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SYNTHESIS OF ANTIMALARIAL ANTIFOLATES

by

DONALD TSWENE SEANEGO

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

Supervisor: Dr Amanda Rousseau

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).

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I am grateful to **God** for the protection, good health and the blessing to complete this research.

LIST OF ABBREVIATIONS

ACT	Artemisinin Combination Therapy
CSIR	Council of Scientific and Industrial Research
DBN	1,5-Diazabicyclo[4.3.0]non-5-ene
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DDT	Dichlorodiphenyltrichloroethane
DHA	Dihydroartemisinin
DHF	Dihydrofolate
DHFR	Dihydrofolate reductase
DHFR-TS	Dihydrofolate reductase-thymidylate synthase
DHPS	Dihydropteroate synthase
DHPT	6-Hydroxymethyl-7,8-dihydropterin
DMF	*N*,*N*-dimethylformamide
DMSO	Dimethyl sulfoxide
EtOAc	Ethyl acetate
FD's	Folate derivatives
GTP	Guanosine triphosphate
HPPK	6-Hydroxymethyl-dihydropterin pyrophosphokinase
HRMS	High resolution mass spectroscopy
IRS	Indoor residual spraying
IR	Infrared
ITN's	Insecticide-treated nets
KHMDS	Potassium bis(trimethylsilyl)amide
LDA	Lithium diisopropyl amide
MCR	Multicomponent coupling reaction

MS	Mass Spectroscopy
NADPH	Nicotinamide adenine phosphate
NMR	Nuclear Magnetic Resonance
pABA	para-aminobenzoic acid
P*f*DHFR	*Plasmodium falciparum*- Dihydrofolate reductase
SHMT	Serine hydroxymethyl transferase
TLC	Thin Layer Chromatography
WHO	World Health Organization

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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**).[8] 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**).

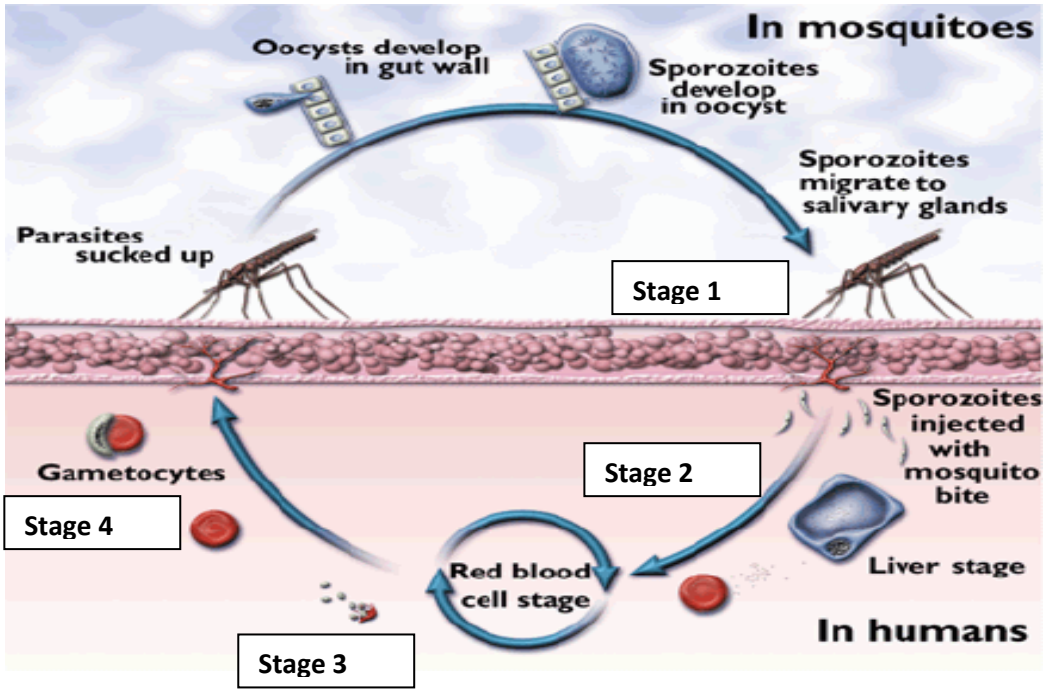


Figure 1: The life cycle of malaria parasite (www.niaid.nih.gov).

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.[9] The highest transmission of malaria is found predominantly in sub-Saharan Africa.[10]

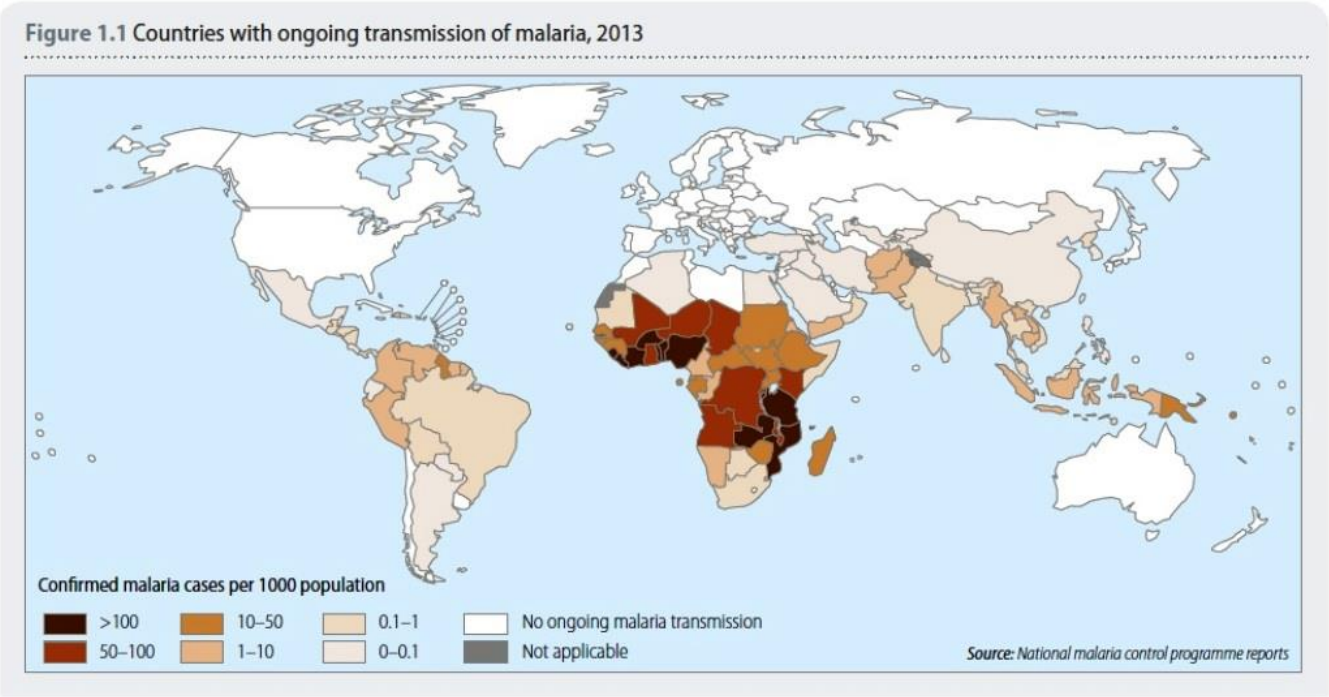


Figure 2: Global distribution of malaria (taken from WHO Malaria Report, 2014)

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.[11]

1.3 Malaria control and prevention

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

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.[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.[14]

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.[15] In countries with ongoing malaria transmission, ITNs are handed out free of charge to all age groups through mass campaigns.[5] Unfortunately, pyrethroid resistance has become common in malaria vectors across Africa.[16]

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.[17]

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.[18] 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.[19] Pattaroyo *et al*. developed a number of vaccines that underwent large-scale clinical trials,[20] one of which was the three-component vaccine (SPf66) which unfortunately did not show clinical effectiveness.[21-22]

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), hepatisis 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.[23] 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.[24] If the vaccines are used in combination, they reduce the spread of parasites which may be resistant to one vaccine.

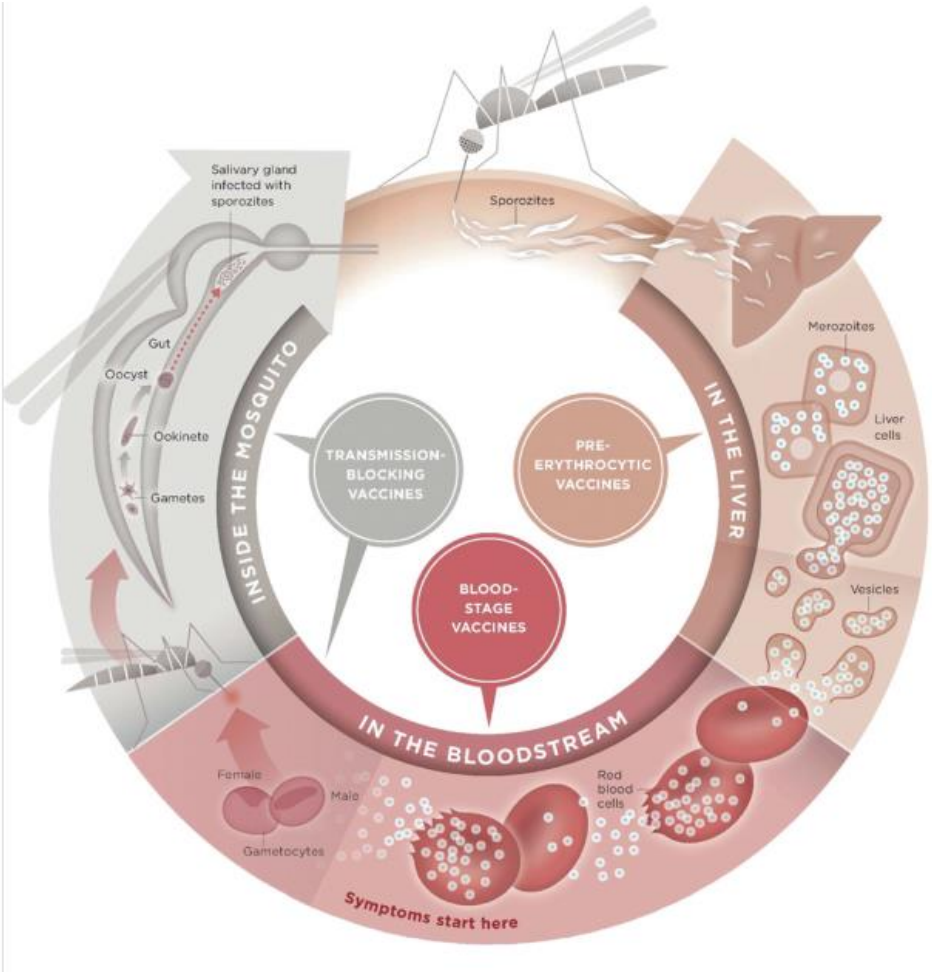


Figure 3: Breaking the cycle with vaccines.[26]

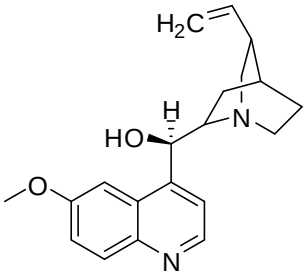
1.3.3 Chemotherapy for *falciparum* malaria

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,[26] 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.[27] 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.[28]



1

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.[29] 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.[30] 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.[31]

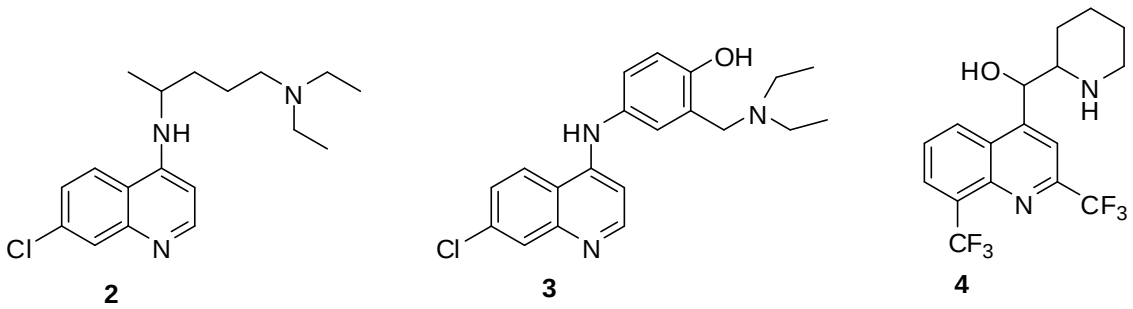


Figure 5: Quinoline-based antimalarial drugs.

Mefloquine (**4**) is an aryl-amino alcohol derivative of quinine, developed by the U.S Army as a replacement for chloroquine.[32] 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.[33] However, its use has been limited due to high costs and the appearance of neuropsychiatric side effects.[34] 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.[35] 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.[36] 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.[37]

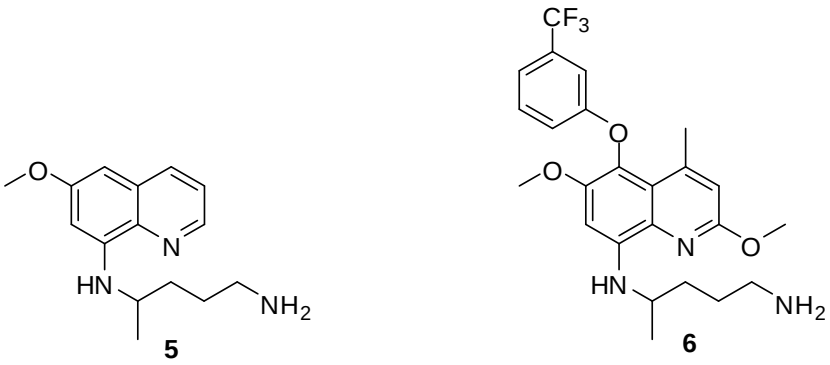


Figure 6: 8-amino quinolines

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.

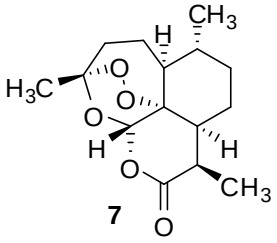


Figure 7: Artemisinin

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).**[34] 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.[38] This analogue is not metabolised to dihydroartemisinin like the above-mentioned artemisinin derivatives.

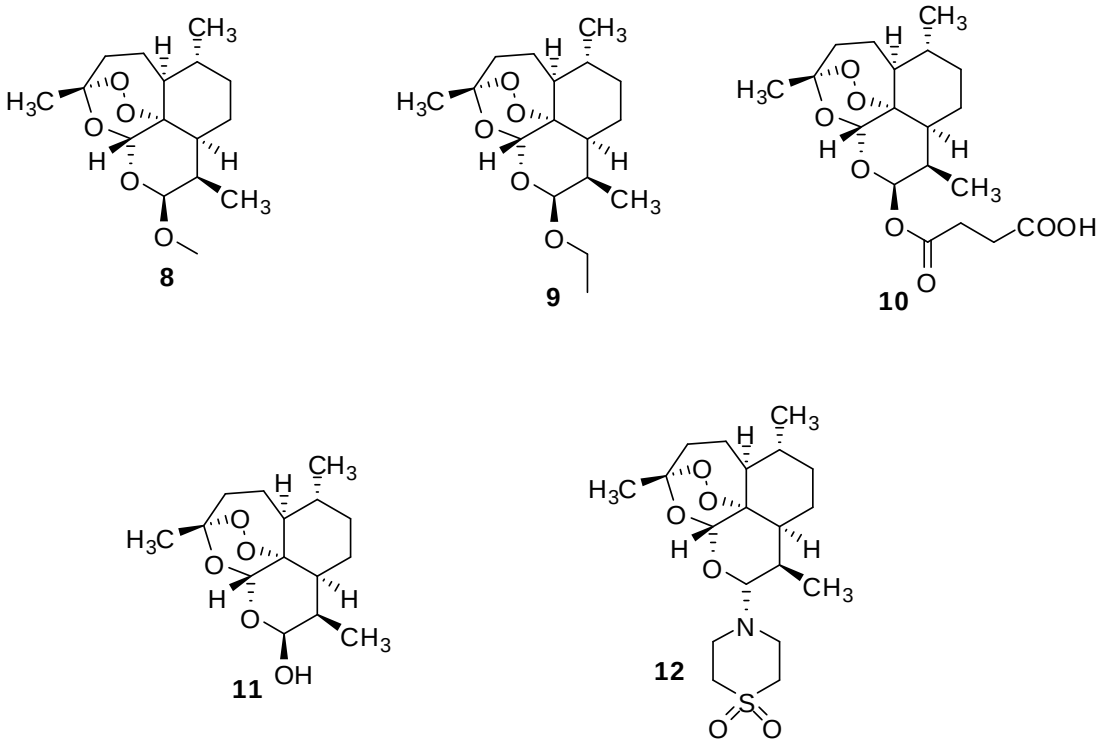


Figure 8: Artemisinin derivatives

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.[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.[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^{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**).

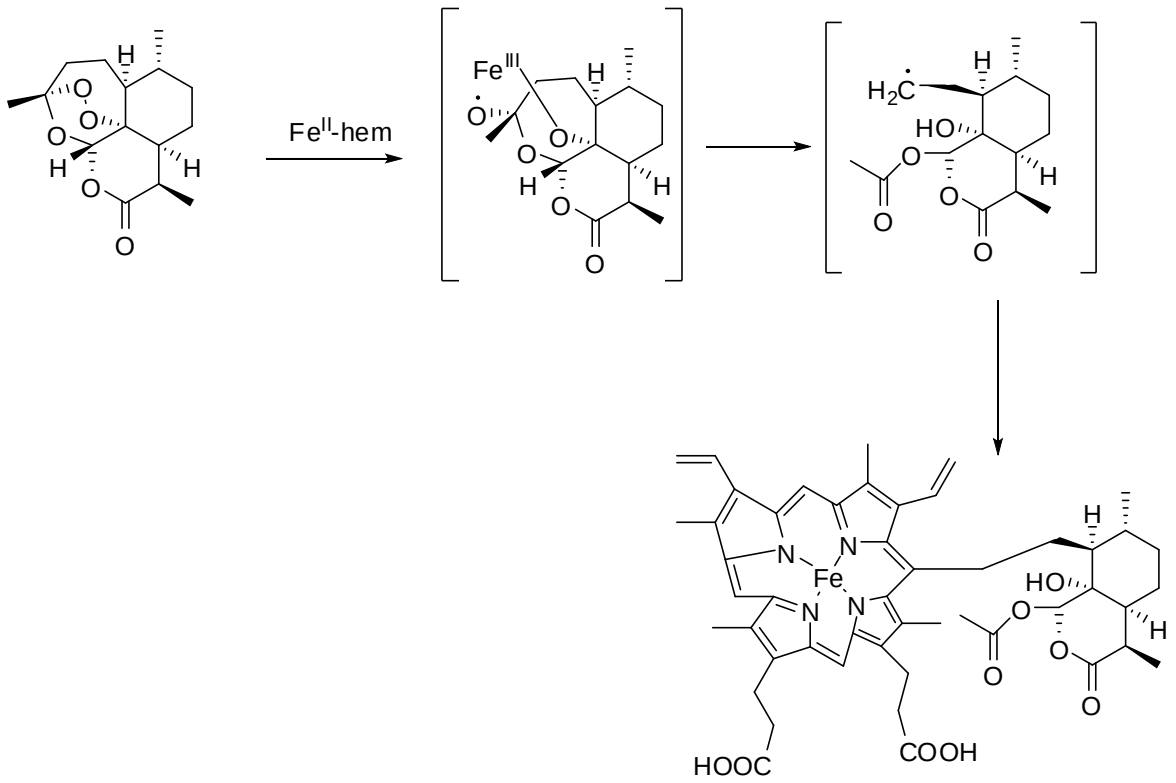


Figure 9: Hypothetical mechanism for the alkylation of heme by artemisinin.[34]

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*f*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**).[41]

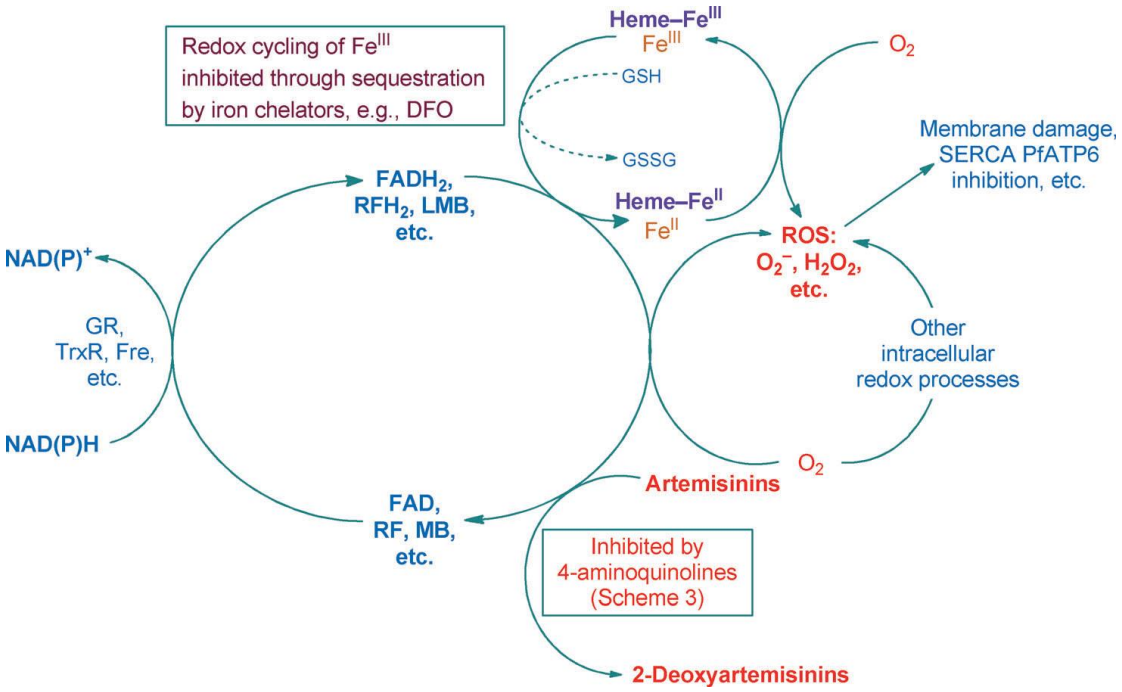


Figure 10: Proposed mechanism for oxidative stress mediated by artemisinin.[41]

1.3.3.3 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.[42] Halofantrine was administered as a racemate, with the enantiomers having comparable *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®).

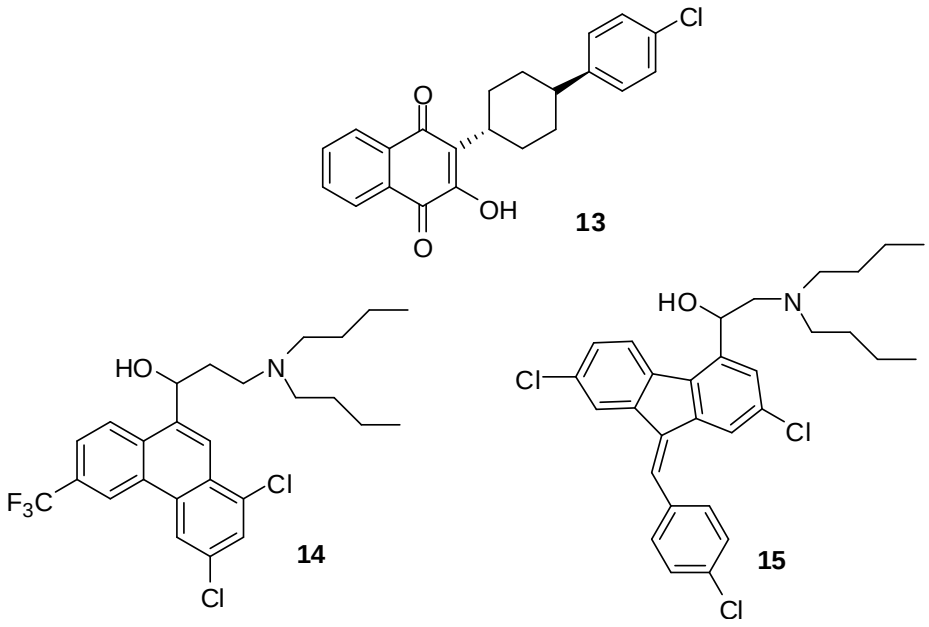


Figure 11: Antimalarial drugs used in combination with other drugs

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.[43] 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.[44] 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.[45]

The malaria parasite is also able to synthesise folate derivatives *de novo*, using a pathway absent in humans. This *de novo* biosynthetic route begins with the GTP cyclohydrolase-catalyzed transformation of guanosine triphosphate (GTP) into dihydroneopterin triphosphate (DHN-PPP) (**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 *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).

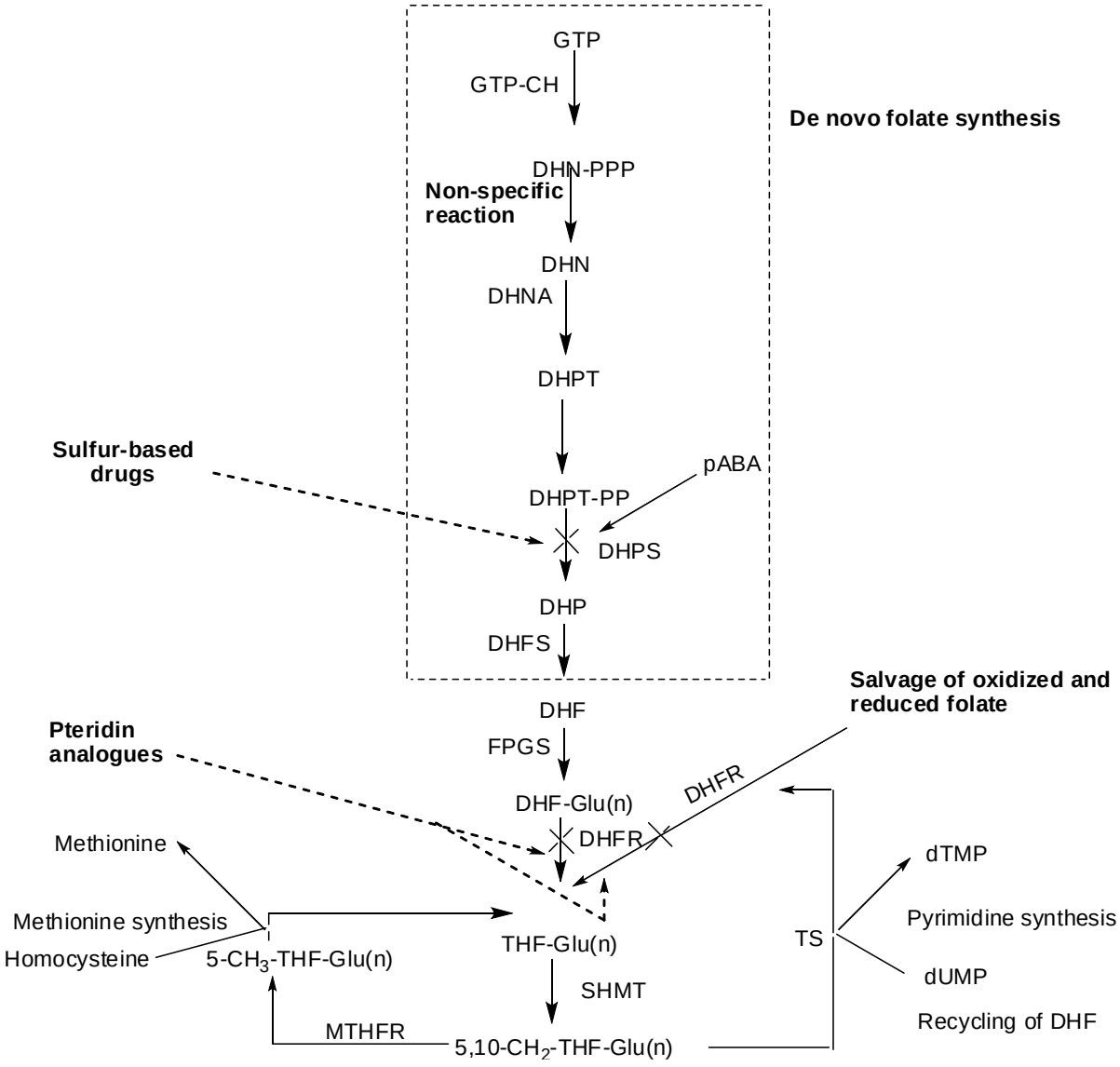


Figure 12: The folate salvage pathway (humans and the malaria parasite) and the *de novo* pathway (malaria parasite).[46]

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.[47] 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

treatment of both *P. falciparum and P. vivax* malaria. Unfortunately, because of its limited effectiveness and high toxicity, development of dapsone was abandoned.[48, 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.[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.[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;[52] hence new effective antimalarials are urgently needed to combat malaria.

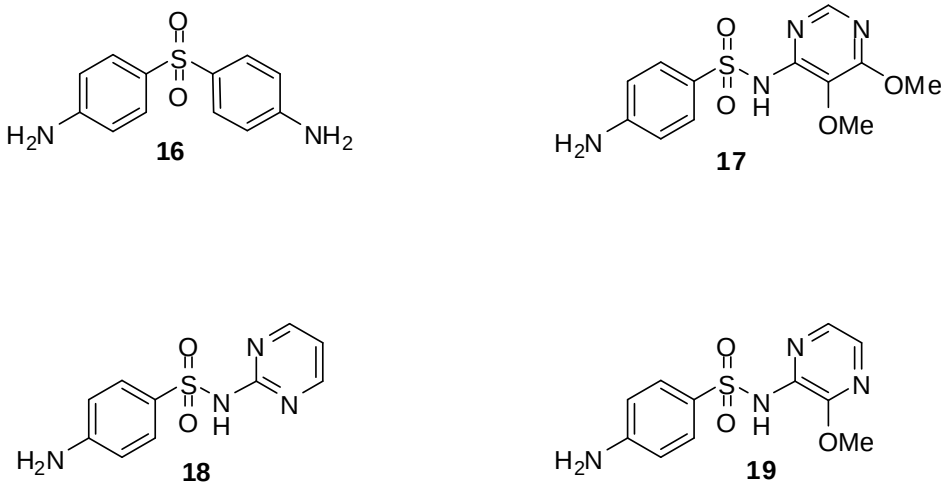


Figure 13: DHPS inhibitors

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.[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),[54] 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.[55]

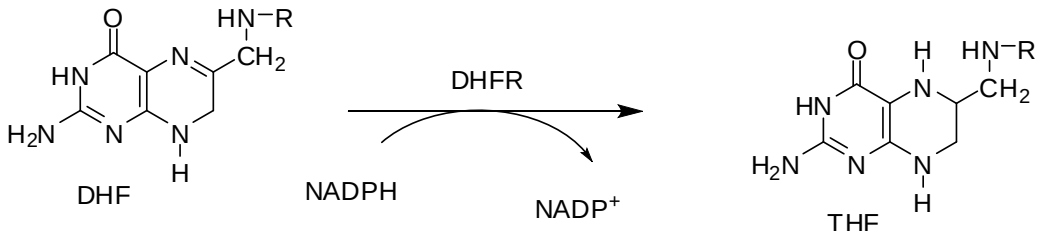


Figure 14: The reduction of DHF to THF catalysed by DHFR.

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.[56] 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**).[57] Chlorproguanil has a higher potency compared to proguanil, and hence it was recommended for prophylaxis at a lower dose.[58]

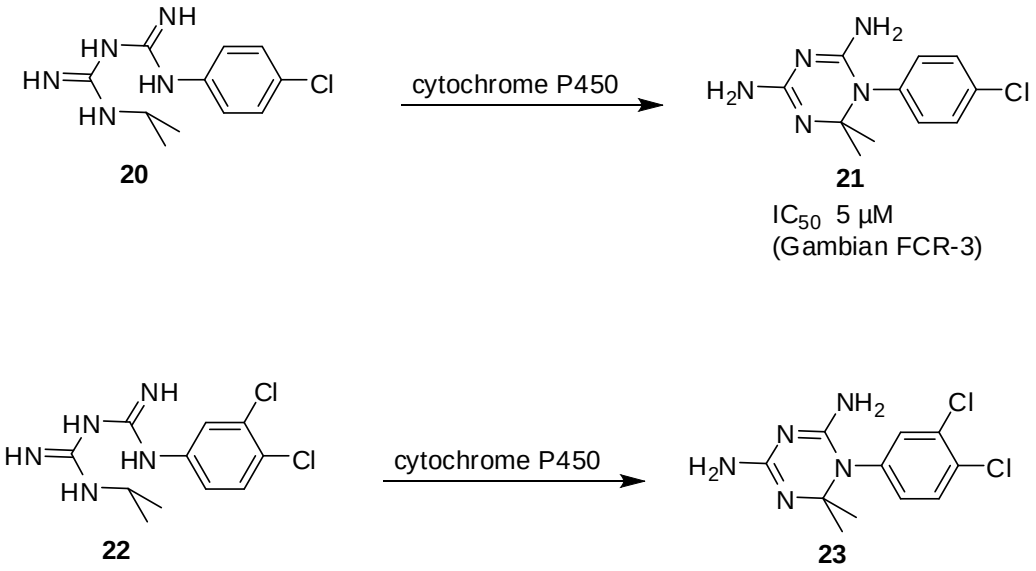


Figure 15: Conversion of proguanil (**20**) and chlorproguanil (**22**) to its metabolite cycloguanil (**21**) and chlorcycloguanil (**23**), respectively.

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.[59] 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.[60]

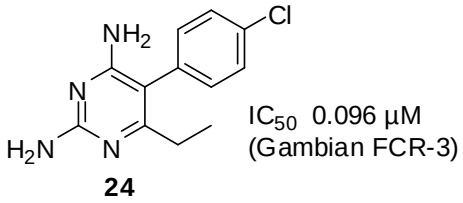


Figure 16: Structure of pyrimethamine

To combat resistant strains, pyrimethamine was used in combination with sulfadoxine for the treatment of malaria.[61] 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.[62] 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]

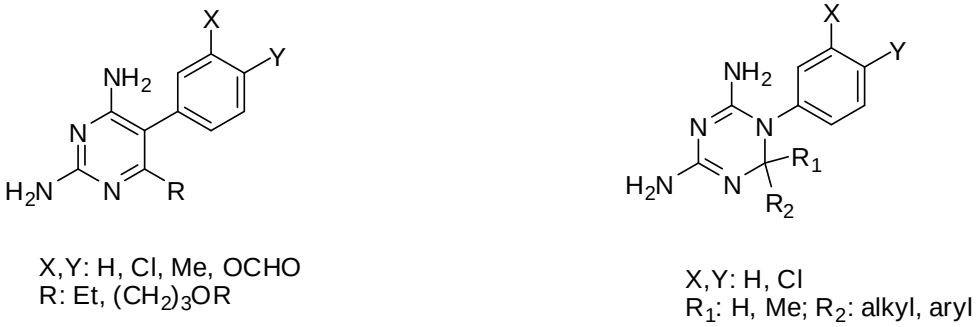
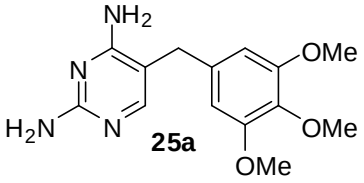


Figure 17: Analogues of pyrimethamine and cycloguanil

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_{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]



25a

Figure 18: Trimethoprim

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.[67] To date, however, none of these compounds have been developed as drugs.

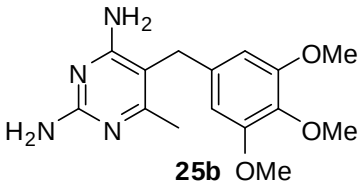


Figure 19: Trimethoprim analogue

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.[68] 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**).[69]

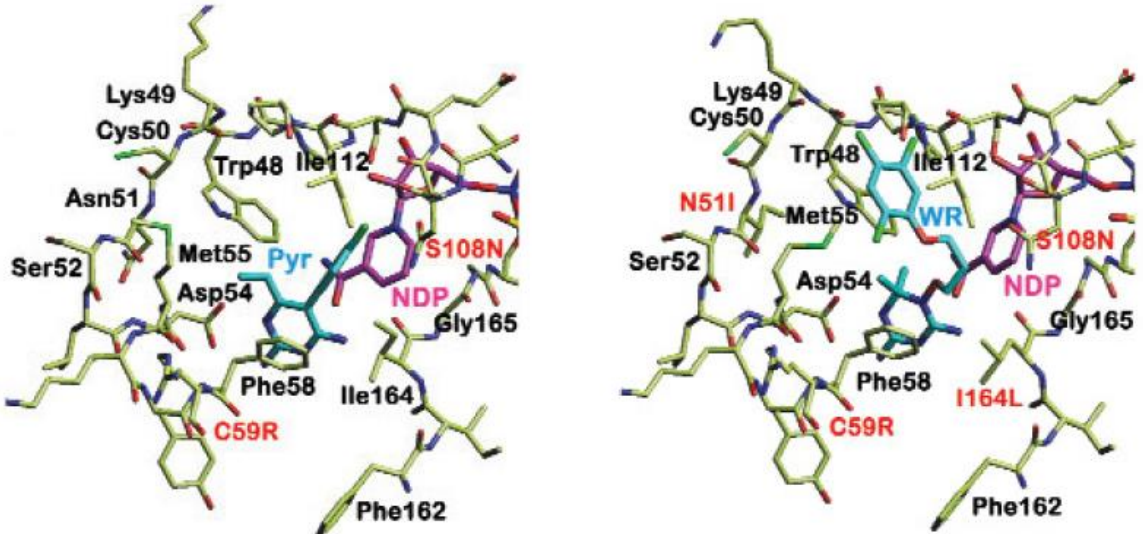


Figure 20: Comparison of interactions at the active sites of mutant PfDHFR-TS with flexible and non flexible antifolates. The enzyme on the left is complexed with pyrimethamine and NADPH. The enzyme on the right is complexed with WR99210 and NADPH. The flexible tail of WR99210 allows it to avoid the 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.[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 *in vivo* models.[71, 72] However, PS-15 was abandoned due to cross-resistance with cycloguanil and pyrimethamine.[73]

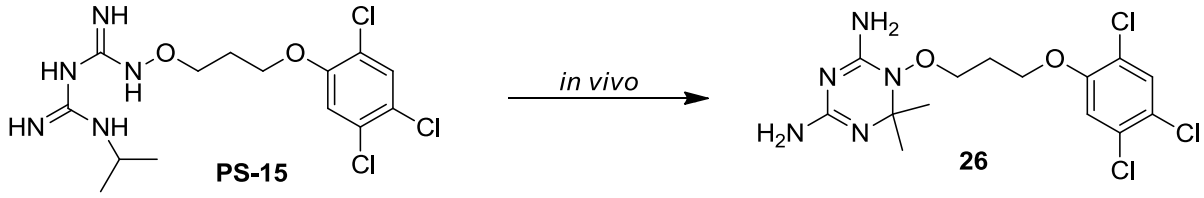
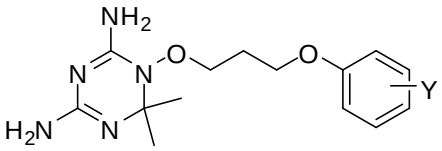


Figure 21: Conversion of a prodrug, PS-15, to its metabolite WR99210.

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.[74] Substituents on the phenyl ring were varied in order to minimise the metabolic degradation of these analogues.



27: Y = 4-OMe
28 = 4-NO_2

Figure 22: Structural analogues of WR99210

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.[75,76] 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.[77-79] 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.[80] 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.[81] 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.[82] 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.[83]

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.[84] 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,[85] 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[86] 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.[87,88] However, clinical trials of Lapdap in Southeast Asia showed that this drug was ineffective in the treatment of uncomplicated malaria.[89]

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.[90] 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.[91]

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.[92] 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 **29**. 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.[93]

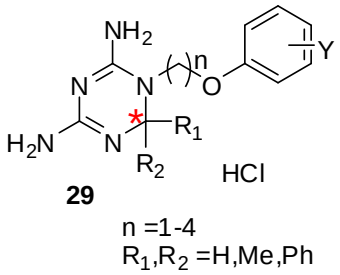
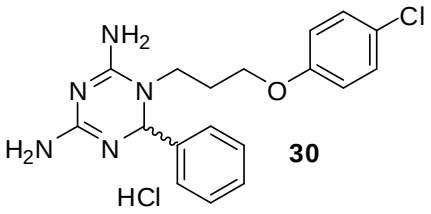


Figure 23: Flexible cycloguanil analogues prepared previously at CSIR

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.[92] 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).



30

IC_{50} 33nM (Gambian FCR-3)
IC_{50} 80nM (D10)
K_i 4-9 (WT); 8.2 nM (FCR-3)

Figure 24: The dihydrotriazine hit compound

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**).

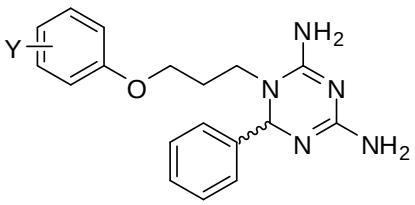


Figure 25: Previously prepared dihydrotriazine compounds

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).

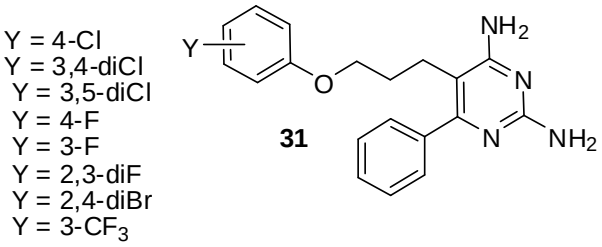
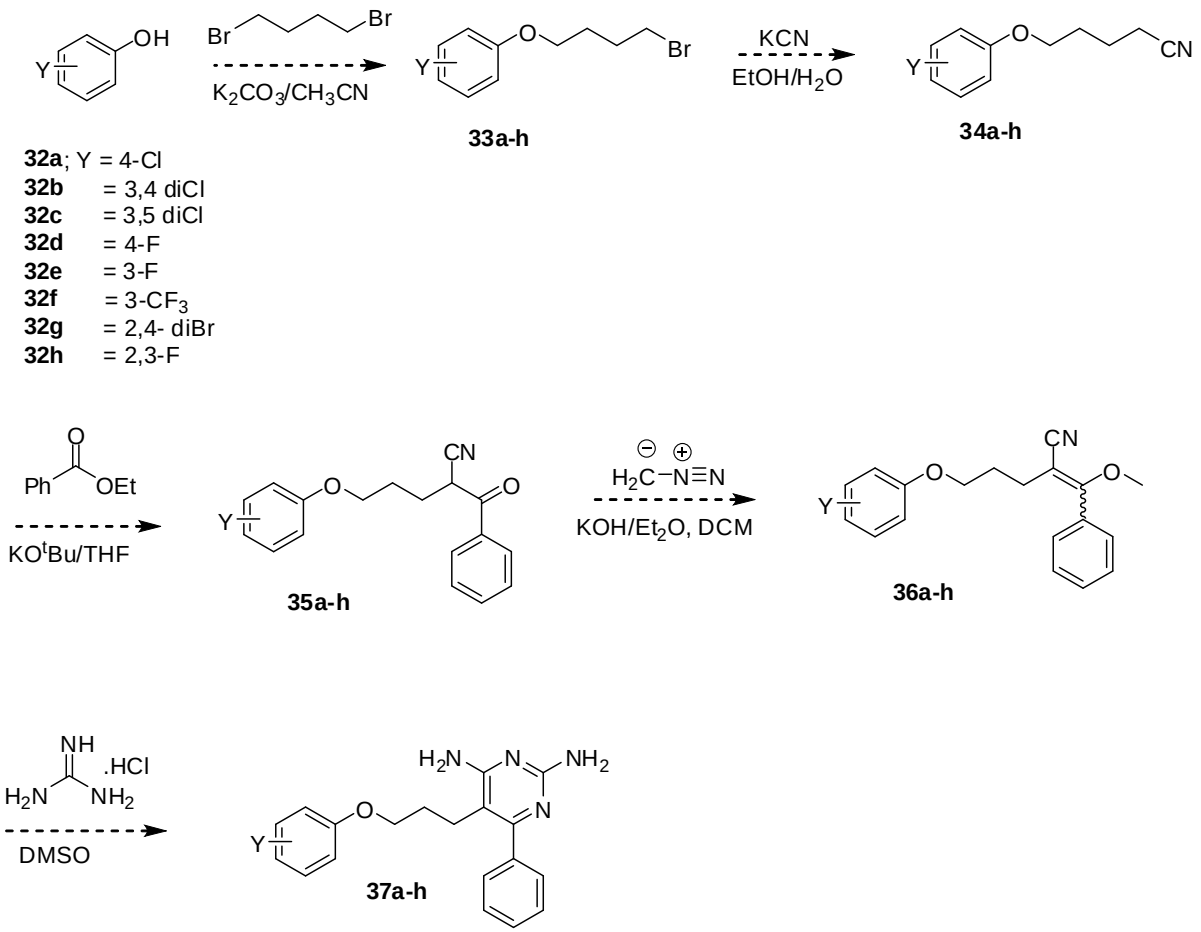


Figure 26: Proposed flexible pyrimidine analogues based on the dihydrotriazine equivalents.

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.

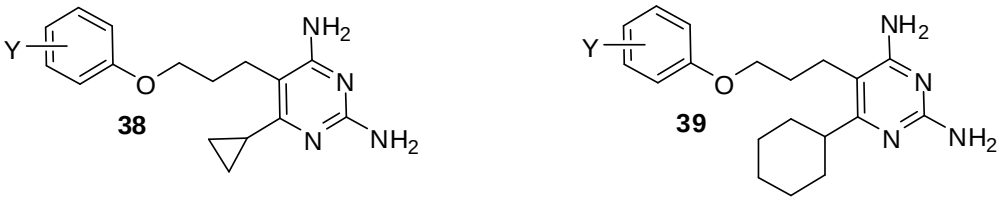


Figure 27: Proposed pyrimidine equivalents bearing a non-aromatic side chain

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.[94]

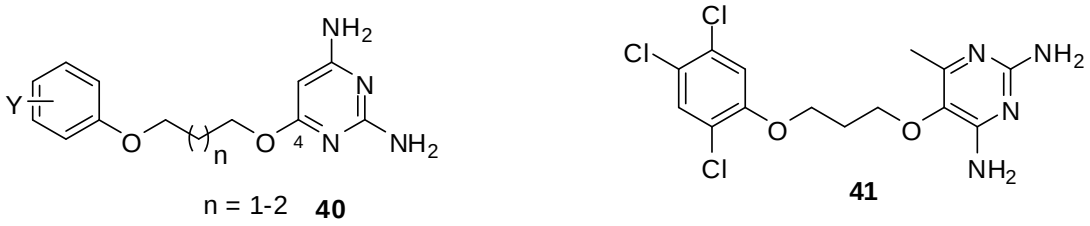
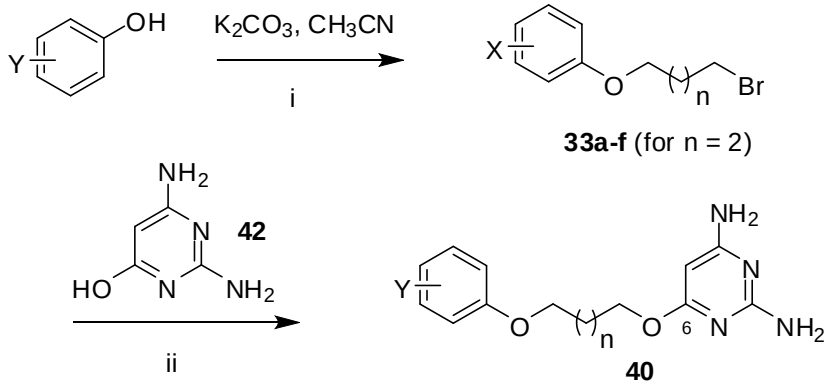


Figure 28: Flexible pyrimidine, P65, (**41**) and proposed pyrimidine analogues (**40**)

These compounds could be prepared by alkylating 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.



Scheme 2: Reagents and conditions: i) For n =1: 1,3-dibromopropane, reflux; for n = 2: 1,4-dibromobutane, reflux; ii) K_2CO_3, CH_3CN, reflux.

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).

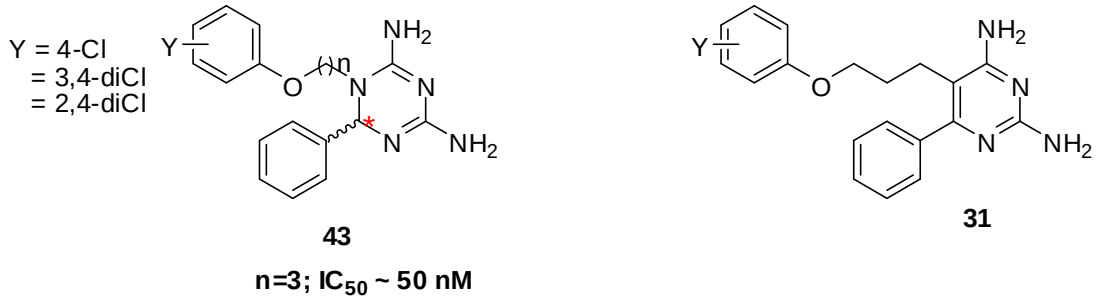


Figure 29: Dihydrotriazine compound prepared previously (**43**) and the proposed pyrimidine equivalents (**31**)

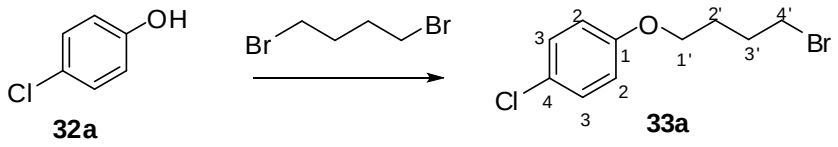
Although the dihydrotriazines displayed potent antimalarial activity *in vitro*, they were isolated as a mixture of two enantiomers from a challenging, low yielding synthetic route. In this project, we will prepare pyrimidine equivalents that are fully aromatic (general structure **31**, **Figure 29**), 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

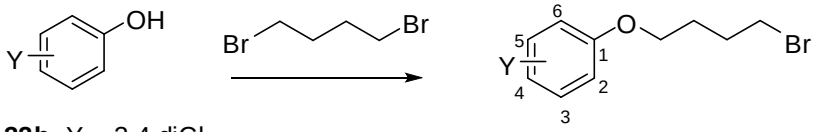


Scheme 3: Reagents and conditions: K_2CO_3, CH_3CN, reflux, 20 h, 98%.

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 $-CH_2Br$ 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.



32b; Y = 3,4 diCl
32c = 3,5 diCl
32d = 4-F
32e = 3-F
32f = 3-CF_3
32g = 2,4- diBr
32h = 2,3-F

33b; Y = 3,4 diCl, 92%
33c: = 3,5 diCl, 88%
33d: = 4-F, 63%
33e: = 3-F, 86%
33f: = 3-CF_3, 89%
33g: = 2,4- diBr , 80%
33h: = 2,3-diF, 84%

Scheme 4: Reagents and conditions: K_2CO_3, CH_3CN, reflux, 20 h.

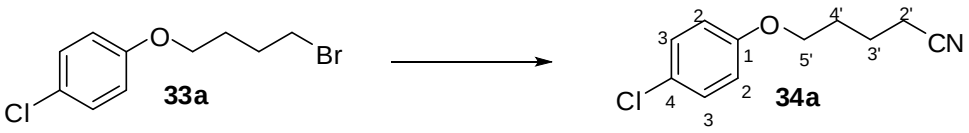
Table 1: Key signals in the ^{1}H NMR spectra of bromoethers **33b-33h**.

	Aromatic region (ppm)	-OCH_2- (ppm)	CH_2Br (ppm)	H2' & H3'(ppm)
Compound **33b**	7.50 (d, H5), 7.22 (d, H2), 6.95 (dd, H6)	3.92 (t)	3.45 (t)	2.11 – 1.80 (m)
Compound **33c**	6.91 (t, H4), 6.75 (d, H2 and H6)	3.92 (t)	3.45 (t)	2.11 – 1.80 (m)
Compound **33d**	7.01 – 6.92 (m, H3), 6.84 – 6.72 (m, H2).	3.96 (t)	3.44 (t)	2.11 – 1.88 (m)
Compound **33e**	7.22 – 7.15 (m, H4), 6.66 – 6.55 (m, H2, H5, H6)	3.95 (t)	3.45 (t)	1.99 – 1.67 (m)
Compound **33f**	7.45 – 7.31 (m, H4), 7.21 – 7.00 (m, H2, H5 and H6)	4.01 (t)	3.48 (t)	2.16 – 1.87 (m)
Compound **33g**	7.66 (d, H3), 7.36 (dd, H5), 6.74 (d, H6)	4.04 (t)	3.46 (t)	2.09 – 1.80 (m)
Compound **33h**	6.97 – 6.92 (m, H5), 6.80 – 7.70 (m, H4, H6),	4.07 (t)	3.50 (t)	2.15 – 1.91 (m)

Table 2: Key signals in the ^{13}C NMR spectra of bromoethers **33b-33h**.

Compound	Aromatic region (ppm)	-OCH_2- (ppm)	CH_2Br (ppm)	C-2' (ppm)	C-3' (ppm)
33b	157.1 (C-1), 134.3 (C-3), 131.4 (C-5), 124.7 (C-4), 116.3 (C2 and C-6).	67.60	27.98	17.01	22.53
33c	159.7 (C-1), 135.5 (C-3), 121.3 (C-4), 114.0 (C-2).	67.32	27.98	17.00	22.33
33d	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).	67.41	33.38	27.88	29.41
33e	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).	67.70	27.91	17.00	22.52
33f	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	33.29	27.82	29.42
33g	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	27.91	17.06	22.50
33h	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).	68.71	33.34	27.82	29.27

2.1.2 Synthesis of 5-(4-chlorophenoxy)pentanenitrile and analogues

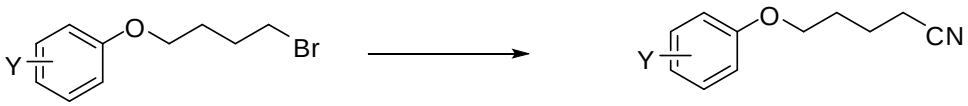


Scheme 5: Reagents and conditions: KCN, EtOH/H_2O, reflux, 2 days, 89%.

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 $C_{11}H_{12}ClNONa$: 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).



34b; Y = 3,4 diCl, 86%
34c: = 3,5 diCl, 71%
34d: = 4-F, 62%
34e: = 3-F, 72%
34f: = 3-CF_3, 74%
34g: = 2,4- diBr , 73%
34h: = 2,3-diF, 86%

Scheme 6: Reagents and conditions: KCN, EtOH/H_2O, reflux, 2 days.

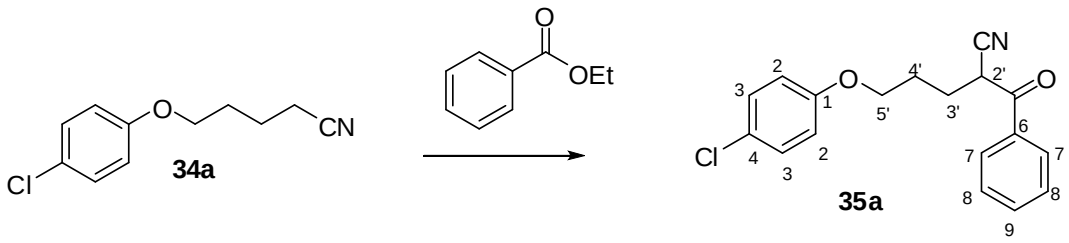
Table 3: Key signals in the ^{1}H NMR spectra of pentanenitriles **34b-34h**.

	-CH_2-CN (ppm)
Compound **34b**	1.85 (t)
Compound **34c**	2.45 (t)
Compound **34d**	2.39 (t)
Compound **34e**	2.41 (t)
Compound **34f**	2.42 (t)
Compound **34g**	2.50 (t)
Compound **34h**	2.46 (t)

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

	-CH_2-CN (ppm)	-C≡N (ppm)
Compound **34b**	17.4	119.1
Compound **34c**	17.00	119.3
Compound **34d**	17.00	119.5
Compound **34e**	17.00	119.3
Compound **34f**	17.01	119.2
Compound **34g**	17.06	119.5
Compound **34h**	17.4	119.3

2.1.3 Synthesis of 2-benzoyl-5-(4-chlorophenoxy)pentanenitrile and analogues.

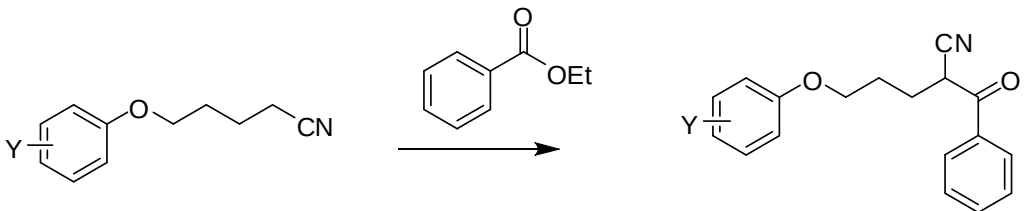


Scheme 7: Reagents and conditions: KO^{t}Bu, THF, rt, 18 h, 81%.

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 R_f 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_2CN 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 $C_{18}H_{16}ClNO_2Na$ 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).



35b; Y = 3,4 diCl, 94%
35c: = 3,5 diCl, 91%
35d: = 4-F, 83%
35e: = 3-F, 87%
35f: = 3-CF_3, 63%
35g = 2,4- diBr , 79%
35h: = 2,3-diF, 81%

Scheme 8: Reagents and conditions: KO^{t}Bu, THF, rt, 18 h.

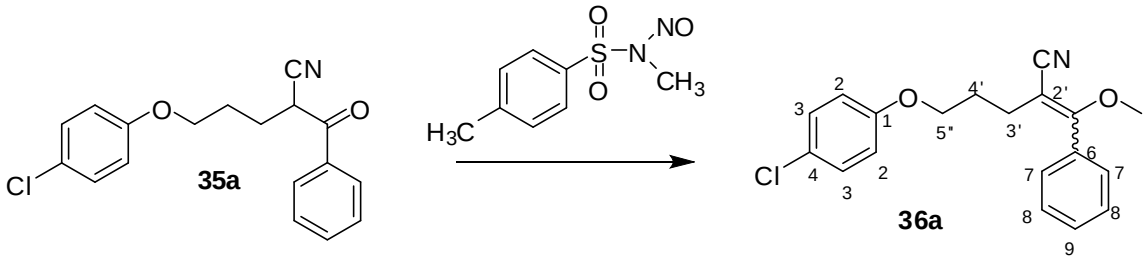
Table 5: Key signal in the ^{1}H NMR spectra of α-cyano ketones **35b-35h**.

	-CH-CN (ppm)
Compound **35b**	4.46 (dd)
Compound **35c**	4.46 (dd)
Compound **35d**	4.50 (dd)
Compound **35e**	4.50 (dd)
Compound **35f**	4.51 (dd)
Compound **35g**	4.64 (dd)
Compound **35h**	4.72 – 4.60 (m)

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

	-C=O (ppm)	-CH-CN (ppm)
Compound **35b**	179.4	30.43
Compound **35c**	190.2	39.25
Compound **35d**	190.4	39.41
Compound **35e**	190.5	39.40
Compound **35f**	190.5	39.41
Compound **35g**	190.6	39.70
Compound **35h**	190.5	39.40

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

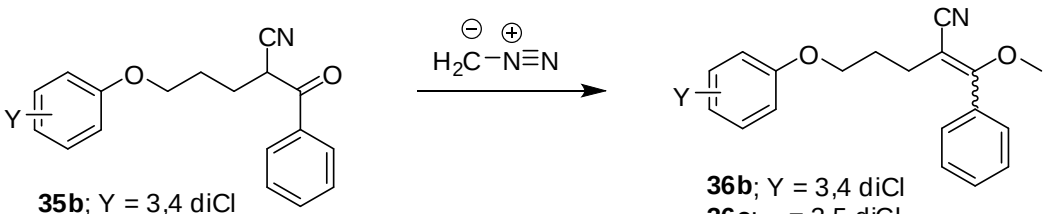


Scheme 9: Reagents and conditions: KOH, Et_2O, carbitol, DCM, rt, 18 h, quant.

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 ^{1}H NMR and ^{13}C NMR spectra. The ^{1}H 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 ^{13}C 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 ^{1}H NMR and ^{13}C NMR spectra of the products were observed.

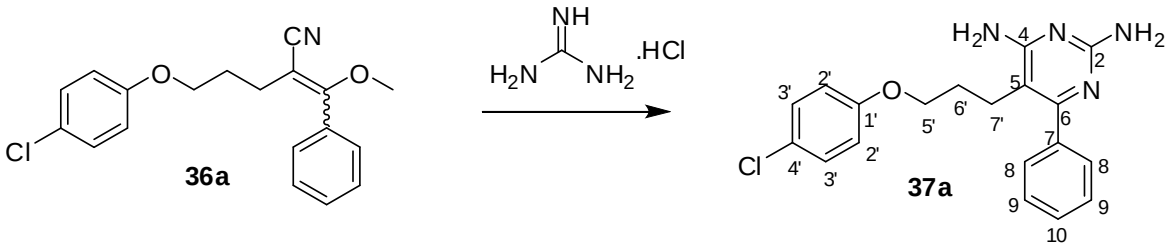


35b; Y = 3,4 diCl
35c: = 3,5 diCl
35d: = 4-F
35e: = 3-F
35f: = 3-CF_3
35g = 2,4- diBr
35h: = 2,3-diF

36b; Y = 3,4 diCl
36c: = 3,5 diCl
36d: = 4-F
36e: = 3-F
36f: = 3-CF_3
36g = 2,4- diBr
36h: = 2,3-diF

Scheme 10: Reagents and conditions: KOH, Et_2O, carbitol, DCM, rt, 18 h, quant.

2.1.5 Synthesis of 5-(3-(4-chlorophenoxy)propyl)-6-phenylpyrimidine-2,4-diamine and analogues.

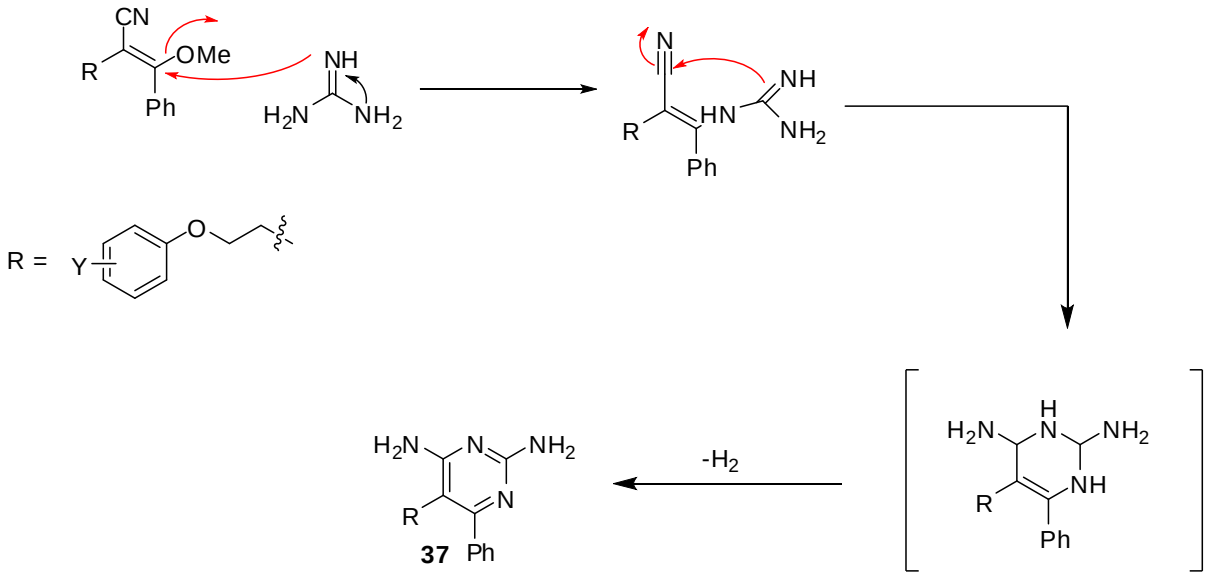


36a **37a**

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.[95] 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.

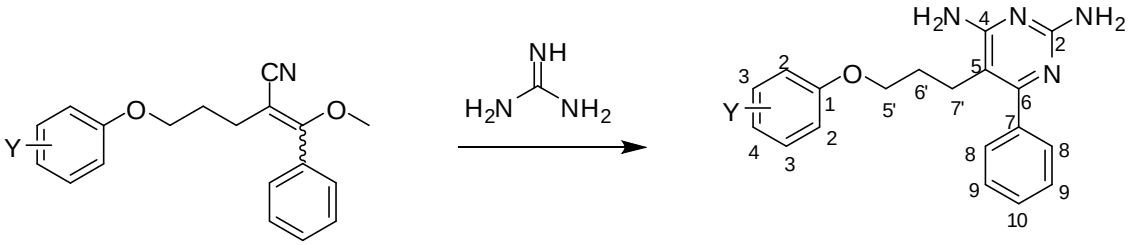


Scheme 12: Proposed mechanism for the formation of pyrimidine **37**.

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 [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.

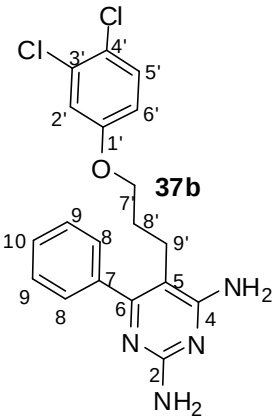


36b: Y = 3,4 diCl,
36c: = 3,5 diCl,
36e: = 3-F

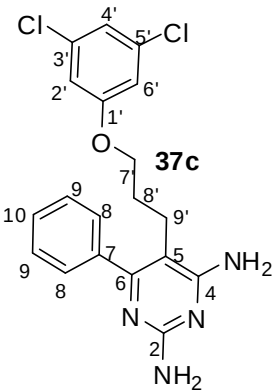
37b: Y = 3,4 diCl, 11%
37c: = 3,5 diCl, 9%
37e: = 3-F, 9%

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

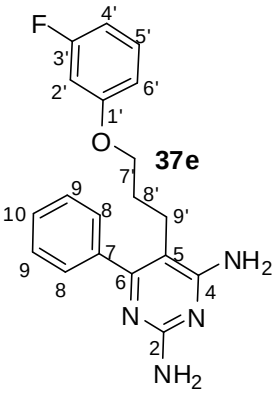
The ^{1}H NMR spectrum of the final compound **37b** contained 6 signals in total. Again as expected, the methoxy signals of the starting material at 3.48 ppm and 3.45 ppm had disappeared. Two triplets at 2.52 ppm and 1.31 ppm integrating for two protons each, were assigned to H7' (-OCH_2-) and H9'. 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 $[M+H]^+$ 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 $C_{19}H_{19}Cl_2N_4O$).



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

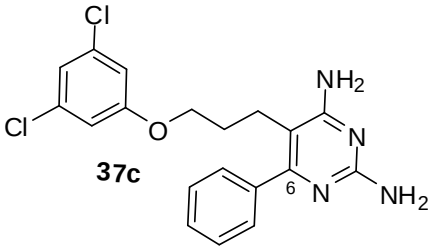
The pyrimidine compounds synthesised were tested for antimalarial activity in an *in vitro P. falciparum* screen on a Gambian FCR-3 strain (chloroquine and cycloguanil resistant strain) at the Wits School of Pharmacy and Pharmacology by Prof R van Zyl. The *P. falciparum* (FCR-3) strain was cultured *in vitro* at 37°C in 3% O_2, 5% CO_2, 92% N_2 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.**

Compound	IC_{50} (µM)	n
37a	3.69 ± 0.35	3
37b	14.80 ± 2.97	3
37c	0.09 ± 0.01	3
37e	Not obtained	0
Quinine	2.81 ± 0.57	3
DHA	0.00614 ± 0.00111	3
Methotrexate	0.23 ± 0.18	3

The most active compound from the pyrimidine series tested was **37c**, with an IC_{50} 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.



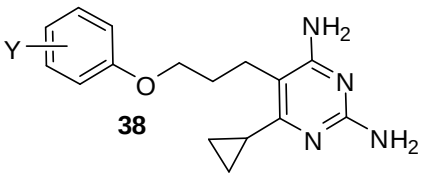
37c

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

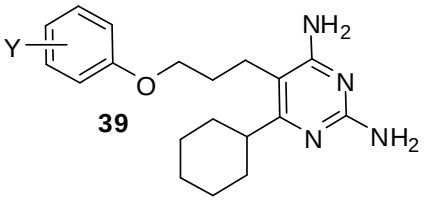
Further assessment of **37c** will be considered for future work in order to better understand these results. This will include toxicity, molecular modelling, and a biochemical DHFR assay.

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**.



38

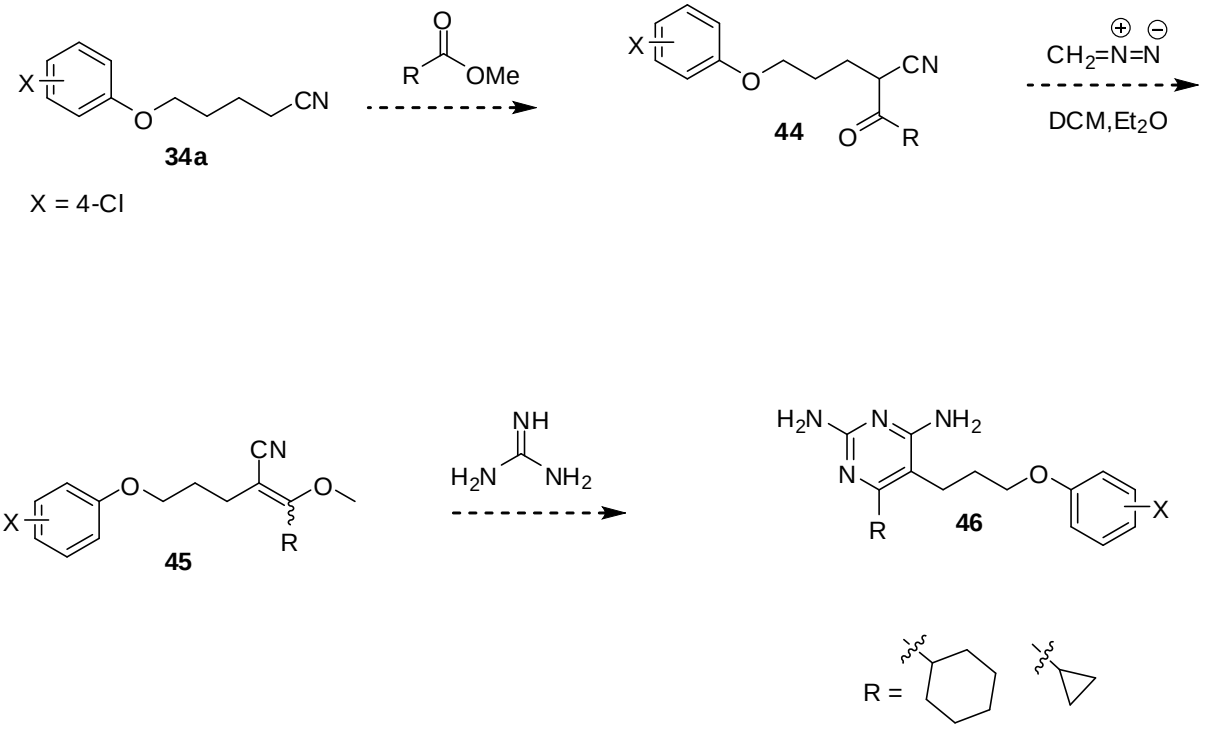


39

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



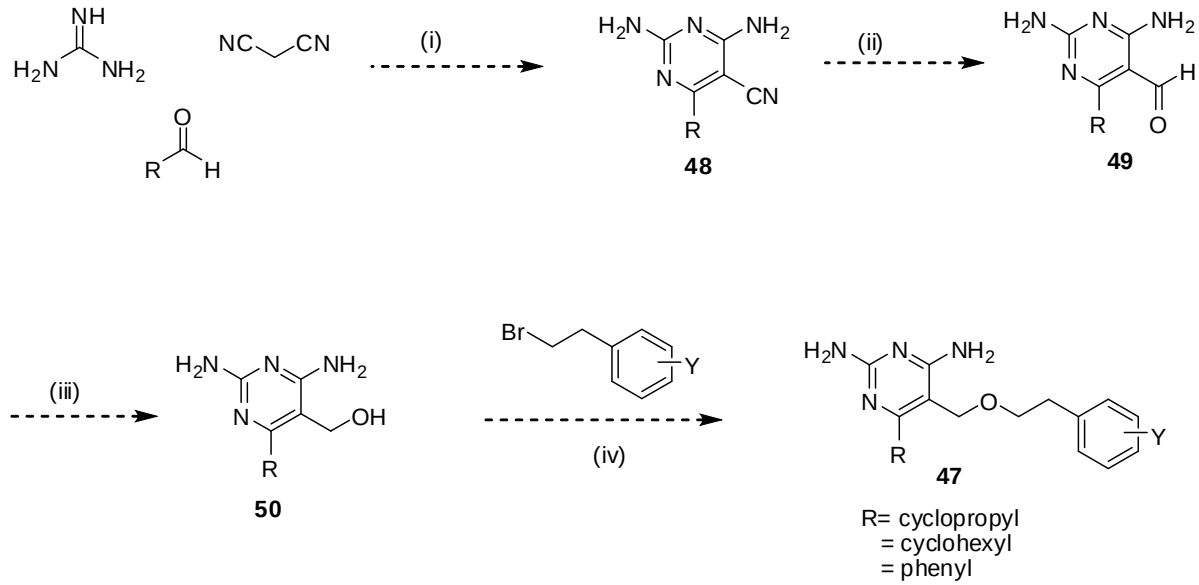
Scheme 14: Planned synthetic approach to 5-(3-(4-chlorophenoxy)propyl)-6-cyclopropyl/cyclohexyl pyrimidine-2,4-diamine.

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).

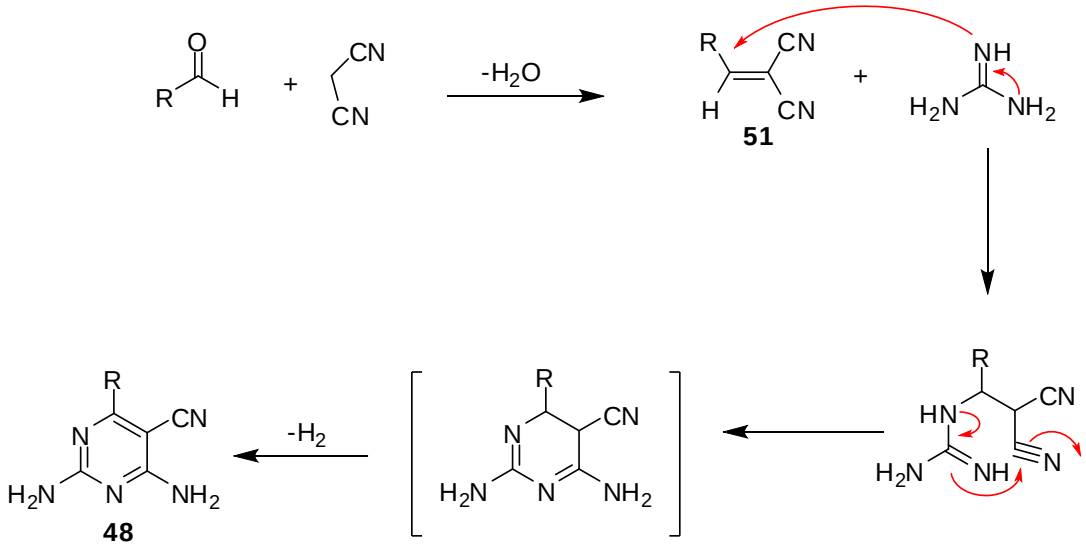
Recently, multicomponent reactions have become a valuable strategy in the preparation of structurally-diverse chemical libraries of drug-like heterocyclic compounds.[96-97] MCRs are chemical reactions in which three or more reagents are mixed together to yield a single product, in a cascade process. 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.[98] 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, H_2O/ethanol, reflux, 18 h; ii) H_2; Pd/C, aq. H_2SO_4, rt; iii) $NaBH_4$, MeOH, rt; iv) KO^tBu, 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**.

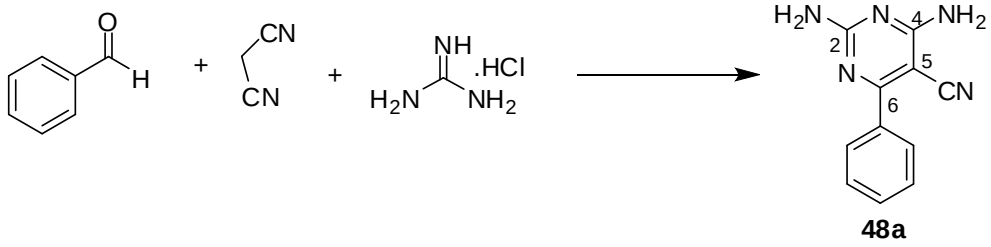
The mechanism for the formation of 5-cyano pyrimidine **48** is shown in Scheme 16. The first step is a Knoevenagel condensation between an aldehyde and malononitrile with its acidic α-hydrogens, to produce adduct **51**. This intermediate then undergoes nucleophilic attack by nitrogen of guanidine,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



Scheme 16: Proposed mechanism of formation of **48** via multicomponent coupling reaction.

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.



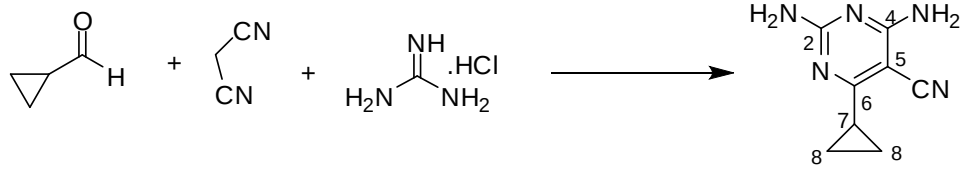
Scheme 17: Reagents and conditions: NaOAc, H_2O/ethanol, reflux, 18 h, 44%.

The ^{1}H 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.



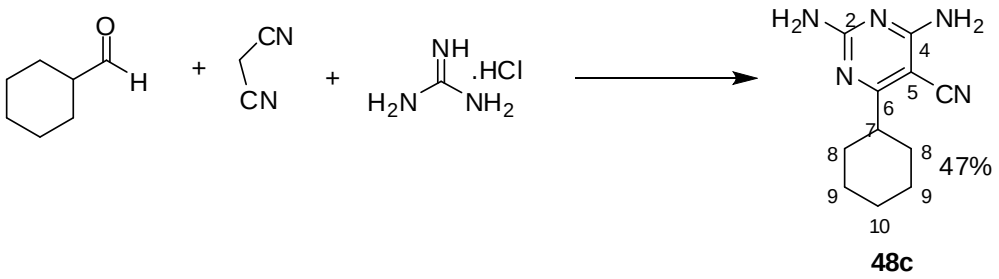
48b

Scheme 18: Reagents and conditions: NaOAc, H_2O/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

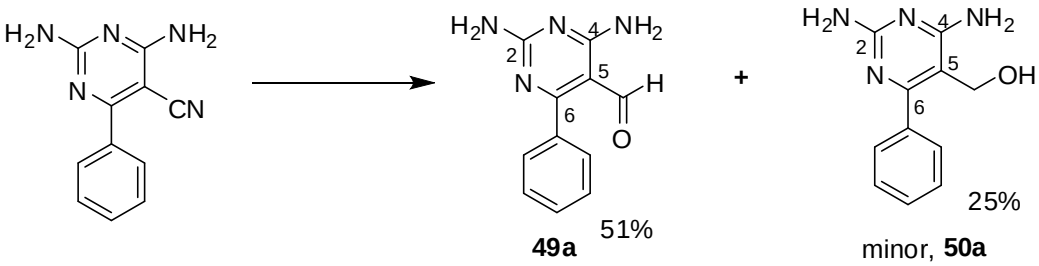
characteristic signal of C-5 of the pyrimidine ring visible at 77.02 ppm. The success of the reaction was also indicated by a stretching band at 2203 cm^{-1} in the IR spectrum, which is an indication of the CN group. The mass spectrum also corresponded well with the expected mass of the product.



Scheme 19: Reagents and conditions: NaOAc, H_2O/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

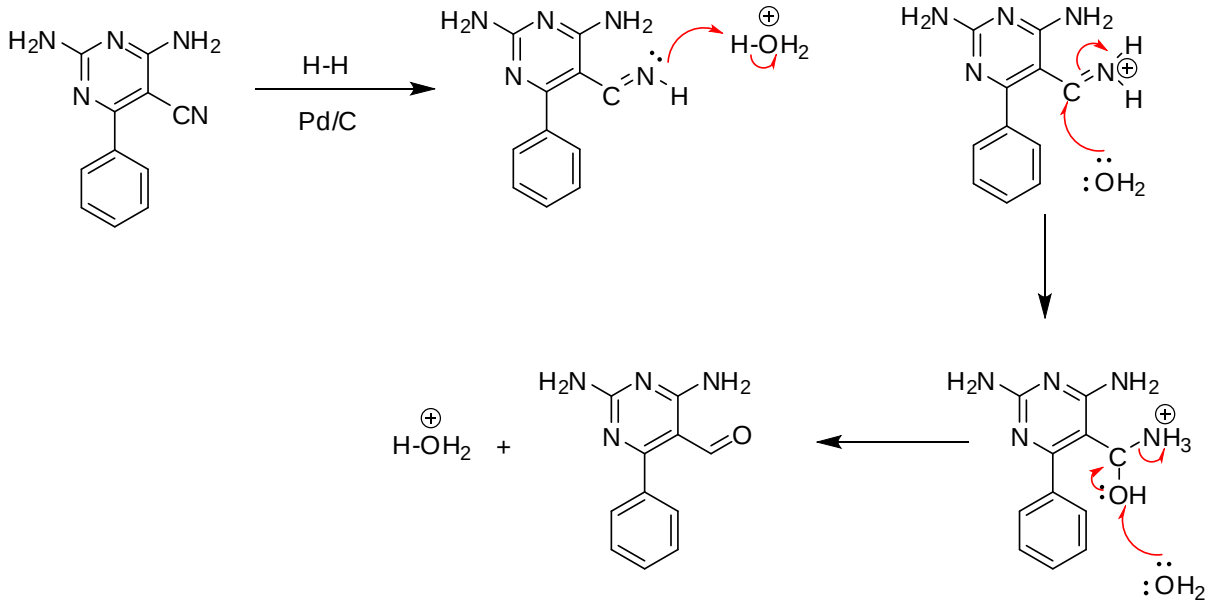


Scheme 20: Reagents and conditions: H_2; Pd/C, H_2O/H_2SO_4, rt, 18 h.

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.

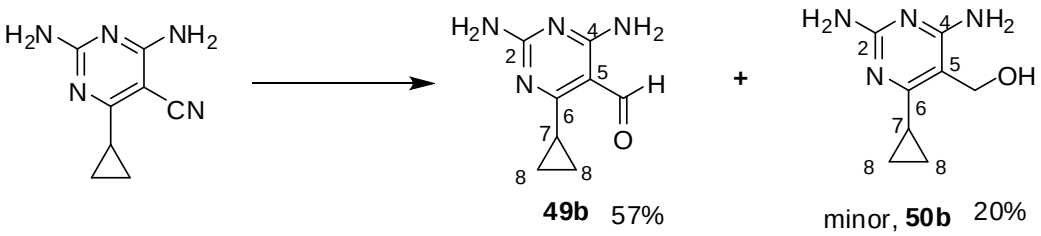
Compound **50a** was also isolated and characterised using NMR spectroscopy. The ^{1}H NMR spectrum confirmed that it was indeed an alcohol **50a** with a triplet at 4.83 ppm and a doublet at 4.20 ppm due to OH and CH_2 groups, respectively. The ^{13}C NMR spectrum further confirmed this with a signal at 56.94 ppm due to CH_2OH.



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).

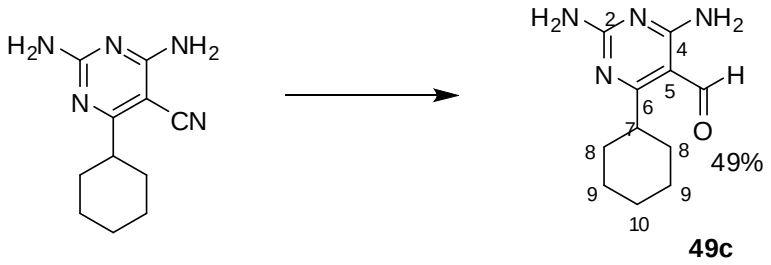


Scheme 22: Reagents and conditions: H_2; Pd/C, H_2O/H_2SO_4, rt, 18 h.

The ^{1}H 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 ^{13}C 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.

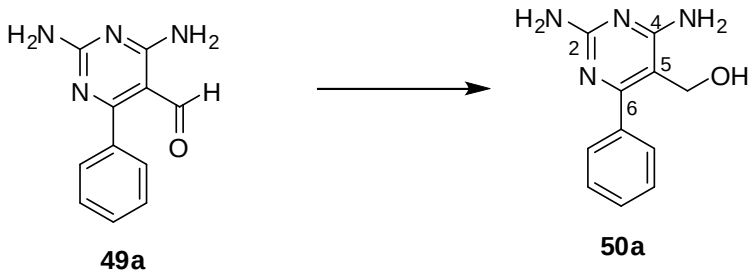
The cyclohexyl analogue **49c** was prepared in a similar manner (Scheme 23). Again as expected, the ^{1}H NMR spectrum of the resultant product **49c** contained a downfield singlet at 10.08 ppm, characteristic of the aldehyde proton. A signal at 117.7 ppm due to the CN group had disappeared. The corresponding 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 $C_{11}H_{17}N_4O$: 221.1401).



49c

Scheme 23: Reagents and conditions: H_2; Pd/C, H_2O/H_2SO_4, rt, 18 h, 49%.

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



49a **50a**

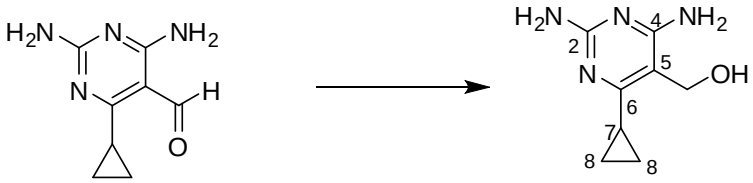
Scheme 24: Reagents and conditions: $NaBH_4$, methanol, rt, 3 h, 88%.

The next step in our synthetic sequence was the reduction of the aldehyde group in 2,4-diamino-6-phenylpyrimidine-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 $NaBH_4$ 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 CH_2 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 ^{13}C NMR spectrum was assigned to CH_2OH.

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



49b **50b**

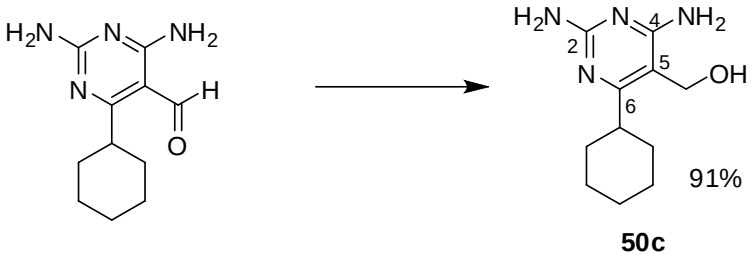
Scheme 25: Reagents and conditions: $NaBH_4$, methanol, rt, 3 h, 84%.

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 CH_2 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 CH_2 groups, respectively. The signal at 10.08 ppm due

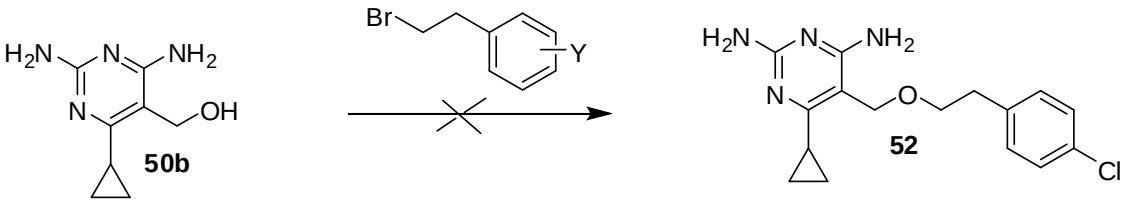
to the aldehyde proton had disappeared. The success of the reaction was also indicated by a stretching band at 3159 cm^{-1} in the IR spectrum, due to the OH group.



50c

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



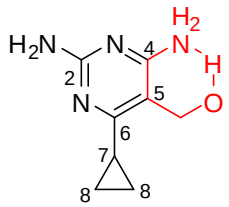
50b **52**

Scheme 27: Reagents and conditions: KO^tBu, 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.

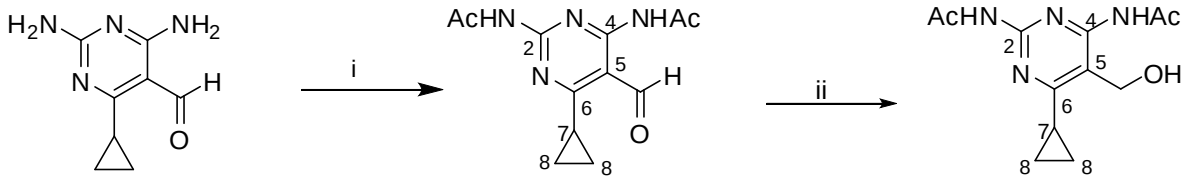
After this time, TLC analysis showed only starting materials present, with no sign of a new product being formed. The reaction was then warmed up to 90°C overnight. When no change was observed, the reaction mixture was quenched and the organic material isolated by extraction and purified by silica gel chromatography. NMR spectroscopic analysis confirmed that the two spots identified were the two starting materials. Several bases (NaH, K_2CO_3 and Et_3N), 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.



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]



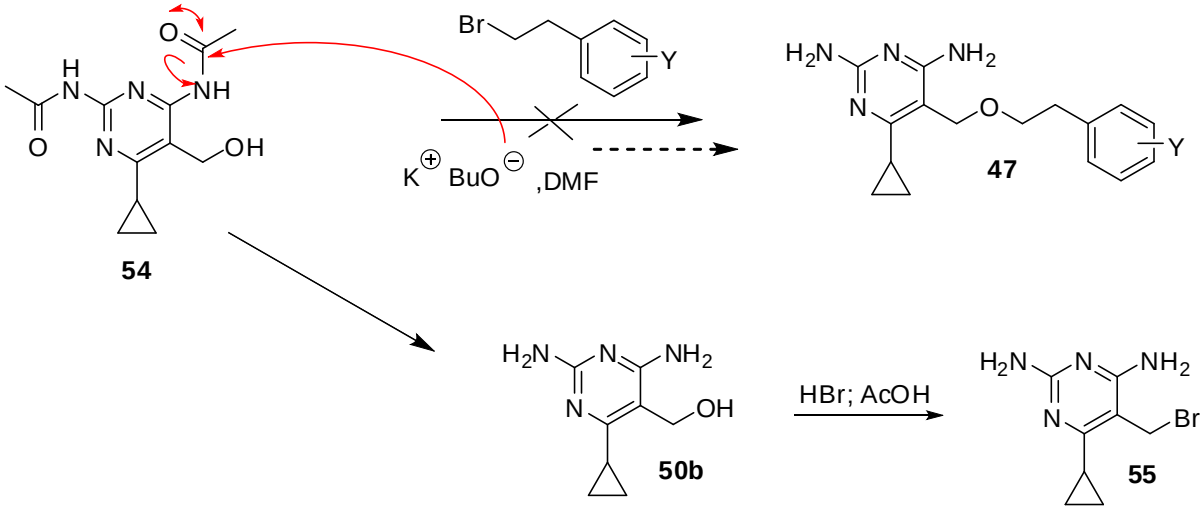
49b **53** **54**

Scheme 28: Reagents and conditions: i) Ac_2O, 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.

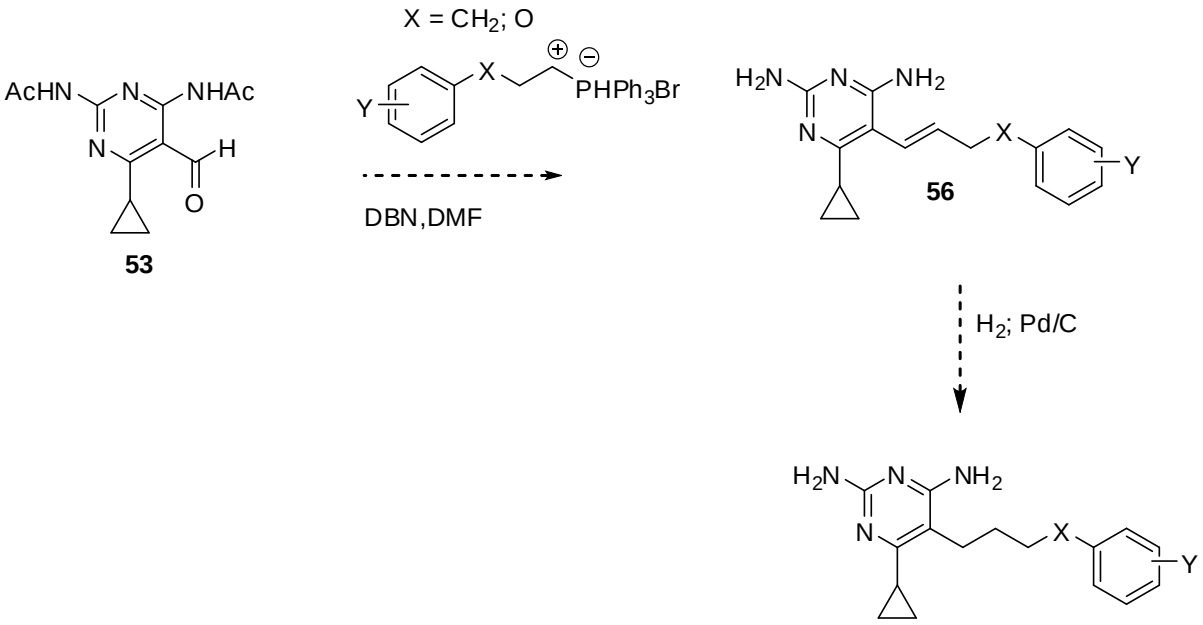


Scheme 29

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.[99] However, to our disappointment, this reaction was also unsuccessful.

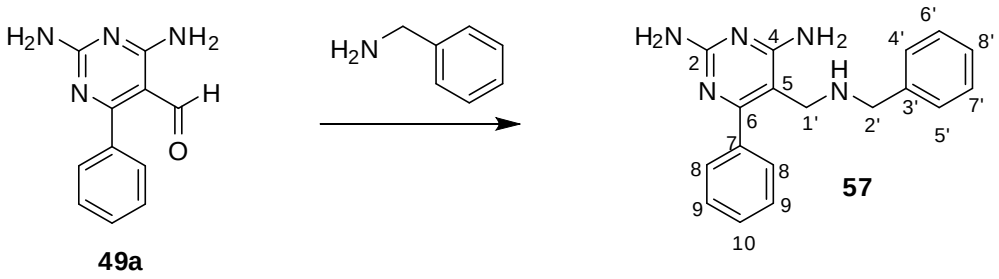


Scheme 30: Wittig approach towards flexible pyrimidine analogues.

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.

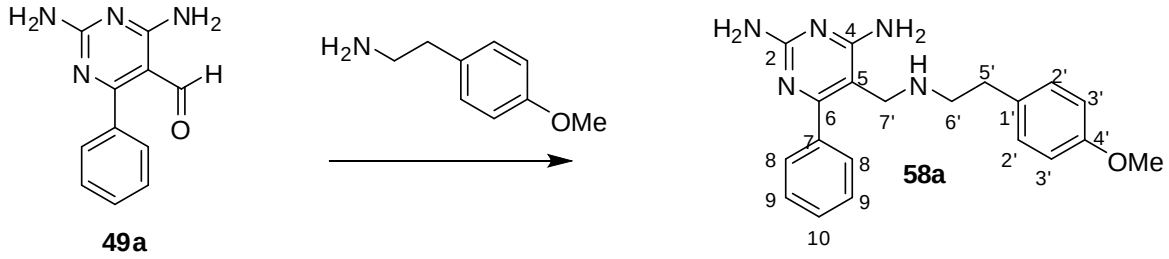


Scheme 31: Reagents and conditions: Ethanol, AcOH, $NaCNBH_3$, reflux, 18 h, 47%.

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 $C_{18}H_{20}N_5$).

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

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



Scheme 32: Reagents and conditions: Ethanol, AcOH, $NaCNBH_3$, reflux, 18 h, 31%.

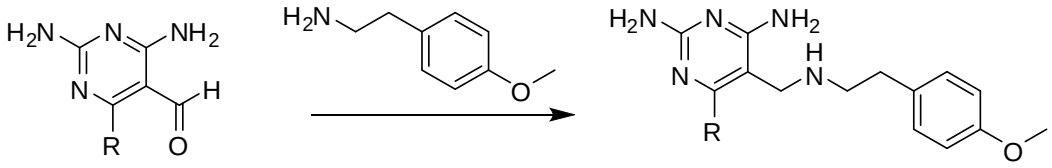
Aldehyde **49a** was therefore treated with 2-(4-methoxyphenyl)ethanamine as described above, to afford **58a** as a yellow solid in a yield of 31%.

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.

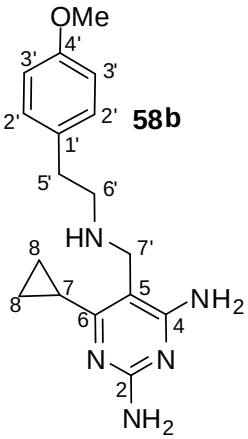
The two amino groups exchanged with the solvent used for spectroscopic analysis. The presence of C-5 of the pyrimidine ring was evident in the ^{13}C NMR spectrum at 102.9 ppm. The ^{13}C NMR spectrum was also convincing, with signals at 55.62 ppm and 45.94 ppm assigned to the CH_2 groups of the alkyl 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.

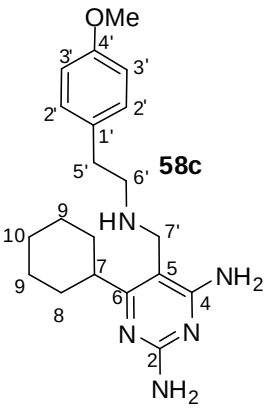


Scheme 33: Reagents and conditions: Ethanol, AcOH, $NaCNBH_3$, reflux, 18 h.

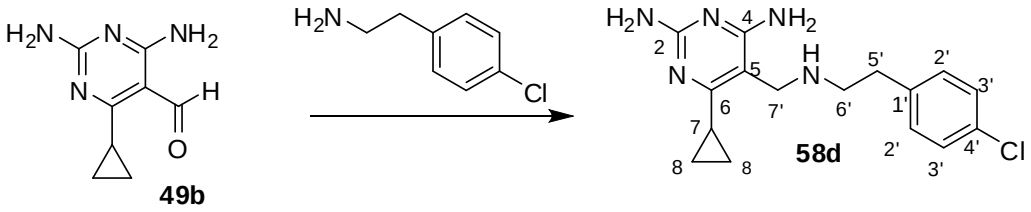


In both the ^{1}H NMR and ^{13}C NMR spectra of the pyrimidine product **58b**, the relevant aldehyde signal had disappeared. In the ^{1}H NMR spectrum, two doublets at 7.11 ppm and 6.86 ppm were assigned to H2' and H3', respectively. The broad signal at 2.81 ppm integrated for four protons due to the 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 $C_{20}H_{29}N_5ONa$).



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



Scheme 34: Reagents and conditions: Ethanol, AcOH, $NaCNBH_3$, reflux, 18 h, 33%.

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.**

Compound	IC_{50} (µM)	n
57	1.94 ± 0.27	3
58a	28.37 ± 5.74	3
58b	18.77 ± 2.18	3
58c	15.68 ± 3.24	3
58d	0.034 ± 0.007	3
Quinine	2.81 ± 0.57	3

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.

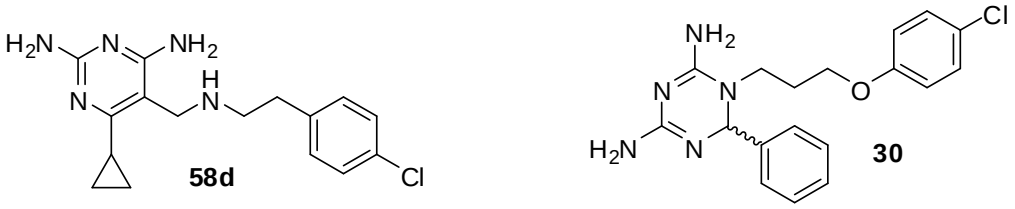
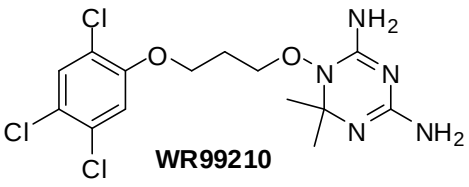
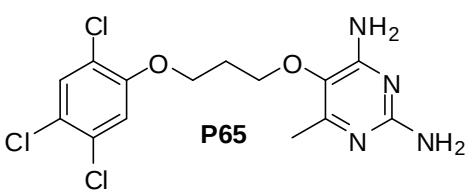


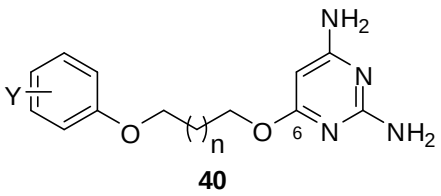
Figure 32: Pyrimidine analogue that displayed better antimalarial activity from the second generation of compounds bearing a flexible side chain.

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

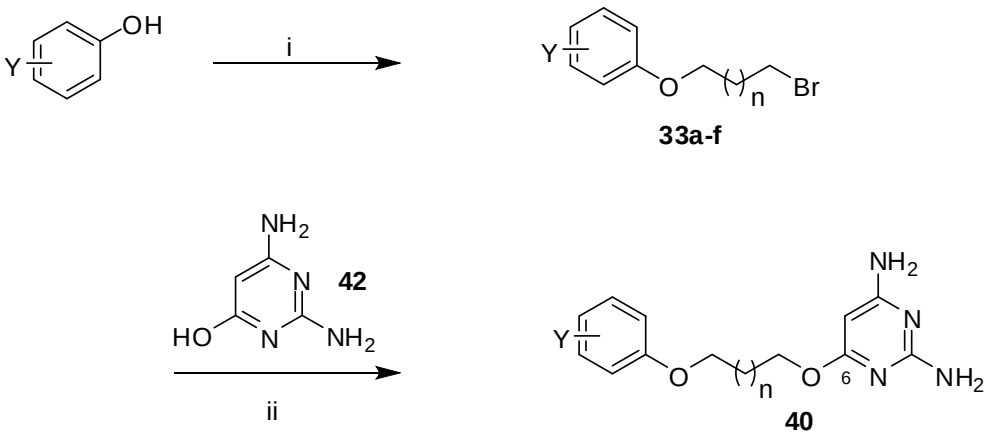
In another aspect of this project, we wanted to assess the effect of moving the flexible side chain on the pyrimidine ring from C-5 to C-6. We realised this could be readily achieved by simple substitution of a 4-hydroxypyrimidine. This would afford a series of compounds with an oxygenated side chain, 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.







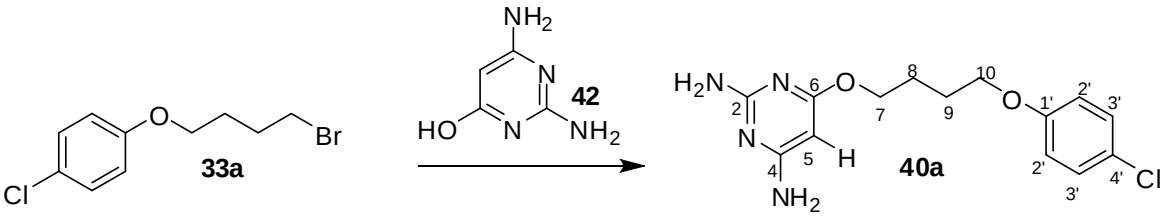
The scheme shown below outlines our planned synthesis of these products. Starting from commercially available substituted phenols, alkylation could be achieved using 1,3-dibromopropane or 1,4-dibromobutane under basic conditions by the procedure described previously (**2.1.1)**. The bromoethers would 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**.[101]



Scheme 35: Reagents and conditions: i) For n = 1, 1,3-dibromopropane, K_2CO_3, CH_3CN, reflux; for n = 2, 1,4-dibromobutane, reflux; ii) K_2CO_3, CH_3CN, 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.

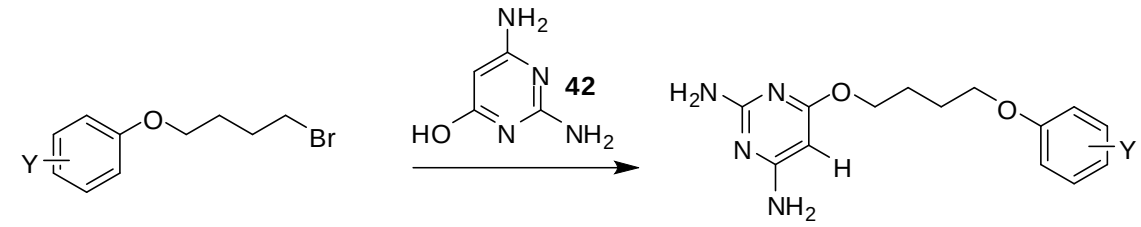


Scheme 36: Reagents and conditions: K_2CO_3, CH_3CN, reflux, 20 h, 50%.

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-CF_3

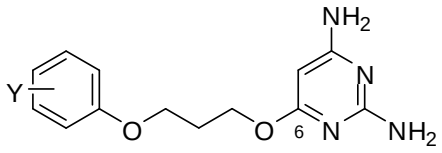
40b; Y = 3,4-diCl, 45%
40c: = 3,5-diCl, 55%
40d: = 4-F, 33%
40e: = 3-F, 40%
40f: = 3-CF_3, 46%

Scheme 37: Reagents and conditions: K_2CO_3, CH_3CN, reflux, 20 h.

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

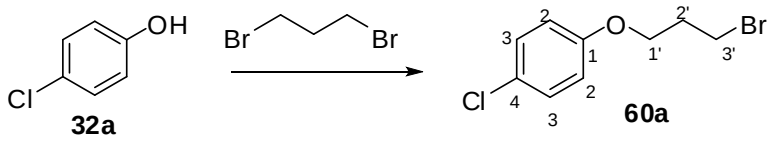
	2 × (NH_2), ppm	-OCH_2 (H7), ppm	-OCH_2 (H10), ppm
Compound **40b**	5.99, 5.82	4.15 – 4.11 (m)	4.05 – 4.01 (m)
Compound **40c**	5.98, 5.82	4.15 – 4.10 (m)	4.06 – 4.00 (m)
Compound **40d**	5.99, 5.81	4.13 (t)	3.98 – 3.94 (m)
Compound **40e**	6.02, 5.86	4.15 (t)	4.01 (t)
Compound **40f**	6.01, 5.86	4.16 (t)	4.09 (t)

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**.



59

2.6.2 Synthesis of 1-(3-bromopropoxy)-4-chlorobenzene and analogues.

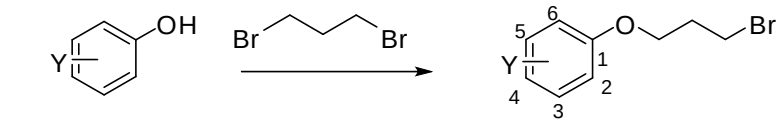


Scheme 38: Reagents and conditions: K_2CO_3, CH_3CN, reflux, 20 h, 99%.

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_2Br 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.



32b; Y = 3,4-diCl
32c: = 3,5-diCl
32d: = 4-F

60b; Y = 3,4-diCl, 95%
60c = 3,5-diCl, 97%
60d = 4-F, 87%

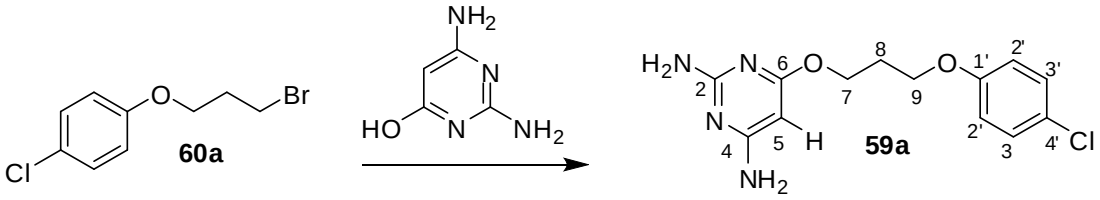
Scheme 39: Reagents and conditions: K_2CO_3, CH_3CN, reflux, 20 h.

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

	Aromatic region (ppm)	-OCH_2- (ppm)	H-2' (ppm)	CH_2Br (ppm)
Compound **60b**	6.97 (dd, H2)	4.00 (t)	2.18 – 1.99 (m)	3.45 (t)
Compound **60c**	6.91 (t, H1), 6.75 (d, H3)	3.92 (t)	1.90 – 1.77 (m)	3.45 (t)
Compound **60d**	7.08 – 6.94 (m, H2), 6.85 – 6.81 (m, H3).	4.02 (t)	1.90 – 1.76 (m)	3.45(t)

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.



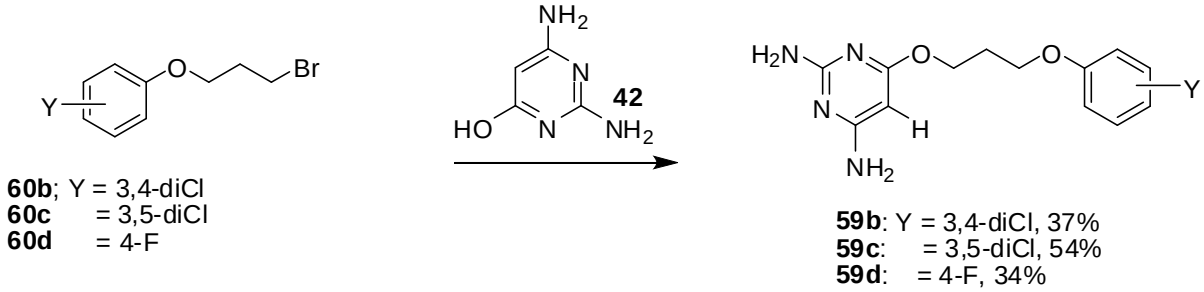
Scheme 40: Reagents and conditions: K_2CO_3, CH_3CN, reflux, 20 h, 35%.

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 $C_{13}H_{16}ClN_4O_2$).

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 ^1H NMR and ^{13}C NMR spectroscopy and HRMS. Diagnostic signals in the ^1H NMR spectra of pyrimidines **59b-d** are listed in Table 11 below.



60b; Y = 3,4-diCl
60c = 3,5-diCl
60d = 4-F

59b: Y = 3,4-diCl, 37%
59c: = 3,5-diCl, 54%
59d: = 4-F, 34%

Scheme 41: Reagents and conditions: K_2CO_3, CH_3CN, reflux, 20 h.

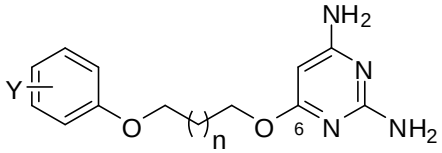
Table 11: Key signals in the ^1H NMR spectra of pyrimidines **59b-59d**.

	2 × (NH_2) (ppm)	-OCH_2 (H7), (ppm)	-OCH_2 (H9) (ppm)
Compound **59b**	5.99, 5.82	4.22 (t)	4.12 (t)
Compound **59c**	6.00, 5.84	4.21(t)	4.12 (t)
Compound **59d**	5.99, 5.85	4.22 (t)	4.12 – 3.95 (m)

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.

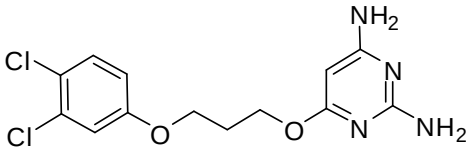
Table 12: Results of *in vitro* antimalarial assay for compounds **40a-f** and **59a-d.**



40: for n = 2
59: for n = 1

Compound	IC_{50} (µM)	n
40a	44.88 ± 7.76	3
40b	11.10	3
40c	7.66	1
40d	54.74 ± 10.91	3
40e	11.18	3
40f	18.02 ± 2.80	3
59a	83.45 ± 8.36	3
59b	4.46 ± 0.29	3
59c	22.80	1
59d	14.96	1
Quinine	2.81 ± 0.57	
DHA	0.00614 ± 0.00111	
Methotrexate	0.23 ± 0.18	

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.



60b

Figure 33: Most active compound from the series.

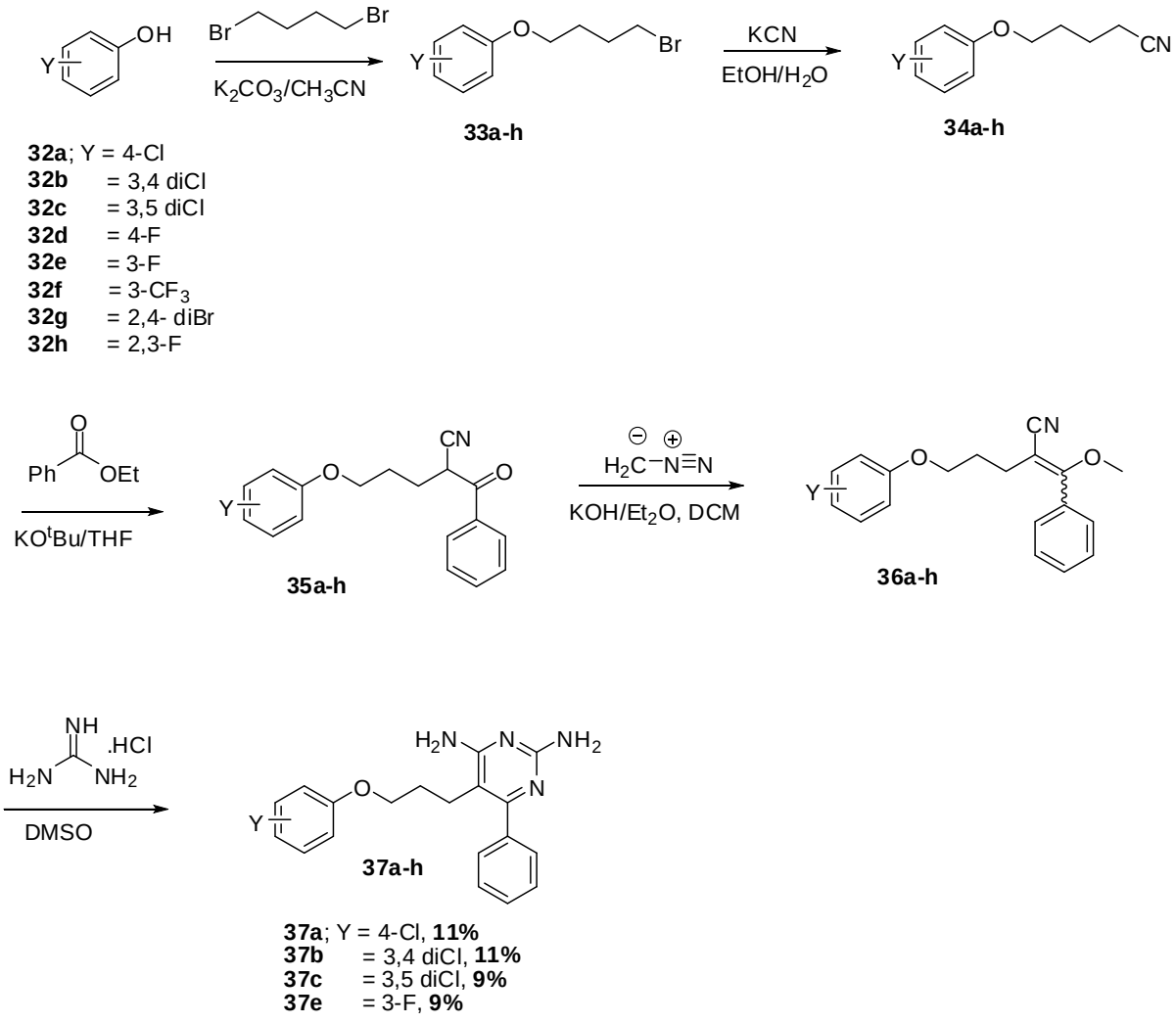
CHAPTER 3: CONCLUSION AND FUTURE WORK

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 α-cyano ketones **35a-h** in good yields of 63-94%. The α-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.

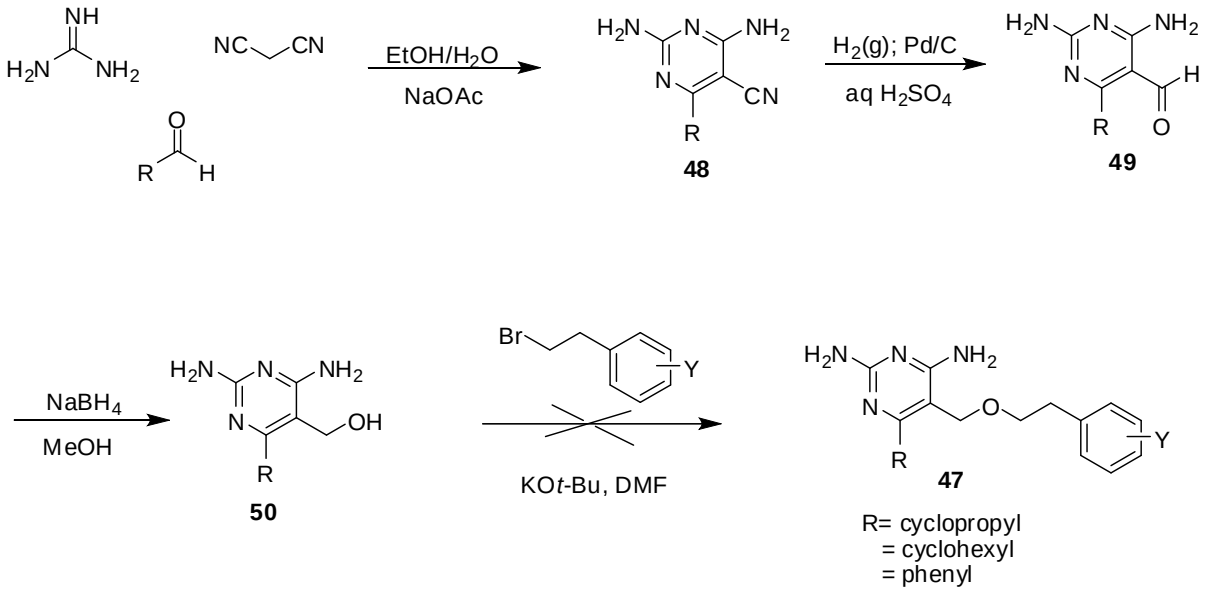
Based on these biological results, we embarked on the synthesis of analogues containing a smaller or more flexible non-aromatic substituent at the 6-position of the pyrimidine ring. The methodology used to prepare pyrimidines **37** was attempted in preparing these compounds, but unfortunately this was not successful.



Scheme 42

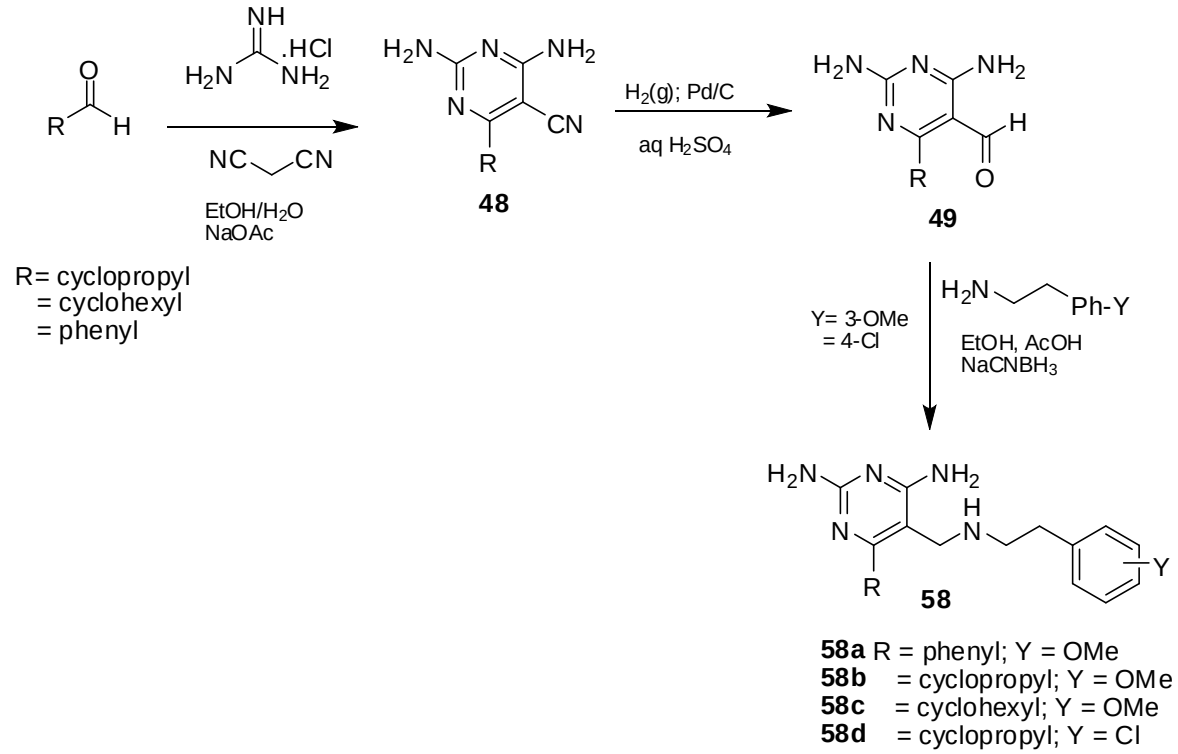
We then made use of a multicomponent coupling reaction for the synthesis of the flexible pyrimidines bearing a non-aromatic side chain. Starting from commercially available malononitrile, an aldehyde and guanidine hydrochloride, 5-cyanopyrimidines **48a-c** was formed in average yields of 41-55%. The 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-NH_2 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.



Scheme 43

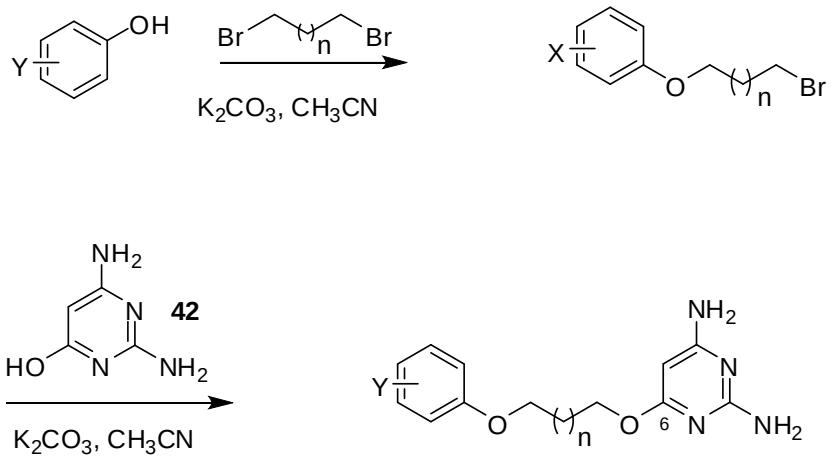
At this point, we focused our attention on functionalisation of the aldehyde group of **49a-c** to synthesise pyrimidines with a flexible linker via reductive amination. This methodology was initially tested using benzylamine and aldehyde **49a** and proved to be successful. We then reacted aldehydes **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 44

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_{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_{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).



40a n = 2; Y = 4-Cl
40b n = 2; Y = 3,4 diCl
40c n = 2; Y = 3,5 diCl
40d n = 2; Y= 4-F
40e n = 2; Y= 3-F
40f n = 2; Y = 3-CF_3

59a n = 1; Y = 4-Cl
59b n = 1; Y = 3,4 diCl
59c n = 1; Y = 3,5 diCl
59d n = 1; Y= 4-F

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 (IC_{50} 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.

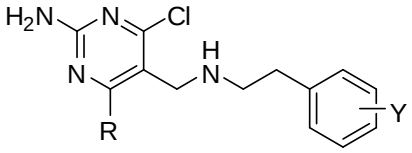


Figure 34

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, F_{254}.

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

^{1}H 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 ($CDCl_3$), the solvent contained tetramethylsilane (TMS, 0.05% v/v) as internal standard. For others, the residual solvent signal was used for referencing. ^{13}C 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.

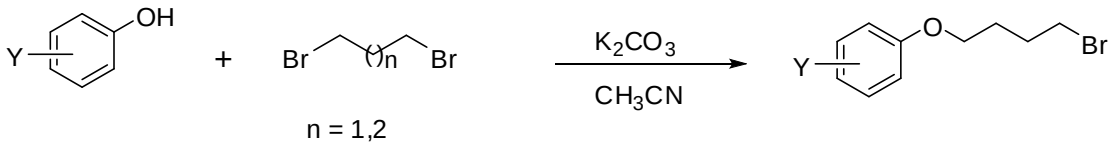
Infra-red spectra were recorded on a Bruker Tensor-27 Fourier Transform spectrometer.

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

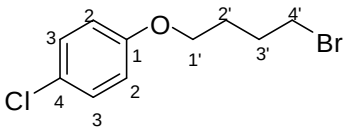


n = 1,2

Y = 4-Cl
= 3,4 diCl
= 3,5 diCl
= 4-F
= 3-F
= 2,3-diF
= 2,4- diBr
= 3-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:

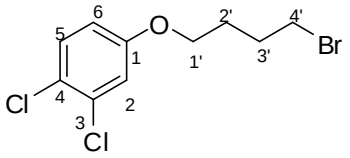
4.2.1 Synthesis of 1-(4-bromobutoxy)-4-chlorobenzene[102] 33a



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, $CDCl_3$)**: δ 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_2Br), 2.63 – 1.59 (4H, m, H2' and H3'). **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 157.3 (C-1), 129.3 (C-3), 125.6 (C-4), 115.7 (C-2), 67.0 (OCH_2), 33.3 (CH_2Br), 29.3 (C-3'), 27.8 (C-2'): **IR (ν_{max}/cm^{-1}):** 2958 (=C-H); 1593, 1578 (C=C); 1104 (C-O); 823 (C-Cl).

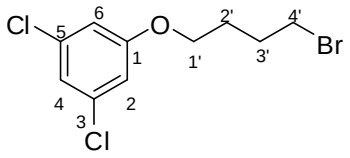
4.2.2 Synthesis of 1-(4-bromobutoxy)-3,4-dichlorobenzene[102] 33b



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, $CDCl_3$)**: 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, OCH_2), 3.45 (2H, t, *J* = 6.4 Hz, CH_2Br), 2.11 – 1.80 (4H, m, H2' and H3'). **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 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_2Br), 22.53 (C-3'), 17.01 (C-2'): **IR (ν_{max}/cm^{-1})**: 3041 (=C-H); 1578 (C=C); 1145 (C-O).

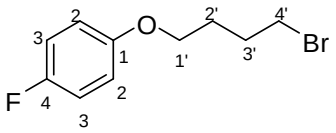
4.2.3 Synthesis of 1-(4-bromobutoxy)-3,5-dichlorobenzene[102] 33c



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, $CDCl_3$)**: δ 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_2Br), 2.11 – 1.80 (4H, m, H2' and H3').**^{13}C NMR (75 MHz, $CDCl_3$)**: δ 159.7 (C-1), 135.5 (C-3), 121.3 (C-4), 114.0 (C-2), 67.32 (OCH_2), 27.98 (CH_2Br), 22.33 (C-3'), 17.00 (C-2'). **IR (ν_{max}/cm^{-1}):** 2953 (=C-H); 1581, 1576 (C=C); 1141 (C-O).

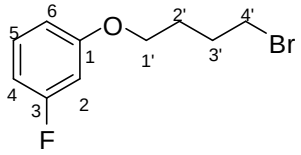
4.2.4 Synthesise of 1-(4-bromobutoxy)-4-fluorobenzene 33d



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% EtOAc/hexane) 0.82. *mp*: 32°C. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 7.01 – 6.92 (2H, m, H3), 6.85 – 6.79 (2H, m, H2), 3.96 (2H, t, J = 5.6 Hz, OCH_2), 3.44 (2H, t, J = 6.4 Hz, CH_2Br), 2.11 – 1.88 (4H, m, H2' and H3'). **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 157.3 (d, $J_{C\text{-}F}$ = 238.3 Hz, C-4), 154.9 (d, $J_{C\text{-}F}$ = 2.0 Hz, C-1), 115.8 (d, $J_{C\text{-}F}$ = 23.1 Hz, C-3), 115.4 (d, $J_{C\text{-}F}$ = 8.0 Hz, C-2), 67.41 (OCH_2), 33.38 (CH_2Br), 29.41 (C-3'), 27.88 (C-2'). **IR (ν_{max}/cm^{-1}):** 2930 (=C-H); 1599, 1578 (C=C); 1133 (C-O). ***HRMS*** *m/z*: calculated for $C_{10}H_{12}BrFONa$: 268.9956, found: $[M + Na]^+$ 268.9955.

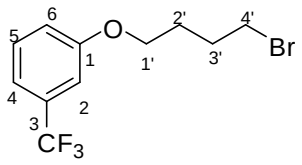
4.2.5 Synthesis of 1-(4-bromobutoxy)-3-fluorobenzene[103] 33e



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% EtOAc/hexane) 0.74. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 7.22 – 7.15 (1H, m, H4), 6.66 – 6.55 (3H, m, H2, H5 and H6), 3.95 (2H, t, J = 5.6 Hz, OCH_2), 3.45 (2H, t, J = 6.4 Hz, CH_2Br), 1.99 – 1.67 (4H, m, H2' and H3'). **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 163.6 (d, $J_{C\text{-}F}$ = 245.3 Hz, C-3), 159.9 (d, $J_{C\text{-}F}$ = 10.8 Hz, C-1), 130.3 (d, $J_{C\text{-}F}$ = 10.0 Hz, C-5), 110.2 (d, $J_{C\text{-}F}$ = 2.9 Hz, C-6), 107.8 (d, $J_{C\text{-}F}$ = 21.3 Hz, C-4), 102.2 (d, $J_{C\text{-}F}$ = 24.8 Hz, C-2), 67.70 (OCH_2), 27.91 (CH_2Br), 22.52 (C-3'), 17.00 (C-2'): **IR (ν_{max}/cm^{-1})**: 2956 (=C-H); 1593, 1577 (C=C); 1084 (C-O).

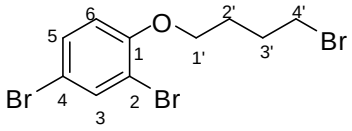
4.2.6 Synthesis of 1-(4-bromobutoxy)-3-(trifluoromethyl)benzene[104] 33f



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, $CDCl_3$)**: δ 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_2Br); 2.16 – 1.87 (4H, m, H2' and H3'). **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 159.1 (C-1), 131. 9 (q, $J_{C\text{-}F}$ = 32.2 Hz, C-CF_3), 130.0 (C-5), 124.1 (q, $J_{C\text{-}F}$ = 272.4 Hz, CF_3), 118.0 (C-6), 117.4 (q, $J_{C\text{-}F}$ = 3.8 Hz, C-4), 111.3 (q, $J_{C\text{-}F}$ = 3.7 Hz, C-2), 67.19 (OCH_2), 33.29 (CH_2Br), 29.42 (C-3'), 27.82 (C-2'): **IR (ν_{max}/cm^{-1})**: 2963 (=C-H); 1594 (C=C); 1066 (C-O); 1224 (C-F).

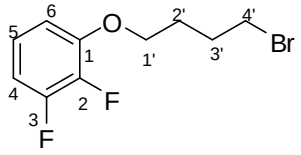
4.2.7 Synthesis of 2,4-dibromo-1-(4-bromobutoxy)benzene 33g



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, $CDCl_3$)**: δ 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_2Br), 2.09 – 1.80 (4H, m, H2' and H3'). **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 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_2Br), 22.50 (C-3'), 17.06 (C-2') : **IR (ν_{max}/cm^{-1})**: 3042 (=C-H); 1583 (C=C); 1095 (C-O). ***HRMS*** *m/z*: calculated for $C_{10}H_{11}Br_3ONa$: 406.8260, found: $[M + Na]^+$ 406.8266.

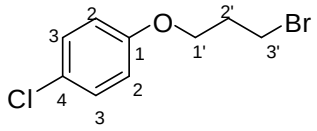
4.2.8 Synthesis of 1-(4-bromobutoxy)-2,3-difluorobenzene[104] 33h



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% EtOAc/hexane) 0.70. *mp*: 28°C. **^{1}H NMR (300 MHz, $CDCl_3$)**: 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_2), 3.50 (2H, t, *J* = 6.4 Hz, CH_2Br), 2.15 – 1.91 (4H, m, H2' and H3'). **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 151.5 (dd, $J_{C\text{-}F}$ = 246.8, 10.4 Hz, C-3), 148.6 (dd, $J_{C\text{-}F}$ = 7.9, 3.2 Hz, C-1), 141.5 (dd, $J_{C\text{-}F}$ = 247.3, 14.1 Hz, C-2), 123.2 (dd, $J_{C\text{-}F}$ = 8.7, 5.2 Hz, C-5), 109.9 (d, $J_{C\text{-}F}$ = 2.9 Hz, C-6), 109.2 (dd, $J_{C\text{-}F}$ = 17.7, 9.5 Hz, C-4), 68.77 (OCH_2), 33.34 (CH_2Br), 29.27 (C-3'), 27.82 (C-2'); **IR (ν_{max}/cm^{-1})**: 2955 (=C-H); 1571 (C=C); 1113 (C-O).

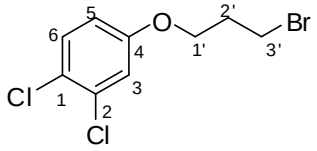
4.2.9 Synthesis of 1-(3-bromopropoxy)-4-chlorobenzene[102] 60a



4-Chlorophenol (6.50 g, 50.6 mmol) was dissolved in acetonitrile (300 ml). To the clear solution, was added 1,3-dibromopropane (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% EtOAc/hexane) 0.83. *mp:* 28°C. **^{1}H NMR (300 MHz, $CDCl_3$)**: 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_2), 3.46 (2H, t, *J* = 6.5 Hz, CH_2Br), 2.19 – 1.95 (2H, m, H2'). **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 157.3 (C-1), 129.3 (C-3), 125.6 (C-1), 115.7 (C-2), 67.2 (OCH_2), 33.3 (CH_2Br), 27.8 (C-2'). **IR (ν_{max}/cm^{-1}):** 2958 (=C-H); 1593, 1578 (C=C); 1104 (C-O).

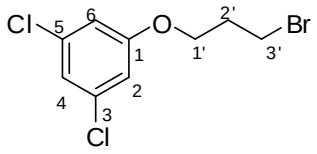
4.2.10 Synthesis of 4-(3-bromopropoxy)-1,2-dichlorobenzene[102] 60b



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, $CDCl_3$)**: δ 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_2), 3.45 (2H, t, *J* = 6.4 Hz, CH_2Br), 2.18 -1.99 (2H, m, H2'). **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 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_2), 25.19 (CH_2Br), 14.08 (C-2'): **IR (ν_{max}/cm^{-1})**: 3041 (=C-H); 1578 (C=C); 1145 (C-O).

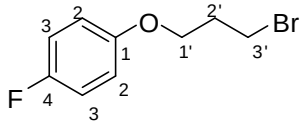
4.2.11 Synthesis of 1-(3-bromopropoxy)-3,5-dichlorobenzene[102] 60c



3,5-Dichlorophenol (5.00 g, 30.7 mmol) was dissolved in acetonitrile (200ml). 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, $CDCl_3$)**: δ 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_2), 3.45 (2H, t, *J* = 6.4 Hz, CH_2Br), 1.90-1.77 (2H, m, H2'); **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 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_2), 27.98 (CH_2Br), 22.33 (C-2'): **IR (ν_{max}/cm^{-1}):** 2953 (=C-H); 1581, 1576 (C=C); 1141 (C-O).

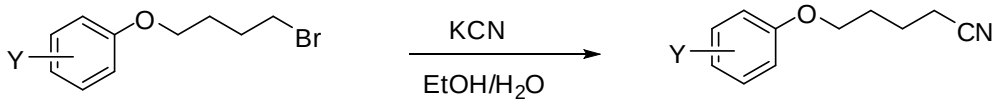
4.2.12 Synthesis of 1-(3-bromopropoxy)-4-fluorobenzene[103] 60d



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. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 7.08 – 6.94 (2H, m, H3), 6.85 – 6.81 (2H, m, H2), 4.02 (2H, t, J = 5.6 Hz, OCH_2), 3.45 (2H, t, J = 6.4 Hz, CH_2Br), 2.15 – 2.07 (2H, m, H2'). **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 157.3 (d, $J_{C\text{-}F}$ = 238.3 Hz, C-4), 154.9 (d, $J_{C\text{-}F}$ = 2.0 Hz, C-1), 115.8 (d, $J_{C\text{-}F}$ = 23.1 Hz, C-3), 115.4 (d, $J_{C\text{-}F}$ = 8.0 Hz, C-2), 66.00 (OCH_2), 30.78 (CH_2Br), 14.15 (C-2'): **IR (ν_{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.

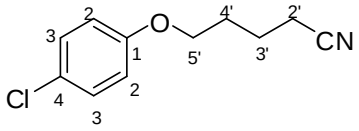


Y = 4-Cl
= 3,4 diCl
= 3,5 diCl
= 4-F
= 3-F
= 2,3-diF
= 2,4- diBr
= 3-CF_3

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 $MgSO_4$, 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:

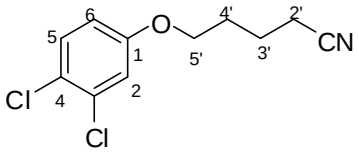
4.3.1 Synthesis of 5-(4-chlorophenoxy)pentanenitrile 34a



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. **^{1}H NMR (300 MHz, $CDCl_3$)**: 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, OCH_2), 2.41 (2H, t, *J* = 6.8 Hz, CH_2CN), 2.63 – 1.59 (4H, m, H3' and H4'). **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 157.3 (C-1), 129.3 (C-3), 125.6 (C-4), 119.5 (CN), 115.7 (C-2), 66.96 (OCH_2), 28.09 (C-4'), 22.35 (C-3'), 16.92 (CH_2CN): **IR (ν_{max}/cm^{-1})**: 3084, 2965 (=C-H); 2252 (CN); 1585, 1570 (C=C). ***HRMS*** *m/z*: calculated for $C_{11}H_{12}ClNONa$: 232.0507, found: $[M + Na]^+$ 232.0500

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

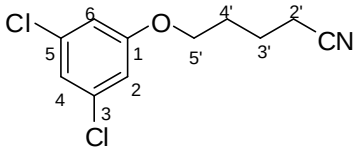


1-(4-Bromobutoxy)-3,4-dichlorobenzene (5.05 g, 16.9 mmol) was dissolved in ethanol/water (160 ml), and treated with potassium cyanide (1.21 g, 18.6 mmol). The product was isolated as pale yellow oil after purification by column chromatography. (3.55 g, 86%).

R_f (30% EtOAc/hexane) 0.66. **^{1}H NMR (300 MHz, $CDCl_3$)**: 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,

OCH_2), 1.85 (2H, t, *J* = 6.4 Hz, CH_2CN), 1.80 – 1.76 (4H, m, H4' and H3').**^{13}C NMR (75 MHz, $CDCl_3$)**: 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 (OCH_2), 28.71 (C-4'); 21.2 (C-3'), 17.4 (CH_2CN): **IR (ν_{max}/cm^{-1})**: 3083, 2964 (=C-H); 2250 (CN); 1599, 1574 (C=C). ***HRMS*** *m/z*: calculated for $C_{11}H_{11}Cl_2NONa$: 266.0118, found: $[M + Na]^+$ 266.0115.

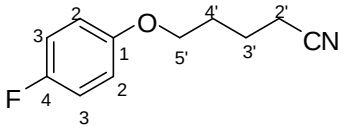
4.3.3. Synthesis of 5-(3,5-dichlorophenoxy)pentanenitrile 34c



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%).

R_f (30% EtOAc/hexane) 0.68. *mp*: 52-53°C. **^{1}H NMR (300 MHz, $CDCl_3$)**: 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, OCH_2), 2.45 (2H, t, *J* = 6.8 Hz, CH_2CN), 2.05 – 1.64 (4H, m, H3' and H4'). **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 159.7 (C-1), 135.5 (C-3), 121.3 (C-4), 119.3 (CN), 113.6 (C-2), 67.32 (OCH_2), 27.98 (C-4'); 22.33 (C-3'), 17.00 (CH_2CN): **IR (ν_{max}/cm^{-1})**: 3083, 2966 (=C-H); 2253 (CN); 1585, 1570 (C=C). ***HRMS*** *m/z*: calculated for $C_{11}H_{11}Cl_2NONa$: 266.0118, found: $[M + Na]^+$ 266.0115.

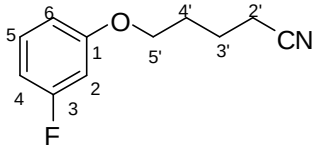
4.3.4 Synthesis of 5-(4-fluorophenoxy)pentanenitrile 34d



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%).

R_f (30% EtOAc/hexane) 0.59. *mp*: 26-27°C. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 7.08 – 6.83 (2H, m, H3), 6.84 – 6.72 (2H, m, H2), 3.91 (2H, t, *J* = 5.6 Hz, OCH_2), 2.39 (2H, t, *J* = 6.6 Hz, CH_2CN), 1.98 – 1.63 (4H, m, H4' and H3'). **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 157.3 (d, $J_{C\text{-}F}$ = 238.3 Hz, C-4), 154.8 (d, $J_{C\text{-}F}$ = 2.0 Hz, C-1), 119.5 (CN), 115.8 (d, $J_{C\text{-}F}$ = 23.1 Hz, C-3), 115.4 (d, $J_{C\text{-}F}$ = 8.0 Hz, C-2), 67.31 (OCH_2), 28.22 (C-4'); 22.45 (C-3'), 17.00 (CH_2CN): **IR (ν_{max}/cm^{-1})**: 2960, 2933 (=C-H); 2240 (CN); 1596, 1503 (C=C). ***HRMS*** *m/z*: calculated for $C_{11}H_{12}FNONa$: 216.0803, found: $[M + Na]^+$ 216.0809.

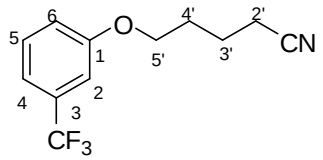
4.3.5 Synthesis of 5-(3-fluorophenoxy)pentanenitrile 34e



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_f (20% EtOAc/hexane) 0.44. **^{1}H NMR (300 MHz, $CDCl_3$)**: 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, OCH_2), 2.41 (2H, t, *J* = 6.8 Hz, CH_2CN), 1.99 – 1.67 (4H, m, H4' and H3'). **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 163.60 (d, $J_{C\text{-}F}$ = 245.3 Hz, C-3), 159.87 (d, $J_{C\text{-}F}$ = 10.8 Hz, C-1), 130.30 (d, $J_{C\text{-}F}$ = 10.0 Hz, C-5), 119.3 (CN), 110.15 (d, $J_{C\text{-}F}$ = 2.9 Hz, C-6), 107.80 (d, $J_{C\text{-}F}$ = 21.3 Hz, C-4), 102.17 (d, $J_{C\text{-}F}$ = 24.8 Hz, C-2), 67.7 (OCH_2), 28.4 (C-4'); 22.24 (C-3'), 17.00 (CH_2CN): **IR (ν_{max}/cm^{-1})**: 2961, 2932 (=C-H); 2239 (CN); 1596, 1503 (C=C). ***HRMS*** *m/z*: calculated for $C_{11}H_{12}FNONa$: 216.0803, found: $[M + Na]^+$ 216.0807.

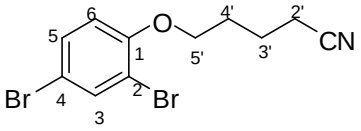
4.3.6 Synthesis of 5-(3-(trifluoromethyl)phenoxy)pentanenitrile 34f



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%).

R_f (30% EtOAc/hexane) 0.65. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 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); 2.42 (2H, t, J = 6.8 Hz, CH_2CN); 2.01 – 1.72 (4H, m, H4' and H3'). **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 159.1 (C-1), 131.9 (q, $J_{C\text{-}F}$ = 32.2 Hz, C-CF_3), 129.8 (C-5), 124.5 (q, $J_{C\text{-}F}$ = 272.4 Hz, CF_3), 119.2 (CN), 118.0 (C-6), 117.4 (q, $J_{C\text{-}F}$ = 3.8 Hz, C-4), 111.4 (q, $J_{C\text{-}F}$ = 3.7 Hz, C-2), 67.75 (OCH_2), 28.33 (C-4'); 22.77 (C-3'), 17.01 (CH_2CN): **IR (ν_{max}/cm^{-1})**: 3039, 2930 (=C-H); 2239 (CN); 1599 (C=C). ***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

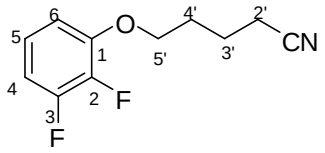


2,4-Dibromo-1-(4-bromobutoxy)benzene (5.00 g, 12.9 mmol) was dissolved in ethanol/water (160 ml). To the ethanoic solution, was added potassium cyanide (1.01 g, 15.5 mmol, 1.2 eq). The product was isolated as white solid after purification by column chromatography using 20% EtOAc/hexane as eluent. (3.12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

R_f (30% EtOAc/hexane) 0.67. *mp*: 49-50°C. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 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), 2.50 (2H, t, J = 6.7 Hz, CH_2CN), 2.09 – 1.80 (4H, m, H3' and H4'). **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 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_2CN): **IR**

(ν_{max}/cm^{-1}): 3091, 2948 (=C-H); 2253 (CN); 1580, 1480 (C=C). ***HRMS*** *m/z*: calculated for $C_{11}H_{11}Br_2NONa$: 353.9107, found: $[M + Na]^+$ 353.9108.

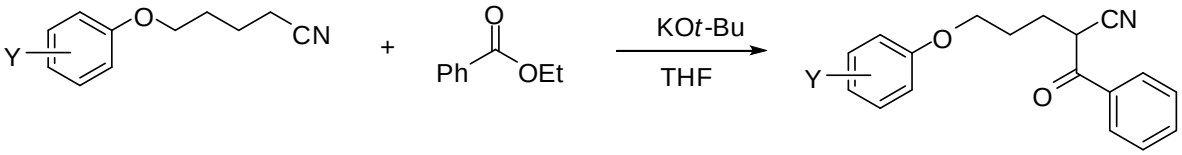
4.3.8 Synthesis of 5-(2,3-difluorophenoxy)pentanenitrile 34h



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%).

R_f (30% EtOAc/hexane) 0.67. *mp*: 48°C. **^{1}H NMR (300 MHz, CDCl$_3$)**: 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_2), 2.46 (2H, t, *J* = 6.8 Hz, CH_2CN), 2.08 – 1.75 (4H, m, H3' and H4'). **^{13}C NMR (75 MHz, CDCl$_3$)**: δ 151.46 (dd, $J_{C\text{-}F}$ = 246.8, 10.4 Hz, C-3), 148.57 (dd, $J_{C\text{-}F}$ = 7.9, 3.2 Hz, C-1), 141.48 (dd, $J_{C\text{-}F}$ = 247.3, 14.1 Hz, C-2), 123.17 (dd, $J_{C\text{-}F}$ = 8.7, 5.2 Hz, C-5), 119.3 (CN), 109.85 (d, $J_{C\text{-}F}$ = 2.9 Hz, C-6), 109.20 (dd, $J_{C\text{-}F}$ = 17.7, 9.5 Hz, C-4), 68.8 (OCH_2), 28.4 (C-4'); 21.6 (C-3'), 17.4 (CH_2CN). **IR (ν_{max}/cm^{-1})**: 2961, 2932 (=C-H); 2239 (CN); 1596, 1503 (C=C). ***HRMS*** *m/z*: calculated for $C_{11}H_{11}F_2NONa$: 234.0709, found: $[M + Na]^+$ 234.0706.

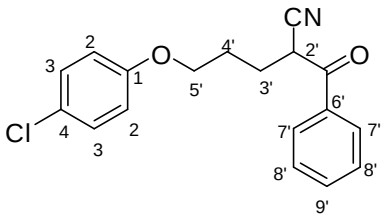
4.4 General synthesis of 2-benzoyl-5-(4-chlorophenoxy)pentanenitrile and analogues.



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-CF_3

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 (NH_4Cl) 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 $MgSO_4$ 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

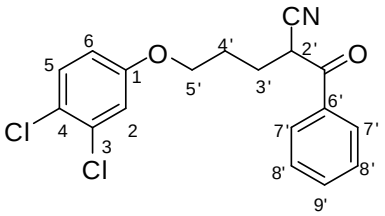


Following the same procedure as above, compound **34a** (2.32 g, 11.1 mmol) was dissolved in dry THF (50 ml). To the clear solution was added potassium *tert*-butoxide (3.72 g, 33.2 mmol) followed by ethyl benzoate (6.33 ml, 44.3 mmol). The product was isolated as yellow

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. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 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, H4' and H3'), **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 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 (ν_{max}/cm^{-1})**: 2878 (=C-H); 2253 (CN); 1694 (C=O); 1596, 1580 (C=C). ***HRMS*** *m/z*: calculated for $C_{18}H_{16}ClNO_2Na$: 336.0770, found: $[M + Na]^+$ 336.0757.

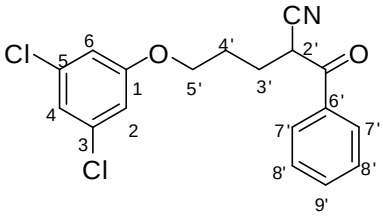
4.4.2 Synthesis of 2-benzoyl-5-(3,4-dichlorophenoxy)pentanenitrile 35b



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. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 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'). **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 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 (ν_{max}/cm^{-1})**: 3041, 2954 (=C-H); 2252 (CN); 1695 (C=O); 1586, 1574 (C=C). ***HRMS*** *m/z*: calculated for $C_{18}H_{15}Cl_2NO_2Na$: 370.0380, found: $[M + Na]^+$ 370.0377.

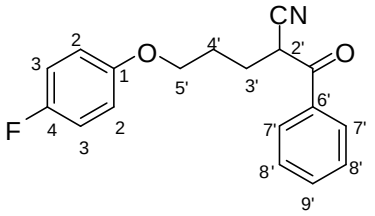
4.4.3 Synthesis of 2-benzoyl-5-(3,5-dichlorophenoxy)pentanenitrile 35c



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% EtOAc/hexane) 0.40. *mp*: 45°C. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 7.97 (2H, d, *J* = 7.1 Hz, H7'), 7.74 – 7.61 (1H, m, H9'), 7.53 (2H, dd, *J* = 8.3, 7.0 Hz, H8'), 6.95 (1H, t, *J* = 1.8 Hz, H4), 6.72 (2H, d, *J* = 1.8 Hz, H2), 4.46 (1H, dd, *J* = 8.1, 5.9 Hz, CHCN), 4.06 – 3.88 (2H, m, OCH_2), 2.38 – 1.73 (4H, m, H4' and H3'). **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 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'): **IR (ν_{max}/cm^{-1})**: 2889 (=C-H); 2255 (CN); 1697 (C=O); 1582, 1570 (C=C). ***HRMS*** *m/z*: calculated for $C_{18}H_{15}Cl_2NO_2Na$: 370.0380, found: $[M + Na]^+$ 370.0381.

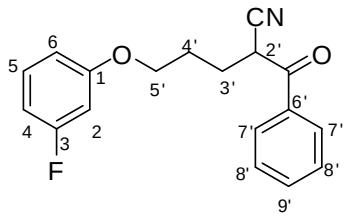
4.4.4 Synthesis of 2-benzoyl-5-(4-fluorophenoxy)pentanenitrile 35d



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, 32.1 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, $CDCl_3$)**: δ 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, $CDCl_3$)**: δ 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), 134.6 (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 (ν_{max}/cm^{-1})**: 2956, 2930 (=C-H); 2252 (CN); 1703 (C=O); 1586, 1577 (C=C). ***HRMS*** *m/z*: calculated for $C_{18}H_{16}FNO_2Na$: 320.1065, found: $[M + Na]^+$ 320.1100.

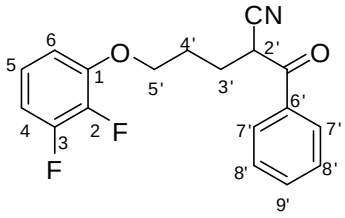
4.4.5 Synthesis of 2-benzoyl-5-(3-fluorophenoxy)pentanenitrile 35e



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, $CDCl_3$)**: δ 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, $CDCl_3$)**: δ 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 (ν_{max}/cm^{-1})**: 2876 (=C-H); 2254 (CN); 1689 (C=O); 1596, 1576 (C=C). ***HRMS*** *m/z*: calculated for $C_{18}H_{16}FNO_2Na$: 320.1065, found: $[M + Na]^+$ 320.1063.

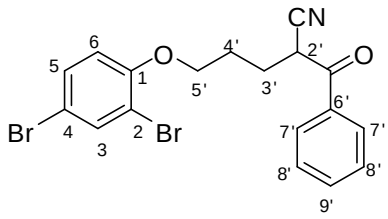
4.4.6 Synthesis of 2-benzoyl-5-(2,3-difluorophenoxy)pentanenitrile 35f



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, $J_{C\text{-}F}$ = 246.8, 10.4 Hz, C-3), 148.6 (dd, $J_{C\text{-}F}$ = 7.9, 3.2 Hz, C-1), 141.5 (dd, $J_{C\text{-}F}$ = 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, $J_{C\text{-}F}$ = 8.7, 5.2 Hz, C-5), 117.2 (CN), 109.9 (d, $J_{C\text{-}F}$ = 2.9 Hz, C-6), 109.2 (dd, $J_{C\text{-}F}$ = 17.7, 9.5 Hz, C-4), 66.89 (OCH_2), 39.40 (CHCN) , 26.73 (C-3') , 26.44 (C-4'). **IR (ν_{max}/cm^{-1})**: 3039, 2930 (=C-H); 2248 (CN); 1705 (C=O); 1594, 1580 (C=C).

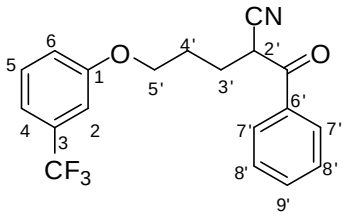
4.4.7 Synthesis of 2-benzoyl-5-(2,4-dibromophenoxy)pentanenitrile 35g



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, $CDCl_3$)**: δ 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'). **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 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'): **IR (ν_{max}/cm^{-1})**: 3010 (=C-H); 2255 (CN); 1699 (C=O); 1578, 1569 (C=C). ***HRMS*** *m/z*: calculated for $C_{18}H_{15}Br_2NO_2Na$: 457.9270, found: $[M + Na]^+$ 457.9370.

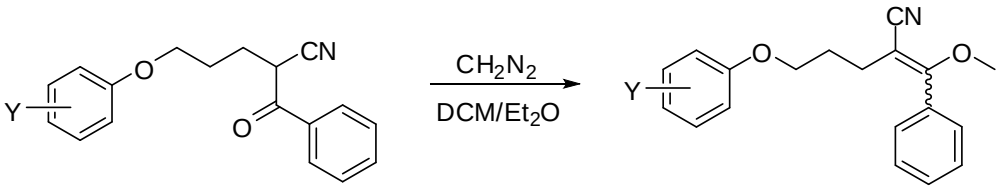
4.4.8 Synthesis of 2-benzoyl-5-(3-(trifluoromethyl)phenoxy)pentanenitrile 35h



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, $CDCl_3$)**: δ 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'). **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 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'); **IR (ν_{max}/cm^{-1})**: 2941 (=C-H); 2249 (CN); 1696 (C=O); 1592, 1571 (C=C). ***HRMS*** *m/z*: calculated for $C_{19}H_{17}F_3NO_2$: 348.1213, found: $[M + H]^+$ 348.1227.

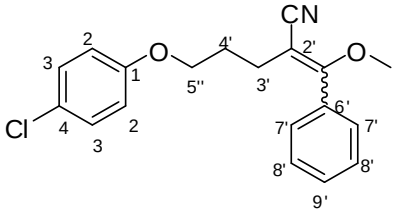
4.5 General procedure for the synthesis of E/Z -5-(4-chlorophenoxy)-2-(methoxy(phenyl)methylene)pentanenitrile and similar compounds



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

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)pentanenitr-ile 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)

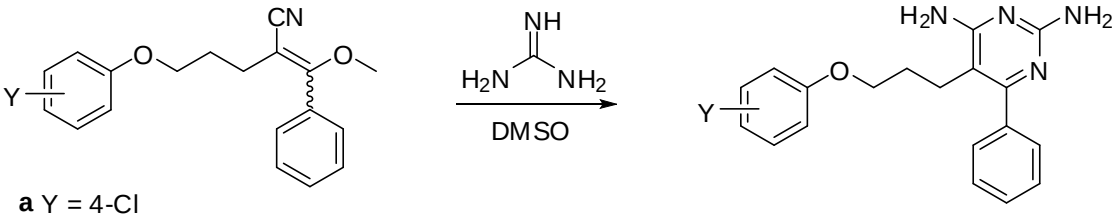
was passed into a solution of **35a** (21.00 g, 3.19 mmol), in dry DCM. This afforded **36a** as a viscous oil in quantitative yield. The product was isolated as a mixture of *E* and *Z* isomers in a ratio of 1.4:1. Owing to the instability of the enol ether, the product was treated as an intermediate and no further characterisation was done.

^{1}H NMR (300 MHz, $CDCl_3$): Major-isomer: δ 7.56 – 7.33 (4H, m, Ar-H), 7.23 – 7.18 (1H, m, Ar-H), 7.23 (2H, d, *J* = 9.1 Hz, H3), 6.84 (2H, d, *J* = 9.0 Hz, H2), 4.00 (2H, t, *J* = 6.1 Hz, OCH_2), 3.43 (3H, s, OCH_3), 2.57 (2H, dd, *J* = 8.2, 6.7 Hz, H3'), 2.11 – 2.01 (2H, m, H4'); **^{13}C NMR (75 MHz, $CDCl_3$)**: Major-isomer: δ 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').

^{1}H NMR (300 MHz, $CDCl_3$): Minor-isomer: δ 7.56 – 7.33 (4H, m, Ar-H), 7.23 – 7.18 (1H, m, Ar-H), 7.18 (2H, d, *J* = 9.1 Hz, H3), 6.66 (2H, d, *J* = 8.9 Hz, H2), 3.85 (2H, t, *J* = 5.9 Hz, OCH_2), 3.47 (3H, s, OCH_3), 2.23 (2H, dd, *J* = 8.1, 6.5 Hz, H3'), 1.99 – 1.87 (2H, m, H4'); **^{13}C NMR (75 MHz, $CDCl_3$)**: Minor-isomer: δ 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 ^{1}H and ^{13}C NMR spectroscopy before being used immediately in the subsequent ring closing reaction.

4.6 General procedure for the synthesis of flexible pyrimidine analogues

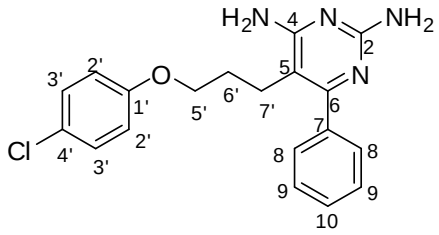


a Y = 4-Cl
b = 3,4 diCl
c = 3,5 diCl
d = 3-F

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.

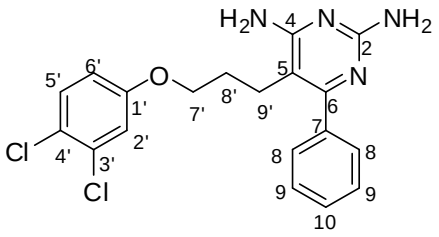
4.6.1 Synthesis of 5-(3-(4-chlorophenoxy)propyl)-6-phenylpyrimidine-2,4-diamine 37a



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%).

R_f (80% EtOAc/hexane) 0.18. *mp*: 130-132°C. **^{1}H NMR (300 MHz, $CDCl_3$)**: 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, NH_2), 5.15 (2H, s, NH_2), 3.84 (2H, t, *J* = 5.6 Hz, OCH_2), 2.53 (2H, t, *J* = 8.6 Hz, H7'), 1.93 – 1.84 (2H, m, H6'). **^{13}C NMR (75 MHz, $CDCl_3$)**: 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 (OCH_2), 28.58 (C-6'), 22.24 (C-7'): **IR (ν_{max}/cm^{-1})**: 3328, 3140 (NH_2); 2929 (=C-H); 1637 (C=N); 1583, 1555 (C=C). ***HRMS*** *m/z*: calculated for $C_{19}H_{20}ClN_4O$: 355.1327, found: $[M + H]^+$ 355.1326

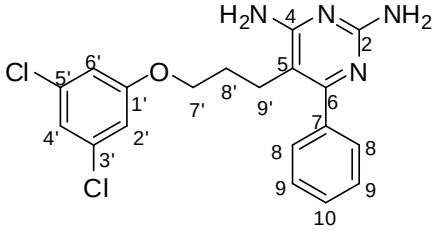
4.6.2 Synthesis of 5-(3-(3,4-dichlorophenoxy)propyl)-6-phenylpyrimidine-2,4-diamine 37b



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 (ν_{max}/cm^{-1})**: 3430, 3164, (N-H); 2866 (=C-H); 1636 (C=N); 1582, 1554 (C=C): ***HRMS*** *m/z*: calculated for $C_{19}H_{19}Cl_2N_4O$: 389.0938, found: $[M + H]^+$ 389.0936

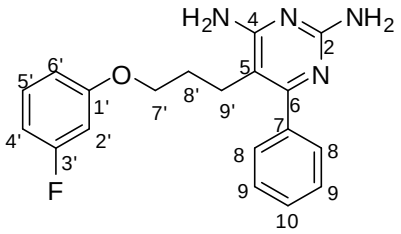
4.6.3 Synthesis of 5-(3-(3,5-dichlorophenoxy)propyl)-6-phenylpyrimidine-2,4-diamine 37c



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 (OCH_2), 27.17 (C-8'), 21.10 (C-9'): **IR (ν_{max}/cm^{-1})**: 3430, 3164 (N-H); 2956 (=C-H); 1637 (C=N); 1599, 1575 (C=C). ***HRMS*** *m/z*: calculated for $C_{19}H_{19}Cl_2N_4O$: 389.0938, found: $[M + H]^+$ 389.0936

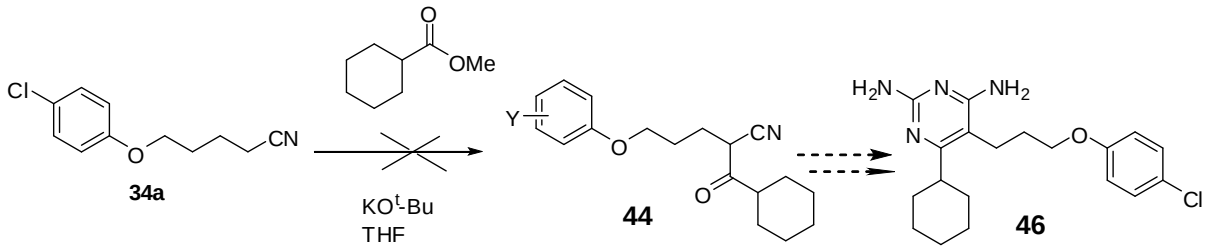
4.6.4 Synthesis of 5-(3-(3-fluorophenoxy)propyl)-6-phenylpyrimidine-2,4-diamine 37e



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%).

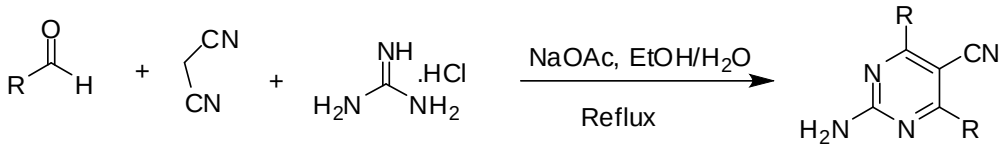
R_f (80% EtOAc/hexane) 0.14. *mp*: 104-105°C. **^{1}H NMR (300 MHz, MeOD)**: δ 6.23 – 5.82 (5H, m, Ar-H), 5.45 – 5.10 (4H, m, H2', H4', H5' and H6'), 2.53 (2H, t, *J* = 5.9 Hz, OCH_2), 1.27 (2H, dd, *J* = 8.6, 6.6 Hz, H9'), 0.67 – 0.53 (2H, m, H8'): **^{13}C NMR (75 MHz, MeOD)**: δ 191.5 (C-6), 188.1 (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 (OCH_2), 27.57 (C-8'), 21.93 (C-9'): **IR (ν_{max}/cm^{-1})**: 3479, 3112, (N-H); 2875 (=C-H); 1612 (C=N); 1576, 1505 (C=C): ***HRMS*** *m/z*: calculated for $C_{19}H_{20}FN_4O$: 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 R_f value as the starting material, the reaction was quenched with a saturated ammonium chloride (NH_4Cl) 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 $MgSO_4$ 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.

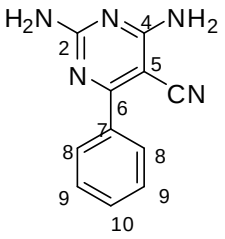


48

R = phenyl
= cyclohexyl
= cyclopropyl

The equimolar mixture of cyclic aldehyde (1.0 eq), malononitrile (1.0 eq) and NaOAc (1.0 eq) in (60 ml) H_2O/(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 × 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

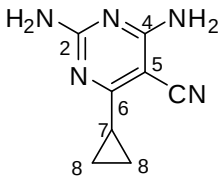


Benzaldehyde (4.63 ml, 45.4 mmol), malononitrile (3.00 g, 45.4 mmol), sodium acetate (3.73 g, 45.4 mmol) and guanidine hydrochloride (4.34 g, 45.4 mmol) afforded, after extraction and column chromatography using 40% EtOAc/hexane as eluent, **48a** (4.23 g, 44%) as a yellow solid.

R_f (80% EtOAc/hexane) 0.81. *mp*: 234 °C. **^{1}H NMR (300 MHz, DMSO-d_6)**: δ 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)**: δ 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 (ν_{max}/cm^{-1})**: 3374 (N-H); 3146 (=C-H); 2205 (CN); 1609 (Ar-C=C).

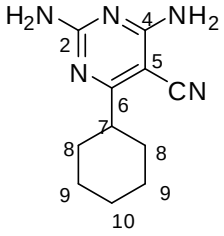
4.8.2 Synthesis of 2,4-diamino-6-cyclopropylpyrimidine-5-carbonitrile 48b



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% EtOAc/hexane) 0.78. *mp*: 190-191°C. **^{1}H NMR (300 MHz, DMSO-d$_6$)**: δ 6.90 (2H, s, NH_2), 6.68 (2H, s, NH_2), 2.04 (1H, p, H7), 0.81 – 0.78 (4H, m, H8). **^{13}C NMR (75 MHz, DMSO-d$_6$)**: δ 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). **IR (ν_{max}/cm^{-1})**: 3498, 3426 (N-H); 2203 (C=N); 1615 (C=C); 1280, 1133 (CH_2). ***HRMS*** *m/z*: calculated for $C_8H_{10}N_5$: 176.0938, found: $[M + H]^+$ 176.0934.

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

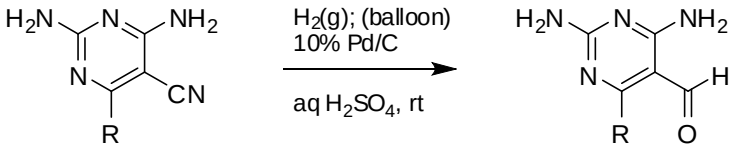


Cyclohexanecarbaldehyde (4.60 mL, 37.8 mmol), malononitrile (2.50 g, 37.8 mmol), sodium acetate (3.10 g, 37.8 mmol) and guanidine hydrochloride (3.62 g, 37.8 mmol) afforded, after extraction and column chromatography using 40% EtOAc/hexane as eluent, **48c** (3.89 g, 47%) as a yellow solid.

R_f (80% EtOAc/hexane) 0.92. *mp*: 201-202 °C. **^{1}H NMR (300 MHz, DMSO-d$_6$)**: δ 6.91 (2H, s, NH_2), 6.78 (2H, s, NH_2), 2.63 (1H, p, Ha), 1.82 – 1.48 (7H, m, cyclohex), 1.21 – 1.35 (3H, m, cyclohex). **^{13}C NMR (75 MHz, DMSO-d$_6$)**: δ 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): **IR**

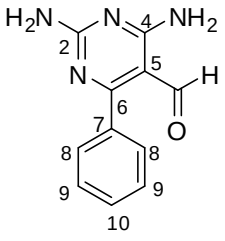
(ν_{max}/cm^{-1}): 3445, 3390 (N-H); 2203 (CN); 1608 (C=C); 1438, 1270, 1014 (CH_2). ***HRMS*** *m/z*: calculated for $C_{11}H_{16}N_5$: 218.1407, found: $[M + H]^+$ 218.1406

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



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 × 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[105] 49a

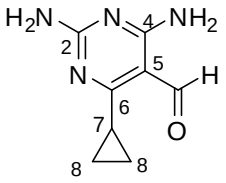


Compound **48a** (1.00 g, 4.73 mmol) in the presence of H_2(g) and 10% palladium/carbon (0.05 g), afforded, after extraction and column chromatography using 60% EtOAc/hexane as eluent, **49a** (0.52 g, 51%) as a light-yellow solid.

R_f (80% EtOAc/hexane) 0.80. **^{1}H NMR (300 MHz, DMSO-d$_6$)**: δ 9.45 (1H, s, CHO), 7.52 – 7.48 (m, 5H, H8, H9 and H10), 7.24-7.13 (4H, m, 2 ×NH_2). **^{13}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).

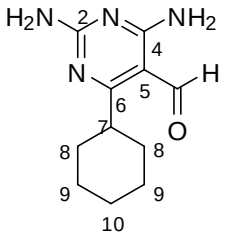
4.9.2 Synthesis of 2,4-diamino-6-cyclopropylpyrimidine-5-carbaldehyde 49b



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, H7), 1.05 – 0.99 (4H, m, H8). **^{13}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 (ν_{max}/cm^{-1})**: 3221 (N-H); 1747 (C=O), 1598 (C=C); 1260, 892 (CH_2).

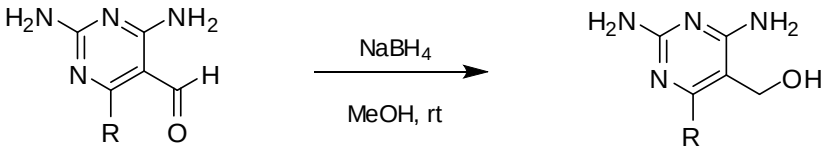
4.9.3 Synthesis of 2,4-diamino-6-cyclohexylpyrimidine-5-carbaldehyde 49c



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.

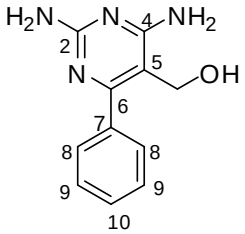
R_f (80% EtOAc/hexane) 0.82. *mp*: 85°C. **^{1}H NMR (300 MHz, DMSO-d$_6$)**: δ 10.08 (1H, s, CHO), 6.98 (2H, s, NH_2), 6.85 (2H, s, NH_2), 2.83 (1H, p, H7), 1.82 – 1.48 (7H, m, cyclohex), 1.21 – 1.35 (3H, m, cyclohex). **^{13}C NMR (75 MHz, DMSO-d$_6$)**: δ 187.8 (C=O); 164.8 (C-6); 164.1 (C-2); 163.6 (C-4); 101.9 (C-5); 44.41 (C-7); 32.02 (C-8); 30.96 (C-10). 26.15 (C-9); **IR ν_{max}/cm^{-1}**: 3431, 3314 (N-H); 1610 (C=C); 1546 (C=O); 1261, 803 (CH_2). ***HRMS*** *m/z*: calculated for $C_{11}H_{17}N_4O$: 221.1401, found: $[M + H]^+$ 221.1397.

4.10 General procedure for the synthesis of (2,4-diamino-6-phenylpyrimid in-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_2O (15 ml). The mixture was then concentrated *in vacuo*, and the aqueous residue was extracted with EtOAc (3 × 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:

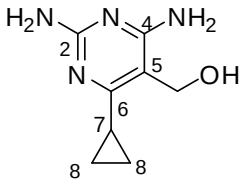
4.10.1 Synthesis of (2,4-diamino-6-phenylpyrimidin-5-yl)methanol[105] 50a



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)**: δ 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)**: δ 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 (ν_{max}/cm^{-1})**: 3490, 3415, 3361 (N-H); 3153 (O-H); 1609, 1555 (C=C); 976 (C-O).

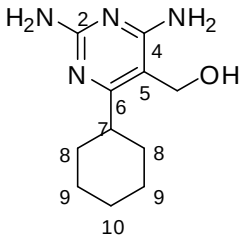
4.10.2 Synthesis of (2,4-diamino-6-cyclopropylpyrimidin-5-yl)methanol 50b



Compound **49b** (0.40 g, 2.24 mmol) and $NaBH_4$ (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$_6$)**: δ 5.92 (2H, s, NH_2), 5.59 (2H, s, NH_2), 4.65 (1H, t, *J* = 5.0 Hz, OH), 4.44 (2H, d, *J* = 5.0 Hz, CH_2); 2.12-2.03 (1H, m, H7), 0.89 – 0.73 (4H, m, H8): **^{13}C NMR (75 MHz, DMSO-d$_6$)**: δ 166.1 (C-6); 163.1 (C-4); 161.9 (C-2); 103.9 (C-5); 54.7 (CH_2); 11.9 (C-7); 8.21 (C-8): **IR (ν_{max}/cm^{-1})**: 3342 (N-H); 3161 (O-H); 1560 (C=C); 980 (C-O). ***HRMS*** *m/z*: calculated for $C_8H_{13}N_4O$: 181.1091, found: $[M + H]^+$ 181.1089.

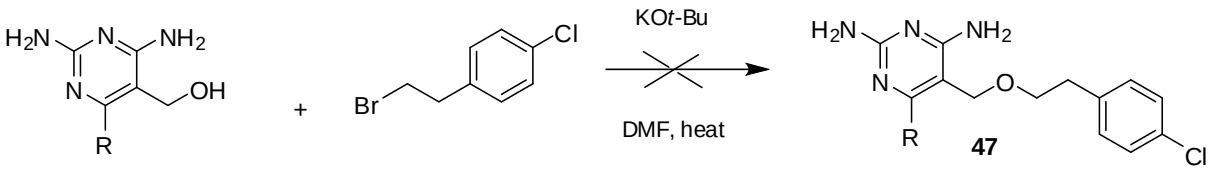
4.10.3 Synthesis of (2,4-diamino-6-cyclohexylpyrimidin-5-yl)methanol 50c



Compound **49c** (0.35 g, 1.59 mmol) and $NaBH_4$ (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$_6$)**: δ 6.89 (2H, s, NH_2), 6.37 (2H, s, NH_2), 4.65 (1H, t, *J* = 5.0 Hz, OH), 4.31 (2H, d, *J* = 5.0 Hz, CH_2); 1.84 – 1.48 (7H, m, cyclohex), 1.41 – 1.35 (3H, m, cyclohex). **^{13}C NMR (75 MHz, DMSO-d$_6$)**: δ 164.2 (C-4 and C-6); 161.9 (C-2); 103.4 (C-5); 57.16 (CH_2OH); 41.12 (C-7); 31.70 (C-8); 26. 44 (C-9); 25.89 (C-10); **IR (ν_{max}/cm^{-1})**: 3303 (N-H); 3159 (O-H); 1578 (C=C); 1000.2 (C-O). ***HRMS*** *m/z*: calculated for $C_{11}H_{19}N_4O$: 223.1561, found: $[M + H]^+$ 223.1549.

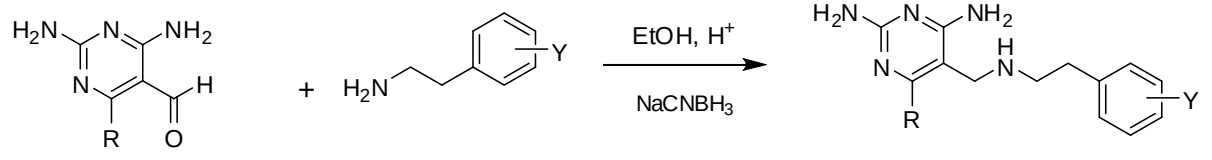
4.11 Attempted synthesis of flexible pyrimidines bearing a non-aromatic side chain



R = phenyl
= cyclohexyl
= cyclopropyl

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 $MgSO_4$, 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.



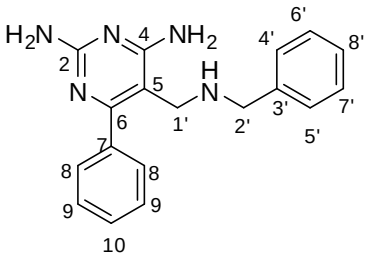
R = phenyl
= cyclohexyl
= cyclopropyl

Y = 4-Cl
= 4-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 $MgSO_4$ 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:

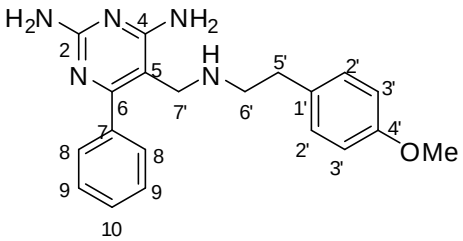
4.12.1 Synthesis of 5-((benzylamino)methyl)-6-phenylpyrimidine-2,4-diamine 57



Compound **49a** (0.12 g, 0.56 mmol), $NaCNBH_3$ (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-d$_6$)**: δ 7.44 – 7.26 (10H, m, Ar-H), 6.55 (2H, s, NH_2), 5.86 (2H, s, NH_2), 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-d$_6$)**: 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 $C_{18}H_{20}N_5$: 306.1720, found: $[M + H]^+$ 306.1714.

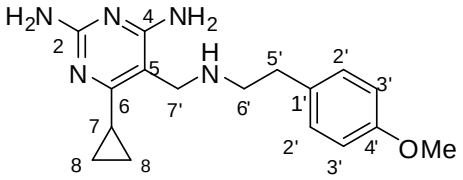
4.12.2 Synthesis of 5-((4-methoxyphenethylamino)methyl)-6-phenylpyrimidine-2,4-diamine 58a



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, $CDCl_3$)**: δ 8.04 (2H, d, *J* = 8.6 Hz, NH_2), 7.65 (2H, d, *J* = 8.9 Hz, H2'), 7.16 (2H, d, *J* = 8.6 Hz, H3'), 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, H7'), 3.80 (3H, s, OCH_3), 3.70 – 3.65 (2H, m, H6'), 2.87 (2H, t, *J* = 6.9 Hz, H5'): **^{13}C NMR (125 MHz, $CDCl_3$)**: δ 183.1 (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'), 34.90 (C-5'): **IR (ν_{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

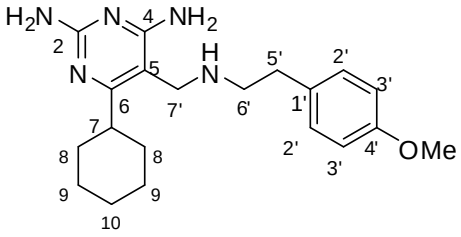


Compound **49b** (0.12 g, 0.67 mmol), $NaCNBH_3$ (0.13 g, 2.0 mmol) and 2-(4-methoxyphenyl)ethanamine (0.12 ml, 0.81 mmol), afforded, after extraction and purification by column chromatography using 80% EtOAc/hexane as eluent, **58b** (0.068 g, 32%) as a white solid.

R_f (5% MeOH/EtOAc) 0.24. *mp*: 101-102°C. **^{1}H NMR (300 MHz, DMSO-d_6)**: δ 7.11 (2H, d, *J* = 8.6 Hz, H2'), 6.86 (2H, d, *J* = 8.8 Hz, H3'), 3.80 (3H, s, OMe), 3.53 – 3.43 (2H, m, H6'), 2.97 (1H, p, *J* = 6.8 Hz, H7), 2.81 (4H, 2 × NH_2), 2.76 (2H, t, *J* = 7.0 Hz, H5'), 2.05 (1H, s, NH), 1.94 (2H, s, H7'), 1.64 – 0.98 (4H, m, H8): **^{13}C NMR (125 MHz, DMSO-d_6)**: δ 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 (ν_{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 $C_{17}H_{24}N_5O$: 314.1983, found: $[M+H]^+$ 314.1957.

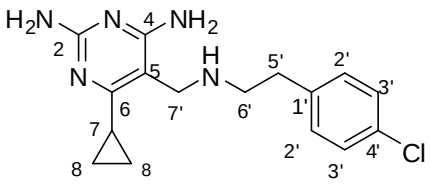
4.12.4 Synthesis of 6-cyclohexyl-5-((4-methoxyphenethylamino)methyl)pyrimidine-2,4-diamine 58c



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, $CDCl_3$)**: δ 7.16 (2H, d, *J* = 8.8 Hz, H2'), 6.90 (2H, d, *J* = 8.9 Hz, H3'), 6.88 – 6.78 (2 ×NH_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, $CDCl_3$)**: δ 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 (ν_{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 $C_{20}H_{30}N_5O$: 356.2452, found: $[M+H]^+$ 356.2262.

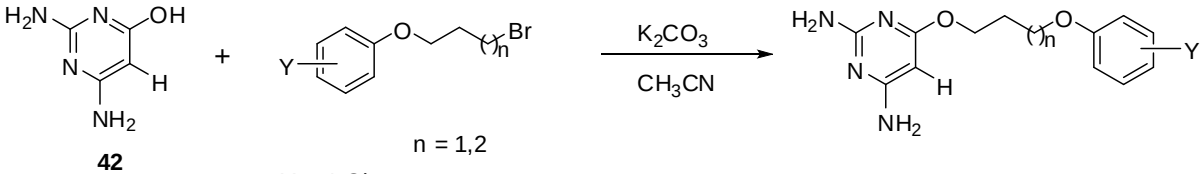
4.12.5 Synthesis of 5-((4-chlorophenethylamino)methyl)-6-cyclopropylpyrimidine-2,4-diamine 58d



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 (ν_{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



42

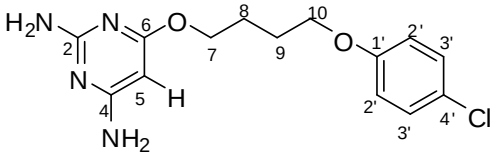
n = 1,2

Y = 4-Cl
= 3,4 diCl
= 3,5 diCl
= 4-F
= 3-F
= 3-CF_3

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.

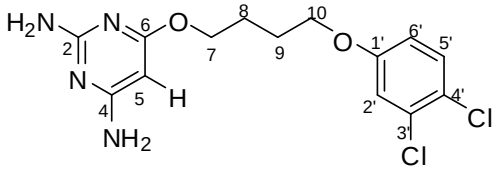
4.13.1 Synthesis of 6-(4-(4-chlorophenoxy)butoxy)pyrimidine-2,4-diamine 40a



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. **^{1}H NMR (300 MHz, DMSO-d$_6$)**: δ 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$_6$)**: δ 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 (ν_{max}/cm^{-1})**: 3520, 3359 (NH_2); 3011 (=C-H); 1576 (C=C); 1124 (C-O); 791 (C-Cl). ***HRMS*** *m/z*: calculated for $C_{14}H_{18}ClN_4O_2$: 309.1120, found: $[M + H]^+$ 309.1117

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

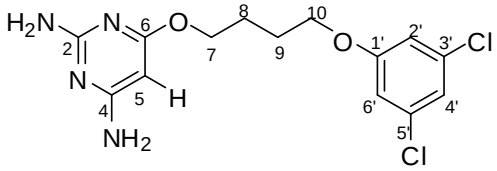


Reaction of 2,4-diamino-6-hydroxy pyrimidine (0.43 g, 3.42 mmol), potassium carbonate (0.71 g, 5.13 mmol) and compound **33b** (1.02 g, 3.42 mmol) as described above afforded, after filtration and column chromatography using 60% EtOAc/hexane as eluent, **40b** (0.53 g, 45%) as a white solid.

R_f (80% EtOAc/hexane) 0.23. *mp*: 136-137°C. **^{1}H NMR (300 MHz, DMSO-d$_6$)**: 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'), 5.99

(2H, s, NH_2), 5.82 (2H, s, NH_2), 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-d$_6$)**: 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 (ν_{max}/cm^{-1})**: 3520, 3359 (NH_2); 3011 (=C-H); 1576 (C=C); 1124 (C-O); 791 (C-Cl). ***HRMS*** *m/z:* calculated for $C_{14}H_{17}Cl_2N_4O_2$: 343.0730, found: $[M + H]^+$ 343.0729.

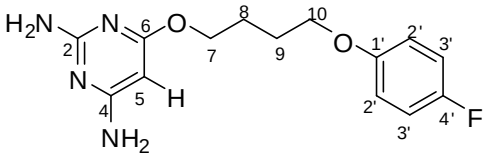
4.13.3 Synthesis of 6-(4-(3,5-dichlorophenoxy)butoxy)pyrimidine-2,4-diamine 40c



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.

R_f (80% EtOAc/hexane) 0.25. *mp*: 106-107°C. **^{1}H NMR (300 MHz, DMSO-d$_6$)**: 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, NH_2), 5.82 (2H, s, NH_2), 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-d$_6$)**: δ 170.1 (C-6); 165.9 (C-4); 163.0 (C-1'); 160.1 (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 (ν_{max}/cm^{-1})**: 3440, 3327 (NH_2); 3008 (=C-H); 1560 (C=C); 1141 (C-O); 828, 791 (C-Cl). ***HRMS*** *m/z*: calculated for $C_{14}H_{17}Cl_2N_4O_2$: 343.0730, found: $[M + H]^+$ 343.0724.

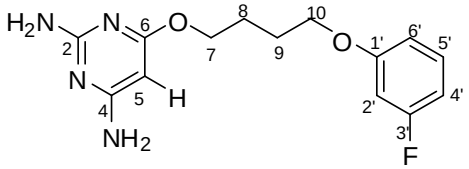
4.13.4 Synthesis of 6-(4-(4-fluorophenoxy)butoxy)pyrimidine-2,4-diamine 40d



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% EtOAc/hexane) 0.23. *mp*: 138°C. **^{1}H NMR (300 MHz, DMSO-d$_6$)**: δ 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}C NMR (75 MHz, DMSO-d$_6$)**: δ 170.1 (C-6), 165.9 (C-4) , 162.9 (C-2), 156.4 (d, $J_{C\text{-}F}$ = 238.3 Hz, C-4'), 154.8 (d, $J_{C\text{-}F}$ = 2.0 Hz, C-1'), 115.8 (d, $J_{C\text{-}F}$ = 23.1 Hz, C-3'), 115.6 (d, $J_{C\text{-}F}$ = 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 (ν_{max}/cm^{-1})**: 3478 (NH_2); 2957 (=C-H); 1577 (C=C); 1216, 1199 (C-O); 1075 (C-F). ***HRMS*** *m/z*: calculated for $C_{14}H_{18}FN_4O_2$: 293.1416, found: $[M + H]^+$ 293.1411.

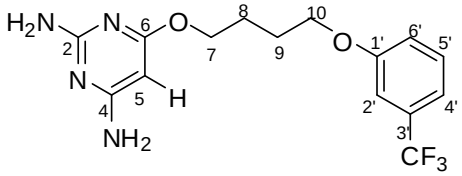
4.13.5 Synthesis of 6-(4-(3-fluorophenoxy)butoxy)pyrimidine-2,4-diamine 40e



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% EtOAc/hexane) 0.20. *mp*: 98-99°C. **^{1}H NMR (300 MHz, DMSO-d$_6$)**: δ 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}C NMR (75 MHz, DMSO-d$_6$)**: δ 170.1 (C-6), 165.9 (C-4) , 162.8 (C-2), 160.2 (d, $J_{C\text{-}F}$ = 245.3 Hz, C-3'), 159.87 (d, $J_{C\text{-}F}$ = 10.8 Hz, C-1'), 130.6 (d, $J_{C\text{-}F}$ = 10.0 Hz, C-5'), 110.8 (d, $J_{C\text{-}F}$ = 2.9 Hz, C-6'), 106.9 (d, $J_{C\text{-}F}$ = 21.3 Hz, C-4'), 101.9 (d, $J_{C\text{-}F}$ = 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 (ν_{max}/cm^{-1})**: 3512, 3344 (NH_2); 2939 (=C-H); 1569 (C=C); 1145 (C-O); 1016 (C-F). ***HRMS*** *m/z*: calculated for $C_{14}H_{18}FN_4O_2$: 293.1416, found: $[M + H]^+$ 293.1413.

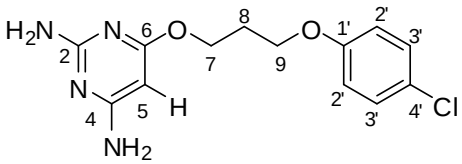
4.13.6 Synthesis of 6-(4-(3-(trifluoromethyl)phenoxy)butoxy)pyrimidine-2,4-diamine 40f



Reaction of 2,4-diamino-6-hydroxy pyrimidine **42** (0.51 g, 4.04 mmol), potassium carbonate (0.84 g, 6.06 mmol) and compound **33f** (1.20 g, 4.04 mmol) as described afforded, after filtration and column chromatography using 60% EtOAc/hexane as eluent, **40f** (0.64 g, 46%) as a white solid.

R_f (80% EtOAc/hexane) 0.20. *mp*: 100-101°C. **^{1}H NMR (500 MHz, DMSO-d$_6$)**: δ 7.53 – 7.50 (1H, m, H2'), 7.34-7.17 (3H, m, H4', H5' and H6'), 6.01 (2H, s, NH_2), 5.86 (2H, s, NH_2), 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). **^{13}C NMR (125 MHz, DMSO-d$_6$)**: δ 170.6 (C-6), 166.4 (C-4) , 163.4 (C-2) , 159.4 (C-1'), 131.10 – 130.31 (m, C-CF_3), 124.50 (q, $J_{C\text{-}F}$ = 272.4 Hz, CF_3), 119.2 (C-6'), 117.4 (q, $J_{C\text{-}F}$ = 3.8 Hz, C-4'), 111.4 (q, *J* = 3.7 Hz, C-2'), 76.56 (C-5), 68.17 (C-10) , 64.62 (C-7) , 25.78 (C-8 and C-9): **IR (ν_{max}/cm^{-1})**: 3512 (NH_2); 2939 (=C-H); 1569 (C=C); 1216, 1141 (C-O); 1015 (C-F): ***HRMS*** *m/z*: calculated for $C_{15}H_{18}F_3N_4O_2$: 343.1384, found: $[M + H]^+$ 343.1377

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

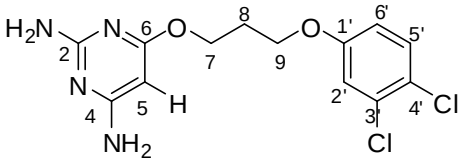


Reaction of 2,4-diamino-6-hydroxy pyrimidine **42** (0.51 g, 4.01 mmol), potassium carbonate (0.83 g, 6.01 mmol) and compound **60a** (1.00 g, 4.01 mmol) as described afforded, after filtration and column chromatography using 60% EtOAc/hexane as eluent, **59a** (0.41 g, 35%) as shiny white crystals.

R_f (80% EtOAc/hexane) 0.27. *mp*: 81-82°C. **^{1}H NMR (300 MHz, DMSO-d$_6$)**: δ 7.31 (2H, d, *J* = 9.0 Hz, H3'), 6.96 (2H, d, *J* = 9.0 Hz, H2'), 6.00 (2H, s, NH_2), 5.85 (2H, s, NH_2), 5.06 (1H, s, H5), 4.22 (2H, t, *J* = 6.4 Hz, H7), 4.05 (2H, t, *J* = 6.3 Hz, H9), 2.07 (2H, p, *J* = 6.3 Hz, H8). **^{13}C NMR (75 MHz, DMSO-d$_6$):** δ 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 (ν_{max}/cm^{-1})**: 3520, 3359 (NH_2); 3011 (=C-H); 1576 (C=C); 1125 (C-O); 791 (C-Cl). **HRMS** *m/z*: calculated for $C_{13}H_{16}ClN_4O_2$: 295.0964, found: $[M + H]^+$ 295.0956.

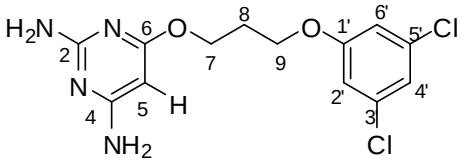
4.13.8 Synthesis of 6-(3-(3,4-dichlorophenoxy)propoxy)pyrimidine-2,4-diamine 59b



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$_6$)**: 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'), 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$_6$)**: δ 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 (ν_{max}/cm^{-1})**: 3529, 3343 (NH_2); 3010 (=C-H); 1581 (C=C); 1101 (C-O): ***HRMS*** *m/z:* calculated for $C_{13}H_{15}Cl_2N_4O_2$: 329.0574, found: $[M + H]^+$ 329.0565.

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

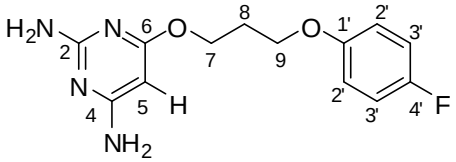


Reaction of 2,4-diamino-6-hydroxy pyrimidine **42** (0.89 g, 7.04 mmol), potassium carbonate (1.46 g, 10.6 mmol) and compound **60c** (2.00 g, 7.04 mmol) as described afforded, after filtration and column chromatography using 60% EtOAc/hexane as eluent, **59c** (1.26 g, 54%) as a creamy-white solid.

R_f (80% EtOAc/hexane) 0.28. *mp*: 105-107°C. **^{1}H NMR (300 MHz, DMSO-d$_6$)**: δ 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,

NH_2), 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). **^{13}C NMR (75 MHz, DMSO-d$_6$)**: 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); 28.31 (C-8); **IR (ν_{max}/cm^{-1})**: 3541, 3362 (NH_2); 3035 (=C-H); 1588 (C=C); 1144 (C-O); 799 (C-Cl): ***HRMS*** *m/z:* calculated for $C_{13}H_{15}Cl_2N_4O_2$: 329.0574, found: $[M + H]^+$ 329.0572.

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



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.

R_f (80% EtOAc/hexane) 0.26. *mp*: 69-71°C. **^{1}H NMR (300 MHz, DMSO-d$_6$)**: δ 7.18 – 7.01 (2H, m, H3'), 6.99 – 6.87 (2H, m, H2'), 5.99 (2H, s, NH_2), 5.85 (2H, s, NH_2), 5.04 (1H, s, H5), 4.22 (2H, t, *J* = 6.4 Hz, H7), 4.12 – 3.95 (2H, m, H9), 2.06 (2H, p, *J* = 6.3 Hz, H8). **^{13}C NMR (75 MHz, DMSO-d$_6$)**: δ 170.0 (C-6); 166.0 (C-4); 163.0 (C-2); 157.32 (d, J_{C-F} = 238.3 Hz, C-4'), 154.84 (d, J_{C-F} = 2.0 Hz, C-1'), 115.84 (d, J_{C-F} = 23.1 Hz, C-3'), 115.41 (d, J_{C-F} = 8.0 Hz, C-2'), 76.11 (C-5); 64.95 (C-7); 61.40 (C-9); 28.57 (C-8): **IR (ν_{max}/cm^{-1})**: 3539, 3332 (NH_2); 3032 (=C-H); 1586 (C=C); 1153 (C-O): ***HRMS*** *m/z*: calculated for $C_{13}H_{16}FN_4O_2$: 279.1310, found: $[M + H]^+$ 279.1252.

REFERENCES

1. Wahlgren, M., Bejarano, M.T., *Nature*, **1999**, *400*, 506.
2. (a) Kumar, A., Katiyar, S.B., Agarwal, A., Chauhan, P.M.S., *Curr. Med. Chem.*, **2003**, *10*, 1137; (b) Shrivastava, S.K., Chauhan, P.M.S., *Curr. Med. Chem*, **2001**, *8*, 1535.
3. Kumar, A., Katiyar, S.B., Agarwal, A., Chauhan, P.M.S., *Drugs Future*, **2003**, *28*, 243.
4. World Health Organization. World malaria report **2010**; 2010 [Geneva 27, Switzerland]
5. World Health Organization. World malaria report **2014**; 2014 [Geneva 27, Switzerland]
6. Guerra, C.A., Snow, T.W, Hay, S.I., *Trends Parasitol.*, **2006,** *22*, 353.
7. Cox-Singh, J., Davis, T.M.E., Lee, K., Shamsul, S.S.G., Matusop, A., Ratman, S., Rahman, H.A., Conway, D.J., Singh, B., *Clin. Infect. Dis.*, **2008**, *46*, 165.
8. Vaughan, A.M., Aly, A.S.I., Kappe, S.H.I., *Cel. Hos. & Mic.*, **2008**, *4*, 209.
9. www.cdc.gov/malaria distribution
10. Breman J.G., *Am. J. Trop. Med. Hyg.*, **2001**, *64*, 1.
11. Malaria Cases per Year, National Department of Health, www.doh.gov.za**, 2003**.
12. Harrison, G., *Third. World. Q.*, **1978**, *1*, 174.
13. D'Alessandro, U., Olaleye, B.O., McGuire, W., Thomson, M.C., Langerock, P., Bennett, S., Greenwood, B.M., *Trans. R. Soc. Trop. Med. Hyg.*, **1995**, *89*, 596.
14. Malaria Centre Report, London School of Hygiene & Tropical Medicine, **2010**, **2011**.
15. WHO, Indoor residual spraying, use of indoor residual spraying for scaling up global malaria control and elimination., **2006b**, 1112.
16. Dash, A.P., Adak, T., Raghavendra, K., Singh, O.P., *Curr. Sci*., **2007**, *92*, 1574.
17. Gahan,, J.B., Travis, B.V., Morton, F.A., Lindquist, A.W., *J. Econ. Entomol*., **1945**, *38*, 223.
18. Facer C.A., Tanner M., *Adv. Parasitol.*, **1997**, *39*, 1.
19. Carter R., Mendis K.N., Miller L.H., Mollineaux L., Saul A., *Nat. Med*., **2000**, *6*, 241.
20. The RTS, S Clinical Trials Partnership., *N. Engl. J. Med*., **2011**, *365*, 1863.

21. Patarroyo, M.E., Amador, R., Clavijo, P., Moreno, A., Guzman, F., Romero, P., Tascon, R., Franco A., Murrilo, L.A., Ponton, G., *Nature*., **1988**, *332*, 158.

22. D'Alessandro, U., Leach, A., Drakeley, C.J., Bennett, S., Olaleye, B.O., Fegan, G.W., Jawara, M., Langerock, P., George, M.O., Targett, G.A., *Lancet*., **1995**, *346*, 462.

23. Nosten, F., Luxemburger, C., Kyle, D.E., *Lancet*., **1996**, *348*, 701.

24. The malERA Consultative Group on Vaccines., *Vaccines. PLoS. Med*., **2011**, *8(1)*, 24.

25. Breman J.G., Brandling-Bennett, A.D., *Vaccine*., **2011**, *29*, 97.

26. White N.J., *New. Engl. J. Med.*, **1996**, *335*, 800.

27. Winstanley, P.A., *Parasitol. Today.*, **2000**, *16*, 146.

28. Farber, P.M., Arscott, L.D., Williams Jr, C.H., Becker, K., Schirmer, R.H., *FEBS Lett*., **1988**, *422*, 311.

29. Wells, T.N., Poll, E.M., *Discov. Med.*, **2010**, *9*, 389.

30. Olliaro, P., *Pharm. Ther*., **2001**, *89*, 207.

31. Foye, W.O., Lemke, T.L., Williams, D.A., *Princi. Med. Chem*., **1995**, Chap 32.

32. Peters, W., *Adv. Parasitol*. **1998**, *41*, 1.

33. Shapiro, T.A., Goldberg, D.E., P*harmacol. Ther*., **2006,** 1021.

34. Wiesner, J., Ortmann, R., Jomaa, H., Schlitzer, M., *Angew. Chem. Int. Ed*., **2003**, *42*, 5274.

35. Baccanari, D.P., Tansik, R.L., Paterson, S.J., Stone, D., *J. Biol. Chem.*, **1984**, *259*, 12291.

36. Norman, P., *Curr. Opin. Invest. Drugs*, **2001**, *2*, 1611.

37. Gangjee, A., Yu, J., McGuire, J.J., Cody, V., Galitsky, N., Kisliuk, R.L., Queener, S.F., *J. Med. Chem*., **2000**, *43*, 3837.

38. Ramharter, M., Burkhardt, D., Nemeth, J., Adegnika, A.A., Kremsner, P.G., *Am. J. Trop. Med. Hyg*., **2006**, *75*, 637.

39. White, N.J., *Antimicrob. Agents Chemother*., **1997**, *41*, 1414.

40. Wootton, D., Opara, H., Dube-mbeye, Q., *Acta. Trop.*, **2005**, *95*, 189.

41. Haynes, R.K., Cheu, K.W., Chan, H.W., Wong, H.N., Li, K.Y., Tang, M.M.K., Chen, M.J., Guo, Z.F., Guo, Z.H., Sinniah, K., Witte, A.B., Coghi, P., Monti, D., *Chem. Med. Chem.*, **2012**, *7*, 2204.
42. Olliaro, P.L., Milhous, W.K., *Humana Press*., **2001**, 219.
43. Olliaro, P., *Pharmacol. Ther.*, **2001**, *89*, 207.
44. Sirawaraporn, W., *Drug. Res.*, **1998**, *1*, 397.
45. Sharma, M., Chauhan, P.M.S., *Fut. Med. Chem*., **2012**, *4*, 1335.
46. Nzila, A., *Drug. Disc. Today*, **2006**, *11*, 19.
47. Olliaro, P., Yuthavong, Y., *Pharmacol. Ther.* **1999**, *81*, 91.
48. Rieckmann K.H., Brewer, G.J., Powell, R.D., *Trans. R. Soc. Trop. Med. Hyg*., **1968**, 62, 649.
49. Sheehy T.W., Reba R.C., Neff T.A., Gaintner, J.R., Tigertt, W.D., *Arch. Intern. Med*., **1967**,*119*, 561.
50. Nzila, A., Kokwaro, G., Winstanley, P., *Trends. Parasitol.*, **2004**, *20*, 109.
51. Macreadie, I., Ginsburg, H., Sirawaraporn, W., Tilley, L., *Parasitol. Today*., **2000**, *16*, 438.
52. Reinaldo, T., Delfino, O.A., Santos, F., José, D., Figueroa, V., *J. Braz. Chem. Soc.*, **2002**, *13*, 727.
53. Rastelli, G., Sirawaraporn, W., Sompornpisut, P., Vilaivan, T., Kamchonwongpaisan, S., Quarrel, R., Lowe, G., Thebtaranonth, Y., Yuthavong, Y., *Bioorg. Med. Chem.* **2000**, *8*, 1117.
54. Kraut, J., Matthews, D.A.,: Jurnak, F.A., McPherson, A., eds., John Wiley & Sons Ltd.: New York, USA, **1987**, *3*, Ch. 1.
55. Zuccotto, F., Martin, A.C.R., Laskowski, R.A., Thornton, J.M., Gilbert, I.H., *J. Comput. Aided Mol. Des.* **1998**, *12*, 241.
56. Curd, F.H.S., Davey, D.G., Rose, F.L., *Ann. Trop. Med. Parasitol*., **1945**, *39*, 208.
57. Wernsdorfer, W.H., *Med. Trop. (Mars).*, **1990**, *50*, 119.
58. Watkins W.M., Brandling-Bennett A.D., Oloo, A.J., Howells, R.E., Gilles, H.M., Koech, D.K., *Lancet*., **1987**, 125.
59. Hitchings, G.H., Elion, G.B., Falco, E.A., Singer, S., *J. Biol. Chem*., **1950**, *183*, 1.

60. Coatney, G.R., Cooper, W.C., Culwell, W.B., White, W.C., *J. Nat. Malar. Soc.*, **1950**, *9*, 183.
61. Hyde, J. E., *Pharmacol. Ther.*, **1990**, *48*, 45.
62. Sirawaraporn, W., Sathitkul, T., Sirawaraporn, R., Yuthavong, Y., Santi, D.V., *Proc. Natl. Acad. Sci.*, **1997**, *94*, 1124.
63. Kamchonwongpaisan, S., Quarrel, R., Charoensetakul, N., Ponsinet, R., Vilaivan, T., Vanichtanankul, J., Tarnchompoo, B., Sirawaraporn, W., Lowe, G., Yuthavong, Y., *J. Med. Chem.*, **2004**, *47*, 673.
64. Newton, P., White, N., *Ann. Rev. Med.*, **1999**, *50*, 179.
65. Tarnchompoo, B., Sirichaiwat, C., Phupong, W., Intaraudom, C., Sirawaraporn, W., Kamchonwongpaisan, S., Vanichtanankul, J., Thebtaranonth, Y., Yuthavong, Y., *J. Med. Chem.*, **2002**, *45*, 1244.
66. Wyss, P.C., Gerber, P., Hartman, P.G., Hubschwerlen, C., Locher, H., Marty, H.P., Stahl, M., *J. Med. Chem.*, **2003**, *46*, 2304.
67. Winstanley, P.A., Mberu, E.K., Szwandt, I.S., Breckenridge, A.M., Watkins, W.M., *Antimicrob. Agents. Chemother.*, **1995**, *39*, 948.
68. Warhust, D.C., *Drug. Discov. Today*, **1998**, *3*, 538.
69. Yuvaniyama J., Chitnumsub, P., Kamchonwongpaisan, S., *Nat. Struct. Biol.*, **2003**, *10*, 357.
70. Canfield, C.J., Milhous, W.K., Ager, A.L., Rossan R.N., Sweeney, T.R., Lewis, N.J., Jacobus, D.P., *Am. J. Trop. Med. Hyg.* **1993**, *49*, 121.
71. Rieckmann, K.H., Yeo, A.E., Edstein, M.D., *Trans. R. Soc. Trop. Med. Hyg.*, **1996**, *90*, 568.
72. Edstein, M.D., Bahr, S., Kotecka, B., Shanks, G.D., Rieckmann, K.H., *Antimicrob. Agents. Chemother.*, **1997**, *41*, 2300.
73. Mital, A., *Curr. Med. Chem.*, **2007**, *14*, 759.
74. Jensen, N.P., Ager, A.L., Bliss, R.A., Canfield, C.J., Kotecka, B.M., Rieckmann, K.H., Jacobus, D.P., Terpinski, J., *J. Med. Chem.*, **2001**, *44*, 3925.
75. Cowman, A.F., Morry, M.J., Biggs, B.A., Cross, G.A.M., Foote, S.J., *Proc. Natl. Acad. Sci. USA.*, **1988**, *85*, 9109.

76. Peterson, D.S., Walliker, D., Wellems, T.E., *Proc. Natl. Acad. Sci. USA*., **1988**, *85*, 9114.

77. Olliaro, P., *Pharmacol. Ther.*, **2001**, *89*, 207.

78. Plowe, C.V., Cortese, J.F., Djimde, A., Nwanyanwu, O.C., Watkins, W.M., Winstanley, P.A., Estrada-Franco, J.G., Mollinedo, R.E., Avila, J.C., Cespedes, J.L., Carter, D., Doumbo, O.K., *J. Infect. Dis*., **1997**, *176*, 1590.

79. Nzila, A.M., Mberu, E.K., Sulo, J., Dayo, H., Winstanley, P.A., Sibley, C.H., Watkins, W.M., *Antimicrob. Agents. Chemother*., **2000**, *44*, 991.

80. D'Alessandro U., Buttiens H., *Trop. Med. Int. Health*., **2001**, *6*, 845.

81. Foley M., Tilley L., *Pharmacol. Ther*., **1998**, *79*, 55.

82. Noedl H., Se, Y., Schaecher, K., Smith, B.L., Socheat, D., Fukuda, M.M., *New. Engl. J. Med*., **2008**, *359*, 2619.

83. Noedl H., *Trends Parasitol*., **2005**, *21*, 404.

84. Olliaro, P., *Pharmacol. Ther.*, **2001**, *89*, 207.

85. Winstanley, P. A., *Parasitol. Today.*, **2000**, *16*, 146.

86. Watkins W.M., Mberu E.K., Winstanley P.A., Plowe, C.V., *Parasitol. Today* ., **1997**, *13*, 459.

87. Curtis, J., Duraisingh, M.T., Warhurst, D.C., *J. Infect. Dis*, **1988**, *177*, 1429.

88. Nzila, A.M., Nduati, E., Mberu, E.K., Hopkins Sibley, C., Monks, S.A., Winstanley, P.A., Watkins, W.M., *J. Infect. Dis*., **2000**, *181*, 2023.

89. Wilairatana, P., Kyle D.E., Looareesuwan, S., Chinwongprom, K., Amradee, S., White, N.J., Watkins, W.M., *Ann. Trop. Med. Parasitol.*, **1997**, *91*, 125.

90. Winstanley, P.A., Mberu, E.K., Szwandt, I.S., Breckenridge, A.M., Watkins, W.M., *Antimicrob. Agents. Chemother*., **1995**, *39*, 948.

91. Kain, K.C., *BioDrug*s., **2003**, *17*, 23.

92. Rousseau, A.L., Gravestock, D., Lourens, A., van Zyl, R., van Hoppe, H., Nkabinde, L., Kamchonwongpaisan, S., Manuscript in preparation.

93. Rousseau, A., Gravestock, D., Lourens, A., WO2011018742, PCT Application PCT/IB2010/053570, **2010**.

94. Yuthavong, Y., Tarnchompoo, B., Yuvaniyama, J., Matthews, D., *PNAS*, **2012**, *109*, 16823.

95. Tarnchompoo, B., Sirichaiwat, C., Phupong, W., Intaraudom, C., Sirawaraporn, W., Kamchonwongpaisan, S., Vanichtanankul, J., Thebtaranonth, Y., Yuthavong, Y., *J. Med. Chem.*, **2002**, *45*, 1244.

96. Tejedor, D., Garcia-Tellado, F., *Chem. Soc. Rev.*, **2007**, *36*, 484.

97. Domling, A., *Chem. Rev*., **2006**, *106*, 17.

98. Raghuvanshi, D.S., Singh, K.N., *J. Heterocyclic. Chem.*, **2011**, *48*, 582.

99. Baker, B.R., Meyer, R.B., *J. Med. Chem*., **1969**, *12*, 224.

100. Gangjee, A., Adair, O.O., Queener, S.F., *J. Med. Chem.*, **2003**, *46*, 5074.

101. Tarnchompoo, B., Yuthavong, Y., Kamchonwongpaisan, S., Yuvaniyama, J., Charman, S., Katiyar, S.B., **2009**, WO 2009/048957, PCT/US2008/079210.

102. Gravestock, D., Rousseau, A.L., Lourens, A.C., Moleele, S.S., van Zyl, R.L., Steenkamp, P.A., *Eur. J. Med. Chem*., **2011**, *46*, 2022.

103. Ratouis, R., Boissier, J.R., Dumont, C., *J. Med. Chem*.,**1965**, *8*, 271.

104. Ma, H.J., Xie, R.L., Zhao, Q.F., Mei, X.D., Ning, J., *J. Agric. Food Chem*., **2010**, *58*, 12817.

105. Rong, L., Han, H., Gao, L., Dai, Y., Cao, M., Tu, Shujiang., *Synth. Comm*., **2010**, *40*, 504.

APPENDIX

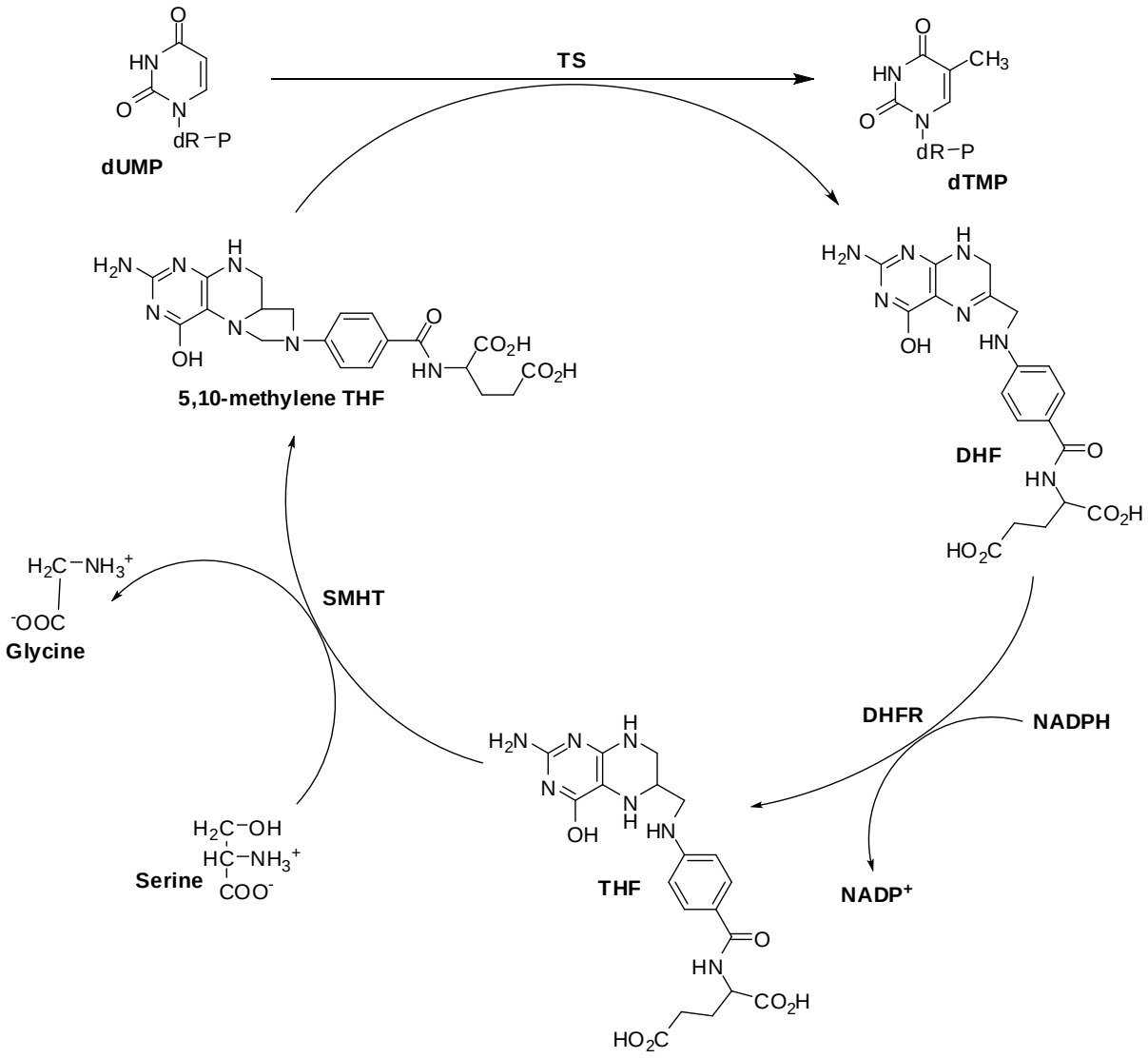


Figure 35: Folate pathway catalysed by three important enzymes.

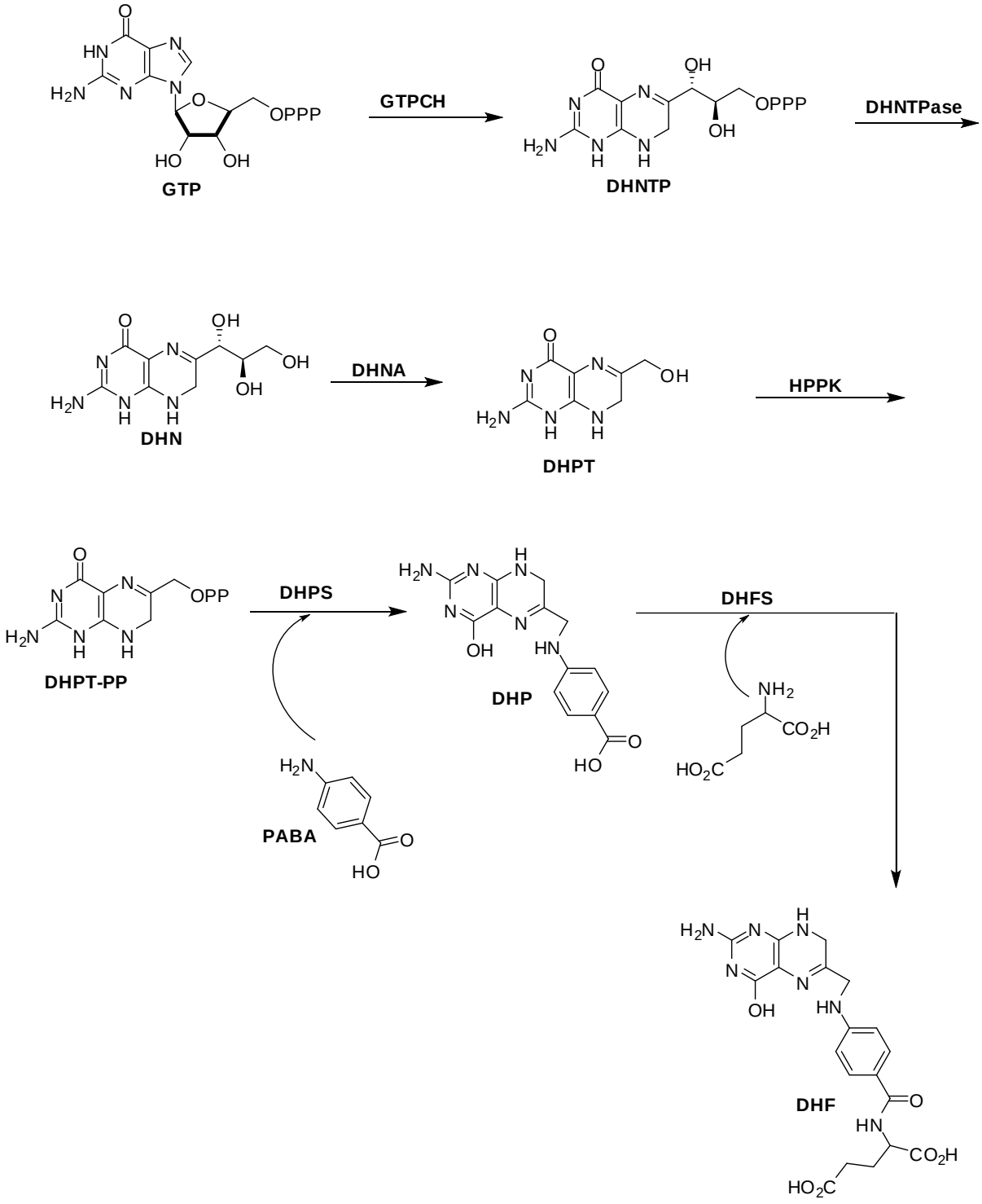
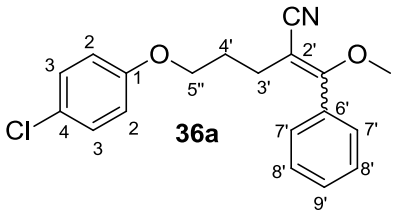
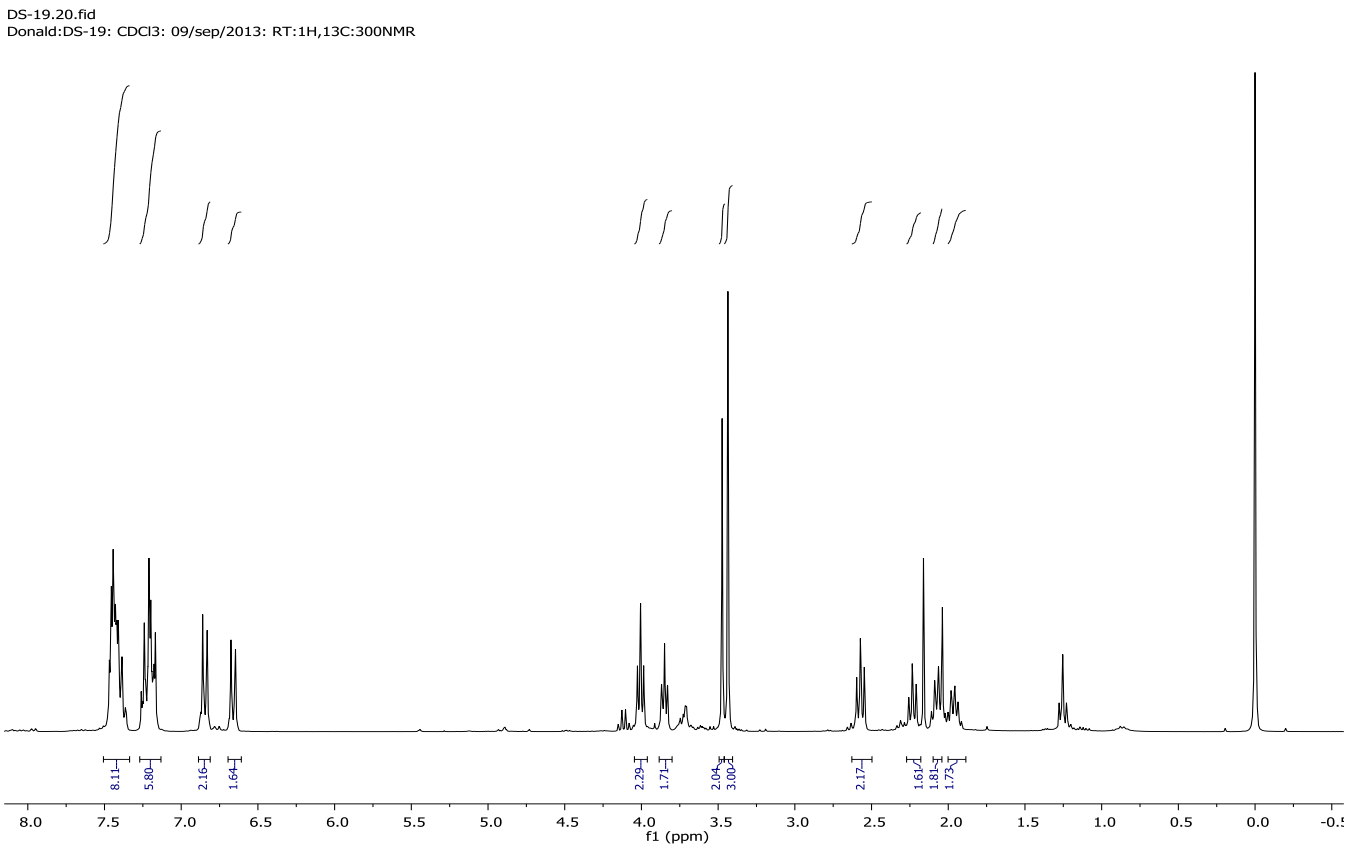


Figure 36: *de novo* Pathway present only in the parasite.

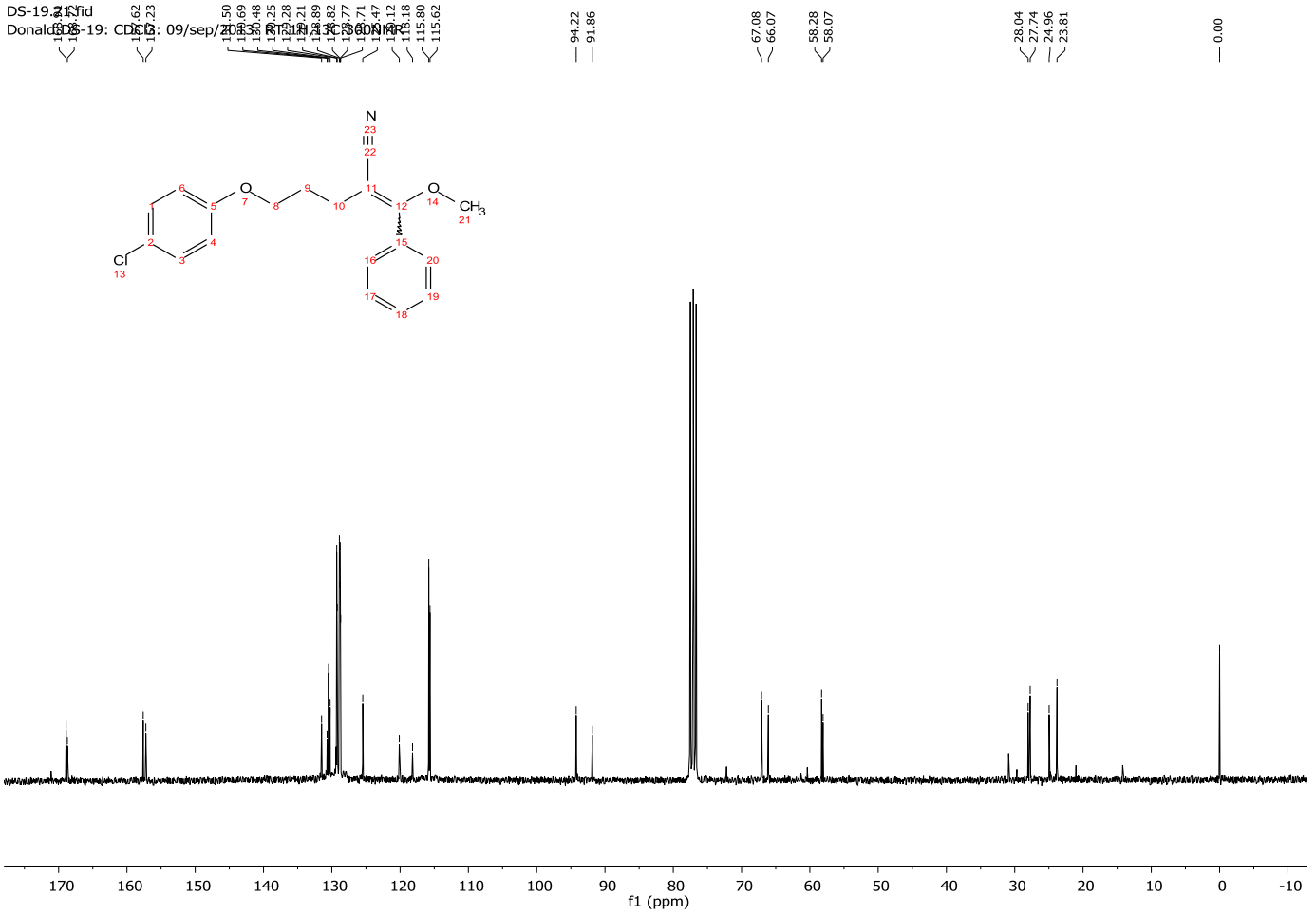
Spectra of selected compounds:

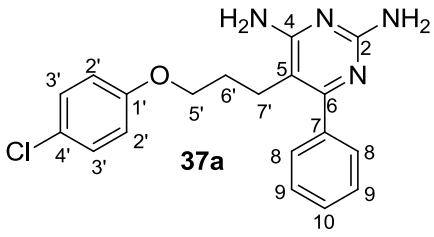


^{1}H NMR of 36a



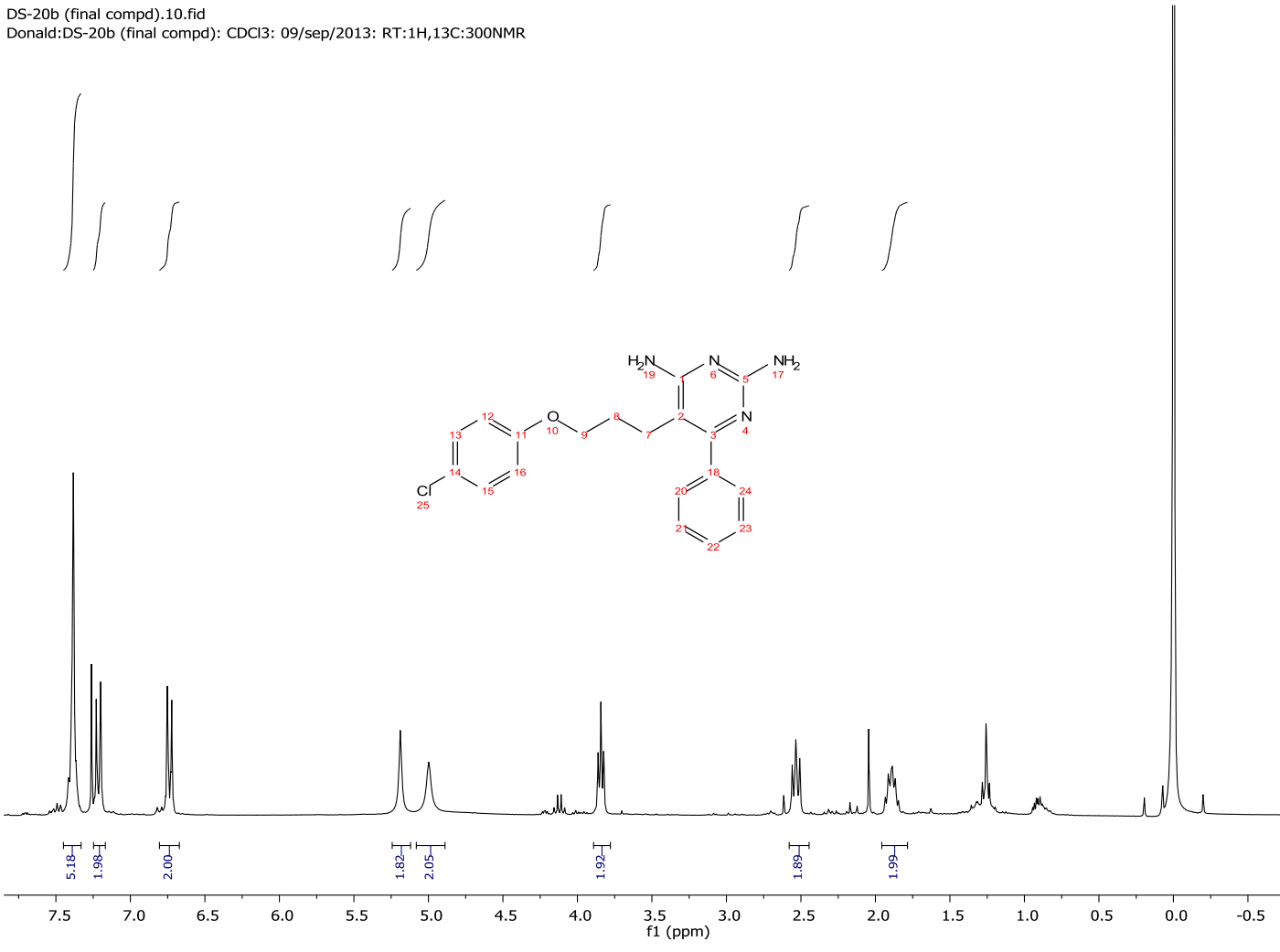
^{13}C NMR of 36a



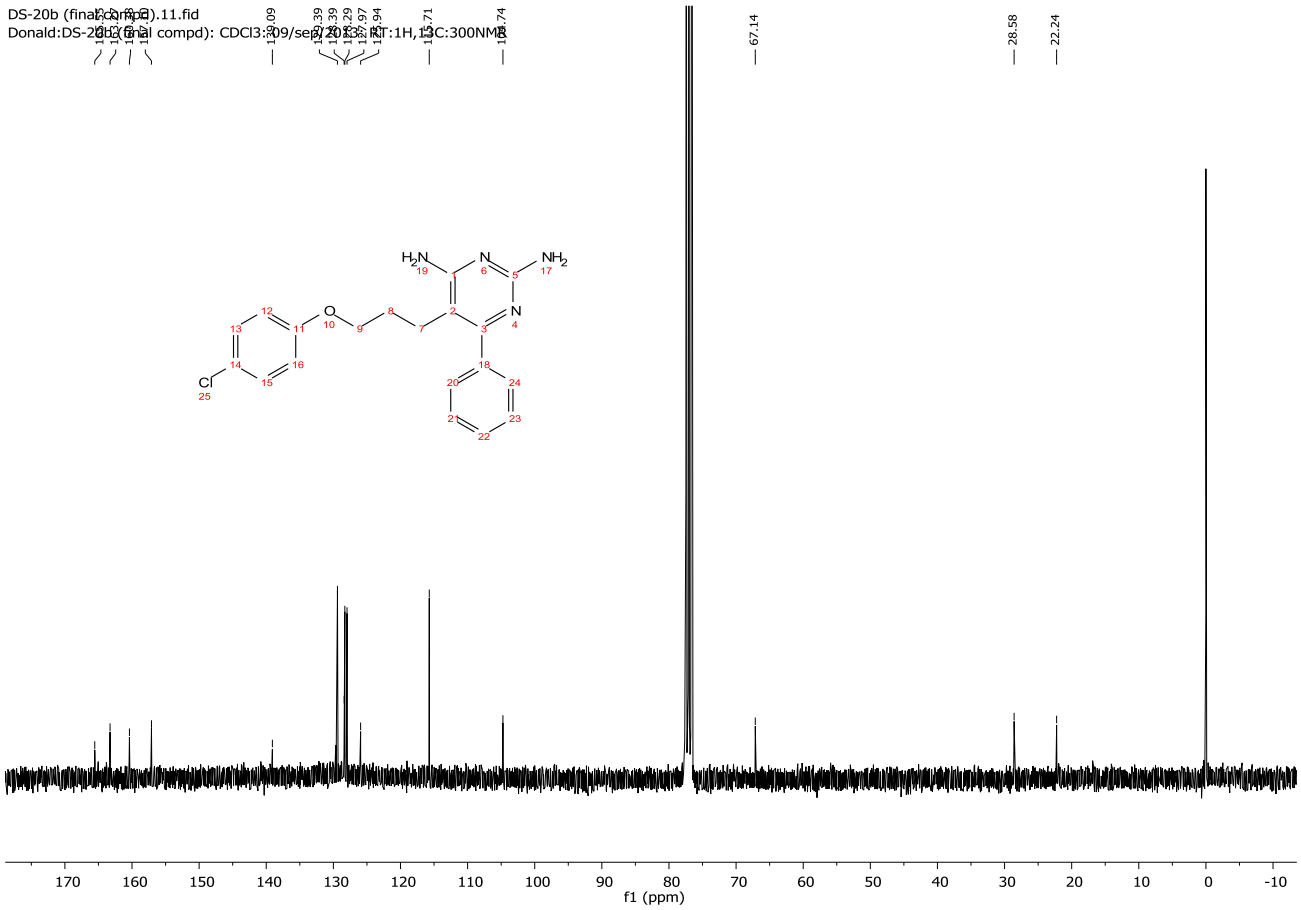


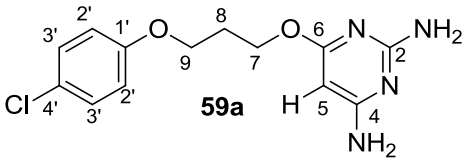
37a

^{1}H NMR of 37a

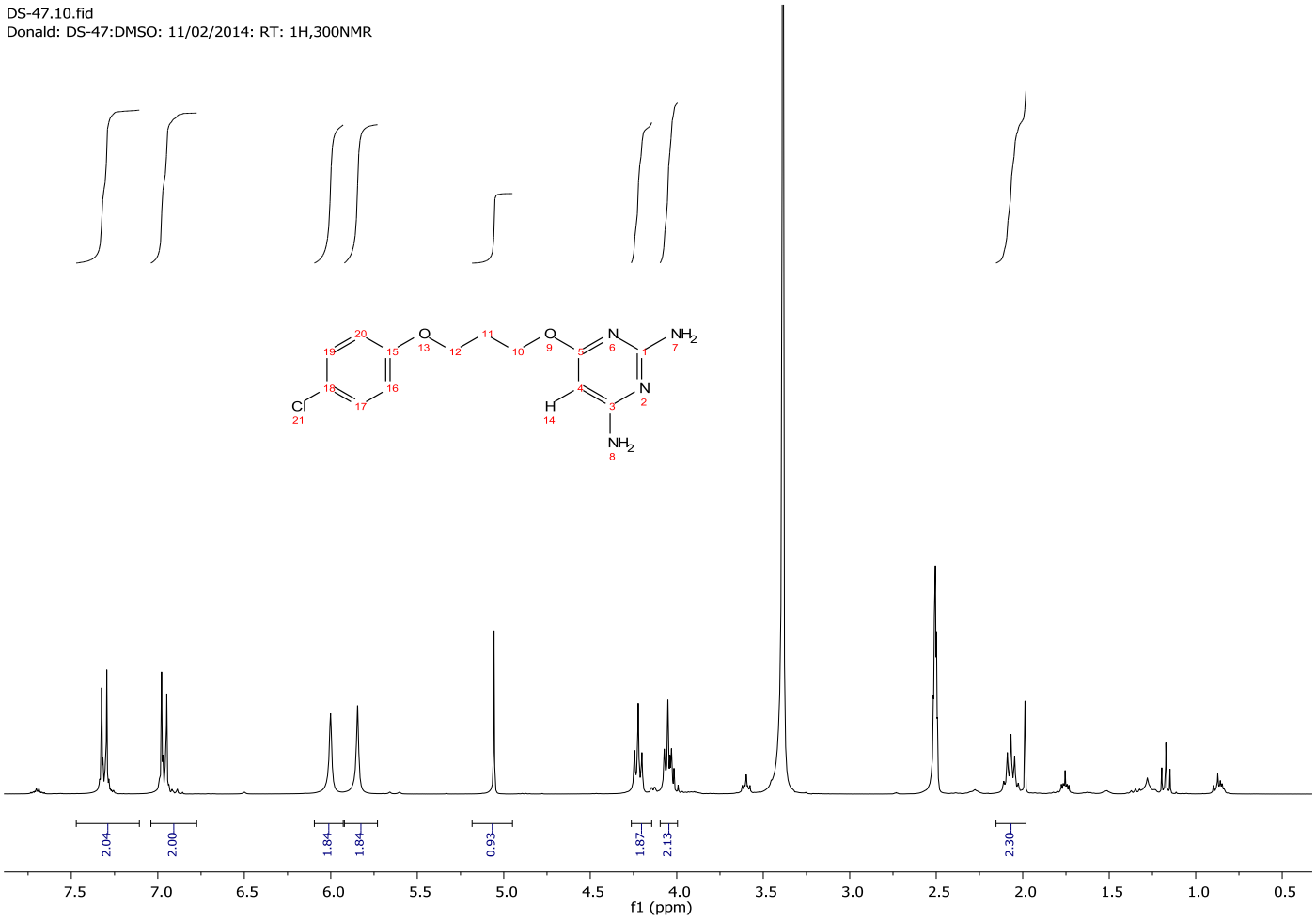


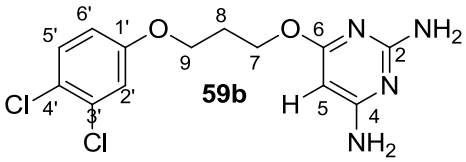
^{13}C NMR of 37a




2'
1'
O
8
O
6
N
NH2
3'
9
7
2
N
Cl
4'
2'
59a
H
5
3'
4
NH2

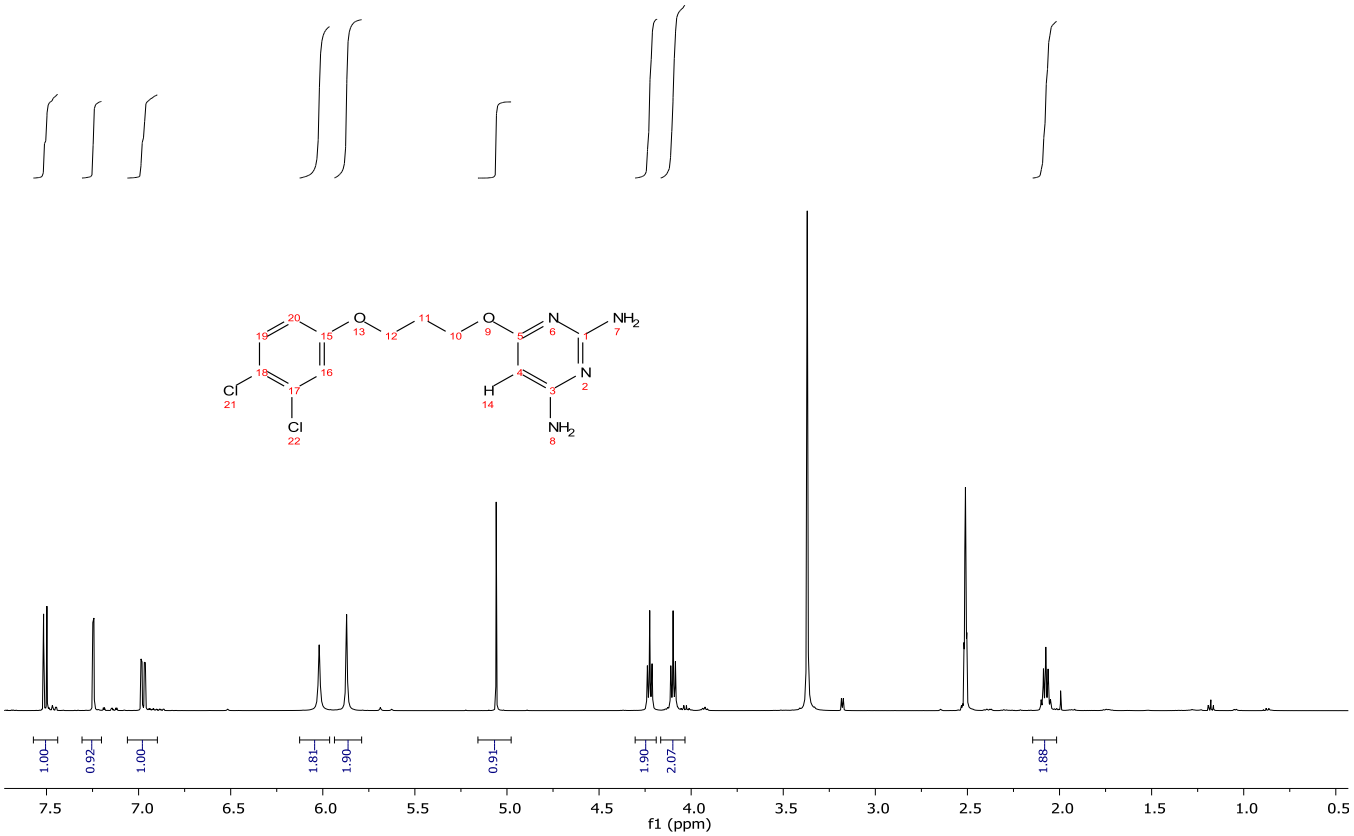
^{1}H NMR of 59a


DS-47.10.fid
Donald: DS-47:DMSO: 11/02/2014: RT: 1H,300NMR
2.04
2.00
1.84
1.84
0.93
1.87
2.13
2.30
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

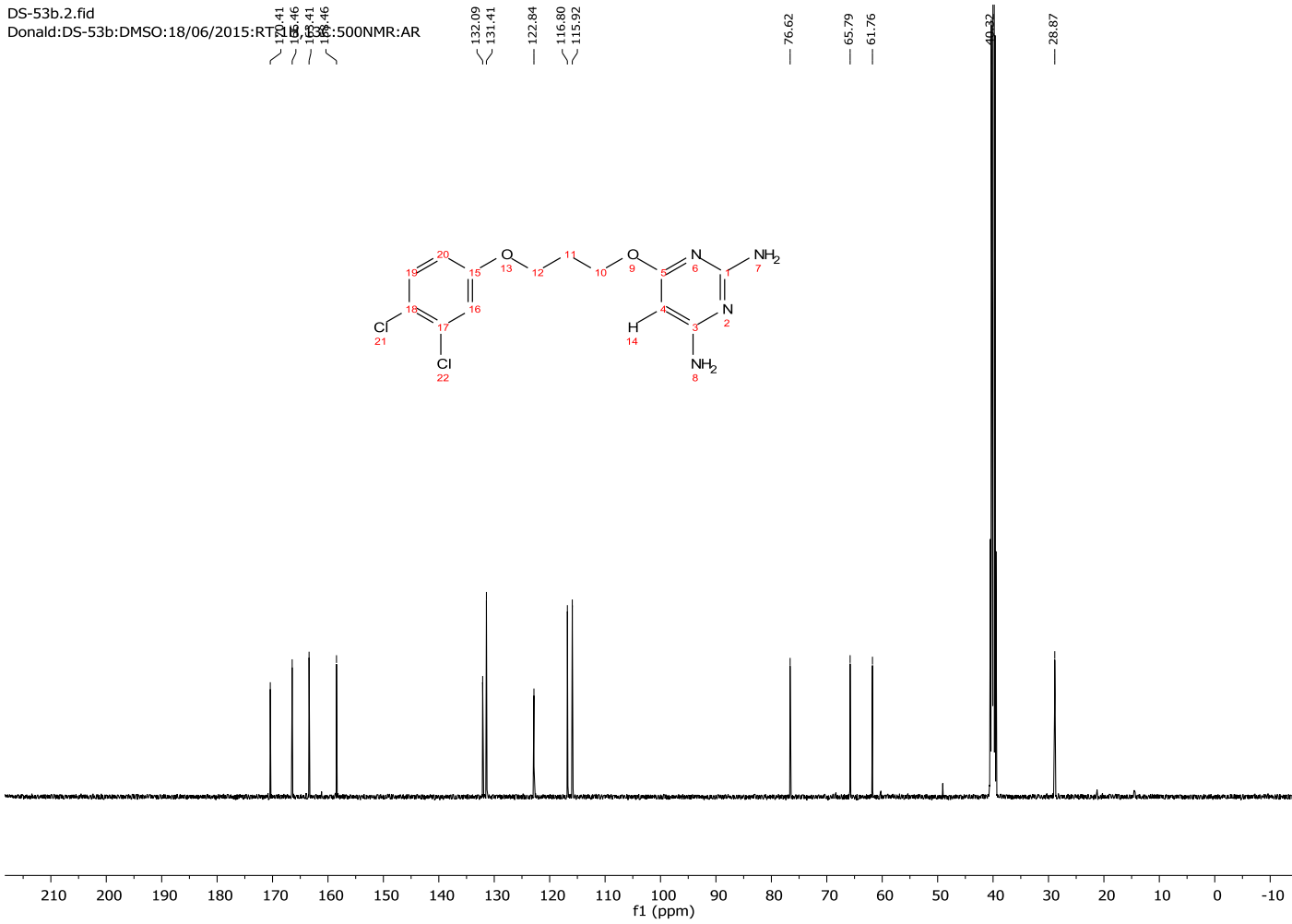


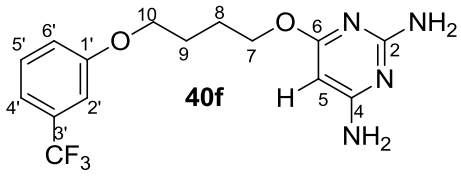
^{1}H NMR of 59b

DS-53b.1.fid
Donald:DS-53b:DMSO:18/06/2015:RT:1H,13C:500NMR:AR



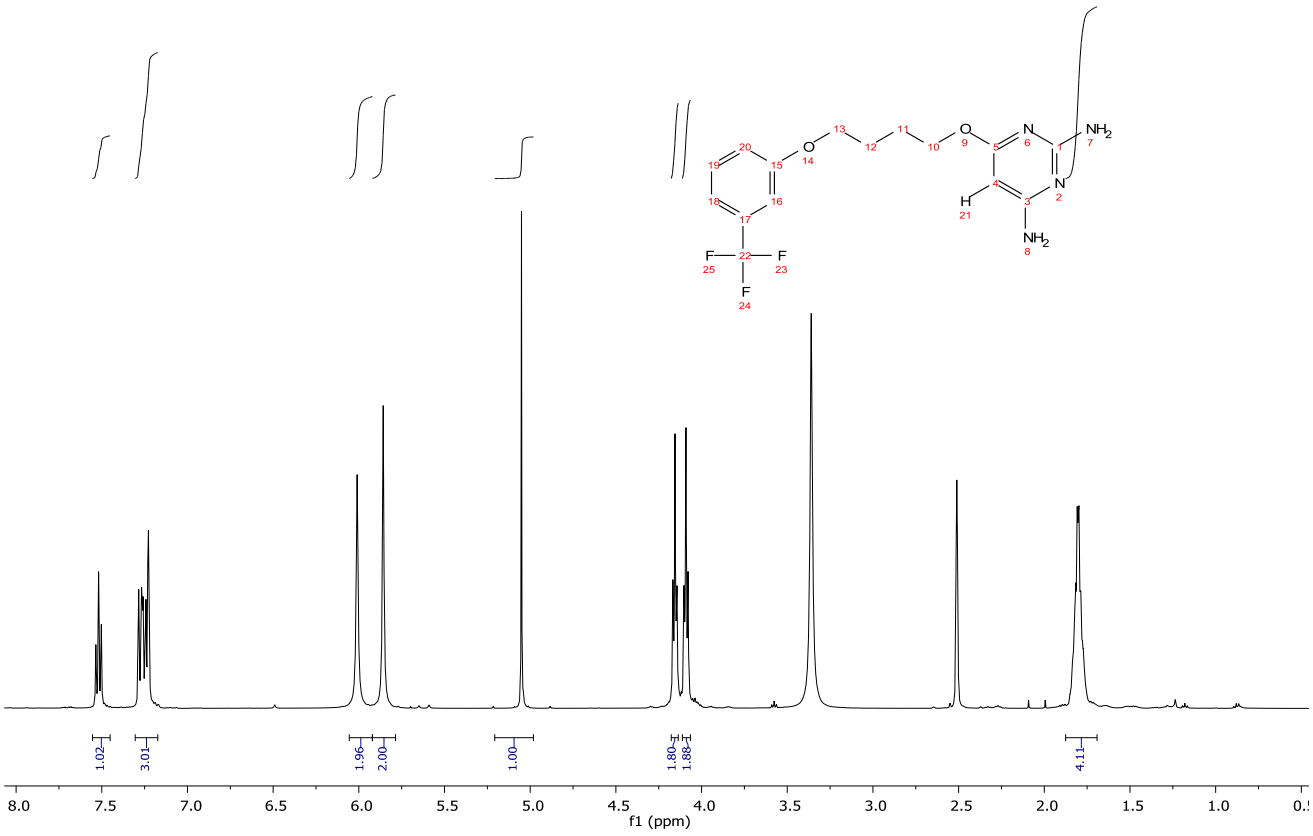
^{13}C NMR of 59b



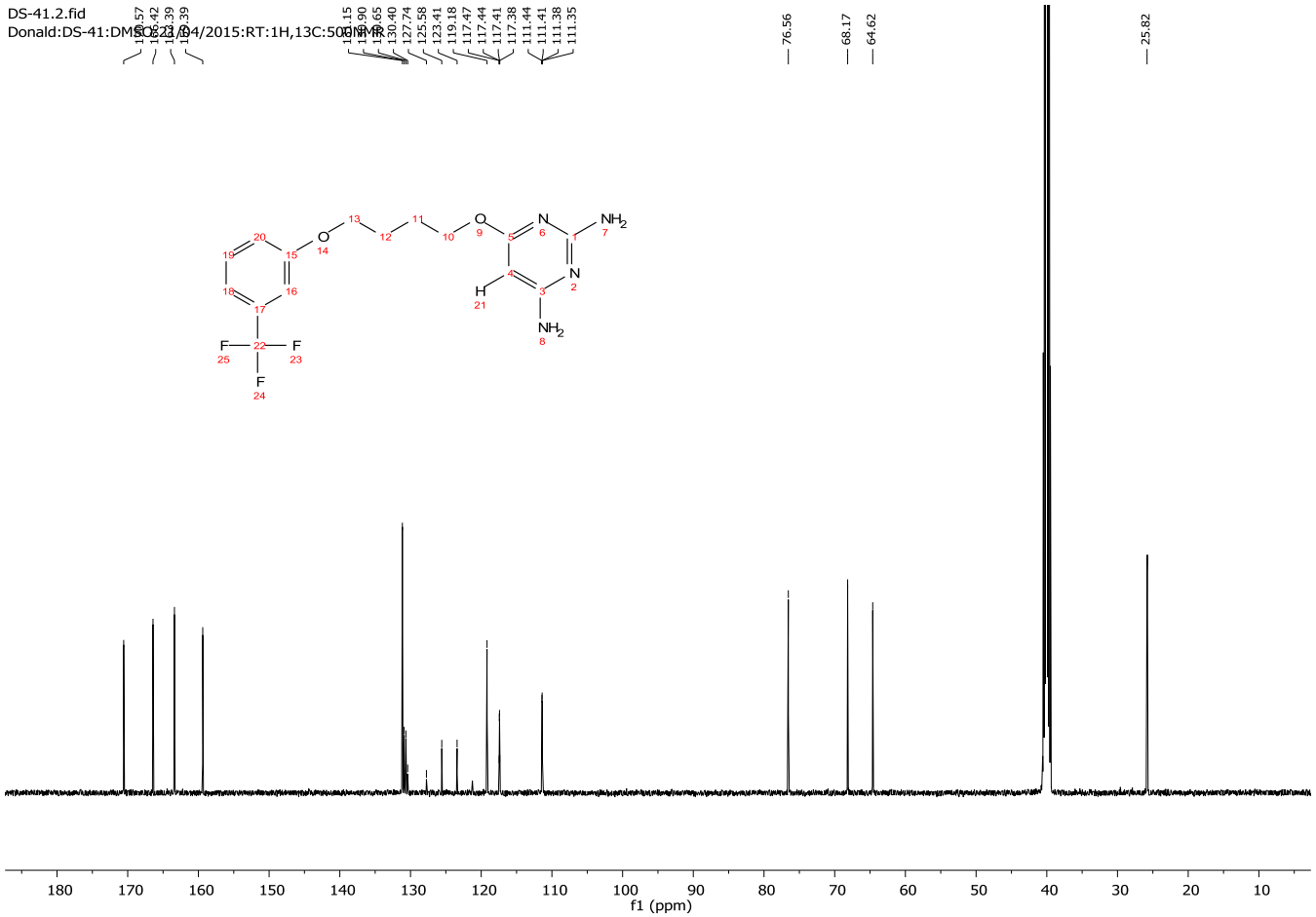


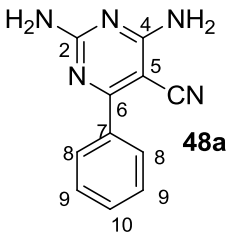
^{1}H NMR of 40f

DS-41.1.fid
Donald:DS-41:DMSO:21/04/2015:RT:1H,13C:500NMR



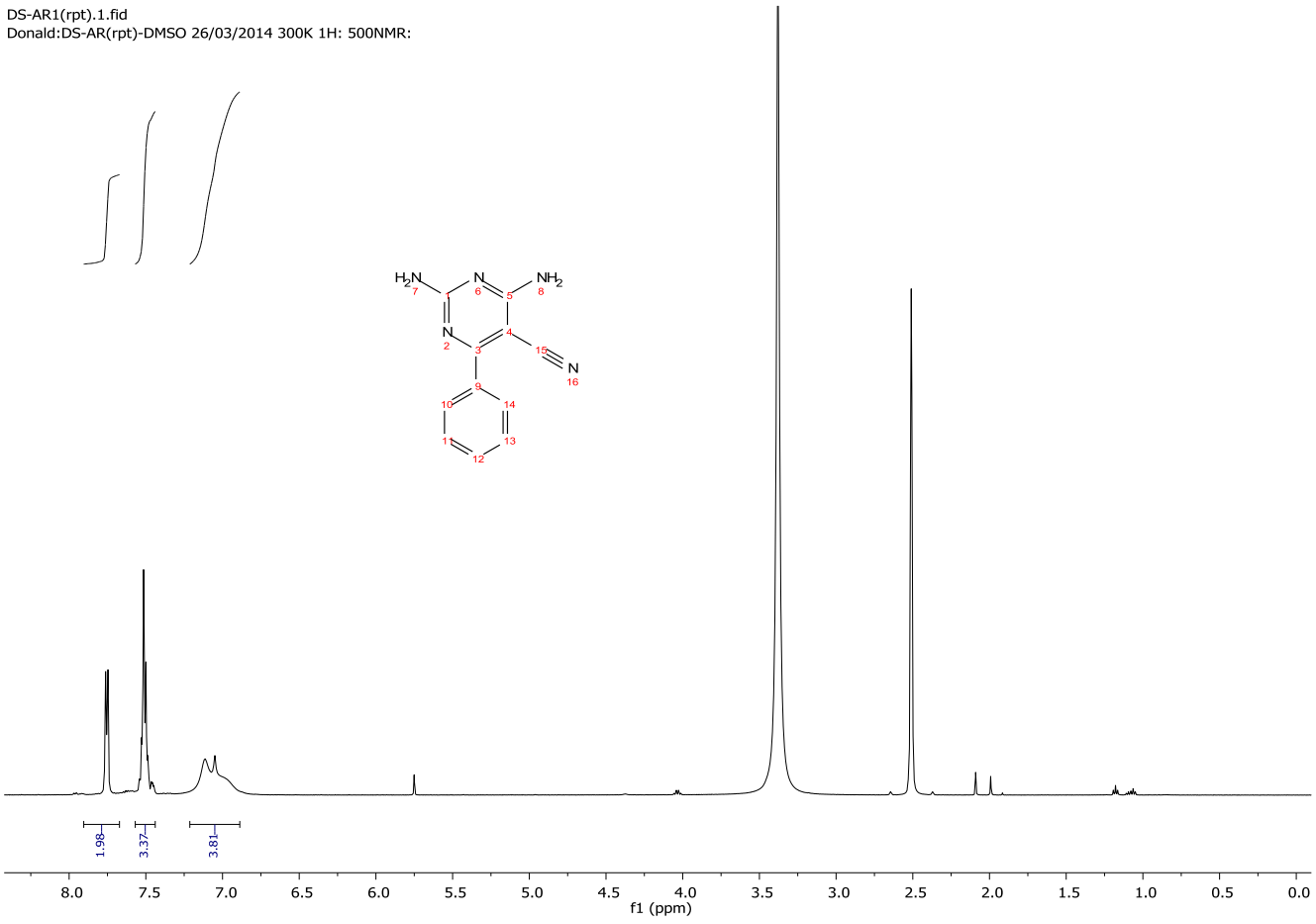
^{13}C NMR of 40f



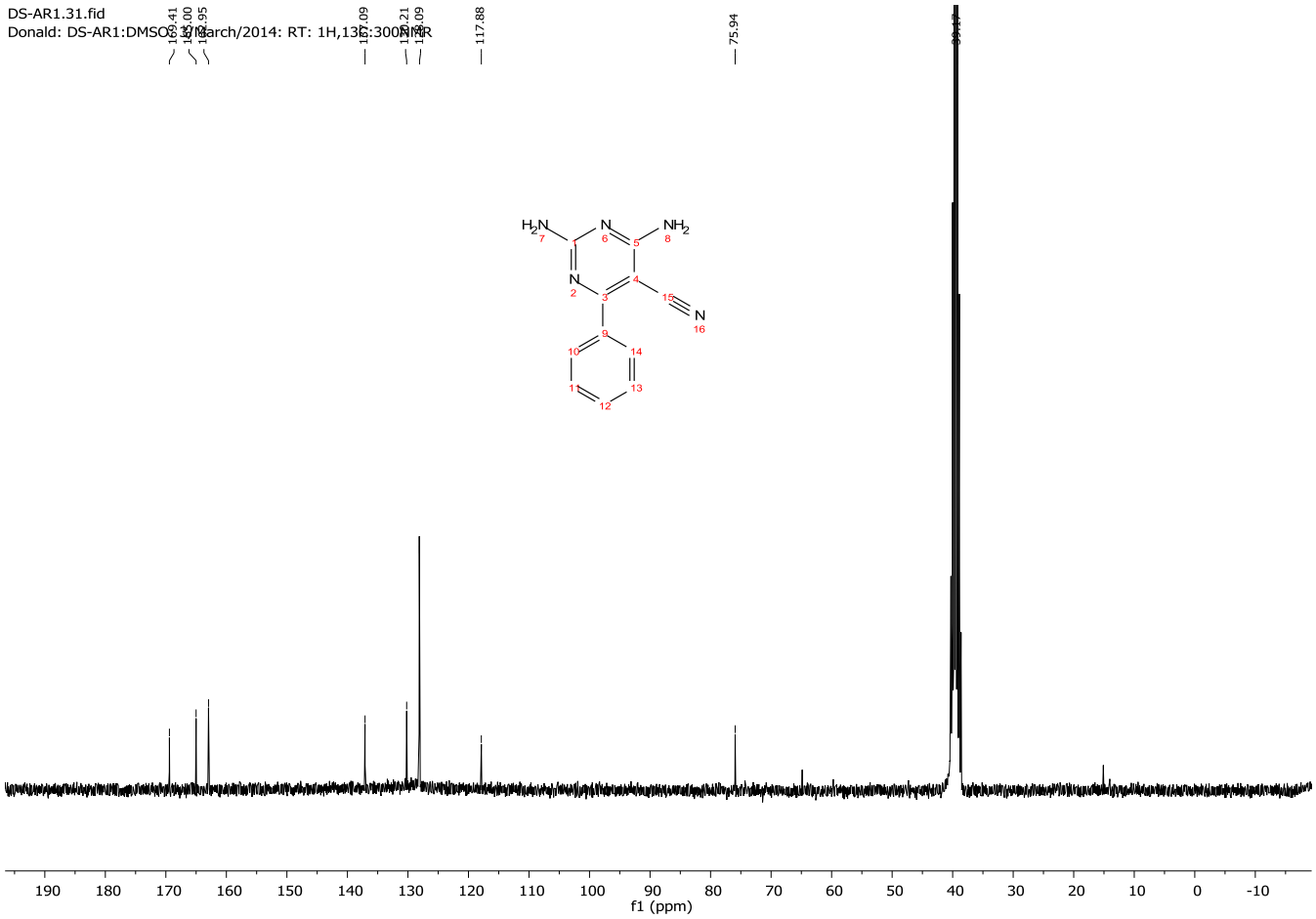


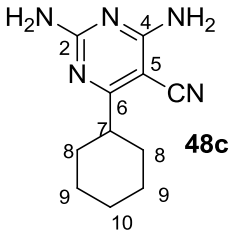
48a

^{1}H NMR of 48a



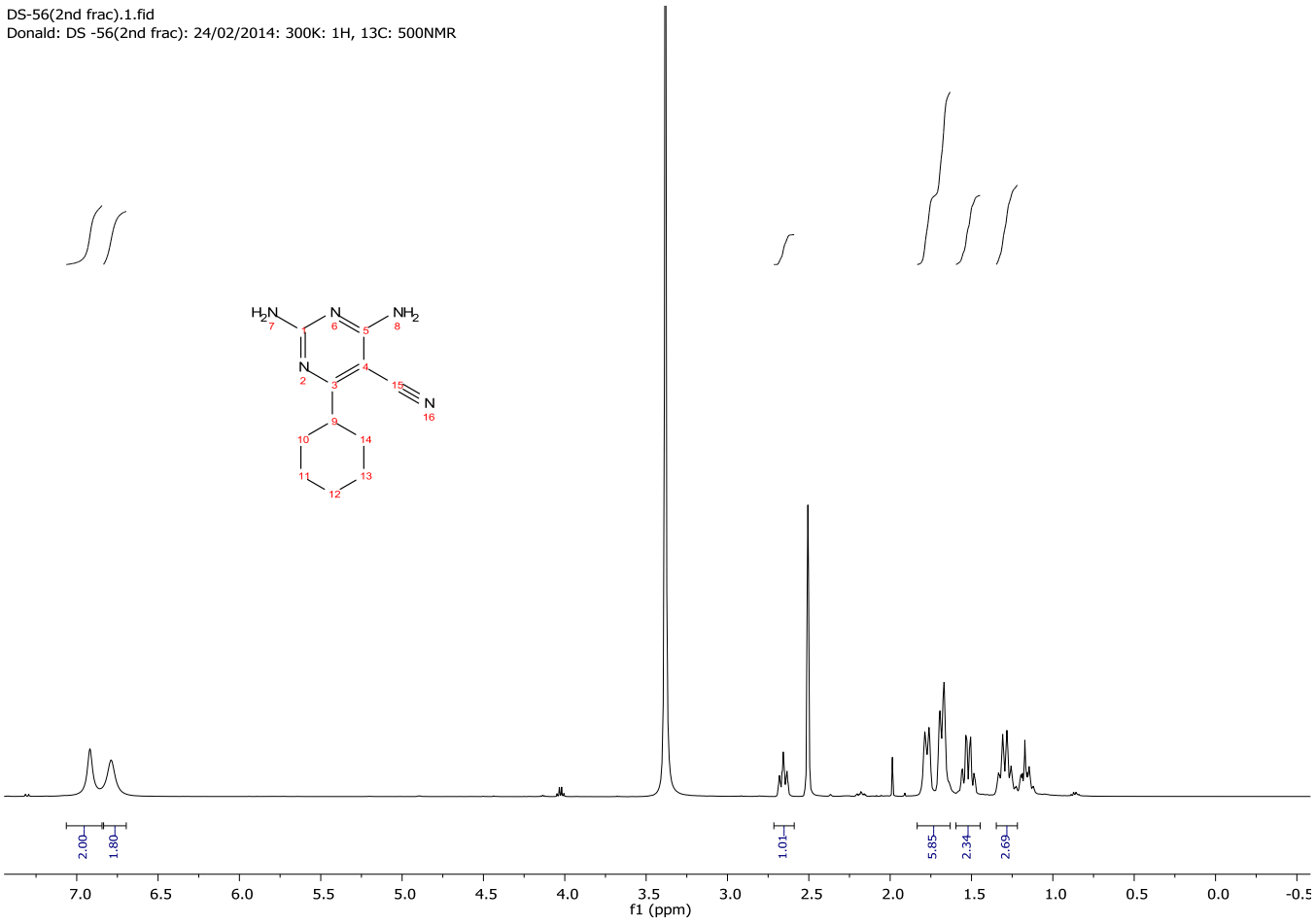
^{13}C NMR of 48a



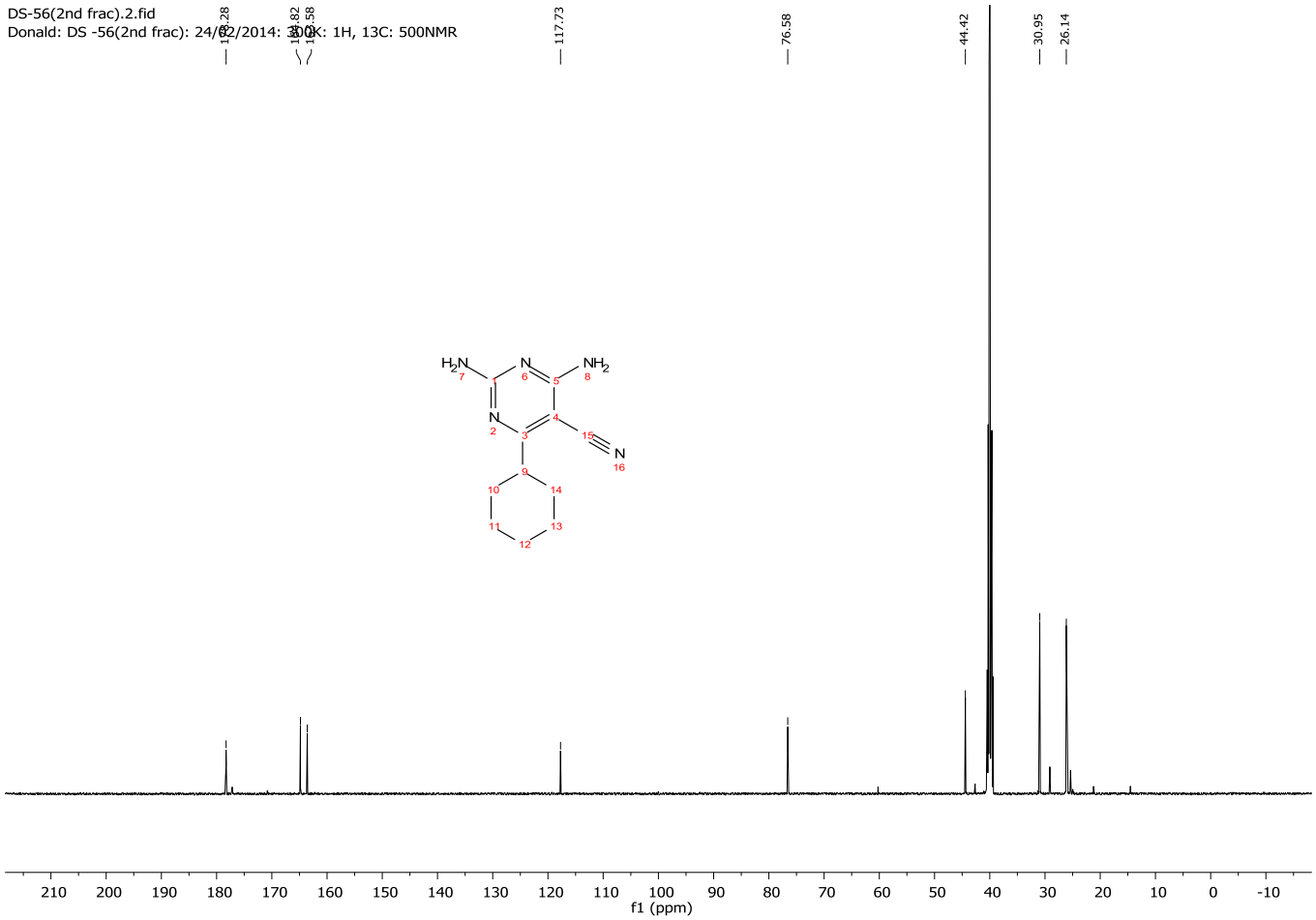


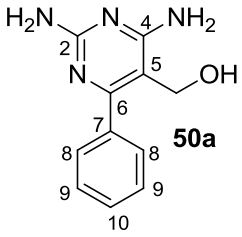
48c

^{1}H NMR of 48c



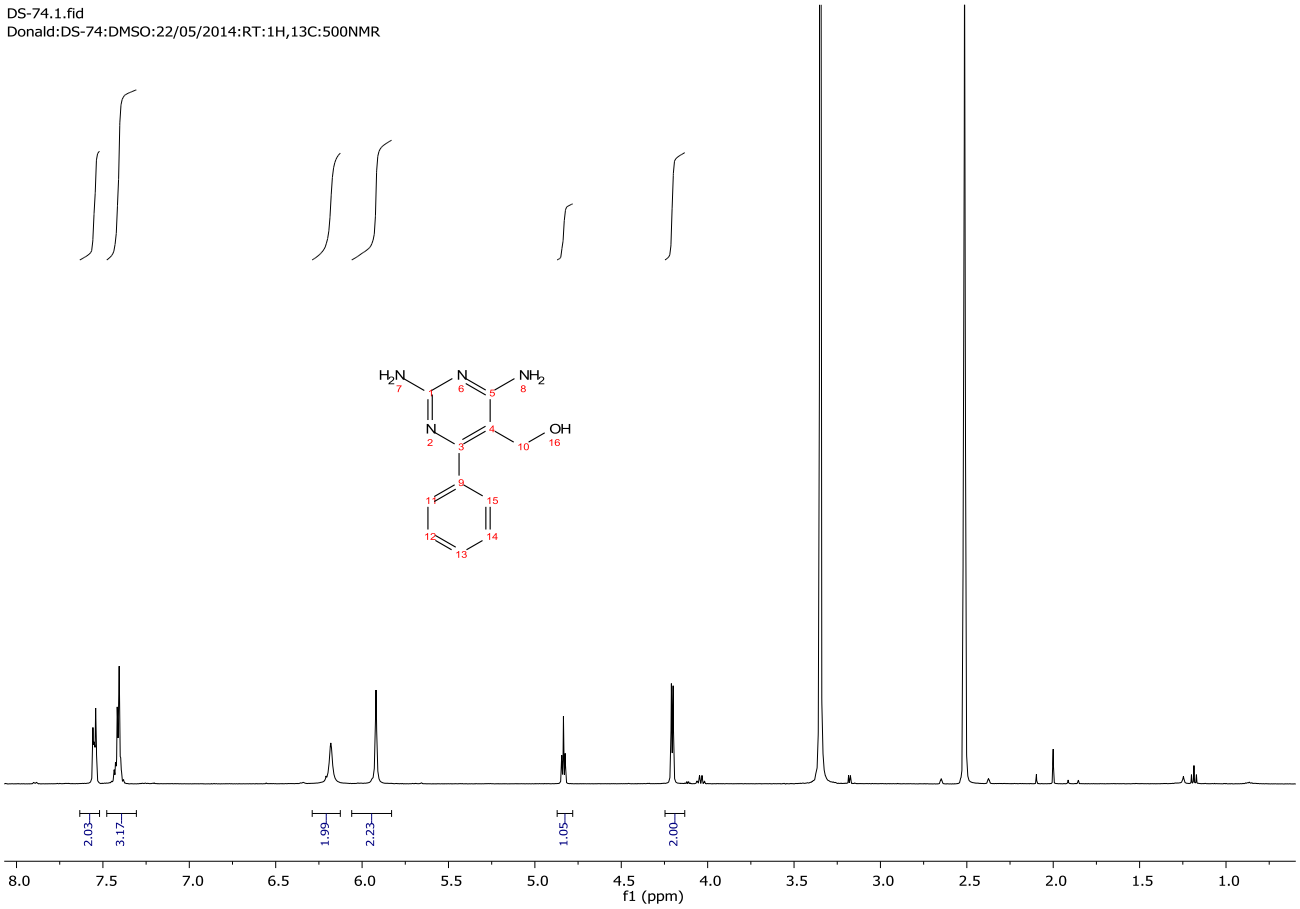
^{13}C NMR of 48c



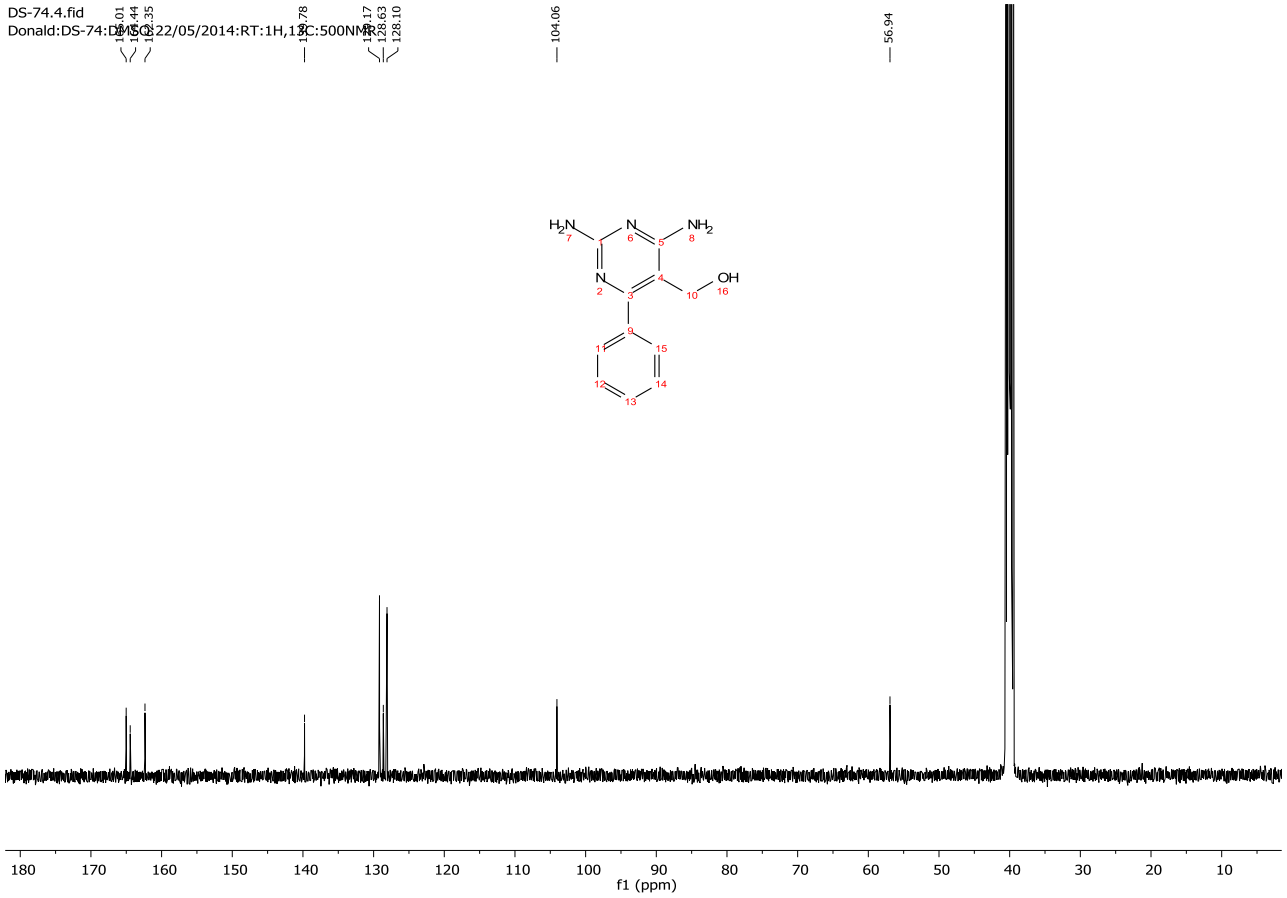


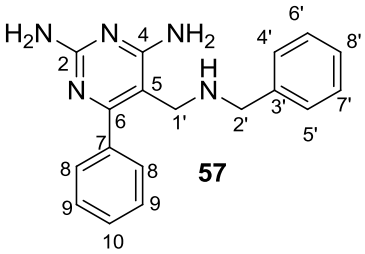
50a

^{1}H NMR of 50a

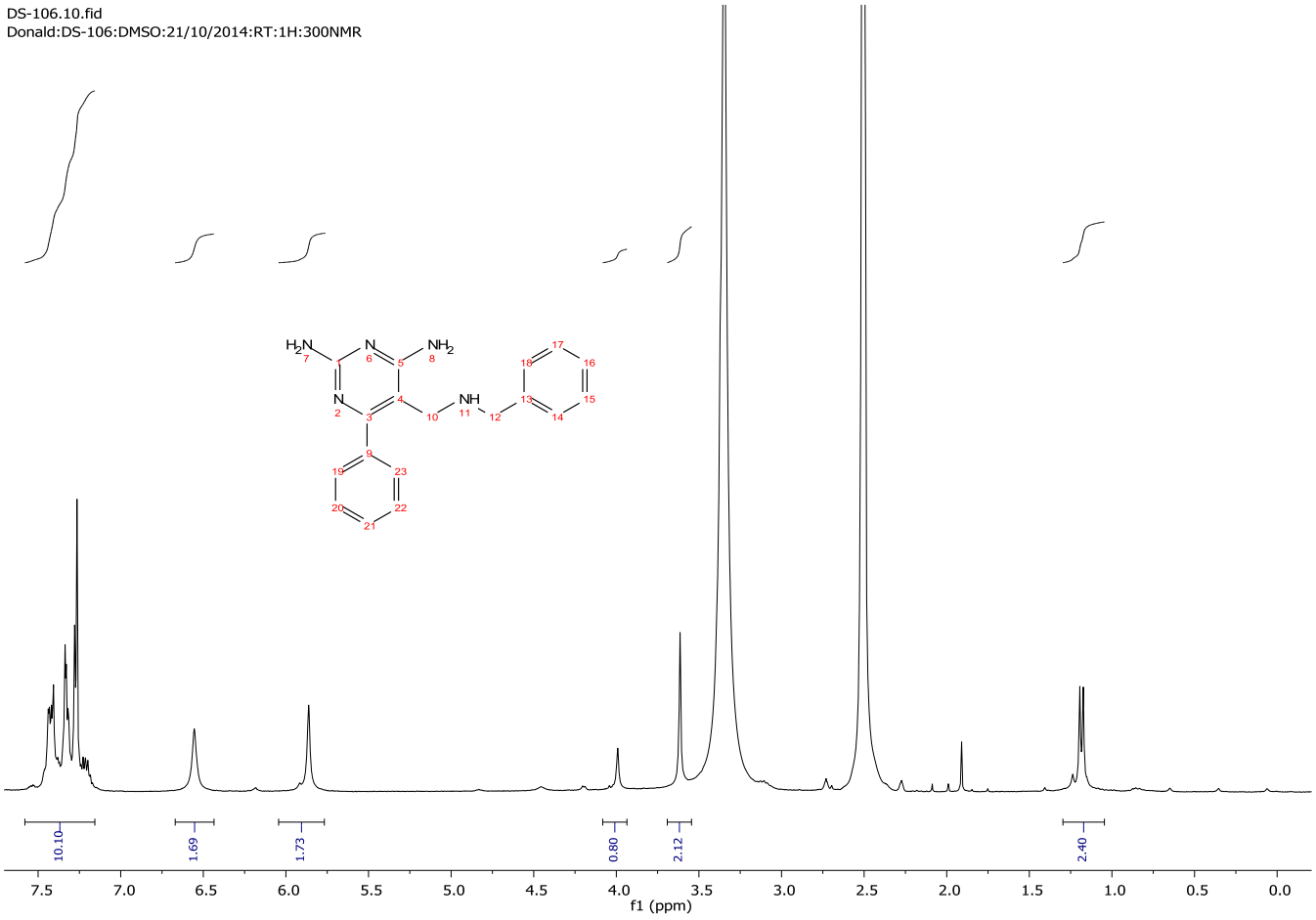


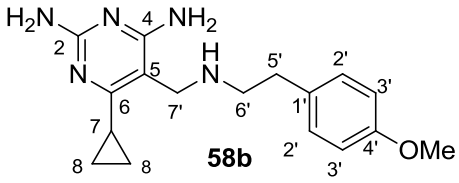
^{13}C NMR of 50a




H_2N
N
4
NH_2
2
N
5
H
N
6
7
1'
2'
3'
4'
5'
6'
7'
8'
8
9
9
10
57

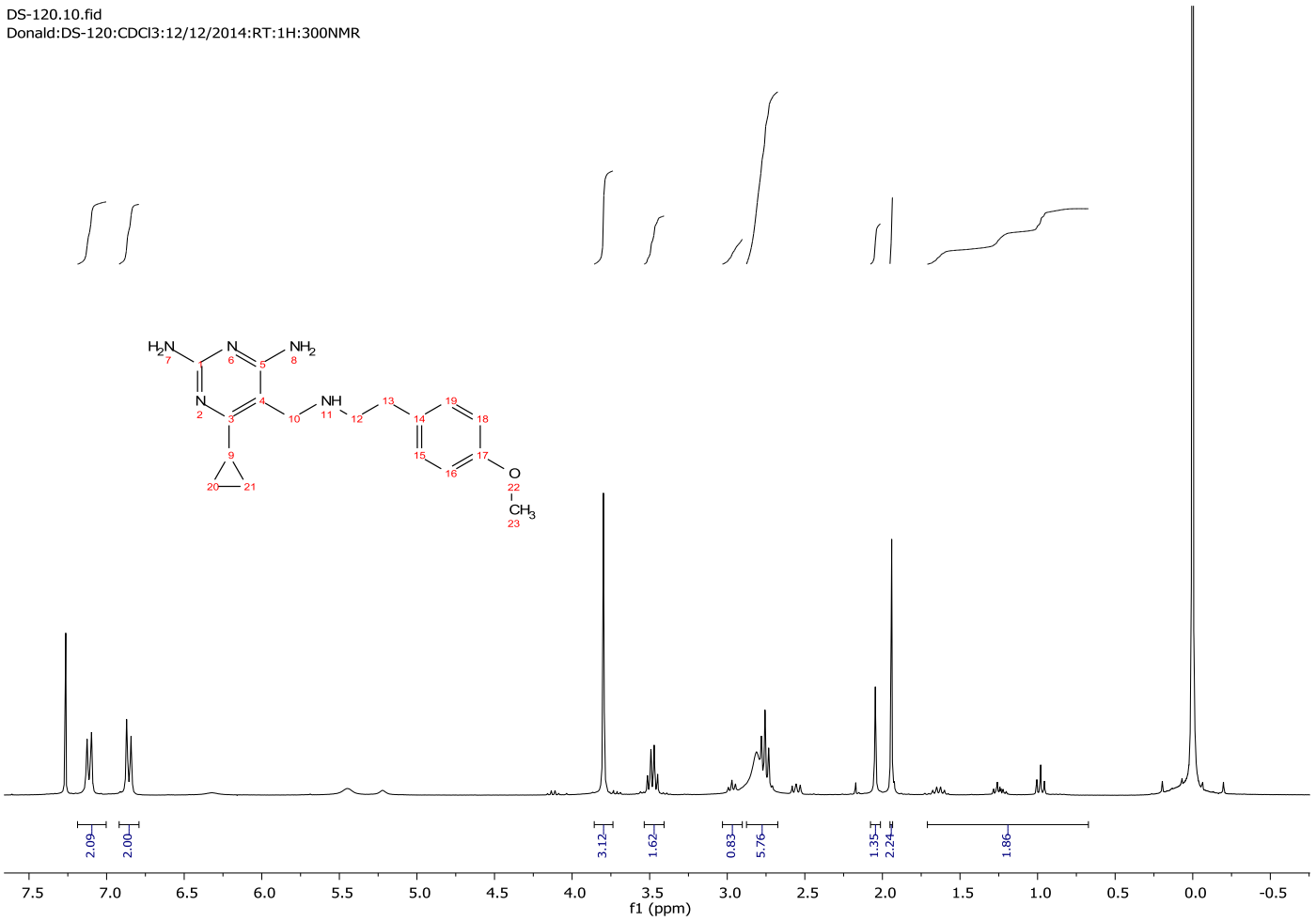
^{1}H NMR of 57


DS-106.10.fid
Donald:DS-106:DMSO:21/10/2014:RT:1H:300NMR
10.10
1.69
1.73
0.80
2.12
2.40
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

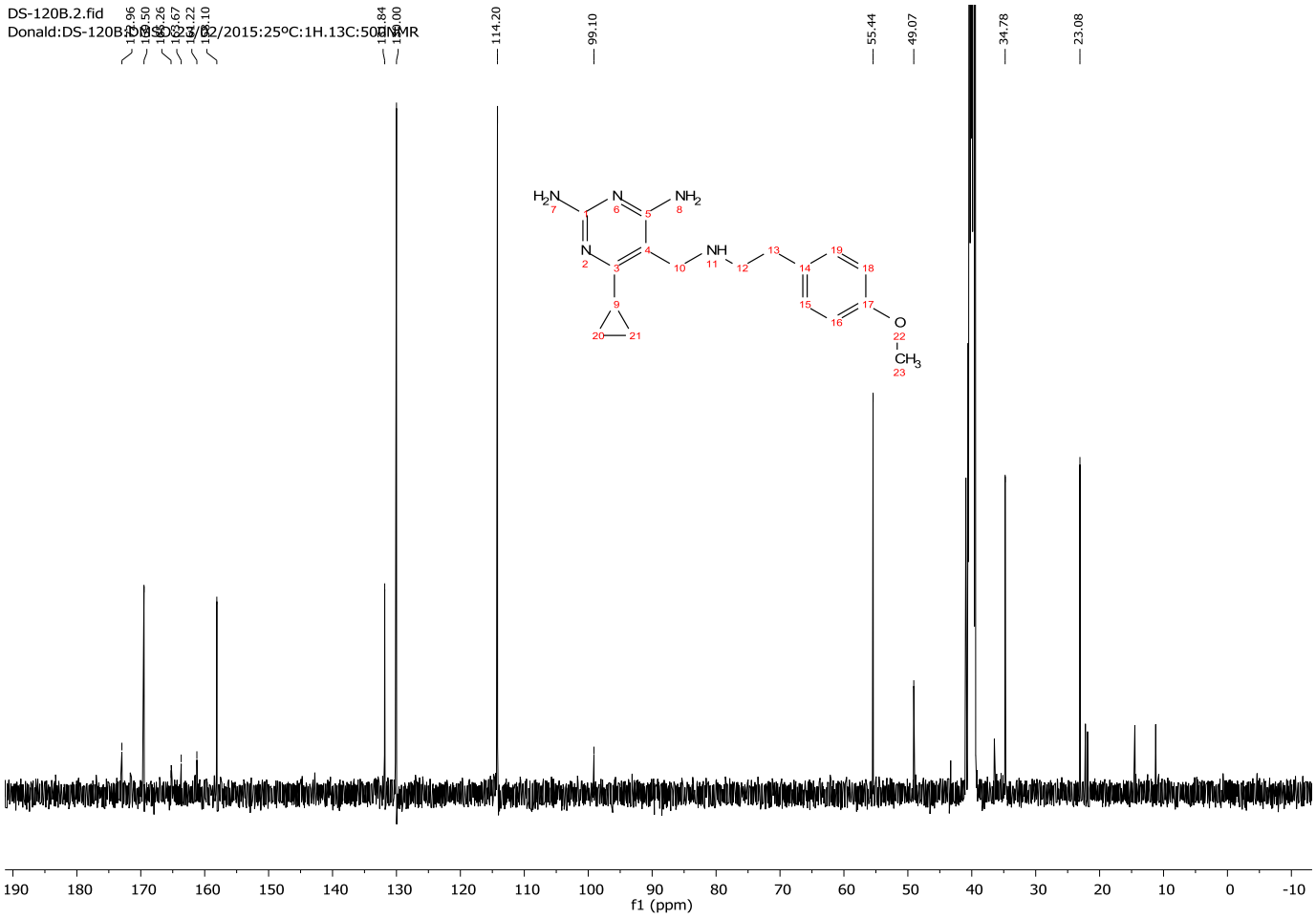


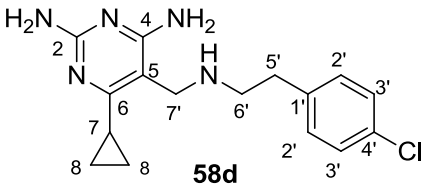
58b

^{1}H NMR of 58b



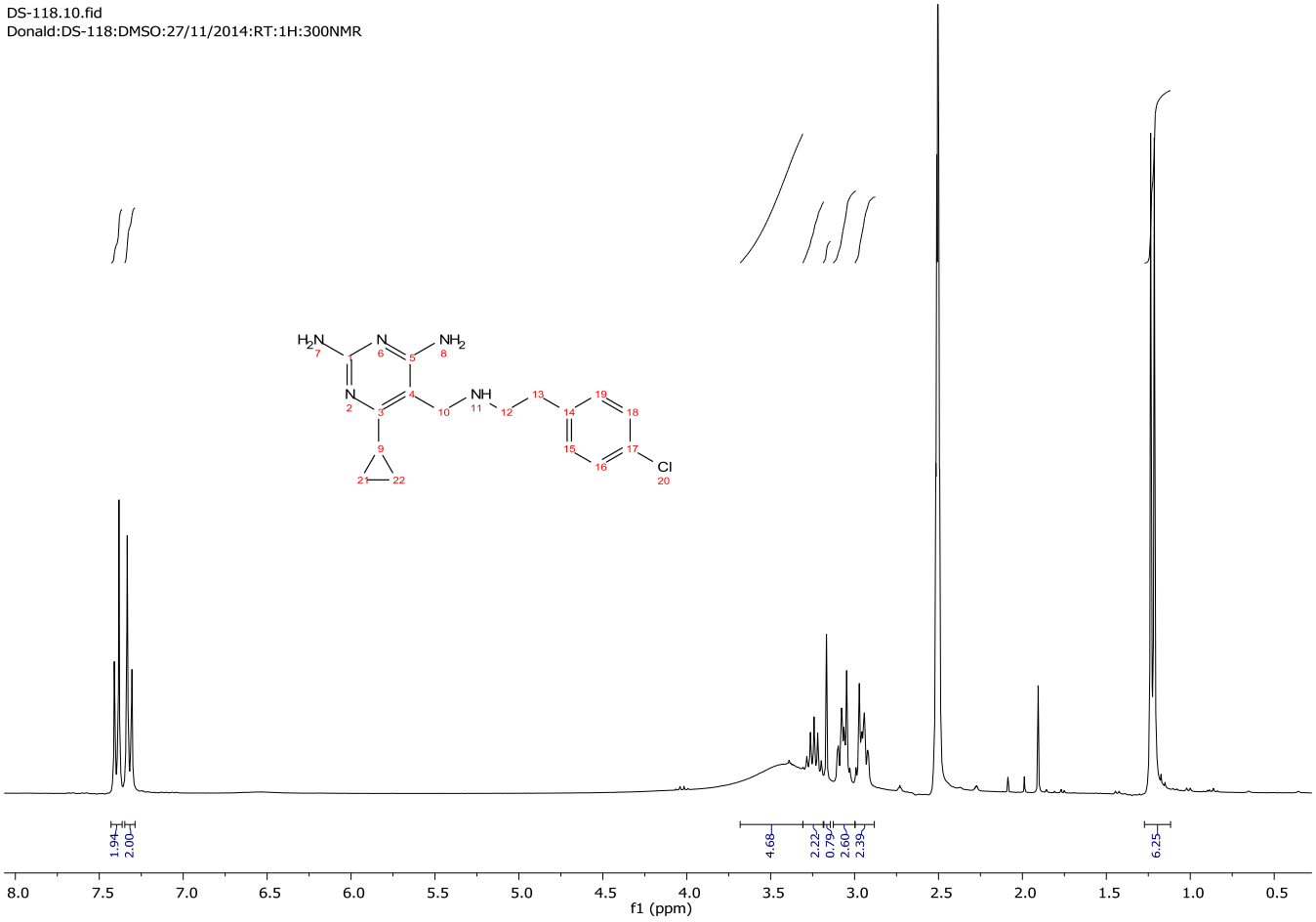
^{13}C NMR of 58b





58d

^{1}H NMR of 58d


DS-118.10.fid
Donald:DS-118:DMSO:27/11/2014:RT:1H:300NMR
1.94
2.00
4.68
2.22
0.79
2.60
2.39
6.25
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)