Open Source Drug Discovery: Multiple Step Synthesis and Evaluation of Antimalarial Drug Candidate OSM-S-106

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Preface

i. Statement of Academic Integrity

The idea of using open source science for antimalarial drug development was conceived by Dr. Matthew Todd. The antimalarial drug candidate, OSM-S-106, at focus in this dissertation, was identified by the Medicines for Malaria Venture as being a useful drug candidate. The hits considered by the Medicines for Malaria Venture were identified from a high throughput screen performed by GlaxoSmithKline, Tres Cantos.

Data for melting points, retention factors and infrared spectra was acquired by me. Nuclear magnetic resonance and liquid chromatography mass spectrometry were performed by Ms Mathamsanqa Bhebhe and me.

I confirm that the work presented in this dissertation is my own. Where information has been derived from other sources, I confirm that this has been indicated in the dissertation.

_____

Signature and Date

ii. Acknowledgement

I would first like to express my thanks to Prof Matthew Todd for the chance to work in his lab, and for his supervision over the last summer in the School of Pharmacy at UCL. Throughout my time here he was always helpful, patient and approachable and showed a lot of faith in me.

A huge appreciation goes to Tha Bhebe and Dr Fahima Idiris for their help and patience from the beginning of this project, with which I have learned a great deal and have improved considerably as a chemist. Big thanks to the rest of the G25 group for their lab advice and for generally being enjoyable to work with; honorary mentions go to Paul King and Uzma Jaffry for good office and lab chats. I'd also like to thank my course mates who have helped me get through this MSc, and to my brothers, Thomas and Huw, for always acting interested in my studies.

Finally, a huge thank you to my parents, Will and Gill Parry, for always being supportive of me, for funding my move to London, and for the proofreading of this dissertation at its various stages – however tedious it may have been.

iii. Abbreviations

ACT	Artemisinin Combination Therapy
α	Alpha
Anal	Elemental Analysis
Anhyd	Anhydrous
Aq	Aqueous
β	Beta
Bu	Butyl
CA	Carbonic Anhydrase
Calcd	Calculated
cLogP	Calculated 1-Octanol/Water Coefficient
Concd	Concentrated
Da	Daltons
d	Doublet
DDT	Dichlorodiphenyltrichloroethane
DNDi	Drugs for Neglected Tropical Diseases Initiative
Dppf	1,1'-Bis(diphenylphosphino)ferrocene
dt	Doublet of Triplets
eg	Example
equiv	Equivalents
Et	Ethyl
FT	Fourier Transform
γ	Gamma

g	Gram
GSK	GlaxoSmithKline
HMBC	Heteronuclear Multiple Bond Coherence
h	Hour
IR	Infrared
J	^{1}H- ^{1}H coupling constant in Hertz
Ki	Equilibrium Constant
LCMS	Liquid Chromatography Mass Spectrometry
LDA	Lithiumdiisopropylamine
Lit	Literature Value
MP	Melting Point
m	Multiplet
M	Molar Concentration
MW	Molecular Weight
m	Meta
Me	Methyl
mg	Milligram
MHz	Megahertz
mL	Millilitre
mm	Millimetre
mmol	Millimole
mp	Melting Point
MVI	Malaria Vaccine Initiative
NA	No Activity

NBS	N-Bromosuccinimide
nm	Nanometre
nM	Nanomolar
NMR	Nuclear Magnetic Resonance
NTD	Neglected Tropical Disease
o	*Ortho*
OSM	Open Source Malaria
p	*Para*
P	*Plasmodium*
Ph	Phenyl
Ppm	Parts per Million
RBC	Red Blood Cells
rt	Room Temperature
s	Singlet
SAR	Structure Activity Relationship
SGC	The Structural Genomics Consortium
SM	Starting Material
t	Triplet
TCAMS	Tres Cantos Anti-Malarial Screen
TCOLF	Tres Cantos Open Lab Foundation
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
tPSA	Topological Polar Surface Area

UCSD	University of California San Diego
µM	Micromolar
UV	Ultra-violet
V	Volume
WHO	World Health Organisation

iv. List of Figures

Figure 1 Prevalence of severe anaemia meeting the threshold requiring blood transfusion according to WHO (<7 g/dL) in children aged under 5 years in sub-Saharan Africa, 2015–2017, by age and malaria infection status. RDT: Rapid Drug Test. Reproduced from World Health Organisation's World malaria report 2018. Licence: CC BY-NC-SA 3.0 IGO.

Figure 2 Lifecycle stages of the Malaria parasite. Reproduced from the Malaria Vaccine Initiative website at www.malariavaccine.org, 2015.

Figure 3 Drug compounds used for the treatment and prevention of Malaria (from 1-8: Quinine, Chloroquine, DDT, Proguanil, Pyrimethamine, Sulfadoxine, and Artemisinin)

Figure 4 3D plot of selected novel chemically diverse compounds identified in TCAMS. Compounds are represented by coloured spheres plotted against their assigned molecular framework number, chemical fingerprint cluster number and estimated anti-plasmodial potency. Included structures are models of drug-like molecules not previously described to have activity against the *Plasmodium* parasite. Reproduced with permission (License number – 4650801449133).

Figure 5 Potent Antimalarial OSM-S-106 and its physiochemical and biological data. cLogP = partition coefficient. MW = molecular weight. tPSA = total polar surface area. 3D7 = resistant strain of *P. falciparum*. HepG2 = human liver cancer cell line.

Figure 6 left to right – α (monomer), β (dimer) , and γ (trimer) carbonic anhydrases found in animals, plants and bacteria respectively.

Figure 7 Catalytic mechanism of CA.

Figure 8 Inhibitors of CA, sulfanilamide **10** and dorzolamide **11**.

Figure 9 CA assay negative control OSM-S-123.

Scheme 1 Retrosynthesis of compound OSM-S-106 **9**.

Scheme 2 Retrosynthesis of compound OSM-S-123 **12**.

Table 1 Compounds synthesised via the bromination of 4-chlorothieno[3,2-*d*]pyrimidine

Figure 10 Assignment of positions of 4-chlorothieno[3,2-d]pyrimidine and similar compounds.

Scheme 3 Bromination of **13**

Scheme 4 Bromination of **17** using a) 1 equivalence of NBS; b) 2 equivalence of NBS; c) bromine in chloroform; d) n-BuLi and bromine.

Scheme 5 Bromination of **17** using 1 equivalence of NBS.

Scheme 6 Bromination of **17** using 2 equivalences of NBS.

Scheme 7 Bromination of **17** using bromine in chloroform

Scheme 8. Bromination of **17** using n-BuLi and bromine.

Scheme 9 Synthesis of **21**.

Scheme 10 Hypothesised Chichibabin mechanism for the synthesis of **21**.

Scheme 11 More likely S_NAr mechanism for the synthesis of **21**.

Scheme 12. Suzuki-Miryaura cross coupling mechanism where the structures of Ar^1 and Ar^2 are shown in table 2. **O.E.** Oxidative addition; **N.L.E.** Nucleophilic Ligand Exchange; **T.M.** Transmetalation; **R.E.** Reductive elimination.

Table 2. Structures of Ar^1 and Ar^2.

Scheme 13 Synthesis of **24**.

Scheme 14. Synthesis of **9**.

Scheme 15 Synthesis of **12**.

Figure 11. Known CA inhibitor, acetazolamide **25**, and analogue of compound **9** with possible increased potency, **26**.

Figure 12. Drugs sulfadoxine **7** and dapsone **28** used for the treatment against the *Plasmodium* parasite family.

Scheme 16 Synthesis of **16** where i) THF, LDA; ii) 1,2-dibromo-1,1,2,2-tetrafluoroethane.

v. Abstract

Malaria kills approximately half a million people worldwide every year – two thirds being children under 5 years old. The disease, which can be treated by antimalarial therapies, is caused by the transmission of the *Plasmodium* parasite which are increasingly showing signs of resistance towards drugs used in treatment. The GSK funded Tres Cantos Antimalarial Screen (TCAMS) saw approximately 13 500+ hit compounds against the parasite, 25% of which possessing the appropriate drug-like properties. Open Source Malaria (OSM) has previously worked towards the resynthesis of a number of these compounds to modify and optimise potency. Open source science is an area of research were data obtained is shared publicly to promote discussion and ideas regarding the research. This study reports the efforts to resynthesize and evaluate one of TCAMS' hit compounds, in the hope to provide information of the drug target of the compound using a negative control which was also synthesised and evaluated in this project. Where problems in the efficiency of the synthetic route occurred, a model study was completed to investigate possible replacements for the reagents used.

Chapter 1: Introduction

1.1. Malaria

Malaria is one of the most common and deadly diseases worldwide and has played a key role throughout human history.[1] Reports of the distinguishable fevers, nausea, and muscle pain all characteristic of the disease can be found in the Hippocratic Corpus in ancient Greece and the Nei Ching (Canon of International Medicine) in ancient China as early as the first millennium BC.[2,3] Only when the parasites responsible for the disease were observed in a deceased malaria patients' blood smear by Charles Louis Alphonse Laveran in 1880 was it understood that parasitic protozoans, which he named *Plasmodium*, were the cause of malaria.[1,4] Avian malaria was subsequently found to be caused as a result of mosquito transmission of the *Plasmodium* parasite in 1897 by Ronald Ross who later won a Nobel Peace prize for his work.[5,6] Later in 1898, Italian scientist Giovanni Battista Grassi was the first to explain the life cycle of the human malarial parasite.[7]

In 2017 there were 219 million cases of malaria with an estimated 435 000 deaths worldwide - over two-thirds of these deaths occurring in children under 5 years old.[8] This number compares to 451 000 estimated deaths in 2016, and 607 000 in 2010. Of all malaria cases, 92% were seen in Africa, 5% in South East Asia, and 2% in Eastern Europe. Encouragingly, these figures have decreased by almost 40% worldwide since the year 2000. Recent reports however have shown a plateau in this downward trend which may suggest the current medication and strategies to prevent the disease have had their impact and now efforts must be done to continue the initial trend.[9]

The disease occurs via the transmission of the *Plasmodium* parasite to humans from an infected female *Anopheles* mosquito.[10] There are five different *Plasmodium* species which have been found to cause malaria in humans; *P. falciparum, P. malariae, P. ovale, P. vivax*, and the simian *P. knowlesi*. Of these five, infections are most common from the *Plasmodium falciparum* and *Plasmodium vivax* parasites.[10] *P. falciparum* is responsible for *ca.* 99.7% of all malaria cases in Africa, 72% in the Western Pacific, 69% in the Eastern Mediterranean, and 63% in South-East Asia in 2017.[8] *P. vivax* is the most widespread parasite that effects South

America with 74% cases of malaria in this region being caused by this species. Without treatment, malaria caused by *P. falciparum* can collect in the capillaries in the brain which would eventually lead to organ failure, coma, and death.[11]

Data collected in 16 high risk African countries between 2015 and 2017 showed that 79% of children under 5 years old that suffered from malaria also were anaemic (Figure 1).[8] Another study explored the risk of malaria to travellers and found that the risk of death was highest in the elderly and in tourists from countries where malaria was rare.[12] The disease is especially life threatening to pregnant women and their unborn children as it contributes to medical conditions such as anaemia which may result in premature birth. Although anaemia is seen as a direct and indirect outcome of the disease, its prevalence among those at risk is not something that has been reported reliably in recent years.[8,13]

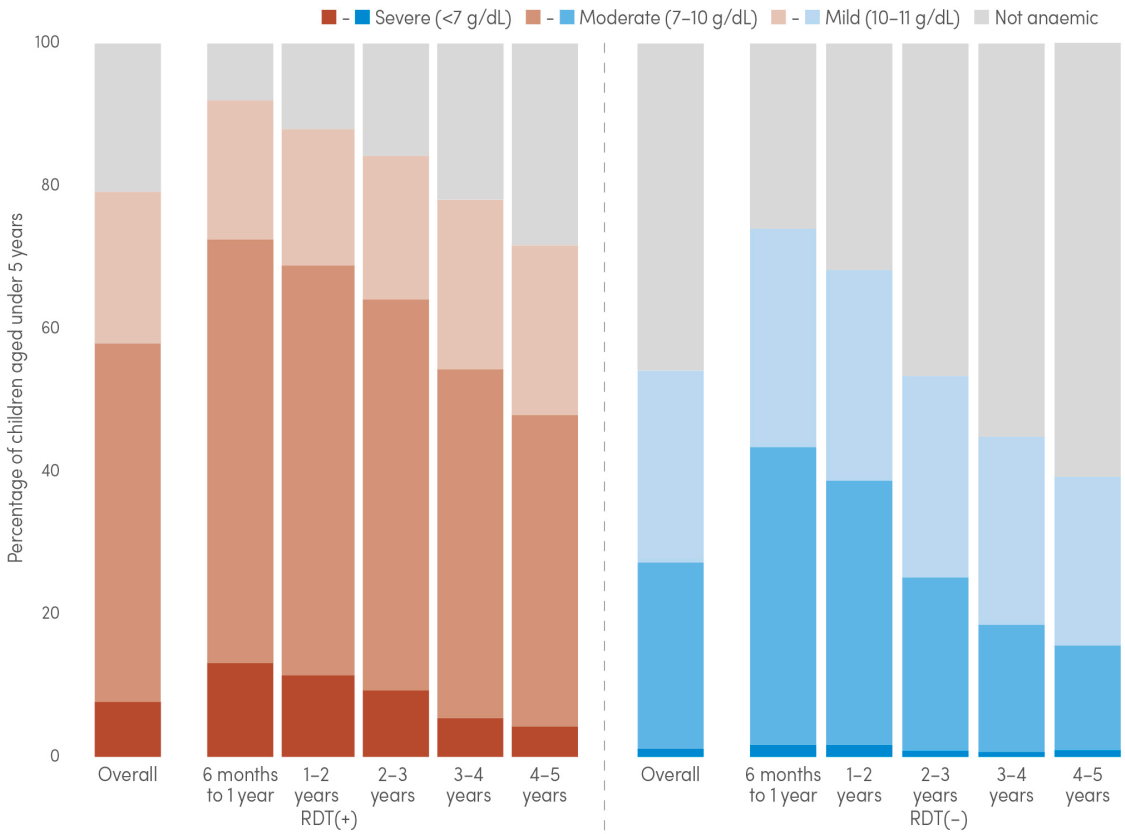


Figure 1. Prevalence of severe anaemia meeting the threshold requiring blood transfusion according to WHO (<7 g/dL) in children aged under 5 years in sub-Saharan Africa, 2015–2017, by age and malaria infection status. RDT: Rapid Drug Test. Reproduced from World Health Organisation's World malaria report 2018. Licence: CC BY-NC-SA 3.0 IGO.[14]

1.2. Lifecycle of the Malaria parasite

Before starting to develop ways to target the *Plasmodium* parasite we must first understand the way the parasite works. Understanding the lifecycle of the parasite could provide us with information that could facilitate more effective target control strategies and even possibly reverse the drug-resistance seen in some parasites today. The *Plasmodium* species of parasites are genetically complex which leads to thousands of antigens being presented to the human immune system from each infection. The parasite experiences a number of different biological stages throughout its life cycle (Figure 2) with different antigens being exhibited at each stage.

In the liver:

When a female mosquito of the *Anopheles* species with the malaria infection bites a human, it transfers the *Plasmodium* parasites (as sporozoites) into the bloodstream which quickly circulate the body, ending up in the liver.[13] Two species of the parasite, *P. vivax* and *P. ovale*, may stay dormant here and cause the patient to relapse months after the initial infection.[15] Throughout the 7-10 days following the initial infection the sporozoites undergo asexual reproduction to multiply in numbers – the human infected will not experience any symptoms at this point. The parasites, now in the developmental stage called merozoites, travel in vesicles from the liver cells to the lungs via the heart where they infect the lung capillaries.[16]

In the bloodstream:

The merozoites multiply inside red blood cells resulting in the rupture of the cells. This enables them to rapidly proliferate and repeat this cycle attacking more and more red blood cells causing the human to experience a fever as the parasites invade the cells.[13] This is the first sign of symptoms the patient will show and might not be observed until over 2 weeks following the initial infection. For some of the merozoites, they stop invading the blood cells and instead grow into their sexual forms, male or female gametocytes, and are released back into the bloodstream.

In the mosquito:

Upon a bite of an infected human by a female anopheline mosquito, the ingested gametocytes mature further into gametes (mature sex cells). Ookinetes that develop from fertilized female gametes forms an oocyst on the exterior of the gut wall inside the mosquito. When the oocyst bursts it releases thousands of sporozoites that had developed during the growth of the cyst.[17] These sporozoites are released into the body of the mosquito and make their way to the salivary glands ready to repeat the cycle once the mosquito bites another human.

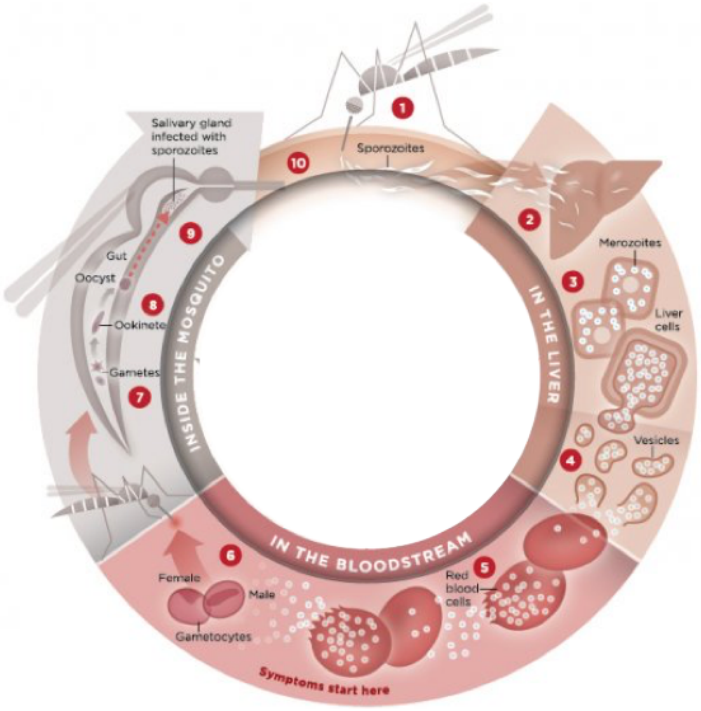


Figure 2. Lifecycle stages of the Malaria parasite. Reproduced from the Malaria Vaccine Initiative website at www.malariavaccine.org, 2015.[13]

1.3. Antimalarial Drug Development and Resistance

Drugs for treating malaria can be traced as far back as the 17th century (Figure 3). The bark of the cinchona tree was found to contain an alkaloid, quinine **1**, which was one of the first natural products found to display antimalarial properties. Quinine was first isolated in 1820, prior to this, the bark had been ground and used to treat patients with the disease. Quinine was the primary drug for treating malaria for over 100 years until chloroquine **2**, a synthetic analogue of the natural compound, was developed in 1934 by Hans Andersag. Following

World War II, chloroquine and the insecticide DDT **3** were used as the first in line attack against malaria in WHO's campaign against global eradication.[18] DDT was however banned in the UK in 1986 and later by the Stockholm Convention worldwide in 2001 due to the harmful long-term health and environmental effects. People with high levels of DDT in their blood were found to be 4 times more likely to develop Alzheimer's disease, although the relationship between the insecticide and the disease was subject to many factors. In 1974, mefloquine **4**, a drug developed by a collaboration between the US Army Medical Research and Development Command, WHO and Roche, was seen to have a high efficacy against malaria on a frequent dose.[18] Alongside the development of these antimalarials, proguanil **5** also surfaced, the success of this pyrimidine drug in treating malaria in humans led to the subsequent development of pyrimethamine **6**. Pyrimethamine was approved in the US in 1981 to treat malaria in combination with sulfadoxine **7**, an ultra-long-lasting sulfonamide.[18]

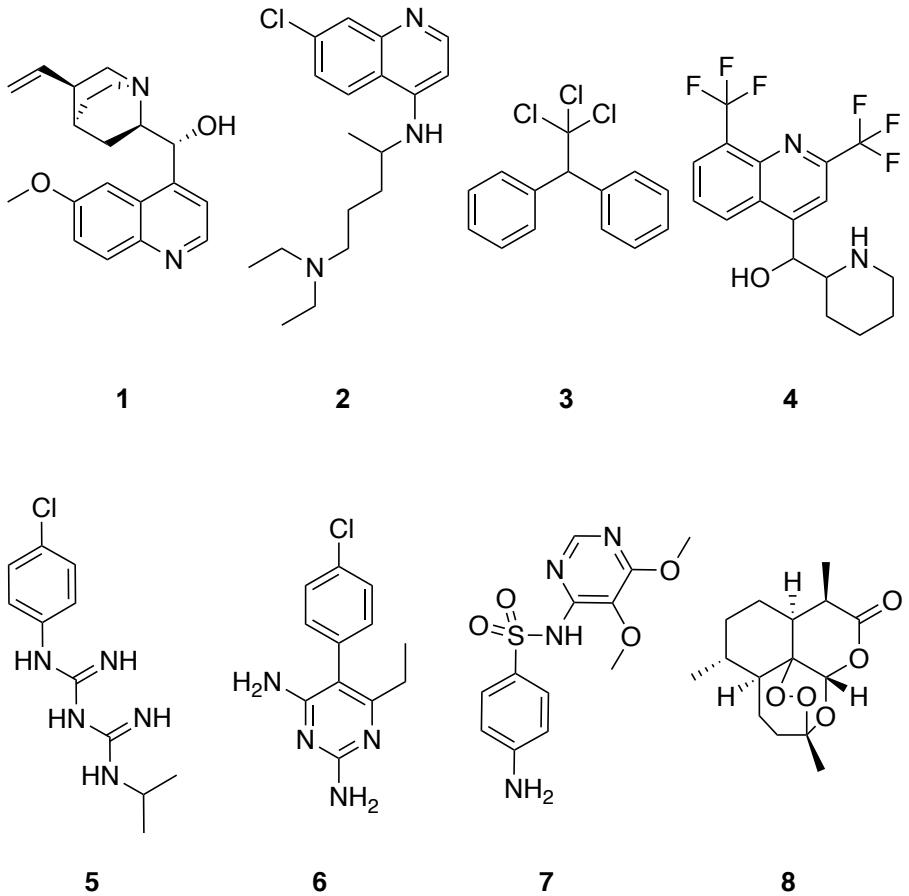


Figure 3. Drug compounds used for the treatment and prevention of Malaria (from **1**-**8**: Quinine, Chloroquine, DDT, Proguanil, Pyrimethamine, Sulfadoxine, and Artemisinin)

Despite the number of compounds proven to be successful in the treatment of malaria, full eradication of the disease has been prevented by the rise in resistant *P. falciparum* malaria. The extensive use of chloroquine in the mid 1900s saw the emergence in these resistant forms of the parasite were first seen in 1957 in Thailand before spreading to Africa and South America by the 1970s. Resistance to the pyrimidine drugs proguanil and pyrimethamine appeared almost within a year of development and mefloquine resistance started to appear when the drug went to market in 1985, just over 10 years after it was first developed. This rapid spread in resistance was difficult to combat and as a result was responsible for an increase in malaria related deaths.[19] A vaccine would be able to target different stages of the parasite lifecycle, the pre-erythrocytic (liver stage) vaccine, blood-stage vaccine, and transmission-blocking (mosquito stage) vaccine. A successful vaccine targeting *P. falciparum* and *P. vivax* malaria has still not reached the market however RTS,S, a vaccine targeting the liver stage of the parasite life cycle, has shown promising Phase 3 trial results and is expected to be available to populations at risk of the disease before 2030.[13]

Artemisinin **8** is a natural compound which was can be isolated from the plant *Artemisia annua*, a herb used in Chinese traditional medicine. It was first discovered in 1972 by Chinese chemist Tu Youyou and was found to be as potent as chloroquine.[20] It was also seen that combining a pre-existing malaria drug with artemisinin improved the efficacy and lowered the chance of resistance developing due to reduced treatment duration.[21] As a result, artemisinin-based combination therapy (ACT) has become one of the main defences against drug resistant malaria in South-East Asia. In 2009, ACTs have been reported to take longer and longer to treat patients with the disease; this could suggest that resistance is emerging to artemisinin and artemisinin-like molecules.[22] If this is the case, without the development of a drug with a novel mode of action, we could be left with untreatable malaria.

Although there is a high level of importance for treating malaria it is equally important to prevent the initial transmission of the disease. Prevention of the disease has conventionally been carried out in two main ways: protection against bites, and chemoprophylaxis.[23] Protection against bites includes vector controls to reducing the interaction between the mosquito vector and humans - this could be by the use of insecticides and bed nets/screens. Malaria chemoprophylaxis involves the use of drugs to prevent the disease from developing.

The drug used depends on several factors such as travel location, duration of exposure, seasonality of transmission, age and pregnancy.[23]

1.4. Open Source Development

The expression "open source" refers to a broad set of products or projects which the creator makes accessible to the public allowing it to be modified and shared in a community-based development.[24,25] This differs from most "closed source" projects where the ability to legally modify and share the data belongs exclusively to the creator. An important difference between open and closed source development in computer programming is the approach of handling bugs and other problems with the software.[26] Programmers for closed-source development often spend excessive time dealing with bug reports which are also often hindered by deadline and budget problems - this time could otherwise be spent on working on future developmental ideas. Open source is able to reduce these problems by including the users of the software with the involvement of the developmental process, often at times allowing the users to construct systems themselves.[27]

Although open source was originally intended for use by computer software development, it has since spread into increasing numbers of applications.[25] In the pharmaceutical industry, open source development has led to the founding of the Drugs for Neglected Tropical Diseases Initiative (DNDi) and the Open Source Drug Discovery for Malaria Consortium.[28] Open Source Malaria (OSM) focuses on using drug molecules originally screened by Big Pharma to have anti-malarial properties and improving them in a process referred to as open source drug discovery.[29] Anyone, whether they are chemistry or biology based, are able to give an input into a number of projects via wiki or GitHub or even post self-obtained data to the open lab notebooks. Participation in an OSM project is open to anyone regardless of expertise as long as six rules are followed, these being:

First Law: All data is open, and all ideas are shared.

Second Law: Anyone can take part at any level of the project.

Third Law: There will be no patents.

Fourth Law: Suggestions are the best form of criticism.

Fifth Law: Public discussion is much more valuable than private email.

Sixth Law: The project is bigger than, and is not owned by, any given lab.[29]

1.5. The GlaxoSmithKline Tres Cantos Antimalarial Screen

GlaxoSmithKline's research and development facility in Tres Cantos, Spain was established in 2001 and has since been devoted to synthesising and evaluating new treatments for diseases of the developing world.[30] In 2010, GSK opened the facility further to motivate and encourage the study of neglected diseases where research is lacking. The Tres Cantos Open Lab Foundation (TCOLF) has since funded and supported researchers from universities to industry in the hopes of advancing ideas that could result in the development of medicines to treat diseases in developing countries. Supported researchers are encouraged to share data and broadcast their work to make sure their research is available to other researchers. To aid the launch of the TCOLF, GSK provided a £5 million investment to support the objectives of the Foundation - two years later, in 2012, the funding doubled. Since the founding of the TCOLF, 60 research projects have been initiated, half of them now being complete.

The Tres Cantos Anti-Malarial Screen (TCAMS) saw nearly 2 million compounds screened from GSK's chemical library for inhibitory properties of the *P. falciparum* parasite.[31] Of these compounds, 13 533 were seen to inhibit the parasite by at least 80% with a concentration of 2 mM. Almost 60% of these confirmed inhibitors were active against the multidrug resistant (3D7) strain of the parasite and just over 80% were new to the malaria research community. The purpose of this was to aid the discovery of new and diverse compounds potent against *P. falciparum* in order to both black the transmission of malaria and to treat the symptoms of clinical malaria. The TCAMS library is the largest screen completed using the inhibition of *P. falciparum* gamete formation as the endpoint.[32]

A later study in 2016 by Miguel-Blanco *et al.* looked into the chemical diversity of the TCAMS compounds and the activity against asexual and stage 5 female gametocytes.[32] The screen

found a total of 405 compounds (Figure 4) which could be used as drug starting points for blocking malaria transmission, 205 of these being novel gametocyte-targeting molecules. Almost 25% of the identified hit compounds have the fitting physiochemical properties (meaning no more than 5 hydrogen bond donors and 10 hydrogen acceptors, a partition coefficient of no more than 5, and a molecular weight no more than 500 Da) and could therefore be studied further in the hopes to be taken forward in development.[32,33] The hits without these properties could be used as starting compounds which can be modified in order to pinpoint the mode of action in preventing *P. falciparum* malaria transmission.

Members of almost all of currently used classes of antimalarials were observed in the screen which therefore validated the accuracy of the assay used. These classes included diaminopyrimidines (e.g. pyrimethamine), 4-aminoquinolines (e.g. chloroquine), and methanol-quinolines (e.g. quinine), however compounds from known antimalarial classes only made up 10% of all hits suggesting the majority of hits may target novel drug targets and pathways.[31]

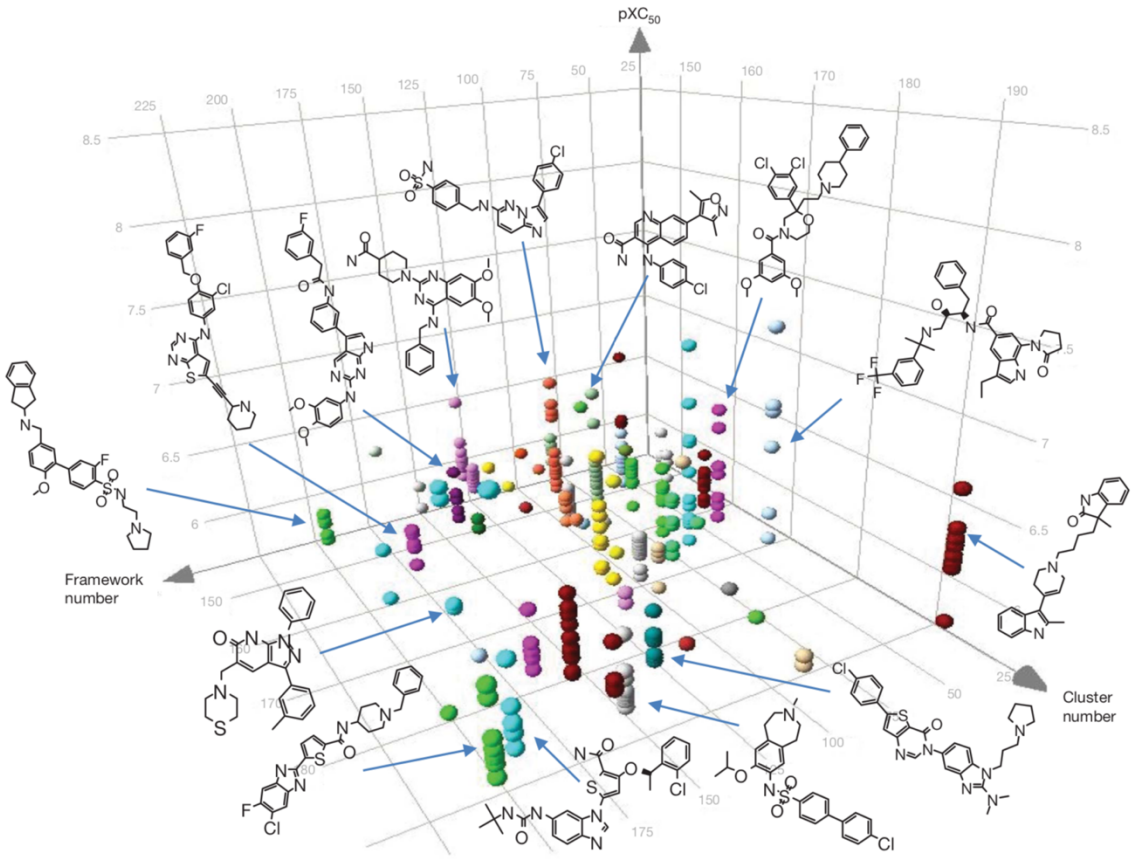
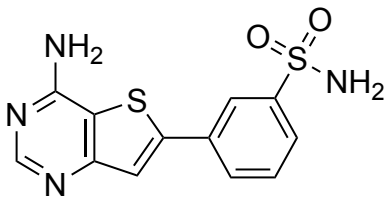


Figure 4. 3D plot of selected novel chemically diverse compounds identified in TCAMS. Compounds are represented by coloured spheres plotted against their assigned molecular framework number, chemical fingerprint cluster number and estimated anti-plasmodial potency. Included structures are models of drug-like molecules not previously described to have activity against the *Plasmodium* parasite. Reproduced with permission (License number – 4650801449133).[31]

1.6. Series 3 OSM and OSM-S-106.

OSM series 3 is the third collection of compounds from TCAMS and is centred around the aminopyrimidine core present in these compounds. OSM-S-106 **9** is a member of series 3 and was the original hit from the TCAMS screen resulting in it being selected by OSM for follow-up studies. The compound has been shown to selectively act on a phenotype specific to the malaria parasite and not human cells. The compound can be seen to exhibit the appropriate physiochemical properties to be orally active, suggesting it would be well-suited to be an antimalarial drug (Figure 5).



9
cLogP: 1.40
MW: 306.36
tPSA: 148.57

IC_{50} (3D7): 151 nM
%Inhibition HepG2 (10 μM): 13

Figure 5. Potent Antimalarial OSM-S-106 and its physiochemical and biological data. cLogP = partition coefficient. MW = molecular weight. tPSA = total polar surface area. 3D7 = resistant strain of *P. falciparum*. HepG2 = human liver cancer cell line.

At the time of writing (Aug 19) the target for OSM-S-106 has still not been identified. In Autumn 2018, data was received by the OSM consortium from the Winzeler Lab (UCSD) regarding studies carried out on the mechanism of action of the drug via the generation of resistant mutants. This data suggested that asparagine-tRNA ligase was the novel target however upon analysis at SGC-UNICAMP, no inhibition or interaction between OSM-S-106 and the enzyme was observed. Several suggestions received via GitHub and Twitter suggested carbonic anhydrase (CA) to be a possible target, however the inhibitory activity of OSM-S-106 on CA has not previously been studied.[34,35]

1.7. Carbonic Anhydrase

Carbonic anhydrases (CA) are ubiquitous zinc-containing enzymes found in most, if not all, organisms which catalyses the carbon dioxide hydration to bicarbonate and hydrogen ions: $CO_2 + H_2O \rightarrow HCO_3^- + H^+$.[36,37,38] The metalloenzyme was first discovered in cow red blood cells in 1933 and has since been found in abundance in all animals, plants and bacteria.[39] There are several families of CAs (α, β, γ, δ and ζ) (Figure 6) which, despite their lack of structural similarity, all accomplish the same task and require the binding of a zinc ion which suggest the occurrence of convergent evolution.[37,40] Inhibitors of the enzymes however show activity against all families due to them binding to the zinc ion in the active site.[41] CAs are usually found confined on membranes or extracellular spaces, this position aids the ability for the enzymes to speed up the intracellular CO_2 and H^+ diffusion. This shift in proton concentration means that CAs are able to alter pH gradients across a membrane helping the maintenance of a consistent pH throughout the cell.[42]

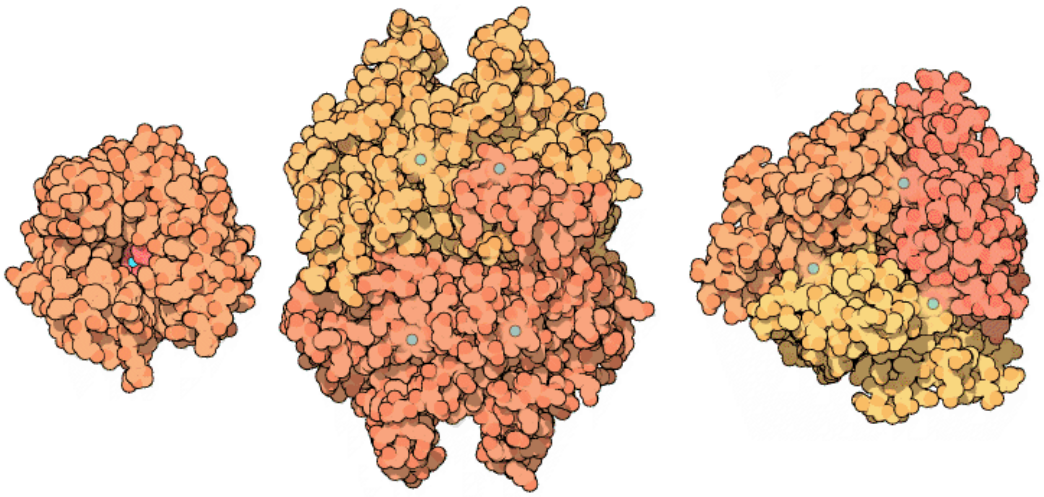


Figure 6. left to right – α (monomer), β (dimer) , and γ (trimer) carbonic anhydrases found in animals, plants and bacteria respectively.[39]

Different families of CAs are found in different families of organisms. Alpha CAs are found in animals, algae, and some bacteria and can be split into four subgroups: three cystolic isoforms (I, II, and III), one mitochondrial (V), one secreted (VI), and five membrane-associated CAs (IV, VII, IX, XII, and XIV).[43] All of these subgroups are comprised of several isoforms which are displayed in different tissues varying from red blood cells (CA1), kidney (CA4), liver (CA5A) and saliva (CA6).[43] The beta family of CAs are mostly found in prokaryotic organisms and

plant chloroplasts and contains an amino acid sequence far more complex than that of the alpha family and can form hexamers and octamers.[39] Finally, the gamma family of CAs which is found in abundance in methanogens and archaea such as *E.coli* and follows a similar catalytic mechanism as alpha-CAs.[44]

The mechanism of CAs involves the attack of a zinc-bound hydroxide ion to a molecule of CO_2 bound in a hydrophobic region of the enzyme (Figure 7).[37] This produces a zinc-bound bicarbonate ion which is then displaced by a molecule of water. The rate-determining step (step 1) consists of an intramolecular proton transfer from the water molecule to a histidine residue which shuttles the proton between the zinc centre and buffer molecules.

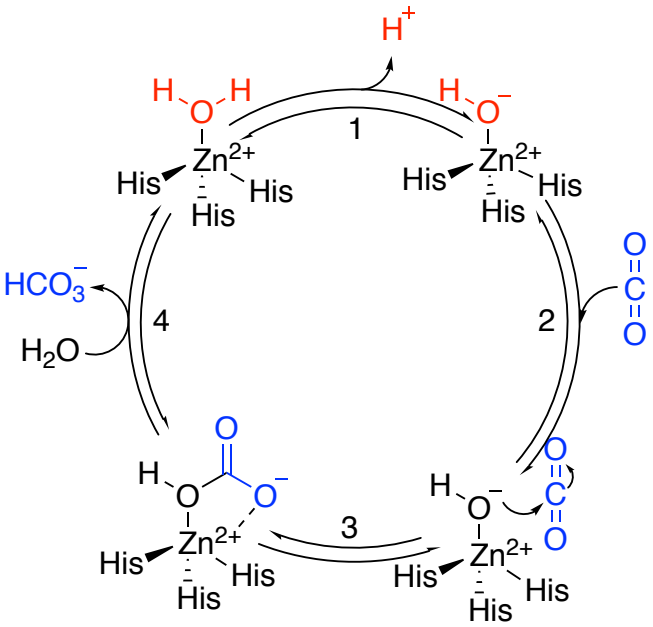
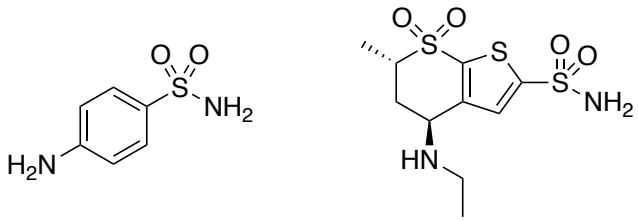


Figure 7. Catalytic mechanism of CA.

CA inhibitors supress the action of these enzymes which in turn suppresses the interactions of the water molecule bound to the zinc ion in the active site with CO_2.[36] This absence of interactions affects the balance of ions across a membrane. Shortly after the first discovery of the enzyme it was also realised that sulfanilamide **10** was a potent inhibitor.[40] This led to further studies on human erythrocytes which were completed in the 1970s in the hopes to discover the first synthetic imidazole inhibitor. More recently, the CA inhibitor dorzolamide **11** has been used to treat a condition, called glaucoma, caused by a fluid build-up in the eye which damages the optic nerve.[45] By affecting ion balance, the inhibitor is able to shift the fluid from the patient's eye which reduces pressure and as a result prevents blindness.



10 **11**

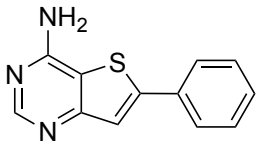
Figure 8. Inhibitors of CA, sulfanilamide **10** and dorzolamide **11**.

Genes coding the alpha-CA enzyme have been found in the *P. falciparum* parasite which displays almost identical catalytic properties as the human host CA1 and 2 isoforms.[46] CAs assists the transport of CO_2 across the plasma membrane of red blood cells which is crucial for the growth of malaria parasites to regulate intracellular pH and through CO_2 fixation to synthesize pyrimidine. A study by Sein and Aikawa in 1998 showed that that malaria parasites in red blood cells have localised CA which, if inhibited, may reduce the concentration of parasite in the blood.[47]

A number of aromatic sulfonamides derived from sulfanilamide have been observed to display inhibitory effects for the malarial CA enzyme with Ki values ranging from 80 – 0.5 µM.[46] The potency of these sulfonamide derivatives on both malarial and human CA are affected by several factors, the main factor being the groups branching off from the central aromatic fragment.[46] As a primary aromatic sulfonamide, there is a great possibility that OSM-S-106's antimalarial properties are due to the fact it binds to plasmodial CA in the malaria parasite. Compound OSM-S-123 is almost identical to OSM-S-106, the difference being the lack of the primary sulfonamide at the meta position of the benzene ring.

Aromatic sulfonamides have also been found to accumulate in RBCs.[48,49] This could lead to an increased concentration of OSM-S-106 in RBCs where the blood stage *Plasmodium* inhabit resulting in the drug being in an appropriate position to target the parasite. This red cell carriage has also been shown to decrease hepatic clearance of the drug.[50] If this enzyme is in fact the target of OSM-S-106, this lack of functional group would therefore result in a lack of binding to the CA enzyme which would decrease the inhibitory activity observed. Evaluation of OSM-S-106 **9** in a CA assay using OSM-S-123 **12** as a negative control would confirm or disprove this hypothesis and provide crucial information towards the mode of action of the

drug. If this hypothesis is correct and the antimalarial activity of OSM-S-106 is caused by targeting this novel target, we will become once step closer to combatting drug-resistant strains of the malaria parasite.



12

Figure 9. CA assay negative control OSM-S-123.

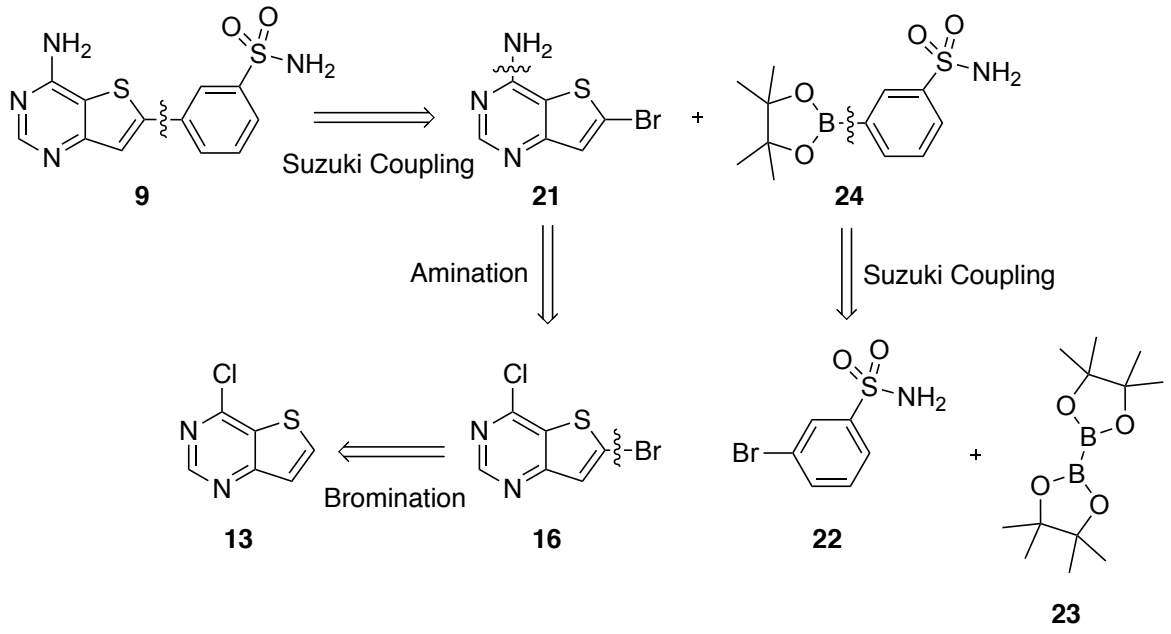
1.8. Aims of the Current Project

Information regarding the synthesis of **9** has only been recorded through research by the OSM group using an range of conditions and obtaining a wide spectrum of yields. High throughput methods which were originally used in the TCAMS have shown the potent antimalarial properties of the compound, however the exact target has not yet been found. This study will look in to optimising the synthesis of **9** to be tested in a CA assay to determine whether this enzyme is the drug target. This will require the synthesis of a novel compound **12** to be used as a negative control due to its lack of a sulfonamide functional group. Characterisation will be done to obtain Rf values, MP, ^{1}H and ^{13}C NMR, FTIR and LCMS. Biological testing will be carried out to verify of the potency of the compounds against the malaria parasite and the target will be explored using a CA assay.

Chapter 2: Results and Discussion

2.1. Synthetic Analysis

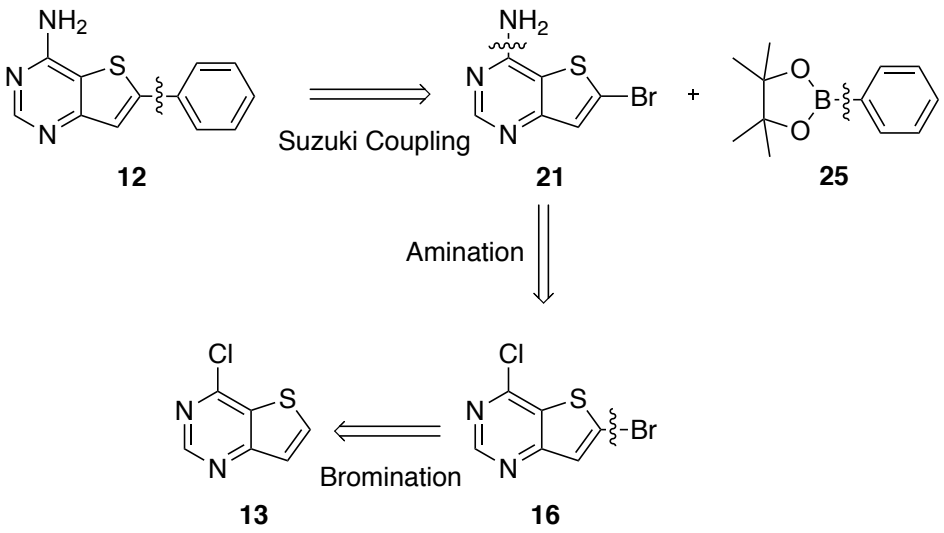
Synthesis of OSM-S-106 **9** has not previously been recorded in literature however previous students have undergone attempts at synthesising the compound with varying success. Knowledge of which reactions were successful, and which were not, led to the development of a retrosynthetic plan to follow (Scheme 1). The primary reaction involved the bromination of commercially available 4-chlorothieno[3,2-*d*]pyrimidine **13** to produce the 6-bromothieno[3,2-*d*]pyrimidine **16** which upon a nucleophilic amine substitution would synthesise the 6-bromothieno[3,2-d]pyrimidin-4-amine **21**.[†] The pinacolyl boronic ester **24** could be synthesised via the coupling of 3-bromobenzenesulfonamide **22** and bis(pinacolatodiborane) **23**. The final compound **9** could then be produced by a Suzuki coupling between the aminated species **21** and boronic ester **24**.



Scheme 1: Retrosynthesis of compound OSM-S-106 **9**.

[†] - This chapter refers heavily to IUPAC nomenclature, please see Appendix.

Synthesis of OSM-S-123 **12** has not previously been recorded in literature or by previous students however due to the similarity of the structure with that of OSM-S-106, an almost identical synthetic route was followed (Scheme 2). The only difference in the two routes would be the final Suzuki coupling as the phenylboronic acid pinacol ester **25** is commercially available and would not need to be synthesised. Similar to the synthesis of **9**, the final compound **12** is produced by a Suzuki coupling between the aminated species and phenylboronic acid pinacol ester.



Scheme 2: Retrosynthesis of compound OSM-S-123 **12**.

2.2. Bromine Substitution on Thienopyrimidine Ring

The synthesis of **9** and **12** began with the effort to substitute a hydrogen on the thiol ring of **13** with a bromine. Initially, the starting material core had been made by another student but the core has since become commercially available so for the sake of obtaining a reasonable final yield **13** was bought rather than synthesised.[51]

The synthesis of **16** from **13** has been recorded in literature by Woodring *et al.* This method used two flasks, flask one containing THF and lithiumdiisopropylamine (LDA) (1.2 equiv) at 0 ^{o}C, and flask two containing **13** (1 equiv) in THF. Flask 2 was added dropwise to flask 1 at -78 ^{o}C before the addition of 1,2-dibromo-1,1,2,2-tetrafluoroethane (1.4 equiv) and left stirring at room temperature for 12 hours to obtain the product in 94% yield.[52,53] For the sake of

replicability, a method carried out by a previous student was followed using **13** (1 equiv), THF and *n*-BuLi (1.3 equiv) before the dropwise addition of bromine (1 equiv).

In the first attempt that this method was followed it was found that after 1 hour of stirring there was still unreacted starting material, so the mixture was left stirring for another 18 hours. Upon NMR analysis it was seen that there were several compounds present in the crude product. Further analysis of the unknown compounds obtained following purification suggested the compounds could be those shown in table 1. Analysis of the ^{1}H NMR spectra obtained was used in the attempt of identifying these unknown compounds. To know for definite if these predictions are correct, another form of analysis such as LCMS will need to be carried out. The identity of the product **16** was confirmed by the use of ^{1}H NMR and HMBC. The ^{1}H NMR spectra for the possible 6-bromo product show two singlet peaks at 8.9 and 7.6 ppm, if the bromine had substituted on the 7 position a singlet at 9.1 ppm would be in replace of the 7.6 ppm peak due to the proton being adjacent to an electronegative sulfur resulting in a higher shift.[54] HMBC spectra show strong correlations between protons and carbons separated by three bond. Analysis of the HMBC spectra of **16** shows a correlation between the proton on the 7 position and the carbon at the 4a position (position assignment shown in figure 10), three bonds away. Comparing this to the HMBC of 6-bromothieno[3,2-*d*]pyrimidine which shows two correlations to 4a and 7a, both three bonds away, can confirm the identity of **16**.

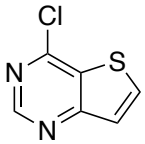
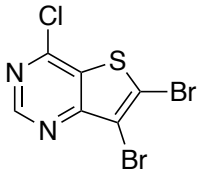
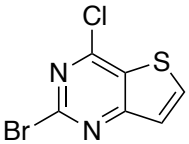
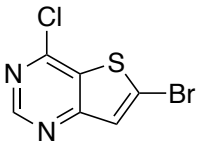
Compound				
	13	**14**	**15**	**16**
Yield	4%	18%	4%	20%

Table 1. Compounds synthesised via the bromination of 4-chlorothieno[3,2-*d*]pyrimidine

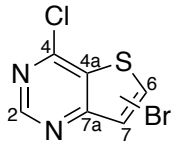
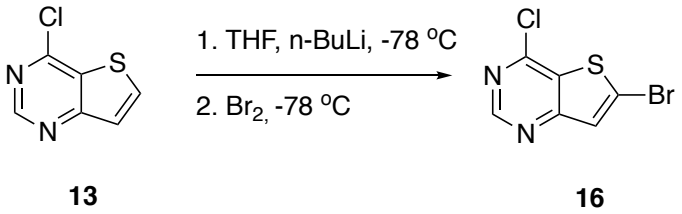


Figure 10. Assignment of positions of 4-chlorothieno[3,2-d]pyrimidine and similar compounds.

The presence of these additional products suggested that the reaction had been left for too long a time. As a result, for the second attempt the reaction was only left for 1 hour following the addition of the bromine. The shorter reaction time for the second attempt resulted in a lack of unwanted side products however there was still 43% unreacted **13** present. The yield for the product **16** stayed the same as the previous attempt at 20%, this compares to a 30% yield of the desired product recorded by a previous student from the OSM consortium.

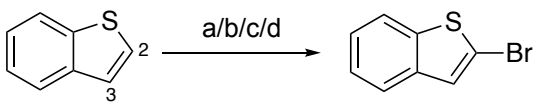


Scheme 3. Bromination of **13**

2.3. Bromine Selectivity on Benzo[*b*]thiophene – Model Study

Several model studies were carried out to report the outcomes of using different reagents and conditions to brominate a heterocyclic ring similar in structure to **13**. These were carried out in the hope to both increase the yield obtained in the synthesis and to find a method that didn't require the use of the highly reactive organolithium reagent to obtain the 2-bromo substituted product.

A benzo[*b*]thiophene ring **17** was used as a simplified model compound to brominate in these set of reactions (Scheme 4). Different equivalences of NBS were used for one set of reactions whilst another reaction studied the selectivity of using bromine in chloroform. The reaction of benzo[*b*]thiophene with *n*-BuLi was also carried out to study whether this synthesised similar side products to the original reaction.

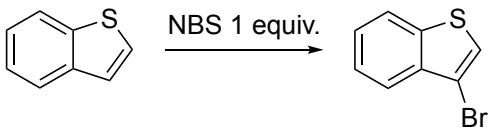


17 **18**

Scheme 4. Bromination of **17** using a) 1 equivalence of NBS; b) 2 equivalence of NBS; c) bromine in chloroform; d) n-BuLi and bromine.

2.3.1. Electrophilic Substitution using 1 Equivalence of NBS

The bromination of a thiophene ring on the 2 position had previously been recorded in a procedure by Kellog *et al.* This procedure used the direct bromination of 3-phenylthiophene using NBS (1.25 equiv) under acidic conditions (maintained using 1:1 (V/V) mixture of chloroform and acetic acid) to obtain a yield of 94% for the 2-bromo-3-phenylthiophene.[55] This procedure was later followed by Dit Chabert *et al.* using benzo[*b*]thiophene **17** in replace of 3-phenylthiophene however it was found that this produced only the 3-bromobenzo[*b*]thiophene **18** as a red oil with a 100% yield.[56] Upon completing the reaction for this study, a colourless oil with a of 56% was obtained. This reaction was done on a 1 g scale whilst Dit Chabert *et al.* completed their reaction on a 10 g scale, this could be a reason for the noticeably lower yield obtained for this study. Confirmation of the identity of the product was done using ^{1}H NMR with the integration of the peaks showing a decrease in protons from 6 in the spectra for **17** to 5 in the spectra for **19**. The position of which the ring was brominated as can be determined again by higher ppm of the singlet due to the proton being adjacent to the electronegative sulfur, which also was in agreement to data found in literature. HMBC was carried out however due to multiple aromatic protons, it did not help in defining the bromine position.

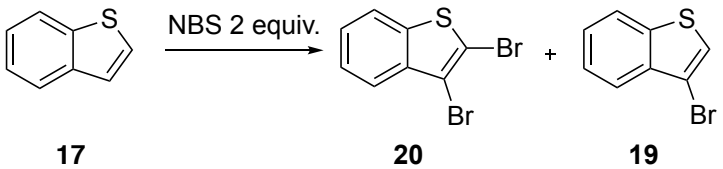


17 **19**

Scheme 5. Bromination of **17** using 1 equivalence of NBS.

2.3.2. Electrophilic substitution using 2 equivalences of NBS

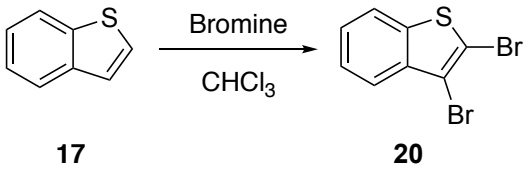
This reaction followed a method from a previous which first used 1 equiv of NBS to synthesise the **18** in a method comparable to that described in by Dit Chabert *et al*. This student found that after stirring at 0 °C for 15 minutes post the addition of NBS (1 equiv) the reaction had not completed so an additional 0.85 equiv of NBS was added. This obtained 18% **19** and 21% **18** with a ratio of 0.85:1 respectively. As this method used a total of 1.85 equivalences of NBS an investigation was conducted in this study looking at the resulting products when the amount of NBS used was increased to 2 equivalences. When this reaction was completed, using the integration of the proton peaks, it was found to produce 38% **19** and 10% **20** with a ratio of 1:0.26 respectively was no traces of 2-bromo.



Scheme 6. Bromination of **17** using 2 equivalences of NBS.

2.3.3. Bromine and Chloroform

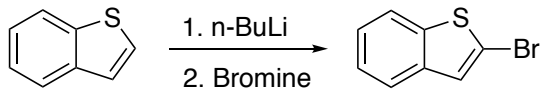
In efforts to selectively synthesise the 2-bromo product, the effects of using bromine and chloroform on benzo[*b*]thiophene was investigated. This synthetic process has been done several times in literature on a 10 g scale and found that it almost exclusively synthesised the 2,3-dibromo product in yields ranging from 90-94%.[57–59] Despite the evidence showing this reaction would only make the 2,3-dibromo product, the procedure was repeated to rule out the possibility that 2-bromo substitution on the thiophene ring could be synthesised. This synthesis however agreed with previous studies as only the 2,3-dibromo product was synthesised with a yield of 91%. Confirmation of the identity of the product was carried out using ^{1}H NMR with number of protons from 6 in the spectra for **17** to 4 in the spectra for **20**. The data obtained from the ^{1}H NMR was also in agreement to those found in literature and therefore provides further confirmation.



Scheme 7. Bromination of **17** using bromine in chloroform.

2.3.4. BuLi and Bromine

Due to the excess in side products formed in the synthesis of **16**, it was of interest to use the same conditions and reagents for the bromination of **17**. This reaction of the bromination using *n*-BuLi and bromine has been recorded by both Inouchi *et al.* and Krajewski *et al.* and synthesised **18** with a 67% and 66% yield respectively.[60,61] Following the procedure let to the synthesis of **18** with a yield of 59% for this model study. The data obtained from ^{1}H NMR of the product was in agreement to those recorded by Inouchi *et al.* and Krajewski *et al.* confirming the correct identification of the product.[60,61] This was the only set of reagents used that selectively substituted the hydrogen at the 2 position of the ring and as a result the bromination of **13** wasn't repeated using the other reagents studied.



17 **18**

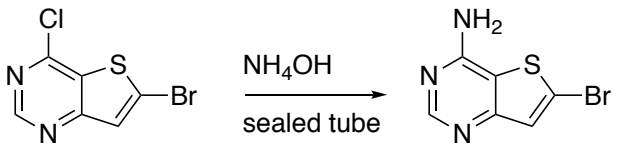
Scheme 8. Bromination of **17** using n-BuLi and bromine.

2.4. Amination of thienopyrimidine ring

Although the amination of 2-bromo-4-chlorothieno[3,2-*d*]pyrimidine itself has not been recorded in literature, thieno[3,2-*d*]pyrimidin-4-amine has been recorded to be synthesised from 4-chlorothieno[3,2-*d*]pyrimidine using ammonium hydroxide in ethanol at 60 °C with a yield of 81%.[62] This reaction subjected the 4-chlorothieno[3,2-*d*]pyrimidine to nucleophilic substitution in a sealed tube so as to not allow the ammonia gas to escape and be unable to react. Methods carried out by past students using the 2-bromo-4-chlorothieno[3,2-*d*]pyrimidine that was required in this study were discovered in the OSM consortium with yields of around 80%.[51]

Due to the lack of appropriately sized of microwaveable vials, it was necessary to scale the reaction down (from 200 mg to 40 mg) for the first several attempts at this reaction in order to maintain safety. The yield of the reaction was left unaffected at 91, 75, 82, and 83% despite this change in scale. Upon purchasing the appropriately sized vials it was possible to scale up the procedure 36x. Although more convenient to carry out, the yield, 86%, was not

significantly different suggesting that this yield is probably nearly at the maximum value under these conditions.

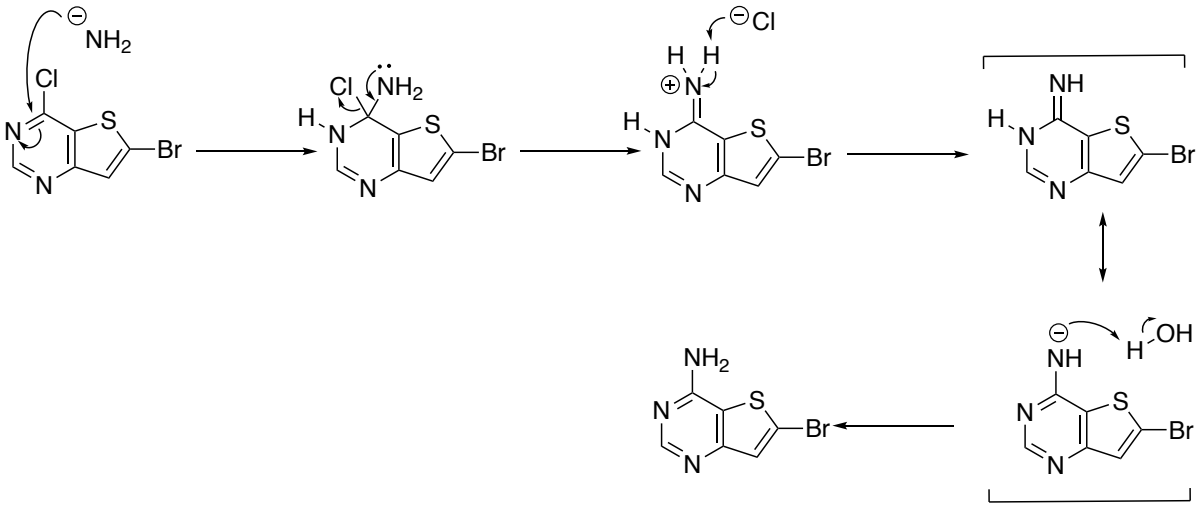


16 **21**

Scheme 9. Synthesis of **21**.

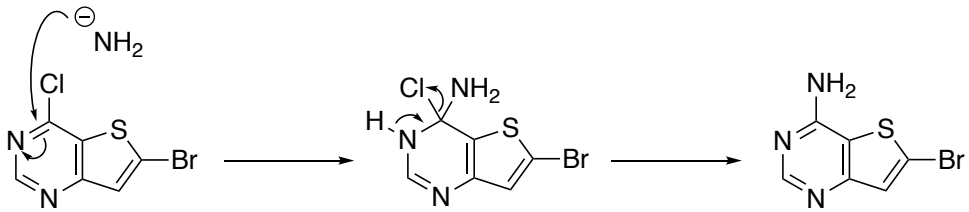
The reaction can be seen to be complete by comparing the proton NMR spectra of the product and the starting material - the extra singlet at 7.5 ppm corresponding to the additional protons from the primary amine group. The crude product was seen to be relatively pure so was not purified further as an attempt to minimise product loss. This purity is based on the NMR spectroscopic analysis and comparison of the melting points from this product (243.5 – 247°C) vs those obtained previously for exhaustively-characterised product (240 – 241 °C).[51]

There has been no proposed mechanism for the amine substitution. The presence of the electron withdrawing nitrogen present in the thienopyrimidine ring may suggest this reaction proceeds with a mechanism similar to the Chichibabin reaction, an addition-elimination reaction that proceeds through a Meisenheimer adduct intermediate (Scheme 10).[63]



Scheme 10. Hypothesised Chichibabin mechanism for the synthesis of **21**.

Another suggestion could be a simple nucleophilic aromatic substitution (S_NAr) route where the amine ion from the ammonium hydroxide displaces the chloride leaving group (Scheme 11). Due to there being only three proton environments in the ^1H NMR spectra and not 4, which would be seen for the Meisenheimer adduct intermediate, it is assumed that the it is the S_NAr mechanism that this reaction follows.



Scheme 11. More likely S_NAr mechanism for the synthesis of **21**.

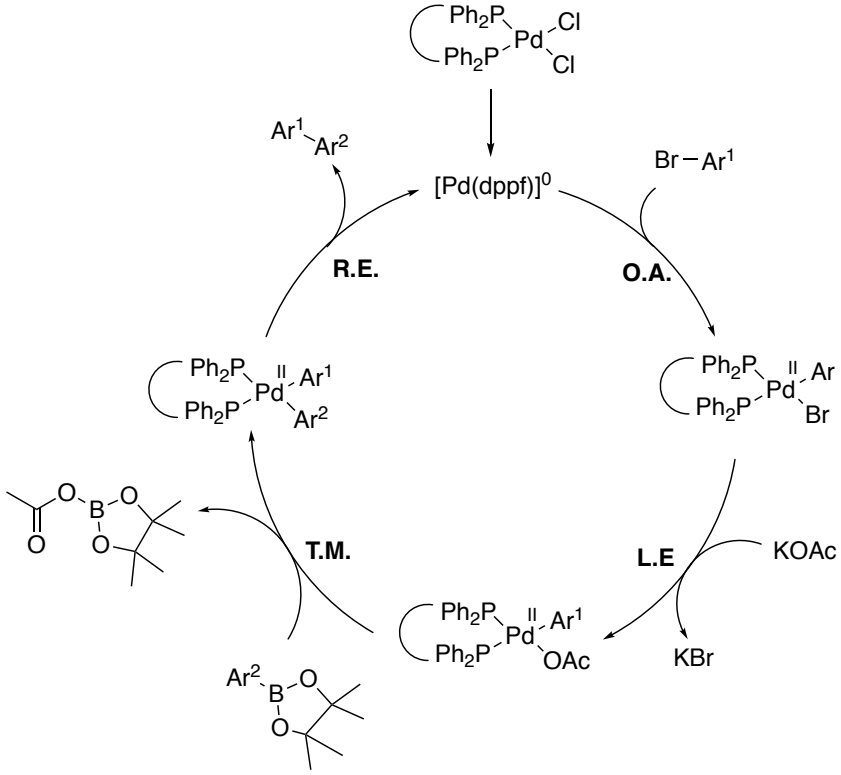
2.5. Suzuki Coupling Reactions

Palladium catalysed reactions forming C-C bonds have been of great importance towards the synthesis of complex organic molecules in the last number of decades.[64] Cross-coupling reactions may be carried out using a variety of organometallic reagents however recent attention has focused on organoborons bonding to the palladium(II) halide catalyst. These reactions have been found to proceed with little problem in the presence of an aqueous base and due to their convenient properties, organoborons are safe to handle without extra precaution both for laboratory and industrial scale reactions.[65] Although non-precious metal (Ni, Cu, Co and Fe) catalysed cross-coupling reactions have also been reported, these generally involve higher temperatures and catalyst loading than their corresponding Pd-catalysed reaction.[46-55]

The first publication of the Suzuki-Miryaura coupling reaction was in 1979 by Japanese chemist, Akira Suzuki, who showed the coupling of an organohalide and boronic acid with the use of a palladium catalyst.[77] The Suzuki-Miryaura coupling reaction has since been used widely in the pharmaceutical industry as a cost efficient way to synthesise intermediates at large scales.[78] This type of coupling reaction is largely favoured over other coupling reactions as they require milder conditions and boronic acids are readily available and are less toxic than alternative compounds such as organostannane and organozinc compounds.[79,80] Problems have sometimes occurred in SM couplings due to incompatibilities with synthetic

reagents caused by unstable boronic acids.[81] This can however be resolved by the use of the boronic ester version of the acid which are stable despite the C-B bond being marginally longer than a C-C bond. Boronic esters have gained increasing attention throughout organic synthesis due to their ability to undergo cross-coupling reactions to synthesise unsymmetrical biaryls with excellent yields.[82,83]

The mechanism for the Suzuki-Miryaura coupling reaction is best viewed from the perspective of the palladium catalyst (Scheme 12).[84] The first step involves the oxidative addition of the arylhalide to the palladium to form the organo-palladium species. Reaction of this organo-palladium species with a base gives a palladium (II) intermediate which goes on to form the second organo-palladium species following transmetalation with the boron complex produced by reaction of the boronic acid, or in this instance a boronic ester, with a base$_{(aq)}$. The desired product is removed via a reductive elimination which also restores the original palladium catalyst, completing the catalytic cycle. The rate determining step of this catalytic cycle can either be the oxidative addition or the transmetalation depending on the reagents.



Scheme 12. Suzuki-Miryaura cross coupling mechanism where the structures of Ar^1 and Ar^2 are shown in table 2. **O.E.** Oxidative addition; **N.L.E.** Nucleophilic Ligand Exchange; **T.M.** Transmetalation; **R.E.** Reductive elimination.

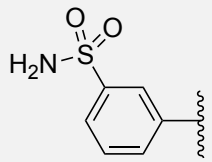
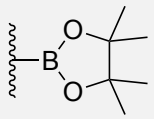
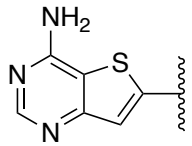
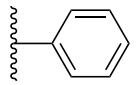
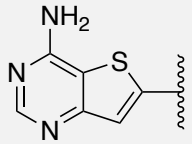
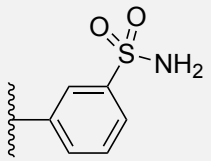
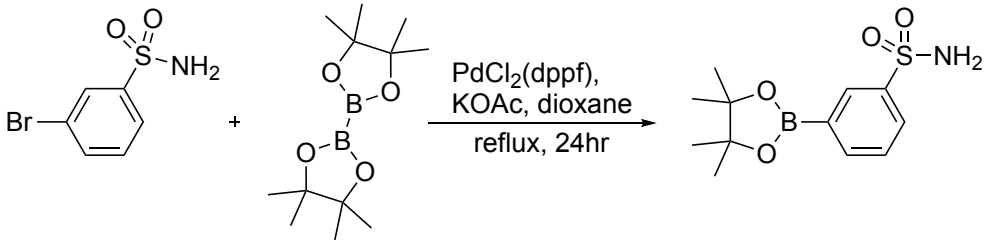
Synthesis of:	Ar^1	Ar^2
24		
12		
9		

Table 2. Structures of Ar^1 and Ar^2.

2.6. Suzuki coupling reaction of 3-bromobenzenesulfonamide and bis(pinacolatodiborane)

The synthesis of **24** has been carried out by Abdelsamie *et al.* in efforts to develop a 17β-hydroxysteroid dehydrogenase inhibitor. This route coupled 3-bromobenzenesulfonamide **22** with bis(pinacolato)diboron **23** in the presence of potassium acetate and the palladium catalyst, $Pd(dppf)Cl_2$. This reaction however was quenched, and the mixture was used without further purification or characterisation due to the compound being relatively unstable.[85] Synthetic routes using similar reagents and conditions have been completed by students with the OSM consortium and obtained a range of yields from 26-55%.[51] Completion of this reaction for this study obtained a yield of 39% for the product **24** with **23** being recovered with a yield of 33%.

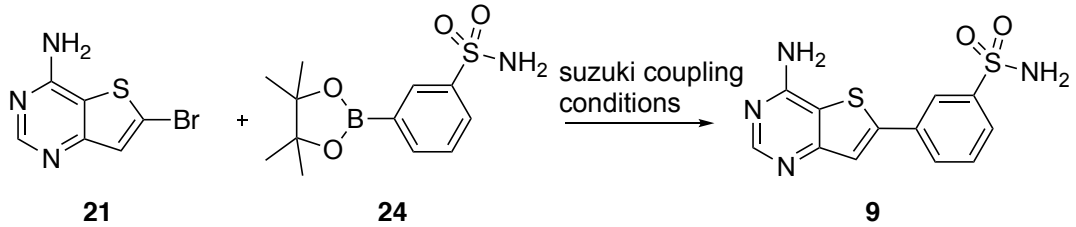


22 **23** **24**

Scheme 13. Synthesis of **24**.

2.7. Synthesis of OSM-S-106

The synthesis of OSM-S-106 **9** using the coupling of **21** and **24** has not yet been recorded in literature however reports documenting the attempts of the synthesis have been recorded, with varying success, by previous students working with the OSM consortium. One student performed the coupling using catalytic $Pd(PPh_3)_4$, caesium fluoride, and sodium bicarbonate in 1,4-dioxane/H_2O under reflux under argon atmosphere but to no avail.[86] Another student recorded the successful coupling using $PdCl_2$(dppf) and potassium carbonate solution in isopropanol under argon atmosphere to synthesise **9** with a 27% yield.[51] This successful procedure was followed in this study and obtained the product in a 22% yield. Confirmation of the identity of the product was carried out by ^{1}H NMR, ^{13}C NMR, and LCMS, the intense singlet at 1.31 ppm in the spectra for **24** corresponding to the four CH_3 groups of **24** was not present in the ^{1}H NMR spectra for the pure product suggesting that there was no boronic ester starting material present in the product. The number of proton and carbon environments are were in agreement to the structure of **9**. If a brominated species was present in the product the LCMS would show two peaks side by side corresponding to the two stable bromine isotopes, ^{79}Br and ^{81}Br, the spectrum however showed no signs of this. The analytical data obtained was also in agreement with those recorded by previous students.

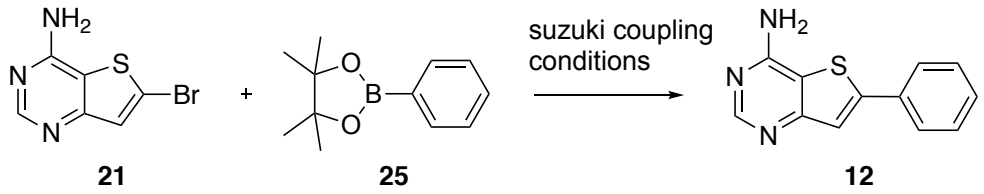


Scheme 14. Synthesis of **9**.

2.8. Synthesis of OSM-S-123

The synthesis of **12** has previously been recording by Morwick *et al.* in the search for an inhibitor of IκB Kinase-β.[87] This procedure involved the amination of a thienopyrimidone core using concentrated ammonium hydroxide to obtain the product in a 46% yield. Due to the similarity of the structure of **12** with the structure of **9** it was decided to follow the same procedure rather than that recorded by Morwick *et al.* For this reaction, commercially available boronic ester **25** was used to synthesise the desired product with a 38% yield. Again,

confirmation of the identity of the product was carried out both by ^{1}H NMR, ^{13}C NMR, and LCMS. The number of proton and carbon environments are were in agreement to the structure of **12** with no CH_3 singlet at 1.31 ppm present for the pure product, the LCMS also didn't show any peaks suggesting a brominated species was present. The analytical data obtained was also in agreement with those recorded by Morwick *et al*.



Scheme 15. Synthesis of **12**.

Chapter 3: Conclusion and Future Work

3.1. Conclusion

The evaluation of the mechanism of action for the anti-malarial hit compound, OSM-S-106, was conduction by firstly resynthesising the compound using methods to optimise overall yield and secondly sending the compound to be biologically evaluated against the potential carbonic anhydrase target. This was compared against OSM-S-123 as a negative control, synthesised via a similar reaction route.

OSM-S-106 and OSM-S-123 were synthesised using a short synthetic route adapted from methods recorded by previous students and literature. The use of the lithium/bromine exchange on 4-chlorothieno[3,2-*d*]pyrimidine proved challenging due to the generation of a number of unwanted side products which majorly impacted the yield of 6-bromo-4-chlorothieno[3,2-*d*]pyrimidine. This led to a follow-up model study where bromination of a benzo[*b*]thiophene was studied using a selection of reagents and conditions. A more efficient method to synthesise the required 6-bromo substituted heterocycle could not be identified. The following amination to synthesise 6-bromothieno[3,2-*d*]pyrimidin-4-amine upheld strong yields. Suzuki coupling reactions were used throughout the synthesis of these compounds

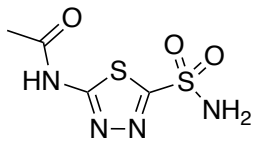
At the time of writing (Aug 2019) the two compound, OSM-S-106 and OSM-S-123, have been sent for biological evaluation regarding their activity against malaria in vitro and their activity against CA. The results of these have not yet been received.

3.2. Future work

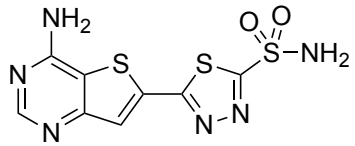
Future research on the target of **9** rely heavily on the arrival of the biological data from the carbonic anhydride assay obtained for OSM-S-106 **9** and OSM-S-123 **12**. If **9** is found to have inhibitory activity and **12** does not, there is a high possibility that carbonic anhydrase is the target. If **9** is not found to inhibit the enzyme, more research will be needed to be done to determine the target of the drug. Optimisation of the synthesis of **9** would increase the overall yield in large scale reactions for further biological testing.

3.2.1. Presence of inhibitory activity

If the carbonic anhydride assay results show that **9** has inhibitory activity and **12** does not, the enzyme may be assumed to be the target of the drug. This could initiate research to optimising the SAR of the compound by analysing small lipophilic analogues of the compound. Structures of compounds known to inhibit the enzyme such as acetazolamide **25** may provide information required to optimise the SAR.[88] In doing so it will be possible to synthesise analogues of **9** such as **26** with increased activity against carbonic anhydrase and *P. falciparum*. In the future, investigating the *in vivo* effects of the drug will prove important. Experiments in mice to demonstrate the potency and stability *in vivo* whilst determining the effective and lethal dose of the drug.



25



26

Figure 11. Known CA inhibitor, acetazolamide **25**, and analogue of compound **9** with possible increased potency, **26**.

3.2.2. Absence of inhibitory activity

If OSM-S-106 doesn't in fact inhibit carbonic anhydrase as expected, more testing will be needed to consider the possibility of other proteins being the target of the drug. The possibility of *P. falciparum* Asparaginyl tRNA Synthetase (*Pf*AsnRS) being the target was investigated however no activity could be detected for both the positive and negative control.[89] Compounds containing a sulfonamide group are used considerably in the treatment of many parasitic diseases, including those from the *Plasmodium* species of parasites.[90] Drugs

in this group of compounds, such as sulfadoxine **7** and dapsone **28** (Figure 12), act by inhibiting dihydropteroate synthase, an enzyme involved in the folate synthesis pathway.[91] These drugs are commonly used in the treatment for malaria alongside pyrimethamine **6,** a drug which acts by inhibiting dihydrofolate reductase (DHFR). Parasites in the P*lasmodium* family produce most of their folates *de novo*, therefore by inhibiting DHPS and DHFR it would be possible to decrease DNA synthesis, resulting in cell death, treating the disease.[92]

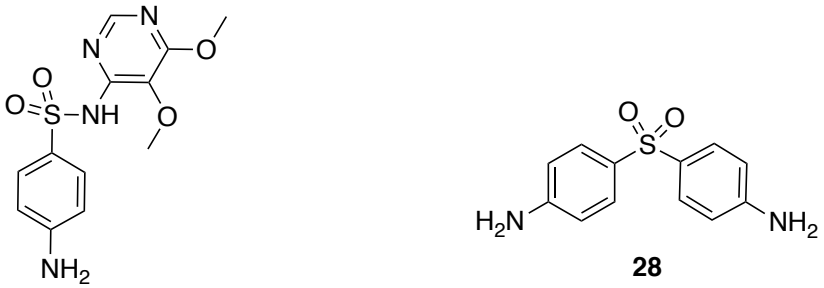
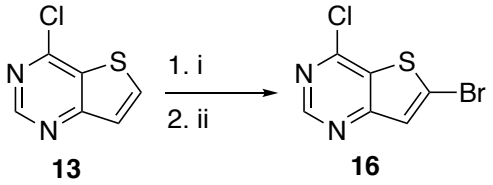


Figure 12. Drugs sulfadoxine **7** and dapsone **28** used for the treatment against the *Plasmodium* parasite family.

3.2.4. Synthesis optimisation

It is always important to ensure that the highest yield and purity are obtained when synthesising a drug compound. As it was found that the method of brominating **13** at the 6 position to synthesise **16** produced a number of side products leaving the yield of the wanted product to be much lower than desired. Therefore, investigating a more efficient method of brominating **13** would be useful to produce OSM-S-106 both selectively and with a high yield. Due to time constraints it was not possible to complete the reaction recorded in the literature by Woodring *et al.* to determine whether this process can selectively synthesise **16** so this would be desirable for future work.[53] It would also be worthwhile to repeat this experiment with the same conditions in this study to fully characterise the side products.



Scheme 16. Synthesis of **16** where i) THF, LDA; ii) 1,2-dibromo-1,1,2,2-tetrafluoroethane.

Chapter 4: Experimental

4.1. General Experimental Details

Commercially available reagents were purchased from either Sigma-Aldrich or Fisher Scientific. Butyllithium reagent was titrated prior to use to determine concentration using the method described in the Chemistry Experimental Details below. No further purification was used on any reagent unless otherwise specified. To maintain anhydrous conditions when required glassware was oven dried and assembled hot before being allowed cool to room temperature. When inert atmospheric conditions were required the reaction was performed under Ar and continually mixed with a magnetic stirrer bar.

Room temperature was 15-25 °C. Reactions under elevated temperatures were heated either using a metal hot plate and stand or silicon oil bath, while being monitored using a temperature probe. A temperature of -46 °C was maintained with an acetonitrile/dry ice bath. A temperature of -78 °C was maintained with an acetone/dry ice bath. TLC was performed on Merck Silica Gel 60 F_{254} aluminium sheets and visualised by UV irradiation (254 nm) using an appropriate stain if necessary. Column chromatography was carried out either A) manually using Aldrich silica gel 40-63 µm using a hand pump to maintain a controlled flow of solvent though the stationary phase or B) using the Biotage Selekt. The compound to be purified was either applied as a solution in EtOAc/Hexane or pre-adsorbed onto silica.

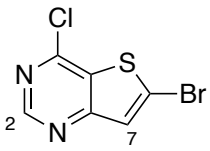
^{1}H and ^{13}C NMR spectra were obtained on the Bruker 400 Advance. Chemical shifts (δ) are reported in ppm with respect to either TMS or residual solvent. ^{1}H NMR signals are reported with multiplicity, relative integrals, coupling constants J (Hz) and assignments. All samples were dried in vacuo prior to NMR spectroscopy. LCMS was performed using the Agilent 1260 Infinity II. Melting points were measured using the Stuart SMP10 melting point system. IR spectra were obtained on the Agilent Cary 630 FTIR.

4.2. Chemistry Experimental Details

Titration of *n*-BuLi

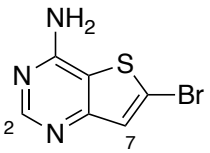
Titration was performed according to a literature procedure.[93] N-benzylbenzamide (200 mg, 0.95 mmol) was dissolved in dry THF (10 mL). The solution was stirred at -46 °C before the dropwise addition of *n*-BuLi. The addition continued until a constant pale blue colour was observed. Total *n*-BuLi added: 0.84 mL, 0.95 mmol, 1 eq., concentration = 1.13 M.

6-Bromo-4-chlorothieno[3,2-*d*]pyrimidine (16)



4-Chlorothieno[3,2-*d*]pyrimidine (5.53 g, 32.39 mmol, 1.00 equiv) was dissolved in dry THF (270 mL) and stirred at -78 °C under Ar. *n*-Butyllithium (1.13 M, 36.00 mL, 40.68 mmol, 1.26 equiv) was added dropwise to the reaction at -78 °C. Bromine (1.60 mL, 31.20 mmol, 1.00 equiv) was added and stirring was continued at -78 °C for 1 h before the mixture was allowed to warm to rt and left stirring overnight. After 18 h the reaction mixture was washed with saturated sodium thiosulfate (150 mL) and ethyl acetate (150 mL). The organic layers were washed with water (100 mL) and brine (100 mL) and the organic layers were dried with anhydrous $MgSO_4$ and concentrated under reduced pressure to give the crude compound; purified by flash chromatography (0-20% EtOAc in hexane) to give **16** as a pale yellow fluffy solid (0.9532g, 12%). $\mathbf{R_f}$ = 0.35 in 10% ethyl acetate in hexane. **mp** 139-140 °C (lit.[94] 138.1–138.9 °C). **^{1}H NMR** (500 MHz, $CDCl_3$) δ_H 8.96 (s, 1H, H^2), 7.64 (s, 1H, H^7) ppm. **^{13}C NMR** (500 MHz, $CDCl_3$) δ 163.5 (*C*-Cl), 154.5 (CH, C^2), 153.3 (C, *C*-N), 131.9 (C, *C*-S), 127.5 (C, *C*-Br), 126.7 (CH, C^7) ppm. **FTIR** (ν_{max}/cm^{-1}) 3070 (C-H), 1513 (C=C), 1360 (C-N). **LCMS** (ESI+) m/z calcd. for $C_6H_3{}^{79}BrClN_2S$ $[MH]^+$ 248.89, $C_6H_3{}^{81}BrClN_2S$ $[MH]^+$ 250.89, found 249.0 $[MH]^+$, 251.0 $[MH]^+$. Analytical data (MP, ^{1}H NMR, LCMS) in agreement to those recorded in literature.[94, 53]

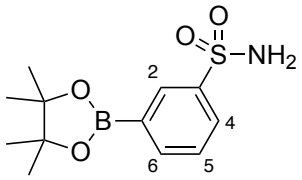
6-Bromothieno[3,2-*d*]pyrimidin-4-amine (21)



Ammonium hydroxide solution (28%, 2.66 mL) and 2-propanol (1.33 mL) were added to 4-chloro-6 bromothieno[3,2-*d*]pyrimidine (126.2 mg, 0.51 mmol, 1.00 equiv) and stirred in a sealed tube at 50 °C for 21 h. The reaction was washed with ethyl acetate (100 mL) The organic layers were washed with water (100 mL) and brine (100 mL) and the combined aqueous layers were washed with ethyl acetate (75

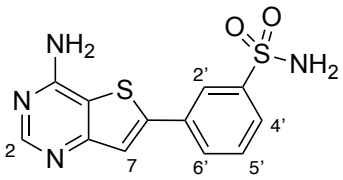
mL). The organic layers were combined, dried with anhydrous $MgSO_4$ and concentrated under reduced pressure to give the **21** as a tan solid (97.10 mg, 83%). $\mathbf{R_f}$ = 0.16 (50% ethyl acetate in hexane). **mp** 243.5 – 247 °C. **^{1}H NMR** (500 MHz, DMSO-d_6) δ_H 8.32 (s, 1H, H^2), 7.56 (s, 1H, H^7), 7.49 (s, 2H, NH_2) ppm. **^{13}C NMR** (126 MHz, DMSO-d_6) δ_C 159.6 (C, *C*-NH_2), 157 (C, C^2), 155.3 (C, *C*=N), 127.5 (C, C^7), 122.4 (C, *C*-Br), 115.5 (C, *C*-S) ppm. **FTIR** (ν_{max}/cm^{-1}) 3265 (N-H), 1669 (C=C), 1278 (C-N). **LCMS** (ESI+) m/z calcd. for $C_6H_5{}^{79}BrN_3S$ $[MH]^+$ 229.9, $C_6H_5{}^{81}BrN_3S$ $[MH]^+$ 231.9, $C_6H_3{}^{79}BrN_3S$ $[MH]^-$ 227.9, $C_6H_3{}^{81}BrN_3S$ $[MH]^-$ 229.9, found 230.1 $[MH]^+$, 232.1 $[MH]^+$, 228.1 $[MH]^-$, 230.0 $[MH]^-$. Analytical data was in agreement with those recorded by previous students but was not found to be recorded in literature.

3-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzenesulfonamide (24)



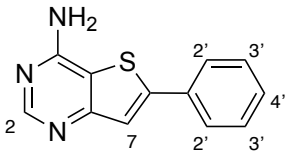
3-Bromobenzenesulfonamide (2.27 g, 9.60 mmol, 1.00 equiv), bis(pinacolatodiborane) (3.02 g, 11.88 mmol, 1.20 equiv), and dry potassium acetate (2.87 g, 29.21 mmol, 3.10 equiv) were dissolved in dioxane (98 mL) and purged with Ar. $PdCl_2$(dppf) (827 mg, 0.11 mmol, 0.01 equiv) was added and the reaction was stirred at reflux for 18 h to form a black reaction mixture. The reaction was allowed to cool to rt and washed with ethyl acetate and water (1:1, 200 mL). The organic layer was dried with anhydrous $MgSO_4$ and concentrated under reduced pressure to give a brown solid (3.30 g). Which was purified by flash chromatography, (20-100% ethyl acetate in hexane) to give the **24** as a light brown flaky solid (1.06g, 39%). $\mathbf{R_f}$ = 0.17 (25% ethyl acetate in hexane). **mp** 212 – 215 °C. **^{1}H NMR** (500 MHz, DMSO-d_6) δ_H 8.14 (s, 1H, H^2), 7.93 (d, J = 8.2 Hz, 1H, H^4), 7.85 (d, J = 7.4 Hz, 1H, H^6), 7.59 (t, J = 7.6 Hz, 1H, H^5), 7.36 (s, 2H, NH_2), 1.31 (s, 12H, 4xCH_3) ppm. **^{13}C NMR** (126 MHz, DMSO-d_6) δ_C 143.7 (C, *C*-S), 137.4 (CH, C^6), 131.3 (CH, C^2), 128.6 (CH, C^5), 128.4 (CH, C^4), 84.2 (C, 2 x $C(CH_3)_2$), 24.6 (CH_3, 4xCH_3) ppm. **FTIR** (ν_{max}/cm^{-1}) 3317 (N-H), 2978 (C-H), 1412 (S=O), 1133 (C-O). **LCMS** (ESI+) m/z calcd. for $C_{12}H_{19}BNO_4S$ $[MH]^+$ 284.1, $C_{12}H_{17}BNO_4S$ $[MH]^-$ 282.1 found 284.1 $[MH]^+$, 282.3 $[MH]^-$. Analytical data (^{1}H NMR, LCMS) in agreement to those recorded in literature.[95, 96]

3-(4-Aminothieno[3,2-*d*]pyrimidin-6-yl)benzenesulfonamide (OSM-S-106) (9)



6-Bromothieno[3,2-*d*]pyrimid-4-amine (40.33 mg, 0.18 mmol, 1.00 equiv) and (3-sulfamoylphenyl)boronic acid pinacol ester (61.34 mg, 0.22 mmol, 1.24 equiv) were dissolved in 2-propanol (4.50 mL) and potassium carbonate solution (1.00 M, 0.5 mL). The mixture was purged with argon and $PdCl_2$(dppf) (25.65 mg, 0.04 mmol, 0.20 equiv) was quickly added. The reaction mixture was covered with a blanket of argon and the reaction was heated at 50 °C for 3.5 h in a sealed vessel. The mixture diluted with ethyl acetate (50 mL), filtered through celite, and washed with water (50 mL) and brine (50 mL). The organic layers were dried with anhydrous $MgSO_4$ and concentrated under reduced pressure to afford crude material as a brown solid (9.50 mg) which was purified by flash chromatography (Biotage, 0-10% MeOH in EtOAc) affording the **9** as a tan solid (6.4 mg, 22%). $\mathbf{R_f}$ = 0.23 in 100% ethyl acetate. **mp** 266-268 °C. **^{1}H NMR** (500 MHz, DMSO-d_6) δ_H 8.40 (s, 1H, H^2), 8.22 (s, 1H, $H^{2'}$), 8.12 (d, 1H, $H^{4'}$), 7.90 (d, 1H, $H^{6'}$), 7.89 (s, 1H, H^7), 7.74 (t, 1H, $H^{5'}$), 7.54 (2, 2H, NH_2), 7.52 (s, 2H, S-NH_2) ppm. **^{13}C NMR** (500 MHz, DMSO-d_6) δ 159.2 (C, *C*-NH_2),158.4 (C, *C*=N), 155.1 (CH, C^2), 147.0 (C, *C*-C-$C^{2'}$), 144.9 (C, *C*-S=O), 132.5 (C, *C*-$C^{2'}$), 130.2 (C, $C^{5'}$), 131.1 (C, $C^{4'}$), 127.3 (C, C^7), 123.4 (C, $C^{2'}$), 121.7 (C, $C^{6'}$), 113.7 (C, *C*-S) ppm **FTIR** (ν_{max}/cm^{-1}) 3395 (N-H), 3121 (C-H), 1569 (C=C), 1334 (S=O), 1155 (C-N). **LCMS** (ESI+) m/z calcd. for $C_{12}H_{11}N_4O_2S_2$ $[MH]^+$ 307.0, $C_{12}H_9N_4O_2S_2$ $[MH]^-$ 305.0 found 307.2 $[MH]^+$, 305.2 $[MH]^+$. Analytical data was in agreement with those recorded by previous students but was not found to be recorded in literature.

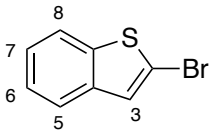
6-Phenylthieno[3,2-*d*]pyrimidin-4-amine (OSM-S-123) (12)



6-Bromothieno[3,2-*d*]pyrimid-4-amine (337.70 mg, 1.47 mmol, 1.00 equiv) and phenylboronic acid pinacol ester (372.30 mg, 1.82 mmol 1.20 equiv) were dissolved in 2-propanol (29 mL), followed by the addition of potassium carbonate solution (1 M, 3 mL). The mixture was purged with Ar and $PdCl_2$(dppf) (108.2 mg, 0.15 mmol, 0.10 equiv) was quickly added. The reaction mixture was covered with a blanket of Ar and heated to 80 °C for 3.5 h in a sealed vessel. The mixture was filtered through celite and washed with water (50 mL) and brine (50 mL). The organic layers were dried over anhydrous $MgSO_4$and concentrated under reduced pressure to afford crude material as a brown solid (198.00 mg) which was purified by flash chromatography in a glass pipette (100% EtOAc) to afford the **12** as a cream/brown solid, (126.00 mg, 38%). $\mathbf{R_f}$ = 0.23 in

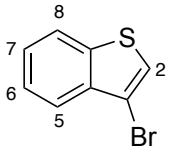
100% ethyl acetate. **mp** 278-282°C (lit.[87] 283-284°C). **^{1}H NMR** (500 MHz, DMSO-d_6) δ_H 8.37 (s, 1H, H^2), 7.85 (d, 2H, $H^{2'}$), 7.80 (s, 1H, H^7), 7.53 (t, 2H, $H^{3'}$), 7.48 (s, 1H, H4'), 7.45 (s, 2H, NH_2) ppm. **^{13}C NMR** (126 MHz, DMSO-d_6) δ_C 160.60 (C, *C*=N), 157.95 (C, *C*-NH_2), 155.23 (C, S-C-*C*=N), 148.77 (C, $C^{2'}$=*C*-S), 132.82 (C, *C*-$C^{2'}$), 129.55 (C, $C^{4'}$), 129.38 (2C, $C^{3'}$), 126.23 (2C, $C^{2'}$), 120.47 (C, C^2), 113.28 (CH, C^7) ppm. **FTIR** (ν_{max}/cm^{-1}) 3291 (N-H), 1666 (C=C), 1069 (C-N). **LCMS** (ESI+) m/z calcd. for $C_{12}H_{10}N_3S$ $[MH]^+$ 228.1, $C_{12}H_8N_3S$ $[MH]^-$ 226.1 found 228.1 $[MH]^+$. Analytical data (MP, ^{1}H NMR) in agreement to those recorded in literature.[87]

2-Bromobenzo[*b*]thiophene (18)



Benzo[*b*]thiophene (1.00 g, 7.45 mmol, 1.00 equiv) was dissolved in THF (36 mL) and stirred at -60°C for 10 minutes before the drop wise addition of *n*-BuLi (1.13 M, 7.25 mL, 1.10 equiv). The reaction was stirred at -60°C for 45 min. Bromine (0.38 mL, 7.45 mmol, 1.00 equiv) was added dropwise and stirred at -60°C for 10 minutes and allowed to stir at rt for 4 h. Saturated sodium thiosulfate solution (100 mL) was added and the mixture was washed with ethyl acetate. The organic extracts were combined and washed with brine solution (100 mL) and dried over anhydrous $MgSO_4$. The mixture was concentrated under reduced pressure to give clear brown oil (1.01 g). The oil was purified by flash chromatography (eluent: hexane) to give SM (64.6 mg, 6%) and the **18** as a pale yellow solid (0.94 g, 59%). **R_f** = 0.82 in 100% hexane **mp** 43-45 °C (lit.[97] 38–40 °C, lit.[98] 46–47 °C) **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.69-7.75 (m, 2H, H^{5+8}), 7.31-7.37 (m, 3H, $H^{3,6+7}$) ppm. **FTIR** (ν_{max}/cm^{-1}) 3082 (C-H stretch), 1420 (C-H bend), 928.1 (C=C), 746 (C-Br). Analytical data (MP, ^{1}H NMR) in agreement to those recorded in literature.[97]

3-Bromobenzo[*b*]thiophene (19)



- **Method 1**

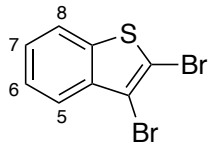
Benzo[*b*]thiophene (1.33 g, 9.88 mmol, 1.00 equiv) and NBS (1.80 g, 10.11 mmol, 1.00 equiv) were dissolved in chloroform (13 mL) and acetic acid (16 mL) and left stirring at rt for 43 h. The reaction mixture was diluted with chloroform (150 mL) and washed with saturated sodium thiosulfate solution (150 mL). The aqueous phase was extracted with chloroform (100 mL). The combined organic layers were washed with saturated sodium carbonate solution (100 mL) and dried over anhydrous $MgSO_4$. The mixture was concentrated under reduced pressure to give a purple oil (1.98 g) that was dissolved in

hexane and purified by plug chromatography leaving **19** as a clear colourless oil (1.18 g, 56.3%). **R_f** = 0.80 in 100% hexane **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.86-7.89 (m, 2H, H^{5+8}), 7.42-7.53 (m, 2H, H^{6+7}), 7.47 (s, 1H, H^2) ppm. **FTIR** (ν_{max}/cm^{-1}) 3060 (C-H stretch), 1420 (C-H bend), 928.1 (C=C), 749 (C-Br). Analytical data (^{1}H NMR, FTIR) in agreement to those recorded in literature.[99]

- **Method 2**

Benzo[*b*]thiophene (1.43 g, 10.68 mmol, 1.00 equiv) and NBS (3.79 g, 21.27 mmol, 2.00 equiv) were dissolved in Chloroform (13 mL) and Acetic acid (16 mL) and left stirring at RT for 43 h. Chloroform (150 mL) was added to the reaction mixture and washed with sodium thiosulfate solution (150 mL). The aqueous phase was extracted with chloroform (100 mL). The organic extracts were combined and washed with saturated sodium carbonate solution. The mixture was dried over anhydrous $MgSO_4$ and filtered before being concentrated under reduced pressure to give a purple oil (2.11 g). The crude product was dissolved in hexane and purified by silica column chromatography leaving a clear pale pink oil (1.19 g) determined by ^{1}H NMR peak integration to be **20** (0.3244 g, 10%) and **19** (0.8666 g, 38%) as an inseparable mixture.

2,3-Dibromobenzo[*b*]thiophene (20)



Benzo[*b*]thiophene (1.42 g, 10.60 mmol) was dissolved in chloroform (20 mL). Bromine (1.2 mL, 23.42 mmol) in chloroform (20 mL) was added in portions over 20 minutes at rt before being left stirring for 21 h in the dark at rt. Solid sodium hydrogen carbonate (5 g) was added to the reaction mixture. The reaction mixture was washed with water (100 mL), the aqueous phase was washed with chloroform (100 mL). The organic extracts were combined and washed with saturated sodium thiosulfate (100 mL) and dried with anhydrous $MgSO_4$. The mixture was concentrated under reduced pressure to give a light brown oil which solidified upon cooling to room temperature to give an off **20** as a white solid (2.82g, 91%). **mp** 57-59 ^oC (lit.[100] 58-59 ^oC). **^{1}H NMR** (400 MHz, $CDCl_3$) δ_H 7.69-7.75 (m, 2H, H^{5+8}), 7.36-7.44 (m, 2H, $H^{,6+7}$) ppm. **FTIR** (ν_{max}/cm^{-1}) 3056 (C-H stretch), 1423 (C-H bend), 980 (C=C), 738 (C-Br). Analytical data (MP, ^{1}H NMR, FTIR) in agreement to those recorded in literature.[100]

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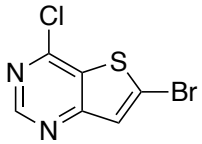
Appendix A) Fused Heterocycle Nomenclature

The heterocyclic ring-systems described in Results and Discussion are named using IUPAC nomenclature, the rules of which are described below using 4-chloro-6-bromo[3,2-*d*]pyrimidine as an example.[†]



1) Both the constituents of the ring system are named - pyrimidine and thiophene.

2) The parent component is identified as the ring system with the highest position in the hierarchy and placed last in the bicyclic structure name (i.e. thienopyrimidine). This hierarchy is described below:

   a) The component containing the heteroatom occurring earliest in the order of: N,F, Cl, Br, I, O, S, Se, Te, P, As, Sb, Bi, Si, Ge, Sn, Pb, B, Hg.

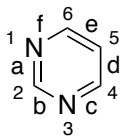
   b) The component containing the largest ring.

   c) A component containing the largest number of heteroatoms.

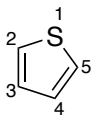
   d) A component containing the greater variety of heteroatoms.

3) The attached component is added as a prefix to the parent compound, and the prefix '-ene' is changed to '-eno'. For example, 'thiene' to 'thieno' - 'thieno' is one of a few acceptable abbreviations in replacement of 'thiopheno'. This determines the fused name to be thienopyrimidine.

4) The atoms of the parent component are numbered whilst the bonds lettered in order, starting with the bond between atoms 1 and 2:

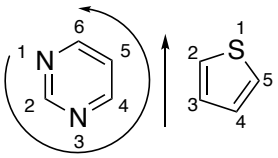


5) The attached component is numbered:



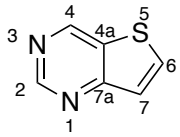
[†] G. P. Moss. Nomenclature of Fused and Bridged Fused Ring Systems, Pure Appl. Chem. 70, 143 (1998). DOI: https://doi.org/10.1351/pac199870010143

6) The atom numbers where both rings fuse are placed within brackets between the two components of the fused name. The order of which is determined by the direction of the parent component numbering:

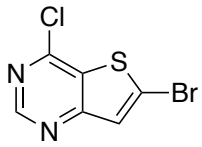


7) The bond letter of the parent compound where the rings fuse is italicized and placed in the brackets after the atom numbers - thieno[3,2-*d*]pyrimidine.

8) The numbering of the fused system starts at an atom adjacent to a fusion carbon and numbered ensuring the heteroatoms receive the smallest possible number. Fusion carbon atoms are given the number of the preceding position and have a letter attached:



Finally resulting in the name of the compound to be 4-chloro-6-bromo[3,2-*d*]pyrimidine:



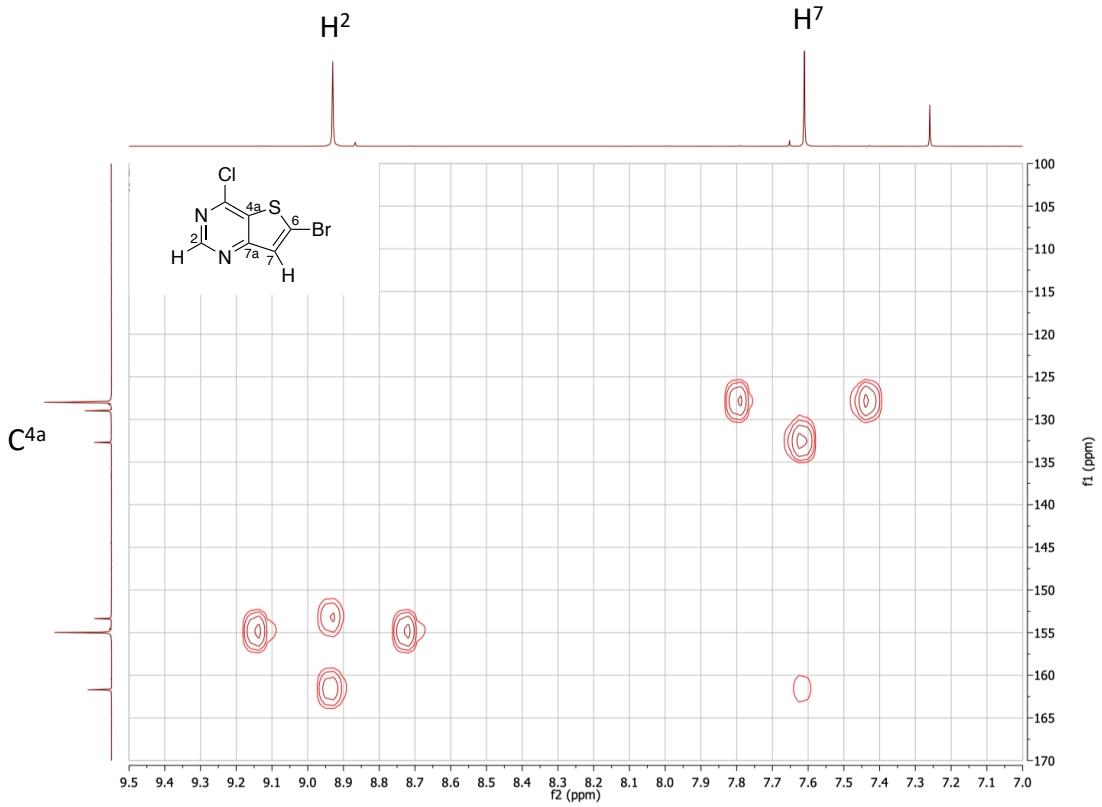
v) HMBC of 6-bromo-4-chlorothieno[3,2-d]pyrimidine (**16**) compared to that of 7-bromo-4-chlorothieno[3,2-d]pyrimidine.

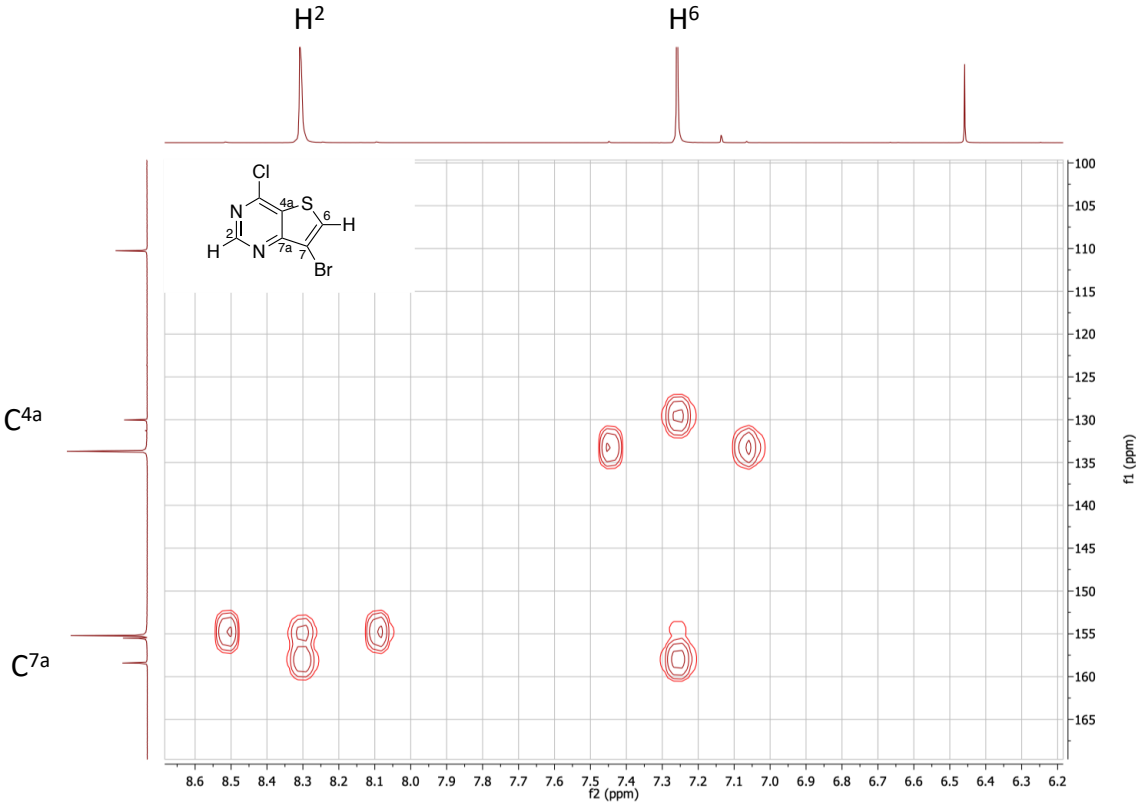
vi) Comparison of the ^{1}H NMR spectra for 6-Bromothieno[3,2-*d*]pyrimidin-4-amine (**21**) and 6-bromo-4-chlorothieno[3,2-d]pyrimidine (**16**)

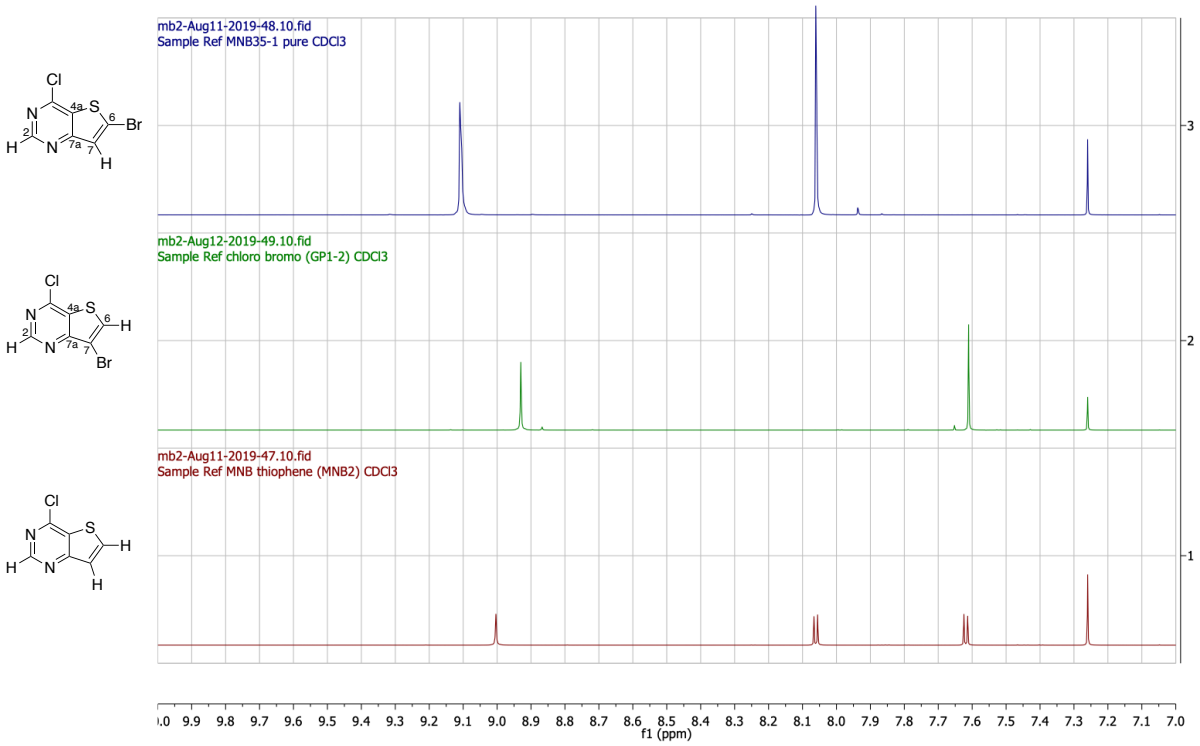
vii) Analytical data obtained for 3-(4-Aminothieno[3,2-*d*]pyrimidin-6-yl)benzenesulfonamide (OSM-S-106) (**9**)6-Bromothieno[3,2-*d*]pyrimidin-4-amine (**21**)

viii) Analytical data obtained for 6-Phenylthieno[3,2-*d*]pyrimidin-4-amine (OSM-S-123) (**12**)

i) HMBC of 6-bromo-4-chlorothieno[3,2-d]pyrimidine (**16**) compared to that of 7-bromo-4-chlorothieno[3,2-d]pyrimidine.



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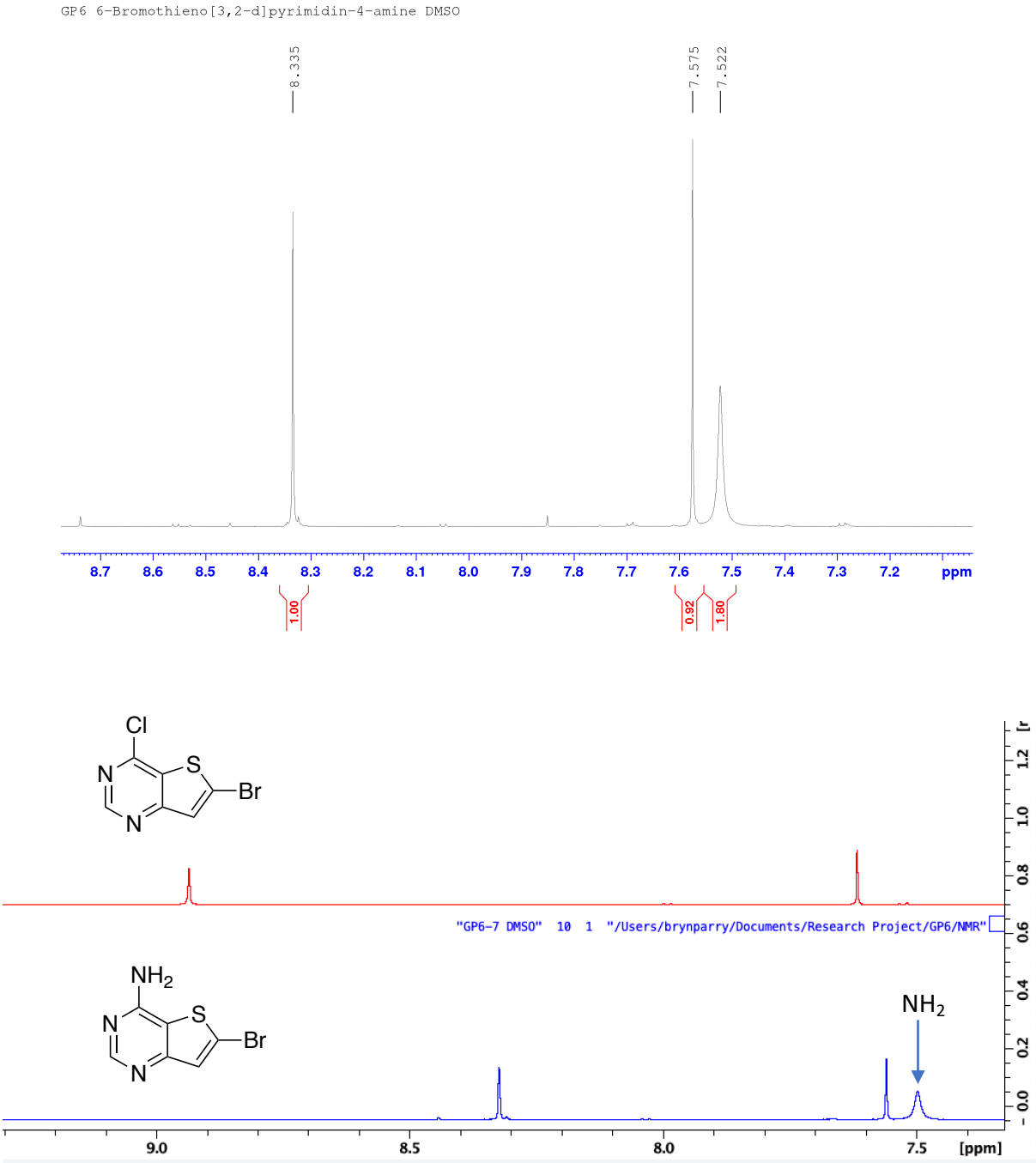
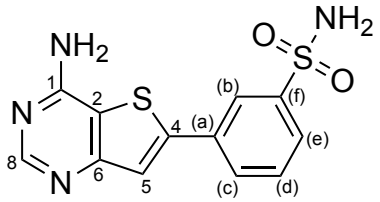
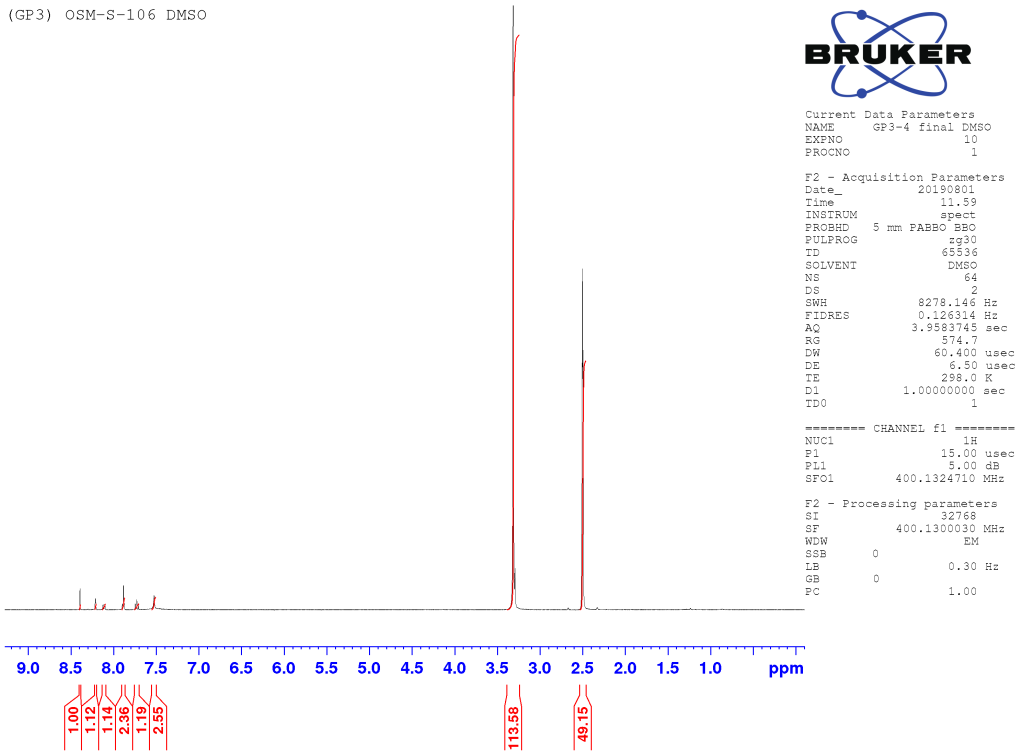
mb2-Aug12-2019-49.10.fid
Sample Ref chloro bromo (GP1-2) CDCl3
mb2-Aug11-2019-47.10.fid
Sample Ref MNB thiophene (MNB2) CDCl3
f1 (ppm)

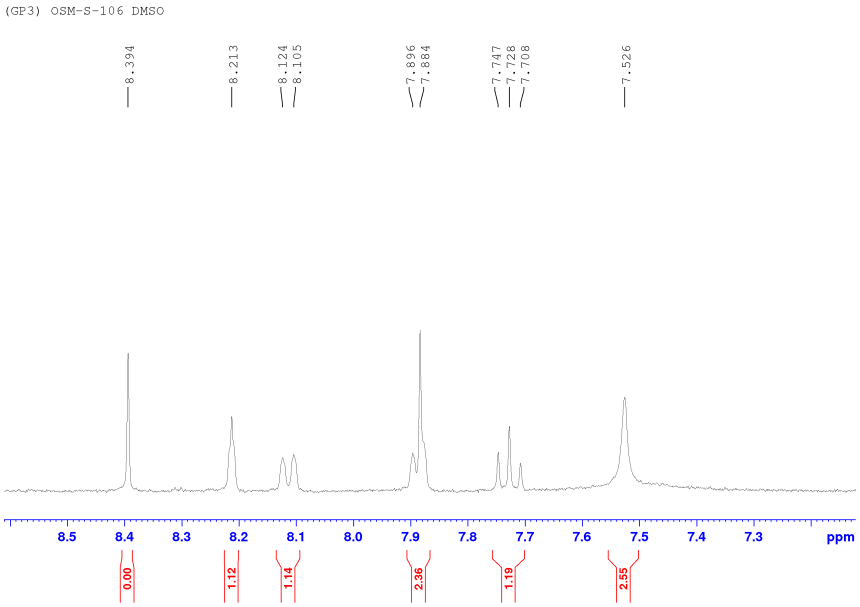
ii) Comparison of the ^{1}H NMR spectra for 6-Bromothieno[3,2-*d*]pyrimidin-4-amine (**21**) and 6-bromo-4-chlorothieno[3,2-d]pyrimidine (**16**)

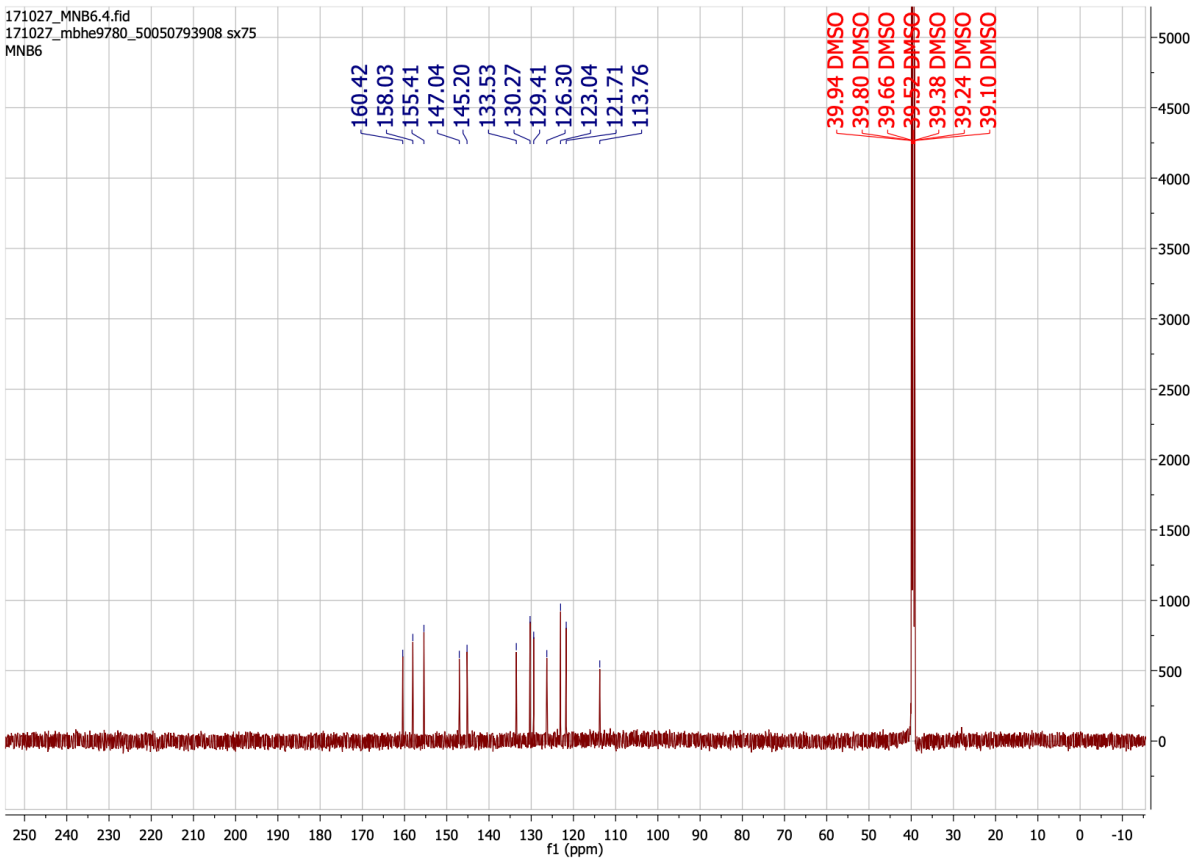


iii) Analytical data obtained for 3-(4-Aminothieno[3,2-*d*]pyrimidin-6-yl)benzenesulfonamide (OSM-S-106) (**9**)





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158.03
155.41
147.04
145.20
133.53
130.27
129.41
126.30
123.04
121.71
113.76
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39.66 DMSO
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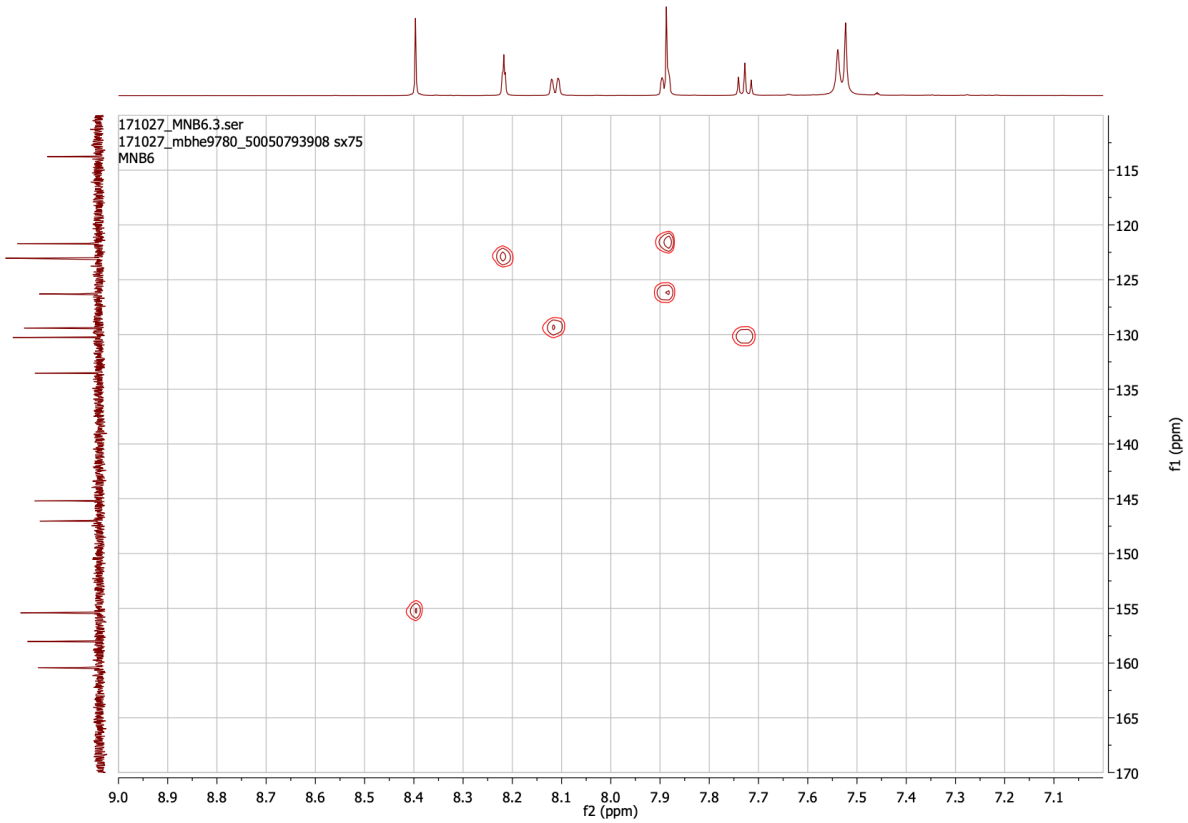
39.38 DMSO
39.24 DMSO
39.10 DMSO
f1 (ppm)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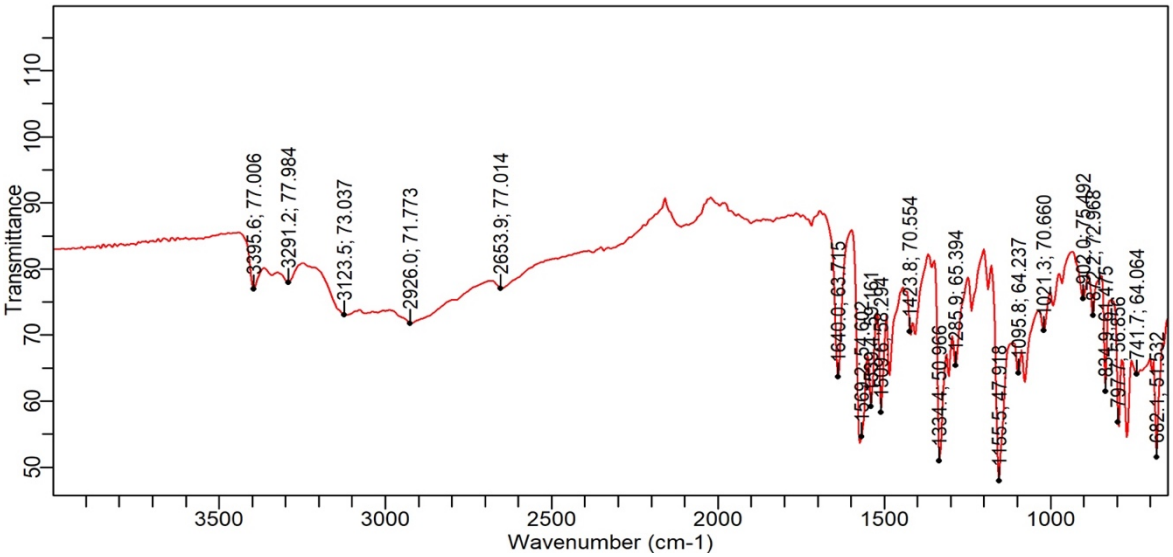
f2 (ppm)
f1 (ppm)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3291.2; 77.984
3123.5; 73.037
2926.0; 71.773
2653.9; 77.014
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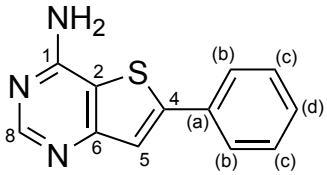
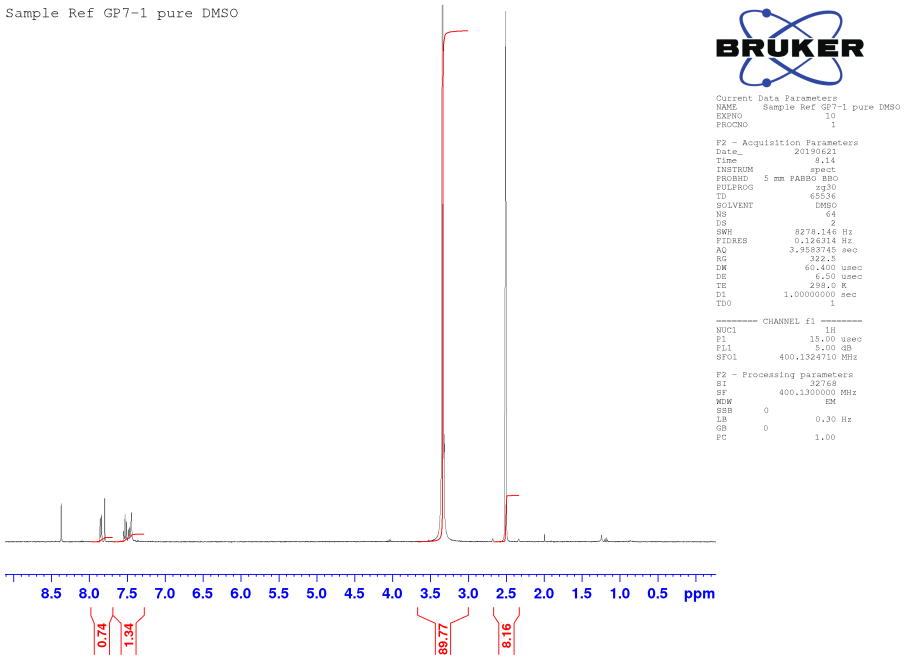
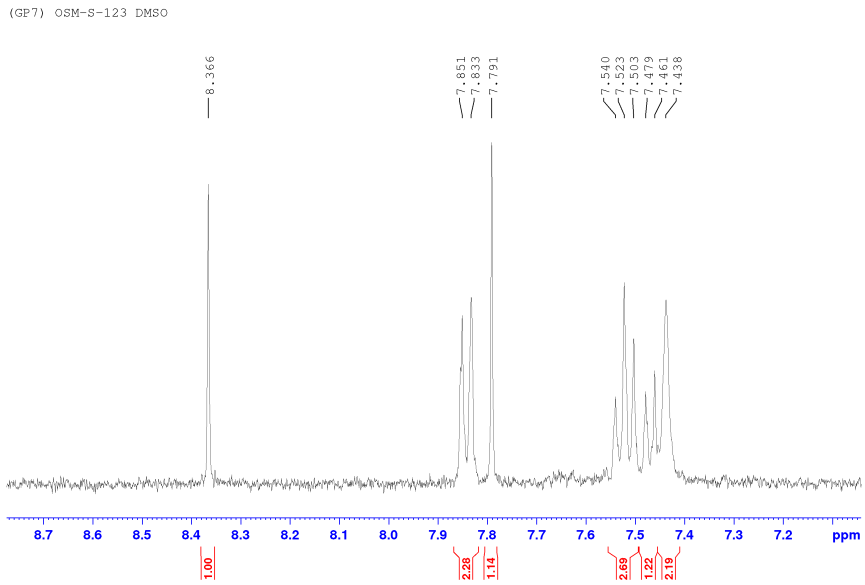
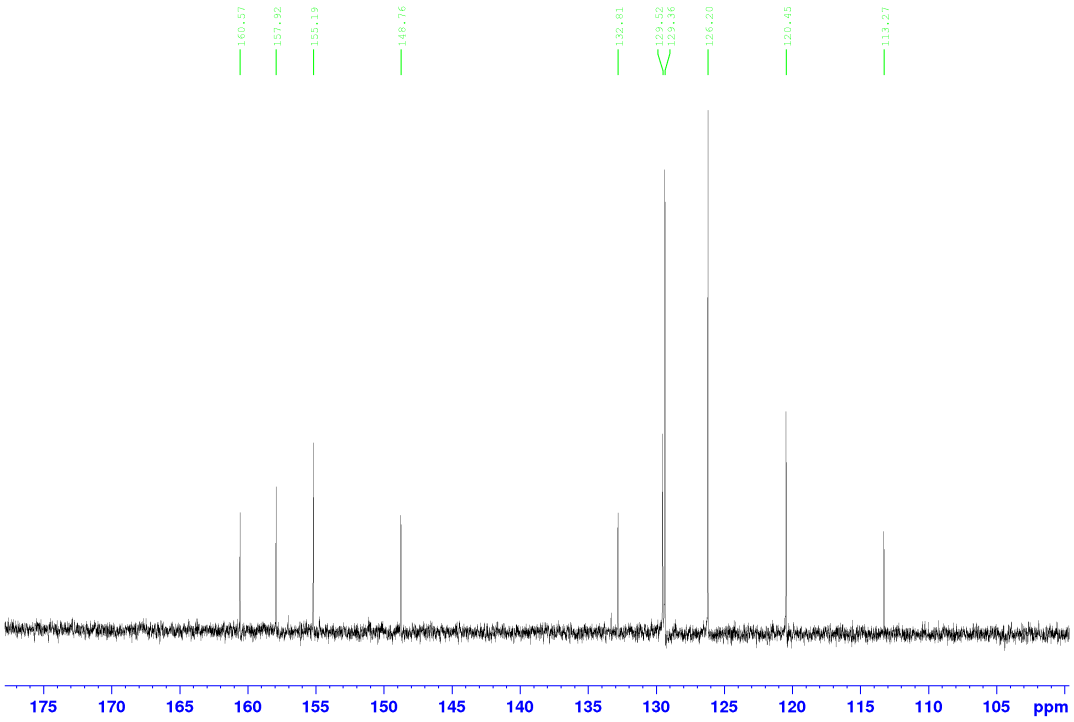
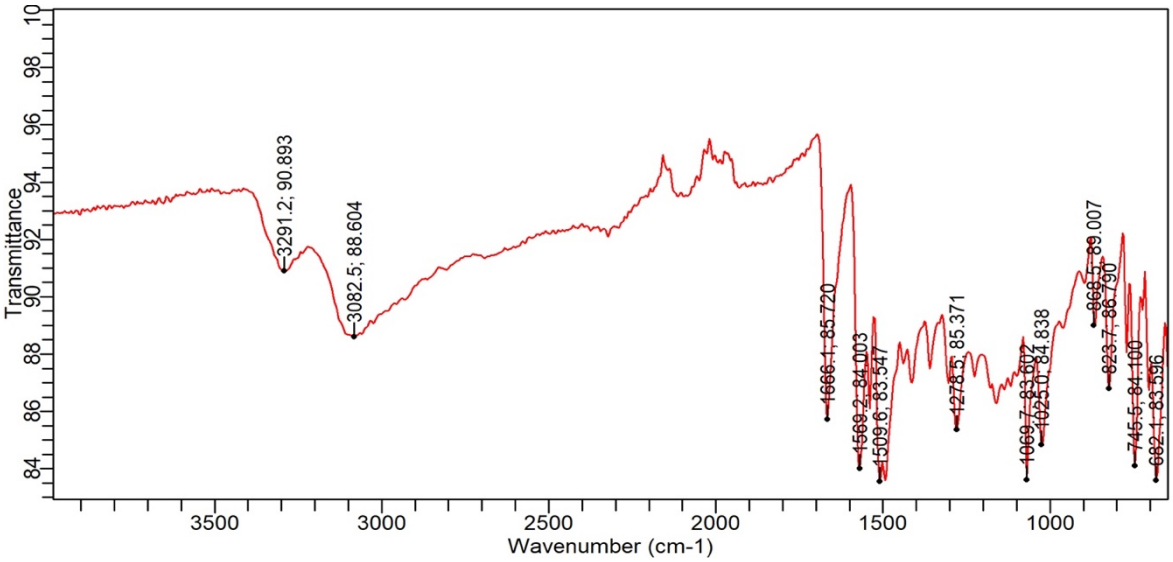
1423.8; 70.554
1334.4; 50.966
1285.9; 65.394
1155.5; 47.918
1095.8; 64.237
1021.3; 70.660
741.7; 64.064
Transmittance
Wavenumber (cm-1)

iv) Analytical data obtained for 6-Phenylthieno[3,2-*d*]pyrimidin-4-amine (OSM-S-123) (**12**)



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