THE DEVELOPMENT OF NOVEL PTERIN CHEMISTRY LEADING TO
POTENTIAL DIHYDROFOLATE REDUCTASE INHIBITORS WITH
POTENTIAL ANTIMALARIAL ACTIVITY

Winston Nxumalo

A thesis submitted to the Faculty of Science, University of the Witwatersrand,
Johannesburg, in fulfillment of the requirements for the degree of Doctor of Philosophy.

October 2011
Declaration

I declare that the work presented in this thesis was carried out exclusively by myself under the supervision of Dr A Dinsmore. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

[Signature]

(Signature of candidate)

[17th day of October 2011]
Abstract

This thesis describes the application pteridine chemistry in various aspects of methodology development and natural product synthesis. The introductory chapter sets the scene by describing naturally occurring pteridines, their applications in biological systems, and recent synthetic strategies.

Firstly, Sonogashira coupling reactions employing benzenesulfonyloxy-O-pteridine (27) and terminal alkynes to give various 6-substituted pteridines are described. This methodology allowed for the total synthesis of a natural occurring pteridine, Sepiapterin-C (46).

Negishi coupling reactions involving benzenesulfonyloxy-O-pteridine (27) and various Zn-reagents are also reported. This methodology, representing the first Negishi coupling on a pteridine nucleus, allowed for the introduction of both aryl- and heteroaryl- substituents at the 6-position of the pteridine ring. The use of methanesulfonyloxy-O-pteridine (26) as a coupling partner is also described.

Selective deprotection and hydrolysis of the formamidine protecting groups to give either the 6-substituted 2,4-diaminopterine or 2-amino-4-oxo-pteridine (pterin), is described.

The synthesized structures are supported by NMR and mass spectral data and melting points where applicable. Novel compounds are verified by NMR spectroscopy, infrared and mass spectrometry.
Dedication

This thesis is dedicated to my Family,

my late father Sonto

my late mother Rose and

my brothers Nyiko, Conrad, Newton and Brian
Acknowledgements

The author wishes to express his sincere gratitude to:

- Dr Andrew Dinsmore for his guidance and assistance;

- his colleagues and friends for their support;

- Professors J.P. Michael, C de Koning, W van Otterlo and Dr S Pelly for their assistance;

- Mr R Mampa for his assistance in the running and processing of the nuclear magnetic resonance spectra;

- Mrs M Ferreira for her assistance in running HPLC-UV-APCI-MS and for accurate mass determinations;

- Mr T Dzara and Mr S Gannon for their glassblowing expertise;

- Prof E Nordlander for hosting me in Sweden;

- The University of the Witwatersrand and National Research Foundation, for financial support.
Table of Contents

CHAPTER 1 INTRODUCTION.........................................................................................1

1.1 Nomenclature and Literature...........................................................................2

1.2 Introduction to the natural occurrence of pteridines...........................................3
  1.2.1 Non-redox active pterins............................................................................4
  1.2.2 Redox co-factors incorporating pterins.......................................................5
    1.2.2.1 The biochemistry of folic acid.............................................................7
    1.2.2.2 Antifolates as DHFR inhibitors.........................................................11

1.3 Synthetic pteridines.......................................................................................13
  1.3.1 Current methodology................................................................................13
  1.3.2 Choice of protecting groups......................................................................19
  1.3.3 Modelling of pterin..................................................................................20

1.4 Characterization of pteridines.........................................................................22

1.5 References....................................................................................................23

CHAPTER 2 SONOGASHIRA COUPLING OF HETEROCYCLIC SULFONATES.......23

2.1 Introduction.....................................................................................................27

2.2 Results and Discussion..................................................................................31
  2.2.1 Sonogashira coupling of sulfonyloxy-quinazoline.....................................31
    2.2.1.2 Effect of temperature and mesylate leaving group.................................36
    2.2.1.3 Sonogashira coupling on sulfonyloxy-heterocycles...............................38
    2.2.1.4 Attempted Sonogashira coupling on sulfonyloxy-benzene substrates........42

  2.2.2 Sonogashira coupling on pteridine-O-sulfonates......................................44
2.2.3 Hydrolysis of protected pteridines.........................................................56

2.2.3.1 Hydrolysis to 2,4-diaminopteridines................................................. 56
2.2.3.2 Hydrolysis to 2-amino-4-oxo-pteridines (pterins)......................... 58
2.2.3.3 Use of HPLC-MS in monitoring the hydrolysis of 6-ethynyl-pteridine to 6-ethynylpterin..........................................................59

2.3 Total synthesis of Sepiapterin-C...............................................................65

2.4 Experimental.........................................................................................69

2.4.1 General...............................................................................................69
2.4.2 Synthetic procedure on quinoxaline.....................................................69
2.4.3 Synthetic procedure on pteridines.......................................................76

2.5 References............................................................................................83

CHAPTER 3 NEGISHI COUPLING OF QUINOXALINE AND PTERIDINE-O- SULFONATES.................................................................86

3.1 Introduction............................................................................................87

3.2 Results and Discussion........................................................................91

3.2.1 Negishi coupling of quinoxaline-o-sulfonates with alkyl- and aryl-Zn substrates.................................................................................91
3.2.2 Negishi coupling of quinoxaline-o-sulfonates with hetero-aryl substrates ......95
3.2.3 Negishi coupling of pteridine-O-sulfonates............................................102
3.2.4 Conversion of Pteridines to Pterins.......................................................108
3.2.5 Conclusion........................................................................................111

3.3 Experimental........................................................................................111

3.3.1 Preparation of Zn-reagents.................................................................111
3.3.2 Negishi coupling of quinoxalines........................................................114
3.3.3 Negishi coupling of pteridines..............................................................118
3.4 References ................................................................................................. 121

CHAPTER 4 AN INVESTIGATION OF SUZUKI COUPLING ON QUINOXALINE- AND PTERIDINE-SULFONATES ............................................................. 123

4.1 Introduction ............................................................................................... 124

4.2 Results and Discussion ............................................................................ 126

4.3 Experimental ............................................................................................ 129

4.4 References ............................................................................................... 129

CHAPTER 5 TETRAMETHYLFORMAMIDINE CHLORIDE (TMFAC) AS A POTENTIAL DIAMINOCARBENE ................................................................. 131

5.1 Introduction ............................................................................................... 132

5.2 Results and Discussion ............................................................................ 134

5.3 References ............................................................................................... 136
### List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>FH₄</td>
<td>Tetrahydrofolate</td>
</tr>
<tr>
<td>Moco</td>
<td>Molybdenum cofactor</td>
</tr>
<tr>
<td>GTP</td>
<td>Guanosine triphosphate</td>
</tr>
<tr>
<td>cPMP</td>
<td>cyclic pyranopterin monophosphate</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>DHF</td>
<td>Dihydrofolate</td>
</tr>
<tr>
<td>NADPH</td>
<td>Nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>FD</td>
<td>Folate deficiency</td>
</tr>
<tr>
<td>PABA</td>
<td>para-aminobenzoic acid</td>
</tr>
<tr>
<td>DHFR</td>
<td>Dihydrofolate reductase inhibitors</td>
</tr>
<tr>
<td>MXT</td>
<td>Methotrexate</td>
</tr>
<tr>
<td>6FP</td>
<td>6-Formylpterin</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoracetic acid</td>
</tr>
<tr>
<td>Ac₂O</td>
<td>Acetic anhydride</td>
</tr>
<tr>
<td>NPE</td>
<td>p-Nitrophenylethyl</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>IR</td>
<td>Infra-red</td>
</tr>
<tr>
<td>UV-VIS</td>
<td>Ultra violet-visible spectroscopy</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>FAB</td>
<td>Fast atom bombardment</td>
</tr>
<tr>
<td>EI</td>
<td>Electron impact</td>
</tr>
<tr>
<td>APCI</td>
<td>Atmospheric pressure chemical ionization</td>
</tr>
<tr>
<td>ESI</td>
<td>Electro spray ionization</td>
</tr>
<tr>
<td>TMS</td>
<td>Trimethyl silyl</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethyl formide</td>
</tr>
<tr>
<td>DMAP</td>
<td>Dimethylamino pyridine</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>TIC</td>
<td>Total ion count</td>
</tr>
<tr>
<td>RT</td>
<td>Retention time</td>
</tr>
<tr>
<td>HPLC</td>
<td>Hi-performance liquid chromatography</td>
</tr>
<tr>
<td>TMFAC</td>
<td>Tetramethylformamidine chloride</td>
</tr>
<tr>
<td>NHC</td>
<td>N-Heterocyclic carbenes</td>
</tr>
<tr>
<td>NHAC</td>
<td>N-Heteroacyclic carbenes</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction
Chapter 1  Introduction

The work described in this Thesis deals with approaches to the synthesis of 6-substituted pterins and pteridines. This chapter reviews different types of naturally occurring pterins and their roles in biological systems, and the development of various pteridine derivatives which have found applications in medicinal chemistry. The chapter also describes the various methods developed for the synthesis of pterin molecules, their scope and limitations.

In the chapters to follow, novel routes to the synthesis of 6-substituted pterins are discussed, starting with Sonogashira coupling chemistry in Chapter 2 and Negishi coupling in Chapter 3.

1.1 Nomenclature and Literature

Pteridines are a group of heterocyclic compounds composed of fused pyrimidine and pyrazine rings and with the numbering system shown below (Figure 1.1). There are three main classes of naturally occurring pteridines namely, lumazines, isoalloxazine and pterins. Lumazines and isoalloxazines have oxo substituents at the 2- and 4- positions with the difference being a phenyl ring annealed in the 6- and 7- position on the isoalloxazine. The most common class of naturally occurring pteridines are the pterins which have an amino group at the 2-position and an oxo-group at the 4-position.

Figure 1.1

Pteridines have been known to exist from as early as the 19th century, with the first synthesis and isolation reported in 1857 and 1889, respectively. However, the exact structure of these pteridines were only resolved in 1940 and this discovery subsequently led to (a) the isolation,
characterization and synthesis of natural occurring pteridines; (b) the synthesis of derivatives of natural pteridines used as drugs for various diseases; and (c) the initiation of interdisciplinary collaboration between synthetic chemistry and biological chemistry, giving birth to medicinal chemistry.

A number of books and many publications in the field of pteridine chemistry come from the medical and biochemical area of research where the pteridines are first isolated from natural sources and their functions well understood before their total synthesis is achieved. A recent Web of Science search (January 2011) revealed over 1629 publications describing the chemistry and biology of pteridines with 958 of these publications coming from the biochemistry and molecular biology journals, while 751 publications were from chemistry journals. Due to the complex nature of pteridine chemistry, the thesis will generally refer to secondary literature in the introduction sections describing the functions of pteridines, and more emphasis will be given to the synthetic chemistry of these compounds.

1.2 Introduction to the natural occurrence of pteridines

Long before their structures were fully resolved, pteridines were identified as yellow pigments in butterflies and insects due to their brightly colored nature. In 1889, two natural pteridines were isolated by Frederick Hopkins from wings of Brimstone butterflies. The structure of these pteridines were later resolved to be xanthopterin and leucopterin (Figure 1.2). Further investigation of the Brimstone butterflies pigments led to the isolation of a third pteridine, isoxanthopterin in 1933. However, due to difficulties with elemental analysis, the constitution of all three compounds remained unknown until 1940 when the pteridine nucleus was finally resolved. The total synthesis of these pteridines were achieved shortly after by Robert Purrrmann.

2
Since then over 50 other naturally occurring pteridines have been isolated and characterised, and the total synthesis of some have been achieved. Naturally occurring pteridines have been isolated from (a) insects, examples include xanthopterin and isoxanthopterin; (b) plants, an example being folic acid; and (c) mammals, an example being neopterin. Most of the pteridines isolated belonged to the pterin family and some were realised to be incorporated in a number of redox cofactors. The remainder of this chapter will focus more on the pterin family of pteridines.

1.2.1 Non-redox active pterins

Many natural occurring pterins are incorporated in a number of redox active cofactors and are believed to be redox active themselves, although the mechanism is not fully understood. There are however, pterins that have not been associated with redox reactions and their biological functions, if any, are largely unknown. In some cases these pterins have been classified as pigments. Examples of these pigments are xanthopterin and sepiapterin-C (isolated from insects, Figure 1.3). More recently, xanthopterin, oncopterin and neopterin have been identified as useful bio-markers for human disease states.

Xanthopterin has been found in butterfly wings and in the urine of mammals. It is the end product of a non-conjugated pteridine compound and inhibits the growth of lymphocytes.
produced by concanavalin. High levels of xanthopterin are found in patients with liver diseases and hemolysis, and can be used to detect the presence of these diseases.

Sepiapterin -C is found along with biopterin (Figure 1.4) and is believed to be a by-product in the biopterin metabolic pathway. No biochemical function of sepiapterin-C has been reported to date.

Neopterin has been found to serve as a bio-marker of mammalian cellular immune system activation. High levels of neopterin are associated with increased production of reactive oxygen species, commonly found in diseases like HIV, cancer, arthritis and bacterial infections. Measuring the amount of neopterin in blood fluids or urine gives an indication on the progression of these diseases. There are a large number of non redox active pterins which will not be discussed in this chapter due to the complex nature of field.

1.2.2 Redox cofactors incorporating pterins

Pterins are incorporated in a number of redox active cofactors which are essential to all life, examples include tetrahydrobiopterin (BH$_4$), the molybdenum cofactors (Moco), flavin and folic acid (Figure 1.4). All these pterins have a rich redox chemistry which take place mainly at the pyrazine ring, where the 5,6- and 7,8- positions can be reduced and oxidised to facilitate electron transfer reactions forming new products. Folic acid, for an example, undergoes reduction at the 7,8-position to dihydrofolate which can also be reduced at the 5,6-position to tetrahydrofolate. However, the redox chemistry of most pterins is still not clear as to whether it goes via a 2-electron or 1-electron transfer pathway and this is still being investigated to date.
Figure 1.4

Tetrahydrobiopterin (BH$_4$) is the reduced form of biopterin which is biosynthesised from guanosine triphosphate (GTP) in an enzyme catalysed reaction. Tetrahydrobiopterin functions as the cofactor in enzymes responsible for the production of monoamine neurotransmitters (epinephrine, norepinephrine, dopamine, serotonin) and nitric oxide production. Conditions such as Alzheimer’s disease, Parkinson’s disease, depression, autism and schizophrenia are believed to arise due to deficiency in BH$_4$. Patients suffering from these diseases are treated with synthetic BH$_4$.

The molybdenum cofactor is biosynthesised from guanosine triphosphate (GTP) and is required in human sulfite oxidase, xanthine oxidoreductase and aldehyde reductase enzymes. These enzymes are found in all forms of life, except streptomyces, and are responsible for the oxidation of toxic substances into less toxic and easily excretable compounds. Sulfite oxidase is responsible for the oxidation of sulfites into sulfates which are easily excreted. Absence of Moco results in accumulation of toxic levels of sulfites and this results in death within months of birth, underlying the vital role of the pterin. People suffering from molybdenum cofactor deficiency have recently been treated with a daily dose of cyclic pyranopterin monophosphate (cPMP) which is the precursor in the biosynthesis of Moco.

Folic acid, which is not biosynthesised by humans, is an essential vitamin responsible for mediating the transfer of one-carbon units. It acts as both the acceptor and donor of one-carbon units in a variety of reactions critical in the metabolism of nucleic acids and amino acids.
nucleic acid metabolism, folic acid is vital for the synthesis of DNA from its precursors, thymidine and purines, it is also involved in the methylation of various sites within DNA and RNA. Folic acid is by far the most widely studied and understood pteridine to date and its metabolic pathway is well understood.

1.2.2.1 The biochemistry of folic acid

The chemistry of folic acid is as follows: Folic acid or folate is converted to dihydrofolate (DHF) in the presence of Nicotinamide adenine dinucleotide phosphate (NADPH), dihydrofolate is then converted to tetrahydrofolate by the enzyme dihydrofolate reductase (DHFR). Tetrahydrofolate \((\text{FH}_4)\) is the most active form of the folate family and is responsible for the production and maintenance of new cells, for DNA synthesis and RNA synthesis (Scheme 1.1).\(^{9-11}\)

Tetrahydrofolate is a substrate in a number of single-carbon-transfer reactions. \(\text{FH}_4\) is converted to methylene-\(\text{FH}_4\) by the addition of a methylene group from one of three carbon donors; formaldehyde, serine or glycine. The methylene-\(\text{FH}_4\) is then converted to methyl-\(\text{FH}_4\) by reduction with NADPH. The methyl group is then transferred to Vitamin-\(\text{B}_{12}\) cobalamin which in turn is transferred to another acceptor, homocysteine, generating methionine which is an amino acid. The methylene-\(\text{FH}_4\) is also used to methylate the pyrimidine base uracil to thymine which is essential for DNA synthesis (Scheme 1.2).
FH$_4$ is very essential in DNA and amino acid synthesis and its concentration is regulated by the presence of folate and also the activity of DHFR. Folate deficiency (FD) results in many health problems such as neural tube defects in developing embryos and also cancer formation due to lack of nucleotides required for DNA synthesis and repair. During early stages of pregnancy, FH$_4$ is required for proper development of the brain, skull and spinal cord.

The association of neural birth defects with FD have long been realised and folic acid supplements are given to pregnant women, and women who plan to get pregnant are advised to take folic acid supplements three months in advance. Research studies have shown a sharp
decrease in birth defects in women who took folic acid supplements during or before pregnancy when compared to the control.\textsuperscript{12-13} The success of these studies led to treatment of other diseases associated with FD.

Folate deficiency can be avoided by taking folic acid supplements along with the daily diet, however, this is limited to first world countries as many people in developing countries cannot afford to buy these supplements or folate rich food products. Another approach to avoiding FD is by fortification, where folic acid is added to flour in order to increase the levels of folic acid in the diet. Studies have shown that people who eat folate fortified food generally have an increased level of folic acid and are less likely to suffer from diseases associated with FD.\textsuperscript{13-18} Although fortification of flour and or grains has proved success in a number of countries, not all countries are performing this practice.

The practice of fortification is so far limited to first world countries, primarily due to cost implications, but other countries are refusing to fortify their food products as there are concerns which link cancer to high levels of folic acid. Studies have shown that different race groups absorb folic acid differently and as a result high levels of folic acid are found in their blood streams.\textsuperscript{19} Although there is no conclusive evidence, high levels of folic acid have been shown to worsen the vitamin $\text{B}_{12}$ deficiency.\textsuperscript{20} Other studies have also shown that high levels of folic acid in late pregnant women resulted in an increase number of babies developing childhood asthma.\textsuperscript{21}

Because folic acid or folate is essential for cell growth and development, diseases such as cancer, bacterial infection and viral infections also require folate to grow and spread, and in these cases, the down regulation of folate is required. All these diseases are characterized by high rates of cell division and growth and thus requires large quantities of folate. Unlike humans, microorganisms and plants have a biosynthetic pathway for folate (Scheme 1.3) and it came as no surprise that the synthetic compounds to inhibit the production of folate were some of the first therapeutic antibacterial agents.
Scheme 1.3

The first generations of drugs targeting the folic acid pathway were the sulphonamides, which were the first synthetic anti-bacterial drugs (Figure 1.5). The sulphonamides inhibit the synthesis of folic acid in bacteria thus hindering its growth. These drugs act by competing with para-aminobenzoic acid (PABA), effectively blocking the addition of L-glutamate which is the final step in the formation of folate. The sulphonamides are still used till this date and over 1000 variations of sulphonamides have been prepared and are used for various bacterial infections.

Figure 1.5

The sulphonamides were ineffective in treatment of cancer since cancer cells are almost identical to normal human cells and do not have a folate biosynthetic pathway. Cancer cells differ from normal cells in that they have a high rate of cell division and growth, and these require relatively large quantities of folate. Down regulation of folate in these cells will result in reduced cell growth and division. The second generation of drugs targeting the folic acid pathway were the
anti-folates which were targeting the conversion of DHF to the active FH₄ and are commonly known as dihydrofolate reductase inhibitors (DHFR inhibitors).

1.2.2.2 Antifolates as DHFR inhibitors

Antifolates act by competing with DHF for the binding site in the DHFR enzyme and thus reduce the amount of FH₄ that can be produced. For antifolates to be effective competitors of DHF, they must have similar structural features that enable them to bind to the active site of the enzyme. Once these antifolates bind the active site, DHF is prevented from binding and therefore no FH₄ can be produced. The first antifolate drug prepared was methotrexate (MXT) which has close structural resemblance to folic acid but is biologically inactive in the folic acid metabolism pathway (Figure 1.6). Methotrexate has since been used for the treatment of various cancer diseases and it is still used till this day.

![methotrexate](image)

Figure 1.6

The success story of MXT led to the development of various antifolates which resembled folic acid, some of the compounds being aminopterin and alimta (Figure 1.7). However these compounds were shown to have severe side effects and this led to development of antifolates that resemble folic acid on the pyrimidine ring. Examples of these new generation drugs are pyrimethamine and trimethoprim (Figure 1.7), which were shown to be effective antifolates and are used for the treatment of malaria and bacterial infections respectively.²⁴
While it has been shown that understanding the structure and function of naturally occurring pterins can lead to improvement of life, folic acid still receive the lion’s share of the research where most of the synthesised pteridines are folic acid derivatives. This is primarily due to the fact that the total synthesis of folic acid is well established and hence it is easy to introduce variations into the synthesis leading to folic acid derivatives. The synthesis starts with 6-formylpterin (6FP) as the starting material, similar to the bacterial biosynthesis of folate, and the pterin ring is reacted with various nucleophiles leading to folic acid or its derivatives (Scheme 1.4).

Figure 1.7
Scheme 1.4

Synthesis of other naturally occurring pterins could not be achieved via the folic acid synthetic route and this led many research groups around the world to come up with synthetic strategies that will lead to the synthesis of different naturally occurring pterins and their derivatives. Most naturally occurring pterins have substituents at the 6-position, thus a synthetic strategy that lead to 6-substituted pterins is of paramount importance.

1.3 Synthetic pteridines

The synthesis of heterocyclic systems can be considered to be best divided into those where an existing ring is functionalized or the ring is constructed from acyclic components (“classical ring synthesis”). In addition to the already established procedure which uses 6FP as starting material, few methods have been developed leading to 6-substituted pterins.

1.3.1 Current methodology

Of the few synthetic approaches leading to 6-substituted pterins, the most notable method is the one developed by E.C. Taylor. Oxidation of pterin with trifluoroacetic acid/ hydrogen peroxide (TFA/H₂O₂) and treatment of the resultant 8-oxide with trifluoroacetic acid/acetyl chloride (TFA/AcCl) gives good yields of 6-chloropterin²⁵ in an unusual Katada rearrangement (Scheme
The chloride is displaced by thiols via nucleophilic substitution reactions and acetylenes under Sonagishira coupling conditions, giving 6-substituted pterins.

Scheme 1.5

The most classical synthetic approach to 6-substituted pterins is of the “ring synthesis” variety, in which an existing ring, usually the pyrimidone or pyrimidine, is used for the construction of the appropriately substituted pyrazine ring. The original classic ring synthesis can be divided into three main reactions, named after their inventors.

The Gabriel-Colman synthesis involves the condensation of a 2,4,5,6-tetraaminopyrimidine ring with dialdehyde, glyoxal, aldehydoketone and diketones, giving rise to a wide range of 6- and 7-substituted pteridines. The problems encountered in this route are the mixture of products in which both the 6- and 7-substituted pterins are obtained (Scheme 1.6a).

The Timmis synthesis entails the condensation of 5-nitroso-2,4,6-pyrimidinetriamine with an α-carbonylmethylene compound to give substituted pteridines (Scheme 1.6 b). The products obtained from this route are normally unambiguous, but the need to prepare the 5-nitroso group is the drawback.
The Boon synthesis involve the condensation of aminoacetone with 2-amino-6-chloro-5-phenylazo-4(3H)-pyrimidinone. Reduction of the azo group to an amine, spontaneously leads to cyclization, and oxidation with oxygen gives 6-methylpterin (Scheme 1.6c). Products are obtained having an unambiguously assigned structure.¹

![Diagram of the Boon synthesis]

Scheme 1.6

Modifications into the classical ring synthesis have been reported recently and the scope has been extended to total synthesis of natural products.²⁶⁻³⁰ Natural pterin compounds synthesised from this procedure are from the biopterin family which include limipterin, tepidopterin (both pterin glycosides), Sepiapterin and Sepiapterin-C (Figure 1.8).³¹
Other modifications to the classical ring synthesis include the method pioneered by Taylor et al. which start by making the substituted pyrazine ring then reacting it with guanidine to give exclusively the 6-substituted pterin (Scheme 1.7). Substituents such as aryl, alkyl and heterocycles were introduced exclusively at the 6-position using this method.
Scheme 1.7

Pterin derivatives can also be made from pteridine (2,4-diaminopteridine) derivatives which are easily hydrolysed to the desired pterin under hydrolytic conditions (Scheme 1.8). The pteridine ring can be constructed by a method similar to the way pterin ring is constructed, i.e. using the above mentioned methods.

![Diagram](image)

**2,4,-diaminopteridine** (R=H)

Scheme 1.8

The route which provides the starting point for the work described in this thesis was pioneered by Dinsmore and Joule et al. The initial proof of concept involved the readily available 2,4-diamino-6-oxopteridine 2, which can be made from tetra-aminopyrimidine and glyoxylic acid (Scheme 1.9). The oxopteridine 2 can be converted into the corresponding pterin, xanthopterin 3. However oxopteridine 2 also has readily differentiated substituents at the 4 and 6-positions. Taking advantage of this, the next step in the synthesis was easily accomplished by tosylation of the 6-oxo group. Thus oxopteridine 2 can be viewed as a masked form of xanthopterin 3, in which differentiation between the (masked) 4-oxo and 6-oxo groups has been achieved. The regio-selective tosylation also provided a potential leaving group, an O-tosylate at the 6-position.

The solubility problems associated with 2 can be overcome by adding an N-protecting group, the Bredereck’s reagent, to give dimethylformamidine derivative 4. Once the tosyl group is on the 6-position, the resulting 6-tosyloxypterin 5 was shown to undergo a Stille type coupling reaction with 1-ethoxyvinyl-1-tributylstannane. The intention for this route was to complete the synthesis of the natural product sepiapterin-C, but the final step was never achieved.
Scheme 1.9

The above mentioned synthetic routes had to overcome the solubility problems associated with pteridines. The extensive H-bonding within pterin molecules, related as they are to DNA base guanosine, is responsible for their insoluble properties. The combination of the amide function and an amino group which are considered weakly acidic and weakly basic, respectively, promote favourable interactions. Consequently, protection of the ND or OD atom of the amide function and the ND atom of the amino group increases their solubility in water and few organic solvents.

Pterin has a water solubility of 1: 57000 g/ml at room temperature, whereas the N(3)-methyl derivative has water solubility of 1: 100 g/ml. Acylation of the C(2)-NH₂ group leading to 2-(acetylamino) derivative gives solubility of 1: 450 g/ml at room temperature. Similarly, pteridine has a water solubility of 1:1350 g/ml and successive substitution of the NH₂ group in the 2- and 4- positions increases the solubility to 1: 320 g/ml and 1: 2.5 g/ml, respectively. Although the methyl protecting groups are good solubilising agents, they are not easily removed and there is thus a need to develop better protecting groups.
1.3.2 Choice of protecting groups

Pterin has two sites available for protection i.e. C(2)-NH₂, N(3)- which are reacted with various protecting groups to enhance their solubility. The C(2)-NH₂-position is acylated with acetic anhydride (Ac₂O) to give the 2-(acetylamino) derivative (Scheme 1.10).³⁷,³⁸ Pivaloyl chloride is also used leading to 2-pivaloyl derivatives.²⁹,³⁰ More recently 2-dimethylformamide dimethyl acetal has been used as a the protecting group generating 2-(N,N-dimethylaminomethyleneamino) derivatives. These can also be achieved by protection with tert-butoxy-bis(dimethylamino)-methane known as the Bredereck’s reagent.

![Scheme 1.10](image)

Scheme 1.10

In addition, the pterin ring possesses an oxygen atom at the 4-position available for protection. The most common protecting groups for the N(3)- and O- atoms are the butyl and p-nitrophenylethyl NPE groups (Figure 1.9), introduced using Mitsunobu conditions. These groups are easily removed under basic conditions.²⁶,²⁷,³⁷

![Figure 1.9](image)
2,4-Diaminopteridine has two sites available for protection, i.e. N(2)- and N(4)-position. Because both sites contain NH$_2$ groups, one protection group can be used for both sites and this limits the number of steps during synthesis. Dinsmore and Joule et al.$^{36}$ used Bredereck’s reagent for the pteridine protection (Scheme 1.9). An observation, made from the work of Joule, is that the two N-protected groups have different stability where one protecting group was shown to fall off during the coupling reaction, no explanation has been given for this observation.$^{36}$

As already mentioned, pterin molecules are synthesised using various methods: (i) a pterin or pteridine ring is functionalised and substituents added thereafter (Scheme 1.5 and 1.9) and (ii) a pterin or pteridine ring is constructed from its precursors with the substituent already attached to the reactants (Scheme 1.6 and 1.7). Conventional C-C bond forming reactions are readily performed in the latter method since most of the substrates e.g. pyrazine, are soluble in organic solvents. In the former method, however, a limited number of C-C bond forming reactions have been reported. Sonogashira coupling was reported on 6-chloropterin (Scheme 1.5) by Taylor et al.$^{30}$ while Joule et al.$^{36}$ reported the Stille coupling (Scheme 1.9), these reactions suffer from a limitation in the number of substituents that can be attached and hence a need to explore the scope of C-C bond forming reactions is required.

### 1.3.3 Modelling of pteridines

The insoluble character of pterin and pteridine has rendered them difficult molecules to work with and as a result simple models that resemble part of the pterin ring have been developed. Most of pterin reactions occur at the pyrazine ring so the pyrimidone ring can replaced by an organic soluble ring. Joule et al.$^{36,39-41}$ and Goswami et al.$^{42}$ have used quinoxaline as a model for the pterin ring (Figure 1.10). The quinoxaline ring has a benzene ring fused with a pyrazine ring and it soluble in common organic solvents.

![Quinoxaline](image)

**Figure 1.10**
The quinoxaline ring system has been used to model the total synthesis of molybdenum cofactor (Moco) using various reactions. Sonogashira and Stille coupling (Scheme 1.11 A) were reported by Joule et al\textsuperscript{26,39} while condensation of 2-aminoanaline with an appropriate substrate leading to quinoxaline ring formation (Scheme 1.11 B) has been reported by Goswami et al.\textsuperscript{42}

Scheme 1.11

The chemistry of quinoxaline and pterin is not always identical, some reactions are possible in the quinoxaline ring but cannot be extended to the pterin ring. The total synthesis of Moco has not been reported to date while the quinoxaline analogue has been reported using various methods (Figure 1.11). The most reported problems with the total synthesis of Moco have been in the formation of pyran ring and the reduction step at the 5,6 and 7,8-positions.

Figure 1.11

Modelling of the pterin ring is still a useful approach in an effort to widen the scope of C-C bond forming reactions due to the ease of working with these organic soluble compounds and ease of characterization when compared to pterins.
1.4 Characterisation of pteridines

Pteridine molecules are generally characterised by conventional methods, e.g. nuclear magnetic resonance (NMR) spectroscopy, infra-red spectroscopy (IR), ultra violet-visible spectroscopy (Uv-Vis), fluorescence spectroscopy and mass spectroscopy. Because the pterin ring has an equal number of carbons and heteroatoms i.e. six carbons, five nitrogen and one oxygen, it is sometimes difficult to get much information from NMR spectroscopy. There are few hydrogen atoms present in the pterin ring and some are involved in hydrogen bonding. The solubility problems associated with pterin limit the number of deuteriated solvents that can be used for NMR analysis.

The most common solvent used is dimethyl sulfoxide (DMSO) which has a disadvantage of being hygroscopic, the presence of moisture in the solvent diminishes the N-H signals due to hydrogen bonding and thus few signals are observed. Other solvents used are either basic solvent such as sodium methoxide (NaOMe) or acidic solvent such as trifluoroacetic acid (TFA). These solvents also attract moisture and also diminish signals of protons involved in hydrogen bonding. The $^1H$ NMR spectra of Sepiapterin-C in TFA only has two hydrogen environments.\cite{31}

The use of IR spectroscopy is limited to pterin molecules having functional groups such as –OH, -NH$_2$ and carbonyl groups. Infra-red is also used to follow reactions which undergo functional group addition or elimination. Not much information regarding the exact structure of the pterin can be obtained from an IR spectrum.

Pterins are characterised by their bright colours, usually yellow, and are good chromophores i.e. able to absorb wavelengths of visible light. Molecules are known to have unique absorption properties and the same apply to pterin molecules. Absorption spectra can also be used to monitor the reactions of pterin by observing the change in the absorbance peaks. UV-vis studies are also used to determine the rate of a reaction e.g. the reduction or oxidation of pterin. The use of UV-vis spectroscopy is one of the oldest techniques that was and still is used to characterise pterin molecules. UV-vis spectroscopy can be used to measure the ionisation constants of pterin molecules since neutral pterin molecules have different absorption spectra to charged pterins.

Fluorescence spectroscopy is used to detect fully oxidised pteridines in solution. It is the most sensitive detective method (limit of detection $\sim 10^{-18}$M, in some cases) and is often used to detect
pterins which serve as biomarkers for certain diseases states e.g. neopterin. A number of fluorescent spectra have been reported for pure pterins and this serves as a reference for detecting pterins in solution even at low concentrations.

Mass spectroscopy is mainly used to confirm the presence of pterin in the pure sample or solution where the molecular ion peak corresponding to the mass of compound is obtained. Due to the high melting point associated with pterins, usually >300°C, there is low volatility of these compounds and less information can be obtained from this technique. Fragmentation of the molecular ion is rarely observed. High energy ionisation techniques such as electron impact (EI) and fast atom bombardment (FAB), and Low ionisation techniques such as atmospheric pressure chemical ionisation (APCI) and electro spray ionisation (ESI) are commonly used as mass detection instruments.

1.5 References


Chapter 2

Sonogashira coupling of heterocyclic sulfonates
Chapter 2  Sonogashira coupling of heterocyclic sulfonates

2.1 Introduction

The Sonogashira coupling, first reported in 1975 by Sonogashira and Hagihara, involves the coupling of terminal alkynes with an aryl, heteroaryl or vinyl group possessing a good leaving group, mainly halogens and trifluoromethanesulfonyl (triflates). The scope of the reaction has been widely investigated and over 24 000 hits were found involving Sonogashira coupling reactions (Reaxys search engine, February 2011). The reactions are performed on electron rich ring systems and also electron deficient ring systems.

\[ \text{A}ryl \text{ or Vinyl} - X + \text{R} \stackrel{\text{Pd}}{\longrightarrow} \text{R} \rightarrow \text{Ar}yl \text{ or Vinyl} \]

Scheme 2.1

The Sonogashira coupling is one of the most common methods for C-C bond formation and has been widely used in organic synthesis. The resultant di-substituted acetylene can be functionalized in different ways, e.g. reduced to an alkene or alkane, halogenated and hydrolyzed, leading to a variety of products (Scheme 2.2).

\[ \text{R}^2 \longrightarrow \text{R}^1 \]
\[ \text{R}^2 \longrightarrow \text{R}^1 \]  
\[ \text{R}^2 \longrightarrow \text{R}^1 \]  
\[ \text{Cl} \]
\[ \text{Cl} \]
\[ \text{Cl} \]

Scheme 2.2

The acetylene also serves as a substrate to ring forming reactions like (a) Diels-Alder reaction, where a 6-membered ring is formed (Scheme 2.2); (b) the Larock indole synthesis, where an indole can be constructed from an aniline possessing an ortho-acetylene (Scheme 2.3 A); (c) more recently, in a one pot reaction procedure, the Sonogashira coupling reactions has been
coupled with the carbopalladative cyclization reaction, where the acetylene substrate is cyclized to form a variety substituted indene substrates (Scheme 2.3 B). Also, alkynes have been found incorporated into natural products (the ene-diynes) which have significant antibiotic and antitumor activity.

\[
\text{\begin{align*}
\text{A} & \quad \begin{array}{c}
\text{NH}_2
\end{array} \quad \begin{array}{c}
Pd
\end{array} \quad \begin{array}{c}
H
\end{array} \\
\text{B} & \quad \begin{array}{c}
\text{OTBS}
\end{array} \quad \begin{array}{c}
\text{Br}
\end{array} \quad \begin{array}{c}
\text{B}
\end{array} \quad \begin{array}{c}
\text{O} \quad \text{H}
\end{array} \quad \begin{array}{c}
\text{B}
\end{array} \quad \begin{array}{c}
\text{R}^1 \quad \text{R}^2
\end{array} \quad \begin{array}{c}
Pd(0)
\end{array} \\
& \quad \begin{array}{c}
\text{OTBS}
\end{array} \quad \begin{array}{c}
\text{R}^1
\end{array} \quad \begin{array}{c}
\text{R}^2
\end{array}
\end{align*}
\]

Scheme 2.3

The reactions are catalyzed by palladium (Pd) and copper (Cu) in the presence of mild base, usually amines, under inert conditions. Recent developments have shown that a nickel (Ni) catalyst can also be used in place of Pd, but the reactions require high temperatures (reflux in dioxane). Another modification on the Sonogashira coupling conditions is the Cu-free conditions, which has shown the reactions to proceed without affecting the yield. The use of a copper catalyst alone has also been reported but high temperatures are required. Metal free Sonogashira coupling reactions have been reported to occur under UV radiation.

Table 2.1 lists the coupling partners employed in Sonogashira coupling reactions arranged in order of the conjugate acid strength and with the number of published occurrences shown in row three (Reaxys database January 2011). The most common halogens are chlorine (Cl), bromine (Br) and iodine (I), no mention of fluorine (F) as a leaving group was found. Atoms or groups that are good leaving groups are usually conjugates of strong acids, hydrogen iodide (Pka -10) is a strong acid while hydrochloric acid (Pka -6) is a relatively weak acid, and hence iodide is a better leaving group than chloride. Triflate, the conjugate anion of trifluoromethanesulfonic acid (Pka -14.9) and is a better leaving group than the methanesulfonyl (mesylate) group, the
conjugate of methanesulfonic acid (PKa -1.9). Recently, though not shown in Table 2.1, the Sonogashira coupling reaction has been reported with an aryl-sulfonyl chloride.3,4

Table 2.1

<table>
<thead>
<tr>
<th>Leaving group</th>
<th>-OSO$_2$CF$_3$</th>
<th>-I</th>
<th>-Br</th>
<th>-Cl</th>
<th>-OSO$_2$Ph</th>
<th>-OSO$_2$Tol</th>
<th>-OSO$_2$Me</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKa of conjugate acid</td>
<td>14.9</td>
<td>10</td>
<td>8</td>
<td>6</td>
<td>5.9</td>
<td>5.3</td>
<td>1.9</td>
</tr>
<tr>
<td>Number of reported SC$^*$</td>
<td>12973</td>
<td>6144</td>
<td>1578</td>
<td>2400</td>
<td>0</td>
<td>120</td>
<td>6</td>
</tr>
</tbody>
</table>

$^*$Sonogashira reactions found by a Reaxys database search Jan 2011

Triflates are the most common in the sulfonyl group family (12973 reactions) while the mesylate group has been rarely employed in Sonogashira coupling reactions (six reactions). The toluenesulfonyl (tosylate) group has gained much attention recently and has been shown to undergo Sonogashira coupling on many aryl systems (over 100 reactions reported on Reaxys database). The yields are lower when compared to most triflate system but are comparable to the halogenated aryl systems. Triflates are used for both electron rich and deficient aryls,2-5 while tosylate groups have only been reported to work on electron rich aryl groups.6 and 9 The presence of an electron-withdrawing group or atom in the aryl or vinyl substrate enhances the leaving group ability, hence electron deficient systems readily undergo Sonogashira coupling.1

The chlorine leaving group, which is a conjugate of HCl, which has a PKa of -6 comparable to the PKa of benzenesulfonic acid -5.9. It comes as a surprise that the use of benzenesulfonate as a leaving group has not been exploited while there are a number of examples that employ a tosylate leaving group in coupling reactions (Reaxys database search September 2010).

Sonogashira coupling reactions have been reported on pterin and pteridine ring systems on four occasions by Taylor et al17-19 (Chapter 1, Scheme 1.4, page 14) and Joule et al.20 Three of the reactions were towards the total synthesis of Moco but attempts to functionalize the acetylene group were unsuccessful.17,20 In all the reactions, the pterin ring had a chlorine leaving group and moderate to good yields were reported. A pteridine ring system with a tosylate leaving group has
been reported by Joule et al., but the substrate was never used for Sonogashira coupling, and shown to undergo a Stille coupling.

A drawback associated with Sonogashira coupling reactions and which has limited the industrial applications, is the need to prepare the R-Hal or R-OSO₂R’ substrates. The halogens are usually prepared by one of three common methods.

Treating the aryl or vinyl group with a strong base, often a lithium alky or amide, and quenching the resulting Li-organometallic with a halogen (Hal⁺) source (Scheme 2.4 A). This may require an ortho-directing and metallating (DOM) group to facilitate a clean reaction. Halogen sources commonly include iodine, di-iodoethane, N-iodo-, bromo-, chloro-succinimide, TMS-iodide or CCl₄. Organo-lithium intermediates require the use of low temperature and inert atmosphere and have poor functional group tolerance.

When feasible, halogens can be activated by for instance PPh₃ or a halophilic Lewis acid such as AlCl₃ to form species that are attacked by some moderately rich electron systems such as pyrroles, furans and sometimes benzene (Scheme 2.4 B).

Amides and suitable heterocyclic systems can be converted to halides using reagents such as PCl₅, POCl₃, SOCl₂ or oxalyl chloride/DMF (Scheme 2.4 C). The requirements for expensive and often hazardous bases and high molecular weight halogen containing reagents, limit the atom economy of the reactions and often come at a high cost.

\[
\begin{align*}
A & : \text{Aryl–H} \xrightarrow{\text{R–Li}} \text{Aryl–Li} \xrightarrow{\text{X}_2} \text{Aryl–X} \\
B & : \text{Aryl–H} + [\text{Hal}]^+ \xrightarrow{\text{[Hal]}^+} \text{Aryl–X} \\
C & : \text{R–NH}_2 \xrightarrow{\text{POCl}_3} \text{R–Cl}
\end{align*}
\]

Scheme 2.4

Aryl and vinyl sulfonates are usually prepared by reacting a sulfonic acid chloride or sulfonic anhydride with a phenol, ketone or amide (Scheme 2.5). These reactions also require the presence of base. The current cost of, for instance, triflic anhydride (~R14 per gram for an order
of 1 Kg, source Aldrich chemical catalogue, Feb. 2011) limits the industrial application, and is considerably higher than that of mesyl or benzenesulfonyl chloride (∼R1 and R0.37 per gram respectively, for orders of 1 Kg).

Scheme 2.5

With the advantages and disadvantages of the Sonogashira coupling reactions identified, we were interested in investigating the potential of pteridine-6-sulfonates as coupling partners in the Sonogashira reactions. Quinoxaline-2-sulfonate, a pteridine model, has been shown to undergo Sonogashira coupling by Joule et al\textsuperscript{22} towards the modeling of the total synthesis of Moco. The reactions employed a triflate leaving group however, and no mention of other sulfonates attached to the quinoxaline or pteridine ring systems have been reported.

In this chapter, we report Sonogashira coupling reactions on pteridine ring system possessing both a benzenesulfonyl and a mesylate leaving group. The discussions start with the modeling of the pteridine ring with the quinoxaline, developing the methodology and then extending to a pteridine. The chapter also discusses the conversion of 2,4-diaminopteridines to the pterins. Finally we discuss the total synthesis of Sepiapterin-C, a natural compound, employing our Sonogashira coupling pathway.

2.2 Results and Discussion

2.2.1 Sonogashira coupling of sulfonyloxy-quinoxaline

Quinoxaline was chosen as a model for the pteridine ring system because it is soluble in common organic solvents and possesses the pyrazine ring of pteridine. The work began by preparing
quinoxalinone 6 from o-phenylenediamine and glyoxylic acid in acetic acid to give the desired product in 70% yield, following recrystallization of the crude product from DMF (Scheme 2.6). The melting point and IR spectra were consistent with those previously reported.\textsuperscript{22} By this route, quinoxalinone 6 was readily prepared in gram quantities.

We initially investigated the benzenesulfonate as potential leaving group in Sonogashira coupling. No previous synthesis of quinoxaline-2-benzenesulfonate has been reported. The synthesis of the corresponding tosylate has however been reported by reaction of quinoxalinone 6 with tosylchloride in the presence of triethyl amine (Et\textsubscript{3}N) and dimethylamino pyridine (DMAP).\textsuperscript{23} Employing these conditions, substituting benzenesulfonyl chloride for tosyl chloride, stirring the reaction for 1 hour and monitoring the reaction on TLC. The reaction was stopped by quenching with sat. NaHCO\textsubscript{3} after TLC showed full consumption of starting material and formation of one new less polar compound. Purification of the crude products on flash silica gave the desired 2-benzenesulfonyloxyquinoxaline 7 in 95% yield.

The product was characterized using NMR and mass spectrometry and was easily prepared in gram quantities. When comparing the characterization spectra of the previously reported quinoxaline-2-tosylate with that of the product isolated from our reaction, strong similarities were observed. The presence of a downfield singlet, at δ 8.67 ppm, was assigned to the 3-H proton. The tosylate-quinoxaline is reported as exhibiting a 3-H shift at 8.65 ppm.\textsuperscript{23} This peak, at δ 8.67 ppm, was used to integrate the other signals and the \textsuperscript{1}H NMR spectra showed the presence of 10H, consistent with our proposed structure. The mass spectrum of the sample gave a peak at \textit{m/z} 262 (M\textsuperscript{+}), consistent with the calculated mass of the desired sulfonate 7.

\begin{center}
\begin{align*}
\text{NH}_2 & \quad \text{O} \\
\text{NH}_2 & \quad \text{O}
\end{align*}
\end{center}

\begin{center}
\begin{align*}
\text{AcOH} & \quad 0 \text{ deg} \\
\text{PhSO}_2\text{Cl} & \quad \text{Et}_3\text{N} \\
\text{DCM} & \quad 0 \text{ deg}
\end{align*}
\end{center}

\textbf{Scheme 2.6}

With a potential coupling partner in hand, we investigated the ability of the quinoxaline-2-benzenesulfonate to undergo Sonogashira coupling. Typical Sonogashira coupling conditions,
employing catalytic Pd and Cu catalysts and Et$_3$N, were selected based on their applications in a wide range of substrates.$^{1-12}$ Treatment of sulfonate 7 with phenylacetylene in the presence of palladium (PdCl$_2$PPh$_3$, 5 mol%), Cul (5 mol%) and Et$_3$N (2 eq.) in DMF at 80°C under an Ar atmosphere for 90 minutes, after which TLC showed no trace of starting material and one new product formed (Scheme 2.7 and Table 2.2, entry 1). The reaction mixture was concentrated in vacuo and purification of the crude on flash silica to give 2(2-phenylethynyl)quinoxaline 8 in 95% yield. The $^1$H NMR spectra of the product was consistent with that of previously reported 8,24 with the 3-H protons at δ 8.99 ppm used as a basis for the integration of the signals. The total number of protons integrated to be 10H. The mass spectrum of the product gave a peak at m/z 230 (M$^+$) consistent with previously reported data.24

The success of this reaction was an encouraging result and we set about exploring the scope of the benzenesulfonate-quinoxaline 8 in Sonogashira coupling with various terminal acetylenes. The results are discussed below.

Treatment of sulfonate 7 with ethynyltrimethylsilane under the above mentioned conditions gave 2-[2-(trimethylsilyl)ethynyl]quinoxaline 9 in 74% yield (Table 2.2, entry 2). The product was obtained as oil due to the presence of the silyl group, also reported in previous publications. The $^1$H NMR spectra obtained was identical to those previously reported with the silyl peak in the upfield position of δ 0.34 ppm and the 3-H proton downfield at δ 8.90 ppm. The mass spectrum of the sample was in agreement with literature and calculated value, where a peak at m/z 226 was observed.

The use of THF as alternative solvent in the Sonogashira coupling reactions was then investigated. Tetrahydrofuran (THF) is a low boiling, hydrophobic and relatively cheap solvent and this makes it a relatively easy solvent to work with compared to DMF. Applying our Sonogashira coupling conditions but changing the solvent from DMF to THF in the coupling of phenylacetylene with sulfonate 7 gave the quinoxaline 8 in 96% yield when the reaction was refluxed for two hours and monitored on TLC. The $^1$H NMR and mass spectra of the product was identical to that previously obtained and literature data.24

When applying the Sonogashira coupling on sulfonate 7 with TMS-acetylene in THF, refluxing for two hours and monitoring the reaction on TLC gave the TMS-quinoxaline 9 in 72%. From
these results, it was concluded that changing the solvent from DMF to THF does not affect the yield of the reactions and therefore the cheaper and low boiling THF can be used for Sonogashira couplings. We then investigated Sonogashira coupling reactions of the sulfonate 7 with various terminal acetylenes in THF, and the results are summarized below and Table 2.2.

Treatment of sulfonate 7 with propargyl alcohol under the above mentioned conditions gave 3-(quinoxalin-2-yl)prop-2-yl-1-ol 10 in 70% yield, following recrystallization of the crude product in acetone (Table 2.2, entry 3). No starting material was visible on TLC after 90 minutes, and the mass balance of the product could have been lost during the crystallization step. The $^1$H NMR spectra of the product was consistent with previously reported data, with the downfield proton at $\delta$ 8.90 ppm and the alcohol proton at $\delta$ 2.96 ppm.

Table 2.2 lists the different products 8-14 of the Sonogashira coupling with different substrates. The NMR spectra, melting points and mass spectra of all but two were in agreement with those previously reported.24,25 There is no previous mention of quinoxalines 11 (Table 2.2, entry 4) and 12 (entry 5) suggesting that the compounds are novel.
Scheme 2.7

Table 2.2

<table>
<thead>
<tr>
<th>Entry</th>
<th>$R=$</th>
<th>Product (yield)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td><img src="image" alt="Product 8 (96)" /></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td><img src="image" alt="Product 9 (72)" /></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td><img src="image" alt="Product 10 (70)" /></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td><img src="image" alt="Product 11 (95)" /></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td><img src="image" alt="Product 12 (83)" /></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td><img src="image" alt="Product 13 (79)" /></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td><img src="image" alt="Product 14 (74)" /></td>
</tr>
</tbody>
</table>

$^a$THF used as solvent
Quinoxaline 11 differs from quinoxaline 10 (entry 3) by a methyl group on the oxygen atom and their $^1$H NMR spectra are very similar. The downfield protons are at 8.90 ppm for 10 and 8.83 ppm for 11, while the –OH proton at 2.96ppm is replaced by –CH$_3$ group at 3.45 ppm as shown by integration with the 3H proton. The melting point of product 11 is 61°C which is much lower than that of the alcohol 10 (139°C), consistent with loss of hydrogen bonding. Accurate mass measurement of the molecular ion evident in the EI mass spectrum of quinoxaline 11 gave a value of 198.07978 amu. This compared well to the calculated value of 198.07930 (C$_{12}$H$_{10}$N$_2$O) and confirms the composition of our proposed structure. These results led us to conclude that the desired product has been synthesized.

Quinoxaline 12 is very similar to quinoxaline 13 (entry 6) and they only differ by one –CH$_2$D group. The downfield protons are very similar, with quinoxaline 12 at δ 8.84 ppm and quinoxaline 13 at δ 8.81 ppm, and quinoxaline 13 has two more hydrogen than quinoxaline 12 consistent with the extra –CH$_2$D group on quinoxaline 13. The IR spectra of quinoxaline 12 gave a peak at $\nu_{\text{max}}$ 3332 cm$^{-1}$ which is a strong indication of the presence of an –OH group, this was also observed in the IR spectrum of quinoxaline 13. Finally the EI mass spectrum gave the molecular ion peak of $m/z$ 212 amu which is consistent with the calculated value of the desired product. From these results we concluded that the desired product was synthesized.

In conclusion, it has been shown that the Sonogashira coupling of quinoxaline-benzenesulfonate proceeded readily with both aryl and alkyl acetylenes to give C-C bond coupled products in good to excellent yield under the normal coupling conditions. We now investigated the effects changing the temperature and leaving group will have on the reaction yields.

**2.2.1.1 Effect of temperature and mesylate leaving group**

We were interested in investigating the effect lowering the temperature of the reaction to room temperature under our Sonogashira coupling conditions. We began by subjecting sulfonate 7 and phenylacetylene to our Sonogashira coupling conditions but performing the reaction at room temperature. The yield of quinoxaline 8 dropped from 95% to 53% when doing the coupling reaction at room temperature and running the reaction for 90 minutes. TLC showed the presence of starting material after 90 minutes implying that the reaction is slow at room temperature. The
reaction still does not go to completion when left to stir overnight with starting material still visible on the TLC plate. Purification of the crude mixture gave 100 mg (when starting with 300 mg) of recovered starting material giving the yield of 75% based on recovered starting material, a yield lower than that obtained at high temperature.

Similar results were observed on the TMS-quinoxaline 9 where the yield dropped from 74% to 35% when running the reaction for 90 minutes at room temperature. Increasing the reaction time to 24 hours gave no improvement on the yield, with starting material still visible on the TLC and 120 mg of starting material (when starting with 300 mg), recovered upon purification on flash silica giving a yield of 56% based on recovered starting material.

From these results it can be seen that lowering the temperature of the reaction results in reduced yield of products and full conversion of starting material to products is never achieved even after longer reaction times. Future work might involve adding more catalyst to the reaction mixture in an effort to drive the reaction to completion.

With benzenesulfonate shown to be a good leaving group in Sonogashira coupling reactions, we turned our investigations into the cheaper sulfonate, methanesulfonate. The 2-mesyloxyquinoxaline\textsuperscript{26} 15 was prepared in 84% yield using the same conditions of making benzenesulfonate 7 but replacing benzenesulfonyl chloride with mesylchloride (Scheme 2.8). The \textsuperscript{1}H NMR spectrum integrated for 8H, with the downfield proton at δ 8.74 ppm, consistent with 3-DH chemical shift of quinoxaline and finally the methyl protons were upfield at δ 3.49 ppm.\textsuperscript{26}

Applying our Sonogashira coupling conditions to sulfonate 15 with phenylacetylene gave the quinoxaline 8 in 67% (a drop from 95% when using sulfonate 7) after running the reaction for two hours (Scheme 2.8, R = Ph). The TLC showed the presence of starting material to be present when the reaction was stopped. Complete conversion of starting material was only achieved when the reaction was allowed to run for 24 hours with an isolated yield of 75% obtained, suggesting that decomposition of starting material might be occurring. The NMR and \textit{MS} spectra of the product were identical to those reported in the literature,\textsuperscript{24} and to those of quinoxaline 8 prepared earlier.
Similarly the yield of TMS-quinoxaline 9 dropped from 70% to 54% and quinoxaline 10 dropped from 74% to 53% (Scheme 2.8). The presence of starting material was observed on TLC when the reactions were stopped after two hours, and full conversion of starting materials to respective products was only achieved when running the reactions for 24 hours or longer. The $^1$H NMR and mass spectra of both products were identical to previously reported data.\(^{24}\)

These results can be explained by comparing the relative acidity of the sulfonic acids. Benzenesulfonic acid has a $P_{ka}$ of 5.9 compared to methanesulfonic acid’s -1.9. Good leaving groups are conjugates of strong acids, therefore benzenesulfonate is a better leaving groups than mesylate as evidenced in our results.

In conclusion, we had established that quinoxaline-benzenesulfonate 7 is a viable partner in Sonogashira coupling reactions with aryl and alkyl acetylenes. While it was not our initial stated goal, we decided to attempt the Sonogashira reactions with other heterocycles containing the benzenesulfonate leaving group.

### 2.2.1.2 Sonogashira coupling on sulfonyloxy-heterocycles

To investigate the scope of benzenesulfonyl substrates in Sonogashira coupling reactions, we attempted to prepare pyrimidine, pyridine and quinoline substrates bearing a benzenesulfonate leaving group. These nitrogen containing rings were to be prepared using the same method leading to sulfonate 7 and sulfonate 15 from their corresponding hydroxy derivatives.

Treating quinoline-2-ol with benzenesulfonyl chloride in the presence of Et$_3$N and DMAP with DCM as solvent, gave quinolin-2-yl benzenesulfonate 16 in 60% yield (Figure 2.2). The melting
point obtained from our results (m.p. 89°C) was in agreement with previously reported data of 90°C.  

Pyridin-2-yl benzenesulfonate 17 was prepared from pyridine-2-ol in 68% yield, following the same procedure for making 16 (Figure 2.2). The product was obtained as an oil as previous, and the 1H NMR and mass spectra of the product were in agreement with reported data.  

Treating pyrimidin-2-ol with benzenesulfonyl chloride did not give the desired pyrimidin-2-yl benzenesulfonate 18 and no previous synthesis of the product has been reported.

![Figure 2.2](image)

We then applied our Sonogashira coupling conditions (PdCl₂(PPh₃)₂, Cul and Et₃N) to the above substrate, choosing phenylacetylene as coupling partner. Treatment of the quinoline-sulfonate 16 with phenylacetylene in refluxing THF and monitoring the reaction on TLC gave undesired results. TLC analysis of the crude showed the presence of starting material and a formation of one new product, however, 1H NMR and mass spectral analysis of the new product was not in agreement with that of the proposed structure. The EI mass spectrum gave a peak at m/z 202 amu, while the 1H NMR spectrum integrated for 10H instead of the expected 11H. When increasing the reaction time from two to 24 hours, similar results were obtained. Increasing the temperature of the reaction by refluxing in DMF also proved to be unsuccessful. Changing the catalyst from PdCl₂(PPh₃)₂ to the more reactive Pd(0) catalyst, Pd₂(dba)₃CHCl₃, could yield the desired coupled product.

Turning our attention to the pyridine-sulfonate 17, we attempted the Sonogashira coupling using our optimized conditions, choosing phenylacetylene as the coupling partner with DMF as solvent.
and monitoring the reaction on TLC. Monitoring the reaction after 2 hours showed no conversion of starting material and the reaction was allowed to run for 24 hours. Purification of the crude mixture on flash silica gave three fractions which were analyzed by $^1$H NMR and mass spectroscopy.

The first fraction was identical to the undesired product obtained in the quinoline-sulfonate 16 coupling, where a peak at $m/z$ 202 amu was obtained in the mass spectrum. The second fraction was an oil which had $^1$H NMR and mass spectra that were consistent with the desired coupled product 2-(2-phenylethynyl)pyridine$^{29}$ 19 (Scheme 2.9) where a peak at $m/z$ 179 amu was obtained from the mass spectrum. The $^1$H NMR spectrum integrated for 9H, with a downfield singlet at $\delta$ 8.62 ppm in agreement with reported data.$^{29}$ The product was obtained in 20% isolated yield and a yield of 66% based on recovered starting material. The third fraction had a NMR and mass spectra identical to those of starting material and was thus assigned to be the starting material. Attempts to improve the yield by changing the catalyst to the more reactive Pd$_2$(dba)$_3$CHCl$_3$ were unsuccessful.

The $^1$H NMR and mass spectra of the undesired product obtained was consistent with the homocoupled product of two phenylacetylene molecules, i.e. 4-phenylbuta-1,3-diyn-1-yl)benzene 20, previously reported to occur in a Cu catalyzed Sonogashira coupling reaction of two phenylacetylene substrates.$^{29}$ Given these results, the earlier coupling using only palladium catalyst with no added CuI was attempted.

![Scheme 2.9](image)

Treatment of the quinoxaline-sulfonate 7 with phenylacetylene under normal Sonogashira coupling conditions but omitting CuI, gave quinoxaline 8 in 96% yield after purification on flash
These results show that CuI does not play an important role in this reaction and can thus be omitted. When using these modified conditions on quinoline-sulfonate 16 and phenylacetylene in DMF, running the reaction for 24 hours and monitoring the reaction on TLC. When analyzing the crude mixture by EI mass spectrometry, the peak at m/z 202 was no longer evident, but no peak corresponding to the desired product was observed. Instead the starting material peak was shown to be the dominant peak. Purification of the crude mixture gave recovered starting material in quantitative amounts. The lack of formation of bis-phenylacetylene 20 under Cu-free Sonogashira coupling strongly suggests that the homo-coupling is Cu catalyzed.

Subjecting pyridine-sulfonate 17 to the Cu-free Sonogashira coupling with phenylacetylene in DMF for 24 hours and monitoring the reaction on TLC gave two fractions after purification on flash silica. The first fraction was assigned to be the desired coupled product, 2-(2-phenylethynyl)pyridine 19 after its 1H NMR and mass spectra were identical to those previously reported and those obtained from our previous synthesis. The second fraction was identified to be the starting material following 1H NMR and mass spectroscopy characterization. The peak at m/z 202 amu was no longer evident, further supporting our claim that the homo-coupling is Cu catalyzed.

An interesting observation was noted on the state of pyridine-sulfonate 17 where upon standing for a number of days, a phase change is observed. The sulfonate was obtained as an oil and this was in agreement with previously reported data. The mass spectrum of the isolated solid was identical to that of the oil and there were no notable differences in their 1H NMR spectrum. Heating the oil in ethanol afforded more of the solid which were collected and subjected to the Cu-free Sonogashira coupling conditions with phenylacetylene. When monitoring the reaction on TLC, no desired coupled product was observed but, a new spot with similar Rf value to the one observed from pyridine-sulfonate 17 (oil) was observed along with the solid starting material. Purification of the crude on flash chromatography gave fractions which confirmed the TLC observations. From these results we postulate that the crystals and the oil are isomers and are likely to be pyridine-o-benzenesulfonate 17a and pyridinone-N-benzenesulfonate 17b(Scheme 2.10)
Previous work has shown that there exists an equilibrium between N-sulfonated and O-sulfonated products at high temperatures for compounds having the 1,2 (N, O) relationship. The N-sulfonyl isomer does not react under Sonogashira coupling conditions and its formation will result in low yields of the desired products. The same isomerization observed on pyridine-sulfonate 17 could be happening on quinoline-sulfonate 16, hence explaining the lack of formation of coupled products. Due to high temperatures required for the Sonogashira coupling, and also high temperatures favoring isomerisation, the substrates 16 and 18 become generally unreactive.

In recently reported Sonogashira coupling reactions quinoline- and pyridine-tosylates, the coupling was only reported to proceed at the 3-positions. The only reported Sonogashira coupling reactions at the 2-position of pyridine and quinoline were performed on substrates having the triflate leaving group. Triflates is a better leaving group than benzenesulfonate or tosylate, suggesting that the rate of coupling is much faster than the rate of isomerization when working with triflate bearing substrates. Further work needs to be done to substantiate these claims.

### 2.2.1.3 Attempted Sonogashira coupling on sulfonyloxy-benzene substrates

In previous publications, Sonogashira coupling reactions were reported on phenyl substrates possessing a triflate and more recently, substrates possessing a tosylate and mesylate leaving groups. In the latter publications, modified Sonogashira coupling conditions were employed where a strong base, Cs₂CO₃, was used and bulky phosphine ligands were also employed. We were interested in employing our simple Sonogashira coupling conditions on phenyl compounds possessing the benzenesulfonate leaving group.
The benzene substrates (benzenesulfonyloxy)benzene 21 and 1-(benzenesulfonyloxy)-3-methoxybenzene 22 (Figure 2.3), were both prepared, according to the method of Choi et al., by treating the corresponding phenols with Et$_3$N and benzenesulfonyl chloride in tetrahydrofuran and stirring the mixture for 48 hours after which TLC showed no trace of starting material and a new product appeared to have formed. Both products were obtained as oils, following aqueous HCl workup and concentration of the crude organic layers, and the yields were 96% for [PhOSO$_2$Ph] 21 and 99% for [3-MeO-PhOSO$_2$Ph] 22. The NMR and mass spectra of both these products were in good agreement with literature data. \(^3\)

Treating the benzene-substrate 21 to our Sonogashira coupling conditions in the presence of phenylacetylene and monitoring the reaction on TLC gave the following observations. Analysis of the crude by TLC showed the presence of starting material even when the reactions were run for 24 hours or longer. Purification of the reaction mixture gave only the unchanged starting material as judged by NMR and mass spectral analysis. Changing the temperature of the reaction from 80°C to 120°C gave no improvement. Changing the catalyst from PdCl$_2$(PPh$_3$)$_2$ to Pd$_2$(dba)$_3$CHCl$_3$ also gave no improvements. Similar observations were obtained when the methoxy-benzene substrate 22 was used.

![Figure 2.3](image)

The lack of detectable Sonogashira coupled products under our conditions is in contrast to the success of the Sonogashira coupling with the quinoxaline sulfonates. The benzene substrates investigated can be classified as electron-rich while the quinoxaline and pyridine substrates can be considered to be electron-deficient. We then concluded that Sonogashira coupling reactions on benzenesulfonyl substrates proceeds smoothly on electron-deficient systems, while electron-rich systems will require the presence of special phosphine ligands and a strong base. Possible future work might involve attempted synthetic coupling of pyridine-, quinoline- and benzene-sulfonates, employing Cs$_2$CO$_3$ and bulky phosphine ligands conditions.
2.2.2 Sonogashira coupling of pteridine-O-sulfonates

With conditions for the Sonogashira Coupling of heterocyclic substrates in hand, we turned to the challenge of extending the scope of this procedure to a pteridine system. Our chosen starting point was an O-sulfonate derived from the bis-protected pteridine 25 (Scheme 2.11). Previous investigations by Joule et.al.\textsuperscript{21} showed the substrate to undergo Stille coupling as described in Chapter 1, section 1.3.1.

The pteridine ring was constructed, according to the method of Pfleiderer,\textsuperscript{34} from tetraaminopyrimidine (TAP) and glyoxylic acid in sulfuric acid (H\textsubscript{2}SO\textsubscript{4}) to give 2,4-diaminopteridine-6-one 23 in 74\% yield. The product was characterized by UV-vis spectrum which was consistent with that reported by Pfleiderer. The product was insoluble in common NMR solvents and no NMR data was obtained. The reaction was performed in an acidic medium so as to promote formation of the 6-oxo-pteridine over the 7-oxo-pteridine. The mechanistic explanation for the regioselectivity is that under acidic conditions, the N(1)- and N(5)- atoms are protonated, making the N(6)-atom more reactive towards electrophiles and thus reacts with the more reactive aldehyde of the glyoxylic acid (Scheme 2.11)

There was no evidence of any detectable formation of the 7-oxo-pteridine during all our subsequent investigations, where all the products derived from the 6-oxo-pteridine were in agreement with those previously reported. Those results are explained below.

Bredereck’s reagent [t-BuO(Me\textsubscript{2}N)\textsubscript{2}CH]\textsubscript{24}, was chosen to introduce the dimethyl(formamidine) protecting and solubilising group, following previous work by Joule et.al.\textsuperscript{21} The Bredereck’s reagent was prepared by treating tetramethylformamidine chloride (TMFAC) with potassium tertiary-butoxide (KtBuO) in tetrahydrofuran.

Dimethylcarbamoyl chloride (DMCC) and dimethylformamide (DMF) were heated at 120\(^\circ\text{C}\) in a flask fitted with an air-bubbler until production of CO\textsubscript{2} was no longer visible (3 days). The excess DMF was removed under high vacuum to give a brown solid with a \textsuperscript{1}H NMR and mass spectra similar to that of TMFAC.\textsuperscript{21} The product was obtained in 91\% yield (Scheme 2.11).

TMFAC and KtBuO were suspended in THF at room temperature until all solid has dissolved (4 days). Following the removal of solvent and purification by distillation, a clear oil with NMR

44
and mass spectra similar to that of the Bredereck’s reagent was obtained in 79% yield (Scheme 2.11).

Scheme 2.11

Following the procedure reported by Joule et al., reacting pteridinone 23 and Bredereck’s reagent 24 in DMF at 60 °C afforded the more soluble pteridine, 2,4-di(N,N-dimethylaminomethyleneamino)pteridine-6-one 25 in 90% yield (Scheme 2.11). The $^1$H NMR spectrum was in agreement to that reported by Joule, with a downfield singlet at $\delta$ 8.76 ppm corresponding to the 7(H) proton and the upfield singlets at $\delta$ 3.07, 3.08, 3.10 and 3.15 ppm corresponding to the four methyl groups on the amidine protecting groups. The difference in chemical shifts between the methyls shows hindered rotation and suggests a significant double bond character to the amidine bond, causing the methyl-C to be in different environments. The total number of protons was integrated to be 14, consistent with the proposed product. The mass spectral data gave a molecular ion peak at $m/z$ 288 amu. The product was obtained as a pale yellow solid that displayed bright yellow fluorescence under long wave UV lamp, or in dilute solution when exposed to sunlight.

The dimethylformamidine protecting groups have been used previously on the 2,4-diaminopteridine ring. On two occasions, the pteridine possessed a chlorine atom at the six
position\textsuperscript{35-36} and on one occasion it had an oxygen atom at the six position.\textsuperscript{21} These substrates were used to synthesize a number of six-substituted pteridines, some of which were tested for anti-cancer activity.\textsuperscript{35}

2,4-Di(\(N,N\)-dimethylaminomethyleneamino)-6-mesyloxopteridine 26 (Figure 2.4A) was prepared by treatment of pteridinone 25, mesylchloride and \(\text{Et}_3\text{N}\) in DCM in a similar method for preparing quinoxaline 7. Following recrystallization from EtOH, the product was obtained as a yellow solid in 65% yield. Spectroscopic data of the compound compared closely with the structurally related tosylate, previously prepared by Joule.\textsuperscript{21} There are down field singlets at around \(\delta\) 8.76 ppm (1H) and \(\delta\) 9.03 ppm (2H), corresponding to the 7(H) proton and the two formamidine protons, respectively (Figure 2.4 B). The APCI mass spectrum of the product gave a [M + H\(^+\)] at \(m/z\) 367 amu that was in agreement with that calculated for C\textsubscript{13}H\textsubscript{19}N\textsubscript{8}O\textsubscript{3}\textsuperscript{32}S.

2,4-Di(\(N,N\)-dimethylaminomethyleneamino)-6-benzenesulfonyloxopteridine 27 (Figure 2.4A) was prepared in 75% yield in a similar manner to the mesylate-pteridine 26. The \(^1\)H NMR spectrum of the product showed a downfield singlet at \(\delta\) 8.56 ppm corresponding to the 7(H) and the four upfield singlets between \(\delta\) 3.19 and 3.30 ppm. The EI mass spectrum gave a peak at \(m/z\) 428 amu [M]\(^+\), consistent with that calculated for C\textsubscript{18}H\textsubscript{20}N\textsubscript{8}O\textsubscript{3}\textsuperscript{32}S.

\begin{center}
\textbf{Figure 2.4 A}
\end{center}
With a protected pteridine-6-sulfonate in hand, we were in a position to attempt the Sonogashira coupling reactions on these substrates. Subjecting the benzenesulfonate-pteridine 27 and phenylacetylene to our Cu-free Sonogashira coupling conditions previously found optimal for quinoxaline 7 (PdCl₂(PPh₃)₂, Et₃N, DMF, 80°C) gave, after two hours, a mixture of two close running new products as judged by TLC, with no starting material observed. Both products had a blue fluorescence under long wave UV light, suggesting incorporation of a pteridine nucleus. Purification of the crude mixture on flash silica only partially separated these new products. Analysis of the close running products employing HPLC-UV-APCI-MS also showed the compounds to be close running. The APCI mass spectra of these two closely eluting fractions gave peaks at m/z 373 and 318 amu. The peak at m/z 373 amu was assigned to be that of the desired Sonogashira coupled product, 2,4-di(N,N-dimethylaminomethyleneamino)-6-(2-phenylethynyl)pteridin-4-yl 28 (Scheme 2.12). The peak at m/z 318 amu, 55 less than molecular weight of the desired coupled product 28, corresponding to a loss of one protecting group was
tentatively assigned to be the mono-deprotected product 4-amino-2-(N,N-dimethylaminomethyleneamino)-6-(2-phenylethynyl)pteridin-4-yl 29. A similar formation of a mono-deprotected pteridine was reported by Joule during the Stille coupling reaction of the tosylate derivative.\textsuperscript{21}

The $^1$H NMR spectrum of the combined fractions gave peaks that were consistent with the coupled products, however, overlapping of peaks was observed on both the upfield and downfield signals. Two downfield singlets δ 8.76 and 9.06 ppm, corresponding to the 7(H) and the formamidine (NCH$^\text{N}$) respectively, from both compounds, were observed. The four downfield singlets between δ 3.01 and 3.12 ppm, were consistent with the amidine methyl groups from both molecules. Integration of the methyl peaks gave a product distribution of ~1:1 but this ratio varied slightly when repeating the reaction and chromatographic purification.

Further purification of the two close running fractions, by flash chromatography, gave a product distribution that favored the mono-deprotected product 29 in a 1:9 ratio, when integrating the methyl peaks. The ratio of the products can also be calculated by integrating the downfield protons at δ 8.76 and 9.06 ppm.

![Scheme 2.12](image)

The general mass yield of these reactions were that, from one gram of sulfonate 27, 0.65 gram of mixed products were obtained and further purification lead to 0.55g of mainly mono-deprotected 29 in an overall yield of 74%. Accurate mass measurements of the sample gave a peak at $m/z$ 317.13849 amu which is in good agreement to the calculated value for C$_{17}$H$_{15}$N$_7$ of 317.13889 amu. From these results we were confident that the Sonogashira coupled product from pteridine-O-benzenesulfonate with phenylacetylene was achieved. The desired product however, loses one protecting group either during the reaction or during purification. This result shows the first Sonogashira coupling reaction involving pteridine-O-sulfonates.
We examined briefly, the effect of varying the catalyst by employing Pd$_2$dba$_3$.CHCl$_3$ instead of PdCl$_2$(PPh$_3$)$_2$. There was no apparent change in the rate of the reaction as judged by TLC analysis and purification of the crude on flash silica gave the two closely running fractions in similar mass yield (72%) to that obtained before. $^1$H NMR of the combined fractions gave a product distribution that favored the desired coupled pteridine 28 in 2:1 ratio, however, further purification of the fractions on flash silica gave product distribution that favored the mono-deprotected pteridine 29 in a 1:9 ratio as observed before. HPLC-UV-APCI-MS confirmed the main component coming from the second purification to be the mono-deprotected pteridine 29. The APCI mass spectrum showed the peak at $m/z$ 318 amu to be the dominant peak with minor peaks at 373 and 263, corresponding to fully protected coupled product 28 and fully deprotected product, 2,4-diamino-6-(phenylethynyl)pteridine 35 (Figure 2.5). In conclusion, it appears that the Pd source did not significantly affect the reaction yield in this case.

![Figure 2.5](image-url) HPLC-UV, TIC chromatograms and APCI-MS of the main component

We then tried to understand how one protecting group is lost during either the reaction, or upon purification by flash chromatography. The removal of the protecting groups can be explained as follows; the protecting groups used are removed by acid hydrolysis and alkaline hydrolysis, thus
the presence of base or acid in the reaction mixture can result in the removal of the protecting groups with the more reactive site being easily displaced.

The reactions are performed in the presence of Et$_3$N, a hindered base, and the side product generated in the Sonogashira coupling is benzenesulfonic acid. Because the formation of the benzenesulfonyl-pteridine 27 is in the presence of Et$_3$N, we eliminated the possibility of the Et$_3$N to remove the protecting group. Benzenesulfonic acid on the contrary is a strong acid with similar strength to HCl and could explain the removal of the protecting group. However, with Et$_3$N used in excess during the reaction, any benzenesulfonic acid formed will be neutralized by the base, thus making the sulfonic acid an unlikely candidate to deprotect the pteridine.

During purification by chromatography, the solvent system used were MeOH/DCM, methanol can act as an acceptor while the silica gel can act as an acid leading to further hydrolysis. This could explain the observation of pteridine-29 becoming the more dominant product upon further purification. To further support our claims that MeOH/DCM in silica is responsible for the deprotection, we ran a 2-D TLC of the two close running fractions. A visible off-diagonal spot was apparent, clearly showing that deprotection of the di-protected 28 to give the mono-protected pteridine 29 was occurring under these chromatographic conditions.

While it was likely that silica gel could provide an acidic surface capable of catalyzing the hydrolysis of the more labile PG, we also investigated the possibility that methanol itself would cause deprotection. We prepared a solution of the starting material, benzenesulfonyl-O-pteridine 27, in a MeOH/DCM mixture (9/1) and analyzing on HPLC-MS. The eluting solvent system chosen was water/methanol running a gradient for 12 minutes starting with 100% water, changing to 100% methanol over 6 minutes. The HPLC-UV-MS run gave a retention time (RT)
of 8.8 minutes, a peak of 429 \([\text{M+H}]^+\) and \(\lambda_{\text{max}}\) 253, 288 and 387nm (Figure 2.6).

Figure 2.6 TIC and extracted UV and mass spectra, from HPLC analysis of the benzenesulfonate 27.

When running the same sample after 24 hours, different results where the original peak at RT 8.84 minutes was replaced by two new peaks at RT 8.43 and 8.87 minutes, were observed. The two new peaks had \(m/z\) 374 and 319 amu, and were tentatively assigned to be the monodeprotected and fully deprotected benzenesulfonyl-pteridines 27b and 27c, suggesting that methanol has the ability to deprotect (Figure 2.7). The isotopic distribution of the molecular ion peaks further supports our proposed structural assignments of these components. Both peaks have \([\text{M+1}]^+\) and \([\text{M+2}]^+\) signals coming from the \(^{13}\text{C}\) isotope and \(^{34}\text{S}\) isotope. In addition to the peak at \(m/z\) 374 amu, a peak at 375 amu which has 17% intensity relative to the 374 peak, and a
peak at 376 which has 6% intensity relative to the 374 peak are observed (Figure 2.7). These peaks correspond to a molecule having 17 carbon atoms and one sulfur atom as expected from our proposed structure. Similar isotopic distributions were observed on the m/z 319 amu peak, where peaks at m/z 320 (14%) and 321(6%) amu were observed.

![Figure 2.7 TIC resulting from HPLC analysis of the pteridine 27, in MeOH / DCM solution for 24 hours, and summed MS spectra over both eluting peaks.](image)

It is also likely that the residual moisture present in both MeOH and silica gel could be responsible for the deprotection. Evidence for this claim was observed on the ¹H NMR spectra of the compounds obtained from the two reactions, where residual DMF peaks were visible.

In conclusion, it appeared that while the Sonogashira coupling of the O-sulfonate 27 and phenylacetylene occurred readily, loss of the one amidine protecting group was also occurring either during the reaction itself, if an adventitious acceptor was present and during the chromatographic purification. While this in itself did not affect the overall yield, it did
complicate analysis of both the crude and purified product. This side reaction was found to accompany many of the reactions to be described below.

Having shown that the pteridine-O-benzenesulfonate and phenylacetylene would undergo Sonogashira coupling, we investigated varying the acetylenes to those previously used in the coupling with quinoxaline-O-benzenesulfonate 7. Treating benzenesulfonate-pteridine 27 with ethynyltrimethylsilane under our previously optimized conditions, running the reaction for two hours and monitoring the reaction on TLC, showed consumption of starting material 27 and afforded a mixture of two new products, following flash purification on silica gel. Analysis of the close running products by HPLC-MS gave peaks at $m/z$ 369 and 314 amu (both presumed [M+H]$^+$). We tentatively assigned these peaks to come from the 6-TMS-ethyl-pteridines 30 and 31 (Figure 2.8) The $^1$H NMR of the mixture gave similar observations as with phenylethynyl-pteridine 28 and 29 (Scheme 2.12), where roughly equal amounts of products are present, and further purification on flash silica results in the formation of more TMS-pteridine 31. General mass yield of these reactions were that, from one gram of sulfonate 27, 0.56 g of product mixture were obtained and further purification gave 0.46 gram of mainly 31 (1:9) in an overall yield of 63%.

![Figure 2.8](image)

Treating benzenesulfonate-pteridine 27 with propargyl alcohol under the Sonogashira coupling conditions, running the reaction for two hours and monitoring the reaction on TLC, did not yield the desired coupled product, propargyl-pteridine 32 (Scheme 2.13). No starting materials were recovered suggesting a side reaction has taken place. Starting with one gram of sulfonate 27, 654 mg of orange precipitates, with the fluorescent character of pteridines, were isolated from the reaction flask and their characterization by APCI mass spectroscopy gave a peak at $m/z$ 319 amu.
The peak was assigned to be that of fully deprotected starting material, benzenesulfonyl-pteridine 27c (Figure 2.7), suggesting that deprotection had taken place. We postulated that the protecting groups on the pteridine ring were removed by the free hydroxyl group on the propargyl alcohol. This result is consistent with the observations above, which showed that benzenesulfonate-pteridine 27 can be fully deprotected in MeOH at room temperature over 24 hours (section 2.2.3.1).

Since the free hydroxyl group appeared to be incompatible with the protecting groups under our optimized Sonogashira coupling conditions, we attempted to mask the free hydroxyl group on propargyl alcohol with a methyl group. 3-Methoxypropyne was obtained by treating propargyl alcohol with dimethyl sulfate in a 13M NaOH solution. The product was obtained as a clear oil, which boiled at 65 °C in keeping with the literature value. The $^1$H NMR spectrum of the product was identical to that reported previously.

Treating benzenesulfonate-pteridine 27 with 3-methoxypropyne under the optimized, Cu-free Sonogashira coupling conditions, running the reaction for two hours and monitoring the reaction on TLC, gave a mixture of two close running coupled products, after purification on flash silica. Partial separation employing HPLC-UV-MS showed two closely eluting components to be present which gave peaks at $m/z$ 286 and 341amu, both presumed to be [M + H]$^+$. The peaks were tentatively assigned to be the methoxypropynyl-pteridines 33 and 34 (Scheme 2.13). $^1$H NMR showed the product mixture to be roughly 80% fully protected pteridine 33 and 20% mono-deprotected pteridine 34. The mass yield for this reaction was that, from one gram of benzenesulfonate-pteridine 27, 0.5 gram of mixed products but with methoxypropynyl-pteridine 33 as the dominant product, was obtained. Comparing this successful result to that obtained above clearly supports the suggestion that an adventitious acceptor (the OH of propargyl alcohol in this case) allows a fast deprotection of the O-sulfonate amidine groups, under the reaction conditions employed here.
We then turned our attention to the synthetic utility of the mesylate-pteridine 26 which offers the advantages of being relatively cheap to prepare and atom economic during the coupling reactions. Subjecting the mesylate-pteridine 26 to our optimized Cu-free Sonogashira coupling conditions with phenylacetylene, running the reaction for two hours and monitoring the reaction on TLC, showed two spots corresponding to the Sonogashira coupling products obtained above and unreacted O-mesylate 26. Workup and purification by flash chromatography gave the coupled pteridine products 28 and 29 in 7% yield (84% based on recovered starting material). Increasing the reaction time to 24 hours did not improve the conversion.

No reactions occurred when either 3-methoxypropyne or ethynyltrimethylsilane were used in an attempted Sonogashira coupling with mesylate 26. TLC showed the presence of starting material even when the reactions were run for longer than 24 hours. When purifying the crude mixture on flash silica, starting material was recovered in almost quantitative amounts and trace amounts of fully the deprotected starting material 27c were detected on the mass spectrum. These results led to the conclusion that Sonogashira coupling reactions on the pteridine ring proceed smoothly with 6-O-benzenesulfonyl leaving group ability, while the 6-O-mesylate leaving group is unreactive under our Sonogashira coupling conditions.
In conclusion, we have demonstrated that a pteridine-O-benzenesulfonate 27 is a useful substrate for Sonogashira coupling reactions using simple Pd catalyzed conditions.

2.2.3 Hydrolysis of protected pteridines

2.2.3.1 Hydrolysis to 2,4-diaminopteridines

With quantities of 6-acetylene-pteridines in hand, we turned to the issue of the hydrolysis. We intended that, under appropriate conditions, hydrolysis could be tuned to give initially the 2,4-diaminopteridine, a much investigated motif in the area of medicinal chemistry, and under stronger hydrolytic conditions the corresponding 2-amino-4-oxo-pteridine (pterin) could be obtained.

2,4-Diaminopteridines have previously been hydrolyzed into 2-amino-4-oxopteridines (pterin) under basic conditions or acidic conditions. A one molar sodium hydroxide (1M NaOH) and ethanol (EtOH) solutions are generally used for the basic hydrolysis while a six molar hydrochloric acid (6M HCl) solution is used in the acid hydrolysis.

We anticipated, from both the behavior described above and previous literature, discussed in Chapter one, that removal of both the dimethylformamidine protecting groups would be facile. Treating the mixture of the phenylacetylene-pteridines 28 and 29 with ammonia solution (1M), gently refluxing the mixture for 16 hours and precipitation by addition of acetic acid, gave the expected 2,4-diamino-6-(phenylethynyl)pteridine 35 in 77% yield (Scheme 2.14). The product was obtained as a yellow solid and melting point, NMR and mass spectral data were consistent with both the proposed structure and data previously reported. The downfield singlet at δ 8.86 ppm is consistent with the 7(H) from pteridine and a peak at m/z 263.1 amu [M+H]⁺, obtained as the sole peak from APCI-MS. HRESI gave a peak at m/z 263.10340 which is in agreement with that calculated for C₁₄H₁₁N₆.
Scheme 2.14

Similarly the mixture of the TMS-acetylene-pteridines 30 and 31 gave 2,4-diamino-6-(ethynyl)pteridine 36 as a yellow solid in 76% yield, with the \(^1\)H NMR and mass spectral data identical to literature data (Scheme 2.14). A peak at \(m/z\) 187 [M+H]\(^+\) was obtained from the APCI mass spectrum, confirming the formation of product (Figure 2.9, a small [2M+H]\(^+\) is also evident).

Figure 2.9 TIC chromatogram and APCI mass spectrum of 2,4-diamino-6-(ethynyl)pteridine.
Finally, 2,4-Diamino-6-[(methoxymethyl)ethynyl]pteridine 37 was prepared, as a yellow solid in 74% yield from the mixture of 33 and 34 (Scheme 2.14). The NMR and mass spectra were identical to that reported previously. No deprotection of the methoxy group was noted as judged by the $^1$H NMR spectrum which gave a singlet at δ 3.35 ppm (3H) and a singlet at δ 4.40 ppm (2H) corresponding the the -CH$_3$ and -CH$_2$ groups respectively. The APCI mass spectrum gave a molecular ion peak at $m/z$ 231 amu [M+H$^+$] in agreement with that calculated for C$_{10}$H$_{11}$N$_6$O.

In conclusion, the ammoniolysis proceeded smoothly as judged by the chromatographic homogeneity of the three diaminopteridines described above. We then addressed the hydrolysis to the corresponding pterin derivatives.

2.2.3.2 Hydrolysis to 2-amino-4-oxo-pteridines (pterins)

Hydrolysis of 2,4-diaminopteridines to their corresponding pterin derivatives have been previously reported to proceed smoothly in a 1M NaOH/Ethanol solution under reflux, and obtaining the products by precipitation with AcOH. Treating the mixture of the phenylethynylpteridines 28 and 29 with 1M NaOH/ EtOH solution and refluxing for 18 hours and precipitation by acidifying the solution with AcOH, gave the desired 6D(phenylethynyl)pterin 38 in 73% yield (Scheme 2.15). The product was isolated as a yellow solid and its $^1$H NMR spectrum was identical to the one reported by Taylor. Accurate mass measurement of the sample gave a molecular ion at 263.08092 amu, in good agreement with that calculated for C$_{14}$H$_9$N$_5$O calculated to be 263.08071 amu.
Applying the same alkaline hydrolytic conditions to the TMS-ethynyl-pteridines 30 and 31 resulted in the solution turning from yellow to black. Attempts to precipitate the product by addition of AcOH resulted in black precipitates forming, however, these precipitates were not soluble in any organic solvent and could not be characterized by $^1$H NMR. An APCI mass spectrum of the crude dark solution did not have the desired molecular ion peak of 187 amu (or 188 if [M+H]$^+$), instead a peak at $m/z$ 178 amu was dominant.

Similarly, treatment of the mixtures of methoxypropynyl-pteridines 33 and 34 with the alkaline hydrolytic conditions, resulted in the solution changing color from yellow to black upon refluxing and the reaction mixtures could not be analyzed by $^1$H NMR. A peak at $m/z$ 177 amu was obtained as the dominant peak from the APCI spectrum. The retention time of this peak was very similar to the one obtained in the attempted TMS-acetylene hydrolysis, suggesting that it could be the same product.

We then turned our attention into monitoring these unsuccessful reactions by partial separation with HPLC and characterization with APCI mass spectroscopy. In addition, HPLC-MS was used to analyze the purity of the products and respective starting material, and also the relative rates of the hydrolysis reactions. The results are discussed below.

### 2.2.3.3 Use of HPLC-MS in monitoring the hydrolysis of 6-ethynyl-pteridine to 6-ethynylpterin.

In investigating the deprotection and hydrolysis of TMS-ethynylpteridine 30 and 31 in the presence of NaOH, we prepared a solution of the two acetylenes in DCM/MeOH (1:9). Before treating the solution with base, HPLC-MS analysis was run to confirm the presence of the two products where two close RT values of 7.34 and 7.49 minutes were obtained (Figure 2.10). The mass spectra of the samples however, were not in agreement with the molecular ion peak of [M+H]$^+$ 369 and 314 amu, instead $m/z$ 241 and 296 amu were observed (Figure 2.10), corresponding to the loss of the trimethylsilyl (TMS) group. These results suggest that the TMS group is labile and does not require the presence of acid, as in many cases, to be removed.
Figure 2.10 HPLC-UV and TIC chromatograms, along with the APCI-MS spectra of the ethynyl-pteridines 30 and 31.

Treating the mixture of 30 and 31 with NaOH/EtOH at reflux temperature and monitoring the reaction at different time intervals gave the following results; an HPLC-MS run of the reaction mixture after one hour showed one compound to be present in solution, which was tentatively assigned to be 6-ethynylpteridine 36 (RT 4.60 min, m/z 187 [M+H]^+, Scheme 2.16), suggesting that the deprotection step is relatively fast.

When sampling the reaction mixture after three hours we observed two new peaks with RT 5.93, 5.99 minutes and m/z 188, 233 amu (both presumed to be [M+H]^+), respectively, along with the 6-ethynylpteridine 36. The species with m/z 188 amu was tentatively assigned to be the desired 6-(ethynyl)pterin 36b. The peak at m/z 233 amu, 46 amu up on ethyne 36, was tentatively assigned to the ethoxyene-pteridine 38. It is likely that this results from the addition of an ethanol molecule across the triple bond of pteridine 36. In support of our structural assignment of the ethoxyene-pteridine 38, the 13C isotope peak at m/z 234 amu had an intensity of ~11% that of the
peak at 233 which was is in agreement with a molecule having ten C atoms, as is the case with our proposed structure (Scheme 2.16).

Similar additions of an ethanol group across a triple bond were observed on pterin 36b after four hours to give 6-ethoxyene-pterin 39 (RT 7.38 min, m/z 234 amu [M+H]+). A peak at m/z 235 amu, with ~11% intensity to that of peak at 234, was observed. At this point the species present in solution had m/z 188, 233 and 234 amu.

Further addition of ethanol groups on pteridine 38 and pterin 39 were observed after six hours, where the only peaks observed were at m/z 278 and 279 amu and were tentatively assigned to be 6-[(diethoxy)ethyl]pteridine 38 and 6-[(diethoxy)ethyl]pterin 39, respectively. The latter two peaks gradually disappear over ten hours to give a new peak with RT 5.08 min and m/z 178 amu, which we tentatively assigned to be 6-(methyl)pterin 42. These results are summarized in Scheme 2.16.

![Scheme 2.16 Proposed scheme for the alkaline hydrolysis of the ethynyl-pteridines 30 and 31. All mass peaks are assumed to be [M+H]+.](image-url)
To investigate further that ethanol was adding twice to the triple bond, the solvent was changed from ethanol to methanol, while following the same reaction procedure and sampling the reaction mixture at same intervals. The peaks at RT 5.99 min., \( m/z \) 233 and RT 7.38 min., \( m/z \) 234 amu were replaced by RT 6.21 min., \( m/z \) 218 and RT 7.45 min., \( m/z \) 219 amu, respectively, while peaks at \( m/z \) 278 and 279 amu were replaced by \( m/z \) 263 and 264 amu, respectively. All the new masses are 15 amu less, which corresponds to the mass difference expected when changing from ethanol to methanol. This strongly supported the mechanism proposed in Scheme 2.16. While the structural assignments are tentative, we could not think of any other likely mechanism.

These results suggest that the ethynyl group is reactive towards nucleophiles, in our case ethoxide or methoxide, in a Michael addition-type reaction. Upon heating the acetal group cleaves off in a mechanism unknown to us but that most likely involves breaking of the required C-C bond through anomeric assistance of an acetal group, as illustrated in Scheme 2.17. The leaving group is then the tautomer of the 6-methylpterin. We found little precedent for this mechanism. In a possible related report, a methoxide nucleophile adds to a 6-ethynylpurine.\(^{38}\) The intermediate acetal, which was not isolated, then cleaves to give a 6-methylpurine.

It is also possible that, given the rich radical chemistry that pterin systems are known to undergo, a single electron mechanism can also account for our observations. Future work might involve an exploration of the mechanism by which this process occurs and an unequivocal synthesis of 6-methylpterin in order to confirm the structure of the final hydrolysis product.

![Scheme 2.17](image)

**Scheme 2.17**

To prove that we did not have 3-N-(methyl)pterin as product, we obtained a sample of \([2-(N,N\text{-dimethylaminomethyleneamino})]-3-N-(methyl)pterin\) 43 and subjecting it to the alkaline hydrolysis conditions, mentioned above, gave \( N\text{-}(methyl)pterin\) 44 which have \( m/z \) 178 amu \([M+H]^+\), but different Uv-vis spectra to 42 (Scheme 2.18 and Figure 2.11).
After obtaining an insight into the alkaline hydrolysis of the ethynylpteridines, we attempted to hydrolyze the ethynylpteridines 30 and 31 to the corresponding pterin using 6M HCl. The use of 6M HCl to hydrolyze 2,4-diaminopteridines to their pterins derivatives has been previously reported to proceed smoothly under reflux.\textsuperscript{38} Treatment of the TMS-ethylpteridine 30 and 31 with 6M HCl/EtOH at room temperature for 18 hours and analysis of the solution using HPLC-MS suggested that one principal species was present. This species had an RT 4.60 min, similar to
diamino-6-ethylpteridine 36 detected during the attempted alkaline hydrolysis, and a molecular ion at m/z 187 amu. No further changes were observed when the reaction was analyzed after 24 hours.

When repeating the above reaction, but increasing the temperature to reflux and monitoring the progression after six hours on HPLC-MS, a peak eluting with RT 4.55 min and m/z 223 amu was obtained along with a peak at m/z 187 amu, previously assigned to be 6-ethylpteridine 36. We tentatively assigned the peak at m/z 223 amu to be 6-(1-chloroethyl)-2,4-(diamino)pteridine 45, corresponding to the addition of HCl across the triple bond to give presumably, the Markovnikov product (Figure 2.12). The isotopic distribution of the molecular ion peak was consistent with the addition of one chlorine atom which has two isotopes, $^{35}$Cl (76%) and $^{37}$Cl (24%). In addition to m/z 223 amu, a peak at m/z 225 amu, with 24% relative intensity to the peak at 223, was observed confirming the presence of chlorine atom in the molecule.

![Figure 2.12](image)

We then replaced HCl with a strong organic acid, CF$_3$COOH, hoping to avoid the side reaction of HCl adding across the triple bond. Treatment of the ethynylpteridines 30 and 31 with CF$_3$COOH and refluxing the reaction for 18 hours while monitoring the reaction by HPLC-MS, only afforded the diamino-6-ethylpteridine 36. The deprotection was observed in the first
hour, however, full deprotection was only achieved after 18 hours, indicating the relative slowness of the reaction.

When using a strong non-chlorinated inorganic acid, 2 M sulfuric acid (H$_2$SO$_4$), on a small scale we did manage to get the desired 6-(ethynyl)pterin 36b as observed from HPLC-MS analysis where peaks at RT 5.99 minutes and m/z 188 were obtained. However, when scaling up the reaction, the desired product could not be isolated in a pure form. Attempts to precipitate the product out of solution gave a dark solid which appeared to contain mainly the desired pterin 36b, but substantial amounts of impurities were noted on the HPLC chromatogram.

In conclusion, the selective hydrolysis to give the natural pterin substituents occurs cleanly when the phenylacetylene Sonogashira coupling products 28 and 29 were exposed to alkaline hydrolysis conditions. When the TMS-ethynylpteridines 30 and 31, and the methoxymethyl-ethynylpteridines 33 and 34, were subjected to the alkaline hydrolysis conditions, side reactions occurred. The resultant by-products appear to result from Michael-type addition of the alkoxide solvent and ultimate C-C bond cleavage to give 6-methylpterin. With these substrates, selective hydrolysis to the corresponding pterin derivatives requires careful monitoring under alkaline conditions and acid hydrolysis employing a non-nucleophilic anion for which sulfuric acid appears appropriate.

2.3 Total Synthesis of Sepiapterin-C

In order to find useful applications for our Sonogashira coupling methodology, we looked for a natural product which might constitute a target. Sepiapterin-C 46, a natural pterin, has been isolated from the eye pigments of the mutant wild type Drosophila melanogaster as a minor component along with sepiapterin and isosepiapterin.$^{39}$ The three pterin molecules were isolated as yellow compounds and are believed to be responsible for the yellow eye color pigments, no other information is known about the biological role of sepiapterin-C. The total synthesis of sepiapterin-C has been reported by Murata et al.$^{40}$ where a classical ring synthesis approach was employed (Scheme 2.19).
Scheme 2.19

Our initial proposed synthesis of sepiapterin-C was to start with the mixed protected 6-(ethynyl)pteridine 30 and 31, deprotection and hydrolysis with NaOH, addition of water across the triple bond, which we intended be facilitated by silver oxide (HgO), to give 6-methylketone-pteridine, and a dissolving metal/amalgam reduction of the 7-D imine employing the conditions described by Murata. However, this route was abandoned due to the side reactions associated with the deprotection/hydrolysis step, described in scheme 2.16.

Having shown that sulfuric acid can be used in the hydrolysis of protected 6-ethynylpteridines 30 and 31 to 6-ethynylpterin, we turned to hydrolysis of ethyne to a methyl ketone. Hydrolysis of a heterocyclic ethyne to a methyl ketone has previously been achieved using the combination CF₃COOH and HgO. We investigated conditions where we might achieve the hydrolysis of the protected ethynylpteridines 30 and 31 to give the methylketone derivative. To this end, the readily available model system, consisting of the quinoxaline 9 was initially employed.

The addition of water across the triple bond was first investigated on the 2-[2-(trimethylsilyl)ethynyl]quinoxaline 9 which was treated with CF₃COOH, HgO and water, and stirred at 50 °C for 18 hours to give 1-(quinoxalin-2-yl)ethan-1-one 47 in 55% yield (Scheme 2.20). The melting point of the product was found to be 78 °C, in agreement with the literature value of 77.5 °C. The IR spectra gave a sharp peak at 1688 cm⁻¹ which is consistent with a C=O stretch. The NMR and mass spectra were in good agreement with those reported previously.
We then attempted to combine the deprotection/hydrolysis step with H$_2$SO$_4$, coupling the reaction with the addition of water on the triple bond by the addition of HgO. We then treated the mixed ethynylpteridines 30 and 31 with 2M H$_2$SO$_4$ and HgO, warming the reaction to 80°C for 18 hours while monitoring by HPLC-MS. Two principal peaks at RT 4.69 min, m/z 205 amu and RT 6.15 min, m/z 206 amu, were obtained with the latter peak more dominant. We tentatively assigned the peak at RT 6.15 min, m/z 206 amu to be the desired 6-acetyl-2-amino-3,4-dihydropteridin-4-one 48 and the peak at RT 4.69 min, m/z 205 amu to be the 6-acetyl-pteridine 48a. The reaction was allowed to run for another two hours at 80°C, after which HPLC-MS analysis of the crude showed only one peak at RT 6.15 min, m/z 206 to be present. (Scheme 2.21).

Neutralization of the solution with 1M NaOH resulted in precipitates forming, and after collection by centrifugation, the product was obtained as yellow solid in 66% yield. The $^1$H NMR spectrum gave a downfield singlet at δ 9.13 ppm, which we tentatively assigned to be 7(H), and an upfield singlet at δ 2.46 ppm tentatively assigned to be the methyl singlet.

The reduction of the 7,8 C=N to form the desired natural pterin has been previously reported by Murata to proceed smoothly by a dissolving aluminium (Al) reduction in aqueous ammonia.\textsuperscript{40} We subjected our 6-acetyl-pterin 48 to the conditions of dissolving Al in NH$_3$, warming the reaction to 55°C for 16 hours, followed by purification on a florisil column to give a yellow solid. The $^1$H NMR, mass and UV spectra of the compound was in agreement with data previously reported by Murata for Sepiapterin-C 46 (Scheme 2.21 and Figure 2.13). The product was isolated in 57% yield, with an overall yield of 24% when starting from benzenesulfonate-
pteridine 27 over three steps.

Scheme 2.21

Figure 2.13 HPLC-UV and TIC chromatogram of Sepiapterin-C, with extracted UV-vis and mass spectra.
The synthesis of Sepiapterin-C 46 can be achieved in a one-pot reaction starting from the mixture of ethynyl-pteridines 30 and 31 where the crude product of 48 was treated, without purification, with Al/aq. NH₃ to give the desired product in overall yield of 14% over two steps when starting from benzenesulfonate-pteridine 27. The characterization spectra of the product were in agreement with those found earlier and reported in the literature.39,40

In conclusion, we have successfully achieved the total synthesis of Sepiapterin-C from a precursor obtained from a Sonogashira coupling reaction. These results further demonstrate the wide range of transformations that can be accomplished on Sonogashira coupled products.

2.4 Experimental

2.4.1 General

a) Purification of reagents and solvents

- THF was distilled from sodium/benzophenone
- Dichloromethane and triethylamine were distilled from calcium hydride
- DMF and ethanol were stored over molecular sieves (3Å)

b) Spectroscopic and physical data

- Melting points were obtained using a Stuart melting point apparatus, and are uncorrected.
- ¹H and ¹³C spectra were reported on Bruker Avance-300 spectrometer at 300 MHz. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane as internal standard (in the case of the ¹H NMR at 7.27 ppm for CDCl₃ and 2.45 ppm for DMSO, and in the case of ¹³C NMR spectra at 77 ppm). The ¹H NMR chemical shifts are reported: value (number of hydrogens, description of signal, coupling constant(s) in hertz (Hz) where applicable). Abbreviations used: s = singlet, d = doublet, t = triplet, q = quartet and m = multiplet.
Infrared spectra were obtained on a Bruker Tensor 27 spectrometer. Samples were placed on a diamond thin film. The absorptions are reported on the wave number (cm\(^{-1}\)) scale, in the range 400 – 4000 cm\(^{-1}\).

High resolution mass spectra were recorded on a Thermo-Finigan DFS instrument. Data are quoted : m/z value (relative abundance).

Low resolution mass spectra were recorded on Thermo-Finigan LXQ instrument using either atmospheric pressure chemical ionization (APCI) or electro spray ionization (ESI) techniques.

HPLC was run on a Thermo-Finigan instrument using a C18 (2) Phenomenex column with 150 x 4.60 mm dimensions. The samples were eluted with a water-methanol solvent system over 10 minutes using a gradient program: starting with 95% water for 2 minutes, changing to 100% methanol over the next 4 minutes and running for 4 minutes with 100% methanol. Detection of eluting components was achieved by a photodiode array and APCI-MS detector.

c) Nomenclature and numbering of compounds

The compounds prepared during the course of this project are named in the following experimental sections according to systematic nomenclature. However, the numbering system used to illustrate the diagrams of these compounds is one adopted for convenience and is not meant to reflect systematic numbering of these compounds.

2.4.2 Synthetic Procedures on quinoxaline

Synthesis of 2-quinoxalinone (6)

\(o\)-Phenylenediamine (10.01g, 0.092 mol) was dissolved in acetic acid (10 ml) and methanol (10 ml), and the solution cooled to -15°C with stirring. Glyoxylic acid (8.50g, 0.092 mol) in water (20 ml) was added drop-wise over 30 minutes to the solution, maintaining the temperature at -15°C. The final solution was allowed to warm up to 0 °C over 90 minutes, filtered, the filtrate washed with water (15 ml) then methanol (15 ml), and air dried to give a dark grey solid.
Recrystallization from DMF gave the 2-quinazolinone as a tan solid (9.40 g, 70%); m.p 267°C (Lit. 266-267°C)\(^{40}\), \(\delta_H\) (300 MHz, DMSO) 7.25–7.34 (2 H, m), 7.54 (1 H, m), 7.77 (1 H, m) and 8.16 (1 H, s). Spectroscopic data agree with those reported elsewhere.\(^{23,40}\)

**Synthesis of 2-benzenesulfonyloxyquinoxaline (7)**

In a round bottom flask, quinoxalinone 6 (1.25 g, 8.5 mmol), DMAP (0.104 g, 0.85 mmol) and benzenesulfonyl chloride (2.18 ml, 17 mmol) were dissolved in dry DCM (20 ml), cooled to 0 °C and stirred for 5 minutes. Et\(_3\)N (3 ml, 22 mmol) was added drop-wise over 5 minutes, the solution allowed to stir at room temperature for 1 hour, reaction quenched with sat. NaHCO\(_3\) (20 ml), the two layers separated and the aqueous layer washed with DCM (2 x 15 ml). The combined organic layers were dried over Na\(_2\)SO\(_4\), concentrated and purified on flash silica eluting with DCM to give 2-benzenesulfonyloxyquinoxaline as a brown solid (2.3g, 95%); m.p. 91°C; R\(_f\) 0.4; \(\delta_H\) (CDCl\(_3\), 300 MHz) 7.58 - 7.63 (2H, m), 7.69 – 7.77 (3H, m), 7.87 – 7.90 (1H, m), 8.09 – 8.17 (3H, m) and 8.67 ppm (1H, s); \(\delta_C\) (CDCl\(_3\), 100 MHz) 128.5, 128.8, 129.0, 129.2, 129.5, 131.2, 134.7, 136.4, 139.1, 139.7, 141.3 and 150.9 ppm, we expected 14C environments but only 12 were observed on the spectrum. It is likely that the symmetry on the phenyl ring could be responsible for few C signals; \(m/z\) (APCI +ve) 287 amu [M+H\(^+\), 100]

**Synthesis of 2-mesyloxyquinoxaline (15)\(^{26}\)**

In a round bottom flask, quinoxalinone (1.25 g, 8.5 mmol), DMAP (0.104 g, 0.85 mmol) and methanesulfonyl chloride (1.32 ml, 17 mmol) were dissolved in dry DCM (20 ml), cooled to 0 °C and stirred for 5 minutes. Et\(_3\)N (3 ml, 22 mmol) was added drop-wise over 5 minutes, the solution allowed to stir at room temperature for 1 hour, and the reaction quenched with sat. NaHCO\(_3\) (20 ml), the two layers separated and the aqueous layer washed with DCM (2 x 15 ml). The combined organic layers were dried over Na\(_2\)SO\(_4\), concentrated and purified on flash silica eluting with DCM to give 2-mesyloxyquinoxaline as a grey solid (1.581 g, 82%); m.p. 91°C (Lit. 92-93°C)\(^{26}\); \(\delta_H\) (300 MHz, CDCl\(_3\)) 3.49 (3H, s), 7.78 – 7.87 (2H, m), 7.91 – 8.08 (1H, m), 8.11 – 8.22 (1H, m) and 8.74 ppm (1H, s); \(\delta_C\) (CDCl\(_3\), 100 MHz) 41.3, 128.4, 129.4, 130.0,
131.4, 139.3, 139.5, 141.5 and 151.2 ppm. Spectroscopic data agree with those reported elsewhere.\textsuperscript{26}

**Synthesis of Pyridin-2-yl benzenesulfonate (17)\textsuperscript{28}**

In a round bottom flask, pyridin-2-ol (2 g, 21 mmol), DMAP (0.132 g, 2.1 mmol) and benzenesulfonyl chloride (3.23 ml, 25.2 mmol) were dissolved in dry DCM (20 ml), cooled to 0 °C and stirred for 5 minutes. Et\textsubscript{3}N (3 ml, 25.2 mmol) was added drop-wise over 5 minutes, the solution allowed to stir at room temperature for 1 hour, and the reaction quenched with sat. NaHCO\textsubscript{3} (20 ml), the two layers separated and the aqueous layer washed with DCM (2 x 15 ml). The combined organic layers were dried over Na\textsubscript{2}SO\textsubscript{4}, concentrated and purified on flash silica eluting with 20% EtOAc/ hexane to give pyridin-2-yl benzenesulfonate as a clear oil (3.313 g, 68%); R\textsubscript{f} 0.31; \(\delta\)\textsubscript{H} (CDCl\textsubscript{3}, 300 MHz) 7.11 – 7.14 (1H, dd, \(J = 7.8, 0.8\) Hz), 7.20 – 7.27 (1H, m), 7.53 – 7.58 (2H, m), 7.65 – 7.70 (1H, m), 7.75 – 7.81 (1H, dt, \(J = 0.8, 8\) Hz), 8.02 (2H, m) and 8.25 ppm (1H, dd, 7.8, 0.8 Hz); m/z (APCI) 236.1 [M+H\textsuperscript{+}, 100]. Spectroscopic data agree with those reported elsewhere.\textsuperscript{28}

**General procedure for the Sonogashira coupling on 2-benzenesulfonyloxyquinoxaline (method 1)**

In a 50 ml 3 neck round bottom flask, equipped with a stirrer bar and under Ar, 2-benzenesulfonyloxyquinoxaline (0.301 g, 1.05 mmol), PdCl\textsubscript{2}(PPh\textsubscript{3})\textsubscript{2} (38. 0 mg, 0.052 mmol, 5 mol%), Et\textsubscript{3}N (0.2 ml, 2.1 mmol, 2 eq.) and the ethynyl substrate (1.26 mmol, 1.2 eq.) were dissolved in dry THF (10 ml). The solution was heated at reflux for 90 minutes, partitioned between EtOAc/water (20 ml, 3:2), filtered through celite, the layers separated and aqueous layer washed with EtOAc (15 ml). The combined organic layers were dried over Na\textsubscript{2}SO\textsubscript{4}, concentrated and purified on flash silica.

**Synthesis of 2(2-phenylethynyl)quinoxaline (8)\textsuperscript{24}**
According to method 1, 2-benzenesulfonylquinoxaline (0.30 g) was treated with phenylacetylene (154 µl, 1.26 mmol, 1.2 eq.). Purification on flash silica, eluting with DCM gave 2-(2-phenylethynyl)quinoxaline as tan crystals (0.234 g, 95%); m.p. 64–65°C (Lit. 65–66.5°C)\(^{24}\); R\(_f\) 0.35; \(\delta\)\(_H\) (CDCl\(_3\), 300 MHz) 7.43 – 7.44 (3H, m), 7.68 – 7.71 (2H, m), 7.77 – 7.81 (2H, m), 8.10 – 8.13 (2H, m) and 8.99 ppm (1H, s); \(\delta\)\(_C\) (CDCl\(_3\), 100 MHz) 86.9, 93.7, 121.4, 128.6, 129.2, 129.3, 129.8, 130.4, 130.7, 132.4, 139.6, 140.9, 142.2, and 147.4 ppm; m/z (EI) 230.03 (M\(^+\), 100%), 204.0 (40), 202.99 (100), 201.98 (30), 175.94 (15), 126.90 (42), 114.89 (35) and 102.85 (32) (HREI found: 230.08445. C\(_{16}\)H\(_{10}\)N\(_2\) requires 230.08440). Physical and spectroscopic data agree with those reported elsewhere.\(^{24}\)

**Synthesis of 2-[2-(trimethylsilyl)ethynyl]quinoxaline (9)\(^{24}\)**

According to method 1, 2-benzenesulfonylquinoxaline (0.301 g) was treated with ethynyltrimethylsilane (178 µl, 1.26 mmol, 1.2 eq.). Purification on flash silica, eluting with DCM gave 2-[2-(trimethylsilyl)ethynyl]quinoxaline as a brown oil (177 mg, 74%); R\(_f\) 0.45; \(\delta\)\(_H\) CDCl\(_3\)) 0.34 (9H, s), 7.74 – 7.82 (2H, m), 8.07 – 8.11 (2H, m) and 8.90 ppm (1H, s); \(\delta\)\(_C\) (CDCl\(_3\), 100 MHz) 0.01, 100.8, 102.0, 129.6, 129.7, 130.9, 131.1, 139.6, 141.4, 142.4 and 147.8 ppm; m/z (EI) 226.14 (M\(^+\), 23%), 211.09 (100) (HREI found: 226.09185. C\(_{13}\)H\(_{14}\)N\(_2\)Si requires 226.09262). Spectroscopic data agree with those reported elsewhere.\(^{24}\)

**Synthesis of 3-(quinoxalin-2-yl)prop-2-yl-1-ol (10)\(^{24}\)**

According to method 1, 2-benzenesulfonylquinoxaline (0.300 g) was treated with propargyl alcohol (73.3 µl, 1.26 mmol, 1.2 eq.). Crystallization of the crude product from acetone gave 3-(quinoxalin-2-yl)prop-2-yl-1-ol as a light brown solid (131 mg, 70%); M.p. 139°C (Lit. 141-142°C); \(v\)\(_{\text{max}}\)/ cm\(^{-1}\) 3271 (O-H) \(\delta\)\(_H\) (300 MHz, CDCl\(_3\)) 2.96 (1H, s), 4.64 (2H, s), 7.75 – 7.83 (2H, m), 8.06 – 8.11 (2H, m) and 8.90 ppm (1H, s); \(\delta\)\(_C\) (100 MHz, CDCl\(_3\)) 51.3, 82.9, 92.2, 128.6, 129.1, 130.8, 132.2, 138.8, 141.1, 142.0 and 146.9 ppm; m/z (EI) 184.05 (M\(^+\), 94%), 155.01 (100) and 128.96 (32) (HREI found: 184.06313. C\(_{11}\)H\(_{8}\)N\(_2\)O requires 184.06366). Spectroscopic data agree with those reported elsewhere.\(^{24}\)
Synthesis of 2-(3-methoxyprop-1-yn-1-yl)quinoxaline (11)

According to method 1, 2-benzenesulfonylquinoxaline (0.301 g) was treated with 3-methoxyprop-1-yn (88.2 mg, 1.26 mmol, 1.2 eq.). Purification on flash silica, eluting with DCM gave 2-(3-methoxyprop-1-yn-1-yl)quinoxaline as a light brown solid (180 mg, 95%); m.p. 61°C; R_f 0.24; δ_H (300 MHz, CDCl_3) 3.45 (3H, s), 4.37 (2H, s), 7.70 – 7.80 (2H, m), 7.99- 8.04 (2H, m) and 8.83 ppm (1H, s); δ_C (100 MHz, CDCl_3) 58.2, 60.2, 83.9, 89.7, 129.3, 130.6, 130.7, 130.8, 138.9, 141.1, 142.1 and 147.1 ppm; m/z (EI) 198.21 (M^+ , 25%), 183.18 (30), 168.19 (100), 155.19 (30) and 129.17 (32) (HREI found: 198.07978. C_{12}H_{10}N_2O requires 198.07930).

Synthesis of 5-(quinoxalin-2-yl)pent-4-yn-1-ol (12)

According to method 1, 2-benzenesulfonylquinoxaline was treated with 4-pentyn-1-ol (117 µl, 1.26 mmol, 1.2 eq.). Purification on flash silica, eluting with 5% MeOH/DCM gave 5-(quinoxalin-2-yl)pent-4-yn-1-ol as a brown oil (175 mg, 83%); R_f 0.23; ν_\text{max} / cm^{-1} 3332 (O-H); δ_H (300 MHz, CDCl_3) 1.97 (2H, quintet, J = 6.9 Hz), 2.70 (2H, t, J = 6.9 Hz), 3.89 (2H, t, J = 6 Hz), 7.75 – 7.78 (2H, m), 8.04 – 8.09 (2H, m) and 8.84 ppm (1H, s); δ_C (100 MHz, CDCl_3) 16.1, 30.9, 61.4, 79.2, 95.2, 129.1, 129.2, 130.2, 130.6, 139.8, 140.8, 142.0 and 147.3; m/z (EI) 212 (M^+, 100), 181 (70), 171 (25) and 147 (70) (HRESI found 213.10281, C_{13}H_{13}N_2O requires 212.10224).

Synthesis of 6-(quinoxalin-2-yl)hex-5-yn-1-ol (13)^{25}

According to method 1, 2-benzenesulfonylquinoxaline (0.302 g) was treated with 5-hexyn-1-ol (138 µl, 1.26 mmol, 1.2 eq.). Purification on flash silica, eluting with 5% MeOH/DCM gave 6-(quinoxalin-2-yl)hex-5-yn-1-ol as a brown solid (176 mg, 79%); m.p. 65°C; R_f 0.25; ν_\text{max} / cm^{-1} 3335 (O-H); δ_H (CDCl_3, 300 MHz) 1.80 (4H, quintet, J = 2.7Hz), 2.59 (2H, t, J = 6.3 Hz), 3.75 (2H, t, 6.3 J = 6.3 Hz), 7.71 – 7.78 (2H, m), 8.02 – 8.07 (2H, m) and 8.81 ppm (1H, s); δ_C (CDCl_3, 100 MHz) 19.4, 24.5, 31.7, 62.0, 79.1, 95.9, 128.9, 129.0, 130.1, 130.5, 138.9, 140.7,
141.9 and 147.2 ppm; \( m/z \) (EI) 226 (M\(^+\), 70%), 221 (35), 197 (65), 184 (90), 181 (100), 169 (70) and 144 (50) (HREI found: 226.11028. \( C_{14}H_{14}N_2O \) requires 226.11060). Spectroscopic data agree with those reported elsewhere.\(^{25}\)

**Synthesis of 4-[2-(quinoxalin-2-yl)ethyl]aniline (14)\(^{24}\)**

According to method 1, 2-benzenesulfonylquinoxaline (0.299 g) was treated with 4-ethynylaniline (143 \( \mu l \), 1.26 mmol, 1.2 eq.). Purification on flash silica, eluting with DCM gave 4-[2-(quinoxalin-2-yl)ethyl]aniline as a yellow solid (190 mg, 74%); m.p. 179°C (Lit. 180°C); \( R_f \) 0.20; \( \delta_H \) (DMSO, 300 MHz) 5.63 (2H, broad s, -DNH\( _2 \)), 6.52 (2H, t, \( J = 7.5 \) Hz), 6.72 (2H, d, \( J = 8.1 \) Hz), 7.11 (2H, t, \( J = 7.2 \) Hz), 7.23 (2H, d, \( J = 7.5 \) Hz) and 7.8 ppm (1H, s); \( \delta_C \) (DMSO, 100 MHz) 78.7, 80.5, 114.1, 114.3, 115.9, 116.8, 125.6, 128.9, 130.5, 132.1, 132.5, 134.7, 142.8, 150.9, 151.4 and 188.5 ppm; \( m/z \) (APCI) 246 \[ M+H^+ \], 100]. Physical and spectroscopic data agree with those reported elsewhere.\(^{24}\)

**Synthesis of 2-(2-phenylethynyl)pyridine (19)\(^{29}\)**

Pyridin-2-yl benzenesulfonate (0.3 g, 1.27 mmol), PdCl\(_2\)(PPh\(_3\))\(_2\) (46.5 mg, 0.064 mmol, 5 mol%), Et\(_3\)N (0.24 ml, 2.54 mmol, 2 eq) and the phenylacetylene (186 \( \mu l \), 1.52 mmol, 1.2 eq.) were dissolved in dry DMF (10 ml). The solution was refluxed under argon for 4 hours, filtered through celite, concentrated and purified on flash silica eluting with 20% EtOAc/hexane to give 2-(2-phenylethynyl)pyridine as a yellow oil (55 mg, 25%); \( R_f \) 0.35; \( \delta_H \) (CDCl\(_3\), 300 MHz) 7.21 – 7.25 (1H, m), 7.36 – 7.38 (3H, m), 7.52 – 7.54 (1H, dd, \( J = 7.8 \), 0.8 Hz), 7.60 – 7.62 (2H, m), 7.68 – 7.71 (1H, dt, \( J = 7.7 \), 1.8 Hz) and 8.61 - 8.63 ppm (1H, d, \( J = 4.8 \) Hz); \( \delta_C \) (CDCl\(_3\), 100 MHz) 88.6, 89.3, 122.8, 127.2, 128.6, 129.0, 129.1, 132.1, 136.2, 143.4 and 150.0 ppm; \( m/z \) (EI) 179.05 (M\(^+\), 100), 151.0 (10) (HREI found: 179.07327. \( C_{13}H_{9}N \) requires 179.07350). Spectroscopic data agree with those reported elsewhere.\(^{29}\)

**Synthesis of 1-(quinoxalin-2-yl)ethan-1-one (47)\(^{39}\)**
2-[2-(trimethylsilyl)ethynyl]quinoxaline (205 mg, 0.895 mmol) in CF₃COOH (10 ml) was treated with HgO (64 mg, 0.295 mmol), the solution warmed to 50 °C and stirred for 18 hours, concentrated and diluted with water (10 ml). The solution was neutralized with 1M NH₄OH, extracted with chloroform (3 x 15 ml), the combined organic layers dried over Na₂SO₄, concentrated and purified on flash silica eluting with 5% EtOAc/hexane to give I-(quinoxalin-2-yl)ethan-1-one as a brown solid (87 mg, 55%); m.p. 78 °C (Lit. 77.5 °C); Rf 0.18; v max/cm⁻¹ 1688 (C=O); δ H (D₂O, 300 MHz) 2.87 (3H, s), 7.84 – 7.93 (2H, m), 8.15 – 8.22 (2H, dd, 7.8, 0.8 Hz) and 9.51 ppm (1H, s); δ C (CDCl₃, 100 MHz) 25.6, 129.4, 130.5, 130.7, 132.2, 141.1, 143.1, 143.9, 146.6 and 199.8 ppm; m/z (EI) 172.02 (M⁺, 95%), 144.0 (46), 129.97 (100), 102.91 (45) (HREI found: 172.06312. C₁₀H₈N₂O requires 172.06366). Physical and spectroscopic data agree with those reported elsewhere.³⁹

2.4.3 Synthetic procedures on pteridines

Synthesis of tetramethylformamidine chloride (TMFAC)

To a 500 ml pre-weighed round bottom flask, connected to a condenser and a bubbler, was added DMF (100 ml, 1.292 mol) and dimethylcarbamoyl chloride (48 ml, 0.522 mol). The flask was heated in an oil bath maintained at 120 °C and stirred for 44 hours. The initial rate of effervescence produced ~1-2 bubbles per second. This rate slowly dropped as the reaction proceeded and no gas evolution was detectable after ~40 hours. The condenser and bubbler were replaced by a short distillation head, attached to a water condenser and receiver flask. The apparatus was slowly evacuated (to high vacuum) at such a rate as to avoid bumping and the remaining DMF was distilled. After condensation of DMF was no longer visible in the water condenser, heating at 120 °C was maintained for a further two hours, to give the tetramethylformamidine chloride (71.25 g, 91%) as a tan crystalline solid; δ H (D₂O, 300 MHz) 3.24 (6H, s), 3.36 (6H, s) and 7.65 ppm (1H, s)

Synthesis of the Bredereck’s reagent [t-BuO(Me₂N)₂CH] (24)
In a 500 ml 2-neck round bottom flask, equipped with a stirrer bar and under positive pressure of Ar, TMFAC (40 g, 0.3 mol) and KtOBu (36 g, 0.32 mol) were suspended in dry THF (400 ml), stirred at room temperature for 4 days. The solution was filtered, cautiously concentrated in vacuo and purified by distillation (water pump) to give Bredereck’s reagent (41.9 g, 79%) as a clear oil; b.p. 52°C at 20 mmHg; δH (CD6, 300 MHz) 1.26 (9H, s), 2.41 (12H, s) and 4.15 ppm (1H, s); δC (CD6, 100 MHz) 29.1, 36.8, 72.0 and 100.8 ppm.

Synthesis of 2,4-diaminopteridin-6-one (23)34

In a 1L flask fitted with a stirrer bar, was added 2M H2SO4 (550 ml) and the flask warmed to 80°C. To the flask was added tetraaminopyrimidine (21.17 g, 0.136 mol) and stirring continued for 10 minutes. Glyoxylic acid (16.35 g, 0.272 mol, 2 eq.) was added, the solution stirred for a further 15 min then allowed to cool to room temperature and left to stir for 2 hours. The resultant suspension was filtered, and the mustard solid washed with water (3 x 50 ml). The solid was suspended in water (150 ml) and neutralized with sat. NaHCO3 (cautiously added in 10 ml portions until effervescence stopped, ca. ~150 ml was required), the solid filtered and washed with water (5 x 60 ml) then MeOH (3 x 50 ml), air dried, and then dried under reduced pressure over P2O5 to give 2,4-diaminopteridin-6-one (17.952 g, 74%) as a mustard solid. m.p. (dec.) >300°C; m/z (ESI) 179 (M+H+, 100); λmax (1M NaOH) 410 and 265 nm. Physical and spectroscopic data agree with those reported elsewhere.34

Synthesis of 2,4-Di(N,N-dimethylaminomethyleneamino)pteridine-6-one (25)31

2,4-Diaminopteridin-6-one (4.02 g, 0.0225 mol) and Bredereck’s reagent (14 ml, 0.0675 mol, 3 eq.) were stirred together in DMF (50 ml), under an Ar atmosphere and warmed to 65°C for 3 hours, and allowed to cool to room temperature. The yellow precipitates were filtered, washed with little cold DMF then Et2O, air dried and finally dried under reduced pressure over P2O5 to give 2,4-di(N,N-dimethylaminomethyleneamino)pteridine-6-one (6.20 g, 91%); m.p. (dec.) 270°C; δH (300 MHz, CDCl3) 3.07 (3H, s), 3.08 (3H, s), 3.10 (3H, s), 3.15 (3H, s), 8.15 (1H, s),
8.48 (1H, s) and 8.76 ppm (1H, s); m/z (EI) 288 (M⁺, 100), 273 (45), 244 (19) and 232 (45).

Spectroscopic data agree with those reported elsewhere.²¹

Synthesis of 2,4-Di(N,N-dimethylaminomethyleneamino)-6-mesyloxopteridine (26)

2,4-Di(N,N-dimethylaminomethyleneamino)pteridine-6-one (2.0 g, 7 mmol), DMAP (0.09 g, 0.7 mmol) and mesyl chloride (1.08 ml, 14 mmol, 2eq.) were dissolved in DCM (25 ml) and cooled to 0°C. Et₃N (2.5 ml, 18 mmol, 2.5 eq.) was added drop-wise into the mixture, and the final solution was allowed to warm to room temperature and stirred for 1 hour. The reaction was quenched with sat. NaHCO₃ (10 ml), the organic layer separated and the aqueous layer washed with DCM (2 x 15 ml). The combined organic layers were dried over MgSO₄, and the solvent evaporated to dryness to give a dark yellow solid which was recrystallized from EtOH to give 2,4-Di(N,N-dimethylaminomethyleneamino)-6-mesyloxopteridine (1.65 g, 62%) as a yellow solid; m.p. (dec) 270°C; δH (CDCl₃, 300 MHz) 3.21 (3H, s), 3.24 (6H, overlapping s), 3.27 (3H, s), 3.69 (3H, s), 8.76 (1H, s), 9.01 (1H, s) and 9.04 ppm (1H, s); m/z (FAB) 367 [M + H⁺, 100%], 289.1 (15) and 136 (30). (HREI found: 366.1236, C₁₃H₁₈N₈O₃S requires 366.1223).

Synthesis of 2,4-Di(N,N-dimethylaminomethyleneamino)-6-benzenesulfonyloxopteridine (27)

2,4-Di(N,N-dimethylaminomethyleneamino)pteridine-6-one (4.160 g, 14.5 mmol), DMAP (0.2 g, 1.45 mmol) and benzenesulfonyl chloride (3.8 ml, 29 mmol, 2eq.) were dissolved in DCM (40 ml) and cooled to 0°C. Et₃N (3.7 ml, 29 mmol, 2 eq.) was added drop-wise into the mixture, and the final solution was allowed to warm to room temperature and stirred for 1 hour. The reaction was quenched with sat. NaHCO₃ (15 ml), the organic layer separated and the aqueous layer washed with DCM (2 x 20 ml). The combined organic layers were dried over MgSO₄, and the solvent evaporated to dryness to give a dark yellow solid which was recrystallized from EtOH to give 2,4-Di(N,N-dimethylaminomethyleneamino)-6-benzenesulfonyloxopteridine (4.62 g, 75%) as a yellow solid; m.p. (dec) 203 – 205°C; δH (CDCl₃, 300 MHz) 3.19 (3H, s), 3.24 (6H, overlapping s), 3.27 (3H, s), 3.69 (3H, s), 7.53 – 7.79 (3H, m), 8.25 – 8.28 (2H, dd, J = 7.8, 0.8 Hz), 8.56 (1H, s) and
8.95 – 8.97 ppm (2H, overlapping singlet); δC (CDCl3, 100 MHz) 35.4, 41.4, 125.2, 129.0, 129.4, 134.4, 136.3, 144.4, 148.6, 155.6, 158.3, 159.8, 166.5 and 168.5 ppm, we expected 18C environments but only observed 14 on the spectrum, it is likely that the symmetry on the phenyl ring and the dimethylamine could be responsible for the few C signals observed; m/z (EI) 428 (M+, 21%), 413 (40), 288.2 (100), 273.1(45), 232 (40), 217 (80) and 162.9 (35) (HREI found: 428.13775. C18H20N8O3S requires 428.13791).

Synthesis of 4-amino-2-(N,N-dimethylaminomethyleneamino)-6-(2-phenylethynyl)pterin-4-yl (29)

In a 2-neck 50 ml round bottom flask, equipped with a magnetic stirrer bar and positive pressure of argon, 2,4-di(N,N-dimethylaminomethyleneamino)-6-benzenesulfonyloxopteridine (1.0 g, 2.34 mmol), PdCl2(PPh3)2 (80 mg, 0.11 mmol), phenylacetylene (350 µl, 2.808 mmol) and Et3N (0.5 ml, 4.68 mmol, 2eq.) were dissolved in DMF (15 ml), warmed to 80°C and stirred for 2 hours. The flask was cooled to room temperature, filtered through celite, concentrated in vacuo and purified on flash silica (eluting with 5% MeOH/DCM) to give two close running fractions (650 mg); m/z (APCI) 373 [M+H+, 60%], 318 [M-55, 70%]+; LCRT 7.01 and 7.05 minutes. Further purification of the close running fractions by flash chromatography on flash silica gave 4-Amino-2-(N,N-dimethylaminomethyleneamino)-6-(2-phenylethynyl)pterin-4-yl (550 mg, 74%) as a yellow solid; m.p. (dec) 201 – 203°C; Rf 0.25; δH (d6-DMSO, 300 MHz) 3.01 (3H, s), 3.12 (3H, s), 7.46 – 7.51 (3H, m), 7.58 – 7.69 (2H, m), 8.03 (2H, broad s), 8.76 (1H, s) and 9.06 ppm (1H, s); m/z (EI) 317 (M+, 30%), 302 (50) and 262 (100) (HREI found: 317.13849. C17H15N7 requires 317.13889).

Synthesis of 4-amino-2-(N,N-dimethylaminomethyleneamino)-6-[2-(trimethylsilyl)ethynyl]-pteridin-4-yl (31)

In a 2-neck 50 ml round bottom flask, equipped with a magnetic stirrer bar and positive pressure of argon, 2,4-di(N,N-dimethylaminomethyleneamino)-6-benzenesulfonyloxopteridine (1.0 g, 2.34 mmol), PdCl2(PPh3)2 (80 mg, 0.11 mmol), TMSCl (395 µl, 2.808 mmol) and Et3N (0.5 ml,
4.68 mmol, 2eq.) were dissolved in DMF (15 ml), warmed to 80°C and stirred for 2 hours. The flask was cooled to room temperature, filtered through celite, concentrated in vacuo and purified on flash silica (eluting with 5% MeOH/DCM) to give two close running fractions (650 mg) as dark yellow solid; m/z (APCI) 369 [M+H⁺, 45%], 314 [M-55]; LCRT 7.34 and 7.45 minutes. Further purification by flash chromatography on flash silica gave 4-amino-2-(N,N-dimethylaminomethyleneamino)-6-[2-(trimethylsilyl)ethyl]-pteridin-4-yl (460 mg, 63%) as a yellow solid; m.p. (dec) 253°C; Rf 0.29; δH (d6-DMF, 300 MHz) 0.29 (9H, s), 3.23 (3H, s), 3.29 (3H, s), 8.88 (1H, s) and 9.03 ppm (1H, s); m/z (EI) 313 (M⁺, 90%), 298 (30), 269 (70), 258 (94), 243 (100) and 201 (40) (HRESI Found: 313.14542, C14H19N7Si requires 313.14712).

Synthesis of 2,4-Di(N,N-dimethylaminomethyleneamino)-6-(3-methoxyprop-1-yn-1-yl)pteridine-4-yl (33)

In a 2-neck 50 ml round bottom flask, equipped with a magnetic stirrer bar and positive pressure of argon, 2,4-di(N,N-dimethylaminomethyleneamino)-6-benzenesulfonyloxopteridine (1.0 g, 2.34 mmol), PdCl2(PPh3)2 (80 mg, 0.11 mmol), propargyl alcohol (198 mg, 2.808 mmol) and Et3N (0.5 ml, 4.68 mmol, 2eq.) were dissolved in 15 ml DMF, warmed to 80°C and stirred for 2 hours. The flask was cooled to room temperature, filtered through celite, concentrated and purified on flash silica (eluting with 5% MeOH/DCM) to give 2,4-di(N,N-dimethylaminomethyleneamino)-6-(3-methoxyprop-1-yn-1-yl)pteridine-4-yl (500 mg, 63%) as a yellow solid; m.p. (dec) 196°C; Rf 0.20; δH (CDCl3, 300 MHz) 3.19– 3.22 (12H, overlapping s), 3.50 (3H, s), 4.40 (2H, s), 8.88 (1H, s) and 9.01– 9.03 ppm (2H, overlapping s); δC (CDCl3, 100 MHz) 35.4, 35.8, 41.4, 41.6, 58.0, 60.2, 84.2, 88.8, 123.5, 128.8, 135.1, 153.6, 158.5, 162.5, 167.1 and 168.6 ppm; m/z (APCI) 341.2 [M + H⁺, 100%]; (HRESI found: 341.18460, C16H21N7O requires 341.1833).

Synthesis of 2,4-diamino-6-(phenylethynyl)pteridine (35)17

4-Amino-2-(N,N-dimethylaminomethyleneamino)-6-(2-phenylethynyl)pterin-4-yl (150 mg, 0.473 mmol) was treated with 10 ml EtOH/1M NH3 (1:1) mixture, gently refluxed for 16 hours,
filtered, the solid washed sequentially with water (5 ml), EtOH (5 ml) and Et₂O (5 ml), and dried under Hi-vac. at 70°C to give 2,4-diamino-6-(phenylethynyl)pteridine (95 mg, 77%) as a yellow solid; m.p. (dec) >300°C; δ_H (d_6-DMSO, 300 MHz) 6.91 – 7.01 (2H, broad s), 7.46 – 7.48 (3H, m), 7.61 – 7.62 (2H, m), 7.84 – 7.87 (2H, broad s) and 8.86 ppm (1H, s); m/z (APCI) 263.1 [M + H⁺, 100](HRESI found: 263.10340, C_{14}H_{10}N_{6} requires 263.10397). Spectroscopic data agree with those reported elsewhere.¹⁷

### Synthesis of 2,4-diamino-(ethynyl)pteridine (36)

4-Amino-2-(N,N-dimethylaminomethyleneamino)-6-[2-(trimethylsilyl)ethynyl]-pteridin-4-yl (200 mg, 0.64 mmol) was suspended in 25% NH₃ solution (10 ml) and gently refluxed for 18 hours, filtered through celite, concentrated to a small volume (ca. ~3 ml), acidified with glacial AcOH (~1 ml) and stored at 2°C for 24 hours. The precipitates were collected by centrifugation, washed sequentially with water (5 ml), acetone (5 ml) and Et₂O (5 ml) to give 2,4-diamino-(ethynyl)pteridine (70 mg, 76%) as yellow solid; m.p. (dec) >300°C; ν_max/cm⁻¹ 3410 (-NH), 3310 (-NH₂), 3016, 2926; δ_H (d_6-DMSO, 300 MHz) 7.09 – 7.11 (2H, broad s), 7.95 – 8.17 (2H, broad s) and 9.16 ppm (1H, s); m/z (APCI) 186 [M + H⁺, 100]. Attempts to get high resolution mass were unsuccessful in both HREI and HRESI instruments.

### Synthesis of 2,4-Diamino-6-[(methoxymethyl)ethynyl]pteridine (37)¹⁷

2,4-Di(N,N-dimethylaminomethyleneamino)-6-(3-methoxyprop-1-yn-1-yl)pteridin-4-yl (130 mg, 0.381 mmol) was dissolved in 8 ml EtOH/1M NH₃ (1:1) mixture, gently refluxed for 8 hours, allowed to cool to room temperature, acidified with AcOH and stored at 2°C for 24 hours. The precipitates were filtered, washed sequentially with water (5 ml), EtOH (5 ml) and Et₂O (5 ml), and dried under High vac. at 60°C to give 2,4-Diamino-6-[(methoxymethyl)ethynyl]pteridine (65 mg, 74%) as a yellow solid; m.p. (dec) >300°C; δ_H (d_6-DMSO, 300 MHz) 3.35 (3H, s), 4.40 (2H, s), 6.87 – 6.99 (2H, broad s), 7.83 (2H, broad s) and 8.74 ppm (1H, s); δ_C (d_6-DMSO, 100 MHz) 57.2, 59.3, 83.4, 87.9, 122.5, 129.5, 152.6, 154.8, 163.4 and 172.3 ppm; m/z (APCI)
Synthesis of 6-(phenylethynyl)pterin (38)

According to the method of Taylor, 17 4-amino-2-(N,N-dimethylaminomethyleneamino)-6-(2-phenylethynyl)pterin-4-yl (200 mg, 0.631 mmol) was treated with 10 ml EtOH/1M NaOH (1:1) mixture, gently refluxed for 16 hours, filtered through celite, concentrated to small volume (~3 ml), acidified with AcOH (~1.5 ml), and stored at 2°C for 18 hours. The yellow precipitates were collected by centrifugation, washed sequentially with water (5 ml), acetone (5 ml) and Et₂O (5 ml), and dried under reduced pressure at 60°C to give 6-(phenylethynyl)pterin (120 mg, 73%) as a yellow solid; m.p. (dec) >300°C; v max /cm⁻¹ 1737 (C=O); δ H (d6-DMSO, 300 MHz) 7.34 – 7.48 (3H, m), 7.63 (2H, m) and 8.72 ppm (1H, s); m/z (APCI) 264 [M + H⁺, 100] (HREI Found: 263.08092. C₁₄H₉N₅O requires 263.08071). Spectroscopic data agree with those reported elsewhere. 17

Synthesis of 6-acetyl-2-amino-3,4-dihydropteridin-4-one (6-acetylpterin) (48)

4-Amino-2-(N,N-dimethylaminomethyleneamino)-6-[2-(trimethylsilyl)ethynyl]-pteridin-4-yl (300 mg, 0.958 mmol) was dissolved in 2M H₂SO₄ (3 ml) and treated with HgO (100 mg, 0.461 mmol), heated to 60°C and stirred at this temperature for 16 hours. 1M NaOH (3 ml) was added and the final solution stirred for 3 hours at 60°C, allowed to cool to room temperature, filtered through celite, concentrated and neutralized by drop-wise addition of 1M NaOH until the pH, as measured by universal indicator strips, was ~7.0. The yellow precipitates that formed, were isolated by centrifugation, washed with 50% MeOH/water (10 ml), dried under reduced pressure at 60°C to give 203 mg of yellow residue. Extraction of the yellow residue with hot MeOH (3 x 15 ml), followed by evaporation, gave 6-acetyl-2-amino-3,4-dihydropteridin-4-one (130 mg, 66%) as a yellow solid; m.p. (dec) >300°C; δH (CF₃COOD, 300 MHz) 2.46 (3H, s) and 9.13 ppm (1H, s); m/z (APCI) 206 [M + H⁺, 100%]; LC RT 6.10 minutes. Attempts to get high resolution mass were unsuccessful in both HREI and HRESI instruments.
Synthesis of Sepiapterin-C (46)\textsuperscript{40}

According to the method of Murata,\textsuperscript{40} 6-acetyl-2-amino-3,4-dihydropteridin-4-one (90 mg, 0.439 mmol) was dissolved in 1M NH\textsubscript{3} (150 ml) and treated with aluminium tunings (200 mg, 7.41 mmol) [pre-treated by immersing in HgCl\textsubscript{2} solution (50 mg in 10 ml water) for 10 minutes and washed with water (3 x 5 ml)], warmed to 55\textdegree{}C, and stirred under Ar for 16 hours, filtered, concentrated to ca. 40 ml, charged through florisil column, washed with water (500 ml) and eluted with 10% aq. Acetone, collecting the yellow eluting fraction. The collected fraction was evaporated to dryness and the residue extracted with hot methanol (3 x 15 ml) to give sepiapterin-C (52 mg, 57%) as yellow solid; m.p. (dec) 252\textdegree{}C; \(\delta\)\textsubscript{H} (CF\textsubscript{3}COOD, 300 MHz) 2.28 (3H, s) and 4.09 ppm (2H, s); \(\nu\textsubscript{max}/\text{cm}^{-1}\) 3183 (broad), 1738; \(m/z\) (APCI) 207 (M\textsuperscript{+}, 100)

Spectroscopic data agree with those reported elsewhere.\textsuperscript{40}

2.5 References

Chapter 3

Negishi coupling of quinoxaline and pteridine-O-sulfonates
Chapter 3  Negishi coupling of quinoxaline and pteridine-O-sulfonates

3.1 Introduction

The Negishi coupling chemistry was first reported in 1977 by Negishi et.al.\textsuperscript{1} where a cross-coupling reaction between an organozinc compound (R-ZnX) and organic halide (R\textsuperscript{1}-X) catalyzed by palladium was observed (Scheme 3.1). Over the past three decades, the chemistry has received much attention and many variations and improvements to suit a particular group of compounds. The variations include the replacement of palladium with a nickel catalyst, the replacement of the halogen leaving group with oxygen containing groups such as triflates and phosphor-ester groups, and also replacing the triphenylphosphine ligands with more bulky electron-rich ligands. A recent search on Reaxys database (Nov 2010) has revealed over 100,000 hits of reactions involving Negishi coupling. The chemistry has been used in key steps toward the total synthesis of various natural products such as vitamin-A, carotenes, reveromycin-B and many more.\textsuperscript{2} The pioneer of this chemistry, Professor Ei-ichi Negishi, was awarded the Nobel prize in chemistry in 2010 along with R.F Heck and A. Suzuki for their contribution in C-C bond forming reactions. The Negishi coupling has also led to the development of other coupling reactions such as Suzuki and Stille coupling, where the zinc is replaced by boron (B) and tin (Sn) respectively.\textsuperscript{2}

\[ \text{R-ZnX} + \text{R}\textsuperscript{1}-X' \xrightarrow{\text{Pd or Ni}} \text{R-R}\textsuperscript{1} \]

\[ X = \text{Cl, Br} \]
\[ X' = \text{Cl, Br, I, OTf, OPO(OR)}_2 \]

\textbf{Scheme 3.1}

The advantages of using Negishi cross-coupling reactions over other C-C bond formation reactions like the Suzuki and Stille are; (i) the reactions are usually performed at room temperature due to the ease in the transmetallation reaction between Zn and Pd while Suzuki and
Stille couplings generally require high temperature, (ii) the reactions do not require hydrolytic base to proceed, as required in the Suzuki coupling reactions, and (iii) the zinc reagents are known to tolerate a wide range of functional groups and in some cases the reactions can be performed in water, an example being the Reformatsky reaction.\(^3\)

Zinc reagents were the first organometallic reagents used in C-C bond forming reactions, dating back to the 19\(^{th}\) century. However, these reactions were generally slow, leading to the development of more reactive organometallic reagents having magnesium (Grignard reagents) and lithium metal ions. The use of Grignard and Li-reagents in C-C bond forming reactions has reduced the use of Zn-reagents to largely Negishi coupling reactions.

The drawback with the Negishi coupling, like other C-C bond coupling reactions, is the need to prepare the Zn-reagent. Zn reagents are usually prepared by one of three general methods, the first of which involves transmetallation reactions between ZnCl\(_2\) and a Li-reagent or Mg-reagent (Scheme 3.2 number 1). The Li- and Mg-reagents are known to be highly reactive and not compatible with many functional groups, thus preparing Zn-reagents from Li- or Mg-reagents might result in functional group incompatibility.

A second method for preparing Zn-reagents is by metal-halogen exchange reaction, where diethyl-zinc (Et\(_2\)Zn) is reacted with a halogenated compound leading to the desired Zn-reagent (Scheme 3.2 number 2).

\[
\begin{align*}
1. & \quad \text{R} \underset{\text{Li or MgX}}{\longrightarrow} \text{ZnCl}_2 \quad \text{R} \underset{\text{Zn}}{\longrightarrow} \text{ZnX} \\
2. & \quad \text{R} \underset{\text{X}}{\longrightarrow} \text{Et}_2\text{Zn} \quad \text{R} \underset{\text{Zn}}{\longrightarrow} \text{ZnX} \\
3. & \quad \text{R} \underset{\text{X}}{\longrightarrow} \text{Zn} \quad \text{R} \underset{\text{Zn}}{\longrightarrow} \text{ZnX}
\end{align*}
\]

Scheme 3.2

A third method for preparing Zn-reagents is by inserting an activated Zn metal into a C-halogen bond (Scheme 3.2 number 3). This is perhaps the most functional group tolerant method available for preparing Zn-reagents but, the need for a halogen atom in the molecule is
drawback. Reagents for the Reformatsky and Barbier reactions are prepared via this method, and more recently Zn-reagents for ‘Negishi like coupling’ reactions has been reported to be prepared \textit{in situ} in water.\textsuperscript{4}

Unlike the Suzuki coupling reactions, Negishi coupling reactions allow for coupling between a wide range groups, including aryl, heteroaryl, alkenyl and alky substituents.\textsuperscript{1,3-10} Coupling between two electron-rich groups, two electron-deficient groups, and electron-rich-electron-deficient groups have been reported to proceed in high yields. Highly stereospecific reactions $C_{sp}^{3}$-$C_{sp}^{2}$ Negishi coupling reactions with a wide range of substituted alky- and alkenyl-zinc compounds have been reported recently (Scheme 3.3).\textsuperscript{4,5}

![Scheme 3.3](image)

The most common leaving groups in Negishi coupling are the halogens (Cl, Br and I) with iodine containing electrophiles being the most reactive and chlorine containing electrophiles being less reactive.\textsuperscript{2} Oxygen containing leaving groups are mainly the sulfonyl groups (triflates, tosylate etc.) with triflates being the more reactive and widely used.\textsuperscript{2,11,12} The various methods usually employed to prepare these coupling partners have already been described in Chapter 2.
Recently the less expensive tosylate group has received much attention and has been shown to undergo Negishi coupling when attached to an electron-deficient system.\textsuperscript{13} However, only three examples of heteroaryl-O-arylsulfonate undergoing Negishi coupling were found (Sci-finder search December 2010).\textsuperscript{13,14} The ring system in which these Negishi coupling were reported are the cumarin and quinolinone ring system bearing an arylsulphonate leaving group (Scheme 3.4). No mention of a mesylate leaving group undergoing Negishi coupling was found (Reaxys and Sci-finder database, December 2010).

\textbf{Scheme 3.4}

To our knowledge, no Negishi coupling reactions have been reported on pteridine or pterin ring systems but, two reactions have been reported on the quinoxaline ring system. Joule \textit{et. al.} reported coupling between 2-chloroquinoxaline and diethyl zinc in 94\% yield,\textsuperscript{15} while Hocek reported coupling between 2-chloroquinoxaline and (benzoyloxymethyl)zinc iodide in 65\% yield (Scheme 3.5).\textsuperscript{16}

\textbf{Scheme 3.5}

The mechanism involved in the Negishi coupling reaction is not fully understood but, it is believed to be similar to that of Stille coupling reaction. The oxidative addition of the halogenated or sulfonated compound is believed to be the first step, followed by transmetallation between Zn and Pd, and finally the reductive elimination giving the desired coupled product (Scheme 3.6).
Scheme 3.6

The oxidative addition step is believed to be the rate determining step (RDS) while the transmetallation is relatively fast due to similar electronic properties between Pd and Zn. Recent studies have shown that electron withdrawing groups attached to the halogenated compound, increases the rate of the oxidative addition step. Electron donating groups attached to the Zn-reagent, on the contrary, increases the rate of the transmetallation step.\textsuperscript{6}

In this chapter, we report Negishi coupling of both quinoxaline and pteridine ring systems possessing benzenesulfonyl and mesylate leaving groups. Hydrolysis of the product pteridine to give various 6-substituted pterin is also reported.

3.2 Results and Discussion

3.2.1 Negishi coupling of quinoxaline-O-sulfonates with alkyl- and aryl-Zn substrates

With recent results showing Negishi coupling of heterocycles bearing a tosylate leaving group,\textsuperscript{13,15} we were interested in investigating if the quinoxaline and pteridine-O-sulfonate substrates prepared in our laboratory, would participate in Negishi coupling reactions.
We started our investigations by treating benzenesulfonyl quinoxaline 7 with diethyl zinc (Et₂Zn) in THF, with 5 mol % of PdCl₂(PPh₃)₂ catalyst and stirring the reaction at room temperature for six hours, when no trace of starting material was detected on TLC. Quenching the reaction with sat. NaHCO₃ and purification on flash silica gave the desired 2-ethylquinoxaline 49 in a 15% yield (Scheme 3.7). The product was isolated as an oil as previously reported and the ¹H NMR and mass spectra were identical to those reported.¹⁵ No other fractions were obtained from the column suggesting that decomposition or unidentified side reactions, giving polar products could be responsible for the low yield and unaccounted mass balance.

Recent mechanistic investigation of Negishi coupling of aryl-halogenes, described in section 3.1, suggest that the RDS may be the insertion of a Pd-species into the C-Hal bond.⁶ It appeared that the insertion of Pd may have been limiting the rate at which our sulfonate system could also undergo Negishi coupling.

In an effort to improve the yield and possibly increase the rate of the oxidative addition step, the reaction was performed at reflux temperature for six hours with TLC showing no trace of starting material. Normal work-up and purification gave an improved yield of 35%. When increasing the reaction time to 18 hours with reflux, the yield improved to 60% with the ¹H NMR and mass spectra identical to those obtained by Joule.¹⁵ The yields were lower compared to the reaction starting with 2-chloroquinoxaline, where 94% yield at room temperature was reported by Joule et.al.¹⁵ suggesting that chlorine is a better leaving group than benzenesulfonate, under these conditions.

This successful Negishi coupling reaction was very encouraging. However, it appeared as though alkyl zinc reagents would be sluggish substrates in Negishi coupling reactions with heteroarylsulfonates, so we turned to the zinc-aryl as possible coupling partners. The Zn-aryl are relatively electron rich substrates when compared to Zn-alkyls and should, according to recent studies,⁶ be able to undergo relatively faster transmetallation with Pd.

Shifting the focus to aromatic-zinc substrates, we began by preparing a solution containing a phenyl-Zn species. For this, we prepared a stock solution of PhMgBr, calculated to be ~ 1.1 M, by standard Grignard methodology. Aliquots of this were used to form the zinc phenyl, by simple addition of a molar equivalent of ZnCl₂, either as a pre-prepared stock solution in THF or
as a solid, under a dry argon atmosphere. No precipitation was observed, and the resultant solution was assumed to be ~1.1M in PhZn⁺, Mg²⁺ and bromide, and ~ 2.2M in chloride.

The bromobenzene was employed in slight excess (1.1 equivalent) during the preparation of the Grignard and it was likely that some remained in this solution. During the subsequent work employing this solution, we did not detect any bi-phenyl products, which might have resulted from Pd-catalyzed reactions of PhZn with residual PhBr.

Subjecting phenyl-ZnCl and benzenesulfonyl quinoxaline 7 to our optimized Negishi coupling conditions, i.e. reflux in THF for 18 hours, quenching with sat.NaHCO₃ and purification on flash silica, gave 2-phenylquinoxaline 50 in 95%. The NMR and mass spectra of the product were consistent with the desired product and were in good agreement with previously reported data (Scheme 3.7).

When the same reaction was performed at room temperature, the reaction yield dropped to 50% with starting material observed on TLC. Refluxing the reaction for 6 hours gave a reduced yield of 60% (76% based on recovered starting material).

Scheme 3.7

Toluene-2-zinc species have been previously prepared by lithiation of 2-bromotoluene with n-BuLi, followed by transmetallation with ZnCl₂ and been used in Negishi coupling reactions.
We prepared the 2-toluenyl-ZnBr according to literature procedure and attempted Negishi coupling with our quinoxaline 7.

Subjecting 2-toluenyl-ZnBr and quinoxaline 7 to our Negishi coupling conditions, followed by quenching with sat.NaHCO$_3$, workup and after purification on flash silica, two fractions were collected. The first eluting fraction had a $^1$H NMR consistent with that of proposed structure and reported literature of 2-toluenequinoxaline 51. The mass spectrum gave a molecular ion peak at m/z 221 amu further supporting our proposed structure.

The second eluting fraction was unreacted quinoxaline 7, as judged by the NMR and mass spectra. The isolated yield was 55% (or 73% based on recovered quinoxaline 7). When the reaction was allowed to run for longer than 24 hours, no full conversion of starting material was observed as judged on TLC and no improvement on the yield was achieved. The low yield of tolylquinoxaline 51 could be attributed to the steric crowding around the reactive site, which might slow the transmetallation step between Pd and Zn.

The substituted 3,4,5-trimethoxybenzene motif occurs extensively in natural products derived via the Shikimic acid route, examples being mescaline and reserpine, and it has also found extensive use in the field of medicinal chemistry. Almost all synthetic approaches to this system have made use of Suzuki coupling conditions where, most commonly a halogenated trimethoxybenzene acts as precursor to a boronic acid, or is a coupling partner with an arylboronic acid. To our knowledge, the zincyl-trimethoxybenzene has never been reported.

We prepared the zincyl-trimethoxybenzene, following the same procedure used to prepare 2-toluenyl-ZnBr, where we started by the lithiation of 3,4,5-trimethoxy-bromobenzene with n-BuLi, followed by transmetallation with ZnCl$_2$. Subjecting the Zn-substrate and quinoxaline 7 to our Negishi coupling conditions, a normal work-up and following purification on flash silica, one fraction was isolated. The compound was isolated as a yellow solid and had a melting point in the range 82 – 83°C, a molecular ion peak at m/z 296 amu was obtained, while the $^1$H NMR spectrum gave peaks that were consistent with the proposed structure. A downfield singlet at δ 9.30 ppm (tentatively assigned to the 3-H proton), a singlet at δ 7.44 ppm (tentatively assigned to be the trimethoxyphenyl protons) and two upfield singlets at δ 3.90 and 4.03 ppm (tentatively assigned to be the methoxy protons), led us to conclude that we had prepared 2-(3,4,5-
trimethoxybenzene)quinoxaline 52 (Scheme 3.7). The product was obtained in 80% yield and its synthesis has never been reported.

In conclusion, we have demonstrated Negishi coupling reactions involving benzenesulfonyloxy-2-quinoxaline with various phenyl-Zn substrates in moderate to excellent yields. The phenyl-Zn substrates were shown to be better coupling partners when compared to the alky-Zn substrate. We then turned our attention in investigating heteroaryl-Zn substrates as coupling partners in Negishi coupling reactions.

**3.2.2 Negishi coupling of quinoxaline-O-sulfonates with hetero-aryl substrates**

We started our investigation of heteroaryl-Zn substrates with 3-zincyl-thiophene. The thiophene occurs extensively in natural products, examples being tiletamin, isobutylthiphene and terthiphene, and has found extensive use in the field of medicinal chemistry. Many of the reactions leading to thiophene substrates have been achieved via the Suzuki coupling approach. A few examples of Negishi coupling employing zincyl-thiophenes, prepared by insertion of Rieke zinc into the C-Cl bond, were found. We were interested in investigating 3-zincyl-thiophene as a coupling partner in Negishi coupling reactions.

We investigated the preparation 3-zincyl-thiophene, from 3-bromothiophene by, lithiation with n-BuLi, and transmetallation with ZnCl2. The putative Zn-substrate and quinoxaline 7 were subjected to our Negishi coupling conditions, and following separation, by flash chromatography on flash silica, two fractions were isolated and analyzed by NMR and mass spectroscopy.

The first eluting fraction, a yellow solid (m.p. 91-92°C), had two molecular ion peaks of roughly equal intensity at m/z 290 amu [M]+ and 292 amu [M+2]+ evident in the EI-mass spectrum. The roughly equal intensity of these molecular ion peaks suggests the presence of a bromine atom in the molecule. Bromine is known to exist as 79Br and 81Br in equal proportions and hence a molecular ion of a mono-brominated molecule is characterized by a characteristic pattern. The peak at m/z 292 is slightly bigger than the peak at 290, and this could be attributed to the presence of 34S atom in the molecule which has 6% natural abundance.

The 1H NMR spectrum integrated for 7H, with a downfield singlet at δ 9.78 ppm, suggesting incorporation of a quinoxaline ring. Two downfield doublets at δ 7.15 and 7.50 ppm, with
identical coupling constants (J) of 5.4 Hz, suggesting 3-bond coupling (J^3), were observed. Two multiplet signals, integrating for two protons each, were observed at δ 7.77 and 8.11 ppm. These peaks were previously assigned to correspond to the 5-, 6-, 7- and 8-H protons in the quinoxaline ring, from reported quinoxaline structures. From these NMR and mass spectral data we tentatively assigned the structure of the compound to be 2-(3’-bromothiophen-2’-yl)quinoxaline 53 (Scheme 3.8).

The second eluting fraction, also a yellow solid (m.p. 112-113°C), had a ^1H NMR and mass spectra that appeared consistent with the coupled structure and that reported in literature of 2-(thiophen-3’-yl)quinoxaline 54 (Scheme 3.8).^23 The two products were each isolated 30% yield.

Scheme 3.8

These results suggest that there is a competition between the metal-halogen exchange reaction, involving 3-bromothiophene and n-BuLi, and a deprotonation of the hydrogen at the 2-position of the 3-bromothiophene. The equal product distribution suggests that the two competing reactions are of comparable rates. Research has shown that 3-lithiothiophene can be obtained exclusively from 3-bromothiophene when the reaction is performed at -78°C.^24 However, repeating these reactions in our laboratory, resulted in equal product distribution to that described above. We attempted varying the lithiation temperature from -78°C to -20°C but, no change in product distribution and yield were observed.

It is likely that the α-acidifying effect of the thiophene sulfur, combined with a small ortho-directing ability of the bromine allows the formation of the thiophene-2-zincyl to compete with production of thiophene-3-zincyl. The facile nature of this side reaction might suggest why the
production of 3-zincylthiophenes, employing a BuLi insertion into a C-Hal bond, has not been reported before.

With these unexpected results, we investigated a chemically similar motif to 3-bromothiophene, 3-bromofuran. Using our previously attempted method of preparing the zincyl-thiophenes, 3-bromofuran was lithiated with n-BuLi followed by transmetallation with ZnCl₂, to give a solution containing the putative 3-zincyl-furan. To this solution was added quinoxaline 7 and catalytic PdCl₂(PPh₃)₂, and heating at reflux maintained for 18 hours. Following aqueous work-up and separation by flash chromatography on flash silica, one product was isolated.

Mass spectral analysis of the compound gave two molecular ion peaks at m/z 275 and 277amu in roughly equal intensities, suggesting the presence of a bromine atom in the molecule. The ¹H NMR spectrum gave similar patterns to those observed in the spectrum of 2-(3-bromothiophen-2-yl)quinoxaline 53, where two downfield doublets at δ 6.71 and 7.65 ppm with equal coupling constants (J = 1.8 Hz), were observed. We tentatively assigned the structure of this compound to be 2-(3’-bromofuran-2’-yl)quinoxaline 55 (Scheme 3.9), and the product was isolated in 21% yield. No trace of the desired 2-(furan-3’-yl)quinoxaline 56 was detected.

Scheme 3.9

Similar results of competition between metal-halogen exchange at the 3-position, and deprotonation at the 2-position on furan ring has been reported by Marcos et al.²⁵ where a 1:1 ratio of n-BuLi and 3-bromofuran favoured metal-halogen exchange, while a 1:2 ratio favoured deprotonation. When attempting the conditions of Marcos et al.²⁵, favouring exclusively the metal-halogen exchange, followed by transmetallation with ZnCl₂, and subjecting the putative 3-
zincyl-furan to our Negishi coupling conditions, no desired coupled product 56 was obtained. TLC analysis showed dark material settling at the base line, and we believe that they could polymers.

We then attempted the conditions that favoured, exclusively, the deprotonation of 3-bromofuran, and transmetallation of the putative 2-lithio-3-bromofuran with ZnCl$_2$. Subjecting this substrate to our Negishi coupling conditions gave the coupled product 2-(3-bromofuran-2-yl)quinoxaline 55 in 20% yield. The quinoxaline starting material 7 was recovered along with substantial amounts of polymeric material.

We postulate that competition reaction between deprotonation and metal-halogen exchange is taking place but, polymerization of the putative 3-zincyl-furan or the coupled product from this precursor is occurring, resulting in the absence of 56. No polymerization of the 3-lithiofuran has been reported in literature, and we did not observe any polymerization during the transmetallation reaction, leading us to conclude that the Negishi product 56 forms but under the reaction conditions, polymerization of this product occurs. No quinoxaline 7 was detected on TLC, when the putative 2-zincyl-3-bromofuran is used, further supporting our claims. The mechanism of this polymerization however, is still unknown to us. Further studies might involve monitoring the progression of the reaction on HPLC-MS spectrometer to identify the species being formed during the reaction.

We then investigated the direct lithiation of furan in the two position, preceding a transmetallation and an attempt at Negishi coupling with the quinoxaline 7. Starting with furan, deprotonation with n-BuLi and transmetallation with ZnCl$_2$ gave a solution containing a putative 2-zincyl-furan. Subjecting the Zn-substrate and quinoxaline 7 to our Negishi coupling conditions, normal work-up and purification on flash silica led to the isolation of one fraction. The $^1$H NMR and mass spectra of the compound was consistent with both the structure and data reported for 2-(furan-2-yl)quinoxaline 57 (Scheme 3.10),$^{26}$ which was obtained in 73% yield. No polymer formation was observed on the TLC plate and reaction flask, further suggesting that 2-(3-furyl)-quinoxaline is undergoing polymerization or other side reactions giving polar and insoluble products.
Scheme 3.10

Having some success of Negishi coupling with oxygen and sulfur containing heteroaryl-Zn substrates, we then investigated the N-heterocyclic substrates as potential Negishi coupling partners with the quinoxaline 7. N-heterocyclic motifs, such as pyrrole, indole and pyridine are incorporated into many of naturally occurring compounds, and have found extensive use in medicinal chemistry.

We began our investigations with N-benzenesulfonyl pyrrole. Lithiation with n-BuLi followed by transmetallation with ZnCl₂, according to previous work, gave a solution containing a putative zincated-pyrrole. Following our Negishi coupling conditions, the Zn-substrate, Pd catalyst and quinoxaline 7 were refluxed in THF for 18 hours, purified on flash silica to give one isolated product. The mass spectrum of the sample gave a molecular ion peak at m/z 336 amu, consistent with the calculated molecular weight of the desired product. The ¹H NMR spectra of the compound integrated for 13 H, with a downfield singlet at δ 9.07 ppm, consistent with the 3-H proton from quinoxaline. Signals at δ 6.48 and 6.77 ppm were tentatively assigned to be two of the 3- and 4-pyrrole-H, as previously found in our laboratory. From these results, we assigned the structure of the product as 2-[1-(benzenesulfonyl)-1H-pyrrol-2-yl]quinoxaline 58, obtained in 10% yield (Scheme 3.11). No starting materials was observed on TLC or isolated from the column during purification but, dark material was evident on the base line following TLC analysis of the crude product, suggesting that polymerization could have occurred, and explaining the poor yield of the quinoxaline 58.
Attempts to improve the yield of quinoxaline 58 by either an increase in reaction time or by increasing the mole equivalents of the Zn-pyrrole with respect to the quinoxaline 7, did not improve the reaction yield, where 7% and 9% yields were obtained respectively. Polymerization was observed in both attempts, as evidenced by significant baseline polar material.

We then investigated an indole substrate as a potential Negishi coupling partner to quinoxaline 7. The N-tosyl-indole was treated sequentially n-BuLi and ZnCl₂ to give a THF solution containing the putative zincyl-indole derivative. Subjecting the quinoxaline 7 and the zincyl-indole to our Negishi coupling conditions, following the reaction by TLC where consumption of starting material and formation of one new product was observed after 18 hours. Following separation by flash chromatography on silica gel, one new compound was isolated. The mass spectrum of the sample gave a molecular ion peak at $m/z$ 400 amu, in agreement with that calculated for desired product. The $^1$H NMR spectrum integrated for 19 H, with a an upfield singlet (3H) at $\delta$ 2.28 ppm, tentatively assigned to be the methyl protons from the tosyl group, and downfield singlet (1H) at $\delta$ 9.27 ppm, consistent with a 3-H of the quinoxaline proton. With these results, we assigned the structure of the compound as 2-{1-[(4-methylbenzene)sulfonyl]-1H-indol-2-yl}quinoxaline 59 (Scheme 3.12), obtained in 15% yield. TLC analysis of the crude product had again showed dark material on the baseline, suggesting that polymerization had occurred, and which might have accounted for the poor mass recovery.

Scheme 3.11

Scheme 3.12
The attempted coupling of an electron deficient pyridine ring system was investigated. Production of 2-lithiopyridine has previously been reported and we undertook to repeat this procedure, prior to addition of ZnCl$_2$ which we intended would provide a partner for a Negishi coupling reaction using our optimal conditions. Attempts to prepare 2-zincyl-pyridine, starting from pyridine, were unsuccessful when using the common procedure for preparing Zn-reagents as judged by TLC and HPLC-MS analysis of the crude Negishi coupling reaction mixture, which showed starting the quinoxaline starting material 7 to be main species present. Separation of the crude mixture on flash chromatography recovered 95% of the quinoxaline starting material 7. Due to time constraints, this was not pursued further and we cannot say if the putative zinc-pyridyl was present, and would not undergo Negishi coupling or perhaps that the zinc-pyridine was not present in solution.

In conclusion, we have demonstrated Negishi coupling reactions to occur between benzenesulfonyloxy-quinoxaline 7 with various heteroaryl-Zn substrates. Competition reactions between metal-halogen exchange and deprotonation with n-BuLi were observed when using 3-bromofuran and 3-bromothiophene, resulting in low reaction yields. As judged by both poor mass recovery and the presence of polar baseline material, we suggest that polymerization of cross-coupled products was observed in a number of reactions, resulting in low isolated yields of these desired products, in some cases none at all. Future work might involve further investigation of the stability of the cross-coupled products to, for instance, stoichiometric zinc cation and catalytic Pd, under the conditions of our optimized Negishi coupled reactions.

Finally we investigated the ability of quinoxaline-O-mesylate as a leaving group in the Negishi coupling reactions as it carries the advantage of being relatively cheap and more atom economic that the corresponding O-benzenesulfonate leaving group.

Treatment of the mesyloxy-quinoxaline 15 with phenyl-Zn, under our Negishi coupling conditions, showed consumption of quinoxaline 15 and formation of one principal new compound after heating at reflux for 18 hours. Following aqueous work-up and purification by flash chromatography, the desired 2-phenylquinoxaline 50 was obtained in 91% yield (Scheme 3.13 a). The melting point, $^1$H, $^{13}$C NMR and mass spectra were identical to those obtained from samples of quinoxaline 50 obtained previously from benzenesulfonate 7, as described above.
Similarly 2-(3,4,5-trimethoxybenzene)quinoxaline 52 was obtained in an 80% yield, when coupling 2-mesyloxy quinoxaline 15 with zincyl-trimethoxybenzene under our Negishi coupling conditions (Scheme 3.13 b). The yield is identical to the yield obtained when starting with benzenesulfonyloxy-quinoxaline 7. The product had a $^1$H NMR and mass spectra that were identical to our previously obtained results.

Having shown the success of Negishi coupling reaction employing benzenesulfonyloxy-quinoxaline 7 and mesyloxy-quinoxaline 15 as coupling partners with alkyl-, aryl- and heteroaryl-Zn substrates, we then investigated the benzenesulfonyloxy-pteridine and mesyloxy-pteridines as potential coupling partners in Negishi coupling reactions.

3.2.3 Negishi coupling of pteridine-O-sulfonates

When working with the pteridines, two challenges were faced; firstly that the amidine protected pteridine-O-sulfonates were not soluble in THF and secondly that the amidine protecting groups are easily removed by both mild acid and mild base, as we have discovered during the investigation of Sonogashira coupling in chapter 2.

The Negishi coupling conditions which were successful with the quinoxaline-O-sulfonates would expose the amidine protected pteridine to conditions involving a stoichiometric organometallic reagent. Both the amidine protecting groups and the electron deficient pteridine nucleus possess electrophilic sites which would potentially react with the polarized Zn-carbon bond. To make matters worse, the Lewis acid nature of the zinc cation could further activate the pteridine by coordination. On paper at least, the fully protected pteridine appeared to offer a number of bi-

Scheme 3.13  a) PhZn; b) $(MeO)_3PhZn$

Similarly 2-(3,4,5-trimethoxybenzene)quinoxaline 52 was obtained in an 80% yield, when coupling 2-mesyloxy quinoxaline 15 with zincyl-trimethoxybenzene under our Negishi coupling conditions (Scheme 3.13 b). The yield is identical to the yield obtained when starting with benzenesulfonyloxy-quinoxaline 7. The product had a $^1$H NMR and mass spectra that were identical to our previously obtained results.

Having shown the success of Negishi coupling reaction employing benzenesulfonyloxy-quinoxaline 7 and mesyloxy-quinoxaline 15 as coupling partners with alkyl-, aryl- and heteroaryl-Zn substrates, we then investigated the benzenesulfonyloxy-pteridine and mesyloxy-pteridines as potential coupling partners in Negishi coupling reactions.

3.2.3 Negishi coupling of pteridine-O-sulfonates

When working with the pteridines, two challenges were faced; firstly that the amidine protected pteridine-O-sulfonates were not soluble in THF and secondly that the amidine protecting groups are easily removed by both mild acid and mild base, as we have discovered during the investigation of Sonogashira coupling in chapter 2.

The Negishi coupling conditions which were successful with the quinoxaline-O-sulfonates would expose the amidine protected pteridine to conditions involving a stoichiometric organometallic reagent. Both the amidine protecting groups and the electron deficient pteridine nucleus possess electrophilic sites which would potentially react with the polarized Zn-carbon bond. To make matters worse, the Lewis acid nature of the zinc cation could further activate the pteridine by coordination. On paper at least, the fully protected pteridine appeared to offer a number of bi-
dentate coordination modes some of which are shown in Figure 3.1. The prospects did not appear good.

![Figure 3.1](image1)

With these formidable issues in mind we began examining the reaction of benzenesulfonyl pteridine 27 with phenyl-Zn under the Negishi coupling conditions in refluxing THF under dry argon. It appeared that the bulk of the pteridine 27 dissolved, to give a dark solution, but within an hour a bulky precipitate was evident. After two hours at reflux, the flask was cooled and the precipitate and supernatant separated by filtration. HPLC-MS analysis of both the precipitates and the mother liquor suggested the main components present to be the deprotected pteridines derived from the pteridine-O-sulfonate (27b and 27c) and significantly, a trace amount of what we tentatively assigned as the mono-deprotected cross-coupled product 61 as judged by an apparent molecular ion at m/z 294 amu [M+H+] (Figure 3.2).

![Figure 3.2](image2)
Encouragingly too, we did not detect any products which might have been expected had addition of the PhZn reagent occurred to either the amidine protecting group or the pteridine nucleus. Possible structures for such byproducts might, we reasoned, be benzaldehyde (following hydrolysis of an aminal resulting from addition of phenyl to the amidine) or the 7-phenyl-7,8-dihydropteridine shown in Scheme 3.14

\[
\begin{align*}
&\begin{array}{c}
\text{NH}_2 \\
\text{H}_2\text{N} \\
\text{N} \\
\text{N} \\
\text{OSO}_2\text{Ph}
\end{array} \\
&\begin{array}{c}
\text{NH}_2 \\
\text{H}_2\text{N} \\
\text{N} \\
\text{N} \\
\text{OSO}_2\text{Ph}
\end{array} \\
&\begin{array}{c}
\text{NH}_2 \\
\text{H}_2\text{N} \\
\text{N} \\
\text{N} \\
\text{OSO}_2\text{Ph}
\end{array}
\end{align*}
\]

**Scheme 3.14** a) reaction on the amidine; b) reaction on the 7-C atom

It appeared likely, given the absence of proton donors under the Negishi coupling conditions, that simple precipitation of, presumably a different crystalline isomorph, of the starting pteridine-O-sulfonate 27 was occurring under our Negishi coupling conditions. The hydrolysis products were those formed during the HPLC-MS analysis, which we had already confirmed could occur, as described in Chapter 2. If this was the case, then we would require a solvent which would dissolve the pteridine and allow the Negishi coupling reaction to occur.

Dichloromethane was investigated as an alternative solvent to THF due to its ability to solubilise protected pteridines. The quinoxaline model was chosen to investigate the Negishi coupling in DCM, where quinoxaline 7 was treated with phenyl-Zn (prepared in THF, as previously described, followed by removal of solvent *in vacuo* and dissolving the salt in dry DCM). After refluxing in DCM for 18 hours, aqueous work and purification on flash silica, the desired 2-phenylquinoxaline 50, identical as judged by 1H NMR and mass spectral analysis to that obtained before, was obtained in 65% yield. No other side products were detected.

We then prepared the PhZn reagent in DCM and added the pteridine 27 and Pd catalyst, and the mixture was heated at reflux for 18 hours. No precipitates were observed, however, HPLC-MS
analysis of the reaction mixture showed the main component to be starting material and a trace amount of desired coupled product. From these results it was evident that the Zn-reagent was not interfering with the protecting groups of the pteridines as observed from HPLC-MS analysis.

We then investigated solvent combinations of DCM/THF where the pteridine and Pd were dissolved in DCM and the PhZn prepared in THF, prior to mixing. Precipitation was observed within the first hour and HPLC-MS analysis of the crude mixture and precipitates showed the presence of both starting material and deprotected starting material, with no desired coupled products detected.

Increasing the temperature of the reaction by performing the reaction in DCM in a sealed tube did not yield any desired Negishi coupled product either. When using chloroform as solvent, apparent polymerization was observed as judged by the formation of precipitates which proved insoluble in all solvent systems, and no trace of Negishi coupling product or starting pteridine 27 were observed on HPLC-MS analysis of the supernatant. Changing the Zn-reagent to 3,4,5-trimethoxybenzene-ZnCl gave similar results to those obtained when using phenyl-ZnCl.

A dipolar aprotic solvent appeared as a possible choice and to investigate this, we attempted the Negishi coupling reactions in DMF. We dissolved quinoxaline 27 and Pd catalyst in DMF, and to this solution was added PnZn in THF. The reaction was warmed to 80°C under dry Ar for two hours after which TLC showed complete consumption of pteridine 27 and the formation of new species. After purification of the crude mixture on flash chromatography, two close eluting fractions were isolated. HPLC-MS analysis of the mixture showed the products to be desired coupled phenyl-pteridine 60 along with the mono-deprotected phenyl-pteridine 61 (Scheme 3.15), as judged by the peaks at m/z 349 and 294 amu [both M+H⁺]. Further purification of the mixed products employing flash chromatography gave a product mixture that was mainly pteridine 61 (>90%), with ¹H NMR showing downfield proton at δ 9.60 ppm and two methyl peaks at δ 3.08 (3H) and 3.19 ppm (3H). The products were isolated in an overall yield of 80%, with no recovered starting material.
Scheme 3.15

Having had Negishi coupling success with PhZn and pteridine 27, we investigated the Negishi coupling between the zincyl-trimethoxybenzene and pteridine 27 employing our new conditions. The zincyl-trimethoxybenzene was prepared in THF, as previously reported, and the solution was added to a DMF solution containing the pteridine 27 and catalytic Pd, warmed to 80°C under Ar and stirred for two hours. Following full consumption of starting material and formation of a new product, as judged by TLC, the crude mixture was purified by flash chromatography, and two close running fractions were isolated. HPLC-MS analysis of the two close fractions gave peaks at m/z 439 and 384 amu [both M+H⁺] as the only peaks. Further purification by flash chromatography, gave product mixture that had the m/z 384 amu peak. ¹H NMR spectrum gave a downfield singlet (1H) at δ 9.59 ppm, consistent with 7-H pteridine signal, and four upfield singlets between δ 3.08 and 3.94 ppm (15H) consistent with the methyl protons on the protecting group and the benzene ring. A singlet in the aromatic region at δ 7.66 (2H) was consistent with the two equivalent protons in the phenyl ring. We then assigned the structures of the compound to be the coupled trimethoxyphenyl-pteridine 62 along with the mono-deprotected pteridine 63. The overall yield of the reaction was 65%.
Figure 3.3

We then investigated the Negishi coupling between pteridine 27 and 2-zincyl-furan. The solution containing zincyl-furan, prepared as described previously, was added to a DMF solution containing the pteridine 27 and catalytic Pd, and final solution warmed to 80°C under Ar and stirred for two hours. Following full consumption of starting material and formation of new product, as judged by TLC, the crude mixture was purified by flash chromatography, and two close running fractions were isolated. HPLC-MS and 1H NMR analysis of the mixture, showed the two close fractions to contain the desired coupled furyl-pteridine 65 along with mono-deprotected furyl-pteridine 65 (Figure 3.4), as the only components. Further purification on flash chromatography gave a product mixture that contained more mono-deprotected furyl-pteridine 65, with an overall yield of 67%.

Figure 3.4

Investigating the ability of mesylate as a leaving in pteridine system, we treated the mesyl-pteridine 26 with phenyl-Zn under the coupling conditions and obtained the mixed coupled phenyl-pteridines 60 and 61 in 78% yield, results similar to those obtained in the quinoxaline experiments. The 1H NMR and HPLC-MS spectral analysis of the products were identical to those of phenyl-pteridines obtained from benzenesulfonate 27.
However, the attempted coupling of the zincated-trimethoxyphenyl and furan rings with the mesyloxy-pteridine 26 were unsuccessful as judged by the TLC and HPLC-UV-APCI-MS. This could be due to the presence of lithium (Li) ions in solutions that have been reported to be poisonous to the palladium catalyst in certain cross-coupling reactions. The furan-2-ylzinc and trimethoxyphenylzinc are prepared by transmetallation from their corresponding lithio-derivatives and are never purified meaning that Li ions are still present, in stoichiometric amounts, in solution during the Negishi coupling reaction. The extent of poisoning by Li and sometimes Mg is not fully understood and is also dependant of the substrates attached to the zinc metal and also the leaving group.

From these results, we postulate that although the yields in Negishi couplings obtained when using benzenesulfonate-substrate and mesylate-substrate are almost identical, the benzenesulfonate-substrates are more reactive than the mesylate. When using benzenesulfonyl-pteridine 27 as substrate, the reactions proceed smoothly in the presence of a potential poison, lithium, while mesyl-pteridine 26 does not give desired products in the presence of Li ions. This might imply that the rate of coupling is faster in benzenesulfonyl-pteridine 27 when compared to poisoning while in mesyl-pteridine 26, rate of poisoning is faster than rate of coupling. More work needs to be done to substantiate which of the possible explanations for the failure of the attempted Negishi couplings under these conditions.

In conclusion, we have demonstrated Negishi coupling reactions between pteridine-O-sulfonates and aryl or heteroaryl-Zn substrates in moderate to good yields. The reactions were performed in a potentially reactive solvent, DMF, but remarkably, no side reactions with the DMF or the amidine protecting groups were observed. The success of these reactions allow for the introduction of various aryl- and heteroaryl-substituents at the 6-position of the pteridine nucleus. We then investigated the conversion of these amidine protected 6-(hetero)arylppteridines into the corresponding 2,4-diaminopteridine and pterin derivatives.

### 3.2.4 Conversion of Pteridines to Pterins

In Chapter 2, methods for removing the protecting groups to give the 2,4-diamino-6-ethynyl-pteridines and pterins were discussed. With our Negishi coupled products in hand, we
investigated their deprotection and hydrolysis to the corresponding 2,4-diamino-6-(hetero)arylpteridines and pterins, respectively.

Treatment of the protected phenyl-pteridines 60 and 61 with 1M NaOH/EtOH mixture, heating at a gentle reflux for 8 hours and precipitation by addition of AcOH gave, after filtration, a yellow solid. $^1$H NMR and mass spectral analysis of the compound were consistent with those previously reported for 6-phenyl-pterin 66, with a $m/z$ peak at 239 [M-1] obtained on the APCI negative mode spectrum, and a downfield singlet at $\delta$ 9.26 ppm obtained from the $^1$H NMR spectrum (Scheme 3.16). The product was obtained in 61% yield.

Scheme 3.16

In a similar manner, the protected trimethoxypteridines 62 and 63 were treated with with 1M NaOH/EtOH mixture, with gentle reflux for 8 hours and precipitation by addition of AcOH to give a yellow solid. The APCI mass spectrum of the compound gave a [M+H]$^+$ at $m/z$ 330 amu and the $^1$H NMR spectrum gave a downfield singlet at $\delta$ 9.23 ppm (1H) and two upfield singlets at $\delta$ 3.72 (3H) and 3.89 ppm (6H). We assigned the structure of the compound to that of 6-(3,4,5-trimethoxyphenyl)pterin 67, obtained in 59% yield (Figure 3.5).

The mixture of the protected furanyl-pteridines 64 and 65 were treated with with 1M NaOH/EtOH mixture, with gentle reflux for 8 hours and precipitation by addition of AcOH to give a yellow solid. The $^1$H NMR spectrum integrated for four hydrogens, with a downfield singlet at $\delta$ 8.97 ppm, consistent with 7-H pterin proton, and upfield singlet at $\delta$ 6.68 ppm, consistent with the furan ring. The APCI mass spectrum gave a peak at $m/z$ 230 amu [M+H$^+$] which is in agreement with the molecular ion calculated for 6-(furan-2-yl)pterin 68 (Figure 3.5). The product was obtained in 63% yield.
Treatment of the protected phenyl-pteridines \( \text{60} \) and \( \text{61} \) with 1M NH\(_3\)/EtOH mixture, with gentle reflux for 8 hours and precipitation by addition of AcOH gave, after filtration, a yellow solid. \(^1\)H NMR and mass spectral analysis of the compound were consistent with those previously reported for 2,4-diamino-6-(phenyl)pteridine \( \text{69} \), with a \( m/z \) peak at 239 \([M+H^+]\) obtained on the APCI spectrum, and a downfield singlet at \( \delta \) 9.30 ppm obtained from the \(^1\)H NMR spectrum (Scheme 3.17).\(^{28}\)

![Scheme 3.17](image)

Similar results were observed when the furanylpteridines \( \text{64} \) and \( \text{65} \) were refluxed in 1M NH\(_3\)/EtOH solution, where 6-furan-2-yl-pterin \( \text{68} \) was obtained, as judged by NMR and mass spectral analysis, instead of the 2,4-diaminopteridine derivative.

These results suggest that the groups attached at the 6-position have a direct effect in the reactivity of the pterin ring. When electron rich substituents, relative to phenyl, are attached at the 6-position, the hydrolysis of the 4-amino group is easily achieved under weak basic conditions such as ammonia. It is likely that the pteridine ring becomes more basic and the first
step will be the protonation of the ring, followed by an attack by a weak nucleophile (water) and the loss of ammonia gives the pterin ring.

3.2.5 Conclusion

We have demonstrated that Negishi coupling chemistry can be performed on quinoxalines and pteridines bearing a benzenesulfonate and mesylate leaving groups. Different substituents i.e. Aryl, heterocycles, and alkyl, can be attached at the 2-position for quinoxaline, and 6-position for pteridines. There seem to be little difference in yields when changing the leaving group from benzenesulfonate to mesylate in the quinoxaline system but the presence Li ions does affect the Negishi coupling when mesylate is a leaving group on pteridines. This method allows for new pterin molecules, possessing various substituents e.g. heterocycles, at the six position to be synthesized and can open new routes towards the total synthesis of natural pterin molecules.

3.3 Experimental

3.3.1 Preparation of Zn-reagents

Phenyl-Zn

To a flame dried 3-neck 250 ml round bottom flask equipped with a stirrer bar, reflux condenser and a positive pressure of dry argon and containing magnesium turnings (2.02 g, 83.33 mmol) was transferred a solution of bromobenzene (9.70 ml, 91.45 mmol, 1.1 eq.) in THF (20 ml) and stirred at room temperature until reaction starts (3 – 5 minutes). Gentle reflux was maintained by cooling in a room temperature water bath and after the reaction had begun to subside (20 minutes) further THF (25 ml) added, and reaction stirred for 2 hours. Further THF (25 ml) was added, the fine precipitates allowed to settle, and the supernatant transferred to a flame dried 150 ml schlenk tube using a canular. The concentration of the phenyl-MgBr was assumed to be 1.15 M.

A typical procedure for preparing PhZn was undertaken as follows ZnCl₂ (anhydrous grade, Aldrich chemical company 99.99%) (0.214 g, 1.575 mmol) in THF (5 ml) was placed in a 3-neck
25 ml r.b. flask, equipped with a stirrer bar under an atmosphere of dry argon. The flask was evacuated to a high vacuum and cautiously heated with a Bunsen flame, concentrating the heat on the solid ZnCl$_2$ until fusion occurred, accompanied by some sublimation (CAUTION, see safety note 1, below). The flask was then allowed to cool to room temperature and a positive pressure of argon applied. Dry THF (5ml) was added and flask stirred until complete dissolution. Phenyl-MgBr (1.3 ml, 1.514 mmol, of the ~1.15 M solution prepared as described above) was added by syringe to the ZnCl$_2$ solution, and the final solution was stirred for 30 minutes to give a solution assumed to contain Phenyl-Zn (1.514 mmol).

Note 1. CAUTION, IMPLOSION AND INJURY IS VERY POSSIBLE USING THIS PROCEDURE. ALL THE FOLLOWING SAFETY ADVICE MUST BE FOLLOWED. The round bottom flask should only be used if free from star cracks and imperfections. It is not advised that any larger volume flask be subjected to this heating regime. If possible, a transparent blast proof screen should be used, but as a minimum requirement, a blast resistant full face visor and thick protective gloves must be worn by the experimenter. Excessive heating should be avoided, and slow warming of the flask body is preferred. The m.p. of a good grade of ZnCl$_2$ is $\sim$ 350°C and heating to just attain the fusion point, in a high vacuum, is sufficient, in our experience to provide an entirely anhydrous sample.

During the course of the work described in Chapter 3, we investigated a number of methods preparing and handling zinc salts and reagents. The preparations described above describe our optimum available anhydrous conditions. We later found that the ZnCl$_2$ could be kept anhydrous in an oven, maintained at $\sim$ 150°C and rapidly weighed before being added as a solid to a prepared lithium (hetero)aryl without any apparent compromise in recovered yield. These variations are described below.

**Toluenyl-ZnCl**

In a flame dried 2-neck r.b. flask, equipped with a stirrer bar and under dry argon, 2-bromotoulene (190 µl, 1.575 mmol) in THF (5ml) (cooled in cryostat / acetone bath maintained at -78°C) was treated with n-BuLi (1.2 ml, 1.89 mmol, 1.6 M solution in hexanes) at -78°C and allowed to warm to room temperature over 15 minutes. ZnCl$_2$ (0.214 g, 1.575 mmol, taken
directly from oven) was added to the flask and final solution stirred for 30 minutes to give a solution of which was presumed contain 2-toluenyl-ZnCl (1.575 mmol), ready for use.

3,4,5-Trimethoxyphenyl-ZnCl

In a flame dried 2-neck r.b. flask, equipped with a stirrer bar and under dry argon, 3,4,5-trimethoxybromobenzene (389 mg, 1.575 mmol) in THF (5ml) (cooled in cryostat / acetone bath maintained at -78°C) was treated with n-BuLi (1.2 ml, 1.89 mmol, 1.6 M solution in hexanes) at -78°C and allowed to cool to room temperature over 15 minutes. ZnCl$_2$ (0.216 g, 1.575 mmol, taken directly from oven) was added to the flask and final solution stirred for 30 minutes to give a solution which was presumed to contain 3,4,5-trimethoxyphenyl-ZnCl (1.575 mmol).

Furan-2-yl-ZnCl

In a flame dried 2-neck r.b. flask, equipped with a stirrer bar and under dry argon, furan (115 µl, 1.575 mmol) in THF (5ml) (cooled in cryostat / acetone bath maintained at -78°C) was treated with n-BuLi (1.2 ml, 1.89 mmol, 1.6 M in hexanes) at -78°C and allowed to cool to room temperature over 15 minutes. ZnCl$_2$ (0.214 g, 1.575 mmol, taken directly from oven) was added to the flask and final solution stirred for 30 minutes to give a solution presumed to contain furan-2-yl-ZnCl (1.575 mmol).

Thiophen-3-yl-ZnCl and 2-zincyl-3-bromothiophene

In a flame dried 2-neck r.b. flask, equipped with a stirrer bar and under dry argon, 3-bromothiophene (148 µl, 1.575 mmol) in THF (5ml) (cooled in cryostat / acetone bath maintained at -78°C) was treated with n-BuLi (1.2 ml, 1.89 mmol, 1.6 M in hexanes) at -78°C and allowed to cool to room temperature over 15 minutes. ZnCl$_2$ (0.214 g, 1.575 mmol) was added to the flask and final solution stirred for 30 minutes to give a solution containing thiophene-3-yl-ZnCl and 3-bromothiophen-2-yl-ZnCl (1.575 mmol), as judged by the Negishi coupled products obtained.
[1-(Benzenesulfonyl)-1H-pyrrol-2-yl]ZnCl

In a flame dried 2-neck r.b. flask, equipped with a stirrer bar and under dry argon, 1-(benzenesulfonyl)pyrrole (351 mg, 1.575 mmol) in THF (5 ml) was treated with n-BuLi (1.2 ml, 1.89 mmol, 1.6 M in hexanes) at -78°C and allowed to cool to room temperature over 15 minutes. ZnCl₂ (0.214 g, 1.575 mmol) was added to the flask and final solution stirred for 30 minutes to give a solution presumed to contain [1-(benzenesulfonyl)-1H-pyrrol-2-yl]ZnCl (1.575 mmol).

{1-[(4-Methylbenzene)sulfonyl]-1H-indol-2-yl}ZnCl

In a flame dried 2-neck r.b. flask, equipped with a stirrer bar and under dry argon, 1-(benzenesulfonyl)pyrrole (214 mg, 0.788 mmol) in THF (5 ml) was treated with n-BuLi (0.76 ml, 0.788 mmol, 1.04 M in hexanes) at -78°C and allowed to cool to room temperature over 15 minutes. ZnCl₂ (0.107 g, 0.788 mmol) was added to the flask and final solution stirred for 30 minutes to give a solution presumed to contain {1-[(4-methylbenzene)sulfonyl]-1H-indol-2-yl}ZnCl (0.788 mmol).

3.3.2 Negishi coupling of quinoxalines

General procedure for the Negishi coupling

To a solution of Zn-substrate (1.575 mmol) in THF (5 ml) was added 2-benzenesulfonylquinoxaline (300 mg, 1.05 mmol) and PdCl₂(PPh₃)₂ (38.0 mg, 0.052 mmol, 5 mol%). The solution was heated to reflux and stirred under Ar for 18 hours, allowed to cool to room temperature, diluted with EtOAc (10 ml), filtered through a pad of silica gel and the pad washed further with aliquots of EtOAc (3×10 ml). The combined filtrate and washings were evaporated in vacuo on a rotary evaporator and subjected to flash chromatography on flash silica gel, eluting with 5% EtOAc/hexane.
2-Ethylquinoxaline (49)\textsuperscript{15}

Diethyl zinc (0.5 ml, 1.575 mmol, 2.86 M in toluene) was treated with 7 according to the general method and after purification by flash chromatography, eluting with 5% EtOAc/hexane, gave 2-ethylquinoxaline as a clear oil (97 mg, 58%); R\textsubscript{f} 0.40; δ\textsubscript{H} (CDCl\textsubscript{3}, 300 MHz) 1.45 (3H, t, J = 7.8 Hz), 3.06 (2H, q, J = 7.5 Hz), 7.73 (2H, m), 8.07 (2H, m) and 8.77 (1H, s); δ\textsubscript{C} (CDCl\textsubscript{3}, 100 MHz) 13.4, 29.6, 128.8, 128.9, 129.1, 129.9, 141.2, 142.2, 145.6 and 158.5. Physical and spectroscopic data agree with those reported elsewhere.\textsuperscript{15}

2-Phenylquinoxaline (50)\textsuperscript{17}

Phenyl-ZnCl (1.575 mmol) was treated with 7 according to the general method and after purification by flash chromatography, eluting with 5% EtOAc/hexane, gave 2-phenylquinoxaline as a white solid (209 mg, 95%); R\textsubscript{f} 0.35; m.p 73\textdegree C (Lit. 73-74\textdegree C); δ\textsubscript{H} (CDCl\textsubscript{3}, 300 MHz) 7.53 – 7.59 (3H, m), 7.75 – 7.81 (2H, m), 8.12 – 8.22 (4H, m) and 9.34 (1H, s); δ\textsubscript{C} (CDCl\textsubscript{3}, 100 MHz) 127.6, 129.1, 129.2, 129.6, 129.7, 130.2, 130.3, 136.8, 141.6, 142.3, 143.4 and 151.9, we expected 14C environments but only 12 were evident from the spectrum. It is likely that the symmetry on the phenyl ring is responsible for the few C environment on the spectra; m/z (LREI) 206.1 (M\textsuperscript{+}, 100). Physical and spectroscopic data agree with those reported elsewhere.\textsuperscript{17}

2-(o-Toluenyl)quinoxaline (51)\textsuperscript{19}

2-Toluenyl-ZnCl (1.575 mmol) was treated with 7 according to the general method and after purification by flash chromatography, eluting with 5% EtOAc/hexane, gave 2-(o-toluenyl)quinoxaline as a light yellow solid (123 mg, 55%); R\textsubscript{f} 0.34; m.p. 80 – 81\textdegree C (Lit. 81-82\textdegree C); δ\textsubscript{H} (CDCl\textsubscript{3}, 300 MHz) 2.48 (3H, s), 7.30 – 7.31 (3H, m), 7.47 (1H, m), 7.71 – 7.74 (2H, m), 8.07 – 8.11 (2H, m) and 8.94 (1H, s); δ\textsubscript{C} (CDCl\textsubscript{3}, 100 MHz) 20.4, 126.4, 129.0, 129.2, 129.5, 130.0, 130.1, 130.4, 131.3, 136.6, 137.0, 140.7, 142.0, 145.6 and 155.0; HRESI found 221.10742, C\textsubscript{15}H\textsubscript{13}N\textsubscript{2} requires 221.10733. Physical and spectroscopic data agree with those reported elsewhere.\textsuperscript{19}
2-(3,4,5-Trimethoxyphenyl)quinoxaline (52)

3,4,5-Trimethoxyphenyl-ZnCl (1.575 mmol) was treated with 7 according to the general method and after purification by flash chromatography, eluting with 5% EtOAc/hexane, gave 2-(3,4,5-trimethoxyphenyl)quinoxaline as a yellow solid (230 mg, 80%); Rf 0.28; m.p. 82 – 83°C; δH (CDCl3, 300 MHz) 3.90 (3H, s), 4.03 (6H, s), 7.44 (2H, s), 7.73 – 7.83 (2H, m), 8.12 – 8.20 (2H, m) and 9.30 (1H, s); δC (CDCl3, 100 MHz) 56.3, 61.0, 104.9, 129.0, 129.4, 129.5, 130.5, 132.1, 140.3, 141.3, 142.1, 143.0, 151.4 and 153.9; m/z (APCI, +ve) 298 [M+H+, 100]; (HREI Found: 297.12336. C17H17N2O3 require 297.12337)

2-(3-bromothiophen-2-yl)quinoxaline (53) and 2-(Thiophen-3-yl)quinoxaline (54)23

Thiophene-3-yl-ZnCl and 3-bromothiophen-2-yl-ZnCl (1.575 mmol) was treated with 7 according to the general method and after purification by flash chromatography, eluting with 5% EtOAc/hexane, gave 2-(3-bromothiophen-2-yl)quinoxaline as a brown solid (90 mg, 30%); Rf 0.30; m.p. 113°C; δH (CDCl3, 300 MHz) 7.15 (1H, d, J = 5.4 Hz), 7.50 (1H, d, J = 5.4 Hz), 7.77 (2H, m), 8.11 (2H, m) and 9.78 (1H, s); δC (CDCl3, 100 MHz) 109.7, 111.0, 127.1, 129.3, 129.4, 130.0, 130.5, 130.0, 141.2, 142.2, 142.9 and 146.9; m/z (LR-FAB) 291 (M+, 100), 293 (M+ + 2, 100).

and 2-(Thiophen-3-yl)quinoxaline as a yellow solid (60 mg, 30%); Rf 0.27; m.p. 91 – 92°C (Lit. 91°C); δH (CDCl3, 300 MHz) 7.21 (1H, dd, J = 5.1 and 3.6 Hz), 7.56 (1H, dd, J = 5.1 and 1.2 Hz), 7.74 (2H, m), 7.88 (1H, dd, J = 5.1 and 1.2 Hz), 8.10 (2H, m) and 9.25 (1H, s); δC (CDCl3, 100 MHz) 127.1, 128.5, 129.0, 129.1, 129.3, 130.0, 130.6, 141.1, 141.9, 142.1, 142.2 and 147.4; Physical and spectroscopic data agree with those reported elsewhere.23

2-(3-Bromofuran-2-yl)quinoxaline (55)

3-Bromofuran-2-yl-ZnCl (1.575 mmol) was treated with 7 according to the general method and after purification by flash chromatography, eluting with 5% EtOAc/hexane, gave 2-(3-Bromofuran-2-yl)quinoxaline as a yellow solid (60 mg, 21%); Rf 0.31; m.p. 92°C; δH (CDCl3,
300 MHz) 6.71 (1H, d, \( J = 1.8 \) Hz), 7.65 (1H, d, \( J = 1.8 \) Hz), 7.78 (2H, m), 8.17 (2H, m) and 9.56 (1H, s); \( \delta_C \) (CDCl\(_3\), 100 MHz) 102.3, 117.3, 129.0, 129.2, 130.1, 130.7, 140.7, 142.1, 142.2, 143.6, 144.8 and 146.6; \( m/z \) (LR-FAB) 274.8 (M\(^+\), 100), 276.8 (M\(^+\) + 2, 100).

2-(Furan-2-yl)quinoxaline (57)\textsuperscript{26}

Furan-2-yl-ZnCl (1.575 mmol) was treated with 7 according to the general method and after purification by flash chromatography, eluting with 5% EtOAc/hexane, gave 2-(Furan-2-yl)quinoxaline as an orange solid (150 mg, 73%); R\(_f\) 0.28; m.p. 100°C (Lit. 97-98°C); \( \delta_H \) (CDCl\(_3\), 300 MHz) 6.64 (1H, dd, \( J = 3.3 \) and 1.5 Hz), 7.33 (1H, d, \( J = 3.3 \) Hz), 7.70 – 7.75 (3H, m), 8.10 (2H, m) and 9.26 (1H, s); \( \delta_C \) (CDCl\(_3\), 100 MHz) 111.9, 112.5, 129.2, 129.4, 130.5, 141.3, 142.1, 143.9, 145.1 and 151.6; (Found: 196.06357, C\(_{12}\)H\(_8\)N\(_2\)O requires 196.06366). Physical and spectroscopic data agree with those reported elsewhere.\textsuperscript{26}

2-[1-(Benzenesulfonyl)-1H-pyrrol-2-yl]quinoxaline (58)

[1-(Benzenesulfonyl)-1H-pyrrol-2-yl]ZnCl (1.575 mmol) was treated with 7 according to the general method and after purification by flash chromatography, eluting with 5% EtOAc/hexane, gave 2-[1-(benzenesulfonyl)-1H-pyrrol-2-yl]quinoxaline as a brown solid (46 mg, 10%); R\(_f\) 0.31; m.p. 180°C; \( \delta_H \) (CDCl\(_3\), 300 MHz) 6.48 (1H, apparent t, \( J = 3.6 \) Hz), 6.77 (1H, dd, \( J = 3.6 \) and 1.5 Hz), 7.51 (2H, m), 7.63 (2H, m), 7.77 – 7.87 (5H, m), 8.12 (1H, m) and 9.07 (1H, s); \( \delta_C \) (CDCl\(_3\), 100 MHz) 112.9, 119.3, 126.9, 127.2, 128.9, 129.1, 129.3, 129.9, 130.3, 132.5, 133.8, 139.5, 141.1, 141.4 and 145.8, we expected 18C environments but only 15 were evident from the spectrum. It is likely that the symmetry on the phenyl ring is responsible for the few C environment on the spectra and some quaternary carbons might not be visible; (HRESI Found: 336.08136, C\(_{18}\)H\(_{14}\)N\(_3\)O\(_2\)\textsuperscript{32}S requires 336.08012)
-\{1-[(4-Methylbenzene)sulfonyl]-1H-indol-2-yl\}quinoxaline (59)

\{1-[(4-Methylbenzene)sulfonyl]-1H-indol-2-yl\}ZnCl (0.788 mmol) was treated with 7 according to the general method and after purification by flash chromatography, eluting with 5% EtOAc/hexane, gave -\{1-[(4-Methylbenzene)sulfonyl]-1H-indol-2-yl\}quinoxaline as a brown solid (36 mg, 15%); R_f 0.28; m.p. 191°C; \(\delta_H\) (CDCl_3, 300 MHz) 2.28 (3H, s), 7.11 (3H, m), 7.31 (2H, m), 7.41 – 7.54 (5H, m), 7.83 - 7.85 (2H, m), 8.15 – 8.27 (3H, m) and 9.27 (1H, s); \(\delta_C\) (CDCl_3, 100 MHz) 21.6, 116.3, 117.8, 121.8, 124.8, 126.2, 127.0, 129.4, 129.5, 130.2, 130.3, 130.4, 133.7, 138.2, 138.5, 141.5, 141.7, 145.1, 147.1 and 147.3, we expected 23C environments but only 20 were evident from the spectrum. It is likely that the symmetry on the phenyl ring is responsible for the few C environment on the spectra and some quaternary carbons might not be visible; (Found 400.11162, C_{23}H_{18}N_3O_2S requires 400.11142).

3.3.3 Negishi coupling on pteridines

General method 2

To the solution of Zn-reagent (3.15 mmo) in DMF/THF mixture (15 ml, 2:1) was added benzenesulfonyl-pteridine 27 (2.1 mmol, 900 mg), Pd_{2}dba_{3}CHCl_{3} (125 mg, 0.1575 mmol, 5 mmol%) and PPh_{3} (125 mg, 0.477 mmol). The final solution was warmed to 80°C and stirred under Ar for 2 hours, concentrated on a rotary evaporator and purified on flash silica, eluting with 5% MeOH/DCM.

4-Amino-2-(N,N-dimethylaminomethyleneamino)-6-(phenyl)pterin-4-yl (61)

Phenyl-ZnCl (3.15 mmol) was treated with 27 according to general method 2 to give 850 mg of mixed 60 and 61. Further purification on flash silica, eluting with 5% MeOH/DCM gave 4-amino-2-(N,N-dimethylaminomethyleneamino)-6-(phenyl)pterin-4-yl as an orange solid (494 mg, 80%); R_f 0.28; m.p. 180°C; \(\delta_H\) (d_6-DMSO, 300 MHz) 3.08 (3H, s), 3.19 (3H, s), 7.51 – 7.53 (3H, m), 8.41 – 8.43 (2H, m), 8.76 (1H, s), 9.60 (1H, s), the –NH_2 signal was too broad to be
assigned and a clean $^{13}$C NMR could not be obtained; $m/z$ (APCI) 294 (M + H$^+$, 100), 239 (80) (HRESI +ve mode Found: 294.14736, C$_{13}$H$_{16}$N$_7$ requires 294.14620)

4-Amino-2-(N,N-dimethylaminomethyleneamino)-6-(3,4,5-trimethoxyphenyl)pterin-4-yl (63)

3,4,5-Trimethoxyphenyl-ZnCl (3.15 mmol) was treated with 27 according to general method 2 to give 672 mg of mixed products 62 and 63. Further purification on flash silica, eluting with 5% MeOH/DCM gave 4-amino-2-(N,N-dimethylaminomethyleneamino)-6-(3,4,5-trimethoxyphenyl)pterin-4-yl as an orange solid (520 mg, 65%); R$_f$ 0.25; m.p. 160°C; $\delta$$_H$ (d$_6$-DMSO, 300 MHz) 3.08 (3H, s), 3.18 (3H, s), 3.74 (3H, s), 3.94 (6H, s), 7.66 (2H, s), 8.76 (1H, s), 9.59 (1H, s), the –NH$_2$ signal was too broad to be assigned and a clean $^{13}$C NMR could not be obtained; $m/z$ (APCI) 384 (M + H$^+$, 100), 329 (40) (HRESI +ve mode found: 384.17772, C$_{18}$H$_{22}$N$_7$O$_3$ requires 384.17786).

4-Amino-2-(N,N-dimethylaminomethyleneamino)-6-(furan-2-yl)pterin-4-yl (65)

Furan-2-yl-ZnCl (3.15 mmol) was treated with 27 according to general method 2 to give 700 mg of mixed products 64 and 65. Further purification on flash silica, eluting with 5% MeOH/DCM gave 4-amino-2-(N,N-dimethylaminomethyleneamino)-6-(furan-2-yl)pterin-4-yl as a dark-red solid (400 mg, 67%); R$_f$ 0.27; m.p.(dec) 300°C; $\delta$$_H$ (d$_6$-DMSO, 300 MHz) 3.06 (3H, s), 3.16 (3H, s), 6.75 (1H, m), 7.31 (1H, m), 7.42 (1H, m), 8.73 (1H, s) and 9.22 (1H, s), the –NH$_2$ signal was too broad to be assigned and a clean $^{13}$C NMR could not be obtained; $m/z$ (APCI) 284 (M + H$^+$, 100), 229 (30) (HRESI +ve mode Found: 284.1232, C$_{13}$H$_{14}$N$_7$O requires 284.1254).

2,4-Diamino-6-(phenyl)pteridine (69)

4-Amino-2-(N,N-dimethylaminomethyleneamino)-6-(phenyl)pterin-4-yl 61 (150 mg, 0.512 mmol) was treated with 1M NH$_3$/EtOH (8 ml, 1:1) and gently refluxed for 8 hours, allowed to cool to room temperature, acidified with AcOH and placed in a fridge (2°C) for 18 hours. The solution was filtered, crystals washed sequentially with water, EtOH and Et$_2$O to give 2,4-
diamino-6-(phenyl)pteridine as a dark green solid (70 mg, 57%); m.p. > 300°C; δ_H (d_6-DMSO, 300 MHz) 7.50 (3H, m), 8.18 (2H, m) and 9.30 (1H, s), the two –NH_2 signals were too broad to be assigned and a clean ^13C NMR could not be obtained; m/z (APCI) 239 (M + H^+, 100) (HRESI Found: 239.1046, C_{12}H_{11}N_6 requires 239.10397). Physical and spectroscopic data agree with those reported elsewhere.\(^{28}\)

6-(Phenyl)pterin (66)\(^ {27}\)

4-Amino-2-(N,N-dimethylaminomethyleneamino)-6-(phenyl)pterin-4-yl 61 (150 mg, 0.512 mmol) was treated with 1M NaOH/EtOH (8 ml, 1:1) and gently refluxed for 8 hours, allowed to cool to room temperature, acidified with AcOH and placed in a fridge (2°C) for 18 hours. The solution was filtered, crystals washed sequentially with water, EtOH and Et_2O to give 6-(phenyl)pterin as a yellow solid (60 mg, 61%); m.p. > 300°C; \(\lambda_{\text{max}}\) (nm) 296, 369; δ_H (d_6-DMSO, 300 MHz) 7.51 (3H, m), 8.12 (2H, m) and 9.26 (1H, s), the –NH_2 and –NH signals were too broad to be assigned and a clean ^13C NMR could not be obtained; m/z (APCI) 239 (M^+, 100). Physical and spectroscopic data agree with those reported elsewhere.\(^ {27}\)

6-(3,4,5-Trimethoxyphenyl)pterin (67)

4-amino-2-(N,N-dimethylaminomethyleneamino)-6-(3,4,5-trimethoxyphenyl)pterin-4-yl (63) (150 mg, 0.391 mmol) was treated with 1M NaOH/EtOH (8 ml, 1:1) and gently refluxed for 8 hours, allowed to cool to room temperature, acidified with AcOH and placed in a fridge (2°C) for 18 hours. The solution was filtered, crystals washed sequentially with water, EtOH and Et_2O to give 6-(3,4,5-Trimethoxyphenyl)pterin as a green-yellow solid (74 mg, 59%); m.p. > 300°C; δ_H (d_6-DMSO, 300 MHz) 3.72 (3H, s), 3.89 (6H, s), 7.38 (2H, s) and 9.23 (1H, s), the –NH_2 and –NH signals were too broad to be assigned and a clean ^13C NMR could not be obtained; \(\lambda_{\text{max}}\) (nm) 305, 379; m/z (APCI) 330 (M + H^+). Attempts to get high resolution mass measurements were unsuccessful in both HREI and HRESI instruments.
\[ 6-(\text{furan-2-yl})\text{pterin (68)} \]

4-amino-2-(\(N,N\)-dimethylaminomethyleneamino)-6-(\text{furan-2-yl})pterin-4-yl (65) (150 mg, 0.530 mmol) was treated with 1M NaOH/EtOH (8 ml, 1:1) and gently refluxed for 8 hours, allowed to cool to room temperature, acidified with AcOH and placed in a fridge (2°C) for 18 hours. The solution was filtered, crystals washed sequentially with water, EtOH and Et\(_2\)O to give \(6(\text{furan-2-yl})\text{pterin}\) as a brown solid (76 mg, 63%); m.p. > 300°C; δ\(_H\) (d\(_6\)-DMSO, 300 MHz) 6.68 (1H, apparent s), 7.14 (1H, apparent s), 7.86 (1H, apparent s) and 8.97 (1H, s), the –NH\(_2\) and –NH signals were too broad to be assigned and a clean \(^{13}\)C NMR could not be obtained; \(\lambda_{\text{max}}\) (nm) 309, 395; \(m/z\) (APCI) 230 (M + H\(^+\), 100). Attempts to get high resolution mass measurements were unsuccessful in both HREI and HRESI instruments.

### 3.4 References


Chapter 4

An investigation of Suzuki coupling of quinoxaline and pteridine-O-sulfonates
Chapter 4 An investigation of Suzuki coupling of quinoxaline and pteridine-O-sulfonates

4.1 Introduction

The Suzuki cross-coupling reaction, sometimes referred to as Suzuki-Miyaura reaction, was first reported in 1979 by Suzuki and Miyaura\(^1\) as a cross-coupling reaction between an aryl- or vinylboronic acid with an aryl- or vinyl-halide, catalyzed by a palladium (0) complex. The reactions have received much attention and have been used to produce olefins, styrenes, substituted biphenyls and bi-aryls. Many key steps in natural product synthesis have included the Suzuki coupling reaction.\(^2,3\) A recent Reaxys database search (December 2010) revealed over 100 000 hits of reactions employing the Suzuki coupling conditions and the pioneer of the reaction Professor Akira Suzuki was awarded the Nobel prize in chemistry for 2010.

\[
\begin{align*}
R\underset{\text{B(OH)}_2}{\xrightarrow{\text{Pd(0)}}} R'X' & \quad \xrightarrow{\text{Pd(0)}} \quad R\underset{\text{R'}}{\xrightarrow{\text{X}' = \text{Cl, Br, I, OTf}}} \end{align*}
\]

**Scheme 4.1**

The Suzuki reaction was reported shortly after the discovery of the Kumada\(^4\) and Negishi\(^5\) coupling reactions where it was demonstrated that organomagnesium and organozinc substrates can be coupled, in the presence of palladium catalyst, with organohalide substrates. The replacements of the metals (Zn and Mg) with metalloid boron (B) lead to the development of the Suzuki reaction. Over the past three decades, much attention to improve the Suzuki reaction has been given by many research groups. The boronic acid group has been replaced by boronic esters, which made the organoboron substrates more reactive and thus improving the yields.\(^2,3,6,7\) The ligands for the palladium complex (usually triphenylphosphine) is replaced by more bulky, electron-rich phosphine ligands\(^8-13\) (Figure 4.1) which have been shown to improve the reaction rates and allowed for coupling reactions between less reactive substrates.
Figure 4.1 Electron rich phosphines for Suzuki coupling

The use of sulfonyloxy ligands as alternative to the halogens and triflate leaving group has received recent attention. Aryl-sulfonates and mesylates have been shown to undergo Suzuki coupling reactions with the less reactive boronic acids in high yield reactions\(^8\text{-}\text{12}\) and in some cases the reactions have been shown to occur in water.\(^\text{14}\) In all these reactions, the bulky electron-rich phosphine ligands were required to achieve these results. With these recent developments, we were interested in investigating Suzuki coupling reactions on benzenesulfonyl-pteridine 27 where model studies will first be performed on the benzenesulfonyl-quinoxaline 7. To our knowledge there are no Suzuki coupling reactions involving pteridine or pterin substrates, reported to date (Reaxys database search Nov 2010).

The reaction conditions for Suzuki coupling and those of previously reported coupling reactions, i.e. Sonogashira and Negishi, are similar. All these reactions can be performed in THF and DMF, they all require palladium catalyst and all require similar leaving groups i.e. halogens and sulfonates. The rate determining step in Suzuki reactions is the transmetallation step between palladium and boron, and this step is very slow compared to transmetallation on Negishi reactions. Suzuki reactions are usually performed at temperatures above 80°C while Negishi reactions are usually performed at room temperatures. Suzuki reactions have the advantage of being tolerant to wide range of functional groups unlike the Negishi and Kumada coupling reactions.

The drawback in Suzuki reactions, like most coupling reactions, is that the boronic acid-reagents usually have to be prepared from Li-reagents. This might limit the type of functional groups to be attached on the boronic acid-reagents. In this chapter, we report Suzuki coupling reactions on
benzenesulfonyl-quinoxaline and the challenges faced when attempting the coupling on benzenesulfonyl-pteridine.

4.2 Results and Discussion

The Suzuki coupling reactions usually require the presence of a relatively strong base such as NaOH and potassium carbonate (K₂CO₃), to make the organoboranes more reactive. The negatively charged base coordinates with the boron atom and increase the nucleophilic character of the organic group attached and thus increases its reactivity.¹⁻⁹ The presence of inorganic base will not be suitable for the pteridine reactions as they have been shown to remove the protecting groups and also hydrolyze pteridines to pterins. We were interested in using Et₃N, a mild base, used for Sonogashira coupling reactions to avoid the problem of deprotection. Research has shown the use of Et₃N as base in a number of Suzuki coupling reaction but, these reactions employed Pd catalysts possessing bulky phosphine ligands.¹⁵

Starting with the benzenesulfonyl quinoxaline 7 and treating with phenylboronic acid, Pd₂(dba)₃·CHCl₃, PPh₃ and Et₃N and refluxing in THF under Ar for 20 hours when no trace of starting material was detected on TLC. Purification by flash chromatography gave one isolated compound which had ¹H NMR and mass spectra identical to those previously found for 2-phenylquinoxaline 50. The product was obtained in 78% (Scheme 4.2). The results were very promising as the conditions used are very similar to those used for Sonogashira coupling reactions (Chapter 2, section 2.2.1).

Using the same conditions, quinoxaline 7 was treated with 2-formylphenyl boronic acid to give 2-(quinoxalin-2-yl)benzaldehyde 70 in 20% yield after refluxing in THF for 20 hours, when consumption of starting material was evident by TLC analysis. ¹H NMR and mass spectral analysis of the compound were in agreement with the desired structure. The accurate mass measurement gave a molecular ion peak at m/z 234.0785 amu which is in agreement to that calculated for C₁₅H₁₀N₂O, while the ¹H NMR gave two downfield protons at 8.10 and 9.85 ppm which are consistent with the 3(H) and aldehyde proton, respectively. Attempts to improve the yields included changing the catalyst to (Pd(PPh₃)₄, changing the phosphine ligands and changing the solvent but, no improvements were observed.
Scheme 4.2

We investigated variation of the Pd-catalyst. When the coupling of the quinoxaline 7 and phenylboronic acid was repeated employing Pd(PPh$_3$)$_4$ as the catalyst, the yield of 50 was reduced to 50% yield. Replacing triphenylphosphine with a phosphonic acid diester ligand, 2-oxo-4,4,5,5-tetramethyl-1,2,3-dioxaphospholane 71 (Figure 4.2),$^{16}$ shown to be a better ligand in a Kumada coupling reaction,$^{17}$ did not improve the yields. The yield of 50 was reduced to 40%. Changing the solvent from THF to DMF also resulted in a reduced yield where 50 was obtained in 50% yield. In all these reactions, no starting materials were detected on TLC and were never recovered during purification.

Figure 4.2

We then investigated the benzenesulfonyloxy-O-pteridine 27 as a coupling partner in Suzuki reactions. Treatment of the benzenesulfonyl pteridine 27 with phenylboronic acid, in the above mentioned conditions, did not give the desired 6-phenylpteridine 60. Increasing the reaction time to 4 days could not improve on the yields. HPLC-MS studies on the reaction mixture showed the presence of two main species, i.e. fully deprotected starting material (m/z 319, M + H) and fully deprotected product (m/z 243, M + H), with the fully deprotected starting material as main
component (Figure 4.3). With these results, we concluded that Suzuki coupling reactions can not be performed on pteridines under these conditions. The mild basic conditions did not give the desired coupled products and we envisage that the use strong base such as NaOH will result in hydrolysis of the amidine protecting group on the pteridine and no further investigations were performed.

Figure 4.3 HPLC-UV and TIC chromatograms for the attempted Suzuki coupling of pteridine 27, along with the extracted APCI mass spectrum.

For future work, an attempt to perform the Suzuki coupling reaction in aqueous base medium will be investigated. In these reactions, fully deprotected pteridines will be used and they have been shown to be soluble in basic and acidic mediums. For Suzuki reactions to occur, an inorganic base is required to make the boronic acid more reactive and this in turn make the solvent medium to be basic. The success of these reactions will eliminate the use of protecting groups.
4.3 Experimental

2-Phenylquinoxaline 50

2-Benzensulfonylquinoxaline 7 (100 mg, 0.35 mmol), Pd$_2$dba$_3$CH$_3$Cl (20.11 mg, 1.75 x 10$^{-5}$ mmol, 5 mol%), PPh$_3$ (8.75 x 10$^{-5}$ mol, 23 mg), phenylboronic acid (64 mg, 0.525 mmol, 1.5 eq.) and Et$_3$N (0.2 ml), were dissolved in dry THF (5 ml). The final solution was heated to reflux and stirred under Ar for 20 hours, allowed to cool to room temperature, diluted with EtOAc (10 ml), filtered through silica gel and purified on flash silica, eluting with 5% EtOAc/hexane to give pure 2-phenylquinoxaline as a white solid (42 mg, 78%). The material had identical m.p., $^1$H NMR and mass spectra to the 2-phenylquinoxaline prepared earlier and the full characterization in Chapter 3.

2-(Quinoxalin-2-yl)benzaldehyde (70)

2-Benzensulfonylquinoxaline 7 (200 mg, 0.70 mmol), Pd$_2$dba$_3$CH$_3$Cl (41 mg, 3.5 x 10$^{-5}$ mmol, 5 mol%), PPh$_3$ (17.5 x 10$^{-5}$ mol, 46 mg), phenylboronic acid (157.4, 1.05 mmol, 1.5 eq.) and Et$_3$N (0.2 ml), were dissolved in dry THF (5 ml). The final solution was heated to reflux and stirred under Ar for 20 hours, allowed to cool to room temperature, diluted with EtOAc (10 ml), filtered through silica gel and purified on flash silica, eluting with 5% EtOAc/hexane to give pure 2-(quinoxalines-2-yl)benzaldehyde as an off-white solid (30 mg, 20%); R$_f$ 0.30; $^\nu_{\max}$ / cm$^{-1}$ 1670; $^\delta_H$ (CDCl$_3$, 300 MHz) 7.35 – 7.43 (4H, m), 7.61 – 7.72 (4H, m), 8.10 (1H, dd, $J$ = 7.5 and 1.2 Hz) and 9.85 (1H, s); $^\delta_C$ (CDCl$_3$, 100 MHz) 125.5, 128.4, 128.6, 129.0, 130.5, 131.7, 133.4, 134.6, 141.2, 143.3 and 191.0; m/z (LREI) 234 (M$^+$, 70), 181 (100) and 147 (40) (Found 234.0785. C$_{15}$H$_{10}$N$_2$O requires 234.0793).

4.4 References

Chapter 5

Tetramethylformamididine Chloride (TMFAC) as a potential diaminocarbene
Chapter 5  Tetramethylformamidine Chloride (TMFAC) as a potential diaminocarbene

5.1 Introduction

Carbenes are defined as neutral compounds of divalent carbon where the carbon atom has only six valence electrons, instead of eight. Carbenes are classified as either singlets or triplets depending on their electronic structure. The triplet carbenes have an sp-hybridized carbon and a linear geometry while the singlet carbenes have an sp$^2$-hybridized carbon and a non-linear geometry. Triplet carbenes have two unpaired electrons and are sometimes referred to as diradicals while singlet carbenes have paired electrons and are relatively more stable than triplets. Factors that favor triplets over singlets include steric and electronic effects of the substituents at the carbene carbon atom. The singlet state is stabilized by σ-electron withdrawing (X), generally more electronegative substituents while σ-electron donating (Z) triplet state. Substituents that can donate π-electrons to the carbene carbon stabilize the singlet state with the opposite applying to the triplet state. The most common atoms or substituents that stabilize the singlet state are the halogens, oxygen-containing and nitrogen-containing groups with the later most common. Triplets state is commonly stabilized by Li, BH$_2$ and BeH.

\[ \text{R} \sim \text{R} \]

\( \text{R} = \text{Z, triplet} \)
\( \text{R} = \text{X, singlet} \)

Figure 5.1

The first generations of carbenes were the simple methylene and dichloromethylene carbenes which were and still used in the formation of cyclopropanes. Methylene is generated in situ from diazomethane under heat or UV radiation while dichloromethylene is generated in situ from the treatment of chloroform with a strong base (Scheme 5.1). These carbenes, however, are never isolated and are only studied in situ. Introduction of stabilizing groups on the carbene carbon atom i.e. σ-electron withdrawing and π-electron donating, led to the formation of more stable
carbenes with half lives of more than 1 minute. The nitrogen containing carbenes, in particular the N-heterocyclic carbenes, were widely explored and the first isolated carbene was imidazol-2-ylidene in 1991.\(^5\)

\[
\text{Scheme 5.1}
\]

N-Heterocyclic carbenes (NHC) have been shown to make stable metal complexes and these complexes have found many applications in C-C cross-coupling reactions,\(^6\)–\(^10\) ring opening polymerization reactions\(^11\) and many other reactions\(^1\). NHC have been used as an alternative to phospnine ligands in Suzuki\(^6,7\) and Negishi\(^8\) cross-coupling reactions and in all these reactions, the NHC were shown to be superior ligands. The catalyst loading, reaction time and temperature are also reduced by the introduction of NHC. Perhaps the most notable use of NHC have been in the olefin metathesis reactions, which have been a subject of comprehensive review articles over the last years.\(^12,13\) The development of carbene-metal complexes for olefin metathesis proved to be an organometallic success story leading to the award of the 2005 Nobel Prize to Chauvin, Grubbs and Schrock.

NHC have been widely studied due to their relative stability and ease of preparation, however, recent studies have shown the N-heteroacyclic carbenes (NHAC) carbenes to be better ligands than NHC and much work is currently underway to prepare stable NHAC.\(^14\)–\(^18\) Challenges that are faced with the preparations of NHAC is the relative ease of dimerization of the carbenes in solution resulting in low concentrations of metal-carbene complexes. Tetraalkylformamidinium salts have been used to prepare carbenes with the more stable carbenes coming from bulky alkyl groups while dimer formation is prevalent with the small alkyl groups. In this chapter we report attempts to prepare bis(dimethylamino)carbene \(^72\) from tetramethylformamidinium chloride (TMFAC) \(^73\) using reported methods for the preparation of carbenes.
5.2 Results and Discussion

The most common procedure for preparing metal-carbene complexes is by treatment of the carbene precursor with silver oxide (Ag₂O) to generate silver-carbene complex then transmetallation with transition metal of choice to give the required complex. The reactions proceed smoothly in THF and DCM but, to our knowledge, these reactions have only been reported on NHC. Mechanistic studies have shown the Ag₂O to act as a base generating the carbene which is then complexed by the generated silver ion. Treatment of 73 with Ag₂O in DMF under Ar did not yield the desired carbene complex and no starting material was recovered. Changing the temperature, counter ion and reaction times gave no improvement. The use of THF as solvent also gave similar observations. The absence of starting material and carbene-complex could imply that 72 could be forming and quickly dimerize, and the dimerized product lost during the work-up stages. The dimerized product, tetrakis-(dimethylamino)ethylene 74 is a low boiling liquid which is very reactive towards oxygen and water making it difficult to isolate. We did not have instrument techniques that enabled the study of the reaction in situ under inert conditions and thus we could not validate our hypothesis.

Scheme 5.2
Another common procedure for preparing carbenes is treatment of the carbene precursor with a strong base, usually alkyl-lithium bases, and complexing the carbene with metal of choice to give the required complex. NHAC have been prepared using this method, although free carbenes have never been isolated however, reported *in situ* studies have shown the relative stability and rate of dimerization of these carbenes.\textsuperscript{15,16} Bis(dimethylamino)carbene 72 has been reported by Alder\textsuperscript{15} to be stable in THF solution for up to 1 minute but no complexation to transition metals was reported. We subjected 73 to the same conditions reported by Alder by treatment with n-BuLi at \(-78^\circ\text{C}\) to generate the carbene and added various metals to the solution but no desired carbene-metal complexes were isolated. The metals investigated were palladium, silver, nickel and copper. No starting material was recovered suggesting that dimerization could have occurred as previously shown by Alder that dimerization occurs easily on tetraalkylaminocarbenes.\textsuperscript{15}

Finally, we investigated the use of Bredereck’s reagent 24 as precursor for the formation of carbene 72. The studies were inspired by the work of Waymouth *et al.*\textsuperscript{25} where the 24 was used as catalyst in a ring-opening polymerization and tranesterification reactions. The proposed mechanism was that an equilibrium exists at high temperatures, between the carbene 72 and 24 (Scheme 5.3) with the carbene responsible for ring-opening.

![Scheme 5.3](image)

The Bredereck’s reagent was heated with PdCl\(_2\) in THF under Ar for 18 hours but no metal-carbene complex was formed and no products were isolated. Attempts to prepare complexes with Ni, Ag and Cu were unsuccessful with no products isolated.

In conclusion, attempts to synthesize bis(diamino)carbene 72 from TMFAC and the Bredereck’s reagent were unsuccessful.
5.3 References


