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SYNTHESIS AND EVALUATION OF ANTIMALARIAL AGENTS AS INHIBITORS OF *PLASMODIUM FALCIPARUM* CALCIUM-DEPENDENT PROTEIN KINASES

Tswene Donald Seanego

A thesis submitted to the Faculty of Science, University of the Witwatersrand Johannesburg

In fulfilment of the requirements for the Degree of Doctor of Philosophy

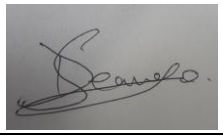
Supervisor: Dr Amanda L. Rousseau

Co-Supervisor: Prof. Charles de Koning

11 March 2019

Declaration

I declare that the work presented in this thesis was carried out exclusively by me under the supervision of Dr Amanda L. Rousseau and Professor Charles B. de Koning. 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.



Donald Tswene Seanego

11 March 2019

Abstract

The world suffers under a serious threat of malaria with about 584 000 deaths reported each year and most of these fatalities being children under five years of age. Malaria is caused by the protozoan parasite of the genus *Plasmodium*. Five different malaria species infect humans and cause disease: *P. vivax, P. malariae, P. ovale, P. knowlesi* and the cause of most malaria deaths, *P. falciparum.* Several drugs have been used for the treatment of malaria since the 17th century. The drugs in use act on different stages of the malaria life cycle. However, most antimalarial drugs target the asexual stage of malaria, but not the gametocytes. Hence, new strategies are urgently needed to disrupt parasite reproduction, thus breaking the malaria life cycle. Studies have shown that calcium-dependent protein kinases (CDPKs) are an attractive drug target because of their uniqueness to plants and apicomplexans. Since the CDPKs are absent from humans (host), this makes them a potential target for drug development. *Plasmodia* species have five CDPKs that share the characteristic domain architecture of plant CDPKs, and studies have shown that these CDPKs play a vital role in various stages of the life cycle of plasmodia and other apicomplexan parasites.

Pyrrolo[2,3-*d*]pyrimidine and pyrimidine are two of the most important heterocycles commonly found in natural products and medicinal products. These two scaffolds have received much attention from researchers and found applications in several areas of pharmaceutical and agrochemical research. Studies have shown that *P. falciparum* CDPK1 (*Pf*CDPK1) is involved in the regulation of parasite motility and controls zygote development and transmission, while *Pf*CDPK4 is required for male gametocyte exflagellation and sexual reproduction of the parasite.

The work detailed in this PhD involved three aspects of research. The first part of this project involved using the recently solved X-ray crystal structure of P*f*CDPK4 to design inhibitors with either a pyrrolo[2,3-*d*]pyrimidine or branched pyrimidine scaffold. Flexible docking protocols were developed and compounds displaying the best binding interactions were synthesised.

The second part of this thesis involved the synthesis of pyrrolo[2,3-*d*]pyrimidine derivatives that are potential inhibitors of P*f*CDPK4 and P*f*CDPK1. These novel compounds were characterised by NMR spectroscopy (where possible) and high resolution mass spectrometry (HRMS) and tested in a whole cell antiplasmodium assay. The most promising compound of this series was 5-(2-ethoxynaphthalen-6-yl)-7-(2-morpholinoethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine, which exhibited antimalarial activity in the low micromolar range (IC_{50} = 8.2 μM).

The third part of this PhD project involved the synthesis of branched pyrimidine analogues in an attempt to identify a simpler scaffold for the development of kinase inhibitors. We have successfully managed to prepare a library of 10 analogues containing the pyrimidine ring. Sequential mono nucleophilic displacement followed by a sealed tube reaction and final Sonogashira cross coupling reaction allowed for the smooth preparation of these branched pyrimidine analogues. Once again, the antimalarial activity of the compounds prepared was assessed in an *in vitro P. falciparum* screen on the drug sensitive strain. N^4-(Cyclopropylmethyl)-5-[2-(2-methoxyphenyl)ethynyl]pyrimidine-4,6-diamine was the only compound in this series to display antimalarial activity (IC_{50} = 21.1 μM).

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First and foremost, I would like to thank the **Almighty God** for giving me life. I am very grateful for the protection, good health and the blessing to complete this research.

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I would like to thank **Prof. Heinrich Hoppe** at Rhodes University for the extensive biological testing performed on my compounds. Thank you for the collaboration!

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

Ke a leboga Mohwaduba Maphaka Monare!

List of Abbreviations

ACT	Artemisinin Combination Therapy
ATP	Adenosine triphosphate
Boc	*Tert*-butoxycarbonyl
CDKs	Cyclin-dependent kinases
DCM	Dichloromethane
DDT	Dichlorodiphenyltrichloroethane
DHFR	Dihydrofolate reductase
DHPS	Dihydropteroate synthase
DIPEA	*N*, *N*-Diisopropylethylamine
DME	1,2-Dimethoxyethane
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethyl sulfoxide
Dtbpy	4,4'-Di-tert-butyl-2,2'-dipyridyl
EC_{50}	Half maximal effective concentration
EGFR	Epidermal growth factor receptor
EtOAc	Ethyl acetate
Et_3N	Triethyl amine
FAK	Focal adhesion kinase
Gsk3	Glycogen synthase kinase 3
hERG	Human ether-a-go-go related gene
Her 4	Human epidermal growth factor receptor 4
HRMS	High resolution mass spectroscopy
IC_{50}	Half maximal inhibitory concentration
IRS	Indoor residual spraying

IR	Infrared
$[Ir(COD)OMe]_2$	Bis(1,5-cyclooctadiene)di-μ-methoxydiiridium(I)
KO_tBu	Potassium *tert*-butoxide
KDR	Kinase insert domain containing receptor
NaOMe	Sodium methoxide
NBT	Nitroblue tetrazolium
$Ni(dppp)Cl_2$	Dichloro[1,3-bis(diphenylphosphino)propane]nickel
NIS	*N*-Iodosuccinimide
NMP	*N*-Methylpyrrolidinone
OTf	Trifluoromethanesulfonate
PES	Phenazine ethosulfate
$Pd(dppf)Cl_2$	[1,1'-Bis(diphenylphosphino)ferrocene]palladium(II) dichloride
$PPd(PPh_3)_4$	Tetrakis(triphenylphosphine)palladium
$Pd(PPh_3)_2Cl_2$	Bis(triphenylphosphine)palladium chloride
RPMI	Roswell Park Memorial Institute
RSCB	Research Collabratory for Structural Bioinformatics
TLC	Thin Layer Chromatography
TBAF	Tetrabutylammonium fluoride
TsCl	4-Toluenesulfonyl chloride
TMS	Trimethylsilyl
TMSCl	Trimethylsilyl chloride
TFA	Triflouroacetic acid
TFAA	Triflouroacetic anhydride
THF	Tetrahydrofuran
SEM	(Trimethylsilyl)ethoxymethyl

Dedication

I would like to dedicate this PhD thesis to my family,

My mother **Agnes Mokibelo Seanego**

My father **Caiphus Masilo Seanego**

And all my brothers and sisters (**Bakgalaka**)

Thank you for your support, prayers, guidance and encouragement.

Kodumela moepa thutse, gao lehumo le tšwang kgauswi!

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CHAPTER 1: BACKGROUND

1.1 Nitrogen-containing heterocycles and their biological significance

Heterocyclic compounds constitute the largest and most diverse family of organic compounds. They are cyclic compounds which contain at least one heteroatom. The most common heteroatoms are nitrogen, sulfur and oxygen, but heterocyclic compounds containing other heteroatoms are also commonly known.[1] Examples of simple heterocyclic compounds are pyridine, pyrrole, thiophene, and furan (**Figure 1**). A molecule of pyridine possesses a ring containing six atoms: five carbon atoms and one nitrogen atom. Pyrrole, thiophene, and furan molecules each contain five-membered rings, composed of four carbon atoms and one atom of nitrogen, sulfur, or oxygen, respectively. Pyridine and pyrrole are both nitrogen heterocycles and were discovered in the 1850s in an oily mixture formed by strong heating of bones.[2] Pyridine and pyrrole rings occur in many important compounds including pharmaceuticals, vitamins and porphyrins, etc.

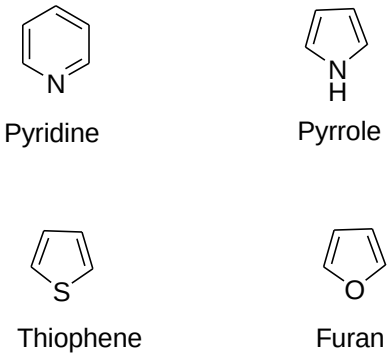
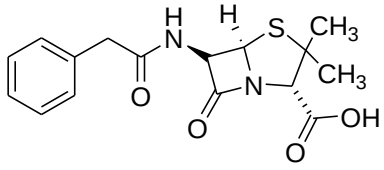


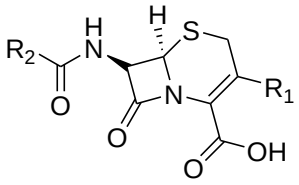
Figure 1: Simple heterocyclic compounds known.

Heterocyclic compounds have a significant role in many scientific fields including medicinal chemistry and biochemistry. Biological molecules such as DNA and RNA, haemoglobin, chlorophyll, vitamins and many more, contain a heterocyclic ring.[3] Medicinal chemistry is an important field in chemistry that draws on classical branches of science: chemistry, biology and some areas of physics, in order to study diseases and find treatments.[4] This branch of chemistry originated when chemists, pharmacists and physicians isolated and purified biologically active materials from plants, micro-organisms and their fermentation products. There are an enormous number of pharmacologically active heterocyclic compounds which are in regular clinical use.

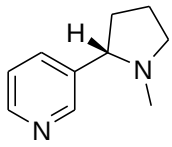
Some of these are naturally-occurring products, for example, antibiotics such as penicillin **1** and cephalosporins **2**, alkaloids[5] such as nicotine **3**, morphine **4** and reserpine **5**, shown in **Figure 2**. The majority are heterocyclic compounds which have found extensive use in pharmaceutical applications as anti-cancer agents, antibacterial agents, antimalarial agents, analgesics, antidepressants, etc. Other heterocyclic compounds have a wide range of applications as pesticides, insecticides, herbicides, corrosion inhibitors, dyestuffs and antioxidants.[6]



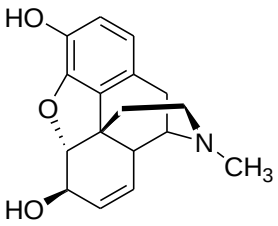
Penicillin G 1



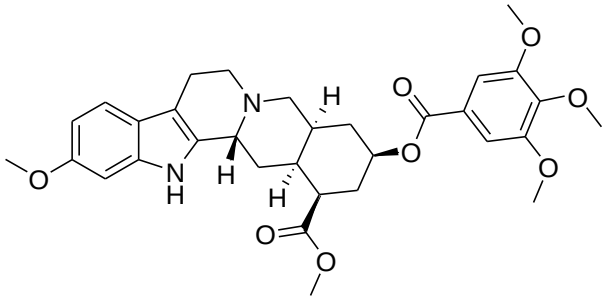
Cephalosporins 2



Nicotine 3



Morphine 4



Reserpine 5

Figure 2: Some heterocyclic compounds which are in regular clinical use.

Nitrogen containing heterocyclic compounds are fundamental building blocks used to synthesise compounds of medicinal or biological interest to chemists. In the section that follows, some key biological activities of heterocyclic compounds related to this project will be discussed.

1.2 Pyrrolo[2,3-*d*]pyrimidine

One of the heterocycles of interest in this project was the fused bicyclic heteroaromatic pyrrolo[2,3-*d*]pyrimidine, or 7-deazapurine. Pyrrolo[2,3-*d*]pyrimidine is one of the most

important heterocycles commonly found in natural products and medicinal products. The skeleton has received much attention from researchers and in several areas of pharmaceutical and agrochemical research.[7]

For example, 7-deazapurines have demonstrated antibacterial, antitumor, antiviral, anti-inflammatory and antihyperglycemic activities.[7] Due to their structural resemblance to purines and pyrimidines, pyrrolo[2,3-*d*]pyrimidines are used as alternatives for canonical constituents of DNA and RNA and are also employed in nucleic acid sequencing.[8]

Pyrrolo[2,3-*d*]pyrimidines are named according to systematic nomenclature as shown below (**Figure 3**). The pyrrolo[2,3-*d*]pyrimidine skeleton contains one nitrogen atom in the five-membered pyrrole ring and two nitrogen atoms in the six-membered pyrimidine ring where the two rings are fused to afford the title compound. Hence, these bicyclic compounds represent structural analogues of biogenic purines and they can be considered as potential antimetabolites in nucleic acid metabolism. Pyrrolo[2,3-*d*]pyrimidine and its derivatives also display strong UV-blue fluorescence and are used as fluorescent functional resources.[9]

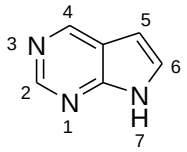


Figure 3: 7*H*-pyrrolo[2,3-*d*]pyrimidine

1.2.1 Naturally occurring pyrrolo[2,3-*d*]pyrimidines

There are a few naturally occurring pyrrolo[2,3-*d*]pyrimidines. For example, tubercidin **6**, sangivamycin **7** and toyocamycin **8** (**Figure 4**), were isolated from *Streptomyces* cultures.[10] Cytotoxic studies of these 7-deazapurine nucleosides were conducted in the 1960s, and all the compounds displayed potent cytotoxic activity against cancer cell lines.[11] Although these natural 7-deazapurines have structural similarities, their modes of action are different. These pyrrolo[2,3-*d*]pyrimidines possess different substituents at the C-5 position of the pyrrole ring of

the pyrrolo[2,3-*d*]pyrimidine nucleus, and this appears to have an influence on their biological activity.[10]

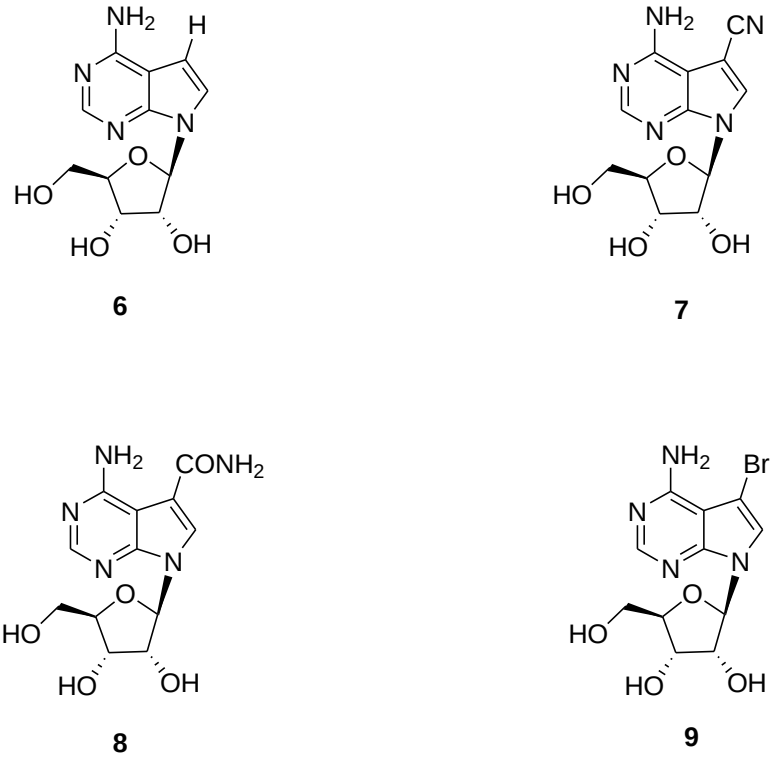


Figure 4: Naturally occurring 7-deazapurines.

Compounds **6**-**8** are phosphorylated by cellular kinases to their mono-, di-, and triphosphate forms and the resultant nucleotides get incorporated both into RNA and DNA which causes inhibition of proteosynthesis and DNA damage, respectively. Tubercidin damages numerous cellular processes such as pre-mRNA processing, mitochondrial respiration, purine synthesis, rRNA processing, and methylation of tRNA.[12] In addition, tubercidin **6** also inhibits *S*-adenosylhomocysteine hydrolase. On the other hand, compound **6** has been identified as a potent inhibitor of protein kinase C, and this is how it exerts its cytotoxic effect.[13] Recently, by comparison, Wakao and co-workers have studied the mechanism of action of sangivamycin **7**, and have shown that it acts through inhibition of Akt and Erk in primary effusion lymphoma cells.[14] Despite these promising results, none of the naturally occurring 7-deazapurine nucleoside compounds have proceeded to clinical trials.[15]

Structural modifications of these natural 7-deazapurine nucleosides have been reported. For example the brominated counterpart **9** of tubercidin has been shown to reversibly inhibit the synthesis of cellular RNA (IC_{50} = 15−30 μM).[15] The brominated derivative also inhibits adenosine kinase and the synthesis of viral DNA but it has been shown to be ineffective against RNA viruses. The cytotoxic effect of bromotubercidin results from incorporation of its triphosphate form into cellular RNA and inhibition of RNA synthesis.[16]

1.2.2 Pyrrolo[2,3-*d*]pyrimidines as kinase inhibitors

The pyrrolo[2,3-*d*]pyrimidine scaffold makes the same hydrophobic and hydrogen-bonding contacts with the hinge region of kinases as the adenine ring of adenosine triphosphate (ATP) as shown below in **Figure 5**. Hence, pyrrolo[2,3-*d*]pyrimidines represent an attractive scaffold for the development of ATP analogues and for the synthesis of novel derivatives by modification of the substitution pattern.[7]

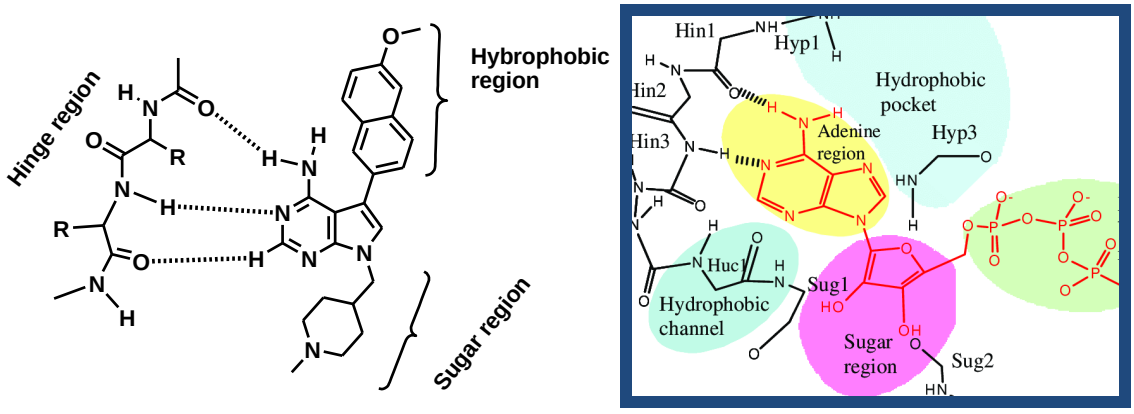
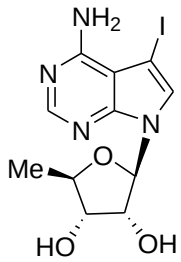


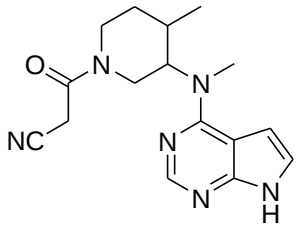
Figure 5: Schematic representation of pyrrolo[2,3-*d*]pyrimidine scaffold binding site (left). ATP binding site, with different regions highlighted (right).[17]

Adenosine is an extracellular purine nucleoside which controls many physiological processes. Adenosine kinase (ADK) is an enzyme that catalyzes the conversion of adenosine to adenosine-5′-*O*-monophosphate (AMP). Inhibition of this enzyme leads to increased adenosine levels.[18] 5′-Deoxy-5-iodotubercidin **10**, which is also a naturally occurring 7-deazapurine nucleoside isolated from a marine red alga, is a potent inhibitor of mammalian adenosine kinases (IC_{50} = 9

nM against human ADK) and does not bind to adenosine receptors (**Figure 6**). The pyrrolopyrimidines were also found to be powerful inhibitors of protein kinases, such as the enzyme Janus kinase 3 (JAK 3). JAK 3 belongs to a family of cytoplasmic protein tyrosine kinases which play an important role in cytokine-triggered signalling events by activators of transcription (STAT) proteins *via* tyrosine phosphorylation.[18] The association of JAK 3 with cytokine signalling pathways makes it an important target for therapeutic intervention in the treatment of inflammatory diseases, autoimmune disorders and organ transplant rejection. Hence, inhibition of this kinase by pyrrolopyrimidines can be useful for the treatment of several immunological syndromes. For example, CP-690,550 **11** is a JAK 3 inhibitor from Pfizer that has already progressed into Phase II clinical trials for acute rejection in kidney transplant patients.[19]



10



11, CP-690,550

Figure 6: Pyrrolopyrimidine kinase inhibitors.

Tofacitinib **12** and Ruxolitinib **13** (**Figure 7**) are 4-substituted pyrrolopyrimidine kinase inhibitors which received FDA approval in 2011 and 2012, respectively.[20] Tofacitinib inhibits Janus kinase 1 (JAK1) and Janus kinase 3 (JAK 3) and is used for the treatment of rheumatoid arthritis, while Ruxolitinib is an inhibitor of JAK1 and JAK2 and is used for the treatment of myelofibrosis.[21]

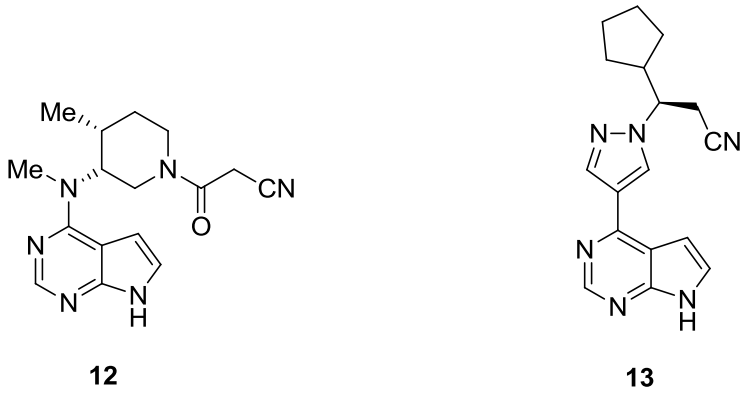


Figure 7: FDA approved 4-substituted pyrrolopyrimidines.

The treatment of chronic pain remains a major challenge in healthcare, with currently used pain drugs suffering from poor efficacy and dose-limiting toxicity. Studies have shown that the voltage-gated sodium ion channel ($Na_v1.7$) is an important regulator of human pain, and inhibition of this channel by selective agents may result in a new class of pain relief therapeutics.[22] Hence, Chakka and co-workers have synthesised a series of 4-substituted pyrrolo[2,3-*d*]pyrimidines as potent and state-dependent inhibitors of voltage-gated sodium ion channel $hNa_v1.7$ for the treatment of chronic pain (**Figure 8**).[22] Compound **14** displayed a hNav1.7 PX (PatchXpress) IC_{50} value of 0.11 μM, hNav1.5 PX IC_{50} of 0.16 μM, human liver microsome (HLM) clearance of 130 μL/min/mg, and >100× selectivity against human ether-a-go-go related gene (hERG), while compound **15** displayed a hNav1.7 PX (PatchXpress) IC_{50} value of 3.6 μM and human liver microsome (HLM) clearance of 61 μL/min/mg.

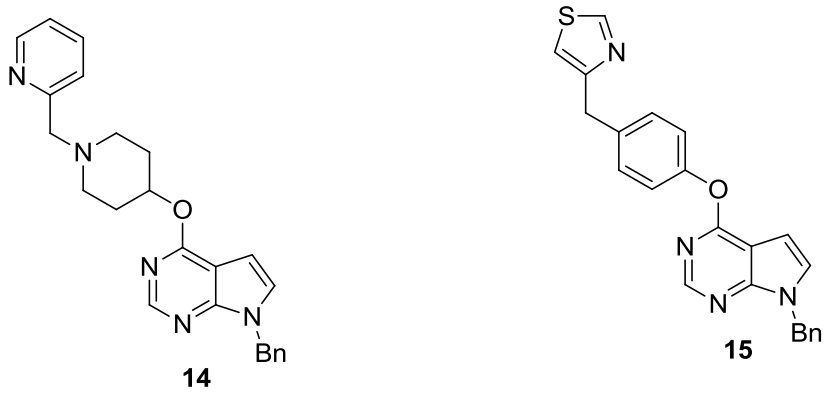


Figure 8: Pyrrolo[2,3-*d*]pyrimidines used for the treatment of chronic pain.

Figure 9 below shows the different structural types of pyrrolopyrimidines (deazapurines), which includes 5-deazapurines, 6-deazapurines and 7-deazapurines. The position of the nitrogen atom in the pyrrolopyrimidine heterocyclic core influences the properties of the corresponding deazapurine. In this project, the heterocycle of interest is 7-deazapurine and in the following section the synthesis of 7-deazapurines will be discussed.

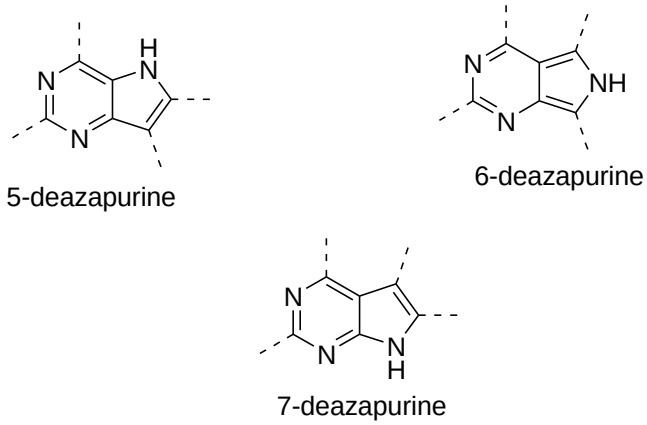


Figure 9: Pyrrolopyrimidine scaffolds.

1.3 Synthesis of pyrrolo[2,3-*d*]pyrimidines

Considering the widespread applications of pyrrolo[2,3-*d*]pyrimidines, various methods have been reported in the literature for the synthesis of this interesting scaffold. Traditionally, two methodologies toward the synthesis of pyrrolo[2,3-*d*]pyrimidines prevail. One approach for the preparation of pyrrolo[2,3-*d*]pyrimidines is that the pyrimidine ring is constructed onto a suitably substituted pyrrole *via* two consecutive amide bonds, using urea derivatives, formamides, esters, or their retro-synthetic analogues, e.g., ortho-esters or isocyanates as C1-building blocks (route A, **Figure 10**).[23]

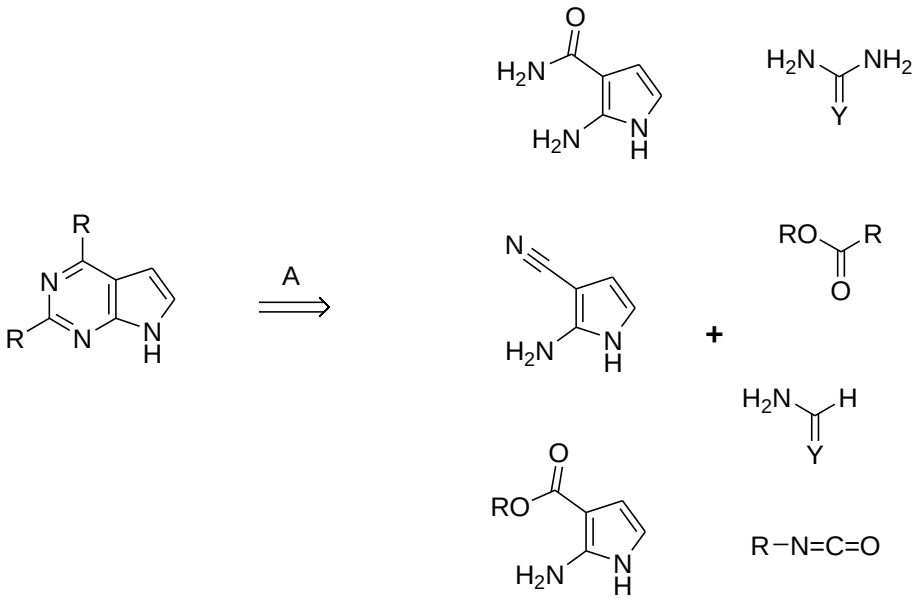


Figure 10: Methodology for the synthesis of the pyrrolo[2,3-*d*]pyrimidine scaffold.

The other approach is the synthesis of 4-aminopyrimidines, containing a substituent at the 5-position. In this case, the pyrrole ring is constructed onto the substituted six-membered pyrimidine ring to form the pyrrolo[2,3-*d*]pyrimidine scaffold. The amino group on the 4-position and the substituent at the 5-position of the pyrimidine undergo heteroannulation to form the desired fused pyrrole (route B, **Figure 11**).[23]

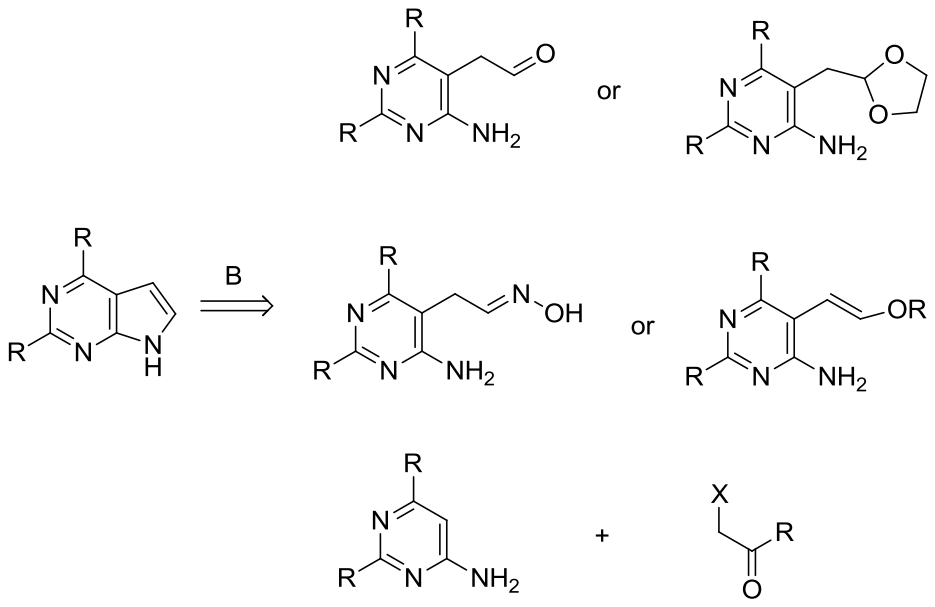
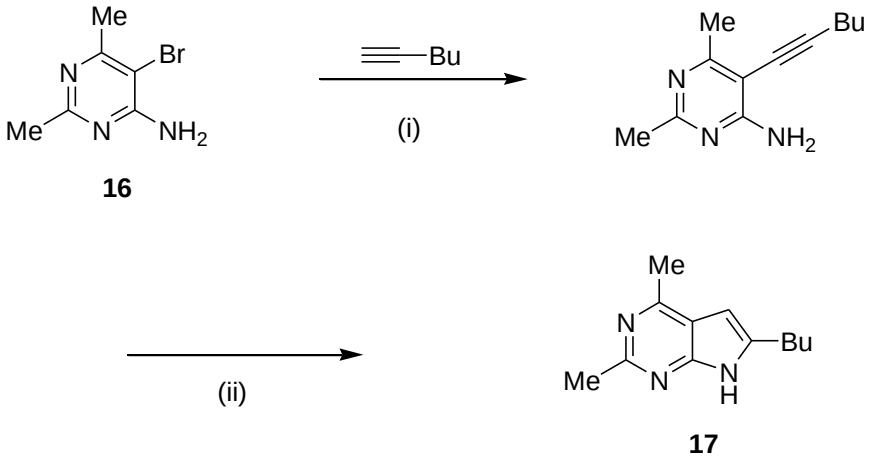


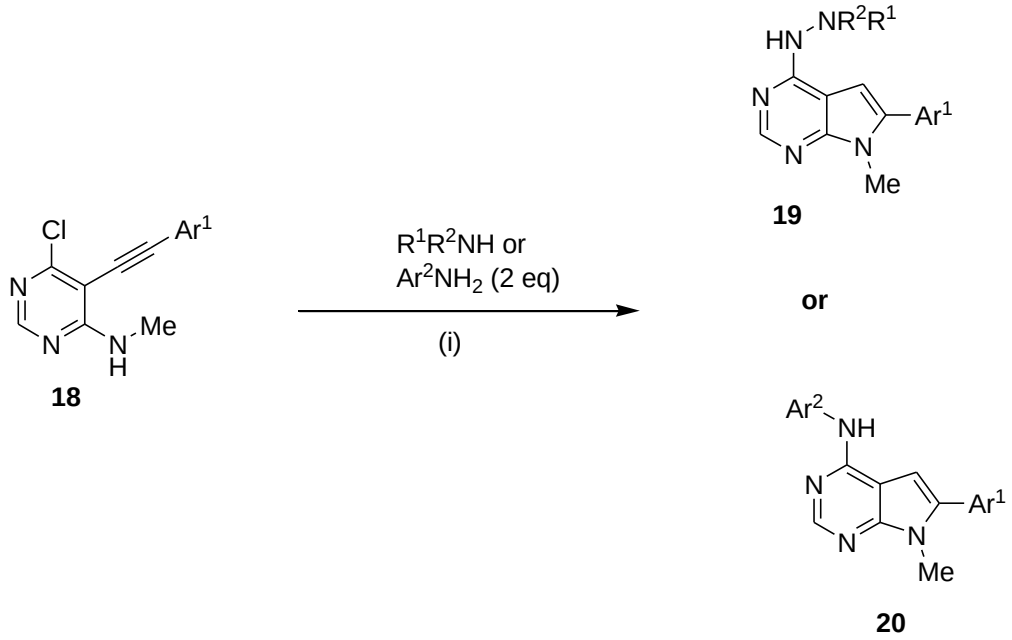
Figure 11: Methodology for the synthesis of the pyrrolo[2,3-*d*]pyrimidine scaffold.

Apart from the two synthetic approaches mentioned above, a variety of entries to the scaffold have been reported in the literature. For example, Knochel and co-workers reported the use of a base-mediated procedure for the synthesis of the 5-membered ring *via* a 5-*endo*-*dig* cyclization.[24] The 5-halogenated (bromo or iodo) derivative **16** was coupled with an alkyne by Sonogashira reaction, and the resulting product was treated with a strong base (potassium *tert*-butoxide) at room temperature, furnishing the substituted pyrrolo[2,3-*d*]pyrimidine **17** through a favoured 5-*endo*-*dig* cyclization in a yield of 61% (Scheme 1).



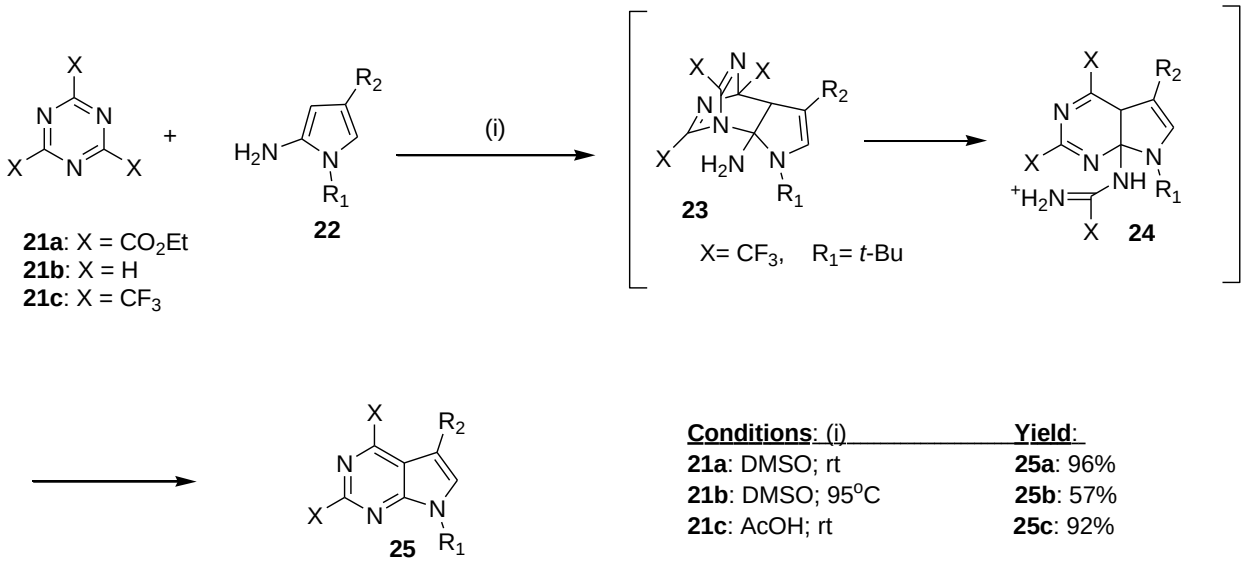
Scheme 1: Reagents and conditions: (i) Pd^0, (ii) KOtBu (1.3 eq), NMP, 60 °C, 5 h, 61%.[24]

Another method for the preparation of the scaffold from similar substrates includes a one-pot procedure in which the scaffold is constructed and a substitution reaction is effected. The synthesis involves the use of microwave irradiation. For example, when 6-chloropyrimidine **18** is treated with alkylamines, hydrazine, or anilines and potassium *tert*-butoxide in acetonitrile, the corresponding 6-substituted pyrrolopyrimidines **19** or **20** are obtained in moderate to good yields (Scheme 2).[25-26]



Scheme 2: Reagents and conditions: (i) KOtBu, CH_3CN, MW, 100 °C, 5-60 min, 27-99%.[25]

The pyrrolo[2,3-*d*]pyrimidine scaffold can also be made from 1,3,5-triazines with aminopyrroles *via* a retro-Diels−Alder approach.[27] This approach falls into the category discussed previously where the pyrimidine ring is constructed onto a substituted pyrrole. For example, when 1,3,5-triazine **21** undergoes a nonconcerted reaction with the *N*-alkylated 2- aminopyrrole **22**, to form the cycloadduct **23**, it eliminated a nitrile through a retro-Diels−Alder reaction.[28-29] The nitrile then reacted with the free amino group of the pyrrole, forming an amidinium ion **24**, which by final elimination and aromatisation produced the pyrrolo[2,3-*d*]pyrimidine **25** in good yields (Scheme 3).[29] Dang and co-workers have reported that electron-withdrawing substituents such as trifluoromethyl and esters increase the reactivity of the triazine, allowing the reaction to occur at room temperature.[31]



Scheme 3: Tandem Diels−Alder approach to form pyrrolo[2,3-*d*]pyrimidine scaffold.[27-31]

1.4 Methodology for functionalization of the pyrrolo[2,3-*d*]pyrimidine scaffold

An efficient method to introduce different substituents onto heterocyclic scaffolds is by direct carbon–carbon bond forming reactions. During the past 40 years, transition metals and directed *ortho* lithiation chemistry have been employed for the functionalization of the pyrrolo[2,3-*d*]pyrimidine scaffold.[32]

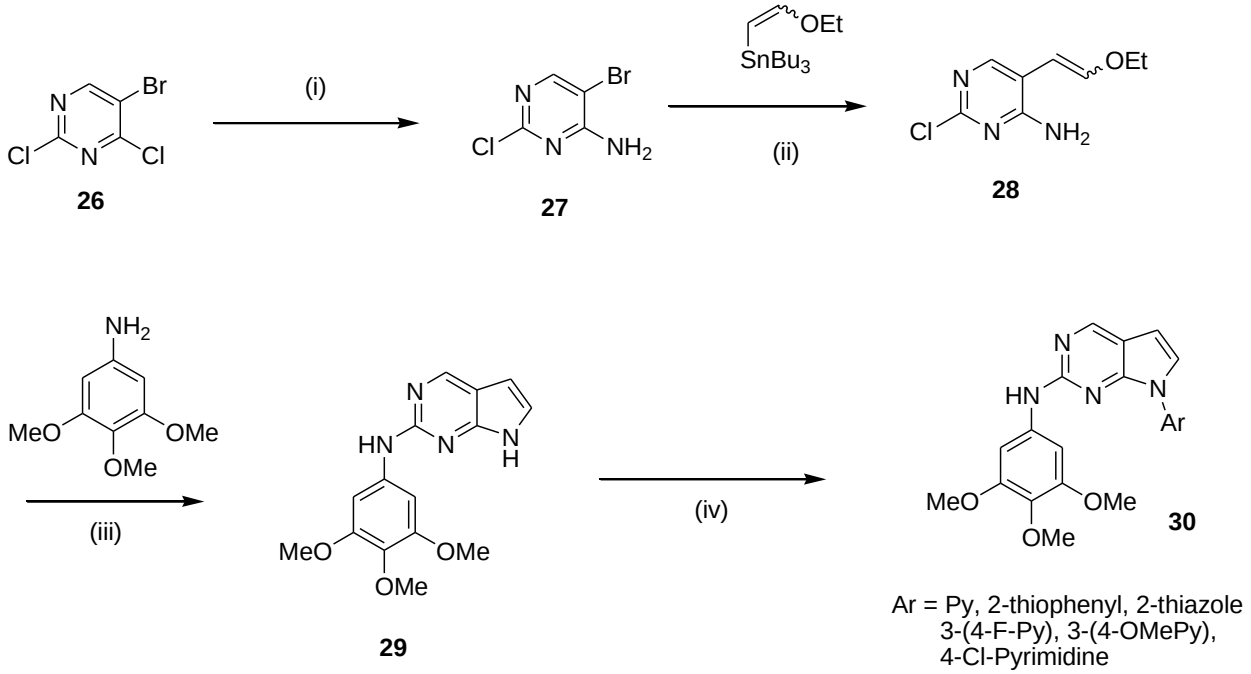
Palladium is one of the most widely used transition metals in the synthesis of functionalized pyrrolo[2,3-*d*]pyrimidines. There are a variety of palladium-catalyzed methods for the synthesis of substituted pyrrolo[2,3-*d*]pyrimidines. One such method is the Suzuki-Miyaura cross-coupling reaction between organoboron compounds and various aromatic halides or triflates in the presence of a suitable base. These coupling reactions offer several advantages of: (1) mild reaction conditions and high product yields; (2) use of a small amount of catalyst; (3) application in one-pot synthesis; (4) water stability; (5) tolerance of a wide range of functional groups; (6) easy separation of inorganic boron by products; (7) environmentally friendliness.[33]

Other coupling reactions, including Sonogashira, Stille and Heck reactions, have been extensively applied not only in academic laboratories, but also in industrial processes.[33] In cross-coupling reactions, cheap and readily available aryl and heteroaryl halides are typically used as starting materials.

In the section that follows, the use of these methodologies for the functionalization of the pyrrolo[2,3-*d*]pyrimidine scaffold will be discussed.

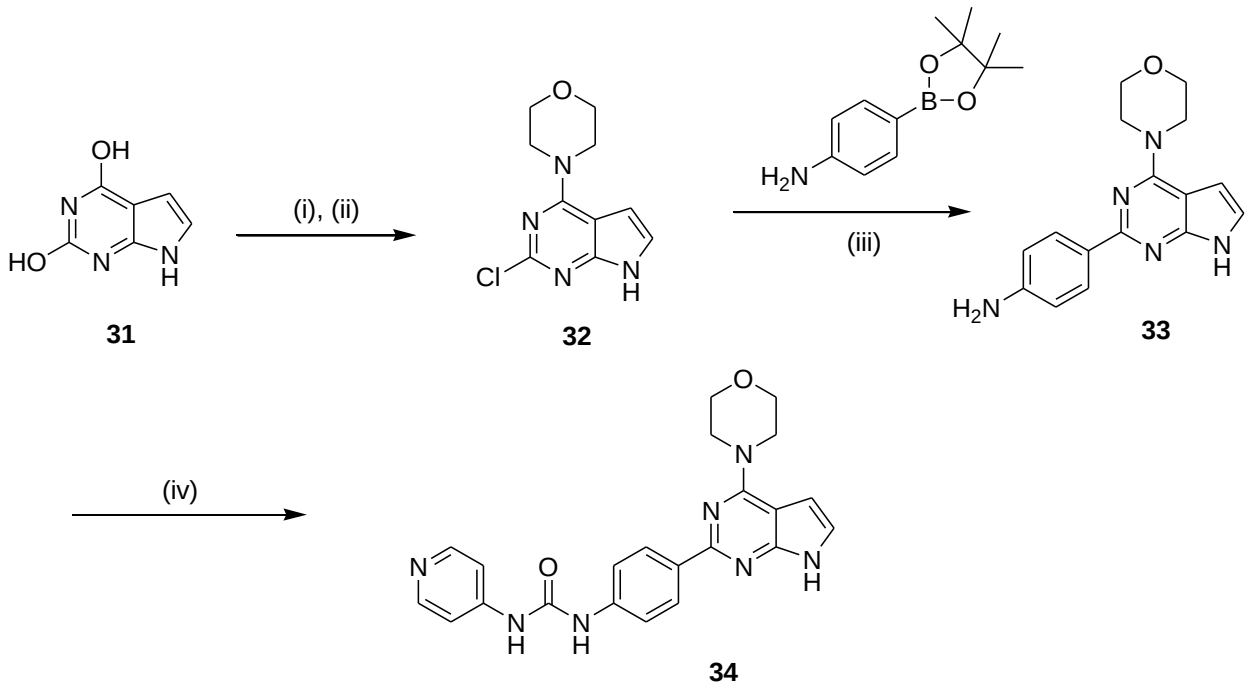
1.4.1 Synthesis of 2-substituted pyrrolo[2,3-*d*]pyrimidines

Numerous reactions for the functionalization of the pyrrolo[2,3-*d*]pyrimidines scaffold have been reported in the literature, but there are only a few on the synthesis of 2-substituted pyrrolo[2,3-*d*]pyrimidines. Choi and co-workers have reported the synthesis of a series of 2-substituted pyrrolo[2,3-*d*]pyrimidines as inhibitors of focal adhesion kinase (FAK).[34] FAK is a protein-tyrosine kinase found at the cell membrane that mediates focal adhesion, regulates processes such as cell migration and anchorage-dependent proliferation, and is thus related to metastasis. Their synthesis started with ammonolysis of trihalogenated pyrimidine **26** to afford amine **27**, followed by a palladium-catalysed Stille coupling reaction to give vinyl ether **28**. Cyclization in refluxing HCl solution in the presence of trimethoxyaniline furnished the key intermediate **29**, providing the 2-substituted pyrrolo[2,3-*d*]pyrimidine in excellent yield. Substituents at *N*-7 were introduced by Ullman-type couplings and gave compounds **30** in good to excellent yields (Scheme 4).[35]



Scheme 4: Reagents and conditions: (i) NH_3 (aq), THF, 25 °C, 94%; (ii) $Pd(PPh_3)_4$, toluene, reflux, 58%; (iii) HCl, BuOH, 90%; (iv) Ar-Br, CuI, K_3PO_4, *trans*-1,2-diamino-cyclohexane, 40-90%.[34-35]

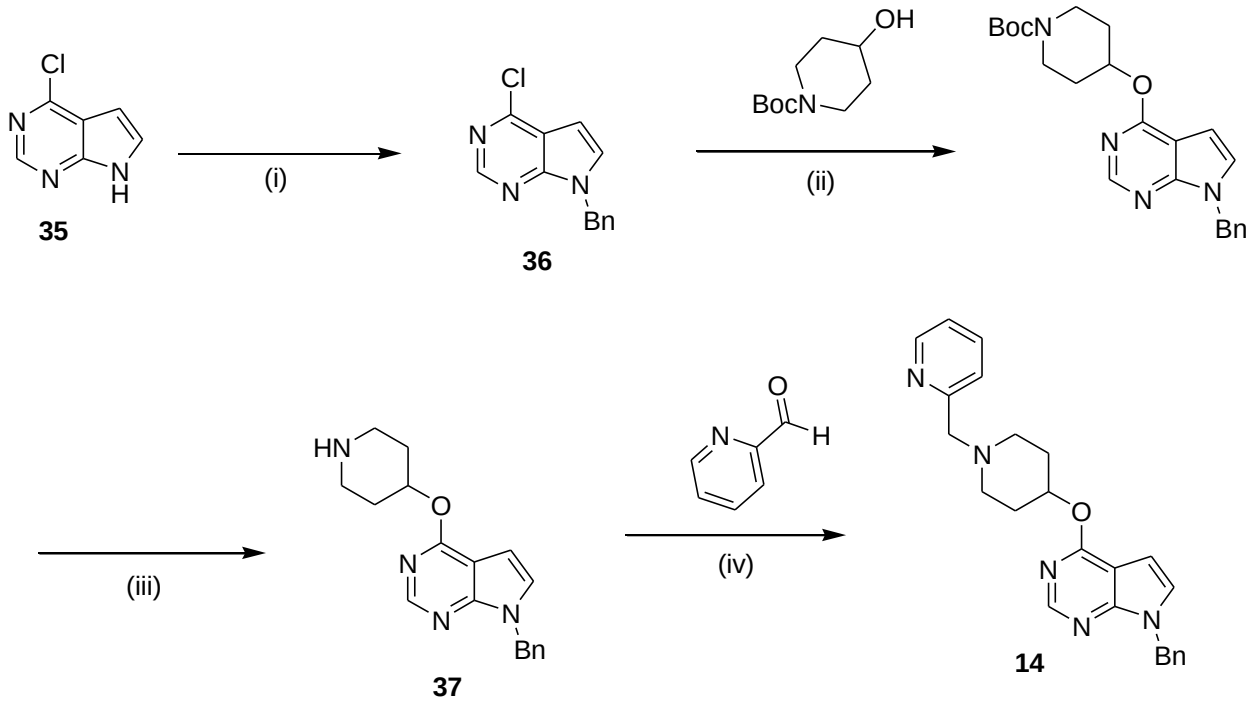
In other papers, palladium-catalyzed reactions of 2-chloropyrrolo[2,3-*d*]pyrimidine with arylboronic acids were investigated in order to obtain potent phosphatidylinositol-3-kinases (PI3Ks). Chen and co-workers have synthesised and evaluated a series of 4-(morpholin-4-yl)pyrrolo[2,3-*d*]pyrimidines, bearing different structural units in position 2 of the scaffold as PI3Ks inhibitors.[36] The synthesis started with chlorination of compound **31** by heating with phosphorus oxychloride ($POCl_3$) followed by selective nucleophilic substitution with the morpholino group at position 4 to give intermediate **32**. Suzuki cross-coupling of **32** with the pinacol ester of 4-aminophenylboronic acid in DME in the presence of $Pd(PPh_3)_4$ at 130 °C under microwave irradiation gave compound **33**. This was converted to the urea derivative **34** by reaction with triphosgene in the presence of a base, followed by treatment with appropriate amines (4-aminopyridine in this case) (Scheme 5).



Scheme 5: Reagents and conditions: (i) $POCl_3$, 120 °C, microwave, 30 min; (ii) morpholine (1.5 eq), Et_3N (3 eq), EtOH, rt, 68%; (iii) 4-aminophenylboronic acid pinacol ester (1.3 eq), $Pd(PPh_3)_4$ (5 mol %), DME, 2 M Na_2CO_3, 130 °C, microwave, 30 min, 83%; (iv) triphosgene (0.6 eq), Et_3N (3 eq), 4-aminopyridine (5 eq), CH_2Cl_2, rt, 12 h, 16%.[36]

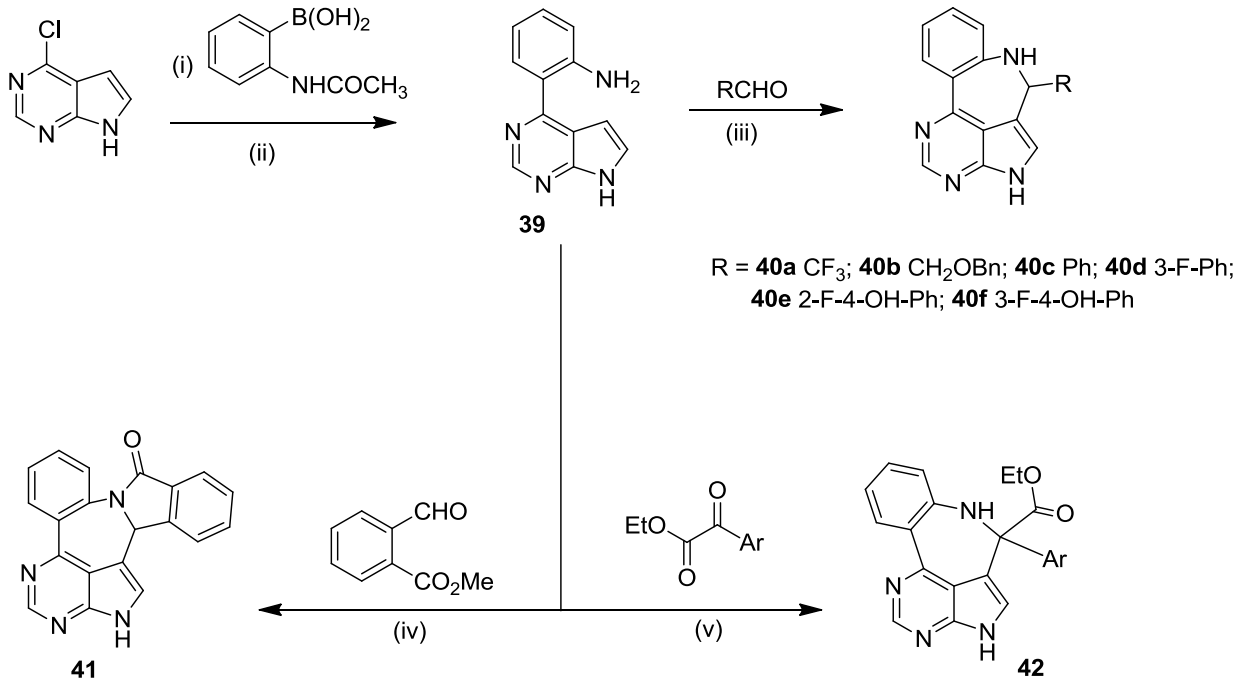
1.4.2 Synthesis of 4-substituted pyrrolo[2,3-*d*]pyrimidines

As previously mentioned in section **1.2.2**, Chakka and co-workers synthesised a series of 4-substituted pyrrolo[2,3-*d*]pyrimidines as inhibitors of voltage-gated sodium ion channel $hNa_v1.7$ for the treatment of chronic pain.[22] The synthesis of these types of compounds started with *N*-benzylation of commercially available 4-chloropyrrolo[2,3-*d*]pyrimidine **35** using benzyl bromide in acetonitrile under basic conditions to afford compound **36** in excellent yield (Scheme 6). Compound **36** was then subjected to nucleophilic aromatic substitution with Boc-protected 4-hydroxypiperidine using potassium *tert*-butoxide as the base, followed by Boc group deprotection using trifluoroacetic acid (TFA) in dichloromethane to afford compound **37** in a yield of 99%. Reductive amination with 2-formyl pyridine and sodium cyanoborohydride ($NaBH_3(CN)$) gave the desired 4-substituted pyrrolo[2,3-*d*]pyrimidine **14**.[22]



Scheme 6: Reagents and conditions: (i) BnBr, K_2CO_3, CH_3CN, rt, 2 h, 99%; (ii) KOtBu, CH_3CN, rt, 2 h, 56%; (iii) TFA, CH_2Cl_2, rt, 2 h, 99%; (iv) $NaBH_3(CN)$, 1,2-dichloroethane, rt, 16 h, 48%.[22]

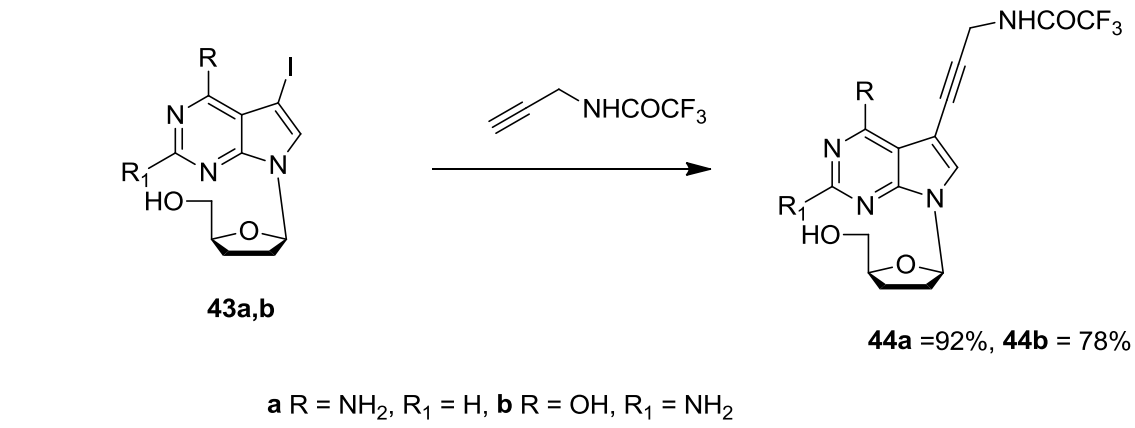
In 2010, Bennani and co-workers reported the synthesis of alternative analogues targeting JAK2. Their synthesis started from a Suzuki coupling of chlorinated pyrrolopyrimidines with 2-acetamidophenylboronic acid using $Pd(PPh_3)_4$ as the catalyst, followed by acyl deprotection with HCl to give the intermediate **39** (Scheme 7). Condensation of compound **39** with several aldehydes in methanol and HCl-dioxane was examined, affording tetracyclic compounds **40a-f** in good yields. Condensation of compound **39** with methyl *o*-formylbenzoate and ethyl 2-oxo-2-phenylacetates gave the amide **41** and the ester **42**, respectively (Scheme 7).[37]



Scheme 7: Reagents and conditions: (i) $Pd(PPh_3)_4$, K_2CO_3, DME, 130 °C, 18 h, 90%. (ii) 12M HCl, reflux, 84%. (iii) MeOH, 4M HCl-dioxane, 100 °C, 30 min, >90%; (iv) 4M HCl-dioxane, 100 °C, 30 min, >90%; (v) 4M HCl-dioxane, 100°C, 30 min, >90%.[37]

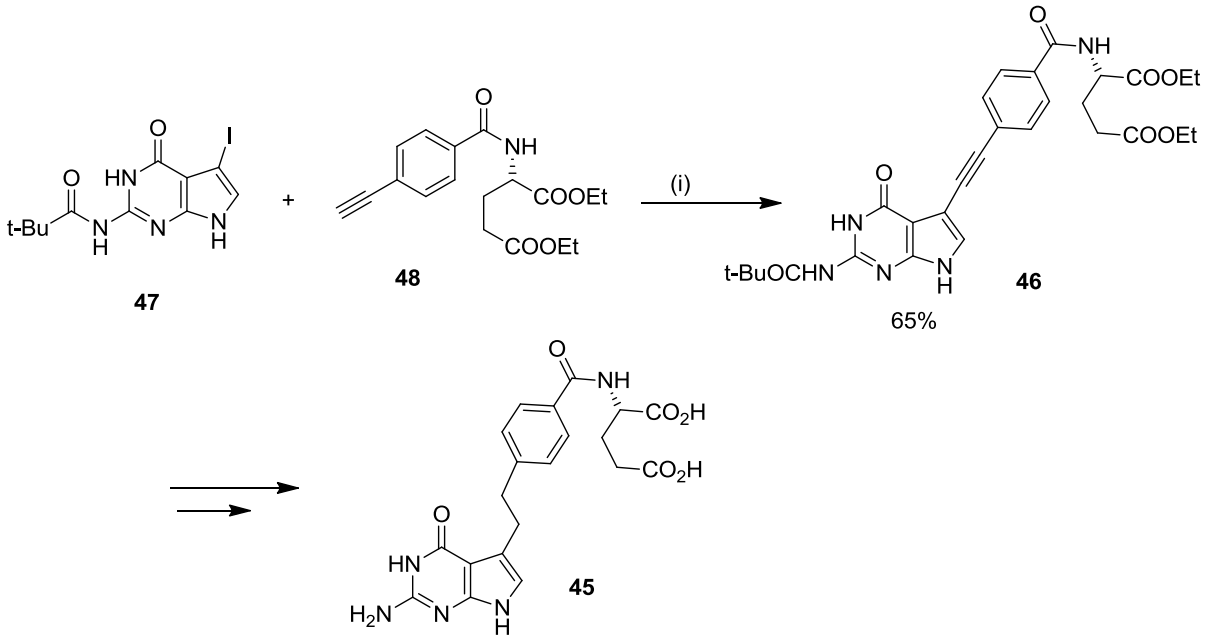
1.4.3 Synthesis of 5-substituted pyrrolo[2,3-*d*]pyrimidines

The first synthesis of 5-substituted pyrrolo[2,3-*d*]pyrimidine nucleosides using a palladium-catalysed cross coupling reaction was in 1989 by F. W. Hobbs.[38] Commercially available 5-iodopyrrolo[2,3-*d*]pyrimidine nucleoside analogues **43** were treated with *N*-(prop-2-yn-1-yl)trifluoroacetamide under Sonogashira reaction conditions in DMF and the desired 5-alkynylpyrrolo[2,3-*d*]pyrimidines **44** were obtained in good yields (Scheme 8). From their observation, the ratio of palladium(0) to copper(I) was found to be very significant. No reaction occurred when the ratio of Pd(0) to Cu(I) was 1:1. The best results were obtained when the Sonogashira cross coupling reaction was carried out in DMF and the ratio of Pd(0) to Cu(I) was 1:2.[38]



Scheme 8: Reagents and conditions: (i) cat. $Pd(PPh_3)_4$, CuI, NEt_3, DMF, rt, 4 h.[38]

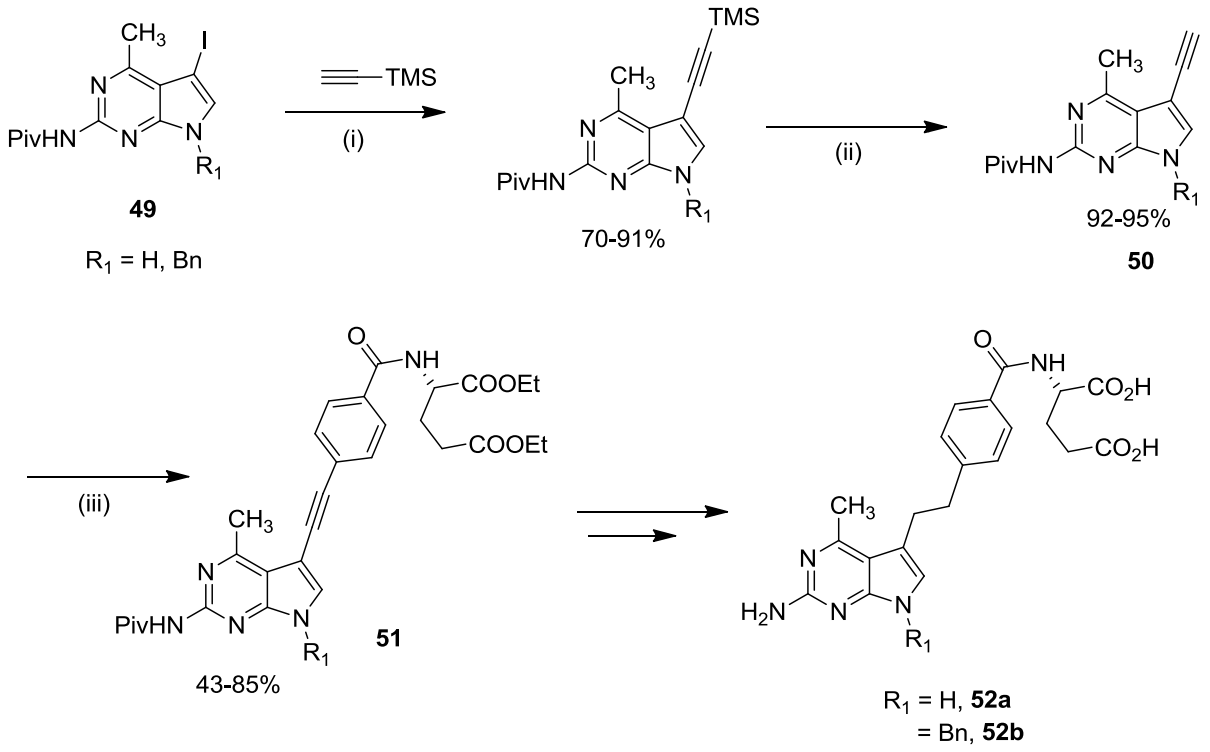
Aryl 5-substituted pyrrolo[2,3-*d*]pyrimidines have been synthesized as anticancer and antibacterial agents. Taylor *et al.* developed a synthetic approach for the potent thymidylate synthase (TS) and dihydrofolate reductase (DHFR) inhibitor Pemetrexed **45**. The Sonogashira reaction was used in the synthesis of the key intermediate **46** from 5-iodopyrrolo[2,3-*d*]pyrimidine **47** and diethyl *N*-(4-ethynylbenzoyl)-L-glutamate **48** (Scheme 9).[39]



Scheme 9: Reagents and conditions: (i) cat. $Pd(PPh_3)_4$, CuI, NEt_3, DMF, rt, 2 h.[39]

In 2000 Gangjee *et al.* reported an alternative method for the synthesis of Pemetrexed analogues.[40] 5-Iodopyrrolo[2,3-*d*]pyrimidines **49** were treated with trimethylsilylacetylene, and

the resulting compounds were subjected to a fluoride ion source to facilitate silyl deprotection to furnish 5-ethynylpyrrolo[2,3-*d*]pyrimidines **50**. Compounds **50** were then successfully coupled with 4-iodobenzoyl-L-glutamate under Sonogashira reaction conditions to give diesters **51** in good yields of up to 85%. Subsequent catalytic hydrogenation and hydrolysis afforded the corresponding antifolates **52a** and **52b** (Scheme 10).[40]

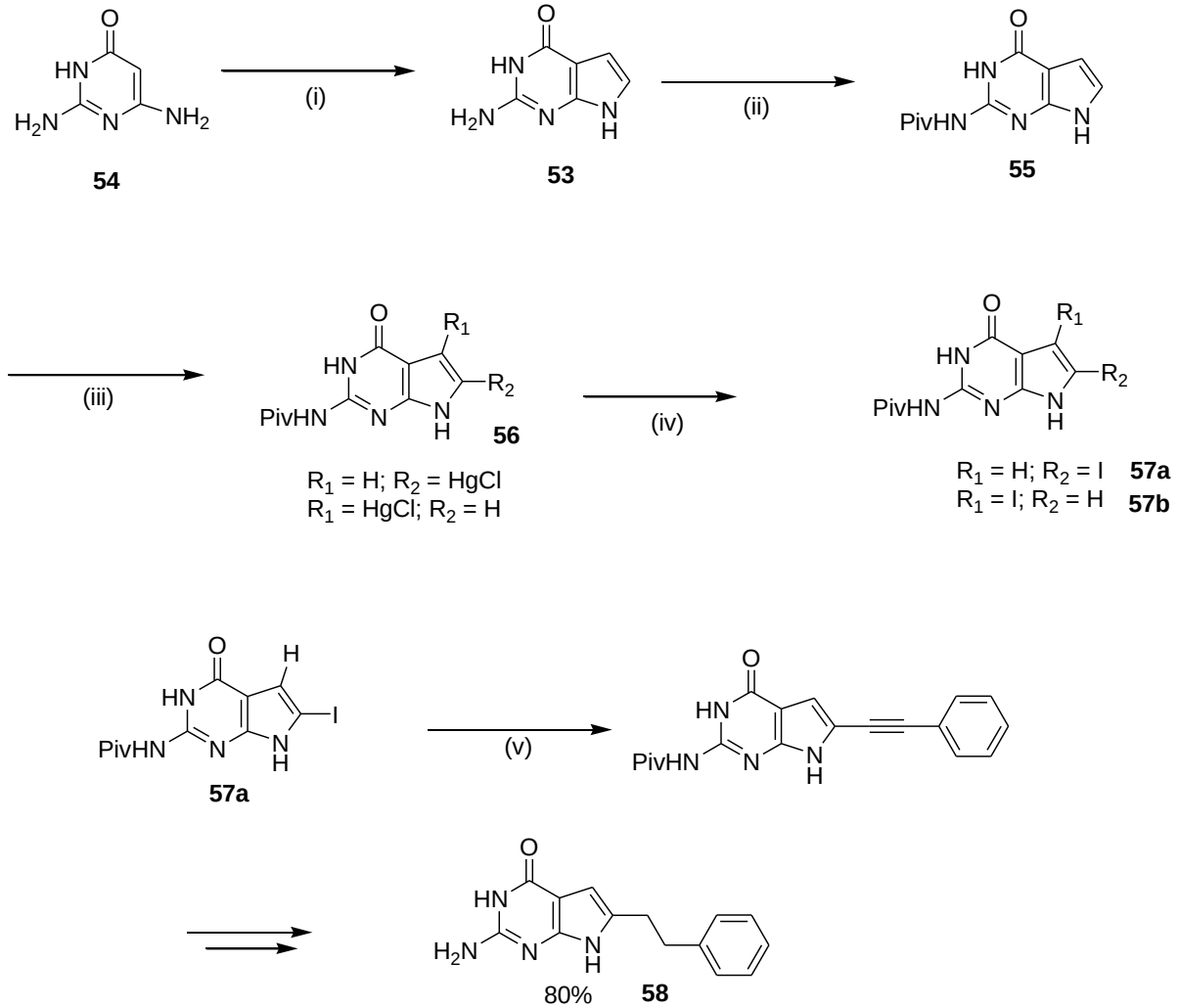


Scheme 10: Reagents and conditions: (i) cat. $Pd(PPh_3)_4$, CuI, NEt_3, DMF, rt; (ii) TBAF, THF, rt; (iii) diethyl *N*-(4-iodobenzoyl)-L-glutamate, cat. $Pd(PPh_3)_4$, CuI, NEt_3, THF, rt.[40]

1.4.4 Synthesis of 6-substituted pyrrolo[2,3-*d*]pyrimidines

There are many synthetic methods for the formation of pyrrolo[2,3-*d*]pyrimidines, but only a few methods are available for the preparation of 6-substituted pyrrolo[2,3-*d*]pyrimidines. However, to address this shortage, Gangjee *et al.* designed and synthesized a series of 6-substituted pyrrolo[2,3-*d*]pyrimidines from key intermediate **53** accessed from commercially available 2,4-diamino-6-hydroxypyrimidine **54** (Scheme 11).[41] Hence, compound **54** was treated with

chloroacetaldehyde in the presence of aqueous sodium acetate followed by treatment with pivaloyl chloride in pyridine, which resulted in the formation of **55**. Compound **55** was then subjected to chloromercuration using mercuric acetate in glacial acetic acid, followed by treatment with a saturated sodium chloride solution to give the desired product **56** as a mixture of regioisomers in 50% overall yield. The resultant mixture was treated with iodine in dichloromethane to afford the corresponding iodo derivatives **57a** and **57b**. Purification using silica gel column chromatography gave the desired 6-iodo compound **57a** in 64% yield.

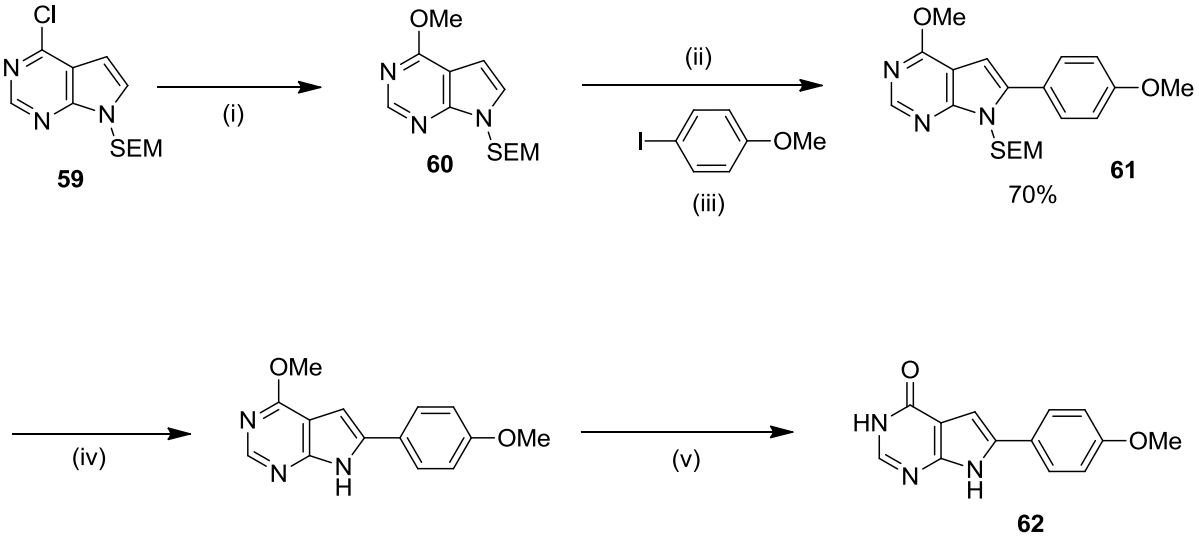


Scheme 11: Reagents and conditions: (i) $ClCH_2CHO$, NaOAc, H_2O, 100 °C; (ii) PivCl, pyridine, 120-130 °C; (iii) (1): $Hg(OAc)_2$, (2): NaCl, (iv) I_2, (v) Phenylacetylene, cat. $Pd(PPh_3)_4$, CuI, EtN_3, DMF, rt.[41]

The next step was a palladium-catalyzed Sonogashira coupling reaction of compound **57a** with the appropriate phenylacetylenes (one example shown in Scheme 11) followed by reduction of

the triple bond using 5% palladium on charcoal (Pd/C) as the catalyst under an atmosphere of hydrogen. A few drops of concentrated ammonium hydroxide were added to the reaction to prevent reduction of the pyrrole ring. Finally, deprotection of the 2-aminopivaloyl group was accomplished using 1 M sodium hydroxide to afford the desired 6-substituted pyrrolo[2,3-*d*]pyrimidine **58** in yields ranging from 44-80% (Scheme 11).[41]

Numerous C−H activation reactions have been reported to access functionalized pyrrolo[2,3-*d*]pyrimidines. C–H activation reactions are currently one of the newest fields in organic synthesis and they are becoming an essential tool in the modification of heterocycles including pyrrolo[2,3-*d*]pyrimidines. For example, Hocek and co-workers have synthesized 6-arylpyrrolopyrimidines by iridium-catalyzed C–H borylation of substituted pyrrolo[2,3-*d*]pyrimidine with iodoarenes.[42] Reaction of SEM-protected 4-chloropyrrolo[2,3-*d*]pyrimidine **59** with NaOMe in methanol gave the nucleophilic substitution product **60**, which was subjected to C–H borylation/Suzuki cross-coupling with 4-iodoanisole in a one-pot reaction to give the desired SEM-protected 6-arylated pyrrolo[2,3-*d*]pyrimidine **61** in 70% yield (Scheme 12).

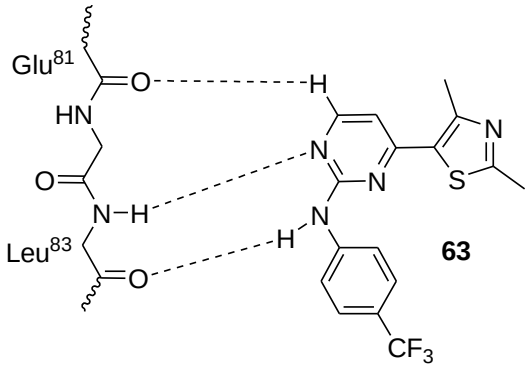


Scheme 12: Reagents and conditions: (i) NaOMe, MeOH, 18 h, 99%; (ii) Bispinacolatodiboron, $[Ir(COD)OMe]_2$ (5 mol%), dtbpy (10 mol%), THF, 80 °C; (iii) $Pd(dppf)Cl_2$ (5 mol%), K_2CO_3, DMF, 90 °C, 1 h; (iv) (1) TFA, rt, 1 h, (2) aq. NH_3, rt, 18 h, 90%; (v) TMSCl (5 eq), NaI (5 eq), CH_3CN, 80 °C, 18 h, 85%.[42-43]

Compound **61** was then SEM-deprotected and *O*-demethylated using trifluoroacetic acid (TFA) followed by aqueous ammonia and iodotrimethylsilane generated *in situ* (from TMSCl and NaI) in acetonitrile, respectively, to furnish 6-aryl-pyrrolo[2,3-*d*]pyrimidin-4-one **62** (Scheme 12).[43]

1.5 4,6-Diamino pyrimidines

Other heterocyclic compounds of interest in this project are 4,6-diaminopyrimidines. The pyrimidine nucleus is present in many kinase inhibitors. 2,4-Disubstituted pyrimidines are a class of compounds that have been extensively studied as inhibitors of diverse kinases, including CDKs, Aurora, p38, KDR, and Gsk3.[44] Crystallographic studies have shown that 2,4-dianilinopyrimidines typically form a hydrogen bonding interaction with the amino acids in the hinge region of many kinases using the pyrimidine N1 and the aniline NH at the C2 position (see structure **63** below; e.g for CDK2 enzyme in **Figure 12**).[45] In contrast, the 4,6-disubstituted pyrimidines represent a class of kinase inhibitors that has been less explored because they are usually not as potent as their corresponding 2,4-regioisomers, due to the lack of attaining the s-*cis* conformation required for the hinge region interaction.[46] However, they have been shown to display potent activity against the epidermal growth factor receptor (EGFR) for the treatment of a range of cancers.



63

Figure 12: Hydrogen-bonding interactions of 2,4-disubstituted pyrimidines with the backbone of enzyme residues Glu81 and Leu83 of CDK2.

EGFR is one of the first receptor tyrosine kinases to be identified by the pharmaceutical industry for drug development. This is due to its universal overexpression in a variety of tumours. Zhang

and co-workers at the Genomics Institute of the Novartis Research Foundation synthesised a library of 4,6-disubstituted pyrimidines as potent inhibitors of EGFR.[47] The 4,6-disubstituted pyrimidine shown below (**Figure 13**) has displayed selectivity against EGFR when evaluated against a panel of 55 recombinant kinases at a concentration of 10 μM. This 4,6-disubstituted pyrimidine inhibitor possesses an enzymatic IC_{50} value of 21 nM against EGFR kinases *in vitro* and blocks receptor autophosphorylation in cells. Inhibitor **64** was coincidentally discovered using a cell-based reporter gene assay (RGA) for modulators of protein stability. Zhang and co-workers have hypothesized that the EGFR selectivity of inhibitor **64** results from its ability to form three hydrogen-bonding interactions with the hinge region while occupying a hydrophobic pocket made accessible due to the small gatekeeper threonine 766. This was observed using the known EGFR co-crystal structures. In addition, compound **64** displayed strong inhibition of two EGFR mutants associated with a clinical response to gefitinib: L861Q (IC_{50} = 4 nM) and L858R (IC_{50} = 63 nM). Unfortunately, 4,6-disubstituted pyrimidine compound **64** showed a much weaker activity against Her 4 (IC_{50} = 7640 nM).[47]

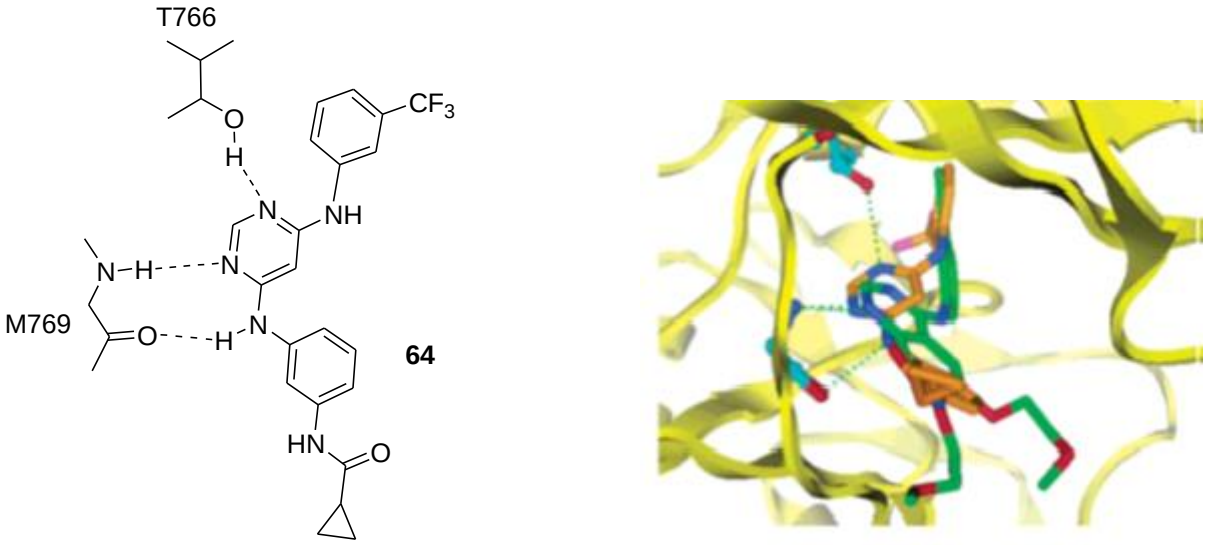
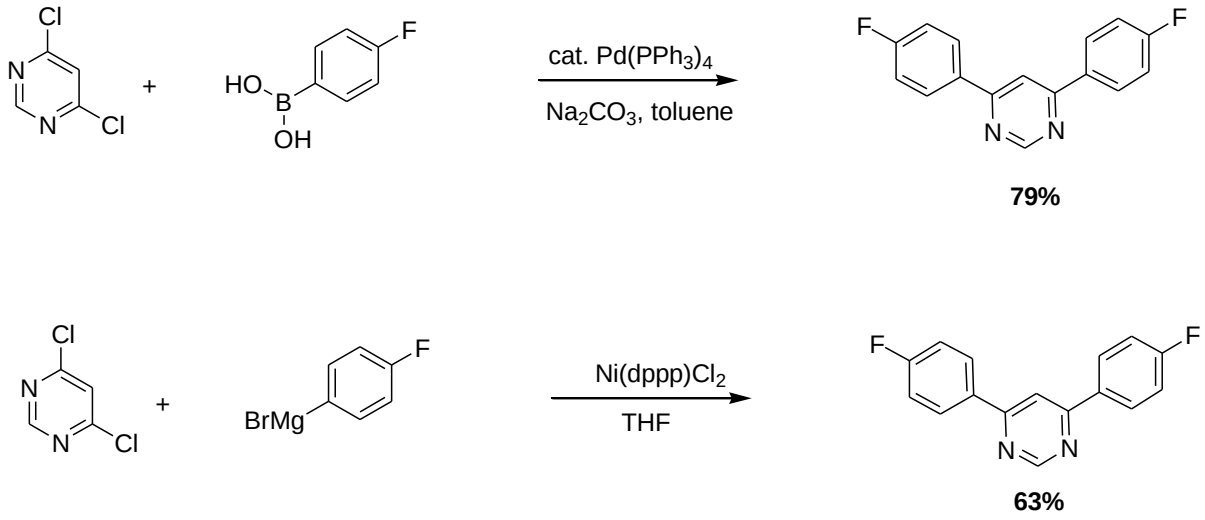


Figure 13: Inhibitor **64** bound to the ATP site of the EGFR kinase domain (left). Superimposition of inhibitor **64** with erlotinib in the ATP site of EGFR kinase domain (right).

In the following paragraphs, several methods used to prepare disubstituted pyrimidines will be explored.

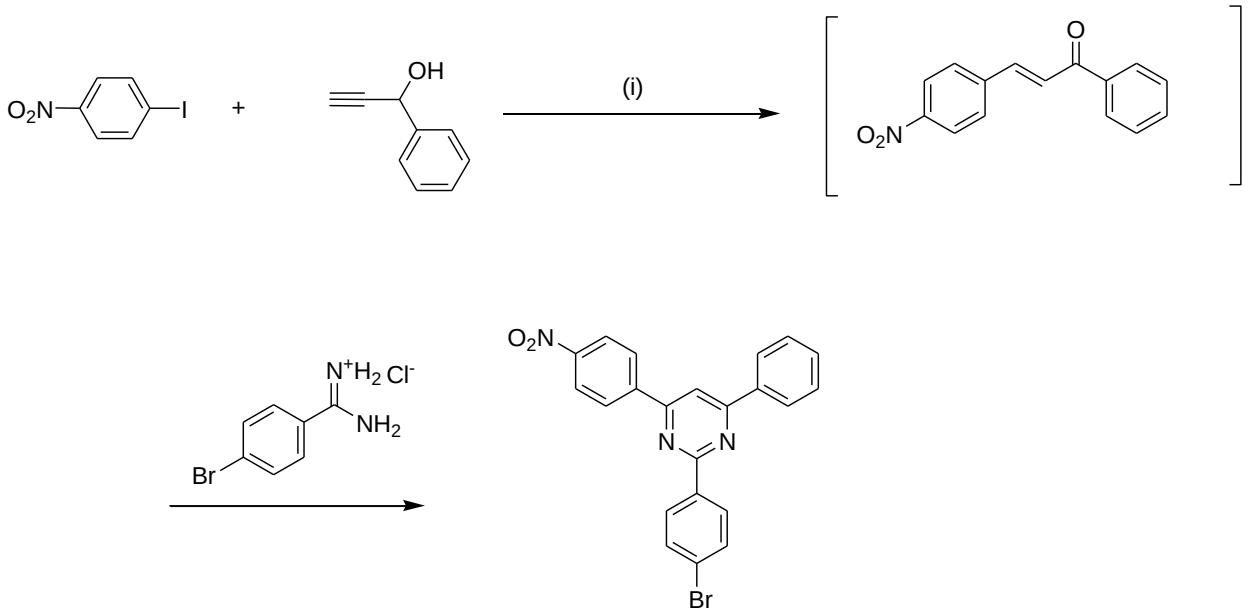
1.5.1 Preparations of substituted pyrimidine derivatives using metal-catalysed reactions

Palladium-catalyzed Suzuki-Miyaura cross-coupling reactions have been widely used in organic chemistry for the formation of carbon-carbon bonds. Of particular relevance to this project, Qing and co-workers have used the Suzuki cross coupling reaction for the preparation of 4,6-disubstituted pyrimidine derivatives.[48] When 4,6-dichloropyrimidine was treated with two equivalents of a boronic acid under Suzuki coupling conditions, using $Pd(PPh_3)_4$ as the catalyst, the expected 4,6-disubstituted pyrimidines were afforded in excellent yields (Scheme 13). However, when the same reaction was performed under Kumada cross coupling conditions, using $Ni(dppp)Cl_2$ in THF, the desired product was obtained in slightly lower yield.[48]



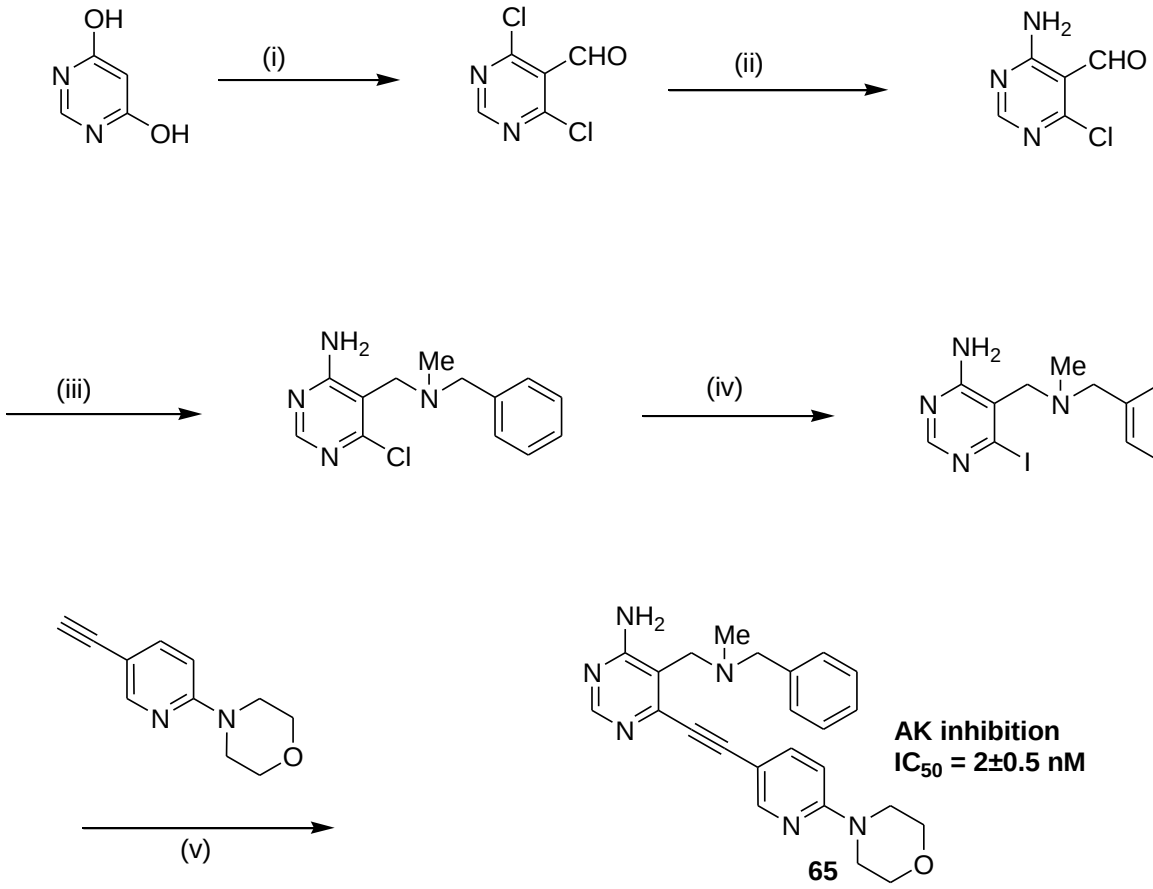
Scheme 13: Synthesis of 4,6-disubstituted pyrimidines *via* Suzuki and Kumada coupling reaction.[48]

In other example, 2,4,6-tri(hetero)aryl-substituted pyrimidines were synthesised using a one-pot palladium-catalysed reaction by Muller and co-workers (Scheme 14).[49] The authors found that palladium catalysed reaction of an electron-poor (hetero)aryl halide and a terminal propargyl alcohol proceeds *via* a coupling-isomerization sequence. Subsequent cyclocondensation reaction of this intermediate with an amidinium salt furnishes the 2,4,6-tri(hetero)aryl-substituted pyrimidines in moderate to excellent yields.[49] It was found that the electron-withdrawing nature of the (hetero)aryl halide is critical for the successful coupling-isomerization step.



Scheme 14: Reagents and conditions: (i) 2% $Pd(PPh_3)_2Cl_2$, 1% CuI, EtN_3, THF, reflux, 56%.[49]

Apart from using the above-mentioned reactions to access substituted pyrimidine derivatives, their preparation could also be achieved over a number of steps which include the Sonogashira coupling reaction. Gomtsyan and co-workers have reported the synthesis and biological activity of substituted pyrimidines as potent adenosine kinase (AK) inhibitors.[50] Adenosine is an extracellular purine nucleoside which controls many physiological processes. Intra- and extracellular adenosine concentrations can be controlled by the inhibition of adenosine kinase.[51-53] Gomtsyan *et al.* have reported the synthesis of adenosine kinase inhibitors that contain a 4-amino-6-[6-(4-morpholino)-3-pyridinylethynyl]pyrimidine core.[50] Bennet and co-workers also reported that the substituent at the 5-position of the pyrimidine ring plays an important role for adenosine kinase activity because it is responsible for the hydrophobic interactions with the protein.[54-57] Compounds with 2- to 3-atom linkers in the 5-position exhibited the highest AK inhibition. For example compound **65** with a 3-atom linker exhibited good efficacy (ED_{50} = 5-7 μM/kg) in an animal model. The substituted pyrimidine derivatives were obtained in moderate to good yields (Scheme 15).



Scheme 15: Reagents and conditions: (i) $POCl_3$, DMF, 1 h, 0 °C, stir 30 minutes at rt, and then reflux, 3 h, 55%. (ii) NH_3, toluene, 50-60 °C, 0.5 h, TLC monitored, 67%. (iii) *N*-Methylbenzylamine, AcOH, $NaB(OAc)_3H$, CH_2Cl_2, rt, 15 h, 46%. (iv) NaI, 40% HI, 70 °C, 10 minutes, and then Na_2CO_3 work-up, 67%. (v) cat. $Pd(PPh_3)_2Cl_2$, CuI, MeCN-Et_3N, rt, 1.5 h, 62%.[50,54-57]

1.6 Closing remarks

The pyrrolo[2,3-*d*]pyrimidine core represents an interesting scaffold for drug discovery, owing to its prevalence in a number of biologically active compounds including antibiotics, anti-inflammatory agents, antiviral agents and anticancer agents. The pyrrolo[2,3-*d*]pyrimidine scaffold makes the same hydrophobic and hydrogen-bonding contacts with the hinge region of kinases as the adenine ring of adenosine triphosphate (ATP), hence the development of new effective analogues that are affordable can be achieved by modification of the scaffold. 4,6-Diaminopyrimidines have also been shown to act as inhibitors of diverse kinases, including

CDKs, Aurora, p38, KDR, and EFGR. As discussed above, there are efficient methods for the preparation and modification of these scaffolds. We planned to use similar approaches in our synthesis to generate a library of compounds containing both the pyrrolo[2,3-*d*]pyrimidine and 4,6-diaminopyrimidines scaffolds and evaluate them for antiplasmodial activity, as will be outlined later in the thesis.

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CHAPTER 2: MALARIA TREATMENT, KINASES AND KINASE INHIBITORS

2.1 Introduction

Malaria, one of the most significant infectious diseases in the world, is caused by the protozoan parasite of the genus *Plasmodium*. In 2016, the World Health Organization (WHO) reported that about 3.2 billion people are at risk of contracting malaria, and close to 216 million cases of malaria and more than 400,000 deaths occurred in the same year. This life-threatening infectious disease is caused by five different *Plasmodium* species, and among them *Plasmodium falciparum* is documented to be the most severe form of malaria and causes the most human infections.[1-4]

The principal way in which malaria spreads from one person to another is by the bites of female mosquitoes of the *Anopheles* species. There are more than 480 species of *Anopheles* mosquitoes, but only about 50 species are capable of transmitting malaria. In the human bloodstream, the sporozoites migrate and replicate to yield merozoites (**Figure 14**). On rupture of the host cells, the merozoites invade red blood cells (RBCs). A cycle of asexual replication occurs, which results in the release of increasing numbers of merozoites into the bloodstream every 48 hours. Some of the merozoites in these cells undergo sexual replication resulting in the formation of male and female gametocytes, which circulate in the bloodstream.[5-7]

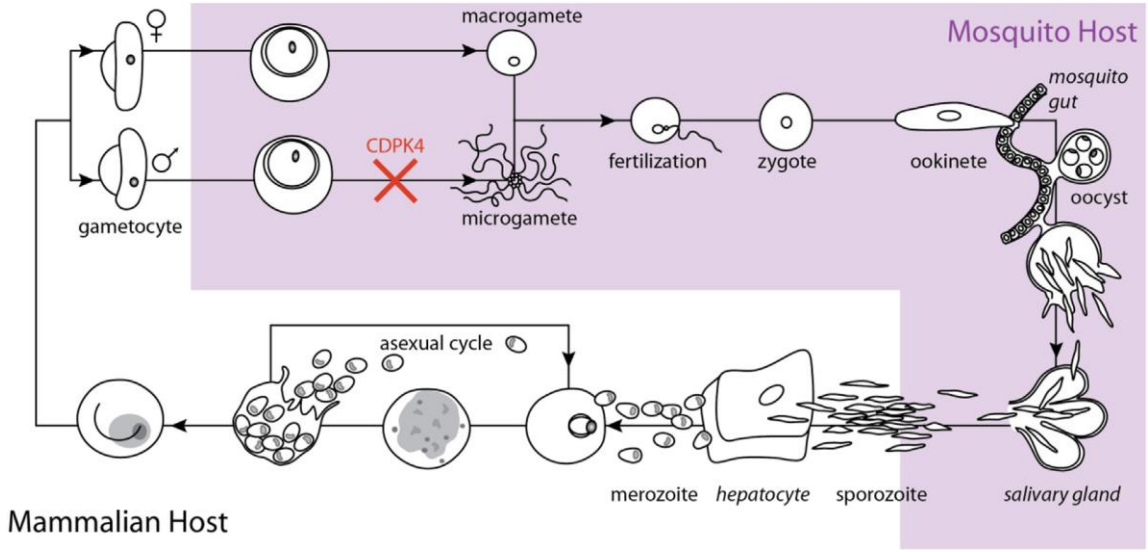


Figure 14: A pictorial illustration of the life cycle of the malaria parasite.[7]

2.2 Distribution of the malaria parasite

Malaria is distributed worldwide, depending mainly on climatic factors such as temperature, rainfall and humidity. The malaria parasite is transmitted in tropical and subtropical regions, where the *Anopheles* mosquitoes can survive, multiply and complete their growth cycles. Generally, at low temperatures, *P. falciparum* cannot complete its growth cycle in the *Anopheles* mosquito, thus transmission is not possible.[8] The highest malaria transmission is found predominantly in sub-Saharan Africa (**Figure 15**).[9]

Since 2000, South Africa has been successful in substantially reducing the burden of malaria morbidity and mortality. Within South Africa's borders, malaria transmission is encountered in north-eastern KwaZulu-Natal and low altitude regions of Limpopo and Mpumalanga. Malaria transmission normally occurs between the months of September and May (rainy season).[10]

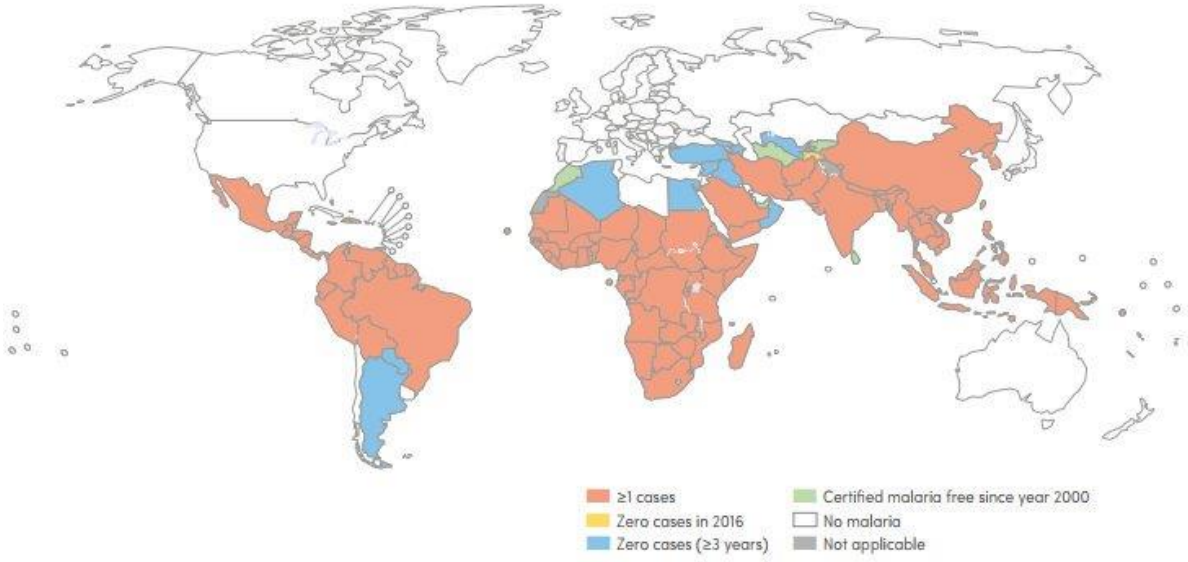


Figure 15: Global distribution of malaria (taken from WHO Malaria Report, 2017).[11]

2.3 Malaria control and prevention

There have been various strategies adopted to combat malaria in many parts of the world including vector control; the use of bednets, indoor residual spraying (IRS) of insecticides and more recently by the promising vaccine development. Mosquito bednets are effective as they

form a physical barrier between the *Anopheles* mosquitoes and man, thus preventing malaria transmission. IRS is applied by spraying the interior surfaces of houses with insecticides and reducing the life span of female mosquitoes. This strategy continues to be of significant value because it slows malaria transmission.[12] Unfortunately, the development of vector resistance to insecticides has been identified, and as a result, malaria vaccines have gained much attention. They target different stages of the malaria life cycle. There are so- called transmission blocking vaccines, which interrupt the transmission of the parasite to mosquitoes in the gamete-stage. Pattaroyo *et al*. developed a number of vaccines that underwent large-scale clinical trials,[13] one of which was the three-component vaccine (SPf66) which unfortunately did not show clinical effectiveness.[14-15] RTS'S [central repeat region of the *Plasmodium falciparum* (R), T-cell epitopes (T), hepatitis B surface antigen (S)] is a pre-erythrocytic vaccine which prevents the infection of red blood cells, the cause of severe forms of malaria. The RTS'S is now in Phase III clinical trials, and it has shown a 50% protective efficiency against clinical disease.[16] However, malaria eradication continues to rely heavily on the use of effective antimalarial drugs.[17]

2.3.1 Antimalarial drugs and resistance

Several drugs have been used for the treatment of malaria since the 17th century. Most existing antimalarial drugs have lost their effectiveness due to the emergence of drug resistance. This has made malaria control and treatment more complicated.[18] Quinine (**66, Figure 16**) is an alkaloid which was isolated as the active compound from the bark of the cinchona tree in 1820. It was used widely for the treatment of malaria until World War II, and is still used today for the treatment of cerebral malaria. Due to toxicity issues, efforts were made in the search for synthetic antimalarial drugs that were structurally related to quinine. The following quinoline derivatives were synthesised and have found widespread use for the treatment of malaria; mefloquine (**67**) chloroquine (**68**) and amodiaquine (**69**). Chloroquine (**68**) has been widely used for both the treatment and prophylaxis of *P. falciparum* malaria since its discovery in 1946. Unfortunately, drug resistance has made this antimalarial largely ineffective. *P. falciparum* resistance to chloroquine first emerged in Thailand in 1957. Its resistance was seen in South America and sub-Saharan Africa in the 1970s. Many factors caused resistance to chloroquine, these include uncontrolled long-term treatment regimes. However, in countries where

chloroquine is still effective, it is used for the treatment of *P. vivax* malaria.[19-22] Mefloquine (**67**) has recently been a topic of media attention due to occurrence of serious psychiatric side effects in some patients. For example in 2018, it was reported that a number of Australian Defence Force personnel deployed in East Timor had suffered such serious side effects. Mefloquine has a long half-life *in vivo*; hence it is an excellent prophylactic drug amongst non-immune travellers because of the once-a-week dosage.[23] Amodiaquine (**69**) belongs to the same family as chloroquine, but it is less efficient, more expensive and more toxic than chloroquine. Its use has been limited since the mid 1980s owing to the appearance of occasional agranulocytosis in travellers taking this drug.[24]

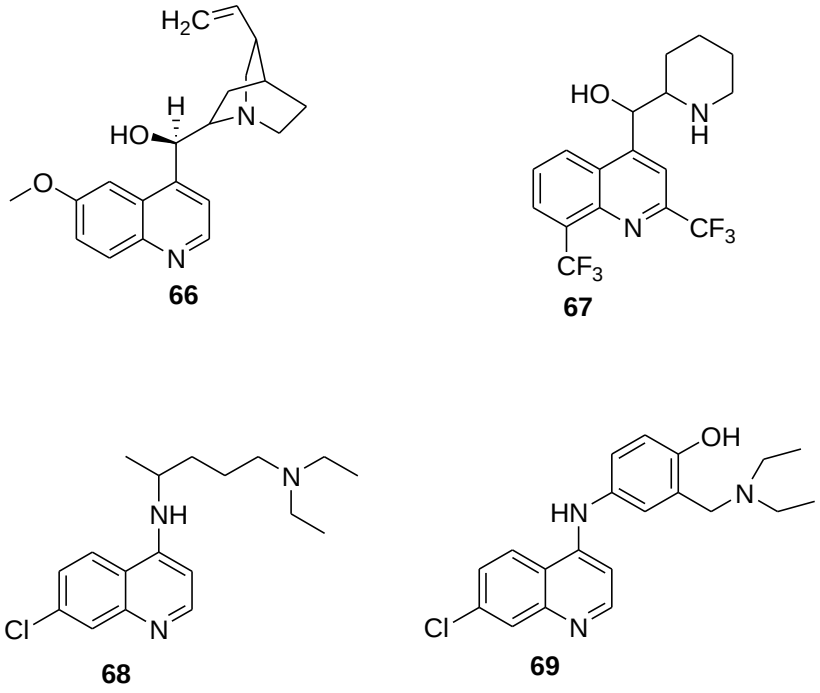


Figure 16: Structure of antimalarial drugs.

Antimalarial antifolates are another class of compounds that has the potential for both the treatment and prevention of malaria and other infectious diseases, as well as cancer. These interesting compounds are also known as folate antagonists and they belong to the group of nucleic acid biosynthesis inhibitors.[25] Proguanil (**70, Figure 17**) was the first antimalarial antifolate that was discovered in 1945 and was found to be more active than quinine against

avian malaria. Proguanil is a prodrug which is metabolized *in vivo* to its triazine form, cycloguanil (**71**) which is an inhibitor of the parasite dihydrofolate reductase (DHFR). Pyrimethamine (**72**) is a 2,4- diaminopyrimidine and belongs to a family of class II antifolates. In 1940, Hitchings and co-workers reported the synthesis of this class of compounds as antimalarials and tested them as analogues of folic acid in the treatment of tumours. Pyrimethamine has been widely used in combinations with sulfadoxine (**73**), which inhibits a different enzyme, DHPS, in the treatment of malaria. Pyrimethamine-sulfadoxine, known as PS or Fansidar, has been highly effective in most malaria endemic areas. However, PS has lost its effectiveness in many countries due to the development of drug resistance of *P. falciparum* caused by point mutations in the target enzymes DHFR and DHPS.[26-28]

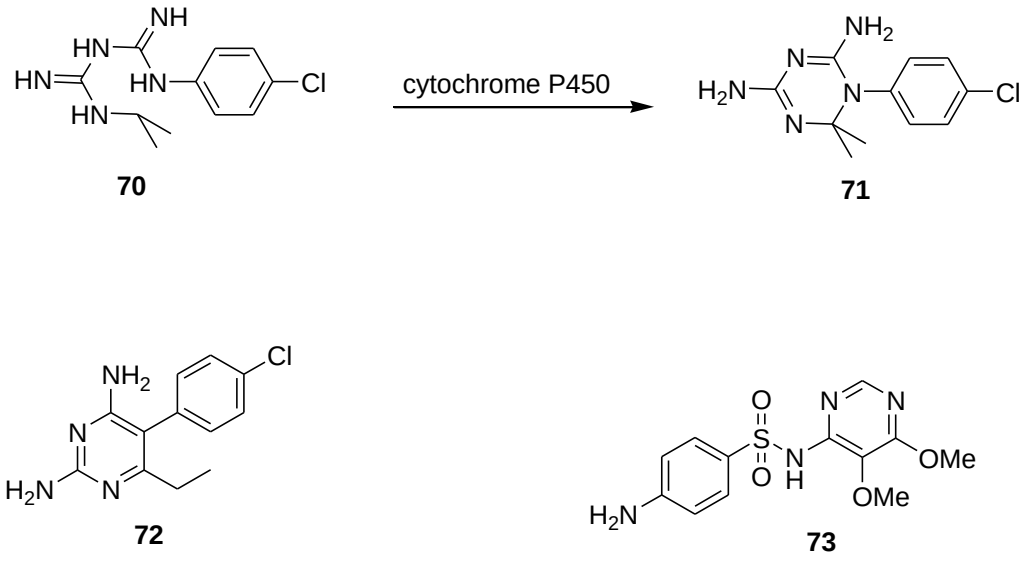


Figure 17: Structures of antimalarial antifolates.

Artemisinin (**74, Figure 18**) is a sesquiterpene lactone (three isoprene units bound to cyclic organic esters) first isolated from a sweet wormwood plant (*Artemisia annua*) in the late 1970s. It has been found to have potent activity against *Plasmodium* species. Studies have shown that artemisinin and its derivatives act by release of free radicals into the parasite vacuoles. They exhibit higher rates of reduction of parasitemia, hence they can be used for the treatment of both complicated and uncomplicated malaria, unlike other antimalarials.[29] Semisynthetic artemisinin

derivatives that have been developed include artemether (**75**), arteether (**76**) and artemisone (**77**). Artemisone is the most recently developed semisynthetic artemisinin derivative, with the best pharmacokinetic/pharmacodynamic profile. It exhibited, compared to other derivates, better efficacy and lack of neurotoxicity in preclinical testing.[30] This analogue is not metabolized *in vivo* to the bioactive compound, dihydroartemisinin (**78**), like the other artemisinin derivatives. Artemisinin and its derivatives have been used in monotherapy, but because of their short duration of action there is a high rate of disease relapse associated with these drugs. Hence, they are usually used in combination therapy with other longer-acting antimalarial drugs. Unfortunately in some areas of the world, the parasite has developed resistance to artemisinin and its derivatives.[31] At this stage, the resistance is not widespread.

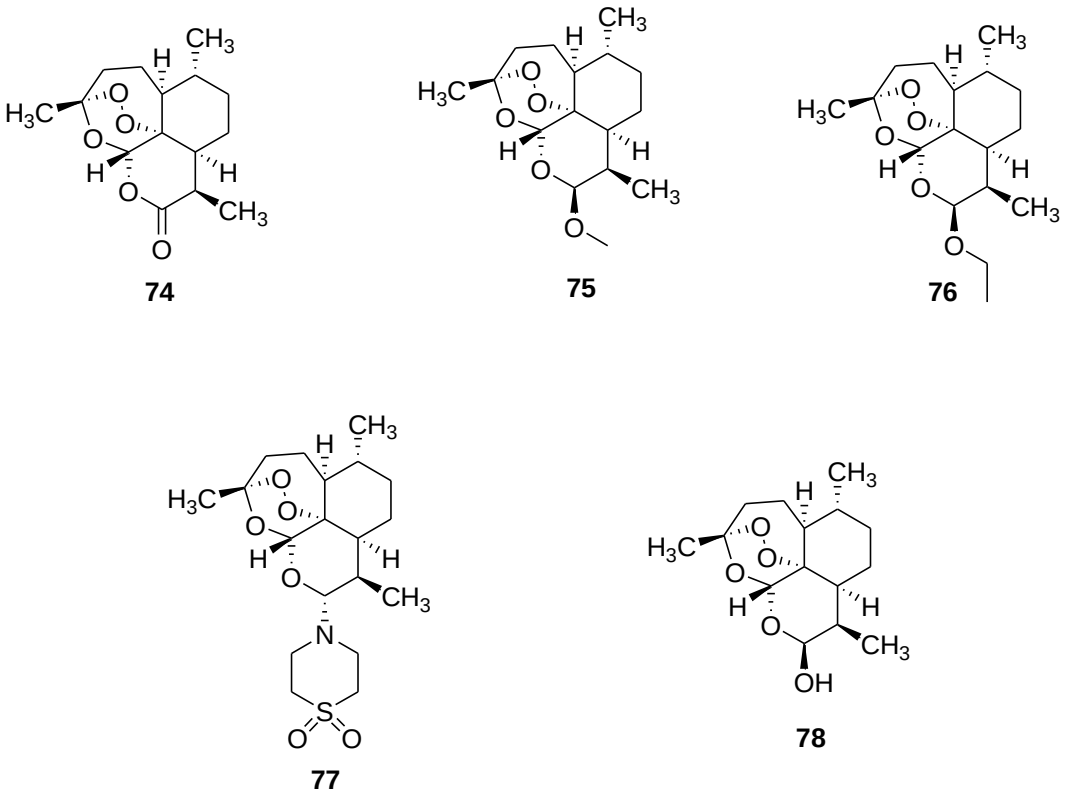


Figure 18: Artemisinin and its derivatives.

Most antimalarial drugs target the asexual stage of malaria, but not the gametocytes. Gametocytes are sexual stages that are formed in the human erythrocytes, which are critical in

the spread of the disease. Artemisinin combination therapy (ACT, e.g artemether **75** and lumefantrine **79** shown below, **Figure 19**) and primaquine **80** have antigametocyte activity. ACT only kills immature gametocytes, while primaquine kills mature but not immature gametocytes. The two drugs are therefore imperfect for preventing malaria transmission to mosquitoes.[32] Hence, new strategies are urgently needed to disrupt parasite reproduction, thus breaking the malaria life cycle.

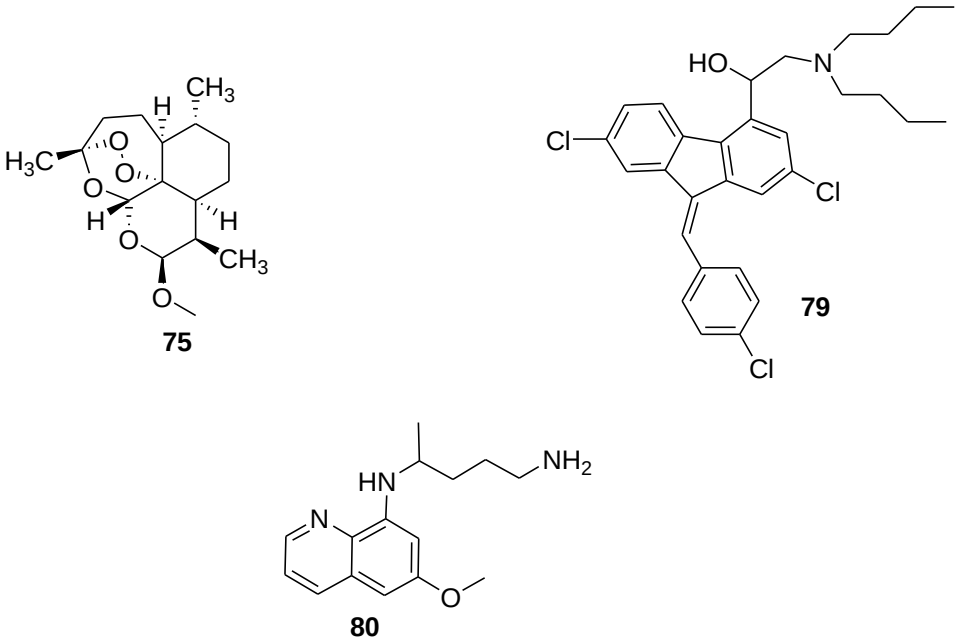


Figure 19: Structures of artemether **75**, lumefantrine **79** and primaquine **80**.

The development of new, effective, affordable antimalarial drugs needs a deep understanding of the basis of resistance. Current antimalarials drugs are rapidly losing their effectiveness against *P. falciparum* due to resistance. Hence, there is an urgent need for new drugs that are cheap, safe and effective against both wild type and mutant strains of *Plasmodium* species. The new drugs should ideally be able to block transmission of the parasite from one infected person to another via the mosquito and thus break the cycle of infection.

The *P. falciparum* kinases have been shown to be essential for both sexual and asexual phases of the multifaceted parasitic life cycle. Hence, they provide potential targets that could be pursued in the search for antimalarials.[33-34] Interestingly, it has been shown that artemisinin inhibits *P.*

falciparum phosphatidylinositol-3-kinase (*Pf*PI3K), and an increase in levels of *Pf*PI3K with a C580Y mutation has been observed in resistant strains.[35]

2.4 Protein kinases and kinase inhibitors

Protein kinases are enzymes that play an essential role in catalysing the transfer of the γ-phosphate group from adenosine triphosphate (ATP) to substrates that usually contain a tyrosine, serine or threonine residue (**Figure 20**). Out of the known amino acids, only threonine, serine, and tyrosine have the appropriate functional group (hydroxyl, OH) to accommodate phosphorylation. Protein kinase phosphorylation is a common mechanism of regulation that is important in almost every activity of eukaryotic cells, including gene expression, motility, proliferation and apoptosis.[36]

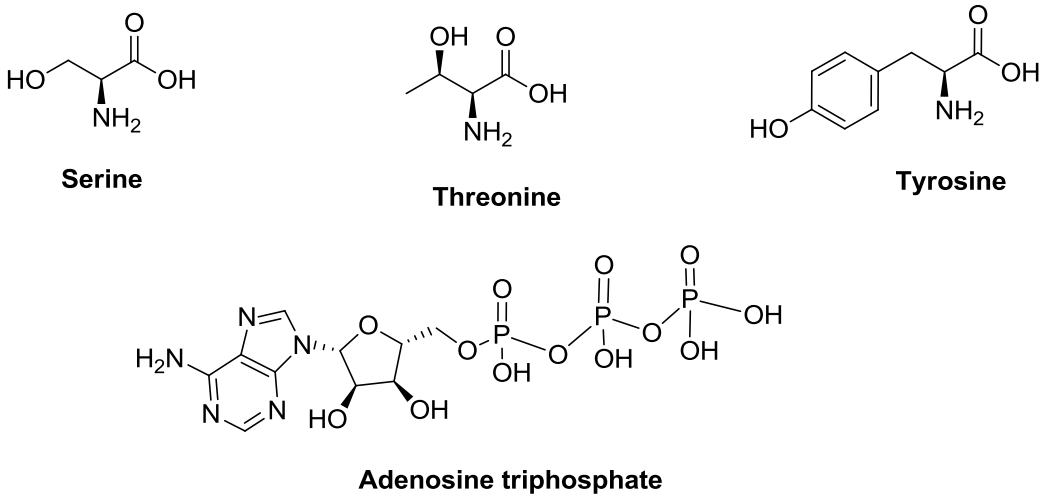


Figure 20: Structure of ATP with known amino acids essential for protein phosphorylation.

2.4.1 Kinase inhibitors

Kinase inhibitors are distinguished by their mode of binding in the ATP binding site.

Type I inhibitors. This type of inhibitors targets the ATP binding site when it is in its active conformation. Type I inhibitors or ATP-competitive inhibitors contain a heterocyclic scaffold which serves to form the same hydrophobic contacts as ATP and makes similar hydrogen bonds with the kinase hinge region, as can be seen in **Figure 21a**.[37]

Type II inhibitors. Type II inhibitors recognize the inactive conformation of the kinase and occupy the hydrophobic site that is directly adjacent to the ATP binding pocket. Type II inhibitors are also called non-ATP competitive inhibitors. The inactive conformation is sometimes referred to as DFG-out motif because of the rearrangement of the activation loop (**Figure 21b**). Hubbard and co-workers have stated that the amino acids surrounding this pocket are less conserved compared to those in the ATP binding pocket, thus it may be easier to achieve kinase selectivity with type II inhibitors. Type II inhibitors occupy an additional site called the allosteric site, and this provides an advantage over Type I inhibitors.[38]

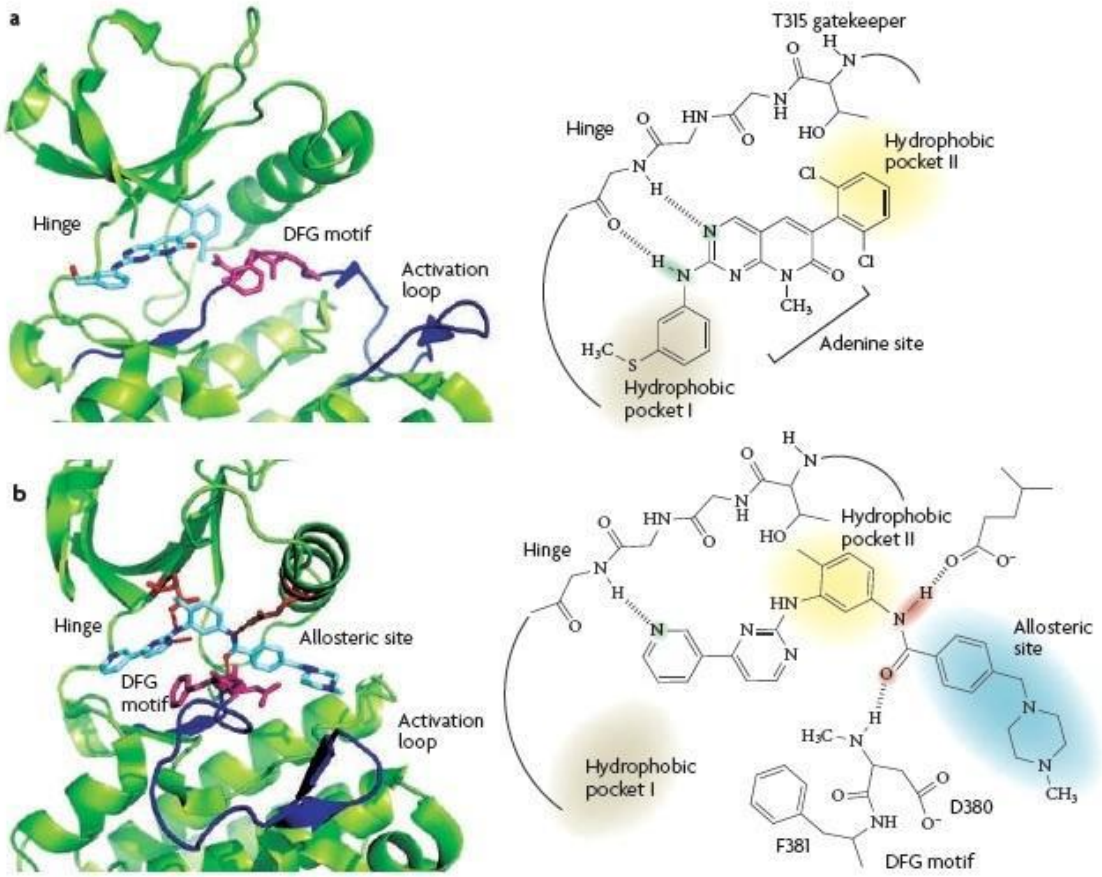


Figure 21: Binding modes of kinase inhibitor type I and II. (taken from *Nature Reviews*, **2009**, 9.)[37]

The other type of kinase inhibitors are **Type III (irreversible or covalent inhibitors).** This type of inhibitors irreversibly inhibit their target protein by forming a covalent bond to a nucleophilic cysteine residue located in the ATP active site. The irreversible inhibitors consist of a heterocyclic core (driving group) which forms the same weak hydrogen bond interactions with the hinge region, just like the reversible counterparts. However, the covalent inhibitors have the advantage of carrying a suitable electrophilic functionality known as a "warhead", which forms a covalent bond with a nucleophilic cysteine residue, usually by undergoing a Michael addition reaction, as shown below in **Figure 22**. The structure of an irreversible inhibitor, Ibrutinib (**81**) is also shown below (**Figure 23**). The number of covalent inhibitors entering anti-cancer clinical trial studies is gradually increasing. To date, five irreversible kinase inhibitors of the epidermal growth factor receptor (EGFR), are being assessed in lung cancer clinical trials.[39-40]

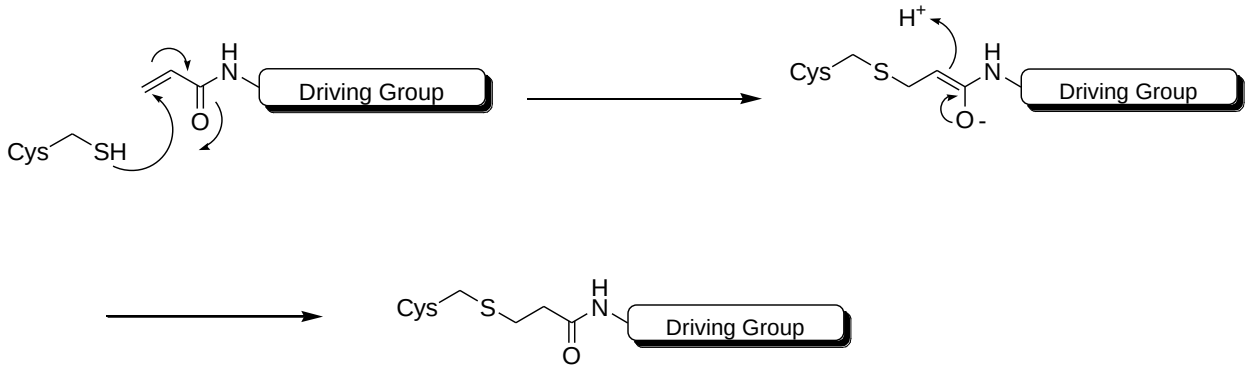


Figure 22: Reaction mechanisms of irreversible inhibitors with cysteine residue.

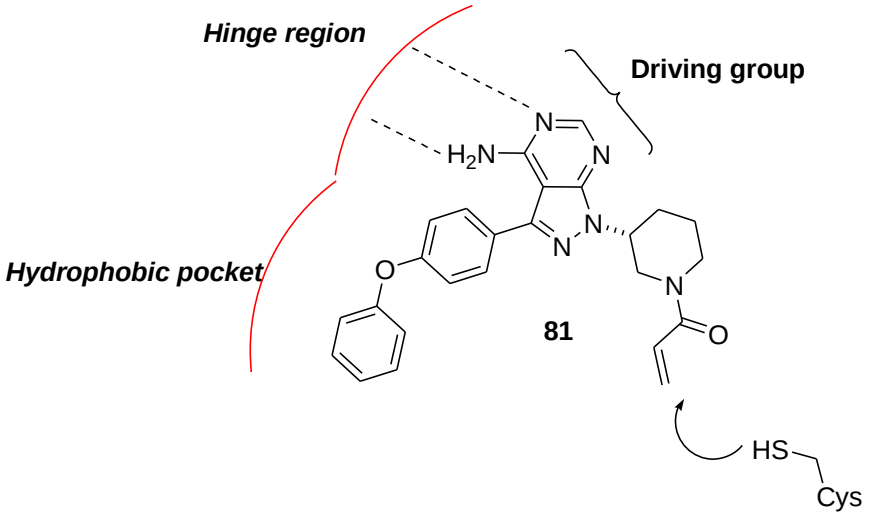


Figure 23: Diagram showing binding sites of the irreversible or covalent inhibitor, ibrutinib.

The DFG-motif, which is a sequence of three residues, Aspartate (D)-Phenylalanine (F)-Glycine (G) (**Figure 24**), has been used in drug discovery to design novel generations of protein kinase inhibitors. Protein kinases may adopt two important conformations; DFG-in (active form) and DFG-out (inactive form). In the DFG-in conformation, the aspartate residue (D) points into the ATP binding pocket and the phenylalanine is rotated away from the ATP binding site. The phenylalanine points in the opposite direction and creates or exposes an additional hydrophobic binding site adjacent to the ATP pocket for inhibitors to utilise in the DFG-out conformation. Some inhibitors make use of a specific conformation of a protein kinase for ligand binding. For example, Type II (allosteric inhibitors) and type III (irreversible inhibitors) make use of, and stabilize, the DFG-out conformational state, whilst Type I inhibitors (ATP competitive) make use of, and stabilize, the DFG-in conformation. The DFG-out conformation is an attractive target of several kinase inhibitors.[41]

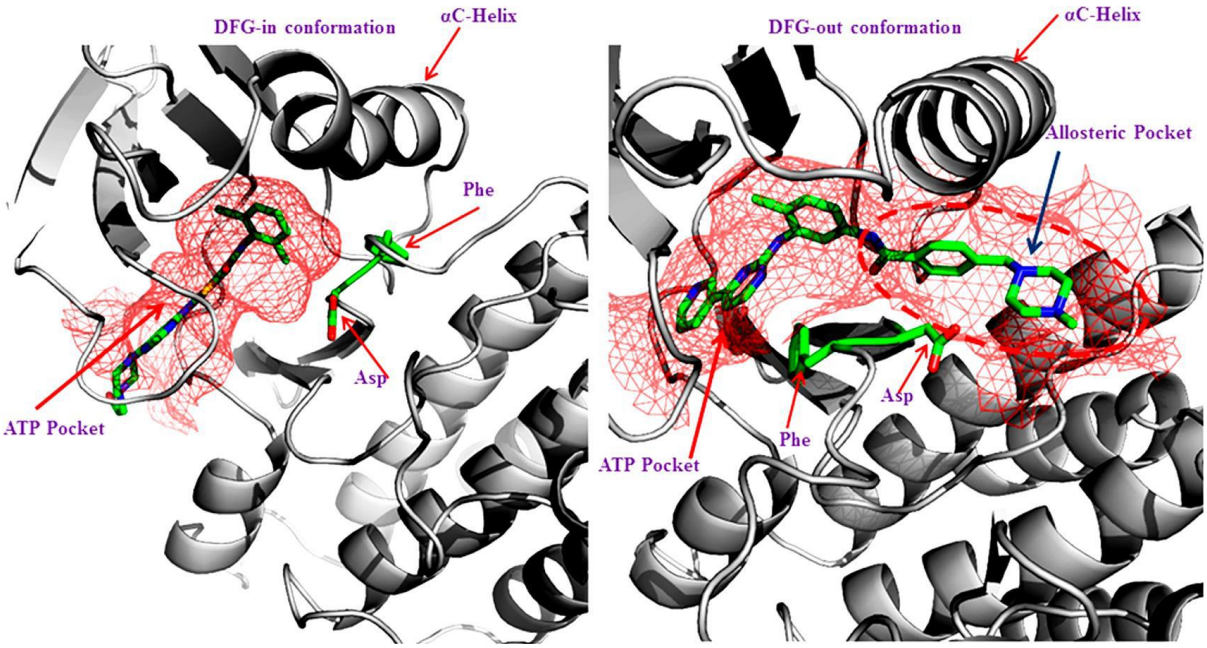


Figure 24: DFG-in conformation (left panel) and DFG-out conformation (right panel).[34]

2.5 Calcium-dependent protein kinases (CDPKs)

Studies have shown that calcium-dependent protein kinases (CDPKs) are an attractive drug target because of their uniqueness to plants and apicomplexans. Calcium controls a wide variety of biological processes in the malaria parasite, such as cell invasion, gametogenesis, circadian rhythms, gliding motility and migration. These CDPKs consist of an *N*-terminus (catalytic domain), a junctional domain and a sequence of calcium binding known as EF hands. The junctional and calmodulin-like regions together are known as the calcium activation domain (CAD, **Figure 25**). According to the biochemical studies done by Harper *et al* on plant CDPKs, the CDPK activity is regulated by the junctional domain by interacting with both the kinase domain and the C-terminal Calmodulin-like domain.[42]

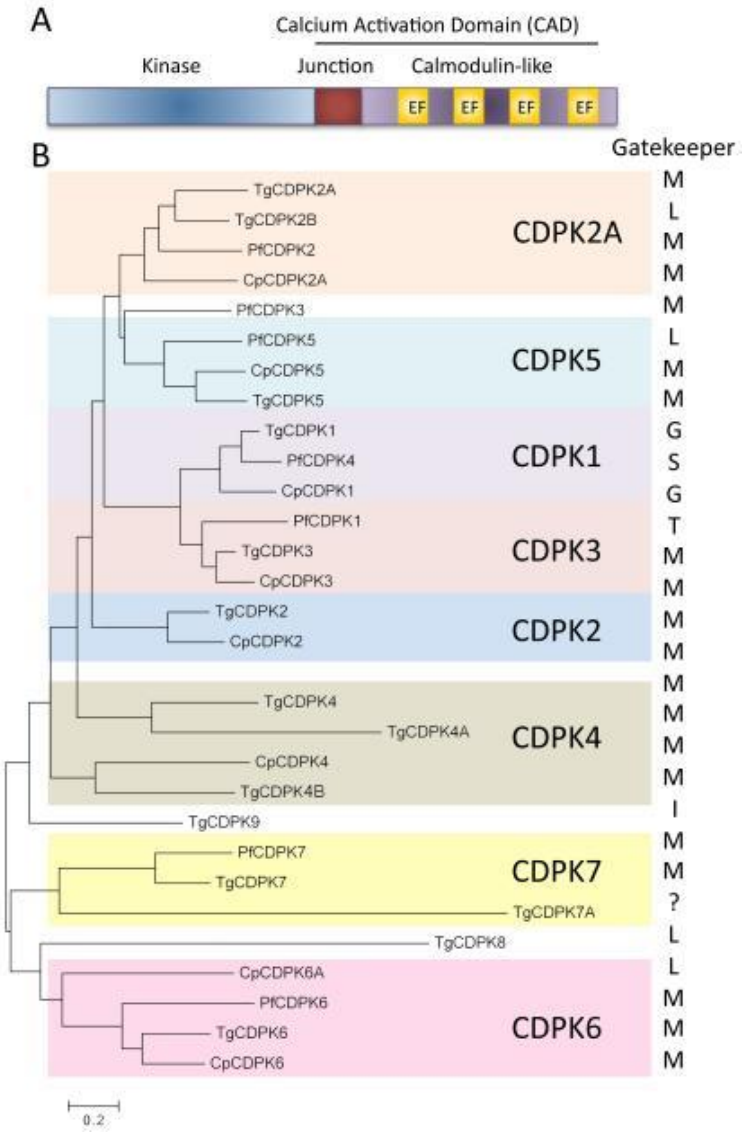


Figure 25: Schematic representation of CDPKs.[42]

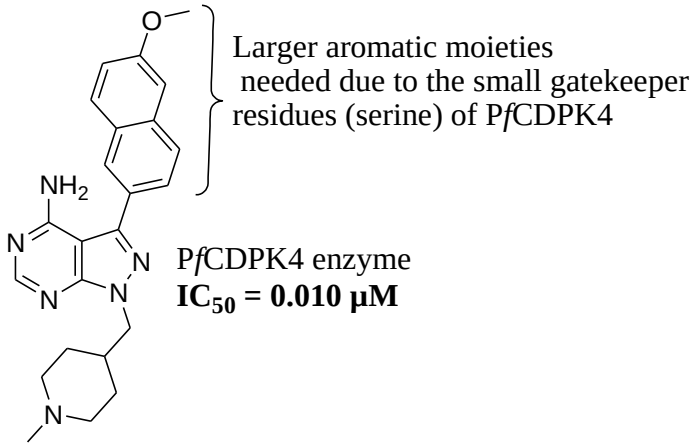
Since the CDPKs are absent from humans (host), this makes them a potential target for drug development. *Plasmodium* has five CDPKs that share the characteristic domain architecture of plant CDPKs, and studies have shown that these CDPKs play a vital role in various stages of the parasitic life cycle of plasmodia and other apicomplexan parasites. Sebastian and co-workers have reported that *P. falciparum* CDPK1 (*Pf*CDPK1) is involved in the regulation of parasite motility and controls zygote development and transmission.[43] Furthermore, CDPK1 gene disruption in *P. falciparum* has not been possible, suggesting that this enzyme is essential for parasite survival. *Pf*CDPK5 has been found to be important for malaria parasite egress regulation

from erythrocytes while *Pf*CDPK3 has an important function in ookinete gliding motility and invasion of mosquito midguts.[43]

In contrast, *Pf*CDPK4 is required for male gametocyte exflagellation and sexual reproduction of the parasite. It has been shown that disrupting the gene of CDPK4 in *P. berghei* causes severe imperfections in mosquito transmission and sexual reproduction.[43] Hence, these studies demonstrated the significance of this enzyme in *Plasmodium* biology and suggested that *Pf*CDPK4 may serve as a target for transmission-blocking drugs. When a mosquito bites a person infected with the malaria parasite, gametocytes can be taken up with blood and allowed to mature in the mosquito gut. Researchers at the University of Washington have shown that this maturation can be blocked by inhibition of *Plasmodium falciparum* calcium-dependent protein kinase 4 (*Pf*CDPK4).[44] Among the five different types of Plasmodial CDPKs discussed above, both *Pf*CDPK1 and *Pf*CDPK4 enzymes play a vital role in the parasitic life cycle and only a few inhibitors against these two enzymes are reported in the literature. *Pf*CDPK1 and *Pf*CDPK4 have not been studied broadly as targets in antimalarial drug development. Due to the structural similarity of these two kinases, inhibitors of P*f*CDPK4 are also often inhibitors of P*f*CDPK1.

Ojo and co-workers have synthesized transmission-blocking compounds (bumped kinase inhibitors) which selectively and potently inhibit *Pf*CDPK4 without being toxic to mammalian cells. These compounds contain a pyrazolo[3,4-*d*]pyrimidine scaffold which makes the same hydrophobic and hydrogen-bonding contacts with the hinge region as the adenine ring of ATP.[44] They have introduced large aromatic groups at the C-3 position of the pyrazolopyrimidine nucleus, because *Pf*CDPK4 has a small gatekeeper residue, serine, which is smaller compared to the larger gatekeeper residues found in human kinases. The bulky group at the C-3 position, which is sterically hindered, occupies the hydrophobic pocket adjacent to this gatekeeper residue and imparts selectivity for P*f*CDPK4 over most kinases that have a larger residue at this position. Ojo *et al*. reported that compound 1294 (**Figure 26**) is a synthetic bumped kinase inhibitor of P*f*CDPK4 and it is capable of preventing malaria transmission.[45] Compound 1294 contains a larger liphophilic group which can be accommodated in the hydrophobic pocket of the ATP binding site owing to a smaller gatekeeper residue, thus it can provide selectivity for plasmodial kinases. It is also reported that compound 1294 has a better bioavailability and longer half-life (4–14 hours, depending on dose) when compared to other precursors; hence this transmission-blocking agent could be used in combination with current treatments such as ACT to eradicate

malaria. Compound 1294 did not show signs of toxicity when examined in mice after high dose administration.



Compound 1294

Figure 26: Structure of compound 1294.

Ojo and co-workers further showed that compound 1294 blocks malaria transmission by inhibition of *Pf*CDPK4 using structure activity relationship (SAR) studies and by generating a drug-resistant *P. falciparum* NF54 strain that has a methionine residue as the gatekeeper of *Pf*CDPK4. Hence, this *Pf*CDPK4 inhibitor, (compound 1294) can be used as a lead to discover new antimalarial drugs that have the potential for both treatment of malaria and prevention of malaria transmission from mosquitoes to humans.

2.6 Discovering new kinase inhibitors

The search for *P. falciparum* kinase inhibitors with better potency and plasmodial selectivity constitutes more than half of current research efforts in antimalarial drug development.[46] As only a small portion of the human kinome can currently be targeted by a selective and potent inhibitor, there is an urgent need for medicinal chemists to develop strategies for the effective discovery and optimization of new kinase inhibitors. However, studies have demonstrated that protein kinases are difficult to target.[47] Protein kinases that are involved in replication, cellular proliferation and apoptosis have been inhibited by a number of natural products from plant or

microbial sources. Examples of natural products that are selective and potent inhibitors include the alkaloid staurosporine (**82**), the purine olomoucine (**83**) and the flavonoid rohitukine (**84**). Several therapeutic inhibitors of protein kinases (**85**, **86**, **87** and **88, Figure 27**) that are structurally related to natural products such as these are currently in the various stages of clinical testing.[48]

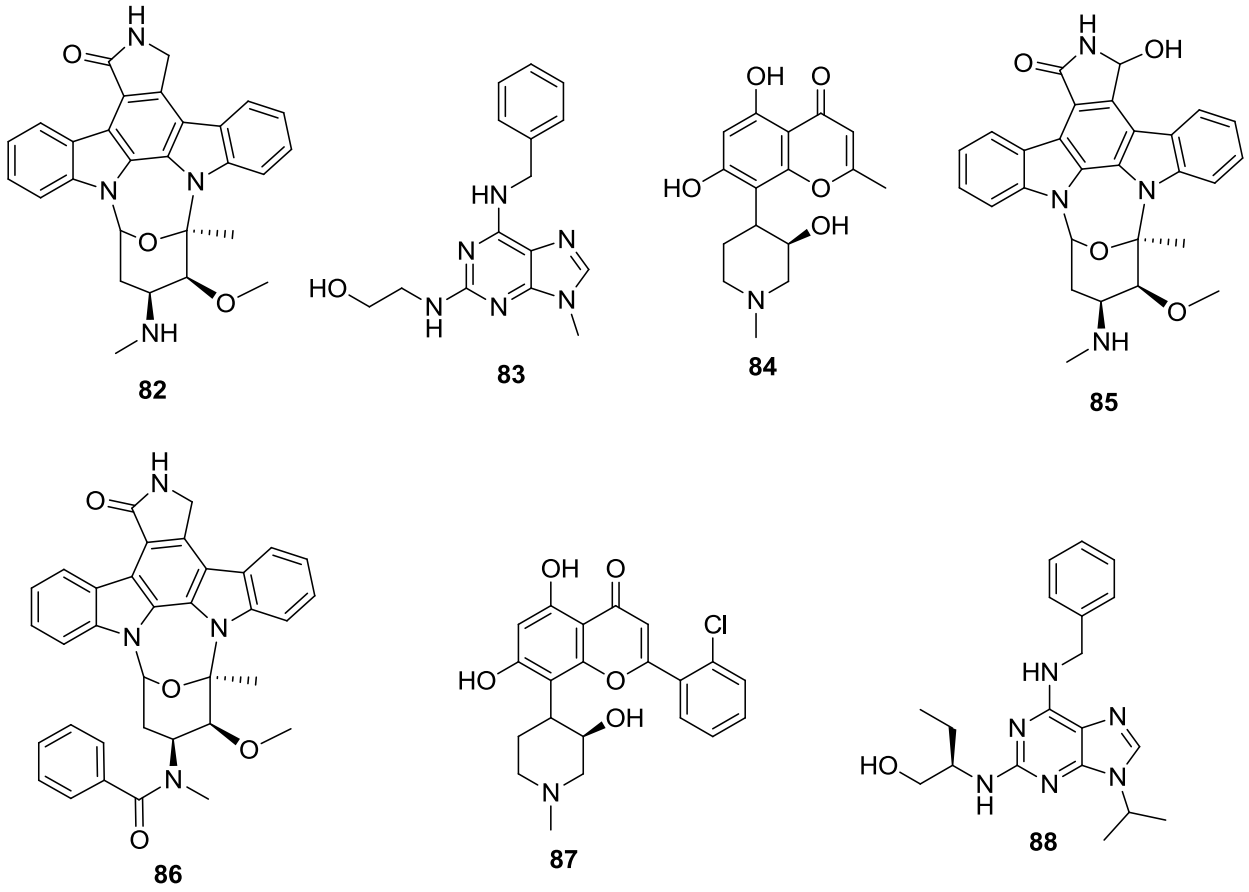


Figure 27: Natural product-based protein kinase inhibitors.

To date, eight synthetic therapeutic protein kinase inhibitors (**89-96, Figure 28**) have been approved by the FDA (Food and Drug Administration) in the US.[49] All of the eight are indicated for the treatment of oncological diseases. These US FDA-approved, small-molecule inhibitors can generally be classified depending on the protein kinase they are targeting. For example,

Gleevec (Imatinib, Novartis), dasatinib and nilotinib inhibit BCR-ABL fusion protein kinase, in chronic myeloid leukemia.

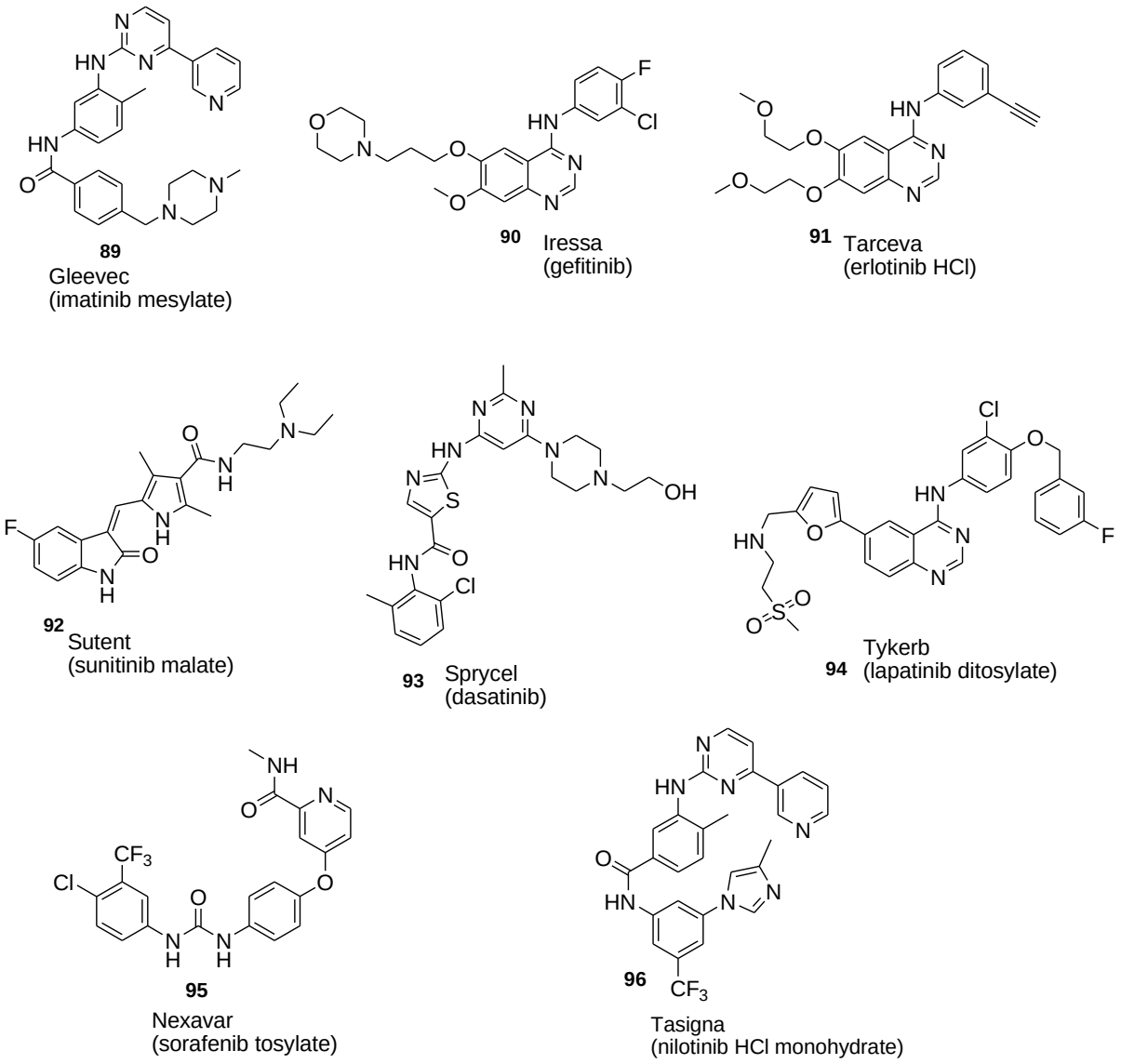


Figure 28: FDA approved protein kinase inhibitors.

The discovery of these therapeutic protein kinase inhibitors and other kinase inhibitors in clinical trials has been possible due to the significant understanding of how they bind to their target kinases, in either a classical or nonclassical manner. These US-FDA-approved small molecules, bind at or near the ATP pocket. Chemical modification or analogue synthesis was performed to

identify inhibitors of a new kinase target. These efforts have led to the discovery of many scaffolds that recognise the ATP-binding site, including pyrimidines, purines, quinazolines and quinolines. To date, only a few therapeutic protein kinase inhibitors have been developed using the different approaches discussed above.

2.7 Aims of this PhD project

The first part of this project involved using the recently solved X-ray crystal structure of P*f*CDPK4 to design inhibitors with either a pyrrolo[2,3-*d*]pyrimidine or branched pyrimidine scaffold. Initially, Biovia Discovery Studio software, and later, Schrodinger, available from the Centre for High Performance Computing (CHPC) in South Africa, were utilized for this purpose. Flexible docking protocols were developed and compounds displaying the best binding interactions were considered for synthesis.

The second part of this project describes the synthesis of pyrrolo[2,3-*d*]pyrimidine derivatives (**Figure 29**) that are potential inhibitors of P*f*CDPK4 and P*f*CDPK1. The application of microwave (MW) assisted ring closure in combination with Suzuki coupling were employed as key steps in the synthesis of suitably substituted pyrrolo[2,3-*d*]pyrimidines. Different functional groups were included at the N-7 of the pyrrolo[2,3-*d*]pyrimidine nucleus.

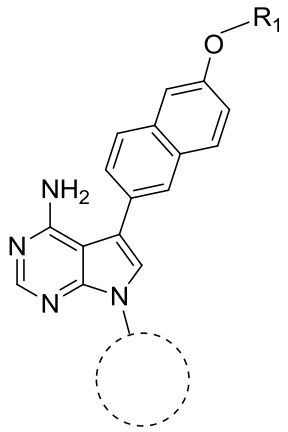
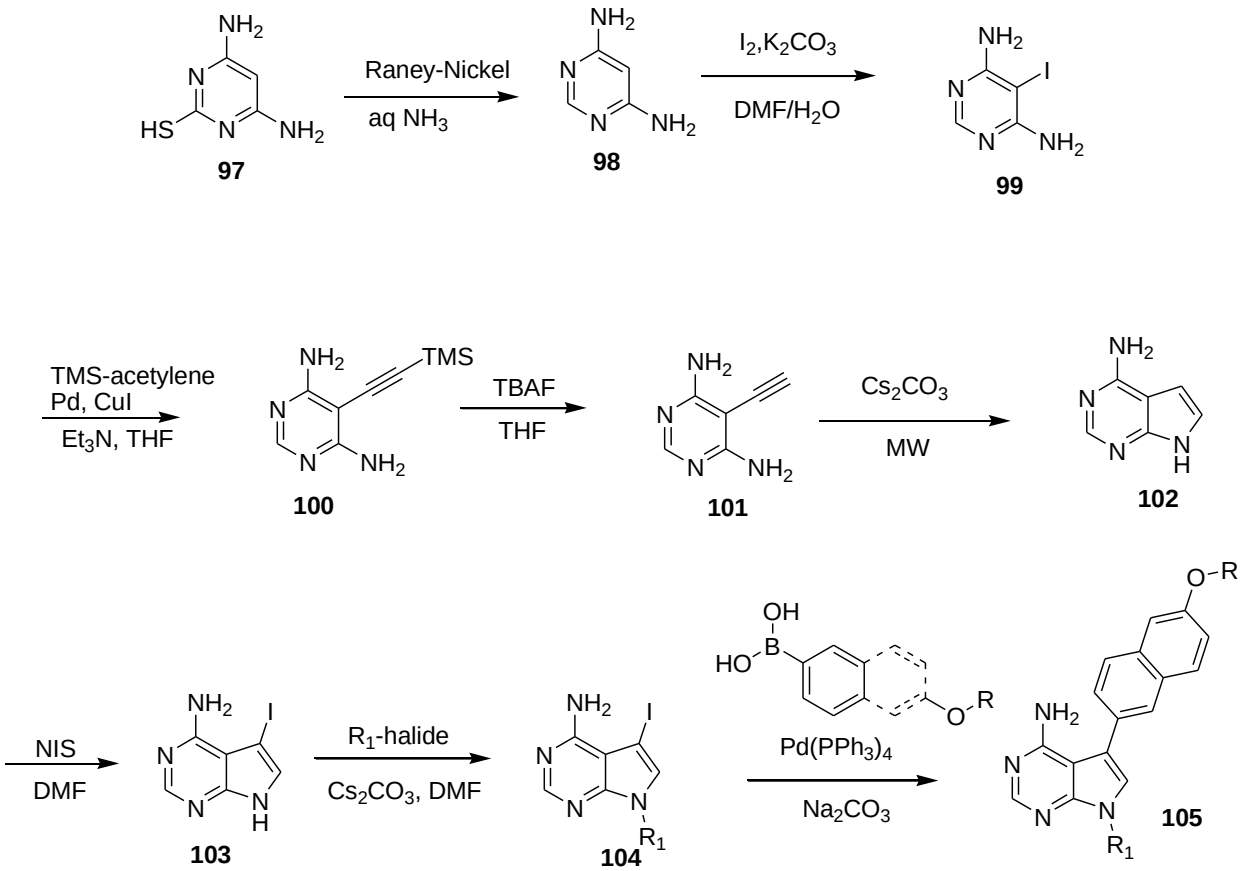


Figure 29: Proposed structure of pyrrolo[2,3-*d*]pyrimidine derivatives.

Our proposed synthetic route (Scheme 16) to these compounds involved reduction of the commercially available 4,6-diaminopyrimidine-2-thiol **97** in the presence of Raney nickel to afford pyrimidine-4,6-diamine **98**. Iodination at C-5 would be achieved with iodine and potassium carbonate in dimethylformamide (DMF) and water. The halogenated compound **99** would then be subjected to Sonogashira coupling conditions using TMS acetylene in the presence of catalytic $Pd(PPh_3)_2Cl_2$ to give product **100** followed by tetrabutylammonium fluoride (TBAF) deprotection in tetrahydrofuran to give a terminal alkyne **101**. The next step would be a ring closure using microwave irradiation with cesium carbonate in dimethyl sulfoxide (DMSO) to afford 7-deazapurine **102**. While compound **102** is commercially available, it is expensive to purchase and sold in small quantities. Hence, we planned to synthesise this intermediate.

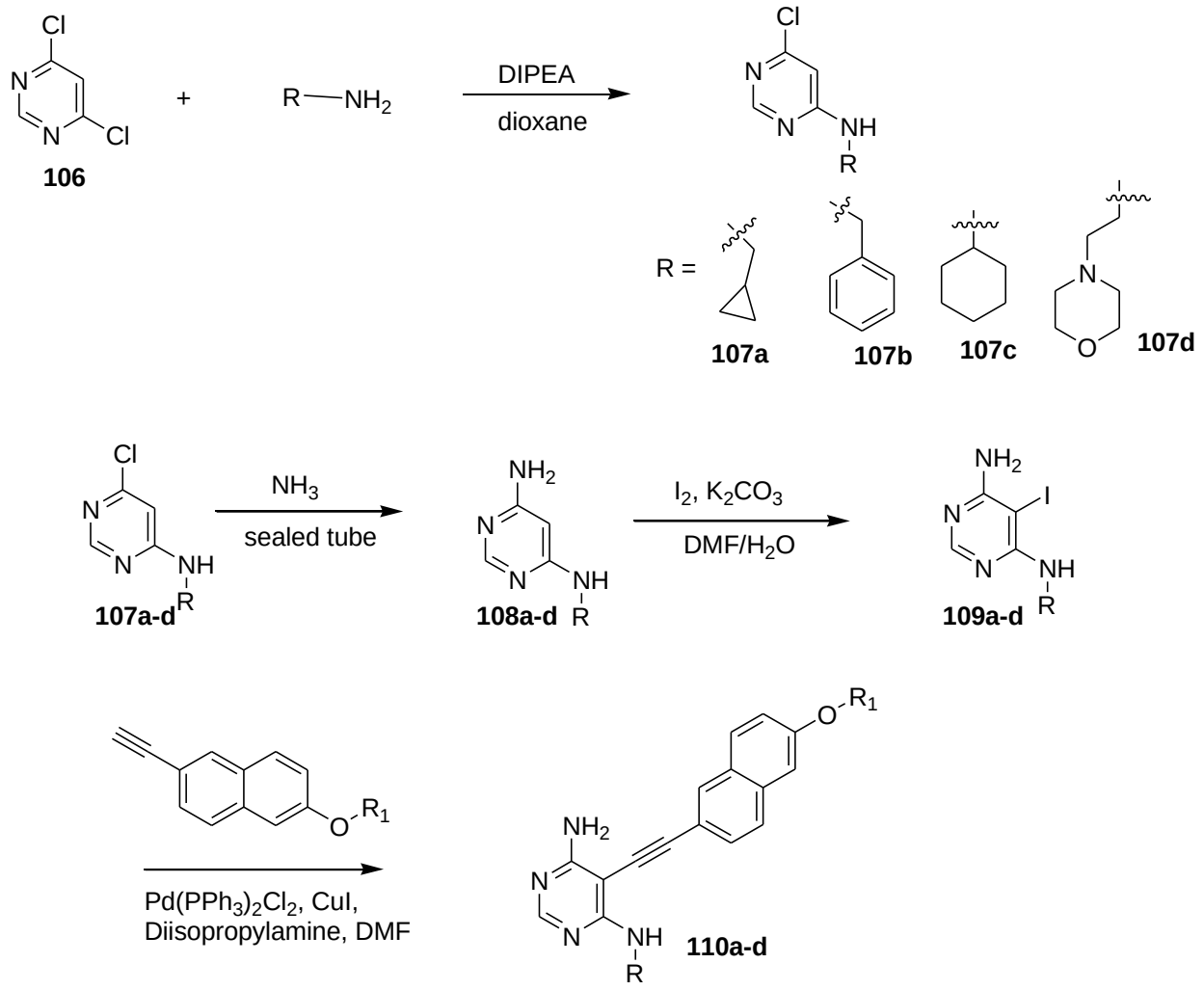


Scheme 16: Proposed route for synthesis of targeted pyrrolo[2,3-*d*]pyrimidines.

With this key intermediate **102** in hand, iodination at C-5 of the pyrrolo[2,3-*d*]pyrimidine core would be accomplished using *N*-iodosuccinimide (NIS) to afford the desired compound **103**, and this would be followed by reaction with appropriately substituted alkyl (R_1) bromides or

tosylates in dimethylformamide to afford compound **104**. The final step in the synthesis of these potential kinase inhibitors **105** would be the introduction of appropriate aryl substituents at C-5 *via* Suzuki-Miyaura coupling with boronic acids.

The third part of this PhD project involved the synthesis of branched pyrimidine analogues in an attempt to identify a simpler scaffold for the development of kinase inhibitors. In this aspect of the project we wished to use the Sonogashira cross coupling reaction to gain access to the kinase inhibitors containing the pyrimidine ring. Our proposed synthetic route (Scheme 17) to these compounds is shown below. These compounds could be prepared by mono nucleophilic displacement of chlorine as our starting point on 4,6-dichloropyrimidine **106** to afford compounds **107a-d**. With compounds **107a-d** in hand, our next synthetic step would be to perform the second nucleophilic displacement of the remaining chlorine atom using aqueous ammonia in ethanol to give the desired products **108a-d**. The third synthetic step would be to prepare the iodopyrimidines **109a-d** using iodine in water and DMF. Thereafter, Sonogashira coupling reaction with different alkynyl derivatives would be performed to possibly construct the 4,5,6-trisubstituted pyrimidine derivatives **110a-d** as potential protein kinase inhibitors.



Scheme 17: Proposed route towards synthesis of branched pyrimidine.

We planned to screen all final compounds containing the pyrrolo[2,3-*d*]pyrimidine or branched pyrimidine scaffold for biological activity in a series of assays conducted at Wits University and Rhodes University. This included both enzymatic assay *Pf*CDPK4 and a whole cell antiplasmodium assay. Active compounds that displayed promising biological activity would be assessed further for cytotoxicity. Unfortunately, we were not able to obtain the results of enzymatic assay in time before submission of this thesis. However, the preliminary whole cell results indicating antiplasmodial activity will be discussed.

2.8 References

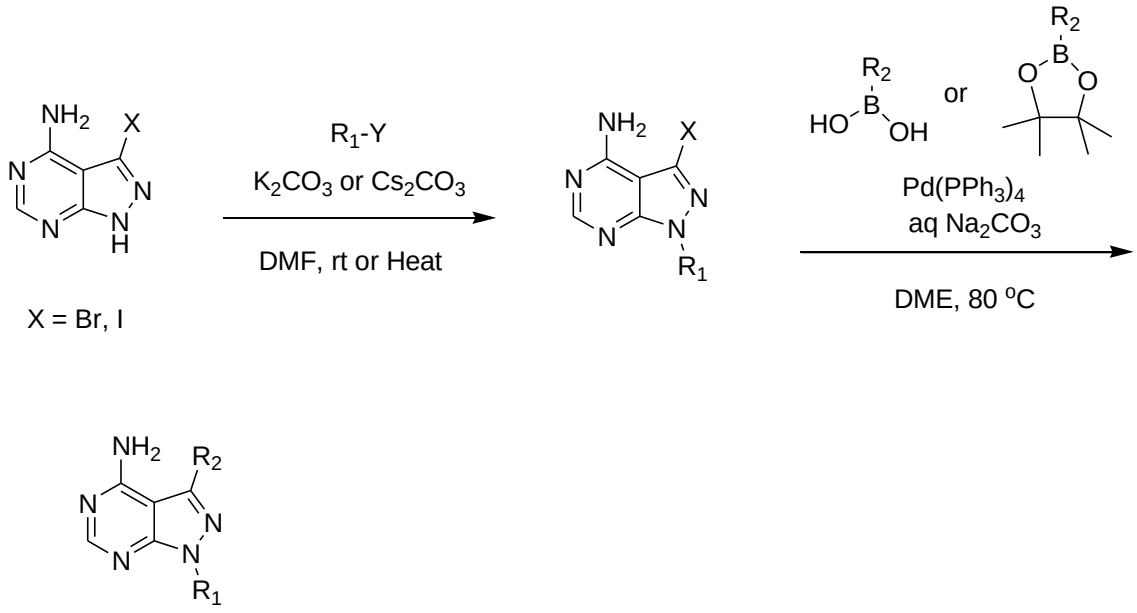
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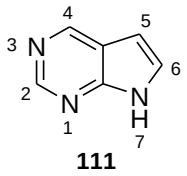
CHAPTER 3: SYNTHESIS OF PYRROLO[2,3-*d*]PYRIMIDINE ANALOGUES.

As discussed in Chapter 2, the aim of this project was to synthesise novel pyrrolo[2,3-*d*]pyrimidine analogues as potential inhibitors of P*f*CDPK4 and P*f*CDPK1. It has been reported that inhibition of *Plasmodium falciparum* calcium-dependent protein kinase 4 (*Pf*CDPK4), enzyme responsible for sexual stage development in the mosquito, blocks gametocytes maturation in the mosquito.[1] Vidadala and co-workers have shown that structural features can be exploited in *Pf*CDPK4's ATP binding site to achieve highly selective inhibitors.[1] Hence, this suggests that *Pf*CDPK4 represents a promising drug target for the development of malaria transmission blocking agents. Pyrazolopyrimidines have been synthesized as transmission-blocking compounds by Ojo and co-workers.[2] These compounds contain bulky groups at the C-3 position of the pyrazolopyrimidine nucleus, due to the small gatekeeper residue, serine, of *Pf*CDPK4. The bulky groups occupy the hydrophobic pocket adjacent to this gatekeeper residue and impart selectivity for P*f*CDPK4 over most kinases that have a larger residue at this position (Scheme 18).[2]



Scheme 18: Synthesis of pyrazolopyrimidine compounds used by Ojo and co-workers.[2]

Due to the structural similarity of these two kinases, P*f*CDPK4 and P*f*CDPK1, and the availability of a crystal structure of *Pf*CDPK4, we hoped to design and synthesize a compound with the potential to inhibit both *Pf*CDPK1 and *Pf*CDPK4. We planned to develop a dual P*f*CDPK1/P*f*CDPK4 inhibitor, which would be suitable for both treatment of malaria and prevention of transmission. We planned to do this by synthesising a series of compounds that contain the pyrrolo[2,3-*d*]pyrimidine nucleus **111** (**Figure 30**), which is different to the scaffold reported by Ojo and co-workers. These compounds were designed based on Compound 1294 (**Figure 26,** shown in the previous chapter), which is a synthetic inhibitor of P*f*CDPK4 and it inhibits exflagellation of gametocytes. Compound 1294 has a better bioavailability and longer half-life when compared to precursors; hence this transmission-blocking agent could be used in combination with current treatments such as ACT to eradicate malaria.[3] We wanted to explore the possibility of using a different heterocyclic core for this purpose.



111

Figure 30: 7*H*-pyrrolo[2,3-*d*]pyrimidine.

3.1 Molecular modelling

In order to rationally design novel inhibitors targeting CDPKs, we have used molecular modelling of the recently solved X-ray crystal structure of P*f*CDPK4 (4QOX from the RSCB protein data bank). Pyrrolo[2,3-*d*]pyrimidine derivatives were docked into the ATP-binding site of the *Pf*CDPK-4 model to identify the key interacting residues using Biovia Discovery Studio software initially; and then later Schrodinger (2017-4, Maestro 11.4). The change in software came about as a result of changes in licensing rights to the Centre for High Performance Computing (CHPC) and academic institutions in South Africa. Using a combination of modelling, the results obtained by Ojo and co-workers; and depending on commercial availability of suitable fragments, we planned to design and synthesise a library of pyrrolo[2,3-*d*]pyrimidines bearing a variety of substituents at N-7 and C-5. We considered a

series of aromatic substituents for C-5; including 2-methoxyphenyl and 3,4-dimethoxyphenyl (owing to the commercial availability of these boronic acids), as well as bulkier naphthalenes to bind in the hydrophobic pocket of the binding site (**Figure 31**).

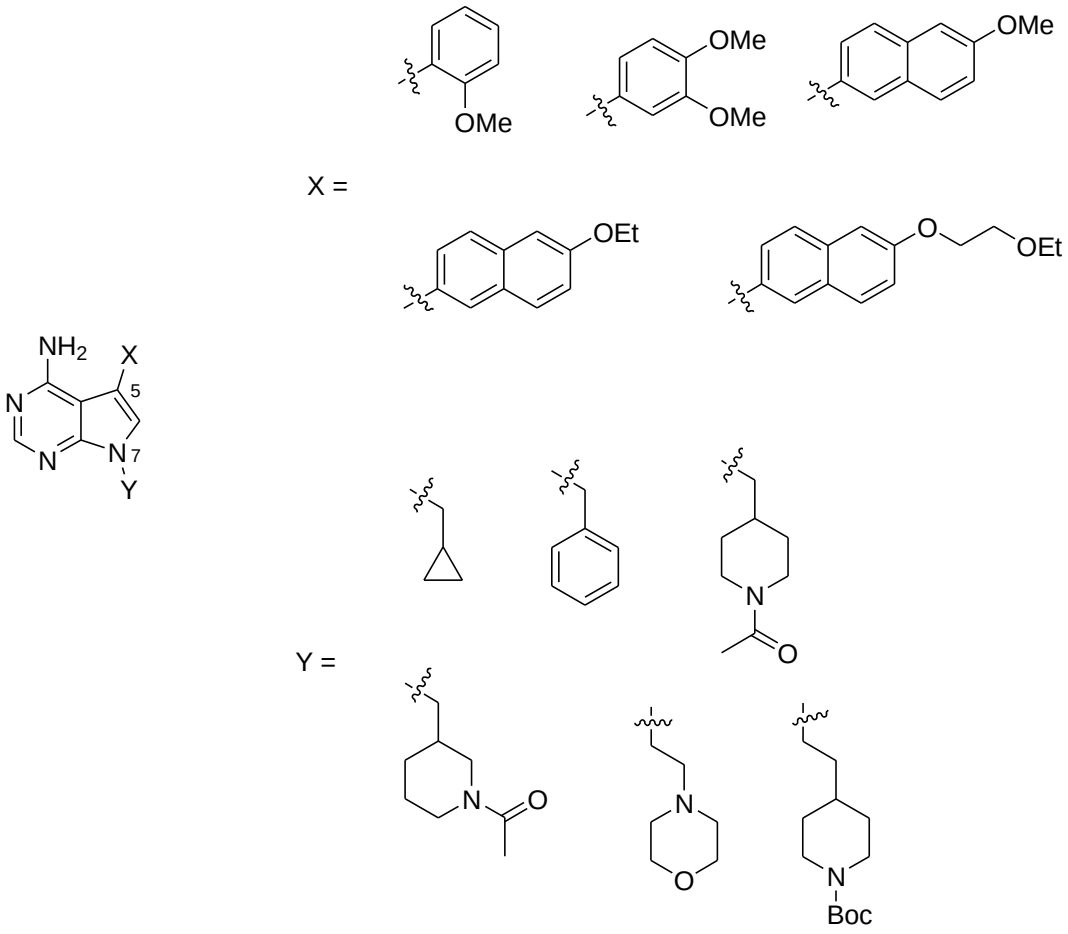


Figure 31: Proposed pyrrolo[2,3-*d*]pyrimidines bearing a variety of substituents at N-7 and C-5.

For N-7 of the pyrrolo[2,3-*d*]pyrimidine nucleus, we wanted to include both hydrophobic groups and those with the potential to form hydrogen-bonds with Glu154 in the sugar pocket. As there is an additional hydrophobic pocket near the carbohydrate binding region; both hydrophobic and hydrophilic groups have the potential to form good binding interactions in this region. We therefore included cyclopropylmethyl, *N*-acetyl piperidinyl methyl, *N*-Boc piperidinyl ethynyl and 2-morpholinoethyl groups in this position. We then docked the pyrrolo[2,3-*d*]pyrimidines bearing these functional groups at N-7 and C-5 into the *Pf*CDPK4 active site (using glide; in Schrodinger) and ligands were ranked according to their docking scores and glide scores. Docked poses of each compound were assessed visually to identify whether the expected

hydrogen-bonding interactions with key amino acid residues in the hinge region were present; notably Asp148 and Tyr150. See for example, **Figure 32**; which shows hydrogen-bonding of the pyrrolo[2,3-*d*]pyrimidine core to Asp148 and Tyr150.

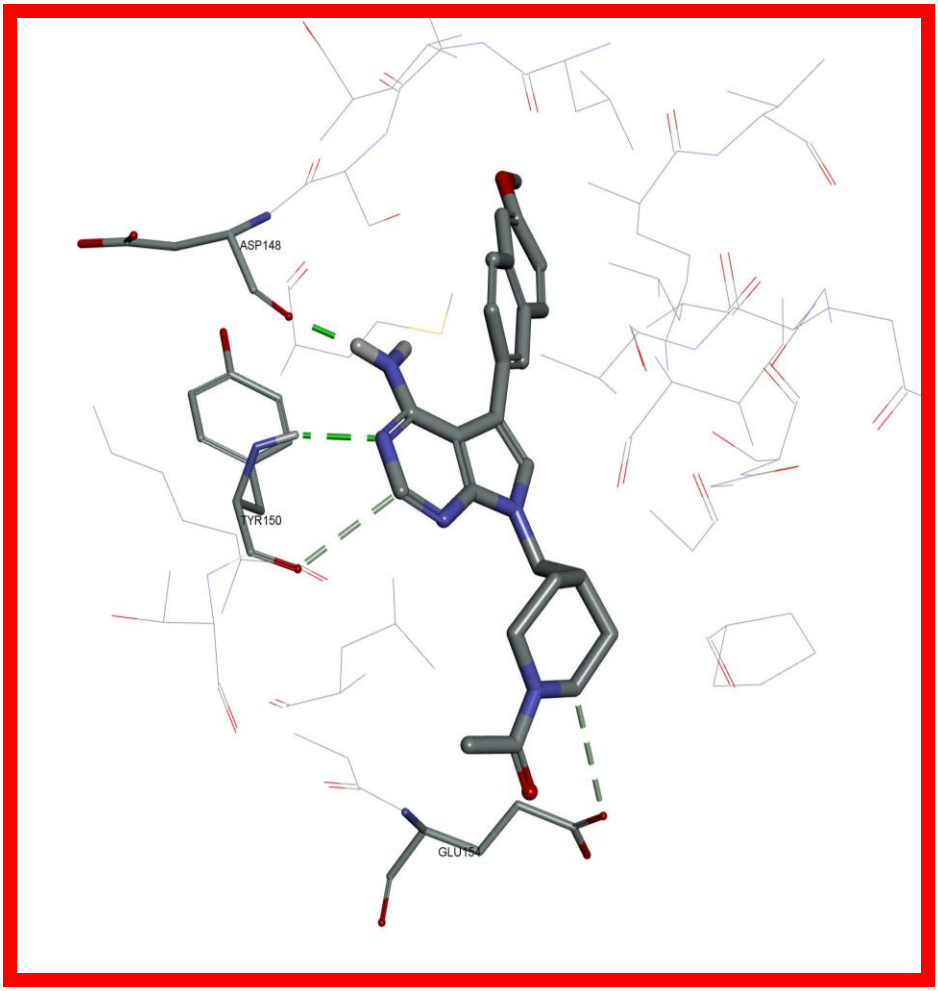


Figure 32: A picture from the molecular modelling showing hydrogen bond interactions with the piperidine moiety and the pyrrolo[2,3-*d*]pyrimidine core.

Interestingly; pyrrolo[2,3-*d*]pyrimidines bearing cyclopropylmethyl at N-7 gave rise to poses with the best docking and gliding scores; followed by those bearing piperidinyl groups. The results also showed that a 3,4-dimethoxyphenyl substituent at C-5 is better than the 2-methoxyphenyl substituent in general. The 3,4-dimethoxyphenyl compounds can form an additional H-bond with Asp215 (**Figure 33, a**); while pi interaction with Lys99 is observed for 2-methoxyphenyl compounds (**Figure 33, b**). However the pyrrolo[2,3-*d*]pyrimidines bearing

naphthalene substituents at C-5 showed better binding overall than either of the 2-methoxyphenyl compounds. The naphthyl substituents are able to fill the large hydrophobic pocket in this region better than the phenyl substituents (**Figure 33, c**). We also noticed that compounds with a methoxy substituent on the C-5 naphthalene group seemed to show better results than those bearing longer ethers, e.g. ethoxy and ethoxyethoxy substituents.

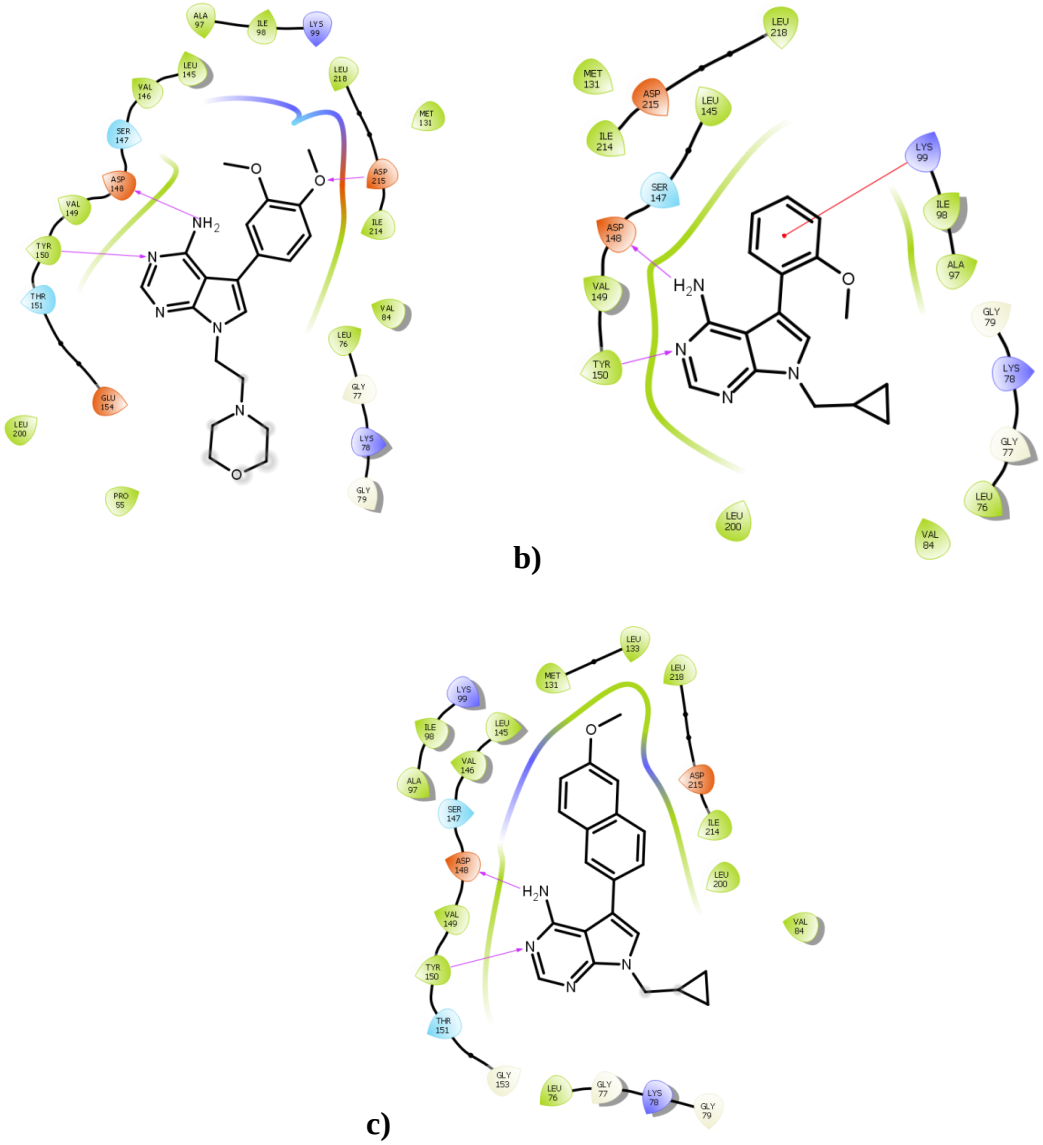


Figure 33: A picture from the molecular modelling showing comparison between 3,4-dimethoxyphenyl compounds and 2-methoxyphenyl compounds; as well as hydrogen bond interactions with compounds bearing the naphthalene group on C-5.

Compounds bearing the piperidine moiety also showed good hydrogen bond acceptor/donor interactions. Additional hydrogen bonding was also observed when this moiety was protected by an acyl or Boc-protecting group. The results from our modelling studies showed that 3-piperidine compounds gave lower docking scores than the 4-piperidine counterparts suggesting better binding interactions (**Figure 34, a**). Extending the alkyl chain by an additional carbon atom was also promising; and additional H-bonding interactions could be identified (**Figure 34, b**).

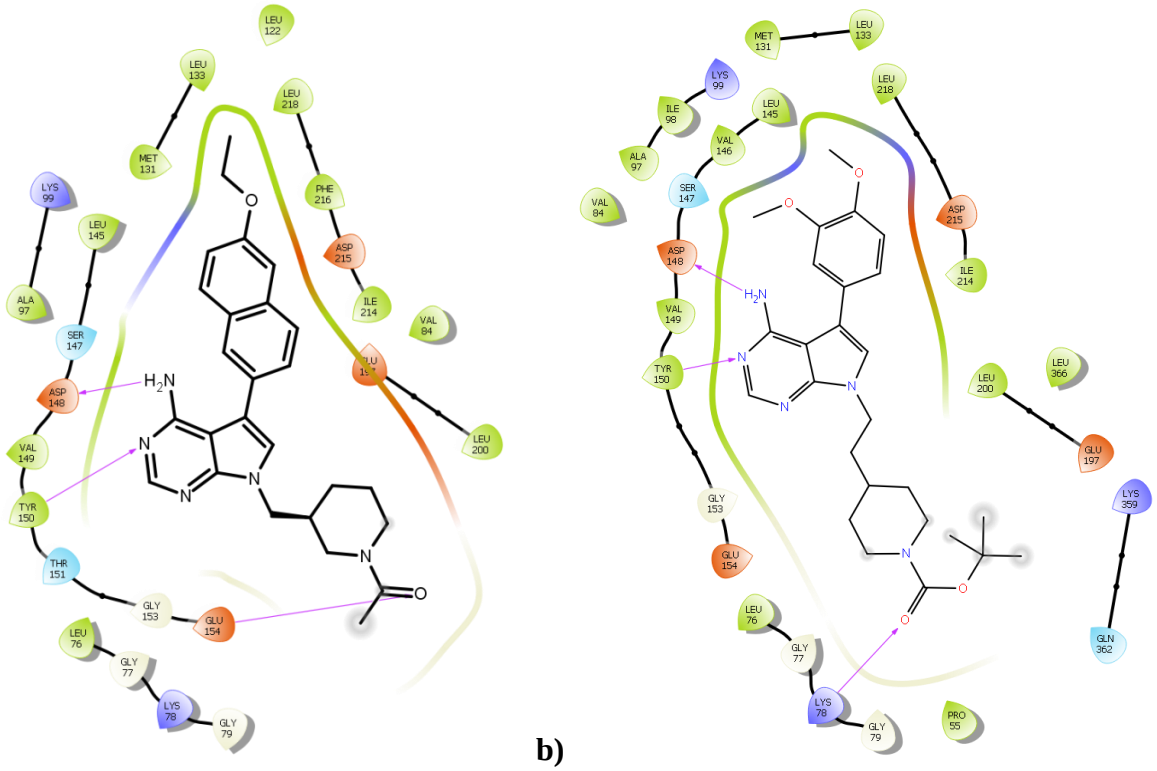


Figure 34: A picture from the molecular modelling showing hydrogen bond interactions with the 3-piperidine moiety, ethyl piperidine-1-carboxylate and the pyrrolo[2,3-*d*]pyrimidine core.

A number of hydrogen bond acceptor/donor interactions were seen between the compounds bearing the 2-morpholinoethyl side chain and key amino acids residues (Asp148 and Tyr150) in the hinge region; but the expected additional H-bond with Glu154 in the carbohydrate binding region was not observed (**Figure 35, a**). However; we did not explore induced fit docking of these compounds to increase the potential for this interaction. Compounds with a benzyl group at N-7 docked as expected in the active site, but generally gave higher docking scores; indicating weaker binding (**Figure 35, b**).

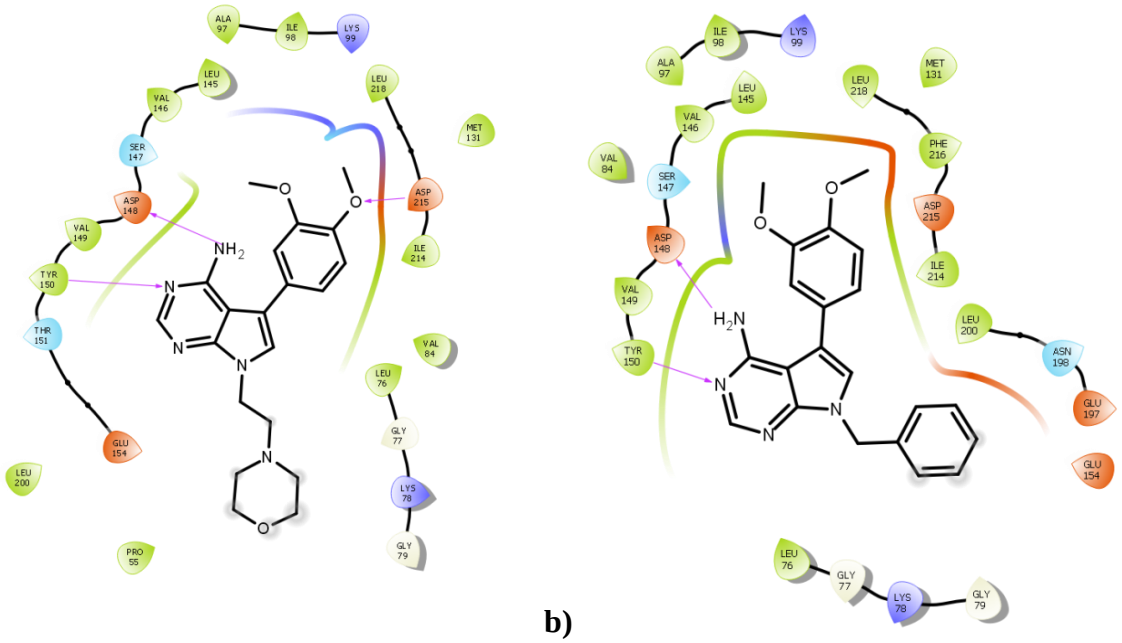


Figure 35: A picture from the molecular modelling showing hydrogen bond interactions with key amino acids residues.

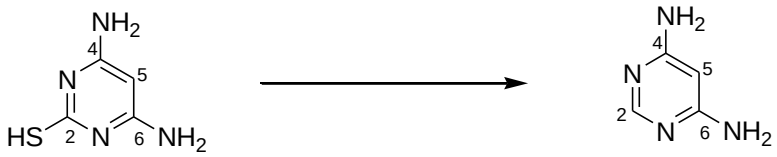
The results from our modelling studies showed that these pyrrolo[2,3-*d*]pyrimidines bearing benzyl, cyclopropylmethyl, (N-acetylpiperidin-4-yl)methyl, 2-(1-Boc-piperidin-4-yl)ethyl and 2-(morpholin-4-yl)ethyl on N-7 of the scaffold could potentially act as inhibitors of *Pf*CDPK4. Key hydrogen-bonding interactions were observed between Tyr150 and Asp148 in the hinge region of *Pf*CDPK4 and N3 and 4-NH_2 groups of the pyrrolo[2,3-*d*]pyrimidine scaffold. As discussed in the previous chapter, the interaction shown by the Asp148 and Tyr150 residues with the N-3, 4-NH and H-2 of pyrrolo[2,3-*d*]pyrimidine heterocycle is crucial for the interaction with the kinase domain of the protein. We therefore embarked on the synthesis of a library of compounds bearing these groups.

3.2 Synthesis of novel pyrrolo[2,3-*d*]pyrimidine analogues as kinase inhibitors of *Plasmodium falciparum* calcium-dependent protein kinases.

The proposed synthetic route towards novel pyrrolo[2,3-*d*]pyrimidine analogues (Scheme 16, shown in previous chapter) involves reduction of the commercially available 4,6-diaminopyrimidine-2-thiol in the presence of Raney nickel to afford pyrimidine-4,6-diamine, which would be subjected to iodination followed by Sonogashira coupling reaction with TMS

acetylene in the presence of $Pd(PPh_3)_2Cl_2$ to give a terminal alkyne. Subsequent ring closure using microwave irradiation with cesium carbonate gives the desired 7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine. With this key intermediate in hand, iodination at C-5 would be accomplished using *N*-iodosuccinimide (NIS), and this would be followed by reaction with appropriately substituted alkyl (R_1) bromides or tosylates in dimethylformamide. The final step to these kinase inhibitors would be the introduction of appropriate aryl substituents at C-5 *via* Suzuki-Miyaura coupling with boronic acids. Our synthesis of potential protein kinase inhibitors followed this ten step process.

3.2.1 Synthesis of pyrimidine-4,6-diamine[4]

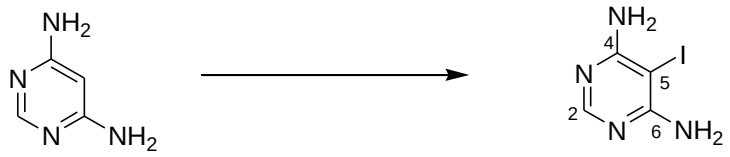


Scheme 19: Reagents and conditions: Raney Ni, aq NH_3, H_2O, reflux, 2 h, 96%.

Our synthetic route to these compounds started with commercially available 4,6-diaminopyrimidine-2-thiol **97**. Our first synthetic step (Scheme 19), was to perform reduction of 4,6-diaminopyrimidine-2-thiol using freshly prepared Raney nickel. The reaction was conducted by heating to reflux in an aqueous ammonia solution for 2 hours. The pyrimidine-4,6-diamine product was formed as a single spot on TLC indicating complete consumption of 4,6-diaminopyrimidine-2-thiol. Excess catalyst was filtered off (not filtering to dryness) under vacuum using a Buchner funnel. After removal of the solvent *in vacuo*, the reduced product was isolated as a light yellow solid in 96% yield.

The formation of **98** was confirmed by both ^{1}H NMR and ^{13}C NMR spectroscopy. In the ^{1}H NMR spectrum, the only significant difference from the starting material was the signal at 7.81 ppm due to H2 and the absence of an S-H signal. In the ^{13}C NMR spectrum the signal due to C-2 shifted to 157.8 ppm from 175.6 ppm indicating the success of the reaction.

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



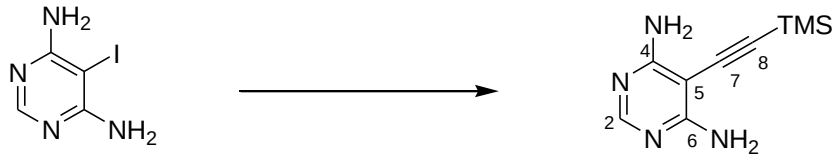
98 **99**

Scheme 20: Reagents and conditions: I_2, K_2CO_3, DMF/H_2O, 45 °C, 4 h, 76%.

Iodination reactions of pyrimidines are known to afford 5-iodinated products. This reaction is completely regioselective and typically none of the 2-iodinated products are isolated.[5] Thus, compound **98** was dissolved in a mixture of DMF and water and to this solution was added potassium carbonate followed by iodine (Scheme 20). The resulting reaction mixture was heated at low temperatures ranging from 40 – 45 °C according to the literature procedure,[6] and the progress of the reaction was monitored by TLC analysis. After complete conversion of all of the starting material to the product, the reaction was quenched with sodium thiosulfate. The desired product **99** precipitated out of solution and was collected by filtration as a yellow solid in a good yield of 76%.

In the ^{1}H NMR spectrum, the singlet signal at 5.38 ppm had disappeared and in the ^{13}C NMR spectrum the signal due to C-5 shifted to 55.21 ppm from 82.6 ppm indicating successful iodination at this position. The proton signal due to H2 between the two nitrogen atoms was still visible at 7.72 ppm in the ^{1}H NMR spectrum, indicating that no substitution had taken place at this position.

3.2.3 Synthesis of 5-[(trimethylsilyl)ethynyl]pyrimidine-4,6-diamine by utilisation of the Sonogashira reaction



99 **100**

Scheme 21: Reagents and conditions: cat. $Pd(PPh_3)_4$, CuI, diisopropylamine, THF, 70 °C, 4 h, 48%.

The Sonogashira coupling reaction is an extremely useful reaction in organic chemistry for the coupling of aryl halides and terminal alkynes in the presence of a palladium(0) or palladium(II) catalyst, copper(I) co-catalyst, and an amine base in a solvent such as DMF or THF.[7-9]

The catalytic cycle (**Figure 36**) starts with an oxidative addition of the Pd^0 **112** to the RX to form four-coordinated palladium complex **113**. The next step in the cycle involves intersection of the palladium cycle with the copper cycle to form a palladium acetylide. This step is called transmetallation and is often the rate determining step in the catalytic cycle.[10] *Trans/cis* rearrangement occurs next, and the final step is the bond formation between the two organic moieties. This step is called reductive elimination.

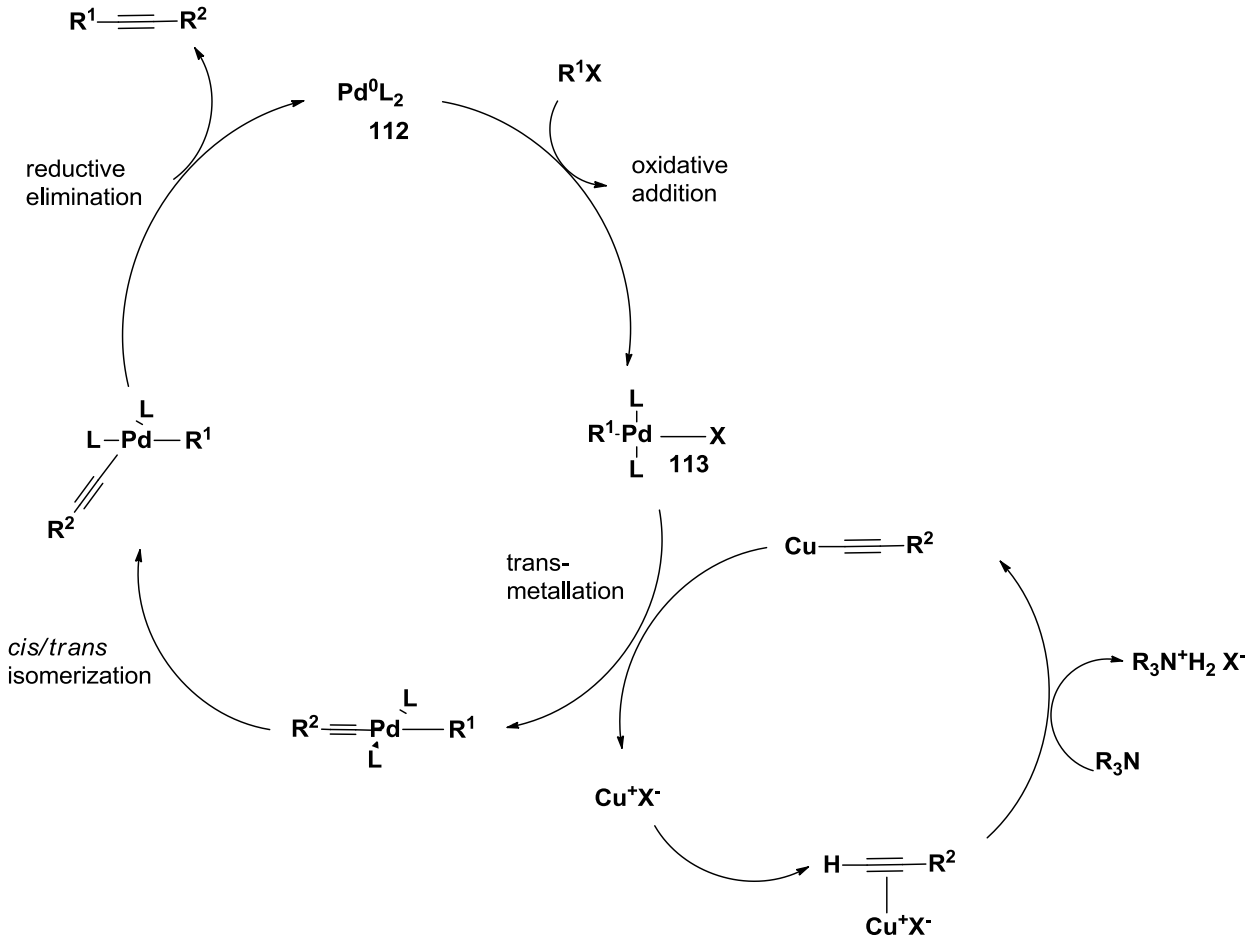
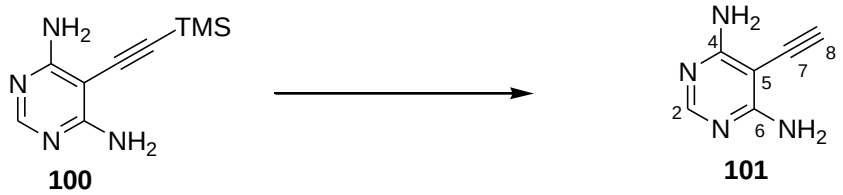


Figure 36: Mechanism for the Sonogashira cross-coupling reaction.

For the formation of compound **100**, 5-iodopyrimidine-4,6-diamine **99**, copper(I) iodide and $Pd(PPh_3)_4$ were placed in a two neck round bottom flask and degassed using N_2 gas and vacuum sequentially for 10 – 15 minutes (Scheme 21). A degassed solution of THF, the base diisopropylamine and excess ethynyltrimethylsilane was discharged into the reaction medium *via* a dropping funnel. The resulting mixture was heated at 70 °C for 4 hours under a nitrogen atmosphere. After workup and purification by silica gel column chromatography, 5-[(trimethylsilyl)ethynyl]pyrimidine-4,6-diamine **100** was obtained in an average yield of 48%.

Three signals were observed in the ^{1}H NMR spectrum of **100**. A singlet in the aromatic region was assigned to H2 and a broad singlet at 6.36 ppm integrating for four protons was characteristic of the NH_2 protons. In the aliphatic region, a singlet at 0.23 ppm integrating for nine protons was assigned to the trimethylsilyl group. A close examination of the ^{13}C NMR spectrum showed two signals at 99.4 ppm and 97.2 ppm indicating the presence of the alkyne functionality. IR analysis showed a stretching band at 2136 cm^{-1}, which is an indication of the C≡C bond of the alkynyl group.

3.2.4 Synthesis of 5-ethynylpyrimidine-4,6-diamine



Scheme 22: Reagents and conditions: TBAF, THF, 0 °C - rt, 3 h, 85%.

The next step in the synthesis was to remove the trimethylsilyl group to afford the terminal alkyne. According to the literature, deprotection of the trimethylsilyl group could be achieved by using potassium carbonate in methanol[11] or tetrabutylammonium fluoride (TBAF) in THF[12]. We decided to use the TBAF method due to shorter reaction times. The desilylation reaction was carried out by dissolving the starting material **100** in THF at 0 °C. TBAF was then added to the reaction flask drop-wise (Scheme 22). The resulting mixture was warmed to room temperature and stirred for three hours. After normal workup, the crude product was purified by column chromatography to furnish the desired product **101** as a yellow solid in a good yield of 85%.

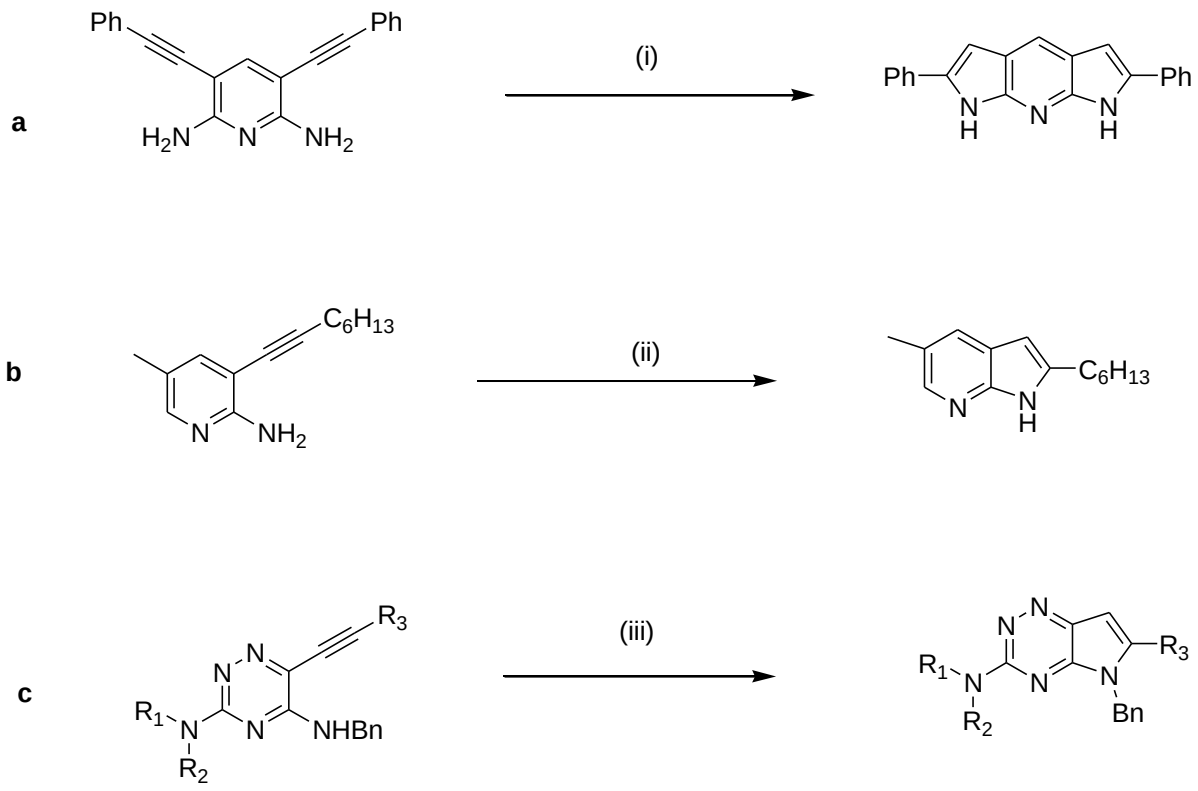
The disappearance of the characteristic upfield singlet at 0.23 ppm in the ^{1}H NMR spectrum due to the trimethylsilyl group and the appearance of a new signal at 4.57 ppm due to terminal alkyne H8 confirmed the formation of the desired product. The signal previously found at 0.00 ppm due to the trimethylsilyl group in the ^{13}C NMR spectrum had also disappeared. This further confirmed the formation of the desired product, 5-ethynylpyrimidine-4,6-diamine **101**. The alkynyl carbon signal also shifted to 77.4 ppm and 76.1 ppm for C8 and C7, respectively.

3.2.5 Heteroannulation to afford pyrrolo[2,3-*d*]pyrimidine.

3.2.5.1 Literature methods for the heteroannulation of terminal alkynes.

There are numerous methods in the literature that have been employed with the aim of converting heteroaromatic Sonogashira coupling products into indoles, pyrrolopyrimidines and azaindoles.[13] This includes the use of reagents ranging from bases to metal salts in order to achieve these conversions. This type of reaction between a terminal alkyne and an amino group to form a fused-pyrrole ring by an *endo-dig* cyclisation reaction is highly favoured according to Baldwin's rules.[14]

Koradin and co-workers treated a 2,6-aminopyridine derivative with potassium *tert*-butoxide in *N*-methylpyrrolidinone (NMP) at room temperature to afford the *bis*-7-azaindole derivative[15] in an excellent yield of 95% (Scheme 23, **a**). Other researchers have reported the use of microwave irradiation assisted synthesis for the conversion of Sonogashira products using bases and water as the solvent to form a fused-pyrrole ring (Scheme 23, **b**).[16]

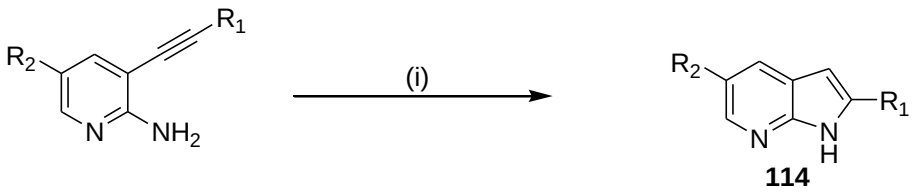


Scheme 23: Reagents and conditions: (i) KOtBu, NMP, rt, 95%, (ii) $NaHCO_3$, H_2O, 200 ^oC, 200 W, 52%, (iii) CuI (10 mol%), Et_3N, MeOH, reflux, 3h, 50 - 82%. [15-17]

Moreover, in 2007, Nyffenegger and co-workers reported the synthesis of pyrrolo[2,3-*e*]triazines by Sonogashira/copper(I)-catalysed heteroannulation in refluxing methanol (Scheme 23, **c**). As shown below, they have obtained these biologically important heterocycles in good yields.[17]

However, for the purpose of this PhD project we wanted to form the pyrrolo[2,3-*d*]pyrimidine ring by Sonogashira heteroannulation. As mentioned previously, there are various methodologies that involve substrates containing a pyridine ring but very limited ones for those containing a pyrimidine ring. In the sections that follow, our attempts at effecting this ring closing reaction will be discussed.

3.2.5.2 Attempted synthesis of 7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine using TFAA/TFA mixture

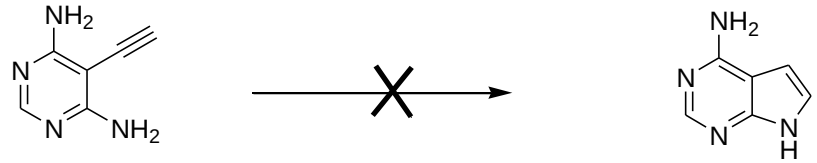


114

Scheme 24: Reagents and conditions: (i) TFA, TFAA, MeCN, 100 °C.

During his tenure at the University of the Witwatersrand, Dr Leboho and co-workers synthesised 7-azaindole derivatives of general structure **114** (Scheme 24).[18] They used an acid-catalysed reaction employing 1 equivalent of trifluoroacetic acid (TFA) and 1.3 equivalents of trifluoroacetic anhydride (TFAA) in acetonitrile to facilitate this transformation. The above reaction worked well for their synthesis and the 7-azaindole derivatives were obtained in good yields.

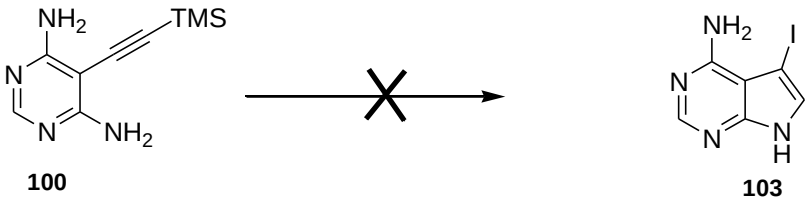
We tested this methodology using the same conditions (Scheme 25) in an attempt to form our 7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine ring **102**. Unfortunately, the methodology used to prepare 7-azaindole derivatives was not successful in our case. After workup and purification by column chromatography, the starting material and various products, not including **102**, were isolated in low yield. This might be due to the difference in the reactivity of the two substrates, pyridine vs pyrimidine. It has been reported in the literature that the 2-and 4-amino groups of pyrimidine are relatively non-nucleophilic under normal conditions.[19] This could explain why the reaction shown below in Scheme 25 was not successful.



101 **102**

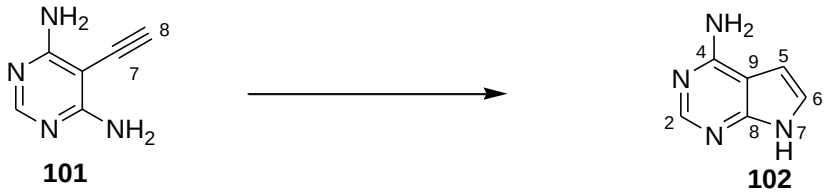
Scheme 25: Reagents and conditions: TFA, TFAA, MeCN, 100 °C.

Further attempts to facilitate the ring closure reaction included conducting the reaction using compound **100** in the presence of iodine and acetonitrile as a solvent, followed by treatment with potassium carbonate in methanol as described by Rao and co-workers (Scheme 26).[4] Unfortunately, this attempt was not successful, with only 5-ethynylpyrimidine-4,6-diamine **101** isolated as the product instead of the desired **103**.



Scheme 26: Reagents and conditions: (1) I_2, K_2CO_3, CH_3CN; (2) MeOH, K_2CO_3.

3.2.5.3 Synthesis of 7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine using microwave irradiation



Scheme 27: Reagents and conditions: Cs_2CO_3, DMSO, MW, 100 W, 180 °C, 15 min, 50%.

As we had not been successful in effecting ring closure to afford our desired pyrrolopyrimidine, we next attempted the use of microwave irradiation to perform our ring closure step. The use of microwave irradiation has been successfully applied in organic chemistry. The reaction times are short and minimal amounts of solvent are required. Hence, it is considered a green method in organic synthesis.[16]

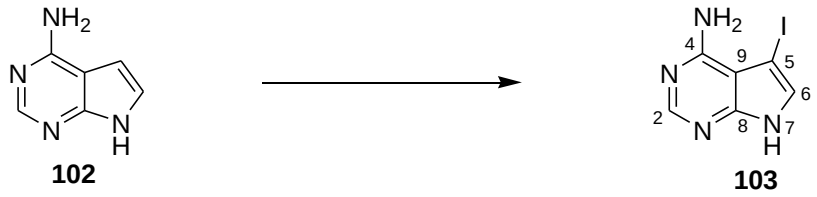
To this end, 5-ethynylpyrimidine-4,6-diamine **101** was placed in a microwave tube and treated with DMSO and cesium carbonate (Scheme 27). The resulting reaction mixture was irradiated at

100 W and 180 °C for 15 minutes and after workup and purification by silica gel column chromatography, 7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **102** was isolated in 50% yield.

The formation of the product was confirmed using NMR and infrared spectroscopy. In the ^{1}H NMR spectrum, the singlet signal at 4.57 ppm due to H8 had disappeared. The ^{1}H NMR spectrum of the product **102** contained two doublet of doublets signals at 7.05 ppm and 6.51 ppm, integrating for one proton each, accounting for H6 and H5 respectively. The broad signal at 11.42 ppm integrating for one proton was due to N-H. In the ^{13}C NMR spectrum, two signals at 77.4 and 76.1 ppm due to the C≡C bond had disappeared. The success of the reaction was further confirmed by the disappearance of the stretching band previously found at 2092 cm^{-1} in the IR spectrum due to the alkyne group.

Several other experimental methods were attempted for this ring closure in an attempt to improve the yield of product, but we were unfortunately unsuccessful in preparing the 7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine ring from 5-ethynylpyrimidine-4,6-diamine by any other method. This included the use of potassium *tert*-butoxide in *N*-methylpyrrolidinone (NMP),[15] potassium carbonate in methanol (MeOH)[20] and CuI (2 mol%) in *N*,*N*-dimethylformamide (DMF).[17] In some cases, the starting material, 5-ethynylpyrimidine-4,6-diamine, was recovered, while under other reaction conditions, unknown products were isolated.

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



102 **103**

Scheme 28: Reagents and conditions: NIS, DMF, 60 °C, 4 h, 57%.

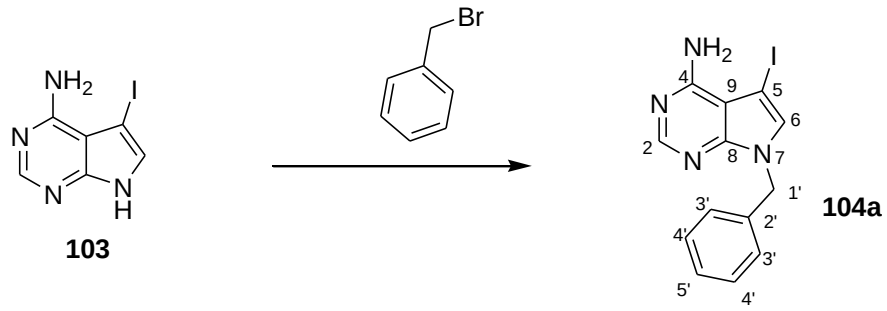
Having successfully prepared the key intermediate **102**, our next synthetic step was to functionalise the C-5 position for subsequent reaction. *N*-iodosuccinimide (NIS) is an effective iodinating agent that is used for electrophilic iodinations and as a source of iodine. 7*H*-Pyrrolo[2,3-*d*]pyrimidin-4-amine **102** was treated with NIS in DMF according to a literature procedure[20] affording the iodinated compounds **103** in 57% yield.

It is important to mention that the 5-position of the 7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine skeleton is the most reactive towards electrophilic substitution. This can be explained on the basis of π electron density, frontier electron density and localization energy.[22] Hence, iodination of this moiety will take place at the preferred 5-position.

Four signals were observed in the ^{1}H NMR spectrum of the product compared to the five signals that were previously observed in the ^{1}H NMR spectrum of the starting material. In the ^{1}H NMR spectrum, a doublet of doublets at 6.51 ppm corresponding to H5 had disappeared. A signal at 50.1 ppm in the ^{13}C NMR spectrum corresponding to C-I further confirmed the formation of the desired product. The molecular ion was confirmed by HRMS to be [M+H] 260.9641 which was consistent with a molecular mass of 260.9639, calculated for $C_6H_6IN_4$.

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

With our iodinated pyrrolopyrimidine in hand; we were now in a position to introduce functionality at N-7 and C-5.

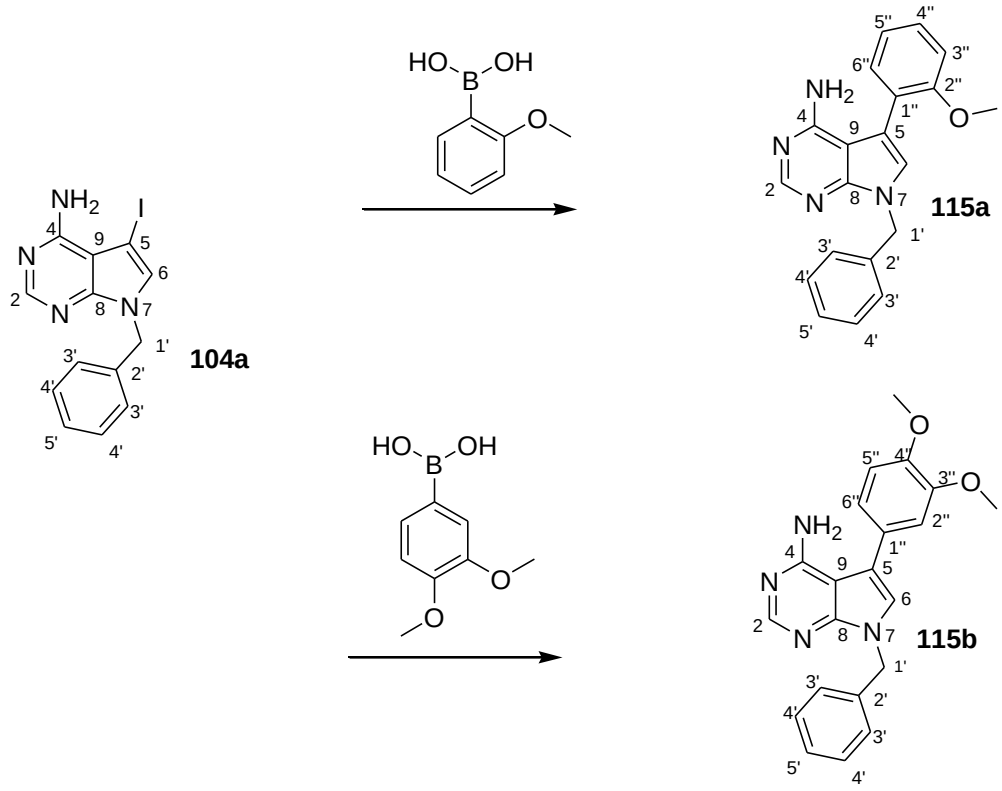


Scheme 29: Reagents and conditions: Cs_2CO_3, DMF, 70 °C, 18 h, 59%.

We initially tested the *N*-alkylation with benzyl bromide and compound **103**. It is worth mentioning that the compounds bearing the benzyl moiety at N-7 were not optimal in molecular modelling studies. However, we used this series as a model study for the development of the methodology for this library of compounds. To this end, 5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **103** was dissolved in DMF and treated with benzyl bromide. The resulting reaction mixture was heated at 70 °C for 18 hours under basic conditions (**Scheme 29**). After normal extraction and purification by column chromatography, compound **104a** was isolated as a light brown solid in an average

yield of 59%. ^{1}H NMR and ^{13}C NMR spectroscopy confirmed formation of compound **104a**. In the ^{1}H NMR spectrum, the phenyl protons appeared as a multiplet at 7.32 – 7.22 ppm integrating for five protons. A singlet at 5.32 ppm was assigned to H-1'. The mass spectrum also corresponded well with the expected mass of the product.

3.2.8 Synthesis of 7-benzyl-5-(2-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine and analogues by utilisation of the Suzuki – Miyaura cross coupling reaction.



Scheme 30: Reagents and conditions: $Pd(PPh_3)_4$, aq Na_2CO_3, DME, 18 h, 45% - 48%.

Having successfully prepared compound **104a**, we were now set for the Suzuki coupling reaction with commercially available 2-methoxyphenylboronic acid (Scheme 30) and 3,4-dimethoxyphenylboronic acid.

The Suzuki-Miyaura coupling reaction involves formation of a carbon-carbon bond. In essence, this reaction facilitates the coupling of aromatic moieties, forming a biaryl bond between these

two moieties. The reaction is usually catalysed by a palladium catalyst and it involves coupling between halide or triflate and an organoboronic acid or ester under basic conditions.[23-24] This C-C bond formation reaction finds extensive application for the preparation of pharmaceutical drugs and in the total synthesis of natural products.

The Suzuki-Miyaura cross coupling reaction begins with an oxidative addition where the aryl halide couples with the palladium catalyst Pd(0) to yield an organopalladium(II) complex (**Figure 37**). In this catalytic cycle, the oxidative addition is often the rate-determining step.[25] In general, the oxidative addition step is quicker for iodo-derivatives and much slower for chloro-compounds, roughly, following the trend: I>OTf>Br>>Cl.[26]

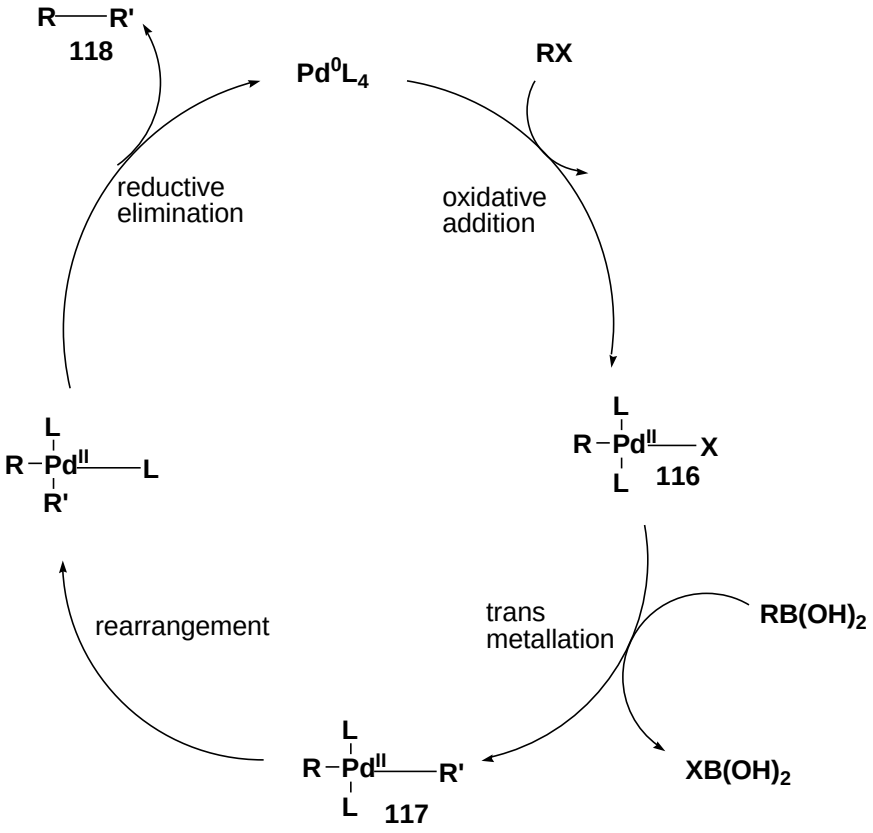


Figure 37: Mechanism of the Suzuki-Miyaura cross-coupling reaction.

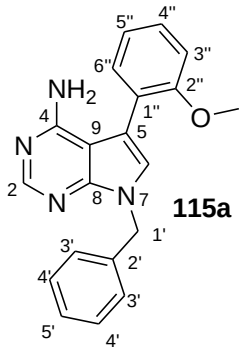
Once formed, intermediate **116** reacts with the organoboron compound to give palladium(II) complex **117**, where palladium maintains its oxidation state of +2. This step is called

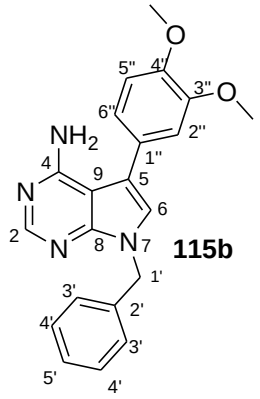
transmetallation. Once *trans/cis* rearrangement is complete, it is followed by the final step (reductive elimination) to furnish the desired product **118** and regenerates the Pd(0) species, which re-enters the catalytic cycle.

With the two moieties in hand required for the Suzuki-Miyaura coupling reaction, we were now in a position to test the final step for the preparation of potential protein kinase inhibitors containing the pyrrolo[2,3-*d*]pyrimidine core, as shown in Scheme 30.

To this end; compound **104a** was treated with catalytic $Pd(PPh_3)_4$ and commercially available boronic acids in DME at 80 °C. In each case; a new product was isolated in moderate yields. **Table 1** below shows the key diagnostic signals found in both ^{1}H NMR and ^{13}C NMR spectra for each of the products. The HRMS results of the products are also given in the table. In each case, the dehalogenated starting material was recovered from the reaction, accounting for the moderate yields of the product. C5 in compounds **115a-b** was now observed at approximately 126 ppm.

Table 1: Key spectroscopic signals for Suzuki coupling products **115a-b** found in both ^{1}H NMR and ^{13}C NMR spectra.

Entry	Product	Yield (%)	NMR spectroscopic information/ ppm (^{1}H and ^{13}C)	HRMS
1	 **115a**	48	^{1}H: 8.13 (s, H2), 7.66 – 7.58 (m, H6'), 3.78 (s, OMe). ^{13}C: 158.3 (C-4), 151.7 (C-2), 125.5 (C-5), 101.4 (C-6), 56.0 (OMe).	Calculated for $C_{20}H_{19}N_4O$: 331.1561, found: $[M + H]^+$ 331.1534.

2	 **115b**	45	^{1}H: 8.16 (1H, s, H2), 6.15 (2H, s, NH_2), 3.79 (s, OMe), 3.789 (s, OMe). ^{13}C: 157.7 (C-4), 152.2 (C-2), 127.6 (C-5), 56.1 (OMe), 55.9 (OMe), 47.6 (C-1').	Calculated for $C_{21}H_{21}N_4O_2$: 361.1666, found: $[M + H]^+$ 361.1622.

As mentioned above, results from the molecular modelling showed that the benzyl moiety is not accommodated particularly well in the active site of the enzyme. Hence, only two final compounds of this series were prepared. However, the methodology we have developed for the synthesis of substituted pyrrolopyrimidines appears to work well, and as such we embarked on the synthesis of analogues. To this end, a number of boronic acids were prepared which could be utilised in our synthetic route.

3.2.9 Preparation of 6-methoxynaphthalen-2-yl-2-boronic acid and analogues.[27-28]

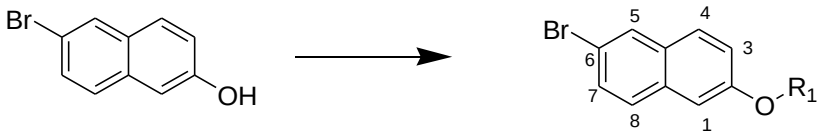
The last step of the synthesis of our novel protein kinase inhibitors containing the pyrrolo[2,3-*d*]pyrimidine skeleton is to perform a Suzuki coupling reaction using a palladium catalyst and an appropriate boronic acid or boronate pinacol ester. While some boronic acids are commercially available, we had to prepare a range of boronic acids containing a naphthalene skeleton. Boronate pinacol esters can be prepared from bis(pinacolato)diboron, Pd(II)Cl_2(dppf), KOAc in DMSO or dioxane at 85 °C,[29] whereas boronic acids can be prepared by utilising conditions (*n*-butyllithium, triisopropyl borate in THF) well known in the literature.[30-31] Although both methods are easy to perform, we chose to prepare the boronic acids due to economic reasons.

Hence, a number of aryl boronic acids were prepared by first alkylating 6-bromonaphthalen-2-ol **119** using alkyl halides. This reaction was performed by dissolving 6-bromonaphthalen-2-ol in acetone and to this was added the base potassium carbonate (K_2CO_3) followed by the alkyl halide (Scheme 31). The reaction mixture was refluxed for 18 hours. The progress of the reaction

was monitored by TLC (20% EtOAc/hexane). After filtration and removal of the solvent *in vacuo*, the crude product was purified by silica gel chromatography. In the synthesis of 2-bromo-6-methoxynaphthalene **120a**, compound **119** was treated as described with methyl iodide to afford the product as a cream white solid in 93% yield.

The success of the reaction was confirmed by NMR spectroscopy. In the ^{1}H NMR spectrum, the aromatic region contained six signals as expected. In the aliphatic region, a definitive signal that confirmed the success of the reaction was the singlet visible at 3.89 ppm due to the methyl group. The ^{13}C NMR spectrum showed a signal at 55.3 ppm due to the methoxy carbon.

Two other analogues **120b-120c** were prepared in a similar manner using the above procedure (Scheme 31). **Table 2** below shows the results obtained for the different alkyl halides used together with the NMR spectroscopic information of each of the products.

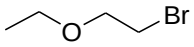
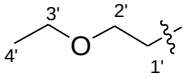


119 **120a-c**

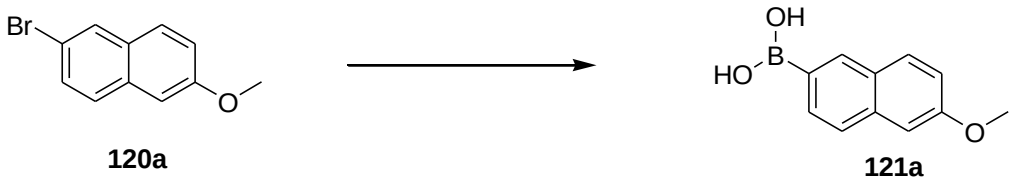
Scheme 31: Reagents and conditions: K_2CO_3, alkyl halide, acetone, reflux, 85 - 93%.

Table 2: Key signals in the ^{1}H NMR spectra of alkylated compounds **120a-120c**.

Entry	Alkyl halide used	R_1	Product (% yield)	NMR spectroscopic information/ ppm (^{1}H)
1	CH_3—I	CH_3– **120a**	93	^{1}H: 7.89 (d, *J* = 2.0 Hz, H5), 3.89 (3H, s, OMe)
2	CH_3CH_2—Br	CH_3CH_2— $H^{2'}$ $H^{1'}$ **120b**	88	^{1}H: 4.11 (q, *J* = 7.0 Hz, H1'), 1.46 (3H, t, *J* = 7.0 Hz, H2')

3		 **120c**	85	^{1}H: 3.84 – 3.82 (2H, m, H2'), 3.61 (2H, q, *J* = 7.0 Hz, H3'), 1.25 (3H, t, *J* = 7.0 Hz, H4')

For our specific needs, as a precursor to the Suzuki-Miyaura coupling reaction, it was essential to synthesise the boronic acids from the previously prepared 2-bromo-6-methoxynaphthalene **120a** and analogues. The preparation of an aryl boronic acid is achieved by firstly forming an organolithium moiety from an aromatic or heteroaromatic halide. The organolithium intermediate is then treated with trimethyl borate or triisopropyl borate. The newly formed aryl-borate ester is then hydrolysed to the corresponding boronic acid using dilute acid.

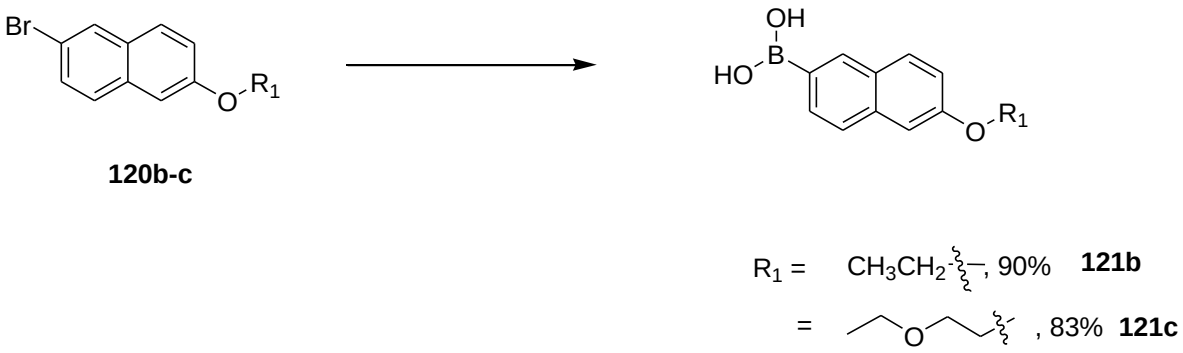


120a **121a**

Scheme 32: Reagents and conditions: *n*-BuLi, $B(O^iPr)_3$, dry THF, -78 °C, then HCl (aq), 4 h, 89%.

Using this protocol, 6-methoxynaphthalen-2-yl-2-boronic acid **121a** was prepared by treating 2-bromo-6-methoxynaphthalene **120a** with *n*-BuLi (2.0 M/THF) in dry THF at -78 °C under a nitrogen atmosphere (Scheme 32). To this was added an excess of triisopropyl borate *via* a syringe. The reaction was then treated with 10% aqueous hydrochloric acid to hydrolyse the borate ester to the corresponding boronic acid. TLC analysis showed the formation of the boronic acid as it is more polar than the starting material 2-bromo-6-methoxynaphthalene. However, 6-methoxynaphthalen-2-yl-2-boronic acid **121a** was not purified further due to instability (decomposes on silica) and was used as isolated in the next step.

Two other aryl boronic acid analogues **121b-121c** were prepared using the above procedure (Scheme 33). All boronic acid analogues were used in the subsequent coupling step without purification.

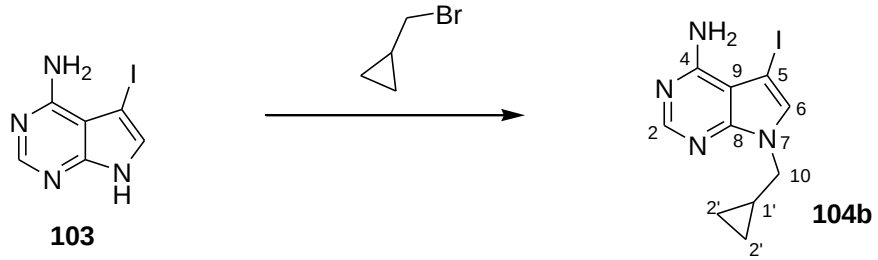


Scheme 33: Reagents and conditions: (i) *n*-BuLi, $B(O^iPr)_3$, dry THF, -78 °C, the HCl (aq), 4 h.

3.2.10 Synthesis of 7-(cyclopropylmethyl)-5-(2-methoxynaphthalen-6-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine and analogues.

With the successful synthesis of compound **103** completed, we were now able to continue with the next step of our synthesis.

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



Scheme 34: Reagents and conditions: Cs_2CO_3, DMF, 70 °C, 18 h, 57%.

The heterocyclic compound **103** was treated with a series of alkyl bromides/tosylates. In the first instance, the reaction was carried out by dissolving the starting material **103** in dry DMF. Cesium carbonate (Cs_2CO_3), was then added to the reaction flask in one portion. This was followed by a dropwise addition of (bromomethyl)cyclopropane *via* a syringe (Scheme 34). The resultant reaction mixture was heated at 70 °C for 18 hours and the progress of the reaction was

monitored by thin layer chromatography (TLC) until complete consumption of the starting material was observed. After normal extraction, removal of the organic solvent *in vacuo* on a rotary evaporator and purification by silica gel column chromatography, compound **104b** was obtained as a light orange solid in an average yield of 57%.

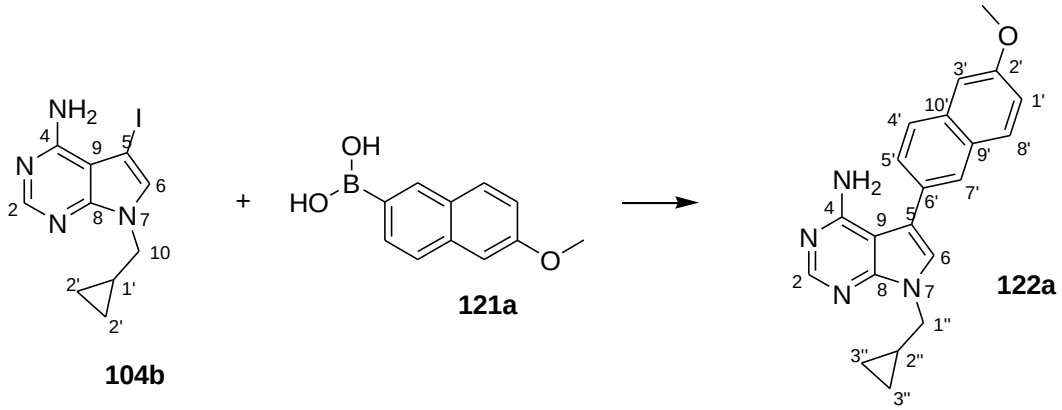
In the ^{1}H NMR spectrum, the singlet signal due to the N-H proton of the starting material at 11.95 ppm had disappeared. The multiplet at 0.51 – 0.35 ppm was assigned to the two CH_2 groups of the cyclopropyl group. A broad signal at 6.59 ppm that integrated for two protons was assigned to the amino group. A signal at 48.7 ppm in the ^{13}C NMR spectrum was assigned to C-5 of the pyrrolopyrimidine ring. Aliphatic carbon signals were observed at 49.8 ppm, 12.1 ppm and 4.1 ppm. The molecular ion was confirmed by HRMS to be [M+H] 315.0118 which was consistent with a molecular mass of 315.0108 (calculated for $C_{10}H_{12}IN_4$).

Alkylation to introduce other moieties onto the N-7 nitrogen atom of the pyrrolo[2,3-*d*]pyrimidine skeleton will be discussed later.

3.2.10.2 Suzuki-Miyaura coupling to synthesise 7-(cyclopropylmethyl)-5-(2-methoxynaphthalen-6-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine and analogues.

With the two moieties in hand required for the Suzuki-Miyaura coupling reaction, we were now in a position to prepare the desired final compounds, potential protein kinase inhibitors containing the pyrrolo[2,3-*d*]pyrimidine core, as shown in Scheme 35. Hence, 7-(cyclopropylmethyl)-5-(2-methoxynaphthalen-6-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **122a** was synthesised by placing 7-(cyclopropylmethyl)-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **104b**, $Pd(PPh_3)_4$ and 6-methoxynaphthalen-2-yl-2-boronic acid **121a** in a flame dried 2-neck round bottom flask under a nitrogen atmosphere. The dropping funnel containing the aqueous sodium carbonate solution and 1,2-dimethoxyethane was degassed for 10-15 minutes and discharged into the reaction vessel. The resultant mixture was then heated at 80 °C for 18 hours

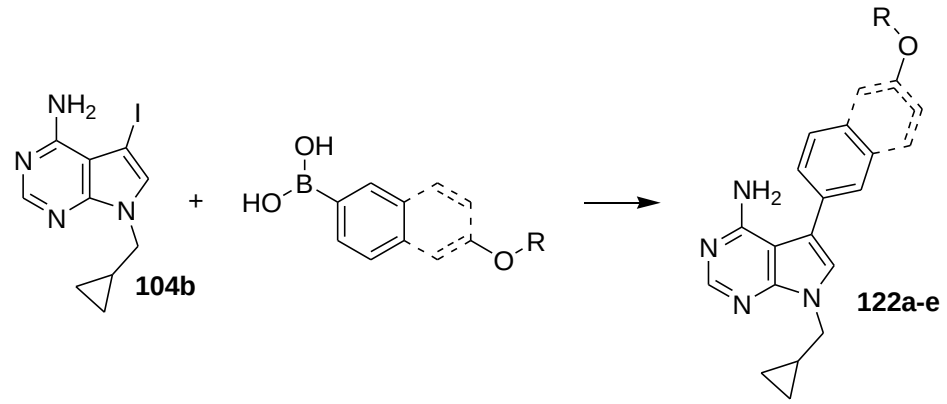
under a nitrogen atmosphere. After workup and purification by silica gel column chromatography the desired coupled product **122a** was obtained in a yield of 55%.



Scheme 35: Reagents and conditions: cat. $Pd(PPh_3)_4$, aq Na_2CO_3, DME, 18 h, 55%.

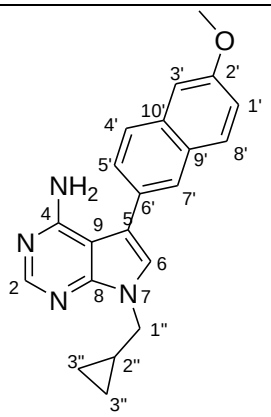
The formation of 7-(cyclopropylmethyl)-5-(2-methoxynaphthalen-6-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine was confirmed by both ^{1}H NMR and ^{13}C NMR spectroscopy. In the ^{1}H NMR spectrum, eight signals were observed in the aromatic region as expected. This is due to the six protons from the naphthalene moiety and two protons (H2 and H6) from the pyrrolo[2,3-*d*]pyrimidine core. Two multiplets at 0.65 – 0.46 ppm each integrating for two protons were assigned to the two CH_2 groups (H3") of the cyclopropyl group. Aliphatic carbon signals were observed at 52.9 ppm, 47.1 ppm, 9.6 ppm and 1.4 ppm. The IR spectrum showed a broad, strong N-H stretch at 3462 cm^{-1}. The molecular ion was confirmed by HRMS to be [M+H] 345.1694 which was consistent with a molecular mass of 345.1717, calculated for $C_{21}H_{21}N_4O$.

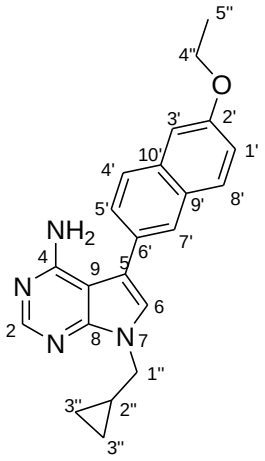
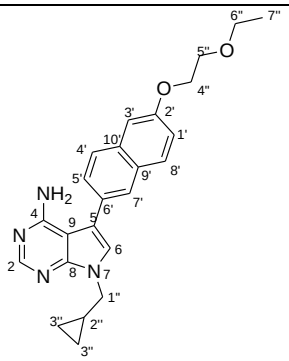
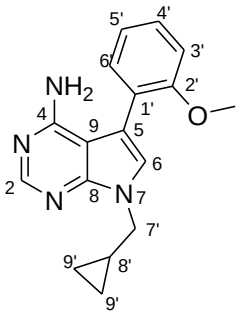
Two other analogues **122b-122c** were prepared in a similar manner using the above procedure (Scheme 36). In addition; the reaction was carried out with commercially available boronic acids; 2-methoxyphenylboronic acid and 3,4-dimethoxyphenylboronic acid to afford products **122d-e**. **Table 3** below shows the results obtained for the different boronic acids used together with key spectroscopic data of each of the products.

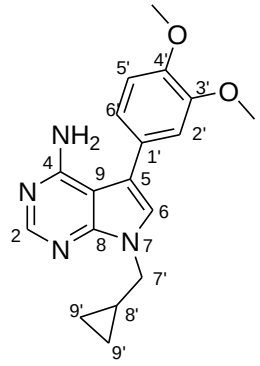


Scheme 36: Reagents and conditions: $Pd(PPh_3)_4$, aq Na_2CO_3, DME, 18 h, 35 - 69%.

Table 3: Spectroscopic data of the Suzuki coupling reaction products **122a-e**.

Entry	Product	Product (% yield)	Key NMR spectroscopic signals/ppm (^{1}H and ^{13}C)	HRMS
122a		55	^{1}H: 8.17 (s, H2), 7.91 – 7.88 (m, H4' and H7'), 3.94 (s, OMe), 0.65 – 0.59 (m, H3''), 0.49 – 0.46 (m, H3''). ^{13}C: 156.5 (C-4), 155.7 (C-2), 148.9 (C-2'), 148.0 (C-8), 52.9 (OMe), 9.6 (C-2''), 1.4 (C-3'').	Calculated for $C_{21}H_{21}N_4O$: 345.1717, found: $[M + H]^+$ 345.1694.

122b		60	^{1}H: 8.18 (H2), 6.19 (s, NH_2), 4.17 (q, *J* = 6.9 Hz, H4"), 1.41 (t, *J* = 6.9 Hz, H5"). ^{13}C: 155.7 (C-4), 149.1 (C-2), 47.1 (C-1"), 12.2 (C-5"), 9.6 (C-2").	Calculated for $C_{22}H_{23}N_4O$: 359.1874, found: $[M + H]^+$ 359.1848.
122c		35	^{1}H: 8.19 (H2), 4.29 – 4.26 (m, H4"), 3.90 – 3.87 (m, H5"). ^{13}C: 155.6 (C-4), 147.9 (C-2), 65.8 (C-4"), 64.9 (C-6"), 12.5 (C-7"), 9.6 (C-2").	Calculated for $C_{24}H_{27}N_4O$: 403.2136, found: $[M + H]^+$ 403.2116.
122d		55	^{1}H: 8.02 (H2), 3.72 (OMe). ^{13}C: 161.6 (C-4), 160.9 (C-2'), 52.5 (OMe).	Calculated for $C_{17}H_{19}N_4O$: 295.1561, found: $[M + H]^+$ 295.1548.

122e		69	^{1}H: 7.98 (H2), 3.66 (s, OMe), 3.65 (s, OMe). ^{13}C: 157.7 (C-4), 151.9 (C-2), 150.4 (C-8), 149.4 (C-4'), 56.1 (OMe), 55.9 (OMe).	Calculated for $C_{18}H_{21}N_4O_2$: 325.1666, found: $[M + H]^+$ 325.1672.

Analysis of the results represented in **Table 3** shows that these novel compounds were obtained in average to good yields of 35 – 69%. As observed in Table 3, HRMS also confirmed the formation of compounds **122a-122e**. Yields of some final products, especially those below 50% could still be improved by revisiting the conditions. Nonetheless, of importance would be the biological screening of these substituted 7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amines for antiplasmodial activity in an *in vitro* screen and activity against *Pf*CDPK4 in a biochemical enzyme assay. Optimisation of the reaction conditions for each product was therefore not carried out at this stage.

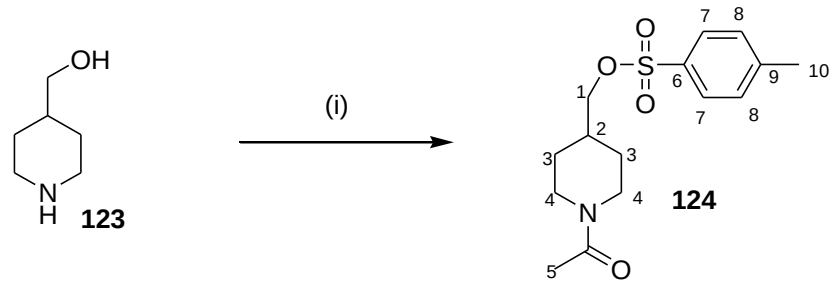
3.2.11 Synthesis of 1-{4-[(4-amino-5-(2-methoxynaphthalen-6-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone and analogues.

Based on the promising results from our molecular modelling studies, we decided to prepare inhibitors bearing the piperidine moiety at N-7 of the 7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine core.

3.2.11.1 Synthesis of (1-acetylpiperidin-4-yl)methyl 4-methylbenzenesulfonate and 1-{4-[(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone

To this end, (1-acetylpiperidin-4-yl)methyl 4-methylbenzenesulfonate was prepared in two steps by firstly treating (piperidin-4-yl)methanol **123** with one equivalent of acetic anhydride in dichloromethane at room temperature for 4 hours under basic conditions.[2] It should be noted that

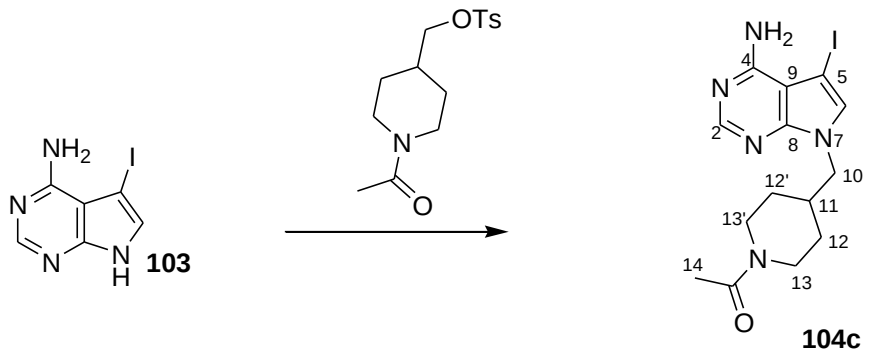
under these conditions the reaction takes place at the more nucleophilic N-H group and not the OH group. The acylated product that formed was not isolated but rather reacted with 4-toluenesulfonyl chloride (TsCl) in order to convert the OH group into a better leaving group (-OTs). To this end, 4-toluenesulfonyl chloride was added slowly to the same reaction vessel and stirred for 5 hours at room temperature. Thin layer chromatography (TLC) showed formation of a new product spot. After workup with water and ethyl acetate, removal of the organic solvent *in vacuo* on a rotary evaporator and purification by silica gel column chromatography, compound **124** was obtained as a viscous oil in an average yield of 48% (Scheme 37).



Scheme 37: Reagents and conditions: (i): (a) Ac_2O, Et_3N, DCM, 4 h, rt, (b) TsCl, 5 h, rt, 48%.

The formation of **124** was confirmed by both ^{1}H NMR and ^{13}C NMR spectroscopy. The ^{1}H NMR spectrum contained two doublets at 7.78 ppm and 7.36 ppm due to H7 and H8, respectively. In the aliphatic region, a singlet integrating for three protons at 2.06 ppm was due to H5. The ^{13}C NMR spectrum of the product showed eleven signals in total, as expected. Notably, a signal at 168.8 ppm was due to the carbonyl carbon, and four signals in the aromatic region were indicative of the phenyl group.

Compound **124** was then subjected to the *N*-alkylation reaction already described with compound **103** using cesium carbonate as the base in dry DMF. The product of this reaction, 1-{4-[(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone **104c** was obtained as a yellow solid in 79% yield (Scheme 38).

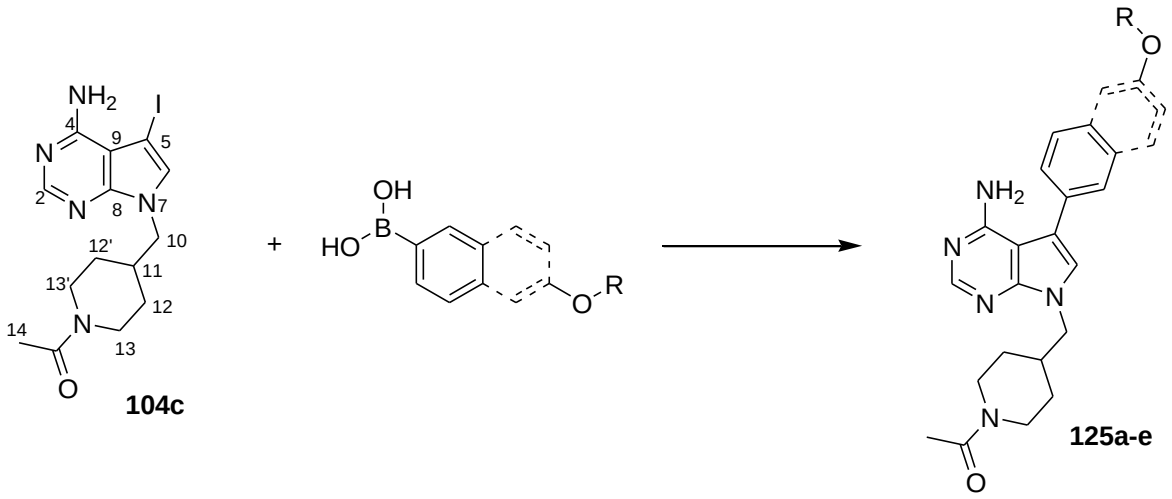


Scheme 38: Reagents and conditions: Cs_2CO_3, DMF, 80 °C, 18 h, 79%.

^{1}H NMR and ^{13}C NMR spectra confirmed the formation of this product. In the ^{1}H NMR spectrum, three signals were observed due to H2, H6 and NH_2 as expected. The piperidine moiety was observed as a series of signals at 4.31 – 0.95 ppm. The IR spectrum also confirmed the presence of the C=O group with a signal visible at 1649 cm^{-1}. The molecular ion was confirmed by HRMS to be $[M + H]^+$ 400.0597 which was consistent with a molecular mass of 400.0636, calculated for $C_{14}H_{19}IN_5O$.

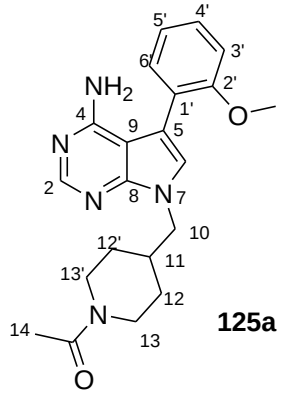
3.2.11.2 Suzuki-Miyaura coupling to synthesise 1-{4-[(4-amino-5-(2-methoxynaphthalen-6-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone and analogues.

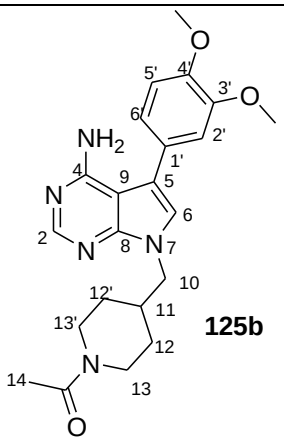
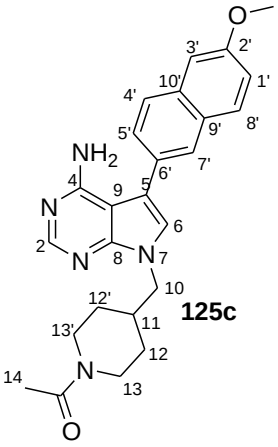
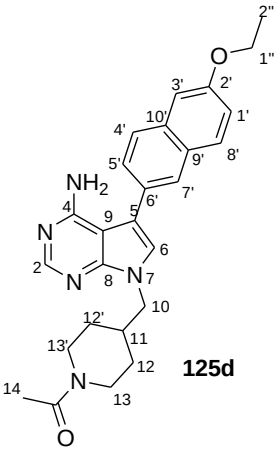
Having successfully prepared compound **104c**, we were now set for the Suzuki-Miyaura coupling reaction with different boronic acids (Scheme 39). The reaction was carried out as described for compounds **115a-b** in section **3.2.8**. The success of this coupling reaction in each case was confirmed by NMR and IR spectroscopy and HRMS of the products isolated. Diagnostic signals for the novel potential kinase inhibitors **125a-125e** are tabulated below (**Table 4**).

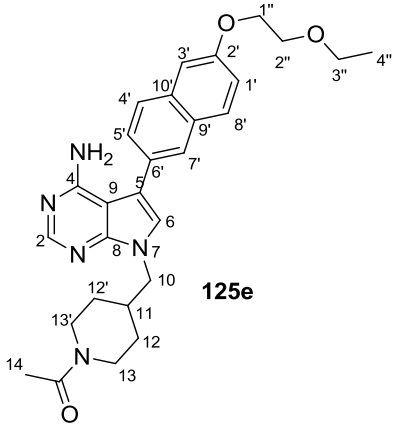


Scheme 39: Reagents and conditions: $Pd(PPh_3)_4$, aq Na_2CO_3, DME, 18 h, 54 - 76%.

Table 4: Key spectroscopic signals for Suzuki products **125a-e**.

Product	Yield (%)	NMR spectroscopic information/ ppm (^{1}H and ^{13}C)	HRMS
 **125a**	54	^{1}H: 8.14 (s, H2), 3.83 (s, OMe), 2.10 (s, H14). ^{13}C: 170.1 (C=O), 157.7 (C-4), 49.3 (OMe), 19.8 (C-14).	Calculated for $C_{21}H_{26}N_5O_2$: 380.2088, found: $[M + H]^+$ 380.2044.

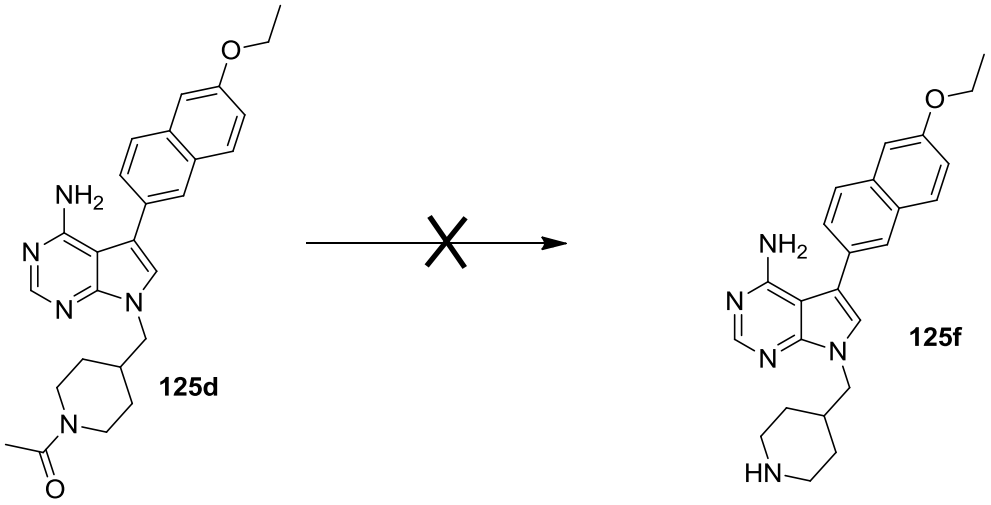
 **125b**	66	^{1}H: 8.04 (s, H2), 4.02 (d, *J* = 7.3 Hz, H10), 3.78 (s, OMe), 3.77 (s, OMe). ^{13}C: 170.0 (C=O), 157.4 (C-4), 49.2 (OMe), 45.9 (OMe).	Calculated for $C_{22}H_{28}N_5O_3$: 410.2194, found: $[M + H]^+$ 410.2153.
 **125c**	74	^{1}H: 8.20 (s, H2), 7.92 − 7.89 (m, H4' and H7'), 2.10 (s, H14). ^{13}C: 170.1 (C=O), 158.1 (C-4), 157.1 (C-2'), 19.8 (C-14).	Calculated for $C_{25}H_{28}N_5O_2$: 430.2245, found: $[M + H]^+$ 430.2214.
 **125d**	76	^{1}H: 8.20 (s, H2), 7.89 – 7.80 (m, H4' and H7'), 1.49 (t, *J* = 7.0 Hz, H2"). ^{13}C: 170.0 (C=O), 157.3 (C-4), 157.2 (C-2'), 19.8 (C-14), 13.7 (C-2").	Calculated for $C_{26}H_{30}N_5O_2$: 444.2401, found: $[M + H]^+$ 444.2375.

 **125e**	54	^{1}H: 4.29 – 4.20 (m, H1"), 4.20 (d, *J* = 7.3 Hz, H10), 3.90 – 3.89 (m, H2"), 2.11 (s, H14). ^{13}C: 168.3 (C=O), 156.9 (C-2'), 21.8 (C-14), 15.6 (C-4").	Calculated for $C_{28}H_{34}N_5O_3$: 488.2663, found: $[M + H]^+$ 488.2622.

Products **125a-e** were obtained in moderate yields. The formation of these products was confirmed using NMR and IR spectroscopy and HRMS. In this case, the ^{1}H NMR spectra played a very important role especially by the presence of new signals in the aromatic region of the newly formed products. In the ^{13}C NMR spectra, the carbonyl carbon (C=O) signal was visible at approximately 170.0 ppm for each product. As the piperidine moiety prefers a chair conformation similar to cyclohexane, the protons gave rise to signals grouped according to their axial or equatorial positions. The equatorial and axial protons for H13 were visible at approximately 4.00 ppm and 3.00 ppm, respectively. While the equatorial and axial protons for H12 were visible at approximately 1.60 ppm and 1.25 ppm, respectively.

From our molecular modelling studies, we hoped to prepare analogues with the potential to form a H-bond with Glu154 in the sugar binding pocket. While the acyl group acts as a H-bond acceptor to the Glu154 NH, there is also the potential for a H-bond to the Glu154 carbonyl group. We therefore considered removing the acyl group on the piperidine moiety for this purpose.

We attempted to remove the acyl group using sodium hydroxide in methanol for 18 hrs (Scheme 40).[32] However, this attempt to get the deprotected compound **125f** was unsuccessful. The majority of the starting material (95%) was recovered in this case.

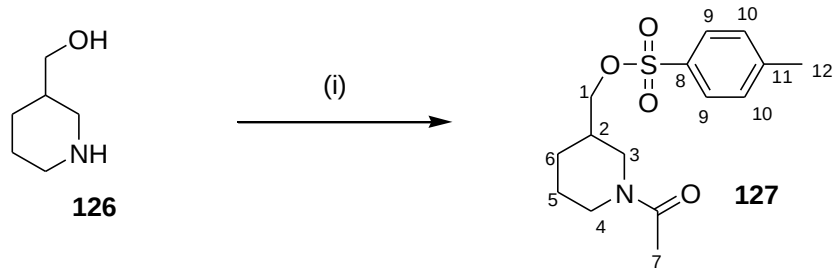


Scheme 40: Reagents and conditions: NaOH, methanol, rt 18 h.

3.2.12 Synthesis of 1-{3-[(4-amino-5-(2-ethoxynaphthalen-6-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone and analogues.

The results from our molecular modelling studies also showed that the hydrogen bonding interaction can be improved when (piperidin-3-yl)methanol is used. Hence, the potential kinase protein inhibitors bearing this moiety at N-7 were prepared in a similar manner to that of (piperidin-4-yl)methanol described above (Scheme 37).

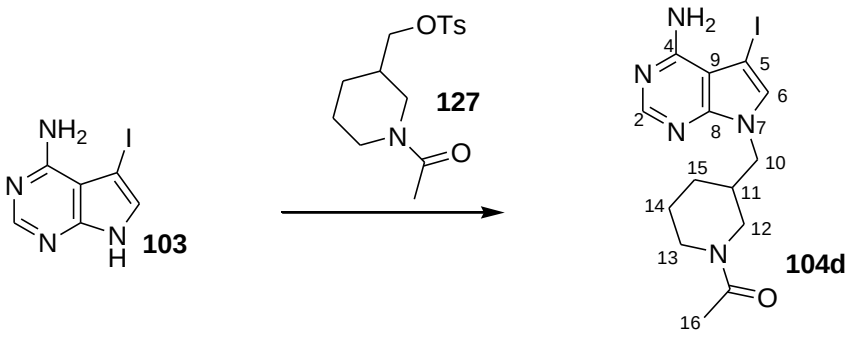
3.2.12.1 Synthesis of (1-acetylpiperidin-3-yl)methyl 4-methylbenzenesulfonate and 1-{3-[(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone



Scheme 41: Reagents and conditions: (i): (a) Ac_2O, Et_3N, DCM, 4 h, rt, (b) TsCl, 5 h, rt, 52%.

To this end, (1-acetylpiperidin-3-yl)methyl 4-methylbenzenesulfonate was prepared in two steps by firstly treating (piperidin-3-yl)methanol **126** with one equivalent of acetic anhydride in dichloromethane at room temperature for 4 hours under basic conditions. The acylated product that formed was not isolated but rather reacted with 4-toluenesulfonyl chloride (TsCl) in order to convert the OH group into a better leaving group (-OTs). To this end, 4-toluenesulfonyl chloride was added slowly to the same reaction vessel and stirred for 5 hours at room temperature. Thin layer chromatography (TLC) showed formation of a new product spot. After normal extraction, removal of the organic solvent *in vacuo* on a rotary evaporator and purification by silica gel column chromatography, compound **127** was obtained as a viscous oil in an average yield of 52% (Scheme 41). Similar changes were also observed in the NMR spectra of the product obtained in this case. The formation of **127** was confirmed by both ^{1}H NMR and ^{13}C NMR spectroscopy. The ^{1}H NMR spectrum contained two doublets at 7.78 ppm and 7.36 ppm due to H9 and H10, respectively. In the aliphatic region, a singlet integrating for three protons at 2.06 ppm was due to H7. In the ^{13}C NMR spectrum, a signal at 168.8 ppm was an indicative of the carbonyl carbon.

Compound **127** was then subjected to the *N*-alkylation reaction already described with compound **103** using cesium carbonate as the base in dry DMF. The product of this reaction, 1-{3-[(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone **104d** was obtained as a yellow solid in 63% yield (Scheme 42).

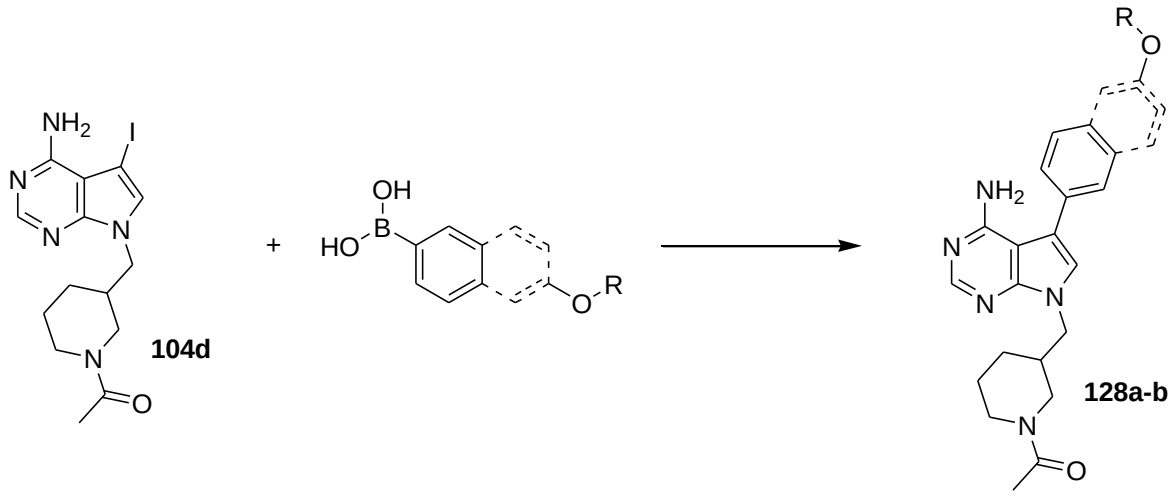


Scheme 42: Reagents and conditions: Cs_2CO_3, DMF, 80 °C, 18 h, 63%.

^{1}H NMR and ^{13}C NMR spectra confirmed the formation of this product. In the ^{1}H NMR spectrum, the piperidine moiety was observed as a series of signals at 3.37 – 0.51 ppm. In the ^{13}C NMR spectrum, a signal at 170.3 ppm due to the carbonyl was visible. The IR spectrum also confirmed the presence of the C=O group with a signal visible at 1649 cm^{-1}. The molecular ion was confirmed by HRMS to be $[M + H]^+$ 400.0597 which was consistent with a molecular mass of 400.0636 (calculated for $C_{14}H_{19}IN_5O$).

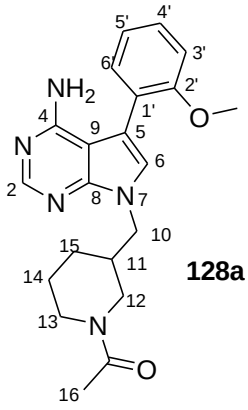
3.2.12.2 Suzuki-Miyaura coupling to synthesise 1-{3-[(4-amino-5-(2-ethoxynaphthalen-6-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone and analogues.

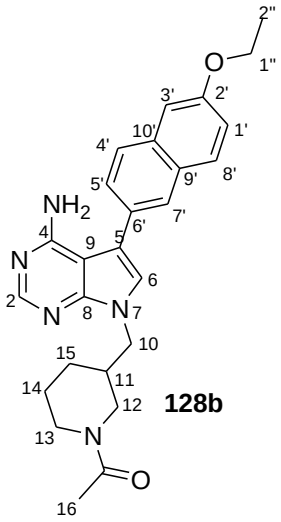
Having successfully prepared compound **104d**, we were now set for the Suzuki-Miyaura coupling reaction with two different boronic acids (Scheme 43). The reaction was carried out as described for compounds **115a-b** in section **3.2.8** above. The success of this coupling reaction in each case was confirmed by NMR and IR spectroscopy and HRMS of the products isolated. Diagnostic signals for the novel potential kinase inhibitors **128a-128b** are tabulated below (**Table 5**). The acyl signal for both compounds **128a-128b** was still visible at 1.28 ppm. The formation of compound **128b** was also confirmed by the appearance of aromatic signals at 7.10 – 6.36 ppm.



Scheme 43: Reagents and conditions: cat. $Pd(PPh_3)_4$, aq Na_2CO_3, DME, 18 h, 44 - 49%.

Table 5: Key spectroscopic signals for Suzuki products **128a-b**.

Product	Yield (%)	NMR spectroscopic information/ ppm (^{1}H and ^{13}C)	HRMS
 **128a**	49	^{1}H: 8.14 (s, H2), 3.83 (s, OMe), 1.27 (s, H16). ^{13}C: 170.1 (C=O), 49.3 (OMe), 19.8 (C-16).	Calculated for $C_{21}H_{26}N_5O_2$: 380.2088, found: $[M + H]^+$ 380.2044.

 **128b**	44	^{1}H: 7.40 (s, H2), 6.52 – 6.43 (m, H3' and H6), 1.28 (s, H16). ^{13}C: 170.3 (C=O), 157.2 (C-4), 150.9 (C-2'), 19.8 (C-16), 13.7 (C-2'').	Calculated for $C_{26}H_{30}N_5O_2$: 444.2401, found: $[M + H]^+$ 444.2375.

Only two compounds bearing the 3-piperidin-1-yl side chain were prepared. This is due to the fact that the Suzuki-Miyaura coupling reaction with this derivative did not go to completion when using the other boronic acids prepared in our laboratories. The products were also difficult to purify because they typically had the same retention factor (R_f) as the staring material. Unfortunately, all attempts to get the reaction to go to completion; and alternative purification protocols (e.g. preparative layer chromatography and recrystallization) were not successful.

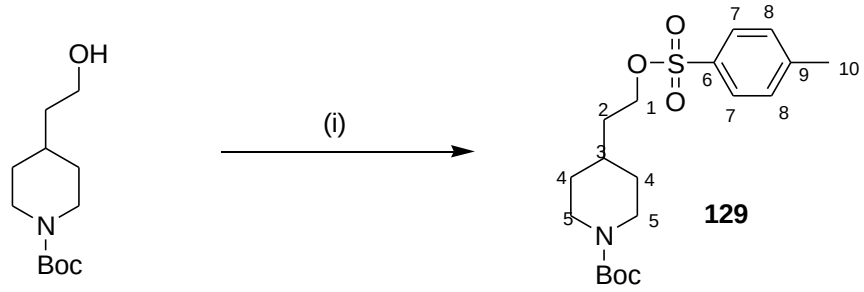
3.2.13 Synthesis of *tert*-butyl 4-{2-[4-amino-5-(3,4-dimethoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl]ethyl}piperidine-1-carboxylate and analogues

In our continued search for the potential kinase protein inhibitors, we were interested in lengthening the chain of the moiety at N-7 by one carbon to see the effect this would have on biological activity. Ideally, we would like to target an additional H-bonding interaction with Glu154 in the active site of *Pf*CDPK4. In the modelling studies, the moieties bearing a two carbon-linker appear to be within proximity to interact with the hydrophobic residues in the ATP-binding site by means of van der Waals interactions. It should also be noted that these types of inhibitors still form hydrogen bonding with the Asp148 and Tyr150 residues *via* the 7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine core. Taking into consideration what is commercially available, we decided to use *tert*-butyl 4-(2-hydroxyethyl)piperidine-1-carboxylate and 4-(2-

chloroethyl)morpholine hydrochloride for this purpose. We also hoped to be able to remove the Boc protecting group to allow for potential H-bonding.

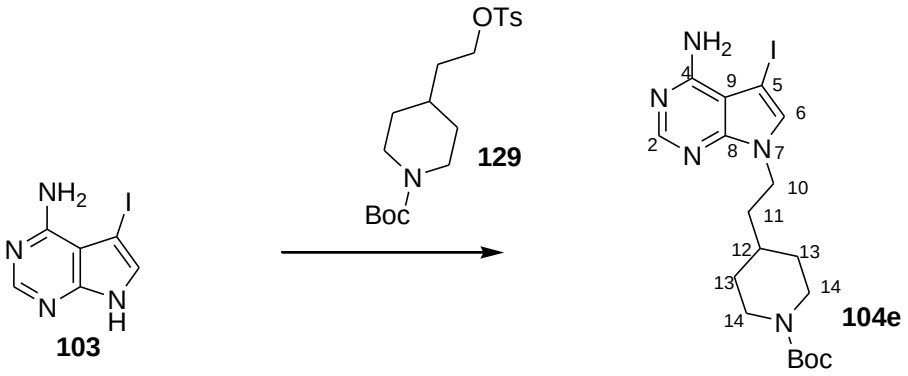
3.2.13.1 Synthesis of 2-[1-(*tert*-butoxycarbonyl)piperidin-4-yl]ethyl 4-methylbenzenesulfonate and *tert*-butyl 4-[2-(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)ethyl]piperidine-1-carboxylate

t*ert*-Butyl 4-(2-hydroxyethyl)piperidine-1-carboxylate was introduced onto the 7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine core by firstly reacting it with 4-toluenesulfonyl chloride in dichloromethane for 5 hours under basic conditions (Scheme 44). The reaction was monitored by thin layer chromatography using 80% EtOAc/hexane. 2-[1-(*tert*-Butoxycarbonyl)piperidin-4-yl]ethyl 4-methylbenzenesulfonate **129** (1.85 g, 55%) had the same retention factor (R_f) as the starting material, hence it was used in the next step without purification. However; from the ^{1}H NMR spectrum key signals for Boc and tosyl groups were visible.



Scheme 44: Reagents and conditions: (i) TsCl, 5 h, rt, 55%.

2-[1-(*Tert*-butoxycarbonyl)piperidin-4-yl]ethyl 4-methylbenzenesulfonate **129** was then reacted with 5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **103** in dry DMF under basic conditions. The resultant reaction mixture was heated at 70 °C for 18 hours and the progress of the reaction was monitored by thin layer chromatography (TLC) until complete consumption of the starting material. After normal extraction, removal of the organic solvent *in vacuo* on a rotary evaporator and purification by silica gel column chromatography, compound **104e** was obtained in a good yield of 66% (Scheme 45).



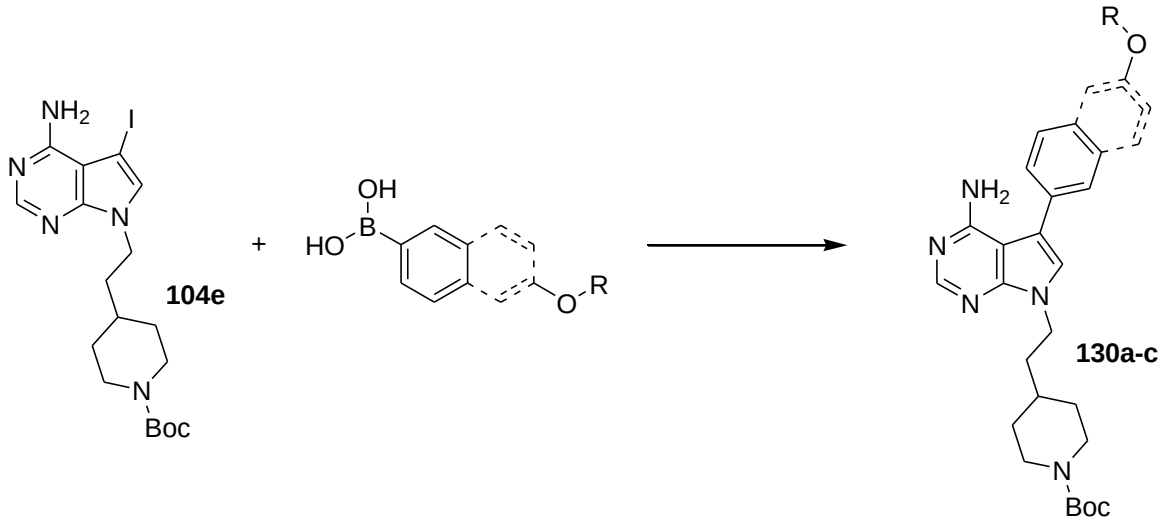
Scheme 45: Reagents and conditions: Cs_2CO_3, DMF, 18 h, 80 °C, 66%.

The formation of compound **104e** was confirmed by NMR, and IR spectroscopy and HRMS. In the ^{1}H NMR spectrum, the singlet signal due to the N-H proton at 11.95 ppm had disappeared. A multiplet at 4.16 – 4.13 ppm integrating for two protons was assigned to H10. A singlet signal at 1.39 ppm integrating for nine protons was consistent with the presence of the Boc group. The ^{13}C NMR spectrum contained a new signal at 157.6 ppm accounting for the carbonyl carbon of the Boc group. Aliphatic carbon signals were observed at 78.9 – 14.8 ppm. The molecular ion was confirmed by HRMS to be $[M + H]^+$ 418.1210 which was consistent with a molecular mass of 418.1211 (calculated for $C_{18}H_{27}IN_5O_2$).

3.2.13.2 Suzuki-Miyaura coupling to synthesise *tert*-butyl 4-{2-[4-amino-5-(3,4-dimethoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl]ethyl}piperidine-1-carboxylate and analogues

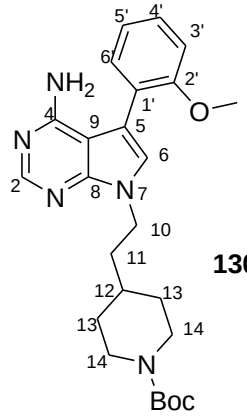
With the heteroaromatic compound **104e** in hand, we embarked on the final step of the synthesis which is the Suzuki-Miyaura coupling reaction with different boronic acids. The Suzuki-Miyaura coupling procedure described in section **3.2.8** was used to prepare these novel potential kinase protein inhibitors (Scheme 46). This was successful with the commercially available boronic acids and **121a**. Although we attempted Suzuki-Miyaura coupling reaction of compound **104e** with other boronic acids, we were not able to isolate the pure compounds. Reactions did not go to completion, and the starting material **104e** and our desired compounds had the same retention factor (R_f) value. Attempts to optimise the reaction conditions to get the reaction to go to

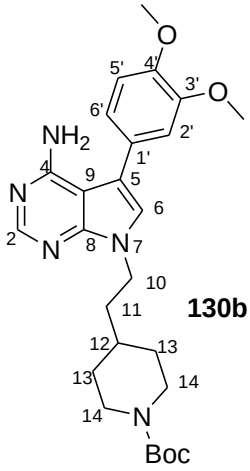
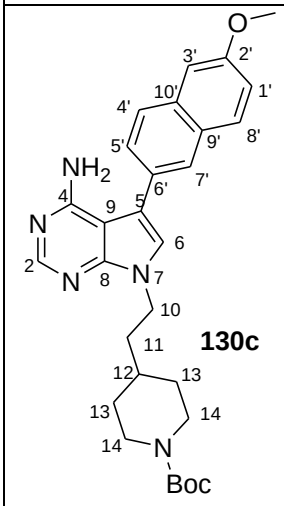
completion were not successful. A variety of alternative purification methods were also attempted, including preparative thin layer chromatography. At this stage, no further work was done on these compounds. **Table 6** below shows the results obtained for the reactions that were successful, including the different boronic acids used together with the spectroscopic information of each of the products.



Scheme 46: Reagents and conditions: $Pd(PPh_3)_4$, aq Na_2CO_3, DME, 18 h, 46 - 48%.

Table 6: Key spectroscopic signals for Suzuki products **130a-c**.

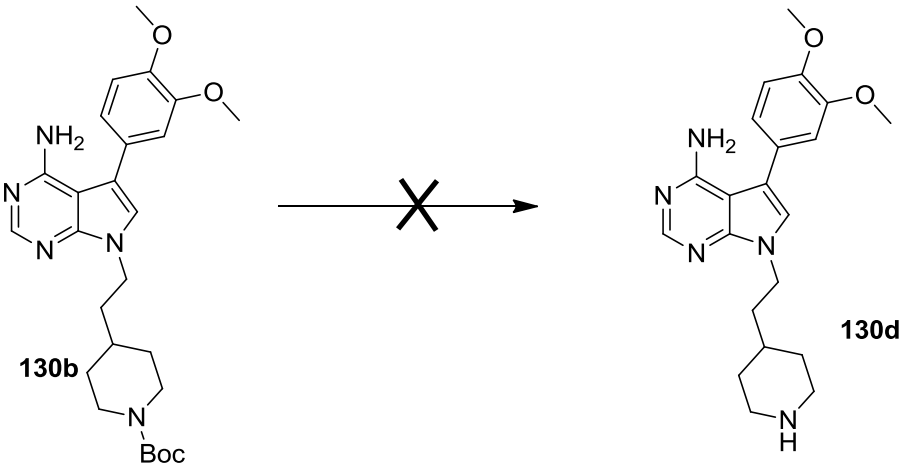
Product	Yield (%)	NMR spectroscopic information/ ppm (^{1}H and ^{13}C)	HRMS
 **130a**	46	^{1}H: 8.10 (1H, s, H2), 4.19 (t, *J* = 7.2 Hz, H10), 3.76 (s, OMe), 1.39 (s, H12 and C(C***H***$_3$)$_3$). ^{13}C: 157.9 (Boc-C=O), 156.9 (C-4), 154.3 (C-2'), 28.6 (3×CH_3).	Calculated for $C_{25}H_{34}N_5O_3$: 452.2663, found: $[M + H]^+$ 452.2648.

 **130b**	48	^{1}H: 8.04 (s, H2), 6.97 – 6.93 (m, Ar-H), 3.78 (s, 2 × OMe), 1.34 (s, H12 and C(C***H***$_3$)$_3$). ^{13}C: 158.0 (C=O), 155.1 (C-4), 150.4 (C-2), 27.3 (3×CH_3).	Calculated for $C_{26}H_{36}N_5O_4$: 482.2769, found: [M + H]$^+$ 482.2743.
 **130c**	46	^{1}H: 8.21 (s, H2), 7.90 (d, *J* = 2.3 Hz, H7'), 3.95 (s, OMe), 1.46 (10H, s, H12 and C(C***H***$_3$)$_3$). ^{13}C: 158.0 (Boc-C=O), 155.2 (C-4), 154.3 (C-2'), 27.3 (3×CH_3).	Calculated for $C_{29}H_{36}N_5O_3$: 502.2820, found: [M + H]$^+$ 502.2818.

In analysing the ^{1}H NMR spectra, the presence of the Boc group and the aromatic protons helped us to confirm that we had indeed formed the desired products **130a-c**. For example, the ^{1}H NMR spectrum of product **130a**, showed the presence of the Boc moiety by a signal at 1.39 ppm integrating for ten protons due to overlap with H12. The aromatic protons appeared as signals at 7.37 – 7.04 ppm. In addition, IR spectroscopy also confirmed the formation of the desired products **130a-130c**. For example, the C=O peak appeared as a signal at approximately 1678 cm^{-1} in each case.

We also attempted the deprotection of compound **130b** using 50% TFA/dichloromethane (Scheme 47).[2] The aim was to get a free amine in order to maximise the H-bonding affinity of the molecule in the active site of the enzyme. TLC showed formation of the new spot together

with that of the starting material; however attempts to isolate compound **130d** using column chromatography and preparative thin layer chromatography were not successful. This deprotection was also attempted on **130a** without success. Unfortunately due to time constraints; no further work was done on these compounds.

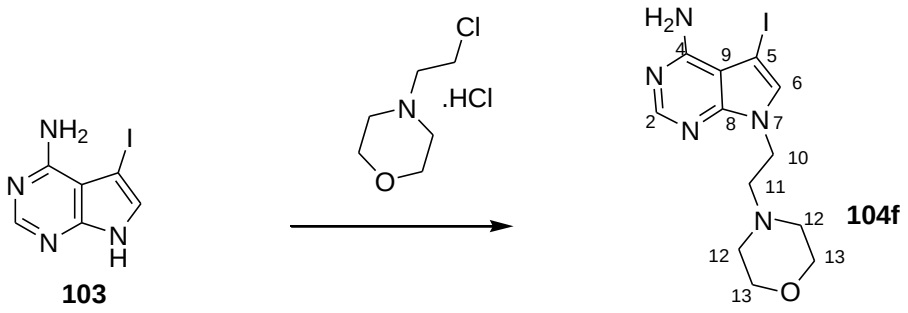


Scheme 47: Reagents and conditions: 50% TFA/DCM, rt.

3.2.14 Synthesis of 5-(3,4-dimethoxyphenyl)-7-(2-morpholinoethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine and analogues

As described earlier, we hoped to prepare analogues with a longer linker and the potential to H-bond in the active site of *Pf*CDPK4 and commercially available morpholinoethyl hydrochloride was identified as a suitable fragment for this purpose.

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



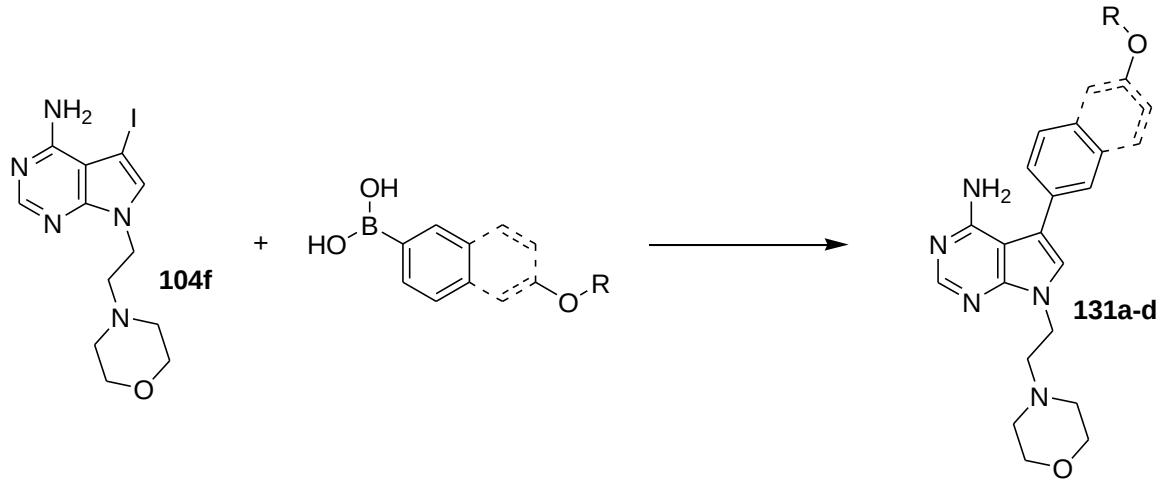
Scheme 48: Reagents and conditions: Cs_2CO_3, DMF, 18 h, 80 °C, 63%.

5-Iodo-7-(2-morpholinoethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **104f** was therefore prepared using the same *N*-alkylation procedure to that of compound **104a** in section **3.2.7**. 5-Iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **103** was dissolved in dry DMF and treated with 4-(2-chloroethyl)morpholine hydrochloride at 80 °C for 18 hours under basic conditions. Cesium carbonate was used as the base for this reaction. After this time, purification of the crude product by silica gel column chromatography using 10% MeOH/chloroform gave compound **104f** as a yellow solid in a yield of 63%. The ^{1}H NMR spectrum of **104f** showed the presence of the two methylene protons H10 and H11 at 4.19 ppm and 2.63 ppm, respectively. The presence of the morpholine group was also observed in the aliphatic region of the ^{1}H NMR spectrum with signals between 3.56 – 2.37 ppm. The molecular ion was confirmed by HRMS to be $[M + H]^+$ 374.0479 which was consistent with a molecular mass of 374.0480, calculated for $C_{12}H_{17}IN_5O$.

3.2.14.2 Suzuki-Miyaura coupling to synthesise 5-(3,4-dimethoxyphenyl)-7-(2-morpholinoethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine and analogues

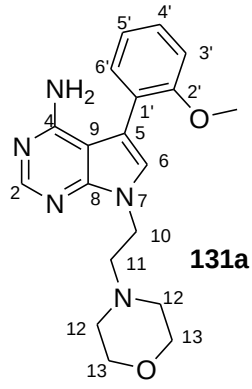
Having successfully prepared compound **104f**, we were now set for the Suzuki-Miyaura coupling reaction with different boronic acids (Scheme 49). The reaction was carried out as described for compounds **115a-b** in section **3.2.8**. The reaction was tested using commercially available boronic acids and **121b**. The other two compounds bearing a methoxy and ethoxyethoxy groups on the naphthalene moiety (analogues of **125c** and **125e**) were not prepared due to time constraints. Nevertheless, we were still delighted with the results because the ethoxy counterpart was prepared and can be used for structure-activity relationship studies. The success of this

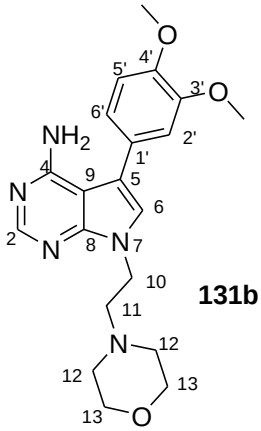
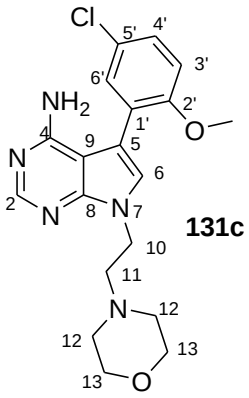
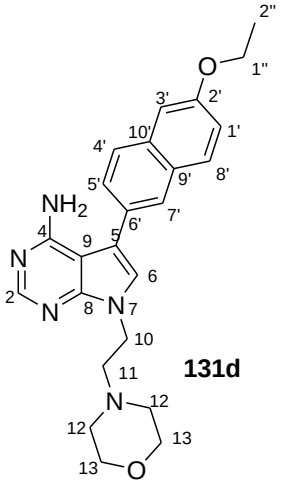
coupling reaction in each case was confirmed by NMR and IR spectroscopy and HRMS of the products isolated. Diagnostic signals for the novel potential kinase inhibitors **131a-131d** are tabulated below (**Table 7**).



Scheme 49: Reagents and conditions: $Pd(PPh_3)_4$, aq Na_2CO_3, DME, 18 h, 51 - 67%.

Table 7: Key spectroscopic signals for Suzuki products **131a-d**.

Product	Yield (%)	NMR spectroscopic information/ ppm (^{1}H and ^{13}C)	HRMS
 **131a**	52	^{1}H: 8.10 (s, H2), 4.27 (t, *J* = 6.6 Hz, H10), 3.76 (s, OMe). ^{13}C: 157.4 (C-4), 156.4 (C-2'), 57.6 (OMe), 55.3 (C-11).	Calculated for $C_{19}H_{24}N_5O_2$: 354.1932, found: $[M + H]^+$ 354.1930.

 **131b**	66	^{1}H: 8.13 (s, H2), 4.28 (t, *J* = 6.6 Hz, H10), 3.81 (s, OMe), 3.80 (s, OMe). ^{13}C: 157.7 (C-4), 155.7 (C-2), 149.5 (C-3'), 130.8 (C-4'), 57.6 (2 × OMe).	Calculated for $C_{20}H_{26}N_5O_3$: 384.2037, found: [M + H]$^{+}$ 384.2003.
 **131c**	67	^{1}H: 8.02 (s, H2), 3.70 (s, OMe), 2.69 (t, *J* = 6.6 Hz, H11). ^{13}C: 151.9 (C-4), 150.6 (C-2), 149.4 (C-2'), 58.2 (OMe), 56.1 (C-11), 48.2 (C-10).	Calculated for $C_{19}H_{23}ClN_5O_2$: 388.1542, found: [M + H]$^{+}$ 388.1508.
 **131d**	51	^{1}H: 8.10 (s, H2), 4.08 (q, *J* = 6.9 Hz, H1''), 1.37 (t, *J* = 7.0 Hz, H2''). ^{13}C: 157.7 (C-4), 157.2 (C-2'), 133.9 (C-10'), 34.8 (C-10), 13.7 (C-2'').	Calculated for $C_{24}H_{28}N_5O_2$: 418.2245, found: [M + H]$^{+}$ 418.2240.

The successful synthesis of compounds **131a-131d** was confirmed by using NMR, and IR spectroscopy and HRMS. The potential kinase protein inhibitors **131a-131d** shown in **Table 7**

were obtained in moderate yields. H2 for compound **131c** is at 8.02 ppm, quite shielded compared to all the others at approximately 8.10 ppm. Also C4 in compound **131c** is at 151.9 ppm compared to all the others at approximately 157 ppm.

3.3 Closing remarks

We were able to prepare a small library of compounds bearing a variety of substituents at N-7 and C-5 of the pyrrolo[2,3-*d*]pyrimidine skeleton. Difficulties were encountered with the purification of some substrates; however, we were able to prepare several compounds in each series. The compounds were tested for antiplasmodial activity in an *in vitro* whole cell assay (Chapter 5). Although not available at present; we also intend to assess the potential for these compounds to inhibit *Pf*CDPK4 and *Pf*CDPK1, in enzyme inhibition studies. This will form part of the future work in this project (Chapter 6).

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CHAPTER 4: SYNTHESIS OF POTENTIAL PROTEIN KINASE INHIBITORS WITH A SUBSTITUTED PYRIMIDINE CORE.

For the purpose of this PhD study, we wished to develop methodology for the synthesis of novel 4,5,6-trisubstituted pyrimidine derivatives as alternatives to the pyrrolo[2,3-*d*]pyrimidine as kinase inhibitors. The synthesis of 4,5,6-trisubstituted pyrimidine derivatives **110** utilized commercially available 4,6-dichloropyrimidine as the starting material. Sequential mono nucleophilic displacement of each chlorine atom afforded our suitably substituted diaminopyrimidines. Thereafter, Sonogashira coupling reaction with different alkynyl derivatives would be performed to possibly construct the 4,5,6-trisubstituted pyrimidine derivatives as potential protein kinase inhibitors (**Figure 38**). We planned to introduce similar functional groups onto the pyrimidine core as had been done on the pyrrolo[2,3-*d*]pyrimidine scaffold. To this end, cyclopropylmethanamine, benzylamine, cyclohexylamine and 2-morpholinoethanamine were used to prepare the 4,5,6-trisubstituted pyrimidine derivatives as potential protein kinase inhibitors.

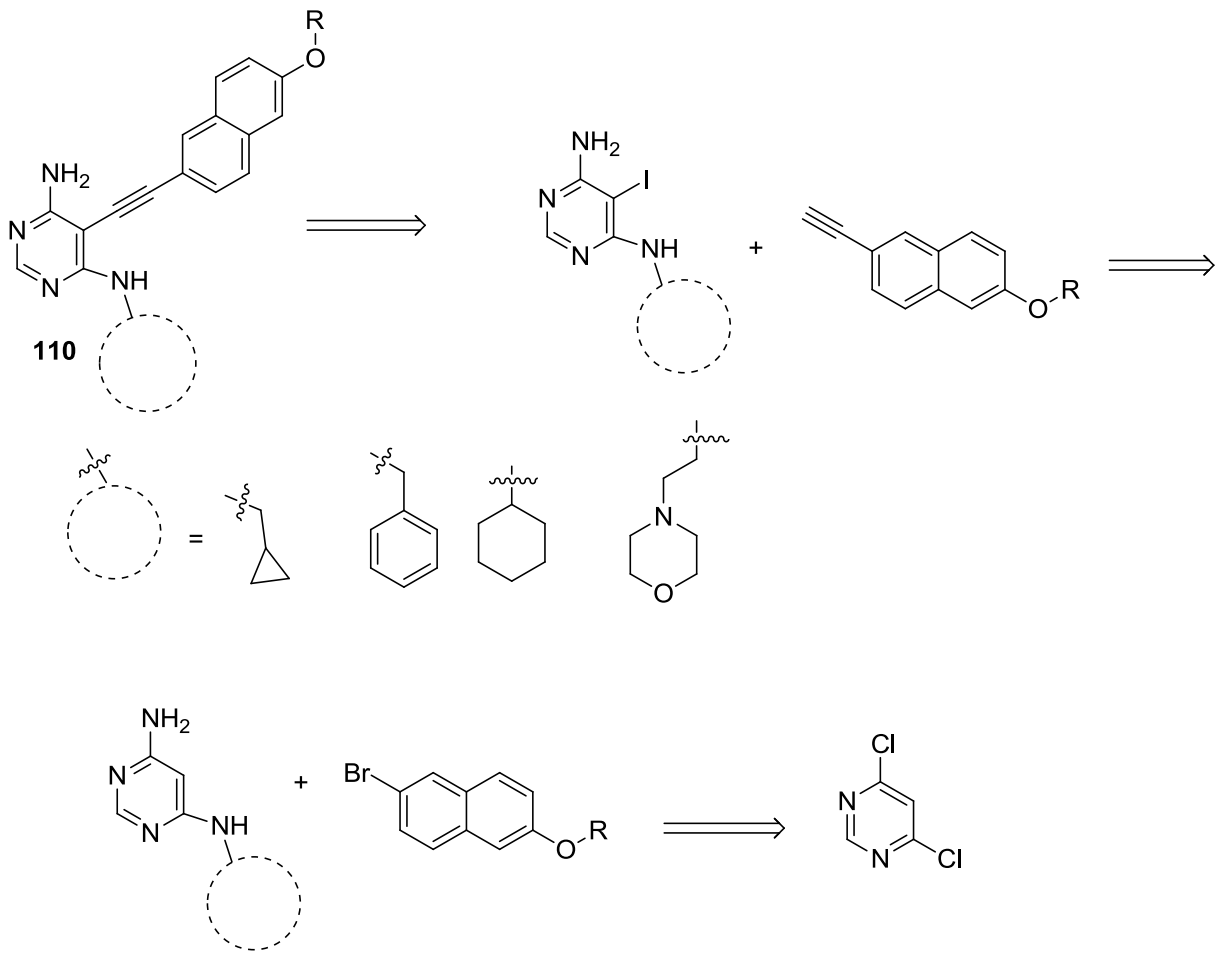


Figure 38: Retrosynthesis of 4,5,6-trisubstituted pyrimidine derivatives from 4,6-dichloropyrimidine.

The proposed pyrimidines **110** were assessed *in silico* using the same docking protocol used for the pyrrolo[2,3-*d*]pyrimidines described in chapter 3. We noted that these compounds could also potentially bind in the active site of *Pf*CDPK4. Key hydrogen-bonding interactions were observed between Tyr150 and Asp148 in the hinge region of *Pf*CDPK4 and N3 and 4-NH_2 groups of the pyrimidine scaffold. However, the expected additional H-bond with Glu154 in the sugar binding region was not observed (**Figure 39**). In general, these compounds gave higher docking scores than the pyrrolo[2,3-*d*]pyrimidine counterparts, indicating weaker binding. Nevertheless, we embarked on the synthesis of a library of compounds bearing these groups.

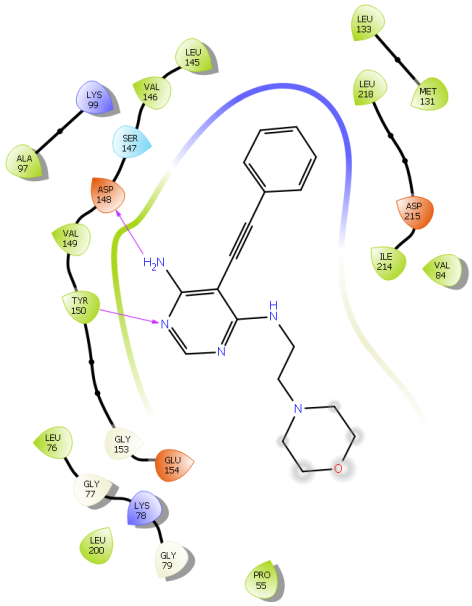
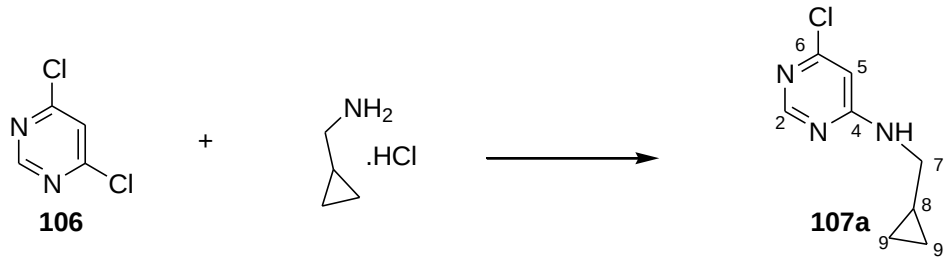


Figure 39: A diagram from the molecular model showing hydrogen bond interactions with key amino acid residues.

4.1 Synthesis of 6-chloro-*N*-(cyclopropylmethyl)pyrimidin-4-amine and analogues

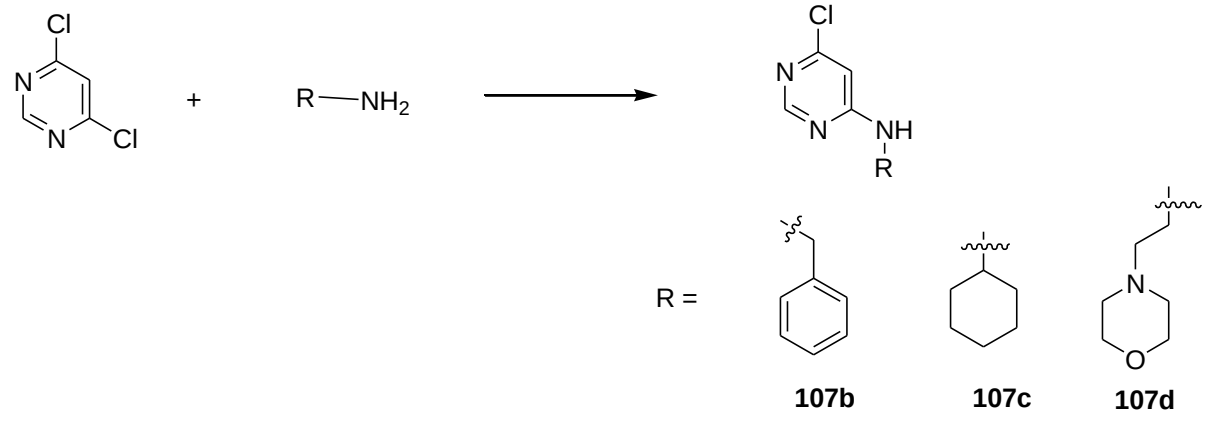
Our route towards the synthesis of the substituted pyrimidine ring derivatives started with the nucleophilic displacement of a chlorine atom on 4,6-dichloropyrimidine **106** .This was done by dissolving **106** in dioxane and treating the resultant solution with the appropriate amine and either *N*,*N*-diisopropylethylamine or triethylamine as the base, according to a literature reported method.[1] The reaction mixture was heated at 90 °C for 18 hours. When the reaction was complete as evident from TLC analysis, the mixture was allowed to cool to room temperature. In the synthesis of 6-chloro-*N*-(cyclopropylmethyl)pyrimidin-4-amine **107a**, compound **106** was treated with cyclopropylmethanamine hydrochloride to afford the product in an excellent yield of 80% (Scheme 50).



Scheme 50: Reagents and conditions: DIPEA, dioxane, 90 °C, 18 h, 80%.

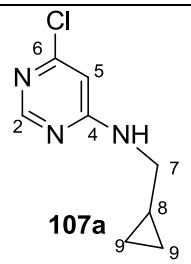
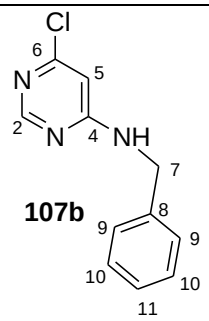
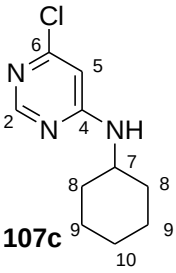
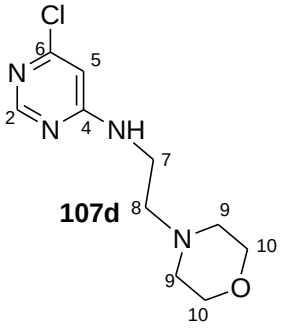
The formation of the product **107a** was confirmed using NMR and infrared spectroscopy. In the ^{1}H NMR spectrum, a peak at 1.00 ppm integrating for one proton and multiplets at 0.46 – 0.19 ppm integrating for four protons were indicative of the cyclopropyl moiety. The ^{1}H NMR spectrum also showed the presence of a singlet at 8.24 ppm and another singlet at 7.82 ppm due to H2 and NH, respectively. A singlet at 3.18 ppm integrating for two protons was assigned to H7. In the IR spectrum, a stretching band was observed at 3230 cm^{-1} due to NH.

Analogues **107b-107d** were prepared in a similar manner using the above procedure (Scheme 51). In each case, the products were isolated in good yields and were characterised using NMR spectroscopy. **Table 8** below gives a summary of the NMR spectroscopic data for the products **107b-d**. As observed from **Table 8**, compounds **107a-107d** were obtained in higher yields of 75 - 85%.

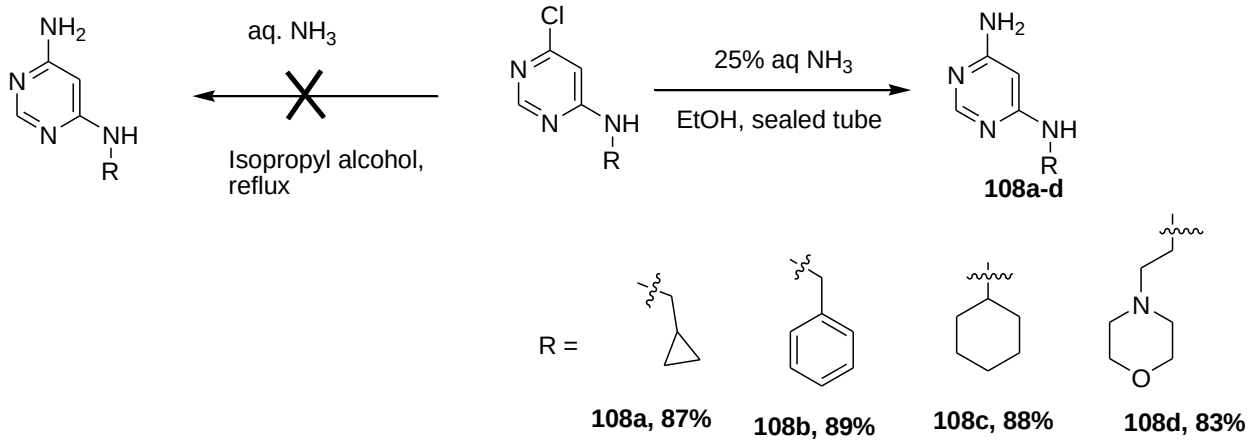


Scheme 51: Reagents and conditions: DIPEA/Et_3N, dioxane, 90 °C, 18 h, 75 - 85%.

Table 8: Key signals in the NMR spectra of compounds **107a-107d**.

Entry	Product	Product (% yield)	NMR spectroscopic information/ ppm (^{1}H and ^{13}C)
1	 **107a**	80	^{1}H: 8.24 (s, H2), 7.82 (s, NH) ^{13}C: 163.0 (C-4), 158.5 (C-6), 103.5 (C-5), 44.6 (C-7)
2	 **107b**	85	^{1}H: 8.25 – 8.22 (m, H2 and NH), 7.38 – 7.22 (m, Ar-H). ^{13}C: 163.1 (C-4), 158.5 (C-6), 103.7 (C-5), 43.6 (C-7).
3	 **107c**	75	^{1}H: 8.22 (s, H2), 1.88 – 1.05 (m, cyclohexyl-H). ^{13}C: 162.3 (C-4), 158.5 (C-6), 25.1 (C-10), 24.4 (C-9).
4	 **107d**	84	^{1}H: 8.26 (s, H2), 3.57 (t, *J* = 4.7 Hz, H10), 3.45 – 3.42 (m, H7). ^{13}C: 163.6 (C-4), 158.9 (C-6), 66.6 (C-10), 57.5 (C-8), 53.8 (C-9).

With the synthesis of compounds **107a-107d** completed (Scheme 50 and 51), our next synthetic step was to perform the second nucleophilic displacement of the remaining chlorine atom using aqueous ammonia (Scheme 52). When compounds **107a-107d** were treated separately with aqueous ammonia in isopropanol overnight at different temperatures (room temperature to reflux), thin layer chromatography analysis did not show the formation of a new spot. We assumed that this was due to the slightly poorer electrophilicity of the amino substituted pyrimidine ring. Hence, we employed forcing conditions for the displacement of the second chlorine moiety. This displacement reaction was accomplished by placing compounds **107a-d** in a sealed tube with ethanol and aqueous ammonia and heating at 170 °C for 48 hours.[1] After this time, the reaction vessel was cooled to room temperature, and the reaction mixture was concentrated *in vacuo* and purified by silica gel column chromatography. In the first instance, this gave compound **108a** as an orange solid in an excellent yield of 87%. In this case, ^{1}H NMR spectroscopy played an important role in confirming formation of the desired product in the appearance of a broad signal at 6.33 ppm due to the newly added NH_2 group. Furthermore, infrared spectroscopy also showed the stretching band due to NH and NH_2 groups. Interestingly, a significant shift in the signal due to C6 in the ^{13}C NMR spectrum was observed from 158.5 ppm to 172.6 ppm.

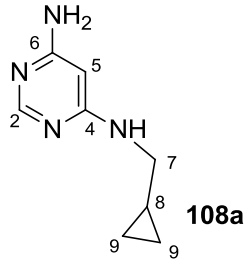
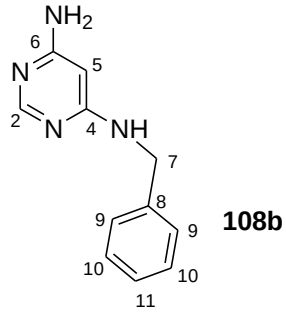
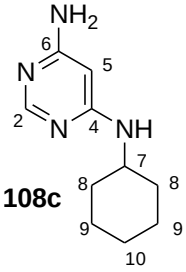


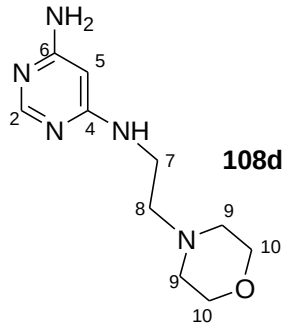
Scheme 52: Ammonolysis of the chloropyrimidine derivative using different conditions.

Analogues **108b-d** were prepared in the same manner and the products displayed similar spectroscopic changes as expected. **Table 9** below gives a summary of the NMR spectroscopic

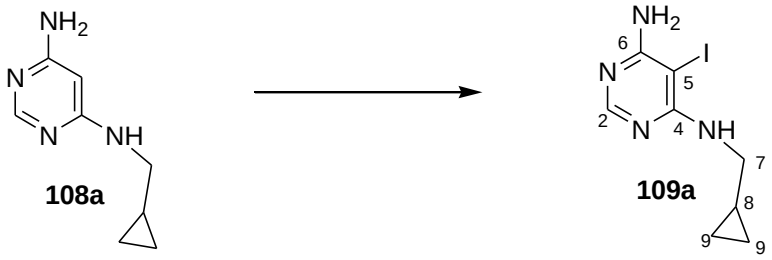
data for the products **108b-d**, which were isolated in good yields. As observed from **Table 9**, a broad signal in the ^{1}H NMR spectra of compounds **108a-d** at 6.04 ppm – 6.35 ppm confirmed the success of the reaction. The significant shift observed for C6 in compound **108a** was not seen for compounds **108b-d**.

Table 9: Key spectroscopic signals for ammonolysis products **108a-d**.

Entry	Product	Product (% yield)	NMR spectroscopic information/ ppm (^{1}H and ^{13}C)
1	 **108a**	87	^{1}H: 8.23 (s, H2), 6.33 (s, NH_2), 6.12 (t, J = 5.7 Hz, NH) ^{13}C: 172.6 (C-6), 162.0 (C-4), 81.3 (C-5), 44.5 (C-7)
2	 **108b**	89	^{1}H: 7.86 (s, H2), 7.10 (t, J = 6.3 Hz, NH), 6.04 (s, NH_2) ^{13}C: 162.5 (C-6), 160.8 (C-4), 56.4 (C-5), 44.2 (C-7)
3	 **108c**	88	^{1}H: 7.82 (s, H2), 6.05 (s, NH_2), 5.40 (d, J = 8.0 Hz, NH) ^{13}C: 165.4 (C-6), 162.5 (C-4), 82.6 (C-5), 51.1 (C-7)

4	 **108d**	83	^{1}H: 7.93 (s, H2), 6.84 (s, NH), 6.35 (s, NH_2) ^{13}C: 162.2 (C-4), 161.9 (C-6), 79.2 (C-5), 65.5 (C-10), 52.8 (C-9)

4.2 Synthesis of N^4-(cyclopropylmethyl)-5-iodopyrimidine-4,6-diamine and analogues



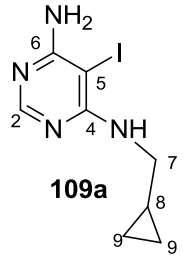
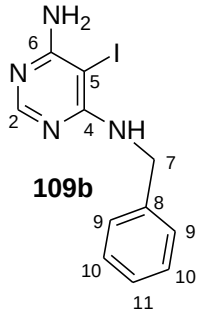
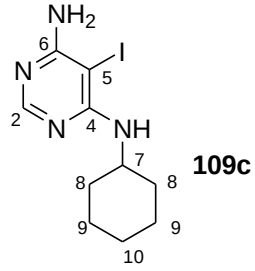
Scheme 53: Reagents and conditions: I_2, K_2CO_3, DMF/H_2O, 40 – 45 °C, 4 h, 83%.

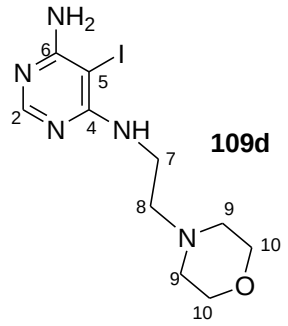
The third step in our synthesis of potential protein kinase inhibitors was to prepare a Sonogashira precursor, by iodinating our 4,6-diaminopyrimidines **108a-108d**.

Hence, compounds **109a-109d** were synthesised using the previously described methodology, employing iodine as the reagent. Therefore, **109a** was prepared by treating a suspension of N^4-(cyclopropylmethyl)pyrimidine-4,6-diamine **108a** in water and DMF with potassium carbonate followed by iodine. The resulting purple mixture was heated at a mild temperature for 4 hours according to a literature procedure.[2] Upon completion of the reaction, the mixture was quenched and filtered to give product **109a** in an excellent yield of 83%. Once again, ^{1}H NMR spectroscopy played an important role in confirming the formation of compound **109a**. The singlet signal previously appearing at 6.50 ppm due to H-5 had disappeared. The success of the reaction was also indicated by a shift in the C-5 signal, from 81.3 ppm to 56.5 ppm in the ^{13}C NMR spectrum. The molecular ion was confirmed by HRMS to be [M+H] 290.1002 which was consistent with a molecular mass of 290.1042, calculated for $C_8H_{12}IN_4$.

Three other analogues **109b-109d** were prepared in a similar manner and similar changes were observed in both NMR and IR spectra. Successful iodination to afford compounds **109b-109d** was also confirmed by HRMS spectroscopy. **Table 10** below gives a summary of the NMR spectroscopic data for the products **109a-d**. As observed in **Table 10**, a significant shift for C5 was seen for all the other compounds **109b-d**.

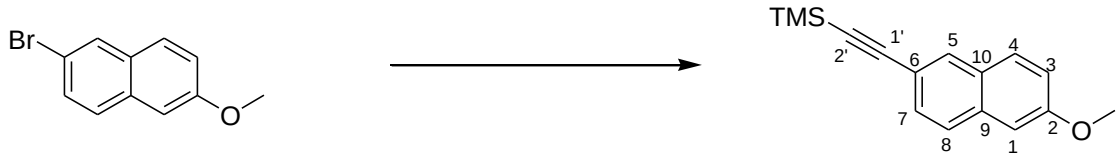
Table 10: Key spectroscopic signals for iodination products **109a-d**.

Entry	Product	Yield (%)	NMR spectroscopic information/ ppm (^{1}H and ^{13}C)	HRMS
1	 **109a**	83	1H: 7.80 (s, H2), 6.30 (s, NH_2) 13C: 162.3 (C-4), 160.7 (C-6), 56.5 (C-5), 45.4 (C-7)	Calculated for $C_8H_{12}IN_4$: 290.1042, found: $[M + H]^+$ 290.1002.
2	 **109b**	84	^{1}H: 7.77 (s, H2), 6.34 (s, NH_2), 4.57 (d, *J* = 6.1 Hz, H7) ^{13}C: 162.5 (C-4), 160.8 (C-6), 56.4 (C-5), 44.2 (C-7).	Calculated for $C_{11}H_{12}IN_4$: 327.0108, found: $[M + H]^+$ 327.0095.
3	 **109c**	89	^{1}H: 7.80 (s, H2), 6.32 (s, NH_2) ^{13}C: 162.4 (C-4), 159.9 (C-6), 57.0 (C-5), 49.6 (C-7).	Calculated for $C_{10}H_{16}IN_4$: 319.0421, found: $[M + H]^+$ 319.0415.

4	 **109d**	76	^{1}H: 7.89 (s, H2), 6.56 (dd, *J* = 14.3, 8.6 Hz, NH_2 and NH) ^{13}C: 162.4 (C-4), 160.8 (C-6), 56.8 (C-5)	Calculated for $C_{10}H_{17}IN_5O$: 350.0480, found: $[M + H]^+$ 350.0475.

Now that we had successfully prepared the aromatic iodide, our next step was to prepare the second Sonogashira precursor, the terminal alkyne, in order to gain access to our desired substituted pyrimidine ring derivatives as potential protein kinase inhibitors.

4.3. Synthesis of [2-(2-methoxynaphthalen-6-yl)ethynyl]trimethylsilane and analogues[3]



120a **132a**

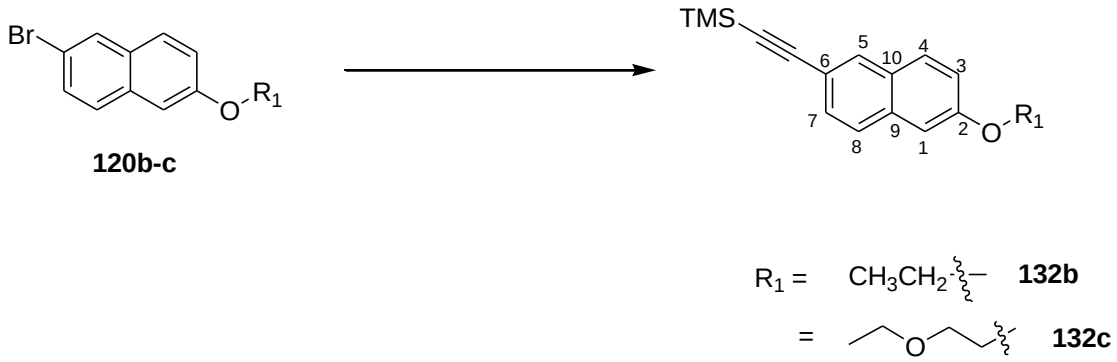
Scheme 54: Reagents and conditions: Trimethylsilylacetylene, $Pd(PPh_3)_2Cl_2$, CuI, diisopropylamine, DMF, 70 °C, 5 h, 79%.

For the synthesis of terminal alkynes, we utilised naphthalenes **120a-c** prepared previously by reaction of commercially available 6-bromonaphthalen-2-ol with different alkyl halides, as discussed in section **3.2.9**. We commenced our synthesis towards the aromatic terminal alkyne by firstly performing the Sonogashira coupling reaction on compounds **120a-c** using a palladium catalyst and copper(I) iodide. To this end, a degassed mixture of 2-bromo-6-methoxynaphthalene **120a**, copper(I) iodide and $Pd(PPh_3)_2Cl_2$ was treated with a degassed solution of trimethylsilylacetylene, diisopropylamine and DMF (Scheme 54). The resulting reaction mixture was heated at 70 °C for 5 hours under a nitrogen atmosphere. After workup extraction and

purification by column chromatography (20% EtOAc/hexane), the desired product **132a** was isolated as a yellow solid (1.68 g, 79%).

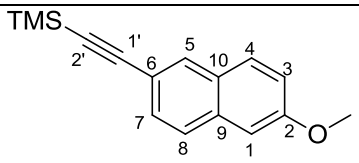
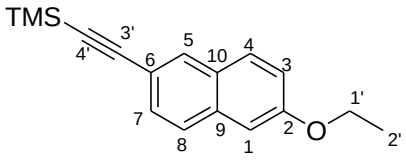
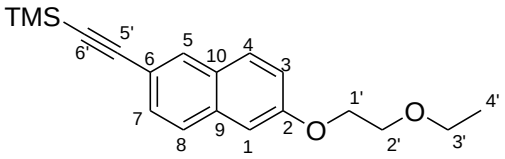
In the ^{1}H NMR spectrum of **132a**, the upfield singlet at 0.26 ppm was assigned to the trimethylsilyl group. The ^{13}C NMR spectrum of the product showed alkyne signals at 106.4 ppm and 93.5 ppm due to C-1' and C-2', respectively. The success of the reaction was further confirmed by a stretching band visible at 2110 cm^{-1} in the IR spectrum, which is an indication of the C≡C group.

Two other analogues **132b-c** were prepared in a similar manner using the above procedure and similar changes were observed in both NMR and IR spectra (Scheme 55). **Table 11** below gives a summary of the NMR spectroscopic data for the products **132a-c**. The alkyne signals of compounds **132b-c** were also observed at 106.4 ppm and 93.5 ppm.

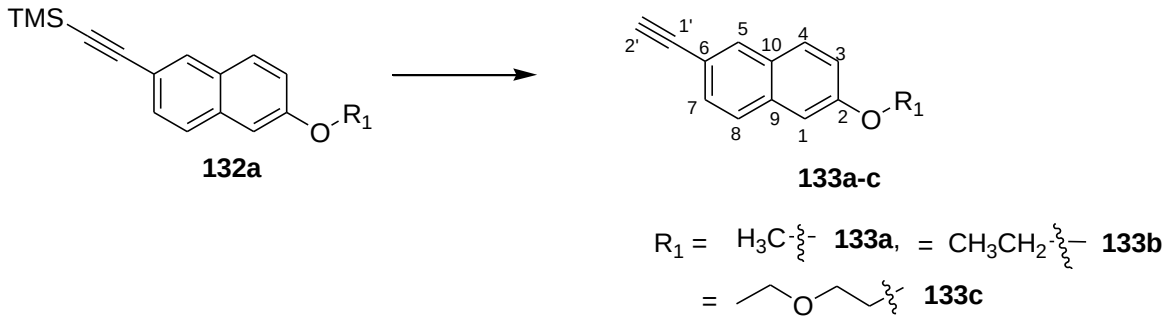


Scheme 55: Reagents and conditions: Trimethylsilylacetylene, $Pd(PPh_3)_2Cl_2$, CuI, diisopropylamine, DMF, 70 °C, 5 h, 73 – 76%.

Table 11: Key signals in the NMR spectra of the Sonogashira coupling reaction products **132a-132c.**

Product	Product (% yield)	NMR spectroscopic information/ ppm (^{1}H and ^{13}C)
 **132a**	79	^{1}H: 7.91 (d, *J* = 1.3 Hz, H5), 3.88 (s, OMe), 0.26 (s, TMS). ^{13}C: 106.4 (C-1'), 93.5 (C-2'), 0.0 (TMS).
 **132b**	73	^{1}H: 7.91 (d, *J* = 1.3 Hz, H5), 1.47 (t, *J* = 7.0 Hz, H2'), 0.27 (s, TMS). ^{13}C: 106.4 (C-3'), 105.7 (C-1), 93.5 (C-4'), 63.5 (C-1'), 0.0 (TMS).
 **132c**	76	^{1}H: 3.84 – 3.82 (m, H2'), 3.61 (q, *J* = 7.0 Hz, H3'), 0.27 (s, TMS). ^{13}C: 106.4 (C-5'), 93.5 (C-6'), 68.8 (C-3'), 15.2 (C-4'), 0.0 (TMS).

4.4 Synthesis of 2-ethynyl-6-methoxynaphthalene[4] and analogues

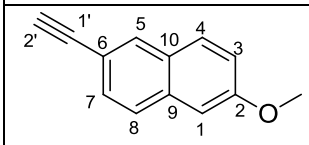
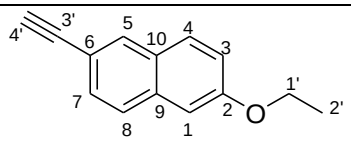
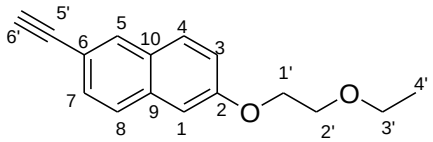


Scheme 56: Reagents and conditions: TBAF, THF, 0 °C - rt, 3 h, 77 - 88%.

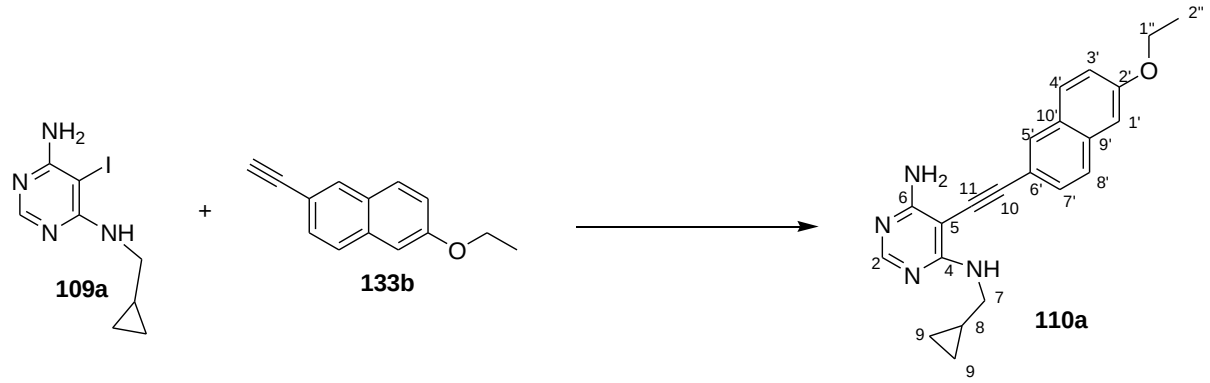
Our next synthetic step was to remove the TMS protecting group in compounds **132a-132c** in order to gain access to the terminal alkyne required for our final step (Scheme 56). There are numerous methods in the literature used for the removal of silyl protecting groups.[5-7] In this case, we decided to use tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) due to the short reaction times. Hence, compounds **132a-132c** were dissolved in THF at 0 °C. TBAF was then added to the reaction flask and the resulting mixture was warmed to room temperature and stirred for three hours. The progress of the reaction was monitored by TLC. When complete, the reaction mixture in each case was quenched with saturated aqueous ammonium chloride and extracted with ethyl acetate. Purification by silica gel column chromatography furnished compounds **133a-133c** in excellent yields of 77 – 88%.

In the ^{1}H NMR spectrum of **133a**, the upfield singlet at 0.26 ppm due to the trimethylsilyl group had disappeared, and a singlet integrating for one proton at 3.10 ppm confirmed the formation of the desired product. In the ^{13}C NMR spectrum, the alkyne signals were visible at 84.2 and 63.5 ppm. Similar changes in the ^{1}H and ^{13}C NMR spectra of analogues **133b-133c** were observed as expected. **Table 12** below gives a summary of the NMR spectroscopic data for the products **133b-c**. As observed in Table 12, the terminal proton for compounds **133b-c** was also seen at 3.10 ppm.

Table 12: Key spectroscopic signals for TMS deprotection products **133a-c**.

Product	Product (% yield)	NMR spectroscopic information/ ppm (^{1}H and ^{13}C)
 **133a**	88	^{1}H: 7.95 (s, H5), 3.10 (s, H2') ^{13}C: 157.9 (C-2), 84.2 (C-1'), 63.5 (C-2')
 **133b**	87	^{1}H: 7.94 (s, H5), 4.14 (q, *J* = 7.0 Hz, H1'), 3.10 (s, H4'), 1.48 (t, *J* = 7.0 Hz, H2'). ^{13}C: 157.7 (C-2), 84.2 (C-3'), 63.5 (C-4'), 14.8 (C-2').
 **133c**	77	^{1}H: 7.89 (d, *J* = 2.0 Hz, H5), 4.19 (dd, *J* = 5.7, 4.0 Hz, H1'), 3.61 (q, *J* = 7.0 Hz, H3'), 3.10 (s, H6'). ^{13}C: 157.1 (C-2), 84.3 (C-5'), 67.5 (C-1'), 63.5 (C-6'), 15.2 (C-4').

4.5 Synthesis of substituted 4,5,6-trisubstituted pyrimidines using Sonogashira coupling reaction of 109a-109d with various alkynes



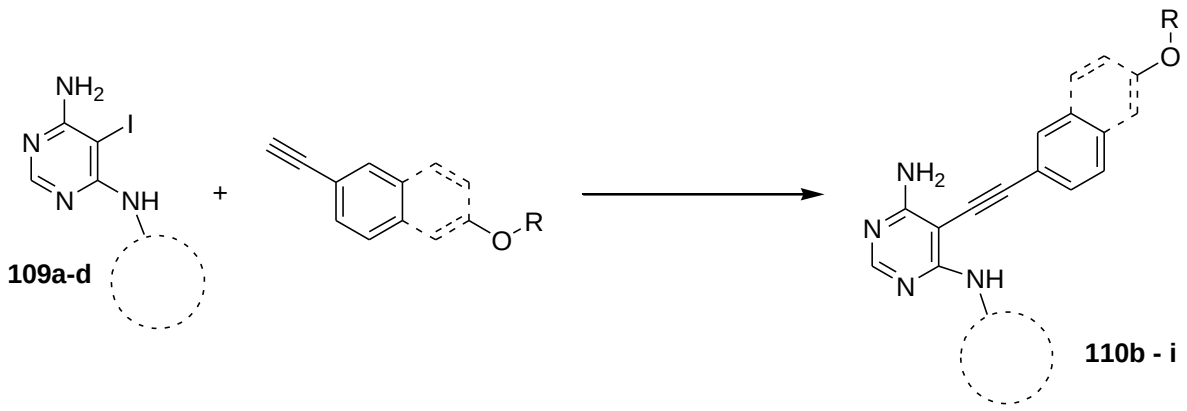
Scheme 57: Reagents and conditions: cat. $Pd(PPh_3)_2Cl_2$, CuI, diisopropylamine, DMF, 70 °C, 5 h, 48%.

The second last step in our synthesis of substituted pyrimidine ring compounds as potential protein kinase inhibitors involved the Sonogashira coupling of our iodinated pyrimidines **109a-d** and alkynes **133a-c**. As mentioned earlier, Sonogashira reactions allow for the formation of C-C bonds by reacting aromatic halides with terminal alkynes, using a palladium complex as a catalyst and copper(I) iodide as the co-catalyst. Diisopropylamine was used as the base. Different solvents (THF, DMF, DMSO) can be used to perform this reaction.[8-10] However, in this case we have used *N*,*N*-dimethylformamide (DMF) due to the poor solubility of compounds **109a**-**109d** in THF. Therefore, to a degassed mixture of N^4-(cyclopropylmethyl)-5-iodopyrimidine-4,6-diamine **109a**, copper(I) iodide, catalytic $Pd(PPh_3)_2Cl_2$ and 2-ethoxy-6-ethynylnaphthalene **133b** was added a degassed solution of diisopropylamine and DMF. The resulting reaction mixture was heated at 70 °C for 5 hours under a nitrogen atmosphere. After complete reaction, as indicated by TLC, the reaction mixture was worked up and the crude product purified by silica gel column chromatography. N^4-(Cyclopropylmethyl)-5-[2-(2-ethoxynaphthalen-6-yl)ethynyl]pyrimidine-4,6-diamine **110a** was isolated in an average yield of 48% (Scheme 57).

The ^{1}H NMR spectrum confirmed the formation of compound **110a** by the appearance of signals due to the naphthalene moiety at 7.81 – 7.19 ppm. A quartet integrating for two protons and a triplet integrating for three protons in the aliphatic region were assigned to H1" and H2", respectively. The cyclopropyl moiety was still visible in the aliphatic region of the ^{1}H NMR

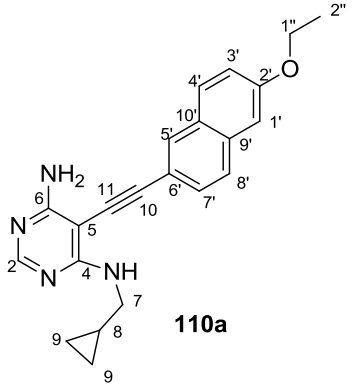
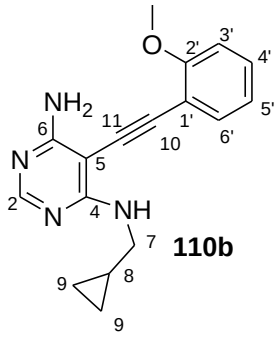
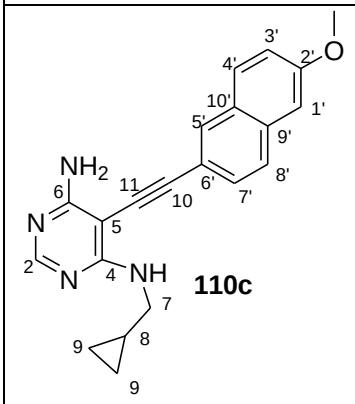
spectrum. The success of the reaction was further confirmed by the alkyne signals at 80.9 and 79.0 ppm in the ^{13}C NMR spectrum. The molecular ion was confirmed by HRMS to be [M+H] 359.1881 which was consistent with a molecular mass of 359.1874 (calculated for $C_{22}H_{23}N_4O$).

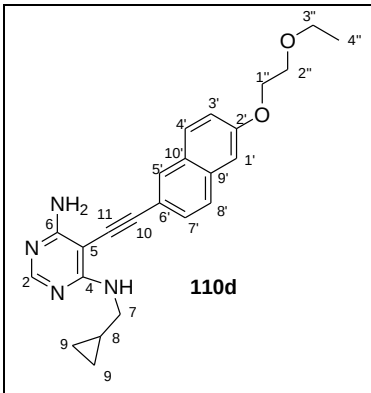
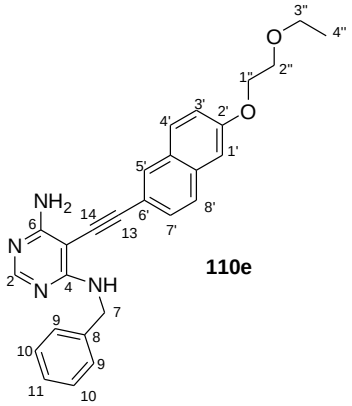
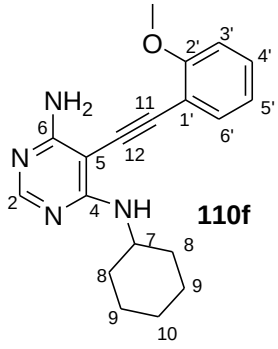
Other analogues **110b-110i** were prepared in a similar manner using the above procedure (Scheme 58). **Table 13** below shows the results obtained for the different alkynes (including both commercially available alkynes and those prepared in our laboratories) used together with the spectroscopic data of the product.

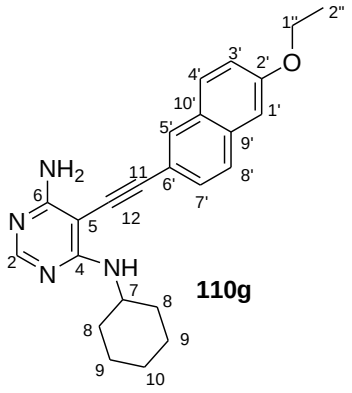
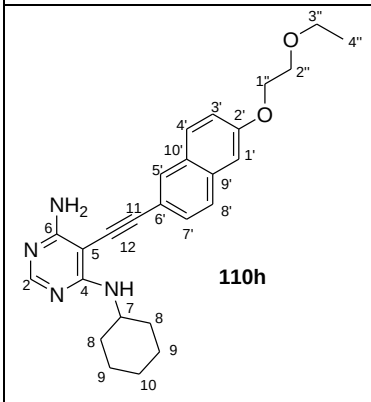
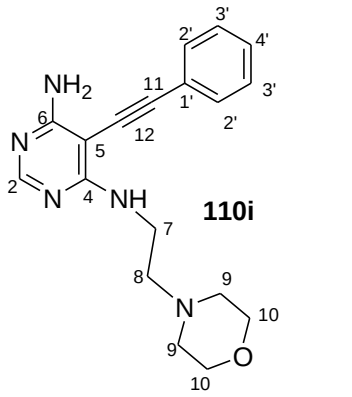


Scheme 58: Reagents and conditions: cat. $Pd(PPh_3)_2Cl_2$, CuI, diisopropylamine, DMF, 70 °C, 5 h, 49 - 79%.

Table 13: The results of the Sonogashira coupling reaction with various alkynes, together with key signals in the ^{1}H NMR and ^{13}C NMR spectra, and HRMS analysis.

Product	Product (% yield)	NMR spectroscopic information/ ppm (^{1}H and ^{13}C)	HRMS
 **110a**	48	^{1}H: δ 7.93 (s, H2), 6.58 (s, NH_2), 4.16 (q, *J* = 7.5 Hz, H1"). ^{13}C: 162.1 (C-6), 156.9 (C-2), 80.9 (C-10), 79.0 (C-11)	Calculated for $C_{22}H_{23}N_4O$: 359.1874, found: $[M + H]^+$ 359.1881.
 **110b**	54	^{1}H: δ 7.96 (s, H2), 6.54 (s, NH_2), 3.91 (s, OMe). ^{13}C: 163.1 (C-6), 161.8 (C-2'), 86.5 (C-10), 79.2 (C-11), 56.3 (C-7), 45.2 (OMe).	Calculated for $C_{17}H_{19}N_4O$: 295.1561, found: $[M + H]^+$ 295.1548.
 **110c**	49	^{1}H: δ 7.93 (s, H2), 7.83 – 7.81 (m, H4' and H8'). ^{13}C: 162.1 (C-6), 157.6 (C-4 and C-2'), 80.9 (C-10), 79.0 (C-11), 55.8 (OMe).	Calculated for $C_{18}H_{21}N_4O_2$: 325.1666, found: $[M + H]^+$ 325.1672.

 **110d**	60	^{1}H: 7.93 (s, H2), 7.80 (d, *J* = 8.7 Hz, H4' and H8'), 4.23 – 4.22 (m, H1''), 3.78 – 3.76 (m, H2''). ^{13}C: 156.9 (C-2), 80.9 (C-10), 79.0 (C-11), 78.9 (C-5), 12.0 (C-8), 3.8 (C-9).	Calculated for $C_{24}H_{27}N_4O$: 403.2136, found: $[M + H]^+$ 403.2116.
 **110e**	65	^{1}H: δ 8.21 (d, *J* = 1.4 Hz, H5'), 7.31 – 7.29 (m, Ar-H). ^{13}C: δ 162.1 (C-6), 157.5 (C-4 and C-2'), 80.9 (C-13), 79.1 (C-14), 66.2 (C-3''), 43.9 (C-7), 15.6 (C-4'').	Calculated for $C_{27}H_{27}N_4O_2$: 439.2136, found: $[M + H]^+$ 439.2115.
 **110f**	74	^{1}H: 6.75 – 6.60 (m, H2 and H6'), 3.15 (s, OMe). ^{13}C: 165.2 (C-6), 161.1 (C-2'), 80.9 (C-11), 78.9 (C-12), 56.3 (OMe).	Calculated for $C_{19}H_{23}N_4O$: 323.1874, found: $[M + H]^+$ 323.1873.

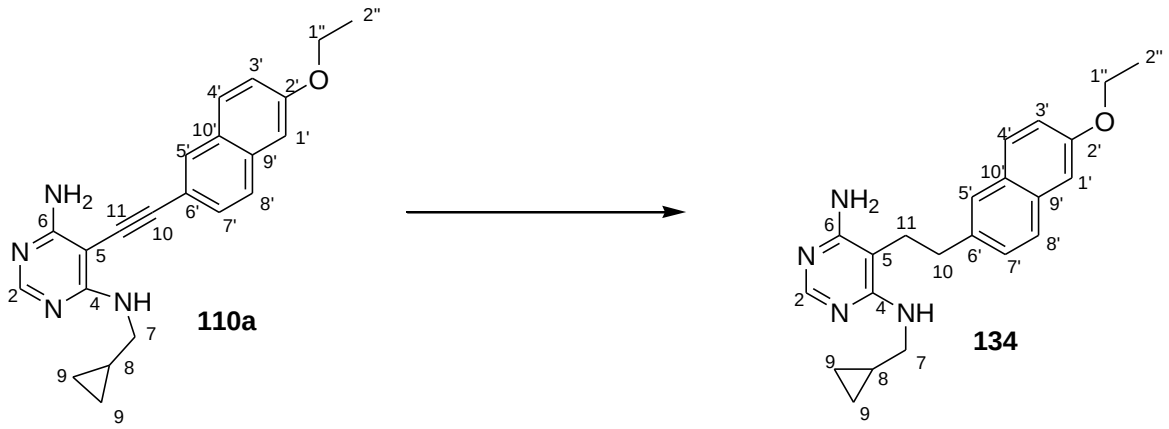
 **110g**	64	^{1}H: δ 8.17 (d, *J* = 1.6 Hz, H5'), 7.95 (s, H2), 7.80 (d, *J* = 8.9 Hz, H4' and H8'), 4.15 (q, *J* = 7.0 Hz, H1"). ^{13}C: δ 163.0 (C-6), 80.9 (C-11), 78.9 (C-12), 63.2 (C-1"), 14.6 (C-2").	Calculated for $C_{24}H_{27}N_4O$: 387.2187, found: $[M + H]^+$ 387.2177.
 **110h**	61	^{1}H: δ 7.84 – 7.79 (m, H2 and H4'), 3.53 (q, *J* = 7.0 Hz, H3"). ^{13}C: δ 163.0 (C-6), 160.7 (C-4), 157.0 (C-2'), 80.9 (C-11), 78.9 (C-12), 65.6 (C-3"), 49.0 (C-7).	Calculated for $C_{26}H_{31}N_4O_2$: 431.2449, found: $[M + H]^+$ 431.2445.
 **110i**	79	^{1}H: δ 7.94 (s, H2), 7.69 – 7.66 (m, H2'), 2.49 – 2.40 (m, H8 and H9). ^{13}C: 162.4 (C-6), 80.9 (C-11), 79.0 (C-5), 78.9 (C-12).	Calculated for $C_{18}H_{22}N_5O$: 324.1826, found: $[M + H]^+$ 324.1824.

All the 4,5,6-trisubstituted pyrimidine products shown in **Table 13** were obtained in moderate to good yields. The formation of these products was confirmed using NMR and IR spectroscopy and HRMS. In the ^{13}C NMR spectra, the alkyne moiety was visible as signals appearing at approximately 80.9 and 79.0 ppm. The products bearing a cyclopropyl, benzyl or cyclohexyl moiety were easier to prepare than those bearing the morpholino moiety. Only one compound bearing the morpholinoethyl side chain was prepared. This is due to the fact that the Sonogashira

coupling reaction with this derivative did not go to completion when using the ethynylnaphthalenes prepared in our laboratories. The products were also difficult to purify because they typically had the same retention factor (R_f) as the staring material. Several separation methods were attempted, including preparative TLC but we were unfortunately unsuccessful in isolating our desired products in pure form.

The above compounds **110a-110i** were assessed for antiplasmodial activity in an *in vitro* screen and will be tested for activity against *Pf*CDPK4 and *Pf*CDPK1 in biochemical enzyme assays. The biological results obtained from compounds **110a-i** will be discussed in the next chapter.

4.6 Synthesis of N^4-(cyclopropylmethyl)-5-[2-(2-ethoxynaphthalen-6-yl)ethynyl]pyrimidine-4,6-diamine 134



Scheme 59: Reagents and conditions: Pd/C, H_2 (g) balloon, EtOH, rt, 18 h, 70%.

In the absence of enzymatic inhibition data, we sought to introduce increased structural flexibility by reduction of the alkyne functionality of one example of compounds **110**. To this end, treatment of an ethanoic solution of compound **110a** with hydrogen in the presence of Pd/C catalyst at ambient temperature for 18 hours gave, after purification by silica gel chromatography, compound **134** in 70% yield.

The ^{1}H NMR spectrum confirmed the formation of compound **134** by the appearance of two triplets at 2.96 ppm and 2.66 ppm due to H10 and H11, respectively. In the ^{13}C NMR spectrum,

two new signals were visible in the aliphatic region at 33.0 ppm and 14.8 ppm due to H10 and H11, respectively. A signal previously appearing at 2187 cm^{-1} in the IR spectrum due to the C≡C stretch had disappeared.

4.7 Closing remarks

A second library of compounds, containing a pyrimidine core as alternative to the pyrrolo[2,3-*d*]pyrimidines template, as potential kinase inhibitors, was prepared. Once again, difficulties were encountered in the purification of some analogues. Nonetheless, a variety of substituted 4,6-diaminopyrimidines were prepared for biological assessment. It was also demonstrated that the alkyne functional group could be reduced to afford more flexible analogues, should this be required.

4.8 References

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CHAPTER 5: BIOLOGICAL EVALUATION OF PYRROLO[2,3-*d*]PYRIMIDINE AND BRANCHED PYRIMIDINE ANALOGUES PREPARED.

The pyrrolo[2,3-*d*]pyrimidines and 4,5,6-trisubstituted pyrimidines synthesized were sent to Prof Heinrich Hoppe at Rhodes University for biological testing. As mentioned earlier, we were not able to obtain the results of the *Pf*CDPK4 enzymatic assay in time before submission of this thesis. However, all of the final compounds synthesised were evaluated in a whole cell antiplasmodium assay using the parasite lactate dehydrogenase (pLDH) assay. Compounds that displayed antiplasmodial activity *in vitro* were assessed for cytotoxicity in a HeLa cell assay. The biological results from the two assays are discussed in the following sections.

5.1 Results and Discussion

The compounds prepared in this study were designed using the crystal structure of *Pf*CDPK4. Inhibition of *Pf*CDPK4 does not, however, result in parasite death as this enzyme is not essential for parasite survival. By comparison; *Pf*CDPK1 is essential for parasite survival, although the parasite has demonstrated the ability to compensate for this to some extent in recent studies.[1] We had hoped to design inhibitors that would be effective against both *Pf*CDPK4 and *Pf*CDPK1, owing to the sequence similarity of these enzymes. Any antiplasmodial activity observed *in vitro* is therefore potentially due to *Pf*CDPK1 inhibition. From work reported by Ojo and co-workers, potent inhibitors of *Pf*CDPK1 displayed moderate antiplasmodial activity *in vitro* (low micromolar range).[2]

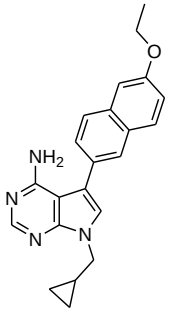
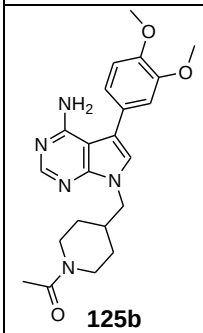
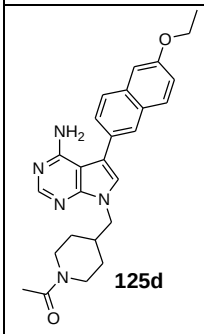
5.1.1 pLDH (Malaria) assay- single concentration screening

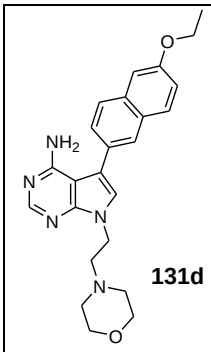
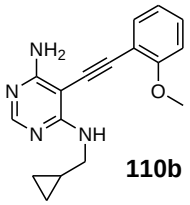
Firstly, malaria parasites (*Plasmodium falciparum* strain 3D7) were maintained in RPMI 1640 medium containing 2mM L-glutamine and 25mM Hepes (Lonza). The medium was further supplemented with 5% Albumax II, 20 mM glucose, 0.65 mM hypoxanthine, 60 µg/mL gentamycin and 2-4% hematocrit human red blood cells. The parasites were cultured at 37 ^{o}C under an atmosphere of 5% CO_2, 5% O_2, 90% N_2 in sealed T25 or T75 culture flasks.

For screening compounds against malaria parasites, compounds at 20 µM were added to parasite cultures in 96-well plates and incubated for 48 h in a 37 ^{o}C CO_2 incubator. After 48 h, the plates were removed from the incubator. Twenty µL of culture was removed from each well and mixed with 125 µL of a mixture of Malstat solution and NBT/PES solution in a fresh 96-well plate. These solutions measure the activity of the pLDH enzyme in the cultures. A purple product was formed when pLDH was present, and this product was quantified in a 96-well plate reader by absorbance at 620 nm (Abs_{620}). The Abs_{620} reading in each well was thus an indication of the pLDH activity in that well and also the number of viable parasites in that well.

For each compound concentration, **% parasite viability** – the pLDH activity in compound-treated wells relative to untreated controls – was calculated. Compounds were tested in duplicate wells, and a standard deviation (SD) was derived. Only the compounds that reduced pLDH activity to less than 25% parasite viability after dosing for 48 hours were carried through to obtain IC_{50} values. Pyrrolo[2,3-*d*]pyrimidines **122b**, **125b**, **125d**, **131d** and 4,5,6-trisubstituted pyrimidine **110b** had a significant effect on the viability of *P. falciparum* at 20 µM. Hence, they were evaluated further to determine IC_{50} values. Compounds for which IC_{50} values were obtained are shown in **Table 14**, with a comparison to chloroquine (an antimalarial drug) used as a drug standard. Of the five compounds that showed activity; four were from the pyrrolo[2,3-*d*]pyrimidine series. Each of these were substituted with either an ethoxynaphthyl group at C-5; or a substituent at N-7 which could potentially form an additional H-bond to Glu154 in the sugar pocket (piperidinyl and morpholine substituents). Significantly, none of these compounds displayed cytotoxicity at a single concentration of 20 µM against a HeLa cell line. None of the compounds bearing a methoxynaphthyl substituent at C-5 showed antiplasmodial activity *in vitro*.

Table 14: Results of *in vitro* antimalarial assay and cytotoxicity for selected pyrrolo[2,3-*d*]pyrimidine and branched pyrimidine compounds.

Compound	**Parasite Viability %**	**IC_{50} values (µM) pLDH assay**	**HeLa assay 20 µM % cell viability**
Chloroquine	0.011	0.016	
 **122b**	24.1 ±3.81	18.7 ± 0.8	79.8
 **125b**	2.74 ± 3.87	12.1 ± 11.4	79.3
 **125d**	-2.29 ±1.83	14.2 ± 1.7	71.7

 **131d**	-12.0 ±2.6	8.2	47.0
 **110b**	9.1 ± 2.4	21.1	HeLa IC_{50} 2.6 µM (18.8% cell viability)

The most active compound from the pyrrolo[2,3-*d*]pyrimidine series bearing a cyclopropylmethyl substituent at N-7 was **122b**, with an IC_{50} value of 18.7 µM against the drug sensitive strain. While we were happy with this outcome; we were surprised to find that this was the only compound of this series bearing a cyclopropylmethyl substituent which showed promising antimalarial activity. The other compounds with this group at N-7 performed poorly against the malaria parasite. Compounds **115a** and **115b** bearing a benzyl group did not reduce the pLDH activity to less than 25%, hence they were not evaluated further for their IC_{50} values. Compounds with a (piperidin-1-yl)ethanone moiety at N-7, showed better parasite inhibition, with the best activity obtained from compounds **125b** and **125d** giving the % parasite viability of 2.74 and -2.29, respectively, and IC_{50} values of 12.1 µM and 14.2 µM, respectively. However, both were less potent than the existing antimalarial drug, chloroquine with an IC_{50} value of 0.016 µM. However, the observed activity could be attributed to the methyl(piperidin-1-yl)ethanone substituent on N-7 possibly hydrogen bonding to Glu154. Just like compound **122b**, compound **125d** also has an ethoxynaphthyl substituent at C-5. Hence, it can be concluded that an ethoxy group influenced the activity of these compounds, as the methoxynaphthyl analogues were not active. Surprisingly, compound **125e** with a longer chain on the 2-position of the naphthyl moiety did not display promising activity. The pyrrolo[2,3-*d*]pyrimidine derivative **131d** bearing a morpholinoethyl substituent at C-5 showed strongest activity against *Plasmodium falciparum in vitro* with an IC_{50} value of 8.2 µM. Once more, compound **131d** has an ethoxy substituent on

the 2-position of the naphthalene moiety. Hence, there was no doubt that this group influenced the activity of these compounds.

As observed in **Table 14**, compound **110b** was the only compound from the branched pyrimidine series which showed activity against *Plasmodium falciparum in vitro*. Interestingly, this compound did not bear an ethoxynaphthyl substituent or a substituent that could H-bond to Glu154 at N-7. This was also the only compound that showed significant cytotoxicity at 20 µM; and therefore an IC_{50} value was obtained of 2.6 µM against the HeLa cell lines. It is not clear whether this was the cause of the apparent antiplasmodial activity or not (i.e. whether the red blood cells in which the antimalarial assay is performed were affected by the compound rather than the parasite). All other branched pyrimidine final compounds did not reduce pLDH activity to less than 25%, hence they were not evaluated further for IC_{50} values.

In summary, the results obtained in this study are very promising. Some compounds showed moderate activity against *Plasmodium falciparum in vitro*, which could suggest inhibition of *Pf*CDPK1. We also noticed that the presence of an ethoxynaphthyl group at C-5 of the pyrrolo[2,3-*d*]pyrimidine derivatives played a very important role in their activity. This ethoxynaphthyl functional group resulted in some of the compounds being potential inhibitors of the enzyme. Moreover, structure activity relationships revealed that pyrrolo[2,3-*d*]pyrimidine is a better scaffold than the branched pyrimidine. This can be seen by comparing the activity of compound **122b** and **110a** (% parasite viability of 88.8). In this case, it may be worthwhile to consider reducing the alkyne functional group to afford more flexible analogues.

All the final compounds containing the pyrrolo[2,3-*d*]pyrimidine or branched pyrimidine scaffold will be screened for biological activity in a *Pf*CDPK4 enzymatic assay once the assay is available in our collaborators research groups (Wits University and Rhodes University).

5.2 Reference

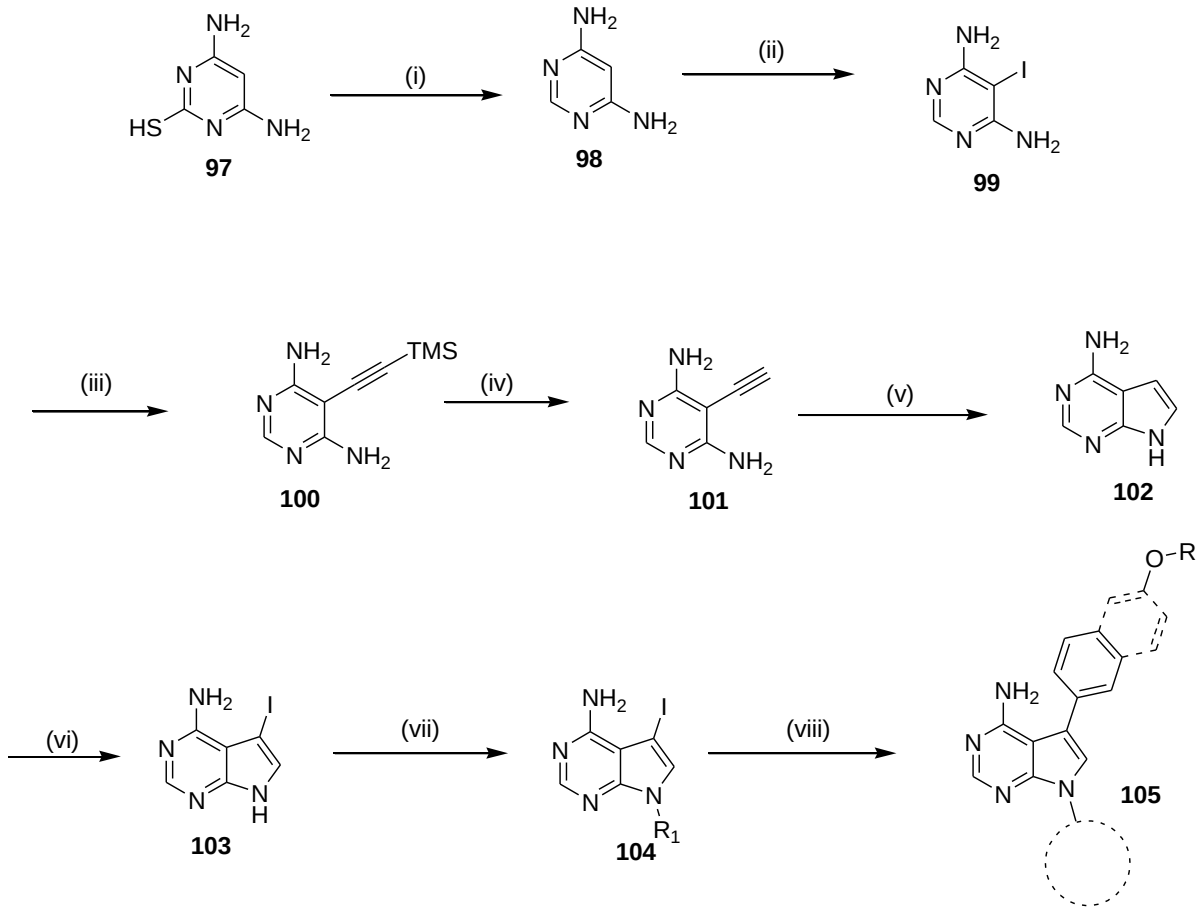
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CHAPTER 6: CONCLUSION AND FUTURE WORK

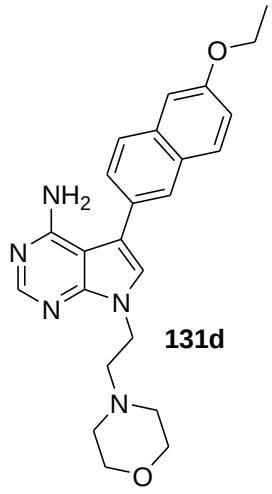
6.1 Conclusions and future work pertaining to Chapter 3

In conclusion, we have successfully prepared a series of suitably substituted pyrrolo[2,3-*d*]pyrimidines to be evaluated for biological activity. The synthesized compounds were analysed by means of ^{1}H NMR and ^{13}C NMR spectroscopy and high resolution mass spectrometry (HRMS). This first generation of compounds containing a pyrrolo[2,3-*d*]pyrimidine scaffold were synthesised in twelve steps (Scheme 60). Our synthesis commenced with reduction of the commercially available 4,6-diaminopyrimidine-2-thiol in the presence of Raney nickel to afford pyrimidine-4,6-diamine **98**. Selective iodination at C-5 was achieved using iodine in dimethylformamide (DMF) and water. The resulting halogenated compound was subjected to a Sonogashira coupling reaction to afford compound **100**, which was then deprotected using a source of fluoride ion, TBAF, to give a terminal alkyne **101** in good yield of up to 85%. The key step in this synthesis was the formation of the pyrrolo[2,3-*d*]pyrimidine ring **102**, and this was prepared by the use of microwave irradiation in dimethyl sulfoxide (DMSO) under basic conditions. With this key intermediate in hand, iodination at C-3 was accomplished using *N*-iodosuccinimide (NIS), followed by reaction with appropriately substituted alkyl (R_1) bromides or tosylates in dimethylformamide. The final step in the synthesis of these potential kinase inhibitors was the introduction of appropriate aryl substituents at C-3 *via* Suzuki-Miyaura coupling reactions with different boronic acids. This gave the suitably substituted pyrrolo[2,3-*d*]pyrimidine analogues **115a-b, 122a-e, 125a-e, 128a-b, 130a-c** and **131a-d** in yields ranging from 35-76% (Scheme 60).



Scheme 60: Reagents and conditions: (i) Raney Ni, aq NH_3, H_2O, reflux, 2 h, 96%. (ii) I_2, K_2CO_3, DMF/H_2O, 45 °C, 4 h, 76%. (iii) TMS-acetylene, cat. $Pd(PPh_3)_4$, CuI, diisopropylamine, THF, 70 °C, 4 h, 48%. (iv) TBAF, THF, 0 °C - rt, 3 h, 85%. (v) Cs_2CO_3, DMSO, MW, 100 W, 180 °C, 15 min, 50%. (vi) NIS, DMF, 60 °C, 4 h, 57%. (vii) R_1-X, Cs_2CO_3, DMF, 70 °C, 18 h, 57% - 79%. (viii) $ArB(OH)_2$,$Pd(PPh_3)_4$, aq Na_2CO_3, DME, 18 h, 35% - 76%.

Antimalarial activity of the pyrrolo[2,3-*d*]pyrimidine compounds prepared was evaluated in a whole cell antiplasmodium assay using the parasite lactate dehydrogenase (pLDH) assay. The pyrrolo[2,3-*d*]pyrimidine series bearing a methylcyclopropyl substituent at N-7 (**122b**) showed some activity in the antiplasmodium assay (IC_{50} 18.7 ± 3.81 µM). Additionally, pyrrolo[2,3-*d*]pyrimidine **131d** was the most potent compound of this series; displaying an IC_{50} of 8.2 µM (**Figure 40**). Further evaluation of this compound will be carried out in order to better understand this result. Unfortunately, all of our pyrrolo[2,3-*d*]pyrimidine compounds were found to be significantly less potent than the drug standard, chloroquine (IC_{50} value of ~0.016 µM).



131d

Figure 40: Pyrrolo[2,3-*d*]pyrimidine showing the greatest antimalarial activity.

Whilst the IC_{50} values of these compounds are not in the nanomolar range and cannot compete with chloroquine, a number of motivating conclusions and future work can be derived from these results. In the absence of biochemical enzyme assay data, the whole cell antiplasmodial assay has given us an indication of potential *Pf*CDPK1 inhibition. Compound **131d** was shown to be one of the most promising compounds synthesized in this series. With the use of molecular modelling against a homology model of *Pf*CDPK1 we could assess whether synthetic modification to the molecule could result in a lower IC_{50} value. Further investigation into compounds **122b, 125b** and **125d** through molecular modelling could suggest suitable modifications that could be made to the substituents of the pyrrolo[2,3-*d*]pyrimidine scaffold, perhaps increasing efficacy of these compounds.

In future, we would like to use the methodology developed in this project to modify the structural features associated with the compounds prepared in this first series. The synthesis of more potent inhibitors with the aid of molecular modelling forms part of this future work. The encouraging results obtained from the biological screening together with the molecular modelling would be undertaken to determine the preferred synthetic modifications for generation of a library of 2nd generation compounds. Importantly; enzyme inhibition assays are needed in order to prepare a second generation of potential inhibitors with improved biological activity.

The results from our modelling studies showed that these compounds could potentially act as inhibitors of *Pf*CDPK4. For example, compound **125d** which contains the 4-(methyl)piperidin-1-

yl)ethanone moiety at N-7 was observed to make a hydrogen bond with key amino acids residues (Asp148 and Tyr150) in the hinge region of *Pf*CDPK4 and with Glu154 in the sugar pocket region of *Pf*CDPK4 (**Figure 41**). The fact that this compound docked well in the active site of *Pf*CDPK4 *in silico*, and also showed moderate activity against *P. falciparum in vitro*, suggests that there is potential to develop a dual *Pf*CDPK1/*Pf*CDPK4 inhibitor; which could prevent malaria transmission.

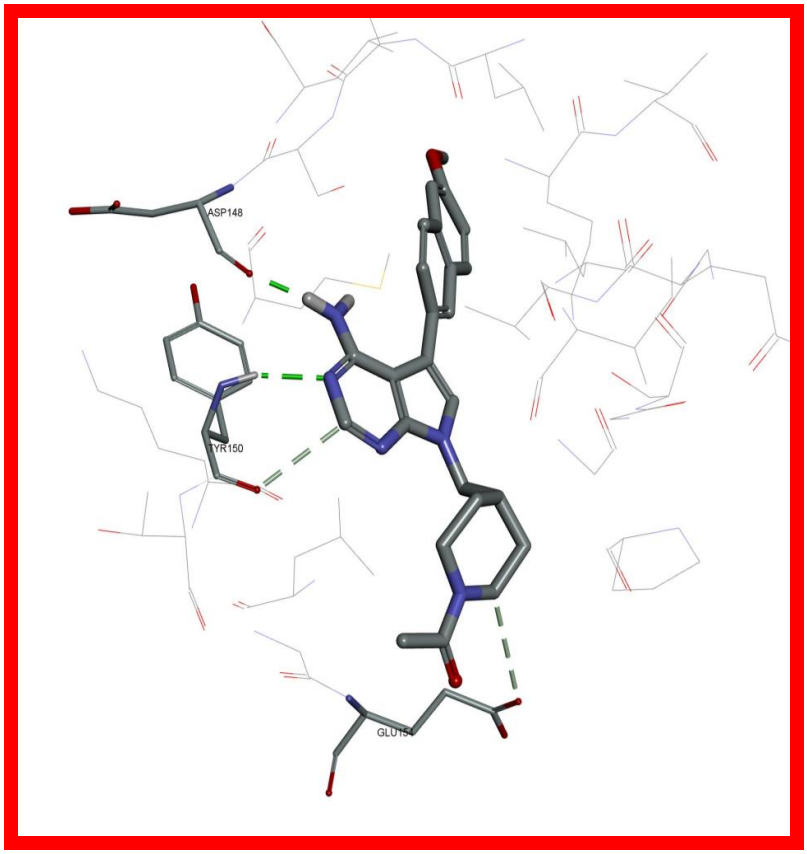
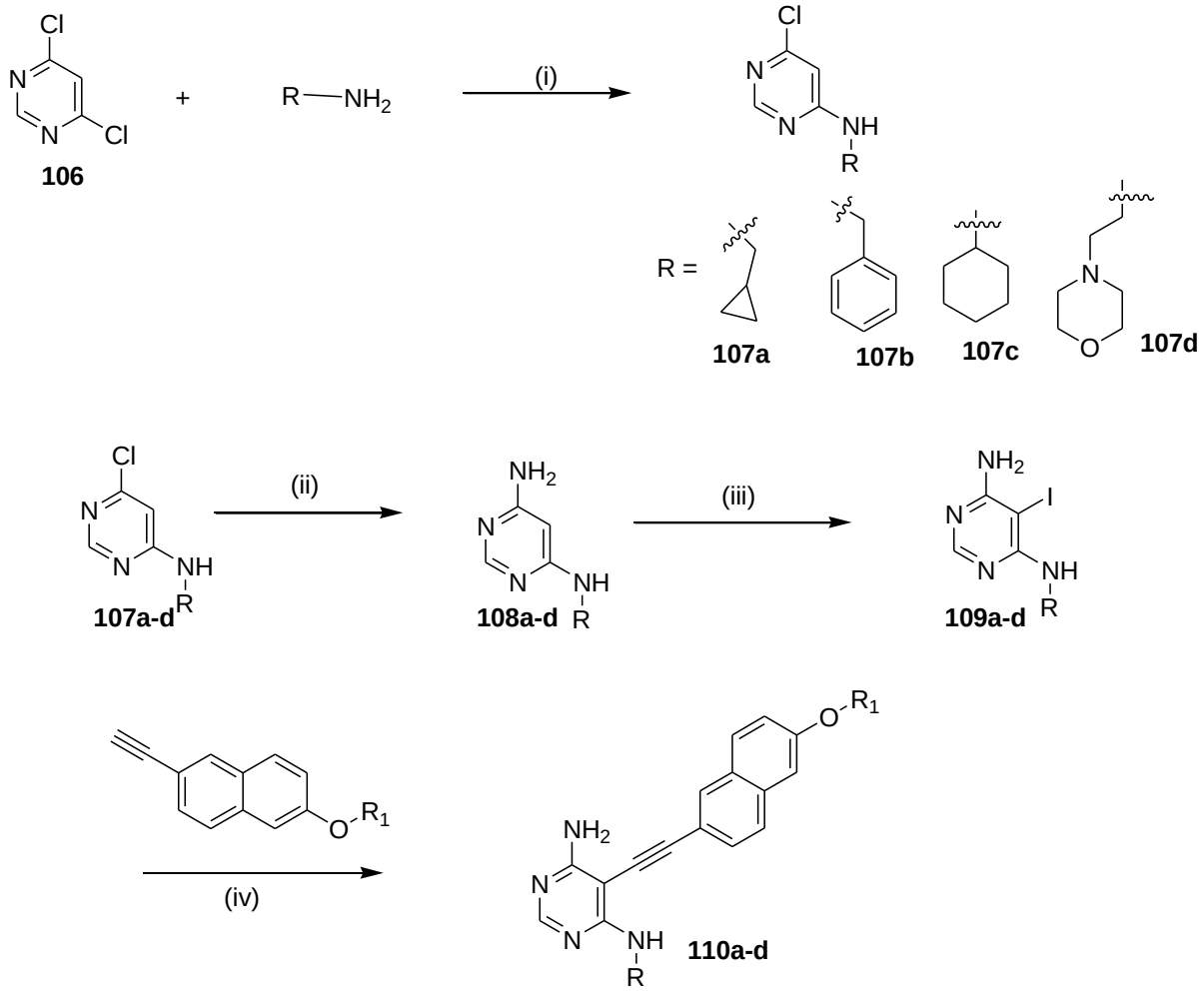


Figure 41: Structure of potential kinase inhibitor **125d** showing hydrogen bond with key amino acids residues (Asp148 and Tyr150).

6.2 Conclusions and future work pertaining to Chapter 4

While awaiting the biological results of the first series of compounds, we embarked on the synthesis of branched pyrimidine analogues as potential protein kinase inhibitors. Our route towards the synthesis of 4,5,6-trisubstituted pyrimidine derivatives **110** started with the sequential mono-nucleophilic displacement of each chlorine atom on 4,6-dichloropyrimidine to

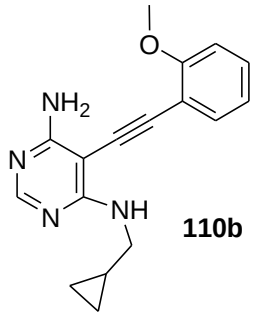
afford suitably substituted diaminopyrimidines **108a-d** (Scheme 61). Thereafter, a Sonogashira coupling reaction with different alkynyl derivatives was performed to construct the 4,5,6-trisubstituted pyrimidine derivatives **110a-i** in yields of 48 – 79% (**Scheme 61**).



Scheme 61: Reagents and conditions: (i) DIPEA, dioxane, 90 °C, 18 h, 75% - 85%. (ii) 25% aq NH_3, EtOH, sealed tube, 48 h, 70 °C, 83 - 89%. (iii) I_2, K_2CO_3, DMF/H_2O, 40 – 45 °C, 4 h, 83 - 89%. (iv) cat. $Pd(PPh_3)_2Cl_2$, CuI, diisopropylamine, DMF, 70 °C, 5 h, 48 - 79%.

Once again, all the 4,5,6-trisubstituted pyrimidine derivatives synthesised were tested for antimalarial activity in a whole cell antiplasmodium assay using the parasite lactate dehydrogenase (pLDH) assay. Compound **110b** was the only one from the branched pyrimidine series which showed significant activity in the antiplasmodium assay (IC_{50} 21.1 ± 2.40 μM, **Figure 42**). However, this was found to be significantly less potent than the drug standard (chloroquine), IC_{50} ~0.016 μM. Furthermore, the compound was shown to be cytotoxic at the

therapeutic concentration. This biological result suggests that pyrrolo[2,3-*d*]pyrimidine is a better scaffold than branched pyrimidine.



110b

Figure 42: Branched pyrimidine showing the greatest antimalarial activity.

In future, we would like to use the methodology developed in this project to modify the structural features of the first series of compounds prepared. Once again, the synthesis of more potent inhibitors with the aid of molecular modelling forms part of this future work. The results from our modelling studies showed that these compounds could also bind in the active site of *Pf*CDPK4. For example, key hydrogen-bonding interactions were observed between Tyr150 and Asp148 in the hinge region of *Pf*CDPK4 and N3 and 4-NH_2 groups of the pyrimidine scaffold (**Figure 43**). However, it may be beneficial to reduce the alkyne functional group to improve these interactions.

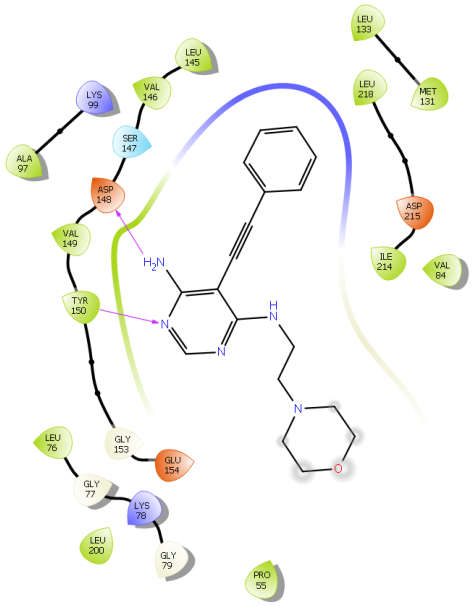


Figure 43

The effect of this change to afford flexible analogues of the 4,5,6-trisubstituted pyrimidines (**Figure 44**) on biological activity will also be assessed in the future. The flexibility of these compounds may result in increased binding affinity with the amino acid residues in the active site, and could potentially enhance the biological activity of these compounds.

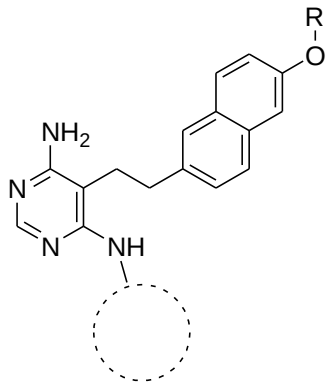


Figure 44

CHAPTER 7: EXPERIMENTAL SECTION

7.1 General Procedures

All reagents were purchased from Sigma-Aldrich (South Africa) or Merck KGaA (South Africa) and were used as received. All solvents for reactions were of analytical grade quality purchased from Sigma-Aldrich (South Africa), Merck KGaA (South Africa) or Minema Chemicals (South Africa). Solvents for column chromatography (ethyl acetate and hexanes) and acetone for cleaning were purchased from Protea Chemicals (South Africa) and were distilled before use to remove non-volatile components. Acetonitrile was distilled from calcium hydride and tetrahydrofuran (THF) was distilled from sodium/ benzophenone ketyl both under a nitrogen atmosphere. *N*,*N*-Dimethylformamide (DMF) was distilled from calcium hydride and stored over molecular sieves. Dichloromethane, chloroform, ethanol and methanol were purchased and used as received.

Column chromatographic purification was done on Macherey-Nagel silica gel 60 (particle size 0.063 mm to 0.200 mm). Thin Layer Chromatographic analysis was done on Merck Aluminium foil backed plates coated with silica gel 60, F_{254}.

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

^{1}H Nuclear Magnetic Resonance (NMR) data were acquired on a Bruker 300 or 500 MHz spectrometer at room temperature, using the specified deuterated solvent. For those compounds soluble in deuterated chloroform ($CDCl_3$), the solvent contained tetramethylsilane (TMS, 0.05% v/v) as internal standard. For others, the residual solvent signal was used for referencing. ^{13}C Nuclear Magnetic Resonance data were acquired on the same instruments. Data processing was done using MestreNova Software under license from Mestrelab Research, CA, USA.

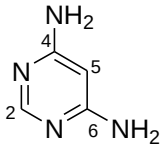
The following abbreviations are used to designate the multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, dd = doublet of doublets, ddd = doublet of doublet of doublets, ddt = doublet of doublet of triplets, td = triplet of doublets, H_a = axial proton, H_e = equatorial proton.

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

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

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

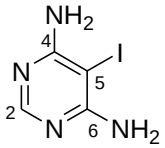
7.2.1 Synthesis of pyrimidine-4,6-diamine[1] 98



To a mixture of 4,6-diaminopyrimidine-2-thiol (5.00 g, 35.2 mmol) in water (500 ml) and 25% aqueous ammonia (20.0 ml) was added Raney nickel (10.0 g) in portions. The resulting mixture was heated at reflux for 2 hours after which it was cooled to room temperature, and the catalyst was removed by filtration on a Buchner funnel. The filtrate was evaporated to dryness on a rotary evaporator to furnish pyrimidine-4,6-diamine **98** (3.70 g, 96%) as a light yellow solid.

R_f (10% MeOH/dichloromethane) 0.32. *mp*: 271-272 °C. **^{1}H NMR (300 MHz, DMSO-d$_6$)**: δ 7.81 (1H, s, H2), 6.02 (4H, s, 2 × NH_2), 5.38 (1H, s, H5); **^{13}C NMR (75 MHz, DMSO-d$_6$)**: δ 163.5 (C-4 and C-6), 157.8 (C-2), 82.6 (C-5); **IR (ν_{max}/cm^{-1})**: 3445, 3303 (N-H); 3092 (=C-H); 1634 (C=N); 1475 (C=C).

7.2.2 Synthesis of 5-iodopyrimidine-4,6-diamine[2] 99

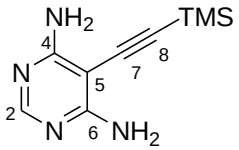


To a suspension of pyrimidine-4,6-diamine **98** (3.00 g, 27.2 mmol) in water (70.0 ml) and DMF (20.0 ml) was added potassium carbonate (5.65 g, 40.9 mmol, 1.5 eq) and iodine (13.8 g, 54.4 mmol, 2 eq). The resulting reaction mixture was heated at 40 - 45 °C for 4 hours. TLC analysis showed consumption of the starting material. The reaction mixture was cooled to room

temperature and quenched with 2 M aqueous sodium thiosulfate (40.0 ml) to give a clear solution. The product that formed was collected by filtration and washed with water (3 × 25 ml) to give 5-iodopyrimidine-4,6-diamine **99** (4.91 g, 76%) as a yellow solid.

Rf (10% MeOH/dichloromethane) 0.36. *mp*: 160 °C. **^{1}H NMR (300 MHz, DMSO-d$_6$)**: δ 7.72 (1H, s, H2), 6.31 (4H, s, 2 × NH_2); **^{13}C NMR (75 MHz, DMSO-d$_6$)**: δ 162.8 (C-4 and C-6), 156.9 (C-2), 55.2 (C-5); **IR (ν_{max}/cm^{-1})**: 3455, 3277 (N-H); 3067 (=C-H); 1627 (C=N); 1459 (C=C); 626.6 (C-I).

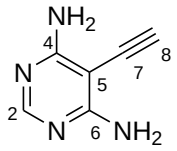
7.2.3 Synthesis of 5-[(trimethylsilyl)ethynyl]pyrimidine-4,6-diamine[3] 100



To a degassed mixture of 5-iodopyrimidine-4,6-diamine **99** (500 mg, 2.12 mmol), copper(I) iodide (12.0 mg, 6.36 × 10^{-2} mmol, 3 mole %) and $Pd(PPh_3)_4$ (122 mg, 0.106 mmol, 5 mole %) was added a degassed solution of ethynyltrimethylsilane (3.02 ml, 21.2 mmol), diisopropylamine (3.00 ml, 21.2 mmol, 10 eq) and tetrahydrofuran (20.0 ml). The resulting brown mixture was heated at 70 °C for 4 hours under a nitrogen atmosphere. After consumption of the starting material, the reaction mixture was quenched with a saturated aqueous ammonium chloride (NH_4Cl) solution and the THF was removed on a rotary evaporator. The remaining aqueous residue was washed three times with ethyl acetate (100 ml) and the organic layers were combined, dried over $MgSO_4$ and filtered through celite and excess solvent removed on a rotary evaporator. The crude product was purified by column chromatography (80% EtOAc/hexane) to furnish 5-[(trimethylsilyl)ethynyl]pyrimidine-4,6-diamine **100** (0.21 g, 48%). as a light yellow solid.

R_f (80% EtOAc/hexane) 0.64. *mp*: 190-192 °C. **^{1}H NMR (300 MHz, DMSO-d$_6$)**: δ 7.82 (1H, s, H2), 6.36 (4H, s, 2 × NH_2), 0.23 (9H, s, TMS); **^{13}C NMR (75 MHz, DMSO-d$_6$)**: δ 163.4 (C-4 and C-6), 156.5 (C-2), 99.4 (C-7), 97.2 (C-8), 78.2 (C-5), 0.00 (TMS); **IR (ν_{max}/cm^{-1})**: 3457, 3291 (N-H); 3089 (=C-H); 2136 (C≡C), 1625 (C=N); 1463 (C=C).

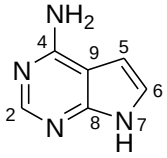
7.2.4 Synthesis of 5-ethynylpyrimidine-4,6-diamine[4] 101



To a solution of 5-[(trimethylsilyl)ethynyl]pyrimidine-4,6-diamine **100** (200 mg, 0.969 mmol) in tetrahydrofuran (20.0 ml) was added tetrabutylammonium fluoride, 1.0 M in THF, (0.561 ml, 1.94 mmol, 2 eq) at 0 °C. The reaction mixture was then allowed to stir at room temperature for 3 hours. TLC analysis showed consumption of the starting material. The reaction mixture was quenched with saturated aqueous ammonium chloride and was extracted with ethyl acetate (3 × 25 ml). The combined organic extracts were dried with $MgSO_4$, filtered through celite and excess solvent was removed on a rotary evaporator. The crude product was purified by column chromatography to give 5-ethynylpyrimidine-4,6-diamine **101** (0.11 g, 85%) as a yellow solid.

R_f (80% EtOAc/hexane) 0.58. *mp*: 202-203 °C. **^{1}H NMR (300 MHz, DMSO-d_6)**: δ 7.82 (1H, s, H2), 6.42 (4H, s, 2 × NH_2), 4.57 (1H, s, H8); **^{13}C NMR (75 MHz, DMSO-d_6)**: δ 163.9 (C-4 and C-6), 156.5 (C-2), 91.1 (C-5), 77.4 (C-8), 76.1 (C-7); **IR (ν_{max}/cm^{-1})**: 3551, 3252 (N-H); 3158 (=C-H); 2092 (C≡C), 1628 (C=N); 1470 (C=C).

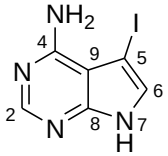
7.2.5 Synthesis of 7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine[5] 102



A mixtute of 5-ethynylpyrimidine-4,6-diamine **101** (300 mg, 2.24 mmol), cesium carbonate (1.46 g, 4.47 mmol) and DMSO (4.00 ml) in a 10.00 ml microwave tube was irradiated at 100 W and 180 °C for 15 minutes. After this time, the reaction mixture was cooled to room temperature and poured into a separating funnel containing 100 ml water and 100 ml ethyl acetate. The aqueous layer was extracted with EtOAc (2 × 100 ml). The combined organic extracts were dried with $MgSO_4$, filtered through celite and excess solvent was removed *in vacuo*. Purification by column chromatography (80% EtOAc/hexane - 10% MeOH/CH_2Cl_2) gave 7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **102** (0.15 g, 50%) as an off-white solid.

R_f (10% MeOH/dichloromethane) 0.28. *mp*: 257-259 °C. **^{1}H NMR (300 MHz, DMSO-d$_6$)**: δ 11.42 (1H, s, NH), 8.01 (1H, s, H2), 7.05 (1H, dd, *J* = 3.5, 2.1 Hz, H6), 6.85 (2H, s, NH_2), 6.51 (1H, dd, *J* = 3.4, 1.8 Hz, H5); **^{13}C NMR (75 MHz, DMSO-d$_6$)**: δ 157.8 (C-4), 152.0 (C-2), 151.1 (C-8), 121.3 (C-6), 102.7 (C-9), 99.3 (C-5); **IR (ν_{max}/cm^{-1})**: 3414, 3291 (N-H); 3086 (=C-H); 1640 (C=N); 1475 (C=C).

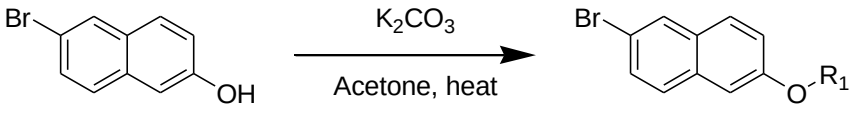
7.2.6 Synthesis of 5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine[6] 103



7*H*-Pyrrolo[2,3-*d*]pyrimidin-4-amine **102** (200 mg, 1.49 mmol) was dissolved in dry DMF (5.00 ml) and to this was added *N*-iodosuccinimide (0.402 g, 1.79 mmol). The resulting brown mixture was heated at 60 °C for 4 hours. The reaction progress was monitored by TLC (80% EtOAc/hexane). When complete, the reaction mixture was cooled to room temperature, quenched with water and extracted with ethyl acetate (2 × 100 ml). The combined organic layers were dried over $MgSO_4$, filtered through celite and excess solvent was removed on a rotary evaporator. The residue was purified by silica gel column chromatography (80% EtOAc/hexane as eluent) to give the desired product **103** (0.22 g, 57%) as viscous oil.

Rf (10% MeOH/dichloromethane) 0.56. **^{1}H NMR (300 MHz, DMSO-d$_6$)**: δ 11.95 (1H, s, NH), 8.05 (1H, s, H2), 7.36 (1H, d, *J* = 1.6 Hz, H6), 6.54 (2H, s, NH_2); **^{13}C NMR (75 MHz, DMSO-d$_6$)**: δ 156.9 (C-4), 151.7 (C-2), 150.5 (C-8), 126.7 (C-6), 102.6 (C-9), 50.1 (C-5); **IR (ν_{max}/cm^{-1})**: 3411, 3288 (N-H); 3085 (=C-H); 1632 (C=N); 1460 (C=C). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_6H_6IN_4$: 260.9639, found: $[M + H]^+$ 260.9641.

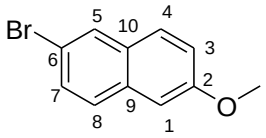
7.3 General procedure for alkylation of 6-bromonaphthalen-2-ol.



120a-c

To a suspension of 6-bromonaphthalen-2-ol **119** in acetone (200 ml) was added potassium carbonate (2 eq) and alkyl halide (1.1 eq). The resultant mixture was refluxed overnight and reaction progress monitored by thin layer chromatography. After completion, the reaction medium was cooled to room temperature, filtered on a Buchner funnel and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (10% - 20% EtOAc/hexane) to give **120a-120c** in good yields. The following products were prepared using this methodology:

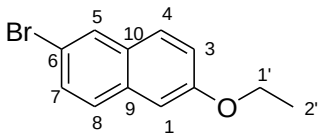
7.3.1 Synthesis of 2-bromo-6-methoxynaphthalene[7] 120a



6-Bromonaphthalen-2-ol **119** (5.00 g, 22.4 mmol) was dissolved in acetone (200 ml). To the clear solution was added potassium carbonate (6.20 g, 44.8 mmol) and methyl iodide (1.53 ml, 24.7 mmol). The pure product **120a** (4.95 g, 93%) was isolated as a cream white solid.

R_f (20% EtOAc/hexane) 0.82. *mp*: 105 °C. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 7.89 (1H, d, *J* = 2.0 Hz, H5), 7.89 – 7.56 (2H, m, H4 and H8), 7.48 (1H, dd, *J* = 8.7, 2.0 Hz, H7), 7.14 (1H, dd, *J* = 8.9, 2.5 Hz, H3), 7.07 (1H, d, *J* = 2.5 Hz, H1), 3.89 (3H, s, OMe); **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 157.9 (C-2), 133.0 (C-9), 130.0 (C-7), 129.6 (C-5), 129.63 (C-4), 128.5 (C-8), 128.4 (C-10), 119.7 (C-3), 117.0 (C-6), 105.8 (C-1), 55.3 (OMe); **IR (ν_{max}/cm^{-1})**: 2967 (=C-H); 1262 (C=C); 1030 (C-O).

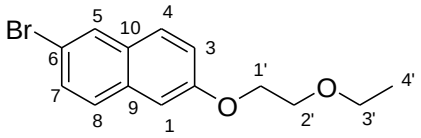
7.3.2 Synthesis of 2-bromo-6-ethoxynaphthalene[7] 120b



6-Bromonaphthalen-2-ol **119** (8.00 g, 35.9 mmol) was dissolved in acetone (200 ml). To the clear solution was added potassium carbonate (9.91 g, 71.7 mmol) and ethyl bromide (2.92 ml, 39.5 mmol). The pure product **120b** (7.93 g, 88%) was isolated as a cream white solid.

R_f (20% EtOAc/hexane) 0.82. *mp*: 96 °C. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 7.89 (1H, d, *J* = 2.0 Hz, H5), 7.63 – 7.57 (2H, m, H4 and H8), 7.47 (1H, dd, *J* = 8.7, 1.9 Hz, H7), 7.14 (1H, dd, *J* = 8.9, 2.5 Hz, H3), 7.06 (1H, d, *J* = 2.4 Hz, H1), 4.11 (2H, q, *J* = 7.0 Hz, H1'), 1.46 (3H, t, *J* = 7.0 Hz, H2'); **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 157.2 (C-2), 133.1 (C-9), 129.9 (C-7), 129.6 (C-5), 129.5 (C-4), 128.4 (C-8), 128.3 (C-10), 120.0 (C-3), 116.9 (C-6), 106.5 (C-1), 63.5 (C-1'), 14.8 (C-2'); **IR (ν_{max}/cm^{-1})**: 2985 (=C-H); 1453 (C=C); 1115 (C-O).

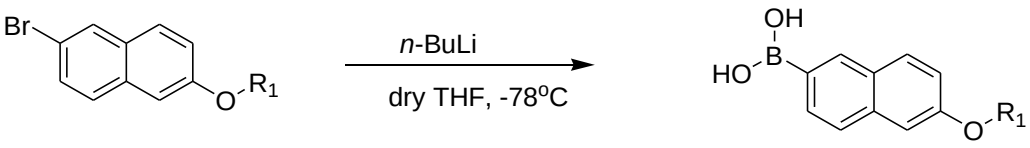
7.3.3 Synthesis of 2-(2-ethoxyethoxy)-6-bromonaphthalene 120c



To a solution of 6-bromonaphthalen-2-ol **119** (5.00 g, 22.4 mmol) in acetone (200 ml) was added potassium carbonate (6.20 g, 44.8 mmol) and 1-bromo-2-ethoxyethane (2.78 ml, 24.7 mmol). A light brown solid product **120c** (5.65 g, 85%) was isolated.

Rf (20% EtOAc/hexane) 0.82. *mp*: 76 °C. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 7.88 (1H, d, *J* = 2.0 Hz, H5), 7.62 – 7.53 (2H, m, H4 and H8), 7.46 (1H, dd, *J* = 8.8, 2.0 Hz, H7), 7.19 (1H, dd, *J* = 9.0, 2.5 Hz, H3), 7.07 (1H, d, *J* = 2.5 Hz, H1), 4.19 (2H, dd, *J* = 5.7, 4.0 Hz, H1'), 3.84 – 3.82 (2H, m, H2'), 3.61 (2H, q, *J* = 7.0 Hz, H3'), 1.25 (3H, t, *J* = 7.0 Hz, H4'); **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 157.1 (C-2), 132.9 (C-9), 130.1 (C-7 and C-5), 129.5 (C-4), 128.4 (C-8 and C-10), 120.1 (C-3), 117.1 (C-6), 106.7 (C-1), 68.8 (C-3'), 67.5 (C-1'), 66.9 (C-2'), 15.2 (C-4'); **IR (ν_{max}/cm^{-1})**: 2875 (=C-H); 1452 (C=C); 1061 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{14}H_{16}{}^{79}BrO_2$: 295.0335, found: $[M + H]^+$ 295.0312.

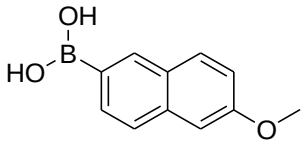
7.4 General procedure for the preparation of boronic acids.



Each of compounds **120a - 120c** was dissolved in freshly distilled, dry tetrahydrofuran (30 ml) in a two necked round bottom flask and degassed for about 15 minutes. The resultant reaction

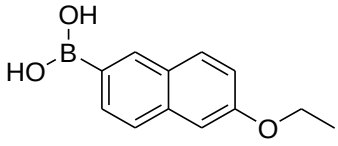
mixture was then cooled to -78 °C under nitrogen atmosphere and *n*-butyllithium (2.0 M/THF, 2.0 eq) was added dropwise to the reaction medium via a syringe. The resulting yellowish reaction mixture was stirred further at the same temperature for one hour. Freshly distilled triisopropyl borate (3.0 eq) was then slowly added to the reaction mixture. The resulting reaction mixture was stirred at -78 °C for another one hour; then slowly allowed to warm up to room temperature over two hours. The reaction mixture was then quenched with 10% aqueous hydrochloric acid (50 ml) and poured into a separating funnel containing diethyl ether (50 ml). The aqueous layer was extracted with diethyl ether (2 × 30 ml). The organic extracts were dried over $MgSO_4$, filtered through celite and excess solvent was allowed to evaporate in the fume hood to give a cream white solid in good yield. Due to decomposition on silica gel, the boronic acids **121a - 121c** prepared were used in the next step without further purification or characterisation. However, crude ^{1}H NMR spectra were obtained showing the desired product as the major product in each case.

7.4.1 Synthesis of 6-methoxynaphthalen-2-yl-2-boronic acid 121a



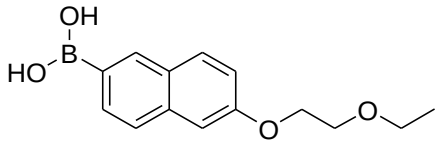
2-Bromo-6-methoxynaphthalene **120a** (2.00 g, 8.44 mmol) was dissolved in THF (30 ml) and cooled to -78 °C under nitrogen atmosphere. *n*-Butyllithium (1.60 ml, 16.9 mmol, 2.0 eq) was then added, followed by triisopropyl borate (6.00 ml, 25.3 mmol, 3.0 eq). 6-Methoxynaphthalen-2-yl-2-boronic acid **121a** (1.52 g, 89%) was isolated as an off-white solid.

7.4.2 Synthesis of 6-ethoxynaphthalen-2-yl-2-boronic acid 121b



2-Bromo-6-ethoxynaphthalene **120b** (2.00 g, 7.96 mmol) was dissolved in THF (30 ml) and cooled to -78 °C under nitrogen atmosphere. *n*-Butyllithium (1.50 ml, 15.9 mmol, 2.0 eq) was then added, followed by triisopropyl borate (5.51 ml, 23.9 mmol, 3.0 eq). 6-Ethoxynaphthalen-2-yl-2-boronic acid **121b** (1.55 g, 90%) was isolated as an off-white solid.

7.4.3 Synthesis of 6-(2-ethoxyethoxy)naphthalen-2-yl-2-boronic acid 121c

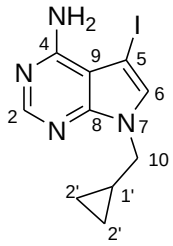


2-(2-Ethoxyethoxy)-6-bromonaphthalene **120c** (2.00 g, 6.78 mmol) was dissolved in THF (30 ml) and cooled to -78 °C under nitrogen atmosphere. *n*-Butyllithium (1.30 ml, 13.6 mmol, 2.0 eq) was then added, followed by triisopropyl borate (4.69 ml, 20.3 mmol, 3.0 eq). 6-(2-Ethoxyethoxy)naphthalen-2-yl-2-boronic acid **121c** (1.46 g, 83%) was isolated as a brown solid.

7.5 Synthesis of 5-substituted 7-(cyclopropylmethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amines.

With our iodinated pyrrolopyrimidine **103** in hand; preparation of potential protein kinase inhibitors bearing a cyclopropylmethyl moiety at N-7 of 7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine will be explained.

7.5.1 Synthesis of 7-(cyclopropylmethyl)-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine 104b



A mixture of 5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **103** (100 mg, 0.385 mmol), cesium carbonate (0.250 g, 0.769 mmol, 2 eq) and (bromomethyl)cyclopropane (0.0410 ml, 0.423 mmol, 1.1 eq) in dry DMF (10.0 ml) was heated at 70 °C for 18 hours. After formation of a new product spot, visible by TLC, the reaction mixture was cooled to room temperature and poured into a separating funnel containing water (100 ml) and ethyl acetate (100 ml). The aqueous layer was extracted with EtOAc (2 × 100 ml). The combined organic extracts were dried over $MgSO_4$, filtered through celite and excess solvent was removed *in vacuo*. Purification by column

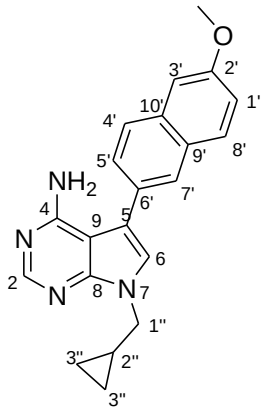
chromatography (80% EtOAc/hexane) gave 7-(cyclopropylmethyl)-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **104b** (0.069 g, 57%) as a light orange solid.

R_f (80% EtOAc/hexane) 0.21. *mp*: 250-252 °C. **^{1}H NMR (500 MHz, DMSO-d_6)**: δ 8.09 (1H, s, H2), 7.53 (1H, s, H6), 6.59 (2H, s, NH_2), 3.96 (2H, d, *J* = 7.2 Hz, H10), 1.23 – 1.20 (1H, m, H1'), 0.51 – 0.35 (4H, m, H2'); **^{13}C NMR (125 MHz, DMSO-d_6)**: δ 157.6 (C-4), 152.2 (C-2), 149.9 (C-8), 129.8 (C-9), 103.3 (C-6), 49.8 (C-10), 48.7 (C-5), 12.1 (C-1'), 4.1 (C-2'); **IR (ν_{max}/cm^{-1})**: 3420, 3292 (N-H); 3080 (=C-H); 1641 (C=N). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{10}H_{12}IN_4$: 315.0108, found: $[M + H]^+$ 315.0118.

7.5.2 General procedure for Suzuki-Miyaura cross coupling

To a flame dried 2-necked round bottom flask (100 ml) under nitrogen gas containing 7-(cyclopropylmethyl)-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **104b**, tetrakis(triphenylphosphine) palladium(0) (0.1 eq) and the relevant boronic acid (2 eq), was added a degassed solution of 1,2-dimethoxyethane (30.0 ml) and 2 M aqueous sodium carbonate (4 eq). The resulting mixture was then heated to 80 °C for 18 hours under nitrogen atmosphere. After completion, the reaction mixture was allowed to cool and quenched with water (50 ml) and transferred to a separating funnel. The organic compound was extracted with ethyl acetate (3 × 100 ml). The organic layers were combined, dried over $MgSO_4$, filtered through celite and excess solvent was removed on a rotary evaporator. Purification by silica gel column chromatography using an ethyl acetate/hexane mixture (50 – 80%) gave title compounds **122a-122e** in good yields. The following products were prepared using this methodology:

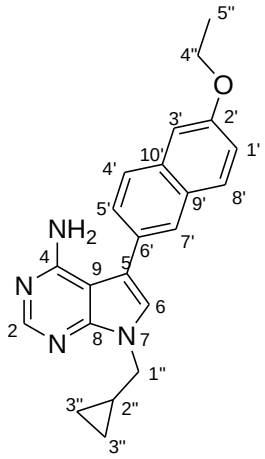
7.5.2.1 Synthesis of 7-(cyclopropylmethyl)-5-(2-methoxynaphthalen-6-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine 122a



To a mixture of 7-(cyclopropylmethyl)-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **104b** (60.0 mg, 0.191 mmol), $Pd(PPh_3)_4$ (0.0221 g, 0.0191 mmol), and 6-methoxynaphthalen-2-yl-2-boronic acid (77.0 mg, 0.382 mmol) was added a solution of DME (30.0 ml) and 2 M aqueous Na_2CO_3 (0.400 ml, 4 eq). The product **122a** (0.036 g, 55%) was isolated as a yellow solid after purification by column chromatography (80% EtOAc/hexane).

R_f (10% MeOH/EtOAc) 0.48. *mp*: 215-217 °C. **^{1}H NMR (300 MHz, MeOD)**: δ 8.17 (1H, s, H2), 7.91 − 7.88 (2H, m, H4' and H7'), 7.81 (1H, d, *J* = 9.0 Hz, H8'), 7.59 (1H, dd, *J* = 8.3, 1.9 Hz, H5'), 7.35 (1H, s, H6), 7.30 (1H, d, *J* = 2.5 Hz, H3'), 7.19 (1H, dd, *J* = 9.0, 2.5 Hz, H1'), 4.11 (2H, d, *J* = 7.1 Hz, H1"), 3.94 (3H, s, OMe), 1.37 – 1.28 (1H, m, H2"), 0.65 – 0.59 (2H, m, H3"), 0.49 – 0.46 (2H, m, H3"); **^{13}C NMR (75 MHz, MeOD)**: δ 156.5 (C-4), 155.7 (C-2), 148.9 (C-2'), 148.0 (C-8), 132.3 (C-10'), 128.0 (C-6' and C-9'), 127.7 (C-5 and C-8'), 127.5 (C-4'), 125.7 (C-7'), 125.3 (C-5'), 121.9 (C-1'), 115.2 (C-6), 103.9 (C-3' and C-9), 52.9 (OMe), 47.1 (C-1"), 9.6 (C-2"), 1.4 (C-3"); **IR (ν_{max}/cm^{-1})**: 3462 (N-H); 3060 (=C-H); 1500 (C=C); 1454 (CH_2); 1020 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{21}H_{21}N_4O$: 345.1717, found: $[M + H]^+$ 345.1694.

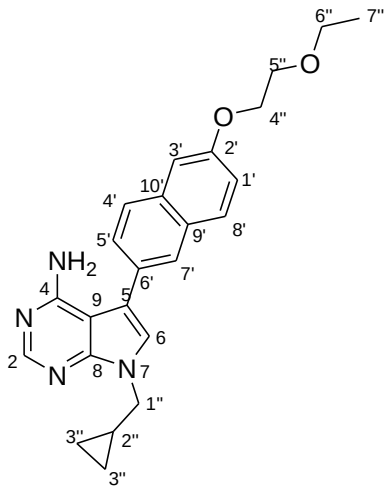
7.5.2.2 Synthesis of 7-(cyclopropylmethyl)-5-(2-ethoxynaphthalen-6-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine 122b



To a mixture of 7-(cyclopropylmethyl)-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **104b** (80.0 mg, 0.255 mmol), $Pd(PPh_3)_4$ (30.0 mg, 0.0255 mmol), and 6-ethoxynaphthalen-2-yl-2-boronic acid (0.110 g, 0.509 mmol) was added a solution of DME (30.0 ml) and 2 M aqueous Na_2CO_3 (0.500 ml, 4 eq). The product **122b** (0.055 g, 60%) was isolated as a light yellow solid after purification by column chromatography (80% EtOAc/hexane).

R_f (10% MeOH/EtOAc) 0.48. *mp*: 215-216 °C. **^{1}H NMR (300 MHz, DMSO-d_6)**: δ 8.18 (1H, s, H2), 7.92 – 7.86 (3H, m, H4', H5' and H8'), 7.62 (1H, d, *J* = 4.4 Hz, H7'), 7.51 (1H, s, H6), 7.36 (1H, d, *J* = 2.5 Hz, H3'), 7.19 (1H, dd, *J* = 8.9, 2.5 Hz, H1'), 6.19 (2H, s, NH_2), 4.17 (2H, q, *J* = 6.9 Hz, H4"), 4.06 (1H, d, *J* = 7.2 Hz, H1"), 1.41 (3H, t, *J* = 6.9 Hz, H5"), 1.37 – 1.28 (1H, m, H2"), 0.55 – 0.42 (4H, m, H3"); **^{13}C NMR (75 MHz, DMSO-d_6)**: δ 155.7 (C-4), 153.0 (C-2'), 149.1 (C-2), 148.0 (C-8), 133.9 (C-10'), 130.2 (C-6'), 130.1 (C-9'), 129.0 (C-8'), 127.6 (C-5), 127.0 (C-4'), 125.8 (C-7'), 125.3 (C-5'), 117.9 (C-1'), 107.3 (C-6), 104.7 (C-3' and C-9), 61.7 (C-4"), 47.1 (C-1"), 12.2 (C-5"), 9.6 (C-2"), 1.4 (C-3"); **IR (ν_{max}/cm^{-1})**: 3464 (N-H); 3062 (=C-H); 1455 (C=C); 1222 (CH_2); 1020 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{22}H_{23}N_4O$: 359.1874, found: $[M + H]^+$ 359.1848.

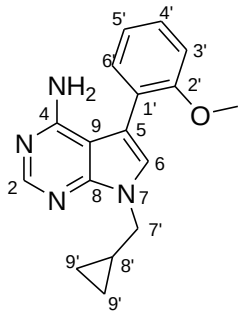
7.5.2.3 Synthesis of 5-[2-(2-ethoxyethoxy)naphthalen-6-yl]-7-(cyclopropylmethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine 122c



To a mixture of 7-(cyclopropylmethyl)-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **104b** (80.0 mg, 0.255 mmol), $Pd(PPh_3)_4$ (30.0 mg, 0.0255 mmol), and 6-(2-ethoxyethoxy)naphthalen-2-yl-2-boronic acid (0.132 g, 0.509 mmol) was added a solution of DME (30.0 ml) and 2 M aqueous Na_2CO_3 (0.500 ml, 4 eq). The product **122c** (0.036 g, 35%) was isolated as a light yellow solid after purification by column chromatography (80% EtOAc/hexane). Yield

R_f (10% MeOH/EtOAc) 0.48. *mp*: 216-218 °C. **^{1}H NMR (300 MHz, MeOD)**: δ 8.19 (1H, s, H2), 7.91 − 7.88 (2H, m, H4' and H7'), 7.83 (1H, d, *J* = 9.0 Hz, H8'), 7.60 (1H, dd, *J* = 8.5, 1.7 Hz, H5'), 7.38 (1H, s, H6), 7.33 (1H, d, *J* = 2.5 Hz, H3'), 7.23 (1H, dd, *J* = 8.9, 2.5 Hz, H1'), 4.29 – 4.26 (2H, m, H4''), 4.13 (2H, d, *J* = 7.1 Hz, H1''), 3.90 – 3.87 (2H, m, H5''), 3.66 (2H, q, *J* = 7.0 Hz, H6''), 1.37 – 1.27 (4H, m, H2'' and H7''), 0.64 – 0.59 (2H, m, H3''), 0.50 – 0.45 (2H, m, H3''); **^{13}C NMR (75 MHz, MeOD)**: δ 155.6 (C-4), 148.7 (C-2' and C-8), 147.9 (C-2), 132.2 (C-10'), 130.9 (C-6'), 130.1 (C-9'), 128.1 (C-8'), 127.7 (C-5), 127.6 (C-4'), 125.7 (C-7'), 125.3 (C-5'), 122.1 (C-1'), 115.2 (C-6), 104.9 (C-3'), 99.1 (C-9), 67.3 (C-5''), 65.8 (C-4''), 64.9 (C-6''), 47.1 (C-1''), 12.5 (C-7''), 9.6 (C-2''), 1.4 (C-3''); **IR (ν_{max}/cm^{-1})**: 3443, 3277 (N-H); 3065 (=C-H); 1631 (C=N); 1062 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{24}H_{27}N_4O$: 403.2136, found: $[M + H]^+$ 403.2116.

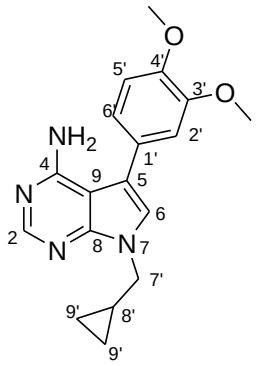
7.5.2.4 Synthesis of 7-(cyclopropylmethyl)-5-(2-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine 122d



To a mixture of 7-(cyclopropylmethyl)-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **104b** (60.0 mg, 0.191 mmol), $Pd(PPh_3)_4$ (0.0221 g, 0.0191 mmol), and 2-methoxyphenylboronic acid (60.0 mg, 0.382 mmol) was added a solution of DME (30.0 ml) and 2 M aqueous Na_2CO_3 (0.400 ml, 4 eq). The product **122d** (0.031 g, 55%) was isolated as a light yellow solid after purification by column chromatography (80% EtOAc/hexane).

R_f (10% MeOH/EtOAc) 0.48. *mp*: 221-222 °C. **^{1}H NMR (300 MHz, DMSO-d$_6$)**: δ 8.02 (1H, s, H2), 7.30 (1H, ddd, *J* = 8.4, 7.3, 1.8 Hz, H4'), 7.22 (1H, dd, *J* = 7.5, 1.8 Hz, H6'), 7.12 (1H, s, H6), 7.03 (1H, d, *J* = 8.5 Hz, H3'), 6.95 (1H, td, *J* = 7.5, 1.1 Hz, H5'), 3.99 (2H, d, *J* = 7.1 Hz, H7'), 3.72 (3H, s, OMe), 0.82 – 0.78 (1H, m, H8'), 0.52 – 0.48 (2H, m, H9'), 0.36 – 0.34 (2H, m, H9'); **^{13}C NMR (75 MHz, DMSO-d$_6$)**: δ 161.6 (C-4), 160.9 (C-2'), 154.3 (C-2), 152.9 (C-8), 127.9 (C-4'), 127.2 (C-6'), 125.3 (C-5), 124.7 (C-1'), 116.0 (C-5'), 115.1 (C-3'), 107.4 (C-6), 105.9 (C-9), 58.6 (C-7'), 52.5 (OMe), 15.0 (C-8'), 6.8 (C-9'); **IR (ν_{max}/cm^{-1})**: 3445, 3225 (N-H); 3115 (=C-H); 1632 (C=N); 1060 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{17}H_{19}N_4O$: 295.1561, found: $[M + H]^+$ 295.1548.

7.5.2.5 Synthesis of 7-(cyclopropylmethyl)-5-(3,4-dimethoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine 122e



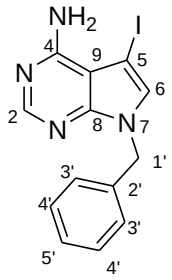
To a mixture of 7-(cyclopropylmethyl)-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **104b** (100 mg, 0.318 mmol), $Pd(PPh_3)_4$ (0.0368 g, 0.0318 mmol), and 3,4-dimethoxyphenylboronic acid (0.120 g, 0.637 mmol) was added a solution of DME (30 ml) and 2 M aqueous Na_2CO_3 (0.700 ml, 4 eq). The product **122e** (0.071 g, 69%). was isolated as a light yellow solid after purification by column chromatography (80% EtOAc/hexane).

R_f (10% MeOH/EtOAc) 0.48. *mp*: 201-202 °C. **^{1}H NMR (500 MHz, DMSO-d$_6$)**: δ 7.98 (1H, s, H2), 7.21 (1H, s, H6), 6.92 – 6.88 (2H, m, H2' and H6'), 6.82 (1H, dd, *J* = 8.1, 2.0 Hz, H5'), 5.92 (2H, s, NH_2), 3.86 (2H, d, *J* = 7.1 Hz, H7'), 3.66 (3H, s, OMe), 3.65 (3H, s, OMe), 1.15 – 1.09 (1H, m, H8'), 0.37 – 0.26 (4H, m, H9'); **^{13}C NMR (125 MHz, DMSO-d$_6$)**: δ 157.7 (C-4), 151.9 (C-2), 150.4 (C-8), 149.4 (C-4'), 148.3 (C-3'), 127.9 (C-1'), 123.3 (C-5), 120.9 (C-6'), 115.5 (C-5'), 112.9 (C-2'), 112.8 (C-6), 100.5 (C-9), 56.1 (OMe), 55.9 (OMe), 48.5 (C-7'), 12.1 (C-8'), 4.1 (C-9'); **IR (ν_{max}/cm^{-1})**: 3444, 3202 (N-H); 3108 (=C-H); 1632 (C=N); 1061 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{18}H_{21}N_4O_2$: 325.1666, found: $[M + H]^+$ 325.1672.

7.6 Synthesis of 5-substituted 7-benzyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amines.

In the following sections, preparation of potential protein kinase inhibitors bearing benzyl moiety at N-7 of 7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine will be discussed.

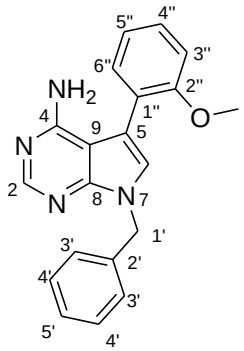
7.6.1 Synthesis of 7-benzyl-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine 104a



Compound **104a** was synthesized from 5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **103** (100 mg, 0.385 mmol), cesium carbonate (0.250 g, 0.769 mmol, 2 eq) and benzyl bromide (0.0500 ml, 0.423 mmol, 1.1 eq) in dry DMF (10.0 ml) using the procedure of 7**.5.1**. After extraction and purification by column chromatography using 80% EtOAc/hexane; 7-benzyl-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **104a** (0.08 g, 59%) was isolated as a light brown solid

R_f (80% EtOAc/hexane) 0.37. *mp*: 220-221 °C. **^{1}H NMR (300 MHz, DMSO-d$_6$)**: δ 8.12 (1H, s, H2), 7.54 (1H, s, H6), 7.32 – 7.22 (5H, m, Ar-H), 6.63 (2H, s, NH_2), 5.32 (2H, s, H1'); **^{13}C NMR (75 MHz, DMSO-d$_6$)**: δ 158.2 (C-4), 152.2 (C-2), 150.7 (C-8), 138.6 (C-2'), 129.0 (C-3'), 127.9 (C-4'), 127.6 (C-5'), 112.8 (C-6), 100.5 (C-9), 48.7 (C-5), 47.6 (C-1'); **IR (ν_{max}/cm^{-1})**: 3452, 3275 (N-H); 3064 (=C-H); 1630 (C=N); 1461 (C=C); 626.8 (C-I). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{13}H_{12}IN_4$: 351.0108, found: $[M + H]^+$ 351.0104.

7.6.2 Synthesis of 7-benzyl-5-(2-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine 115a



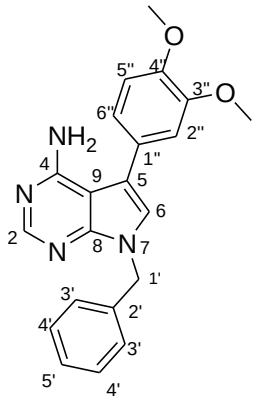
To a mixture of 7-benzyl-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **104a** (40.0 mg, 0.114 mmol), $Pd(PPh_3)_4$ (0.0132 g, 0.0114 mmol), and 2-methoxyphenylboronic acid (347 mg, 0.228 mmol) was added a solution of DME (30.0 ml) and 2 M aqueous Na_2CO_3 (0.300 ml). Using

general procedure **7.5.2** product **115a** (0.018 g, 48%) was isolated as a light yellow solid after purification by column chromatography (80% EtOAc/hexane).

R_f (10% MeOH/EtOAc) 0.50. *mp*: 240-241 °C. **^{1}H NMR (300 MHz, MeOD)**: δ 8.13 (1H, s, H2), 7.66 – 7.58 (1H, m, H6''), 7.36 – 7.22 (6H, m, Ar-H and H4''), 7.11 − 7.04 (2H, m, H3'' and H6), 7.02 (1H, t, *J* = 7.4 Hz, H5''), 5.41 (2H, s, H1'), 3.78 (3H, s, OMe); **^{13}C NMR (75 MHz, MeOD)**: δ 158.3 (C-4 and C-2''), 151.7 (C-2), 150.8 (C-8), 138.9 (C-2'), 132.8 (C-4''), 130.5 (C-3'), 130.1 (C-4'), 128.8 (C-6''), 125.5 (C-5 and C-5'), 122.2 (C-1'' and C-5''), 112.6 (C-3''), 101.4 (C-6), 100.1 (C-9), 56.0 (OMe), 47.6 (C-1'); **IR (ν_{max}/cm^{-1})**: 3476 (N-H); 3107 (=C-H); 1581 (C=C); 1452 (CH_2); 1026 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{20}H_{19}N_4O$: 331.1561, found: $[M + H]^+$ 331.1534.

7.6.3 Synthesis of 7-benzyl-5-(3,4-dimethoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine

115b



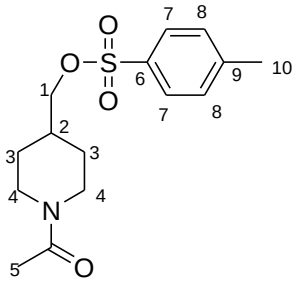
Reaction of 7-benzyl-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **104a** (500 mg, 0.143 mmol), $Pd(PPh_3)_4$ (0.0165 g, 0.0143 mmol), 3,4-dimethoxyphenylboronic acid (0.0520 g, 0.286 mmol) and 2 M aqueous Na_2CO_3 (0.300 ml, 4 eq) as described in general procedure **5.5.2** afforded, after filtration and column chromatography using 80% EtOAc/hexane as eluent, 7-benzyl-5-(3,4-dimethoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **115b** (0.023 g, 45%) as a yellow solid.

R_f (10% MeOH/EtOAc) 0.50. *mp*: 242-244 °C.**^{1}H NMR (300 MHz, DMSO-d$_6$)**: δ 8.16 (1H, s, H2), 7.37 (1H, s, H6), 7.31 (5H, m, Ar-H), 7.06 – 7.01 (2H, m, H2'' and H5''), 6.96 (1H, dd, *J* = 8.1, 2.0 Hz, H6''), 6.15 (2H, s, NH_2), 5.38 (2H, s, H1'), 3.79 (3H, s, OMe), 3.789 (3H, s, OMe);

^{13}C NMR (75 MHz, DMSO-d_6): δ 157.7 (C-4), 152.2 (C-2), 150.7 (C-8), 149.4 (C-3"), 148.3 (C-4"), 138.6 (C-2'), 129.0 (C-3'), 127.9 (C-4'), 127.9 (C-1"), 127.6 (C-5 and C-5'), 123.4 (C-6"), 120.9 (C-5"), 116.1 (C-2"), 112.8 (C-6), 100.5 (C-9), 56.1 (OMe), 55.9 (OMe), 47.6 (C-1'); **IR (ν_{max}/cm^{-1})**: 3425 (N-H); 3108 (=C-H); 1579 (C=C); 1029 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{21}H_{21}N_4O_2$: 361.1666, found: $[M + H]^+$ 361.1622.

7.7 Synthesis of 5-substituted 7-piperidin-4-yl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amines

7.7.1 Synthesis of (1-acetylpiperidin-4-yl)methyl 4-methylbenzenesulfonate[8] 124

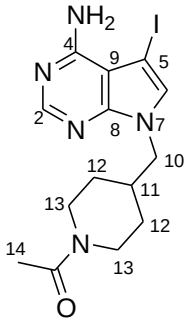


(Piperidin-4-yl)methanol **123** (2.00 g, 17.4 mmol), acetic anhydride (1.64 ml, 17.4 mmol, 1.0 eq), and triethylamine (6.05 ml, 43.4 mmol, 2.5 eq) in (20.0 ml) dichloromethane were stirred at room temperature for 4 hours. 4-Toluenesulfonyl chloride (4.97 g, 26.0 mmol, 1.5 eq) was added slowly to the same reaction medium and stirred for 5 hours at room temperature. The reaction mixture was diluted with ethyl acetate (100 ml), washed with dilute aqueous $NaHCO_3$ (50.0 ml), 1 M aqueous HCl and brine (50.0 ml). The organic layers were combined, dried over $MgSO_4$, filtered through celite and excess solvent was removed on a rotary evaporator. (1-Acetylpiperidin-4-yl)methyl 4-methylbenzenesulfonate **124** (2.60 g, 48%) was obtained as a light yellow viscous oil after purification using 80% EtOAc/hexane solvent.

R_f (80% EtOAc/hexane) 0.15. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 7.78 (2H, d, *J* = 8.3 Hz, H7), 7.36 (2H, d, *J* = 8.1 Hz, H8), 4.69 – 4.47 (1H, m, H4), 3.90 – 3.83 (3H, m, H1 and H4), 3.11 – 2.94 (1H, m, H4), 2.50 – 2.46 (4H, m, H4 and H10), 2.06 (3H, s, H5), 1.97 – 1.87 (1H, m, H2), 1.76 (2H, t, *J* = 13.2 Hz, H3), 1.27 – 1.03 (2H, m, H3); **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 168.8 (C=O), 145.0 (C-9), 132.8 (C-6), 129.9 (C-8), 127.9 (C-7), 73.7 (C-1), 45.9 (C-4), 29.3 (C-3), 21.7 (C-10), 21.4 (C-5), 20.9 (C-2); **IR (ν_{max}/cm-^{1})**: 2936 (C-H); 1621 (C=O); 1440 (CH_2); 1188 (S=O);

1151 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{15}H_{22}NO_4S$: 312.1271, found: $[M + H]^+$ 312.1261.

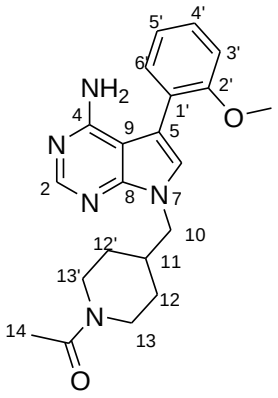
7.7.2 Synthesis of 1-{4-[(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone 104c



Compound **104c** was prepared from the reaction of 5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **103** (70.0 mg, 0.270 mmol), cesium carbonate (0.175 g, 0.538 mmol, 2 eq) and (1-acetylpiperidin-4-yl)methyl 4-methylbenzenesulfonate **124** (0.109 g, 0.349 mmol, 1.3 eq) using the procedure of **7.5.1**. Purification of the crude product by silica gel column chromatography (10% MeOH/chloroform) gave 1-{4-[(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone **104c** (0.085 g, 79%) as a yellow solid.

R_f (10% MeOH/chloroform) 0.28. *mp*: 210-212 °C. **^{1}H NMR (500 MHz, DMSO-d$_6$)**: δ 8.09 (1H, s, H2), 7.44 (1H, s, H6), 6.59 (2H, s, NH_2), 4.31 (1H, d, *J* = 13.0 Hz, H_e13), 4.00 (2H, d, *J* = 7.2 Hz, H10), 3.76 (1H, d, *J* = 13.1 Hz, H_e13), 2.97 – 2.87 (1H, m, H_a13), 2.44 (1H, td, *J* = 12.7, 2.9 Hz, H_a13), 2.04 (1H, td, *J* = 7.6, 3.8 Hz, H11), 1.95 (3H, s, H14), 1.45 – 1.42 (2H, m, H_e12), 1.16 – 0.95 (2H, m, H_a12). **^{13}C NMR (125 MHz, DMSO-d$_6$)**: δ 168.4 (C=O), 157.6 (C-4), 152.2 (C-2), 150.3 (C-8), 130.5 (C-9), 103.3 (C-6), 49.55 (C-10), 45.80 (C-5), 36.79 (C-13), 30.21 (C-11), 29.46 (C-14), 21.74 (C-12); **IR (ν_{max}/cm^{-1})**: 3427 (N-H); 3058 (=C-H); 1649 (C=O); 1440 (C=C); 1249 (C-N); 940.9 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{14}H_{19}IN_5O$: 400.0636, found: $[M + H]^+$ 400.0597.

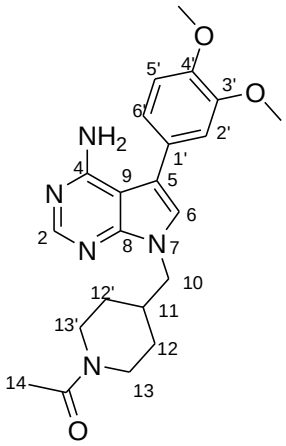
7.7.3 Synthesis of 1-{4-[(4-amino-5-(2-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone 125a



Reaction of 1-{4-[(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone **104c** (70.0 mg, 0.175 mmol), $Pd(PPh_3)_4$ (20.0 mg, 0.0175 mmol), 2-methoxyphenylboronic acid (533 mg, 0.351 mmol) and 2 M aqueous Na_2CO_3 (0.400 ml, 4 eq) as described in general procedure **7.5.2** afforded, after filtration and column chromatography using 10% MeOH/chloroform as eluent, 1-{4-[(4-amino-5-(2-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone **125a** (0.036 g, 54%) as a light yellow solid.

R_f (10% MeOH/chloroform) 0.28. *mp*: 215-217 °C. **^{1}H NMR (500 MHz, MeOD)**: δ 8.14 (1H, s, H2), 7.42 (1H, ddd, *J* = 8.3, 7.4, 1.8 Hz, H4'), 7.33 (1H, dd, *J* = 7.5, 1.8 Hz, H6'), 7.15 (1H, dd, *J* = 8.4, 1.0 Hz, H3'), 7.13 (1H, s, H6), 7.07 (1H, td, *J* = 7.6, 1.1 Hz, H5'), 4.55 – 4.51 (1H, m, H_e13), 4.15 (2H, d, *J* = 7.3 Hz, H10), 3.96 – 3.92 (1H, m, H_e13), 3.83 (3H, s, OMe), 3.08 (1H, ddd, *J* = 13.8, 12.4, 2.8 Hz, H_a13), 2.61 (1H, td, *J* = 12.9, 2.9 Hz, H_a13), 2.23 – 2.18 (1H, m, H11), 2.10 (3H, s, H14), 1.71 – 1.65 (2H, m, H_e12), 1.35 – 1.25 (2H, m, H_a12); **^{13}C NMR (125 MHz, MeOD)**: δ 170.1 (C=O), 157.7 (C-4), 156.9 (C-2'), 150.5 (C-2), 149.4 (C-8), 131.5 (C-4'), 129.1 (C-6'), 124.7 (C-5), 123.0 (C-1'), 120.8 (C-5'), 112.1 (C-3'), 111.2 (C-6), 102.4 (C-9), 54.7 (C-10), 49.3 (OMe), 45.9 (C-13), 41.2 (C-13'), 36.9 (C-11), 29.8 (C-12), 29.1 (C-12'), 19.8 (C-14); **IR (ν_{max}/cm^{-1})**: 3419 (N-H); 2933 (=C-H); 1633 (C=O); 1474 (C=C); 1252 (C-N); 1027 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{21}H_{26}N_5O_2$: 380.2088, found: $[M + H]^+$ 380.2044.

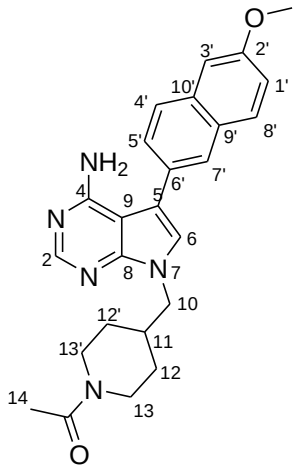
7.7.4 Synthesis of 1-{4-[(4-amino-5-(3,4-dimethoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone 125b



Reaction of 1-{4-[(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone **104c** (80.0 mg, 0.200 mmol), $Pd(PPh_3)_4$ (0.0232 g, 0.0200 mmol), 3,4-dimethoxyphenylboronic acid (73.0 mg, 0.400 mmol) and 2 M aqueous Na_2CO_3 (0.400 ml, 4 eq) as described in general procedure **7.5.2** afforded, after filtration and column chromatography using 10% MeOH/chloroform as eluent, **125b** (0.054 g, 66%) as a yellow solid.

R_f (10% MeOH/chloroform) 0.28. *mp*: 215-216 °C. **^{1}H NMR (500 MHz, MeOD)**: δ 8.04 (1H, s, H2), 7.07 (1H, s, H6), 6.97 – 6.91 (3H, m, H2', H5' and H6'), 4.43 – 4.39 (1H, m, H_e13), 4.02 (2H, d, *J* = 7.3 Hz, H10), 3.82 (1H, m, H_e13), 3.78 (3H, s, OMe), 3.775 (3H, s, OMe), 2.95 (1H, ddd, *J* = 13.7, 12.3, 2.8 Hz, H_a13), 2.48 (1H, td, *J* = 12.9, 2.9 Hz, H_a13), 2.10 (1H, ddt, *J* = 11.5, 7.7, 3.9 Hz, H11), 1.97 (3H, s, H14), 1.54 (2H, tdd, *J* = 11.8, 4.0, 2.2 Hz, H_e12), 1.21 – 1.11 (2H, m, H_a12); **^{13}C NMR (125 MHz, MeOD)**: δ 170.0 (C=O), 157.4 (C-4), 150.8 (C-2), 149.7 (C-8), 149.4 (C-4'), 148.7 (C-3'), 127.3 (C-1'), 123.6 (C-5), 121.0 (C-6'), 116.5 (C-5'), 112.5 (C-2'), 112.1 (C-6), 100.6 (C-9), 62.9 (C-10), 49.2 (OMe), 45.9 (OMe), 41.1 (C-13 and C-13'), 36.9 (C-11), 29.8 (C-12), 29.1 (C-12'), 19.8 (C-14); **IR (ν_{max}/cm^{-1})**: 3468 (N-H); 2923 (=C-H); 1612 (C=O); 1440 (C=C); 1231 (C-N); 1023 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{22}H_{28}N_5O_3$: 410.2194, found: $[M + H]^+$ 410.2153.

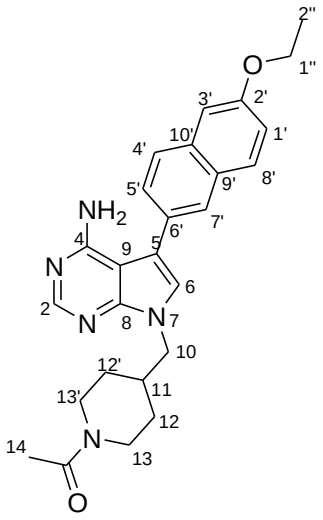
7.7.5 Synthesis of 1-{4-[(4-amino-5-(2-methoxynaphthalen-6-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone 125c



To a mixture of 1-{4-[(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone **104c** (80.0 mg, 0.200 mmol), $Pd(PPh_3)_4$ (0.0232 g, 0.0200 mmol), 6-methoxynaphthalen-2-yl-2-boronic acid (0.0810 g, 0.401 mmol) was added a degassed solution of DME (30 ml) and 2 M aqueous Na_2CO_3 (0.400 ml, 4 eq). The product, 1-{4-[(4-amino-5-(2-methoxynaphthalen-6-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone **125c** (0.064 g, 74%), was isolated as a yellow solid after purification by column chromatography (10% MeOH/chloroform).

R_f (10% MeOH/chloroform) 0.28. *mp*: 214-216 °C. **^{1}H NMR (500 MHz, MeOD)**: δ 8.20 (1H, s, H2), 7.92 − 7.89 (2H, m, H4' and H7'), 7.83 (1H, d, *J* = 9.0 Hz, H8'), 7.60 (1H, dd, *J* = 8.4, 1.8 Hz, H5'), 7.32 (1H, d, *J* = 2.6 Hz, H3'), 7.31 (1H, s, H6), 7.21 (1H, dd, *J* = 9.0, 2.5 Hz, H1'), 4.54 (1H, dt, *J* = 13.0, 2.1 Hz, H_e13), 4.19 (2H, d, *J* = 7.3 Hz, H8''), 3.96 (3H, s, OMe), 3.94 – 3.93 (1H, m, H_e11), 3.09 (1H, td, *J* = 13.5, 2.7 Hz, H_a11), 2.62 (1H, td, *J* = 12.9, 2.9 Hz, H_a11), 2.28 − 2.22 (1H, m, H_e11), 2.10 (3H, s, H14), 1.72 − 1.66 (2H, m, H_e12''), 1.35 – 1.23 (2H, m, H_a12''); **^{13}C NMR (125 MHz, MeOD)**: δ 170.1 (C=O), 158.1 (C-4), 157.1 (C-2'), 150.3 (C-2), 149.8 (C-8), 133.8 (C-10'), 129.3 (C-6' and C-9'), 129.2 (C-8'), 128.9 (C-5), 127.4 (C-4'), 126.8 (C-7'), 124.2 (C-5'), 119.1 (C-1'), 105.4 (C-6), 100.6 (C-3' and C-9), 54.5 (C-10), 49.3 (OMe), 45.9 (C-13), 41.2 (C-13'), 36.9 (C-11), 29.8 (C-12), 29.1 (C-12'), 19.8 (C-14); **IR (ν_{max}/cm^{-1})**: 3300 (N-H); 2917 (=C-H); 1606 (C=O); 1440 (C=C); 1259 (C-N); 1026 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{25}H_{28}N_5O_2$: 430.2245, found: $[M + H]^+$ 430.2214.

7.7.6 Synthesis of 1-{4-[(4-amino-5-(2-ethoxynaphthalen-6-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone 125d

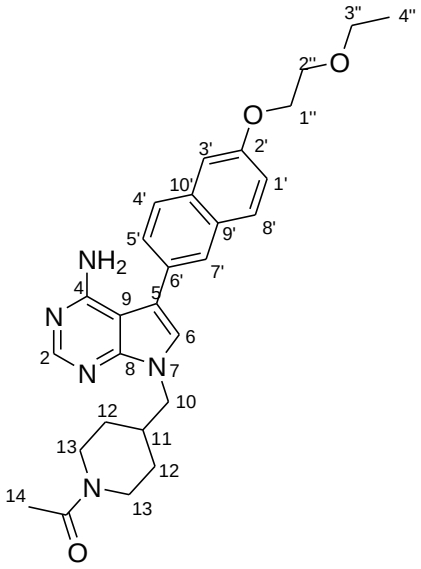


To a mixture of 1-{4-[(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone **104c** (100 mg, 0.251 mmol), $Pd(PPh_3)_4$ (289 mg, 0.0251 mmol), 6-ethoxynaphthalen-2-yl-2-boronic acid (0.108 g, 0.501 mmol) was added a degassed solution of DME (30 ml) and 2 M aqueous Na_2CO_3 (0.500 ml, 4 eq). The product, 1-{4-[(4-amino-5-(2-ethoxynaphthalen-6-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone **125d** (0.084 g, 76%), was isolated as a yellow solid after purification by column chromatography (10% MeOH/chloroform).

R_f (10% MeOH/chloroform) 0.28. *mp*: 215-217 °C. **^{1}H NMR (500 MHz, MeOD)**: δ 8.20 (1H, s, H2), 7.89 – 7.80 (2H, m, H4' and H7'), 7.81 (1H, d, *J* = 8.9 Hz, H8'), 7.59 (1H, dd, *J* = 8.5, 1.7 Hz, H5'), 7.29 − 7.285 (2H, m, H3' and H6), 7.19 (1H, dd, *J* = 8.9, 2.5 Hz, H1'), 4.54 (1H, ddt, *J* = 13.3, 4.5, 2.4 Hz, H_e13), 4.22 – 4.16 (4H, m, H10 and H1''), 3.95 − 3.91 (1H, m, H_e13), 3.08 (1H, ddd, *J* = 13.7, 12.3, 2.7 Hz, H_a13), 2.61 (1H, td, *J* = 12.9, 2.9 Hz, H_a13), 2.26 – 2.21 (1H, m, H11), 2.10 (3H, s, H14), 1.71 – 1.65 (2H, m, H_e12), 1.49 (3H, t, *J* = 7.0 Hz, H2''), 1.36 – 1.29 (2H, m, H_a12''); **^{13}C NMR (125 MHz, MeOD)**: δ 170.0 (C=O), 157.3 (C-4), 157.2 (C-2'), 150.3 (C-2), 149.9 (C-8), 133.9 (C-10'), 129.2 (C-6' and C-9'), 129.1 (C-8'), 128.9 (C-5), 127.3 (C-4'), 126.8 (C-7'), 124.2 (C-5'), 119.4 (C-1'), 106.2 (C-6), 100.6 (C-3' and C-9), 63.2 (C-1''), 49.3 (C-10), 45.9 (C-13), 41.1 (C-13'), 36.9 (C-11), 29.8 (C-12), 29.1 (C-12'), 19.8 (C-14), 13.7 (C-2'');

IR (ν_{max}/cm^{-}1): 3468 (N-H); 2923 (=C-H); 1612 (C=O); 1440 (C=C); 1231 (C-N); 1023 (C-O). ***HRMS (ES^{+})*** *m/z*: calculated for $C_{26}H_{30}N_5O_2$: 444.2401, found: $[M + H]^+$ 444.2375.

7.7.7 Synthesis of 1-{4-{[5-(2-(2-ethoxyethoxy)naphthalen-6-yl)-4-amino-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl]methyl}piperidin-1-yl}ethanone 125e



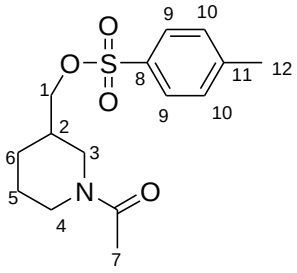
Reaction of 1-{4-[(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone **104c** (70.0 mg, 0.175 mmol), $Pd(PPh_3)_4$ (0.0203 g, 0.0175 mmol), 6-(2-ethoxyethoxy)naphthalen-2-yl-2-boronic acid (0.0912 g, 0.351 mmol) and 2 M aqueous Na_2CO_3 (0.400 ml, 4 eq) as described in general procedure **7.5.2** afforded, after filtration and column chromatography using 10% MeOH/chloroform as eluent, 1-{4-{[5-(2-(2-ethoxyethoxy)naphthalen-6-yl)-4-amino-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl]methyl}piperidin-1-yl}ethanone **125e** (0.046 g, 54%) as a light brown viscous oil.

R_f (10% MeOH/chloroform) 0.28. *mp*: 215-216 °C. **^{1}H NMR (500 MHz, MeOD)** δ 8.20 (1H, s, H2), 7.92 − 7.90 (2H, m, H4' and H7'), 7.85 (1H, d, *J* = 9.0 Hz, H8'), 7.61 (1H, dd, *J* = 8.5, 1.8 Hz, H5'), 7.34 (1H, d, *J* = 2.6 Hz, H3'), 7.32 (1H, s, H6), 7.25 (1H, dd, *J* = 8.9, 2.5 Hz, H1'), 4.29 – 4.20 (2H, m, H1"), 4.20 (2H, d, *J* = 7.3 Hz, H10), 3.95 (1H, d, *J* = 13.6 Hz, H_e13), 3.90 – 3.89 (2H, m, H2"), 3.69 – 3.65 (3H, m, H_a13 and H3"), 3.10 (1H, td, *J* = 13.5, 2.8 Hz, H_e13), 2.62 (1H, td, *J* = 13.0, 3.0 Hz, H_a13), 2.25 (1H, ddt, *J* = 15.3, 7.7, 3.8 Hz, H11), 2.11 (3H, s, H14), 1.72 – 1.67 (2H, m, H_e12), 1.36 – 1.30 (2H, m, H_a12), 1.27 (3H, t, *J* = 7.0 Hz, H4"); **^{13}C NMR**

(125 MHz, MeOD): δ 168.3 (C=O), 157.7 (C-4), 156.9 (C-2'), 152.0 (C-2), 151.0 (C-8), 133.5 (C-10'), 130.3 (C-6'), 129.8 (C-9'), 129.2 (C-8'), 128.0 (C-5), 127.9 (C-4'), 126.9 (C-7'), 124.6 (C-5'), 119.7 (C-1'), 107.2 (C-6), 100.4 (C-3' and C-9), 68.8 (C-1''), 67.8 (C-2''), 66.2 (C-3''), 49.4 (C-10), 45.9 (C-13), 30.4 (C-11), 29.6 (C-12), 21.8 (C-14), 15.6 (C-4''); **IR (ν_{max}/cm^{-1})**: 3301 (N-H); 3108 (=C-H); 1644 (C=O); 1473 (C=C); 1272 (C-N); 1029 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{28}H_{34}N_5O_3$: 488.2663, found: $[M + H]^+$ 488.2622.

7.8 Synthesis of 5-substituted 7-piperidin-3-yl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amines

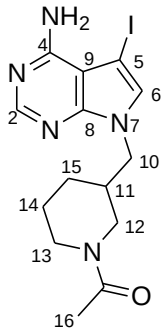
7.8.1 Synthesis of (1-acetylpiperidin-3-yl)methyl 4-methylbenzenesulfonate 127



Following the procedure of **7.7.1**, (1-acetylpiperidin-3-yl)methyl 4-methylbenzenesulfonate **127** (2.8 g, 52%) was isolated as a light yellow viscous oil after purification by silica gel column chromatography using 80% EtOAc/hexane as eluting solvent.

R_f (80% EtOAc/hexane) 0.15. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 7.78 (2H, d, *J* = 8.3 Hz, H9), 7.36 (2H, d, *J* = 8.1 Hz, H10), 4.69 – 4.47 (1H, m, H3), 3.90 – 3.83 (3H, m, H1 and H3), 3.11 – 2.94 (1H, m, H4), 2.50 – 2.46 (4H, m, H4 and H12), 2.06 (3H, s, H7), 1.97 – 1.87 (2H, m, H5), 1.76 – 1.65 (1H, m, H2), 1.27 – 1.03 (2H, m, H6); **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 168.8 (C=O), 145.0 (C-11), 132.8 (C-8), 129.9 (C-10), 127.9 (C-9), 73.7 (C-1), 45.9 (C-3), 44.8 (C-4), 29.3 (C-2), 29.3 (C-6), 22.9 (C-5), 24.3 (C-12), 21.7 (C-7); **IR (ν_{max}/cm^{-1})**: 2936 (C-H); 1621 (C=O); 1440 (CH_2); 1188 (S=O); 1151 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{15}H_{22}NO_4S$: 312.1271, found: $[M + H]^+$ 312.1261.

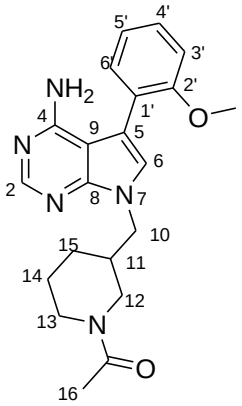
7.8.2 Synthesis of 1-{3-[(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone 104d



Compound **104d** was prepared from the reaction of 5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **103** (80.0 mg, 0.308 mmol), cesium carbonate (200 mg, 0.615 mmol, 2 eq) and (1-acetylpiperidin-4-yl)methyl 4-methylbenzenesulfonate **127** (0.124 g, 0.399 mmol, 1.3 eq) using the procedure of **7.5.1**. Purification of the crude product by silica gel column chromatography (10% MeOH/chloroform) gave 1-{3-[(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone **104d** as a yellow solid (0.077 g, 63%).

R_f (10% MeOH/chloroform) 0.28. *mp*: 210-212 °C. **^{1}H NMR (300 MHz, MeOD)**: δ 7.37 (1H, s, H2), 6.52 (1H, s, H6), 3.37 (2H, d, *J* = 7.3 Hz, H10), 2.93 (1H, d, *J* = 11.3 Hz, piperidine-H), 1.97 (2H, dd, *J* = 24.6, 14.8 Hz, piperidine-H), 1.28 (3H, s, H16), 1.01 (3H, d, *J* = 10.2 Hz, piperidine-H), 0.74 – 0.51 (3H, m, piperidine-H); **^{13}C NMR (125 MHz, MeOD)**: δ 170.3 (C=O), 157.2 (C-4), 150.6 (C-2), 149.9 (C-8), 130.5 (C-9), 106.2 (C-6), 57.8 (C-10), 49.2 (C-5), 46.0 (C-12), 44.7 (C-13), 29.9 (C-11), 29.4 (C-15), 22.9 (C-14), 21. 2 (C-16); **IR (ν_{max}/cm^{-1})**: 3427 (N-H); 3058 (=C-H); 1649 (C=O); 1440 (C=C); 1249 (C-N); 940.9 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{14}H_{19}IN_5O$: 400.0636, found: $[M + H]^+$ 400.0597.

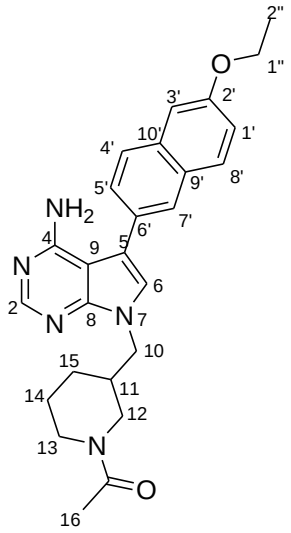
7.8.3 Synthesis of 1-{3-[(4-amino-5-(2-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone 128a



Reaction of 1-{3-[(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone **104d** (70.0 mg, 0.175 mmol), $Pd(PPh_3)_4$ (20.0 mg, 0.0175 mmol), 2-methoxyphenylboronic acid (533 mg, 0.351 mmol) and 2 M aqueous Na_2CO_3 (0.400 ml, 4 eq) as described in general procedure **7.5.2** afforded, after filtration and column chromatography using 10% MeOH/chloroform as eluent, 1-{3-[(4-amino-5-(2-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone **128a** (0.033 g, 49%) as a viscous oil.

R_f (10% MeOH/chloroform) 0.28. **^{1}H NMR (500 MHz, MeOD)**: δ 8.14 (1H, s, H2), 7.42 (1H, ddd, *J* = 8.3, 7.4, 1.8 Hz, H4'), 7.16 (1H, dd, *J* = 8.4, 1.1 Hz, H6'), 7.13 (1H, s, H6), 7.07 – 6.83 (1H, m, H3' and H5'), 3.83 (3H, s, OMe), 3.49 – 3.32 (2H, m, H10), 2.95 (1H, d, *J* = 11.3 Hz, piperidine-H), 1.97 (2H, dd, *J* = 24.6, 14.8 Hz, piperidine-H), 1.27 (3H, s, H16), 1.02 (3H, d, *J* = 10.2 Hz, piperidine-H), 0.74 – 0.51 (3H, m, piperidine-H); **^{13}C NMR (125 MHz, MeOD)**: δ 170.1 (C=O), 157.7 (C-4), 156.9 (C-2'), 150.5 (C-2), 149.4 (C-8), 131.5 (C-4'), 129.1 (C-6'), 124.7 (C-5), 123.0 (C-1'), 120.8 (C-5'), 112.1 (C-3'), 111.2 (C-6), 102.4 (C-9), 54.7 (C-10), 49.3 (OMe), 45.9 (C-12), 44.8 (C-13), 41.2 (C-14), 36.9 (C-11), 29.8 (C-15), 19.8 (C-16); **IR (ν_{max}/cm^{-1})**: 3419 (N-H); 2933 (=C-H); 1633 (C=O); 1474 (C=C); 1252 (C-N); 1027 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{21}H_{26}N_5O_2$: 380.2088, found: $[M + H]^+$ 380.2044.

7.8.4 1-{3-[(4-amino-5-(2-ethoxynaphthalen-6-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone 128b



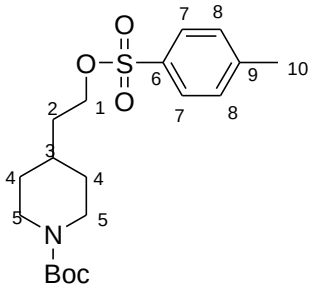
Reaction of 1-{3-[(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone **104d** (90.0 mg, 0.226 mmol), $Pd(PPh_3)_4$ (30.0 mg, 0.0226 mmol), 6-(2-ethoxyethoxy)naphthalen-2-yl-2-boronic acid (90.0 mg, 0.451 mmol) and 2 M aqueous Na_2CO_3 (0.500 ml, 4 eq) as described in general procedure **7.5.2** afforded, after filtration and column chromatography using 10% MeOH/chloroform as eluent, 1-{3-[(4-amino-5-(2-ethoxynaphthalen-6-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl]piperidin-1-yl}ethanone **128b** (0.044 g, 44%) as a light yellow solid.

R_f (10% MeOH/chloroform) 0.28. *mp*: 215-217 °C. **^{1}H NMR (300 MHz, MeOD)**: δ 7.40 (1H, s, H2), 7.10 – 7.00 (2H, m, H4' and H7'), 6.99 (1H, d, *J* = 9.0 Hz, H8'), 6.77 (1H, d, *J* = 8.3 Hz, H5'), 6.52 – 6.43 (2H, m, H3' and H6), 6.36 (1H, d, *J* = 8.9 Hz, H1'), 3.50 – 3.31 (4H, m, H10 and H1''), 2.93 (1H, d, *J* = 11.3 Hz, piperidine-H), 1.97 (2H, dd, *J* = 24.6, 14.8 Hz, piperidine-H), 1.28 (3H, s, H16), 1.01 (3H, d, *J* = 10.2 Hz, piperidine-H), 0.74 – 0.51 (6H, m, piperidine-H and H2''); **^{13}C NMR (125 MHz, MeOD)**: δ 170.3 (C=O), 157.2 (C-4), 150.9 (C-2'), 150.6 (C-2), 149.9 (C-8), 133.8 (C-10'), 129.1 (C-6' and C-9'), 129.0 (C-8'), 127.4 (C-5), 127.3 (C-4'), 126.8 (C-7'), 124.0 (C-5'), 119.4 (C-1'), 106.2 (C-6), 100.6 (C-3' and C-9), 63.2 (C-1''), 59.2 (C-10), 49.7 (C-12), 44.8 (C-13), 29.4 (C-11), 29.1 (C-15), 22.9 (C-14), 19.8 (C-16), 13.7 (C-2''); **IR (ν_{max}/cm^{-1})**: 3304 (N-H); 2928 (=C-H); 1607 (C=O); 1438 (C=C); 1261 (C-N); 1041 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{26}H_{30}N_5O_2$: 444.2401, found: $[M + H]^+$ 444.2375.

7.9 Synthesis of 5-substituted 7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine *N*-boc derivatives

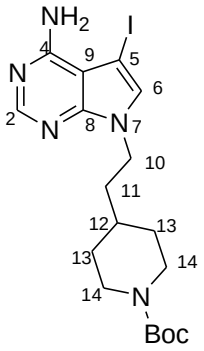
7.9.1 Synthesis of 2-[1-(*tert*-butoxycarbonyl)piperidin-4-yl]ethyl 4-methylbenzenesulfonate

129



To a reaction of *tert*-butyl 4-(2-hydroxyethyl)piperidine-1-carboxylate (2.00 g, 8.72 mmol) and triethylamine (3.04 ml, 21.8 mmol, 2.5 eq) in dry dichloromethane (20.0 ml) was added 4-toluenesulfonyl chloride (2.49 g, 13.1 mmol, 1.5 eq) portion-wise. The resulting brownish solution was stirred at room temperature for 5 hours. After this time, TLC analysis showed the formation of a new product, and then the reaction mixture was diluted with ethyl acetate (100 ml), washed with dilute aqueous $NaHCO_3$ (50.0 ml) and brine (50.0 ml). The organic layers were combined, dried over $MgSO_4$, filtered through celite and excess solvent was removed on a rotary evaporator. 2-[1-(*tert*-Butoxycarbonyl)piperidin-4-yl]ethyl 4-methylbenzenesulfonate **129** (1.85 g, 55%) had the same retention factor (R_f) as the starting material, hence it was used in the next step without purification.

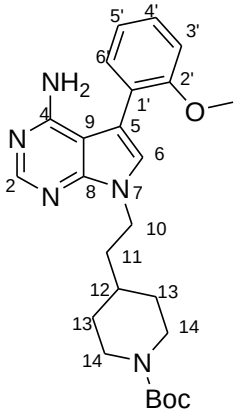
7.9.2 Synthesis of *tert*-butyl 4-[2-(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)ethyl]piperidine-1-carboxylate 104e



A mixture of 5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **103** (100 mg, 0.385 mmol), cesium carbonate (0.250 g, 0.769 mmol, 2 eq) and 2-[1-(*tert*-butoxycarbonyl)piperidin-4-yl]ethyl 4-methylbenzenesulfonate **129** (0.193 g, 0.499 mmol, 1.3 eq) in dry DMF (7.00 ml) was heated at 70 °C for 18 hours. The reaction was monitored by TLC (80% EtOAc/hexane). When complete, the reaction mixture was cooled to room temperature, filtered and poured into a separating funnel containing 100 ml water and 100 ml ethyl acetate. The aqueous layer was extracted with EtOAc (2 × 100 ml). The combined organic extracts were dried with $MgSO_4$, filtered through celite and excess solvent was removed *in vacuo*. Purification by column chromatography using 80% EtOAc/hexane gave *tert*-butyl 4-[2-(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)ethyl]piperidine-1-carboxylate **104e** (0.120 g, 66%) as a light brown viscous oil.

R_f (80% EtOAc/hexane) 0.48. **^{1}H NMR (500 MHz, DMSO-d$_6$)**: δ 8.10 (1H, s, H2), 7.50 (1H, s, H6), 6.59 (2H, s, NH_2), 4.16 – 4.13 (2H, m, H10), 3.88 (2H, d, J = 13.0 Hz, H_e14), 2.65 – 2.60 (2H, m, H_a14), 1.70 – 1.66 (4H, m, H11 and H_e13), 1.39 (9H, s, C(C***H***$_3$)$_3$), 1.30 (1H, tt, J = 7.3, 3.8 Hz, H12), 1.04 – 0.96 (2H, m, H_a13); **^{13}C NMR (125 MHz, DMSO-d$_6$)**: δ 157.6 (Boc-C=O), 154.3 (C-4), 152.2 (C-2), 150.1 (C-8), 129.8 (C-9), 103.3 (C-6), 78.9 (–***C***CH_3), 50.0 (C-10), 49.9 (C-5), 41.9 (C-14), 36.7 (C-11), 33.1 (C-13), 28.6 (3×CH_3), 14.8 (C-12); **IR (ν_{max}/cm^{-1})**: 3485, 3277 (N-H); 2930 (=C-H); 1679 (C=O); 1453 (C=C). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{18}H_{27}IN_5O_2$: 472.1211, found: $[M + H]^+$ 472.1210.

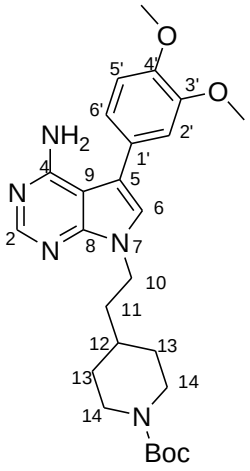
7.9.3 Synthesis of *tert*-butyl 4-{2-[4-amino-5-(2-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl]ethyl}piperidine-1-carboxylate 130a



To a mixture of *tert*-butyl 4-[2-(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)ethyl]piperidine-1-carboxylate **104e** (80.0 mg, 0.169 mmol), $Pd(PPh_3)_4$ (0.0196 g, 0.0169 mmol), and 2-methoxyphenylboronic acid (516 mg, 0.339 mmol) was added a solution of DME (30 ml) and 2 M aqueous Na_2CO_3 (0.400 ml). The product, *tert*-butyl 4-{2-[4-amino-5-(2-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl]ethyl}piperidine-1-carboxylate **130a** (0.035 g, 46%), was isolated as a viscous oil after purification by column chromatography (80% EtOAc/hexane).

R_f (80% EtOAc/hexane) 0.48; **^{1}H NMR (300 MHz, DMSO-d$_6$)**: δ 8.10 (1H, s, H2), 7.37 (1H, ddd, *J* = 8.3, 7.3, 1.8 Hz, H4'), 7.26 (1H, dd, *J* = 7.5, 1.8 Hz, H6'), 7.22 (1H, s, H6), 7.14 (1H, dd, *J* = 8.4, 1.1 Hz, H3'), 7.04 (1H, td, *J* = 7.4, 1.1 Hz, H5'), 5.84 (2H, s, NH_2), 4.19 (2H, t, *J* = 7.2 Hz, H10), 3.90 (2H, d, *J* = 13.1 Hz, H_e14), 3.76 (3H, s, OMe), 2.64 (2H, s, H_a14), 1.77 – 1.69 (4H, m, H11 and H_e13), 1.39 (10H, s, H12 and C(C***H***$_3$)$_3$), 1.10 – 0.95 (2H, m, H_a13): **^{13}C NMR (75 MHz, DMSO-d$_6$)**: δ 157.9 (Boc-C=O), 156.9 (C-4), 154.3 (C-2'), 151.8 (C-2), 150.1 (C-8), 132.0 (Ar-C), 129.3 (Ar-C), 124.2 (Ar-C), 123.9 (Ar-C), 121.3 (Ar-C), 112.1 (Ar-C), 111.2 (C-6), 102.3 (C-9), 78.9 (–**C**CH_3), 55.8 (C-10 and OMe), 41.7 (C-14), 36.7 (C-13), 33.3 (C-11), 28.6 (3×CH_3), 14.9 (C-12); **IR (ν_{max}/cm^{-1})**: 3485 (N-H); 2930 (=C-H); 1679 (C=O); 1453 (C=C); 1161 (C-N); 759.9 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{25}H_{34}N_5O_3$: 452.2663, found: $[M + H]^+$ 452.2648.

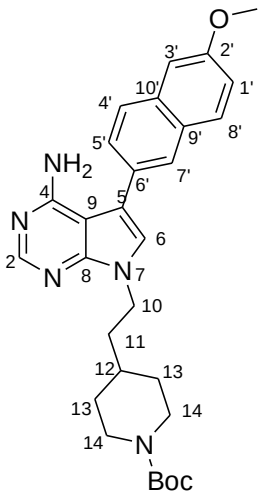
7.9.4 Synthesis of *tert*-butyl 4-{2-[4-amino-5-(3,4-dimethoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl]ethyl}piperidine-1-carboxylate 130b



Compound **130b** was synthesized from a mixture of *tert*-butyl 4-[2-(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)ethyl]piperidine-1-carboxylate **104e** (90.0 mg, 0.191 mmol), $Pd(PPh_3)_4$ (20.0 mg, 0.0191 mmol), and 3,4-dimethoxyphenylboronic acid (695 mg, 0.382 mmol) using Suzuki - Miyaura coupling procedure described in **7.5.2**. To this mixture was added a degassed solution of DME (30.0 ml) and 2 M aqueous Na_2CO_3 (0.400 ml). The product **130b** (0.0440 g, 48%) was isolated as brown oil after purification by column chromatography (80% EtOAc/hexane).

R_f (80% EtOAc/hexane) 0.48. **^{1}H NMR (500 MHz, MeOD)**: δ 8.04 (1H, s, H2), 7.10 (1H, s, H6), 6.97 – 6.93 (3H, m, Ar-H), 4.17 (2H, t, *J* = 7.3 Hz, H10), 3.93 (2H, dt, *J* = 14.6, 3.6 Hz, H_e14), 3.78 (6H, s, 2 × OMe), 2.61 – 2.59 (2H, m, H_a14), 1.69 – 1.62 (4H, m, H11 and H_e13), 1.34 (10H, s, H12 and C(C***H***$_3$)$_3$), 1.05 – 1.02 (2H, m, H_a13); **^{13}C NMR (125 MHz, MeOD)**: δ 158.0 (C=O), 155.1 (C-4), 150.4 (C-2), 149.4 (C-8), 148.6 (C-3' and C-4'), 127.3 (C-1'), 123.0 (C-5), 120.9 (C-6'), 116.7 (C-2' and C-5'), 112.5 (C-6), 112.1 (C-9), 79.6 (–***C***CH_3), 55.1 (2 × OMe and C-10), 41.8 (C-14), 36.5 (C-11), 33.3 (C-13), 27.3 (3×CH_3), 14.8 (C-12); **IR (ν_{max}/cm^{-1})**: 3485 (N-H); 2862 (=C-H); 1678 (C=O); 1453 (C=C); 1244 (C-N); 865.3 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{26}H_{36}N_5O_4$: 482.2769, found: $[M + H]^+$ 482.2743.

7.9.5 Synthesis of *tert*-butyl 4-{2-[4-amino-5-(2-methoxynaphthalen-6-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl]ethyl}piperidine-1-carboxylate 130c



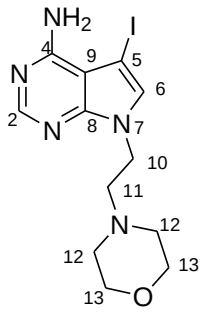
Reaction of *tert*-butyl 4-[2-(4-amino-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)ethyl]piperidine-1-carboxylate **104e** (100 mg, 0.212 mmol), $Pd(PPh_3)_4$ (0.0245 g, 0.0212 mmol), 6-methoxynaphthalen-2-yl-2-boronic acid (857 mg, 0.424 mmol) and 2 M aqueous Na_2CO_3 (0.400 ml, 4 eq) as described in general procedure **7.5.2** afforded, after filtration and column chromatography using 80% EtOAc/hexane as eluent, *tert*-butyl 4-{2-[4-amino-5-(2-methoxynaphthalen-6-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl]ethyl}piperidine-1-carboxylate **130c** (0.0490 g, 46%) as a light brown viscous oil.

R_f (80% EtOAc/hexane) 0.48. **^{1}H NMR (500 MHz, MeOD)**: δ 8.21 (1H, s, H2), 7.90 (1H, d, *J* = 2.3 Hz, H7'), 7.82 (1H, dd, *J* = 9.1, 2.3 Hz, H5'), 7.81 – 7.63 (2H, m, H4' and H8'), 7.37 (1H, d, *J* = 2.5 Hz, H3'), 7.32 (1H, s, H6), 7.22 – 7.19 (1H, m, H1'), 4.35 (2H, t, *J* = 7.3 Hz, H10), 4.06 (2H, d, *J* = 13.5 Hz, H_e14), 3.95 (3H, s, OMe), 2.73 (2H, s, H_a14), 1.84 (4H, m, H11 and H_e13), 1.46 (10H, s, H12 and C(C***H***$_3$)$_3$), 0.91 – 0.89 (2H, m, H_a13); **^{13}C NMR (125 MHz, MeOD)**: δ 158.0 (Boc-C=O), 155.2 (C-4), 154.3 (C-2'), 152.2 (C-2), 150.1 (C-8), 133.9 (C-10'), 132.4 (C-6'), 130.8 (C-9'), 129.0 (C-8'), 127.4 (C-5), 127.1 (C-4'), 126.8 (C-7'), 123.7 (C-5'), 119.2 (C-1'), 107.2 (C-6), 105.4 (C-9 and C-3'), 79.6 (–***C***CH_3), 54.5 (C-10 and OMe), 36.5 (C-14), 33.3 (C-11), 29.3 (C-13), 27.3 (3×CH_3), 14.8 (C-12); **IR (ν_{max}/cm^{-1})**: 3459 (N-H); 2871 (=C-H); 1623 (C=O); 1453 (C=C); 1209 (C-N); 852.6 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{29}H_{36}N_5O_3$: 502.2820, found: $[M + H]^+$ 502.2818.

7.10 Synthesis of 5-substituted pyrrolo[2,3-*d*]pyrimidin-4-amine morpholinoethyl derivatives

With our iodinated pyrrolopyrimidine **103** in hand; *N*-alkylation with commercially available morpholinoethyl hydrochloride followed by Suzuki-Miyaura coupling reactions with different boronic acids will be discussed.

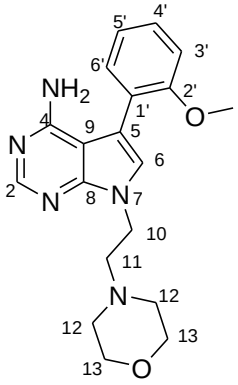
7.10.1 Synthesis of 5-iodo-7-(2-morpholinoethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine 104f



Compound **104f** was prepared from the reaction of 5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **103** (0.280 g, 1.08 mmol), cesium carbonate (0.701 g, 2.15 mmol, 2 eq) and 4-(2-chloroethyl)morpholine hydrochloride (0.240 g, 1.29 mmol, 1.2 eq) using the procedure of **7.5.1**. Purification of the crude product by silica gel column chromatography (10% MeOH/chloroform) gave 5-iodo-7-(2-morpholinoethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **104f** (0.253 g, 63%) as a yellow solid.

R_f (10% MeOH/chloroform) 0.26; *mp*: 215-218 °C. **^{1}H NMR (500 MHz, MeOD)**: δ 7.99 (1H, s, H2), 7.27 (1H, s, H6), 4.19 (2H, t, *J* = 6.5 Hz, H10), 3.56 – 3.50 (4H, m, H13), 2.63 (2H, t, *J* = 6.5 Hz, H11), 2.44 – 2.37 (4H, m, H12); **^{13}C NMR (125 MHz, MeOD)**: δ 157.3 (C-2 and C-4), 151.1 (C-8), 149.5 (C-9), 103.5 (C-6), 66.5 (C-13), 60.2 (C-5), 57.7 (C-11), 53.3 (C-12), 41.4 (C-10); **IR (ν_{max}/cm^{-1})**: 3330, 3175 (N-H); 3066 (=C-H); 1560 (C=C); 1242 (C-N); 1061 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{12}H_{17}IN_5O$: 374.0480, found: $[M + H]^+$ 374.0479.

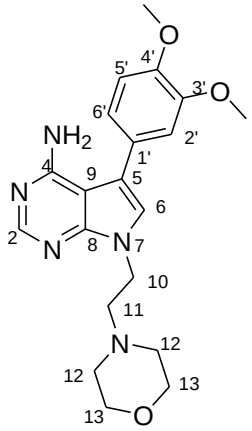
7.10.2 Synthesis of 5-(2-ethoxyphenyl)-7-(2-morpholinoethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine 131a



Reaction of compound **104f** (210 mg, 0.563 mmol), $Pd(PPh_3)_4$ (65.0 mg, 0.0563 mmol) and 2-methoxyphenylboronic acid (0.171 g, 1.13 mmol) in DME (30.0 ml) as described in general procedure **7.5.2**, afforded 5-(2-methoxyphenyl)-7-(2-morpholinoethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **131a** (73.2 mg, 52%) as a light yellow solid after purification by silica gel column chromatography (10% MeOH/chloroform).

R_f (10% MeOH/chloroform) 0.26; *mp*: 221-223 °C. **^{1}H NMR (300 MHz, DMSO-d$_6$)**: δ 8.10 (1H, s, H2), 7.38 – 7.35 (1H, m, H5'), 7.27 − 7.24 (2H, m, H3' and H6), 7.14 (1H, dd, *J* = 8.4, 1.1 Hz, H6'), 7.04 (1H, td, *J* = 7.4, 1.1 Hz, H4'), 5.85 (2H, s, NH_2), 4.27 (2H, t, *J* = 6.6 Hz, H10), 3.76 (3H, s, OMe), 3.55 (4H, t, *J* = 4.7 Hz, H13), 2.74 – 2.68 (2H, m, H11), 2.47 – 2.44 (4H, m, H12); **^{13}C NMR (125 MHz, DMSO-d$_6$)**: δ 157.4 (C-4), 156.4 (C-2'), 151.2 (C-2), 149.7 (C-8), 131.4 (C-4'), 128.8 (C-6'), 124.3 (C-5), 123.3 (C-1'), 120.8 (C-5'), 111.6 (C-3'), 110.6 (C-6), 101.8 (C-9), 66.1 (C-13), 57.6 (OMe), 55.3 (C-11), 53.1 (C-12), 40.7 (C-10); **IR (ν_{max}/cm^{-1})**: 3327, (N-H); 3056 (=C-H); 1694 (C=N); 1460 (C=C); 1436 (CH_2); 1115 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{19}H_{24}N_5O_2$: 354.1932, found: $[M + H]^+$ 354.1930.

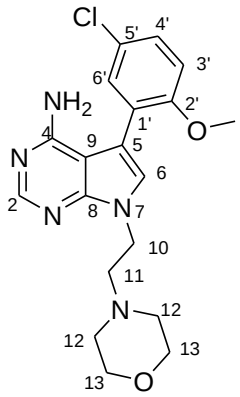
7.10.3 Synthesis of 5-(3,4-dimethoxyphenyl)-7-(2-morpholinoethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine 131b



Reaction of compound **104f** (100 mg, 0.268 mmol), $Pd(PPh_3)_4$ (30.9 mg, 0.0268 mmol) and 3,4-dimethoxyphenylboronic acid (97.5 mg, 0.536 mmol) in DME (30.0 ml) as described in general procedure **7.5.2**, afforded compound **131b** (68.0 mg, 66%) as a yellow solid.

R_f (10% MeOH/chloroform) 0.26. *mp*: 220-221 °C. **^{1}H NMR (300 MHz, DMSO-d$_6$)**: δ 8.13 (1H, s, H2), 7.33 (1H, s, H6), 7.08 – 6.95 (3H, m, H2', H5' and H6'), 6.09 (2H, s, NH_2), 4.28 (2H, t, *J* = 6.6 Hz, H10), 3.81 (3H, s, OMe), 3.80 (3H, s, OMe), 3.54 (4H, t, *J* = 4.7 Hz, H13), 2.70 (2H, t, *J* = 6.6 Hz, H11), 2.47 – 2.44 (4H, m, H12); **^{13}C NMR (75 MHz, DMSO-d$_6$)**: δ 157.7 (C-4), 155.7 (C-2), 150.7 (C-8), 149.5 (C-3'), 130.8 (C-4'), 128.4 (C-1'), 125.4 (C-5), 125.0 (C-6'), 124.9 (C-5'), 112.6 (C-2'), 110.9 (C-6), 102.3 (C-9), 66.5 (C-13), 57.6 (2 × OMe), 55.0 (C-11), 53.3 (C-12), 41.3 (C-10); **IR (ν_{max}/cm^{-1})**: 3230, 3082 (N-H); 3020 (=C-H); 1566 (C=C); 1234 (C-N); 1068 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{20}H_{26}N_5O_3$: 384.2037, found: $[M + H]^+$ 384.2003.

7.10.4 Synthesis of 5-(5-chloro-2-methoxyphenyl)-7-(2-morpholinoethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine 131c

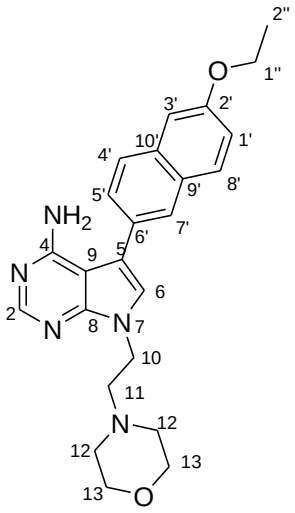


Compound **131c** was synthesized from **104f** (120 mg, 0.322 mmol), $Pd(PPh_3)_4$ (37.2 mg, 0.0322 mmol) and 5-chloro-2-methoxyphenylboronic acid (0.120 g, 0.643 mmol) in DME (50.0 ml) and purified by column chromatography (10% MeOH/chloroform) to afford **131c** (83.5 mg, 67%) as a yellow solid.

R_f (10% MeOH/chloroform) 0.26; *mp*: 220-222 °C. **^{1}H NMR (300 MHz, MeOD)**: δ 8.02 (1H, s, H2), 7.25 (1H, dd, *J* = 8.8, 2.7 Hz, H4'), 7.19 (1H, d, *J* = 2.7 Hz, H6'), 7.10 (1H, s, H6), 6.99 (1H, d, *J* = 8.8 Hz, H3'), 4.24 (2H, t, *J* = 6.6 Hz, H10), 3.70 (3H, s, OMe), 3.56 – 3.53 (4H, m, H13), 2.69 (2H, t, *J* = 6.6 Hz, H11), 2.42 (4H, dd, *J* = 5.6, 3.7 Hz, H12); **^{13}C NMR (75 MHz, MeOD)**: δ 151.9 (C-4), 150.6 (C-2), 149.4 (C-2'), 148.2 (C-8), 127.8 (C-4'), 123.8 (C-6'), 120.8 (C-5' and C-1'), 115.5 (C-5), 112.8 (C-3' and C-6), 100.4 (C-9), 66.7 (C-13), 58.2 (OMe), 56.1

(C-11), 53.7 (C-12), 48.2 (C-10); **IR (ν_{max}/cm^{-1})**: 3330, 3175 (N-H); 3066 (=C-H); 1560 (C=C); 1242 (C-N); 1061 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{19}H_{23}ClN_5O_2$: 388.1542, found: $[M + H]^+$ 388.1508.

7.10.5 Synthesis of 5-(2-ethoxynaphthalen-6-yl)-7-(2-morpholinoethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine 131d



Compound **131d** was synthesized from **104f** (0.250 g, 0.670 mmol), $Pd(PPh_3)_4$ (77.4 mg, 0.0670 mmol) and 6-ethoxynaphthalen-2-yl-2-boronic acid (0.291 g, 1.34 mmol) in DME (40.0 ml) and purified by column chromatography (10% MeOH/chloroform) to afford **131d** (85.3 mg, 51%) as a viscous oil.

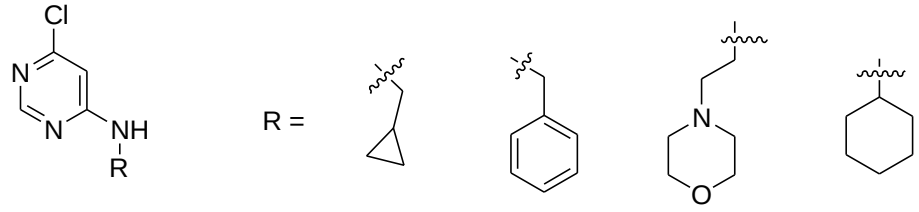
R_f (10% MeOH/chloroform) 0.26. **^{1}H NMR (300 MHz, MeOD)**: δ 8.10 (1H, s, H2), 7.80 – 7.68 (3H, m, H4', H7' and H8'), 7.46 (1H, dd, *J* = 8.6, 1.8 Hz, H5'), 7.22 (1H, s, H6), 7.17 (1H, d, *J* = 2.6 Hz, H3'), 7.07 (1H, dd, *J* = 8.9, 2.5 Hz, H1'), 4.29 (2H, t, *J* = 6.5 Hz, H10), 4.08 (2H, q, *J* = 6.9 Hz, H1"), 3.57 – 3.54 (4H, m, H13), 2.76 – 2.71 (2H, m, H11), 2.49 – 2.44 (4H, m, H12), 1.37 (3H, t, *J* = 7.0 Hz, H2"); **^{13}C NMR (75 MHz, MeOD)**: δ 157.7 (C-4), 157.2 (C-2'), 155.7 (C-2), 150.7 (C-8), 133.9 (C-10'), 129.9 (C-9'), 129.2 (C-6'), 129.1 (C-8'), 127.3 (C-5), 127.1 (C-4'), 126.8 (C-7'), 124.2 (C-5'), 119.4 (C-1'), 106.2 (C-6), 100.6 (C-3' and C-9), 66.4 (C-13), 63.2 (C-1"), 55.1 (C-11), 53.2 (C-12), 34.8 (C-10), 13.7 (C-2"); **IR (ν_{max}/cm^{-1})**: 3233, 3079 (N-H);

2990 (=C-H); 1560 (C=C); 1070 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{24}H_{28}N_5O_2$: 418.2245, found: $[M + H]^+$ 418.2240.

PART 2: Synthesis of novel 4,5,6-trisubstituted pyrimidine derivatives.

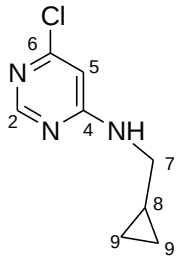
7.11 Synthesis of 5-substituted 4,6-diamino pyrimidines

7.11.1 General procedure for nucleophilic displacement reactions of 4,6-dichloropyrimidine



To a solution of 4,6-dichloropyrimidine in dioxane (50.0 ml) was added *N*,*N*-diisopropylethylamine or triethylamine (2 eq) and an appropriate amine (1.1 eq). The resulting mixture was heated at 90 °C for 18 hours. The reaction progress was monitored by TLC (20% EtOAc/hexane). After consumption of the starting material in each case, the reaction mixture was poured into a separating funnel containing water (100 ml) and ethyl acetate (100 ml). The mixture was shaken to partition the contents. The combined organic extracts for each reaction were dried over $MgSO_4$, filtered through celite and solvent was removed on a rotary evaporator. The crude products were purified by silica gel column chromatography (20% EtOAc/hexane as eluent). The following products were prepared using this methodology:

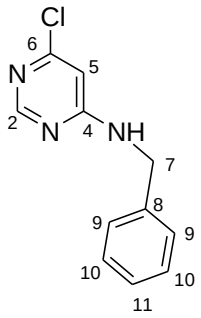
7.11.1.1 Synthesis of 6-chloro-*N*-(cyclopropylmethyl)pyrimidin-4-amine[9] 107a



Reaction of 4,6-dichloropyrimidine **106** (2.00 g, 13.4 mmol), *N,N*-diisopropylethylamine (4.58 ml, 26.9 mmol), and cyclopropylmethanamine hydrochloride (1.59 g, 14.8 mmol) gave 6-chloro-*N*-(cyclopropylmethyl)pyrimidin-4-amine **107a** (1.98 g, 80%) as a yellow solid product after purification by column chromatography (20 - 50% EtOAc/hexane).

R_f (50% EtOAc/hexane) 0.54. *mp*: 81-82 °C. **^{1}H NMR (500 MHz, DMSO-d$_6$)**: δ 8.24 (1H, s, H2), 7.82 (1H, s, NH), 6.51 (1H, s, H5), 3.18 (2H, s, H7), 1.00 (1H, ddt, *J* = 10.2, 7.2, 3.6 Hz, H8), 0.46 – 0.43 (2H, m, H9), 0.22 – 0.19 (2H, m, H9); **^{13}C NMR (125 MHz, DMSO-d$_6$)**: δ 163.0 (C-4), 158.5 (C-6), 156.8 (C-2), 103.5 (C-5), 44.6 (C-7), 10.4 (C-8), 3.3 (C-9); **IR (ν_{max}/cm^{-1})**: 3230, 3082 (N-H); 3020 (=C-H); 1566 (C=C); 1327 (C-N). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_8H_{11}ClN_3$: 184.0643, found: $[M + H]^+$ 184.0641.

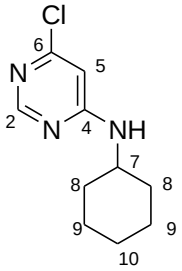
7.11.1.2 Synthesis of N-benzyl-6-chloropyrimidin-4-amine[9] 107b



Reaction of 4,6-dichloropyrimidine **106** (2.00 g, 13.4 mmol), DIPEA (4.58 ml, 26.9 mmol), and benzylamine (1.61 ml, 14.8 mmol) gave *N*-benzyl-6-chloropyrimidin-4-amine **107b** (2.51 g, 85%) as a yellow solid product after purification by column chromatography (50% EtOAc/hexane).

R_f (50% EtOAc/hexane) 0.69. *mp*: 85-86 °C. **^{1}H NMR (300 MHz, DMSO-d_6)**: δ 8.25 – 8.22 (2H, m, H2 and NH), 7.38 – 7.22 (5H, m, Ar-H), 6.59 (1H, s, H5), 4.57 – 4.55 (2H, m, H7); **^{13}C NMR (125 MHz, DMSO-d_6)**: δ 163.1 (C-4), 158.5 (C-6), 157.1 (C-2), 138.9 (C-8), 128.4 (C-10), 127.3 (C-11), 126.9 (C-9), 103.7 (C-5), 43.6 (C-7); **IR (ν_{max}/cm^{-1})**: 3212 (N-H); 3066 (=C-H); 1590 (C=C); 1217 (C-N).

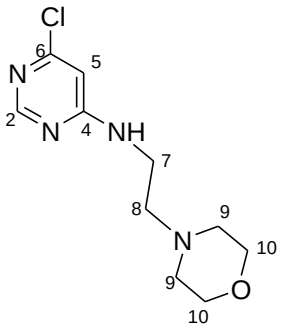
7.11.1.3 Synthesis of 6-chloro-N-cyclohexylpyrimidin-4-amine[10] 107c



Reaction of 4,6-dichloropyrimidine **106** (4.00 g, 26.8 mmol), Et_3N (7.48 ml, 53.7 mmol), and cyclohexylamine (3.38 ml, 29.5 mmol) gave 6-chloro-*N*-cyclohexylpyrimidin-4-amine **107c** (4.24 g, 75%) as an off-white solid product after purification by column chromatography (20% EtOAc/hexane).

R_f (20% EtOAc/hexane) 0.20. *mp*: 80-81 °C. **^{1}H NMR (300 MHz, DMSO-d_6)**: δ 8.22 (1H, s, H2), 7.60 (1H, d, *J* = 6.2 Hz, NH), 6.46 (1H, s, H5), 3.80 (1H, s, H7), 1.88 – 1.50 (5H, m, cyclohexyl-H), 1.31 – 1.05 (5H, m, cyclohexyl-H); **^{13}C NMR (75 MHz, DMSO-d_6)**: δ 162.3 (C-4), 158.5 (C-6), 156.8 (C-2), 103.5 (C-5), 48.8 (C-7), 32.1 (C-8), 25.1 (C-10), 24.4 (C-9); **IR (ν_{max}/cm^{-1})**: 3253 (N-H); 2930 (=C-H); 1587 (C=C); 1449 (CH_2); 1337 (C-N).

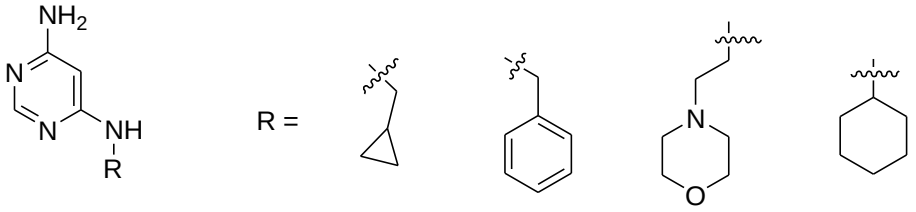
7.11.1.4 Synthesis of 6-chloro-N-(2-morpholinoethyl)pyrimidin-4-amine 107d



Reaction of 4,6-dichloropyrimidine **106** (4.00 g, 26.8 mmol), *N*,*N*-diisopropylethylamine (9.16 ml, 53.7 mmol), and 2-morpholinoethanamine (3.87 ml, 29.5 mmol) furnished 6-chloro-*N*-(2-morpholinoethyl)pyrimidin-4-amine **107d** (5.48 g, 84%) as a yellow solid product after purification by silica gel column chromatography (20% EtOAc/hexane).

R_f (80% EtOAc/hexane) 0.79. *mp*: 85-86 °C. **^{1}H NMR (500 MHz, DMSO-d$_6$)**: δ 8.26 (1H, s, H2), 7.62 (1H, s, NH), 6.57 (1H, s, H5), 3.57 (4H, t, *J* = 4.7 Hz, H10), 3.45 – 3.42 (2H, m, H7), 2.46 − 2.39 (6H, m, H8 and H9); **^{13}C NMR (125 MHz, DMSO-d$_6$)**: δ 163.6 (C-4), 158.9 (C-6), 157.4 (C-2), 104.2 (C-5), 66.6 (C-10), 57.5 (C-8), 53.8 (C-9), 37.7 (C-7); **IR (ν_{max}/cm^{-1})**: 3299, 3140 (N-H); 2848 (=C-H); 1589 (C=C); 1020 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{10}H_{16}ClN_4O$: 243.1014, found: $[M + H]^+$ 243.1011.

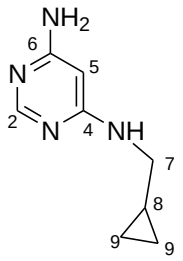
7.12 Synthesis of substituted 4,6-diaminopyrimidines



7.12.1 General procedure for ammonolysis of 4-amino-6-chloropyrimidines

To a solution of each of compounds **107a - 107d** in ethanol (5.00 ml) was added aqueous ammonia (40.0 ml). The resulting reaction mixtures were placed separately in a sealed tube and heated at 170 °C for 48 hours. After this time, the reaction mixture in each case was cooled and then concentrated *in vacuo*. The resulting residue from each reaction was purified with silica gel column chromatography using 50 - 80% EtOAc/hexane as eluent, to give the following products:

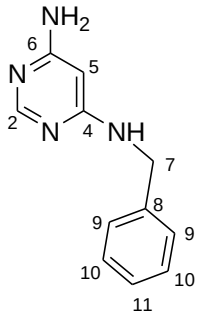
7.12.1.1 Synthesis of N4-(cyclopropylmethyl)pyrimidine-4,6-diamine 108a



Reaction of 6-chloro-*N*-(cyclopropylmethyl)pyrimidin-4-amine **107a** (2.00 g, 10.9 mmol) with ammonia (40.0 ml) in ethanol (5.00 ml), as described in general procedure **5.12.1**, afforded compound **108a** (1.56 g, 87%) as an orange solid.

R_f (80% EtOAc/hexane) 0.26. *mp*: 92-93 °C. **^{1}H NMR (500 MHz, DMSO-d$_6$)**: δ 8.23 (1H, s, H2), 6.50 (1H, s, H5), 6.33 (2H, s, NH_2), 6.12 (1H, t, *J* = 5.7 Hz, NH), 3.18 (2H, s, H7), 1.00 (1H, ddt, *J* = 10.2, 7.2, 3.6, Hz, H8), 0.46 – 0.43 (2H, m, H9), 0.22 – 0.19 (2H, m, H9); **^{13}C NMR (125 MHz, DMSO-d$_6$)**: δ 172.6 (C-6), 162.0 (C-4), 156.3 (C-2), 81.3 (C-5), 44.5 (C-7), 10.7 (C-8), 3.2 (C-9); **IR (ν_{max}/cm^{-1})**: 3230, 3082 (N-H); 3020 (=C-H); 1566 (C=C); 1327 (C-N). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_8H_{13}N_4$: 165.1142, found: $[M + H]^+$ 165.1144.

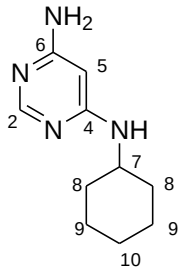
7.12.1.2 Synthesis of N4-benzylpyrimidine-4,6-diamine 108b



Following the same procedure as above, compound **107b** (2.00 g, 9.10 mmol) was dissolved in ethanol (5.00 ml). To the light yellow solution was added aqueous ammonia (40.0 ml). The product **108b** (1.62 g, 89%) was isolated as a light orange solid after purification with column chromatography (2% MeOH/ethyl acetate).

R_f (2% MeOH/ethyl acetate) 0.29. *mp*: 95-96 °C. **^{1}H NMR (300 MHz, DMSO-d$_6$)**: δ 7.86 (1H, s, H2), 7.34 – 7.20 (5H, m, Ar-H), 7.10 (1H, t, *J* = 6.3 Hz, NH), 6.04 (2H, s, NH_2), 5.37 (1H, s, H5), 4.38 (2H, d, *J* = 6.3 Hz, H7); **^{13}C NMR (75 MHz, DMSO-d$_6$)**: δ 162.5 (C-6), 160.8 (C-4), 156.7 (C-2), 140.5 (C-8), 128.1 (C-10), 126.8 (C-9), 126.4 (C-11), 56.4 (C-5), 44.2 (C-7); **IR (ν_{max}/cm^{-1})**: 3466, 3410 (N-H); 3123 (=C-H); 1570 (C=C); 1242 (C-N). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{11}H_{13}N_4$: 201.1142, found: $[M + H]^+$ 201.1131.

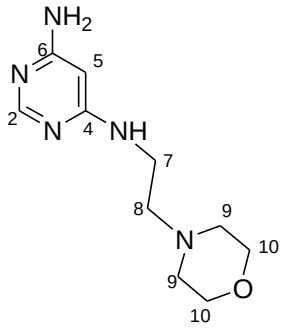
7.12.1.3 Synthesis of N4-cyclohexylpyrimidine-4,6-diamine 108c



Following the same procedure as above, compound **107c** (3.00 g, 14.2 mmol) was dissolved in ethanol (7.00 ml). To the light yellow solution was added aqueous ammonia. The product **108c** (2.39 g, 88%) was isolated as a light orange solid after purification with column chromatography (50% EtOAc/hexane).

R_f (50% EtOAc/hexane) 0.35. *mp*: 90-91 °C. **^{1}H NMR (300 MHz, DMSO-d$_6$)**: δ 7.82 (1H, s, H2), 6.05 (2H, s, NH_2), 5.40 (1H, d, *J* = 8.0 Hz, NH), 5.37 (1H, s, H5), 1.80 – 1.51 (5H, m, cyclohexyl-H), 1.38 – 1.05 (6H, m, cyclohexyl-H); **^{13}C NMR (75 MHz, DMSO-d$_6$)**: δ 165.4 (C-6), 162.5 (C-4), 155.4 (C-2), 82.6 (C-5), 51.1 (C-7), 33.4 (C-8), 28.2 (C-10), 23.7 (C-9); **IR (ν_{max}/cm^{-1})**: 3393, 3303 (N-H); 2930 (=C-H); 1570 (C=C); 1157 (C-N). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{10}H_{17}N_4$: 193.1455, found: $[M + H]^+$ 193.1450.

7.12.1.4 Synthesis of N4-(2-morpholinoethyl)pyrimidine-4,6-diamine 108d



Following the same procedure as above, compound **107d** (2.00 g, 8.24 mmol) was dissolved in ethanol (5.00 ml). To the light yellow solution was added aqueous ammonia. The product **108d** (1.53 g, 83%) was isolated as a light orange solid after purification with silica gel column chromatography (10% MeOH/chloroform).

R_f (10% MeOH/chloroform) 0.26. *mp*: 95-97 °C. **^{1}H NMR (300 MHz, DMSO-d$_6$)**: δ 7.93 (1H, s, H2), 6.84 (1H, s, NH), 6.35 (2H, s, NH_2), 5.46 (1H, s, H5), 3.63 (4H, s, H10), 3.34 (2H, s, H7), 2.65 − 2.49 (6H, m, H8 and H9); **^{13}C NMR (75 MHz, DMSO-d$_6$)**: δ 162.2 (C-4), 161.9 (C-6), 156.3 (C-2), 79.2 (C-5), 65.5 (C-10), 56.9 (C-8), 52.8 (C-9), 36.9 (C-7); **IR (ν_{max}/cm^{-1})**: 3383, 3329 (N-H); 2861 (=C-H); 1596 (C=C); 1445 (CH_2); 1093 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{10}H_{18}N_5O$: 224.1513, found: $[M + H]^+$ 224.1511.

7.13 Synthesis of N^4-(cyclopropylmethyl)-5-iodopyrimidine-4,6-diamine and analogues

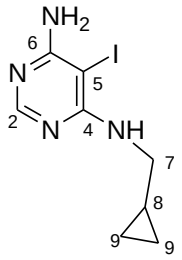
In the following sections, iodination of compounds **109a-d** will be discussed using the previously described methodology.

7.13.1 General procedure for iodination

To a suspension of starting material **108a - 108d** in water (50.0 ml) and DMF (10.0 ml) was added potassium carbonate (1.5 eq) and iodine (2 eq). The resulting reaction mixture was heated at 40 - 45 °C for 4 hours. TLC analysis showed consumption of the starting material in each case. After this time, the reaction mixture was cooled down to room temperature and then quenched with 2 M aqueous sodium thiosulfate (40.0 ml). In each case, the iodinated product

precipitated out of solution and was collected by filtration, washed with water (3 × 25 ml) to give the following compounds **109a - 109d** in good yields.

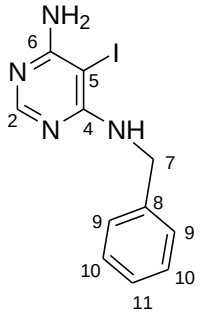
7.13.1.1 Synthesis of N^4-(cyclopropylmethyl)-5-iodopyrimidine-4,6-diamine 109a



Reaction of N^4-(cyclopropylmethyl)pyrimidine-4,6-diamine **108a** (1.92 g, 11.7 mmol), potassium carbonate (2.42 g, 17.5 mmol), and iodine (5.94 g, 23.4 mmol) in water (50.0 ml) and DMF (20.0 ml) gave N^4-(cyclopropylmethyl)-5-iodopyrimidine-4,6-diamine **109a** (2.8 g, 83%) as a light yellow solid after drying with high vacuum.

R_f (80% EtOAc/hexane) 0.25. *mp*: 105-106 °C. **^{1}H NMR (300 MHz, DMSO-d_6)**: δ 7.80 (1H, s, H2), 6.30 (2H, s, NH_2), 6.12 (1H, t, J = 5.7 Hz, NH), 3.24 – 3.15 (2H, m, H7), 1.20 − 1.00 (1H, m, H8), 0.42 – 0.31 (2H, m, H9), 0.25 – 0.16 (2H, m, H9); **^{13}C NMR (75 MHz, DMSO-d_6)**: δ 162.3 (C-4), 160.7 (C-6), 156.8 (C-2), 56.5 (C-5), 45.4 (C-7), 11.3 (C-8), 3.2 (C-9); **IR (ν_{max}/cm^{-1})**: 3455, 3280 (N-H); 3073 (=C-H); 1627 (C=N); 1487 (C=C); 1455 (CH_2). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_8H_{12}IN_4$: 291.1042, found: $[M + H]^+$ 291.1002.

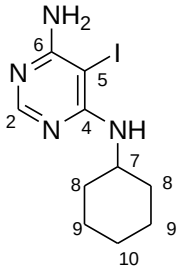
7.13.1.2 Synthesis of N^4-benzyl-5-iodopyrimidine-4,6-diamine 109b



Following the same procedure as above, compound **108b** (900 mg, 4.49 mmol) was dissolved in water (50.0 ml) and DMF (20.0 ml). To the purple solution was added potassium carbonate (0.932 g, 6.74 mmol) followed by iodine (2.28 g, 8.98 mmol). N^4-Benzyl-5-iodopyrimidine-4,6-diamine **109b** (1.24 g, 84%) was isolated as yellow solid.

R_f (2% MeOH/ethyl acetate) 0.48. *mp*: 107-109 °C. **^{1}H NMR (300 MHz, DMSO-d$_6$)**: δ 7.77 (1H, s, H2), 7.32 – 7.17 (5H, m, Ar-H), 6.77 (1H, t, *J* = 6.1 Hz, NH), 6.34 (2H, s, NH_2), 4.57 (2H, d, *J* = 6.1 Hz, H7); **^{13}C NMR (75 MHz, DMSO-d$_6$)**: δ 162.5 (C-4), 160.8 (C-6), 156.7 (C-2), 140.5 (C-8), 128.1 (C-10), 126.8 (C-9), 126.4 (C-11), 56.4 (C-5), 44.2 (C-7); **IR (ν_{max}/cm^{-1})**: 3465, 3408 (N-H); 3121 (=C-H); 1571 (C=C); 1241 (C-N). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{11}H_{12}IN_4$: 327.0108, found: $[M + H]^+$ 327.0095.

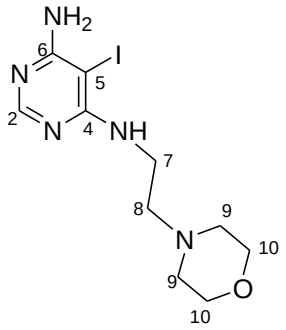
7.13.1.3 Synthesis of N^4-cyclohexyl-5-iodopyrimidine-4,6-diamine 109c



Following the same procedure as above, compound **108c** (1.00 g, 5.20 mmol) was dissolved in water (50.0 ml) and DMF (20.0 ml). To the purple solution was added potassium carbonate (1.08 g, 7.80 mmol) followed by iodine (2.64 g, 10.4 mmol). N^4-Cyclohexyl-5-iodopyrimidine-4,6-diamine **109c** (1.47 g, 89%) was isolated as yellow solid.

R_f (50% EtOAc/hexane) 0.40. *mp*: 102-103 °C. **^{1}H NMR (300 MHz, DMSO-d$_6$)**: δ 7.80 (1H, s, H2), 6.32 (2H, s, NH_2), 5.40 (1H, d, *J* = 8.0 Hz, NH), 1.80 – 1.51 (5H, m, cyclohexyl-H), 1.38 – 1.05 (6H, m, cyclohexyl-H); **^{13}C NMR (75 MHz, DMSO-d$_6$)**: δ 162.4 (C-4), 159.9 (C-6), 156.8 (C-2), 57.0 (C-5), 49.6 (C-7), 32.4 (C-8), 25.2 (C-10), 24.7 (C-9); **IR (ν_{max}/cm^{-1})**: 3395, 3303 (N-H); 2925 (=C-H); 1578 (C=C); 1471 (CH_2); 1157 (C-N). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{10}H_{16}IN_4$: 319.0421, found: $[M + H]^+$ 319.0415.

7.13.1.4 Synthesis of 5-iodo-N^4-(2-morpholinoethyl)pyrimidine-4,6-diamine 109d



Reaction of N^4-(2-morpholinoethyl)pyrimidine-4,6-diamine **108d** (2.30 g, 10.3 mmol), potassium carbonate (2.14 g, 15.5 mmol), and iodine (5.23 g, 20.6 mmol) in water (50.0 ml) and DMF (20.0 ml) gave 5-iodo-N^4-(2-morpholinoethyl)pyrimidine-4,6-diamine **109d** (2.75 g, 76%) as a yellow solid.

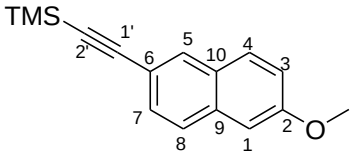
R_f (10% MeOH/ethyl acetate) 0.28. *mp*: 111-113 °C. **^{1}H NMR (300 MHz, DMSO-d_6)**: δ 7.89 (1H, s, H2), 6.56 (3H, dd, *J* = 14.3, 8.6 Hz, NH_2 and NH), 3.82 (4H, t, *J* = 4.7 Hz, H10), 3.66 (2H, q, *J* = 5.8, Hz, H7), 3.31 – 3.24 (6H, m, 6H); **^{13}C NMR (125 MHz, DMSO-d_6)**: δ 162.4 (C-4), 160.8 (C-6), 156.3 (C-2), 63.5 (C-10), 56.8 (C-5), 56.2 (C-8), 51.5 (C-9), 36.1 (C-7); **IR (ν_{max}/cm^{-1})**: 3413, 3291 (N-H); 3032 (=C-H); 1582 (C=C); 1456 (CH_2); 1070 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{10}H_{17}IN_5O$: 350.0480, found: $[M + H]^+$ 350.0475.

7.14 General procedure for the synthesis of (2-(2-methoxynaphthalen-6-yl)ethynyl)trimethylsilane and derivatives by Sonogashira coupling reaction

To a degassed mixture of compounds **120a - 120c**, copper(I) iodide (3 mole %) and $Pd(PPh_3)_2Cl_2$ (5 mole %) was added a degassed solution of ethynyltrimethylsilane (10 eq), diisopropylamine (10 eq) and tetrahydrofuran or DMF (20.0 ml). The resulting black mixture was heated at 70 °C for 5 hours under nitrogen atmosphere. After consumption of the starting material determined by TLC, the reaction mixture was quenched with a saturated aqueous ammonium chloride (NH_4Cl) solution and the THF was removed on a rotary evaporator. The aqueous residue was washed three times with ethyl acetate (100 ml) and the organic layers were combined, dried over $MgSO_4$, filtered through celite and excess solvent removed on a rotary evaporator. Each of the crude

products was purified by column chromatography (20% EtOAc/hexane as eluent) to furnish the following products, **132a – 132c**, in good yields.

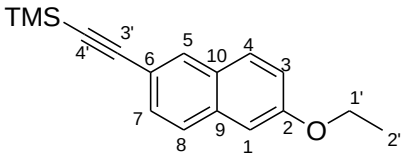
7.14.1 Synthesis of [2-(2-methoxynaphthalen-6-yl)ethynyl]trimethylsilane[11] 132a



To a mixture of 2-bromo-6-methoxynaphthalene **120a** (2.00 g, 8.44 mmol), copper(I) iodide (480 mg, 0.253 mmol), and $Pd(PPh_3)_2Cl_2$ (0.296 g, 0.422 mmol) under nitrogen atmosphere, was added a degassed solution of trimethylsilylacetylene (11.9 ml, 84.4 mmol), diisopropylamine (11.9 ml, 84.4 mmol) and DMF (10.0 ml). The product **132a** (1.68 g, 79%) was isolated as a yellow solid after purification by column chromatography (20% EtOAc/hexane).

R_f (20% EtOAc/hexane) 0.95. *mp*: 96 °C. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 7.91 (1H, d, *J* = 2.0 Hz, H5), 7.89 – 7.54 (2H, m, H4 and H8), 7.48 (1H, dd, *J* = 8.7, 2.0 Hz, H7), 7.13 (1H, dd, *J* = 8.9, 2.5 Hz, H3), 7.07 (1H, d, *J* = 2.5 Hz, H1), 3.88 (3H, s, OMe), 0.26 (9H, s, TMS); **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 157.9 (C-2), 133.0 (C-9), 130.0 (C-7), 129.6 (C-5), 129.6 (C-4), 128.5 (C-8), 128.4 (C-10), 119.7 (C-3), 117.0 (C-6), 106.4 (C-1'), 105.8 (C-1), 93.5 (C-2'), 55.3 (OMe), 0.0 (TMS); **IR (ν_{max}/cm^{-1})**: 3285 (=C-H); 2110 (C≡C); 1635 (C=C); 1040 (C-O).

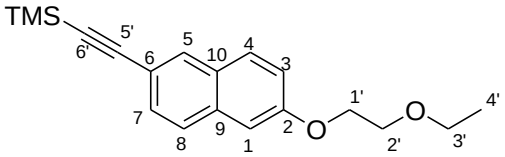
7.14.2 Synthesis of [2-(2-ethoxynaphthalen-6-yl)ethynyl]trimethylsilane[11] 132b



To a mixture of 2-bromo-6-ethoxynaphthalene **120b** (2.00 g, 7.96 mmol), copper(I) iodide (46.0 mg, 0.239 mmol), and $Pd(PPh_3)_2Cl_2$ (0.279 g, 0.398 mmol) under nitrogen atmosphere, was added a degassed solution of trimethylsilylacetylene (11.3 ml, 79.6 mmol), diisopropylamine (11.3 ml, 79.6 mmol) and DMF (10.0 ml). The product **132b** (1.56 g, 73%) was isolated as an off-white solid after purification by column chromatography (20% EtOAc/hexane).

R_f (20% EtOAc/hexane) 0.95. *mp*: 96 °C. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 7.91 (1H, d, *J* = 1.3 Hz, H5), 7.70 − 7.63 (2H, m, H4 and H8), 7.46 (1H, dd, *J* = 8.4, 1.7 Hz, H7), 7.13 (1H, dd, *J* = 8.9, 2.5 Hz, H3), 7.07 (1H, d, *J* = 2.5 Hz, H1), 4.14 (2H, q, *J* = 7.0 Hz, H1'), 1.47 (3H, t, *J* = 7.0 Hz, H2'), 0.27 (9H, s, TMS); **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 157.6 (C-2), 134.2 (C-9), 131.8 (C-7), 129.0 (C-5), 128.2 (C-4), 126.6 (C-8 and C-10), 119.6 (C-3), 117.8 (C-6), 106.4 (C-3'), 105.7 (C-1), 93.5 (C-4'), 63.5 (C-1'), 14.7 (C-2'), 0.0 (TMS); **IR (ν_{max}/cm^{-1})**: 3271 (=C-H); 2104 (C≡C); 1621 (C=C); 1044 (C-O).

7.14.3 Synthesis of {2-[2-(2-ethoxyethoxy)naphthalen-6-yl]ethynyl}trimethylsilane 132c



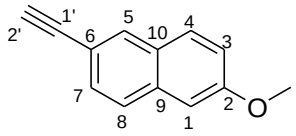
To a mixture of 2-(2-ethoxyethoxy)-6-bromonaphthalene **120c** (3.00 g, 10.2 mmol), copper(I) iodide (0.0580 g, 0.305 mmol), and $Pd(PPh_3)_2Cl_2$ (0.357 g, 0.508 mmol) under nitrogen atmosphere, was added a degassed solution of trimethylsilylacetylene (14.4 ml, 102 mmol), diisopropylamine (14.4 ml, 102 mmol) and DMF (10.0 ml). The product **132c** (1.63 g, 76%) was isolated as a brown solid after purification by column chromatography (20% EtOAc/hexane).

R_f (20% EtOAc/hexane) 0.95. *mp*: 101°C. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 7.89 (1H, d, *J* = 2.0 Hz, H5), 7.70 − 7.63 (2H, m, H4 and H8), 7.45 (1H, dd, *J* = 8.8, 2.0 Hz, H7), 7.19 (1H, dd, *J* = 9.0, 2.5 Hz, H3), 7.07 (1H, d, *J* = 2.5 Hz, H1), 4.19 (2H, dd, *J* = 5.7, 4.0 Hz, H1'), 3.84 – 3.82 (2H, m, H2'), 3.61 (2H, q, *J* = 7.0 Hz, H3'), 1.25 (3H, t, *J* = 7.0 Hz, H4'), 0.27 (9H, s, TMS); **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 157.1 (C-2), 132.9 (C-9), 130.1 (C-7), 129.5 (C-4 and C-5), 128.4 (C-8 and C-10), 120.1 (C-3), 117.1 (C-6), 106.7 (C-1), 106.4 (C-5'), 93.5 (C-6'), 68.8 (C-3'), 67.5 (C-1'), 66.9 (C-2'), 15.2 (C-4'), 0.0 (TMS); **IR (ν_{max}/cm^{-1})**: 2931 (=C-H); 2190 (C≡C); 1621 (C=C); 1040 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{19}H_{25}O_2Si$: 313.1626, found: $[M + H]^+$ 313.1620.

7.15 General procedure for the deprotection of [2-(2-methoxynaphthalen-6-yl)ethynyl)trimethylsilane and analogues using TBAF

[2-(2-Methoxynaphthalen-6-yl)ethynyl]trimethylsilane and analogues were dissolved separately in dry tetrahydrofuran (30.0 ml) and cooled to 0 °C. Tetrabutylammonium fluoride (1.2 eq) was added slowly to the reaction medium and the reaction mixture turned brown. The resultant brown mixture was allowed to warm to room temperature and stirred for 3 hours. TLC analysis showed consumption of the starting material. The reaction mixture was quenched with a saturated aqueous ammonium chloride solution and was extracted with ethyl acetate (3 × 25 ml). The organic extracts were dried with $MgSO_4$, filtered through celite and excess solvent was removed on a rotary evaporator. The crude product in each case was purified by column chromatography (20% EtOAc/hexane as eluent) to furnish the following products:

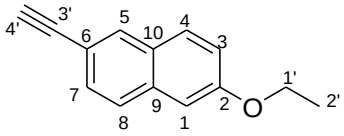
7.15.1 Synthesis of 2-ethynyl-6-methoxynaphthalene[12] 133a



Following the same procedure as above, compound **132a** (1.00 g, 3.93 mmol) was dissolved in dry THF (30.0 ml). After complete dissolution, TBAF (1.37 ml, 4.72 mmol) was added. The terminal alkyne product **133a** (0.63 g, 88%) was isolated as a light orange solid after purification by column chromatography (20% EtOAc/hexane).

R_f (20% EtOAc/hexane) 0.98. *mp*: 102 °C. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 7.95 (1H, s, H5), 7.71 – 7.66 (2H, m, H4 and H8), 7.49 (1H, dd, *J* = 8.5, 1.6 Hz, H7), 7.16 (1H, dd, *J* = 9.0, 2.5 Hz, H3), 7.11 (1H, d, *J* = 2.5 Hz, H1), 3.93 (3H, s, OMe), 3.10 (1H, s, H2'); **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 157.9 (C-2), 133.0 (C-9), 130.0 (C-7), 129.6 (C-5), 129.6 (C-4), 128.5 (C-8), 128.4 (C-10), 119.7 (C-3), 117.0 (C-6), 105.8 (C-1), 84.2 (C-1'), 63.5 (C-2'), 55.3 (OMe); **IR (ν_{max}/cm^{-1})**: 3256 (=C-H); 2105 (C≡C); 1625 (C=C); 1063 (C-O).

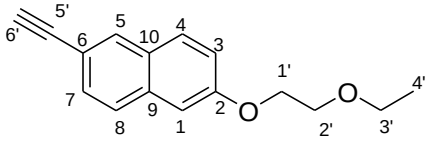
7.15.2 Synthesis of 2-ethoxy-6-ethynylnaphthalene[12] 133b



[2-(2-Ethoxynaphthalen-6-yl)ethynyl]trimethylsilane **132a** (1.50 g, 5.59 mmol) was dissolved in dry THF (30.0 ml). After complete dissolution, TBAF (1.94 ml, 6.71 mmol) was added to the reaction mixture. 2-Ethoxy-6-ethynylnaphthalene **133b** (0.95 g, 87%) was isolated as a yellow solid after purification by column chromatography (20% EtOAc/hexane).

R_f (20% EtOAc/hexane) 0.98. *mp*: 102 °C. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 7.94 (1H, s, H5), 7.70 –7.63 (2H, m, H4 and H8), 7.47 (1H, dd, *J* = 8.5, 1.6 Hz, H7), 7.15 (1H, dd, *J* = 8.9, 2.5 Hz, H3), 7.08 (1H, d, *J* = 2.4 Hz, H1), 4.14 (2H, q, *J* = 7.0 Hz, H1'), 3.10 (1H, s, H4'), 1.48 (3H, t, *J* = 7.0 Hz, H2'); **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 157.7 (C-2), 134.4 (C-9), 132.1 (C-7), 129.3 (C-5), 129.1 (C-4), 128.2 (C-8), 126.8 (C-10), 119.8 (C-3), 116.8 (C-6), 106.5 (C-1), 84.2 (C-3'), 63.5 (C-4'), 63.5 (C-1'), 14.8 (C-2'); **IR (ν_{max}/cm^{-1})**: 3271 (=C-H); 2104 (C≡C); 1621 (C=C); 1044 (C-O).

7.15.3 Synthesis of 2-(2-ethoxyethoxy)-6-ethynylnaphthalene 133c



Following the same procedure as above**,** {2-[2-(2-ethoxyethoxy)naphthalen-6-yl]ethynyl}trimethylsilane **132c** (1.00 g, 3.20 mmol) was dissolved in dry THF (30.0 ml). After complete dissolution, TBAF (1.11 ml, 3.84 mmol) was added to the reaction mixture. 2-(2-ethoxyethoxy)-6-ethynylnaphthalene **133c** (0.59 g, 77%) was isolated as a yellow solid after purification by column chromatography (20% EtOAc/hexane).

R_f (20% EtOAc/hexane) 0.98. *mp*: 115 °C. **^{1}H NMR (300 MHz, $CDCl_3$)** δ 7.89 (1H, d, *J* = 2.0 Hz, H5), 7.70 – 7.66 (2H, m, H4 and H8), 7.45 (1H, dd, *J* = 8.8, 2.0 Hz, H7), 7.19 (1H, dd, *J* = 9.0, 2.5 Hz, H3), 7.07 (1H, d, *J* = 2.5 Hz, H1), 4.19 (2H, dd, *J* = 5.7, 4.0 Hz, H1'), 3.84 – 3.82

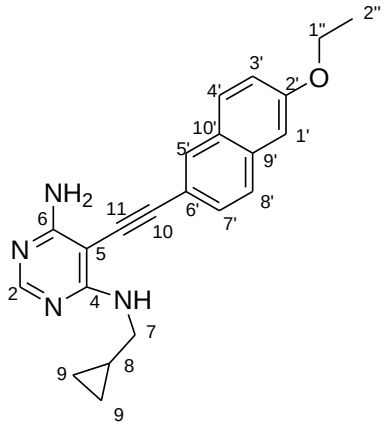
(2H, m, H2'), 3.61 (2H, q, *J* = 7.0 Hz, H3'), 3.10 (1H, s, H6'), 1.25 (3H, t, *J* = 7.0 Hz, H4'); **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 157.1 (C-2), 132.9 (C-9), 130.1 (C-7 and C-5), 129.5 (C-4), 128.4 (C-8 and C-10), 120.1 (C-3), 117.1 (C-6), 106.7 (C-1), 84.3 (C-5'), 68.8 (C-3'), 67.5 (C-1'), 66.9 (C-2'), 63.5 (C-6'), 15.2 (C-4'); **IR (ν_{max}/cm^{-1})**: 3246 (=C-H); 2196 (C≡C); 1596 (C=C); 1063 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{16}H_{17}O_2$: 241.1230, found: $[M + H]^+$ 241.1232.

7.16 Synthesis of 4,5,6-trisubstituted pyrimidine derivatives

7.16.1 General procedure for the preparation of compounds 110 using Sonogashira cross coupling reaction

To a flame dried 2-neck round bottom flask (100 ml) under nitrogen gas containing each of compounds **109a – 109d**, CuI (5 mole %) and $Pd(PPh_3)_2Cl_2$ (5 mole %) was added a degassed solution of diisopropylamine (10 eq) and DMF (10.0 ml) using a dropping funnel. The resultant reaction mixture in each case was heated at 70 °C for 5 hours under nitrogen atmosphere. After this time, each reaction mixture was cooled to room temperature and poured into a separating funnel containing water (100 ml) and ethyl acetate (100 ml). The aqueous layer was extracted twice with 100 ml ethyl acetate. The combined organic layers for each reaction were dried over magnesium sulfate, filtered through celite and excess solvent was removed on a rotary evaporator, followed by purification using silica gel column chromatography (50% EtOAc/hexane – 10% MeOH/EtOAc). Each of the following products **110a-110i** was prepared using this method:

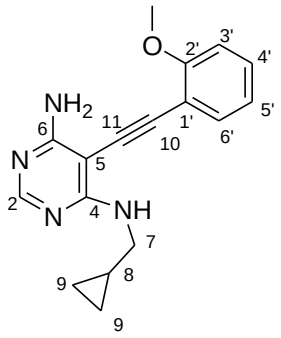
7.16.1.1 Synthesis of N^4-(cyclopropylmethyl)-5-[2-(2-ethoxynaphthalen-6-yl)ethynyl]pyrimidine-4,6-diamine 110a



Reaction of N^4-(cyclopropylmethyl)-5-iodopyrimidine-4,6-diamine **109a** (230 mg, 0.793 mmol), copper(I) iodide (7.55 mg, 0.0396 mmol), $Pd(PPh_3)_2Cl_2$ (27.8 mg, 0.0396 mmol) and compound **133b** (0.311 g, 1.59 mmol) as described afforded, after extraction and column chromatography using 50% EtOAc/hexane as eluent, **110a** (0.136 g, 48%) as a pale yellow solid.

R_f (50% EtOAc/hexane) 0.24. *mp*: 212-214 °C. **^{1}H NMR (500 MHz, DMSO-d_6)**: δ 8.19 (1H, s, H5'), 7.93 (1H, s, H2), 7.81 – 7.73 (2H, m, H4' and H8'), 7.74 (1H, d, *J* = 8.7 Hz, H7'), 7.33 (1H, d, *J* = 2.6 Hz, H1'), 7.19 (1H, dd, *J* = 8.8, 2.6 Hz, H3'), 6.69 (1H, t, *J* = 5.9 Hz, NH), 6.58 (2H, s, NH_2), 4.16 (2H, q, *J* = 7.5 Hz, H1''), 3.29 (2H, d, *J* = 7.3 Hz, H7), 1.40 (3H, t, *J* = 6.9 Hz, H2''), 1.15 − 1.13 (1H, m, H8), 0.42 – 0.41 (2H, m, H9), 0.28 – 0.27 (2H, m, H9); **^{13}C NMR (125 MHz, DMSO-d_6)**: δ 162.1 (C-6), 157.6 (C-4 and C-2'), 156.9 (C-2), 134.1 (C-9'), 131.9 (C-5'), 130.8 (C-7'), 129.6 (C-4'), 129.3 (C-10'), 127.0 (C-8'), 120.0 (C-3'), 118.7 (C-6'), 107.3 (C-1'), 80.9 (C-10), 79.0 (C-11), 78.9 (C-5), 63.7 (C-1''), 45.0 (C-7), 15.1 (C-2''), 12.0 (C-8), 3.8 (C-9); **IR (ν_{max}/cm^{-1})**: 3478, 3422 (N-H); 3056 (=C-H); 2187 (C≡C); 1570 (C=C); 1436 (CH_2); 1117 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{22}H_{23}N_4O$: 359.1874, found: $[M + H]^+$ 359.1881.

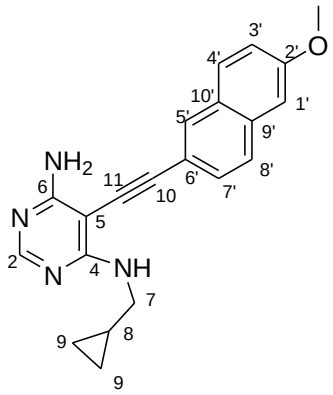
7.16.1.2 Synthesis of N^4-(cyclopropylmethyl)-5-[2-(2-methoxyphenyl)ethynyl]pyrimidine-4,6-diamine 110b



Reaction of N^4-(cyclopropylmethyl)-5-iodopyrimidine-4,6-diamine **109a** (200 mg, 0.689 mmol), copper(I) iodide (6.56 mg, 0.0345 mmol), $Pd(PPh_3)_2Cl_2$ (24.2 mg, 0.0345 mmol) and 1-ethynyl-2-methoxybenzene (0.182 g, 1.38 mmol) as described afforded, after extraction and column chromatography using 50% EtOAc/hexane as eluent, **110b** (0.11 g, 54%) as a viscous oil.

R_f (50% EtOAc/hexane) 0.24. **^{1}H NMR (500 MHz, DMSO-d_6)**: δ 7.96 (1H, s, H2), 7.65 – 7.63 (1H, m, H5'), 7.36 (1H, ddd, *J* = 8.9, 7.5, 1.8 Hz, H4'), 7.11 (1H, d, *J* = 8.3 Hz, H6'), 7.01 (1H, td, *J* = 7.6, 1.0 Hz, H3'), 6.54 (2H, s, NH_2), 6.34 (1H, t, *J* = 5.8 Hz, NH), 3.91 (3H, s, OMe), 3.31 (2H, d, *J* = 6.4 Hz, H7), 1.13 – 1.08 (1H, m, H8), 0.48 – 0.45 (2H, m, H9), 0.28 – 0.25 (2H, m, H9); **^{13}C NMR (125 MHz, DMSO-d_6)**: δ 163.1 (C-6), 161.8 (C-2'), 159.3 (C-4), 157.0 (C-2), 132.0 (C-6'), 129.2 (C-4'), 120.9 (C-5'), 112.5 (C-3'), 111.5 (C-1'), 97.9 (C-5), 86.5 (C-10), 79.2 (C-11), 56.3 (C-7), 45.2 (OMe), 11.8 (C-8), 3.7 (C-9): **IR (ν_{max}/cm^{-1})**: 3467, 3402 (N-H); 3078 (=C-H); 2197 (C≡C); 1042 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{17}H_{19}N_4O$: 295.1561, found: $[M + H]^+$ 295.1548.

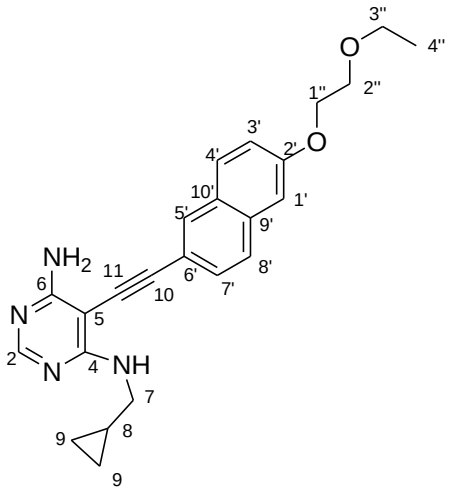
7.16.1.3 Synthesis of N^4-(cyclopropylmethyl)-5-[2-(2-methoxynaphthalen-6-yl)ethynyl]pyrimidine-4,6-diamine 110c



Reaction of N^4-(cyclopropylmethyl)-5-iodopyrimidine-4,6-diamine **109a** (120 mg, 0.414 mmol), copper (I) iodide (3.94 mg, 0.0207 mmol), $Pd(PPh_3)_2Cl_2$ (14.5 mg, 0.0207 mmol) and compound **133a** (0.151 g, 0.827 mmol) as described afforded, after extraction and column chromatography using 50% EtOAc/hexane as eluent, **110c** (0.070 g, 49%) as a light yellow oil.

R_f (50% EtOAc/hexane) 0.24. **^{1}H NMR (500 MHz, DMSO-d_6)**: δ 8.20 – 8.19 (1H, m, H5'), 7.93 (1H, s, H2), 7.83 – 7.81 (2H, m, H4' and H8'), 7.75 (1H, dd, *J* = 8.5, 1.7 Hz, H7'), 7.34 (1H, d, *J* = 2.7 Hz, H1'), 7.20 (1H, dd, *J* = 8.9, 2.6 Hz, H3'), 6.70 (1H, t, *J* = 6.0 Hz, NH), 6.58 (2H, s, NH_2), 3.89 (3H, s, OMe), 3.29 (2H, d, *J* = 6.5 Hz, H7), 1.16 – 1.11 (1H, m, H8), 0.44 – 0.37 (2H, m, H9), 0.28 – 0.26 (2H, m, H9); **^{13}C NMR (125 MHz, DMSO-d_6)**: δ 162.1 (C-6), 157.6 (C-4 and C-2'), 156.9 (C-2), 134.1 (C-9'), 131.9 (C-5'), 130.8 (C-7'), 129.6 (C-4'), 129.3 (C-10'), 127.0 (C-8'), 120.0 (C-3'), 118.7 (C-6'), 107.3 (C-1'), 80.9 (C-10), 79.0 (C-11), 78.9 (C-5), 55.8 (OMe), 45.0 (C-7), 12.0 (C-8), 3.8 (C-9); **IR (ν_{max}/cm^{-1})**: 3476, 3430 (N-H); 3066 (=C-H); 2116 (C≡C); 1573 (C=C); 1436 (CH_2); 1056 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{18}H_{21}N_4O_2$: 325.1666, found: $[M + H]^+$ 325.1672.

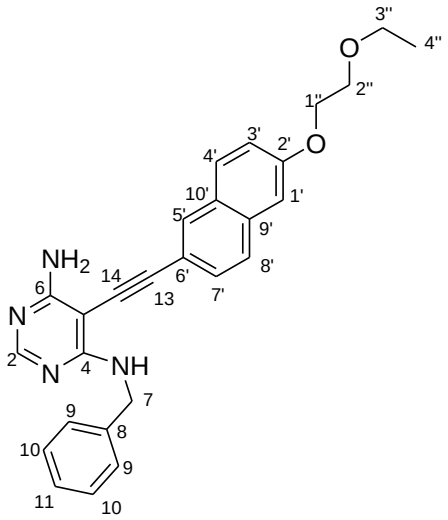
7.16.1.4 Synthesis of 5-{2-[2-(2-ethoxyethoxy)naphthalen-6-yl]ethynyl}-N^4-(cyclopropylmethyl)pyrimidine-4,6-diamine 110d



Reaction of N^4-(cyclopropylmethyl)-5-iodopyrimidine-4,6-diamine **109a** (80.0 mg, 0.276 mmol), copper (I) iodide (2.63 mg, 0.0138 mmol), $Pd(PPh_3)_2Cl_2$ (9.68 mg, 0.0138 mmol) and compound **133c** (0.133 g, 0.552 mmol) as described afforded, after extraction and column chromatography using 50% EtOAc/hexane as eluent, **110d** (0.067 g, 60%) as a pale yellow oil.

R_f (50% EtOAc/hexane) 0.24. **^{1}H NMR (500 MHz, DMSO-d_6)**: δ 8.19 (1H, d, *J* = 1.5 Hz, H5'), 7.93 (1H, s, H2), 7.80 (2H, d, *J* = 8.7 Hz, H4' and H8'), 7.74 (1H, dd, *J* = 8.4, 1.6 Hz, H7'), 7.35 (1H, d, *J* = 2.5 Hz, H1'), 7.22 (1H, dd, *J* = 8.9, 2.5 Hz, H3'), 6.70 (1H, t, *J* = 6.0 Hz, NH), 6.58 (2H, s, NH_2), 4.23 – 4.22 (2H, m, H1''), 3.78 – 3.76 (2H, m, H2''), 3.54 (2H, q, *J* = 7.0 Hz, H3''), 3.29 (2H, d, *J* = 4.9 Hz, H7), 1.15 (4H, t, *J* = 7.0 Hz, H8 and H4''), 0.44 – 0.39 (2H, m, H9), 0.28 – 0.25 (2H, m, H9); **^{13}C NMR (125 MHz, DMSO-d_6)**: δ 162.1 (C-6), 157.6 (C-4 and C-2'), 156.9 (C-2), 134.1 (C-9'), 131.9 (C-5'), 130.8 (C-7'), 129.6 (C-4'), 129.3 (C-10'), 127.0 (C-8'), 120.0 (C-3'), 118.7 (C-6'), 107.3 (C-1'), 80.9 (C-10), 79.0 (C-11), 78.9 (C-5), 68.7 (C-1''), 67.8 (C-2''), 66.2 (C-3''), 45.0 (C-7), 15.6 (C-4''), 12.0 (C-8), 3.8 (C-9); **IR (ν_{max}/cm^{-1})**: 3481, 3430 (N-H); 3061 (=C-H); 2188 (C≡C); 1574 (C=C); 1115 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{24}H_{27}N_4O$: 403.2136, found: $[M + H]^+$ 403.2116.

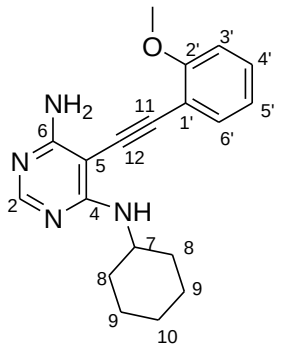
7.16.1.5 Synthesis of 5-{2-[2-(2-ethoxyethoxy)naphthalen-6-yl]ethynyl}-N^4-benzylpyrimidine-4,6-diamine 110e



To a mixture of N^4-benzyl-5-iodopyrimidine-4,6-diamine **109b** (100 mg, 0.307 mmol), copper(I) iodide (2.92 mg, 0.0153 mmol), and $Pd(PPh_3)_2Cl_2$ (10.8 mg, 0.0153 mmol) and compound **133c** (0.147 g, 0.613 mmol) under nitrogen atmosphere, was added a degassed solution of diisopropylamine (0.434 ml, 3.07 mmol) and DMF (10.0 ml). The product **110e** was isolated as brown oil (0.087g, 65%) after purification by column chromatography (80% EtOAc/hexane).

R_f (80% EtOAc/hexane) 0.45. **^{1}H NMR (500 MHz, DMSO-d_6)**: δ 8.21 (1H, d, *J* = 1.4 Hz, H5'), 7.78 (3H, m, H2, H4' and H8'), 7.35 (1H, d, *J* = 2.5 Hz, H1'), 7.31 – 7.29 (5H, m, Ar-H), 7.29 – 7.19 (2H, m, H7' and H3'), 6.66 (2H, s, NH_2), 4.67 (2H, d, *J* = 6.3 Hz, H7), 4.23 – 4.21 (2H, m, H1''), 3.77 – 3.76 (2H, m, H2''), 3.53 (2H, q, *J* = 7.0 Hz, H3''), 1.14 (3H, t, *J* = 7.0 Hz, H4''); **^{13}C NMR (125 MHz, DMSO-d_6)**: δ 162.1 (C-6), 157.5 (C-4 and C-2'), 156.8 (C-2), 141.2 (C-8), 134.0 (C-9'), 131.9 (C-5'), 130.8 (C-7'), 129.6 (C-4'), 129.4 (C-10), 129.3 (C-9), 128.6 (C-10'), 126.9 (C-8' and C-11), 119.9 (C-3'), 118.8 (C-6'), 107.4 (C-1'), 101.6 (C-5), 80.9 (C-13), 79.1 (C-14), 68.7 (C-1''), 67.8 (C-2''), 66.2 (C-3''), 43.9 (C-7), 15.6 (C-4''); **IR (ν_{max}/cm^{-1})**: 3476 (N-H); 2972 (=C-H); 2116 (C≡C); 1571 (C=C); 1056 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{27}H_{27}N_4O_2$: 439.2136, found: $[M + H]^+$ 439.2115.

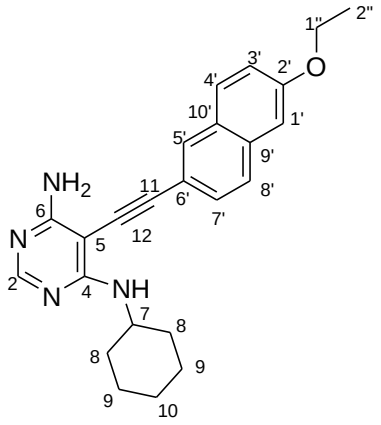
7.16.1.6 Synthesis of N^4-cyclohexyl-5-[2-(2-methoxyphenyl)ethynyl]pyrimidine-4,6-diamine 110f



Reaction of N^4-cyclohexyl-5-iodopyrimidine-4,6-diamine **109c** (110 mg, 0.346 mmol), copper(I) iodide (3.29 mg, 0.0173 mmol), $Pd(PPh_3)_2Cl_2$ (12.1 mg, 0.0173 mmol) and 1-ethynyl-2-methoxybenzene (0.0914 g, 0.691 mmol) as described afforded, after extraction and column chromatography using 50% EtOAc/hexane as eluent, **110f** (0.12 g, 74%) as a brown oil.

R_f (50% EtOAc/hexane) 0.38. **^{1}H NMR (300 MHz, MeOD)**: δ 6.75 – 6.60 (2H, m, H2 and H6'), 6.59 – 6.49 (1H, m, H4'), 6.26 (1H, d, *J* = 8.3 Hz, H3'), 6.16 (1H, td, *J* = 7.5, 0.8 Hz, H5'), 3.15 (3H, s, OMe), 1.30 – 0.83 (6H, m, cyclohexyl-H), 0.64 – 0.45 (5H, m, cyclohexyl-H); **^{13}C NMR (75 MHz, MeOD)**: δ 165.2 (C-6), 161.1 (C-2'), 159.1 (C-4), 157.1 (C-2), 133.6 (C-6'), 132.8 (C-4'), 130.0 (C-5'), 112.5, (C-3'), 111.5 (C-1'), 80.9 (C-11), 79.0 (C-5), 78.9 (C-12), 56.3 (OMe), 49.2 (C-7), 33.4 (C-8), 25.7 (C-10), 25.1 (C-9); **IR (ν_{max}/cm^{-1})**: 3334, 3310 (N-H); 2960 (=C-H); 2116 (C≡C); 1567 (C=C); 1090 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{19}H_{23}N_4O$: 323.1874, found: $[M + H]^+$ 323.1873.

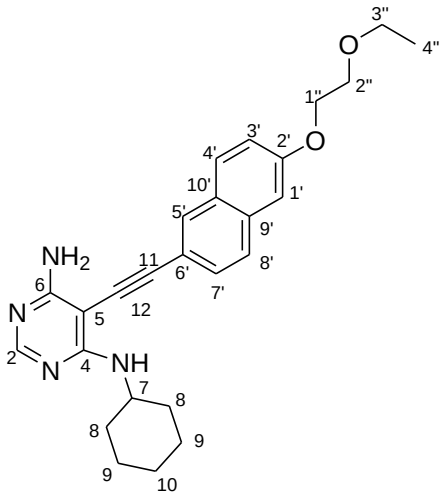
7.16.1.7 Synthesis of N^4-cyclohexyl-5-[2-(2-ethoxynaphthalen-6-yl)ethynyl]pyrimidine-4,6-diamine 110g



Reaction of N^4-cyclohexyl-5-iodopyrimidine-4,6-diamine **109c** (200 mg, 0.629 mmol), copper(I) iodide (5.99 mg, 0.0314 mmol), $Pd(PPh_3)_2Cl_2$ (22.1 mg, 0.0314 mmol) and compound **133b** (0.247 g, 1.26 mmol) as described afforded, after extraction and column chromatography using 50% EtOAc/hexane as eluent, **110g** (0.16 g, 64%) as a brown oil.

R_f (50% EtOAc/hexane) 0.38. **^{1}H NMR (300 MHz, DMSO-d_6)**: δ 8.17 (1H, d, *J* = 1.6 Hz, H5'), 7.95 (1H, s, H2), 7.80 (2H, d, *J* = 8.9 Hz, H4' and H8'), 7.71 (1H, dd, *J* = 8.4, 1.6 Hz, H7'), 7.32 (1H, d, *J* = 2.5 Hz, H1'), 7.19 (1H, dd, *J* = 8.9, 2.5 Hz, H3'), 6.62 (2H, s, NH_2), 6.02 (1H, d, *J* = 8.3 Hz, NH), 4.15 (2H, q, *J* = 7.0 Hz, H1''), 3.98 – 3.94 (1H, m, H7), 1.87 (2H, dd, *J* = 8.7, 4.7 Hz, cyclohexyl-H), 1.74 – 1.58 (3H, m, cyclohexyl-H), 1.46 – 1.17 (8H, m, cyclohexyl-H and H2''); **^{13}C NMR (125 MHz, DMSO-d_6)**: δ 163.0 (C-6), 160.7 (C-4), 157.8 (C-2'), 156.4 (C-2), 133.6 (C-9'), 130.3 (C-5'), 129.1 (C-10'), 128.8 (C-4'), 126.6 (C-8'), 119.5 (C-3'), 117.9 (C-6'), 106.2 (C-1'), 80.9 (C-11), 79.0 (C-5), 78.9 (C-12), 63.2 (C-1''), 49.0 (C-7), 32.5 (C-8), 25.2 (C-10), 24.9 (C-9), 14.6 (C-2''); **IR (ν_{max}/cm^{-1})**: 3290, 3285 (N-H); 2960 (=C-H); 2196 (C≡C); 1560 (C=C); 1210 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{24}H_{27}N_4O$: 387.2187, found: $[M + H]^+$ 387.2177.

7.16.1.8 Synthesis of 5-{2-[2-(2-ethoxyethoxy)naphthalen-6-yl]ethynyl}-N^4-cyclohexylpyrimidine-4,6-diamine 110h

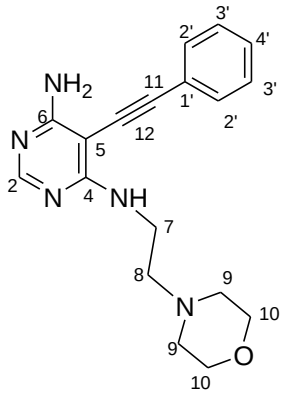


Reaction of N^4-cyclohexyl-5-iodopyrimidine-4,6-diamine **109c** (160 mg, 0.503 mmol), copper(I) iodide (4.79 mg, 0.0251 mmol), $Pd(PPh_3)_2Cl_2$ (17.6 mg, 0.0251 mmol) and compound **133c** (0.242 g, 1.01 mmol) as described afforded, after extraction and column chromatography using 50% EtOAc/hexane as eluent, **110h** (0.13 g, 61%) as a light brown oil.

R_f (50% EtOAc/hexane) 0.38. **^{1}H NMR (300 MHz, DMSO-d_6)**: δ 8.18 – 8.17 (1H, m, H5'), 7.84 – 7.79 (2H, m, H2 and H4'), 7.72 (1H, dd, *J* = 8.5, 1.6 Hz, H7'), 7.63 – 7.57 (1H, m, H8'), 7.36 (1H, d, *J* = 2.5 Hz, H1'), 7.22 (1H, dd, *J* = 9.0, 2.5 Hz, H3'), 6.62 (2H, s, NH_2), 6.03 (1H, d, *J* = 8.3 Hz, NH), 4.24 – 4.21 (2H, m, H1"), 4.00 – 3.95 (1H, m, H7), 3.78 – 3.75 (2H, m, H2"), 3.53 (2H, q, *J* = 7.0 Hz, H3"), 1.88 (2H, d, *J* = 10.7 Hz, cyclohexyl-H), 1.75 – 1.59 (3H, m, cyclohexyl-H), 1.43 – 1.34 (3H, m, cyclohexyl-H), 1.20 – 1.12 (5H, m, cyclohexyl-H and H4"); **^{13}C NMR (125 MHz, DMSO-d_6)**: δ 163.0 (C-6), 160.7 (C-4), 157.0 (C-2'), 156.4 (C-2), 133.6 (C-9'), 130.3 (C-5'), 129.2 (C-10'), 128.8 (C-4'), 128.7 (C-8'), 119.4 (C-3'), 118.1 (C-6'), 106.9 (C-1'), 80.9 (C-11), 79.0 (C-5), 78.9 (C-12), 68.2 (C-2"), 67.3 (C-1"), 65.6 (C-3"), 49.0 (C-7), 32.5 (C-8), 25.2 (C-10), 24.9 (C-9), 15.1 (C-4"); **IR (ν_{max}/cm^{-1})**: 3331, 3305 (N-H); 2965 (=C-H); 2190 (C≡C); 1560 (C=C); 1230 (C-O). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{26}H_{31}N_4O_2$: 431.2449, found: $[M + H]^+$ 431.2445.

7.16.1.9 Synthesis of N^4-(2-morpholinoethyl)-5-(2-phenylethynyl)pyrimidine-4,6-diamine

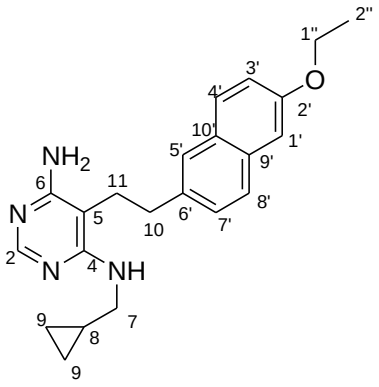
110i



Reaction of 5-iodo-N^4-(2-morpholinoethyl)pyrimidine-4,6-diamine **109d** (100 mg, 0.286 mmol), copper (I) iodide (2.73 mg, 0.0143 mmol), $Pd(PPh_3)_2Cl_2$ (10.1 mg, 0.0143 mmol) and phenylacetylene (0.0630 ml, 0.573 mmol) as described afforded, after extraction and column chromatography using 10% MeOH/ethyl acetate as eluent, **110i** (0.073 g, 79%) as a brown solid.

R_f (10% MeOH/ethyl acetate) 0.20. *mp*: 205-207 °C. **^{1}H NMR (300 MHz, DMSO-d_6)**: δ 7.94 (1H, s, H2), 7.69 – 7.66 (2H, m, H2'), 7.44 – 7.35 (3H, m, H3' and H4'), 6.62 (2H, s, NH_2), 6.48 (1H, t, *J* = 5.4 Hz, NH), 3.56 (4H, t, *J* = 4.6 Hz, H10), 3.49 (2H, d, *J* = 5.9 Hz, H7), 2.49 – 2.40 (6H, m, H8 and H9); **^{13}C NMR (75 MHz, DMSO-d_6)**: δ 162.4 (C-6), 160.8 (C-4), 156.3 (C-2), 132.2 (C-2'), 128.4 (C-4'), 128.3 (C-3'), 122.7 (C-1'), 80.9 (C-11), 79.0 (C-5), 78.9 (C-12), 63.5 (C-10), 56.2 (C-8), 51.5 (C-9), 36.1 (C-7); **IR (ν_{max}/cm^{-1})**: 3334, 3310 (N-H); 2976 (=C-H); 2190 (C≡C); 1560 (C=C). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{18}H_{22}N_5O$: 324.1826, found: $[M + H]^+$ 324.1824.

7.16.1.10 Synthesis of N^4-(cyclopropylmethyl)-5-[2-(2-ethoxynaphthalen-6-yl)ethynyl]pyrimidine-4,6-diamine 134



N^4-(cyclopropylmethyl)-5-[2-(2-ethoxynaphthalen-6-yl)ethynyl]pyrimidine-4,6-diamine **110a** (180 mg, 0.502 mmol) was dissolved in ethanol (10 ml) before adding 10% wet Pd/C (17.6 mg, 33 mole %). Oxygen was removed from this system by vacuum before introducing hydrogen gas from a balloon; this step was repeated 3 times before the mixture was left to stir at room temperature for ~18 hours. The reaction was stopped upon completion and the Pd/C was filtered over a bed of celite followed by the evaporation of the solvent *in vacuo*. The product **134** (0.128 mg, 70%) was purified by preparative TLC (50% EtOAc/hexane), and was isolated as a light yellow oil.

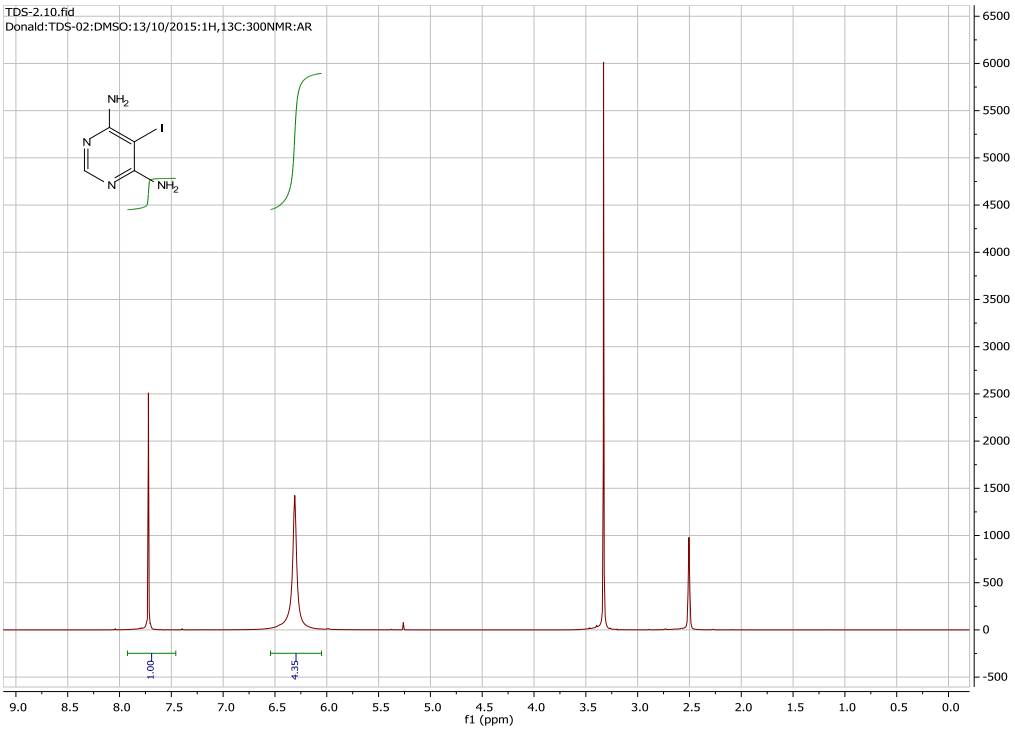
R_f (50% EtOAc/hexane) 0.22. **^{1}H NMR (300 MHz, $CDCl_3$)**: δ 8.09 (1H, s, H5'), 7.67 – 7.61 (2H, m, H4' and H8'), 7.53 (1H, s, H2), 7.22 (1H, dd, *J* = 8.4, 1.7 Hz, H7'), 7.13 (1H, dd, *J* = 8.9, 2.5 Hz, H3'), 7.09 (1H, d, *J* = 2.3 Hz, H1'), 4.37 (2H, s, NH_2), 4.22 (1H, d, *J* = 4.5 Hz, NH), 4.14 (2H, q, *J* = 7.0 Hz, H1"), 3.07 (2H, dd, *J* = 7.0, 5.1 Hz, H7), 2.96 (2H, t, *J* = 7.1 Hz, H10), 2.66 (2H, t, *J* = 7.1 Hz, H11), 1.48 (3H, t, *J* = 7.0 Hz, H2"), 0.82 – 0.71 (1H, m, H8), 0.41 – 0.29 (2H, m, H9), 0.20 – 0.00 (2H, m, H9); **^{13}C NMR (75 MHz, $CDCl_3$)**: δ 160.4 (C-6), 159.9 (C-4), 156.8 (C-2'), 155.6 (C-2), 136.1 (C-6'), 133.4 (C-9'), 129.1 (C-10'), 128.9 (C-4'), 127.3 (C-7'), 127.3 (C-5'), 126.4 (C-8'), 119.4 (C-3'), 106.5 (C-5), 94.8 (C-1'), 63.5 (C-1"), 46.4 (C-7), 33.0 (C-10), 26.8 (C-11), 14.8 (C-2"), 10.7 (C-8), 3.2 (C-9). ***HRMS (ES$^+$)*** *m/z*: calculated for $C_{22}H_{27}N_4O$: 363.2187, found: $[M + H]^+$ 363.2185.

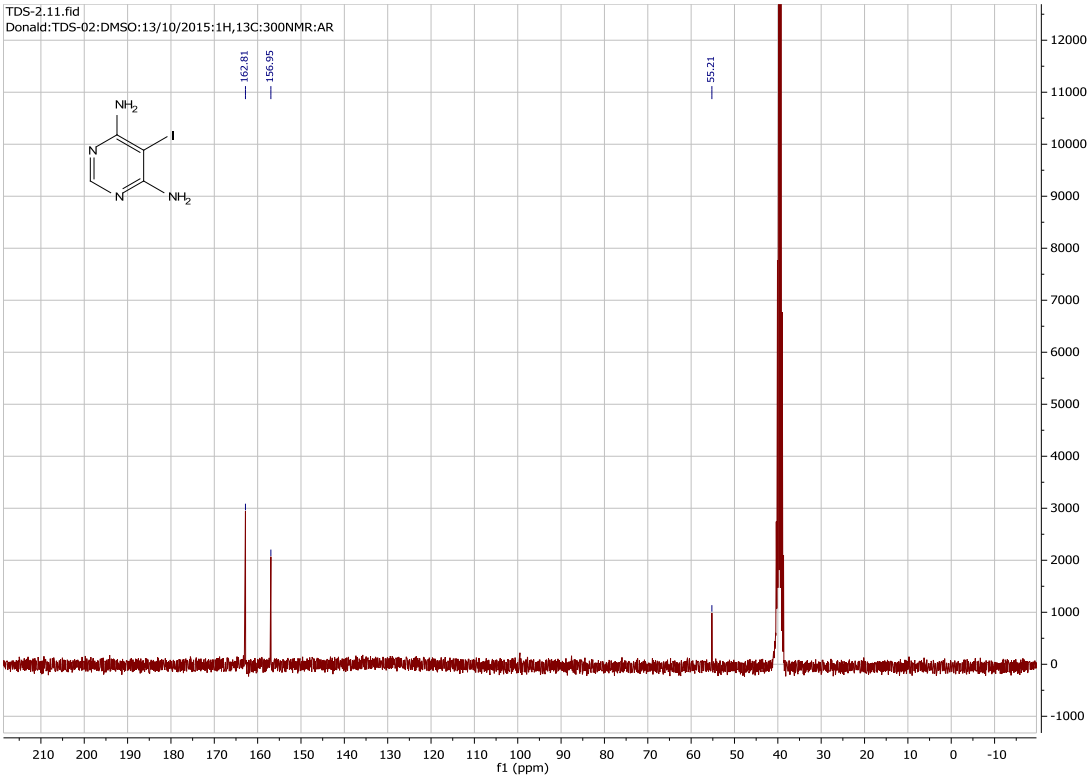
7.17 References

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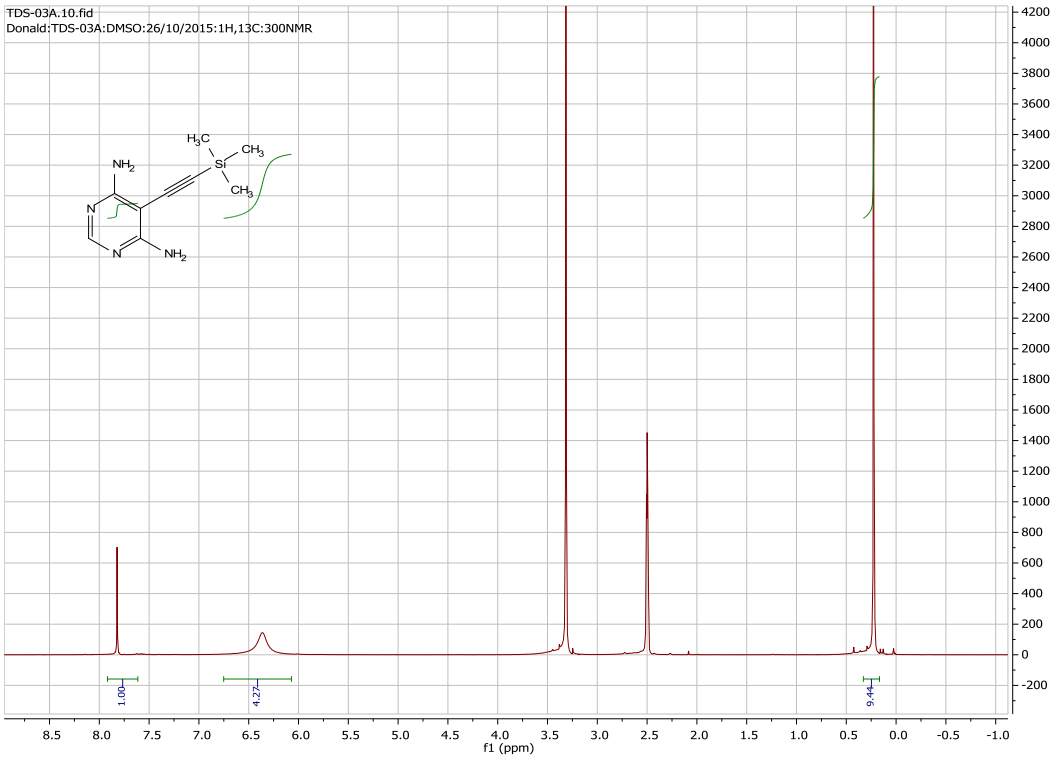
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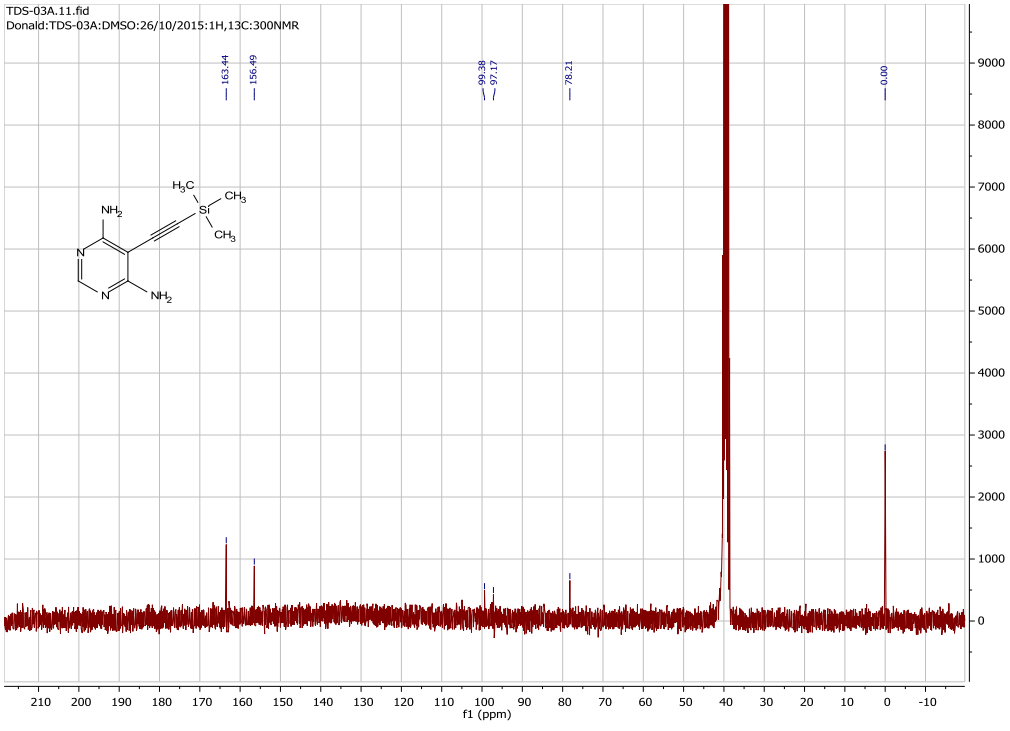
^{1}H and ^{13}C NMR spectra for Compound 99



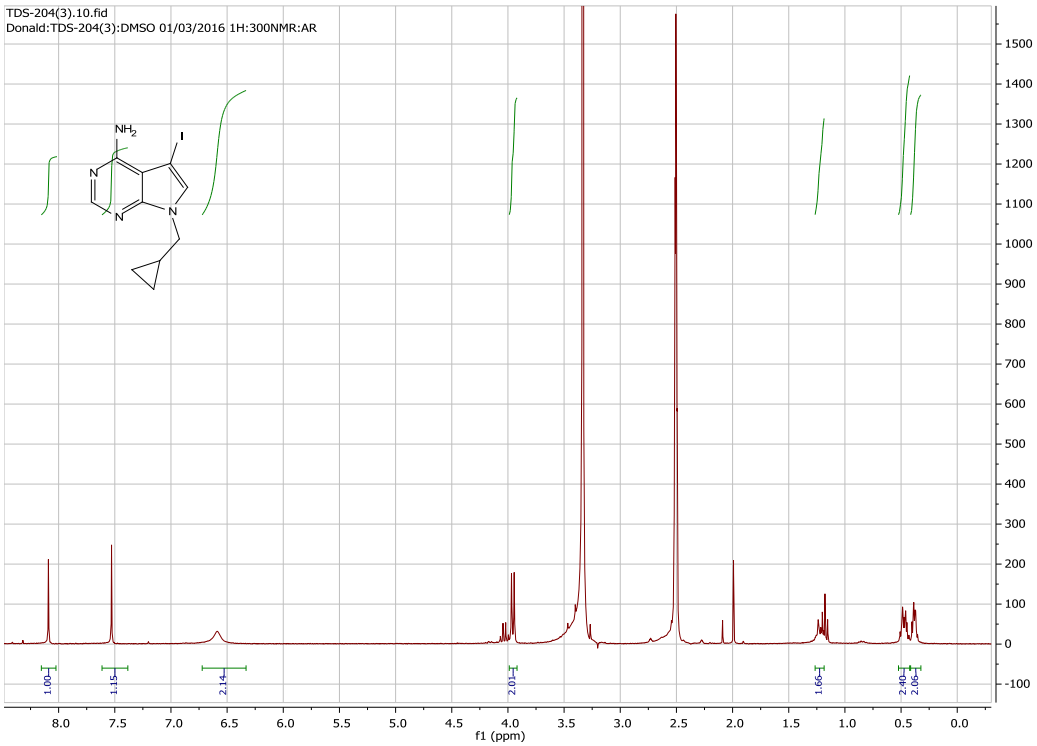


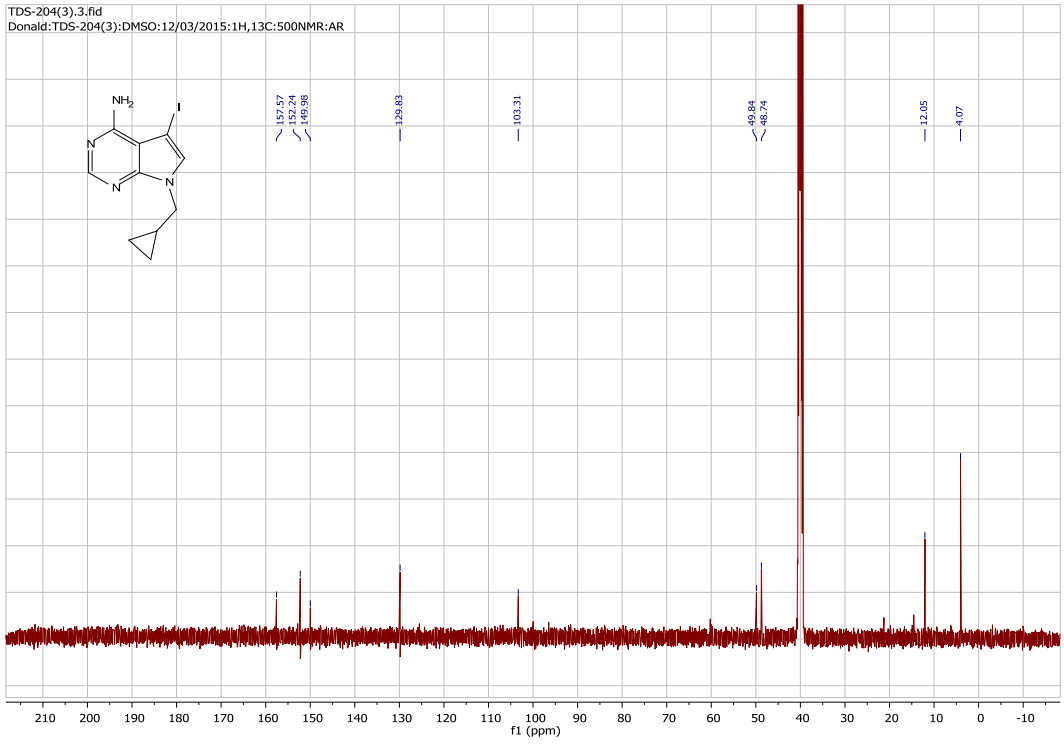
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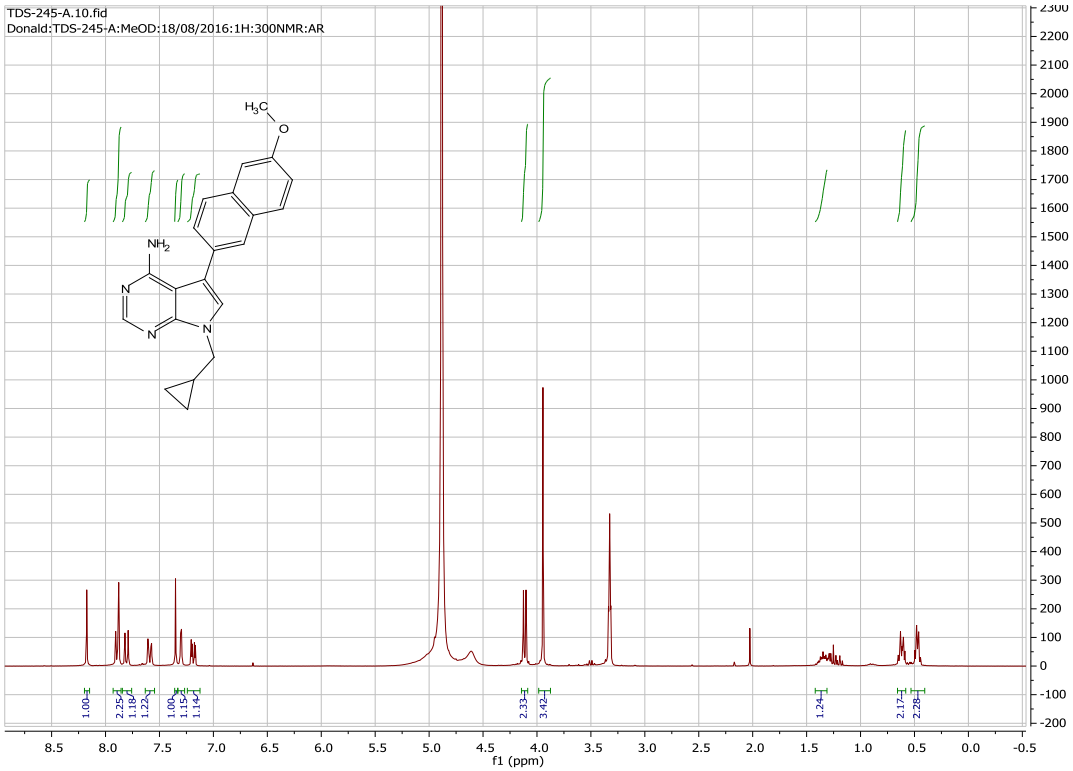


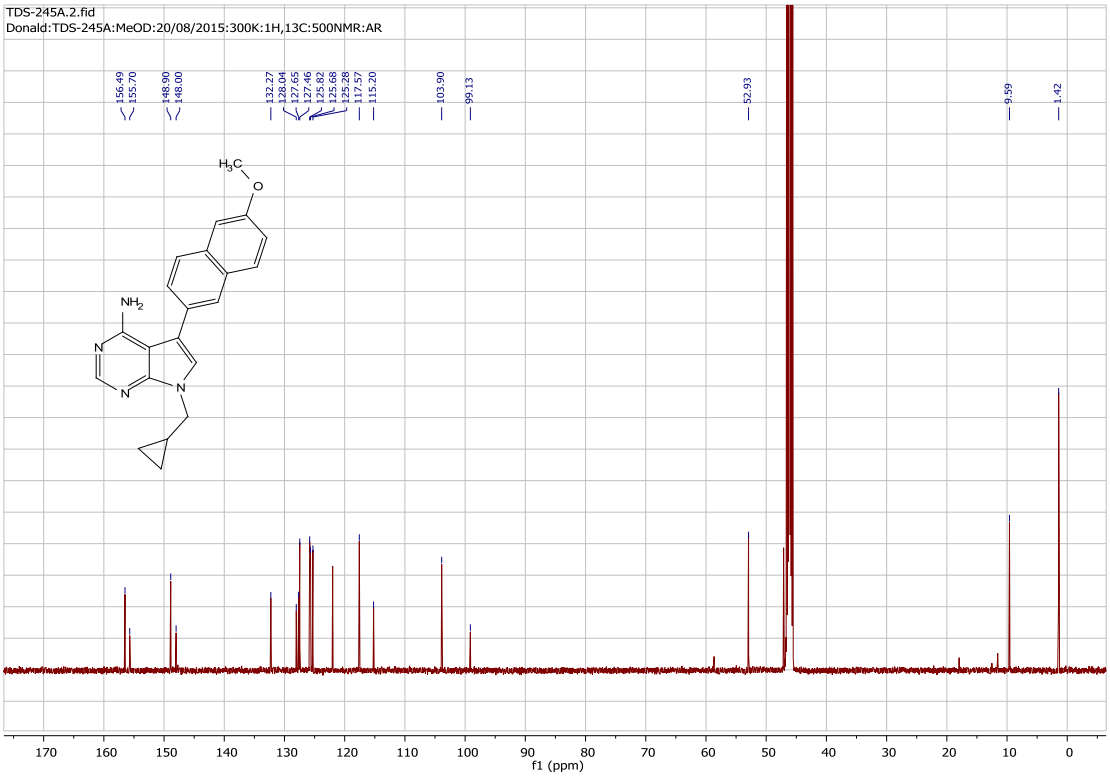
^{1}H and ^{13}C NMR spectra for Compound 104b



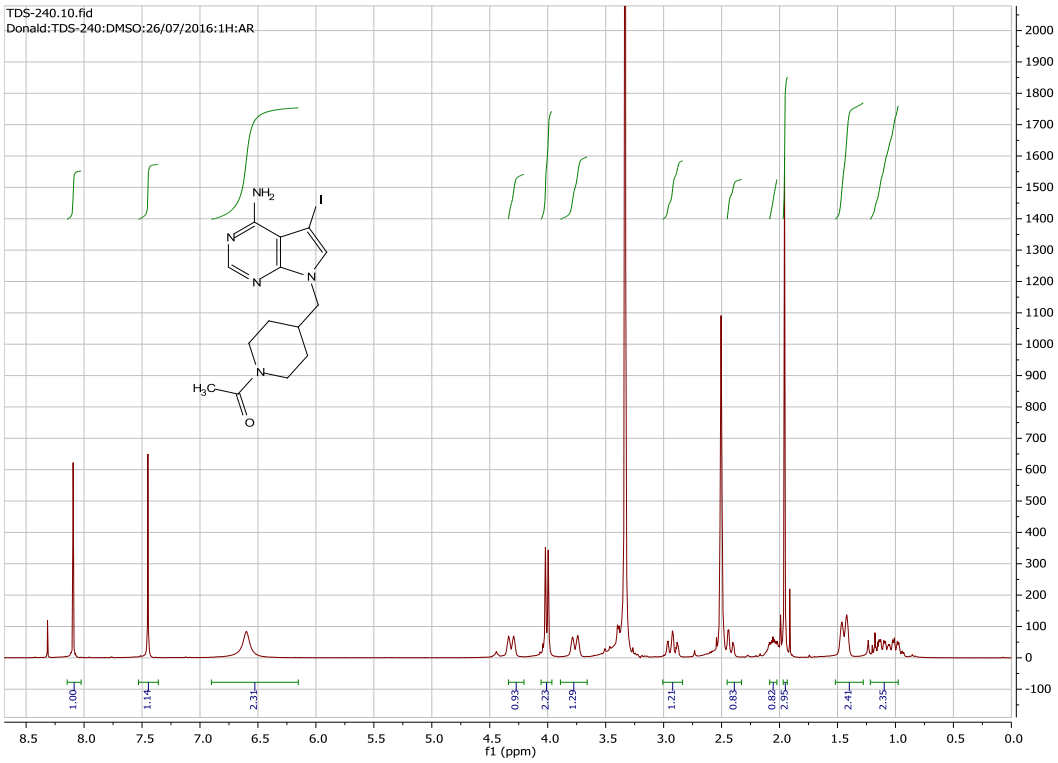


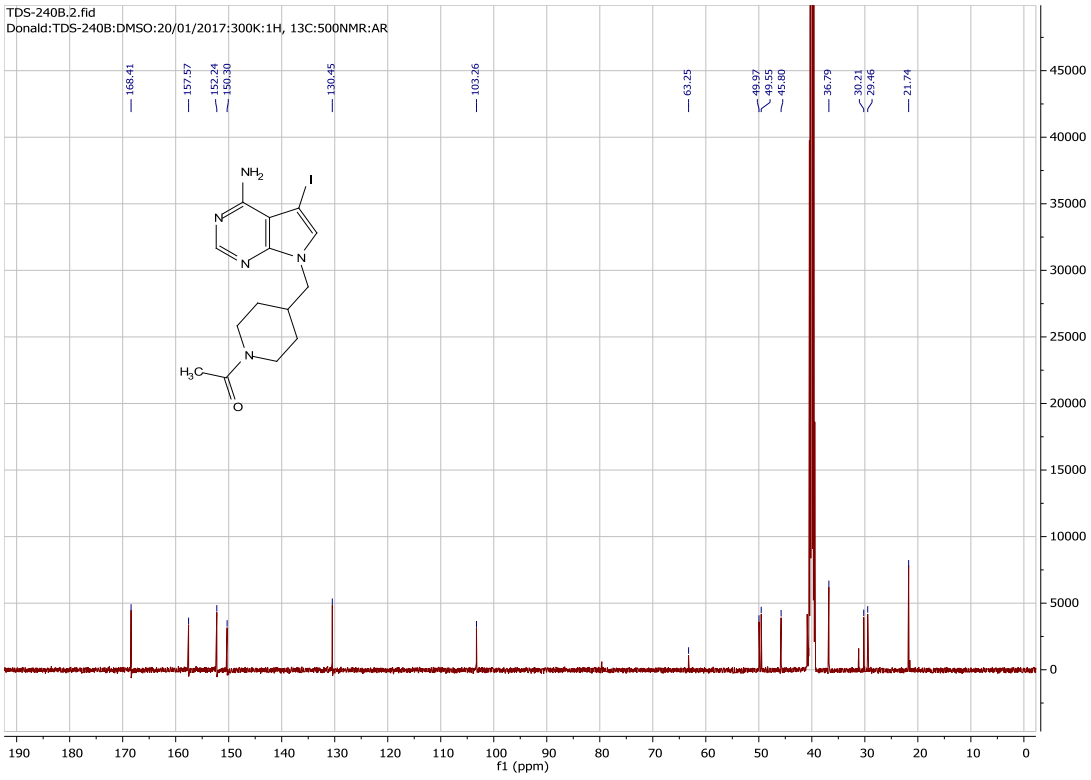
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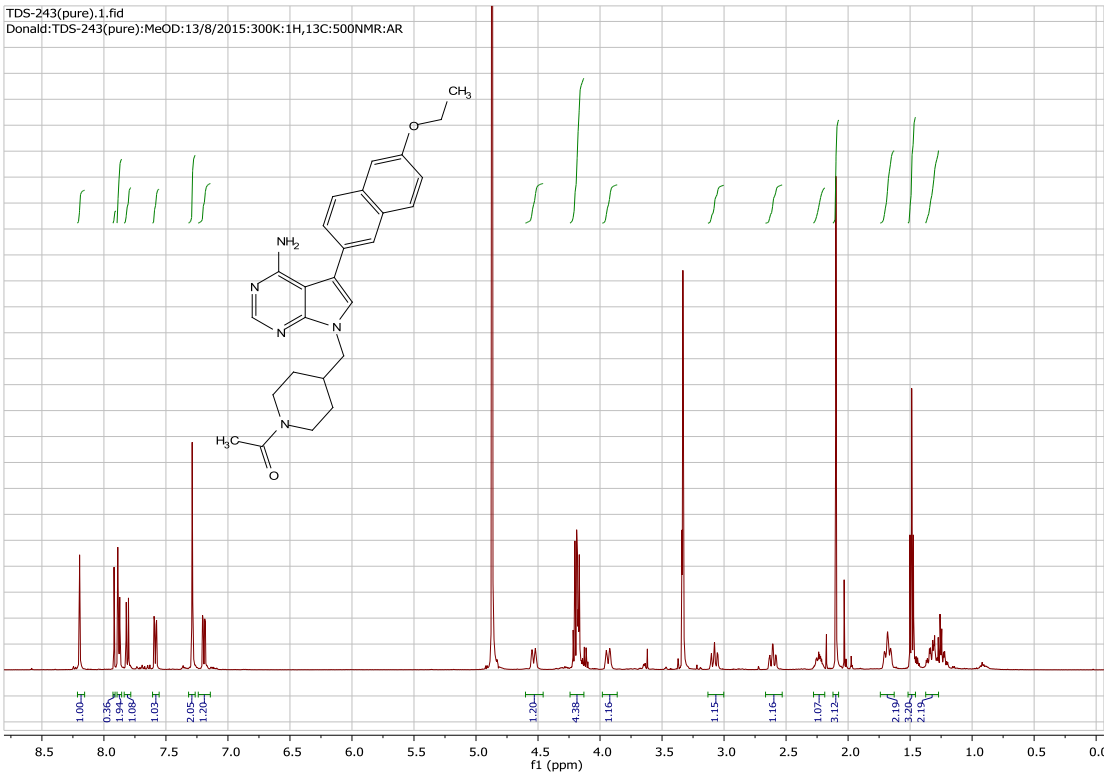


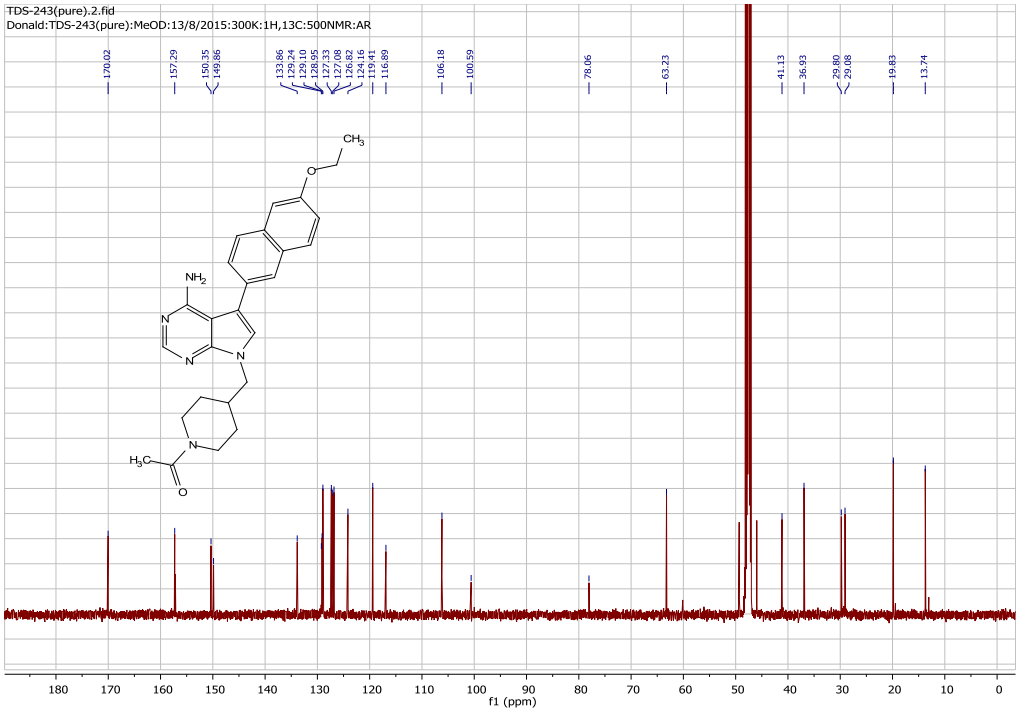
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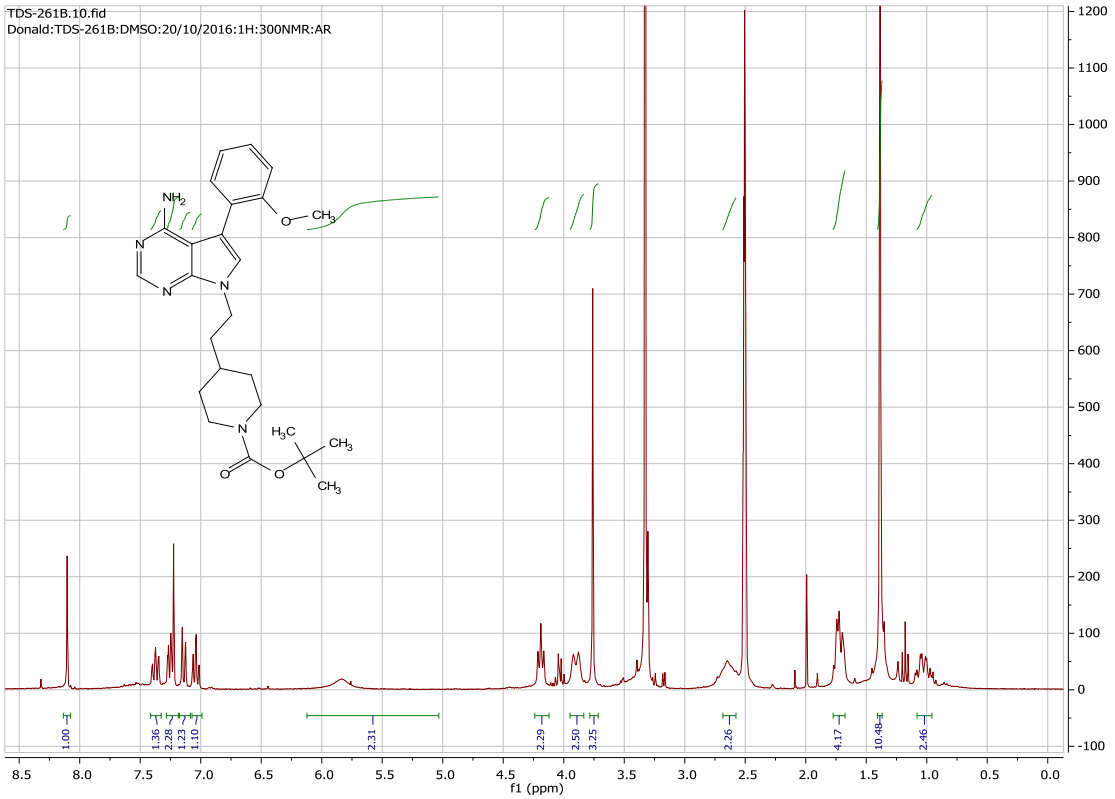


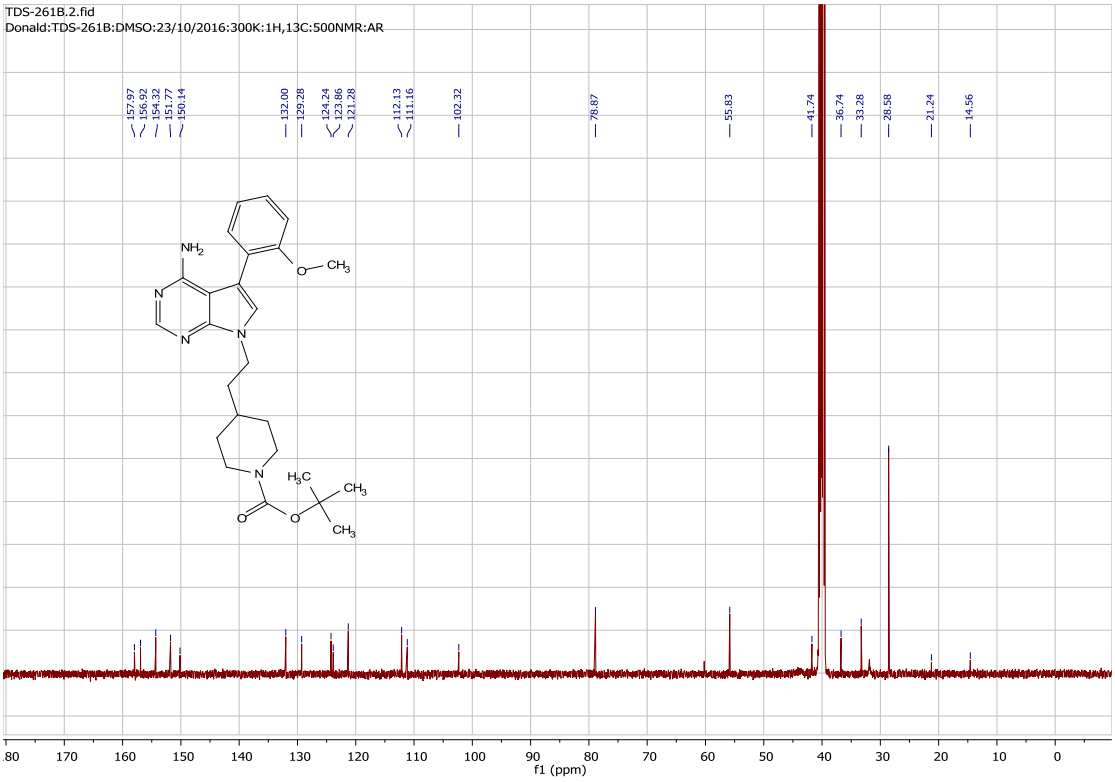
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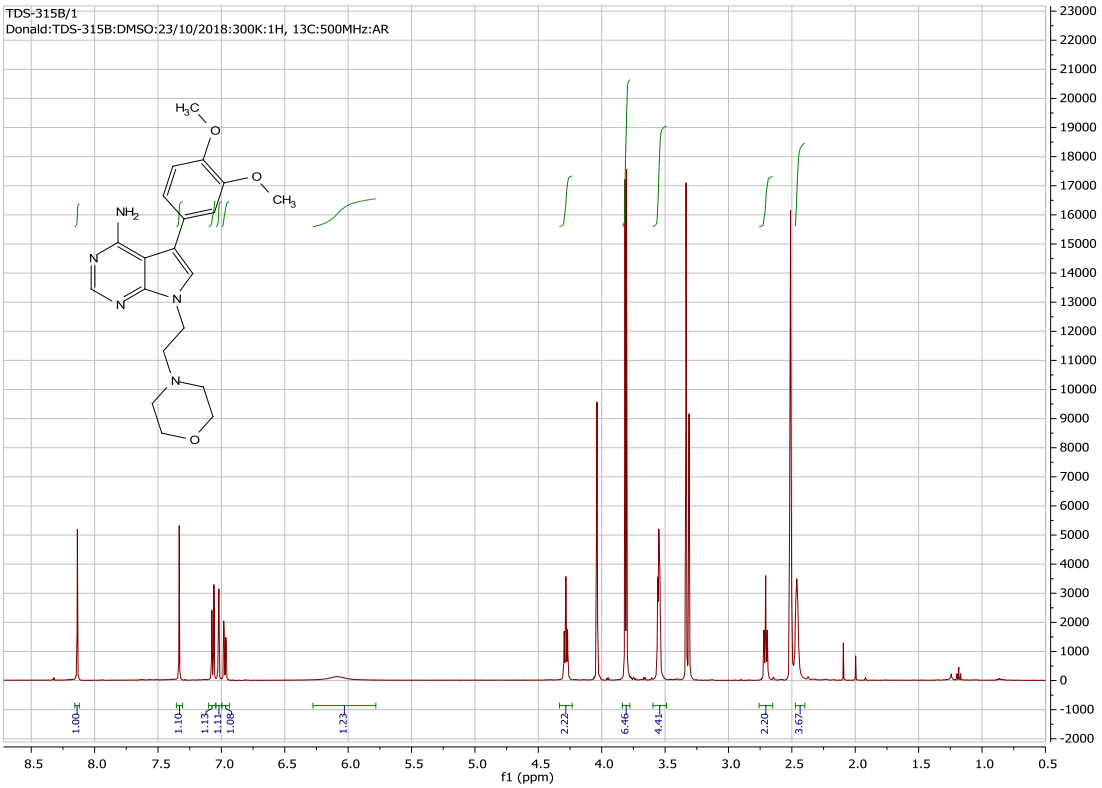


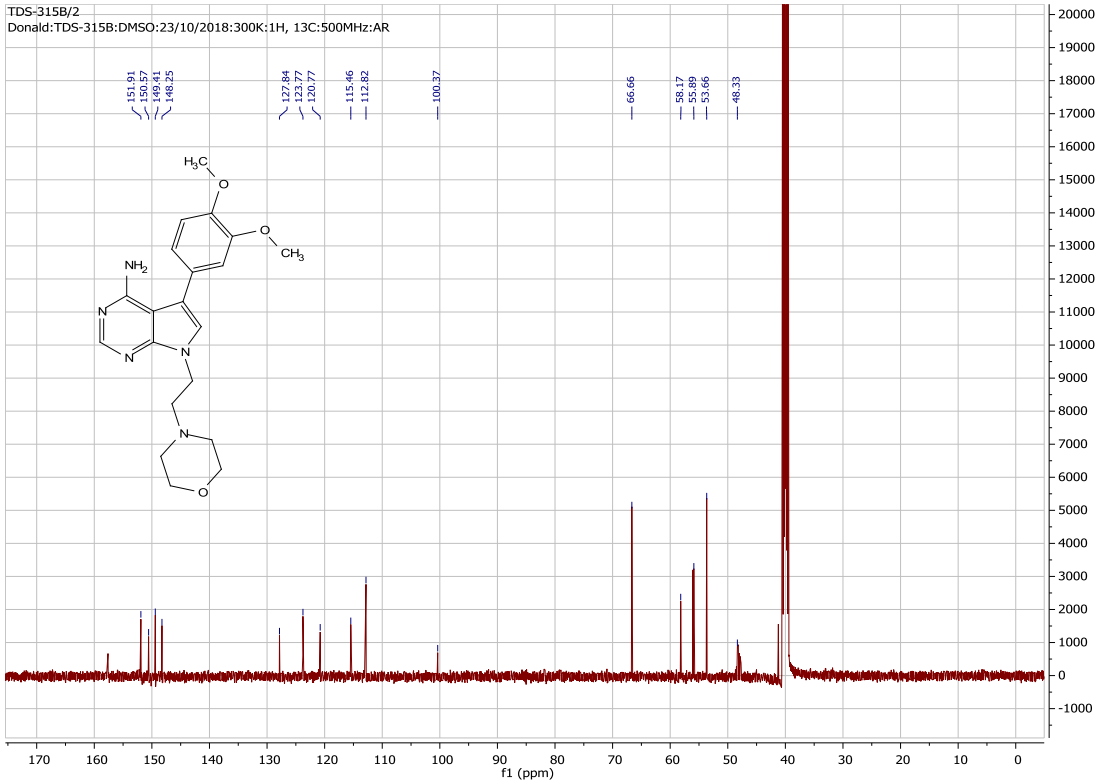
^{1}H and ^{13}C NMR spectra for Compound 130a



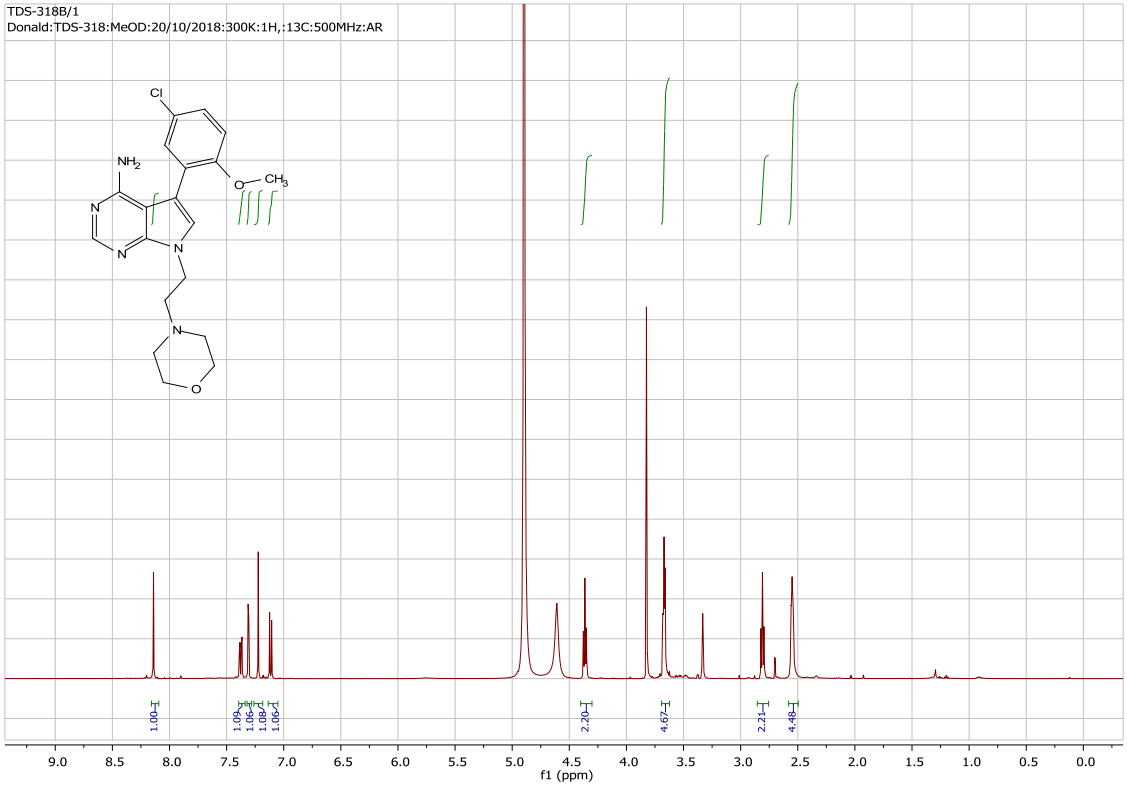


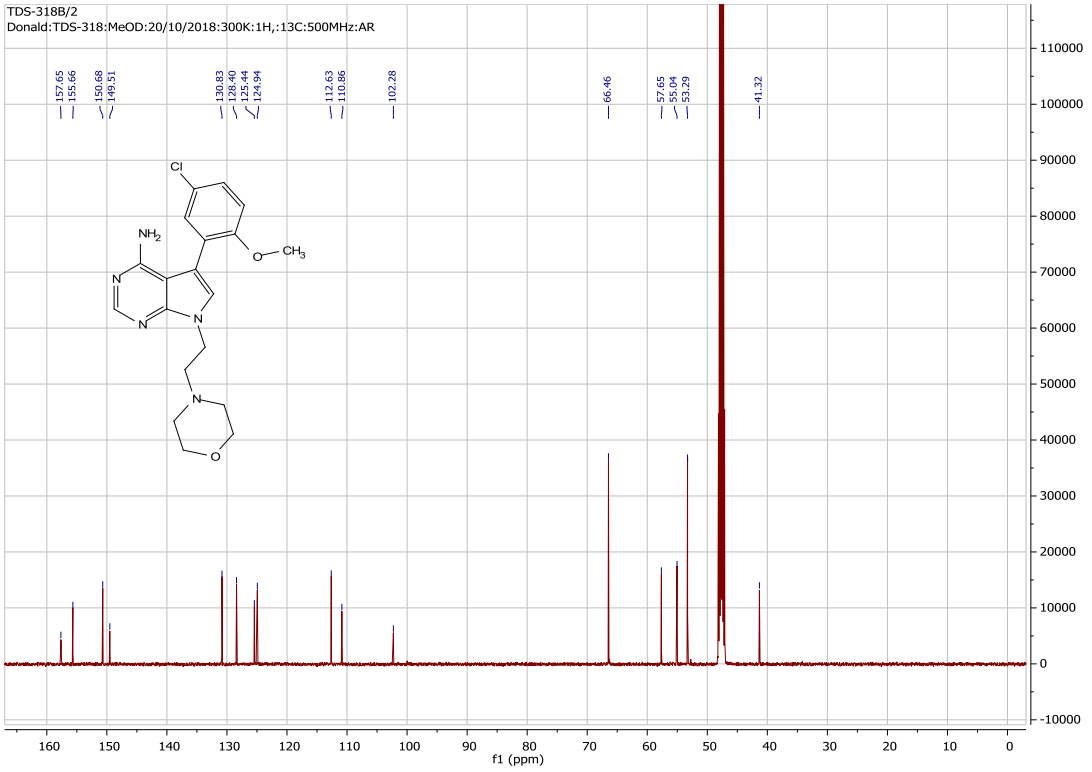
^{1}H and ^{13}C NMR spectra for Compound 131b



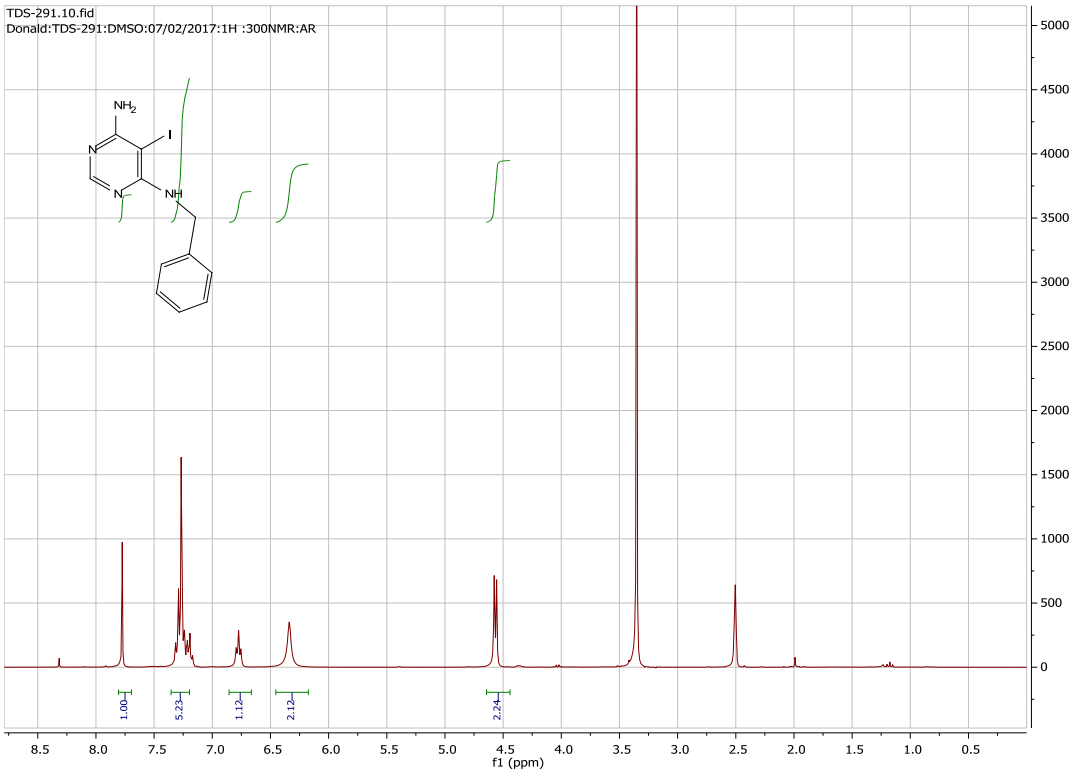


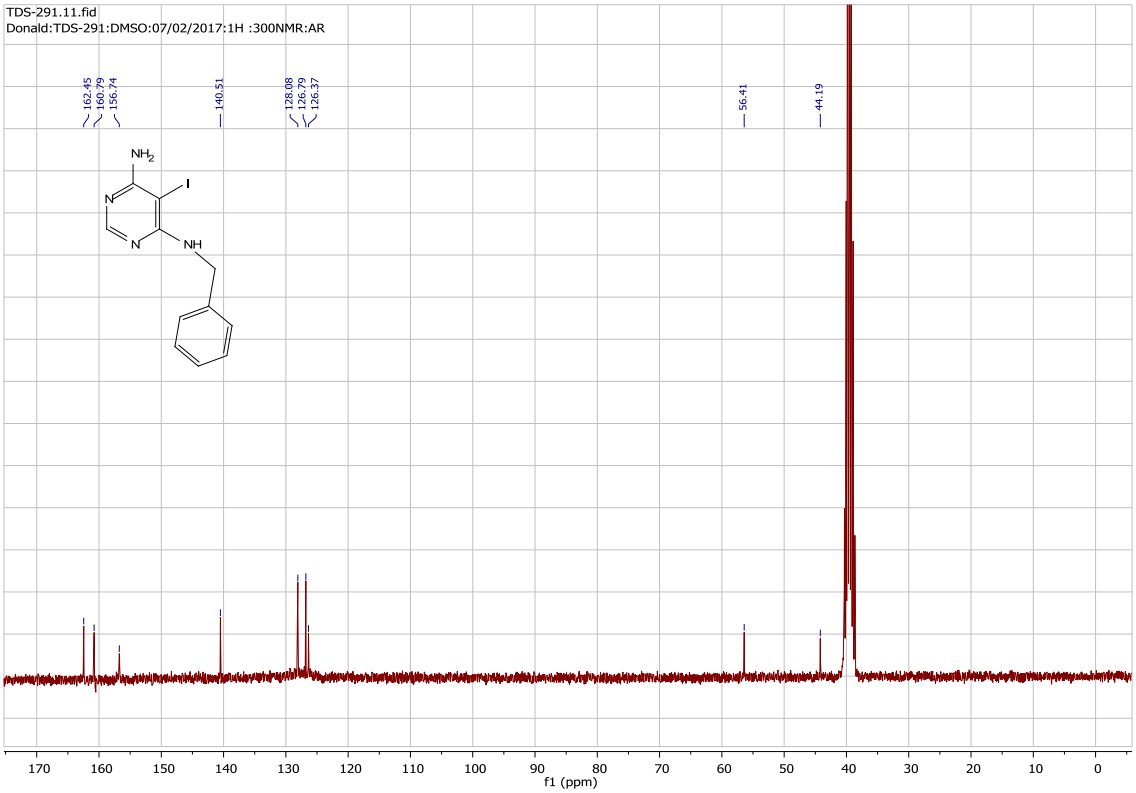
^{1}H and ^{13}C NMR spectra for Compound 131c



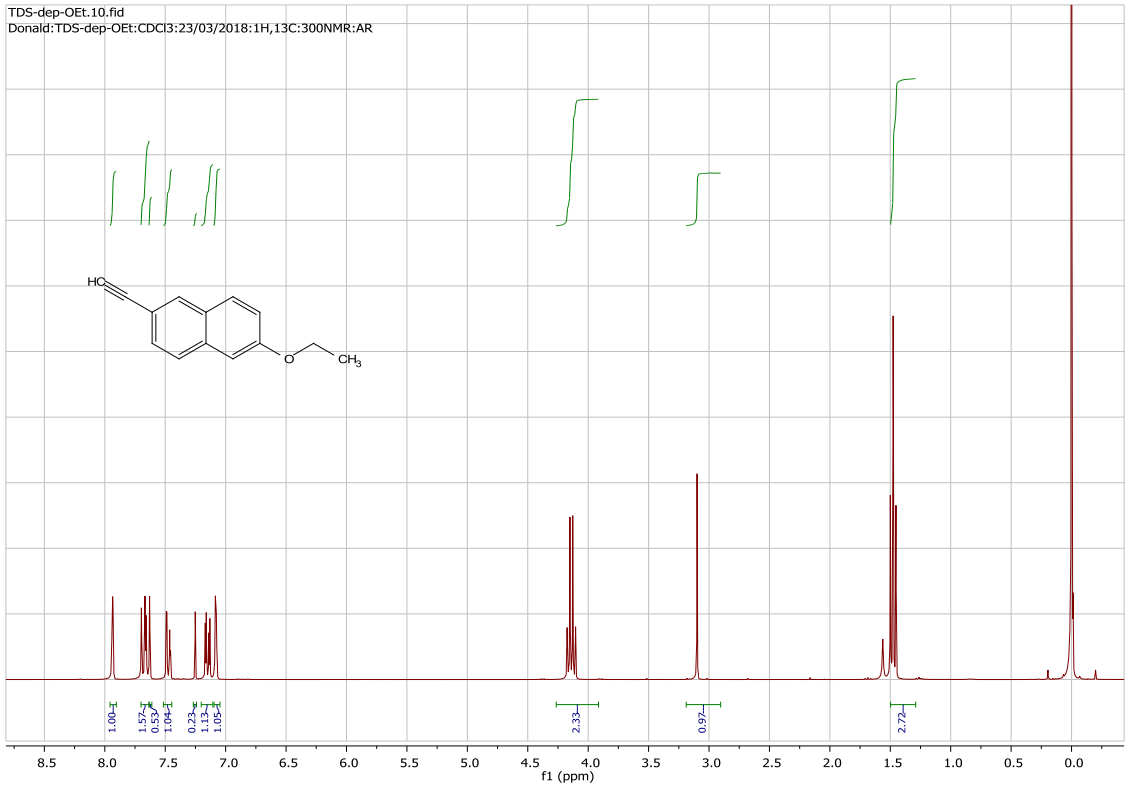


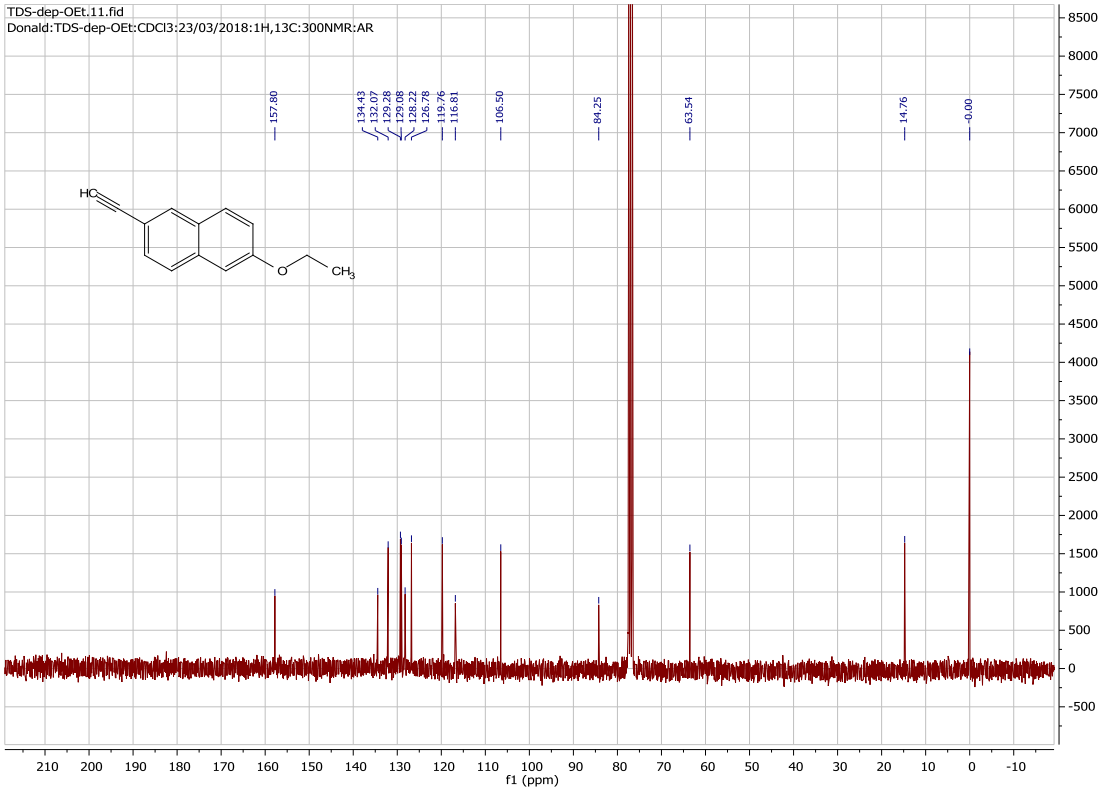
^{1}H and ^{13}C NMR spectra for Compound 109b



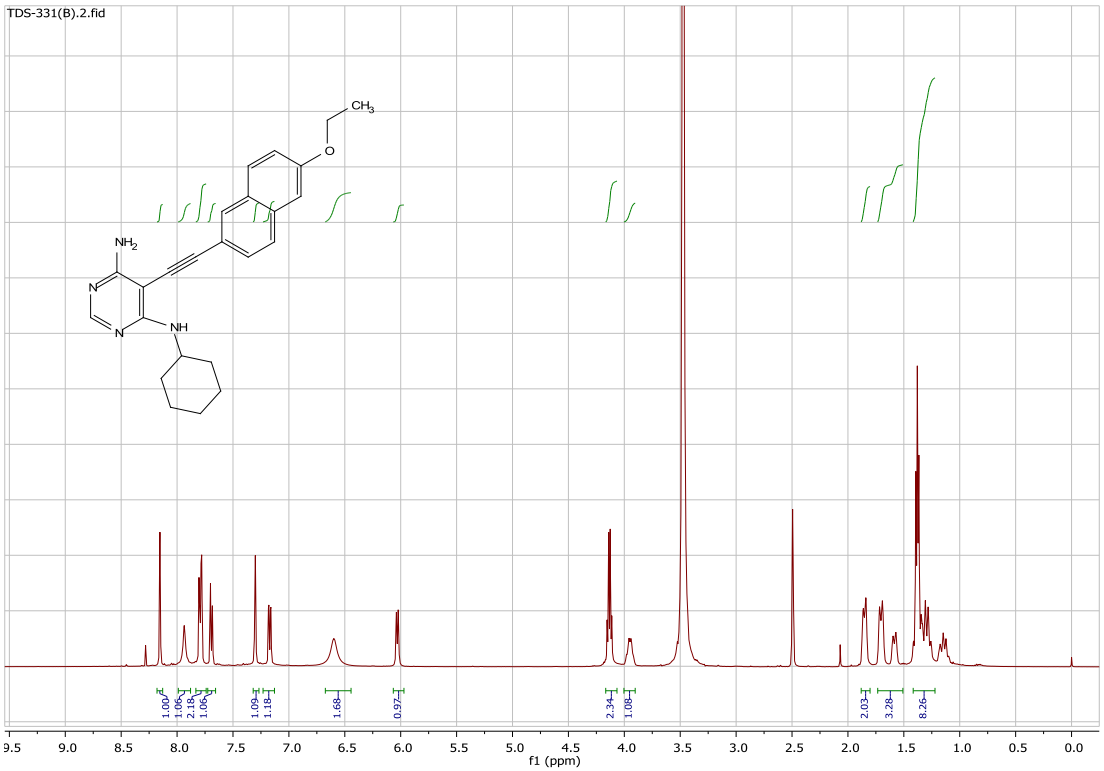


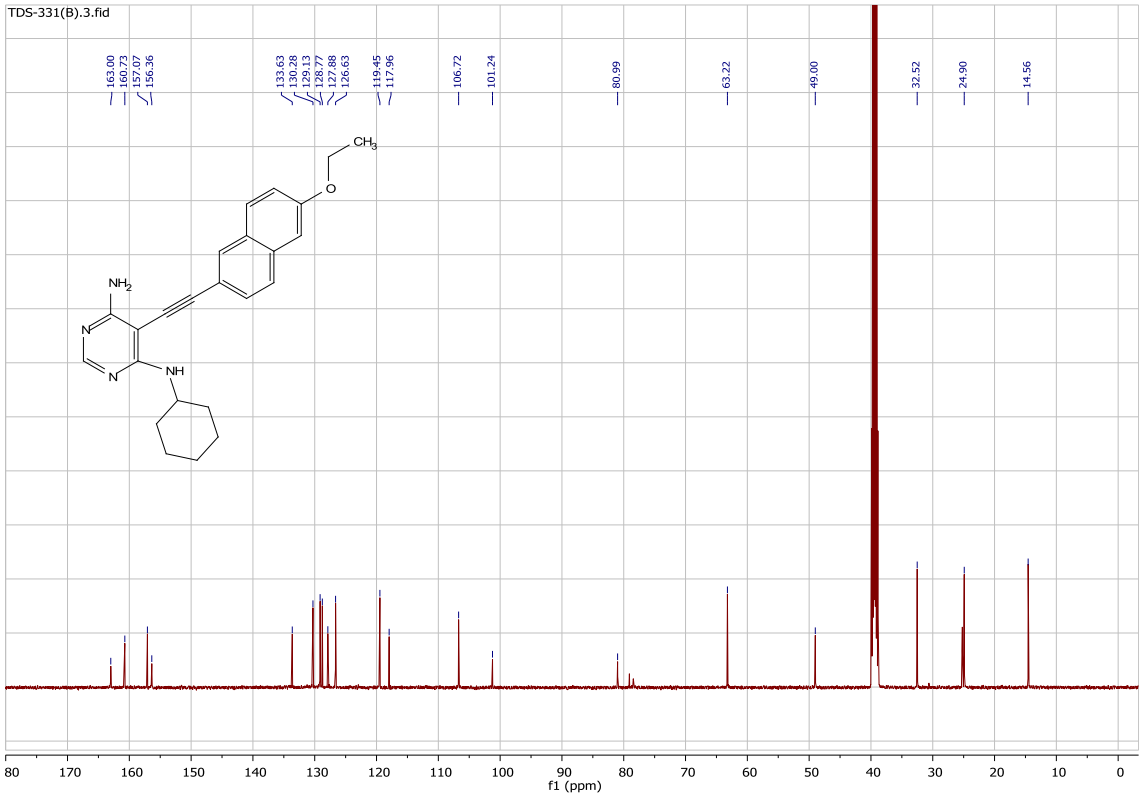
^{1}H and ^{13}C NMR spectra for Compound 133b





^{1}H and ^{13}C NMR spectra for Compound 110g





^{1}H and ^{13}C NMR spectra for Compound 134

