SYNTHESES OF ANTIMALARIAL ANTFOLATES

by

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A thesis submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg
In fulfilment of the requirements for the Degree of Master of Science

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Co-Supervisor: Prof. Charles de Koning

June, 30, 2015
Declaration

I declare that the work presented in this thesis was carried out exclusively by me under the supervision of Dr A.L Rousseau and Professor C.B. de Koning. It is being submitted for the degree of Master of Science in the University of the Witwatersrand, Johannesburg, and has not been submitted before for any degree or examination in any other university.

___________________________

Donald Tswene Seanego
ABSTRACT

The world suffers under a serious threat of malaria with about 584 000 deaths reported each year and most of these fatalities being children under five years of age. Malaria is caused by the protozoan parasite of the genus *Plasmodium*. Five different malaria species infect humans and cause disease: *P. vivax, P. malariae, P. ovale, P. knowlesi* and the cause of most malaria deaths, *P. falciparum*. The main reason for this disturbing situation is the emergence of drug resistance which reduces the effectiveness of most antimalarials. Hence, there is an urgent need for new drugs that will possibly be effective against both wild type and mutant strains of *Plasmodium* species. Pyrimethamine, a dihydrofolate reductase (DHFR) inhibitor, has been used most widely as an antimalarial antifolate drug for the treatment of malaria. However, rapid development of parasite resistance to this drug occurred because of its rigidity. Parasitic resistance to antimalarial antifolates arises from single mutations at various amino acid residues surrounding the PfDHFR active site.

In this project, we aimed to design and synthesise a novel series of flexible pyrimidine analogues of a dihydrotriazine hit compound prepared in a previous study. These compounds were designed to target folate metabolism in the malaria parasite. The initial series of compounds prepared in this project were synthesised over 5 steps in an overall yield of 10%. The flexible pyrimidine analogues were screened for antimalarial activity in an *in vitro P. falciparum* screen on the Gambian FCR-3 strain (chloroquine and cycloguanil resistant strain) with dihydroartemisinin, methotrexate and quinine as controls. 5-(3-(3,5-Dichlorophenoxy)propyl)-6-phenylpyrimidine-2,4-diamine displayed the best antimalarial activity (IC$_{50}$ = 0.09 µM) of the compounds in this series. Surprisingly; this was the only compound prepared in this series that proved to be as effective as our original hit dihydrotriazine (IC$_{50}$ ~50 nM).

In the second generation of compounds prepared in this study, we used a multicomponent coupling approach to synthesise three flexible pyrimidines bearing a non-aromatic side chain at the 6-position of the pyrimidine ring. For comparison, two analogues bearing a phenyl group at the 6-position of the pyrimidine ring were also prepared. Once again; only one compound of this series [5-((4-chlorophenethylamino)methyl)-6-cyclopropylpyrimidine-2,4-diamine, (IC$_{50}$ = 0.03 µM)] showed activity comparable with our original hit compound.
Finally, ten substituted pyrimidines bearing a flexible side chain at the 6-position of the pyrimidine ring, were prepared. These compounds are structurally similar to P65, [6-methyl-5-(3-(2,4,5-trichlorophenoxy)propoxy)pyrimidine-2,4-diamine] an analogue of a potent antifolate, WR99210, found to have good oral bioavailability in rats. Once again, the antimalarial activity of the compounds prepared was assessed in an in vitro P. falciparum screen on the Gambian FCR-3 strain. The most promising compound of this series was 6-(3-(3,4-dichlorophenoxy)propoxy)pyrimidine-2,4-diamine, which exhibited antimalarial activity in the low micromolar range (IC$_{50}$ = 4.46 µM).
ACKNOWLEDGEMENTS

First and foremost, I would like to convey my special gratitude to my supervisor, Dr Amanda Rousseau. Thank you for your support, kindness, sweetness and encouragement for the past two years. Without you this project would not have been successful. Thank you for giving me the opportunity to work with and learn from you. I am looking forward to work with you in future.

Prof Charles de Koning, my co-supervisor, thank you for sharing your invaluable experience, your time with me. You are easily approachable and always willing to help. It was a glory having you as my co-supervisor.

I am also grateful to Prof Jo Michael and Dr Moira Bode, for their help, suggestion and support; especially during the group meetings.

Dr Myron Johnson and Dr Izak Kotzè, thank you for running countless NMR spectra for me. Without your spectrometer, we (organic chemists) are clueless.

Wits organic group {Kennedy, Jean, Memory, Jimmy, Peter, Kamogelo, Fatima, Hendrik, Charles, Xolani, Allan, Dubekile, Robyn and William} thank you for the fun we had in the past two years. Big Up to you!

I am extremely thankful to University of the Witwatersrand for providing me with the facilities to do my research and National Research Foundation for financial support.

Mom (Agnes), Dad (Caiphus) and the whole family, thank you for your support, prayers, guidance and encouragement.

I am grateful to God for the protection, good health and the blessing to complete this research.
# LIST OF ABBREVIATIONS

<table>
<thead>
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<th>Description</th>
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<tbody>
<tr>
<td>ACT</td>
<td>Artemisinin Combination Therapy</td>
</tr>
<tr>
<td>CSIR</td>
<td>Council of Scientific and Industrial Research</td>
</tr>
<tr>
<td>DBN</td>
<td>1,5-Diazabicyclo[4.3.0]non-5-ene</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-Diazabicyclo[5.4.0]undec-7-ene</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DDT</td>
<td>Dichlorodiphenyltrichloroethane</td>
</tr>
<tr>
<td>DHA</td>
<td>Dihydroartemisinin</td>
</tr>
<tr>
<td>DHF</td>
<td>Dihydrofolate</td>
</tr>
<tr>
<td>DHFR</td>
<td>Dihydrofolate reductase</td>
</tr>
<tr>
<td>DHFR-TS</td>
<td>Dihydrofolate reductase-thymidylate synthase</td>
</tr>
<tr>
<td>DHPS</td>
<td>Dihydropteroate synthase</td>
</tr>
<tr>
<td>DHPT</td>
<td>6-Hydroxymethyl-7,8-dihydropterin</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>EtOAc</td>
<td>Ethyl acetate</td>
</tr>
<tr>
<td>FD’s</td>
<td>Folate derivatives</td>
</tr>
<tr>
<td>GTP</td>
<td>Guanosine triphosphate</td>
</tr>
<tr>
<td>HPPK</td>
<td>6-Hydroxymethyl-dihydropterin pyrophosphokinase</td>
</tr>
<tr>
<td>HRMS</td>
<td>High resolution mass spectroscopy</td>
</tr>
<tr>
<td>IRS</td>
<td>Indoor residual spraying</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>ITN’s</td>
<td>Insecticide-treated nets</td>
</tr>
<tr>
<td>KHMDS</td>
<td>Potassium bis(trimethylsilyl)amide</td>
</tr>
<tr>
<td>LDA</td>
<td>Lithium diisopropyl amide</td>
</tr>
<tr>
<td>MCR</td>
<td>Multicomponent coupling reaction</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>MS</td>
<td>Mass Spectroscopy</td>
</tr>
<tr>
<td>NADPH</td>
<td>Nicotinamide adenine phosphate</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>pABA</td>
<td>para-aminobenzoic acid</td>
</tr>
<tr>
<td>P/fDHFR</td>
<td><em>Plasmodium falciparum</em> Dihydrofolate reductase</td>
</tr>
<tr>
<td>SHMT</td>
<td>Serine hydroxymethyl transferase</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin Layer Chromatography</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
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CHAPTER 1: MALARIA AND ITS TREATMENT

1.1 Introduction

Malaria continues to be a major global health problem, despite being studied for many years since its discovery in human blood in 1880 by Charles Laveran. The world suffers under the enormous threat of malaria, with approximately 500 million people infected worldwide annually, and close to 584 000 deaths reported each year due to lack of adequate treatment.\(^1\)\(^-\)\(^3\) Malaria in humans is caused by five different species of *Plasmodium* parasites: *P. vivax*, *P. malariae*, *P. ovale*, *P. knowlesi* and *P. falciparum*. *P. vivax* and *P. falciparum* are the two species that predominate as threats to public health in Asia and Africa respectively.

*P. falciparum* causes the most severe form of malaria and results in the majority of the disease burden worldwide. It is responsible for 561 000 reported deaths annually, with most of these fatalities being children under five years of age.\(^4\) The geographic distribution of *P. vivax* is wider than *P. falciparum* because *P. vivax* can survive at higher altitudes and at lower temperatures. However, although *P. vivax* occurs throughout Africa, the risk of infection is low because of the absence of the Duffy gene, which generates a protein essential for *P. vivax* invasion of red blood cells, in many African countries.\(^5\) *P. ovale* denominates in western Africa. Currently, *P. knowlesi* cases have been restricted to Malaysia.\(^6\)\(^-\)\(^7\)

*P. falciparum* is spread from one person to another by female mosquitoes of the *Anopheles* species. There are about 400 species of *Anopheles* mosquitoes, but only 30 are capable of transmitting malaria in humans. *Anopheles gambiae*, found in Africa, is one of the best known malaria vectors. It prefers feeding on human blood, and lives in areas near human habitation. Prior to transmission, *P. falciparum* resides within the salivary glands of the mosquito in the sporozoite stage of the parasitic life cycle (Figure 1, stage 1). As the mosquito takes its blood meal from a human, it injects a small amount of saliva into the skin wound. The saliva of the mosquito contains anti-hemostatic and anti-inflammatory enzymes that disrupt the clotting process and inhibit the pain reaction. Each infected bite contains approximately 5-200 sporozoites which proceed to infect the human host. Within minutes of being introduced into the human bloodstream, the sporozoites infect the liver cells (Figure 1, stage 2).
After invading the liver cells, the sporozoites replicate and infect hepatocytes, which in turn ruptures and release thousands of merozoites into the host’s bloodstream, which then invade the erythrocytes (Figure 1, stage 3). In the red blood cells, a cycle of asexual replication occurs, with the release of increasing numbers of merozoites into the bloodstream every 48 hours. Some of the merozoites in these cells undergo sexual replication to form male and female gametocytes, which circulate in the bloodstream (Figure 1, stage 4).

**Figure 1:** The life cycle of malaria parasite (www.niaid.nih.gov).

### 1.2 Distribution of the malaria parasite

Malaria is distributed globally, depending largely on climatic factors such as humidity, rainfall and temperature. As a result of these factors, the malaria parasite is primarily transmitted in tropical and subtropical regions, where the *Anopheles* mosquitoes can survive and multiply. Generally, at high temperatures, *P. falciparum* can effectively complete its growth cycle in the *Anopheles* mosquito, thus transmission will be more intense. The highest transmission of malaria is found predominantly in sub-Saharan Africa.
Within South Africa’s borders, malaria is encountered largely in part of the Mpumalanga province, KwaZulu-Natal, and the border areas of the Limpopo and North West provinces. Incidence of malaria cases increase enormously during the rainy season that lasts from September to May.\textsuperscript{11}

\section*{1.3 Malaria control and prevention}

The main approach adopted to combat malaria in various parts of the world is by vector control,\textsuperscript{12} and prevention is achieved through prophylaxis, indoor residual spraying of insecticides, the use of bednets and more recently by the promising vaccine development.

\subsection*{1.3.1 Vector control for malaria}

Dichlorodiphenyltrichloroethane (DDT) was discovered as the first synthetic organic insecticide to control malaria in the late 1940s. DDT is more affordable than current chemicals used for mosquito control, such as the pyrethroids, but is unfortunately toxic. It prevented many deaths that would have occurred due to malaria when used in global spraying.\textsuperscript{13} The development of insecticide resistance resulted in failure of the DDT approach.
to interrupt malaria transmission completely in many countries in the 1980’s and malaria resurged to higher levels as other eradication programmes deteriorated. Currently, although different strategies are available for vector control, the most successful are indoor residual spraying (IRS) of insecticides and the use of insecticide-treated nets (ITNs). Mosquito nets efficiently prevent malaria transmission by forming a physical barrier between anopheles mosquitoes and man. ITNs are impregnated with pyrethroids and decrease the man-mosquito contact by prevention, and also kill the mosquito with its residual insecticidal activity. In countries with ongoing malaria transmission, ITNs are handed out free of charge to all age groups through mass campaigns. Unfortunately, pyrethroid resistance has become common in malaria vectors across Africa.

IRS with insecticides continues to be of significant value in malaria control. It is applied by spraying the interior surfaces of houses with insecticides, and it slows malaria transmission by reducing the life span of female mosquitoes.

1.3.2 Vaccines

Resistance of the vectors to insecticides is becoming more common, thus the development of a malaria vaccine is of significant importance. Malaria vaccines target different stages of the parasite cycle, each with discrete antigenic selection. Gamete-stage vaccines interrupt transmission of the parasite to mosquitoes that feed on an infected individual. These are known as transmission blocking vaccines, and while they contribute to protection of the human population, they do not protect the vaccinated individual. Pattaroyo et al. developed a number of vaccines that underwent large-scale clinical trials, one of which was the three-component vaccine (SPf66) which unfortunately did not show clinical effectiveness.

The pre-erythrocytic vaccines prevent the infection of red blood cells, the cause of severe forms of malaria. The most advanced successful malaria vaccine to date is known as RTS’S [central repeat region of the Plasmodium falciparum (R), T-cell epitopes (T), hepatitis B surface antigen (S)]. The RTS’S is now in Phase III clinical trials, and it has shown a 50% protective effectiveness against clinical disease. Other vaccines are developed to combat the disease at the asexual blood stage once the infection has already entered the bloodstream of
the human host. If the vaccines are used in combination, they reduce the spread of parasites which may be resistant to one vaccine.

**Figure 3**: Breaking the cycle with vaccines.

1.3.3 **Chemotherapy for falciparum malaria**

The treatment of malaria started in the 17th century, and several drugs have been used to treat this disease. The drugs in use act on different stages of the malaria life cycle. It is of interest to note that an antimalarial drug could be useful against one *Plasmodium* species and completely ineffective against another.

Antimalarial drugs are used for both the treatment of malaria and in prevention of malaria infection. Most antimalarial drugs target the erythrocytic stages of malaria infection, which is the phase of infection that causes symptomatic illness.
1.3.3.1 Quinoline-derivatives

Quinine (1) has been used as a first-line drug for the treatment of malaria. It was isolated as the active compound from the bark of the cinchona tree in 1820. As a result of this, malaria was one of the first diseases to be treated with a pure chemical compound. After its discovery, quinine was used to cure malaria globally. Before the development of artemisinin-based therapies, quinine played an important role in the treatment of multiresistant malaria, despite its relatively low efficacy and high toxicity. Due to its high solubility, quinine was given intravenously when patients were unable to tolerate oral medication.

![Structure of quinine](image)

**Figure 4:** Structure of quinine

The quinoline derivatives are structurally related to quinine and contain a quinoline moiety. The 4-amino quinoline, chloroquine (2) (Figure 5), was discovered in 1946 and, until recently, played an important role in both the treatment and prophylaxis of *falciparum* malaria in countries where transmission is severe. Unfortunately, widespread resistance to chloroquine has made the drug largely ineffective. However, chloroquine is still used in some countries, and in areas where chloroquine is still effective, it is used to treat *P. vivax* malaria. There is a continued interest in quinoline-containing drugs, although their mode of action is poorly understood. Amodiaquine (3) (Figure 5) belongs to the same family as chloroquine, but it is less efficient, more expensive and more toxic than chloroquine. Its use has been limited since the mid 1980s owing to the appearance of occasional agranulocytosis in travellers taking this drug.
Figure 5: Quinoline-based antimalarial drugs.

Mefloquine (4) is an aryl-amino alcohol derivative of quinine, developed by the U.S Army as a replacement for chloroquine. Mefloquine has a long half-life *in vivo*; hence it is an excellent prophylactic drug amongst non-immune travellers because of the once-a-week dosage. However, its use has been limited due to high costs and the appearance of neuropsychiatric side effects. In addition, cross-resistance has also developed against this drug.

The 4-amino quinolines impede the detoxification of free heme (ferriprotoporphyrin IX) in the parasite-infected red blood cell. The free heme is generated during the degradation of hemoglobin by the parasite, and it is toxic to the parasite. Accumulation of free heme within the food vacuole, which occupies 3-5% of the volume of the infected erythrocyte, could reach the 200-500 mM concentration level. The malaria parasite deals with this problem by polymerising the heme into nontoxic hemozoin (malaria pigment). 4-Aminoquinolines disrupt this polymerisation process, resulting in the accumulation of free heme.

Chemical modification of existing drugs offers new possibilities for drug discovery. Several antimalarial drugs in use today were developed in this manner. For instance, primaquine (5) is an 8-amino quinoline which has been used for eradication of liver stages in *P. vivax* infections. However, it was found to be inactive against blood stages at pharmacological concentrations. Chemical modification of primaquine led to a less toxic derivative, tafenoquine (6), which has a longer half-life of 2-3 weeks. It is active against erythrocytic stages of both chloroquine- and multiresistant strains. However, its mode of action is largely unknown.
1.3.3.2 Artemisinin and its derivatives

Artemisinin (7) is a sesquiterpene lactone first isolated from a Chinese plant (Artemisia annua) in the late 1970’s, and has potent activity against Plasmodium species. Extracts of the plant were used for the treatment of malaria in Asia for centuries before the active compound was identified.

Semisynthetic artemisinin derivatives with greater antimalarial potency have been developed; and include artemether (8), arteether (9) and artesunate (10) (Figure 8). These drugs are metabolised in vivo to the bioactive compound, dihydroartemisinin (11). Artemisone (12) is the most recently developed semisynthetic artemisinin derivative, with the best pharmacokinetic/pharmacodynamic profile. It exhibited, compared to other derivates, better efficacy and lack of neurotoxicity in preclinical testing. This analogue is not metabolised to dihydroartemisinin like the above-mentioned artemisinin derivatives.
Artemisinin and artemisinin derivatives act faster than any other currently available antimalarial drug; exhibiting higher rates of reduction of parasitemia. Hence, they can be used for treating complicated and uncomplicated malaria. The artemisinins are active against the sexual parasite stage (gametocytes), which is a significant advantage over other antimalarials.\textsuperscript{39} As a consequence of their very short plasma half-lives, artemisinin drugs are increasingly used in combination with other antimalarials with longer half-lives to prevent the development of resistance.\textsuperscript{40} The mechanism of action of these compounds is not well understood, but one hypothesis is that their antimalarial activity depends on the cleavage of the peroxide bridge after contact with the Fe\textsuperscript{II} present in the food vacuole. The peroxide undergoes reductive cleavage to generate oxygen-centred radicals, potent hydrogen-abstracting agents. The oxygen-centred radicals generate carbon-centred radicals by intramolecular hydrogen-atom abstraction. The C-centred radicals are postulated to react with biomolecules, and can alkylate heme (Figure 9).
More recently, work by Haynes et al. suggested that artemisinins disrupt redox homeostasis in the parasite, by maintaining oxidized forms of reduced flavin cofactors, which are important for the functioning of flavoenzymes. Flavoenzymes are responsible for maintaining the reduced glutathione, which is present in high concentration in erythrocytes. Reactive oxygen species (ROS) are generated during oxidation of Fe$^{II}$. Artemisinins are also capable of generating ROS. The generation of ROS by artemisinins explain their ability to provoke membrane damage and to affect the parasite SERCA P/ATP6 Ca$^{2+}$ transporter. By maintaining high levels of ROS in the erythrocyte and preventing the cell from removing these through glutathione and related pathways, artemisinins effectively disrupt redox homeostasis resulting in parasite death (Figure 10).
Figure 10: Proposed mechanism for oxidative stress mediated by artemisinin.\textsuperscript{41}

1.3.3 Other antimalarial agents

Atovaquone (13) is an antimalarial that was previously used for treatment and prevention of malaria. Unfortunately, the parasite developed resistance against this drug. It is now employed in combination with other antimalarial drugs. The administration of halofantrine (14) has been limited due to toxicity and its potential to induce heart arrhythmia.\textsuperscript{42} Halofantrine was administered as a racemate, with the enantiomers having comparable \textit{in vitro} antimalarial activity. Its analogue lumefantrine (15) was developed by chemical modification of this drug and it is now used in combination with artemether (Coartem\textsuperscript{®}).
1.3.3.4 Antimalarial Antifolate drugs

An interesting class of compounds that show promise for the treatment and prevention of malaria and other diseases, including cancer, are antifolates. Antifolates, also known as folate antagonists, constitute a class of antimalarials belonging to the group of nucleic acid biosynthesis inhibitors; according to Olliaro’s classification. Antifolates can be divided into two groups; class I and class II antifolates, depending on their site of action.

Folate metabolism is an interesting target for the treatment of malaria because it offers many possibilities for selective inhibition of biochemical processes that are required for parasite survival. The malaria parasite relies heavily on folate derivatives (FDs) as cellular cofactors for a number of processes, including the initiation of protein synthesis, and the biosynthesis of purines, pyrimidines and some amino acids. FDs can be produced via the folate salvage pathway or via a de novo folate-synthesis pathway.

The folate salvage pathway, which is also present in mammalian cells, represents a powerful target for the treatment of malaria. The key targeted enzymes include bifunctional dihydrofolate reductase – thymidylate synthase (DHFR-TS), which catalyses the NADPH-
dependent reduction of dihydrofolate (DHF) to tetrahydrofolate (THF). THF in turn is necessary for the formation of methylene THF, catalysed by serine hydroxymethyl transferase (SHMT). Methylene THF is necessary for the biosynthesis of deoxythimidine monophosphate (dTMP), a DNA building block. This reaction regenerates DHF which can then re-enter the salvage pathway. Inhibition of one of these enzymes leads to disruption of DNA synthesis.\(^4\)

The malaria parasite is also able to synthesise folate derivatives \textit{de novo}, using a pathway absent in humans. This \textit{de novo} biosynthetic route begins with the GTP cyclohydrolase-catalyzed transformation of guanosine triphosphate (GTP) into dihydroneopterin triphosphate (DHN-PPP) (\textbf{Figure 12, see Appendix for structures}). The latter is then dephosphorylated to dihydroneopterin (DHN) and 6-hydroxymethyl-7,8-dihydropterin (DHPT) by the enzymes dihydroneopterin triphosphate pyrophosphohydrolase and dihydroneopterin aldolase. DHPT is transformed by 6-hydroxymethyl-dihydropterin pyrophosphokinase (HPPK) into dihydropteridine pyrophosphate (DHPT-PP), which condenses with p-aminobenzoic acid (pABA) to generate 7,8-dihydropteroate (DHP), a reaction catalyzed by dihydropteroate synthase (DHPS). DHPS is the only enzyme of the \textit{de novo} folate synthesis pathway that is used as an antimalarial drug target. Sulfur-based drugs, mimics of pABA, are the inhibitors of this enzyme and are also widely used as antibacterial agents. Finally, DHP is glutamated by the key enzyme dihydrofolate synthase (DHFS) generating DHF, which is further glutamated by folypolyglutamate synthase (FPGS).
1.3.3.4.1 Class I antifolates

Class I antifolates are sulfones and sulfonamides, whose structures are similar to p-aminobenzoic acid (pABA). They act by inhibiting dihydropteroate synthase (DHPS), an enzyme present only in the de novo pathway of the parasite. Inhibition of DHPS slows down the formation of 7,8-dihydropteroate, which is a precursor of DHF. Dapsone (16) is the most effective DHPS inhibitor of malaria to date. It has been used in the past for the

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**Figure 12:** The folate salvage pathway (humans and the malaria parasite) and the *de novo* pathway (malaria parasite).[^46]
treatment of both *P. falciparum* and *P. vivax* malaria. Unfortunately, because of its limited effectiveness and high toxicity, development of dapsone was abandoned.\textsuperscript{38, 49}

Other DHPS inhibitors include sulfadoxine (17), sulfadiazine (18) and sulfalene (19) (Figure 13). However, as the parasite can efficiently use exogenous folates, either as folic acid or folinic acid by the folate salvage pathway, blockage of the *de novo* biosynthetic pathway induced by Class 1 antifolates is not entirely efficient.\textsuperscript{50} As a result, these drugs are commonly used in combination therapy. Resistance to sulfadoxine appears to arise from point mutations in the *dhps* domain of the *dhps-pppk* gene.\textsuperscript{51} Hence, it is has been used in combination with pyrimethamine for the treatment of malaria in many African countries. Unfortunately, resistance to this combination is slowly developing;\textsuperscript{52} hence new effective antimalarials are urgently needed to combat malaria.

![Figure 13: DHPS inhibitors](image)

1.3.3.4.2 **Class II antifolates**

This project focuses on the design of class II antifolates that inhibit dihydrofolate reductase (DHFR). The DHFR domain of the bifunctional dihydrofolate reductase-thymidylate synthase (DHFR-TS) is one of the few well-defined targets in malaria chemotherapy.\textsuperscript{53}
Dihydrofolate reductase (DHFR) is an important target for drug development against a variety of infectious diseases. Its function is to catalyze the reduction of 7,8-dihydrofolate (DHF) to 5,6,7,8-tetrahydrofolate (THF), in a biochemical reaction whose coenzyme is the reduced form of nicotinamide adenine phosphate (NADPH) (Figure 14). As already described, THF and other reduced folates are essential cellular cofactors required by the parasite for a number of key processes, including the initiation of protein synthesis, and the biosynthesis of deoxythymidylate (dTMP), methionine, purine nucleotides and other essential metabolites. Inhibition of DHFR thus prevents biosynthesis of DNA, leading to cell death. This metabolic role has made DHFR a highly successful and popular target for drugs against cancer, bacteria, the malaria parasite and other eukaryotic pathogens.  

![Figure 14: The reduction of DHF to THF catalysed by DHFR.](image)

The first antimalarial antifolate, known as proguanil, was discovered in 1945. Proguanil (20) was found to be more active than quinine against avian malaria and to have a better therapeutic index in animal models. It was used in the late 1940s for prophylaxis for malaria in plantation workers in Southeast Asia and elsewhere.

Studies demonstrated that proguanil is a prodrug and metabolizes in vivo to its triazine form, cycloguanil (21), which is an inhibitor of the parasite DHFR. Chlorination of the phenyl ring of proguanil led to its analogue, chlorproguanil (22). Similar to proguanil, it is converted in vivo to its active metabolite chlorcycloguanil (23). Chlorproguanil has a higher potency compared to proguanil, and hence it was recommended for prophylaxis at a lower dose.
Pyrimethamine (24) is also a class II antifolate, and belongs to the family of 2,4-diaminopyrimidines (Figure 16). This antifolate has a similar structure to cycloguanil, although it contains a fully aromatic pyrimidine ring. Interest in these compounds as antimalarials was sparked in the late 1940s when Hitchings and co-workers synthesized and tested them as analogues of folic acid in the treatment of tumours. Pyrimethamine, a selective inhibitor of pfDHFR, has been used most widely as an antimalarial antifolate drug, known as Daraprim. However, rapid development of parasite resistance to this drug occurred shortly after its introduction.

To combat resistant strains, pyrimethamine was used in combination with sulfadoxine for the treatment of malaria. This combination is known as Fansidar. Unfortunately, the malaria parasite rapidly developed resistance to the pyrimethamine-sulfadoxine combination. This has rendered Fansidar ineffective for the treatment of uncomplicated malaria in some areas like South America and Southeast Asia, as the clinical response is slow. The development
of resistance seen in the DHFR inhibitors, pyrimethamine and cycloguanil, has led to the synthesis of analogues of these drugs that avoid point mutations in the enzyme active site. New compounds were prepared that have high affinity for the C59R+S108N double mutant of DHFR. Novel analogues of pyrimethamine and cycloguanil (Figure 17) were synthesized by Kamchonwongpaisan and co-workers in 2004 and tested against pfDHFR carrying mutations responsible for antifolate resistance.63

![Figure 17: Analogues of pyrimethamine and cycloguanil](image)

These analogues were designed to avoid steric clashes in the active site of mutant enzymes. Some of these inhibitors have shown good antiplasmodial activity when tested against resistant strains of *P. falciparum in vitro* and have relatively low toxicity. These compounds were found to be more active than their parent drug against drug resistant strains, displaying IC\textsubscript{50} values at low micromolar level.64 However, these compounds were still not effective enough to be considered as drug candidates.

Trimethoprim (25a) was developed as a more flexible analogue of pyrimethamine. Trimethoprim has a similar structure to pyrimethamine, the difference between the two antifolates being the existence of a methylene group between the two aromatic rings. This allows torsional freedom within the DHFR active site.65 However, due to its slow action, trimethoprim never showed any advantage over pyrimethamine. It was however used in combination with other drugs.66

![Figure 18: Trimethoprim](image)
There is strong evidence to suggest that flexible antifolates are more effective against *P. falciparum* strains resistant to pyrimethamine and cycloguanil. These observations have led to the synthesis of trimethoprim analogues, such as 25b (Figure 19). These compounds showed good antimalarial activity when tested against *P. falciparum in vitro* and they bind strongly to the wild type DHFR and strains containing S108N and C59R+S108N mutations. To date, however, none of these compounds have been developed as drugs.

![Figure 19: Trimethoprim analogue](image)

Studies have demonstrated that an increase in the length of the linker between the phenyl ring and the diaminopyrimidine/dihydrotriazine ring up to 5 atoms increases the level of torsional flexibility of the compound, thus increasing the potency of antifolates. These studies led to the discovery of WR99210 (26), which contains a flexible linker between the two rings. WR99210 resembles the flexibility seen in the natural substrate dihydrofolate (DHF). Yuvaniyama *et al.* showed that WR99210 could avoid steric clashes with mutant amino acid residues in the active site of DHFR (such as Ser-108-Asn) because of its flexible side chain (Figure 20).
Figure 20: Comparison of interactions at the active sites of mutant PfDHFR-TS with flexible and non flexible antifolates. The enzyme on the left is complexed with pyrimethamine and NADPH. The enzyme on the right is complexed with WR99210 and NADPH. The flexible tail of WR99210 allows it to avoid the pyrimethamine-resistant mutations labelled in red.

However, the development of WR99210 as an antimalarial drug has been terminated because of its poor oral bioavailability owing to poor intestinal permeability.\textsuperscript{70} Based on this, the prodrug of WR99210, PS-15, was developed by Rieckmann and co-workers in 1996 and it has shown better pharmacokinetic properties and is more effective than WR99210 in \textit{in vivo} models.\textsuperscript{71, 72} However, PS-15 was abandoned due to cross-resistance with cycloguanil and pyrimethamine.\textsuperscript{73}

![Conversion of a prodrug, PS-15, to its metabolite WR99210.](image)

These studies have led to the development of analogues of WR99210 and PS-15 such as 27 and 28 (shown below), and some are in preclinical studies.\textsuperscript{74} Substituents on the phenyl ring were varied in order to minimise the metabolic degradation of these analogues.
1.3.3.5 Antimalarial drug resistance

The emergence of drug resistance is reducing the effectiveness of most antimalarials. Drug resistance to antimalarials can be caused by different modifications in the parasite cell. For example, low membrane permeability of the drug can reduce its absorption or the rate of elimination of the drug can be rapid, resulting in a lower accumulation of the drug in the organism. Parasitic resistance to DHFR and DHPS inhibitors arises from single mutations at various amino acid residues surrounding the PfDHFR active site. Researchers have confirmed that resistance to pyrimethamine is related to a mutation at residue 108 of pfDHFR, where a serine is substituted by asparagine. Additional mutations in residues Asn51 or Cys59 generate double mutants (51+S108N and C59R+S108N), and this decreases sensitivity of the drug even further. The mutations in DHPS that infer drug resistance have been reported in the literature, and include A581G, S436F, A613T, A613S, S436A, A437G and K540E. Combination therapies utilising both DHFR and DHPS inhibitors attempted to overcome this problem of resistance. However, resistance to combination therapies may also occur.

The first occurrence of chloroquine resistance was reported in the late 1950s. Resistance to chloroquine was caused by many factors including uncontrolled long-term treatment regimes. The concentration of chloroquine inside the digestive vacuole is reduced in parasite-resistant strains; hence, the accumulation mechanism of chloroquine becomes less effective. Artemisinin-based combination therapies are favoured in areas where *falciparum* malaria is widespread. ACTs have shown high effectiveness in the treatment of malaria in Southeast Asia, where transmission is typically low. However, the emergence of clinical resistance to artemisinin was reported in the Thai–Cambodian border in 2006. Since artemisinin derivatives are responsible for the reduction in the parasite burden, resistance to these drugs can have major implications for the control of malaria in affected countries.
An understanding of the basis of resistance is important for the development of new effective antimalarial drugs that are affordable. Currently used antimalarials are rapidly losing their effectiveness against *P. falciparum* due to resistance. There is a need for new drugs, which must be effective against both wild type and mutant strains of *Plasmodium* species. Hence, there is an urgent need for drugs that will possibly block transmission of the parasite from one infected person to another *via* the mosquito and thus break the cycle of infection.

1.3.3.6 Combination therapy

Combinations of drugs have become an advisable strategy for the treatment of malaria in an attempt to slow the development of drug resistance.\(^8^4\) Combination therapy usually involves drugs that inhibit different targets. The most widely used combination is undoubtedly pyrimethamine-sulfadoxine, known as PS or Fansidar. PS has been highly effective in most of Africa,\(^8^5\) but resistance to PS due to point mutations has rendered the drug ineffective in many countries. Combination of chlorproguanil and dapsone (Lapdap\(^®\)) was associated with a decrease in efficacy\(^8^6\) in the presence of point mutation at amino acid 164 of DHFR that rendered PS useless. It was shown in clinical studies that resistance to chlorproguanil-dapsone developed much more slowly than resistance to PS.\(^8^7,8^8\) However, clinical trials of Lapdap in Southeast Asia showed that this drug was ineffective in the treatment of uncomplicated malaria.\(^8^9\)

In addition, artemisinin-derivatives are also used in combination therapy with antifolate drugs. Recently, a combination of proguanil/dapsone and artesunate has been developed, however resistance has already been reported.\(^9^0\) 4-Amino quinolines are also used in combination therapy. The combination of atovaquone-proguanil (Malarone\(^®\) or Malanil) is still widely used for both the treatment and the prevention of malaria. Atovaquone-proguanil is an inhibitor of parasitic electron-transport chain.\(^9^1\)
1.4 Our approach to the search for new antifolates

During her tenure at the Council for Scientific and Industrial Research (CSIR), Dr Amanda Rousseau and co-workers were responsible for a project involving the design and synthesis of a novel series of flexible cycloguanil analogues (Figure 23) as inhibitors of PfDHFR. They used crystallographic data of wild-type PfDHFR and mutant PfDHFR complexed with WR99210 and pyrimethamine to prepare flexible antimalarial agents based on cycloguanil. In their synthesis, they varied the length of a flexible linker from 1-5 atoms between the substituted phenyl ring and the 1,2-dihydro-1,3,5-triazine-4,6-diamine heterocycle. Different groups were also introduced at the stereogenic centre compared with cycloguanil. Significantly, the N-O labile bond of WR99210 was replaced with the more stable N-C bond.

![Chemical structure of compound 29](image)

**Figure 23:** Flexible cycloguanil analogues prepared previously at CSIR

Their initial series of compounds (bearing a 4 atom linker between the two rings) showed good antimalarial activity in the nanomolar range comparable with that of cycloguanil against the drug sensitive *P. falciparum* strain. However, the hit compound (30) also displayed whole cell activity in the nanomolar range against a chloroquine/cycloguanil resistant strain of *Plasmodium falciparum* (Gambian FCR-3 strain, IC$_{50}$ 33 nM). The hit compound was found to be 150 times more active than cycloguanil against the drug-resistant strain. The other compounds prepared (bearing flexible linkers of 1, 2, 3 and 5 atoms) only showed activity comparable with cycloguanil against the drug resistant *Plasmodium falciparum* strain (2.0-11 µM).
However, the synthesis of these compounds was not simple and the final product was isolated as a racemic mixture. The final compounds prepared were screened as a mixture of two enantiomers. Hence, an alternative approach was sought to address both the problems associated with the synthesis of these compounds, and the formation of enantiomeric mixtures of products.

1.5 Aims of this project

In this project, we aim to synthesize flexible pyrimidine analogues of cycloguanil with the potential to display antimalarial activity. The pyrimidine analogues are based on the potent dihydrotriazine antifolate compound which was synthesized previously by researchers at the CSIR. As discussed, the series of alkylated dihydrotriazine compounds prepared showed better activity than cycloguanil against drug resistant strains of P. falciparum in vitro. Compounds bearing a 4-atom linker between the substituted phenyl and the 1,2-dihydro-1,3,5-triazine-4,6-diamine heterocycle were found to be particularly active and displayed antimalarial activity in the low nanomolar range against the drug resistant strains (Figure 25).

Figure 25: Previously prepared dihydrotriazine compounds

However, these compounds were isolated as a mixture of two enantiomers. Efforts made to resolve the enantiomers were not successful. As such, the antimalarial activity of the individual enantiomers is not known. Thus, we wanted to eliminate the problem of enantiomeric mixtures by preparing the fully aromatic pyrimidine equivalents of these
compounds, such as 31. These compounds are anticipated to act as inhibitors of dihydrofolate reductase (DHFR).

\[
\begin{align*}
Y &= 4-\text{Cl} \\
Y &= 3,4-\text{diCl} \\
Y &= 3,5-\text{diCl} \\
Y &= 4-\text{F} \\
Y &= 3-\text{F} \\
Y &= 2,3-\text{diF} \\
Y &= 2,4-\text{diBr} \\
Y &= 3-\text{CF}_3
\end{align*}
\]

**Figure 26:** Proposed flexible pyrimidine analogues based on the dihydrotriazine equivalents.

Our proposed synthetic route (Scheme 1) to these compounds involves alkylation of the commercially available substituted phenols 32a-h with 1,4-dibromobutane in the presence of a base to afford bromoethers 33a-h. Functional group interconversion of the alkyl bromide to the corresponding nitriles 34a-h would be achieved with potassium cyanide in an ethanoic solution. The nitriles would then be treated with ethyl benzoate under basic conditions to furnish the α-cyano ketones 35a-h. The enol ethers 36a-h would be prepared by reacting the α-cyano ketone compounds with the alkylating agent, diazomethane. The final step to flexible pyrimidines would be a ring closure using guanidine hydrochloride in dimethyl sulfoxide (DMSO) to afford the desired products 37a-h.

All compounds synthesised will be tested for antimalarial activity in an *in vitro P. falciparum* screen on a Gambian FCR-3 strain (chloroquine and cycloguanil resistant strain). Active compounds that display promising biological activity will be assessed further for cytotoxicity.
Scheme 1: Proposed route towards synthesis of flexible pyrimidine analogues

Biological data associated with the first series of compounds prepared will assist with the design and the synthesis of the second generation of antimalarials, where structural features associated with antimalarial activity will be combined in the second generation. Pyrimidine analogues bearing a non-aromatic side on the 4-position of the pyrimidine ring 38 and 39 (Figure 27) will also be prepared using this methodology.

Figure 27: Proposed pyrimidine equivalents bearing a non-aromatic side chain
We also wish to explore the effect of moving the flexible side chain from the 5-position of the pyrimidine to the 4-position of the pyrimidine ring, to afford analogues of general structure 40. These compounds are structurally similar to P65 (41), an analogue of WR99210 that was synthesized by Yuthavong and co-workers in 2012 and found to be active.\textsuperscript{94}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure28}
\caption{Flexible pyrimidine, P65, (41) and proposed pyrimidine analogues (40)}
\end{figure}

These compounds could be prepared by alkylation commercially available substituted phenols with 1,3-dibromopropane or 1,4-dibromobutane under basic conditions to afford bromoethers. The bromoether would then react with 2,4-diaminopyrimidin-6-ol using an appropriate base to give the desired product 40 (Scheme 2). We hope that substitution with bromoether compounds will occur at the 4-hydroxy group as the 2- and 4-amino groups of compound 42 are relatively non-nucleophilic.

\begin{scheme}
\centering
\includegraphics[width=\textwidth]{scheme2}
\caption{Reagents and conditions: i) For \( n = 1 \): 1,3-dibromopropane, reflux; for \( n = 2 \): 1,4-dibromobutane, reflux; ii) \( \text{K}_2\text{CO}_3, \text{CH}_3\text{CN} \), reflux.}
\end{scheme}

Through the synthesis of these analogues, we therefore hope to explore the chemical space in the active site of PfDHFR, and identify new molecular scaffolds with promising antimalarial activity.
CHAPTER 2: RESULTS AND DISCUSSION

As discussed in Chapter 1, the aim of this project is to prepare novel flexible pyrimidine analogues as potential antimalarial agents. As part of research into antimalarial antifolates that started at the CSIR, we were interested in preparing a series of pyrimethamine-like compounds that possess a flexible linker between the substituted phenyl and pyrimidine rings. These compounds were designed based on a series of cycloguanil-like compounds that possessed a flexible tether between the 1,2-dihydro-1,3,5-triazine-4,6-diamine heterocycle and the substituted phenyl ring, with potent antimalarial activity (general structure 43, Figure 29). In particular, compounds bearing a 4-atom linker were found to be more active than cycloguanil against drug resistant strains and displayed antimalarial activity in the nanomolar range (IC$_{50}$ ~ 50 nM).

![Figure 29](image)

**Figure 29:** Dihydrotriazine compound prepared previously (43) and the proposed pyrimidine equivalents (31)

Although the dihydrotriazines displayed potent antimalarial activity *in vitro*, they were isolated as a mixture of two enantiomers from a challenging, low yielding synthetic route. In this project, we will prepare pyrimidine equivalents that are fully aromatic (general structure 31, Figure 29), thus eliminating the problem of enantiomeric mixtures and determine the effect of this modification on biological activity. We anticipated that the pyrimidine equivalents 31 would also be easier to synthesise than the dihydrotriazine counterparts 43.

The proposed synthetic route towards flexible pyrimidines (Scheme 2) involves alkylation of commercially available substituted phenols with 1,4-dibromobutane in the presence of a base to afford bromoethers. A functional group interconversion to a nitrile then enables formation
of α-cyano ketones. This is followed by conversion to an enol ether and subsequent ring closure to the desired pyrimidine product.

2.1 Synthesis of flexible pyrimidine analogues as DHFR inhibitors

2.1.1 Synthesis of 1-(4-bromobutoxy)-4-chlorobenzene and analogues

![Chemical structure](image)

**Scheme 3:** Reagents and conditions: K$_2$CO$_3$, CH$_3$CN, reflux, 20 h, 98%.

The synthesis of pyrimethamine analogues followed this five step process. We envisaged preparing a range of flexible pyrimidines starting from commercially available phenols. The synthetic route was first tested using 4-chlorophenol 32a as the substrate. In the first synthetic step (Scheme 3), readily available 1,4-dibromobutane was reacted with 4-chlorophenol at 90°C under basic conditions. The reaction was conducted under dilute conditions and in the presence of excess dibromoalkane so as to prevent the formation of dimers. Hence, three molar equivalents of dibromoalkane were utilised in the reaction. The bromoether product was formed as a single spot on TLC, indicating complete consumption of the 4-chlorophenol. Excess 1,4-dibromobutane was distilled off under high vacuum to furnish the alkylated product 33a as a white solid in 98% yield.

Five signals were observed in the $^1$H NMR spectrum of 33a. Two doublets in the aromatic region were assigned to H2 and H3. The aliphatic region contained three signals; two triplets at 3.93 ppm and 3.46 ppm each integrating for two protons and a multiplet at 2.63 – 1.59 ppm integrating for four protons, characteristic of the methylene protons of the alkyl chain. The two triplets were due to the -OCH$_2$- and -CH$_2$Br methylene protons respectively. The multiplet integrating for four protons was due to H2’ and H3’. The $^{13}$C NMR spectrum of the product 33a showed eight signals in total, as expected. Four signals were visible in the aromatic region and four in the aliphatic region, the latter owing to carbons of the alkyl chain.
Seven other analogues 33b-33h were prepared in a similar manner (Scheme 4). The $^1$H NMR and $^{13}$C NMR spectra of the products were similar, as expected. Key signals for the compounds are described in Tables 1 and 2 below.

![Chemical structure](image)

**Scheme 4**: Reagents and conditions: $\text{K}_2\text{CO}_3$, CH$_3$CN, reflux, 20 h.

**Table 1**: Key signals in the $^1$H NMR spectra of bromoethers 33b-33h.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Aromatic region (ppm)</th>
<th>-OCH$_2$- (ppm)</th>
<th>CH$_2$Br (ppm)</th>
<th>H2’ &amp; H3’(ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>33b</td>
<td>7.50 (d, H5), 7.22 (d, H2), 6.95 (dd, H6)</td>
<td>3.92 (t)</td>
<td>3.45 (t)</td>
<td>2.11 – 1.80 (m)</td>
</tr>
<tr>
<td>33c</td>
<td>6.91 (t, H4), 6.75 (d, H2 and H6)</td>
<td>3.92 (t)</td>
<td>3.45 (t)</td>
<td>2.11 – 1.80 (m)</td>
</tr>
<tr>
<td>33d</td>
<td>7.01 – 6.92 (m, H3), 6.84 – 6.72 (m, H2).</td>
<td>3.96 (t)</td>
<td>3.44 (t)</td>
<td>2.11 – 1.88 (m)</td>
</tr>
<tr>
<td>33e</td>
<td>7.22 – 7.15 (m, H4), 6.66 – 6.55 (m, H2, H5, H6)</td>
<td>3.95 (t)</td>
<td>3.45 (t)</td>
<td>1.99 – 1.67 (m)</td>
</tr>
<tr>
<td>33f</td>
<td>7.45 – 7.31 (m, H4), 7.21 – 7.00 (m, H2, H5 and H6)</td>
<td>4.01 (t)</td>
<td>3.48 (t)</td>
<td>2.16 – 1.87 (m)</td>
</tr>
<tr>
<td>33g</td>
<td>7.66 (d, H3), 7.36 (dd, H5), 6.74 (d, H6)</td>
<td>4.04 (t)</td>
<td>3.46 (t)</td>
<td>2.09 – 1.80 (m)</td>
</tr>
<tr>
<td>33h</td>
<td>6.97 – 6.92 (m, H5), 6.80 – 7.70 (m, H4, H6),</td>
<td>4.07 (t)</td>
<td>3.50 (t)</td>
<td>2.15 – 1.91 (m)</td>
</tr>
</tbody>
</table>
Table 2: Key signals in the $^{13}$C NMR spectra of bromoethers **33b-33h**.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Aromatic region (ppm)</th>
<th>-OCH$_2$ (ppm)</th>
<th>CH$_2$Br (ppm)</th>
<th>C-2’ (ppm)</th>
<th>C-3’ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>33b</strong></td>
<td>157.1 (C-1), 134.3 (C-3), 131.4 (C-5), 124.7 (C-4), 116.3 (C2 and C-6).</td>
<td>67.60</td>
<td>27.98</td>
<td>17.01</td>
<td>22.53</td>
</tr>
<tr>
<td><strong>33c</strong></td>
<td>159.7 (C-1), 135.5 (C-3), 121.3 (C-4), 114.0 (C-2).</td>
<td>67.32</td>
<td>27.98</td>
<td>17.00</td>
<td>22.33</td>
</tr>
<tr>
<td><strong>33d</strong></td>
<td>157.3 (d, $J_{C,F} = 238.3$ Hz, C-4), 154.9 (d, $J_{C,F} = 2.0$ Hz, C-1), 115.84 (d, $J_{C,F} = 23.1$ Hz, C-3), 115.4 (d, $J_{C,F} = 8.0$ Hz, C-2).</td>
<td>67.41</td>
<td>33.38</td>
<td>27.88</td>
<td>29.41</td>
</tr>
<tr>
<td><strong>33e</strong></td>
<td>163.6 (d, $J_{C,F} = 245.3$ Hz, C-3), 159.9 (d, $J_{C,F} = 10.8$ Hz, C-1), 130.3 (d, $J_{C,F} = 10.0$ Hz, C-5), 110.2 (d, $J_{C,F} = 2.9$ Hz, C-6), 107.8 (d, $J_{C,F} = 21.3$ Hz, C-4), 102.2 (d, $J_{C,F} = 24.8$ Hz, C-2).</td>
<td>67.70</td>
<td>27.91</td>
<td>17.00</td>
<td>22.52</td>
</tr>
<tr>
<td><strong>33f</strong></td>
<td>159.1 (C-1), 131.9 (q, $J_{C,F} = 32.2$ Hz, C-CF$<em>3$), 130.0 (C-5), 124.1 (q, $J</em>{C,F} = 272.4$ Hz, CF$<em>3$), 118.0 (C-6), 117.4 (q, $J</em>{C,F} = 3.8$ Hz, C-4), 111.3 (q, $J_{C,F} = 3.7$ Hz, C-2).</td>
<td>67.19</td>
<td>33.29</td>
<td>27.82</td>
<td>29.42</td>
</tr>
<tr>
<td><strong>33g</strong></td>
<td>154.4 (C-1), 136.0 (C-3), 131.3 (C-5), 114.2 (C-6), 113.2 (C-2), 113.1 (C-4)</td>
<td>68.28</td>
<td>27.91</td>
<td>17.06</td>
<td>22.50</td>
</tr>
<tr>
<td><strong>33h</strong></td>
<td>151.5 (dd, $J_{C,F} = 246.8$, 10.4 Hz, C-3), 148.6 (dd, $J_{C,F} = 7.9$, 3.2 Hz, C-1), 141.5 (dd, $J_{C,F} = 247.3$, 14.1 Hz, C-2), 123.2 (dd, $J_{C,F} = 8.7$, 5.2 Hz, C-5), 109.9 (d, $J_{C,F} = 2.9$ Hz, C-6), 109.2 (dd, $J_{C,F} = 17.7$, 9.5 Hz, C-4).</td>
<td>68.71</td>
<td>33.34</td>
<td>27.82</td>
<td>29.27</td>
</tr>
</tbody>
</table>
2.1.2 Synthesis of 5-(4-chlorophenoxy)pentanenitrile and analogues

Scheme 5: Reagents and conditions: KCN, EtOH/H$_2$O, reflux, 2 days, 89%.

With a range of bromoethers 33a-33h in hand, we were now in a position to prepare the desired nitriles 34a-h. Reaction of the bromoether 33a using potassium cyanide in an ethanol/water mixture furnished the corresponding nitrile 34a. The reaction was conducted by heating to reflux under a nitrogen atmosphere for two days. The bromoether impurities were removed by silica gel column chromatography to give 34a as a pale-yellow oil in 89% yield.

In the $^1$H NMR spectrum, the only significant difference from the starting material was the observed shift of the triplet signal from 3.46 ppm to 2.41 ppm, due to the shielding effect of the nitrile group. The other four signals were similar to that of the starting material, both in chemical shift and multiplicity. The $^{13}$C NMR spectrum of the product showed one extra signal at 119.5 ppm due to the presence of the CN group. The CH$_2$-CN signal shifted upfield to 16.92 ppm due to the less deshielding effect of the nitrile group compared to that of the bromine atom. The success of the reaction was further confirmed by a stretching band visible at 2252 cm$^{-1}$ in the IR spectrum, which is an indication of the CN group. The mass spectrum also corresponded well with the expected mass of the product (calculated for C$_{11}$H$_{12}$ClNO Na: 232.0507, found: [M + Na] 232.0500).

Analogues 34b-34h were prepared in a similar manner (Scheme 6). The $^1$H NMR and $^{13}$C NMR spectra of the products were similar as expected, and key diagnostic signals are tabulated below (Tables 3 and 4).
Scheme 6: Reagents and conditions: KCN, EtOH/H$_2$O, reflux, 2 days.

Table 3: Key signals in the $^1$H NMR spectra of pentanenitriles 34b-34h.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$^{1}$H NMR (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound 34b</td>
<td>1.85 (t)</td>
</tr>
<tr>
<td>Compound 34c</td>
<td>2.45 (t)</td>
</tr>
<tr>
<td>Compound 34d</td>
<td>2.39 (t)</td>
</tr>
<tr>
<td>Compound 34e</td>
<td>2.41 (t)</td>
</tr>
<tr>
<td>Compound 34f</td>
<td>2.42 (t)</td>
</tr>
<tr>
<td>Compound 34g</td>
<td>2.50 (t)</td>
</tr>
<tr>
<td>Compound 34h</td>
<td>2.46 (t)</td>
</tr>
</tbody>
</table>

Table 4: Key signals in the $^{13}$C NMR spectra of pentanenitriles 34b-34h.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$^{1}$H NMR (ppm)</th>
<th>$^{1}$C NMR (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound 34b</td>
<td>17.4</td>
<td>119.1</td>
</tr>
<tr>
<td>Compound 34c</td>
<td>17.00</td>
<td>119.3</td>
</tr>
<tr>
<td>Compound 34d</td>
<td>17.00</td>
<td>119.5</td>
</tr>
<tr>
<td>Compound 34e</td>
<td>17.00</td>
<td>119.3</td>
</tr>
<tr>
<td>Compound 34f</td>
<td>17.01</td>
<td>119.2</td>
</tr>
<tr>
<td>Compound 34g</td>
<td>17.06</td>
<td>119.5</td>
</tr>
<tr>
<td>Compound 34h</td>
<td>17.4</td>
<td>119.3</td>
</tr>
</tbody>
</table>
2.1.3 Synthesis of 2-benzoyl-5-(4-chlorophenoxy)pentanenitrile and analogues.

Scheme 7: Reagents and conditions: KOtBu, THF, rt, 18 h, 81%.

The third step in our synthesis of flexible pyrimidines was the addition of ethyl benzoate to the nitrile derivatives 34a-h. This reaction takes advantage of the acidity of the protons (pKa = 25) alpha to the nitrile group, which are abstracted by strong base to generate an anion. This reacts with the electrophilic carbonyl carbon of ethyl benzoate, followed by loss of ethoxide ion, to give the desired α-cyano ketones 35a-h. In our test reaction; starting material 34a was dissolved in anhydrous THF, to which potassium tert-butoxide was added. The reaction mixture changed colour from light yellow to dark brown. Ethyl benzoate was then added to the reaction mixture after several minutes, and the resulting mixture was stirred at room temperature under a nitrogen atmosphere overnight. The Rf of the product decreased slightly due to the hydrogen bonding of the carbonyl oxygen with silica gel on TLC. After extraction, purification by silica gel column chromatography and recrystallization, the product 35a was obtained as yellow crystals in a good yield of 81%.

The $^1$H NMR spectrum of the product 35a showed five additional signals accounting for the newly added phenyl moiety in the region 8.05 ppm to 7.52 ppm. In the aliphatic region, the triplet due to CH$_2$CN had disappeared and was replaced by a doublet of doublets integrating for one proton at 4.48 ppm owing to CHCN. This was an indication of the success of the reaction. The $^{13}$C NMR spectrum further supported this and showed 14 signals in total. Notably, a new signal at 190.4 ppm due to the carbonyl carbon, and four new signals in the aromatic region were indicative of the phenacyl group. The IR spectrum also confirmed the presence of the C=O group with a signal visible at 1694 cm$^{-1}$. The molecular ion was confirmed by HRMS to be [M+Na$^+$] 336.0757 which was consistent with the mass calculated for C$_{18}$H$_{16}$ClNO$_2$Na of 336.0770.
The other α-cyano ketone analogues 35b-35h were prepared using the above procedure (Scheme 8). Once again, $^1$H NMR and $^{13}$C NMR spectra of the analogues were similar, as expected. Diagnostic signals for the analogues 35b-35h are tabulated below (Tables 5 and 6).

![Scheme 8: Reagents and conditions: KO'Bu, THF, rt, 18 h.](image)

**Table 5: Key signal in the $^1$H NMR spectra of α-cyano ketones 35b-35h.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>-CH-CN (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>35b</td>
<td>4.46 (dd)</td>
</tr>
<tr>
<td>35c</td>
<td>4.46 (dd)</td>
</tr>
<tr>
<td>35d</td>
<td>4.50 (dd)</td>
</tr>
<tr>
<td>35e</td>
<td>4.50 (dd)</td>
</tr>
<tr>
<td>35f</td>
<td>4.51 (dd)</td>
</tr>
<tr>
<td>35g</td>
<td>4.64 (dd)</td>
</tr>
<tr>
<td>35h</td>
<td>4.72 – 4.60 (m)</td>
</tr>
</tbody>
</table>

**Table 6: Key signals in the $^{13}$C NMR spectra of α-cyano ketones 35b-35h.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>-C=O (ppm)</th>
<th>-CH-CN (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>35b</td>
<td>179.4</td>
<td>30.43</td>
</tr>
<tr>
<td>35c</td>
<td>190.2</td>
<td>39.25</td>
</tr>
<tr>
<td>35d</td>
<td>190.4</td>
<td>39.41</td>
</tr>
<tr>
<td>35e</td>
<td>190.5</td>
<td>39.40</td>
</tr>
<tr>
<td>35f</td>
<td>190.5</td>
<td>39.41</td>
</tr>
<tr>
<td>35g</td>
<td>190.6</td>
<td>39.70</td>
</tr>
<tr>
<td>35h</td>
<td>190.5</td>
<td>39.40</td>
</tr>
</tbody>
</table>
2.1.4 Synthesis of E/Z -5-(4-chlorophenoxy)-2-(methoxy(phenyl)methylene) pentanenitrile and analogues.

Scheme 9: Reagents and conditions: KOH, Et₂O, carbitol, DCM, rt, 18 h, quant.

We were now in a position to prepare the required enol ethers 36a-36h derived from the α-cyano ketones 35a-h. In each case, the α-cyano ketone prepared in the previous step was treated with diazomethane generated from diazald. The diazomethane apparatus containing potassium hydroxide in a diethyl ether/carbitol solution was lowered into a water bath at 70-80°C. As diazomethane is explosive, care was taken when doing this reaction. Diazomethane gas was distilled into a flask containing the starting material 35a dissolved in dry dichloromethane. The reaction mixture was stirred at room temperature and, upon completion of the reaction, excess diazomethane was quenched with a few drops of acetic acid and the mixture concentrated to dryness, in vacuo. The enol ether intermediate 36a was isolated as a mixture of E/Z isomers in a ratio of 60:40 (E:Z). The crude product was isolated as a viscous yellow oil in quantitative yields, and used in the next step without further purification.

The enol ether intermediate 36a derived from 35a indeed formed as a mixture of E/Z isomers. This was evident by the doubling up of each signal in the ¹H NMR and ¹³C NMR spectra. The ¹H NMR spectrum contained two singlets at 3.47 ppm and 3.43 ppm due to the methoxy groups of each isomer. The presence of the methoxy groups confirmed the success of the reaction, as we expected the conversion of the α-cyano ketone 35a to the desired enol ether product 36a. The signal due to CHCN at 4.48 ppm was no longer visible, and a shift in the methylene signal was observed from 2.41 – 1.85 ppm to 2.57 ppm. The signal at 190.4 ppm in the ¹³C NMR spectrum due to the carbonyl carbon (C=O) also disappeared, as expected, and two new signals at 168.9 ppm and 94.22 ppm were observed for the alkene carbons.

Seven other analogues 36b-36h were prepared in the same manner (Scheme 10). Similar changes in the ¹H NMR and ¹³C NMR spectra of the products were observed.
2.1.5 Synthesis of 5-(3-(4-chlorophenoxy)propyl)-6-phenylpyrimidine-2,4-diamine and analogues.

Scheme 10: Reagents and conditions: KOH, Et₂O, carbitol, DCM, rt, 18 h, quant.

Scheme 11: Reagents and conditions: NaOMe, DMSO, 90°C, 24 h, 11%.

The last step of the synthesis involved ring closure using guanidine hydrochloride to form our desired pyrimidine product. Tarnchompoo and co-workers prepared substituted pyrimidines from an enol ether and guanidine. They obtained their products in good yields of 56-90%, but all products contained a biaryl axis at C-5 of the pyrimidine ring. We hoped to apply this methodology to the synthesis of our flexible pyrimidine products. This step was once again tested on the 4-Cl analogue 36a. Sodium metal was dissolved in methanol to prepare a solution of sodium methoxide. Guanidine hydrochloride salt was then dissolved in the prepared sodium methoxide solution to furnish free guanidine after filtration of sodium chloride and removal of methanol in vacuo on a rotary evaporator. The enol ether intermediate 36a dissolved in dry DMSO was then treated with guanidine and heated at 90°C overnight under a nitrogen atmosphere. The reaction was monitored by TLC and to our delight, the formation of a new product was observed as a highly fluorescent blue spot on the
baseline of the TLC plate. However, the reaction did not go to completion, even when the mixture was left to stir for an extended period at 90°C. After extraction and purification by silica gel column chromatography, compound 37a was obtained as a yellow solid, albeit in a low yield of 11%. The majority of starting material (65%) was recovered. Attempts to drive the reaction to completion or improve the yield by conducting the reaction in a microwave or increasing the reaction temperature were not successful. The mechanism for the formation of the pyrimidine ring is shown in Scheme 12. The first step involves nucleophilic attack on the enol ether by nitrogen of guanidine, which kicks off the methoxy group. This is followed by subsequent ring closure to form the heterocyclic ring. Atmospheric oxygen is responsible for oxidation to the aromatic pyrimidine.

Scheme 12: Proposed mechanism for the formation of pyrimidine 37.

The $^1$H NMR spectrum of pyrimidine 37a contained 8 signals in total. As expected, the methoxy signals of the starting material at 3.47 ppm and 3.43 ppm had disappeared, indicating consumption of both $E$ and $Z$ isomers. Two broad singlets at 5.29 ppm and 5.15 ppm, integrating for two protons each, were assigned to the amino groups on the pyrimidine ring. The aliphatic region of the $^1$H NMR spectrum clearly showed two triplets and a multiplet integrating for two protons each at 3.84 ppm, 2.53 ppm and 1.93 – 1.84 ppm, respectively, for the methylene groups in the alkyl chain. The $^{13}$C NMR spectrum was also convincing, as formation of the pyrimidine ring was confirmed by a signal at 104.8 ppm, characteristic of C-5 of the pyrimidine ring. The CN signals at 120.1 ppm and 118.2 ppm in the starting material had also disappeared. The IR spectrum showed broad, strong N-H
stretches at 3328 cm\(^{-1}\) and 3140 cm\(^{-1}\), which is an indication of the formation of our desired product. Success of the reaction was further confirmed by the disappearance of the C=N band at 2253 cm\(^{-1}\) in the IR spectrum. The molecular ion was confirmed by HRMS to be \([\text{M+H}]^+ 355.1326\) which was consistent with a molecular mass of 355.1327.

Three other analogues 37b, c and e were prepared in a similar manner, although all in disappointingly low yields (Scheme 13). The synthesized flexible pyrimidines 37b, c and e were validated by means of \(^1\)H NMR and \(^{13}\)C NMR spectroscopy and HRMS.

Scheme 13: Reagents and conditions: NaOMe, DMSO, 90°C, 24 h.

The \(^1\)H NMR spectrum of the final compound 37b contained 6 signals in total. Again as expected, the methoxy signals of the starting material at 3.48 ppm and 3.45 ppm had disappeared. Two triplets at 2.52 ppm and 1.31 ppm integrating for two protons each, were assigned to H7’ (-OCH\(_2\)-) and H9’. The two amino groups exchanged with the solvent used for spectroscopic analysis. The \(^{13}\)C NMR spectrum was also convincing, with signals at 66.87 ppm, 27.17 ppm and 21.10 ppm assigned to the CH\(_2\) groups of the alkyl chain. A signal at 105.3 ppm in the \(^{13}\)C NMR spectrum was assigned to C5 of the pyrimidine ring. The IR spectrum showed stretching bands at 3430 and 3164 cm\(^{-1}\), which is an indication of the NH\(_2\) groups. The molecular ion was confirmed by HRMS to be \([\text{M+H}]^+ 389.0936\) which was consistent with a molecular mass of 389.0938.

In the \(^1\)H NMR spectrum of pyrimidine 37c, a triplet and a doublet at 5.68 ppm and 5.36 ppm were assigned to H4’ and H2’, respectively.
Two triplets and a multiplet integrating for two protons each appeared in the $^1$H NMR spectrum at 2.52 ppm, 1.31 ppm and 0.60 – 0.55 ppm, respectively for the methylene groups in the alkyl chain. The two amino groups were not observable due to solvent exchange. A signal at 105.3 ppm in the $^{13}$C NMR spectrum was assigned to C-5 of the pyrimidine ring, this further confirmed success of the reaction. The formation of product 37c was further confirmed by HRMS with a molecular ion of [M+H] 389.0936 which was consistent with a molecular mass of 389.0938, (calculated for C$_{19}$H$_{19}$Cl$_2$N$_4$O).

The $^1$H NMR spectrum of pyrimidine 37e contained two triplets and a multiplet integrating for two protons each at 2.53 ppm, 1.27 ppm and 0.67 – 0.53 ppm respectively, for the methylene groups in the alkyl chain. The two amino groups exchanged with the solvent used for spectroscopic analysis. The $^{13}$C NMR spectrum was also convincing, as formation of the pyrimidine ring was confirmed by the characteristic signal at 104.8 ppm due to C-5 of the pyrimidine ring. Absorption bands in the IR spectrum at 3479 cm$^{-1}$ and 3112 cm$^{-1}$ validated the presence of the N-H groups. The molecular ion was confirmed by HRMS to be [M+H] 339.1621 which was consistent with a molecular mass of 339.1703.

The other analogues 36d, f, g and h were also carried through to the last ring closure step, but we were unable to isolate the desired final products 37d, f, g and h from these reactions. Several experimental methods were attempted, but we were unfortunately unsuccessful in preparing the flexible pyrimidines from these substrates. In some cases, the starting material was recovered, while under other conditions, starting material decomposed during the reaction.

Varied experimental conditions tested included the use of different bases (NaH) and solvents (DMF). Further attempts to facilitate the reaction included conducting the reaction in the microwave using 1,4-dioxane as a solvent. However, all the attempted methods were not successful in forming the desired pyrimidine, with either starting material recovered from the reaction, or starting material decomposing during the reaction.
2.2 Biological evaluation of pyrimidine analogues prepared

The pyrimidine compounds synthesised were tested for antimalarial activity in an in vitro *P. falciparum* screen on a Gambian FCR-3 strain (chloroquine and cycloguanil resistant strain) at the Wits School of Pharmacy and Pharmacology by Prof R van Zyl. The *P. falciparum* (FCR-3) strain was cultured in vitro at 37°C in 3% O₂, 5% CO₂, 92% N₂ and adjusted to a 0.625% parasitaemia/ 1.25% haematocrit before being incubated along with the compounds for 72 hours. The plates were frozen overnight and, once thawed, incubated in the dark for 1 hour at room temperature with buffered SYBR green I. The fluorescence was read in a microplate reader with excitation and emission wavelength bands centered at 485 and 528 nm, respectively. The percentage inhibition was calculated taking the untreated and dihydroartemisinin control into account. Dihydroartemisinin (DHA) was used as the positive control. At least three independent experiments were conducted for each sample. The results of the biological screening are shown in Table 7.

**Table 7: Results of in vitro antimalarial assay for compounds 37a-c, e.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ (µM)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>37a</td>
<td>3.69 ± 0.35</td>
<td>3</td>
</tr>
<tr>
<td>37b</td>
<td>14.80 ± 2.97</td>
<td>3</td>
</tr>
<tr>
<td>37c</td>
<td>0.09 ± 0.01</td>
<td>3</td>
</tr>
<tr>
<td>37e</td>
<td>Not obtained</td>
<td>0</td>
</tr>
<tr>
<td>Quinine</td>
<td>2.81 ± 0.57</td>
<td>3</td>
</tr>
<tr>
<td>DHA</td>
<td>0.00614 ± 0.0011</td>
<td>3</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>0.23 ± 0.18</td>
<td>3</td>
</tr>
</tbody>
</table>

The most active compound from the pyrimidine series tested was 37c, with an IC₅₀ of 0.09 µM against the drug resistant strain (Figure 30). While we were happy with this result; we were surprised to find that this was the only compound of the series which showed promising antimalarial activity. The pyrimidine analogue of our hit compound 30, by comparison, was
significantly less potent with an IC$_{50}$ of 3.69 µM in the same assay. This could be due to the fact that our pyrimidine compounds now also contain a rigid biaryl axis at C-6. This was not the case in the dihydrotriazine series, where the phenyl substituent at C-6 was not rigid and planar as the dihydrotriazine ring is not aromatic. Based on these results, we modified our target series of compounds to include non-aromatic side chains.

![Figure 30](image)

**Figure 30**: Most active compound from the first generation of synthesis.

Further assessment of 37c will be considered for future work in order to better understand these results. This will include toxicity, molecular modelling, and a biochemical DHFR assay.

### 2.3 Planned approach towards the second generation of compounds bearing a non-aromatic side chain

Biological assessment of the first series of pyrimidines 37a-c, e prepared, showed that these compounds were less active than the dihydrotriazine counterparts prepared previously. As discussed, this might be due to the sterically constrained linkage between the phenyl substituent and the pyrimidine ring. Hence, in the second generation of compounds, we embarked on the synthesis of analogues containing a smaller or more flexible non-aromatic substituent at the 6-position of the pyrimidine ring, as shown below in two examples in Figure 31.

![Figure 31](image)

**Figure 31**: Flexible pyrimidines bearing a non-aromatic substituent.
We planned to use the same approach adopted in the synthesis of pyrimidines 37a-c, and e, utilising a non-aromatic ester in the generation of the α-cyano ketone 44 (Scheme 14). We hoped to be able to generate the enol ether 45 and convert this to the desired pyrimidines 46 by reaction with guanidine hydrochloride (Scheme 14).

2.3.1  Synthesis of 5-(3-(4-chlorophenoxy)propyl)-6-cyclopropyl/cyclohexyl pyrimidine-2,4-diamine

\[ X = 4-\text{Cl} \]
Scheme 14: Planned synthetic approach to 5-(3-(4-chlorophenoxy)propyl)-6-cyclopropyl/cyclohexyl pyrimidine-2,4-diamine.

We began our synthesis from the advanced precursor 34a prepared for the synthesis of pyrimidine 37a, and treated it with methyl cyclohexanoate (R = cyclohexyl) in the presence of potassium tert-butoxide in dry THF at room temperature under a nitrogen atmosphere (Scheme 14). The reaction was monitored by TLC, and after several hours only the starting material was detected. The reaction was also tested using methyl cyclopropanecarboxylate (R = cyclopropyl) in the presence of potassium tert-butoxide. Unfortunately, all attempts to prepare α-cyano ketones 44 failed using this methodology.

A series of bases (NaH, KHMDS, NaOMe, and LDA) and different reaction conditions were tested in an attempt to facilitate formation of 44 without success. As a result, we were therefore unable to prepare analogues with a non-aromatic side chain such as 46 using this approach.

2.4 Planned approach towards synthesis of flexible pyrimidines using a multicomponent coupling reaction (MCR).

Recently, multicomponent reactions have become a valuable strategy in the preparation of structurally-diverse chemical libraries of drug-like heterocyclic compounds. MCRs are chemical reactions in which three or more reagents are mixed together to yield a single product, in a cascade process. Raghuvanshi and co-workers have reported the synthesis of pyrimidines by a three-component condensation of an aromatic aldehyde, malononitrile and guanidine hydrochloride in ethanol. We planned to use this MCR approach in an alternative synthesis of flexible pyrimidines bearing a non-aromatic side chain. Shown below in Scheme 15 is our proposed, alternative route to synthesise the desired products 47. It should be noted that the position of the oxygen atom in the flexible linker will now be in a different position to that of the pyrimidines 37a-d synthesized in the first series. The effect of this change on biological activity would also have to be assessed. As such, the analogue bearing a phenyl group at C-6 will also be prepared for direct comparison.
Scheme 15: Reagents and conditions: i) NaOAc, H₂O/ethanol, reflux, 18 h; ii) H₂; Pd/C, aq. H₂SO₄, rt; iii) NaBH₄, MeOH, rt; iv) KO'Bu, THF, 18 h.

The first synthetic step involves a multicomponent coupling reaction (MCR) of aldehyde, malononitrile and guanidine hydrochloride in an ethanolic solution to afford the desired 5-cyano pyrimidine 48. The nitrile group of 48 will be converted to the aldehyde 49 by a Pd/C catalysed hydrogenation in aqueous acid. This would then be followed by reduction of the carbonyl group to the corresponding alcohol 50, and reaction of the alcohol with substituted phenethylene bromides would hopefully afford the desired flexible pyrimidines 47.

The mechanism for the formation of 5-cyano pyrimidine 48 is shown in Scheme 16. The first step is a Knoevenagel condensation between an aldehyde and malononitrile with its acidic α-hydrogens, to produce adduct 51. This intermediate then undergoes nucleophilic attack by nitrogen of guanidine, followed by subsequent ring closure to form the heterocyclic ring. Atmospheric oxygen is responsible for oxidation to the aromatic 5-cyano pyrimidine 48.
2.4.1 Synthesis of 2,4-diamino-6-phenylpyrimidine-5-carbonitrile

In our test reaction; as shown in Scheme 17, benzaldehyde and malononitrile were stirred together for at least 10 minutes to form the condensation adduct. Guanidine hydrochloride was then added to the reaction mixture and the reaction changed in colour, from white to dark brown. The reaction mixture was then heated at reflux overnight, and then cooled to room temperature. After work-up, the product and other impurities precipitated out of solution. Following column chromatography, the product 48a was obtained as a yellow solid in 44% yield.

Scheme 17: Reagents and conditions: NaOAc, H2O/ethanol, reflux, 18 h, 44%.

The 1H NMR spectrum of the product 48a contained a broad signal integrating for four protons at 7.13 ppm due to the two amino groups. The phenyl protons appeared as a multiplet
at 7.75 – 7.40 ppm. The $^{13}$C NMR spectrum was also convincing, as formation of the pyrimidine ring was confirmed by a signal at 75.94 ppm, characteristic of C-5 of the pyrimidine ring, while the CN signal appeared at 117.9 ppm. The IR spectrum showed a stretching band at 3374 cm$^{-1}$, which is an indication of the N-H groups, while the C=N stretching band appeared at 2205 cm$^{-1}$.

We were now in a position to attempt the preparation of flexible pyrimidines bearing non-aromatic side chains using this route.

### 2.4.2 Synthesis of 2,4-diamino-6-cyclopropylpyrimidine-5-carbonitrile and 2,4-diamino-6-cyclohexylpyrimidine-5-carbonitrile.

![Scheme 18](image)

**Scheme 18:** Reagents and conditions: NaOAc, H$_2$O/ethanol, reflux, 18 h, 41%.

The MCR approach was then repeated using cyclopropanecarbaldehyde, in the first synthetic step towards a flexible antifolate. This key step involved the reaction of cyclopropanecarboxaldehyde, malononitrile and guanidine hydrochloride in aqueous ethanol. The aldehyde and malononitrile were stirred together for at least 10 minutes to form the condensation adduct 51 (R = cyclopropyl). The reaction changed colour from white to light brown after addition of guanidine hydrochloride. The reaction mixture was then heated at reflux overnight, and then cooled to room temperature. TLC analysis showed formation of the product 48b which precipitated out of solution after work-up with ethyl acetate and water. Following column chromatography, the product was obtained as a yellow solid in 41% yield.

Four signals were observed in the $^1$H NMR spectrum of the product. The two broad singlets at 6.90 ppm and 6.68 ppm were assigned to the amino groups at C-4 and C-2 of the pyrimidine ring, respectively. A pentet at 2.04 ppm integrating for one proton and a multiplet at 0.81 – 0.78 ppm integrating for four protons were indicative of the cyclopropyl moiety. The $^{13}$C NMR spectrum also confirmed the formation of the expected product, with the
characteristic signal of C-5 of the pyrimidine ring visible at 77.02 ppm. The success of the reaction was also indicated by a stretching band at 2203 cm\(^{-1}\) in the IR spectrum, which is an indication of the CN group. The mass spectrum also corresponded well with the expected mass of the product.

**Scheme 19:** Reagents and conditions: NaOAc, H\(_2\)O/ethanol, reflux, 18 h, 47%.

The cyclohexyl analogue 48c was prepared in a similar manner (Scheme 19). The \(^1\)H NMR spectrum of the product 48c contained 4 signals in total. The two broad singlets at 6.91 ppm and 6.78 ppm were assigned to the amino groups at C-4 and C-2 of the pyrimidine ring, respectively. A pentet at 2.63 ppm integrating for one proton and a multiplet at 1.82 ppm – 1.35 ppm integrating for ten protons were indicative of the cyclohexyl moiety. The \(^{13}\)C NMR spectrum also confirmed the formation of the expected product, with the characteristic signal of C-5 of the pyrimidine ring visible at 76.59 ppm while the CN group appeared at 117.7 ppm. The success of the reaction was also indicated by a stretching band at 2203 cm\(^{-1}\) in the IR spectrum, which is an indication of the CN group. The molecular ion was confirmed by HRMS to be [M+H] 218.1406 which was consistent with a molecular mass of 218.1407.

### 2.4.3 Synthesis of 2,4-diamino-6-phenylpyrimidine-5-carbaldehyde

**Scheme 20:** Reagents and conditions: H\(_2\); Pd/C, H\(_2\)O/H\(_2\)SO\(_4\), rt, 18 h.
Having successfully prepared the nitrile derivative, we continued with the second step of the synthesis; the formation of the pyrimidine carbaldehyde 49a. To this end, the starting material 48a was dissolved in dilute aqueous sulfuric acid, and the palladium catalyst was added to the reaction mixture. The flask was evacuated and then placed under an atmosphere of hydrogen. Conversion of the nitrile to the aldehyde goes via the imine formed by nitrile reduction, followed by hydrolysis of the imine in mild acid to afford the aldehyde (Scheme 21). The reaction mixture was left to stir at room temperature for 18 hours under a hydrogen atmosphere. TLC analysis showed the disappearance of the nitrile starting material and the formation of a product spot 49a, along with a spot for the alcohol 50a which resulted from reduction of the aldehyde. The formation of the aldehyde 49a was confirmed on TLC by means of a 2,4-dinitrophenyl hydrazine (DNPH) stain. After filtration, extraction and purification by silica gel column chromatography, the product was obtained as a light-yellow solid in a reasonable yield of 51%.

In the $^1$H NMR spectrum, the appearance of the downfield signal at 9.45 ppm integrating for one proton was due to the aldehyde proton. The $^{13}$C NMR spectrum contained a new signal at 188.5 ppm accounting for the carbonyl carbon of the aldehyde. The C-5 signal shifted from 75.94 ppm to 102.9 ppm due to the shielding effect of the carbonyl group.

Compound 50a was also isolated and characterised using NMR spectroscopy. The $^1$H NMR spectrum confirmed that it was indeed an alcohol 50a with a triplet at 4.83 ppm and a doublet at 4.20 ppm due to OH and CH$_2$ groups, respectively. The $^{13}$C NMR spectrum further confirmed this with a signal at 56.94 ppm due to CH$_2$OH.
Scheme 21: Proposed mechanism for the formation of aldehyde 49a from cyanopyrimidine in mild acid.

2.4.4 Synthesis of 2,4-diamino-6-cyclopropylpyrimidine-5-carbaldehyde and 2,4-diamino-6-cyclohexylpyrimidine-5-carbaldehyde

With the successful synthesis of phenyl carbaldehyde 49a completed, we now applied this methodology to our precursors bearing flexible side chains, 48b and 48c. The cyclopropyl analogue was synthesised in a moderate yield of 57% using this methodology (Scheme 22).

Scheme 22: Reagents and conditions: H₂; Pd/C, H₂O/H₂SO₄, rt, 18 h.

The ¹H NMR spectrum of the cyclopropyl product 49b was relatively simple. The appearance of the characteristic downfield singlet at 10.1 ppm due to the aldehyde proton confirmed the formation of the desired product. The corresponding signal in the ¹³C NMR spectrum due to the carbonyl carbon (C=O) was visible at 189.7 ppm. The signal for C-5 now appeared at 99.17 ppm due to the deshielding effect of the carbonyl group. The IR spectrum further
confirmed the success of the reaction by the presence of a stretching band at 1747 cm\(^{-1}\), which is an indication of the C=O group.

The cyclohexyl analogue 49c was prepared in a similar manner (Scheme 23). Again as expected, the \(^1\)H NMR spectrum of the resultant product 49c contained a downfield singlet at 10.08 ppm, characteristic of the aldehyde proton. A signal at 117.7 ppm due to the CN group had disappeared. The corresponding signal in the \(^13\)C NMR spectrum due to the carbonyl carbon (C=O) was visible at 187.8 ppm. The molecular ion was confirmed by HRMS to be [M+H] 221.1397 which was consistent with the expected molecular mass of 221.140, (calculated for C\(_{11}\)H\(_7\)N\(_4\)O: 221.1401).

Scheme 23: Reagents and conditions: H\(_2\); Pd/C, H\(_2\)O/H\(_2\)SO\(_4\), rt, 18 h, 49%.

2.4.5 Synthesis of (2,4-diamino-6-phenylpyrimidin-5-yl)methanol

Scheme 24: Reagents and conditions: NaBH\(_4\), methanol, rt, 3 h, 88%.

The next step in our synthetic sequence was the reduction of the aldehyde group in 2,4-diamino-6-phenylpyrimidin-5-carbaldehyde 49a to the corresponding alcohol 50a. The reaction was carried out by dissolving the starting material 49a in methanol at 0° C. Sodium borohydride (NaBH\(_4\)), was then added to the reaction flask portion-wise. The reaction was then allowed to stir at room temperature under inert conditions for 3 hours and the progress of the reaction was monitored by TLC analysis. After conversion of all of the starting material
to the product, the unreacted NaBH₄ was quenched with water. This was followed by work-up and purification by silica gel column chromatography to furnish the desired product 50a as a white solid in a good yield of 88%.

The ¹H NMR spectrum of the product 50a contained a triplet at 4.83 ppm and a doublet at 4.20 ppm due to OH and CH₂ groups, respectively. As expected, the aldehyde signal in the ¹H NMR spectrum at 9.45 ppm had disappeared. In addition, a signal at 56.94 ppm in the ¹³C NMR spectrum was assigned to CH₂OH.

2.4.6 Synthesis of (2,4-diamino-6-cyclopropylpyrimidin-5-yl)methanol and (2,4-diamino-6-cyclohexylpyrimidin-5-yl)methanol

Scheme 25: Reagents and conditions: NaBH₄, methanol, rt, 3 h, 84%.

The methodology tested on phenyl carbaldehyde 49a was then applied to our cyclopropyl and cyclohexyl analogues; 49b and 49c. The cyclopropyl analogue was isolated as a white solid in a good yield of 84% using this procedure (Scheme 25).

Two changes were observed in the ¹H NMR spectrum of the product: firstly, the signal at 10.1 ppm due to the aldehyde proton had disappeared, and secondly, a triplet at 4.65 ppm and a doublet at 4.44 ppm appeared, due to the newly formed OH and CH₂ groups, respectively. The success of the reaction was also indicated by a stretching band at 3161 cm⁻¹ in the IR spectrum, which is an indication of the OH group. The stretching band for the amino groups was still visible at 3342 cm⁻¹. The molecular ion was confirmed by HRMS to be [M+H] 181.1089 which was consistent with a molecular mass of 181.1091.

The cyclohexyl analogue was prepared in similar manner (Scheme 26). The ¹H NMR spectrum of the resultant product 50c contained a triplet at 4.65 ppm and a doublet at 4.31 ppm, due to the newly formed OH and CH₂ groups, respectively. The signal at 10.08 ppm due
to the aldehyde proton had disappeared. The success of the reaction was also indicated by a stretching band at 3159 cm\(^{-1}\) in the IR spectrum, due to the OH group.

Scheme 26: Reagents and conditions: NaBH\(_4\), methanol, rt, 3 h, 91%.

2.4.7 Attempted synthesis of 5-((4-chlorophenethoxy)methyl)-6cyclopropylpyrimidine-2,4-diamine

Scheme 27: Reagents and conditions: KO\(^{t}\)Bu, THF, 18 h.

Having successfully prepared the precursor for the final step of our planned synthetic sequence, we hoped to subject the pyrimidinyl methanols 50a-c to a substitution reaction with a variety of phenethylene bromides in the presence of a suitable base. To this end, (2,4-diamino-6-cyclopropylpyrimidin-5-yl)methanol 50b was dissolved in dry DMF, and potassium tert-butoxide was added to it. After a few minutes, a solution of 1-(2-bromoethyl)-4-chlorobenzene in DMF was added to the reaction flask. The reaction mixture was left to stir overnight at room temperature under atmospheric conditions.

After this time, TLC analysis showed only starting materials present, with no sign of a new product being formed. The reaction was then warmed up to 90°C overnight. When no change was observed, the reaction mixture was quenched and the organic material isolated by extraction and purified by silica gel chromatography. NMR spectroscopic analysis confirmed that the two spots identified were the two starting materials. Several bases (NaH, K\(_2\)CO\(_3\) and Et\(_3\)N), solvents and different reaction conditions were tested in order to facilitate the desired
substitution of the alcohol 50b, but these were unsuccessful. This inactivity may be due to intramolecular hydrogen bonding that could exist between the C4-NH$_2$ and the OH groups, forming a very stable six-membered ring as shown below.

![Diagram of 50b]

In order to test this hypothesis; we decided to acetylate the amino groups in an attempt to minimise the hydrogen bonding. We therefore acetylated the amino groups in the 2- and 4-positions of aldehyde 49b using acetic anhydride in dimethyl formamide (DMF), following the procedure of Baker et al.$^{99}$

![Scheme 28]

**Scheme 28:** Reagents and conditions: i) Ac$_2$O, DMF, reflux, 1 h, 75%, ii) NaBH$_4$, MeOH, rt, 2 h, 60%.

This was followed by reduction of the aldehyde with sodium borohydride in methanol to the corresponding alcohol 54. However, all attempts to react the alcohol 54 with alkyl bromides in the presence of a suitable base were unsuccessful. All the bases used resulted in the conversion of the amide back to the amine 50b, rather than the desired nucleophilic displacement of an alkyl halide by the alcohol 54. Formation of alcohol 50b from compound 54 was confirmed by $^1$H NMR spectroscopy.

We also attempted the bromination of the alcohol 50b using 37% hydrogen bromide in acetic acid, as described by Gangjee and co-workers to afford 55.$^{100}$ The aim was to displace the resulting bromide with a suitably substituted alcohol, but this was also not successful. We
therefore abandoned this approach to flexible pyrimidines 47, and sought alternative ways to functionalise our pyrimidine with a flexible side chain.

Scheme 29

2.4.8 Preparation of flexible pyrimidines by functionalisation of a pyrimidine carbaldehyde.

2.4.8.1 Wittig reaction

As we had been successful in carrying out functional group interconversions on aldehydes 49a-c, we focused our attention on using the aldehyde functional group as a handle to build our flexible side chain. Initially, we considered using a Wittig approach, which would afford pyrimidines 56. The Wittig reaction was initially tested on acetylated pyrimidine 53 using methyl triphenylphosphonium bromide and a variety of bases (DBN, DBU, and KHMDS) using the method described by Baker. However, to our disappointment, this reaction was also unsuccessful.
Scheme 30: Wittig approach towards flexible pyrimidine analogues.

2.4.8.2 Reductive amination

Undeterred, we next attempted reductive amination on aldehyde 49b with suitably substituted amines. This methodology would result in the introduction of a nitrogen atom in the flexible linker via reductive amination. Once again, the effect of this change on biological activity would have to be assessed. As such; the analogue bearing a phenyl ring at C-6 will also be prepared.

We initially tested the reductive amination with benzylamine and aldehyde 49a. To this end, aldehyde 49a was dissolved in absolute ethanol to which a few drops of glacial acetic acid were added. The role of the acid was to protonate the carbonyl oxygen, making the carbonyl carbon more susceptible to nucleophilic attack. Benzylamine was then added to the reaction flask and the mixture was heated to reflux for 18 hours. After this time, TLC analysis showed the formation of a new product, which we assumed was the imine intermediate. Sodium cyanoborohydride was then added to the reaction mixture, which was left to stir at reflux overnight under a nitrogen atmosphere. To our delight, TLC analysis showed the formation of a new product. After workup and purification by silica gel column chromatography, the product 57 was isolated in 47% yield.
**Scheme 31:** Reagents and conditions: Ethanol, AcOH, NaCNBH₃, reflux, 18 h, 47%.

The $^1$H NMR spectrum of the product 57 contained two broad singlets at 6.55 ppm and 5.86 ppm, integrating for two protons each, accounting for the two amino groups. A doublet at 1.18 ppm was assigned to H-1'. In the $^{13}$C NMR spectrum, the aldehyde signal at 188.5 ppm had disappeared, as expected. The aliphatic region contained two signals at 52.75 ppm and 45.94 ppm due to C-2' and C-1', respectively. The molecular ion was confirmed by HRMS to be [M+H] 306.1714 which was consistent with a molecular mass of 306.1720, (calculated for C₁₈H₂₀N₅).

With this positive result in hand, we tested the methodology using a number of substituted phenethylenamine amines.

### 2.4.8.2.1 Synthesis of 5-((4-methoxyphenethylamino)methyl)-6-phenylpyrimidine-2,4-diamine

**Scheme 32:** Reagents and conditions: Ethanol, AcOH, NaCNBH₃, reflux, 18 h, 31%.

Aldehyde 49a was therefore treated with 2-(4-methoxyphenyl)ethanamine as described above, to afford 58a as a yellow solid in a yield of 31%.

The $^1$H NMR spectrum of final compound 58a contained two doublets at 7.65 ppm and 7.16 ppm due to H2’ and H3’, respectively. The phenyl protons appeared as a multiplet at 7.01 – 6.80 ppm. A singlet integrating for three protons at 3.80 ppm was due to the methoxy group.
The two amino groups exchanged with the solvent used for spectroscopic analysis. The presence of C-5 of the pyrimidine ring was evident in the $^{13}$C NMR spectrum at 102.9 ppm. The $^{13}$C NMR spectrum was also convincing, with signals at 55.62 ppm and 45.94 ppm assigned to the CH$_2$ groups of the alkyl chain.

2.4.8.2.2 Synthesis of 6-cyclopropyl-5-((4-methoxyphenethylamino)methyl)pyrimidine-2,4-diamine and 6-cyclohexyl-5-((4-methoxyphenethylamino)methyl)pyrimidine-2,4-diamine.

Two other flexible analogues 58b-58c bearing a non-aromatic side chain were prepared in a similar manner (Scheme 30). The $^1$H and $^{13}$C NMR spectra of the products were similar, as expected.

![Scheme 33: Reagents and conditions: Ethanol, AcOH, NaCNBH₃, reflux, 18 h.](image)

In both the $^1$H NMR and $^{13}$C NMR spectra of the pyrimidine product 58b, the relevant aldehyde signal had disappeared. In the $^1$H NMR spectrum, two doublets at 7.11 ppm and 6.86 ppm were assigned to H2’ and H3’, respectively. The broad signal at 2.81 ppm integrated for four protons due to the two amino. A singlet integrating for two protons at 1.94 ppm was due to H7’. The multiplet at 1.64 – 0.98 ppm was assigned to the two CH$_2$ groups of the cyclopropyl group. The characteristic signal due to C-5 of the pyrimidine ring was visible at 99.11 ppm in the $^{13}$C NMR spectrum. Signals at 49.07 ppm and 36.47 ppm were assigned to the methylene groups in the alkyl chain. The IR spectrum showed a broad, strong N-H stretch at 3284 cm$^{-1}$ which is an indication of the formation of our desired product. The molecular ion was confirmed by HRMS to be [M$^+$] 313.1901 which was consistent with a molecular mass of 313.1903.
Similarly for product 58c, the aldehyde signal in both the $^1$H NMR and $^{13}$C NMR spectra had disappeared. In the $^1$H NMR spectrum, two doublets at 7.16 ppm and 6.90 ppm were assigned to H2’ and H3’, respectively. A multiplet at 3.68 – 3.65 ppm and a triplet at 2.87 ppm integrating for two protons each were assigned to H6’ and H5’, respectively. The cyclohexyl protons gave rise to a multiplet at coupled together at 1.30 – 1.26 ppm. The IR spectrum showed a broad, strong N-H stretch at 3313 cm$^{-1}$. The molecular ion was confirmed by HRMS to be [M+Na] 378.1912 which was consistent with a molecular mass of 378.2272, (calculated for C$_{20}$H$_{29}$N$_5$ONa).

2.4.8.2.3 Synthesis of 5-((4-chlorophenethylamino)methyl)-6-cyclopropylpyrimidine-2,4-diamine

Aldehyde 49b was then treated with 4-chlorophenethylamine in a similar manner to that described above. The product was isolated in a moderate yield of 33%. In the $^1$H NMR spectrum, two doublets at 7.40 ppm and 7.32 ppm were assigned to H3’ and H2’, respectively. The broad signal at 3.39 ppm integrated for four protons due to the two amino groups. A pentet integrating for one proton at 3.24 ppm was due to the cyclopropyl H7. Two multiplets at 3.11 – 2.89 ppm were assigned to the two CH$_2$ groups of the alkyl chain. The signals due to H7’ hydrogens and H-8 of the cypropyl group overlapped at 1.30 – 1.26 ppm. A signal at 99.11 ppm in the $^{13}$C NMR spectrum was assigned to C-5 of the pyrimidine ring. Aliphatic carbons were observed at 45.44 ppm, 31.88 ppm, 21.65 ppm and 19.41 ppm.
2.5 Biological assessment of the pyrimidine analogues bearing a flexible side chain.

Once again, the pyrimidine compounds synthesised were tested for antimalarial activity in an *in vitro* *P. falciparum* screen on a Gambian FCR-3 strain (chloroquine and cycloguanil resistant strain) by Prof R van Zyl at the Wits School of Pharmacy and Pharmacology, as described before. Quinine was used as the positive control. The results of the biological screening are shown in Table 8.

**Table 8:** Results of *in vitro* antimalarial assay for compounds 57, 58a-58d.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ (µM)</th>
<th>n</th>
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<tbody>
<tr>
<td>57</td>
<td>1.94 ± 0.27</td>
<td>3</td>
</tr>
<tr>
<td>58a</td>
<td>28.37 ± 5.74</td>
<td>3</td>
</tr>
<tr>
<td>58b</td>
<td>18.77 ± 2.18</td>
<td>3</td>
</tr>
<tr>
<td>58c</td>
<td>15.68 ± 3.24</td>
<td>3</td>
</tr>
<tr>
<td>58d</td>
<td>0.034 ± 0.007</td>
<td>3</td>
</tr>
<tr>
<td>Quinine</td>
<td>2.81 ± 0.57</td>
<td>3</td>
</tr>
</tbody>
</table>

The most active compound from the second generation of compounds was 58d with an IC$_{50}$ of 0.030 µM against the drug resistant strain (Figure 32). This is comparable with the activity of our hit dihydrotriazine 30 (IC$_{50}$ ~50 nM). Once again, although we were pleased with this result, it is not clear why this is the only active compound in this series. In particular, the observed antimalarial activity of 58b is significantly poorer than that observed for 58d. Further compounds will have to be prepared and molecular modelling done in order to understand this observation. Further biological evaluation of these compounds will also be carried out in order to determine whether 58d is a DHFR inhibitor or whether it has a different mode of action against the parasite.
Figure 32: Pyrimidine analogue that displayed better antimalarial activity from the second generation of compounds bearing a flexible side chain.

2.6 Planned approach towards pyrimidine analogues that are structurally similar to P65.

In another aspect of this project, we wanted to assess the effect of moving the flexible side chain on the pyrimidine ring from C-5 to C-6. We realised this could be readily achieved by simple substitution of a 4-hydroxypyrimidine. This would afford a series of compounds with an oxygenated side chain, much like that present in WR99210 and P65. P65, or 6-methyl-5-(3-(2,4,5-trichlorophenoxy)propoxy)pyrimidine-2,4-diamine, was discovered as an analogue of WR99210 by Yuthavong and co-workers in 2012.94 P65 was found to have good oral bioavailability in rats compared to WR99210. However, P65 has its flexible side chain at C-5 of the pyrimidine ring. We decided to prepare a series of pyrimidines of general structure 40 bearing a flexible side chain at the 6-position of the pyrimidine ring, in order to assess the effect of this change on the biological activity of the compounds.
The scheme shown below outlines our planned synthesis of these products. Starting from commercially available substituted phenols, alkylation could be achieved using 1,3-dibromopropane or 1,4-dibromobutane under basic conditions by the procedure described previously (2.1.1). The bromoethers would then react with 2,4-diaminopyrimidin-6-ol using an appropriate base to give the desired products 40. As the 2-and 4-amino groups are relatively non-nucleophilic, substitution is likely to occur only at the 6-hydroxy group of 42.

![Diagram](image)

**Scheme 35:** Reagents and conditions: i) For n = 1, 1,3-dibromopropane, K$_2$CO$_3$, CH$_3$CN, reflux; for n = 2, 1,4-dibromobutane, reflux; ii) K$_2$CO$_3$, CH$_3$CN, reflux.

Bromoethers 33a-f, prepared previously for the synthesis of pyrimidines 37, were used in this synthetic sequence to prepare analogues bearing a 6-atom linker at the 6-position of the pyrimidine ring.

### 2.6.1 Synthesis of 6-(4-(4-chlorophenoxy)butoxy)pyrimidine-2,4-diamine and analogues.

![Diagram](image)

**Scheme 36:** Reagents and conditions: K$_2$CO$_3$, CH$_3$CN, reflux, 20 h, 50%.
The final step of the synthesis of this series of flexible pyrimidines was tested with 4-chlorobromoether 33a. Hydroxypyrimidine 42 was therefore dissolved in dry acetonitrile and potassium carbonate was added to the reaction flask. To this mixture, bromoether 33a dissolved in acetonitrile was added. The reaction mixture was heated to reflux overnight under a nitrogen atmosphere. The reaction mixture changed from colourless to white during the course of the reaction. TLC analysis indicated that a new major product had formed along with another faint spot. Following filtration and workup, the crude product was purified by silica gel column chromatography to furnish the product 40a as a white solid in a mediocre yield of 50%.

The $^1$H NMR spectrum of the product contained two broad signals integrating for two protons each at 5.99 ppm and 5.82 ppm due to the two amino groups. A multiplet at 4.15 – 4.11 ppm and a triplet at 3.98 ppm were assigned to H10 (-OCH$_2$-) and H7 (-OCH$_2$-). Finally, a definitive signal that confirmed the success of the reaction was the singlet visible at 5.04 ppm due to H5. In the aromatic region, two doublets at 7.31 ppm and 6.95 ppm were assigned to H3’ and H2’, respectively. The $^{13}$C NMR spectrum was also convincing, with signals at 67.54 ppm, 64.13 ppm and 25.32 ppm assigned to the CH$_2$ groups of the alkyl chain. The pyrimidine carbons appeared in the upfield region at 170.1 ppm, 165.9 ppm, 162.9 ppm and 76.10 ppm (C-5). The IR spectrum showed stretching bands at 3520 and 3359 cm$^{-1}$, which is an indication of the N-H groups, while the C-O stretching band appeared at 1124 cm$^{-1}$. The molecular ion was confirmed by HRMS to be [M+H]$^+$ 309.1117 which was consistent with a molecular mass of 309.1120.

Five other analogues 40b-40f were prepared in a similar manner (Scheme 37). Structures of the synthesized flexible pyrimidines 40b-40f were confirmed by means of $^1$H NMR and $^{13}$C NMR spectroscopy and high resolution mass spectrometry (HRMS). Key $^1$H NMR spectroscopic signals for each of these products are given in Table 9 below.
33b; Y = 3,4-diCl
33c: = 3,5-diCl
33d: = 4-F
33e: = 3-F
33f: = 3-CF₃

40b; Y = 3,4-diCl, 45%
40c: = 3,5-diCl, 55%
40d: = 4-F, 33%
40e: = 3-F, 40%
40f: = 3-CF₃, 46%

**Scheme 37:** Reagents and conditions: K₂CO₃, CH₃CN, reflux, 20 h.

**Table 9:** Key signals in the ¹H NMR spectra of pyrimidines 40b-40f.

<table>
<thead>
<tr>
<th>Compound</th>
<th>2 × (NH₂), ppm</th>
<th>-OCH₂ (H7), ppm</th>
<th>-OCH₂ (H10), ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound 40b</td>
<td>5.99, 5.82</td>
<td>4.15 – 4.11 (m)</td>
<td>4.05 – 4.01 (m)</td>
</tr>
<tr>
<td>Compound 40c</td>
<td>5.98, 5.82</td>
<td>4.15 – 4.10 (m)</td>
<td>4.06 – 4.00 (m)</td>
</tr>
<tr>
<td>Compound 40d</td>
<td>5.99, 5.81</td>
<td>4.13 (t)</td>
<td>3.98 – 3.94 (m)</td>
</tr>
<tr>
<td>Compound 40e</td>
<td>6.02, 5.86</td>
<td>4.15 (t)</td>
<td>4.01 (t)</td>
</tr>
<tr>
<td>Compound 40f</td>
<td>6.01, 5.86</td>
<td>4.16 (t)</td>
<td>4.09 (t)</td>
</tr>
</tbody>
</table>

We also prepared a series of pyrimidines bearing a 5-atom linker between the pyrimidine ring and the phenyl ring (general structure 59), starting from substituted phenols 32a-32h.
2.6.2 Synthesis of 1-(3-bromopropoxy)-4-chlorobenzene and analogues.

Scheme 38: Reagents and conditions: K$_2$CO$_3$, CH$_3$CN, reflux, 20 h, 99%.

The synthetic route towards flexible pyrimidines bearing three carbon atoms between the oxygen atoms was again tested using 4-chlorophenol as the substrate. In the first synthetic step (Scheme 38), readily available 1,3-dibromopropane was reacted with 4-chlorophenol at 110 °C under basic conditions. As before, the reaction was conducted under dilute conditions with an excess of the dibromoalkane so as to prevent the formation of dimers. The bromoether product was formed as a single spot on TLC, indicating complete consumption of the 4-chlorophenol. Excess 1,3-dibromopropane was distilled off under high vacuum to furnish the alkylated product 60a as a white solid in 99% yield.

Five signals were observed in the $^1$H NMR spectrum of 60a. Two doublets in the aromatic region were assigned to H2 and H3. The aliphatic region contained three signals; two triplets at 4.02 ppm and 3.46 ppm each integrating for two protons and a multiplet at 2.19 – 1.95 ppm integrating for two protons, characteristic of the methylene protons of the alkyl chain. The two triplets were due to the -OCH$_2$- and -CH$_2$Br methylene protons respectively. The multiplet integrating for two protons was due to H5. The $^{13}$C NMR spectrum of the product showed seven signals in total, as expected. Four signals were visible in the aromatic region and three in the aliphatic region, the latter owing to carbons of the alkyl chain.

Bromoethers 60b-60d were prepared in a similar manner (Scheme 39). Trends in the $^1$H NMR and $^{13}$C NMR spectra of the products were observed, as expected. Key signals in the $^1$H NMR spectrum of each product is highlighted in Table 10 below.
Scheme 39: Reagents and conditions: K₂CO₃, CH₃CN, reflux, 20 h.

Table 10: Key signals in the $^1$H NMR spectra of bromoethers 60b-60d.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Aromatic region (ppm)</th>
<th>-OCH₂- (ppm)</th>
<th>H-2’ (ppm)</th>
<th>CH₃Br (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound 60b</td>
<td>6.97 (dd, H2)</td>
<td>4.00 (t)</td>
<td>2.18 – 1.99 (m)</td>
<td>3.45 (t)</td>
</tr>
<tr>
<td>Compound 60c</td>
<td>6.91 (t, H1), 6.75 (d, H3)</td>
<td>3.92 (t)</td>
<td>1.90 – 1.77 (m)</td>
<td>3.45 (t)</td>
</tr>
<tr>
<td>Compound 60d</td>
<td>7.08 – 6.94 (m, H2), 6.85 – 6.81 (m, H3)</td>
<td>4.02 (t)</td>
<td>1.90 – 1.76 (m)</td>
<td>3.45(t)</td>
</tr>
</tbody>
</table>

Purified bromoethers 60a-60d were then used in the subsequent step to prepare analogues bearing a 5-atom linker at the 6-position of the pyrimidine ring.

2.6.3 Synthesis of 6-(3-(4-chlorophenoxy)propoxy)pyrimidine-2,4-diamine and analogues.

Scheme 40: Reagents and conditions: K₂CO₃, CH₃CN, reflux, 20 h, 35%.

The final step of the synthesis of this series flexible pyrimidines was tested with 4-chlorobromoether 60a. The procedure of 2.6.1 was followed to prepare our flexible pyrimidines 59a-d.

The $^1$H NMR spectrum of the product 59a contained two broad signals integrating for two protons each at 6.00 ppm and 5.85 ppm due to the two amino groups. Two triplets at
4.22 ppm and 4.05 ppm were assigned to H7 (-OCH$_2$-) and H9 (-OCH$_2$-). Finally, the singlet visible at 5.06 ppm due to H5 confirmed the success of the reaction. In the aromatic region, two doublets at 7.31 ppm and 6.96 ppm were assigned to H3’ and H2’, respectively. The $^{13}$C NMR spectrum further supported formation of the desired product, with signals at 64.77 ppm, 61.36 ppm and 28.48 ppm assigned to the CH$_2$ groups of the alkyl chain. The pyrimidine carbons appeared in the upfield region at 170.0 ppm, 165.9 ppm 163.0 ppm and 76.12 ppm (C-5). The IR spectrum showed stretching bands at 3520 and 3359 cm$^{-1}$, which is an indication of the N-H groups while the C-O stretching band appeared at 1125 cm$^{-1}$. The molecular ion was confirmed by HRMS to be [M+H] 295.0956 which was consistent with a molecular mass of 295.0964, (calculated for C$_{13}$H$_{16}$ClN$_4$O$_2$).

Three other analogues 59b-59d were prepared in a similar manner to that described for 59a (Scheme 41). The synthesized flexible pyrimidines 59b-59d were characterised by means of $^1$H NMR and $^{13}$C NMR spectroscopy and HRMS. Diagnostic signals in the $^1$H NMR spectra of pyrimidines 59b-d are listed in Table 11 below.

![Scheme 41: Reagents and conditions: K$_2$CO$_3$, CH$_3$CN, reflux, 20 h.](image)

**Table 11:** Key signals in the $^1$H NMR spectra of pyrimidines 59b-59d.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$2 \times (\text{NH}_2)$ (ppm)</th>
<th>-OCH$_2$ (H7), (ppm)</th>
<th>-OCH$_2$ (H9) (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound 59b</td>
<td>5.99, 5.82</td>
<td>4.22 (t)</td>
<td>4.12 (t)</td>
</tr>
<tr>
<td>Compound 59c</td>
<td>6.00, 5.84</td>
<td>4.21(t)</td>
<td>4.12 (t)</td>
</tr>
<tr>
<td>Compound 59d</td>
<td>5.99, 5.85</td>
<td>4.22 (t)</td>
<td>4.12 – 3.95 (m)</td>
</tr>
</tbody>
</table>
2.7 Biological evaluation of pyrimidine analogues structurally similar to P65

All the pyrimidine compounds synthesised were tested for antimalarial activity in an in vitro P. falciparum screen on a Gambian FCR-3 strain (chloroquine and cycloguanil resistant strain) by Prof R van Zyl at the Wits School of Pharmacy and Pharmacology, as described before. Dihydroartemisinin (DHA), methotrexate and quinine were used as the positive controls. As before, at least three independent experiments were conducted for each sample. The results of the biological screening are shown in Table 12.

Table 12: Results of in vitro antimalarial assay for compounds 40a-f and 59a-d.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC_{50} (µM)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>40a</td>
<td>44.88 ± 7.76</td>
<td>3</td>
</tr>
<tr>
<td>40b</td>
<td>11.10</td>
<td>3</td>
</tr>
<tr>
<td>40c</td>
<td>7.66</td>
<td>1</td>
</tr>
<tr>
<td>40d</td>
<td>54.74 ± 10.91</td>
<td>3</td>
</tr>
<tr>
<td>40e</td>
<td>11.18</td>
<td>3</td>
</tr>
<tr>
<td>40f</td>
<td>18.02 ± 2.80</td>
<td>3</td>
</tr>
<tr>
<td>59a</td>
<td>83.45 ± 8.36</td>
<td>3</td>
</tr>
<tr>
<td>59b</td>
<td>4.46 ± 0.29</td>
<td>3</td>
</tr>
<tr>
<td>59c</td>
<td>22.80</td>
<td>1</td>
</tr>
<tr>
<td>59d</td>
<td>14.96</td>
<td>1</td>
</tr>
<tr>
<td>Quinine</td>
<td>2.81 ± 0.57</td>
<td></td>
</tr>
<tr>
<td>DHA</td>
<td>0.00614 ± 0.00111</td>
<td></td>
</tr>
<tr>
<td>Methotrexate</td>
<td>0.23 ± 0.18</td>
<td></td>
</tr>
</tbody>
</table>
The most active compound from the series tested was 59b, with an IC$_{50}$ of 4.46 µM against the drug resistant *P. falciparum* strain (Figure 33). Therefore, this series of compounds did not prove to be as active as the original hit dihydrotriazine compound. Moving the side chain to C-6 did not improve the activity of these compounds.

*Figure 33:* Most active compound from the series.
CHAPTER 3: CONCLUSION AND FUTURE WORK

3.1 Conclusions

In conclusion, we have successfully prepared a series of flexible pyrimidine analogues of our potent dihydrotriazine compound. The synthesized compounds were analysed by means of $^1$H NMR and $^{13}$C NMR spectroscopy and high resolution mass spectroscopy (HRMS). The first generation of compounds bearing a phenyl substituent at C-6 of the pyrimidine ring, were synthesised in five steps. The bromoether compounds 33a-h were prepared by alkylation of commercially available substituted phenols, which then underwent a functional group interconversion to afford the corresponding nitriles 34-h in an ethanoic solution. The nitrile in each case was treated with ethyl benzoate under basic conditions to furnish the $\alpha$-cyano ketones 35a-h in good yields of 63-94%. The $\alpha$-cyano ketone compounds were reacted with the alkylating agent, diazomethane, to form the enol ether products 36a-h in quantitative yields. The final step of the synthesis was a ring closure to form the pyrimidine ring, which involved the reaction of the enol ether intermediates with guanidine hydrochloride in dimethyl sulfoxide (DMSO). This gave the flexible pyrimidine analogues 37a-c, e in yields ranging from 9-11% (Scheme 42). We were unable to prepare the desired pyrimidines 37d, f, g, and h from the corresponding enol ethers.

Antimalarial activity of the pyrimidine compounds prepared was evaluated in an in vitro P. falciparum screen on a Gambian FCR-3 strain (chloroquine and cycloguanil resistant strain). The pyrimidine 37a was found to display antimalarial activity in the low micromolar range ($IC_{50}$ 3.69 ± 0.43 µM). Unfortunately, this was found to be significantly less potent than our original dihydrotriazine, ($IC_{50}$ ~50 nM). The drop in activity could be due to the fact that our pyrimidine compound now also contains a rigid biaryl axis at C-6, when compared with the non-aromatic dihydrotriazine. Surprisingly, pyrimidine 37c was the most potent compound of this series; displaying an $IC_{50}$ of 90 nM. Further evaluation of this compound will be carried out in order to better understand this result.

Based on these biological results, we embarked on the synthesis of analogues containing a smaller or more flexible non-aromatic substituent at the 6-position of the pyrimidine ring. The methodology used to prepare pyrimidines 37 was attempted in preparing these compounds, but unfortunately this was not successful.
We then made use of a multicomponent coupling reaction for the synthesis of the flexible pyrimidines bearing a non-aromatic side chain. Starting from commercially available malononitrile, an aldehyde and guanidine hydrochloride, 5-cyanopyrimidines 48a-c was formed in average yields of 41-55%. The nitrile group of 48a-c was converted to an aldehyde by a Pd/C catalysed hydrogenation in aqueous sulfuric acid to afford 49a-c, as shown in Scheme 42. The corresponding alcohols 50a-c were successfully obtained by reduction of the carbonyl group using sodium borohydride. Unfortunately, the nucleophilic displacement of an alkyl halide by the benzylic alcohols 50a-c did not furnish the desired flexible pyrimidine analogues 47. This lack of reactivity could be attributed to the intramolecular hydrogen bonding that may exist between the C4-NH2 and the OH groups, forming a very stable six-
membered ring. Attempts to disrupt this by N-acetylation were also not successful. Attempts were also made to convert the OH group into a bromide, followed by displacement with a suitably substituted alcohol, but this was also not successful. Therefore, the synthesis of our flexible pyrimidines 47 using this route had to be abandoned.

At this point, we focused our attention on functionalisation of the aldehyde group of 49a-c to synthesise pyrimidines with a flexible linker via reductive amination. This methodology was initially tested using benzylamine and aldehyde 49a and proved to be successful. We then reacted aldehydes 49a-c with a number of substituted phenethylene amines in ethanol and glacial acetic acid to synthesise flexible pyrimidines 58a-d in average yields of 30-33% (Scheme 44).

Scheme 43
All the pyrimidine compounds prepared were also assessed for antimalarial activity in the *in vitro* *P. falciparum* screen on the Gambian FCR-3 strain. Gratifyingly, the flexible pyrimidine 58d exhibited antimalarial activity in the nanomolar range (IC\textsubscript{50} = 30nM). However, this was the only compound of this series to show such potent activity. In particular, we anticipated that compound 58b would also have similar antimalarial properties, but this was not the case. (IC\textsubscript{50} = 18.77 µM). This might be due to the fact that it (58b) does not fit well into the active site of the enzyme. Further investigations will be carried out.

Finally, a series of pyrimidines bearing a flexible side chain at the 6-position of the pyrimidine ring were prepared in two steps (Scheme 45).
Once again, all synthesised pyrimidine compounds were assessed for antimalarial activity in the *in vitro* *P. falciparum* screen on the Gambian FCR-3 strain. The pyrimidine 59b was found to display antimalarial activity in the low micromolar range (IC₅₀ 4.46 ± 0.29 µM). This biological result suggests that moving the side chain to the 6-position reduces the antimalarial activity of these compounds.

### 3.2 Future work

In future, we would like to use the methodology developed in this project to modify the structural features associated with compounds prepared in the first and second generation of study. The synthesis of more flexible pyrimidines bearing a non-aromatic side chain forms part of the future work. Compounds 37a, 37b and 58d will be assessed further for cytotoxicity and their mode of action will also be evaluated. The use of molecular modelling will also be worthwhile, in order to see how these compounds fit into the active site of the DHFR enzyme.
We also plan to assess the effect of halogenations of the pyrimidine ring on biological activity, by preparing 2-amino-4-chloropyrimidines, as shown in Figure 34. This 4-amino group usually binds close to amino acid residues in the active site that are frequently mutated. We anticipate that this variation may reduce possible development of resistance.
CHAPTER 4: EXPERIMENTAL SECTION

4.1 General Procedures

All reagents were purchased from Sigma-Aldrich (South Africa) or Merck KGaA (South Africa) and were used as received. All solvents for reactions were of analytical grade quality purchased from Sigma-Aldrich (South Africa), Merck KGaA (South Africa) or Minema Chemicals (South Africa). Solvents for column chromatography (ethyl acetate and hexanes) and acetone for cleaning were purchased from Protea Chemicals (South Africa) and were distilled before use to remove non-volatiles.

Column chromatographic purification was done on Macherey-Nagel silica gel 60 (particle size 0.063 mm to 0.200 mm). Thin Layer Chromatographic analysis was done on Merck Aluminium foil backed plates coated with silica gel 60, F254.

Melting points were recorded on a Stuart SMP10 apparatus and are uncorrected.

1H Nuclear Magnetic Resonance data were acquired on a Bruker 300 or 500 MHz spectrometer at room temperature, using the specified deuterated solvent. For those compounds soluble in deuterated chloroform (CDCl3), the solvent contained tetramethylsilane (TMS, 0.05% v/v) as internal standard. For others, the residual solvent signal was used for referencing. 13C Nuclear Magnetic Resonance data were acquired on the same instrument. Data processing was done using MestreNova Software under license from Mestrelab Research, CA, USA.

The following abbreviations are used to designate the multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, dd = doublet of doublets.

Infra-red spectra were recorded on a Bruker Tensor-27 Fourier Transform spectrometer.

Mass Spectra (High Resolution) were recorded on a SYNAPT G2 HDMS mass spectrometer (ESI) at Stellenbosch University.

Acetonitrile was distilled from calcium hydride and; tetrahydrofuran (THF) was distilled from sodium/ benzophenone both under a nitrogen atmosphere. N,N-Dimethylformamide
(DMF) was distilled from calcium hydride and stored over molecular sieves. Dichloromethane, chloroform, ethanol and methanol were purchased and used as is.

4.2 General procedure for the synthesis of 1-(4-bromobutoxy)-4-chlorobenzene and analogues

\[
\begin{align*}
\text{Y} & \quad \text{OH} \\
& + \quad \text{Br} \quad \text{+n} \quad \text{Br} \\
& \quad \text{n} = 1.2 \\
\end{align*}
\]

\[\text{Y} = 4-\text{Cl} \]
\[= 3,4 \text{ diCl} \]
\[= 3,5 \text{ diCl} \]
\[= 4-\text{F} \]
\[= 3-\text{F} \]
\[= 2,3-\text{diF} \]
\[= 2,4-\text{diBr} \]
\[= 3-\text{CF}_3 \]

To a solution of substituted phenol in dry acetonitrile was added 1,4-dibromobutane (3 eq) or 1,3-dibromopropane (3 eq) and potassium carbonate (1.5 eq). The resultant mixture was heated in an oil bath at 90-100 °C overnight under nitrogen atmosphere. TLC analysis showed consumption of the starting material. The reaction mixture was allowed to cool to room temperature and filtered through celite. The filtrate was concentrated on a rotary evaporator and excess 1,4-dibromobutane or 1,3-dibromopropane was recovered by distillation under high vacuum to give the following products:

4.2.1 Synthesis of 1-(4-bromobutoxy)-4-chlorobenzene \(^{102}\) 33a

4-Chlorophenol (5.00 g, 38.9 mmol) was dissolved in dry acetonitrile (250 ml). To the clear solution, was added 1,4-dibromobutane (13.9 ml, 117 mmol) and potassium carbonate (8.06 g, 58.3 mmol). The pure product was isolated as a white crystalline solid (10.0 g, 98%).
$R_f$ (30% EtOAc/hexane) 0.84. mp: 29°C. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 7.21 (2H, d, $J = 8.9$ Hz, H3), 6.74 (2H, d, $J = 8.9$ Hz, H2), 3.93 (2H, t, $J = 6.0$ Hz, OCH$_2$), 3.46 (2H, t, $J = 6.5$ Hz, CH$_2$Br), 2.63 − 1.59 (4H, m, H2' and H3'). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 157.3 (C-1), 129.3 (C-3), 125.6 (C-4), 115.7 (C-2), 67.0 (OCH$_2$), 33.3 (CH$_2$Br), 29.3 (C-3'), 27.8 (C-2').

$R_f$ (20% EtOAc/hexane) 0.73. mp: 25°C. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 7.50 (1H, d, $J = 8.9$ Hz, H5), 7.22 (1H, d, $J = 2.9$ Hz, H2), 6.95 (1H, dd, $J = 8.9$, 2.9 Hz, H6), 3.92 (2H, t, $J = 5.9$ Hz, OCH$_2$), 3.45 (2H, t, $J = 6.4$ Hz, CH$_2$Br), 2.11 − 1.80 (4H, m, H2' and H3'). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 157.1 (C-1), 134.3 (C-3), 131.4 (C-5), 124.7 (C-4), 116.3 (C2 and C-6), 67.60 (OCH$_2$), 27.98 (CH$_2$Br), 22.53 (C-3'), 17.01 (C-2').

IR ($v_{\text{max}/\text{cm}^{-1}}$): 2958 (=C-H); 1593, 1578 (C=C); 1104 (C-O); 823 (C-Cl).

4.2.2 Synthesis of 1-(4-bromobutoxy)-3,4-dichlorobenzene$^{102}$ 33b

3,4-Dichlorophenol (5.05 g, 31.0 mmol) was dissolved in acetonitrile (250 ml). To the clear solution, was added 1,4-dibromobutane (11.1 ml, 92.9 mmol) and potassium carbonate (6.42 g, 46.5 mmol). The pure product was isolated as a white solid (8.50 g, 92%).

$R_f$ (20% EtOAc/hexane) 0.73. mp: 25°C. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 7.50 (1H, d, $J = 8.9$ Hz, H5), 7.22 (1H, d, $J = 2.9$ Hz, H2), 6.95 (1H, dd, $J = 8.9$, 2.9 Hz, H6), 3.92 (2H, t, $J = 5.9$ Hz, OCH$_2$), 3.45 (2H, t, $J = 6.4$ Hz, CH$_2$Br), 2.11 − 1.80 (4H, m, H2' and H3'). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 157.1 (C-1), 134.3 (C-3), 131.4 (C-5), 124.7 (C-4), 116.3 (C2 and C-6), 67.60 (OCH$_2$), 27.98 (CH$_2$Br), 22.53 (C-3'), 17.01 (C-2'). IR ($v_{\text{max}/\text{cm}^{-1}}$): 3041 (=C-H); 1578 (C=C); 1145 (C-O).

4.2.3 Synthesis of 1-(4-bromobutoxy)-3,5-dichlorobenzene$^{102}$ 33c

3,5-Dichlorophenol (5.00 g, 30.7 mmol) was dissolved in acetonitrile (250 ml). To the clear solution, was added 1,4-dibromobutane (10.9 ml, 92.0 mmol) and potassium carbonate (6.36 g, 46.0 mmol). The pure product was isolated as a white crystalline solid (8.02 g, 88%).

$R_f$ (30% EtOAc/hexane) 0.85. mp: 26°C. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 6.91 (1H, t, $J = 1.8$ Hz, H4), 6.75 (2H, d, $J = 1.9$ Hz, H2 and H6), 3.92 (2H, t, $J = 5.9$ Hz, OCH$_2$), 3.45 (2H, t, $J = 6.4$ Hz, CH$_2$Br), 2.11 − 1.80 (4H, m, H2' and H3'). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 159.7 (C-1), 135.5 (C-3), 121.3 (C-4), 114.0 (C-2), 67.32 (OCH$_2$), 27.98 (CH$_2$Br), 22.33 (C-3'), 17.00 (C-2'). IR ($v_{\text{max}/\text{cm}^{-1}}$): 2953 (=C-H); 1581, 1576 (C=C); 1141 (C-O).
4.2.4 Synthesis of 1-(4-bromobutoxy)-4-fluorobenzene 33d

\[
\begin{align*}
\text{F} & \quad \text{4} & \quad \text{3} & \quad \text{2} & \quad \text{1}\nonumber \\
\text{Br} & \quad \text{2'} & \quad \text{3'} & \quad \text{4'} & \quad \text{1'}
\end{align*}
\]

4-Fluorophenol (5.02 g, 44.8 mmol) was dissolved in acetonitrile (250 ml). To the brownish solution, was added 1,4-dibromobutane (16.0 ml, 134 mmol) and potassium carbonate (9.28 g, 67.2 mmol). The product was isolated as a white solid (6.92 g, 63%).

\[ R_f (30\% \text{ EtOAc/hexane}) 0.82. \text{mp:} 32^\circ \text{C.} \]

\[ ^1\text{H NMR (300 MHz, CDCl}_3\]): \delta 7.01 – 6.92 (2H, m, H3), 6.85 – 6.79 (2H, m, H2), 3.96 (2H, t, \text{J} = 5.6 \text{ Hz, OCH}_2), 3.44 (2H, t, \text{J} = 6.4 \text{ Hz, CH}_2\text{Br}), 2.11 – 1.88 (4H, m, H2' and H3'). \]

\[ ^{13}\text{C NMR (75 MHz, CDCl}_3\)): \delta 157.3 \text{ (d, } J_{\text{C-F}} = 238.3 \text{ Hz, C-4), 154.9 \text{ (d, } J_{\text{C-F}} = 2.0 \text{ Hz, C-1), 115.8 \text{ (d, } J_{\text{C-F}} = 23.1 \text{ Hz, C-3), 115.4 \text{ (d, } J_{\text{C-F}} = 8.0 \text{ Hz, C-2), 67.41 \text{ (OCH}_2\text{), 33.38 \text{ (CH}_2\text{Br), 29.41 \text{ (C-3'), 27.88 \text{ (C-2')} \text{. IR (v}_\text{max/cm}^{-1}) 2930 \text{ (=C-H); 1599, 1578 \text{ (C=C); 1133 \text{ (C-O). HRMS m/z: calculated for C}_{10}\text{H}_{12}\text{BrFONa: 268.9956, found: [M + Na]}^+ 268.9955.} \]

4.2.5 Synthesis of 1-(4-bromobutoxy)-3-fluorobenzene 33e

\[
\begin{align*}
\text{F} & \quad \text{6} & \quad \text{5} & \quad \text{3} & \quad \text{2}\nonumber \\
\text{Br} & \quad \text{2'} & \quad \text{3'} & \quad \text{4'} & \quad \text{1'}
\end{align*}
\]

3-Fluorophenol (5.00 g, 44.6 mmol) was dissolved in acetonitrile (250 ml). To the brown solution, was added 1,4-dibromobutane (15.9 ml, 134 mmol) and potassium carbonate (9.25 g, 66.9 mmol). The pure product was isolated as a brown oil (9.51 g, 86%).

\[ R_f (20\% \text{ EtOAc/hexane}) 0.74. \text{H NMR (300 MHz, CDCl}_3\): \delta 7.22 – 7.15 (1H, m, H4), 6.66 – 6.55 (3H, m, H2, H5 and H6), 3.95 (2H, t, \text{J} = 5.6 \text{ Hz, OCH}_2), 3.45 (2H, t, \text{J} = 6.4 \text{ Hz, CH}_2\text{Br), 1.99 – 1.67 (4H, m, H2' and H3').} \]

\[ ^{13}\text{C NMR (75 MHz, CDCl}_3\)): \delta 163.6 \text{ (d, } J_{\text{C-F}} = 245.3 \text{ Hz, C-3), 159.9 \text{ (d, } J_{\text{C-F}} = 10.8 \text{ Hz, C-1), 130.3 \text{ (d, } J_{\text{C-F}} = 10.0 \text{ Hz, C-5), 110.2 \text{ (d, } J_{\text{C-F}} = 2.9 \text{ Hz, C-6), 107.8 \text{ (d, } J_{\text{C-F}} = 21.3 \text{ Hz, C-4), 102.2 \text{ (d, } J_{\text{C-F}} = 24.8 \text{ Hz, C-2), 67.70 \text{ (OCH}_2\text{), 27.91 \text{ (CH}_2\text{Br), 22.52 \text{ (C-3'), 17.00 \text{ (C-2')} \text{. IR (v}_\text{max/cm}^{-1}) 2956 \text{ (=C-H); 1593, 1577 \text{ (C=C); 1084 \text{ (C-O).} \]
4.2.6 Synthesis of 1-(4-bromobutoxy)-3-(trifluoromethyl)benzene$^{104}$ 33f

![Chemical Structure](attachment:image)

3-(Trifluoromethyl)phenol (7.00 g, 43.2 mmol) was dissolved in acetonitrile (250 ml). To the yellow solution, was added 1,4-dibromobutane (15.5 ml, 126 mmol) and potassium carbonate (8.95 g, 64.8 mmol). The product was isolated as a pale-yellow oil (11.5 g, 89%).

$R_f$(30% EtOAc/hexane) 0.85. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 7.45 – 7.31 (1H, m, H4); 7.21 – 7.00 (3H, m, H2, H5 and H6); 4.01 (2H, t, $J = 6.0$ Hz, OCH$_2$); 3.48 (2H, t, $J = 6.6$ Hz, CH$_2$Br); 2.16 – 1.87 (4H, m, H2' and H3'). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 159.1 (C-1), 131.9 (q, $J_{C-F} = 32.2$ Hz, C-CF$_3$), 130.0 (C-5), 124.1 (q, $J_{C-F} = 272.4$ Hz, CF$_3$), 118.0 (C-6), 117.4 (q, $J_{C-F} = 3.8$ Hz, C-4), 111.3 (q, $J_{C-F} = 3.7$ Hz, C-2), 67.19 (OCH$_2$), 53.29 (CH$_2$Br), 29.42 (C-3').

4.2.7 Synthesis of 2,4-dibromo-1-(4-bromobutoxy)benzene 33g

![Chemical Structure](attachment:image)

2,4-Dibromophenol (5.00 g, 19.8 mmol) was dissolved in acetonitrile (260 ml). To the brownish solution, was added 1,4-dibromobutane (7.11 ml, 59.5 mmol) and potassium carbonate (4.12 g, 30.0 mmol). The product was isolated as a light-brown solid (6.13 g, 80%).

$R_f$(30% EtOAc/hexane) 0.81. mp: 30°C. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 7.66 (1H, d, $J = 2.4$ Hz, H3), 7.36 (1H, dd, $J = 8.8, 2.4$ Hz, H5), 6.74 (1H, d, $J = 8.8$ Hz, H6), 4.04 (2H, t, $J = 5.4$ Hz, OCH$_2$), 3.46 (2H, t, $J = 6.5$ Hz, CH$_2$Br), 2.09 – 1.80 (4H, m, H2' and H3'). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 154.4 (C-1), 136.0 (C-3), 131.3 (C-5), 114.2 (C-6), 113.2 (C-2), 113.1 (C-4), 68.28 (OCH$_2$), 27.91 (CH$_2$Br), 22.50 (C-3'), 17.06 (C-2') : IR ($\nu_{\text{max}}$/cm$^{-1}$): 3042 (=C-H); 1583 (C=C); 1095 (C-O). HRMS m/z: calculated for C$_{10}$H$_{11}$Br$_3$ONa: 406.8260, found: [M + Na]$^+$ 406.8266.
4.2.8 Synthesis of 1-(4-bromobutoxy)-2,3-difluorobenzene\textsuperscript{104} 33h

\[
\begin{array}{c}
\text{F} \\
\text{Br} \\
\end{array}
\]

2,3-Difluorophenol (5.00 g, 38.4 mmol) was dissolved in acetonitrile (250 ml). To the brown solution, was added 1,4-dibromobutane (13.8 ml, 115 mmol) and potassium carbonate (7.97 g, 57.7 mmol). The product was isolated as an off-white solid (9.29 g, 84\%).

\( R_f (20\% \text{ EtOAc/hexane}) 0.70. \) \textit{mp:} 28°C. 1\textsuperscript{H} NMR (300 MHz, CDCl\textsubscript{3}): δ 6.97 – 6.92 (1H, m, H5), 6.80 – 7.70 (2H, m, H4 and H6), 4.07 (2H, t, J = 5.9 Hz, OCH\textsubscript{2}), 3.50 (2H, t, J = 6.4 Hz, CH\textsubscript{2}Br), 2.15 – 1.91 (4H, m, H2’ and H3’). 13\textsuperscript{C} NMR (75 MHz, CDCl\textsubscript{3}): δ 151.5 (dd, J\textsubscript{C-F} = 246.8, 10.4 Hz, C-3), 148.6 (dd, J\textsubscript{C-F} = 7.9, 3.2 Hz, C-1), 141.5 (dd, J\textsubscript{C-F} = 247.3, 14.1 Hz, C-2), 123.2 (dd, J\textsubscript{C-F} = 8.7, 5.2 Hz, C-5), 109.9 (d, J\textsubscript{C-F} = 2.9 Hz, C-6), 109.2 (dd, J\textsubscript{C-F} = 17.7, 9.5 Hz, C-4), 68.77 (OCH\textsubscript{2}), 33.34 (CH\textsubscript{2}Br), 29.27 (C-3’), 27.82 (C-2’); IR (\( \nu_{\text{max}}/\text{cm}^{-1} \)): 2955 (=C-H); 1571 (C=C); 1113 (C-O).

4.2.9 Synthesis of 1-(3-bromopropoxy)-4-chlorobenzene\textsuperscript{102} 60a

\[
\begin{array}{c}
\text{Cl} \\
\text{Br} \\
\end{array}
\]

4-Chlorophenol (6.50 g, 50.6 mmol) was dissolved in acetonitrile (300 ml). To the clear solution, was added 1,3-dibromopropene (15.4 ml, 152 mmol) and potassium carbonate (10.5 g, 75.8 mmol). The pure product was isolated as a white solid (12.5 g, 99\%).

\( R_f (30\% \text{ EtOAc/hexane}) 0.83. \) \textit{mp:} 28°C. 1\textsuperscript{H} NMR (300 MHz, CDCl\textsubscript{3}): δ 7.23 (2H, d, J = 8.9 Hz, H3), 6.81 (2H, d, J = 8.9 Hz, H2), 4.02 (2H, t, J = 5.7 Hz, OCH\textsubscript{2}), 3.46 (2H, t, J = 6.5 Hz, CH\textsubscript{2}Br), 2.19 – 1.95 (2H, m, H2’). 13\textsuperscript{C} NMR (75 MHz, CDCl\textsubscript{3}): δ 157.3 (C-1), 129.3 (C-3), 125.6 (C-1), 115.7 (C-2), 67.2 (OCH\textsubscript{2}), 33.3 (CH\textsubscript{2}Br), 27.8 (C-2’). IR (\( \nu_{\text{max}}/\text{cm}^{-1} \)): 2958 (=C-H); 1593, 1578 (C=C); 1104 (C-O).
4.2.10 Synthesis of 4-(3-bromopropoxy)-1,2-dichlorobenzene\textsuperscript{102} 60b

\[
\begin{align*}
\text{Cl} & \quad 6 \quad 5 \quad O \quad 2' \quad \text{Br} \\
1 & \quad 2 & \quad 3 & \quad 4 & \quad 1' & \quad 3'
\end{align*}
\]

3,4-Dichlorophenol (5.40 g, 33.1 mmol) was dissolved in acetonitrile (250 ml). To the clear solution, was added 1,3-dibromopropane (10.1 ml, 99.4 mmol) and potassium carbonate (6.87 g, 49.7 mmol). The product was isolated as a white solid after distillation (8.96 g, 95%).

\(R_f\) (30\% EtOAc/hexane) 0.84. \(mp\): 29°C. \(^1\)H NMR (300 MHz, CDCl\textsubscript{3}): \(\delta\) 7.31 (1H, d, \(J = 8.9\) Hz, H6), 6.97 (1H, d, \(J = 2.9\) Hz, H3), 6.73 (1H, dd, \(J = 8.9, 2.9\) Hz, H5), 4.00 (2H, t, \(J = 5.8\) Hz, OCH\textsubscript{2}), 3.45 (2H, t, \(J = 6.4\) Hz, CH\textsubscript{2}Br), 2.18 - 1.99 (2H, m, H2'). \(^{13}\)C NMR (75 MHz, CDCl\textsubscript{3}): \(\delta\) 157.4 (C-4), 132.7 (C-2), 130.7 (C-6), 124.2 (C-1), 116.4 (C-3), 114.5 (C-5), 65.99 (OCH\textsubscript{2}), 25.19 (CH\textsubscript{2}Br), 14.08 (C-2'). IR (\(v_{\text{max}}/\text{cm}^{-1}\)): 3041 (=C-H); 1578 (C=C); 1145 (C-O).

4.2.11 Synthesis of 1-(3-bromopropoxy)-3,5-dichlorobenzene\textsuperscript{102} 60c

\[
\begin{align*}
\text{Cl} & \quad 5 \quad 6 \quad O \quad 2' \quad \text{Br} \\
4 & \quad 3 & \quad 2 & \quad 1 & \quad \text{Cl}
\end{align*}
\]

3,5-Dichlorophenol (5.00 g, 30.7 mmol) was dissolved in acetonitrile (200 ml). To the clear solution, was added 1,3-dibromopropane (9.34 ml, 92.0 mmol) and potassium carbonate (6.36 g, 46.0 mmol). The product was isolated as a white solid (8.46 g, 97%).

\(R_f\) (30\% EtOAc/hexane) 0.83. \(mp\): 27°C. \(^1\)H NMR (300 MHz, CDCl\textsubscript{3}): \(\delta\) 6.91 (1H, t, \(J = 1.8\) Hz, H4), 6.75 (2H, d, \(J = 1.9\) Hz, H3), 3.92 (2H, t, \(J = 5.9\) Hz, OCH\textsubscript{2}), 3.45 (2H, t, \(J = 6.4\) Hz, CH\textsubscript{2}Br), 1.90 - 1.77 (2H, m, H2'); \(^{13}\)C NMR (75 MHz, CDCl\textsubscript{3}): \(\delta\) 159.7 (C-1), 135.5 (C-3 and C-5), 121.3 (C-4), 114.0 (C-2 and C-6), 67.32 (OCH\textsubscript{2}), 27.98 (CH\textsubscript{2}Br), 22.33 (C-2'). IR (\(v_{\text{max}}/\text{cm}^{-1}\)): 2953 (=C-H); 1581, 1576 (C=C); 1141 (C-O).
4.2.12 Synthesis of 1-(3-bromopropoxy)-4-fluorobenzene\textsuperscript{103} 60d

4-Fluorophenol (4.00 g, 35.7 mmol) was dissolved in acetonitrile (200 ml). To the brownish solution, was added 1,3-dibromopropane (10.9 ml, 107 mmol) and potassium carbonate (7.40 g, 53.5 mmol). The pure product was isolated as a light brown solid (7.26 g, 87%).

\( R_f \) (20% EtOAc/hexane) 0.69, \( mp: \) 30°C. \( ^1H \) NMR (300 MHz, CDCl\textsubscript{3}): \( \delta \) 7.08 – 6.94 (2H, m, H3), 6.85 – 6.81 (2H, m, H2), 4.02 (2H, t, \( J = 5.6 \) Hz, OCH\textsubscript{2}), 3.45 (2H, t, \( J = 6.4 \) Hz, CH\textsubscript{2}Br), 2.15 – 2.07 (2H, m, H2’). \( ^13C \) NMR (75 MHz, CDCl\textsubscript{3}): \( \delta \) 157.3 (d, \( J_{C-F} = 238.3 \) Hz, C-4), 154.9 (d, \( J_{C-F} = 2.0 \) Hz, C-1), 115.8 (d, \( J_{C-F} = 23.1 \) Hz, C-3), 115.4 (d, \( J_{C-F} = 8.0 \) Hz, C-2), 66.00 (OCH\textsubscript{2}), 30.78 (CH\textsubscript{2}Br), 14.15 (C-2’): IR (\( \nu \text{max/cm}^{-1} \)): 2956 (=C-H); 1593, 1577 (C=C); 1084 (C-O).

4.3 General procedure for the synthesis of 5-(4-chlorophenoxy)pentanenitrile and analogues.

Compounds 33a-h were dissolved separately in an ethanol/water solution (3:1) and treated with potassium cyanide (1.1 eq.). The resulting heterogeneous mixture in each case was stirred and heated at reflux under a nitrogen atmosphere for two days. The reaction progress was monitored by TLC (40% EtOAc/hexane). When complete, each reaction mixture was
cooled to room temperature and quenched with an aqueous NaOH solution (0.1 M, 100 ml). Each mixture was concentrated on a rotary evaporator and the residue was extracted with DCM (3 × 100 ml). The combined organic layers for each reaction were dried with MgSO₄, filtered through celite and excess solvent was removed on a rotary evaporator. Each of the crude products was purified by silica gel column chromatography (10% EtOAc/hexane as eluent). The following products were prepared using this methodology:

### 4.3.1 Synthesis of 5-(4-chlorophenoxy)pentanenitrile 34a

![Chemical structure](image)

1-(4-Bromobutoxy)-4-chlorobenzene (4.00 g, 15.2 mmol) was dissolved in ethanol/water (160 ml). To the clear solution was added potassium cyanide (1.10 g, 16.7 mmol). The product was isolated as pale yellow oil after purification by column chromatography (10% EtOAc/hexane). Yield (2.85 g, 89%).

$R_f$ (30% EtOAc/hexane) 0.69. $^1H$ NMR (300 MHz, CDCl₃): δ 7.21 (2H, d, $J = 9.0$ Hz, H3), 6.79 (2H, d, $J = 9.0$ Hz, H2), 3.94 (2H, t, $J = 5.6$ Hz, OCH₂), 2.41 (2H, t, $J = 6.8$ Hz, CH₂CN), 2.63 – 1.59 (4H, m, H3’ and H4’). $^{13}$C NMR (75 MHz, CDCl₃): δ 157.3 (C-1), 129.3 (C-3), 125.6 (C-4), 119.5 (CN), 115.7 (C-2), 66.96 (OCH₂), 28.09 (C-4’), 22.35 (C-3’), 16.92 (CH₂CN): IR ($v_{max}$/cm⁻¹): 3084, 2965 (=C-H); 2252 (CN); 1585, 1570 (C=C). HRMS m/z: calculated for C₁₁H₁₂ClNO: 232.0507, found: [M + Na]+ 232.0500

### 4.3.2 Synthesis of 5-(3,4-dichlorophenoxy)pentanenitrile 34b

![Chemical structure](image)

1-(4-Bromobutoxy)-3,4-dichlorobenzene (5.05 g, 16.9 mmol) was dissolved in ethanol/water (160 ml), and treated with potassium cyanide (1.21 g, 18.6 mmol). The product was isolated as pale yellow oil after purification by column chromatography. (3.55 g, 86%).

$R_f$ (30% EtOAc/hexane) 0.66. $^1H$ NMR (300 MHz, CDCl₃): δ 7.50 (1H, d, $J = 8.9$ Hz, H5), 7.22 (1H, d, $J = 2.9$ Hz, H2), 6.95 (1H, dd, $J = 8.9, 2.9$ Hz, H6), 3.92 (2H, t, $J = 5.9$ Hz,
OCH₂), 1.85 (2H, t, J = 6.4 Hz, CH₂CN), 1.80 – 1.76 (4H, m, H4’ and H3’).

**13C NMR (75 MHz, CDCl₃)**: δ 157.1 (C-1), 134.3 (C-3), 131.4 (C-5), 124.7 (C-4), 119.1 (CN), 116.3 (C-2 and C-6), 67.6 (OCH₂), 28.71 (C-4’), 21.2 (C-3’), 17.4 (CH₂CN): IR (v max/cm⁻¹): 3083, 2964 (=C-H); 1599, 1574 (C=C).

**HRMS m/z**: calculated for C₁₁H₁₁Cl₂NO Na: 266.0118, found: [M + Na]⁺ 266.0115.

### 4.3.3 Synthesis of 5-(3,5-dichlorophenoxy)pentanenitrile 34c

![Structure of 5-(3,5-dichlorophenoxy)pentanenitrile](image)

1-(4-Bromobutoxy)-3,5-dichlorobenzene (4.20 g, 14.1 mmol) was dissolved in ethanol/water (160 ml). To the ethanoic solution, was added potassium cyanide (1.01 g, 15.5 mmol). The product was isolated as a white solid after purification by column chromatography using 20% EtOAc/hexane as eluent. Yield (2.45 g, 71%).

Rᵣ (30% EtOAc/hexane) 0.68. mp: 52-53°C. **1H NMR (300 MHz, CDCl₃)**: δ 6.96 (1H, t, J = 1.8 Hz, H4), 6.78 (2H, d, J = 1.8 Hz, H2 and H6), 3.98 (2H, t, J = 5.6 Hz, OCH₂), 2.45 (2H, t, J = 6.8 Hz, CH₂CN), 2.05 – 1.64 (4H, m, H3’ and H4’). **13C NMR (75 MHz, CDCl₃)**: δ 159.7 (C-1), 135.5 (C-3), 121.3 (C-4), 119.3 (CN), 113.6 (C-2), 67.32 (OCH₂), 27.98 (C-4’); 22.33 (C-3’), 17.00 (CH₂CN): IR (v max/cm⁻¹): 3083, 2966 (=C-H); 2250 (CN); 1585, 1570 (C=C). **HRMS m/z**: calculated for C₁₁H₁₁Cl₂NO Na: 266.0118, found: [M + Na]⁺ 266.0115.

### 4.3.4 Synthesis of 5-(4-fluorophenoxy)pentanenitrile 34d

![Structure of 5-(4-fluorophenoxy)pentanenitrile](image)

1-(4-Bromobutoxy)-4-fluorobenzene (4.00 g, 16.2 mmol) was dissolved in ethanol/water (160 ml), and treated with potassium cyanide (1.20 g, 17.8 mmol). The product was isolated as a white solid after purification by column chromatography (20% EtOAc/hexane) (1.93 g, 62%).
4.3.5 Synthesis of 5-(3-fluorophenoxy)pentanenitrile 34e

1-(4-Bromobutoxy)-3-fluorobenzene (5.00 g, 20.2 mmol) was dissolved in ethanol/water (160 ml). To the ethanoic solution, was added potassium cyanide (1.58 g, 24.3 mmol). The product was isolated as brown oil after purification by column chromatography using 20% EtOAc/hexane as eluent. (3.22 g, 72%).

*R* (20% EtOAc/hexane) 0.44. **H NMR (300 MHz, CDCl₃):** δ 7.26-7.16 (1H, m, H₄), 6.72-6.48 (3H, m, H₂, H₅ and H₆), 3.95 (2H, t, *J* = 5.6 Hz, OCH₂), 2.41 (2H, t, *J* = 6.8 Hz, CH₂CN), 1.99-1.67 (4H, m, H₄' and H₃'). **C NMR (75 MHz, CDCl₃):** δ 163.60 (d, *J*ₐₕ = 245.3 Hz, C-3), 159.87 (d, *J*ₐₕ = 10.8 Hz, C-1), 130.30 (d, *J*ₐₕ = 10.0 Hz, C-5), 119.3 (CN), 110.15 (d, *J*ₐₕ = 2.9 Hz, C-6), 107.80 (d, *J*ₐₕ = 21.3 Hz, C-4), 102.17 (d, *J*ₐₕ = 24.8 Hz, C-2), 67.7 (OCH₂), 28.4 (C-4'); 22.24 (C-3'), 17.00 (CH₂CN): **IR (υ<sub>max</sub>/cm⁻¹):** 2961, 2932 (=C-H); 2239 (CN); 1596, 1503 (C=C). **HRMS m/z:** calculated for C₁₁H₁₂FNONa: 216.0803, found: [M + Na]<sup>+</sup> 216.0807.
4.3.6 Synthesis of 5-(3-(trifluoromethyl)phenoxy)pentanenitrile 34f

\[
\begin{array}{c}
\text{CF}_3 \\
\text{O} \\
\text{5} \\
\text{4} \\
\text{1} \\
\text{2} \\
\text{3} \\
\text{6} \\
\text{2'} \\
\text{3'} \\
\text{4'} \\
\text{5'} \\
\text{CN}
\end{array}
\]

1-(4-Bromobutoxy)-3-(trifluoromethyl)benzene (6.00 g, 20.2 mmol) was dissolved in ethanol/water (160 ml), and treated with potassium cyanide (1.45 g, 22.2 mmol). The product was isolated as yellow oil after purification by column chromatography using 20% EtOAc/hexane as eluent. (3.65 g, 74%).

\[\text{R}_f(30\% \text{ EtOAc/hexane) 0.65.} \]

\[\text{H NMR (300 MHz, CDCl}_3\text{)}: \delta 7.40 - 7.35 (1H, m, H2); 7.23 - 7.01 (2H, m, H4, H5 and H6); 4.01 (2H, t, J = 5.7 Hz, OCH}_2\text{}); 2.42 (2H, t, J = 6.8 Hz, CH}_2\text{CN}); 2.01 - 1.72 (4H, m, H4' and H3'). \]

\[\text{13C NMR (75 MHz, CDCl}_3\text{)}: \delta 159.1 (C-1), 131.9 (q, J_C-F = 32.2 Hz, C-CF}_3\), 129.8 (C-5), 124.5 (q, J_C-F = 272.4 Hz, CF}_3\), 119.2 (CN), 118.0 (C-6), 117.4 (q, J_C-F = 3.8 Hz, C-4), 111.4 (q, J_C-F = 3.7 Hz, C-2), 67.75 (OCH}_2\), 28.33 (C-4'), 22.77 (C-3'), 17.01 (CH}_2\text{CN}); \text{IR (v max/cm}^{-1}\text{): 3039, 2930 (C-H); 2239 (CN); 1599 (C=C).} \]

\[\text{HRMS m/z: calculated for C}_{12}H_{12}F_3NONa: 266.0771, found: [M + Na]^+ 266.0773} \]

4.3.7 Synthesis of 5-(2,4-dibromophenoxy)pentanenitrile 34g

\[
\begin{array}{c}
\text{Br} \\
\text{5} \\
\text{4} \\
\text{3} \\
\text{6} \\
\text{2'} \\
\text{3'} \\
\text{4'} \\
\text{5'} \\
\text{CN}
\end{array}
\]

2,4-Dibromo-1-(4-bromobutoxy)benzene (5.00 g, 12.9 mmol) was dissolved in ethanol/water (160 ml). To the ethanoic solution, was added potassium cyanide (1.01 g, 15.5 mmol, 1.2 eq). The product was isolated as white solid after purification by column chromatography using 20% EtOAc/hexane as eluent. (3.12 g, 73%).

\[\text{R}_f(30\% \text{ EtOAc/hexane) 0.67. mp: 49-50°C.} \]

\[\text{H NMR (300 MHz, CDCl}_3\text{)}: \delta 7.66 (1H, d, J = 2.4 Hz, H3), 7.36 (1H, dd, J = 8.8, 2.4 Hz, H5), 6.74 (1H, d, J = 8.8 Hz, H6), 4.04 (2H, t, J = 5.4 Hz, OCH}_2\text{}); 2.50 (2H, t, J = 6.7 Hz, CH}_2\text{CN}); 2.09 - 1.80 (4H, m, H3' and H4'). \]

\[\text{13C NMR (75 MHz, CDCl}_3\text{)}: \delta 154.4 (C-1), 135.6 (C-5), 131.3 (C-3), 119.5 (CN), 114.2 (C-6), 113.2 (C-2), 113.1 (C-4), 68.28 (OCH}_2\), 27.91 (C-4'); 22.50 (C-3'), 17.06 (CH}_2\text{CN); IR} \]
(ν<sub>max/cm</sub>-1): 3091, 2948 (=C-H); 2253 (CN); 1580, 1480 (C=C). **HRMS m/z**: calculated for C<sub>11</sub>H<sub>10</sub>Br<sub>2</sub>N: 353.9107, found: [M + Na]<sup>+</sup> 353.9108.

**4.3.8  Synthesis of 5-(2,3-difluorophenoxy)pentanenitrile 34h**

![](image)

1-(4-Bromobutoxy)-2,3-difluorobenzene (7.05 g, 26.6 mmol) was dissolved in ethanol/water (160 ml). To the clear solution, was added potassium cyanide (1.91 g, 29.3 mmol). The product was isolated as an off-white solid after purification by column chromatography using 20% EtOAc/hexane as eluent. Yield (4.83 g, 86%).

*RF* (30% EtOAc/hexane) 0.67. *mp*: 48°C. **<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):** δ 7.04- 6.92 (1H, m, H5), 6.82 – 6.67 (2H, m, H4 and H6), 4.07 (2H, t, J = 5.7 Hz, OCH<sub>2</sub>), 2.46 (2H, t, J = 6.8 Hz, CH<sub>2</sub>CN), 2.08 – 1.75 (4H, m, H3’ and H4’). **<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>):** δ 151.46 (dd, J<sub>CF</sub> = 246.8, 10.4 Hz, C-3), 148.57 (dd, J<sub>CF</sub> = 7.9, 3.2 Hz, C-1), 141.48 (dd, J<sub>CF</sub> = 247.3, 14.1 Hz, C-2), 123.17 (dd, J<sub>CF</sub> = 8.7, 5.2 Hz, C-5), 119.3 (CN), 109.85 (d, J<sub>CF</sub> = 2.9 Hz, C-6), 109.20 (dd, J<sub>CF</sub> = 17.7, 9.5 Hz, C-4), 68.8 (OCH<sub>2</sub>), 28.4 (C-4’); 21.6 (C-3’), 17.4 (CH<sub>2</sub>CN). **IR (ν<sub>max/cm</sub>-1):** 2961, 2932 (=C-H); 2239 (CN); 1596, 1503 (C=C). **HRMS m/z**: calculated for C<sub>11</sub>H<sub>10</sub>F<sub>2</sub>N: 234.0709, found: [M + Na]<sup>+</sup> 234.0706.
4.4 General synthesis of 2-benzoyl-5-(4-chlorophenoxy)pentanenitrile and analogues.

![Chemical Structure](image)

a: Y = 4-Cl  
b = 3,4 diCl  
c = 3,5 diCl  
d = 4-F  
e = 3-F  
f = 2,3-diF  
g = 2,4-diBr  
h = 3-CF₃

To a solution of each of compounds 34a-h (1 eq) dissolved separately in dry THF was added potassium tert-butoxide (3 eq.) and ethyl benzoate (4 eq.) Each reaction mixture was stirred at room temperature under a nitrogen atmosphere overnight. The reaction progress was monitored by TLC (30% EtOAc/hexane). After consumption of the starting material in each case, the reaction was quenched with a saturated aqueous ammonium chloride (NH₄Cl) solution and the THF was removed on a rotary evaporator. The remaining aqueous residue of each reaction was washed three times with ethyl acetate (100 ml) and the organic layers were combined, dried over MgSO₄ and filtered through celite. Each of the crude products was purified by column chromatography (20% EtOAc/hexane) to furnish the following products, 35a-h.

4.4.1 Synthesis of 2-benzoyl-5-(4-chlorophenoxy)pentanenitrile 35a

![Chemical Structure](image)

Following the same procedure as above, compound 34a (2.32 g, 11.1 mmol) was dissolved in dry THF (50 ml). To the clear solution was added potassium tert-butoxide (3.72 g, 33.2 mmol) followed by ethyl benzoate (6.33 ml, 44.3 mmol). The product was isolated as yellow
crystals after purification by column chromatography (10% EtOAc/hexane) and recrystallization from ethyl acetate/hexanes. (2.82 g, 81%)

$R_f$ (30% EtOAc/hexane) 0.54, $mp$: 124-126°C. 1H NMR (300 MHz, CDCl$_3$): $\delta$ 8.05 – 7.82 (2H, m, H7’), 7.75 – 7.56 (1H, m, H9’), 7.52 (2H, dd, $J = 8.3, 6.9$ Hz, H8’), 7.22 (2H, d, $J = 9.0$ Hz, H3), 6.77 (2H, d, $J = 9.0$ Hz, H2), 4.48 (1H, dd, $J = 8.4, 5.7$ Hz, CHCN), 4.04 -3.99 (2H, m, OCH$_2$), 2.41 – 1.85 (4H, m, H3’ and H4’).

1H NMR (300 MHz, CDCl$_3$): $\delta$ 8.05 – 7.82 (2H, m, H7’), 7.75 – 7.56 (1H, m, H9’), 7.52 (2H, dd, $J = 8.3, 6.9$ Hz, H8’), 7.22 (2H, d, $J = 9.0$ Hz, H3), 6.77 (2H, d, $J = 9.0$ Hz, H2), 4.48 (1H, dd, $J = 8.4, 5.7$ Hz, CHCN), 4.04 -3.99 (2H, m, OCH$_2$), 2.41 – 1.85 (4H, m, H3’ and H4’).

13C NMR (75 MHz, CDCl$_3$): $\delta$ 190.4 (C=O), 157.1 (C-1), 134.6 (C-9’), 133.9 (C-6’), 129.4 (C-3), 129.2 (C-7’), 128.8 (C-8’), 125.9 (C-4), 117.1 (CN), 115.7 (C-2), 66.96 (OCH$_2$), 39.37 (CHCN), 26.72 (C-3’), 26.51 (C-4’): IR ($v_{max}/cm^{-1}$): 2878 (=$C$-H); 2253 (CN); 1694 (C=O); 1596, 1580 (C=C). HRMS m/z: calculated for C$_{18}$H$_{16}$ClNO$_2$Na: 336.0770, found: [M + Na]$^+$ 336.0757.

4.4.2 Synthesis of 2-benzoyl-5-(3,4-dichlorophenoxy)pentanenitrile 35b

Following the same procedure as above, compound 34b (2.50 g, 10.2 mmol) was dissolved in dry THF (60 ml). To the yellow solution was added potassium tert-butoxide (3.45 g, 30.7 mmol) followed by ethyl benzoate (5.89 ml, 40.9 mmol). The product was isolated as a white solid after purification by column chromatography (10% EtOAc/hexane). (3.34 g, 94%).

$R_f$ (30% EtOAc/hexane) 0.51. $mp$: 43°C. 1H NMR (300 MHz, CDCl$_3$): $\delta$ 8.02 – 7.87 (2H, m, H7’), 7.74 – 7.60 (1H, m, H6); 7.57 – 7.39 (2H, m, H8’), 7.38 – 7.21 (1H, m, H9’); 6.92 (1H, d, $J = 2.9$ Hz, H2); 6.69 (1H, ddd, $J = 8.9, 2.9, 0.6$ Hz, H5); 4.46 (1H, dd, $J = 8.1$ Hz, 5.8 Hz, CHCN); 4.06 – 3.87 (2H, m, OCH$_2$); 2.38 – 1.90 (4H, m, H3’ and H4’). 13C NMR (75 MHz, CDCl$_3$): $\delta$ 179.4 (C=O), 157.6 (C-1), 133.9 (C-6’), 132.8 (C-3), 131.2 (C-5), 130.0 (C-9’), 129.2 (C-7’), 128.8 (C-8’), 125.9 (C-4), 119.4 (CN), 116.5 (C-2), 114.3 (C-6), 67.20 (OCH$_2$), 30.43 (CHCN), 27.97 (C-3’), 24.10 (C-4’): IR ($v_{max}/cm^{-1}$): 3041, 2954 (=$C$-H); 2252 (CN); 1695 (C=O); 1586, 1574 (C=C). HRMS m/z: calculated for C$_{18}$H$_{16}$ClNO$_2$Na: 370.0380, found: [M + Na]$^+$ 370.0377.
4.4.3 Synthesis of 2-benzoyl-5-(3,5-dichlorophenoxy)pentanenitrile 35c

Following the same procedure as above, compound 34c (1.98 g, 8.11 mmol) was dissolved in dry THF (40 ml). To the yellow solution was added potassium tert-butoxide (2.73 g, 24.3 mmol) followed by ethyl benzoate (4.64 ml, 32.4 mmol). The product was isolated as a light yellow solid after purification by column chromatography (10% EtOAc/hexane). (2.80 g, 91%).

\[ R_f(20\% \, \text{EtOAc/hexane}) \, 0.40. \, mp: \, 45^\circ \text{C}. \]

\[ ^1H \, \text{NMR (300 MHz, CDCl}_3\): \delta 7.97 \, (2H, d, J = 7.1 \, \text{Hz, H7}'), 7.74 – 7.61 \, (1H, m, H9'), 7.53 \, (2H, dd, J = 8.3, 7.0 \, \text{Hz, H8}'), 6.95 \, (1H, t, J = 1.8 \, \text{Hz, H4}), 6.72 \, (2H, d, J = 1.8 \, \text{Hz, H2}), 4.46 \, (1H, dd, J = 8.1, 5.9 \, \text{Hz, CHCN}), 4.06 – 3.88 \, (2H, m, OCH}_2)\].

\[ ^13C \, \text{NMR (75 MHz, CDCl}_3\): \delta 190.2 \, (C=O), 159.5 \, (C-1), 135.5 \, (C-3 and C-5), 134.7 \, (C-9'), 133.9 \, (C-6'), 129.2 \, (C-7'), 128.8 \, (C-8'), 121.3 \, (C-4), 117.0 \, (CN), 113.5 \, (C-2 and C-6), 67.27 \, (OCH}_2), 39.25 \, (CHCN), 26.49 \, (C-3'), 26.37 \, (C-4'): \]

\[ \text{IR (v_{max}/cm}^{-1})\, : \, 2889 \, (=C-H); 2255 \, (CN); 1697 \, (C=O); 1582, 1570 \, (C=C). \]

HRMS m/z: calculated for C\textsubscript{18}H\textsubscript{15}Cl\textsubscript{2}NO\textsubscript{2}Na: 370.0380, found: [M + Na]\textsuperscript{+} 370.0381.

4.4.4 Synthesis of 2-benzoyl-5-(4-fluorophenoxy)pentanenitrile 35d

Following the same procedure as above, compound 34d (1.55 g, 8.02 mmol) was dissolved in dry THF (45 ml). After complete dissolution, potassium tert-butoxide (2.70 g, 24.1 mmol) was added to the mixture followed by ethyl benzoate (4.60 ml, 32.4 mmol). The product was isolated as a yellow solid after purification by column chromatography (10% EtOAc/hexane). (1.99 g, 83%).
$R_f$ (20% EtOAc/hexane) 0.49. mp: 86°C. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 8.04 – 7.90 (2H, m, H7’), 7.72 – 7.57 (1H, m, H9’), 7.51 (2H, dd, $J = 8.4$, 7.1 Hz, H8’), 7.03 – 6.90 (2H, m, H3), 6.87 – 6.70 (2H, m, H2), 4.50 (1H, dd, $J = 8.5$, 5.7 Hz, CHCN), 4.08 – 3.93 (2H, m, OCH$_2$), 2.34 – 1.78 (4H, m, H3’ and H4’). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 190.4 (C=O), 157.4 (d, J$_{C-F}$ = 238.7 Hz, C-4), 154.6 (d, J$_{C-F}$ = 2.1 Hz, C-1), 154.6 (C-9’), 133.9 (C-6’), 129.1 (C-7’), 128.8 (C-8’), 117.2 (CN), 115.8 (d, J$_{C-F}$ = 23.1 Hz, C-3), 115.4 (d, J$_{C-F}$ = 8.0 Hz, C-2), 67.27 (OCH$_2$), 39.41 (CHCN), 26.80 (C-3’), 26.59 (C-4’): IR ($\nu_{max}$/cm$^{-1}$): 2956, 2930 (=C-H); 2252 (CN); 1703 (C=O); 1586, 1577 (C=C). HRMS m/z: calculated for C$_{18}$H$_{16}$FNO$_2$Na: 320.1065, found: [M + Na]$^+$ 320.1100.

4.4.5 Synthesis of 2-benzoyl-5-(3-fluorophenoxy)pentanenitrile 35e

Following the same procedure as above, compound 34e (4.88 g, 25.3 mmol) was dissolved in dry THF (70 ml). After complete dissolution, potassium tert-butoxide (8.50 g, 75.8 mmol) was added followed by ethyl benzoate (14.4 ml, 101 mmol). The product was isolated as a yellow solid after purification by column chromatography (10% EtOAc/hexane). (6.53 g, 87%).

$R_f$ (30% EtOAc/hexane) 0.49. mp: 32°C. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 7.96 (2H, d, $J = 7.1$ Hz, H7’), 7.70 – 7.59 (1H, m, H9’), 7.51 (2H, dd, $J = 8.4$, 7.1 Hz, H8’), 7.28 – 7.12 (1H, m, H5), 6.73 – 6.46 (3H, m, H2, H4 and H6), 4.50 (1H, dd, $J = 8.4$, 5.7 Hz, CHCN), 4.04 – 3.99 (2H, m, OCH$_2$), 2.36 – 1.99 (4H, m, H3’ and H4’). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 190.5 (C=O), 163.6 (d, $J = 245.3$ Hz, C-3), 159.9 (d, $J = 10.8$ Hz, C-1), 134.6 (C-9’), 133.9 (C-6’), 130.3 (d, $J = 10.0$ Hz, C-5), 129.1 (C-7’), 128.8 (C-8’), 117.2 (CN), 110.2 (d, $J = 2.9$ Hz, C-6), 107.8 (d, $J = 21.3$ Hz, C-4), 102.2 (d, $J = 24.8$ Hz, C-2), 66.89 (OCH$_2$), 39.40 (CHCN), 26.73 (C-3’), 26.44 (C-4’): IR ($\nu_{max}$/cm$^{-1}$): 2876 (=C-H); 2254 (CN); 1689 (C=O); 1596, 1576 (C=C). HRMS m/z: calculated for C$_{18}$H$_{16}$FNO$_2$Na: 320.1065, found: [M + Na]$^+$ 320.1063.
4.4.6 Synthesis of 2-benzoyl-5-(2,3-difluorophenoxy)pentanenitrile 35f

Following the same procedure as above, compound 34h (1.88 g, 8.90 mmol) was dissolved in dry THF (50 ml). After complete dissolution, potassium tert-butoxide (3.00 g, 26.7 mmol) was added followed by ethyl benzoate (5.10 ml, 35.6 mmol). The α-cyano ketone product was isolated as a yellow solid after purification by column chromatography (10% EtOAc/hexane). (2.27 g, 81%).

$R_f$ (30% EtOAc/hexane) 0.48. mp: 51-52°C. $^1$H NMR (300 MHz, CDCl$_3$): δ 8.00 – 7.95 (2H, m, H7’), 7.66 – 7.47 (3H, m, H8’ and H9’), 7.08 – 6.83 (1H, m, H5), 6.84 – 6.56 (2H, m, H4 and H6), 4.72 – 4.60 (1H, m, CHCN), 4.24 – 3.95 (2H, m, OCH$_2$), 2.57 – 1.71 (4H, m, H3’ and H6’). $^{13}$C NMR (75 MHz, CDCl$_3$): δ 190.5 (C=O), 151.5 (dd, $J_{C-F} = 246.8$, 10.4 Hz, C-3), 148.6 (dd, $J_{C-F} = 7.9$, 3.2 Hz, C-1), 141.5 (dd, $J_{C-F} = 247.3$, 14.1 Hz, C-2), 134.6 (C-9’), 133.9 (C-6’), 129.1 (C-7’), 128.8 (C-8’), 123.2 (dd, $J_{C-F} = 8.7$, 5.2 Hz, C-5), 117.2 (CN), 109.9 (d, $J_{C-F} = 2.9$ Hz, C-6), 109.2 (dd, $J_{C-F} = 17.7$, 9.5 Hz, C-4), 66.89 (OCH$_2$), 39.40 (CHCN), 26.73 (C-3’), 26.44 (C-4’). IR ($v_{max}$/cm$^{-1}$): 3039, 2930 (=C-H); 2248 (CN); 1705 (C=O); 1594, 1580 (C=C).

4.4.7 Synthesis of 2-benzoyl-5-(2,4-dibromophenoxy)pentanenitrile 35g

Following the same procedure as above, compound 34g (2.94 g, 8.83 mmol) was dissolved in dry THF (50 ml). After complete dissolution, potassium tert-butoxide (2.97 g, 26.5 mmol) was added followed by ethyl benzoate (5.05 ml, 35.3 mmol). The product was isolated as a yellow solid after purification by column chromatography (10% EtOAc/hexane). Yield (3.03 g, 79%).
$R_f$ (20\% EtOAc/hexane) 0.41.  $mp$: 35°C. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 7.98 (2H, d, $J = 7.1$ Hz, H7'), 7.64 - 7.54 (2H, m, H3 and H9'), 7.50 (2H, dd, $J = 8.5$, 7.1 Hz, H8'), 7.35 (1H, dd, $J = 8.7$, 2.4 Hz, H5), 6.74 (1H, d, $J = 8.8$ Hz, H6), 4.64 (1H, dd, $J = 8.5$, 5.6 Hz, CHCN), 4.10 – 4.01 (2H, m, OCH$_2$), 2.37 – 1.91 (4H, m, H4' and H3'). $^1$3C NMR (75 MHz, CDCl$_3$): $\delta$ 190.6 (C=O), 154.2 (C-1) 135.5 (C-3), 134.5 (C-9'), 133.9 (C-6'), 131.3 (C-5), 129.1 (C-7'), 128.8 (C-8'), 117.2 (CN), 114.2 (C-6), 113.4 (C-4), 68.35 (OCH$_2$), 39.70 (CHCN), 26.83 (C-3'), 26.25 (C-4'). $\text{IR (}v_{\text{max}}/\text{cm}^{-1}\text{): 3010 (=C-H); 2255 (CN); 1699 (C=O); 1578, 1569 (C=C).}$ $\text{HRMS } m/z$: calculated for C$_{18}$H$_{15}$Br$_2$NO$_2$: 457.9270, found: [M + Na]$^+$ 457.9370.

4.4.8 Synthesis of 2-benzoyl-5-(3-(trifluoromethyl)phenoxy)pentanenitrile 35h

Following the same procedure as above, compound 34f (2.50 g, 10.3 mmol) was dissolved in dry THF (60 ml). To the clear solution was added potassium tert-butoxide (3.46 g, 30.8 mmol) followed by ethyl benzoate (5.88 ml, 41.1 mmol). The product was isolated as a yellow viscous oil after purification by column chromatography (10\% EtOAc/hexane). Yield (2.24 g, 63%).

$R_f$ (20\% EtOAc/hexane) 0.42. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 8.12 – 7.92 (2H, m, H7'), 7.65 – 7.33 (4H, m, H2, H8' and H9'), 7.23 – 6.92 (3H, m, H4, H5 and H6), 4.51 (1H, dd, $J = 8.4$, 5.8 Hz, CHCN), 4.12 – 3.94 (2H, m, OCH$_2$), 2.35 – 1.92 (4H, m, H3' and H4'). $^1$3C NMR (75 MHz, CDCl$_3$): $\delta$ 190.5 (C=O), 158.7 (C-1), 134.7 (C-6'), 133.9 (C-9'), 131.9 (q, $J = 32.3$ Hz, C-CF$_3$),130.1 (C-5), 129.6 (C-7'), 129.2 (C-8'), 123.9 (q, $J = 272.4$ Hz, CF$_3$), 117.9 (C-6), 117.8 (CN), 117.7 (q, $J = 3.9$ Hz, C-4), 113.3 (q, $J = 3.9$ Hz, C-2), 66.94 (OCH$_2$), 39.41 (C-CN), 26.69 (C-3'), 26.47 (C-4'); $\text{IR (}v_{\text{max}}/\text{cm}^{-1}\text{): 2941 (=C-H); 2255 (CN); 1696 (C=O); 1592, 1571 (C=C).}$ $\text{HRMS } m/z$: calculated for C$_{19}$H$_{17}$F$_3$NO$_2$: 348.1213, found: [M + H]$^+$ 348.1227.
4.5 General procedure for the synthesis of E/Z-5-(4-chlorophenoxy)-2-(methoxy(phenyl)methylene)pentanenitrile and similar compounds

Carbitol, diethyl ether and aqueous potassium hydroxide (10.7 M, 20.0 eq) were placed in a diazomethane apparatus fitted with a condenser and a dropping funnel containing diazald (3.0 eq) dissolved in diethyl ether. The diazomethane apparatus was lowered into a water bath at 70-80°C. Diazomethane gas, generated from the reaction of diazald and the above reaction mixture was passed into a flask containing the starting material 35a-g (1.00 eq), in dry dichloromethane (50 ml). Diethyl ether (10 ml) was used to wash the dropping funnel. After distillation was complete, the reaction mixture was left to stir at room temperature overnight. Any excess diazomethane was quenched with glacial acetic acid. The reaction was evaporated to dryness in vacuo to give the crude product, which was taken to the next step without further purification. Partial characterisation of the intermediate enol ethers was done by $^1$H and $^{13}$C NMR spectroscopy.

4.5.1 Synthesis-of-E/Z-5-(4-chlorophenoxy)-2-(methoxy(phenyl)methylene)pentanenitrile 36a and analogues.

Diazomethane gas, produced from the reaction of diazald (2.05 g, 9.56 mmol), potassium hydroxide solution (10.7 M, 6 ml), in a mixture of carbitol (4 ml) and diethyl ether (20 ml)
was passed into a solution of 35a (21.00 g, 3.19 mmol), in dry DCM. This afforded 36a as a viscous oil in quantitative yield. The product was isolated as a mixture of E and Z isomers in a ratio of 1.4:1. Owing to the instability of the enol ether, the product was treated as an intermediate and no further characterisation was done.

\[ ^1H \text{NMR (300 MHz, CDCl}_3\]: Major-isomer: \[ \delta 7.56 - 7.33 \text{ (4H, m, Ar-H), 7.23 - 7.18 (1H, m, Ar-H), 7.23 (2H, d, } J = 9.1 \text{ Hz, H3), 6.84 (2H, d, } J = 9.0 \text{ Hz, H2), 4.00 (2H, t, } J = 6.1 \text{ Hz, OCH}_2\), 3.43 (3H, s, OCH}_3\), 2.57 (2H, dd, } J = 8.2, 6.7 \text{ Hz, H3’), 2.11 - 2.01 (2H, m, H4’); \]

\[ ^13C \text{NMR (75 MHz, CDCl}_3\]: Major-isomer: \[ \delta 168.9 (=C-OCH}_3\), 157.6 (C-1), 131.5 (C-6’), 130.5 (C-3), 129.3 (C-8’), 128.9 (C-7’), 128.8 (C-9’), 125.5 (C-4), 120.1 (CN), 115.8 (C-2), 94.22 (C-2’), 67.09 (OCH}_2\), 58.28 (OCH}_3\), 28.05 (C-4’), 24.97 (C-3’). \]

\[ ^1H \text{NMR (300 MHz, CDCl}_3\]: Minor-isomer: \[ \delta 7.56 - 7.33 \text{ (4H, m, Ar-H), 7.23 - 7.18 (1H, m, Ar-H), 7.18 (2H, d, } J = 9.1 \text{ Hz, H3), 6.66 (2H, d, } J = 8.9 \text{ Hz, H2), 3.85 (2H, t, } J = 5.9 \text{ Hz, OCH}_2\), 3.47 (3H, s, OCH}_3\), 2.23 (2H, dd, } J = 8.1, 6.5 \text{ Hz, H3’), 1.99 - 1.87 (2H, m, H4’); \]

\[ ^13C \text{NMR (75 MHz, CDCl}_3\]: Minor-isomer: \[ \delta 168.7 (=C-OCH}_3\), 157.2 (C-1), 130.7 (C-6’), 130.3 (C-3), 129.2 (C-8’), 128.8 (C-7’), 128.7 (C-9’), 125.5 (C-4), 118.2 (CN), 115.6 (C-2), 91.88 (C-2’), 66.10 (OCH}_2\), 58.06 (OCH}_3\), 27.74 (C-4’), 23.81 (C-3’). \]

Enol ethers 36b-h were prepared in a similar manner and analysed by \(^1H\) and \(^13C\) NMR spectroscopy before being used immediately in the subsequent ring closing reaction.

4.6 General procedure for the synthesis of flexible pyrimidine analogues

\[ Y = 4-\text{Cl} \]
\[ b = 3,4 \text{ diCl} \]
\[ c = 3,5 \text{ diCl} \]
\[ d = 3-\text{F} \]

Sodium metal (2.0 eq) was dissolved in methanol (20 ml) to make sodium methoxide. Guanidine hydrochloride salt was then dissolved in the prepared sodium methoxide to furnish free guanidine after filtration and evaporation of methanol on a rotary evaporator. The enol ether starting material 36a-d (1 eq) was dissolved in dry DMSO and then added to the flask
containing free guanidine. The reaction mixture was then heated at 80-100°C overnight. After formation of a new product spot, visible by TLC, the reaction mixture was heated to 120°C in order to remove DMSO under vacuum. The solid residue was dissolved in a methanol-ethyl acetate mixture and then purified by column chromatography to give the desired pyrimidine product.

4.6.1 Synthesis of 5-(3-(4-chlorophenoxy)propyl)-6-phenylpyrimidine-2,4-diamine 37a

Compound 37a was synthesized from 36a (0.55 g, 1.68 mmol, 1.0eq) and free guanidine (0.32 g, 3.36 mmol, 2.0 eq) in dry DMSO (5.0 ml) and purified by column chromatography (80% EtOAc/hexane) to afford a yellow solid (0.065 g, 11%).

Rf (80% EtOAc/hexane) 0.18. mp: 130-132°C. 1H NMR (300 MHz, CDCl3): δ 7.43 – 7.33 (5H, m, Ar-H), 7.21 (2H, d, J = 8.9 Hz, H3’), 6.74 (2H, d, J = 8.9 Hz, H2’), 5.29 (2H, s, NH2), 5.15 (2H, s, NH2), 3.84 (2H, t, J = 5.6 Hz, OCH2), 2.53 (2H, t, J = 8.6 Hz, H7’), 1.93 – 1.84 (2H, m, H6’). 13C NMR (75 MHz, CDCl3): δ 165.6 (C-6), 163.3 (C-2), 160.4 (C-4), 157.1 (C-1’), 129.7 (C-7), 129.4 (C-3’), 128.4 (C-9), 128.3 (C-10), 128.0 (C-8), 125.9 (C-4’), 115.7 (C-2’), 104.8 (C-5), 67.14 (OCH2), 28.58 (C-6’), 22.24 (C-7’): IR (νmax/cm⁻¹): 3328, 3140 (NH2); 2929 (=C-H); 1637 (C=N); 1583, 1555 (C=C). HRMS m/z: calculated for C19H20ClN4O: 355.1327, found: [M + H]⁺ 355.1326
4.6.2  Synthesis of 5-(3-(3,4-dichlorophenoxy)propyl)-6-phenylpyrimidine-2,4-diamine 37b

![Chemical Structure of 37b]

Compound 37b was synthesized from 36b (0.50 g, 1.38 mmol, 1.0 eq) and free guanidine (0.26 g, 2.76 mmol, 2.0 eq) in dry DMSO (5.0 ml) and purified by column chromatography (80% EtOAc/hexane) to afford a light-yellow solid (0.057 g, 11%).

R$_f$(80% EtOAc/hexane) 0.17. mp: 102-104°C. $^1$H NMR (300 MHz, MeOD): δ 7.50 (1H, d, J = 8.9 Hz, H5'), 7.22 (1H, d, J = 2.9 Hz, H2'), 6.95 (1H, dd, J = 8.9, 2.9 Hz, H6'), 6.23 – 6.00 (5H, m, Ar-H), 2.52 (2H, t, J = 5.8 Hz, OCH$_2$), 1.31 (2H, t, J = 7.4 Hz, H9'), 1.93 – 1.83 (2H, m, H8'); $^{13}$C NMR (75 MHz, MeOD): δ 165.0 (C-6), 160.0 (C-2), 157.7 (C-1' and C-4), 135.4 (C-3'), 135.0 (C-7), 129.1 (C-9), 128.2 (C-10), 127.5 (C-8), 120.2 (C-4'), 113.2 (C-2' and C-6'), 105.3 (C-5), 66.87 (OCH$_2$), 27.17 (C-8'), 21.10 (C-9'); IR ($v_{max}$/cm$^{-1}$): 3430, 3164, (N-H); 2866 (=C-H); 1636 (C=N); 1582, 1544 (C=C); HRMS m/z: calculated for C$_{19}$H$_{19}$Cl$_2$N$_4$O: 389.0938, found: [M + H]$^+$ 389.0936

4.6.3  Synthesis of 5-(3-(3,5-dichlorophenoxy)propyl)-6-phenylpyrimidine-2,4-diamine 37c

![Chemical Structure of 37c]

Compound 37c was synthesized from 36c (0.50 g, 1.68 mmol, 1.0 eq) and free guanidine (0.32 g, 3.36 mmol, 2.0 eq) in dry DMSO (5.0 ml) and purified by column chromatography (80% EtOAc/hexane) to afford a brown solid (0.048 g, 9%). R$_f$(80% EtOAc/hexane) 0.18. mp: 105°C. $^1$H NMR (300 MHz, MeOD): δ 6.23 – 6.00 (5H, m, Ar-H), 5.68 (1H, t, J = 1.8 Hz, H4'), 5.36 (2H, d, J = 1.8 Hz, H2' and H6'), 2.52 (2H, t, J = 5.8 Hz, OCH$_2$), 1.31 (2H, t, J = 7.4 Hz, H9'), 0.60 – 0.55 (2H, m, H8'); $^{13}$C NMR (75 MHz, MeOD): δ 164.7 (C-6), 159.9 (C-2), 157.7 (C-2 and C-1'), 135.5 (C-3'), 130.8 (C-7), 128.3 (C-8), 127.9 (C-9), 127.5
(C-10), 120.2 (C-4’), 113.2 (C-2’ and C-6’), 105.3 (C-5), 66.88 (OCH₃), 27.17 (C-8’), 21.10 (C-9’): IR (v_max/cm⁻¹): 3430, 3164 (N-H); 2956 (=C-H); 1637 (C=N); 1599, 1575 (C=C). HRMS m/z: calculated for C₁₉H₁₉Cl₂N₄O: 389.0938, found: [M + H]⁺ 389.0936

4.6.4 Synthesis of 5-(3-(3-fluorophenoxy)propyl)-6-phenylpyrimidine-2,4-diamine 37e

![Structure of Compound 37e](image)

Compound 37e was synthesized from 37e (0.45 g, 1.45 mmol, 1.0 eq) and free guanidine (0.28 g, 2.89 mmol, 2.0 eq) in dry DMSO (5.0 ml) and purified by column chromatography (80% EtOAc/hexane) to afford a creamy-white solid (0.043 g, 9%).

R_f (80% EtOAc/hexane) 0.14. mp: 104-105°C. ¹H NMR (300 MHz, MeOD): δ 6.23 – 5.82 (5H, m, Ar-H), 5.45 – 5.10 (4H, m, H2’, H4’, H5’ and H6’), 2.53 (2H, t, J = 5.9 Hz, OCH₃), 1.27 (2H, dd, J = 8.6, 6.6 Hz, H9’), 0.67 – 0.53 (2H, m, H8’): ¹³C NMR (75 MHz, MeOD): δ 191.5 (C-6), 188.1 (C-2), 183.3 (C-4 and C-1’), 157.7 (C-4 and C-2’), 145.7 (C-7), 128.1 (C-5’ and C-9), 125.5 (C-8 and C-10), 112.5 (C-6’), 109.9 (C-5), 94.45 (C-4’), 81.20 (C-2’), 56.02 (OCH₃), 27.57 (C-8’), 21.93 (C-9’): IR (v_max/cm⁻¹): 3479, 3112, (N-H); 2875 (=C-H); 1612 (C=N); 1576, 1505 (C=C): HRMS m/z: calculated for C₁₉H₂₀F₄N₄O: 339.1703, found: [M + H]⁺ 339.1621
4.7 Attempted synthesis of second generation of compounds bearing a non-aromatic side chain

To a mixture of 34a (2.04 g, 9.73 mmol, 1 eq) dissolved in dry THF (30 ml) was added potassium tert-butoxide (3.28 g, 29.2 mmol, 3 eq.) and methyl cyclohexanecarboxylate (5.56 ml, 38.9 mmol, 4 eq.). The reaction mixture was stirred at room temperature under a nitrogen atmosphere overnight. The reaction was monitored by TLC, and no new spot for the expected product 44 was detected. In order to determine if the product has the same Rf value as the starting material, the reaction was quenched with a saturated ammonium chloride (NH₄Cl) solution and the THF was removed on a rotary evaporator. The remaining aqueous residue was washed three times with ethyl acetate (100 ml) and the organic layers were combined, dried over MgSO₄ and filtered through celite. The crude product was purified by column chromatography (20% EtOAc/hexane) to give a pale-yellow oil, but this was not the desired product. Spectroscopic analysis showed that the product isolated was actually the starting material 34a. As a result we were unable to prepare analogues with a non-aromatic side chain 46 using this methodology. This was also attempted on 34d without success.
4.8 General procedure for the synthesis of 2,4-diamino-6-cyclopropylpyrimidine-5-carbonitrile and similar compounds using a multi-component coupling approach.

\[
\text{R}H_2O + \text{CN} + \text{NH}_2\text{HCl} \xrightarrow{\text{NaOAc, EtOH/H}_2\text{O}} \text{Reflux} \quad 48
\]

The equimolar mixture of cyclic aldehyde (1.0 eq), malononitrile (1.0 eq) and NaOAc (1.0 eq) in (60 ml) H\(_2\)O/(10 ml) EtOH was stirred mechanically for at least 10 minutes, then guanidine hydrochloride (1.0 eq) was added to the above reaction mixture and the reaction mixture was refluxed for overnight. After completion of the starting material, the reaction mixture was cooled down to room temperature and poured to a separating funnel containing 100 ml ethyl acetate. The aqueous layer was extracted twice with (2 \(\times\) 150 ml) EtOAc. The extracts were dried with MgSO\(_4\), filtered through celite and excess solvent was removed on a rotary evaporator. The crude product was purified by column chromatography (40% EtOAc/hexane). The following products were synthesized by this method:

4.8.1 Synthesis of 2,4-diamino-6-phenylpyrimidine-5-carbonitrile\(^{105}\) 48a

Benzaldehyde (4.63 ml, 45.4 mmol), malononitrile (3.00 g, 45.4 mmol), sodium acetate (3.73 g, 45.4 mmol) and guanidine hydrochloride (4.34 g, 45.4 mmol) afforded, after extraction and column chromatography using 40% EtOAc/hexane as eluent, 48a (4.23 g, 44%) as a yellow solid.

\(R\)\(_f\) (80% EtOAc/hexane) 0.81. \(mp\): 234 °C. \(^1H\) NMR (300 MHz, DMSO-\(d_6\)): \(\delta\) 7.75 – 7.71 (2H, m, H8), 7.57 – 7.40 (3H, m, H9 and H10), 7.13 (4H, br. s, C2NH\(_2\) and C4NH\(_2\)). \(^{13}C\) NMR (75 MHz, DMSO-\(d_6\)): \(\delta\) 169.4 (C-4); 165.0 (C-2); 162.9 (C-6); 137.1 (C-7); 130.2 (C-
10; 128.1 (C-9); 128.1 (C-8); 117.9 (CN); 75.94 (C-5); IR \( (v_{\text{max}}/\text{cm}^{-1}) \): 3374 (N-H); 3146 (=C-H); 2205 (CN); 1609 (Ar-C=C).

### 4.8.2 Synthesis of 2,4-diamino-6-cyclopropylpyrimidine-5-carbonitrile 48b

![Chemical Structure](image)

Reaction of cyclopropanecarbaldehyde (3.39 ml, 45.4 mmol), malononitrile (3.00 g, 45.4 mmol), sodium acetate (3.73 g, 45.4 mmol) and guanidine hydrochloride (4.34 g, 45.4 mmol) as described afforded, after extraction and column chromatography using 40% EtOAc/hexane as eluent, 48b (3.25 g, 41%) as a yellow solid. 

\[ R_f (80\% \text{ EtOAc/hexane}) 0.78. \text{ mp: } 190-191^\circ\text{C}. \]

\[ ^1H \text{ NMR (300 MHz, DMSO-}d_6\): } \delta 6.90 (2H, s, N-H), 6.68 (2H, s, N-H), 2.04 (1H, p, H7), 0.81 – 0.78 (4H, m, H8). \]

\[ ^{13}\text{C NMR (75 MHz, DMSO-}d_6\): } \delta 174.7 (C-6); 163.8 (C-4); 163.1 (C-2); 117.6 (CN); 77.02 (C-5); 14.94 (C-7); 9.51 (C-8). \]

\[ \text{IR (}v_{\text{max}}/\text{cm}^{-1}) : 3498, 3426 (N-H); 2203 (C=N); 1615 (C=C); 1280, 1133 (CH}_2). \]

\[ \text{HRMS m/z: calculated for C}_{8}\text{H}_{10}\text{N}_5: 176.0938, \text{ found: } [M + H]^+ 176.0934. \]

### 4.8.3 Synthesis of 2,4-diamino-6-cyclohexylpyrimidine-5-carbonitrile 48c

![Chemical Structure](image)

Cyclohexanecarbaldehyde (4.60 mL, 37.8 mmol), malononitrile (2.50 g, 37.8 mmol), sodium acetate (3.10 g, 37.8 mmol) and guanidine hydrochloride (3.62 g, 37.8 mmol) afforded, after extraction and column chromatography using 40% EtOAc/hexane as eluent, 48c (3.89 g, 47%) as a yellow solid.

\[ R_f (80\% \text{ EtOAc/hexane}) 0.92. \text{ mp: } 201-202^\circ\text{C}. \]

\[ ^1H \text{ NMR (300 MHz, DMSO-}d_6\): } \delta 6.91 (2H, s, N-H), 6.78 (2H, s, N-H), 2.63 (1H, p, H7), 1.82 – 1.48 (7H, m, cyclohex), 1.21 – 1.35 (3H, m, cyclohex). \]

\[ ^{13}\text{C NMR (75 MHz, DMSO-}d_6\): } \delta 178.3 (C-6); 164.8 (C-4); 163.6 (C-2); 117.7 (CN); 76.59 (C-5); 44.42 (C-7); 30.95 (C-8); 26.14 (C-9); 26.12 (C-10). \]

\[ \text{IR} \]
\(v_{\text{max}}/\text{cm}^{-1}\): 3445, 3390 (N-H); 2203 (CN); 1608 (C=C); 1438, 1270, 1014 (CH\(_2\)). \textbf{HRMS} \\
m/z: \text{calculated for C}_{11}H_{16}N_5: 218.1407, \text{found: [M + H]}^+ 218.1406

4.9 General procedure for the synthesis of 2,4-diamino-6-phenylpyrimidine-5-carbaldehyde and other analogues 48b-c

![Diagram of the reaction](attachment:image.png)

10% Palladium/carbon (0.1 eq) was added to a two-necked, round-bottom flask containing starting material (one of 48a-c, 1.0 eq) dissolved in aqueous sulphuric acid (2.0 M). The reaction flask was evacuated and filled with hydrogen gas. A balloon filled with hydrogen gas, was then fitted to the flask. The reaction mixture was stirred at room temperature for 18 hours under a hydrogen atmosphere. The reaction was monitored by TLC (80% EtOAc/hexane). When complete, the reaction mixture was filtered and neutralised with a 2M aqueous NaOH solution. The aqueous solution was extracted with EtOAc (3 x 100 ml). The organic layers were combined and dried with MgSO\(_4\), filtered through celite and excess solvent removed on a rotary evaporator. The crude product in each case was purified by column chromatography (60% EtOAc/hexane) to furnish the following products:

4.9.1 Synthesis of 2,4-diamino-6-phenylpyrimidine-5-carbaldehyde\(^\text{105}\) 49a

![Diagram of the product](attachment:image.png)

Compound 48a (1.00 g, 4.73 mmol) in the presence of H\(_2\)(g) and 10% palladium/carbon (0.05 g), afforded, after extraction and column chromatography using 60% EtOAc/hexane as eluent, 49a (0.52 g, 51%) as a light-yellow solid.

\(R_f\) (80% EtOAc/hexane) 0.80. \(^1\text{H NMR (300 MHz, DMSO-}d_6\)): \(\delta\) 9.45 (1H, s, CHO), 7.52 – 7.48 (m, 5H, H8, H9 and H10), 7.24-7.13 (4H, m, 2 xNH\(_2\)). \(^{13}\text{C NMR (75 MHz, DMSO-}d_6\):}
δ 188.5 (C=O); 174.1 (C-2); 170.3 (C-4), 163.2 (C-6); 136.7 (C-7); 130.2 (C-10); 129.3 (C-9); 128.0 (C-8); 102.9 (C-5).

4.9.2 Synthesis of 2,4-diamino-6-cyclopropylpyrimidine-5-carbaldehyde 49b

\[
\begin{array}{c}
\text{H}_2N\text{N} \\
\text{N} \quad \text{O}
\end{array}
\]

Compound 48b (1.00 g, 5.71 mmol) in the presence of H\(_2\) (g) and 10% palladium/carbon (0.06 g added) as described, afforded, after extraction and column chromatography using 60% EtOAc/hexane as eluent, 49b (0.58 g, 57%) as a light-yellow solid.

\(R_f\) (80% EtOAc/hexane) 0.69. mp: 83°C. \(^1\)H NMR (300 MHz, DMSO-d\(_6\)): δ 10.1 (1H, s, CHO), 5.56 (2H, s, NH\(_2\)), 5.30 (2H, s, NH\(_2\)), 1.81 – 1.68 (1H, m, H\(_7\)), 1.05 – 0.99 (4H, m, H\(_8\)). \(^1^3\)C NMR (75 MHz, DMSO-d\(_6\)): δ 189.7 (C=O); 174.7 (C-6); 163.8 (C-4); 163.1 (C-2); 99.17 (C-5); 23.20 (C-7); 13.96 (C-8). IR (\(\nu_{\text{max}}/\text{cm}^{-1}\)): 3221 (N-H); 1747 (C=O), 1598 (C=C); 1260, 892 (CH\(_2\)).

4.9.3 Synthesis of 2,4-diamino-6-cyclohexylpyrimidine-5-carbaldehyde 49c

\[
\begin{array}{c}
\text{H}_2N\text{N} \\
\text{N} \quad \text{O}
\end{array}
\]

Compound 48c (0.75 g, 3.45 mmol) in the presence of H\(_2\) (g) and 10% palladium/carbon (0.05 g) as described, afforded, after extraction and column chromatography using 60% EtOAc/hexane as eluent, 49c (0.37 g, 49%) as a light-yellow solid.

\(R_f\) (80% EtOAc/hexane) 0.82. mp: 85°C. \(^1\)H NMR (300 MHz, DMSO-d\(_6\)): δ 10.08 (1H, s, CHO), 6.98 (2H, s, NH\(_2\)), 6.85 (2H, s, NH\(_2\)), 2.83 (1H, p, H\(_7\)), 1.82 – 1.48 (7H, m, cyclohex), 1.21 – 1.35 (3H, m, cyclohex). \(^1^3\)C NMR (75 MHz, DMSO-d\(_6\)): δ 187.8 (C=O); 164.8 (C-6); 164.1 (C-2); 163.6 (C-4); 101.9 (C-5); 44.41 (C-7); 32.02 (C-8); 30.96 (C-10). 26.15 (C-9). IR (\(\nu_{\text{max}}/\text{cm}^{-1}\)): 3431, 3314 (N-H); 1610 (C=C); 1546 (C=O); 1261, 803 (CH\(_2\)). HRMS m/z: calculated for C\(_{11}\)H\(_{17}\)N\(_4\)O: 221.1401, found: [M + H]\(^+\) 221.1397.
4.10 General procedure for the synthesis of (2,4-diamino-6-phenylpyrimidin-5-yl)methanol and its analogues 50b-c

The starting material (each of 49a-c, 1.0 eq) was dissolved in methanol (30 ml) and cooled in an ice-bath. Sodium borohydride (2.0 eq) was then added to the reaction mixture portion-wise. The reaction was stirred at room temperature for 3 hours. The reaction was monitored by TLC (80% EtOAc/hexane). After consumption of the starting material, the reaction was quenched with H$_2$O (15 ml). The mixture was then concentrated in vacuo, and the aqueous residue was extracted with EtOAc (3 x 100 ml). The extracts were dried with MgSO$_4$, filtered through celite and excess solvent was removed on a rotary evaporator. The crude product in each case was purified by silica gel column chromatography. The following products were prepared using this method:

4.10.1 Synthesis of (2,4-diamino-6-phenylpyrimidin-5-yl)methanol$^{105}$ 50a

Compound 49a (0.50 g, 2.33 mmol) and NaBH$_4$ (0.18 g, 4.67 mmol, 2.0 eq) in methanol (30 ml), afforded, after extraction and purification by column chromatography (80% EtOAc/hexane), 50a (0.44 g, 88%) as a white solid.

$R_f$ (10% MeOH/EtOAc) 0.26. $^1$H NMR (300 MHz, DMSO-d$_6$): $\delta$ 7.61 – 7.48 (2H, m, H9), 7.40-7.29 (3H, m, H8 and H10), 6.17 (2H, s, NH$_2$), 5.91 (2H, s, NH$_2$), 4.83 (1H, t, $J$ = 5.0 Hz, OH), 4.20 (2H, d, $J$ = 5.0 Hz, CH$_2$): $^{13}$C NMR (75 MHz, DMSO-d$_6$): $\delta$ 165.0 (C-6); 164.5 (C-4); 162.4 (C-2); 139.8 (C-7); 129.2 (C-9); 128.6 (C-10); 128.1 (C-8); 104.1 (C-5); 56.94 (CH$_2$): IR ($\nu_{max}$/cm$^{-1}$): 3490, 3415, 3361 (N-H); 3153 (O-H); 1609, 1555 (C=C); 976 (C-O).
4.10.2 Synthesis of (2,4-diamino-6-cyclopropylpyrimidin-5-yl)methanol 50b

![Chemical structure of 50b](image)

Compound 49b (0.40 g, 2.24 mmol) and NaBH₄ (0.17 g, 4.49 mmol, 2.0 eq) in methanol (35 ml), afforded, after extraction and purification by column chromatography (80% EtOAc/hexane), 50b (0.34 g, 84%) as a white solid.

\( R_f \) (10% MeOH/EtOAc) 0.25. mp: 80°C. \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \( \delta \) 5.92 (2H, s, NH₂), 5.59 (2H, s, NH₂), 4.65 (1H, t, \( J = 5.0 \) Hz, OH), 4.44 (2H, d, \( J = 5.0 \) Hz, CH₂); 2.12-2.03 (1H, m, H7), 0.89 – 0.73 (4H, m, H8): \(^{13}\)C NMR (75 MHz, DMSO-\(d_6\)): \( \delta \) 166.1 (C-6); 163.1 (C-4); 161.9 (C-2); 103.9 (C-5); 54.7 (CH₂); 11.9 (C-7); 8.21 (C-8): IR (\( \nu_{\text{max}}/\text{cm}^{-1} \)): 3342 (N-H); 3161 (O-H); 1560 (C=C); 980 (C-O). HRMS \( m/z \): calculated for C₈H₁₃N₄O: 181.1091, found: [M + H]\(^+\) 181.1089.

4.10.3 Synthesis of (2,4-diamino-6-cyclohexylpyrimidin-5-yl)methanol 50c

![Chemical structure of 50c](image)

Compound 49c (0.35 g, 1.59 mmol) and NaBH₄ (0.12 g, 3.18 mmol, 2.0 eq) in methanol (30 ml), afforded, after extraction and purification by column chromatography (80% EtOAc/hexane), 50c (0.32 g, 91%) as a white solid.

\( R_f \) (10% MeOH/EtOAc) 0.24. mp: 96-97°C \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \( \delta \) 6.89 (2H, s, NH₂), 6.37 (2H, s, NH₂), 4.65 (1H, t, \( J = 5.0 \) Hz, OH), 4.31 (2H, d, \( J = 5.0 \) Hz, CH₂); 1.84 – 1.48 (7H, m, cyclohex), 1.41 – 1.35 (3H, m, cyclohex), \(^{13}\)C NMR (75 MHz, DMSO-\(d_6\)): \( \delta \) 164.2 (C-4 and C-6); 161.9 (C-2); 103.4 (C-5); 57.16 (CH₂OH); 41.12 (C-7); 31.70 (C-8); 26.44 (C-9); 25.89 (C-10): IR (\( \nu_{\text{max}}/\text{cm}^{-1} \)): 3303 (N-H); 3156 (O-H); 1578 (C=C); 1000.2 (C-O). HRMS \( m/z \): calculated for C₁₁H₁₀N₄O: 223.1561, found: [M + H]\(^+\) 223.1549.
4.11 Attempted synthesis of flexible pyrimidines bearing a non-aromatic side chain

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{N} & \quad \text{N} & \quad \text{NH}_2 \\
\quad & \quad \text{R} & \quad \quad & \\
\text{H}_2\text{N} & \quad \text{N} & \quad \text{N} & \quad \text{NH}_2 \\
\quad & \quad \text{R} & \quad \quad & \\
\end{align*}
\]

\[\rightarrow\]

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{N} & \quad \text{N} & \quad \text{NH}_2 \\
\quad & \quad \text{R} & \quad \quad & \\
\text{H}_2\text{N} & \quad \text{N} & \quad \text{N} & \quad \text{NH}_2 \\
\quad & \quad \text{R} & \quad \quad & \\
\end{align*}
\]

\[\text{KOT-Bu, DMF, heat}\]

To a suspension of 50a (0.22 g, 1.02 mmol, 1 eq) in anhydrous DMF (5.00 ml) was added potassium tert-butoxide (0.17 g, 1.53 mmol, 1.5 eq). 1-(2-Bromoethyl)-4-chlorobenzene (0.16 ml, 1.12 mmol, 1.1 eq) was dissolved in (1.0 ml) DMF and added to the reaction flask. The reaction mixture was heated at 110 °C overnight. The reaction progress was monitored by TLC (80% EtOAc/hexane). When no change was observed, the reaction was left to stir for another 18 hours. The reaction mixture was then transferred into a separating funnel and treated with water (2 × 100 ml) and extracted with ethyl acetate (2 × 100 ml). The extracts were dried with MgSO₄, filtered through celite and excess solvent was removed on a rotary evaporator. After purification by silica gel column chromatography, the two starting materials were isolated. Hence, the flexible DHFR inhibitor 47 was not prepared by this method. Subsequently, several bases were tested in this reaction, but without success.

4.12 General procedure for the synthesis of flexible pyrimethamine analogues by reductive amination.

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{N} & \quad \text{N} & \quad \text{NH}_2 \\
\quad & \quad \text{R} & \quad \quad & \\
\text{H}_2\text{N} & \quad \text{N} & \quad \text{N} & \quad \text{NH}_2 \\
\quad & \quad \text{R} & \quad \quad & \\
\end{align*}
\]

\[\text{EtOH, H+}\]

\[\text{NaCNBH}_3\]

\[\text{R = phenyl}\]
\[= \text{cyclohexyl}\]
\[= \text{cyclopropyl}\]

\[\text{Y = 4-Cl}\]
\[= 4-\text{OMe}\]

To a suspension of aldehyde (each of 49a-c, 1 eq) in absolute ethanol (40.00 ml), was added glacial acetic acid (5 drops). A suitably substituted phenethylamine (1.2 eq) and sodium
cyanoborohydride (3.0 eq) were then added to the reaction mixture. The reaction mixture was heated at reflux overnight under a nitrogen atmosphere. The reaction was monitored by TLC (80% EtOAc/hexane). After consumption of the starting materials and the appearance of a product spot, the reaction mixture was cooled to room temperature and then concentrated in vacuo. The resulting residue was extracted with water (2 × 100 ml) and ethyl acetate (2 × 150 ml), and the organic layers were combined, dried over MgSO₄ and filtered through celite. The organic solvent was evaporated in vacuo and the residue in each case was purified by silica gel column chromatography. The following compounds were prepared using this method:

### 4.12.1 Synthesis of 5-((benzylamino)methyl)-6-phenylpyrimidine-2,4-diamine 57

![Chemical Structure](image)

Compound 49a (0.12 g, 0.56 mmol), NaCNBH₃ (0.11 g, 1.7 mmol) and benzyl amine (0.073 ml, 0.67 mmol), afforded, after purification by column chromatography (80% EtOAc/hexane), 57 (0.081 g, 47%) as a white solid.

$R_f$ (5% MeOH/EtOAc) 0.25. $mp$: 134-136°C. $^1$H NMR (500 MHz, DMSO-d₆): δ 7.44 – 7.26 (10H, m, Ar-H), 6.55 (2H, s, NH₂), 5.86 (2H, s, NH₂), 3.99 (1H, s, NH), 3.61 (2H, s, H₂'), 1.18 (2H, d, J = 6.4 Hz, H₁'). $^{13}$C NMR (125 MHz, DMSO-d₆): δ 165.4 (C-6); 164.3 (C-4); 161.9 (C-2); 140.8 (C-3'); 129.8 (C-7); 129.0 (C-9), 128.9 (C-10), 128.7 (C-6' and 7'), 128.6 (C-4' and C-5'), 128.5 (C-8), 128.4 (C-8'), 102.1 (C-5); 52.75 (C-2'); 45.94 (C-1'). IR (ν max/cm⁻¹): 3492, 3298, 3088 (N-H); 2842 (=C-H); 1620 (C=N); 1585, 1553 (C=C). HRMS m/z: calculated for C₁₈H₂₇N₅: 306.1720, found: [M + H]+ 306.1714.
4.12.2 Synthesis of 5-((4-methoxyphenethylamino)methyl)-6-phenylpyrimidine-2,4-diamine 58a

![Chemical structure of 58a]

Compound 49a (0.15 g, 0.70 mmol), NaCNBH$_3$ (0.13 g, 2.10 mmol) and 2-(4-methoxyphenyl)ethanamine (0.12 ml, 0.84 mmol), afforded, after purified by column chromatography using 80% EtOAc/hexane as eluent, 58a (0.075 g, 31%) as a yellow solid. 

$R_f$ (10% MeOH/EtOAc) 0.32. mp: 97-98°C. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 8.04 (2H, d, $J = 8.6$ Hz, NH$_2$), 7.65 (2H, d, $J = 8.9$ Hz, H$_2$'), 7.16 (2H, d, $J = 8.6$ Hz, H$_3$'), 6.95 (2H, d, $J = 8.6$ Hz, NH$_2$), 6.92 – 6.83 (5H, m, Ar-H), 3.88 (1H, s, NH), 3.84 (2H, s, H$_3$'), 3.80 (3H, s, OCH$_3$), 2.87 (2H, t, $J = 6.9$ Hz, H$_5$'). $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 172.9 (C-6), 172.3 (C-2 and C-4), 161.9 (C-2'), 157.8 (C-4'), 135.0 (C-7'), 129.8 (C-2' and C-9), 128.6 (C-8), 123.7 (C-1'), 114.5 (C-3'), 102.6 (C-5), 55.29 (OCH$_3$), 48.20 (C-6' and C-7'). IR ($v_{\text{max}}$/cm$^{-1}$): 3438, 3338, (N-H); 2839 (=C-H); 1625 (C=N); 1580, 1562 (C=C):

4.12.3 Synthesis of 6-cyclopropyl-5-((4-methoxyphenethylamino)methyl)pyrimidine-2,4-diamine 58b

![Chemical structure of 58b]

Compound 49b (0.12 g, 0.67 mmol), NaCNBH$_3$ (0.13 g, 2.0 mmol) and 2-(4-methoxyphenyl)ethanamine (0.12 ml, 0.81 mmol), afforded, after extraction and purification by column chromatography using 80% EtOAc/hexane as eluent, 58b (0.068 g, 32%) as a white solid.

$R_f$ (5% MeOH/EtOAc) 0.24. mp: 101-102°C. $^1$H NMR (300 MHz, DMSO-d$_6$): $\delta$ 7.11 (2H, d, $J = 8.6$ Hz, H$_2$'), 6.86 (2H, d, $J = 8.8$ Hz, H$_3$'), 3.80 (3H, s, OMe), 3.53 – 3.43 (2H, m, H$_6$'), 2.97 (1H, p, $J = 6.8$ Hz, H7), 2.81 (4H, 2 × NH$_2$), 2.76 (2H, t, $J = 7.0$ Hz, H$_5$'), 2.05 (1H, s, NH), 1.94 (2H, s, H$_7$'), 1.64 – 0.98 (4H, m, H8). $^{13}$C NMR (125 MHz, DMSO-d$_6$): $\delta$ 172.9
(C-4), 169.5 (C-6), 161.2 (C-2); 158.1 (C-4’), 131.9 (C-1’), 130.1 (C-2’), 114.2 (C-3’), 99.11 (C-5), 55.44 (OCH$_3$), 49.07 (C-7’), 40.92 (C-6’), 36.47 (C-5’), 23.08 (C-8), 22.22 (C-7): IR ($\nu_{\text{max}}$/cm$^{-1}$): 3284 (N-H); 2932 (C-H); 1637 (C=N); 1553, 1508 (C=C); 1298, 1246 (CH$_2$).

**HRMS m/z:** calculated for C$_{17}$H$_{24}$N$_5$O: 314.1983, found: [M+H]$^+$ 314.1957.

### 4.12.4 Synthesis of 6-cyclohexyl-5-((4-methoxyphenethylamino)methyl)pyrimidine-2,4-diamine 58c

Compound 49c (0.11 g, 0.50 mmol), NaCNBH$_3$ (0.094 g, 1.50 mmol) and 2-(4-methoxyphenyl)ethanamine (0.10 ml, 0.60 mmol), afforded, after extraction and purification by column chromatography using 80% EtOAc/hexane as eluent, 58c (0.054 g, 30%) as a light-yellow solid.

$R_f$ (5% MeOH/EtOAc) 0.32. mp: 123-124°C. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 7.16 (2H, d, $J = 8.8$ Hz, H2’), 6.90 (2H, d, $J = 8.9$ Hz, H3’), 6.88 – 6.78 (2 xNH$_2$), 3.84 (2H, s, H7’), 3.82 (1H, s, NH), 3.80 (3H, s, OMe), 3.68 – 3.65 (2H, m, H6’), 3.54 (1H, p, $J = 6.7$ Hz, H7), 2.87 (2H, t, $J = 6.9$ Hz, H5’), 1.30 – 1.26 (10H, m, cyclohexane): $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ 166.9 (C-4 and C-6), 159.8 (C-2); 158.0 (C-4’), 129.8 (C-1’), 128.8 (C-2’), 114.4 (C-3’), 113.6 (C-5), 55.58 (OCH$_3$), 53.63 (C-6’ and C-7’), 41.47 (C-5’), 32.92 (C-8), 31.01 (C-7), 26.00 (C-10), 25.00 (C-9): IR ($\nu_{\text{max}}$/cm$^{-1}$): 3313, 3084, (N-H); 2836 (=C-H); 1673 (C=N); 1583, 1554 (C=C); 1297 (C-O): HRMS m/z: calculated for C$_{26}$H$_{30}$N$_5$O: 356.2452, found: [M+H]$^+$ 356.2262.
4.12.5 Synthesis of 5-((4-chlorophenethylamino)methyl)-6-cyclopropylpyrimidine-2,4-diamine 58d

![Chemical structure](image)

Compound 49b (0.14 g, 0.79 mmol), NaCNBH$_3$ (0.15 g, 2.4 mmol) and 2-(4-chlorophenyl)ethanamine (0.13 ml, 0.94 mmol), afforded, after purification with column chromatography using 80% EtOAc/hexane as eluent, 58d (0.083 g, 33%) as clear crystals.

$R_f$(5% MeOH/EtOAc) 0.28. *mp*: 130-131°C. $^1$H NMR (500 MHz, DMSO-d$_6$): δ 7.40 (2H, d, J = 8.4 Hz, H3’), 7.32 (2H, d, J = 8.5 Hz, H2’), 3.39 (4H, br. s, 2 × NH$_2$), 3.24 (1H, p, J = 6.5 Hz, H7), 3.17 (1H, s, NH), 3.11 – 3.01 (2H, m, H6’), 3.01 – 2.89 (2H, m, H5’), 1.30 – 1.26 (6H, d, J = 6.5 Hz, H7’ and H8’).

$^{13}$C NMR (125 MHz, DMSO-d$_6$): δ 172.6 (C-4 and C-6); 171.0 (C-2), 137.0 (C-1’), 131.8 (C-4’), 131.1 (C-2’), 128.9 (C-3’), 106.8 (C-5), 49.94 (C-7’), 45.46 (C-6’), 31.86 (C-5’), 21.65 (C-7), 19.39 (C-8): IR ($v_{\text{max}}$/cm$^{-1}$): 2975 (N-H); 2738 (=C-H); 1604 (C=N); 1493 (C=C). HRMS m/z: calculated for C$_{16}$H$_{21}$ClN$_5$: 318.1487, found: [M+H]$^+$ 318.1488.

4.13 General procedure towards flexible pyrimidine analogues that are chemically similar to P65, which is the 2,4-diaminopyrimidine analogue of WR99210

![Chemical structure](image)

To a stirred solution of hydroxy pyrimidine 42 in dry acetonitrile (20 ml) was added potassium carbonate (1.5 eq) as the base. To this, was added a suitably substituted bromoether reported in general procedure 4.2, in dry acetonitrile (30 ml). The reaction was heated to reflux under an atmosphere of nitrogen overnight. TLC analysis showed
consumption of the bromoether starting material. The reaction mixture was allowed to cool to room temperature, filtered through celite and the filtrate was concentrated in vacuo to give an oily crude product. The crude product was purified by column chromatography (60% EtOAc/hexane) to furnish the desired product.

4.13.1 Synthesis of 6-(4-(4-chlorophenoxy)butoxy)pyrimidine-2,4-diamine 40a

Reaction of 2,4-diamino-6-hydroxy pyrimidine 42 (0.48 g, 3.79 mmol), potassium carbonate (0.79 g, 5.69 mmol) and compound 33a (1.00 g, 3.79 mmol) as described afforded, after filtration and column chromatography using 60% EtOAc/hexane as eluent, 40a (0.58 g, 50%) as a white solid.

$R_f$ (80% EtOAc/hexane) 0.25. $mp$: 97-98°C. $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ 7.31 (2H, d, $J = 8.9$ Hz, H3’), 6.95 (2H, d, $J = 9.0$ Hz, H2’), 5.98 (2H, s, NH$_2$), 5.82 (2H, s, NH$_2$), 5.04 (1H, s, H5), 4.15 – 4.11 (2H, m, H7), 3.98 (2H, t, $J = 3.5$ Hz, H10), 1.81 – 1.68 (4H, m, H8 and H9). $^{13}C$ NMR (75 MHz, DMSO-$d_6$): $\delta$ 170.1 (C-6); 165.9 (C-4); 162.9 (C-2); 157.4 (C-1’); 129.2 (C-3’); 124.1 (C-4’); 116.2 (C-2’); 76.10 (C-5); 67.54 (C-10); 64.13 (C-7); 25.32 (C-8 and C-9): IR ($v_{\text{max}}$/cm$^{-1}$): 3520, 3359 (NH$_2$); 3011 (=C-H); 1576 (C=C); 1124 (C-O); 791 (C-Cl). HRMS m/z: calculated for C$_{14}$H$_{18}$ClN$_4$O$_2$: 309.1120, found: [M + H]$^+$ 309.1117

4.13.2 Synthesis of 6-(4-(3,4-dichlorophenoxy)butoxy)pyrimidine-2,4-diamine 40b

Reaction of 2,4-diamino-6-hydroxy pyrimidine (0.43 g, 3.42 mmol), potassium carbonate (0.71 g, 5.13 mmol) and compound 33b (1.02 g, 3.42 mmol) as described above afforded, after filtration and column chromatography using 60% EtOAc/hexane as eluent, 40b (0.53 g, 45%) as a white solid.

$R_f$ (80% EtOAc/hexane) 0.23. $mp$: 136-137°C. $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ 7.50 (1H, d, $J = 8.9$ Hz, H5’), 7.22 (1H, d, $J = 2.9$ Hz, H2’), 6.95 (1H, dd, $J = 8.9$, 2.9 Hz, H6’), 5.99
(2H, s, NH₂), 5.82 (2H, s, NH₂), 5.04 (1H, s, H5), 4.15 – 4.11 (2H, m, H7), 4.05 – 4.01 (2H, m, H10), 1.78 – 1.74 (4H, m, H8 and H9). \(^{13}\)C NMR (75 MHz, DMSO-d₆): δ 170.6 (C-6); 166.5 (C-4); 163.4 (C-2); 158.6 (C-1’); 132.1 (C-3’); 131.4 (C-5’); 122.7 (C-4’); 116.8 (C-2’); 115.9 (C-6’); 76.57 (C-5); 68.54 (C-10); 64.57 (C-7); 25.70 (C-8 and C-9): IR (νₘₐₓ/cm\(^{-1}\)): 3520, 3359 (NH₂); 3011 (=C-H); 1576 (C=C); 1124 (C-O); 791 (C-Cl). HRMS m/z: calculated for C\(_{14}\)H\(_{17}\)Cl\(_2\)N\(_4\)O\(_2\): 343.0730, found: [M + H]\(^+\) 343.0729.

### 4.13.3 Synthesis of 6-(4-(3,5-dichlorophenoxy)butoxy)pyrimidine-2,4-diamine 40c

![Structure](image)

Reaction of 2,4-diamino-6-hydroxy pyrimidine 42 (0.40 g, 3.15 mmol), potassium carbonate (0.65 g, 4.73 mmol) and compound 33c (0.94 g, 3.15 mmol) as described above afforded, after filtration and column chromatography using 60% EtOAc/hexane as eluent, 40c (0.59 g, 55%) as a white solid.

Rₜ (80% EtOAc/hexane) 0.25. mp: 106-107°C. \(^{1}\)H NMR (300 MHz, DMSO-d₆): δ 7.13 (1H, t, J = 1.8 Hz, H4’), 7.03 (2H, d, J = 1.8 Hz, H2’ and H6’), 5.98 (2H, s, NH₂), 5.04 (1H, s, H5), 4.15 – 4.10 (2H, m, H7), 4.06 – 4.00 (2H, m, H10), 1.83 – 1.68 (4H, m, H8 and H9). \(^{13}\)C NMR (75 MHz, DMSO-d₆): δ 170.1 (C-6); 165.9 (C-4); 163.0 (C-1’); 161.0 (C-2); 134.5 (C-3’ and C-5’); 120.1 (C-4’); 113.8 (C-2’ and C-6’); 76.09 (C-5); 68.19 (C-10); 64.04 (C-7); 25.22 (C-8); 25.12 (C-9): IR (νₘₐₓ/cm\(^{-1}\)): 3440, 3327 (NH₂); 3008 (=C-H); 1576 (C=C); 1141 (C-O); 828, 791 (C-Cl). HRMS m/z: calculated for C\(_{14}\)H\(_{17}\)Cl\(_2\)N\(_4\)O\(_2\): 343.0730, found: [M + H]\(^+\) 343.0724.

### 4.13.4 Synthesis of 6-(4-(4-fluorophenoxy)butoxy)pyrimidine-2,4-diamine 40d

![Structure](image)

Reaction of 2,4-diamino-6-hydroxy pyrimidine 42 (0.23 g, 1.86 mmol), potassium carbonate (0.39 g, 2.79 mmol) and compound 33d (0.46 g, 1.86 mmol) as described above afforded, after filtration and column chromatography using 60% EtOAc/hexane as eluent, 40d (0.18 g, 33%) as a white solid.
\[ R_f (80\% \text{ EtOAc/hexane}) \] 0.23. \[ mp: \] 138°C. \[ ^1\text{H NMR (300 MHz, DMSO-\text{d}_6)}: \] \[ \delta \] 7.12 – 7.06 (2H, m, H3’), 6.99 – 6.84 (2H, m, H2’), 5.99 (2H, s, NH$_2$), 5.81 (2H, s, NH$_2$), 5.06 (1H, s, H5), 4.13 (2H, t, \( J = 3.1 \) Hz, H7), 3.98 – 3.94 (2H, m, H10), 1.78 – 1.74 (4H, m, H8 and H9). \[ ^{13}\text{C NMR (75 MHz, DMSO-\text{d}_6)}: \] \[ \delta \] 170.1 (C-6), 165.9 (C-4), 162.9 (C-2), 156.4 (d, \( J_{C:F} = 238.3 \) Hz, C-4’), 154.8 (d, \( J_{C:F} = 2.0 \) Hz, C-1’), 115.8 (d, \( J_{C:F} = 23.1 \) Hz, C-3’), 115.6 (d, \( J_{C:F} = 8.0 \) Hz, C-2’), 76.13 (C-5), 67.73 (C-10), 64.23 (C-7), 25.33 (C-8 and C-9): IR \( (v_{\text{max/cm}^{-1}}): \) 3478 (NH$_2$); 2957 (\( =C-H \)); 1577 (C=C); 1216, 1199 (C-O); 1075 (C-F). \[ HRMS m/z: \] calculated for C$_{14}$H$_{18}$FN$_4$O$_2$: 293.1416, found: [M + H]$^+$ 293.1411.

### 4.13.5 Synthesis of 6-(4-(3-fluorophenoxy)butoxy)pyrimidine-2,4-diamine 40e

![Synthesis of 6-(4-(3-fluorophenoxy)butoxy)pyrimidine-2,4-diamine 40e](image)

Reaction of 2,4-diamino-6-hydroxy pyrimidine (0.27 g, 2.14 mmol), potassium carbonate (0.44 g, 3.22 mmol) and compound 33e (0.53 g, 2.14 mmol) as described above afforded, after filtration and column chromatography using 60% EtOAc/hexane as eluent, 40e (0.25 g, 40%) as a light brown solid.

\[ R_f (80\% \text{ EtOAc/hexane}) \] 0.20. \[ mp: \] 98-99°C. \[ ^1\text{H NMR (300 MHz, DMSO-\text{d}_6)}: \] \[ \delta \] 7.34 – 7.26 (1H, m, H4’), 6.83 – 6.71 (3H, m, H2’, H5’ and H6’), 6.02 (2H, s, NH$_2$), 5.86 (2H, s, NH$_2$), 5.04 (1H, s, H5), 4.15 (2H, t, \( J = 6.0 \) Hz, H7), 4.01 (2H, t, \( J = 3.7 \) Hz, H10), 1.79 – 1.77 (4H, m, H8 and H9). \[ ^{13}\text{C NMR (75 MHz, DMSO-\text{d}_6)}: \] \[ \delta \] 170.1 (C-6), 165.9 (C-4), 162.8 (C-2), 160.2 (d, \( J_{C:F} = 245.3 \) Hz, C-3’), 159.87 (d, \( J_{C:F} = 10.8 \) Hz, C-1’), 130.6 (d, \( J_{C:F} = 10.0 \) Hz, C-5’), 110.8 (d, \( J_{C:F} = 2.9 \) Hz, C-6’), 106.9 (d, \( J_{C:F} = 21.3 \) Hz, C-4’), 101.9 (d, \( J_{C:F} = 24.8 \) Hz, C-2’), 76.07 (C-5), 67.59 (C-10), 64.15 (C-7), 25.33 (C-8), 25.27 (C-9); IR \( (v_{\text{max/cm}^{-1}}): \) 3512, 3344 (NH$_2$); 2939 (\( =C-H \)); 1577 (C=C); 1216, 1199 (C-O); 1075 (C-F). \[ HRMS m/z: \] calculated for C$_{14}$H$_{18}$FN$_4$O$_2$: 293.1416, found: [M + H]$^+$ 293.1413.
4.13.6 Synthesis of 6-(4-(3-(trifluoromethyl)phenoxy)butoxy)pyrimidine-2,4-diamine 40f

\[
\text{Rf} \ (80\% \ \text{EtOAc/hexane}) \ 0.20. \ \text{mp:} \ 100-101^\circ C. \ \text{\textsuperscript{1}H NMR } (500 \text{ MHz, DMSO-}\text{d}_6): \ \delta \ 7.53 \ - 7.50 \ (1H, m, H2'), 7.34-7.17 \ (3H, m, H4', H5' and H6'), 6.01 \ (2H, s, NH2), 5.86 \ (2H, s, NH2), 5.05 \ (1H, s, H5), 4.16 \ (2H, t, J = 5.9 Hz, H7), 4.09 \ (2H, t, J = 5.9 Hz, H10), 1.89 - 1.71 \ (4H, m, H8 and H9). \ \text{\textsuperscript{13}C NMR } (125 \text{ MHz, DMSO-}\text{d}_6): \ \delta \ 170.6 \ (C-6), 166.4 \ (C-4) , 163.4 \ (C-2), 159.4 \ (C-1'), 131.10 - 130.31 \ (m, C-CF\textsubscript{3}), 124.50 \ (q, J_{C-F} = 272.4 \text{ Hz, CF}_3), 119.2 \ (C-6'), 117.4 \ (q, J_{C-F} = 3.8 \text{ Hz, C-4'}), 111.4 \ (q, J = 3.7 \text{ Hz, C-2'}), 76.56 \ (C-5), 68.17 \ (C-10), 64.62 \ (C-7), 25.78 \ (C-8 and C-9): \text{IR } (v_{\text{max}}/\text{cm}^{-1}): \ 3512 \ (\text{NH}_2); 2939 \ (=C-\text{H}); 1569 \ (C=\text{C}); 1216, 1141 \ (C-O); 1015 \ (C-F): \text{HRMS} \ m/z: \ \text{calculated for C}_{15}H_{18}F_{3}N_{4}O_{2}: \ 343.1384, \ \text{found: } [\text{M + H}]^+ \ 343.1377

4.13.7 Synthesis of 6-(3-(4-chlorophenoxy)propoxy)pyrimidine-2,4-diamine 59a

\[
\text{Rf} \ (80\% \ \text{EtOAc/hexane}) \ 0.27. \ \text{mp:} \ 81-82^\circ C. \ \text{\textsuperscript{1}H NMR } (300 \text{ MHz, DMSO-}\text{d}_6): \ \delta \ 7.31 \ (2H, d, J = 9.0 \text{ Hz, H3'}), 6.96 \ (2H, d, J = 9.0 \text{ Hz, H2'}), 6.00 \ (2H, s, NH2), 5.85 \ (2H, s, NH2), 5.06 \ (1H, s, H5), 4.22 \ (2H, t, J = 6.4 \text{ Hz, H7}), 4.05 \ (2H, t, J = 6.3 \text{ Hz, H9}), 2.07 \ (2H, p, J = 6.3 \text{ Hz, H8}). \ \text{\textsuperscript{13}C NMR } (75 \text{ MHz, DMSO-}\text{d}_6): \ \delta \ 170.0 \ (C-6); 165.9 \ (C-4); 163.0 \ (C-2); 157.3 \ (C-1');
129.2 (C-3’); 124.2 (C-4’); 116.2 (C-2’); 76.12 (C-5); 64.77 (C-7); 61.36 (C-9); 28.48 (C-8);
**IR** ($\nu_{\text{max}}$/cm$^{-1}$): 3520, 3359 (NH$_2$); 3011 (=C-H); 1576 (C=C); 1125 (C-O); 791 (C-Cl).
**HRMS m/z**: calculated for C$_{13}$H$_{15}$ClN$_4$O$_2$: 295.0964, found: [M + H]$^+$ 295.0956.

4.13.8 Synthesis of 6-(3-(3,4-dichlorophenoxy)propoxy)pyrimidine-2,4-diamine 59b

![Chemical Structure](structure1.png)

Reaction of 2,4-diamino-6-hydroxy pyrimidine 42 (0.44 g, 3.52 mmol), potassium carbonate (0.73 g, 5.28 mmol) and compound 60b (1.00 g, 3.52 mmol) as described afforded, after filtration and column chromatography using 60% EtOAc/hexane as eluent, 59b (0.43 g, 37%) as light a yellow solid.

$R_f$ (80% EtOAc/hexane) 0.28. $mp$: 134-135°C. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 7.50 (1H, d, $J = 8.9$ Hz, H5’), 7.22 (1H, d, $J = 2.9$ Hz, H2’), 6.95 (1H, dd, $J = 8.9$, 2.9 Hz, H6’), 5.99 (2H, s, NH$_2$), 5.82 (2H, s, NH$_2$), 5.04 (1H, s, H5), 4.22 (2H, t, $J = 6.4$ Hz, H7), 4.12 (2H, t, $J = 6.2$ Hz, H9), 2.06 (2H, p, $J = 6.3$ Hz, H8). $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 170.4 (C-6); 166.5 (C-4); 163.4 (C-2); 158.5 (C-1’); 132.1 (C-3’); 131.4 (C-5’); 122.8 (C-4’); 116.8 (C-2’); 115.9 (C-6’); 76.62 (C-5); 65.79 (C-7); 61.76 (C-9); 28.87 (C-8). **IR** ($\nu_{\text{max}}$/cm$^{-1}$): 3529, 3343 (NH$_2$); 3010 (=C-H); 1581 (C=C); 1101 (C-O): **HRMS m/z**: calculated for C$_{13}$H$_{15}$ClN$_4$O$_2$: 329.0574, found: [M + H]$^+$ 329.0565.

4.13.9 Synthesis of 6-(3-(4-fluorophenoxy)propoxy)pyrimidine-2,4-diamine 59c

![Chemical Structure](structure2.png)

Reaction of 2,4-diamino-6-hydroxy pyrimidine 42 (0.89 g, 7.04 mmol), potassium carbonate (1.46 g, 10.6 mmol) and compound 60c (2.00 g, 7.04 mmol) as described afforded, after filtration and column chromatography using 60% EtOAc/hexane as eluent, 59c (1.26 g, 54%) as a creamy-white solid.

$R_f$ (80% EtOAc/hexane) 0.28. $mp$: 105-107°C. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 7.14 (1H, t, $J = 1.8$ Hz, H4’), 7.06 (2H, d, $J = 1.8$ Hz, H2’ and H6’), 6.00 (2H, s, NH$_2$), 5.84 (2H, s,
NH₂), 5.05 (1H, s, H5), 4.21 (2H, t, J = 6.4 Hz, H7), 4.12 (2H, t, J = 6.2 Hz, H9), 2.11 – 2.02 (2H, m, H8). ¹³C NMR (75 MHz, DMSO-d₆): δ 170.0 (C-6); 166.0 (C-4); 163.0 (C-1’); 160.0 (C-2); 134.6 (C-3’ and C-5’); 120.3 (C-4’); 113.9 (C-2’ and C-6’); 76.13 (C-5); 65.46 (C-7); 61.19 (C-9); 2.11 – 2.02 (2H, m, H8).

4.13.10 Synthesis of 6-(3-(4-fluorophenoxy)propoxy)pyrimidine-2,4-diamine 59d

![Chemical Structure](image)

Reaction of 2,4-diamino-6-hydroxy pyrimidine 42 (0.27 g, 2.15 mmol), potassium carbonate (0.44 g, 3.22 mmol) and compound 60d (0.50 g, 2.15 mmol) as described afforded, after filtration and column chromatography using 60% EtOAc/hexane as eluent, 59d (0.20 g, 34%) as a white solid.

Rf(80% EtOAc/hexane) 0.26. mp: 69-71°C. ¹H NMR (300 MHz, DMSO-d₆): δ 7.18 – 7.01 (2H, m, H3’), 6.99 – 6.87 (2H, m, H2’), 5.99 (2H, s, NH₂), 5.85 (2H, s, NH₂), 5.04 (1H, s, H5), 4.21 (2H, t, J = 6.4 Hz, H7), 4.12 – 3.95 (2H, m, H9), 2.06 (2H, p, J = 6.3 Hz, H8). ¹³C NMR (75 MHz, DMSO-d₆): δ 170.0 (C-6); 166.0 (C-4); 163.0 (C-2); 157.32 (d, J₈₋₆ = 238.3 Hz, C-4’), 154.84 (d, J₈₋₆ = 2.0 Hz, C-1’), 115.84 (d, J₈₋₆ = 23.1 Hz, C-3’), 115.41 (d, J₈₋₆ = 8.0 Hz, C-2’), 76.11 (C-5); 64.95 (C-7); 61.40 (C-9); 28.57 (C-8). IR (vₘₚₑₙ/cm⁻¹): 3539, 3332 (NH₂); 3032 (=C-H); 1586 (C=C); 1153 (C-O). HRMS m/z: calculated for C₁₃H₁₅F₄N₄O₂: 329.0574, found: [M + H]⁺ 329.0572.
REFERENCES


9. www.cdc.gov/malaria distribution


15. WHO, Indoor residual spraying, use of indoor residual spraying for scaling up global malaria control and elimination., 2006b, 1112.


**APPENDIX**

Figure 35: Folate pathway catalysed by three important enzymes.
Figure 36: *de novo* Pathway present only in the parasite.
Spectra of selected compounds:

\[ \text{H NMR of 36a} \]

\[ \text{1H NMR of 36a} \]

DS-19.20.8d
Donald DS-19; CDCl3; 09/sep/2013; RT-1H,13C:300NMR
$^1$H NMR of 37a
$^{13}$C NMR of 37a
\( ^1H \) NMR of 59b
$^{13}$C NMR of 59b
$^1$H NMR of 40f
$^{1}H$ NMR of 48a
$^{13}$C NMR of 48a
$^1$H NMR of 48c

DS-56(2nd frac).1.fid
Donald: DS -56(2nd frac): 24/02/2014: 300K; 1H, 13C; 500NMR
$^{13}$C NMR of 48c

[Chemical structure image]
$^1$H NMR of 50a
$^{13}$C NMR of 50a
$^1$H NMR of 57
$^1$H NMR of 58b
$^{13}$C NMR of 58b
$^1$H NMR of 58d