Open Source Drug Discovery: Efforts Toward the Synthesis and Evaluation of Two Antimalarial Drug Candidates

A thesis submitted in partial fulfilment of the requirement for admission to the degree of

Bachelor of Science (Honours)

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Preface

1. Statement of Contribution of the Student

The concept of applying an open source approach to antimalarial drug development was conceived by Dr. Matthew Todd. The two antimalarial drug candidates that are the focal point of this work were identified as worthwhile drug candidates by the Medicines for Malaria Venture. The hits considered by the Medicines for Malaria Venture were identified from a high throughput screen performed by GlaxoSmithKline, Tres Cantos. Medicinal chemistry advice pertaining to the development of the two drug candidates was given by Dr. Paul Willis, of the Medicines for Malaria Venture, and by Dr. Matthew Todd. *In silico* identification of commercial analogues was performed by Dr. Iain Wallace and myself. Advice on how to obtain these commercial analogues was provided by Dr. Christopher Southan. These analogues were checked for known inactivity against malaria by Felix Calderon of GlaxoSmithKline, Tres Cantos. Biological testing of samples was performed by Professor Vicky Avery and Dr. Sabine Mangold of Griffith University.

Low resolution mass spectrometry was performed by Dr. Nick Proschogo, Mr. Christopher Phippen, Dr. Paul Ylioja and myself. High resolution mass spectrometry was performed by Dr. Nick Proschogo. Assistance with acquiring mass spectra was provided by Dr. Nick Proschogo. Nuclear magnetic resonance spectra were obtained by Ms. Althea Tsang, Mr. Rob Thompson and myself. Assistance with acquiring nuclear magnetic resonance spectra was provided by Dr. Ian Luck. Infrared spectra were obtained by Mr. Angus Jones and myself. Elemental analysis was performed by Mr. Bob McAllister at the Campbell Microanalytical Laboratory at the University of Otago, New Zealand. Experimental equipment and facilities were maintained by Mr. Bruce Dellit and Mr. Carlo Piscicelli. Proof reading of this thesis was performed by Dr. Matthew H. Todd, Dr. Paul Ylioja, Ms. Althea Tsang and Dr. Alice Williamson.

The suggestion to use *N*-iodosuccinimide to carry out iodination of thieno[3,2-*d*]pyrimid-4-one was provided by Dr. Stuart Wepplo, Dr. Stuart Mickel and other anonymous sources at http://www.chemicalforums.com. The Buchwald-Hartwig cross coupling reaction was suggested as a source of interference with Suzuki couplings by anonymous sources at the same website. Methods for producing chlorine gas were suggested by Alastair Donald, *via* Twitter, and

authenticated by anonymous sources at http://www.sciencemadness.org and http://www.youtube.com

I certify that this report contains work carried out by myself except where otherwise acknowledged

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Signature and Date

2. Acknowledgements

A most sincere appreciation is extended to Dr. Matthew H. Todd for the guidance I was afforded throughout the year. It was a pleasure to undertake this project with you. Particular thanks are given for the advice on how to be more scientific, and less alchemic, in my approach to chemistry, and for the lessons on how to transform my floral writing style – developed during a healthy education in the humanities department – into something resembling a more succinct, scientific approach. Finally, thanks are given for always responding to my oftentimes rude and highly-strung personal communications in a polite and courteous manner, even when my own nervous and stressed self could not respond in kind.

An enormous debt of gratitude is owed to Dr. Paul Ylioja. It can be said with certainty that this project would surely have been a failure without your insight, scholarship and advice. The assistance you provided throughout the year went far beyond the call of duty, and for that, I am very grateful. Your departure from the Todd group, and from academia, represents the departure of a genuine asset that will be sorely missed. I look forward to the day when we can engage in debate over libertarian politics once more. I wish you the very best for the future and I hope that you have a wonderful life with your new family.

To Dr. Murray Robertson and to Ms. Althea Tsang, a most warm thank you is given. I am deeply indebted to the both of you for your collective wisdom, time and (perhaps begrudging) willingness to lend out glassware. It was wonderful to have four ears to which I could address my pent up rage, and to have two mouths which spoke to me of the same ills. Without our tandem nagging and passive-aggressive behaviour the laboratory would be an inhospitable jungle of gorilla behaviour. To Mr. Angus Jones is extended a special thank you, for without your friendship my honours year would surely have been far less enjoyable, far too quiet, and not hilarious enough. Keep up with your tutoring job and avoid violence gangs. Generous thanks are owed to Dr. Alice Williamson for the writing tips, and for her handy chemical phraseology, of which a more thorough explanation may be elsewhere found. Mr. Bruce Dellit and Mr. Carlo Piscicelli both warrant a mention for the hospitality and warmth they afforded me throughout the year. Special thanks is directed toward Dr. Nick Proschogo, for his warmth and willingness to run high resolution mass spectra for me at a moment's notice A motion of thanks is extended to the other – and I spent fifteen minutes coming up with this – Todd pod members (*Messrs* and *Mesdames* Anthony, Kat, Ming Feng, Nilupa, Soo, Matin, Cale, Matthew, Sarah and Zoe), with whom I shared the laboratory with this year. Thanks are also given to the

wider audience of this open source project, for the time spent reading project reports and openly posted problems.

I owe a tremendous amount of gratitude to my parents, Mr. Steven Cronshaw and Mrs. Shona Cronshaw, for their financial and emotional support this year, which formed the bedrock of this project. At last count I believe that you paid for part of my plane ticket back from Canada, the costs of associated with my moving out of home, three repairs for three separate mechanical problems with my car, my groceries for a good chunk of the year and numerous electricity and fuel bills. Let there be no doubt over how sincere my thanks to you both are. A final thankyou is extended to Ms. Sheila Amos, who despite asking for none of this, still put up with it at tremendous personal cost. This honours project generated significant stress in your life, which you didn't need, and I apologise for any pain you endured throughout the year. It is my sincere belief that my own perseverance throughout this year is owed entirely to the collective efforts of this trio, to whom this entire body of work is hereby dedicated, with love.

3. Abbreviations

2D	2-Dimensional
Å	Ångstrom
Ac	Acyl
ACT	Artemisinin Combination Therapy
Anal	Elemental Analysis
Anhyd	Anhydrous
APCI	Atmospheric-Pressure Chemical Ionisation
Aq	Aqueous
ATR	Attenuated Total Reflectance
AUD	Australian Dollars
Bn	Benzyl
Boc	*tert*-butyloxycarbonyl
br	Broad
Bu	Butyl
Calcd	Calculated
cLogP	Calculated 1-Octanol/Water Coefficient
Concd	Concentrated
d	Doublet
DDT	Dichlorodiphenyltrichloroethane
DMAP	4-Dimethylaminopyridine
DMF	*N,N*-Dimethylformamide
dppf	1,1'-Bis(diphenylphosphino)ferrocene
DPPIV	Dipeptidyl peptidase IV
dt	Doublet of Triplets
eg	Example
eq	Equivalents
ESI	Electrospray Ionisation
Et	Ethyl
FGI	Functional Group Interconversion
FT	Fourier Transform
FTICR	Fourier Transform Ion Cyclotron Resonance
g	Gram

GSK	GlaxoSmithKline
h	Hour
HEK 293	Human Embryonic Kidney 293 Cells
HEPG2	Human Hepatocellular Carcinoma
HMBC	Heteronuclear Multiple-Bond Correlation
HRMS	High Resolution Mass Spectrometry
HSQC	Heteronuclear Single Quantum Coherence
HTS	High Throughput Screen
Hz	Hertz
IC_{50}	Half Maximal Inhibition Concentration
IR	Infrared
IUPAC	International Union of Pure and Applied Chemistry
J	^{1}H-^{1}H coupling constant in Hertz
LCMS	Liquid Chromatography Mass Spectrometry
Lit	Literature Value
LRMS	Low Resolution Mass Spectrometry
m	Multiplet
M	Molecular Ion
m	Meta
Me	Methyl
mg	Milligram
MHz	Megahertz
mL	Millilitre
mm	Millimetre
mmol	Millimole
mp	Melting Point
MVI	Malaria Vaccine Initiative
n	Normal
NA	No Activity
NBS	*N*-Bromosuccinimide
NIS	*N*-Iodosuccinimide
Nm	Nanometre
nM	Nanomolar
NMR	Nuclear Magnetic Resonance

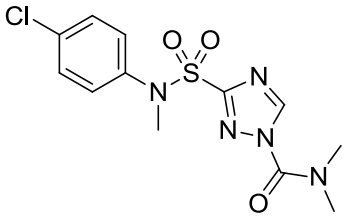
NR	No Reaction
NTD	Neglected Tropical Disease
o	Ortho
p	Para
P	*Plasmodium*
petrol	Petroleum ether
Ph	Phenyl
ppm	Parts Per Million
quant	Quantitative
rt	Room temperature
s	Singlet
SAR	Structure-Activity Relationship
S_EAr	Electrophilic Aromatic Substitution
sec	Secondary
SM	Starting Material
S_NAr	Nucleophilic Aromatic Substitution
t	Triplet
T	Tesla
t	Tertiary
TCAMS	Tres Cantos Anti Malarial Set
TCMDC	Tres Cantos Malaria Data Compound
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Trimethylsilane
tPSA	Topological Polar Surface Area
TSL	The Synaptic Leap
US	United States
UV	Ultra-violet
WHO	World Health Organisation
μL	Microlitre
μM	Micromolar

4. Abstract

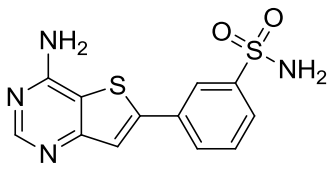
Malaria kills nearly a million people each year, most of them children. The development of resistance against the last, widely efficacious antimalarial treatments (the artemisinins) necessitates the development of new chemotherapies to treat malaria. GlaxoSmithKline expedited the search for new antimalarials by openly publishing the results of a screen of almost two million compounds against the parasite. Two potent hits from this screen have been identified, which appear suitable for a lead development campaign.

Open source science involves publicly sharing all data, ideas and discussions, and allowing anyone to participate. Solving the problems inherent to neglected tropical diseases is a task well suited to an open source approach. Herein, our efforts to synthesise and evaluate these hit compounds using the philosophy of open source science are described.

We report the use of a short, scalable synthetic route toward one of the hit compounds (**9**). The possible mechanism of formation of a crucial reactive species formed during this synthesis is also described. The biological evaluation of this hit indicated that it was more potent than originally reported by GSK, and validated the suitability of this compound for a lead development campaign. We describe the difficulties encountered during the attempted synthesis of the other hit compound (**10**), and the use of open discussion to find solutions to these problems. We describe the preliminary structure-activity relationship of this hit, based on the biological evaluation of several synthetic precursors.



9 (TCMDC 134395)
IC_{50} (3D7): 767 nM
%Inhibition HepG2 (10 μM): 7



10 (TCMDC 135294)
IC_{50} (3D7): 151 nM
%Inhibition HepG2 (10 μM): 13

Chapter 1: Introduction

1. Malaria

Despite a long history involving attempts at control and eradication, malaria remains an important neglected tropical disease (NTD).[1] In 2010, there were an estimated 216 million clinical cases of malaria that resulted in the deaths of 655,000 people, 86% of which were children aged 5 years and younger. The disease primarily affects populations in the world's poorest regions, with 81% of cases and 91% of deaths occurring in Africa.

The disease is caused by protozoa belonging to the *Plasmodium* genus. Five species of *Plasmodium* are capable of infecting humans (*P. falciparum*, *P. vivax, P. ovale, P. malariae* and *P. knowlesi*). Malaria caused by *P. falciparum* is the most deadly and most prevalent, and predominates in Africa, however *P. vivax* is particularly prevalent elsewhere and is estimated to cause as much as 25 – 40% of the global malaria burden.[2]

The symptoms of malaria are caused by the proliferation of *Plasmodium* protozoa in the human host.[3] Infection is caused by a bite from a female *anopheles* mosquito carrying malaria sporozoites, which, upon human transmission, mature to sexual stage gametocytes. These gametocytes are taken up by female *anopheles* mosquitoes during blood meals, and in the mosquito gut the gametocytes mature to form sporozoites, thereby completing the lifecycle (**Figure 1**).[4]

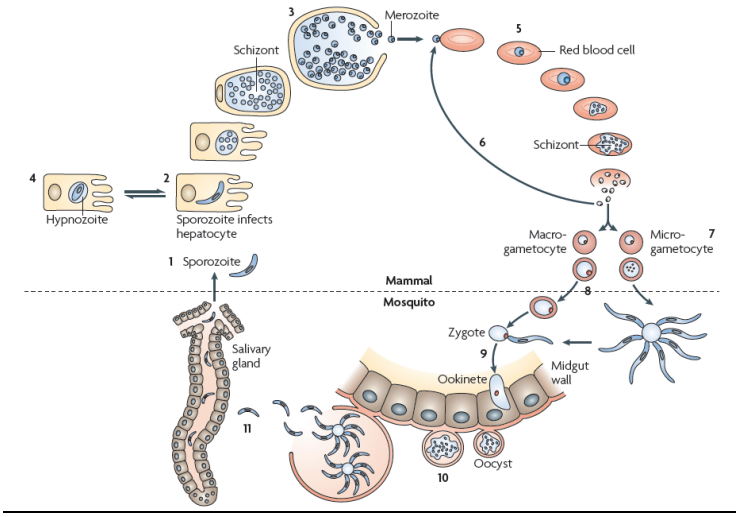
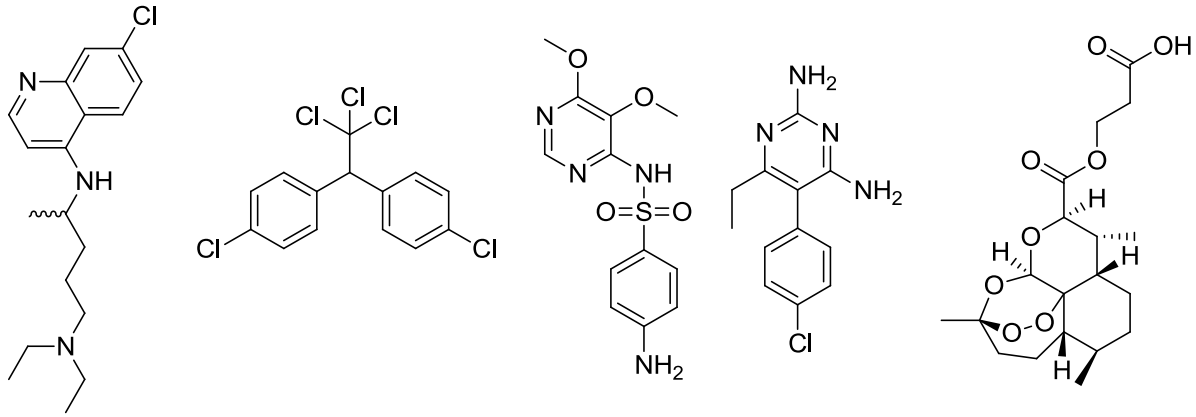


Figure 1: Lifecycle of the Malaria Parasite[4]

Each species of *Plasmodium* has a subtly different lifecycle. In the case of *P. falciparum* and *P. malariae*, infection is followed by rapid asexual division of the protozoa, resulting in the release of large numbers of merozoites into the bloodstream. In *P. vivax* and *P. ovale* some of the parasites become dormant in the liver for a period, which can be between 3 weeks or several years, before the protozoa are reactivated.

At present all forms of malaria are completely preventable and treatable. Various chemotherapies have been used to treat malaria in the past century (**Figure 2**). The combination of chloroquine (**1**) as a treatment for malaria, alongside the use of the insecticide DDT (**2**) as a vector control in the mid-20th century, proved to be a successful combination, generating hope within the WHO that malaria might be eradicated in the future.[5] Unfortunately, the heavy use of chloroquine generated resistance against its effects. Medicines developed subsequently such as sulfadoxine (**3**) and pyrimethamine (**4**) have also succumbed to the effects of resistance.[5] The exclusive use of these three medicines is now discouraged by the WHO as a result.[6] The artemisinin type drugs (such as artesunate, **5**) are newly developed antimalarials that posses a safe and efficacious profile.[7] In clinical practice an artemisinin is frequently paired with an antimalarial possessing a different mode of action (eg: chloroquine, **1**). These 'artemisinin combination therapies' (ACT's) are now recommended by the WHO as the front line treatment for uncomplicated malaria.



1 **2** **3** **4** **5**

Figure 2: Treatments Used for the Treatment and Prophylaxis of Malaria (from left to right: Chloroquine, DDT, Sulfadoxine, Pyrimethamine, and Artesunate)

The artemisinins are the last remaining, widely efficacious antimalarial treatment. Resistance has been observed in Southeast Asia and this has terrifying implications for those most at risk of infection.[8,7] Surveillance of dosage regiment compliance amongst those receiving ACTs and increased efforts to contain the spread of resistant strains are needed.[9]

No new chemical class of antimalarials has entered into clinical practice since 1996.[10] This necessitates a new armamentarium of chemotherapies with which to treat malaria. The current pipeline of antimalarial agents being developed (**Figure 3**) contains just under 60 individual projects at various stages of the drug development process.

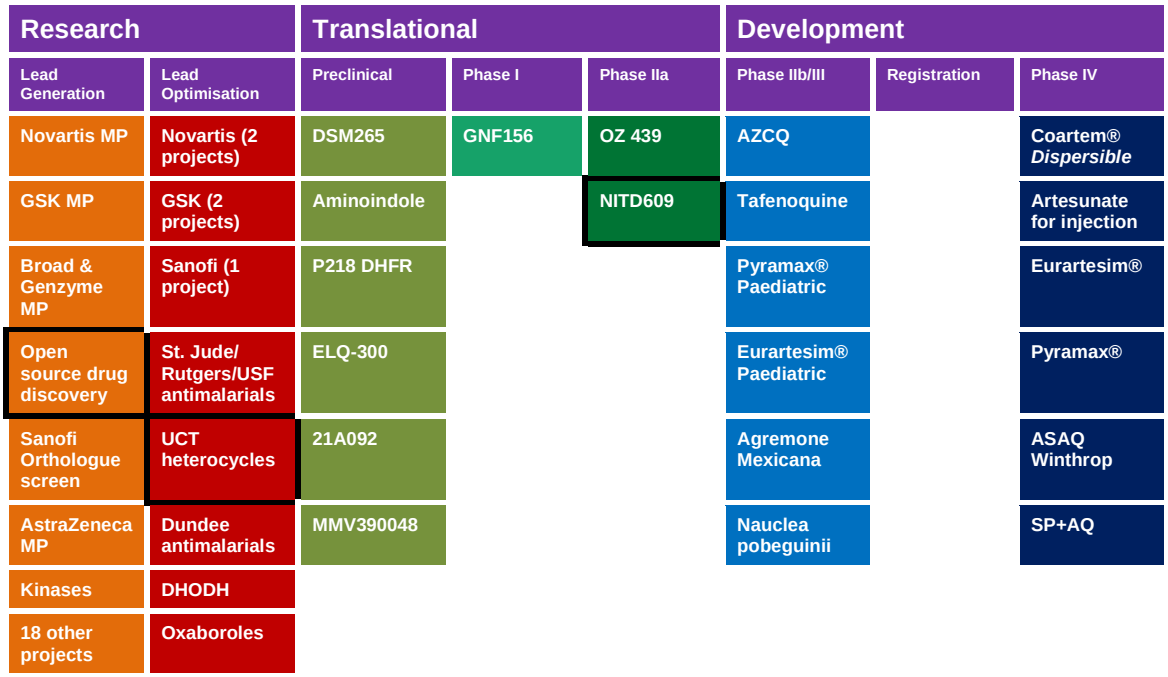
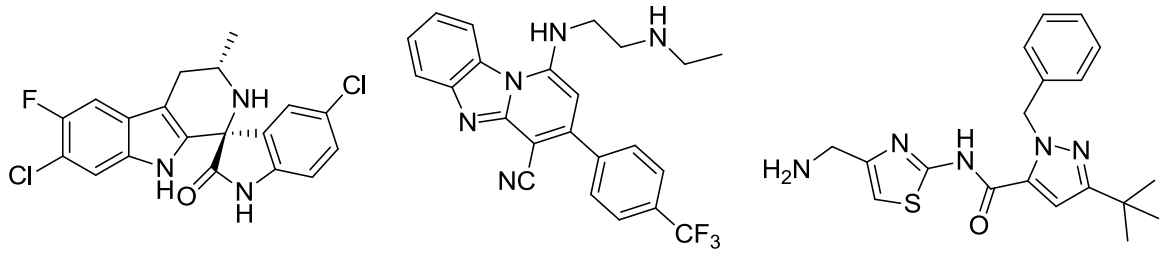


Figure 3: Current Condensed Malaria Pipeline (projects mentioned hereafter encased within a black border)[11]

Representative of the projects in the antimalarial pipeline is NITD609 ("NITD609" in **Figure 3**). In 2010, a team from Novartis published the results of a high throughput screen (HTS) of approximately 12,000 synthetic compounds and natural products against malaria parasites. 17 hits were identified as possessing activity against both the 3D7 (chloroquine sensitive) and Dd2 (multidrug resistant) *P. falciparum* strains at sub micromolar concentrations.[12] The 17 hits were evaluated against the Medicines for Malaria Venture (MMV) compound progression criteria, and of these 17 hits, NITD609 (**6**) was by far the most impressive.[13] It was capable of being produced through a short and simple synthetic route, safe (displaying no cytotoxicity, genotoxicity or cardiotoxicity), compatible with a once daily oral dose schedule and capable of

killing parasite blood stages. It was determined that NITD609 killed malaria parasites through a novel mode of action. Furthermore, the mechanism through which malaria parasites developed resistance toward NITD609 was also found to be novel. The compound has shown great promise and is now in phase IIa clinical trials.

A substantial quantity of lead generation and optimisation has been carried out by the Chibale group of The University of Cape Town ("UCT Heterocycles" in **Figure 3**). A HTS of 1440 non-proprietary compounds found that the pyrido[1,2-*a*]benzimidazoles had potent antimalarial activity with no accompanying cytotoxicity *in vitro*. The relatively slow action exhibited *in vivo* by the most active of these compounds (compound **7**) was worrying.[14] A HTS performed on a larger library of compounds by the Chibale group lead to the development of a novel series of orally active aminomethylthiazole pyrazole carboxamides with good *in vitro* activity against *P. falciparum*. Unfortunately, the lead compound of this series **8** also exhibited moderate toxicity potential *in vitro*.[15] Though the UCT heterocycles and NITD609 projects generated substantial potency *in vitro* and *in vivo*, there is a need for further drug discovery and development.



6 **7** **8**

Figure 4: Three Promising Antimalarial Treatments in the Current Pipeline

Small molecules are not the only type of antimalarial therapy being explored. The development of a malaria vaccine has long been a goal of the WHO. As of 2011, a vaccine targeting *P. falciparum* developed by GlaxoSmithKline (GSK), and the Malaria Vaccine Initiative (MVI) and the Bill & Melinda Gates Foundation is in phase III clinical trials, with 20 other malaria vaccines in phase I or II clinical trials.[1] Historically, antimalarial vaccines have been beset by complications. The 1970's saw the development of the first efficacious antimalarial vaccine, that exhibited a 90% protection rate.[16] Unfortunately, the vaccine was impractical owing to the need for hundreds of mosquito bites before the vaccine became effective.[17] Alterative antimalarial vaccines have been beset by the need for the subcutaneous delivery of a prohibitively large number of sporozoites. These therapies have generally been poorly immunogenic in humans. This tactic also requires conditions that sporozoites do not readily survive, namely, cryogenic

preservation outside of the host. Despite ongoing research there is currently no vaccine available for the clinical treatment of malaria.[4]

Vector controls such as insecticide treated bed nets and insecticide spraying, in addition to community education, are important methods of curtailing the spread of malaria.[6] These do not treat infected patients though, and the development of small molecules for the treatment of malaria remains an important strategy in the fight against this disease. The challenges faced by teams that are developing the aforementioned UCT Heterocycles and NITD609 projects exemplify the profound scientific challenge involved in the optimisation of a HTS hit into a compound suitable for clinical usage.[18]

2. Antimalarial Drug Development

The challenge of drug development is aided somewhat by a large body of literature that enables medicinal chemists to focus their labour on more drug-like regions of chemical space. The need to increase potency whilst limiting lipophilicity limits the drug-like regions of chemical space, however compounds identified within this space have a high probability of good oral bioavailability.[19] Many guidelines exist to help medicinal chemists explore the sensible regions of chemical space. Perhaps the best known amongst these are the Lipinski rules, which state that the probability of a compound having good bioavailability is enhanced if the compound has: a) not more than 5 hydrogen bond donors; b) not more than 10 hydrogen bond acceptors; c) a molecular weight of less than 500 Daltons, and; d) a LogP not greater than 5.[20] Limiting the number of phenyl rings, by elimination or replacement with a heteroaromatic bioisostere, has also been identified as a good method for increasing bioavailability.[21] Other studies have highlighted the importance of topological polar surface area (tPSA) as a good indicator of bioavailability.[19] Also important is the avoidance of functional groups known to frequently give rise to false hits in screening campaigns.[22] There are significant exceptions to these guidelines. Natural products are often cited as examples of such exceptions.[23] Nevertheless, the widespread adoption of these empirically derived heuristics by medicinal chemists is indicative of their success, especially when applied to small molecules.

A number of other considerations are also crucial for antimalarial drug development. Pharmacophores present in new antimalarial chemotherapies should be novel to the parasite to avoid chemical interactions to which the malaria parasite has already developed resistance. The

use of novel pharmacophores, and thus novel chemical interactions, results in a greatly diminished chance of resistance development.[18,24] The concentration of malaria amongst the young and poor necessitates the development of new therapies that are affordable. As benchmarks, Chloroquine (**1**) costs US$0.10 – 0.20 per adult course whereas the ACT's are slightly more expensive, costing in the range of US$1.20 – 3.50 per adult course. An African child can experience more than 10 episodes of malaria per year, meaning that the effects of chronic human use must be known.[24] A thorough safety profile of new antimalarials is therefore vital.

Perhaps the most crucial requirement for a new antimalarial is for it to be better than existing treatments. If this requirement cannot be fulfilled then there is no reason for its use in clinical practice. The fact that no new chemical class of antimalarials has entered into clinical practice since 1996 is illustrative of the difficulties inherent to this field.[10]

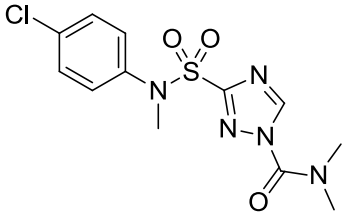
3. The GlaxoSmithKline Tres Cantos Antimalarial Screen

In 2010, GSK expedited the search for new antimalarial treatments by openly and freely publishing the results of an antimalarial HTS of almost two million compounds in the GSK corporate collection.[25] The identity and associated biological data of 13,533 confirmed inhibitors of *P. falciparum* in the Tres Cantos Anti Malarial Screen (TCAMS) dataset were made publicly available, free of charge, on the ChEMBL NTD database.[26] A year later, GSK identified the 47 most attractive series amongst the 13,533 compounds in the TCAMS set.[27] These series were the top scoring series in a points based ranking system. Compounds that displayed potent inhibition of *P. falciparum* (both 3D7 and Dd2 strains) with no cytotoxicity were ranked highly, as were compounds that possessed drug like structures. It was reasoned that compounds that matched to many structurally similar compounds in the screen would be more easily undergo a SAR based lead development campaign than a compound with fewer structurally similar compounds present in the screen. Compounds with structurally similar neighbours were also assigned points, and all confirmed hits were ranked. The net result was 3414 ranked compounds. To minimise the development of resistance to any of these hits, those compounds possessing known antimalarial pharmacophores were eliminated, as were those compounds that possessed functional groups with known toxicity and/or stability issues. The 552 compounds that remained made up the 47 series that GSK identified as the most attractive.

Until recently rational drug design was the predominant approach to antimalarial drug discovery.[28] This approach involves identification of a target, such as a known enzyme or cell receptor, and subsequent development of a compound that is best able to inhibit it. The GSK TCAMS, like the Novartis campaign that led to the identification of NITD609 (**6**), was a phenotypic screen rather than a rational drug design screen. Phenotypic screens such as these aim to find compounds that are capable of killing the malaria parasite, with attention paid to the molecular target at a later date. Hits identified from phenotypic screens have an advantage over hits obtained from rational approaches, in that they are already known to be bioavailable compounds. The GSK TCAMS led to identification of thousands of diverse compounds that, despite not having known targets in the parasite (at the time of publishing), are still highly amenable to SAR based chemical modifications aimed at increasing the potency of the hits. In addition, the unknown mechanisms of action leave open the possibility of the hit compounds hitting multiple targets, thereby limiting their liability to resistance.

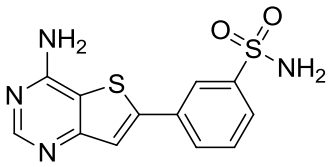
4. TCMDC 134395 and TCMDC 135294

The ranking system used by GSK left open the possibility of promising compounds being overlooked. Two such examples of compounds not prioritised by GSK are TCMDC 134295 (**9**) and TCMDC 135294 (**10**) (**Figure 5**). These compounds displayed potent antimalarial activity in the TCAMS HTS, but were not included in the top 500 compounds as ranked by GSK. The basis for this exclusion is unknown, although one possibility is that both of these compounds were 'singletons' (having no more than 3 structurally similar compounds in the screen[25]). MMV medicinal chemists identified these two compounds as promising structures from the 15,533 inhibitors of *P. falciparum*. The compounds exhibited no cytotoxicity in HepG2 cells, which showed that the antimalarial activity was a phenotype specific to the parasite. The two structures appeared to occupy a region of chemical space well suited to orally bioactive drugs, and no duplication of effort was possible since, to the knowledge of collaborators at the MMV and GSK, no other research was being performed on these compounds.



9 (TCMDC 134395)
cLogP: 1.89
MW: 343.79
tPSA: 96.77

IC_{50} (3D7): 767 nM
%Inhibition HepG2 (10 μM): 7



10 (TCMDC 135294)
cLogp: 1.40
MW: 306.36
tPSA: 148.57

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

Figure 5: Two Potential Antimalarials and their Associated Data

Although the molecules satisfied all of the requirements for screening hits as set out by the MMV progression criteria,[13] the high throughput nature of the GSK screen meant that there was a significant possibility of a false positive. In addition, there was no information concerning the synthetic origin, chemical characterisation and purity of the hits, a feature anathema to academia but common in industrial HTS campaigns. To satisfy the MMV progression criteria these compounds had to be resynthesised and retested biologically before a lead development campaign could begin.

5. Open Source Science

The notion of open source began in software development, and eventually led to the development of software packages such as *Linux* and *Firefox*.[29] Such activity proceeds by allowing the open sharing of ideas online and by allowing anyone to contribute. This process has generated solutions to complex problems in science. For example, players of the protein folding game *FoldIt* discovered a way of increasing the activity of a computationally designed Diels-Alderase protein 18 fold.[30] The possibility of a completely open source science project was demonstrated by the development of an improved synthesis of a widely used pharmaceutical in a collaboration between the Todd group and the WHO.[31] In this project, research was accelerated through the spontaneous participation of people unknown to the core team at the outset. In cases such as these the term 'open source' refers not to the source code, but to the open origin of contributors and to the open discussion of ideas.[32]

The open source approach is particularly applicable to NTD research. The cost of bringing a new molecular entity to the drug market was recently estimated at approximately US$1.8 billion.[33] Pharmaceutical corporations cannot recover these staggering costs in the NTD market, which is primarily made up of the world's poorest citizens. Since there is no profit to be had, there is no reason to prevent the release of intellectual property.[34] The open release of the TCAMS (which contained 82% proprietary compounds) by GSK is a testament to this. The lack of a profit incentive also means that there is no reason to withhold data from publication, or to file restrictive patents, as is often the case in both academia and industry.[31] In the absence of economic barriers, solutions to NTDs are held back only by the speed at which projects can develop. Use of the open source approach in solving the problems of NTDs would involve the open dissemination of research data to anyone, and the collaboration amongst anyone who wishes to participate in quickly finding solutions to terrible diseases such as malaria.

6. Aims of the Current Project

Two compounds from the GSK TCAMS antimalarial HTS, TCMDC 134395 (**9**) and TCMDC 135294 (**10**), have been identified as potent hits that look promising from a drug development perspective. Before these compounds can progress to a lead development campaign, they must be resynthesised to confirm their biological activity, as stipulated by the MMV compound progression criteria.[13]

The aim of this project is to carry out this task using an open source approach. Analogues of the hits will be synthesised, and obtained through commercial suppliers, to develop a preliminary SAR. Following biological evaluation, the decision of whether to forward the hits into the lead development stage will be made.

A project description will be maintained on a wiki (OpenWetWare).[35] An electronic lab book will be maintained online.[36, 37] A web presence will be maintained to generate interest in the project, by posting updates on Twitter[38], Google+[39] and elsewhere as the needs arise. Project status updates will regularly be posted online at The Synaptic Leap.[40] The open source approach will be guided by six principles:[41]

i) All data are open and all ideas are shared
ii) Anyone can take part at any level of the project
iii) There will be no patents

iv) Suggestions are the best form of criticism

v) Public discussion is much more valuable than private email

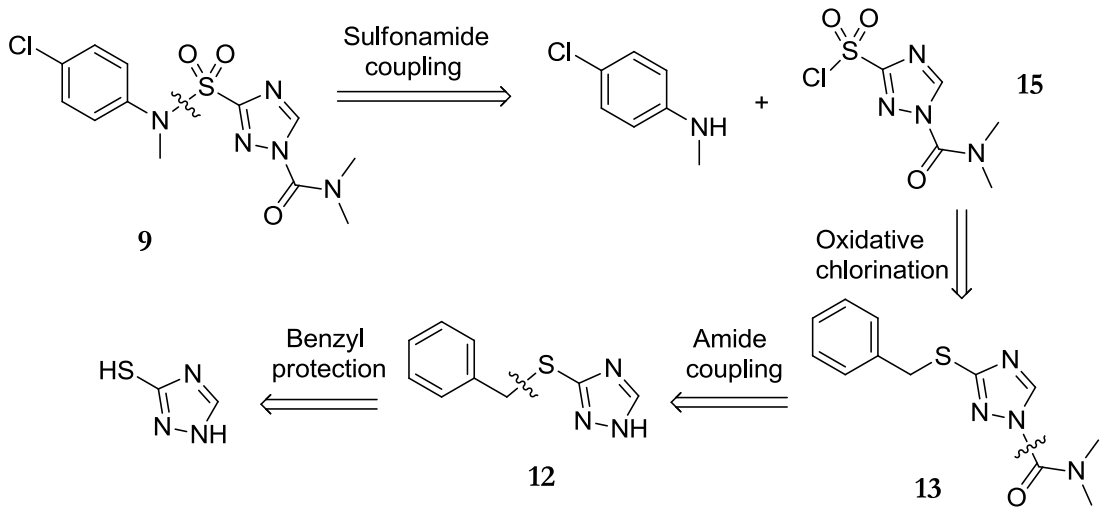
vi) The project is bigger than, and is not owned by, any given lab. The aim is to find a good drug for malaria, by whatever means, as quickly as possible.

Chapter 3: Results and Discussion

1. TCMDC 134395: Synthesis and Characterisation

i) Retrosynthetic Analysis

A survey of prior art revealed that Yasuda *et al.*[42] had synthesised and patented the use of TCMDC 134395 (**9**) for the treatment and prophylaxis of diseases affected by DDPIV (Dipeptidyl peptidase-4) inhibitors (eg: obesity and diabetes). To the best of our knowledge TCMDC 134395 had not been evaluated as an antimalarial prior to the GSK TCAMS HTS. A retrosynthetic analysis (**Scheme 1**) developed by the patent authors indicated that **9** could be synthesised in four steps from commercially available starting materials. The patent provided few experimental details and the compounds reported were characterised only by ^{1}H NMR spectroscopy. Neverrtheless, it was decided to follow this synthetic plan as the approach appeared sound.



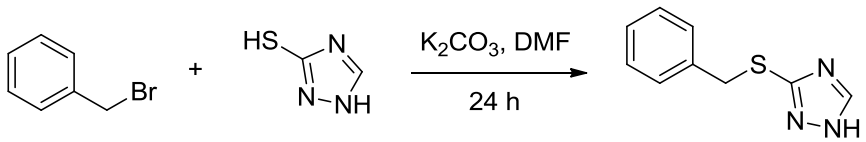
Scheme 1: Retrosynthesis of TCMDC 134395 (**9**)

TCMDC 134395 (**9**) would be generated by coupling the commercially available 4-chloro-*N*-methylaniline and the sulfonyl chloride **15**. An oxidative chlorination would be used to generate **15** from the benzyl protected triazolourea **13** in a functional group interconversion (FGI) that combines three reactions (a debenzylation, oxidation and chlorination) into a single, atom efficient reaction. The benzyl protected triazolourea **13** could be formed from the commercially

available dimethylcarbamoyl chloride and the benzyl protected triazole **12**, which in turn could be produced by benzylation of the commercially available 3-mercapto-1*H*-1,2,4-triazole (**11**).

ii) Benzyl Protection

The synthesis began with attempts to install a benzyl group on the thiol of **11**. Presumably, this strategy was used by Yasuda *et al.* to avoid competing reactivity arising from the thiol later in the synthesis of TCMDC 134395 (**9**). The patent being followed suggested that the protection could be carried out by stirring **11** and benzyl bromide in DMF overnight without the addition of a base, however when attempted this approach gave no conversion of starting materials. The use of excess benzyl bromide in a repeated attempt at this reaction also failed to generate the desired product, and starting materials were recovered (53%). When the reaction was performed with the addition of a base, the protection reaction was successful and **12** was produced in 48% yield (**Scheme 2**). This indicated that a base was required, contrary to the literature report. A recrystallisation was attempted for synthetic convenience. Washing the crude solid in petrol before dissolution in hot petrol with subsequent drops of EtOH resulted in the formation **12** as a crystalline solid in 66% yield. This method proved time consuming and a quicker method was found whereby the crude solid was recrystallised from 50% toluene/petrol to give **12** in 48% yield. This protection reaction could therefore be carried out on a gram scale with an efficient, non-chromatographic purification method.



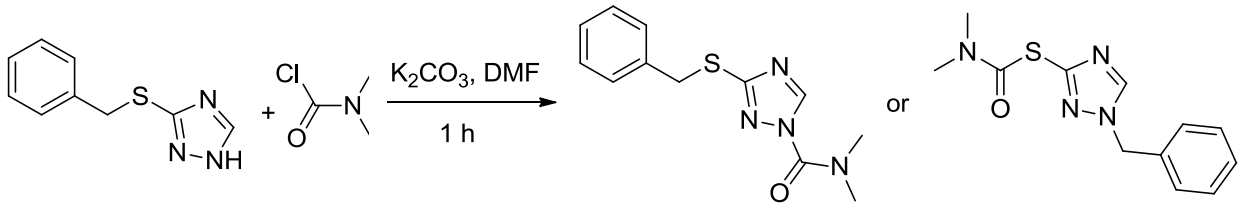
11 **12** (48%)

Scheme 2: Benzyl Protection of **11** to give **12**

iii) Carbamoylation

The benzyl protected triazole **13** was synthesised in 92% yield by stirring **12**, dimethylcarbamoyl chloride and K_2CO_3 in DMF in accordance with literature precedent (**Scheme 3**). This reaction proved amenable to scale up: a 10 mmol scale reaction gave **13** in 86% yield. The characterisation of **13** was straightforward, though the data were also consistent with the structure of the possible isomer **14**. This possibility was excluded by the ultimate synthesis of TCMDC 134395 (**9**). Purification of the compound was difficult: despite extensive

chromatography the compound remained impure by ^{1}H NMR spectroscopy. Compound **13** was therefore used without further purification in subsequent reactions.

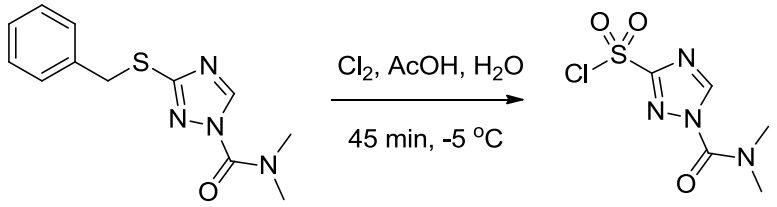


12 **13** (92%) **14**

Scheme 3: Two Possible Isomers Formed in the Carbamoylation of **12**

iv) Oxidative Chlorination

The penultimate step in the synthesis of TCMDC 134395 (**9**) was the synthesis of the sulfonyl chloride **15**. Sulfonamides are a ubiquitous functionality in many pharmaceuticals and are frequently generated from sulfonyl chlorides.[43] In the absence of functional groups sensitive to halogens, the most common method of preparing sulfonyl chlorides is *via* an oxidative chlorination reaction using $Cl_2/H_2O/H^+$.[44] Yasuda *et al.* used this reaction to produce the sulfonyl chloride **15** from the benzyl protected triazolourea **13** (**Scheme 4**).



13 **15**

Scheme 4: Synthesis of the Sulfonyl Chloride **15** *via* Oxidative Chlorination

Preparation of sulfonamides without the use of an oxidative chlorination typically involves the multi-step process of deprotection, oxidation and chlorination, all performed in separate reactions.[45] The oxidative chlorination is a one pot approach that offers significant advantages in terms of atom economy and synthetic ease. An investigation of the literature revealed only one alternative preparation of sulfonyl chlorides from 3-merpcato-1*H*-1,2,4-triazoles, involving a two-step process of oxidation by H_2O_2 followed by chlorination with Cl_2.[46] Though the synthetic route seemed valid, the patent procedure was poorly described and involved a significant hazard in the handling of noxious Cl_2.

The ease of *in situ* production of Cl_2 was investigated. Addition of concentrated HCl to $KMnO_4$ was one possibility, though production of synthetically useful Cl_2 from this method necessitates a rigorous purification and drying process to remove damp HCl gas.[47] A public plea for help on Twitter[48] was re-communicated though accounts belonging to Dalton Transactions and Nature Chemistry. This approach netted a suggestion to use a combination of NaClO or $Ca(ClO)_2$ and concentrated HCl to provide Cl_2.[49]. An alternative suggestion, from ScienceInsight[50] pointed us toward a procedure detailed on YouTube that produced Cl_2 from trichloroisocyanuric acid and HCl (**Scheme 5**).[51] A post on a well trafficked chemistry forum vouched for the purity of Cl_2 produced through this procedure.[52] The affordability of this method was notable. Aldrich offers 6 kg cylinders of Cl_2 for AU$2910.00, whereas 2 kg of pool chlorine (85% trichloroisocyanuric acid) from a hardware store was priced at AU$24.95. This method of Cl_2 production was pursued in awareness that advice had come from unorthodox and in some cases anonymous sources.

Scheme 5: Production of Chlorine Gas from Trichloroisocyanuric Acid

Use of Cl_2 required the design and construction of an apparatus in which to safely perform the reaction. An apparatus was duly constructed with three reaction chambers: one for production of Cl_2, a second for carrying out the oxidative chlorination reaction, and a third for quenching excess Cl_2. A positive pressure of N_2 enabled the Cl_2 to flow through the apparatus. Flasks were placed between each reaction chamber to prevent contamination from backflow (**Figure 6**).

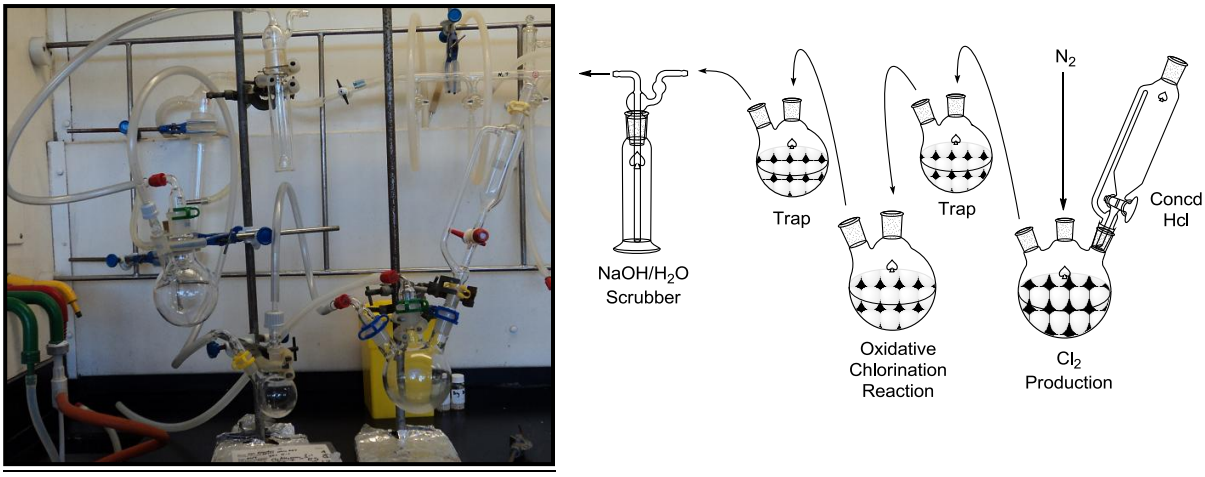


Figure 6: Oxidative Chlorination Apparatus (left: actual apparatus with one trap missing; right: schematic of the apparatus)

Compound **13** was dissolved in AcOH/H_2O and treated with Cl_2 for 1.5 h based upon the patent procedure (albeit with longer reaction times) to give the sulfonyl chloride **15** as a viscous oil (**Scheme 4**; note different reaction times). One new product was formed in a complete reaction according to TLC analysis. This product was successfully characterised after the solvents were evaporated under a stream of N_2 gas for 48 h. Aqueous workup of **15** (following concentration under N_2), as suggested by the patent was not followed owing to suspicion that **15** would be labile to hydrolysis. Initially, **15** did not undergo workup prior to subsequent reactions, but this later gave unsatisfactory results. The aqueous workup suggested by the patent was followed, but this gave **15** in only 25% yield (lit: 81% yield).[42] When **15** was isolated by rotary evaporation it was unsuitable for subsequent reactions. Addition of **15**, concentrated *via* rotary evaporation, to K_2CO_3 in a subsequent reaction resulted in vigorous effervescence. This indicated that residual acids (AcOH/HCl) would need to be removed from **15** prior to subsequent use. Isolation of **15** under high vacuum for 5.5 h with gentle heating proved to effective, although residual benzyl chloride and AcOH prevented an accurate yield from being obtained.

The prevalence of sulfonyl chlorides in the literature notwithstanding, there remains a dearth of mechanistic information regarding this reaction. This information was critical to an explanation of the reaction as we encountered it. Prior to 2010 the majority of mechanistic information related to the so-called 'sulfohaloform reaction' pathway[44], whereby sulfur substituents are exhaustively chlorinated prior to cleavage, followed by transformation of the resultant sulfenyl chloride into a sulfonyl chloride.[53] In 2010 Barnwell *et al.* proposed that this pathway is only

followed when sulfur substituents are capable of forming very stable carbocations (eg: acyl, *t*-butyl). Another pathway was proposed for sulfur substituents that are capable of forming a carbocation of intermediate stability (eg: benzyl, isopropyl) (hereafter termed the 'Barnwell pathway') (**Figure 7**).

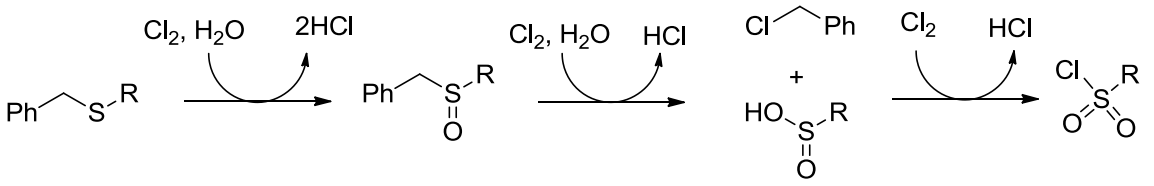


Figure 7: Oxidative Chlorination *via* the Barnwell Pathway

The relatively moderate stability of the benzyl carbocation formed during the synthesis of the sulfonyl chloride **15** suggested that this reaction proceeds selectively *via* the Barnwell pathway. The reaction does not lead to cleavage of the bond between the sulfur and the triazole ring of **13** since that would form an unstable aromatic carbocation. ^{1}H NMR spectroscopy of the crude reaction mixture indicated the presence of **15** alongside peaks corresponding to benzyl chloride and AcOH. Peaks consistent with other products such as the hypothesised sulfinic acid and sulfoxide were not identified in either the crude ^{1}H NMR spectrum or low resolution mass spectrum. This was consistent with TLC analysis, which showed that the reaction was complete. No mechanism has been proposed for the oxidative chlorination reaction that proceeds through the Barnwell pathway. A novel mechanism based on the Barnwell pathway and the oxidative chlorination as encountered in this body of work (**Scheme 4**) is therefore proposed in **Figure 8**.

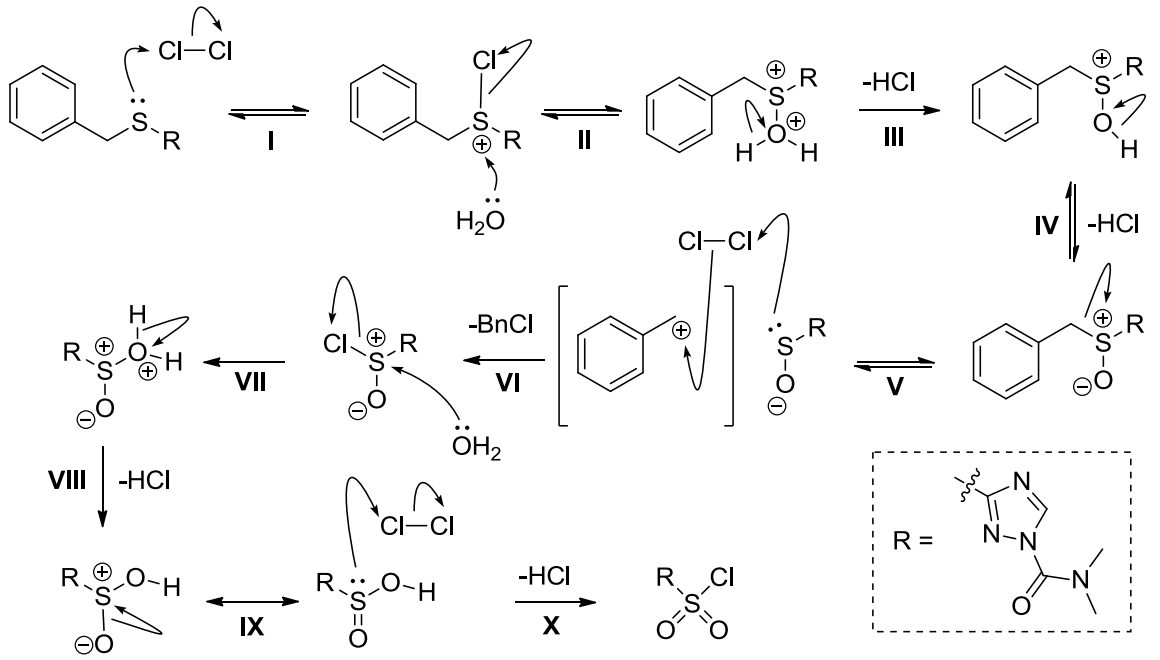


Figure 8: Suggested Mechanism for the Oxidative Chlorination of **13**

Sulfur is oxidised by Cl_2 (**I**) before the chlorine is displaced by H_2O (**II**), possibly with simultaneous loss of H^+ to avoid the formation of a dication. The loss of HCl gives the sulfoxide (**III** – **IV**). The polar protic solvents enable S_N1 type dissociation of the sulfur-benzyl bond (**V**). Though reversible, this is pushed to completion by chlorination of the carbocation and sulfide, with the loss of BnCl (**VI**). The sulfur is oxidised again by Cl_2 (**V**), and again H_2O displaces Cl (**VII**). The loss of HCl gives the sulfinic acid (**VIII**), with the lone pair on this species attacking Cl_2 to give the sulfonyl chloride with further loss of HCl (**X**). The mechanism is analogous to the oxidation of sulfides by H_2O_2,[54] albeit with Cl_2 activating the sulfur, thereafter permitting participation by H_2O.

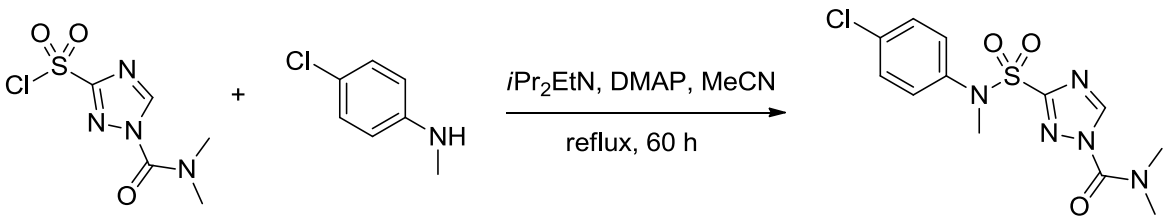
vi) Sulfonamide Coupling

The patent being followed generated TCMDC 134395 (**9**) through the coupling of commercially available 4-chloro-*N*-methylaniline and **15**, in MeCN at 50 °C in the presence of K_2CO_3. This procedure failed to convert starting materials by TLC analysis (**Table 1**, **Entry 1**). Various reaction conditions for the formation of TCMDC 134395 (**9**) were therefore assayed (**Table 1**).

Table 1: Reaction Conditions Assayed for the Synthesis of TCMDC 134395 (**9**)

Entry	Method for Purifying 15	Reaction Conditions	Yield of 9
1	Nil	K_2CO_3, MeCN, 50 °C, 60 h	SM
2	Rotary Evaporation	K_2CO_3, MeCN, 50 °C, 60 h	SM
3	Aq Workup	K_2CO_3, MeCN, 50 °C, 60 h	13%
4	High Vacuum	Et_3N, MeCN, 50 °C, 60 h	16%
5	High Vacuum	*i*Pr_2EtN, MeCN, DMAP, reflux, 60 h	28%

The difficulties inherent to the purification of **15** had a bearing on the outcome of the coupling reaction. No conversion of starting materials was observed by TLC when **15** was blown down by N_2 and used as a crude product (**Table 1**, **Entry 1**). Vigorous effervescence was observed when **15** (isolated by rotary evaporation) was added to a suspension of K_2CO_3 in MeCN (**Table 1**, **Entry 2**). A sample of **15** was isolated through aqueous workup. Coupling of this sample to 4-chloro-*N*-methylaniline as per the patent procedure generated TCMDC 134395 (**9**) in 13% purified yield (**Table 1**, **Entry 3**). Isolation of crude **15** under high vacuum generated a batch of purified **15**, which was coupled with 4-chloro-*N*-methylaniline in the usual conditions, but with K_2CO_3 substituted by Et_3N. This method gave TCMDC 134395 (**9**) in 16% purified yield (**Table 1**, **Entry 4**). The same batch of **15** was coupled with 4-chloro-*N*-methylaniline but: i) Et_3N was substituted with *i*Pr_2EtN; ii) 0.1 eq DMAP was added, and; iii) the MeCN solution was heated at reflux for 60 hr. This strategy furnished TCMDC 134395 (**9**) in 28% purified yield (**Table 1**, **Entry 5**; **Scheme 6**).



15 TCMDC 134395 (**9**) (28%)

Scheme 6: Final Step in the Synthesis of TCMDC 134395 (**9**)

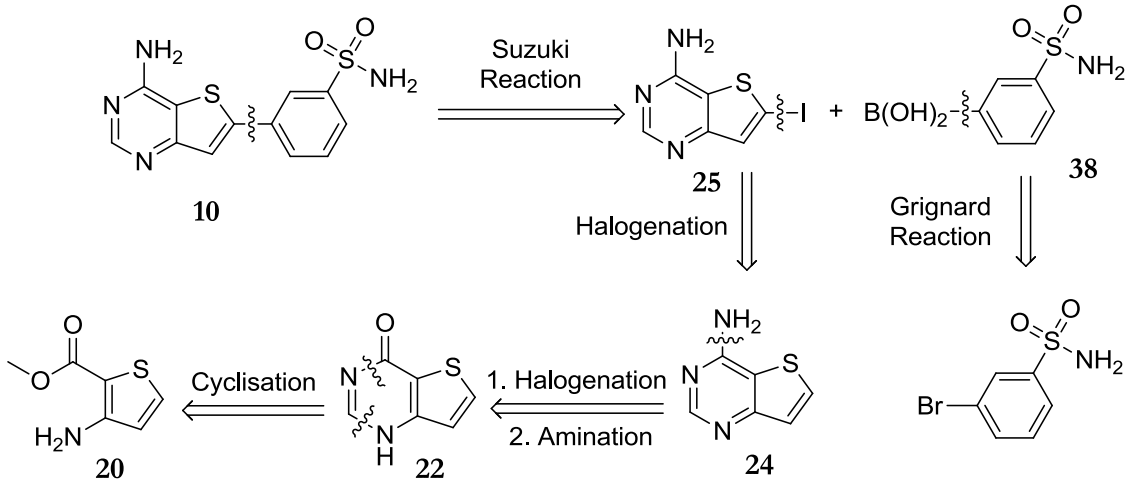
TCMDC 134395 (**9**) was originally evaluated biologically as its HCl salt. In the presence of the buffer used in the GSK screen, the molecule is presumably in equilibrium with its free base in solution. Attempts to convert TCMDC 134395 (**9**) from its freebase form into its isolable hydrochloride salt using HCl gas, generated from $CaCl_2$ and 32% aqueous HCl, were unsuccessful and returned starting material quantitatively. The synthesis of TCMDC 134395 (**9**)

in its freebase form was complete though, and this compound was confirmed to be more than 90% pure by elemental analysis before being sent for biological evaluation (*vide infra*).

2. TCMDC 135294: Synthesis and Characterisation

i) Retrosynthetic Analysis

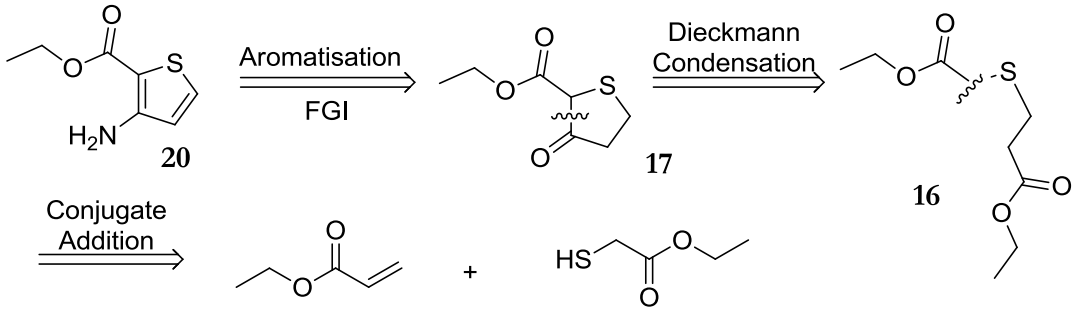
No complete syntheses of TCMDC 135294 (**10**) were uncovered from a survey of prior art. The desire for a convergent synthesis guided the development of a novel retrosynthetic plan (**Scheme 7**). The final reaction would be a Suzuki coupling between the iodinated aminothieno[3,2-*d*]pyrimidine **25** and the boronic acid **38**. The latter could be formed from the commercially available 4-bromobenzenesulfonamide in a Grignard reaction. The iodinated thieno[3,2-*d*]pyrimidin-4-amine (**25**) could be produced *via* halogenation of thieno[3,2-*d*]pyrimidin-4-amine (**24**). This aminated species could be produced from thieno[3,2-*d*]pyrimidone **22** though trivial functional group manipulation operating on thieno[3,2-*d*]pyrimidin-4(1*H*)-one (**22**), which could be generated though a ring closure of the commercially available reagents, formamide and methyl 3-aminothiophene-2-carboxylate (**20**).



Scheme 7: Convergent Retrosynthesis of TCMDC 135294 (**10**)

Starting materials were ordered from commercial suppliers. The synthesis of the aminothiophene starting material was attempted while waiting for delivery. This required a separate, simple retrosynthetic strategy (**Scheme 8**). Formation of ethyl 3-aminothiophene-2-carboxylate could be carried out through the dual aromatisation/FGI of tetrahydrothiophene **17**. This compound could be formed through a Dieckmann condensation of the conjugate

product **16**, which in turn could be manufactured through a conjugate addition between ethyl mercaptoacetate and ethyl acrylate. The use of ethyl side chains in lieu of methyl side chains (determined by availability in house) was not a cause for concern as these groups were expected to be sacrificial in later steps.



Scheme 8: Retrosynthesis of Methyl 3-aminothiophene-2-carboxylate (**20**)

This approach would permit easy access to a versatile range of analogues at a later date (**Figure 9**). Variation around the thieno[3,2-*d*]pyrimidine core would be achieved by use of different arylboronic acids in the Suzuki coupling (**A**).† These arylboronic acids could be varied prior to the Suzuki coupling (**B**). Various primary or secondary amines could be prepared and substituted on 4-chlorothieno[3,2-*d*]pyrimidine by S_NAr of chlorine at 4-position (**C**). It was envisioned that modification at the 2-position of the core ring system could be achieved by substituting various amides for formamide in the assembly of the thieno[3,2-*d*]pyrimidone ring (**D**). Finally, variation of the thienopyrimidine ring system itself could be achieved initially (**E** and **F**).

† This chapter makes extensive use of fused bicyclic ring nomenclature. Refer to **Appendix B** for a review of these naming conventions.

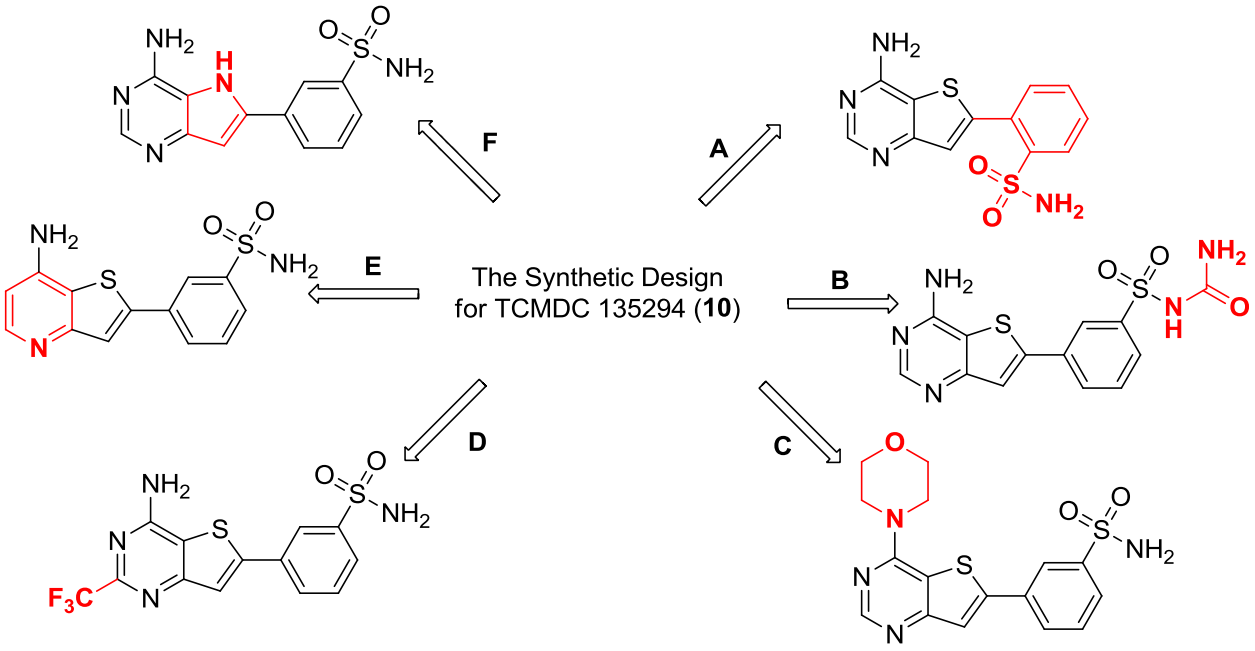
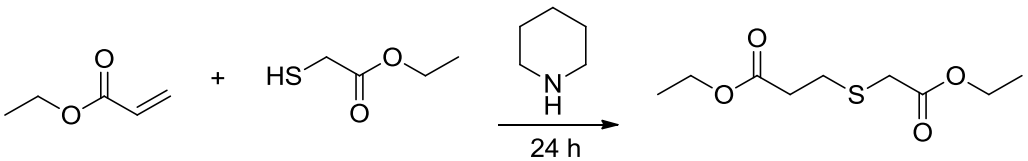


Figure 9: Variations in Structure Permitted by the Synthetic Design of TCMDC 135294 (**10**)

ii) Conjugate Addition

The conjugate addition of ethyl mercaptoacetate to ethyl acrylate according to a literature procedure gave the conjugate addition product **16** in 97% yield (**Scheme 9**).[55] Characterisation of this product was complicated by the lack of spectroscopic data in the literature. Therefore, nearly complete characterisation of **16** was carried out. A high resolution mass spectrum and a boiling point were not obtained due to the intense stench of the product.



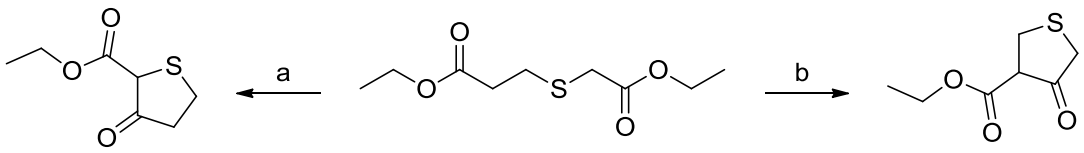
16 (97%)

Scheme 9: Formation of **16** *via* Conjugate Addition

iii) Dieckmann Condensation

It was hoped that the Dieckmann condensation of **16** would generate **17**, however, this condensation could also lead to the isomeric product **18**. In 1980, Duus had reported that the formation of either of the two products could be selective when reaction temperatures were controlled.[56] A half century prior, Woodward and Eastman had interpreted this result in terms

of kinetic versus thermodynamic control (albeit for the methyl-, rather than the ethyl-diesters), and Duus applied this conclusion to the ethyl-diesters (**Scheme 10**).[57]



17 **16** **18**

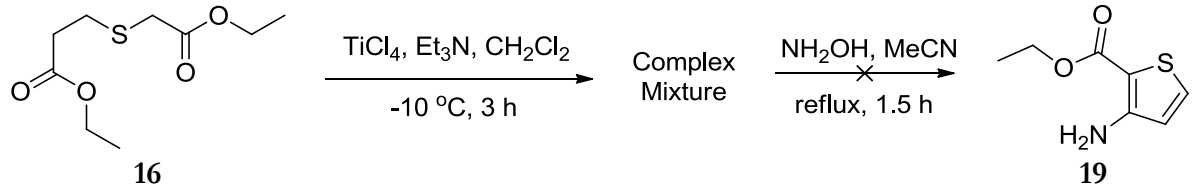
Scheme 10: The Kinetic (left) and Thermodynamic (right) Products of a Dieckmann Condensation on the Conjugate Addition Product **16**. *Reagents and Conditions:* a) NaOEt, EtOH, 0 °C, 6 h. b) NaOEt, PhMe, reflux, 6 h

There was suggestion that the kinetic formation of **17** occurred due to the proton releasing effect imparted on the –SC*H*CO– proton by the combined electronegativities of sulfur and the carbonyl group.[58] Woodward and Eastman rejected this hypothesis, noting that the relatively more electronegative oxygen displayed a weaker kinetic effect when it was substituted for sulfur.[57] They instead suggested that the kinetic effect might be due to the ability of sulfur to accommodate excess electron density in its valence shell. Several years later, Hromatka *et al.* concluded that the formation of **18** under thermodynamic control depended on the ability of the kinetic product **17** to undergo temperature dependent retro-Claisen cleavage, anion isomerisation and alternative recyclisation.[56, 59] Hrotmatka *et al.* offered no additional explanation for the formation of the kinetic product.

With this in mind, the results of the present study were surprising. Selective formation of the kinetic isomer **17** was difficult. Use of NaOMe and NaOEt as bases at low temperatures as per the literature procedure did not yield any conversion of starting material.[56] When the reaction was performed using a fresh batch of NaOEt in dried EtOH, starting material was consumed to give multiple products by TLC. Comparison of the crude ^{1}H NMR spectrum with known literature values indicated that both the thermodynamic product **18** and the kinetic product **17** had formed, alongside other products which could not be identified owing to the complexity of the spectrum.[56] No ions with identifiable molecular formulae with relevance to the reaction were visible in the low resolution mass spectrum. The lack of selectivity and the failure to observe identifiable ions in the mass spectrum led to abandonment of this approach.

A regioselective method for generating the tetrahydrothiophene **17** was desired for simplicity. Deshmukh *et al.* reported that the use of $TiCl_4$ in a Dieckmann type condensation allowed for

regioselective formation of the tetrahydrothiophene **17** from **16**.[60] This procedure was followed and showed completion by TLC. ^{1}H NMR spectroscopy of the crude reaction mixture indicated the presence of the tetrahydrothiophene **17**, with no concomitant formation of the proposed thermodynamic product. Trace contamination by the corresponding thiophene was expected, and observed. [60] The effects of keto-enol tautomerism was also evident in the spectrum. Attempted purification of the crude product *via* column chromatography lead to loss of the product on the column. This reaction was reattempted and purified *via* column chromatography, with significant mass loss, to afford three different compounds as determined by ^{1}H NMR and IR spectroscopy and low resolution mass spectrometry. The spectra of these products were not consistent with those expected for **16**, **17** or **18** and these products were not able to be identified. Attempts to convert these unknown products into the thiophene **19** by the action of hydroxylamine hydrochloride at in refluxing MeCN led to complete recovery of starting materials (**Scheme 11**).



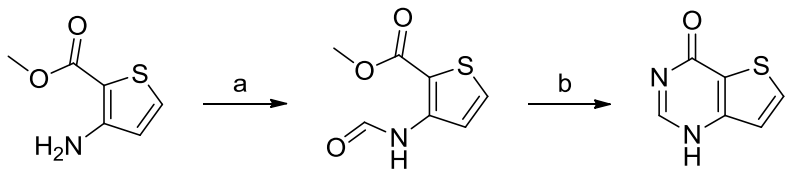
Scheme 11: Failed Conversion of the Tetrahydrothiophene **17** into the Thiophene **19**

The arrival of the commercially sourced starting material **20** led to the abandonment of the synthesis of the thiophene **19**. Though this study yielded no purified product, interesting results were nevertheless observed. Maintenance of low temperatures was found to be an insufficient method of controlling the selectivity in the Dieckmann condensation of **16**. A $TiCl_4$ mediated Dieckmann condensation offered the requisite selectivity according to ^{1}H NMR spectroscopy of the crude mixture in one instance, though this product could not be isolated for characterisation.

iv) Synthesis of Thieno[3,2-*d*]pyrimdin-4-amine Core

The synthesis of the key heterocycle **22** began with the attempted condensation of methyl 3-aminothiophene-2-carboxylate (**20**) with formamide at 180 °C, according to a literature procedure.[61] Although the reaction appeared incomplete by TLC after 8 h, the reaction was purified due to time constraints. Substantial mass loss occurred in the workup, but some starting material (10%) was isolated from the organic extracts. The aldehyde **21** was also formed and

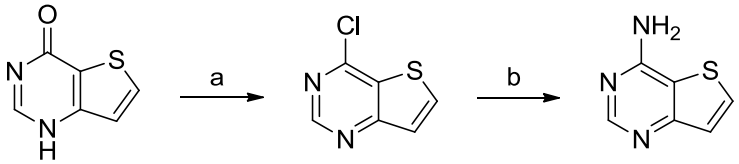
purified in trace yield. The known decomposition of formamide into CO_2, CO, H_2O and HCN at elevated temperatures might account for the low yield.[62] An alternative method of generating **22** involved two independent condensation reactions.[63] Methyl 3-aminothiophene-2-carboxylate (**20**) and NH_4OAc were stirred in refluxing formic acid for 7 h, to produce the aldehyde **21** in 60% yield following recrystallisation from aqueous EtOH, in accordance with the literature precedent.[63] Ammonium formate and **21** were then stirred in formamide at reflux overnight to afford **22** as a crystalline solid in 42% yield, as dictated by the same literature reference (**Scheme 12**).



20 **21** (60%) **22** (42%)

Scheme 12: Assembly of the Thieno[3,2-*d*]pyrimidone **22**. *Reagents and Conditions:* a) NH_4OAc, formic acid, reflux, 3 h; b) ammonium formate, formamide, reflux, 20 h.

It was subsequently found that the thienopyrimidone **22** could be formed in a one pot reaction when the thiophene **20**, formic acid and ammonium formate were dissolved in formamide and heated at 140 °C for 20 h. This strategy gave thienopyrimidone **22** as a crystalline solid in 53% yield following recrystallisation from 50% $EtOH/H_2O$. The 4-position of the thienopyrimidone **22** was chlorinated in a S_NAr type reaction by refluxing **22** dissolved in neat $POCl_3$ under N_2 for 1.5 h following literature precedent.[64] This process formed the chlorinated thienopyrimidine **23** in good yield (78%). Formation of the chlorinated thieno[3,2-*d*]pyrimidine **23** permitted access to a thieno[3,2-*d*]pyrimidine scaffold with a good leaving group at the 4-position, which thereby enabled further S_NAr type chemistry (and the production of analogues at a later date). No literature method could be found where Cl at the 4-position on compound **23** was replaced with NH_2. A literature method utilised NH_4OH/H_2O at 120 °C at elevated pressures to carry out this substitution on a pyrimidine.[65] After confirming that this substitution would not occur at 40 °C at ambient pressure, the use of a sealed tube was investigated. Once the sealed tube was confirmed to be able to withstand the procedure, the literature precedent was followed to generate thieno[3,2-*d*]pyrimidin-4-amine (**24**) as a yellow solid (**Scheme 13**).



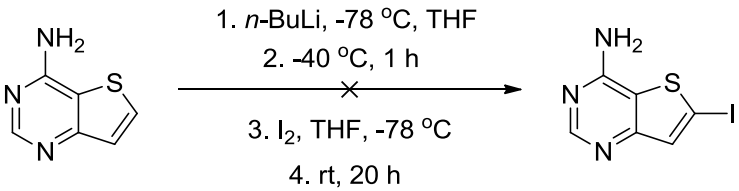
22 **23** (78%) **24**

Scheme 13: Assembly of Thieno[3,2-*d*]pyrimidin-4-amine **24**. *Reagents and Conditions*: a) $POCl_3$, reflux, 1.5 h; b) NH_4OH/H_2O, 120 °C, sealed tube.

The reaction was worked up as per the literature protocol. In difference to the literature procedure, the product was not purified *via* column chromatography since it appeared sufficiently pure by ^1H NMR spectroscopy. Substantial H_2O remained in the crude reaction mixture despite extensive attempts at drying the crude product. With retrospect it is possible that residual H_2O could explain the failures of subsequent deprotonation reactions, though this was not apparent at the time.

v) Halogenation of the Thieno[3,2-*d*]pyrimidine core (I)

It was intended that the proton at the 6-position of thieno[3,2-*d*]pyrimidin-4-amine (**24**) would undergo selective lithiation and subsequent halogenation (**Scheme 14**) at the 6-position. The literature precedent being followed had the amine at the 4-position masked by a morpholine group, and the 2-position was protected by a chlorine atom such that the 6-position was the only site of this compound liable to electrophilic attack.[66,67] Attempted lithiation and halogenation of thieno[3,2-*d*]pyrimidin-4-amine (**24**) resulted in extensive decomposition by ^1H NMR.



24 **25**

Scheme 14: Attempted Halogenation of **24**

A deprotonation study was performed on thieno[3,2-*d*]pyrimidin-4-amine (**24**) with variation in temperature and base to investigate whether the deprotonation of a thienopyrimidine ring system carrying a primary amine could proceed at all,. In this investigation, deprotonation of thieno[3,2-*d*]pyrimidin-4-amine (**24**) would be followed by a D_2O quench in varied conditions.

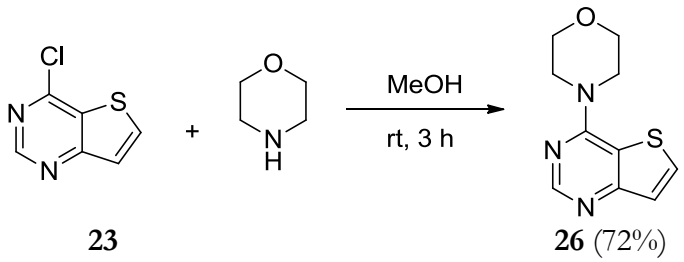
Any hydrogen that had been replaced by deuterium would be rendered invisible in the ^{1}H NMR spectrum. The results of this investigation are summarised in **Table 2**.

Table 2: Variation of Deprotonation Reaction Conditions

Reagent	Maximum Temperature (°C)	Result
n-BuLi (3 eq)	-40	SM recovery
n-BuLi (3 eq)	-15	SM recovery
sec-BuLi (3 eq)	-40	SM recovery
sec-BuLi (3 eq)	-15	SM recovery
sec-BuLi (7 eq)	-15	Double deprotonation

According to ^{1}H NMR spectroscopy, selective deprotonation was not possible under the conditions assayed. Deprotonation of **24** was observed only at -15 °C using excess *sec*-BuLi, and without the requisite selectivity. Although S_EAr usually occurs at the 6-position of thiophene rings, attack at the 7-position of thieno[3,2-*d*]pyrimidines is not unheard of.[68]

An *N*-protected derivative of thieno[3,2-*d*]pyrimidin-4-amine (**24**) was sought to avoid the problems of the free amine. Stirring chlorinated thienopyrimidine **23** and morpholine in MeOH at room temperature generated the morpholine derivative **26** in 72% yield (**Scheme 15**).

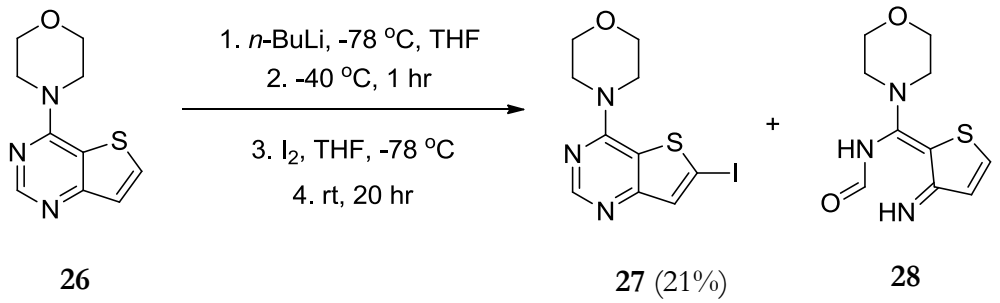


23 **26** (72%)

Scheme 15: Synthesis of Morpholine Derivative **26**

Compound **26** was subjected to *sec*-BuLi at -40 °C followed by a D_2O quench. Signals consistent with the desired D_2O quench were observed in the ^{1}H NMR spectrum alongside starting material and one decomposition product. Based on this success, halogenation of **26** using I_2 and fresh *n*-BuLi at -78 to -40 °C was performed. The iodinated morpholine derivative **27** was generated in 10% yield following column chromatography (**Scheme 16**). Extensive decomposition products were observed in the crude reaction mixture. The ^{1}H NMR spectrum of the purified decomposition product indicated that the thiophene ring had remained intact.

Three singlets were observed in the downfield region of the ^{1}H NMR spectrum. This indicated that the fragmentation of the pyrimidine ring had occured. The origin of the new atoms required to form this decomposition product is unknown, though several possibilities exist (eg: residual H_2O, H_2O quench, etc.). One such decomposition product is suggested (**Scheme 16**), though it is difficult to rationalise a mechanism of formation. To limit decomposition the reaction was attempted at -78 °C, and this generated the iodinated morpholine derivative **27** in 21% purified yield (**Scheme 16**).



26 **27** (21%) **28**

Scheme 16: Lithiation/Halogenation of the Morpholine Derivative **26**

Despite sub-optimal yields this series of reactions proved that deprotonation and halogenation at the 6-position on a thieno[3,2-*d*]pyrimidine scaffold was possible. This was a unique achievement, as all 16 instances of this in the literature feature substitution at the 2-position, and 8 of those 16 instances feature substitution at the 7-position.

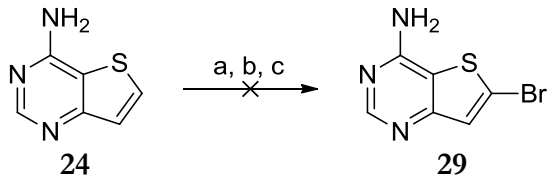
vi) Electrophilic Aromatic Substitution of the Thieno[3,2-*d*]pyrimidine Core

Morpholine protection at the 4-position of **27** was not expected to be readily removable (though the compound could serve as a viable analogue in the medicinal chemistry campaign), so the generation of a thieno[3,2-*d*]pyrimidine scaffold with an amino group at the 4-position and an iodine at the 6-position required a different strategy. A plea for assistance was sought from the wider online chemistry community since strategies to overcome this synthetic hurdle were not forthcoming from the literature. A post at the highly trafficked Chemistry Forums website generated (as of October 18, 2012) 18 unique responses and 1054 views.[69] One suggestion, from two users with extensive research backgrounds,‡ was to use NIS/NBS in a direct S_EAr on

‡ Users: "Discodermolide" (Stuart Mickel, independent chemistry consultant) and "orgopete" (Peter Wepplo, ex-faculty, Monmouth University), released here with permission.

the thieno[3,2-*d*]pyrimidine core. This chemical approach would be advantageous since it would avoid the use of alkyl lithiums which carry with them a requirement for anhydrous conditions and the apparent risk of compound decomposition.

The use of NBS/NIS in DMF at room temperature is a known method for halogenating the 2- and 5-positions of thiophenes, however these conditions were found to be ineffective, and starting material (**24**) was recovered (57%) (**Scheme 17**, **a**).[70] A biphasic reaction system of catalytic $HClO_4$ in NBS/CCl_4 was located in the literature and attempted, albeit in slightly altered reaction conditions (for safety), however this also gave negative results (**Scheme 17**, **b**).[71] The use of Br_2 and 33% HBr/AcOH in refluxing Et_2O/H_2O was another known literature method, but this also led to recovery of starting material (36%) (**Scheme 17**, **c**).[72] From these reactions it was concluded that S_EAr was not a viable method of halogenating the 6-position of **24**.

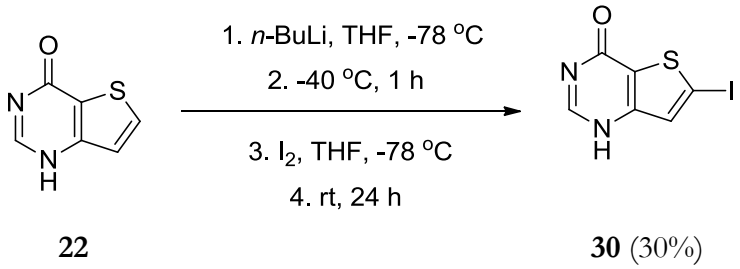


24 **29**

Scheme 17: Failed Halogenation of **24**. *Reagents and Conditions*: a) NBS, DMF, rt, 24 h; b) NBS, cat. H_2SO_4, $CHCl_3$, rt, 24 h; c) Br_2, 33% HBr/AcOH, Et_2O, H_2O, reflux, 6 h.

vii) Halogenation of the Thieno[3,2-*d*]pyrimidine Core (II)

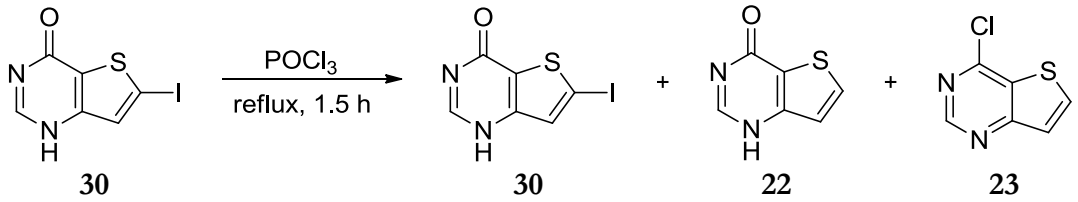
The failures of S_EAr type reactions on the thieno[3,2-*d*]pyrimidine core prompted a return to direct lithiation/halogenation. The possibility of carrying this out on the thieno[3,2-*d*]pyrimidone **22** was investigated. This approach was sensible, since the same trivial functional group manipulations that generated **24** could be used on a halogenated thieno[3,2-*d*]pyrimidine scaffold to generate a product suitable for the envisaged Suzuki couplings. Deprotonation of the thienopyrimidone **22** with *n*-BuLi at -78 to -40 °C followed by iodination at -78 °C gave the iodinated species **30** in 30% yield following column chromatography (**Scheme 18**). Monitoring of the reaction by TLC indicated that no conversion of starting material took place following the addition of iodine, indicating that any lithiated species had been quenched during the iodination.



22 **30** (30%)

Scheme 18: Iodination of the Thieno[3,2-*d*]pyrimidone **22**

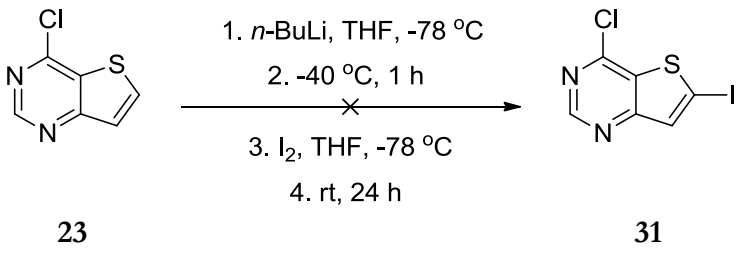
Attempts to chlorinate the 4-position of the iodinated thieno[3,2-*d*]pyrimidone **30** under the same conditions ($POCl_3$, reflux) that gave the chlorinated thieno[3,2-*d*]pyrimidine **23** were unsuccessful. The starting material (**30**) and the chlorinated thieno[3,2-*d*]pyrimidine **23** were observed by ^1H NMR spectroscopy and low resolution mass spectrometry. The presence of signals in the 8.0 – 9.5 ppm region of the ^1H NMR spectrum indicated decomposition had occurred. These difficulties prompted the use of alternative strategies for the synthesis of thieno[3,2-*d*]pyrimidine scaffolds suitable for use in Suzuki coupling reactions.



30 **30** **22** **23**

Scheme 19: Products from Chlorination of the Iodinated Thieno[3,2-*d*]pyrimidone **30**

It was envisaged that the lithiation/iodination of **23** would furnish 4-chloro-6-iodothieno[3,2-*d*]pyrimidine (**31**). Treating a solution of **23** in THF with *n*-BuLi from -78 °C to -40 °C resulted in a drastic colour change in the solution, from yellow to violet over the course of the warming period. Starting material was recovered (57%) following the addition of I_2 and an aqueous workup (**Scheme 20**). The origin of the extensive colour change is unclear, but the low yield implies significant decomposition occured.

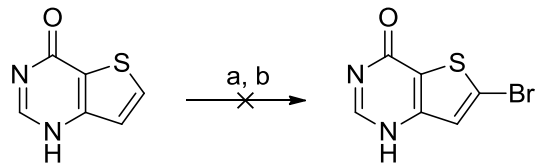


23 **31**

Scheme 20: Failed Iodination of the Chlorinated Thieno[3,2-*d*]pyrimidine **23**

viii) Halogenation of the Thieno[3,2-*d*]pyrimidine Core (III)

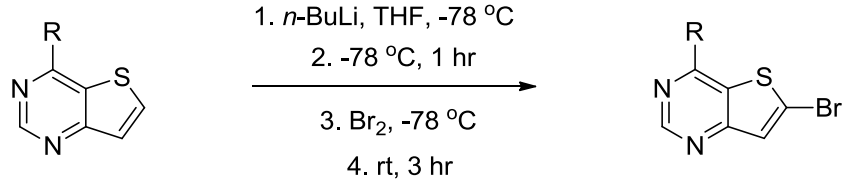
The iodinated thieno[3,2-*d*]pyrimidine scaffolds that were subsequently used in Suzuki couplings (*vide infra*) failed to undergo the desired cross-couplings. These iodinated substrates were generally formed in poor yield. These failings lead to the synthesis of brominated substrates for use in later Suzuki couplings. Compound **22** was treated to the same conditions that generated **30**, but with Br_2 substituted for I_2. This led to quantitative recovery of starting materials. The use of 1,2-dibromoethane analogues as halogen sources for lithiation-substitution reactions on thieno[3,2-*d*]pyrimidine scaffolds is known.[73,74] The use of this reagent was tested in the same conditions that generated **30**, but starting materials were again recovered quantitatively (**Scheme 21**).



22 **32**

Scheme 21: Failed Bromination of the Thieno[3,2-*d*]pyrimidone **22**. *Reagents and Conditions*: a) i) *n*-BuLi, THF, -78 °C, ii) - 40 °C, 1 h, iii) Br_2, -78 °C, iv) rt, 3 h; b) i) *n*-BuLi, THF, -78 °C, ii) – 40 °C, 1 h, iii) CH_2BrCH_2Br, -78 °C, iv) rt, 3 h.

Ni *et al.* had successfully substituted the 6-position of the chlorinated thienopyrimidine **23** by maintaining the reaction temperature at -78 °C until addition of Br_2/I_2 was complete.[75] Mimicry of these reaction conditions furnished the purified brominated 4-chlorothienopyrimidone **33** in a 50% purified yield at 1 mmol scale and 53% purified yield at 5 mmol scale (**Scheme 22**). Replication of this methodology on the morpholine derivative **26** furnished the purified brominated morpholine derivative **34** in 83% yield (**Scheme 22**).



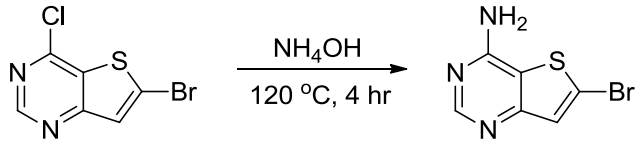
23 R = Cl
26 R = *N*-Morpholino

33 R = Cl (53%)
34 R = *N*-Morpholino(83%)

Scheme 22: Bromination of Thienopyrimidines **23** and **26**

Variation in the electronic nature of ring substituents did not have a noticeable effect since low yields and decomposition products were observed when substrates containing both electron donating (eg: NH_2 and morpholine) and electron withdrawing groups (eg: O, Cl) were used. The higher yields obtained when lithiation/halogenations were carried out at lower temperatures indicated that the maintenance of lower temperatures was crucial for these reactions to proceed in good yield. This hypothesis is supported by the fact that decomposition occurred more readily in the syntheses of **27**, **31**, and **24** when these reactions were carried out at higher temperatures. In contrast, the iodination of the morpholine derivative **26** showed only a modest increase in yield (10% to 21%) when performed at lower temperatures, and a large increase in yield when Br_2 was used in place of I_2. These results indicate that the use of Br_2 and the maintenance of lower temperatures provide the greatest chance of delivering products of these reactions in good yield.

Installation of an NH_2 at the 4-position was completed using the same methodology that was used to furnish thieno[3,2-*d*]pyrimidin-4-amine (**24**), giving **29** in modest yield (43%) (**Scheme 23**). This compound, along with the other thieno[3,2-*d*]pyrimidine based compounds that underwent halogenation at the 6-position, were taken on to Suzuki couplings and the ultimate synthesis of TCMDC 135294 (**10**).



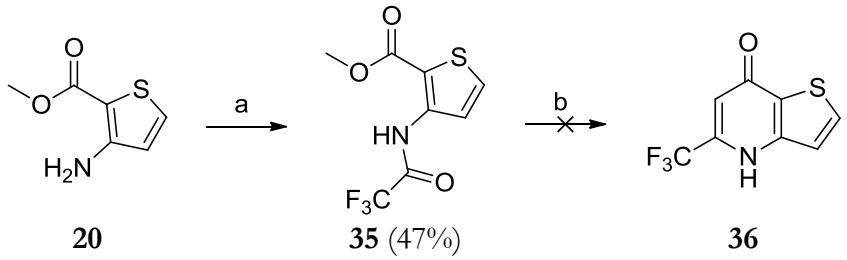
33 **29** (43%)

Scheme 23: Synthesis of 6-Bromothieno[3,2-*d*]pyrimidin-4-amine (**29**)

ix) Fluorination at the 2-position of the Thieno[3,2-*d*]pyrimidine Scaffold

A method for generating 2-substituted analogues was desired to probe the SAR of TCMDC 135294 (**10**). Additionally, a 2-substituted thieno[3,2-*d*]pyrimidine scaffold might better withstand the hypothesised decomposition observed under strongly basic conditions (**28**). A trifluoromethyl group was chosen to test the possibility of 2-substitution, owing to the ready availability of TFA. The condensation of **20** and TFA did not occur by TLC and starring materials were recovered quantitatively. Conversely, condensation of **20** and TFAA proceeded according to a literature procedure to give the desired trifluoromethyl derivative **35** in 47% yield (**Scheme 24**, **a**).[76] It was hoped that the use of the same ring closing methodology, albeit with the addition of DMF to promote solubility of the trifluoromethylated species, that was used to

form **22** (from **21**) could be used in a ring closing reaction to generate **36**. These conditions did not lead to conversion of starting materials by TLC (**Scheme 24**, **b**). Reattempting the synthesis of **36**, through the exact replication of the experimental method that gave **22** (refluxing formamide) was being evaluated at the time of writing.

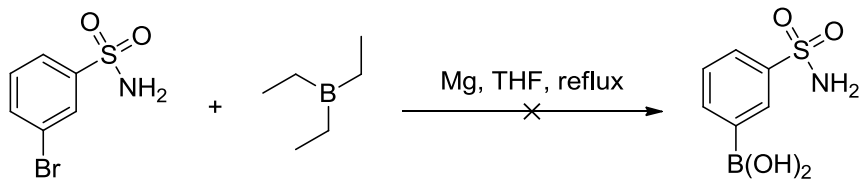


20 **35** (47%) **36**

Scheme 24: Failed Synthesis of a 2-Substituted Thienopyrimidine Scaffold. *Reagents and Conditions*: a) TFAA, pyridine, MeCN, 0 °C, 3 h; b) ammonium formate, formamide, DMF, 140 °C, 24 h.

x) Synthesis of Boronic Acid Derivatives

The assembly of TCMDC 135294 (**10**) required a Suzuki coupling of 6-bromothieno[3,2-*d*]pyrimidin-4-amine (**29**) with 3-sulfamoylphenylboronic acid (**38**). It was envisaged that the boronic acid **38** could be generated *via* a Grignard reaction (**Scheme 25**), according to a known procedure for the assembly of phenylboronic acid derivatives.[77] The magnesium was to be activated with the addition of iodine according to a known procedure.[78] When performed, this reaction failed to generate the boronic acid **38** and starting material was recovered (80%) following a quench with H_2O.

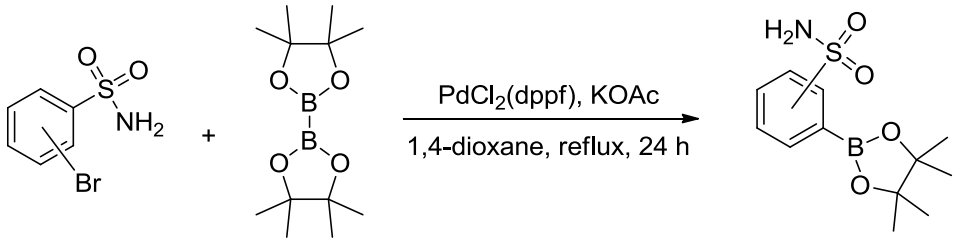


37 **38**

Scheme 25: Failed Generation of the Boronic Acid **38** *via* a Grignard Reaction

The telltale signs of exothermic activation of magnesium were not observed, suggesting that the activation of magnesium had not occurred. However, it was also possible that the triethylborane used was unreactive owing to the age of the sample used. The use of pinacolyl boronate esters in lieu of boronic acids in Suzuki couplings is well known.[79] The *m*-boronate ester **41** was generated and purified *via* column chromatography in 62% yield according to a literature

procedure based on similar substrates. The same methodology was used on *p*-bromobenzenesulfonamide (**39**) to afford the *p*-substituted boronate ester **42** in 56% yield following column chromatography. The synthesis of the *o*-substituted boronate ester using this methodology was unsuccessful.



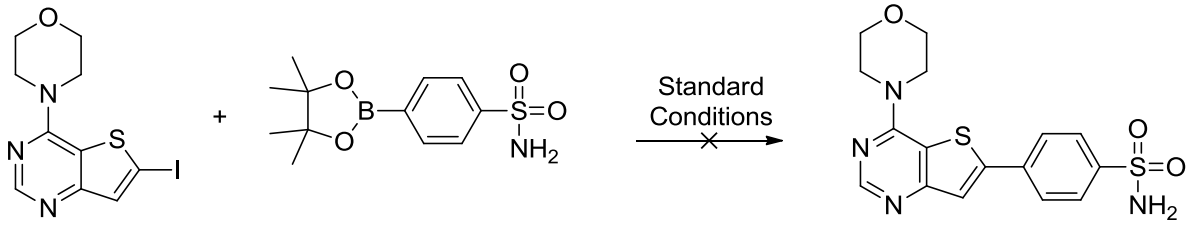
37: *m*-isomer
39: *p*-isomer
40: *o*-isomer

41: *m*-isomer (62%)
42: *p*-isomer (56 %)

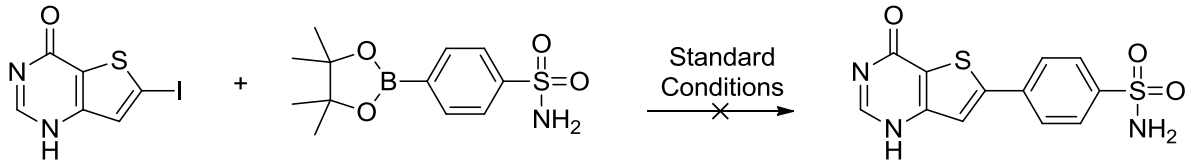
Scheme 26: Synthesis of the Boronate Esters **41** and **42**.

xi) Suzuki Couplings

The application of the Suzuki reaction to couple thienopyrimidines and phenylboronic acid derivatives is known chemistry and it was envisaged that the synthesis of TCMDC 135294 (**10**) would be completed using this chemistry.[80] Unfortunately a microwave reactor, used in most literature couplings of these substrates, was not readily accessible for the present study. An experimental method that performed a Suzuki coupling on these substrates without a microwave reactor was found in the literature, but it lacked experimental detail.[75] A patent that gave suitable conditions for relevant substrates was found.[81] A procedure (hereinafter termed the "standard conditions") which performed the coupling of the substrates using catalytic $Pd(PPh_3)_4$, CsF (4 eq), $NaHCO_3$ (2 eq) in refluxing 1,4-dioxane/H_2O (2:1) under argon for 24 hours was drawn from the combination of these syntheses. The *p*-boronate ester **42** was not novel and could be bought commercially. It was decided to use **42** rather than the required *m*-boronate ester **41** in initial Suzuki couplings to validate the synthetic approach. Coupling of **27** with **42**, and of **30** with **42** was attempted using the standard conditions (**Scheme 27**)



27 **42** **43**

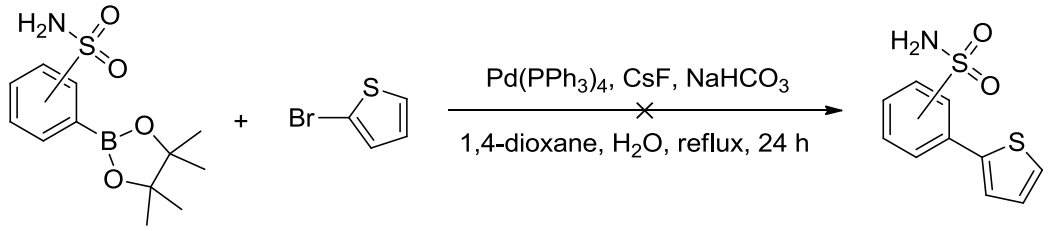


30 **42** **44**

Scheme 27: Attempted Suzuki coupling of **30** and **42**, and of **27** and **42**

Both reactions showed complete consumption of starting material by TLC, and were purified *via* column chromatography following aqueous workup. The ^{1}H NMR spectrum that was obtained from the purified product from reaction generating **43** was more complex than expected. No ions with identifiable molecular formulae of relevance to the reaction were visible in the low resolution mass spectrum. Purification of the products obtained for the synthesis of **44** returned starting material (**42**, 10%) as well as a product exhibiting the same ^{1}H NMR spectrum that was obtained for the unsuccessful synthesis of **43** (albeit without morpholine signals). Mass spectrometry of this product showed peaks corresponding to the H and Na ions of **30**, implying that either the purification was incomplete or that the unknown product decomposed to form **42** in the mass spectrometer. Alternatively, the two reactions were generating the same by-product which interfered with the desired Suzuki reaction.

A model system was invoked to investigate whether the thieno[3,2-*d*]pyrimidine substrates were causing issue for the Suzuki coupling. In this, **41** and **42** were coupled with 2-bromothiophene under the standard conditions (**Scheme 28**).



41 *m*-isomer
42 *p*-isomer

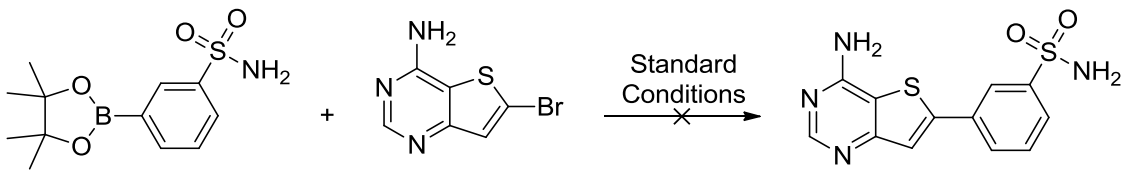
45 *m*-isomer
46 *p*-isomer

Scheme 28: Model Suzuki Couplings of 2-Bromothiophene and **41** or **42**

Both reactions were incomplete by TLC, but had nevertheless formed a new substance of low polarity, and so were purified *via* column chromatography. The ^{1}H NMR spectrum of the product formed from the intended synthesis of **46** was identical to that observed for the product obtained for the attempted syntheses of **43** and **44**. The similarity between these three reactions suggested that the unexpected behaviour of the Suzuki reaction occurred without the

presence of a pyrimidine ring. Generation of the *m*-isomer **45** initially proceeded under the same conditions as for the *p*-isomer **46**, though unfortunately the reaction boiled dry after 3 hours. The ^{1}H NMR spectrum of the purified product showed unexpected clarity in the aromatic region (3 triplets, 1 singlet, and 2 doublets) which did not correspond to the spectrum of starting materials or the expected spectrum of the desired product. In addition, no ions with identifiable molecular formulae with relevance to the reaction were visible in the mass spectrum. The homocoupling of substrates was also ruled out by these spectra.

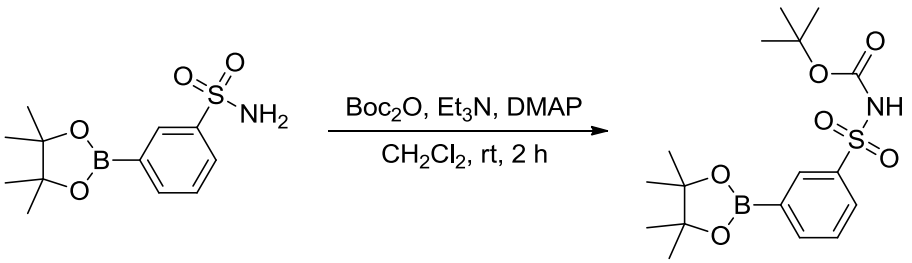
An attempt was made to couple **29** with the *m*-isomer **41** under the standard conditions, even though Suzuki reactions had hitherto failed to generate desired products. Aqueous workup and chromatographic purification led to the isolation of starting material **29** (22%) and another product that, by ^{1}H NMR spectroscopy, was the same product that was generated in the attempted syntheses of **43**, **44** and **46** (**Scheme 29**).



41 + **29** → **47**

Scheme 29: Failed Synthesis of the Desired Suzuki Product **47**

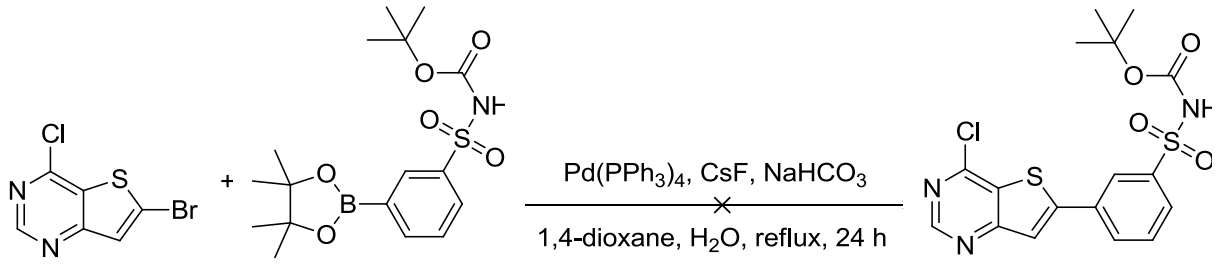
Literature searches were made alongside a public, online appeal for help. The latter yielded advice (anonymously given) which suggested that the NH_2 and aryl halide groups on many of the substrates under consideration might generate competition between Buchwald-Hartwig and Suzuki couplings.[82] Presumably, the complex ^{1}H NMR spectra that had been observed in the syntheses of **43**, **44**, **46** and **47** could be explained through multiple linkages forming through Buchwald-Hartwig and Suzuki reactions forming a macromolecule of some kind. The use of a protected amine, such as **48**, in Suzuki reactions could eliminate Buchwald-Hartwig reactivity and still permit a straightforward synthesis of the final product (**10**). It was important to test whether a relatively easily installed *Boc* protecting group could be installed before moving on to protecting groups that are harder to install but would lead to complete elimination of amine reactivity.[83] The *Boc*-protected boronate ester **48** was synthesised according to a literature procedure for generation of similar *Boc*-proteted benzenesulfonamides (**Scheme 30**).[84]



41 **48** (40%)

Scheme 30: Synthesis of the *Boc*-Protected *m*-Substituted Boronate Ester **48**

Coupling of the *Boc*-protected, *m*-substituted boronate ester **41** with 6-bromo-4-chlorothieno[3,2-*d*]pyrimidine (**33**) was performed under the standard conditions. ^{1}H NMR spectroscopy of the crude reaction mixture revealed peaks corresponding to the starting material **48**, and the same unknown patterns observed during ^{1}H NMR spectroscopy of products from the syntheses of **43**, **44**, **46** and **47**. The crude mixture was not purified since the products had already been identified through comparison to relevant ^{1}H NMR spectra.

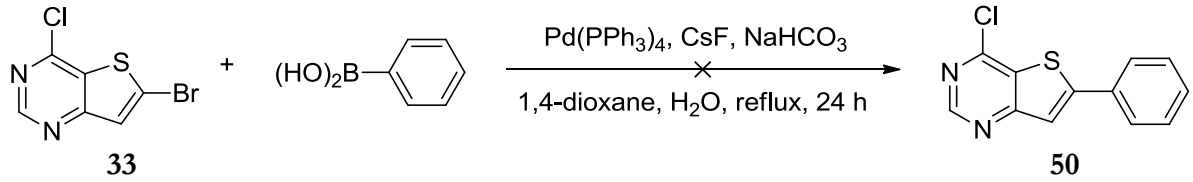


33 **48** **49**

Scheme 31: Failed Suzuki Coupling of *Boc*-protected Sulfonamide **48**

Suzuki coupling of **33** and phenylboronic acid under the standard conditions was attempted to determine whether the benezenesulfonamide moiety was causing problems for the Suzuki coupling and whether the more reactive boronic acid was required (**Scheme 32**). The reaction was difficult to monitor *via* TLC due to multiple spots, but aqueous workup and chromatographic purification were performed after 24 hours at reflux. Examination of the product by ^{1}H NMR spectroscopy showed an absence of complex splitting characteristic of the presence of a phenyl group. This indicated the Suzuki coupling had not proceeded. Four distinct environments, each with a singlet and an apparent shoulder singlet (in a 2:1 ratio), were observed in the region of the ^{1}H NMR spectrum characteristic of thiophene protons. The presence of only one pyrimidine signal suggested a deficiency of pyrimidine protons. The formation of a macromolecule linked by linkages primarily through the pyrimidine rings would

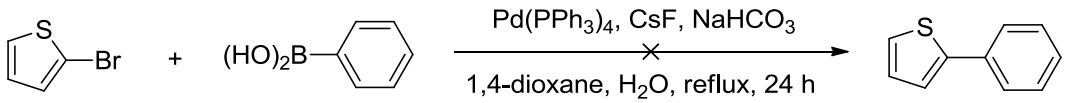
account for this, but this does not fully explain the observed spectrum and no realistic structure could be proposed for this molecule, the characterisation of which is ongoing.



33 **50**

Scheme 32: Attempted Suzuki Coupling of **33** and Phenylboronic Acid

These results were unexpected, since neither thiophenes or pyrimidines are known to cause difficulty for use in Suzuki couplings.[80] The successful use of CsF in Suzuki reactions has been demonstrated previously.[85,86] The standard conditions were drawn from a patent, and a journal communication lacking any experimental detail, so there was some reason to doubt their suitability in the present synthesis. A model Suzuki reaction was used to determine the suitability of the standard conditions for Suzuki couplings. The attempted coupling of 2-bromothiophene and phenylboronic acid under the standard conditions lead to conversion of starting materials by TLC, however, the signals observed in the ^{1}H NMR spectrum of the purified product did not correspond to known values of the desired product (**Scheme 33**).[87]



Scheme 33: Failed Suzuki Coupling of 2-Bromothiophene and Phenylboronic Acid

Although no definitive conclusions could be drawn from these results, they nevertheless generated some insight. The trivial Suzuki coupling of 2-bromothiophene and phenylboronic acid did not generate the desired product, which indicated that the standard conditions might be causing the observed difficulties. The attempted synthesis of **50** did not generate the unknown product that was generated in the attempted syntheses of **43**, **44**, **46**, **47** and **48**. This indicated that the absence of the sulfonamide moiety, or the use of a boronic acid (rather than a boronate ester) might be vital to a successful synthesis of TCMDC 135294 (**10**).The identity of the unknown product formed during the attempted syntheses of **43**, **44**, **46**, **47** and **48** could not be established but characterisation of this compound, through crystallisation of a bulk sample, is underway at the time of writing. The only commonly observed side reaction of Suzuki couplings is the migration of phenyl groups from the palladium catalyst.[88] This possibility could be tested by adoption of a different catalyst. The use of the catalyst $PdCl_2$(dppf), which successfully

generated the boronate esters **41** and **42**, was one possibility being explored at the time of writing.

xii) Acquisition of Commercially Synthesised Analogues

The development of a preliminary SAR for TCMDC 135294 (**10**) by the acquisition of compounds made synthetically synthetic means could be complemented by acquisition of commercial analogues for biological testing. Many commercial organisations sell small quantities of custom compounds, sourced from academic and industrial settings. Commercially available analogues of **10** were thus identified through *in silico* searches of over 5.4 million compounds on eMolecules.[89] Computational similarity calculations and database searches were performed by Dr. Iain Wallace (Novartis, formerly of ChEMBL) as a spontaneous contribution to the project. This netted the identification of commercially available compounds with a Tanimoto coefficient greater than 0.3. A thorough review of the Tanimoto coefficient is beyond the scope of the present study, and a more detailed description may be found elsewhere.[90,91] In brief, the analysis deconstructed **10** into constituent parts (eg a benzene ring) to form a 'molecular fingerprint', which is then compared to the molecular fingerprints belonging to every molecule available on the database. The similarity between two compounds is given by the following, where M^1 and M^2 are the two molecules under consideration:

$$Tanimoto\ (M^1, M^2) = \frac{n(Fingerprints\ in\ common\ between\ M^1\ and\ M^2)}{Total\ fingerprints\ in\ M^1\ and\ M^2}$$

Six commercially available compounds with a Tanimoto coefficient greater than 0.3 were identified. Although the Tanimoto coefficient used was low by cheminformatics standards,[90] the compounds nevertheless appeared by eye to be suitable for use in developing a SAR based on **10**. Some of these commercially available compounds became unavailable during the period of analysis. The eMolecules database was again searched for compounds with similarity to **10**, but this time a Tanimoto coefficient minimum of 0.55 was used. This search returned 18 commercially available analogues. In the interests of simplicity, a single supplier of compounds was sought. Fortunately, 13 of these 18 compounds were available from one source (Enamine Ltd). GSK publicly confirmed that they had not evaluated any of these compounds as antimalarial agents. As part of a public consultation aimed at identifying which compounds should be purchased, Dr. Paul Willis of the MMV advocated an exclusive focus on compounds bearing a thienopyrimidine scaffold.[92] The exclusion of compounds with a cLogP value greater

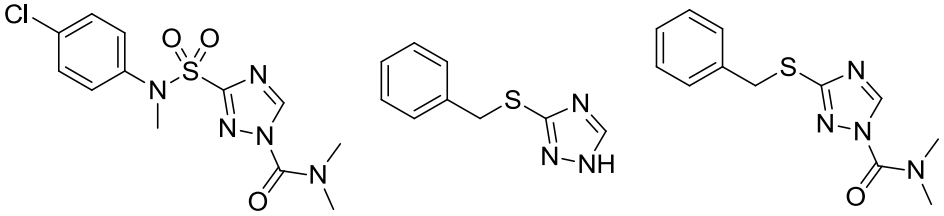
than 4.0 returned 9 compounds (viewable in **Appendix B**). These were purchased from Enamine, and sent for biological evaluation.

3. Biological Evaluation of Compounds

i) Biological Evaluation of TCMDC 134395 (9) and TCMDC 135294 (10)

Resynthesised TCMDC 134395 (**9**) was evaluated for antimalarial activity *in vitro*, alongside the synthetic bench stable precursors (**12**, **13**), to obtain a preliminary SAR Active compounds were screened for cytotoxicity against HEK 293 cells *in vitro* to ensure that any observed activity was a phenotype unique to the parasite. These data are shown in **Table 3**.

Table 3: Biological Data for TCMDC 134395 (**9**) and its Synthetic Precursors

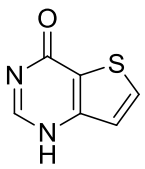
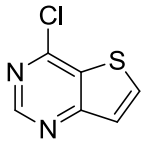
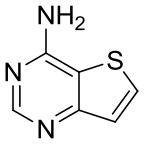
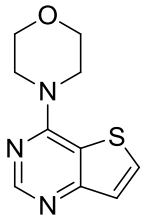
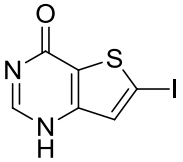


	Compound 9	12	13
3D7 IC_{50}	333 nM	NA	438 nM
HEK 293 (% Inhibition)	No	-	No

The data obtained confirm that TCMDC 124395 (**9**) is a potent antimalarial with no associated cytotoxicity. The observed potency was higher than that reported in the original study.[25] The synthetic precursor (**13**) also displayed potent activity against the parasite. These results show that TCMDC 124395 (**9**) is of great interest for a lead development campaign.

Although the synthesis of TCMDC 135294 (**10**) was not complete, there were several bench stable precursors to this compound (**22**, **23**, **24**, **26** and **30**) that were available for testing. These were also despatched for testing in order to obtain a preliminary SAR. These data are shown in **Table 4**.

Table 4: Biological Data for Synthetic Precursors to TCMDC 135294 (**10**)

					
	22	**23**	**24**	**26**	**30**
3D7 IC_{50}	NA	9.4 μM (haemolysis)	NA	NA	NA
HEK 293 (% Inhibition)	-	No	-	-	-

The suitability of TCMDC 135294 (**10**) for a lead development campaign cannot be determined based only upon these results. The data obtained indicate that the thieno[3,2-*d*]pyrimidine on its own is insufficient to generate antimalarial activity. Variation of the substitution present on the scaffold failed to substantially effect antimalarial activity *in vitro*. Only compound **23** showed potency, however this was accompanied by haemolysis.

Due to unusually long customs clearance delays and a synchronicity problem in the parasites used for evaluation of antimalarial activity, biological data for the commercially available analogues was not obtained. These biological data will be released by collaborators at Griffith University on November 6th, one day after submission of this thesis.

Incoming data for commercially obtained analogues, in addition to biological data for TCMDC 135294 (**10**) (once resynthesised), will permit a more detailed investigation regarding the suitability of TCMDC 135294 (**10**) for a lead development campaign

Chapter 4: Conclusions and Future Work

1. Conclusions

The prosecution of TCMDC 134395 (**9**) and TCMDC 135294 (**10**) for a lead development campaign depended on confirmation of their activity against malaria *in vitro*. We proposed that the task of resynthesising and evaluating these hits could be carried out using an open source approach, whereby all information and discussions would be carried out openly, and where anyone would be welcome to contribute.

TCMDC 134395 (**9**) was generated using a short synthetic strategy drawn from the literature. Efficient purification processes were developed for two reactions, and another was optimised through an assay of reaction conditions. The use of the oxidative chlorination presented a challenge in arriving at a safe method for carrying it out. Although the overall pathway through which this reaction occurs is known, the mechanism is not. A mechanism for this reaction was suggested. Testing of the hit (**9**) for antimalarial activity showed that it was more than twice as potent as was originally reported by GSK. The precursor **13** was also active against the parasite. Neither compound demonstrated cytotoxicity. The compound presents an attractive starting point for a lead development campaign.

The synthesis of TCMDC 135294 (**10**) is ongoing. Synthesis of the thiophene **20** was not possible using our methods, however it could be obtained commercially. The assembly of a thieno[3,2-*d*]pyrimidin-4-amine scaffold built upon **20** was simple, but halogenation on this scaffold was not. A method for carrying out lithium/halogen exchange on thieno[3,2-*d*]pyrimidines was developed. A selection of boronate esters for Suzuki couplings was generated, in the hope that these could be used to furnish TCMDC 135294 (**10**). These Suzuki couplings failed to generate the desired product. Multiple Suzuki reactions appeared to generate the same product by ^{1}H NMR spectroscopy, however the identity of this product could not be established at the time of writing. Conversely, other Suzuki reactions appeared to generate different products, also awaiting identification. Several synthetic precursors to TCMDC 135294 (**10**) were evaluated in lieu of the final product. The only precursor that displayed substantial activity was **26**, which also led to haemolysis. This preliminary SAR suggests that any antimalarial activity demonstrated by TCMDC 135294 (**10**) depends on the substituent at the 6-position of the thieno[3,2-*d*]pyrimidine scaffold.

The project was hastened by the use of an open source approach. Assistance regarding the oxidative chlorination, halogenation reactions and the Buchwald-Hartwig cross coupling came from various online sources from contributors with a range of backgrounds. The acquisition of commercial analogues of **10** was hastened by assistance from Dr. Iain Wallace, who identified a simple method for identifying structurally similar analogues of **10** online. These analogues have been purchased and are currently being biologically evaluated.

2. Future Work

TCMDC 134395 (**9**), having been confirmed as a having potent activity against malaria *in vitro*, is now a suitable option for a lead development campaign, pending confirmation from GSK and the MMV that no other work is being performed on TCMDC 134395 (**9**). Any future work done on the compound should be carried out as per the MMV compound progression criteria.[13] *In vivo* experiments in mice may begin. These experiments should include the demonstration of potency and stability *in vivo*. Initial SAR determination may begin with the synthesis of small, lipophobic analogues. These may be prepared through the variation of the amine coupled with the sulfonyl chloride **15**, and/or through the variation of the acyl chloride coupled to the benzyl protected triazole **12** (**Figure 10**). A clue pertaining to the SAR of this hit compound has already been determined through the antimalarial potency exhibited by a synthetic precursor (**13**). Analogues may be acquired from commercial entities, through the same processes by which commercial analogues of TCMDC 135294 (**10**) were obtained.

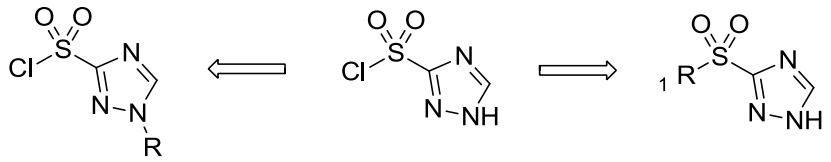
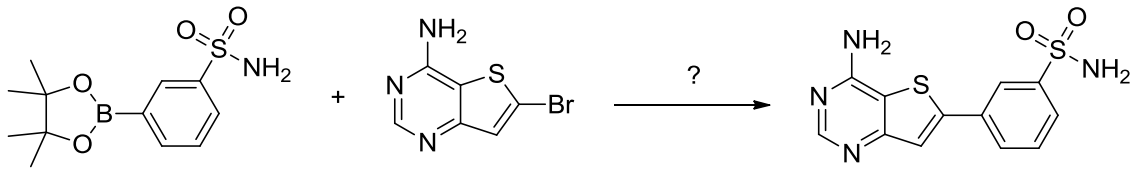


Figure 10: Possible Methods to Synthesise TCMDC 134395 (**9**) Analogues

A successful methodology for the Suzuki coupling of the boronate ester (**41**) and the brominated thieno[3,2-*d*]pyrimidin-4-amine (**29**) is needed to complete the synthesis of TCMDC 135294 (**10**). A screen of palladium catalysts is one possible approach to developing such a methodology. The successful use of $PdCl_2$(dppf) in the synthesis of the boronate esters **41** and **42** indicates that this might be a successful catalyst for use on these substrates. Identification of the product common to the attempted syntheses of **43**, **44**, **46**, **47** and **48** is vital. Knowledge of

this product will provide important clues concerning the problems inherent to the present Suzuki reaction. Lastly, variations in the standard reaction conditions may furnish the desired Suzuki product (**Scheme 35**).



Scheme 34: The Synthesis of TCMDC 135294 (**10**) Depends on the Development of Conditions Appropriate to the Reaction

The arrival of biological data for commercially obtained analogues of TCMDC 135294 (**10**) will permit the elucidation of a preliminary SAR. Once TCMDC 135294 (**10**) has been reevaluated *in vitro*, a decision can be made as to whether this compound should be advanced to a lead development campaign.

Chapter 6: Experimental

1. General Experimental Details

Commercially available reagents were purchased from either Sigma-Aldrich or Alfa Aesar, with the exception of trichloroisocyanuric acid, which was obtained as HY CLOR: HY TECH pool chlorine (85% trichloroisocyanuric acid) from Bunnings Warehouse. Aged alkyllithium reagents were titrated to determine concentration prior to use. All reagents were used without further purification unless otherwise specified. When anhydrous conditions were required glassware was oven dried, assembled hot and allowed to cool under a positive pressure of N_2. THF was distilled over Na wire/benzophenone ketyl radical. CH_2Cl_2, when used as a reaction solvent, was distilled over CaH_2. Dried solvent was stored over Ajax molecular sieves, 3Å (1.5 – 2.5 mm). Et_3N and *i*Pr_2EtN were stored over Ajax molecular sieves, 3Å (1.5 – 2.5 mm) prior to use. All reactions were performed under an inert atmosphere of N_2 or Ar and mixed with continuous magnetic stirring. Room temperature is taken to be between 15 and 30 °C. Reactions requiring elevated temperatures were heated using a silicone oil bath on a magnetic stirrer and monitored using a Yellowline TC3 temperature probe. Temperatures in the range of -5 °C to -15 °C were achieved with an ice and sodium chloride slurry. Cooling to -40 °C was achieved with a MeCN and liquid N_2 bath. Cooling to -78 °C was achieved with an acetone and dry ice bath. TLC was performed on Merck Silica Gel 60 F254 pre-coated aluminium plates (0.2 mm). Visualisation was by UV irradiation (254 nm) and an appropriate stain prepared according to group guidelines.[93] Column chromatography was carried out using Davasil Chomatographic Silica Media, LC60A 40 – 63 μm, using a positive pressure of N_2 to force solvent flow though the stationary phase at a controlled pace. The compound to be purified was either applied as a solution in CH_2Cl_2 or pre-adsorbed onto silica.

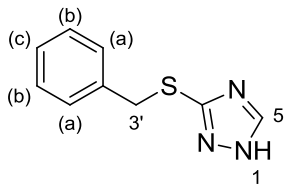
^1H and ^{13}C NMR spectra were obtained on either Bruker Avance DPX200 (200.13 MHz and 50.33 MHz respectively), DPX300 (300.13 MHz and 75.48 MHz respectively) or DPX400 (400.13 MHz and 100.48 MHz respectively). ^{19}F NMR spectra were obtained on a Bruker Avance DPX300 (282 MHz). Chemical shifts (δ) are reported in ppm with respect to either TMS or residual solvent. ^1H NMR signals are reported with multiplicity, relative integrals, coupling constants *J* (Hz) and assignments. Assignments are numbered according to IUPAC convention. When IUPAC convention is unsuitable, assignments are designated with a prime or

by enclosure within parentheses. When required, assignments were determined HSQC and HMBC NMR spectroscopy. All samples were dried *in vacuo* prior NMR spectroscopy. LRMS was performed using ESI+, ESI- or APCI on a Finnigan quadrupole ion trap mass spectrometer. HRMS was performed on a Bruker 7T FTCIR mass spectrometer. LCMS was performedon a Shimadzu LCMS 2020 (equipped with photodiode array) using aWaters XTerra MS C18 2.1 × 150 mm column (5 μm). A binary, linear MeCN/H_2O gradient at 0.2 mL min^{-1} was used, with 0.1% v/v formic acid as a mobile phase modifier. Elemental analysis was performed at the University of Otago, New Zealand using a Carbo-Erba EA 1108 instrument. When elemental analysis gave unsatisfactory results, various equivalents of solvent are quoted to account for the discrepancy between calculated and observed values. Melting points were measured using an Optimelt Automated Melting Point System. IR spectra were obtained on a Bruker Alpha-E FT-IR by ATR. Absorption with maxima are reported in wavenumbers (cm^{-1}). Films were obtained by evaporating acetone from dissolved samples.

Entries with underlined compound titles are novel. All other entries are known compounds.

2. Experimental Chemistry

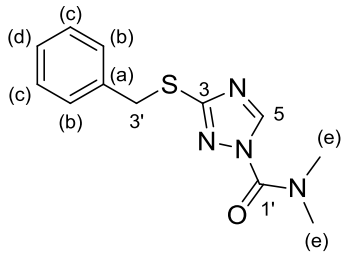
3-Benzylthio-1*H*-1,2,4-triazole (12)



3-Mercapto-1,2,4-triazole **11** (3.00 g, 29.6 mmol, 1.0 eq) and finely powdered anhyd K_2CO_3 (8.65 g, 62.6 mmol, 2.1 eq) were stirred in DMF (30 mL). BnBr (3.85 mL, 32.4 mmol, 1.1 eq) was added dropwise with stirring. The mixture was stirred at rt for 96 h. To the mixture were added EtOAc (50 mL) and saturated aq $NaHCO_3$ (50 mL). The organic layer was separated, washed with H_2O (2 × 30 mL) and brine (30 mL), dried with $MgSO_4$ and concd *in vacuo* to yield a murky white solid (3.39 g). This crude solid was dissolved in boiling 50% PhMe/petrol (30 mL) and heated gently until complete dissolution occurred. The solution was allowed to cool to rt before the filtrand was washed repeatedly with chilled 50% PhMe/petrol with manual crushing before being dried *in vacuo* to yield the title compound as white needles (2.72 g, 14.3 mmol, 48%).

m.p: 77 °C – 78 °C (lit: 79 °C – 81 °C)[94]. ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.36 (2H, s, $H^{3'}$), 7.26-7.33 (5H, m, H^a, H^b, H^c), 8.09 (1H, s, H^5). LRMS (ESI+): *m*/*z* 191.2 ($[MH]^+$ 100%). Spectroscopic data match those in the literature.[42]

3-(Benzylthio)-*N*,*N*-dimethyl-1*H*-1,2,4-triazole-1-carboxamide (13)



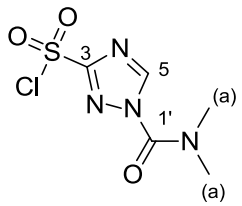
Benzyl protected triazole **12** (2.23 g, 11.7 mmol, 1.0 eq) and finely powdered anhyd $NaHCO_3$ (6.76 g, 48.9 mmol, 4.2 eq) were stirred in DMF (25 mL) at 0 °C. To the stirring mixture was added dimethylcarbamoyl chloride (4.3 mL, 48 mmol, 4 eq) dropwise. The mixture was allowed to warm to rt and stirred at rt for 2 h. The reaction was quenched with H_2O (10 mL). To the solution was added EtOAc (75 mL). The organic layer was separated, washed with H_2O (2 × 50 mL) and brine (50 mL), dried over $MgSO_4$ and concd *in vacuo* to yield the desired compound as viscous oil (2.27 g, 10.4 mmol, 86%).

^{1}H NMR (200 MHz, $CDCl_3$): δ 3.14 (6H, br s, $H^{e'}$), 4.37 (2H, s, $H^{3'}$), 7.18-7.41 (5H, m, H^b, H^c, H^d), 8.72 (1H, s, H^5). ^{13}C NMR (75 MHz, $CDCl_3$): δ 36.0 ($C^{3'}$), 38.9 (C^e, br), 127.4 (C^d), 128.5 (C^b), 128.8 (C^c), 136.9 (C^a), 147.2 (C^5), 149.3 ($C^{1'}$), 162.1 (C^3). IR (film): ν_{max}/cm^{-1} 1697, 1478, 1452, 1398, 1375, 1236, 1185, 997, 908. LRMS (ESI+): m/z 284.8 ([MNa]$^+$, 85%). HRMS (ESI+): m/z calcd for [MNa]$^+$ 285.07860, found 285.07803.

Chlorine gas

Cl—Cl Finely powdered trichloroisocyanuric acid (12.85 g, 47.0 mmol, 1.0 eq) was stirred under N_2 before 32% aq HCl (14.0 mL, 142 mmol, 3.0 eq) was added dropwise to yield the title compound as a yellow-green gas (theoretical yield: 3.15 L, 141 mmol). This product was subsequently used without further purification or characterisation.

1-(Dimethylcarbamoyl)-1*H*-1,2,4-triazole-3-sulfonyl chloride (15)

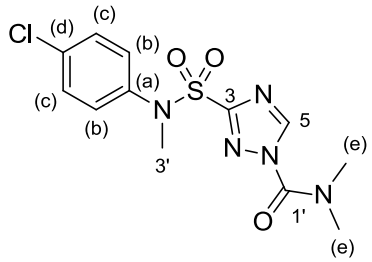


Benzyl protected triazolourea **13** (2.72 g, 10.4 mmol, 1.0 eq) was dissolved in glacial AcOH (10.0 mL, 174 mmol, 17 eq) and H_2O (3.8 mL, 210 mmol, 20 eq) and cooled to -5 °C with stirring. Cl_2 (9.1 L, 406 mmol, 39 eq) was bubbled though this stirring solution at -5 °C over three 0.5 h periods before the reaction vessel was flushed with N_2. The residue was concd *in vacuo* over 5.5 h to yield the title compound as viscous yellow oil (2.69 g).

^{1}H NMR (200 MHz, $CDCl_3$): δ 3.23 (3H, br s, H^a), 3.36 (3H, br s, H^a), 8.96 (1H, s, H^5). ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.9 (C^a), 39.5 (C^a), 148.4 (C^5), 163.0 (C^3), 170.9 ($C^{1'}$). LRMS

(APCI): *m*/*z* 239.2 ([MNa]$^+$, ^{35}Cl, 100%), 241.3 ([MNa]$^+$, ^{37}Cl, 44%). HRMS (ESI+): *m*/*z* calcd. for ([MNa]$^+$, ^{35}Cl) 260.98521, ([MNa]$^+$, ^{37}Cl) 262.97956, found 260.98205, 262.97897. Anal. was not obtained due to expected hydrolysis. An IR spectrum was not obtained due to the presence of residual acids.

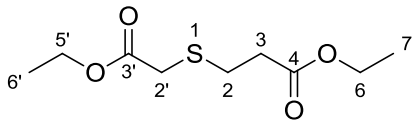
3-(*N*-(4-Chlorophenyl)-*N*-methylsulfamoyl)-*N*,*N*-dimethyl-1*H*-1,2,4-triazole-1-carboxamide (9)



Sulfonyl chloride **15** (726 mg, 3.04 mmol, 1 equiv.) and DMAP (40 mg, 0.30 mmol, 0.1 eq) were dissolved in MeCN (10 mL) and stirred at rt. To the stirring solution were added *i*Pr$_2$EtN (1.2 mL, 6.9 mmol, 2.3 eq) and 4-chloro-*N*-methylaniline (0.42 mL, 3.5 mmol, 1.1 eq). The solution was heated at reflux for 60 h. The reaction solution was allowed to cool to rt. To the solution were added EtOAc (100 mL) and H_2O (40 mL). The organic layer was separated, washed with H_2O (3 × 30 mL) and brine (30 mL), dried over $MgSO_4$ and concd *in vacuo* to yield a brown liquid (904 mg). The residue was purified *via* column chromatography (5% EtOAc/petrol → 100% EtOAc) to give a maroon paste which was dissolved in boiling 50% EtOH/H_2O. The solution was left undisturbed at rt for 2 days. The filtrand was washed with H_2O (10 mL) to give the title compound as radiating maroon crystals (296 mg, 0.86 mmol, 28%).

m.p. 120 °C – 121 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.17 (6H, s, H^e), 3.46 (3H, s, H$^{3'}$), 7.13-7.37 (4H, m, H^a, H^b, H^c, H^d), 8.87 (1H, s, H^5). ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.7 (C$^{3'}$), 39.6 (C^e), 128.4 (C^b), 129.5 (C^d), 133.9 (C^c), 139.1 (C^a), 147.9 (C^5), 148.5 (C^3), 161.0 (C$^{1'}$). IR (film): ν_{max}/cm^{-1} 1717, 1484, 1368, 1182, 1150. LRMS (ESI+): *m*/*z* 366.3 ([MNa]$^+$, ^{35}Cl, 100%), 368.2 ([MNa]$^+$, ^{37}Cl, 40%). HRMS (ESI+): *m*/*z* calcd. for ([MNa]$^+$, ^{35}Cl) 366.04036, ([MNa]$^+$, ^{37}Cl) 368.03741, found 366.03953, 368.03690. Anal calcd for $C_{12}H_{14}ClN_5O_3S$: C, 41.92; H, 4.10; N, 20.37; S, 9.33, found: C, 42.20; H, 4.27; N, 20.23; S, 9.06.

Ethyl 3-((2-ethoxy-2-oxoethyl)thio)propanoate (16)

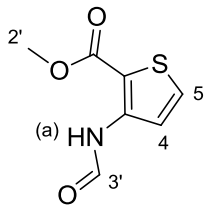


To ethyl 2-mercaptoacetate (2.19 mL, 18.2 mmol, 1.0 eq) was added piperidine (0.10 mL, 1.01 mmol, 0.1 eq) with stirring. Ethyl acrylate (1.94 mL, 18.2 mmol, 1.0 equiv.) was added dropwise over 2 h. The solution was stirred at rt overnight. To the stirring solution were added additional ethyl acrylate (0.20 mL, 1.87 mmol, 0.1 equiv.) and piperidine (2 drops). The

solution was stirred at rt for 6 h. To the solution were added EtOAc (30 mL) and H_2O (30 mL). The organic layer was separated, washed with H_2O (3 × 30 mL) and brine (30 mL), dried over $MgSO_4$ and concd *in vacuo* to yield the title compound as a colourless, foul smelling oil (3.40 g, 17.7 mmol, 97%).

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.27 (3H, t, *J* 7.2, $H^{7'}$), 1.29 (3H, t, *J* 7.0, $H^{6'}$), 2.64 (2H, t, *J* 7.2, $H^{3'}$), 2.92 (2H, t, *J* 7.3, H^{2}), 3.24 (2H, s, $H^{2'}$), 4.18 (4H, apparent dt, *J* 7.4 ($H^{5'}$) 7.0 (H^{6})). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.2 ($C^{7'}$, $C^{6'}$), 27.6 (C^{2}), 33.7 (C^{3}), 34.4 ($C^{2'}$), 60.7 ($C^{5'}$), 61.7 (C^{6}), 170.2 ($C^{3'}$), 171.7 (C^{4}). IR (oil): ν_{max}/cm^{-1} 1726, 1270, 1247, 1125, 1027. LCMS (ESI+): *m*/*z* 221.3 ([MH]$^+$, 100%). ^{1}H NMR data match those reported in the literature.[56]

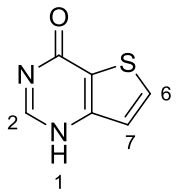
Methyl 3-formamidothiophene-2-carboxylate (21)



Methyl-3-aminothiophene-2-carboxylate **20** (300 mg, 2.11 mmol, 1.0 eq) and NH_4OAc (226 mg, 2.93 mmol, 1.4 eq) were dissolved in formic acid (1.00 mL, 26.5 mmol, 12.6 eq). The solution was heated at gentle reflux for 7 h before being allowed to cool to rt. A brown solid formed which was tipped onto ice, filtered, and washed with H_2O (50 mL) to yield a pale brown powder (575 mg). The powder was dissolved in boiling 50% EtOH/H_2O and filtered. The filtrate was collected and dried *in vacuo* to give the title compound as fluffy, white crystals (237 mg, 1.27 mmol, 60%).

m.p: 93 °C – 94 °C (lit: 82 °C)[95]. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.90 (3H, s, $H^{2'}$), 7.49 (1H, d, *J* 5.4, H^{4}), 8.11 (1H, d, *J* 5.4, H^{5}), 8.42 (1H, s, $H^{3'}$), 10.10 (1H, br s, H^{a}). LRMS (APCI): *m*/*z* 637.6 (3[MK]$^+$, 10%), 607.9 (3[(M-CO)K]$^+$, 100%), 579.9 (3[(M-2(CO))K]$^+$, 95%), 551.9 (3[(M-3(CO))K]$^+$, 25%. ^{1}H NMR data match those reported in the literature.[96]

Thieno[3,2-*d*]pyrimidin-4(1*H*)-one (22)

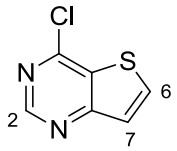


Method A. Methyl 3-formamidothiophene-2-carboxylate **21** (589 mg, 3.18 mmol, 1.0 eq) and ammonium formate (710 mg, 11.3 mmol, 3.5 eq) were dissolved in formamide (1.80 mL, 45.2 mmol, 14.2 eq). The slurry was heated at 140 °C for 24 h. The reaction mixture was allowed to cool to rt (40 mL). To the solution was added acetone (40 mL). The solution was concd *in vacuo* until only a little acetone remained to give a brown solution, which was left at rt for 1 h. The orange crystals that formed were collected *via* filtration and washed repeatedly with chilled H_2O. The crystals were dried *in vacuo* to yield the title compound (202 mg, 1.32 mmol, 42%).

Method B. Methyl-3-aminothiophene-2-carboxylate **21** (2.53 g, 16.1 mmol, 1.0 eq), ammonium formate (1.10 g, 17.5 mmol, 1.1 eq) and formic acid (0.64 mL, 17.0 mmol, 1.1 eq) were dissolved in formamide (3.4 mL, 85 mmol, 5.2 eq). The slurry was heated at 140 °C for 20 h. The reaction mixture was allowed to cool to rt. Brown solid was collected *via* filtration and washed with chilled H_2O. This brown powder was recrystallised from boiling 50% EtOH/H_2O to give the title compound as fluffy brown crystals (1.30 g, 8.52 mmol, 53%).

m.p: 219 °C – 220 °C (lit: 220 °C). ^{1}H NMR (200 MHz, $(CD_3)_2SO$): δ 7.42 (1H, d, *J* 5.2, H^7), 8.17 (1H, s, H^6), 8.20 (1H, d, *J* 5.4, H^2), 12.49 (1H, br, H^1). LRMS (APCI): *m*/*z* 153.3 ($[MH]^+$, 100%), 185 ($[MNa]^+$, 70%). Spectroscopic data match those reported in the literature.[97]

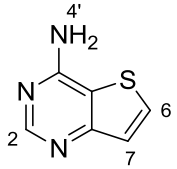
4-Chlorothieno[3,2-*d*]pyrimidine (23)



To thieno[3,2-*d*]pyrimidin-4(1*H*)-one **22** (600 mg, 3.44 mmol, 1.0 eq) was added $POCl_3$ (3.60 mL, 38.9 mmol, 11.3 eq) dropwise under N_2. The solution was heated to reflux for 1.5 h under N_2. The solution was allowed to cool to rt before being quenched by saturated aq $NaHCO_3$ until effervescence ceased. To the solution was added Et_2O (80 mL). The organic layer was separated, washed with H_2O (2 × 20 mL) and brine (2 × 20 mL), dried over $MgSO_4$ and concd *in vacuo* to yield the title compound as a pale yellow solid (458 mg, 2.68 mmol, 78%).

m.p: 124 °C – 126 °C (lit[98]: 123 °C - 124 °C). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.62 (1H, d, *J* 5.4, H^7), 8.06 (1H, d, *J* 5.6, H^6), 9.00 (1H, s, H^2). LRMS (APCI): *m*/*z* 171.4 ($[MH]^+$, ^{35}Cl, 100%), 173.4 ($[MH]^+$, ^{37}Cl, 100%). Spectoscopic data match those reported in the literature.[99]

Thieno[3,2-*d*]pyrimidin-4-amine (24)



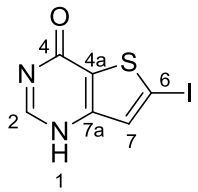
4-Chlorothieno[3,2-*d*]pyrimidine **23** (274 mg, 1.61 mmol, 1 eq) and 28% aq NH_4OH (5.0 mL, 14 M, 74 mmol, 74 eq) were heated at 120 °C for 4 h in a sealed tube. The reaction was allowed to cool to rt before MeOH (5 mL) was added. Residual solids were dissolved with shaking before the solution was concd *in vacuo* to a yellow solid, which was dissolved in boiling 80% EtOH/acetone. The solution was left undisturbed at rt for 24 h before being filtered. The filtrand was washed with chilled EtOH to yield the title compound as orange flakes (247 mg, 1.59 mmol, 94%).

m.p: 222 °C (lit[95]: 226 °C). ^{1}H NMR (200 MHz, $(CD_3)_2SO$): δ 7.35 (1H, d, *J* 5.4, H^7), 7.52 (2H, br s, $H^{4'}$), 8.11 (1H, d, *J* 5.2, H^6), 8.37 (1H, s, H^2). LRMS (APCI): *m*/*z* 152.4 ($[MH]^+$, 100%). Spectroscopic data match those reported in the literature.[100]

Titration of *n*-BuLi

Titration was performed according to a literature procedure.[101] Diphenylacetic acid (212 mg, 1.00 mmol, 1.0 eq) was dissolved in THF (8 mL). The solution was stirred at rt before *n*-BuLi was added dropwise. This addition continued until a permament yellow colourisation was observed. Example: total *n*-BuLi added: 1.35 mL, 2 mmol, 2 eq., giving concentration of *n*-BuLi = 1.48 M.

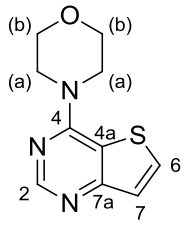
6-Iodothieno[3,2-*d*]pyrimidin-4(1*H*)-one (30)



Thieno[3,2-*d*]pyrimidin-4(1*H*)-one **22** (1.52 g, 10.0 mmol, 1.0 equiv.) was dried *in vacuo* and dissolved in THF (75 mL) with heating and sonication. After stirring the solution at -78 °C for 10 min, *n*-BuLi solution (1.48 M in hexanes, 13.5 mL, 20.0 mmol, 2.0 equiv.) was added dropwise with stirring. The reaction was stirred at -78 °C for 1 h, allowed to warm to -40 °C, and then stirred for a further 1 h. The stirring solution was cooled to -78 °C before I_2 (5.08 g, 20.0 mmol, 2 equiv.) in THF (45 mL) was added dropwise. The reaction solution was allowed to warm to rt and was then stirred at rt for a further 20 h. To the solution were added H_2O (40 mL) and $CHCl_3$ (100 mL). The biphasic solution was washed and shaken with saturated aq $Na_2S_2O_3$ until the colouration disappeared. The organic layer was separated, washed with H_2O (3 × 70 mL) and brine (70 mL), dried over $MgSO_4$ and concd *in vacuo* to yield a yellow solid (925 mg). The solid was recrystallised from hot EtOH/H_2O/acetone (2:2:1) to yield the title compound as pale yellow flakes (833 mg, 3.00 mmol, 30%).

Sublimes at 258 °C. Residual solid mp: 274 °C – 275 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 7.59 (1H, s, H^4), 8.08 (1H, s, H^1). ^{13}C-NMR (100 MHz, CD_3OD): δ 89.5 (C^6), 130.0 (C^{4a}), 135.8 (C^7), 138.9 (C^{7a}), 148.0 (C^2), 159.8 (C^4). IR (film): ν_{max}/cm^{-1} 1657, 1590. LRMS (ESI+): *m*/*z* 279.3 ($[MH]^+$, 50%). HRMS (ESI+): *m*/*z* calcd. for $[MNa]^+$ 300.89085, found 300.89041. Anal calcd for $C_6H_3IN_2OS$: C, 25.92; H, 1.09; N, 10.07, anal calcd for $C_6H_3IN_2OS$ + 0.1 eq EtOH: C, 26.34; H, 1.28; N, 9.91, found: C, 26.49; H, 1.13; N, 9.95.

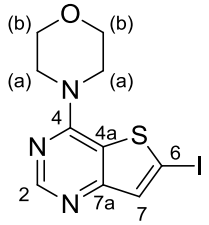
4-(Thieno[3,2-*d*]pyrimidin-4-yl)morpholine (26)



4-Chlorothieno[3,2-*d*]pyrimidine **23** (758 mg, 4.44 mmol, 1 equiv.) was dissolved in MeOH (20 mL) and stirred at rt. To the stirring solution was added morpholine (0.76 mL, 8.78 mmol, 1.97 equiv.) dropwise. The solution was stirred for 3 h at rt before being cooled on ice and filtered. The filtrand was washed with chilled MeOH (20 mL) and H_2O (20 mL), dissolved in acetone (10 mL) and concentrated *in vacuo* to yield the title compound as a white powder (233 mg, 1.05 mmol). The filtrate was collected and concentrated *in vacuo* to yield a white gel. To the gel were added EtOAc (50 mL) and H_2O (20 mL). The organic layer was separated, washed with H_2O (2 × 20 mL) and brine (20 mL), dried over $MgSO_4$ and concd *in vacuo* to give the title compound as a pale yellow powder (473 mg, 2.13 mmol) (Total yield: 706 mg, 3.18 mmol, 72%).

m.p: 135 °C – 136 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.87 (4H, d, *J* 4.6, H^a), 4.00 (4H, d, *J* 4.8, H^b), 7.45 (1H, d, *J* 5.6, H^7), 7.75 (1H, d, *J* 5.6, H^6), 8.61 (1H, s, H^2). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 46.4 (C^a), 66.9 (C^b), 114.6 (C^{4a}), 125.5 (C^6), 131.7 (C^7), 154.4 (C^{7a}), 158.5 (C^2), 161.8 (C^4). IR (film): ν_{max}/cm^{-1} 3482, 3057, 1552, 1517, 1491, 1452, 1436, 1119, 1018, 906. LRMS (ESI+): *m*/*z* 222.6 ([MH]$^+$, 100%). HRMS (ESI+): *m*/*z* calcd. for [MH]$^+$ 222.07011, found 222.06955. Anal calcd for $C_{10}H_{11}N_3OS$: C, 54.28; H, 5.01; N, 18.99, found: C, 54.29; H, 4.96; N, 18.95.

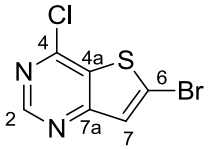
4-(6-Iodothieno[3,2-*d*]pyrimidin-4-yl)morpholine (27)



4-(Thieno[3,2-*d*]pyrimidin-4-yl)morpholine **26** (134 mg, 0.60 mmol, 1 eq.) was dried *in vacuo* before being dissolution in THF (10 mL). The solution was stirred at -78 °C for 10 min before *n*-BuLi solution (2.5 M in hexanes, 0.40 mL, 1.00 mmol, 1.6 equiv.) was added dropwise. The solution was stirred at -78 °C for 15 min before a solution of I_2 (189 mg, 0.74 mmol, 1.2 equiv.) in THF (10 mL) was added dropwise. The solution was stirred at -78 °C for 1 h, allowed to warm to rt, and stirred for 1 h. To the solution were added H_2O (5 mL) and $CHCl_3$ (50 mL). The biphasic solution was washed and shaken with saturated aq $Na_2S_2O_3$ until colourisation disappeared. The organic layer was separated, washed with H_2O (30 mL) and brine (30 mL), dried over $MgSO_4$ and concd *in vacuo* to yield a yellow solid (168 mg). The solid was purified *via* column chromatography (100% hexane → 40% EtOAc/hexane) and the residue recrystallised from 50% EtOH/H_2O to give the title compound as fibrous yellow needles (71 mg, 0.2 mmol, 33%).

m.p: 204 °C. ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 3.74 (4H, d, *J* 5.2, H^a), 3.83 (4H, d, *J* 5.2, H^b), 7.75 (1H, s, H^7), 8.42 (1H, s, H^2). ^{13}C-NMR (100 MHz, $(CD_3)_2SO$): δ 45.7 (C^a), 65.9 (C^b), 90.4 (C^6), 118.7 (C^4), 133.8 (C^7), 154.3 (C^{4a}), 156.3 (C^2), 161.9 (C^{7a}). IR (film): ν_{max}/cm^{-1} 1554, 1518, 1492, 1439. LRMS (APCI): *m*/*z* 348.0 ([MH]$^+$, 60%). HRMS (ESI+): *m*/*z* calcd. for [MH]$^+$ 347.96675, found 347.96625. Anal calcd for $C_{10}H_{10}IN_3OS$: C, 34.60; H, 2.90; N, 12.10, anal calcd for $C_{10}H_{10}IN_3OS$ + 0.1 eq EtOH: C, 34.83; H, 3.04; N, 11.94, found: C, 35.19; H, 2.86; N, 12.04.

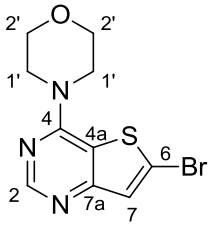
6-Bromo-4-chlorothieno[3,2-*d*]pyrimidine (33)



4-Chlorothieno[3,2-*d*]pyrimidine **23** (914 mg, 5.36 mmol, 1.0 equiv.) was dried *in vacuo* before being dissolved in THF (20 mL). The solution was stirred at -78 °C for 10 min before *n*-BuLi (2.5 M in hexanes, 2.2 mL, 5.5 mmol, 1.0 equiv.) was added dropwise. This solution was stirred at -78 °C for 30 min before Br_2 (0.3 mL, 5.8 mmol, 1.1 equiv.) was added dropwise. This reaction was allowed to warm to rt before being stirred at rt for 2 h. To the solution were added H_2O (45 mL) and EtOAc (100 mL). The biphasic solution was washed with saturated aq $Na_2S_2O_3$ until the colourisation disappeared. The organic layer was separated, washed with H_2O (2 × 50 mL) and brine (30 mL), dried over $MgSO_4$ and concd *in vacuo* to yield an orange solid. The solid was recrystallised from 50% EtOH/H_2O with drops of acetone to yield the title chromatography as a pale, off-white paste (708 mg, 2.84 mmol, 53%).

m.p: 135 °C – 136 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 (1H, s, H^7), 8.93 (1H, s, H^2). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 128.0 (C^7), 129.0 (C^6), 132.7 (C^{4a}), 153.4 (C^{7a}), 155.0 (C^2), 161.7 (C^4). IR (film): ν_{max}/cm^{-1} 1552, 1514, 1426, 1360, 1302, 976. LRMS (APCI): *m*/*z* 249.1 ([MH]$^+$, (^{35}Cl, ^{79}Br), 65%), 250.9 ([MH]$^+$, (^{35}Cl, ^{81}Br)/(^{37}Cl, ^{79}Br), 100%), 253.1 ([MH]$^+$, (^{37}Cl, ^{81}Br), 25%). HRMS (ESI+): *m*/*z* calcd. for ([MH]$^+$, (^{35}Cl, ^{79}Br)) 248.88888, ([MH]$^+$, (^{35}Cl, ^{81}Br)/(^{37}Cl, ^{79}Br)) 250.88684, ([MH]$^+$, (^{37}Cl, ^{81}Br)) 252.88389, found 248.88834, 250.88617, 252.88332.

4-(6-Bromothieno[3,2-*d*]pyrimidin-4-yl)morpholine (34)

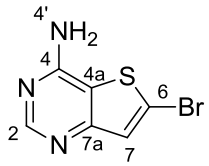


4-(Thieno[3,2-*d*]pyrimidin-4-yl)morpholine **26** (100 mg, 0.45 mmol, 1.0 eq) was dried *in vacuo* before being dissolved in THF (10 mL) and stirred at -78 °C. To the stirring solution was added *n*-BuLi (2.5 M in hexanes, 0.18 mL, 4.5 mmol, 9.9 eq) dropwise at -78 °C with stirring. The solution was stirred

at -78 °C for 0.5 h before Br_2 (0.1 mL, 1.9 mmol, 4.1 equiv.) was added dropwise at -78 °C. The solution was allowed to warm to rt before being stirred at rt overnight. To the solution were added H_2O (5 mL) and EtOAc (70 mL). The biphasic solution was washed with saturated aq $Na_2S_2O_3$ until colourisation disappeared. The organic layer was separated, washed with H_2O (30 mL) and brine (20 mL), dried over $MgSO_4$ and concd *in vacuo* to give an orange solid (122 mg). This solid recrystallised from boiling 50% EtOH/H_2O to yield the title product as long orange rods (112 mg, 0.37 mmol, 83%).

m.p: 139 °C – 140 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.85 (4H, d, *J* 4.4, $H^{1'}$), 3.91 (4H, d, *J* 4.4, $H^{2'}$), 7.46 (1H, s, H^7), 8.53 (1H, s, H^2).^{13}C-NMR (75 MHz, $CDCl_3$): δ 46.5 ($C^{1'}$), 66.9 ($C^{2'}$), 108.9 (C^6), 123.5 (C^{4a}), 127.9 (C^7), 154.5 (C^{7a}), 157.1 (C^2), 160.8 (C^4). IR (film): ν_{max}/cm^{-1} 1555, 1523, 1494, 899. LRMS (APCI): *m*/*z* 300.0 ([MH]$^+$, ^{79}Br, 100%), 301.9 ([MH]$^+$, ^{81}Br, 99%). HRMS (ESI+): *m*/*z* calcd. for ([MH]$^+$, ^{79}Br) 299.98062, ([MH]$^+$, ^{81}Br) 301.97857, found 299.98003, 301.97790. Anal calcd for $C_{10}H_{10}BrN_3OS$: C, 40.01; H, 3.36; N, 14.00, anal calcd for $C_{10}H_{10}BrN_3OS$ + 0.2 eq EtOH: C, 40.37; H, 3.65; N, 13.58, found: C, 40.67; H, 3.45; N, 13.58.

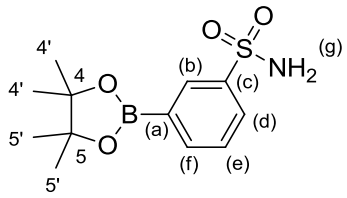
6-bromothieno[3,2-*d*]pyrimidin-4-amine (29)



4-Chlorothieno[3,2-*d*]pyrimidine **26** (143 mg, 0.84 mmol, 1.0 eq) and 28 % aq NH_4OH (5.0 mL, 14.8 M, 74 mmol, 87 eq) were heated at 120 °C for 3 h in a sealed tube. To the solution was added MeOH (5 mL). The solution was concentrated *in vacuo* to yield a yellow solid. EtOAc (30 mL) and H_2O (10 mL) were added to the yellow solid. The organic layer was separated, washed with H_2O (10 mL) and brine (10 mL), dried over $MgSO_4$ and concd *in vacuo* to yield the title compound as a yellow solid (83 mg, 0.36 mmol, 43%).

Decomposes at 243 – 245 °C, mp (residual solid): 250 °C – 252 °C. ^{1}H NMR (400 MHz, CH_3OD): δ 7.40 (1H, s, H^7), 8.33 (1H, s, H^2), ($H^{4'}$ not observed).^{13}C-NMR (100 MHz, CH_3OD): δ 117.6 (C^6), 125.0 (C^{4a}), 128.0 (C^7), 156.0 (C^{7a}), 158.9 (C^2), 160.0 (C^4). IR (film): ν_{max}/cm^{-1} 3150, 1676, 1578, 1533, 1514, 822. LRMS (APCI): *m*/*z* 230.0 ([MH]$^+$, ^{79}Br, 98%), 232.0 ([MH]$^+$, ^{81}Br, 100%). HRMS (ESI+): *m*/*z* calcd. for ([MH]$^+$, ^{79}Br) 229.93876, ([MH]$^+$, ^{81}Br) 231.93671, found 229.93813, 231.93604. Anal calcd for $C_6H_4BrN_3S$: C, 31.32; H, 1.75; N, 18.26, found: C, 31.58; H, 1.82; N, 17.99.

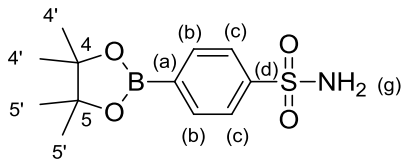
3-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzenesulfonamide (41)



3-bromobenzenesulfonamide **37** (590 mg, 2.50 mmol, 1.0 eq), dried KOAc (912 mg, 9.29 mmol, 3.7 eq) and bis(pinacolato)diboron (1.21 g, 4.78 mmol, 1.9 eq) were dissolved in 1,4-dioxane (25 mL). N_2 gas was bubbled through the suspension for 1.5 h. To this degassed suspension was added $PdCl_2$(dppf) (20.0 mg, 0.03 mmol, 1 mol %). The suspension was heated at reflux for 24 h. To the solution were added EtOAc (100 mL) and H_2O (30 mL). The biphasic solution was sonicated and filtered through a pad of celite with washing by EtOAc (2 × 30 mL). The organic layer was separated, washed with brine (40 mL), dried over $MgSO_4$ and concd *in vacuo* to yield a brown and white solid (1.36 g). This solid was purified *via* column chromatography (40% EtOAc/petrol → 100% EtOAc) and recrystallised from 50% EtOH/H2O to yield the title compound as gold flakes (435 mg, 1.54 mmol, 62%).

m.p: 222 °C – 224 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.36 (12H, s, H^9), 4.77 (2H, s, H^g), 4.94 (2H, s, H^7), 7.53 (1H, t, *J* 3.7, H^4), 7.93 (1H, d, *J* 7.2, H^5), 8.00 (1H, d, *J* 7.8, H^3), 8.28 (1H, s, H^1).^{13}C-NMR (100 MHz, $CDCl_3$): δ 25.0 ($C^{4'}$, $C^{5'}$), 84.6 (C^4, C^5), [128.6, 129.0, 132.6, 139.1 (C^b, C^d, C^e, C^f)], 141.6 (C^c), (C^a not visible). IR (film): ν_{max}/cm^{-1} 1600, 1414, 1357, 1164, 1142, 1122, 1106, 860, 840. LRMS (ESI+): *m*/*z* 589.0 ([2MNa]$^+$, 55%). HRMS (ESI+): *m*/*z* calcd. for [MNa]$^+$ 306.09473, found 306.09431. Anal calcd for $C_{12}H_{18}BNO_4S$: C, 50.90; H, 6.41; N, 4.95, found: C, 50.87; H, 6.42; N, 4.92.

4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzenesulfonamide (42)

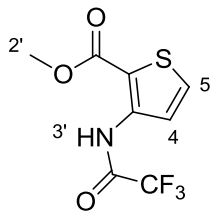


4-bromobenzenesulfonamide **39** (579 mg, 2.45 mmol, 1.0 eq), dried KOAc (883 mg, 8.99 mmol, 3.7 eq) and bis(pinacolato)diboron (1.21 g, 4.76 mmol, 1.9 eq) were dissolved in 1,4-dioxane (25 mL). N_2 gas was bubbled through the suspension for 1.5 h. To the degassed suspension was added $PdCl_2$(dppf) (20.0 mg, 0.03 mmol, 1 mol %). The suspension was heated to reflux for 24 h. To the solution were added EtOAc (100 mL) and H_2O (30 mL). The biphasic solution was sonicated before the solution was filtered through a pad of celite. The celite was washed with EtOAc (2 × 30 mL). The organic layer was separated, washed with brine (40 mL), dried over $MgSO_4$ and concd *in vacuo* to yield a brown and white solid (1.27 g). The solid was purified *via* column chromatography (10%

EtOAc/petrol → 75% EtOAc/petrol) and recrystallised from 75% acetone/petrol to yield the title compound as golden prisms (389 mg, 1.37 mmol, 56%).

m.p: 240 – 241 °C (lit[102]: 240 – 242 °C). ^{1}H NMR (200 MHz, $(CD_3)_2SO$): δ 1.31 (12H, s, $H^{4'}$, $H^{5'}$), 7.41 (2H, s, H^{g}), 7.85 (4H, apparent s, H^{b}, H^{c}). ^{13}C NMR (125 MHz, $(CD_3)_2SO$): δ 24.7 ($C^{4'}$, $C^{5'}$), 84.1 (C^{4}, C^{5}), 124.9 (C^{c}), 134.8 (C^{b}), 146.6 (C^{d}), (C^{a} not visible). IR (film): ν_{max}/cm^{-1} 3259, 1391, 1359, 1330, 1163, 1141, 1077, 917, 856, 832. LRMS (ESI+): m/z 589.1 ([MNa]$^{+}$, 60%). Spectral data match those reported in the literature.[102]

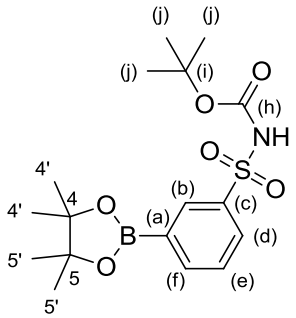
Methyl 3-(2,2,2-trifluoroacetamido)thiophene-2-carboxylate (35)



Methyl-3-aminothiophene-2-carboxylate (1.56 g, 9.92 mmol, 1.0 eq) was dissolved in pyridine (20 mL) and MeCN (25 mL) and stirred at 0 °C for 10 min before TFAA (1.50 mL, 10.6 mmol, 1.1 eq) was added dropwise. The solution was stirred at 0°C for 2 h before being allowed to warm to rt. Volatiles were removed *in vacuo* to yield an orange liquid. To the liquid were added Et_2O (50 mL) and H_2O (20 mL). The organic layer was separated, washed with H_2O (2 × 30 mL) and brine (30 mL), dried over $MgSO_4$ and concd *in vacuo* to yield an orange solid (2.13 g). This solid was recrystallised with 50% EtOH/H_2O to yield the title compound as yellow crystals (1.19 g 4.70 mmol, 47%).

m.p: 75 °C (lit: 76 – 77 °C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.94 (3H, s, $H^{2'}$), 7.57 (1H, d, *J* 5.4, H^{4}), 8.07 (1H, d, *J* 5.4, H^{5}). ^{19}F-NMR (282 MHz, $CDCl_3$): δ -75.93 . IR (film): ν_{max}/cm^{-1} 1735, 1689, 1583, 1197, 1153. HRMS (ESI+): m/z calcd for [MNa]$^{+}$ 275.99182, found 275.99127. Anal calcd for $C_8H_6F_3NO_3S$: C, 37.95; H, 2.39; N, 5.53, found: C, 38.22; H, 2.34; N, 5.58. Spectral data match those reported in the literature.[103]

***tert*-Butyl (3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonylcarbamate (48)**



3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzenesulfondamide **41** (142 mg, 0.50 mmol, 1.0 eq) and *Boc*$_2$O (103 mg, 0.47 mmol, 0.9 eq), DMAP (10.0 mg, 0.08 mmol, 0.2 eq) and Et_3N (0.08 mL, 0.57 mmol, 1.1 eq) were dissolved in CH_2Cl_2 (10 mL) and stirred at rt for 24 h. To the solution were added EtOAc (50 mL) and H_2O (50 mL). The organic layer was separated, washed with H_2O (2 × 30 mL) and brine (20 mL), dried over $MgSO_4$ and concd *in vacuo* to yield

a yellow oil (119 mg). This oil was purified via column chromatography (EtOAc 10% → $MeOH/CH_2Cl_2$) to yield the title compound as a yellow oil (77.0 mg, 0.20 mmol, 40%).

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.25 (9H, s, H^j), 1.35 (12H, s, $H^{4'}$, $H^{5'}$), 7.54 (1H, t, *J* 7.6, H^e), 8.05 (1H, d, *J* 7.4, H^f), 8.10 (1H, d, *J* 8.0, H^d), 8.42 (1H, s, H^b).^{13}C-NMR (100 MHz, $CDCl_3$): δ 25.0 ($C^{4'}$, $C^{5'}$), 28.0 (C^j), 84.1 (C^i), 84.6 (C^4, C^5), [128.3, 130.9, 134.2, 139.8 (C^b, C^d, C^e, C^f)], 138.9 (C^c), 149.6 (C^h), (C^a not visible). IR (oil): ν_{max}/cm^{-1} 1746, 1357, 1143, 1088, 842. LRMS (ESI+): *m/z* 405.9 ($[MNa]^+$, 20%), 788.8 ($[2MNa]^+$, 100%). HRMS (ESI+): *m/z* calcd. for $[MNa]^+$ 406.14716, found 406.14692.

3. Experimental Biology

Inhibition of *P. falciparum* was measured by Dr. Sabine Mangold of Griffith University using a 384-well, high throughput imaging assay, using materials and methods described in detail elsewhere.[104] The fluorescence based *P. falciparum* growth inhibition assay used the DNA-intercalating dye, 4',6-diamidino-2-phenylindole, to monitor changes in parasite number. Fluorescent images were acquired on the PerkinElmer Opera High Throughput confocal imaging system and analyzed with a spot detection algorithm using the Acapella data processing software. Inhibition is reported as half maximal inhibition concentration (IC_{50}) as a percentage with respect to the IC_{50} values of artemisinin as a control, except when inactive (**Table 5**). Graphical data was provided only in low resolution format, which may be found on the electronic lab book.[37]

Cytotoxicity was measured by Dr. Sabine Mangold of Griffith University using materials and methods described in detail elsewhere.[105] Compounds were added to assay wells containing 3000 12 hour adherent cells/well (HEK 293) in an assay volume of 45µl. The plates were incubated for 72 hours at 37°C and 5% CO_2. After incubation the supernatant was removed and 40 µl of 10% Alamar blue added per well. Plates were incubated for a further 5-6 hours and measured for fluorescence. Data as a reported as either demonstrating measurable cytotoxicity (Yes) or not demonstrating measurable cytotoxicity (No) (**Table 5**). Graphical data was provided only in low resolution format, which may be found on the electronic lab book.[37]

Table 5: Inhibition and Cytotoxicity Measurements of Synthetic Compounds

Compound	% Inhibition *P. falciparum* ($IC_{50}/IC_{50}^{control}$)	Inhibition HEK 293 ($\%IC_{50}/\%IC_{50}^{puromycin}$)
9	330 nM	No
12	NA	-
13	438 nM	No
22	NA	-
23	9.4 μM	No (haemolysis)
23	NA	-
24	NA	-
26	NA	-
30	NA	-

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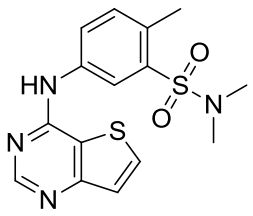
Appendix A: Analogue Structures

Key: **Compound number** (Enamine Catalogue Number)



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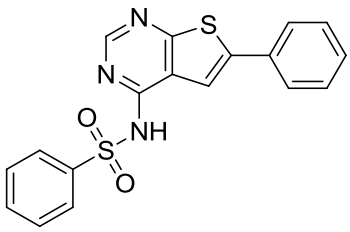

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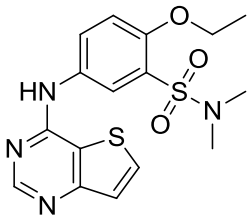

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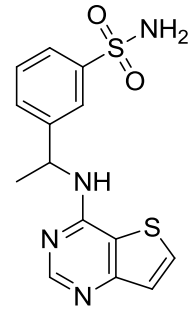

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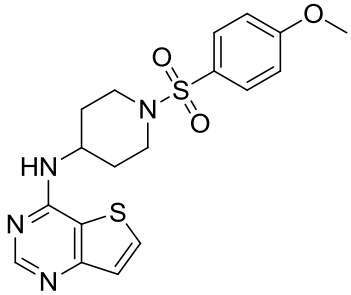

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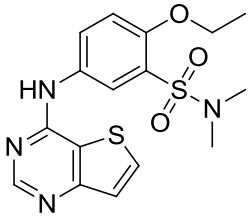

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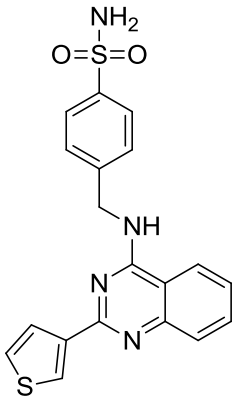
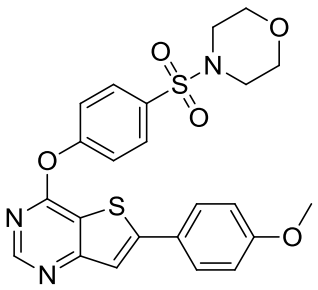
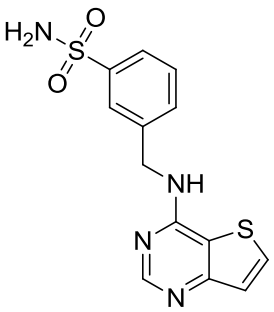


57: Z86248675



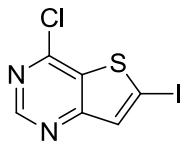
58: Z127173570



59: Z367550756

Appendix B: Nomenclature of Fused Heterocycles

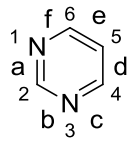
The ring systems described in Chapter 3, Part 2 are named according to nomenclature prescribed by the IUPAC, which, for convenience, is described below.[1A]



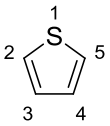
31

4-Chloro-6-iodo[3,2-*d*]pyrimidine will be used as an illustrative example. These heterocycles are named according to the following rules:

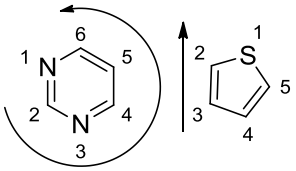
1. Each individual component of the bicyclic ring system is named (i.e. pyrimidine and thiophene)
2. The parent component is identified and placed last in the bicyclic structure name (i.e. thieno*pyrimidine*).
    a. The parent component is the one with the highest position in the hierarchy described hereat:
        i. A heterocyclic 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 (in this case the parent compound is pyrimidine since its constituent heteroatom, nitrogen, comes first).
        ii. A component containing the largest ring.
        iii. A component containing the largest number of heteroatoms.
        iv. A component containing the greater variety of heretoatoms.
    b. The attached component (i.e. thiophene) is added as a prefix to the parent compound, and the prefix '-ene' is changed to '-eno'. 'Thieno' as the abbreviated form of 'thiopheno' is one of a few acceptable abbreviations, thus yielding the fused name: thienopyrimidine.
    c. The atoms of the parent component are numbered. The bonds of the parent component are lettered in order, starting with the bond between atoms 1 and 2. I.e:



d. The attached component is numbered as usual. I.e.

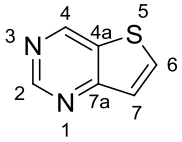


e. The atom numbers of the attached component that form the fusion carbons are placed within brackets between the two components of the fused name. The order of these numbers is determined by following the direction of numbering in the parent constituent. I.e. [3,2], not [2,3]:



f. The bond of the parent compound that forms fusion bond (I.e. bond 'd') is italicized, placed within the brackets after the atom numbers and separated from these by a hyphen. I.e: thieno[3,2-*d*]pyrimidine

g. The numbering of the fused system is carried out anew, starting at an atom adjacent to a fusion carbon, and numbered such that the heteroatoms receive the smallest possible number. Fusion carbon atoms are given the number of the preceding position and have a roman letter attached as a suffix. I.e.



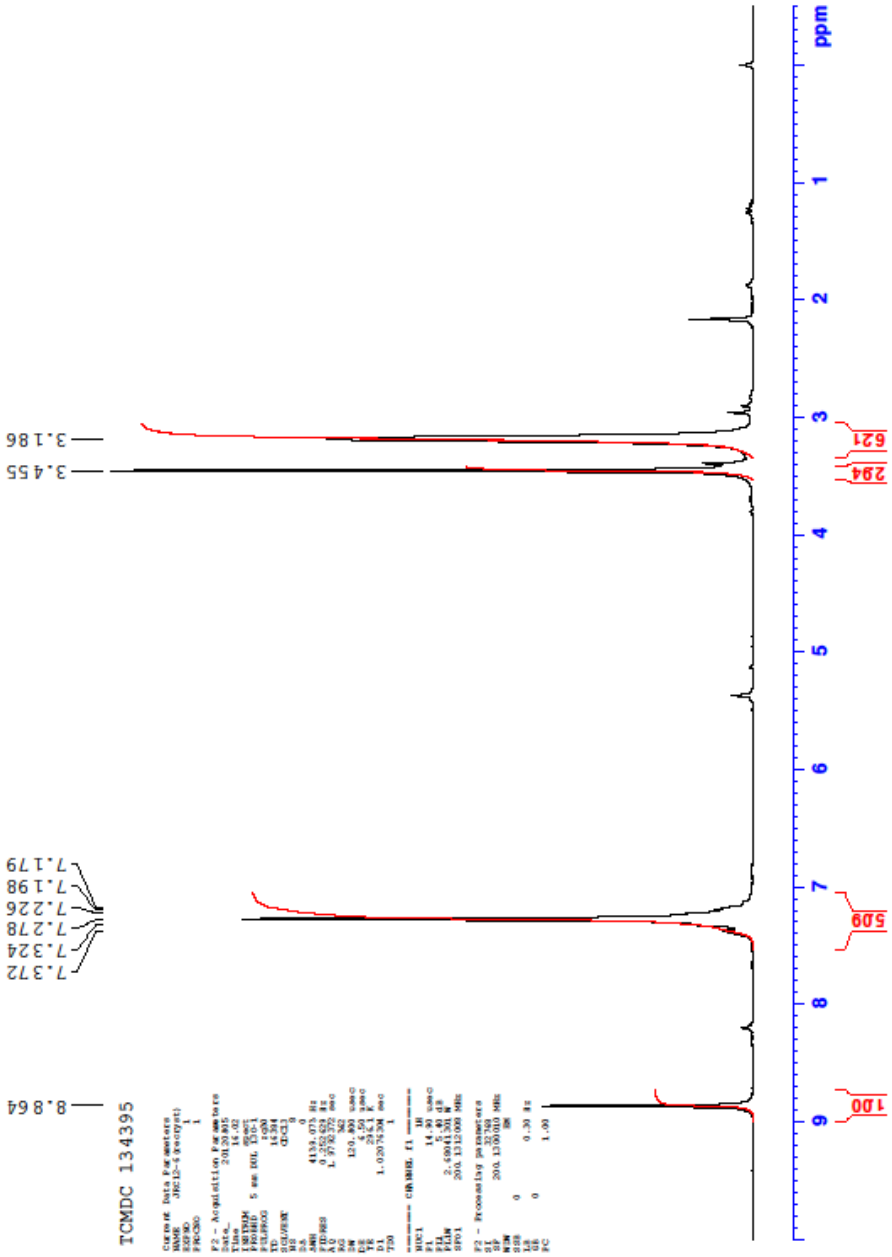
Attached substituents are numbered according to standard IUPAC nomenclature. I.e. 4-chloro-6-iodothieno[3,2-*d*]pyrimidine for the compound at the beginning of this appendix.

References:

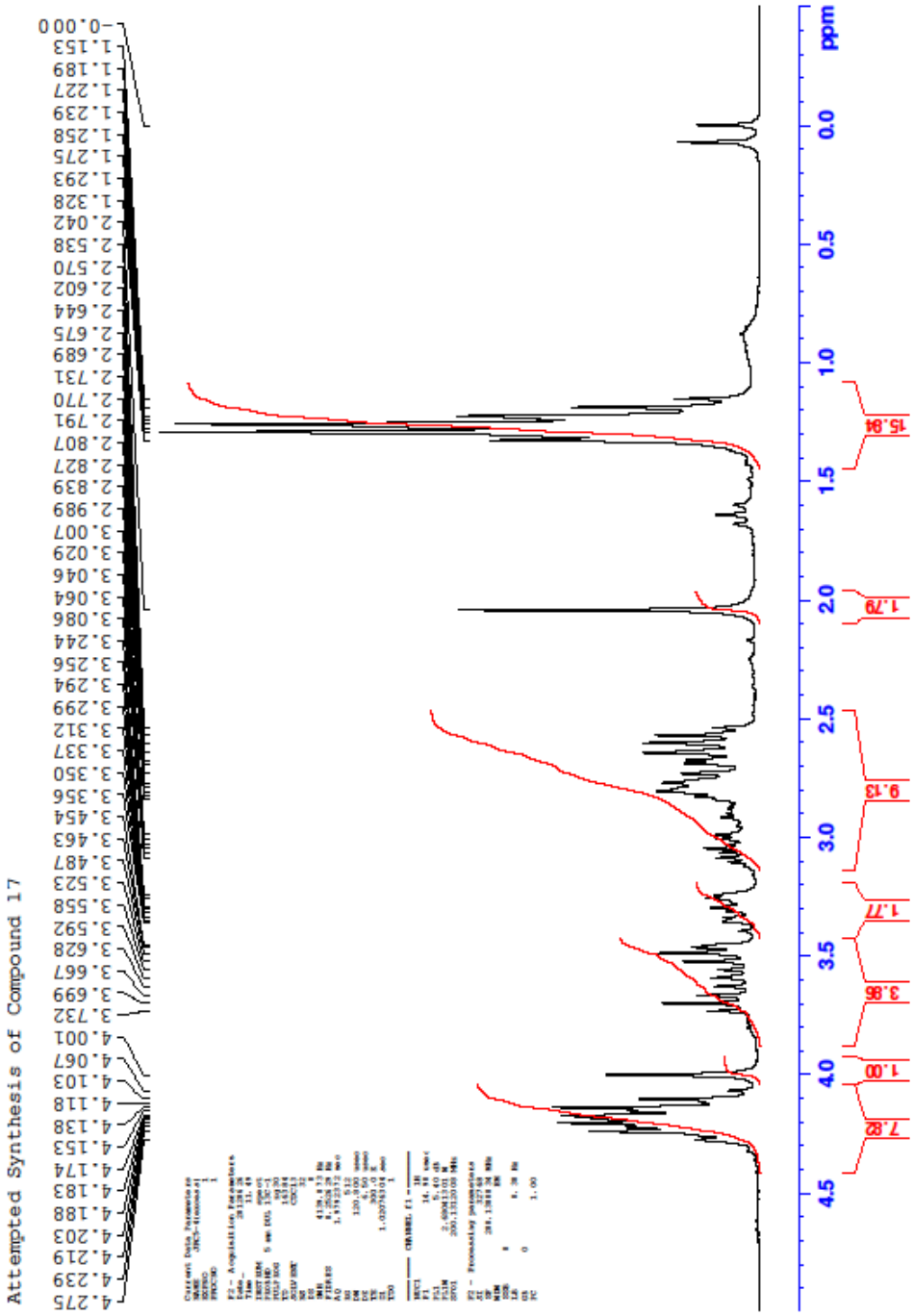
1A. Moss, G. P., Nomenclature of fused and bridged fused ring systems (IUPAC Recommendations 1998). *Pure Appl. Chem.* **1998,** *70* (1), 143-216.

Appendix C: Selected ^{1}H NMR Spectra

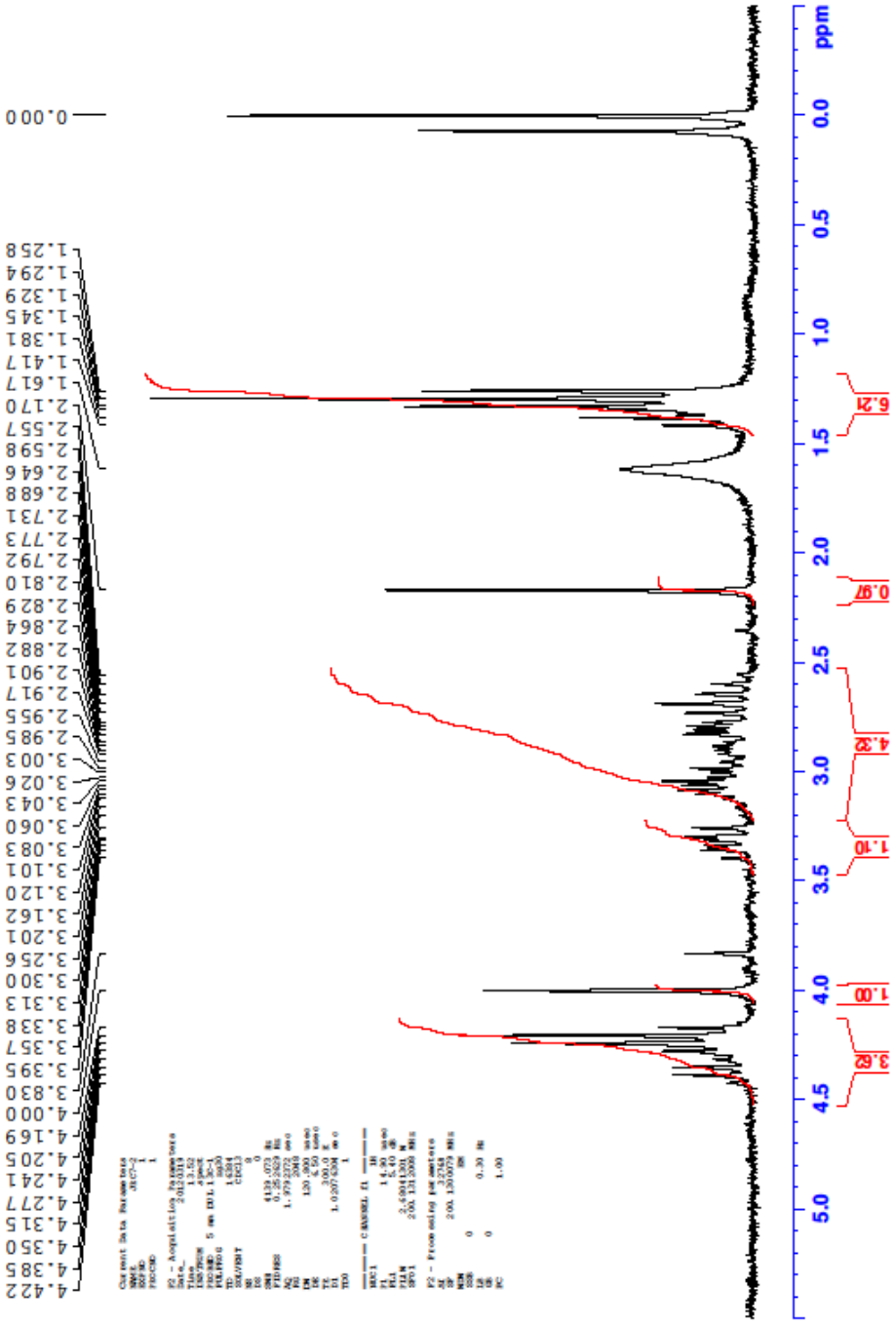
1. TCMDC 134295 (9)



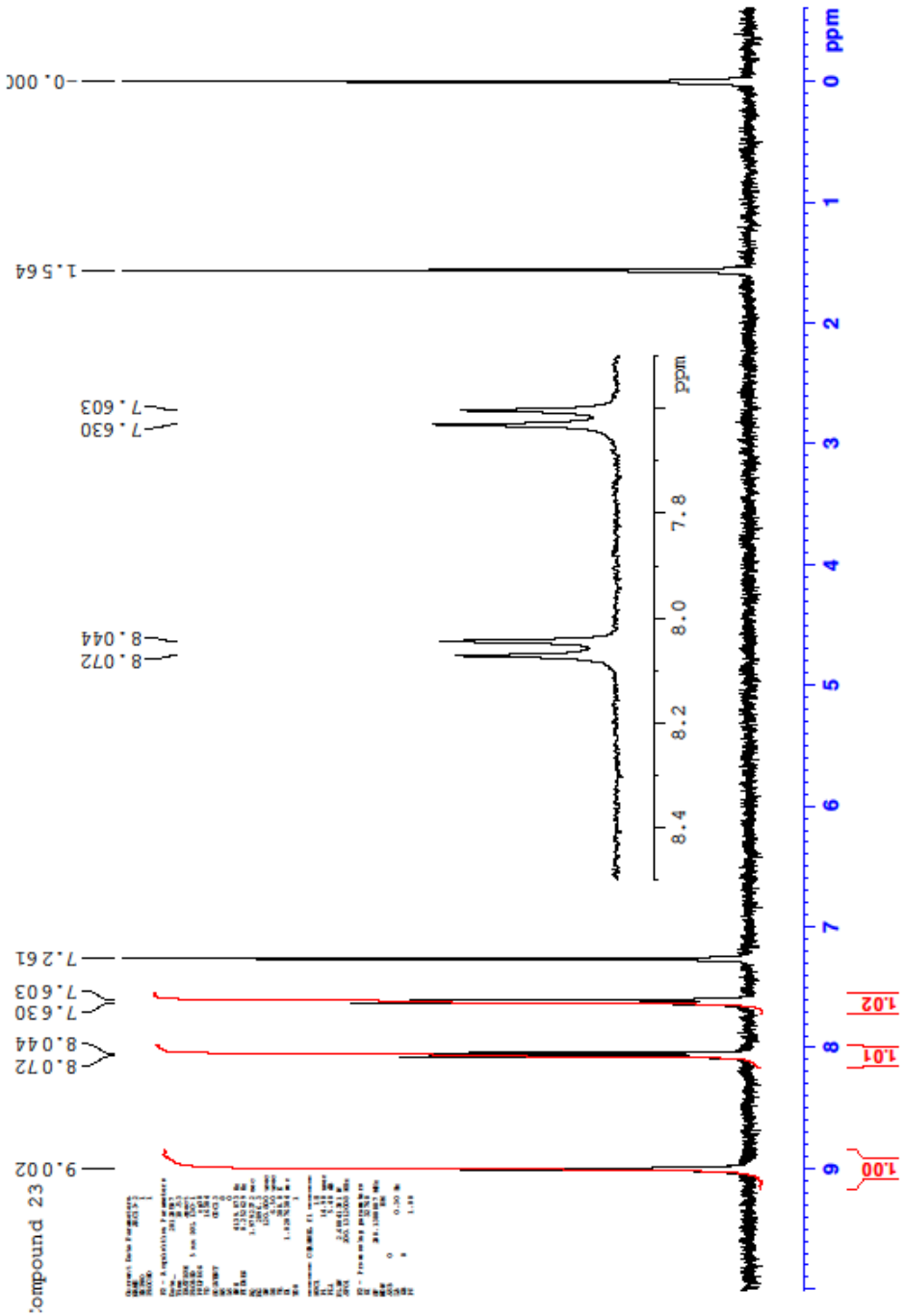
2. Attempted Synthesis of Compound 17 using NaOEt/EtOH



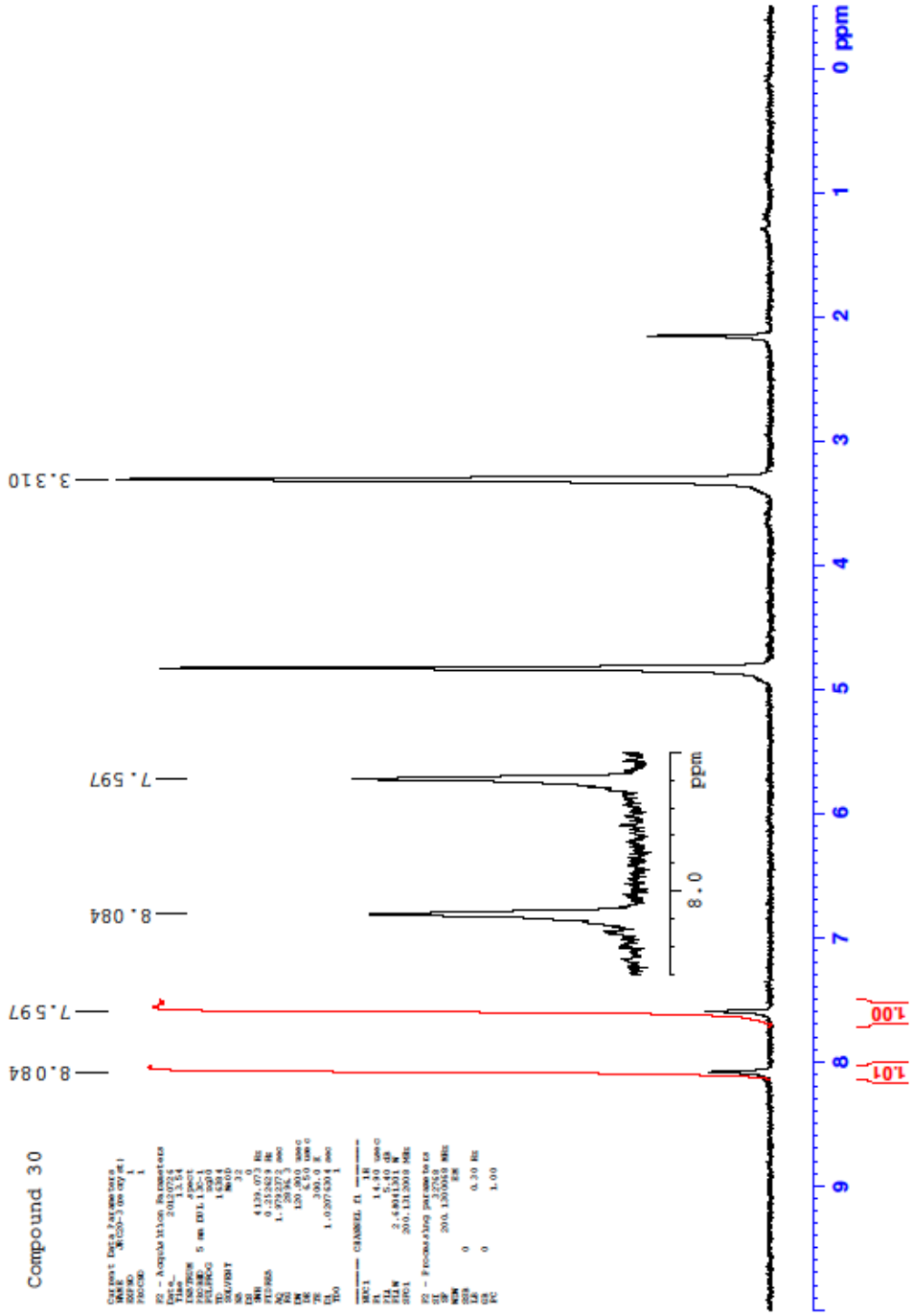
3. Attempted Synthesis of Compound 17 using $TiCl_4$



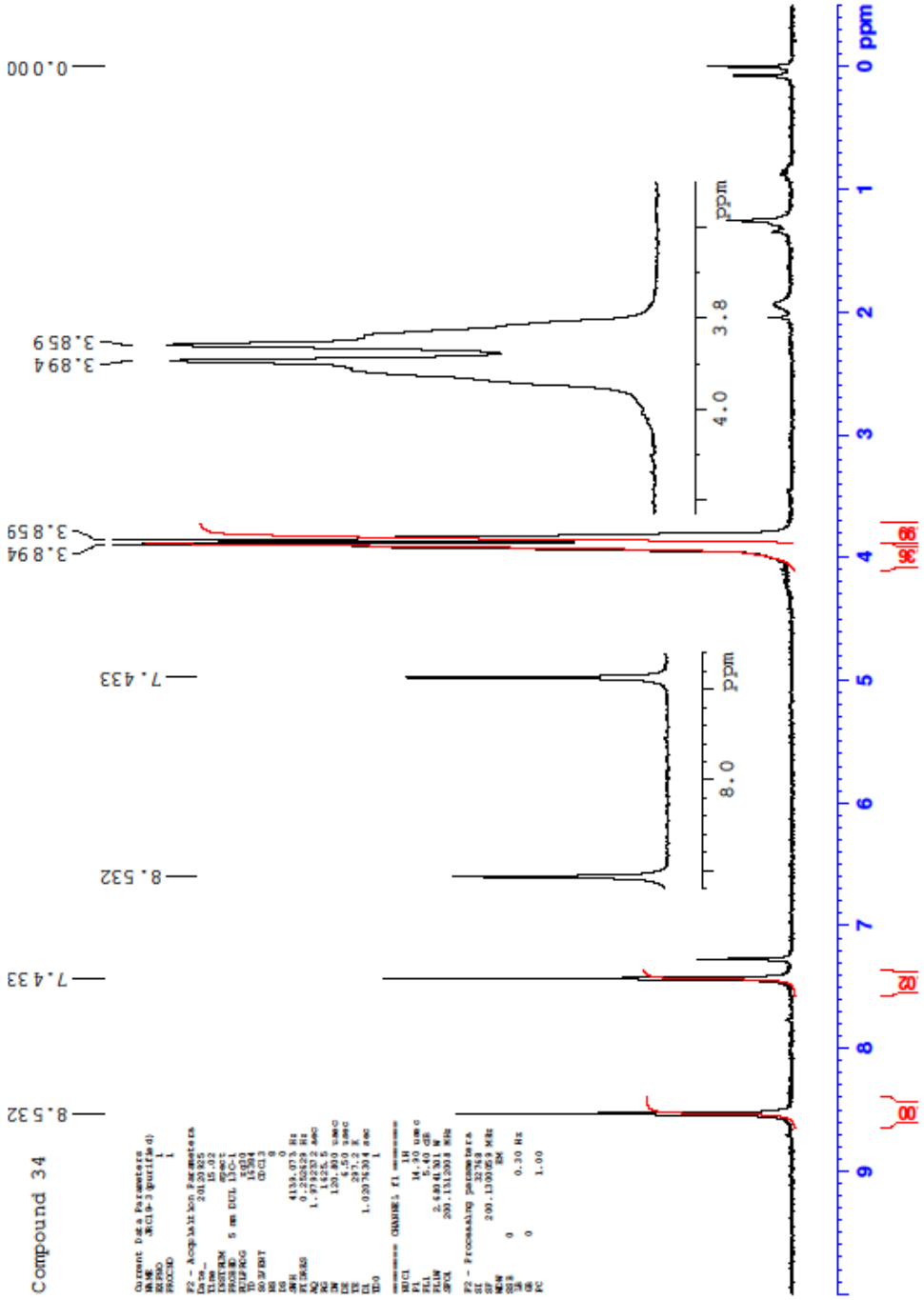
4. Compound 23



5. Compound 30



6. Compound 34



6. Unknown Product from the Attempted Syntheses of 43, 44, 46, 47 and 48