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SYNTHESIS AND BIOLOGICAL EVALUATION OF
PLASMODIUM FALCIPARUM CALCIUM-DEPENDENT
PROTEIN KINASE INHIBITORS FOR MALARIA
TREATMENT AND TRANSMISSION-BLOCKING

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Declaration

I declare that the work presented in this dissertation was carried out exclusively by myself under the supervision of Prof Amanda L. Rousseau, Prof Charles B. de Koning, and Dr Hendrik Henning. It is being submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg and has not been submitted before for any degree or examination at any other university.



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Abstract

Malaria is a mosquito-borne disease that was responsible for an estimated 249 million cases and 608 000 mortalities within 85 malaria endemic countries and areas in 2022, with children under the age of 5-years most burdened. Only five of the *Plasmodium* parasites are known to infect humans, with *P. falciparum* causing the most severe form of malaria. Several drugs have been used in the treatment of malaria, however, due to the emergence of parasite resistance, the efficacy of most available drugs has been reduced, prompting a need for the development of novel antimalarial agents to prevent an increase in malaria incidences and mortalities. Ideal novel antimalarial agents are those that target both the asexual and sexual forms of the *Plasmodium* parasite in the human host, to both treat and prevent the transmission of malaria. Two *P. falciparum* calcium-dependent protein kinases (*Pf*CDPKs), *Pf*CDPK1 and *Pf*CDPK4, have emerged as attractive targets for such antimalarial compounds owing to their involvement in both the asexual and sexual blood stages of the parasite, and their absence in the human kinome. In this PhD thesis, we explore the pyrrolo[2,3-*d*]pyrimidine derivatives and their ring open analogues, the 4,6-diaminopyrimidine derivatives, as potential inhibitors of *Pf*CDPK1 and *Pf*CDPK4 towards the treatment of malaria and blocking of parasite transmission from human host to the mosquito.

The first part of the thesis involves the design and *in silico* study of the targeted compounds, the pyrrolo[2,3-*d*]pyrimidine and the 4,6-diaminopyrimidine derivatives. We assessed the binding affinities of these compounds *in silico* against the *Pf*CDPK4 model prepared from the crystal structure 4QOX from the RCSB Protein Data Bank, and the *Pf*CDPK1 model prepared through homology modelling. The compounds were docked into the active site of the enzyme and interacted in the binding site in a similar way as the adenine ring of the natural substrate, adenosine triphosphate (ATP). Substituents important for selectivity and potency were chosen, and shown to occupy additional binding pockets, suggesting that our target compounds are potential inhibitors of *Pf*CDPK1 and *Pf*CDPK4. A representative docking pose from each series of compounds was chosen to evaluate the compound-enzyme stability using molecular dynamics and we found that the afforded poses are relatively stable with the hinge region interaction between the enzymes and the ligands playing a central role. Furthermore, we evaluated the compounds' pharmacokinetic properties and toxicity potential, and results indicated that the molecules have good oral bioavailability and poor potential to cause neurological side effects but could cause cardiac-based complications.

The second part of the research focuses on the synthesis and biological evaluation of the targeted pyrrolo[2,3-*d*]pyrimidine and 4,6-diaminopyrimidine-based compounds. The synthesis of the pyrrolo[2,3-*d*]pyrimidine compounds was achieved using a synthetic route consisting of nine synthetic steps which includes the formation of the pyrrolo[2,3-*d*]pyrimidine ring and the addition of side-groups that confer potency and selectivity to the compounds. We modified some of the synthetic steps using microwave irradiation, including the Sonogashira coupling reaction. Three compounds were found to be exclusively active against recombinant *Pf*CDPK4 in the micromolar range, while one was active against *Pf*CDPK1, also in the micromolar range.

The 4,6-diaminopyrimidine derivatives bearing a two-atom linker to a bulky naphthalene substituent were divided into three classes the ethyne-, ethene-, and ethane-linked compounds. The ethyne- and ethene-linked compounds were afforded through at least four synthetic steps starting from 4,6-dichloropyrimidine. Three of these synthetic steps were modified using microwave irradiation. We could not prepare the target ethane-linked compounds by our planned hydrogenation of the corresponding alkyne precursors, as this reaction afforded only alkene products. This could be due to steric hindrance, although alternative reduction methods were not attempted. Unfortunately, the compounds from these series are yet to be tested for biological activity against *Pf*CDPK1 and *Pf*CDPK4.

A second series of 4,6-diaminopyrimidine derivatives bearing a one-atom linker to a bulky naphthalene substituent were divided into two classes: those bearing a carbon-linker, and heteroatom-linked compounds. We developed the synthetic protocol for the synthesis of carbon-linked 4,6-diaminopyrimidine compounds on a model study as far as the formation of 5-benzyl-6-chloropyrimidin-4-amine through five synthetic steps; with only one synthetic step remaining to afford an analogue of the target compounds. We have yet to apply the developed protocol in the synthesis of the targeted carbon-linked 4,6-diaminopyrimidine compounds and test them for biological activity.

The development of a synthetic route to afford heteroatom-linked 4,6-diaminopyrimidine derivatives went as far as four synthetic steps of the planned six synthetic steps. Microwave irradiation played a central role in affording three of the intermediates, significantly reducing the synthesis time and solvent quantities. We have yet to complete and apply the synthetic route to our targeted compounds which will be tested for biological activity against *Pf*CDPK1 and *Pf*CDPK4.

Molecular modelling has proved to be a viable tool in the design of our ligands and prediction of their binding potential in the active site of the targeted enzymes. To the scope of our research, the

models we developed have shown good accuracy in predicting the binding affinity of the ligands *in vivo* as confirmed by experimental data. It gives us confidence that the biological results from the targeted 4,6-diaminopyrimidine derivative will agree with *in silico* data to a great degree. Also, it justifies the further use of molecular modelling in the design of new ligands and improvement of current ligands towards finding molecules that are potent inhibitors of the targeted enzymes.

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Dedication

To my dearest loving mother – Mrs MK Chavalala, late father – Mr RS Chavalala,
and siblings – Carol, Mariam, Grace, Mavis, Rueben, and Joel.
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Chapter 1: Background

1.1 Introduction

Malaria is a mosquito-borne parasitic disease that is contracted by humans through the saliva of an *Anopheles* mosquito during a blood meal. Malaria is characterised by symptoms such as fever, headaches, and muscle aches, to name a few, which are not unique to the disease making it difficult to diagnose and treat early. In 2022, malaria was responsible for an estimated 249 million cases and 608 000 mortalities within 85 malaria endemic countries [1]. The World Health Organization (WHO) African region accounted for most of these cases and mortalities with children under the age of 5-years most burdened [1]. The causative agent of malaria, the *Plasmodium* parasite, can infect various species of vertebrates, however, four are known to infect humans exclusively. These are *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. The fifth, *P. knowlesi*, has recently been found to also infect humans and can be transmitted from one human to another via the *Anopheles* mosquito vector [2].

Among the five human infecting *Plasmodium* parasites, *P. falciparum* causes the most severe form of malaria, followed closely by *P. vivax* [3]. The observed prolonged sequestration of erythrocytes infected with *P. falciparum* to endothelial cells and healthy erythrocytes is central to the virulent nature of *P. falciparum* malaria. Consequences of this phenomenon includes, but is not limited to, macrovascular occlusion which may lead to tissue infarction and cerebral malaria [4, 5]. Sequestration in *P. vivax* (and other forms of human malaria, but not *P. falciparum*) is not prolonged and thus, it is often subjected to parasite clearance through the spleen reducing blood parasitaemia [6]. The prevalence of *P. falciparum* in the WHO African region is one factor contributing to the observed disproportionately high malaria-based cases and mortalities, complemented by the emergence of parasite resistance to both insecticides and antimalarial drugs

The *Plasmodium* parasite survival and proliferation depends on two hosts, the *Anopheles* mosquitoes (also serving as a vector), and humans. Understanding the parasite's lifecycle (**Figure 1.1**) and biological processes undertaken is important in deriving strategies and solutions for the prevention, treatment, and eradication of malaria. Humans contract the *Plasmodium* parasite in the form of sporozoites from the *Anopheles* mosquito saliva secreted during a blood meal. The sporozoites migrate to the liver through the blood stream where they invade the hepatocytes in which they multiply into merozoites. *P. vivax* and *P. ovale* sporozoites remain dormant for weeks and sometimes months before being activated to form merozoites, causing malaria recurrence after

treatment [7]. Merozoites are then released back into the bloodstream where they invade erythrocytes starting the intraerythrocytic cycle. The growth of the parasite inside the erythrocytes proceeds through various stages starting with the development of young trophozoites (ring forms) which evolve into mature trophozoites. This is followed by the erythrocytic schizont stage, initially characterised by immature schizonts which develop into mature schizonts. Through erythrocytic schizogony, each mature schizont fabricates new merozoites which overwhelms the infected cell resulting in its destruction. The newly released merozoites invade healthy erythrocytes starting another intraerythrocytic cycle which persists until the parasites are cleared by the immune system or by therapy.

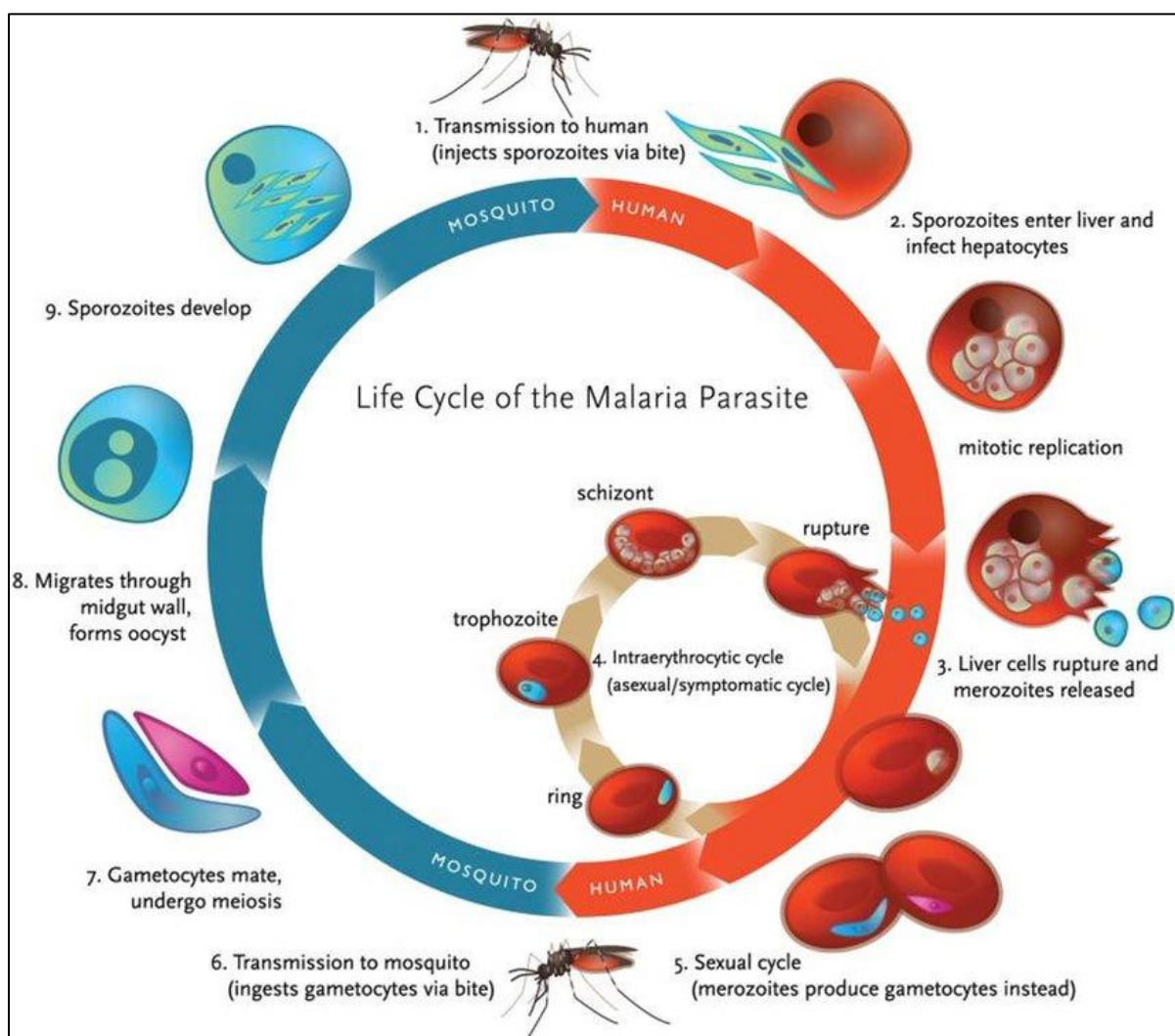


Figure 1.1. The malaria parasite lifecycle between the human host and the female *Anopheles* mosquito host [8].

A portion of the merozoites differentiate into male and female gametocytes (the sexual forms of the parasite) which are taken up by a female *Anopheles* mosquito during a blood meal. Within the mosquito midgut the female gametocytes mature into macrogametes while the male gametocytes

undergo exflagellation to form microgametes. The macrogametes are then fertilised by microgametes forming a zygote that evolves into an ookinete. The latter penetrates the mosquito midgut wall forming an oocyst, which matures producing sporozoites. The sporozoites are released from the oocyst and they migrate into the mosquito salivary glands where they are stored, completing the parasite lifecycle. The cycle is initiated again when the infected mosquito inoculates a healthy human with sporozoites during a blood meal.

1.2 Malaria prophylaxis and treatment

A typical strategy employed in the prevention and treatment of malaria involves the disruption of one or more stages of the malaria parasite lifecycle. These range from preventing physical contact between the vector and the host to targeting biological processes undertaken by the parasite for maturation and proliferation. Prophylactic agents target the vector or the development of the sporozoites inside the vector. They also target liver stage parasites preventing them from successfully forming merozoites that will initiate the intraerythrocytic parasite cycle – a stage where symptoms of malaria and complications manifest. In essence, prophylactic agents prevent the onset of symptomatic malaria in the human by targeting stages of the malaria lifecycle that precede the intraerythrocytic cycle. Treatment of malaria involves the use of chemotherapeutic agents that target the parasite involved in the intraerythrocytic stage. The agents can be used in monotherapy, or as a combination of drugs with at least one responsible for clearing the blood stage parasitaemia.

1.2.1 Vector-targeted interventions

Long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) of insecticides have played an important role in reducing malaria transmission. LLINs provide a physical barrier between the mosquito and humans and kills mosquitoes that land on the net through the insecticide. IRS targets mosquitoes that rest indoors after/before a blood meal. Pyrethroid was the common insecticide used with bed nets (owing to its relative safety when in contact with humans for longer durations), however, the emergence of resistant mosquitoes to pyrethroids prompted the use of other insecticides or a combination of insecticides. The World Health Organisation currently recommends the use of pyrethroid-chlorfenapyr, pyrethroid-pyriproxyfen, and pyrethroid-piperonyl butoxide in place of pyrethroids alone [1]. IRS alone or in combination with LLINs has been shown to reduce the malaria parasite prevalence, especially when non-pyrethroid-like insecticides are used [9].

1.2.2 Vaccines

Vaccines are biologically derived agents that induce the formation of antibodies that fight against a particular pathogen, usually a pathogen whose antigen is part of the vaccine's formulation. In malaria prophylaxis, vaccines can be designed to induce antibodies that fight against specific forms of the malaria parasite expressed in different stages of the malaria lifecycle. These are sporozoite (and merozoite at the liver; pre-erythrocytic stage), trophozoite, schizonts, and merozoites (erythrocytic stage forms), and gametocytes (in the human bloodstream or inside the vector). Only two malaria vaccines are recommended for use in malaria prophylaxis thus far [10]. These are the RTS,S/AS01 and R21/Matrix-M vaccines. Both are circumsporozoite protein-based vaccines; they target the pre-erythrocytic stage of the malaria parasite, therefore, preventing the formation of malaria disease forming parasites, and gametocytes (essential for transmission). The RTS,S/AS01 vaccine has an efficacy of 36% against clinical malaria and 32% against severe malaria when administered with a booster to children of 5-17 months [11] tested after 48 months. R21/Matrix-M vaccine's efficacy was found to be 75% and 68% at sites with seasonal and annual transmission after 12 months from time of administration [12]. After the successful pilot implementation of the vaccines in malaria endemic countries including Ghana, Malawi, and Kenya through the Malaria Vaccine Implementation Programme (MVIP), the vaccines are now used in five additional countries including Benin, Burkina Faso, Cameroon, Liberia, and Sierra Leone as part of the childhood immunisation programme against *P. falciparum* malaria [10].

1.2.3 Antimalarial drugs

Antimalarial drugs can be used to treat symptomatic malaria or as prophylactic agents. They are generally divided into classes depending on their chemical structure and mode of action. Quinine **1.1**, the first compound to be used for treating malaria, is an arylaminoalcohol compound (**Figure 1.2**) along with mefloquine **1.2**, and lumefantrine **1.3**. It is currently recommended in the treatment of severe malaria as are some artemisinin compounds (see later). Mefloquine retains its relevance in the treatment of malaria because it is active against most *Plasmodium* strains that are resistant to chloroquine (**1.4, Figure 1.2**) [13]. The drop in the efficiency of mefloquine in monotherapy treatment, as observed in some regions in Asia, due to resistance, raises concern. Mefloquine resistance also reduces the effectiveness of other antimalarial drugs like artesunate when used in combination therapy [14]. Lumefantrine is useful both in monotherapy and combination therapy in treating malaria [15].

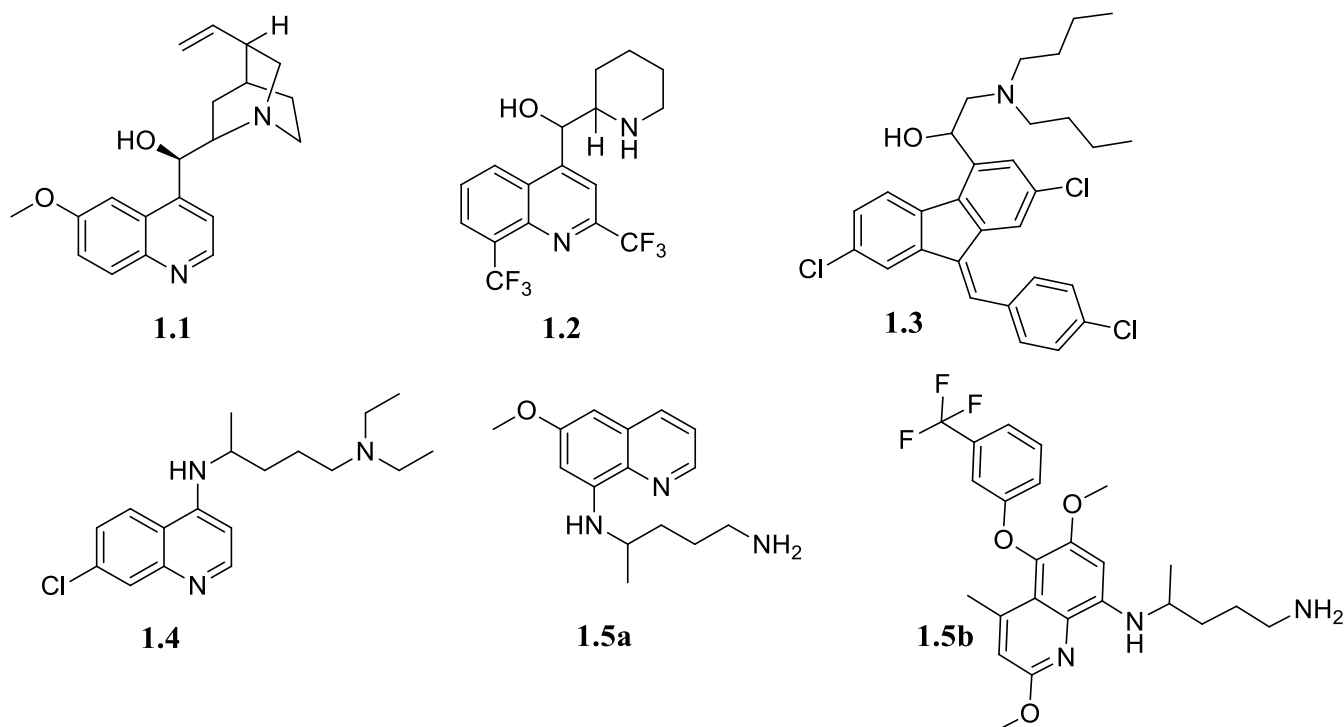


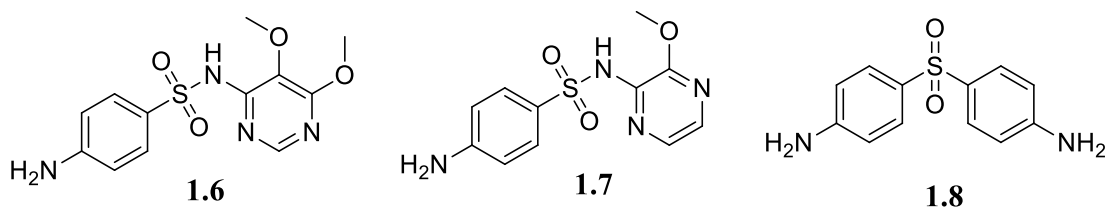
Figure 1.2. Chemical structure of clinically-approved antimalarial drugs: Arylaminoalcohol (1.1-1.3) and aminoquinoline (1.4 and 1.5) compounds.

Chloroquine, primaquine **1.5a**, and tafenoquine **1.5b** are aminoquinoline compounds (**Figure 1.2**), used in the treatment and prophylaxis of malaria. Chloroquine has been the most successful agent used in monotherapy for the treatment of malaria prior to the emergence of resistant parasites. It is still used today in areas where chloroquine resistant parasites are not pronounced. Chloroquine forms a drug-heme complex that is incorporated into hemozoin through the processes of polymerisation. The incorporation caps the hemozoin stopping further polymerisation, depriving the parasite of its food source [16]. Additionally, the heme accumulates in the food vacuole becoming toxic to the parasite. Primaquine is the only compound that has activity against dormant liver parasites. It is therefore the only compound used in the radical cure for *P. vivax* malaria [17], preventing the relapse of malaria post blood parasitaemia clearance. It is also the only antimalarial agent with gametocidal activity against late-stage gametocytes making it a transmission blocking agent [18] that can be used with other antimalarial drugs that clear asexual forms of the parasites. Primaquine usage in Asia and Africa is limited by its side effect (hemolysis) in humans with glucose-6-phosphate-dehydrogenase (G6PD) deficiency [19]. Tafenoquine **1.5b** offered an improvement to primaquine with a half-life of 16 days (compared to 4-9 hours half-life of primaquine), however, it is also contraindicated in patients with G6PD deficiency.

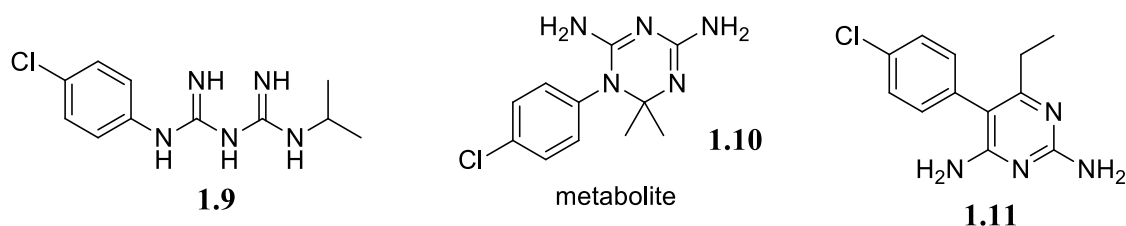
Antifolates are a group of compounds that act as prophylactic agents by disrupting the parasite's folate metabolic pathway. The parasite's folate pathway is essential in the maturation and

proliferation of parasites. Antifolates are divided into two classes depending on whether they inhibit dihydropteroate synthase (DHPS, class I antifolates) or dihydrofolate reductase (DHFR, class II antifolates). Principal class I and class II antifolate compounds used in malaria treatment (including the metabolite of prodrug proguanil) are illustrated in **Figure 1.3**. Proguanil **1.9**, whose metabolite is a class II antifolate, was discovered in 1945 at which time it was found to perform better than quinine against avian malaria with a better therapeutic index [20]. It can be used in monotherapy as a prophylactic agent, in which case its metabolite cycloguanil **1.10** is the active agent, or in combination with atovaquone **1.12** (Malarone[®]) [21, 22]. Proguanil works synergistically with atovaquone by enhancing atovaquone's ability to inhibit cytochrome *bc1* complex or complex III in the mitochondrial electron transport chain, while it is converted to cycloguanil *in vivo*, a metabolite that inhibits DHFR [23]. The use of proguanil with dapsone **1.8** (class I antifolate) and/or artesunate (**1.15**, **Figure 1.4**) has been limited by the toxicity of dapsone and the reduction in cycloguanil activity. Pyrimethamine **1.11** is another class II antifolate that finds its application in combination therapies with class I antifolates such as sulfadoxine **1.6** and sulfalene **1.7** for treating malaria. Pyrimethamine was initially studied in the treatment of tumours and later found its use as an antimalarial agent following the observation (and subsequent antimalarial activity screening) made by Falco *et al.* that pyrimethamine is analogous to cycloguanil [24]. Sulfadoxine/pyrimethamine (Fansidar[®]) combination therapy is used to treat uncomplicated malaria in areas where chloroquine resistant *P. falciparum* parasites are identified. Unfortunately, the development of mutations in the active site of the target enzymes DHPS and DHFR has given rise to resistant parasites reducing the effectiveness of antifolates and, thus, their application, as antimalarial agents [25, 26].

Class I antifolates



Class II antifolates



Naphthoquinone drug used in combination with antifolates.

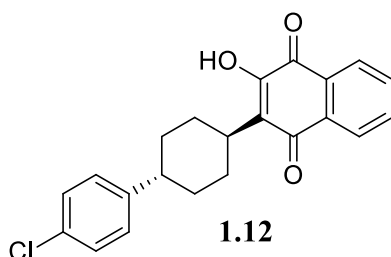


Figure 1.3. Antifolate compounds (**1.6-1.11**) and atovaquone **1.12**.

Artemisinin (**1.13**, **Figure 1.4**) and its derivatives (collectively called artemisinins, **Figure 1.4**) have played a crucial role in the treatment of uncomplicated and severe malaria since their inception. They are the only known class of compounds that have antimalarial activity on both asexual (both the small and large ring stage) and sexual (early-stage gametocytes) malaria parasites with the asexual parasite most susceptible [27]. Artemisinins are characterised by a sesquiterpene lactone structure with an endoperoxide moiety which is central in the antimalarial activity of these drugs [28, 29]. The parent molecule, artemisinin, is an active ingredient isolated in 1972 from the extract of the sweet wormwood tree (*Artemisia annua*). The hydroreduction of artemisinin's lactone group into a lactol affords the first semisynthetic derivative dihydroartemisinin **1.14** from which other widely used artemisinins such as artesunate **1.15**, and artemether **1.16** can be derived. These however are metabolised back to dihydroartemisinin, which is important in exerting prolonged antimalarial activity owing to a longer half-life when compared to parent molecules [29]. Generally, artemisinins have a shorter half-life than other antimalarial drugs. As a result, they are often applied in combination with long lasting antimalarial drugs such as lumefantrine, as artemisinin-combination therapy (ACT). Artemether, an orally available drug owing to its

lipophilicity [30], is combined with lumefantrine under the name Coartem® or Riamet®. Artesunate finds its application in combination with either mefloquine, or sulfadoxine-pyrimethamine. Typically, the artemisininins clear most of the erythrocytic parasites and upon elimination the partner drugs clear the remaining parasites.

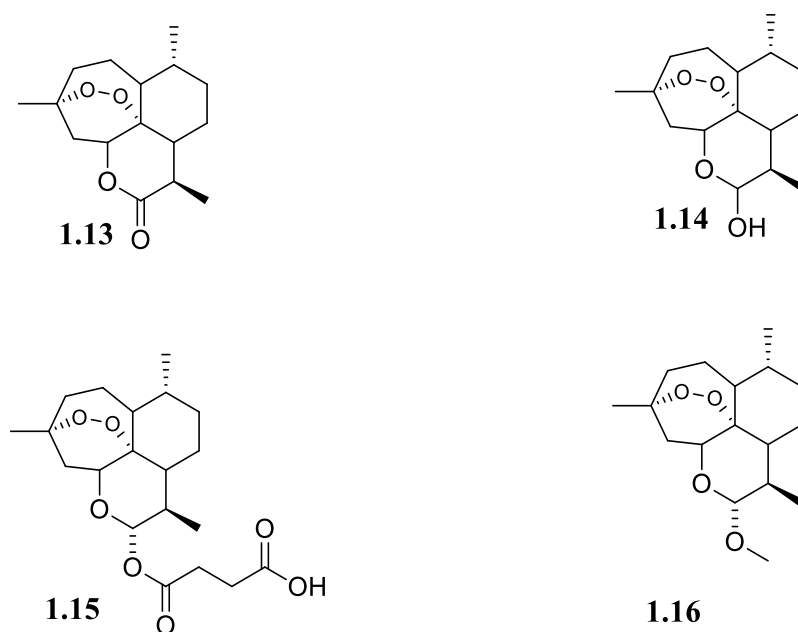


Figure 1.4. Structures of artemisinin **1.13** and its derivatives **1.14-1.16**.

1.3 Drug resistance and strategic approach to novel antimalarial agents

The delayed eradication of malaria worldwide is primarily attributed to the development of resistance by the vector (the female *Anopheles* mosquito) to insecticides and the *Plasmodium* parasites to antimalarial agents. *P. falciparum* has developed resistance to arylaminoalcohol, aminoquinoline, and antifolate compounds. Artemisininins are currently the only antimalarial agents limiting the devastating effect of multi-drug resistant *falciparum* malaria. Unfortunately, resistance to artemisininins has been observed in Southeast Asia and is emerging in Africa [31, 32]. Worldwide/regionwide distribution of resistant parasites is hastened by parasite transmission and currently there is no antimalarial agent that is effective in preventing transmission. There is an urgent need, therefore, for agents with novel mode of action that will prevent the rise in malaria cases and deaths upon the collapse of artemisininins. Ideally, these agents should discourage the emergence of resistant parasites and their spread in addition to treating symptomatic malaria.

An ideal antimalarial agent will be one that will target both the asexual and sexual forms of the *Plasmodium* parasite in the human host and in doing so treat malaria and prevent its transmission. Currently, there is no single antimalarial compound that can effectively clear both asexual and

sexual forms of the parasite in humans. Theoretically, a combination of drugs, each with activity against either asexual or sexual stages can be used in clearing of blood parasites. Unfortunately, there is no such combination currently available. Potentially, primaquine can be combined with artemisinins to achieve blood parasite clearance of both asexual and sexual stages. Primaquine would target late-stage gametocytes while artemisinins target early-stage gametocytes in addition to asexual forms of the parasites. However, the use of this combination is hindered by primaquine toxicity; it causes severe haemolysis in patients with G6PD deficiency. G6PD is important in protecting red blood cells (RBCs) from oxidation damage. Patients with G6PD deficiency have their RBCs breaking down prematurely. The haemolysis of RBCs is intensified by primaquine at a rate faster than the body's ability to replace lost RBCs resulting in severe haemolysis that can lead to death.

A strategic approach to novel antimalarial agents would be finding targets (or homologous targets) that are expressed in every stage of the malaria parasite lifecycle or at least in the sexual and asexual parasite stages. The agents would interfere with the normal functioning of the target(s) and therefore, simultaneously cure and block parasite transmission. Plasmepsins protease are a good example of such targets. Plasmepsins (PM) are *Plasmodium* species aspartic proteases that are essential for the development of the parasite – they are expressed at different stages of the malaria parasite lifecycle (**Figure 1.5**). Of the available 10 plasmepsins (PMI-PMX), PMX is most attractive because it is expressed in the liver, blood, and transmission stages. Inhibition of PMX is accompanied by the parasite's inability to egress infected cells, or invade healthy cells and the mosquito midgut [33].

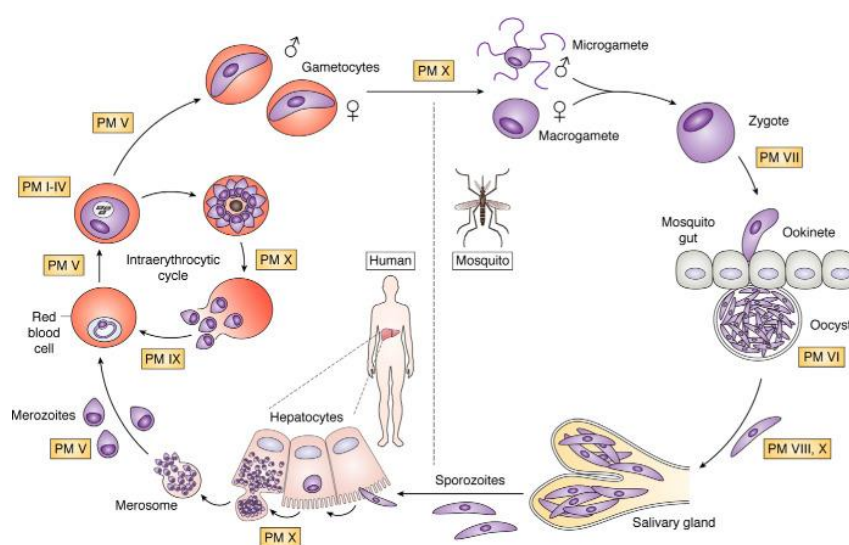


Figure 1.5. Malaria parasite lifecycle with points where the plasmepsin proteases are expressed [33].

Plasmodium protein kinases are other potential targets for antimalarial agents that could both treat malaria and block parasite transmission. They are essential in the development and growth of the parasite at various stages of the lifecycle. Of the various protein kinase families, calcium-dependent protein kinases (CDPKs) carry the potential of being selective owing to their uniqueness to plants and protists; they are not found in humans [34]. Two *Plasmodium falciparum* CDPKs (*Pf*CDPK) are of relevance to the considered strategic approach, these are *Pf*CDPK1 and *Pf*CDPK4. The former is active in the blood stages while the latter is expressed in the transmission stages. Due to their amino acid identity (39-56%), there is a chance that one agent can inhibit both enzymes resulting in both the treatment of malaria and blocking of parasite transmission. In fact, there are studies that demonstrate that the inhibition of both *Pf*CDPK1 and *Pf*CDPK4 by a single antimalarial agent is possible [35]. In this research, our interest is in the *Pf*CDPKs as potential targets for novel antimalarial agents that will treat malaria disease and prevent parasite transmission.

1.4 Protein kinases as potential targets

Protein kinases (PKs) are a family of kinases that are responsible for catalysing the transfer of a phosphate group from a phosphate-donating molecule like adenosine triphosphate (**Figure 1.6**) to a protein substrate. They are classified based on the amino acid residue they phosphorylate, for example, kinases that phosphorylate serine and threonine residues are termed serine/threonine PKs while those that phosphorylate tyrosine are termed tyrosine PKs. PKs play an important role in intracellular signal transmission by activating proteins that regulate important cellular processes such as metabolism, apoptosis, and proliferation. A typical example is the serine/threonine phosphorylation of the death-associated protein, that leads to the translocation of apoptosis-inducing kinase 2 from the cytoplasm to the nucleus, in which apoptosis of B and T cells is induced [36].

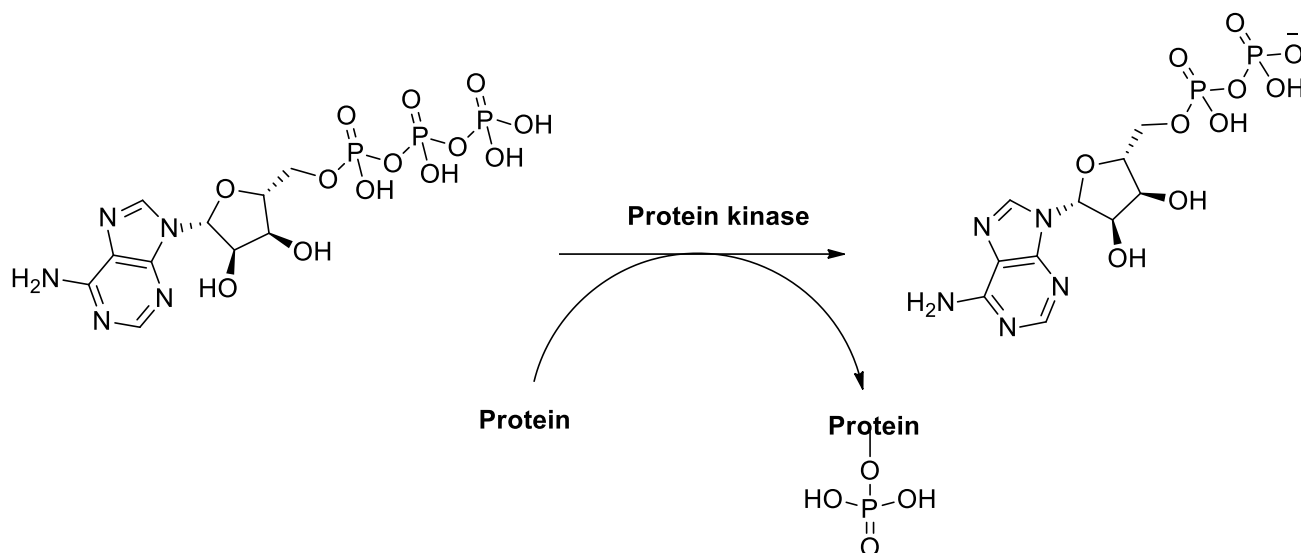


Figure 1.6. Phosphorylation of a protein catalysed by a protein kinase.

1.4.1 Protein kinase binding site geometry

Protein kinases (PKs) have a conserved structural core that consists of two lobes (N- and C-lobes) made up of β -sheets and α -helices (**Figure 1.7**). One of the α -helices found on the N-lobe domain (α C-helix) has an important regulatory function [37]. The lobes are connected by an ordered amino acid sequence linker making up the hinge region of the binding site which forms hydrogen bonds with the adenine ring of the cofactor ATP. The cleft between the lobes and the hinge region makes up the binding site of ATP. PKs also consist of the activation loop that starts with a conserved tripeptide motif (aspartate-phenylalanine-glycine motif or DFG motif) followed by 20-30 amino acids [38]. It is involved in protein substrate recognition, phosphorylation, and regulates the activity of the kinase.

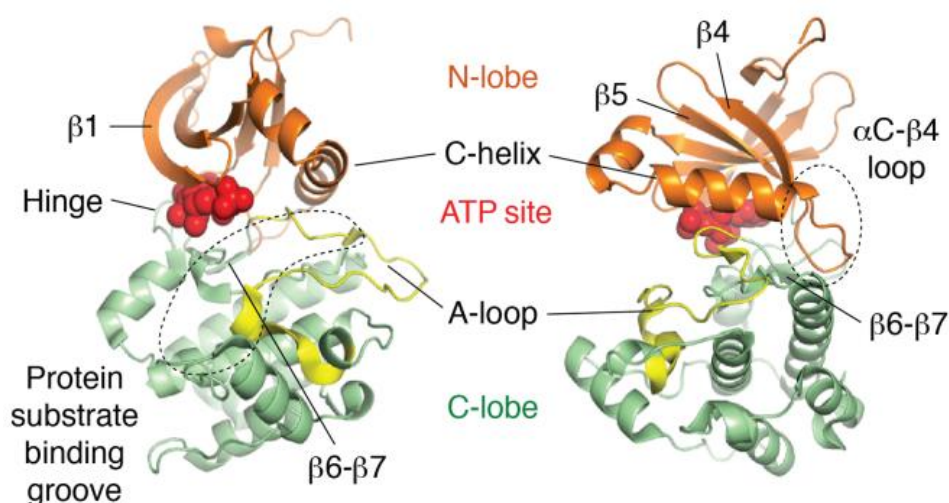


Figure 1.7. The structural elements of protein kinases (retrieved from [40]).

The DFG-motif can assume various conformations of which the two common extremes are the DFG-in and DFG-out conformations [39]. These can be expressed in both the active and inactive conformations of the kinase. In a DFG-in conformation, the activation loop is positioned towards the α C-helix along with the phenylalanine side chain of the DFG motif (**Figure 1.8a**). The opposite is observed in the DFG-out conformation; the activation loop moves away from the α C-helix and the phenylalanine side chain points towards the ATP binding site (**Figure 1.8b**). The conformation of the DFG motif and the α C-helix (α C-in or α C-out) affect the geometry of the binding site, by either exposing or closing potential binding pockets. The DFG-out conformation exposes a pocket that is otherwise not available in the DFG-in conformation due to occupation by the phenylalanine side chain. Likewise, an additional pocket can be created when the α C-helix moves away from the ATP site (α C-out), instead of towards the ATP site (α C-in) (**Figure 1.8**). Generally, PKs have two pockets that can be exploited in the design of effective and selective inhibitors in addition to the ATP site. The first pocket, whose entrance is facilitated by the gatekeeper residue, is adjacent to the ATP binding site separated by one amino acid residue (available in the α C-out conformation). The second pocket is at the proximity of the DFG motif and its availability is dependent on the DFG-out conformation (**Figure 1.8**).

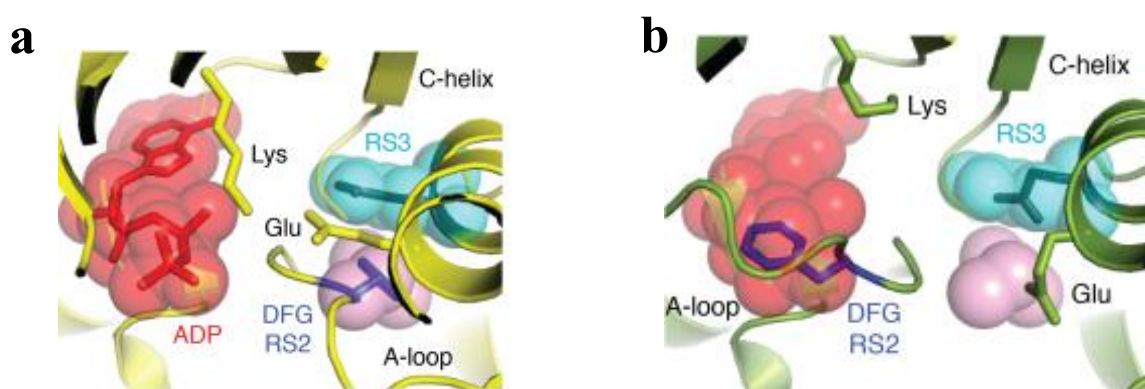


Figure 1.8. a) DFG-in conformation with the activation loop (A-loop) towards the α C-helix and the phenylalanine side chain (purple) occupying the hydrophobic pocket (pink) that is otherwise not occupied in the b) DFG-out conformation [40]. The translucent blue spheres represent the third potential binding pocket available in the C-out conformation of the α C-helix.

1.4.2 Types of kinase inhibitors

There are various types of kinase inhibitors (Type I-VI; reviewed in [41-43]) and here we will limit our discussion to inhibitors that target pockets in the vicinity of the ATP site including the ATP site itself (ATP site and adjacent pockets). Such inhibitors range from type I to type III. Type I inhibitors bind in the active form of the kinase with DFG-in and α C-in conformations. They are confined in the ATP site and mimic the hydrophobic bonds and hydrogen bonds typically observed

with ATP at the hinge region; they are ATP competitive inhibitors. Their activity and selectivity take advantage of the nature of the gatekeeper residue (shape, size, and polarity) [43]. FDA approved type I inhibitors include gefitinib **1.17**, ceritinib **1.18** and palbociclib **1.19** to name a few (**Figure 1.9**). All these are used in cancer treatment, and they make similar interactions to ATP in the active site. For example, gefitinib is an epidermal growth factor receptor (EGFR) inhibitor whose quinazoline ring forms hydrogen bonding interactions at the hinge region via the pyrimidine ring nitrogen atom, in addition to hydrophobic interactions [44]. Also, the 3-chloro-4-fluorophenyl group interacts with the gatekeeper residue and surrounding residues.

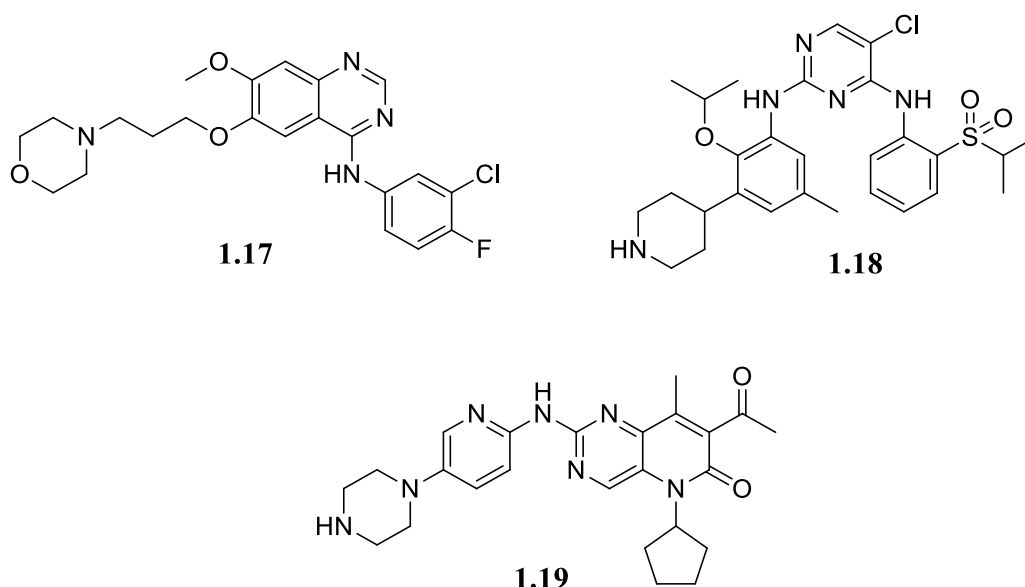


Figure 1.9. Type I protein kinase inhibitors.

Type I_2^1 inhibitors are agents that bind in an inactive form of the protein kinase with DFG-in and α C-out conformations (sometimes in the α C-in conformations). They compete with ATP at the binding pocket and make similar hydrophobic and hydrogen bonding interactions at the hinge region. The α C-out conformation exposes a pocket that is poorly conserved and can be exploited in the design of potent and selective inhibitors. The size and shape of the pocket is governed by the nature of the gatekeeper residue; residues with small side chains result in large pockets that can be occupied by large molecular moieties improving the inhibitor's overall binding affinity. Type I_2^1 can be subdivided into two classes depending on whether they occupy the hydrophobic pocket or not. Inhibitors that make use of the hydrophobic pocket are termed type I_2^1A inhibitors, while those that do not occupy the pocket are termed type I_2^1B inhibitors. Lapatinib **1.20** and vemurafenib **1.21** are FDA approved type I_2^1 inhibitors used in the treatment of breast cancer and melanoma, respectively (**Figure 1.10**). Their bicyclic cores occupy the ATP site while the chloro-

aniline moiety of lapatinib and the difluoro-phenyl group of vemurafenib occupy the hydrophobic pocket.

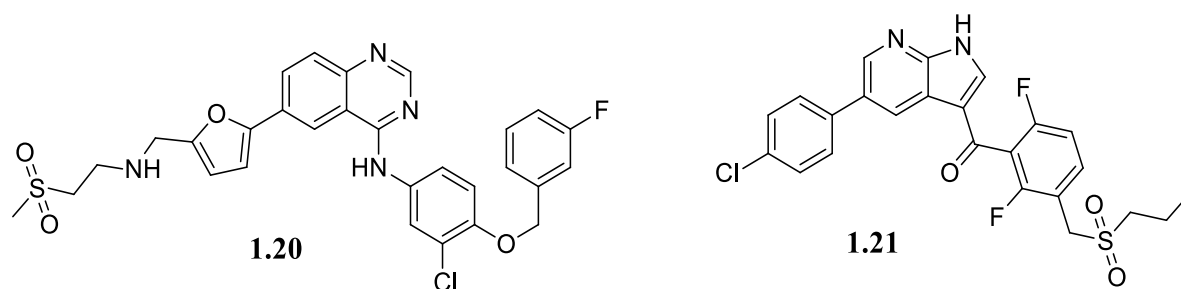


Figure 1.10. Type I₂¹ inhibitors lapatinib **1.20** and vemurafenib **1.21**.

Type II inhibitors bind to an inactive conformation of the kinase with a DFG-out conformation and either α C-in or α C-out helix conformations. They occupy the ATP site and extend to a cavity that is exposed because of the DFG-out conformation. In a DFG-out conformation, the side chain of the phenylalanine residue moves towards the ATP site (occupies the sugar/phosphate pockets) exposing a cavity near the DFG motif, adjacent to the ATP site. Occupation of this pocket stabilises the enzyme in an inactive conformation, thus preventing phosphorylation. Type II inhibitors can be either type IIA or type IIB, depending on whether they occupy the exposed pocket or not. Occupation of the pocket is a characteristic of type IIA inhibitors. Sorafenib (**1.22**, **Figure 1.11**), an anticancer drug, is a typical example of a type II inhibitor. Its picolinamide moiety participates in bonding at the hinge region while the trifluoromethylphenyl moiety occupies the exposed DFG hydrophobic pocket. The drug appreciates additional hydrogen bonding through the urea moiety with the residues on the α C-helix and DFG moieties.

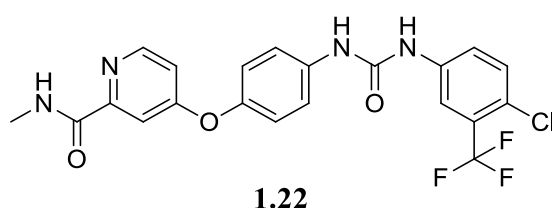


Figure 1.11. Chemical structure of a type II inhibitor sorafenib.

Type III inhibitors are allosteric inhibitors. They do not compete with ATP for binding in the active site. Instead, they occupy either pocket that becomes available in the DFG-out and α C-out conformations. The occupied allosteric sites are poorly conserved; therefore, type III inhibitors can be selective.

Each protein kinase inhibitor type has a set of advantages and disadvantages. For example, type III inhibitors are not affected by the intracellular concentration of ATP because they bind in the

allosteric site while ATP-competitive inhibitors (e.g. type I) are affected. Inhibitor types that are most attractive are type I_2^1 and type II inhibitors, particularly inhibitors of subtype A (type I_2^1A and type IIA). The additional pockets that these inhibitors occupy are poorly conserved, therefore, they can be targeted for selectivity. Also, the additional interactions afforded in these pockets positively contribute to the overall binding affinities of the inhibitors and their residence time in the active site. Subtype A of type I_2^1 and II inhibitors have been found to bind longer in the active site compared to subtype B inhibitors [41].

Knowledge on a target's function and binding site geometry is essential in structure-based drug design. Such knowledge is exploited in the design of potent and selective drugs. Protein kinases are attractive drug targets owing to their importance in the regulation of cell processes such as cell proliferation, and the presence of weakly conserved binding pockets that can be exploited for selectivity. PKs have been well explored and this is evidenced by the numerous kinase inhibitors that are FDA approved to date [45]. However, there are no kinase inhibitors that are approved for the treatment or prophylaxis of malaria. In fact, most approved inhibitors (approx. 86%) are used for the treatment of various cancers. Research on various kinases is currently undertaken in the search for effective antimalarial agents [46]. Among these kinases are calcium-dependent protein kinases (CDPK). They serve as potential targets for potent and selective antimalarial agents because of their importance in parasite survival and absence in humans.

1.4.3 *Plasmodium falciparum* calcium-dependent protein kinases and inhibitors

Calcium-dependent protein kinases (CDPK) are protein kinases whose activation is dependent on the intracellular concentration of calcium cations (Ca^{2+}), serving as second messengers. They are made up of five structural domains including the N-terminal domain, kinase/catalytic domain, autoinhibitory/junction domain, the regulatory domain, and the C-terminal domain [47]. The catalytic/kinase domain is where ATP binding and phosphorylation of protein substrates occurs. The junction domain contains a pseudo-protein substrate that inhibits the activity of the enzyme. The regulatory domain is a calmodulin-like domain with four EF-hand motifs on which Ca^{2+} binds. The activation of the protein is thought to occur in two possible ways [48, 49]; both require the binding of Ca^{2+} into the EF-hand motifs which in turn bind with the autoinhibitory domain, which releases the pseudo-protein substrate from the kinase, activating the enzyme.

While CDPKs have been identified in plants and protists, none have been found in humans [50], making them attractive targets for drug development against protozoan-caused diseases like malaria. The focus in antimalarial drug development is directed towards the *Plasmodium*

falciparum species because it causes the deadliest form of malaria. The *P. falciparum* parasite kinome contains seven calcium-dependent protein kinases (*Pf*CDPK1-*Pf*CDPK7) of which five (*Pf*CDPK1-*Pf*CDPK5) have the typical protein kinase domain structure. They are expressed in all three stages of the parasite lifecycle, highlighting their importance in the survival of the parasite. *Pf*CDPK1 and *Pf*CDPK5 have been shown to be important for cell egress and invasion during the intraerythrocytic cycle [51-54] while suppression of *Pf*CDPK2 and *Pf*CDPK4 effectively blocked the transmission of the malaria parasite from the human host to the mosquito [55-57]. Inhibition of at least two of these kinases, one from each parasite stage, by one agent, would be ideal for malaria treatment given the inevitable development of resistant parasites. However, this may not be possible owing to minor structural differences like the gatekeeper residue and therefore, the size of the hydrophobic pocket, which makes it near impossible for one agent (especially type I₂ inhibitors) to inhibit all the kinases. The only promising target combination is that of *Pf*CDK1 and *Pf*CDPK4, owing to their structural similarity. The relatively small gatekeeper residues and high sequence homology (ca. 53%) [58] of *Pf*CDPK1 and *Pf*CDPK4 compared to other *Pf*CDPKs make them potential targets for dual-inhibitor antimalarial agents.

Several compounds have been studied by various research groups on their ability to inhibit either *Pf*CDPK1 or *Pf*CDPK4. *Pf*CDPK1 inhibitors include, but are not limited to, the bisindolocarbazole K252a (**1.23**), the imidazopyridazine-based compound (**1.24**), and purfalcamine (**1.25**, **Figure 1.12**). In a study carried out by Green et al., K252a **1.23** was demonstrated to be a potent inhibitor of *Pf*CDPK1; at intermediate concentrations (~0.4 µM) of K252a they observed that erythrocyte invasion by the merozoites was reduced by 65% [51]. Compound **1.24** was identified as a result of a series of structural modifications that followed the discovery of imidazopyridazine as a potential scaffold for inhibitors of *Pf*CDPK1 following the screening of 35000 compounds [59]. The compound has *Pf*CDPK1 IC₅₀ value of 12 nM (*in vitro* enzymatic assay) and it reduced parasitaemia in *P. bergeri* mouse model by up to 51% - an improvement compared to its predecessors [60]. Purfalcamine was the most potent compound against *Pf*CDPK1 *in vitro* (IC₅₀=17 nM) discovered by Kato *et al.* within a series of 2,6,9-trisubstituted purines, identified after screening 20000 compounds on *Pf*CDPK1 [61].

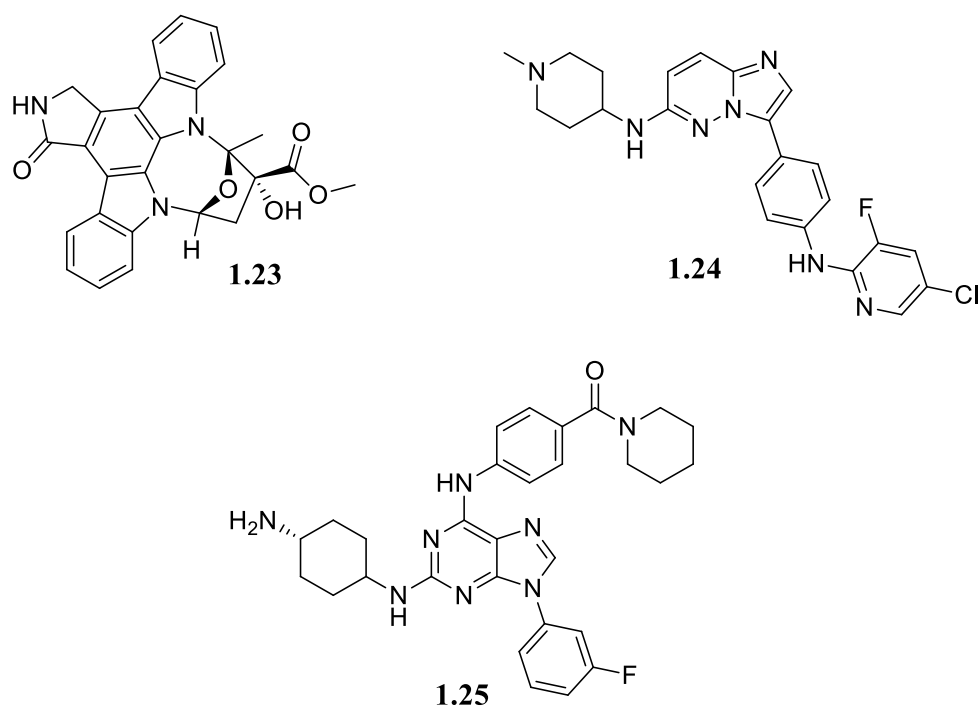


Figure 1.12. Compounds that have demonstrated inhibitory potential against *Pf*CDPK1.

Bumped kinase inhibitor **1.26** (BKI-1, **Figure 1.13**) has been identified as a potent inhibitor of *Pf*CDPK4 ($IC_{50}=0.004 \mu M$). Ojo et al. demonstrated that there is a correlation between the inhibition of *Pf*CDPK4 and the exflagellation ability of microgametocytes [62]. This suggested that the *Pf*CDPK4 may be linked to exflagellation of microgametocytes *in vivo*. This was later confirmed by Ojo et al., using chemical-genetic evidence; parasites with a mutated *Pf*CDPK4 retained their exflagellation ability while those bearing a wild-type kinase had reduced exflagellation [57]. Exflagellation is a process that occurs inside the mosquito gut; to impair transmission, the drug must be available at effective concentrations in the human blood during a blood meal. Unfortunately, BKI-1 does not have a satisfactory pharmacokinetics (PK) profile and as a result, modification processes took place to improve the PK profile, yielding compound 1294 **1.27** (**Figure 1.13**). Methylation of the secondary amine of the piperidine moiety of BKI-1 to afford compound 1294 was accompanied by better metabolic stability and improved blood level exposure without compromising *Pf*CDPK4 inhibitory activity ($IC_{50}=0.010 \mu M$) and anti-exflagellation ability ($EC_{50}=0.047 \mu M$) [57]. Furthermore, it was demonstrated that compound 1294 blood concentration of $3 \mu M$ was enough to completely block transmission, with lower concentration ($0.1 - 1 \mu M$) able to reduce oocyst infectivity between 15% and 38%. Unfortunately, compound 1294's remarkable safety profile is interrupted by its activity against hERG at concentrations necessary for inhibition. It forms, however, a good starting point for the development of potent and selective *Pf*CDPK4 inhibitors.

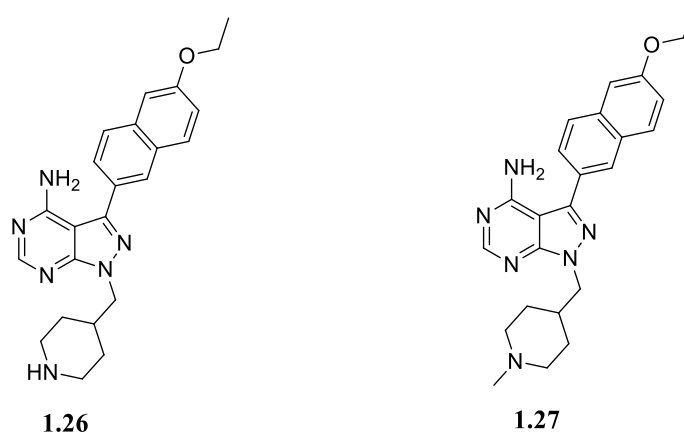


Figure 1.13. Structures of bumped kinase inhibitors: BK1-1 (**1.26**) and compound 1294 (**1.27**).

The most comprehensive work done on the inhibition of *Plasmodium* kinases is that presented by Vidadala and coworkers [35]. Not only did they demonstrate potent inhibitors of *Pf*CDPK4, but they also showed that inhibitors of *Pf*CDPK4 can potently inhibit *Pf*CDPK1. Furthermore, they demonstrated structural features of the *Pf*CDPK4 binding site that can be exploited to obtain potent and selective inhibitors, highlighting the advantages of type I₂¹ kinase inhibitors. Vidadala et al. explored two scaffolds in their study; the pyrazolo[2,3-*d*]pyrimidine and imidazo[1,5-*a*]pyrazine scaffolds (**Figure 1.14**) [35]. They started their investigation by varying the R¹ substituent on the pyrazolo[2,3-*d*]pyrimidine scaffold while keeping the R² substituent between the isopropyl and tertiary butyl moieties. They incorporated bulky groups such as phenyl, naphthyl and quinolone to take advantage of the large hydrophobic pocket of the kinase, that is rarely observed in human kinases, for selectivity. *In vitro* inhibition results indicated that 2-naphthyl and 6-quinolino-substituents were good substituents for R¹ (IC₅₀<1 μM). Also, results showed that in 67% of cases the difference in inhibitory efficacy between *Pf*CDPK4 and *Pf*CDPK1 is less than 3-fold. It was expected that inhibition of *Pf*CDPK1 would be less potent owing to the kinase's larger threonine gatekeeper residue. However, this was not entirely the case since some compounds showed better efficacy for *Pf*CDPK1 than for *Pf*CDPK4.

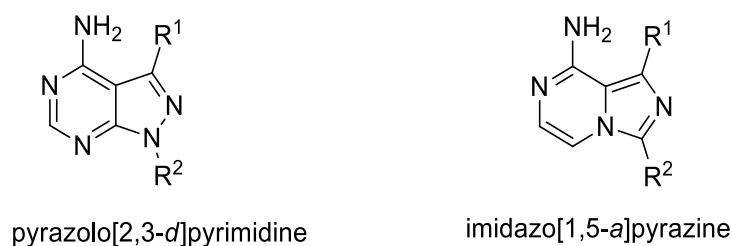


Figure 1.14. Pyrazolo[2,3-*d*]pyrimidine and imidazo[1,5-*a*]pyrazine scaffolds.

Having identified the best R¹ substituents, Vidadala and coworkers moved their attention to identifying ideal R² substituents [35]. They discovered that the best R² substituents are those bearing a methylene linkage to the scaffold. A greater than 100-fold increase in potency against *Pf*CDPK4 was observed with compounds bearing the 4-piperidinemethylene group. Likewise, such an increase was observed against *Pf*CDPK1, however to a lesser extent (10-fold). Selectivity toward *Pf*CDPK4 over Src human kinase was also improved (1300 to 5000-fold) by such substituents. These results show the intricate interplay between the R¹ and R² substituents in conferring potency and selectivity. *In vitro* results of analogues bearing the imidazo[1,5-*a*]pyrazine scaffold were comparable to their pyrazolo[2,3-*d*]pyrimidine counterpart. The exception was for compounds bearing the 4-piperidinemethylene moiety where pyrazolo[2,3-*d*]pyrimidines were more potent than the imidazo[1,5-*a*]pyrazine compounds [35].

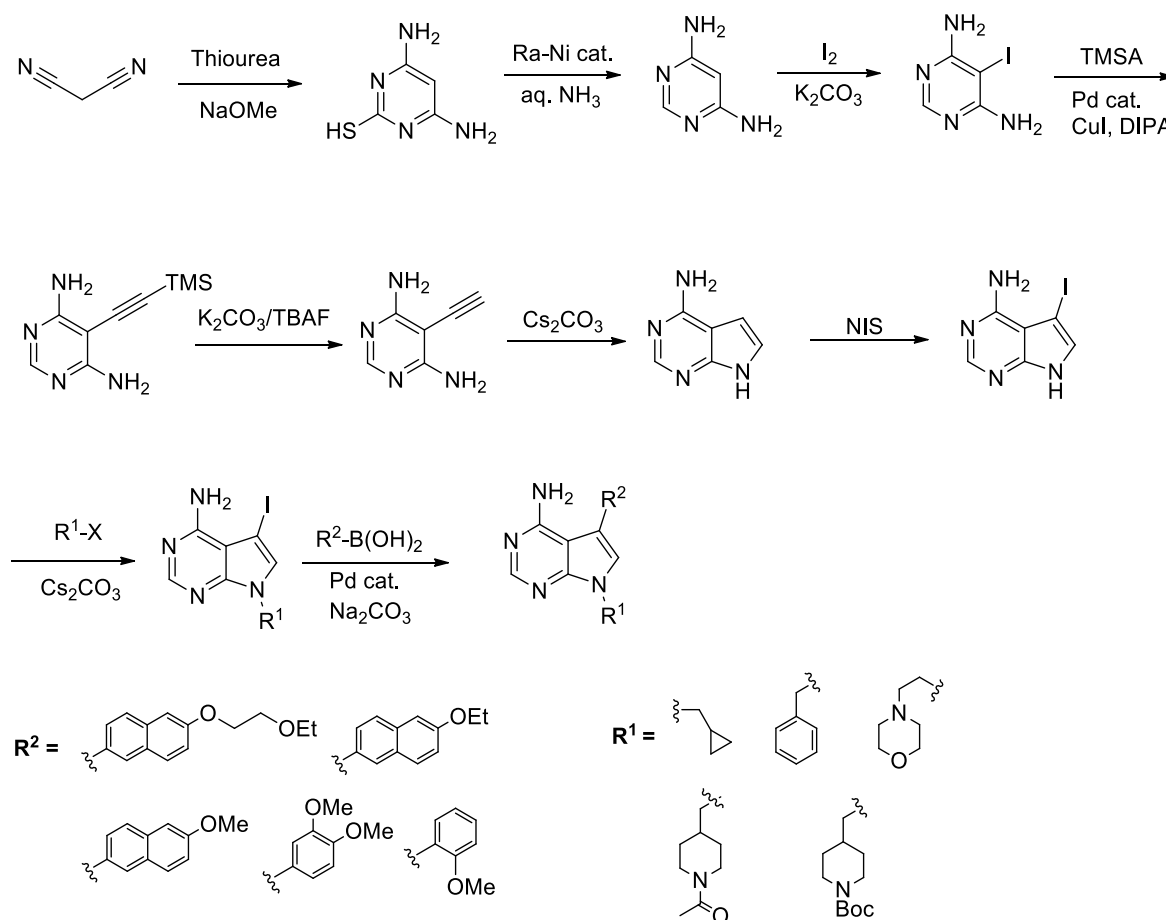
In this project, we explore the pyrrolo[2,3-*d*]pyrimidine derivatives (**Scheme 1.1**) and their ring open analogues, the 4,6-diaminopyrimidine derivatives (**Scheme 1.2** and **1.3**), as potential inhibitors of *Pf*CDPK1 and *Pf*CDPK4. These scaffolds make the same hydrophobic and hydrogen bonding interactions at the binding site as the adenine ring of ATP. We wanted to see if these scaffolds will perform better, similarly, or worse than the pyrazolo[2,3-*d*]pyrimidines and imidazo[1,5-*a*]pyrazine compounds explored by Vidadala and coworkers. We study their potential to inhibit the target enzyme *in silico* and then synthesize them to evaluate their inhibitory potential in biological assays.

1.5 Aim of the project

We aimed to synthesise type I₂¹ kinase inhibitors that would interact with both *Pf*CDPK1 and *Pf*CDPK4 towards malaria treatment and transmission-blocking. This was carried out in three parts, starting with molecular modelling, followed by synthesis, and ending with biological evaluations. The first part of the project included the design and *in silico* study of targeted compounds where we designed compounds that resemble a type I₂¹ kinase inhibitor structure. Type I₂¹ inhibitors are competitive agents that occupy the ATP site in the kinase active site making hydrogen bond and hydrophobic interactions like the adenine ring of the cofactor ATP. Additionally, and most importantly, they occupied the hydrophobic pocket adjacent to the ATP site that has been proven to be important in imparting selectivity over the compounds towards parasite kinases. Using the crystal structure of *Pf*CDPK4 and a homology model of *Pf*CDPK1 we developed, we evaluated the targeted compounds binding poses and interactions with these kinases. The best scoring compounds within each class were further studied using molecular

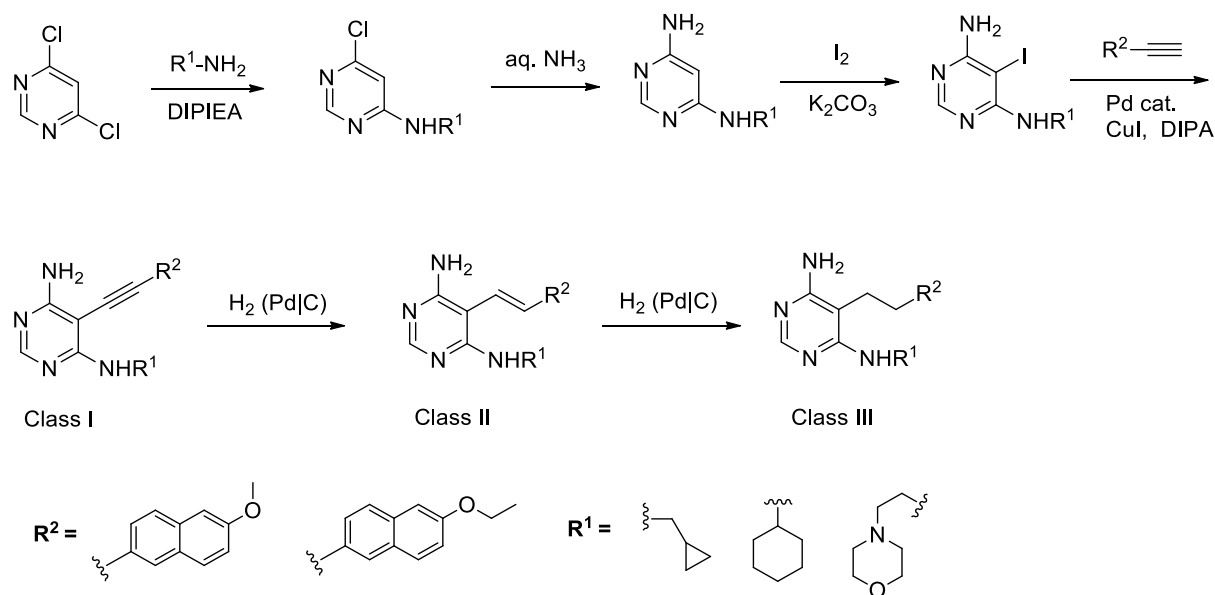
dynamics, assessing their conformational stability and interactions. Lastly, we assessed the targeted compounds for pharmacokinetic properties and potential toxicity.

The second part of the project involved the synthesis of the targeted kinase inhibitors bearing the pyrrolo[2,3-*d*]pyrimidine and 4,6-diaminopyrimidine cores. The synthesis of pyrrolo[2,3-*d*]pyrimidine derivatives started with the formation of 4,6-diaminopyrimidine-2-thiol from malononitrile and thiourea, followed by the removal of the thiol moiety using Raney-Nickel and aqueous ammonia (Scheme 1.1). The 5-position of the resulting 4,6-diaminopyrimidine was iodinated in preparation for the Sonogashira coupling reaction that introduced trimethylsilylacetylene (TMSA). The acetylene was deprotected using either tetra-*n*-butylammonium fluoride (TBAF) or potassium carbonate. Using caesium carbonate (Cs_2CO_3) as a base, the terminal alkyne structure was transformed into a two-fused-ring structure, the pyrrolo[2,3-*d*]pyrimidine ring. From here the R groups were introduced by alkylating the secondary amine with an alkyl halide (R^1) and via the Suzuki-Miyaura coupling reaction with the R^2 groups boronic acid intermediates.



Scheme 1.1. The synthesis of pyrrolo[2,3-*d*]pyrimidine compounds.

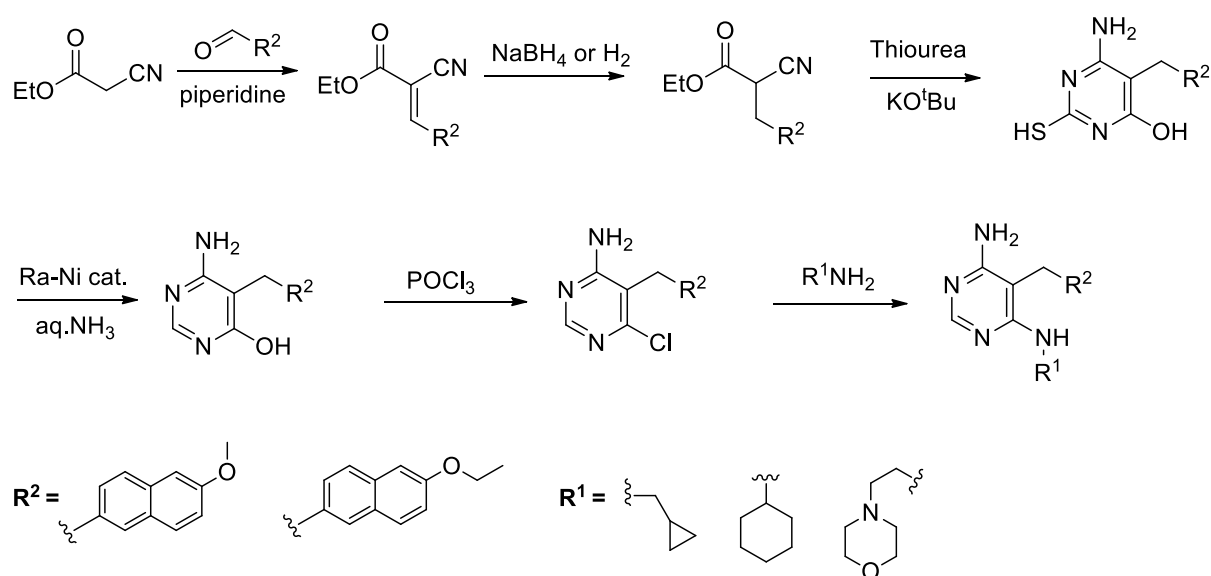
The synthesis of 4,6-diaminopyrimidine derivatives bearing a two-atom linkage between the scaffold and the R^2 substituents was carried out as outlined in **Scheme 1.2**. Three classes of inhibitors would be afforded in this part. They differ in flexibility dependent on the nature of the two-atom linkage. The linker could either be an acetylene (class I), an ethylene (class II), or an ethane (class III) moiety. We wanted to see the effect of linker flexibility in the occupation of the hydrophobic pocket important for selectivity; we hypothesised that more flexible linkers will be more favourable owing to their potential to assume more conformations compared to less flexible linkers. The synthesis began by the amination of the commercially available 4,6-dichloropyrimidine introducing R^1 moieties. This was followed by the displacement of the second chlorine using aqueous ammonia at high temperatures and pressure. The afforded diamino compound would be iodinated at the 5-position followed by the introduction of the acetylene-containing R^2 moiety via the Sonogashira coupling reaction. The transformation would afford class I of inhibitors in this series. Class II and class III would be afforded by a subsequent hydrogenation of class I compounds in the presence of an appropriate reducing agent.



Scheme 1.2. The synthetic route of two-atom linker 4,6-diaminopyrimidine compounds.

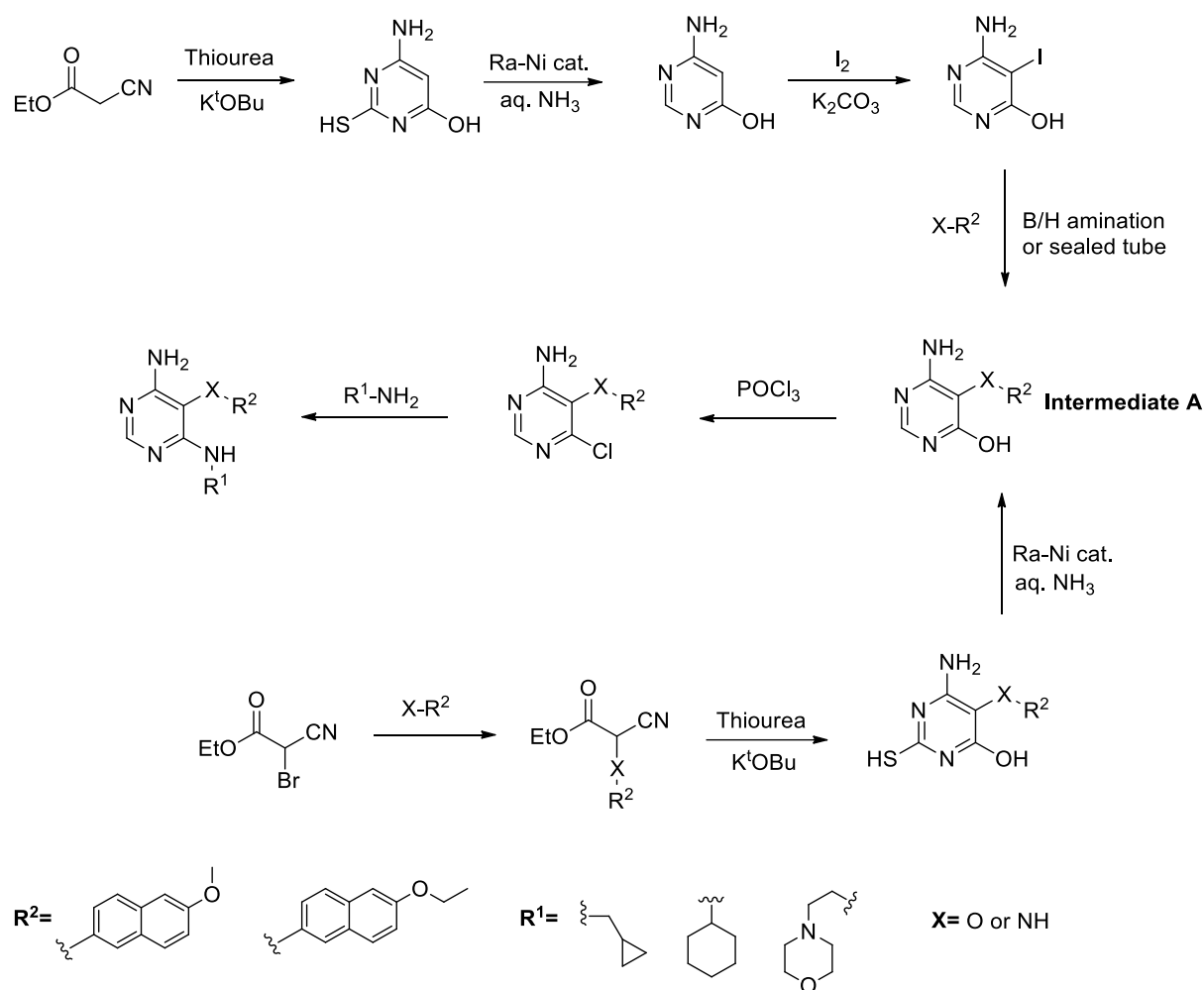
The proposed synthesis of 4,6-diaminopyrimidine derivatives bearing a one-atom linkage between the scaffold and the R^2 substituents was divided into two synthetic schemes depending on the nature of the linker. This linkage can either be a carbon atom, a nitrogen atom, or an oxygen atom. The varied molecular geometry afforded by these atoms would confer unique presentation of the R^2 groups in the hydrophobic pocket. We wanted to identify the conformation that will return the best binding interaction with the kinases.

The synthesis of the carbon linker class of 4,6-diaminopyrimidine derivatives (**Scheme 1.3**) started with a condensation reaction between ethylcyanoacetate and an aldehyde-bearing R^2 group to afford a Michael acceptor type compound. The compound would then be reduced using either sodium borohydride or hydrogen gas followed by a ring forming reaction with thiourea affording an amine, hydroxy, and thiol substituted pyrimidine ring. The synthetic sequence would proceed with the removal of the thiol functionality using Raney-Nickel catalyst, and the substitution of the hydroxy moiety with a chlorine. Subsequent amination of the chloro-intermediates would afford targeted compounds.



Scheme 1.3. Synthesis of the carbon linker 4,6-diaminopyrimidine derivatives.

We envisaged the synthesis of heteroatom linked 4,6-diaminopyrimidine derivatives via two related approaches (**Scheme 1.4**). The first approach involved the preparation of the pyrimidine ring prior to introducing the R^1 and R^2 groups. The pyrimidine ring is formed from ethylcyanoacetate and thiourea. The thiol would be removed using Raney-Nickel catalyst and the 5-position of the ring was iodinated. From the intermediate a Buchwald-Hartwig (B/H) or high-pressure reaction would be carried out to introduce the R^2 groups affording intermediate A (**Scheme 1.4**). Alternatively, the same intermediate could be afforded by first brominating the ethylcyanoacetate followed by the introduction of the R^2 group. In this approach, when the pyrimidine ring is formed the R^2 group would already be incorporated. The removal of the thiol group would afford intermediate A. From intermediate A, our target compounds could be achieved following chlorination using $POCl_3$ and amination with secondary amines.



Scheme 1.4. Approaches to the synthesis of heteroatom linked 4,6-diaminopyrimidine derivatives.

The third and last part of the project included biological evaluation of our synthesised compounds. Enzymatic (*Pf*CDPK1 and *Pf*CDPK4) and whole cell antiparasmodial assays would be performed. All the biological screening and testing would be carried out by our collaborators at Rhodes University and the University of Washington.

1.6 References

1. *World Malaria Report 2023*. 2023, World Health Organisation: Geneva.
2. White, N.J., *Plasmodium knowlesi: The Fifth Human Malaria Parasite*. Clinical Infectious Diseases, 2008. **46**(2): p. 172-173.
3. Dayananda, K.K., R.N. Achur, and D.C. Gowda, *Epidemiology, drug resistance, and pathophysiology of Plasmodium vivax malaria*. J Vector Borne Dis, 2018. **55**(1): p. 1-8.
4. Sherman, I.W., S. Eda, and E. Winograd, *Cytoadherence and sequestration in Plasmodium falciparum: defining the ties that bind*. Microbes Infect, 2003. **5**(10): p. 897-909.
5. Idro, R., et al., *Cerebral malaria: mechanisms of brain injury and strategies for improved neurocognitive outcome*. Pediatr Res, 2010. **68**(4): p. 267-74.
6. Milner, D.A., *Malaria Pathogenesis*. Cold Spring Harbor Perspectives in Medicine, 2018. **8**(1).

7. Battle, K.E., et al., *Geographical variation in Plasmodium vivax relapse*. Malar J, 2014. **13**: p. 144.
8. Klein, E.Y., *Antimalarial drug resistance: a review of the biology and strategies to delay emergence and spread*. International Journal of Antimicrobial Agents, 2013. **41**(4): p. 311-317.
9. Pryce, J., N. Medley, and L. Choi, *Indoor residual spraying for preventing malaria in communities using insecticide-treated nets*. Cochrane Database Syst Rev, 2022. **1**(1): p. Cd012688.
10. *WHO recommends R21/Matrix-M vaccine for malaria prevention in updated advice on immunization*. 2023 [cited 2024; Available from: <https://www.who.int/news/item/02-10-2023-who-recommends-r21-matrix-m-vaccine-for-malaria-prevention-in-updated-advice-on-immunization>].
11. *Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial*. Lancet, 2015. **386**(9988): p. 31-45.
12. Datto, M.S., et al., *Safety and efficacy of malaria vaccine candidate R21/Matrix-M in African children: a multicentre, double-blind, randomised, phase 3 trial*. Lancet, 2024. **403**(10426): p. 533-544.
13. Ringwald, P., et al., *In vitro activities of pyronaridine, alone and in combination with other antimalarial drugs, against Plasmodium falciparum*. Antimicrob Agents Chemother, 1999. **43**(6): p. 1525-7.
14. Price, R.N., et al., *Mefloquine resistance in Plasmodium falciparum and increased pfmdr1 gene copy number*. Lancet, 2004. **364**(9432): p. 438-447.
15. Omari, A.A., C. Gamble, and P. Garner, *Artemether-lumefantrine for uncomplicated malaria: a systematic review*. Trop Med Int Health, 2004. **9**(2): p. 192-9.
16. Sullivan, D.J., Jr., et al., *On the molecular mechanism of chloroquine's antimalarial action*. Proc Natl Acad Sci U S A, 1996. **93**(21): p. 11865-70.
17. Arnold, J., et al., *The effect of continuous and intermittent primaquine therapy on the relapse rate of Chesson strain vivax malaria*. J Lab Clin Med, 1954. **44**(3): p. 429-38.
18. White, N.J., *Primaquine to prevent transmission of falciparum malaria*. Lancet Infect Dis, 2013. **13**(2): p. 175-81.
19. Taylor, W.R. and N.J. White, *Antimalarial drug toxicity: a review*. Drug Saf, 2004. **27**(1): p. 25-61.
20. Curd, F.H.S., D.G. Davey, and F.L. Rose, *Studies on Synthetic Antimalarial Drugs*. Annals of Tropical Medicine & Parasitology, 1945. **39**(3-4): p. 208-216.
21. Kain, K.C., G.D. Shanks, and J.S. Keystone, *Malaria Chemoprophylaxis in the Age of Drug Resistance. I. Currently Recommended Drug Regimens*. Clinical Infectious Diseases, 2001. **33**(2): p. 226-234.
22. Watkins, W.M., D.G. Sixsmith, and J.D. Chulay, *The activity of proguanil and its metabolites, cycloguanil and p-chlorophenylbiguanide, against Plasmodium falciparum in vitro*. Annals of Tropical Medicine & Parasitology, 1984. **78**(3): p. 273-278.
23. Srivastava, I.K. and A.B. Vaidya, *A mechanism for the synergistic antimalarial action of atovaquone and proguanil*. Antimicrob Agents Chemother, 1999. **43**(6): p. 1334-9.
24. Falco, E.A., et al., *2:4-diaminopyrimidines- a new series of antimalarials*. Br J Pharmacol Chemother, 1951. **6**(2): p. 185-200.
25. Gregson, A. and C.V. Plowe, *Mechanisms of Resistance of Malaria Parasites to Antifolates*. Pharmacological Reviews, 2005. **57**(1): p. 117-145.
26. Naidoo, I. and C. Roper, *Mapping 'partially resistant', 'fully resistant', and 'super resistant' malaria*. Trends Parasitol, 2013. **29**(10): p. 505-15.

27. Terkuile, F., et al., *Plasmodium falciparum: In Vitro Studies of the Pharmacodynamic Properties of Drugs Used for the Treatment of Severe Malaria*. Experimental Parasitology, 1993. **76**(1): p. 85-95.
28. Eckstein-Ludwig, U., et al., *Artemisinins target the SERCA of Plasmodium falciparum*. Nature, 2003. **424**(6951): p. 957-61.
29. Krishna, S., A.-C. Uhlemann, and R.K. Haynes, *Artemisinins: mechanisms of action and potential for resistance*. Drug Resistance Updates, 2004. **7**(4): p. 233-244.
30. Kokwaro, G., L. Mwai, and A. Nzila, *Artemether/lumefantrine in the treatment of uncomplicated falciparum malaria*. Expert Opin Pharmacother, 2007. **8**(1): p. 75-94.
31. Noedl, H., D. Socheat, and W. Satimai, *Artemisinin-Resistant Malaria in Asia*. New England Journal of Medicine, 2009. **361**(5): p. 540-541.
32. Uwimana, A. and E. Legrand, *Emergence and clonal expansion of in vitro artemisinin-resistant Plasmodium falciparum kelch13 R561H mutant parasites in Rwanda*. 2020. **26**(10): p. 1602-1608.
33. Nasamu, A.S. and A.J. Polino, *Malaria parasite plasmepsins: More than just plain old degradative pepsins*. 2020. **295**(25): p. 8425-8441.
34. Harper, J.F. and A. Harmon, *Plants, symbiosis and parasites: a calcium signalling connection*. Nat Rev Mol Cell Biol, 2005. **6**(7): p. 555-66.
35. Vidadala, R.S.R., et al., *Development of potent and selective Plasmodium falciparum calcium-dependent protein kinase 4 (PfCDPK4) inhibitors that block the transmission of malaria to mosquitoes*. European Journal of Medicinal Chemistry, 2014. **74**: p. 562-573.
36. Kuwahara, H., M. Nishizaki, and H. Kanazawa, *Nuclear localization signal and phosphorylation of Serine350 specify intracellular localization of DRAK2*. J Biochem, 2008. **143**(3): p. 349-58.
37. Jura, N., et al., *Catalytic control in the EGF receptor and its connection to general kinase regulatory mechanisms*. Mol Cell, 2011. **42**(1): p. 9-22.
38. Vijayan, R.S.K., et al., *Conformational Analysis of the DFG-Out Kinase Motif and Biochemical Profiling of Structurally Validated Type II Inhibitors*. Journal of Medicinal Chemistry, 2015. **58**(1): p. 466-479.
39. Modi, V. and R.L. Dunbrack, Jr., *Defining a new nomenclature for the structures of active and inactive kinases*. 2019. **116**(14): p. 6818-6827.
40. Arter, C., et al., *Structural features of the protein kinase domain and targeted binding by small-molecule inhibitors*. J Biol Chem, 2022. **298**(8): p. 102247.
41. Roskoski, R., Jr., *Classification of small molecule protein kinase inhibitors based upon the structures of their drug-enzyme complexes*. Pharmacol Res, 2016. **103**: p. 26-48.
42. Zuccotto, F., et al., *Through the "Gatekeeper Door": Exploiting the Active Kinase Conformation*. Journal of Medicinal Chemistry, 2010. **53**(7): p. 2681-2694.
43. Fabbro, D., S.W. Cowan-Jacob, and H. Moebitz, *Ten things you should know about protein kinases: IUPHAR Review 14*. Br J Pharmacol, 2015. **172**(11): p. 2675-700.
44. Yun, C.H., et al., *Structures of Lung Cancer-Derived EGFR Mutants and Inhibitor Complexes: Mechanism of Activation and Insights into Differential Inhibitor Sensitivity*. Cancer Cell, 2007. **11**(3): p. 217-227.
45. Roskoski, R., *Properties of FDA-approved small molecule protein kinase inhibitors: A 2024 update*. Pharmacological Research, 2024. **200**: p. 107059.
46. Mustière, R., P. Vanelle, and N. Primas, *Plasmodial Kinase Inhibitors Targeting Malaria: Recent Developments*. Molecules, 2020. **25**(24).
47. Harmon, A.C., *Calcium-regulated protein kinases of plants*. Gravit Space Biol Bull, 2003. **16**(2): p. 83-90.
48. Christodoulou, J., et al., *Evidence for differing roles for each lobe of the calmodulin-like domain in a calcium-dependent protein kinase*. J Biol Chem, 2004. **279**(28): p. 29092-100.

49. Harper, J.F., G. Breton, and A. Harmon, *Decoding Ca(2+) signals through plant protein kinases*. Annu Rev Plant Biol, 2004. **55**: p. 263-88.
50. Sanders, D., et al., *Calcium at the crossroads of signaling*. Plant Cell, 2002. **14 Suppl**(Suppl): p. S401-17.
51. Green, J.L., et al., *The motor complex of Plasmodium falciparum: phosphorylation by a calcium-dependent protein kinase*. J Biol Chem, 2008. **283**(45): p. 30980-9.
52. Bansal, A., et al., *Characterization of Plasmodium falciparum calcium-dependent protein kinase 1 (PfCDPK1) and its role in microneme secretion during erythrocyte invasion*. J Biol Chem, 2013. **288**(3): p. 1590-602.
53. Azevedo, M.F., et al., *Inhibition of Plasmodium falciparum CDPK1 by conditional expression of its J-domain demonstrates a key role in schizont development*. Biochem J, 2013. **452**(3): p. 433-41.
54. Dvorin, J.D., et al., *A plant-like kinase in Plasmodium falciparum regulates parasite egress from erythrocytes*. Science, 2010. **328**(5980): p. 910-2.
55. Bansal, A., et al., *Plasmodium falciparum Calcium-Dependent Protein Kinase 2 Is Critical for Male Gametocyte Exflagellation but Not Essential for Asexual Proliferation*. mBio, 2017. **8**(5): p. e01656-17
56. Kumar, S., M.T. Haile, and M.R. Hoopmann, *Plasmodium falciparum Calcium-Dependent Protein Kinase 4 is Critical for Male Gametogenesis and Transmission to the Mosquito Vector*. 2021. **12**(6): p. e0257521.
57. Ojo, K.K., et al., *A Specific Inhibitor of PfCDPK4 Blocks Malaria Transmission: Chemical-genetic Validation*. The Journal of Infectious Diseases, 2013. **209**(2): p. 275-284.
58. Ghartey-Kwansah, G., et al., *Calcium-dependent Protein Kinases in Malaria Parasite Development and Infection*. Cell Transplantation, 2020. **29**: p. 0963689719884888.
59. Ansell, K.H., et al., *Biochemical and antiparasitic properties of inhibitors of the Plasmodium falciparum calcium-dependent protein kinase PfCDPK1*. Antimicrobial Agents and Chemotherapy. 2014. **58**(10): p. 6032-43.
60. Chapman, T.M., et al., *Optimization of an imidazopyridazine series of inhibitors of Plasmodium falciparum calcium-dependent protein kinase 1 (PfCDPK1)*. J Med Chem, 2014. **57**(8): p. 3570-87.
61. Kato, N., et al., *Gene expression signatures and small-molecule compounds link a protein kinase to Plasmodium falciparum motility*. Nat Chem Biol, 2008. **4**(6): p. 347-56.
62. Ojo, K.K., et al., *Transmission of malaria to mosquitoes blocked by bumped kinase inhibitors*. The Journal of Clinical Investigation, 2012. **122**(6): p. 2301-2305.

Chapter 2: Design and *in silico* studies of pyrrolo[2,3-*d*]pyrimidine and *N*⁴- and 5-substituted-4,6-diaminopyrimidine derivatives

2.1 Introduction

There are several phases that are involved in the development of drugs from the laboratory to the market. These include the discovery, design, and development of hit and lead compounds to molecules with drug-like properties, followed by the preclinical and clinical stages where the drug is tested for attributes like efficacy and toxicity *in vitro* and *in vivo*, and then on human subjects, respectively. Lead compounds can be discovered by way of serendipity, high throughput screening of natural products and ligand libraries, from existing drugs or natural ligands, or by computer-aided designs to name a few [1, 2]. The antihypertensive drug, clonidine **2.1** and the antibacterial drug penicillin G **2.2** are examples of commercially available drugs whose discovery involves serendipity, whereas amoxycillin **2.3** was derived from an existing drug (penicillin) and oseltamivir **2.4** was identified by computer-aided design (**Figure 2.1**) [3-6].

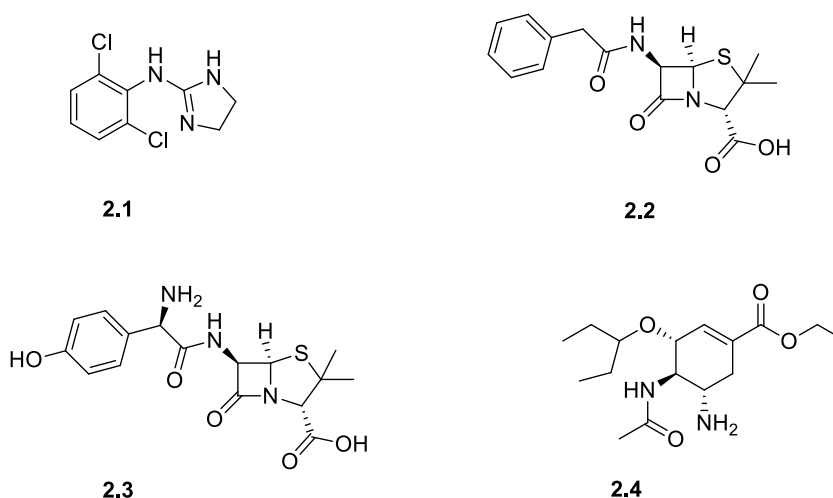


Figure 2.1. Chemical structures of clonidine **2.1** (antihypertensive drug), penicillin G **2.2** (antibacterial drug), amoxycillin **2.3** (antibacterial drug), and oseltamivir **2.4** (anti-influenza drug).

Advances in technology and science have rendered computer-aided drug design (CADD) a useful tool for both academia and pharmaceutical sectors in visualizing, designing, and optimizing lead compounds into effective drugs. CADD tools can be used to provide insight on the behavior of both small molecules and macromolecules, predict how they interact with each other, and give a forecast on the probable pharmacological and side effects associated with studied ligands [7]. Since its inception there have been reports on its success not only in designing drugs with desired

activity but also in reducing both the time and costs associated with the early studies of drug discovery [8, 9]. However, it is not without disadvantages. CADD heavily relies on the presence and quality of experimental data on which predictive algorithms are applied - poor data produces results of poor accuracy. Furthermore, theories on which algorithms are based have a long way to go before they can address the complexities of biological systems, the success of which will yield computer-generated models and prediction of high accuracy [10].

CADD can either be structure-based or ligand-based depending on the available structural information on the targets and ligands. Structure-based drug design (SBDD) makes use of the 3-dimensional (3D) data of the target acquired experimentally or by way of homology modelling [11]. It analyses the data to identify key structural features on the target molecule like the binding sites on which ligands are docked and assessed for affinity. Ligand-based drug design (LBDD) is the go-to approach when there is limited to no information on the biological target of interest save that of the ligands that produce the desired biological response. A relationship between structure and activity is then studied and a model is created that is used to design ligands with the desired biological effect [12].

Pharmacodynamics, how a drug interacts with a biological target to produce a pharmacological effect, is the primary focus in drug design and development. However, it does not exist in isolation because for a drug to be useful as a clinical agent it needs to reach the target. Therefore, alongside pharmacodynamics, a pharmacokinetics study must be carried out. Pharmacokinetics, defined as how the body interacts with the drug, or simply what the body does to the drug, involves four focus areas which are absorption, distribution, metabolism/biotransformation, and excretion (ADME) [13]. Executing pharmacodynamics and pharmacokinetics studies in the early stages of drug design and discovery is a good strategy (especially through CADD) in reducing research costs and the likelihood of drug failure further up the drug discovery pipeline.

In this research we employ a SBDD approach to evaluate the potential performance and interaction of our compounds with the targeted enzymes (pharmacodynamics studies). Our choice is informed by the available structural information of both targets – the crystal structure of *Pf*CDPK4 and amino acid sequence of *Pf*CDPK1. We also look at computer-generated ligand-based properties from which the pharmacokinetic attributes of our compounds can be inferred.

2.2 Structural design rationale

Our target molecules share structural features with adenosine triphosphate (ATP), a natural substrate of the targeted kinases, *Pf*CDPK4 and *Pf*CDPK1. They both have the amino-pyrimidine

ring which forms H-bonding at the hinge region of the binding site. This helps in orienting the molecule such that essential groups, like the triphosphate in ATP, are in the correct region of space. Additionally, these intermolecular forces keep the molecule bound long enough in the pocket for a catalytic reaction to occur or a competitive inhibition. The potential of pyrazolo[2,3-*d*]pyrimidine **2.5** and imidazo[1,5-*a*]pyrazine **2.6** scaffolds to inhibit *Pf*CDPK4 and *Pf*CDPK1 have been studied [14]. Thus, we decided to explore the pyrrolo[2,3-*d*]pyrimidine **2.7** fused-ring system as a scaffold for potential inhibitors (**Figure 2.2**).

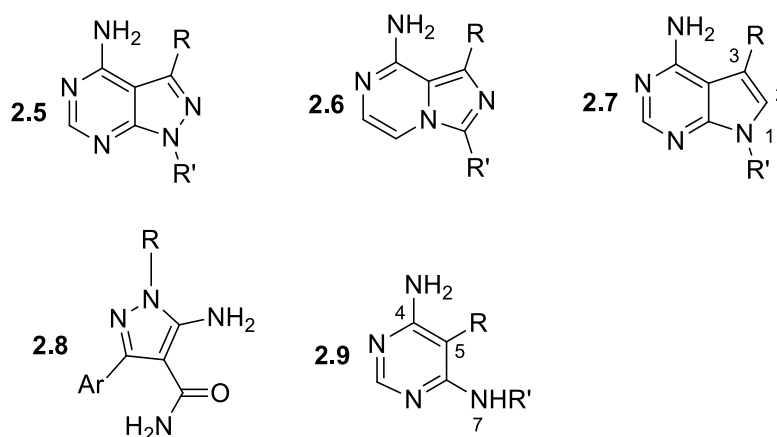


Figure 2.2. General structures of compounds that have been studied as potential inhibitors of *Pf*CDPK4 (**2.5**, **2.6** & **2.8**) and compounds that are studied in this research as potential inhibitors of *Pf*CDPK4 and *Pf*CDPK1 (**2.7** & **2.9**).

Insight gained from work by Huang et al. [15] where they explored 5-aminopyrazole-4-carboxamide analogues **2.8** as inhibitors of *Pf*CDPK4, highlights the possibility of using a simpler scaffold in the development of inhibitors. We wanted to preserve the H-bonding observed at the hinge region of the active site, therefore, we decided to explore derivatives of 4,6-diaminopyrimidine **2.9** as potential inhibitors of the targeted kinase (**Figure 2.2**).

Having decided which scaffolds to use, we moved on to choosing substituents that will be incorporated at the C3 and N1 positions of pyrrolo[2,3-*d*]pyrimidines, and the C5 and N7 positions of 4,6-diaminopyrimidines. Our work was made easier by the research done by Vidadala *et al.* where they observed that having a bulky aromatic moiety at the C3 position of their pyrazolopyrimidine compounds yielded selective inhibitors; the large aryl moiety took advantage of the large hydrophobic pocket of *Pf*CDPK4 due to a relatively small gatekeeper residue compared to most human kinases [14]. We decided then to incorporate the naphthalene derivatives at the C3 and C5 positions of the pyrrolo[2,3-*d*]pyrimidine and 4,6-diaminopyrimidine scaffolds, respectively. We added the 2-methoxyphenyl and 3,4-dimethoxyphenyl groups for the pyrrolo[2,3-*d*]pyrimidines because the boronic acid precursors of these groups are available commercially.

The *N*-substituents on either scaffold is varied in both their hydrophobic character, size, and linker-length. We limited our focus on five *N*-substituents, the methylenecyclopropane, methylenebenzene, cyclohexane, ethylenemorpholine and piperidine (acyl and *tert*-butyloxycarbonyl (boc) protected), as these were available in our laboratory. These substituents occupy a binding pocket (ribose pocket) that is available for the sugar moiety of ATP. It is through an interplay between the *N*-substituents and aromatic moieties that an optimum binding affinity is achieved. The explored compounds are illustrated in **Figure 2.3**, both the pyrrolo[2,3-*d*]pyrimidine (**Figure 2.3a**) and 4,6-diaminopyrimidine (**Figure 2.3b**) series.

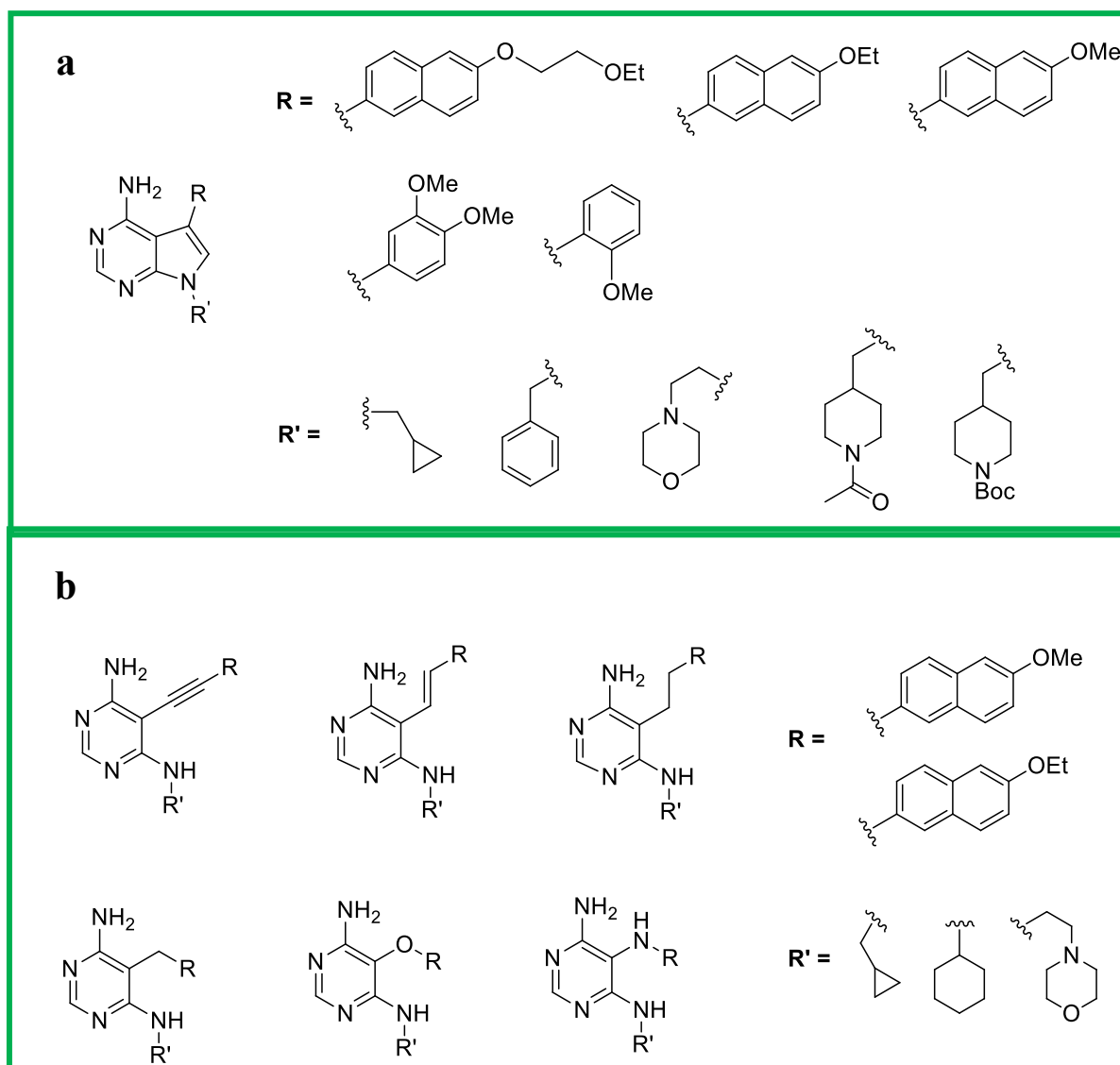


Figure 2.3. Chemical structures of the targeted **a**) pyrrolo[2,3-*d*]pyrimidine and **b**) 4,6-diaminopyrimidine compounds.

In an interest to explore the nature of the hydrophobic pocket on which the large aromatic groups bind, we varied the atom type and linker-length between the 4,6-diaminopyrimidine scaffold and the naphthalene moieties affording two series of compounds. The first class contain a linker with two atoms between the scaffold and the aromatic group made up of carbon atoms with a varied degree of unsaturation – single, double, and triple bond (**Figure 2.3b** – top row). We hypothesised that reducing the triple bond to a double and single bond will make the bulky aromatic moiety more flexible and therefore able to assume multiple conformations within the binding site, one of which will be an ideal or favourable conformation. The second class of the 4,6-diaminopyrimidines contain a linker with one atom between the scaffold and the naphthalene moieties that is varied between a carbon, oxygen, or a nitrogen atom (**Figure 2.3b**– bottom row). The different bonding angle afforded by each atom between the scaffold and the aryl group will confer a unique conformation on each ligand in the active site and thus a unique binding affinity. Overall, the data obtained on these classes will help us understand the effects of linker length, flexibility, and atom type on inhibitory activity.

2.3. Molecular modelling and molecular docking

2.3.1. *Plasmodium falciparum* Calcium-Dependent Protein Kinase 4 studies

We started our *in silico* study by preparing the crystal structure of PfCDPK4 (4QOX, from <https://www.rcsb.org>) using the Protein Preparation Wizard from the Schrödinger suite (2022-2). Protein preparation ensures that issues inherent to the imported crystal structure such as missing hydrogen atoms, missing side chain residues, steric clashes, and bond length deviation, to name a few, are addressed. Important residues to note from the model are Ser147, Asp148, and Tyr150. Asp148 and Tyr150 make up the hinge region of the binding site, whereas Ser147 is the gatekeeper residue to the hydrophobic pocket essential for selectivity. Low energy/minimized 3D models of our targeted compounds and their ionized counterparts (at a physiological pH of 7 ± 2) were also prepared via LigPrep [16]. Preparing accurate models of the enzyme and ligands at the beginning of modeling studies is important as the accuracy of subsequent simulations like molecular docking and molecular dynamics is dependent on this.

We then looked at docking our compounds of interest into the prepared protein to study how they interact with the enzyme. The first protocol we considered was rigid receptor docking (RRD) using the Glide tool [17]. During RRD the only flexible entity is the ligand. In other words, the receptor remains rigid – as the name implies – while the ligand maneuvers around the binding site to find a low energy conformation. With RRD, a receptor grid defining the binding site on which the

ligands will be sampled is prepared prior to docking. We found RRD to be inappropriate for our ligands mainly because the binding site's hydrophobic pocket was defined by a relatively small aromatic moiety (bromobenzyl) of the 4QOX cognate ligand (**Figure 2.4a**) when compared to our aromatic moieties. Setting up a larger receptor grid, therefore, did not change the pocket size.

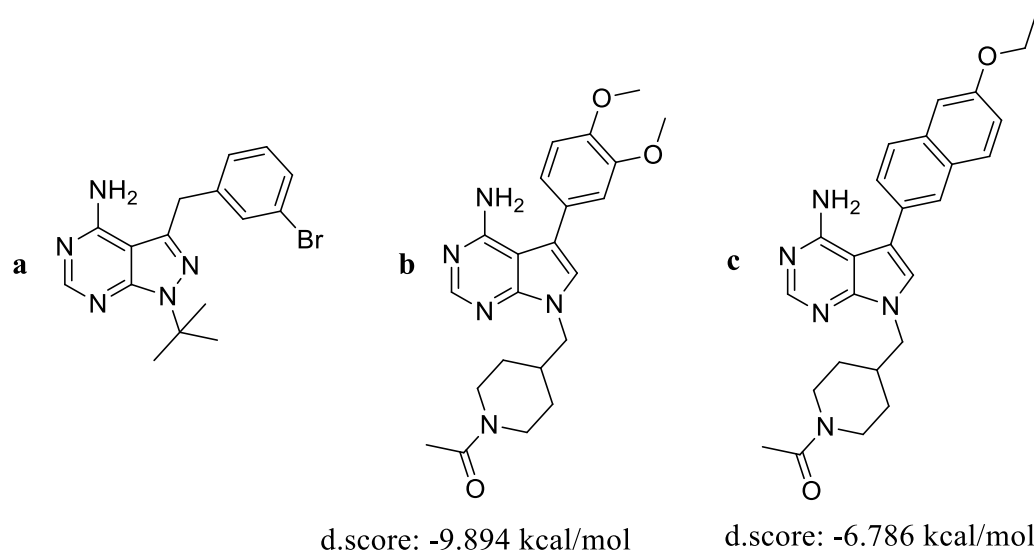


Figure 2.4. Illustration of ligands with their different sizes and docking scores with **a**) the cognate ligand, **b**) a ligand slightly larger than cognate ligand and **c**) a much larger ligand than the cognate ligand.

Structures with aromatic groups of similar size to the bromobenzene moiety of the cognate ligand gave a higher docking score (-9.894 kcal/mol) as they experienced more favorable interaction than bad interactions (**Figure 2.4b**). Ligands with larger aromatic groups (the naphthalene moiety), however, had lower docking scores (e.g. -6.786 kcal/mol) as they experienced unfavorable interaction within the small, predefined hydrophobic pocket (**Figure 2.4c**). Because of this, some ligands with the naphthalene moiety forwent the essential H-bonding at the hinge region to circumvent the unfavorable interactions (**Figure 2.5**). They assumed a conformation that resulted in higher binding affinity.

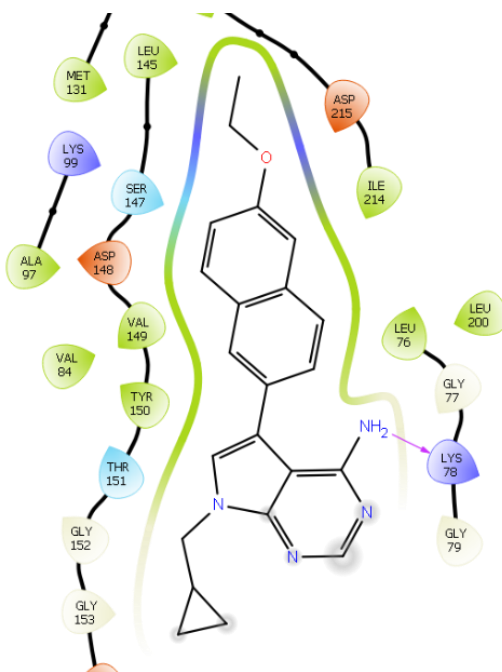


Figure 2.5. Illustration of an incorrectly bound ligand because of a small hydrophobic pocket of the target enzyme as defined by the cognate ligand.

Induced fit docking (IFD) protocol addressed the issues experienced with RRD owing to the additional step performed with Prime [18]. After the initial docking step by Glide, residues near the binding site are rearranged to minimize unfavorable interactions redefining the binding pocket. Ligands are then redocked into their corresponding redefined binding space, sampling different conformations and producing multiple binding poses with varying binding affinity. A hydrogen bond constraint was added to the docking protocol forcing ligands to H-bond with either Tyr150 or Asp148 residues of our enzyme model.

The IFD protocol was validated by redocking the cognate ligand and measuring the root mean square deviation (RMSD) of the conformations before and after docking. The afforded RMSD value was 1.8 Å, an acceptable number given that it is below 3 Å (values above 3 Å are associated with a significant change in structural conformation). The ligand-receptor interactions were also assessed. We observed that the redocked ligand had the desired H-bonding at the hinge region as would be seen with the adenosine ring of ATP (**Figure 2.6a**). It lacked however, the pi-cation and halogen interactions from the initial ligand (**Figure 2.6b**). This difference is likely due to the conformational change undergone by the protein after crystallisation, induced by the removal of the solvent.

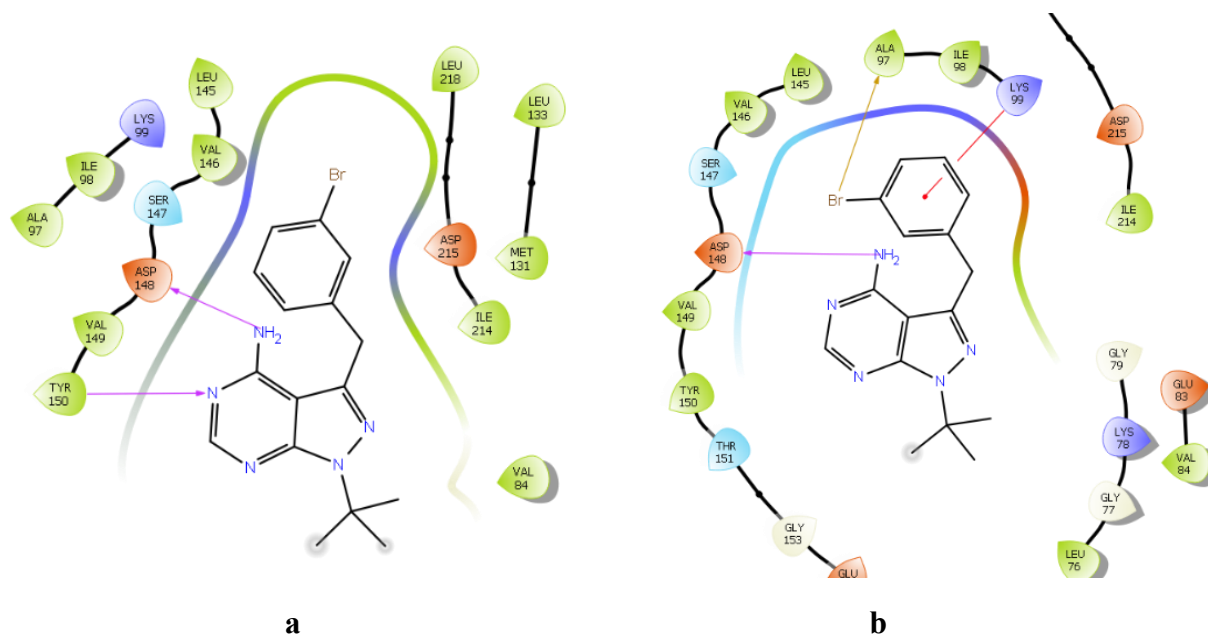
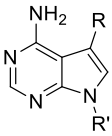
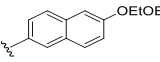
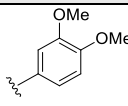
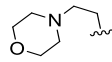
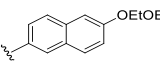
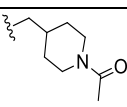
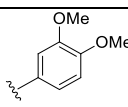
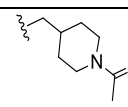
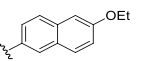
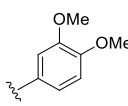
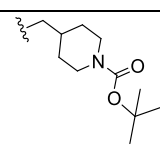
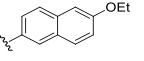
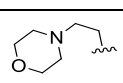
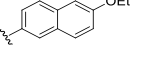
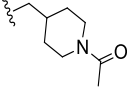
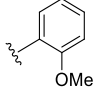
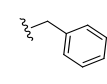
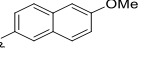
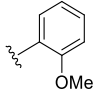
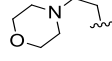
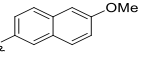
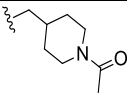
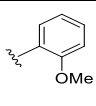
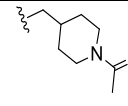
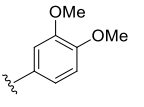
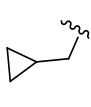
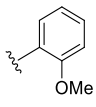
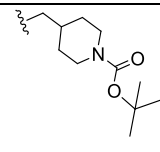
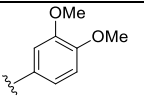


Figure 2.6. 2D poses of the cognate ligand **a)** before docking and **b)** after docking.

After validating our IFD protocol we applied the method on our targeted pyrrolo[2,3-*d*]pyrimidine compounds. The pyrrolo[2,3-*d*]pyrimidine compounds interact with the enzyme in a similar fashion to the adenine ring of the cognate ligand (**Figure 2.6b**). They all participate in the essential H-bonding with residues Tyr150 and Asp148 making up the hinge region. All the bulky aromatics were admitted inside the hydrophobic pocket adjacent to the hinge region. Molecules with the naphthalene moiety scored better than molecules with the dimethoxyphenyl group (**Table 2.1**). This is likely caused by additional Van der Waals interaction afforded by the larger ring.

Table 2.1. Docking score (in kcal/mol) results of the pyrrolo[2,3-*d*]pyrimidine compounds from an IFD experiment with the *Pf*CDPK4 enzyme model.

							
Entry	R	R'	Docking score	Entry	R	R'	Docking score
1			-13.617	10			-9.697
2			-9.682	11			-10.227
3			-12.787	12			-10.013
4			-13.976	13			-11.117
5			-13.037	14			-11.271
6			-13.083	15			-10.992
7			-13.634	16			-11.873
8			-10.823	17			-9.715
9			-12.503				

The dimethoxybenzene moiety participated in hydrogen bonding with either Lys99, Asp215, or the gatekeeper residue Ser147 (**Figure 2.7a**). These hydrogen bonding interactions were not seen with the methoxybenzene moiety, likely because of the ortho position of the methoxy compared to the meta and para positions in the dimethoxy moiety. Unique to the methoxybenzene moiety is the pi-cation interaction between the aromatic ring and Lys99 (**Figure 2.7b**). This pi-cation interaction is not seen on other bulky aromatics save for one of the ethoxyethoxy-naphthalene derivatives (**Figure 2.7b**). However, the interaction may be present in a dynamic situation where molecules are in constant motion, as is the case in real life. The methoxy, ethoxy, and ethoxyethoxy functionalities on the naphthalene rings did not participate in any traditional hydrogen bonding; they participated in aromatic hydrogen bonding, an interaction encountered on all the bulky aromatic groups. The piperidine (both acyl and *tert*-butyloxycarbonyl (boc) protected) and morpholine moieties hydrogen bond with either Ser80, Gly153, or Lys231 (**Figure 2.7c**) in the sugar pocket of the binding site contributing to the overall binding affinity of the compounds. Hydrogen bonding was not observed in any of the cyclopropyl and benzyl containing compounds owing to them lacking heteroatoms.

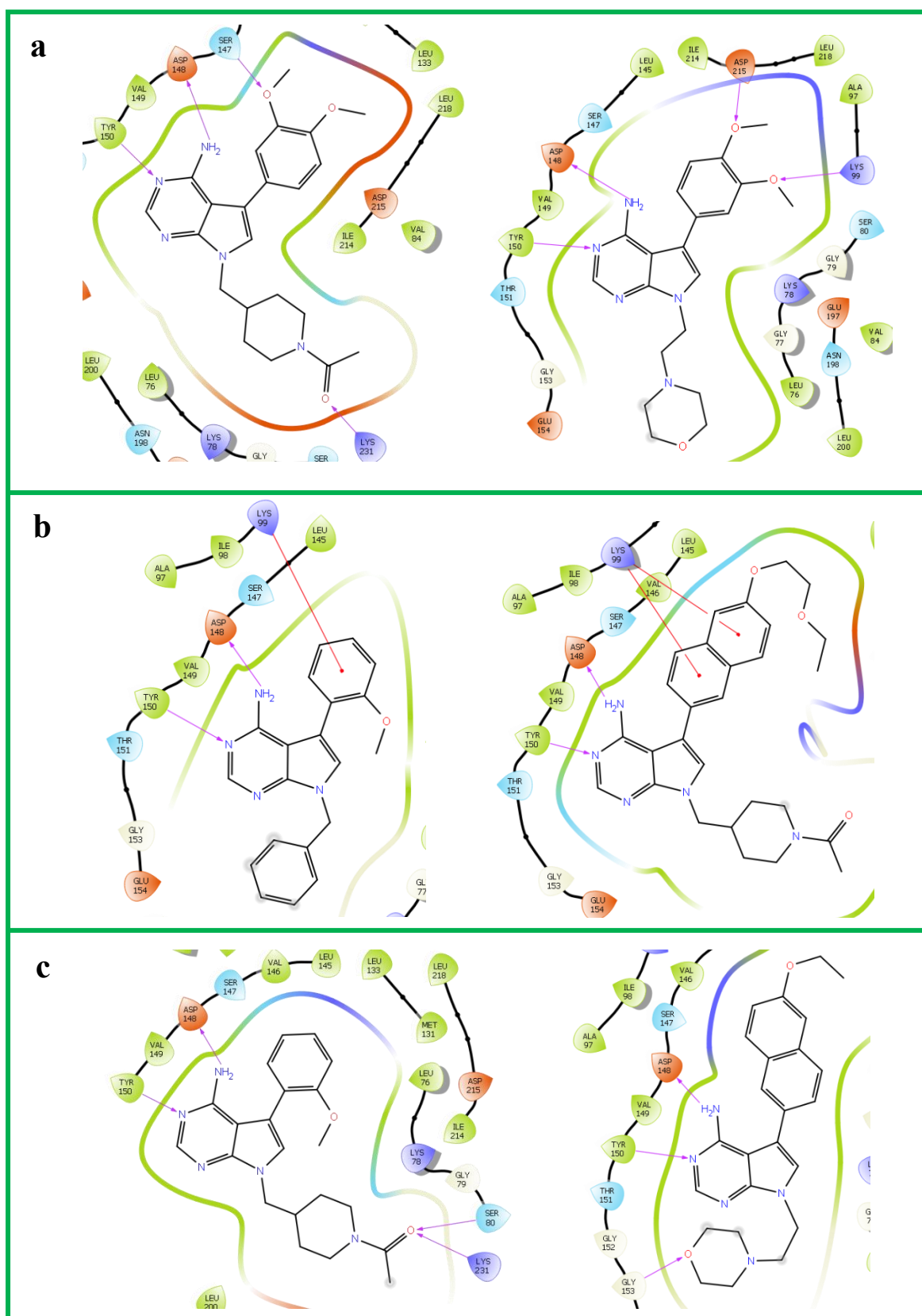
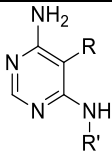
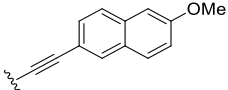
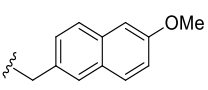
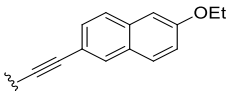
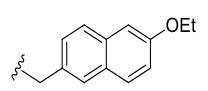
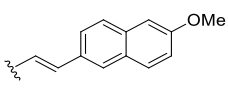
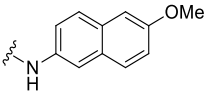
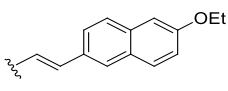
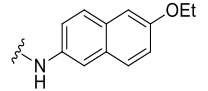
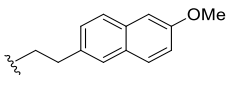
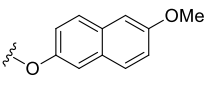
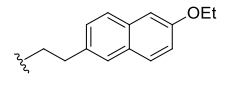
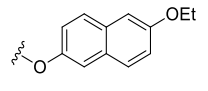


Figure 2.7. 2D ligand interaction diagram of the pyrrolo[2,3-*d*]pyrimidines showing **a)** H-bonding in the hydrophobic region by the dimethoxybenzene group, **b)** pi-cation interaction by the methoxybenzene (left) and ethoxyethoxynaphthalene moiety (right) in the hydrophobic pocket, and **c)** H-bonding by the acylpiperidine and morpholine moiety at the ribose pocket.

Table 2.2. Docking score results (in kcal/mol) of the 4,6-diaminopyrimidine compounds from an IFD experiment with the *Pf*CDPK4 enzyme model.

							
Entry	R	R'	Docking score	Entry	R	R'	Docking score
1			-9.276	7			-11.751
			-10.885				-11.549
			-12.585				-12.268
2			No Pose	8			-11.494
			No Pose				-12.334
			-10.142				-11.168
3			-11.099	9			-12.230
			-11.461				-7.443
			-11.416				-12.352
4			-10.876	10			-11.915
			-9.038				-12.197
			-9.334				-12.936
5			-12.140	11			-11.596
			-12.012				-11.456
			-13.531				-11.564
6			-12.835	12			-11.897
			-13.426				-11.456
			-9.475				-11.866

After docking and analysing the pyrrolo[2,3-*d*]pyrimidine compounds, we docked the 4,6-diaminopyrimidine derivatives to the prepared *Pf*CDPK4 model, and then assessed the obtained results. The ethane-linked naphthalene compounds of the 4,6-diaminopyrimidine series (Entries 5 & 6 of **Table 2.2**) scored better than the acetylene- and ethene-linked compounds (entries 1 & 2 and 3 & 4, respectively of **Table 2.2**). The acetylene derivatives did not fit well inside the binding pocket owing to linear bond angle resulting from the carbon-carbon triple bond. The linker could not bend any lower than 170° to fit the naphthalene ring inside the targeted hydrophobic pocket while maintaining optimal H-bonding at the hinge region (**Figure 2.8a**). The methoxy-naphthalene compounds in this series (entry 1) performed better than the ethoxy-naphthalene molecules (entry 2) because of their relatively small size. The additional -CH₂ from the ethoxy made it harder to fit the naphthalene in the hydrophobic pocket while maintaining optimal interactions at the hinge region. In fact, only one pose of the ethoxy-naphthalene molecules was returned, evidently benefitting from H-bonding, and bond rotations afforded by the ethyl-morpholine moiety (**Figure 2.8b**). The cyclopropyl and cyclohexyl ethoxynaphthalene derivatives did not have a pose that complied with the H-bonding constraint set when running the IFD experiments (entry 2, **Table 2.2**).

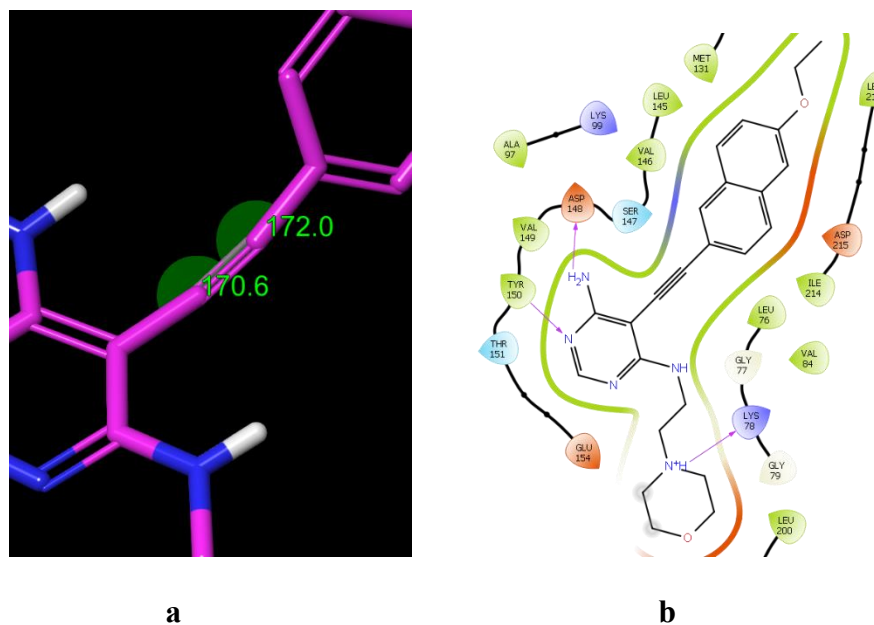


Figure 2.8. a) an enlarged picture of the best docking acetylene-linked naphthalene compound displaying bond distortion. b) 2D ligand interaction diagram demonstrating additional H-bonding afforded by the morpholine moiety.

Reducing the triple bond to a double or single bond resulted in poses with better docking scores overall (**Table 2.2**). The bulky aromatic groups were accommodated inside the hydrophobic pocket without compromising the pyrimidine interaction at the hinge region. All the compounds formed the essential hinge region H-bonding in addition to the aromatic ring H-bonding by the naphthalene moiety (**Figure 2.9a**). The afforded H-bonding and salt bridge interaction observed with the morpholine moiety generally improved the binding affinity of the ligands as reflected by the docking scores (**Table 2.2**). Any low scoring compound of the morpholine moiety had their naphthalene moiety poorly or not at all fitted into the hydrophobic pocket (**Figure 2.9b**). The results from the series bearing the two-atom linker between the pyrimidine and naphthalene rings highlight the importance of balancing rigidity and flexibility when designing ligands. Rigid compounds had poor interactions in the binding pocket while flexible compounds offered improvements, although with a risk of an unpreferred conformation being favorable.

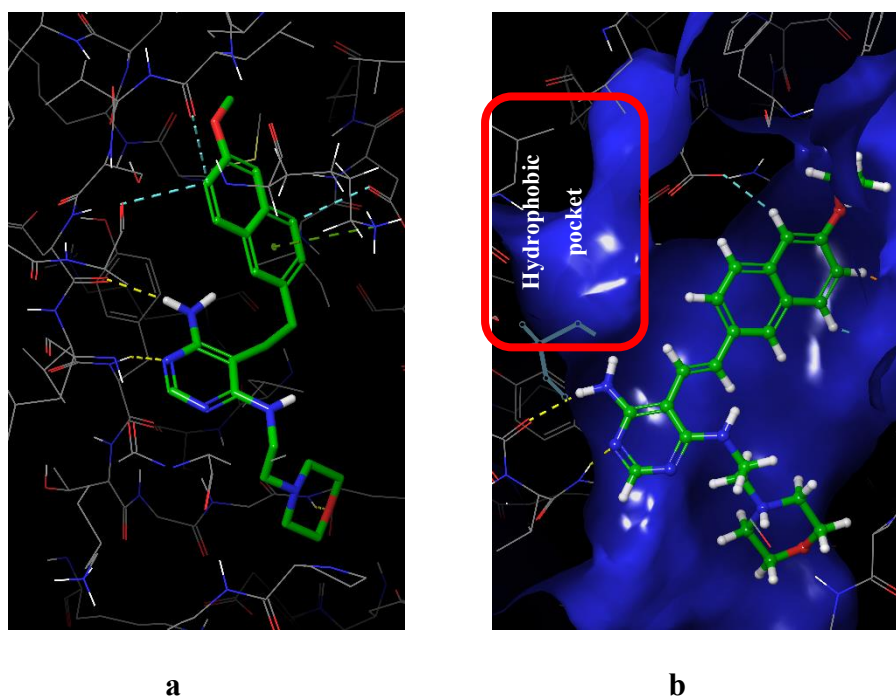


Figure 2.9. a) 3D ligand interaction diagram showing the H-bonding at the hinge region and aromatic H-bonding at the naphthalene moiety. b) 3D ligand interaction diagram with the binding site surface illustrating the missed hydrophobic pocket by the naphthalene ring.

All the one-atom linker compounds (entries 7-12; **Table 2.2**) formed H-bonding at the hinge region as required. Additionally, the naphthalene rings participated in aromatic H-bonding at the hydrophobic pocket (**Figure 2.10a**). The expected hydrogen bonding by the heteroatom's linker was not observed since the linkers are located at the middle of the binding pocket far away from the amino acid residues. Heteroatoms prone to H-bonding are those located at or near the terminal sides of the molecule, closer to the receptor's residues. The morpholine moiety is an example of

such groups which engage in H-bonding (**Figure 2.10b**). There is no pharmacodynamic advantage afforded by heteroatoms as a linker over a carbon atom, however, these may improve the pharmacokinetic properties of the compounds (e.g. the partition coefficient, solubility). The different naphthalene moieties (methoxy- and ethoxy-) perform roughly the same. The docking score of the one-atom linked naphthalene compounds ranges from -11.168 to -12.352, with one outlier at -12.936 due to a molecule with a morpholine moiety (**Figure 2.10b**).

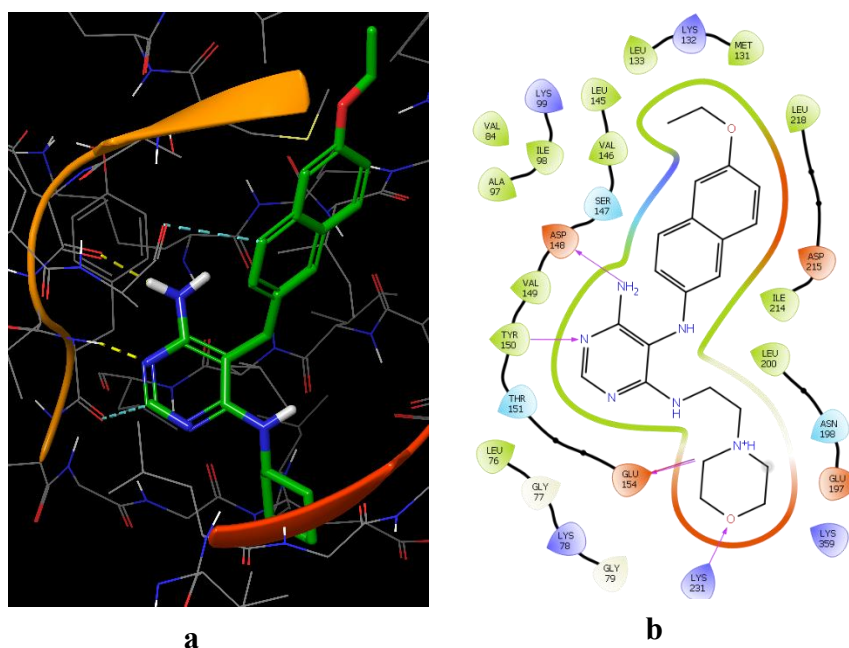


Figure 2.10. a) 3D representation of binding site interactions seen on all one-atom linked 4,6-diaminopyrimidine compounds. b) The best scoring compound of the one-atom linked naphthalene 4,6-diaminopyrimidine compounds. It has a morpholine moiety which forms H-bonding and salt bridge interactions.

In general, the ethane-linked naphthalene compounds are the best performing within the 4,6-diaminopyrimidine series followed closely by the one-atom linked molecules. The one-atom linked naphthalene compounds (entry 7-12; **Table 2.2**) all performed the same. The ethene- and acetylene-linked compounds ranked last with the former having a slight advantage (**Figure 2.11**).

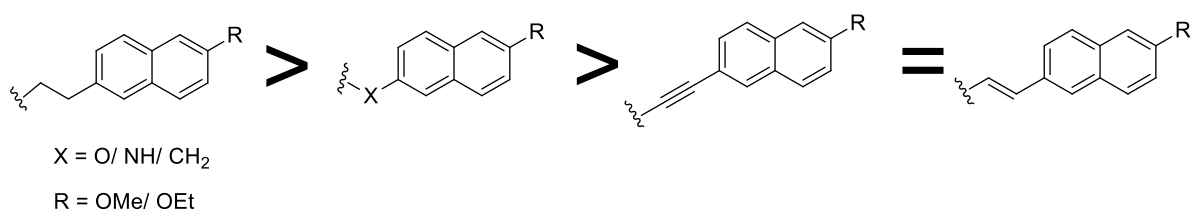


Figure 2.11. 2D shortened structural drawing of the 4,6-diaminopyrimidine derivatives showing the order of docking performance from best to worst.

All our targeted compounds presented the essential H-bonding at the hinge region defined by residues Tyr150 and Asp148. The bulky aromatics were moderately to perfectly accommodated at

their respective hydrophobic pocket without compromising H-bonding at the hinge region, except for the acetylene-linked naphthalene compounds owing to their overall inflexible structure. The piperidine, morpholine, cyclohexane, and cyclopropane moieties played a unique role in both anchoring the molecule in the binding site and imparting additional ligand-receptor interactions affecting ligand conformation and binding affinity, respectively.

2.3.2. *Plasmodium falciparum* Calcium-Dependent Protein Kinase 1 studies

We started our molecular modelling by building the 3D structure of *Pf*CDPK1 from its amino acid sequence using the homology modelling process given that the crystal structure is not available yet. The amino acid sequence of our target kinase was imported into the Schrödinger multiple sequence viewer from which a process of building a homology model was undertaken. The BLAST algorithm was used to search for homologs of the target from the protein database and a list of homologs was compiled with varying sequence identity. The homolog 3Q5I had the highest sequence identity at 93% so it was selected as a template for our target sequence. 3Q5I is an accession number for the protein kinase of *Plasmodium bergeri*, a unicellular parasite that causes rodent malaria. The amino acid sequence of the homolog and target were aligned, and a homology model was created with the homolog cognate ligand added. The afforded model was then prepared using the protein preparation wizard to ensure that issues like steric clashes were addressed and the structure was minimized.

The validity of the prepared model was assessed using the Ramachandran plot (**Figure 2.12**). The red regions represent the favored region for the backbone dihedral angles/ torsional angles while the yellow and white regions represent the allowed and forbidden regions respectively. The favored region is associated with protein tripeptides where there are no steric clashes, and the forbidden regions have protein structure presentation that are sterically forbidden except for glycine since it has no side chain [19]. Most of the dihedral angles (presented as black dots) fall within the favored region of the plot and a few are in the allowed region. The dihedral angles on the forbidden region are ignored since they are caused by glycine residues. The plot affirms that our structure is valid and therefore can be used for further modelling simulations.

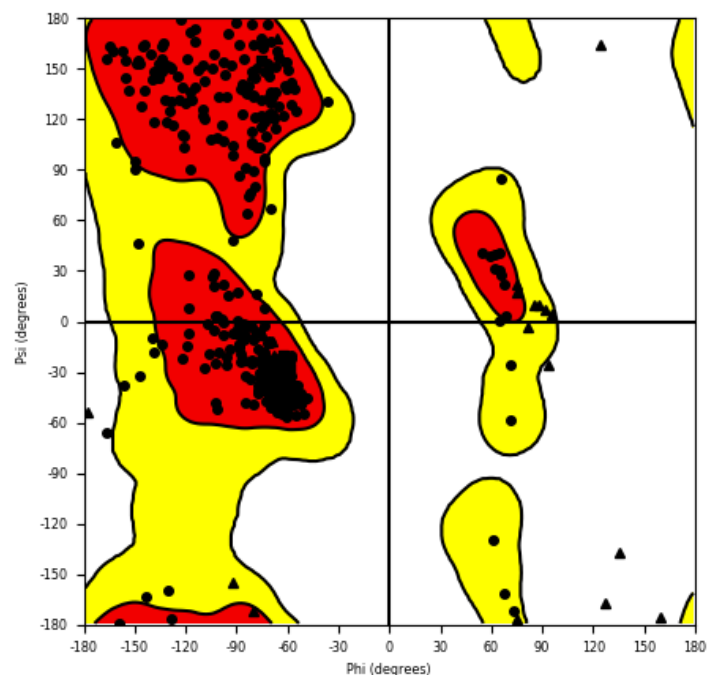
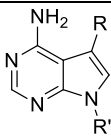
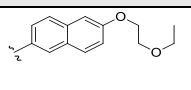
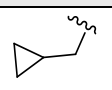
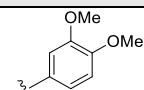
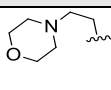
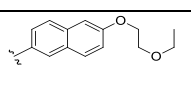
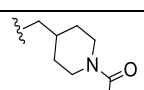
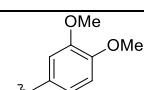
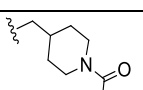
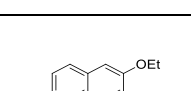
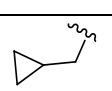
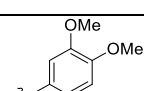
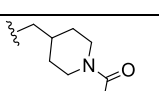
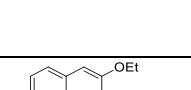
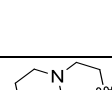
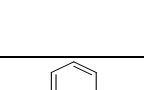
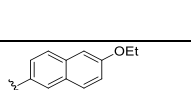
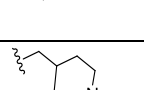
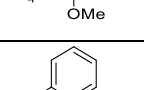
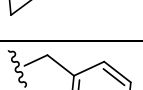
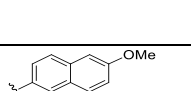
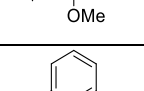
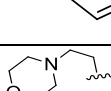
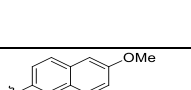
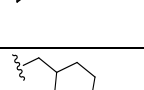
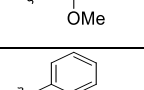
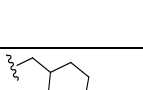
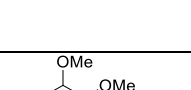
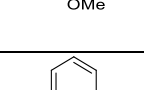
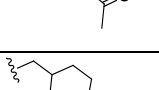
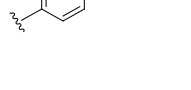


Figure 2.12. The Ramachandran plot of the prepared homology model of *Pf*CDPK1 based on the homolog 3Q5I.

We initially docked the pyrrolo[2,3-*d*]pyrimidine series of compounds into the prepared *Pf*CDPK1 homology model to assess their potential binding affinity. The obtained docking score results, which reflect the relative binding affinity of the compounds, are summarized in **Table 2.3**. The pyrrolo[2,3-*d*]pyrimidine compounds formed hydrogen bonds at the hinge region with Glu146 and Tyr148 as required. All the bulky aromatic groups were fitted in the hydrophobic pocket. The ethoxy- (entries 3-5, **Table 2.3**) and methoxynaphthalene derivatives (entry 6 & 7, **Table 2.3**) scored better than the ethoxyethoxynaphthalene derivatives (entry 1 & 2, **Table 2.3**).

Table 2.3. Docking score results (in kcal/mol) of the pyrrolo[2,3-*d*]pyrimidine compounds from an IFD experiment with the *Pf*CDPK1 enzyme model.

							
Entry	R	R'	Docking score	Entry	R	R'	Docking score
1			-8.607	10			-9.910
2			-9.524	11			-11.113
3			-10.575	12			-10.013
4			-11.005	13			-9.168
5			-10.882	14			-9.063
6			-10.446	15			-8.936
7			-10.368	16			-10.259
8			-9.516	17			-9.121
9			-9.478				

The longer ethoxyethoxy moiety on the naphthalene ring resulted in unfavorable interactions in the hydrophobic pocket (**Figure 2.13a**). It also disturbed the optimal position of the aminopyrimidine ring in the hinge region resulting in a bad interaction (**Figure 2.13a**). The dimethoxybenzene derivatives (entries 8-12, **Table 2.3**) had better binding affinity in the active site compared to their monomethoxybenzene counterparts (entries 13-17, **Table 2.3**). While the dimethoxybenzene's methoxy functionality is positioned at the 3 and 4 positions of the benzene ring, the methoxybenzene's methoxy functionality is positioned at the 2-position of the benzene ring. This arrangement results in unfavorable interactions between the methoxy and residue in the hydrophobic pocket; the bulkiest part of the methoxybenzene moiety is positioned next to the gatekeeper residue (i.e. the narrow part of the hydrophobic pocket, **Figure 2.13b**). The piperidine and morpholine containing derivatives have the potential to hydrogen bond in the ribose pocket adding to the overall binding affinity of the compounds (**Figure 2.13c**). The best scoring compound within the pyrrolopyrimidine series, the dimethoxybenzene bearing compound (entry 11, **Table 2.3**), enjoys optimal interactions in the hydrophobic pocket (which is smaller in *Pf*CDPK1 than in *Pf*CDPK4) because of the smaller ring size and additional hydrogen bonding afforded by the methoxy moieties.

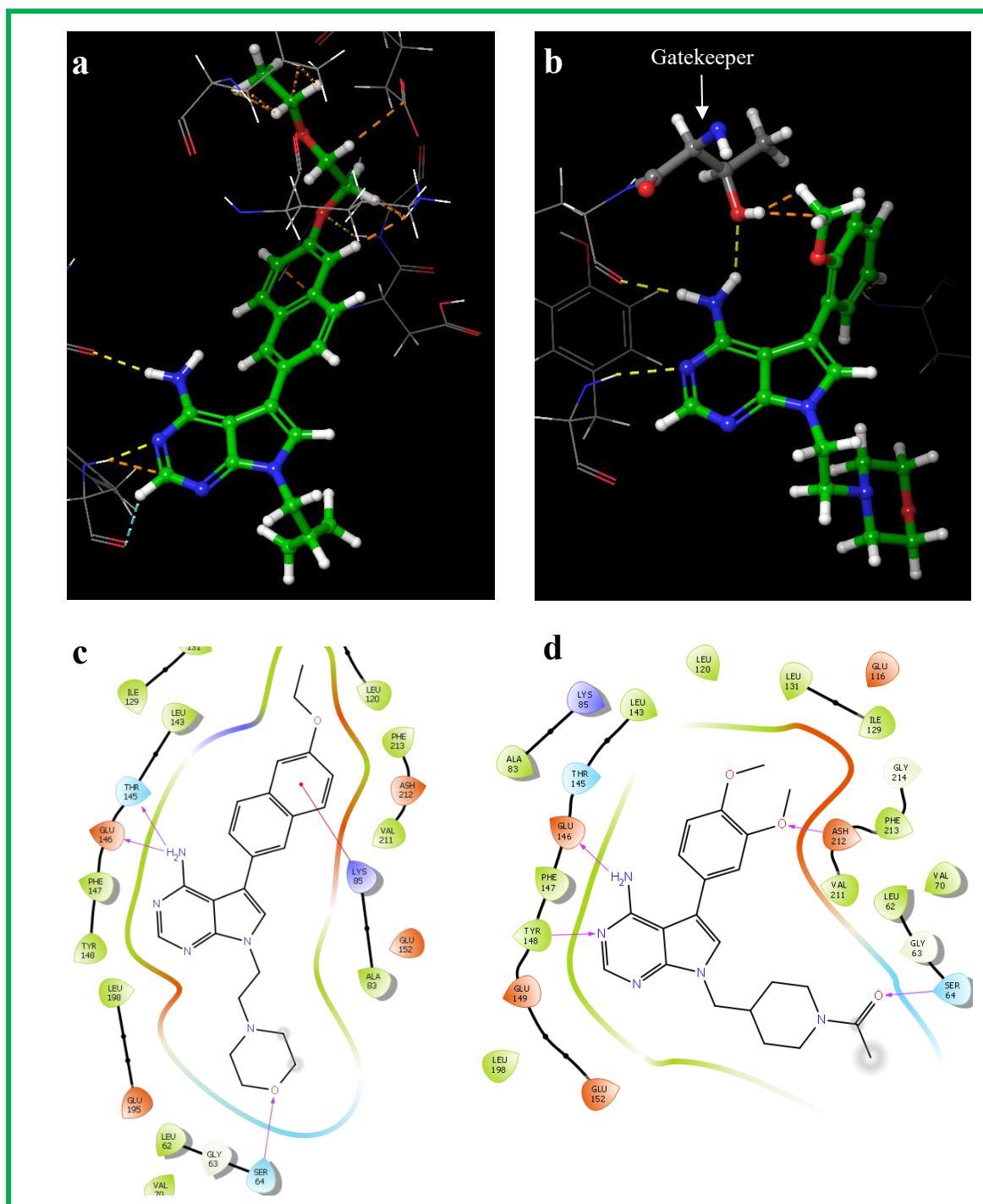
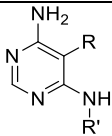
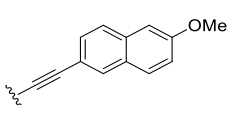
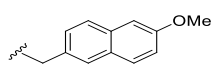
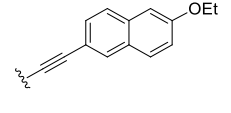
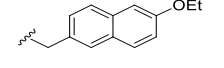
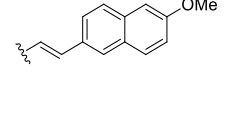
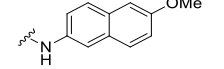
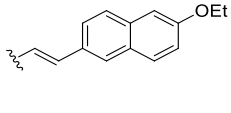
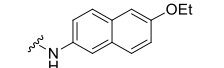
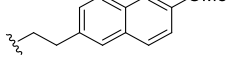
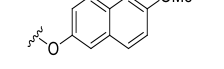
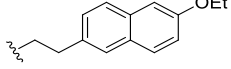
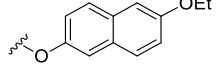


Figure 2.13. 3D diagrams showing the hinge region interactions with **a)** unfavourable interactions (orange dash lines) by the ethoxyethoxy moiety at the hydrophobic pocket and **b)** bad interaction (orange dash lines) through the methoxy, also in the hydrophobic pocket. 2D ligand interaction diagrams representing the **c)** morpholine bearing compound H-bonding at the ribose pocket, and **d)** a best scoring compound with the piperidine moiety H-bonding at the ribose pocket.

We then docked the 4,6-diaminopyrimidine compounds in the active site of the homology model of *Pf*CDPK1. The docking score results which reflect the binding affinities of the compounds are published in **Table 2.4**. The acetylene-linked naphthalene compounds (entry 1-2; **Table 2.4**) hydrogen bonded via the pyrimidine ring at the hinge region of the binding site. However, the naphthalene groups were not admitted in the hydrophobic pocket, instead they occupied a solvent exposed region adjacent to the pocket (**Figure 2.14a**). The approximately linear angle between the scaffold and the naphthalene ring is one of the contributing factors leading to the observed conformation. The rigidity of the linker prevents the naphthalene ring from entering the pocket. Another factor is the small hydrophobic pocket caused by the large gatekeeper residue (Thr145); the larger aromatic rings experience steric hindrance when they approach the pocket. The morpholine derivatives generally have higher docking scores owing to their ability to hydrogen bond and form salt bridge interactions (**Figure 2.14b**). Similar binding was observed with the ethene-linked naphthalene compounds (entries 3-4; **Table 2.4**). The scaffold partakes in H-bonding while the bulky aromatic moieties dock at a solvent exposed region as an alternative to the size reduced hydrophobic pocket. Reducing the triple bond to a double bond linker did not improve the conformation and binding affinity of the molecules.

Table 2.4. Docking score results (in kcal/mol) of the 4,6-diaminopyrimidine compounds from an IFD experiment with the *Pf*CDPK1 enzyme model.

							
Entry	R	R'	Docking score	Entry	R	R'	Docking score
1			-8.974	7			-9.707
			-8.326				-9.344
			-9.030				-9.860
2			-9.392	8			-10.328
			-9.909				-9.754
			-9.813				-10.784
3			-7.978	9			-8.866
			-9.632				-9.178
			-10.187				-9.982
4			-9.057	10			-9.867
			-9.542				-8.873
			-9.513				-9.494
5			-6.826	11			-9.609
			-8.705				-9.087
			-8.227				-8.855
6			-9.465	12			-9.666
			-9.058				-9.624
			-10.080				-9.924

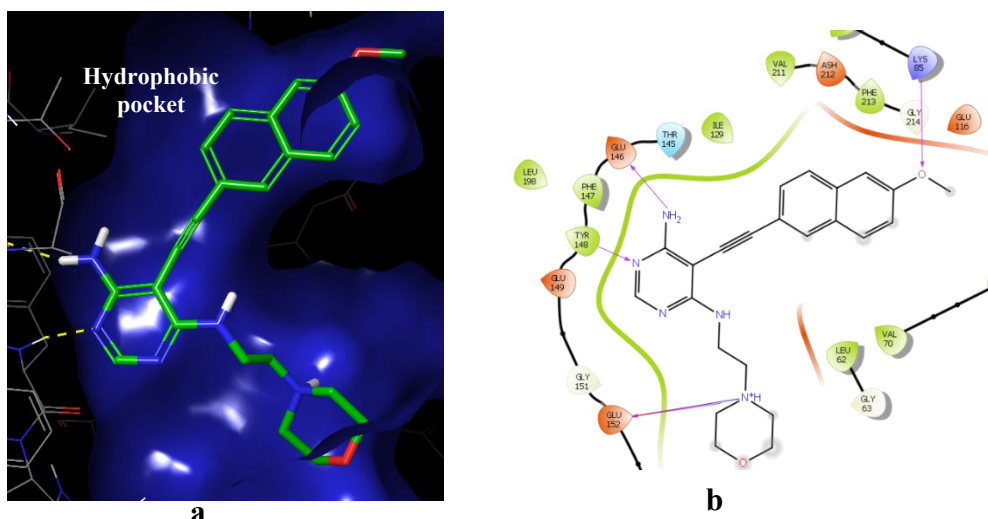


Figure 2.14. General docking pose of the acetylene-linked naphthalene compounds in **a**) 3D ligand interaction (binding pocket in blue) and **b**) 2D ligand interaction diagram.

Likewise, none of the ethane-linked compounds (entry 5-6; **Table 2.4**) had their naphthalene moieties docked inside the hydrophobic pocket (**Figure 2.15a**) save for one. The adequately docked compound benefits from the slightly enlarged pocket caused by the gatekeepers' side chain directing the hydrogen and methyl group towards the pocket instead of the hydroxy and methyl group combination (**Figure 2.15b**). The morpholine derivative boosts the binding affinity of the compounds by forming additional H-bonding as evidenced by the high docking scores, followed closely by the cyclohexane derivatives (**Table 2.4**).

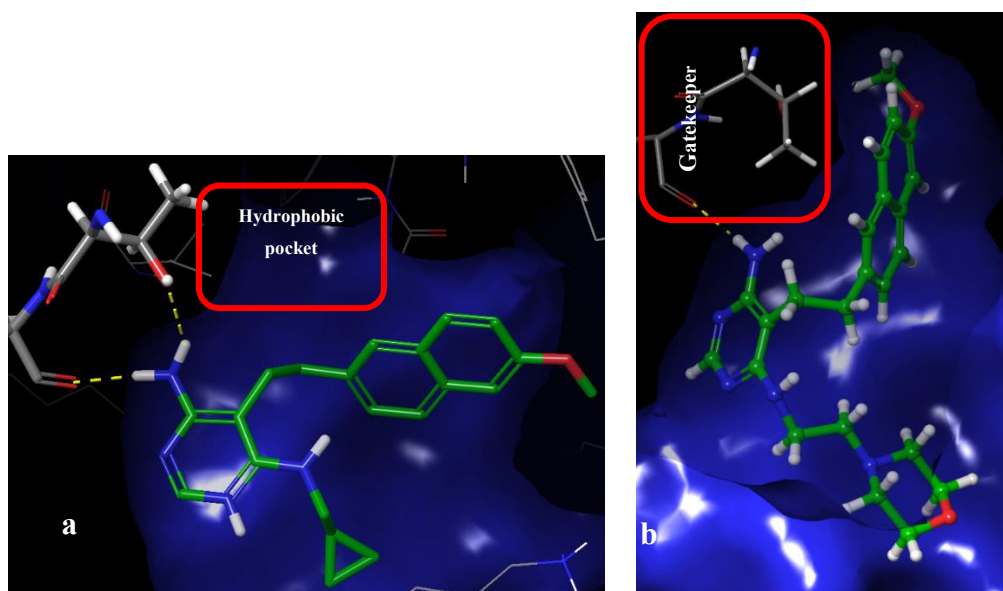


Figure 2.15. **a**) General docking poses of the ethane-linked compounds and **b**) adequately docked compound benefitting from the gatekeeper residue side chain orientation.

site of *Pf*CDPK4 resulting in less interactions between the ligands and the enzyme. *Pf*CDPK1 hydrophobic pocket widens before the second ring of the naphthalene moiety (turning point, **Figure 2.17a**). This reduced the hydrophobic interactions that the naphthalene ring forms in the hydrophobic pocket. It also creates an alternative binding space, that is easily accessible, for the bulky aromatic groups, especially those of the 4,6-diaminopyrimidine derivatives with a two-atom linker. The hydrophobic pocket of *Pf*CDPK4 widens closer to the pyrrolo[2,3-*d*]pyrimidine scaffold (turning point, **Figure 2.17b**) creating an elongated pocket that can form hydrophobic interaction with both rings of the naphthalene moiety, thus, adding positively to the overall binding affinity of the compounds. The narrow hydrophobic pocket size of *Pf*CDPK1 and the geometry of the binding site make the compounds score worse than when they are docked in the *Pf*CDPK4 active site.

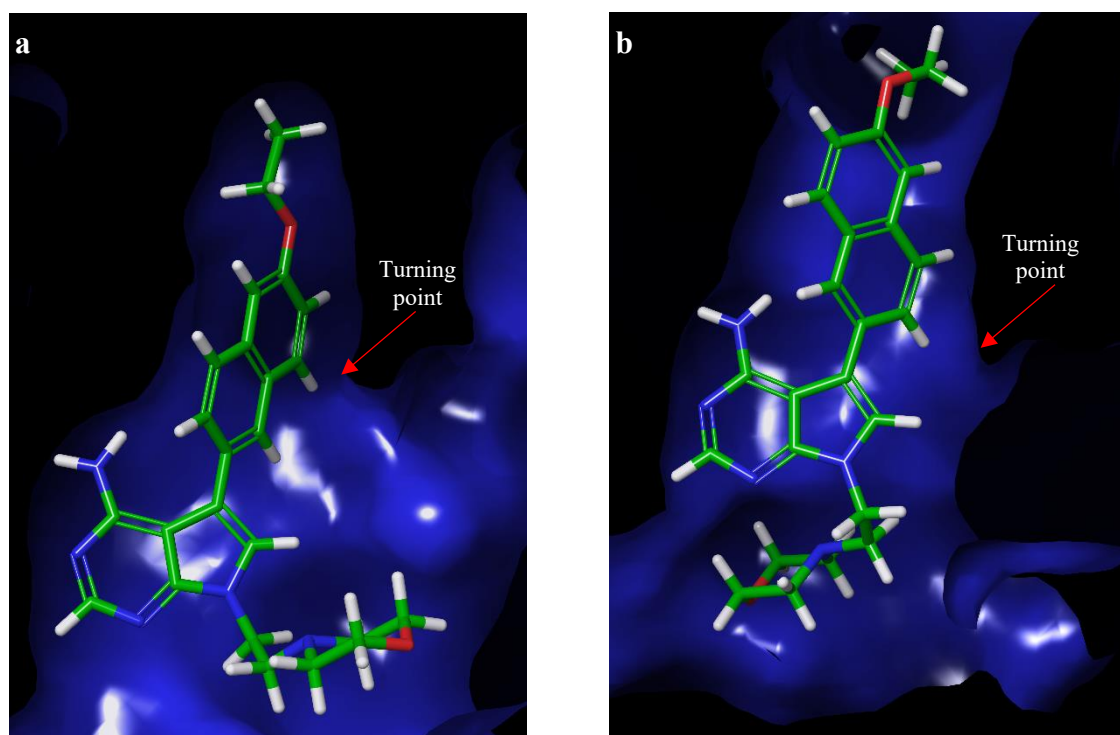


Figure 2.17. The binding site surface (blue) diagrams of (a) *Pf*CDPK1 and (b) *Pf*CDPK4.

2.4 Molecular dynamics (post-docking)

Macromolecules such as enzymes are in constant motion in biological systems. Most of these motions are essential for biological processes such as ligand docking, signal transductions, and catalysis. Understanding how these macromolecules move within a fine temporal resolution can aid in the design of molecules that can interact with them to give a desired response. Experimentally, studying macromolecules movement is not possible. Computationally, however, this is possible by a method called molecular dynamics (MD). MD is a computer-aided simulation

method used to analyse the physical movement of atoms and molecules. It can be applied in three major areas of structure-based design including structure determination and modelling, docking and ligand design, and free-energy calculations [20]. We use MD for post-docking analysis, where we analyse generated ligand-enzyme complexes for conformational stability. Under dynamic conditions other ligand-enzyme interactions that were not reported in docking experiments can be identified. This can either improve the binding mode of the initial complex or reveal instability that leads to the rejection of the ligand in the binding site, or a significant conformational change.

Molecular dynamics simulations were run using the Desmond program from the Schrodinger suite (2022-1) [21]. The process started with the solvation of the complexes using the single point charge (SPC) solvation model with a cubic boundary of 10 Å. The system's net charge was neutralized using either Na⁺ ions or Cl⁻ ions for a negative and positive net charge, respectively, and a 0.15M NaCl salt was added. The MD simulations were run under an NPT ensemble class, where the number of particles (N), pressure (P) and temperature (T) are kept constant. The temperature was set to 310K and pressure at 1.01325 bar. The model was relaxed prior to the simulation using the provided default protocol. The simulation time was set to 50 ns with each of the 1000 frames recorded at 50 ps intervals.

The best performing compounds in both conformation and docking score were selected for further study using MD (**Figure 2.18**) From each IFD experiment (*Pf*CDPK1 and *Pf*CDPK4) a compound was selected from the pyrrolo[2,3-*d*]pyrimidine, two atom linker, and the one atom linker series. Three features were viewed when analysing the results which are the conformational stability of the protein structure and the ligand, stability of the binding site residues, and the protein ligand interactions.

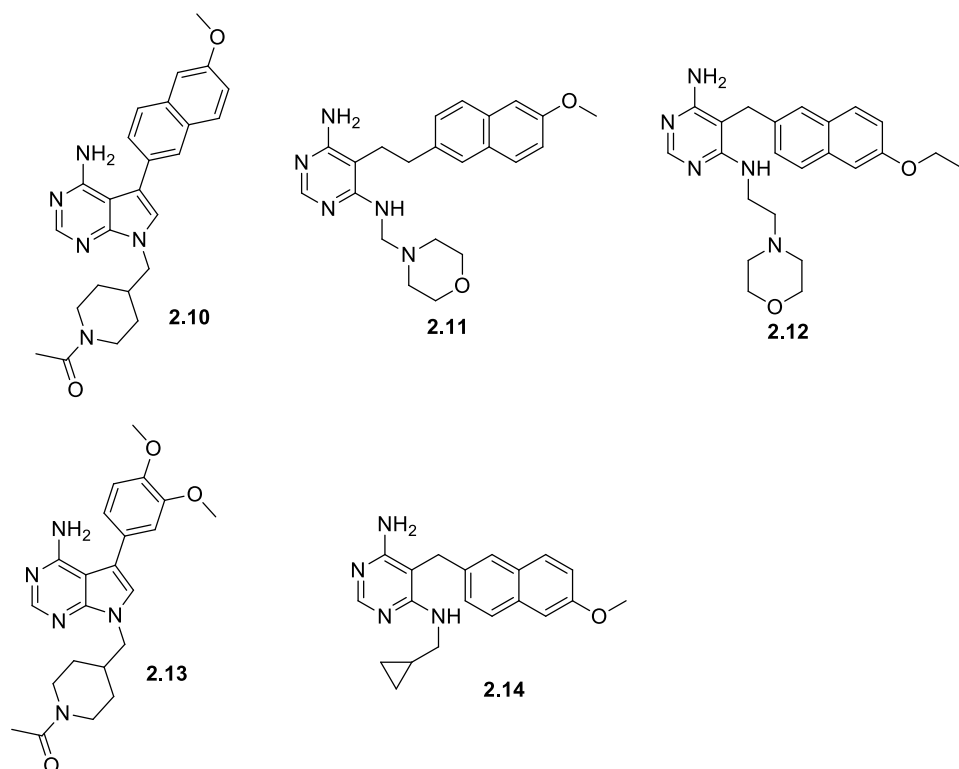


Figure 2.18. Compounds selected from *Pf*CDPK4 (**2.10-2.12**) and *Pf*CDPK1 (**2.11, 2.13-2.14**) IFD experiments for further study in MD.

2.4.1 Molecular dynamics result: *Pf*CDPK4 IFD poses.

The protein and ligand of the pyrrolo[2,3-*d*]pyrimidine compound **2.10** reach conformational stability within 60 ns of the MD simulation. This is indicated by the root mean square deviation (RMSD) values that oscillate between 2.0-2.8 Å for the protein (C-alpha) and 2.4-3.2 Å for the ligand (aligned to the protein) – significant conformational changes occur when RMSD values exceed 3 Å (**Figure 2.19a**). This is also true for most residues of the protein, especially those making up the binding site (identified by green vertical bars in **Figure 2.19b**), their root mean square fluctuation (RMSF) values are less than 1 Å. The exception to this is residue Ser333 and Lys334 showing a RMSF value greater than 4 Å. This is because both residues undergo significant conformational changes since they make up the C-terminus of the protein and are located at a solvent exposed region – nothing is therefore restricting their movement. However, this is of no consequence to the conformation of the binding site residues.

The trajectories of each experiment reveal that the ligand remains inside the binding pocket for the duration of the experiment (100 ns) with the conformations of the core and aromatic groups more conserved than that of the sugar moieties/ *N*-substituents. This is supported by the ligands' atom RMSF that is less than 1 Å for both the core (atoms index: 1-10) and aromatic moiety (atom index: 11, 14-25).

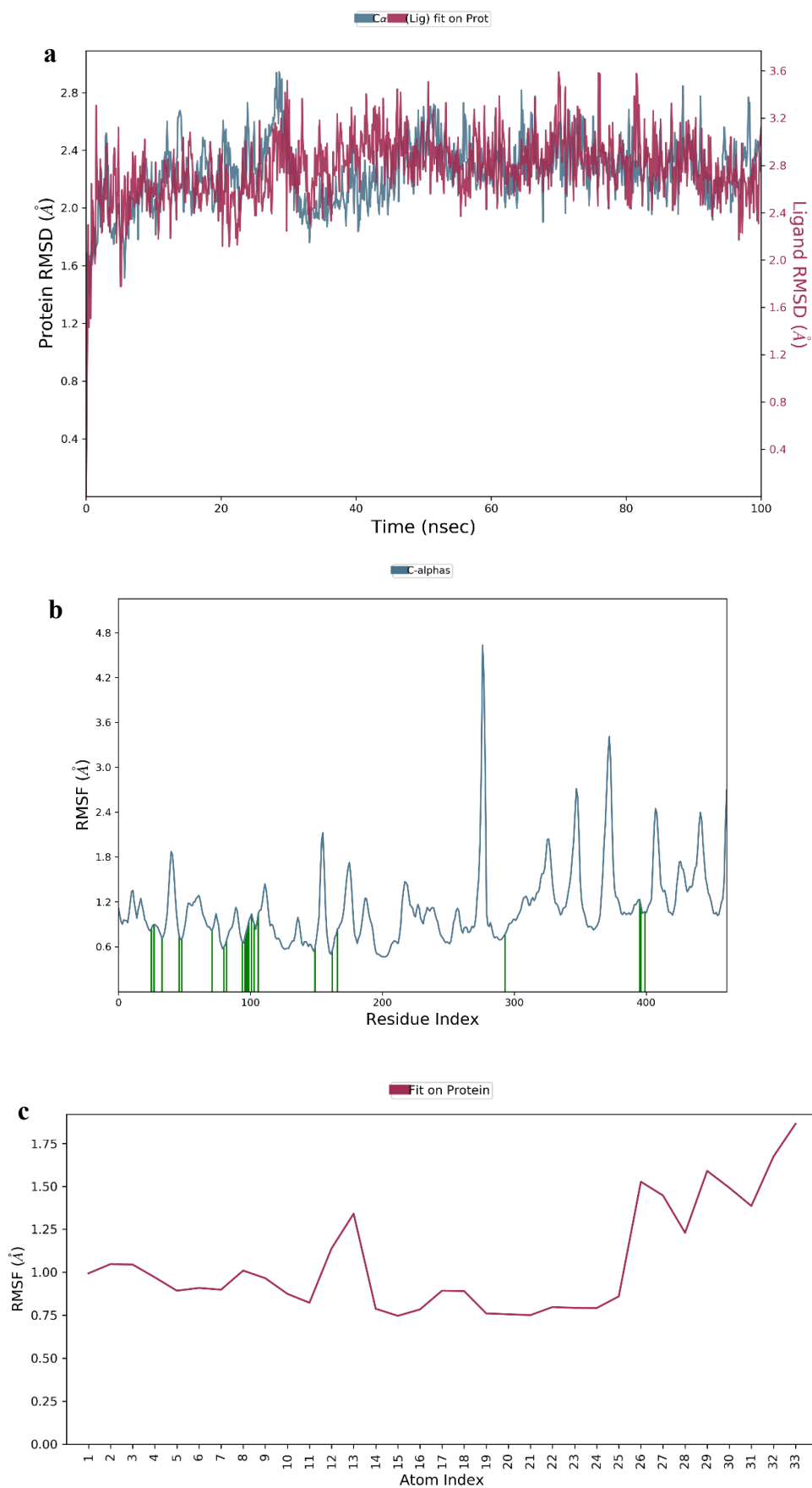


Figure 2.19. Root mean square deviation and root-mean-square fluctuation charts of the pyrrolo[2,3-*d*]pyrimidine compound **2.10** in *Pf*CDPK4.

However, the *N*-substituent – the piperidine – has more pronounced changes (atom index: 12-13, 26-33), although, within the acceptable range - less than 3 Å (**Figure 2.19c**). Furthermore, the dominant ligand-receptor intermolecular interactions observed are the H-bonding at the hinge region and pi-cation interaction at the hydrophobic pocket. The overall interaction between the ligand and the protein during the 100 ns simulation time are summarized in the histogram (**Figure 2.20**).

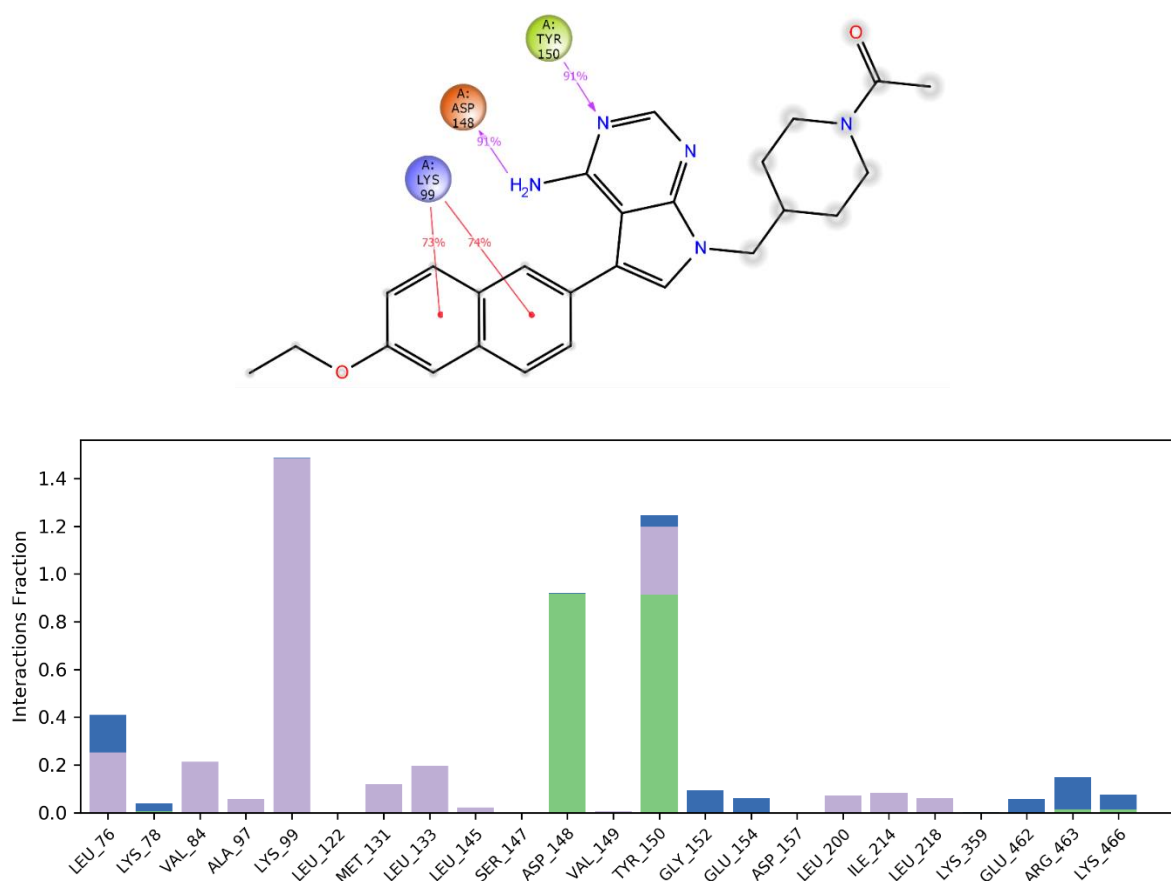


Figure 2.20. Ligand-protein contact diagram and histogram (Green = H-bond; purple = hydrophobic bonding; blue = water bridge) of **2.10** in *Pf*CDPK4.

The trajectory of a complex involving **2.11** shows that the ligand remains in the binding site for the duration of the experiment. The protein C-alphas experience minor oscillations after 60 ns with the difference of approximately 1.1 Å indicating that the assumed conformation is the same as the one afforded on the IFD experiment (**Figure 2.21a**). The ligand's average increase in RMSD towards the end of the simulation, when aligned with the protein, is not accompanied by a conformational change. The RMSD is constant below 3 Å when aligned to the ligand (pink curve; in **Figure 2.21a**). The binding pocket residues are conserved relative to their starting positions,

RMSF ≤ 3 Å (**Figure 2.21b**). The hinge region H-bonding forms the strongest and most dominant interaction between the ligand and protein (**Figure 2.21c**) keeping the ligand stable in the pocket.

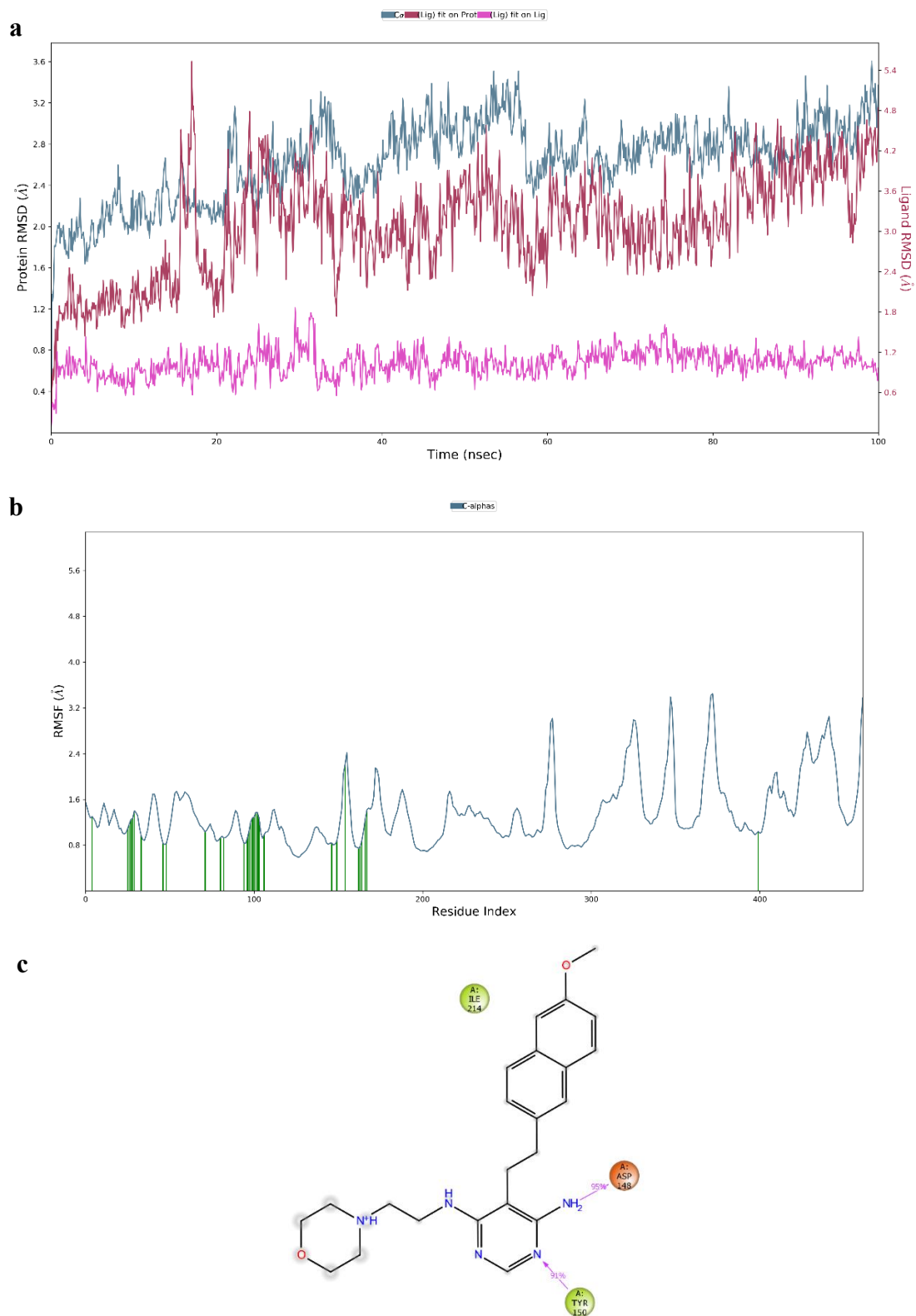


Figure 2.21. **a**) The protein and ligand RMSD plot. **b**) The protein residues RMSF plot with binding residues indicated by green vertical lines. **c**) The 2D ligand interaction diagram showing the strongest and dominant intermolecular forces between **2.11** and *Pf*CDPK4.

The protein and ligand conformations of a complex involving **2.12** reach equilibrium after 80 ns confirmed by RMSD (**Figure 2.22a**). There are no significant changes in the positions of the residues making up the binding site (**Figure 2.22b**) all return an RMSF below 3 Å. The H-bonding by the scaffold at the hinge region, and the hydrophobic interactions by naphthalene ring with Val84, Lys99, and Tyr150 make-up the dominant intermolecular forces during the simulation time (**Figure 2.23**).

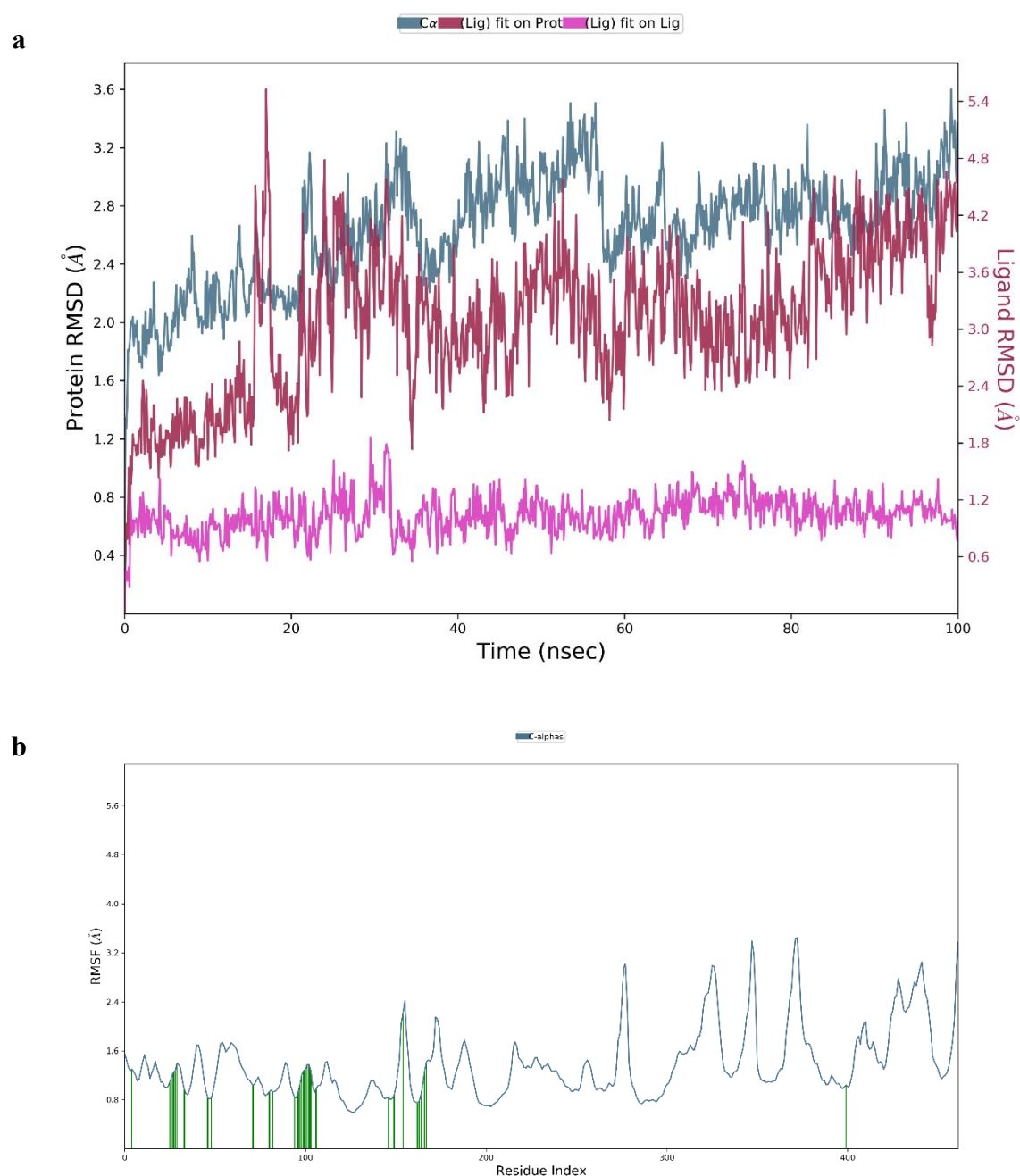


Figure 2.22. a) Protein and ligand RMSD curve and **b)** the protein residues RMSF plot of **2.12** in *Pf*CDPK4.

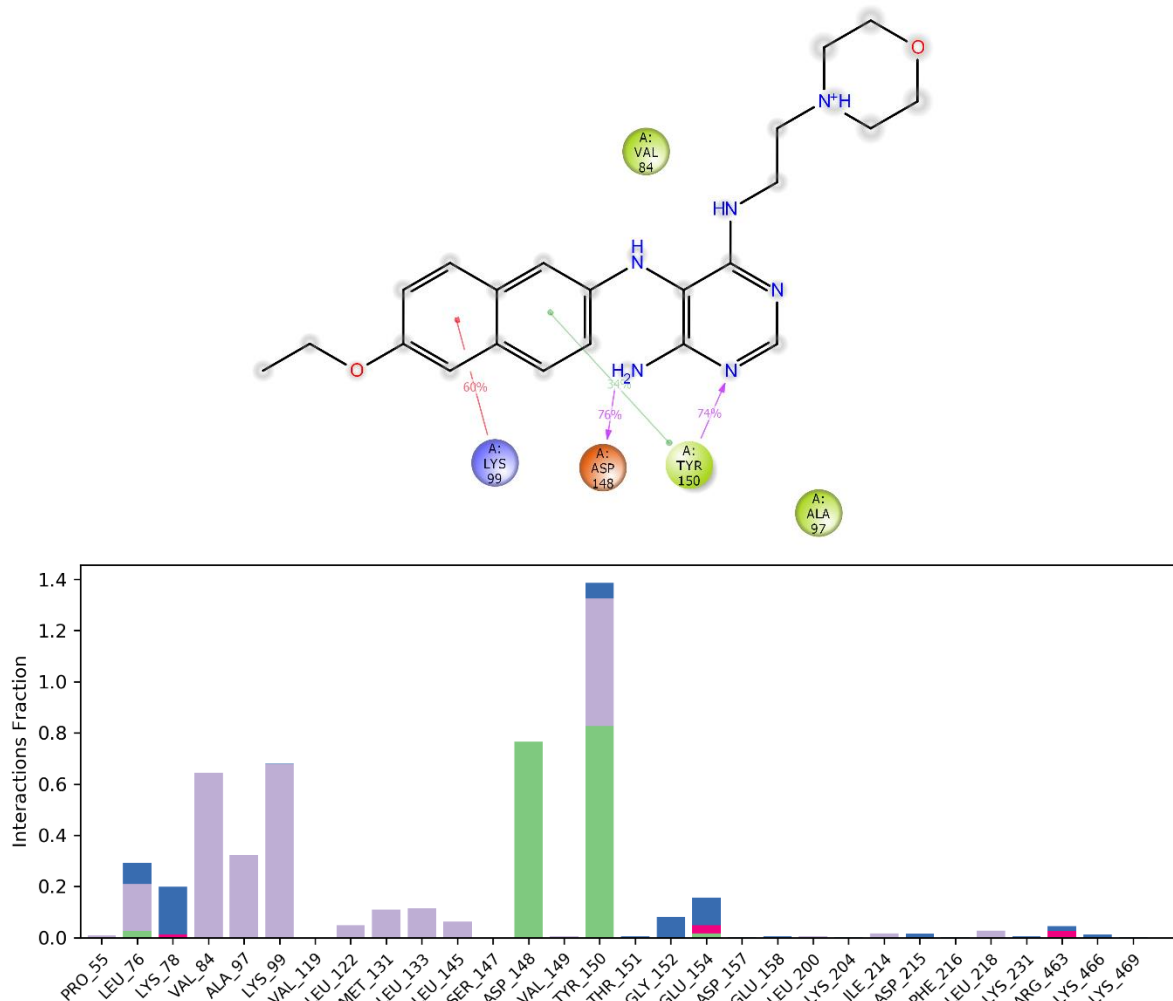


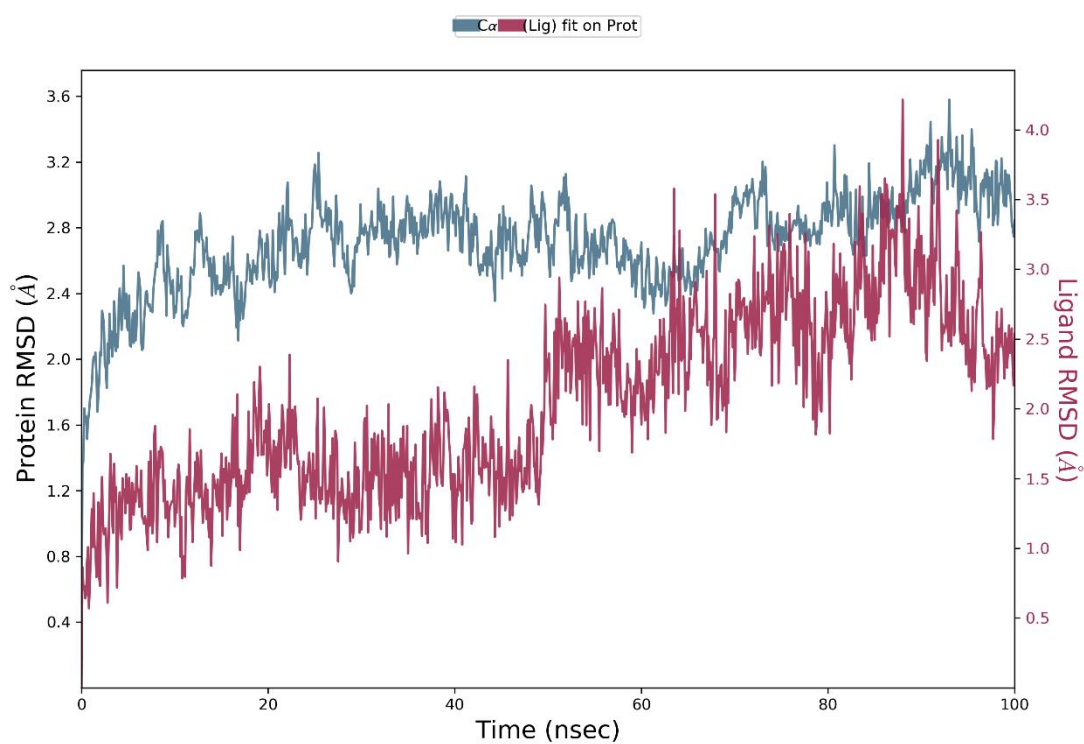
Figure 2.23. The 2D ligand interaction diagram and histogram (Green = H-bond; purple = hydrophobic bonding; blue = water bridge) of **2.12** in *Pf*CDPK4.

2.4.2 Molecular dynamics result: *Pf*CDPK1 IFD poses.

The RMSD of the C-alphas on the protein complex with **2.13** reaches equilibrium after 80 ns of the simulation time with changes that are less than 3 Å (**Figure 2.24a**). This indicates that the protein structure conformation is undergoing minor structural changes. The ligand RMSD also reaches equilibrium after 60 ns with changes that are less than 3 Å indicating that there are no major changes on the ligand conformation when fitted to the protein (**Figure 2.24a**). In other words, the ligand remains in the binding pocket assuming relatively the same conformation afforded during the IFD experiment. Furthermore, the protein residues RMSF show that the residues interacting with the ligand undergo minor displacement during the simulation – the binding site is relatively conserved – except for Ala 66 (**Figure 2.24b**). The latter interacts with the piperidine functionality of **2.13** forming weak H-bonding that is sometimes mediated by a water molecule. It does not form a major part of the binding site given that it is located at the entrance of the binding site far away from the hinge region and the hydrophobic pocket. Thus, its

conformational change does not have a significant impact on the overall geometry of the binding site.

a



b

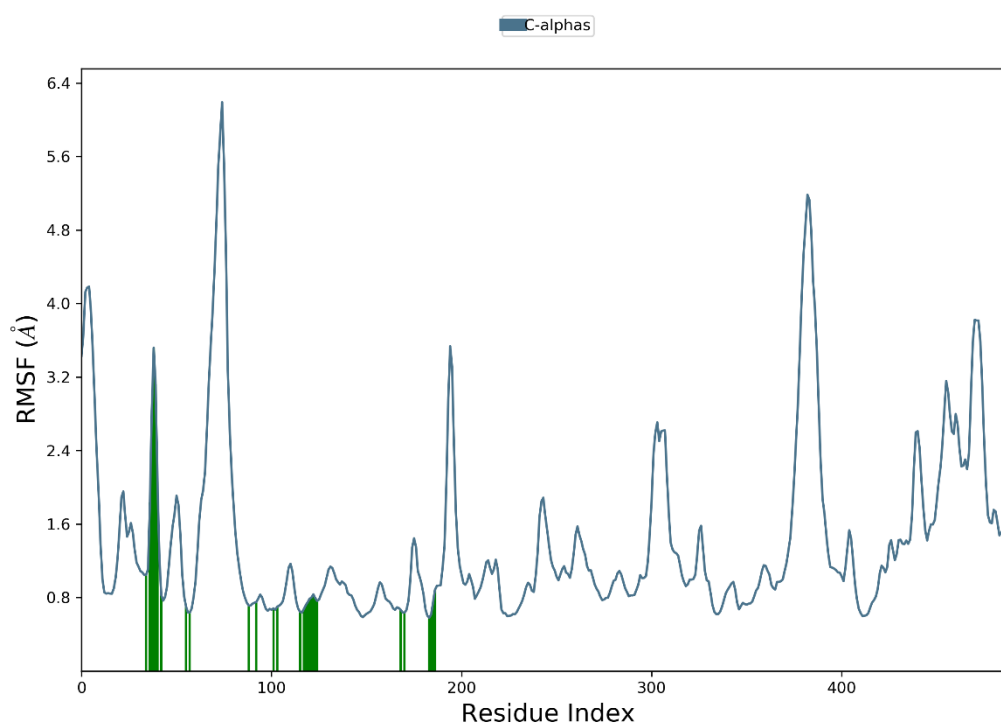
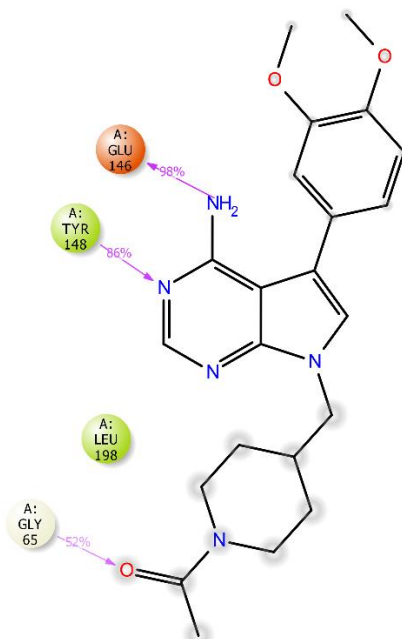


Figure 2.24 a) Protein-ligand RMSD curve and b) protein residue RMSF curve of **2.13** in *Pj*/CDPK1.

The ligand is kept in the binding site primarily by the H-bonding at the hinge region. The residues Tyr148 and Glu146 interact with the pyrimidine ring 86% and 98% of the simulation time, respectively (**Figure 2.25a**). Another interaction maintained at least 40% of the time is H-bonding between Gly65 and the carbonyl functionality of the piperidine moiety. A summary of all the interactions encountered during the simulation time and their respective duration is presented in the histogram (**Figure 2.25b**).

a



b

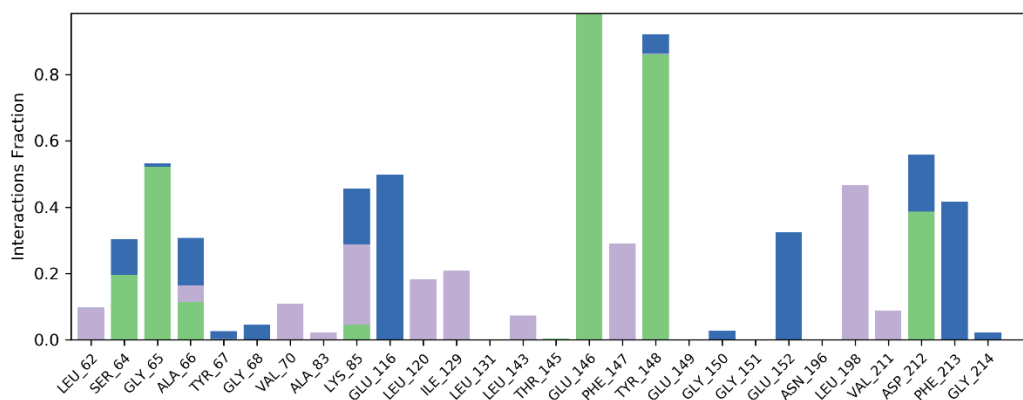


Figure 2.25. **a**) 2D ligand interaction diagram showing interactions that are maintained 40% of the time and **b**) histogram (Green = H-bond; purple = hydrophobic bonding; blue = water bridge) of **2.13** in *P/CDPK1*.

The RMSD of both the *P/CDPK1* protein C-alphas and ligand **2.11** reaches equilibrium after 60 ns which is then followed by an average increase in RMSD from 90 ns (**Figure 2.26a**). During equilibrium, the afforded ligand conformation resembles that afforded from the IFD experiment, where the naphthalene is docked in the hydrophobic pocket and the pyrimidine ring participates in H-bonding at the hinge region. The subsequent increase in RMSD is associated with the change in

the ligand's conformation – the ligand is lowered down the binding site towards the solvent exposed region. This results in the withdrawal of the naphthalene ring from the hydrophobic pocket, and the pyrimidine ring moves away from the hinge region. The protein conformation remains conserved for the duration of the simulation. The increase in RMSD at 90 ns, possibly induced by the change in the ligand's conformation, remains below 3.6 Å. In fact, this increase is immediately followed by a decrease towards the equilibrium position (RMSD $\approx$ 2.8). The protein conserved conformation is further supported by the low RMSF of the protein residues (**Figure 2.26b**). The displacement of the protein residues during simulation is not pronounced (RMSF $\leq$ 3 Å) apart from the N- and C- terminus residues and some solvent exposed surface residues such as Tyr100 and Asn101.

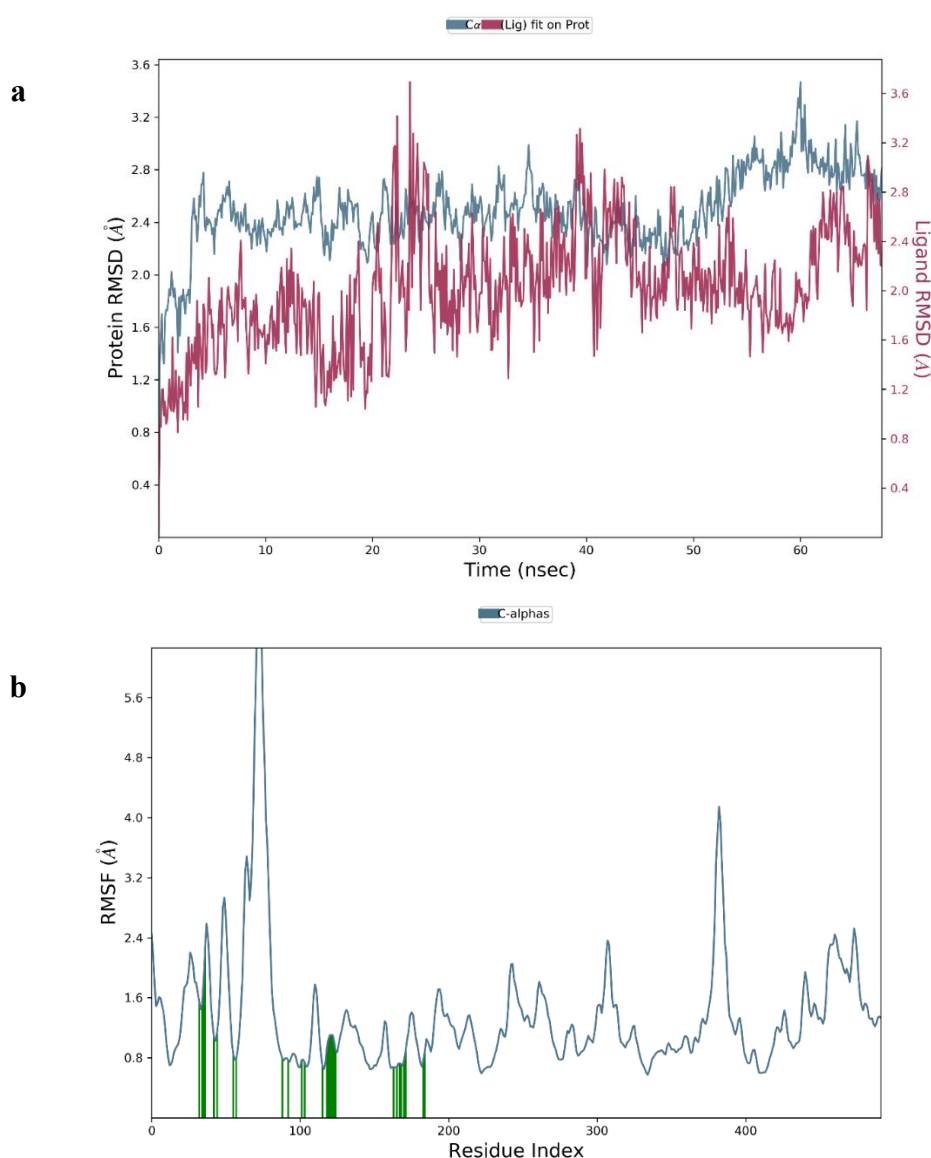


Figure 2.26. a) Protein-ligand RMSD curve and b) protein residue RMSF curve of **2.11** in *PfCDPK1*.

The key H-bonding interactions at the hinge region are maintained less than 50% of the time and often bridged by a water molecule (**Figure 2.27**). This causes the pyrimidine core to occasionally move from the desired region. Typically, the amine on the pyrimidine ring interacts exclusively with Tyr148. However, in this case, it interacts with either Tyr146 or Glu148 but not both, and sometimes interacts with neither. The combined hydrophobic interactions are apparently not enough to supplement the H-bonding interactions in keeping the ligand stable in the binding site, hence the pronounced change in the ligand conformation.

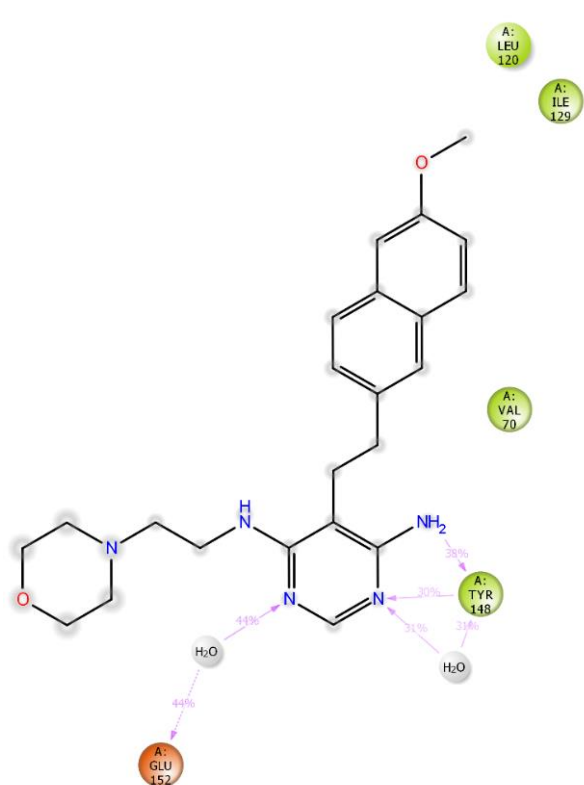


Figure 2.27. 2D protein-ligand interaction diagram of **2.11** with *P/CDPK1*.

The ligand conformation of **2.14** (aligned to the protein) is conserved as evidenced by the RMSD that is predominantly below 3 Å (**Figure 2.28a**). There is an average increase in the deviation from the beginning of the simulation until 33 ns, triggered by the ligands search for a more stable conformation. This is followed by a gradual decrease until equilibrium is reached at 70 ns with the average RMSD value of 2.5 Å. The accompanying protein structure (C-alphas) RMSD reaches stability at 56 ns and thereafter oscillates around 3.5 Å. The observed large RMSD (>3 Å) is associated with an insignificant change in the protein conformation, it does not affect the binding site residues conformation. The residues making up the binding site and interacting with the ligand remain conserved from their initial state. This is supported by the observed RMSF values that are below 1.6 Å (**Figure 2.28b**).

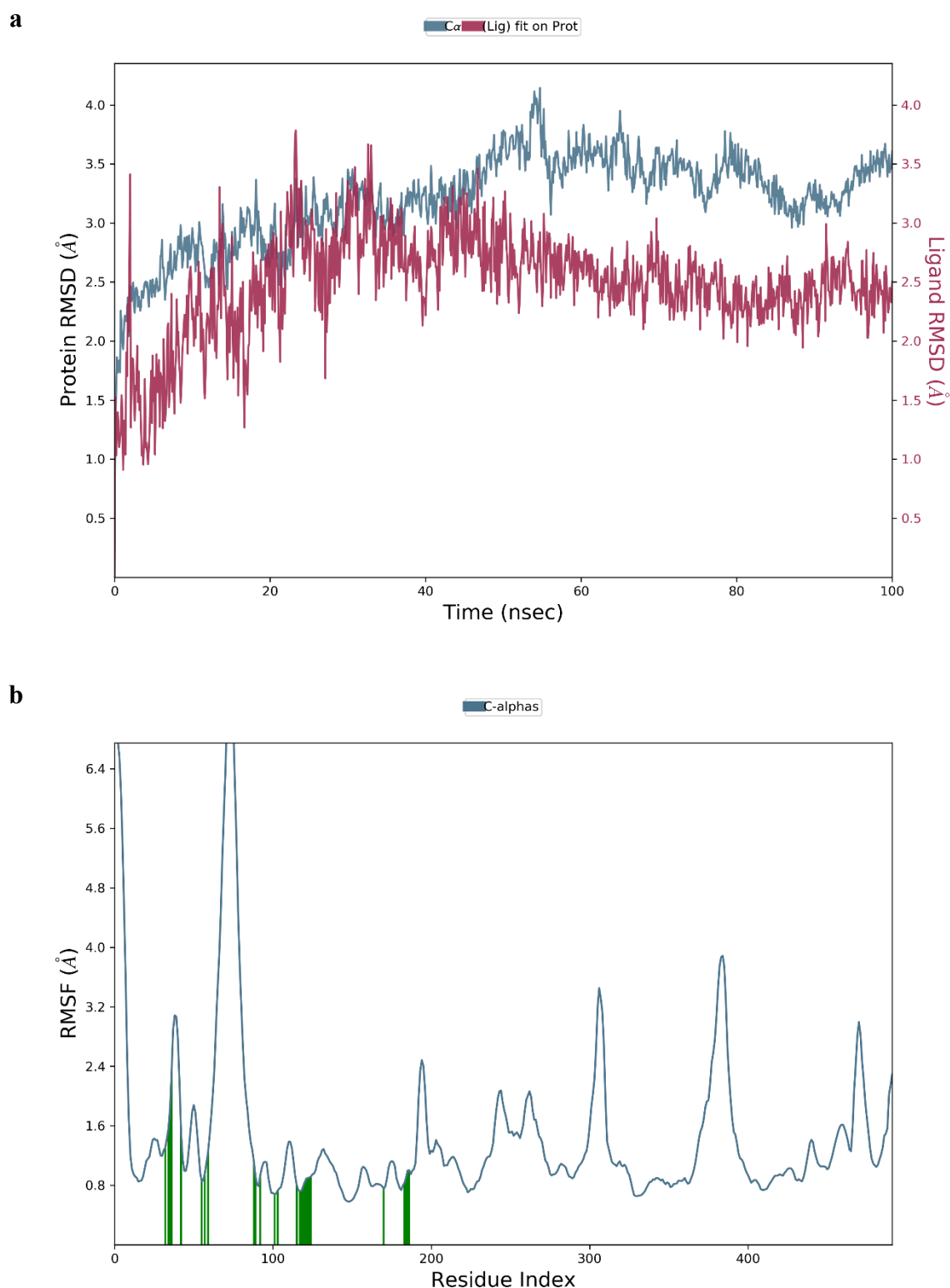


Figure 2.28. a) Protein-ligand RMSD curve and **b)** protein residue RMSF curve of 2.14 in *Pf*CDPK1.

The residue Glu146 and Tyr148 maintain H-bonding with the aminopyrimidine ring for 93% and 92% of the time, respectively (**Figure 2.29a**). This is vital in keeping the ligand inside the binding site with the aryl moiety projected into the hydrophobic pocket. Other encountered interactions

play a minor role in the binding affinity of the drug; the best of these by Gly150 only happen 34% of the time and is water mediated (**Figure 2.29b**).

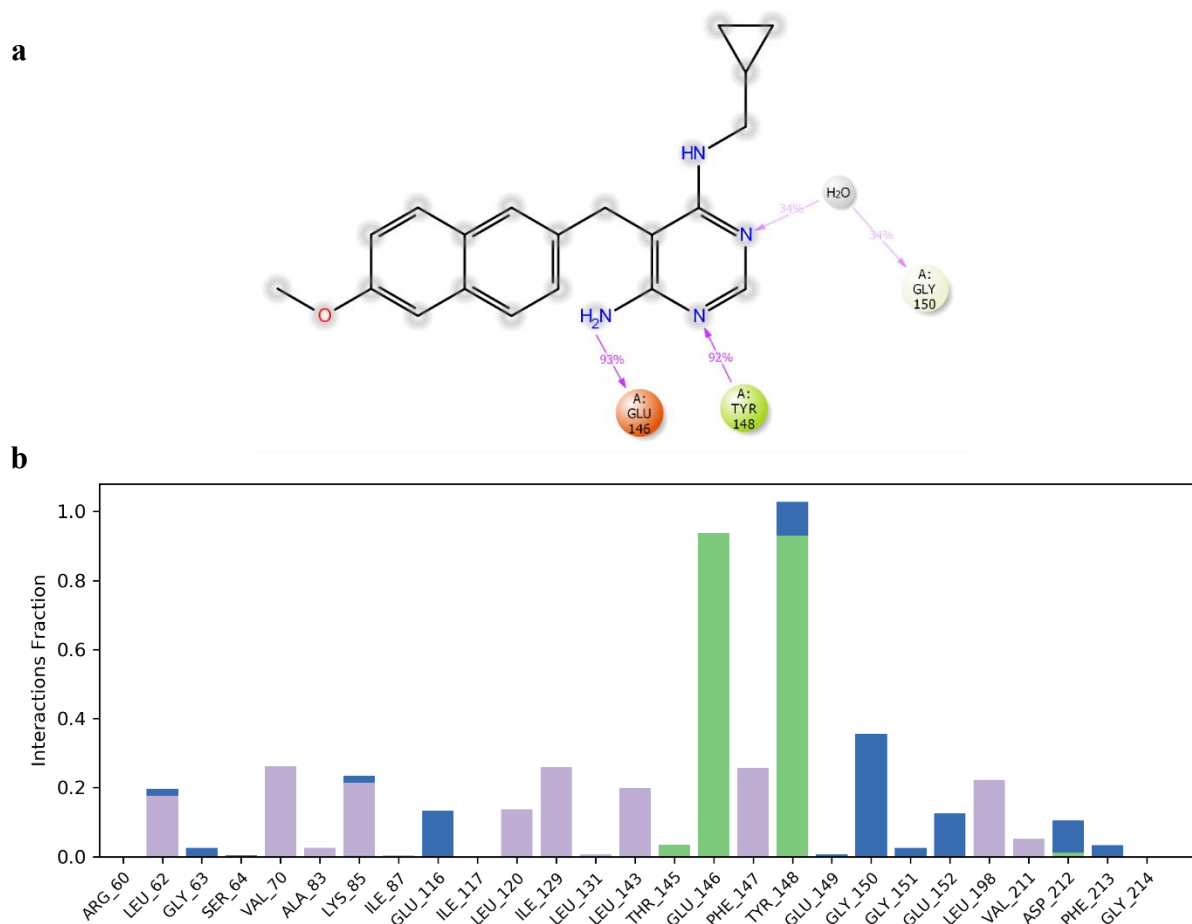


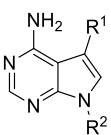
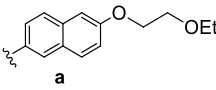
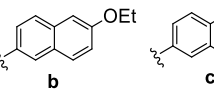
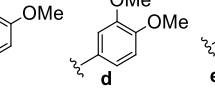
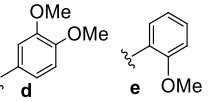
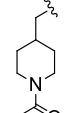
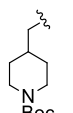
Figure 2.29. **a**) 2D protein- ligand interaction diagram and **b**) interaction histogram (Green = H-bond; purple = hydrophobic bonding; blue = water bridge) of **2.14** with *P/CDPK1*.

2.5. Drug-like properties

We assessed our target compounds, both the pyrrolo[2,3-*d*]pyrimidine and 4,6-diaminopyrimidine analogues for potential oral bioavailability, and toxicity (cardiac and central nervous system [CNS] related). Molecular properties necessary for the pharmacokinetics study were generated using the Schrodinger suite QikProp tool (**Table 2.5** and **Table 2.6**) [22]. The oral bioavailability potential of our compounds was assessed using Lipinski's and Veber's rules. Lipinski et al. stated that a compound is likely to have better absorption and permeation if it has at least four of the following molecular properties: a molecular weight less than 500 Da, no more than 5 hydrogen bond donors (HBD), no more than 10 hydrogen bond acceptors (HBA) and a partition coefficient (QPlogP_{o/w}) not greater than 5 [23]. Veber et al. realized that oral bioavailability can be predicted using molecular flexibility and polar surface area (PSA) [24]. They observed that a molecule with high flexibility, measured by the number of rotatable bonds (#rb), and a high polar surface area (PSA)

generally had poor permeation. They suggested, therefore, that for a molecule to have good oral bioavailability it must not exceed 10 rotatable bonds and a PSA of 140 Å². All our target molecules adhere to both Lipinski's and Veber's rules; therefore, they display the potential of having good oral bioavailability.

Table 2.5. QikProp generated molecular properties of pyrrolo[2,3-d]pyrimidine derivatives.

  a  b  c  d  e  1  2  3  4  5											
Entry	R ¹	R ²	MWt (g/mol)	HBD	HBA	QPlogPo/w	#rb	PSA (Å ²)	^a QPlogHERG	^b QPlogBB	^c QPlogKhsa
1	a	1	402.50	2	6	5.26	8	65	-6.73	-0.81	0.88
2		4	487.60	2	8	4.56	8	96	-5.60	-1.41	0.61
3	b	1	358.44	2	3	4.97	5	57	-6.17	-0.56	0.91
4		3	417.51	2	7	3.77	6	70	-6.89	-0.23	0.55
5		4	443.55	2	6	4.31	5	87	-5.18	-1.12	0.67
6	c	1	344.42	2	3	4.63	4	57	-6.00	-0.42	0.80
7		4	429.52	2	6	3.87	4	87	-4.83	-0.97	0.53
8	d	1	324.38	2	4	3.98	5	65	-5.71	-0.57	0.53
9		2	360.42	2	4	4.76	5	64	-6.70	-0.53	0.76
10		3	383.45	2	8	2.61	6	80	-6.02	-0.21	0.13
11		4	409.49	2	7	3.06	5	95	-4.09	-1.02	0.21
12		5	467.57	2	7	5.26	6	97	-5.96	-1.02	1.11
13	e	1	294.36	2	3	3.60	4	56	-4.96	-0.40	0.42
14		2	330.39	2	3	4.38	4	55	-6.00	-0.37	0.64
15		3	353.42	2	7	2.40	5	70	-5.61	-0.09	0.09
16		4	379.46	2	6	2.98	4	86	-4.06	-0.89	0.20
17		5	437.54	2	6	5.20	5	88	-5.97	-0.90	1.11

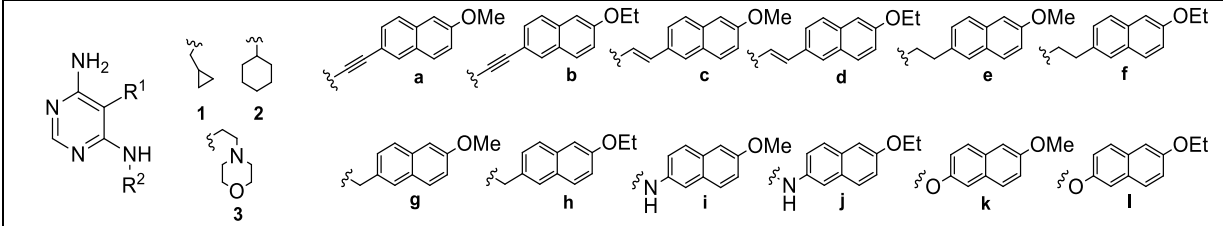
^aQPlogHERG: Predicted IC₅₀ values for the blockage of HERG K⁺ channels.

^bQPlogBB: Predicted blood/brain partition coefficient.

^cQPlogKhsa: Prediction of binding to the human serum albumin.

Cardiac related side effects were assessed using the predicted IC₅₀ values for the blockage of HERG K⁺ channels (QPlogHERG). The predicted values for our target compounds were predominantly below -5, save for a few (entry 7, 11, 13, and 16 **Table 2.5** and entry 24 and 25 **Table 2.6**). Molecules with the potential to block the HERG K⁺ channels generally have values that are below -5; To avoid cardiac related side effects, structural modification of such molecules during the lead optimization stage is necessary. Our molecules do not display any potential to cause CNS related side effects. This is indicated by the value of QPlogBB which fall within the recommended range (-3.0 to 1.2). They also do not bind strongly to the human serum albumin. The predicted values of QPlogKhsa fall within the recommended range (-1.5 to 1.5). Compounds that bind strongly to the human serum albumin tend to reach the target site in lower quantity. A competitive inhibitor must be available at the target site in high concentrations to compete effectively with the natural substrate, therefore, compounds that bind strongly to the human serum albumin will not be competent.

Table 2.6. QikProp generated molecular properties of 4,6-diaminopyrimidine derivatives.

											
Entry	R ¹	R ²	MWt	HBD	HBA	QPlogPo/w	#rb	PSA	^a QPlogHERG	^b QPlogBB	^c QPlogKhsa
1		1	372.47	3	4	4.92	6	66	-6.49	-0.74	0.84
2	a	2	344.42	3	4	4.39	7	68	-6.50	-0.88	0.60
3		3	403.48	3	7	3.30	8	83	-7.37	-0.60	0.31
4		1	386.50	3	4	5.39	7	65	-6.93	-0.88	1.00
5	b	2	358.44	3	4	4.85	8	67	-7.03	-1.07	0.75
6		3	417.51	3	7	3.35	9	79	-6.73	-0.65	0.34
7		1	376.50	3	4	4.93	7	63	-5.50	-0.50	0.79
8	c	2	348.45	3	4	4.45	8	65	-5.95	-0.75	0.57
9		3	407.51	3	7	2.93	9	77	-5.60	-0.39	0.18
10		1	390.53	3	4	5.44	8	64	-6.33	-0.79	0.98
11	d	2	362.47	3	4	4.81	9	66	-6.16	-0.93	0.70
12		3	421.54	3	7	3.27	10	79	-6.19	-0.57	0.27
13	e	1	374.49	3	4	4.87	7	62	-6.16	-0.75	0.79

14		2	346.43	3	4	4.38	8	64	-6.22	-0.88	0.55
15		3	405.50	3	7	3.25	9	78	-6.66	-0.49	0.26
16		1	388.51	3	4	5.24	8	63	-6.38	-0.91	0.92
17	f	2	360.46	3	4	4.83	9	64	-6.65	-0.96	0.68
18		3	419.53	3	7	3.48	10	77	-6.80	-0.64	0.32
19		1	362.47	3	4	4.52	6	61	-5.57	-0.55	0.68
20	g	2	334.42	3	4	3.95	7	64	-5.34	-0.64	0.42
21		3	393.49	3	7	2.83	8	78	-6.01	-0.35	0.16
22		1	376.50	3	4	5.06	7	61	-6.20	-0.65	0.85
23	h	2	348.45	3	4	4.52	8	63	-6.09	-0.77	0.60
24		3	407.51	3	7	2.69	9	75	-4.97	-0.32	0.11
25		1	364.45	3	4	3.84	6	73	-4.88	-0.65	0.47
26	i	2	336.39	3	4	3.65	7	73	-5.51	-0.65	0.29
27		3	395.46	3	8	2.53	8	88	-6.38	-0.45	0.04
28		1	378.47	3	4	4.55	7	71	-5.75	-0.71	0.67
29	j	2	350.42	3	4	3.74	8	74	-5.21	-0.86	0.35
30		3	409.49	3	8	3.02	9	88	-6.88	-0.51	0.15
31		1	363.46	4	4	3.90	6	74	-5.69	-0.76	0.46
32	k	2	335.41	4	4	3.13	7	77	-5.09	-0.87	0.16
33		3	394.48	4	8	2.33	8	91	-6.58	-0.67	0.01
34		1	377.49	4	4	4.22	7	75	-5.82	-0.93	0.58
35	l	2	349.44	4	4	3.52	8	76	-5.48	-0.99	0.27
36		3	408.50	4	8	2.38	9	91	-5.86	-0.59	0.02

^a**QPlogHERG**: Predicted IC₅₀ values for the blockage of HERG K⁺ channels.

^b**QPlogBB**: Predicted blood/brain partition coefficient.

^c**QPlogKhsa**: Prediction of binding to the human serum albumin.

2.6. Conclusion

Our *in silico* studies show that the pursued ligands can potentially inhibit the *Pf*CDPK4 enzyme. Docking studies indicated that the ligands hydrogen bonded at the hinge region and the large aromatic moieties were accommodated in the hydrophobic pocket essential for potency and selectivity. The ethane linker derivatives afforded better conformation compared to the ethene and acetylene linker compounds owing to the relative flexibility of the linker. They are followed by the one atom linker compounds which gave similar docking results within the series independent

on the identity of the linker (C, N, or O). The presence of the piperidine and morpholine moiety in the ligand improved the docking scores due to additional hydrogen bonding at the sugar region via the heteroatoms. Molecular dynamics studies revealed that the IFD afforded conformations of both the ligand and protein that were stable (low energy conformations) – the afforded poses were maintained. It also revealed that the hinge region hydrogen bonding by the scaffolds – the pyrrolo[2,3-*d*]pyrimidine and the 4,6-diaminopyrimidine – played an important role in both keeping the molecule in the binding site and projecting aromatic groups and *N*-substituents to their respective pockets.

In silico studies on the *Pf*CDPK1 enzyme showed less promising results. The enzyme has a larger gatekeeper residue that creates a sharp angle between the hydrophobic pocket and hinge region in addition to narrowing the hydrophobic pocket. This made it almost impossible for large aromatic groups to occupy the pocket. Ligands bearing a two-atom linker between the 4,6-diaminopyrimidine scaffold and the aromatic ring were especially inconvenienced. They preferred docking their aromatic moieties on the region adjacent to the hydrophobic pocket. The pyrrolo[2,3-*d*]pyrimidines docked better overall, however, a ligand with a small aromatic moiety (dimethoxybenzene) had the best binding affinity. Molecular dynamics studies indicated that the pyrrolo[2,3-*d*]pyrimidine and one atom linker compounds maintained their positions in the active site owing to the defined hydrogen bonding at the hinge region; both the protein and ligand conformation were conserved. The two-atom linker compound suffered a significant conformational change resulting from poor H-bonding at the hinge region.

The ligand-based pharmacokinetics properties generated suggest that our ligands have good oral bioavailability and poor CNS toxicity. Of concern is their potential to cause cardiac related toxicity by inhibiting the HERG K⁺ channels, an issue that must be resolved via structural modifications. Generally, the pyrrolo[2,3-*d*]pyrimidine compounds show the potential of being dual type I₂¹ inhibitors of *Pf*CDPK4 and *Pf*CDPK1, followed by the one-atom linker diaminopyrimidine compounds. The two-atom linker diaminopyrimidine compounds (ethane bridged) will work best as type I₂¹ inhibitors of *Pf*CDPK4 and Type II inhibitors of *Pf*CDPK1.

In silico studies have shown that our target ligands, the pyrrolo[2,3-*d*]pyrimidines and the 4,6-diaminopyrimidines can act as potential inhibitors of *Pf*CDPK1 and *Pf*CDPK4. We, therefore, ventured into the synthesis and biological evaluation of these ligands, the results of which are discussed in the next chapters. Chapter 3 discusses the synthesis and biological evaluation of pyrrolo[2,3-*d*]pyrimidine derivatives and their respective biological inhibitory data. In Chapter 4

we discuss the synthesis of 4,6-diaminopyrimidine derivatives, both the one-atom linkage compounds and the two-atom linkage compounds. Unfortunately, the biological data on the 4,6-diaminopyrimidine compounds was not attained in time before the submission of the thesis.

2.7. References

1. Doytchinova, I., *Drug Design-Past, Present, Future*. Molecules, 2022. **27**(5): p. 9.
2. Patrick, G.L., *An Introduction to Medicinal Chemistry*. 5th ed. An Introduction to Medicinal Chemistry. 2013, Oxford, United Kingdom: Oxford University Press.
3. Gaynes, R., *The Discovery of Penicillin-New Insights After More Than 75 Years of Clinical Use*. Emerging Infectious Diseases, 2017. **23**(5): p. 849-853.
4. Stähle, H., *A historical perspective: development of clonidine*. Best Practice & Research Clinical Anaesthesiology, 2000. **14**(2): p. 237-246.
5. Vonitzstein, M., et al., *Rational design of potent sialidase-based inhibitors of influenza-virus replication*. Nature, 1993. **363**(6428): p. 418-423.
6. Sutherland, R., E.A. Croydon, and G.N. Rolinson, *Amoxycillin: a new semi-synthetic penicillin*. Br Med J, 1972. **3**(5817): p. 13-6.
7. Niazi, S.K. and Z. Mariam, *Computer-Aided Drug Design and Drug Discovery: A Prospective Analysis*. Pharmaceuticals, 2024. **17**(1). p: 22.
8. von Itzstein, M., *The war against influenza: Discovery and development of sialidase inhibitors*. Nature Reviews Drug Discovery, 2007. **6**(12): p. 967-974.
9. Sliwoski, G., et al., *Computational methods in drug discovery*. Pharmacol Rev, 2014. **66**(1): p. 334-95.
10. Gayathiri, E., et al., *Computational approaches for modeling and structural design of biological systems: A comprehensive review*. Progress in Biophysics & Molecular Biology, 2023. **185**: p. 17-32.
11. Mandal, S., M. Moudgil, and S.K. Mandal, *Rational drug design*. Eur J Pharmacol, 2009. **625**(1-3): p. 90-100.
12. Acharya, C., et al., *Recent advances in ligand-based drug design: relevance and utility of the conformationally sampled pharmacophore approach*. Curr Comput Aided Drug Des, 2011. **7**(1): p. 10-22.
13. KD, T., *Essentials of Medical Pharmacology*. 7th ed. 2013, New Delhi, India: Jaypee Brothers Medical Publisher (P) LTD.
14. Vidadala, R.S.R., et al., *Development of potent and selective Plasmodium falciparum calcium-dependent protein kinase 4 (PfCDPK4) inhibitors that block the transmission of malaria to mosquitoes*. European Journal of Medicinal Chemistry, 2014. **74**: p. 562-573.
15. Huang, W.L., et al., *5-Aminopyrazole-4-carboxamide analogues are selective inhibitors of Plasmodium falciparum microgametocyte exflagellation and potential malaria transmission blocking agents*. Bioorganic & Medicinal Chemistry Letters, 2016. **26**(22): p. 5487-5491.
16. *LigPrep*. 2022, Schrödinger, LLC: New York, NY.
17. *Glide*. 2022, Schrödinger, LLC: New York, NY.
18. *Prime*. 2022, Schrödinger, LLC: New York, NY.
19. Wiltgen, M., *Algorithms for Structure Comparison and Analysis: Homology Modelling of Proteins*, in *Encyclopedia of Bioinformatics and Computational Biology*, S. Ranganathan, et al., Editors. 2019, Academic Press: Oxford. p. 38-61.
20. Sottriffer, C.A., *Molecular Dynamics Simulations in Drug Design*, in *Encyclopedic Reference of Genomics and Proteomics in Molecular Medicine*. 2006, Springer Berlin Heidelberg: Berlin, Heidelberg. p. 1153-1160.

21. *Desmond*. 2022, D.E. Shaw Research: New York, NY.
22. *QikProp*. 2022, Schrödinger, LLC: New York, NY.
23. Lipinski, C.A., et al., *Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings*. *Adv Drug Deliv Rev*, 2001. **46**(1-3): p. 3-26.
24. Veber, D.F., et al., *Molecular Properties That Influence the Oral Bioavailability of Drug Candidates*. *Journal of Medicinal Chemistry*, 2002. **45**(12): p. 2615-2623.

Chapter 3: Synthesis and biological evaluation of pyrrolo[2,3-*d*]pyrimidine derivatives as potential inhibitors of *Pf*CDPK1 and *Pf*CDPK4

The aim of this PhD is to synthesize compounds that will effectively interact with both *Pf*CDPK1 and *Pf*CDPK4 towards malaria treatment and transmission-blocking. In light of the work that was done by Vidadala and co-workers [1] in their development of *Pf*CDPK4 inhibitors, we have designed pyrrolo[2,3-*d*]pyrimidine derivatives that are potential inhibitors of *Pf*CDPK1 and *Pf*CDPK4. We wanted to compare the activity of our ligands with the explored pyrazolo[2,3-*d*]pyrimidine and imidazo[1,5-*a*]pyrazine inhibitors already described [1]. Our compounds contain structural moieties that are ideal for potency and selectivity in addition to being recognized in the ATP binding site. *In silico* studies done (Chapter 2) have shown that the pyrrolo[2,3-*d*]pyrimidine derivatives can bind in the active site of the enzymes occupying the ATP site and two additional pockets that are necessary for potency and selectivity. In this chapter we discuss the synthesis and biological evaluation of pyrrolo[2,3-*d*]pyrimidine compounds validating the results obtained from molecular modelling.

Our synthesis of the pyrrolo[2,3-*d*]pyrimidine derivatives was done concurrently with another student in our laboratory. As a result, our synthesis scope was reduced to four target compounds (**Figure 3.1**). We, however, endeavored to improve the synthetic protocols employed, and these improvements in the synthetic strategy are also described.

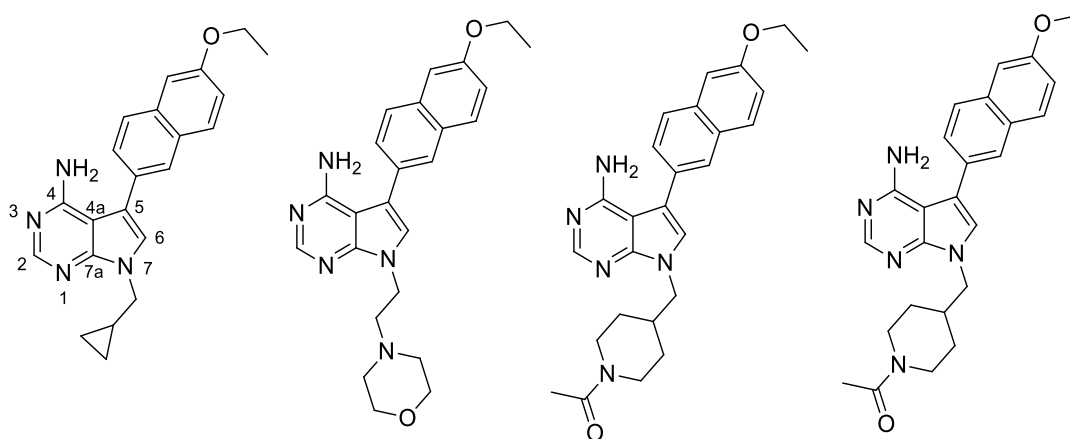
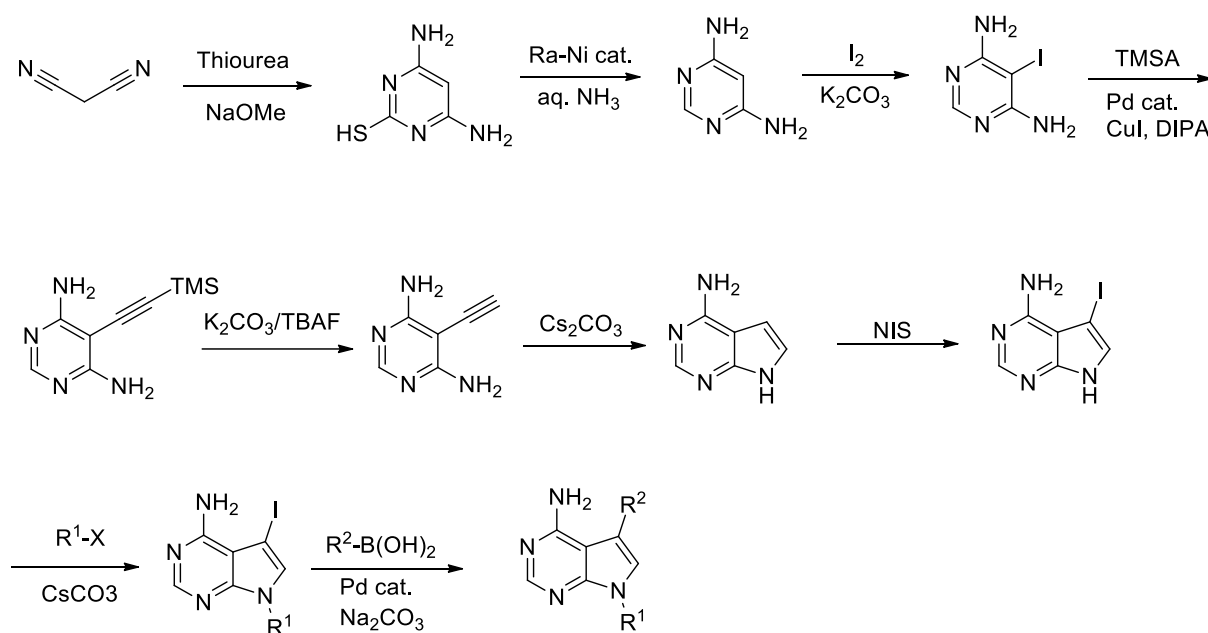


Figure 3.1. Four of the pyrrolo[2,3-*d*]pyrimidine compounds targeted for synthesis.

3.1 Synthesis of pyrrolo[2,3-*d*]pyrimidine derivatives

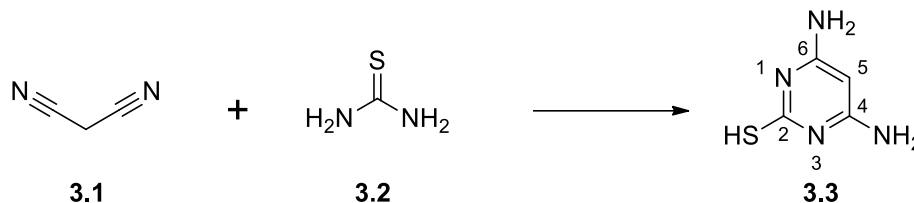
The designed pyrrolo[2,3-*d*]pyrimidine derivatives are made up of three structural components which play an important role in the inhibition of the parasite kinases, *Pf*CDPK1 and *Pf*CDPK4. These include the pyrrolo[2,3-*d*]pyrimidine scaffold, the bulky aromatic moiety located at the C5 position of the scaffold, and the ribose substituents at the N7 position (**Figure 3.1**). We planned the synthesis of the pyrrolo[2,3-*d*]pyrimidine derivatives starting by the formation of the pyrimidine ring from malononitrile and thiourea (**Scheme 3.1**). This would be followed by the removal of a thiol functionality and the iodination of the C5 position to prepare the pyrimidine ring for the addition of trimethylsilylacetylene (TMSA) via the Sonogashira coupling reaction. After the addition of TMSA, the trimethylsilyl group would be removed and the pyrrolo[2,3-*d*]pyrimidine compound would be formed. From this point the ribose substituents (R^1) and bulky aromatic groups (R^2) would be afforded utilising conventional alkylating procedures and the Suzuki-Miyaura coupling reaction, respectively. The experimental work undertaken to afford the targeted pyrrolo[2,3-*d*]pyrimidine compounds (**Figure 3.1**) is described in the following sections.



Scheme 3.1. Proposed synthetic route for the synthesis of pyrrolo[2,3-*d*]pyrimidine derivatives.

3.1.1 Part one: Preparation of the pyrrolo[2,3-*d*]pyrimidine scaffold

3.1.1.1 Synthesis of 4,6-diaminopyrimidine-2-thiol [2]

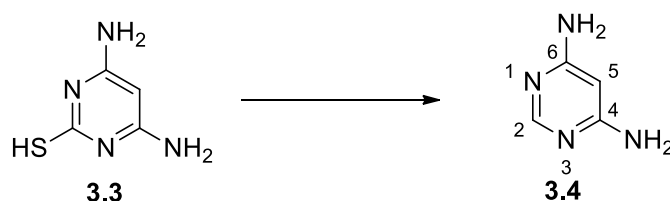


Scheme 3.2. Synthesis of 4,6-diaminopyrimidine-2-thiol **3.3**. Reagents and conditions: NaOMe, MeOH, 65°C, 24 hrs, 53%.

The 4-aminopyrimidine core is an essential component for ATP competitive inhibitors. The precursor of the pyrimidine ring, 4,6-diaminopyrimidine-2-thiol **3.3**, is assembled by the reaction of malononitrile **3.1** and thiourea **3.2** in the presence of sodium methoxide (**Scheme 3.2**). The reaction mixture was heated in methanol at reflux for 24 hours before cooling to room temperature and concentrating *in vacuo*. The resulting crude material was dissolved in water and the solution's pH was adjusted to ~7 using glacial acetic acid at which point a precipitate formed. The precipitate was separated by filtration using a glass sintered funnel and dried *in vacuo* to afford the product as a light brown to white solid in 53% yield.

The formation of **3.3** was confirmed using both ¹H NMR and ¹³C NMR spectroscopy. The proton NMR spectrum displayed three signals at 11.12, 6.74, and 5.06 ppm integrating for 1, 4, and 1 proton(s), respectively. The signal at 11.12 ppm is typical for an aromatic thiol functional group, while the signal at 6.74 ppm is caused by the two primary amine protons owing to the symmetry of the structure, hence the integration of 4. The last signal at 5.06 is due to the C5 proton. The ¹³C NMR spectrum reveal three signals at 177.5 ppm and 74.7 ppm. As an effect of structural symmetry and similarity in the carbon environment, the former signal is due to C2, C4, and C6 while the latter signal is due to C5.

3.1.1.2 Synthesis of pyrimidine-4,6-diamine [3]

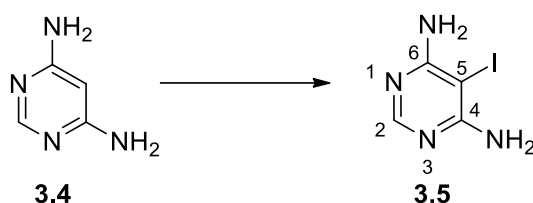


Scheme 3.3. Synthesis of pyrimidine-4,6-diamine **3.4**. Reagents and conditions: Raney-Ni, aq.NH₃, 100°C, 2 hrs, 90%.

The 4,6-diaminopyrimidine ring **3.4** was afforded after the reduction of precursor **3.3** using Raney-nickel (**Scheme 3.3**). This was achieved in aqueous ammonia with heating for 2 hours at 100°C. The reaction can be carried out in water; the addition of aqueous ammonia was necessary to encourage the dissolution of the starting substrate **3.3**. At the end of the reaction, the catalyst was recovered by filtration and the filtrate was concentrated *in vacuo* to afford **3.4** as an off-white solid in 90% yield without further purification.

The ^1H NMR spectrum of **3.4** was like that of **3.3** however, with a signal at 7.80 ppm due to H2 in place of the thiol functionality. Additionally, the ^{13}C NMR spectrum C2 signal initial observed at 177.5 ppm was shifted to 157.8 ppm confirming successful reduction of **3.3**, while C4 and C6 gave rise to a signal at 163.5 ppm. Infrared spectrum gave signals at 3445 and 3090 cm^{-1} due to the NH and $\text{sp}^2\text{-CH}$.

3.1.1.3 Synthesis of 5-iodopyrimidine-4,6-diamine [4]



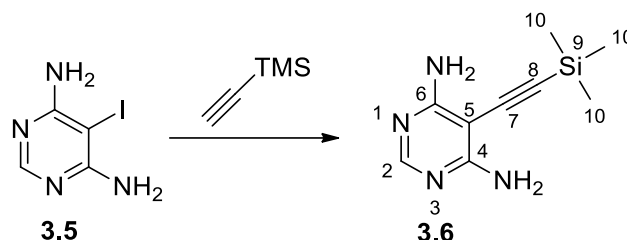
Scheme 3.4. Synthesis of 5-iodopyrimidine-4,6-diamine. Reagents and conditions: I_2 , K_2CO_3 , DMF/ H_2O , 100°C, 1 hr, 68%.

The intermediate **3.5** was prepared for the Sonogashira cross-coupling reaction with the acetylene functionality by iodinating the C5 position of **3.4** using molecular iodine in the presence of potassium carbonate (**Scheme 3.4**). To ensure complete dissolution of both the substrates and reagents a mixture of DMF and water was used as a solvent. The product can be afforded by conventional heating at 40-45°C for 4 hours affording **3.5** in a yield of 65% [4]. We, however, used microwave irradiation (MWI) heating at 100°C for 1 hour affording 68% yield after work-up. The afforded yields from both approaches are comparable. Benefits of using microwave irradiation are reduced reaction time and solvent quantities. The only limiting factor when using MWI heating in a research laboratory is the scale in which the reaction can be done. The microwave vessel can take a reaction setup defined by at most 3 grams of the starting material **3.4**.

Work-up of the reaction mixture included dilution with water followed by quenching iodine using sodium thiosulfate pellets added in small amounts while stirring until no colour change was observed. The formed precipitate was collected by filtration and dried *in vacuo* affording the product **3.5** as a yellow solid. Further purification was not necessary.

The signal that was previously due to H5 on the starting material **3.4** was absent from the ^1H NMR spectrum of **3.5**, indicating successful iodination of the C5 carbon. Additionally, an upfield shift of the signal due to C5 from 82.5 ppm to 55.7 ppm was observed.

3.1.1.4 Synthesis of 5-((trimethylsilyl)ethynyl)pyrimidine-4,6-diamine [5]

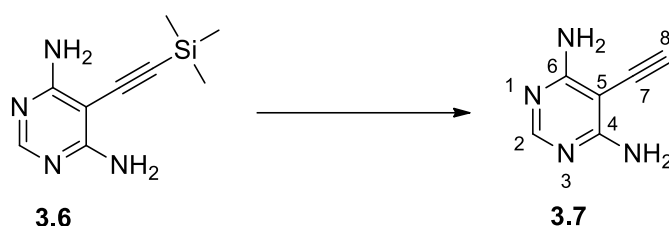


Scheme 3.5. Synthesis of 5-((trimethylsilyl)ethynyl)pyrimidine-4,6-diamine. Reagents and conditions: cat. $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$, CuI , DIPA, 2-MeTHF, 80°C , 30 minutes, 61%.

The iodine in **3.5** was then substituted by trimethylsilylacetylene using the Sonogashira coupling reaction (**Scheme 3.5**). The Sonogashira coupling reaction is palladium and copper catalysed reaction between aryl or alkenyl halides or triflates and terminal alkynes to form a C-C bond [6]. In our study we used bis(triphenylphosphine)palladium(II) dichloride and copper iodide as catalysts. Originally, the reaction was done using conventional heating at 70°C in tetrahydrofuran (THF) for 4 hours under inert conditions using excess (10 equivalents) trimethylsilylacetylene (TMSA) and diisopropylamine (DIPA). We, however, improved the reaction by using MWI heating at 80°C in 2-methyltetrahydrofuran (2-MeTHF, a green solvent) for 30 minutes. Also, we lowered the number of equivalents of TMSA and DIPA down to 2 equivalents each. We found that it was necessary to increase the equivalents of the palladium catalyst from 5% to 10%, to prolong its usefulness since the reaction was done in air, and not under an inert atmosphere. After work-up and purification, the product **3.6** was afforded as an off-white solid in 61% yield – an improvement from 48% obtained under conventional method [7].

The presence of a trimethylsilylacetylene moiety was confirmed in the ^1H NMR spectrum by a signal at 0.00 ppm integrating to 9 protons due to H10. In addition, the ^{13}C NMR spectrum had two signals at 91.6 ppm and 77.8 ppm due to C8 and C7, respectively. A downfield shift of C5 from 55.67 ppm to 76.62 ppm was also observed. Furthermore, the molecular ion $(\text{M}+\text{H})^+$ was confirmed by HRMS to be 207.1063 which corresponded to the theoretical mass of 207.1061, calculated for $\text{C}_9\text{H}_{15}\text{N}_4\text{Si}^+$.

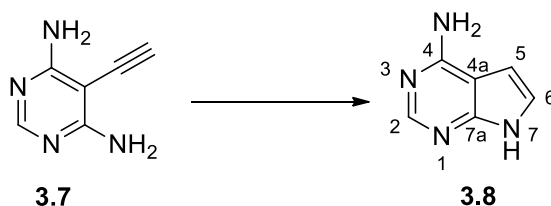
3.1.1.5 Synthesis of 5-ethynylpyrimidine-4,6-diamine [7]



Scheme 3.6. Synthesis of 5-ethynylpyrimidine-4,6-diamine. Reagents and conditions: K_2CO_3 , methanol, rt., 2hrs, 64%.

The afforded intermediate **3.6** was deprotected using potassium carbonate in methanol to afford **3.7** as a white solid after purification (**Scheme 3.6**). The formation of the product was confirmed by the absence of the signal previously due to H10 of **3.6** at 0.00 ppm and the appearance of a peak at 4.36 ppm due to H8. The molecular ion of **3.7** ($\text{C}_6\text{H}_7\text{N}_4^+$) was confirmed by HRMS to be 135.0665, corresponding to the theoretical value of 135.0667.

3.1.1.6 Synthesis of 7H-pyrrolo[2,3-d]pyrimidin-4-amine [7]



Scheme 3.7. Synthesis of 7H-pyrrolo[2,3-d]pyrimidin-4-amine. Reagents and conditions: Cs_2CO_3 , DMSO, 180°C MWI (150 W), 15 minutes, 50%.

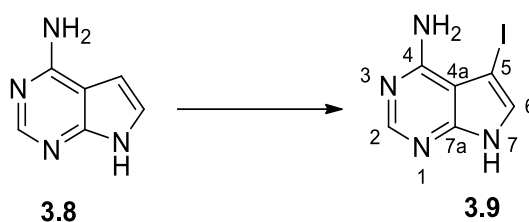
The pyrrolo[2,3-d]pyrimidine scaffold **3.8** was afforded following the intramolecular reaction of **3.7** mediated by caesium carbonate (**Scheme 3.7**). The mixture of the starting material **3.7** and caesium carbonate in DMSO was heated for 15 minutes at 180°C by microwave irradiation to afford **3.8** in 50% yield following reaction workup and purification.

The formation of the product was confirmed by both ^1H NMR and ^{13}C NMR spectroscopy. The proton NMR spectroscopic signals integrated for 6 protons as expected. The key signals were those due to H7, H6 and H5 appearing at 11.44, 7.05, and 6.50 ppm, respectively, showing that a five membered ring was successfully formed. The carbon NMR spectrum had 6 peaks typical of the formed product **3.8**. Signals that were previously due to C8, C5, and C7 of **3.7** now appear at 121.2, 102.7, and 99.3 ppm as C6, C4a, and C5 of **3.8**, respectively. The downfield shift of these signals indicates a change in the chemical environment which is, in this case, the formation of a five membered aromatic ring.

Compound **3.8** is available commercially as 6-amino-7-deazapurine and synthesis of some pyrrolo[2,3-*d*]pyrimidine derivatives has started at this point. However, we saw it fit to synthesize the compound ourselves because of the expensive cost and available quantity (1 grams per unit) of 6-amino-7-deazapurine.

3.1.1.7 Synthesis of 5-iodo-7*H*-pyrrolo[2, 3-*d*]pyrimidin-4-amine [8]

At this point of the synthesis, we have successfully made the pyrrolo[2,3-*d*]pyrimidine scaffold on which the R¹ and R² substituents can be incorporated (**Figure 3.1**). From here, there are 3 potential routes that can be taken to achieve our targeted compound all of which have the same synthetic protocols but in different order. We found that the route that starts with iodinating the C5 position allowed for the preparation of an advanced common intermediate on which varied R¹ and R² substituents will be introduced, reducing the overall synthesis effort required to get the final compounds. We, therefore, decided to start by iodinating C5 before incorporating the R¹ and R² substituents (**Scheme 3.7**).



Scheme 3.8. Synthesis of 5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine. Reagents and conditions: NIS, CHCl₃, 62°C, 2 hrs, 90%.

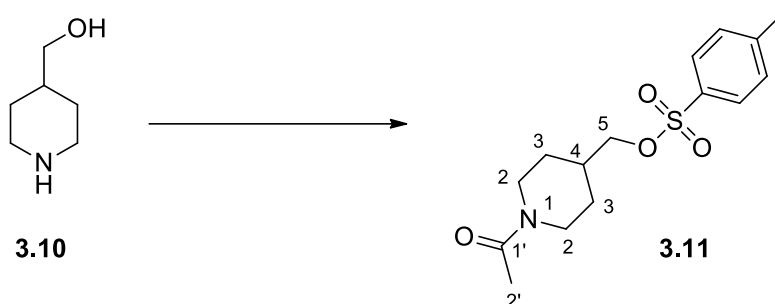
The C5 position was functionalised with iodine (**Scheme 3.8**) to prepare the scaffold for the introduction of large aryl moieties. The reaction was performed using dimethylformamide (DMF) as the solvent initially. A solution of 6-amino-7-deazapurine **3.8** and *N*-iodosuccinimide (NIS) in DMF was heated at 60°C for 4 hours before diluting with water at which point a precipitate formed. The precipitate was collected by filtration and purified to afford the product **3.9** as a yellow solid in 81% yield. We found, however, that higher yields can be afforded with less reaction time when using chloroform as a solvent instead of DMF. Heating the reaction in chloroform at 60°C for 2 hours afforded the product in 90% yield after work up and purification.

The success of the reaction was verified by the absence of the typical H5 proton signal of **3.8** in the ¹H NMR spectrum of **3.9**. As expected, there was a shift in the position of the C5 signal of the product from 99.3 in **3.8** to 50.1 ppm in the ¹³C NMR spectrum of **3.9**. The molecular ion of **3.9** (C₆H₆IN₄⁺) was confirmed by HRMS to be 260.9641 corresponding to the theoretical value of 260.9632.

At this point, we had prepared a molecule that cannot distinguish between human kinases and parasite kinases. To make the molecule selective towards parasite kinases we had to functionalise the N7 and C5 positions of the 5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidine molecule with an alkyl moiety and a bulky aryl moiety, respectively. These, as discussed previously, are essential in making the compounds potent and selective. The next two sections, section 3.1.2 and section 3.1.3, discuss the synthetic procedures adopted in functionalising the pyrrolo[2,3-*d*]pyrimidine scaffold to afford compounds that are more likely to be selective towards the parasite kinases.

3.1.2 Part two: Incorporating the alkyl moiety at the N7-position of the pyrrolo[2,3-*d*]pyrimidine scaffold

3.1.2.1 Synthesis of (1-acetylpiperidin-4-yl)methyl 4-methylbenzenesulfonate [7]



Scheme 3.9. Synthesis of (1-acetylpiperidin-4-yl)methyl 4-methylbenzenesulfonate. Reagents and conditions: (i) Acetic anhydride, triethylamine, DCM, r.t., 4hrs. (ii) 4-Toluenesulfonfylchloride, r.t., 12 hrs.

We started by introducing alkyl moieties at the N7 position of compound **3.9** that will occupy the sugar ribose pocket of the targeted kinases. This can be achieved by alkylating the N7 position of **3.9** using precursors with good leaving groups such as halogens or the toluenesulfonyl group, to name but a few. Most of these precursors are commercially available, however, a precursor bearing the *N*-acylpiperidine moiety was prepared starting from 4-piperidinemethanol **3.10** (**Scheme 3.9**). To a solution of **3.10**, triethylamine, and DCM cooled to 0°C was carefully added an acylating agent, acetic anhydride. After the addition, the mixture was warmed to room temperature and then stirred for 4 hours. This procedure adds the acyl moiety at the N position of the piperidine ring. N-alkylation took precedence over O-alkylation because the secondary amine nitrogen is more nucleophilic than the methanol oxygen atom. Also, to ensure a controlled addition of the acyl group to the nitrogen atom we used one equivalent of acetic anhydride

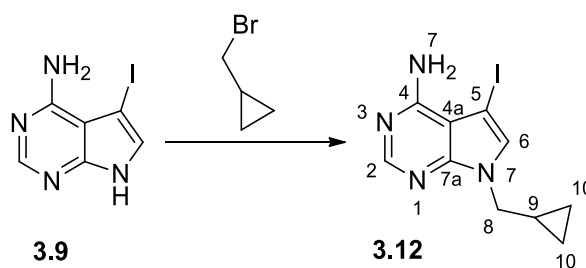
After four hours stirring at room temperature, we cooled the solution down to 0°C, followed by the addition of 4-toluenesulfonylchloride. We then proceeded by warming the mixture to room temperature and stirring for a further 12 hours. The reaction mixture was diluted with ethyl acetate

and washed with aqueous NaHCO₃, 1M HCl, and brine successively to neutralise residues of the base. After concentrating the organic layer *in vacuo*, we isolated the product using silica gel column chromatography affording the product as a translucent yellow oil in 43% yield.

The toluene moiety of the product **3.11** was easily identified from the ¹H NMR spectrum by two doublet signals at 7.78 ppm and 7.36 ppm typical of *p*-substituted aromatic protons, and the signal at 2.07 ppm due to the aromatic CH₃ protons. These signals integrate for 2, 2, and 3 protons respectively. The addition of the acyl group was confirmed by a multiplet signal at 2.58 - 2.41 ppm due to H2' overlapping with signals due to H2. Overall, a total of 21 protons were identified from the spectrum, as expected for product **3.11**. Likewise, the ¹³C NMR spectroscopic signals that were due to the aromatic group were easily identifiable – four signals between 145.0 ppm and 127.9 ppm – in addition to the carbonyl carbon C1' at 168.8 ppm. The ¹³C NMR spectrum returned 13 signals as expected of the product. Further identification was done using infrared spectroscopy (IR) confirming the presence of the carbonyl (1644 cm⁻¹), sulfoxide (1348 cm⁻¹) and sp²-carbon protons (3071 cm⁻¹). HRMS confirmed the molecular ion of **3.11** (C₁₅H₂₂NO₄S⁺) to be 312.1261, consistent with the theoretical value of 312.1271.

With all the alkylating agents on hand, we went on with alkylating the N7 position of the pyrrolo[2,3-*d*]pyrimidine intermediate **3.9** to get one step closer to the target compounds.

3.1.2.2 Synthesis of 7-(cyclopropylmethyl)-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine [7]

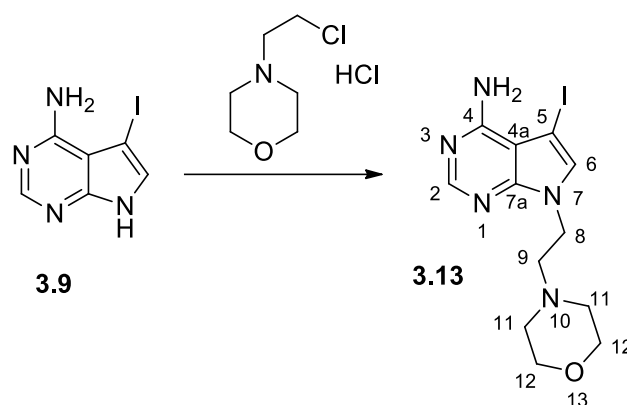


Scheme 3.10. Synthesis of 7-(cyclopropylmethyl)-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine. Reagents and conditions: Cs₂CO₃, DMF, 80°C, 18 hrs, 74%.

Compound **3.12** was prepared by heating a mixture of 5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **3.9**, (bromomethyl)cyclopropane, and caesium carbonate in DMF for 18 hours at 80°C before cooling to room temperature and diluting with ethyl acetate. The solution was washed with brine multiple times to extract DMF, HBr and Cs₂CO₃. The dried organic materials were concentrated and purified by silica gel chromatography to afford the product as a light-yellow solid in 74% yield.

The product **3.12** was confirmed by both ^1H NMR and ^{13}C NMR spectroscopy. The key changes observed in the ^1H NMR spectrum were the absence of the H7 proton and the appearance of peaks at 3.95 ppm, 1.21 ppm, and a multiplet signal at 0.50-0.34 ppm due to H8, H9, and H10, respectively. The ^{13}C NMR spectrum had 3 additional peaks in the aliphatic region due to the methylcyclopropane moiety at 48.74 ppm (C8), 12.05 ppm (C9), and 4.07 ppm (C10). Furthermore, HRMS confirmed the molecular ion of **3.12**, $\text{C}_{10}\text{H}_{12}\text{IN}_4^+$, to be 315.0118 corresponding to the calculated value of 315.0101 for the same ion.

3.1.2.3 Synthesis of 5-iodo-7-(2-morpholinoethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine [7]

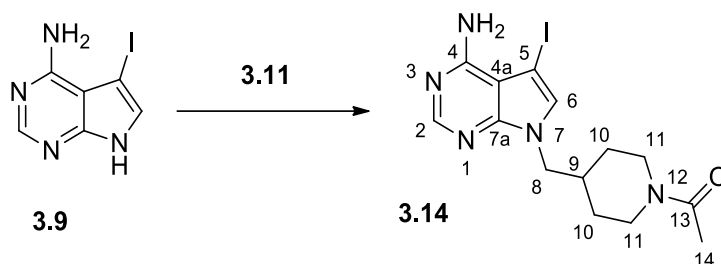


Scheme 3.11. Synthesis of 5-iodo-7-(2-morpholinoethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine. Reagents and conditions: Cs_2CO_3 , DMF, 80°C , 18 hrs, 59%.

Compound **3.13** was afforded following the same procedure outlined in the synthesis of **3.12**, however, using 4-(2-chloroethyl)morpholine hydrochloride as an alkylating agent (**Scheme 3.11**). The product was afforded in 59% yield as a light-brown solid.

The ethylmorpholine moiety of the product was confirmed by the ^1H NMR spectrum with signals appearing at 4.22 ppm and 3.51 ppm due to H8 and H9, respectively. These signals are the only two that have a triplet multiplicity integrating for two protons. Morpholine ring protons (H12 and H11) were also identified at 3.51 and 2.41 ppm. The ^{13}C NMR spectrum showed 4 additional peaks in the aliphatic region (excluding C5 at 49.84 ppm) that were due to C12, C9, C11, and C8 between 66.59 ppm and 41.93 ppm, as expected. The molecular ion of **3.13**, $\text{C}_{12}\text{H}_{17}\text{IN}_5\text{O}^+$, was confirmed to be 374.0479 by HRMS corresponding to the theoretical value of 374.0433 for the same molecular ion.

3.1.2.4 Synthesis of 1-{4-[(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethenone [7]



Scheme 3.12. Preparation of 1-{4-[(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethenone. Reagents and conditions: Cs₂CO₃, DMF, 80°C, 18 hrs, 48%.

A mixture consisting of substrate **3.9**, the alkylating agent **3.11**, and caesium carbonate was heated for 18 hours to afford **3.14** after work-up and purification as an off-white solid in 48% yield (**Scheme 3.12**). Like other alkylation reactions, the proton NMR spectrum was lacking the H7 proton indicating successful alkylation. The acylpiperidine moiety was confirmed by the presence of a doublet peak at 4.01 ppm due to H8, a multiplet peak at 2.10-2.02 ppm due to H9, and a singlet peak integrating for 3 protons at 1.96 ppm due to H14. The carbon NMR spectrum contained a deshielded peak at 168.3 ppm due to the carbonyl carbon C13, and aliphatic peaks including those at 49.55 ppm and 36.79 ppm due to C8 and C9, respectively.

3.1.3 Part three: Incorporating the bulky aryl moieties at the C5 position of the pyrrolo[2,3-*d*]pyrimidine scaffold using the Suzuki-Miyaura reaction

The last part in the synthesis of the target pyrrolopyrimidine derivatives involved the incorporation of a large aryl moiety at the C5 position of the scaffold. These aromatic moieties occupy the hydrophobic pocket in the active site adjoined to the ATP binding site, whose size is determined by a gatekeeper residue. These large aromatic moieties are unable to occupy human kinases because these kinases have a small pocket caused by a large gatekeeper residue. As a result, the moiety imparts selectivity to the derivatives toward parasite kinases because of their large hydrophobic pocket caused by small gatekeeper residues.

We envisaged linking the scaffold and the aromatic moieties using the Suzuki-Miyaura coupling reaction. The Suzuki-Miyaura coupling reaction is a carbon-carbon bond forming reaction between aromatic halides and aromatic boronic acids, catalysed by palladium in the presence of a base. The mechanism begins by oxidative addition of the organohalide to the palladium (0) species to form a palladium (II) complex (**Figure 3.2**). The halide on the palladium complex is then replaced by an anion originating from the base, which is then replaced, through transmetalation,

by the aromatic moiety from the boronic acid. The boronic acid aromatic moiety is made more nucleophilic by the addition of an anion to boron prior to transmetalation. The afforded palladium complex, following transmetalation, undergoes reductive elimination, coupling the aryl substituents together to form the final product. Compound **3.12**, **3.13** and **3.14** form part of the aromatic halides we plan to use in the Suzuki-Miyaura coupling reaction. The boronic acids of interest for our project were prepared starting from the aromatic halide **3.15** (Scheme 3.13). The synthesis of the required boronic acids is summarised in the following sections.

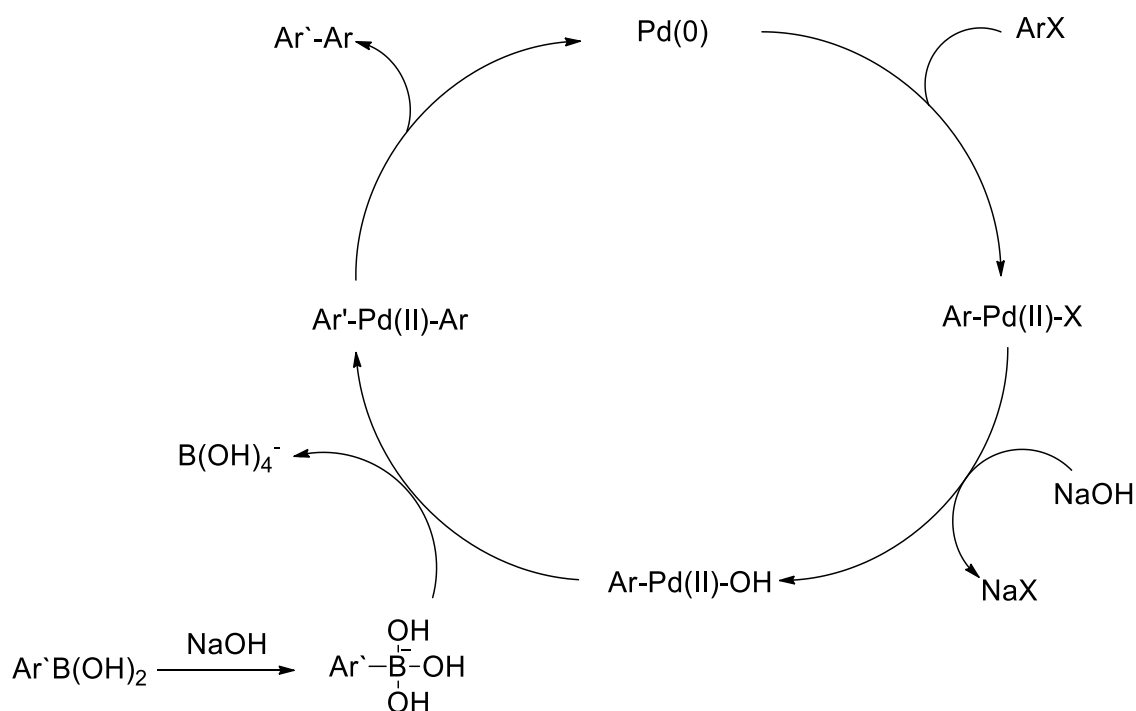
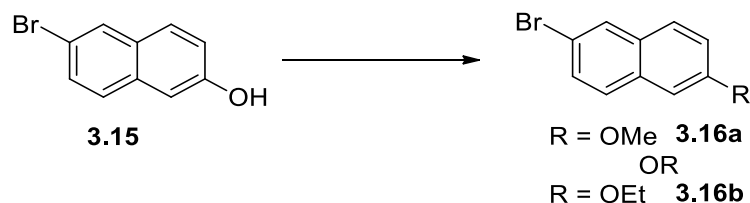


Figure 3.2. Suzuki-Miyaura coupling reaction mechanism.

3.1.3.1 Synthesis of 2-bromo-6-methoxynaphthalene and 2-bromo-6-ethoxynaphthalene [7]



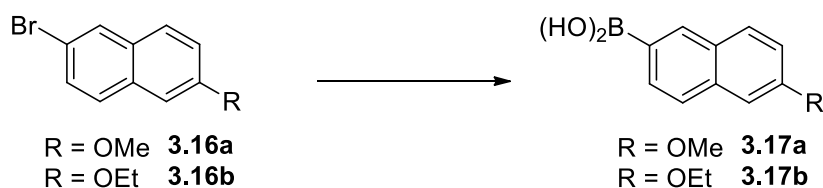
Scheme 3.13. Synthesis of 2-bromo-6-methoxynaphthalene and 2-bromo-6-ethoxynaphthalene. Reagents and conditions: dimethylsulfate or ethylbromide, K₂CO₃, acetone, 60 °C, 12 hrs.

The naphthalene halides we planned to use to afford the desired compounds were not commercially available, therefore, we prepared them from 6-bromonaphthalen-2-ol **3.15**. We started by alkylating the hydroxy moiety of **3.15** using alkylating agents (dimethylsulfate for **3.16a**, and

ethylbromide for **3.16b**), and potassium carbonate in acetone at reflux overnight. The solvent was concentrated *in vacuo* and the achieved crude matter was diluted with water. The products were afforded after work up and silica gel chromatography purification as white solids in excellent yields (99% **3.16a**; 75% **3.16b**).

Key signals in the ^1H NMR spectra confirming the formation of the products were the appearance of signals due to the CH_3 of **3.16a** at 3.91 ppm and the appearance of a quartet and triplet signal due to OCH_2 and CH_3 at 4.13 ppm and 1.48 ppm, respectively for **3.16b**. Six aromatic signals between 7.91-7.08 ppm were also observed confirming the presence of the naphthalene moiety. A total of 11 ^{13}C NMR spectroscopic signals were seen with one due to CH_3 (55.65 ppm) appearing in the aliphatic region for **3.16a**. Likewise, 12 ^{13}C NMR spectroscopic signals were seen with two peaks due to the CH_2 (63.54 ppm) and CH_3 (14.78 ppm) appearing in the aliphatic region for **3.16b**.

3.1.3.2 Synthesis of (6-methoxynaphthalen-2-yl)boronic acid and (6-ethoxynaphthalen-2-yl)boronic acid [7]

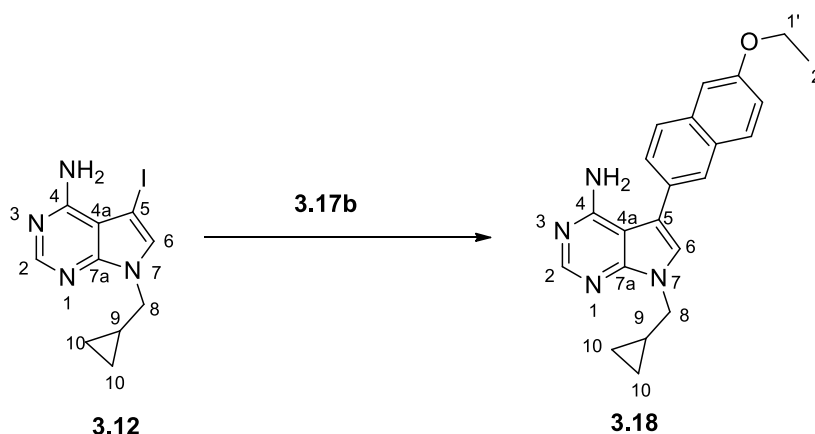


Scheme 3.14. Preparation of boronic acid from a bromonaphthalene intermediate. Reagents and conditions: (i) *n*-Butyllithium (1.6M in hexane), THF, -78°C , 30 minutes. (ii) Triisopropyl borate, -78°C to r.t., 2 hrs, 55% (**3.17a**) and 62% (**3.17b**).

With the alkyl halides in hand, we were now able to prepare the desired boronic acids for a Suzuki-Miyaura coupling reaction. The preparations of boronic acid **3.17a** and **3.17b** was carried out following the same synthetic protocol starting with the appropriate substrate, **3.16a** and **3.16b**, respectively (**Scheme 3.14**). To a solution of the relevant bromonaphthalene starting material in dry THF at -78°C was added *n*-butyllithium followed by triisopropylborate after 30 minutes of stirring. The solution was then stirred at room temperature for 2 hours before quenching with 2M aqueous HCl, hydrolysing the boronic ester to the corresponding boronic acid. Attempts were made to purify the compounds using silica gel chromatography; however, the products decomposed on silica. We managed to purify the products by taking advantage of the different solubilities of the starting materials and products in hexane. We added hexane to the crude material and sonicated the mixture to encourage the dissolution of the starting material. The products were recovered by filtration as white solids in moderate yields (55% **3.17a** and 62% **3.17b**).

The boronic acids were characterised using ^1H and ^{13}C NMR spectroscopy. A characteristic signal in the ^1H NMR spectra of the boronic acid products is that due to the hydroxyl moieties. This signal is not always observed but with **3.17b** it appeared at 8.06 ppm; it was not visible in the NMR spectrum of **3.17a**. The ^{13}C NMR spectra of both products has the correct number of signals, but noteworthy is the ppm positions of the aromatic peaks due to the products, it is different to that of the respective starting materials, suggesting that a different molecule is formed.

3.1.3.3 Synthesis of 7-(cyclopropylmethyl)-5-(6-ethoxynaphthalen-2-yl)-7H-pyrrolo[2,3-*d*]pyrimidin-4-amine [7]



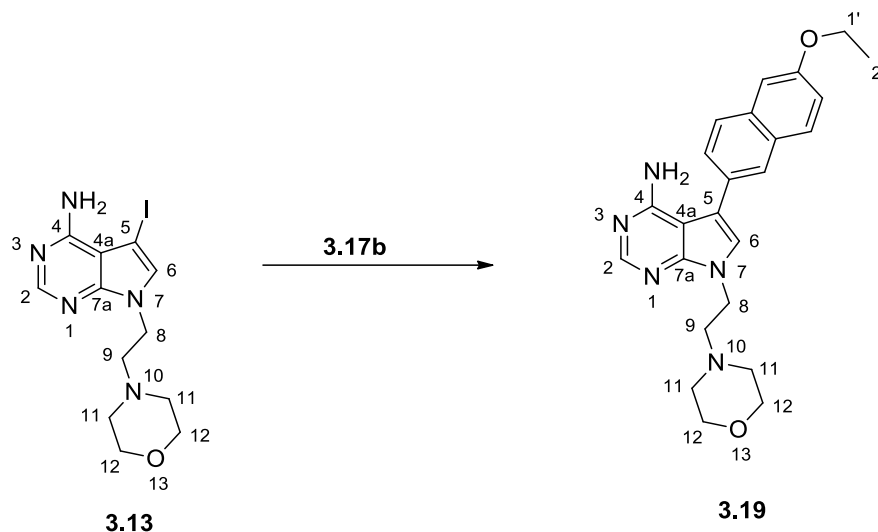
Scheme 3.15. Preparation of 1 7-(cyclopropylmethyl)-5-(6-ethoxynaphthalen-2-yl)-7H-pyrrolo[2,3-*d*]pyrimidin-4-amine. Reagents and conditions: cat $\text{Pd}(\text{PPh}_3)_4$, aq. Na_2CO_3 , 1,2-dimethoxyethane, 90-100°C, 18 hrs, 39%.

Having prepared the naphthalene boronic acid precursors we were now able to substitute the C5 position of the pyrrolo[2,3-*d*]pyrimidine scaffold using the Suzuki-Miyaura coupling reaction. The reaction allowed us to attain our targeted compounds starting from the appropriate alkylated intermediate. Compound **3.18** was afforded starting with iodinated pyrrolo[2,3-*d*]pyrimidine **3.12** (Scheme 3.15). A mixture of **3.12**, **3.17b** and catalytic $\text{Pd}(\text{PPh}_3)_4$ in an inert environment was added a degassed solution of 1,2-DME and aqueous Na_2CO_3 . The reaction mixture was heated at reflux for 18 hours before diluting with brine and extracting with ethyl acetate. The final compound **3.18** was obtained as a yellow-orange solid after silica gel chromatography in 39% yield.

Seven aromatic signals were observed from the ^1H NMR spectrum between 8.34 ppm and 7.15 ppm. Signal due to the ethoxy moiety of the naphthalene structure appeared at 4.19 and 1.51 ppm as a quartet and triplet, due to H1', and H2', respectively. The cyclopropyl moiety was confirmed by three multiplet signals between 0.91 and 0.61 ppm integrating for 1, 2, and 2 protons, due to H9, H10, and H10, respectively. The carbon spectrum had 21 signal consistent with **3.18** with 12 in the aromatic region (two overlapping) and 5 in the aliphatic region. The molecular ion,

$C_{22}H_{23}N_4O^+$, of **3.18** was confirmed to be 359.1848 by HRMS consistent with the theoretical value of 359.1886 for the same molecular ion.

3.1.3.4 Synthesis of 5-(6-ethoxynaphthalen-2-yl)-7-(2-(tetrahydro-2*H*-morpholin-4-yl)ethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine [7]

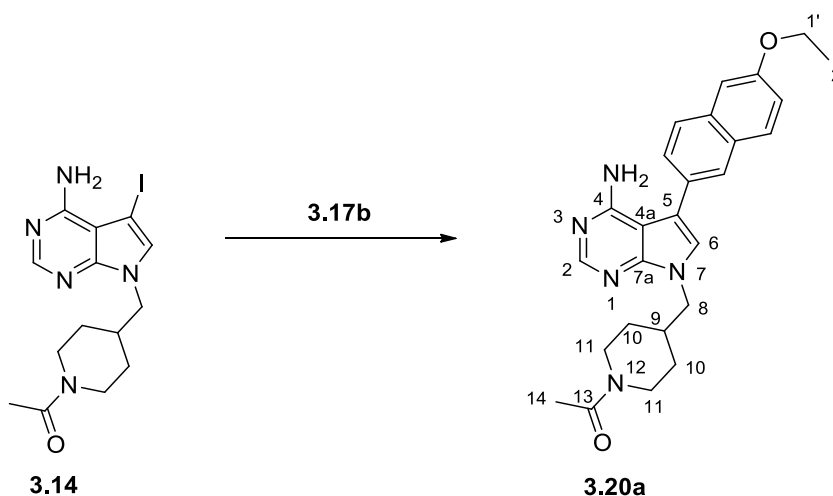


Scheme 3.16. Preparation of 5-(6-ethoxynaphthalen-2-yl)-7-(2-(tetrahydro-2*H*-morpholin-4-yl)ethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine. Reagents and conditions: $Pd(PPh_3)_4$, aq. Na_2CO_3 , 1,2-dimethoxyethane, 90-100°C, 18 hrs, 32%.

Targeted compound **3.19** was obtained following the same synthetic protocol used for **3.18**, using iodinated pyrrolo[2,3-*d*]pyrimidine substrate **3.13** and boronic acid **3.17b** (Scheme 3.16). The product was afforded as an off-white solid in 32% yield after purification by silica gel chromatography.

The formation of the product **3.19** was confirmed by ^1H NMR spectroscopy with 8 aromatic and 17 aliphatic signals observed in the ^1H NMR spectrum of the product. The morpholine ring peaks appeared at 2.55 and 3.70 ppm while peaks due to H1' and H2' appeared at 4.19 and 1.51 ppm, respectively. The carbon NMR spectrum had 21 peaks with 15 in the aromatic region (two signals overlapped) and 6 in the aliphatic region as expected. HRMS found the molecular ion of the product **3.19** ($C_{24}H_{28}N_5O_2^+$) to be 418.2240 which correspond to the calculated value of 418.2238 for the same molecular ion, further confirming the formation of the product.

3.1.3.5 Synthesis of 1-(4-((4-amino-5-(6-ethoxynaphthalen-2-yl)-7H-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl)piperidin-1-yl)ethanone and analogue [7]

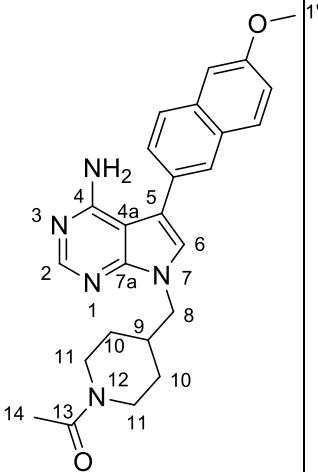


Scheme 3.17. Preparation of 1-(4-((4-amino-5-(6-ethoxynaphthalen-2-yl)-7H-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl)piperidin-1-yl)ethanone. Reagents and conditions: Pd(PPh₃), aq. Na₂CO₃, 1,2-dimethoxyethane, 90-100°C, 18 hrs, 41%.

The iodo-intermediate **3.14**, boronic acid **3.17b** and aqueous Na₂CO₃ were heated in 1,2-DME for 18 hours to afford the product **3.20a** in 41% yield after purification by silica gel chromatography. The compound was characterised using ¹H and ¹³C NMR spectroscopy and HRMS. The presence of the pyrrolopyrimidine core was confirmed by the signals at 8.33, 5.33, and 6.96 ppm due to H2, NH₂ and H6 in the proton NMR spectrum of the product. The presence of the naphthalene ring was confirmed by the six aromatic peaks between 7.86 and 7.15 ppm. The acylpiperidine moiety was confirmed by the signal at 168.8 ppm due to C13 in the carbon NMR spectrum. All aromatic carbons were accounted for (16 in total) between 157.23 and 101.4 ppm. HRMS confirmed the presence of a molecular ion, C₂₆H₂₉N₅O₂⁺, due to the product **3.20a** to be 444.2375 which is consistent with the theoretical value of 444.2394.

An analogue of compound **3.20a** (compound **3.20b**), was prepared following the same synthetic procedure but with boronic acid **3.17a** and then characterised in a similar manner. Key NMR spectroscopic peaks identifying the product, HRMS mass to charge ratio, and yield of **3.20b**, are reported in the table below (**Table 3.1**).

Table 3.1. Key NMR spectroscopic signals and HRMS m/z ratio of compound **3.20b**

Entry	Compound	Structure	Key ^1H and ^{13}C NMR spectroscopic signals/ppm and HRMS	Yield
1	3.20b		^1H : 8.33 (s, 1H, H2), 7.87 – 7.79 (m, 2H, ArH), 7.77 (d, $J = 8.7$ Hz, 1H, ArH), 7.57 (dd, $J = 8.4, 1.7$ Hz, 1H, ArH), 7.23 – 7.17 (m, 2H, ArH), 6.96 (s, 1H, H6), 5.37 (s, 2H, NH_2). ^{13}C : 168.81 (C13), 157.91 (ArC), 157.26 (C4), 152.09 (C2), 150.87 (C7a), 133.54 (ArC), 129.71 (ArC), 129.28 (ArC), 129.05 (ArC), 127.60 (C6), 127.55 (ArC), 127.12 (ArC), 123.22 (ArC), 119.63 (ArC), 116.38 (C5), 105.75 (ArC), 101.25 (C4a). HRMS $[\text{C}_{25}\text{H}_{28}\text{N}_5\text{O}_2]^+$: Found 430.2214; calculated 430.2238.	65%

3.2 Biological evaluation of pyrrolo[2,3-*d*]pyrimidine derivatives.

We synthesised pyrrolo[2,3-*d*]pyrimidine derivatives with the aim of developing molecules that will selectively inhibit *Pf*CDPK1 and *Pf*CDPK4 over human kinases. Inhibition of these enzymes has been shown to halt malaria parasite proliferation in the human host and vector (*Anopheles* mosquito) with the effect of treating malaria and blocking effective transmission of the parasite from humans to mosquito. We assessed these molecules *in silico* and found that they can act as potential inhibitors of both enzymes (*Pf*CDPK1 and *Pf*CDPK4, Chapter 2). To validate the obtained *in silico* results, we subjected all the synthesised compounds including those prepared by a fellow student to biological assays including enzyme activity assays and cytotoxicity assay. Biological assays were carried out by our collaborators at Rhodes University and University of Washington. Results acquired from the assays are discussed in the next sections.

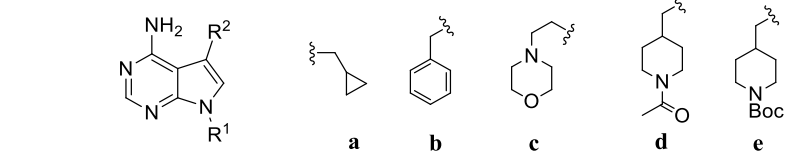
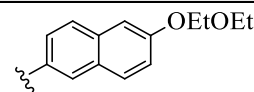
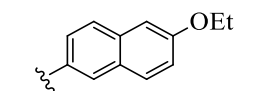
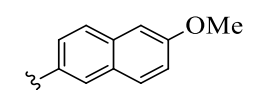
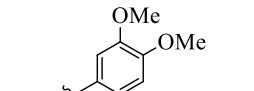
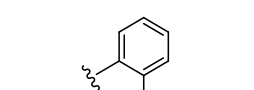
3.2.1 Enzyme activity assay

To find out the inhibitory potential of our compounds against the enzymes of interest, our collaborators at the University of Washington tested them on recombinant *Pf*CDPK1 and *Pf*CDPK4 proteins. The proteins were prepared and purified following published procedures where recombinant expression was carried out in Rosetta Oxford *Escherichia coli* using Studier auto-induction method at 20°C, and purified by immobilised metal affinity chromatography (IMAC) and size exclusion chromatography [9]. Kinase phosphorylation reactions were carried out in a medium containing 20mM HEPES buffer (pH 7.5, adjusted by KOH), 0.1% bovine serum albumin, 10nM MgCl₂, $\pm$ 1 mM CaCl₂, and 1mM EGTA (pH 7.2) in three replicates. The reaction was initiated by the addition of ATP (10 μ M) in a mixture of the Syntide-2 peptide substrate (40 μ M, with sequence: PLARTLS VAGLPGKK), *Pf*CDPK1 (10 nM), *Pf*CDPK4 (180 nM), and the synthesised pyrrolo[2,3-*d*]pyrimidine derivatives, and then terminated after 90 minutes incubation at 30°C. The catalytic activity of the recombinant kinases was determined using a Kinase Glo luciferase assay (Promega) that measured the depletion of ATP in the presence of the peptide substrate. Luminescence-based readout as counts per second were acquired using the Envision Multimode Plate Reader (PerkinsElmer). Enzymatic inhibitory results (IC₅₀) due to the prepared compounds are presented in **Table 3.2** (mean $\pm$ SD). Note that all the compounds tested, as well as those prepared but not tested, are included here for comparison with our molecular modelling study. The compounds prepared in this project are explicitly shown.

Generally, all the compounds had poor activity against the *Pf*CDPK4 enzyme (IC₅₀>2 μ M) save for three (entries 3, 5, & 7). All active compounds had the naphthalene moiety as the R² substituent on the pyrrolo[2,3-*d*]pyrimidine scaffold suggesting that larger moieties at this position are ideal for potency; none of the mono- or dimethoxyphenyl substituents were active against *Pf*CDPK4. Furthermore, the methoxy- and ethoxynaphthalene bearing compounds had better activity compared to their ethoxyethoxynaphthalene compounds. These results are consistent with the data obtained from our *in silico* studies. From our *Pf*CDPK4 docking studies of the pyrrolo[2,3-*d*]pyrimidine derivatives, we concluded that the compound bearing the naphthalene moiety made better interaction in the active site of the enzyme than the phenyl counterparts. Also, methoxy- and ethoxynaphthalene compounds had good docking scores compared to the ethoxyethoxynaphthalene compounds, with the longer side chain forming unfavourable interactions in the hydrophobic pocket. The obtained results shed light on the predictive accuracy of the developed *Pf*CDPK4 model. So far, the experimental results correlate well with our

predicted results. The docking scores of the active compounds will be used as a base score in designing molecules with improved potency against the target kinases.

Table 3.2. Biological assays data including *Pf*CDPK1 and *Pf*CDPK4 enzymatic inhibitory concentrations (IC₅₀), and HeLa percent cell viability [7].

					
Entry	R ²	R ¹	<i>Pf</i> CDPK4 IC ₅₀ /μM	<i>Pf</i> CDPK1 IC ₅₀ /μM	Hela % cell viability at 20 μM
1		a	—	—	73.0 ± 4.1
2		d	>2	>2	94.4 ± 9.3
3 (3.18)		a	0.3694±0.1381	>2	79.8 ± 3.3
4 (3.19)		c	—	—	47.0 ± 7.0
5 (3.20a)		d	0.5298±0.1847	>2	92.8 ± 3.1
6		a	—	—	93.6 ± 7.1
7 (3.20b)		d	0.2104±0.0680	>2	92.1 ± 3.1
8		a	>2	0.5885±0.2059	88.8 ± 0.7
9		b	—	—	95.4 ± 0.5
10		c	>2	>2	88 ± 11.8
11		d	>2	>2	79.3 ± 0.8
12		e	>2	>2	82.4 ± 1.4
13		a	>2	>2	62.0 ± 2.1
14		b	—	—	97.0 ± 12.5
15		c	>2	>2	112 ± 2.8
16		d	>2	>2	93.3 ± 3.2
17		e	—	—	78.9 ± 0.1

Likewise, none of the synthesised compounds were active against the *Pf*CDPK1 enzyme except for one (entry 8, **Table 3.2**). The active compound has a smaller R² substituent which was predicted by the *in silico* model to better occupy the kinase small hydrophobic pocket, typical of the *Pf*CDPK1 enzyme – the small size of the pocket does not favour large aromatic groups like the naphthalene moiety. The model also predicted that the monomethoxyphenyl bearing compounds, although small, had poor activity owing to the ortho-position of the methoxy side chain. This arrangement caused bad interaction between the methoxy side chain of the phenyl moiety and the gatekeeper residue in the binding site. The observation is consistent with the inhibitory data obtained – monomethoxyphenyl bearing compounds were not active against *Pf*CDPK1 (IC₅₀ > 2 μM). Based on *in silico* docking results it was expected that the best inhibitor of *Pf*CDPK1 will have the dimethoxyphenyl R² substituent and the acylpiperidine moiety as the R¹ substituent. However, experimentally the best inhibitor of *Pf*CDPK1 has the methylcyclopropane moiety as the R¹ substituent. This suggest that the ribose pocket component of the developed *Pf*CDPK1 model needs adjustment. This can be achieved by further refining the model using molecular dynamics or using a different homolog as a template.

By comparison, our pyrrolo[2,3-*d*]pyrimidine inhibitors performed worse than the pyrazolo[2,3-*d*]pyrimidine and imidazo[1,5-*a*]pyrazine compounds developed by Vidadala and coworkers [1] against the target enzymes *Pf*CDPK1 and *Pf*CDPK4. The additional nitrogen on the scaffolds they explored seems to be an important determinant of the molecules active/potent conformation.

3.3.2 Cytotoxicity assay

Our collaborators at Rhodes University also tested our compounds for potential toxicity to human cells using the HeLa cells line. It is advised that toxicity studies be performed early in the design and development of medicinal drugs to avoid financial losses that are associated with the rejection of the drug later on due to poor safety profiles. HeLa cells prepared in 96 well plates at a density of 2×10^4 cells per well in Dulbecco's modified eagle medium (DMEM) with 10% fetal bovine serum (FBS), 2 mM L-glutamine and antibiotics (penicillin, streptomycin, and amphotericin B) were incubated in a 5% CO₂ incubator overnight at 37°C. The prepared target compounds, the pyrrolo[2,3-*d*]pyrimidine derivatives, were then added to the final concentration of 20 μM and incubation was continued for an additional 48 hours. To quantify the remaining viable cells resazurin was added to the wells to a final concentration of 50 μM and incubation was continued for 4 hours before measuring resorufin fluorescence (Ex₅₆₀/Em₅₉₀) using a Spectramax M3 plate reader. Fluorescence reading were corrected by subtracting background readings due to wells without cells and then converted to percentage (%) viability relative to untreated control wells.

HeLa percent cell viability for each pyrrolo[2,3-*d*]pyrimidine derivative is presented in **Table 3.2** (mean $\pm$ SD).

Obtained cytotoxicity assay results indicate that all the compounds have poor activity against human cells, except for one (entry 4, **Table 3.2**) which shows moderate cell toxicity. Most of the compounds have cell viability of at least 76% indicating that the compounds have minimal cytotoxic activity. This suggests that minor structural modification will have to be made to improve the safety profile of the compounds. Since the assay is done on whole cells, we cannot be sure that the toxic activity is caused by the inhibition of human kinases. Although probable, other non-kinase cell apoptosis mechanisms may be responsible. To ascertain if the toxic activity of the compounds is by inhibiting the human kinases, further studies like human kinases selectivity profiling will have to be carried out.

3.4 References

1. Vidadala, R.S.R., et al., *Development of potent and selective Plasmodium falciparum calcium-dependent protein kinase 4 (PfCDPK4) inhibitors that block the transmission of malaria to mosquitoes*. European Journal of Medicinal Chemistry, 2014. **74**: p. 562-573.
2. Chhabria, M.T., et al., *Synthesis and α 1-Adrenoceptor Antagonistic Activity of Some 4-Amino-5,7-dimethyl-2-(substituted) aminopyrido(2,3-*d*) pyrimidines*. Arzneimittelforschung, 2002. **52**(11): p. 792-796.
3. Brown, D.J., *Improved syntheses in the pyrimidine series .1. The use of pyrimidine-thiols*. Journal of the Society of Chemical Industry-London, 1950. **69**(12): p. 353-356.
4. Perner, R.J., et al., *Synthesis and biological evaluation of 6,7-disubstituted 4-aminopyrido[2,3-*d*]pyrimidines as adenosine kinase inhibitors*. Bioorganic & Medicinal Chemistry Letters, 2005. **15**(11): p. 2803-2807.
5. Maiti, M., et al., *Influence of the Nucleobase and Anchimeric Assistance of the Carboxyl Acid Groups in the Hydrolysis of Amino Acid Nucleoside Phosphoramidates*. Chemistry – A European Journal, 2012. **18**(3): p. 857-868.
6. Chinchilla, R. and C. Nájera, *The Sonogashira Reaction: A Booming Methodology in Synthetic Organic Chemistry*. Chemical Reviews, 2007. **107**(3): p. 874-922.
7. Seanego, T.D. and H.E. Chavalala, *7H-Pyrrolo[2,3-*d*]pyrimidine-4-amines as Potential Inhibitors of Plasmodium falciparum Calcium-Dependent Protein Kinases*. ChemMedChem. 2022. **17**(22): p. e202200421.
8. Li, Y., et al., *Identification of 5-(2,3-Dihydro-1H-indol-5-yl)-7H-pyrrolo[2,3-*d*]pyrimidin-4-amine Derivatives as a New Class of Receptor-Interacting Protein Kinase 1 (RIPK1) Inhibitors, Which Showed Potent Activity in a Tumor Metastasis Model*. Journal of Medicinal Chemistry, 2018. **61**(24): p. 11398-11414.
9. Ojo, K.K., et al., *A Specific Inhibitor of PfCDPK4 Blocks Malaria Transmission: Chemical-genetic Validation*. The Journal of Infectious Diseases, 2013. **209**(2): p. 275-284.

Chapter 4: Synthesis of 4,6-diaminopyrimidine derivatives as potential inhibitors of *Pf*CDPK1 and *Pf*CDPK4

Results from molecular modelling studies have suggested that 4,6-diaminopyrimidine bearing derivatives have the potential to interact with the targeted enzymes *Pf*CDPK1 and *Pf*CDPK4. The docking models have shown that the pyrimidine moiety makes similar hydrogen bonding interactions to the natural substrate (adenosine triphosphate) of the targeted kinase at the binding site, and the sugar substituents and the bulky aromatic substituents necessary for potency and selectivity were projected to the correct pockets (**Figure 4.1**). We, therefore, in this chapter discuss the synthesis of 4,6-diaminopyrimidine derivative as potential inhibitors of *Pf*CDPK1 and *Pf*CDPK4.

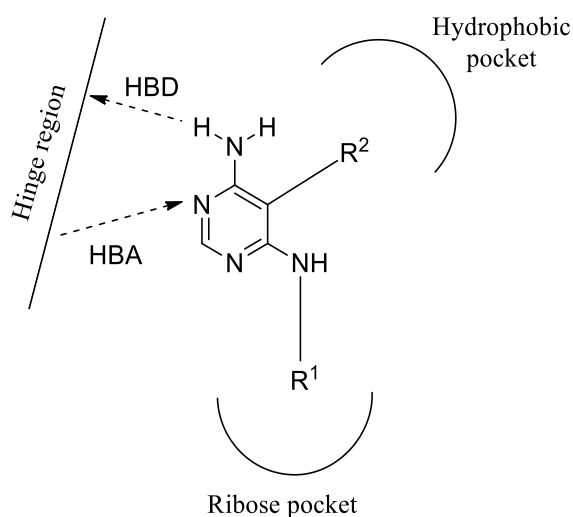


Figure 4.1. Overview of the binding interactions between the 4,6-diaminopyrimidine derivatives and the target CDPK enzymes.

The targeted 4,6-diaminopyrimidine derivatives are generally divided into two broad categories, the two-atom linkage and the one-atom linkage compounds, named after the number of atoms, that link the pyrimidine moiety and the bulky fragment R² making up the targeted compounds (**Figure 4.2**). The categories are further divided into at least two classes each depending on the nature of the linker. For the two-atom linkage compounds, the linker could either be an acetylene (class I compounds), an ethene (class II compounds), or an ethane (class III compounds). The on-atom linkage compounds are divided into the carbon series and heteroatom series after the nature of the atom (X) that links the pyrimidine and the bulky fragments R².

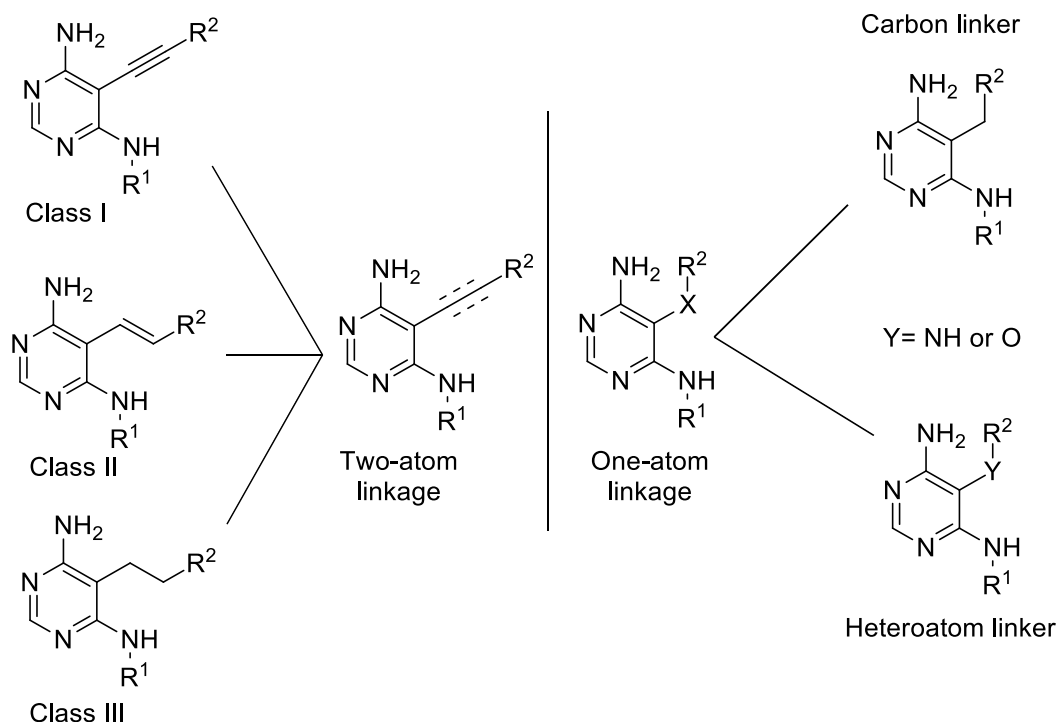
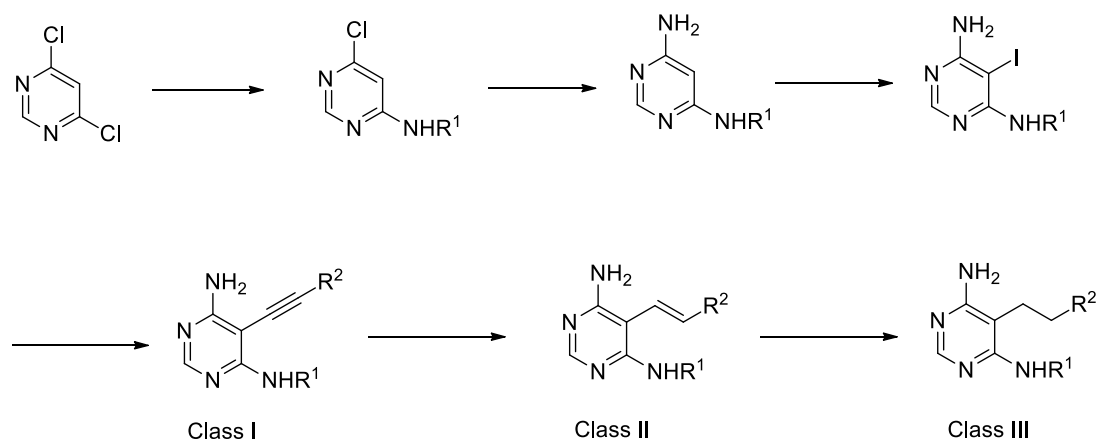


Figure 4.2. Division of the targeted 4,6-diaminopyrimidine compounds.

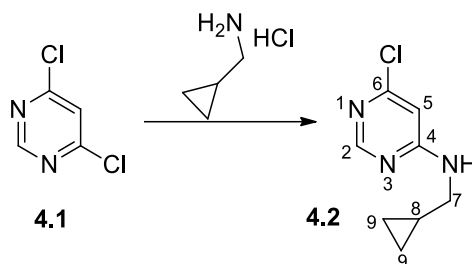
4.1 Preparation of two-atom linkage 4,6-diaminopyrimidine derivatives

We planned the synthesis of 4,6-diaminopyrimidine compounds starting from 4,6-dichloropyrimidine (**Scheme 4.1**). To the latter a nucleophilic substitution takes place with suitably substituted amines adding the NHR¹ substituent on the ring. The next step was to substitute the remaining chlorine substituent with an amino group using ammonia. From here we prepared for the addition of the bulky R² group by iodination at the C5 position of the pyrimidine ring followed by a Sonogashira coupling reaction. This would afford the Class I series of compounds. Subsequent reduction would afford the remaining Class II and III compounds.



Scheme 4.1. Proposed synthetic route for two-atom linkage 4,6-diaminopyrimidine compounds

4.1.1 Synthesis of 6-chloro-*N*-(cyclopropylmethyl)pyrimidin-4-amine [1]

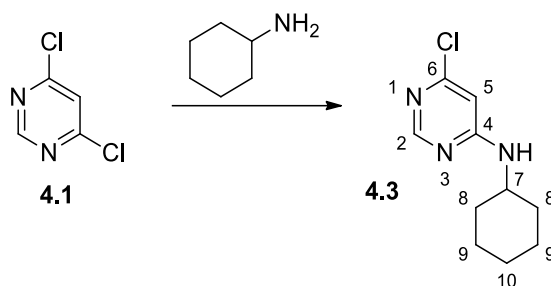


Scheme 4.2. Preparation of 6-chloro-*N*-(cyclopropylmethyl)pyrimidin-4-amine. Reagents and conditions: DIPEA, 2-MeTHF, 80°C, 18 hours, 75%.

We started the synthesis of 4,6-diaminopyrimidine derivatives by displacing the chlorine in the commercially available 4,6-dichloropyrimidine **4.1** using alkylamines to introduce the R¹ groups to the pyrimidine scaffold. The chosen R¹ groups were the methylcyclopropane, cyclohexane and ethylmorpholine groups. The methylcyclopropane bearing compound **4.2** was afforded after heating a mixture of **4.1**, cyclopropanemethylamine hydrochloride, and *N,N*-diisopropylethylamine (DIPEA) in 2-methyltetrahydrofuran (2-MeTHF) at 80°C for 18 hours (**Scheme 4.2**). The product was then purified using liquid/liquid extraction and subsequently silica gel chromatography giving a white solid in 75% yield. Initially we employed dioxane and toluene as the solvents for the synthesis, however, we later saw a need to change the solvents owing to their toxicity. We found 2-MeTHF to be a suitable solvent because it has low toxicity and is derived from agricultural waste. Changing the solvent to 2-MeTHF gave yields that were comparable to the yields afforded when using dioxane or toluene as solvents.

The successful formation of the product was confirmed using ¹H and ¹³C NMR spectroscopy. The presence of the pyrimidine ring was confirmed by singlet proton signals appearing at 8.34 ppm and 6.34 ppm due to H2 and H5, respectively. Protons due to the cyclopropanemethylamine moiety appeared at 5.34, 3.15, 1.08, 0.61, and 0.28 ppm due to NH, H7, H8, H9 and H9', respectively, with H8 and H9 signals appearing as multiplets. The carbon NMR spectrum showed a total of 7 signals typical of the product with a signal due to C6 and C4 appearing at 163.1 ppm and a signal due to C2 appearing at 158.5 ppm. Carbon signals due to C7, C8, and C9 were seen at 46.5, 10.4 and 3.6 ppm. Furthermore, infrared spectrum of the product has a signal due to the N-H bond at 3314 cm⁻¹.

4.1.2 Synthesis of 6-chloro-*N*-cyclohexylpyrimidin-4-amine [2]

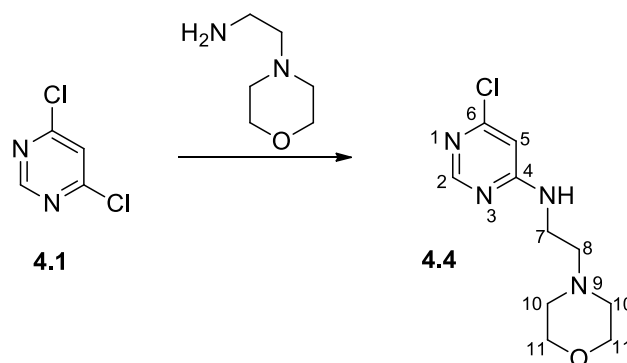


Scheme 4.3. Preparation of 6-chloro-*N*-cyclohexylpyrimidin-4-amine. Regents and conditions: DIPEA, 2-MeTHF, 80°C, 18 hours, 84%.

The cyclohexyl containing compound **4.3** was afforded in a similar manner described for **4.2** but using cyclohexylamine. The reaction mixture consisting of **4.1**, cyclohexylamine, DIPEA, and 2-MeTHF heated for 18 hours afforded the desired product **4.3** as a white solid in 84% yield after purification (**Scheme 4.3**).

The presence of the pyrimidine core was identified by proton signals with a singlet multiplicity appearing at 8.32 ppm and 6.31 ppm resulting from H2 and H5, respectively. The amine proton appeared at 5.25 ppm and H10 appeared between 1.31-1.16 ppm overlapping with H9. The remaining cyclohexane protons appeared as either equatorial or axial resulting in two signals for one chemical environment. Signals due to H8 appeared at 2.01 ppm and 1.78 ppm as doublets of triplets, and H9 signals appeared between 1.47-1.34 ppm and 1.31-1.16 ppm as multipeaks. The carbon NMR spectrum had 4 peaks appearing in the aliphatic region corresponding to the cyclohexane moiety. The remaining two signals were in the aromatic region at 162.4 ppm due to C4 and C6, while C2 appeared as a peak at 158.5 ppm. The formation of the product **4.3** was further confirmed by infrared spectroscopy via the signal appearing at 3242 cm⁻¹ due to N-H.

4.1.3 Synthesis of 6-chloro-*N*-(2-morpholinoethyl)pyrimidin-4-amine [3]

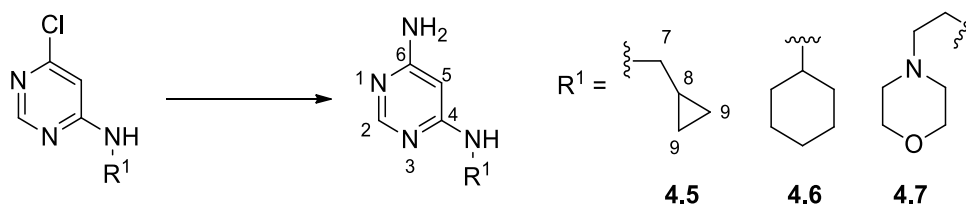


Scheme 4.4. Preparation of 6-chloro-*N*-(2-morpholinoethyl)pyrimidin-4-amine. Regents and conditions: DIPEA, 2-MeTHF, 80°C, 18 hours, 84%

The morpholine bearing intermediate **4.4** was afforded from a reaction of **4.1** and 4-(2-aminoethyl)morpholine mediated by DIPEA in 2-MeTHF (**Scheme 4.4**). After heating for 18 hours the solvent was concentrated, and the resulting crude residue was diluted with water. The product was extracted in ethyl acetate and purified using silica gel chromatography to afford the product as a white solid in 97% yield.

The proton signals due to H2 and H5 as singlets appearing at 8.35 ppm and 6.38 ppm in the ^1H NMR spectrum confirmed the presence of the pyrimidine moiety on the product. The aminoethylmorpholine moiety was confirmed by a singlet peak at 5.83 ppm due to NH, a triplet at 3.73 ppm due to H11, a singlet at 3.39 ppm due to H7, a triplet at 2.62 ppm due to H8, and another triplet at 2.49 ppm due to H10. The carbon NMR spectrum had the characteristic peaks in the aromatic region due to the pyrimidine core, and 4 peaks in the aliphatic region corresponding to the ethylmorpholine moiety. Carbon NMR spectroscopic signals due to the ethyl C7 and C8 appeared at 37.3 ppm and 56.3 ppm respectively, while C10 and C11 appeared at 53.2 ppm and 66.8 ppm, respectively. The presence of the aminoethylmorpholine moiety was further confirmed by an infrared spectroscopic signal due to N-H appearing at 3297 cm^{-1} .

4.1.4 Synthesis of *N*⁴-(cyclopropylmethyl)pyrimidine-4,6-diamine and related compounds [4]



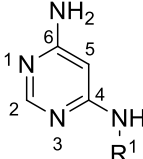
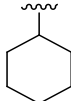
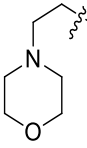
Scheme 4.5. Preparation of *N*⁴-substitutedpyrimidine-4,6-diamine compounds. Reagents and conditions: 25% aq. NH_3 , ethanol, 170°C , 48 hrs, 54-92%.

The second step in the synthesis of 4,6-diaminopyrimidine derivatives was to displace the second chloro substituent with ammonia to afford a 4,6-diaminopyrimidine scaffold (**Scheme 4.5**). To achieve this, we dissolved the starting materials in ethanol in a pressure tube and added excess aqueous ammonia. The reaction was heated at 170°C for 48 hours to afford the products after workup and purification. The product **4.5**, was afforded starting from intermediate **4.2**. After heating for 48 hours the solvent was concentrated *in vacuo* and the afforded crude product was diluted with water. The acidic solution was neutralised using potassium carbonate and the product was extracted with ethyl acetate. Silica gel column chromatography was used to purify the product affording a white solid in 68% yield.

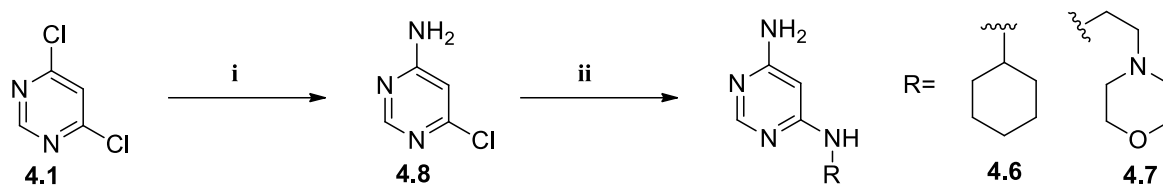
Key signals identifying the product **4.5** from the ^1H NMR spectrum were those due to the newly installed NH_2 substituent appearing at 4.61 ppm, and H5 appearing at an upfield chemical shift of 5.38 ppm. A marked change was observed in the aromatic region of the ^{13}C NMR spectrum with a total of 4 signals visible. These signals are due to C6, C4, C2, and C5 at 163.0, 162.9, 158.3, 81.9 ppm respectively. Substituting chlorine with an amine caused a slight downfield shift of C6. Other proton and carbon spectroscopic signals present in the product maintained roughly the same chemical shift as in the starting material. The infrared spectrum had two signals due to NH at 3464, and 3306 cm^{-1} indicating the presence of more than one NH on the product or at least one NH_2 . A molecular ion $\text{C}_8\text{H}_{13}\text{N}_4^+$, due to the product was confirmed by HRMS to be 165.1051 consistent with the theoretical value of 165.1032 for the same molecular ion.

Compounds **4.6**, and **4.7** were afforded using the same synthetic protocol as in the synthesis of **4.5**, however, starting from the appropriate intermediates. The key ^1H and ^{13}C NMR signals identifying the afforded products are presented in **Table 4.1** along with the melting points and yields.

Table 4.1. Key NMR spectroscopic signal and yield corresponding to the products **4.6** and **4.7**.

				
Entry	R ¹	Key NMR signals /ppm	Mp. (°C)	Yield
1	 4.6	^1H : δ 5.38 (s, 1H, H5), 4.58 (s, 2H, NH_2), ^{13}C : δ 162.7(C6), 161.9(C4), 158.0 (C2), 53.4 (C5).	210–211	92%
2	 4.7	^1H : δ 5.39 (s, 1H, H5), 4.83 (s, 2H, NH_2). ^{13}C : δ 163.2 (C6), 162.9 (C4), 158.8 (C2), 82.2 (C5).	155–157	54%

4.1.5 Alternative synthesis of *N*⁴-substituted pyrimidine-4,6-diamine compounds [5]



Scheme 4.6. Alternative synthesis of *N*⁴-substituted pyrimidine-4,6-diamine intermediates. Reagents and conditions: **i**) isopropyl alcohol, aq. ammonia, rt, 1hr, 70%. **ii**) alkylamine, toluene, 200°C MWI (150 W), 1 hour.

An alternative approach to the synthesis of *N*⁴-substituted-pyrimidine-4,6-diamine is by introducing the amine first, followed by an NHR¹, in the opposite order to how we have described the synthesis of the target intermediates so far (**Scheme 4.6**). We explored this approach because we wanted to reduce the use of large quantities of solvent and reaction time by using microwave irradiation, and the number of synthetic steps, as all products could be derived from the same **4.8** intermediate (chloro amino pyrimidine). Using microwave irradiation while having aqueous ammonia as a reagent could potentially lead to explosions due to rapid increase in pressure, as the temperature of the reaction rise while heating. However, with reagents such as cyclopropylmethylamine hydrochloride, 4-(2-aminoethyl)morpholine, or cyclohexylamine, a rapid pressure build-up is not an issue because they have high boiling points (>180°C).

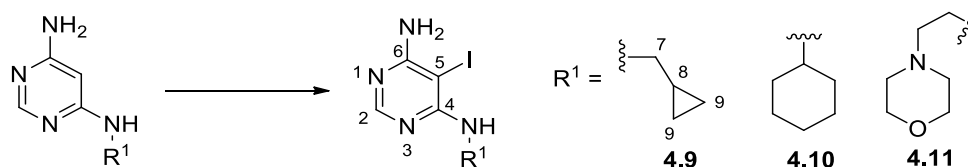
6-Chloropyrimidin-4-amine **4.8** was afforded using a similar synthetic procedure employed by Zhan and coworkers where they dissolved the substrate **4.1** in isopropyl alcohol, added aqueous ammonia, and stirred the resulting solution at room temperature for one hour [5]. We followed the procedure, however with heating at 100°C for 1 hour to afford **4.8** as a white solid in 70% yield after isolation by filtration. When the reaction was done at room temperature, we recovered unreacted starting material after an hour of stirring. The formation of the product was confirmed using ¹H NMR spectroscopy and the key signal in the spectrum was that due to NH₂ appearing at 7.24 ppm. A key signal in the ¹³C NMR spectrum is that due to C5, appearing upfield at 103.14 ppm as expected. The product **4.8** melts at 204-207°C which is higher than the melting point of **4.1** (65-67°C), indicating that a different molecule had formed from the starting substrate.

From this point we could introduce the appropriate alkylamine by heating **4.8** in the presence of the amine precursor at high temperatures, similar to how we introduced the alkylamines at the C6 position previously. This approach has been employed by Whitehead et al. when they were synthesising potential diuretics and it afforded products with yields ranging from 13% to 99% [6]. We used the same methodology, however with microwave irradiation. We loaded a microwave vessel with the substrate **4.8**, two molar equivalents of the alkylamine precursors, and small

quantities of toluene. The mixture was then heated at 200°C for 1 hour at which point no further conversion occurred; heating further than 1 hour did not afford higher yields.

The reaction between **4.8** and cyclohexylamine afforded the product **4.6** in 69% yield after purification. A reaction between **4.8** and 4-(2-aminoethyl)morpholine afforded the product **4.7** as a white solid in 92% yield following purification by recrystallisation. Unfortunately, at a time when this synthesis was tested, we did not have cyclopropanemethylamine hydrochloride at hand to evaluate the protocol on all our targeted NHR¹ groups. The synthetic approach serves as a good alternative to the synthesis of *N*⁴-substituted pyrimidine-4,6-diamine derivatives, offering advantages such as reduced overall reaction time and synthetic steps, and minimal solvent use.

4.1.6 Synthesis of *N*⁴-(cyclopropylmethyl)-5-iodopyrimidine-4,6-diamine and related compounds



Scheme 4.7. Iodination of *N*⁴-substituted-5-pyrimidine-4,6-diamine compounds. Reagents and conditions: I₂, K₂CO₃, DMF/water (1:7), MWI, 60°C, 0.5 hrs, 50%-96%.

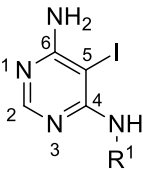
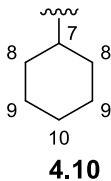
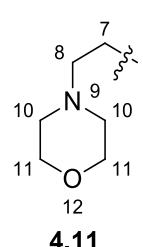
The afforded intermediates **4.5**, **4.6**, and **4.7** were iodinated in the C5 position to prepare them for the introduction of large aryl groups via the Sonogashira coupling reaction (**Scheme 4.7**). To a mixture of the starting material, molecular iodine, and potassium carbonate was added water and DMF. The solvent combination is used to ensure that both the substrate and the reagents go into solution. The resulting mixture was heated for 4 hours at 45°C before cooling down to room temperature and diluting with water. The excess iodine was quenched by adding small amounts of sodium thiosulfate with stirring until no further colour change was observed. During the quenching process, a precipitate formed that was collected by filtration to afford the products. We later modified the synthetic protocol by using microwave irradiation heating at 60°C for 1 hour. The methodology returned yields that were comparable or, as was observed with most cases, improved. We, therefore, found the microwave assisted synthesis of *N*⁴-substituted-5-iodopyrimidine-4,6-diamine to be a better approach than the conventional method.

The synthesis of *N*⁴-(cyclopropylmethyl)-5-iodopyrimidine-4,6-diamine **4.9**, followed the procedure as described starting from intermediate **4.5** to afford the product as a yellow solid in 62% yield by conventional methodology and 75% yield by microwave assisted synthesis. Key changes identifying the success of the reaction in the ¹H NMR spectrum was the absence of the

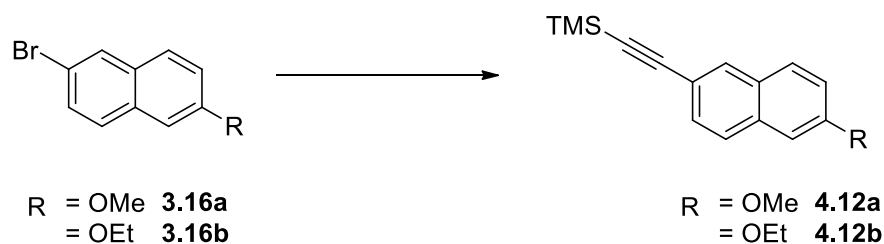
signal that was previously due to H5. We also noted that the carbon signal due to C5 in the ^{13}C NMR spectrum move further upfield to 58.2 ppm. All other proton and carbon spectroscopic signals on the products NMR spectra appeared as observed on the starting substrate **4.5**.

The products **4.10** and **4.11** were afforded in a similar manner starting with the appropriate substrates – **4.6** and **4.7**, respectively. The NMR spectroscopic signals identifying the different structural components making up the products and HRMS spectrometric data are presented in **Table 4.2** along with the yield afforded by conventional (conv.) and microwave (mw) method.

Table 4.2. Key NMR spectroscopic signals, HRMS molecular ion m/z , and yields corresponding to the products **4.10** and **4.11**

			
Entry	R ¹	Characteristic NMR signals /ppm and HRMS	Yield
1	 4.10	^1H: δ 7.98 (s, 1H, H2), 5.12 (s, 2H, NH₂), 4.98 (d, J = 8.0 Hz, 1H, NH), 4.03 – 3.89 (m, 1H, H7), 2.07 – 1.96 (m, 2H, H8), 1.79 – 1.70 (m, 2H, H9), 1.69 – 1.60 (m, 1H, H10), 1.50 – 1.37 (m, 2H, H9), 1.30 – 1.17 (m, 3H, H8 and H10). ^{13}C: δ 161.54 (C6), 160.0 (C4), 157.3 (C2), 58.5 (C5), 50.1 (C7), 33.3 (C8), 25.7 (C10), 24.8 (C9). HRMS [C₁₀H₁₆IN₄]⁺: Found 319.0220; calculated 319.0312.	mw: 96% conv.: 51%
2	 4.11	^1H: δ 7.99 (s, 1H, H2), 5.94 (s, 1H, NH), 5.00 (s, 2H, NH₂), 3.76 (t, J = 4.5 Hz, 4H, H11), 3.52 (q, J = 5.6 Hz, 2H, H7), 2.62 (t, J = 6.1 Hz, 2H, H8), 2.52 (t, J = 4.6 Hz, 4H, H10). ^{13}C: δ 161.5 (C6), 160.8 (C4), 157.5 (C2), 67.2 (C11), 58.2 (C5), 56.4 (C8), 53.1 (C10), 38.0 (C7). HRMS [C₁₀H₁₇IN₅O]⁺: Found 350.0258; calculated 350.0370.	mw: 50% conv.: 65%

4.1.7 Synthesis of [(6-methoxynaphthalen-2-yl)ethynyl]trimethylsilane and [(6-ethoxynaphthalen-2-yl)ethynyl]trimethylsilane [7, 8]



Scheme 4.8. Sonogashira coupling reaction. Reagents and conditions: TMSA, Pd(PPh₃)₂Cl₂, CuI, DIPA, 91% -97%.

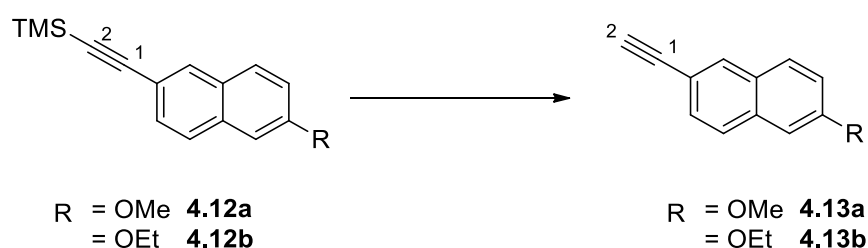
Thus far, we have described the synthesis of intermediates that are made up of the diaminopyrimidine scaffold iodinated at the C5 position – and substituted with R¹ substituents. What remains to afford the first class of 4,6-diaminopyrimidine inhibitors (acetylene linkage derivatives) is to incorporate the bulky R² groups using the Sonogashira coupling reaction. The bulky R² groups that we plan to incorporate are substituted naphthalenes, and in order to couple these to the pyrimidine core via a two-atom linker, we needed to first substitute these with an acetylene moiety. These terminal alkene precursors needed for the Sonogashira coupling reaction were not commercially available, therefore, we prepared them from intermediates **3.16a** and **3.16b** whose synthesis was described in chapter 3 (**Scheme 4.8**). The mixture of the intermediate, Pd(PPh₃)₂Cl₂, and copper (I) iodide was placed in an inert atmosphere before adding a degassed solution of diisopropylamine (DIPA) and trimethylsilylacetylene (TMSA) in dry THF. The resulting solution was heated for 5 hours at 60°C followed by work up and purification to afford products, **4.12a** and **4.12b**, in excellent yields of 95% and 91%, respectively.

We later modified the synthetic protocol by using microwave irradiation (MWI) to heat the reaction. As is typical of MWI reactions, we appreciated reduced reaction times and used smaller volumes of solvent and fewer equivalents of some reagents. The reaction was done for 30 minutes with heating at 70°C in 2-MeTHF, with reduced amounts of the base DIPA and the substrate TMSA (~2 molar equiv. each). The conventional Sonogashira coupling reaction protocol requires that DIPA and TMSA be added in excess; in our case we used 10 molar equivalents for each. After quenching the reaction with saturated aqueous ammonia solution and purification, the products **4.12a** and **4.12b** were achieved in excellent yields of 97% and 95%, respectively. We, therefore, found the MWI reaction to be a better approach in the Sonogashira coupling of our substrates.

The afforded products were accompanied by a signal at ~0.00 ppm integrating for 9 protons due to the trimethyl groups (TMS), in addition to the 6 aromatic protons appearing between 7.92 ppm

and 7.08 ppm in the ^1H NMR spectra. The product **4.12a** was further characterized by a singlet signal at 3.92 ppm due to the methoxy CH_3 . For product **4.12b** these unique signals appear at 4.14 ppm and 1.48 ppm as a quartet and triplet due to the ethoxy CH_2 and CH_3 , respectively. The ^{13}C NMR spectra of both products had three additional signals compared to the respective starting substrates due to the trimethylsilylacetylene moiety of the products. HRMS confirmed the molecular ion of **4.12a** ($\text{C}_{16}\text{H}_{19}\text{OSi}^+$) to be 255.1130 consistent with the calculated value of 255.1127, while the molecular ion of **4.12b** ($\text{C}_{17}\text{H}_{21}\text{OSi}^+$) was confirmed to be 296.1122, corresponding to 269.1129.

4.1.8 Synthesis of 2-ethynyl-6-methoxynaphthalene and 2-ethoxy-6-ethynylnaphthalene [8]

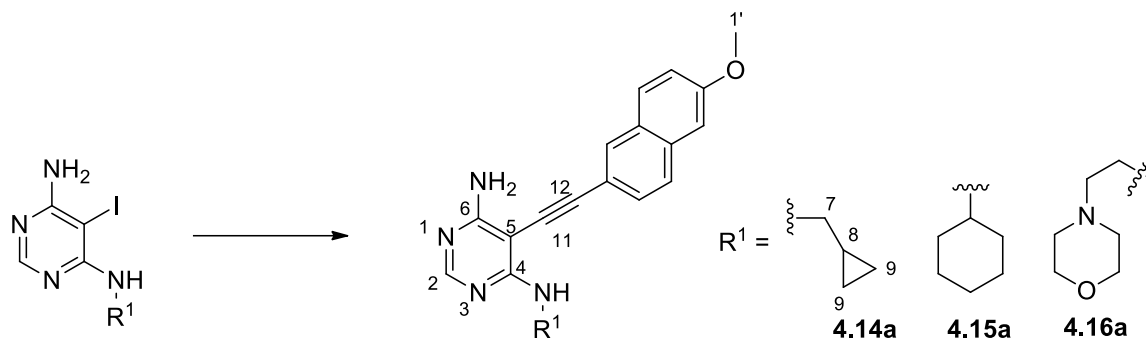


Scheme 4.9. Formation of the terminal alkene. Reagent and conditions: K_2CO_3 , MeOH, 1 hr, 74%-98%.

We completed the preparation of the naphthalene intermediate required to form class I compounds by deprotecting the silyl group (**Scheme 4.9**). This was achieved by employing two synthetic protocols which differ in the base and solvent used. The first protocol involved the use of tetrabutylammonium fluoride with THF as a solvent. To a mixture of the substrate and dry THF was added tetrabutylammonium fluoride at -78°C followed by stirring overnight at room temperature. The reaction was quenched using saturated aqueous ammonium chloride solution and afforded the deprotected products as white solids after purification in moderate yields (85% for **4.13a** and 74% for **4.13b**). The second procedure employed potassium carbonate and methanol. The reaction mixture was stirred at room temperature for 1 hour to afford the products, **4.13a** and **4.13b** in yields of 93% and 98%, respectively. Evidently, using potassium carbonate as the base and methanol as the solvent afforded higher yields in significantly reduced time.

The products were characterised by a singlet peak appearing at 3.10 ppm due to H_2 in the ^1H NMR spectrum of each product. The signals that were due to the TMS group were absent on both the ^{13}C and ^1H NMR spectra. There was an observable shift in the position of the signal caused by C2 upfield to ~ 84.2 ppm for both products indicating a change in the acetylene environment.

4.1.9 Synthesis of *N*⁴-(cyclopropylmethyl)-5-[(6-methoxynaphthalen-2-yl)ethynyl]pyrimidine-4,6-diamine and related compounds (class I compounds)



Scheme 4.10. Preparation of acetylene linkage 4,6-diaminopyrimidine derivatives (class I compounds) from *N*⁴-substituted-5-iodopyrimidine-4,6-diamine. Reagent and conditions: Pd(PPh₃)₂Cl₂, CuI, DIPA, 2-MeTHF, 70°C, 1 hr.

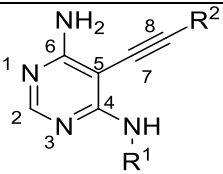
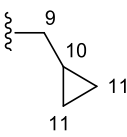
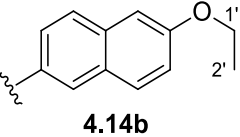
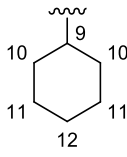
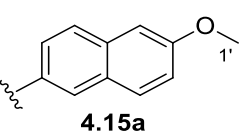
At this point of the synthesis, we had in hand the precursors needed to afford the first class (class I) of the targeted two atom linked 4,6-diaminopyrimidine derived compounds. We, therefore, employed the Sonogashira coupling reaction to afford the desired compounds (**Scheme 4.10**) utilising MWI heating, as we found the approach to be economical and better yielding. The synthesis of targeted compound **4.14a** proceeded by heating a mixture of the substrate, dichlorobis(triphenylphosphine)palladium(II), copper(I) iodide, and diisopropylamine in 2-MeTHF for 60 minutes. The product was afforded after silica gel chromatography purification in 80% yield.

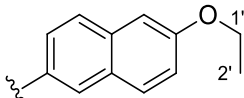
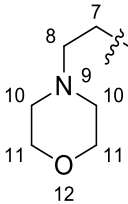
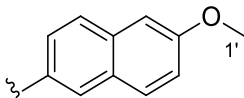
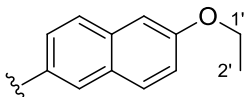
Both the ¹H and ¹³C NMR spectroscopy were used to characterise the product. The presence of the aminopyrimidine ring was confirmed by two singlet signals appearing at 8.15 and 5.18 ppm due to H2 and NH₂, respectively, in the ¹H NMR spectrum. The methylcyclopropane moiety was confirmed by proton signals at 3.39, 1.13, 0.57 and 0.30 ppm due to H7, H8, H9 and H9' respectively. The naphthalene moiety was confirmed by aromatic protons signals appearing between 7.96 ppm and 7.12 ppm and a singlet signal due to the methoxy protons (H1') at 3.96 ppm. The ¹³C NMR spectrum confirmed the presence of the pyrimidine ring via the C2 signal at 156.78 ppm, the naphthalene moiety via the C1' signal at 55.4 ppm and the acetylene bridge by signals appearing at 101.2 ppm and 79.5 ppm due to H12 and H11. The molecular ion of **4.14a** (C₂₁H₂₁N₄O⁺) was confirmed by HRMS to be 345.1711 corresponding to the calculated value of 345.1710 for the same molecular ion.

Other derivatives bearing the cyclopropyl, cyclohexyl, and morpholino groups with either the methoxynaphthalene or ethoxynaphthalene moiety were prepared in a similar manner starting with the appropriate substrates. Key spectroscopic signals (¹H and ¹³C NMR) confirming the presence

of the aminopyrimidine core, the R¹ substituent and the naphthalene moiety for each attained product as discussed for **4.14a**, including HRMS m/z ratios and the respective yields, are presented in **Table 4.3**.

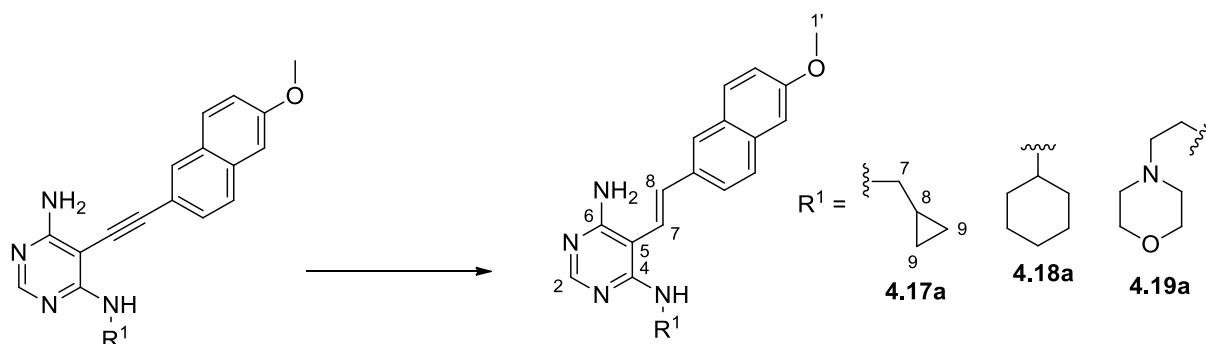
Table 4.3. ¹H and ¹³C NMR spectroscopic signals, HRMS mass to charge ratios and yields of acetylene-linked 4,6-diaminopyrimidines.

				
Entry	R ¹	R ²	Key NMR signals /ppm and HRMS	Yield
1		 4.14b	¹ H: δ 8.15 (s, 1H, H2), 7.95 (s, 1H, ArH), 7.72 (t, <i>J</i> = 7.9 Hz, 2H, ArH), 7.50 (d, <i>J</i> = 8.6 Hz, 1H, ArH), 7.19 (d, <i>J</i> = 9.2 Hz, 1H, ArH), 7.12 (s, 1H, ArH), 5.18 (s, 2H, NH ₂), 4.17 (q, <i>J</i> = 7.2 Hz, 2H, H1'), 3.39 (t, <i>J</i> = 6.4 Hz, 2H, H9), 1.50 (t, <i>J</i> = 7.2 Hz, 3H, H2'), 1.13 (p, <i>J</i> = 6.7, 6.2 Hz, 1H, H9), 0.57 (d, <i>J</i> = 7.7 Hz, 2H, H11), 0.30 (d, <i>J</i> = 4.9 Hz, 2H, H11) ¹³ C: δ 156.8 (C2), 102.2 (C8) 79.5 (C7), 63.6 (C1'), 14.8 (C2'). HRMS [C ₂₂ H ₂₃ N ₄ O] ⁺ : Found 359.1866; calculated 359.1866.	83%
2		 4.15a	¹ H: δ 8.15 (s, 1H, H2), 7.94 (s, 1H, ArH), 7.74 (d, <i>J</i> = 1.4 Hz, 1H, ArH), 7.72 (s, 1H, ArH), 7.50 (dd, <i>J</i> = 8.4, 1.7 Hz, 1H, ArH), 7.19 (dd, <i>J</i> = 8.9, 2.5 Hz, 1H, ArH), 7.13 (d, <i>J</i> = 2.5 Hz, 1H, ArH), 5.24 (d, <i>J</i> = 8.2 Hz, 1H, NH), 5.17 (s, 2H, NH ₂), 4.10 – 3.98 (m, 1H, H9), 3.94 (s, 3H, H1'), 2.11 – 2.02 (m, 2H, H10), 1.81 – 1.71 (m, 2H, H11), 1.69 – 1.60 (m, 1H, H12), 1.53 – 1.37 (m, 2H, H11), 1.32 – 1.20 (m, 3H, H12 & H10).	85%

			^{13}C : δ 156.8 (C2), 102.2 (C8), 79.6 (C5 & C7), 55.4 (C1'), HRMS $[\text{C}_{23}\text{H}_{25}\text{N}_4\text{O}]^+$: Found 373.2024; calculated 373.2023.	
3		 4.15b	^1H : δ 8.15 (s, 1H, H2), 7.96 – 7.86 (m, 1H, ArH), 7.76 – 7.67 (m, 2H, ArH), 7.49 (dd, J = 8.4, 1.7 Hz, 1H, ArH), 7.18 (dd, J = 8.9, 2.4 Hz, 1H, ArH), 7.12 (d, J = 2.5 Hz, 1H, ArH), 5.19 (s, 2H, NH_2), 4.17 (q, J = 7.0 Hz, 2H, H1'), 4.10 – 3.98 (m, 1H, H9), 2.10 – 2.02 (m, 2H, H9), 1.76 (dt, J = 13.5, 3.9 Hz, 2H, H11), 1.65 (d, J = 17.1 Hz, 1H, H12), 1.52 – 1.38 (m, 5H, H2' & H11), 1.33 – 1.17 (m, 3H, H10 & H12). ^{13}C : δ 156.7 (C2), 102.3 (C8), 80.5 (C7), 79.5 (C5), 63.6 (C1'), 14.8 (C2') HRMS $[\text{C}_{24}\text{H}_{27}\text{N}_4\text{O}]^+$: Found 387.2182; calculated 387.2179.	90%
4		 4.16a	^1H : δ 8.17 (s, 1H, H2), 7.95 (s, 1H, ArH), 7.72 (t, J = 9.1 Hz, 2H, ArH), 7.53 (d, J = 8.4 Hz, 1H, ArH), 7.19 (d, J = 9.1 Hz, 1H, ArH), 7.14 (s, 1H, ArH), 6.22 (s, 1H, NH), 5.32 (s, 2H, NH_2), 3.95 (s, 3H, H1'), 3.70 (s, 4H, H11), 3.60 (s, 2H, H8), 2.69 – 2.60 (m, 2H, H11), 2.53 (s, 4H, H10) HRMS $[\text{C}_{23}\text{H}_{26}\text{N}_5\text{O}_2]^+$: Found 404.2084; calculated 404.2081.	79%
5		 4.16b	^1H : δ 8.16 (s, 1H, H2), 7.95 (s, 1H, ArH), 7.71 (d, J = 8.6 Hz, 2H, ArH), 7.51 (dt, J = 8.6, 2.0 Hz, 1H, ArH), 7.19 (dd, J = 9.0, 2.4 Hz, 1H, ArH), 7.12 (d, J = 2.5 Hz, 1H, ArH), 6.20 (s, 1H, NH), 5.23 (s, 2H, NH_2), 4.17 (q, J = 7.1 Hz, 2H, H1'), 3.71 (t, J = 4.7 Hz, 4H,	83%

			H11), 3.62 (d, $J = 5.8$ Hz, 2H, H8), 2.67 (t, $J = 6.3$ Hz, 2H, H7), 2.55 (s, 4H, H10), 1.49 (td, $J = 7.0, 2.8$ Hz, 3H, H2'). ^{13}C : δ 134.3 (C2), 63.6 (C1'), 56.9 (C7), 53.3 (C10), 37.0 (C8), 14.8 (C2'). HRMS $[\text{C}_{24}\text{H}_{28}\text{N}_5\text{O}_2]^+$: Found 418.2239; calculated 418.2238.	
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4.1.10 Synthesis of (*E*)-*N*⁴-(cyclopropylmethyl)-5-(2-(6-methoxynaphthalen-2-yl)vinyl)pyrimidine-4,6-diamine and related compounds



Scheme 4.11. Preparation of ethene linked 4,6-diaminopyrimidine derivatives (class II compounds) from class I compounds. Reagent and conditions: $\text{H}_2(\text{g})$ -6 bar, Pd/C catalyst, ethanol, r.t., 6 hours, 50-77%.

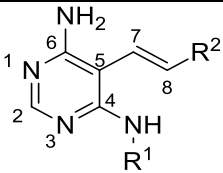
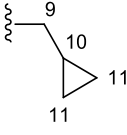
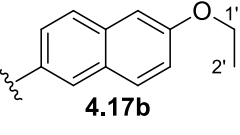
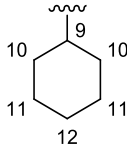
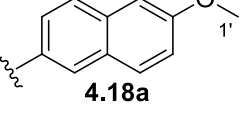
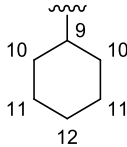
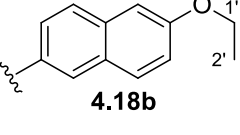
We planned to afford 4,6-diaminopyrimidine derivatives with partially unsaturated and saturated linkers (class II and class III compounds) by reducing class I compounds. We envisioned forming the fully reduced compounds (class III) using hydrogen gas in the presence of a palladium on carbon catalyst. However, to our surprise, these conditions afforded the alkenes (class II compounds) (**Scheme 4.11**). The reaction was carried out by dissolving the substrates in absolute ethanol and then adding 10 wt.% palladium on carbon. To the mixture was added hydrogen gas contained in an inflated balloon while stirring overnight. Analysis of the reaction using TLC revealed that no product had formed, and that the starting material was still present. We then decided to conduct the reaction at the higher pressure in a pressure vessel to encourage the reaction to take place. The strategy afforded the alkene compounds in moderate to good yields when 6 bar hydrogen gas pressure was applied for 6 hours.

The synthesis of **4.17a** was carried out described starting from intermediate **4.15a** to afford the product as a brown solid in 77% yield. The product was confirmed using ^1H , ^{13}C NMR spectroscopy and mass spectrometry. Key spectroscopic signals appeared at 6.93 ppm and 6.20

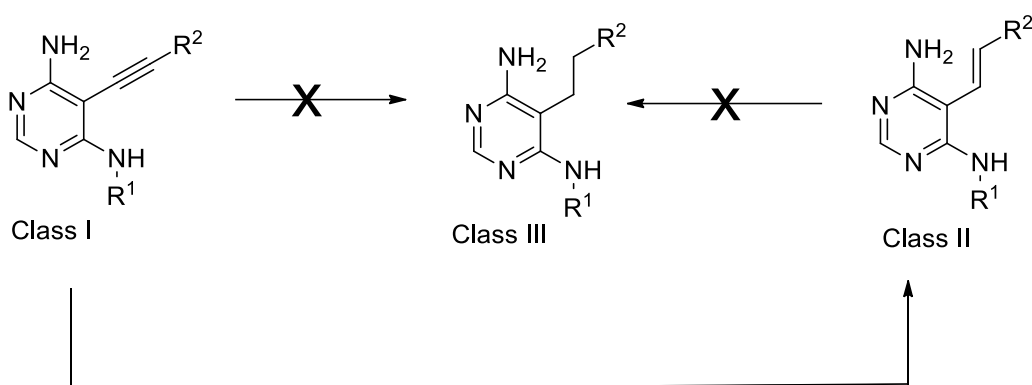
ppm due to H7 and H8 respectively on the proton NMR spectrum. The coupling constants of these peaks (11.9 Hz) indicate that the ethene assumed an entgegen (*E*) geometry. Key ^{13}C NMR spectroscopic signals are those due to C7 and C8 now appearing downfield at 134.9 ppm and 118.6 ppm. HRMS confirmed the molecular ion of **4.17a**, $\text{C}_{21}\text{H}_{23}\text{N}_4\text{O}^+$, to be 347.1868 which is consistent with the theoretical value of 347.1566 calculated for the same molecular ion.

Related compounds **4.17b**, **4.18a**, and **4.18b** were synthesized using the same high-pressure protocol. Key spectroscopic signals confirming the success of the reaction and the yield for each product are presented in **Table 4.4**.

Table 4.4. Yields, key ^1H and ^{13}C NMR spectroscopic signals and HRMS mass to charge ratios of ethene-linked 4,6-diaminopyrimidine compounds.

				
Entry	R ¹	R ²	Key NMR spectroscopic signals /ppm and HRMS molecular ion ratio	Yield
1		 4.17b	^1H : δ 6.94 (d, J = 11.9 Hz, 1H, H7), 6.19 (d, J = 11.9 Hz, 1H, H8). ^{13}C : δ 135.1 (C7), 118.3 (C8). HRMS $[\text{C}_{22}\text{H}_{25}\text{N}_4\text{O}]^+$: Found 361.2024; calculated 361.2023.	68%
2		 4.18a	^1H : δ 6.92 (d, J = 11.9 Hz, 1H, H7), 6.15 (d, J = 11.9 Hz, 1H, H8). ^{13}C : δ 135.2 (C7), 118.5 (C8). HRMS $[\text{C}_{23}\text{H}_{27}\text{N}_4\text{O}]^+$: Found 375.2180; calculated 375.2179	50%
3		 4.18b	^1H : δ 6.92 (d, J = 11.9 Hz, 1H, H7), 6.14 (d, J = 11.9 Hz, 1H, H8). ^{13}C : δ 135.2 (C7), 118.4 (C8). HRMS $[\text{C}_{24}\text{H}_{29}\text{N}_4\text{O}]^+$: Found 389.2338; calculated 389.2297.	63%

4.1.11 Synthesis of ethane-linked 4,6-diaminopyrimidines derivatives



Scheme 4.12: Attempted synthesis of ethane-linked 4,6-diaminopyrimidines from either class I or class II compounds. Reagents and conditions: 6 bar $\text{H}_2(\text{g})$, Pd/C, absolute ethanol, rt., 48 hours.

We had planned to achieve the synthesis of ethane-linked 4,6-diaminopyrimidine derivatives by reducing either the acetylene- or ethene-linked compounds (**Scheme 4.12**). The starting compounds were dissolved in absolute ethanol and the resulting solution was loaded into a hydrogenation reactor. Hydrogen gas was then added at 6 bar pressure while stirring at room temperature for 24 hours. The reaction did not occur when ethene-linked compounds (class II) were used as substrates; the starting substrate was recovered. However, when acetylene-linked compounds (class I) were used as substrates, the reaction afforded class II compounds. We then decided to extend the duration to 48 hours; the results were the same as when the reaction was done for 24 hours. Thin layer chromatography did not show any indication of a different product forming, as a result we decided not to extend the duration of the reaction further than 48 hours.

We hypothesised that steric hindrance may be the primary cause of reaction failure. The conformation adopted by the ethene-linked molecules is such that the linker moiety is moved closer to the pyrimidine and R^1 groups (**Figure 4.3**), making it difficult for the alkene moiety to be adsorbed on the surface of the palladium metal for catalysed reduction to occur. Due to time constraints, other reduction methods were not considered for this project, but will be considered for future work.

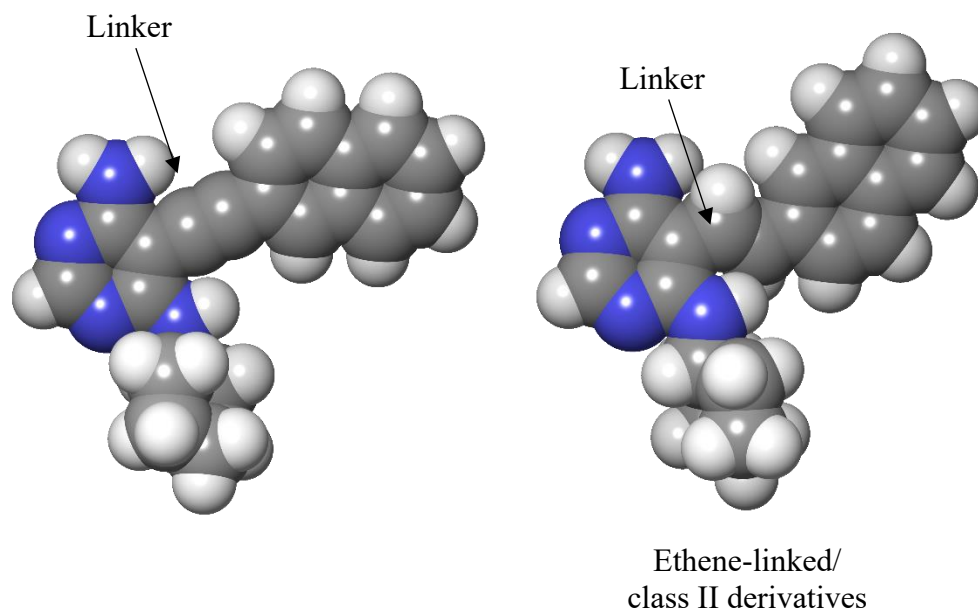


Figure 4.3. 3D model of acetylene- and ethene bridged molecules.

4.2 Preparation of one-atom linkage 4,6-diaminopyrimidine derivatives: carbon-atom linker compounds

As part of our project aim, we decide to explore one-atom linkage derivatives with carbon atom as a linker between the pyrimidine moiety and the bulky R^2 moiety (**Figure 4.4**). These derivatives are structurally different to the two-atom linker compounds by 1) the number of atoms between the pyrimidine moiety and the naphthalene moiety, and 2) the presentation of the naphthalene group relative to the pyrimidine moiety. The different conformations ought to afford unique interactions in the binding sites of the target enzymes, *Pf*CDPK1 and *Pf*CDPK4.

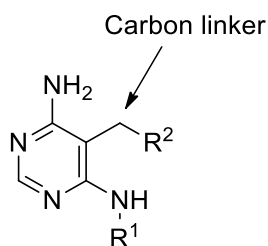
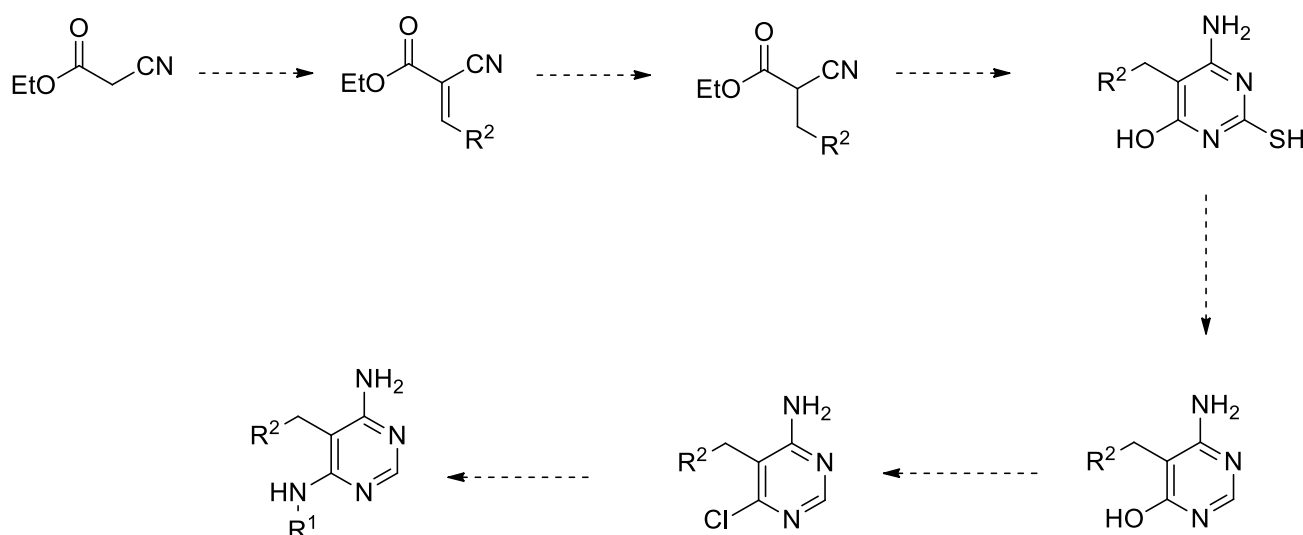


Figure 4.4. General structure of the one carbon-linker 4,6-diaminopyrimidine derivatives.

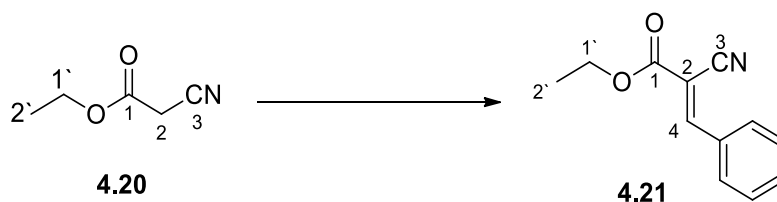
The general approach adopted thus far in the synthesis of our targeted inhibitors has been to prepare the pyrimidine scaffold on which the substituents necessary for potency and selectivity are added. However, with the one carbon-atom linked 4,6-diaminopyrimidine derivatives, we planned to form the pyrimidine ring with the R^2 substituent already in place. We planned to achieve this by adding R^2 groups on ethyl cyanoacetate followed by ring formation between the afforded intermediate and thiourea (**Scheme 4.13**). The resulting molecule would be further modified to afford our target

molecules by removing the thiol and substituting the hydroxy moiety with a chloride to enable the addition of R¹ groups. To test the validity of the proposed synthetic route and synthetic protocols thereof, we used a phenyl moiety at R² in place of the naphthalene groups, as the precursors we would use are commercially available. The development of the synthetic protocols towards the synthesis of the one carbon-linker 4,6-diaminopyrimidine compounds using our model system bearing a phenyl substituent at R², is described in the following sections.



Scheme 4.13: Proposed synthesis of carbon atom-linker 4,6-diaminopyrimidine derivatives.

4.2.1 Synthesis of ethyl (*E*)-ethyl 2-cyano-3-phenylacrylate [9]



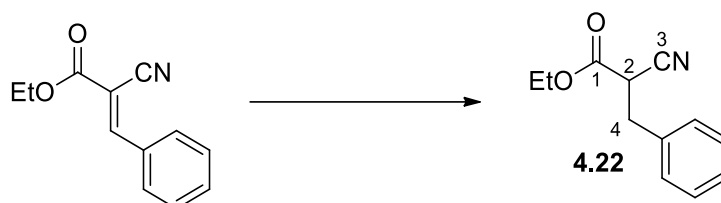
Scheme 4.14. Preparation of ethyl 2-cyano-3-phenylacrylate. Reagents and conditions: benzaldehyde, piperidine, MWI (10 W), 10 minutes, 98%.

The addition of the phenyl group into the C2 position of ethyl cyanoacetate **4.20** was accomplished through two synthetic steps. The first step utilises the Knoevenagel condensation reaction between **4.20** and benzaldehyde (**Scheme 4.14**). Initially, the substrate and reagent were heated for 2 hours in ethanol using microwave irradiation (MWI) to afford the product **4.21** as a white crystalline solid after recrystallisation in yield of 92%. We later performed the reaction neat with constant MWI power of 10 watts for 10 minutes affording the product in 98% yield after recrystallisation.

The product **4.21** was characterized by ¹H and ¹³C NMR spectroscopy. The phenyl moiety on the product was confirmed by peaks between 8.04-7.48 ppm due to aromatic protons and a singlet

peak at 8.26 ppm due to H4 in the ^1H NMR spectrum. The ethyl cyanoacetate moiety was confirmed by a quartet and triplet peaks due to H1' and H2' appearing at 4.39 ppm and 1.41 ppm, respectively. The carbon NMR spectrum had 10 signals as expected from the product, with that due to C1 appearing at 162.5 ppm and that due to C4 appearing at 155.1 ppm confirming the presence of both the ethyl cyanoacetate fragment and the phenyl fragment.

4.2.2 Synthesis of ethyl 2-cyano-3-phenylpropanoate [10]

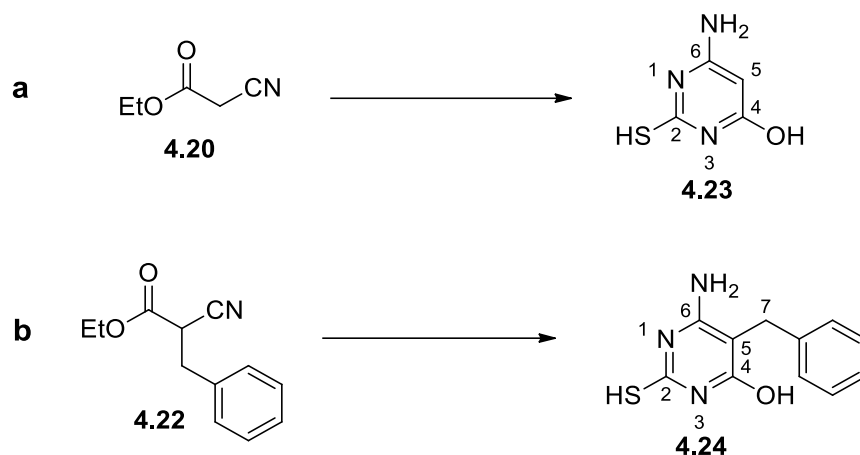


Scheme 4.15. Synthesis of ethyl 2-cyano-3-phenylpropanoate from (*E*)-ethyl 2-cyano-3-phenylacrylate. Reagents and conditions: 1) NaBH_4 , ethanol, 0°C , 5 min, 53%. 2) 6 bar $\text{H}_2(\text{g})$, methanol, rt., 30 minutes, 77%.

The second synthetic step in the synthesis of ethyl 2-cyano-3-phenylpropanoate starting from ethyl cyanoacetate was to reduce the alkene of compound **4.21** (Scheme 4.15). This was initially achieved by using sodium borohydride in ethanol at 0°C within 5 minutes. The product was afforded in 53% yield after work up and purification by silica gel chromatography. The lower yield was due to the formation of side products that were not identified, but likely due to reduction of the nitrile moiety, and although not anticipated, possibly also the ester. We therefore attempted an alternative approach using hydrogen gas catalysed by palladium. The reaction returned a higher yield of 77% within 30 minutes at 6 bar hydrogen gas pressure. In this case we observed that the nitrile moiety was also reduced but to a lesser extent, unless the reaction was allowed to proceed longer than 30 minutes.

Signals on the ^1H NMR spectrum of the product **4.22** integrated to the correct number of hydrogens with the key signals appearing as a doublet of doublets and quartet of doublets at 3.71 ppm and 3.22 ppm due to H2 and H4, respectively. The key ^{13}C NMR spectroscopic signal was that due to C4 which shifted upfield to 35.7 ppm from the aromatic region.

4.2.3 Synthesis of 6-amino-5-benzyl-2-mercaptopyrimidin-4-ol



Scheme 4.16. Preparation of the pyrimidine moiety. Reagents and conditions: thiourea, KO^tBu, constant 10 W MWI, 5-10 minutes, 64% (**4.23**) and 56% (**4.24**).

At this point of the synthesis, we had successfully introduced the R² substituent on one of the precursors required to form the pyrimidine ring. The next step was to synthesize the pyrimidine scaffold and as discussed previously, the R² group will already be part of the ring structure (**Scheme 4.16**). We started by heating a mixture of the substrate **4.22** and thiourea in ethanol with sodium ethoxide as a base for 2 hours following a well described synthetic protocol [11]. This approach, however, did not afford us the desired compound even when the reaction was allowed to proceed for 24 hours. We then decided to explore other synthetic protocols that could yield the desired compound. During this development phase, we used ethyl cyanoacetate **4.20** as a model compound in place of the intermediate **4.22** to test these protocols (**Scheme 4.16 a**).

Following the work done by Goswami et al. where they afforded functionalised pyrimidines using a domestic microwave [12], we prepared a solvent-free mixture of **4.20**, thiourea, and potassium *tert*-butoxide which was radiated under microwave conditions at a constant power of 150 Watts. The reaction resulted in a rapid increase in temperature and build-up of pressure and had to be terminated within 30 seconds. Workup of the reaction and purification resulted in 36% of the desired product. To circumvent the rapid increase in temperature and pressure, we added a high boiling point solvent, *n*-butanol, starting with 1 ml quantity. We were able to do the reaction for 1 minute at 150 Watts before a phase of rapid temperature increase started. The reaction, upon purification gave 40% of the product. Increasing the amount of solvent resulted in lower yields (~20%), but with extended duration. We then lowered the power to 50 Watts, maintaining the 1 ml of *n*-butanol. With this we could afford up to 49% yield within 5 minutes. When the reaction

was done for 10 minutes in 2×5 minutes cycles allowing the reaction mixture to cool down to room temperature after the first cycle. The methodology afforded the product in 67% yield, however, after repeated experiments we noticed that the method is not reproducible. We returned to using solvent free conditions with the power lowered down to 10 Watts and with this the product **4.23** was afforded in 64% yield within 5 minutes. The methodology was repeated at least 5 times, and the afforded yields were consistent ($\sim 65\% \pm 3\%$).

The formation of the product **4.23** was confirmed using ^1H and ^{13}C NMR spectroscopy. The thiol and hydroxy moieties appeared as overlapping singlets between 11.72 -11.47 ppm in the ^1H NMR spectrum. A proton signal integrating for two protons due to NH_2 appeared at 6.40 and the H5 peak was visible at 4.71 ppm as a singlet. The ^{13}C NMR spectrum had the correct number of signals (4 peaks) corresponding to the structure of **4.23**.

We adopted this methodology for the synthesis of the intermediate **4.24** (Scheme 4.16 b). To a well ground mixture of potassium *tert*-butoxide and thiourea was added ethyl 2-cyano-3-phenylpropanoate **4.22** and the resulting mixture was microwave irradiated at 10 Watts for 10 minutes. The resulting solid matter was diluted with water and the solution pH was adjusted to 5 using glacial acetic acid during which time a white precipitate formed. The precipitate was collected by filtration to afford the product without the need for further purification in 56% yield.

The formation of the pyrimidine ring was confirmed by ^1H NMR spectroscopic signals due to the hydroxy, thiol, and amine protons appearing at 11.81, 11.49 and 6.25 ppm, respectively. These signals appeared as singlets integrating to one proton for the thiol and hydroxy moieties and two protons for the amine moiety. The phenyl group was confirmed by a multiplet signal due to aromatic protons between 7.21-7.09 ppm, and a singlet peak integrating to two protons due to H7 at 3.53 ppm. The carbon NMR spectrum had a total of 9 signals typical of the product **4.24** with 7 appearing in the aromatic region and 2 due to C5 and C7 appearing at 89.7 and 27.6 ppm, respectively. HRMS confirmed a molecular ion $[\text{M}+\text{H}]^+$ due to **4.24** to be 234.0698 which is consistent with the theoretical value of 234.0694 calculated for $\text{C}_{11}\text{H}_{12}\text{N}_3\text{OS}^+$.

4.2.4 Synthesis of 6-amino-5-benzylpyrimidin-4-ol



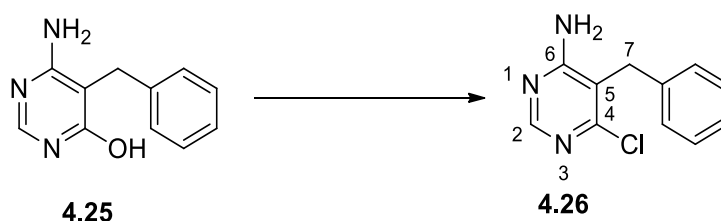
Scheme 4.17. Synthesis of 6-amino-5-benzylpyrimidin-4-ol. Reagents and conditions: Raney-nickel, 25% aqueous ammonia, 100°C, 2 hours, 82%.

The afforded intermediate **4.24** contained a thiol substituent at the C2 position of the ring which is not part of the targeted compound. We, therefore, endeavoured to remove the group and replace it with a proton using Raney-Nickel (**Scheme 4.17**). To achieve this, we prepared a mixture of the substrate **4.24**, and Raney-Nickel in water and heated it for 2 hours. We then proceeded to filter the suspension to separate Raney-nickel from the reaction solution while the solution was hot. Allowing the solution to cool down to room temperature before filtration resulted in the formation of a precipitated which combined with Raney-nickel making it hard to separate. The filtrate was concentrated *in vacuo* to afford the product in 82% yield without further purification.

The key changes in the ^1H NMR are the disappearance of the signal previously due to the thiol proton and the appearance of the signal at 7.73 ppm due to H2 integrating to one proton with a singlet multiplicity. C2 on the ^{13}C NMR also moved upfield to 147.1 ppm from 173.3 ppm.

Synthesis of **4.25** can be achieved directly from **4.22** by using formamidine acetate in place of thiourea. We attempted this approach, and it proved to be low yielding – the desired compound was produced lower yields (at most 5%).

4.2.5 Synthesis of 5-benzyl-6-chloropyrimidin-4-amine



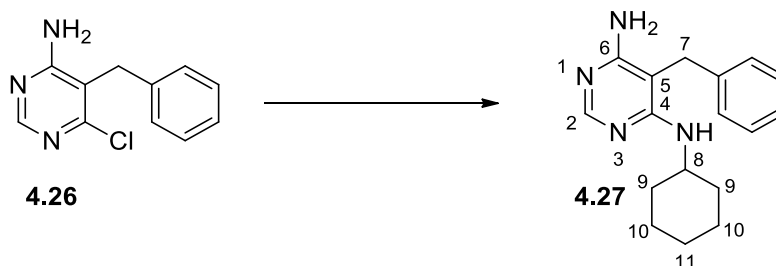
Scheme 4.18. Synthesis of 5-benzyl-6-chloropyrimidin-4-amine. Reagents and conditions: POCl_3 , 100°C, 3 hours, 91%.

The next step in the development of the methodology for the synthesis of the carbon-linker 4,6-diaminopyrimidine derivative was to chlorinate the C4 position in preparation for the addition of the NHR^1 groups (**Scheme 4.18**). This we achieved by substituting the hydroxy moiety with a chloride substituent using POCl_3 . A mixture of the intermediate **4.25** and POCl_3 were loaded inside

a pressure tube followed by heating at 100°C. After 3 hours the excess POCl₃ was quenched in ice water and the resulting acidic solution was neutralised with aqueous ammonia. The product **4.26** was afforded in 91% yield as a yellow solid after purification.

The formation of the product **4.26** was confirmed by the absence of the OH signal on the ¹H NMR spectrum. All other protons were accounted for and appeared at the expected regions. The key observable change on the ¹³C NMR spectrum was that due to C5. The signal moved downfield from 97.3 ppm to 112.9 ppm owing to the change in the chemical environment caused by the addition of the chloride atom at the C4 position. HRMS confirmed **4.26** [M+H]⁺ molecular ion to be 220.0637 which is consistent with the theoretical value of 220.0636 calculated for C₁₁H₁₁N₃Cl⁺.

4.2.6 Synthesis of 5-benzyl-*N*⁴-cyclohexylpyrimidine-4,6-diamine



Scheme 4.19: Synthesis of 5-benzyl-*N*⁴-cyclohexylpyrimidine-4,6-diamine. Reagents and conditions: Cyclohexylamine, toluene, 200°C, 1 hour, MWI (150W), 37%.

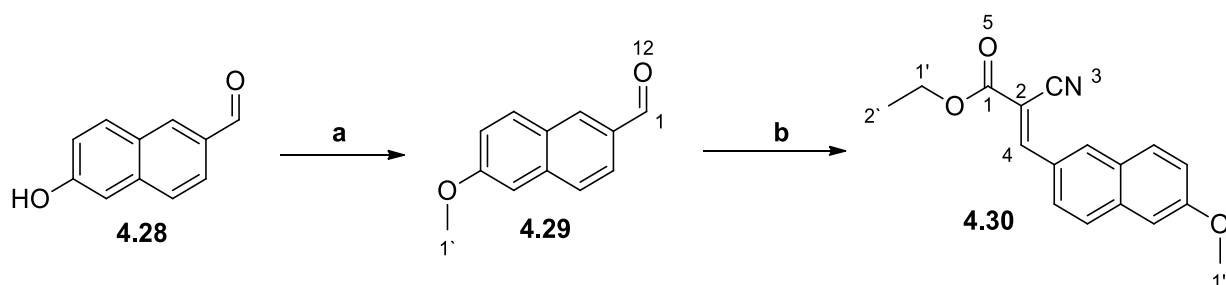
The last step in the synthesis of carbon-linked 4,6-diaminopyrimidines was to introduce the NHR¹ at the C4 position of **4.26**. We achieved this by employing the same synthetic protocol used in the synthesis of *N*⁴-substituted pyrimidine-4,6-diamine compounds (section 4.1.5) where the chloro-substrate and the amine were combined and heated by microwave irradiation for 60 minutes to get the product. To produce **4.27**, compound **4.26** was combined with cyclohexylamine and small quantities of toluene in a microwave tube. The resulting mixture was heated by microwave irradiation for one hour before diluting with methanol. The afforded solution was concentrated on silica, followed by purification using silica gel column chromatography, producing **4.27** as a white solid in 37% yield. An attempt made to improve the yield of **4.27** by increasing the reaction time to 2 hours was not successful; the reaction reaches full conversion after 1 hour. Due to time constraints, we could not try out other strategies to improve the yields, like using a different solvent.

The formation of **4.27** was confirmed using both ¹H and ¹³C NMR spectroscopy. The aromatic protons due to the benzyl moiety appeared between 7.29 and 7.11 ppm as a multiplet integrating to 5 protons in the ¹H NMR spectrum and the -CH₂- protons appeared at 3.72 ppm as a singlet. H2

and NH₂ protons appeared also as singlets at 7.85 and 5.87 ppm, respectively. The NH proton appeared as a doublet integrating to one proton at 5.57 ppm. It couples with H8, which appeared as a multiplet, integrating to one proton, between 3.90 and 3.81 ppm. The remaining protons that further confirms the presence of the cyclohexyl group on the product appeared as multiplets between 1.79 and 1.03 ppm. The ¹³C NMR spectrum had peaks due to C6, C4, C2, and C5 appearing at 161.5, 159.9, 155.9, and 93.8 ppm confirming the presence of the pyrimidine core. The benzyl moiety carbon peaks appeared at 140.1, 128.6, 128.5 and 126.3 ppm and the -CH₂-carbon signal appeared at 28.4 ppm. ¹³C NMR spectroscopic signals for the cyclohexyl moiety appeared at 49.2, 33.2, 25.9 and 25.3 ppm. The product **4.27** had a melting point range of 175-177°C which differs from that of the starting substrate **4.26** (185-188°C), further indicating that the reaction afforded a new compound.

4.2.7 Synthesis of (*E*)-ethyl 2-cyano-3-(6-methoxynaphthalen-2-yl)acrylate [13, 14]

The synthetic methodology described thus far was for a model system bearing a phenyl substituent at R², which, as mentioned above, was chosen since benzaldehyde is commercially available. Our targeted compounds, however, should ideally contain a bulky naphthalene moiety which is key for potency and selectivity towards the target parasitic enzymes. During the development of the synthetic protocol towards the synthesis of our one carbon-linker derivatives on our model system, we managed to make minor progress on the synthesis of the required naphthalene precursors necessary for the synthesis of the target compounds.



Scheme 4.20. Synthesis of (*E*)-ethyl 2-cyano-3-(6-methoxynaphthalen-2-yl)acrylate from 6-hydroxy-2-naphthaldehyde. Reagents and conditions: **a**) dimethylsulfate, K₂CO₃, acetone, 60°C, 2 hr, 74%. **b**) ethyl cyanoacetate, 10 W MWI, 10 minutes, 87%.

The naphthalene aldehyde needed for the synthesis of our targeted compounds was not commercially available and therefore was prepared from the substrate **4.28** (**Scheme 4.20**). A reaction of **4.28** with dimethylsulfate in acetone afforded the product **4.29** in 74% yield after heating at 60°C for 2 hours and purification using silica gel chromatography. The key changes in the ¹H and ¹³C NMR spectra were the appearance of H1' and C1' at 3.96 ppm and 55.5 ppm,

respectively. Other signals confirming the naphthalene and aldehyde fragments of the product were also observed and were consistent with literature reported spectra [14].

The next step was to introduce the intermediate **4.29** on the C2 position of ethyl cyanoacetate via the Knoevenagel condensation reaction. To achieve this a mixture of **4.29**, ethyl cyanoacetate **4.20**, and piperidine were heated by MWI at a constant power of 10W for 5 minutes followed by recrystallisation of the afforded crude to give the product **4.30** as a light-yellow fluffy solid in 87% yield.

We were able to confirm the ethyl cyanoacetate fragment on the product by ^1H NMR spectroscopic signals integrating for two and three protons due to H1' (quartet) and H2' (multiplet), at 4.40 ppm and 1.46-1.37 ppm, and the ^{13}C NMR spectroscopic signals due to C1, C1' and C2' at 163.0 ppm, 62.6, and 14.2 ppm, respectively. The presence of the naphthalene moiety was confirmed by ^1H NMR spectroscopic singlet signal at 8.34 ppm due to H4, 5 aromatic signals appearing between 8.29-7.13 ppm, and a siglet signal integrating to one three protons due to H1'' appearing at 3.96 ppm. Unfortunately, we did not get to test the cyclisation of this precursor with thiourea to form the desired substituted pyrimidine. This will be considered for future work.

4.3 Preparation of one-atom linkage 4,6-diaminopyrimidine derivatives: heteroatom-atom linker compounds

Heteroatom-linker 4,6-diaminopyrimidine derivatives are a variant of carbon-atom linker compounds discussed above, however, with an oxygen or nitrogen atom as the linker (**Figure 4.5**). These were designed based on the hypothesis that the unique geometry conferred by the heteroatom will have a unique binding conformation in the active site of the target enzymes. Biological results arising from the heteroatom-linker 4,6-diaminopyrimidine and the carbon-linker 4,6-diaminopyrimidine derivatives will provide information on which conformation is best suited for inhibition of the target enzymes

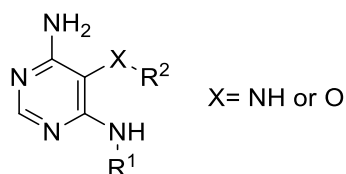
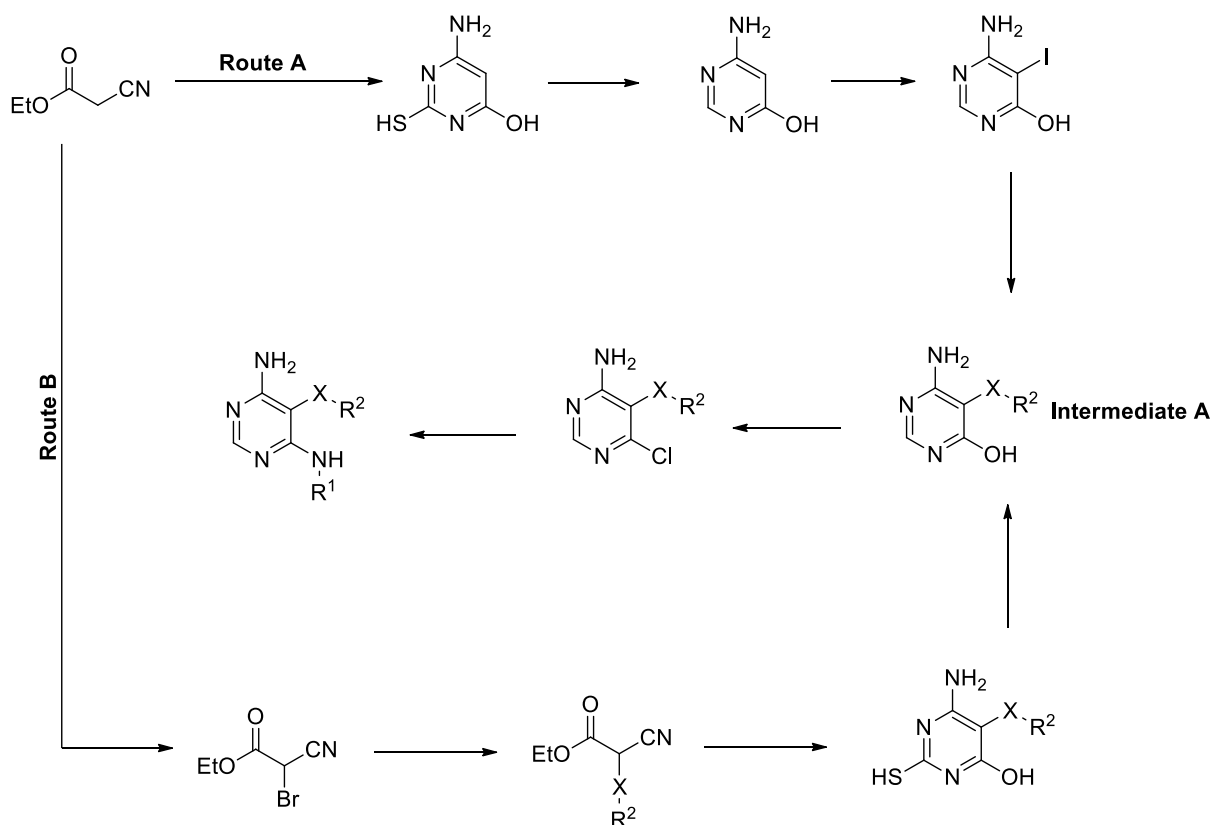


Figure 4.5. The general structure of heteroatom linker derivatives.

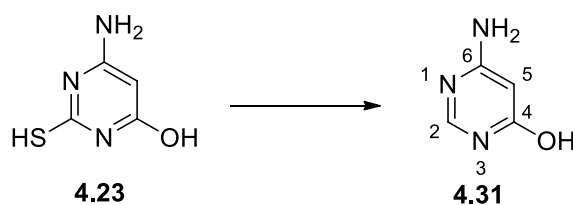
We planned to prepare the targeted compounds via two synthetic approaches. The first included the formation of the pyrimidine ring with the R^2 substituent already in place (**Scheme 4.21**, Route B). Ethyl cyanoacetate would be brominated at the C2 position followed by substitution with

aromatic amine at the same position. The afforded intermediate would then be reacted with thiourea to form the pyrimidine ring, which would, in turn, be modified by the removal of the thiol group to afford the intermediate A. In the second approach we would introduce the R² groups after the pyrimidine ring has been developed to afford intermediate A (**Scheme 4.21**, Route A). This would be accomplished by a metal catalysed coupling reaction (such as the Buchwald-Hartwig amination reaction for X=N). From intermediate A, the compound would be chlorinated at C4 to prepare the scaffold for amination with an alkylamine to afford the targeted compounds.



Scheme 4.21. Proposed synthesis of heteroatom-linker 4,6-diaminopyrimidine derivatives.

4.3.1 Synthesis of 6-aminopyrimidin-4-ol [15]



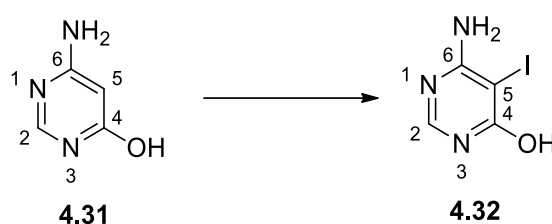
Scheme 4.22. Synthesis of 6-aminopyrimidin-4-ol. Reagents and conditions: Raney-nickel, aqueous NH₃, 100°C, 2 hrs, 78%.

We started the development of our synthetic protocols by exploring synthetic route A, **Scheme 4.21**. The synthesis of the intermediate **4.23** is already described in section 4.2.3. The compound

4.31 was acquired from the reaction of **4.23** with Raney-nickel in aqueous ammonia (**Scheme 4.22**). The substrate and reagent were heated at 100°C for 2 hours before hot filtration to recover the Raney-nickel. The filtrate was concentrated *in vacuo* to afford the product as a white solid in 78% yield.

A key ^1H NMR spectroscopic signal confirming the formation of **4.31** was that appearing as a singlet integrating to one proton due to H2 at 7.78 ppm and a key ^{13}C NMR spectroscopic signal appeared at 150.0 ppm due to C2. The latter signals shifted upfield indicating a change in the chemical environment.

4.3.2 Synthesis of 6-amino-5-iodopyrimidin-4-ol

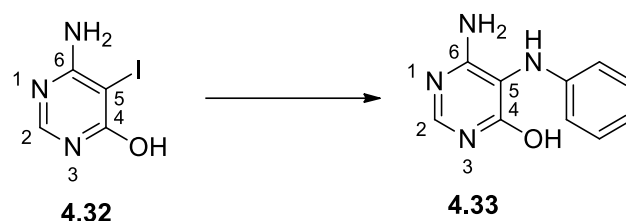


Scheme 4.23. Preparation of 6-amino-5-iodopyrimidin-4-ol. Reagents and conditions: I_2 , K_2CO_3 , DMF/ H_2O , 100°C, 1 hr, 68%.

We then functionalised the C5 position of **4.31** with iodine to prepare an intermediate on which the R^2 substituent will be added (**Scheme 4.23**). This was achieved following a microwave-assisted protocol described in section 4.1.6. To a mixture of **4.31**, iodine, and potassium carbonate was added DMF and water. The resulting suspension was heated at 100°C for 1 hour after which the solution was diluted with water and excess iodine was quenched with sodium thiosulfate. The formed precipitate was collected by filtration affording the product in 68% yield without further purification.

The product's ^1H NMR spectrum was lacking the signal that was previously due to H5, indicating that substitution had taken place. However, signals due to OH, H2, and NH_2 were observed appearing at 11.80, 7.76, and 6.70 ppm, respectively. A key change in the ^{13}C NMR is that due to C5 which moved upfield from 78.6 ppm to 59.0 ppm. HRMS confirmed the molecular ion of the product, $\text{C}_4\text{H}_5\text{IN}_3\text{O}^+$, to be 237.9474 which correspond to the calculated value of 237.9412 for the same molecular ion.

4.3.3 Synthesis of 6-amino-5-(phenylamino)pyrimidin-4-ol



Scheme 4.24. Preparation of 6-amino-5-(phenylamino)pyrimidin-4-ol. Reagents and conditions: aniline, K^tOBu, 180°C, MWI, 68%.

At this point of the synthesis, we were at a position to introduce the aromatic amines at the C5 position of the intermediate **4.32** (Scheme 4.24). We followed the synthetic protocols described by Fang et al. where they explored the nucleophilic substitutions of halouracils using microwave irradiation [16], however, with aniline added at 2 mole equivalents and not in excess. To this end, we tested the reaction by preparing a mixture of **4.32**, aniline, and potassium carbonate in a microwave vessel and heated at 180°C for 10 minutes. Upon cooling a precipitate formed and was collected by filtration to afford the product as a light brown solid in 68% yield requiring no further purification. Anticipating that naphthylamines, which would be required to afford our desired compounds, will be solid; we attempted the reaction using DMF as the solvent. Unfortunately, under these conditions, the reaction did not afford the desired compound. The reaction only worked well when done solvent-free in high concentrations of aniline.

Successful addition of the phenyl aniline moiety was confirmed by aromatic signals appearing as a triplet integrating to two protons at 7.00 ppm, a doublet integrating to two protons at 6.54 and a triplet signal integrating to one proton at 6.48 ppm in the ¹H NMR spectrum. The secondary amine (NH) was confirmed by a singlet peak integrating to one proton appearing at 4.99 ppm. The ¹³C NMR spectrum also accounted for peaks due to the mono-substituted phenyl carbons appearing at 129.2, 116.1, and 114.3 ppm.

We managed to get this far on developing a synthetic route for making heteroatom-linked 4,6-diaminopyrimidine derivatives. What remains is to functionalise the C4 position with targeted X-R¹ groups starting by chlorinating the intermediate **4.33** using POCl₃ followed by amination. This would have to be explored as future work.

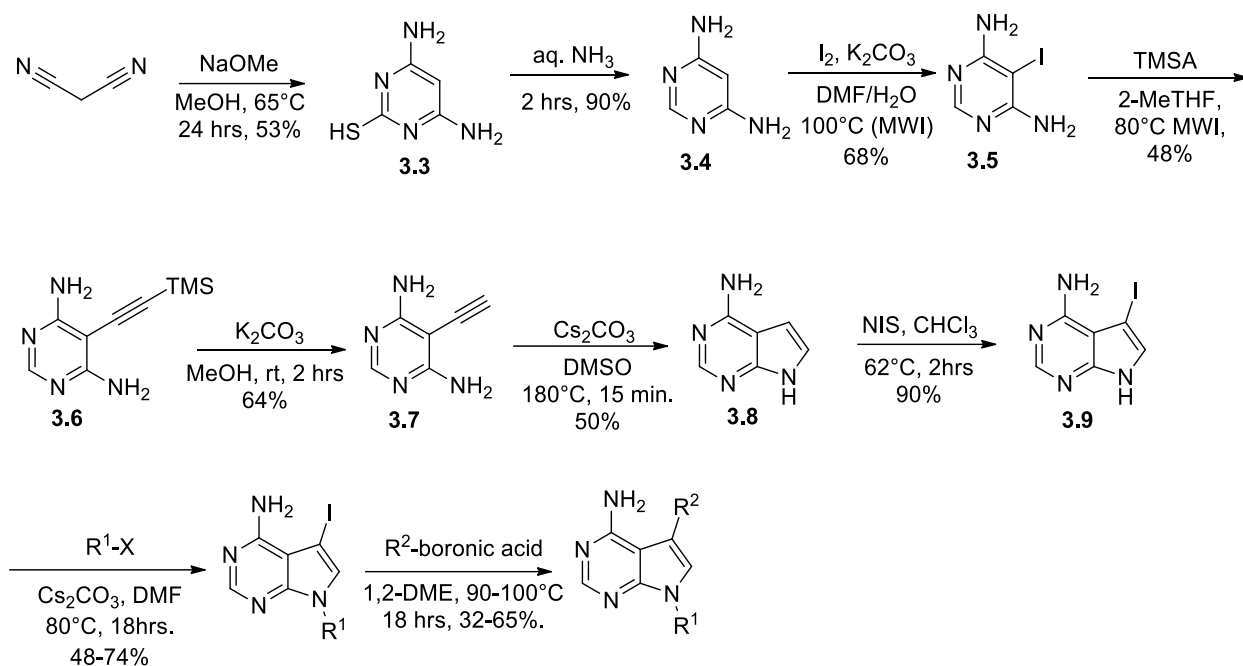
4.4 References

1. Masood, M.A., et al., *Discovery of a series of potent and selective human H4 antagonists using ligand efficiency and libraries to explore structure–activity relationship (SAR)*. Bioorganic & Medicinal Chemistry Letters, 2011. **21**(21): p. 6591-6595.
2. Garner, J., et al., *Identification of Aminopyrimidine Regioisomers via Line Broadening Effects in ¹H and ¹³C NMR Spectroscopy*. Australian Journal of Chemistry, 2004. **57**(11): p. 1079-1083.
3. Deng, X., et al., *Expanding the Diversity of Allosteric Bcr-Abl Inhibitors*. Journal of Medicinal Chemistry, 2010. **53**(19): p. 6934-6946.
4. Whitehead, C.W. and J.J. Traverso, *Diuretics. II. Alkoxymercuration by Mixed Anion Salts of Mercury*. Journal of the American Chemical Society, 1958. **80**(9): p. 2182-2185.
5. Zhan, W., et al., *Integrating docking scores, interaction profiles and molecular descriptors to improve the accuracy of molecular docking: Toward the discovery of novel Akt1 inhibitors*. European Journal of Medicinal Chemistry, 2014. **75**: p. 11-20.
6. Whitehead, C.W. and J.J. Traverso, *Diuretics. III. 4,6-Diaminopyrimidines*. Journal of the American Chemical Society, 1958. **80**(9): p. 2185-2189.
7. Carpita, A., et al., *Synthesis of the racemic forms of carbon–carbon double bond locked analogues of strobilurins which are characterized by a 2-arylcyclopropane ring cis-substituted at C-1 by the methyl (E)-3-methoxypropenoate unit*. Tetrahedron, 2002. **58**(19): p. 3673-3680.
8. Arakawa, Y., et al., *Design of an extremely high birefringence nematic liquid crystal based on a dinaphthyl-diacetylene mesogen*. Journal of Materials Chemistry, 2012. **22**(28): p. 13908-13910.
9. Ayoubi, S.A.-E., F. Texier-Boullet, and J. Hamelin, *Minute Synthesis of Electrophilic Alkenes under Microwave Irradiation*. Synthesis, 1994. **1994**(03): p. 258-260.
10. Che, J. and Y. Lam, *Polymer-Supported Hantzsch 1,4-Dihydropyridine Ester: An Efficient Biomimetic Hydrogen Source for the Reduction of Ketimines and Electron-Withdrawing Group Conjugated Olefins*. Advanced Synthesis & Catalysis, 2010. **352**(10): p. 1752-1758.
11. Röhrkasten, R., et al., *Synthesis of the Caffeine Metabolites 5-Acetyl-amino-6-formyl-amino-3-methyluracil (AFMU) and 5-Acetyl-amino-6-amino-3-methyluracil (AAMU) on a Preparative Scale*. Zeitschrift für Naturforschung B, 1997. **52**(12): p. 1526-1532.
12. Goswami, S., et al., *Microwave-expedited one-pot, two-component, solvent-free synthesis of functionalized pyrimidines*. Australian Journal of Chemistry, 2007. **60**(2): p. 120-123.
13. Zhao, Y., et al., *Wavelength-tunable AIEgens based on 6-methoxy-2-naphthaldehyde: AIE behavior and bioimaging performance*. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2022. **281**: p. 121621.
14. Utley, J.H.P. and G.G. Rozenberg, *Electroorganic reactions. Part 56: Anodic oxidation of 2-methyl- and 2-benzyl-naphthalenes: factors influencing competing pathways*. Tetrahedron, 2002. **58**(26): p. 5251-5265.
15. Brown, D.J., *Improved syntheses in the pyrimidine series .1. The use of lyrimidine-thiols*. Journal of the Society of Chemical Industry-London, 1950. **69**(12): p. 353-356.
16. Fang, W.-P., et al., *Synthesis of substituted uracils by the reactions of halouracils with selenium, sulfur, oxygen and nitrogen nucleophiles under focused microwave irradiation*. Tetrahedron, 2005. **61**(12): p. 3107-3113.

Chapter 5: Conclusion and future work

5.1 Synthesis and biological evaluation of pyrrolo[2,3-*d*]pyrimidine compounds

Molecular modelling studies done in this PhD thesis suggested that pyrrolo[2,3-*d*]pyrimidine compounds were potential inhibitors of the target enzymes *Pf*CDPK1 and *Pf*CDPK4. The compounds showed similar interactions in the active site of the enzymes to those experienced by the natural substrate adenosine triphosphate. Also, *in silico* pharmacokinetics studies revealed that the compounds could be expected to have good bioavailability and carried no anticipated neurological side effects. We therefore embarked on the synthesis of these compounds via a synthetic route that included nine synthetic steps, involving the formation of the pyrrolo[2,3-*d*]pyrimidine ring, followed by the introduction of ribose substituents (R^1) and bulky aryl moieties (R^2 , **Scheme 5.1**). We started the synthesis of the pyrrolo[2,3-*d*]pyrimidine compounds by forming **3.3** from malononitrile and thiourea. The intermediate was then reduced using Raney-nickel to give **3.4**, which was iodinated at the C5 position affording **3.5**. The iodination reaction was improved by using microwave irradiation, which permitted the use of less quantities of solvent and reduced reaction time without compromising the yields. Using the Sonogashira coupling reaction between **3.5** and trimethylsilylacetylene, we afforded compound **3.6** on which we appreciated improved yields (up to 61% from 48%) when microwave irradiation was used. The intermediate **3.6** was deprotected to give **3.7** using potassium carbonate in methanol. The pyrrolo[2,3-*d*]pyrimidine intermediate **3.8** was afforded following a microwave-mediated reaction in DMSO using the base cesium carbonate. The afforded intermediate **3.8** was functionalised at the C3 position with iodine, alkylated to introduce the R^1 substituents, and then subjected to the Suzuki-Miyaura reaction with boronic acids to add bulky aryl groups (R^2 substituents), affording our targeted compounds.



Scheme 5.1. Synthesis of pyrrolo[2,3-*d*]pyrimidine compounds.

We then tested the pyrrolo[2,3-*d*]pyrimidine compounds synthesised along with those made by a fellow student for biological activity against the recombinant enzymes *Pf*CDPK1 and *Pf*CDPK4, and HeLa cell lines. Three of the compounds were active against *Pf*CDPK4 and one was active against *Pf*CDPK1 (**Figure 5.1a**). Compounds active against *Pf*CDPK4 had an ethoxynaphthalene moiety attached to the pyrrolo[2,3-*d*]pyrimidine ring. This was consistent with results afforded from the molecular modelling study where larger aromatic groups had better affinity to *Pf*CDPK4 than smaller aromatic groups. These results attest to the reliability of the *Pf*CDPK4 model we developed. The active compound against *Pf*CDPK1 had a small aromatic moiety, the dimethoxybenzene fragment, which was consistent with the prediction afforded by the *Pf*CDPK1 model *in silico* with one exception. The best scoring compound against *Pf*CDPK1 *in silico* has the acylpiperidine moiety as an R¹ substituent instead of methylcyclopropane. These observations suggest that the *Pf*CDPK1 model requires modification to better predict the binding affinity of compounds, especially the ribose pocket component. Generally, all compounds had displayed minimal cytotoxicity against HeLa cells, save for one which had moderate inhibition (53% cell apoptosis at 20 μM concentration, **Figure 5.1b**).

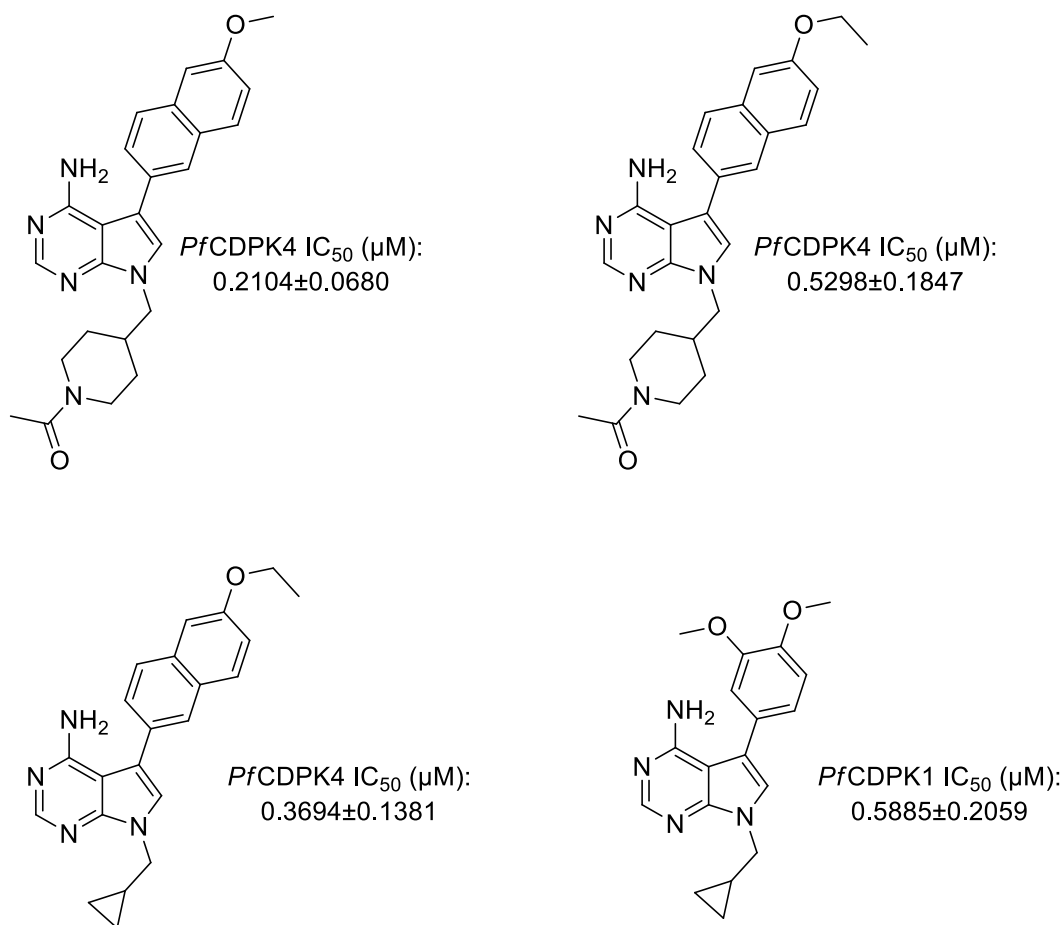
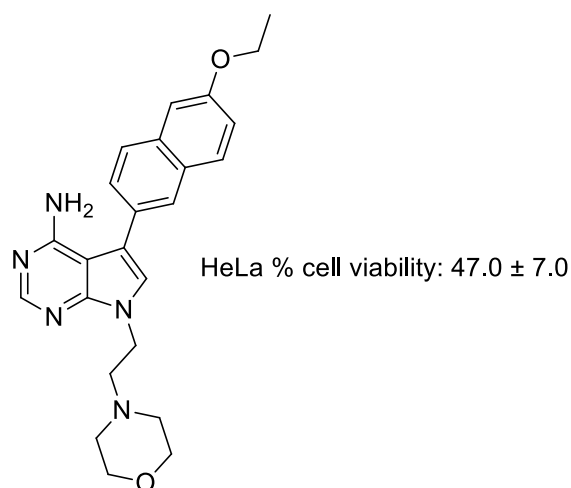
a**b**

Figure 5.1. a) Active pyrrolo[2,3-*d*]pyrimidine compounds against *Pf*CDPK1 and *Pf*CDPK4. b) Compound with moderate cytotoxicity as indicated by inhibition of HeLa cell line.

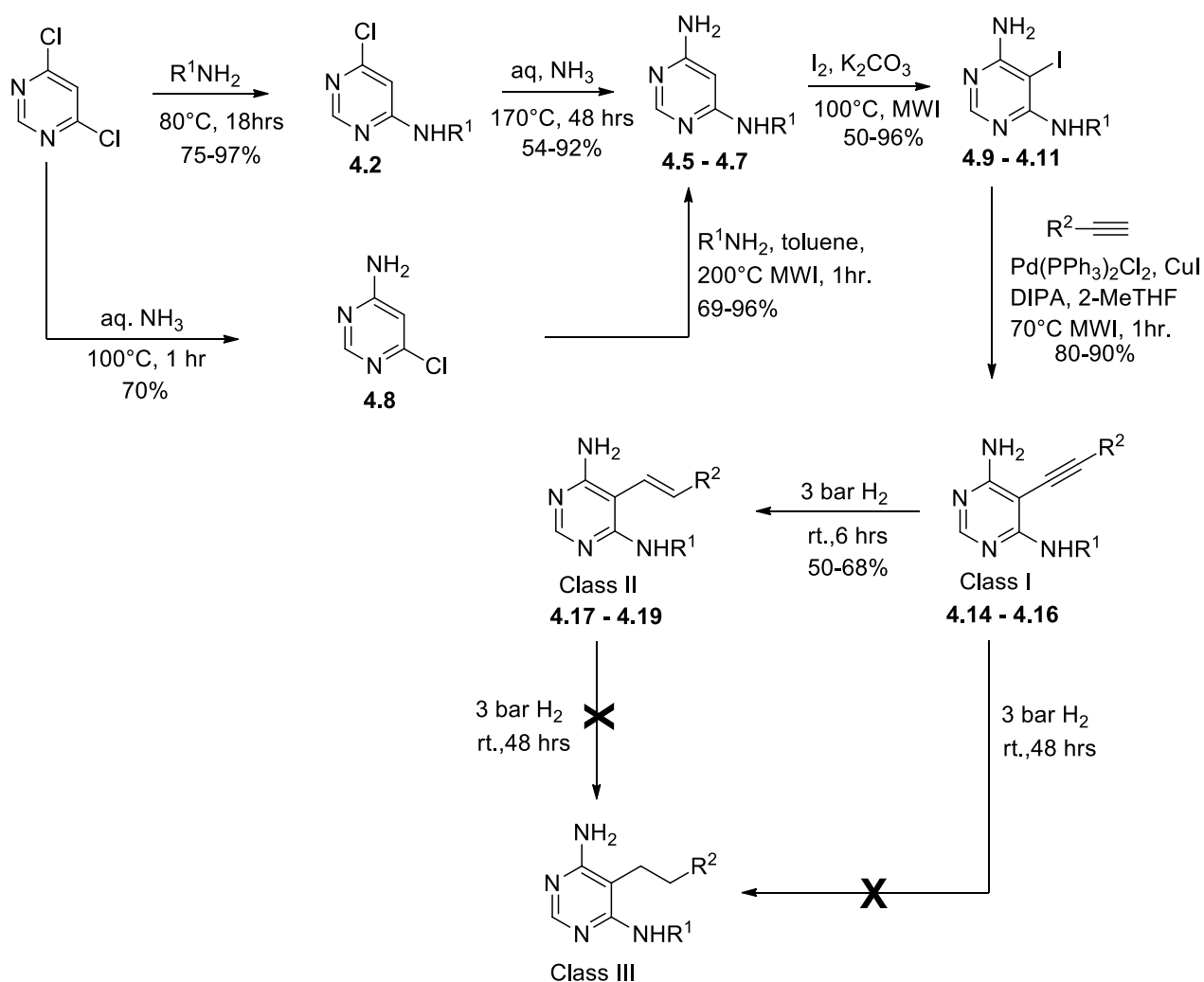
In future, we hope to modify the *Pf*CDPK1 model, especially the ribose region of the binding site, so that the model better predicts the activity of compounds. Also, we want to find a bulky R^1 substituent with a size that is between the naphthalene ring and the benzene ring to ensure that

compounds are better suited to inhibit both *Pf*CDPK1 and *Pf*CDPK4 without compromising selectivity. Active compounds against the targeted enzymes will serve as a valuable starting point in the design of a second generation of pyrrolo[2,3-*d*]pyrimidine inhibitors.

5.2 Synthesis of 4,6-diaminopyrimidine derivatives

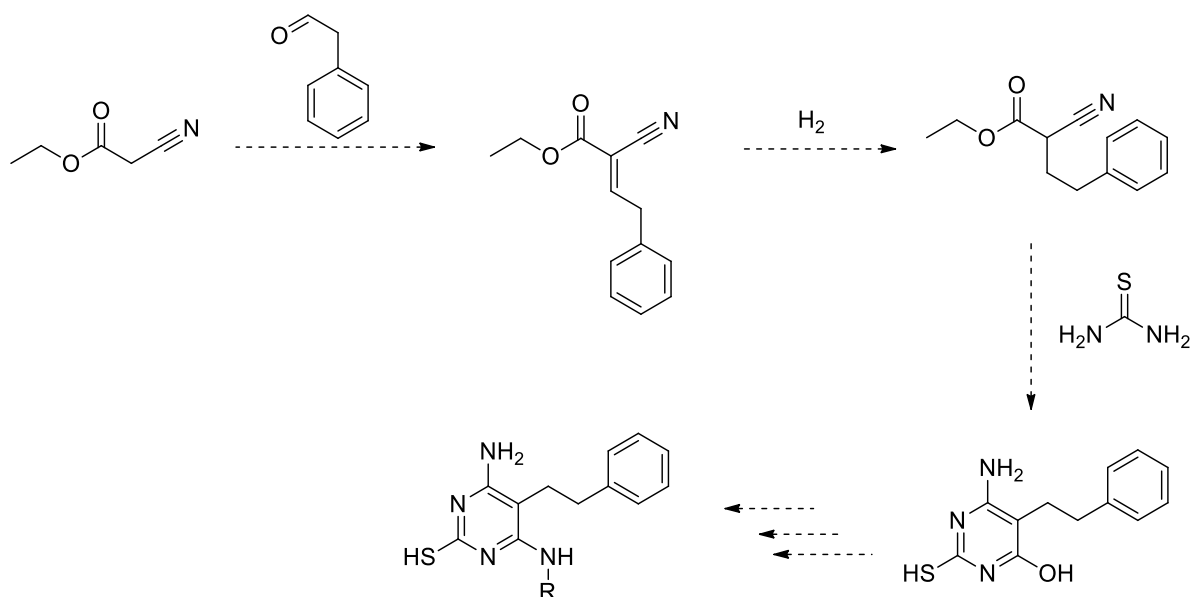
5.2.1 Two-atom linker compounds

We also embarked on the synthesis of 4,6-diaminopyrimidine compounds bearing a two-atom linkage between the pyrimidine moieties and the naphthalene groups. The compounds were divided into three classes depending on the degree of bonding between the linker atoms namely the acetylene (class I), ethene (class II) and ethane-linked compounds (class III, **Figure 5.2**). These were afforded starting with a nucleophilic displacement of the chloro substituents on 4,6-dichloropyrimidine by alkylamines and ammonia successively, to afford diaminopyrimidine intermediates **4.5-4.7**. The same intermediates were afforded starting with the displacement of the chloro substituents with ammonia to give **4.8** and then a microwave mediated nucleophilic displacement using alkylamines. We appreciated reduced reaction time and solvent quantities when the latter approach was used. We then functionalised the C5 position with iodine followed by a Sonogashira reaction to afford the first class of targeted compounds in good yields. Both the iodination reaction and coupling reaction were achieved using microwave irradiation. We then tried to reduce the class I compounds to class III derivatives using hydrogen gas without any success. Instead, class II compounds were afforded in moderate yields. We reasoned that steric hindrance is likely the cause why the acetylene linker was not completely reduced to an alkane.



Scheme 5.2. The synthetic route used in the synthesis of two-atom linker 4,6-diaminopyrimidine compounds.

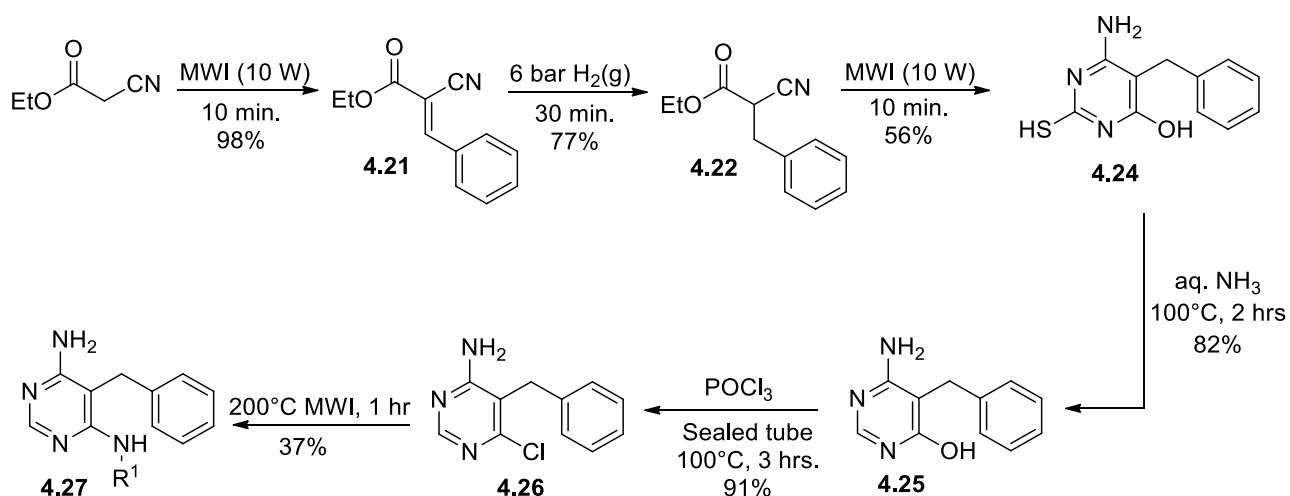
In future, we plan to pursue class III compounds using a different synthetic protocol that allows for the reduction of the acetylene moiety to an alkane before the pyrimidine ring is formed to avoid steric hindrance. One possibility is outlined in **Scheme 5.3**. The aromatic groups are added to ethyl cyanoacetate via the Knoevenagel condensation reaction followed by reduction to give alkane-linked aromatic intermediates. The intermediates will be reacted with thiourea to form a pyrimidine ring, that will then undergo several reactions to get the desired class III compounds. The afforded compounds will be tested for biological activity against *Pf*CDPK1 and *Pf*CDPK4 along with class I and class II compounds.



Scheme 5.3. Proposed route for the synthesis of class III two-linker 4,6-diaminopyrimidine compounds.

5.2.2 One-atom linker compounds (Carbon-linker)

One atom linker compound also showed the potential to inhibit *Pf*CDPK1 and *Pf*CDPK4 *in silico*. We managed to develop synthetic protocols required to afford the desired potential inhibitors which include 6 synthetic steps (**Scheme 5.4**). Starting from ethyl cyanoacetate we afforded **4.21** in good yields through microwave irradiation for 10 minutes. The intermediate was reduced with hydrogen gas to give **4.22** which, via microwave irradiation, reacted with thiourea to afford the pyrimidine intermediate **4.24**. The thiol was then removed using Raney-nickel in aqueous ammonia to give **4.25** and then chlorinated using phosphorus oxychloride affording **4.26**. The final product **4.27** was afforded after heating **4.26** and cyclohexylamine for 1 hour, concluding the development of the synthetic protocols to achieve carbon-linked 4,6-diaminopyrimidines.

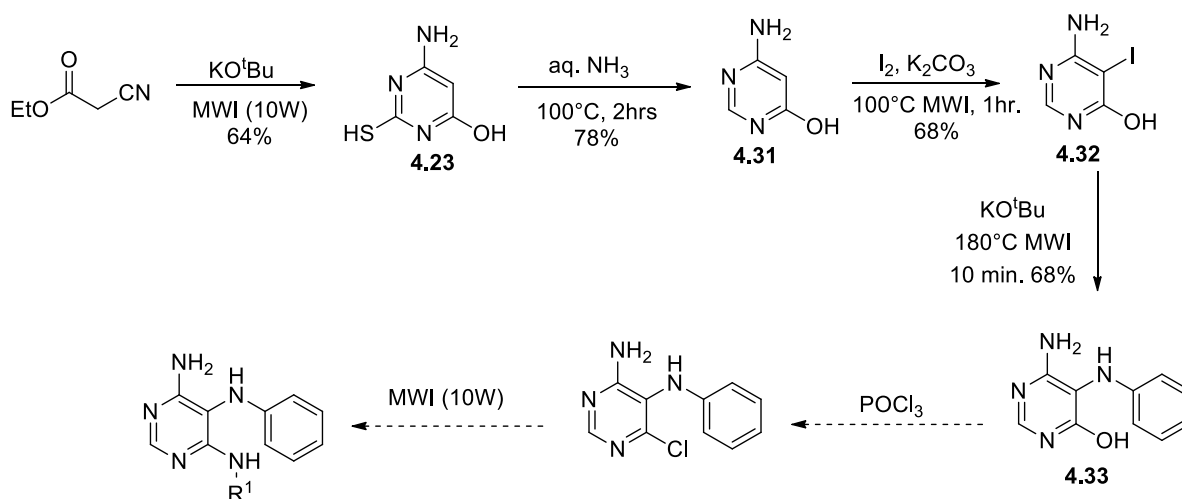


Scheme 5.4. Synthetic protocols used to achieve a carbon-linked 4,6-diaminopyrimidine compound.

In future we want to use the developed synthetic protocols (**Scheme 5.4**) to afford our target compounds. These have a naphthalene fragment in place of the benzyl moiety. The final carbon-linker 4,6-diaminopyrimidine compounds will be tested for activity against *Pf*CDPK4, *Pf*CDPK1, and HeLa cells lines to assess their inhibitory potential and toxicity.

5.2.3 One- atom linker compounds (Heteroatom-linker)

Progress made in the development of a series of synthetic protocols that will afford heteroatom-linked 4,6-diaminopyrimidine compounds go as far as the synthesis of intermediate **4.32** (**Scheme 5.5**). We started by forming the pyrimidine compound **4.23** from ethyl cyanoacetate and thiourea, followed by the removal of the thiol group using Raney-nickel, and functionalising the C5 position with iodine to give **4.31**. Under basic and solvent-free conditions compound **4.32** was afforded in moderate yields.



Scheme 5.5. The synthesis of heteroatom-linked 4,6-diaminopyrimidine compounds.

In future, we hope to complete the synthetic route by adding the R^1 groups via two steps including chlorination and nucleophilic substitution, respectively. We also want to explore other means of adding aromatic amines to **4.32**. The current procedure works because the substrate aniline is a liquid and can be used in excess as a solvent as well. However, with our target aromatic amines using excess quantities will not be practical. Such alternatives include the use of Buchwald-Hartwig amination or forming the pyrimidine ring with the aromatic amine moiety already in place as we did for the synthesis of one carbon-linker 4,6-diaminopyrimidine compounds. The completed synthetic route will then be applied in the synthesis of our target compounds which will then be tested for biological activity and toxicity.

Chapter 6: Experimental section

6.1 General procedures

All reagents used were purchased from Sigma-Aldrich and were used without further purification. Solvents including tetrahydrofuran (THF), diethylether (Et₂O), dichloromethane (DCM), and acetonitrile (MeCN) were distilled under nitrogen atmosphere prior to use. THF and Et₂O were distilled over sodium wire using benzophenone as an indicator. MeCN and THF were distilled over calcium hydride.

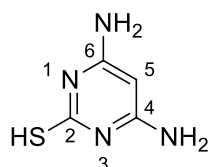
Thin layer chromatography (TLC) performed on pre-coated aluminium plates with 0.25 mm silica gel 60 was used to monitor the progress of the reactions and was visualised under UV light (254 nm) or through potassium permanganate solution. Purification of reaction product was achieved by normal or flash column chromatography utilizing silica gel 60 with 70 – 230 and 230-400 mesh, respectively.

NMR spectra were recorded on Bruker Advance-300, Bruker Advance-400, and Bruker Advance-500 instruments and measured at 300, 400, and 500 MHz, respectively for ¹H NMR and 75, 101 and 125 MHz for ¹³C NMR. Spectra were recorded using deuterated solvent including chloroform (CDCl₃), and dimethylsulphoxide (DMSO-*d*₆) referenced using the internal standard tetramethylsilane (TMS) or residual solvent signal.

Infra-red spectra were recorded on a Bruker Tensor 27 Spectrometer. Mass spectrometry was performed using Bruker Compact Q-TOF High Resolution Spectrometer using electrospray ionization mode (ESI+). Melting points were acquired using Stuart SMP20 melting point apparatus.

6.2 Synthesis of pyrrolo[2,3-*d*]pyrimidine derivatives

6.2.1 Synthesis of 4,6-diaminopyrimidine-2-thiol 3.3 [1]

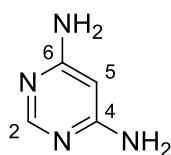


To a freshly prepared solution of sodium methoxide [Sodium (2.8 g, 121.1 mmol) and methanol (100 ml)] was added thiourea (5.1 g, 66.6 mmol) and malononitrile (4.00 g, 60.6 mmol). The mixture was heated at reflux for 24 hours before cooling to room temperature, concentrating *in*

vacuo, and diluting with water. The resulting solution pH was adjusted to 7 with glacial acetic acid and the formed precipitated was collected by filtration affording 4,6-diaminopyrimidine-2-thiol (4.57 g, 53%) as a white solid.

R_f(MeOH): 0.38. **¹H NMR** (400 MHz, DMSO-*d*₆) δ 11.12 (s, 1H, SH), 6.74 (s, 4H, 2×NH₂), 5.06 (s, 1H, H5). **¹³C NMR** (101 MHz, DMSO-*d*₆) δ 177.49 (C2, C4, & C6), 74.66 (C5) .**mp** (°C): decompose at 300°C.

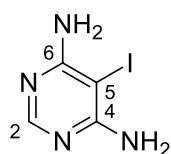
6.2.2 Synthesis of pyrimidine-4,6-diamine 3.4 [2]



To a suspension of 4,6-diaminopyrimidine-2-thiol (2.13 g, 14.95 mmol) in water (250 ml) was added 25% aqueous ammonia (20 ml) followed by Raney-nickel (4.25 g). The mixture was heated at reflux for 2 hours before cooling to room temperature and removing the catalyst via filtration. The precipitate was collected by filtration, washed with water, and dried to afford pyrimidine-4,6-diamine (1.49 g; 90%) as an off-white solid.

R_f(20% MeOH/DCM): 0.28. **¹H NMR** (300 MHz, DMSO-*d*₆) δ 7.80 (d, *J* = 1.0 Hz, 1H, H2), 6.00 (s, 4H, 2×NH₂), 5.37 (d, *J* = 1.1 Hz, 1H, H5). **¹³C NMR** (75 MHz, DMSO-*d*₆) δ 163.5 (C6 & C4), 157.8 (C2), 82.5 (C5). **IR** (v/cm⁻¹): 3445 (NH), 3090 (=C-H). **mp** (°C): decompose at 269 – 270.

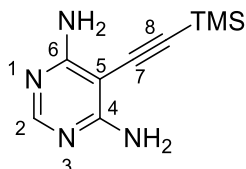
6.2.3 Synthesis of 5-iodopyrimidine-4,6-diamine 3.5 [3]



To a suspension of pyrimidine-4,6-diamine (1.14 g; 10.37 mmol) and water (7.0 ml) was added dimethylformamide (2.0 ml), potassium carbonate (2.867 g, 20.74 mmol) and iodine (5.264 g, 20.74 mmol). The mixture was heated at 100°C for 1 hour by microwave irradiation before cooling down to room temperature and diluting with water (100 ml). Excess iodine was quenched by adding sodium thiosulfate pellets gradually until the solution was clear. The formed precipitate was collected by filtration, washed with water, and dried to afford 5-iodopyrimidine-4,6-diamine (1.95 g; 68%) as a yellow solid.

R_f (20% MeOH/EtOAc): 0.71. **¹H NMR** (300 MHz, DMSO-*d*₆) δ 7.72 (s, 1H, H2), 6.31 (s, 4H, 2×NH₂). **¹³C NMR** (101 MHz, DMSO-*d*₆) δ 163.3 (C4 & C6), 157.5 (C2), 55.7 (C5). **IR** (v/cm⁻¹): 3450 (N–H), 3080 (=C–H), 1626 (C=C), 1312 (C–N), 530 (C–I). **mp** (°C): 230 – 233.

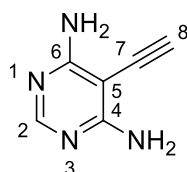
6.2.4 Synthesis of 5-((trimethylsilyl)ethynyl)pyrimidine-4,6-diamine **3.6** [4]



To a microwave vessel loaded with 5-iodopyrimidine-4,6-diamine **3.5** (1.50 g, 6.36 mmol), Pd(PPh₃)₂Cl₂ (0.45 g, 0.64 mmol), and CuI (36 mg, 0.19 mmol) was added a solution of trimethylsilyl acetylene (0.94 ml, 12.71 mmol), diisopropylamine (1.78 ml, 12.71 mmol), and 2-methyltetrahydrofuran (6 ml). The mixture was heated at 80°C for 30 minutes by microwave irradiation before cooling to room temperature and diluting with saturated aqueous ammonium chloride. The organic matter was extracted with ethyl acetate, dried with anhydrous magnesium sulfate, filtered, and then concentrated *in vacuo* before separation by silica gel column chromatography to afford 5-((trimethylsilyl)ethynyl)pyrimidine-4,6-diamine (1.08 g; 61%) as an off-white solid.

R_f (10% MeOH/EtOAc): 0.45. **¹H NMR** (400 MHz, DMSO-*d*₆) δ 7.57 (s, 1H, H2), 6.14 (s, 4H, 2×NH₂), -0.00 (s, 9H, 3×CH₃). **¹³C NMR** (101 MHz, DMSO-*d*₆) δ 164.4 (C6 & C4), 157.0 (C2), 91.6 (C8), 77.8, 76.6. **HRMS** (ESI⁺): Found [M+H]⁺ 207.1063, C₉H₁₅N₄Si⁺ requires 207.1061. **mp** (°C): 189 – 192.

6.2.5 Synthesis of 5-ethynylpyrimidine-4,6-diamine **3.7** [5]

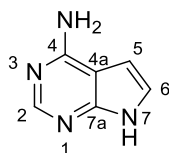


To a solution of 5-((trimethylsilyl)ethynyl)pyrimidine-4,6-diamine **3.6** (0.98g, 4.76 mmol) in methanol (150 ml) was added potassium carbonate (1.32g, 9.53 mmol). The mixture was stirred for 2 hours at room temperature before evaporating the solvent *in vacuo* and diluting with water (100 ml). The organic matter was extracted with ethyl acetate (3×100 ml), dried with anhydrous magnesium sulfate, filtered, and concentrated *in vacuo* to afford 5-ethynylpyrimidine-4,6-diamine

(0.411 g, 64%) as a white solid after purification of the concentrate by silica gel column chromatography.

R_f (10% MeOH/EtOAc): 0.38. **¹H NMR** (400 MHz, DMSO-*d*₆) δ 7.60 (s, 1H, H2), 6.22 (s, 4H, 2×NH₂), 4.36 (s, 1H, H8). **¹³C NMR** (101 MHz, DMSO-*d*₆) δ 164.4 (C6&C4), 157.0 (C2), 91.6 (C8), 77.8 (C7), 76.6 (C5). **HRMS** (ESI⁺): Found [M+H]⁺ 135.0665, C₆H₇N₄⁺ requires 135.0667. **mp** (°C): 145 – 147.

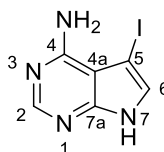
6.2.6 Synthesis of 7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **3.8** [5]



To a solution of 5-ethynylpyrimidine-4,6-diamine **3.7** (0.19 g, 1.42 mmol) in DMSO (3 ml) was added caesium carbonate (0.921 g, 2.83 mmol). The mixture was heated at 180°C for 15 minutes before cooling to room temperature and diluting with ethyl acetate (100 ml). The mixture was then washed with brine (5×100 ml), dried in anhydrous magnesium sulfate, and then concentrated *in vacuo*. The crude was purified using silica gel chromatography to afford 7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (95 mg; 50%) as a white solid.

R_f (20% MeOH/DCM): 0.26. **¹H NMR** (400 MHz, DMSO-*d*₆) δ 11.44 (s, 1H, H7), 8.01 (s, 1H, H2), 7.05 (t, *J* = 2.7 Hz, 1H, H6), 6.87 (s, 2H, NH₂), 6.50 (s, 1H, H5). **¹³C NMR** (101 MHz, DMSO-*d*₆) δ 157.8 (C4), 152.0 (C2), 151.1 (C7a), 121.2 (C6), 102.7 (C4a), 99.3 (C5). **mp** (°C): 264 – 266.

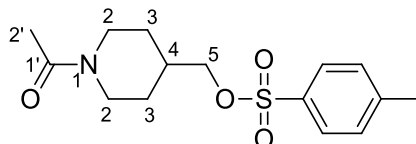
6.2.7 Synthesis of 5-iodo-7*H*-pyrrolo[2, 3-*d*]pyrimidin-4-amine **3.9** [6]



To a stirred suspension of 6-amino-7-deazapurine **3.8** (0.54 g, 4.02 mmol) in 20 ml chloroform was added *N*-iodosuccinimide (1.08 g, 4.82 mmol) and the resulting mixture was refluxed for two hours under nitrogen gas. After cooling, the solvent was removed *in vacuo* and the resulting solid was purified using silica gel column chromatography to afford 5-iodo-7*H*-pyrrolo[2, 3-*d*]pyrimidin-4-amine (0.94 g, 90%) as a yellow solid.

R_f (10% MeOH/DCM): 0.54. **¹H NMR** (300 MHz, DMSO-*d*₆) δ 11.96 (s, 1H, NH), 8.05 (s, 1H, H-2), 7.36 (d, *J* = 1.7 Hz, 1H, H6), 6.54 (s, 2H, NH₂). **¹³C NMR** (75 MHz, DMSO-*d*₆) δ 156.9 (C4), 151.7 (C7a), 150.5 (C2), 126.7 (C6), 102.6 (C4a), 50.1 (C5). **HRMS** (ESI⁺): Found [M+H]⁺ 260.9641, C₆H₆IN₄⁺ requires 260.9632.

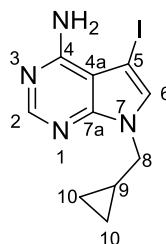
6.2.8 Synthesis of (1-acetylpiperidin-4-yl)methyl 4-methylbenzenesulfonate 3.11 [5]



A solution of 4-piperidinemethanol (2.00 g, 17.4 mmol) and triethylamine (6.1 ml, 43.4 mmol) in 20 ml DCM was cooled to 0 °C before carefully adding acetic anhydride (1.64 ml, 17.4 mmol). The mixture was stirred for 4 hours at room temperature under an inert gas, cooled to 0 °C, and then 4-toluenesulfonyl chloride (4.97, 26.0 mmol) was added slowly. The resulting mixture was stirred overnight at room temperature, diluted with ethyl acetate and washed with 50 ml saturated aqueous NaHCO₃, 1 M aqueous HCl, and brine successively. The organic fractions were dried with magnesium sulfate, filtered through celite and the excess solvent was evaporated *in vacuo*. Purification was achieved using silica gel chromatography to afford (1-acetylpiperidin-4-yl)methyl 4-methylbenzenesulfonate (2.24 g, 43%) as a yellow oil.

R_f (EtOAc): 0.21. **¹H NMR** (300 MHz, CDCl₃) δ 7.78 (d, *J* = 8.3 Hz, 2H, ArH), 7.36 (d, *J* = 8.0 Hz, 2H, ArH), 4.60 (ddt, *J* = 13.3, 4.7, 2.4 Hz, 1H, H2 equatorial), 3.91 – 3.74 (m, 3H, H5, H2 equatorial), 3.10 – 2.93 (m, 1H, H2 axial), 2.58 – 2.41 (m, 4H, H2', H2 axial), 2.07 (s, 3H, CH₃), 1.98 – 1.84 (m, 1H, H4), 1.86 – 1.73 (m, 1H, H3 equatorial), 1.65 (dt, *J* = 13.3, 2.8 Hz, 1H, H3 equatorial), 1.12 (dq, *J* = 24.7, 12.5, 4.4 Hz, 2H, H3 axial). **¹³C NMR** (75 MHz, CDCl₃) δ 168.8 (C1'), 145.0 (ArC), 132.7 (ArC), 129.9 (ArC), 127.9 (ArC), 73.6 (C5), 45.9 (C2), 40.9 (C2), 35.8 (C4), 28.9 (C3), 27.8 (C3), 21.7 (C2'), 21.5 (CH₃). **IR** (ν/cm⁻¹): 3071(=C–H); 2938 (–C–H); 1644 (R₂NC=O); 1348 (S=O); 1170 (C–N). **HRMS** (ESI⁺): Found [M+H]⁺ 312.1261, C₁₅H₂₂NO₄S⁺ requires 312.1271.

6.2.9 Synthesis of 7-(cyclopropylmethyl)-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine 3.12 [5]

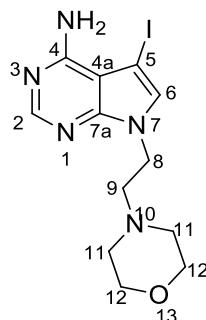


To a stirred solution of 5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **3.9** (0.42 g, 0.77 mmol) in 20 ml DMF was added caesium carbonate (0.501 g, 1.54 mmol) followed by (bromomethyl)cyclopropane (0.125 ml, 0.923 mmol). The resulting mixture was heated at 80°C under nitrogen for 18 hours, after which it was cooled down to room temperature, transferred to a separating funnel containing ethyl acetate (100 ml) and washed with brine (5×100 ml). The washed organic material was dried with magnesium sulfate, filtered through celite and concentrated *in vacuo*. 7-(cyclopropylmethyl)-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (0.18 g, 74%) was afforded as a light-yellow solid after purification of the concentrate with silica gel column.

R_f (60% EtOAc/chloroform): 0.21. **¹H NMR** (300 MHz, DMSO-*d*₆) δ 8.09 (s, 1H, H2), 7.52 (s, 1H, H6), 6.59 (s, 2H, NH₂), 3.95 (d, *J* = 7.2 Hz, 2H, H8), 1.21 (dq, *J* = 12.3, 7.5, 4.8 Hz, 1H, H9), 0.50 – 0.43 (m, 2H, H10), 0.41 – 0.34 (m, 2H, H10). **¹³C NMR** (126 MHz, DMSO-*d*₆) δ 157.6 (C4), 152.2 (C2), 150.0 (C7a), 129.8 (C6), 103.3 (C4a), 49.7 (C5), 48.7 (C8), 12.0 (C9), 4.1 (C10). **HRMS** (ESI⁺): Found [M+H]⁺ 315.0118, C₁₀H₁₂IN₄⁺ requires 315.0101. **mp** (°C): 250 – 252.

The analogues **3.13-3.14** were prepared in a similar way using a respective alkylating agent and silica gel chromatography mobile phase solutions.

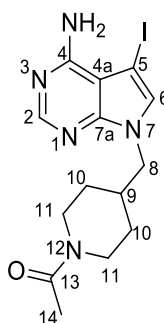
6.2.10 Synthesis of 5-iodo-7-(2-morpholinoethyl)-7H-pyrrolo[2,3-*d*]pyrimidin-4-amine 3.13 [5]



A reaction of 5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **3.9** (0.269 g, 1.14 mmol), caesium carbonate (0.742 g, 2.27 mmol), and 4-(2-chloroethyl)morpholine hydrochloride (0.233 g, 1.25 mmol) in *N,N*-dimethylformamide afforded 5-iodo-7-(2-morpholinoethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (0.250g, 59%) as a white to light brown solid after purification.

R_f (10% MeOH/chloroform): 0.38. **¹H NMR** (300 MHz, DMSO-*d*₆) δ 8.09 (s, 1H, H2), 7.48 (s, 1H, H6), 6.58 (s, 2H, NH₂), 4.22 (t, *J* = 6.5 Hz, 2H, H8), 3.51 (t, *J* = 4.5 Hz, 4H, H12), 2.63 (t, *J* = 6.5 Hz, 2H, H9), 2.41 (t, *J* = 4.5 Hz, 4H, H11). **¹³C NMR** (126 MHz, DMSO-*d*₆) δ 157.5 (C4), 152.2 (C2), 150.2 (C7a), 130.3 (C6), 103.3 (C4a), 66.6 (C12), 58.0 (C9), 53.6 (C11), 49.8 (C5), 41.4 (C8). **HRMS** (ESI⁺): Found [M+H]⁺ 374.0479, C₁₂H₁₇IN₅O⁺ requires 374.0433. **mp** (°C): 215 – 218.

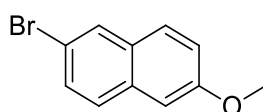
6.2.11 Synthesis of 1-{4-[(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethenone 3.14 [5]



A reaction of 5-iodo-7*H*-pyrrolo[2, 3-*d*]pyrimidin-4-amine **3.9** (0.20 g, 0.77 mmol), caesium carbonate (0.50 g, 1.54 mmol), and 1-acetyl piperidin-4-yl 4-methylbenzenesulfonate (0.29 g, 0.92 mmol) in *N,N*-dimethylformamide afforded 1-{4-[(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethenone (0.15 g; 48%) as an off-white solid after purification.

R_f (10% MeOH/chloroform): 0.34. **¹H NMR** (500 MHz, DMSO-*d*₆) δ 8.10 (s, 1H, H2), 7.45 (s, 1H, H6), 6.60 (s, 2H, NH₂), 4.32 (d, *J* = 13.1 Hz, 1H, H11), 4.01 (d, *J* = 7.3 Hz, 2H, H8), 3.77 (d, *J* = 13.7 Hz, 1H, H11), 2.97 – 2.89 (m, 1H, H11), 2.45 (td, *J* = 12.7, 2.9 Hz, 1H, H11), 2.10 – 2.02 (m, 1H, H9), 1.96 (s, 3H, H14), 1.45 (d, *J* = 13.2 Hz, 2H, H10), 1.12 (qd, *J* = 12.2, 4.2 Hz, 1H, H10), 1.01 (qd, *J* = 12.2, 4.4 Hz, 1H, H10). **¹³C NMR** (126 MHz, DMSO- *d*₆) δ 168.3 (C13), 157.6 (C4), 152.3 (C2), 150.3 (C7a), 130.4 (C6), 103.3 (C4a), 50.0 (C5), 49.6 (C8), 45.8 (C11), 40.9 (C11), 36.8 (C9), 30.2 (C10), 29.5 (C10), 21.1 (C14). **HRMS** (ESI⁺): Found [M+H]⁺ 400.0619, C₁₄H₁₉IN₅O⁺ requires 400.0629. **mp** (°C): 210 – 211.

6.2.12 Synthesis of 2-bromo-6-methoxynaphthalene 3.16a [5]

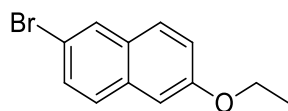


A solution of 6-bromonaphthalen-2-ol (1.00 g, 4.48 mmol) and potassium carbonate (1.24g, 8.97 mmol) in acetone (30 ml) was stirred at room temperature for 30 minutes before adding dimethylsulfate (0.47 ml, 4.93 mmol). The resulting mixture was stirred further overnight under reflux, cooled to room temperature, and transferred into a separating funnel containing brine (100 ml). The organic compounds were extracted with ethyl acetate (3×100 ml), dried with magnesium sulfate, filtered through celite, and concentrated *in vacuo*. The afforded precipitate was purified using silica gel chromatography to afford 2-bromo-6-methoxynaphthalene (1.05 g, 99 %) as a white solid.

R_f (Hexane): 0.21. **¹H NMR** (300 MHz, CDCl₃) δ 7.91 (d, *J* = 2.0 Hz, 1H, ArH), 7.62 (dd, *J* = 11.5, 8.9 Hz, 2H, ArH), 7.50 (dd, *J* = 8.7, 2.0 Hz, 1H, ArH), 7.16 (dd, *J* = 8.9, 2.5 Hz, 1H, ArH), 7.09 (d, *J* = 2.5 Hz, 1H, ArH), 3.91 (s, 3H, OCH₃). **¹³C NMR** (75 MHz, CDCl₃) δ 157.9 (ArC), 133.0 (ArC), 130.0 (ArC), 129.7 (ArC), 129.6 (ArC), 128.5 (ArC), 128.4 (ArC), 119.8 (ArC), 117.2 (ArC), 105.7 (ArC), 55.4 (CH₃). **IR** (v/cm⁻¹): 3060 (=C–H); 2967 (–C–H); 1624 (C=C); 1208 (C–O). **mp** (°C): 110–111.

The analogue **3.16b** was synthesised the same way using the appropriate alkylating agent.

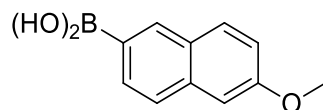
6.2.13 Synthesis of 2-bromo-6-ethoxynaphthalene 3.16b [5]



A reaction of 6-bromonaphthalen-2-ol (2.00 g, 8.97 mmol), potassium carbonate (2.48 g, 17.93 mmol), and ethylbromide (0.74 ml, 9.86 mmol) under acetone (50 ml) reflux afforded 2-bromo-6-ethoxynaphthalene (1.69 g, 75%) as a white solid after purification by silica gel column chromatography.

R_f (Hexane): 0.26. **¹H NMR** (300 MHz, CDCl₃) δ 7.91 (d, J = 1.9 Hz, 1H, ArH), 7.65 – 7.55 (m, 2H, ArH), 7.48 (dd, J = 8.7, 2.0 Hz, 1H, ArH), 7.15 (dd, J = 9.0, 2.5 Hz, 1H, ArH), 7.08 (d, J = 2.5 Hz, 1H, ArH), 4.13 (q, J = 7.0 Hz, 2H, OCH₂-), 1.48 (t, J = 7.0 Hz, 3H, CH₃). **¹³C NMR** (75 MHz, CDCl₃) δ 157.2 (ArC), 133.1 (ArC), 129.9 (ArC), 129.6 (ArC), 129.5 (ArC), 128.4 (ArC), 128.3 (ArC), 120.0 (ArC), 116.9 (ArC), 106.5 (ArC), 63.5 (CH₂), 14.8 (CH₃). **IR** (v/cm⁻¹): 3057 (=C–H); 2937 (–C–H); 1622 (C=C); 1206 (C–O). **mp** (°C): 82-26.

6.2.14 Synthesis of (6-methoxynaphthalen-2-yl)boronic acid 3.17a [5]

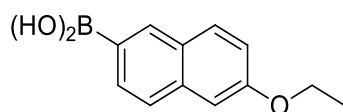


A solution of 2-bromo-6-methoxynaphthalene (1.00 g, 4.22 mmol) in dry THF was degassed for 15 minutes and cooled down to -78°C before adding *n*-butyllithium – 1.6 M in hexane (5.27 ml, 8.44 mmol) and stirring for 30 minutes. Triisopropyl borate (2.92 ml, 12.7 mmol) was then added and the resulting solution was stirred for 2 hours while warming up to room temperature from -78°C and then quenched with 2 M aqueous HCl (100 ml). The organic compounds were extracted with ethyl acetate (100ml), washed with brine (2×100 ml), dried with magnesium sulfate, filtered through celite, and concentrated *in vacuo*. The resulting solid was suspended in hexane, subjected to sonication, and then filtered to (6-methoxynaphthalen-2-yl)boronic acid (0.469 g, 55%) as a white solid.

R_f (10% EtOAc/hexane): 0.13. **¹H NMR** (400 MHz, DMSO-*d*₆) δ 8.29 (s, 1H, ArH), 7.82 (dd, J = 8.6, 5.4 Hz, 2H, ArH), 7.75 (d, J = 8.2 Hz, 1H, ArH), 7.29 (d, J = 2.4 Hz, 1H, ArH), 7.15 (dd, J = 8.9, 2.4 Hz, 1H, ArH), 3.89 (s, 3H, CH₃). **¹³C NMR** (101 MHz, DMSO-*d*₆) δ 158.24 (ArC), 135.90 (ArC), 135.22 (ArC), 131.66 (ArC), 130.37 (ArC), 128.37 (ArC), 125.85 (ArC), 118.73 (ArC), 106.17 (ArC), 55.65 (CH₃).

The boronic acid, **3.17b** was prepared in the same manner.

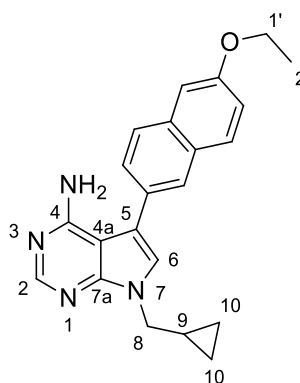
6.2.15 Synthesis of (6-ethoxynaphthalen-2-yl)boronic acid **3.17b** [7]



A reaction between 2-bromo-6-methoxynaphthalene (1.00 g, 3.98 mmol), *n*-butyllithium (0.75 ml, 7.96 mmol), and triisopropylborate (2.76 ml, 11.95 mmol) quenched with 2 M HCl (100 ml) afforded (6-ethoxynaphthalen-2-yl)boronic acid (0.53 g, 62%) as a white solid after purification.

R_f (10% EtOAc/hexane): 0.13. **¹H NMR** (500 MHz, DMSO-*d*₆) δ 8.29 (s, 1H, ArH), 8.06 (s, 2H, OH), 7.84 – 7.79 (m, 2H, ArH), 7.73 (d, *J* = 8.3 Hz, 1H, ArH), 7.27 (d, *J* = 2.5 Hz, 1H, ArH), 7.14 (dd, *J* = 8.9, 2.5 Hz, 1H, ArH), 4.15 (q, *J* = 7.0 Hz, 2H, CH₂), 1.40 (t, *J* = 7.0 Hz, 3H, CH₃). **¹³C NMR** (126 MHz, DMSO-*d*₆) δ 157.5 (ArC), 135.9 (ArC), 135.2 (ArC), 131.6 (ArC), 130.4 (ArC), 128.3 (ArC), 125.8 (ArC), 118.9 (ArC), 106.8 (ArC), 79.6 (ArC), 63.6 (CH₂), 15.1 (CH₃). **mp** (°C): 84-85.

6.2.16 Synthesis of 7-(cyclopropylmethyl)-5-(6-ethoxynaphthalen-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **3.18** [5]



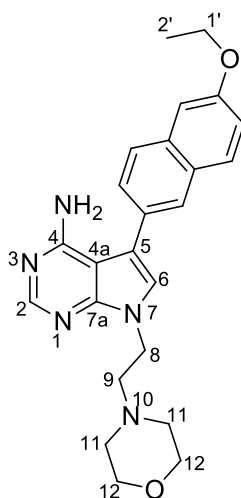
A dried two-necked round bottom flask was charged with 7-(cyclopropylmethyl)-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (0.100 g, 0.318 mmol), (6-ethoxynaphthalen-2-yl)boronic acid (0.103 g, 0.478 mmol), and Pd(PPh₃)₄ (0.037 g, 0.032 mmol). The mixture was put under inert gas for 10 minutes after which a separately degassed 1,2-dimethoxyethane (40 ml) and 2 M aqueous Na₂CO₃ (0.64 ml, 1.27 mmol) in additional water (17 ml) were added. The resulting solution was refluxed for 18 hours under nitrogen and then allowed to cool down to room temperature before concentrating *in vacuo* (only water remained). The remaining solutions was transferred into a separation funnel containing brine (100 ml) and extracted with ethyl acetate (3×100 ml). The

combined organic fractions were dried with magnesium sulfate, filtered through celite, and concentrated *in vacuo*. Purification of the concentrate was achieved using silica gel column chromatography to afford 7-(cyclopropylmethyl)-5-(6-ethoxynaphthalen-2-yl)-7H-pyrrolo[2,3-*d*]pyrimidin-4-amine (0.05 g, 39%) as a yellow-orange solid.

R_f (60 % EtOAc/ CHCl₃): 0.23. **¹H NMR** (400 MHz, CDCl₃) δ 8.34 (s, 1H, H2), 7.87 (s, 1H, ArH), 7.79 (dd, *J* = 16.2, 8.6 Hz, 2H, ArH), 7.58 (dd, *J* = 8.4, 1.7 Hz, 1H, ArH), 7.23 – 7.15 (m, 3H, ArH and H6), 5.26 (s, 2H, NH₂), 4.19 (q, *J* = 7.1 Hz, 2H, H1'), 4.12 (d, *J* = 7.1 Hz, 2H, H8), 1.51 (t, *J* = 6.9 Hz, 3H, H2'), 0.91 – 0.82 (m, 1H, H9), 0.68 – 0.61 (m, 2H, H10), 0.46 (m, 2H, H10). **¹³C NMR** (126 MHz, CDCl₃) δ 157.2 (ArC), 156.8 (C4), 151.4 (C2), 150.5 (C7a), 133.5 (ArC), 129.8 (ArC), 129.3 (ArC), 129.0 (C-6 and ArC), 127.6 (ArC), 127.5 (ArC), 127.1 (ArC), 122.7 (ArC), 119.9 (C5), 106.4 (ArC), 105.5 (C4a), 63.6 (C1'), 49.0 (C8), 14.8 (C2'), 11.4 (C9), 4.1 (C10). **HRMS** (ESI⁺): Found [M+H]⁺ 359.1848, C₂₂H₂₃N₄O⁺ requires 359.1886. **mp** (°C): 215 – 216.

Compounds **3.19**, **3.20a**, and **3.20b** were afforded in a similar manner.

6.2.17 Synthesis of 5-(6-ethoxynaphthalen-2-yl)-7-(2-(tetrahydro-2H-morpholin-4-yl)ethyl)-7H-pyrrolo[2,3-*d*]pyrimidin-4-amine **3.19** [5]

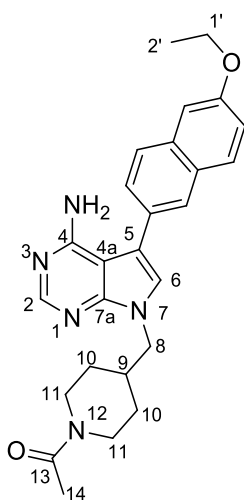


A reaction of 5-iodo-7-(2-morpholinoethyl)-7H-pyrrolo[2,3-*d*]pyrimidin-4-amine (0.080 g, 0.214 mmol), (6-ethoxynaphthalen-2-yl)boronic acid (0.069 g, 0.322 mmol), Pd(PPh₃)₄ (0.025 g, 0.021 mmol), and 2M aqueous Na₂CO₃ (0.43 ml, 0.86 mmol) afforded 5-(6-ethoxynaphthalen-2-yl)-7-(2-(tetrahydro-2H-morpholin-4-yl)ethyl)-7H-pyrrolo[2,3-*d*]pyrimidin-4-amine (0.03g; 32%) as an off-white solid after purification by silica gel column chromatography.

R_f (10% MeOH/ EtOAc): 0.27. **¹H NMR** (500 MHz, CDCl₃) δ 8.34 (s, 1H, H2), 7.85 (d, *J* = 1.7 Hz, 1H, ArH), 7.79 (dd, *J* = 20.0, 8.7 Hz, 2H, ArH), 7.57 (dd, *J* = 8.4, 1.7 Hz, 1H, ArH), 7.21 (dd,

$J = 8.8, 2.5$ Hz, 1H, ArH), 7.18 (d, $J = 2.5$ Hz, 1H, ArH), 7.12 (s, 1H, H6), 5.12 (s, 2H, NH₂), 4.38 (t, $J = 6.6$ Hz, 2H, H8), 4.19 (q, $J = 6.9$ Hz, 2H, H1'), 3.70 (t, $J = 4.6$ Hz, 4H, H12), 2.82 (t, $J = 6.6$ Hz, 2H, H9), 2.55 (t, $J = 4.6$ Hz, 4H, H11), 1.51 (t, $J = 6.9$ Hz, 3H, H2'). **¹³C NMR** (126 MHz, CDCl₃) δ 157.2 (ArC), 156.9 (C4), 151.9 (C2), 150.8 (C7a), 133.6 (ArC), 129.8 (ArC), 129.3 (ArC), 129.0 (C6 and ArC), 127.6 (ArC), 127.1 (ArC), 123.2 (ArC), 119.9 (ArC), 116.3 (C5), 106.5 (ArC), 101.2 (C4a), 67.0 (C12), 63.6 (C1'), 58.3 (C9), 53.7 (C11), 41.7 (C8), 14.8 (C2'). **HRMS** (ESI⁺): Found $[M+H]^+$ 418.2240, C₂₄H₂₈N₅O₂⁺ requires 418.2238.

6.2.18 Synthesis of 1-(4-((4-amino-5-(6-ethoxynaphthalen-2-yl)-7H-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl)piperidin-1-yl)ethenone **3.20a** [5]

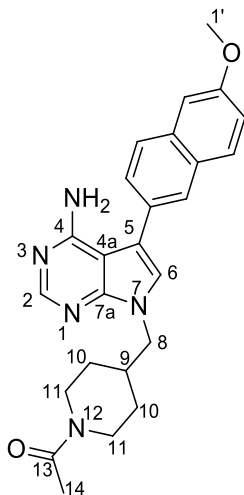


A reaction of 1-(4-((4-amino-5-iodo-7H-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl)piperidin-1-yl)ethenone (0.081 g, 0.20 mmol), (6-ethoxynaphthalen-2-yl)boronic acid (0.076 g, 0.30 mmol), Pd(PPh₃)₄ (0.023 g, 0.020 mmol), and aqueous 2 M Na₂CO₃ (0.41 ml, 0.81 mmol) afforded the 1-(4-((4-amino-5-(6-ethoxynaphthalen-2-yl)-7H-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl)piperidin-1-yl)ethenone (0.037 g; 41%) as a white solid after purification by silica gel column chromatography.

R_f (5% MeOH/ EtOAc): 0.19. **¹H NMR** (400 MHz, CDCl₃) δ 8.33 (s, 1H, H2), 7.86 – 7.73 (m, 3H, ArH), 7.56 (d, $J = 8.4$ Hz, 1H, ArH), 7.24 – 7.15 (m, 2H, ArH), 6.96 (s, 1H, H-6), 5.33 (s, 2H, NH₂), 4.63 (d, $J = 13.4$ Hz, 1H, H11 equatorial), 4.15 (m, 4H, H-8 and H1'), 3.81 (d, $J = 13.8$ Hz, 1H, H11 equatorial), 3.06 – 2.95 (m, 1H, H11 axial), 2.59 – 2.46 (m, 1H, H11-axial), 2.07 (s, 3H, H14), 1.70 (d, $J = 13.2$ Hz, 2H, H10 equatorial), 1.50 (t, $J = 6.9$ Hz, 3H, H2'), 1.33 – 1.18 (m, 3H, H10 axial and H9). **¹³C NMR** (101 MHz, CDCl₃) δ 168.8 (C-13), 157.2 (ArC and C4), 152.1 (C2), 150.9 (C7a), 133.4 (ArC), 129.6 (ArC), 129.2 (ArC), 129.0 (ArC), 127.6 (C6), 127.5 (ArC), 127.1 (ArC), 123.2 (ArC), 120.0 (ArC), 116.4 (C5), 106.5 (ArC), 101.3 (C4a), 63.6 (C1'), 50.0

(C8), 46.1 (C11), 41.2 (C11), 37.2 (C9), 30.4 (C10), 29.5 (C10), 21.5 (C14), 14.8 (C2'). **HRMS** (ESI⁺): Found [M+H]⁺ 444.2375, C₂₆H₂₉N₅O₂⁺ requires 444.2394. **mp** (°C): 215 – 217.

6.2.19 Synthesis of 1-(4-((4-amino-5-(6-methoxynaphthalen-2-yl)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)methyl)piperidin-1-yl)ethenone **3.20b** [5]

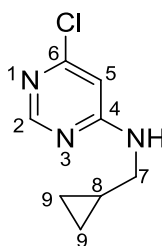


A reaction of 1-{4-[(4-amino-5-iodo-7H-pyrrolo[2,3-d]pyrimidin-7-yl)methyl]piperidin-1-yl}ethenone (0.079 g, 0.20 mmol), (6-methoxynaphthalen-2-yl)boronic acid (0.060 g, 0.30 mmol) Pd(PPh₃)₄ (0.023 g, 0.020 mmol), and aqueous 2 M Na₂CO₃ (0.40 ml, 0.79 mmol) afforded 1-(4-((4-amino-5-(6-methoxynaphthalen-2-yl)-7H-pyrrolo[2,3-d]pyrimidin-7-yl)methyl)piperidin-1-yl)ethenone (0.055 g; 65%) as a white solid after silica gel column chromatography purification.

R_f (5% MeOH/chloroform): 0.20. **¹H NMR** (400 MHz, CDCl₃) δ 8.33 (s, 1H, H2), 7.87 – 7.79 (m, 2H, ArH), 7.77 (d, *J* = 8.7 Hz, 1H, ArH), 7.57 (dd, *J* = 8.4, 1.7 Hz, 1H, ArH), 7.23 – 7.17 (m, 2H, ArH), 6.96 (s, 1H, H6), 5.37 (s, 2H, NH₂), 4.63 (d, *J* = 13.4 Hz, 1H, H11 equatorial), 4.13 (qd, *J* = 13.9, 7.2 Hz, 2H, H8), 3.94 (s, 3H, H1'), 3.81 (d, *J* = 13.9 Hz, 1H, H11 equatorial), 3.05 – 2.95 (m, 1H, H11 axial), 2.57 – 2.47 (m, 1H, H11 axial), 2.07 (s, 3H, H14), 1.70 (d, *J* = 13.0 Hz, 2H, H10 equatorial), 1.32 – 1.19 (m, 3H, H10 axial and H9). **¹³C NMR** (101 MHz, CDCl₃) δ 168.8 (C13), 157.9 (ArC), 157.3 (C4), 152.1 (C2), 150.9 (C7a), 133.5 (ArC), 129.71 (ArC), 129.3 (ArC), 129.1 (ArC), 127.6 (C6), 127.5 (ArC), 127.1 (ArC), 123.2 (ArC), 119.6 (ArC), 116.4 (C5), 105.7 (ArC), 101.2 (C4a), 55.4 (C1'), 50.0 (C8), 46.1 (C11), 41.2 (C11), 37.2 (C9), 30.4 (C10), 29.5 (C10), 21.4 (C14). **HRMS** (ESI⁺): Found [M+H]⁺ 430.2214, C₂₅H₂₈N₅O₂⁺ requires 430.2238. **mp** (°C): 214 – 216.

6.3 Synthesis of 4,6-diaminopyrimidine compounds: Two-atom linker derivatives

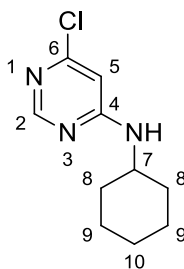
6.3.1 Synthesis of 6-chloro-*N*-(cyclopropylmethyl)pyrimidin-4-amine 4.2 [8]



To a suspension of 4,6-dichloropyrimidine (0.62 g, 4.17 mmol) in 2-methyltetrahydrofuran (100 ml) was added *N,N*-diisopropylethylamine (1.45 ml, 8.35 mmol) and cyclopropanemethylamine hydrochloride (0.54 g, 5.01 mmol). The mixture was refluxed under nitrogen for 18 hours after which it was cooled to room temperature and transferred into a separating funnel containing brine (100 ml). The organic compounds were extracted with ethyl acetate (3×100 ml), dried with anhydrous magnesium sulfate, filtered, and concentrated *in vacuo*. The resulting concentrate was purified using silica gel column chromatography affording 6-chloro-*N*-(cyclopropylmethyl)pyrimidin-4-amine (0.57 g, 75%) as a white solid.

R_f (20 % EtOAc/hexane): 0.10. **¹H NMR** (400 MHz, CDCl₃) δ 8.34 (s, 1H, H2), 6.34 (s, 1H, H5), 5.34 (s, 1H, NH), 3.15 (s, 2H, H7), 1.13 – 1.03 (m, 1H, H8), 0.64 – 0.55 (m, 2H, H9), 0.30 – 0.24 (m, 2H, H9). **¹³C NMR** (101 MHz, CDCl₃) δ 163.1 (C6 & C4), 158.5 (C2), 46.5 (C7), 10.4 (C8), 3.6 (C9). **IR** (ν/cm⁻¹): 3314 (N–H); 3083 (=C–H); 2964 (–C–H); 1599 (C=C); 1327 (C–N). **HRMS** (ESI⁺): Found [M+H]⁺ 184.0539, C₈H₁₁ClN₃⁺ requires 184.0534. **mp** (°C): 106 – 107.

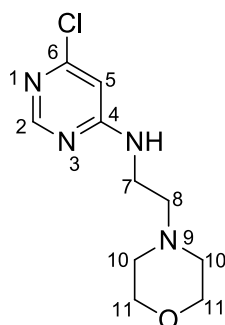
6.3.2 Synthesis of 6-chloro-*N*-cyclohexylpyrimidin-4-amine 4.3 [9]



Following the same method as described above, the reaction of 4,6-dichloropyrimidine (5.00 g, 33.56 mmol), *N,N*-diisopropylethylamine (11.69 ml, 67.12 mmol), and cyclohexylamine (4.61 ml, 40.27 mmol) afforded 6-chloro-*N*-cyclohexylpyrimidin-4-amine (6.00 g; 84%) as an off-white solid after a silica gel column chromatography.

R_f (20 % EtOAc/hexane): 0.38. **¹H NMR** (400 MHz, CDCl₃) δ 8.32 (s, 1H, H2), 6.31 (s, 1H, H5), 5.25 (s, 1H, NH), 2.01 (dt, *J* = 12.5, 3.9 Hz, 2H, H8 equatorial), 1.78 (dt, *J* = 13.3, 3.7 Hz, 2H, H8 axial), 1.67 (dt, *J* = 12.8, 4.0 Hz, 1H, H7), 1.47 – 1.34 (m, 2H, H9 equatorial), 1.31 – 1.16 (m, 4H, H9 axial and H10). **¹³C NMR** (101 MHz, CDCl₃) δ 162.4 (ArC), 158.5 (ArC), 50.1 (C7), 32.8 (C8), 25.4 (C9), 24.7 (C10). **IR** (ν/cm⁻¹): 3242 (N–H); 3033 (=C–H); 2930 (–C–H); 1651 (C=C); 1337 (C–N). **HRMS** (ESI⁺): Found [M+H]⁺ 212.0842, C₁₀H₁₅ClN₃⁺ requires 212.0847. **mp** (°C): 124 – 126.

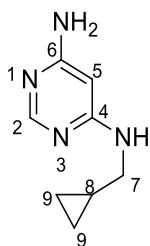
6.3.3 Synthesis of 6-chloro-*N*-(2-morpholinoethyl)pyrimidin-4-amine 4.4 [10]



Following the method described above, the reaction of 4,6-dichloropyrimidine (1.00 g, 6.71 mmol), *N,N*-diisopropylethylamine (2.34 ml, 13.42 mmol), and 4-(2-aminoethyl)morpholine (1.06 ml, 8.05 mmol) afforded 6-chloro-*N*-(2-morpholinoethyl)pyrimidin-4-amine as an off-white solid (1.57 g, 97%) after purification through silica gel column chromatography.

R_f (10 % MeOH/EtOAc): 0.43. **¹H NMR** (400 MHz, CDCl₃) δ 8.35 (s, 1H, H2), 6.38 (s, 1H, H5), 5.83 (s, 1H, NH), 3.73 (t, *J* = 4.6 Hz, 4H, H11), 3.39 (s, 2H, H7), 2.62 (t, *J* = 5.9 Hz, 2H, H8), 2.49 (t, *J* = 4.5 Hz, 4H, H10). **¹³C NMR** (101 MHz, CDCl₃) δ 163.0 (ArC), 158.4 (ArC), 66.8 (C11), 56.4 (C8), 53.2 (C10), 37.3 (C7). **IR** (ν/cm⁻¹): 3297 (N–H); 3140 (=C–H); 2898 (–C–H); 1589 (C=C); 1336 (C–N); 1105 (C–O). **mp** (°C): 61 – 63.

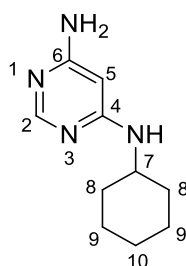
6.3.4 Synthesis of *N*⁴-(cyclopropylmethyl)pyrimidine-4,6-diamine and related compounds 4.5



A solution of 6-chloro-*N*-(cyclopropylmethyl)pyrimidin-4-amine (0.53 g, 2.89 mmol), ethanol (5 ml), and 25% aqueous ammonia (40 ml) was prepared in a sealed tube and heated at 170°C for 48 hours, after which it was allowed to cool down to room temperature and transferred to a separating funnel containing a saturated aqueous potassium carbonate solution. The organic material was extracted into ethyl acetate (3×100 ml), dried with magnesium sulfate, filtered, and concentrated *in vacuo*. The resulting concentrate was purified using silica gel chromatography to afford *N*⁴-(cyclopropylmethyl)pyrimidine-4,6-diamine (0.32 g, 68%) as a white solid.

R_f (10% MeOH/chloroform): 0.24. **¹H NMR** (400 MHz, CDCl₃) δ 8.11 (s, 1H, H2), 5.38 (s, 1H, H5), 4.98 (s, 1H, NH), 4.61 (s, 2H, NH₂), 3.03 (dd, *J* = 7.0, 5.3 Hz, 2H, H7), 1.12 – 1.01 (m, 1H, H8), 0.61 – 0.50 (m, 2H, H9), 0.31 – 0.18 (m, 2H, H9). **¹³C NMR** (101 MHz, CDCl₃) δ 163.04 (C6), 162.92 (C4), 158.28 (C2), 81.92 (C5), 46.53 (C7), 10.48 (C8), 3.48 (C9). **IR** (ν/cm⁻¹): 3464, 3306 (N–H); 3081 (=C–H); 2874 (–C–H); 1650 (C=C); 1326, 1160 (C–N). **HRMS** (ESI⁺): Found [M+H]⁺ 165.1051, C₈H₁₃N₄⁺ requires 165.1032. **mp** (°C): 137 – 139.

6.3.5 Synthesis of *N*⁴-cyclohexylpyrimidine-4,6-diamine 4.6 [11]



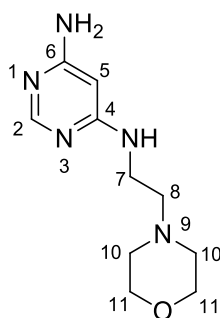
Method 1: Using the method described above, a solution of 6-chloro-*N*-cyclohexylpyrimidin-4-amine **7b** (2.50 g, 10.30 mmol), ethanol (5 ml), and 25% aqueous ammonia (40 ml) afforded *N*⁴-cyclohexylpyrimidine-4,6-diamine (1.82 g, 92%) as a white solid after purification by silica gel column chromatography.

Method 2: A mixture of 6-chloropyrimidin-4-amine (0.29 g, 2.26 mmol) and cyclohexylamine (0.66 ml, 5.33 mmol) in toluene (1.50 ml) was heated at 200°C for 1 hours by microwave

irradiation. The afforded reaction mixture was diluted with methanol and concentrated on silica *in vacuo*. The resulting concentrate was purified using silica gel chromatography to give *N*⁴-cyclohexylpyrimidine-4,6-diamine (0.30 g; 69%) as a white solid.

R_f (10% MeOH/EtOAc): 0.25. **¹H NMR** (400 MHz, CDCl₃) δ 8.09 (s, 1H, H2), 5.38 (s, 1H, H5), 4.76 (s, 1H, NH), 4.58 (s, 2H, NH₂), 3.36 (s, 1H, H7), 2.03 – 1.96 (m, 2H, H8 equatorial), 1.76 (dt, *J* = 13.6, 4.3 Hz, 2H, H8 axial), 1.69 – 1.60 (m, 1H, H10 equatorial), 1.43 – 1.32 (m, 2H, H9 equatorial), 1.29 – 1.16 (m, 3H, H9 axial and H10 axial). **¹³C NMR** (101 MHz, CDCl₃) δ 162.7 (C6), 161.9 (C4), 157.9 (C2), 53.4 (C5), 50.0 (C7), 32.9 (C8), 25.6 (C10), 24.7 (C9). **IR** (v/cm⁻¹): 3297, 3314; (N–H) 3129 (=C–H); 2923(–C–H). **HRMS** (ESI⁺): Found [M+H]⁺ 193.1333, C₁₀H₁₇N₄⁺ requires 193.1345. **mp** (°C): 210 – 211.

6.3.6 Synthesis of *N*⁴-(2-morpholinoethyl)pyrimidine-4,6-diamine 4.7

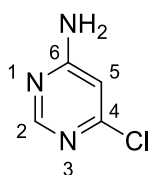


Method 1: A solution of 6-chloro-*N*-(2-morpholinoethyl)pyrimidin-4-amine **7c** (1.10 g, 4.52 mmol), ethanol (5 ml), and 25% aqueous ammonia (40 ml) afforded *N*⁴-(2-morpholinoethyl)pyrimidine-4,6-diamine (0.54 g, 54 %) as a light brown translucent solid after purification by silica gel column chromatography.

Method 2: A mixture of 6-chloropyrimidin-4-amine (0.40 g, 3.10 mmol) and 4-(2-aminoethyl)morpholine (0.81 ml, 6.21 mmol) in toluene (1 ml) was heated by microwave irradiation at 200°C for 1 hour to afford *N*⁴-(2-morpholinoethyl)pyrimidine-4,6-diamine (0.64 g; 92%) as a light brown translucent solid after recrystallization.

R_f (10% MeOH/chloroform): 0.10. **¹H NMR** (400 MHz, CDCl₃) δ 8.10 (s, 1H, H2), 5.45 (t, *J* = 5.0 Hz, 1H, NH), 5.39 (s, 1H, H5), 4.83 (s, 2H, NH₂), 3.71 (t, *J* = 4.6 Hz, 4H, H11), 3.26 (m, 2H, H7), 2.59 (t, *J* = 6.0 Hz, 2H, H8), 2.47 (t, *J* = 4.5 Hz, 4H, H10). **¹³C NMR** (101 MHz, CDCl₃) δ 163.2 (C6), 162.9 (C4), 158.3 (C2), 82.2 (C5), 66.9 (C11), 56.7 (C-8), 53.3 (C-10), 37.6 (C-7). **IR** (v/cm⁻¹): 3383, 3326 (N–H); 3091 (=C–H); 2965 (–C–H); 1146 (C–N); 1109 (C–O). **mp** (°C): 155 -157.

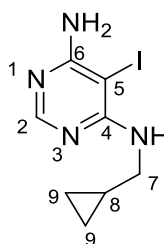
6.3.7 Synthesis of 6-chloropyrimidin-4-amine 4.8 [12]



To a solution of 4,6-dichloropyrimidine (5.00 g, 33.56 mmol) in isopropanol (20 ml) was added 25% aqueous ammonia (50 ml) and the resulting solution was heated at 140°C for 1 hour before cooling down to 0°C. The formed precipitate was collected by filtration and washed with water to afford 6-chloropyrimidin-4-amine as a white solid (3.03 g; 70%).

R_f (EtOAc): 0.50. **¹H NMR** (400 MHz, DMSO-*d*₆) δ 8.20 (d, *J* = 0.9 Hz, 1H, H2), 7.24 (s, 2H, NH₂), 6.44 (s, 1H, H5). **¹³C NMR** (101 MHz, DMSO-*d*₆) δ 165.2 (C6), 159.1 (C4), 158.3 (C2), 103.1 (C5). **mp** (°C): 204 - 207.

6.3.8 Synthesis of *N*⁴-(cyclopropylmethyl)-5-iodopyrimidine-4,6-diamine and related compounds 4.9



Method 1: To a suspension of *N*⁴-(cyclopropylmethyl)pyrimidine-4,6-diamine (0.50 g, 3.04 mmol) in water (50 ml) and *N,N*-dimethylformamide (20 ml) was added potassium carbonate (0.631 g, 4.57 mmol) followed by iodine (1.55 g, 6.09 mmol). The mixture was heated for 3 hours at 45°C after which it was cooled down to room temperature and quenched with 2 M aqueous sodium thiosulfate (40 ml). In a case where a precipitate formed, the mixture was filtered to afford the pure product, otherwise, the organic material was extracted with ethyl acetate (100 ml) and washed with brine (5×100 ml). The ethyl acetate mixture was dried with magnesium sulfate, filtered through celite, and concentrated *in vacuo* to afford *N*⁴-(cyclopropylmethyl)-5-iodopyrimidine-4,6-diamine (0.82 g, 62%) as a yellow solid after purification by silica gel column chromatography.

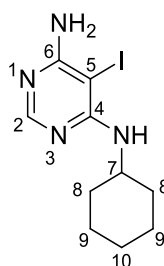
Method 2: To a microwave vessel with *N*⁴-(cyclopropylmethyl)pyrimidine-4,6-diamine (213 mg, 1.30 mmol) dissolved in a mixture of DMF (1 ml) and water (5 ml) was added potassium carbonate (269 mg, 1.95 mmol) followed by iodine (658 mg, 2.59 mmol). The mixture was heated at 60 °C

for 30 minutes before cooling and adding enough saturated aqueous sodium thiosulfate to dissolve the unreacted iodine and extracting the organic material using ethyl acetate (3×100 ml). The ethyl acetate fractions were dried with magnesium sulfate, filtered, and concentrated *in vacuo* to afford *N*⁴-(cyclopropylmethyl)-5-iodopyrimidine-4,6-diamine (0.42 g, 75%) as a yellow solid after purification by silica gel column chromatography.

R_f (80% EtOAc/Hex): 0.43. **¹H NMR** (400 MHz, CDCl₃) δ 7.99 (s, 1H, H2), 5.22 – 5.13 (m, 3H, NH and NH₂), 3.32 (dd, *J* = 7.1, 5.2 Hz, 2H, H7), 1.16-1.02 (m, H8), 0.61 – 0.50 (m, 2H, H9), 0.34 – 0.21 (m, 2H, H9). **¹³C NMR** (101 MHz, CDCl₃) δ 161.6 (C6), 160.7 (C4), 157.3 (C2), 58.2 (C5), 47.0 (C7), 10.8 (C8), 3.4 (C9). **IR** (ν/cm⁻¹): 3404, 3281 (N–H); 3074 (C=C); 1304, 1145 (C–N); 536 (C–I). **mp** (°C): 124 – 127.

Compounds **4.10** and **4.11** were prepared following the procedures described for compound **4.9** above.

6.3.9 Synthesis of *N*⁴-cyclohexyl-5-iodopyrimidine-4,6-diamine **4.10**



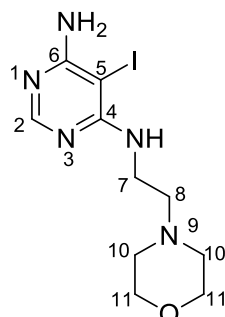
Method 1: A reaction of *N*⁴-cyclohexylpyrimidine-4,6-diamine (0.47g, 2.46 mmol), potassium carbonate (0.51 g, 3.68 mmol) and iodine (1.25 g, 4.91 mmol) afforded *N*⁴-cyclohexyl-5-iodopyrimidine-4,6-diamine as an off-white solid (0.60 g, 51%).

Method 2: A microwave reaction of *N*⁴-cyclohexylpyrimidine-4,6-diamine (0.20 g, 1.03 mmol), potassium carbonate (0.21 g, 1.54 mmol), and iodine (0.52 mg, 2.06 mmol) in DMF/water solvent mixture (1:5) afforded *N*⁴-cyclohexyl-5-iodopyrimidine-4,6-diamine as an off-white solid (0.33 g, 96%).

R_f (80 % EtOAc/hexane): 0.45. **¹H NMR** (400 MHz, CDCl₃) δ 7.98 (s, 1H, H2), 5.12 (s, 2H, NH₂), 4.98 (d, *J* = 8.0 Hz, 1H, NH), 4.03 – 3.89 (m, 1H, H7), 2.07 – 1.96 (m, 2H, H8), 1.79 – 1.70 (m, 2H, two of H9), 1.69 – 1.60 (m, 1H, one of H10), 1.50 – 1.37 (m, 2H, two of H9), 1.30 – 1.17 (m, 3H, H8 and one of H10). **¹³C NMR** (101 MHz, CDCl₃) δ 161.5 (C6), 160.0 (C4), 157.3 (C2), 58.5 (C5), 50.2 (C7), 33.3 (C8), 25.7 (C10), 24.8 (C9). **IR** (ν/cm⁻¹): 3394, 3307 (N–H); 3113 (=C–H);

2924 (–C–H); 1638 (C=N); 638 (C–I). **HRMS** (ESI+): Found $[M+H]^+$ 319.0220, $C_{10}H_{16}IN_4^+$ requires 319.0312. **mp** (°C): 128 – 130.

6.3.10 Synthesis of 5-iodo-*N*⁴-(2-morpholinoethyl)pyrimidine-4,6-diamine 4.11

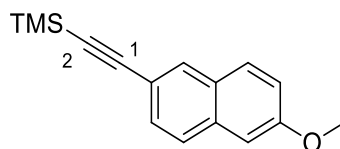


Method 1: A reaction of *N*⁴-(2-morpholinoethyl)pyrimidine-4,6-diamine (0.12 g, 0.56 mmol), potassium carbonate (0.12 g, 0.83), and iodine (0.28 mg, 1.11 mmol) afforded 5-iodo-*N*⁴-(2-morpholinoethyl)pyrimidine-4,6-diamine (0.13 mg, 65%) as an off-white solid.

Method 2: A microwave reaction of *N*⁴-(2-morpholinoethyl)pyrimidine-4,6-diamine (388 mg, 1.74 mmol), potassium carbonate (0.36 g, 2.61 mmol), and iodine (0.88 g, 3.48 mmol) afforded 5-iodo-*N*⁴-(2-morpholinoethyl)pyrimidine-4,6-diamine (0.31 g, 50%) as a white solid after purification

R_f (10% MeOH/EtOAc): 0.24. **¹H NMR** (400 MHz, CDCl₃) δ 7.99 (s, 1H, H2), 5.94 (s, 1H, NH), 5.00 (s, 2H, NH₂), 3.76 (t, *J* = 4.5 Hz, 4H, H11), 3.52 (q, *J* = 5.6 Hz, 2H, H7), 2.62 (t, *J* = 6.1 Hz, 2H, H8), 2.52 (t, *J* = 4.6 Hz, 4H, H10). **¹³C NMR** (101 MHz, CDCl₃) δ 161.5 (C6), 160.8 (C4), 157.5 (C-2), 67.2 (C-11), 58.2 (C-5), 56.4 (C-8), 53.1 (C-10), 38.0 (C-7). **IR** (ν/cm^{−1}): 3345, 3299 (N–H); 3118 (=C–H); 2848 (–C–H); 1638 (C=N); 1577 (C=C); 645 (C–I). **HRMS** (ESI+): Found $[M+H]^+$ 350.0258, $C_{10}H_{17}IN_5O^+$ requires 350.0370. **mp** (°C): decompose at 163 – 165.

6.3.11 Synthesis of [(6-methoxynaphthalen-2-yl)ethynyl]trimethylsilane 4.12a [13]



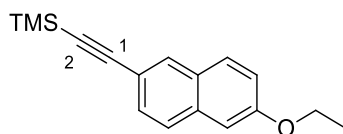
Method 1: To a degassed mixture of 2-bromo-6-methoxynaphthalene (1.00 g, 4.22 mmol), copper(I) iodide (0.024 g, 0.13 mmol), and Pd(PPh₃)₂Cl₂ (0.15 g, 0.21 mmol) was added a degassed solution of ethynyltrimethylsilane (3.11 ml, 42.18 mmol) and diisopropylamine (5.91 ml, 42.18 mmol) in distilled tetrahydrofuran (20 ml). The resulting mixture was heated at 60 °C under

nitrogen for 5 hours after which it was quenched with a saturated aqueous ammonium chloride solution (100 ml) and transferred to a separating funnel where the organic compounds were extracted with ethyl acetate (3×100 ml). The ethyl acetate fractions were dried with magnesium sulfate, filtered through celite, and concentrated *in vacuo*. The resulting crude was purified using silica gel column chromatography to afford [(6-methoxynaphthalen-2-yl)ethynyl]trimethylsilane (1.02 g, 95%) as a yellow solid.

Method 2: To a 35 ml microwave vessel charged with 2-bromo-6-methoxynaphthalene (0.59 g, 2.49 mmol), copper(I) iodide (0.014 g, 0.075 mmol), and Pd(PPh₃)₂Cl₂ (0.087 g, 0.12 mmol) was added a degassed mixture of 2-methyltetrahydrofuran (6 ml), DIPA (3.49 ml, 24.89 mmol) and, ethynyltrimethylsilane (1.84 ml, 24.89 mmol). The resulting mixture was heated for 30 minutes at 70°C by microwave irradiation before cooling down to room temperature and quenching with a saturated aqueous solution of ammonium chloride (100 ml). The organic material was extracted with ethyl acetate (100 ml) and then washed with brine (2×100 ml), dried with magnesium sulfate, and filtered. The concentrated crude was purified using silica gel column chromatography to afford [(6-methoxynaphthalen-2-yl)ethynyl]trimethylsilane (613 mg, 97%) as a yellow solid.

R_f(Hexane): 0.32. **¹H NMR** (400 MHz, CDCl₃) δ 7.64 (dd, *J* = 3.6, 1.8 Hz, 1H, ArH), 7.45 – 7.29 (m, 2H, ArH), 7.21 (ddd, *J* = 10.2, 8.6, 1.8 Hz, 1H, ArH), 6.92 – 6.78 (m, 2H, ArH), 3.64 (d, *J* = 1.0 Hz, 3H, OCH₃), 0.00 (s, 9H, SiCH₃). **¹³C NMR** (101 MHz, CDCl₃) δ 158.3 (ArC), 134.2 (ArC), 131.8 (ArC), 129.3 (ArC), 129.2 (ArC), 128.4 (ArC), 126.6 (ArC), 119.3 (ArC), 117.9 (ArC), 105.7 (ArC), 105.6 (C2), 93.6 (C1), 55.3 (CH₃), 0.0 (Si-C). **IR** (ν/cm⁻¹): 3060 (=C–H) 2958 (–C–H); 2153 (C≡C); 1626 (C=C); 1243 (C–O). **HRMS** (ESI⁺): Found [M+H]⁺ 255.1130, C₁₆H₁₉OSi⁺ requires 255.1127. **mp** (°C): 69 – 71.

6.3.12 Synthesis of [(6-ethoxynaphthalen-2-yl)ethynyl]trimethylsilane 4.12b [14]

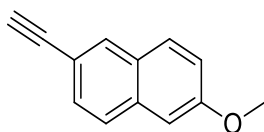


Method 1: A reaction of 2-bromo-6-ethoxynaphthalene (2.00 g, 7.96 mmol), copper(I) iodide (0.046g, 0.239 mmol), Pd(PPh₃)₂Cl₂ (0.28 g, 0.40 mmol), ethynyltrimethylsilane (5.88 ml, 79.6 mmol), and diisopropylamine (11.2 ml, 79.6 mmol) in distilled tetrahydrofuran (20 ml) afforded [(6-ethoxynaphthalen-2-yl)ethynyl]trimethylsilane (1.95 g; 91%) as a yellow solid after a silica gel column chromatography

Method 2: A microwave reaction of 2-bromo-6-ethoxynaphthalene (2.24 g, 8.89 mmol) copper(I) iodide (0.051 mg, 0.28 mmol), and Pd(PPh₃)₂Cl₂ (0.31 mg, 0.45 mmol) with a degassed mixture of 2-methyltetrahydrofuran (6 ml), DIPA (2.49 ml, 17.79 mmol) and, ethynyltrimethylsilane (1.31 ml, 17.79 mmol) afforded [(6-ethoxynaphthalen-2-yl)ethynyl]trimethylsilane (2.28 g, 95%) as a yellow solid.

R_f (Hexane): 0.43. **¹H NMR** (400 MHz, CDCl₃) δ 7.91 (s, 1H, ArH), 7.64 (dd, *J* = 18.9, 8.6 Hz, 2H, ArH), 7.45 (dd, *J* = 8.5, 1.5 Hz, 1H, ArH), 7.14 (dd, *J* = 8.8, 2.4 Hz, 1H, ArH), 7.08 (d, *J* = 2.5 Hz, 1H, ArH), 4.14 (q, *J* = 7.0 Hz, 2H, CH₂), 1.48 (t, *J* = 7.0 Hz, 3H, CH₃), 0.27 (s, 9H, Si-CH₃). **¹³C NMR** (101 MHz, CDCl₃) δ 157.6 (ArC), 134.2 (ArC), 131.7 (ArC), 129.2 (ArC), 129.0 (ArC), 128.2 (ArC), 126.5 (ArC), 119.6 (ArC), 117.8 (ArC), 106.5 (ArC), 105.7 (C2), 93.5 (C1), 63.5 (CH₂), 14.7 (CH₃), 0.0 (Si-C). **IR** (ν/cm⁻¹): 3058 (=C-H); 2979(-C-H); 2151(C≡C); 1621 (C=C); 1208 (C-O). **HRMS** (ESI⁺): Found [M+H]⁺ 269.1122, C₁₇H₂₁OSi⁺ requires 269.1129. **mp** (°C): 110 – 111.

6.3.13 Synthesis of 2-ethynyl-6-methoxynaphthalene 4.13a [14]

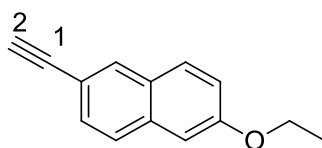


Method 1: A solution of [(6-methoxynaphthalen-2-yl)ethynyl]trimethylsilane (1.30 g, 5.10 mmol) in distilled tetrahydrofuran (30 ml) was cooled down to 0 °C before adding tetrabutylammonium fluoride (1.77 ml, 6.12 mmol) slowly. The resulting mixture was warmed up to room temperature and stirred overnight, after which it was quenched with a saturated aqueous solution of ammonium chloride (100 ml). The organic material in the mixture was extracted with ethyl acetate (100 ml), dried with magnesium sulfate, filtered through celite, and concentrated *in vacuo*. The afforded crude was purified using silica gel chromatography to afford 2-ethynyl-6-methoxynaphthalene (0.79 g, 85%). as a white solid.

Method 2: A mixture of [(6-methoxynaphthalen-2-yl)ethynyl]trimethylsilane (0.79 g, 3.09 mmol) and potassium carbonate (0.85 g, 6.18 mmol) in methanol (50 ml) was stirred at room temperature for 1 hour. The mixture was then diluted with water (100 ml) and extracted in ethyl acetate (3×100 ml) before drying with anhydrous magnesium sulfate, filtering, and concentrating *in vacuo*. The resulting crude was purified using silica gel column chromatography to afford 2-ethynyl-6-methoxynaphthalene (0.52 g; 93%) as a white solid.

R_f(Hexane): 0.18. **¹H NMR** (400 MHz, CDCl₃) δ 7.95 (s, 1H, ArH), 7.68 (t, *J* = 8.8 Hz, 2H, ArH), 7.49 (dd, *J* = 8.4, 1.7 Hz, 1H, ArH), 7.16 (dd, *J* = 8.9, 2.5 Hz, 1H, ArH), 7.11 (d, *J* = 2.5 Hz, 1H, ArH), 3.92 (s, 3H, OCH₃), 3.10 (s, 1H, ≡CH). **¹³C NMR** (101 MHz, CDCl₃) δ 158.5 (ArH), 134.4 (ArH), 132.1 (ArH), 129.3 (ArH), 129.2 (ArH), 128.3 (ArH), 126.8 (ArH), 119.5 (ArH), 117.0 (ArH), 105.8 (ArH), 84.2 (C-2), 77.3-76.7 (C-1), 55.35 (CH₃). **IR** (ν/cm⁻¹): 3265 (≡C-H); 3061 (=C-H), 2970 (-C-H); 2107 (C≡C); 1625 (C=C); 1267 (C-O). **mp** (°C): 106 – 107.

6.3.14 Synthesis of 2-ethoxy-6-ethynynaphthalene 4.13b [14]

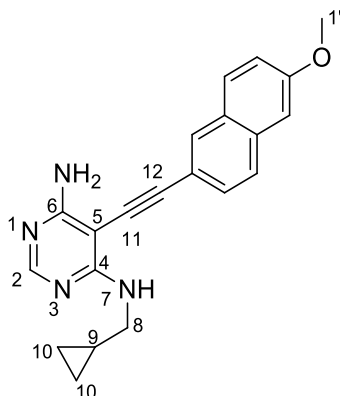


Method 1: A reaction of [(6-ethoxynaphthalen-2-yl)ethynyl]trimethylsilane **10b** (0.770 g, 2.87 mmol) and tetrabutylammonium fluoride (1.00 ml, 3.44 mmol) in distilled tetrahydrofuran (30 ml) afforded 2-ethoxy-6-ethynynaphthalene (0.44 g, 74%) as a white solid after silica gel column chromatography purification.

Method 2: A reaction between [(6-ethoxynaphthalen-2-yl)ethynyl]trimethylsilane (0.94 g, 3.51 mmol) and potassium carbonate (0.97 mg, 7.01 mmol) in methanol (50 ml) afforded 2-ethoxy-6-ethynynaphthalene (0.67 g; 98%) as a white solid after silica gel column chromatography purification.

R_f (Hexane): 0.13. **¹H NMR** (300 MHz, CDCl₃) δ 7.98 – 7.90 (m, 1H, ArH), 7.67 (dd, *J* = 11.7, 8.7 Hz, 2H, ArH), 7.48 (dd, *J* = 8.4, 1.7 Hz, 1H, ArH), 7.16 (dd, *J* = 8.9, 2.5 Hz, 1H, ArH), 7.09 (d, *J* = 2.5 Hz, 1H, ArH), 4.15 (q, *J* = 7.0 Hz, 2H, CH₂), 3.10 (s, 1H, ≡CH), 1.48 (t, *J* = 7.0 Hz, 3H, CH₃). **¹³C NMR** (101 MHz, CDCl₃) δ 157.8 (ArC), 134.4 (ArC), 132.1 (ArC), 129.3 (ArC), 129.1 (ArC), 128.2 (ArC), 126.8 (ArC), 119.8 (ArC), 116.8 (ArC), 106.5 (ArC), 84.2 (C2), 76.6 (C1), 63.6 (CH₂), 14.8 (CH₃). **IR** (ν/cm⁻¹): 3269 (≡C-H); 3054 (=C-H); 2982 (-C-H); 2112 (C≡C), 1621 (C=C). **mp** (°C): 86 – 88.

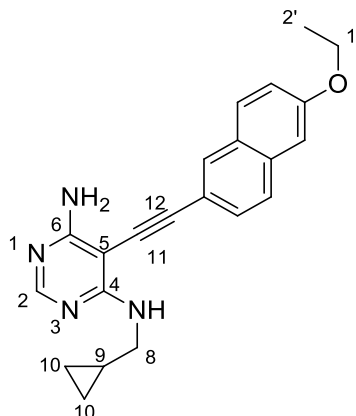
6.3.15 Synthesis of *N*⁴-(cyclopropylmethyl)-5-[(6-methoxynaphthalen-2-yl)ethynyl]pyrimidine-4,6-diamine 4.14a



To a degassed mixture of *N*⁴-(cyclopropylmethyl)-5-iodopyrimidine-4,6-diamine (0.20 g, 0.69 mmol), methoxy-6-ethynylnaphthalene (0.26 g, 1.45 mmol), copper(I) iodide (4.0 mg, 0.021 mmol), and Pd(PPh₃)₂Cl₂ (0.051 g, 0.072 mmol), in a microwave vessel was added a degassed solution of diisopropylamine (0.51 ml, 3.62 mmol) in 2-methyltetrahydrofuran (20 ml). The resulting mixture was heated at 70 °C under nitrogen for 30 minutes after which it was cooled to room temperature and transferred to a separating funnel containing water (100 ml). The organic compounds were extracted in ethyl acetate (3×100 ml) and dried with anhydrous magnesium sulfate, filtered through celite, and concentrated *in vacuo*. The resulting crude was purified using silica gel column chromatography to afford *N*⁴-(cyclopropylmethyl)-5-[(6-methoxynaphthalen-2-yl)ethynyl]pyrimidine-4,6-diamine (0.20 g, 80%) as a brown solid.

R_f (EtOAc): 0.19. **¹H NMR** (400 MHz, CDCl₃) δ 8.16 (s, 1H, H2), 7.96 (s, 1H, ArH), 7.73 (d, *J* = 8.7 Hz, 2H, ArH), 7.52 (d, *J* = 8.7 Hz, 1H, ArH), 7.21 – 7.12 (m, 2H, ArH), 5.47 (s, 1H, NH), 5.12 (s, 2H, NH₂), 3.96 (s, 3H, H1'), 3.38 (d, *J* = 7.3 Hz, 2H, H8), 1.13 (s, 1H, H9), 0.57 (d, *J* = 7.7 Hz, 2H, two of H10), 0.31 (s, 2H, two of H10). **¹³C NMR** (101 MHz, CDCl₃) δ 162.4 (C6), 161.9 (C4), 158.6 (ArC), 156.8 (C2), 134.3 (ArC), 131.1 (ArC), 129.35 (ArC), 128.66 (ArC), 128.43, 127.05 (ArC), 119.7 (ArC), 117.5, 105.9 (ArC), 102.2 (C12), 79.5 (C5 & C11), 55.4 (C1'), 46.0 (C8), 11.0 (C9), 3.4 (C10). **IR** (ν/cm⁻¹): 3480, 3423 (N–H); 3057 (=C–H); 3005 (–C–H); 2190 (C=C); 1637 (C=N); 1210 (C–O). **HRMS** (ESI⁺): Found [M+H]⁺ 345.1711, C₂₁H₂₁N₄O⁺ requires 345.1710. **mp** (°C): 158 – 160.

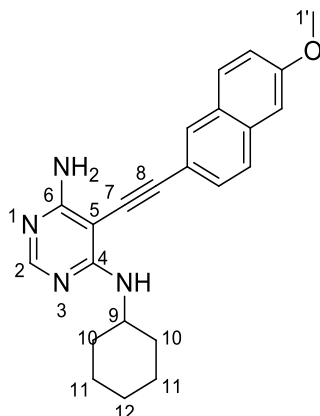
6.3.16 Synthesis of *N*⁴-(cyclopropylmethyl)-5-[(6-ethoxynaphthalen-2-yl)ethynyl]pyrimidine-4,6-diamine 4.14b



To a microwave tube loaded with *N*⁴-(cyclopropylmethyl)-5-iodopyrimidine-4,6-diamine (210 mg, 0.723 mmol), 2-ethoxy-6-ethynylnaphthalene (0.28 mg, 1.45 mmol), Pd(PPh₃)₂Cl₂ (0.051 g, 0.072 mmol), and CuI (4.0 mg, 0.022 mmol) was added a degassed mixture of diisopropylamine (0.51 ml, 0.36 mmol) in 2-methyltetrahydrofuran (5ml). The reaction was heated for 60 minutes at 70 °C before cooling to room temperature, diluting with water (100 ml), and extracting with ethyl acetate. The fractions were dried with magnesium sulfate, concentrated *in vacuo* and purified using a silica gel column with ethyl acetate as an eluent to afford *N*⁴-(cyclopropylmethyl)-5-[(6-ethoxynaphthalen-2-yl)ethynyl]pyrimidine-4,6-diamine (0.22 g, 83%) as a yellow solid.

R_f (EtOAc): 0.28. **¹H NMR** (400 MHz, CDCl₃) δ 8.15 (s, 1H, H₂), 7.95 (s, 1H, ArH), 7.72 (t, *J* = 7.9 Hz, 2H, ArH), 7.50 (d, *J* = 8.6 Hz, 1H, ArH), 7.19 (d, *J* = 9.2 Hz, 1H, ArH), 7.12 (s, 1H, ArH), 5.47 (s, 1H, NH), 5.18 (s, 2H, NH₂), 4.17 (q, *J* = 7.2 Hz, 2H, H1'), 3.39 (t, *J* = 6.4 Hz, 2H, H₈), 1.50 (t, *J* = 7.2 Hz, 3H, H_{2'}), 1.13 (p, *J* = 6.7, 6.2 Hz, 1H, H₉), 0.57 (d, *J* = 7.7 Hz, 2H, two of H₁₀), 0.30 (d, *J* = 4.9 Hz, 2H, two of H₁₀). **¹³C NMR** (101 MHz, CDCl₃) δ 162.5 (C₆), 161.9 (C₄), 157.9 (ArC), 156.8 (C₂), 134.3 (ArC), 131.1 (ArC), 129.3 (ArC), 128.6 (ArC), 128.3 (ArC), 127.0 (ArC), 120.0 (ArC), 117.4 (ArC), 106.6 (ArC), 102.22 (C₁₂), 80.6 (C₅), 79.4 (C₁₁), 63.6 (C_{1'}), 46.0 (C₈), 14.8 (C_{2'}), 11.1 (C₉), 3.4 (C₁₀). **IR** (ν/cm⁻¹): 3478, 3285 (N–H); 3060 (–C–H); 2975 (–C–H); 2187 (C≡C); 1637 (C=C); 1632 (C=N); 1205 (C–O). **HRMS** (ESI⁺): Found [M+H]⁺ 359.1866, C₂₂H₂₃N₄O⁺ requires 359.1866. **mp** (°C): 162 – 164.

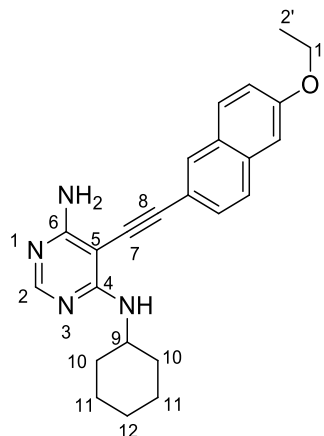
6.3.17 Synthesis of *N*⁴-cyclohexyl-5-[(6-methoxynaphthalen-2-yl)ethynyl]pyrimidine-4,6-diamine 4.15a



A microwave reaction of *N*⁴-cyclohexyl-5-iodopyrimidine-4,6-diamine (0.23 g, 0.72 mmol), 2-methoxy-6-ethynyl naphthalene (0.26 g, 1.43 mmol), copper(I) iodide (4.0 mg, 0.02 mmol), Pd(PPh₃)₂Cl₂ (25.0 mg, 0.036 mmol), and diisopropylamine (1.00 ml, 7.17 mmol) in 2-methyltetrahydrofuran (5 ml) afforded *N*⁴-cyclohexyl-5-[(6-methoxynaphthalen-2-yl)ethynyl]pyrimidine-4,6-diamine (0.23 g, 85%) as a solid after purification by silica gel column chromatography.

R_f (50 % EtOAc/hexane): 0.35. **¹H NMR** (400 MHz, CDCl₃) δ 8.15 (s, 1H, H2), 7.94 (s, 1H, ArH), 7.74 (d, *J* = 1.4 Hz, 1H, ArH), 7.72 (s, 1H, ArH), 7.50 (dd, *J* = 8.4, 1.7 Hz, 1H, ArH), 7.19 (dd, *J* = 8.9, 2.5 Hz, 1H, ArH), 7.13 (d, *J* = 2.5 Hz, 1H, ArH), 5.24 (d, *J* = 8.2 Hz, 1H, NH), 5.17 (s, 2H, NH₂), 4.10 – 3.98 (m, 1H, H9), 3.94 (s, 3H, H1'), 2.11 – 2.02 (m, 2H, H10), 1.81 – 1.71 (m, 2H, two of H11), 1.69 – 1.60 (m, 1H, one of H12), 1.53 – 1.37 (m, 2H, two of H11), 1.32 – 1.20 (m, 3H, one of H12 & two of H10). **¹³C NMR** (101 MHz, CDCl₃) δ 162.4 (C6), 161.3 (C4), 158.5 (ArC), 156.8 (C2), 134.3, 131.01 (ArC), 129.3 (ArC), 128.7 (ArC), 128.4, 127.0 (ArC), 119.7 (ArC), 117.5 (ArH), 105.9 (ArC), 102.2 (C8), 79.6 (C5 & C7), 55.4 (C1'), 49.4 (C9), 33.6 (C10), 25.7 (C12), 24.9 (C11). **IR** (v/cm⁻¹): 3462, 3364 (N–H); 3059 (–C–H); 2925 (–C–H); 2186 (C≡C); 1316 (C–N); 1206 (C–O). **HRMS** (ESI⁺): Found [M+H]⁺ 373.2024, C₂₃H₂₅N₄O⁺ requires 373.2023. **mp** (°C): 151 – 152.

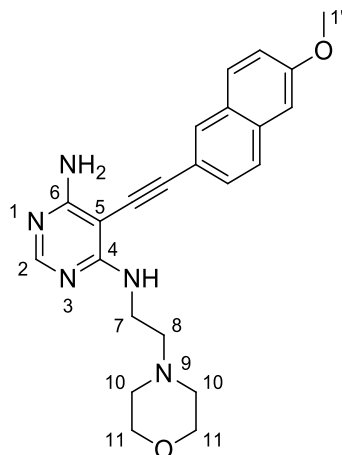
6.3.18 Synthesis of *N*⁴-cyclohexyl-5-[(6-ethoxynaphthalen-2-yl)ethynyl]pyrimidine-4,6-diamine 4.15b



A microwave reaction of *N*⁴-cyclohexyl-5-iodopyrimidine-4,6-diamine (151 mg, 0.474 mmol), 2-ethoxy-6-ethynynaphthalene (186 mg, 0.949 mmol), copper(I) iodide (2.7 mg, 0.014 mmol), Pd(PPh₃)₂Cl₂ (33 mg, 0.047 mmol), and diisopropylamine (0.33 ml, 2.37 mmol) in 2-methyltetrahydrofuran (5 ml) afforded *N*⁴-cyclohexyl-5-[(6-ethoxynaphthalen-2-yl)ethynyl]pyrimidine-4,6-diamine (0.17 g, 90%) as a light brown solid following purification by silica gel column chromatography.

R_f (50 % EtOAc/hexane): 0.38. **¹H NMR** (400 MHz, CDCl₃) δ 8.15 (s, 1H, H2), 7.96 – 7.86 (m, 1H, ArH), 7.76 – 7.67 (m, 2H, ArH), 7.49 (dd, *J* = 8.4, 1.7 Hz, 1H, ArH), 7.18 (dd, *J* = 8.9, 2.4 Hz, 1H, ArH), 7.12 (d, *J* = 2.5 Hz, 1H, ArH), 5.25 (d, *J* = 8.2 Hz, 1H, NH), 5.19 (s, 2H, NH₂), 4.17 (q, *J* = 7.0 Hz, 2H, H1'), 4.10 – 3.98 (m, 1H, H9), 2.10 – 2.02 (m, 2H, two of H10), 1.76 (dt, *J* = 13.5, 3.9 Hz, 2H, two of H11), 1.65 (d, *J* = 17.1 Hz, 1H, one of H12), 1.52 – 1.38 (m, 5H, H2' & two of H11), 1.33 – 1.17 (m, 3H, two of H10 & one of H12). **¹³C NMR** (101 MHz, CDCl₃) δ 162.3 (C6), 161.2 (C4), 157.9 (ArC), 156.6 (C2), 134.3 (ArC), 131.0 (ArC), 129.3 (ArC), 128.6 (ArC), 128.4 (ArC), 127.0 (ArC), 120.0 (ArC), 117.4 (ArC), 106.6 (ArC), 102.3 (C8), 79.4 (C5), 63.6 (C1'), 49.4 (C9), 33.6 (C10), 25.7 (C12), 24.9 (C11), 14.8 (C2'). **IR** (ν/cm⁻¹): 3469, 3295 (N–H); 3058 (=C–H); 2925 (–C–H); 2191 (C≡C); 1624 (C=C); 1204 (C–O). **HRMS** (ESI⁺): Found [M+H]⁺ 387.2182, C₂₄H₂₇N₄O⁺ requires 387.2179. **mp** (°C): 83 – 84.

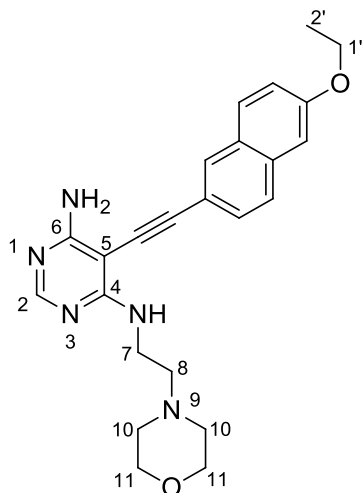
6.3.19 Synthesis of 5-[(6-methoxynaphthalen-2-yl)ethynyl]-*N*⁴-(2-morpholinoethyl)pyrimidine-4,6-diamine 4.16a



A microwave reaction of 5-iodo-*N*⁴-(2-morpholinoethyl)pyrimidine-4,6-diamine (0.50 g, 1.43 mmol), 2-methoxy-6-ethynylnaphthalene (0.52 g, 2.86 mmol), copper(I) iodide (8.2 mg, 0.04 mmol), Pd(PPh₃)₂Cl₂ (0.050 g, 0.07 mmol), and diisopropylamine (2.01 ml, 14.32 mmol) in 2-methyltetrahydrofuran (5 ml) afforded 5-[(6-methoxynaphthalen-2-yl)ethynyl]-*N*⁴-(2-morpholinoethyl)pyrimidine-4,6-diamine (0.46g; 79%) as a solid after purification using silica gel column chromatography.

Rf (80% EtOAc/MeOH): 0.33. **¹H NMR** (400 MHz, CDCl₃) δ 8.17 (s, 1H, H2), 7.95 (s, 1H, ArH), 7.72 (t, *J* = 9.1 Hz, 2H, ArH), 7.53 (d, *J* = 8.4 Hz, 1H, ArH), 7.19 (d, *J* = 9.1 Hz, 1H, ArH), 7.14 (s, 1H, ArH), 6.22 (s, 1H, NH), 5.32 (s, 2H, NH₂), 3.95 (s, 3H, H1'), 3.70 (s, 4H, H11), 3.60 (s, 2H, H8), 2.69 – 2.60 (m, 2H, H11), 2.53 (s, 4H, H10). **HRMS** (ESI+): Found [M+H]⁺ 404.2084, C₂₃H₂₆N₅O₂⁺ requires 404.2081. **mp** (°C): 233-234.

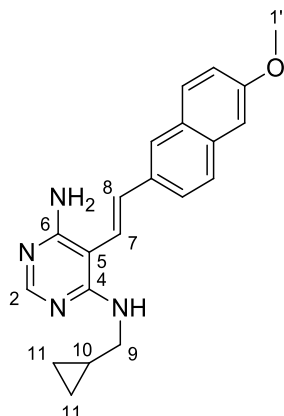
6.3.20 Synthesis of 5-[(6-ethoxynaphthalen-2-yl)ethynyl]-*N*⁴-(2-morpholinoethyl)pyrimidine-4,6-diamine 4.16b



A microwave reaction of 5-iodo-*N*⁴-(2-morpholinoethyl)pyrimidine-4,6-diamine (0.24 g, 0.69 mmol), 2-ethoxy-6-ethynyl-naphthalene (0.27 g, 1.37 mmol), copper(I) iodide (3.9 mg, 0.02 mmol), Pd(PPh₃)₂Cl₂ (0.024 g, 0.03 mmol), and diisopropylamine (0.96 ml, 6.87 mmol) in 2-methyltetrahydrofuran (5 ml) afforded 5-[(6-ethoxynaphthalen-2-yl)ethynyl]-*N*⁴-(2-morpholinoethyl)pyrimidine-4,6-diamine (0.24 g; 83%) as a solid after purification by silica gel column chromatography.

R_f (80% EtOAc/MeOH): 0.38. **¹H NMR** (400 MHz, CDCl₃) δ 8.16 (s, 1H, H₂), 7.95 (s, 1H, ArH), 7.71 (d, *J* = 8.6 Hz, 2H, ArH), 7.51 (dt, *J* = 8.6, 2.0 Hz, 1H, ArH), 7.19 (dd, *J* = 9.0, 2.4 Hz, 1H, ArH), 7.12 (d, *J* = 2.5 Hz, 1H, ArH), 6.20 (s, 1H, NH), 5.23 (s, 2H, NH₂), 4.17 (q, *J* = 7.1 Hz, 2H, H1'), 3.71 (t, *J* = 4.7 Hz, 4H, H11), 3.62 (d, *J* = 5.8 Hz, 2H, H₈), 2.67 (t, *J* = 6.3 Hz, 2H, H₇), 2.55 (s, 4H, H10), 1.49 (td, *J* = 7.0, 2.8 Hz, 3H, H₂'). **¹³C NMR** (101 MHz, CDCl₃) δ 134.3 (C₂), 130.8 (ArH), 129.2 (ArH), 128.4 (ArH), 127.0 (ArH), 120.1 (ArH), 106.6 (ArH), 67.0 (C11), 63.6 (C1'), 56.9 (C₇), 53.3 (C10), 37.0 (C₈), 14.8 (C₂'). **HRMS** (ESI⁺): Found [M+H]⁺ 418.2239, C₂₄H₂₈N₅O₂⁺ requires 418.2238.

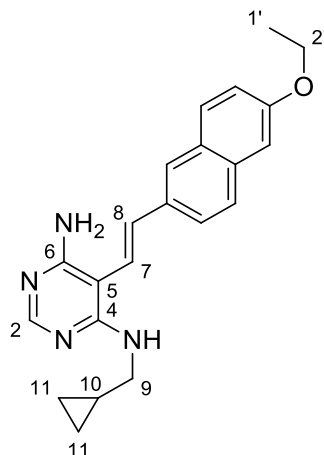
6.3.21 Synthesis of (*E*)-*N*⁴-(cyclopropylmethyl)-5-[2-(6-methoxynaphthalen-2-yl)vinyl]pyrimidine-4,6-diamine 4.17a



A reaction between *N*⁴-(cyclopropylmethyl)-5-[(6-methoxynaphthalen-2-yl)ethynyl]pyrimidine-4,6-diamine (84 mg, 0.24 mmol) and excess hydrogen gas at 6 bar, catalysed with 10 wt% palladium on carbon catalyst (16 mg, 20 wt% of starting material) in ethanol (20 ml) afforded (*E*)-*N*⁴-(cyclopropylmethyl)-5-[2-(6-methoxynaphthalen-2-yl)vinyl]pyrimidine-4,6-diamine (65 mg, 77%) as a light brown solid in 6 hours after purification by silica gel column chromatography.

R_f (50% EtOAc/hexane): 0.31. **¹H NMR** (400 MHz, CDCl₃) δ 8.20 (s, 1H, H₂), 7.67 – 7.62 (m, 2H, ArH), 7.58 (d, *J* = 8.6 Hz, 1H, ArH), 7.36 (dd, *J* = 8.6, 1.8 Hz, 1H, ArH), 7.11 (dd, *J* = 8.9, 2.5 Hz, 1H, ArH), 7.06 (d, *J* = 2.5 Hz, 1H, ArH), 6.93 (d, *J* = 11.9 Hz, 1H, H₇), 6.20 (d, *J* = 11.9 Hz, 1H, H₈), 4.68 (t, *J* = 5.2 Hz, 1H, NH), 4.56 (s, 2H, NH₂), 3.91 (d, *J* = 1.9 Hz, 3H, H_{1'}), 3.16 (dd, *J* = 7.1, 5.1 Hz, 2H, H₉), 0.81 – 0.66 (m, 1H, H₁₀), 0.28 – 0.18 (m, 2H, two of H₁₁), -0.03 – -0.12 (m, 2H, two of H₁₁). **¹³C NMR** (101 MHz, CDCl₃) δ 158.6 (C₆), 158.6 (C₄), 158.3 (ArC), 157.0 (C₂), 134.9 (C₇), 134.5 (ArC), 131.4 (ArC), 129.8 (ArC), 128.6 (ArC), 128.2 (ArC), 127.3 (ArC), 127.0 (ArC), 125.9 (ArC), 119.2 (ArC), 118.6 (C₈), 105.6 (C₇), 93.8 (C₅), 55.3 (C_{1'}), 46.1 (C₉), 10.7 (C₁₀), 3.1 (C₁₁). **IR** (ν/cm⁻¹): 3473, 3292 (N–H); 3143 (=C–H); 2950 (–C–H); 1685 (C=N); 1625 (C–O). **HRMS** (ESI⁺): Found [M+H]⁺ 347.1868, C₂₁H₂₃N₄O⁺ requires 347.1566.

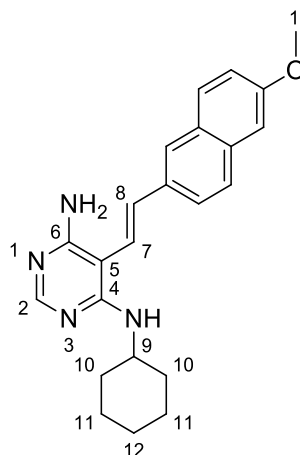
6.3.22 Synthesis of (*E*)-*N*⁴-(cyclopropylmethyl)-5-[2-(6-ethoxynaphthalen-2-yl)vinyl]pyrimidine-4,6-diamine **4.17b**



Using the method described above, the reaction between *N*⁴-(cyclopropylmethyl)-5-[(6-methoxynaphthalen-2-yl)ethynyl]pyrimidine-4,6-diamine (100 mg, 0.28 mmol) and hydrogen gas catalysed by 10 wt% palladium on carbon (10 mg, 10 wt%) afforded (*E*)-*N*⁴-(cyclopropylmethyl)-5-[2-(6-ethoxynaphthalen-2-yl)vinyl]pyrimidine-4,6-diamine (68 mg, 68%) as a light brown solid after purification by silica gel column chromatography.

R_f (50% EtOAc/hexane): 0.35. **¹H NMR** (400 MHz, CDCl₃) δ 8.20 (s, 1H, H2), 7.66 – 7.54 (m, 3H, ArH), 7.34 (dd, *J* = 8.6, 1.9 Hz, 1H, ArH), 7.13 – 7.04 (m, 2H, ArH), 6.94 (d, *J* = 11.9 Hz, 1H, H7), 6.19 (d, *J* = 11.9 Hz, 1H, H8), 4.71 (t, *J* = 5.2 Hz, 1H, NH), 4.66 (s, 2H, NH₂), 4.17 – 4.11 (m, 2H, H2'), 3.16 (dd, *J* = 7.1, 5.1 Hz, 2H, H9), 1.49 – 1.45 (m, 3H, H1'), 0.80 – 0.66 (m, 1H, H10), 0.28 – 0.18 (m, 2H, two of H11), -0.03 – -0.12 (m, 2H, two of H11). **¹³C NMR** (101 MHz, CDCl₃) δ 158.5 (C6), 158.3 (C4), 157.7 (ArC), 156.5 (C2), 135.1 (C7), 134.5 (ArC), 131.3 (ArC), 129.7 (ArC), 128.6 (ArC), 128.3 (ArC), 127.0 (ArC), 125.8 (ArC), 119.5 (ArC), 118.3 (C8), 106. (ArC), 93.8 (C5), 63.5 (C2'), 46.1 (C9), 14.8 (C1'), 10.7 (C10), 3.1 (C11). **IR** (v/cm⁻¹): 3472 (=C-H), 3290 (N-H); 3127 (–C–H); 2981 (–C–H); 1626 (C=C); 1256 (C–O). **HRMS** (ESI⁺): Found [M+H]⁺ 361.2024, C₂₂H₂₅N₄O⁺ requires 361.2023. **mp** (°C): 171 – 173.

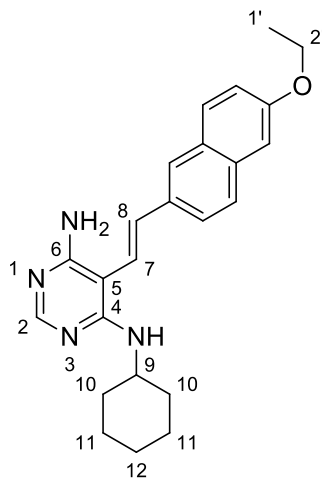
6.3.23 Synthesis of (*E*)-*N*⁴-cyclohexyl-5-[2-(6-methoxynaphthalen-2-yl)vinyl]pyrimidine-4,6-diamine 4.18a



A reaction between *N*⁴-cyclohexyl-5-[(6-methoxynaphthalen-2-yl)ethynyl]pyrimidine-4,6-diamine (68 mg, 0.18 mmol) and hydrogen gas catalysed by 10 wt% palladium on carbon (14 mg) afforded (*E*)-*N*⁴-cyclohexyl-5-[2-(6-methoxynaphthalen-2-yl)vinyl]pyrimidine-4,6-diamine (45 mg, 66%) after silica gel column chromatography.

R_f (50% EtOAc/hexane): 0.21. **¹H NMR** (300 MHz, CDCl₃) δ 8.19 (s, 1H, H₂), 7.66 – 7.56 (m, 3H, ArH), 7.34 (dd, *J* = 8.6, 1.8 Hz, 1H, ArH), 7.12 (dd, *J* = 8.9, 2.5 Hz, 1H, ArH), 7.07 (d, *J* = 2.5 Hz, 1H, ArH), 6.92 (d, *J* = 11.9 Hz, 1H, H₇), 6.15 (d, *J* = 11.9 Hz, 1H, H₈), 4.60 (s, 2H, NH₂), 4.51 (d, *J* = 8.1 Hz, 1H, NH), 3.93 – 3.85 (m, 4H, H_{1'} & H₉), 1.83 – 1.68 (m, 2H, H₁₀), 1.57 – 1.43 (m, 2H, H₁₁), 1.35 – 1.20 (m, 2H, H₁₁), 1.09 – 0.96 (m, 1H, H₁₂), 0.90 – 0.79 (m, 2H, H₁₀ & H₁₂). **¹³C NMR** (101 MHz, CDCl₃) δ 158.3 (C₆), 157.93 (C₄), 156.6 (C₂), 135.1 (C₇), 134.5 (ArC), 131.4 (ArC), 129.8 (ArC), 128.6 (ArC), 128.1 (ArC), 127.1 (ArC), 125. (ArC), 119.2 (ArC), 118.5 (C₈), 105.6 (ArC), 93.8 (C₅), 55.3 (C_{1'}), 49.1 (C₉), 33.3 (C₁₀), 25.6 (C₁₂), 24.7 (C₁₁). **IR** (v/cm⁻¹): 3417, 3300 (N–H); 3131 (=C–H); 2850 (–C–H); 1625 (C=C); 1258 (C–O). **HRMS** (ESI⁺): Found [M+H]⁺ 375.2180, C₂₃H₂₇N₄O⁺ requires 375.2179. **mp** (°C): 78 – 80.

6.3.24 Synthesis of (*E*)-*N*⁴-cyclohexyl-5-[2-(6-ethoxynaphthalen-2-yl)vinyl]pyrimidine-4,6-diamine 4.18b

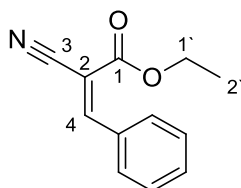


A 10 wt% palladium on carbon (10 mg) catalysed reaction between *N*⁴-cyclohexyl-5-[(6-ethoxynaphthalen-2-yl)ethynyl]pyrimidine-4,6-diamine (52 mg, 0.14 mmol) and hydrogen gas in ethanol (20 ml) afforded (*E*)-*N*⁴-cyclohexyl-5-[2-(6-ethoxynaphthalen-2-yl)vinyl]pyrimidine-4,6-diamine (33 mg, 63%) as a light brown solid after purification via silica gel column chromatography.

R_f (50% EtOAc/hexane): 0.29. **¹H NMR** (300 MHz, CDCl₃) δ 8.19 (s, 1H, H2), 7.63 (dd, *J* = 5.3, 3.6 Hz, 2H, ArH), 7.56 (d, *J* = 8.6 Hz, 1H, ArH), 7.38 – 7.29 (m, 1H, ArH), 7.13 – 7.05 (m, 2H, ArH), 6.92 (d, *J* = 11.9 Hz, 1H, H7), 6.14 (d, *J* = 11.9 Hz, 1H, H8), 4.65 (s, 2H, NH₂), 4.51 (d, *J* = 8.3 Hz, 1H, NH), 4.14 (q, *J* = 7.0, 2.7 Hz, 2H, H2'), 3.98 – 3.79 (m, 1H, H9), 1.80 – 1.72 (m, 2H, two of H10), 1.56 – 1.42 (m, 4H, two of H11 & H2'), 1.32 – 1.17 (m, 2H, two of H11), 1.09 – 0.74 (m, 4H, two of H10 & H12). **¹³C NMR** (101 MHz, CDCl₃) δ 158.2 (C6), 157.9 (C4), 157.6 (ArH), 156.4 (C2), 135.2 (C7), 134.5 (ArH), 131.3 (ArH), 129.73 (ArH), 128.6 (ArH), 128.1 (ArH), 127.1 (ArH), 125.8 (ArH), 119.5 (ArH), 118.4 (C8), 106.4 (ArH), 93.6 (C5), 63.5 (C2'), 49.1 (C9), 33.3 (C10), 25.56 (C12), 24.65 (C11), 14.78 (C1'). **IR** (ν/cm⁻¹): 3430 (=C-H), 3299 (N-H); 3057 (=C-H); 2723 (-C-H); 1628 (C=C); 1254 (C-O). **HRMS** (ESI⁺): Found [M+H]⁺ 389.2338, C₂₄H₂₉N₄O⁺ requires 389.2297. **mp** (°C): 159 – 160.

6.4 Synthesis of 4,6-diaminopyrimidine compounds: Carbon linker derivatives

6.4.1 Synthesis of ethyl-2-cyano-3-phenylacrylate 4.21 [15]

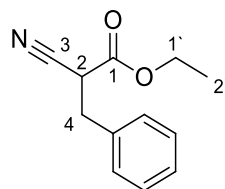


Method 1: A mixture of ethyl cyanoacetate (3.00 ml; 28.11 mmol), benzaldehyde (2.87 ml; 28.11 mmol), and piperidine (3 drops) in absolute ethanol (10 ml) was heated for 2 hours at 80°C by microwave irradiation (150W). Upon cooling to room temperature, a precipitate formed and was collected by filtration. The precipitate was recrystallised with ethanol and water to afford ethyl-2-cyano-3-phenylacrylate (5.22 g; 92%) as a white crystalline solid

Method 2: A mixture of ethyl cyanoacetate (1.00 ml, 9.37 mmol), benzaldehyde (1.00 ml; 9.84 mmol), and piperidine (5 drops) was heated at a constant microwave power of 10 W for 10 minutes. A formed precipitate upon cooling to room temperature was recrystallised with ethanol to give ethyl-2-cyano-3-phenylacrylate (1.85 g; 98%) as a white solid.

R_f (5% EtOAc/Hexane): 0.43. **¹H NMR** (400 MHz, CDCl₃) δ 8.26 (s, 1H, H₄), 8.04 – 7.96 (m, 2H, ArH), 7.59 – 7.48 (m, 3H, ArH), 4.39 (q, *J* = 7.1 Hz, 2H, H_{1'}), 1.41 (t, *J* = 7.1 Hz, 3H, H_{2'}). **¹³C NMR** (101 MHz, CDCl₃) δ 162.5 (C₁), 155.1 (C₄), 133.3 (ArC), 131.5 (ArC), 131.1 (ArC), 129.3 (ArC), 115.5 (C₃), 103.0 (C₂), 62.8 (C_{1'}), 14.2 (C_{2'}). **mp** (°C): 51-54.

6.4.2 Synthesis of ethyl 2-cyano-3-phenylpropanoate 4.22 [16]



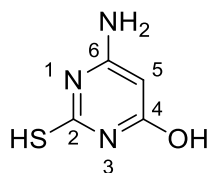
Method 1: To a solution of ethyl-2-cyano-3-phenylacrylate (0.58 g; 2.89 mmol) with absolute ethanol (20 ml) at 0°C was added NaBH₄ (0.13 mg; 3.47 mmol) carefully and then stirred for 5 minutes before quenching with aqueous HCl (0.62 ml of 32% aqueous HCl diluted with 40 ml H₂O; 6.35 mmol). The organic matter was extracted with ethyl acetate (3×100 ml) dried with anhydrous magnesium sulfate, filtered, and concentrated *in vacuo*. Ethyl 2-cyano-3-

phenylpropanoate (0.31 g; 53%) was obtained as colourless oil after purification of the crude by silica gel column chromatography.

Method 2: To a mixture of ethyl-2-cyano-3-phenylacrylate (5.06 g, 25.12 mmol), 10%wt. palladium on carbon (0.51 g), and methanol (20 ml) was added hydrogen gas at 6-bar with stirring for 30 minutes. The catalyst was recovered by filtration and the filtrate was concentrated *in vacuo*. Ethyl 2-cyano-3-phenylpropanoate (3.94 g; 77%) was afforded as a colourless oil after purification of the concentrate by silica gel column chromatography.

R_f (10% EtOAc/ Hexane): 0.33. **¹H NMR** (300 MHz, CDCl₃) δ 7.40 – 7.18 (m, 5H, ArH), 4.21 (q, *J* = 7.1 Hz, 2H, H1'), 3.71 (dd, *J* = 8.3, 5.9 Hz, 1H, H2), 3.22 (qd, *J* = 13.8, 7.1 Hz, 2H, H4), 1.25 (t, *J* = 7.2 Hz, 3H, H2'). **¹³C NMR** (75 MHz, CDCl₃) δ 165.5 (C1), 135.4 (ArC), 129.0 (ArC), 128.9 (ArC), 127.8 (ArC), 116.2 (C3), 62.9 (C1'), 39.7 (C2), 35.7 (C4), 13.9 (C2').

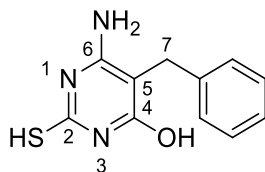
6.4.3 Synthesis of 6-amino-2-mercaptopyrimidin-4-ol 4.23 [17]



To a 35 ml microwave vessel loaded with a ground mixture of thiourea (0.80 g, 10.46 mmol) and potassium *tert*-butoxide (1.17g, 10.46 mmol) was added ethyl cyanoacetate (2.23 ml, 20.91 mmol). The resulting mixture was microwave irradiated at 10 W for 5 minutes before cooling to room temperature and diluting with water (50 ml). The resulting solution's pH was adjusted to 5 using glacial acetic acid, and the formed precipitate was collected by filtration and dried *in vacuo* yielding 6-amino-2-mercaptopyrimidin-4-ol (0.95 g; 64%) as a white solid.

R_f (10% MeOH/EtOAc): 0.55. **¹H NMR** (400 MHz, DMSO-*d*₆) δ 11.72 – 11.47 (m, 2H, -OH and -SH), 6.40 (s, 2H, NH₂), 4.71 (s, 1H, H5). **¹³C NMR** (101 MHz, DMSO-*d*₆) δ 175.0 (C2), 162.2 (C4), 154.8 (C6), 78.6 (C5).

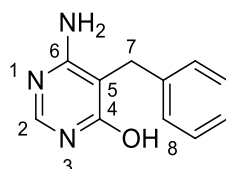
6.4.4 Synthesis of 6-amino-5-benzyl-2-mercaptopyrimidin-4-ol 4.24



A mixture of ethyl 2-cyano-3-phenylpropanoate (2.50g; 12.30 mmol), thiourea (1.03; 13.53 mmol), and potassium *tert*-butoxide (1.52 g; 13.53 mmol) was microwave irradiated at 10 W for 10 minutes before cooling to room temperature and diluting with water (50 ml). The resulting solution's pH was adjusted to 5 using glacial acetic acid, and the formed precipitate was collected by filtration and dried *in vacuo* yielding 6-amino-5-benzyl-2-mercaptopyrimidin-4-ol (1.60 g; 56%) as a white solid.

R_f (10% MeOH/EtOAc): 0.50. **¹H NMR** (400 MHz, DMSO-*d*₆) δ 11.81 (s, 1H, OH), 11.49 (s, 1H, SH), 7.32 – 7.09 (m, 5H, ArH), 6.25 (s, 2H, NH₂), 3.53 (s, 2H, H7). **¹³C NMR** (101 MHz, DMSO-*d*₆) δ 173.3 (C2), 162.2 (C6), 151.6 (C4), 141.0 (ArC), 128.5 (ArC), 128.4 (ArC), 126.1 (ArC), 89.7 (C5), 27.6 (C7). **HRMS** (ESI⁺): Found [M+H]⁺ 234.0698, C₁₁H₁₂N₃OS⁺ requires 234.0694. **mp** (°C): 295-298.

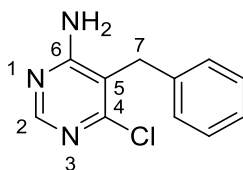
6.4.5 Synthesis of 6-amino-5-benzylpyrimidin-4-ol 4.25



To a mixture of 6-amino-5-benzyl-2-mercaptopyrimidin-4-ol (1.60 g, 6.86 mmol), ammonia (20 ml), and water (100 ml) was added Raney Nickel catalyst (4.10 g). The resulting mixture was refluxed for 2 hours. The catalyst was then recovered by filtration and the filtrate was concentrated to afford 6-amino-5-benzylpyrimidin-4-ol (1.14 g; 82%) as a white solid.

R_f (10% MeOH/EtOAc): 0.25. **¹H NMR** (400 MHz, DMSO-*d*₆) δ 11.54 (s, 1H, OH), 7.73 (s, 1H, H2), 7.30 – 7.17 (m, 4H, ArH), 7.12 (t, *J* = 7.2 Hz, 1H, ArH), 6.26 (s, 2H, NH₂), 3.60 (s, 2H, H7). **¹³C NMR** (101 MHz, DMSO-*d*₆) δ 162.2 (C6), 160.9 (C4), 147.5 (C2), 141.3 (ArC), 128.6 (ArC), 128.3 (ArC), 125.9 (ArC), 97.3 (C5), 28.4 (C7). **mp** (°C): 224-227.

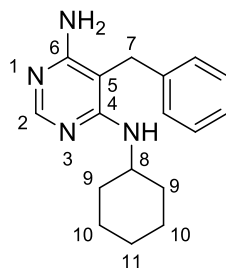
6.4.6 Synthesis of 5-benzyl-6-chloropyrimidin-4-amine 4.26



A mixture of 6-amino-5-benzylpyrimidin-4-ol (0.12 g, 0.79 mmol) and POCl₃ (5.00 ml) contained in a pressure tube was heated at 100°C for 3 hours before cooling down to room temperature and quenching the reaction in ice water (~100 ml). The resulting solution pH was adjusted to 7 using 25% aqueous ammonia and the organic matter was extracted with ethyl acetate (3×100 ml). Acquired fractions were dried with anhydrous sodium sulfate, filtered, and concentrated *in vacuo* to afford 5-benzyl-6-chloropyrimidin-4-amine (0.12 g; 91%) as a yellow solid after purification via silica gel column chromatography.

R_f (10% MeOH/EtOAc): 0.79. **¹H NMR** (400 MHz, DMSO-*d*₆) δ 8.13 (s, 1H, H2), 7.29 (t, *J* = 7.4 Hz, 2H, ArH), 7.24 – 7.15 (m, 5H, ArH & NH₂), 3.97 (s, 2H, H7). **¹³C NMR** (101 MHz, DMSO-*d*₆) δ 164.0 (C6), 158.7 (C4), 156.5 (C2), 138.1 (ArC), 128.8 (ArC), 128.3 (ArC), 126.7 (ArC), 112.9 (C5), 31.9 (C7). **HRMS** (ESI⁺): Found [M+H]⁺ 220.0637, C₁₁H₁₁N₃Cl⁺ requires 220.0636. **mp** (°C): 185-188.

6.4.7 Synthesis of 5-benzyl-*N*⁴-cyclohexylpyrimidine-4,6-diamine 4.27

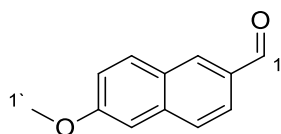


A mixture of 5-benzyl-6-chloropyrimidin-4-amine (0.26 g, 1.17 mmol), cyclohexylamine (0.27 ml, 2.33 mmol), and toluene (1.50 ml) was heated at 200°C for 1 hour by microwave irradiation. The resulting reaction mixture was dissolved with methanol (50 ml) and concentrated on silica gel, *in vacuo*. The afforded concentrate was purified using silica gel column chromatography to afford 5-benzyl-*N*⁴-cyclohexylpyrimidine-4,6-diamine (0.12 g; 37%) as a white solid.

R_f (50% EtOAc/hexane): 0.25. **¹H NMR** (400 MHz, DMSO-*d*₆) δ 7.85 (s, 1H, H2), 7.29 – 7.11 (m, 5H, ArH), 5.87 (s, 2H, NH₂), 5.57 (d, *J* = 8.0 Hz, 1H, NH), 3.90 – 3.81 (m, 1H, H8), 3.72 (s, 2H, H7), 1.79 – 1.70 (m, 2H, *H9), 1.66 – 1.57 (m, 2H, *H10), 1.55 (d, *J* = 12.7 Hz, 1H, 1×H11), 1.32 – 1.03 (m, 5H, 2×H9 & 2×H10 & 1×H11). **¹³C NMR** (101 MHz, DMSO-*d*₆) δ 161.5 (C6), 159.9 (C4), 155.9 (C2), 140.1 (ArC), 128.6 (ArC), 128.5 (ArC), 126.3 (ArC), 93.8 (C5), 49.2 (C8), 33.2 (*C9), 28.4 (C7), 25.9 (C11), 25.3 (*C10). **mp** (°C): 175-177.

*Assignments for these signals have not been confirmed.

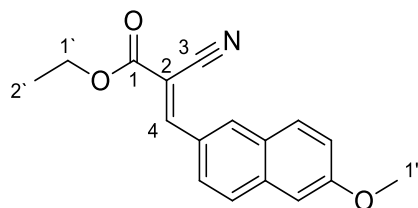
6.4.8 Synthesis of 6-methoxy-2-naphthaldehyde 4.29 [18]



To a mixture of 6-hydroxy-2-naphthaldehyde (2.00 g, 11.61 mmol), and potassium carbonate (3.21 g, 23.23 mmol) in acetone (100 ml) was added dimethylsulfate (2.20 ml, 23.23 mmol) followed by heating at 60°C for 2 hours. The solvent was concentrated *in vacuo* and the afforded crude was diluted with water (100 ml). The organic material was extracted with ethyl acetate (3×100 ml) and purified using silica gel chromatography to afford 6-methoxy-2-naphthaldehyde (1.00 g, 74%) as a yellow solid.

R_f (10% EtOAc/hexane): 0.20. **¹H NMR** (400 MHz, CDCl₃) δ 10.09 (s, 1H, H1), 8.25 (s, 1H, ArH), 7.96 – 7.85 (m, 2H, ArH), 7.81 (d, *J* = 8.5 Hz, 1H, ArH), 7.27 – 7.21 (m, 1H, ArH), 7.18 (d, *J* = 2.5 Hz, 1H, ArH), 3.96 (s, 3H, H1'). **¹³C NMR** (101 MHz, CDCl₃) δ 192.0 (C1), 160.3 (ArC), 138.3 (ArC), 134.2 (ArC), 132.4 (ArC), 131.1 (ArC), 128.00 (ArC), 127.7 (ArC), 123.7 (ArC), 120.0 (ArC), 106.1 (ArC), 55.5 (C1'). **mp** (°C): 80-83.

6.4.9 Synthesis of (*E*)-ethyl 2-cyano-3-(6-methoxynaphthalen-2-yl)acrylate 4.30 [19]

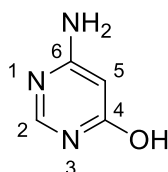


A microwave tube was loaded with ethyl cyanoacetate (0.54 ml, 5.09 mmol), 6-methoxy-2-naphthaldehyde (0.92 g, 5.34 mmol), and piperidine (5 drops). The resulting mixture was heated by microwave irradiation for 5 minutes at a constant power of 10 W before cooling to room temperature. The afforded precipitated was collected by filtration and recrystallised in ethanol to afford (*E*)-ethyl 2-cyano-3-(6-methoxynaphthalen-2-yl)acrylate (1.34 g; 87%) as a light-yellow solid.

R_f (10% EtOAc/Hexane): 0.17. **¹H NMR** (400 MHz, CDCl₃) δ 8.34 (s, 1H, H₄), 8.29 (s, 1H, ArH), 8.17 (d, *J* = 8.7 Hz, 1H, ArH), 7.86 – 7.75 (m, 2H, ArH), 7.24 – 7.13 (m, 2H, ArH), 4.40 (q, *J* = 7.1, 6.7 Hz, 2H, H_{1'}), 3.96 (s, 3H, H_{1''}), 1.46 – 1.37 (m, 3H, H_{2'}). **¹³C NMR** (101 MHz, CDCl₃) δ 163.0 (C₁), 160.4 (ArC), 155.1 (C₄), 137.3 (ArC), 134.2 (ArC), 131.1 (ArC), 128.2, 127.8 (ArC), 126.9 (ArC), 126.1, 120.2 (ArC), 116.1 (C₃), 106.0 (ArC), 101.0 (C₂), 62.5 (C_{1'}), 55.5 (C_{1''}), 14.2 (C_{2'}). **mp** (°C): 124-127.

6.5 Synthesis of 4,6-diaminopyrimidine compounds: heteroatom (N and O) linker derivatives

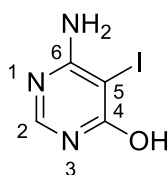
6.5.1 Synthesis of 6-aminopyrimidin-4-ol 4.31 [2]



To a mixture of 6-amino-2-mercaptopyrimidin-4-ol (2.00g, 13.98 mmol), and 25% aqueous ammonia (20 ml) in water (100 ml) was added Raney Nickel (4.00 g). The resulting suspension was heated at reflux for 2 hours followed immediately by filtering to recover Raney-nickel. The filtrate was concentrated *in vacuo* to afford 6-aminopyrimidin-4-ol (1.21 g, 78%) as a white solid.

R_f (10% MeOH/EtOAc): 0.20. **¹H NMR** (400 MHz, DMSO-*d*₆) δ 11.47 (s, 1H, OH), 7.78 (s, 1H, H₂), 6.44 (s, 2H, NH₂), 4.98 (s, 1H, H₅). **¹³C NMR** (101 MHz, DMSO-*d*₆) δ 164.6 (C₆), 162.4 (C₄), 150.0 (C₂), 85.7 (C₅). **mp** (°C): 265-268.

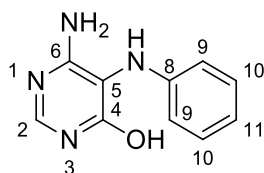
6.5.2 Synthesis of 6-amino-5-iodopyrimidin-4-ol 4.32



To a mixture of 6-aminopyrimidin-4-ol (1.00 g, 9.00 mmol), potassium carbonate (2.49 g, 18.00 mmol), and molecular iodine (4.57 g, 18.00 mmol) in a microwave tube was added water and dimethylformamide. The resulting solution was heated by microwave irradiation at 100°C for 1 hour before cooling down to room temperature and diluting with water (100 ml). The excess iodine was quenched by adding sodium thiosulfate pellets in small quantities while stirring until no further colour change was observed. The formed precipitate was collected by filtration to afford 6-amino-5-iodopyrimidin-4-ol (1.44 g, 68%) as a light brown solid.

R_f (10% MeOH/EtOAc): 0.5. **¹H NMR** (400 MHz, DMSO-*d*₆) δ 11.80 (s, 1H, OH), 7.76 (s, 1H, H2), 6.70 (s, 2H, NH₂). **¹³C NMR** (101 MHz, DMSO-*d*₆) δ 163.9 (C6), 159.8 (C4), 149.2 (C2), 59.0 (C5). **HRMS** (ESI⁺): Found [M+H]⁺ 237.9474, C₄H₅IN₃O⁺ requires 237.9412. **mp** (°C): 228-230.

6.5.3 Synthesis of 6-amino-5-(phenylamino)pyrimidin-4-ol 4.33



A microwave tube with 6-amino-5-iodopyrimidin-4-ol (0.50 g, 2.11 mmol), potassium *tert*-butoxide (0.52 g, 4.64 mmol), and aniline (0.39 ml, 4.22 mmol), was heated at 180°C for 10 minutes by microwave irradiation. Upon cooling the formed precipitate was collected by filtration, washed with water and dried *in vacuo* to 6-amino-5-(phenylamino)pyrimidin-4-ol (0.29 g, 68%) as a white crystalline solid.

R_f (10% MeOH/EtOAc): 0.3. **¹H NMR** (400 MHz, DMSO-*d*₆) δ 11.81 (s, 1H, OH), 7.76 (s, 1H, H2), 7.00 (t, *J* = 7.7 Hz, 2H, ArH), 6.72 (s, 2H, NH₂), 6.54 (d, *J* = 7.8 Hz, 2H, ArH), 6.48 (t, *J* = 7.3 Hz, 1H, H11), 4.99 (s, 1H, NH). **¹³C NMR** (101 MHz, DMSO-*d*₆) δ 163.9 (C6), 159.8 (C4), 149.2 (C2), 149.1 (C8), 129.2 (ArC), 116.1 (C11), 114.3 (ArC), 59.0 (C5). **HRMS** (ESI⁺): Found [M+H]⁺ 203.1010, C₁₀H₁₁N₄O⁺ requires 203.0927. **mp** (°C): 180-184.

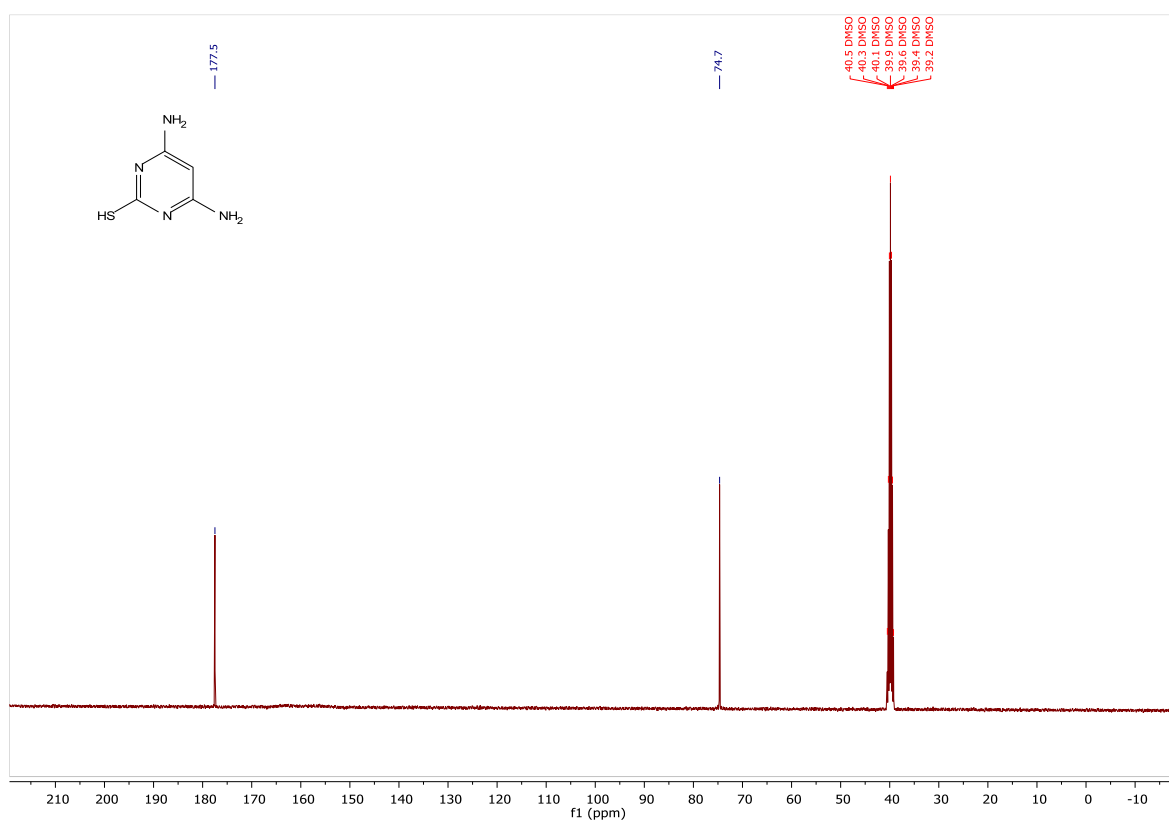
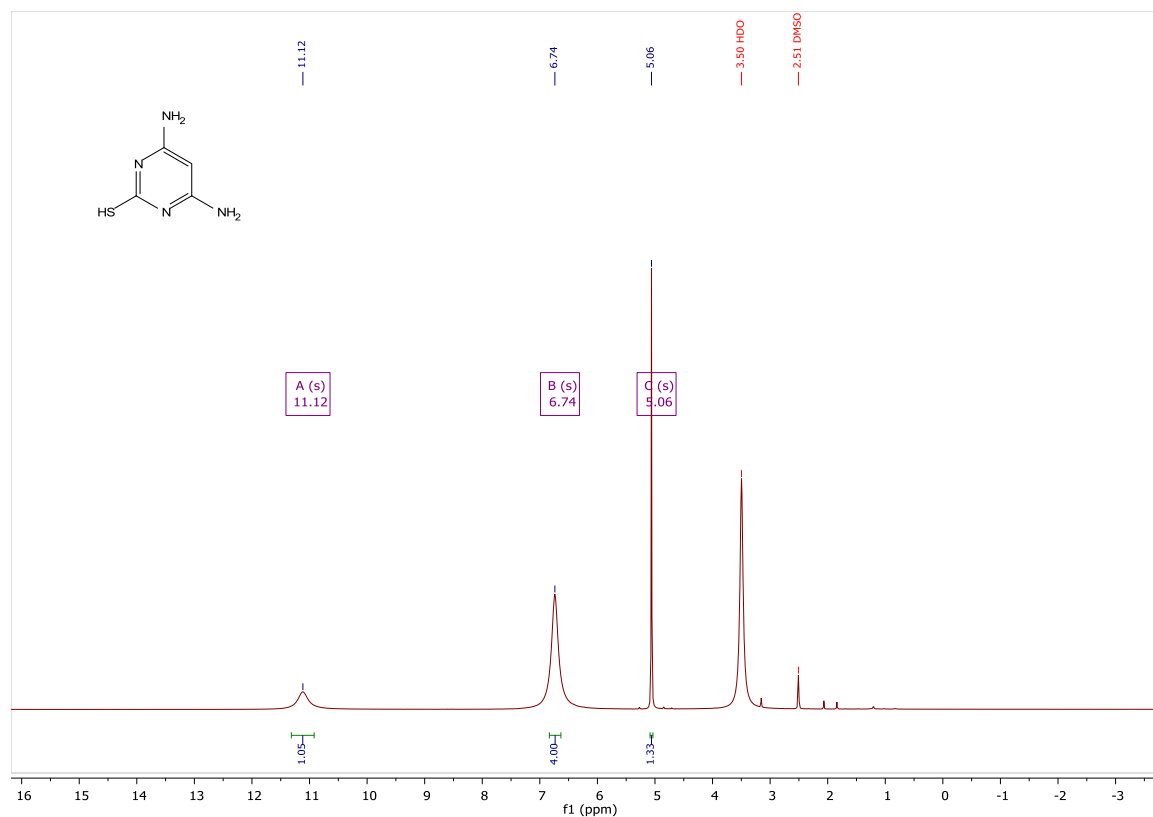
6.6 References

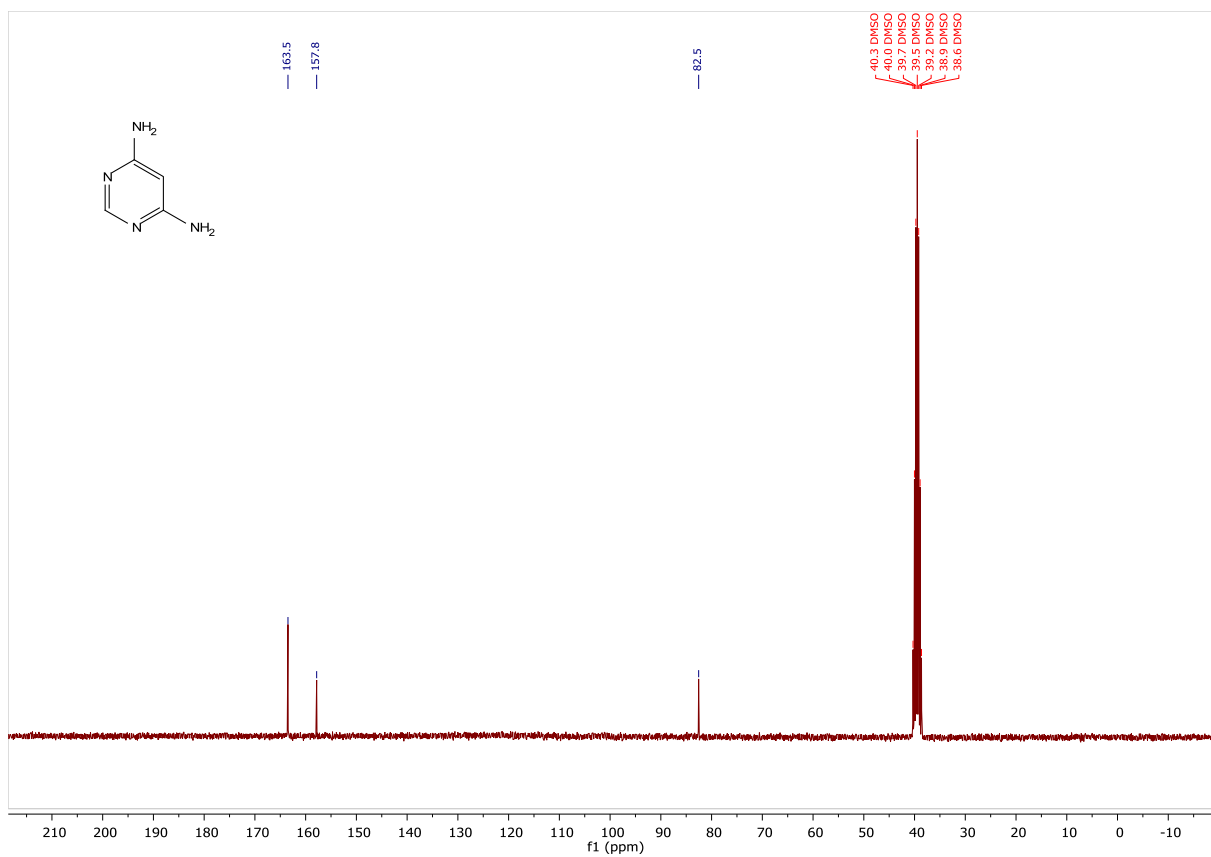
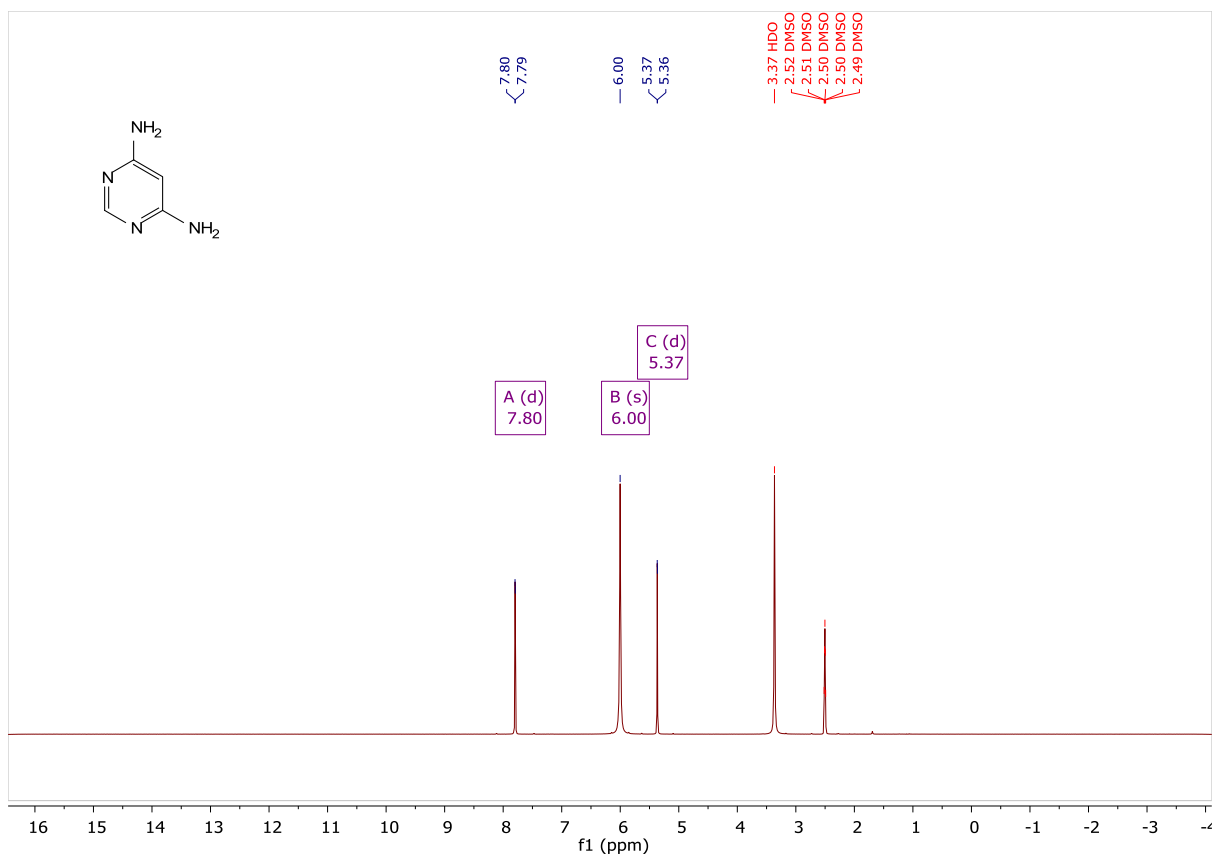
1. Chhabria, M.T., et al., *Synthesis and $\alpha 1$ -Adrenoceptor Antagonistic Activity of Some 4-Amino-5,7-dimethyl-2-(substituted) aminopyrido(2,3-d) pyrimidines*. *Arzneimittelforschung*, 2002. **52**(11): p. 792-796.
2. Brown, D.J., *Improved syntheses in the pyrimidine series .I. The use of pyrimidine thiols*. *Journal of the Society of Chemical Industry-London*, 1950. **69**(12): p. 353-356.
3. Perner, R.J., et al., *Synthesis and biological evaluation of 6,7-disubstituted 4-aminopyrido[2,3-d]pyrimidines as adenosine kinase inhibitors*. *Bioorganic & Medicinal Chemistry Letters*, 2005. **15**(11): p. 2803-2807.
4. Maiti, M., et al., *Influence of the Nucleobase and Anchimeric Assistance of the Carboxyl Acid Groups in the Hydrolysis of Amino Acid Nucleoside Phosphoramidates*. *Chemistry – A European Journal*, 2012. **18**(3): p. 857-868.
5. Seanego, T.D. and H.E. Chavalala, *7H-Pyrrolo[2,3-d]pyrimidine-4-amines as Potential Inhibitors of Plasmodium falciparum Calcium-Dependent Protein Kinases*. *ChemMedChem*. 2022. **17**(22): p. e202200421.
6. Li, Y., et al., *Identification of 5-(2,3-Dihydro-1H-indol-5-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine Derivatives as a New Class of Receptor-Interacting Protein Kinase 1 (RIPK1) Inhibitors, Which Showed Potent Activity in a Tumor Metastasis Model*. *Journal of Medicinal Chemistry*, 2018. **61**(24): p. 11398-11414.
7. Mewshaw, R.E., et al., *ER β Ligands. 3. Exploiting Two Binding Orientations of the 2-Phenylnaphthalene Scaffold To Achieve ER β Selectivity*. *Journal of Medicinal Chemistry*, 2005. **48**(12): p. 3953-3979.
8. Masood, M.A., et al., *Discovery of a series of potent and selective human H4 antagonists using ligand efficiency and libraries to explore structure–activity relationship (SAR)*. *Bioorganic & Medicinal Chemistry Letters*, 2011. **21**(21): p. 6591-6595.
9. Garner, J., et al., *Identification of Aminopyrimidine Regioisomers via Line Broadening Effects in 1H and 13C NMR Spectroscopy*. *Australian Journal of Chemistry*, 2004. **57**(11): p. 1079-1083.
10. Deng, X., et al., *Expanding the Diversity of Allosteric Bcr-Abl Inhibitors*. *Journal of Medicinal Chemistry*, 2010. **53**(19): p. 6934-6946.
11. Whitehead, C.W. and J.J. Traverso, *Diuretics. II. Alkoxymercuration by Mixed Anion Salts of Mercury*. *Journal of the American Chemical Society*, 1958. **80**(9): p. 2182-2185.
12. Zhan, W., et al., *Integrating docking scores, interaction profiles and molecular descriptors to improve the accuracy of molecular docking: Toward the discovery of novel Akt1 inhibitors*. *European Journal of Medicinal Chemistry*, 2014. **75**: p. 11-20.
13. Carpita, A., et al., *Synthesis of the racemic forms of carbon–carbon double bond locked analogues of strobilurins which are characterized by a 2-arylcyclopropane ring cis-substituted at C-1 by the methyl (E)-3-methoxypropenoate unit*. *Tetrahedron*, 2002. **58**(19): p. 3673-3680.
14. Arakawa, Y., et al., *Design of an extremely high birefringence nematic liquid crystal based on a dinaphthyl-diacetylene mesogen*. *Journal of Materials Chemistry*, 2012. **22**(28): p. 13908-13910.
15. Ayoubi, S.A.-E., F. Texier-Boullet, and J. Hamelin, *Minute Synthesis of Electrophilic Alkenes under Microwave Irradiation*. *Synthesis*, 1994. **1994**(03): p. 258-260.
16. Che, J. and Y. Lam, *Polymer-Supported Hantzsch 1,4-Dihydropyridine Ester: An Efficient Biomimetic Hydrogen Source for the Reduction of Ketimines and Electron-Withdrawing Group Conjugated Olefins*. *Advanced Synthesis & Catalysis*, 2010. **352**(10): p. 1752-1758.

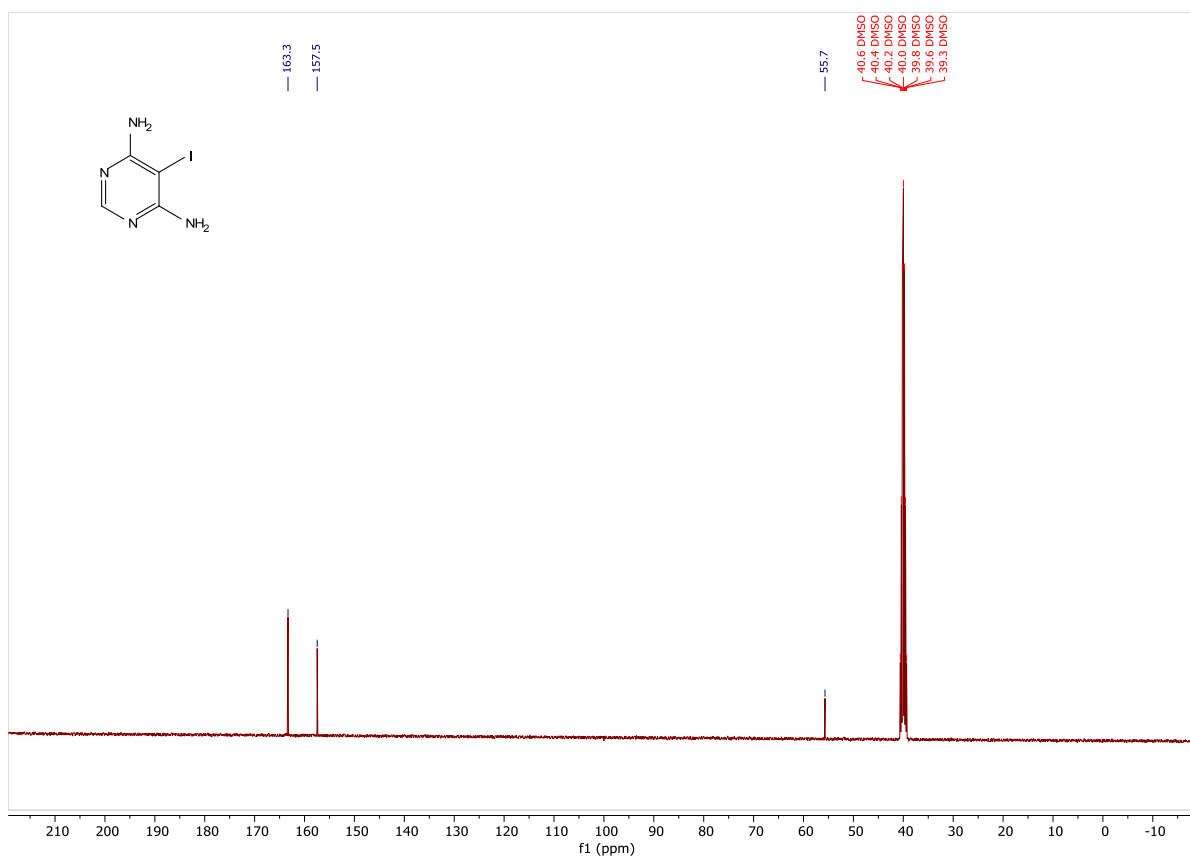
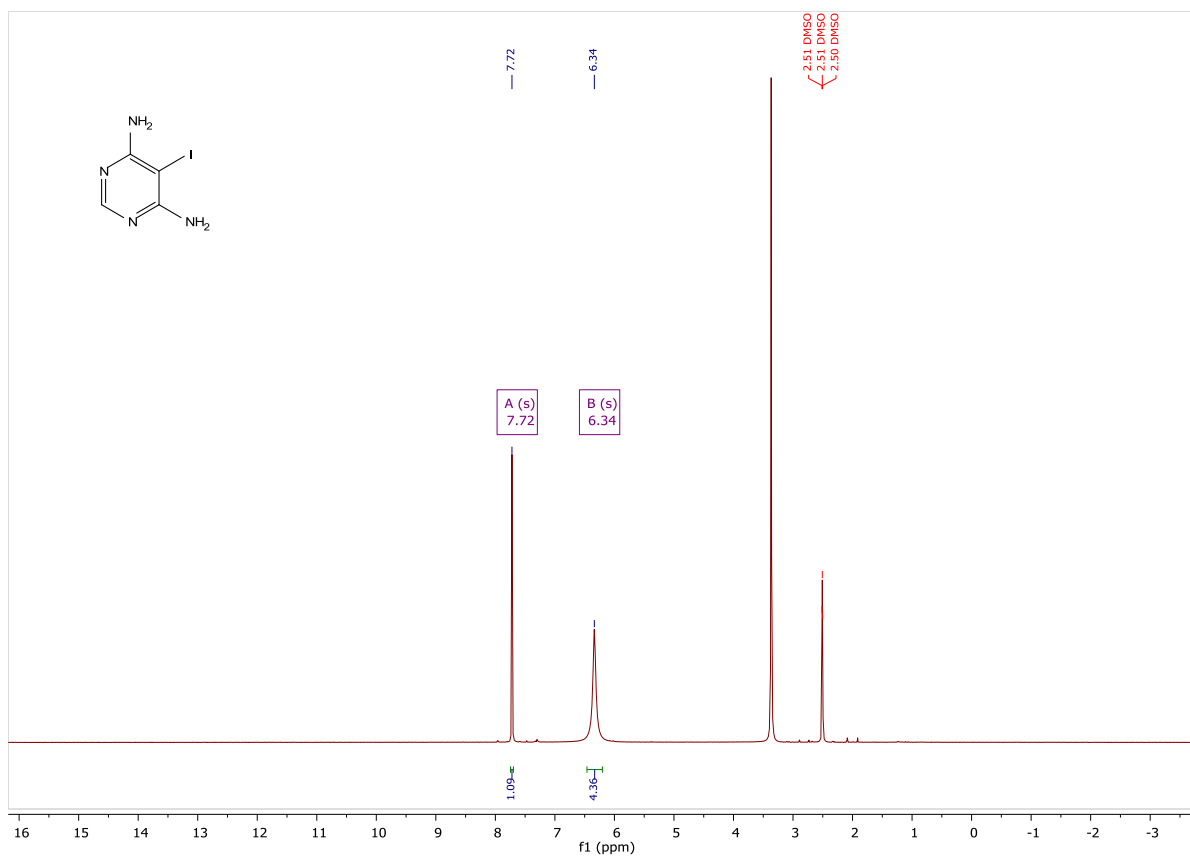
17. Röhrkasten, R., et al., *Synthesis of the Caffeine Metabolites 5-Acetylamino-6-formylamino-3-methyluracil (AFMU) and 5-Acetylamino-6-amino-3-methyluracil (AAMU) on a Preparative Scale*. Zeitschrift für Naturforschung B, 1997. **52**(12): p. 1526-1532.
18. Utley, J.H.P. and G.G. Rozenberg, *Electroorganic reactions. Part 56: Anodic oxidation of 2-methyl- and 2-benzyl-naphthalenes: factors influencing competing pathways*. Tetrahedron, 2002. **58**(26): p. 5251-5265.
19. Zhao, Y., et al., *Wavelength-tunable AIEgens based on 6-methoxy-2-naphthaldehyde: AIE behavior and bioimaging performance*. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2022. **281**: p. 121621.

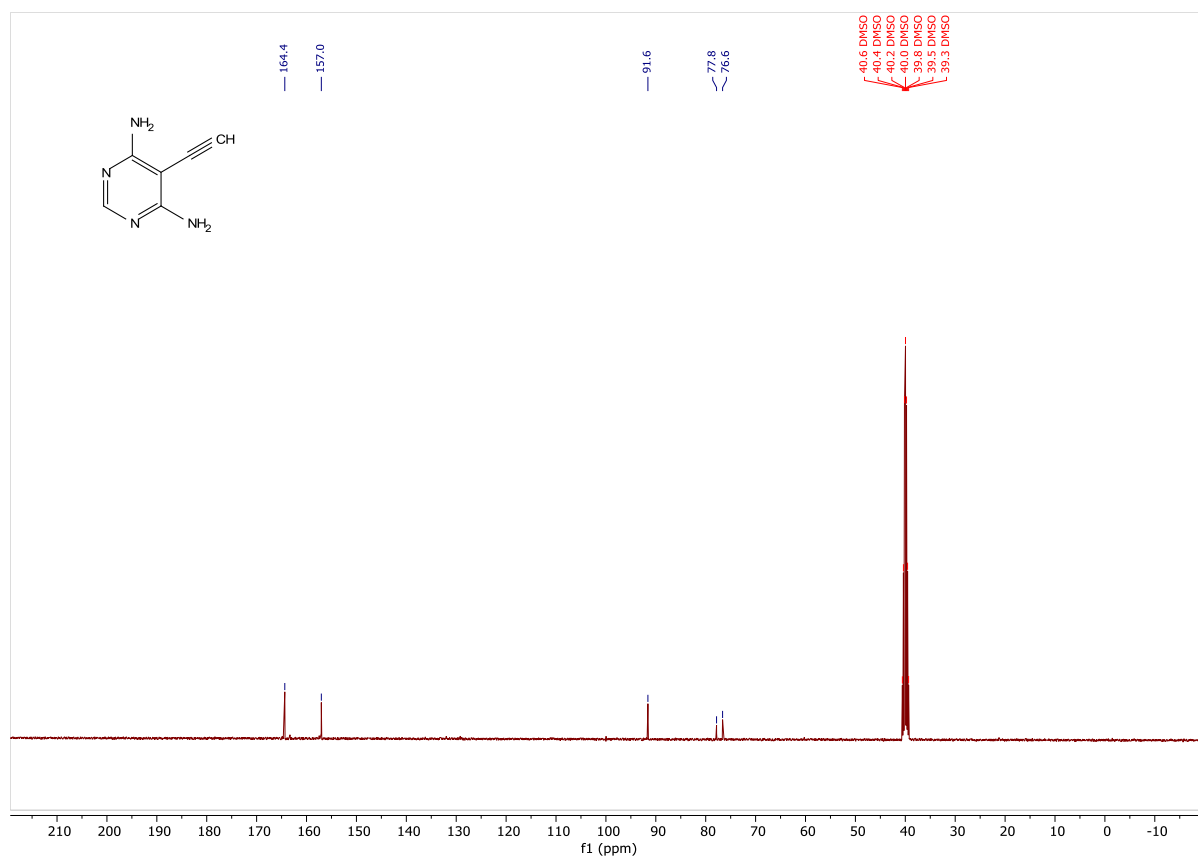
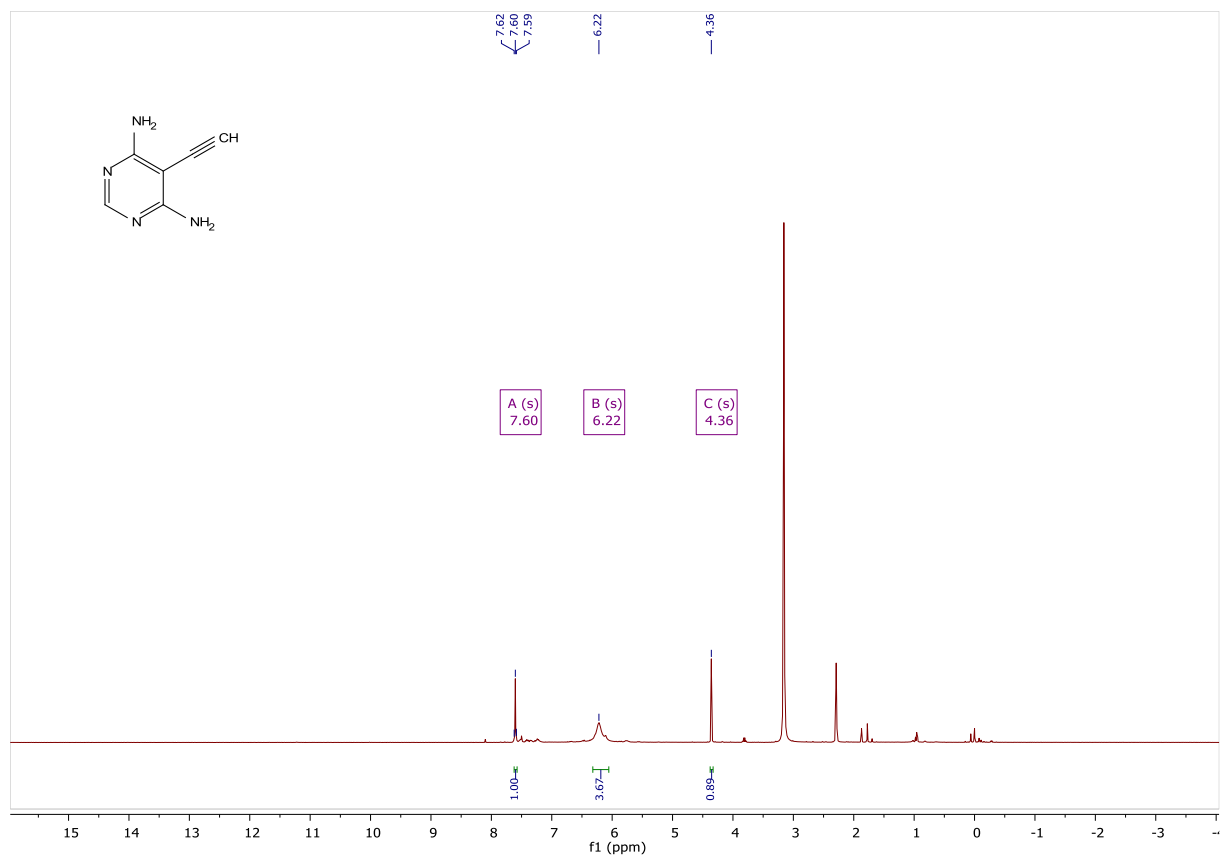
Appendices

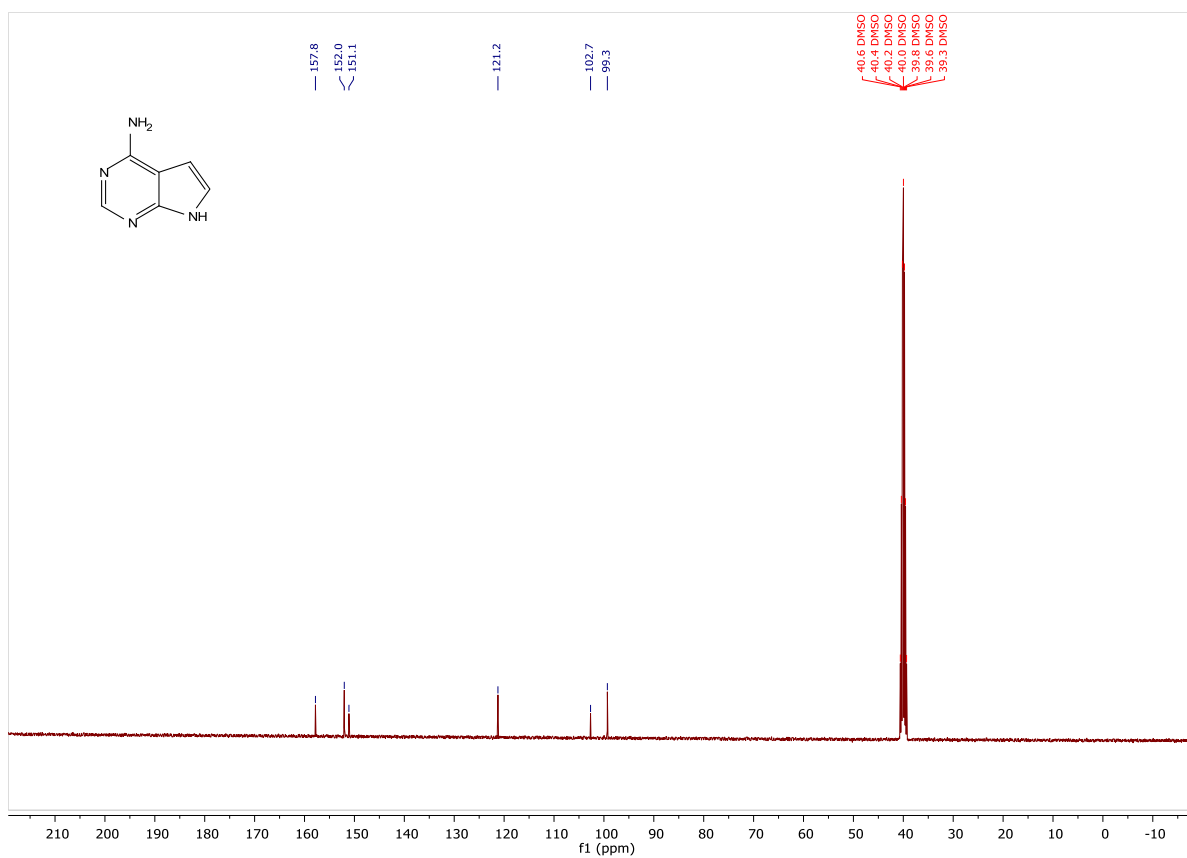
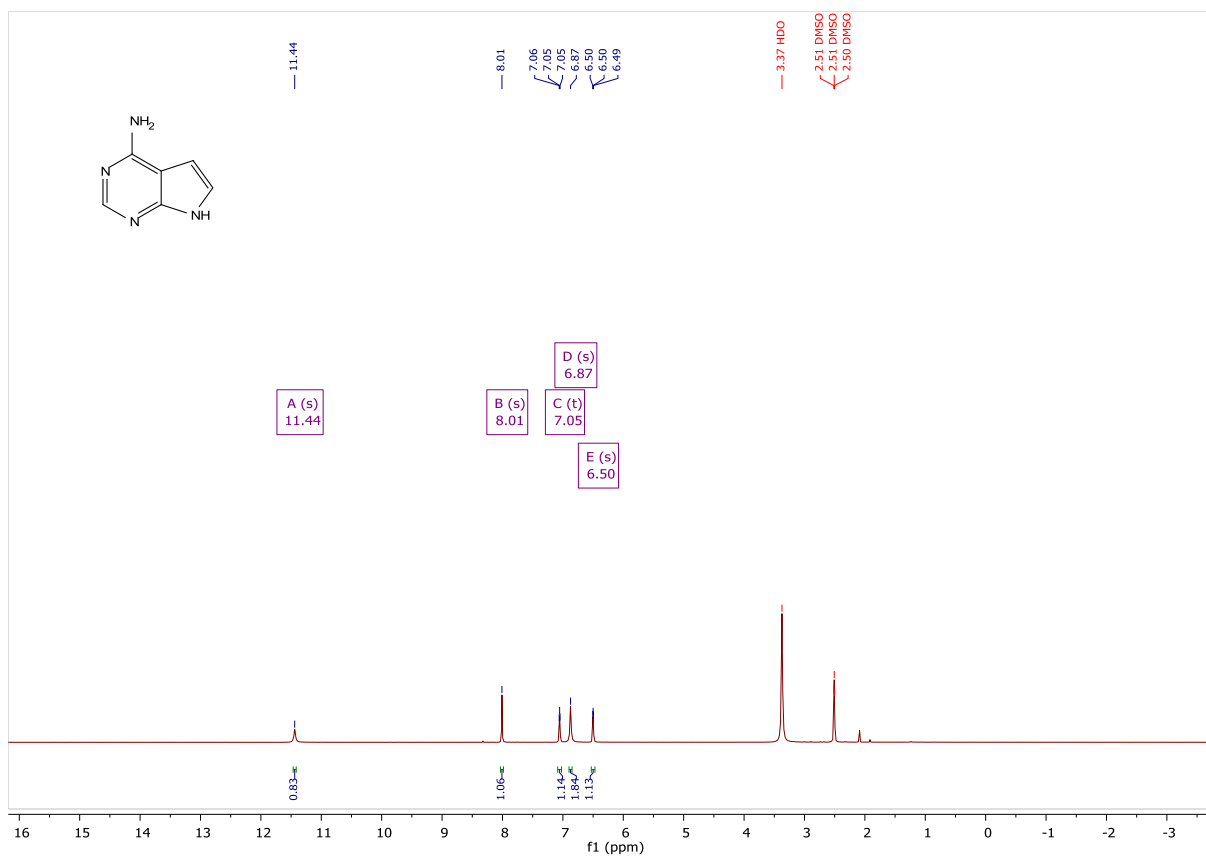
A. Nuclear magnetic resonance (NMR) spectra

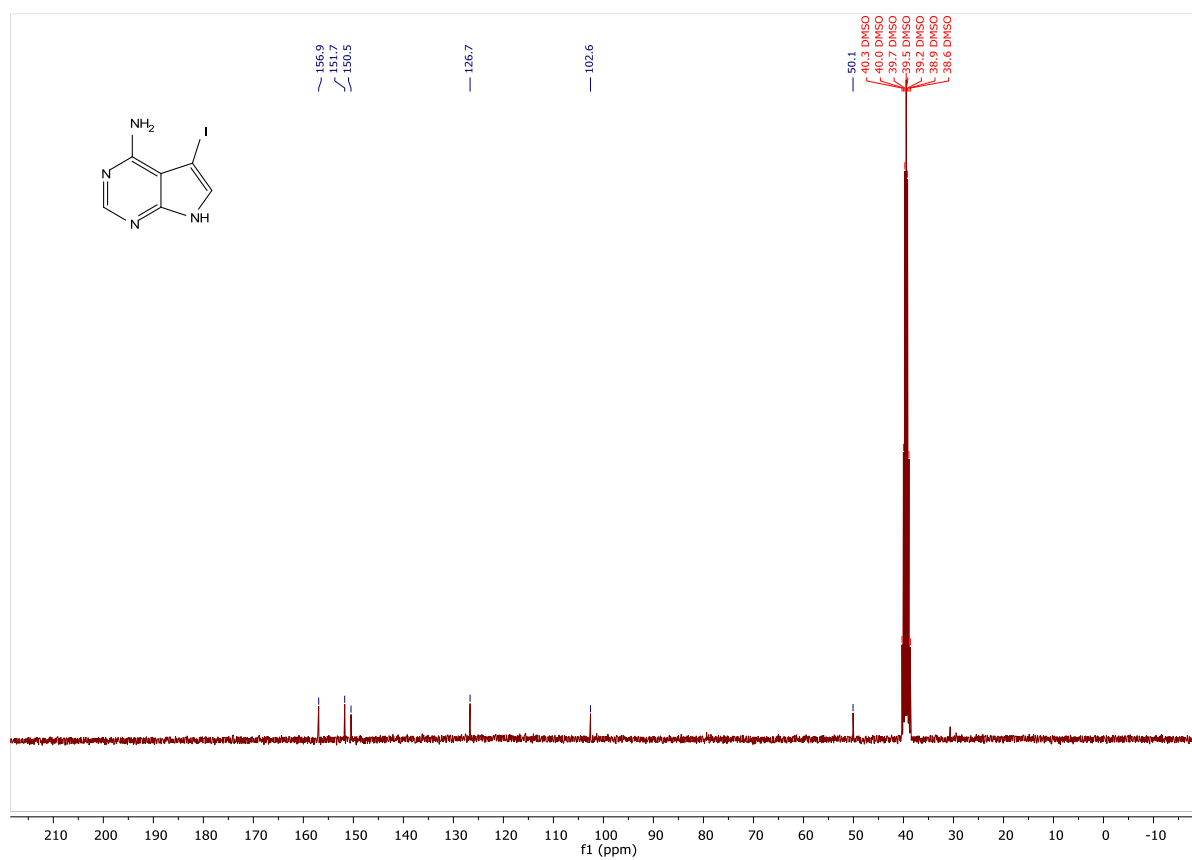
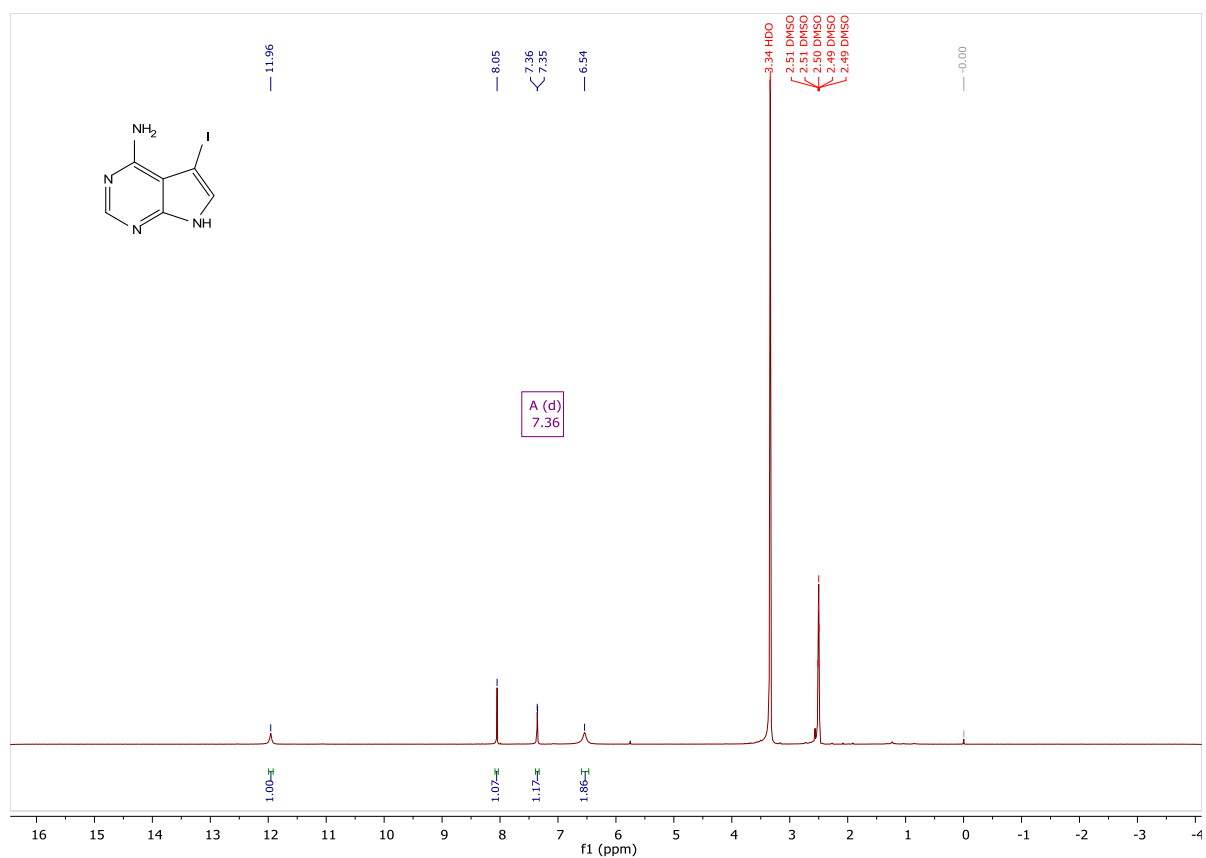


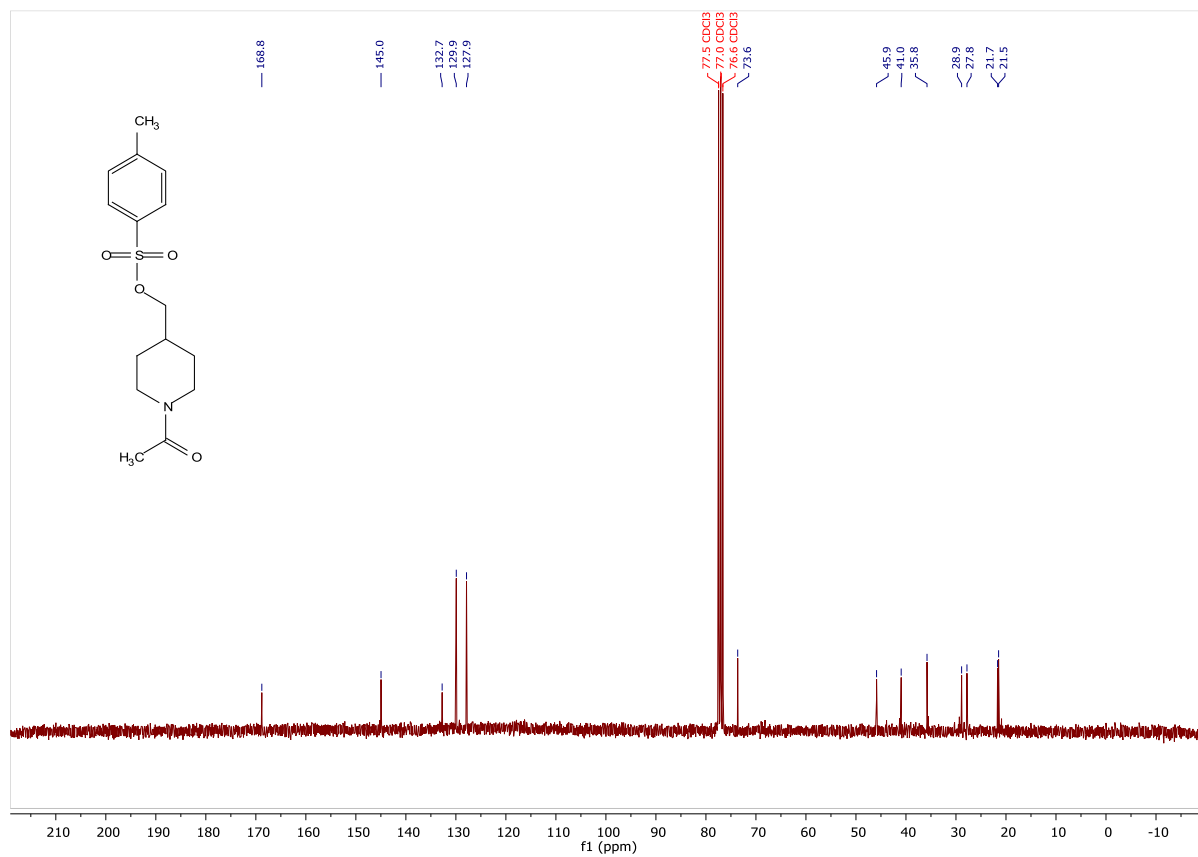
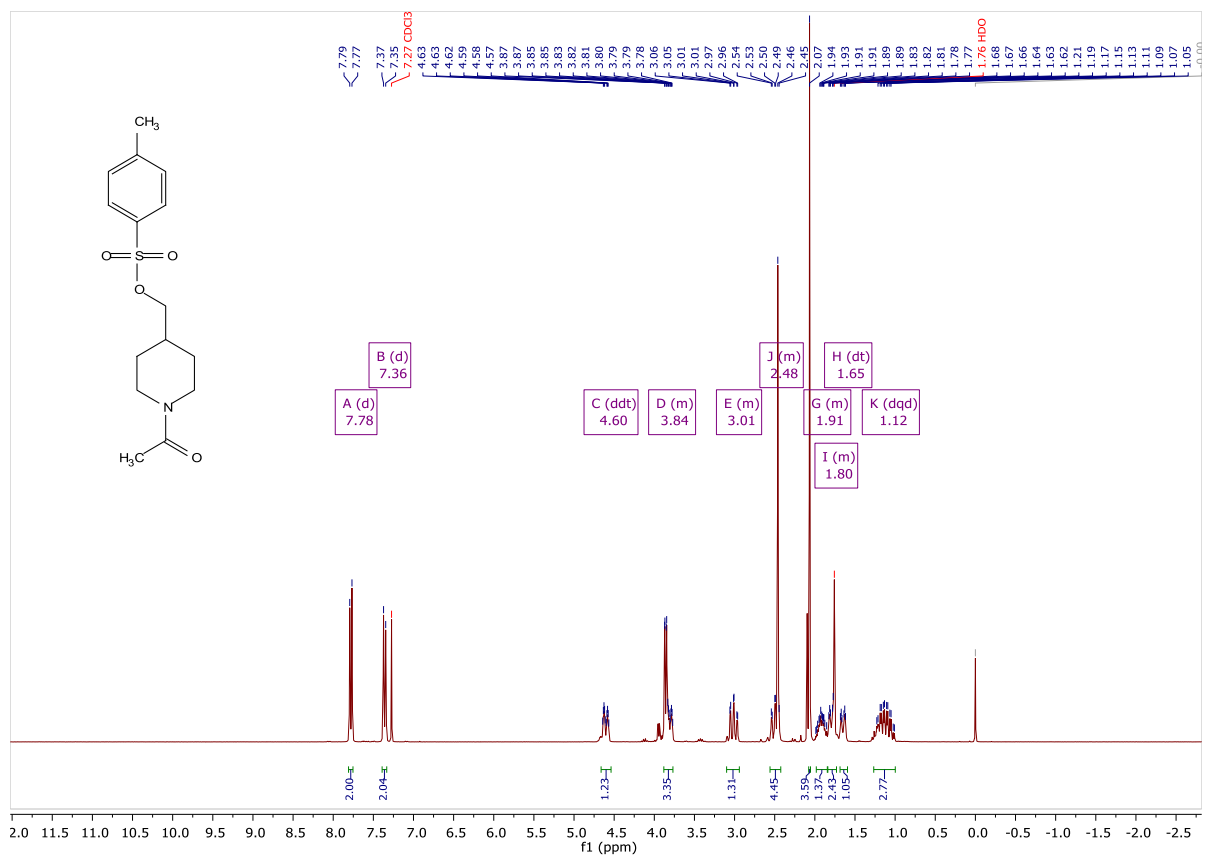


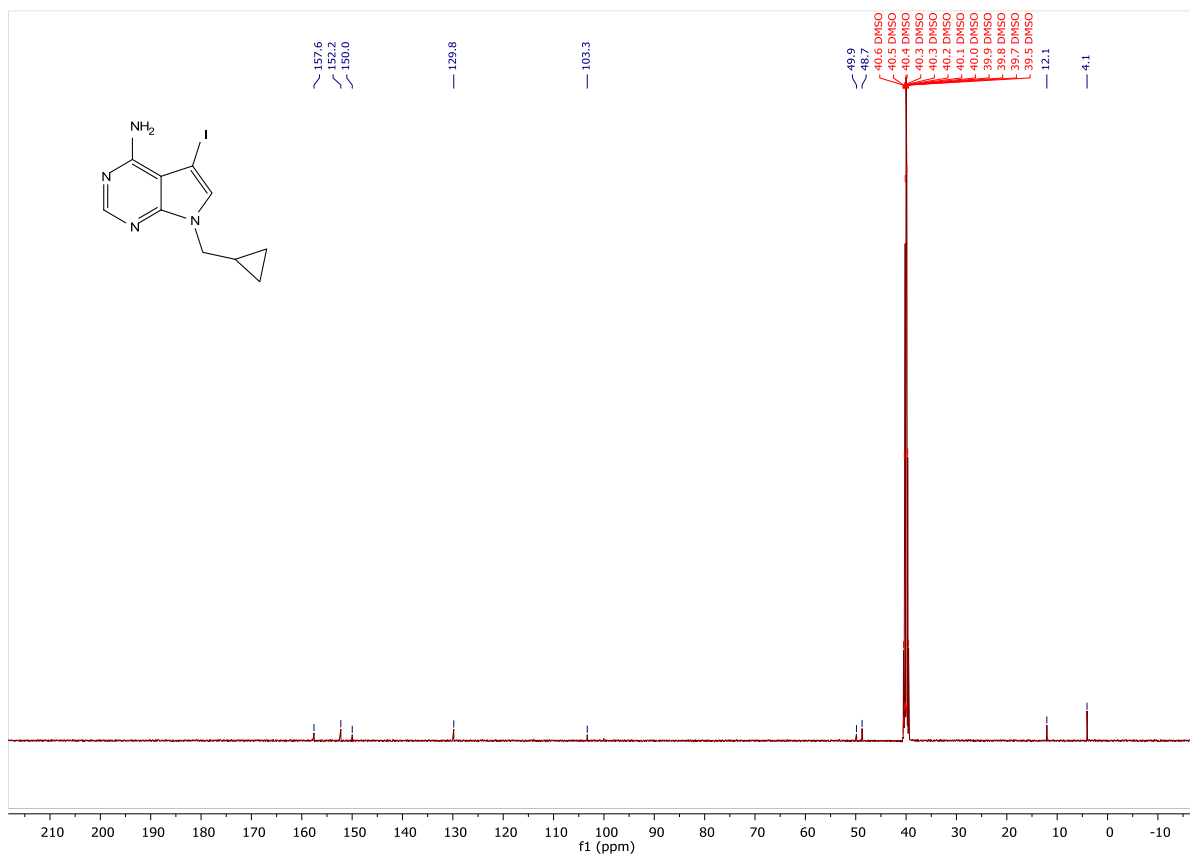
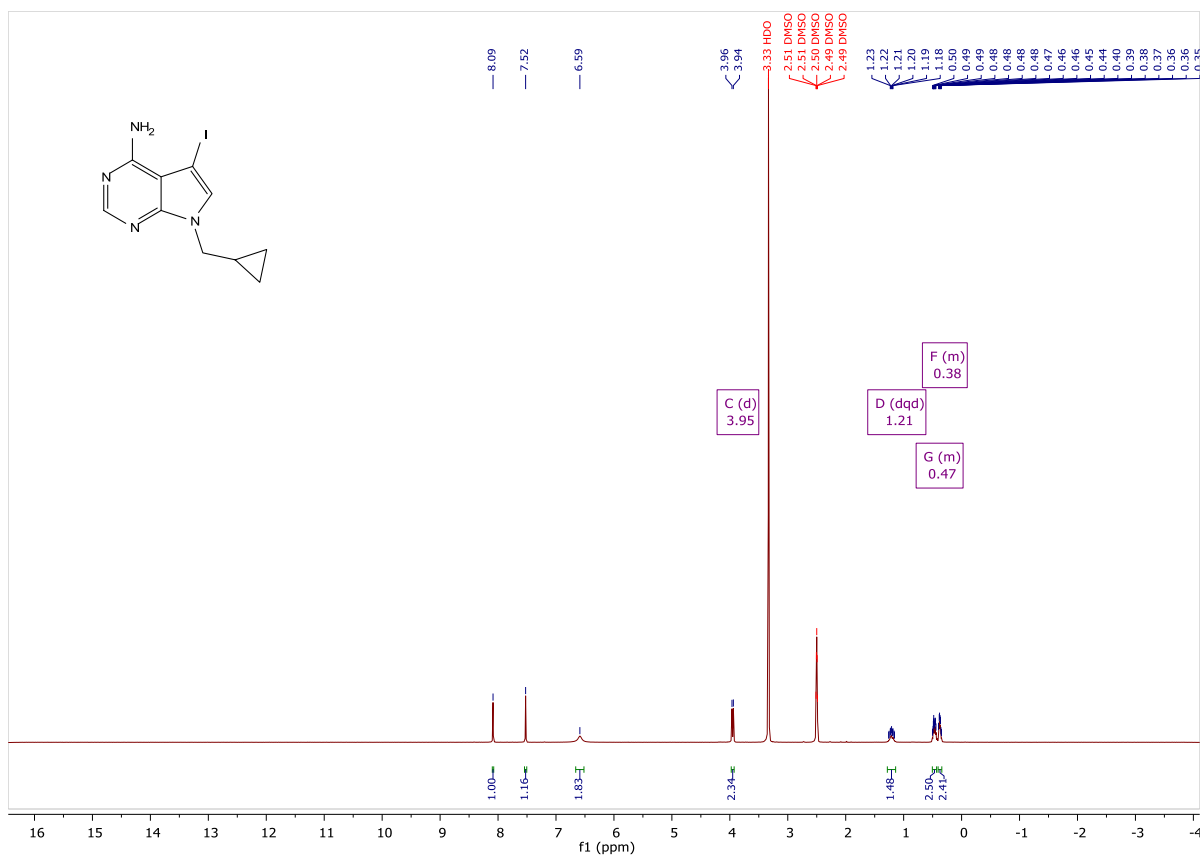


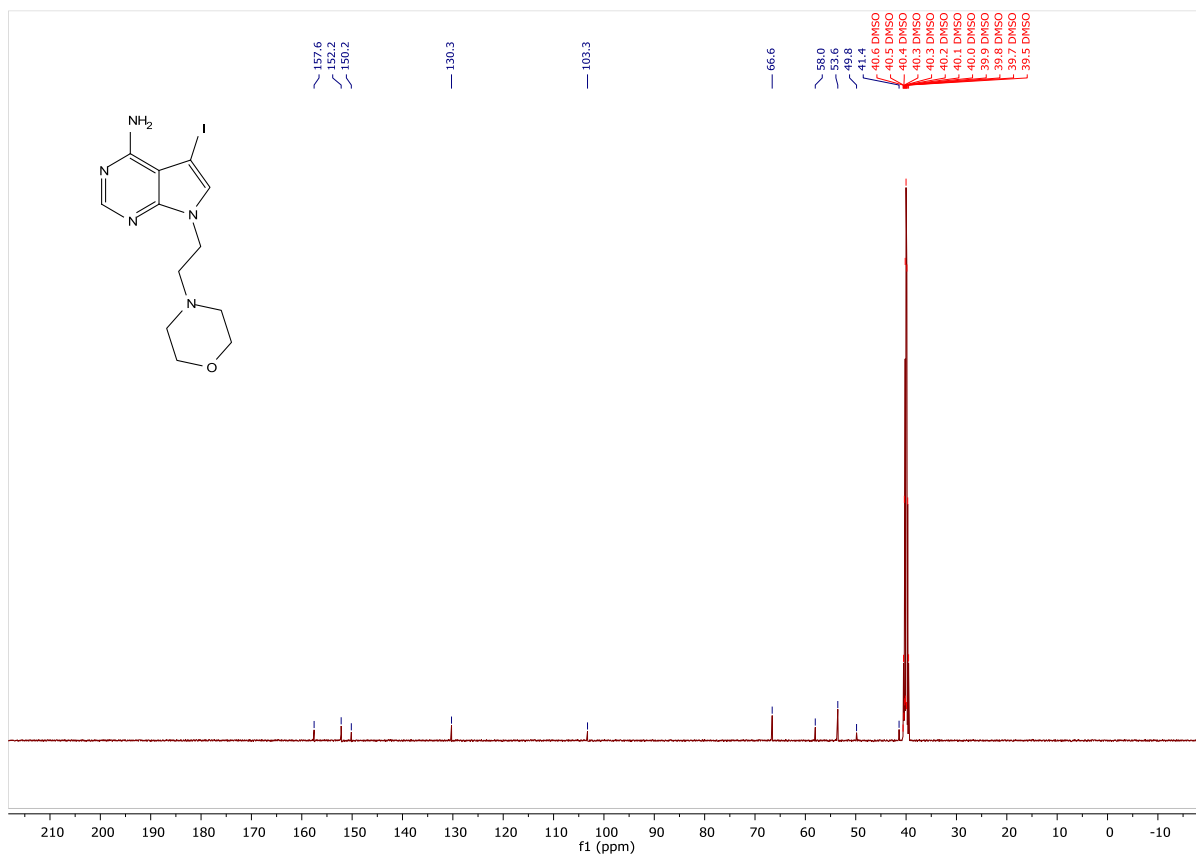
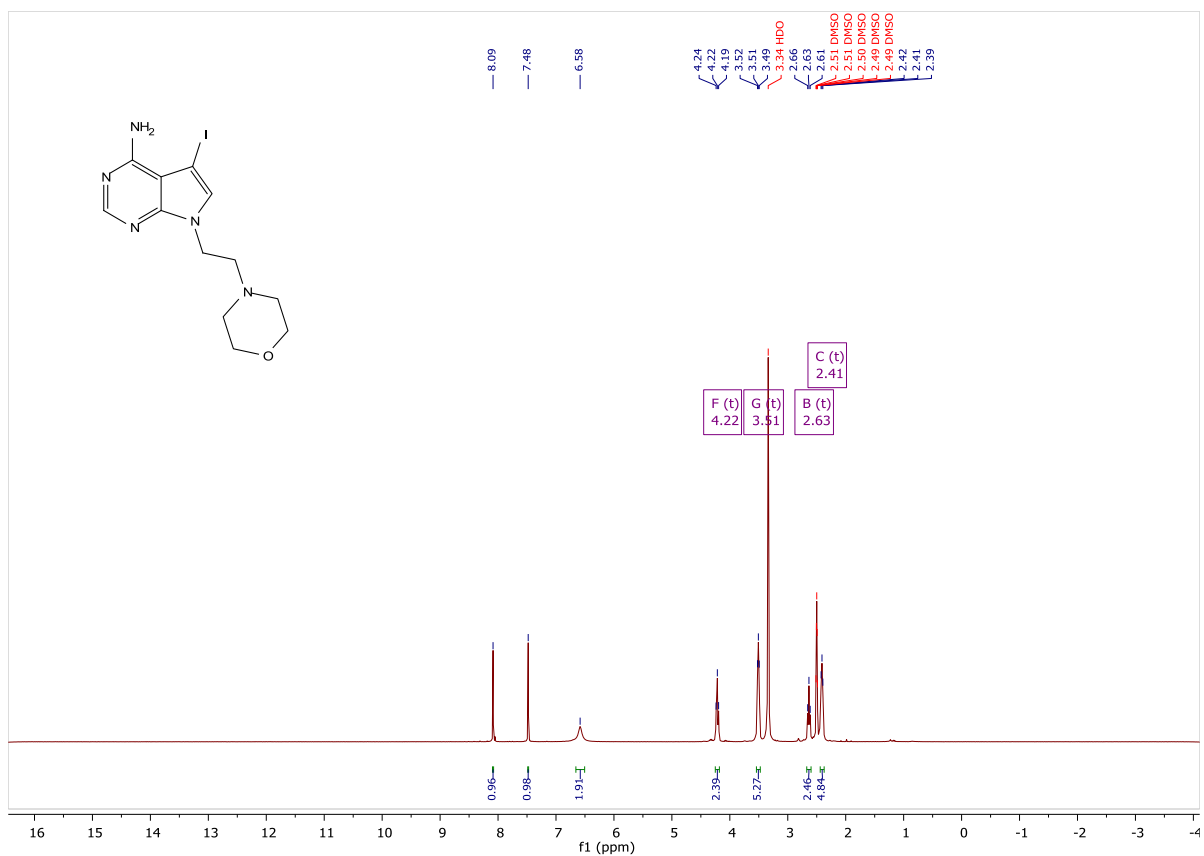


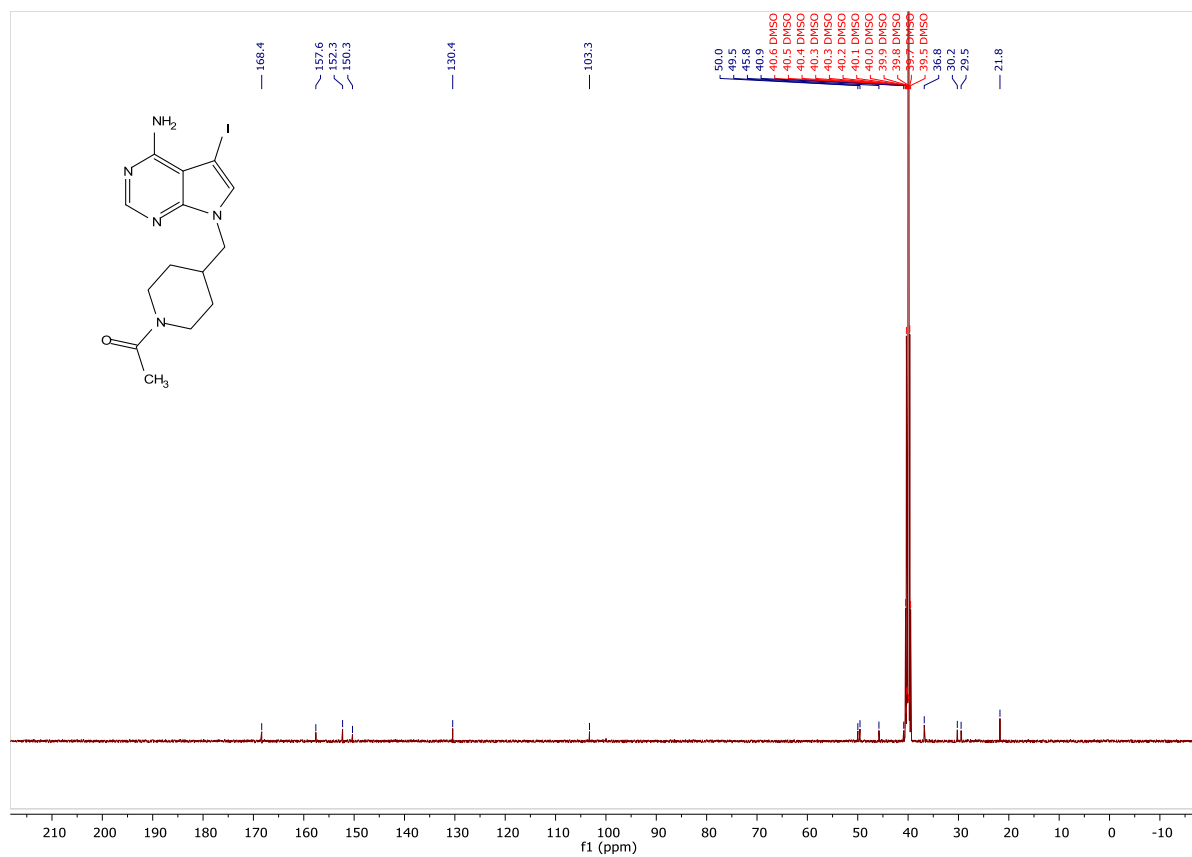
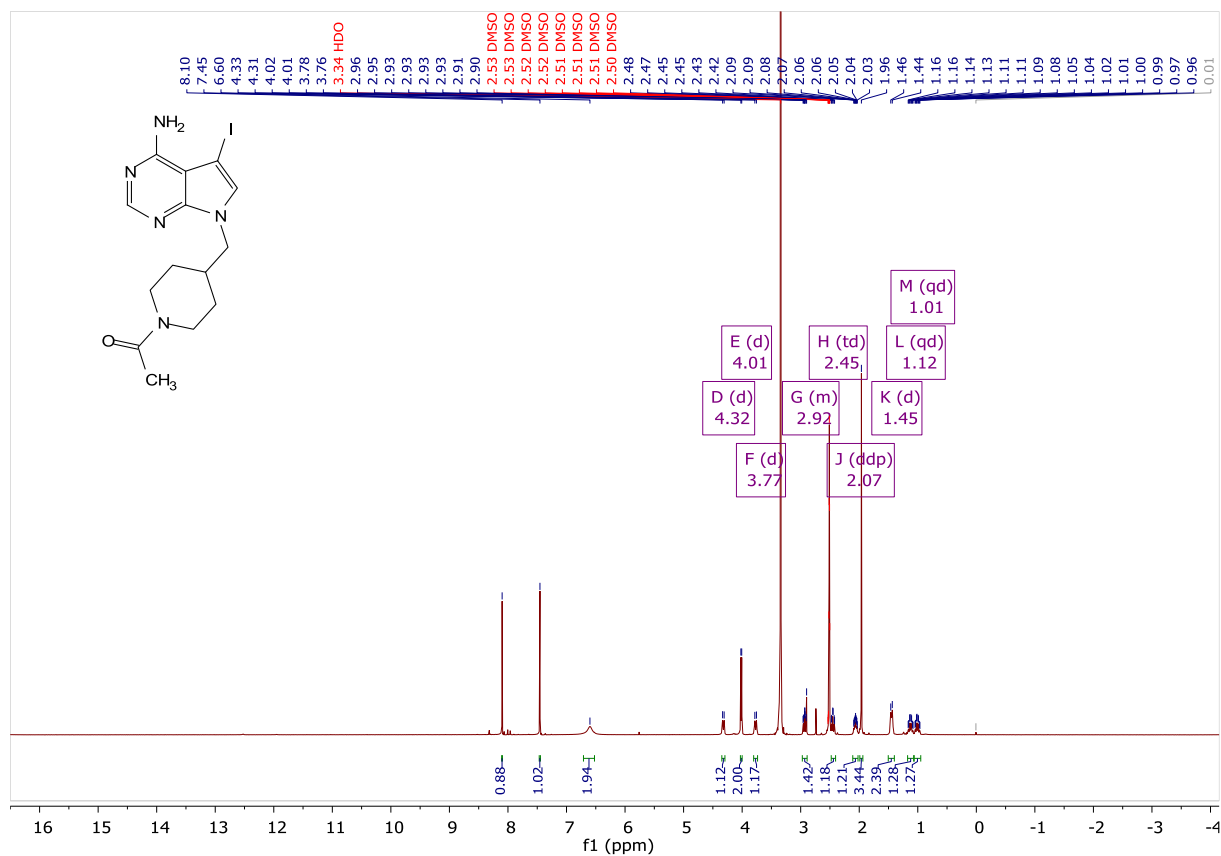


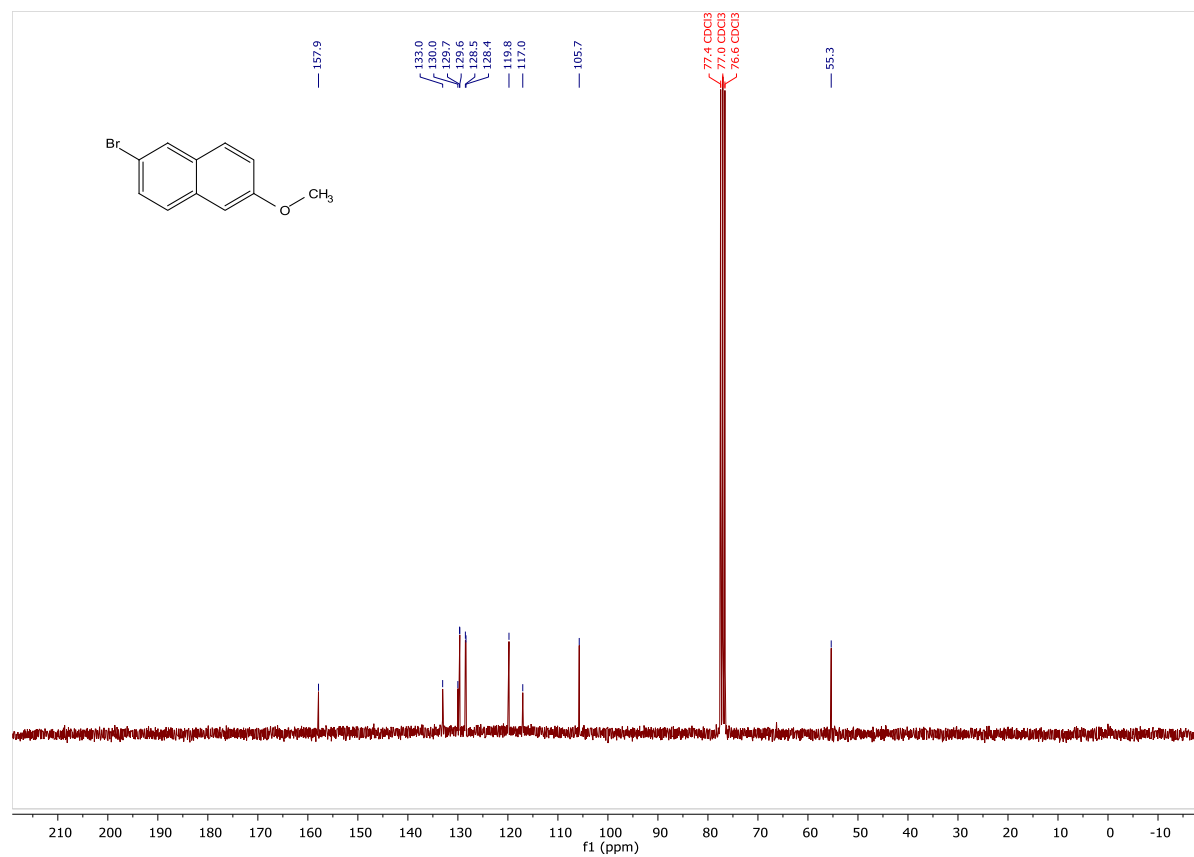
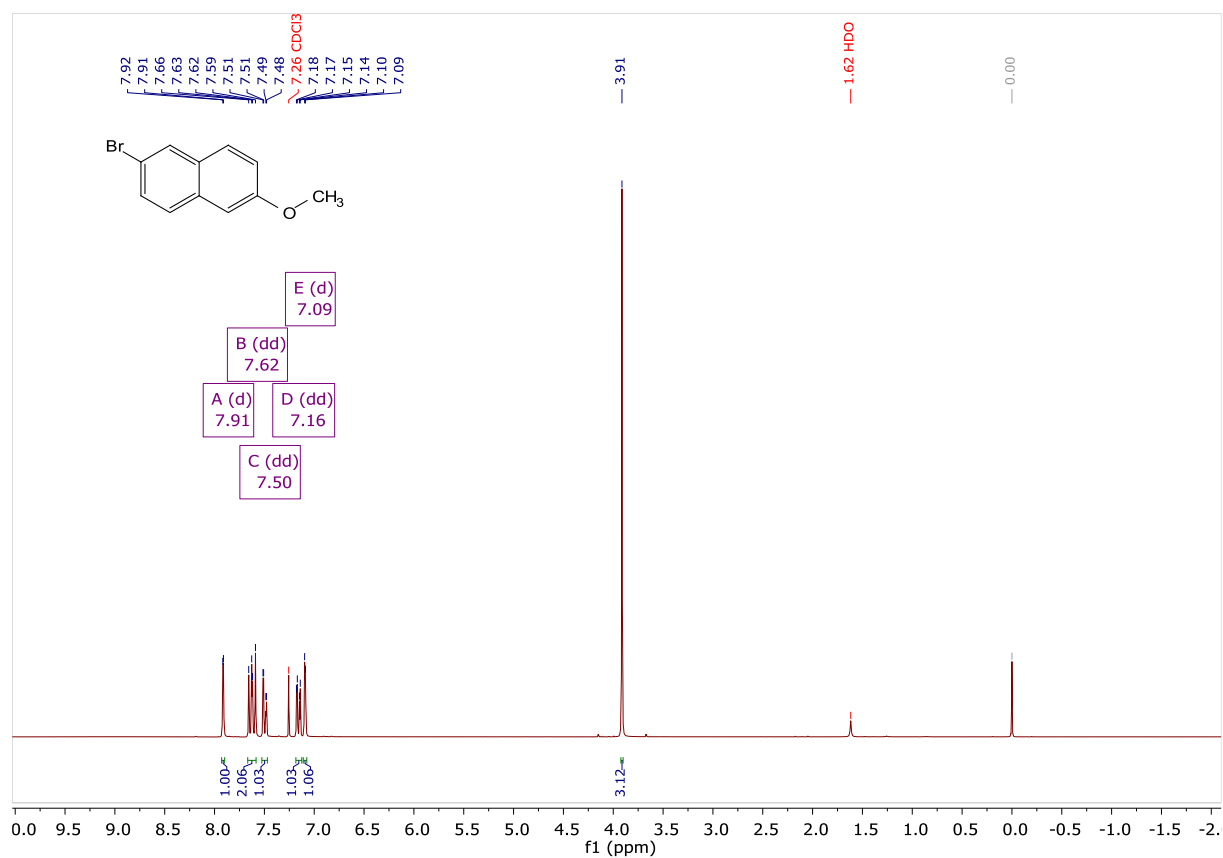


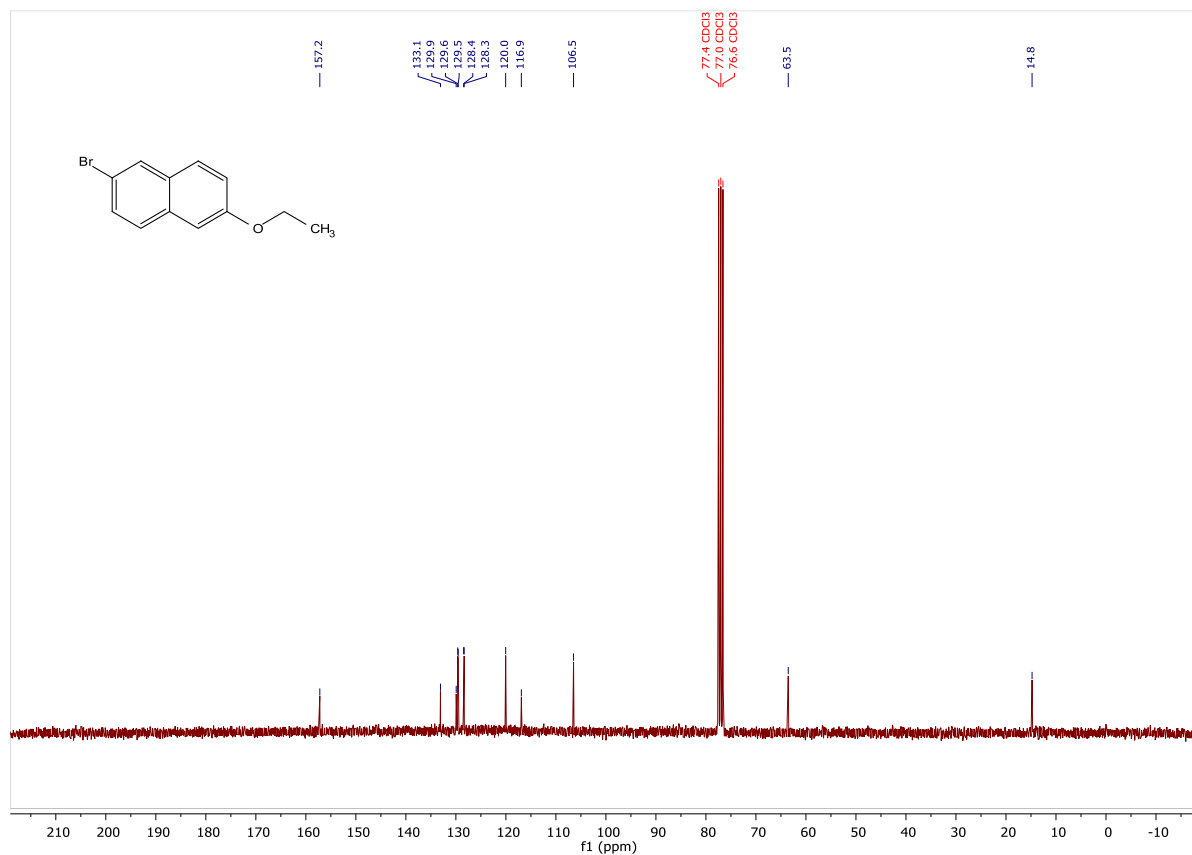
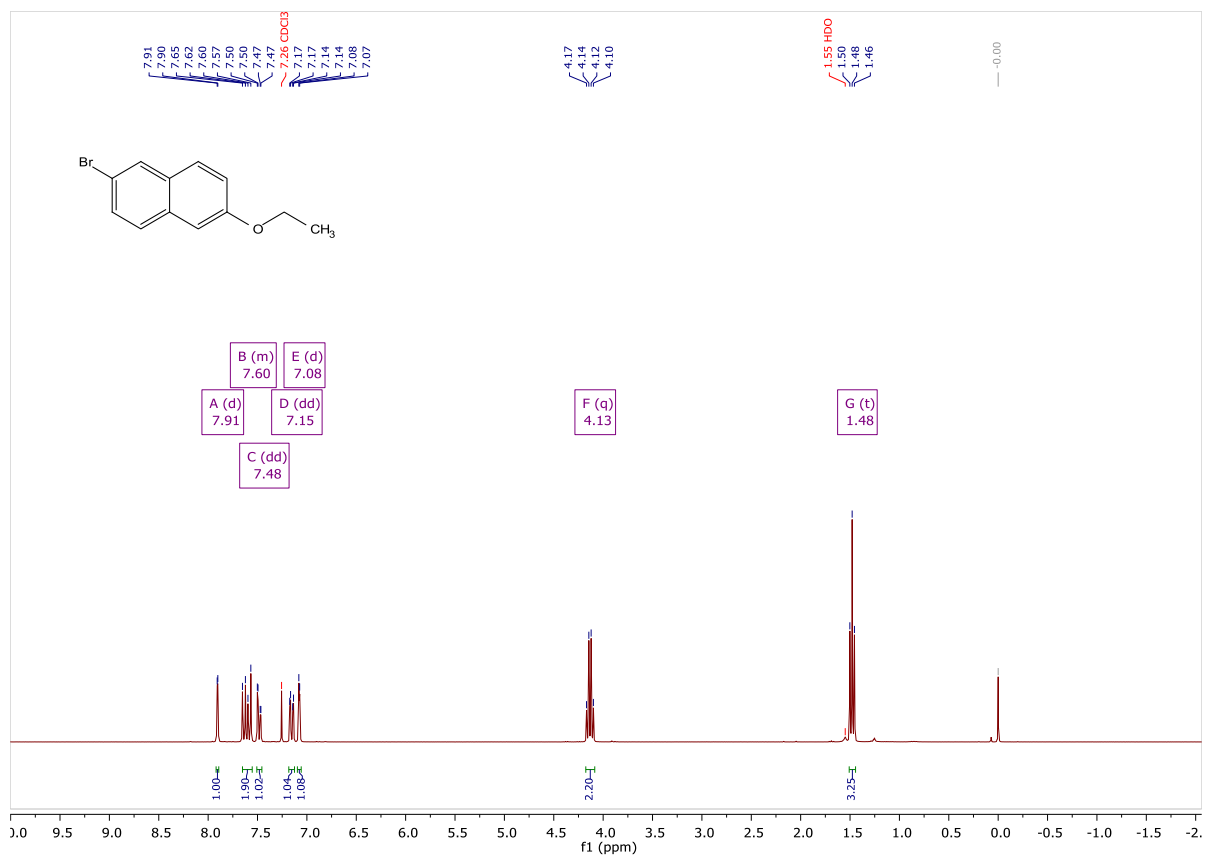


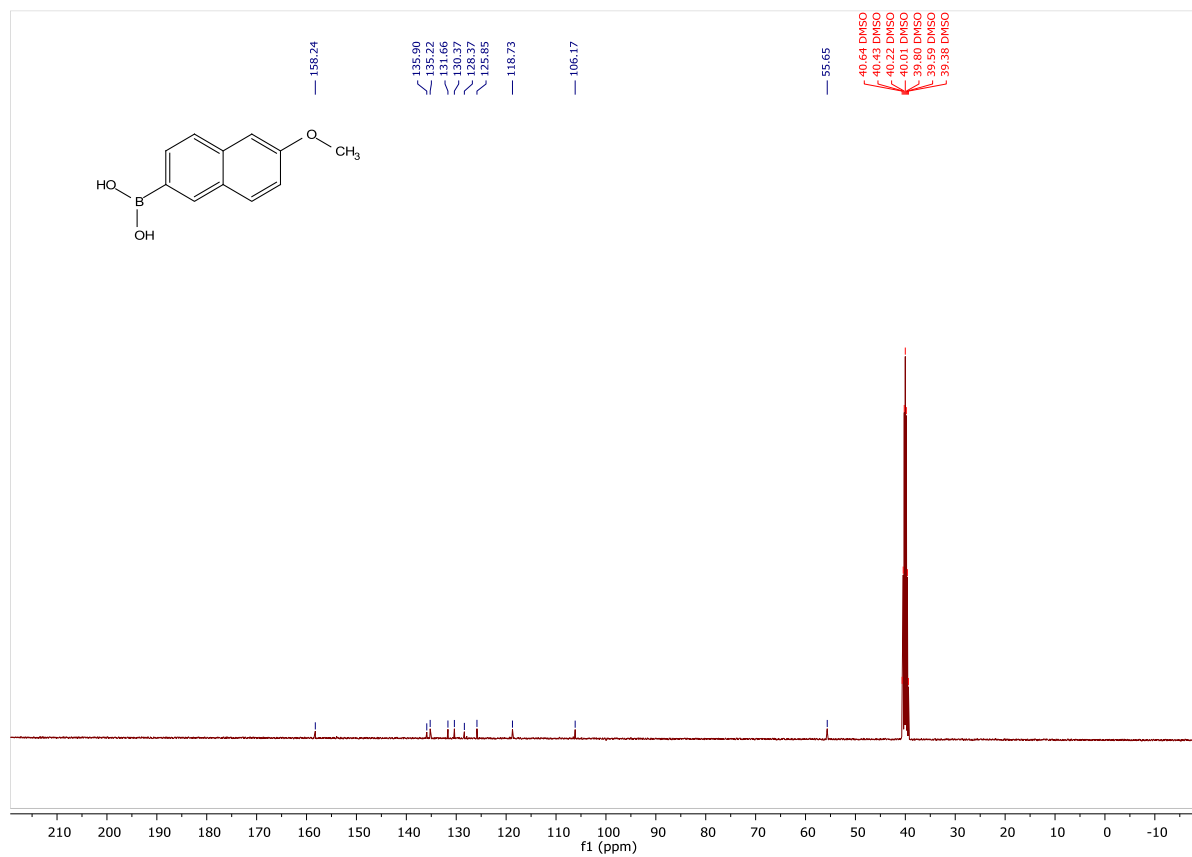
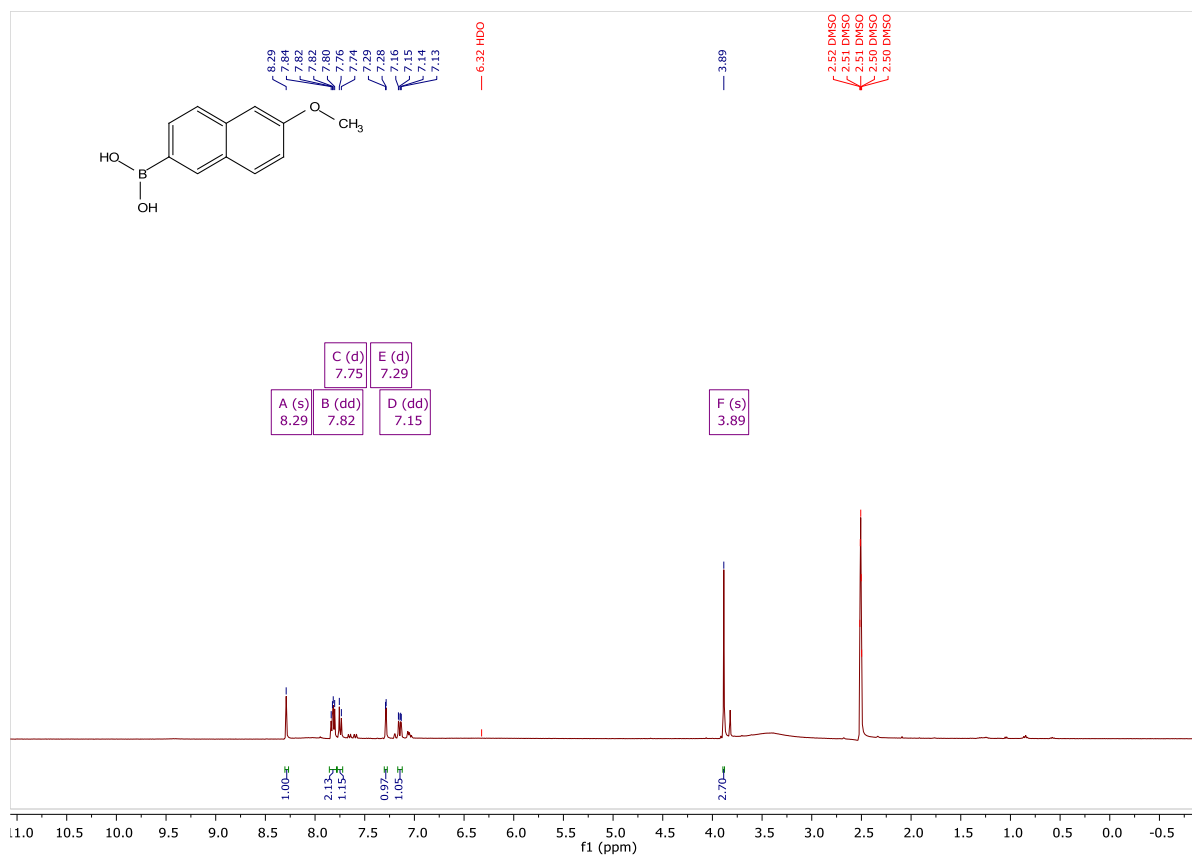


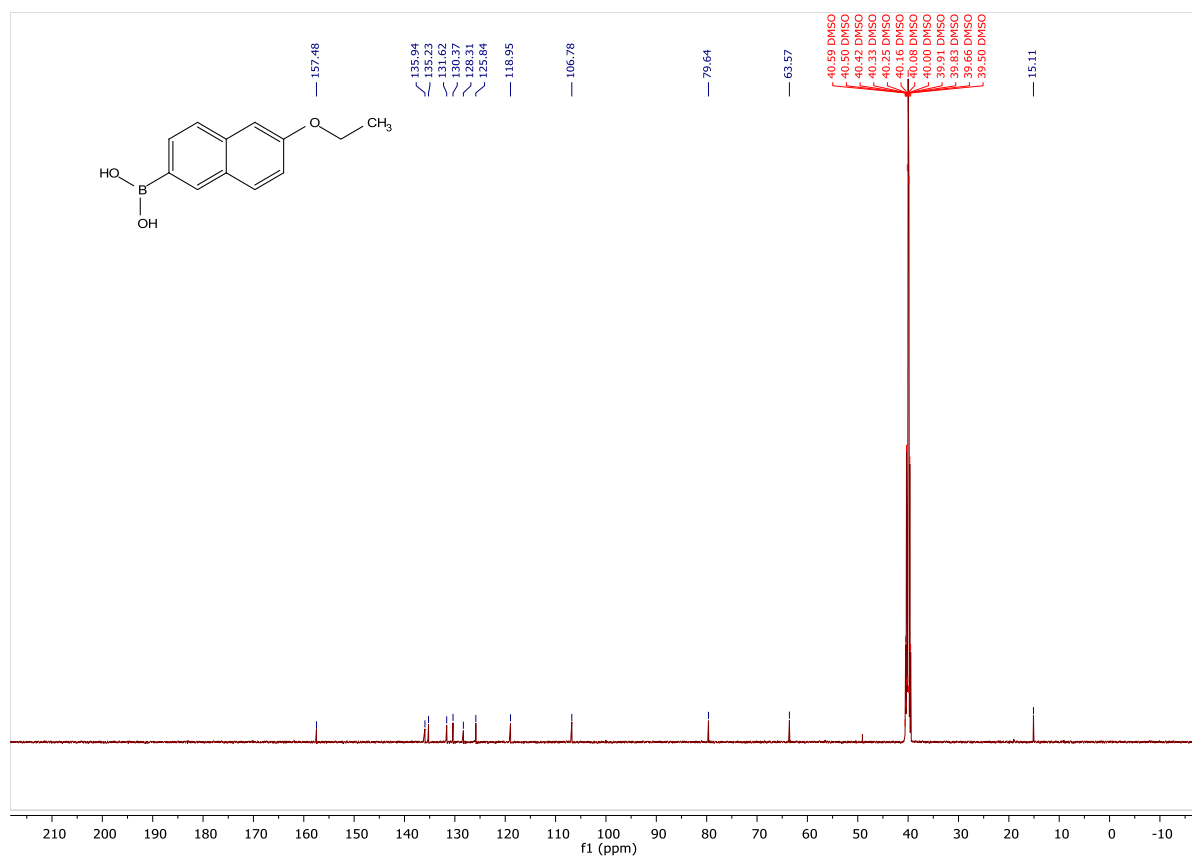
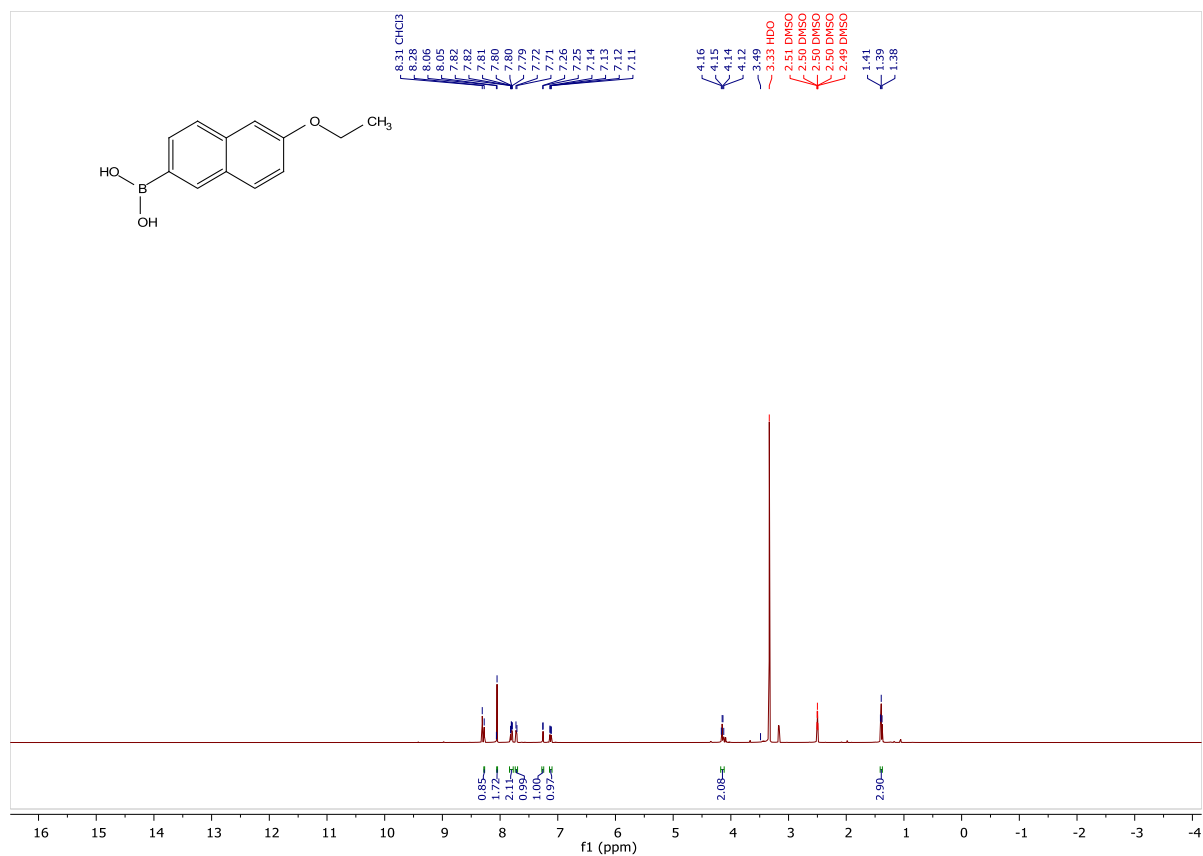


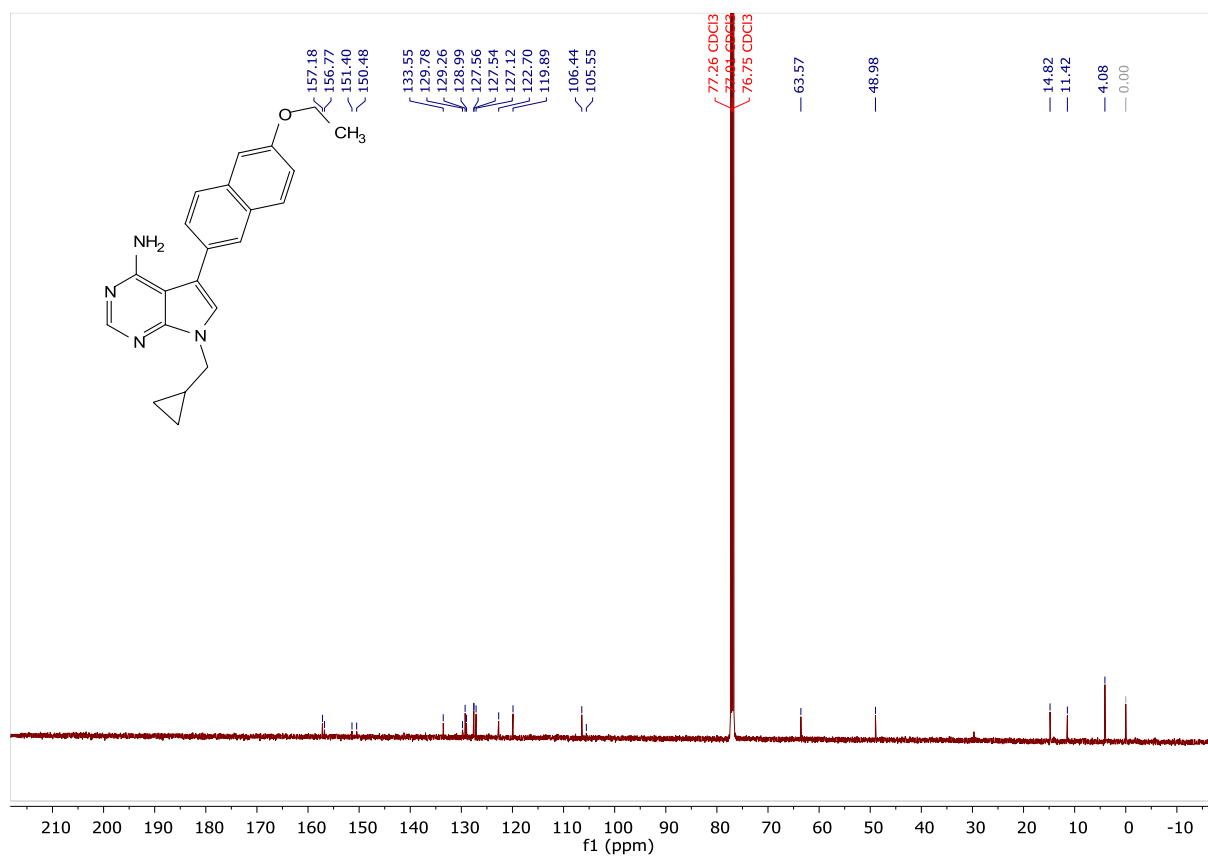
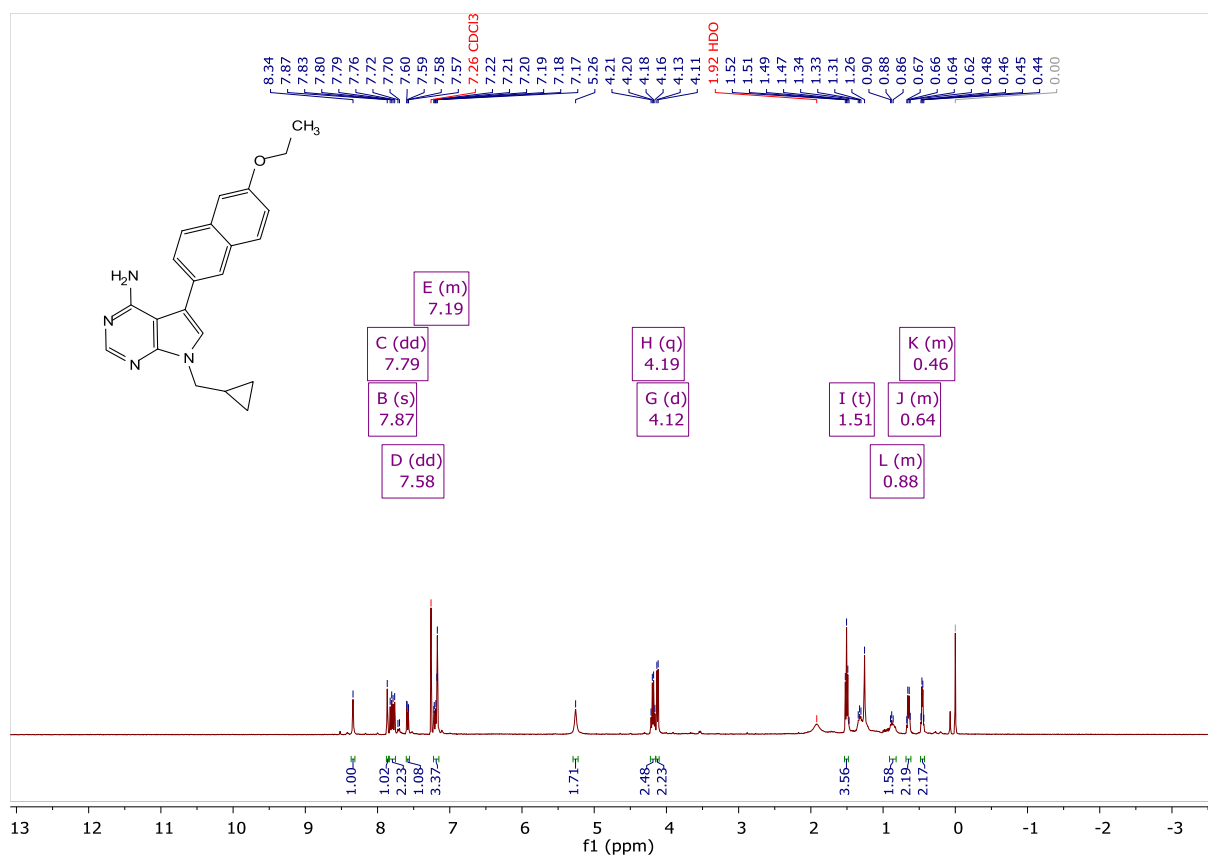


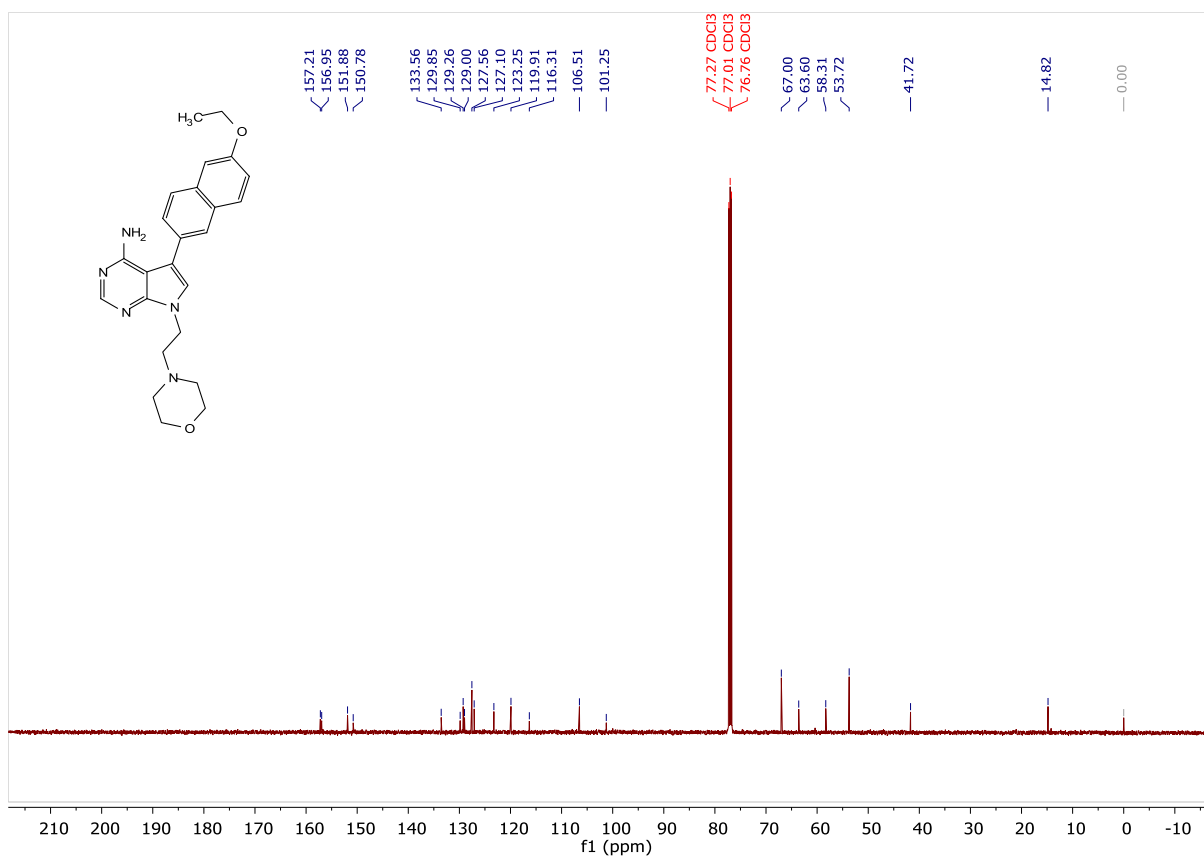
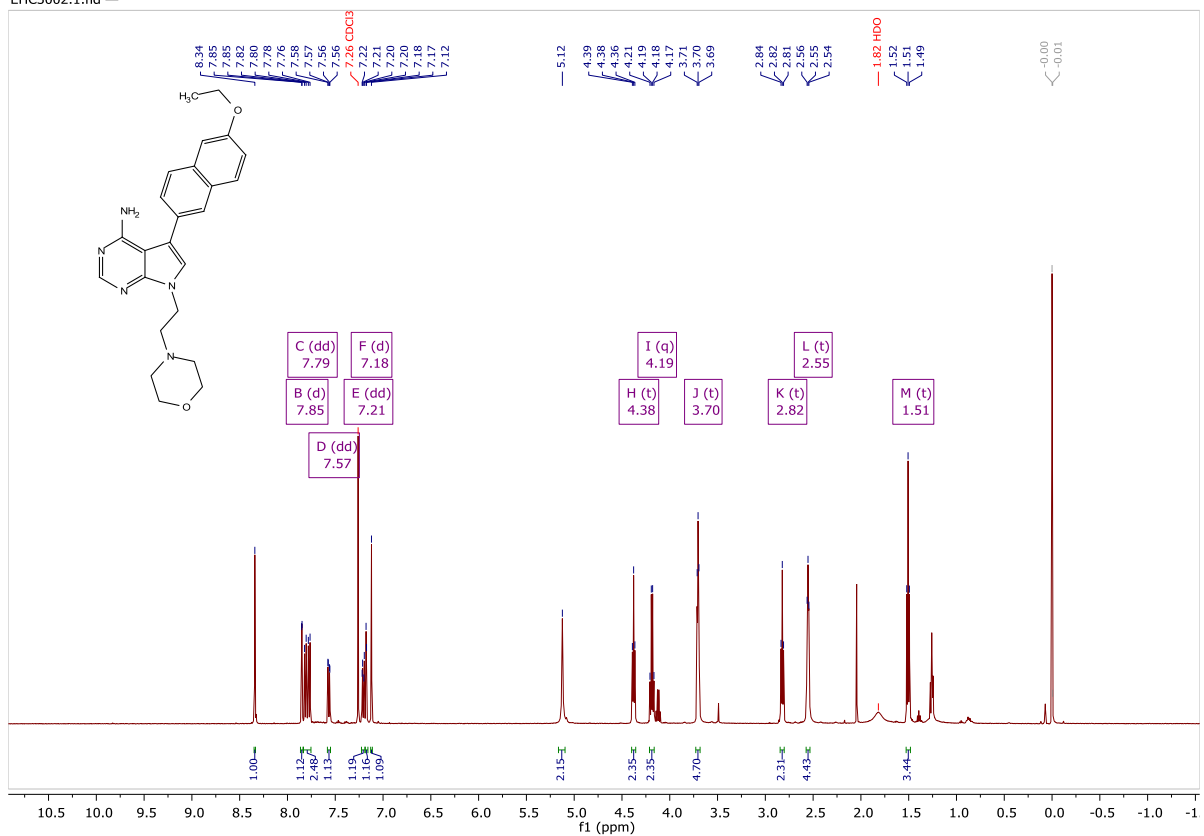


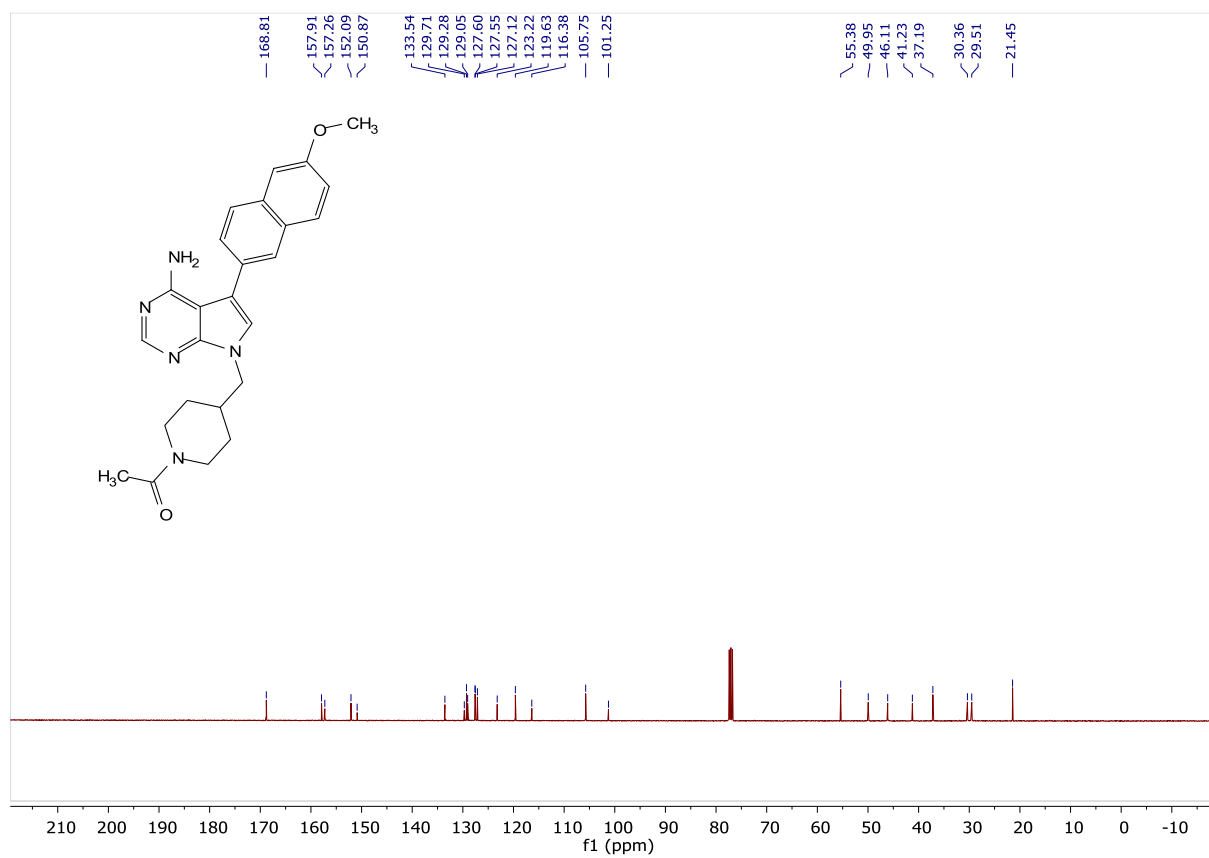
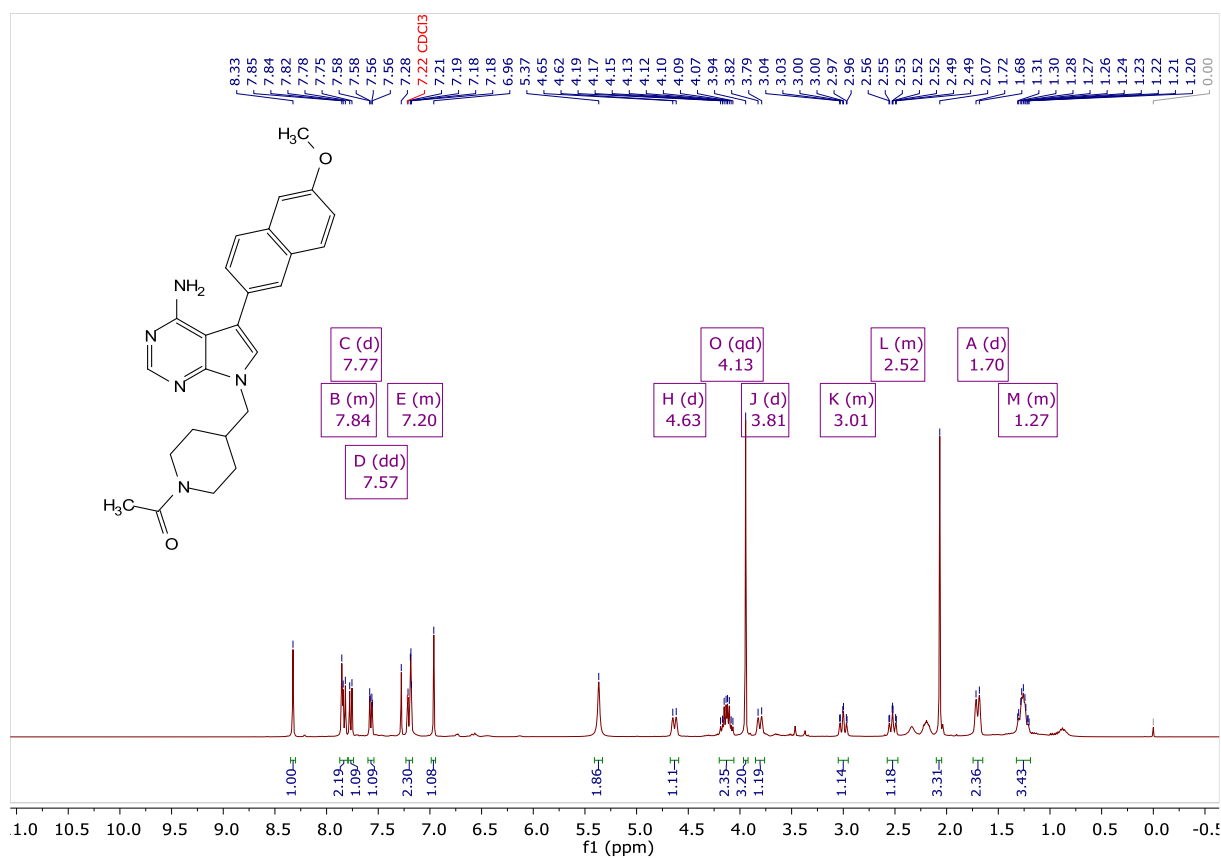


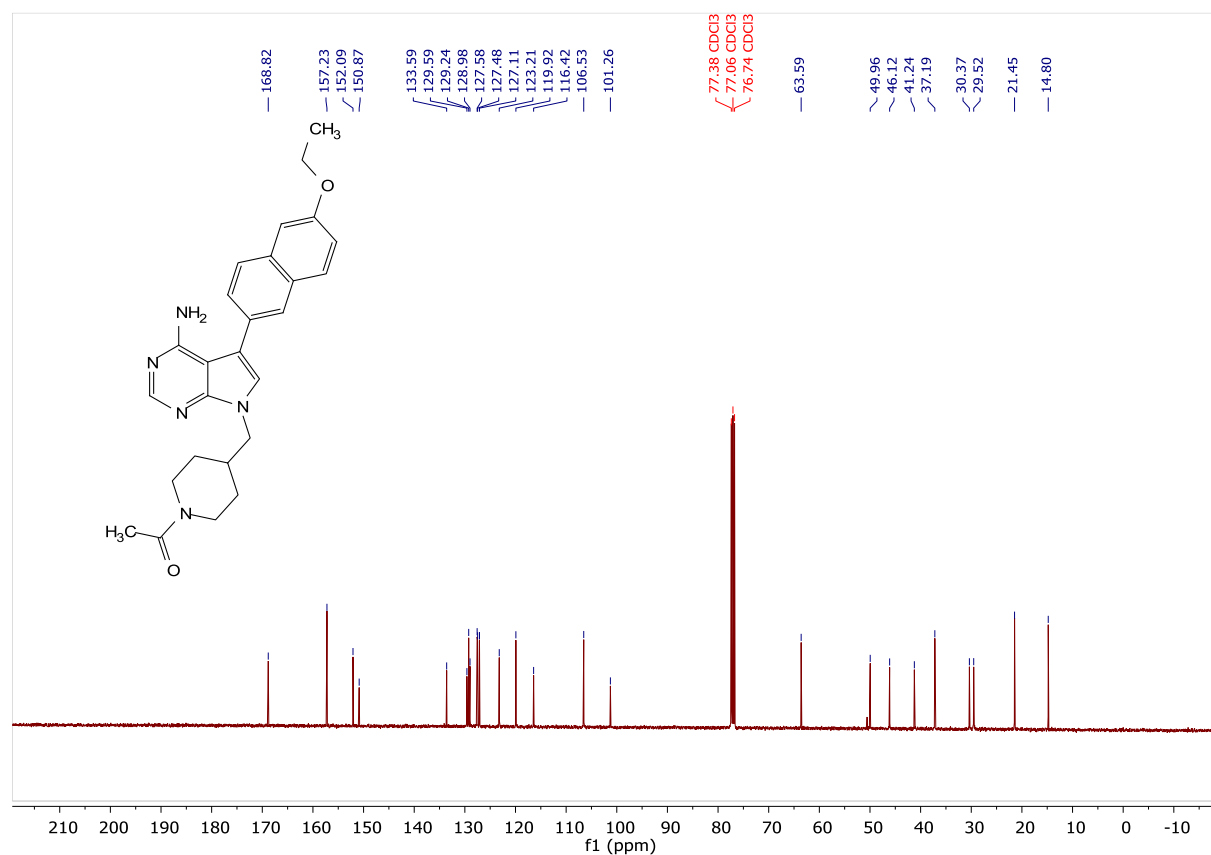
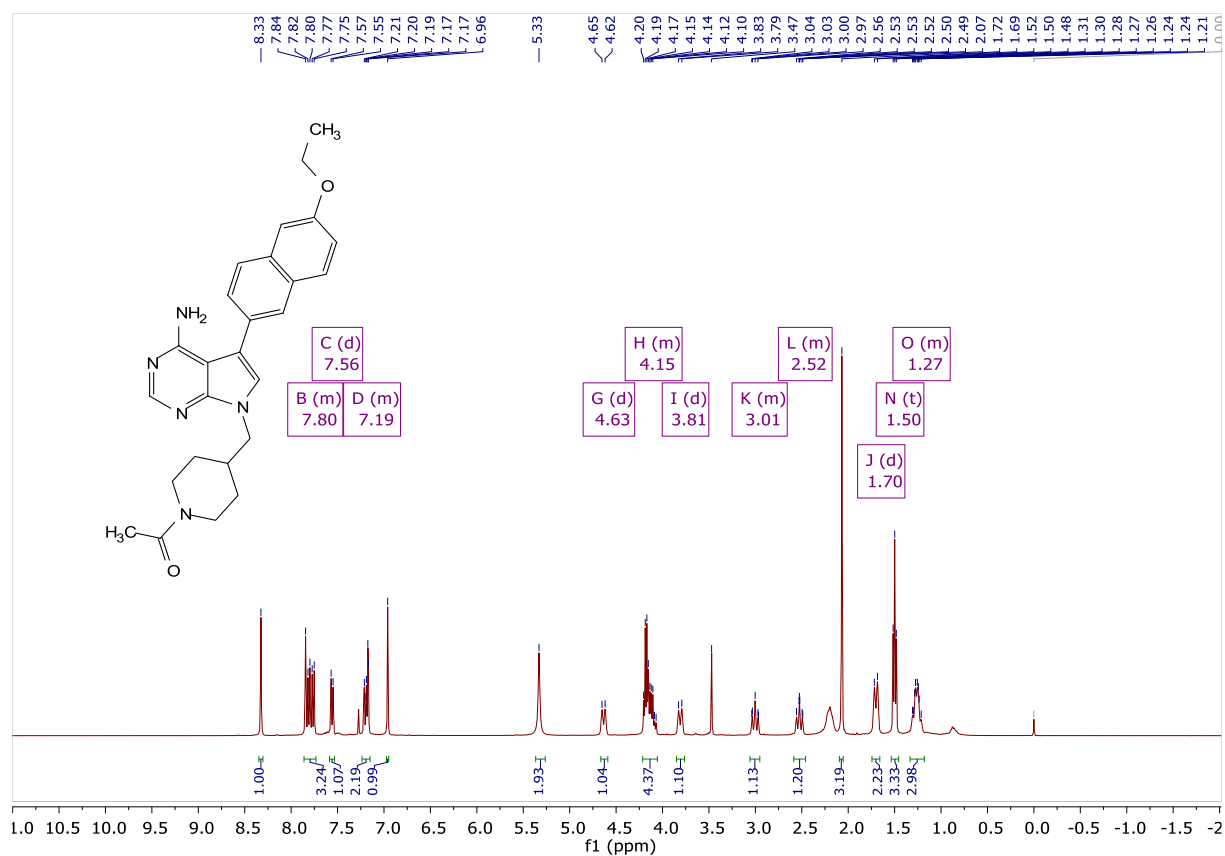


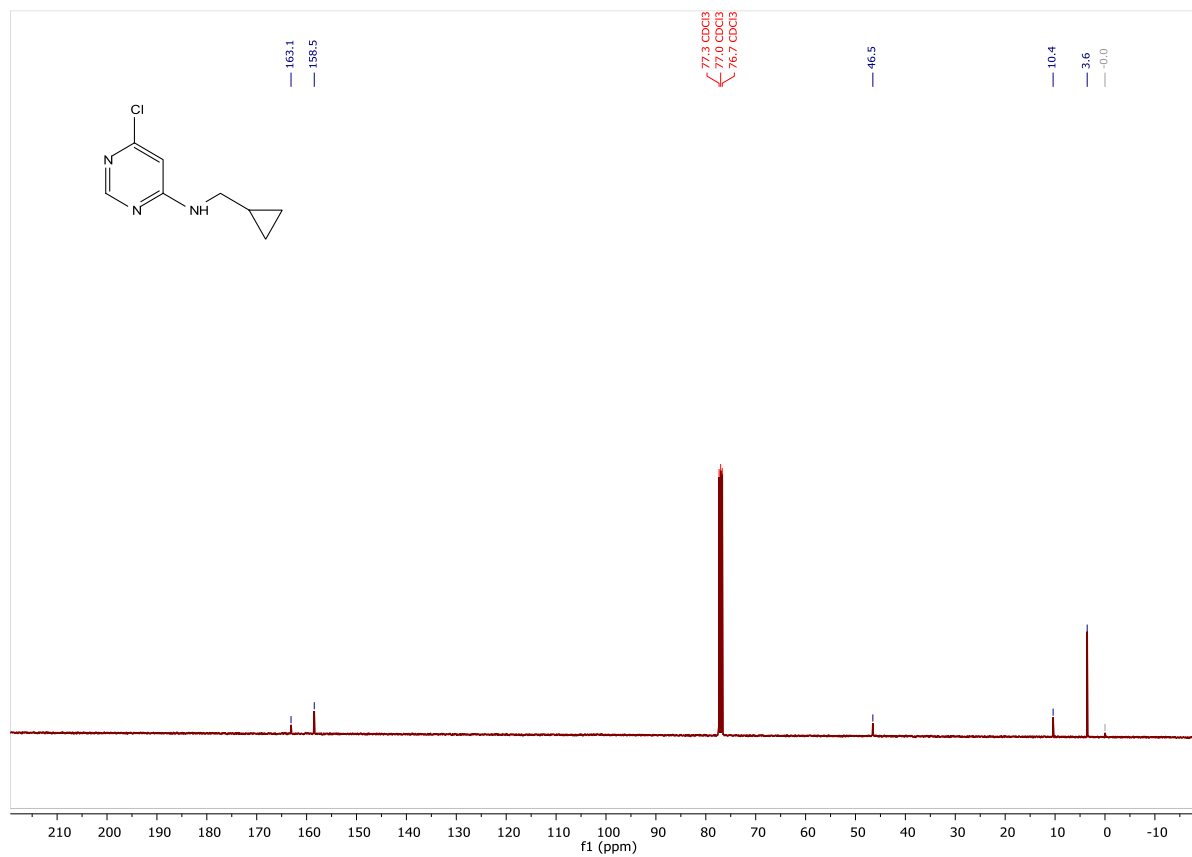
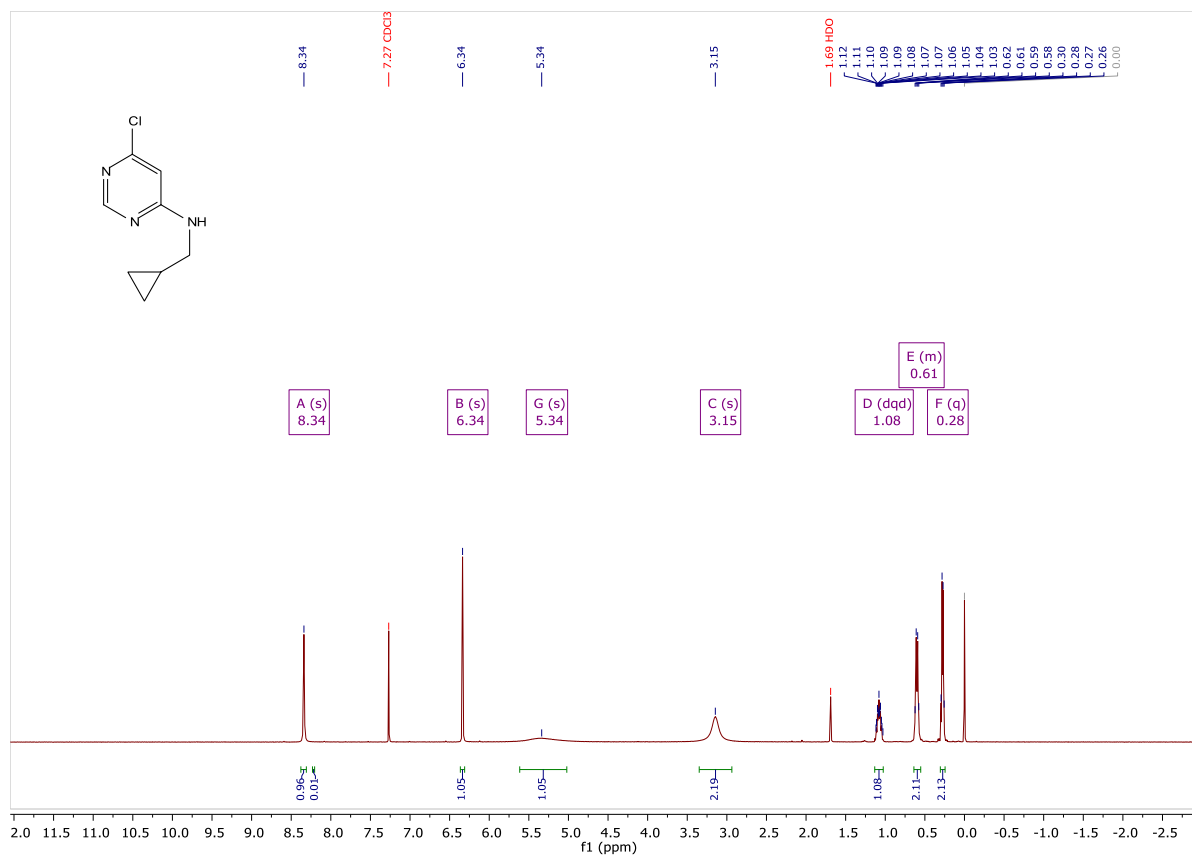


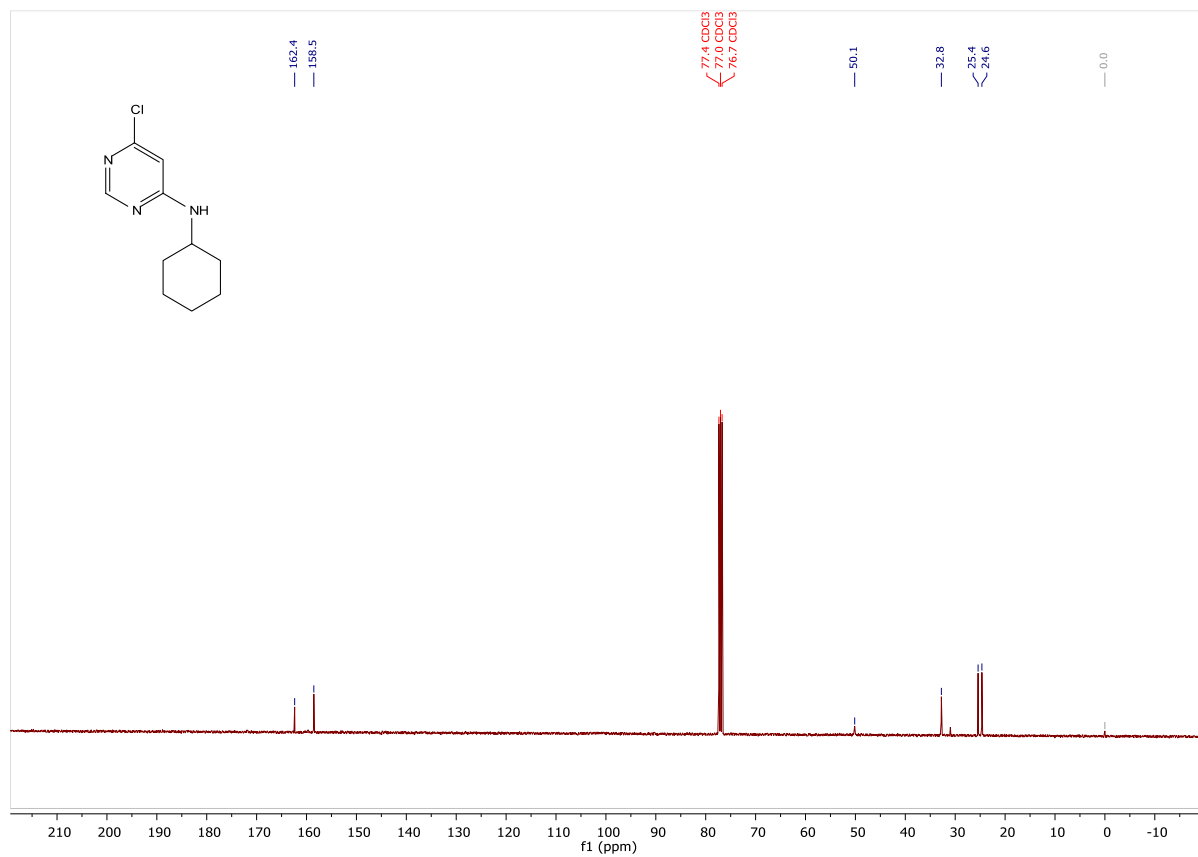
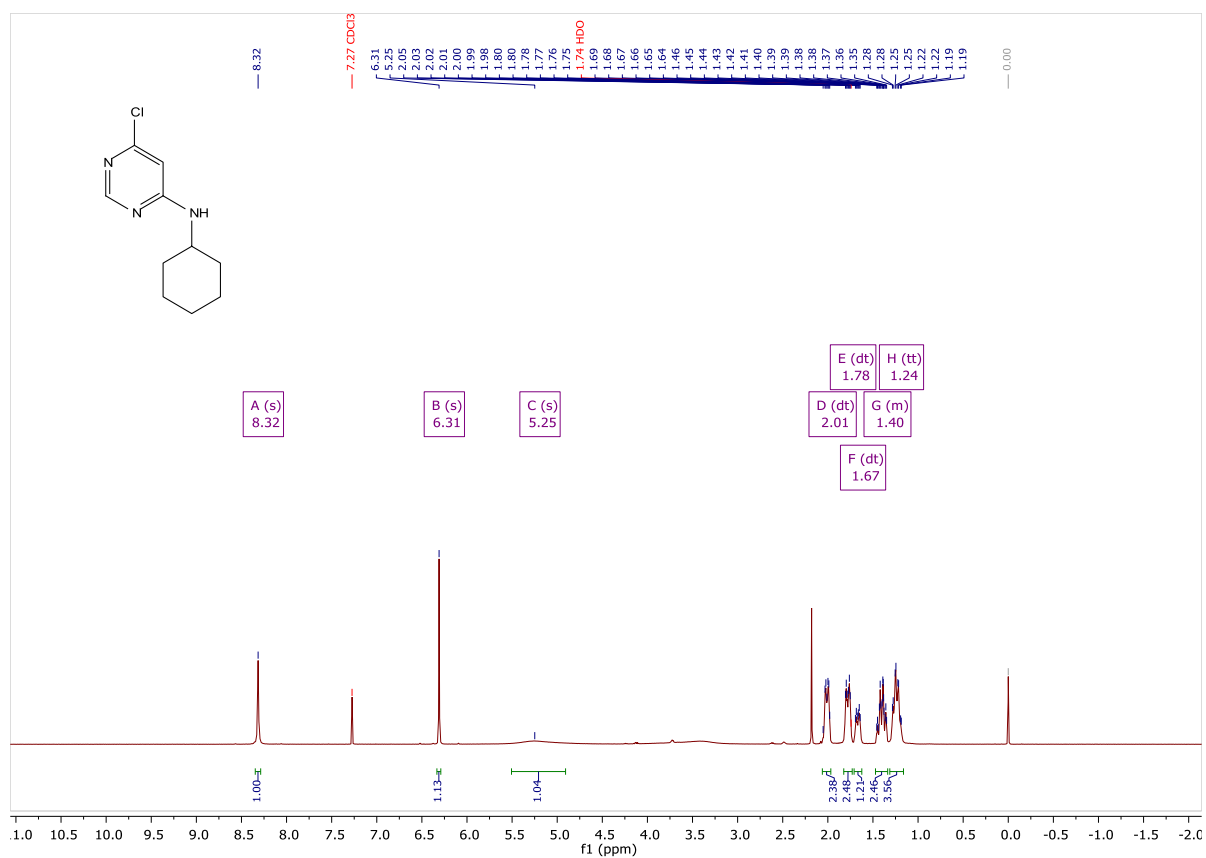


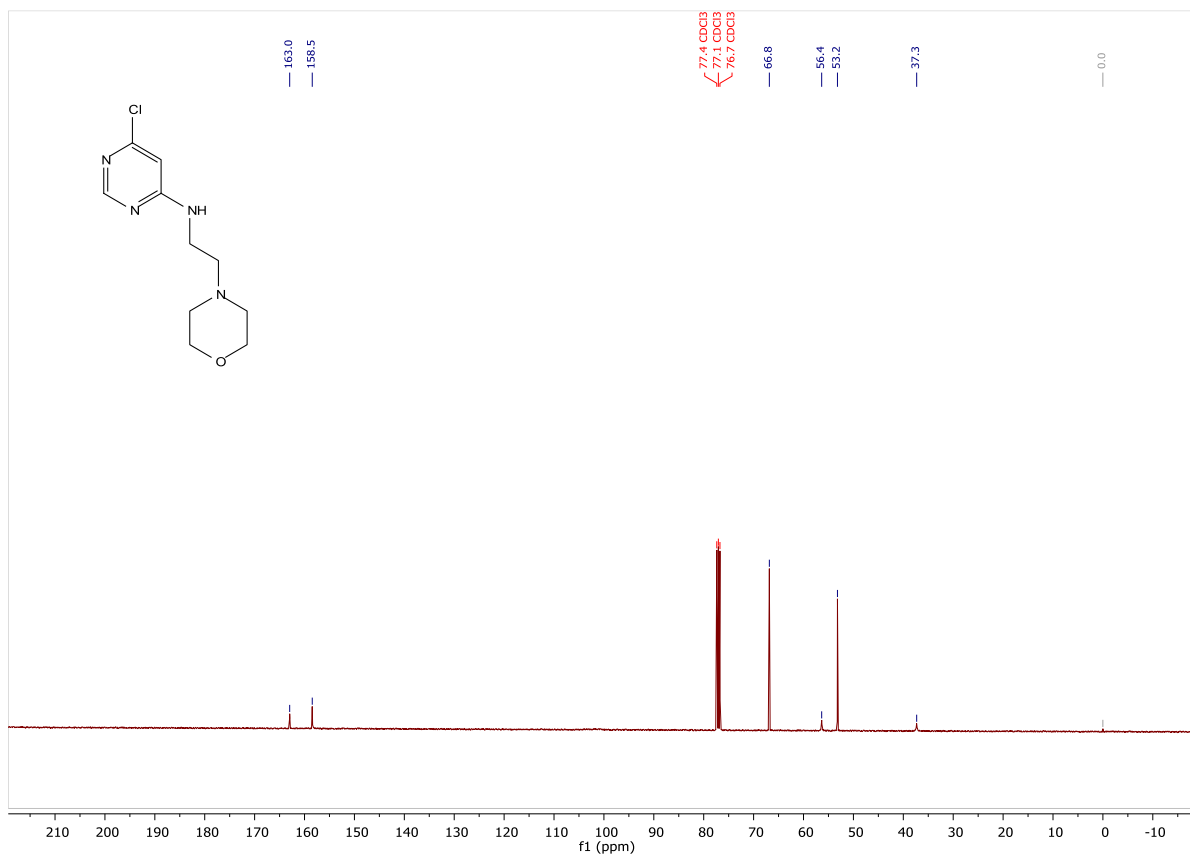
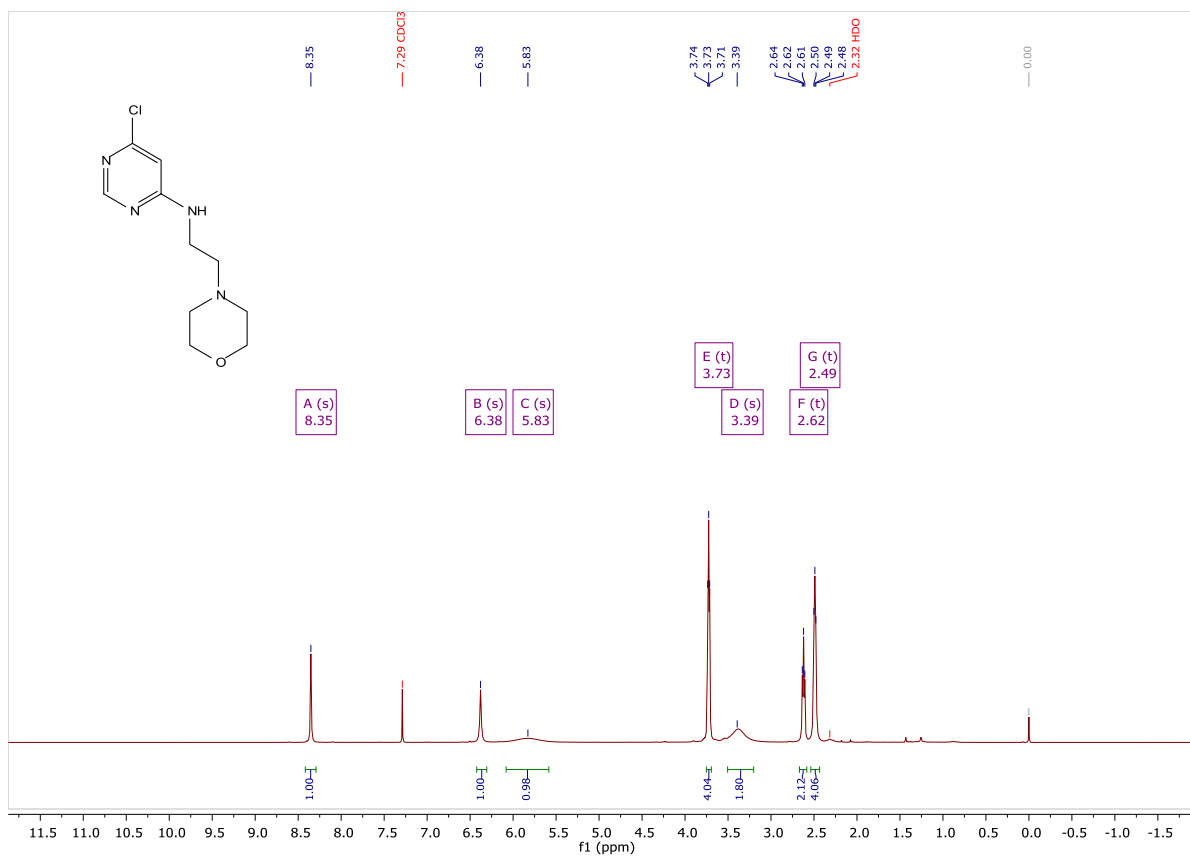


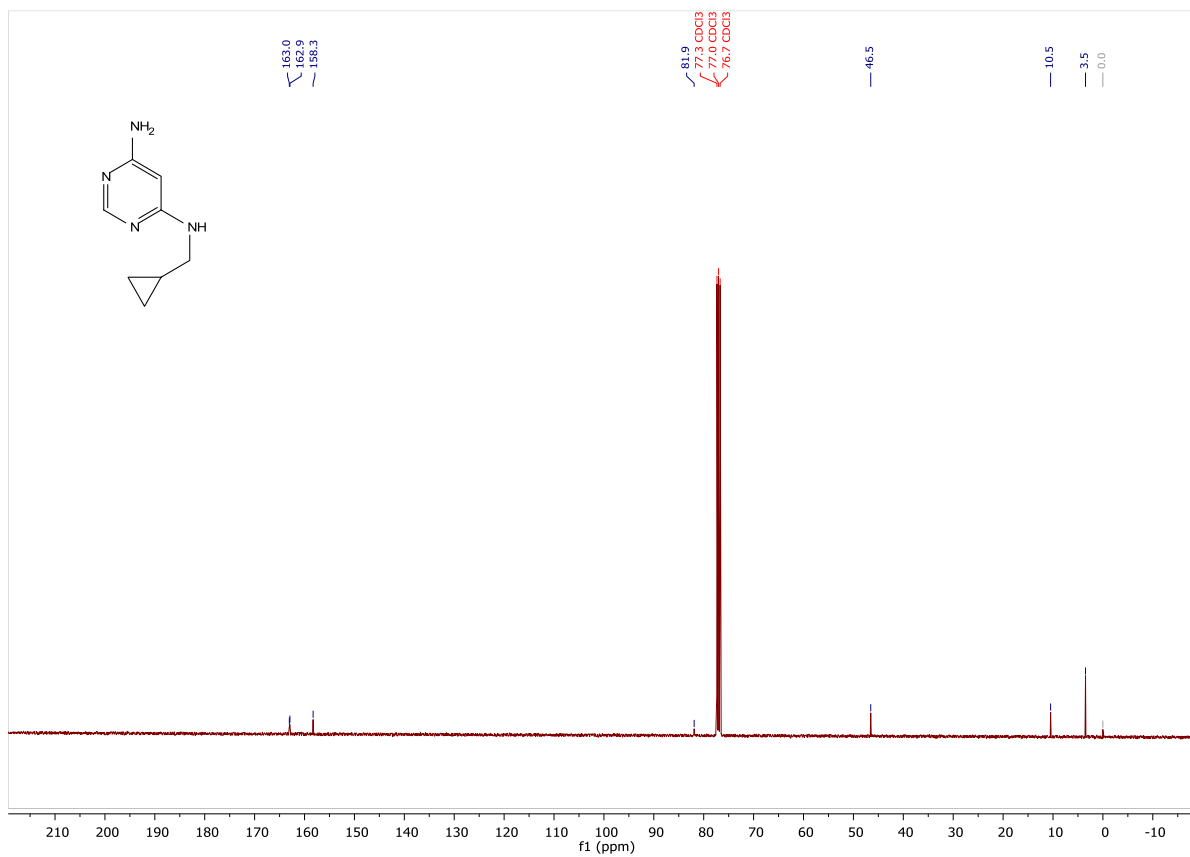
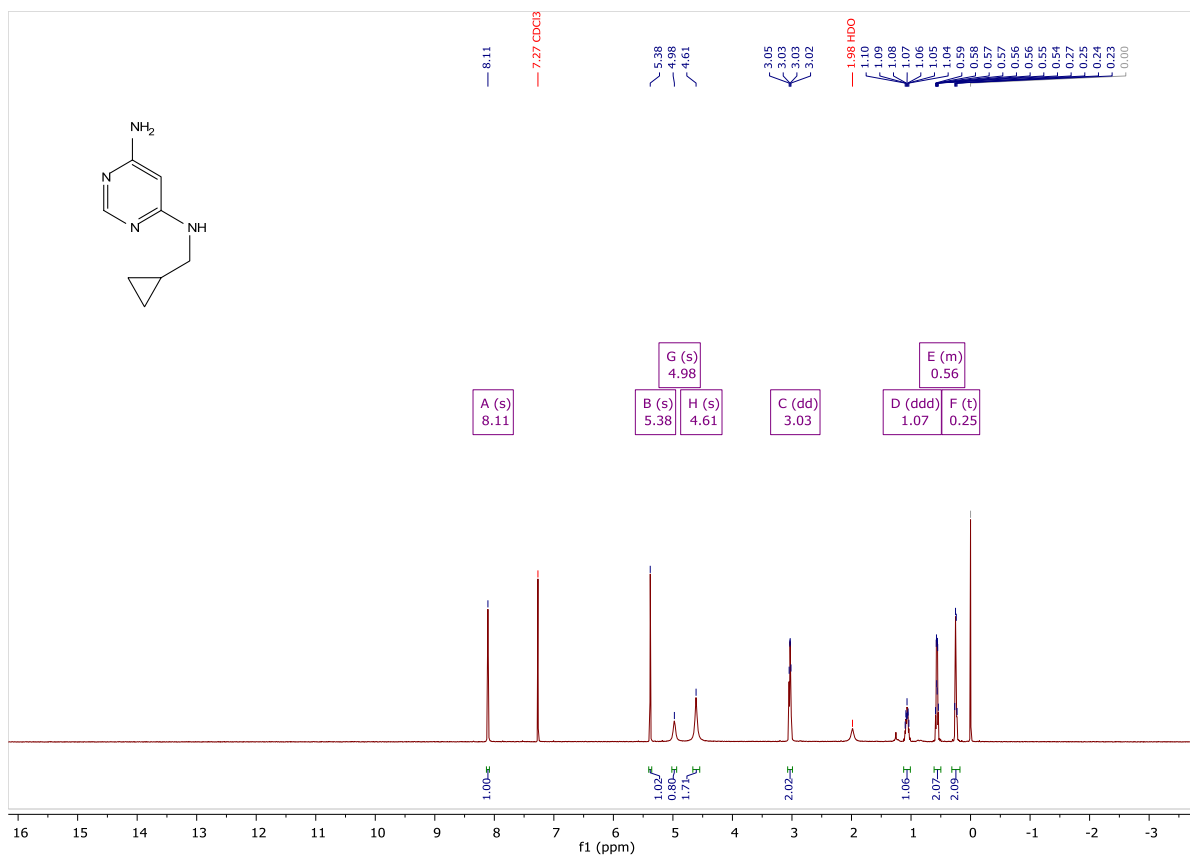


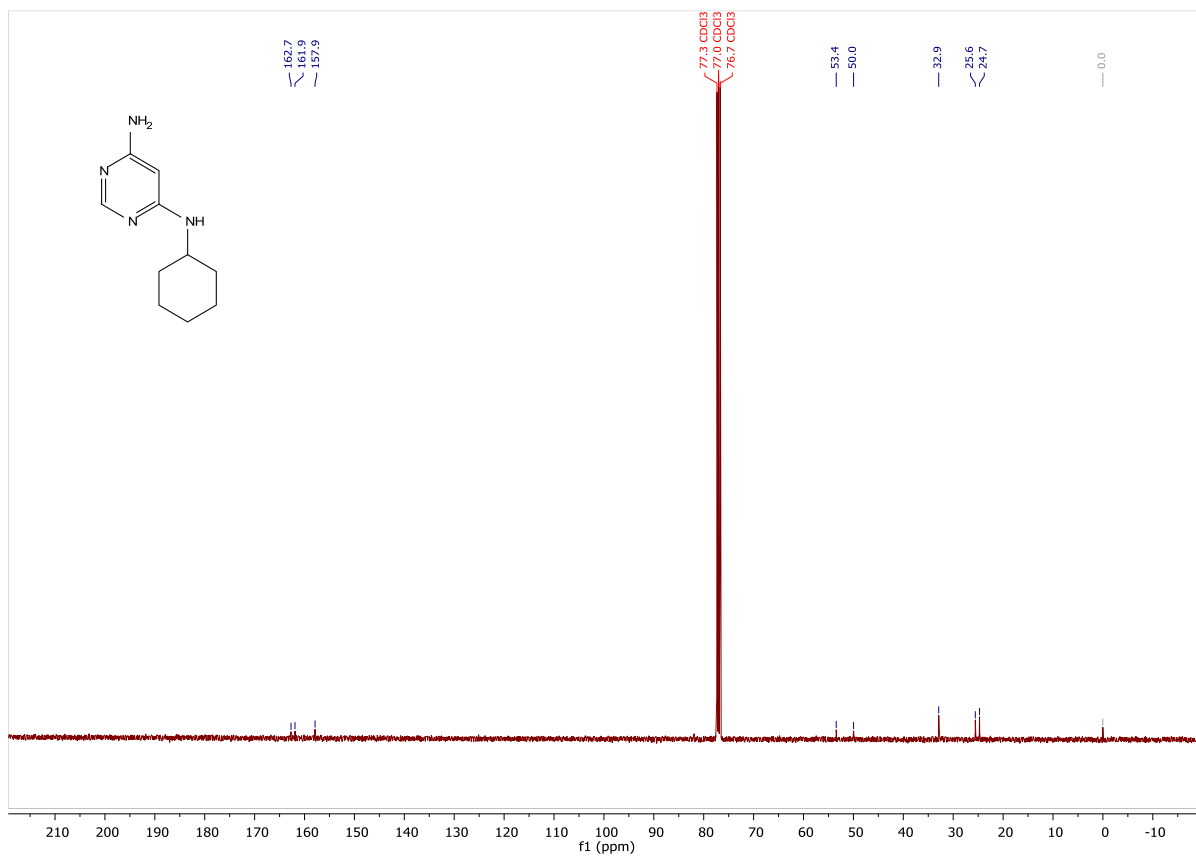
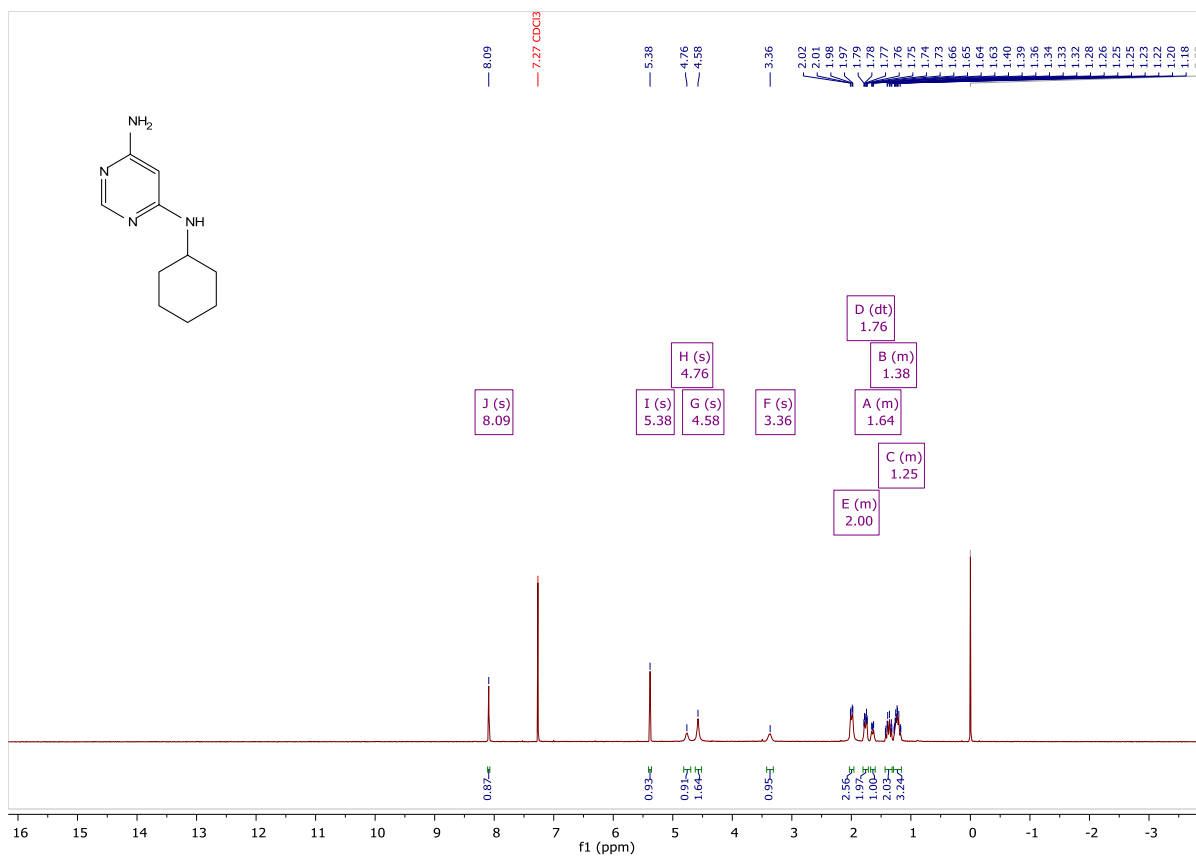


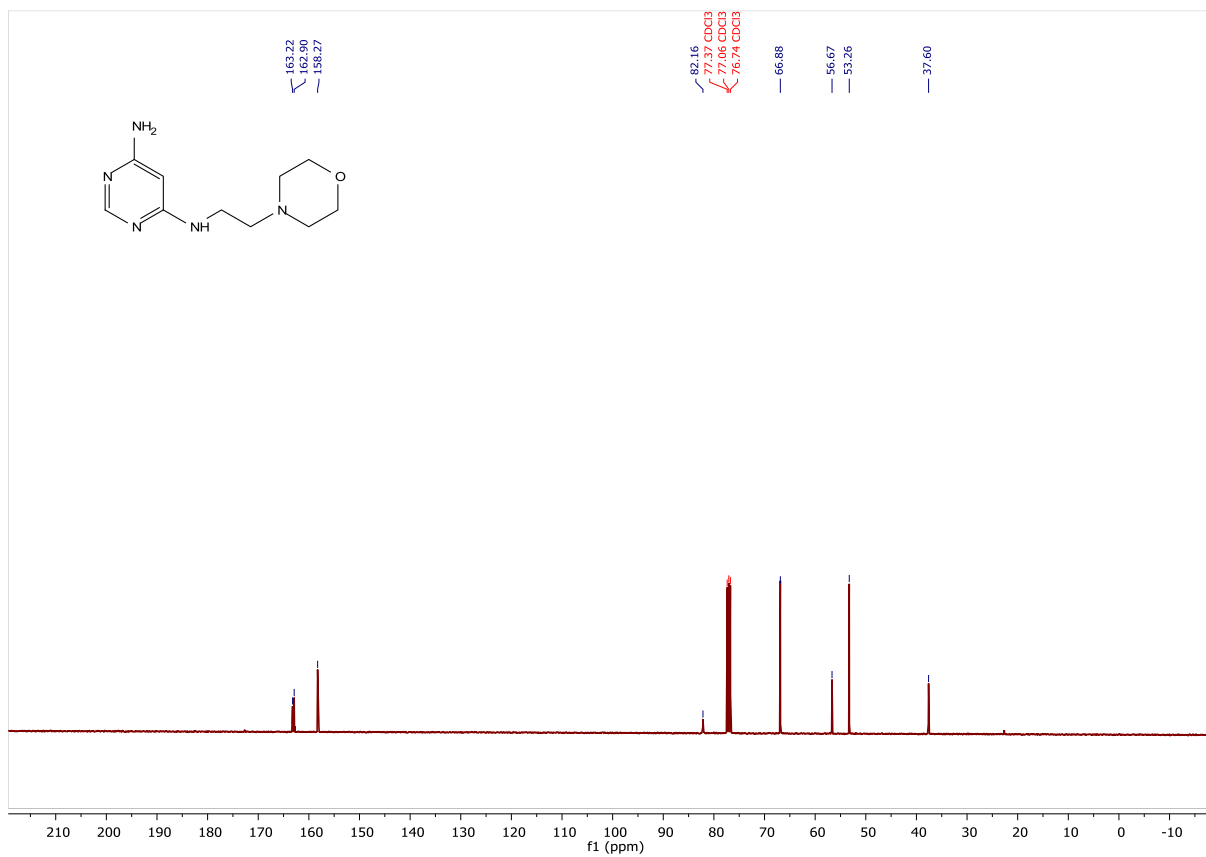
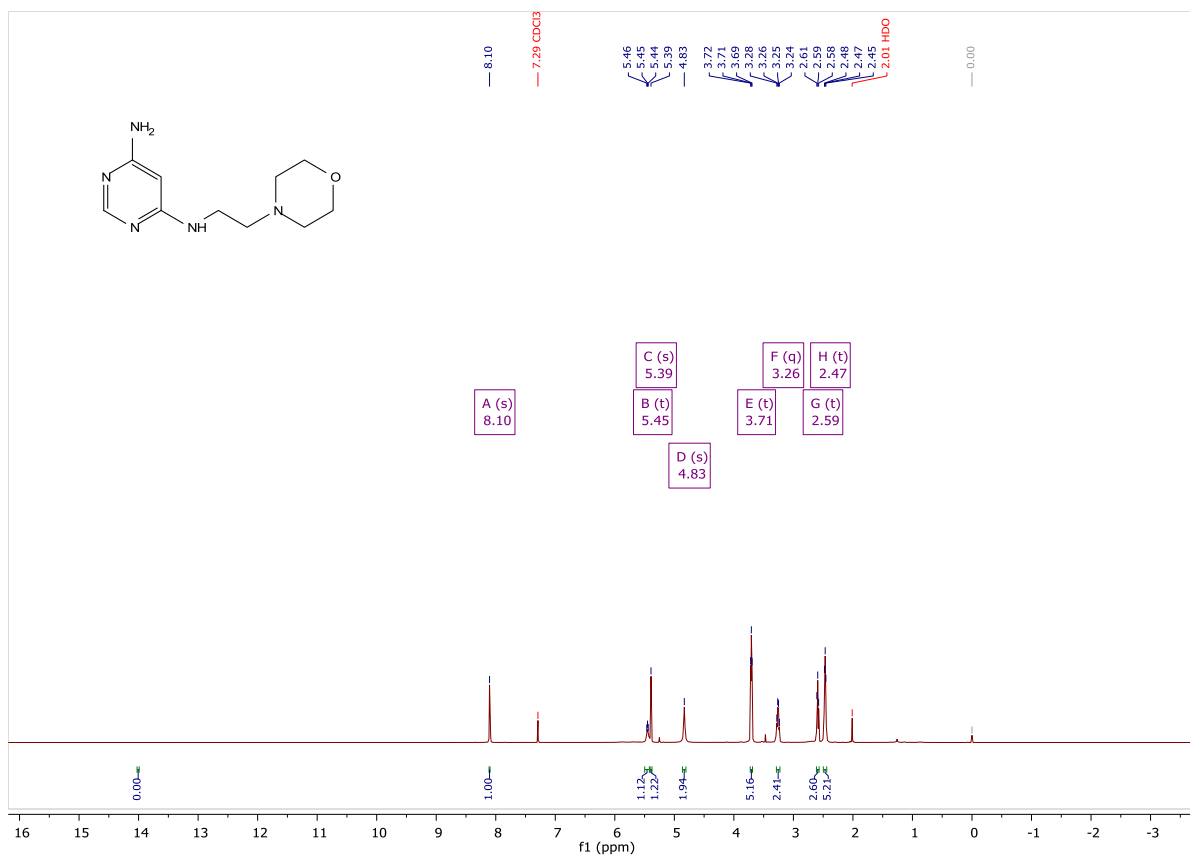


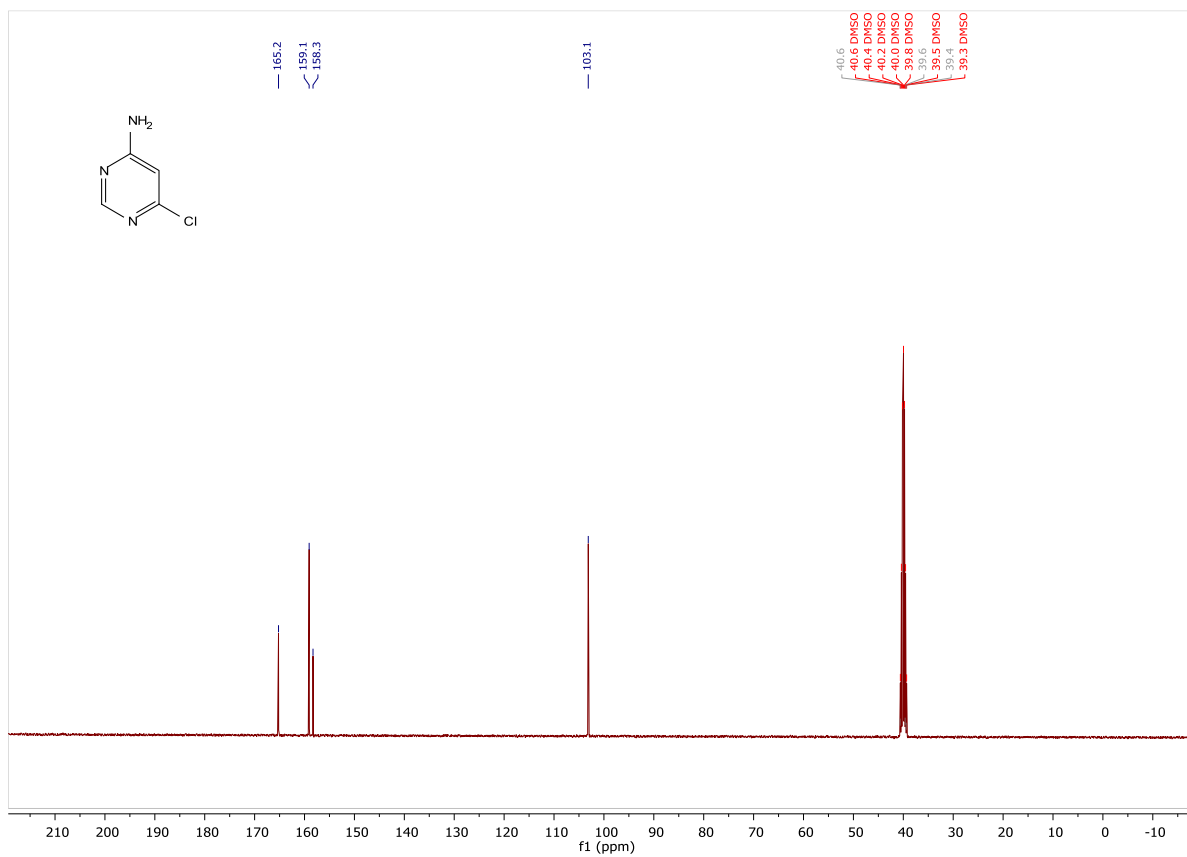
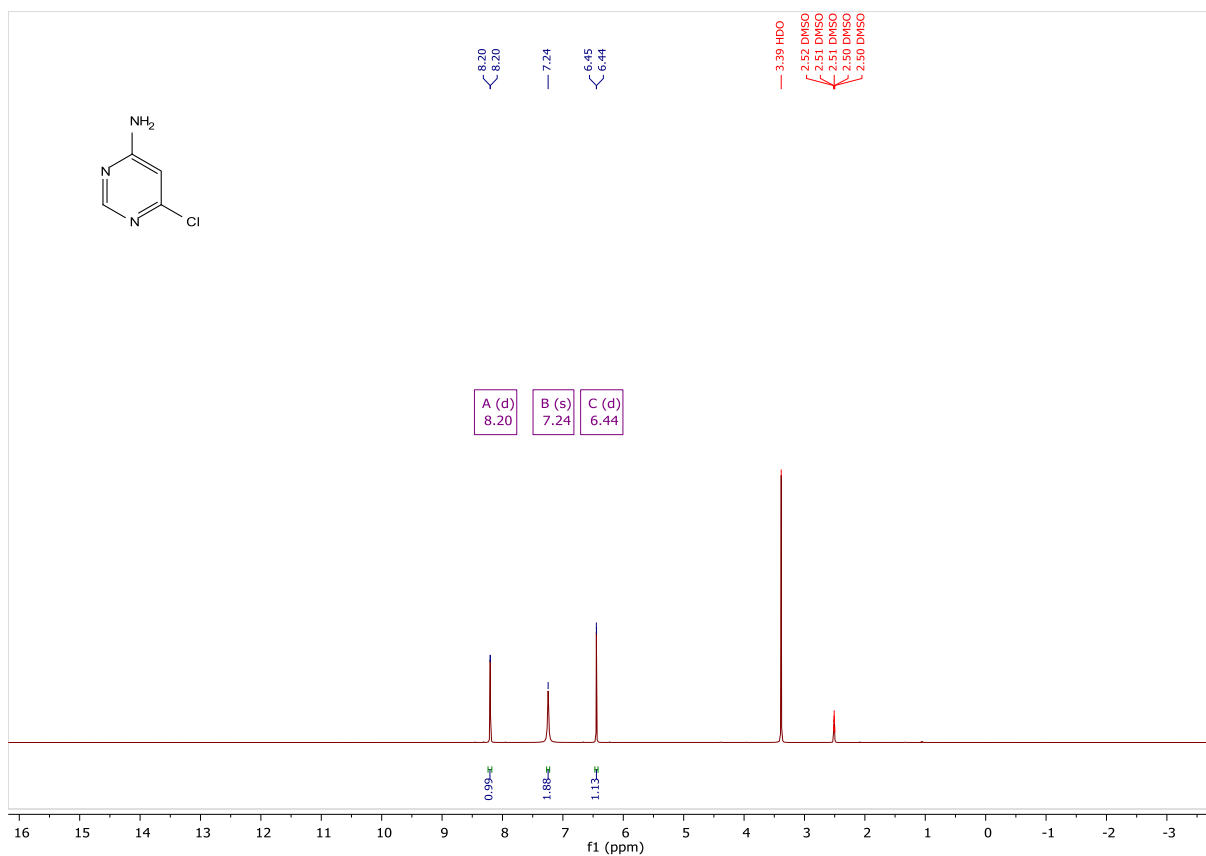


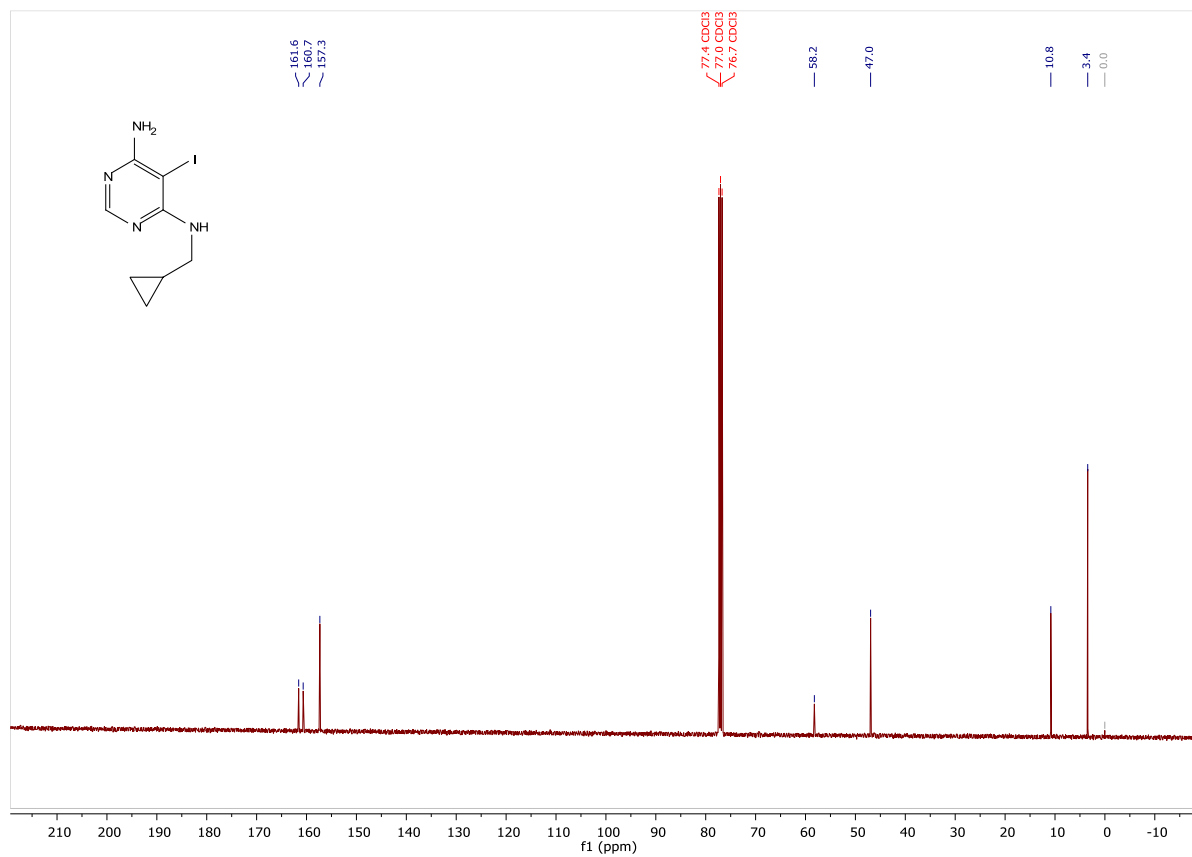
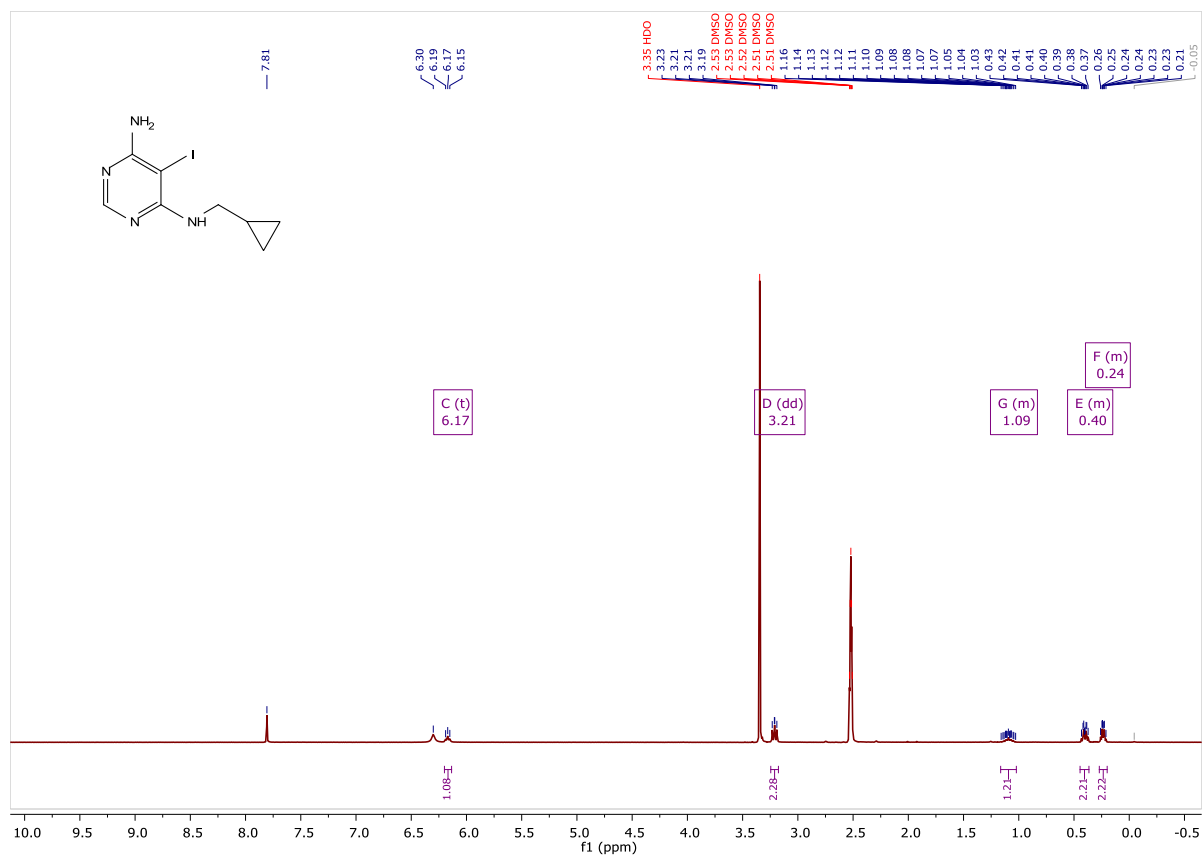


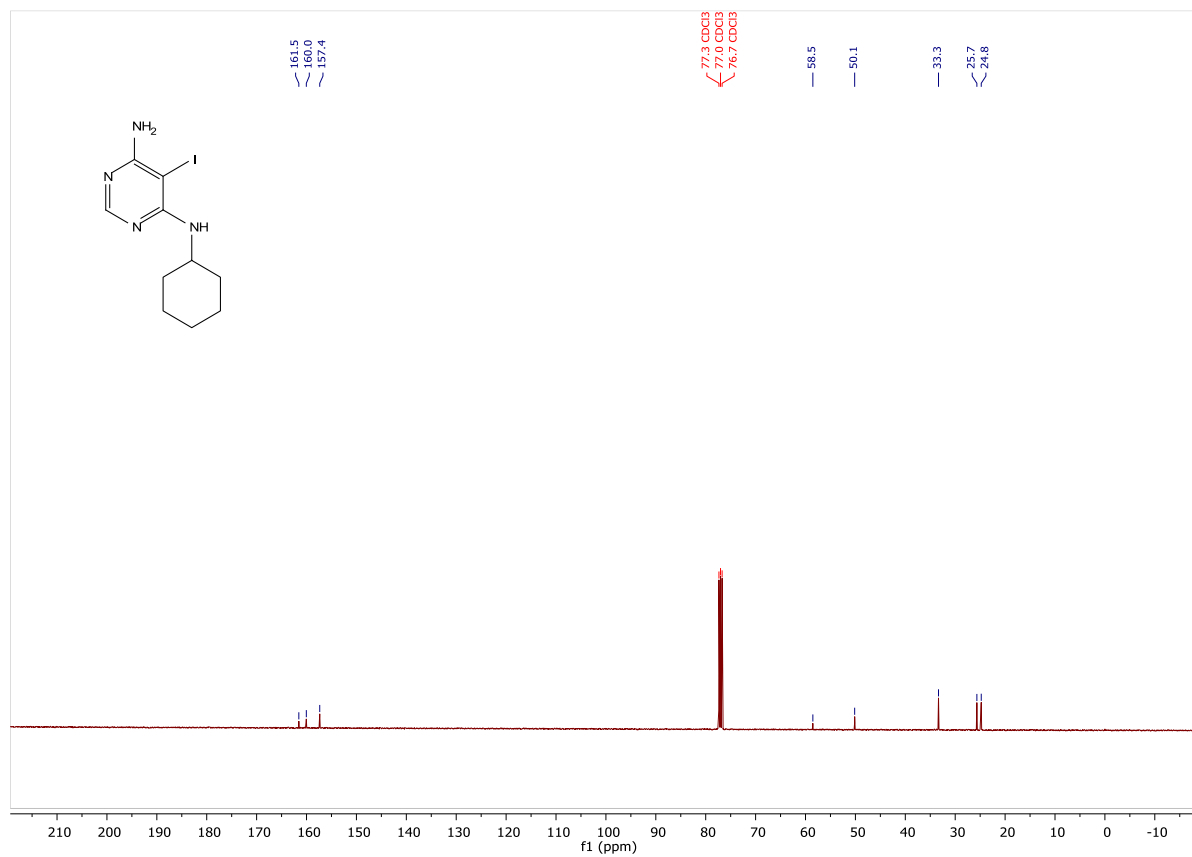
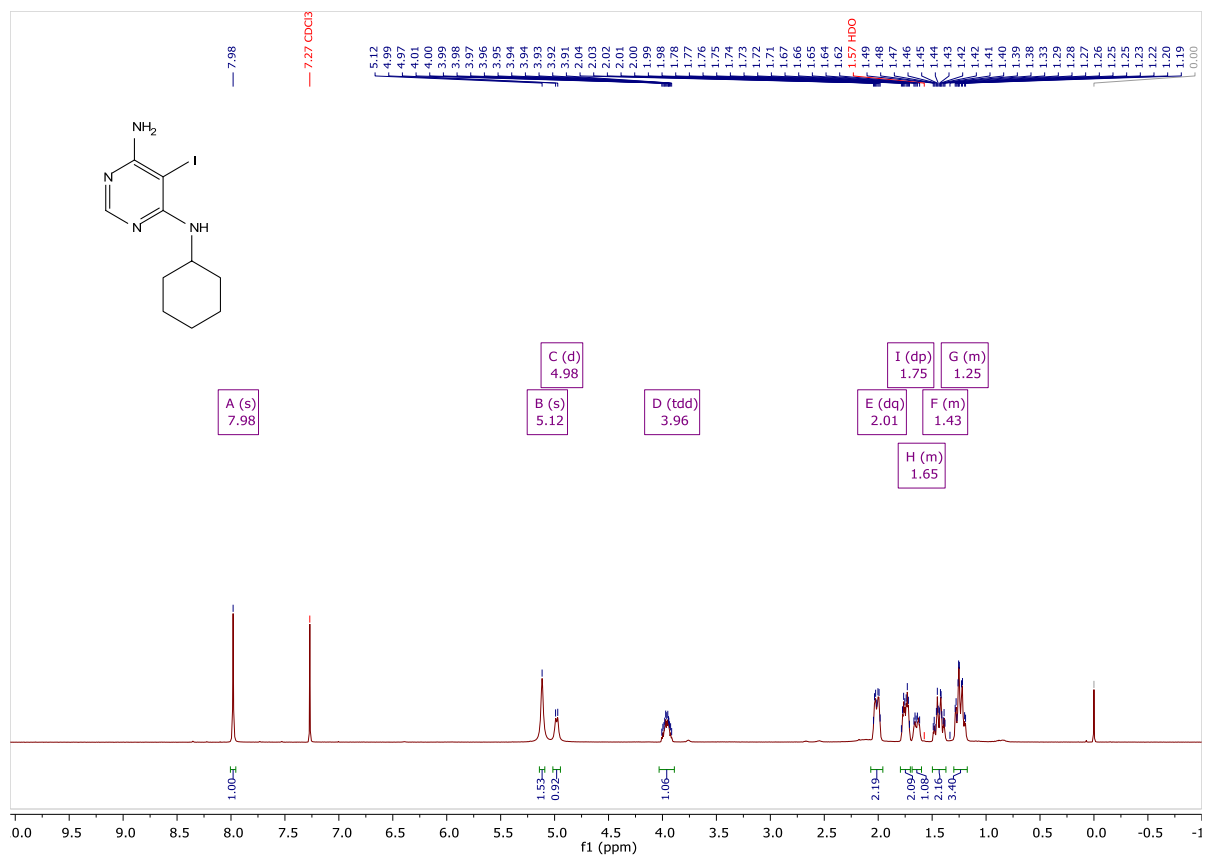


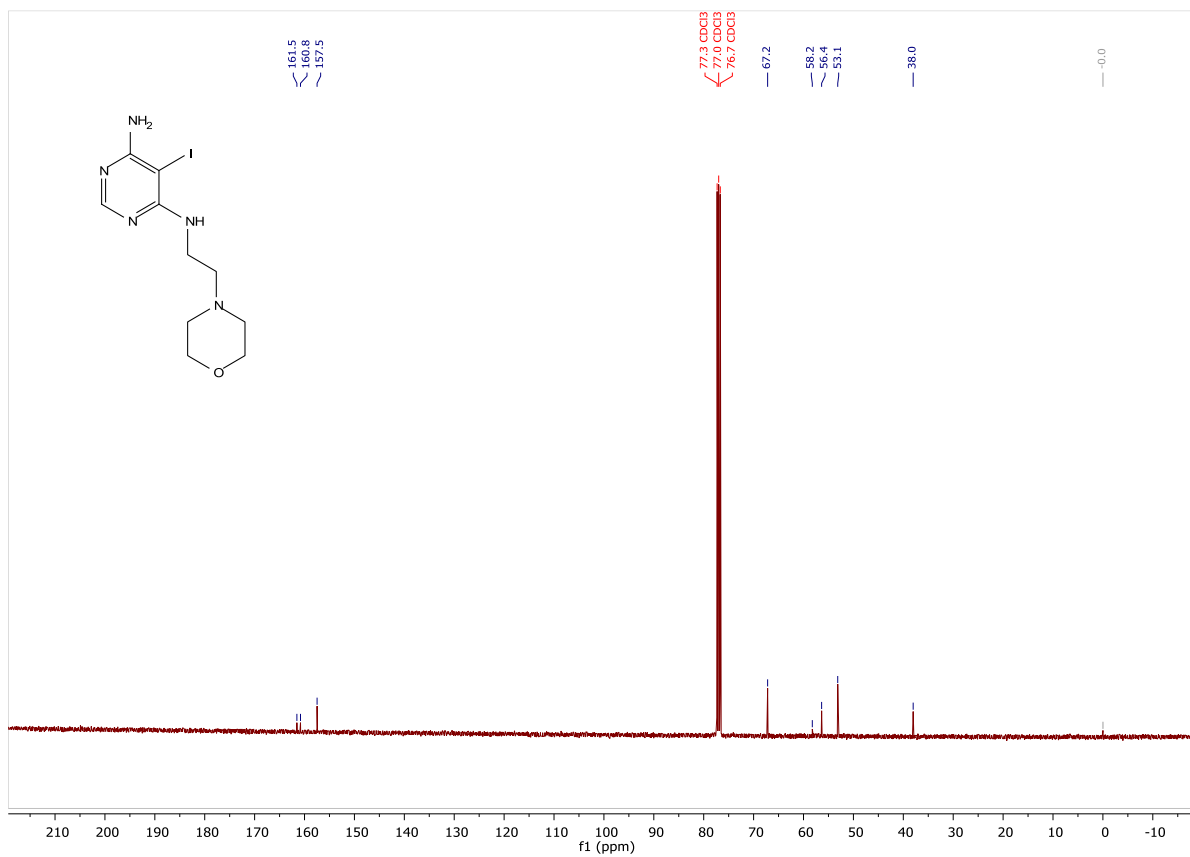
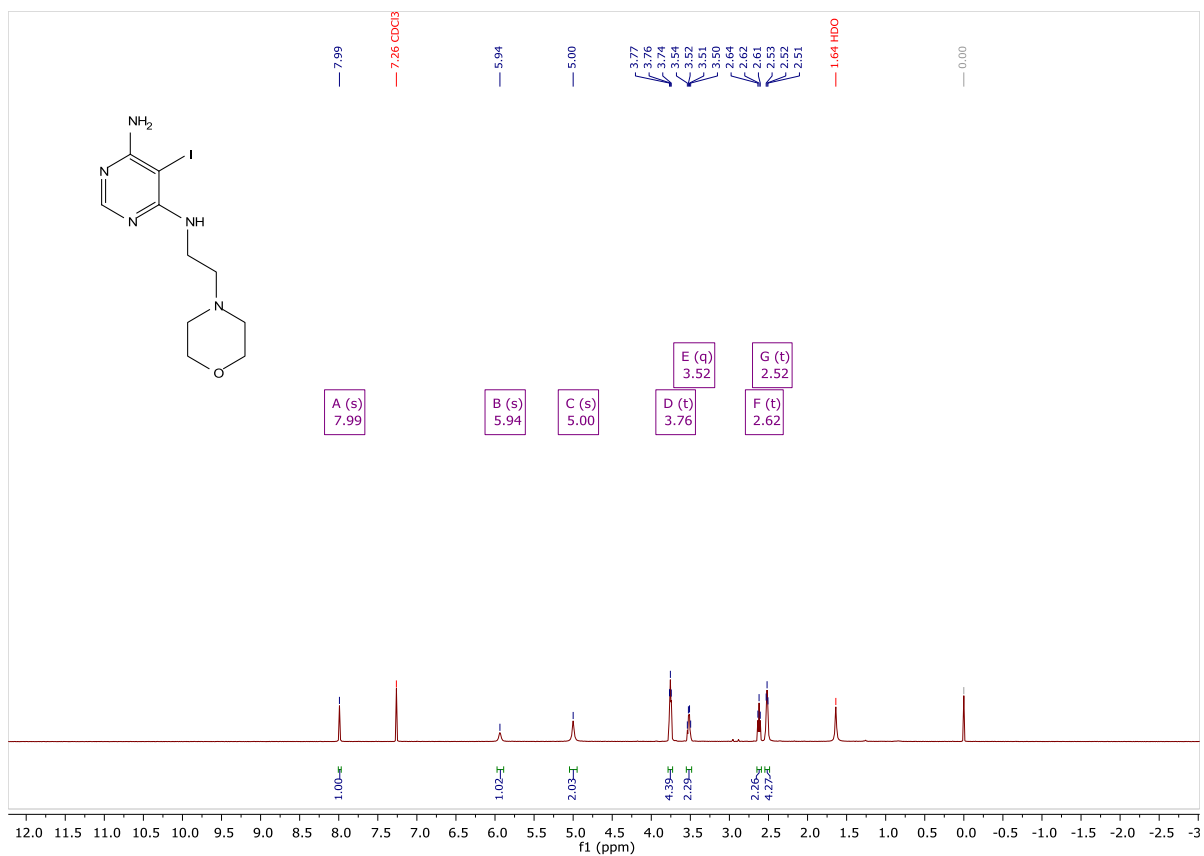


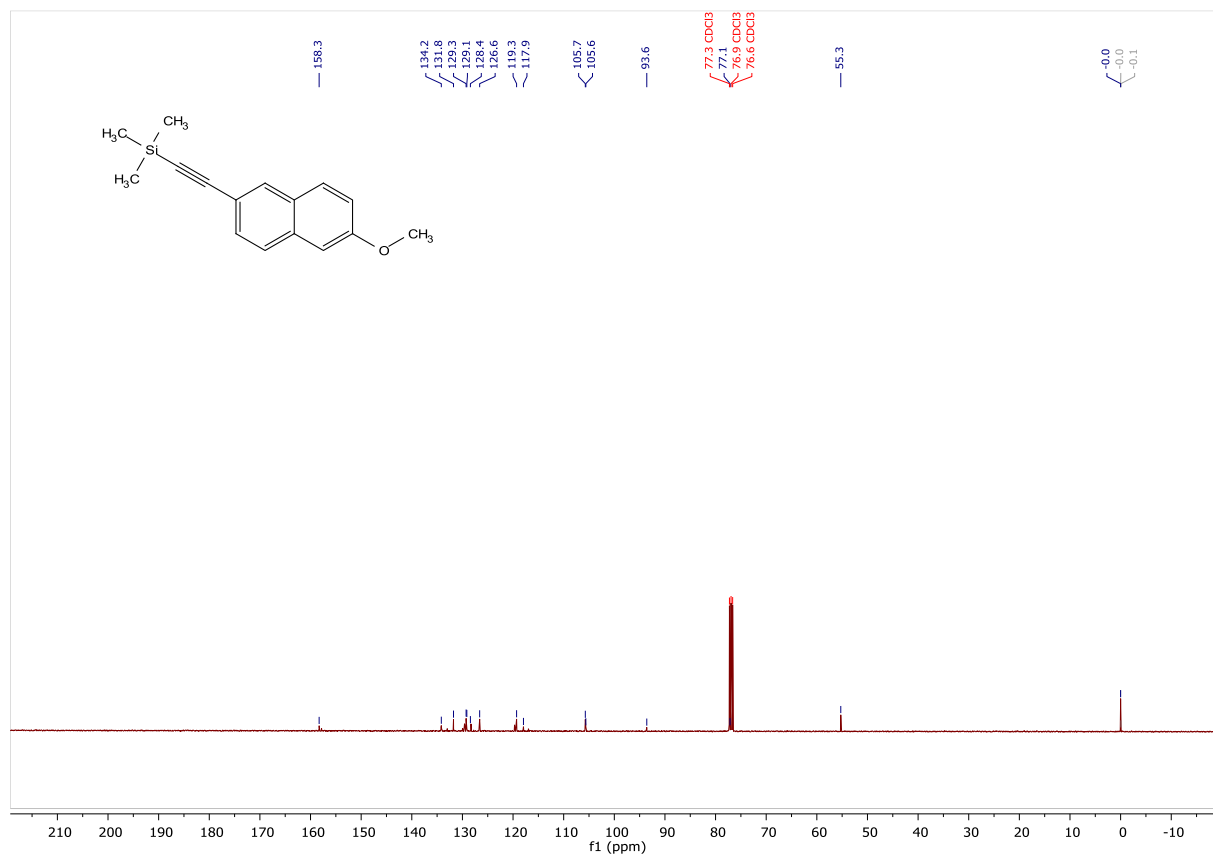
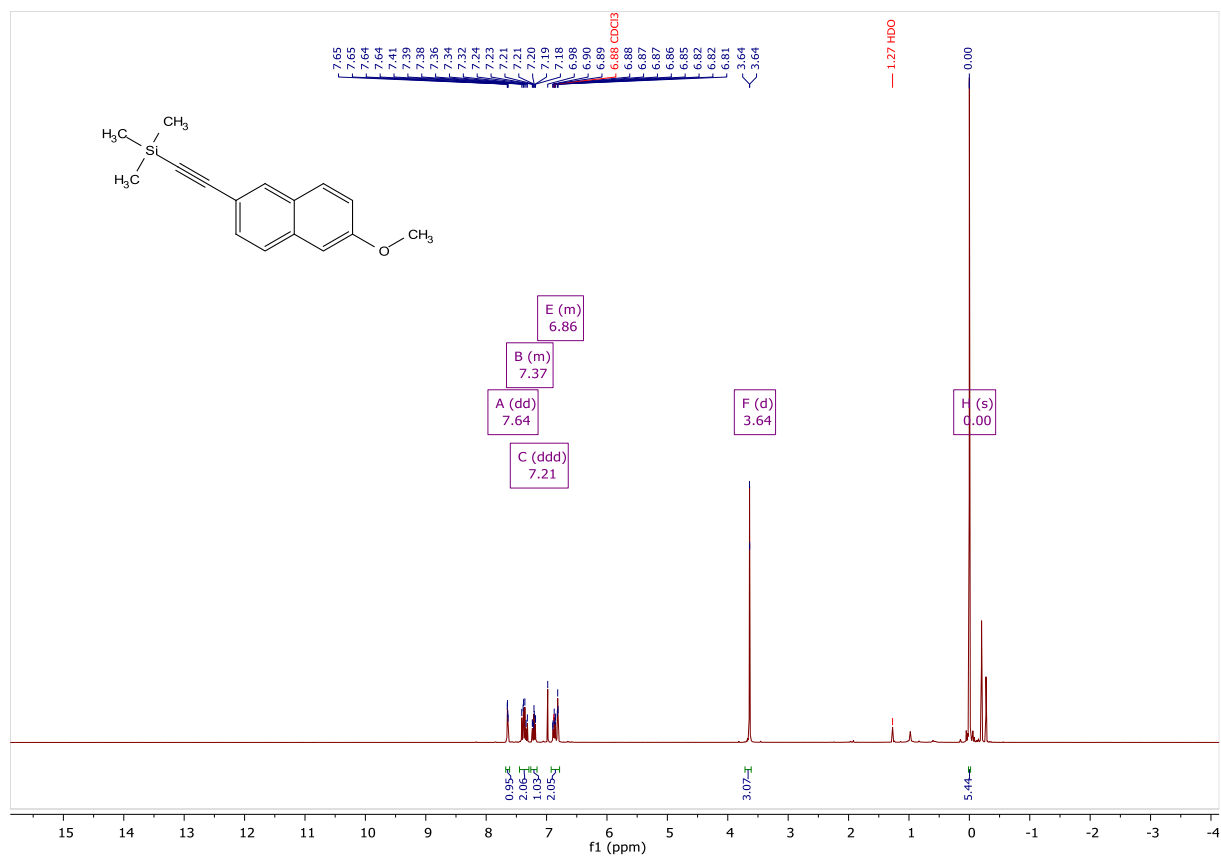


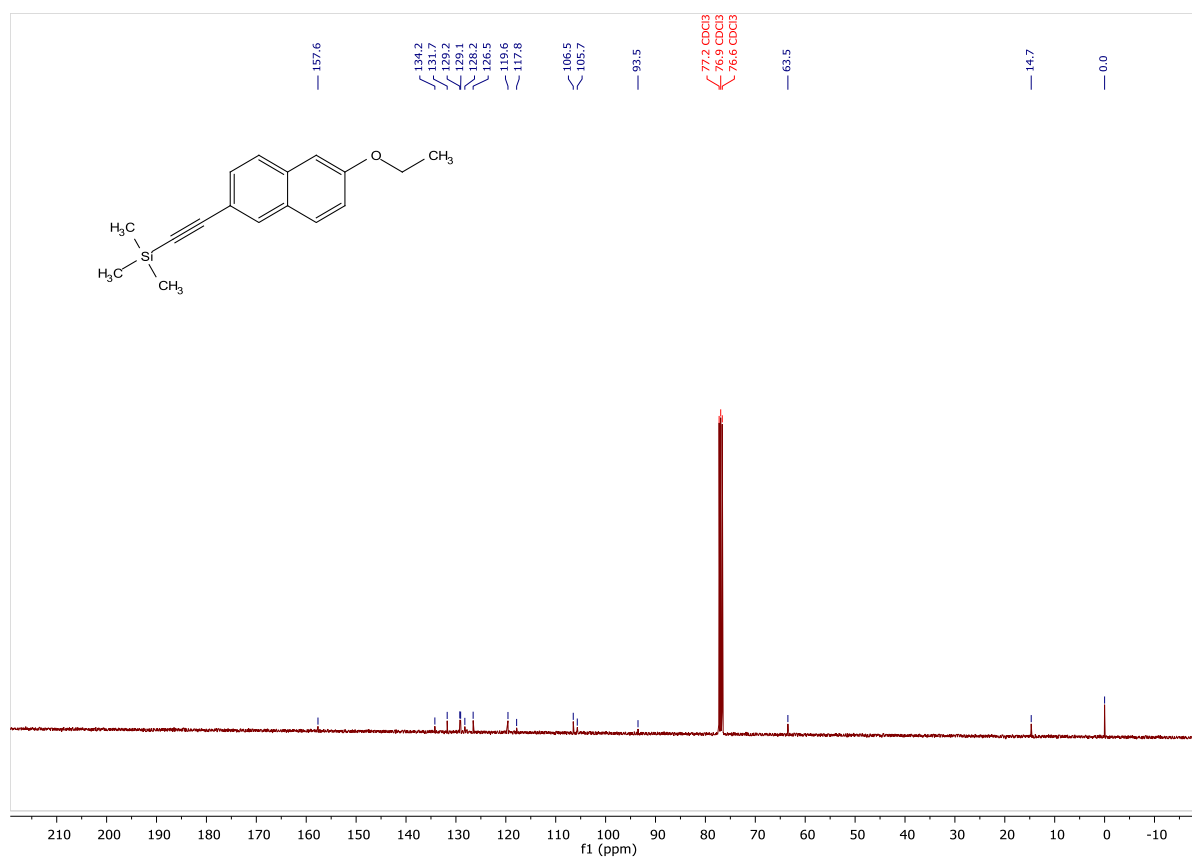
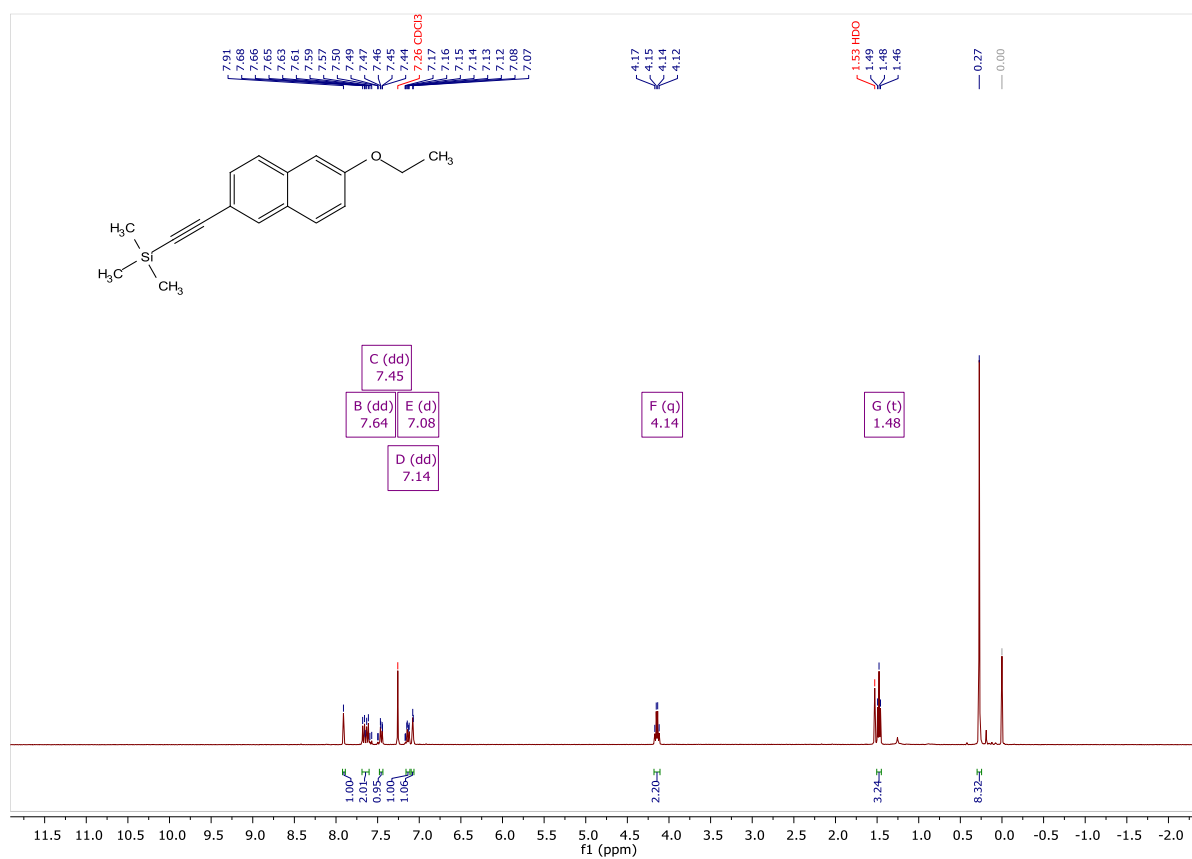


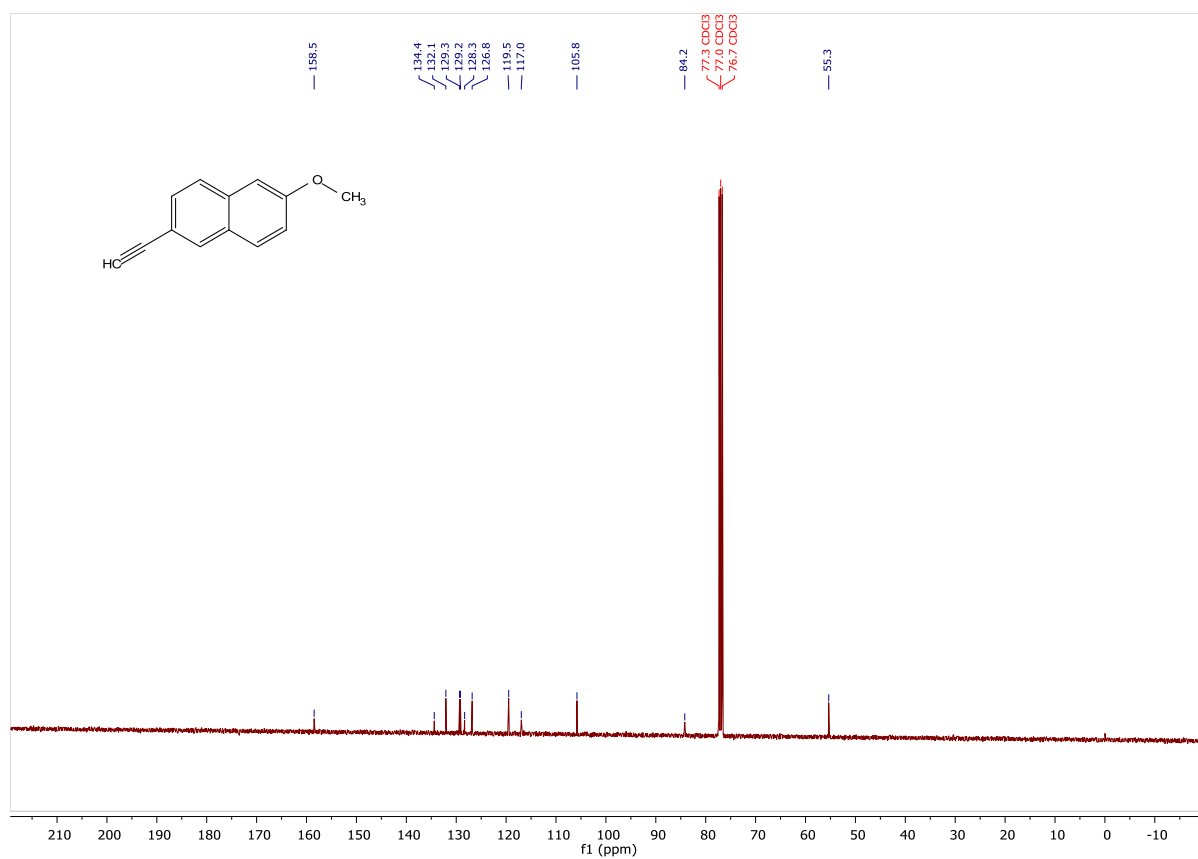
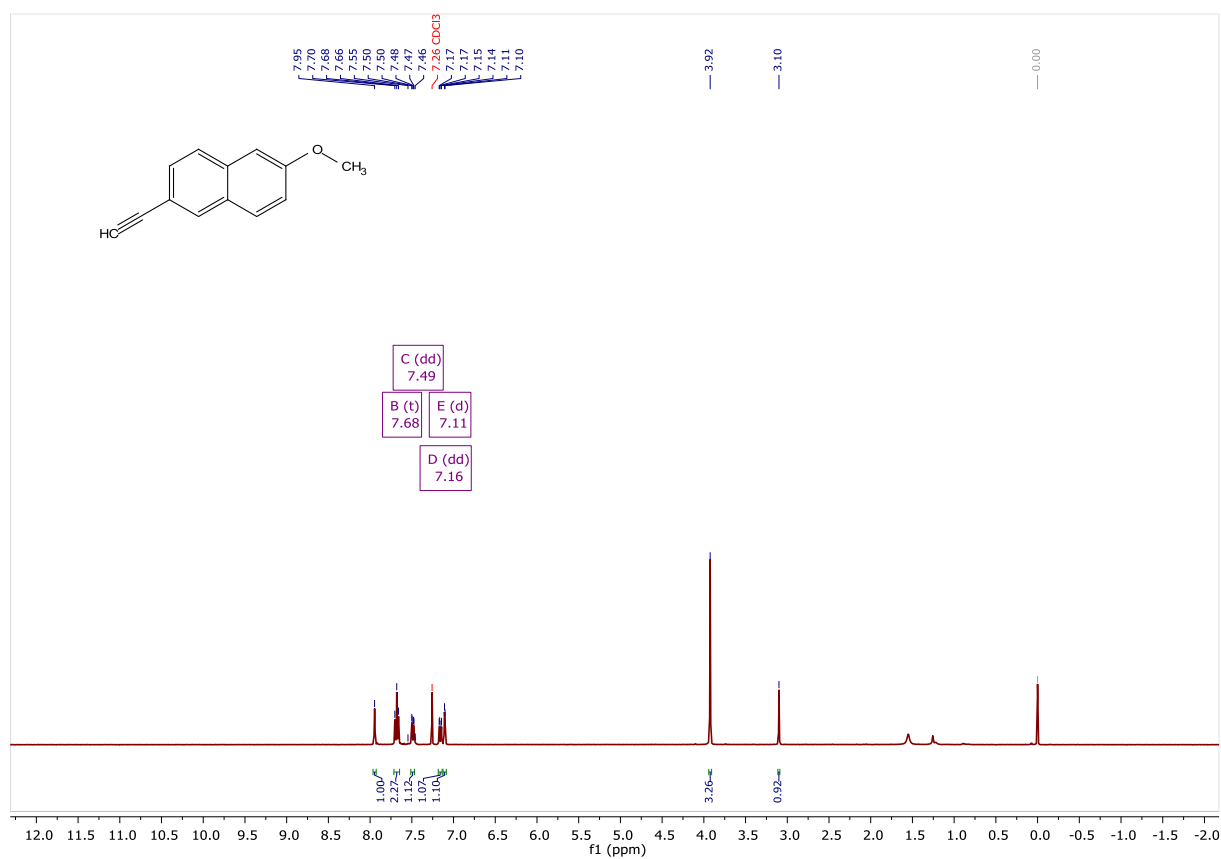


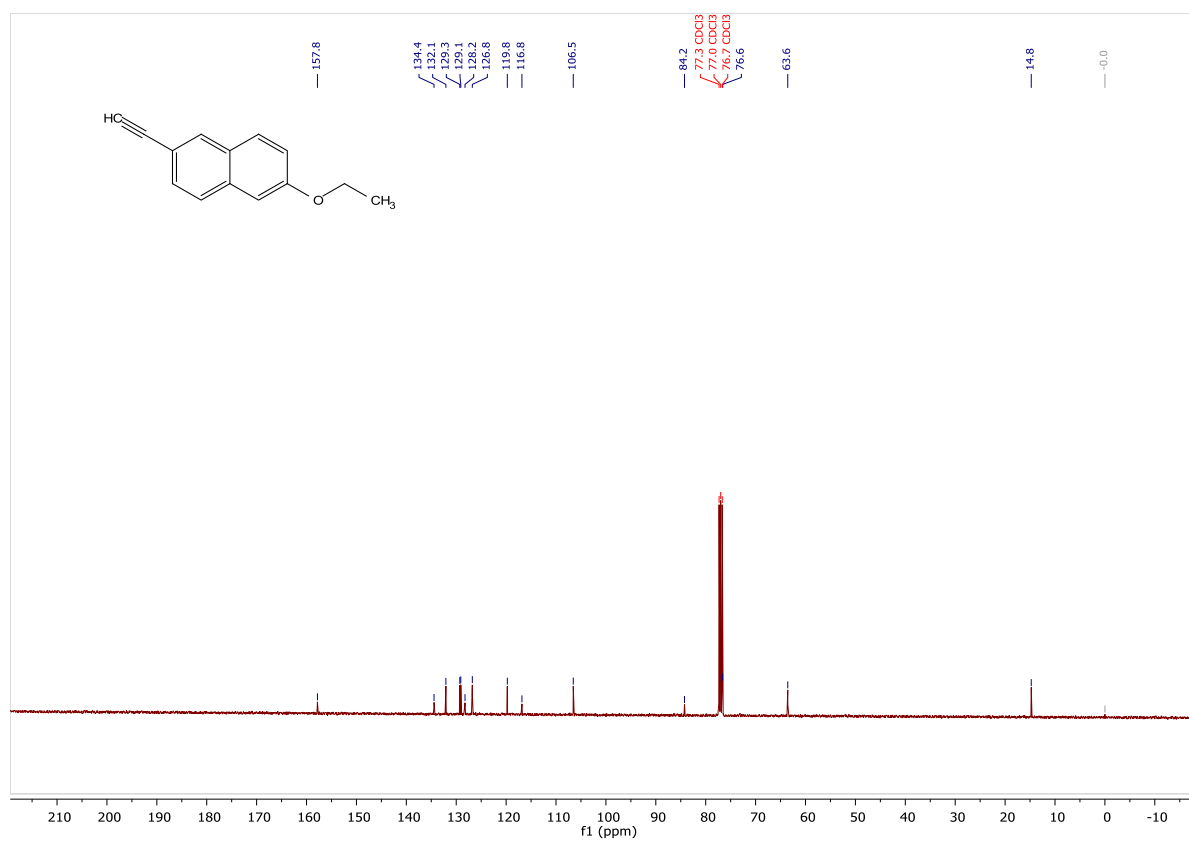
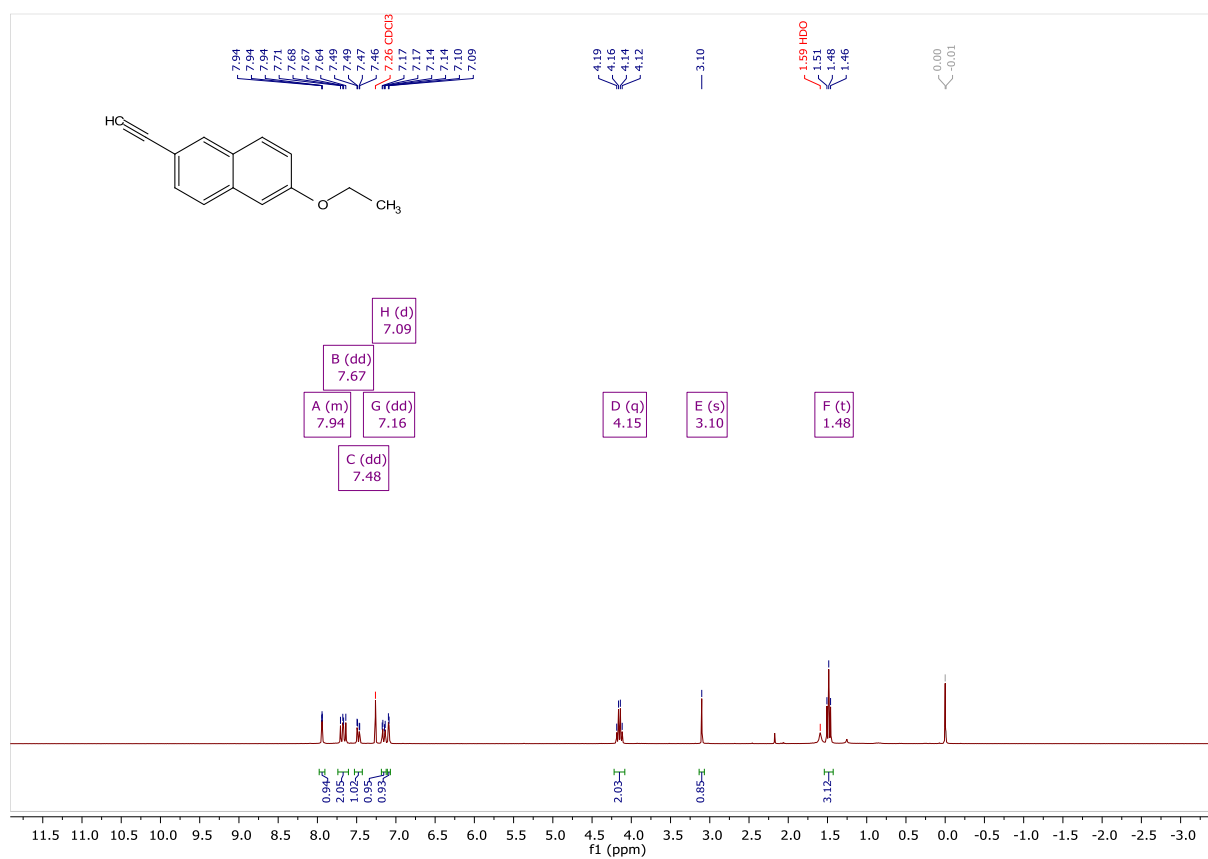


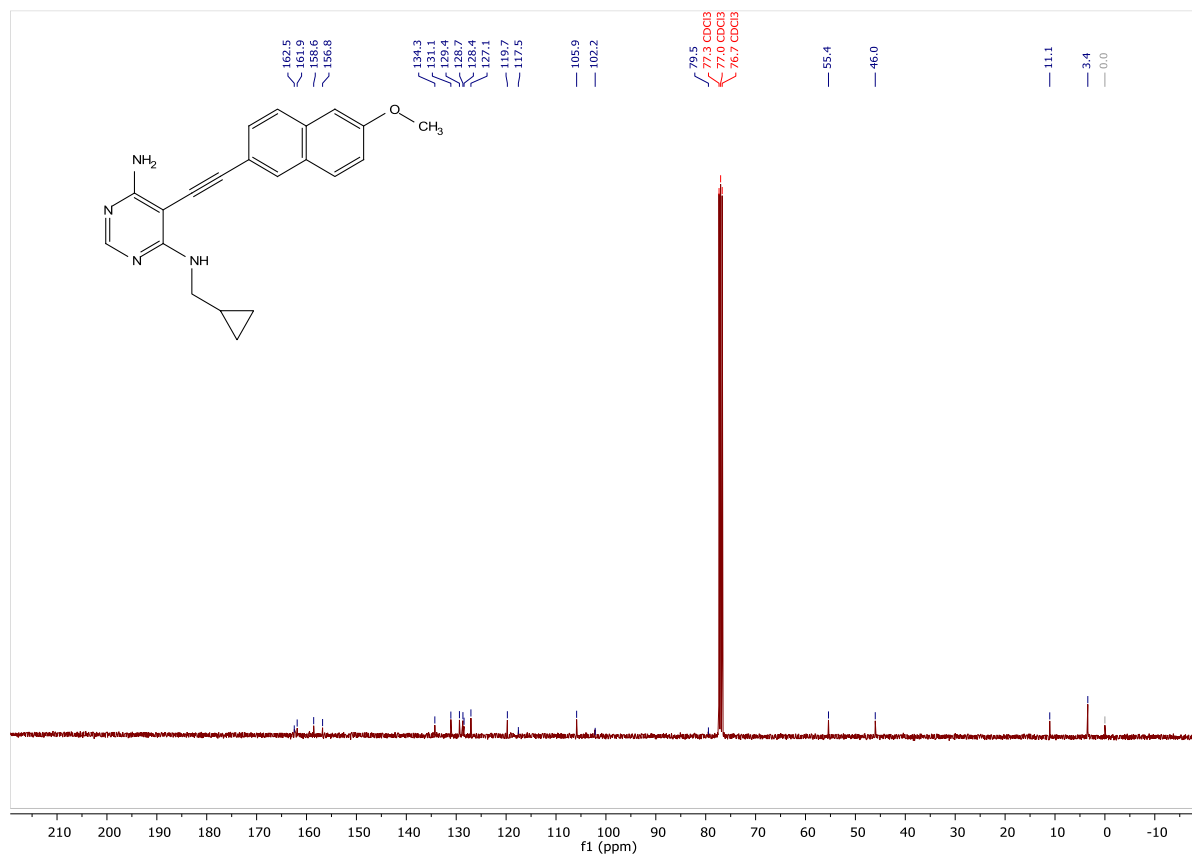
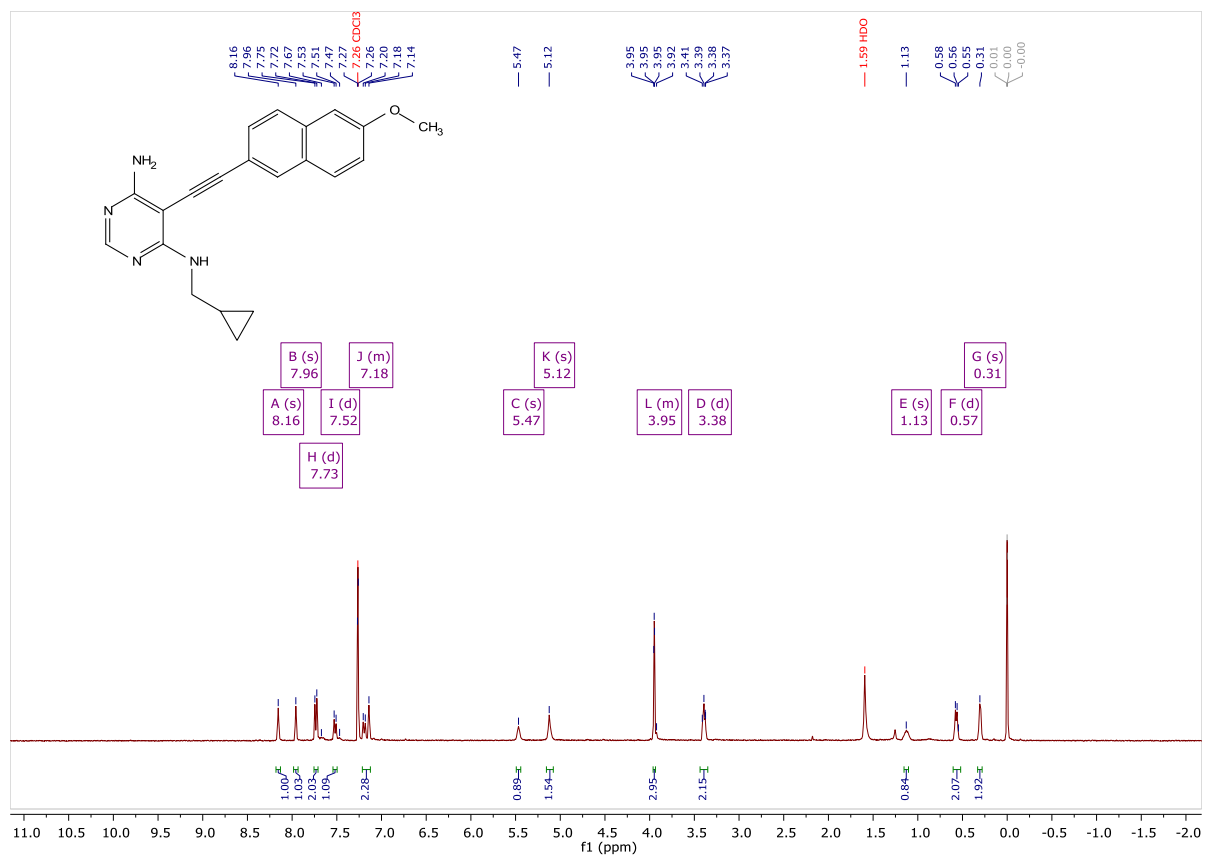


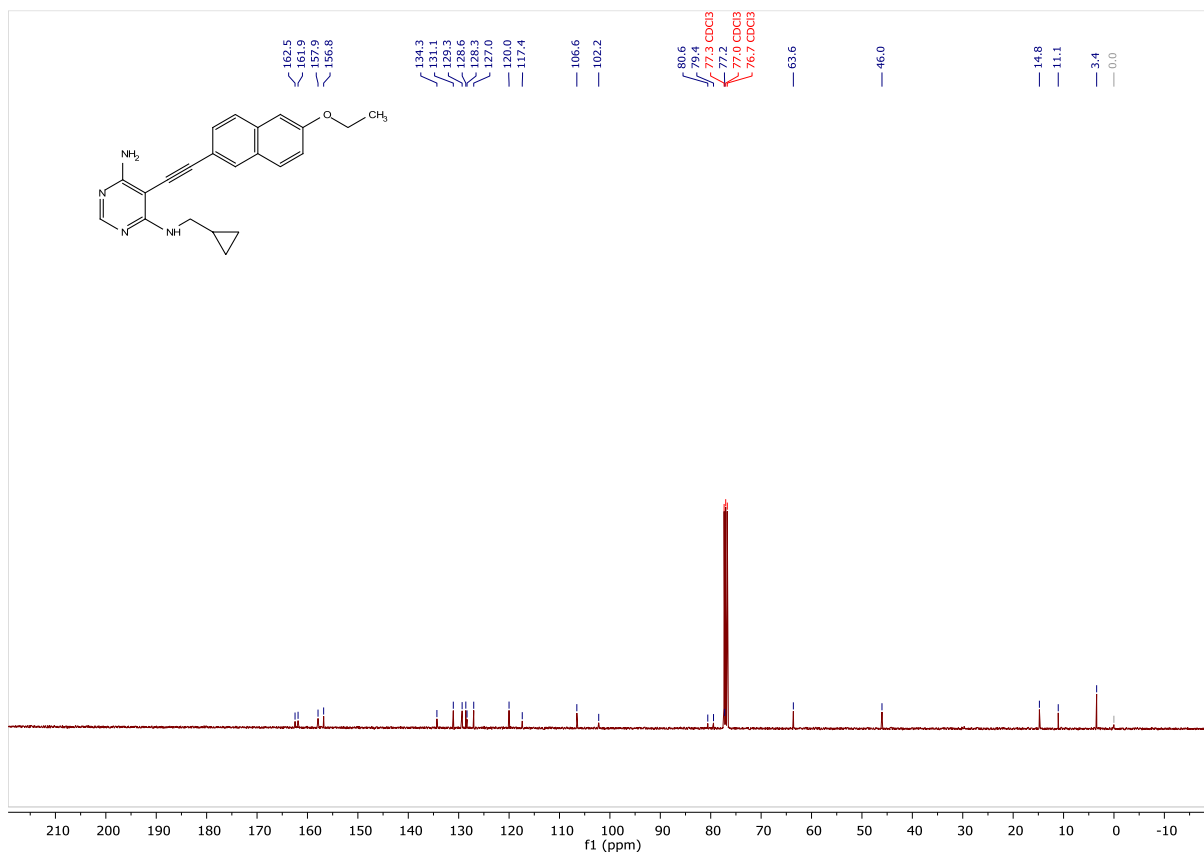
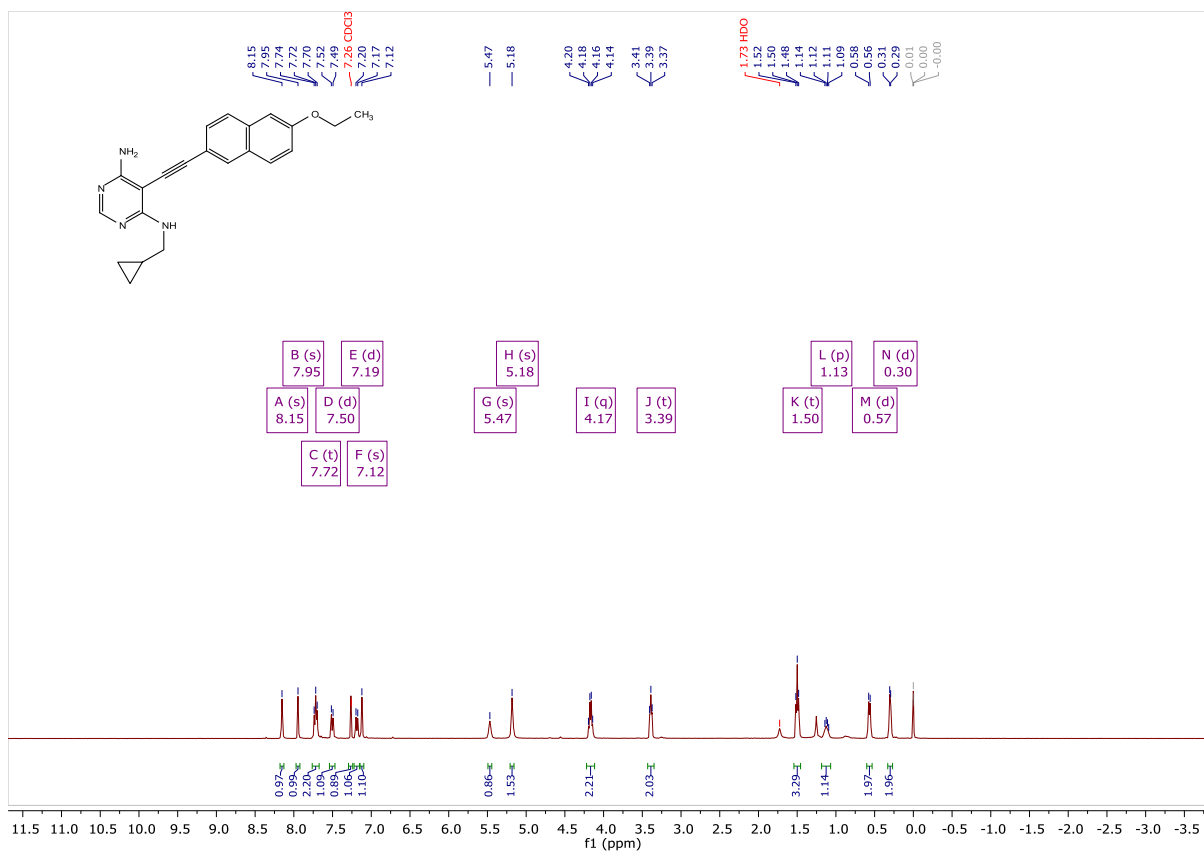


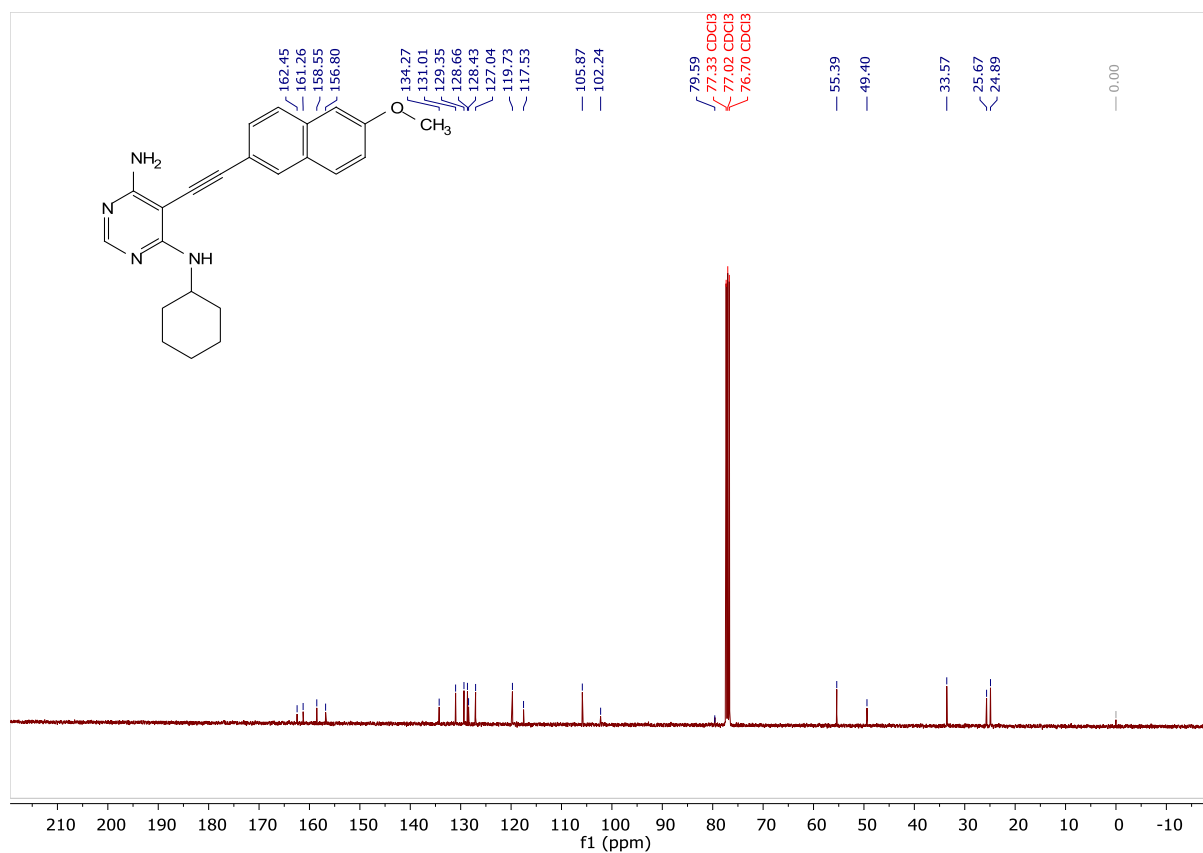
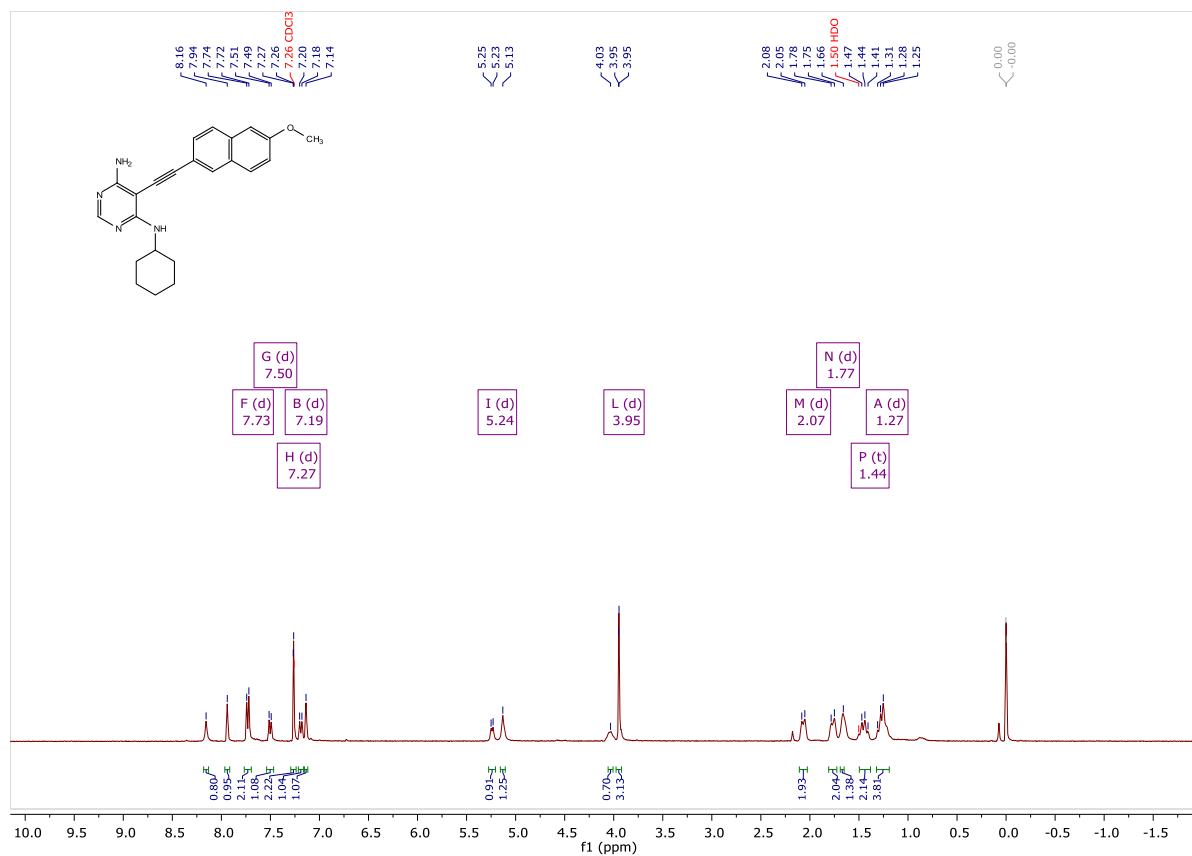


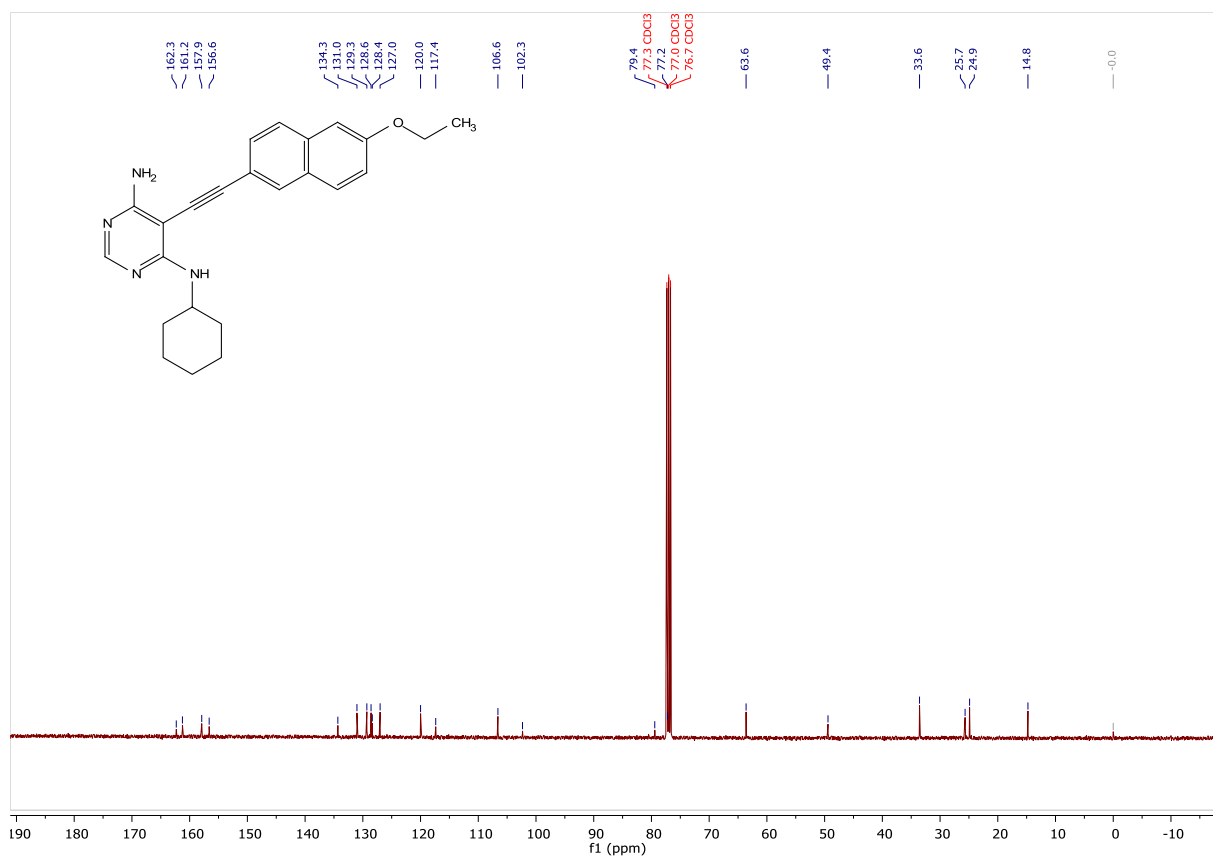
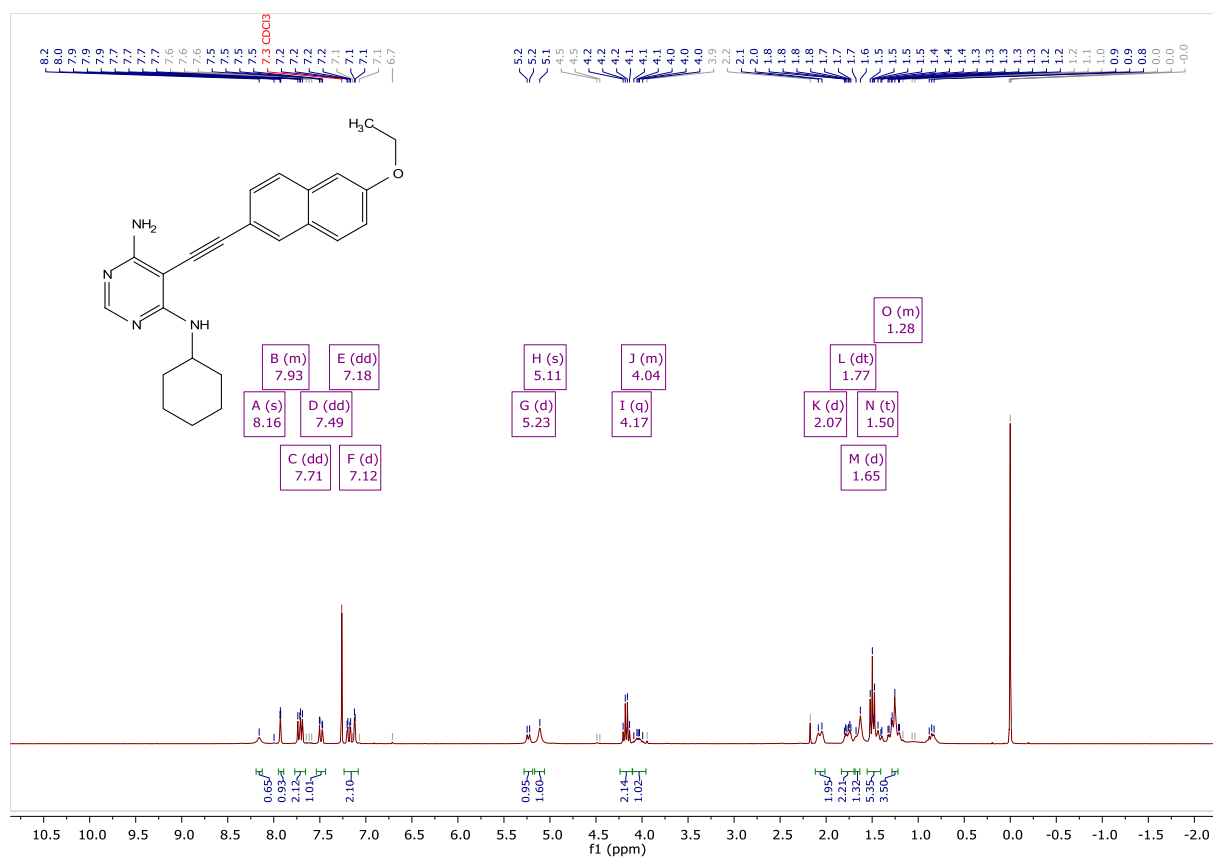


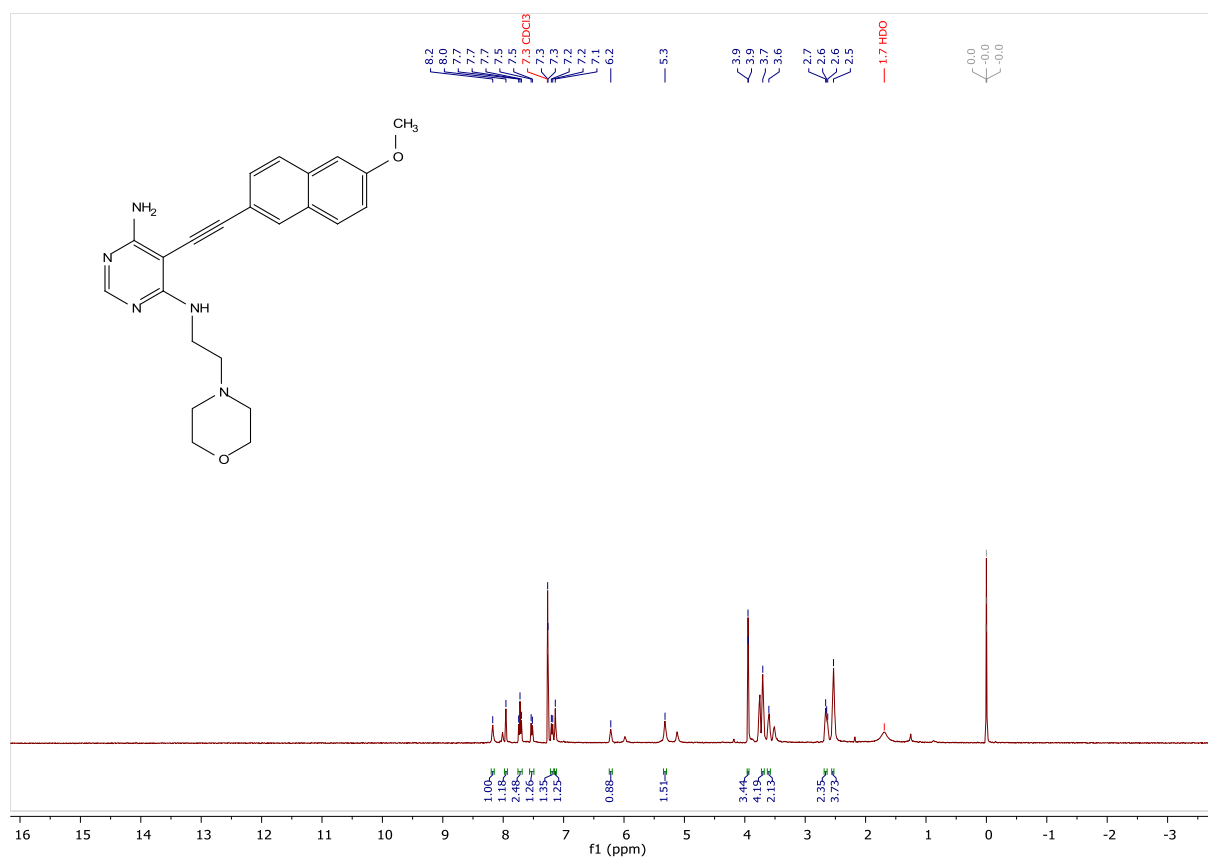


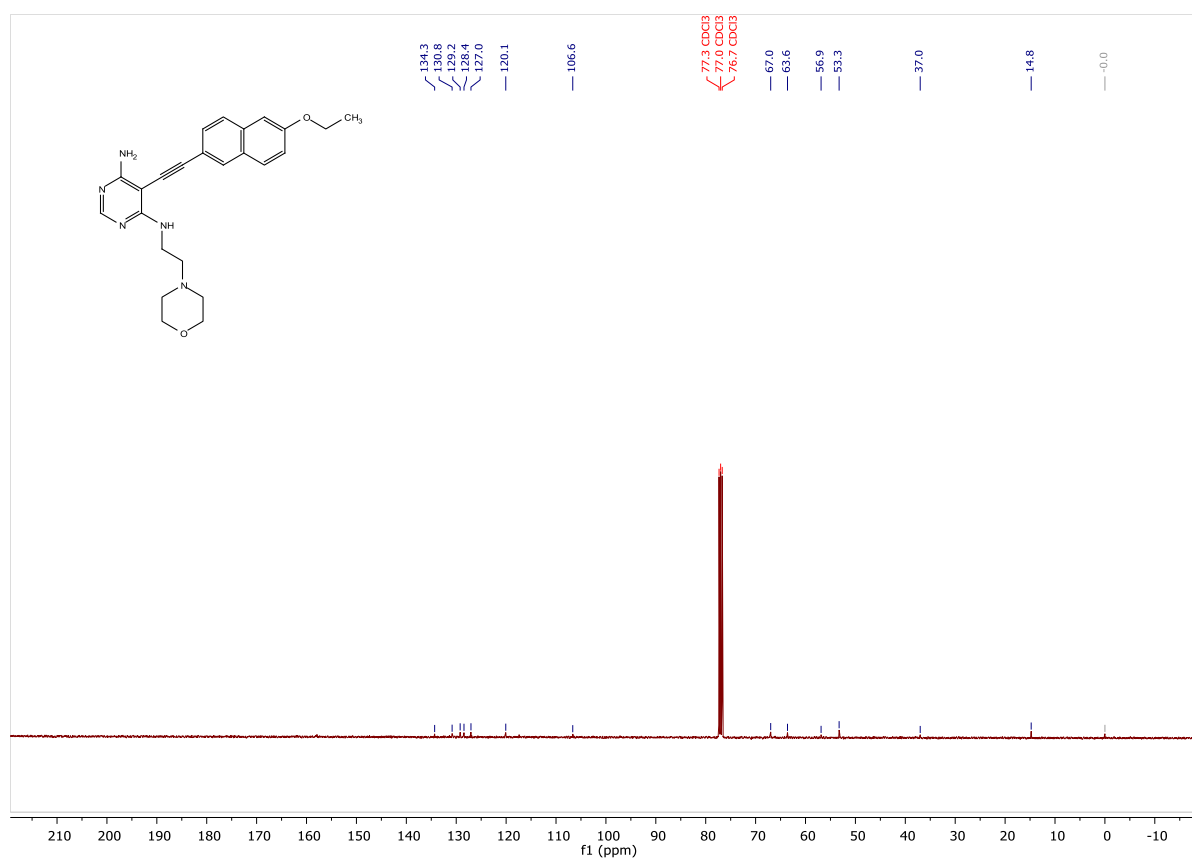
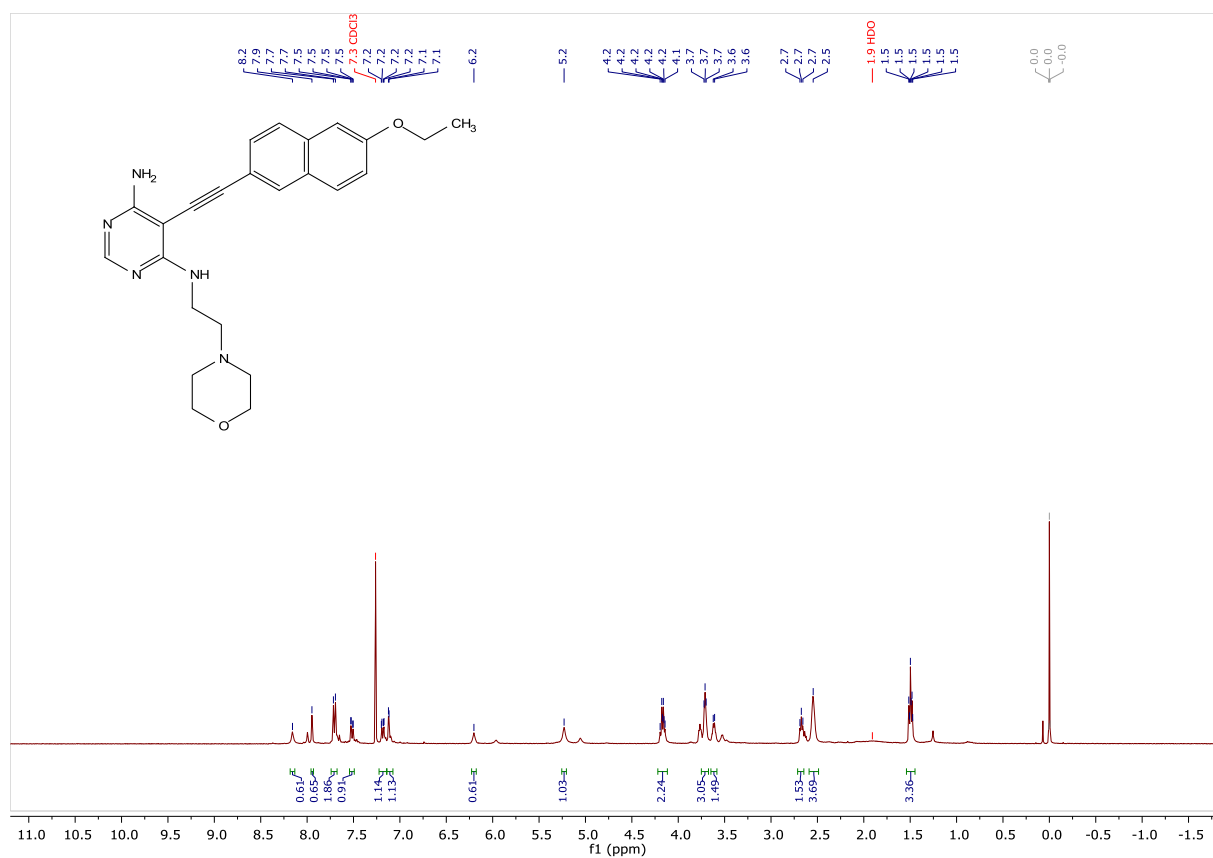


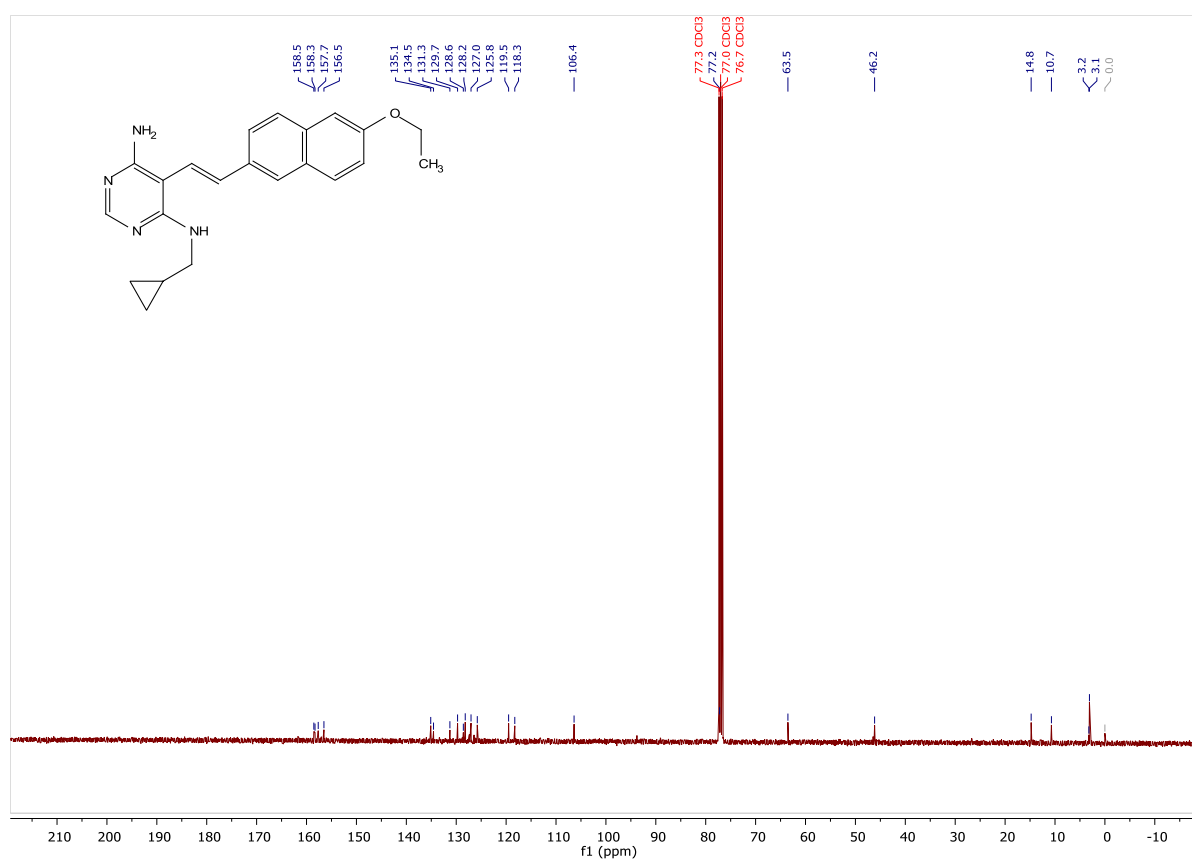
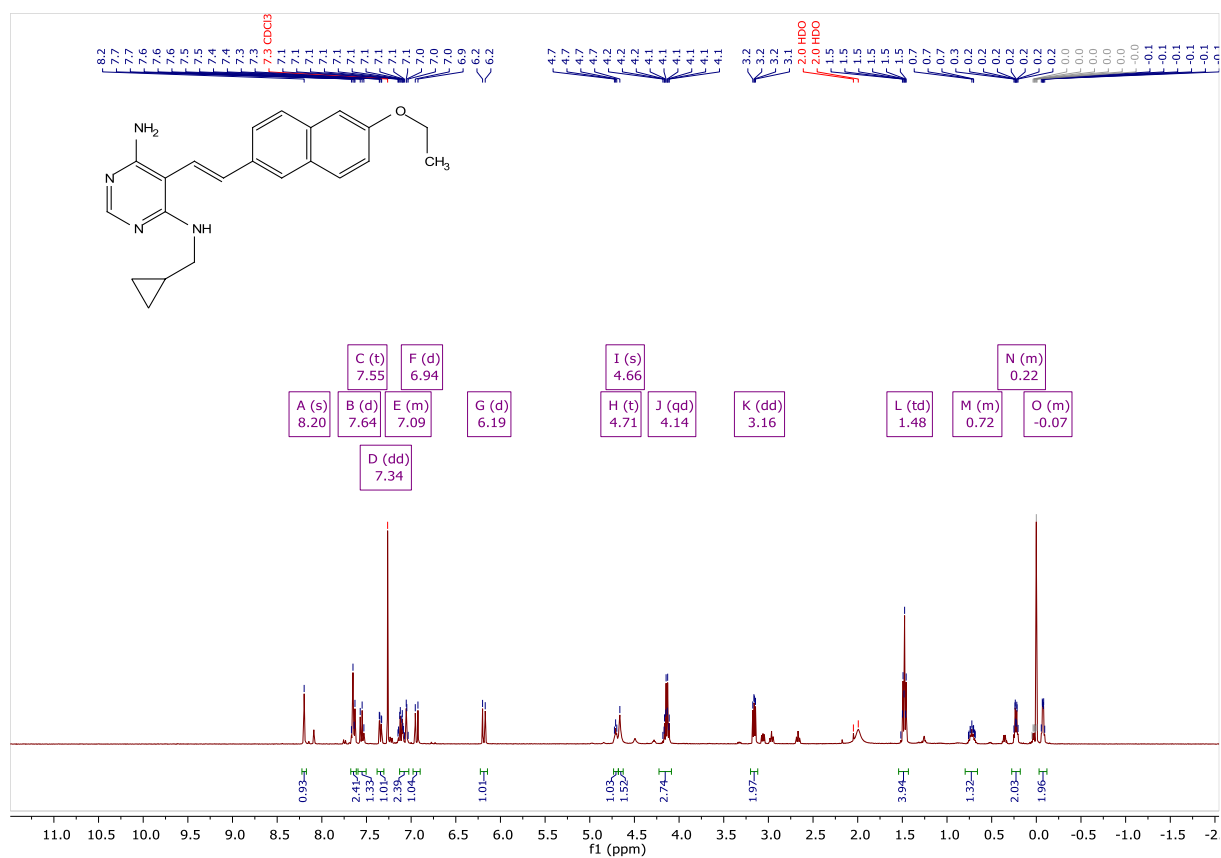


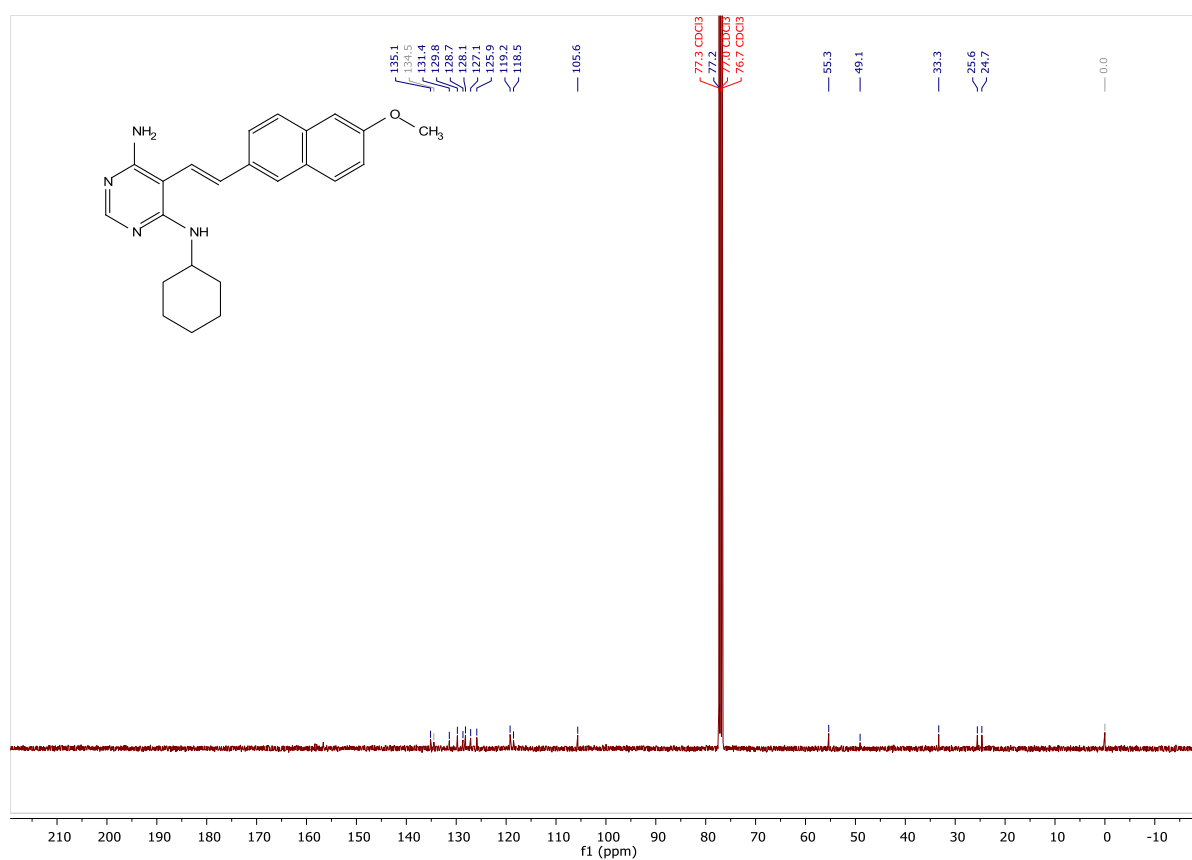
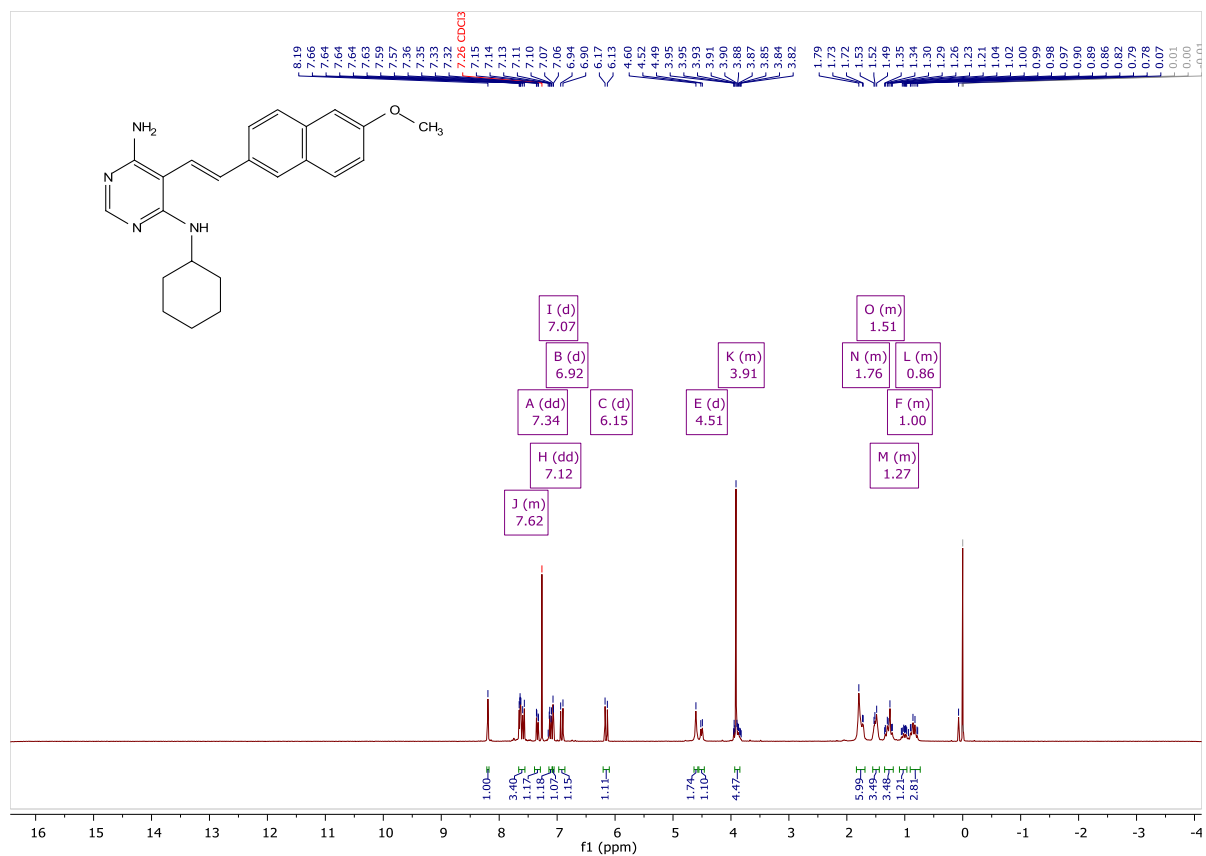


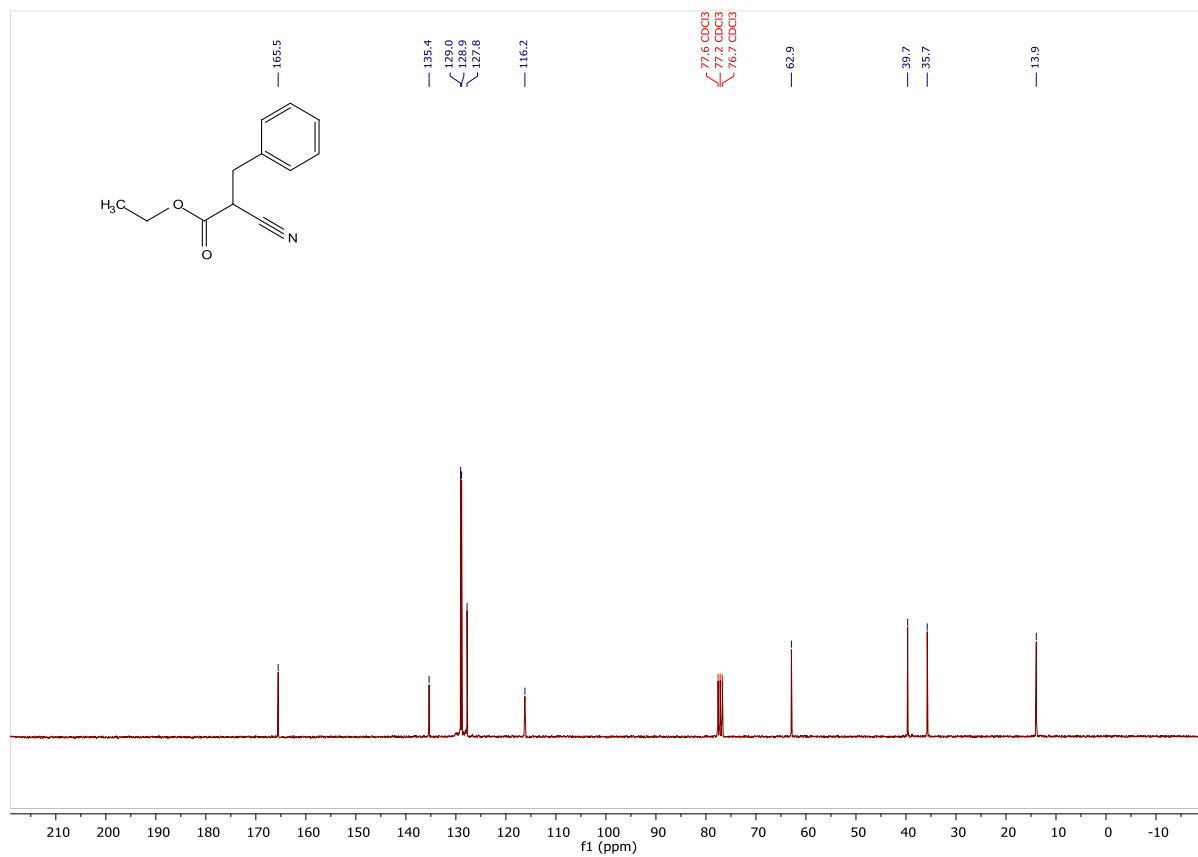
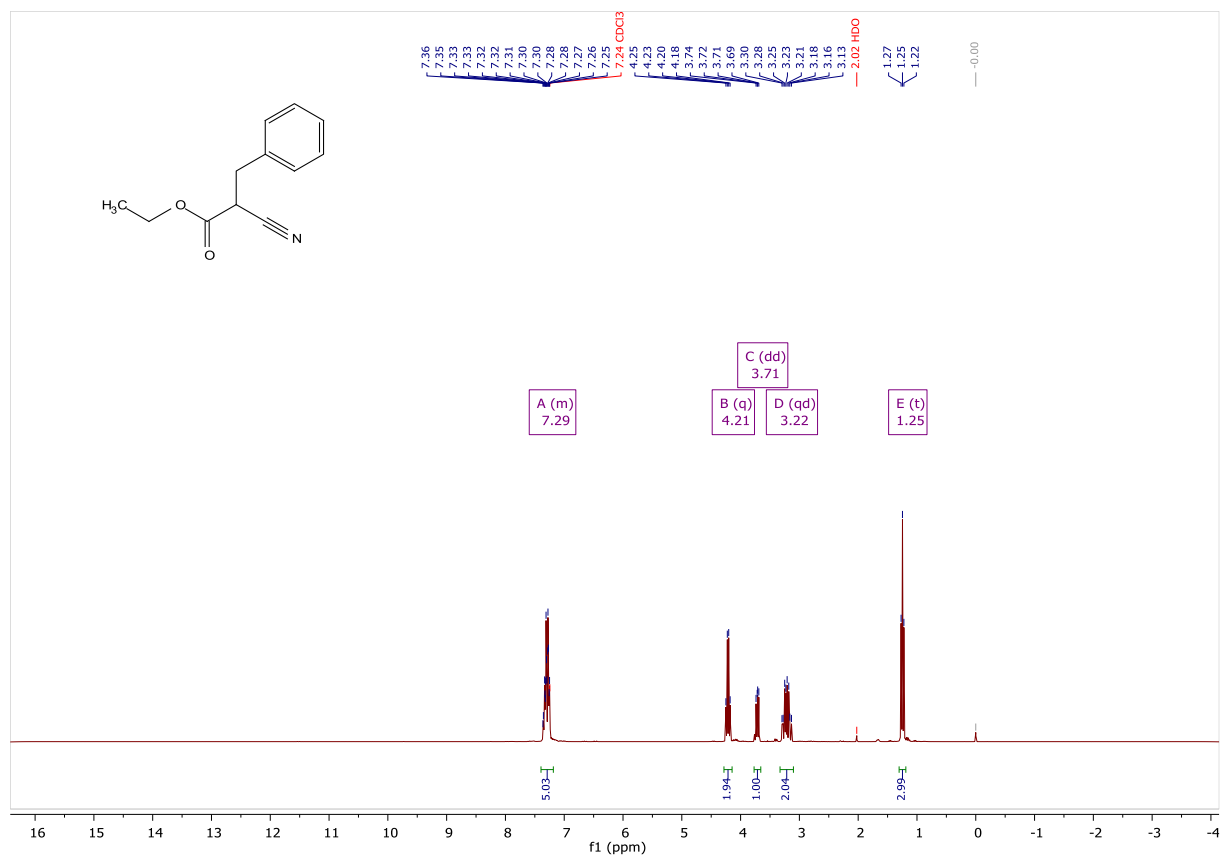


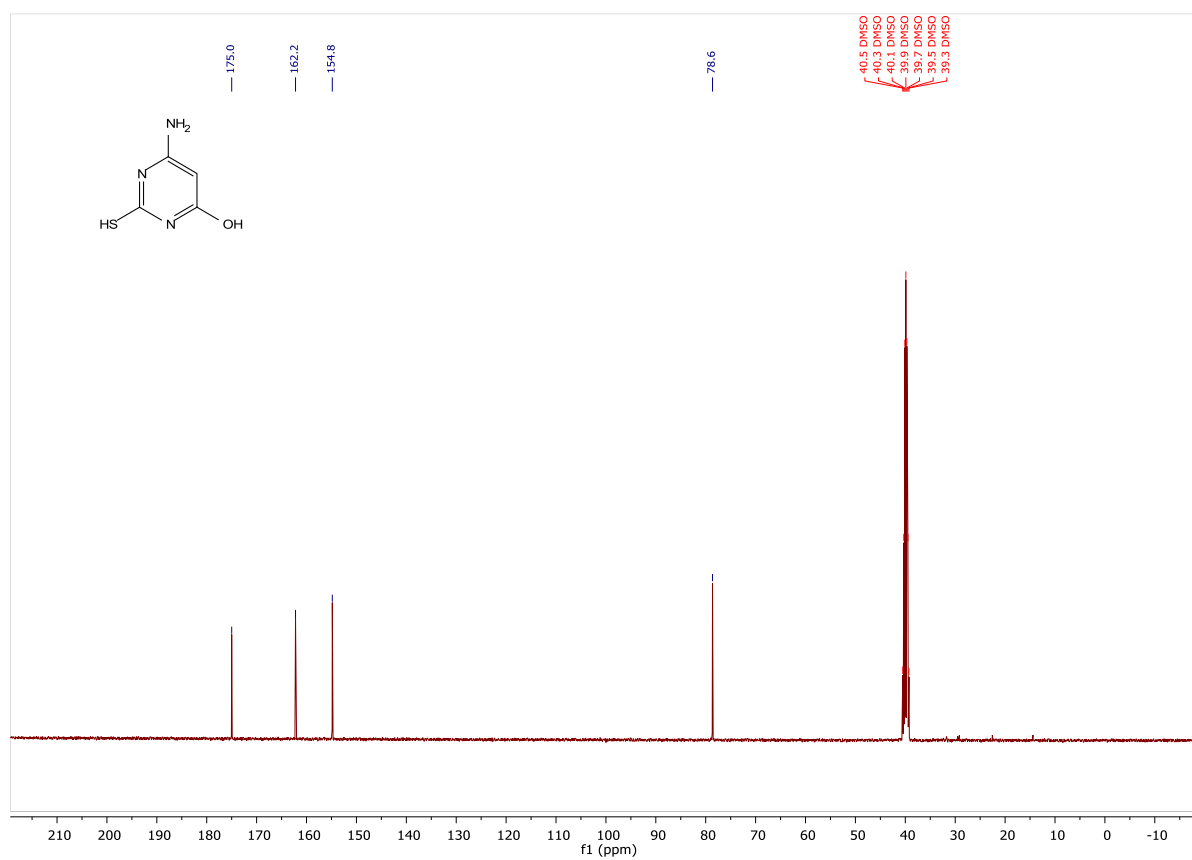
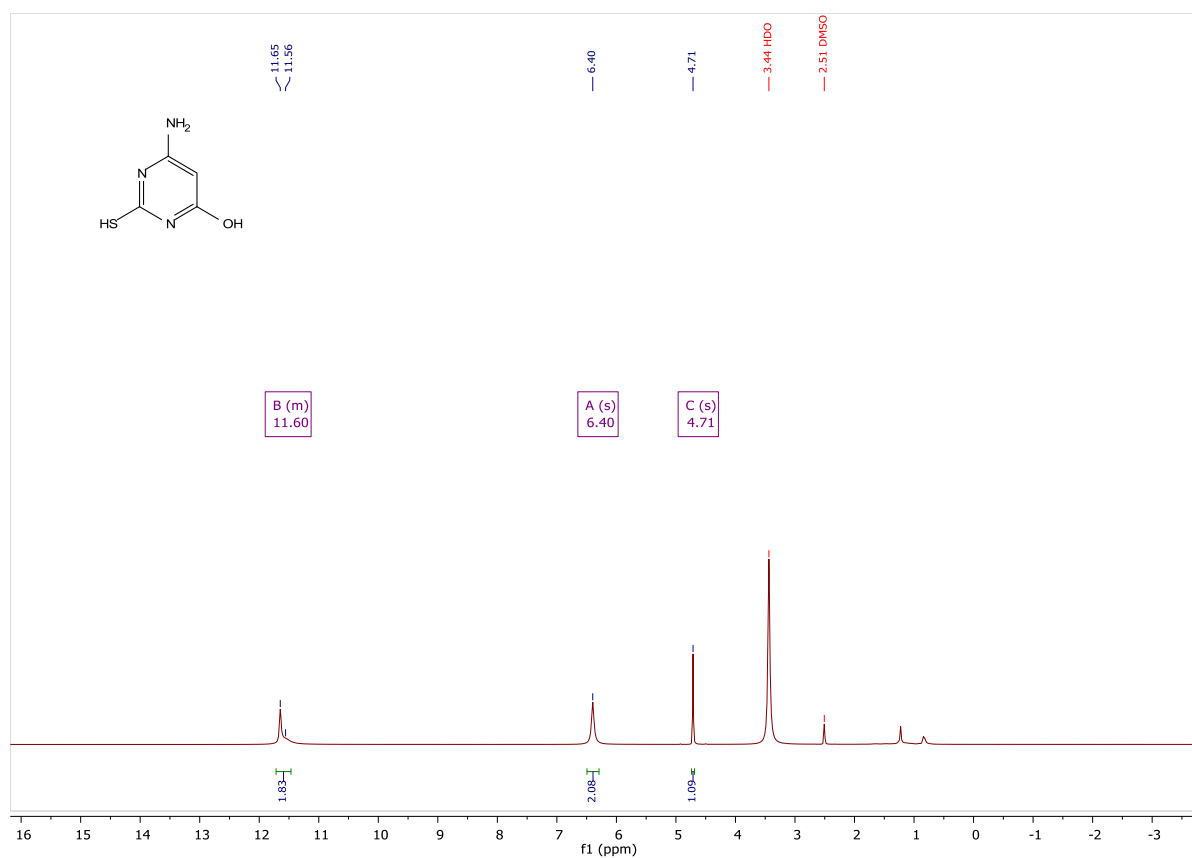


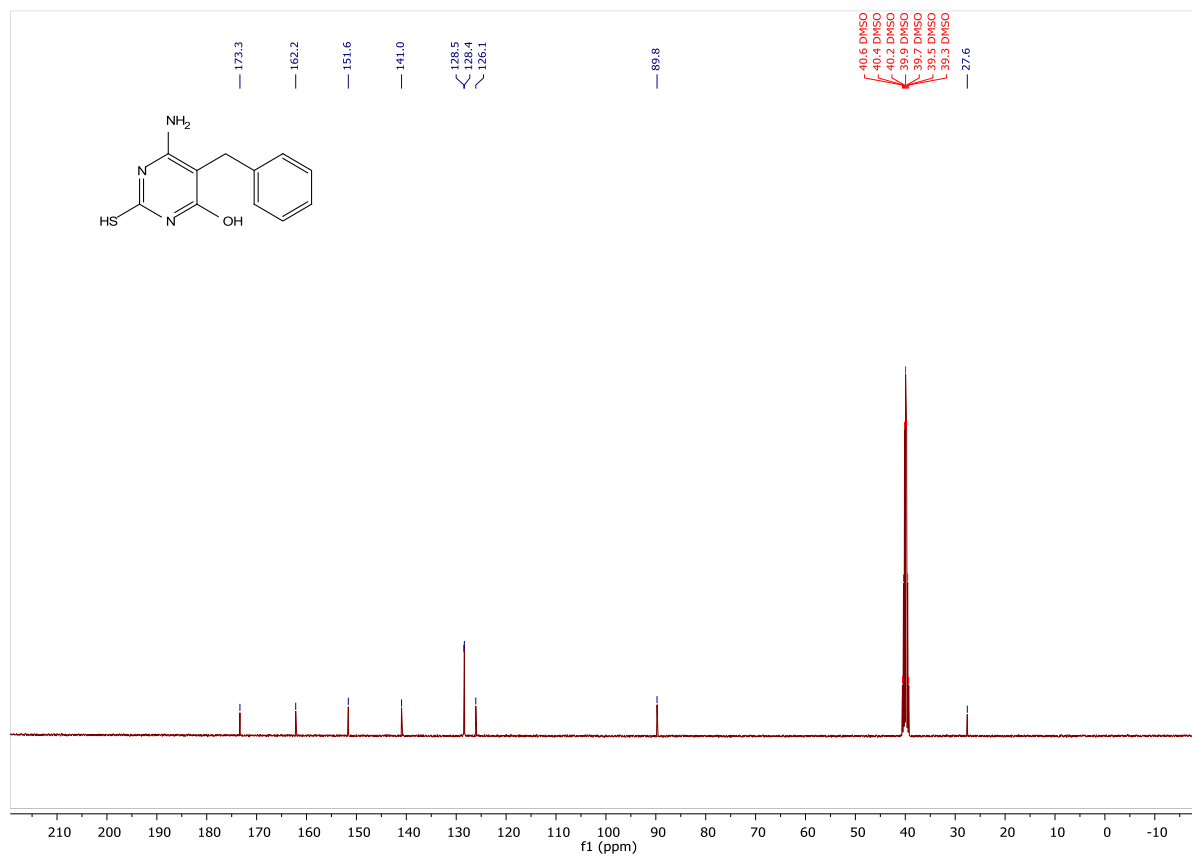
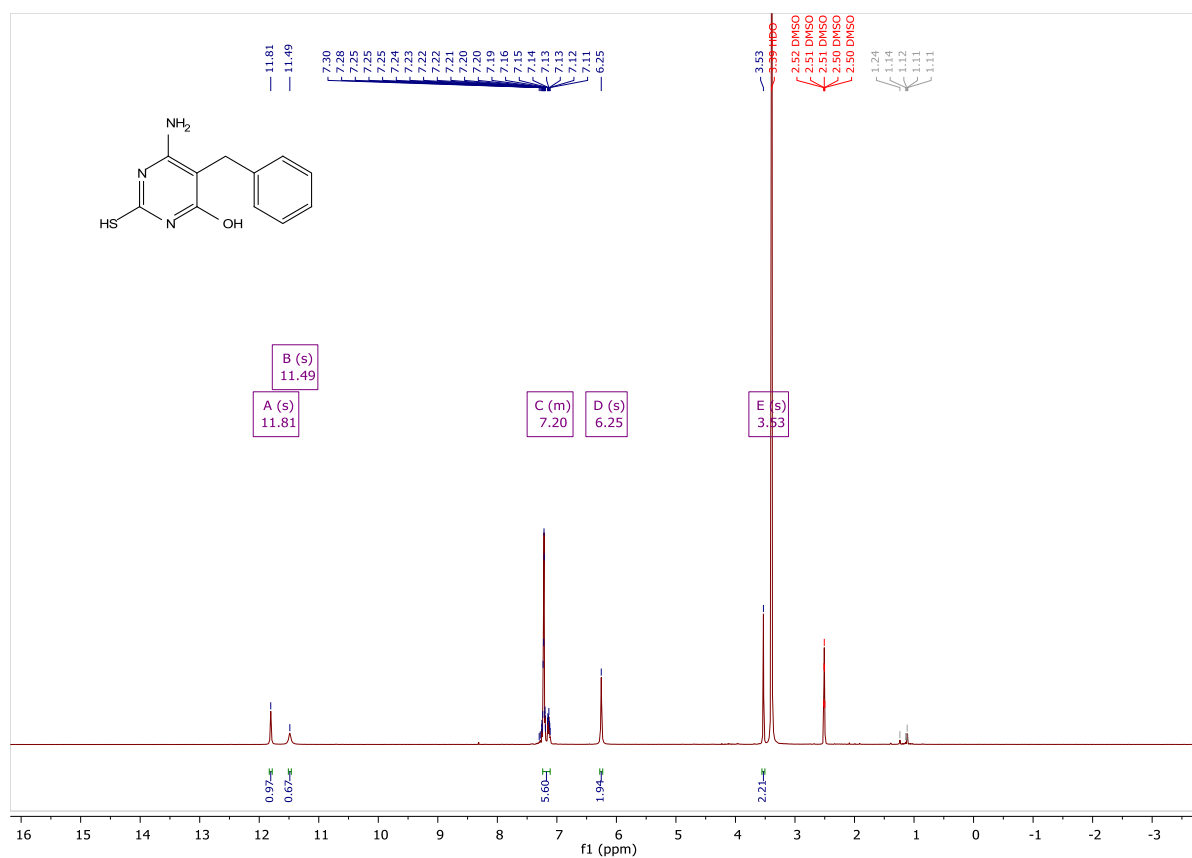


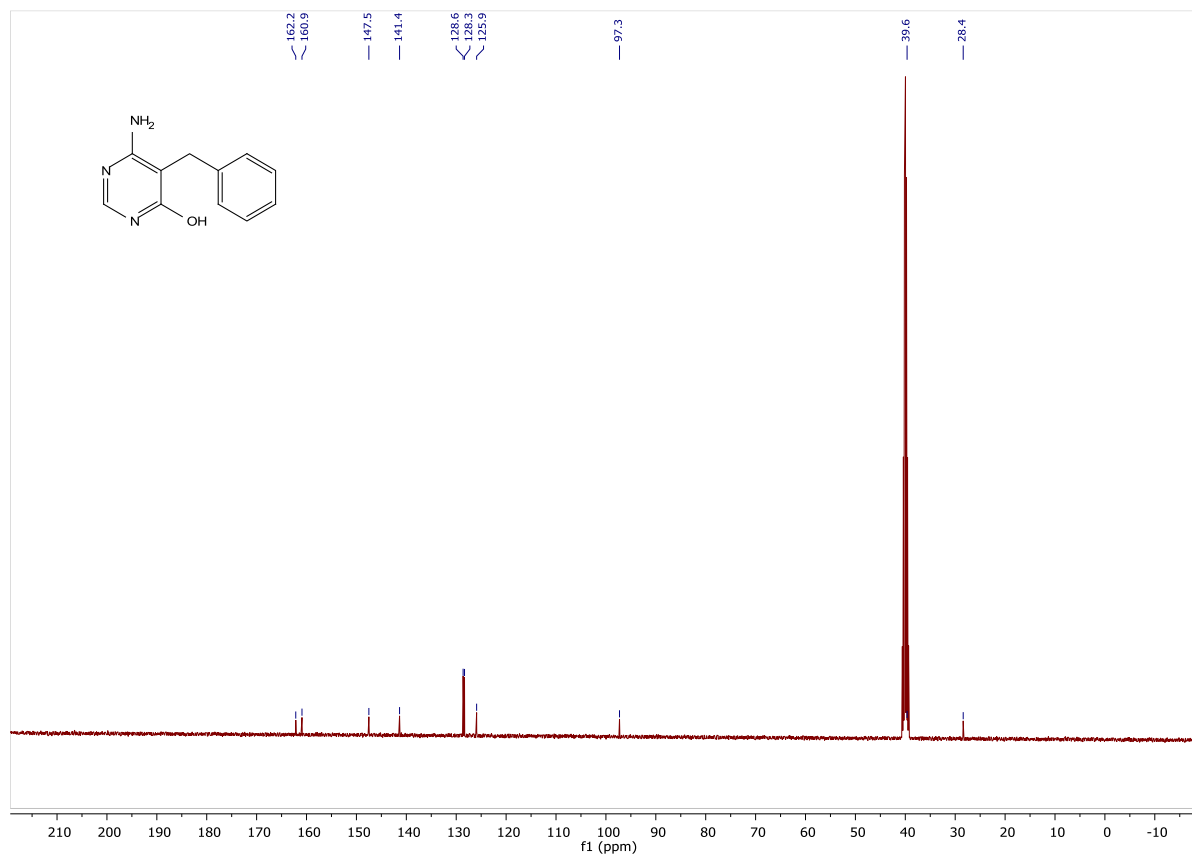
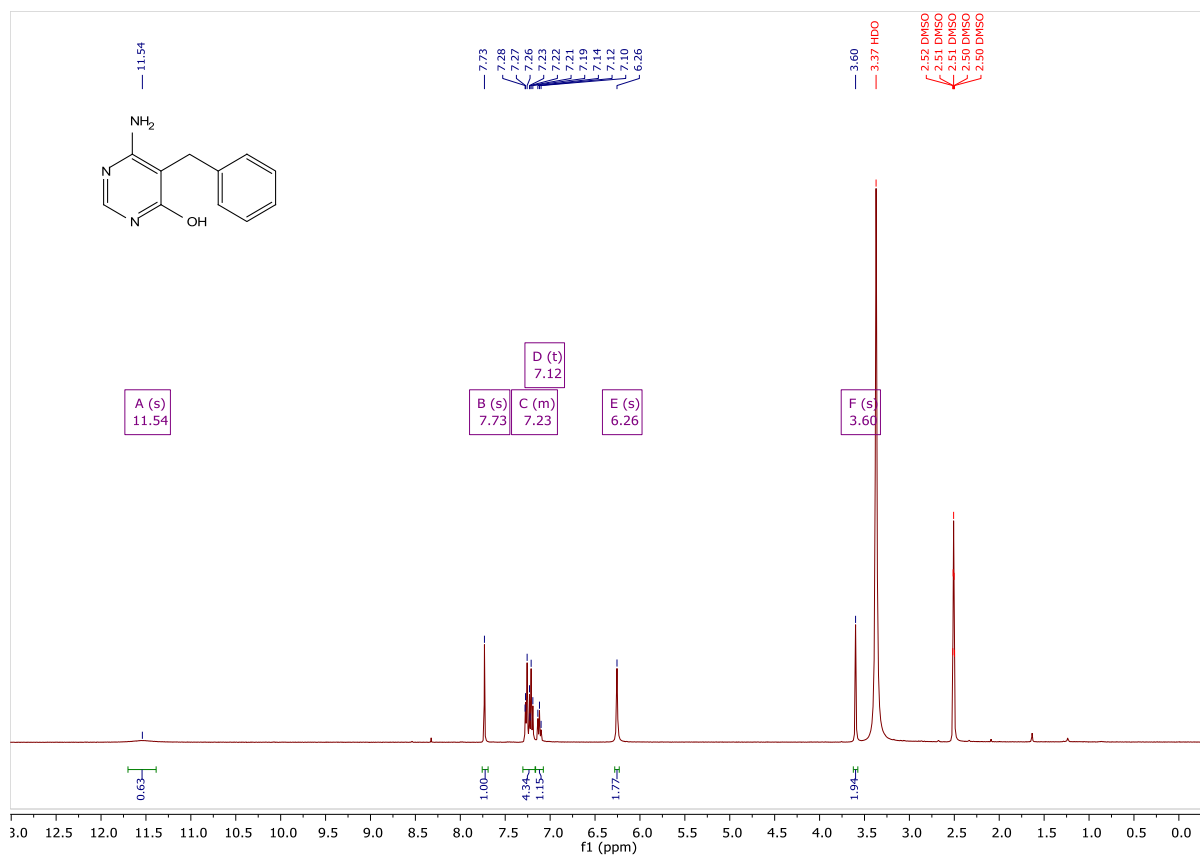


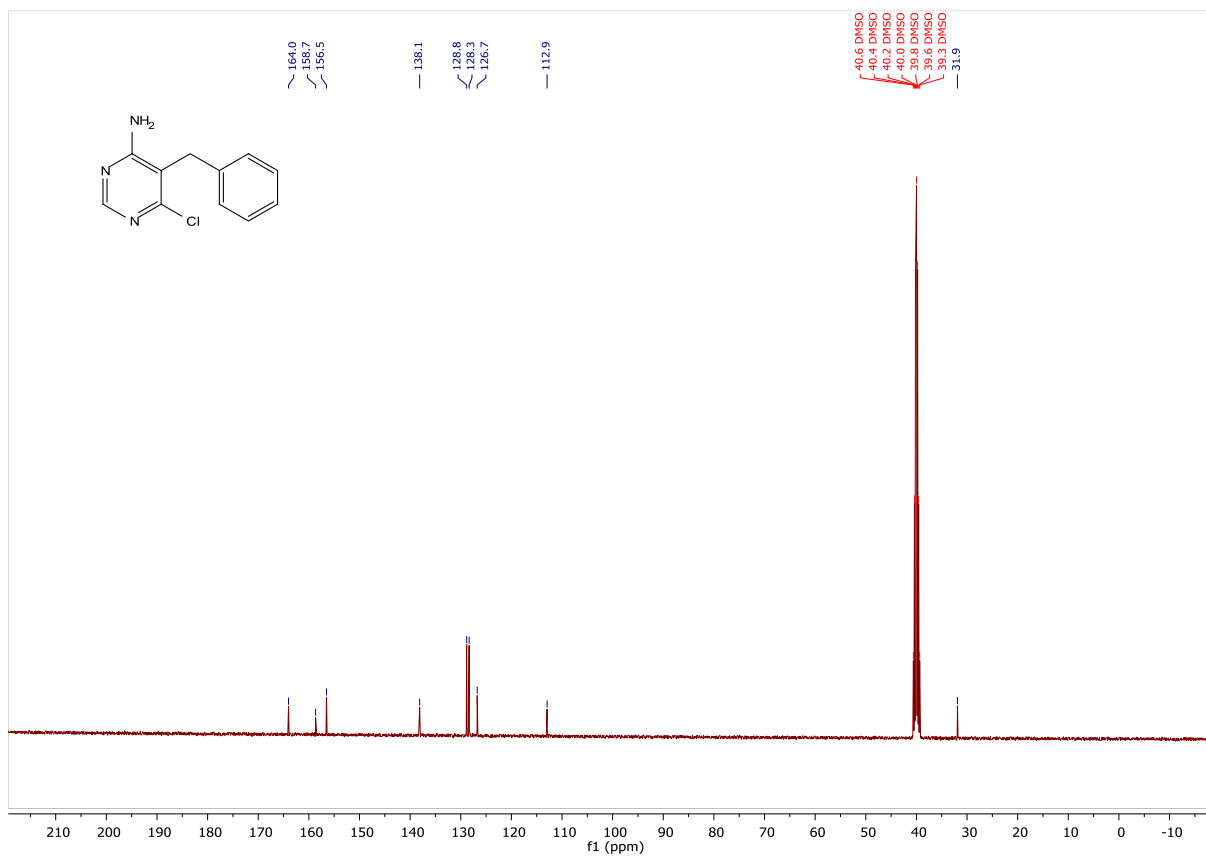
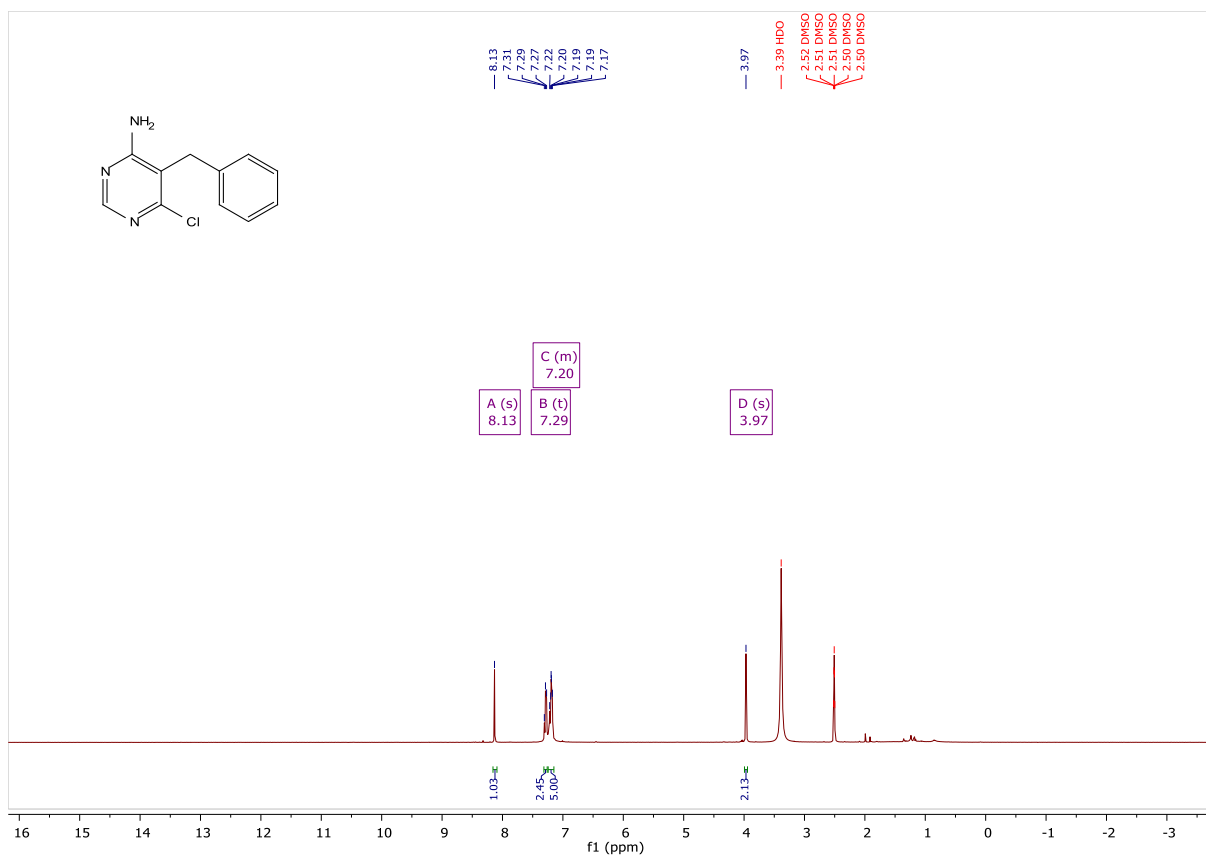


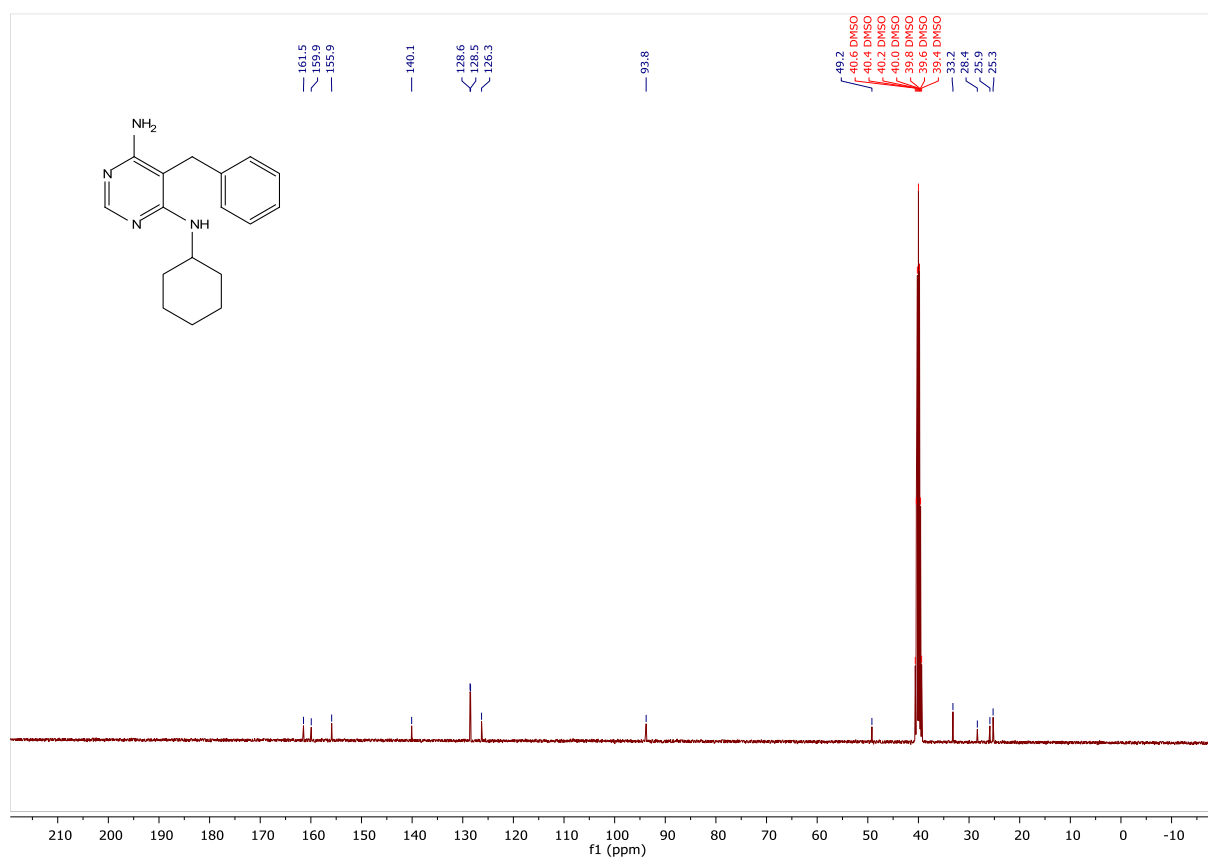
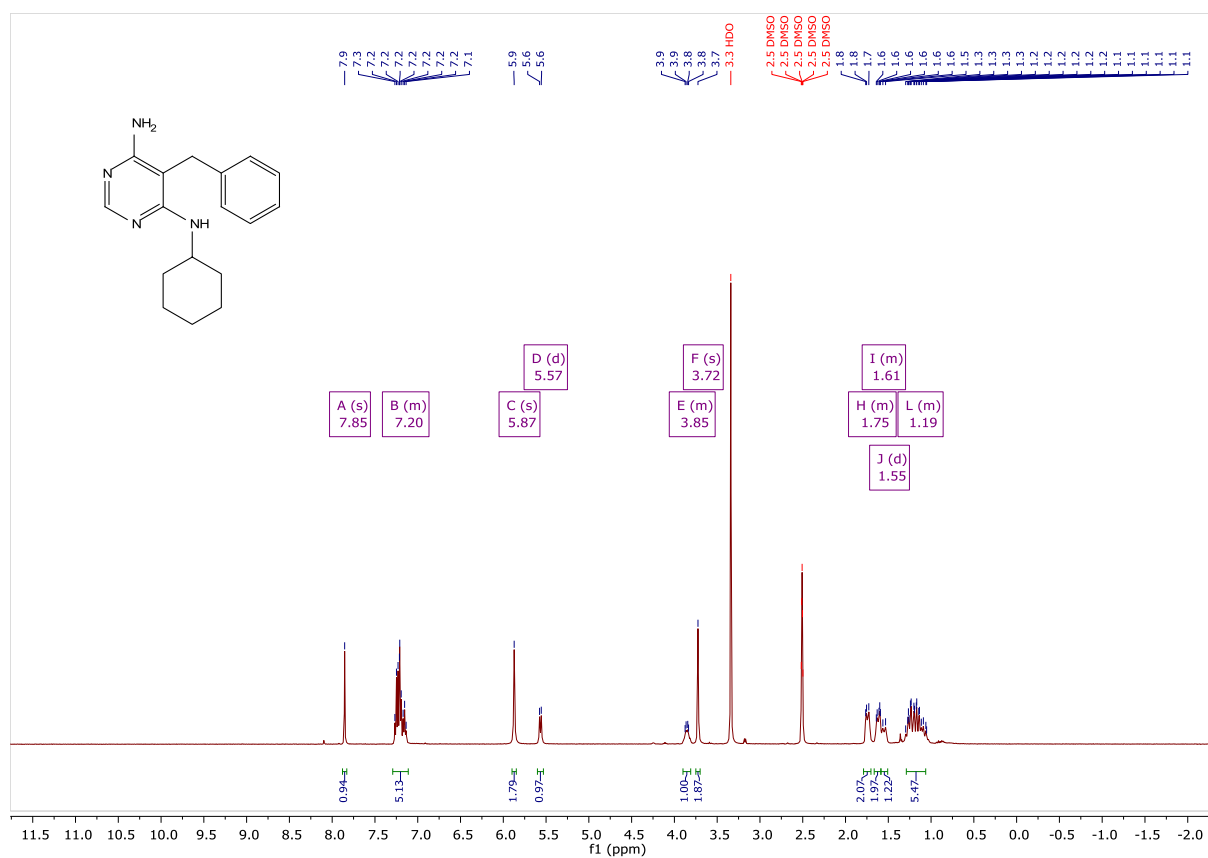


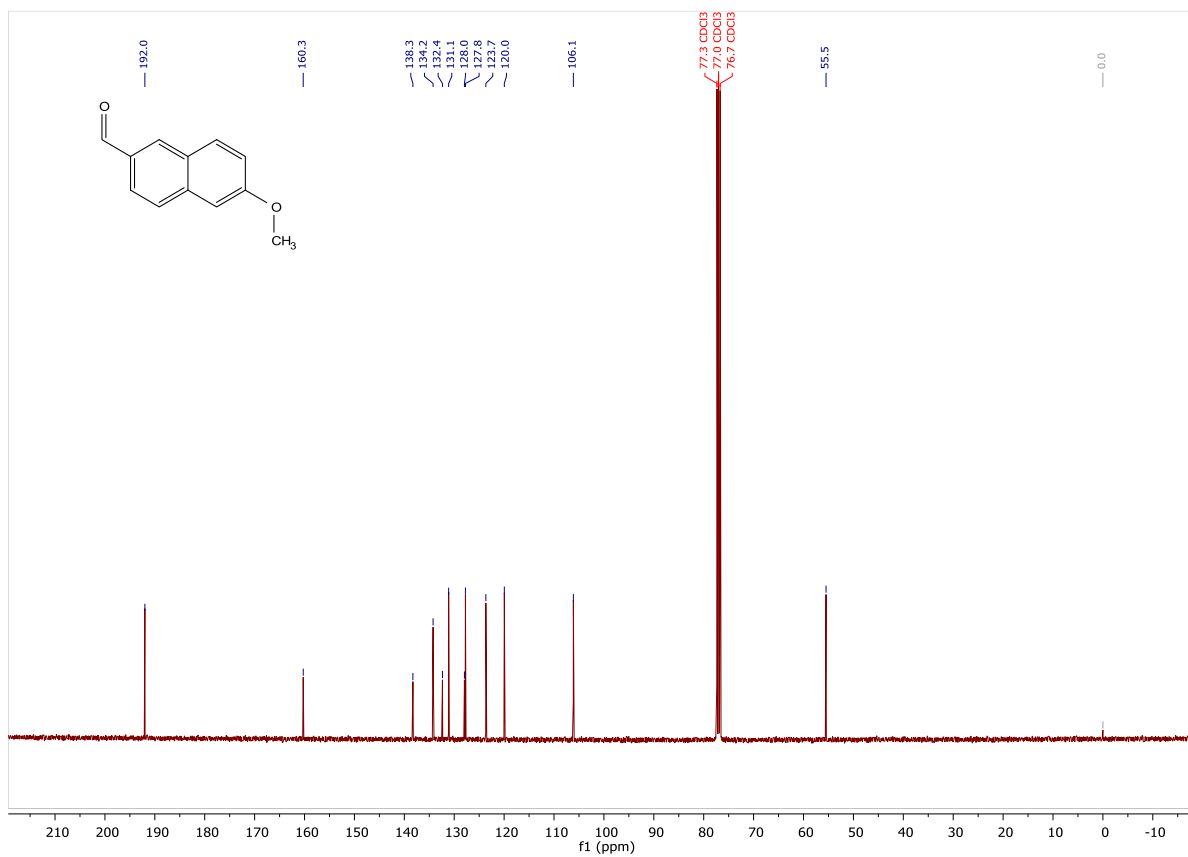
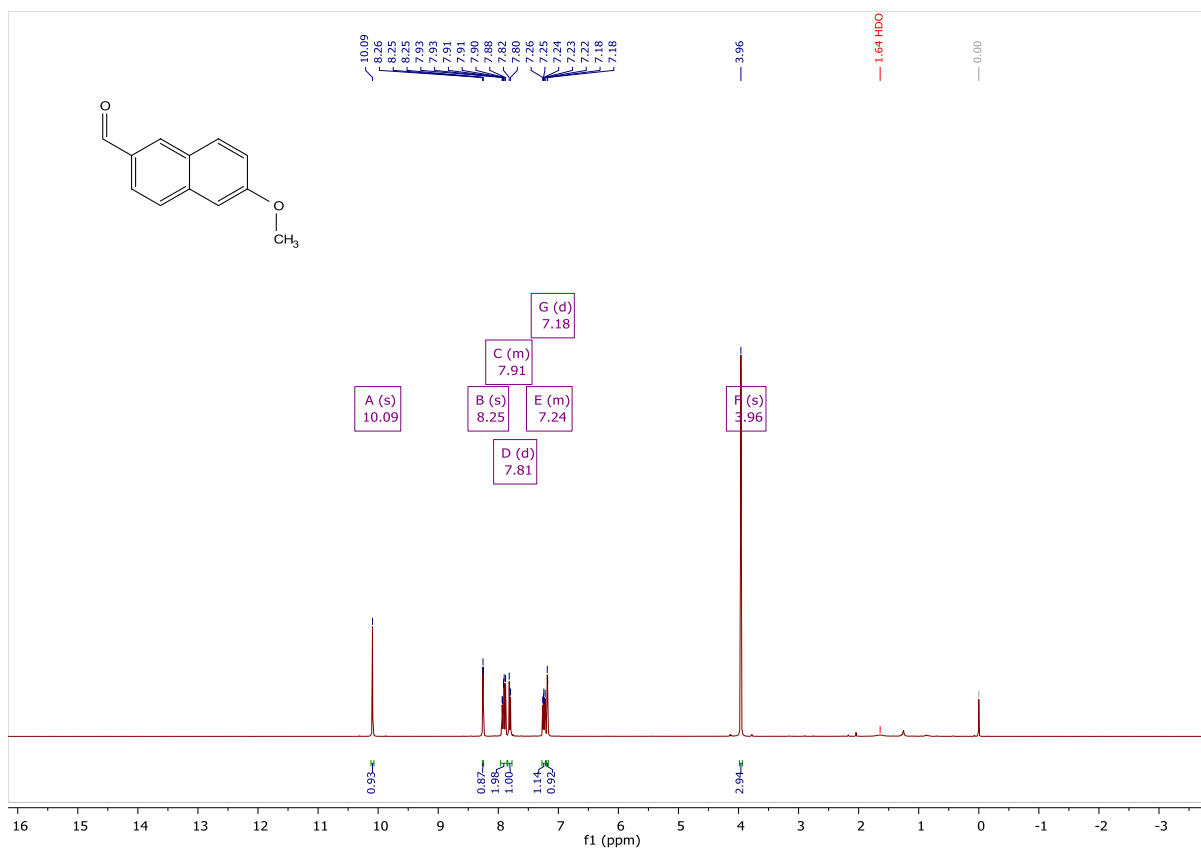


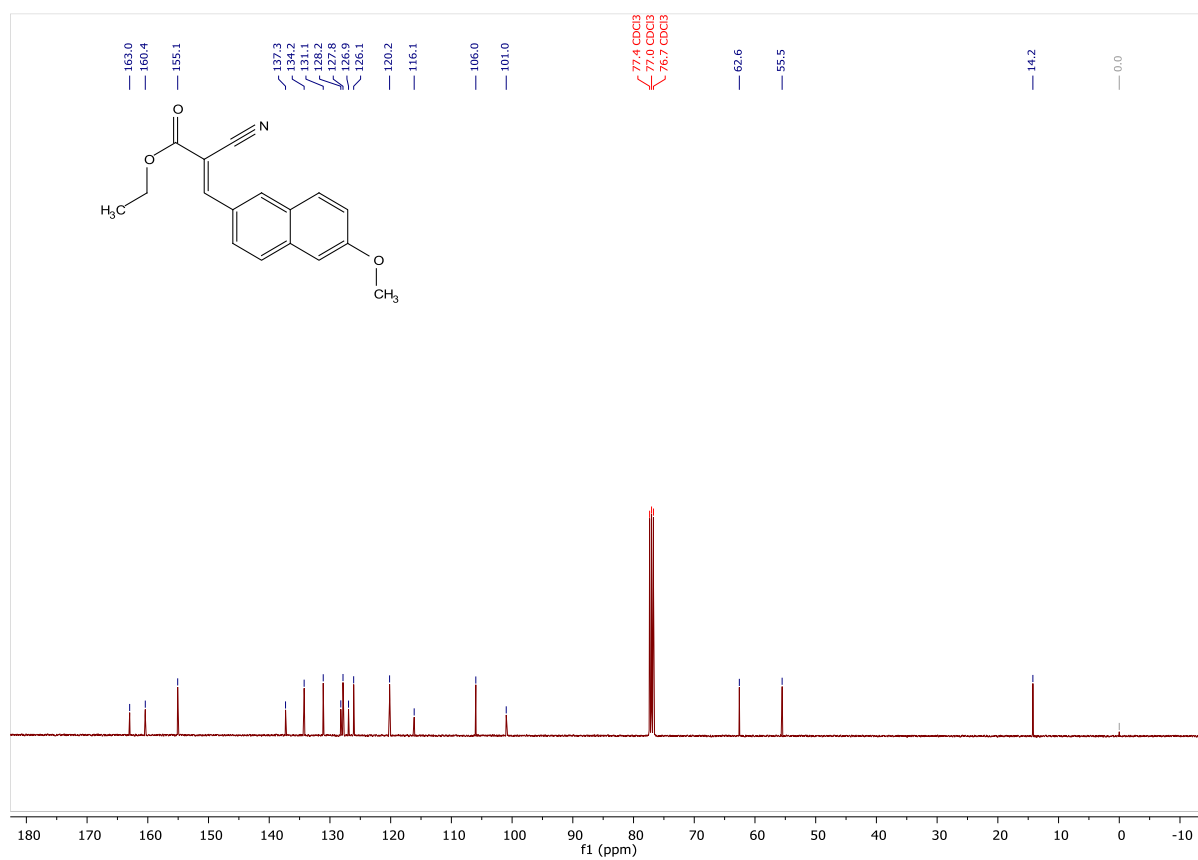
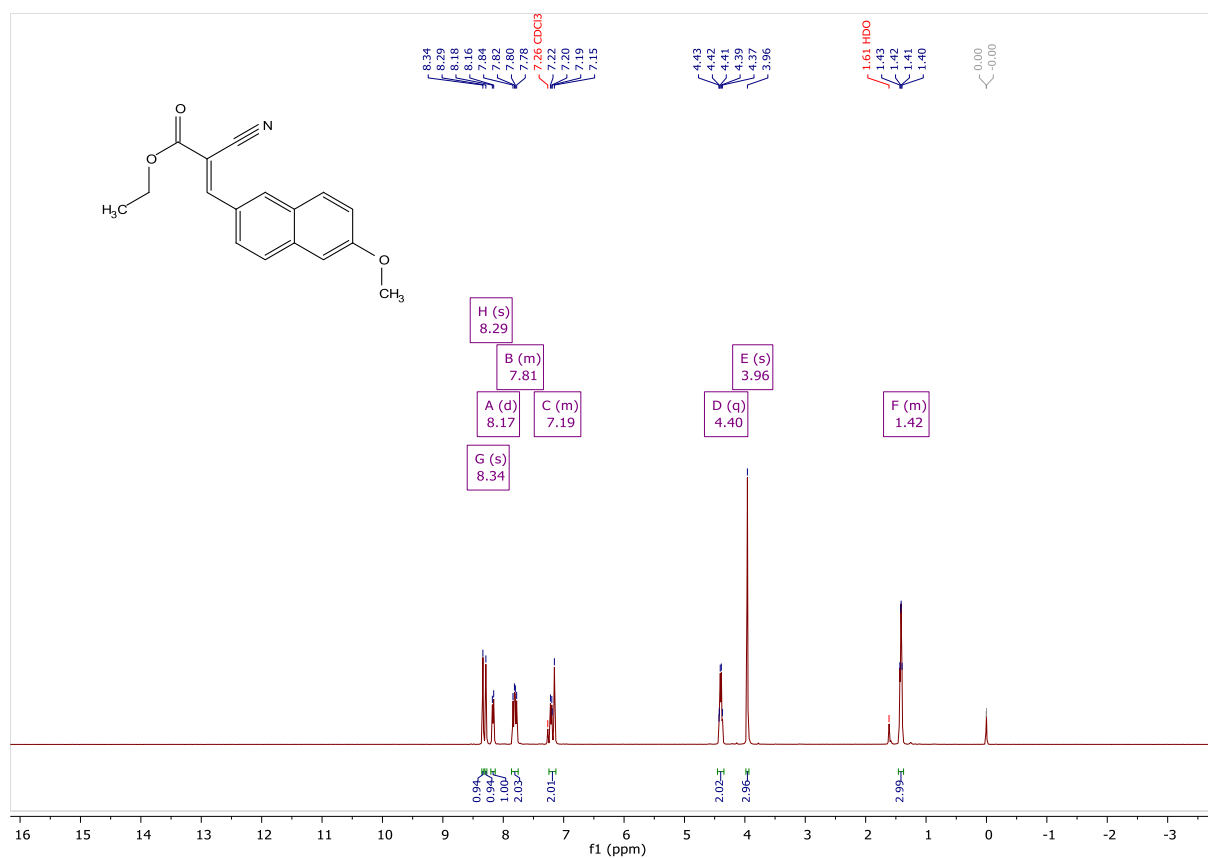


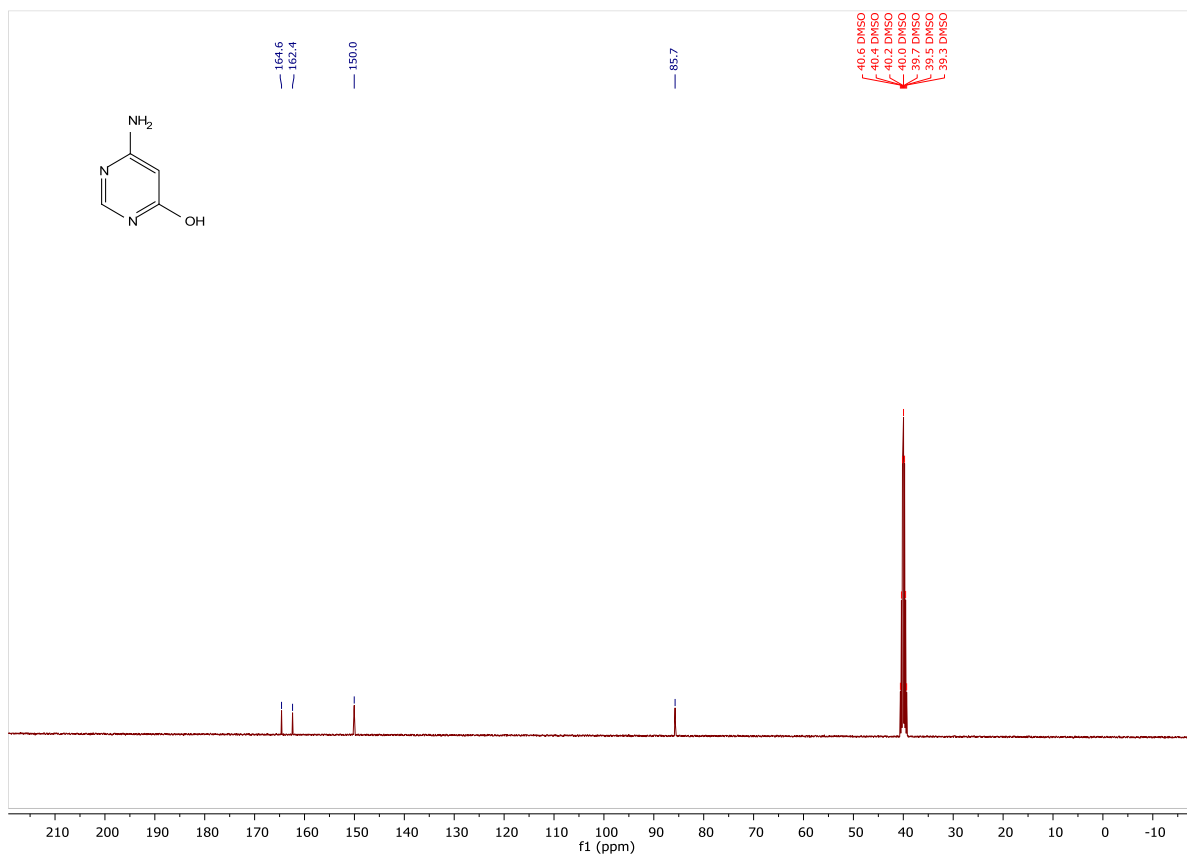
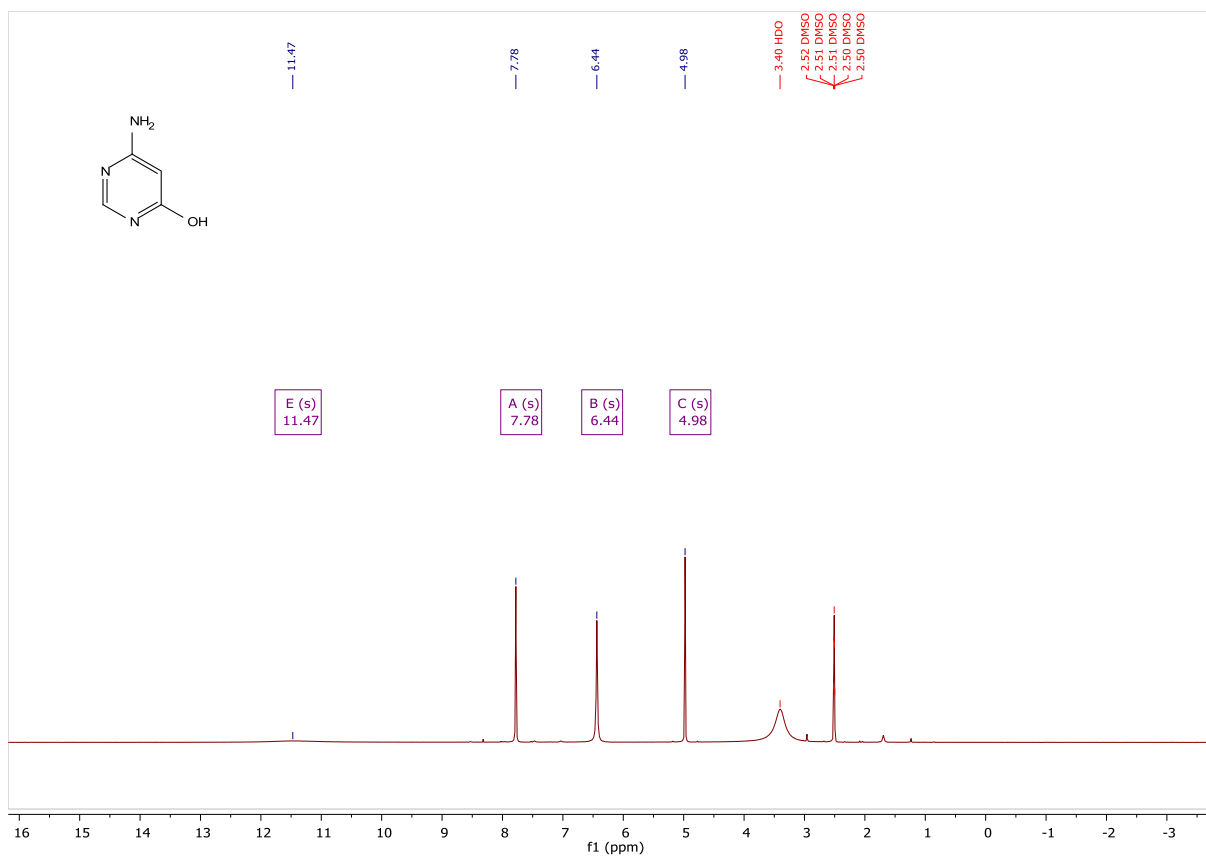


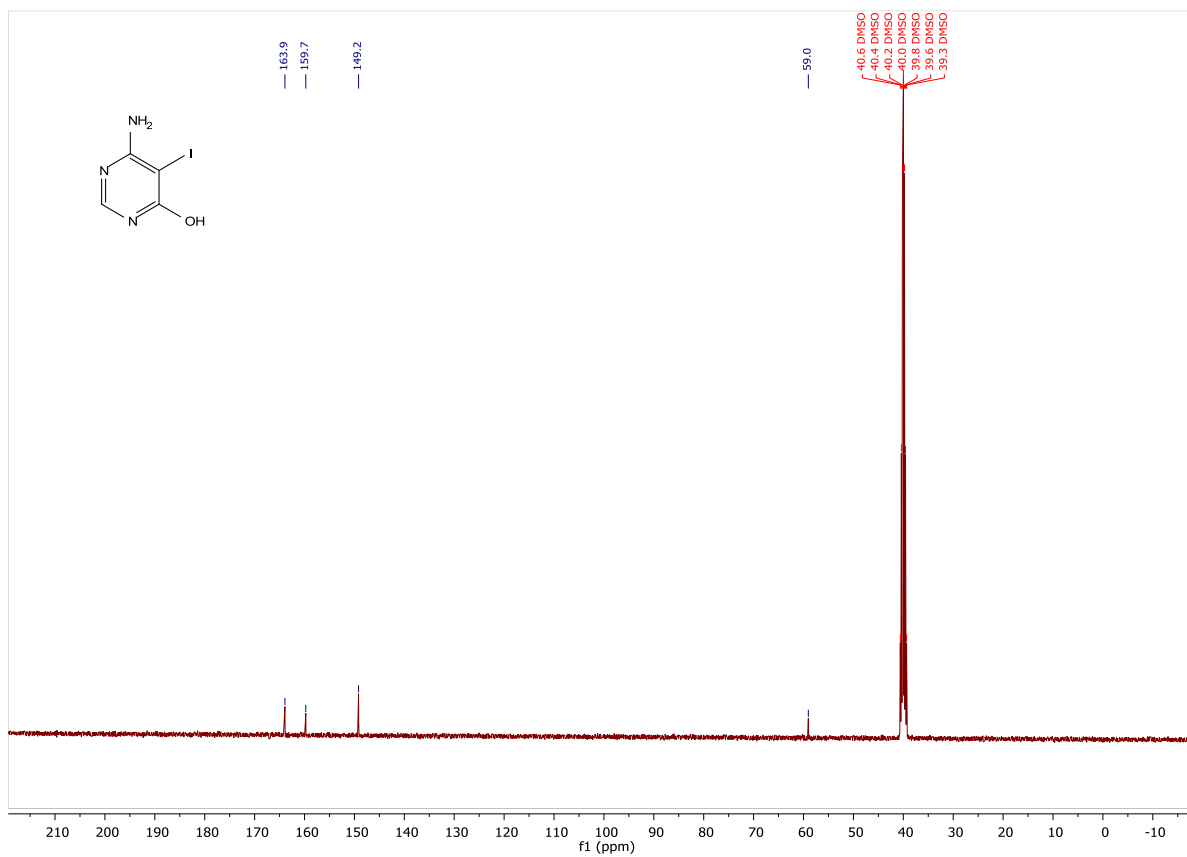
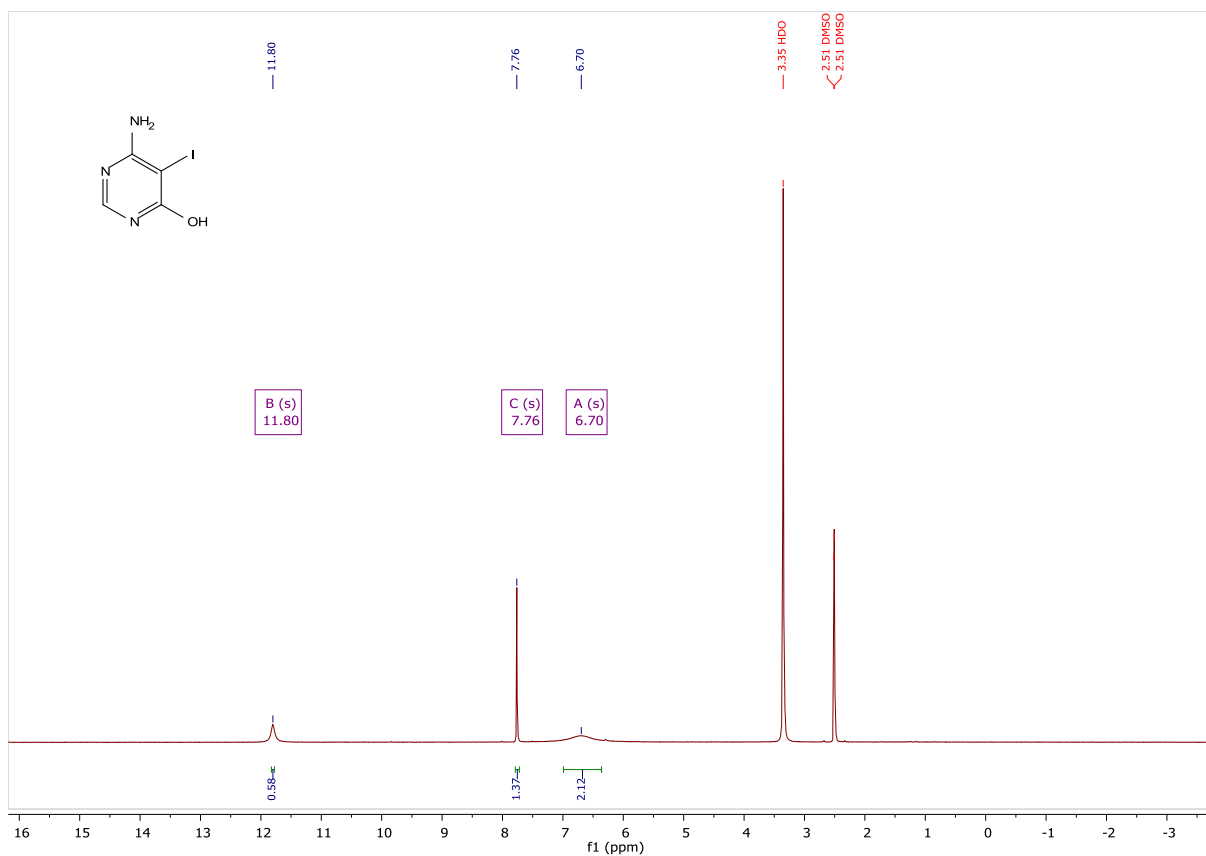


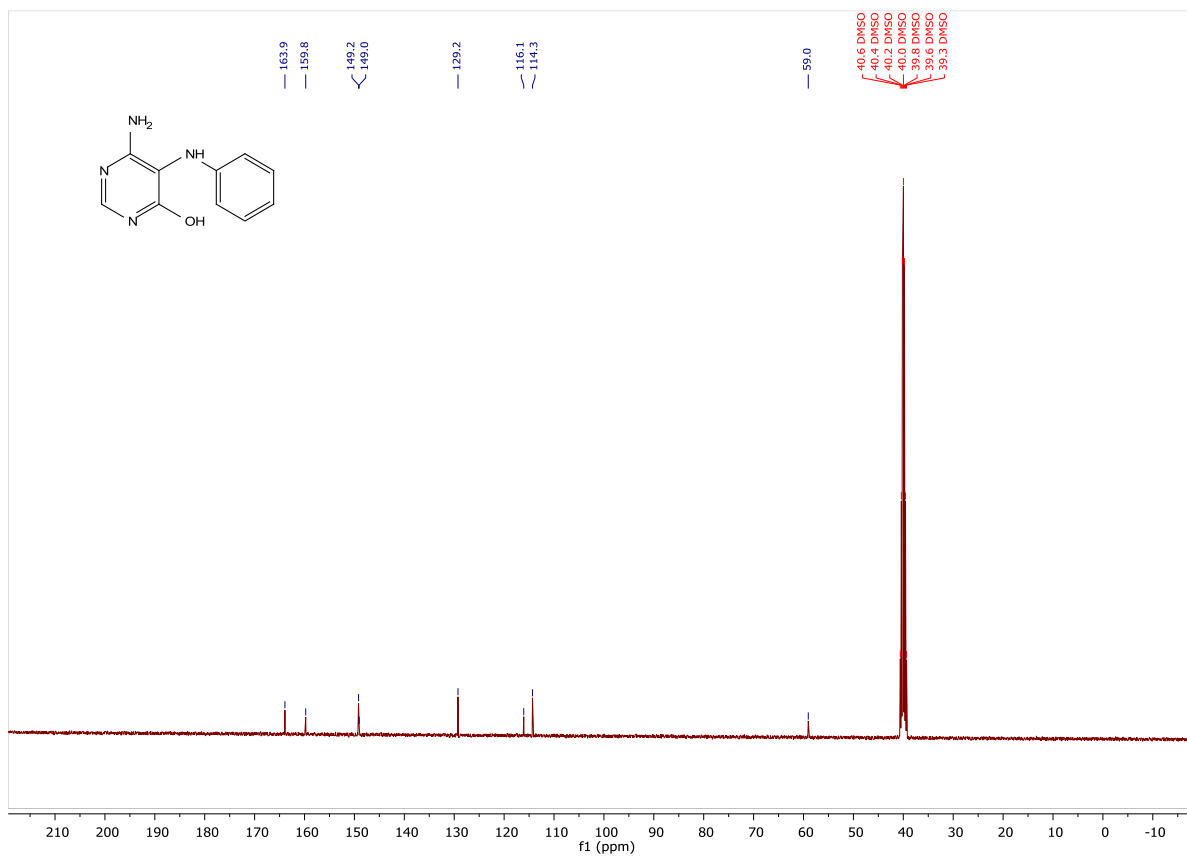
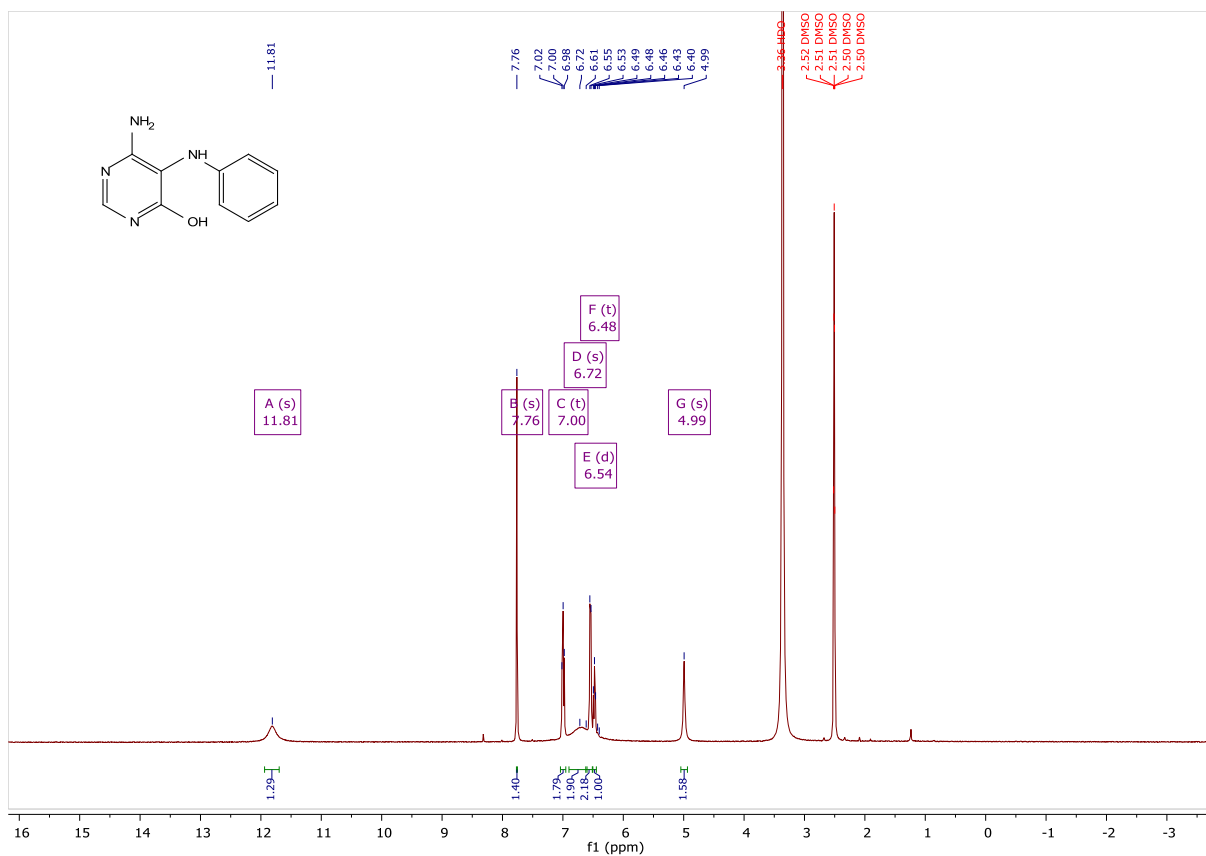






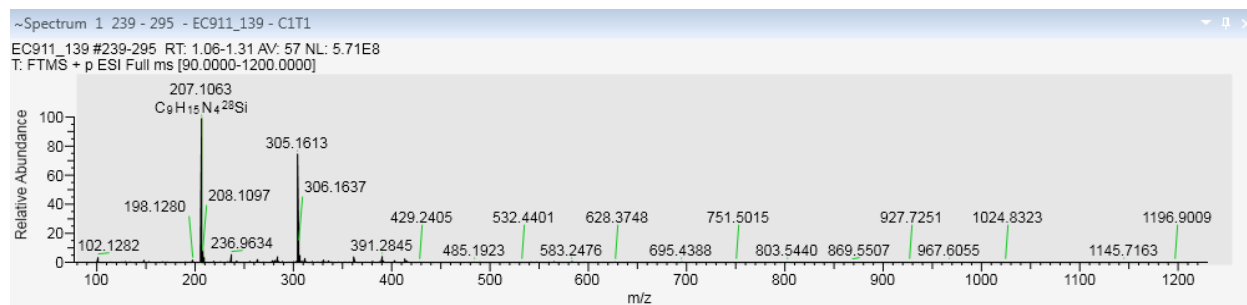




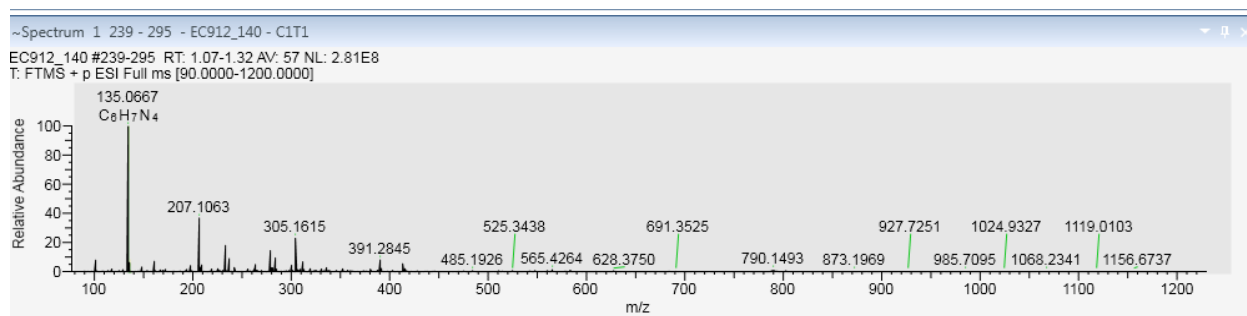


B. High resolution mass spectrometry (HRMS) spectra

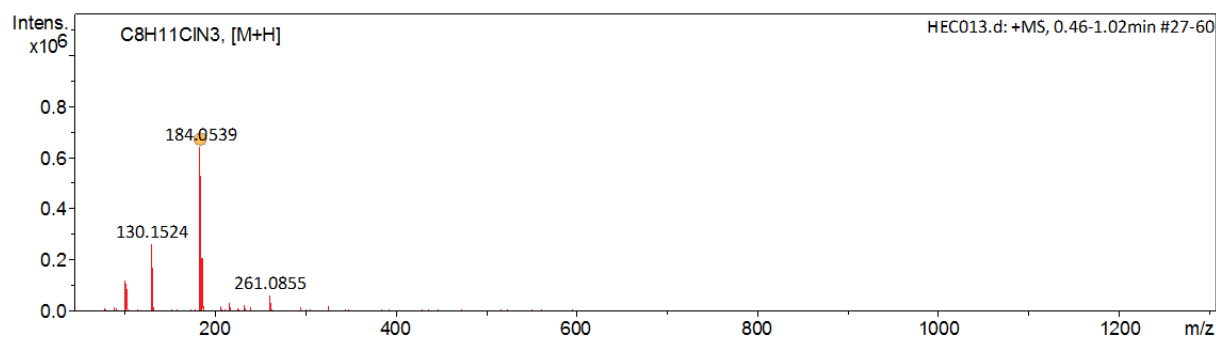
Compound 3.6



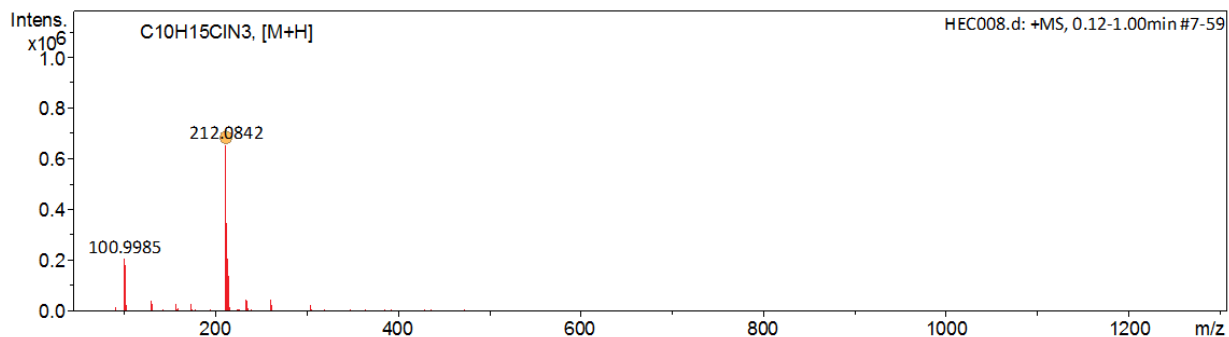
Compound 3.7



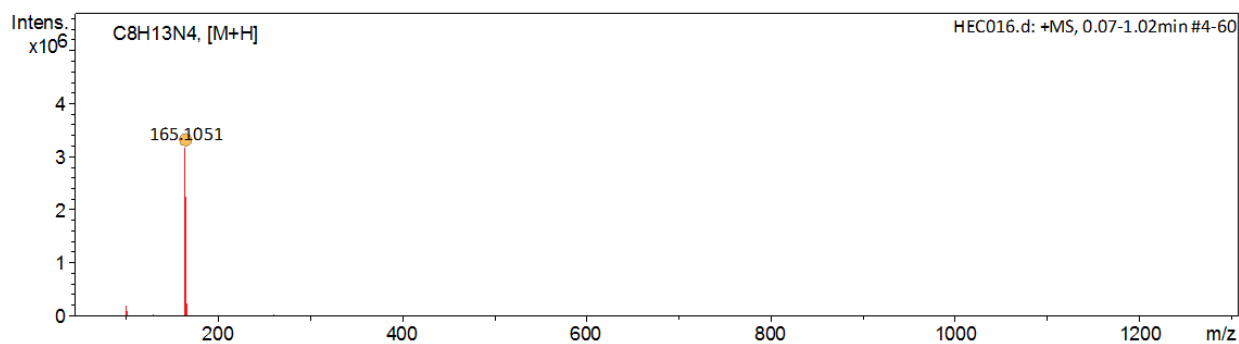
Compound 4.2



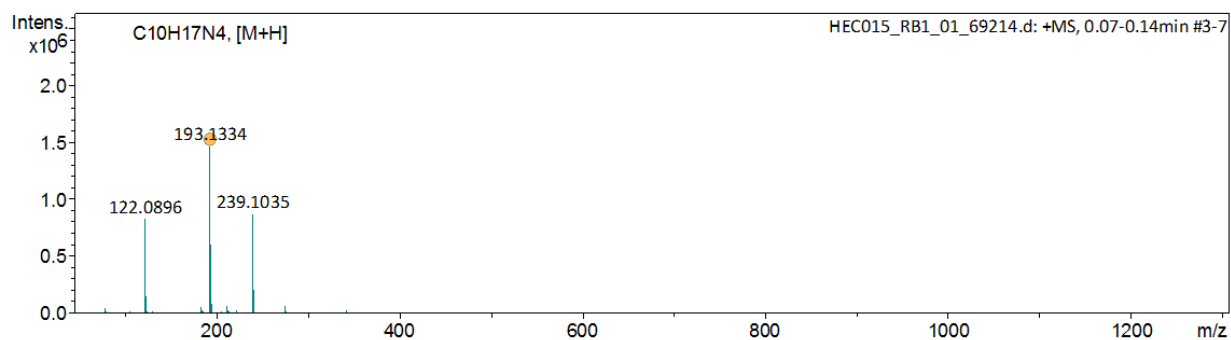
Compound 4.3



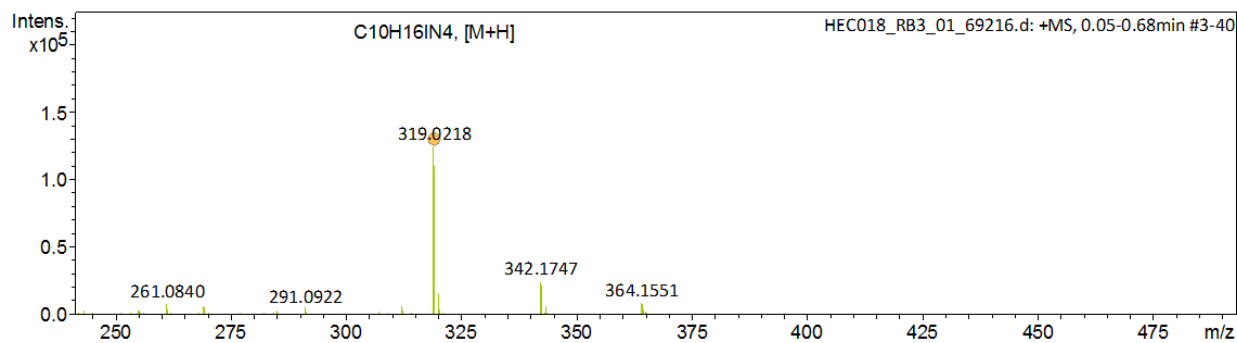
Compound 4.5



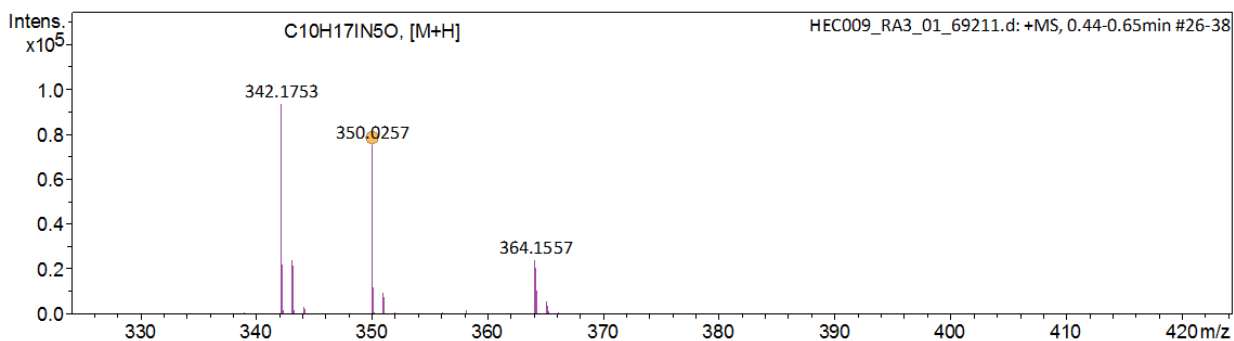
Compound 4.6



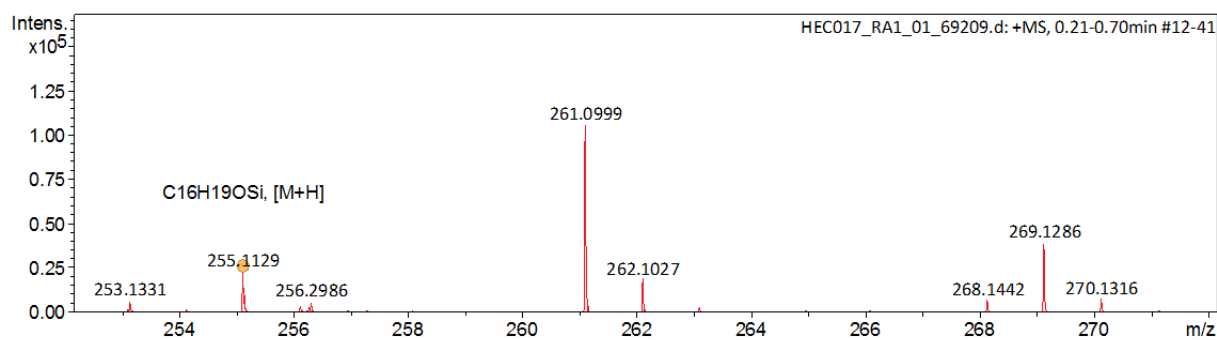
Compound 4.10



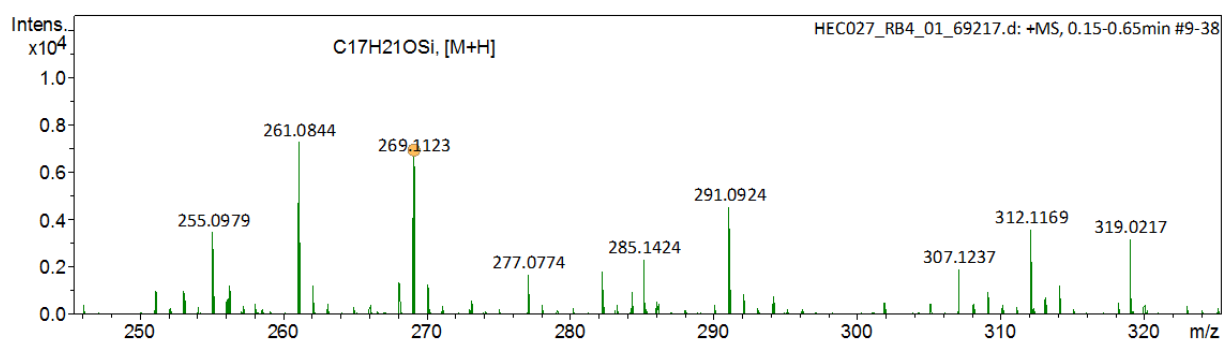
Compound 4.11



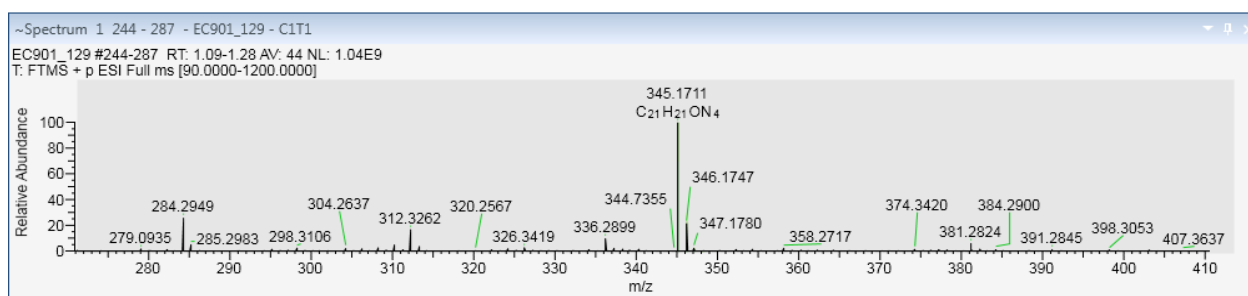
Compound 4.12a



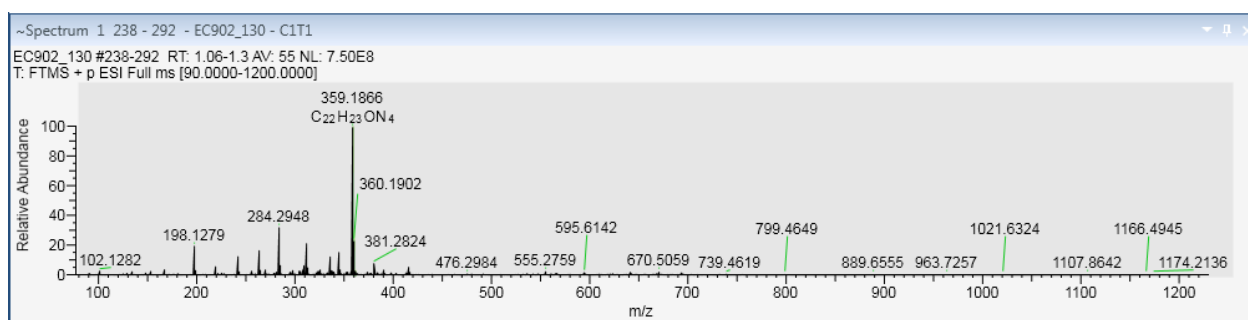
Compound 4.12b



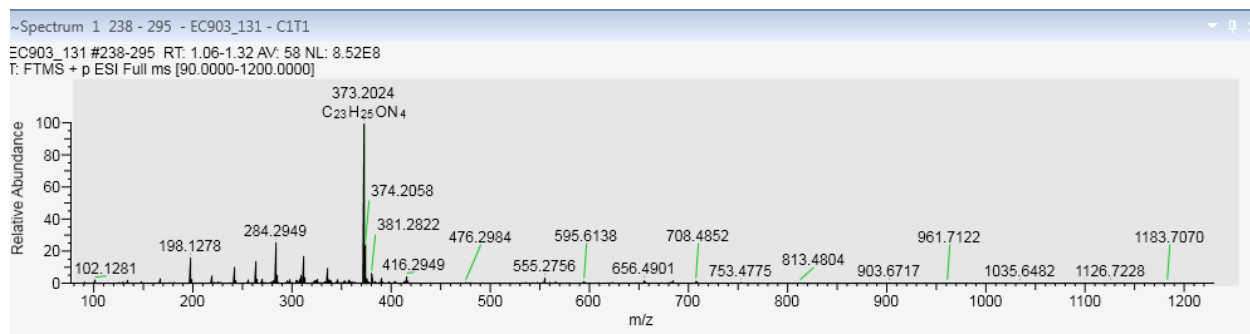
Compound 4.14a



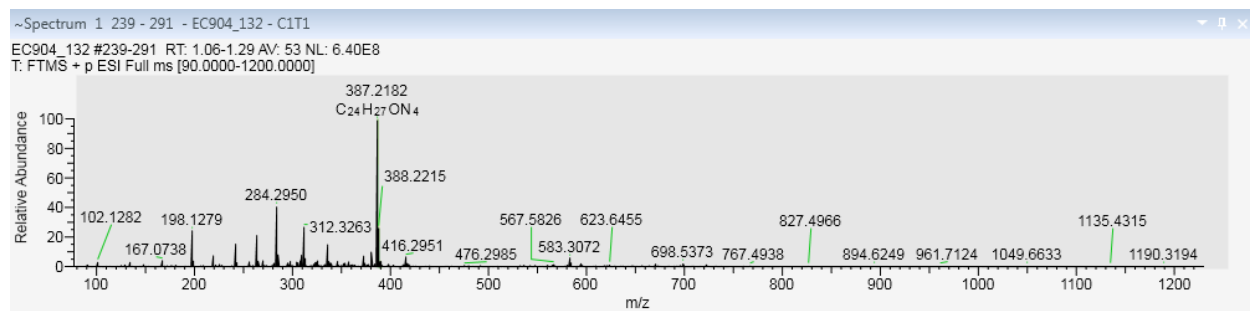
Compound 4.14b



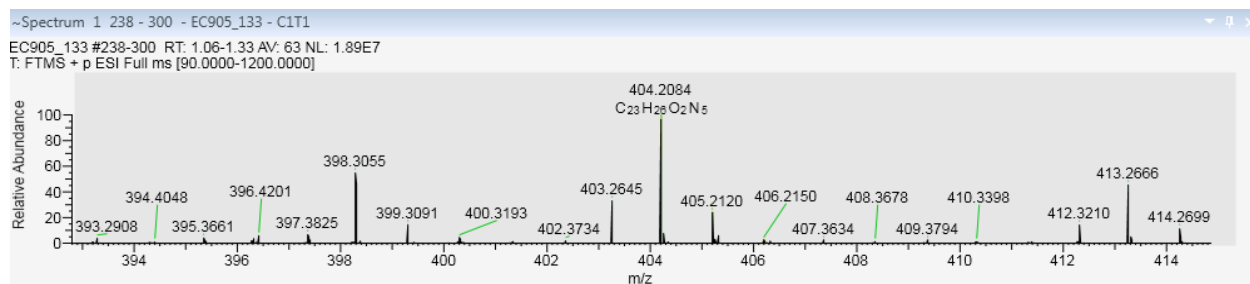
Compound 4.15a



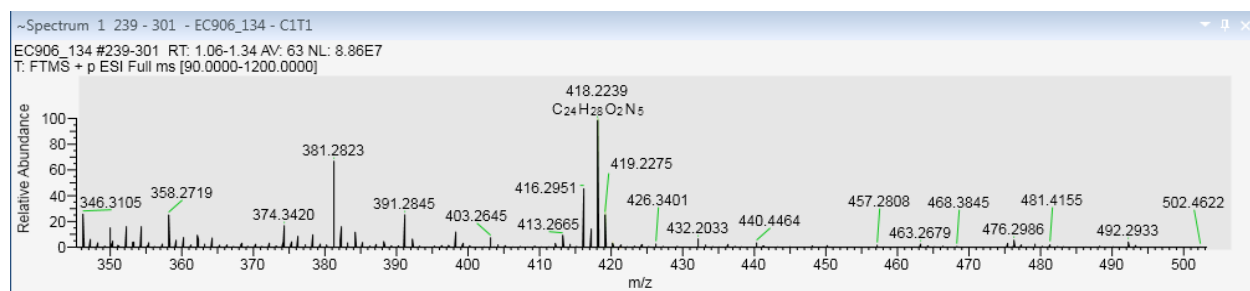
Compound 4.15b



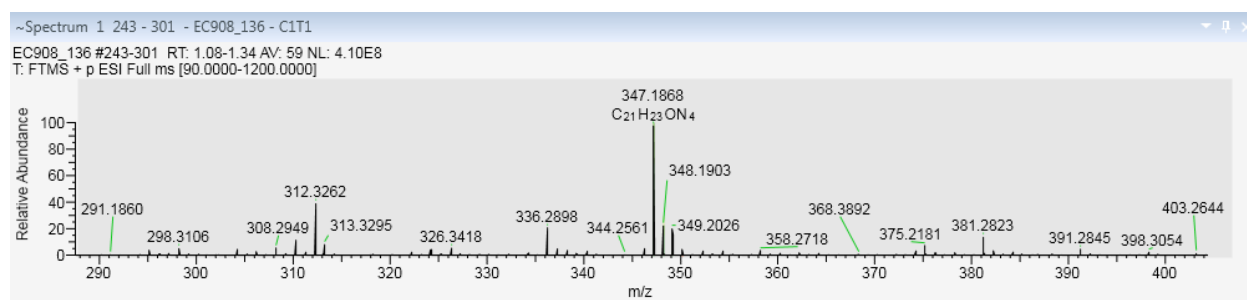
Compound 4.16a



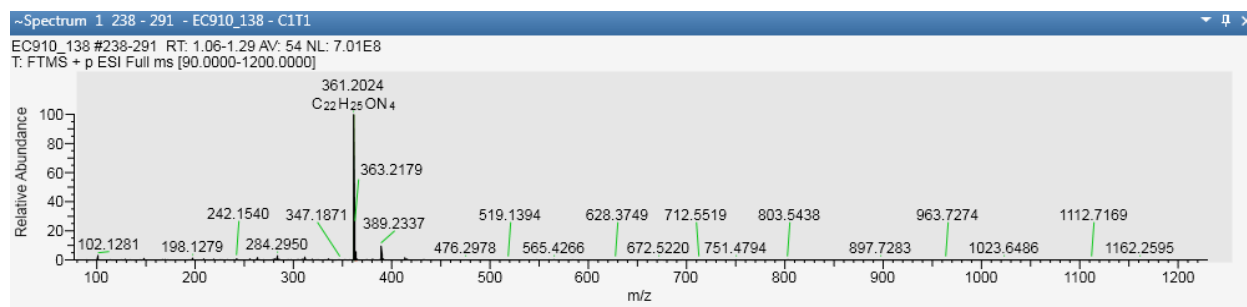
Compound 4.16b



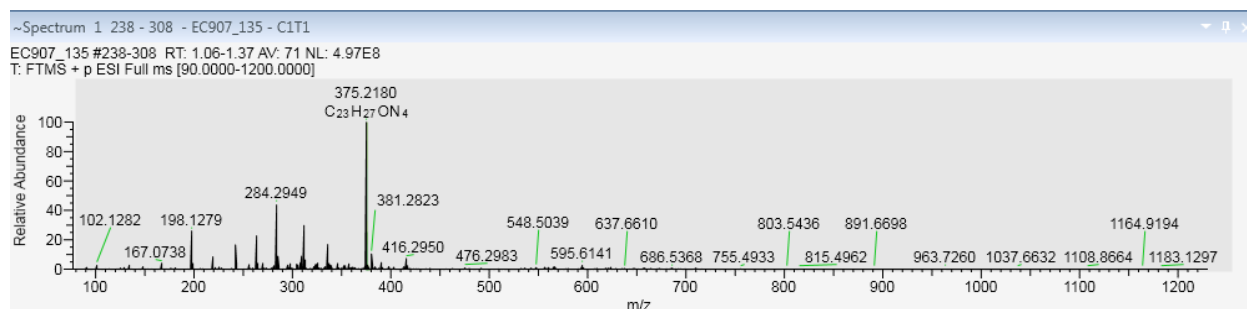
Compound 4.17a



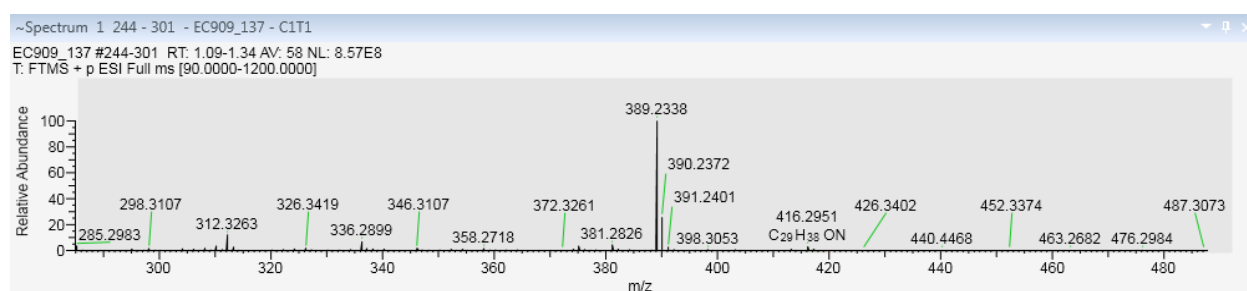
Compound 4.17b



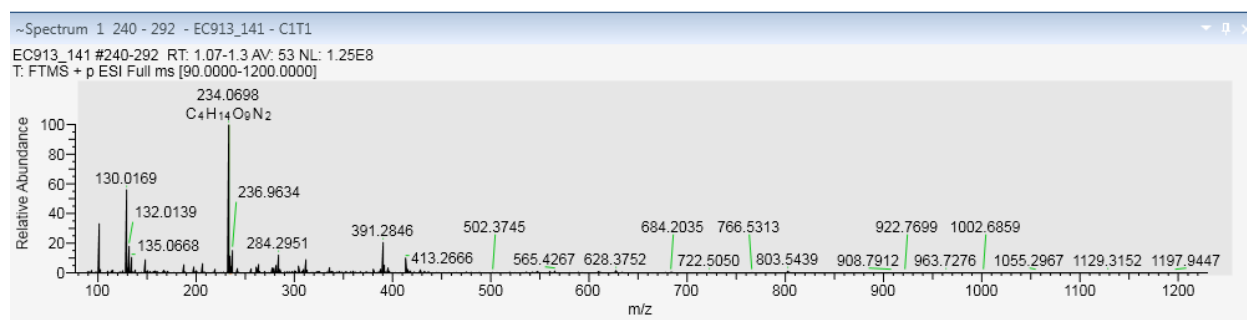
Compound 4.18a



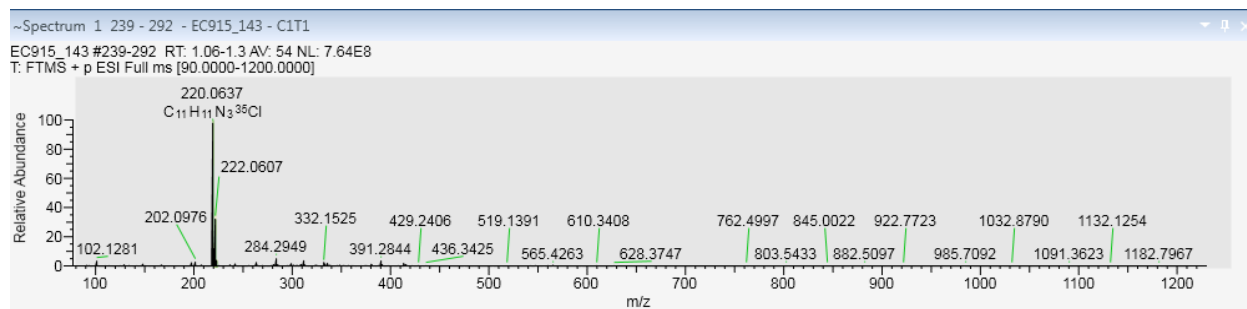
Compound 4.18b



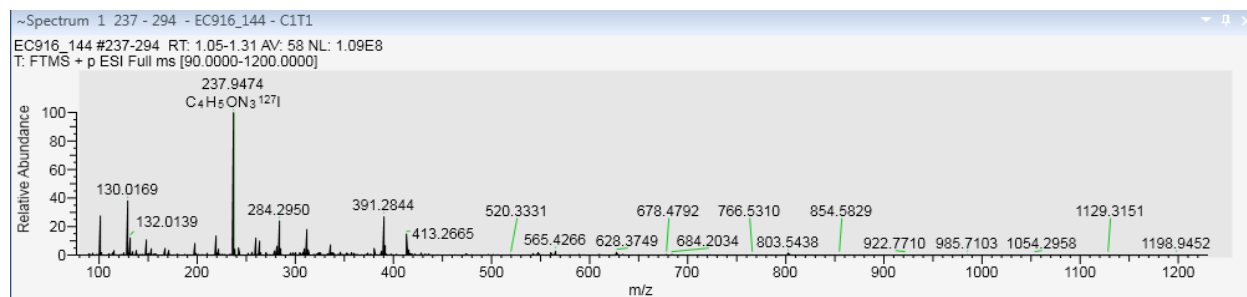
Compound 4.24



Compound 4.26



Compound 4.32



Compound 4.33

