Synthesis and Modification of Antiplasmodial Antifolates

By

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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 Prof. C.B de Koning. It is being submitted for the degree of Master of Science at the University of the Witwatersrand, Johannesburg, and has not been submitted before for any degree or examination in any other institution.

Tebogo Molatsane

Abstract

There are many diseases responsible for the countless deaths in the African continent, one of which is malaria. Malaria is an ancient parasitic disease caused by the parasite of the genus Plasmodium, which is widely transmitted by the anopheles mosquito. This disease has been reported to have claimed millions of lives across the world and urgent solutions have been sought after since the epidemic. In attempts to manage the disease, the anti-malaria community has introduced management approaches which span the scope of environmental control and pest management. Of great importance of these approaches has been the chemotherapeutic approach towards combating the disease. The main issue associated with the chemotherapy approach has been the rise of drug resistance. Considering this drug resistance, ways to salvage the available drug knowledge, methods such as combination therapy have risen and have proven effective to a certain extent. Nonetheless, the journey towards developing drugs which could potentially eradicate malaria continues.

In this two-part project we sought to synthesize compounds which could potentially exhibit DHFR inhibitory properties. The first part of the project consists of the synthesis of flexible pyrimidine analogues from a 3-step synthetic route. This route began with a multicomponent reaction starting with commercially available aldehydes, malononitrile and guanidine hydrochloride to yield a 6-substituted 2,4-diaminopyrimidine carbonitrile. This precursor was further functionalized at the nitrile 5-position by means of a hydrogenation reaction to give an aldehyde. This aldehyde was then coupled to a commercially available phenethylamine by means of a reductive amination reaction to give the desired flexible pyrimidine analogue. Despite successfully completing the synthetic steps, the compounds were difficult to work with and thus we encountered an issue with the purity of the target compounds since purification by conventional methods was not viable.

In the second part of the project we sought to access compounds with a scaffold like the one envisioned in the first part of the project via accessing a rigid intermediate. We accessed the rigid compounds via a coupling reaction between two acetylenes. The library of ten analogues was successfully synthesized and was taken for testing and due to time constraints; we were unable to establish their activity in a whole cell *P. falciparum* assay. One of these rigid intermediates with the butyne linker was then reduced using a hydrogenation reaction conditions to give the flexible analogue.

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List of Abbreviations

ACT	Artemisinin Combination Therapy
CSIR	Council of Scientific and Industrial Research
CDC	Centres for Disease control and Prevention
CHH	Cyclohexyl proton (hydrogen)
DEPT	Distortionless enhancement by polarization transfer
DCM	Dichloromethane
DDT	Dichlorodiphenyltrichloroethane
DHFR	Dihydrofolate reductase
DHFR-TS	Dihydrofolate reductase-thymidylate synthase
DHPS	Dihydropteroate synthase
DMF	*N*,*N*-dimethylformamide
DMSO	Dimethyl sulfoxide
FD's	Folate derivatives
HRMS	High resolution mass spectroscopy
HSQC	Heteronuclear single quantum coherence
IR	Infrared
ITN's	Insecticide-treated nets
MCR	Multicomponent coupling reaction
MS	Mass Spectroscopy
NMR	Nuclear Magnetic Resonance
PfDHFR	Plasmodium falciparum- Dihydrofolate reductase
TLC	Thin Layer Chromatography
WHO	World Health Organization

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Chapter 1

1. Introduction

1.1 History

Malaria is an ancient disease which is caused by protozoan parasites of the genus *Plasmodium.* Moreover, it is defined as a febrile illness which is characterized by fever, headache and diarrhea among other symptoms (1). Five major species have been identified as the main causes of malaria in humans. *P falciparum* is known to be the most infectious in regions of Africa causing the most severe infections and highest mortality and accounting for 90% of all deaths in Africa (2). *P. vivax, P malariae, P ovale and P knowlesi* are among the other major contributors to the perpetuation of the disease in humans across the world (3), with *P. vivax* being the main causative species specifically in the Indian subcontinent (1). The transmission of this parasite is facilitated and sustained through the activities of the female anopheline mosquito, which is commonly observed in tropical regions of the world due to the ideal breeding and living conditions provided by these regions[1]. This in turn has a huge impact on the epidemiological patterns of the disease as observed worldwide (4). Factors such as temperature can play a huge role in the type of *Plasmodium* species that will be observed predominantly in specific parts of the world. *P. vivax* develops with more ease in mosquitoes found in lower temperate regions versus *P. falciparum* whose sporogony development is inhibited when ambient temperature falls below 20 °C (4). As a result, the administration of antimalarial drugs is heavily influenced by the geographic location of the infection and the species in question (1).

Two major clinical syndromes have been identified because of malaria; firstly, malaria with respiratory distress followed by malaria with neurological disturbances or cerebral malaria. Cerebral malaria appears predominantly in children especially under the age of five (5). To much interest one study showed that children who were exposed to malaria before birth developed a tolerance towards *P. falciparum* (5). The persistence of the tolerance towards the parasite continues into their childhood, ultimately reducing the ability of the child's immune system to defend itself against the parasite, which increases the likelihood of the child developing malaria (5).

According to the Centres for Diseases Control and Prevention (CDC), the first observations of malaria or symptoms like those of malaria were noted 4000 years ago as reported in Chinese medical reports (6). This was then succeeded by records emerging from Greece which dated back to the 4th century BC (6). Among other records the Susruta Sanskrit medical treatise quoted symptoms of malaria which were expanded and linked to bites of certain insects, alongside Roman writers who associated the disease with swamps (6).

The connection between malaria and swamps was built on much speculation and superstitious reasoning. It was believed that evil spirits or malaria gods were the rulers of theses marshes which were great breeding grounds for the Anopheles mosquito. However, for a long time the vector was not implicated in the transmission of malaria. Malaria was attributed to Nergal, the Babylonian god of destruction and pestilence by the Roman people (7). It was only at a later stage that the link was made between the mosquito and malaria, resulting in the naming of the mosquito as the "Prince of the Devils" (7). Due to the high rate of malaria incidence in the swamp areas, malaria was later associated with the ingestion of stagnant water, which lead to the genesis of the Roman drainage programs. These programs served as the 1st recorded intervention against malaria.

Drainage of swamps was highly beneficial for many parts of the USA and Europe. This was evident in the rapid decline in malaria morbidity in these areas (6). As a result, more benefits were realized through this process. The exposed ground due to the drained swamps provided good soil for agricultural activity. This subsequently led to more income affording the nations a better standard of life (6). Cultivation of livestock served as a divergent, where fewer humans were first in line for the feeding purposes of the mosquito in the presence of more livestock.

Charles Alphonse Lavern was recognized as the first person to notice parasites in the blood of malaria infected patients, for which he won a Nobel Prize in 1907 (6). Based on his observations, the understanding of malaria has catapulted and advanced immensely. Upon the discovery of the causal agent of malaria it was also noted that there were disparities in the periodicity of the fevers which were now known to be caused by this protozoan parasite, this prompted the urgency for differentiation of the parasites (7). Camillo Golgi, an Italian neurophysiologist established that there were at least 2 forms of the disease based on the differences in intensities of the fever in malaria infected patients (6). This observation prompted the search for the possible species responsible for the differences in symptoms in patients. Giovanni Batista Grassi and Raimondo Filetti were later responsible for the

introduction of the names *Plasmodium vivax* and *malariae* with the introduction of *knowlesi* occurring much later in 1965 (7). Details as to who was responsible for the naming of *P. falciparum* are not apparent in the available literature.

The discovery of the parasite that causes malaria was heavily driven by the rise in peculiar deaths during the 2nd world war, where Charles Alphonse Lavern was stationed in Algeria in his capacity as medical doctor and so happened upon the disease. The driving force behind this discovery was the lethality of the disease and how frequently soldiers were losing their lives off the battlefield (7).

A vast amount of foundational work had been done and was still ongoing when the mode of transmission of the malaria parasite was discovered. This played a significant role in the understanding of the disease and had potential to aid in the mitigation of the disease. The important question of "how" the disease was being transmitted from one person to another needed an answer. And based on the work of multiple researchers in the 1800's the answer was found. The names of Charles Alphonse Lavern and Giovanni Batista Grassi are readily mentioned about the understanding of the transmission of the disease. However, Sir Patrick Manson, also known as the "father of tropical medicine", is believed to have been the first person to successfully demonstrate the link between human, parasite and mosquito in 1878 (6). Based on these findings Sir Ronald Ross was able to further confirm these postulations making a stronger case for transmission of malaria by the anopheles mosquito (7).

The findings of Ronald Ross in 1897 that the vector of malaria was indeed the mosquito was ground breaking and has played a huge role in the shaping of vector control (8). Furthermore, the life cycle of the mosquito became an important area of study to provide answers pertaining to malaria. Understanding mosquito biology and the mosquito-malaria interactions was critical for the advancement of treatments to combat the disease.

1.2 The life cycle of the malaria parasite

A clear understanding of the life cycle of the malaria parasite has shown to be useful in the cultivation of approaches towards the control and eradication of the disease. The intricacy of the malaria parasite biology has been a major hindrance to the development of robust treatment methods. A good working knowledge of the developmental stages of the parasite presents possible targets for the interruption of the life cycle **Figure 1**. The malaria life cycle hinges on the infection of 2 hosts, namely, the human host (asexual phase) and the female anopheles mosquitoes (sexual phase) (4). These two hosts are representative of the 2 separate sub cycles

that make up the entire life cycle of the parasite. In the human host, the cycle begins in the liver cells and then progresses on to the red blood cells (8). It is in the red blood cells where broods of the parasite grow and eventually destroy the red blood cells by releasing daughter parasites, also known as merozoites, which continue the cycle by invading more red blood cells (4). A small percentage of these differentiate into gametocytes.

The beginning of a different cycle is seen when the mosquito has a blood meal and gametocytes, which arise as progeny of the asexual blood stages of the parasite, are picked up during this meal (10). An important point to note is that the mosquito is the vector which only facilitates the transmission of the disease between hosts and does not exhibit any symptoms unless the gametocytes reach the mature stage five unlike the human host (11). Gametocytes are a blood stage of the parasite in the human host which marks the growth and multiplication in the mosquito host (4). This growth spurt occurs over a period of approximately 10-18 days resulting in the occurrence of parasites in the salivary glands of the mosquito known as sporozoites (4). This phase of the malaria parasite life cycle is referred to as the sporogonic cycle (4). These are then injected along with the saliva of the mosquito into a different human host during a blood meal marking the initiation of another cycle.

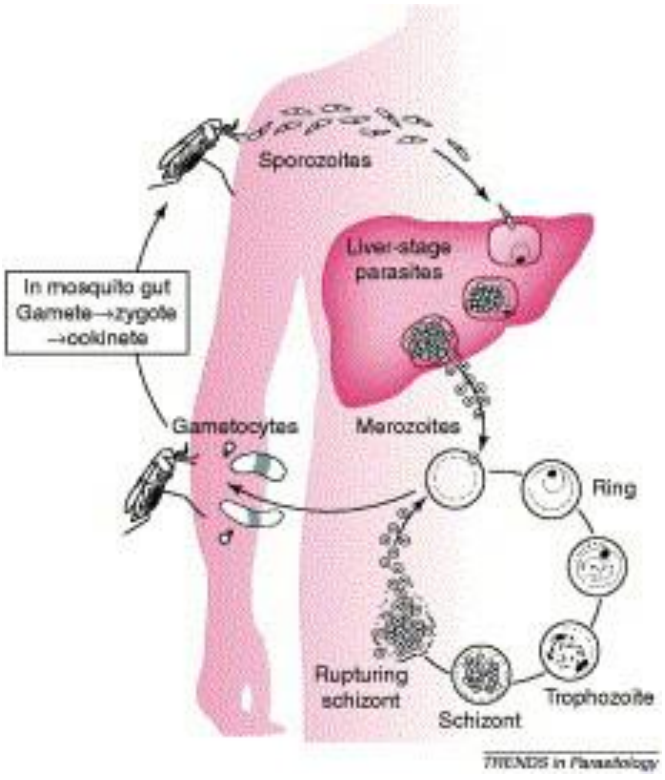


Figure 1: Life cycle of malaria parasite (3).

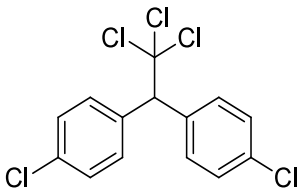
As depicted in the **Figure 1**, the malaria parasite life cycle is intricate but has become well understood over the decades due to persistent research and endless efforts from across the world by the anti-malaria communities. Developmental stages present within the cycle present possible targets for the interruption of the life cycle. The development of vaccines, anti-vector measures and drugs have been pivotal in the prevention of transmission, infection and disease.

1.3 Malaria control through environmental management

In gaining an understanding of the mosquito life cycle it becomes possible to successfully manipulate the insect to reduce transmission (8). The link between the vector and the environment could have only been made upon the realization of the mosquito as the vector (8). Further understanding of mosquito biology and how they reproduce was also pivotal in the initiation and progression of vector control as a means of eradicating malaria, dating back to the early years of the 100th century when the link between mosquitos and swamps was made (12). The water in swamps provided great breeding sites for the mosquito, so in reducing the breeding grounds the mosquito population was anticipated to reduce in size and the outcome was exactly as expected. This phenomenon was later referred to as source reduction, the permanent destruction or removal of mosquito breeding sites (12). But it was later realized that the size of the mosquito population was a more peripheral issue and that the life span of the mosquito was more important when looking at solving the issue of transmission (3). Nonetheless, this marked the beginning of revolutionary acts towards managing the environment as a way of controlling malaria.

These efforts were then later succeeded by using a chemical, dichlorodiphenyltrichloroethane, known as DDT **1**. DDT was the first modern synthetic insecticide developed in the 1940s (13). This organochloride was widely used against many insect borne diseases including malaria and was very successful in controlling the anopheles mosquito, resulting in reduced malaria transmission. Due to the widespread use of this insecticide the development of resistance against DDT was confirmed in 1966 (3, 4). Additionally, the insecticide had undesirable side effects such as human toxicity and environmental drawbacks resulting in the development of regulations regarding the use of DDT (13). The use of DDT was found to pose adverse environmental effects, threatening the wildlife and showing potential of affecting human health (13). Upon these discoveries widespread use of DDT was prohibited as observed in the Stockholm Convention for persistent organic pollutant (POPs) (14). The indoor use of DDT was certified by the WHO in 2006 in countries where malaria was still a major health problem.

Other insecticides have been introduced but were short lived due to the ability of the insect to gain resistance.



1

Figure 2: dichlorodiphenyltrichloroethane, DDT.

An important approach towards continued insecticide usage has been using Insecticide Treated Mosquito-Nets (ITNs). These represent one of the most widely used methods to prevent the transmission of the malaria parasite and fall under the personal protection measures (12). The rise in bed net usage came with the development of pyrethroid insecticides, for example permethrin **2**, which exhibited stability and were able to retain activity for long time periods (10). These were then coupled with various fabrics to serve as means of providing residual insecticides (10). This method of prevention is by far the most affordable and is in widespread use despite the notable limitations (3). Although the nets create a barrier between the mosquito and the human, when not installed correctly or if human skin gets in contact with the net the mosquito can still finds its way to the human (15). In the case of the mosquito managing its way through, the nets require it to probe through the impregnated surface or find another point of entry, upon gaining entry the same mosquito must find a way out which increases the contact time of the mosquito with the treated net (11). With repeated exposure the mosquito will acquire a dose which is enough to warrant its death, mitigating the chances to transmit the parasite (3). In one study the benefits of the pyrethroid-impregnated bed net were tested and found to exhibit very little to no toxicity to humans, had a low volatility, was odorless and reduced indoor biting by mosquitoes (17).

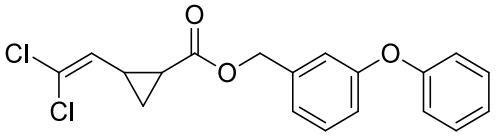


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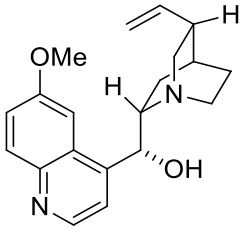
Figure 3: Permethrin, example of a pyrethroid insecticide

Of great importance is the need for collaborative efforts between the various approaches of malaria treatment and prevention as the armory is very limited when used in isolation. The use of insecticides in combination with bed nets has proven to be very effective especially in the impoverished endemic malaria regions. Vector control has its own set of limitations and is unerring and thus demands to be supplemented with chemotherapy. Chemotherapy has shown itself as an important weapon to have access to and has been tantamount to the revolution of malaria eradication.

Contrary to popular belief, most efforts towards combatting malaria are focused on vector control and other short-term approaches (18). This may be owing to the fact these approaches are a lot more affordable when compared to the process of developing a new drug. This necessitates gaining a good understanding of the vector responsible for the transmission of the disease. In the case of malaria, the vector is the female anopheles mosquito which has complete immunity against the disease (19).

1.4 Malaria control through chemotherapy

The treatment of malaria through chemotherapy spans a broad scope of efforts, where numerous years have been spent cultivating permanent treatment/prevention systems. The beginning of the treatment of malaria by chemotherapeutic means was marked using Quinine **3** (**Figure 4)**, which is the oldest most widely utilized compound (20). This compound has been used for medicinal purposes for centuries and occurs naturally in the bark of cinchona trees in South America (18, 20). The usage of Quinine as an antimalarial was succeeded by Artemisinin, which were only introduced into the west at the end of the 20th century. The phasing out of quinine and the takeover of artemisinin-based therapy was separated by the up rise of synthetic quinolines.



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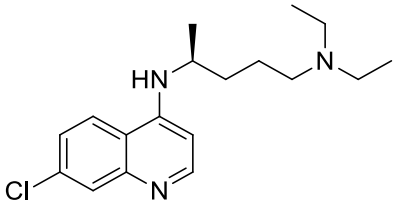

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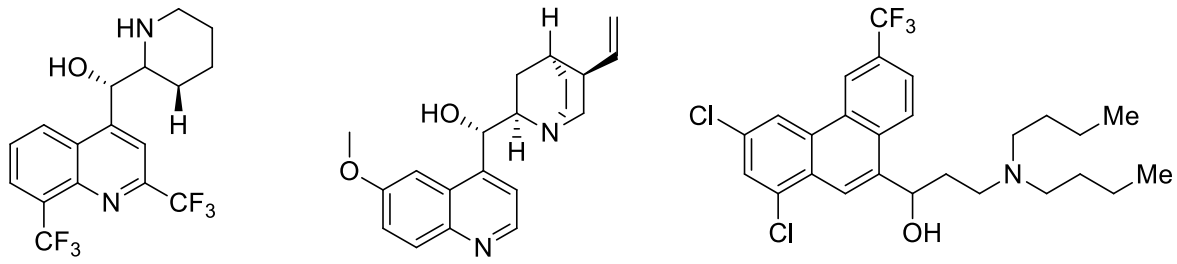
Figure 4: Quinine and Chloroquine

Chloroquine 4 (**Figure 4**), a 4-amino-quinoline compound, first synthesized by I.G Farben industries in the 1930s, is the most discussed synthetic quinoline (21). The rise in resistance against quinine led to the development and subsequent widespread use of chloroquine. In the decades which followed, chloroquine was reported for its efficacious nature towards malaria, but in the late 1950s, resistance towards chloroquine began to reveal itself (22). The exact mechanism of resistance was uncovered after numerous years of investigation. The slower rate of appearance and spread of resistance was suspected to be owing to genetic complexity of the target affected by chloroquine and the level of interaction of the drug with the target active site. Pertinent to this quest was developing an understanding of the mode of action of the drug. Chloroquine was found to act by accumulating to a high level in the acidic food vacuoles of the parasite (23). This compound was found to be capable of diffusing across the vacuolar membrane where protonation would occur, inhibiting the exit of the molecule. This accumulation would result in the inhibition of the proper sequestration of the toxic haem moieties which arose from the digestion of haemoglobin by the parasite in the erythrocytes (23). This would then lead to the death of the parasite from exposure to its own waste.

Related to chloroquine are other arylaminoalcohols such as mefloquine **5**, quinidine **6** and halofantrine **7** which is a phenanthrene alcohol (**Figure 6**). These compounds were also confirmed from morphological studies to act in food vacuoles (23). These were also eventually rendered ineffective with the rise of resistance for example, mefloquine, which was introduced in 1971, and was effective against malaria due to the good prophylactic properties (23). Unfortunately, due to the development of widespread resistance it was rendered ineffective and resulted in its diminishing application over the decades.



5 **6** **7**

Figure 5: Mefloquine **5**, quinidine **6** and halofantrine **7**

Important to the treatment of malaria is the class of compounds known as the naphthoquinones best represented by atovaquone **8 (Figure 6)**, which is best described as a highly substituted naphthoquinone derivative (23). This molecule is also known as a structural analogue of ubiquinone in the mitochondrial electron transport chain (25), has been confirmed to act by interfering with the transfer of electrons generated by pyrimidine synthesis which are important in maintaining the membrane potential of the mitochondrion (23). Despite much success in the use of this molecule against malaria, the appearance of resistance has limited the widespread use of atovaquone.

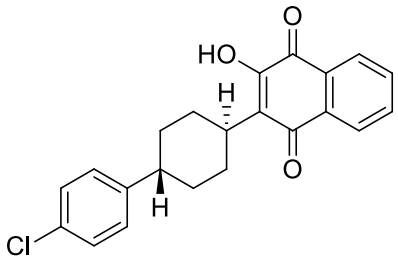


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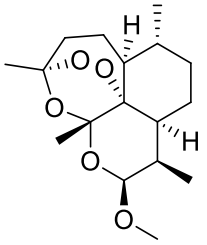
Figure 6: Atovaquone

The use of another naturally occurring compound, artemisinin, was pivotal in the treatment of malaria. This class of drugs originates from the Chinese herb qinghao (*Artemisia annua*) which was used extensively for the treatment of fevers which were suspected to arise due to malaria (26). With the intentions of improving the bioavailability of the molecule, the analogues artemether **9** and artesunate **10** were developed (**Figure 7**). These compounds are classed as sesquiterpene lactones which possess an epoxide bridge across the seven-membered ring and act on the asexual blood stages (27). This class of compounds is effective and has been useful

against multiple drug resistant malaria parasites such as P. *falciparum*, supporting their widespread application (23). This class is deemed superior over other antimalarials because they possess gametocytocidal action, limiting the transmission of the parasite to other hosts (27). The artemisinins can inhibit the parasite at earlier stages in the asexual cycle than the quinoline based drugs thus rendering them superior to the likes of mefloquine and chloroquine. The artemisinin-based therapy was also found to operate by accumulating in the food vacuoles and interact with the haemazoin like the quinolines (28). This was highly disputed over the years and are currently believed to disrupt redox homeostasis in the parasite (27). In the absence of significant resistance reports, cases of recrudescence have been reported when the compounds were used in monotherapy (23). As a result, artemisinins are used in combination with drugs that have a longer acting period like mefloquine (26).



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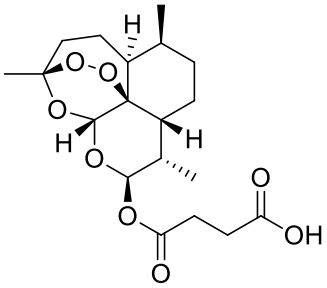

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Figure 7: Artemether and artesunate

The emergence of drug resistance has led to the discovery and cultivation of many classes of drugs for the treatment of malaria. There are mainly two approaches to classifying antimalarial drugs namely, by structure or according to their activity on different stages of the malaria parasite life cycle. The classification of drugs by antimalarial activity gives some insight into the possible mode of action of the drug (8). The five classifications by antimalarial activity are as listed below:

1. Casual prophylactic drugs- this class of drugs acts on the pre-erythrocytic forms of the parasite
2. Schizonticidal drugs (Blood schizonticides) - a class of drugs which act on the sexual erythrocytic forms of all species of malaria. A classic example of this type of drug is Chloroquine, the mode of action of which is well known and understood was considered

as the prototype antimalarial due to its cheap nature, was the most robust drug in the treatment of the disease. Quinine and mefloquine are also blood schizonticides (26).

3. Gametocytocidal drugs- most active on sexual forms of all species of the malaria parasites- an example would include quinine (which has a dual nature) a blood schizonticide with gametocytocidal activity. Quinine is a weak base which is speculated to act by concentrating in the food vacuoles of the parasite
4. Sporontocidal drugs- this class of drugs inhibit the sporogonic phase of the development of the parasite. Primaquine is an example of this class of drugs based on its reported mode of action.
5. Tissue schizonticides (for preventing relapse), mainly act on hypnozoites in the liver. Pyrimethamine is commonly known for its role in the inhibition of dihydrofolate reductase of the plasmodia by blocking the biosynthesis of the purines and pyrimidines, important functionalities in the synthesis of DNA and cell multiplication. Pyrimethamine is an example of a tissue schizonticide.

Most of the drugs known for the treatment of malaria (whether in past or present times) have been seen to have properties that allow for classification in more than one class for example, pyrimethamine and primaquine were reported to exhibit both casual prophylactic and tissue schizonticidal properties (6). It has also been established that some drugs may exhibit specific trends of activity based on the species of the parasite. For example, quinine and chloroquine showed gametocytocidal activity against *P. vivax* and *P. malariae* but not *P. falciparum*. In contrast to quinine and chloroquine, primaquine was observed to show good gametocytocidal activity against *P. falciparum* and all other species (26). Of great importance to this project is a class of compounds known as the antifolates which have played an integral role in the treatment of malaria.

1.5 Antifolates

Antifolates are a class of drugs which are known simply as folate antagonists. Antimalarial antifolates have been reported as central in the prophylaxis and treatment of malaria. Like many other synthetic antimalarials, antifolates were discovered in the early 1900's during the Second World War (29). With an increase in the understanding of the role of folate derivatives in humans, the rise of antifolates ensued. These drugs were originally used for the treatment of leukaemia, but the great success observed in the treatment of tumors prompted the application of these drugs against other rapidly dividing cells such as bacteria and parasites (4).

Methotrexate **11** (**Figure 8**), an aminopterin, was the specific antifolate that was used in the trial that lead to the expansion of use against other diseases.

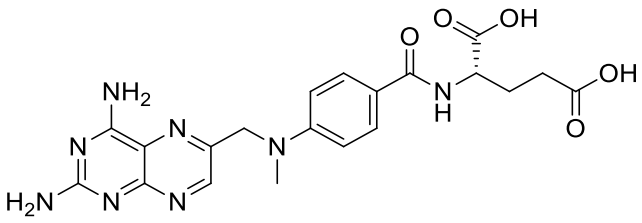

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Figure 8: Methotrexate

1.5.1 Significance of antifolates in malaria treatment

In the literature, much value is placed on folate derivatives (FDs) since these are important cellular cofactors in the synthesis of nucleotides for DNA replication, and for the synthesis and metabolism of certain amino acids (30,31). These functions necessitate the initiation of protein synthesis in mitochondria (30). Tumours, bacteria and malaria parasites rely heavily on the presence of these folate derivatives as these seriously implicate their growth and the efficiency of host invasion (30). Folate antagonism does not only assume a central position in cancer treatment but also extends to malaria treatment (30). Antifolates are used to block a sequential step in the metabolism of folic acids, a pathway which is critical for the survival of the parasite (32). The absence of the *de novo* pathway in higher organisms makes it a very attractive target for drug development (33)

Antifolates are divided into 2 classes:

1. Class 1- inhibitors of dihydrofolate reductase (DHFR)
2. Class 2- inhibitors of dihydropteroate synthase (DHPS)

Both classes of antifolates have been useful and beneficial in the treatment of malaria. However, the combination of anti-DHFR and anti-DHPS inhibitors has shown to be more effective in the treatment of malaria.

1.5.2 Class I inhibitors

DHFR is responsible for the catalysis of the reduction of 7, 8-dihydrofolate (DHF) to 5, 6, 7, 8-tetrahydrofolate (THF) which is a key cofactor for major folate-based reactions. In the parasite THF is obtained by the synthesis of endogenous THF, the salvaging of oxidized forms

of folate by recycling DHF (which arises from the synthesis of deoxythymidine monophosphate (dTMP)) and from exogenous sources (31). The *P. falciparum dhfr* enzyme is expressed as a bifunctional protein with thymidylate synthase (TS) and is known to be an excellent target for antimalarial drugs (31). Due to its central role in the folate pathway, this enzyme has been predominantly targeted for the development of antimalarial drugs.

The *de novo* synthesis of the folate functionality from which folate derivatives are produced does not exist in mammalian cells. As a result, folate derivatives are acquired from other sources including the dietary factors and are then functionalized appropriately. In Plasmodium, the presence of folate derivatives is deduced from the existence of the parasite enzymes that use the various compounds as substrates. DHFR plays a huge role in the occurrence of two major folate-based reactions, one of which is the endogenous synthesis of THF in microorganisms that can produce folates *de novo* as seen with *Plasmodium. Plasmodium* and its human host differ primarily in the way the translation of DHFR-TS (dihydrofolate reductase-thymidylate synthase) is regulated.

1.5.2.1 Proguanil

This was the first reported antimalarial antifolate which was first discovered and used during the Second World War. The usefulness of this drug was reported in 1945 because of its stellar activity which superseded that of the prominent quinine by exhibiting a much better therapeutic index in animal models (8). Proguanil **12** (R=H) is a prodrug, which is metabolized into a triazine form identified as cycloguanil which in turn inhibits DHFR (**Figure 9**). Proguanil is used primarily as a prophylactic agent or in combination with other drugs such as chloroquine or atovaquone (31). These drugs compete with DHF to bind in the active site of DHFR. When used in combination with atovaquone, the drug is referred to as Malarone where atovaquone maintains its role of inhibiting the electron transport to the cytochrome bc1 complex coenzyme Q. Due to much success in the treatment of malaria the search for proguanil analogues was pursued. Resistance to proguanil appears due to a single point mutation in the cytochrome B or the dihydrofolate reductase gene (34,35). The search for newer more potent analogues in the face of rising resistance led to the rise of more analogues (26). In discussions about the more attractive anti-DHFR antifolates, cycloguanil **14** is pertinent considering that it is a metabolite of proguanil and has been closely linked to the antimalarial activity of proguanil. However, due to recent work, a contrast between the mode of action of the analogues has been discovered, where proguanil has been found to behave in synergism with atovaquone whereas cycloguanil plays a more antagonistic role in the presence of the same counterpart.

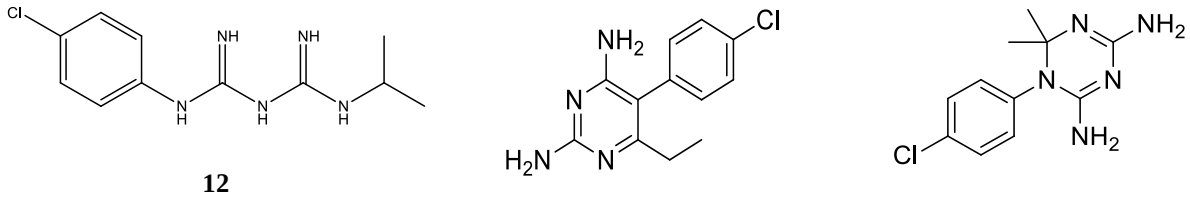


Figure 9: R=H proguanil, R=Cl chlorcycloguanil **12**, Pyrimethamine **13** and cycloguanil **14**

1.5.2.2 Chlorproguanil

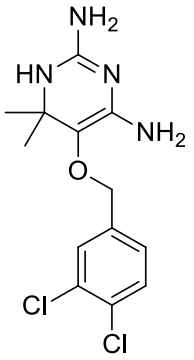
Chlorproguanil **12** (R=Cl) was generated from the chlorination of proguanil (**Figure 9**). This analogue of proguanil is metabolized to the active metabolite chlorcycloguanil (36). The drug is used in combination with Dapsone (diaminodiphenyl sulphone), a DHPS inhibitor, for improved efficacy and higher potency.

1.5.2.3 Pyrimethamine

This drug is a 2,4 diaminopyrimidine derivative, which was originally synthesized and tested as an analogue of folic acid in the treatment of tumours (37). Due to its similarity in structure to cycloguanil, it was hypothesized that 2,4 diaminopyrimidines could display antimalarial activity (23). As speculated, it was found to be true and this subsequently led to pyrimethamine **13** being the most widely used antimalarial antifolate (36). Pyrimethamine **13** is widely described as a competitive inhibitor of the parasite DHFR and has a narrow selectivity window (37). This accounts for some toxicity which was observed in the human host. Resistance to this drug when applied in monotherapy was rapid, following its widespread use (39). As a result, the drug was also observed to exhibit improved activity when used in combination with sulfadoxine or sulfalene and has since been used in combination therapy in many parts of the world.

There are a few unattractive anti-DHFR antifolates with questionable activity based on properties such as bioavailability. Clociguanil **15** is an example of such compounds which is an analogue of chlorcycloguanil with a methylene-oxy linker between the triazine and phenyl ring (40). The drug is reported to have irregular bioavailability and short acting properties which has made its viability for widespread application very risky and thus remains unexplored. BRL 6231 (WR99210) **16** which is tri substituted on the phenyl ring and has a

longer aliphatic linker (**Figure 10**). The issues with this drug are in its low bioavailability which has limited the pursuit for its antimalarial function.



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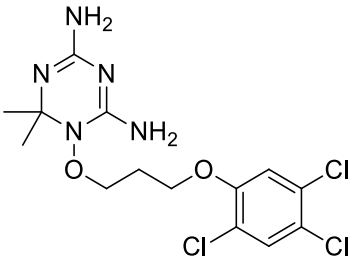

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Figure 10: Clociguanil **15** and WR99210 **16**

The antifolates discussed in this introduction were found to be superiorly useful as antimalarial compounds due to their higher affinity for binding with *P. falciparum.* As result, many researchers have pursued the journey towards understanding the mechanistic details between the drugs and the varying sites of action. There have been observations that show that the parasite enzyme is less readily replenished when targeted by anti-DHFR inhibitors. Despite this rationale the question of selectivity remains.

1.5.3 Class II inhibitors

Sulfadrugs are widely reported for their ability to block the *de novo* folate synthesis pathway. This is notable because of the parasites ability to synthesize folates *de novo*. DHPS is the 2nd most targeted enzyme in the folate pathway of the parasite (41). DHPS is another important enzyme in the *de novo* synthetic folate pathway which catalyzes the addition of *p*-aminobenzoic acid (*p*ABA) to dihydropterin pyrophosphate (DHPP) to form pteroic acid as a key step in folate biosynthesis (42). Sulfadrugs form the 2nd class of antimalarial antifolates and have had very useful applications in this function. Two sulfadrug classes exist, the sulfonamides and the sulfones. There have been very few to no reports of successful application in monotherapy due to efficacy issues and unacceptable toxicity, leading to the complete abandonment of monotherapy attempts (43). However, these have been successful in combination therapies with class I inhibitors (43).

Dapsone **17** (**Figure 11**), the most potent DHPS inhibitor in the treatment of malaria is a good example to demonstrate the limitations of the use of this drug (44). This drug is a sulfone based agent which was found to suppress the growth of various pathogenic agents including mycobacteria and the malaria parasite (44). Its application in the treatment of malaria was limited by its low efficacy and high toxicity (45). The pre-eminence of the class arose when notable synergy between this class (anti-DHPS) and the class I inhibitors was observed. Dapsone in combination with pyrimethamine (Maloprim) was more useful and more tolerable by the host, especially towards the treatment of other diseases such as leprosy and pneumocystic pneumonia (46).

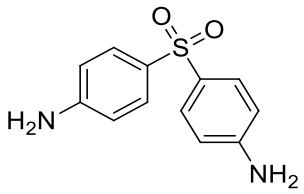

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Figure 11: Dapsone

The success of antifolates against malaria was halted by the emergence of parasite resistance in the 1980s and has since been stagnant. Despite much success with antimalarial antifolates, very little is known about *Plasmodium* folate metabolism. With DHFR being the main enzyme targeted by drugs, followed by DHPS, a search for new drugs targeting other known enzymes in the folate pathway of the parasite continues in the face of rising resistance.

1.6 Addressing the issue of drug resistance

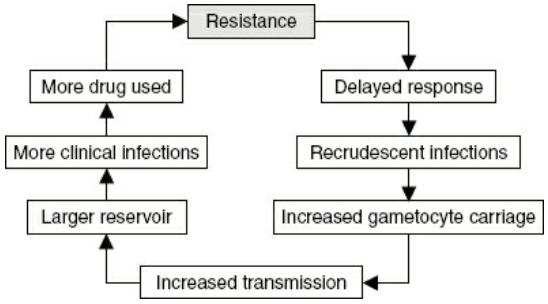


Figure 12: Vicious cycle of antimalarial drug resistance-adapted from Nosten F. (3)

Drug resistance is described as a reduction in the effectiveness of a drug against a disease or a condition, where much emphasis is placed on the phrase "reduction in effectiveness". This reduction in effectiveness is widely discussed in literature due to the amount of time which has been spent towards gaining a better understanding in how it occurs. Resistance occurs as a vicious cycle where recrudescent infections, increased transmission and increased drug use are among the major drivers of the preeminence of resistance (**Figure 12**). Many modes of the development of resistance have been observed in viruses, bacteria, protozoa and even cancers against chemotherapy. But in more instances than one, drug resistance has been heavily attributed to the development of a single point mutation in the target enzyme, which either instantaneously renders the preventative or curative compound ineffective or leads to the gradual or incremental development of resistance in the case of multiple point mutations. In the case of the multiple point mutations, each mutation contributes to the reduction in the effectiveness of the drug which ultimately results in the nullification of the drug. Alongside point mutations, parasite drug resistance is also said to occur due to changes in gene expression which is then propagated by drug selection pressure. Parasite resistance has also been described as the ability of the parasite to survive or multiply despite the administration of a drug in doses equal to or higher than those recommended, within the limits of host tolerability (47). This has been closely attributed to a reduction in the time it would take the drug to clear the parasites from the bloodstream. This in turn results in the issue of recrudescence, which is the recurrence of the disease in the time following a period of parasite inactivity.

In the specific case of antifolates, resistance was observed soon after their application as antimalarials. The resistance to class I antifolates in malaria is owing to the observed changes in the target enzyme, DHFR, which results in a significant decrease in the binding strengths of inhibitors (48). As a result, many efforts have been made towards overcoming the issues fueled by antifolate resistance. The cultivation of new antifolates effective against resistant parasites

is possible, mainly owing to the vast amount of knowledge available about wild-type and mutant DHFR. This information is useful in the development of new antimalarials with high binding affinities to mutant forms of the enzyme (49). In addition, using combinations of drugs also aided in the treatment of drug resistant parasites. The combination of pyrimethamine, a DHFR inhibitor with sulfadoxine, a DHPS inhibitor, was useful in the treatment of drug resistant malaria initially, although resistance was soon reported after its introduction. Fortunately, the combination still maintains its efficacy in intermittent preventative treatment during pregnancy (14).

1.6.1 The role of combination therapy

The main benefit of combination therapy is that its addresses the issue of rising resistance against already existing antimalarial drugs with the much-needed urgency. It offers some form of immediate solution, especially when compared to the process of developing new drugs. The fact that only a handful of new antimalarial drugs have been introduced to the current collection over the past few decades does not help the course of action taken to combat malaria (49). An additional benefit of combination therapy includes the observable reduction in the risk of selecting the resistant mutants of the plasmodial parasites (49). The probability of resistance developing simultaneously to two chemotherapeutic agents with independent mechanisms of action is extremely low; examples include Lapdap (chlorproguanil/dapsone) and Malarone (atovaquone/proguanil) (50). With the rise of combination therapy in the treatment of malaria combinations of artemisinin derivatives with drug partners which have a completely unrelated mode of action has created a new hope for the anti-malaria activists (20). By rapidly decreasing the parasite density, inclusion of artemisinin derivatives has been reported to improve the effectiveness of the drug combinations (19). The approach of combination therapy holds the potential to not only prevent the emergence and spread of resistance, it also holds the potential to interrupt its transmission. Although highly valuable, the use of combination therapy does not rule out the possibility of resistance but a well thought out combination of drugs have been shown to delay the selection of the resistant mutant (20).

Combination therapy significantly reduces the chances of resistance developing towards a drug. Combination therapy is also useful to extend the lifetime of drugs which were observed to lose their efficacy, by using them in conjunction with other drugs. This is beneficial since malaria affects mainly the poor countries in the world, where the disposable capital for development of new treatments is very limited. The principle of drug combination therapy is an old one, where there are records of application to the treatment of bacterial infections such

as tuberculosis, HIV/AIDS and the chemotherapy of certain cancers (51). Earlier examples of combination therapy attempts against malaria include pyrimethamine/sulfadoxine and atovaquone/proguanil. The reason for failure of these combinations is postulated to be owing to the similarities of the modes of action of the drugs in question (16). The other possible reasons are the possibility of already existing resistance against one of the drug partners which is not circumvented by the combination therapy (52). Other reasons such as mismatched pharmacokinetic properties or an overall slow action against the parasite may be responsible for the failure of the combinations (16).

Other combinations of drugs such as Malarone, Maloprim, Lapdap, Fansidar and Metakelfin (sulphadoxine-pyrimethamine), all of which arose from a combination between existing drugs, have had a hand at successful management of malaria in significant or in small ways (53). The possibility of prolonged usage of a drug is very appealing and since the countries which are at the mercy of this disease cannot afford the development of new drugs, combination therapy has been and will continue to be of good service to these communities.

1.6.2 Malaria from a social, economic and political perspective

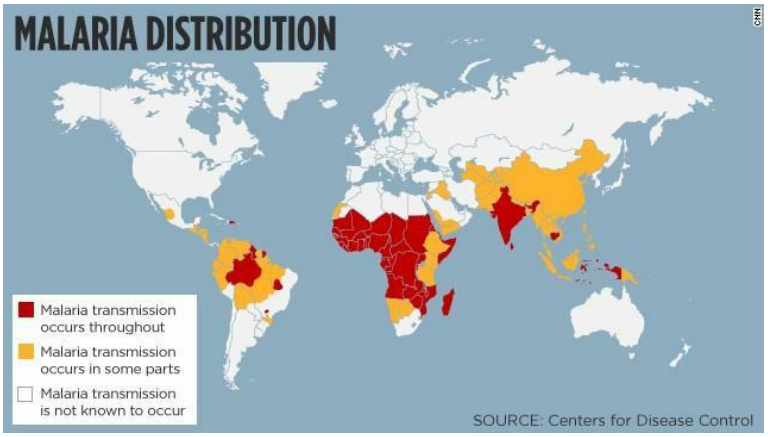


Figure 13: Map depicting malaria distribution-adapted from Centres of Disease control (6)

In addition to the biological factors which greatly aggravate the state of nations which are holoendemic to malaria, there are other factors such as social, economic and political drivers which all have a huge in hand in the impact of malaria on the world. Malaria has been classed as a third world issue with most, if not all the major world economies having no input because those countries are unaffected. Places which are largely affected by malaria are unfortunately those that cannot afford to fight against malaria with monetary armory. As seen in the diagram **(Figure 9)**, the impact of malaria is largely observed in parts of Africa, where many communities are at the mercy of poverty, amid massive political instability which affects the

economies, ultimately impacting the quality of life in these regions. Malaria has been a disease of the poor with most of the major pharmaceutical companies not showing a level of willingness to help diffuse the matter at hand. The allocation of global and local resources plays a huge role in the trends observed in malaria morbidity and mortality especially in the presence of biological factors (55). Low income countries bear a high burden of both infectious and chronic disease, where an inversely proportional relationship exists between socioeconomic development and disease advancement within a population (55).

The WHO has previously identified poverty as the greatest risk factor for malaria (55). This is based mainly on the strong correlation between the absence of funds/resources and poor health outcomes/measures. This is true again as we see that countries with the most robust health systems even within the same continent namely, Africa, are not faced with the same health challenges posed by malaria even given the similarities in the environmental and biological factors. This fact holds even more truth when we compare the impact of malaria in the first world countries which have the most efficient health systems and measures to those of the poorer countries in Africa and others in Asia (39).

The ability of people to gain access to vector control tools implicates many people and individuals in poor countries and makes them more vulnerable to environmental risk. In one study where the authors were looking at the direct and indirect costs of malaria, it was seen that low-income households would have to spend at least 32% of their monthly income to effectively prevent the transmission of malaria (56). This number is unsustainable leaving many people in this income bracket helpless and completely at the mercy of the plight.

The available biological and ecological analyses have been the main shapers of how we view and understand malaria currently, and very little mind has been paid to the ethnographic narratives of people's lived experiences with the disease (55). Not only does malaria affect the mortality and morbidity of people, it also affects how efficient the health sectors and the economic growth of those given countries are. This being evident in the deteriorating response time of the health sectors to disease outbreak and the progressive annual decline in the economic growth (41). The development and social wellbeing of nations is affected by malaria which brings about serious implications. High endemic regions across the globe remain unaffected by eradication measures which are periodically set in place, where the poorest nations are still faced with issues like overly centralized and under developed health infrastructure. There is much work that still needs to be done to aid the investments in public

sanitation and services, to at least put these poorer countries at a more level start point towards eradication.

Perhaps aiming for eradication is overly optimistic instead, aiming to have systems in place which aid in the robust management of the disease could be a lot more meaningful and sustainable from an economic perspective for the developing countries. The WHO made recommendations regarding the use of artemisinin-based combination therapies (ACTs), which have a remarkable 95% cure rate with a low probability of inducing parasite resistance (40). The outcomes of ACTs are impressive due to the significant long-term reduction in the malaria incidences. Despite the positive effect that ACTs have had on malaria trends in some African countries, the high costs associated with the large-scale provision of these combinations poses a large challenge for many African economies. The average life-saving course of treatment is estimated at around 2 to 3 dollars, but this still does not make the drug combinations accessible (41).

In considering the social, economic and political factors which add onto the malaria burden, the role of malaria in the edification of other diseases is often neglected and remains rarely discussed. Anemia, which is largely linked with the deficiency of iron in patients, is one of the main diseases which is instigated and aggravated by the presence of malaria (41). Anemia can be fatal in its most severe form especially in young children, the immuno-compromised, elderly people and pregnant women (41). The progression of anemia often increases the need for blood transfusions which spikes the risk of HIV infection, especially in a continent that faces the challenge of high mortality and morbidity due to HIV/AIDS. Additionally, malaria can increase the viral load of an individual infected with HIV, where HIV can aggravate malaria fevers and ultimately have adverse effects on the host (2). Studies have shown that severe anemia is heavily influenced by the transmission of *P. falciparum* where it was found that 60% of all severe anemia in infants was due to malaria pressure (41). Apart from anemia, malaria is thought to have a significant effect on other diseases such as measles and respiratory infections (41). Economic development has a large influence on health systems but in the absence of this much sought after alternative solutions which match the economic profiles of the affected developing countries are necessary.

1.7 Approaches towards drug discovery

There remains an urgent need for the development of new antimalarial drugs to curb the disease. This becomes even more critical in the face of persisting drug resistance. Multiple approaches have been explored and recorded over the years for example, covalent biotherapy. This is the linking of two distinct chemical moieties which act on separate or the same biological targets via different modes of action (1). Another example is the use of natural products and their derivatives, which have served as a reservoir for new drugs over the decades. These offer molecular frameworks for the development of new agents. Most commonly known and discussed scaffolds include quinine and artemisinin, both extracted from plants and have prompted the synthesis of other potent drugs such as chloroquine and artemether.

The cross application of drugs is one of the important approaches towards the discovery of new drugs. The use of existing drugs which are used against another known disease has proven useful and often serendipitous. A classic example of this is the discovery of the efficacy/activity of antifolates against malaria. This was significant because antifolates were originally used against cancer. The main advantage of this approach is the drugs in question are already clinically approved with established safety and pharmacokinetic profiles (1). This approach significantly reduces the research time and costs associated with drug development.

A more sophisticated approach involves the development of new compounds against novel targets. This approach is scientifically proven and requires the knowledge of genomics and proteomics which could lead to the discovery of novel drug targets (1). Of course, this approach is guaranteed to be long and tedious and is not necessarily one which would address the issue of mortality due to malaria with the required urgency. In one study, researchers show how understanding the folate pathway offers many enzymes which can be explored for the development of new compounds for the successful treatment of malaria (35, 36).

A more feasible approach when considering the issue of urgency is one where existing compounds are improved by chemical modification. Unlike the previous approach, no prior knowledge of the biological target or mode of action is required (59). This approach was used in the modification of quinine with the aim of improving activity which led to the discovery of chloroquine, halofantrine, primaquine and mefloquine (60). A painstaking task but a venture worth exploring since this approach allows for the screening of a compound against various diseases once attained.

1.8 Our approach towards the synthesis of potential antimalarial antifolates

- The journey towards the synthesis of our compounds of interest was initiated at the Council for Scientific and Industrial Research (CSIR), when Dr Amanda Rousseau and her associates embarked on a quest towards the synthesis of a novel series of flexible cycloguanil analogues as inhibitors of *Pf*DHFR(63). This initiative was prompted by the information acquired from crystallographic data of wild-type and mutant *Pf*DHFR complexed with WR99210 and pyrimethamine (63). In their approach towards synthesizing flexible antimalarial agents based on cycloguanil, variations in the number of atoms (1-5) in the linker between the substituted phenyl ring and the 1, 2-dihydro-1, 3, 5-triazine-4, 6-diamine were made (61).

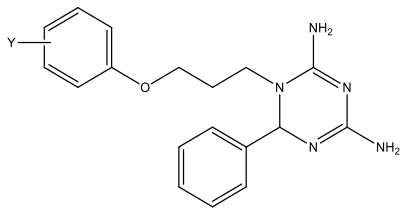


Figure 14: 1,2-dihydro-1,3,5-triazine-4,6-diamine

After these variations were introduced, good antimalarial activity in the nanomolar range was observed for compounds bearing a four-atom linker against a drug sensitive *P. falciparum* strain. Additionally, the hit compound showed whole cell activity against a chloroquine/cycloguanil resistant strain of *P. falciparum* (63). Although highly efficacious, the limiting factor was the ease with which the compounds were obtained. The final products were isolated as a racemic mixture and it remains unknown as to which enantiomer was responsible for the observed activity (63).

This prompted the quest towards the synthesis of the equivalent pyrimidine analogues of cycloguanil with the hopes of retaining the antimalarial activity. This approach was good in theory but the concern of the loss in activity was valid. Although this series of compounds exhibited some activity, it did not match that of the previous hit compound. With this information, we proceeded on to seeking newer variations which could potentially match the prior antimalarial activity or supersede it.

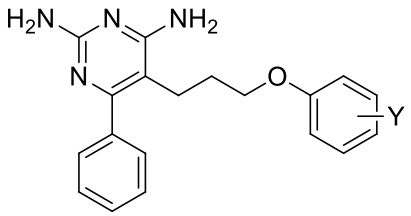
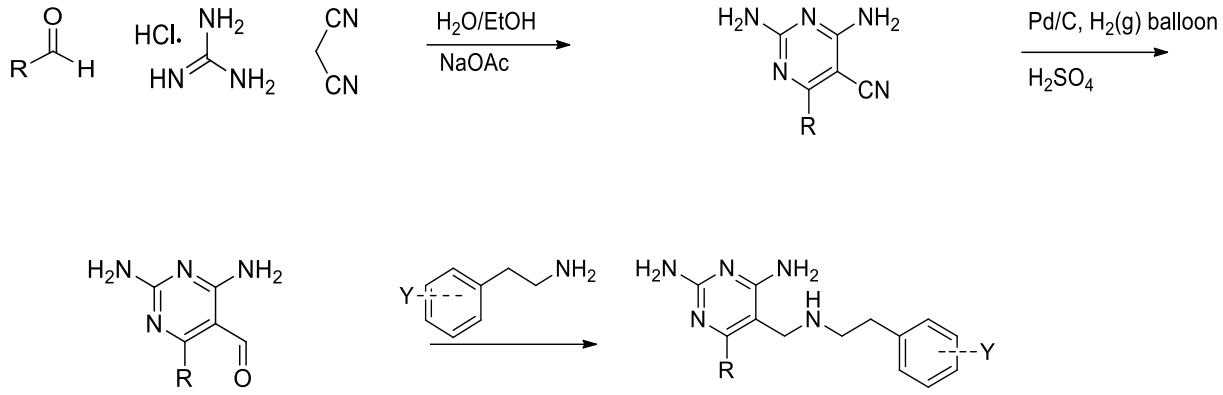


Figure 15: Proposed flexible pyrimidine analogues based on the dihydrotriazine equivalents

1.8.1 The aims of this this project

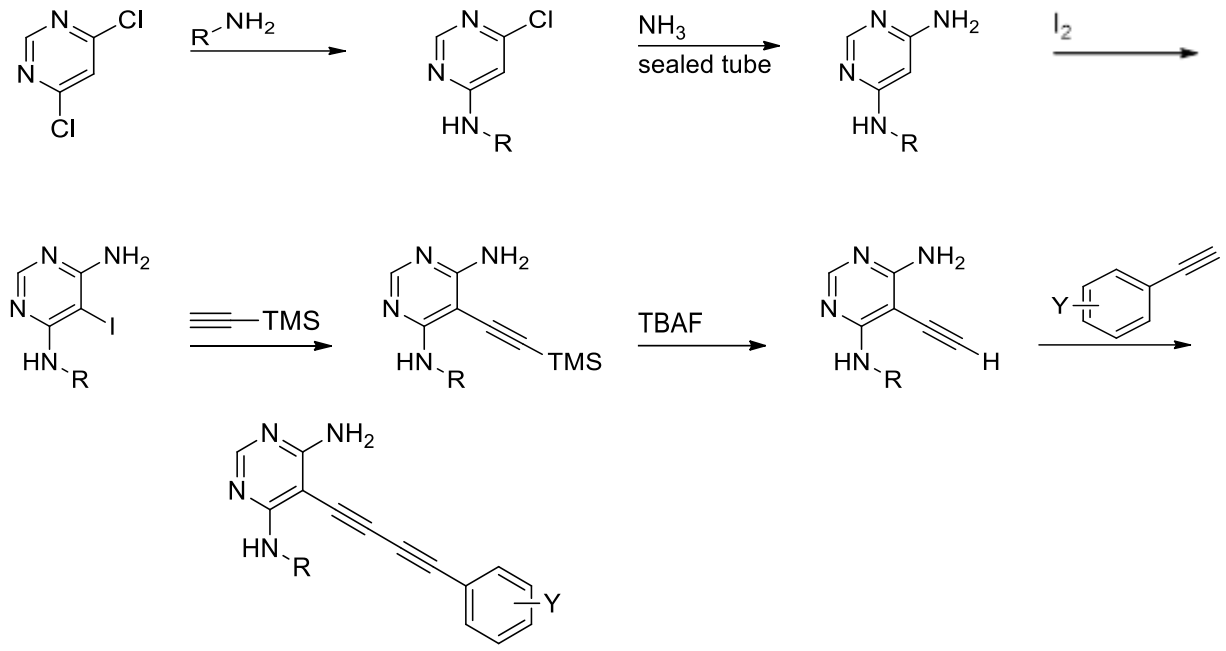
Continuing with the synthesis of flexible pyrimidine analogues of cycloguanil, we aim to expand the library of 2,4-diaminopyrimidine carbonitriles with the hopes of gaining a better understanding of the structure activity relationships of these compounds. The pyrimidine forms the same H-bonds in the active site of DHFR as the natural substrate dihydrofolate. The flexible linker allows the second aromatic ring to form a pi-pi interaction with a phenylanaline residue in the active site, and avoid unfavourable contacts with mutant amino acid residues, for example, asparagine ASN109 replaces serine SER, causing the steric clash responsible for the resistance to cycloguanil and pyrimethamine. In a previous attempt to synthesize these compounds, much difficulty was encountered with the purification. Considering this challenge, we aim to find or development a purification approach for these compounds to enable their testing (this would encompass part 1 of this project). As the first approach had previously proved synthetically challenging, we also plan to develop an alternative approach to related analogues (part 2).

Our proposed synthetic route for part 1 (as depicted in **Scheme 1**) would begin with the reaction of commercially available malononitrile, guanidine hydrochloride and an aldehyde in a solvent combination of water/ethanol (EtOH). This was done to yield a 2, 4- diaminopyrimidine carbonitrile, which is converted to the aldehyde by hydrogenation with Pd/C as the catalyst in 2M H_2SO_4. The resulting carbaldehydes will be reacted with a substituted phenethylamine of choice in the presence of sodium cyanoborohydride ($NaCNBH_3$) in EtOH to afford the desired product.



Scheme 1: General synthetic approach towards the synthesis of substituted 2,4-diaminopyrimidine amines

For part 2 our proposed synthetic route (**Scheme 2**) involves the reaction of 4,6-dichloropyrimidine with an amine of choice to yield a 4-amino-6-chloropyrimidine. With this precursor in hand, a sealed tube reaction in ammonia affords a 4, 6-diaminopyrimidine. This precursor is subjected to conditions enabling an iodination at the 5-position of the pyrimidine core. The iodinated product is then coupled to TMS-acetylene via the Sonogashira coupling reaction. The TMS protected compound will then be deprotected and then reacted with other commercially available acetylenes to afford our desire diyne compounds. The diynes can be reduced using Pd/C in EtOH to give the corresponding pyrimidine with a flexible side chain.



Scheme 2: General synthetic approach towards the synthesis of substituted 4,6-diaminopyrimine diynes

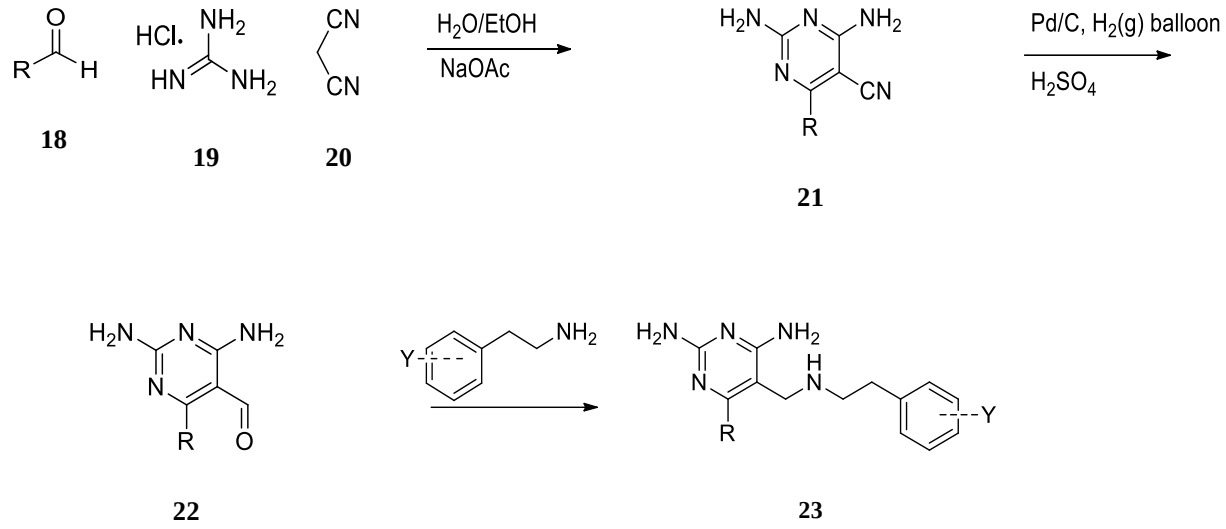
Chapter 2

2. Results and Discussion

2.1 Synthesis of flexible pyrimidine analogues as potential DHFR inhibitors

2.1.1 Synthesis of 6-substituted 2,4-diamino-pyrimidinecarbonitrile

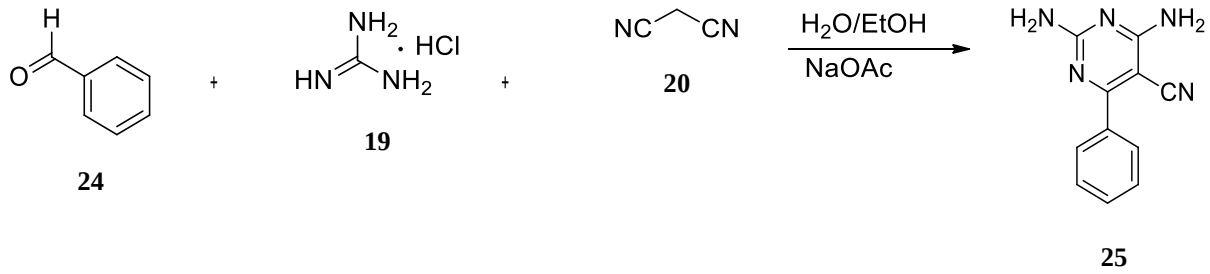
This project began with the need to reach the flexible pyrimidine analogues, as analogues of a series of the triazine compounds that showed good biological activity against the *P. falciparum* parasite. The aim of this project was to expand the library of flexible pyrimidine analogues bearing either an aromatic group at the 6-position or a cyclohexyl or cyclopropyl group, and to optimize the reaction conditions for the synthesis of these compounds. Moreover, we sought to establish the structure activity relationship of the compounds in question with the hopes of discovering new hit compounds. The synthesis of the pyrimidine analogues was achieved via the synthetic route shown in **Scheme 3**:



Scheme 3: Overall synthetic route towards the synthesis of potential flexible pyrimidine DHFR inhibitors, part one**.**

Commercially available aldehyde **18**, guanidine hydrochloride **19** and malononitrile **20** were stirred together in a water and ethanol mixture to give carbonitrile **21** via a multicomponent reaction. The resulting carbonitrile was then functionalized via a functional group interconversion from the carbonitrile **21** to the aldehyde **22.** A reductive amination between aldehyde **22** and a substituted phenethylamine was then performed to access the desire flexible pyrimidine triazine analogues **23.**

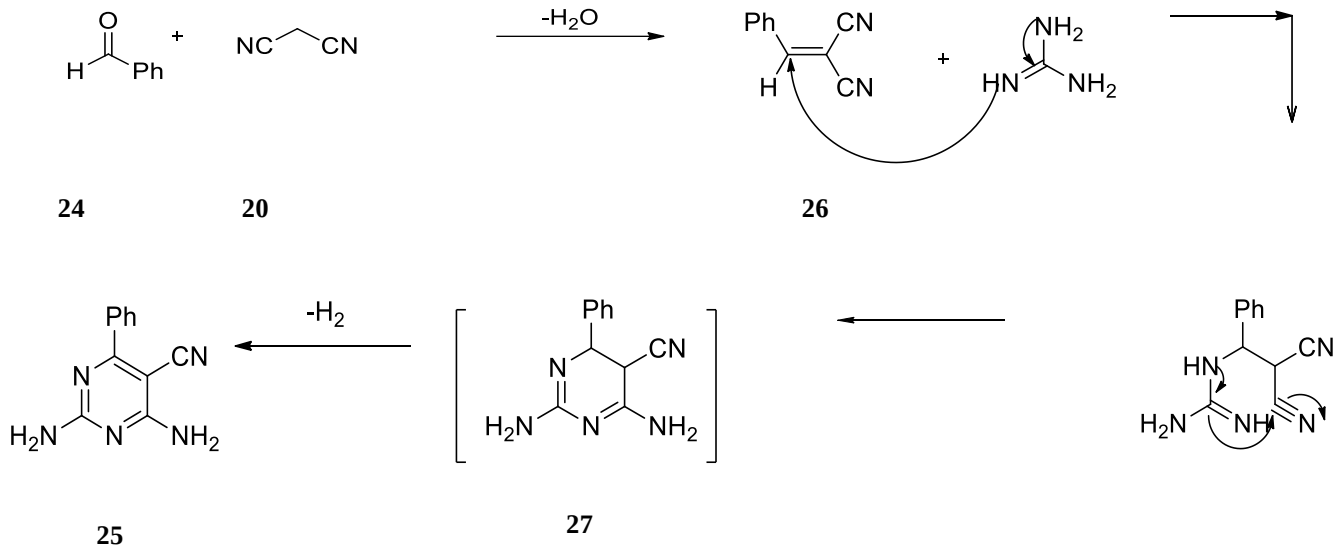
2.1.1.1Synthesis of 2,4-diamino-6-phenylpyrimidine-5-carbonitrile[59]



24 **19** **20** **25**

Scheme 4: Reaction conditions: H_2O/EtOH, NaOAc, refluxing, ~14 hours, 30%

The beginning of our synthetic journey began with the synthesis of 2,4-diamino-phenylpyrimidine-5 carbonitrile **25** via a multicomponent reaction (MCR). The MCR is a valuable tool which grants access to structurally diverse compounds which have many valuable applications (59). The use of this reaction was first reported in 1850 where the Strecker reaction was the 1st reported MCR and is advantageous because it is typically easy to conduct, often results in little to no side product and often affords the desired product in a single step. This makes the reaction very appealing from a synthetic chemistry perspective and thus was used to obtain the desired product **25**.



24 **20** **26** **27** **25**

Scheme 5: Proposed mechanism for the multicomponent reaction, showing the Knoevenagal intermediate[64]

Commercially available benzaldehyde **24**, malononitrile **20** and sodium acetate were stirred together in water and ethanol to allow for the formation of the intermediate benzylidene **26**. Upon the addition of the guanidine hydrochloride **19**, **26** underwent a Knoevenagal condensation to form the pyrimidine core. The Knoevenagal condensation is a very important reaction in organic synthesis, characterized by the formation of a stable intermediate **27** (60). This then undergoes an aromatization to afford the desired product carbonitrile **25.** This reaction has been known since the 19th century and was named after Emil Knoevenagel who first happened upon it.

The ^{1}H NMR spectrum of the product isolated in a low yield of 30% exhibited distinct peaks which indicate the successful formation of the desired product. The signal seen at 7.09 ppm owing to the two NH_2 groups on the pyrimidine core and signals due to aromatic protons between 7.50 and 7.83 ppm. In the ^{13}C NMR spectrum, product formation was characterized by the distinct appearance of signals due to the pyrimidine ring at 163 (C6), 164 (C4) and 174 (C2) ppm. The fourth carbon signal expected for the pyrimidine core is known to appear significantly further upfield than the other peaks and appears at 76 ppm for carbonitrile **25**. The presence of the peak at 117 ppm is attributed to the nitrile carbon, while the presence of signals due to the aromatic phenyl carbons in the region between 120 and 140 ppm confirms the successful synthesis of the desired product. Additionally, from the IR spectrum of **25** a distinct peak appearing at 2203- 2205 cm^{-1} (medium stretch), which can be attributed to the CN group, was observed.

Unfortunately, the major product isolated from the reaction was the benzylidene intermediate **26** at 70% yield or sometimes more. From the ^{1}H NMR spectrum the appearance of a distinct singlet at 6.8 ppm due to the benzylidene proton, which was highly desheilded by the nature of the alkene, was the first important signal noticed. Additionally, the multiplet at 7.65 ppm due to the aromatic protons confirms the structure of **26**. This then posed a challenge as the observed yield of the desired product was particularly low, irrespective of whether the reaction was performed on a small or large scale. This prompted the search for an alternative approach towards acquiring the desired product. We attempted microwave-assisted reactions because of the increasing popularity due to their convenient reaction time frames. The outcome of the microwave-assisted approach was not any different from that of the conventional approach, where the intermediate was still being isolated as the major product.

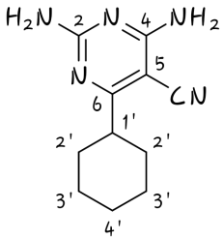
This led to the reevaluation of the reaction conditions; it was thought that the mole ratio of the guanidine hydrochloride being added was not sufficient. The guanidine HCl was then added in excess to assess if this would drive the rate at which the benzylidene intermediate would undergo the Knoevenagal condensation. To much disappointment this attempt was met with the same results as previously observed, where the intermediate was still being isolated as the major product. It was then considered that perhaps leaving the reaction to run for a longer time would allow for the occurrence of the desired reaction. The reaction was left running for 2 days but this was of no aid since some form of decomposition took place and lower yields of the product than what was previously observed were isolated.

Failing to improve the desired product yields we reverted to the conventional method and attempted a different set of reaction conditions, where bismuth nitrate pentahydrate ($Bi(NO_3)_3 .5H_2O$) was used as a catalyst (61). This approach did not improve the yields as we had hoped so we reverted to the original method.

With countless repetition of the reaction much of the intermediate was accumulated and thus led to the search of a method which could convert this precursor to the desired product. The use of $Bi (NO_3)_3 .5H_2O$ had been reported in literature for the successful conversion of benzylidene to pyrimidine carbonitriles (61). This attempt was successful but was limited by low yield due to poor conversion of the starting material.

2.1.1.2 Synthesis of 2,4-diamino-6-cyclohexylpyrimidine-5-carbonitrile

With the phenyl substituted carbonitriles in hand we sought to make analogues with an aliphatic substituent at C-6. To this end we aimed to synthesize 2,4-diamino-6-cyclohexylpyrimidine-5-carbonitrile **28 (Figure 16)**.

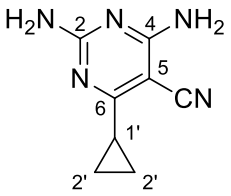


28

Figure 16: 2,4-Diamino-6-cyclohexylpyrimidine-5-carbonitrile

Similarly, the synthesis of **28** was achieved via the MCR and the product formation was confirmed by NMR spectroscopy. From the ^{1}H NMR spectrum five signals were observed as expected, 3 in the aliphatic region owing to the cyclohexyl group at 1-1.8 ppm and 2.7 ppm, and 2 close to the aromatic region due to the NH_2 groups at 6.7 ppm and 6.9 ppm. Furthermore, from the ^{13}C NMR spectrum the aliphatic carbons appeared at 26 ppm (C3'), 30 ppm (C2') and 44 ppm (C1'), in contrast to the carbon NMR spectrum of **25**, only aromatic peaks due to the pyrimidine ring were observed. This analogue was isolated in very poor yields at 12%, being the lowest when compared to the other two analogues.

2.1.1.3 Synthesis of 2,4-diamino-6-cyclopropylpyrimidine-5-carbonitrile



29

Figure 17: 2,4-Diamino-6-cyclopropylpyrimidine-5-carbonitrile

Finally, we prepared the 6-cyclopropylpyrimidine carbonitrile **29 (Figure 12)** using the same method. The successful synthesis of compound **29** recovered at a yield of 22% was confirmed by the appearance of three signals in the ^{1}H NMR spectrum. The signals due to the two NH_2 groups appeared at 6.5 ppm and 6.8 ppm and the signal due to the two H2' groups were observed at 0.8 ppm and 1.8 ppm. The CH proton was not visible on the spectrum, this might have been owing to the orientation due to rapid free rotation of the molecule when the compound was analyzed. Nonetheless the presence of the carbon attached to H1' was

confirmed to appear at 15 ppm. A summary of the important NMR spectroscopic data pertaining to the pyrimidine carbonitriles prepared is given in **Table 1** and **Table 2** below.

Table 1: Summary of the important ^{1}H NMR spectroscopic peaks of the carbonitrile analogues. **25**-R= phenyl, **28**-R=cyclohexyl and **29**- R=cyclopropyl

Analogues	Aromatics/aliphatics ppm	NH_2 groups ppm
25	7.50-7.83	7.09
29	0.8 & 1.8	6.5 & 6.8
28	1-1.8 & 2.7	6.7 & 6.9

Table 2: Summary of the important ^{13}C NMR spectroscopic data of the carbonitrile analogues. **25**-R =phenyl, **28**-R=cyclohexyl and **29**-R=cyclopropyl

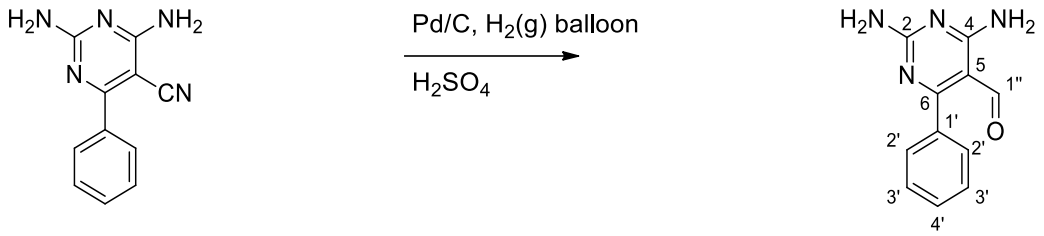
Analogues	Aromatics/Aliphatics ppm	CN ppm	Pyrimidine ppm	C5 ppm
25	76,128, 130 & 137	118	163, 165 & 169	76
29	10 & 15	118	163, 164 & 174	77
28	26, 26.2, 30 & 44	118	163, 164 & 178	77

2.1.2 Synthesis of the 6-substituted pyrimidine-5-carbaldehyde analogues[64]

The successful synthesis of the pyrimidinecarbonitriles was of great importance since it has afforded a scaffold which is heterocyclic and functionalized. Additionally, this scaffold can be functionalized even further to bring us closer to the synthesis of the desired flexible pyrimidine targets. With our nitriles in hand we sought to convert these to the aldehyde equivalents by a

single step reaction which is not commonly used for this type of conversion. The hydrogenation reaction is typically used for the reduction or saturation of organic compounds but can be expanded beyond this function as seen in this project.

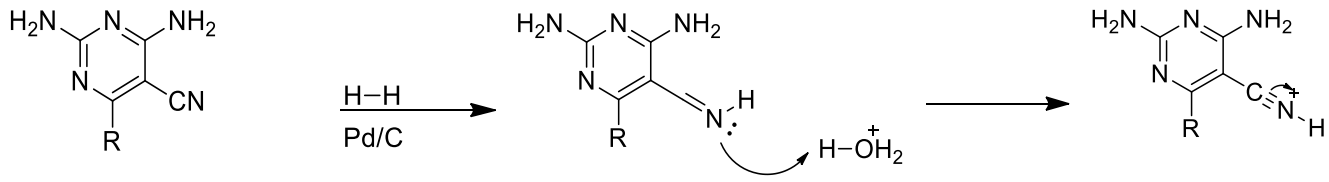
2.1.2.1 Synthesis of 2,4-diamino-6-phenylpyrimidine-5-carbaldehyde

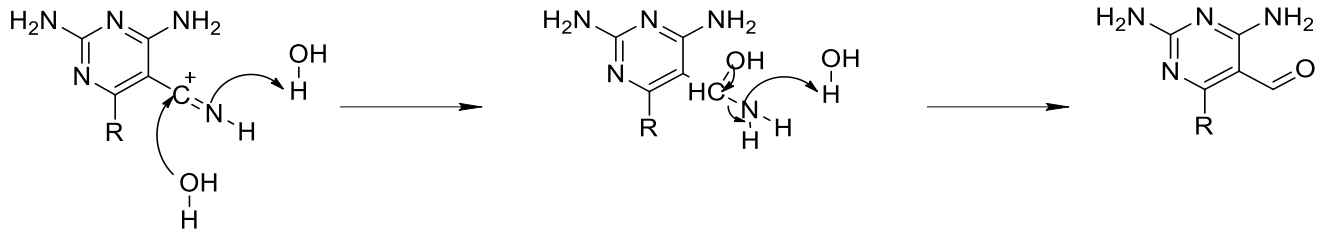


25 **30**

Scheme 6: Reaction conditions: Pd/C, H_2 (g) balloon, 2M aq H_2SO_4, rt, 12-14 h, 48%

The synthesis of **30** (as depicted in **Scheme 6**) was achieved by the dissolution of **25** in dilute aqueous sulfuric acid. The starting material was not readily soluble in the acid solution and thus required a bit of time to stir and a slight heating to speed up the process. The product was obtained by means of catalytic hydrogenation in the presence of Pd/C catalyst which involves the formation of an intermediate imine which was then hydrolyzed to the aldehyde (**Scheme** 7). The method in discussion was chosen because, although there have been reports of likely over reduction, in the case of **30** none was observed (62). Instead complete conversion of the starting material to the aldehyde was observed and confirmed by staining the TLC plate with 2,4 dinitrophenylhydrazine (2,4-DNPH). The product spot turned into a bright yellow colour and gradually darkened upon exposure to the air as expected for aldehydes. The identity of the product was then confirmed by NMR spectroscopy.





Scheme 7: Proposed mechanism for the synthesis of the aldehyde

The ^{1}H NMR spectrum of **30** exhibited a characteristic peak which would be specific for our compound of interest. The CHO peak (H1") was seen at 9.5 ppm, a new peak which appears as result of the successful conversion of CN group to an aldehyde. The rest of the peaks remain the same as previously described for **25**. In the ^{13}C NMR spectrum the appearance of the new peak at 191 ppm owing to the C=O (C1") and the simultaneous disappearance of the CN at 117 ppm confirms the conversion of our nitrile functional group to the aldehyde form. Additionally, a change in chemical shift seen for C5 from 79 ppm to 104 ppm shows how the aldehyde has an overall deshielding effect on the atoms in the vicinity. Furthermore, the IR spectrum data shows the disappearance of the CN peak at 2203 ν/cm^{-1} and the appearance of a C=O stretch at 1658 ν/cm^{-1}.

In previous attempts to synthesize **30**, the challenge of incomplete conversion was encountered. The reaction was previously performed with catalytic amounts of Pd/C, but we found that stoichiometric amounts of Pd/C enabled the complete conversion of the nitrile to the aldehyde. Other attempts towards obtaining the desired product, including the use of Raney Ni was considered and tested but no conversion of the starting material was observed.

Unfortunately, isolated yields of the product were quite low (less than 50%) and was suspected to be owing to the highly polar nature of the carbaldehye. We sought to improve the yields by performing the extraction at a more basic pH. This was useful in that a slight improvement in the reported yields was observed. Other changes, such as the use of more polar solvents for extraction and saturating the aqueous layer with NaCl, did not improve the isolated yield of the product.

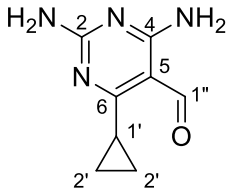
2.1.2.2 Synthesis of 2, 4-diamino-6-cyclohexylpyrimidine-5-carbaldehyde

31

Figure 18: 2,4-Diamino-6-cyclohexylpyrimidine-5-carbaldehyde

The same procedure as stated above for the synthesis of **30** was undertaken for the cyclohexyl analogue **31**. The assessment of the NMR spectroscopic data of this compound was indicative of the successful synthesis of the desired product. From the ^{1}H NMR spectrum, the aldehydic proton was observed at 10 ppm, this peak is significant because it marks the successful conversion of the nitrile to the aldehyde as mentioned for the phenyl analogue. From the ^{13}C NMR spectrum the carbonyl carbon appears at 187 ppm, accompanied by the shift of C5 to 101 ppm from 76 ppm. The overall yield of 43% of this analogue was no different from that of **30**.

2.1.2.3 Synthesis of 2, 4-diamino-6-cyclopropylpyrimidine-5-carbaldehyde



32

Figure 19: 2,4-Diaminocyclopropylpyrimidine-5-carbaldehyde

Finally, in this series we subjected cyclopropyl carbonitrile **29** to the same reaction conditions to afford **32.** This analogue **32** exhibited the best yields despite its propensity to over reduction where the alcohol was also isolated. The reasons for this are unknown and no further work was done to understand this tendency due to time limitations. H1" was observed at 9.9 ppm in the ^{1}H NMR spectrum of **32** with the corresponding carbon found at 187 ppm and an upfield shift of C5 to 102.6 ppm observed in the ^{13}C NMR spectrum.

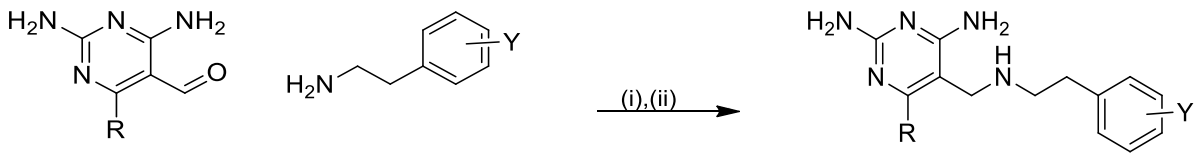
From the collected data it was confirmed that the synthesis of the above stated compounds was successful, and the important peaks are listed below (**Table 3**). In keeping with the overall poor yield, the overall yield of **32** is quoted slightly above 50% as 57%.

Table 3 Summary of the ^{1}H NMR spectroscopic data, aldehydic proton chemical shift and the ^{13}C NMR spectroscopic data.

Compound	Aldehydic proton (ppm)- ^{1}H NMR spectrum	C=O (ppm)- ^{13}C NMR spectrum	C5 (ppm) - ^{13}C NMR spectrum
30	9.5(s)	191	104
32	10 (s)	187	103
31	10 (s)	187	101

2.1.3 Reductive amination between pyrimidinecarbaldehyde and phenethylamines[63]

With the aldehydes **30**-**32** successfully synthesized, we sought to consolidate our synthetic route to reach the desired flexible pyrimidine analogues. This was to be achieved by the reaction of one of the aldehydes with different commercially available phenethylamines via a reductive amination reaction. This reaction was chosen as the reaction conditions are well known and well-studied, making it a convenient route to the synthesis of the target compounds.



33

Y= 2-OMe
3-OMe
4-OMe

R=Ph

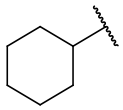
2-F

3-F

2-Cl

3-Cl

4-Cl



Scheme 8: General reaction scheme of the reductive amination reaction: Reaction Condition**s**: (i) ethanol, acetic acid, 12 hours, refluxing. (ii) $NaCNBH_3$, 12 hours, refluxing. R= phenyl, cyclohexyl and cyclopropyl. Y=2,3,4-Cl, F and -OMe

The reaction as represented in **Scheme 8** was performed between previously synthesized aldehydes (**30**, **31** and **32**) and commercially available phenethylamines, to make access towards **33** possible. Using reaction conditions previously attempted in our labs for this reductive amination step we dissolved each of our synthesized aldehydes (**30**, **31** and **32)** in ethanol before adding catalytic amounts of acetic acid. A substituted phenethylamine of choice was then added to each mixture and stirred for 12 hours under an atmosphere of nitrogen at room temperature, to enable the imine formation. At the end of the 12 hours, the reducing agent, sodium cyanoborohydride, was added and stirred with the mixture while refluxing with the aim of attaining the reduced amine.

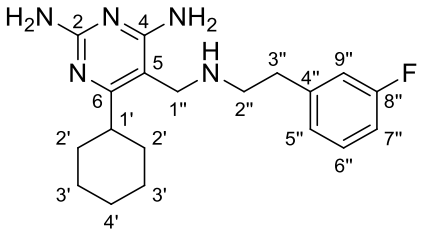
The straightforward reductive amination conditions were ideal for the compounds we were trying to synthesize due to the mildness of the reaction conditions, the easy work up and the availability of the starting materials, but due to unknown reasons the resulting product was isolated as a highly impure mixture and as a result, this made the characterization of these compounds particularly difficult. The purification of the compounds by column chromatography was highly challenging considering the polar nature of the compounds due to the amino substituents.

With this limitation at hand, we then focused our attention on improving the reaction conditions by varying parameters, with the hopes of improving the purity of the resulting compounds upon extraction to make purification by conventional methods a lot more accessible. We tried the reaction in other solvents such as methanol and dichloroethane (DCE), neither of which was useful in this regard. We varied temperature, attempted the reaction at room temperature and other higher temperatures. The reaction was also attempted as a single step reaction where all the necessary reagents were added in one pot. Other reducing agents were attempted such as

sodium triacetoxyborohydride and sodium borohydride but neither of the two were of any usefulness for the task.

In our attempts to optimise the reaction conditions the search of the literature yielded many reaction conditions towards the synthesis of amines like those which we sought to access. These reaction conditions were also tried on a simpler reaction system between benzaldehyde and benzylamine and the target compound was successfully isolated. The main disparity and possibly main point of contention, was that these conditions were largely reported for simpler systems, with none being suitable for heterocyclic systems like the one we were seeking to establish. Nonetheless, we were able to confirm the successful synthesis of the following two compounds, proving that the reaction conditions were feasible but not necessarily viable for the system we were trying to achieve.

2.1.3.1 Synthesis of 6-cyclohexyl-5-(((3-fluorophenethyl)amino)methyl)pyrimidine-2,4-diamine



33a

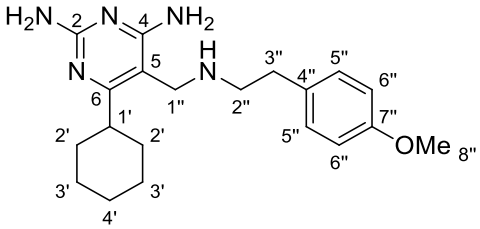
Figure 20: 6-Cyclohexyl-5-(((3-fluorophenethyl)amino)methyl)pyrimidine-2,4-diamine

In ethanol, 2,4-diamino-6-cyclohexylpyrimidine-5-carbaldehyde **31**, was dissolved by stirring at room temperature. To this mixture acetic acid was added and stirred for 10 minutes before adding 3-fluoro-phenethylamine. The mixture was stirred overnight under an atmosphere of nitrogen while refluxing. The stirred mixture was then cooled before introducing $NaCNBH_3$ and stirring for an additional 12 hours. At the end of the 12 hours, the product was extracted several times from the aqueous layer using ethyl acetate to afford the crude product as a bright yellow solid. In a fortuitous attempt to purify **33a** by washing with small amounts of isopropanol alcohol we were able to isolate an off yellow solid. The resulting solid product was then characterized by NMR spectroscopic techniques.

From the ^{1}H NMR spectrum we were able to successfully identify the aromatic protons as a new addition to the former NMR spectrum of the aldehyde **31**. Multiplet signals were observed owing to the various aromatic protons at 7.23 ppm due to H5", 6.88 ppm due to H6", 6.93 ppm due to H7" and 6.98 ppm due to H9". Furthermore, signals appearing as triplets at 2.85 ppm due to H2" and 3.10 ppm due to H3" were observed. H1" appeared as a signal at 3.14-3.21 ppm as a singlet peak H1' was also distinctly visible as a peak at appearing as a triplet of triplets as a part of the cyclohexyl ring.

From the ^{13}C NMR spectrum, the aromatic region had doublet signals due to C-F coupling. The carbon directly attached to the fluorine C8" was observed as a peak at 163.1 ppm with a $_{C\text{-}F}J$ coupling constant of 245.1 Hz. Due to its position on the compound C4" was identified as a doublet signal at 139.2 ppm, exhibiting a $_{C\text{-}F}J$ value of 7.5 Hz. The doublet signal at 130.4 ppm was due to C6" and C7" and C9" were observed as doublet peaks at 113.6 ppm and 115.2 ppm, respectively. The signal due to C5" was seen at 124.3 ppm, completing the aromatic carbons. The carbons in the linker chain were observed in the more aliphatic region of the spectrum with C1", C2" and C3" appearing at 50.6 ppm, 45.6 ppm and 44.7 ppm, respectively. The pyrimidine carbons appeared as signals at 178.7 ppm (C2), 164.9 ppm (C4) and 164.1 ppm (C6), with C5 shifting upfield appearing at 77.6 ppm. Further confirmation of the successful synthesis of **33a** was confirmed by HRMS, the mass found for this compound was 344.2240 which corresponds with the molecular formula $C_{19}H_{26}FN_5$. Additional characterization by IR indicated the presence of the NH_2 groups could be observed at 3391 ν/cm^{-1}and 3109 ν/cm^{-1}.

2.1.3.2 Synthesis of 6-cyclohexyl-5-(((4-methoxyphenethyl)amino)methyl)pyrimidine-2,4-diamine



33b

Figure 21: 6-Cyclohexy-5-(((4-methoxyphenethyl)amino)methyl)pyrimidine-2,4-diamine

Following the same procedure as stated for **33a**, **33b** was synthesized, where 4-methoxy phenethylamine was used as the amine of choice. Several methods towards the purification of the resulting crude product were attempted including column chromatography and

recrystallisation. The product was isolated as a solid after purification by column chromatography and then characterized by NMR spectroscopy.

The ^{1}H NMR spectrum of **33b** exhibited two doublets in the aromatic region due to H5" and H6" with each set of protons appearing at 6.89 ppm and 7.19 ppm respectively. These were accompanied by a singlet more upfield due to the methoxy protons at 3.77 ppm and two triplet signals at 3.13 ppm and 2.89 ppm due to H2" and H3" respectively. The presence of the cyclohexyl ring was evident by the signals in the aliphatic region.

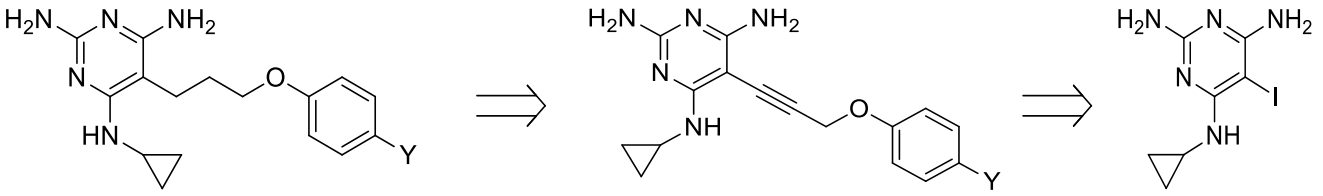
In the ^{13}C NMR spectrum, signals due to C5" and C6" appeared at 129.7 ppm and 113.9 ppm, respectively, as one would expect from prior analyses. The appearance of C4" and C7" was seen as signals at 99.5 ppm and 158.05, respectively. C7" appeared more downfield due to the de-shielding effect of the methoxy group directly attached. We can further confirm the presence of the methoxy group by the appearance of a signal at 55.3 ppm characteristic of the methyl carbon of the methoxy substituents. At a chemical shift of 94.2 ppm we further observed a signal due to C5 which serves as a handle for the linker chain. The exact positions of C1", C2" and C3" were not confirmed by other 2-D experiments but we were able to confirm that the signals at 41,4 ppm, 31.9 ppm and 31.0 ppm were in fact CH_2 signals from DEPT NMR spectroscopic data. The cyclohexyl carbons along with the pyrimidine carbon signals were evident as previously observed in the NMR spectra of the aldehyde. We were then able to confirm that the mass of the compound synthesized was indeed 356.2401 corresponding to a compound with a molecular formula of $C_{20}H_{30}N_5O$ [M + H] as was observed for **33b.**

Considering the difficulty encountered towards the synthesis and purification of the compounds reported we began to look at the possibility of purification by semi preparative HPLC. We have managed to assess some of the other analogues by analytical HPLC methods. This work is still on-going, and we hope to isolate purified samples of the other analogues prepared.

Part 2

2.2 Towards the synthesis of N^4- substituted-5-((phenyl)buta-1,3-diyn-1-yl)pyrimidine-4,6-diamine

In the interests of expanding the scope of the project we sought to introduce a new approach towards synthesizing compounds which were similar in structure to the 2,4-diaminopyrimidine compounds which we had made in part one of the project. This was heavily prompted by the challenges that were faced in the previous project regarding the purification of the compounds. Access to the new library of compounds **34** would be achieved through the hydrogenation of a suitable alkyne precursor **35** as shown in **Scheme 9** by methods which will be explored in the text below.



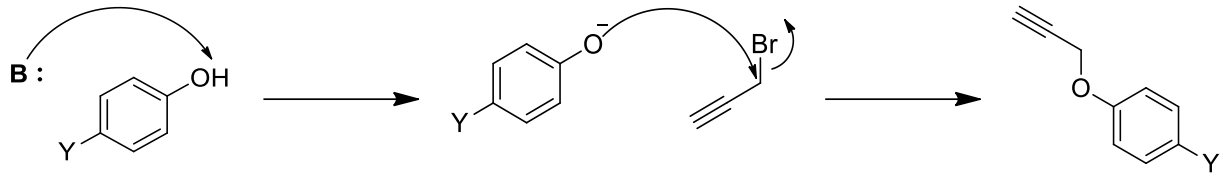
Scheme 9: Proposed structure of new library of compounds and the precursors necessary towards the desired compounds

Disconnection between C-5 (of the pyrimidine) and the acetylene carbon of **35**, results in the two precursors **36** and **37** shown above. The two structures were anticipated to converge by means of a Sonogashira reaction. These can be accessed via simple synthetic routes which involve known reactions which have been widely used for the synthesis of other substrates.

2.2.1 Synthesis of substituted (prop-2-yn-1-yloxy)benzene

To prepare the proposed compounds and the respective analogues, the synthesis of the acetylene precursor **37** was required. Various substituted phenols were reacted with propargyl bromide in acetone with potassium carbonate present, to give the desired products in good

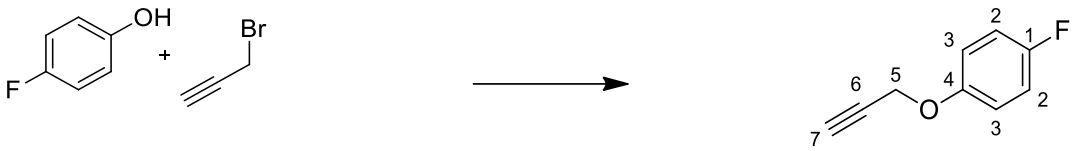
yields. The type of reaction responsible of the product formation is known as an S_N2 reaction where the base potassium carbonate (K_2CO_3) deprotonates the phenol to drive the nucleophilic attack at the alpha carbon to afford the desired acetylene (**Scheme 10)**.



37

Scheme 10: Mechanistic details for the S_N2 reaction

2.2.1.1 Synthesis of 1-fluoro-4-(prop-2-yn-1-yloxy)benzene

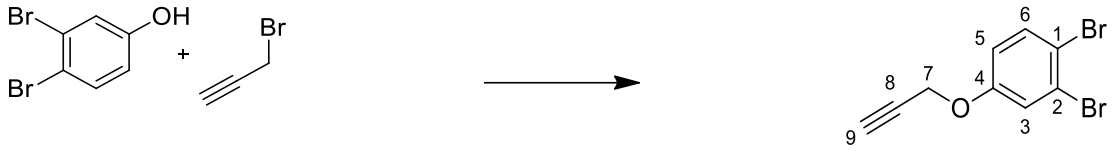


38a

Scheme 11: Reaction conditions: K_2CO_3, acetone, rt.12 hrs, 93%

The synthesis of **38a** (**Scheme 11**) was carried out under the stated conditions and was subsequently characterized using NMR spectroscopic data. The ^{1}H NMR spectroscopic data showed the presence of an acetylenic proton at 2.52 ppm which appeared as a triplet due to splitting by the CH_2 protons two bonds away. The CH_2 protons appear as a doublet signal at 4.59 ppm. Additionally, the aromatic region of the spectrum exhibited two distinct doublets which appear because of the *para* substituted phenol which was used. The doublets owing to H2 and H3 appear at 6.84 ppm and 6.74 ppm, respectively. Furthermore, from the carbon NMR spectrum distinct fluorine carbon coupling was evident where C1 appeared as a signal at 157.8 ppm with a $_{C\text{-}F}J$ of 239.3 Hz, a value typically observed for carbons directly attached to the fluoro-substituent. C2 and C3 appeared as doublets at 115.9 ppm and 116.2 ppm with $_{C\text{-}F}J$ values of 23.2 Hz and 8.1 Hz, respectively. C4 gave rise to a doublet with a $_{C\text{-}F}J$ value of 2.0 Hz which appeared at 153.6 ppm. The acetylic carbons C6 and C7 appeared as signals at 78.4 ppm and 75.7 ppm, respectively, while C5 appeared slightly more upfield at 56.5 ppm, which confirmed the successful synthesis of **38a.**

2.2.1.2 Synthesis of 1,2-dibromo-4-(prop-2-yn-1-yloxy)benzene



38b

Scheme 12: Reaction conditions: K_2CO_3, acetone, rt.12 hrs, 98%

As we sought to further extend the library of acetylenes, using the same reactions conditions as previously reported we synthesized **38b** using a di-substituted phenol as shown in **Scheme 12**. The success of the reaction was confirmed using NMR spectroscopic techniques.

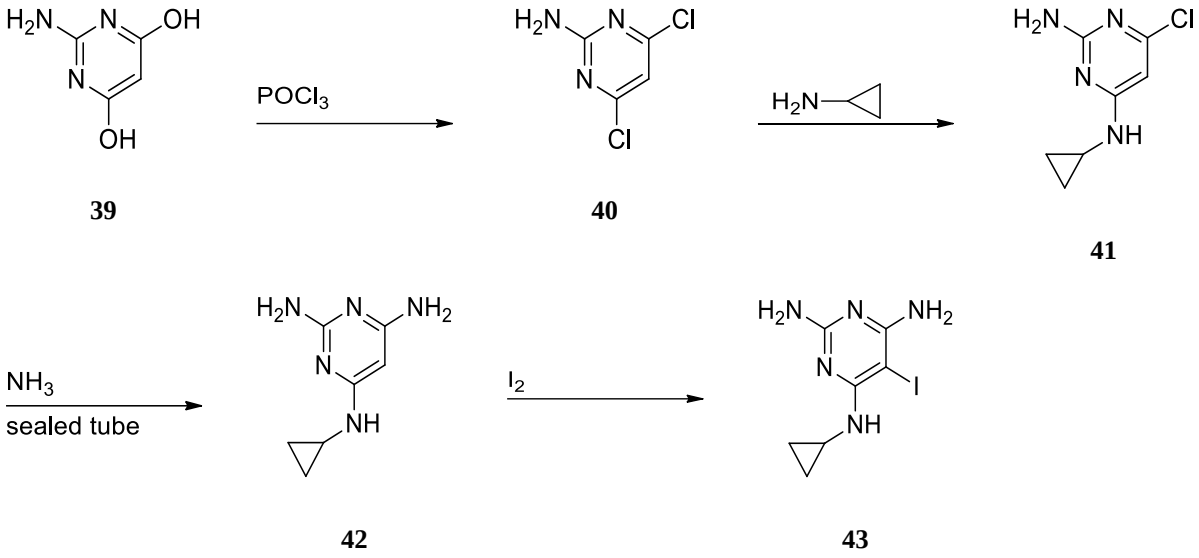
The ^{1}H NMR spectrum exhibited 2 doublets at 7.69 ppm and 6.94 ppm due to H6 and H5, respectively. An additional signal due to H3 was found to appear at 7.38 ppm as a doublet of doublet. As was observed in the spectrum of **38a**, the acetylenic proton for **38b** was observed at 2.55 ppm and the CH_2 protons appeared at 4.75 ppm as a doublet. Additional information confirming the structure of **38b** was obtained from the ^{13}C NMR spectrum. At 153.3 ppm, a signal due to C4 was observed, alongside C6 at 135.7 and C2 at 131.1 ppm. C3 and C5 appeared as signals at 115.4 ppm and 114.2 ppm, respectively, while C1 was appearing as a signal at 113.4 ppm. We would like to conclude that the C9 appears beneath the chloroform (solvent peak) and would appear near C8 which appears as a signal at 77.5 ppm. The signal due to C7 appeared as a signal at 57.0 ppm, which concludes the series of carbon signals of **38b**. Interestingly the reaction was repeatedly found to be high yielding with the product yields ranging between 85% and 99%.

2.2.2 Synthesis of N^4-cyclopropyl-5-iodopyrimidine-2,4,6-triamine

Starting from the commercially available 4,6-dihydroxy-2-aminopyrimidine **39**, the 4,6-dichloro-2-aminopyrimidine **40** was made by displacing both hydroxy-groups with a chloro-group from $POCl_3$. This was followed by the displacement of one of the chloro-groups by an

amine i.e.; cyclopropylamine in *n*-butanol in the presence of Hunig's base to yield **41**. The displacement of the second chloro-group was achieved under harsher conditions in ethanol and excess aqueous ammonia solution in a sealed tube reaction to yield **42**. This was followed by iodination at the 5-position of the pyrimidine ring.

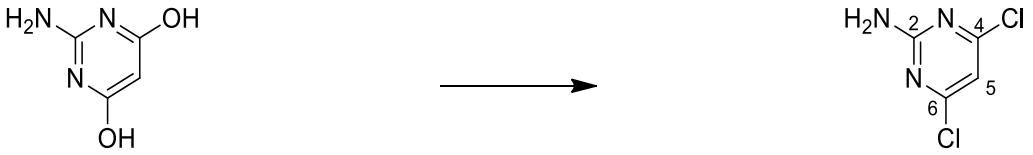
The synthesis of **43** was approached as depicted in the **Scheme 13** below.



39 **40** **41** **42** **43**

Scheme 13: Proposed synthetic approach towards the synthesis of N^4-cyclopropyl-5-iodopyrimidine-2, 4, 6-triamine.

2.2.2.1 Synthesis of 4,6-dichloropyrimidine-2-amine



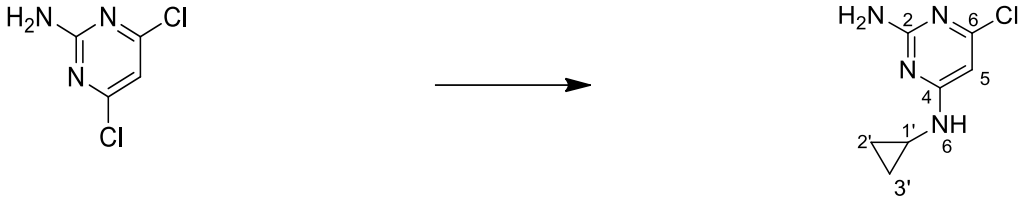
40

Scheme 14: Reaction conditions**:** $POCl_3$, *N, N*-DMA, 60 °C, 4 hrs, 40%

To achieve the synthesis of **40 (Scheme 14)**, commercially available 4,6-dihydroxypyrimidine-2-amine **39** was dissolved in phosphorus oxychloride ($POCl_3$) (5 ml) and stirred for 3-4 hour at 60 °C. The thick pale-yellow mixture was maintained at this temperature upon the dropwise addition of *N*,*N*-dimethylaniline (*N*, *N*-DMA) over an hour, the mixture would brown during this process. The reaction mixture was stirred for an hour and then cooled to 0 °C and quenched with water. The faint brown clay-like precipitate was then filtered off and washed several times with water and dried under vacuum. The product was then crystallized from ethanol. The resulting product was isolated at a yield of 40% as fine bright yellow crystals.

The ^{1}H NMR spectrum showed 2 signals, a broad peak at 4.7 ppm due to the NH_2 group and a sharp singlet at 7.58 ppm due to H5. From the ^{13}C NMR spectrum, the structure of the product was concluded by the signals at 108 ppm (C5), 163 ppm (C4 & C6) and 164 ppm (C2), corresponding to the results seen in literature (66).

2.2.2.2 Synthesis of 6-chloro-*N*^{4}-cyclopropylpyrimidine-2,4-diamine



41

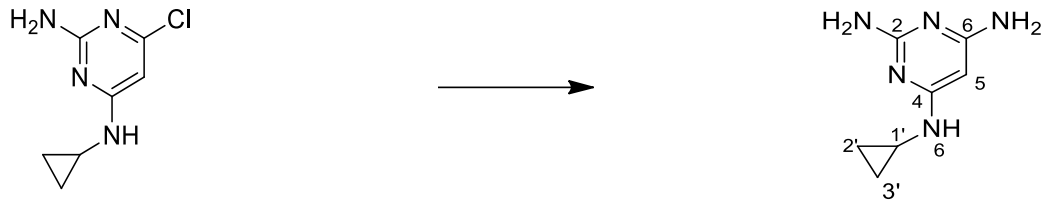
Scheme 15: Reaction conditions: cyclopropylamine, Hunigs base, *n*-BuOH, 95° C, 14 hrs, 53%

The synthesis of **41** was achieved by dissolving **40** in 20 ml *n*-butanol (*n*-BuOH) (**Scheme 15**). Upon complete dissolution, cyclopropylamine was added and stirred before introducing Hunig's base. The reaction mixture was stirred overnight at 95 °C and then stopped upon checking the progress of the reaction by TLC. The crude product was pure enough to proceed without any further purification.

New peaks were observed in the ^{1}H NMR spectrum, and these four new peaks would confirm the synthesis of the product. A new signal due to the NH was seen at 5.7 ppm, with the NH_2 and H5 peaks still present as expected. In the aliphatic region of the spectrum 3 new signals were observed at 0.45 ppm (CH_2), 0.68 ppm (CH_2) and at 2.5 ppm (CH). The peak at 2.5 ppm is not visible due to overlap with the solvent peak (DMSO) but the presence of this peak is confirmed on the ^{13}C NMR spectrum. From this spectrum a new peak at 7.05 ppm was observed

due to the CH_2 on the cyclopropyl ring. At 23.5 ppm another peak is observed due to the cyclopropyl CH, although absent on the proton spectrum we can confidently confirm the presence of this piece of the cyclopropyl ring. The rest of the spectrum remained the same as that of the starting material.

2.2.2.3 Synthesis of N^4-cyclopropylpyrimidine-2,4,6-triamine



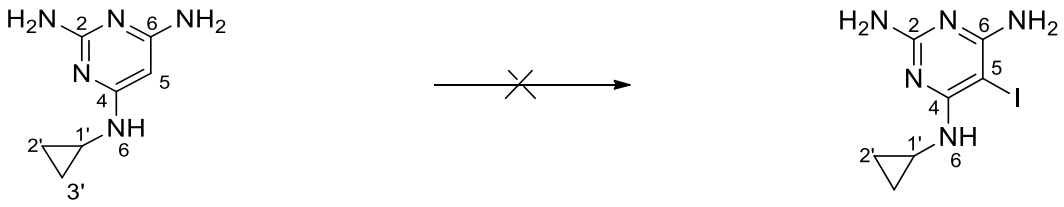
42

Scheme 16: Reaction conditions: 25% ammonia solution, EtOH,170 °C, 2 days, 77%

Displacing the second chloro group is more difficult because the ring is now electron rich due to the newly introduced amine functionality (**Scheme 16**). As such **42** was synthesized by reacting **41** with aqueous ammonia in a sealed tube at 170 °C for 2 days. At the end of the 48 hours the furnace was switched off and left to cool down to room temperature, and the solvent was concentrated *in vacuo*. The resulting crude product was then characterized as only one spot was observed by TLC.

The ^{1}H NMR spectrum showed a new broad peak at 5.48 ppm due to the newly introduced NH_2 group at position 6 on the pyrimidine ring. On the ^{13}C NMR spectrum a slight shift in the signal due to C6 was observed, the peak moves from 163 ppm to 164 ppm, which can be attributed to the presence of the amino group.

2.2.2.4 Attempted synthesis of N^4-cyclopropyl-5-iodopyrimidine-2,4,6-triamine



43

Scheme 17: Reaction conditions: water/DMF, iodine, K_2CO_3, 45 °C, 14 h

We were then in the position to introduce the iodo-substituent at the 5-position of the pyrimidine ring, for subsequent Sonogashira coupling. We hoped to achieve this by reacting **42** in water along with K_2CO_3 before adding dimethyl formamide (DMF) and iodine granules (**Scheme 17**). This mixture was stirred overnight at 45 °C and then stopped the next day. The cooled mixture was then quenched with 2M aqueous sodium thiosulfate ($Na_2S_2O_3$) to quench the iodine. The precipitation of a solid was expected from previous work on a seies of aromatic derivatives, but was not observed in this instance. The reaction was repeated several times and in the absence of a precipitate a normal extraction was undertaken. This attempt was still unsuccessful. We were unable to visualize the starting material by TLC, which meant that some form of conversion had taken place, however we were never able to isolate the desired product.

The reaction was attempted using a different iodine source, *N*-iodosuccinimide (NIS) and DMF as the solvent. This attempt was also unsuccessful, and once again although starting material had been consumed, we were unable to isolate the desired product. The reaction was attempted on another substrate and was proven to be feasible on a similar scaffold, which we will discuss later in the text, confirming the viability of this method for what we were trying to achieve. The aqueous layer showed the presence of a UV active compound by TLC and upon attempts to recover this compound from the aqueous layer by evaporation of the water, an insoluble salt was recovered.

With the difficulty to obtain the desired product we attempted to switch the steps of the synthesis, with the hopes of reaching our target precursor. The ammonolysis step and the iodination step were switched to see if we could access the product. Unfortunately, this was unsuccessful.

Our failure to iodinate at the 5-position led us to attempt the reaction with a different substrate to the one that we were using. Cyclopropylamine was substituted with benzylamine in the first step which afforded us a new series of intermediates. Starting from 4,6-dichloropyrimidine-2-amine we began the synthesis which we hoped would afford us N^4-benzyl-5-iodopyrimidine-2,4,6-triamine **44 (Figure 22)**.

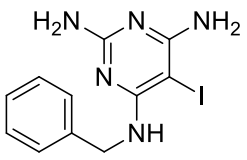
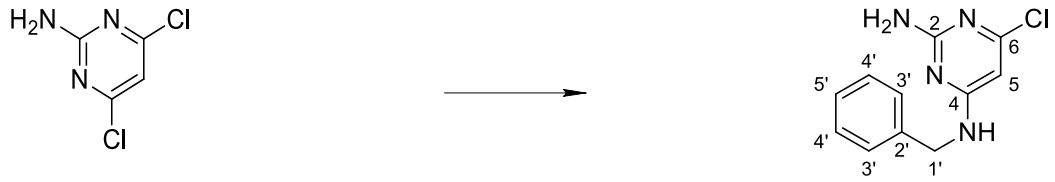


Figure 22: N^4-benzyl-5-iodopyrimidine-2,4,6-triamine

2.3 Towards the synthesis of N^4-benzyl-5-iodopyrimidine-2,4,6-triamine

2.3.1 Synthesis of N^4-benzyl-6-chloropyrimidine-2,4-diamine



39 **45**

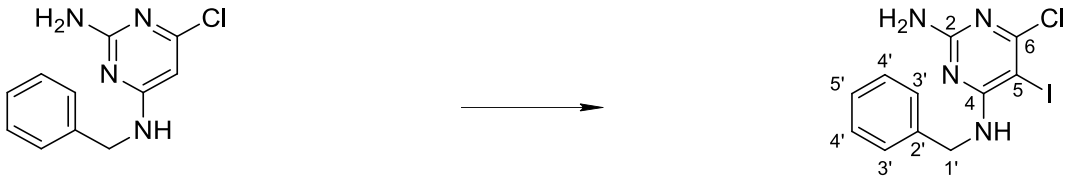
Scheme 18: Reaction conditions: Benzylamine, *n*-BuOH, Hunigs base, 95 °C, 14 h, 60%

The process towards the synthesis of **44** began with the formation of **45** via the same reaction conditions as described for **40**. In *n*-butanol, **39**, benzylamine and Hunig's base were stirred together overnight to afford the desired product **45** in good yield (**Scheme 18**). The product is formed through nucleophilic substitution where the chloro group is replaced by benzylamine which serves as the nucleophile. The Hunig's base is a good base for this reaction since it is non-nucleophilic and thus poses no competition for the desired reaction (67). This reaction was convenient since it required the use of reagents which were readily available, and the product was easily recovered.

Successful synthesis of this product was confirmed by NMR spectroscopy. From the ^{1}H NMR spectrum five signals were observed in total, three were new due to the group introduced in this reaction. Firstly, a signal at 5.8 ppm due to the NH group, then a multiplet at 7.3 ppm due to the aromatic protons and finally a signal at 4.5 ppm due to the benzylic CH_2 all confirmed that we had synthesized the product. The ^{13}C NMR spectrum showed the benzylic CH_2 (C1') at 43.3 ppm, the aromatic carbons appearing as signals at 126.8 ppm (C5'), 127.2 ppm and 128.3 ppm (C3' & C4'), the distinct aromatic C2' which appears at 139.5 ppm and C5 appeared as a signal at 102.4 ppm. The presence of C5 was further confirmed from the ^{1}H NMR spectrum with H5 appearing as a part of the aromatic multiplet.

2.3.2 Synthesis of N^4-benzyl-6-chloro-5-iodopyrimidine-2,4-diamine

Considering the challenge that we had faced with getting the iodination reaction to proceed towards the synthesis of N^4-cyclopropyl-5-iodopyrimidine-2,4,6-triamine **43** we decided to try to switch the order of the reaction steps for **44** as well since the cyclopropyl analogue was not yielding the desired outcome. On this substrate **45** we proceeded to attempt the iodination before the ammonolysis with the hope of attaining the desired product.



45 **46**

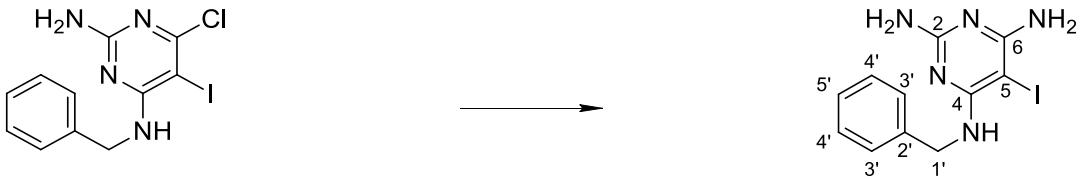
Scheme 19: Reaction conditions: water/DMF, iodine, K_2CO_3, 45 °C, 14 h, 87%

We therefore subjected **45** to our conditions for iodination: potassium carbonate, iodine (solid granules) and DMF/water were added and the mixture was stirred at 45 °C overnight (**Scheme 19**). Upon addition of sodium thiosulfate pentahydrate, the product precipitated out of solution as a yellow powder. The use of a small amount of DMF also makes for the attractiveness of this reaction, no work up is necessary, and the reaction occurs at low temperatures. Although convenient, the reaction was not quantitative or reproducible and in some instances the product would co- precipitate with the starting material or no product would be isolated by precipitation at allfor some peculiar reason. In these cases, the product would be isolated via a normal work up procedure using organic solvents. Despite this, the product was recovered in good yields with the highest observed yield being 87%.

The main feature of interest on the ^{1}H NMR spectrum was the disappearance of the signal due to H5 and the drastic shift in chemical shift upfield of C5. One less proton was observed as part of the aromatic peaks on the ^{1}H NMR spectrum of **46** with the rest of the signals remaining as they were previously. The new chemical shift of C5 in the ^{13}C NMR spectrum was 62.2 ppm

having shifted from 102.3 ppm with the rest of the peak remaining at around the same chemical shifts observed previously confirming the synthesis of **46.**

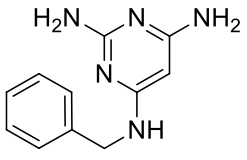
2.3.3 Attempted synthesis of N^4-benzyl-5-iodopyrimidine-2,4,6-triamine



46 **47**

Scheme 20: Reaction conditions: ethanol/ammonia solution, 170 °C, 2 days

We now tested ammonolysis of our iodinated compound **46**. We therefore subjected **46** to our sealed tube conditions described previously (**Scheme 20**). The displacement of the chloro group at C6 necessitates the use of harsh conditions due to the electron rich nature of the diamino pyrimidine core. This process is known to require high amounts of energy we ran the risk of removing both halogens in this reaction. Unfortunately, this was the case that was observed. While the chloro-group had been displaced by the ammonia, the C5- position had subsequently been dehalogenated and we produced **48** instead of **47**.

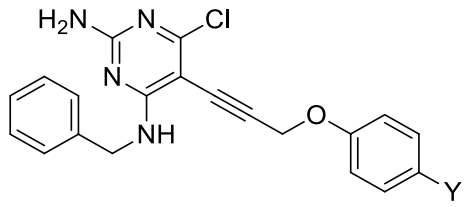


48

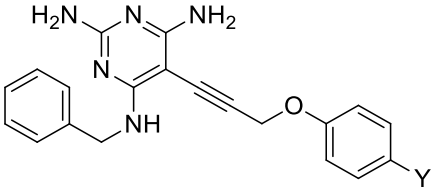
Figure 23: N^4-benzyl-5-pyrimidine-2,4,6-triamine

From the NMR spectroscopic data were able to confirm the successful synthesis of **48**. The NH_2 group and a singlet due to the proton at position 5 confirmed the synthesis **48**.

As we had been able to prepare iodinated pyrimidine **46**, we therefore decided to attempt the Sonogashira coupling on this substrate to afford **49**, with the intentions of converting it to **50**. We then hoped to subject this product to the previously described ammonolysis conditions or by another approach which would not be as harsh with the hopes of attaining **50**.



49

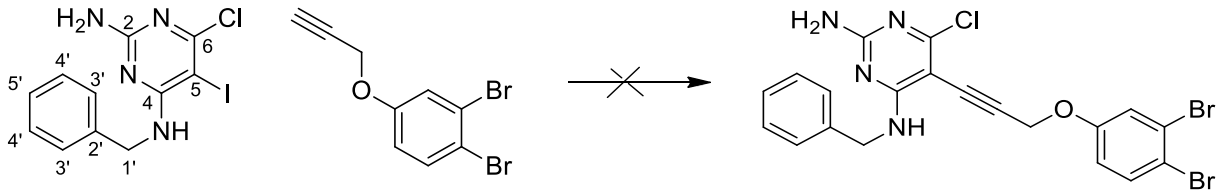


50

Figure 24: Revised targets towards the synthesis of desired compounds

The Sonogashira reaction is an old reaction which has been used in organic synthesis for ages and has been found to be very reliable in the coupling of aryl halides and acetylenes (47). The conditions of this reaction are widely reported and have been since modified to meet the profile of the given nature of the substrates. Due to the widespread use of this reaction we opted to attempt this reaction for our substrates although our aryl halide was in the form of a pyrimidine we anticipated a positive outcome.

2.3.4 Attempted synthesis of N^4-benzyl-6-chloro-5-(3-(3,4-dibromophenoxy)prop-1-yn-1-yl)pyrimidine-2,4-diamine



46 **38b** **49a**

Scheme 21: Failed attempt towards the synthesis of N^4-benzyl-6-chloro-5-(3-(3,4-dibromophenoxy)prop-1-yn-1-yl)pyrimidine-2,4-diamine

Our first attempt at the Sonogashira reaction of **46** and **38b**, used copper iodide, $Pd(PPh_3)Cl_2$ and diisopropylamine at room temperature overnight (**Scheme 21**). After this time the reaction was stopped and the crude product isolated as a brown solid.

Upon characterization of the product by NMR spectroscopy, it was found that the dehalogenated product **45** was formed and not our desired **49a**. This dehalogenated product was thought to arise due to the failure of one of the intermediate steps in the Sonogashira cycle (**Figure 23).** Failure to complete the trans-metalation step could result in the dehalogenated product **45 (2.3.1)**.

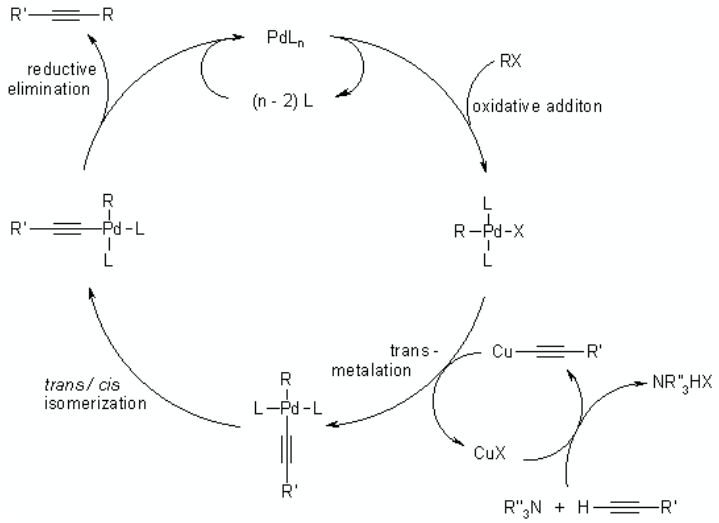
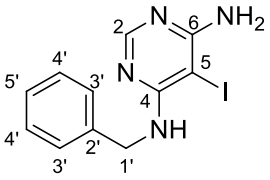


Figure 25: Proposed method for Sonogashira reaction

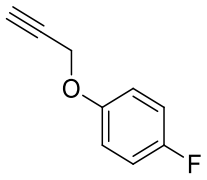
With this information in hand we then considered the reaction conditions and what could be changed to achieve the synthesis of **49a**. In the first attempt of the Sonogashira coupling reaction, $Pd(PPh_3)Cl_2$ with Pd in the 2+ oxidation state was used. We thought that this could play a role in the reaction, and decided to change the palladium source from $Pd(PPh_3)Cl_2$ to $Pd(PPh_3)_4$ (tetrakis (triphenylphosphine)palladium(0)). The reaction was repeated using $Pd(PPh_3)_4$ at room temperature and under reflux. However, only starting material was isolated.

Other variations were introduced to the reaction conditions, including a change in solvent; DMF and THF, changes in the base used; triethylamine and diisopropylamine, the temperature; room temperature to reflux and even changes in the acetylene which was being used. None of

these changes were successful. We therefore decided to test these conditions on a different set of pyrimidines with one less nitrogen substituent with the hopes of making the pyrimidine ring less electron rich.



51



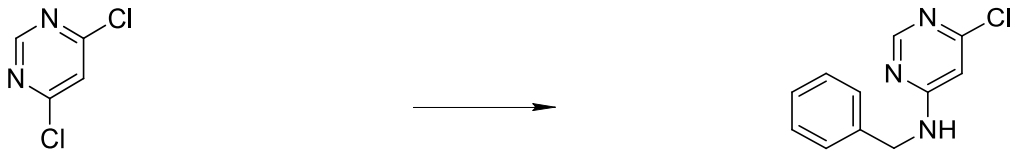
38a

Figure 26: Key intermediates towards the synthesis of target compounds

To achieve this Sonogashira reaction we had synthesized **51** as a part of our test reactions earlier in our synthesis but had not accounted for its synthesis. The synthesis of **51** was achieved as stated below. We also decided to change the acetylene from **38b** to **38a** which only had one substituent in the 4-position. These changes were made with the hopes of optimizing the Sonogashira coupling step between **51** and **38a**.

1.2 Towards the synthesis of N^4-benzyl-5-iodopyrimidne-4,6-diamine

1.2.1 Synthesis of *N*-benzyl-6-chloropyrimidin-4-amine



52 **53**

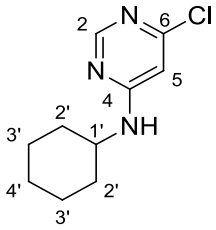
Scheme 22: Reaction conditions: benzylamine, *n*-BuOH, Hunigs base, 95 °C, 14 hrs, 95%

Starting from the commercially available **52** in *n*-butanol, benzylamine and Hunig's base were added and the reaction mixture stirred overnight at 95 °C (**Scheme 22**). The product was readily formed as described previously for earlier analogues **41** and **45**. From the NMR spectroscopic

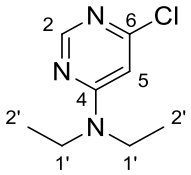
data, product formation was easily confirmed. The product was consistently isolated in good yields.

The ^{1}H NMR spectrum of **53** exhibited four signals, one at 4.5 ppm due to H1, a sharp singlet at 6.4 ppm due to H5, a multiplet at 7.3 ppm due to the aromatic protons and a broad singlet at 8.2 ppm due to H2. The ^{13}C NMR spectrum also exhibited signals which were consistent with **53**, 163.9 ppm (C6), and 158.4 ppm (C2 & C4), with the aromatic carbons (C3, C4', C5') appearing as signals at 127.4 to 129.0 ppm, C2' at 136.9 ppm and C1' at 45.6 ppm. From the HRMS the mass of **53** was confirmed as 220.0723 consistent with the molecular formula of $C_{11}H_{11}N_4{}^{35}Cl$.

With this intermediate in hand we sought to functionalize it further to bring us closer to our desired intermediate **51**.



54



55

Figure 27: Analogues of *N*-benzyl-6-chloropyrimidin-4-amine

We had hoped to extend the library of intermediates like **53** by introducing other aliphatic groups at the 4-position (**Figure 25**). Cyclohexylamine and diethylamine we used to diversify the library of 5-iodo-pyrimidines we had intended on making. This was done to expand the scope of compounds which we envisioned to make via the Sonogashira coupling step.

2.4.1.1 Synthesis of 6-chloro-N-cyclohexylpyrimidine-4-amine

Following the same procedure as stated for **53**, **54** was synthesized in excellent yields (86%). The structure of which was confirmed by NMR spectroscopy. From the ^{1}H NMR spectrum, peaks due to the cyclohexyl protons were apparent at 2.01, 1.72 and a multiplet from 1.53-1.12 ppm, encompassing all 10 protons. Sharp singlets due to H2 and H5 were visible at 8.31. and 6.31 ppm respectively. These were accompanied by another broader singlet at 5.32 ppm due to

the NH proton. The identity of the compound was further confirmed by HRMS, with a major peak of 212.0951 m/z corresponding to the calculated mass of the desired product.

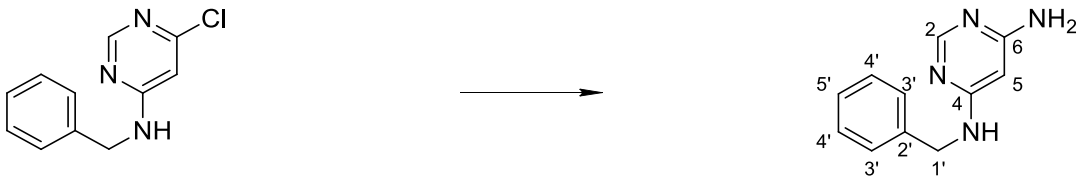
2.4.1.2 Synthesis of 6-chloro-*N, N*-diethylpyrimidine-4-amine

Likewise, **55** was synthesized in excellent yields (96%) and the structure was confirmed by NMR spectroscopy. The ^{1}H NMR spectrum of **55** showed H2 and H5 in the aromatic region at 8.35 and 6.38 ppm respectively. A multiplet from 3.68 to 3.32 ppm due to H1' and a triplet at 1.20 ppm due to the two CH_3 groups were also present in the spectrum.

The reaction was found to be highly reproducible with good yields ranging from 86%- 94%. The resulting product seldom required any further purification making it convenient and easy to proceed to the next step of the synthesis.

With the analogues at had we sought to functionalize the 6-position by displacing the chloro-group with an amino group to bring us closer to achieving the synthesis of our desired products and its analogues. This was to be achieved by ammonolysis as previously described.

1.2.2 Synthesis of N^4-benzylpyrimidin-4,6-diamine



53 **56**

Scheme 23: Reaction conditions: EtOH/ aq NH_3 solution, 170 °C, 2 days, 89%

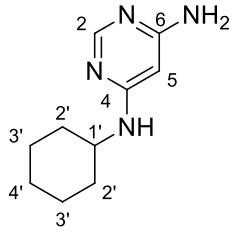
We therefore subjected **53** to our standard ammonolysis reaction conditions for the displacement of the second chloro substituent (**Scheme 23**). To this end **53** was treated with aqueous ammonia solution for 48 hours at 170 °C in a sealed tube. The product was isolated as a tan solid after the reaction was complete and was then characterized by NMR spectroscopy. From the ^{1}H NMR spectrum the appearance of a new signal at 6.1 ppm which integrated for 2 protons due to the NH_2 group was indicative of successful displacement of the chloro group. The ^{13}C NMR spectrum of this compound remained largely the same as that of the previous

precursor, except for the pyrimidine carbons which gave rise to 3 distinct peaks at 163.2 ppm (C6), 162.5 ppm (C4) and 157. 5 ppm (C2).

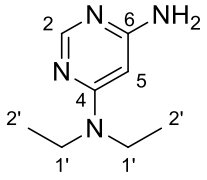
In addition to **56**, two other analogues namely; **57** and **58** were synthesized with the intentions of expanding the library of compounds which were available for further reaction.

1.2.2.1 Synthesis of N^4-substituted-pyrimidine-4,6-diamine

In keeping with the ammonolysis conditions previously stated, **57** was synthesized and isolated in 91% yield. Of great importance on the ^{1}H NMR spectrum was the appearance of a broad singlet owing to the NH_2 group. The peak was observed at a chemical shift of 6.4 ppm. Similarly, the synthesis of **58** (in 90 % yield) was undertaken and the successful synthesis of which was marked by the NH_2 peak at 5.47 ppm. The yields of this reaction for all the stated analogues were good, ranging from 89%- 91%. Additionally, the NH group was observed on the IR spectra appearing at wavenumbers ranging between 3300- 3100.



57

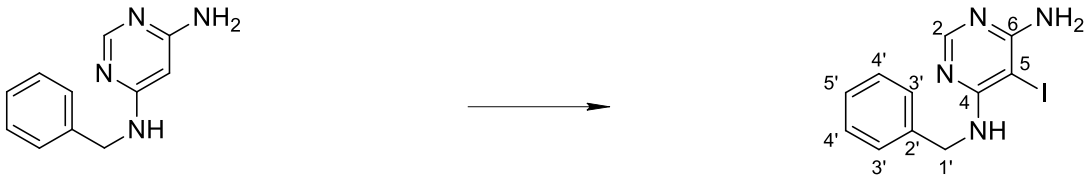


58

Figure 28: Analogues of N^4-substituted-pyrimidine-4,6-diamine

2.4.2.2 Synthesis of N^4-benzyl-5-iodopyrimidin-4,6-diamine

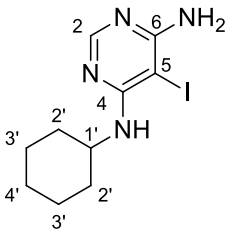
In previous attempts to make N^4-benzyl-5-iodopyrimidine-2,4,6-triamine **44**, this is the step that proved challenging where we struggled to obtain the desired iodinated pyrimidine product. This lead us to attempting the same reaction on this substrate **56** which has one less amino substituent with the hopes of accessing the iodinated product **59**.



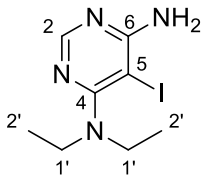
59

Scheme 24: Reaction conditions: water/DMF, iodine, K_2CO_3, 45 °C, 14 h, 49%

To our delight, the synthesis of **59** was achieved when **56** was stirred in water, with potassium carbonate, iodine and DMF at 45 °C overnight (**Scheme 24**). The product was isolated and characterized by NMR spectroscopy. The disappearance of H5 from the ^{1}H NMR spectrum was anticipated and thus observed. This was accompanied by the upfield shift of C5 from between 80- 90 ppm to 59.7 ppm. This change is typically observed in the chemical shifts of carbons which undergo halogenation. The iodination of the C5 position drastically affects the chemical shift of the carbon, and is observed in other analogues as seen in **Table 4**.



60



61

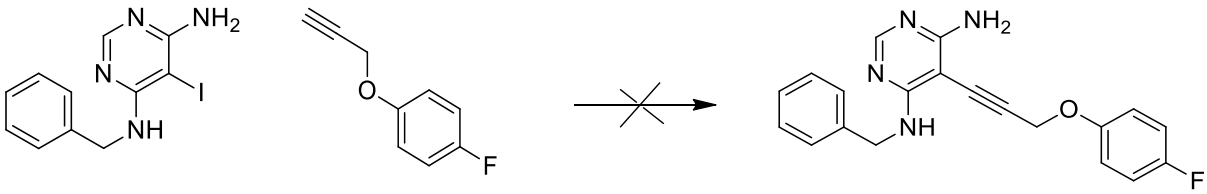
Figure 29: Analogues of N^4-substituted-5-iodopyrimidin-4,6-diamine

In keeping with our attempts to expand the library of compounds which we sought to make as way of introducing diversity **60** and **61** were also synthesized. Although these two analogues were synthesized successfully their use was limited by the low yielding nature of the iodination reaction. In each case, a yield of 1.6% and 4.7% for **60** and **61**, respectively, very little amounts of the product were isolated. As a result, we were not able to continue with subsequent steps and the synthesis was tested with the use of **59.** This leads us to wonder if the aliphatic group affects how the solubility of the product and creates a need for some alternative methods towards the synthesis of the iodinated aliphatic pyrimidines**. Table 4** below includes a summary of the major peaks which confirm the successful synthesis of the series of analogues **59-61.**

Table 4: Summary of the key features of the iodinated analogues of this series **59-61.**

Compound	Aliphatics/aromatics ^{1}H NMR spectrum	C5 ^{13}C NMR spectrum
59	7.34 (m)	59.7
60	1.24, 1.45,1.70 & 2.01 (m)	58.6
61	1.20 & 3.48 (m)	58.3

With the successful synthesis of **59**, we were now able to re-attempt the Sonogashira coupling reaction (**Scheme 27**) on a simpler pyrimidine scaffold with the hopes of achieving a successful synthesis.



59 **38a** **62**

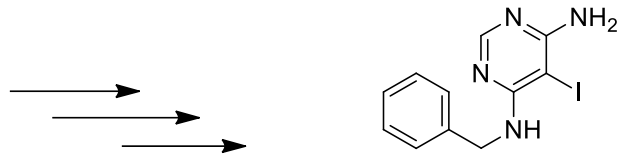
Scheme 25: Reaction conditions: DMF, CuI, $Pd(PPh_3)_4$, TEA, rt-reflux, 14-18 h

With the successful synthesis of **59**, the synthesis of **62** was plausible. In DMF the starting materials were dissolved and stirred overnight (**Scheme 25**). The reaction was stopped the following day and product isolated as a brown slurry which was purified by column chromatography. Unfortunately, only starting material was isolated indicating that the reaction was unsuccessful. The experiment was repeated at least twice and in all instances only starting material was isolated. We suspected that the acetylene might be too electron rich owing to the oxygen substituent. This feature could introduce some complexities in the reactivity of the

substrate. This poses a challenge of compatibility; the pyrimidine and the complex acetylene were not necessarily the best suited substrates for achieving the intended outcome via the Sonogashira coupling reaction.

1.3 Synthesis of N^4-benzyl-5-((trimethylsilyl)ethynyl)pyrimidine-4,6-diamine

In sight of this information and the repeated failure to get the Sonogashira reaction to work we revised our target. We considered continuing our synthetic journey using **59** but with trimethylsilylacetylene (TMS) a simpler acetylene. This would allow us to prepare **63** and its analogues. The deprotection of **63** would make it possible to couple this substrate with acetylene and afford us a rigid pyrimidine analogue **65** which can be further reduced to a flexible pyrimidine compound **66**.



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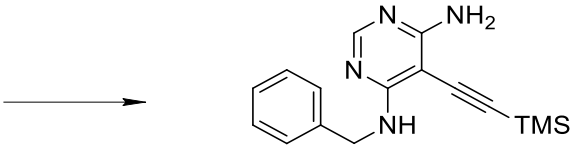

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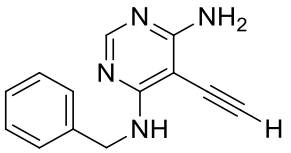
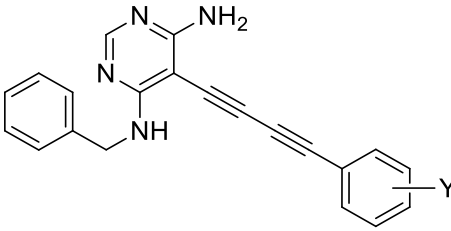
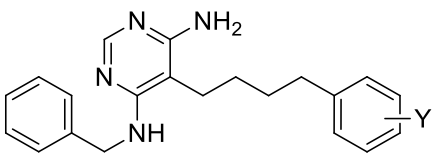


64



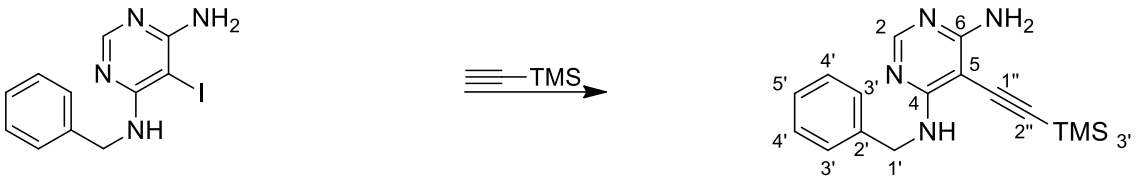
65



66

Scheme 26: Proposed synthetic changes towards the synthesis of the flexible pyrimidine target

With this information in hand we sought to make analogues of **65**. This journey began with the Sonogashira coupling reaction to afford **63**. This intermediate was successfully synthesized which allowed us to proceed with attempts to deprotect the acetylene to create a handle for further reaction.



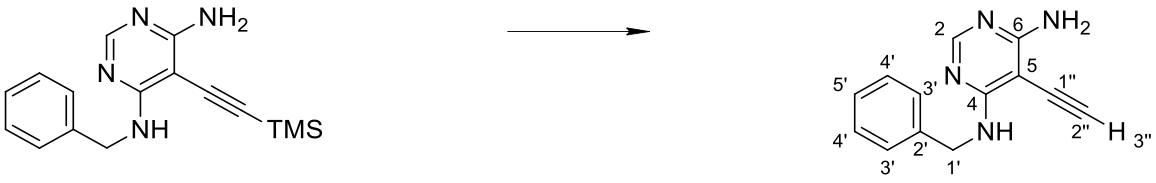
59 **63**

Scheme 27: Reaction conditions: TMS acetylene, CuI, $Pd(PPh_3)_2Cl_2$, TEA, THF, 16 h, rt, 45%

Following the same procedure reported previously for the Sonogashira coupling, **59**, copper iodide and $Pd(PPh_3)_2Cl_2$ were dissolved in THF and treated with triethylamine and TMS acetylene at room temperature (**Scheme 27**). The reaction of TMS acetylene with **59** was straightforward, yielding the desired product in 45% yield without much optimization. The product was isolated and then characterized by NMR spectroscopy.

The ^{1}H NMR spectrum showed a new signal very close to the TMS reference signal in chloroform, a sharp singlet at 0.2 ppm due to the TMS acetylene. From the ^{13}C NMR spectrum, 3 new signals were apparent with the first one appearing at 0.09 ppm near that of the reference TMS. The acetylene signals at 97.0 ppm due to C1'' and another at 108 ppm due to C2'' were observed. The other signals were consistent with those observed in the NMR spectra of **59**. furthermore, we were able to confirm the identity of our product by HRMS which showed a major peak of mass 312.1537, corresponding with the calculated mass of **63**.

1.4 Synthesis of *N*^{4}-benzyl-5-ethynylpyrimidine-4,6-diamine



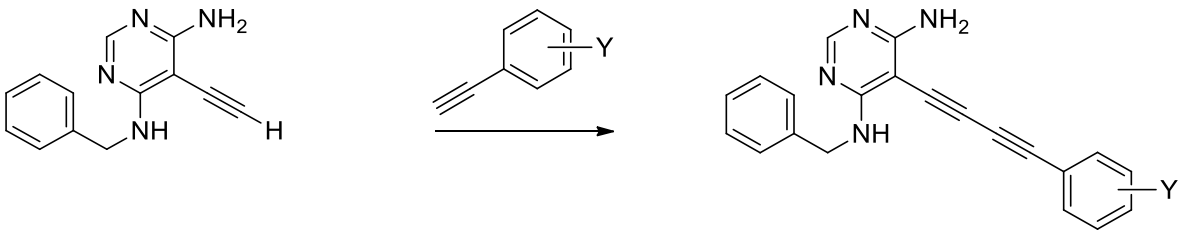
63 **64**

Scheme 28: Reaction conditions: TBAF, THF, rt, 14 h, 17%

The deprotection of **63** to afford **64** was achieved by dissolving **63** in THF then followed by the dropwise addition of tetrabutylammonium fluoride (TBAF) at 5 °C (**Scheme 28**). The reaction was then stirred at room temperature overnight. The use of TBAF for the deprotection of silicon groups is widely reported and is typically considered to be a simple reaction (72).

The emergence of a singlet peak above 3.5 ppm was anticipated in the ^{1}H NMR spectrum of **21**. This was the case where a signal at 3.7 ppm was observed due to the successful deprotection of **63**. In the ^{13}C NMR spectrum of the product, the disappearance of the signals due to TMS group, supports the information observed in the proton spectrum concerning the successful synthesis of **64**. The HRMS further confirmed the mass of the product to be 225.1180 as expected from the calculated mass.

1.5 Copper coupling reaction towards the synthesis of substituted *N*^{4}-benzyl-5-((phenyl)buta-1,3-diyn-1-yl)pyrimidine-4,6-diamine

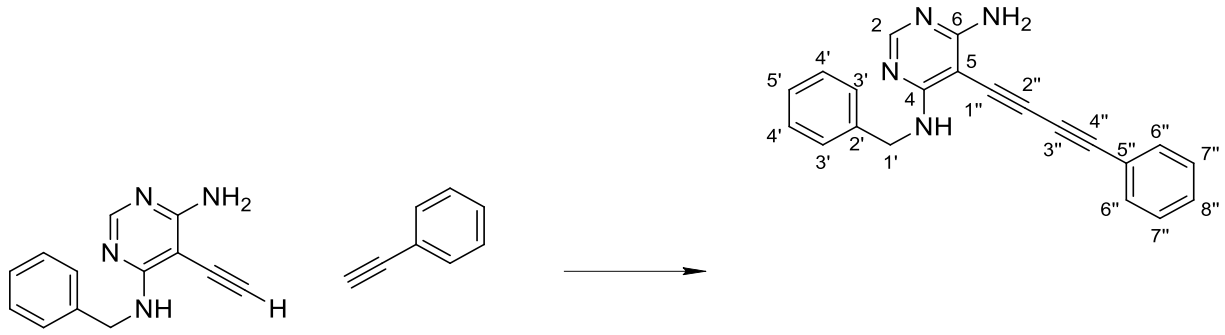


Scheme 29: Reagents and conditions: $Cu(OAc)_2.H_2O$, piperidine, DCM, rt, 16 h, 13-22%

With this intermediate in hand we were now able to attempt the coupling of this intermediate with appropriately substituted aromatic acetylenes (**Scheme 29**). We hoped that these would be less electron rich than the acetylenes we had prepared previously. With the necessary reagents in hand, we were able to attempt the reaction on a model system looking at the coupling of phenethylacetylene and **64**. Methods towards the synthesis of 1,3-conjugated diynes have been developed as these compounds play important roles in synthetic chemistry (52). The early

methods towards attaining these diynes are those of Glaser-Hay and Cadiot-Chodkiewiez which have evolved over the years with the increase in the understanding of the reaction (52). Other metals that have been reported to facilitate this oxidative coupling of terminal alkynes include copper and nickel bimetallic systems (52).

1.5.1 Synthesis of N^4-benzyl-5-((phenyl)buta-1,3-diyn-1-yl)pyrimidine-4,6-diamine (68)



64 **67** **68**

Scheme 30: Reagents and conditions: $Cu(OAc)_2.H_2O$, piperidine, DCM, rt, 16 h, 14%

The use of copper mediated reactions for the coupling of acetylenes is widely reported and known to be robust even at room temperature (**Scheme 30**). Countless reactions have been reported where the diynes of interest were isolated in good yields. We therefore tested the reaction on **64** with phenylacetylene **67** in the presence of copper acetate monohydrate and piperidine at room temperature. Upon completion of the coupling reaction, the reaction mixture was then purified by column chromatography followed by preparative TLC.

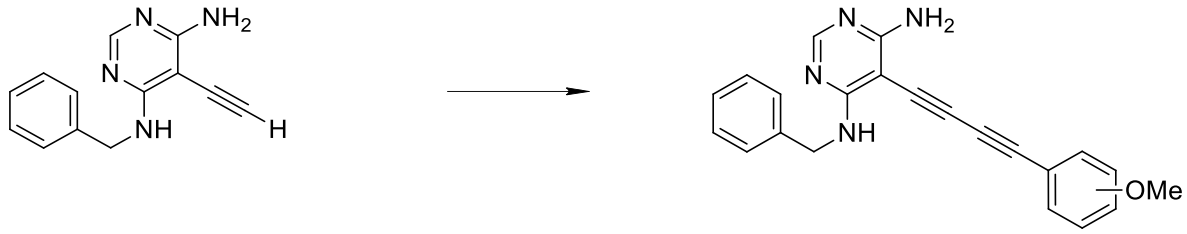
The ^{1}H NMR spectrum of **68** was simple, exhibiting six signals. A singlet due to H2 at 8.2 ppm, a multiplet region at 7.3- 7.5 ppm due to the 10 aromatic protons, a triplet due to the NH group close to the benzylic CH_2, and finally a broad singlet at 5.4 ppm due to NH_2 and a doublet at 4.4 ppm due to the benzylic CH_2. In the ^{13}C NMR spectrum, key features were easily identified such as the benzylic CH_2 carbon which appeared as a signal at 44.9 ppm. The presence of peaks which were observed previously include, those due to the pyrimidine carbons: 164.1 ppm due to C6, 163.1 ppm due to C4 and 157.1 ppm owing to the presence of C2. An interesting feature of this ^{13}C NMR spectrum was the region between 86.6 ppm and 72.6 ppm which was commonly known for the appearance of acetylene carbons. As expected 5 signals were observed in this region with the fifth carbon appearing owing to the pyrimidine C5. This carbon

is known to exhibit varying chemical shifts owing to how it is functionalized. In this specific case C5 was appearing in the same region as the other acetylene carbons at 79.4 ppm where it was previously observed at 59.6 ppm in the carbon NMR spectrum of **64**.

The use of other NMR spectroscopic experiments was helpful in the identification of the acetylene peaks as accurately as possible. From the combined information obtained from the HSQC NMR spectrum and HMBC NMR spectrum we were able to confirm the identities of these carbons. Due to the proximity of C1" to the pyrimidine carbons and the closeness of C4" to C6" we were able to successfully deduce which carbons were responsible for which signals. The carbon identified as C1" was assigned to the peak at 72.6 ppm and C4" was found to appear as a signal at 85.4 ppm. We could only postulate that C2" gave rise to the signal at a chemical shift of 73.4 ppm and C3" at 86.6 ppm due to the arrangement of the carbons. The aromatic carbons which were present previously could be deduced from the information collected from previous spectra. From these previous spectra it was apparent that C3' accounted for the signal at 127.5 ppm and C4' for the signal at 128.5 ppm. It was also deduced that C2' was appearing as a signal at 138.6 ppm as it had been seen previously. C5' was appearing as a signal at 127.5 ppm and C8" at 129.4 ppm. C6" and C7" gave rise to signals appearing at chemical shifts of 132.3 ppm and 128.7 ppm, respectively. The identity of compound **68** was further confirmed by high resolution mass spectrometry which found the mass of 355.4091 *m/z* which was expected for a compound of formula $C_{22}H_{10}N_6$.

With this success at attaining the desired product, we sought to expand the library of rigid pyrimidine analogues. This was useful because these compounds could be converted into flexible pyrimidine analogues which could potentially exhibit anti-DHFR activity by hydrogenation of the triple bonds. The variable acetylenes which were used for the purposes of expanding the library of compounds were limited to commercially available substrates which had methoxy, chloro and fluoro groups in the two, three and four positions and only one di-substituted compound was synthesized.

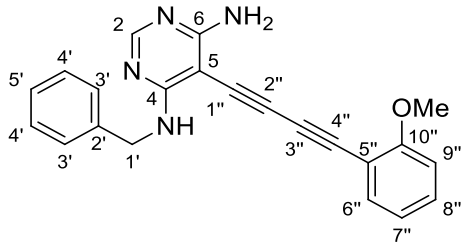
1.5.2 Synthesis of methoxy-substituted rigid pyrimidine analogues



64 **69**

Scheme 31: Reaction conditions: 2-, 3-, 4-ethynylanisole, $Cu(OAc)_2.H_2O$, piperidine, DCM, rt, ~14 h

1.5.2.1 Synthesis of N^4-benzyl-5-((2-methoxyphenyl)buta-1,3-diyn-1-yl)pyrimidine-4,6-diaimine (69a)



6**9a**

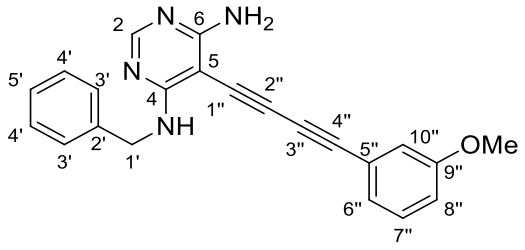
Figure 30: N^4-benzyl-5-((2-methoxyphenyl)buta-1,3-diyn-1-yl)pyrimidine-4,6-diaimine

Following the same procedure as stipulated for **68**, **69a** was synthesized. In DCM the 2-ethynylanisole, **64**, piperidine and $Cu(OAc)_2.H_2O$ were stirred overnight to afford **69a** in 22% yield. The product was purified and then characterized by NMR spectroscopy.

In the ^1H NMR spectrum of **69a**, it was observed that there were more signals in the aromatic region as through this reaction we were introducing unsymmetrical aromatic acetylene. Additionally, the appearance of a sharp singlet at 3.7 ppm owing to the OMe group present on the aromatic acetylene was observed. Despite this information, the absence of protons on the diyne functionality made it impossible to verify the presence of the triple bonds. From the ^{13}C NMR spectrum the presence of the diyne functionality became more apparent. At 72. 7 ppm to 86.9 ppm 5 signals were apparent, the first acetylene carbon identified was C4" which gave rise to a signal at a chemical shift of 81.9 ppm. C3" was then observed as a peak at 86.7 ppm, C2" at 73.2 ppm, C1" at 60.40 ppm and C5 which appears as a signal at 79.51 ppm. These peaks provide insight into the coupled part of the compound implicating a successful synthesis

of the desired compound. Additionally, the presence of the carbon signal owing to the OMe group was visible at 55.3 ppm, and the downfield shift of C10" of the signal due to 161.2 ppm, which is commonly observed in aromatic carbons that have a methoxy group attached, was visible.

1.5.2.2 Synthesis of N^4-benzyl-5-((3-methoxyphenyl)buta-1,3-diyn-1-yl)pyrimidine-4,6-diaimine (69b)

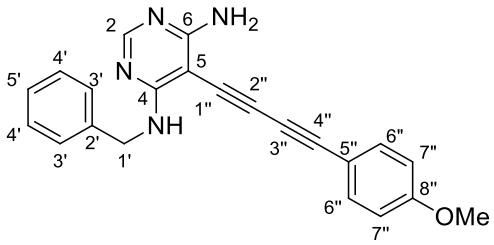


59b

Figure 31: N^4-benzyl-5-((3-methoxyphenyl)buta-1,3-diyn-1-yl)pyrimidine-4,6-diaimine

Following suite, **69b** was successfully synthesized in 13% yield and characterized allowing for the observation of the key features. The acetylenic carbons were the first key feature that we sought to identify by carbon NMR spectroscopy after the previous compound which we had made, as these were a direct indicator of the successful coupling of the acetylenes. From the ^{13}C NMR spectrum, 5 signals were observed between 72. 7 ppm and 86.5 ppm, 4 of which were acetylenic and the fifth signal appearing due to C5 at 79.3 ppm. With this information we sought to further confirm the presence of other key components. The methoxy carbon appeared as a signal at 55.3 ppm, accompanied by a singlet at 3.8 ppm in the ^{1}H NMR spectrum as seen for the previous analogue, and the C10" signal at 159.3 ppm which indicates the successful synthesis of **69b**. The rest of the NMR spectroscopic data was consistent with that of the other analogues. The HRMS data also showed a major peak of 355.1554 *m/z*, confirming the synthesis of the desired compound.

1.5.2.3 Synthesis of N^4-benzyl-5-((4-methoxyphenyl)buta-1,3-diyn-1-yl)pyrimidine-4,6-diaimine (69c)



69c

Figure 32: *N*[4]-benzyl-5-((4-methoxyphenyl)buta-1,3-diyn-1-yl)pyrimidine-4,6-diaimine

Likewise, the synthesis of **69c** was carried out by acetylenic aerobic copper mediated coupling and was isolated in 13% yield. The ^{1}H NMR and ^{13}C NMR spectroscopic data agreed with the predicted structure of the desired compound. From the ^{1}H NMR spectrum two doublets at 7.5 ppm and 6.8 ppm were apparent due to the *para*-substituted nature of the phenylacetylene (**Table 5**). Furthermore, the presence of the acetylenic carbons was confirmed from the ^{13}C NMR spectroscopic data. These carbons were observed at 72.7 ppm, 73.2 ppm, 85.3 ppm and 86.8 ppm alongside the C5 which appeared as a signal is at 79.6 ppm. The HRMS data confirms the mass of the product as expected 355.2013 *m/z* as predicted.

Three analogues were synthesized with the methoxy substituent on the phenylacetylene. The structures were confirmed by NMR spectroscopy and by HRMS. The HRMS data coincided with those predicted. ^{1}H NMR spectra of the analogues are similar and differ only in the aromatic region of the spectrum. The ^{13}C NMR spectroscopic data (**Table 6**) of all 3 analogues exhibit the same pattern of signals with slight disparities in the chemical shifts of the signals due to acetylenic carbons, C5, C-OMe and the methoxy carbon. Although similar, the compound exhibit vastly different melting points which would be an indicator of the effect of the position of the substituent on crystal packing and intermolecular interactions.

Table 5: Summary of the major ^{1}H NMR spectroscopic data of **69a-c**.

Compound	Aromatics (ppm)	OMe (ppm)
69a	6.9, 7.1, 7.2& 7.3	3.7
69b	6.8 & 7.5	3.8

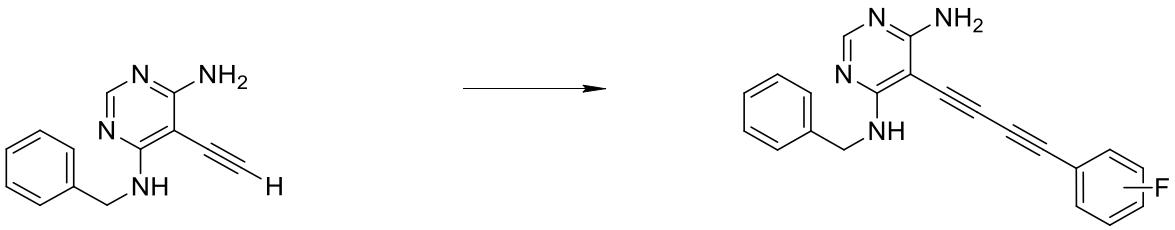
69c	6.8 &7.5	3.8

Table 6: Summary of the major ^{13}C NMR spectroscopic data of **69a-c**.

Compound	O-CH_3 (ppm)	C-OMe (ppm)	Acetylene carbons (ppm)
69a	55.3	159.3	72.7,73.2, 85.3 & 86.5
69b	55.9	161.2	73.2,81.9 & 86.7
69c	55.4	160.6	72.1, 72.3, 85.6 &86.8

1.5.3 Synthesis of fluoro-substituted rigid pyrimidine analogues

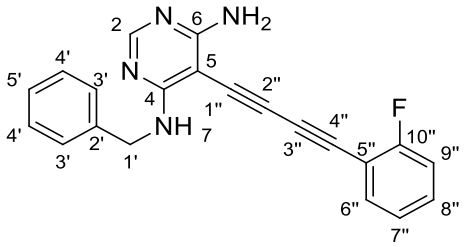
In addition to the methoxy substituted diynes which were synthesized, fluoro substituted analogues were also synthesized. Based on the method used in the synthesis of **68** the library of compounds was extended. Apart from the affordability of the fluoro substituted phenylacetylenes used in this synthesis, the halogen could influence the biological activity of the product. The possibility of synthesizing compounds which could exhibit interesting properties against the parasite made the synthesis of these variants pertinent to the completion of this series of compounds.



64 **70**

Scheme 32: Reaction conditions: 1-ethynyl -2-, 3-, 4- fluorobenzene, $Cu(OAc)_2.H_2O$, piperidine, DCM, rt, ~14 h

1.5.3.1 Synthesis of N^4-benzyl-5-((2-fluorophenyl)buta-1,3-diyn-1-yl)pyrimidine-4,6-diamine (70a)



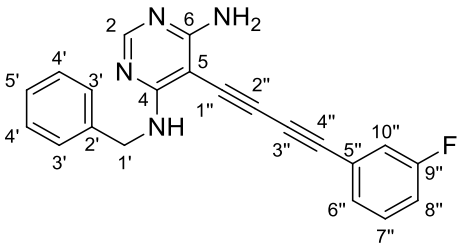
70a

Figure 33: N^4-benzyl-5-((2-fluorophenyl)buta-1,3-diyn-1-yl)pyrimidine-4,6-diamine

The preparation of **70a** was achieved by the reaction of 2-fluoro phenylacetylene, **64**, piperidine and $Cu(OAc)_2.H_2O$ in DCM under aerobic conditions at room temperature. The product was isolated by column chromatography as a pure bright orange solid in 20% yield and then characterized by NMR spectroscopy.

From the ^1H NMR spectrum, the presence of the aromatic protons due to the phenylacetylene was confirmed by the increase in the number of protons the signal integrated for compared to that of the starting material. In addition, the other features such as the benzylic CH_2, NH, NH_2, H2 and the benzyl aromatics gave rise to similar signals as observed previously. As previously observed the ^1H NMR spectrum does not give much information about the acetylenic functionalities since these possess no protons. As such, the ^{13}C NMR spectrum was used to confirm the presence of these carbons which would show the successful coupling of the acetylenes. The acetylenic carbons were observed as 4 signals from 74.0 ppm to 86.3 ppm as expected, with the fifth signal appearing due to C5 as observed for the previous compounds discussed. An additional interesting feature of the ^{13}C NMR spectrum was the fluorine coupling evident in the aromatic carbon signals, appearing as doublets. The carbons affected by this splitting effect were anticipated to be those in closest proximity to C10, which also appeared as a doublet with a coupling constant of 253.4 Hz, characteristic of a carbon directly attached to the fluoro-group in the case of **70a**. The signals at 110.5 and 115.8 ppm displayed fluorine coupling, and these have been identified as C5" and C9" respectively both appearing ortho to the fluoro group. The HRMS data further confirms the identity of **70a** where a mass of 343,1351was found and matched the calculated mass.

1.5.3.2 Synthesis of N^4-benzyl-5-((3-fluorophenyl)buta-1,3-diyn-1-yl)pyrimidine-4,6-diamine (70b)

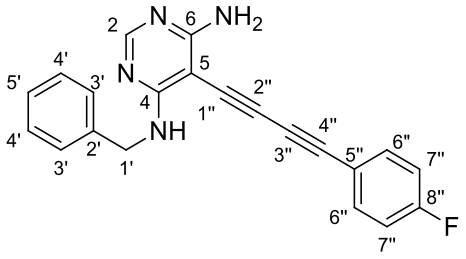


70b

Figure 34: N^4-benzyl-5-((3-fluorophenyl)buta-1,3-diyn-1-yl)pyrimidine-4,6-diamine

The synthesis of our second fluorinated analogue, **70b** was successful. The product was isolated in 16% yield. The H NMR spectrum of **70b** shows an increase in the number of protons in the aromatic region as expected. The ^{13}C NMR spectrum confirms the successful coupling of the acetylenes because of signals due to the acetylenic carbons observed along with C5 from 73.5 ppm to 86.3 ppm. The presence of fluorine coupling is also evident as 2 signals appear as doublets at 117.0 ppm and 119.1 ppm due to C10" and C8" respectively. Furthermore, C9" which is directly attached to the fluoro-group was found with a coupling constant of 247.6 Hz, consistent with the value observed for **70a** and thus confirms that the **70b** was synthesized successfully. The melting point of this analogue from found between 145-147 °C, which was the lowest amongst the fluoro-analogues **(70a-70c**), which could be largely owing to the position of the fluoro-group on the aromatic ring, which could contribute to the strength or the weakening of the intermolecular forces that affect the melting point.

1.5.3.3 Synthesis of N^4-benzyl-5-((4-fluorophenyl)buta-1,3-diyn-1-yl)pyrimidine-4,6-diamine (70c)



70c

Figure 35: *N*[4]-benzyl-5-((4-fluorophenyl)buta-1,3-diyn-1-yl)pyrimidine-4,6-diamine

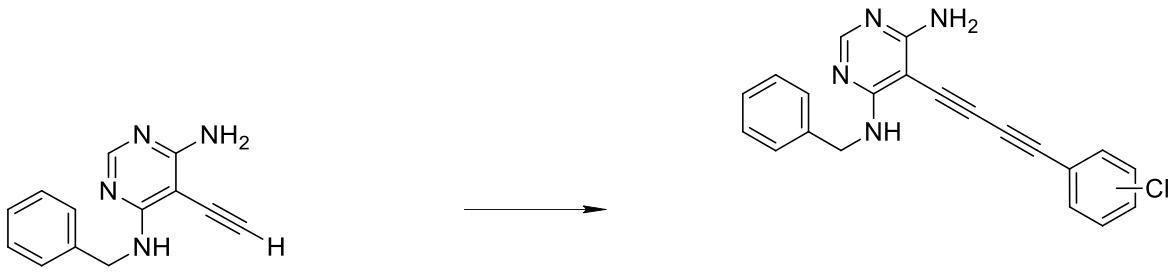
The [1]H NMR spectrum of **70c** was found to be consistent to those of similar analogues but still did not present enough information to conclude the structure if the product. Similar to other analogues **70c** was isolated in 16% yield. With this information at hand further characterization was obtained from the [13]C NMR spectrum. The presence of signals due to the acetylenic carbons confirms the successful coupling of the acetylenes. These acetylenic carbons were observed between 72.7 ppm and 86.4 ppm with C5 appearing at 77.8 ppm. In this instance C2" and C1" appear as a single peak due to unknown reasons, this phenomenon where two signals near each other end up merging has been observed in other substrates. Of great importance was the observation of fluoro coupling which was present even in the [13]C NMR spectrum of **70c.** C8" (carbon directly attached to fluoro group) had a coupling constant of 286.4 Hz (**Table 7**). From the IR data, it was evident that the carbon triple bond stretching, which is an important feature in the identification of the diynes, was present within the same wavenumber region (2200- 2100) for all three analogues.

Table 7: summary of compound defining peaks

Compound	Fluorine coupling (ppm)	C-F (ppm)	Acetylene carbons (ppm)
70a	117.0 & 119.1	130.2	73.5,74.3, 84.0 & 86.3
70b	115.9 & 116.2	161.2	73.2,81.9,86.7& 85.2
70c	115.8 & 110.5	134.0	74.0,78.1, 79.1 & 86.3

1.5.4 Synthesis of chloro-substituted rigid pyrimidine analogues

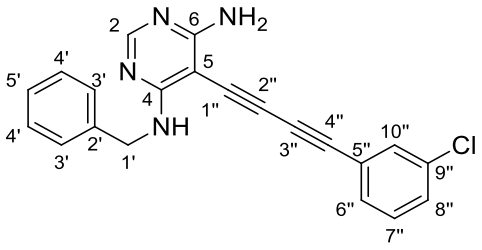
With the intentions of extending the library of 1,3-diynes, analogues with chloro substituents were synthesized. In keeping with the commonly utilized halogens and the aspect of affordability the following 1,3-diynes (**71a-c**) were synthesized and characterized. Compound with aromatic chloro groups have been found to be useful in the treatment of several diseases including malaria, this group has can confer interesting properties to the compound it is introduced to. For this set of compounds, the 3-chloro, 4-chloro and 3,4-dichloro phenylacetylenes were used to prepare our unsymmetrical 1,3-diynes.



64 **71**

Scheme 33: Reaction conditions: 1-ethynyl -3-, 4-, 3.4-chlorobenzene, $Cu(OAc)_2.H_2O$, piperidine, DCM, rt, ~14 h

1.5.4.1 Synthesis of N^4-benzyl-5-((3-chlorophenyl)buta-1, 3-diyn-1-yl)pyrimidine-4,6-diamine (71a)



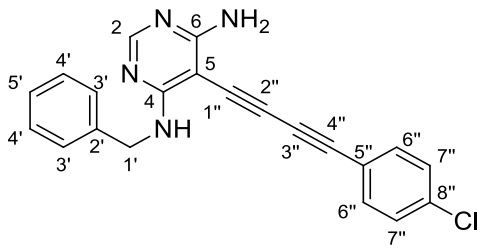
71a

Figure 36: N^4-benzyl-5-((3-chlorophenyl)buta-1, 3-diyn-1-yl)pyrimidine-4,6-diamine

In DCM 55a, 3-chloro phenylacetylene, piperidine and $Cu(OAc)_2.H_2O$ were stirred together overnight at room temperature to achieve the synthesis of 55a. this mixture was purified by column chromatography in 18% yield, followed by further purification by preparative TLC. The product was then characterized by NMR spectroscopy.

The ^{1}H NMR spectrum of **71a** exhibited the H1 singlet at 8.15 ppm, NH singlet at 5.67 ppm, a broad singlet at 5.37 due to the NH_2 and the benzylic CH_2 singlet at 4.70 ppm. In addition to these resident features a new singlet due to H10'' was observed at 7.48 ppm with the rest of the new peaks appearing as a part of the aromatic multiplets. Further confirmation of the structure of 56a was achieved through the assessment of the ^{13}C NMR spectrum. The main features of interest in this spectrum were the acetylenic carbons which appeared as signals between 73.6 ppm and 86.3 ppm including a signal for C5 as expected. Another key feature of the spectrum which confirms the synthesis of our desired product is the aromatic region with signals other than that of the starting material with C9" appearing at 134.4 ppm, C6" at 132.0 ppm, C7" 130.4 ppm, C8"/C10" at 129.8 ppm and C5" at 123.3 ppm. Additionally, the mass of the product was confirmed as 358.1056 *m/z.*

1.5.4.2 Synthesis of N^4-benzyl-5-((4-chlorophenyl)buta-1,3-diyn-1-yl)pyrimidine-4,6-diamine (71b)



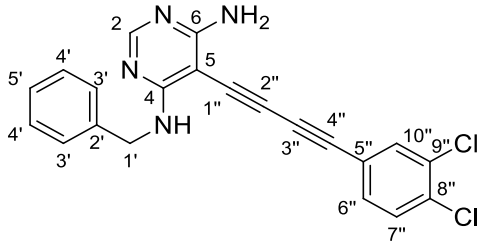
71b

Figure 37: N^4-benzyl-5-((4-chlorophenyl)buta-1,3-diyn-1-yl)pyrimidine-4,6-diamine

With the successful synthesis of **71a**, the same procedure was followed to achieve the synthesis of 55b. 4-chloro phenylacetylene was used in the reaction to afford **71b** in 22% yield. The product was purified and characterized by NMR spectroscopy.

From the ^{1}H NMR spectrum the doublet signals due to H6" and H7" were anticipated but only one doublet was observed and the second was assumed to be overlapping with the benzyl aromatic protons as this signal integrated for 7H. The aromatic region accounted for the correct number of protons and further characterization of the compound was confirmed by ^{13}C NMR spectroscopy. From the ^{13}C NMR spectroscopic data, the presence of the acetylenic carbons were confirmed by the presence of the carbon signals from 73.3 ppm to 86.3 ppm and the C5 carbon which appeared as a signal at 79.2 ppm. The carbon attached to the halogen (C8") also appeared downfield at 135.6 ppm as anticipated. The data from the HRMS confirms the mass of the product as 359.1 *m/z* as predicted.

1.5.4.3 Synthesis of N^4-benzyl-5-((3,4-chlorophenyl)buta-1,3-diyn-1-yl)pyrimidine-4,6-diamine (71c)



71c

Figure 38: N^4-benzyl-5-((3,4-chlorophenyl)buta-1,3-diyn-1-yl)pyrimidine-4,6-diamine

Finally, we were able to synthesize this analogue **71c** using a 3,4-disubstituted-chloro-phenylacetylene. Following the same procedure as stated for the other analogues **71c** was successfully synthesized and isolated in 20% yield as a yellow solid.

From the ^{1}H NMR spectroscopic data it was clear from the aromatic region which integrated for 8 protons in total excluding H2. H3'-H5' appeared as a multiplet at 7.36 ppm, accompanied by a doublet due to H6" at 7.41 ppm, H7" appeared as a doublet in the same region at 7.31 ppm and H10" appeared as a singlet at 7.57 ppm. The other proton signals were consistent with previously stated spectroscopic data. ^{13}C NMR spectrum confirms the presence of the acetylenic carbons C1" to C4" which appeared between 74.1 ppm and 86.1 ppm. Furthermore, C8" and C9" appeared at 134. 0 ppm and 132.9 ppm, respectively, the chemical shift of C8" (120.0 ppm) in **71b** being vastly different from that of C8" in **71c**. This could possibly be because of the presence of the chloro group on C9" in **71c**, having a greater deshielding effect on the adjacent carbons.

The HRMS confirmed the synthesis of a compound with a mass of 393.0667 m/z which corresponds with the mass of compound with a molecular formula of $C_{21}H_{14}N_4{}^{35}Cl_2$. Of the 3 chloro substituted analogues, **71c** exhibited the lowest melting point which is interesting because of its di-substituted nature.

Table 8: Summary of the major ^{13}C NMR spectrum characteristic peak

Compound	C-Cl (ppm)	Acetylene carbons (ppm)
61a	134.4	73.6,74.5, 83.8 & 86.3

61b	135.6	73.3,74.3, 84.2 & 86.3
61c	134.0 & 132.9	74.1,75.2, 82.9 & 86.1

The copper mediated reaction was optimized to give the unsymmetrical 1,3-diynes a-c at room temperature. The products were isolated in low yields due to formation of a side product which occurred with a very similar R_f value to that of the desired product. On TLC 3 spots were visualized and characterized. The top spot was observed as the substituted phenylacetylene in all instances. The second spot was a mixture of products consisting of the product and an unknown side product which we would like to suggest was responsible for the low yielding nature of the reaction. We suspected that there might have been some homo-coupling which was occurring, but the NMR spectroscopic data showed otherwise and nullified the assumption. To this end the identity of this spot is unknown and attempts towards the identification thereof should be considered to get a holistic understanding of the type of reactions which are furnished by these conditions used.

Moreover, the purification of the compounds of interest proved to be tedious since this required both the use of column chromatography and preparative TLC in low polarity solvent combinations (20% EtOAc/hexane). Nonetheless, enough of the product was isolated to carry out the necessary characterization. Additionally, the low yields of this compound could easily be owing to the extensive purification undertaken to achieve good purity of the targets.

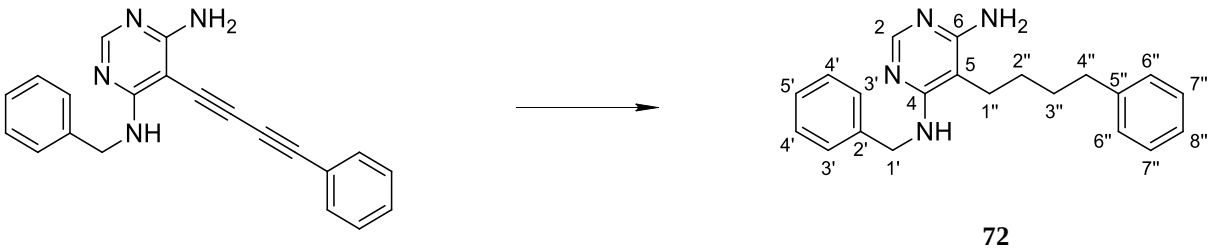
Attempts were made to achieve the synthesis of the diynes using palladium chemistry, but this was unsuccessful. The conditions used for this reaction were the same as those seen for the Sonogashira reaction. The products isolated from this reaction yielded no useful information into what was being formed under these conditions and as such we abandoned this approach.

Despite the successful synthesis of the target compounds, much work remains necessary to help understand the conditions used to achieve the coupling of the two starting materials and whether the nature of the pyrimidine intermediate could be affecting the output of the reaction. It is fair to assume that the pyrimidine core and how it's functionalized heavily affects the success of the reaction. Considering that not much research has been done with these types of compounds, these reactions are often done on normal aromatic acetylenes, seldom the case of pyrimidines. Exploration of other solvents could serve the greater good of this step and the general variation of other aspects of the reaction conditions. For example, the synthesis of the

68 required the use of excess amounts of the phenylacetylene but with repeated attempts of the reaction it was observed that the reaction still proceeds to give the desired product even with lower stoichiometric amounts of the phenylacetylene, thus making it important to further investigate the reaction conditions.

1.6 Synthesis of N^4-benzyl-5-(4-phenylbutyl)pyrimidine-4,6-diamine (72)

With the success observed towards the synthesis of the diynes discussed above, we sought to demonstrate how the further functionalization of these compounds is possible in the event of more flexibility being necessary. The usefulness of the flexible heterocyclic analogues against malaria synthesized in our labs dictate the development of a method which grants access to the flexible pyrimidine analogues from the rigid unsymmetrical 1,3-diyne compounds. Since **68** was used as the test reaction, it made perfect sense to attempt the reduction of the diynes to the complete aliphatic chain as seen in **30** to **32** using methods which have been used in synthesis and have been shown to be efficient. The application of the conditions used in the hydrogenation reaction for the conversion of the nitrile to the aldehyde was attempted for the reduction of the triple bonds found in the diynes.



68 **72**

Scheme 34: Reaction conditions: Pd/C, H_2 (g) balloon, MeOH, rt, 18 h, 29%

In methanol, **68** and Pd/C were stirred together under a nitrogen atmosphere for 18 hours. When the reaction was established as complete the Pd/C was filtered off and the methanol was removed *in vacuo,* leaving behind crude solid which was further purified by preparative TLC. The pure product was then characterized by NMR spectroscopy.

From the ^{1}H NMR spectrum, protons due to the CH_2 groups in the aliphatic linker chain provided prominent characteristic signals which were not present in the precursor **68**. It was

anticipated that the change from **58** to **72** would result in the observation of four new signals in the upfield region of the proton spectrum and such was the case of the pattern observed in the ^{1}H NMR spectrum of **72**. The protons represented as H3" and H2" were anticipated to exhibit multiplets due to the splitting that was expected to take place due to the protons found on either of the adjacent carbons. H1" and H4" were anticipated to exhibit triplets, due to the CH_2 groups on the inner part of the 4-atom linker. The exact position of the protons was confirmed using other 2D NMR spectroscopic experiments and we were able to confirm that H2" and H3" were appearing as multiplets at 1.5 ppm and 1.7 ppm, respectively. While H1" and H4" were found to afford multiplets at 2.2 ppm and 2.6 ppm, respectively. The rest of the spectrum remained largely unchanged as anticipated.

From the ^{13}C NMR spectrum, an upfield shift by the carbons C1"-C4" were indicative of the successful conversion of the diyne to the corresponding phenylbutyl pyrimidine. The carbon signals due to C1" to C4" moved from 70-86 ppm to 24-36 ppm. C1" and C4" were identified as peaks at 24.0 ppm and 35.5 ppm, respectively, and the signals due to C2" and C3" were observed at 26.1 ppm and 31.1 ppm, respectively. The disparity in chemical shift between C1" and C4" may be owing to the nature of the groups attached to these carbons. C4" is attached to aromatic carbons which is electron rich, imposing this property on C4" subsequently affecting the chemical shift of C4", whereas C1" is attached to the more electron rich pyrimidine ring which could be transposing some electron density on C1" providing a more shielding effect on the carbon.

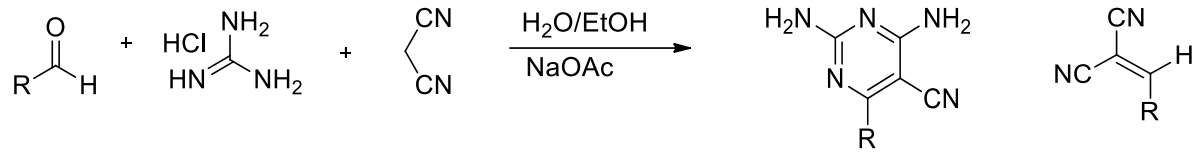
1.7 Biological Testing

A library of 10 rigid analogues of the *N*^{4}-benzyl-5-((phenyl)buta-1,3-diyn-1-yl)pyrimidine-4,6-diamine which we sought to further functionalize to the equivalent flexible analogues were prepared. Although we had intended on testing these compounds in a whole *P. falciparum* assay, were unable to due to time constraints. As a result, the biological testing of these compound will form an integral part of the future work associated with this project.

Chapter 3

3.1 Conclusions and Future work

In conclusion, we were successful in the synthesis of the flexible pyrimidine analogues as potential DHFR inhibitors, but we were met with some limitations. The 3-step synthetic route was followed as originally proposed and each step optimized as intended except for the reductive amination step. This continues to be a work in progress since we were unable to determine which set of conditions would yield the purest form of the desired product. The multicomponent reaction step was confirmed as robust yielding the same outcome with each attempt (**Scheme 35**). The issue of the reaction conditions favoring the alkylidene intermediate **26** remains and more work needs to be done to achieve a higher yield of the desired 2,4-diaminopyrimidine carbonitrile **21**.

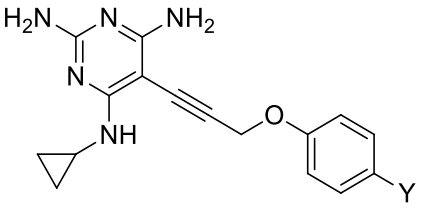


18 **19** **20** **21** **26**

Scheme 35: Multi-component reaction towards the synthesis of 6-subtituted 2,4-diamino-pyrimidinecarbonitrile and alkylidene

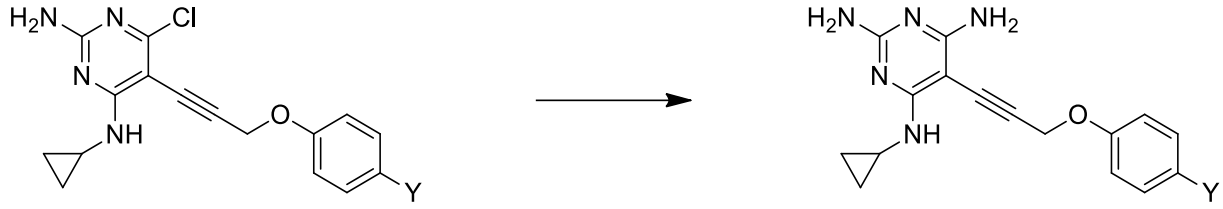
With regards to the hydrogenation step, the desired product was isolated in each case, but the issue of low yields was still in effect even at the end of this project. The outcome of the reductive amination step remains an issue, as we were unable to confidently determine what was causing such a high level of impurity with the compounds which we sought to prepare. From the library of precursor compounds which we synthesized we reported the synthesis of only two analogues as these compounds proved to be difficult to work with. Despite our various attempts to purify the compounds by column chromatography, preparatory TLC, recrystallisation, washing with various solvents and even varying reaction conditions with the hopes of accessing purer compounds we were unable to consolidate the purity of more analogues. More attempts have been recently made to improve the purity of the analogues using semi-preparative HPLC and we hope to accomplish better purity for the remaining compounds. Due to the limitations encountered in this project we then sought to find a different approach towards accessing compounds with a similar scaffold, maintaining the pyrimidine core and the 4-atom linker. This then led to the second part of this project where we explored a different approach.

In the second part of the project we intended on expanding the scope of the project by using a Sonogashira coupling reaction to introduce the flexible side chain. This afforded a set of rigid intermediates which could be subjected to hydrogenation. The original expansion of the project envisioned the synthesis of **34** but due to our inability to access **34** (**Figure 39**) we revised our synthetic route. By switching the order of the synthetic steps, we envisioned accessing **49** **(Figure 40)** with the hopes of converting it to **34** as we had originally planned. But to our misfortune, these attempts failed.



34

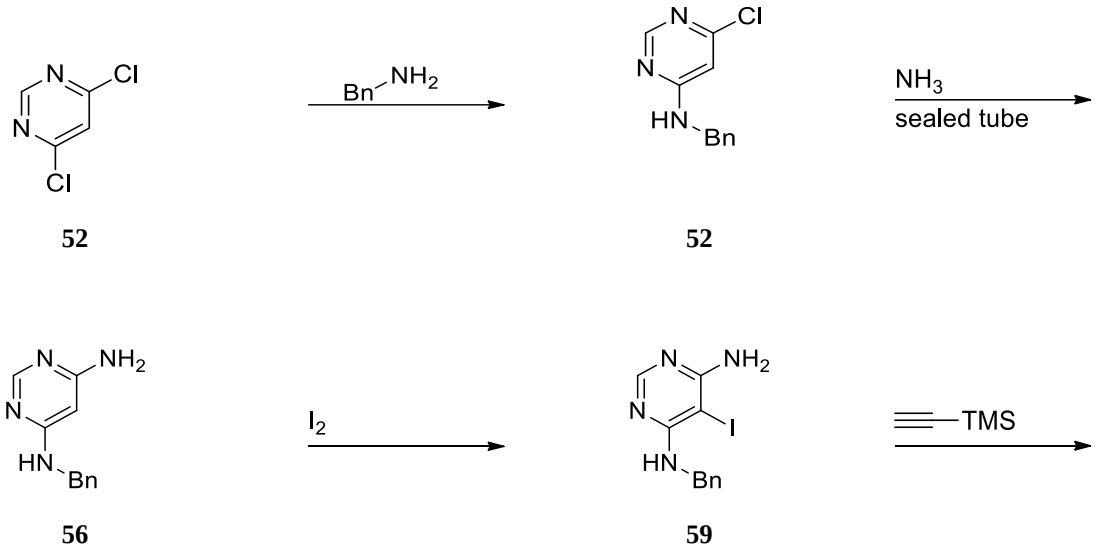
Figure 39: *N*[4]-cyclopropylpyrimidine-2,4,6-triamine



49 **34**

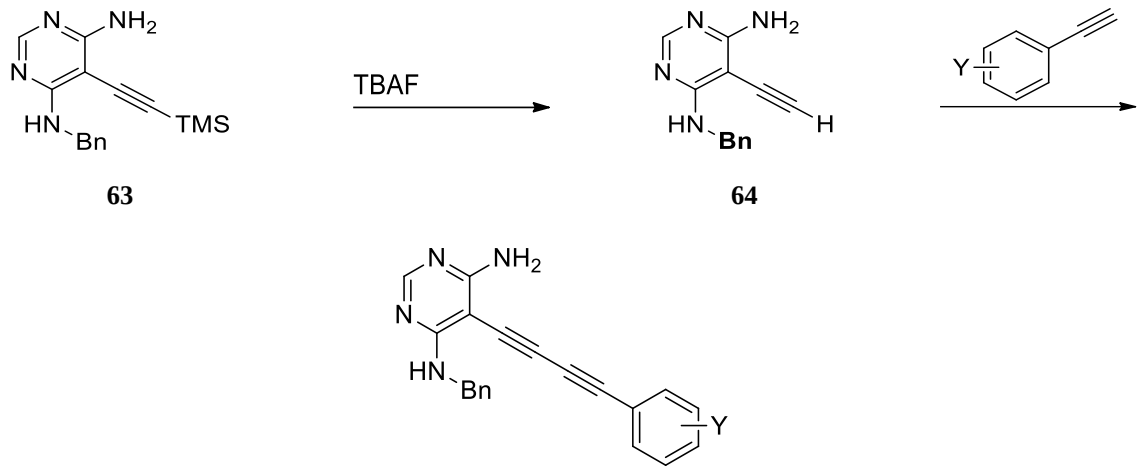
Figure 40: Conversion of chloro-diamine to the equivalent triamine

Finally, in this work we chose to synthesise a series of compounds with reduced functionality such as 65, omitting one of the amino substituents (**Scheme 36**).



52 **52**

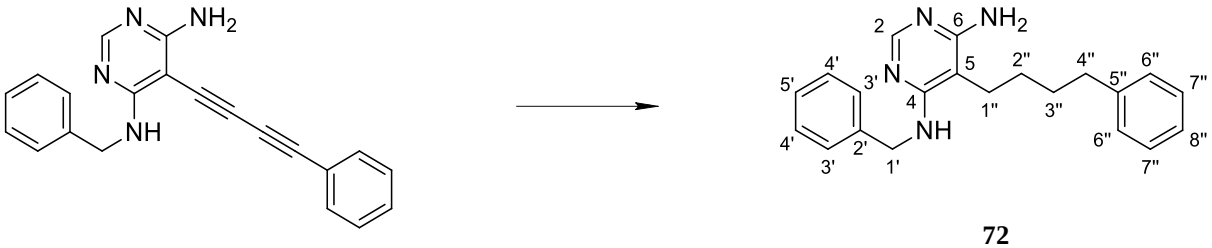
56 **59**



63 **64** **65**

Scheme 36: General synthetic route towards the synthesis of substituted 1,3-diynes

The synthesis of analogues of **65** began with the reaction of the commercially available 4,6-dichloropyrimidine with various amines in *n*-BuOH, in the presence Hunigs base which gave the products **53-55** in yields between 85%-97%. The resulting products (**53-55**) were then converted by ammonolysis in a sealed tube reaction into the diaminopyrimidine (**56-58**) in good yields (89%- 93%). The C5 position was further functionalized by iodination, a reaction which was performed in a water/DMF combination, the mixture quenched with sodium thiosulfate to give a yellow precipitate in poor yields (**59-61**). Due to the low yielding nature of the iodination step we could only continue the synthesis journey towards analogue of **65** using **59** only. The C5 position of **59** was then protected using trimethylsilyl acetylene following a Sonogashira procedure, which was followed by a deprotection step of the TMS acetylene using TBAF in THF to yield **63**. With this precursor at hand, we then executed a reaction where the resulting acetylene was coupled to a collection of commercially available phenylacetylenes, affording a series diynes in low yields (13%- 26%). We then sought to demonstrate that these rigid structures could be converted into more flexible compounds by reducing the triple bonds, since flexible pyrimidines had been found to exhibit potential as DHFR inhibitors. Due to time constraints, this conversion was demonstrated on only one of the diyne compounds, **68**, which was successfully converted from a diyne with a 4-carbon (butyne) linker between the 2 rings to a butane linker. This was achieved by hydrogenation using stoichiometric amounts of Pd/C, in an atmosphere of hydrogen, full conversion was observed. The library of diyne compounds consists of 10 analogues which have been taken for biological testing with the hopes of determining possible opportunities for application.



68 **72**

Scheme 37: Reaction conditions: Pd/C, H_2 (g) balloon, MeOH, rt, 18 h, 29%

For future work purposes to improve the synthesis of the library of diynes and to extend the collection, the currently existing library of diynes can be converted to the flexible equivalents with the hopes of comparing the biological activity of the rigid series versus the flexible series (**Scheme 37**). Furthermore, exploring methods of improving the yields of the acetylene coupling step would be significant as this reaction was found to yield other side products which were not appropriately characterized due to time constraints. Thus, further interrogation of the reaction conditions could be valuable with respect to improving the reaction yields. Additionally, from a cost perspective it would very valuable if a method towards the synthesis of the commercially available substituted phenylacetylenes was found considering how expensive these were found to be. Optimizing the reaction with other amines constituting aliphatic groups (like cyclohexyl, cyclopentyl, diethylamine) at the 4-position would also make for a good comparative basis, expanding the scope of the project even further allowing for a more well-rounded series of compounds.

CHAPTER 4:

4. EXPERIMENTAL PROCEDURE

4.1 General Procedures

4.1.1 Purification of solvent and reagents

Solvents used for reactions were used as purchased. Tetrahydrofuran and dichloromethane were distilled over sodium wire with benzophenone as an indicator and calcium hydride, respectively. Reagents obtained from commercial sources i.e.; Sigma Aldrich, were used with no further purification unless stated otherwise. The solvents used for extraction and chromatographic functions Ethyl acetate (EtOAc) and hexane was purified by distillation prior to utilization.

4.1.2 Chromatography

Purification of compounds was accomplished using Macherey-Nagel silica gel (particle size 0.063 mm to 0.200 mm) as the adsorbent. For flash chromatography, silica gel of particle size 0.040 mm to 0.070 mm was used. Mixtures of ethyl acetate and hexane were used as the mobile phase unless stated otherwise. Reported R_f values are for analytical thin layer chromatography (TLC) performed using aluminum backed Silica gel 60 F_{254}. TLC plate adsorbed compounds were viewed under UV light or visualized by staining in 2,4-dinitrophenylhydrazine (DNPH).

4.1.3 Spectroscopic and physical data

The Bruker AVANCE 300 spectrophotometer was used to record ^{1}H NMR and ^{13}C NMR spectra at 300.13 MHz and 75.47 MHz, respectively. The Bruker Ultrashield 500 Plus

spectrophotometer was used occasionally to record ^{1}H NMR (500.13 MHz) and ^{13}C NMR (125.76 MHz) spectra. Spectra were recorded in deuterated chloroform ($CDCl_3$), dimethyl sulfoxide (d_6-DMSO) or deuterated methanol (MeOD). The spectra were reported in parts per million relative to tetramethylsilane at 0.00 ppm for ^{1}H NMR spectra and the deuterated signals at 77.23 ppm for $CDCl_3$, 39.51 ppm for d_6-DMSO and 49.15 ppm for MeOD for ^{13}C NMR spectra. Coupling constants/ *J* values are given in Hertz (Hz).

Infra-red spectra were recorded on a Bruker Tensor-27 Fourier Transform spectrophotometer. Measurements were made by loading the sample directly onto a diamond cell. The signals are reported on the wavenumber scale (ν/cm^{-1}).

Melting points were recorded using the Stuart SMP10-4 Melting Point Apparatus and are uncorrected.

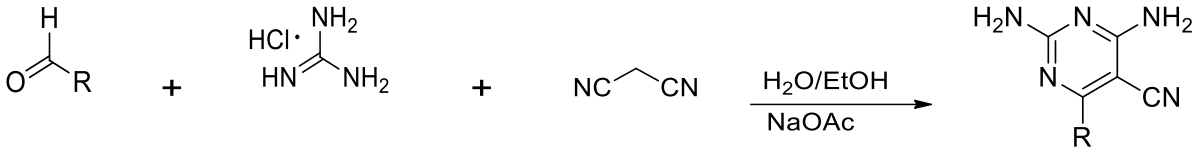
High resolution mass spectra were recorded at the University of the Witwatersrand in either El^+ or El^- mode on a Bruker Compact Q-TOF high resolution mass spectrophotometer.

4.1.4 Other general procedures

All reactions were performed under normal air or under a nitrogen atmosphere as specified. All glassware was used dry. The use of the term *in vacuo* refers to the removal of solvents using a rotary evaporator. Residual solvents were removed under high vacuum pressure (*ca* 0.1mmHg) at ambient temperature after the purification of compounds.

Part 1

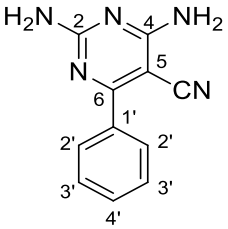
4.2 Preparation of 2,4-diaminopyrimidine-5-carbonitrile



Equimolar amounts of commercially available aldehydes, malononitrile and sodium acetate (NaOAc), were dissolved by stirring for approximately 10 minutes in a mixture of 60 ml water and 10 ml ethanol, before adding guanidine hydrochloride (1 mol eq) and stirring for 12-18 hours while refluxing. The reaction mixture was then allowed to cool to room temperature before the aqueous phase was extracted with an organic solvent. The aqueous layer was extracted 3 times with EtOAc (3 x 150 ml), and the combined organic layers were dried over $MgSO_4$ before filtering over a bed of celite layers. Upon evaporation of the solvent, the crude product was then purified by column chromatography (80% EtOAc/hexane). The following compounds were synthesized using this method:

4.2.1 Synthesis of 2,4-diamino-phenylpyrimidine-5-carbonitrile (25)[59,64]

25 was prepared from, benzaldehyde (5.00 ml, 48.5 mmol), malononitrile (3.40 g, 51.5 mmol), NaOAc (5.00 g, 61.0 mmol) and guanidine hydrochloride (5.60 g, 58.6 mmol) in ethanol/water (70 ml) as described. The product was isolated as an orange powder (2.50 g, 30%).



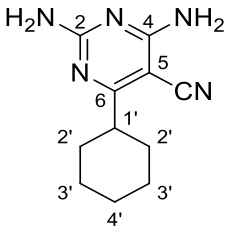
25

R_f (80% EtOAc/hexane) 0.47. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 7.86 – 7.67 (m, 2H, H2'), 7.50 (dt, *J* = 5.3, 2.7 Hz, 3H, H3' and H4'), 7.09 (d, *J* = 34.8 Hz, 4H, NH_2). **^{13}C NMR (126 MHz, DMSO-d_6)** δ 169.4 (C4), 165.0 (C2), 163.0 (C6), 137.1 (C1'), 130.2 (C4'), 128.1 (C2'), 128.1 (C3'), 117.9 (CN),

75.9 (C5). **IR (ν_{max}/cm^{-1}):** 2205 (CN); 3375, 3421 (NH_2), 3154 (=CH)

4.2.2 Synthesis of 2,4-diamino-6-cyclohexylpyrimidine-5-carbonitrile (28)[59,64]

In 60 ml H_2O/10 ml ethanol, cyclohexanecarbaldehyde (5.00 ml, 41.3 mmol), malononitrile (3.00 g, 45.4 mmol), NaOAc (3.50 g, 42.7 mmol) were stirred together for 10 minutes before adding guanidine hydrochloride (4.00 g, 41.9 mmol). The product was isolated as an orange powder (800 mg, 12%).

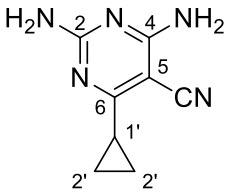


28

R_f (80% EtOAc/hexane) 0.46. **^{1}H NMR (300 MHz, DMSO-*d_6*)** δ 6.92 (s, 2H, NH_2), 6.80 (s, 2H, NH_2), 2.66 (tt, *J* = 11.6, 3.6 Hz, 1H, H1'), 1.85 – 1.43 (m, 7H, H2', H3' and H4'), 1.40 – 1.09 (m, 3H, H2', H3'and H4'). **^{13}C NMR (126 MHz, DMSO-*d_6*)** δ 178.2 (C4), 163.6 (C2), 163.1 (C6), 117.7 (CN), 76.6 (C5), 44.4 (C1'), 31.0 (C2'), 26.2 (C3'), 26.0 (C4'). **IR (ν_{max}/cm^{-1}):** 3445, 3390 (N-H); 2203 (CN); 1608 (C=C); 1438, 1270, 1014 (CH_2).

4.2.3 Synthesis of 2,4-diamino-6-cyclopropylpyrimidine-5-carbonitrile (29)[59,64]

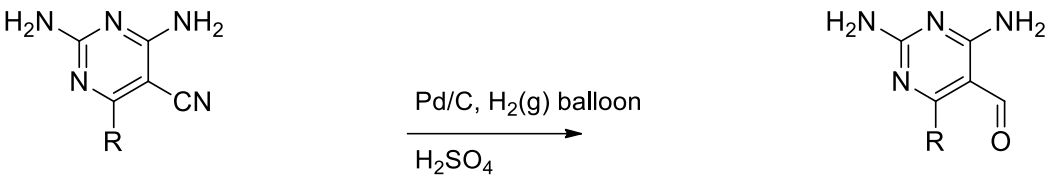
In 60 ml H_2O/10 ml ethanol, cyclopropane-carbaldehyde (3 ml, 40.2 mmol), malononitrile (3 g, 45.4 mmol), NaOAc (4 g, 48.8 mmol) were stirred together for 10 minutes before adding guanidine hydrochloride (5 g, 52.3 mmol). The product was isolated as an orange powder (810 mg, 22%).



29

R_f (80% EtOAc/hexane) 0.44. **^{1}H NMR (300 MHz, DMSO-*d*$_6$)** δ 6.70 (d, *J* = 24.0 Hz, 2H, NH_2), 6.51 (s, 2H, NH_2), 1.94 – 1.77 (m, 1H, H1'), 0.90 – 0.69 (m, 4H, (H2'). **^{13}C NMR (126 MHz, DMSO-*d*$_6$)** δ 174.7 (C4), 163.8 (C2), 163.1 (C6), 117.7 (CN), 77.0 (C5), 15.0 (C1'), 9.55 (C2'). **IR (ν_{max}/cm^{-1}):** 3498, 3426 (N-H); 2203 (C=N); 1615 (C=C); 1280, 1133 (CH_2)

4.3 Towards the synthesis of 2,4-diaminopyrimidine-5-carbaldehyde[64]

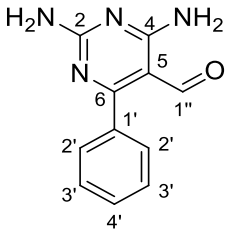


In a 2-necked round bottom flask, the carbonitrile (1 mol eq) was dissolved in 2M aq. H_2SO_4 (25 ml) before adding Pd/C (0.33 mol eq). The reaction vessel was then evacuated at least 3 times at 30 second intervals before introducing H_2 gas from a balloon each time. The reaction mixture was left to stir for 12 hours at room temperature while stirring under an atmosphere of hydrogen. Upon complete conversion of starting material (confirmed by TLC) the reaction was stopped, the Pd/C filtered removed by filtration, followed by the neutralization of the acid by addition of 2M NaOH (30-40 ml) until the pH was neutral. This neutral mixture was then

extracted with EtOAc (3 x 150 ml), the organic layers were combined and then dried over $MgSO_4$ before filtering over a bed of celite. The product was isolated as a pure product as a yellow solid in poor yields.

4.3.1 Synthesis of 2,4-diamino-6-phenylpyrimidine-5-carbaldehyde (30)[64]

In 30 ml of 2M aq. H_2SO_4, **25** (620 mg, 2.89 mmol) was dissolved and then stirred with Pd/C (200 mg) overnight under a hydrogen gas atmosphere. Upon completion, the product was isolated as a yellow solid (300 mg, 48%).

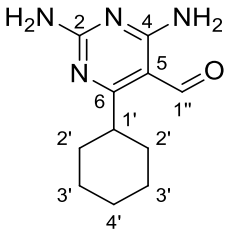


30

R_f (80% EtOAc/hexane) 0.42. **^{1}H NMR (300 MHz, DMSO-*d*$_6$)** δ 9.46 (s, 1H, H1''), 8.37 (s,1H, 1 of NH_2), 7.57 (s, 1H, 1 of NH_2) 7.54 – 7.38 (m, 5H, H2', H3', H4'), 7.31 – 7.05 (m, 2H, NH_2). **^{13}C NMR (126 MHz, DMSO-*d*$_6$)** δ 189.0 (C1''), 174.7 (C4), 164.0 (C2), 163.7 (C6), 137.2 (C1'), 130.0 (C4'), 129.8 (C3'), 128.5 (C2'), 103.4 (C5). **IR (ν_{max}/cm^{-1}):** 3431, 3314 (N-H); 1610 (C=C); 1546 (C=O).

4.3.2 Synthesis of 2, 4-diamino-6-cyclohexylpyrimidine-5-carbaldehyde (30)[64]

In 30 ml of 2M H_2SO_4, **28** (750 mg, 3.41 mmol) was dissolved and then stirred with Pd/C (300 mg) overnight under a hydrogen gas atmosphere. Upon completion, the product was isolated as a yellow solid (500 mg, 43%).

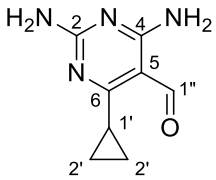


30

R_f (80% EtOAc/hexane) 0.41. **^{1}H NMR (300 MHz, DMSO-d_6)** δ 10.03 (s, 1H, H1''), 6.93 (s, 2H, NH_2), 6.79 (s, 2H, NH_2), 2.66 (tt, *J* = 11.5, 3.6 Hz, 1H, H1'), 1.86 – 1.43 (m, 7H, H2', H3', H4'), 1.43 – 1.05 (m, 3H, H2', H3', H4').**^{13}C NMR (75 MHz, DMSO-d_6)** δ 187.2 (C1''), 177.8 (C4), 164.3 (C2), 163.1 (C6), 76.1 (C5), 43.9 (C1'), 31.5 (C2'), 30.4 (C2'), 25.6 (C3'), 25.5(C4'). **IR (ν_{max}/cm^{-1}):** 1630 (C=O); 3465, 3408 (NH_2); (CH)

4.3.3 Synthesis of 2, 4-diamino-6-cyclopropylpyrimidine-5-carbaldehyde (31)[64]

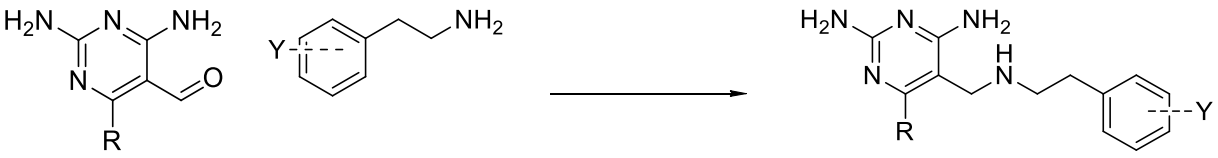
In 40 ml of 2M aq H_2SO_4, **29** (760 mg, 3.50mmol) was dissolved and then stirred with Pd/C (240 mg) overnight under a hydrogen gas atmosphere. Upon completion, the product was isolated as a yellow solid (440 mg, 57%).



31

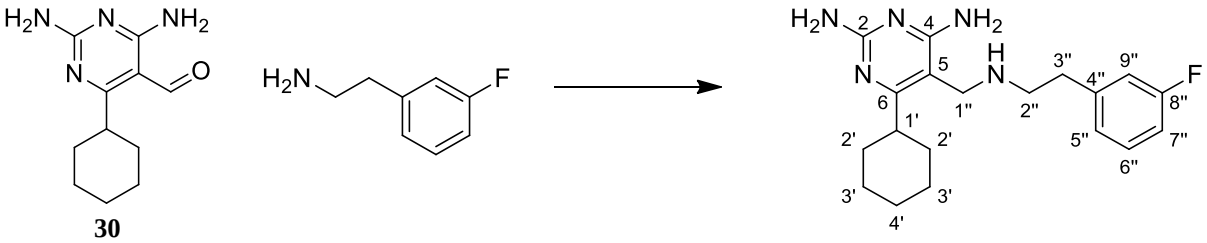
R_f (80% EtOAc/hexane) 0.4.**^{1}H NMR (500 MHz, DMSO-d_6) δ** 9.95 (s, 1H, CHO), 8.37 (s, 1H, 1 of NH_2), 7.38 (s, 1H, 1 of NH_2), 7.00 (d, *J* = 60.8 Hz, 2H, NH_2), 1.61 (h, *J* = 7.4 Hz, 1H, CH), 0.91 (t, *J* = 7.3 Hz, 4H, CH_2). **^{13}C NMR (126 MHz, DMSO-d_6) δ** 187.6 (C=O), 176.6 (C2), 163.4 (C4 & C6), 102.6 (C5), 34.9 (C1'), 22.7 (C2'), 13 .8 (C2'). **IR (ν_{max}/cm^{-1}):** 1630 (C=O); 3465, 3408 (NH_2)

4.4 General approach towards the Reductive Amination[64]



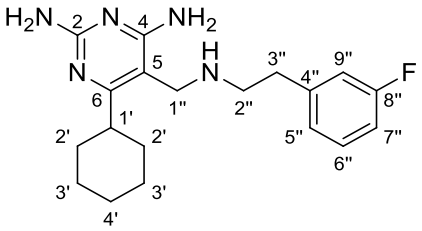
In a round bottom flask, carbaldehye (0.5 mmol) was dissolved in absolute ethanol (10 ml). Upon dissolution, glacial acetic acid (1 ml) was added and the mixture stirred for a short while before addition of substituted phenethylamine. The reaction was left to stir overnight while refluxing under nitrogen. The next day, the reaction mixture cooled to room temperature before adding $NaCNBH_3$ (6 mol eq) in portions. The reaction mixture was left to stir overnight while refluxing an inert atmosphere. Upon completion, the solvent was removed under reduced pressure and the residue diluted with water, followed by the extraction of the organics with EtOAc (2 x150 ml). The organic layer was then dried over $MgSO_4$ followed by filtration over a bed of celite. The solvent was removed *in vacuo* and the crude product was isolated as a solid. Purification was achieved by recrystallization, washing with isopropylamine (ISA) or by column chromatography

4.4.1 Synthesis of 6-cyclohexyl-5-(((3-fluorophenethyl)amino)methyl)pyrimidine-2, 4-diamine(33)[63,64]



30 **33a**

30 (100 mg, 0.5 mmol) was dissolved in 20 ml EtOH before AcOH (1 ml) was added and stirred. The 3-fluoro-phenethylamine (0.15 ml, 1.10 mmol) was then added and stirred overnight. To this mixture $NaCNBH_3$ (200 mg, 3.20 mmol) was added and stirred overnight. The crude product was washed with isopropylamine affording the white solid (30 mg, 10%)



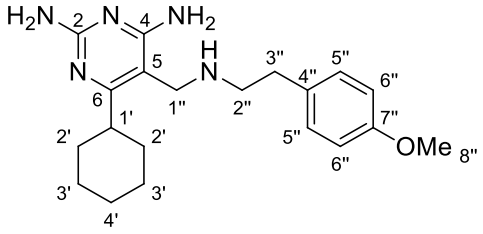
33a

)% EtOAc/hexane): **^{1}H NMR (500 MHz, MeOD)** δ 7.23 (m, 1H, H5”), 6.98 (s, *J* = 7.6 Hz, 1H, H9”), 6.93 (m, 1H, H7”), 6.88 (m, ,1H, H6”), 3.21 – 3.14 (m, 2H, H1”), 3.14 – 3.05 (m, 2H, H3”), 2.85 (m, 2H, H2”), 2.63 (tt, *J* = 11.8, 3.3 Hz, 1H, H1’), 1.71 (m, 2H,CHH), 1.62 (m, 3H, CHH), 1.47 (m, 2H, CHH), 1.25 (m, 1H, CHH), 1.17 – 0.97 (m, 1H, CHH). **^{13}C NMR (126 MHz, MeOD)** δ 178.8 (C2), 164.9 (C4), 164.1 (C6), 163.1 (d, $_{C\text{-}F}J$ = 245.1 Hz) (C8”), 139.2 (d, $_{C\text{-}F}J$ = 7.5 Hz) (C4”), 130.4 (d, $_{C\text{-}F}J$ = 8.4 Hz) (C6”), 124.26 (C5”), 115.2 (d, $_{C\text{-}F}J$ = 21.7 Hz) (C9”), 113.6 (d, $_{C\text{-}F}J$ = 21.2 Hz) (C7”),77.2 (C5), 50.6 (C1”), 48.1 (C2”), 45.6 (C3”), 44.7 (C1’), 30.5 (C2’), 25.9 (C3’), 25.6 (C4’), 17.9. **IR (ν_{max}/cm^{-1})**: 3391, 3109 (NH_2); 2922 (CH_2). ***HRMS*** *m/z:* calculated for $C_{19}H_{26}FN_5Na$: 343.2172, found: $[M+ H + Na]^+$ 344.2240

4.4.2 Synthesis of 6-cyclohexyl-5-(((4-methoxyphenethyl) amino) methyl) pyrimidine-2, 4-diamine (33b)[63,64]

30 **33a**

30 (100 mg, 0.5 mmol) was dissolved in 20 ml EtOH before AcOH (1 ml) was added and stirred. The 4-methoxy-phenethylamine (0.20 ml, 1.4 mmol) was then added and stirred overnight. To this mixture $NaCNBH_3$ (300 mg, 4.80 mmol) was added and stirred overnight. The crude product was washed with isopropylamine affording the white solid (60 mg, 31%)



33b

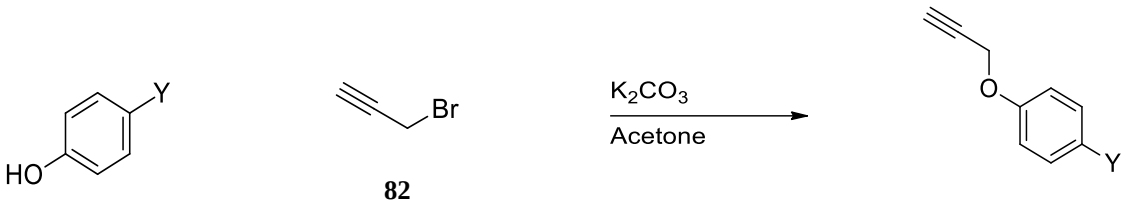
)% EtOAc/hexane): **^{1}H NMR (500 MHz, MeOD)** δ 7.19 (d, *J* = 8.2 Hz, 2H, H6"), 6.89 (d, *J* = 8.3 Hz, 2H, H5"), 3.77 (s, 3H, OMe), 3.13 (t, *J* = 8.1 Hz, 2H, H3"), 2.89 (t, *J* = 8.1 Hz, 2H, H2"), 2.78 (td, *J* = 11.7, 5.8 Hz, 1H, H1'), 1.90 – 1.80 (m, 4H, CHH), 1.77 (d, *J* = 14.0 Hz, 5H, CHH), 1.61 (m, *J* = 11.9, 11.4, 6.2 Hz, 4H, CHH), 1.45 – 1.33 (m, 4H, CHH). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 170.7 (C2), 162.8 (C4), 160.8 (C6), 158.1 (C7"), 129.7 (C5"), 113.9 (C6"), 99.5 (C4"), 94.2 (C5) 55.3 (OMe), 41.4 (C1"), 32.0 (C2"), 31.0 (C3"), 26.6 (C2'), 26.0 (C3'), 10.5 (C4'). **IR (ν_{max}/cm^{-1})**: 3311, 3012 (NH_2); 1540 (C-O) ***HRMS*** *m/z:* calculated for $C_{20}H_{29}N_5ONa$: 355.2372, found: $[M+ H + Na]^+$ 356.2401

Part 2

In our attempts to access **34**, we sought to synthesize the following compounds as precursors to the target.

To a solution of a substituted phenol **81** (1 mol eq) and K_2CO_3 (4 mol eq) in acetone 40 ml (solvent grade), propargyl bromide **82** (1.1 mol eq) (80% w/w in toluene) was added and heated to reflux overnight (14-16 hours). Upon completion, this mixture was then filtered and concentrated to afford **83** as an oil/solid in good too excellent yields.

4.5 Synthesis of substituted (prop-2-yn-1-yloxy)benzene

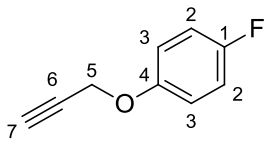


81 **82** **83**

4.5.1 Synthesis of 1-fluoro-4-(prop-2-yn-1-yloxy)benzene (38a)[65]

38a

4-Fluorophenol (2.00 g, 17.8 mmol), K_2CO_3 (10.0 g, 72.3 mmol) and propargyl bromide were stirred together while refluxing for 15 hours. The product was isolated and used with no further purification. The product was isolated as a brown oil (2.4 g, 93%).



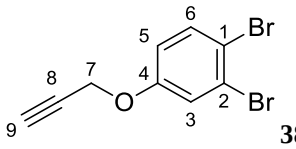
38a

R_f (80% EtOAc/Hexane) 0.72.**^{1}H NMR (300 MHz, $CDCl_3$)** δ 7.03 – 6.84 (m, 2H, H2), 6.74 (m, 2H, H3), 4.59 (dd, *J* = 2.4, 1.3 Hz, 2H, H5), 2.52 (t, *J* = 2.4 Hz, 1H, H7). **^{13}C NMR (75 MHz, $CDCl_3$)** δ 157.8 (d, *J* = 239.3 Hz) (C1), 153.6 (d, *J* = 2.0 Hz) (C4), 116.2 (d, *J* = 8.1 Hz) (C3), 115.9 (d, *J* = 23.2 Hz) (C2), 78.4 (C6), 75.7 (C7), 56.5 (C5). **IR (ν_{max}/cm^{-1}):** 2284 (C=H), 1509 (C-O)

4.5.2 Synthesis of 1,2-dibromo-4-(prop-2-yn-1-yloxy)benzene (38b)[65]

38b

3,4-dibromophenol (2.00 g, 7.94 mmol), K_2CO_3 (4.4 g, 31.8 mmol) and propargyl bromide were stirred together while refluxing for 15 hours. The product was isolated and used with no further purification. The product was isolated as a brown oil (2.3 g, 98%).

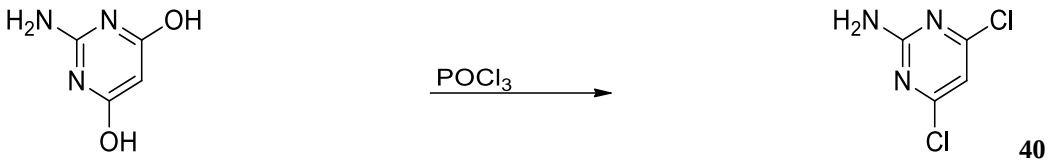


38b

R_f (80% EtOAc/Hexane) 0.78.**^{1}H NMR (500 MHz, $CDCl_3$)** δ 7.69 (d, *J* = 2.4 Hz, 1H, H6), 7.38 (dd, *J* = 8.8, 2.4 Hz, 1H, H3), 6.94 (d, *J* = 8.8 Hz, 1H, H5), 4.75 (d, *J* = 2.4 Hz, 2H, H7), 2.55 (t, *J* = 2.4 Hz, 1H, H9). **^{13}C NMR (75 MHz, $CDCl_3$)** δ 153.3 (C4), 135.7 (C6), 131.1 (C2), 115.4 (C3), 114.2 (C5), 113.4 (C1), 77.5 (C8), 57.0 (C7). **IR (ν_{max}/cm^{-1}):** 2074 (C=H), , 1576 (C-O)

4.6 Experimental work for the synthesis of *N*^{4}-cyclopropyl-5-(3-phenoxy-prop-1-yn-1-yl)pyrimidine- 2, 4, 6-triamine

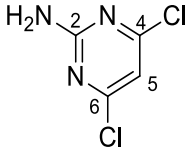
4.6.1 Synthesis of 4,6-dichloropyrimidin-2-amine[66] (40)



40

2-amino-4, 6-dihydroxypyrimidine (1g, 15.7 mmol, 1 eq) was stirred in phosphorous oxychloride (5 ml) at 60 °C for 4 hours before *N*,*N*-dimethylaniline (DMA) (5 ml, 23.6 mmol, 1.5 eq) was added and then stirred for an additional hour at a maintained temperature of 60 °C. At the end of the hour the reaction mixture was then cooled to 0 °C and quenched with water, where the product crashes out of solution. After filtration, the resulting tan/yellow clay-like

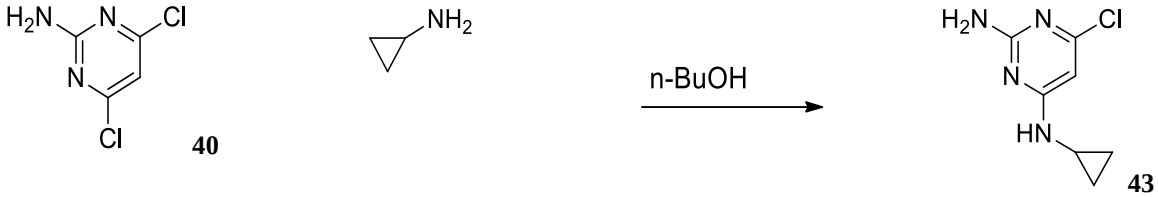
solid was dried before recrystallization from ethanol is performed. The desired product was isolated as a bright yellow solid in 40% yield.



40

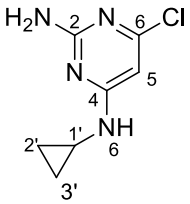
R_f (80% EtOAc/Hexane) 0.42. *mp* 215 °C. **^{1}H NMR (500 MHz, DMSO-*d_6*)** δ 7.58 (s, 2H, NH_2), 6.85 (s,1H, H5). **^{13}C NMR (126 MHz, DMSO-*d_6*)** δ 163.30 (C2), 161.44 (C4&6), 108.01 (C5). **IR (ν_{max}/cm^{-1}):** 3384 (NH_2), 816, 792 (=CCl)

4.6.2 Synthesis of 6-chloro-*N*^{4}-cyclopropylpyrimidine-2,4-diamine[67] (43)



40 **43**

40 (500 mg, 3.10 mmol), was dissolved in 15 ml of *n*-butanol. To this mixture, cyclopropylamine (0.23 ml, 3.40 mmol) was added, followed by the addition of Hunig's base (0.80 ml, 4.6 mmol). The mixture was stirred overnight at 95 °C. Upon completion of the reaction, the solvent was removed by evaporation, leaving a thick oily paste- like solution which was then dissolved in 100 ml of EtOAc. This organic layer was then washed with water (100 ml), brine (100 ml), dried over $MgSO_4$ and then concentrated *in vacuo* to yield a light brown/tan solid product after purification by column chromatography 80 % EtOAc/Hexane 39 (300 mg, 53%).

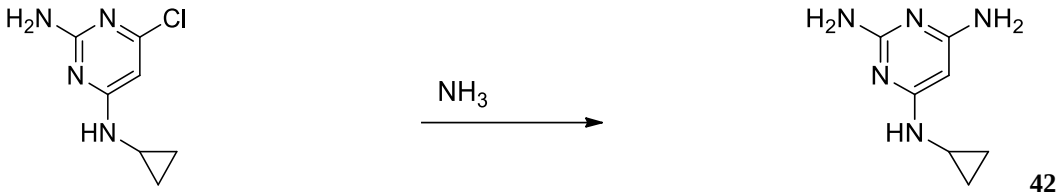


43

R_f (80% EtOAc/Hexane) 0.38. *mp* 154 °C. **^{1}H NMR (300 MHz, DMSO-*d_6*)** δ 7.05 (d, *J* = 2.9 Hz, H5), 6.14 (s, 2H, NH_2), 5.60 (s, 1H, NH), 0.62 (td, *J* = 7.3, 7.2, 4.9 Hz, 1H, H1'), 0.43 (td, *J* = 6.9, 6.7, 4.6 Hz, 2H, H2'), 0.19 (m, 2H, H2'). **^{13}C NMR (75 MHz, DMSO-*d_6*)** δ 165.4 (C2), 162.7 (C4), 159.4 (C6), 99.3 (C5), 23.1

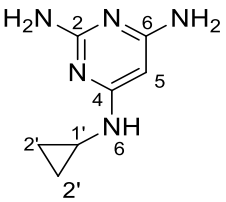
(C1'), 6.53 (C2'). **IR (ν_{max}/cm^{-1}):** 3318 (NH_2), 816, 792 (=CCl)

4.6.3 Synthesis of N^4-cyclopropylpyrimidine-2, 4, 6-triamine (42)[68]



42

41 (150 mg, 0.8 mmol) was dissolved in 5 ml of absolute ethanol before adding 30 ml of ammonia solution to a sealed tube which was heated at 170 °C for 2 days in a furnace. Upon removal of the sealed tube from the furnace the sealed tube was cooled to room temperature before it was cracked open at its apex and the contents transferred into a round bottom flask. The solvent was evaporated off leaving a tan solid. The product was isolated as tan solid (103 mg, 77%).



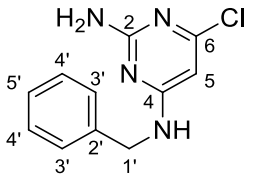
42

R_f (80% EtOAc/Hexane) 0.32, *mp:*185 °C. **^{1}H NMR (500 MHz, DMSO-d_6)** δ 5.99 (br s, *J* = 3.5 Hz, 1H, NH), 5.48 (br s, 2H, NH_2), 5.16 (br s, 2H, NH_2), 4.84 (s, 1H, H5), 2.12 (m, 1H, H1'), 0.60 (m, 2H, H2'), 0.35 (m, 2H, H2'). **^{13}C NMR (126 MHz, DMSO-d_6)** δ 165.3 (C2), 164.9 (C4), 163.0 (C6), 74.3 (C5), 23.6 (C1'), 7.2 (C2'and C3'). **IR (ν_{max}/cm^{-1}):** 3474 (NH_2), 3310 (NH_2).

4.6.4 Towards the synthesis of N^4-benzyl -5-(3-phenoxy-prop-1-yn-1-yl) pyrimidine- 2, 4, 6-triamine

4.6.4.1 Synthesis of 6-chloro-N^4-benzylpyrimidine-2, 4-diamine (45)[67]

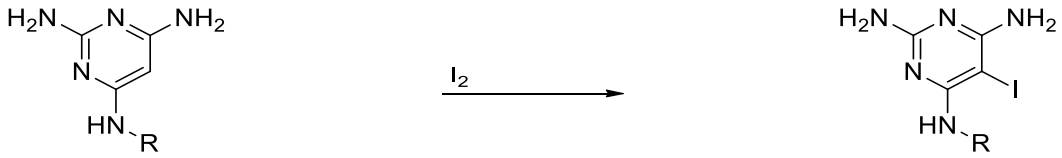
Following the same method as in 4.5.2, **41** (1.00 g, 6.90 mmol) dissolved in *n*-butanol (20ml) to which benzylamine (0.800 ml, 7.60 mmol) and Hunig's base (2.0 ml, 10 mmol) were added and stirred overnight at 95 °C to afford a light brown crude product. The crude was then purified by column chromatography 80% E/H affording a light brown product at (900 mg, 60% yield).



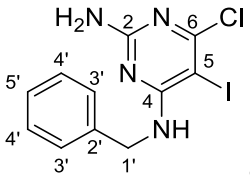
45

R_f (80% EtOAc/hexane) 0.51. *mp* 150-152 °C. **^{1}H NMR (DMSO-d_6, 300 MHz)** δ 7.63 (s, 1H, H5), 7.45 – 7.10 (6H, m, Ar-H), 6.46 (s, 2H, NH_2), 5.82 (s, 1H, NH), 4.60 – 4.34 (s, 2H, H1'). **^{13}C NMR (75 MHz, DMSO-d_6)** δ 164.1 (C2), 162.9 (C4 &C6), 139.5 (C2'), 128.3 (C4'), 127.2 (C3'), 126.8 (C5'), 43.3 (C1'). **IR (ν_{max}/cm^{-1}):** 3484 (NH_2), 2973(CH). ***HRMS m/z:*** calculated for $C_{11}H_{11}N_4$ ^{35}ClNa: 235.6848, found: $[M+Na]^+$ 235.0748

4.6.4.2 Synthesis of N^4-benzyl-6-chloro-5-iodopyrimidine-2,4-diamine (46)[69]



45 (200 mg, 0.850 mmol) was dissolved in H_2O (20 ml), after which K_2CO_3 (180 mg, 1.30 mmol), iodine (430 mg, 1.70 mmol) and DMF (2 ml) were added and stirred overnight to afford **46** as a light brown powder (267 mg,87%). The product was used with no further purification.



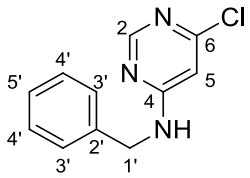
46

R_f (80% EtOAc/hexane) 0.71. *mp* 169 °C. **^{1}H NMR (DMSO-d_6, 300 MHz)** δ 7.37 – 7.26 (m, 4H, H3', H4'), 7.22 (m, 1H, H5'), 7.17 – 7.09 (m,1H, NH), 6.59 (s, 2H, NH_2), 4.55 (d, *J*=6.1 Hz, 2H, H1'). **^{13}C NMR (126 MHz, DMSO)** δ 163.1 (C6), 162.3 (C4), 161.9 (C2), 140.2 (C2'), 128.6 (C5'), 127.7 (C3'), 127.1 (C5)', 62.2 (C5), 44.8 (C1'). **IR (ν_{max}/cm^{-1}):** 3475, 3395 (NH_2), 2944 (CH), 888 (CCl), 750 (CI). ***HRMS*** *m/z:* calculated for $C_{11}H_{10}N_4{}^{35}ClINa$: 360.5814, found: $[M+ Na]^+$ 360.9720

4.6.5 Towards the synthesis of *N*^{4}-benzyl-5-iodopyrimidin-4,6-diamine

4.6.5.1 Synthesis of N-benzyl-6-chloropyrimidine-4-amine (53)[67]

2,4 dichloropyrimidine (1.00 g, 6.70 mmol) was dissolved in *n*-butanol (15 ml) followed by the addition of benzylamine (0.80 ml, 7.3 mmol) and Hunig's base (1.75 ml, 10.0 mmol). This mixture was then stirred at 95 °C overnight open to the atmosphere. Upon completion of the reaction and no further purification, the product was isolated as a tan powder at (1.38 g, 94%).

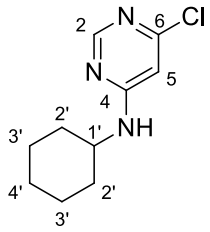


53

R_f (80% EtOAc/hexane) 0.5. *mp* 154 °C.**^{1}H NMR (300 MHz, $CDCl_3$)** δ 8.19 (s, 1H, H2), 7.46 – 7.23 (m, 6H, H3', H4', H5', & H5), 6.35 (d, 1H, *J*=0.9 Hz, NH), 4.50 (s, 2H, H1'). **^{13}C NMR (75 MHz, $CDCl_3$)** δ 163.3 (C6), 158.4 (C4 &C2), 129.0 (C4' & C5'), 128.0 (C2'), 127.5 (C3'), 102.3 (C5), 45.6 (C1'). **IR (ν_{max}/cm^{-1}):** 3033,2990 (=CH), 855 (CCl) ***HRMS*** *m/z:* calculated for $C_{11}H_{11}N_4{}^{35}ClNa$: 219.6702, found: $[M+H+Na]^+$ 220.0641

4.6.5.2 Synthesis of 6-chloro-*N*-cyclohexylpyrimidine-4-amine (54)

2,4 dichloropyrimidine (1.00 g, 6.70 mmol) was dissolved in *n*-butanol (15 ml) followed by the addition of cyclohexylamine (0.84 ml, 7.3 mmol) and Hunig's base (1.80 ml, 10.0 mmol). This mixture was then stirred at 95 °C overnight. Upon completion and purification, the product was isolated as a tan powder at (1. 2 g, 86%)

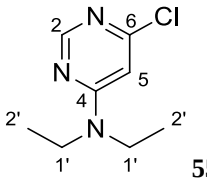


54

R_f (80% EtOAc/hexane) 0.47. *mp* 141 °C.**^{1}H NMR (300 MHz, $CDCl_3$)** δ 8.31 (s, 1H, H2), 6.31 (s, 1H, H5), 5.32 (s, 1H, NH), 2.01 (m, 2H, cyc-H), 1.72 (ddt, *J* = 33.0, 12.5, 4.1 Hz, 3H, cyc-H), 1.53 – 1.12 (m, 5H, cyc-H). **^{13}C NMR (75 MHz, $CDCl_3$)** δ 162.4 (C6), 158.5 (C2 & C4), 50.1 (C1'), 32.8 (C2'), 25.4 (C4'), 24.7 (C3'). **IR (ν_{max}/cm^{-1}):** 3255 (NH), 2930, 2852 (=CH). ***HRMS*** *m/z:* calculated for $C_{10}H_{14}N_3{}^{35}ClNa$: 212.6193, found: $[M+Na]^+$ 212.0951

4.6.5.3 Synthesis of 6-chloro-*N, N*-diethylpyrimidn-4-amine (55)[67]

2, 4 dichloropyrimidine (2 g, 13.4 mmol) was dissolved in *n*-butanol (30 ml) followed by the addition of diethylamine (1.5 ml, 14.8 mmol) and Hunig's base (3.5 ml, 20.1 mmol). This mixture was then stirred at 95 °C overnight. Upon completion of the reaction, no further purification was performed, the product was isolated as a tan powder at (2.7 g, 96%)



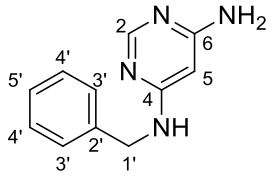
55

R_f (80% EtOAc/hexane) 0.45. *mp* 133 °C.**^{1}H NMR (300 MHz, $CDCl_3$)** δ 8.35 (d,1H, *J*=1.0 Hz, H2), 6.38 (d, 1H, *J*=1.0 Hz, H5), 3.68 – 3.32 (m, 4H, H1'), 1.20 (t, 6H, *J* = 7.1 Hz, H2'). **^{13}C NMR (75 MHz, $CDCl_3$)** δ 161.5 (C6), 159.4 (C4), 158.1 (C2), 100.8 (C5), 42.6 (C1'), 12.6 (C2'). **IR (ν_{max}/cm^{-1}):** 2933, 2803 (CH), 754 (CCl). ***HRMS*** *m/z:* calculated for

$C_{10}H_{14}N_3ClNa$: 185.6540, found: $[M+H+Na]^+$ 186.0951

4.6.5.4 Synthesis of *N*⁴-benzylpyrimidine-4,6-diamine (56)[68]

53 (1.00 g, 0.500 mmol) was dissolved in 10 ml of ethanol. To this mixture ammonia solution (20 ml) was added to the glass tube and sealed. The reaction vessel was heated at 170 °C for 2 days in a furnace. The product was isolated as a pure tan powder at (1.5 g, 89%) yield upon removal of the solvent under vacuum pressure.

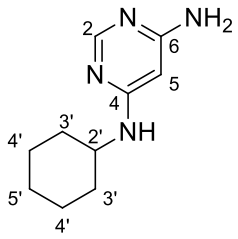


56

)% EtOAc/hexane) 0.45. mp 185°C.1H NMR (300 MHz, DMSO-d6) δ 7.85 (s, 1H, H2), 7.45 – 7.13 (m, 6H, Ar-H, H5), 6.10 (s, 2H, NH2), 5.40 (s, 1H, NH), 4.37 (d, J = 6.0 Hz, 2H, H1'). 13C NMR (75 MHz, DMSO-d6) δ 163.2 (C6), 162.5 (C4), 157.5 (C2), 140.2 (C2'), 128.2 (C4'), 127.0 (C3'), 81.8 (C5), 43.6 (C1'). IR (νmax/cm-1): 3474 (NH2), 3064, 2920 (=CH). HRMS m/z: calculated for $C_{10}H_{14}N_3{}^{35}ClNa$:201.2398, found: [M+Na] + 201.9137

4.6.5.5 Synthesis of *N*⁴-cyclohexylpyrimidine-4, 6-diamine (57)[68]

54 (1.00 g, 0.500 mmol) was dissolved in 10 ml of ethanol. To this mixture ammonia solution (25 ml) was added and sealed and allowed to react at 170 °C for 2 days in a furnace. The product was isolated with no further purification as a pure tan powder at (1.3 g, 91%) yield upon removal of the solvent under vacuum pressure.

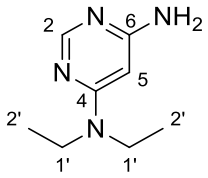


54

R_f (80% EtOAc/hexane) 0.52. *mp* 152 °C.**^{1}H NMR (300 MHz, $CDCl_3$)** δ 8.31 (s, 1H, H2), 6.31 (s, 1H, H5), 5.57 (d, 2H, *J* = 1.1 Hz, NH_2) 5.32 (s, 1H, NH), 2.01 (m, 2H, cyc-H), 1.72 (ddt, *J* = 33.0, 12.5, 4.1 Hz, 3H, cyc-H), 1.53 – 1.12 (m, 5H, cyc-H). **^{13}C NMR (75 MHz, $CDCl_3$)** δ 162.4 (C6), 158.5 (C2 & C4), 50.1 (C1'), 32.8 (C2'), 25.4 (C4'), 24.7 (C3'). **IR (ν_{max}/cm^{-1}):** 3255 (NH), 2930, 2852 (=CH). ***HRMS*** *m/z:* calculated for $C_{10}H_{14}N_3{}^{35}ClNa$: 192.2608, found: $[M+Na]^+$ 193.0951

4.6.5.6 Synthesis of N^4, N^4-diethylpyrimidin*e-4, 6*-diamine (58)[68]

55 (1.00 g, 5.40 mmol) was dissolved in 5 ml of ethanol. To this mixture ammonia solution (30 ml) was added and sealed and allowed to react at 170 °C for 2 days in a furnace. The product was isolated as a pure tan powder at (900 mg, 90%) upon removal of the solvent under vacuum.



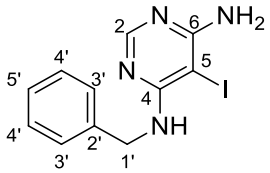
58

R_f (80% EtOAc/hexane) 0.5. *mp* 146-148 °C.**^{1}H NMR (300 MHz, DMSO-d_6)** δ 8.25 (s, 1H, H2), 6.13 (s,1H, H5), 5.47 (d, 2H, *J* = 1.1 Hz, NH_2), 3.36 (q, 4H, *J*=7.0 Hz, H2'), 1.04 (t, 6H, *J*=7.1 Hz, H1').**^{13}C NMR (75 MHz, DMSO-d_6)** δ 163.4 (C6), 160.4 (C4), 157.1 (C2), 80.6 (C5), 41.2 (C1'), 12.8 (C2'). **IR (ν_{max}/cm^{-1}):** 3317 (NH_2), 2933,2803 (=CH).

HRMS *m/z:* calculated for $C_8H_{14}N_4Na$: 166.2236, found: $[M+H+Na]^+$ 167.1292

4.6.5.7 Synthesis of N^4-benzyl-*5*-iodopyrimidin*e-4, 6*-diamine (59)[69]

56 (300 mg, 1.50 mmol) was dissolved in H_2O (12.0 ml), where K_2CO_3 (3.10 g, 2.20 mmol), iodine (780 mg, 3.00 mmol) and DMF (1.50 ml) were and added and stirred overnight to afford **59** as a light brown powder (435 mg, 49%).

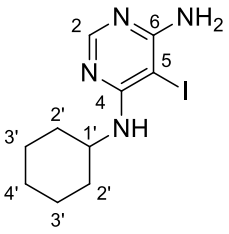


59

R_f (80% EtOAc/hexane) 0.5. *mp* 166 °C.**^{1}H NMR (300 MHz, $CDCl_3$)** δ 8.00 (s, 1H, H2), 7.48 – 7.20 (m, 5H, Bn-H), 5.46 (s, 1H, NH), 5.31 (s, 2H, NH_2), 4.69 (d, *J* = 5.6 Hz, 2H, H1'). **^{13}C NMR (75 MHz, $CDCl_3$)** δ 160.2 (C4 &C6), 157.3 (C2), 138.9 (C2'), 128.7 (C4'), 127.5 (C3'), 127.4 (C5'), 59.7 (C5), 45.8 (C1'). **IR (ν_{max}/cm^{-1}):** 3465, 3408 (NH_2), 3154 (=CH) 789 (CI). ***HRMS*** *m/z:* calculated for $C_{11}H_{11}N_4INa$: 326.1363, found: $[M+H+Na]^+$ 327.0110

4.6.5.8 Synthesis of N^4-cyclohexyl-5-iodopyrimidine-4, 6-diamine (60)[69]

57 (1.00 g, 5.20 mmol) was dissolved in H_2O (15.0 ml), were treated with K_2CO_3 (1.10 g, 7.80 mmol), iodine (2.70 mg, 10.4 mmol) and DMF (1.50 ml) and stirred overnight at 45 °C open to the atmosphere to afford **60** as a light brown powder (27.0 mg, 1.6%).

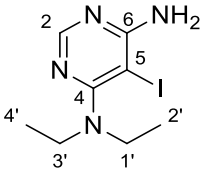


60

R_f (80% EtOAc/hexane) 0.5. *mp (lit).* **^{1}H NMR (300 MHz, $CDCl_3$)** δ 7.97 (s, 2H, H2), 5.23 (s, 2H, NH_2), 4.97 (d, *J*=8.2 Hz, 1H, NH), 4.04 – 3.89 (m, 1H, H1'), 2.09 – 1.92 (m, 2H, CH-H), 1.70 (m, *J*=27.3, 12.7, 4.1 Hz, 3H, CH-H), 1.55 – 1.34 (m, 2H, CH-H), 1.33 – 1.14 (m, 4H, CH-H). **^{13}C NMR (75 MHz, $CDCl_3$)** δ 161.7 (C6), 160.1 (C4), 157.4 (C1), 58.6 (C5), 50.1 (C1'), 33.4 (C2'), 25.7 (C3'), 24.8 (C4'). **IR (ν_{max}/cm^{-1}):** 3375 (NH_2), 3154 (=CH). ***HRMS*** *m/z:* calculated for $C_{11}H_{11}N_4INa$: 318.1574, found: $[M+H+Na]^+$ 319.2110

4.6.5.9 Synthesis of N^4, N^4-diethyl-5-iodopyrimidine-4, 6-diamine (61)[69]

58 (400 mg, 2.40 mmol) was dissolved in H_2O (15 ml), and then treated with K_2CO_3 (500 mg, 3.60 mmol), iodine (1.25 mg, 4.80 mmol) and DMF (1.5 ml) were and added and stirred overnight to afford **61** as a light brown powder (33.0 mg, 4.7%).

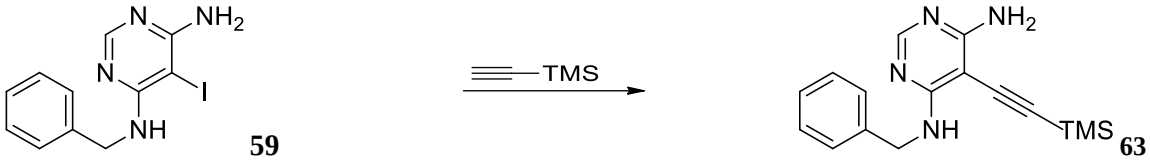


61

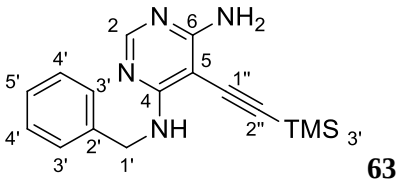
R_f (80% EtOAc/hexane) 0.47.**^{1}H NMR (300 MHz, $CDCl_3$)** δ 7.99 (s, 1H, H2), 5.57 – 5.33 (m, 2H, NH_2), 3.62 – 3.34 (m, 4H, H1' & H3'), 1.20 (m, 6H, H4' & H2'). **^{13}C NMR (75 MHz, $CDCl_3$)** δ 161.72 (C6), 160.69 (C4), 157.29 (C2), 58.27 (C5), 42.00 (C3'), 36.85 (C1'), 15.09 (C4'), 12.85 (C2'). **IR (ν_{max}/cm^{-1}):** 3375 (NH_2), 3154 (=CH). ***HRMS*** *m/z:* calculated for $C_{11}H_{11}N_4INa$: 292.1201, found: $[M+H+Na]^+$ 293.0110

4.7 Synthesis of N^4-benzyl-5-(phenylbuta-1,3- diyn-1-yl) pyrimidine-4,6-diamine

4.7.1 Synthesis of N^4-benzyl-5-((trimethylsilyl)ethynyl)pyrimidine-4,6-diamine (63)[70]



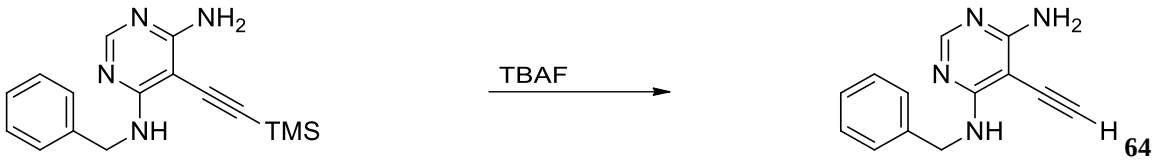
59 (500 mg, 1.50 mmol), CuI (60.0 mg, 3.20 mmol), $Pd(PPh_3)Cl_2$ (100 mg, 0.100 mmol) were degassed under nitrogen in a two-necked flask for ~15 minutes. TMS acetylene (2.20 ml, 15.5 mmol), triethylamine (TEA) (2.00 ml, 14.3 mmol) and THF (10 ml) were also degassed by bubbling nitrogen through this mixture for ~10 minutes. Through a dropping funnel the degassed liquid was introduced to the solids, the resulting mixture was stirred at room temperature for a maximum of 30 minutes before refluxing overnight under nitrogen. Upon completion of the reaction the THF was removed *in vacuo* followed by the purification of the crude by column chromatography in 80% (EtOAc/hexane). The product was isolated as a yellow solid (150 mg, 45%).



63

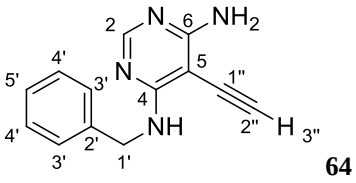
R_f (80% EtOAc/hexane) 0.46. *mp*: 156 °C. **^{1}H NMR (300 MHz, $CDCl_3$)** δ 8.13 (s, 1H, H2), 7.45 – 7.21 (m, 5H, Ar-H), 5.56 (t, *J* = 5.9 Hz, 1H, NH), 5.24 (s, 2H, NH_2), 4.79 – 4.67 (d, J=5.8 Hz 2H, H1'), 0.23 (s, 9H, 3 x CH_3). **^{13}C NMR (75 MHz, $CDCl_3$)** δ 162.8 (C6), 162.2 (C4), 156.8 (C2), 138.8 (C2'), 128.6 (C4'), 127.3 (C3'), 127.2 (C5'), 107.9 (C2''), 96.0 (C1''), 80.6 (C5), 44.7 (C1'), 0.25 (C3''/TMS). **IR (ν_{max}/cm^{-1}):** 3467 (NH_2), 3063, 2958 (=CH), 2131 (C-Si). ***HRMS*** *m/z:* calculated for $C_{16}H_{20}N_4SiNa$: 296.4423, found: $[M+Na]^+$ 296.1537

4.7.2 Synthesis of N^4-benzyl-5-ethynylpyrimidine-4, 6-diamine (64)[71]



64

63 (200 mg, 1.42 mmol) was dissolved in THF (20 ml) at room temperature. The temperature was then reduced to 5 °C in an ice bath while stirring, while TBAF (0.6 ml, 3.20 mmol) was carefully added to the reaction mixture dropwise. After complete addition the reaction mixture was warmed to room temperature was stirring and then left to stir overnight under normal air. Upon completion, no work up was performed, and the reaction mixture was purified by column chromatography 80 % E/H. The product was isolated as pale-yellow solid (40 mg, 17%).

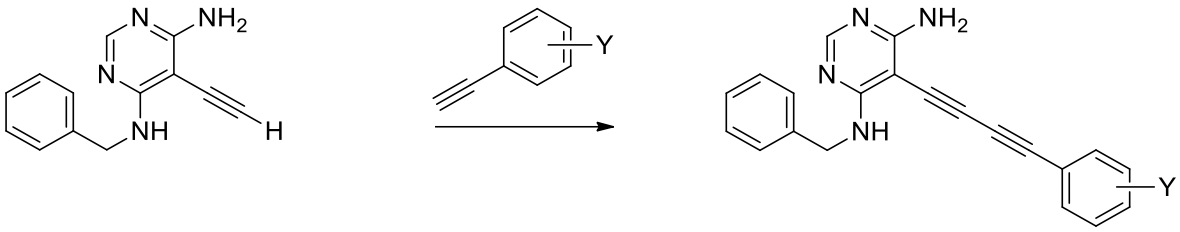


64

R_f (80% EtOAc/hexane) 0.43. *mp*: 188-189 °C. **^{1}H NMR (300 MHz, $CDCl_3$)** δ 7.89 (s, 1H, H2), 7.40 – 7.03 (m, 6H, Bn-H), 5.67 (br s, 1H, NH), 5.45 (s, 2H, NH_2), 4.51 (d, *J* = 5.7, 2.5 Hz, 2H, H1'), 3.73 (s, 1H, H3''). **^{13}C NMR (75 MHz, $CDCl_3$)** δ 160.23 (C6 &C4), 157.29 (C2), 138.94 (C2'), 128.68 (C4'), 127.44 (C3'), 127.37 (C5'), 77.22 (C1'' &C2''), 59.70 (C5), 45.78 (C1'). **IR (ν_{max}/cm^{-1}):** 3469, 3404 (NH_2), 3108 (=CH), 2086 (C=C). ***HRMS*** *m/z:* calculated for $C_{13}H_{12}N_4Na$: 224.2612, found: $[M+H+Na]^+$ 225.1180

4.8 Synthesis of *N*^{4}-benzyl-5-((phenyl) buta-1,3-diyn-1-yl)pyrimidine-4,6-diamine through the copper coupling approach[51]

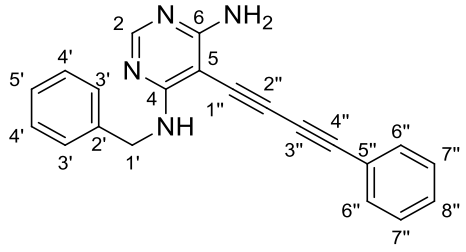
General approach



64 (1 mol eq) was added to DCM followed by the addition of a substituted phenyl acetylene (5 mol eq), piperidine (3 mol eq) and $Cu(OAc)_2.H2O$ (0.1 mol eq). This mixture was stirred at room temperature for 12 hours under air. Upon completion, the solvent was removed by evaporation under reduced pressure and the crude purified by column chromatography (40% EtOAc/hexane to 100% EtOAc). The product fraction was then purified further by preparative TLC (20% EtOAc/hexane to 40% EtOAc/hexane), where the plate was run first in 20% EtOAc/hexane then once in 40% EtOAc/hexane before reverting to 2 more runs in 20% EtOAc/hexane.

4.8.1 Synthesis of *N*^{4}-benzyl-benzyl-5-(phenylbuta-1,3-diyn-1-yl)pyrimidine-4,6-diamine (68)

64 (100 mg, 0.500 mmol) was dissolved in DCM (5 ml) along with phenyl acetylene (0.30 ml, 2.70 mmol), $Cu(OAc)_2.H_2O$ (20 mg, 0.10 mmol) and piperidine (0.13 ml, 0.13 mmol). The reaction was stirred open to atmospheric air at room temperature for 12 hours. The reaction mixture was then concentrated *in vacuo* and the crude residue was purified by column chromatography as described above. The product was isolated as a yellow solid (20 mg, 14%).



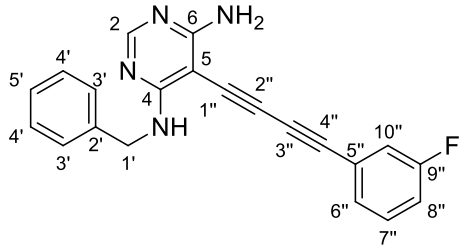
68

R_f (80% EtOAc/hexane) 0.55. *mp*: 140 °C. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 8.15 (s, 1H, H2), 7.54– 7.45 (m, 2H, Ar-H), 7.40 – 7.28 (m, 7H, Ar-H), 5.69 (t, *J* = 5.6 Hz, 1H, NH), 5.43 (s, 2H, NH_2), 4.75 (d, *J* = 5.9 Hz, 2H, H1'). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 164.1 (C6), 163.1 (C4), 157.1 (C2), 138.6 (C2'), 132.3 (C6''), 129.4 (C5'), 128.7 (C7''), 128.5 (C4'), 127.6 (C3'), 127.5 (C8''), 121.5 (C5''), 86.6 (C3''), 85.4 (C4''), 79.4 (C5), 73.4(C2''), 72.6 (C1''), 44.9 (C1'). **IR (ν_{max}/cm^{-1}):** 3375 (NH_2), 3154,

(=CH), 2205 2136 (C triple bond C) ***HRMS** m/z:* calculated for $C_{22}H_{10}N_6Na$: 324.3745, found: $[M+H+Na]^+$ 324.1060

4.8.2 Synthesis of N^4-benzyl -5-((3-fluorophenyl)buta-1,3-diyn-1-yl)pyrimidine-4,6-diamine (70b)

64 (100 mg, 0.500 mmol) was dissolved in DCM (5 ml) along with 1-ethynyl-3 fluorobenzene (0.30 ml, 2.7 mmol), $Cu(OAc)_2.H_2O$ (20 mg, 0.10mmol) and piperidine (0.13 ml, 0.13 mmol). The reaction was stirred in the open atmospheric air at room temperature for 12 hours. The reaction mixture was then concentrated *in vacuo* and the crude product was purified by column chromatography. The product was isolated as a yellow solid (25 mg, 16%).

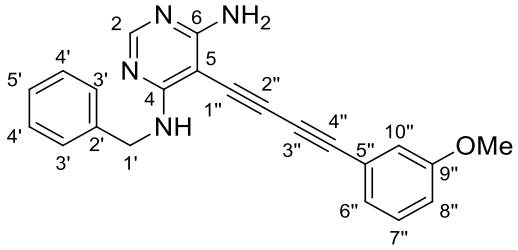


70b

R_f (80% EtOAc/hexane) 0.55. *mp* 145-147 °C.**^{1}H NMR (500 MHz, $CDCl_3$)** δ 8.14 (s, 1H, H2), 7.34 (br s, 2H, H10”), 7.28 (m, 5H, Ar-H), 7.18 (d, *J* = 9.0 Hz, 1H, H8”), 7.08 (t, *J* = 7.8 Hz, 2H, H7” & H6”), 5.69 (s, 1H, NH), 5.46 (s, 2H, NH_2), 4.80 – 4.71 (m, 2H, H1’). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 164.2 (C6), 163.2 (C4), 161.3 (d, J_{C-F} = 247.6 Hz, C9’’), 157.3 (C2), 138.6 (C2’), 130.2 (d, J_{C-F} = 8.6 Hz C5’’), 128.7 (C4’), 128.2 (d, J_{C-F} = 3.1 Hz, C6”), 127.6 (C3’), 127.5 (C5’), 123.3 (d, J_{C-F} = 9.5 Hz, C7’’), 119.1 (d, J_{C-F} = 23.1, C8’’), 117.0 (d, J_{C-F} = 2 Hz, C10”), 86.3 (C3”), 84.0 (C4’’), 79.1 (C5), 74.3 (C2”), 73.5 (C1’’), 44.9 (C1’). **IR (ν_{max}/cm^{-1}):** 3471 (NH_2), 3088, 2919 (=CH), 2204, 2144 (C triple bond C). ***HRMS** m/z:* calculated for $C_{21}H_{15}N_4FNa$: 342.3690, found: $[M+H+Na]^+$ 343.1351

4.8.3 Synthesis of N^4-benzyl-5-((3-methoxyphenyl)buta-1,3-diyn-1-yl)pyrimidine-4,6-diamine (69b)

64 (100 mg, 0.500 mmol) was dissolved in DCM (5 ml) along with 2-ethynylanisole (0.3 ml, 2.7 mmol), $Cu(OAc)_2.H_2O$ (20 mg, 0.10 mmol) and piperidine (0.13 ml, 0.13 mmol). The reaction was stirred open to the atmosphere at room temperature for 12 hours. The reaction mixture was then concentrated in vacuo and the crude was purified by column chromatography. The product was isolated as a yellow/tan solid (20 mg, 13%).

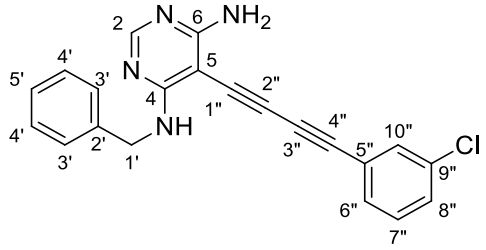


69b

R_f (80% EtOAc/hexane) 0.51. *mp* 151-153 °C.**^{1}H NMR (300 MHz, $CDCl_3$)** δ 8.15 (s, 1H, H2), 7.39 – 7.32 (m, 6H, Ar-H), 7.32 – 7.28 (m, 1H, H7”), 7.26 (t, *J* = 3.9 Hz, 1H, H6”), 7.22 (s, 1H, H8”), 7.01 (dd, *J* = 2.5, 1.4 Hz, 1H, H10”), , 5.69 (t, *J* = 5.7 Hz, 1H, NH), 5.39 (s, 2H, NH_2), 4.75 (d, *J* = 5.9 Hz, 2H, H1’).**^{13}C NMR (75 MHz, CDCl3)** δ 164.2 (C6), 163.1 (C4), 159.3 (C9’’), 157.2 (C2), 138.6 (C2’), 129.6 (C7”), 128.7 (C4’),127.6 (C3’), 127.5 (C5’), 124.9 (C6”), 122.4 (C5’’), 116.9 (C10”), 116.2 (C7''), 86.5 (C3’'), 85.3 (C4’'), 79.3 (5), 73.2 (C2’'), 72.7 (C1’'), 55.3 (OMe), 44.9 (C1’). **IR (ν_{max}/cm^{-1}):** 3375 (NH_2), 3154 (=CH). 2205,2136 (C triple bond C). ***HRMS*** *m/z:* calculated for $C_{22}H_{18}N_4ONa$: 354.4045, found: $[M+H+Na]^+$ 355.1554

4.8.4 Towards the synthesis of N^4-benzyl-5-((3-chlorophenyl)buta-1,3-diyn-1-yl)pyrimidine-4,6-diamine (71a)

64 (100 mg, 0.500 mmol) was dissolved in DCM (5 ml) along with 3-chloro-1-ethynylbenzene (0.30 ml, 2.7 mmol), $Cu(OAc)_2.H_2O$ (20 mg, 0.10 mmol) and piperidine (0.13 ml, 0.13mmol). The reaction was stirred in the open atmospheric air at room temperature for 12 hours. The reaction mixture was then concentrated *in vacuo* and the crude was purified by column chromatography.

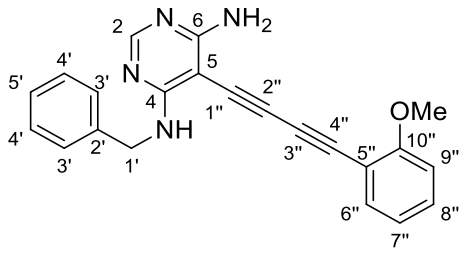


71a

R_f (80% EtOAc/hexane)0.53. *mp* 179-180 °C. **^{1}H NMR (500 MHz, $CDCl_3$) δ** 8.15 (s, 1H, H2), 7.48 (s, 1H, H10''), 7.36 (m, 6H, Ar-H & H7"), 7.32 – 7.22 (m, 2H, H6" & H8"), 5.67 (s, 1H, NH), 5.37 (s, 2H, NH_2), 4.81 – 4.67 (m, 2H, CH_2). **^{13}C NMR (126 MHz, $CDCl_3$) δ** 164.2 (C6), 163.2 (C4), 157.3 (C2), 138.6 (C2'), 134.4 (C9"), 132.0 (C6"), 130.4 (C7"), 129.8 (C8" or 10"), 128.7 (C4'), 127.6 (C3'), 127.5 (C5'), 123.3 (C5"), 86.3 (C3"), 83.8 (C4"), 79.1 (C5), 74.5 (C2"), 73.6 (C1"), 44.9 (C1'). **IR (ν_{max}/cm^{-1}):** 3375 (NH_2), 3154 (=CH), 2210, 2132 (C triple bond C). ***HRMS*** *m/z:* calculated for $C_{22}H_{18}N_4ONa$: 358.8236, found: [M+H+ Na] $^+$ 359.1056

4.8.5 Synthesis of N^4-benzyl-5-((2-methoxyphenyl)buta-1,3-diyn-1-yl)pyrimidine-4,6-diamine (69a)

64 (100 mg, 0.500 mmol) was dissolved in DCM (5 ml) along with 2-ethynylanisole (0.30 ml, 2.7 mmol), $Cu(OAc)_2.H_2O$ (20 mg,0.10 mmol) and piperidine (0.13 ml, 0.13 mmol). The reaction was stirred open to the atmosphere at room temperature for 12 hours. The reaction mixture was then concentrated *in vacuo* and the crude product was purified by column chromatography. The product was isolated as a yellow solid (35 mg, 22%)

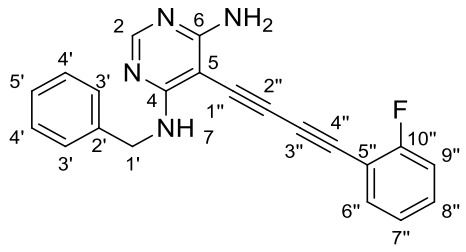


69a

R_f (80% EtOAc/hexane) 0.53. *mp* 148-150 °C. **^{1}H NMR (500 MHz, $CDCl_3$)** δ 8.17 (s, 1H, H2), 7.48 (d, *J* = 7.6 Hz, 1H, H9”), 7.37 (m, 5H, Ar-H), 7.30 (m, 1H, H8”), 7.00 – 6.87 (m, 2H, H6” & H7”), 5.74 (t, *J* = 6.0 Hz, 1H, NH), 5.40 (s, 2H, NH_2), 4.76 (d, *J* = 5.9 Hz, 2H, H1’), 3.92 (s, 3H, OMe).**^{13}C NMR (126 MHz, $CDCl_3$)** δ 164.11 (C6), 163.08 (C4), 161.23 (C10’’), 157.12 (C2), 138.66 (C2’), 134.28 (C6’’), 130.98 (C8”), 128.70 (C4’), 127.58 (C3’), 127.44 (C5’), 120.7 (C9”), 110.71 (C9” &C5”), 86.71 (C3”), 81.94 (C4”), 79.51 (C5), 73.24 (C2”), 60.40 (C1”), 55.88 (OMe), 44.86 (C1’). **IR (ν_{max}/cm^{-1}):** 3375 (NH_2), 3154 (=CH). **IR (ν_{max}/cm^{-1}):** 3375 (NH_2), 3154 (=CH), 2205 2136 (C triple bond C). ***HRMS*** *m/z:* calculated for $C_{22}H_{18}N_4ONa$: 354.4045, found: $[M+H+Na]^+$ 355.1554

4.8.6 Synthesis of N^4-benzyl-5-((2-fluorophenyl)buta-1,3-diyn-1-yl)pyrimidine-4,6-diamine (70a)

64 (100 mg, 0.500 mmol) was dissolved in DCM (5 ml) along with 1-ethynyl-2-fluorobenzene (0.30 ml, 2.7 mmol), $Cu(OAc)_2.H_2O$ (20 mg, 0.10 mmol) and piperidine (0.13 ml, 0.13 mmol). The reaction was stirred open to the atmosphere at room temperature for 12 hours. The reaction mixture was then concentrated *in vacuo* and the crude product was purified by column chromatography. The product was isolated as a yellow solid (30 mg, 20%)



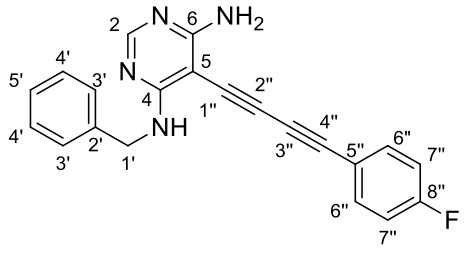
70a

R_f (80% EtOAc/hexane) 0.51. *mp* 158-160 °C.**^{1}H NMR (500 MHz, $CDCl_3$)** δ 8.18 (s, 1H, H2), 7.51 (t, *J* = 7.4 Hz, 1H, H6''), 7.38 (m, , 5H, Ar-H), 7.34 – 7.27 (m, 1H, H7''), 7.13 (m, 2H, H9'' and H6''), 5.73 (t, *J* = 5.9 Hz, 1H, NH), 5.46 (s, 2H, NH_2), 4.78 (d, *J* = 5.9 Hz, 2H, H1'). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 164.3 (C6), 163.2 (C4), 163.5 (d, J_{C-F} = 253.4 Hz, C10") 157.3 (C2), 138.6 (C2'), 134.1 (C6''), 131.1 (d, J_{C-F} = 8.0 Hz C8''), 128.7 (C4'), 127.6 (C3'), 127.5 (C5'), 124.2 (C7''), 115.8 (d, J_{C-F} = 20.6 Hz, C9''), 110.48 (d, J_{C-F} = 15.6 Hz C5''), 86.28 (C3"), 79.07 (C4"), 78.73 (C5), 78.11 (C2"), 73.98 (C1"), 44.88 (C1'). **IR (ν_{max}/cm^{-1}):** 3471 (NH_2), 3088, 2919 (=CH), 2205, 2136 (C triple bond C). ***HRMS*** *m/z:* calculated for $C_{21}H_{15}N_4FNa$: 343.3690, found: $[M+H+Na]^+$ 343.1351

4.8.7 Towards the synthesis of N^4-benzyl -5-((4-fluorophenyl) buta-1,3-diyn-1-yl)pyrimidine-4,6-diamine (70c)

64 (100 mg, 0.500 mmol) was dissolved in DCM (5 ml) along with 1-ethynyl-4-fluorobenzene (0.30 ml, 2.7 mmol), $Cu(OAc)_2.H_2O$ (20 mg, 0.10mmol) and piperidine (0.13 ml, 0.13 mmol). The

reaction was stirred open to the atmospheric at room temperature for 12 hours. The reaction mixture was then concentrated *in vacuo* and the crude residue was purified by column chromatography. The product was isolated as a yellow solid (25 mg, 16%)

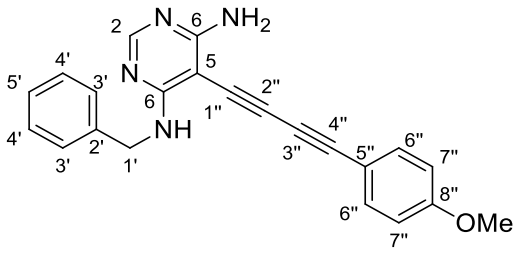


70c

R_f (80% EtOAc/hexane) 0.55. *mp* 167-169 °C.**^{1}H NMR (300 MHz, $CDCl_3$)** δ 8.15 (s, 1H, H2), 7.48 (m, *J* = 10.1, 5.2, 2.5 Hz, 2H, H7''), 7.41 – 7.24 (m, 5H, Ar-H), 7.10 – 6.97 (m, 2H, H6''), 5.68 (t, *J* = 5.6 Hz, 1H, NH), 5.38 (s, 2H, NH_2), 4.75 (d, *J* = 5.9 Hz, 2H, H1'). **^{13}C NMR (75 MHz, $CDCl_3$)** δ 164.17 (C6), 163.13 (C4), 162.3 (d, J_{C-F} = 286.4 Hz, C8") 157.23(C2), 138.60 (C2'), 134.4 (d, J_{C-F} = 8.6 Hz), 128.72 (C4'), 128.21 (d, *J* = 3.1 Hz, C5"), 127.57 (C3'), 127.49 (C5'), 123.35 (d, *J* = 9.5 Hz,C5"), 119.0 (d, J_{C-F} = 23.0 Hz, C7") , 116.9 (d, *J* = 21.3 Hz) (C6''), 86.39 (C3''), 84.32 (C4"), 77.77 (C5), 74.2 (C2"), 73.5 (C1"), 44.88 (C1'). **IR (ν_{max}/cm^{-1}):** 3471 (NH_2), 3088, 2919 (=CH), 2205, 2136 (C triple bond C). ***HRMS*** *m/z:* calculated for $C_{21}H_{15}N_4FNa$: 342.3690, found: $[M+H+Na]^+$ 343.1351

4.8.8 Towards the synthesis of *N*^{4}-benzyl -5-((4-methoxyphenyl) buta-1,3-diyn-1-yl)pyrimidine-4,6-diamine (69c)

64 (100 mg, 0.500 mmol) was dissolved in DCM (5 ml) along with 4-ethynylanisole (0.30 ml, 2.8 mmol), $Cu(OAc)_2.H_2O$ (20 mg, 0.10 mmol) and piperidine (0.13 ml, 0.13 mmol). The reaction was stirred in the open atmospheric air at room temperature for 12 hours. The reaction mixture was then concentrated *in vacuo* and the crude residue was purified by column chromatography. The product was isolated as a yellow/light orange solid (20 mg, 13%)

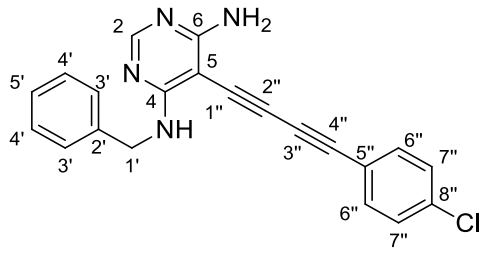


69c

R_f (80% EtOAc/hexane) 0.48. *mp* 149-150 °C.**^{1}H NMR (500 MHz, $CDCl_3$)** δ 8.17 (s, 1H, H2), 7.51 – 7.44 (d, 2H, H6"), 7.42 – 7.34 (m, 4H, Ar-H &), 6.95 – 6.83 (m, 2H, H7"), 5.69 (t, *J* = 5.9 Hz, 1H, NH), 5.38 (s, 2H, NH_2), 4.77 (d, *J* = 5.9 Hz, 2H, H1'), 3.85 (s, 3H, OMe). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 164.04 (C6), 163.07 (C4), 160.58 (C8''), 157.08 (C2), 138.67 (C2'), 134.02 (C6''), 128.71 (C4'), 127.58 (C3'), 127.46 (C5'), 114.27 (C7''), 113.34 (C5''), 86.78 (C3''), 85.66 (C4''), 79.60 (C5), 72.24 (C2''), 72.00 (C1''), 55.37 (OMe), 44.88 (C1'). **IR (ν_{max}/cm^{-1}):** 3375 (NH_2), 3154 (=CH), 2205, 2136 (C triple bond C). ***HRMS*** *m/z:* calculated for $C_{22}H_{18}N_4ONa$: 354.4045, found: $[M+Na]^+$ 355.1554

4.8.9 Towards the synthesis of *N*^{4}-benzyl -5-((4-chlorophenyl) buta-1,3-diyn-1-yl)pyrimidine-4,6-diamine (71b)

64 (100 mg,0.500 mmol) was dissolved in DCM (5 ml) along with 1-chloro-4-ethynylbenzene (0.3 ml, 2.7 mmol), $Cu(OAc)_2.H_2O$ (20 mg, 0.10 mmol) and piperidine (0.13 ml, 0.13 mmol). The reaction was stirred open to the atmosphere at room temperature for 12 hours. The reaction mixture was then concentrated *in vacuo* and the crude residue was purified by column chromatography. The product was isolated as light-yellow solid (35 mg, 22%).

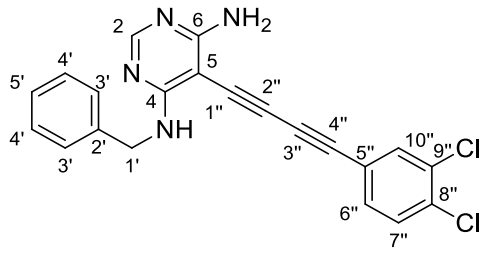


71b

R_f (80% EtOAc/hexane) 0.66. *mp* 185-187 °C.**^{1}H NMR (500 MHz, $CDCl_3$)** δ 8.18 (s, 1H, H2), 7.45 (d, *J* = 8.0 Hz, 2H, H6"), 7.36 – 7.31 (m, 5H, Ar-H), 7.29 (d, *J* = 8.0 Hz, 2H, H7"), 5.69 (s, 1H, NH), 5.34 (s, 2H, NH_2), 4.77 (d, *J* = 5.8 Hz, 2H, H1'). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 164.2 (C6), 163.2 (C4), 157.3 (C2), 138.6 (C2'), 135.7 (1'), 133.5 (C10''), 129.0 (C9''), 128.7 (C4'), 127.6 (C3'), 127.5 (C5'), 120.0 (C8''), 86.3 (C3''), 84.2 (C4''), 79.2 (C5), 74.3 (C2''), 73.3 (C1''), 44.9 (C1'). **IR (ν_{max}/cm^{-1}):** 3375 (NH_2), 3154 (=CH), 2205, 2136 (C triple bond C). ***HRMS*** *m/z:* calculated for $C_{22}H_{18}N_4ONa$: 358.8236, found: $[M+Na]^+$ 359.1056

4.8.10 Towards the synthesis of *N*^{4}-benzyl-5-((3,4-chlorophenyl)buta-1,3-diyn-1-yl)pyrimidine-4,6-diamine (71c)

64 (100 mg, 0.500 mmol) was dissolved in DCM (5 ml) along with 3,4-dichlorophenylacetylene (0.3 ml, 2.7 mmol), $Cu(OAc)_2.H_2O$ (20 mg, 0.10mmol) and piperidine (0.13 ml, 0.13 mmol). The reaction was stirred open to the atmospheric at room temperature for 12 hours. The reaction mixture was then concentrated *in vacuo* and the crude residue was purified by column chromatography. The product was isolated as a yellow solid (25 mg, 20%)



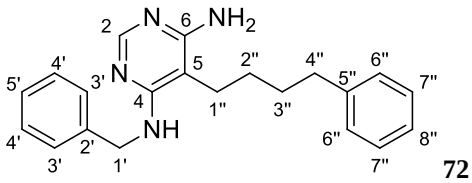
71c

R_f (80% EtOAc/hexane) 0.71. *mp* 165 °C **^{1}H NMR (500 MHz, $CDCl_3$)** δ 8.15 (s, 1H, H2), 7.57 (s, 1H, H10''), 7.41 (d, *J* = 8.3 Hz, 1H, H6''), 7.36 (m, 5H, Ar-H), 7.31 (d, *J* = 8.1 Hz, 1H, H7''), 5.65 (s, 1H, NH), 5.30 (s, 2H, NH_2), 4.75 (d, *J* = 5.8 Hz, 2H, H1').

13C NMR (126 MHz, $CDCl_3$) δ 164.2 (C6), 163.2 (C4), 157.4 (C2), 138.5 (C2'), 134.0 (C8''), 133.7 (C10''), 132.9 (C9''), 131.2 (C6''), 130.6 (C7''), 128.8 (C4'), 127.6 (C3'), 127.5 (C5'), 121.5 (C5''), 86.1 (C3''), 82.9 (C4''), 79.0 (C5), 75.3 (C2''), 74.1 (C1''), 44.9 (C1'). **IR (ν_{max}/cm^{-1}):** 3466, 3396 (NH_2), 3068, 2924 (=CH), 2201, 2129 (C triple bond C). ***HRMS*** *m/z:* calculated for $C_{21}H_{14}N_4Cl_2Na$: 393.2687, found: $[M+ Na]^+$ 393.0667

4.9 Synthesis of *N*^{4}-benzyl-5-(4-phenylbutyl)pyrimidine-4,6-diamine (72)

N^4-benzyl-benzyl-5-(phenylbuta-1,3-diyn-1-yl)pyrimidine-4,6-diamine **68** (1 mol eq) was dissolved in ethanol (10 ml) before adding 10% Pd/C (33 mole %). Oxygen was removed from this system by vacuum before introducing hydrogen gas from a balloon; this step was repeated 3 times before the mixture was left to stir at room temperature for ~14 hours. The reaction was stopped upon completion and the Pd/C was filtered off over a bed of celite followed by the evaporation of the solvent. The product was purified by preparative TLC (40% EtOAc/hexane)



72

68 (100 mg, 0.300 mmol) and 10% Pd/C (30 mg) were stirred together in ethanol before introducing hydrogen gas and stirring overnight. The crude product was purified by PLC (40% EtOAc/hexane) in a developing tank. The resulting product was obtained as oil (30 mg, 29%).

R_f (80% EtOAc/hexane) 0.31. **^{1}H NMR (300 MHz, $CDCl_3$)**δ 8.11 (s, 1H, H2), 7.36 – 7.22 (m, 7H, Ar-H), 7.21 – 7.10 (m, 3H, Ar-H), 4.65 (m, 2H, H1'), 4.61 (m, 1H, NH), 4.54 (s, 2H, NH_2), 2.63 (t, *J* = 7.4 Hz, 2H, H4''), 2.24 (dd, *J* = 8.7, 7.0 Hz, 2H, H1''), 1.80 – 1.62 (m, 2H, H3''), 1.61 – 1.46 (m, 2H, H2''). **^{13}C NMR (75 MHz, $CDCl_3$)** δ 159.9 (C6), 159.9 (C4), 155.7 (C2), 141.7 (C5''), 139.5 (C2'), 128.6 (C6''), 128.4 (C7''), 128.3 (C4'), 127.5 (C3'), 127.2 (C5'), 125.9 (C8''), 95.6 (C5), 45.3 (C1'), 35.5 (C4''), 31.1 (C3''), 26.1 (C2''), 24.0 (C1''). **IR (ν_{max}/cm^{-1}):** 3375 (NH_2), 3154 (=CH). ***HRMS*** *m/z:* calculated for $C_{21}H_{14}N_4Cl_2Na$: 332.4421, found: $[M+ Na]^+$ 332.0667

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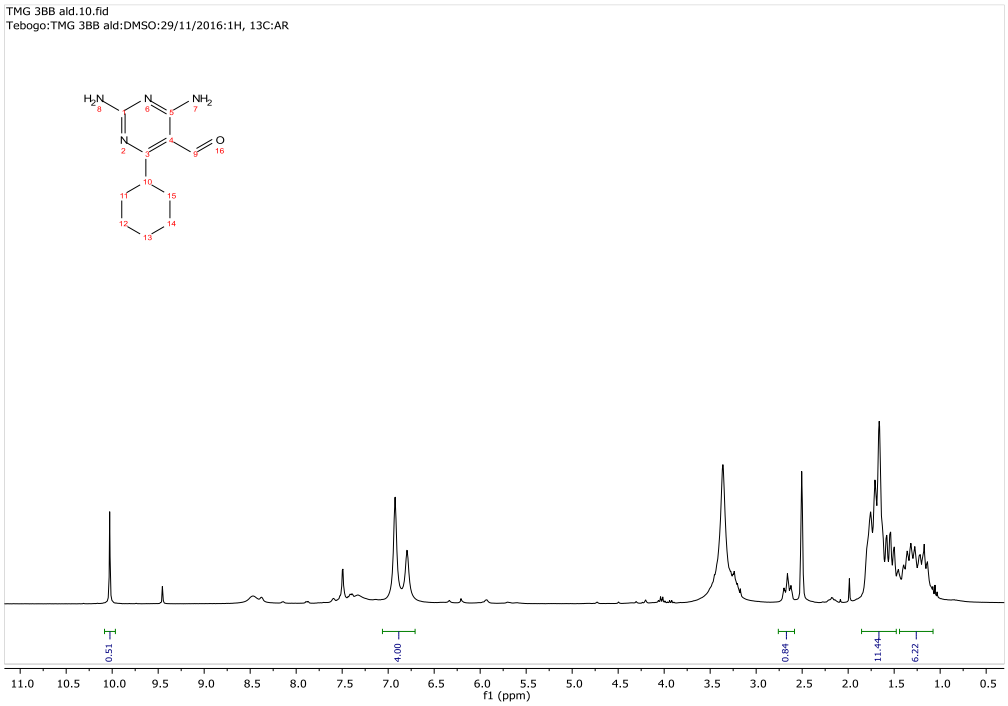
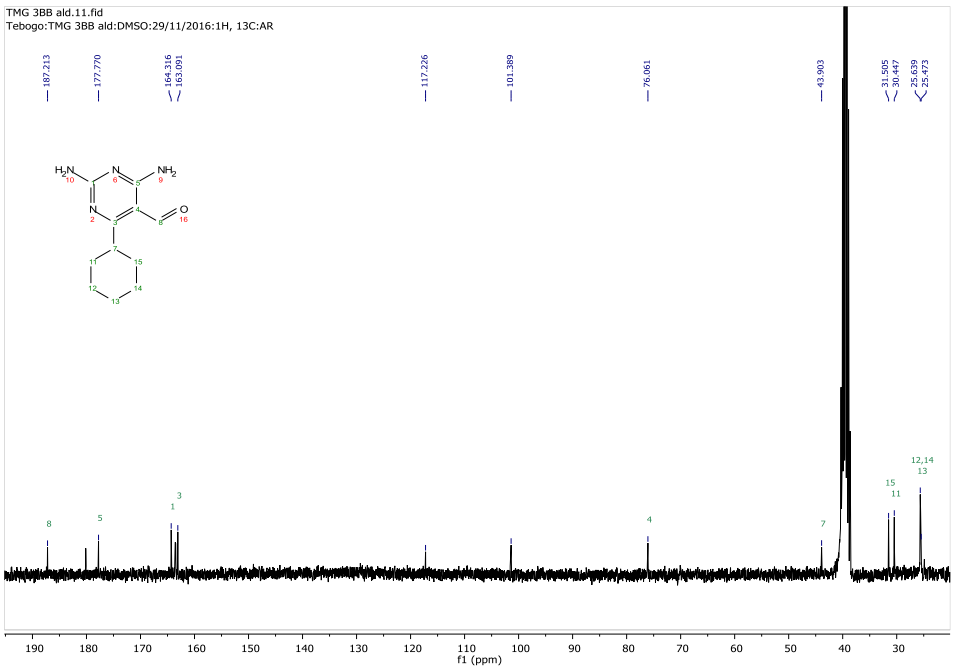
Appendix

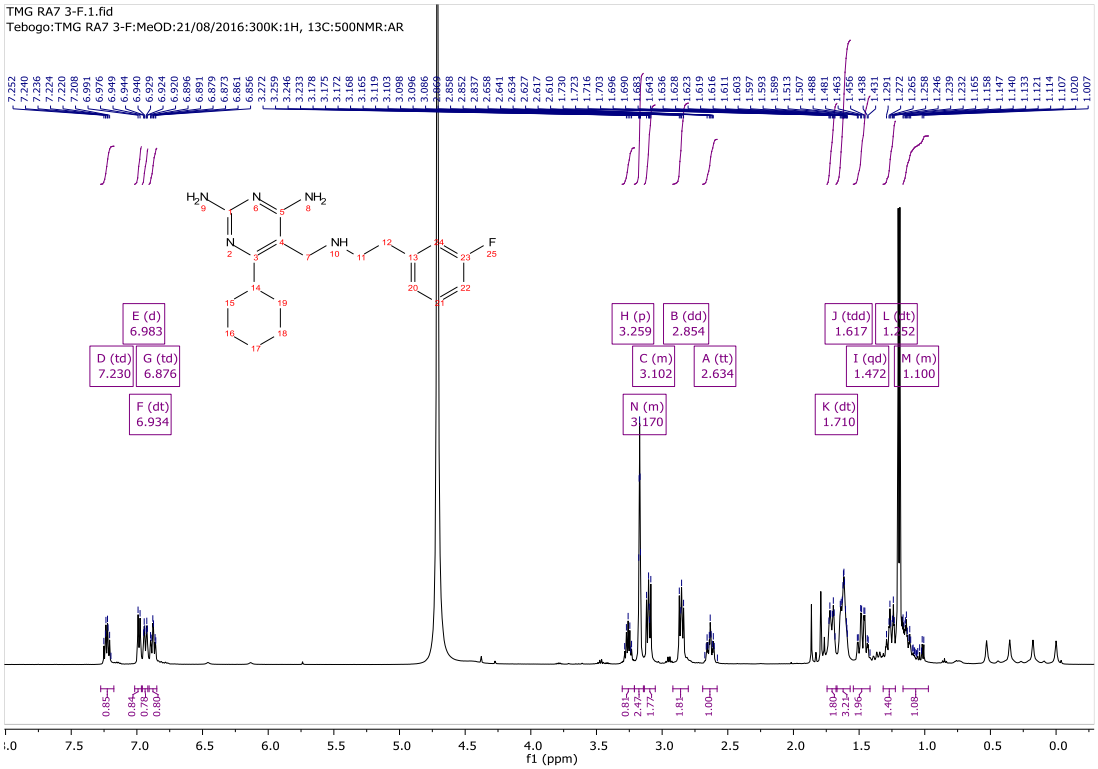
Spectra of selected compounds:

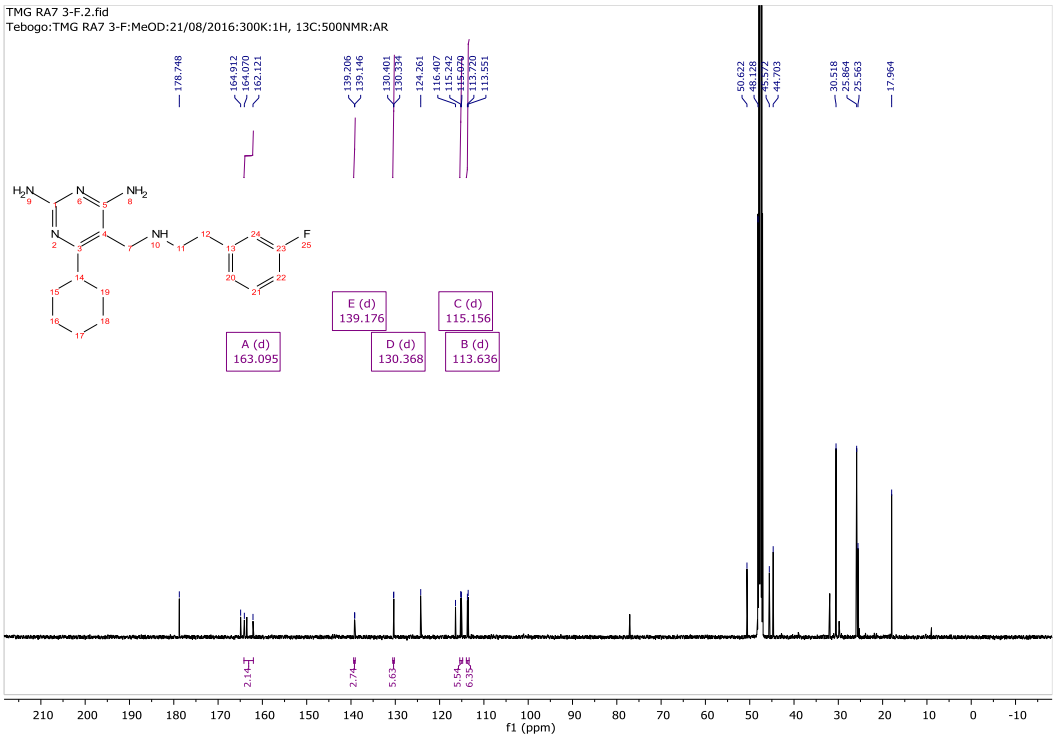
NMR data for 30:



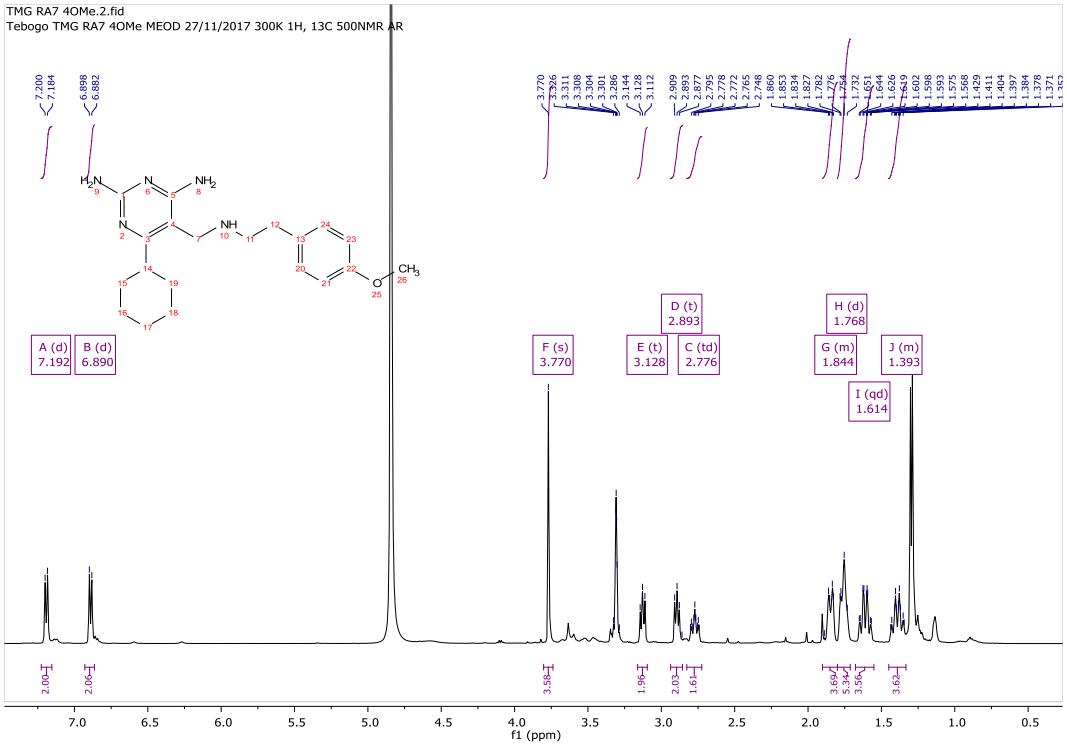


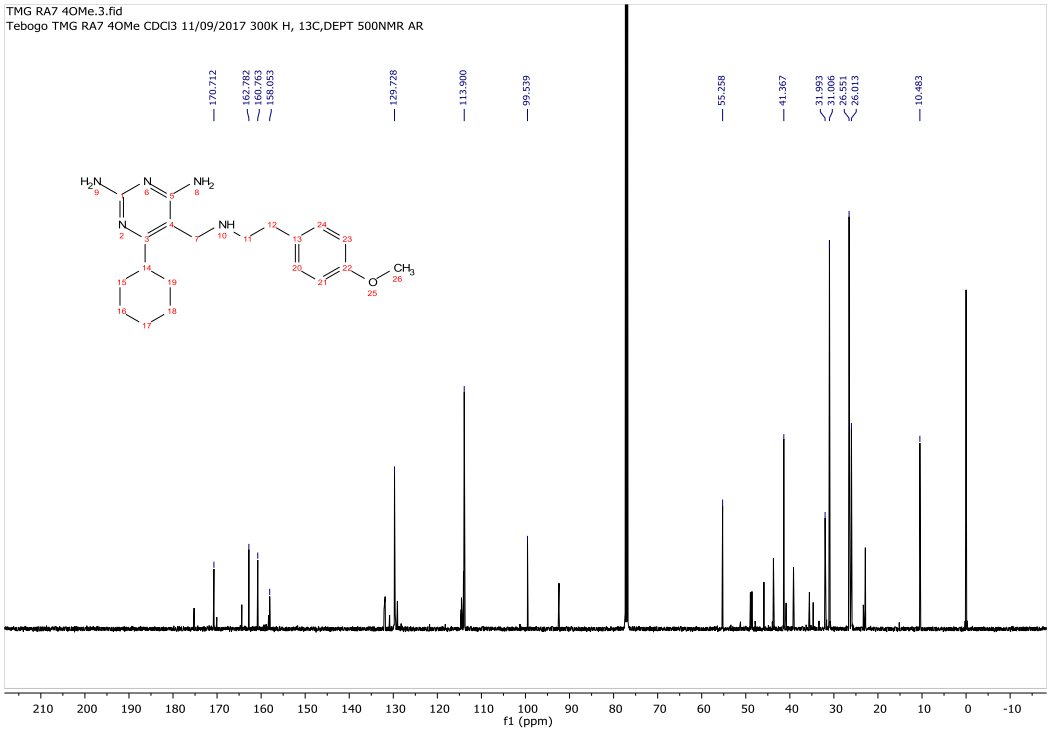
NMR spectra of 33a:





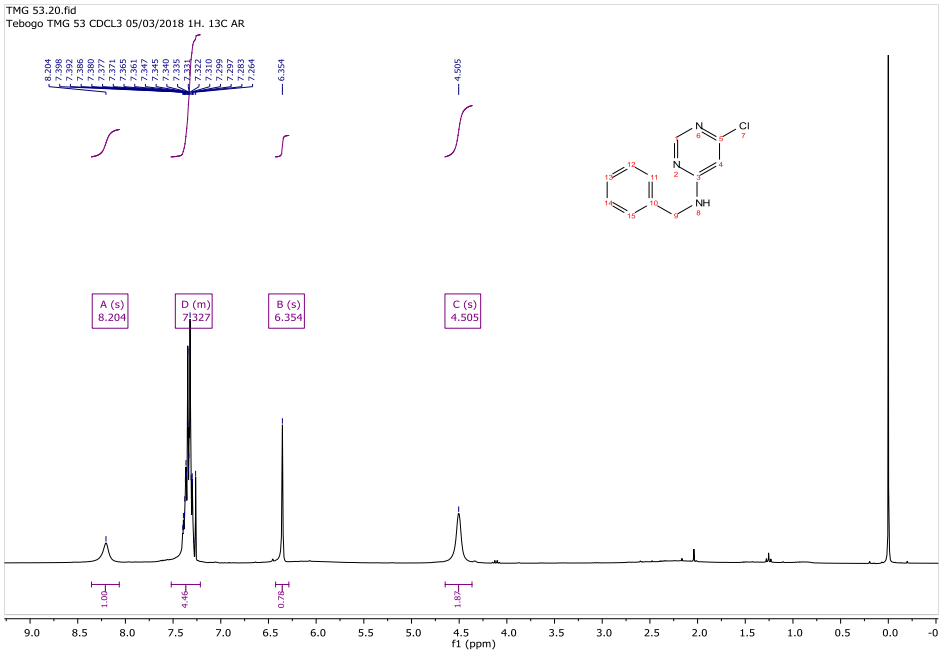
NMR spectra of 33b:

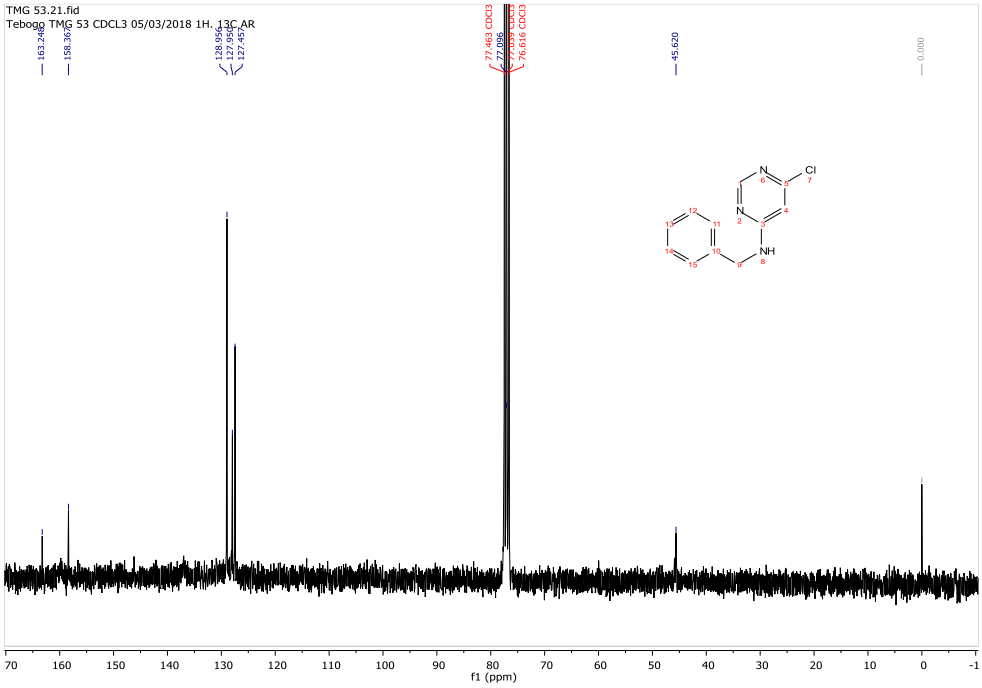




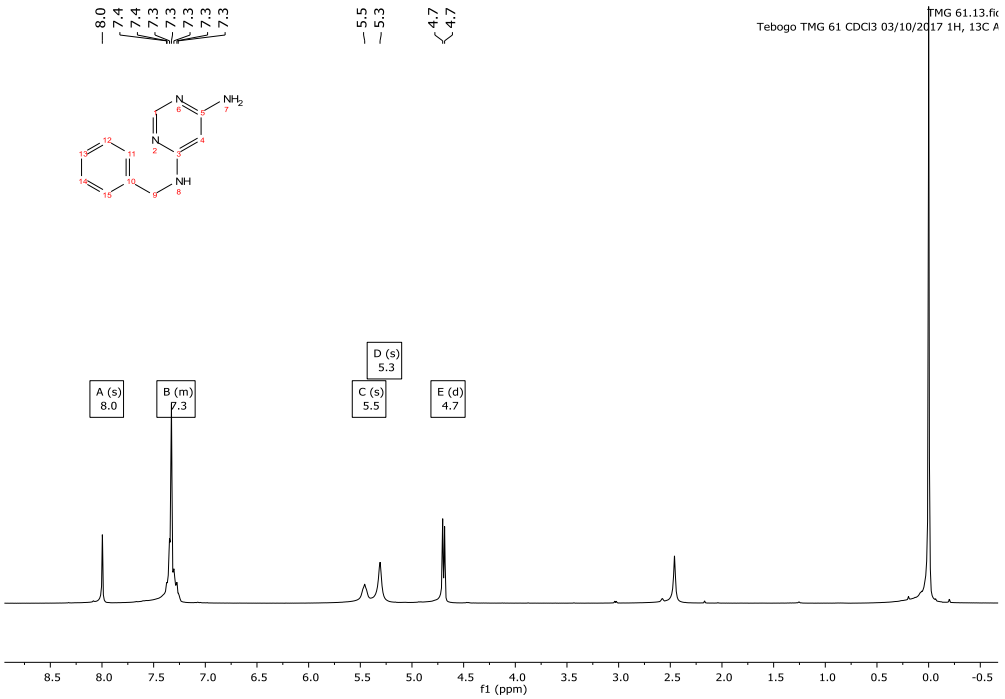
Part 2

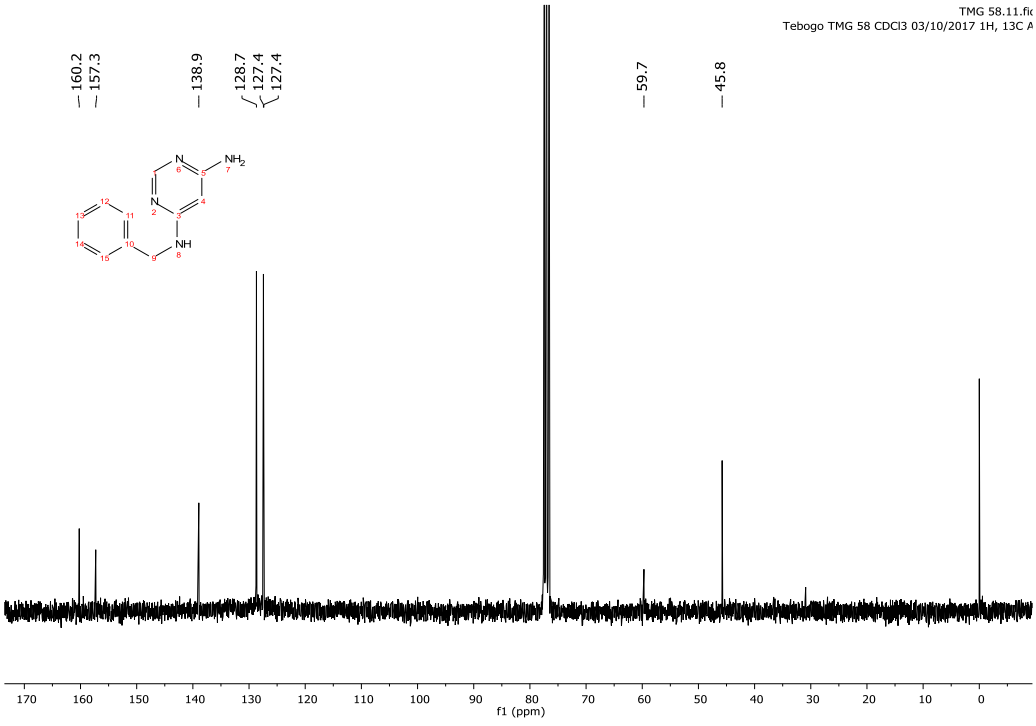
NMR spectra of 53:



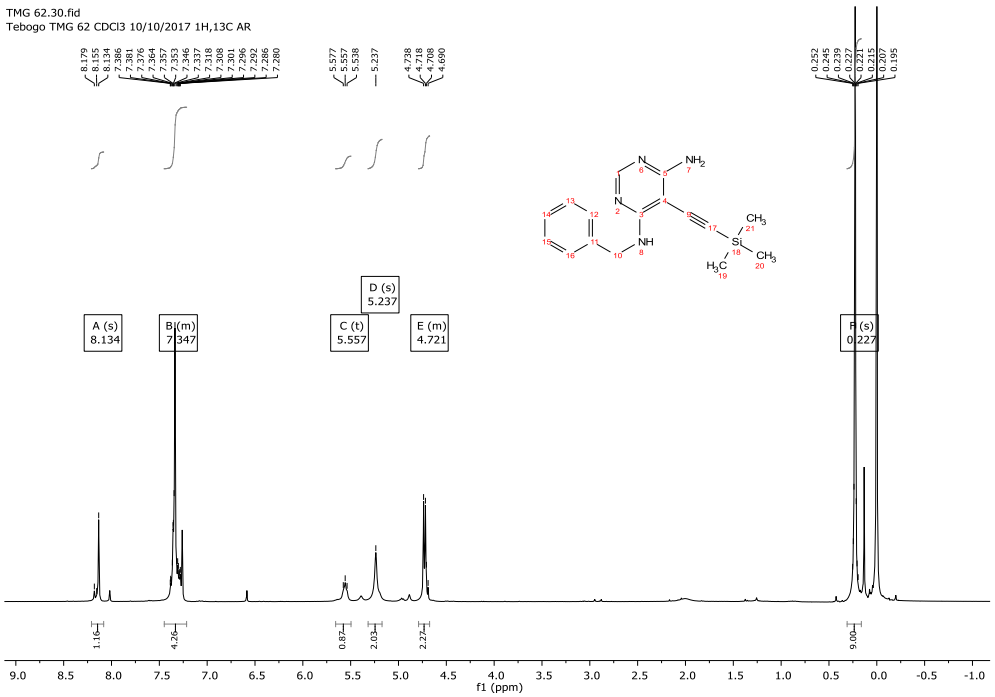


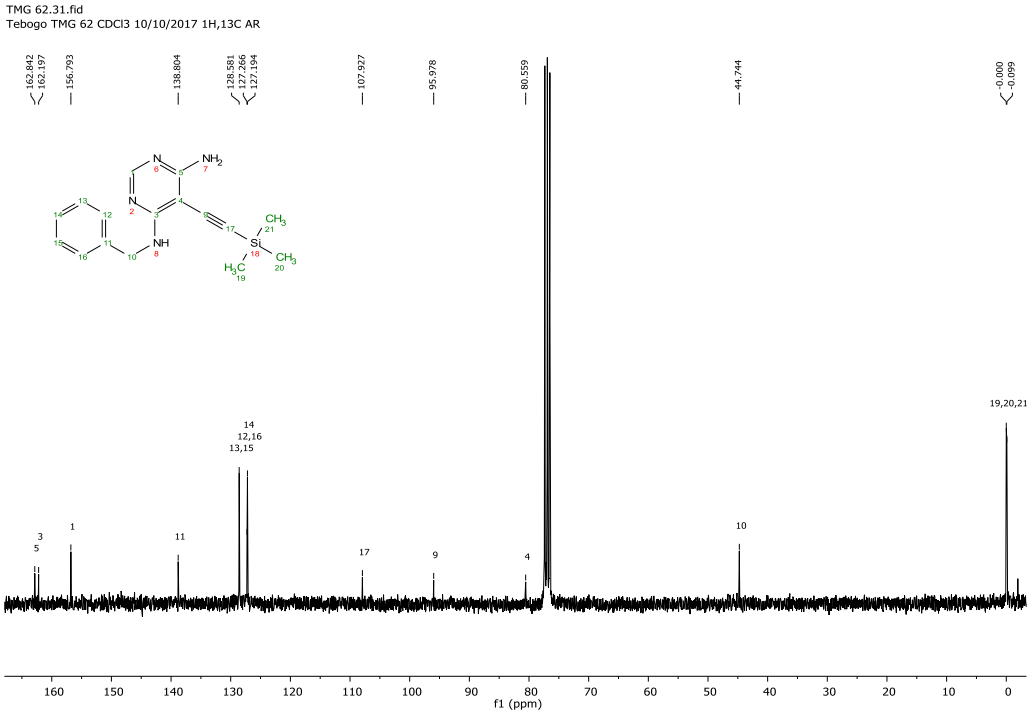
NMR spectra of 56:



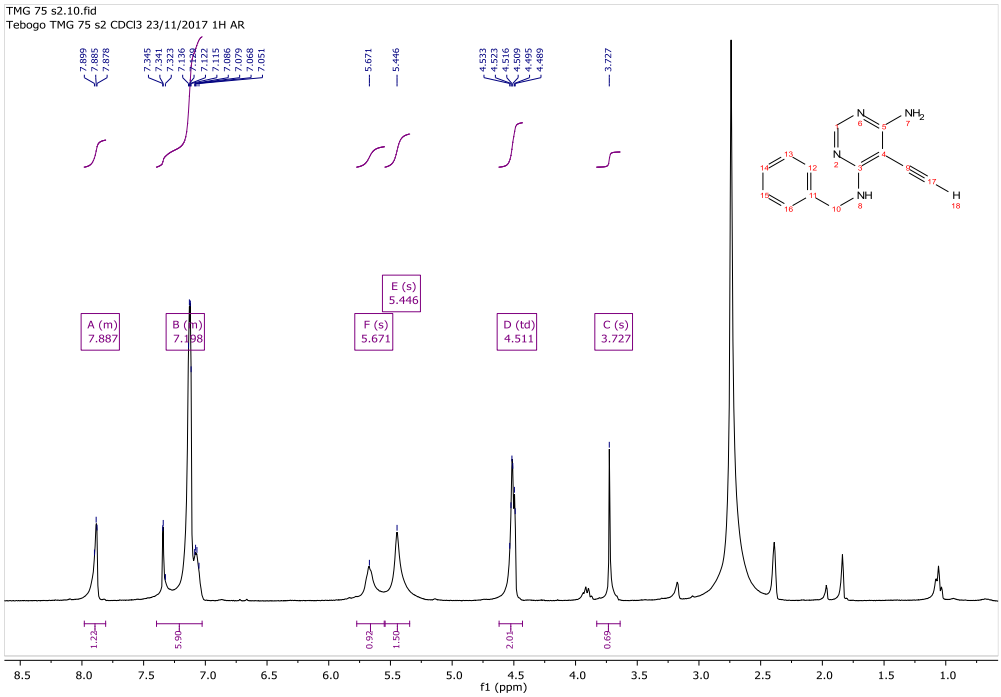


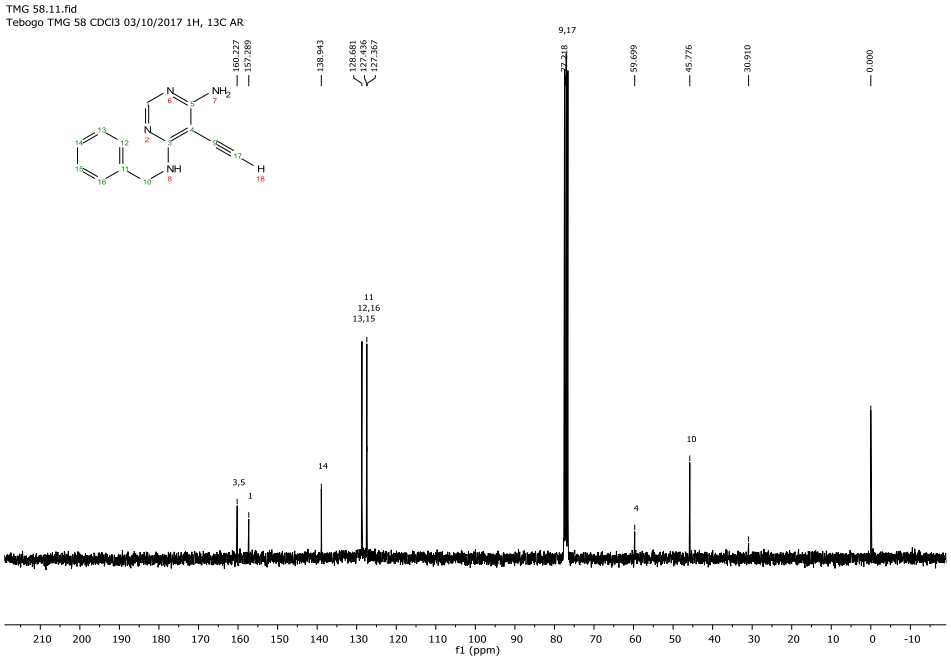
NMR spectra of 63:



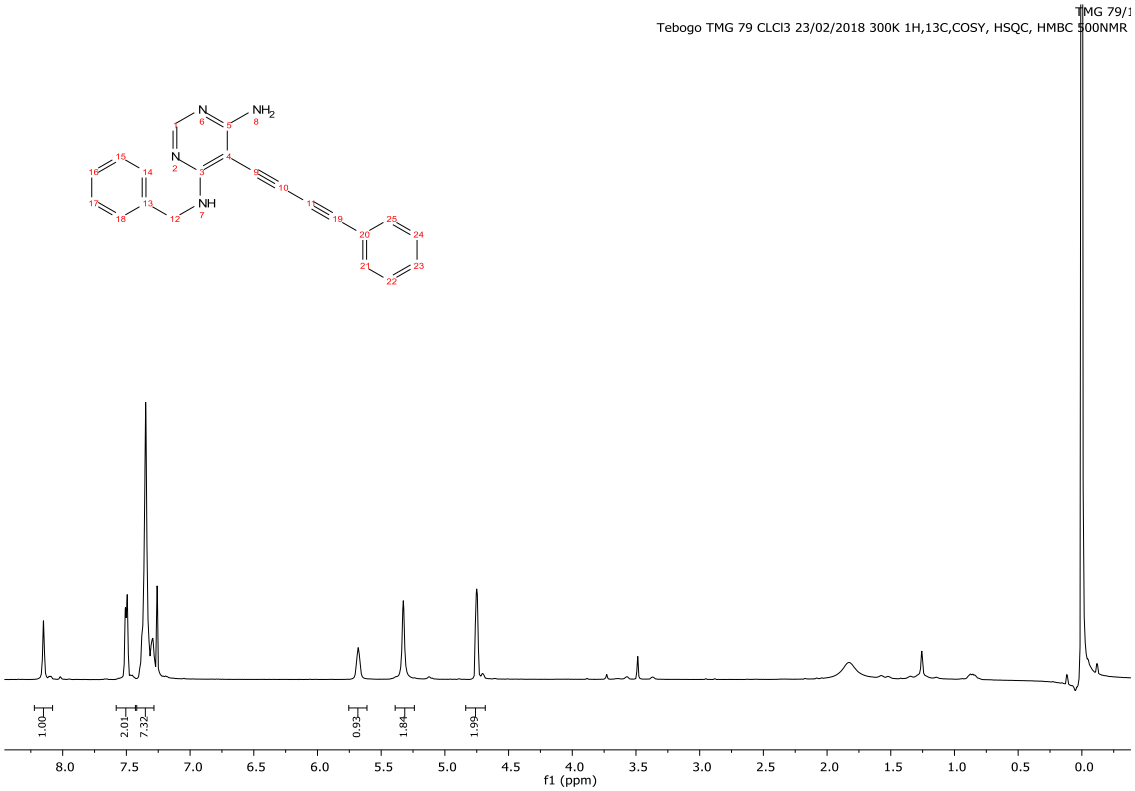


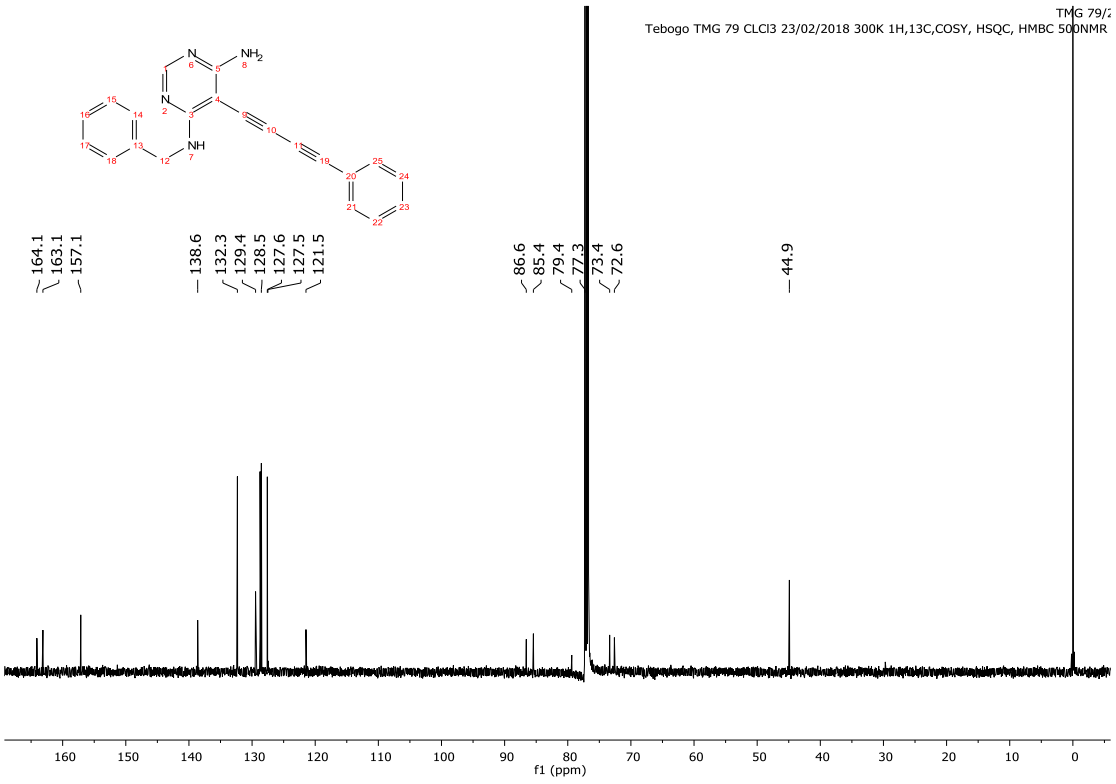
NMR spectra of 64:



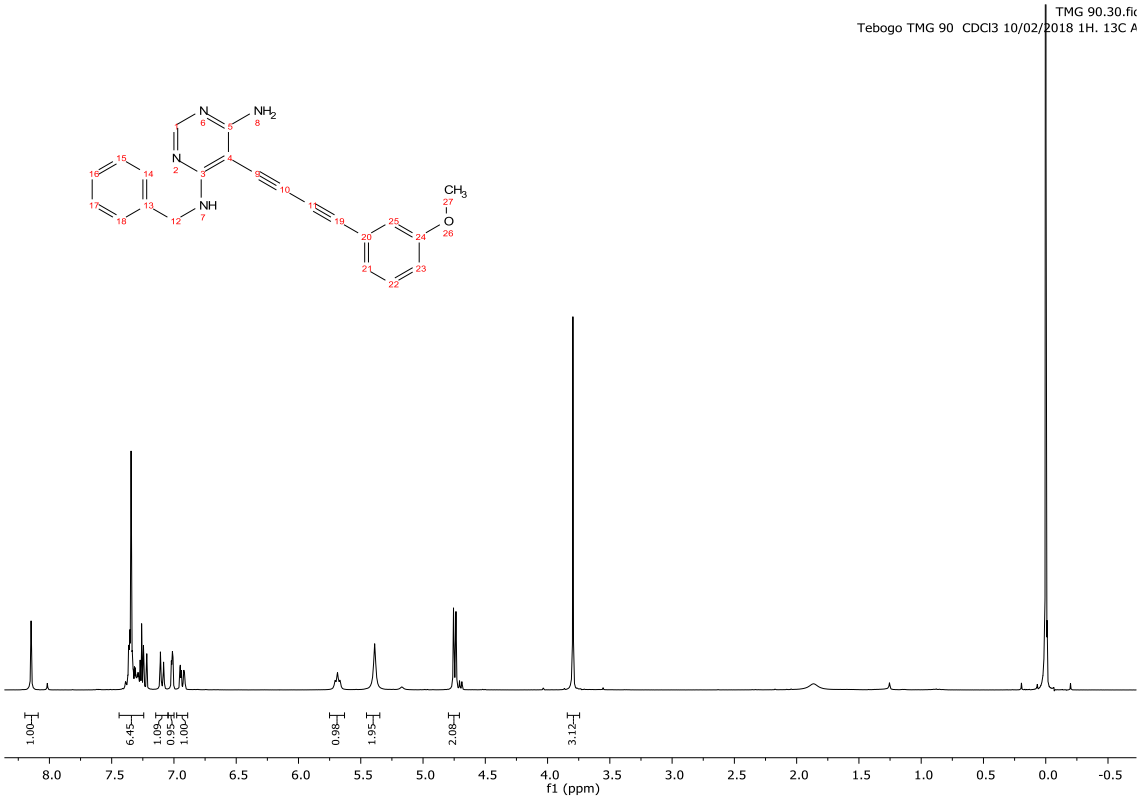


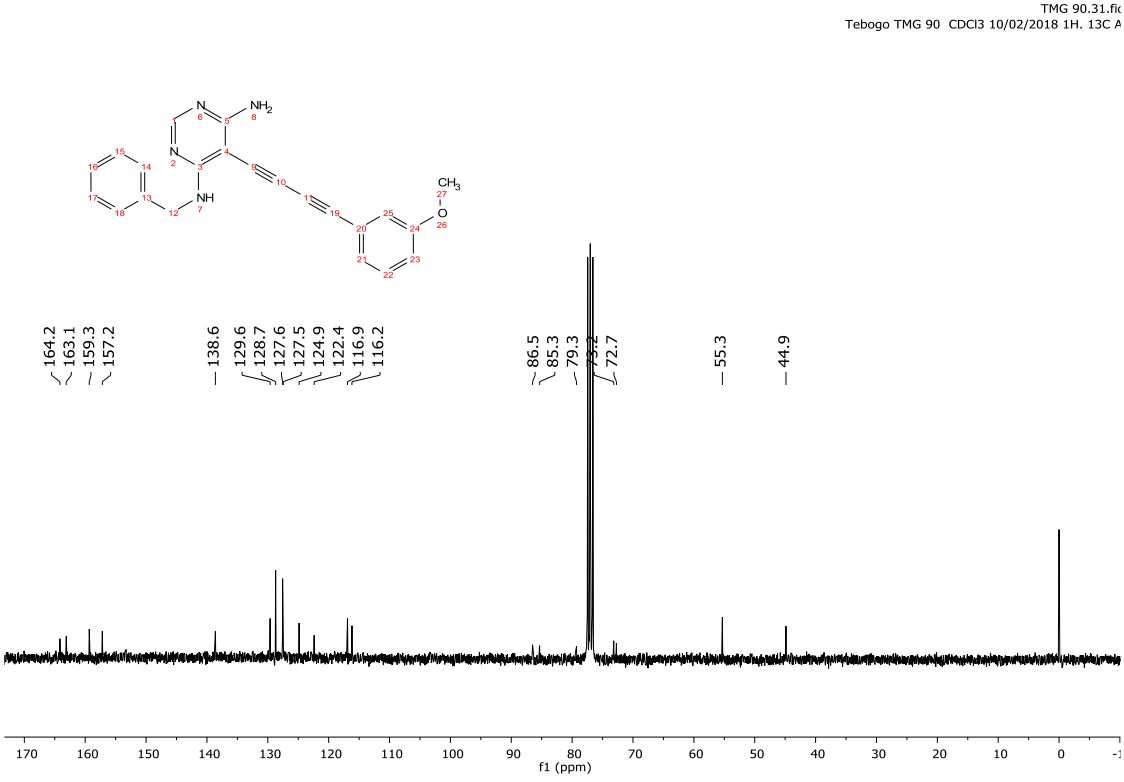
NMR spectra of 68:



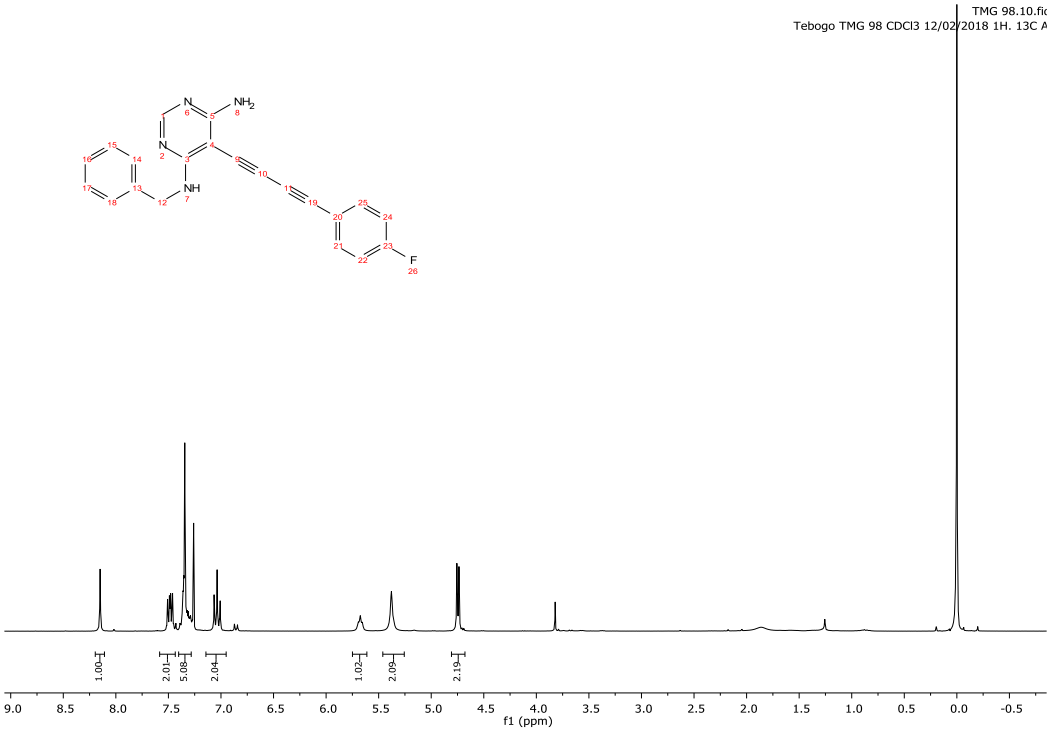


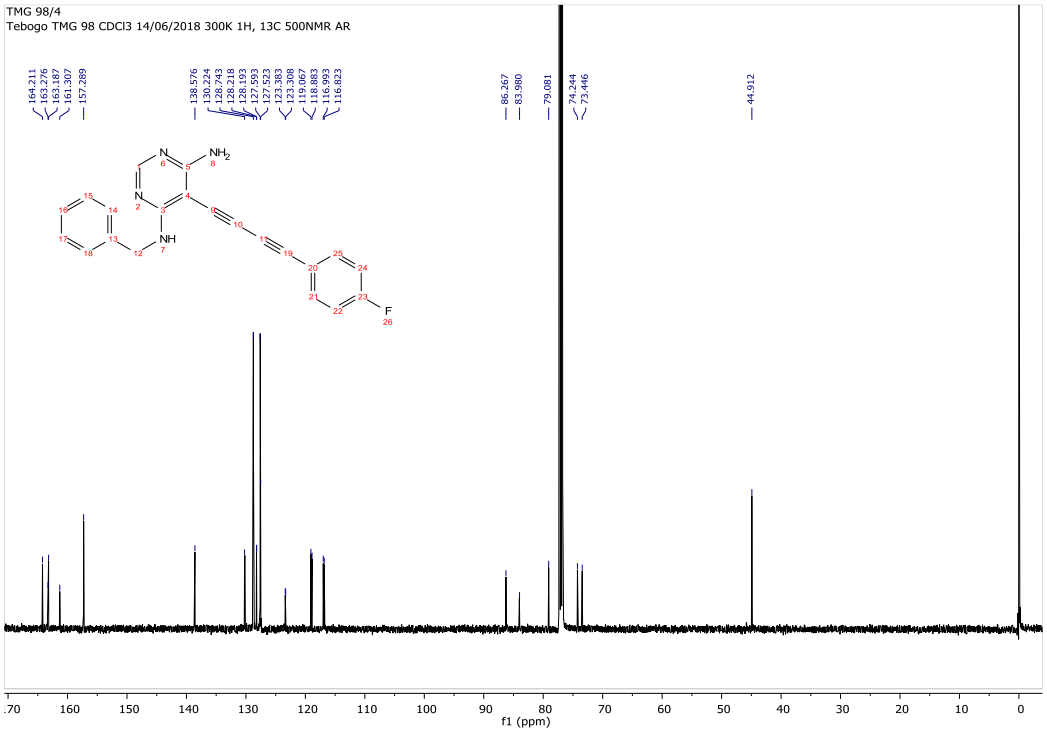
NMR spectra of 69b:



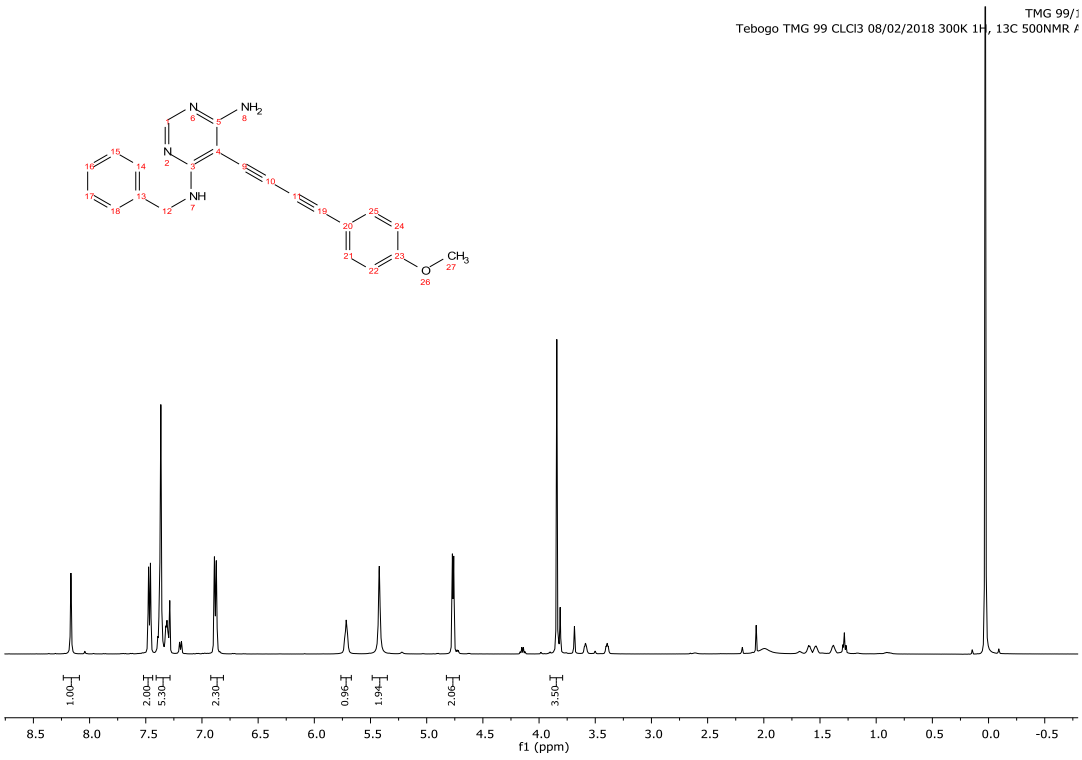


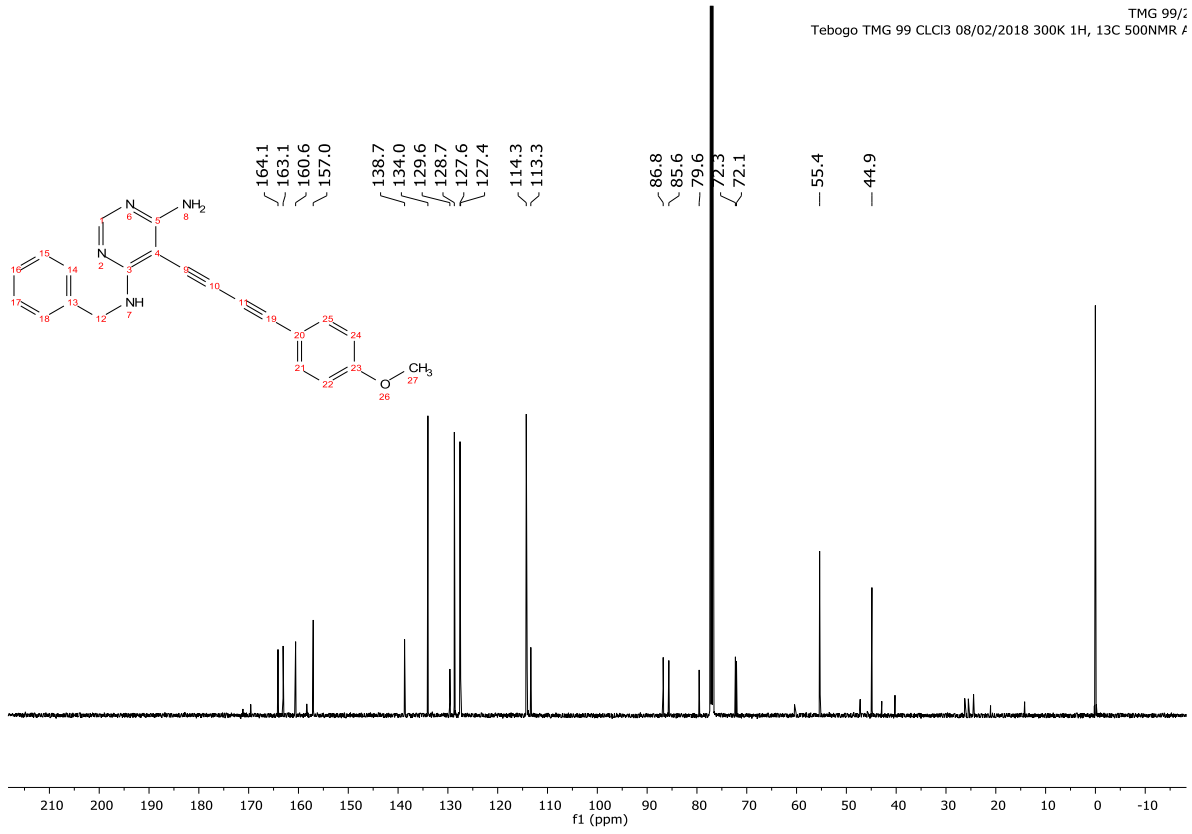
NMR spectra of 70c:



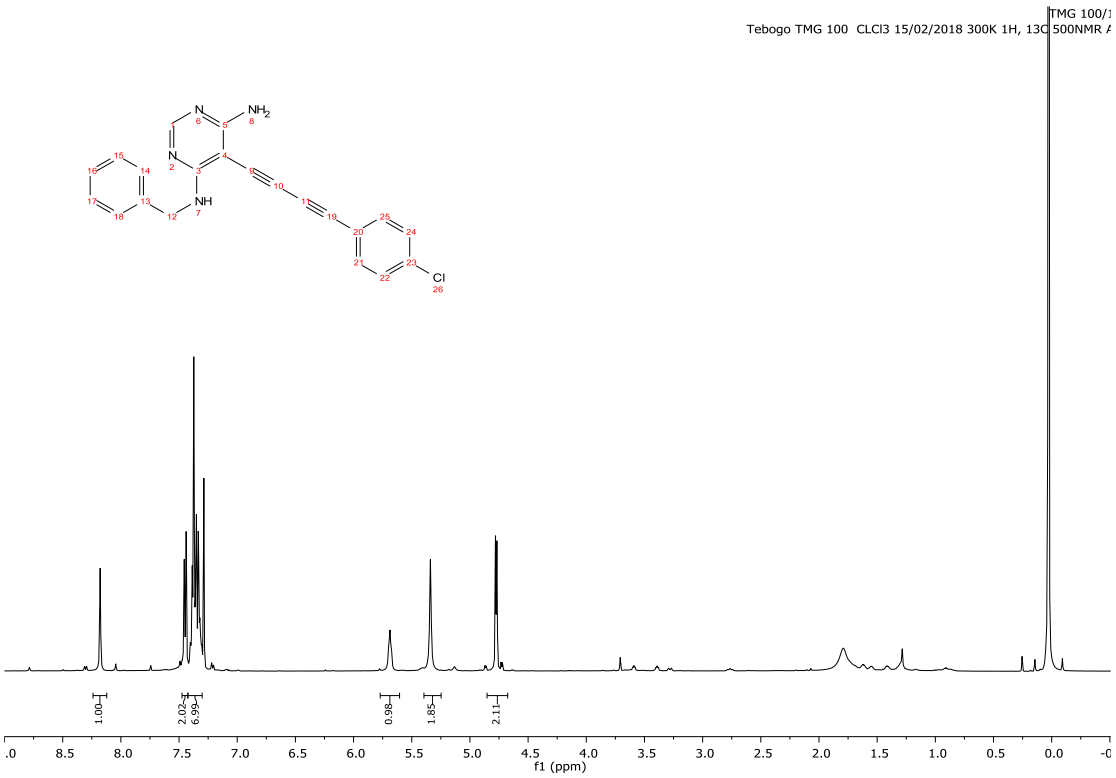


NMR spectra of 70c:





NMR spectra of 71b: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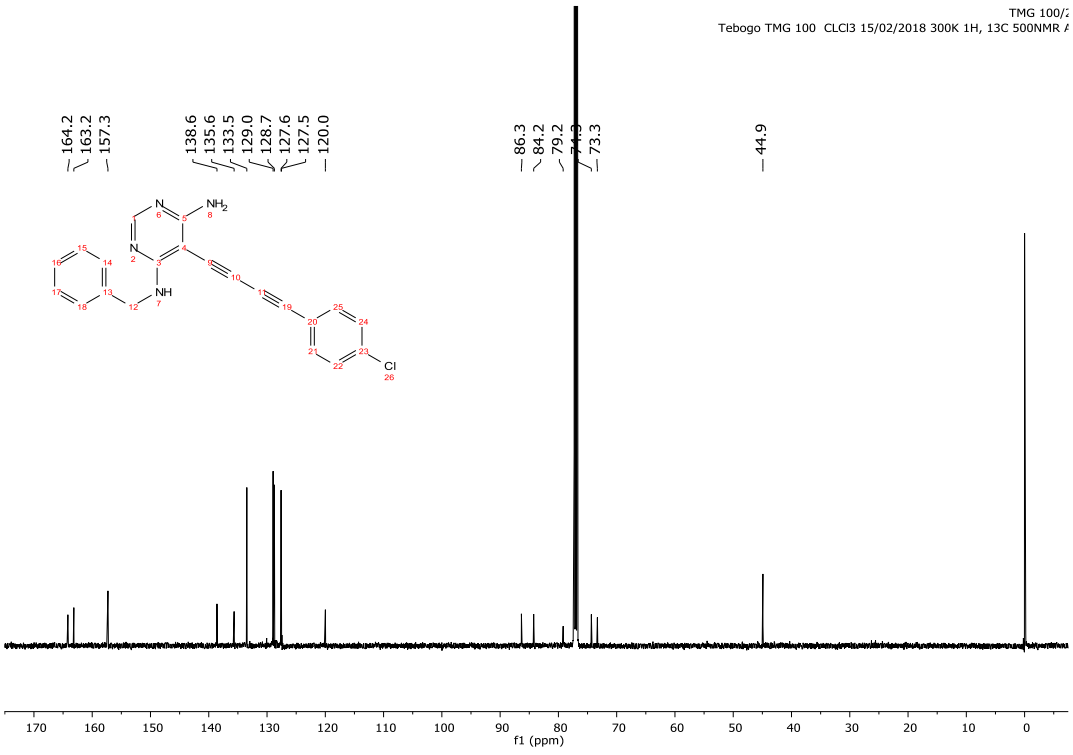
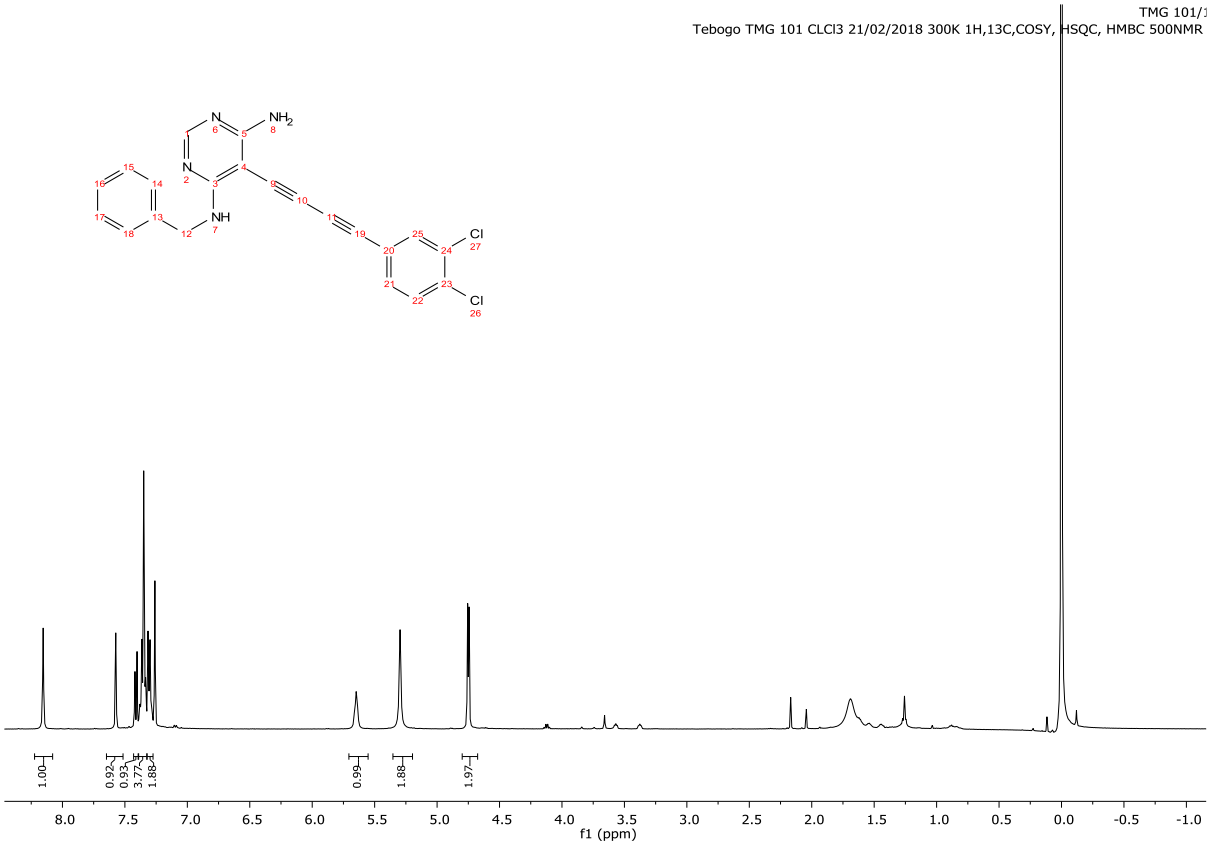
2.02
6.99
0.98
1.85
2.11
f1 (ppm)


TMG 100/2
Tebogo TMG 100 CLCl3 15/02/2018 300K 1H, 13C 500NMR
164.2
163.2
157.3
138.6
135.6
133.5
129.0
128.7
127.6
127.5
120.0
86.3
84.2
79.2
74.3
73.3
44.9
f1 (ppm)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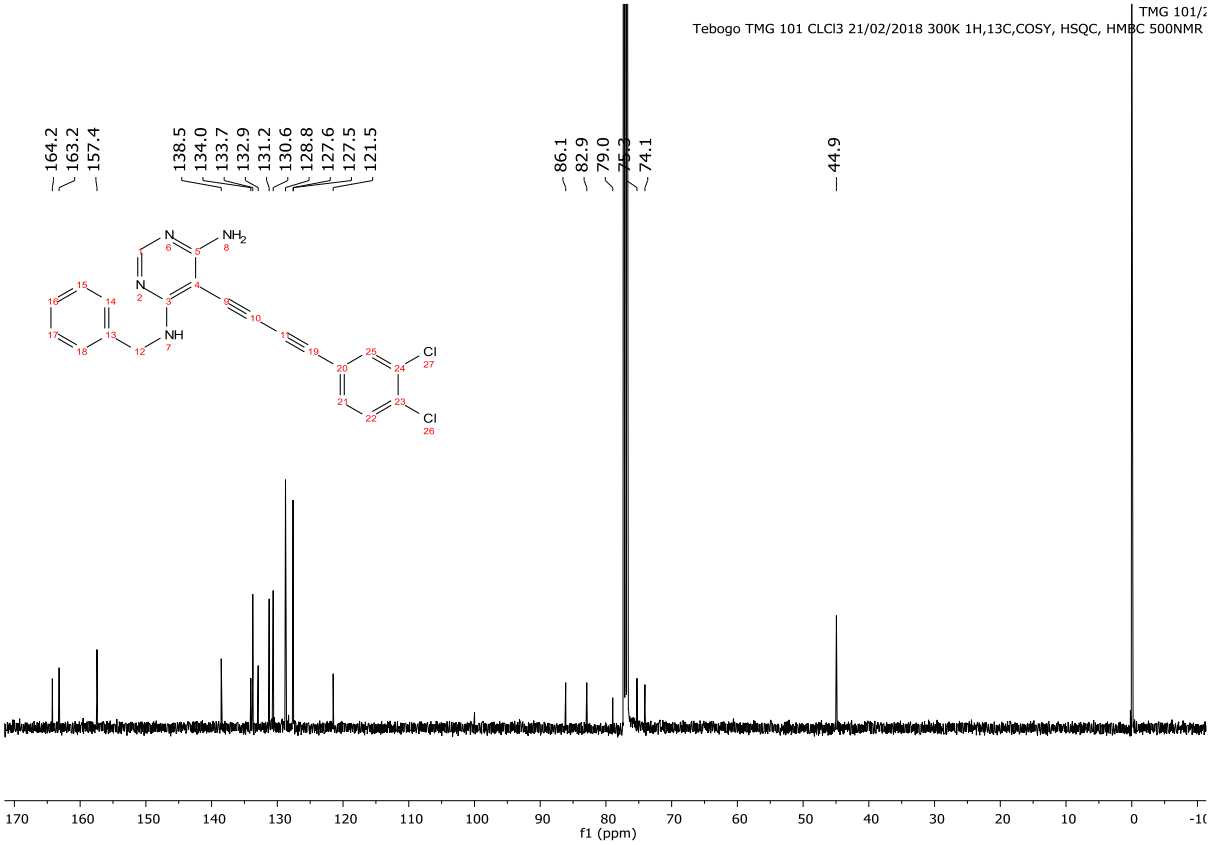
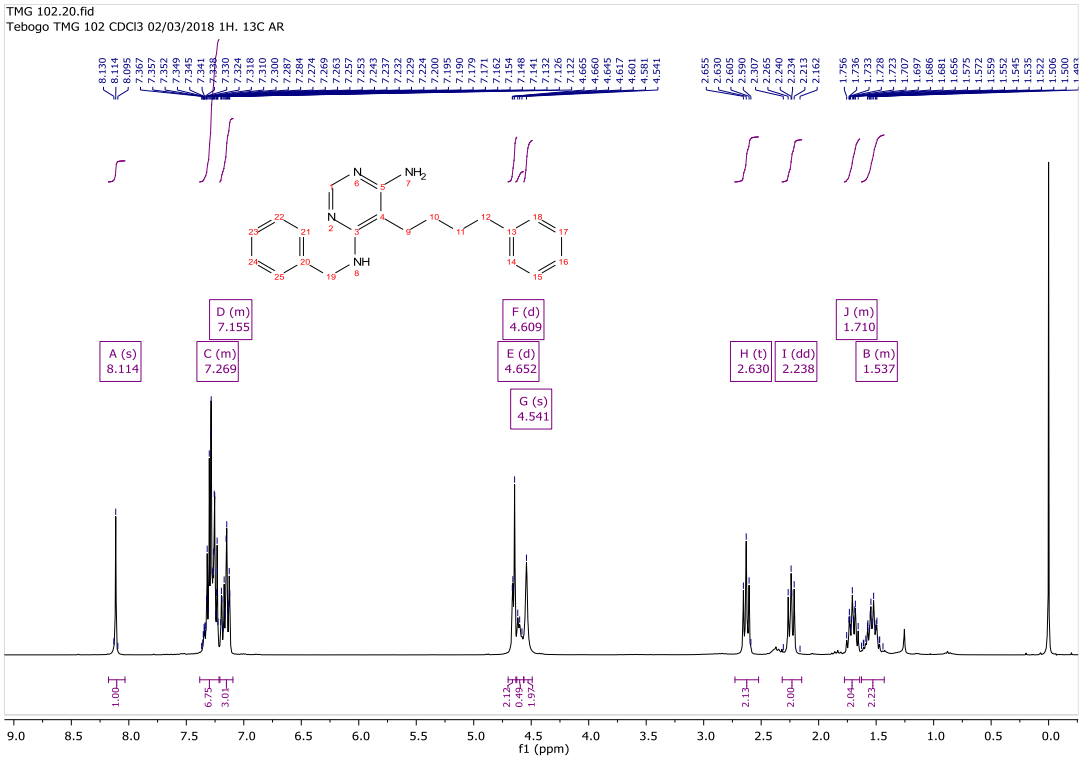
0.93
3.77
1.88
0.99
1.88
1.97
f1 (ppm)


TMG 101/2
Tebogo TMG 101 CLCl3 21/02/2018 300K 1H,13C,COSY, HSQC, HMBC 500NMR
164.2
163.2
157.4
138.5
134.0
133.7
132.9
131.2
130.6
128.8
127.6
127.5
121.5
86.1
82.9
79.0
75.3
74.1
44.9
f1 (ppm)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C (m) 7.269
D (m) 7.155
E (d) 4.652
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I (dd) 2.238
B (m) 1.537
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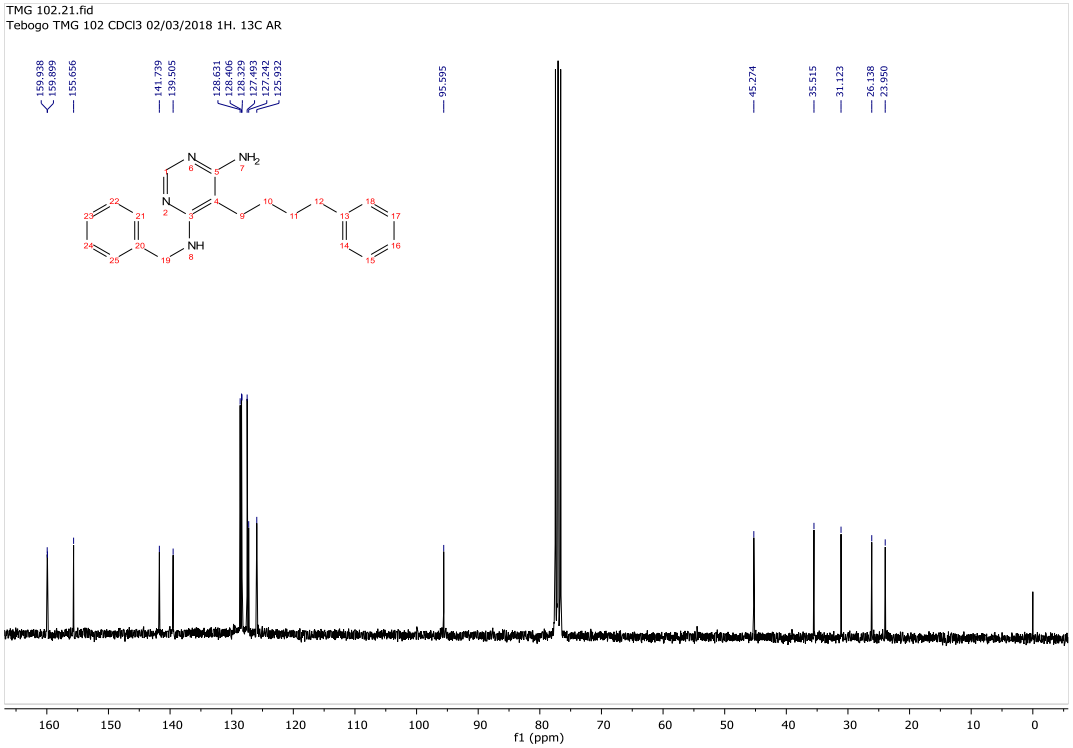
f1 (ppm)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f1 (ppm)