278 nm; ^{1}H NMR (Acetone-d_6) δ 0.06 (s, 6H, CH_3), 0.90 (s, 9H, CH_3), 1.27 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 3.85 (m, 2H, H-5', H-5''), 4.04 (m, 1H, H-4'), 4.81 (dd, 1H, H-3'), 5.28 (dd, 1H, H-2'), 5.57 (d, 1H, H-1'), 5.63 (d, 1H, H-5), 7.37 (d, 1H, H-4''), 7.73 (d, 1H, H-2''), 7.90 (d, 1H, H-5''').

5'-O-tert-Butyldimethylsilyl-2',3'-O-isopropylidene-6-(thiophene-2-yl) uridine (16). Used same method as above, replacing 3-iodothiophene with 2-iodothiophene (0.39 mmol, 1.3 equivalents). Crude product was then purified using silica gel column chromatography (0-30% ethyl acetate:hexanes). Pure product obtained as white solid with 90% yield (0.11 g). UV ($CHCl_3$): λ_{max}=275 nm; ^{1}H NMR ($CDCL_3$) δ 0.06 (s, 6H, CH_3), 0.90 (s, 9H, CH_3), 1.27 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 3.85 (m, 2H, H-5', H-5''), 4.06 (m, 1H, H-4'), 4.81 (dd, 1H, H-3'), 5.28 (dd, 1H, H-2'), 5.69 (d, 1H, H-1'), 5.74 (s, 1H, H-5), 7.26 (dd, 1H, H-4''), 7.51 (dd, 1H, H-3''), 7.81 (dd, 1H, H-5''').

5'-O-tert-Butyldimethylsilyl-6-(furan-2-yl)-2',3'-O-isopropylidene uridine

Used same method as above, replacing 3-iodothiophene with 2-bromofuran (0.39 mmol, 1.3 equivalents). Clean product was not obtained.

5'-O-tert-Butyldimethylsilyl-2',3'-O-isopropylidene-6-(imidazol-4-yl) uridine

Used same method as above, replacing 3-iodothiophene with 4-iodoimidazole (0.39 mmol, 1.3 equivalents). Clean product was not obtained.

5'-O-tert-Butyldimethylsilyl -2',3'-O-isopropylidene-6-(pyrazol-4-yl) uridine (15).

Used same method as above, replacing 3-iodothiophene with 4-iodopyrazole (0.39 mmol, 1.3 equivalents). Crude product was purified using silica gel column chromatography (0-4% MeOH:$CHCl_3$). Pure product was obtained as white solid with 50% yield (0.068 g). UV ($CHCl_3$): λ_{max}=279 nm; ^{1}H NMR ($CDCL_3$) δ 0.04 (s, 6H, CH_3), 0.89 (s, 9H, CH_3),

1.29 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 3.83 (m, 2H, H-5', H-5''), 4.13 (m, 1H, H-4'), 4.83 (dd, 1H, H-3'), 5.21 (dd, 1H, H-2'), 5.67 (d, 1H, H-1'), 5.76 (s, 1H, H-5), 7.93 (s, 2H, H-3'', H-5'''), 9.14 (brs, 1H, -NH); MS (ESI) calculated ($M+Na^+$) 487.2, observed 487.2.

5-Bromo-5'-O-tert-butyldimethylsilyl-2',3'-O-isopropylidene uridine (18). To a stirred suspension of 5'-*O-tert*-butyldimethylsilyl-2',3'-*O*-isopropylidene uridine (**5**) (2.0 g, 5.0 mmol) in 1,2-dimethoxy ethane (40 mL) was added a solution of NaN_3 (1.305 g, 20.1 mmol) in water (6 mL). *N*-Bromosuccinimide (0.982 g, 5.5 mmol) was added and reaction was stirred at room temperature for 24 hours. Crude mixture was concentrated and dissolved in ethyl acetate (20 mL), extracted with water (20 mL) and dried with Na_2SO_4. Reaction mixture was clean with quantitative yield (2.44 g) and was taken directly to next step. UV ($CHCl_3$): λ_{max}=278 nm; ^{1}H NMR ($CDCL_3$) δ 0.06 (s, 6H, CH_3), 0.85 (s, 9H, CH_3), 1.30 (s, 3H, CH_3), 1.53 (s, 3H, CH_3), 3.74 (dd, 1H, H-5''), 3.87 (dd, 1H, H-5'), 4.34 (m, 1H, H-4'), 4.65 (dd, 1H, H-3'), 4.67 (dd, 1H, H-2'), 5.87 (d, 1H, H-1'), 7.86 (s, 1H, H-6), 9.88 (brs, 1H, -NH).

6-Cyano-5'-O-tert-butyldimethylsilyl-2',3'-O-isopropylidene uridine (19). Anhydrous DMF (20 mL) was added to 5-bromo-5'-*O-tert*-butyldimethylsilyl-2',3'-*O*-isopropylidene uridine (**18**) (1.78 g, 3.7 mmol) and NaCN (0.2923 g, 5.96 mmol). The reaction mixture was allowed to stir at room temperature 12 hours, and then was brought to pH 6 with 10% HCl and extracted with ethyl acetate (20 mL) and water (15 mL), washed with brine (20 mL) and dried with Na_2SO_4. The product has the same chromatographic shift on the

TLC as that of starting material, and the product was identified using UV spectroscopy prior to purification. The product was purified using silica gel column chromatography (0-40% ethyl acetate:hexanes), to give 1.01 g (64%) of product as a white solid. UV ($CHCl_3$): λ_{max}=283 nm; ^{1}H NMR ($CDCL_3$) δ 0.04 (s, 6H, CH_3), 0.88 (s, 9H, CH_3), 1.33 (s, 3H, CH_3), 1.51 (s, 3H, CH_3), 3.81 (m, 2H, H-5', H-5''), 4.13 (m, 1H, H-4'), 4.75 (dd, 1H, H-3'), 5.11 (dd, 1H, H-2'), 6.02 (d, 1H, H-1'), 6.27 (s, 1H, H-5), 8.27 (brs, 1H, -NH).

5'-O-tert-Butyldimethylsilyl-2',3'-O-isopropylidene-6-(tetrazol-4-yl) uridine (20) and 2',3'-O-isopropylidene-6-(tetrazol-4-yl) uridine (side product;21). Anhydrous DMF (10 mL) was added to 6-cyano-5'-*O-tert*-butyldimethylsilyl-2',3'-*O*-isopropylidene uridine 19 (0.837 g, 2.0 mmol), NaN_3 (0.1413 g, 2.2 mmol) and NH_4Cl (0.116 g, 2.2 mmol) and mixture was stirred at 90 °C for 2 hours. Reaction mixture was then allowed to return to room temperature and water (30 mL) was added. Since no precipitate was seen, the mixture was then extracted with ethyl acetate, with most of the product going into the aqueous layer. Both layers were evaporated separately and were purified using silica gel column chromatography on a Biotage system (0-20% MeOH:$CHCl_3$). The product was eluted at 15% MeOH:$CHCl_3$. The total yield of the crude product including the side product was 0.475 g (58%) obtained as a white solid. UV (MeOH): λ_{max}= 274 nm; ^{1}H NMR (DMSO-d_6) δ 0.01 (s, 6H, CH_3), 0.84 (s, 9H, CH_3), 1.29 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 3.73 (m, 2H, H-5', H-5''), 3.89 (m, 1H, H-4'), 4.71 (dd, 1H, H-3'), 5.23 (dd, 1H, H-2'), 5.88 (d, 1H, H-1'), 6.76 (s, 1H, H-5), 11.48 (brs, 1H, -NH).

6-(Thiophene-2-yl) uridine (8). 5'-*O*-*tert*-Butyldimethylsilyl-6-(thiophene-2-yl)-2',3'-*O*-isopropylidene uridine (0.3 g, 0.5 mmol) was placed in a flask at 0 °C and 3 mL of a 50% aqueous solution of trifluoroacetic acid was added. After 10 minutes, reaction was allowed to return to room temperature and stirred for an additional 2 hours. The reaction mixture was then concentrated. Crude compound was washed with water, the solvent was evaporated twice more to remove excess TFA. Crude compound was purified using silica gel column chromatography (0-8% MeOH:$CHCl_3$) to obtain a pure product as a very pale yellow solid (0.113 g, 55%). UV (MeOH): λ_{max}=275 nm (ε=6368), ^{1}H NMR (MeOH-d_4) δ 3.55 (m, 1H, H-5''), 3.67 (m, 2H, H-4', H-5'), 4.17 (dd, 1H, H-3'), 4.62 (dd, 1H, H-2'), 5.41 (d, 1H, H-1'), 5.61 (s, 1H, H-5), 7.07 (dd, 1H, H-4''), 7.42 (dd, 1H, H-2''), 7.57 (dd, 1H, H-5''') MS (ESI): Calculated ($M+Na^+$) 349.33, observed 349.05. 97% pure (HPLC).

6-(Thiophene-3-yl) uridine (6). Used same method as above, replacing the starting material with 5'-*O*-(*tert*-butyldimethylsilyl)-2',3'-*O*-isopropylidene-6-(thiophene-3-yl) uridine (0.350 g, 0.58 mmol). Crude compound was purified using silica gel column chromatography (0-8% MeOH:$CHCl_3$) to obtain the pure product (0.167 g, % yield) as a white solid. UV (MeOH): λ_{max}=276 nm (ε=9115), ^{1}H NMR (MeOH-d_4) δ 3.54 (m, 1H, H-5''), 3.65 (m, 2H, H-4', H-5'), 4.14 (dd, 1H, H-3'), 4.59 (dd, 1H, H-2'), 5.24 (d, 1H, H-1'), 5.54 (s, 1H, H-5), 7.23 (dd, 1H, H-4''), 7.47 (dd, 1H, H-2''), 7.71 (dd, 1H, H-5'''). MS (ESI): Calculated ($M+Na^+$) 349.33, observed 349.05. 99% pure (HPLC).

6-(Pyrazol-4-yl) uridine (10). Used same method as above, replacing the starting material with 5'-*O*-(*tert*-butyldimethylsilyl)-2',3'-*O*-isopropylidene-6-(pyrazol-4-yl) uridine (0.160 g, 0.34 mmol). To make crude product soluble in water, 1 M NH_4OH was added dropwise until full solubility was achieved. The resulting mixture was then loaded on a C18 reverse silica gel column. The product came with the solvent front in 100% water. 0.064 g (60%) pure product obtained as white solid. UV (H_2O): λ_{max}= 278 nm (ε=9607), ^{1}H NMR (D_2O with NH_4OH) δ 3.63 (m, 1H, H-5''), 3.74 (m, 2H, H-4', H-5'), 4.18 (dd, 1H, H-3'), 4.77 (peak overlaps D_2O, 1H, H-2'), 5.48 (d, 1H, H-1'), 5.64 (s, 1H, H-5), 7.83 (s, 2H, H-3'', H-5'''). 96% pure (HPLC).

6-(Tetrazol-4-yl) uridine (12). Used same method as above, replacing the starting material with 5'-*O*-(*tert*-butyldimethylsilyl)-2',3'-*O*-isopropylidene-6-(tetrazol-4-yl) uridine (0.200 g, 0.43 mmol). Crude compound purified using C18 reverse phase silica gel, 100% water. Pure compound obtained in quantitative yield (0.134 g, quantitative yield) as a white solid. UV (H_2O): λ_{max} = 274 nm (ε = 9460), ^{1}H NMR (DMSO-d_6) δ 3.31 (dd, 1H, H-5''), 3.46 (dd, 1H, H-5'), 3.56 (m, 1H, H-4'), 3.90 (dd, 1H, H-3'), 4.51 (dd, 1H, H-2'), 5.38 (d, 1H, H-1'), 6.03 (s, 1H, H-5), 11.76 (brs, 1H, -NH). 97% pure (HPLC).

6-(Thiophene-2-yl) uridine-5'-monophosphate (9). Anhydrous CH_3CN (2.5 mL) was added to flask with H_2O (7.57 µL, 0.44 mmol) and pyridine (0.058 mL, 0.75 mmol). Flask was brought to 0 °C and $POCl_3$ (0.060 mL, 0.69 mmol) was added. The reaction mixture was allowed to stir for 10 minutes at 0 °C and then added to 6-(thiophene-2-yl)

uridine (0.051 g, 0.16 mmol) under anhydrous conditions. Mixture was allowed to stir at 0 °C for two hours, and then at room temperature for an additional two hours. Cold water (15 mL) was then added and reaction mixture was allowed to stir at room temperature for another hour. Reaction mixture was then evaporated and crude mixture was run on a dowex ion exchange column (0-6% formic acid:water) to purify. Crude mixture was then concentrated and neutralized at 0 °C with 40% NH_4OH solution. Crude mixture was again concentrated and purified using C18 reverse phase silica gel column, coming in 100% water. Pure product was obtained as white solid (0.026 g, 38%). UV (H_2O): λ_{max}= 275 nm (ε=2428), ^{1}H NMR (D_2O) δ 3.53 (m, 1H, H-5''), 3.59 (m, 1H, H-5'), 3.66 (m, 1H, H-4'), 4.11 (dd, 1H, H-3'), 4.82 (peak overlaps D_2O, 1H, H-2'), 5.35 (d, 1H, H-1'), 5.73 (s, 1H, H-5), 7.00 (dd, 1H, H-4''), 7.25 (dd, 1H, H-3''), 7.50 (dd, 1H, H-5'''). 31% pure (HPLC).

6-(thiophene-3-yl) uridine-5'-monophosphate (7). Used same method as above, replacing starting material with 6-(thiophene-3-yl) uridine (0.051 g, 0.16 mmol). Pure compound obtained as white solid 0.001 g (1.5%). UV (H_2O): λ_{max}= 276 nm (ε=4290), ^{1}H NMR (D_2O) δ 3.69 (m, 1H, H-5''), 3.78 (m, 1H, H-5'), 3.90 (m, 1H, H-4'), 4.18 (dd, 1H. H-3'), 5.24 (d, 1H, H-1'), 5.68 (s, 1H, H-5), 7.11 (dd, 1H, H-4''), 7.43 (dd, 1H, H-2''), 7.62 (dd, 1H, H-5''') MS (ESI): Calculated ($M-2NH_3+Na^+$) 429.01, observed 429.01. Amount insufficient for purity testing.

6-(Pyrazol-4-yl) uridine-5'-monophosphate (11, impure). Used same method as above, replacing starting material with 6-(pyrazol-4-yl) uridine (0.050 g, 0.16 mmol). Concentrated and neutralized crude reaction mixture at 0 °C with 40% NH_4OH solution and purified using C18 reverse phase silica gel column, product coming with 1% acetic acid in water. Impure compound obtained as white solid (0.038 g of impure compound). UV (H_2O): λ_{max} = 280 nm, (ε=791), ^1H NMR (D_2O; obtained for impure compound) δ 3.89 (m, 2H, H-5'', H-5'), 4.02 (m, 1H, H-4'), 4.36 (dd, 1H, H-3'), 5.59 (d, 1H, H-1'), 5.84 (s, 1H, H-5), 7.98 (s, 2H, H-3'', H-5'').

6-(Tetrazol-4-yl) uridine-5'-monophosphate (13, impure). Used same method as above, replacing starting material with 6-(tetrazol-4-yl) uridine (0.100 g, 0.32 mmol). Mixture of product and starting material obtained (0.060 g), unable to be purified using C18 reverse phase silica gel column. Achieved separation on analytical reverse phase HPLC (0-20% CH_3CN:1% HCl aqueous solution). UV (H_2O): λ_{max} = 274 nm, ^1H NMR (D_2O; obtained for impure compound) δ ppm 3.88 (m, 2H, H-5'', H-5'), 4.01 (m, 1H, H-4'), 4.31 (dd, 1H, H-3'), 5.43 (d, 1H, H-1'), 6.02 (s, 1H, H-5).

II.c. Biological Testing

Anticancer Activity

Anticancer activity evaluations were performed by Dr. Danijela Konforte (Prof. Christopher Paige's laboratory) at Ontario Cancer Institute, Princess Margaret Hospital,

Toronto, Ontario, Canada. All successfully synthesized nucleosides were screened for their potential growth inhibition activity on OCI-AML1 cell line isolated from acute myeloid leukemia patients, and was a gift from Dr. M. Minden's lab at the Ontario Cancer Institute in Ontario, Canada. OCI-AML1 cells were grown in Iscove's MDM supplemented with 5% fetal calf serum (FCS). The *in vitro* cell proliferation assay was carried out by WST.1 assay (water soluble tetrazolium salt: 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate), which is a colorimetric assay for the nonradioactive quantification of cell proliferation, cell viability and cytotoxicity. It relies on the cleavage of WST.1 to formazan by cellular enzymes. The amount of formazan dye formed correlates to the number of metabolically active cells in the culture and is readily read in an ELISA microplate reader using an absorbance wavelength of 440 nm. These OD values were plotted against concentrations of different compounds and inhibition constant 50 (IC_{50}) was determined for each compound.

Antimalarial Activity

Antimalarial activities were evaluated in the laboratories of Dr. Ian Crandall (Department of Laboratory Medicine and Pathobiology, University of Toronto) and Dr. Kevin Kain (Department of Medicine, University of Toronto). Assays comparing the antiplasmodial activities of the inhibitors were performed using the SYBR-Green method against *P. falciparum* (3D7) strain. A known concentration of the inhibitor and RPMI-A medium was treated with parasite culture (2% hematocrit, 2% parasitemia) and incubated for 48 hours at 37 °C. Relative fluorescence was then determined using a Fluostar Optima

plate reader. After this, IC_{50} values of the inhibitor could be determined using nonlinear regression analysis of the data using the computer program SigmaPlot.

ODCase Inhibition Activity

Inhibition of ODCase activities was conducted by Ms. Ewa Poduch at Toronto General Research Institute, University Health Network, Toronto, Ontario, Canada. The ODCase inhibitory properties of the synthesized compounds were evaluated using isothermal titration calorimetry (ITC). The reaction buffer typically consisted of 50 mM Tris, 20 mM DTT and 40 mM NaCl, adjusted to pH 7.5. The inhibition assay was initiated by coinjection of the OMP as substrate and the inhibitor into the ITC sample chamber and change in heat was measured as the reaction progressed until the heat returned to baseline, indicating the end of the reaction. The final substrate concentration was typically 40 μM in the reaction cell. The inhibition constant for the inhibitors was calculated either by direct fitting of the linear portions of the thermogram into the fitting function of Origin software or by Dixon plot method.

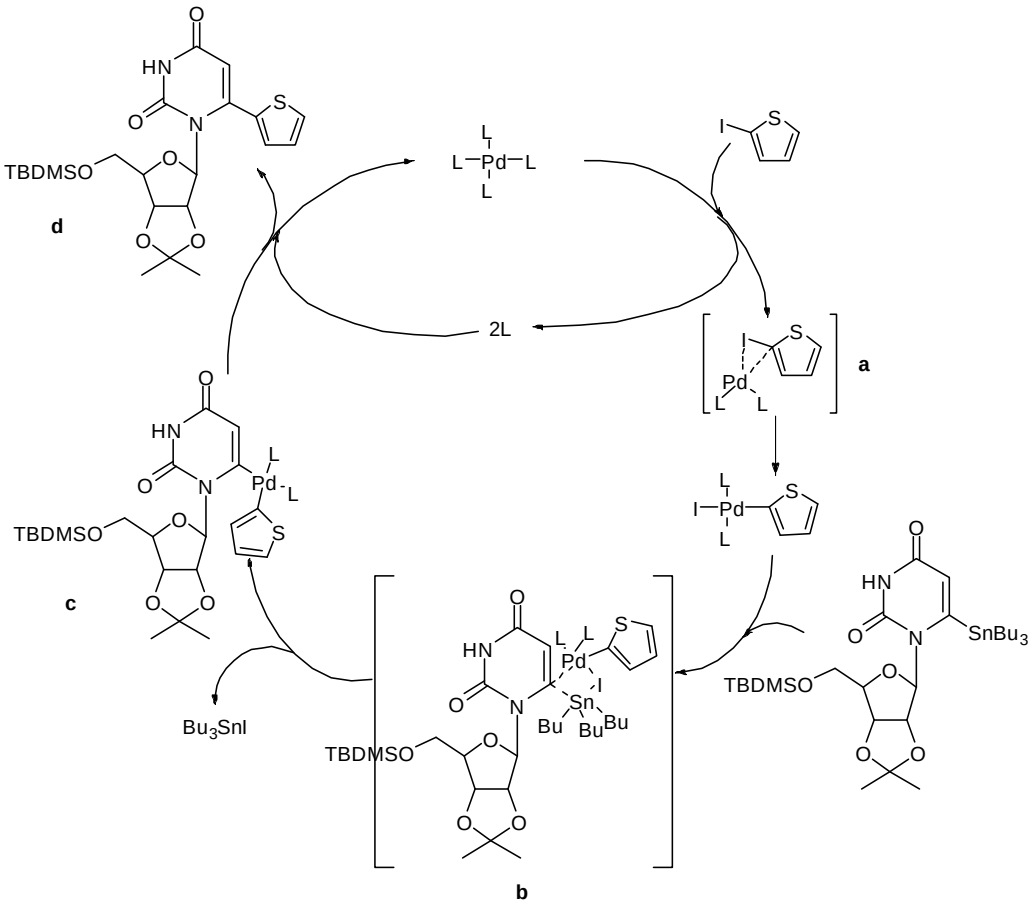
CHAPTER III

RESULTS AND DISCUSSION

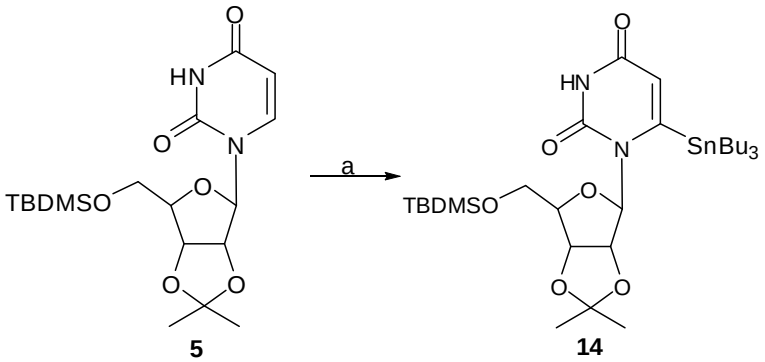
III.a. Stille Coupling

For three of the four final compounds successfully synthesized, 6-(pyrazol-4-yl) UMP (**11**), 6-(thiophen-2-yl) UMP (**9**) and 6-(thiophen-3-yl) UMP (**7**), Stille Coupling was used to establish the C-C bond between C6 of uridine and the heterocycle. Stille-coupling employs the use of a stannane on an aryl halide to produce a C−C bond (Scheme 4). This method was ideal, as the intermediate, 5'-*O*-(*tert*-butyldimethylsilyl)-6-tributyltin-2',3'-*O*-isopropylidene uridine, had been previously synthesized successfully (Scheme 5) (Palmisano, 1993). Another incentive for the use of this method was the versatility, as it was projected to work with any heterocycle containing a halide group. Of the five heterocycles used, three produced the desired product, with two of those three having desirable yields of ≥90%.

To produce the intermediate, 6-tributyltin fully protected uridine (**14**) was synthesized. This was done according to a known procedure reported by Giovanni Palmisano and his group (Palmisano, 1993). The synthesis was successful with a 71% yield. The synthesis for this intermediate is presented in Scheme 5.

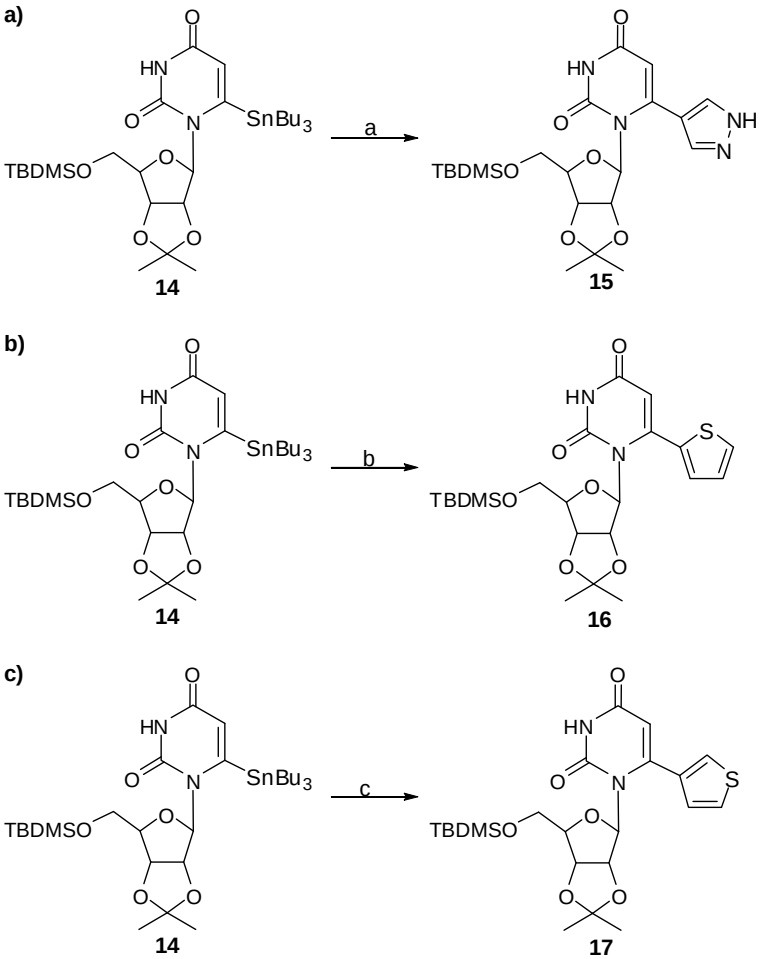


Scheme 4. Reaction mechanism for Stille coupling. L= triphenylphosphine.



Scheme 5. General synthesis for formation of 6-tributyltin intermediate from fully protected uridine. (a) LDA, THF, -78 °C, *n*-Bu_3SnCl.

The three methods using heterocycles that achieved success were using 2-iodothiophene, 3-iodothiophene and 4-iodopyrazole to give the 2-thiophenyl protected uridine (**16),** 3-thiophenyl protected uridine (**17)**, and 4-pyrazolyl protected uridine (**15)**, respectively, seen in Scheme 6. For 2-iodothiophene and 3-iodothiophene (Scheme 4b and 4c, respectively), the method was successful with up to 90% yields. The byproducts produced were very different in polarity, allowing easy separation.



Scheme 6. Schemes for formation of compounds synthesized using Stille coupling. (a) DMF, $Pd(TPP)_4$, 4-Iodopyrazole, CuI, 80 °C (b) DMF, $Pd(TPP)_4$, 2-Iodothiophene, CuI, 80 °C (c) DMF, $Pd(TPP)_4$, 3-Iodothiophene, CuI, 80 °C.

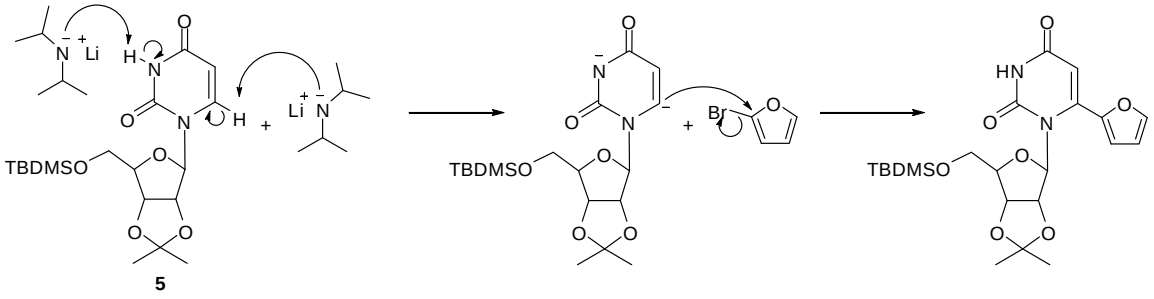
The method of direct nucleophilic attack using LDA was attempted with both 2-iodo and 3-iodothiophene, however the yields were less than 50% in both cases (Bello, 2008). Thus Stille coupling was chosen as a method of choice for subsequent synthesis.

The coupling of 4-iodopyrazole to compound **14** (Scheme 4a) presented challenges, and moderate yields (50-60%) were observed compared to those with 2- or 3-iodothiophene. The first problem was seen when the ^{1}H NMR spectrum was recorded on a presumed pure product isolated from the column chromatography. All desired peaks were present, however there was a set of three extra peaks at ~7.5 ppm (specific ppm values) and the peak areas were in the ratio 2:1:2. After purifying the compound again, the peaks were still present in the same ratio to each other, but in a different ratio to known product peaks, leading to the conclusion that they were an impurity rather than attached to the compound. Based on the location and ratio, the impurity was suspected to be a substituted phenyl group, which was supported by the fact that the catalyst for the reaction was palladium triphenylphosphine. Based on this information, purification solvents were changed from ethyl acetate and hexanes to methanol and chloroform, from 0-4% MeOH:$CHCl_3$. After these efforts, the product was obtained in its pure form. The attempts to purify the nucleotide of this compound are ongoing.

Stille coupling did not yield products when performed with 4-iodoimidazole and 2-bromofuran. When 2-bromofuran was reacted with the fully protected 6-tributyltin uridine, the tributyltin group was lost, yielding fully protected uridine. This led to the conclusion that it was the bromide as the halogen that was impeding the success of this

reaction, and as iodated furans were not available, this method was not useful to prepare the target compounds in this project.

A mechanism involving direct nucleophilic attack (Scheme 7) was also attempted with the 2-bromofuran using LDA, and the TLC indicated that the reaction may be successful. However, as soon as the reaction was quenched or exposed to moisture, the presumed product was spontaneously converted to fully protected uridine (**5**). Several work up procedures were attempted, to reduce the amount of water in the reaction mixture, including a 1:1 mixture of ethyl acetate and saturated NH_4Cl, but all resulted in the same reversion to fully protected uridine.



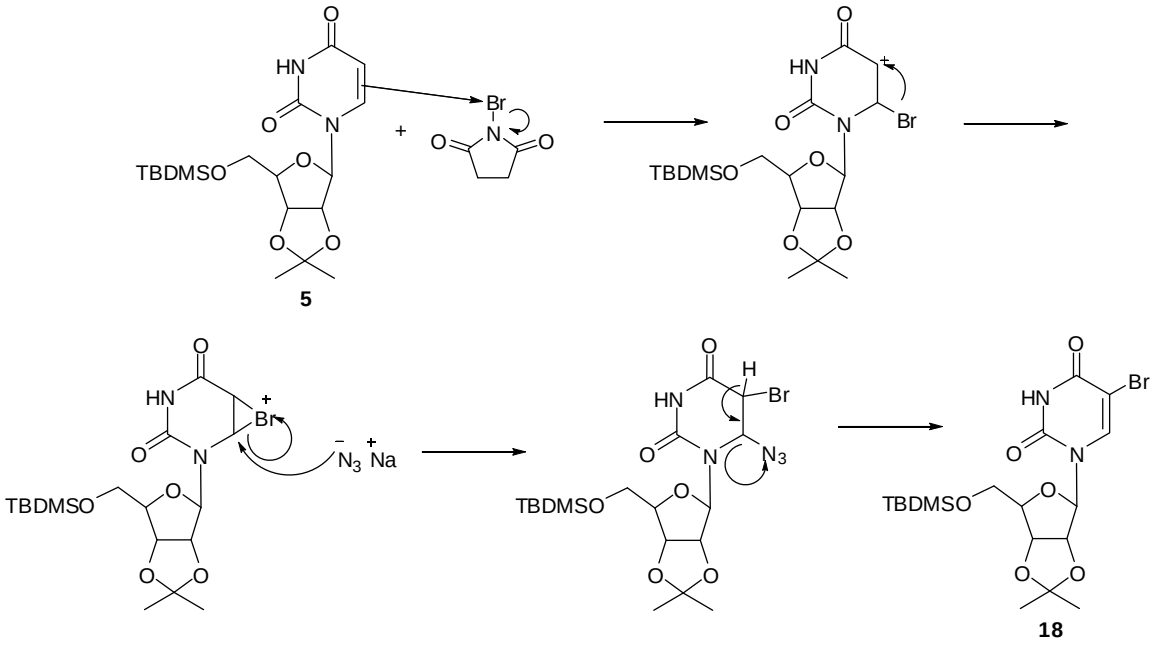
Scheme 7. Mechanism involving direct nucleophilic attack of C-6 lithiated uridine onto 2-bromouridine.

When Stille coupling was attempted with 4-iodoimidazole, the TLCs used to monitor the reaction were extremely messy, containing 4-6 separate spots. This reaction was tried multiple times with similar results. Purification was attempted, and fractions tested using ^{1}H and ^{13}C NMR and UV, but all gave inconclusive results. As two other

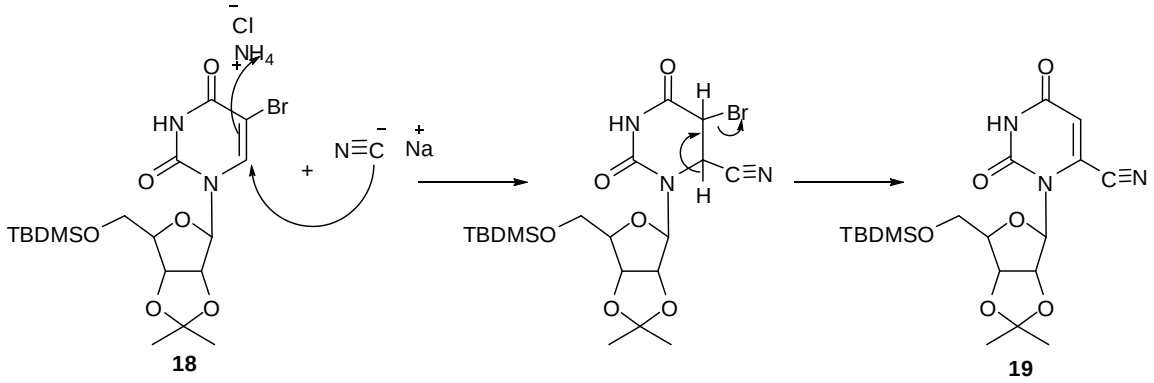
derivatives with nitrogen heterocycles had been successfully prepared, this compound was abandoned at this point.

III.b. Click Chemistry

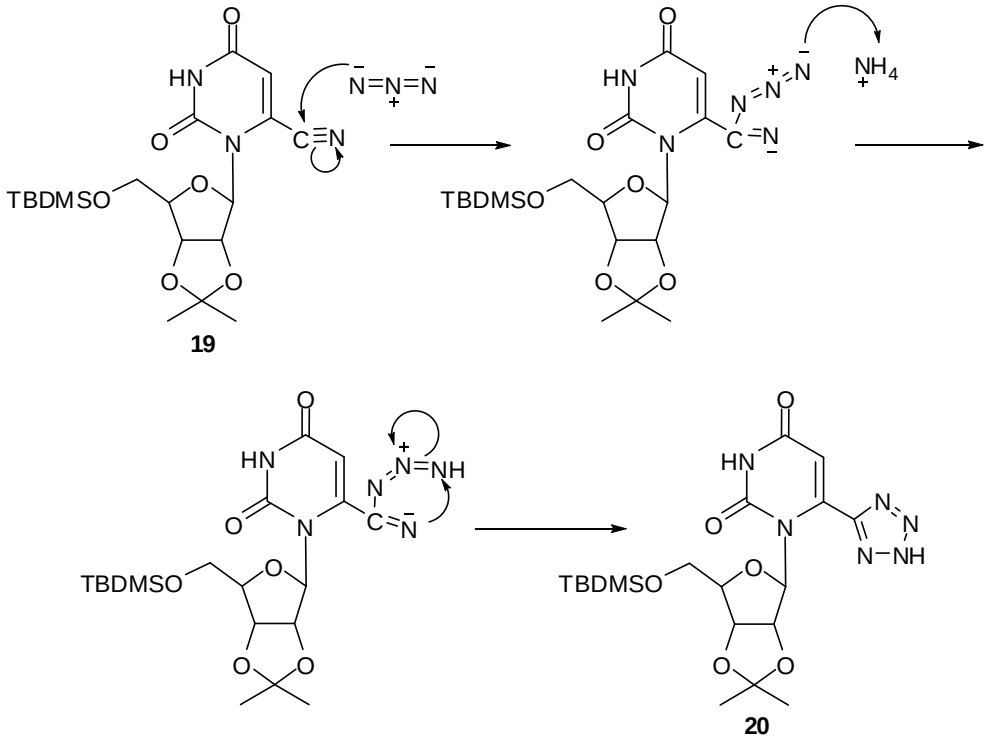
Click chemistry is a term used to describe a set of reliable, quick and selective reactions by joining small reactive units, usually through hetero-atom link, to generate specific substances (Leuven, 2007). The term was first used by H.C. Kolb, M.G. Finn and K.B. Sharpless in 2001 and has three main classes, including nucleophilic opening of spring-loaded electrophiles, condensation reactions of carbonyl compounds and cycloaddition reactions (Kolb, 2001; Leuven, 2007). The mechanism used for the synthesis of 6-tetrazole UMP (Schemes 8-10) falls into the third category. In this mechanism, 5-bromo fully protected uridine (**18**) was prepared, then converted to 6-cyano fully protected uridine (**19**) (Kumar, 1994; Ueda, 1975; Poduch, 2006). This was then cyclized using the Click Chemistry methodology with NaN_3 to form 6-tetrazole fully protected uridine (**20**) (Kumar, 1996; Poduch, 2006). This synthesis can be seen in the following scheme, Scheme 11, and was based on previous synthesis of C6-derivatives by Poduch et al. (Poduch, 2006; and references cited there in).



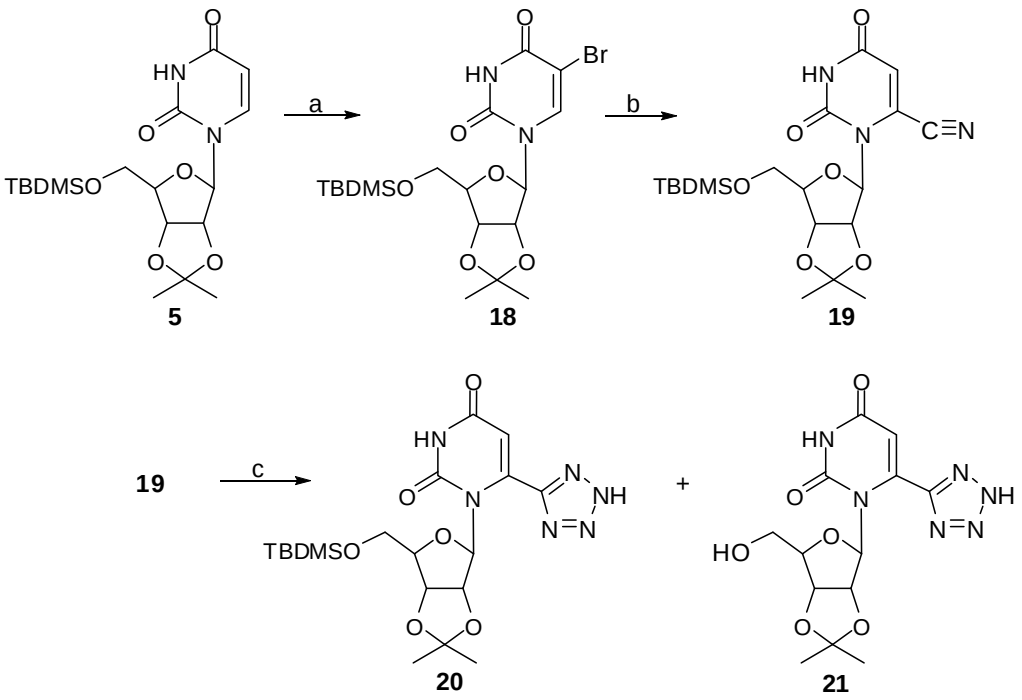
Scheme 8. Reaction mechanism for the formation of 5-bromo from fully protected uridine.



Scheme 9. Reaction mechanism for the formation of 6-cyano from the 5-bromo fully protected uridine derivative.



Scheme 10. Reaction mechanism for the formation of 6-tetrazole from the fully protected 6-cyano uridine derivative.



Scheme 11. Synthesis for the formation of 6-tetrazolyl-UMP using click chemistry methodology. Reaction conditions: (a) 1,2-dimethoxyethane, NaN_3, H_2O, *N*-bromosuccinimide (b) NaCN, DMF (c) NaN_3, NH_4Cl, DMF, 90 °C.

This method of synthesizing 6-tetrazole uridine had previously been successful, but after deprotection, the researchers had been unable to properly purify, and so could not get pure 6-tetrazole uridine or go on to make 6-tetrazole uridine-5'-monophosphate (Bello, 2008). Purification problems first occurred with the fully protected compound. The tetrazole group, which is highly polar, imposed a high polarity on compound when attached, making regular phase separation difficult. However, the TBDMS and isopropylidene protecting groups countered this increased polarity and so reverse phase separation was unsuccessful.

Purification was tried using a manual silica gel column, with solvent gradients from 0-10% MeOH:$CHCl_3$, however triethylamine (up to 5%) had to be used to neutralize the acidic nature of the silica gel. The pure product was obtained by this method, however the triethylamine could not be extracted even after several attempts of neutralization with 1% HCl and extraction with ether.

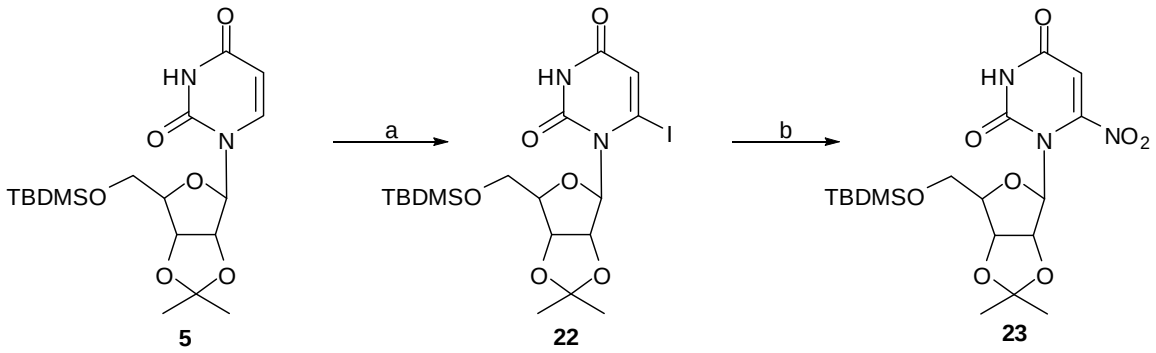
Compound **20** was also purified with manual column using up to 30% MeOH without triethylamine, but this high concentration of MeOH caused the silica gel to dissolve and come through the column with product. Finally, the column was tried on a column instrument (Biotage), which allows control of the flow rate. With the highest flow rate recommended for column size, the compound was able to be successfully separated with only 20% MeOH in $CHCl_3$. After successful purification, it was seen that a partially deprotected side product was also synthesized during the reaction (compound **21**).

After the deprotection of the compound, it was highly polar and able to be separated using reverse phase C18 silica gel column on a Biotage, yielding the pure 6-tetrazole uridine. This was unable to be accomplished with a manual reverse phase column, mainly because of the inability to control flow rate. Once the pure product was obtained successfully, monophosphorylation at the 5' position was feasible. This reaction proceeded smoothly, however the starting material (6-tetrazolyl uridine, **12**) and product (6-tetrazolyl UMP, **13**) could not be separated using the reverse phase C18 column on the Biotage™ separation unit, as both came with the solvent front in 100% water. Solvent gradients using 50% acetonitrile in water and water with 1% acetic acid were used, with

neither producing the desired results. However, separation was achieved using a C18 reverse phase analytical column on HPLC with a gradient of 0-20% acetonitrile in 1% HCl:H_2O solution. Attempts to use this method of separation at the preparatory level and obtain the pure compound are ongoing.

III.c. Preparation of 6-Nitrouridine Analog

While adding a nitro group at the C5 position of UMP has proved to be possible, a method for adding a C6 nitro group is still elusive. The method tried here was done by converting 6-iodouridine to 6-nitrouridine using an Ullmann-type synthesis shown to work previously on aromatic compounds (Scheme 9) (Saito, 2005). This synthesis proceeds using a copper catalyst, ligand, and nitrite salt added to the halide (Scheme 12) (Saito, 2005). It was shown to work on aromatic rings with various substitutions, and since the uracil ring of UMP mimics an aromatic, this seemed a reasonable approach to the synthesis (Saito, 2005). However, when this reaction was tried as shown, the TLCs obtained during and after the reaction were extremely messy, with more than five additional compounds with very poor resolution due to smearing. The starting material was lost during the reaction, leading to the conclusion that a stronger nitrite salt might lead to the desired results, such as sodium nitrite, potassium nitrite or silver nitrite. Also, the temperature could be increased to up to 120 °C without degradation of the starting material. Any of these modifications could potentially produce results, however due to time constraint, they were unable to be tested.



Scheme 12. Attempted synthesis for the formation of 6-nitro from fully protected 6-iodo derivative. Reactions conditions: (a) LDA, THF, iodine, -78 °C (b) DMF, copper powder, tetrabutylammonium nitrite, *N,N'*-dimethylethylenediamine, 100 °C.

III.d. Stability of Compounds

All final compounds that were successfully isolated were stable over the course of up to three days at room temperature, according to ^{1}H NMR obtained over the time period. However, compounds themselves were still stored in airtight containers at 0 °C. One intermediate, the 6-tributyltin uridine derivative, was unstable over several days if left at room temperature. If stored at 0 °C in an airtight container, the compound would last for several weeks. After this, it would slowly break down and produce low yield or completely unsuccessful reactions when used.

III.e. Biological Testing

For the biological testing of the final nucleoside compounds, they were tested against both malaria parasites (*P. falciparum*) and cancer cell lines (OCI-AML1). For this type of testing, the compound must not have the monophosphate, or it will not be able to enter through the cell membrane. The nucleotide derivatives were tested against the pure

enzyme, ODCase, using an ITC_{200}. For both 6-(thiophen-2-yl) uridine and 6-(thiophene-3-yl) uridine, the IC_{50} value for anticancer activity was >50 μM and for antimalarial activity was >300 μM. For the inhibition of ODCase, IC_{50} value of the monophosphates of these two compounds was >200 μM. The biological testing for the 6-(pyrazol-4-yl) and 6-(tetrazol-4-yl) uridine and monophosphate compounds is currently in progress.

CHAPTER IV

CONCLUSIONS AND FUTURE DIRECTIONS

Over the course of the time spent working on this project as research towards a masters degree in chemistry, good accomplishments have been made. The formation of C−C bonds is known to be a stumbling block in many syntheses, although coupling reactions such as Negishi and Stille coupling have made it easier. While the compounds tested did not show the desired antimalarial or anticancer activity, eight novel compounds have been synthesized using old and new methods. These compounds are 6-(thiophen-3-yl) uridine (**6**), 6-(thiophen-2-yl) uridine (**8**), 6-(pyrazol-4-yl) uridine (**10**) and 6-(tetrazol-4-yl) uridine (**12**), along with the corresponding nucleotides, 6-(thiophen-3-yl) uridine-5'-monophosphate (**7**), 6-(thiophen-2-yl) uridine-5'-monophosphate (**9**), 6-(pyrazol-4-yl) uridine-5' monophosphate (**11**) (impure) and 6-(tetrazol-4-yl) uridine-5'-monophosphate (**13**) (impure). These final compounds can be seen in Figure 3. The two impure compounds are in the process of being purified for biological testing.

Some C6-UMP derivatives have already given promising results, and the synthesis of a variety of these derivatives will only increase the likelihood of finding a potent and reliable inhibitor of ODCase that can become a potential malaria drug. The easier it is to create novel, C6 derivative compounds of uridine, the faster this goal can be accomplished. The research presented here supports this in presenting a method that could be used to create many different compounds.

If a method could be found based on the method in this research for the synthesis of 6-nitrouridine and its monophosphate, the potential for that compound would be huge. The continuation of this research, with support from computational design efforts, could potentially produce a new antimalarial, helping to curb this deadly disease and save the lives or improve the lifestyles of millions.

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