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Synthesis, Analysis and Biological Evaluation of Heterocyclic Drugs

A thesis submitted to University of Sussex

By

Mohammed Abdulwahhab M Baashen

In Candidature of

Doctor of Philosophy

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School of Life Sciences

University of Sussex

DECLARATION

This work has not previously been accepted in substance for any degree and is not being concurrently submitted for candidature for any degree.

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ABSTRACT

Chapter 1: Chapter One provides an overview on the Bohlmann-Rahtz pyridine synthesis. New procedures, implementing metal based Lewis acids, Brønsted acids and metal-free Lewis acid catalysts have been used in this process. Also, new one-pot two- and three-component methodologies have been developed for the synthesis of various natural products containing the pyridine motif and these have been compared and contrasted.

This chapter also discusses signalling pathways in Werner syndrome cells. The inhibitor SB203580 has been shown to prevent the phosphorylation of the p38 α kinase in a ATP competitive manner and this implicates this mechanism in accelerated ageing and gives potential to the prospect of targeting this pathway in a drug discovery programme, if better mechanistic understanding can be garnered.

Chapter 2: Chapter Two discusses the Bohlman–Rahtz synthesis of various substituted pyridines. The process has been modified to be simple, involves mild conditions and provides the heterocyclic targets in high yield. We have shown that substituted pyridines could be synthesised efficiently under microwave conditions using a relatively short reaction time. The process was also successful for the production of a range of fused heterocycles containing the pyridine moiety in high yield, including pyrido[2,3-*d*]pyrimidin-4(3*H*)-ones and pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones.

Chapter 3: Chapter Three describes the efficient synthesis of the p38 MAPK inhibitor UR-13756 using a Hantzsch-type three component cyclocondensation. Microwave irradiation of a mixture of 3-amino-1-methylpyrazole hydrochloride, 1-(4-fluorophenyl)-2-(pyridine-4-yl)ethanone and 4-fluorobenzaldehyde for 4 hours in ethanol under acidic catalytic conditions provided UR-13756 in high yield (71%) after purification by column chromatography.

Chapter 4: Chapter Four shows the synthesis of 4-(3-amino-1-(4-methoxyphenyl)-1*H*-pyrazol-4-yl)benzamide in three steps by the use of rigorous experimental procedures under microwave conditions. This technique led to faster heating rates and allowed the rapid optimization of yields. These advantages were observed in all steps and allow formation of products in high yields. Biological study of the inhibitor 4-(3-amino-1-(4-methoxyphenyl)-1*H*-pyrazol-4-yl)benzamide showed, by ELISA analysis, that p38 α signalling was inhibited in control dermal cells. Some progress was made towards the synthesis of 3-amino-4-[1-(3-1*H*-pyrazol-4-yl)]benzamide.

Chapter 5: Chapter Five investigates the synthesis of the chemotherapeutic agent RO3201195, a highly selective inhibitor of p38 α , in seven steps under microwave conditions. The procedure provides a relatively high overall yield of the desired product and all other intermediates involved in individual steps compared with conventional heating methods.

Chapter 6: Chapter Six provides the experimental procedures and various spectroscopic data for the synthesized compounds.

ABBREVIATIONS

A	Anisomycin
app	Apparent
APcI	Atmospheric pressure chemical ionization
Ar	Unspecified aromatic aryl substituent
<i>c</i>	Concentration
CDCl ₃	Deuterated chloroform
Column chromatography	Flash column chromatography
d	Doublet
DMF	<i>N,N</i> -dimethylformamide
DMFDMA	Dimethylformamide dimethyl acetal
DMSO	Dimethyl sulfoxide
EI	Electron Impact
ELISA	Enzyme linked immuno-sorbant assay
Equiv.	Equivalent
ERK	Extracellular signal regulated kinase
ES	Electrospray
g	Gram(s)
GHz	Gigahertz
h	Hour(s)
HSP27	Heat shock protein 27
Hz	Hertz
IC ₅₀	Concentration of an inhibitor that is required for 50% inhibition of an enzyme <i>in vitro</i>
ICAM-1	Adhesion molecules
IL-1β	Interleukin-1β
IR	Infra red

<i>J</i>	Coupling constant (in Hz)
JNKs	Jun–NH ₂ –terminal kinases
<i>k_d</i>	Dissociation constant
L	Laevorotatory
LHMDS	lithium bis-trimethylsilylamide
light petrol	Petroleum ether 40–60 °C
m	Multiplet
<i>m</i>	<i>meta</i>
MAOS	Microwave–assisted organic synthesis
MAP	Mitogen activated protein
MAPK	Mitogen activated protein kinase
MAPKAPK–2 (MK2a)	MAPK activated protein kinase 2a
MAPKK	Mitogen activated protein kinase kinase
MAPKKK	Mitogen activated protein kinase kinase kinase
Me	Methyl
MHz	Megahertz
min	Minute(s)
MK2a (MAPKAPK2)	MAPK–activated protein kinase 2a
MKK3	Mitogen activated protein kinase kinase 3
MKK6	Mitogen activated protein kinase kinase 6
mL	Millilitre
mmol	Millimole(s)
mol	Mole(s)
mp	Melting point
MS	Low–resolution mass spectrometry
MW	Microwave irradiation
nM	Nanomolar

NMR	Nuclear magnetic resonance
<i>p</i>	<i>para</i>
<i>p</i> -TsOH	<i>para</i> -toluenesulfonic acid monohydrate
p38	Protein 38
p38 α MAPK	Protein 38 α mitogen activated protein kinase (isoform α)
p38 α :MK2a	Protein 38 α mitogen activated protein kinase activated protein kinase
Ph	Phenyl
pHSP27	Phosphorylated heat shock protein 27
pp38	Phosphorylated protein 38
R	Specified substituent
R_f	Retention factor
RT	Room temperature
s	Singlet
SAR	Structure–activity relationship
t	Triplet
<i>tert</i>	Tertiary
TFA- <i>d</i>	Deuteriotrifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TNF α	Tumor necrosis factor- α
W	Watt
WRN	Werner gene (defective) encoding the disease
WRNp	Werner protein
WS	Werner Syndrome
vs	versus
μ M	Micromolar

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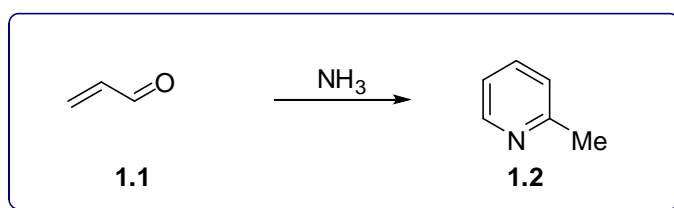
CHAPTER ONE: INTRODUCTION

Chapter One

Introduction

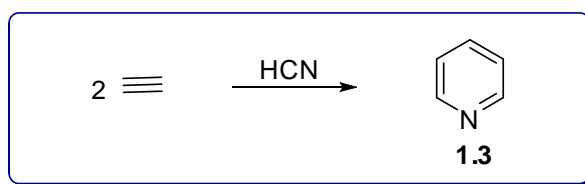
1.1 The discovery of pyridine

In 1846 the first pyridine base, picoline, was isolated from bone oil by Thomas Anderson.¹ The chemistry of pyridine was born when Wilhelm Körner (1869) and James Dewar (1871) independently formulated a monoaza analogue of benzene.² Understanding the structure of pyridine enabled a number synthetic processes to be developed. The synthesis of 2-picoline (**1.2**; Scheme 1.1) albeit in low yield was reported by Baeyer^{3,4} from the reaction of acrolein (**1.1**) with aqueous ammonia (Scheme 1.1).



Scheme 1.1: Synthesis of 2-picoline (**1.2**) from acrolein (**1.1**) and ammonia

In 1876 the laboratory synthesis of pyridine (**1.3**) was discovered by Ramsey.⁵ Reaction of a mixture of acetylene and hydrogen cyanide in a red-hot tube gave the heterocycle **1.3** (Scheme 1.2).⁵ Large quantities of pyridine were obtained from natural sources *via* coal tar distillation.



Scheme 1.2: Synthesis of pyridine (**1.3**) from acetylene and HCN

In the 1930s Elvehjem and Koehn were able to isolate nicotinamide (**1.4**) and nicotinic acid (**1.5**) from vitamin B2 (Figure 1.1).⁶ Their discovery provided treatment for human pellagra, a vitamin deficiency causing dermatitis and dementia. One could chart that from that date, researchers started to pay attention to the synthesis and properties of pyridine derivatives.

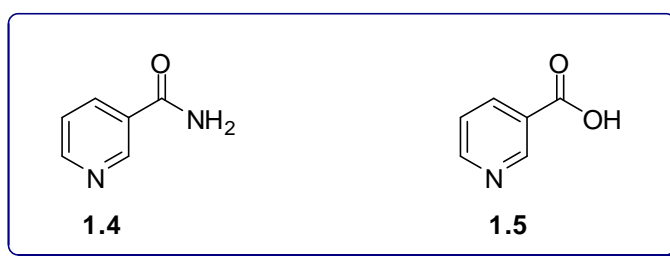


Figure 1.1: Structures of nicotinamide (**1.4**) and nicotinic acid (**1.5**)

The chemistry of pyridine provided fundamental understanding of the chemistry and properties of biological systems, since this heterocycle plays an important role in both biological and chemical coordination. The pyridine ring system is one of the most common heterocyclic motifs that is found to modulate various enzymes of living organisms. For example, nicotinamide adenine dinucleotide phosphate (NADP⁺: **1.6**; Figure 1.2) is intimately involved in various oxidation–reduction processes.⁷

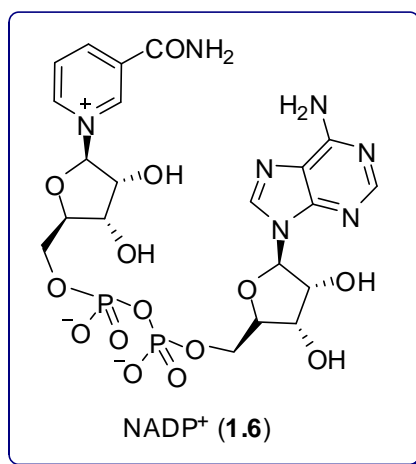


Figure 1.2: Structure of nicotinamide adenine dinucleotide phosphate (NADP⁺: **1.6**)

Also, the pyridine moiety is found in over 7000 pharmaceutical drugs such as the anti-tuberculosis drug **1.7**, the HIV inhibitor L-754,394 (**1.8**), agrochemicals **1.9–1.11** (Figure 1.2)⁸ and a large number of natural products.^{9–11}

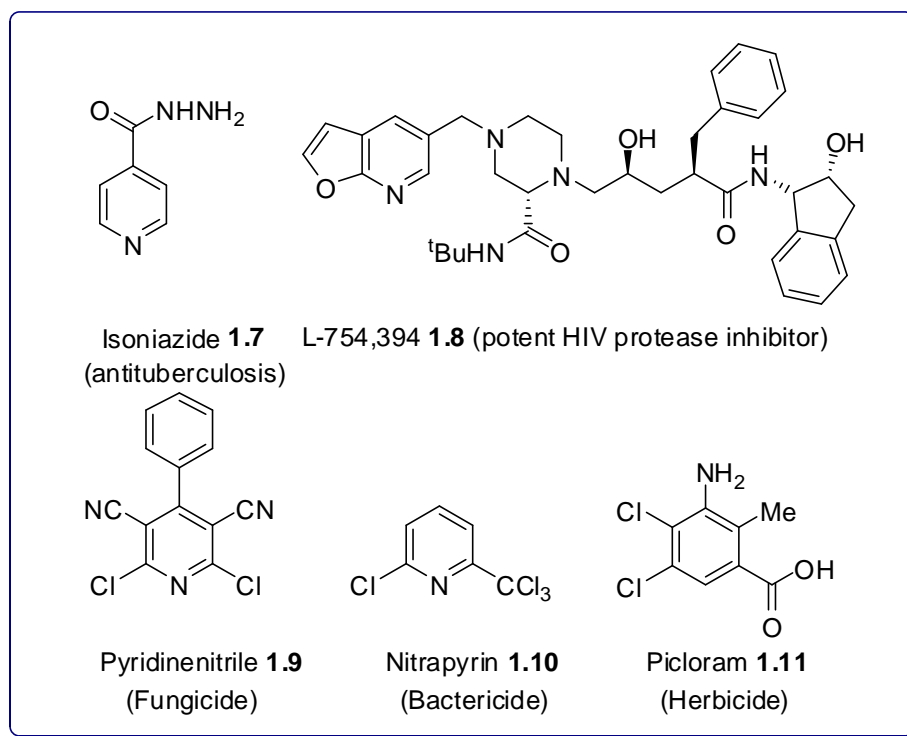


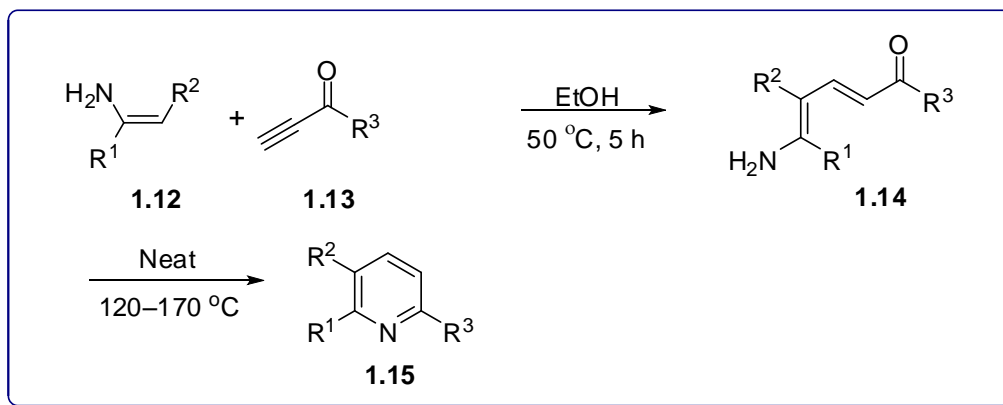
Figure 1.3: Structures of some pyridine pharmaceutical drugs and agrochemicals

1.2 The Bohlmann-Rahtz pyridine synthesis

1.2.1 Discovery

Various methods are available for the synthesis of pyridines.¹² One such method is the Bohlmann-Rahtz pyridine synthesis which was first discovered in 1957.¹³ Substituted pyridines were synthesized in a two-step process by the reaction of enamines **1.12** and alkynyl ketones or aldehydes **1.13** (Scheme 1.3). The first step involves production of the corresponding aminodiene intermediate **1.14** in high yield. The second step involves conversion of intermediates **1.14** to the corresponding

trisubstituted pyridines **1.15** (Scheme 1.3) at high temperature (120–170 °C).¹³ Compounds **1.15** were produced in excellent overall yields.



Scheme 1.3: The traditional Bohlmann-Rahtz pyridine synthesis

1.2.2 Recent improvements in methodology

The Bohlmann-Rahtz pyridine synthesis has been recognised as a valuable method for the synthesis of pyridine derivatives. The Bagley research group has developed various efficient processes for the synthesis of pyridines under microwave conditions for exploration of their biological properties.^{14–16} The Bohlmann-Rahtz process provides various tri- and tetrasubstituted pyridines with total control of regiochemistry. Many attempts have been made to improve the process so that it can be carried out under milder conditions. The following sections will highlight current developments in the Bohlmann–Rahtz synthesis of substituted pyridines.

1.2.2.1 Use of catalysts and solvents

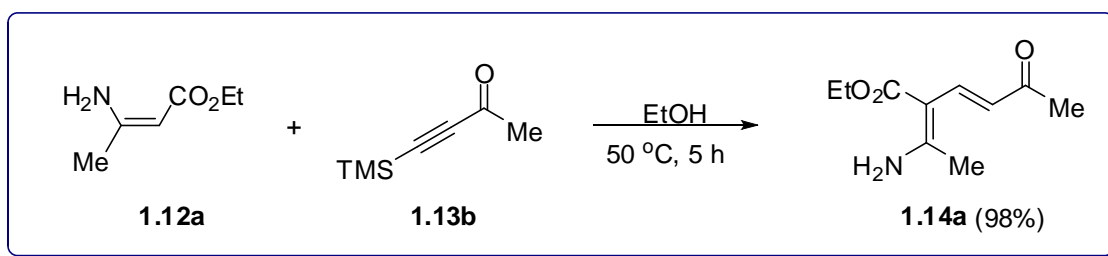
The scope and utility of the Bohlmann-Rahtz reaction¹⁷ has been developed to take place in a single step. In order to investigate these improvements, aminodienone **1.14a** was synthesised by standard Bohlmann-Rahtz methodology,¹⁷ from ethyl

β -aminocrotonate (**1.12a**) and but-3-yn-2-one (**1.13a**) in ethanol (EtOH) at 50 °C to give the corresponding cyclodehydration precursor in 98% yield. However, the availability and volatility of butynone **1.13a** prompted researchers to find alternative approaches for the production of **1.14a**. Therefore, a mixture of ethyl β -aminocrotonate (**1.12a**) and 4-(trimethylsilyl)but-3-yn-2-one (**1.13b**), which is cheaper, readily available and less volatile than **1.13a**, was heated to 50 °C in various solvents (Table 1.1).¹⁷

Table 1.1: Michael addition of **1.12a** and **1.13b** in various solvent at 50 °C

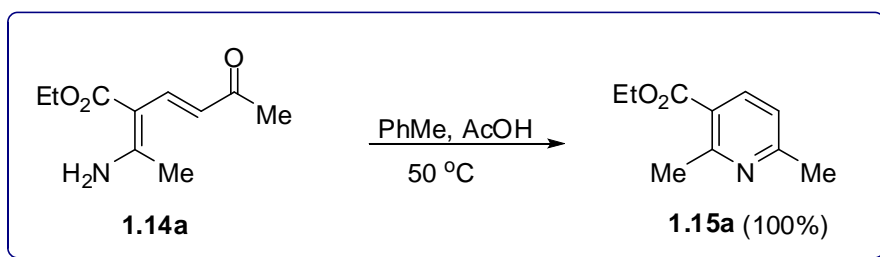
Solvent	Yield of 1.14a (%)	Solvent	Yield of 1.14a (%)
Acetone	No reaction	Diethyl ether	No reaction
Toluene	No reaction	Neat	No reaction
Dichloromethane	No reaction	Dimethylsulphoxide	59
Chloroform	No reaction	Ethanol	98

In most cases no reactions took place. However, when a mixture of **1.12a** and butynone **1.13b** was heated at 50 °C for 5 hours in EtOH (the solvent originally used by Bohlmann and Rahtz) or dimethylsulphoxide (DMSO) the corresponding aminodienone **1.14a** was produced in 98 or 59% yield, respectively (Scheme 1.4).



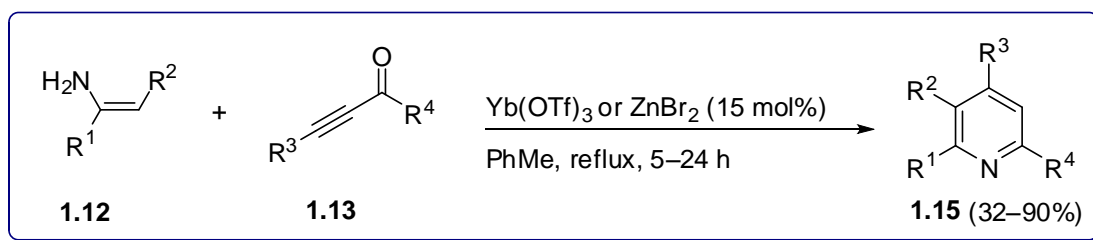
Scheme 1.4: Synthesis of aminodiene **1.14a** using a modified alkynone **1.13b**

The Bohlmann-Rahtz synthesis requires the use of a Michael donor (enamine) and a Michael acceptor (alkynone), therefore, in principle the use of a Brønsted¹⁸ (such as acetic acid, AcOH) or Lewis acid¹⁹ could affect the conjugate addition and the double bond isomerisation of aminodienone **1.14a**, and facilitate spontaneous cyclodehydration to pyridine **1.15a**. In this regard, the use of an acid catalyst could affect the reaction to take place could at lower temperatures than the traditional Bohlmann-Rahtz conditions. It was found that heating aminodienone **1.14a** at 50 °C in a mixture of toluene (PhMe) and acetic acid (in 5:1 by volume) gave pyridine **1.15a** in excellent yield (Scheme 1.5).^{18,19}



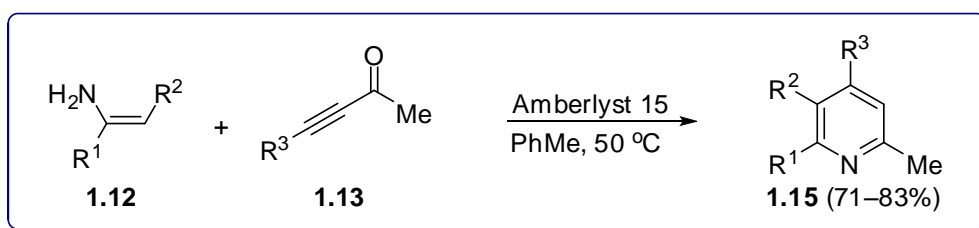
Scheme 1.5: Synthesis of pyridine **1.15a** from aminodienone **1.14a** by Brønsted acid catalysis

A range of Lewis acids (*e.g.* BF₃·OEt₂, FeCl₃, ZnBr₂, Yb(OTf)₃) have been used successfully as an alternative to Brønsted acids in this process.¹⁸ A range of substituted pyridines **1.15** were produced in high yields from aminodienone **1.14** under acidic conditions in a single step in heating at reflux in dichloromethane (Scheme 1.6).¹⁸ It was found that reactions catalyzed by zinc(II) bromide were faster and more efficient than those conducted in the presence of ytterbium(III) triflate.¹⁸



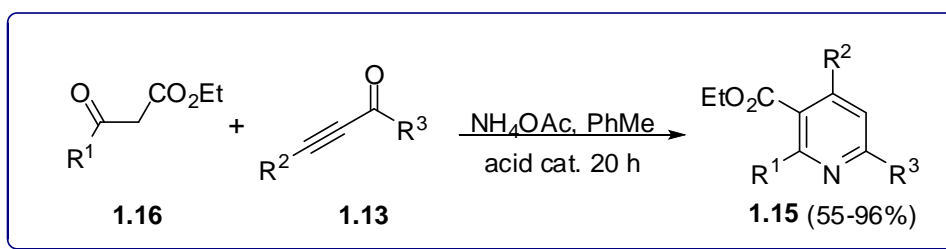
Scheme 1.6: One-step Lewis acid catalysis synthesis of substituted pyridines **1.15**

The conjugate addition/cyclodehydration of enamines **1.12** and alkynones **1.13** in toluene at 50 °C in the presence of Amberlyst-15 ion exchange resin for 26 hours have also been attempted (Scheme 1.7).¹⁸ This procedure was efficient and substituted pyridines **1.15** were produced in high yields (71–83%).¹⁸



Scheme 1.7: Synthesis of pyridines **1.15** using Amberlyst-15 ion exchange resin

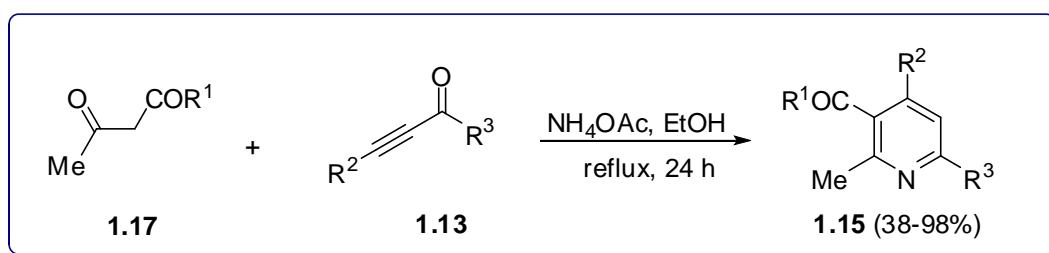
A three-component condensation of a range of β -keto esters **1.16**, alkynones **1.13** and ammonium acetate in excess (Scheme 1.8) provided a much more efficient approach towards a number of polysubstituted pyridines **1.15**.²⁰ The reaction involved the use of two mole equivalents of **1.13** to produce the corresponding substituted pyridine **1.15** in good to excellent yield (55–96%) in a single preparative step with total regiocontrol.²⁰



Scheme 1.8: Three component condensation reaction for the production of substituted pyridines **1.15** under acidic conditions

Clearly the results reported in Scheme 1.8 showed the scope of using cheap and commercially available 1,3-dicarbonyl compounds **1.16** with alkynones **1.13** to produce various substituted pyridines **1.15** in high yield.

A mild acid-free three-component condensation reaction for the production of substituted pyridines **1.15** has also been developed.²¹ Reaction of 1,3-dicarbonyl compounds **1.17** and alkynones **1.13** in ethanol under reflux conditions for 24 hours in the presence of ammonium acetate produced the corresponding substituted pyridines **1.15** in moderate to excellent yields (38–98%) after purification (Scheme 1.9).²¹



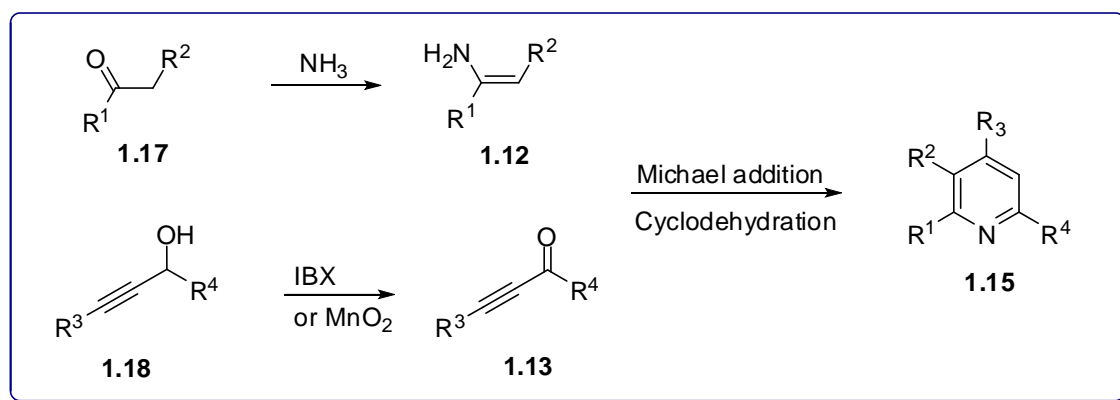
Scheme 1.9: Mild acid-free three-component condensation reaction for the production of substituted pyridines **1.15**

1.2.2.2 Tandem oxidation-heteroannulation Bohlmann-Rahtz reaction

The *in-situ* tandem oxidation-heteroannulation of propargylic alcohols **1.18** with either *o*-iodoxybenzoic acid (IBX) or manganese dioxide provided a new one-pot

tandem route to nitrogen-containing heteroaromatic building blocks. The process involved four separate synthetic transformations in a single preparative step.²² Various heteroannulation reactions provided high yields and with total regiocontrol of the pyridine **1.15** from either enamine **1.12** or β -keto ester **1.17** precursors.

The process involved heating an enamine **1.12**, generated beforehand from the reaction of **1.17** and ammonia, with a one-fold excess of both the propargylic alcohols **1.18** and *o*-iodoxybenzoic acid in dimethylsulphoxide-acetic acid (5:1) at 65 °C overnight to give the corresponding substituted pyridines **1.15** in 20–73% yields after purification (Scheme 1.10).²²



Scheme 1.10: Tandem oxidation-heteroannulation of propargylic alcohols for the production of substituted pyridines **1.15**

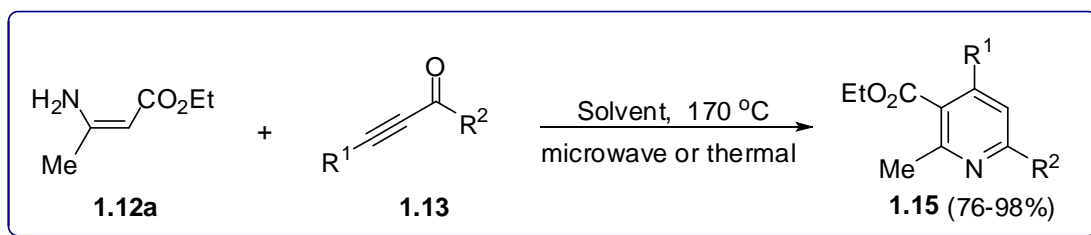
The efficiency of this process was found to be dependent on the nature of the propargylic alcohol. Low yields obtained in some cases could be attributed to oxidative degradation of **1.12** under the reaction conditions. Indeed, when a range of propargylic alcohols **1.18**, β -keto esters **1.17** and ammonium acetate were heated under reflux conditions in a toluene-acetic acid mixture (5:1 by volume) in the presence of manganese dioxide in a one-pot process, this gave the corresponding substituted

pyridines **1.15** (Scheme 1.10), in up to 96% yield and with total regiocontrol. Therefore, generating the enamine *in-situ* from the condensation of the corresponding β -keto ester **1.17** and ammonia was effective in reducing enamine degradation.²² The process represented in Scheme 1.10 is a convenient three component cyclocondensation that involves four transformations in a single preparative step.²²

1.2.2.3 Microwave synthesis

The use of microwave dielectric heating, known as microwave-assisted organic synthesis (MAOS), in synthetic chemistry is a valuable and convenient alternative to the conventional conductive heating methods.²³ Microwave-assisted chemistry can provide faster heating rates and improve reaction rates by carrying out transformations under sealed vessel conditions well above the boiling point of the reaction solvent. The popularity and reproducibility of microwave chemistry has increased dramatically due to recent advances in instrumentation that led to development of new synthetic reactions and rapid optimisation of existing procedures.^{24–29}

The Bohlmann-Rahtz reaction has been investigated under microwave-assisted conditions in a self-tuning single-mode CEM Discover™ Focused Synthesizer.³⁰ For example, reaction of a mixture of ethyl β -aminocrotonate **1.12a** and an excess phenylpropynones **1.13** in toluene or dimethylsulphoxide at 170 °C under microwave conditions gave the corresponding substituted pyridines **1.15** in 76–98% yields (Scheme 1.11) after 10–90 minutes.³⁰



Scheme 1.11: One-pot synthesis of substituted pyridines **1.15** via heteroannulation of enamine **1.12a** with alkynones **1.13** under microwave or thermal conditions

The use of dimethylsulphoxide which is a more polar solvent was found to be more efficient under microwave irradiation and resulted in a shorter reaction time (10 min) compared with the use of toluene (90 min). Reactions conducted in toluene could be accelerated in the presence of zinc(II) bromide (15 mol%) as a Lewis acid catalyst.³⁰ However, the optimum conditions involved the use of a solution of toluene–acetic acid (5:1 by volume) at 170 °C for 10 minutes. The reaction was also successful in some cases when no solvent was used and high yields of the products were obtained.³⁰

Clearly, the microwave-assisted reaction facilitated both Michael addition and cyclodehydration in a single synthetic step and afforded substituted pyridines **1.15** as single regioisomers. Also, all of the reactions carried out under microwave-assisted conditions provided substituted pyridines **1.15** in a higher yield compared with the corresponding processes conducted by traditional conductive heating methods, although rigorous temperature control was not investigated for a formal comparison of specific, non-thermal microwave effects, for which there is no rigorous evidence in this transformation. The only exception was the solventless reaction that gave higher yields in a Carius tube, possibly because of poor energy transfer when a neat reaction mixture was irradiated under microwave conditions.

The reaction represented in Scheme 1.11 showed a one-pot microwave-assisted Bohlmann-Rahtz reaction. The process is a simple and highly-expedient route for the production of tri- and tetra-substituted pyridines which proceeds with total control of regiochemistry.³⁰ Nonetheless the generality of the microwave-assisted process is only viable for substrates that can tolerate the high temperature conditions.

1.2.2.4 Continuous flow reactors

From the early reactions carried out in domestic ovens to the use of multimodal or monomodal instruments designed for organic synthesis, microwave-heating technology has been implemented worldwide and continues to be developed.^{24–29} However, although modern monomodal instruments dedicated for MAOS are very successful in small scale processes, efforts to process this technology in continuous flow (CF) reactors have in the past been frustrated by the physical limitations of microwave heating, with a penetration depth of only a few centimetres and the limited dimensions of the standing wave cavity. Current technology has attempted to overcome these disadvantages with conventional instruments by the use of CF reactors that pump the reagents through a small heated coil that winds in and out of the cavity, with external temperature monitoring using a fibre optic sensor, although alternative methods, such as using a multimode batch or CF reactor, have also been described.

A new method for carrying out MAOS under CF processing using a commercially available monomodal microwave synthesizer has been described by the Bagley research group.³¹ A 10 mL flow cell was inserted into the cavity of a self-tunable monomodal microwave synthesizer, irradiated, and stabilized at the desired reaction temperature through moderation of microwave power before the introduction of reagents into the reactor (Figure 1.4). Aminodienone **1.14b** was dissolved in the

reaction solvent and introduced into the microwave cavity through a small tube (~5 mm internal diameter) *via* a HPLC pump. The mixture of solvent and **1.14b** was passed through a layer of sand (~10 g) to minimize dispersion and create micro-channels within the small tube and coupled with the microwave irradiation in the reaction chamber to exit at the bottom of the reaction tube. As a result of the continuous pressure exerted by the HPLC pump under the action of a back pressure regulator, the reacting mixture passed through the micro-channels in the sand, was forced up the reaction tube and was collected in a flask for purification by column chromatography to produce substituted pyridine **1.15b**.

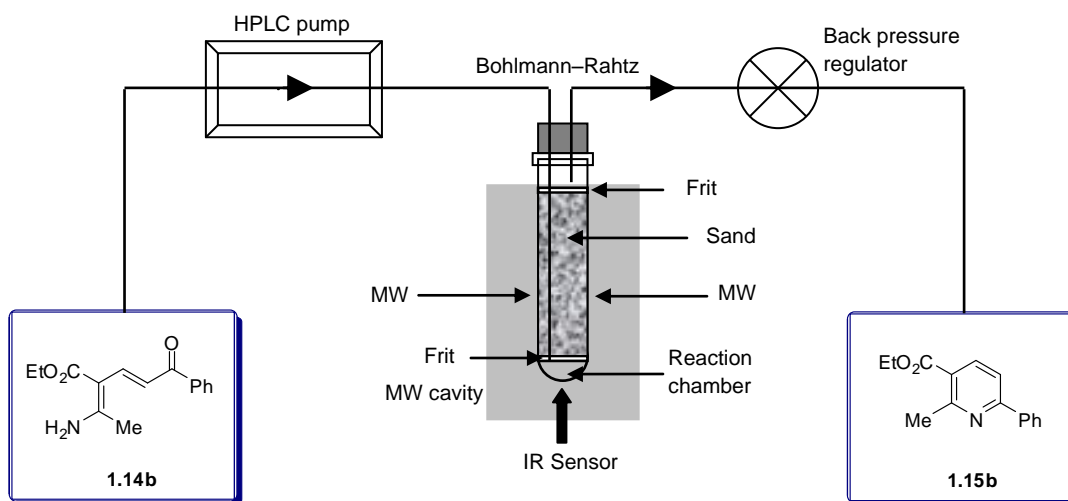
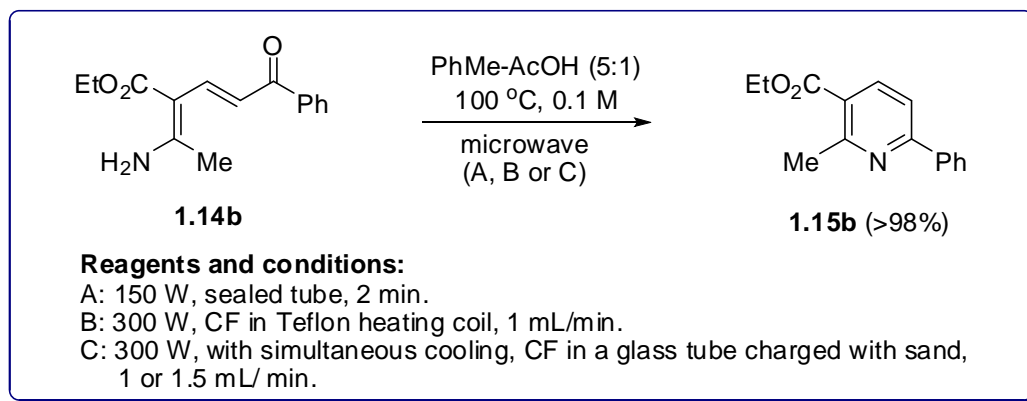


Figure 1.2: Schematic diagram of the CF microwave reactor

The development of a new microwave-assisted CF process shown above for the synthesis of pyridines based upon the Bohlmann-Rahtz reaction was examined in this microwave flow cell.³¹ To this end, aminodienone **1.14b** was subjected to cyclodehydration in a CF process under homogeneous conditions in a mixture of toluene–acetic acid (5:1 by volume) over sand. The results were compared with batch

experiments that were carried out in a sealed tube under conventional microwave synthesis and a homogeneous CF process using a commercially available Teflon heating coil (Scheme 1.12).³¹



Scheme 1.12: Microwave assisted Bohlmann-Rahtz synthesis of pyridine **1.15b**

Conventional microwave synthesis (method A) using the same reaction tube (10 mL) as described for the CF process, in the same solvent mixture and **1.14b**, that was then sealed and irradiated for a fixed time. The product was purified by conventional methods. The shortfall with this batch method, as mentioned earlier, is the fact that only a small amount of material could be processed at any one time because of the small volume (10 mL) of the reaction tube. Further comparisons were also made with the commercially available Teflon heating coil system (method B), which benefits from a continuous spiralling of the tube in the microwave cavity; this was also an open system similar to the one described in Figure 1.4 and is a more direct comparison to the CF system described (method C). Under conditions that gave efficient conversion (>98%) to pyridine **1.15b**, the processing rates of material using the glass tube reactor (method C) were considerably higher.

Moreover, CF reactions run at the same flow rate used less magnetron energy in the glass tube (Method C) than in the Teflon heating coil (Method B), demonstrating that a glass tube CF reactor offers (1) improved heating efficiency; (2) the potential for operation on a large scale; (3) successful transfer from batch (Method A) to CF processing (Method C); and (4) improved performance over commercial Teflon heating coils. As a result of these findings based in part upon the Bohlmann-Rahtz synthesis, the scene has been set to transfer this processing technology to large-scale operations, ultimately leading potentially to an industrial-based process.

Optimization of residence times were established for efficient reaction by the use of a Syrris microreactor platform in which these parameters transferred well to microwave-assisted batch, continuous flow regimes and stop-flow in addition to continuous flow conductive heating apparatus. Small variations in the required residence time could be resulted from the differences in how the temperature of the reactor was measured, being either the external or internal temperature of the cell. Of Microwave-assisted batch processes in a single-mode cavity were found to be the most efficient among the apparatus investigated.¹⁵ They were successfully transferred to continuous stop-flow production in the Voyager system, batch processing in a multimode synthesizer on <4 mmol scale and continuous processing in the single-mode Discover system. Finally, through small adjustments in residence time and reaction dilution, this reaction was successfully transferred to mesoscale production by the use of continuous flow multimode microwave reactor.¹⁵ Clearly, this indicated that present technique does have the means to scale-out or scale-up microwave batch experiments and the Bohlmann–Rahtz cyclodehydration reaction’s parameters could be reliably transferred between different technology platforms for mesoscale production. It is premature to say whether these same design rules hold for other reactions and systems

of study. In a similar fashion, it could be anticipated that discoveries made in microwave batch experiments, especially for homogenous mixtures, will follow similar principles.¹⁵ One would expect the scale up of these methods would be relatively straightforward using a single- or multi-mode microwave flow reactor, a stop-flow microwave platform, or a multimode batch reactor. However, the scale was not much more than a ten-fold increase for a multimode batch reactor. On the other hand, continuous flow conductive heating platforms operating on either meso- or micro-scale production, would seem to give rise to small but significant differences in the chemical efficiency of reaction, which one could attribute to variations in temperature measurement or heating profile.¹⁵ Whether these design rules hold for the scale up of heterogeneous processes, such as reactions over a catalyst, is another matter entirely and one that will continue to provide significant challenges for the continuous flow platforms and microwave-assisted synthesis for some time to come, and the results compared with a glass tube microwave flow reactor.¹⁵

To explore the scale-up of a microwave-assisted procedure in this regard in the first instance to mesoscale production using continuous flow technology, the Bohlmann-Rahtz reaction was investigated using a range of commercial technology platforms.

1.2.3 Applications

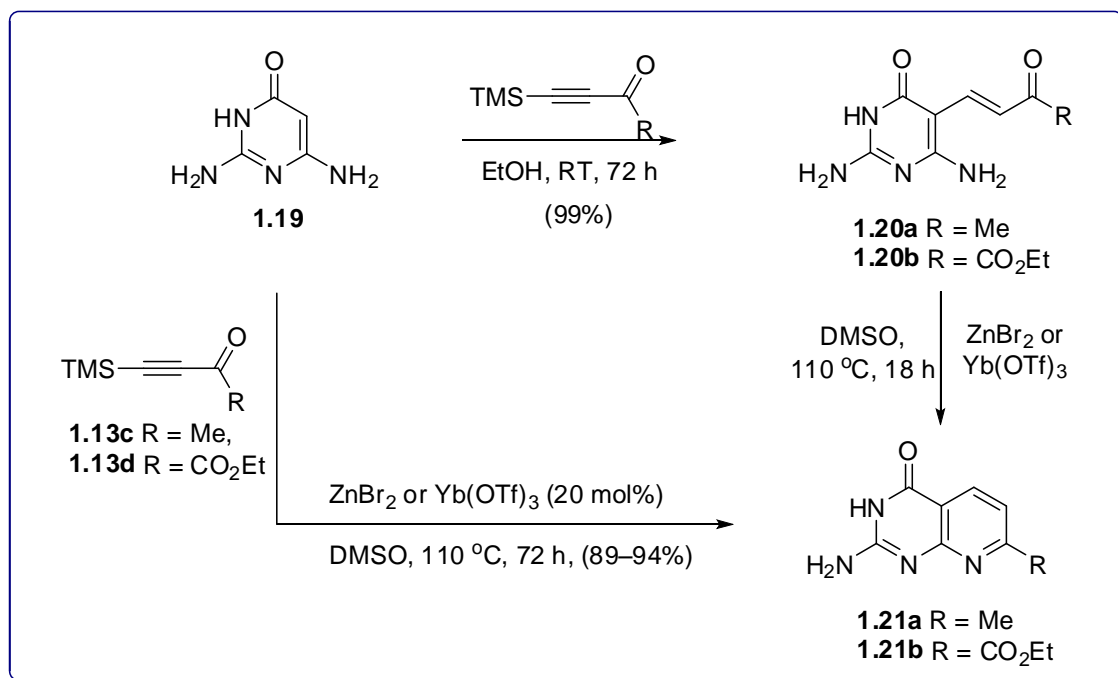
The scope and applications of modified Bohlmann-Rahtz procedures towards the synthesis of various substituted pyridine-containing heterocycles has recieved great attention from chemists. These heterocycles, including substituted pyridines and fused pyridines such as pyrido[2,3-*d*]pyrimidines, play an important role as building blocks in natural product synthesis and so new, efficient and expedient methods are of considerable value.

1.2.3.1 Synthesis of pyrido[2,3-*d*]pyrimidines and uracil derivatives

The synthesis and biological activities of various pyrido[2,3-*d*]pyrimidines (5-deazapteridines) have received relatively little attention in spite of their structural relationship to both pyridines and pterins.^{32–34} In recent years interest in pyrido[2,3-*d*]pyrimidines has increased dramatically as a results of their biological properties and the potential for folate antagonists^{35,36} to elicit highly species-specific responses as antitumour,³⁷ antibacterial,^{38–42} anti-inflammatory⁴³ and insecticidal agents.⁴⁴

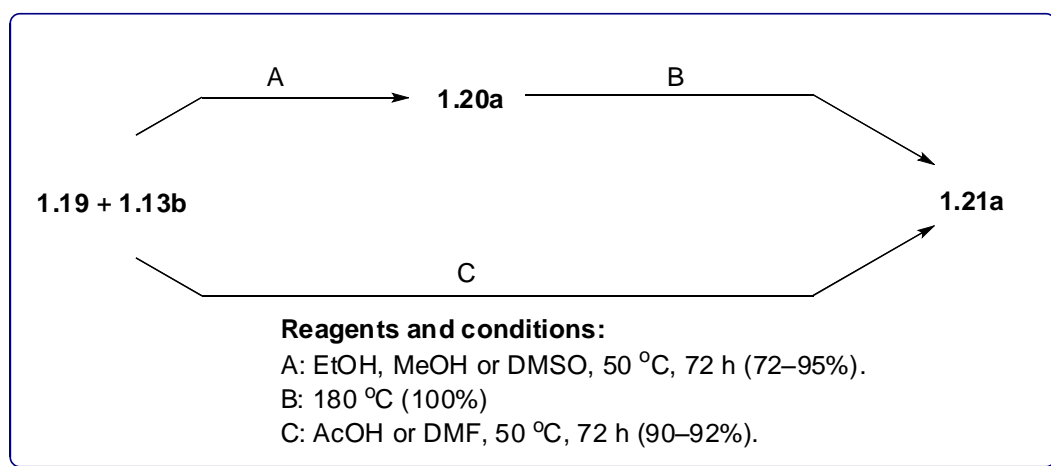
Following the success of various modified Bohlmann-Rahtz conditions for the synthesis of pyridines,^{17–19} it was proposed by Bagley's research group that similar Michael addition-cyclodehydration strategies could be used for the production of highly functionalised 5-deazapteridines.⁴⁵ The first study of this kind investigated the synthesis of pyrido[2,3-*d*]pyrimidines using ethynyl ketones under Lewis acid catalyzed conditions.⁴⁵ For example, the preparation of 2-substituted pyrido[2,3-*d*]pyrimidines **1.21** from the corresponding (diaminopyrimidinyl)propenone **1.20** or in one pot from the reaction of diaminopyrimidinone **1.19** and alkynones **1.13** was reported (Scheme 1.13). When a solution of the pyrimidine derived Bohlmann-Rahtz intermediate **1.20a** or **1.20b** and zinc(II) bromide (20 mol%) in dimethylsulphoxide was heated overnight at 110 °C the heteroannulated product, **1.21a** or **1.21b**, respectively, was produced in quantitative yield (Scheme 1.13).⁴⁵ Furthermore, when a solution of diaminopyrimidinone **1.19** and either alkynone **1.13c** or **1.13d** in dimethylsulphoxide was heated at 110 °C for 72 hours in the presence of zinc(II) bromide (20 mol%) the pure pyrido[2,3-*d*]pyrimidine **1.21a,b** was isolated in 92 and 89% yield, respectively (Scheme 1.13).⁴⁵ When the reaction was catalysed with ytterbium(III) triflate (20 mol%) gave a slightly impure product **1.21a,b** in 94 and 91% yields, respectively (Scheme 1.13).¹⁹ Although these reactions were highly efficient and gave the deazapterin as a

single regioisomer, the prolonged reaction times and high temperature conditions were a significant drawback to high-throughput operation.



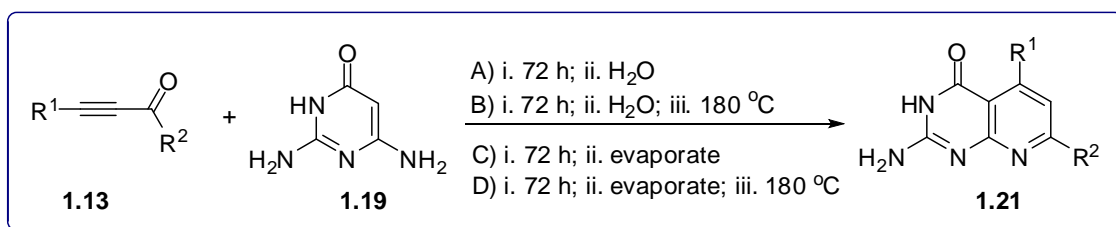
Scheme 1.13: Synthesis of pyrido[2,3-*d*]pyrimidines **1.21a,b** using Lewis acid catalysis

The structural identity of pyrido[2,3-*d*]pyrimidine **1.21a** and regiochemistry of the reactions conducted in acetic acid and *N,N*-dimethylformamide were confirmed by further experimentation.⁴⁵ (3-Oxobut-1-enyl)pyrimidine **1.20a** was heated to 180 °C to facilitate Bohlmann-Rahtz cyclodehydration and the product obtained was identical to pyrido[2,3-*d*]pyrimidine **1.21a** in every respect.⁴⁵ This is an indication that both the one and two-step methods for pyrido[2,3-*d*]pyrimidine synthesis based upon a Bohlmann-Rahtz pyridine annelation, proceeded with total regiocontrol (Scheme 1.14) and in 72–95% yield, depending on the solvent used, by *C*-alkylation and subsequent cyclodehydration of Michael addition product **1.20a**.⁴⁵



Scheme 1.14: Summary of conditions to synthesize **1.21a**

To explore the scope of the process, the treatment of pyrimidinone **1.19** with various 4-substituted alkynones **1.13** in a range of solvents (acetic acid, ethanol, *N,N*-dimethylformamide or dimethylsulphoxide) under four different sets of reaction conditions (A–D), at room temperature or with heating to 180 °C to effect cyclodehydration, was investigated towards the synthesis of a number of pyrido[2,3-*d*]pyrimidines **1.21** (Scheme 1.15).⁴⁵ In many of these cases, isolation of pyridopyrimidines **1.21** could be effected simply by precipitation upon the addition of water.



Scheme 1.15: Synthesis of **1.21** from alkynones **1.13** under different conditions

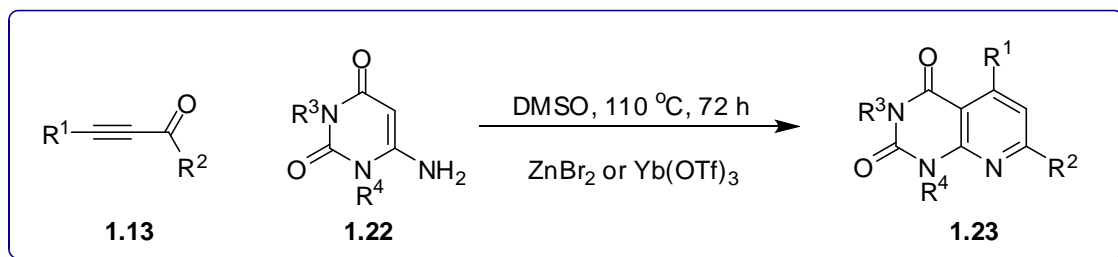
The course of the reaction was found to be dependent on the choice of alkynone and solvent. Terminally substituted alkynones ($R^1 \neq H$) appeared to be slow in reaction,

presumably because of increased steric hindrance in the Michael addition, resulting in starting materials being recovered even after long reaction times (72 hours) at room temperature in DMSO or DMF as a solvent. The purity of pyrido[2,3-*d*]pyrimidines **1.21** synthesised by method A or B in DMSO was much higher than similar reactions conducted in either ethanol or DMF.⁴⁵

The Michael addition and subsequent cyclodehydration of pyrimidinone **1.19** and various alkynones under optimized methods did produce pyrido[2,3-*d*]pyrimidines **1.21** in excellent yields (71–98%). This process was effective in various solvents, although DMSO emerged as the best solvent under these conditions. The experimental procedures were facile, required no further purification and provided the heterocyclic targets in one or two steps with total regiocontrol.⁴⁵

These successful Bohlmann-Rahtz conditions were subsequently adopted for the synthesis of pyridopyrimidines **1.23a** from the 6-aminouracil derivative **1.22a** ($R^3 = R^4 = H$) and 4-(trimethylsilyl)butynone (**1.13e**) in DMSO at 110 °C for 72 hours in the presence of either zinc(II) bromide or ytterbium(III) triflate (20 mol%) as a Lewis acid catalyst to give the products in 60 or 52% yield, respectively (Scheme 1.16).⁴⁶ The reaction was found to be quite general in scope and various 5-deazapterins **1.23** were produced in good yield (Scheme 1.16).⁴⁵ When trimethylsilyl-substituted alkynones **1.13** ($R^1 = SiMe_3$) were reacted with uracil derivatives **1.22**, spontaneous protodesilylation accompanied Michael addition-cyclodehydration to produce pyridopyrimidines **1.23** ($R^1 = H$).⁴⁶ When these reactions were conducted either in the absence of a Lewis acid or at room temperature the efficiency of the reaction was reduced dramatically. The optimum conditions involved heating aminouracil **1.22** and the corresponding alkynone **1.13** at 110 °C in the presence of zinc(II) bromide for 72 h to give the products **1.23** in 60–75% yield.⁴⁶ Replacing zinc(II) bromide with

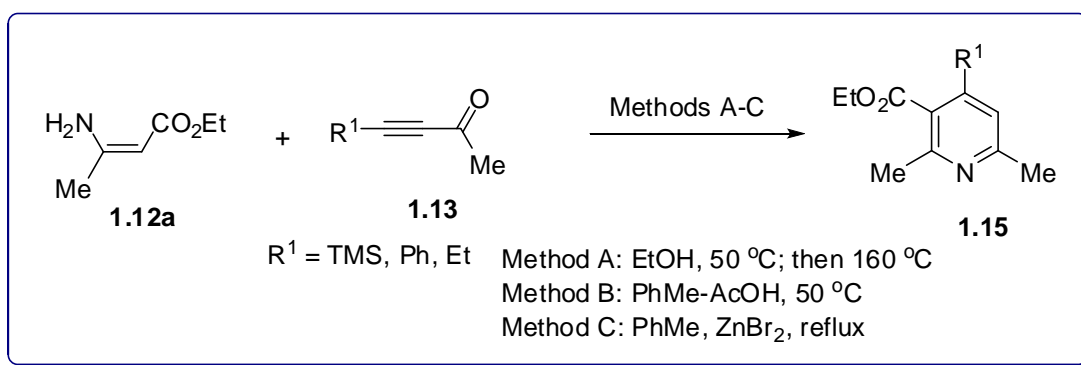
ytterbium(III) triflate caused a small reduction in the efficiency of the cyclocondensation process. It was apparent that the Lewis acid catalyzed cyclocondensation was appropriate for various alkynones and uracil derivatives **1.22** and constituted a reliable method for the synthesis of pyridopyrimidines.⁴⁶



Scheme 1.16: Reaction of 6-aminouracil **1.22** and alkynone **1.13** under Lewis acid catalyzed conditions

1.2.3.2 Combinatorial library synthesis

With the development of new Bohlmann-Rahtz methods for the synthesis of pyridine and related heterocycles, efforts to apply this reaction technology to the combinatorial synthesis of pyridine libraries was carried out. In the first instance, a targeted combinatorial pyridine library was generated with varying product ratios and high library purity using a simple acid-base extraction workup procedure. For example, the reaction of ethyl β -aminocrotonate (**1.12a**) with various alkynones **1.13** produced a small library of pyridines **1.15** (Scheme 1.17) using either Bohlmann-Rahtz conditions in ethanol (Method A), in an acetic acid–toluene mixture (Method B) or in the presence of a Lewis acid in toluene (method C) and the product ratios and library purities were compared.⁴⁷



Scheme 1.17: Combinatorial synthesis of substituted pyridines **1.15**

Heating a mixture of **1.12a** and **1.13** to 50 °C in ethanol followed by isolation and heating the isolated intermediate produced according to the traditional Bohlmann-Rahtz method to 160 °C for 3 h gave the corresponding substituted pyridines **1.15**, with protodesilylation occurring throughout the course of reaction.⁴⁷ The product ratio (R) was large and varied between $1 < R < 25$, although the overall yield and product purity were good.⁴⁷ Some protodesilylation also took place when the reaction was conducted in a mixture of toluene–acetic acid (method B) and although the product ratio (R) improved, varying between $1 < R < 8.3$, the overall yield was low (30%).⁴⁷ However, the Lewis acid-catalyzed conditions (method C) resulted in no protodesilylation and gave **1.15** in high purity and yield.⁴⁷ The product ratio (R) was between $1 < R < 2$, indicating that under these conditions all ethynylketone subset members reacted efficiently to give a diverse combinatorial library of products. It was curious to note that the product ratios varied between the different heteroannulation procedures, as did the identity of the major product, indicating that the alkynones display different reactivity profiles according to the method used.⁴⁷ It was concluded that the Lewis acid-catalyzed methods appeared to provide the best overall yield, product ratios, and library purity for the solution phase combinatorial synthesis of pyridines from ethyl β -aminocrotonate (**1.12a**) in the Bohlmann-Rahtz synthesis.

This approach has the potential to produce highly-substituted and heavily functionalized pyridines directly from readily available starting materials without the need for purification and so should complement other published procedures for the solution phase combinatorial synthesis of pyridine libraries.⁴⁷

Simple low molecular weight heterocycles make ideal scaffolds on which to base the high throughput synthesis of libraries of drug-like compounds. Examples include the use of 1,2,5-thiadiazolidin-3-one-1,1-dioxide and pyrimidine scaffolds in the synthesis of serine proteinase and kinase inhibitors, respectively,⁴⁸ and the use of simple pyridine scaffolds to generate libraries of inhibitors of HIV-1 protease and Factor Xa.⁴⁹ Moody and co-workers developed new heterocyclic scaffolds **1.23** for library synthesis, trisubstituted pyridines **1.24–1.26** containing two points of potential diversity *via* Bohlmann-Rahtz methodology (Figure 1.3).

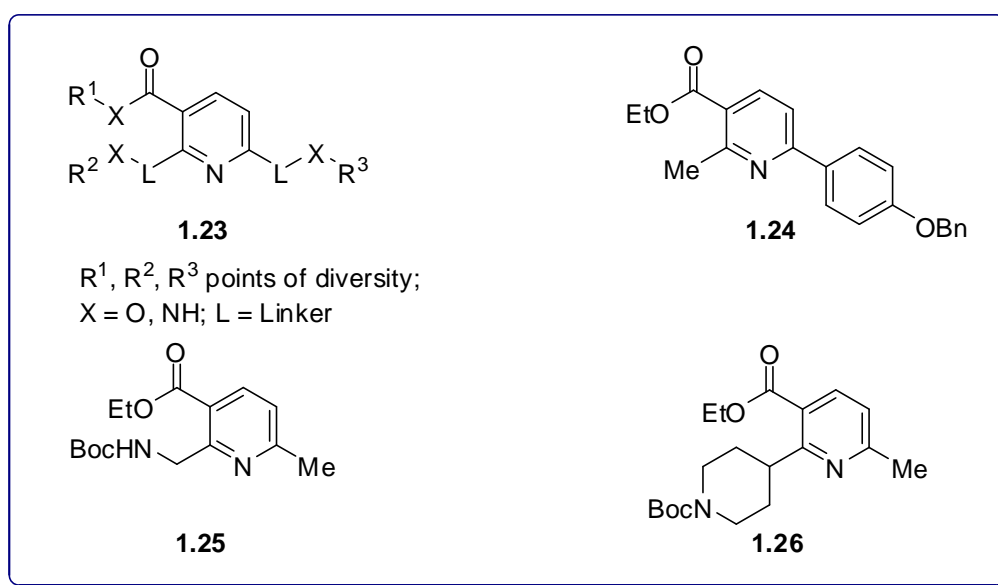
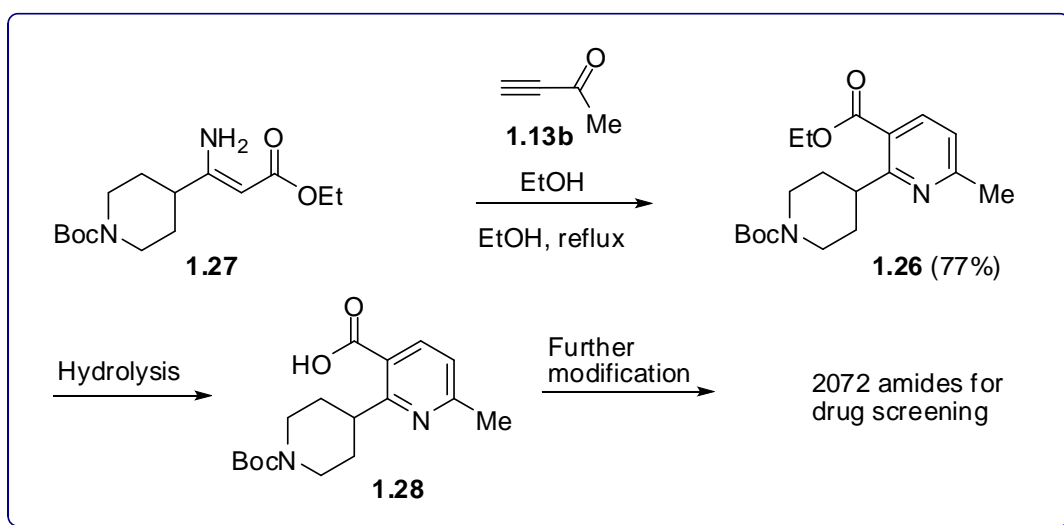


Figure 1.3 Pyridine scaffold **1.23** and the corresponding pyridines **1.24–1.26**

The conditions used for the synthesis of pyridine **1.26** provided, for example, this product in 77% yield by the reaction of enamine **1.27** with butynone **1.13b** in

ethanol under reflux (Scheme 1.18).⁵⁰ Hydrolysis of ester **1.26** gave acid **1.28** (Scheme 1.18). Following a design process directed towards drug-like products, acid **1.28** was coupled with various amines to provide 14 protected intermediates in 61–87% yield on a 100 mmol scale, that were then subjected to further reaction with 148 carboxylic acids on Boc-deprotection to generate 2072 amides for biological screening.

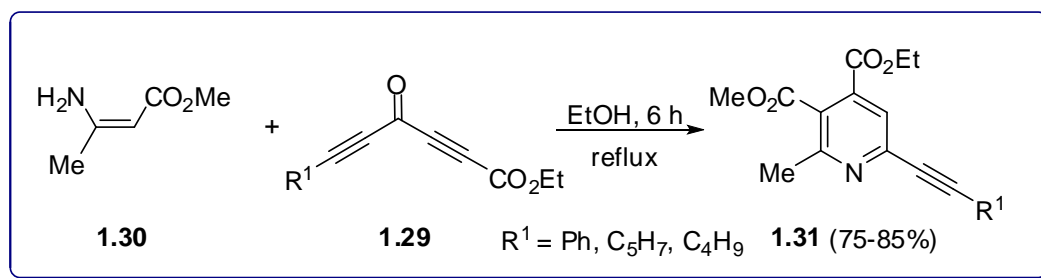


Scheme 1.18: Drug design library synthesis applying the Bohlmann-Rahtz pyridine synthesis

Clearly, the use of modified Bohlmann-Rahtz methodology has been demonstrated in an efficient large scale synthesis of the pyridine scaffold **1.28** containing two points of diversity and applied in library synthesis.⁵⁰

1.2.3.3 Natural product chemistry

The Bohlmann-Rahtz reactions of *bis*-acetylenes **1.29** with enamine **1.30** under traditional conditions, in ethanol under reflux, has also been investigated and afforded the corresponding alkynylpyridines **1.31** in high yield (Scheme 1.19).⁵¹



Scheme 1.19: Bohlmann-Rahtz reaction for the synthesis of substituted pyridines **1.31**

The initial report by Baldwin⁵¹ inspired the synthesis of analogues of various heterocyclic α -amino acids such as azatyrosine **1.32**, mimosine **1.33** and lathyrine **1.34**.⁵² Such compounds were found to display antibiotic, antitumor, wool growth and pollen growth inhibitory activities (Figure 4).⁵²

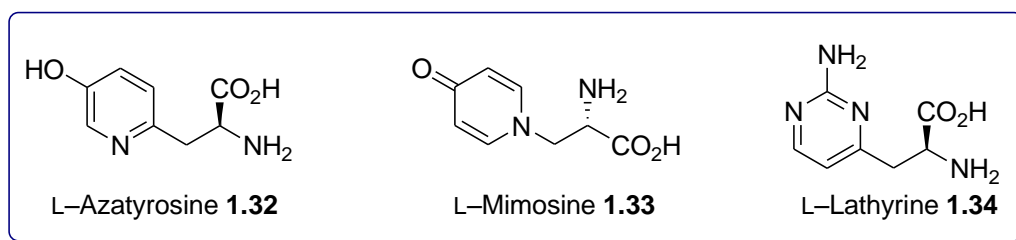
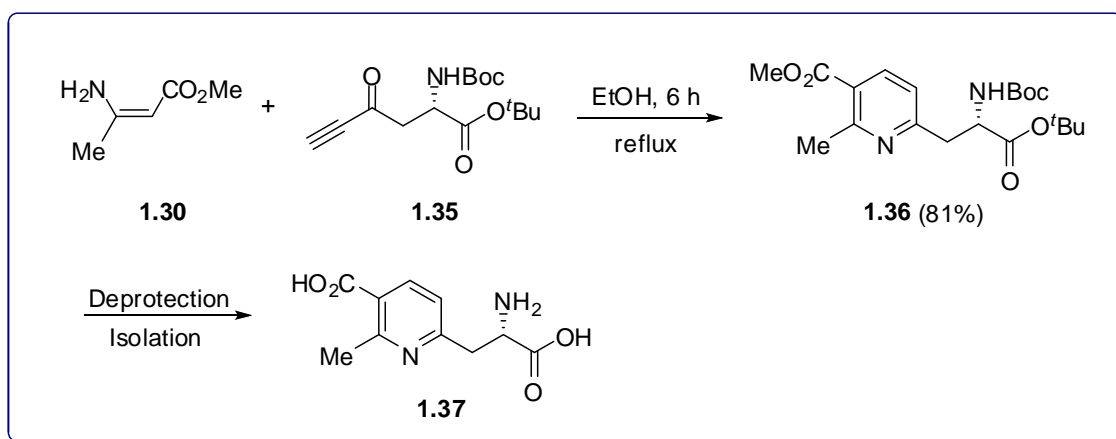


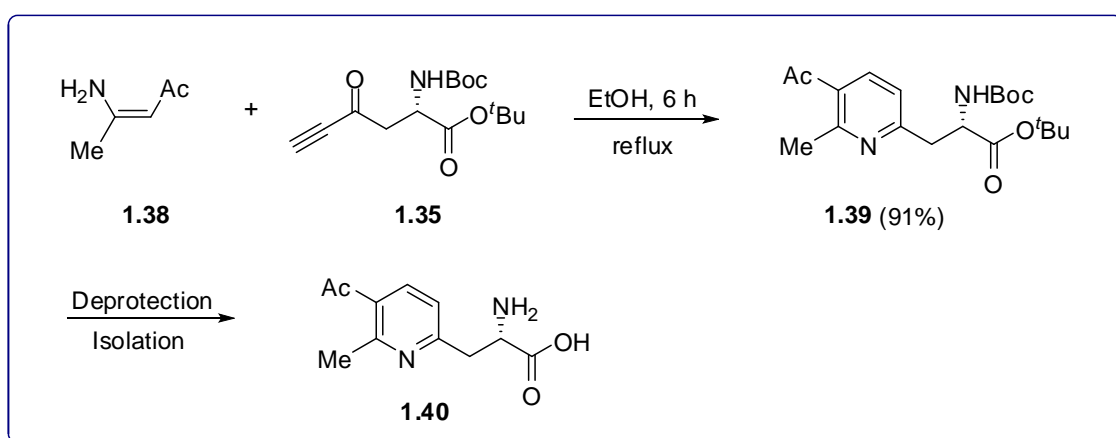
Figure 1.4: Structures of heterocyclic α -amino acids, azatyrosine **1.32**, mimosine **1.33** and lathyrine **1.34**

Reaction of alkynone **1.35** and 3-aminobut-2-enoic acid methyl ester **1.30** in ethanol under reflux gave the corresponding trisubstituted pyridine, β -alanine **1.36** in 81% yield (Scheme 1.20).⁵¹ Subsequent deprotection and purification by ion exchange chromatography afforded the free amino acid **1.37** as an analogue of the α -amino acid L-azatyrosine **1.32** (Scheme 1.20).⁵¹



Scheme 1.20: Bohlmann–Rahtz synthesis of analogue **1.37** of L-azatyrosine **1.32**

In a similar manner condensation of alkynone **1.35** and 4-aminopent-3-en-2-one (**1.38**) gave β-alanine **1.39** in 91% yield (Scheme 1.21).⁵¹ Subsequent deprotection gave the corresponding amino acid **1.40** (Scheme 1.21).⁵¹



Scheme 1.21: Bohlmann–Rahtz synthesis of analogue **1.40** of L-azatyrosine **1.32**

The most significant application of the Bohlmann–Rahtz reaction to be reported to date is in the synthesis of thiopeptide antibiotics. This class of naturally-occurring antimicrobial compounds isolated from bacteria includes, for example, thiostrepton **1.41**



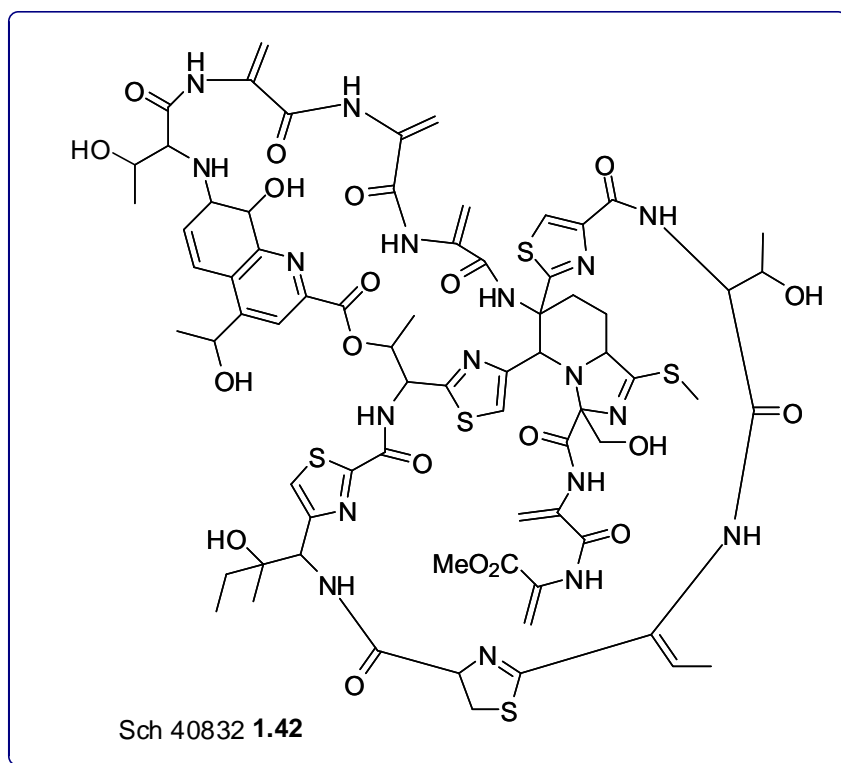


Figure 1.6: Thiopeptide antibiotic 1.42

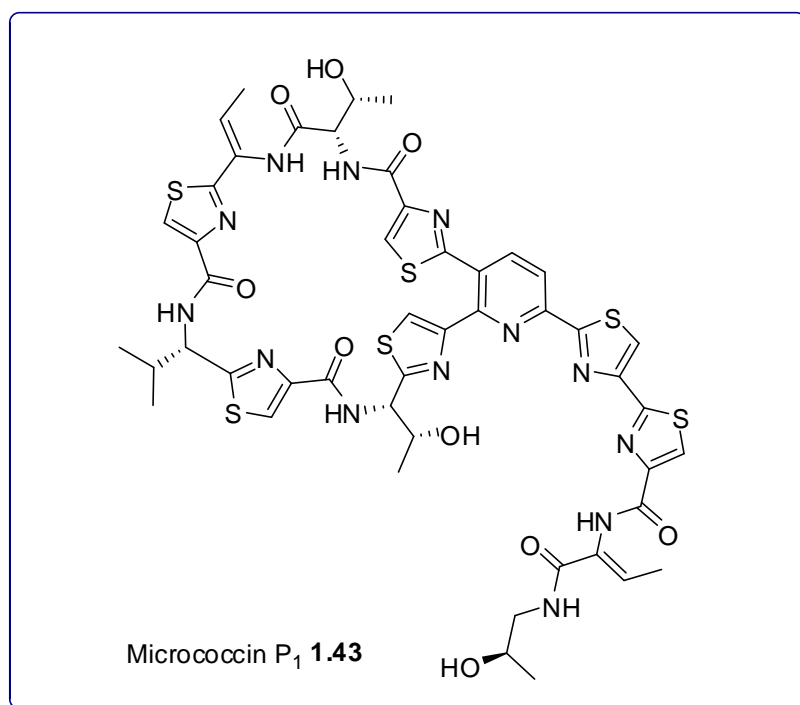


Figure 1.7: Thiopeptide antibiotic 1.43

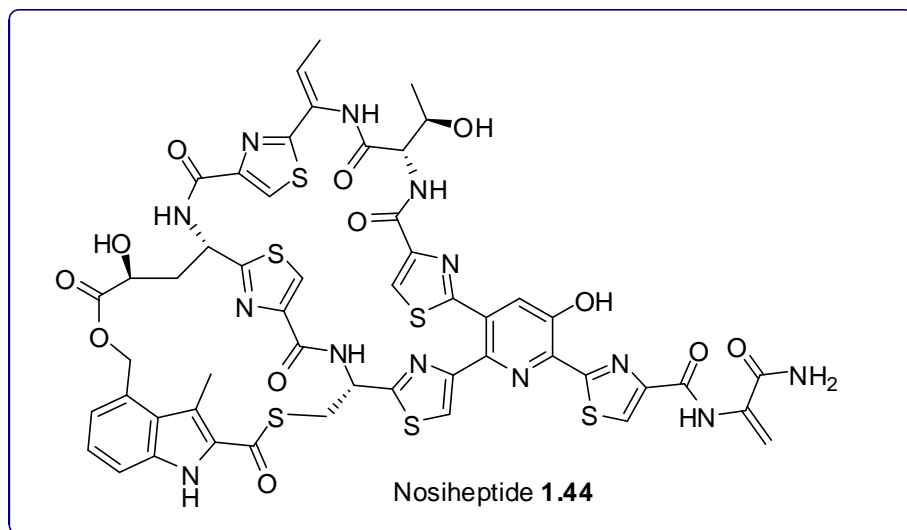
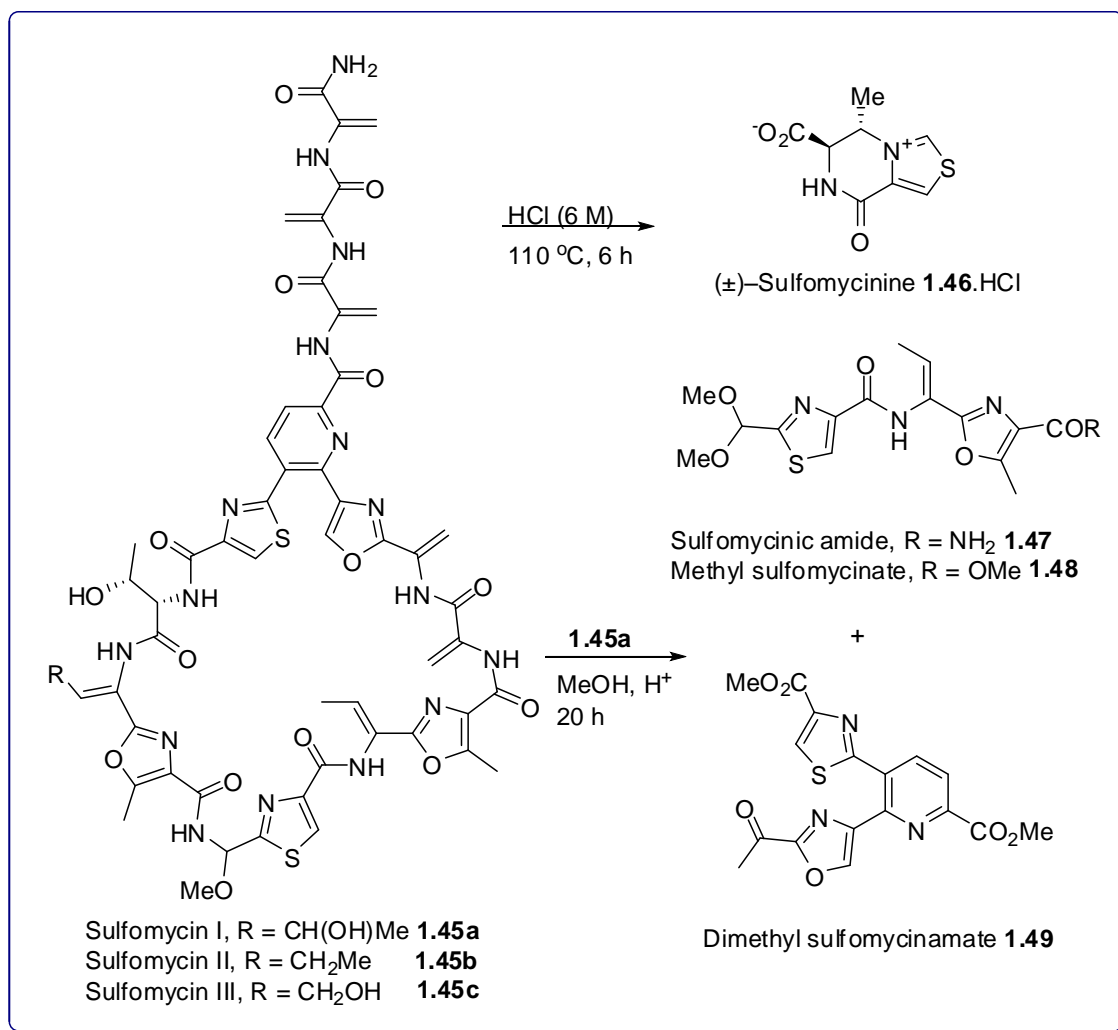


Figure 1.8: Thiopeptide antibiotic **1.44**

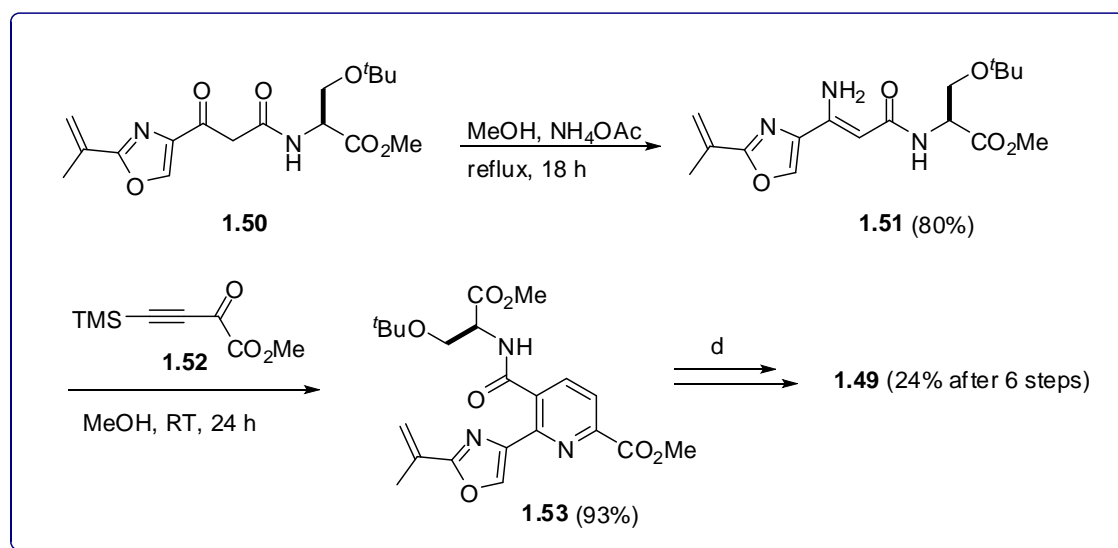
The sulfomycins are one sub-class members of this family of antibiotics, isolated in this instance from *Streptomyces viridochromogenes*. Acidic hydrolysis of sulfomycins **1.45a–c** with 6M HCl at 110 °C for 6 hours gave the chemical degradation product (±)-sulfomycinine hydrochloride **1.46** (Scheme 1.22).⁵⁴ Furthermore when sulfomycin I **1.45a** was heated under reflux in methanol in the presence of Amberlyst-15 ion-exchange resin for 20 hours, this gave the degradation products sulfomycinic amide **1.47**, methyl sulfomycinate **1.48** and dimethyl sulfomycinamate **1.49** (Scheme 1.22), all of which were used collectively to assist the structural elucidation of the natural product.⁵⁴



Scheme 1.22: Degradation of the sulfomycins **1.45a–c**

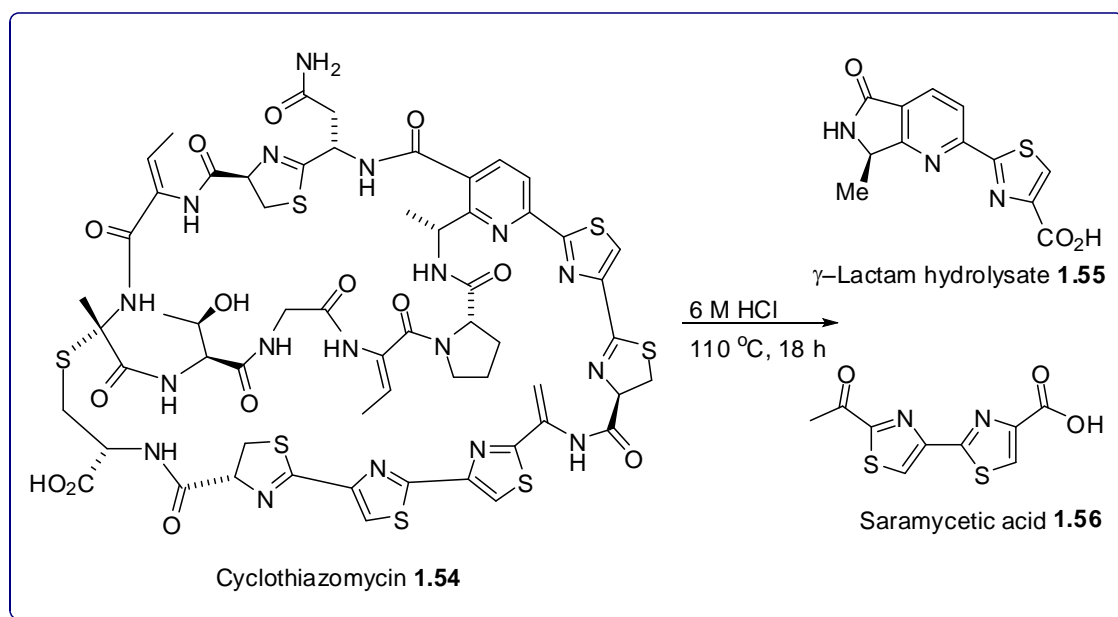
Dimethyl sulfomycinamate (**1.49**; Scheme 1.23) was synthesised from keto amide **1.50** using modified Bohlmann-Rahtz methodology (Scheme 1.23).⁵⁵ When the ketone **1.50** was heated under reflux overnight in methanol in the presence of ammonium acetate, this gave the Bohlmann-Rahtz precursor, enamine **1.51**, in 80% yield (Scheme 1.23).⁵⁵ Bohlmann-Rahtz reaction of enamine **1.51** with methyl oxobutynoate **1.52** in methanol at room temperature gave pyridine **1.53** (Scheme 1.23) in 93% yield as a single regioisomer *via* Michael addition and spontaneous cyclodehydration.⁵⁵ This pyridine synthesis was later improved by the Bagley research group by the introduction of a one-pot three-component process.²¹ Heating β -keto amide

1.49, used as a tautomeric mixture, methyl oxobutynoate **1.52**, and ammonium acetate (10 equiv) under reflux in methanol for 5 hours gave pyridine **1.53** directly in 81% yield.²¹ Elaboration of oxazole-pyridine **1.53** was achieved in a further 6 steps resulting in the synthesis of dimethyl sulfomycinamate **1.49** with total regiocontrol in 13 steps and 8% overall yield, by the Bohlmann-Rahtz heteroannulation of enamine **1.51**, or in 12 steps and 9% overall yield by a three-component cyclocondensation using β -keto amide **1.49** and ammonia in methanol.⁵⁵



Scheme 1.23: Bohlmann-Rahtz synthesis of dimethyl sulfomycinamate (**1.49**)

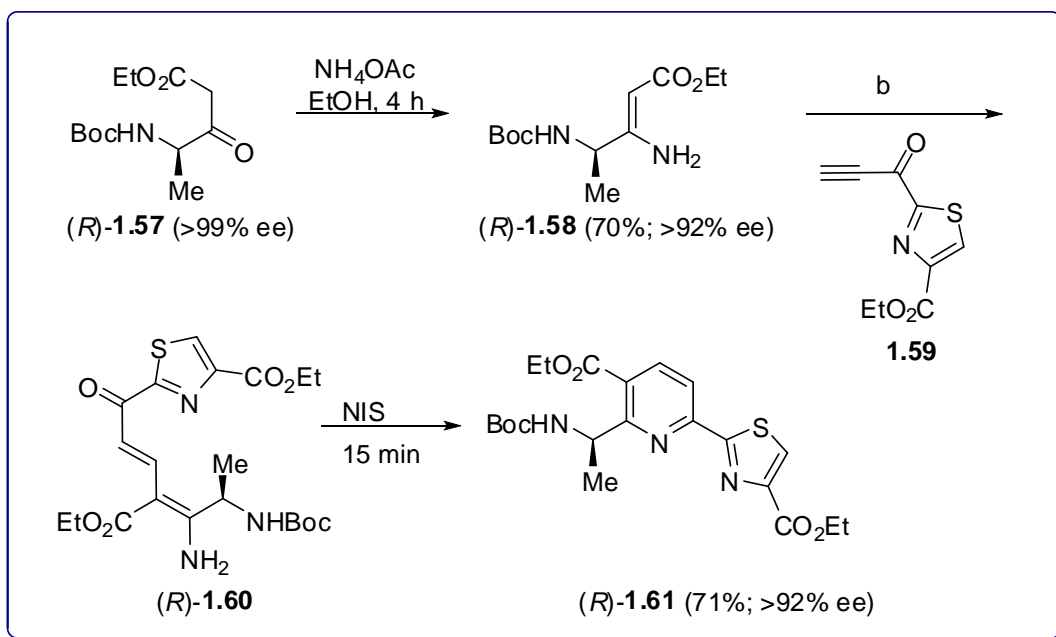
Synthetic efforts using modified Bohlmann-Rahtz methodology has also been directed towards other members of the thiopeptide antibiotics. Cyclothiazomycin (**1.54**) was isolated from the fermentation broth of *Streptomyces* sp. NR0516 obtained from a soil sample collected at Kanagawa, Japan, and has been shown to be a selective inhibitor of human plasma renin at an IC_{50} of $1.7 \mu M$.⁵⁶ Chemical degradation of the natural product **1.54** gave heterocyclic amino acid γ -lactam **1.55** and saramycetic acid I **1.56** (Scheme 1.24).⁵⁷



Scheme 1.24: Degradation products of cyclothiazomycin **1.54**

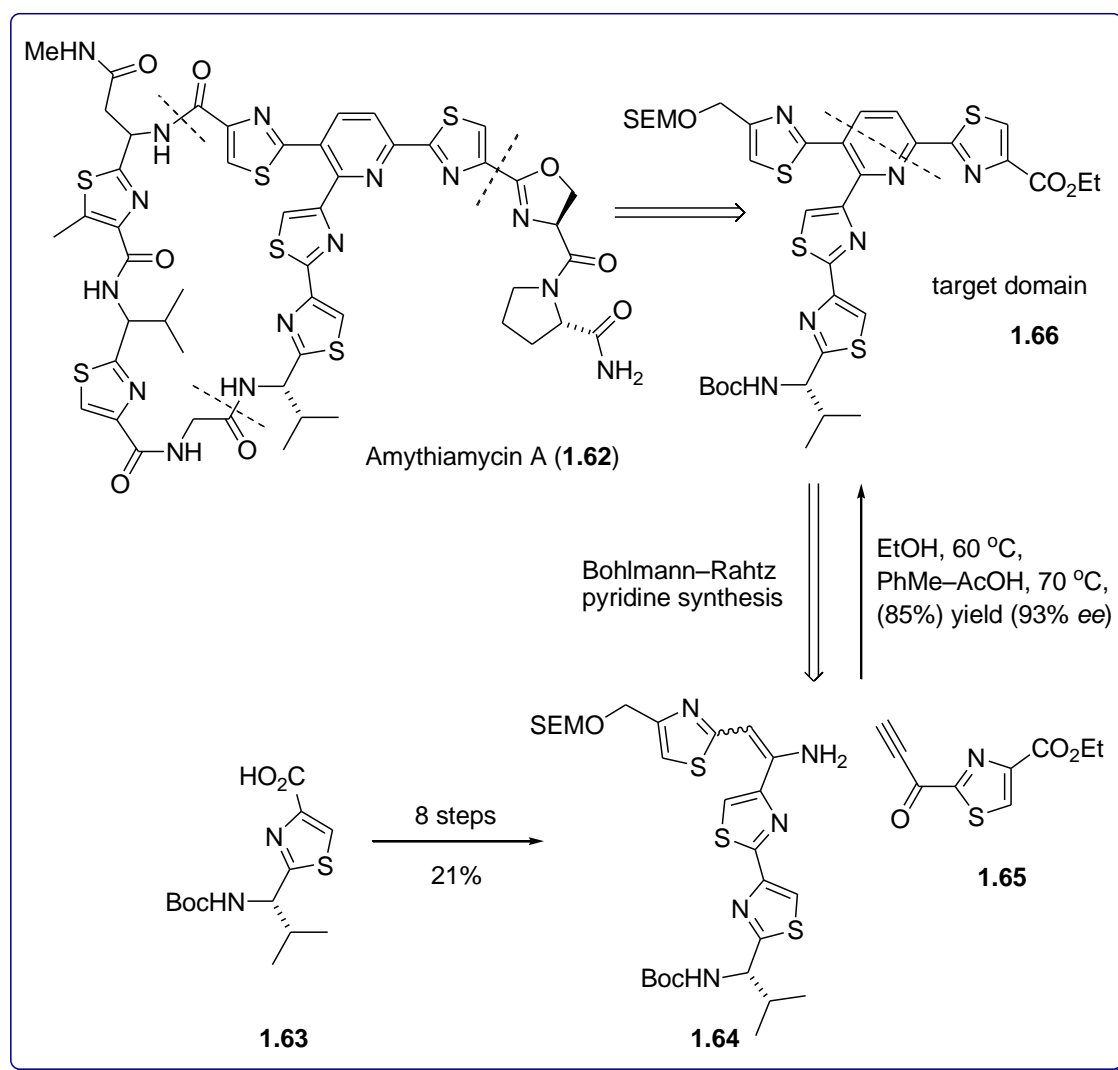
γ -Lactam **1.61** was synthesized starting from the corresponding amino acid **1.57** using the Bohlmann-Rahtz pyridine synthesis (Scheme 1.25).⁵⁸ Reaction of (*R*)- β -keto ester **1.57**⁵⁹ with ammonium acetate gave enamine **1.58** in 70% yield and 92% *ee* when the reaction was carried out at room temperature in ethanol (Scheme 1.25). The Bohlmann-Rahtz reaction of enamine **1.58** and propynone **1.59** gave pyridine **1.61** as a single regioisomer in 73% yield, albeit only in 14% *ee*. Changing the cyclodehydrating agent from a Brønsted acid to *N*-iodosuccinimide, at 0 °C, gave pyridine **1.60** in much improved enantiomeric purity, 92% *ee*.

To improve this process still further, a one-pot process was developed by the addition of ammonium acetate to a solution of (*R*)- β -keto ester **1.57** (>99% *ee*) in ethanol. After 4 hours at room temperature, thiazolylpropynone **1.59** was added and the mixture was stirred to complete the Michael addition. The mixture was then cooled to 0 °C, and *N*-iodosuccinimide was added (Scheme 1.25) to give *C*- and *N*-terminal-protected amino acid **1.61** in 55% yield and 96% *ee* from this one-pot reaction.



Scheme 1.25: Synthesis of cyclothiaomycin central domain **1.61**

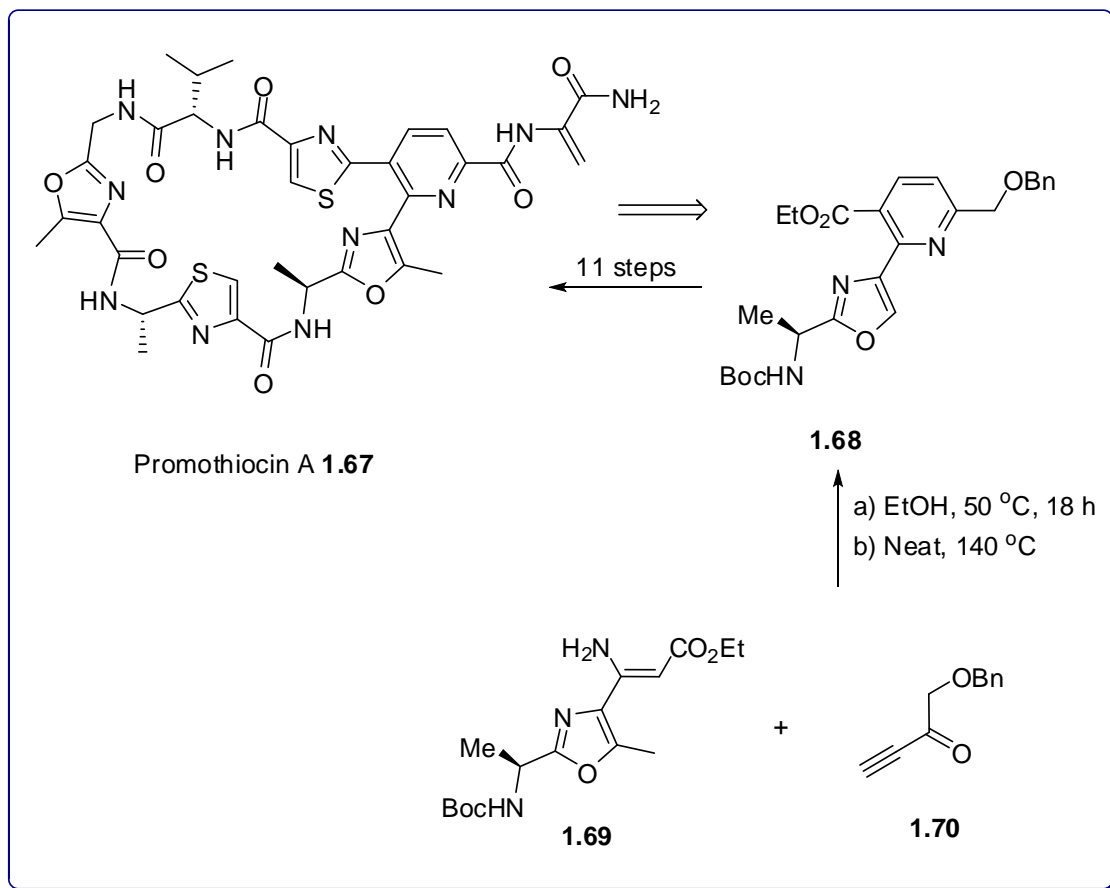
Retrosynthetic studies of the thiopeptide amythiamicin A **1.62** provided a further application of the Bohlmann-Rahtz reaction by Michael addition-cyclodehydration of enamine **1.64** and alkynone **1.65** (Scheme 1.26).⁶⁰ Michael addition of enamine **1.64** and alkynone **1.65** in ethanol followed by acetic acid-catalyzed cyclodehydration at 70 °C gave the amythiamicin heterocyclic domain **1.66** with total regiocontrol in 85% yield and 93% *ee* (Scheme 1.26).⁶⁰ Overall, this heterocyclic cluster **1.66**, related to amythiamicin (**1.62**), was generated in only 9 steps and 18% overall yield from **1.63**.⁶⁰



Scheme 1.26: Retrosynthetic analysis of amythiamycin A (**1.62**) and the forward synthesis of the target domain **1.66**

Promothiocin A (**1.67**) was isolated from *Streptomyces* sp. SF2741 and its structure was proposed in 1994, principally on the basis of NMR spectroscopic analysis.⁶¹ The synthesis of **1.67** reported by Bagley and Moody (Scheme 1.27) confirmed the natural product constitution and stereochemistry, based upon a Bohlmann-Rahtz strategy.⁶² Reaction of enamine **1.69** with alkynone **1.70** in ethanol at 50 °C overnight according to traditional Bohlmann-Rahtz conditions, followed by heating to

high temperature to effect cyclodehydration, gave the pyridine intermediate **1.68** (Scheme 1.27) in 83% yield.



Scheme 1.27: The structure of promothiocin A (**1.67**) and a summary of the total synthesis incorporating a traditional Bohlmann-Rahtz reaction

1.3 Werner syndrome

Werner syndrome (WS) is one of the most well characterized premature ageing disorders. Patients with WS have several symptoms of ageing that present at a younger age than normal. However, not all symptoms of WS resemble the normal ageing process, and WS is best described as a segmental progeroid disorder.⁶³ There are several other premature ageing syndromes such as Cockayne syndrome, Rothmund Thompson

and Hutchinson Gilford progerias. Such conditions are very rare in the general population.⁶⁴

The development of premature ageing in Werner syndrome is characterized by a number of clinical symptoms, including short stature, premature greying of the hair, cataracts, osteoporosis, type II diabetes, high levels of inflammatory diseases and accelerated atherosclerosis, accompanied by a high incidence of cancers, and also arteriosclerosis.⁶⁵ The clinical picture of WS is more complex than a simple global acceleration of age-linked pathology. This disease provides a convincing mimic of the normal ageing phenotype: individuals with WS appear to have essentially normal immune function and lack notable pathology of the central nervous system. The median life expectancy of WS patients is 47 years, with major cause of death being myocardial infarction or mesenchymal neoplasm.⁶⁶

The development of research in this area has focused on defining the cellular role of the Werner protein, WRNp, encoded by the gene defective in the disease, *WRN*. WRNp is a nuclear 1432-amino acid protein.⁶⁷ The WRNp interacts with many proteins involved in DNA replication, transcription, DNA recombination and DNA repair.⁶⁸ The nuclear protein, WRNp, is found predominantly in the nucleoli but relocates to replication foci at S phase of the cell cycle, and to sites of DNA damage in response to DNA-damaging agents such as 4-nitroquinoline 1-oxide.⁶⁹ Primary WS cells display genome instability, manifested by telomere dysfunction, an elevated rate of chromosomal translocation and genomic deletions,⁷⁰ and are hypersensitive to a subset of DNA damaging agents,⁷¹ suggesting that the WRNp plays a key role in the cellular response to specific types of DNA damage. These features have led to WS being classified as a genome instability syndrome.⁷² Cultured cells from normal individuals have a finite capacity to divide, after which they enter a state of permanent cell-cycle

arrest termed ‘replicative senescence’ or also named ‘cellular senescence’. Senescence has been postulated to contribute to normal human ageing and shows acceleration in WS.⁷³ Primary fibroblasts from WS patients show a very abbreviated *in vitro* lifespan; they divide more slowly than normal cells, exit the cell cycle at a higher rate than normal fibroblasts and so senesce more rapidly.⁷⁴ Studies suggest that WRNp participates in biological pathways by acting at telomeric ends. WS cells display some defects in telomere metabolism, including increased rates of telomere shortening⁷⁵ and deficiencies in repair at telomeres.⁷⁶ Both normal and WS fibroblasts use the progressive erosion of chromosomal telomeres as a cell division ‘counter’, but recently it has been shown that this telomere driven senescence synergizes with an additional telomere-independent mechanism in WS.⁷⁷

The accelerated ageing of mitotic tissue *in vivo* has been linked with the premature senescence of cultured primary fibroblasts *in vitro*, which show a very abbreviated lifespan. Cellular senescence in normal human fibroblasts but also in WS fibroblasts appears to be triggered by telomere erosion.⁷⁸ Young WS fibroblasts have telomeres of similar length to young normal cells but they senesce with longer mean telomere lengths. This might indicate that WS cells are more sensitive to variations in telomere length.

1.4. Use of MAPK inhibitors to treat Werner syndrome

Mitogen activated protein kinases (MAPKs) are intracellular signal transduction molecules, a group of protein serine/threonine kinases that are activated in response to a variety of extracellular stimuli and mediate signal transduction from the cell surface to the nucleus. They regulate various physiological and pathological cellular phenomena,

including inflammation, cell cycle, cell differentiation, apoptotic cell death, oncogenic transformation, tumor cell invasion, and metastasis. The MAPK superfamily consists of at least four broad families, namely extracellular-signal-regulated kinase (ERK), Jun-NH₂-terminal kinases (JNKs), ERK5 (or big MAPK1, BMK-1) and p38 MAPK. MAP kinases, in combination with several other signaling pathways, can differentially alter the phosphorylation status of the transcription factors in a pattern unique to a given external signal. The mechanism by which this occurs involves the enzymatic transfer of phosphate groups from high-energy donor molecules, such as a ATP, to specific target molecules (substrate), in a process termed phosphorylation. Specifically p38 MAPK is implicated in transducing the stress signal arising from stalled replication forks, leading to telomere independent senescence. Classical MAP kinase signaling occurs in cascades, involving MAP kinases (MAPK) that are activated by MAPK kinases (MAPKK), which are in turn activated by MAPKK kinases (MAPKKK). The activation of specific MAPKK kinases through extracellular signals, such as endotoxins, stress, inflammatory cytokines TNF- α (tumor necrosis factor- α) and IL-1 β (interleukin-1 β) and growth factors, leads to phosphorylation and activation of downstream MAPK kinases that, in turn, phosphorylate and activate their MAP kinase. The activated MAP kinases can activate downstream kinases and transcription factors leading to stabilized mRNA that encode cytokines and receptors involved in inflammation and immunity, regulation of gene expression, translation, cell cycle progression, proliferation, differentiation, growth inhibition and apoptosis (Figure 1.9).⁷⁹

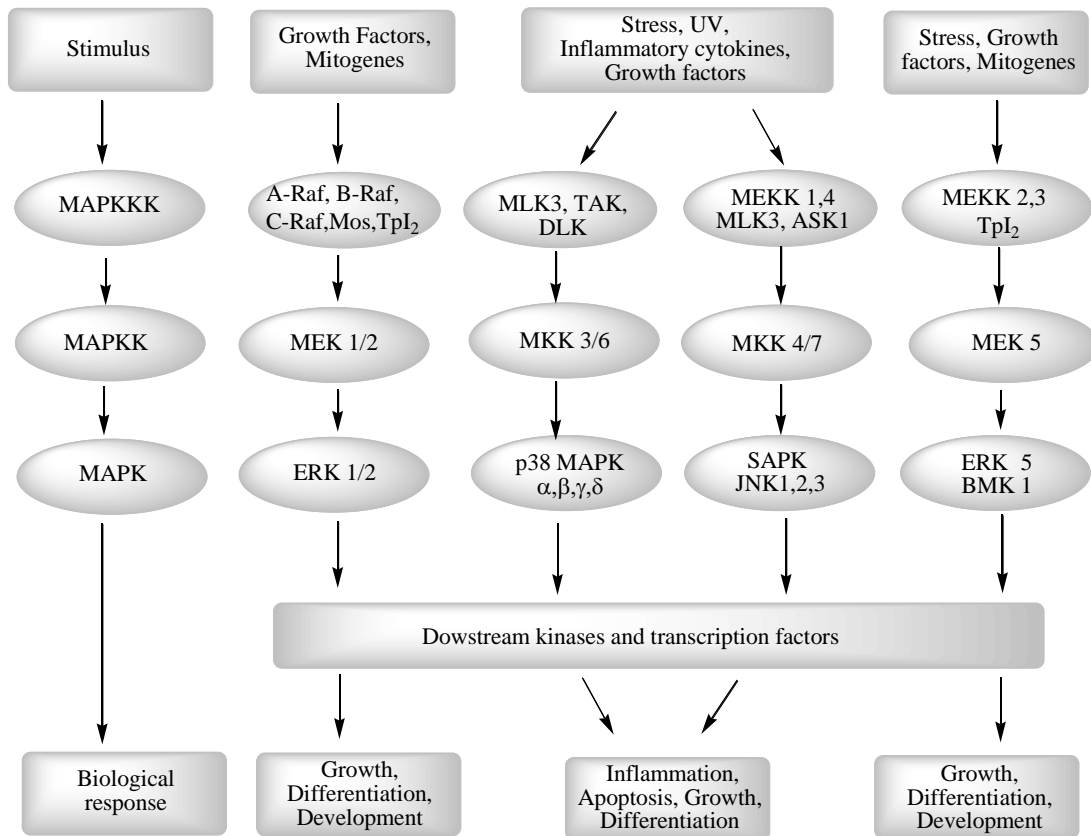


Figure 1.9: Mitogen-activated protein kinase signaling cascades⁷⁹

It has been established that the p38 MAP kinase pathway is activated in response to physical stress signals such as osmotic shock, heat, UV light and in response to such pro-inflammatory cytokines as TNF- α , IL-1 β and growth factors. The p38 kinase is widely expressed in many cell types, including immune, inflammatory and endothelial cells. There are four p38 isoforms known as α , β , γ and δ and each encoded by a separate gene, but the p38 α kinase is believed to be the family member primarily responsible for regulation of inflammation. The p38 α MAPK is activated through *bis*-phosphorylation on a Thr-Gly-Tyr motif located in a region known as the activation loop, leading to increased production of pro-inflammatory cytokines such as TNF- α and IL-1 β . Activation is achieved by dual-specificity serine/threonine MAPK kinases,

MKK3 and MKK6. These kinases serve as upstream MAPK kinases responsible for p38 activation. The primary substrate of p38 MAPK is MAPK activated protein kinase 2 (MAPKAPK-2 or MK2) which in turn phosphorylates the small heat shock protein HSP27. This protein promotes polymerization of actin filaments and maintains integrity of the cytoskeleton (Figure 1.10).⁸⁰ The p38 α MAP kinase is a protein serine-threonine kinase: it plays a central role in numerous pro-inflammatory responses. An important and accepted therapeutic approach for potential drug intervention in inflammatory disease is the reduction of pro-inflammatory cytokines.

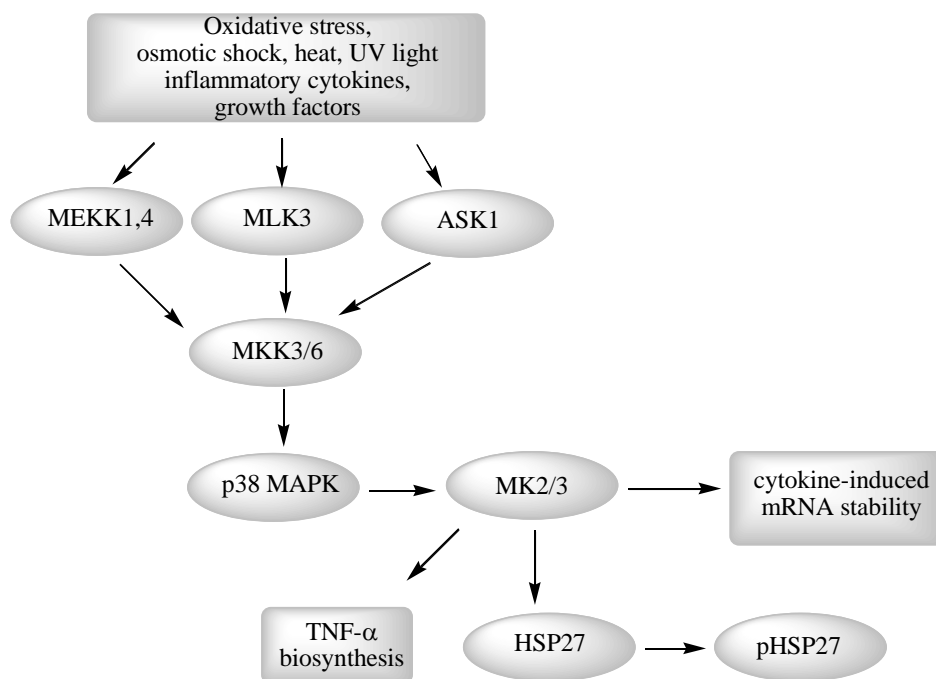


Figure 1.10: The p38 MAPK signalling pathway

The initial discovery in 1994 of p38 as the molecular target for a novel class of cytokine suppressive inhibitors has been exemplified by the prototypical inhibitor, pyridinylimidazole, SB203580 (**1.71**) (Figure 1.11).⁸¹ This is one of a number of

prototypes originally prepared as inflammatory cytokine synthesis inhibitors that subsequently were found to be selective inhibitors of p38 MAP kinase. SB203580 (**1.71**) inhibits the catalytic activity of p38 MAP kinase by competitive binding in the ATP pocket.

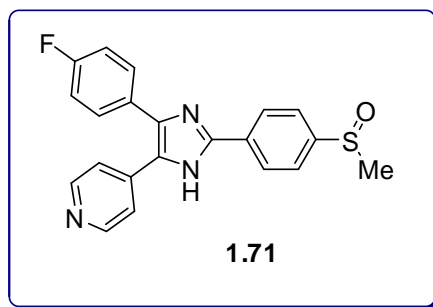


Figure 1.11: The prototypical inhibitor SB203580 (**1.71**)

Studies of p38 MAP kinase show this enzyme possesses a hydrophobic pocket not occupied by ATP (**1.72**). This provides opportunities for the discovery and design of selective small molecule ATP competitive inhibitors imitating ATP-adenine and gaining selectivity by occupying areas unfilled by ATP (**1.72**). Crystallographic mutational and biochemical studies by researchers at Boehringer Ingelheim demonstrated that SB203580 (**1.71**) and related pyridin-4-yl imidazole derivatives bind in the ATP binding site of p38. The pyridine ring nitrogen of the inhibitor forms a hydrogen bond to the backbone NH of Met109 in the linker region. This is a common feature observed in all crystal structures of p38 in complex with respective pyridin-4-yl imidazole derivatives. This H-bond underlines the crucial importance of the pyridine ring for biological activity.^{82,83} While the 4-fluorophenyl ring of SB203580 (**1.71**) binds to a hydrophobic region which in p38 is represented by an unusually spacious pocket, a

second hydrophobic area below the linker region is left unoccupied by simple pyridin-4-yl imidazole inhibitors (Figure 1.12).

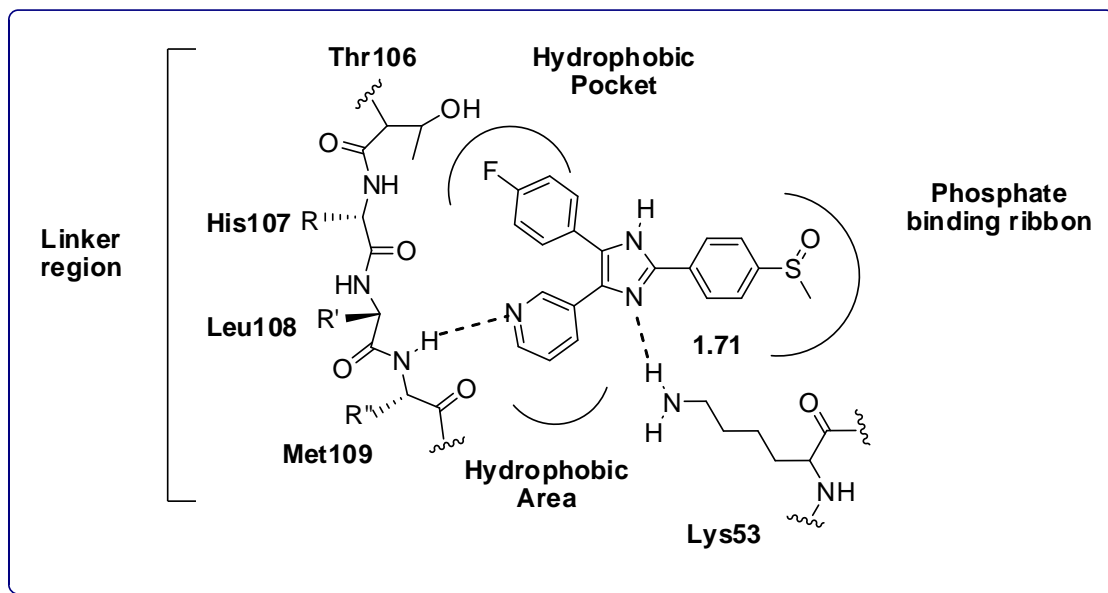


Figure 1.12: SB203580 (1.71) binding modes with p38 α

1.5 Conclusions

The Bohlmann-Rahtz pyridine synthesis has re-emerged as a viable route to substituted pyridines. New procedures, implementing metal based Lewis acid, Brønsted acid and metal free Lewis acid catalysis have been introduced in which various solvents including non-protic (toluene) and protic (ethanol and dimethylsulphoxide) were used. Also, new one-pot two- and three-component methodologies to produce substituted pyridines have been developed. Such procedures have been applied to the synthesis of various natural products and pyridine-containing libraries designed specifically for drug discovery, natural product mimetics and especially in the synthesis and structure elucidation of the thiopeptide antibiotics. The methods have been found to be

regiospecific and highly stereoselective, as well as relatively ecologically benign through promotion by microwave irradiation.

The mutant gene that encodes for the WRN protein has permitted the molecular pathology of Werner syndrome and its biological basis to be uncovered. Primary WS cells show many of the characteristics of cells growing under ‘replication stress’, display genome instability, manifested by telomere dysfunction, an elevated rate of chromosomal translocation and genomic deletions, and are hypersensitive to a subset of DNA damaging agents. These features suggest that the WRNp plays an important role in the cellular response to specific types of DNA damage. The genome instability seen in WS, together with the frequent replication fork stalling, provides a plausible trigger for replication stress in WS cells and a possible involvement for the p38 signalling pathway in inducing a shortened replicative life span. The studies have identified that premature senescence could be the result from activation of the ‘stress-associated p38 mitogen-activated protein kinase’ (MAPK), which is pivotal in orchestrating many stages of inflammation. Various heterocyclic inhibitors of the p38 MAPK signaling pathway have been investigated in Werner syndrome cells, such as SB203580, a cytokine-suppressive anti-inflammatory drug. The results revealed an unexpected reversal of the ageing phenotype of drug-treated WS fibroblasts, which show a much increased replicative life span and a growth rate and morphology that resembles that of young normal cells. The inhibitor SB203580 has been shown to prevent the phosphorylation of p38 α kinase in a ATP competitive manner and this implicates (but does not prove) this mechanism in accelerated ageing and gives potential to the prospect of targeting this pathway in a drug discovery programme, if a better mechanistic understanding can be garnered.

1.6 Goals of the project

The aim of the work was to develop new, rapid microwave-assisted methods for the synthesis of various substituted heterocycles, including pyridine derivatives where upon a Hantzsch-type three-component condensation, or related process, is involved. Also, new methodology for the synthesis of several biologically active inhibitors will be attempted under microwave conditions.

The research aimed to modify the Bohlmann-Rahtz pyridine synthesis to be simple, general, require short reaction times, involve mild conditions and to be high yielding. Such improvements could be achieved by carrying out the reactions under microwave conditions.

The project also aimed to synthesise the p38 MAPK inhibitor UR-13756 using a Hantzsch-type three component cyclocondensation under microwave irradiation conditions. Such procedure if successful should be simple and convenient and could build upon advances in Bohlmann-Rahtz pyridine synthesis in the development of improved methodology.

As an alternative inhibitor chemotype, 4-(3-amino-1-(4-methoxyphenyl)-1*H*-pyrazol-4-yl)benzamide was aimed to be synthesised in three steps under microwave conditions. The biological activities of such inhibitor would be investigated.

As a final inhibitor chemotype, the project also aimed to prepare the chemotherapeutic agent RO3201195, a highly selective inhibitor of P38 α using improved methods. It was planned to synthesise RO3201195 in seven steps under microwave conditions to prepare rapid access to this target for future biological evaluation.

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CHAPTER TWO
SYNTHESIS OF PYRIDINE DERIVATIVES

CHAPTER TWO

SYNTHESIS OF PYRIDINE DERIVATIVES

2.1 Introduction

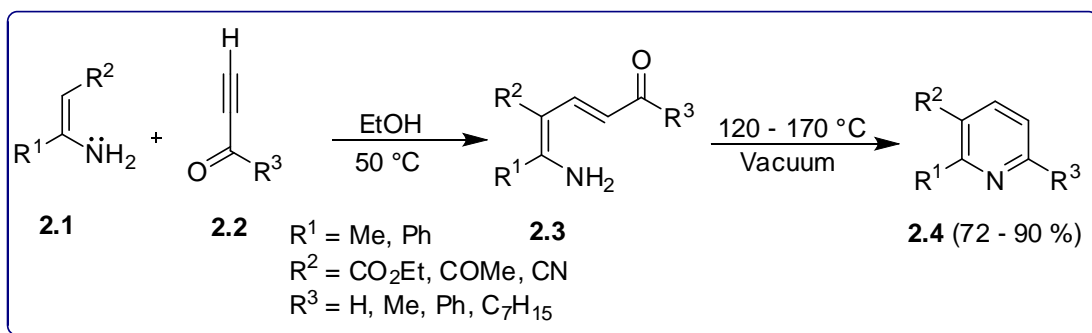
The synthesis of pyridine derivatives continues to attract interest from both chemists and biologists due to their wide range of biological properties.^{1,2} Various methods are available for the synthesis of substituted pyridines. The Bohlmann–Rahtz pyridine synthesis is one such method for the synthesis of pyridine derivatives that can proceed in a single step.³ Condensation of ethynylketones with enamines gave the corresponding aminodiene as an intermediate which underwent cyclodehydration to give the substituted pyridine derivatives.

The Bohlmann–Rahtz reaction is related to the well-known Hantzsch dihydropyridine synthesis. However, no aromatization oxidation step is required. Although the Bohlmann–Rahtz pyridine synthesis is highly versatile, it suffers from drawbacks that have limited its synthetic utility such as the requirement of high temperature for the cyclodehydration. Some of these drawbacks have been overcome to make it a convenient process for the synthesis of pyridines, although its substrate scope remains incompletely defined to date.

2.2 Bohlmann–Rahtz Reaction

Bohlmann and Rahtz first reported the synthesis of substituted pyridines **2.4** in a two step process.³ The first step involves reaction of enamines **2.1** and ethynylketones or aldehydes **2.2** to produce the corresponding aminodienone intermediates **2.3** (Scheme 2.1).³ Intermediates **2.3** were found to be kinetically stable due to the enone *E*-geometry. The second step involves heating **2.3** at 120–170 °C to promote the C=C

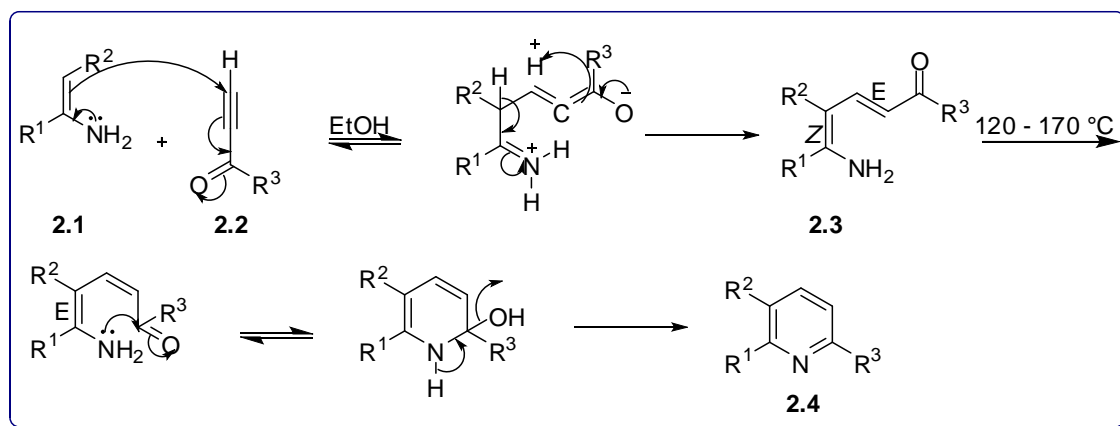
E/Z isomerisation followed by spontaneous cyclodehydration to provide the corresponding substituted pyridines **2.4** in high yield (72–90%; Scheme 2.1).³



Scheme 2.1 Synthesis of 2,3,6–trisubstituted pyridines **2.4** *via* Bohlmann–Rahtz methods³

The reaction represented in Scheme **2.1** is related to the Hantzsch dihydropyridine synthesis,^{4–8} but the use of ethynylketones provided the pyridine directly without the final aromaization step.^{9,10}

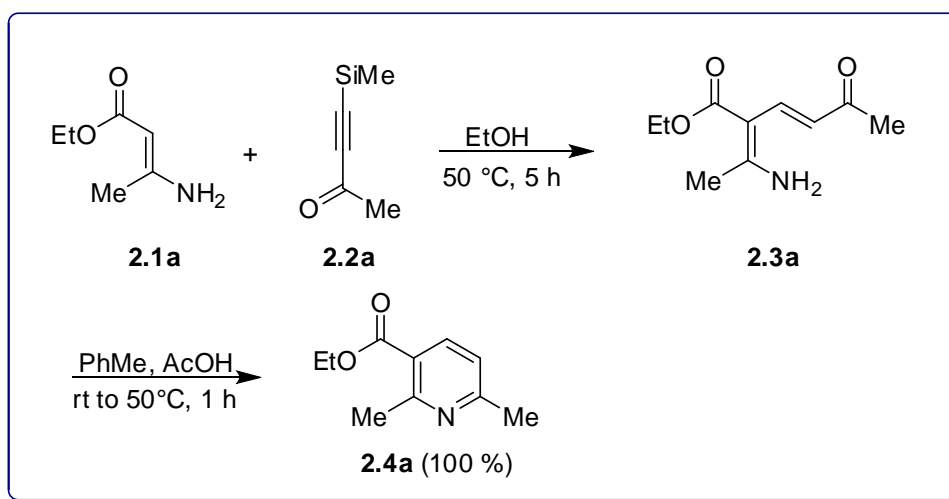
A suggested mechanism for the Bohlmann–Rahtz reaction is represented in Scheme **2.2** which involves a Michael addition followed by cyclodehydration to produce the pyridine derivative.



Scheme 2.2 Suggested mechanism for the two–step Bohlmann–Rahtz synthesis of substituted pyridines **2.4**

It was found that the Bohlmann–Rahtz reaction proceeded well in polar solvents rather than non–polar solvents and favoured polar protic solvents (*e.g.* ethanol) over polar aprotic solvents (*e. g.* dimethylsulfoxide).⁸

It has been reported that the use of acetic acid promoted the conjugate addition and double bond isomerisation of intermediates **2.3** necessary for cyclodehydration to produce pyridine derivatives **2.4**.¹¹ For example, condensation of **2.1a** and **2.2a** gave intermediate **2.3a** in 98% yield which underwent cyclodehydration in acid medium to provide trisubstituted pyridine **2.4a** in quantitative yield (scheme 2.3).¹¹ However, the reaction failed to provide the product when the ethyl ester (CO_2Et) was replaced by a cyano group.¹¹



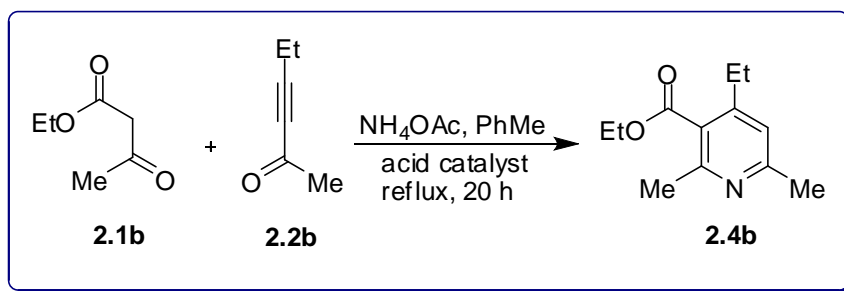
Scheme 2.3 Synthesis of trisubstituted pyridine **2.4a** via Bronsted acid Catalyst

Lewis acid catalysts have been used successfully for the production of substituted pyridines **2.4** in this process.^{11,12} Various Lewis acids such as FeCl_3 , $\text{Sc}(\text{OTf})_3$, ZnBr_2 and $\text{Yb}(\text{OTf})_3$ have been used;^{11,12} however, boron trifluoride etherate failed to produce the corresponding pyridine derivative. Zinc(II) bromide and ytterbium(III) triflate were found to be the most effective Lewis acids for the production of **2.4** in high yield.^{11,12} Generally reactions catalysed by zinc(II) bromide were faster and higher yielding than those conducted in the presence of ytterbium(III) triflate with a few exceptions.

As an alternative, reactions of enamines **2.1** and ethynyl ketones **2.2** in the presence of Amberlyst-15 ion-exchange resin for 26 hours at 50 °C in toluene gave the corresponding tetrasubstituted pyridines in 71–83% yields.¹²

A new multiple-component condensation reaction has been investigated to overcome the poor availability of precursors in this reaction.¹³ Such a process involves a three-component condensation of a β -keto ester **2.5** and alkynone **2.2b** in the presence of ammonium acetate in toluene under reflux condition for 20 hours (Scheme 2.4).¹³ The enamine **2.1** was generated *in-situ* and then reacted with **2.2b** to produce the

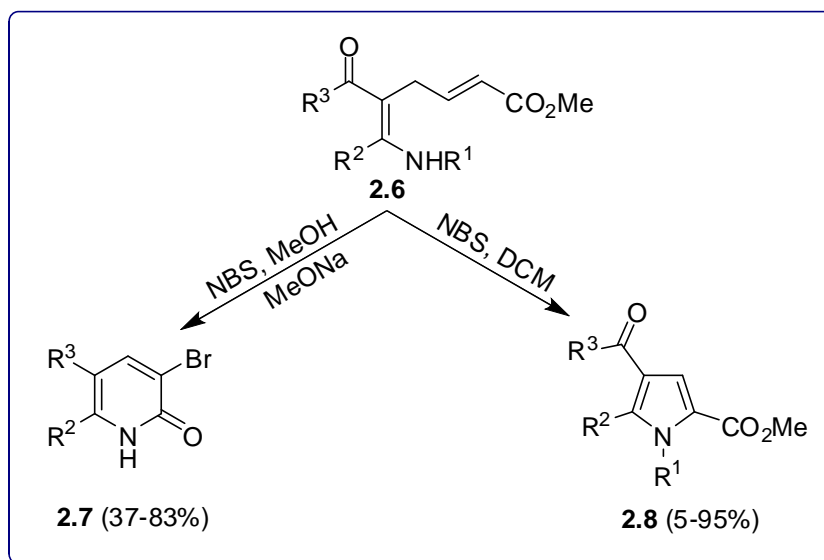
corresponding aminodienone intermediate **2.3b**. Acid catalysed cyclodehydration of **2.3b** afforded the corresponding substituted pyridines **2.4b** (Scheme 2.4) in 67–96% yields.¹³ Acetic acid, zinc (II) bromide and Amberlyst 15 were used successfully as acid catalysts in this 3-component process.¹³



Scheme 2.4 Three-component condensation reaction for the production of substituted pyridine **2.4b**

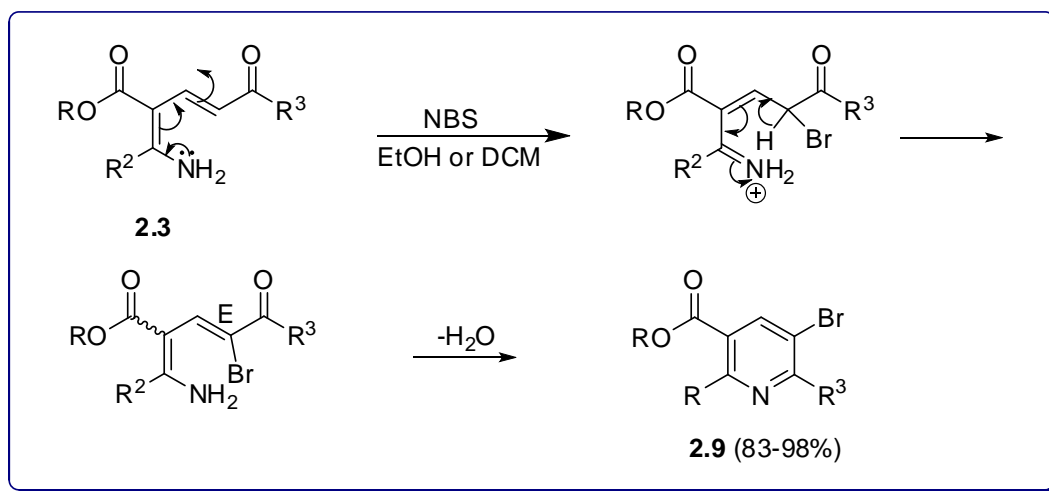
Further modifications of a multistep three-component condensation process have been reported for the synthesis of a series of substituted pyridines **2.4** in 38–98% yield using ammonium acetate in ethanol under reflux conditions for 24 h.¹⁴

In a related process, Dechoux reported a method for the cyclization of δ -dienaminoesters **2.6** to produce pyridinones **2.7** on treatment with NBS under basic conditions (Scheme 2.5)^{15,16}



Scheme 2.5 Synthesis of pyridinones **2.7** and pyrroles **2.8**

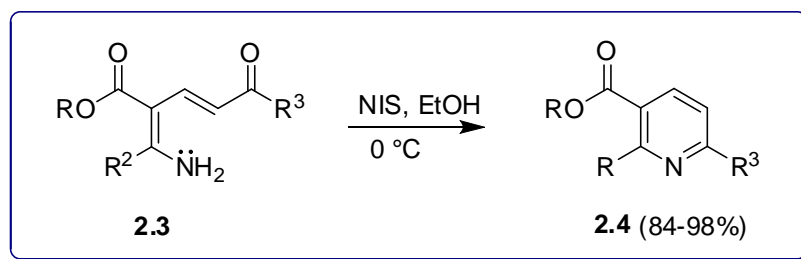
Bagley's research group have investigated the treatment of various Bohlmann-Rahtz aminodienones **2.3** with NBS under Dechoux's conditions and 5-bromopyridines **2.9** were produced in 83–98% yield (Scheme 2.6).¹⁴



Scheme 2.6 Synthesis of substituted pyridines **2.4** via bromination–cyclodehydration

2.3

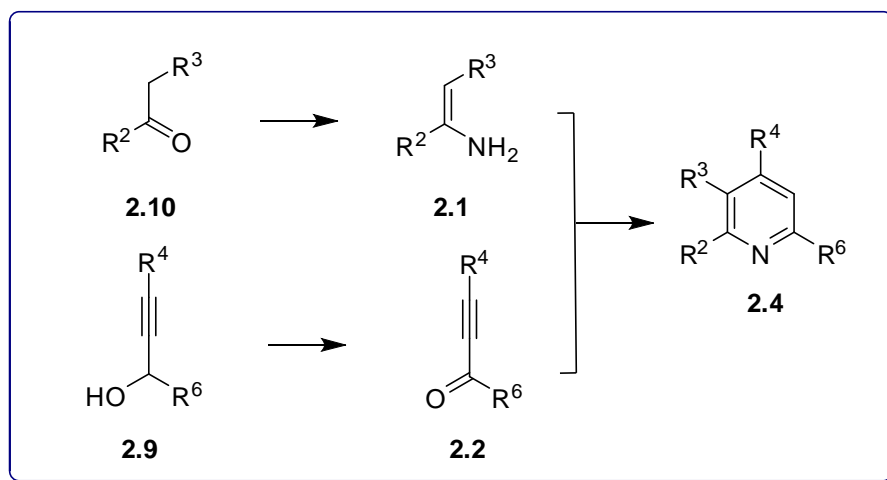
Also, *N*-iodosuccinimide (NIS) was successfully used to produce a range of substituted pyridines **2.4** in 84–98% yields at 0 °C in ethanol (Scheme 2.7).¹⁴ In this case the iodosuccinimide behaved as a Lewis acid rather than a halogenating agent to catalyze the cyclodehydration to the pyridine.



Scheme 2.6 Synthesis of substituted pyridines **2.4** via NIS-mediated cyclodehydration of aminodienones **2.3**

Traces of iodine could have been responsible in the NIS-mediated reaction to give the reported yield of product.¹⁷ In order to probe this possibility, it was found that the use of iodine alone gave a quantitative yield of the corresponding substituted pyridine **2.4**; the reaction was general for various substrates **2.3** and provided high yields (92–98%) of pyridine products **2.4**.¹⁴

A new one-pot tandem oxidation–heteroannellation of propargylic alcohols **2.9** with either *ortho*-iodoxybenzoic acid (IBX) or manganese dioxide (MnO₂) has been found to provide substituted pyridines.¹⁸ This reaction of β-ketoesters **2.10** with propargylic alcohols **2.9** in the presence of ammonium acetate under reflux in a mixture of toluene–acetic acid (5:1) gave the corresponding pyridines **2.4** (Scheme 2.8) in high yield.¹⁸ It was postulated that the enamine **2.1** was produced *in-situ* from condensation of the β-ketoester **2.10** with ammonia.¹⁹



Scheme 2.8 Tandem oxidation–heteroannulation of alcohols **2.9**

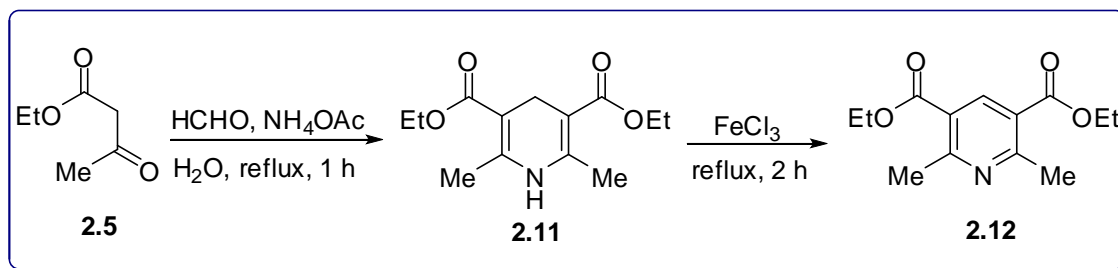
Substituted pyridines **2.4** were also synthesised in low to high yields under microwave conditions.^{19,20} For example, it was found that reactions of β -aminocrotonate **2.1a** with alkynones **2.2** in DMSO at 170 °C for 20 minutes under microwave conditions gave substituted pyridines **2.4** in 24–94% yields (Scheme 2.10).¹⁹ However, the reaction involved the use of very high temperatures which could be considered as a disadvantage. Also, under these conditions, the yield of a number of substituted pyridines **2.4** was low in some cases.

The Bohlmann–Rahtz reaction has been applied in the synthesis of various fused heterocycles containing a pyridine moiety.^{21–24} It has also been used for the production of more complex target molecules and biologically relevant structures.^{25–29}

2.3 Hantzsch dihydropyridine synthesis

The Hantzsch dihydropyridine synthesis was first reported in 1891.⁴ It is a well-known multi-component reaction that involves an aldehyde, β -ketoester (two molar equivalents) and ammonia or ammonium acetate.⁴ For example, reaction of

formaldehyde, ethyl acetate (**2.5**) and ammonium acetate in aqueous medium under reflux conditions gave dihydropyridine **2.11** (Scheme 2.9). Aromatisation of **2.11** with FeCl_3 gave the corresponding pyridine derivative **2.12** in 93% yield (Scheme 2.9).⁴



Scheme 2.9 Hantzsch dihydropyridine synthesis

The reaction proceeded *via* Knoevenagel condensation product **2.13** (Figure 2.1) which is another key intermediate, along with ester enamine **2.14** (Figure 2.1) in this reaction. Product **2.13** was produced from reaction of ethyl acetoacetate with formaldehyde, whilst intermediate **2.14** was generated by condensation of the second equivalent of ethyl acetoacetate (**2.5**) with ammonia. Evidence of the mechanistic course has been provided in studies by Katrizky.⁴¹

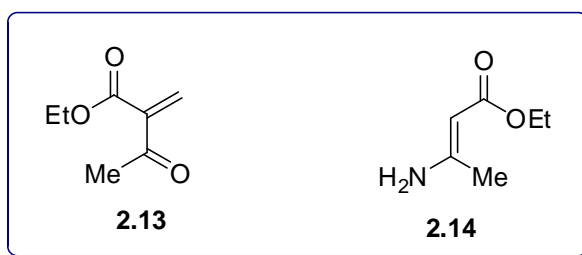
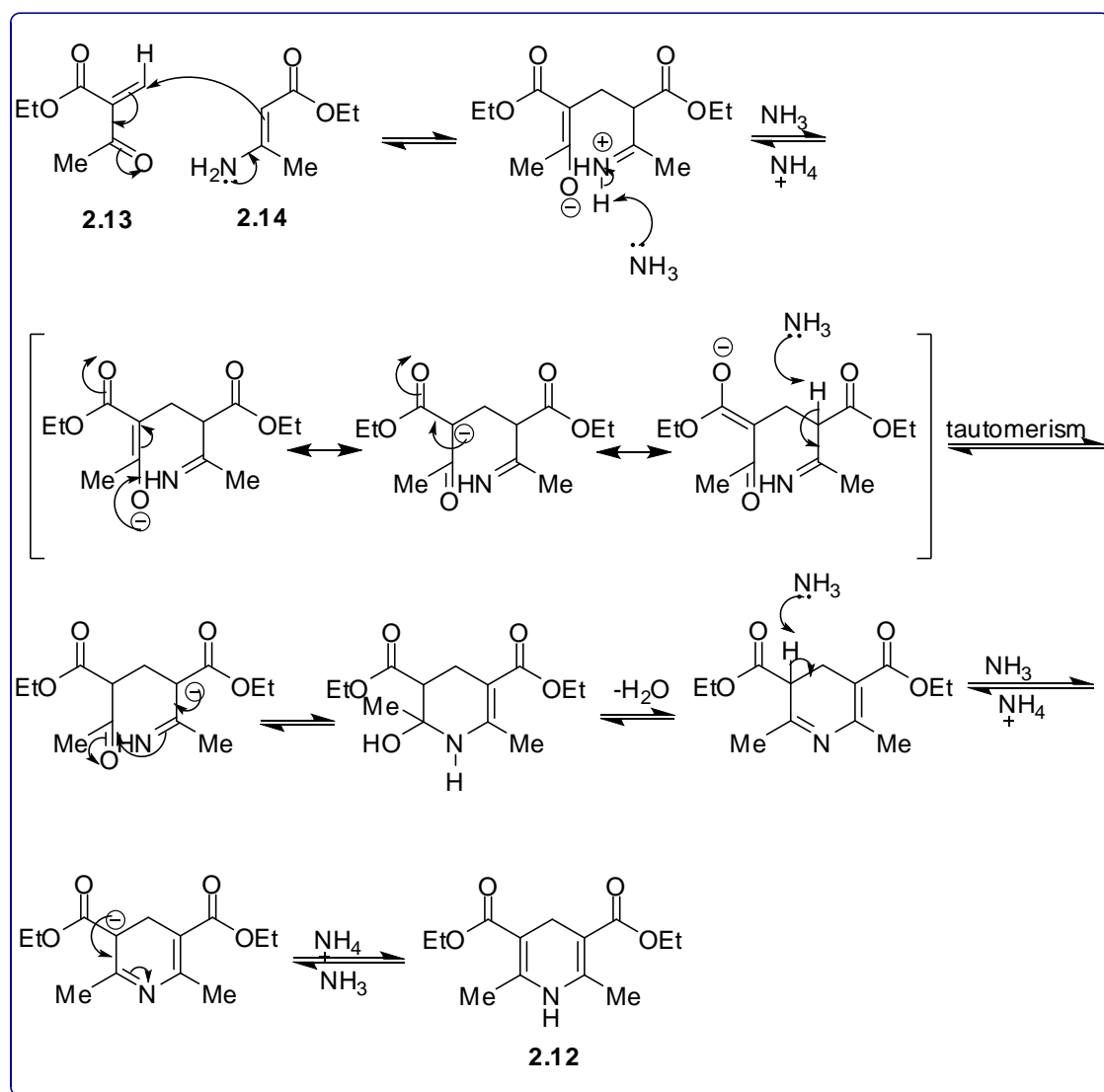


Figure 2.1 Key intermediates **2.13** and **2.14** in Hantzsch dihydropyridine synthesis

The mechanism for Hantzsch reaction is represented in Scheme 2.10.



Scheme 2.10 Suggested mechanism for Hantzsch dihydropyridine synthesis

The Hantzsch synthesis has been applied in the production of various dihydropyridine derivatives where the reaction benefits from use of microwave irradiation and various catalysts.^{30–40}

2.4 Synthesis of substituted pyridine derivatives

The aim of the work reported in this chapter was to develop a new synthetic approach for the synthesis of substituted pyridine derivatives that was applicable to the pyrazolopyridine core of UR-14756 and other fused pyridine-containing heterocyclic motifs. The use of microwave irradiation to facilitate Bohlmann-Rahtz pyridine synthesis had met with some success in previous studies, although yields could be variable. Furthermore, the use of Lewis acids (15 mol% ZnBr_2) or Brønsted acids (AcOH) had been found to be compatible with these conditions.¹⁹ Thus, our initial goal was to establish if by combining the action of microwaves and a catalyst would provide a reliable and rapid route to Bohlmann-Rahtz pyridines that could then be extended to access fused pyridine-containing heterocycles. In particular, since AuCl_3 had been reported to exhibit high efficiency as a catalyst in glucoside synthesis,⁴² based upon its alkynophilicity, its behaviour in the Bohlmann-Rahtz reaction was prioritised as a good initial system of study.

The first target was the synthesis of ethyl 2,6-dimethylpyridine-3-carboxylate (**2.4a**) under microwave conditions to consolidate the pyridine forming step. Compound **2.4a** was previously synthesised within our research group¹¹ from the reaction of ethyl β -aminocrotonate (**2.1a**) and 4-(trimethylsilyl)but-3-yn-2-one (**2.2a**) to produce intermediate **2.3a** (Scheme 2.3).¹¹ Cyclodehydration of **2.3a** under acidic conditions provided **2.4a** in high yields.¹¹

The first task was the synthesis of intermediate **2.3a** under microwave conditions. Various attempts were made to synthesis **2.3a** from the reaction of **2.1a** and **2.2a** in ethanol under microwave irradiation. The results obtained are recorded in Table 2.1 along with those obtained in a comparative process under reflux conditions.

Table 2.1 Synthesis of intermediate **2.3a** under microwave conditions

<p>The reaction scheme shows the synthesis of intermediate 2.3a from 2.1a and 2.2a in EtOH. 2.1a is ethyl 2-amino-3-methylacrylate, and 2.2a is 1-methyl-2-(trimethylsilyl)ethyn-1-one. The product 2.3a is ethyl 2-amino-3-methyl-2-(3-oxobut-1-en-1-yl)acrylate.</p>			
Entry	Reaction conditions		Yield (%)
	Temp (°C)	Time (h)	
1	50, reflux	5	98 ^a
2	60, MW	1	86 ^a
3	60, MW	1	100 ^b

^a Yield of pure product **2.3a** after purification by column chromatography (silica gel; ethyl acetate–petroleum ether in 1:1 by volume).

^b Yield of pure product **2.3a** after crystallization from a mixture of hexane–acetone, 7:1 by volume.

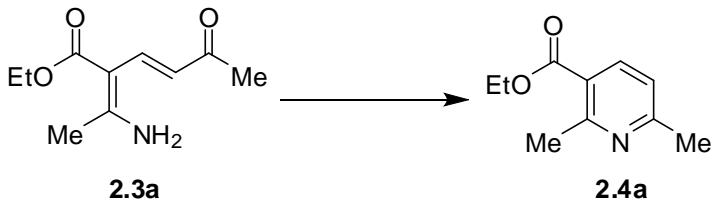
The results reported in Table 2.1 clearly indicated that compound **2.3a** could be produced in quantitative yield under microwave conditions at 60 °C for 1 hour (Table 2.1; Entry 3). Also, no column chromatography was needed to purify the product **2.3a**. This represented the most rapid method reported to date for efficient access to substrates for Bohlmann-Rahtz cyclodehydration and as such should facilitate the study of the second cyclodehydration step.

The structure of **2.3a** was confirmed by various spectroscopic data (see Chapter 6; Section 6.2 for details). The atmospheric pressure chemical ionization mass spectrum of **2.3a** showed a molecular ion peak at $m/z = 197$. The HRMS of the molecular ion peak confirmed the formula as C₁₀H₁₅NO₃. The IR spectrum showed a strong absorption bands at 1740 and 1640 cm⁻¹ due to two carbonyl groups. The ¹H NMR spectrum showed two characteristic doublets ($J = 15$ Hz) at δ 7.45 and 6.38 ppm due to the CH=CH protons. It also showed the presence of three methyl groups that resonated at δ

2.45 (singlet), 2.10 (singlet) and 1.22 (triplet, $J = 7.1$ Hz) ppm. ^{13}C NMR spectroscopic analysis showed the presence of two carbonyl carbons that resonated as singlet signals at δ 199.7 (ketone) and 170.5 (ester) ppm and also showed all other carbons that were consistent with the given structure.

Having successfully synthesised **2.3a** in quantitative yield under microwave conditions, our attention turned next to the synthesis of substituted pyridine **2.4a** under various reaction conditions, including the use of a lewis acid catalyst with high alkynophilicity (AuCl_3). In order to improve and optimize this process, solvent, temperature and catalyst were varied and the isolated yield of the pyridine product was determined, where appropriate. The results obtained are recorded in Table 2.2.

Table 2.2 Synthesis of ethyl 2,6-dimethylpyridine-3-carboxylate **2.4a** under various reaction conditions

<div style="text-align: center;">  <p>2.3a 2.4a</p> </div>						
Entry	Temp (°C)	Time (h)	Solvent	Acid	Catalyst	Yield (%)
1	RT	5	PhMe	AcOH	—	85
2	RT	5	PhMe	AcOH	$\text{HAuCl}_4 \cdot \text{H}_2\text{O}$	99
3 ^a	RT	20	2-propanol	—	CeCl_3	—
4	120, MW	1	PhMe	AcOH	$\text{HAuCl}_4 \cdot \text{H}_2\text{O}$	<i>b</i>

^a NaI was used.

^b An unknown side product was generated but its structure was not established.

Firstly, it was found that the yield of **2.4a** was improved to 99% when $\text{HAuCl}_4 \cdot \text{H}_2\text{O}$ was used as a catalyst in toluene as solvent in the presence of acetic acid

at room temperature for 5 hours (Table 2.2; Entry 2). This compared favourably with the 85% isolated yield when no Au catalyst was used (Table 2; Entry 1) but acetic acid was present. However, in comparison when CeCl_3 was used as a catalyst in 2-propanol the reaction never went to completion even after 20 hours at room temperature (Table 2.2; Entry 3). The reaction was attempted under microwave conditions (120 °C for 1 hour) in a mixture of toluene and acetic acid to accelerate the process, but the desired product **2.4a** was no longer formed (Table 2.2; Entry 4). The NMR spectrum of the crude material showed the presence of a new product but its structure could not be established.

The structure of **2.4a** was confirmed by spectral data from NMR, MS and IR. The APcI mass spectrum of **2.4a** showed the presence of a pseudo molecular ion peak (MH) at $m/z = 180$ and the HRMS confirmed its formula as $\text{C}_{10}\text{H}_{14}\text{NO}_2$. The ^1H NMR spectrum of **2.4a** show two characteristic doublets ($J = 8$ Hz) that resonated at 8.08 and 7.22 ppm which were attributed to H-4 and H-5 of the pyridine ring, respectively. The ^{13}C NMR showed a singlet signal at $\delta = 166.1$ ppm due to the carbonyl carbon. Also, it showed resonances for all pyridine carbons and the two methyl and ethyl groups.

Since microwave conditions were not successful to produce pyridine **2.4a** from **2.3a**, our attention next turned to the synthesis of **2.4a** directly from **2.1a** and **2.2a** without isolation of the intermediate **2.3a**. If successful this would provide a more direct route to the target pyridines. The results obtained are recorded in Table 2.3 carried out in toluene and in the presence and absence of $\text{HAuCl}_4 \cdot \text{H}_2\text{O}$ as catalyst.

Table 2.3 Synthesis of pyridine **2.4a** directly from the reaction of enamine **2.1a** and ethynylketone **2.2a**

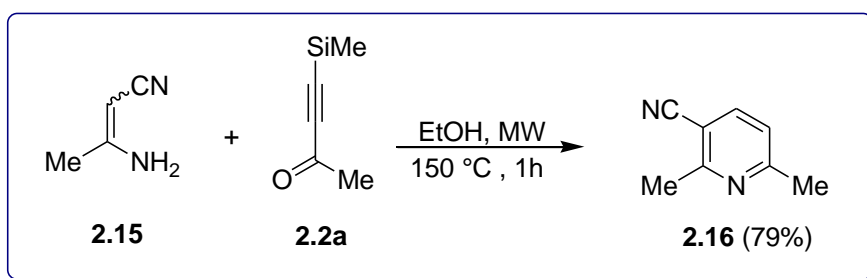
	2.1a		2.2a		2.4a
Temp		Catalyst			
Entry	(°C)	Time (h)	Solvent		Yield (%)
1	RT	5	PhMe	H[AuCl ₄].H ₂ O	—
2	RT	5	PhMe–AcOH	H[AuCl ₄].H ₂ O	^a
3	120, MW	1	PhMe–AcOH	—	62
4	120, MW	1	PhMe–AcOH	HCl	74
5	120, MW	1	PhMe–AcOH	H[AuCl ₄].H ₂ O	96

^a A new product was produced but its structure was not established.

Curiously, it was found that reaction of **2.1a** and **2.2a** under microwave conditions at 120 °C for 1 hour in toluene in the presence of acetic acid and H[AuCl₄].H₂O produced **2.4a** in 96% yield (Table 2.3; Entry 5). This was now in stark contrast to the process conducted at room temperature using the Au catalyst for 5 hours in the presence or absence of acetic acid, neither of which provided the desired product **2.4a** (Table 2.3; Entries 1 and 2). Product **2.4a** (Entry 5) was indistinguishable in all respects from the material produced from intermediate **2.3a** (Table 2.2). To establish if the addition of H[AuCl₄].H₂O had facilitated the reaction two further experiments were carried out: firstly the Au catalyst was omitted and the reaction repeated (Entry 3) and secondly HCl was added to the reaction mixture (Entry 4) to establish if the presence of the Brønsted acid had been responsible for any increase in yield. In both of these cases pyridine **2.4a** was isolated. It was concluded that, in this system of study, AcOH and

HCl may well be behaving as Brønsted acid catalysts for the Bohlmann-Rahtz reaction but the presence of a Lewis acid catalyst with high alkynophilicity may have contributed to the high yield of the process.

Having successfully produced **2.4a** under microwave conditions in a single step and in only 1 h reaction time, our attention next turned to the synthesis of other substituted pyridines to test the generality of the process. However, it was found that reaction of ethyl 3-aminocrotononitrile (**2.15**) and 4-(trimethylsilyl)but-3-yn-2-one (**2.2a**) under the Au-catalyzed conditions this time was not successful, whereas microwave irradiation in dry ethanol at 150 °C for 1 hour in the absence of HAuCl_4 gave 2,6-dimethyl-3-pyridinecarbonitrile (**2.16**) in 79% yield after purification by column chromatography (Scheme 2.11).

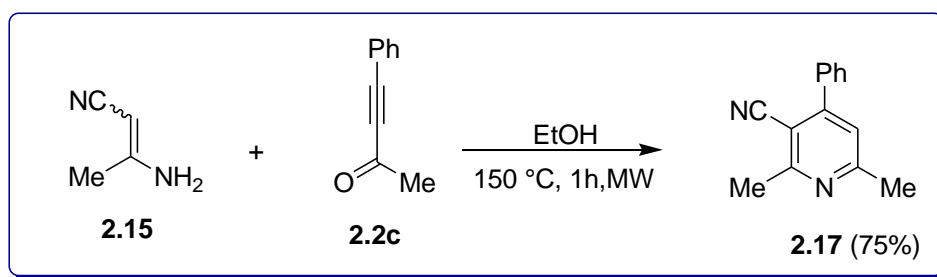


Scheme 2.11 Synthesis of 2,6-dimethyl-3-pyridinecarbonitrile (**2.16**) under microwave conditions

The APcI mass spectrum of **2.16** showed a pseudo molecular ion peak at $m/z = 133$ (MH) and the EI-mass spectrum showed a molecular ion peak at $m/z = 132$ (M^{+}). The HRMS confirmed the formula of the molecular ion as $\text{C}_8\text{H}_8\text{N}_2$. The presence of the $\text{C}\equiv\text{N}$ group was confirmed by both IR and ^{13}C NMR spectroscopy. The IR spectrum showed an absorption band at $\nu = 2190\text{ cm}^{-1}$ due to the $\text{C}\equiv\text{N}$ stretch vibration. The ^{13}C NMR spectrum showed a singlet carbon at $\delta 117.5\text{ ppm}$ due to the nitrile carbon. The

^1H NMR spectrum of **2.16** showed the presence of two doublets ($J = 8$ Hz) at $\delta = 8.05$ and 7.22 ppm due to H-4 and H-5 of the pyridine ring, respectively and two singlet signals at δ 3.32 and 2.61 ppm due to methyl group proton resonances.

Given that for nitrile-containing substrates relatively simple conditions were successful in pyridine formation, our attention turned to the use of a much less-reactive ethynylketone to establish the scope of the process. To that end, the synthesis of 3-cyano-2-methyl-6-phenylpyridine (**2.17**) was attempted from reaction of ethyl 3-aminocrotononitrile (**2.15**) and 4-phenyl-3-buten-2-one (**2.2c**) under microwave conditions (Scheme 2.12).

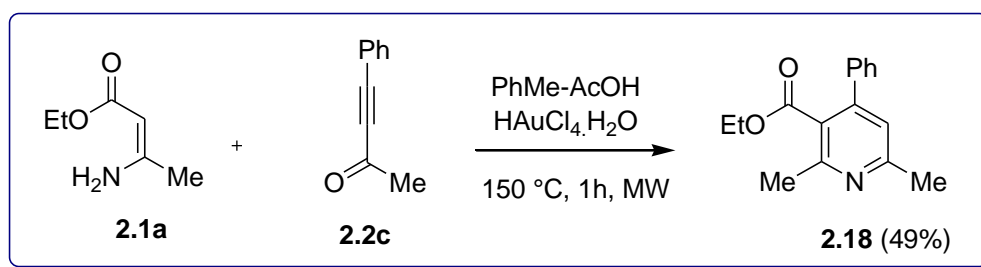


Scheme 2.12 Synthesis of 3-cyano-2-methyl-6-phenyl pyridine (**2.17**) under microwave conditions

Once again, the use of HAuCl_4 gave mixtures of products whereas irradiation in a protic solvent gave the desired pyridine. Compound **2.17** was produced in 75% isolated yield (Scheme 2.12) after purification by column chromatography when the reaction was carried in ethanol under microwave conditions at 150 °C for one hour. It was concluded that even terminally-substituted ethynylketones when reacted with aminocrotononitrile substrates gave good yields of the Bohlmann-Rahtz pyridines in one step under these simple conditions.

The EI-mass spectrum of **2.17** showed a pseudo molecular ion peak at $m/z = 207$ and the HRMS of the pseudo molecular ion confirmed its formula as $C_{15}H_{13}N$ (MH). The IR spectrum of **2.17** showed an absorption band at ν 2290 cm^{-1} due to the $C\equiv N$ group. Furthermore, the NMR spectra of **2.17** were in agreement with the assigned structure.

Given that the crotonate precursors were the substrates that required the presence of the Au catalyst for efficient conversion to the pyridine, we investigated one further reaction of ethyl 3-aminocrotonate (**2.1a**) and the terminally-substituted less-reactive ethynylketone, 4-phenyl-3-butyne-2-one (**2.2c**), under microwave conditions in an attempt to produce ethyl 2,6-dimethyl-4-phenylpyridine-3-carboxylate (**2.18**; Scheme 2.13).



Scheme 2.13 Synthesis of 2,6-dimethyl-4-phenylpyridine-3-carboxylate (**2.18**) under microwave conditions

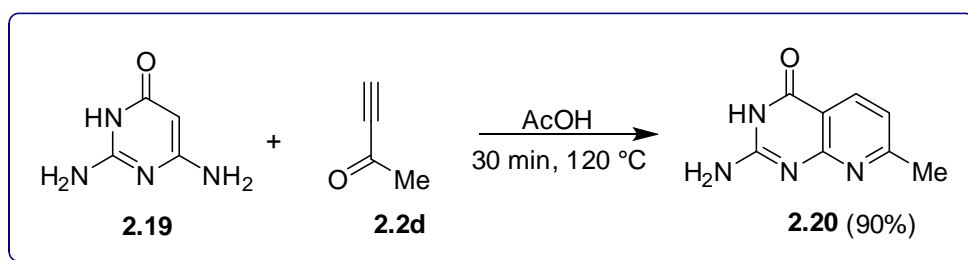
It was found that reaction of **2.1a** and **2.2c** in toluene and acetic acid in the presence of gold hydrochloride as a catalyst under microwave irradiation at 150 °C for 1 hour gave **2.18** in 49% yield (Scheme 2.13) after purification by column chromatography. This was a considerable improvement over a related AcOH catalyzed method (50 °C, 5-6 h) investigated previously which failed to give the desired pyridine.⁴³

The structure of **2.18** was confirmed by IR, NMR and mass spectral data. The IR spectrum of **2.18** showed a strong absorption band at ν 1730 cm^{-1} due to the carboxylate group. The APcI mass spectrum showed a very intense (100%) pseudo molecular ion peak at $m/z = 256$ due to MH. The EI mass spectrum of **2.18** showed the presence of a molecular ion peak $m/z = 255$ and the HRMS confirmed the formula of the molecular ion as $\text{C}_{16}\text{H}_{17}\text{NO}_2$. The NMR spectral data were in agreement with the predicted one collected from Chem Draw.

Having successfully synthesised a range of substituted pyridines our attention was next turned to the synthesis of fused heterocycles containing the pyridine moiety. Given that, for all but the most challenging of substrates, the use of simple conditions in the presence of acetic acid under microwave irradiation gave good yields of the corresponding pyridines, this method was favoured as the procedure of choice. The synthesis of pyrido[2,3-*d*]pyrimidines from uracil derivatives has been reported in the past and serves as a rapid route to the 5-deazapterin motif,⁴⁴ but many of these reactions suffer from low yields, use expensive or not readily available precursors and suffer from limited substrate tolerance. Uracil derivatives are versatile building blocks for the synthesis of a wide range of nitrogen-containing heteroaromatic species.⁴⁵ Pyrazolopyridines,⁴⁶ pyrimidopyrimidines,⁴⁷ pyrazolopyrimidines,⁴⁸ pyridopurines,⁴⁹ and xanthine derivatives⁵⁰ have all been prepared by the functionalisation of these precursors, which provide a ready-assembled 2-aminopyrimidin-4-one motif for incorporation into the target heterocycle. On this basis, we sought to develop a facile and rapid synthesis of pyrido[2,3-*d*]pyrimidines representing a 5-deazapterin unit from commercially available aminouracil derivatives using a heteroannulation process based upon the Bohlmann-Rahtz pyridine synthesis.

2.5 Synthesis of substituted pyrido[2,3-*d*]pyrimidinone derivatives

Reaction of 2,4-diamino-6-hydroxypyridine (**2.19**) with equimolar quantities of 3-butyne-2-one (**2.2d**) in acetic acid under microwave conditions was attempted in an attempt to produce 2-amino-7-methylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**2.20**; Scheme 2.14). The reaction mixture was irradiated at 120 °C for 30 min and submitted for aqueous work-up. The crude mixture was examined by TLC analysis, which showed the formation of a new product and the consumption of both of the starting materials. Product **2.20** was obtained in 90% yield without the need for further purification. Compound **2.20** was previously synthesised in similar yield but in ethanol at 50 °C for 72 h.¹² The new process clearly showed that the use of microwave conditions provided an efficient and simple synthesis of the deazapterin structure **2.20** (See Section 6.7).

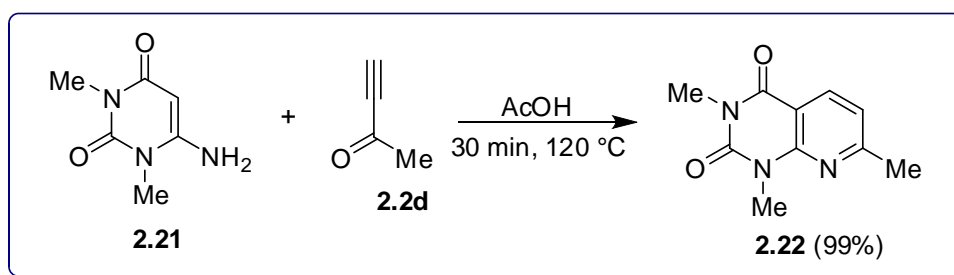


Scheme 2.14 Synthesis of 2-amino-7-methylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**2.20**)

The structure of **2.20** was confirmed by spectroscopic and spectrometric data obtained from NMR, MS and IR. The IR spectrum showed a strong absorption band at $\nu = 1666\text{ cm}^{-1}$ corresponding to the vibration of the carbonyl group. The APcI spectrum showed an intense pseudo molecular ion peak at m/z 177 and the high resolution mass spectrum of this ion confirmed its formula as $\text{C}_8\text{H}_{19}\text{N}_4\text{O} (\text{MH}^+)$. The ^1H NMR spectrum of **2.20** showed two characteristic doublets ($J = 8.1\text{ Hz}$) that resonated at $\delta = 9.05$ and 7.62 ppm due to H-5 and H-6, respectively. The ^{13}C NMR spectrum showed all the

expected signals and was consistent with the suggested structure of **2.20** (See Section 6.7).

In a similar manner, the reaction of aminodimethyluracil **2.21** with equimolar quantities of 3-butyn-2-one (**2.2d**) in acetic acid under microwave conditions gave 7-amino-1,3-dimethylpyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (**2.22**) in 99% isolated yield (Scheme 2.15).

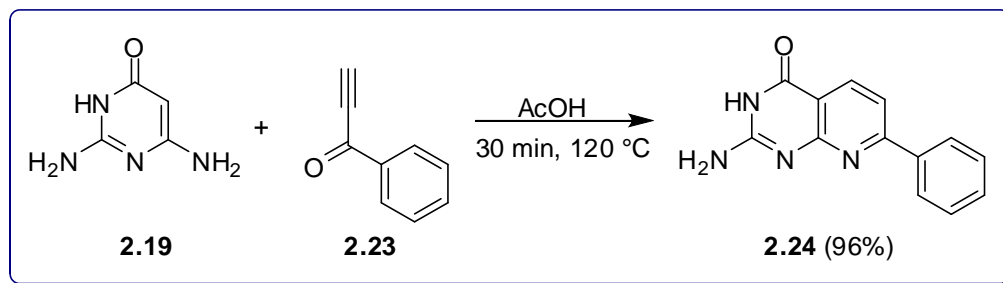


Scheme 2.15 Synthesis of 1,3,7-trimethylpyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (**2.22**)

The structure of **2.22** was confirmed by the spectroscopic data obtained by IR, NMR and MS analysis. The electron impact mass spectrum of **2.22** showed the absence of a molecular ion peak but showed a pseudo molecular ion peak at m/z 190 due to elimination of a methyl radical from the molecular ion ($[M - \text{Me}]^+$). The high resolution mass spectrum of this ion confirmed its formula as $\text{C}_9\text{H}_8\text{N}_3\text{O}_2$. The ^1H NMR spectrum showed the presence of protons due to the three methyl groups and two characteristics doublets ($J = 8.1$ Hz) that resonated at $\delta = 8.32$ and 7.03 ppm due to H-5 and H-6, respectively (See Section 6.8).

Also, it was found that reaction of 2,4-diamino-6-hydroxypyridine (**2.19**) with an equimolar quantity of 1-phenyl-2-propyn-1-one (**2.23**) in acetic acid under

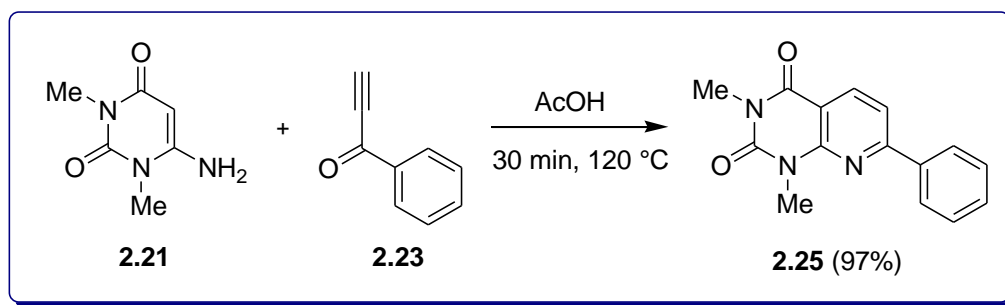
microwave conditions gave 2-amino-7-phenylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**2.24**) in 96% yield (Scheme 2.16).



Scheme 2.16 Synthesis of 2-amino-7-phenylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**2.24**)

The structure of **2.24** was confirmed by the spectroscopic and spectrometric data obtained from NMR and MS. The EI-mass spectrum of **2.24** showed a molecular ion peak at $m/z = 238$ and the HRMS of the molecular ion confirmed its formula as $C_{13}H_{10}N_4O$ (M). The IR spectrum of **2.24** showed an absorption band at $\nu = 3248\text{ cm}^{-1}$ due to NH_2 and NH groups. It also showed a strong band at $\nu = 1666\text{ cm}^{-1}$ due to the $C=O$. The NMR spectra of **2.24** were in agreement with the assigned structure (See Section 6.9).

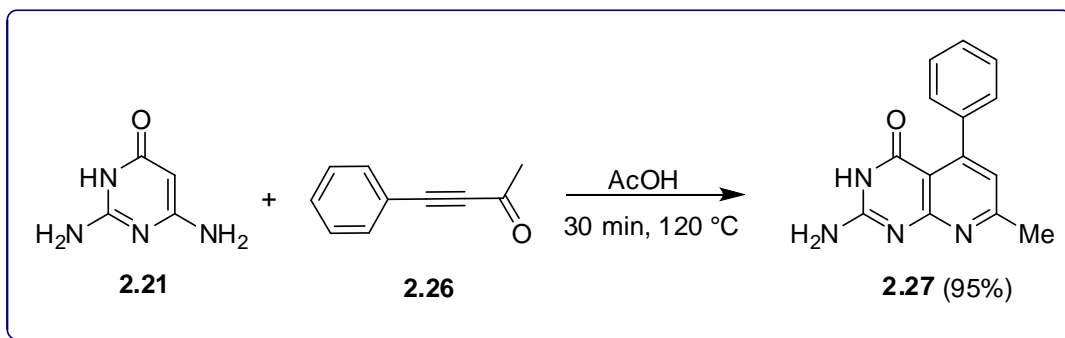
The same method was used for the synthesis of 1,3-dimethyl-7-phenylpyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione. Reaction of dimethyluracil derivative **2.21** with an equimolar quantity of 1-phenyl-2-propyn-1-one (**2.23**) in acetic acid under microwave conditions gave 1,3-dimethyl-7-phenylpyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (**2.25**) in 97% yield (Scheme 2.16).



Scheme 2.16 Synthesis of 1,3-dimethyl-7-phenylpyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (**2.25**)

The structure of **2.25** was confirmed by spectroscopic and spectrometric data obtained from IR, NMR and MS. The EI-mass spectrum of **2.25** showed a pseudo molecular ion peak at $m/z = 267$ ($[M - Me]^+$) and the HRMS of the pseudo molecular ion confirmed its formula as $C_{14}H_{10}N_3O_2$. The IR spectrum of **2.25** showed absorption bands at $\nu = 3248\text{ cm}^{-1}$, due to the NH_2 and NH , $\nu = 1666\text{ cm}^{-1}$, due to the $C=O$, and $\nu = 1581\text{ cm}^{-1}$, characteristic of these motifs. The NMR spectra of **2.25** were in agreement with the assigned structure (See Section 6.10).

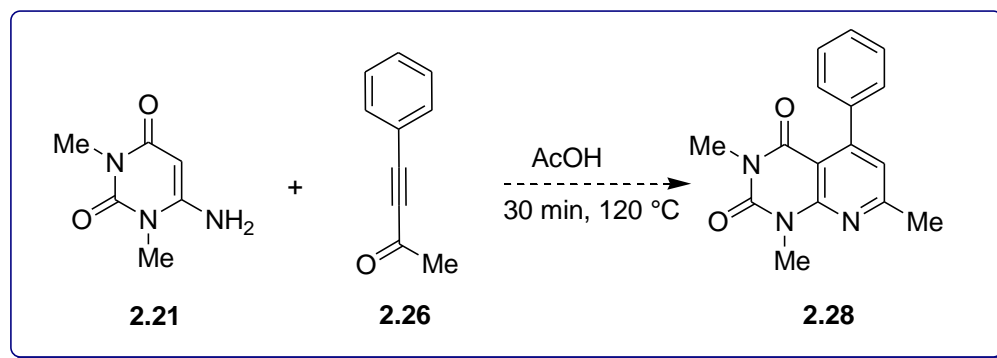
As a further example, the reaction of 2,4-diamino-6-hydroxypyridine (**2.19**) with an equimolar quantity of 4-phenyl-1-butyne-2-one (**2.26**) in acetic acid under microwave conditions produced 2-amino-7-phenylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**2.27**) in 95% yield (Scheme 2.17).



Scheme 2.17: Synthesis of 2-amino-7-phenylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**2.27**)

The structure of **2.27** was confirmed by spectroscopic and spectrometric data obtained from NMR and MS. The EI–mass spectrum of **2.27** showed a molecular ion peak at $m/z = 252$ (M) and the HRMS of the molecular ion confirmed its formula as $C_{14}H_{12}N_4O$. The IR spectrum of **2.27** showed absorption bands at $\nu = 3233$, 1669 and 1590 cm^{-1} due to the NH_2 and NH , $C=O$ and $C=C$, respectively. The NMR spectra of **2.27** were in agreement with the proposed structure (See Section 6.11).

Finally, the synthesis of 2-amino-7-phenylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**2.28**) was attempted under similar conditions (Scheme 2.18). Reaction of uracil derivative **2.21** with an equimolar quantity of 4-phenyl-1-butyne-2-one (**2.26**) in acetic acid under microwave conditions gave a mixture products, from which the desired deazapterin **2.28** could not be isolated, although the signals for the desired product **2.28** could be identified in the 1H NMR spectrum.



Scheme 2.18: Synthesis of 2-amino-7-phenylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**2.28**)

2.5 Conclusions

Recently Bohlmann–Rahtz pyridine synthesis has provided a convenient route for the synthesis of various substituted pyridine products. The process has been modified in this work to be rapid, simple, operate under mild conditions and high yielding. We have shown that substituted pyridines could be synthesized efficiently and

in high yields under microwave conditions from readily-available precursors in what is essentially a relatively short reaction time. The process has been applied successfully to the production of substituted pyrido[2,3-*d*]pyrimidinone derivatives that represent the core of the deazapterins in high yields. Reaction times for these more complex fused targets have been reduce from 72 h to 30 min in what is a remarkably efficient and general process. Given the great success of this methodology, it is anticipated that the microwave-assisted Brønsted acid catalyzed Bohlmann-Rahtz reaction could find further application in the future in the synthesis of pyridine-containing targets.

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CHAPTER THREE

SYNTHESIS OF AMINOPYRAZOLES, PYRAZOLOPYRIMIDINES, PYRAZOLOPYRIDINES AND THE P38 MAPK INHIBITOR UR-13756

CHAPTER THREE

SYNTHESIS OF AMINOPYRAZOLES, PYRAZOLOPYRIMIDINES, PYRAZOLOPYRIDINES AND THE P38 MAPK INHIBITOR UR-13756

3.1. Introduction

The p38 mitogen-activated protein kinases (MAPKs) are involved in cell differentiation with a highly recognised role in cell cycle regulation, senescence, inflammation, tumorigenesis and cell death.¹⁻³ P38 is also responsive to heat shock, ultraviolet radiation and the action of cytokines.

Four isoforms of p38 are known which are p38 α , p38 β , p38 γ and p38 δ , which are also known as MAPK14, MAPK11, MAPK12 and MAPK13, respectively.⁴ The p38 α isoform is the most studied member of this family which participates in cell-signalling cascades, upregulated in many inflammatory diseases in humans, and is involved in the biosynthesis of pro-inflammatory cytokines.⁵ When activated, p38 α MAPK is phosphorylated in an activation loop by dual specificity kinase MKK3 and 6 in response to extracellular stimuli and phosphorylates other kinases, leading to the regulation of target genes. The reduction of pro-inflammatory cytokine levels offers a means for the treatment of inflammatory disorders such as rheumatoid arthritis and so the design of safe and efficacious p38 α inhibitors suitable for clinical investigation remains a compelling therapeutic target.

A range of pyridylimidazoles were originally synthesised as dual cyclooxygenase 15-lipoxygenase inhibitors.⁶ As a result SB203580 (**3.1**; Figure 3.1) was discovered and was found to be an ATP-competitive inhibitor of p38 MAPK,

reducing the activation of the phosphorylation of the small heat-shock protein 27 (HSP27) and of MAPK-activated protein kinase 2 (MK2).⁷

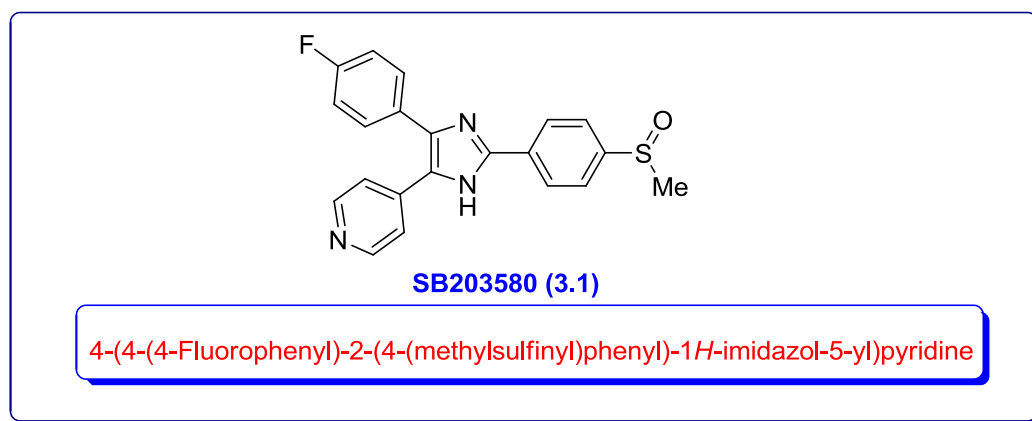


Figure 3.1: Structure of p38 MAPK inhibitor (3.1)

The SB203580 p38 α inhibitor mediates multiple cellular responses, including the production of inflammatory cytokines. Following this discovery, a wide variety of structurally-distinct chemotypes have been uncovered which inhibit this target enzyme with notable differences in their binding motif.

Of similar structure, CAY10571 (**3.2**; Figure 3.2) is an anti-inflammatory pyridinylimidazole derivative that inhibits the production of eicosanoids and suppresses the synthesis of cytokines in human monocytes.⁵

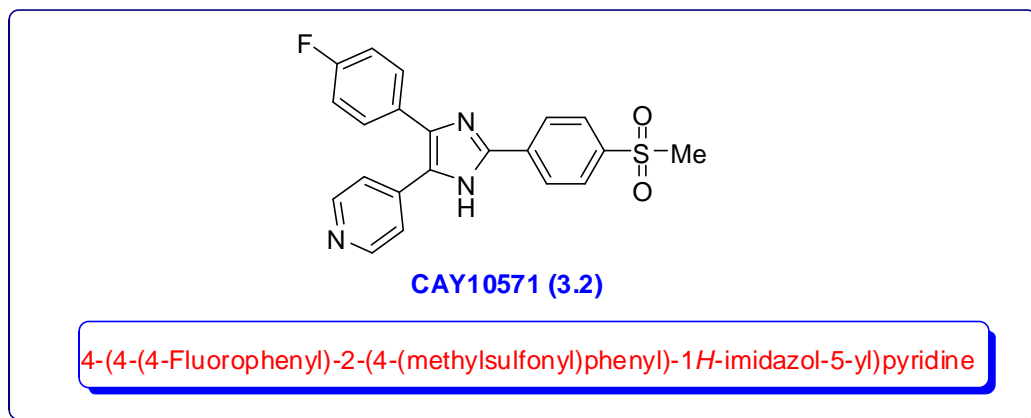


Figure 3.2: Structure of the p38 MAPK inhibitor CAY10571 (3.2)

A wide range of other structurally distinct chemotypes were developed to target p38.⁸⁻¹¹ Recently, our research group developed a synthetic route for the production of BIRB796 (3.3; Figure 3.3), which is a urea-based p38 inhibitor.¹² BIRB796 is similar to SB203580 in terms of its inhibition of the JNKs and C-Raf1.¹³⁻¹⁵ Urea-based inhibitors, including Boehringer Ingelheim's p38 MAPK candidate BIRB796, which was advanced to clinical trials, are one such chemotype, adopting a unique binding mode that is distinct from adenosine 5-triphosphate competitive binders.

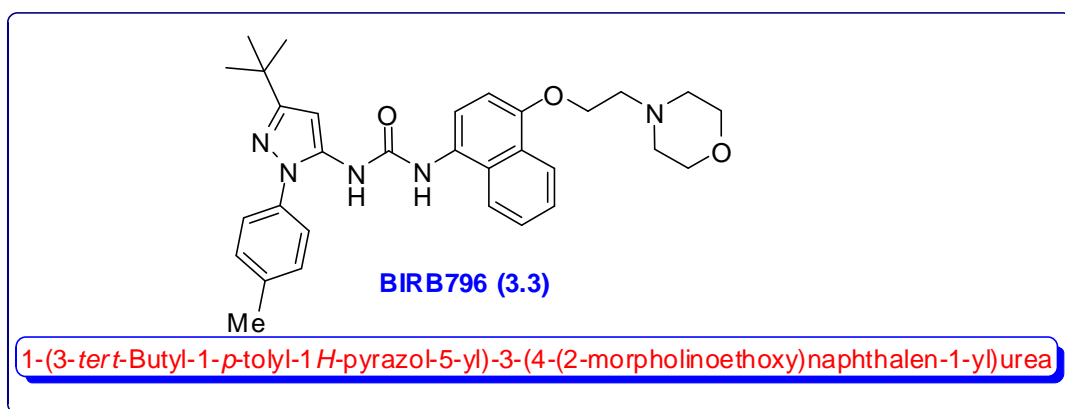


Figure 3.3: Structure of the p38 MAPK inhibitor BIRB796 (3.3)

Biological evaluation of target inhibitors is illustrated by the behaviour of RO3201195 (**3.4**; Figure 3.4) in a cellular assay.¹⁶ The ability of RO3201195 to inhibit p38 α and JNK was tested in human hTERT-immortalized HCA2 cells.¹⁶ Kinase activity was detected using antibodies specific for phosphorylated (S73) HSP27 and antibodies that detect total levels of HSP27, the degree of activation being measured as the ratio of phospho-protein/total protein. In this system p38 activation by anisomycin subsequently activates MK2 that then phosphorylates HSP27. For JNK the phosphorylation status of the JNK substrate c-jun was monitored. RO3201195 (**3.4**) displays excellent kinase selectivity for p38 α MAPK over the related stress-activated kinase JNK.

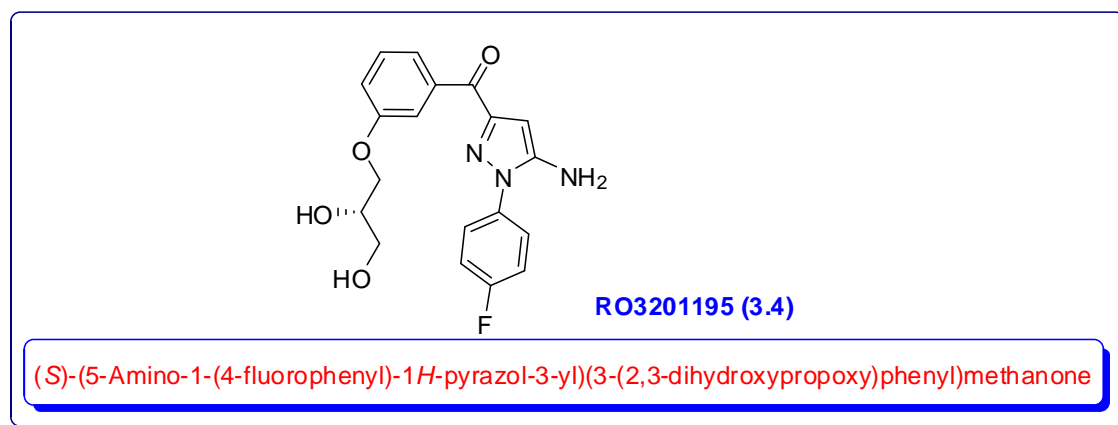


Figure 3.4: Structure of RO3201195 p38 MAPK inhibitor (3.4)

A structurally-related p38 inhibitor pyrazolopyridine chemotype (**3.5**; Figure 3.5) has been developed.¹⁷ This heterocyclic motif provided the potential for structure-activity relationship (SAR) exploration. The nature of R¹ provided a degree of solubility, with potential interactions between R², R³, R⁴ and R⁵ groups and regions of the kinase.¹⁷

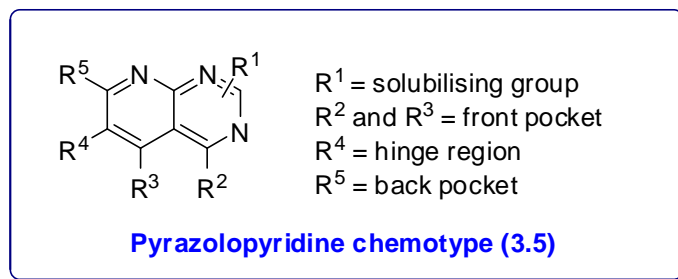


Figure 3.5: Structure-activity relationship of pyrazolopyridine chemotypes (3.5)

VX-745 (**3.6**; Figure 3.6) and its congeners are another particularly interesting inhibitor chemotype, in light of the unprecedented levels of selectivity they exhibit for p38 α over a variety of other closely related kinases. This exquisite selectivity shown by the Vertex class of inhibitors was the inspiration behind their use in WS cells.^{18,19} VX-745 (**3.6**; Figure 3.6) is derived from a pyrimido-pyridazinone core and is characterized by the presence of an extended vinylogous amide system that is critical for functional potency. An isomeric core based upon a pyrido-pyrimidone was envisioned to be a suitable alternative for the development of potential new inhibitors. Scaffolds such as **3.6** were reasoned to be stable in biological systems and hence desirable as substrates for drug design and development.^{18,19}

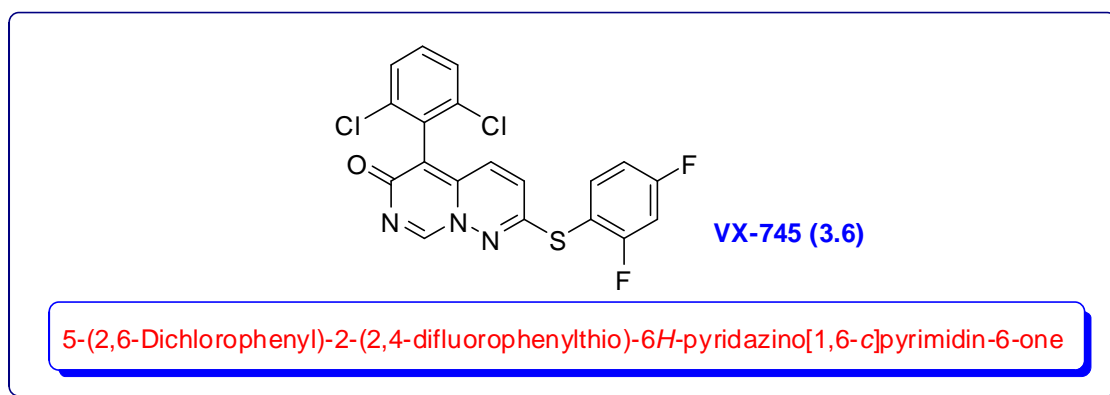


Figure 3.6: Structure of the p38 MAPK inhibitor VX-745 (3.6)

UR-13756 (**3.7**; Figure 3.7) is a potent and selective p38 mitogen-activated protein kinase (MAPK) inhibitor that is based on a pyrazolopyridine core. UR-13756 showed a better kinase selectivity profile than both BIRB796 and SB203580 with regard to the JNKs and C-Raf1 using a kinase panel of 115 kinases.^{20,21} Furthermore, UR-13756 showed good selectivity for the p38 α isoform, compared with BIRB796 and SB203580 which showed equal selectivities for both p38 α and p38 β .²²

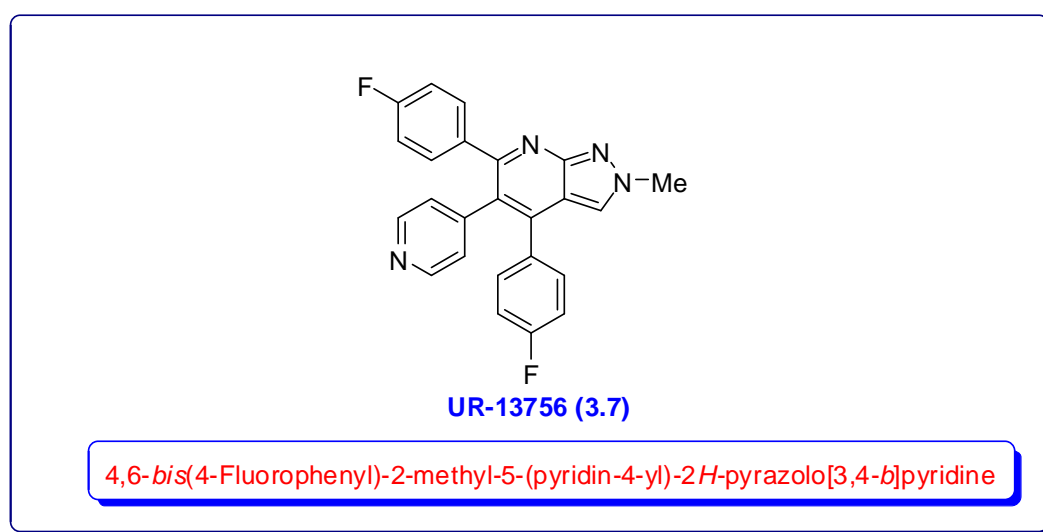
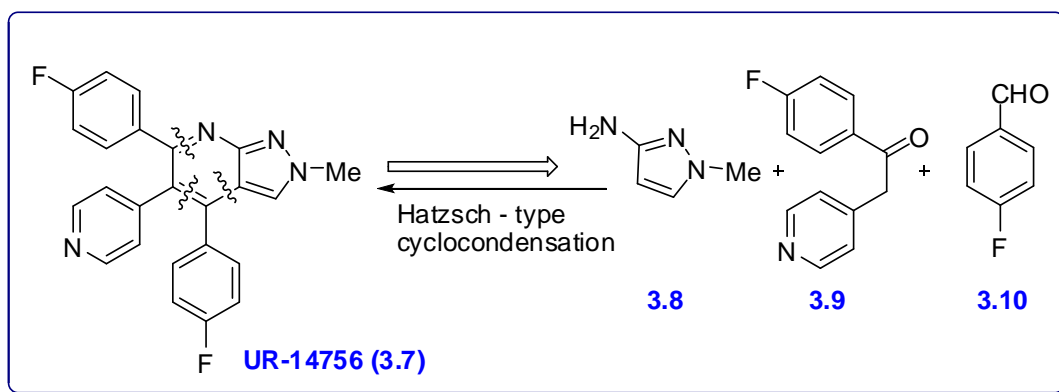


Figure 3.7: Structure of UR-13756 (3.7)

UR-13756 was synthesised within our research group and its activity in Werner syndrome cells was investigated.²³ Therefore, the work described in this chapter aimed to improve the synthesis of UR-13756 (**3.7**), delivering the target inhibitor in high yield and greater quantities using a convenient and rapid method in order to carry out more extensive studies on the effect of this inhibitor on cell proliferation in primary Werner syndrome cells.

The route for the synthesis of UR-13756 (**3.7**) is represented in Scheme 3.1 that involves a three-component cyclocondensation reaction related to Hantzsch

dihydropyridine synthesis. The method involves condensation of 1-methyl-3-aminopyrazole (**3.8**), a ketone **3.9** and 4-fluorobenzaldehyde (**3.10**), based on literature precedent (Scheme 3.1).^{21,23} The first task was therefore the synthesis of **3.8** and **3.9**.

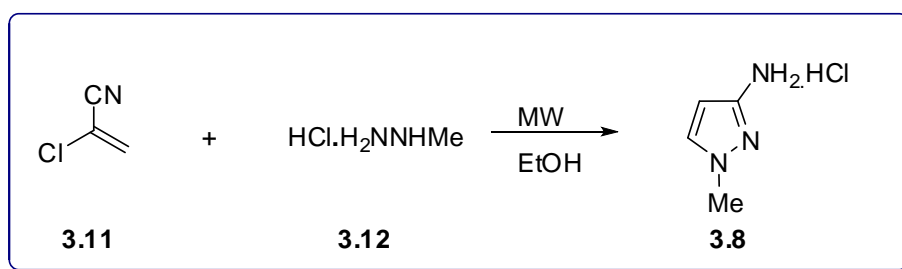


Scheme 3.1: Towards the synthesis of UR-13756 (**3.7**)

3.2. New developments in aminopyrazole synthesis

3.2.1. Synthesis of 3-amino-1-methylpyrazole hydrochloride (**3.8**)

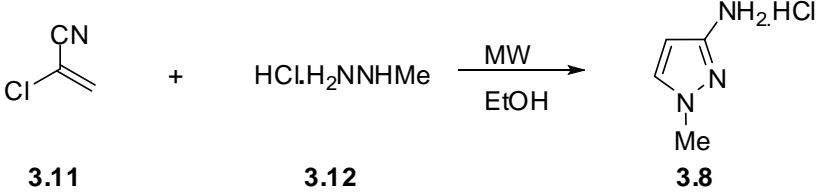
3-Amino-1-methylpyrazole hydrochloride (**3.8**) was previously prepared within our research group in 81% yield from the reaction of chloroacrylonitrile (**3.11**) and methylhydrazine hydrochloride (**3.12**) in ethanol under microwave conditions (Scheme 3.2).²³



Scheme 3.2: Synthesis of **3.8** under microwave conditions

The reaction represented in Scheme 3.2 was attempted under microwave irradiation at 100 °C for 2 minutes, followed by precipitation of the hydrochloride salt of the product, to give **3.8** in 78% yield after purification by recrystallization (Table 1; Entry 2). In an effort to increase the yield of **3.8** by increasing the time, it was found that prolonged irradiation of a mixture of **3.11** and **3.12** caused an increase in the yield of **3.8** (Table 1; Entries 2 and 3). After irradiation for 6 min, a yield of 81% was obtained, whereas the yield of **3.8** could be improved further to 95% following irradiation of 2 minutes at 120 °C.

Table 3.1: Microwave-assisted synthesis of 3-amino-1-methylpyrazole hydrochloride (**3.6**)^a

			
Entry	Reaction conditions ^a		Yield (%) of 3.8 ^b
	Temp (°C)	Time (min)	
1	100	6	81
2	100	2	78
3	120	2	95

^a A solution of **3.11** (0.10 mL; 1.25 mmol) and methylhydrazine hydrochloride (**3.12**; 0.21 g; 2.50 mmol) in EtOH (2 mL) was irradiated at the given temperature.

^b Yield (%) for isolated pure product.

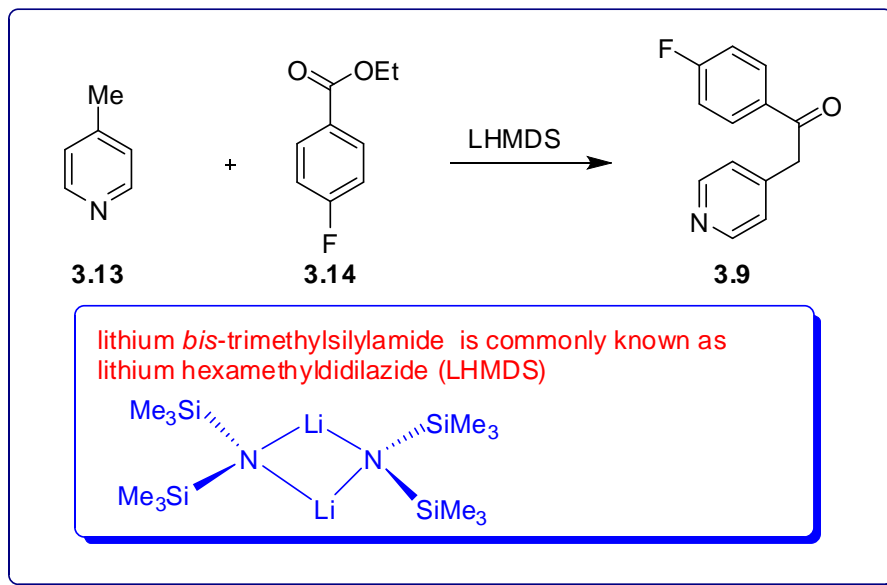
The structure of compound **3.8** was confirmed by various spectroscopic techniques including NMR and infrared spectroscopy, and submitted to mass

spectrometric analysis. The electron-impact (EI) mass spectrum showed a very high intensity (relative intensity 100%) pseudo molecular ion $[M - HCl]^+$ at $m/z = 97$. The accurate mass of the pseudo molecular ion confirmed the formula as $C_4H_7N_3 [M - HCl]^+$, indicating the formation of the target pyrazole ring system **3.8**.

The IR spectrum of compound **3.8** showed no absorption band corresponding to the vibration of the $C\equiv N$ group. It showed absorption bands at ν_{max} 3376 and 3018 cm^{-1} due to the stretching vibrations of the NH_2 group.

The 1H NMR spectrum showed characteristic doublets ($J = 2.3$ Hz) at δ 7.60 and 6.02 ppm due to vicinal coupling of H-5 and H-4 of the pyrazole ring, respectively. It also showed an exchangeable singlet at δ 9.30 ppm due to H_3NCl protons and a singlet at δ 3.77 ppm corresponding to the methyl protons. The structure of **3.8** was confirmed further by the ^{13}C NMR spectroscopic data, which showed characteristic resonances at δ 134.5 and 96.2 ppm due to C-5 and C-4, respectively.

Our attention next turned to the synthesis of 1-(4-fluorophenyl)-2-(pyridine-4-yl)ethanone (**3.9**). Synthesis of ketone **3.9**²⁵ was attempted under various basic reaction conditions which involved Claisen condensation (Scheme 3.3). Reaction of 4-picoline (**3.13**) and ethyl 4-fluorobenzoate (**3.14**) in the presence of lithium *bis*-trimethylsilylamide (LHMDS; Scheme 3.3) at room temperature for 1 hour gave **3.9** in 41% yield after purification.



Scheme 3.3: Synthesis of 1-(4-fluorophenyl)-2-(pyridine-4-yl)ethanone (**3.9**)

Various attempts were made in order to find conditions to improve the yield of **3.9**. Indeed, irradiation of a mixture of **3.13** and **3.14** in the presence of LHMDS at 80 °C for 10 min gave **3.9** in 50% yield after purification. However, it was not possible to increase the yield of **3.9** further by increasing the reaction time due to formation of side-products and/or decomposition of the material.

The structure of compound **3.9** was confirmed by various spectroscopic and spectrometric techniques (See experimental section for details). The electron impact (EI) mass spectrum showed a molecular ion peak (M^+) at $m/z = 215$. Moreover, the positive electrospray (ES^+) mass spectrum showed a pseudo molecular ion $[MH]^+$ peak at $m/z = 216$. The accurate mass of the molecular ion confirmed the formula as $C_{13}H_{10}NOF$. The IR spectrum of **3.9** showed a strong absorption band at $\nu_{\max} = 1670$ cm^{-1} due to the stretching vibration of the C=O group.

The 1H NMR spectrum showed all the expected protons and showed a characteristic singlet at δ 4.49 ppm corresponding to the CH_2 protons. The structure of

3.9 was confirmed further by its ^{13}C NMR proton-decoupled spectroscopic data, which showed two doublets in the aromatic region at 131.5 and 128.3 ppm due to the C-2/C-6 and C-3/C-5 of 4-fluorophenyl group. The carbonyl carbon resonated as a singlet at δ 191.3 ppm, whereas the CH_2 carbon resonated at δ 43.5 ppm.

Having successfully produced both 3-amino-1-methylpyrazole hydrochloride (**3.8**) and 1-(4-fluorophenyl)-2-(pyridin-4-yl)ethanone (**3.9**) our attention next turned to the synthesis of UR-13756 (**3.7**) using these precursors.

3.3. Synthesis of 4,6-bis(4-fluorophenyl)-2-methyl-5-(pyridine-4-yl)-2H-pyrazolo[3,4-*b*]pyridine (UR-13756; **3.7)**

3.3.1. Use of p38 MAPK inhibitors for the treatment of Werner syndrome (WS)

Oho Werner, a German scientist, investigated Werner syndrome (WS), as part of his PhD study. Werner syndrome is defined as a genetic disorder which can be used as a model disease for the investigation of human ageing. This genetic disorder is extremely rare and results from mutation in the WRN gene.^{26,27}

People suffering from Werner syndrome display many clinical features of old age such as early susceptibility to several major age related diseases and as a result their median life expectancy is reduced to 47 years.²⁸ Consequently, WS is widely used as a model disease to investigate the mechanisms underlying normal human ageing. There are several signs of Werner syndrome such as premature greying of hair, cataracts, retarded growth, osteoporosis, and skin sclerosis.²⁸ For humans suffering with Werner syndrome death often occurs as a result of myocardial infarction (20%) or malignancy (80%).²⁸

Several compounds have been synthesised in the group and were found to inhibit the p38 signalling pathway in Werner syndrome cells such as SB203580 (**3.1**; Figure 3.1),⁷ CAY10571 (**3.2**; Figure 3.20),⁵ BIRB796 (**3.3**; Figure 3.3),¹² RO3201195 (**3.4**; Figure 3.4),¹⁶ VX-745 (**3.6**; Figure 3.6)^{18,19} and UR-13756 (**3.7**; Figure 3.7).²⁰⁻²³ In these studies, the effect of RO3201195 (**3.4**; Figure 3.4) on the p38 signalling pathway in WS cells was assessed by immunoblot assay.^{29,30} RO3201195 (**3.4**) can inhibit p38 α -signalling as shown in Figure 3.8. In control cells, low p38 α activity is indicated by low p-HSP27 levels and a low p-HSP27/HSP27 ratio (DMSO columns). Anisomycin treatment activates p38 α that increases p-HSP27 levels and the p-HSP27/HSP27 ratio (columns). RO3201195 pre-treatment increasingly inhibits the anisomycin-induced activity of p38 α , as indicated by the decreasing p-HSP27 levels and the p-HSP27/HSP27 ratio. Maximal inhibition was achieved between 2.5 and 10.0 μ M RO3201195. The IC₅₀ in this system is approximately 180–200 nM, similar to the reported IC₅₀ for RO3201195 inhibition of p38 α induced TNF α , or IL-1 β production in peripheral blood mononucleocytes that have IC₅₀ values of 500 nM and 400 nM, respectively.^{29,30}

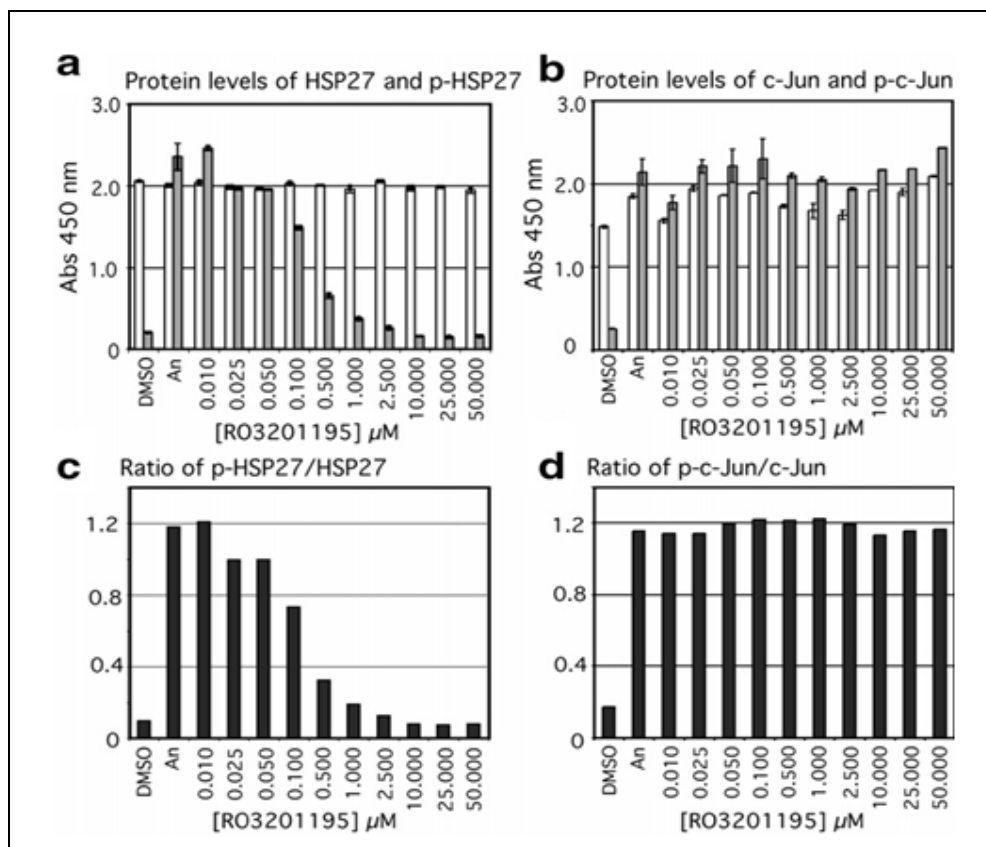
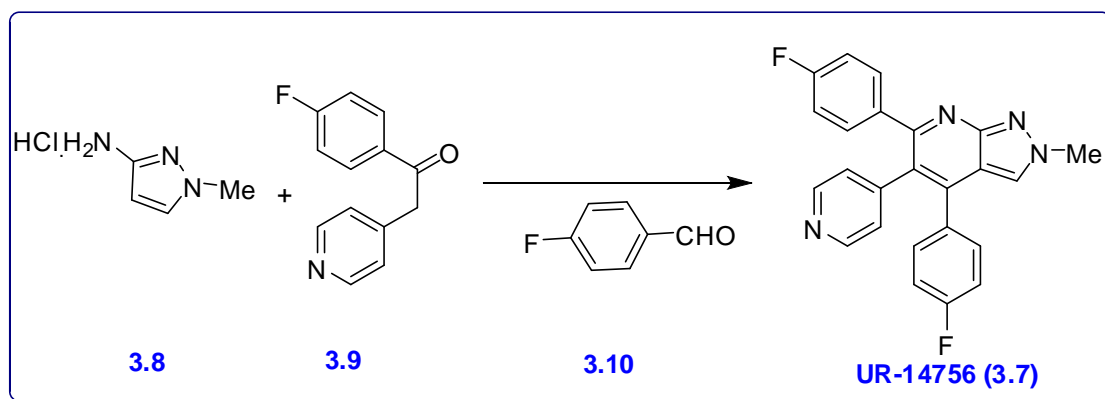


Figure 3.8: Results for the effect of RO3201195 (**3.4**) on p38 α activity (a and c) and on JNK activity (b and d). For the upper panels, total protein is indicated by the white bars, and the phosphorylated protein by the dark grey bars. In the lower panel the ratio of phosphorylated protein to total protein is indicated by the black bars. DMSO are cells with only DMSO treatment, An refers to cells treated with anisomycin, and 0.010–50.000 are cells pre-treated with increasing concentrations of RO3201195 followed by treatment with anisomycin. p-HSP27 and p-c-jun are the phosphorylated forms of HSP27 and c-jun, respectively.

Very recently, vitamin C supplementation was found to reverse the premature ageing and appeared to normalise several age-related molecular markers.³¹ It was found that vitamin C decreases activities in human Werner syndrome and instead increases tissue repair gene activities.³¹

3.3.2. Synthesis of the highly selective P38 MAPK inhibitor UR-13756 (3.7)

Given that an effective route to aminopyrazole **3.8** and ketone **3.9** was secured, our efforts turned to the synthesis of UR-13756 (**3.7**), as represented in Scheme 3.4, based on literature precedent.^{21,23}



Scheme 3.4: Synthesis of UR-13756 (**3.7**)

The process involved a three-component cyclocondensation reaction related to the Hantzsch synthesis of dihydropyridine (Scheme 3.4). The synthesis of UR-13756 (**3.7**) involves use of 3-amino-1-methylpyrazole hydrochloride (**3.8**), as the enamine component, which in reaction with 4-fluorobenzaldehyde (**3.10**) and ketone (**3.9**) would give the pseudo [6.5] bicyclic core of target compound **3.7**.

The Hantzsch-type multicomponent cyclocondensation of **3.8**, **3.9** and **3.10** was reported to produce the UR-13756 (**3.7**) in 80% yield after purification by column chromatography when the reaction was carried out under reflux conditions for 2 days in methoxyethanol as a solvent and hydrochloride acid as the catalyst.²³ Under these reaction conditions, **3.7** was produced in 79% yield as a pure compound (Table 3.2; Entry 1).

Our aim was to accelerate the process of producing **3.7** by carrying the reaction under microwave conditions at a higher temperature. First, the reaction was attempted in 2-methoxyethanol in the presence of HCl at 120 °C under microwave irradiation for 1 h (Table 3.2; Entry 2). Following work-up, TLC analysis showed the formation of **3.7** along with unreacted components **3.8**, **3.9** and **3.10**. Signals for **3.7** were also seen in the ¹H NMR spectrum of the crude reaction mixture.

Various attempts were made to synthesis **3.7** in high yield by irradiation of a mixture of **3.8**, **3.9** and **3.10** (equimolar preparations) at various temperatures in 2-methoxyethanol as the solvent and in presence of hydrochloric acid as a catalyst. The results obtained are summarised in Table 3.2. Several variations were attempted to improve yield of **3.7** in which reaction time and reaction temperatures were varied but none were successful. Also, a reaction was attempted in which a mixture of ethyl acetate and ethanol (1:4 or 5) was irradiated at elevated temperatures (150–170 °C) but this failed to give more than a trace of product. Similar observations were made when ethanol was used as a solvent and acetic acid was used as a catalyst.

Our attention next turned to the use of ethanol as solvent and HCl as the catalyst: the reaction mixture was irradiated at 140 °C for 2 hours. Following this procedure, UR-13756 (**3.7**) was produced in 27% yield after purification by column chromatography on silica (Table 3.3; Entry 1).

Table 3.2: Synthesis of UR-13756 (**3.7**) under various reaction conditions in 2-methoxyethanol in the presence of HCl

<p>The reaction scheme shows the synthesis of UR-14756 (3.7) from three starting materials: 3.8 (1-methyl-4-aminopyrazole hydrochloride), 3.9 (2-(4-fluorophenyl)-1-(pyridin-2-yl)ethan-1-one), and 3.10 (4-fluorobenzaldehyde). The reaction is carried out in 2-methoxyethanol in the presence of HCl. The product, UR-14756 (3.7), is a complex molecule featuring a pyrazolo[1,5-a]pyridine core substituted with a 4-fluorophenyl group, a 4-pyridyl group, and a 4-fluorophenyl group.</p>			
Entry	Reaction conditions		Yield (%) of 3.7 ^a
	Temp (°C)	Time (h)	
1	reflux	48	79 (80) ^b
2	120 MW	1	— ^c
3	140 MW	1	— ^c
4	140 MW	2	— ^c
5	140 MW	3	— ^c
6	160 MW	1	— ^c
7	170 MW	1	— ^c

^a Yield reported for pure product.

^b Reported yield for a similar reaction.²³

^c The TLC and ¹H NMR spectrum of the reaction mixture indicated formation of UR-13756 (**3.7**) along with starting materials. No pure product was isolated.

We next attempted to improve the yield of **3.7** under microwave irradiation in ethanol in which reaction parameters such as temperature, time and catalyst were varied. The results obtained are recorded in Table 3.3.

Table 3.3 Synthesis of UR-13756 (**3.5**) under microwave condition in ethanol

<p>The reaction scheme shows the synthesis of UR-14756 (3.7) from three starting materials: 3.8 (1-methyl-2-aminopyrrole hydrochloride), 3.9 (4-fluorobenzyl pyridine-2-carboxylate), and 3.10 (4-fluorobenzaldehyde). The reaction is carried out in EtOH to yield UR-14756 (3.7).</p>				
Entry	Reaction conditions			Yield (%) of 3.7 ^a
	MW Temp (°C)	Time (h)	Catalyst	
1	140	48	HCl	27
2	150	1	HCl	40
3	150	1	H ₂ SO ₄	33
4 ^b	130	2	HCl	23
5	150	3	HCl	45
6	155	1	HCl	25
7	150	4	HCl	71

^a Yield reported for pure product.

^b BaMnO₄ was added to the reaction mixture.

The results reported in Table 3.3 clearly indicate that ethanol is a much better solvent than 2-methoxyethanol and the yield of **3.7** was improved by increasing the reaction time. The optimum conditions involved irradiation of equimolar quantities of **3.8**, **3.9** and **3.10** in ethanol in the presence of HCl at 150 °C for 4 hours to give UR-13756 (**3.7**) in 71% yield after purification. Such a route to UR-13756 is much more rapid compared to the reported method involving 48 hours reaction time under

reflux conditions. Also, the new process is convenient and simple to produce UR-13756 in high yield.

Clearly, the results revealed that HCl is a much better catalyst than H₂SO₄ and acetic acid and that the reaction temperature and time play an important role in the process (Scheme 3.4).

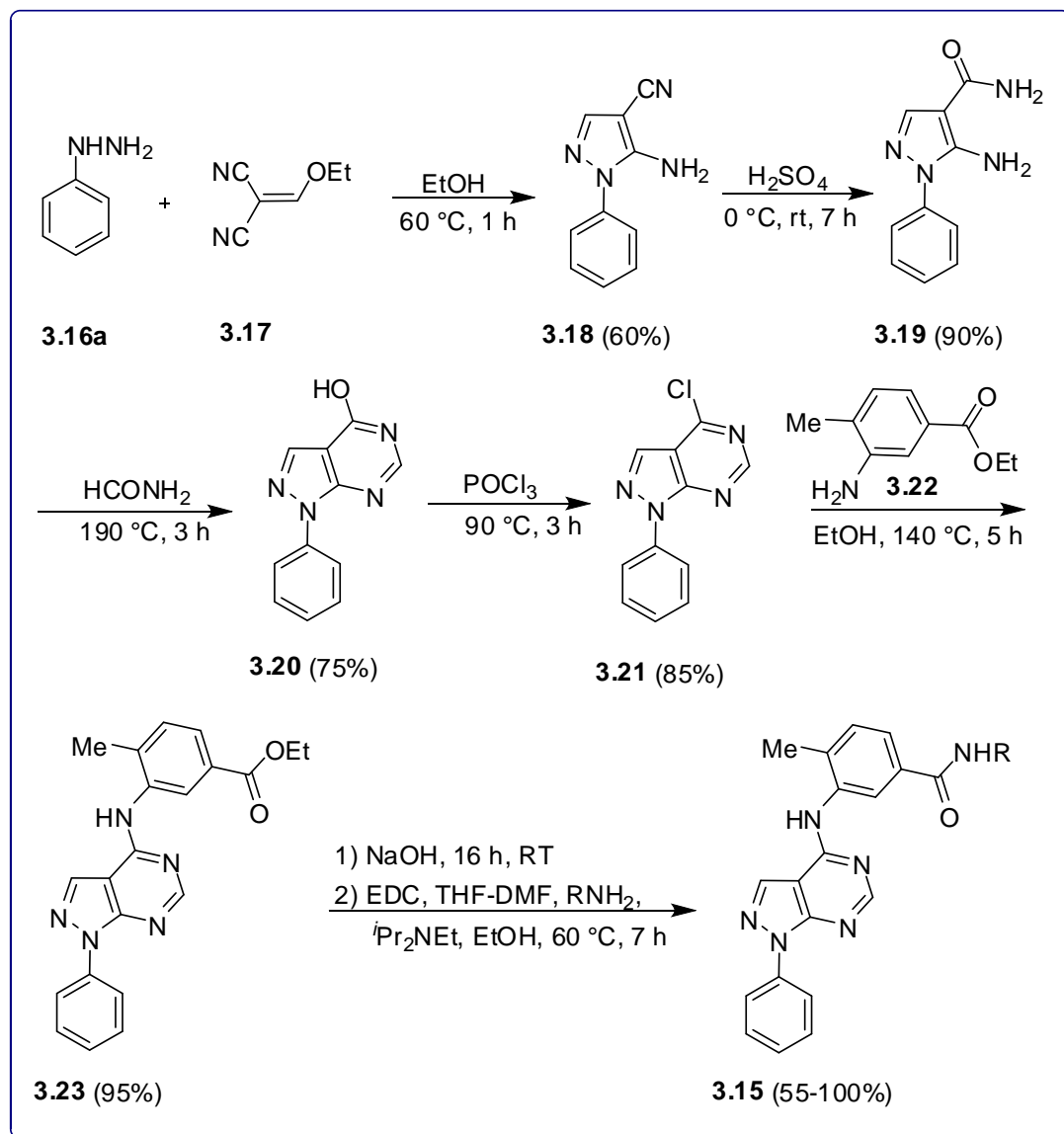
The structure of compound **3.7** was confirmed by various spectroscopic techniques including IR, NMR and mass spectrometric analysis (See experimental section for details). The electron-impact mass spectrum of **3.7** showed a molecular ion peak at $m/z = 398$. The accurate mass of the molecular ion confirmed the formula as C₂₄H₁₇N₄F₂. Also, the positive electrospray (ES⁺) mass spectrum showed very high intensity (100%) pseudo molecular ion (MH)⁺ at $m/z = 399$. The IR spectrum of compound **3.7** showed strong absorption bands at $\nu_{\max} = 1610$ and 1530 cm^{-1} due to the aromatic groups.

The NMR spectra of **3.7** showed all the expected proton and carbon resonances and were in agreement with its structure. The ¹H NMR spectrum showed two doublets ($J = 8.1\text{ Hz}$) corresponding with H-2/H-6 of two 4-fluorophenyl groups and two apparent triplets due to the corresponding H-3/H-5 4-fluorophenyl groups. It also showed a singlet resonance arising from the three methyl protons, whereas H-3 resonated as a singlet at $\delta = 7.43\text{ ppm}$. The ¹³C NMR spectrum showed two doublets ($J = 250\text{ Hz}$) at $\delta = 163.2$ and 162.3 ppm corresponding to C-4 of two 4-fluorophenyl groups, due to coupling of carbons with the fluorine atoms. C-2 and C-6 of two 4-fluorophenyl groups resonated as doublets ($J = 8.8\text{ Hz}$) at $\delta = 130.2$ and 128.9 ppm . All other carbon signals were observed and are agreement with expected values.

This work demonstrated that the use of microwave irradiation can accelerate the synthesis of a known p38 inhibitor for testing in Werner syndrome cells. Our attention next turned to the synthesis of pyrazolopyridine derivatives such as pyrazolopyridine **3.15** under microwave conditions. The use of a different inhibitor would allow us to explore the behaviour of a panel of p38 inhibitors, with different selectivity profiles, to establish the role of p38 inhibition on the WS cell phenotype.

3.4. Towards the synthesis of pyrazolopyrimidine 3.15

In studies towards UR-13756, methods for the rapid synthesis of aminopyrazoles have been established. The aim of this body of work was to utilize these improvements in the synthesis of an alternative p38 inhibitor **3.15**³² (scheme 3.5). The aminopyrazole motif is present in a number of inhibitor chemotypes and this would enable us to test the use of this new methodology. Compound **3.15** was identified as a potent and selective p38 α inhibitor that was found to be highly efficacious *in vivo* in an acute pharmacodynamic model of TNF α production in mice.³² The literature route for the production of **3.15** is represented in Scheme 3.5.³² Such synthetic pathway involves 7 steps, including a cyclocondensation reaction to give an aminopyrazole intermediate **3.18**, and provided **3.15** in 14–28% overall yield. It was hoped that, as a first point of study, the efficiency and facility of this route could be quickly improved using the microwave-mediated pyrazole synthesis to access another chemotype for study of accelerated ageing in WS cells.



Scheme 3.5 Synthetic pathway for the production of pyrazolopyrimidine **3.15**³²

Focussing upon the initial cyclocondensation reaction, the synthesis of **3.18** was attempted under various reaction conditions, including the reported procedures.^{19,33} The yields obtained of the pyrazole product **3.18** are recorded in Table 3.4.

Table 3.4 Synthesis of 5-amino-4-cyano-1-phenyl pyrazole (**3.18**)

<div style="text-align: center;"> <p> <chem>Nc1ccccc1NNH2</chem> (3.16a) + <chem>NC(=C(C#N)C#N)OCC</chem> (3.17) $\xrightarrow{\text{EtOH}}$ <chem>Nc1cc(C#N)cnn1-c2ccccc2</chem> (3.18) </p> </div>			
Entry	Temperature (°C)	Time	Yield (%)
1	60	1 h	9 ^a (23) ^b
2	60, MW	1 h	55 ^c
3	60, MW	2 h	38 ^d (60) ^c
4	60, MW	3 h	95 ^c
5	70, MW	2 h	35 ^e
6	80, MW	1 h	29 ^e
7	120, MW	5 min	45 ^c
8	120, MW	30 min	63 ^c
9	120, MW	45 min	95 ^c

^a After crystallization from a mixture of EtOH and H₂O (1:10 by volume).

^b After crystallization from H₂O.

^c After column chromatography (petroleum ether–ethyl acetate in 5:1 by volume).

^d After extraction with diethyl ether.

^e After filtration of solid obtained followed by washing with diethyl ether.

The results in Table 3.4 showed that use of conductive heating at 60 °C for 1 hour provided low yields of the pyrazole product (9–23%), although the limits of this procedure could in part be attributed to purification. Use of microwave conditions and

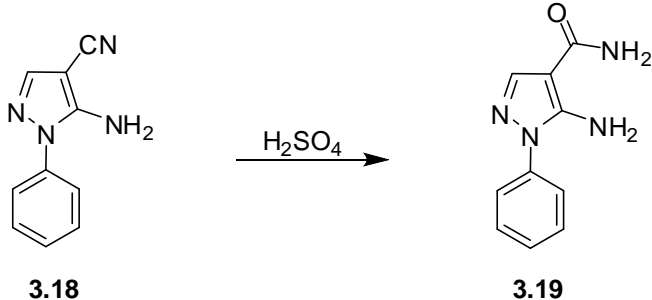
purification by column chromatography, for the same reaction time and temperature, provided an improved yield (55%) of the product **3.18** (Table 3.4; Entry 2). However, when the reaction time or temperature were increased (Table 3.4; Entries 4 and 9, respectively) an excellent yield of the aminopyrazole product **3.18** was obtained.

The structure of **3.18** was confirmed by various spectroscopic techniques including NMR, and IR spectroscopy, and low and high resolution mass spectrometric data (See experimental section for details).

Having successfully produced **3.18** in excellent yield, our attention next turned to the synthesis of 5-amino-4-aminocarbonyl-1-phenyl-1*H*-pyrazole (**3.19**). The synthesis of **3.19** started by following the literature route^{19,33} but this gave the desired product in only 3% yield (Table 3.5; Entry 1). Increasing the reaction temperature (0-50 °C) provided a better yield of **3.19** (64%) after a prolonged reaction time. Carrying out the reaction under microwave irradiation provided high yields of **3.19** (60–85%: Table 3.5; Entries 4–7) in very short time. The results obtained are recorded in Table 3.5.

The results reported in Table 3.5 clearly indicated that the use of microwave heating improved the yield of **3.19** significantly without the need for column chromatography for the purification of the product. This was successful under microwave irradiation on a 0.5 g scale. The structure of **3.19** was confirmed by NMR and IR spectroscopic and mass spectrometric data.

Table 3.5 Synthesis of 5-amino-4-aminocarbonyl-1-phenyl-1*H*-pyrazole (**3.19**)

<div style="text-align: center;">  <p>3.18 3.19</p> </div>			
Entry	Temperature (°C)	Time (h)	Yield (%)
1	0	16	3 ^a
2	0 to rt	5	12 ^a
3	0 to 50	5	64 ^b
4	0 to 50, MW	1	74
5	0 to 50, MW	3	60 ^b
6	0 to 60, MW	3	80 ^c
7	0 to 80, MW	1	84 ^c

^a Product was extracted by diethyl ether and purified by column chromatography.

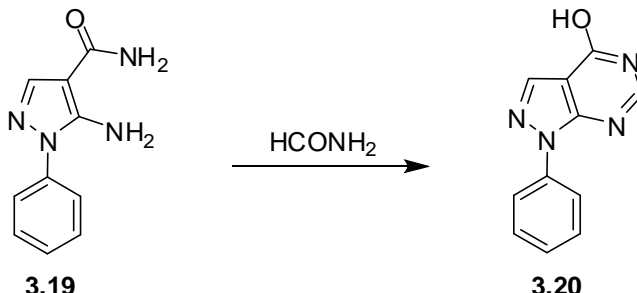
^b Product was extracted with diethyl ether.

^c Product was extracted with chloroform.

Having successfully produced aminopyrazole **3.18** and the hydrolysis product **3.19** in high yield under microwave-assisted conditions, comparing with the literature,^{19,33} we therefore attempted the synthesis of 4-hydroxy-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (**3.20**).

A series of experiments was conducted to convert **3.19** to **3.20** under various reaction conditions. The reaction was also carried out under the reported procedure to for comparison.^{19,33} The yields of **3.20** obtained are recorded in Table 3.6.

Table 3.6 Synthesis of 4-hydroxy-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (**3.20**) under microwave-assisted conditions

<div style="text-align: center;">  <p>3.19 3.20</p> </div>			
Entry	Temperature (°C)	Time (min)	Yield (%) ^a
1	100	60	75
2	150	30	75
3	150	45	97
4	160	45	96
5	190	20	60
6	190	30	82
7	190	40	88
8	180	60	56 ^a

^a Yield obtained followed filtration of product and washing with H₂O.

^b Yield obtained under the reported conditions.^{19,33}

The results showed that the yield of **3.20** has been improved by the use of microwave irradiation compared to the yield obtained using the literature procedure^{19,33} (Table 3.5; Entry 8).

It was found that the yield of **3.20** was dependent on both the reaction temperature and time. For example, the yield of **3.20** was 75% when the temperature was 100 or 150 °C and the reaction time was 60 or 30 minutes, respectively (Table 3.6; Entries 1 and 2). The optimum conditions gave an improved yield of 97% when the temperature was increased to 150 °C and the reaction time was 45 minutes (Table 3.6; Entry 3).

At higher temperatures (190 °C) the yields of **3.20** were reduced somewhat (60-88%) indicating that these conditions may not be as suitable, possibly due to partial decomposition of product.

The positive electrospray (ES⁺) mass spectrum of **3.20** showed the presence of a very intense (100%) molecular ion peak at $m/z = 212$ and high resolution mass spectrometric analysis confirmed the formula of the molecular ion peak as C₁₁H₉N₄O. The ¹H NMR spectrum of **3.20** showed a characteristic exchangeable singlet at $\delta = 9.71$ ppm due to the OH proton and two singlets that resonated at $\delta = 8.23$ and 7.94 ppm due to H-5 and H-2, respectively. Also, the ¹³C NMR spectrum showed all of the expected signals and was consistent with the given structure.

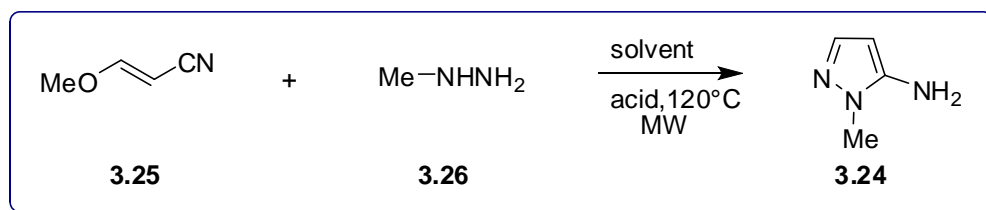
Our attention next turned to the conversion of hydroxypyrazolopyrimidine **3.20** to the corresponding chloride **3.21**.

Various attempts were made to synthesise and improve the conditions for the preparation of **3.21**. However, all attempts failed to produce a pure sample of **3.21** and the purification step turned out to be problematic and so no further attempts were made.

These studies had shown that it was possible to adapt our new method of aminopyrazole synthesis to prepare the 4-cyanopyrazole intermediate **3.18**, using a malononitrile derivative, and convert this to pyrazolopyrimidine **3.20**, all under microwave-assisted conditions. The pyrazole-forming cyclocondensation using an alternative, ethoxyacrylonitrile, substrate had caused a change in the regiochemical outcome, from the 3-aminopyrazole motif to a 5-aminopyrazole. Thus, it was considered whether similar alkoxyacrylonitrile substrates could be used to access a range of 5-aminopyrazoles in a microwave-assisted cyclocondensation reaction.

3.5 Towards the synthesis of pyrazolopyridines from aminopyrazoles

In order to test the cyclocondensation reaction, synthesis of 5-amino-1-methyl-1*H*-pyrazole (**3.24**) was attempted (Scheme 3.6).



Scheme 3.6 Synthesis of 5-amino-1-methyl-1*H*-pyrazole (**3.24**)

Reactions of acrylonitrile **3.25** with methyl hydrazine (**3.26**) in a chosen solvent (ethanol or toluene) under various reaction conditions were attempted. The yields obtained of **3.24** are recorded in Table 3.7.

Table 3.7 Synthesis of 5-amino-1-methyl-1*H*-pyrazole (**3.24**) under various reaction conditions (at 120 °C, MW) according to Scheme 3.6.

Entry	Solvent	Acid	Time (min)	Yield (%)
1	EtOH	—	5	— ^a
2	EtOH	—	10	— ^a
3	EtOH	HCl	5	— ^a
4	EtOH	HCl	45	— ^a
5	EtOH	AcOH	15	— ^b
6	PhMe	HCl	15	— ^b
7	PhMe	AcOH	15	40 ^c
8	PhMe	AcOH	60	97 ^c
9	PhMe	—	60	32 ^c

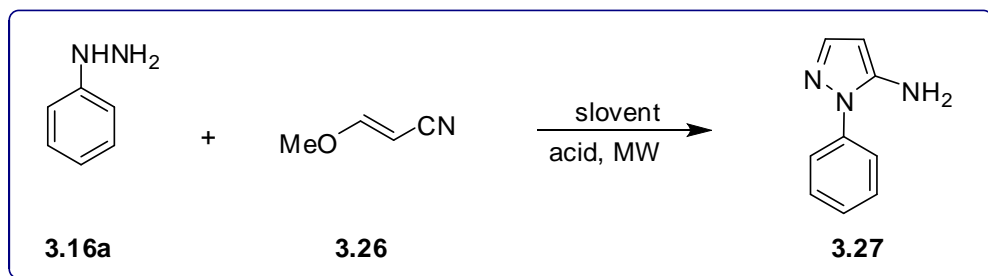
^a No product was isolated.

^b A mixture of two isomeric products was obtained which were difficult to separate by column chromatography.

^c Yields reported were for pure product after purification by crystallisation from hexane–ethyl acetate (3:1 by volume).

The results recorded in table 3.7 showed clearly that 5-aminopyrazole **3.24** was produced as a single regioisomer (to the limits of detection) in 97% yield (Table 3.7; Entry 8) when the reaction was carried out under microwave conditions at 120 °C in toluene in the presence of acetic acid for 1 hour. This regiochemical outcome was in line with our previous studies in the synthesis of pyrazole **3.18**. Also, it was clear that acetic acid was much better as a catalyst compared with HCl under similar reaction conditions.

In order to further explore the scope of the process, a similar target, 5-amino-1-phenyl-1*H*-pyrazole (**3.27**) was synthesised (Scheme 3.7) under various conditions and the yields obtained were compared (Table 3.8).



Scheme 3.7 Synthesis of 5-amino-1-phenyl-1*H*-pyrazole (**3.27**)

Table 3.8 Synthesis of 5-amino-1-phenyl-1*H*-pyrazole (**3.27**) according to Scheme 3.7 under microwave conditions.

Entry	Solvent	Acid	Temp (°C)	Time (min)	Yield (%)
1	EtOH	HCl	120	5	— ^a
2	EtOH	HCl	120	10	— ^a
3	EtOH	—	120	45	— ^a
4	EtOH	AcOH	120	15	— ^a
5	EtOH	AcOH	150	30	— ^a
6	PhMe	HCl	120	15	— ^a
7	PhMe	AcOH	150	30	75
8	PhMe	AcOH	150	45	82 ^b
9	PhMe	AcOH	150	60	92 ^c

^a No pure product was obtained.

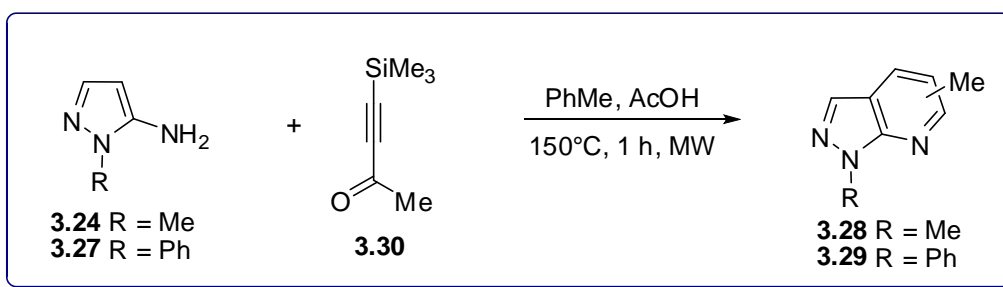
^b Yield reported was for pure product after crystallization from a mixture of toluene and acetone (2:1 by volume).

^c Product was recrystallized from hexane–ethyl acetate mixture (in 3:1 by volume).

Again the results in Table 3.8 showed that compound **3.27** can be produced in 92% yield (Entry 9) under microwave conditions at 150 °C for 1 hour in toluene in the presence of acetic acid as a catalyst and isolated as a single regioisomer.

The structures **3.24** and **3.27** were confirmed by the data collected from IR and NMR spectroscopy, and low and high resolution mass spectrometry and agreed with literature values.³⁴

Having successfully produced **3.24** and **3.27** in high yields, our attention next turned to attempt their conversion to the corresponding pyrazolopyridines **3.28** and **3.29**, respectively (Scheme 3.8). This reaction would employ the 5-aminopyrazole substrate as a new enamine component in a Bohlmann-Rahtz reaction, a hitherto unreported transformation of 5-aminopyrazoles. If successful this would establish a new route to this fused core which could establish an analogue of UR-13756 for biological study.



Scheme 3.8 Attempted synthesis of pyrazolopyridines **3.28** and **3.29**

It was found that reaction of aminopyrazole **3.24** with ethynyl ketone **3.30** in toluene in the presence of acetic acid under microwave irradiation at 150 °C for 1 hour

gave no product at all and only starting materials were recovered quantitatively, indicating that no reaction had taken place.

On the other hand, reaction of phenylpyrazole **3.27** with the same ethynyl ketone **3.30** under similar reaction conditions produced the corresponding imine **3.31** (Figure 3.8) in 35% yield. Clearly, the aminopyrazole under these conditions had demonstrated behaviour as an amine nucleophile, rather than an enamine nucleophile in a Bohlmann-Rahtz cyclocondensation.

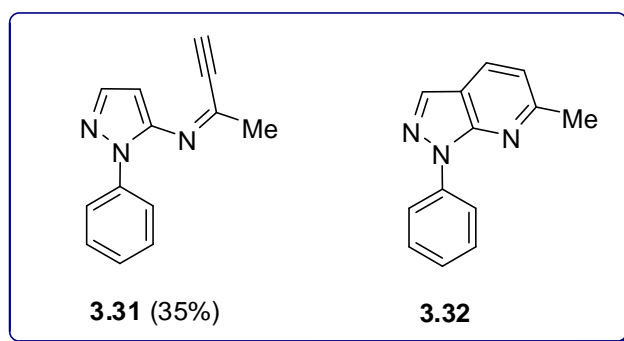
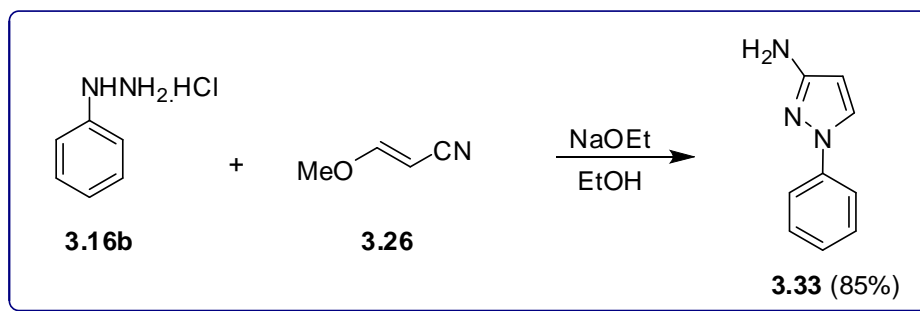


Figure 3.8 Structures of compounds **3.31** and **3.32**

Next, we attempted to convert the imine product of condensation **3.31** to the corresponding pyrazolopyridine **3.32** (Figure 3.8) using a mixture of toluene and acetic acid in the presence of $\text{HAuCl}_4 \cdot \text{H}_2\text{O}$ as catalyst under microwave irradiation at 150 °C for 1 hour. However, no product was obtained and **3.31** were quantitatively recovered. No further attempts were made to find conditions to effect the conversions of **3.31** to **3.32** or **3.24** and **3.27** to the corresponding pyrazolopyridines **3.28** and **3.29**, respectively.

Although the 5-aminopyrazoles had failed to behave as enamine components in this reaction, it was considered that changing the position of the amino group in compound **3.24** or **3.27** might have an effect on this process and could give rise to an

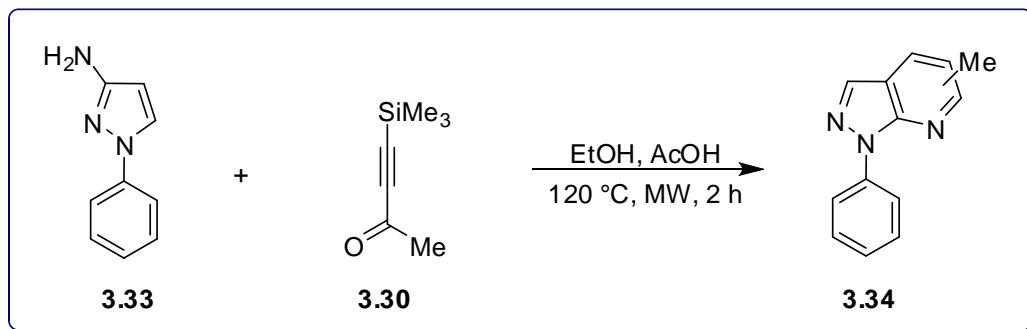
alternative pyrazolopyridine motif. Therefore, the synthesis of 3-amino-1-phenyl-1*H*-pyrazole (**3.33**; Scheme 3.9) was attempted.



Scheme 3.9 Synthesis of 3-amino-1-phenyl-1*H*-pyrazole (**3.33**)

In order to switch the regiochemical outcome of the pyrazole formation, the conditions were altered: instead of carrying the process out in acidic media, reaction of phenylhydrazine hydrochloride (**3.16b**) with 3-methoxyacrylonitrile (**3.26**) in ethanol was studied in the presence of an excess of sodium ethoxide base under microwave heating at 150 °C for two hours. Gratifyingly, this caused a complete switch in the regiochemistry of the product, giving 3-aminopyrazole **3.33** in 85% yield (Scheme 3.9) after purification by column chromatography.

With a route to the alternative regioisomer to hand, next, we attempted the Bohlmann-Rahtz reaction described in Scheme 3.10 in an attempt to convert **3.33** to its corresponding pyrazolopyridine **3.34**. However, no product was obtained under these conditions, confirming again that the aminopyrazoles are not enamine equivalents in this cyclocondensation reaction. No further attempts were made to try to find alternative conditions under which this reaction would proceed.



Scheme 3.10 Attempt synthesis of pyrazolopyridine (**3.34**)

Finally, a one pot synthesis of **3.34** was attempted from the reaction of phenylhydrazine hydrochloride **3.16b**, acrylonitrile **3.26** and the ethynyl ketone **3.30** under microwave conditions at 150 °C for two hours in the presence of $\text{HAuCl}_4 \cdot \text{H}_2\text{O}$. However, no pyrazolopyridine **3.34** was produced and instead the ^1H NMR spectrum of the crude reaction mixture showed the presence of imine **3.31** (Figure 3.8), which was separated and purified by column chromatography and isolated in 67% yield.

3.6. Conclusions

The p38 MAPK inhibitor UR-13756 can be prepared rapidly and efficiently using Hantzsch-type three component cyclocondensation. Microwave irradiation of a mixture of 3-amino-1-methylpyrazole hydrochloride, 1-(4-fluorophenyl)-2-(pyridine-4-yl)ethanone and 4-fluorobenzaldehyde for 4 hours in ethanol under acidic catalytic conditions provided UR-13756 in 71% yield after purification by column chromatography. The method is simple and high yielding but still there is room for modification.

We have also attempted the synthesis of several pyrazolopyrimidines and pyrazolopyridine derivatives under microwave conditions. However, no pure products were obtained under the conditions investigated.

However, of greatest interest, it has been demonstrated that using a single substrate, methoxyacrylonitrile **3.26**, it is possible to isolate a single aminopyrazole regioisomer using a microwave-assisted cyclocondensation reaction. Furthermore, by choice of conditions, selecting either acidic or basic media, it is possible to switch the regiochemical outcome to produce 5-aminopyrazoles or 3-aminopyrazoles, respectively, in excellent yield.

3.5. References

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CHAPTER FOUR

SYNTHESIS OF PYRAZOLE DERIVATIVES AS INHIBITORS OF PROTEIN KINASE MK2

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SYNTHESIS OF PYRAZOLE DERIVATIVES AS INHIBITORS OF PROTEIN KINASE MK2

4.1 Introduction

Tumour necrosis factor- α (TNF- α) is a key cytokine in cutaneous inflammation which is mainly produced by macrophages.¹⁻³ The biosynthesis of TNF- α is initiated on extracellular activation of signalling pathways that signals *via* mitogen-activated protein kinases (MAPKs) as has been shown for p38 MAPK.⁴

Modification of the function of TNF- α was found to be very successful for the treatment of skin inflammation in several diseases.² However, although there are many biological drugs that are known to down regulate TNF- α in the inflammation of skin, their clinical use has been hindered by their severe side effects. Also, the use of TNF- α inhibitory biological drugs has been restricted by their large molecular weight and size. Thus it is desirable to have small molecular weight molecules that elicit a similar response.²

The kinase MK2, which is a direct downstream substrate of p38 involved in the production of TNF- α , is a promising inflammatory disease drug target that could be inhibited using small molecular weight chemical tools.^{5,6}

Several MK2 inhibitors have been synthesized and used in animal models. The coming section will highlight the development in the area of specific MK2-inhibitors for treatment of inflammatory diseases and in particular the most recent ones that show improved selectivity and efficacy profiles.

4.2. Development of specific MK2 inhibitors for the treatment of inflammatory diseases

A series of 3-aminopyrazole based MK2 inhibitors have been synthesized by Velcicky's research group³ and have been proven to be selective inhibitors for intracellular phosphorylation of hsp27 and Lps-induced TNF- α release in cells. For example, the pyrazole derivatives **4.1–4.5** (Figure 4.1) were revealed by a scaffold hopping strategy and have been developed to be selective MK2 kinase inhibitors.³

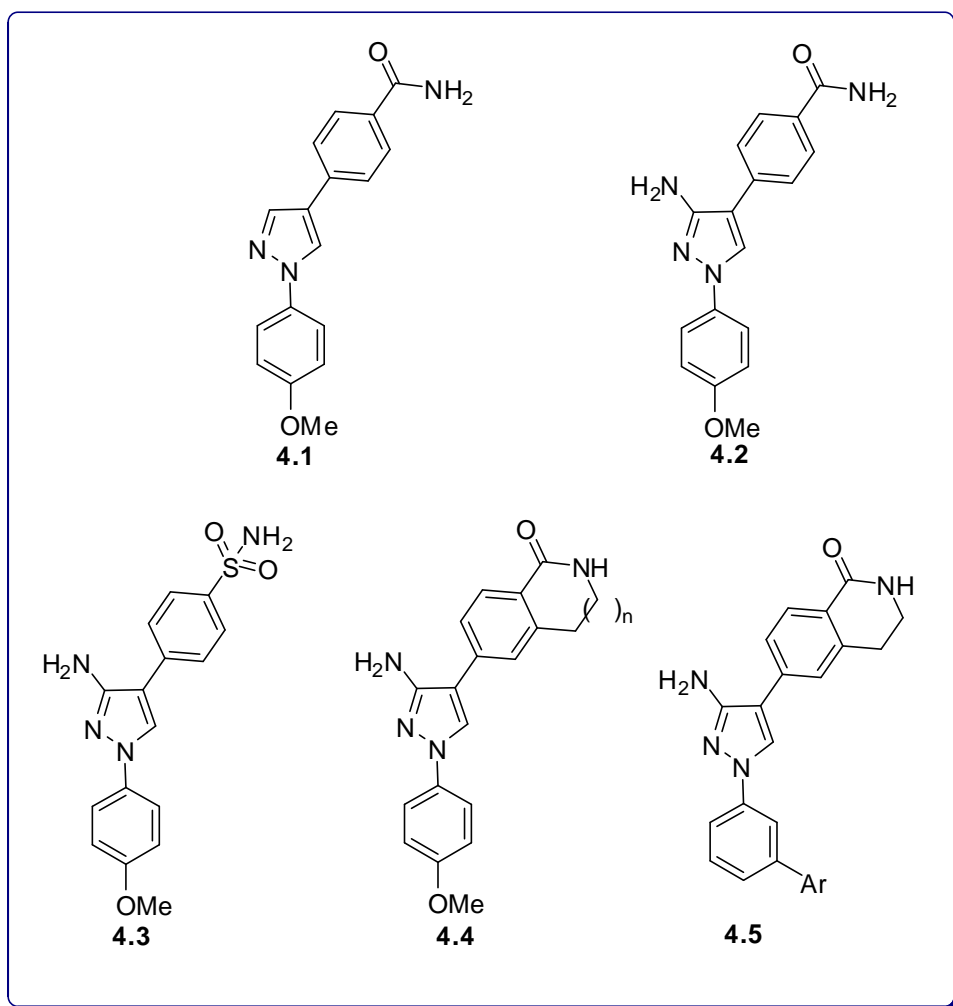
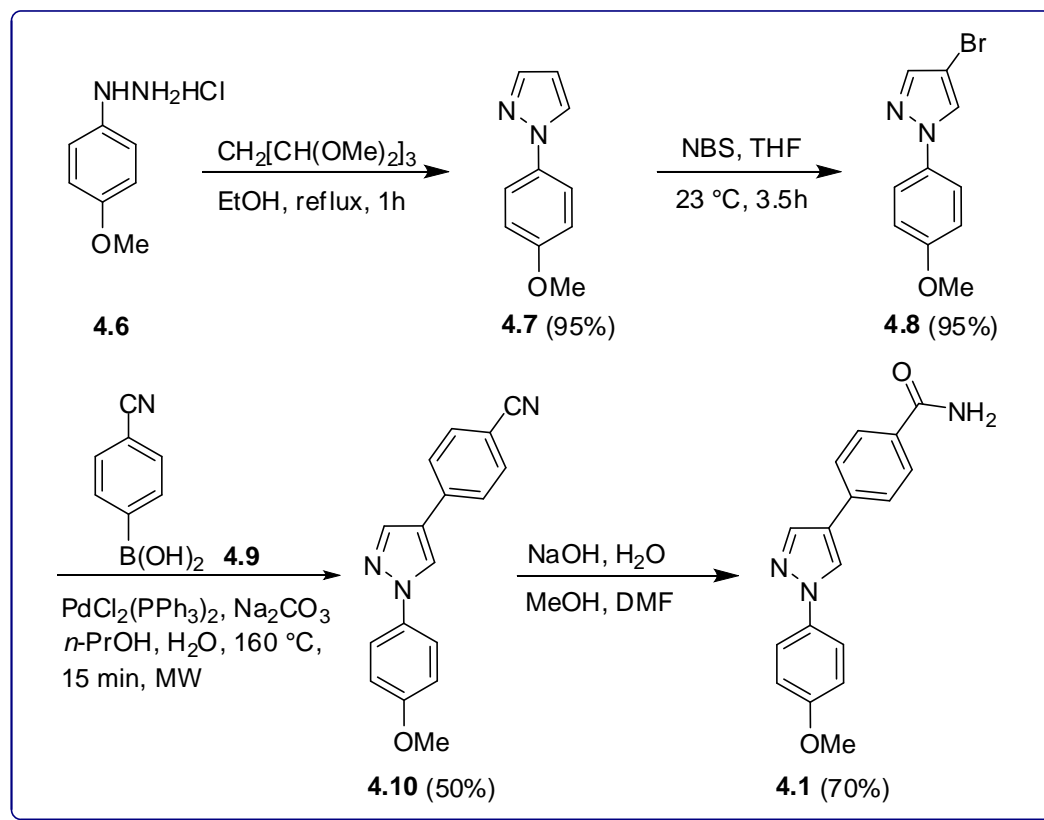


Figure 4.1. Structures of 3-aminopyrazole MK2 inhibitors **4.1–4.5** developed by Velcicky's research group³

4-[1-(4-Methoxyphenyl)-1*H*-pyrazole-4-yl]benzamide (**4.1**) was synthesized in 32% overall yield in four steps as shown in Scheme 4.1.³

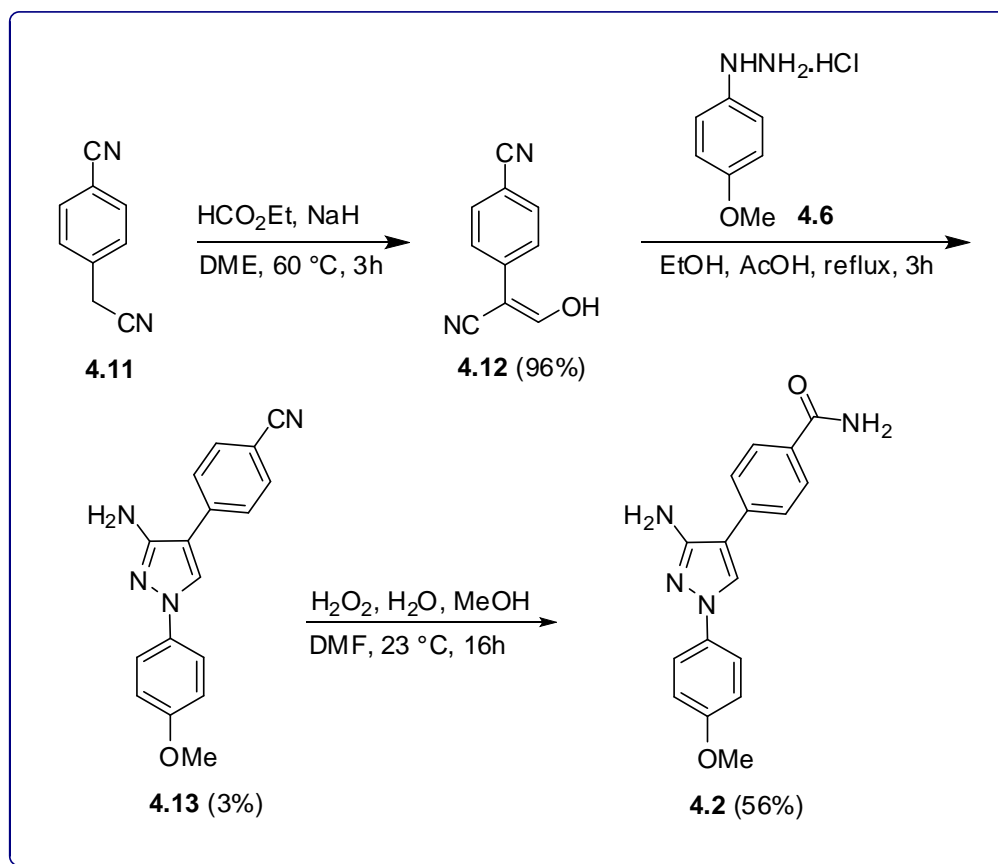


Scheme 4.1. Synthesis of 1*H*-pyrazole **4.1**³

The first step in their route involved the reaction of 4-(methoxyphenyl)hydrazine hydrochloride (**4.6**) with 1,1,3,3-tetramethoxypropane in ethanol (EtOH) under reflux conditions to produce 1-(4-methoxyphenyl)-1*H*-pyrazole (**4.7**) in 95% yield (Scheme 4.1).³ This was followed by bromination of the pyrazole (**4.7**) using *N*-bromosuccinimide (NBS) in tetrahydrofuran (THF) at room temperature to give 4-bromo-1-(4-methoxyphenyl)-1*H*-pyrazole (**4.8**) in 95% yield (Scheme 4.1).³ The bromopyrazole (**4.8**) was an ideal substrate for Suzuki–coupling^{7–11} with 4-cyanophenylboronic acid (**4.9**) in aqueous propanol in the presence of *bis*-(triphenylphosphine)palladium(II) chloride (PdCl₂(PPh₃)₂) and sodium

carbonate at 160 °C for 15 min under microwave conditions to give 4-[1-(4-methoxyphenyl)-1*H*-pyrazole-4-yl]benzonitrile (**4.10**) in 50% yield (Scheme 4.1).³ Finally on hydrolysis amide **4.1** was produced in 70% yield (Scheme 4.1).³

As an alternative, more densely functionalized inhibitor, 4-[3-amino-1-(4-methoxyphenyl)-1*H*-pyrazole-4-yl]benzamide (**4.2**) was synthesized, as represented in Scheme 4.2, in *ca.* 2% overall yield in three steps.³ Reaction of 4-(cyanomethyl)benzonitrile (**4.11**) with ethyl formate in the presence of sodium hydride gave 4-(1-cyano-2-hydroxyvinyl)benzamide (**4.12**) in 96 % yield (Scheme 4.2).³ Treatment of **4.12** with (4-methoxyphenyl)hydrazine hydrochloride (**4.6**) in ethanol in the presence of acetic acid under reflux conditions for 3 hours gave 4-[3-amino-1-(4-methoxyphenyl)-1*H*-pyrazol-4-yl]benzamide (**4.13**), but in only 3% yield (Scheme 4.2).³ Finally, hydrolysis of **4.13** gave the desired product, the aminopyrazole **4.2**, in 56% yield (Scheme 4.2).³



Scheme 4.2. Synthesis of 3-amino-1*H*-pyrazole **4.2**

The overall yield of **4.2** was improved to 45% by a modification of the synthetic procedure represented in scheme 4.2.³ It was found that treatment of **4.11** with ethyl formate produced **4.12** *in-situ* which on treatment with iodomethane in dimethylformamide (DMF) at 40 °C gave the analogue 4-(1-cyano-2-methoxyvinyl)benzamide (**4.14**; Figure 4.2) in 86% yield.³ Reaction of acrylonitrile **4.14** with hydrazine **4.6** in ethanol under basic conditions improved the yield of the pyrazole synthesis and gave the same nitrile intermediate **4.13** in improved yield (93%) that was hydrolysed (56% yield) to give the target inhibitor **4.2** in an improved route.³

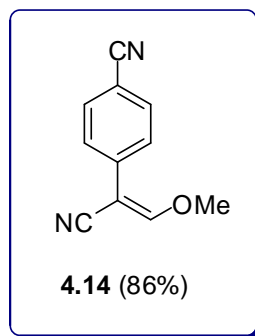
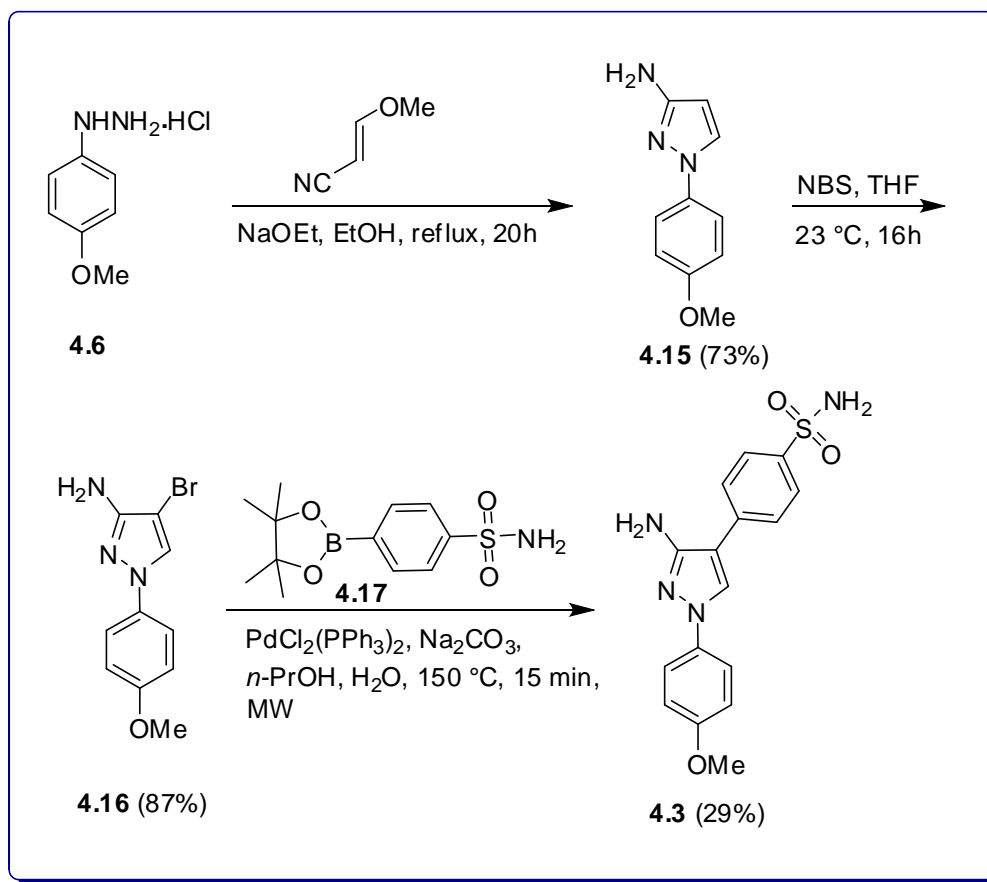


Figure 4.2. Structure of 4-(1-cyano-2-methoxyvinyl)benzamide (**4.14**)

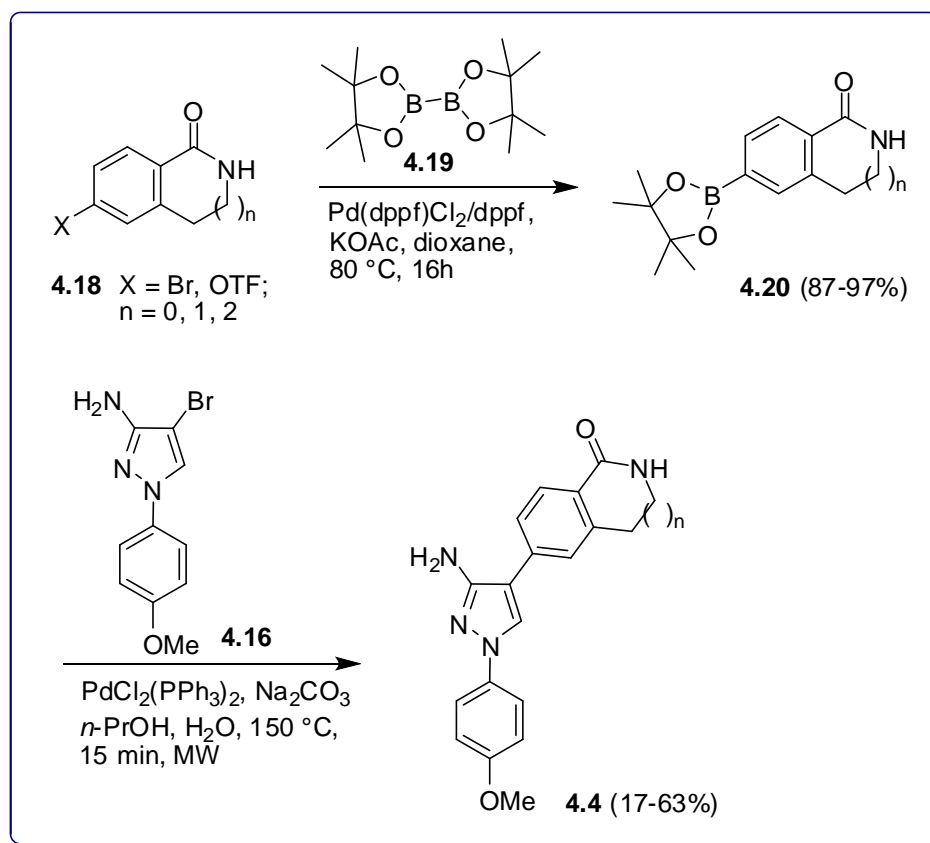
In order to probe the SAR of this chemotype, a number of analogues needed to be prepared. 4-[3-Amino-1-(4-methoxyphenyl)-1*H*-pyrazole-4-yl]benzamide (**4.3**) was synthesised in 18% overall yield in three steps as represented in Scheme 4.3.³ The first step involved reaction of the methoxyphenylhydrazine (**4.6**) with 3-methoxyacrylonitrile in ethanol in the presence of a base under reflux conditions for 20 hours to give 3-amino-1-(4-methoxyphenyl)-1*H*-pyrazole (**4.15**) in 73% yield (Scheme 4.3).³

The second step involved bromination of the pyrazole **4.15** with *N*-bromosuccinimide in THF at room temperature for 16 hours to give 3-amino-4-bromo-1-(4-methoxyphenyl)-1-*H*-pyrazole (**4.16**) in 87% yield (Scheme 4.3).³ Finally Suzuki-coupling⁷⁻¹¹ of **4.16** with 4-bromobenzenesulfonamide pinacol ester (**4.17**) in the presence of *bis*-(triphenylphosphine)palladium(II) chloride and sodium carbonate in aqueous *n*-propanol at 150 °C under microwave conditions for 15 min gave **4.3** in 29% yield (Scheme 4.3).³ It is noteworthy that the yield for this Suzuki coupling is somewhat low, but it is assumed that this is an unoptimized procedure.



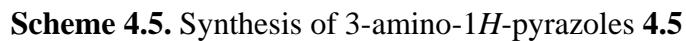
Scheme 4.3. Synthesis of 3-amino-1*H*-pyrazole **4.3**

A series of additional 4-substituted 3-amino-1-(4-methoxyphenyl)-1*H*-pyrazoles **4.4** were also prepared by a similar sequence (Scheme 4.4).³ Reaction of **4.18** with *bis*(pinacolato)diboron (**4.19**) in dioxane at 80 °C for 16 hours in the presence of [1,1-*bis*(diphenylphosphino)ferrocene]palladium(II) dichloride and potassium acetate gave the corresponding analogues **4.20** in 87-97% yields (Scheme 4.4).³ Suzuki-coupling of **4.20** and **4.16**, previously synthesised in Scheme 4.3, in aqueous propanol in the presence of a catalyst gave the target inhibitors **4.4** in 17-63% yields (Scheme 4.4).³



Scheme 4.4. Synthesis of 3-amino-1*H*-pyrazoles **4.4**

The authors also varied the substitution pattern of the *N*-aryl ring (Scheme **4.5**). In the synthesis of 6-[3-amino-1-aryl-1*H*-pyrazole-4-yl]-3,4-dihydroisoquinolin-1(2*H*)-ones **4.5**³ it was found that the reaction of (3-bromophenyl)hydrazine hydrochloride (**4.21**) with 3-methoxyacetonitrile in ethanol in the presence of a base under reflux condition for 20 hours gave 3-amino-1-(3-bromophenyl)-1*H*-pyrazole (**4.22**) in 91% yield (Scheme 4.5).³ Suzuki-couplings⁷⁻¹¹ of bromobenzene **4.22** and a number of arylboronic acids in the presence of a palladium catalyst gave the corresponding 3-amino-1-aryl-1*H*-pyrazoles, which were brominated subsequently using *N*-bromosuccinimide at room temperature to give a subset of 3-amino-1-aryl-4-bromo-1*H*-pyrazoles **4.23** in 51-96% yields over the two steps (Scheme 4.5).³ Finally, Suzuki-couplings of **4.23** with 6-[4,4,5,5-tetramethyl-1,3,2-



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Scheme 4.2) and 4-[1(3-(1*H*-indol-6-yl)phenyl)-3-amino-1*H*-pyrazol-4-yl] benzamide (**4.24**; Figure 4.3) as simple targets to investigate their MK2 inhibition.

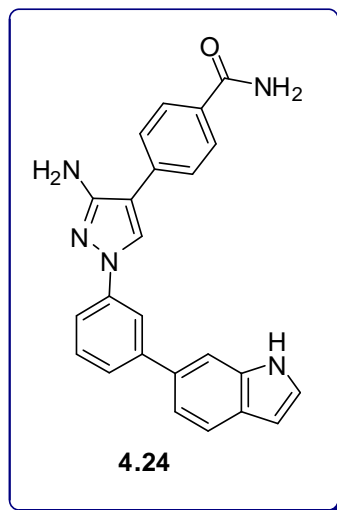
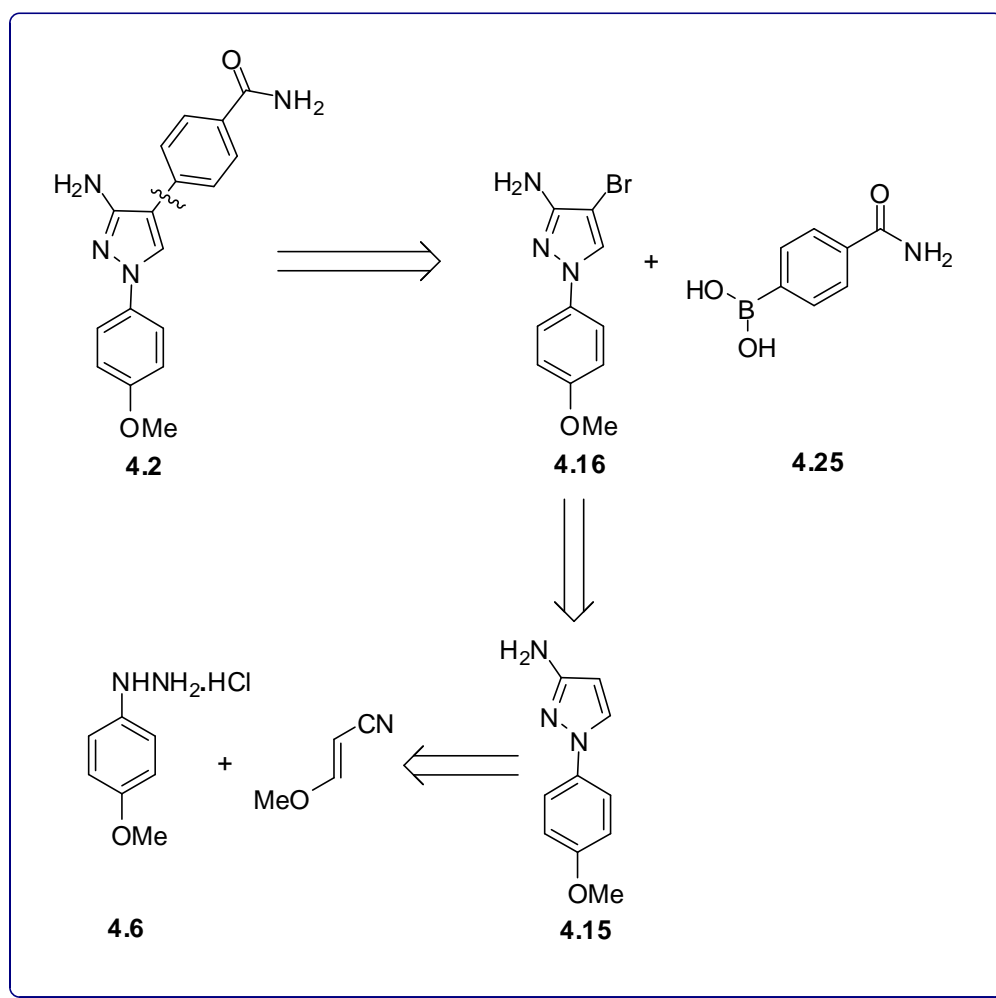


Figure 4.3. Structure of 3-amino-1*H*-pyrazole **4.24**

4.3 Synthesis of 4-[3-amino-1-(4-methoxyphenyl)-1*H*-pyrazol-4-yl]benzamide (**4.2**)

In order to test the role of MK2 in accelerated ageing in Werner syndrome, the aminopyrazole chemotype, exemplified by the simple carboxamide derivative **4.2**, prepared by Velcicky's research group,³ was chosen as having suitable selectivity profile and ready availability based upon these earlier reports. There also seemed interesting regioselectivity issues to explore regarding the regiochemistry of aminopyrazole formation (3-amino- vs. 5-aminopyrazole formation) and the efficiency of many of the Suzuki couplings in the reported routes. Retrosynthetic analysis of **4.2** revealed a boronic acid derivative **4.25** and 3-amino-4-bromo-1-(4-methoxyphenyl)-1*H*-pyrazole (**4.16**), available *via* bromination of 3-amino-1*H*-pyrazole (**4.15**) as shown in Scheme 4.6. It was reported that the 3-aminopyrazole **4.15** could be prepared from reaction of (4-methoxyphenyl)hydrazine

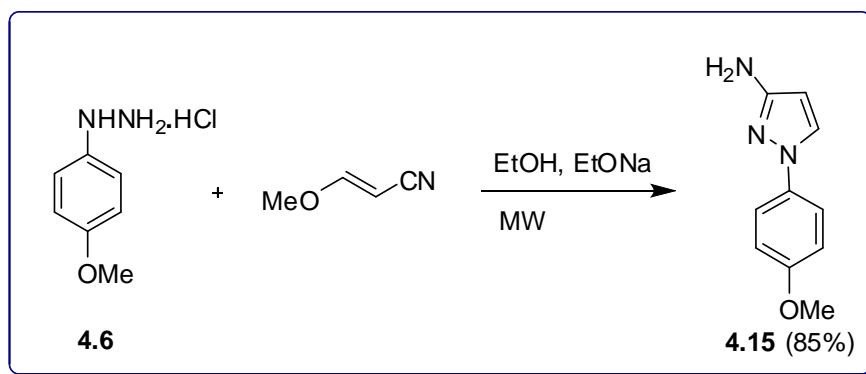
hydrochloride (**4.6**) and 3-methoxyacronitrile (Scheme 4.6) with the regiochemistry determined by carrying out the reaction under strongly basic conditions. Based upon previous studies, this heterocyclization appeared suitable for investigation under microwave irradiation as it should be effective as a rapid and high yielding route to pyrazole targets, providing that the regiochemical outcome could be controlled under the alternative conditions.



Scheme 4.6. Retrosynthesis of 3-aminopyrazole **4.2**

The synthesis of 3-aminopyrazole **4.2** was carried out in three steps. The first step involved reaction of (4-methoxyphenyl)hydrazine hydrochloride (**4.6**; 1 molar equivalent) and 3-methoxyacrylonitrile (two molar equivalents) in ethanol in the presence of sodium ethoxide (4 molar equivalents) under microwave heating (Scheme 4.7).

The optimized conditions were found to involve 2 hours reaction at 150 °C (150 W). The reaction mixture was worked-up by passing compressed air through the microwave cavity followed by the addition of water. The reaction mixture was extracted with ethyl acetate and the organic layer was dried and evaporated under reduce pressure to give the crude product. TLC analysis clearly showed the formation of a new product and the disappearance of the starting material **4.6**. Purification by column chromatography on silica gave 3-amino-1-(4-methoxyphenyl)-1*H*-pyrazole (**4.15**) in 85% yield as a yellow solid.

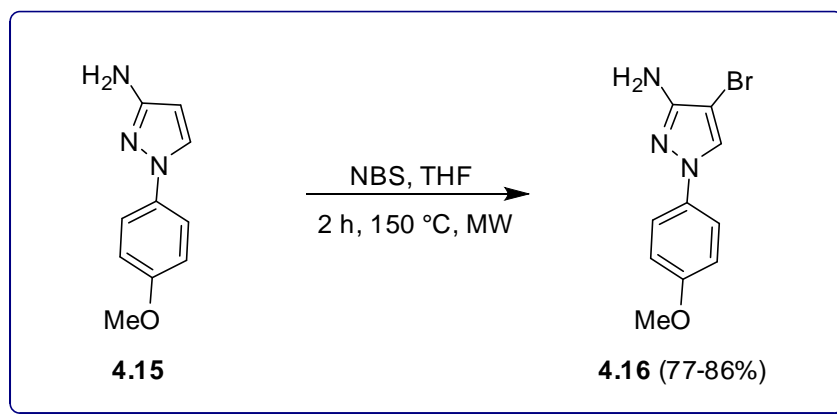


Scheme 4.7. Synthesis of 3-amino-1-(4-methoxyphenol)-1*H*-pyrazole (**4.15**)

The structure of **4.15** was confirmed by various spectroscopic techniques including IR and ^1H and ^{13}C -NMR spectroscopic analysis and low and high resolution mass spectrometry. The electron impact mass spectrum of **4.15** showed the base molecular ion

peak at $m/z = 189$. The accurate mass for the molecular ion confirmed the molecular formula of **4.15** as $C_{10}H_{11}N_3O$. The 1H -NMR spectrum of **4.15** showed two doublets at $\delta = 8.00$ and 5.67 ppm ($J = 2.5$ Hz) corresponding to H-5 and H-4 of the pyrazole ring, respectively. It also showed two doublets (J 9.0 Hz) at $\delta = 7.55$ and 6.95 ppm due to H-2/H-6 and H-3/H-5 of 4-methoxyphenyl ring, respectively. The two singlets resonating at $\delta = 4.96$ (exchanged on addition of D_2O) and 3.76 ppm were also observed and correspond to the amino and methoxy protons, respectively. The ^{13}C -NMR spectrum of **4.15** showed all of the expected signals and was consistent with the given structure.

The second step in the synthesis of **4.2** involved bromination of **4.15** to produce 3-amino-4-bromo-1-(4-methoxyphenyl)-1*H*-pyrazole (**4.16**; Scheme 4.8). It was found that this process could also be accelerated under microwave irradiation. Reaction of equimolar equivalents of **4.15** and *N*-bromosuccinimide in THF for 2 hours at $150^\circ C$ under microwave irradiation gave **4.16** in 77% yield (Scheme 4.8) after purification by column chromatography.

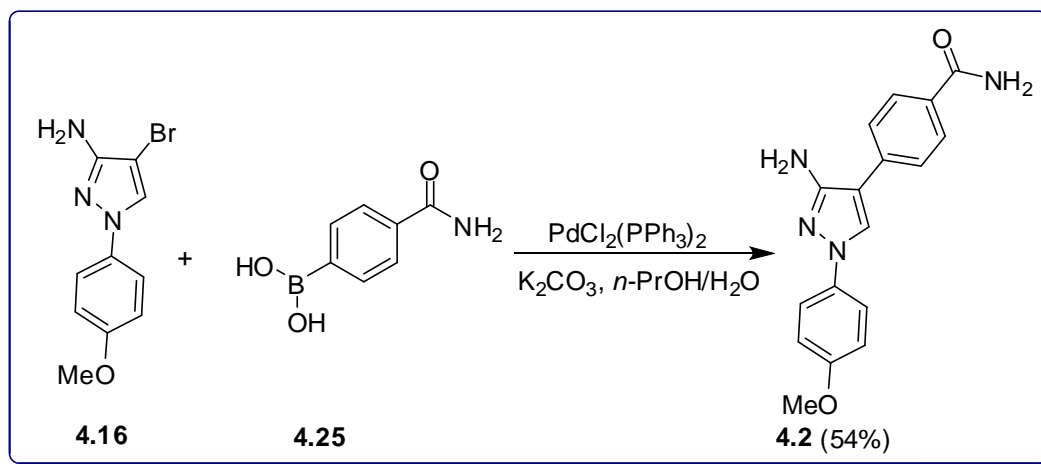


Scheme 4.8. Synthesis of 3-amino-4-bromo-1-(4-methoxyphenyl)-1*H*-pyrazole (**4.16**)

The electron impact mass spectrum of **4.16** showed two molecular ions at $m/z = 269$ and 267 (base peak) for the different Br isotopes. High resolution analysis of the two molecular ions confirmed the molecular formula, $C_{10}H_{10}N_3O^{81}Br$ and $C_{10}H_{10}N_3O^{79}Br$, respectively. The 1H -NMR spectrum of bromide **4.15** showed a characteristic singlet that resonated at $\delta = 8.34$ ppm due to H-5 of the pyrazole ring. Also, ^{13}C -NMR spectroscopic analysis showed a characteristic singlet carbon that resonated at $\delta = 95.3$ ppm corresponding to C-4 of the pyrazole ring.

Several attempts were made to improve the yield of **4.16**. It was found that reaction of **4.15** with *N*-bromosuccinimide in THF at room temperature for 16 hours gave **4.16** in 86% yield after purification by column chromatography, but the microwave-mediated route was used in preference on account of its short reaction time.

The third step involved a Suzuki-coupling⁷⁻¹¹ reaction of **4.16** with 4-carbamoylphenylboronic acid **4.25** (Scheme 4.9). Reaction of **4.16** and boronic acid **4.25** was attempted in aqueous *iso*-propyl alcohol in the presence of potassium carbonate and *bis*-(triphenylphosphine)palladium(II) chloride under microwave heating (Scheme 4.9).



Scheme 4.9. Synthesis of target **4.2**

It was found that reaction of equimolar quantities of **4.16** and **4.25** under microwave conditions gave the target product **4.2** in 54% yield (Scheme 4.9) after purification by column chromatography.

The structure of compound **4.2** was confirmed by IR and NMR spectroscopic and mass spectrometric analysis. The IR spectrum of **4.2** showed a strong absorption band at $\nu = 1653\text{ cm}^{-1}$ corresponding to the amide carbonyl stretch.

The ^1H -NMR spectrum of **4.2** showed three exchangeable singlet resonances at δ 7.96 (1 H), 7.31 (1 H) and 5.18 (2 H) ppm due to the two NH_2 groups, respectively. It also showed all of the expected resonances and their chemical shifts were consistent with the given structure. The ^{13}C NMR spectrum of **4.2** showed a singlet that resonated at very low field at δ 168.2 ppm due to the carbonyl carbon of the amide group. It also showed all of the other expected carbon resonances.

The electron impact mass spectrum of **4.2** showed a very intense molecular ion base peak at m/z 308. The molecular formula of the molecular ion was confirmed as $\text{C}_{17}\text{H}_{16}\text{N}_4\text{O}_2$ by high resolution mass spectrometry.

4.4 Biological behaviour of MK2 inhibitor 4.2 on Werner syndrome cells

MK2 inhibitor **4.2** was considered as a potential inhibitor of the ‘stress signal’ in Werner syndrome (WS). In collaboration with our project partners in the School of Medicine at Cardiff University, the ability of MK2 inhibitor **4.2** was examined in telomerase-immortalized HCA2 dermal cells to establish our premise for restoring the effects of rapid ageing. Using an enzyme linked immune-sorbant assay (ELISA) it was possible to detect the behaviour of target **4.2** at different concentrations and establish its

inhibition of a downstream target, HSP27. ELISA is a method to determine the extent of phosphorylation of specific proteins, and thus to understand the alteration of pathway signalling events. The procedure utilizes antibodies to reveal only the interested phosphorylated proteins, in a protein mixture with high specificity. In particular, in our analysis, the antibody used identifies the total HSP27 (blue columns), heat shock proteins, downstream of activated p38 α , and pHSP27, phosphorylated heat shock proteins (orange columns), which acts as a good marker of p38 activity as it is not activated by other substrates (Figure 4.4).

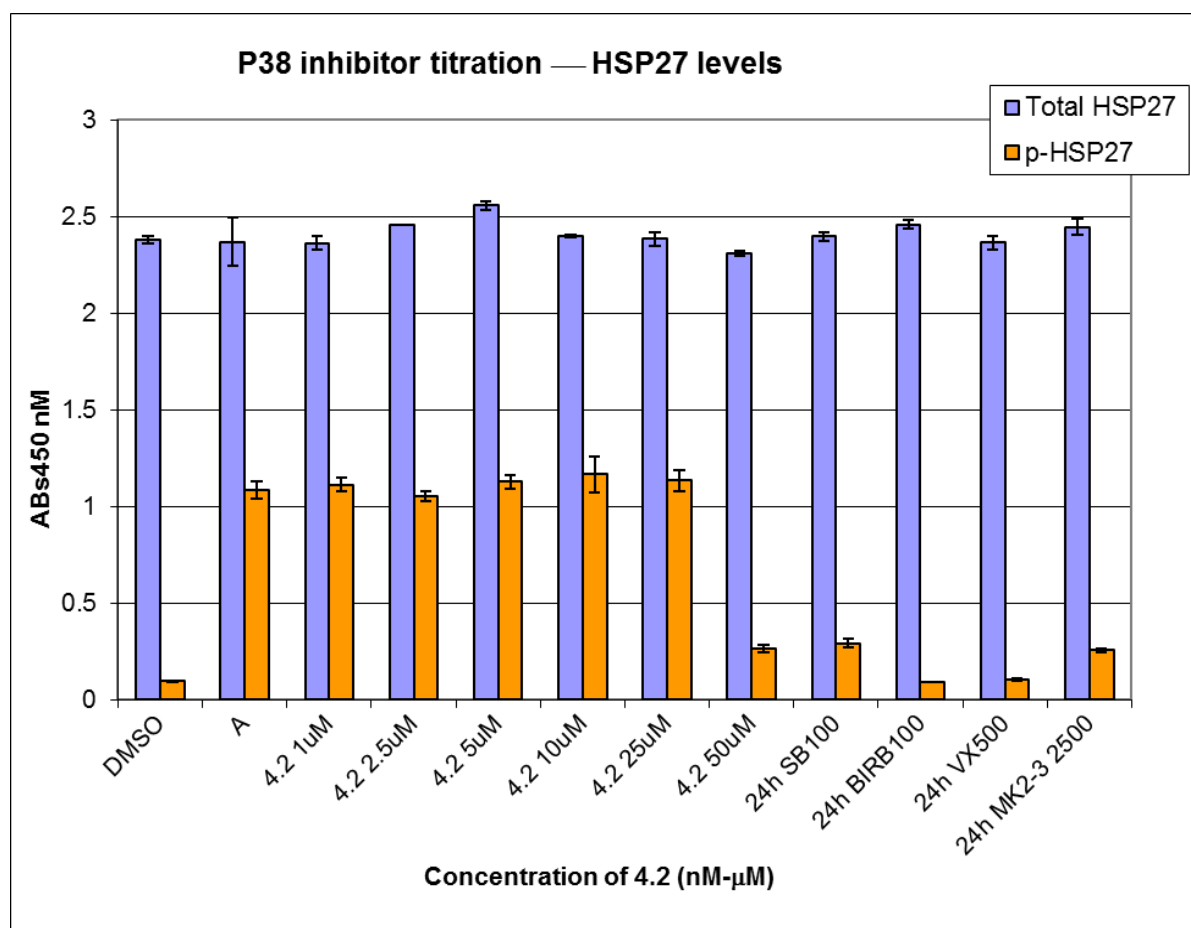


Figure 4.4. MK2 inhibitor **4.2** and its effect on p38 signalling activity

Each level of protein is examined and compared in HCA2 cells in dimethylsulfoxide (DMSO) in the presence of increasing concentrations of **4.2**, from 10 nM to 50 μ M, following treatment with anisomycin (A), which stimulates the phosphorylation of p38. Initially the DMSO, as carrier control, shows a low level of pHSP27 with respect to the total HSP27 protein (DMSO), as expected. The use of anisomycin (A) is an important reference because stimulating the phosphorylation of p38, enables the qualitative effects to be observed with dosage. WS cells treated with anisomycin to up-regulate p38 α confirmed an increase of p38 activity with high level of pHSP27, as expected, while the level of HSP27 was unchanged. The same experiment was repeated but the cells were cultured with the inhibitor **4.2** at different concentrations from 10 nM to 50 μ M. The level of HSP27 was about the same in all samples, visible by the similar heights of the blue columns, contrary to the levels of pHSP27 which changed (orange columns) (Figure 4.4). The initial concentration of the inhibitor **4.2** was 10 nM (not shown) and processed until 500 nM when this last concentration led to a decrease in the level of pHSP27, showing the inhibition of p38 signalling. The ratio of pHSP27/HSP27 is shown in Figure 4.5.

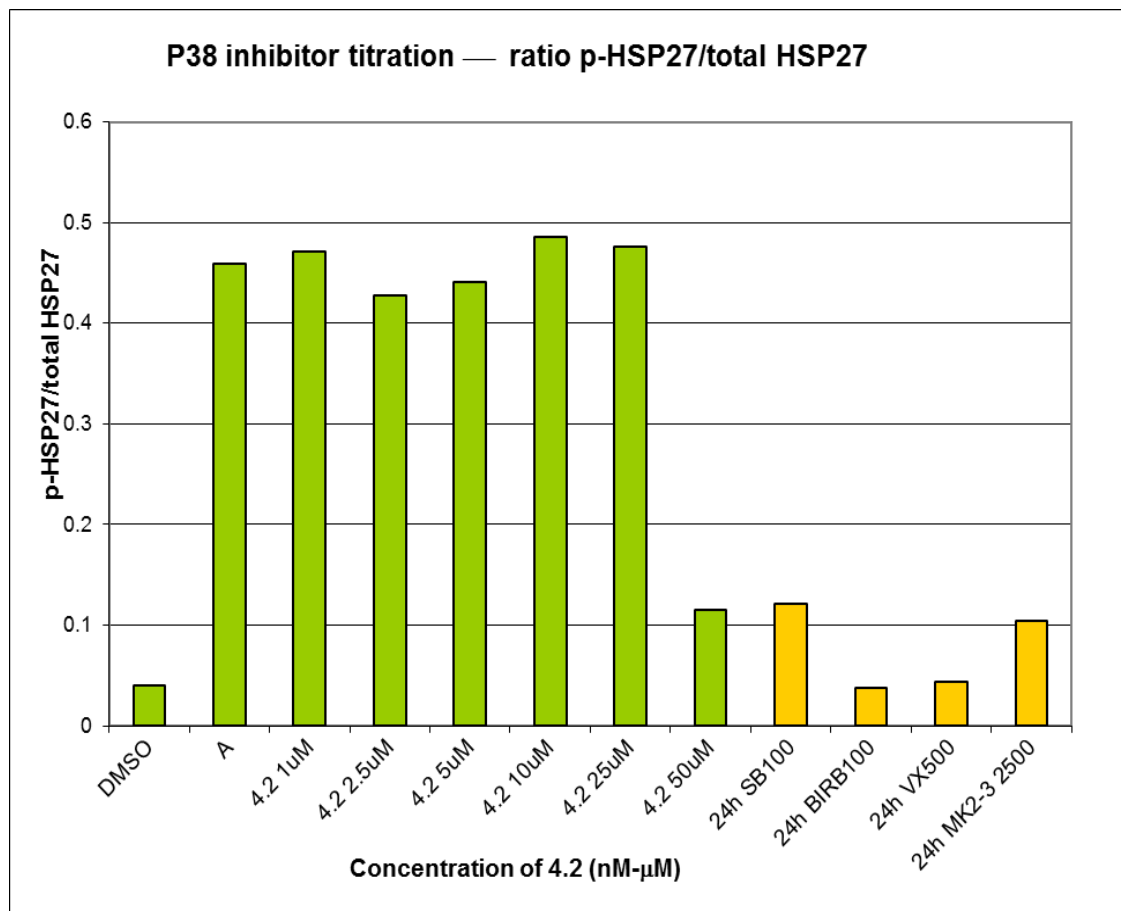
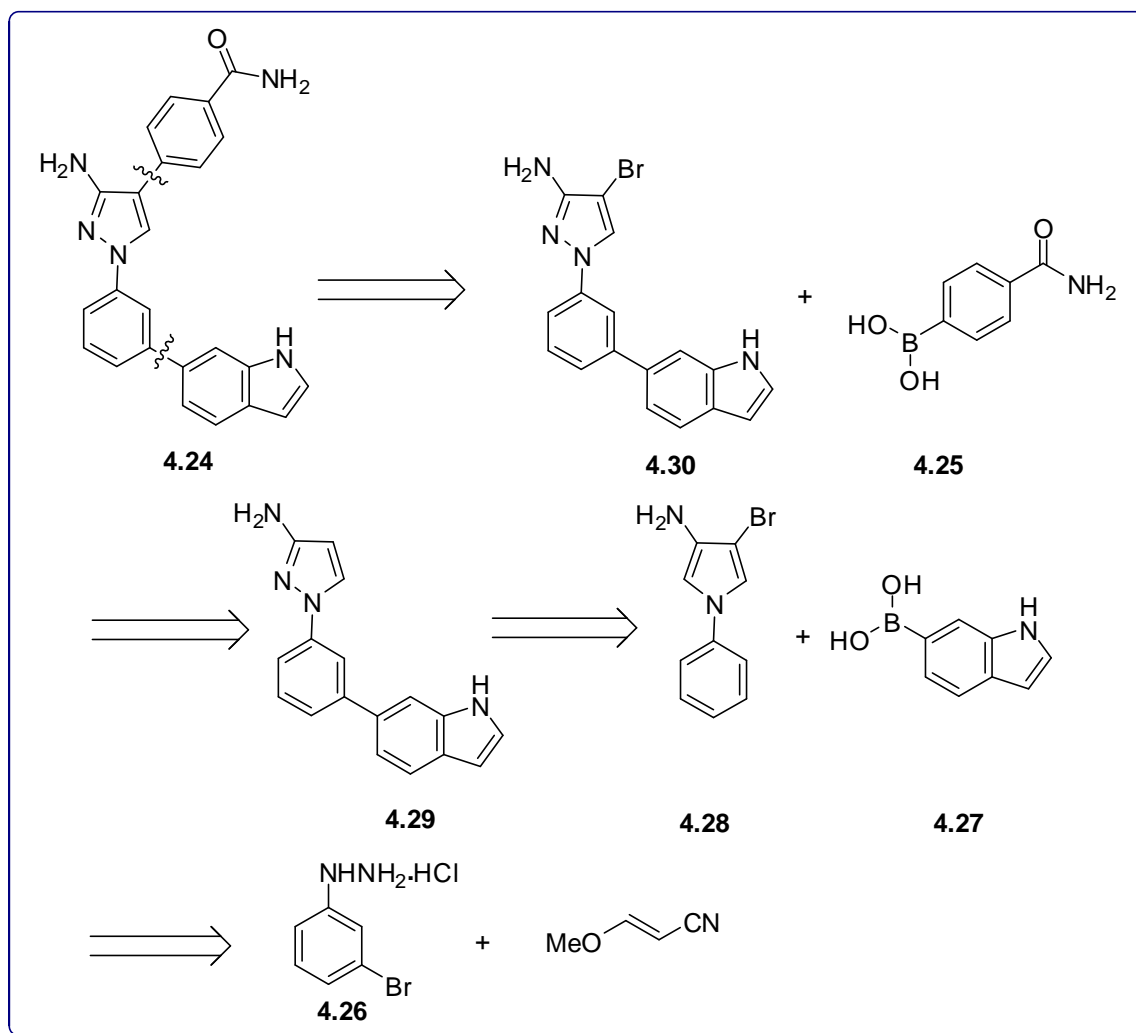


Figure 4.5. The ratio of pHSP27/HSP27 for MK2 inhibitor **4.2**

These experiments show that anisomycin treatment increased the level of pHSP27 by up-regulation of p38, while an increase in the concentration of **4.2** inhibits the process of activation, presumably of MK2, and thus maintains low levels of pHSP27 (Figure 4.5).

4.5 Attempted synthesis of 3-amino-4-[1-(3-1H-pyrazol-4-yl)]benzamide (**4.24**)

In an effort to prepare a more efficacious inhibitor, an alternative and more structurally-complex MK2 inhibitor **4.24** was selected.



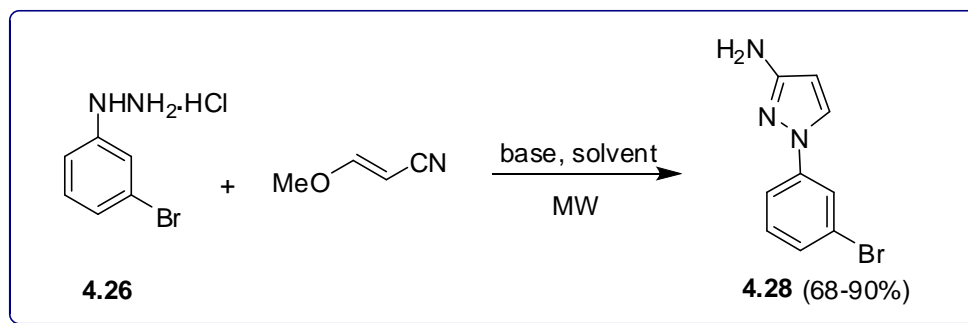
Scheme 4.10. Restorsynthesis of 3-amino-4-[1-(3-1H-pyrazol-4-yl)]benzamide (**4.24**)

Retrosynthetic analysis (Scheme 4.10) of **4.24** revealed a boronic acid derivative **4.25** and 3-amino-4-bromo-1-[3-(1H-indol-6-yl)-1H-pyrazole (**4.30**) as shown. Indole derivative **4.30** could be prepared from bromination of 3-amino-1-[3-(1H-indol-6-yl)phenyl]-1H-pyrazole (**4.29**; Scheme 4.10), which in turn itself could be prepared by Suzuki-coupling of boronic acid derivative **4.27** and 3-amino-1-(3-bromophenyl)-1H-pyrazole (**4.28**) generated from the reaction of (3-bromophenyl)hydrazine hydrochloride (**4.26**) with 3-methoxyacrylonitrile (Scheme 4.10) using a similar strategy to before.

The synthesis of **4.24** could thus take place in four steps. The first step involving the synthesis of 3-amino-1-(3-bromophenyl)-1*H*-pyrazole (**4.28**; Scheme 4.11) under conditions similar to that used in Scheme 4.7, will be discussed first.

4.5.1 Synthesis of 3-amino-1-(3-bromophenyl)-1*H*-pyrazole (**4.28**)

Reaction of (3-bromophenyl)hydrazine hydrochloride (**4.26**; one molar equivalent) with 3-methoxyacrylonitrile (two molar equivalents) in *tert*-butyl alcohol in the presence of potassium *tert*-butoxide as a base under microwave heating for 2 hours at 150 °C gave 3-amino-1-(3-bromophenyl)-1*H*-pyrazole (**4.28**) in 68% yield (Scheme 4.11) after purification by column chromatography.



Scheme 4.11. Synthesis of 3-amino-1-(3-bromophenyl)-1*H*-pyrazole (**4.28**)

The structure of **4.28** was confirmed by IR and NMR spectroscopic and mass spectrometric analysis. The electron impact mass spectrum of **4.28** showed two very intense molecular ion base peaks at m/z 239 and 237 for the different Br isotopes. The high resolution mass spectrum of the molecular ion at m/z 237 confirmed the molecular formula of **4.28** as C₉H₈N₃⁷⁹Br.

The NMR spectra of **4.28** showed all the expected proton and carbon resonances and their chemical shifts were consistent in all respects with the given structure. The ^1H -NMR spectrum of **4.28** showed two characteristic doublets ($J = 2.5$ Hz) at $\delta = 8.21$ and 5.77 ppm due to H-5 and H-4 of the pyrazole ring, respectively. It also showed an exchangeable singlet at $\delta = 5.17$ ppm due to the NH_2 protons. The ^{13}C -NMR spectrum of **4.28** showed a characteristic methine signal at $\delta = 97.6$ ppm corresponding to C-4 of the pyrazole ring.

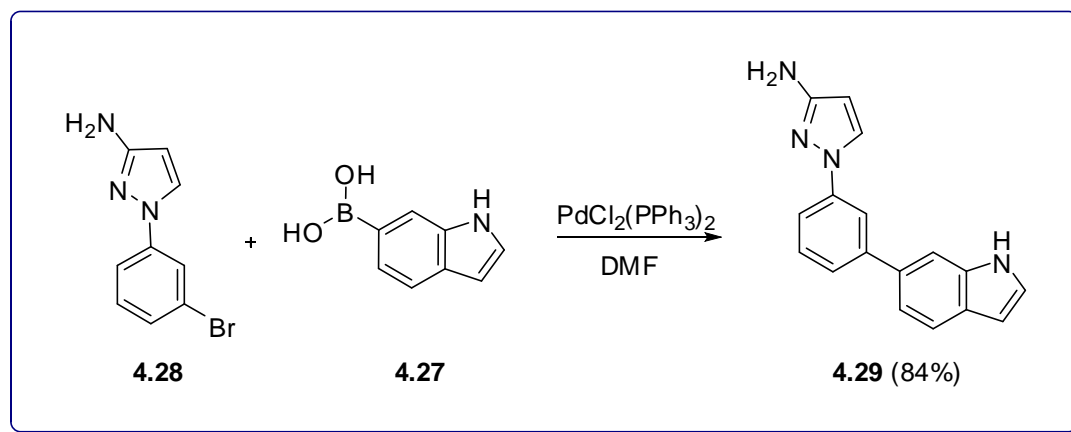
Several experiments were conducted in order to find the optimum conditions under which the yield of **4.28** could be improved. It was found that by reducing the microwave irradiation hold time, reaction of **4.26** and 3-methoxyacrylonitrile, again used in a molar ratio of 1:2, in dry ethanol in the presence of sodium ethoxide under microwave irradiation for 1 hour at 150°C gave **4.28** in 89% yield after purification. The yield of **4.28** was similar (90%) when the reaction was carried out in ethanol under reflux conditions for 20 hours. Under these conditions, chromatographic purification was not employed since the crude product was essentially pure by NMR spectroscopic analysis.

Having successfully produced **4.28** in high yield, our attention next turned to the synthesis of 3-amino-1-[3-(1*H*-indol-6-yl)phenyl]-1*H*-pyrazole (**4.29**).

4.5.2 Synthesis of 3-amino-1-[3-(1*H*-indol-6-yl)phenyl]-1*H*-pyrazole (**4.29**)

Suzuki-coupling⁷⁻¹⁰ of arylbromide **4.28** with 1*H*-indol-6-ylboronic acid (**4.27**) was attempted (Scheme 4.12). Reaction of 3-amino-1-(3-bromophenyl)-1*H*-pyrazole (**4.28**; 3.3 molar equivalents) and the boronic acid derivative **4.27** (one molar equivalent) in *N,N*-dimethylformamide (DMF) was attempted in the presence of cesium carbonate (6.7

molar equivalents) and a catalytic amount of *bis*-(triphenylphosphine)palladium(II) chloride under microwave irradiation for 2 hours at 150 °C (Scheme 4.12). The crude product obtained was analyzed by TLC and this confirmed the formation of a new product. Purification by column chromatography gave pure indole **4.29** in 84% yield.



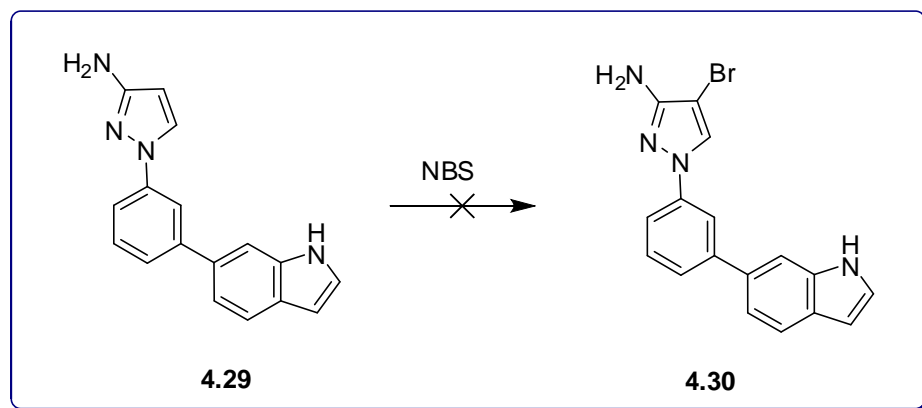
Scheme 4.12. Synthesis of 3-amino-1-[3-(1*H*-indol-6-yl)phenyl]-1*H*-pyrazole (**4.29**)

The structure of **4.29** was confirmed by several spectroscopic techniques including IR and NMR spectroscopy and mass spectrometry (See Chapter six for details). The electron impact mass spectrum of **4.29** showed a very intense molecular ion base peak at m/z 274 and the high resolution mass spectrum confirmed its molecular formula as $\text{C}_{17}\text{H}_{14}\text{N}_2$. The NMR spectra of **4.29** showed all the expected signals for both pyrazole, indole and aryl groups and were consistent with the given structure.

Our attention next turned to the third step in the synthetic pathway for the production of **4.24** - the bromination of **4.29** to produce the corresponding bromo derivative **4.30**.

4.5.3 Synthesis 3-amino-1-(3-(1*H*-indol-6-yl)phenyl)-4-bromo-1*H*-pyrazole (4.30)

Bromination of **4.29** with *N*-bromosuccinimide (NBS) was attempted under various reaction conditions in an attempt to synthesize 3-amino-1-(3-(1*H*-indol-6-yl)phenyl)-4-bromo-1*H*-pyrazole (**4.30**; Scheme 4.13). However, NMR spectroscopic analysis of the product was complex and showed the formation of a mixture of products in which bromination had occurred at various positions of the pyrazole and indole rings. Attempts were made to purify the product but these were not successful. Therefore it was decided to attempt the synthesis of the target inhibitor **4.24** by another route.

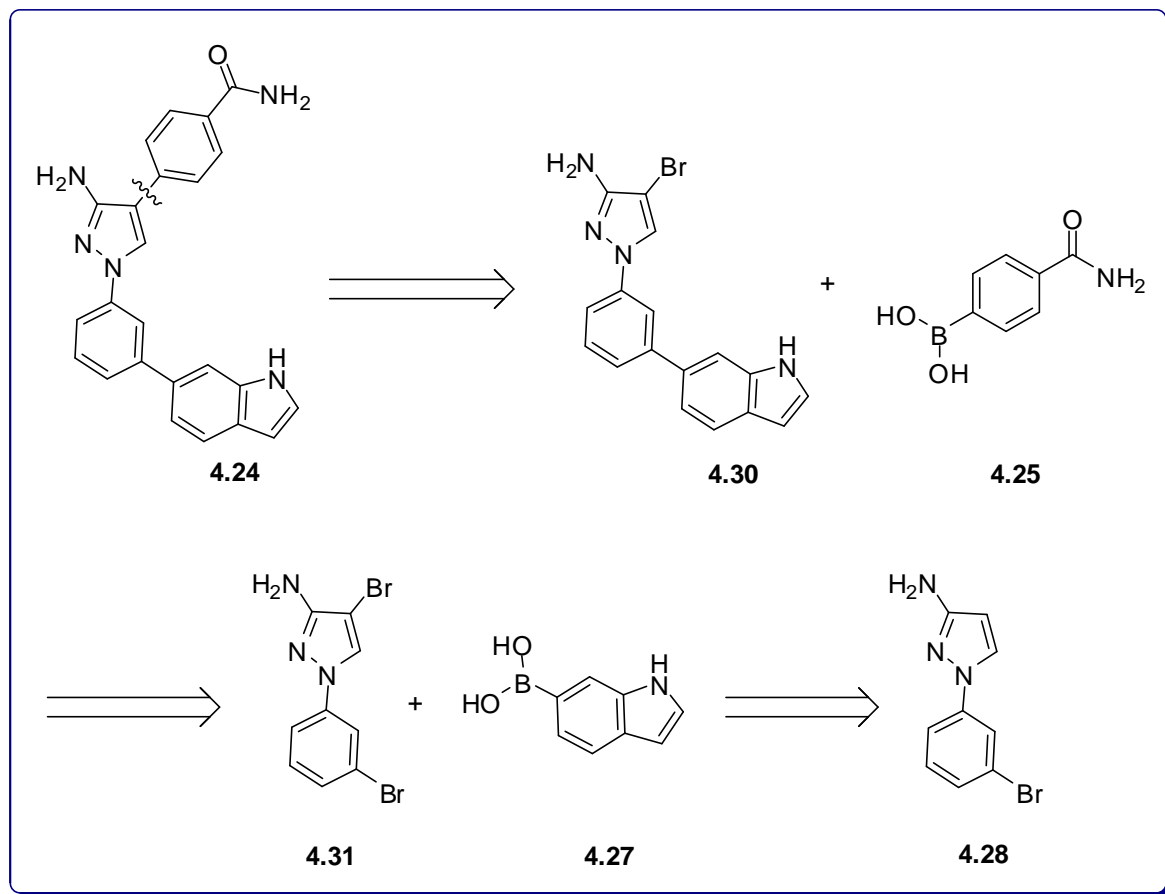


Scheme 4.13. Attempted synthesis of **4.30** by bromination of **4.29**

4.5.4 Alternative attempt at the synthesis of 3-amino-4-[1-(3-1*H*-pyrazol-4-yl)]benzamide (4.24)

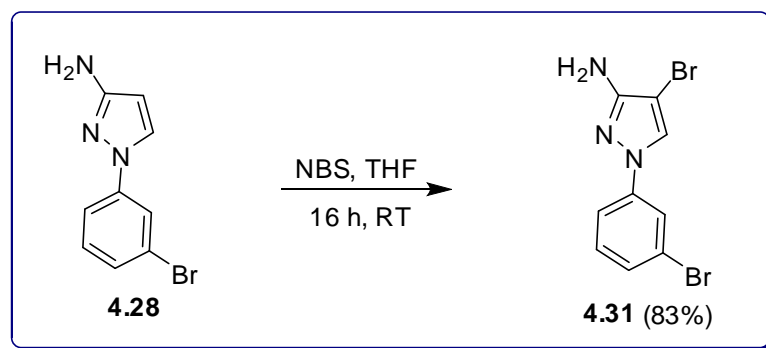
Retrosynthetic analysis of target **4.24** revealed a boronic acid derivative **4.25** and bromide **4.30**, available *via* a selective Suzuki-coupling⁷⁻¹⁰ of boronic acid derivative **4.27** and 3-amino-4-bromo-1-(3-bromophenyl)-1*H*-pyrazole **4.31** (Scheme 4.14). The dibromide substrate for this process, **4.31**, could be produced *via* bromination of bromide **4.28**

(Scheme 4.14). This approach would rely upon the chemoselective reaction of the aryl bromide in preference to the heteroaryl bromide group.



Scheme 4.14. Retrosynthetic pathway for **4.24**

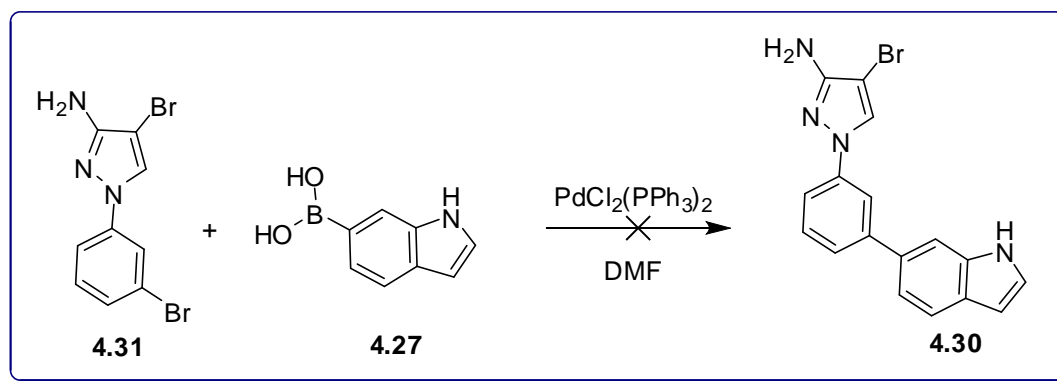
The synthetic pathway represented in Scheme 4.14 involved three steps. The first step involved bromination of 3-amino-1-(3-bromophenyl)-1H-pyrazole (**4.28**) using *N*-bromosuccinimide which was previously prepared as shown in Scheme 4.11. It was found that bromination of **4.28** with NBS in THF at room temperature for 16 hours gave dibromide **4.31** in 83% yield after purification by column chromatography (Scheme 4.15).



Scheme 4.15. Synthesis of 3-amino-4-bromo-1*H*-pyrazole (**4.31**)

The structure of **4.31** was confirmed by IR and NMR spectroscopic and mass spectrometric data (see experimental chapter for details). The electron-impact mass spectrum showed three different Br isotope peaks for the molecular ion and confirmed the molecular formula of **4.31** as C₉H₇Br₂N₃. The ¹H-NMR spectrum of **4.31** showed an exchangeable singlet that resonated at $\delta = 5.38$ ppm due to the NH₂ protons and a characteristic singlet that resonated at $\delta = 8.55$ ppm due to H-5 of the pyrazole ring. The ¹³C-NMR spectrum of **4.31** showed a characteristic singlet at $\delta = 85.2$ ppm and a doublet at $\delta = 127.5$ ppm due to C-4 and C-5 of the pyrazole ring, respectively.

Having successfully synthesised dibromide **4.31**, our attention turned to the synthesis of 3-amino-4-bromo-1-(3-(1*H*-indol-6-yl)phenyl)-1*H*-pyrazole (**4.30**) via chemoselective Suzuki-coupling of **4.31** and boronic acid **4.27** (Scheme 4.16).



Scheme 4.16. Attempt synthesis of **4.30**

The reaction was attempted under various conditions and unfortunately starting material **4.31** was mostly recovered unreacted along with a complex mixture of products. No conditions were found to produce **4.30** cleanly and in high yield. No further attempts were made to find conditions under which **4.30** could be prepared and so production of a more selective or efficacious MK2 inhibitor were halted in favour of alternative inhibitors of study.

4.6 Conclusions

The synthesis of 4-(3-amino-1-(4-methoxyphenyl)-1H-pyrazol-4-yl)benzamide (**4.2**) has been achieved in three steps. Rigorous experimental procedures have been investigated using microwave dielectric heating. This technique led to faster reaction times and allowed the rapid optimization of yields. These advantages were employed in all steps and allowed the rapid formation of the desired targets in high yield. Biological study of one inhibitor 4-(3-amino-1-(4-methoxyphenyl)-1H-pyrazol-4-yl)benzamide (**4.2**) showed, by ELISA analysis, that p38 α signalling was inhibited in control dermal cells. Experiments

can now be carried out, given its inhibitory profile, to explore the role of MK2 signalling in accelerated ageing and these studies are underway in the Kipling laboratories.

Some progress was made towards the synthesis of 3-amino-4-[1-(3-1*H*-pyrazol-4-yl)]benzamide (**4.24**) but the final product was not obtained and so the synthesis of additional inhibitors based upon this chemotype was not pursued further.

4.7 References

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CHAPTER FIVE
SYNTHESIS OF THE p38 INHIBITORS OF RO3201195

CHAPTER FIVE

SYNTHESIS OF THE p38 INHIBITORS OF RO3201195

5.1 Introduction

The development of p38 inhibitors for the treatment of inflammatory diseases has attracted considerable attention from the pharmaceutical industry. P38 MAP kinase plays an important role in the regulation of production of pro-inflammatory cytokines and so an effective therapy for the treatment of many inflammatory diseases could be achieved by blocking p38 MAP kinase.^{1,2} The central role of pro-inflammatory cytokines such as IL-1 β and TNF α and their validation as a target has been proven by successful therapeutic treatment.^{1,2} Various chemotypes have been identified as small molecule p38 mitogen-activated protein kinase inhibitors.^{3,4}

In 1994, SB203580 (**5.1**; Figure 5.1) was discovered as the parent of a class of cytokine suppressive inhibitors.¹ SB203580 (**5.1**) was found to be an inhibitor for the catalytic action of p38 MAP kinase by competitive binding in the ATP pocket.

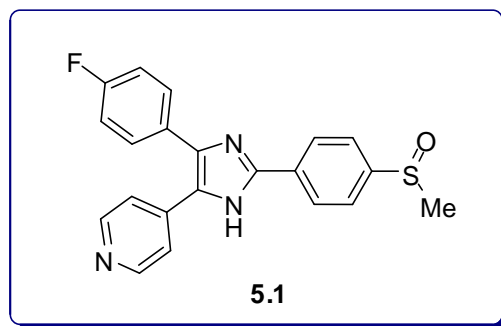


Figure 5.1: The structure of inhibitor SB203580 (**5.1**)

Further developments in the field of protein kinase targeted drug discovery has led to the identification of many inhibitors. Such inhibitors were designed to take advantages of specific interactions that are located within or close to the ATP binding site of p38. The ability of the inhibitor to imitate ATP (**5.2**; Figure 5.1) and occupy regions within the ATP binding cleft and therefore to mimic specific key H-bond interactions was found to be the most important feature in p38 inhibitor and kinase inhibitor design.^{1,2} X-Ray crystallography of other kinases complexed with ATP (**5.2**) shows that this natural ligand interacts directly with the p38 hinge region residue His107 and Met109 to form a pair of hydrogen bonds with the adenine ring of ATP (Figure 5.2).^{5,6}

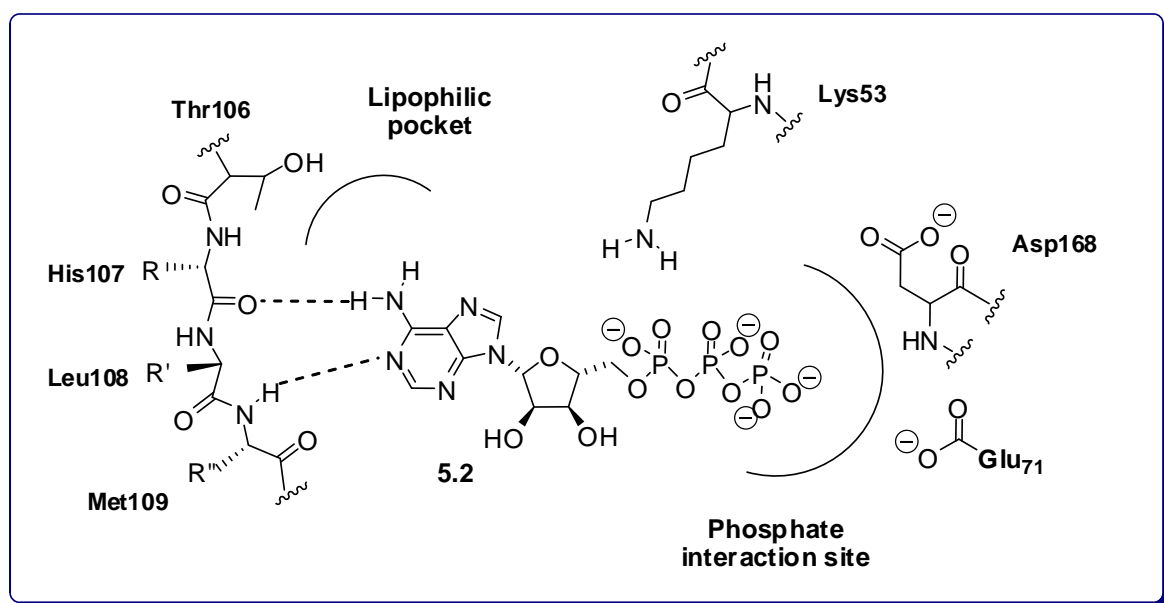


Figure 5.2: ATP binding mode with p38α

The orientation of the triphosphate group in such a way provides efficient catalysis for the γ -phosphorylation transfer to the substrate protein upon p38 MAP kinase activation.⁷

SB203580 (**5.1**) and related pyridin-4-yl imidazoles bind in the ATP binding site of p38 as proven by crystallographic studies. The nitrogen of the pyridine group in **5.1** forms a hydrogen bond with the NH of the backbone of Met109 in the linker region.⁸⁻¹⁰ The 4-fluorophenyl ring in **5.1** binds to hydrophobic region which in p38 is represented by a spacious pocket, a second hydrophobic area below the linker region is left unoccupied by simple pyridin-4-yl imidazole inhibitors (Figure 5.3).^{8,10}

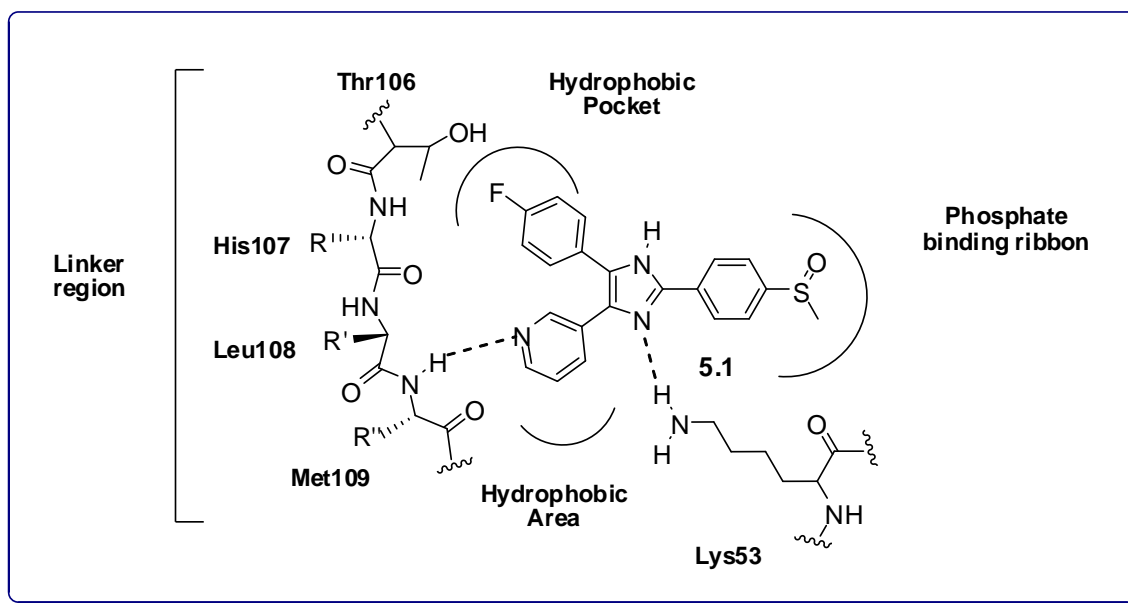


Figure 5.3: SB 203580 (**5.1**) binding modes with p38 α

Efforts have been made to develop various structurally-related p38 inhibitors that could improve potency and minimize liabilities, in particular in selectivity, that have been identified in the pyridine-imidazole series.

5.2 Use of p38 inhibitors for the treatment of Werner syndrome cells

The research group of Kipling from Cardiff University has investigated the role of p38-transduced stress signalling in Werner syndrome cells using SB203580 (**5.1**; Figure 5.1), the cytokine-suppressive anti-inflammatory drug, whose major inhibitory target is p38.¹

The study of genome instability observed in WS, along with frequent replication fork stalling, provides a plausible trigger for replication stress in WS cells and a possible involvement for p38 signalling in inducing the shortened replicative life span.¹

The research groups of Bagley and Kipling, along with others, have investigated the use of BIRB796 (**5.3**; Figure 5.4) as a potent and selective inhibitor of p38 α mitogen-activated protein kinase for the study of accelerated ageing in Werner syndrome cells.^{11–14}

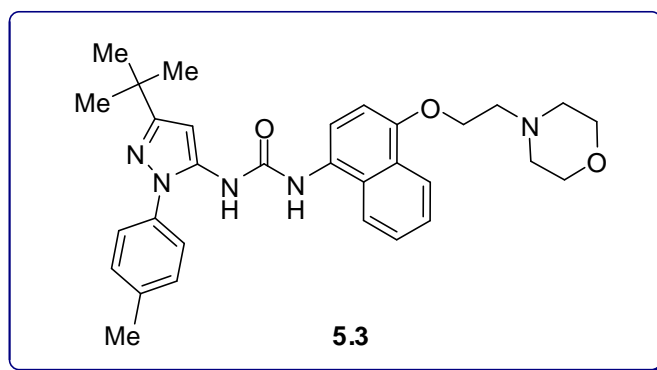


Figure 5.4: Structure of BIRB 796 (**5.3**)

The 5-aminopyrazol-4-yl ketone p38 inhibitor RO3201195 (**5.4**; Figure 5.5) was discovered by Hoffmann-La Roche. RO3201195 (**5.4**) is an orally bioavailable and highly selective inhibitor of both α and β -isoforms of p38 MAP kinase which binds to both the

phosphorylated and unphosphorylated forms of the enzyme, as is common for ATP competitive inhibitors.¹⁵

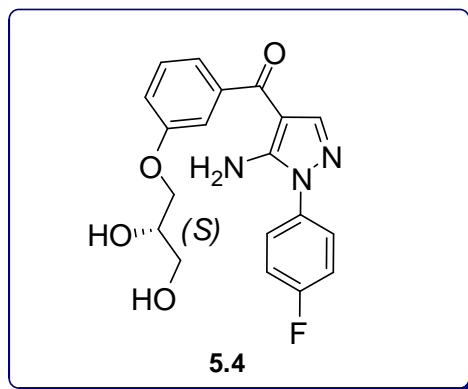


Figure 5.5: The structure of RO3201195 (**5.4**)

X-Ray crystallographic studies showed that RO3201195 (**5.4**) was able to form a hydrogen bond between the benzoyl oxygen and the NH of Met109 of the main chain. Also, it showed an interaction in the ATP binding site.¹⁵ RO3201195 (**5.4**) forms two hydrogen bonds, one with the backbone His107 and one with the side chain hydroxyl group of Thr106.^{15,16} The binding interactions of RO3201195 (**5.4**) with p38 α are shown in Figure 5.6.

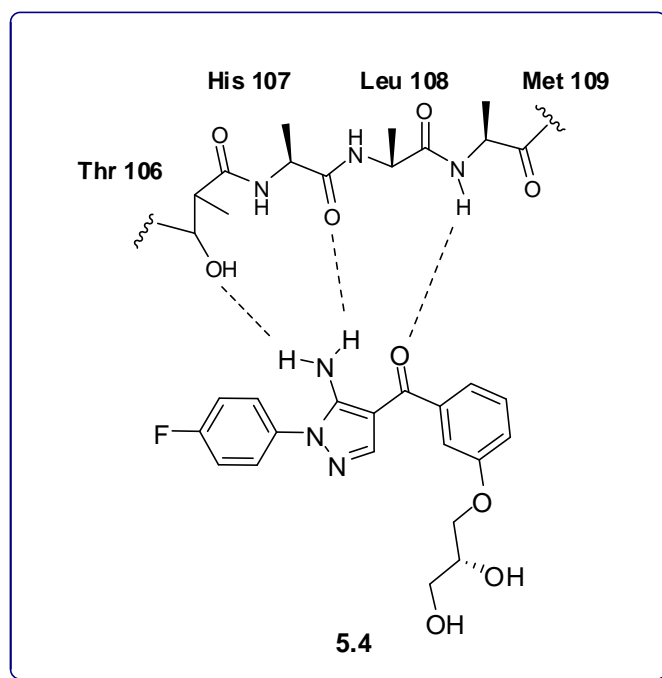


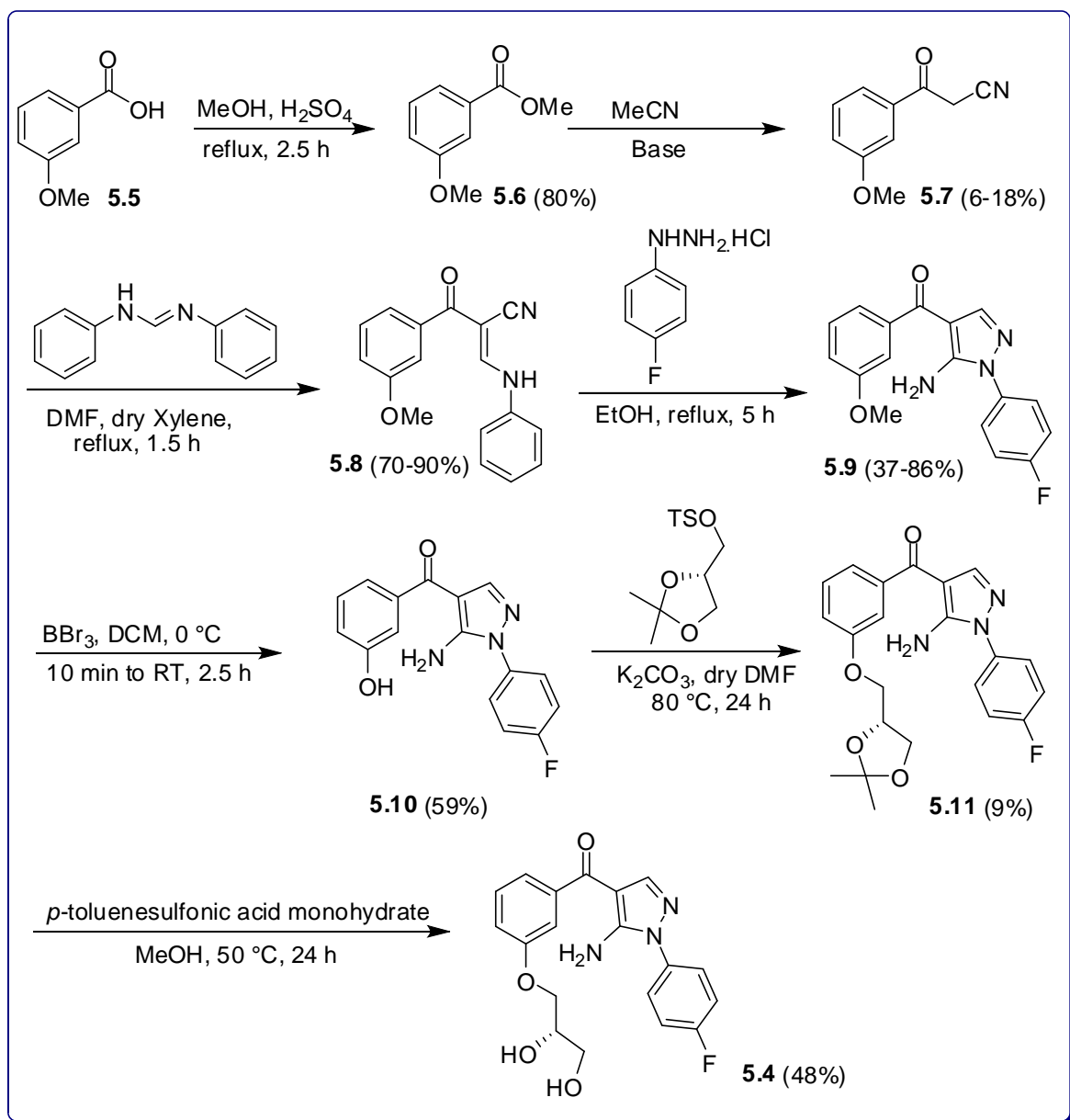
Figure 5.6: Binding interactions of RO3201195 (**5.4**)

The kinase selectivity profile of **5.4** *in vitro* indicated that only 6 kinases were inhibited out of a panel of 105 kinases with inhibition >50% at 10 μ M concentration and only two of those were inhibited at comparable concentrations to its p38 activity, PDGFR β and GAK.¹⁵ The synthesis of RO3201195 (**5.4**) and its ability to inhibit p38 α and JNK was investigated in hTERT-immortalized HCA2 and WS dermal fibroblasts at 200 nM concentration, both by enzyme linked immuno-sorbent assay (ELISA) and immunoblot assay, and displays excellent kinase selectivity for p38 α MAPK over the related stress-activated kinase JNK.¹⁷

5.3 Synthesis of the highly selective RO3201195 (**5.4**)

RO3201195 (**5.4**) was previously synthesised^{15,17} in seven steps as shown in Scheme 5.1. The first step involves esterification of 3-methoxybenzoic acid (**5.5**) with

methanol to give the corresponding methyl ester **5.6** in 80% yield (Scheme 5.1).^{15,17} The second step involves reaction of **5.6** with acetonitrile, in the presence of a base, to give the corresponding nitrile **5.7** in low yields (6–18%) depending upon the type of base used.¹⁸ The third step involves reaction of nitrile **5.7** with *N,N'*-diphenylformamidine under reflux conditions in dry xylene for 1.5 h to give 3-(phenylamino)acrylonitrile (**5.8**) in 85% yield.^{17,18} This yield was improved to 90% when the reaction was carried out under microwave-assisted conditions for 20 minutes at 180 °C (Scheme 5.1).^{15,17,18} Cyclocondensation of **5.8** with 4-fluorophenylhydrazine hydrochloride in ethanol under reflux conditions for 5 hours gave 5-aminopyrazole (**5.9**) in 37% yield (Scheme 5.1).^{15,18} The yield of pyrazole **5.9** formation was improved to 86% when the reaction was carried out under microwave heating for 1 h at 140 °C.^{15,18} Reaction of **5.9** with boron tribromide in dichloromethane 0 °C for 20 minutes followed by room temperature for 2.5 h gave phenol derivative **5.10** in 59% yield (Scheme 5.1). Reaction of **5.10** with (*S*)-*O*-iso-propyldipheneglycerol tosylate under basic conditions gave ketal **5.11** in low yield (9%).¹⁸ The final target RO3201195 (**5.4**) was obtained in 48% yield upon hydrolysis of **5.11** under acidic conditions (Scheme 5.1).¹⁸ Thus, although this route was successful in delivering the target inhibitor, many of these steps were poorly efficient and this precluded the production of the inhibitor in any appreciable quantities, thus preventing further biological study.

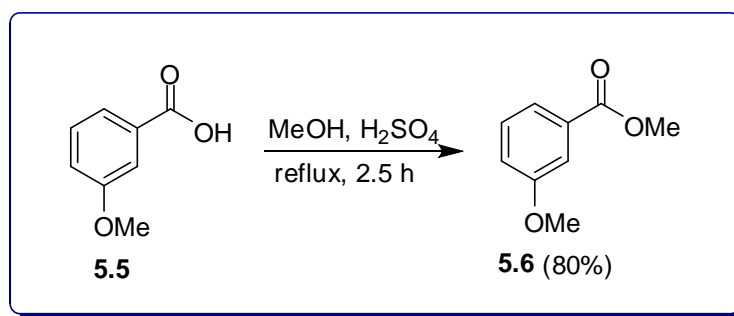


Scheme 5.1: Synthesis of RO3201195 (**5.4**)

The goals of the work reported in this chapter aimed to improve the synthesis of RO3201195 (**5.4**) and obtain the product in sufficient yield for further study. We now report our successful attempts to deliver **5.4** predominantly using microwave heating.^{15,17,18}

5.3.1. Synthesis of methyl 3-methoxybenzoate (5.6)

The synthesis of RO3201195 (**5.4**) was first attempted based on literature procedures.^{15,18} The esterification of the commercially available 3-methoxybenzoic acid (**5.5**) with methanol in the presence of a catalytic amount of concentrated sulphuric acid under reflux condition was attempted. Following work-up and extraction with diethyl ether, methyl 3-methoxybenzoate was produced in 80% yield (Scheme 5.2) as a colourless oil.¹⁹ This was in good agreement with the literature route.



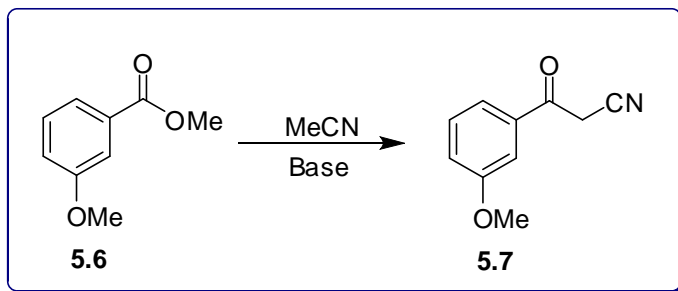
Scheme 5.2: Synthesis of 3-methoxybenzoic acid methyl ester (**5.6**)

The structure of ester **5.6** was confirmed by IR and NMR spectroscopic analysis and mass spectrometric data. The ¹H NMR spectrum of **5.6** showed two singlets at δ = 3.85 and 3.91 ppm corresponding to two methoxy groups. The ¹³C-NMR spectrum showed a singlet signal that resonated at δ = 167.0 ppm corresponding to the carbonyl carbon. Moreover, the APCI mass spectrum of **5.6** showed a very intense signal at m/z = 167 corresponding to the pseudo molecular ion (MH⁺).

5.3.2. Synthesis of 3-methoxybenzoylacetone nitrile (**5.7**)

Having successfully produced methyl ester **5.6**, our attention turned to the conversion of **5.6** to the corresponding nitrile **5.7**. 3-Methoxybenzoylacetone nitrile (**5.7**) was

previously synthesised in very low yield (6–18%) within our research group.¹⁸ The reaction of **5.6** with acetonitrile in the presence of a base was carried out (Scheme 5.3).



Scheme 5.3: Synthesis of 3-methoxybenzoylacetonitrile (**5.7**)

First, the literature procedure for the production of **5.7** was followed in which sodium ethoxide was used as a base.²⁰ A mixture of ester **5.6** and sodium ethoxide was stirred at room temperature and at 80 °C for 5 minutes after which a gelatinous mass was formed which was slowly added to dry acetonitrile. The mixture was heated under reflux conditions for 24 hours and submitted to an aqueous work-up. The residue obtained was purified by column chromatography to give acetonitrile **5.7** in only 9% yield, along with a significant quantity of starting material **5.6** (Table 5.1; Entry 1). Use of sodium methoxide²¹ did not improve the yield of **5.7** under similar conditions to the sodium ethoxide reaction (Table 5.1; Entry 2).

In another experiment, a mixture of **5.6** and sodium hydride in anhydrous tetrahydrofuran (THF) was stirred at room temperature for 10 minutes. The mixture was added in a dropwise manner to anhydrous acetonitrile under a N₂ atmosphere and the resulting mixture was heated under reflux for 1.5 hours. Following aqueous work-up and purification of the crude product, **5.7** was obtained in a slightly better yield (15 %) but this

was still considered low (Table 5.1; Entry 3). The reasons for the low yield of **5.7** obtained in this reaction were not clear although the gelatinous state of the reaction medium no doubt played a part. In an attempt to improve the yield of **5.7** we decided to carry out the reaction under microwave conditions using sodium hydride as the base.

A mixture of **5.6** and sodium hydride in THF was stirred at room temperature for 10 minutes and then added to acetonitrile. The resulting mixture was heated at 150 °C for 1 hour under microwave heating. The reaction mixture was submitted to aqueous work-up and the product was extracted with ethyl acetate to give pure acetonitrile product **5.7** as a colourless solid in 35% yield (Table 5.1; Entry 4). The improvement in yield was significant but still was not satisfactory; therefore, the reaction was attempted under reflux but for a long period of time (24 hours) in an attempt to improve the yield further.

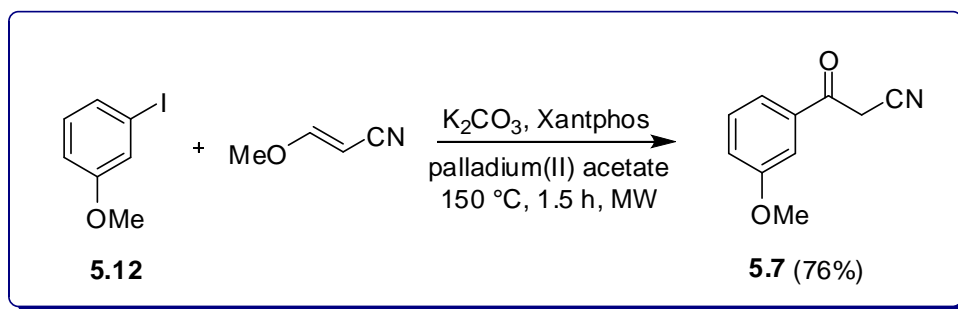
Table 5.1 Synthesis of 3-methoxybenzoylacetonitrile (**5.7**) according to Scheme 5.3

Entry	Base	Condition	Time (h)	Yield (%) ^a
1	NaOEt	Reflux	24	9
2	NaOMe	Reflux	24	9
3	NaH	Reflux	1.5	15
4	NaH	MW, 150 °C	1	35
5	NaH	Reflux	24	75

^a Yield obtained of **5.7** either after purification by column chromatography or solvent extraction using ethyl acetate.

Indeed, stirring a mixture of **5.6** and NaH in THF at room temperature for 20 minutes followed by the addition of acetonitrile then heating the whole mixture at reflux for 24 hours gave the product **5.7** in 75% yield after purification simply by extraction (Table 5.1; Entry 5).

Given the difficulties associated with this transformation and the prolonged reaction time required, efforts were made to access benzoyl acetonitrile **5.7** by a different route. To that end, reaction of 3-iodoanisole (**5.12**) with 3-methoxyacrylonitrile, used to good effect in aminopyrazole synthesis, in the presence of potassium(II) acetate as catalyst under microwave heating at 150 °C for 1.5 hours was attempted (Scheme 5.4). Gratifyingly, product **5.7** was isolated in 76% yield after purification by column chromatography. This material **5.7** produced by this route was identical in all respects (melting point, appearance and spectroscopic data) to the previously obtained sample.



Scheme 5.4: Synthesis of 3-methoxybenzoyl acetonitrile (**5.7**)

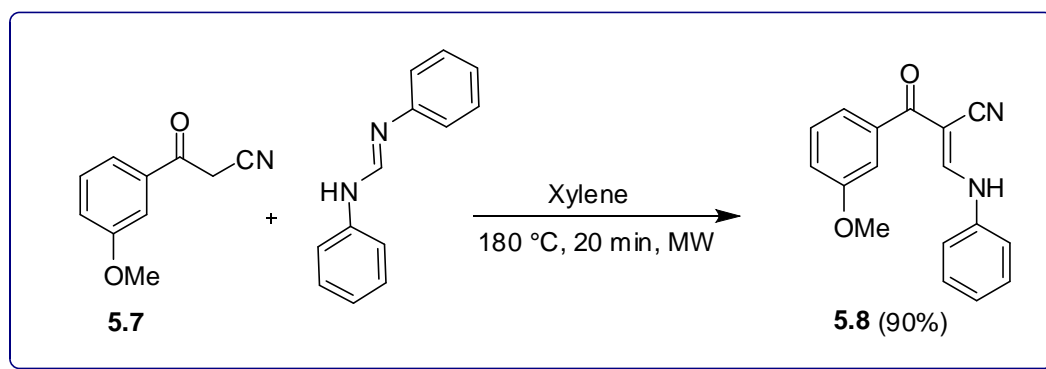
The structure of **5.7** was confirmed by various spectroscopic techniques (See Chapter Six for details). The IR spectrum of **5.7** showed absorption bands at $\nu_{\text{max}} = 2343$ and 1650 cm^{-1} due to $\text{C}\equiv\text{N}$ and $\text{C}=\text{O}$ vibrations, respectively. The ^1H NMR spectrum showed a singlet resonance at $\delta = 3.89 \text{ ppm}$ due to the CH_2 protons. The ^{13}C NMR

spectrum of **5.7** showed a signal that resonated at $\delta = 29.5$ ppm corresponding to CH_2 carbon. The electrospray mass spectrum showed a molecular ion peak at $m/z = 175$. The high resolution mass spectrum of **5.7** confirmed the formula of the molecular ion as $\text{C}_{10}\text{H}_9\text{NO}_2$.

Having successfully synthesised compound **5.7** in high yield (76%), we therefore, proceeded with the subsequent steps towards the synthesis of the target inhibitor **5.4**.

5.3.3 Synthesis of 2-(3-methoxybenzoyl)-3-phenylaminoacrylonitrile (**5.8**)

In previous studies, Knoevenagel condensation of 3-methoxybenzoylacetonitrile (**5.7**) and *N,N'*-diphenylformamidine was attempted in dry xylene under reflux conditions for 1.5 hours (Scheme 5.5).¹⁸ However, we chose to heat the reaction mixture under microwave irradiation at 180 °C for 20 minutes and then allowed the mixture to cool to room temperature and remove the solvent under reduced pressure. The residue obtained was treated with diethyl ether to give pure 2-(3-methoxybenzoyl)-3-phenylaminoacrylonitrile (**5.8**) in 90% yield.

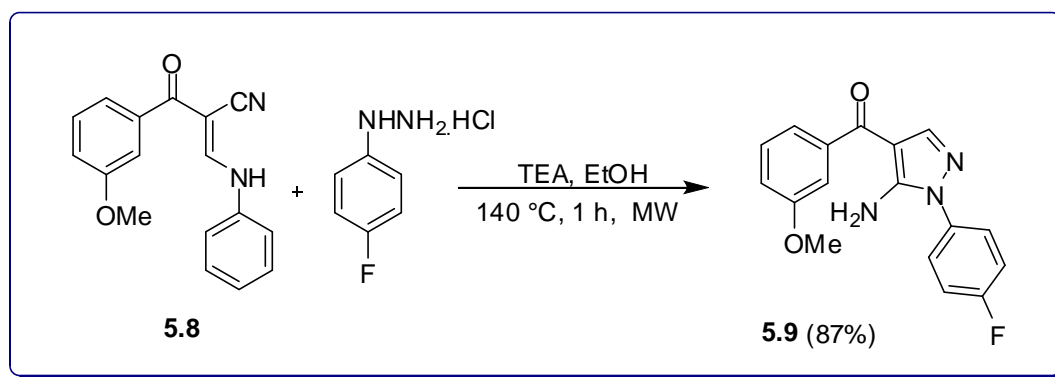


Scheme 5.5: Synthesis of 2-(3-methoxybenzoyl)-3-phenylaminoacrylonitrile (**5.8**)

The electron-impact mass spectrum of **5.8** showed a molecular ion peak at $m/z = 278$ and high resolution mass spectrometric analysis confirmed the formula of the molecular ion as $C_{17}H_{14}N_2O_2$ (M^{+}). The 1H NMR spectrum of **5.8** showed a doublet ($J = 13$ Hz) at $\delta = 8.06$ ppm corresponding to the CH proton and an exchangeable doublet ($J = 13$ Hz) at $\delta = 4.10$ ppm corresponding to the NH proton. The ^{13}C NMR spectrum showed all of the expected signals and was consistent with the structure of **5.8**.

5.3.4 Synthesis of 5-amino-1-(4-fluorophenyl)-1H-pyrazol-4-yl)-3-methoxyphenyl ketone (**5.9**)

Following the successful synthesis of aminoacrylonitrile **5.8**, the next step was its cyclocondensation with 4-fluorophenylhydrazine hydrochloride (Scheme 5.6). The reaction was carried out under microwave heating in ethanol and in the presence of triethylamine (TEA) for one hour at 140 °C to give 5-amino-1-(4-fluorophenyl)-1H-pyrazol-4-yl)-3-methoxyphenyl ketone (**5.9**) in 87% yield (Scheme 5.6), after purification by column chromatography, as a colourless solid.

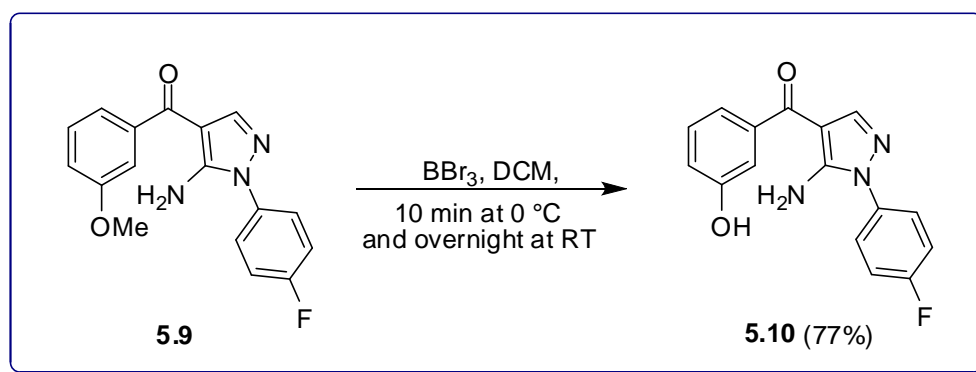


Scheme 5.6: Synthesis of 5-amino-1-(4-fluorophenyl)-1H-pyrazol-4-yl)-3-methoxyphenyl ketone (**5.9**)

The spectroscopic data collected confirmed the structure of **5.9**. The APCI mass spectrum of **5.9** showed a pseudo molecular ion (MH^+) peak at $m/z = 312$ and the formula of MH^+ was confirmed as $\text{C}_{17}\text{H}_{15}\text{N}_3\text{O}_2\text{F}$ by high resolution mass spectrometric analysis. The ^1H NMR spectrum of **5.9** showed a singlet that resonated at $\delta = 7.83$ ppm due to H-3 of the pyrazole ring. It also showed an exchangeable singlet at $\delta = 6.07$ ppm due to the NH_2 protons. The ^{13}C NMR spectrum showed that the C-4 of the 4-fluorophenyl moiety resonated at δ 162.1 ppm ($J = 280$ Hz) and showed all of the characteristic signals for the pyrazole ring as well as all other expected resonances.

5.3.5 Synthesis of 5-amino-1*H*-(pyrazol-4-yl)-3-hydroxyphenyl ketone (**5.10**)

Transformation of the methyl ether of **5.9** to 5-amino-1*H*-(pyrazol-4-yl)-3-hydroxyphenyl ketone (**5.11**) was based on literature precedent.^{15,18} Reaction of 5-aminopyrazole **5.10** with boron triboromide (BBr_3) in dichloromethane was carried out at 0°C for 10 minutes and at room temperature overnight (Scheme 5.7).



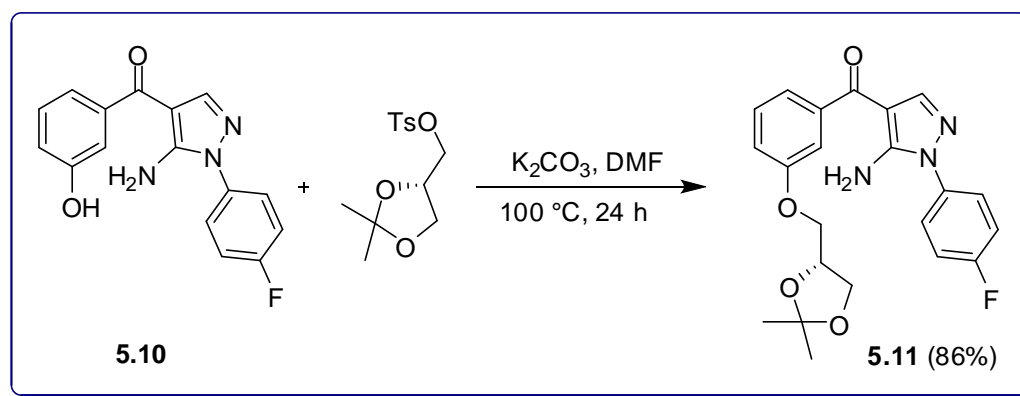
Scheme 5.7: Synthesis of 5-amino-1*H*-(pyrazol-4-yl)-3-hydroxyphenyl ketone (**5.10**)

The reaction mixture was submitted to aqueous work-up and the product was extracted with dichloromethane to give the desired phenol **5.10** in 77% yield as a colourless solid. This was an improvement over the reported yield for **5.10** under similar reaction conditions (59%).¹⁸

The EI mass spectrum of **5.10** showed a molecular ion peak at $m/z = 298$ and the formula of the molecular ion was confirmed as $C_{16}H_{12}N_3O_2F$ (M) by high resolution mass spectrometric analysis. The NMR spectra were in agreement with the suggested structure of **5.10**.

5.3.6 Synthesis of [5-amino-1-(4-fluorophenyl)-1*H*-pyrazol-4-yl]-3-[[*(R)*-2,2-dimethyl-1,3-dioxolan-4-yl]methoxy]phenyl ketone (**5.11**)

The next step in the synthesis of RO3201195 (**5.4**) was the formation of ketal **5.11** via alkylation of **5.10**. Reaction of phenolic compound **5.10** with (*S*)-*O*-iso-propyldieneglycerol tosylate in anhydrous dimethylsulphoxide (DMSO) in the presence of anhydrous potassium carbonate under a N_2 atmosphere for 24 hours at 100 °C was investigated (Scheme 5.8). Following aqueous work-up, the crude product was extracted with ethyl acetate and was purified by crystallization from a hexane–ethyl acetate mixture (3:1 by volume) to give ether **5.11** in 68% yield (Scheme 5.8). This was a considerable improvement compared to 9% as previously reported and so provided substantially more material for study.¹⁸



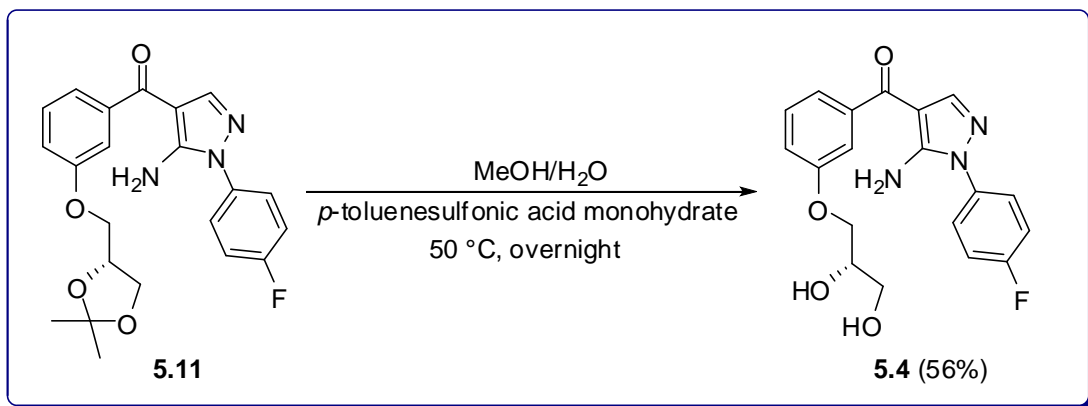
Scheme 5.8: Synthesis of [5-amino-1-(4-fluorophenyl)-1*H*-pyrazol-4-yl]-3-{[(*R*)-2,2-dimethyl-1,3-dioxolan-4-yl]methoxy}phenyl ketone (**5.11**)

The structure of **5.11** was confirmed by the use of various spectroscopic techniques including IR and NMR and low and high resolution mass spectrometric data. The APCI mass spectrum of **5.11** showed a very intense pseudo molecular ion peak (MH^+) at $m/z = 412$ and the formula of the molecular ion was confirmed as $\text{C}_{22}\text{H}_{23}\text{N}_3\text{O}_4\text{F}$ by high resolution mass spectrometric analysis. The NMR spectra of **5.11** showed all of the expected signals and were in agreement with the structure of **5.11**.

5.3.7 Synthesis of 5-amino-1-(4-fluorophenyl)-4-{3-[2(*S*),3-dihydroxypropoxy]-benzoyl}pyrazole (**5.4**)

The final step in the synthesis of RO3201195 (**5.4**) was the deprotection of ketal **5.11** to diol **5.4**. A mixture of **5.11** and *p*-toluenesulfonic acid monohydrate in aqueous methanol was stirred overnight at 50 °C under a N_2 atmosphere (Scheme 5.9). Following

aqueous work-up, the crude product was purified by column chromatography to give RO3201195 (**5.4**) in 56% yield (Scheme 5.9), compared to 48% as previously reported.¹⁸



Scheme 5.9: Synthesis of RO3201195 (**5.4**)

The APCI mass spectrum of **5.4** showed a very intense pseudo molecular ion peak (MH^+) at $m/z = 372$. The high resolution mass spectrum confirmed the formula of the pseudo molecular ion as $C_{19}H_{19}N_3O_4F$. The NMR spectra of **5.4** showed all of the expected signals and were in agreement with the given structure.

5.4 Conclusions

The synthesis of the chemotherapeutic agent RO3201195 (**5.4**), which is a highly selective inhibitor of p38 α , has been achieved in seven steps. The procedure has been modified and now provides a high yield of the desired product, using in many steps microwave heating. The intermediates, 3-methoxybenzoylacetonitrile (**5.7**), 5-amino-1*H*-(pyrazol-4-yl)-3-hydroxyphenyl ketone (**5.10**) and [5-amino-1-(4-fluorophenyl)-1*H*-pyrazol-4-yl]-3-[[*(S)*-2,2-dimethyl-1,3-dioxolan-4-yl]methoxy}phenyl ketone (**5.11**) were

produced in much improved yield (76, 77 and 68%, respectively) compared to earlier reports (18, 59 and 9%, respectively).¹⁸ Also, the final step in the synthetic process provided the target product RO3201195 (**5.4**) in higher yield (56%) compared to the reported one (48%).¹⁸ Prior to this work, RO3201195 was produced in a seven step sequence in an overall yield equal to or less-than 0.2%, with the lowest yielding step of the sequence the protodemethylation (9%). This severely impeded its use as an inhibitor of study in WS cells. Now, in a seven step sequence, RO3201195 can be formed in an overall yield of 18%, with the lowest yielding step the final deprotection/purification sequence. Furthermore, many of the steps now are rapid and promoted by microwave heating, providing expedient access to the inhibitor for biological study.

5.5 References

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CHAPTER SIX
EXPERIMENTAL

CHAPTER SIX

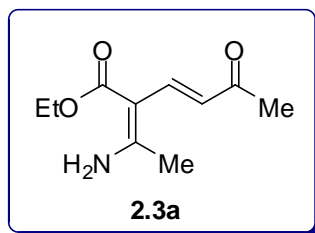
EXPERIMENTAL

6.1 Experimental Techniques

Commercially available reagents were used without further purification; solvents were dried by standard procedures. Light petroleum refers to the fraction with bp 40–60 °C, ether (Et₂O) refers to diethyl ether and EtOAc refers to ethyl acetate. Column chromatography was carried out using Merck Kieselgel 60 H silica or Matrex silica 60. Analytical thin layer chromatography was carried out using aluminium-backed plates coated with Merck Kieselgel 60 GF254 that were visualised under UV light (at 254 and/or 360 nm). Melting points (mp) were determined on a Kofler hot stage apparatus and are uncorrected. Infra-red (IR) spectra were recorded in the range 4000–600 cm⁻¹ on a Jasco FT/IR-660 plus instrument by dissolving the product in chloroform, applying droplets on a NaCl plate and allowing evaporation of the solvent or as KBr disks and are reported in cm⁻¹. Nuclear magnetic resonance (NMR) spectra were recorded in CDCl₃, DMSO-*d*₆ or TFA-*d* at 25 °C using a Bruker AV400 or Bruker AV500 spectrometer operating at 400 or 500 MHz for ¹H and 100 or 125 MHz for ¹³C measurements, respectively. Chemical shifts were reported in ppm relative to TMS; *J* values were recorded in Hz and multiplicities were expressed by the usual conventions (s = singlet, d = doublet, t = triplet, app = apparent, m = multiplet). Assignments of signals are based on integration values, coupling patterns and expected chemical shift values and have not been rigorously confirmed. ¹³C multiplicities were revealed by DEPT signals and are reported as s (C), d (CH), t (CH₂) and q (CH₃). Low-resolution mass spectra (MS) were determined on a Waters GCT Premier Spectrometer using atmospheric pressure chemical ionization (APCI) unless otherwise stated. ES refers to

electrospray ionization, CI refers to chemical ionization (ammonia) and EI refers to electron impact. High-resolution mass spectra were recorded on a Waters LCT Premier XE instrument using the ionisation methods specified. *In vacuo* refers to evaporation at reduced pressure using a rotary evaporator and diaphragm pump, followed by the removal of trace volatiles using a vacuum (oil) pump. Microwave experiments were carried out in a CEM Discover microwave synthesizer at the temperature and initial power stated, with modulation of power to maintain constant reaction temperature as measured by the in-built IR sensor.

6.2 Synthesis of 2-amino-3-ethoxycarbonylhepta-2,4-dien-6-one (2.3a)



A mixture of ethyl β -aminocrotonate (**2.1a**; 0.20 mL, 1.6 mmol) and 4-(trimethylsilyl)but-3-yn-2-one (**2.2a**; 0.52 mL, 3.2 mmol) in dry ethanol (4 mL) was irradiated at 60 °C (initial power 100 W) for 60 min in a sealed pressure-rated reaction tube (10 mL), using a CEM Discover Microwave Synthesizer, cooled and then evaporated *in vacuo*. Purification by recrystallization from hexane–acetone mixture gave the *title compound* as a yellow solid (**2.3a**; 0.31 g, 100%).

mp 123–124 °C (lit.¹ 125.5–126.4 °C) (Found: M^+ , 197.1053. $C_{10}H_{15}NO_3$ [M] requires 197.1052).

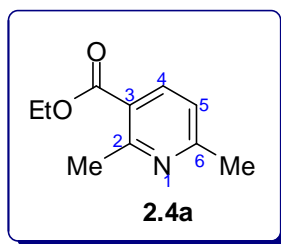
FT-IR (KBr)/ cm^{-1} ν_{max} : 3332, 1740 (C=O), 1554, 1489, 1443, 1352, 1310, 1277, 1200, 1183, 1111 and 1023.

^1H NMR (400 MHz; $\text{DMSO}-d_6$): 9.20 (br s, exch. D_2O , 1 H, 1 H of NH_2), 8.50 (br s, exch. D_2O , 1 H, 1 H of NH_2), 7.45 (d, $J = 15$ Hz, 1 H, $\text{CH}=\text{CHCOCH}_3$), 6.38 (d, $J = 15$ Hz, 1 H, $\text{CH}=\text{CHCOCH}_3$), 4.15 (q, $J = 7.1$ Hz, 2 H, CH_2CH_3), 2.45 (s, 3 H, CH_3), 2.10 (s, 3 H, CH_3) and 1.22 (t, $J = 7.1$ Hz, CH_2CH_3).

^{13}C NMR (100 MHz; $\text{DMSO}-d_6$): δ 199.7 (s, $\text{CH}_3\text{C}=\text{O}$), 170.5 (s, $\text{C}=\text{O}$), 166.5 (s, $\text{C}-\text{NH}_2$), 140.4 (d, $\text{CH}=\text{CHCOCH}_3$), 122.0 (d, $\text{CH}=\text{CHCOCH}_3$), 95.3 (s, $\text{C}=\text{CCOCH}_2\text{CH}_3$), 59.9 (t, CH_2CH_3), 29.2 (q, COCH_3), 23.5 (q, $\text{CH}_3\text{C}=\text{C}$) and 15.3 (q, CH_3CH_2).

APCI-MS: m/z (%) 197 (M^+ , 35), 179 (50), 151 (100) and 108 (85).

6.3 Synthesis of ethyl 2,6-dimethylpyridine-3-carboxylate (2.4a)



6.3.1 Method A:

A mixture of 2-amino-3-ethoxycarbonylhepta-2,4-diene-6-one (**2.3a**; 0.70 g, 3.55 mmol) and a solution of gold hydrochloride ($\text{HAuCl}_4 \cdot \text{H}_2\text{O}$) as (8900 ppm, 0.50 mL) as a catalyst in toluene-acetic acid mixture (15 mL; 5:1 by volume) was stirred at room temperature for 5 hours. The solution was neutralised with saturated aqueous NaHCO_3 solution and then extracted with ethyl acetate (3×25 mL). The combined organic layers were washed successively with water and brine, dried (MgSO_4) and evaporated *in vacuo*. Purification of the crude product was carried out by column chromatography

(silica gel; petroleum ether–ethyl acetate; 3:1 by volume) to give the *title compound* (**2.4a**; 0.61 g, 99%) as a light brown oil.

mp 61–63 °C (lit.² 60 °C) (Found: MH^+ , 180.1024. $C_{10}H_{14}NO_2$ [MH] requires 180.1021).

FT–IR (KBr)/ cm^{-1} ν_{max} : 1760 (C=O), 1640, 1320, 1280, 1191, 1105, 897 and 788.

1H NMR (400 MHz; DMSO- d_6): δ 8.05 (d, J = 8 Hz, 1 H, H-4), 7.22 (d, J = 8 Hz, 1 H, H-5), 4.22 (q, J = 7.1 Hz, 2 H, CH_2), 2.62 (s, 3 H, CH_3), 2.38 (s, 3 H, CH_3) and 1.25 (t, J = 7.1 Hz, 3 H, CH_2CH_3).

^{13}C NMR (100 MHz; DMSO- d_6): δ 166.1 (s, C=O), 161.9 (s, C-2), 159.5 (s, C-6), 136.5 (CH, C-4), 122.1 (s, C-3), 121.5 (d, C-5), 60.8 (t, CH_2), 25.1 (q, CH_3), 25.1 (q, CH_3) and 14.2 (q, CH_2CH_3).

APCI–MS: m/z 180 (MH^+ , 100).

6.3.2 Method B:

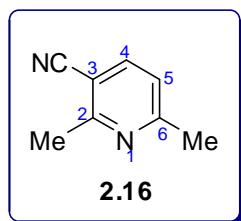
A mixture of ethyl β -aminocrotonate (**2.1a**; 0.10 mL, 0.81 mmol), 4-(trimethylsilyl)but-3-yn-2-one (**2.2a**; 0.25 mL, 1.7 mmol) and a solution of gold hydrochloride (8900 ppm, 0.10 mL) as a catalyst in toluene–acetic acid mixture (5 mL; 5:1 by volume) was irradiated at 120 °C (initial power 100 W) for 60 min in a sealed pressure-rated reaction tube (10 mL), using a CEM Discover Microwave Synthesizer. The solution was neutralised with saturated aqueous $NaHCO_3$ solution and then extracted with ethyl acetate (3×20 mL). The combined organic layers were washed successively with water and brine, dried ($MgSO_4$) and evaporated *in vacuo*. Purification of the crude product was carried out by column chromatography (silica gel; petroleum ether–ethyl acetate;

3:1 by volume) to give the *title compound* (**2.4a**; 0.137 g; 97%) as a light brown oil. The spectral data of the product were consistent with that produced from **Method A** (Section 6.3.1).

6.3.3 Method C:

A mixture of ethyl β -aminocrotonate (**2.1a**; 0.10 mL, 0.81 mmol), 4-(trimethylsilyl)but-3-yn-2-one (**2.2a**; 0.25 mL, 1.7 mmol) and a hydrochloride (0.10 mL) as a catalyst in toluene–acetic acid mixture (5 mL; 5:1 by volume) was irradiated at 120 °C (initial power 100 W) for 60 min in a sealed pressure-rated reaction tube (10 mL), using a CEM Discover Microwave Synthesizer. The solution was neutralised with saturated aqueous NaHCO₃ solution and then extracted with ethyl acetate (3 \times 20 mL). The combined organic layers were washed successively with water and brine, dried (MgSO₄) and evaporated *in vacuo*. Purification of the crude product was carried out by column chromatography (silica gel; petroleum ether–ethyl acetate; 3:1 by volume) to give the *title compound* (**2.4a**; 0.103 g; 72%) as a light brown oil. The spectral data of the product were consistent with that produced from **Method A** (Section 6.3.1).

6.4 Synthesis of 2,6-dimethyl-3-pyridinecarbonitrile (**2.16**)



A solution of ethyl 3-aminocrotononitrile (**2.15**; 0.07 g, 0.80 mmol) and 4-(trimethylsilyl)but-3-yn-2-one (**2.2a**; 0.25 mL, 1.7 mmol) in dry ethanol (2 mL) was irradiated at 150 °C (initial power 200 W) for 60 min in a sealed pressure-rated reaction

tube (10 mL), using a CEM Discover Microwave Synthesizer. After cooling in a flow of compressed air, the solvent removed under reduced pressure to give the crude product, which was purified by column chromatography (petroleum ether–ethyl acetate; 3:1 by volume) to give the *title compound* (**2.16**; 0.08 g, 79%) as a light brown solid.

mp 83–85 °C (lit.³ 82 °C) (Found: MH^+ , 132.0687. $\text{C}_8\text{H}_8\text{N}_2$ requires $[M]$ 132.0687).

FT–IR (KBr)/ cm^{-1} ν_{max} : 3261, 2190 ($\text{C}\equiv\text{N}$), 1600, 1560, 1430, 1398, 1267, 1120, 997 and 873.

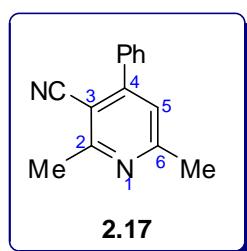
^1H NMR (400 MHz; $\text{DMSO}-d_6$): δ 8.05 (d, $J = 8$ Hz, 1 H, H-4), 7.22 (d, $J = 8$ Hz, 1 H, H-5), 3.32 (s, 3 H, CH_3) and 2.61 (s, 3 H, CH_3).

^{13}C NMR (100 MHz; $\text{DMSO}-d_6$): δ 162.6 (s, C-6), 160.2 (s, C-2), 141.1 (d, C-4), 121.3 (d, C-5), 117.5 (s, $\text{C}\equiv\text{N}$), 105.61 (s, C-3), 24.9 (q, CH_3) and 23.6 (q, CH_3).

EI–MS: m/z (%) 132 (M^+ , 100).

APCI–MS: m/z (%) 174 ($[\text{M} + \text{MeCNH}]^+$, 52) and 133 ($[\text{MH}]^+$, 100).

6.5 Synthesis of 3-cyano-2-methyl-6-phenylpyridine (**2.17**)



A solution of ethyl 3-aminocrotononitrile (**2.15**; 0.07 g, 0.80 mmol) and 4-phenyl-3-buten-2-one (**2.2c**; 0.12 mL, 0.80 mmol) in dry ethanol (3 mL) was irradiated at 150 °C (initial power 200 W) for 60 min in a sealed pressure-rated reaction tube (10 mL) using a CEM Discover Microwave Synthesizer. After cooling in a flow of compressed air, the

solvent was removed under reduced pressure to give the crude product. Purification by column chromatography (petroleum ether–ethyl acetate; 3:1 by volume) gave the *title compound* (**2.17**; 0.08 g, 75%) as a yellow solid.

mp 107–109 °C (lit.⁴ 109–110 °C) (Found: MH^+ , 207.1040. $\text{C}_{15}\text{H}_{13}\text{N}$ [MH] requires 207.1048).

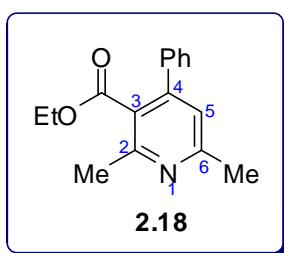
FT–IR (KBr)/ cm^{-1} ν_{max} : 2963, 2290, 1601, 1570, 1310, 1250, 1117, 1030, 962 and 803.

^1H NMR (400 MHz, CDCl_3): δ 7.48 (m, 2 H, H-2'/H-6'), 7.48 (m, 1 H, H-4'), 7.31 (m, 2 H, H-3'/H5'), 5.98 (s, 1 H, H-5), 2.50 (s, 3 H, CH_3) and 2.17 (s, 3 H, CH_3).

^{13}C NMR (100 MHz, CDCl_3): δ 159.5 (s, C-6), 159.1 (s, C-2), 150.5 (s, C-4), 139.2 (s, C-1'), 128.9 (d, C-4'), 128.8 (d, C-3'/C-5'), 128.2 (d, C-2'/C-6'), 126.1 (s, $\text{C}\equiv\text{N}$), 121.6 (d, C-5), 117.8 (s, C-3), 24.0 (q, CH_3) and 23.2 (q, CH_3).

EI–MS: m/z (%) 207 ($[\text{MH}]^+$, 100).

6.6 Synthesis of ethyl 2,6-dimethyl-4-phenylpyridine-3-carboxylate (**2.18**)



A solution of ethyl 3-aminocrotonate (**2.1a**; 0.07 g, 0.50 mmol), 4-phenyl-3-butyne-2-one (**2.2c**; 0.70 mL, 0.50 mmol) and a solution of gold hydrochloride (8900 ppm, 0.050 mL) as catalyst in toluene–acetic acid (3 mL; 5:1 by volume) was irradiated at 150 °C (initial power 200 W) for 60 min in a sealed pressure-rated reaction tube (10 mL), using a CEM Discover Microwave Synthesizer. After cooling in a flow of compressed air, the

solution was neutralised with saturated aqueous NaHCO₃ solution and then extracted with ethyl acetate (3 × 10 mL). The organic layers were combined, washed successively with water and brine, dried (MgSO₄) and evaporated *in vacuo*, to give the crude product. Purification by column chromatography (petroleum ether–ethyl acetate; 3:1 by volume) gave the *title compound* (**2.18**; 0.06 g, 49%) as a light brown oil.

mp 83–85 °C (Found: M⁺, 255.1259. C₁₆H₁₇NO₂ [M] requires 255.1261).

FT–IR (KBr)/cm⁻¹: ν_{max} 3120 (CH), 2980, 1730 (C=O), 1672 (C=O), 1387, 1290, 1178, 1110, 976, 886 and 723.

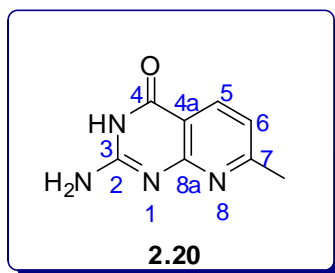
¹H NMR (400 MHz, CDCl₃): δ 7.33 (s, 5 H, Ph), 6.95 (s, 1 H, H-5), 4.02 (q, *J* = 7.2 Hz, 2 CH₂), 2.55 (s, 3 H, CH₃), 2.52 (s, 3 H, CH₃) and 0.91 (t, *J* = 7.2 Hz, CH₂CH₃).

¹³C NMR (100 MHz, CDCl₃): δ 169.5 (s, C=O), 159.9 (s, C-2), 155.8 (s, C-6), 149.1 (s, C-4), 139.3 (s, C-1'), 128.9 (d, C-4'), 128.0 (d, C-3'/C-5'), 126.0 (s, C-3), 121.7 (d, C-2'/C-6'), 61.6 (t, CH₂), 25.1 (CH₃), 22.7 (CH₃) and 14.3 (CH₂CH₃).

EI–MS: *m/z* (%) 255 (M⁺, 73), 237 (12), 226 (14), 210 ([M – EtO]⁺, 100), 184 (39), 167 (20), 146 (15) and 129 (22).

APCI–MS: *m/z* (%) 256 ([MH]⁺, 100).

6.7 Synthesis of 2-amino-7-methylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (2.20)



6.7.1 Method A:

A solution of 2,4-diamino-6-hydroxypyridine (**2.19**; 0.214 g, 1.70 mmol) and 3-butyne-2-one (**2.2d**; 0.133 mL, 1.70 mmol) in acetic acid (4 mL) was irradiated at 120 °C for 30 min in a pressure-rated glass tube (10 mL) using a CEM Discover microwave synthesizer by moderating the initial power (150 W). After cooling in a flow of compressed air the mixture was washed with water, filtered and dried *in vacuo* to give the *title compound* (**2.20**; 0.263 g, 90%) as a colourless solid, mp > 300 °C (lit.⁵ > 330 °C) (Found: MH⁺, 177.0773 C₈H₉N₄O requires MH⁺ 177.0776).

FT-IR (KBr)/cm⁻¹ ν_{\max} : 3248 (NH₂ and NH), 1666 (C=O) and 1581 (C=C).

¹H NMR (500 MHz; TFA-*d*): δ 9.05 (d, *J* = 8.1 Hz, 1 H, H-5), 7.62 (d, *J* = 8.1 Hz, 1 H, H-6) and 3.04 (s, 3 H, CH₃).

¹³C NMR (125 MHz; TFA-*d*): δ 161.6 (s, C-4), 156.4 (d, C-5), 154.6 (s, C-2), 147.3 (s, C-8a), 127.0 (d, C-5), 121.8 (d, C-6), 113.4 (s, C-4a) and 20.6 (q, CH₃).

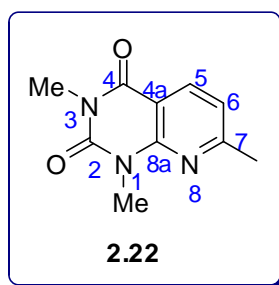
APCI-MS: *m/z* 177 (MH⁺, 91%).

6.7.2 Method B:

A solution of 2,4-diamino-6-hydroxypyridine (**2.19**; 0.214 g, 1.70 mmol), 3-butyne-2-one (**2.2d**; 0.133 mL, 1.70 mmol) and a solution of gold hydrochloride (8900 ppm, 0.10 mL) as catalyst in acetic acid (4 mL) was irradiated at 120 °C for 30 min in a pressure-

rated glass tube (10 mL) using a CEM Discover microwave synthesizer by moderating the initial power (150 W). After cooling in a flow of compressed air the mixture was washed with water, filtered and dried *in vacuo* to give the *title compound* (**2.20**; 0.227 g, 76%) as a light grey solid.

6.8 Synthesis of 1,3,7-trimethylpyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (**2.22**)



A solution of 6-amino-1,3-dimethyluracil (**2.21**; 0.263 g, 1.70 mmol) and 3-butyn-2-one (**2.2d**; 0.133 mL, 1.70 mmol) in acetic acid (4 mL) was irradiated at 120 °C for 30 min in a pressure-rated glass tube (10 mL) using a CEM Discover microwave synthesizer by moderating the initial power (150 W). After cooling in a flow of compressed air the mixture was neutralised with saturated aqueous NaHCO₃ solution. The aqueous layer was extracted with EtOAc (3 × 35 mL) and the organic extracts were combined, washed successively with water and brine, dried (MgSO₄) and evaporated *in vacuo* to give the *title compound* (**2.22**; 0.321 g, 99%) as a creamy solid (Found: [M – Me]⁺, 190.0614. C₉H₈N₃O₂ requires [M – Me] 190.0617).

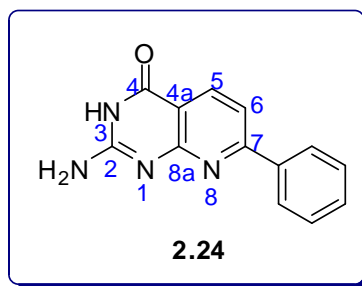
FT-IR (KBr)/cm⁻¹ ν_{max} : 1660 (C=O), 1577 (C=C) and 1500.

¹H NMR (500 MHz; CDCl₃): δ 8.32 (d, *J* = 8.1 Hz, 1 H, H-5), 7.03 (d, *J* = 8.1 Hz, 1 H, H-6), 3.75 (s, 3 H, CH₃), 3.45 (s, 3 H, CH₃) and 2.82 (s, 3 H, CH₃).

^{13}C NMR (125 MHz; CDCl_3): δ 162.1 (s, C-2 and C-4), 159.2 (s, C-8a), 149.5 (s, C-7), 138.3 (d, C-5), 119.5 (d, C-6), 107.5 (s, C-4a), 30.1 (q, CH_3), 29.1 (q, CH_3) and 26.3 (q, CH_3).

EI-MS: m/z (%) 191 ($[\text{MH} - \text{Me}]^+$, 13), 190 ($[\text{M} - \text{Me}]^+$, 100), 164 (33), 163 (46), 110 (7) and 85 (73).

6.9 Synthesis of 2-amino-7-phenylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (2.24)



A solution of 2,4-diamino-6-hydroxypyridine (**2.19**) (0.097 g, 0.77 mmol) and 1-phenyl-2-propyn-1-one (**2.23**) (0.10 g, 0.77 mmol) in acetic acid (4 mL) was irradiated at 120 °C for 30 min in a pressure-rated glass tube (10 mL) using a CEM Discover microwave synthesizer by moderating the initial power (150 W). After cooling in a flow of compressed air, water was added to the mixture and the precipitate was filtered, washed with saturated aqueous NaHCO_3 solution and dried *in vacuo* to give the *title compound* (**2.24**; 0.176 g, 96%) as a yellow solid, mp > 300 °C (Found: M^+ , 238.0852. $\text{C}_{13}\text{H}_{10}\text{N}_4\text{O}$ requires $[\text{M}]$ 238.0855).

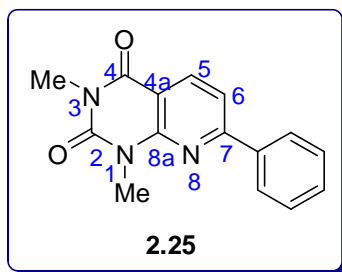
FT-IR (KBr)/ cm^{-1} ν_{max} : 3248 (NH_2 and NH), 1666 ($\text{C}=\text{O}$) and 1581 ($\text{C}=\text{C}$).

^1H NMR (500 MHz; $\text{TFA}-d$): δ 9.16 (d, $J = 8.3$ Hz, 1 H, H-6), 8.07–8.00 (m, 3 H, H-5 and H-2/H-6 of Ph), 7.89 (t, $J = 7.8$ Hz, 1 H, H-4 of Ph) and 7.82 (t, $J = 7.5$ Hz, 2 H, H-3/H-5 of Ph).

^{13}C NMR (125 MHz; TFA-*d*): δ 160.5 (s, C-4), 159.3 (s, C-7), 155.0 (s, C-2), 145.8 (s, C-8a), 134.5 (s, C-1 of Ph), 130.0 (d, C-5), 127.9 (d, C-6), 117.8 (d, C-4 of Ph), 115.3 (d, C-3/C-5 of Ph), 113.2 (d, C-2/C-6 of Ph) and 110.8 (s, C-4a).

EI-MS: m/z (%) 239 ($[\text{MH}]^+$, 12), 238 (M^+ , 100), 212 (22), 170 (33) and 77 (65).

6.10 Synthesis of 1,3-dimethyl-7-phenylpyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (**2.25**)



A solution of 6-amino-1,3-dimethyluracil (**2.21**; 0.120 g, 0.77 mmol) and 1-phenyl-2-propyn-1-one (**2.23**; 0.10 g, 0.77 mmol) in acetic acid (4 mL) was irradiated at 120 °C for 30 min in a pressure-rated glass tube (10 mL) using a CEM Discover microwave synthesizer by moderating the initial power (150 W). After cooling in a flow of compressed air, water was added to the mixture and the precipitate was filtered, washed with saturated aqueous NaHCO_3 solution and dried *in vacuo* to give the *title compound* (**2.25**; 0.199 g, 97%) as a colourless solid, mp > 300 °C (Found: $[\text{M} - \text{Me}]^+$, 267.1008. $\text{C}_{15}\text{H}_{13}\text{N}_3\text{O}_2$ requires $[\text{M} - \text{Me}]$ 267.1008).

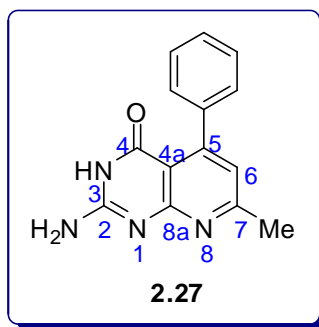
FT-IR (KBr)/ cm^{-1} ν_{max} : 1668 (C=O), 1592 (C=C) and 1524.

^1H NMR (500 MHz; DMSO-*d*₆): δ 8.40 (d, J = 8.1 Hz, 1 H, H-5), 8.21 (d, J = 7.5 Hz, 2 H, H-2/H-6 of Ph), 7.91 (d, J = 8.1 Hz, 1 H, H-6) and 7.55 (m, 3 H, H-3/H-4/H-5 of Ph).

^{13}C NMR (125 MHz; DMSO- d_6): δ 160.2 (s, C-4), 150.9 (s, C-8a), 150.6 (s, C-2), 145.7 (s, C-7), 138.3 (d, C-5), 137.8 (s, C-1 of Ph), 131.0 (d, C-4 of Ph), 129.8 (d, C-3/C-5 of Ph), 128.0 (d, C-2/C-6 of Ph), 113.7 (d, C-6), 109.5 (s, C-4a), 29.5 (q, CH_3) and 28.2 (q, CH_3).

EI-MS: m/z (%) 268 ($[\text{MH} - \text{Me}]^+$, 14), 267 ($[\text{M} - \text{Me}]^+$, 100), 226 (39), 165 (52) and 77 (80).

6.11 Synthesis of 2-amino-5-methyl-7-phenylpyrido[2,3-*d*]pyrimidin-4(3*H*)-one (2.27)



A solution of 2,4-diamino-6-hydroxypyridine (**2.19**; 0.088 g, 0.70 mmol) and 4-phenyl-1-butyn-2-one (**2.26**; 0.10 mL, 0.70 mmol) in acetic acid (4 mL) was irradiated at 120 °C for 30 min in a pressure-rated glass tube (10 mL) using a CEM Discover microwave synthesizer by moderating the initial power (150 W). After cooling in a flow of compressed air, water was added to the mixture and the precipitate was filtered, washed with saturated aqueous NaHCO_3 solution and dried *in vacuo* to give the *title compound* (**2.27**; 0.168 g, 95%) as a brown solid, mp > 300 °C (Found: M^+ , 252.2713. $\text{C}_{14}\text{H}_{12}\text{N}_4\text{O}$ requires $[\text{M}]$ 252.2713).

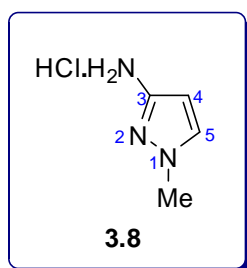
FT-IR (KBr)/ cm^{-1} ν_{max} : 3233 (NH_2 and NH), 1669 ($\text{C}=\text{O}$), 1590 ($\text{C}=\text{C}$) and 1514.

^1H NMR (500 MHz; TFA-*d*): δ 7.70 (t, $J = 7.5$ Hz, 1 H, H-4 of Ph), 7.62 (t, $J = 7.5$ Hz, 2 H, H-3/H-5 of Ph), 7.51 (d, $J = 7.5$ Hz, 2 H, H-2/H-6 of Ph), 7.44 (s, 1 H, H-6) and 2.98 (s, 3 H, CH_3).

^{13}C NMR (125 MHz; TFA-*d*): δ 157.9 (s, C-4), 152.0 (s, C-8a), 149.1 (s, C-7), 148.2 (s, C-2), 128.2 (s, C-5), 127.3 (s, C-1 of Ph), 117.8 (d, C-4 of Ph), 115.6 (d, C-3/C-5 of Ph), 113.2 (d, C-2/C-6 of Ph), 111.0 (d, C-6), 110.1 (s, C-4a) and 18.6 (q, CH_3).

EI-MS: m/z (%) 253 ($[\text{MH}]^+$, 13), 252 (M^+ , 100), 238 (7), 190 (28), 155 (43) and 77 (78).

6.12 Synthesis of 3-amino-1-methyl-1H-pyrazole hydrochloride (3.8)



A solution of 2-chloroacrylonitrile (**3.11**; 0.10 mL, 1.25 mmol) and methylhydrazine hydrochloride (**3.12**; 0.14 mL, 2.5 mmol) in EtOH (2 mL) was irradiated at 120 °C for 2 min in a pressure-rated glass tube (10 mL) using a CEM Discover microwave synthesizer by moderating the initial power (100 W). After cooling in a flow of compressed air, the mixture was kept in refrigerator for overnight. The solid obtained was filtered and washed with ethyl acetate to give the *title compound* (**3.8**; 0.16 g, 95%) as a colourless solid.

mp 163–165 °C (lit.⁶ 161–163 °C) (Found: $[\text{M} - \text{HCl}]^+$, 97.0640. $\text{C}_4\text{H}_7\text{N}_3$ [$\text{M} - \text{HCl}$] requires 97.0637).

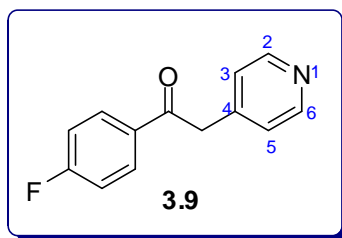
FT-IR (KBr)/cm⁻¹ ν_{\max} : 3376 (NH), 3018 (NH), 2951 (CH), 1570, 1515, 1496, 1458, 1386, 1226 and 1119.

¹H NMR (400 MHz; DMSO-*d*₆): δ 9.30 (s, exch. D₂O, 3 H, NH₂.HCl), 7.68 (d, *J* = 2.3 Hz, 1 H, H-4), 6.02 (d, *J* = 2.3 Hz, 1 H, H-5) and 3.77 (s, 3 H, CH₃).

¹³C NMR (100 MHz; DMSO-*d*₆): δ 143.1 (d, C-3), 134.8 (d, C-5), 96.2 (s, C-4) and 37.5 (q, CH₃).

EI-MS: *m/z* (%) 98 ([M - Cl]⁺, 5), 97 ([M - HCl]⁺, 100) and 96 ([M - HCl - H]⁺, 55).

6.13 Synthesis of 1-(4-fluorophenyl)-2-(4-pyridyl)ethanone (3.9)



6.13.1 Method A:

A solution of 4-picoline (**3.13**; 2.21 mL, 22.73 mmol) and ethyl 4-fluorobenzoate (**3.14**; 3.33 mL, 22.71 mmol) in THF (17 mL) was stirred at 0 °C for 15 min. Lithium *bis*-trimethylsilyl amide (LHMDS; 20 mL) was added dropwise with stirring over an hour at room temperature. The mixture was triturated with light petroleum (20 mL) and the solid obtained was dissolved in HCl (3 M). The solution was neutralised with saturated aqueous NaHCO₃ solution and then extracted with ethyl acetate (3 × 15 mL). The organic layers were combined, washed successively with water and brine, dried (MgSO₄) and evaporated *in vacuo* to give the *title compound* (**3.9**; 1.96 g, 40%) as a yellow solid.

mp 82–84 °C (lit.⁶ 79–81 °C) (Found: 215.0740. C₁₃H₁₀FNO [*M*] requires 215.0746).

FT–IR (KBr)/cm⁻¹ ν_{\max} : 3390, 3015 (CH), 2897, 1670 (C=O), 1610, 1465 and 1305.

¹H NMR (400 MHz; DMSO-*d*₆): δ 8.50 (m, 2 H, H-2 and H-6), 8.13 (m, 2 H, H-2'/H-6'), 7.39 (m, 2 H, H-3'/H-5'), 7.28 (m, 2 H, H-3 and H-5) and 4.49 (s, 2 H, CH₂).

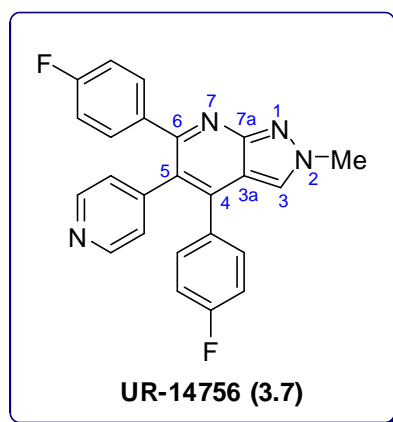
¹³C NMR (100 MHz; DMSO-*d*₆): δ 191.3 (s, C=O), 166.9 (s, C-4'), 147.8 (d, C-2/C-6), 147.7 (s, C-4), 133.1 (s, C-1'), 131.5 (d, C-2'/C-6'), 128.3 (d, C-3/C-5), 119.2 (d, C-3'/C-5') and 43.5 (t, CH₂C=O).

EI–MS: *m/z* (%) 215 (M⁺, 34), 123 ([M – C₆H₅F]⁺, 100), 95 ([C₆H₄F]⁺, 95) and 75 (47).

ES⁺–MS: *m/z* (%) 216 ([MH]⁺, 100), 216 (100) and 217 (20).

6.13.2 Method B:

A solution of 4-picoline (**3.13**; 0.22 mL, 2.27 mmol) and ethyl 4-fluorobenzoate (**3.14**; 0.33 mL, 2.27 mmol) in THF (2.5 mL) was stirred at 0 °C for 15 min. Lithium *bis*-trimethylsilyl amide (LHMDS; 2.2 mL) was added dropwise with stirring for 5 minutes, then irradiated for 10 minutes at 80 °C in a sealed pressure-rated reaction tube (10 mL), using a CEM Discover Microwave Synthesizer. The mixture was triturated with light petroleum (2.5 mL) and the solid obtained was dissolved in HCl (3 M). The solution was neutralised with saturated aqueous NaHCO₃ solution and then extracted with ethyl acetate (3 × 15 mL). The organic layers were combined, washed successively with water and brine, dried (MgSO₄) and evaporated *in vacuo* to give the *title compound* (**3.9**; 0.235 g, 50%). as a beige solid.



A solution of 1-(4-fluorophenyl)-2-(4-pyridyl)ethanone (**3.9**; 0.21 g, 0.97 mmol), 3-amino-1-methyl-1*H*-pyrazole hydrochloride (**3.8**; 0.13 g, 0.97 mmol), 4-fluorobenzaldehyde (**3.10**; 0.10 mL, 0.97 mmol) and hydrochloric acid (0.40 mL, 10 M) in ethanol (4 mL) was irradiated at 150 °C for 4 hours in a pressure-rated glass tube (10 mL) using a CEM Discover microwave synthesizer by moderating the initial power (150 W). After cooling in a flow of compressed air the solution was neutralised with saturated aqueous NaHCO₃ solution and then extracted with ethyl acetate (3 × 20 mL). The organic layers were combined, washed successively with water and brine, dried (MgSO₄) and evaporated *in vacuo*. Purification of the crude product was carried out by column chromatography (silica gel; petroleum ether–ethyl acetate; 1:1 by volume) to give the *title compound* (**3.7**; 0.28 g, 71%) as a colourless solid.

mp 93–95 °C (lit.⁶ 92–94 °C) (Found: MH⁺, 399.1429. C₂₄H₁₇N₄F₂ [MH] requires 399.1421).

FT-IR (KBr)/cm⁻¹ v_{max}: 3557, 1610, 1530, 1402, 1350, 1290, 1245, 1168, 1112 and 1078.

^1H NMR (400 MHz; $\text{DMSO}-d_6$): δ 8.34 (d, $J = 6.0$ Hz, 2 H, H-2'/H-6'), 7.43 (s, 1 H, H-3), 7.42 (dd, $J = 8.8, 5.4$ Hz, 2 H, H-2''/H-6''), 7.31 (dd, $J = 8.8, 5.4$ Hz, 2 H, H-2'''/H-6'''), 7.12 (app t, $J = 8.8$ Hz, 2 H, H-3''/H-5'), 6.97 (app t, $J = 8.8$ Hz, 2 H, H-3'''/H-5'''), 6.95 (d, $J = 6.0$ Hz, 2 H, H-3'/H-5') and 3.78 (s, 3 H, CH_3).

^{13}C NMR (100 MHz; $\text{DMSO}-d_6$): δ 163.2 (s [d, $J = 250$ Hz], C-4'), 162.3 (s [d, $J = 250$ Hz], C-4''), 160.1 (s, C-7a), 158.8 (s, C-4), 152.2 (d, C-2'/C-6'), 149.9 (s, C-6), 143.5 (s, C-1'), 135.6 (s [d, $J = 3.3$ Hz], C-1''), 131.7 (d [d, $J = 3.4$ Hz], C-1'''), 130.2 (d [d, $J = 8.3$ Hz], C-2''/C-6''), 128.9 (d [d, $J = 8.3$ Hz], C-2'''/C-6'''), 126.7 (d, C-5), 125.9 (s, C-3), 123.9 (d, C-3'/C-5'), 116.1 (d [d, $J = 21.7$ Hz], C-3''/C-5''), 113.8 (d [d, $J = 21.6$ Hz], C-3'''/C-5'''), 101.9 (s, C-3a) and 41.1 (q, CH_3).

$\text{ES}^+ - \text{MS}$: m/z (%) 399 ($[\text{MH}]^+$, 100) 399 (100) and 400 (11).

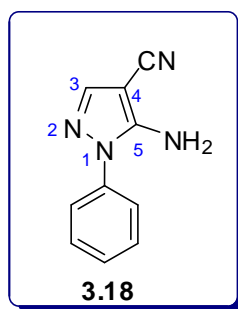
$\text{EI}^+ - \text{MS}$: m/z (%) 398 (M^+ , 12) 397 ($[\text{M} - 1]^+$, 25), 347 (78), 304 (23), 275 (12), 230 (15), 109 (100) and 95 (26).

6.14.2 Method B:

A solution of 1-(4-fluorophenyl)-2-(4-pyridyl)ethanone (0.42 g, 1.95 mmol), 3-amino-1-methyl-1*H*-pyrazole hydrochloride (0.26 g, 1.95 mmol), 4-fluorobenzaldehyde (0.21 mL, 1.95 mmol) and hydrochloride acid (0.12 mL, 10 M) in methyl glycol (6 mL) was heated under reflux for 48 hours. The solution was neutralised with saturated aqueous NaHCO_3 solution and then extracted with ethyl acetate (3×20 mL). The organic layers were combined, washed successively with water and brine, dried (MgSO_4) and the solvent was evaporated *in vacuo* to give the crude product. Purification by column chromatography (silica gel; petroleum ether–ethyl acetate; 1:1 by volume) gave the *title compound* (0.61 g, 79%) as a colourless solid. The NMR spectral data of the product

obtained *via* this method was identical in all respects with data produced from using **Method A** (Section 6.14.1).

6.15 Synthesis of 5-amino-4-cyano-1-phenyl-1H-pyrazole (3.18)



6.15.1 Method A:

A mixture of phenylhydrazine (**3.16a**; 0.45 mL, 4.2 mmol) and ethoxymethylenemalononitrile (**3.17**; 0.51 g, 4.2 mmol) in ethanol (4 mL) was irradiated at 120 °C for 45 min in a pressure-rated glass tube (10 mL) using a CEM Discover microwave synthesizer by moderating the initial power (100 W). After cooling in a flow of compressed air, the solvent was removed under reduced pressure to give the crude product. Purification by column chromatography (petroleum ether–ethyl acetate; 5:1 by volume) gave the *title compound* (**3.18**; 0.74 g, 95%) as light brown solid.

mp 137–139 °C (lit.⁷ 140 °C) (Found: MH^+ , 185.0827. $C_{10}H_9N_4$ requires $[MH]$: 185.0822).

FT–IR (KBr)/ cm^{-1} ν_{max} : 3302 (NH), 3240 (NH), 2230 ($C\equiv N$), 1577, 1530 and 1368.

1H NMR (400 MHz; $CDCl_3$): δ 7.58 (s, 1 H, H-3), 7.49–7.37 (m, 5 H, Ph) and 4.52 (s, exch. D_2O , 2 H, NH_2).

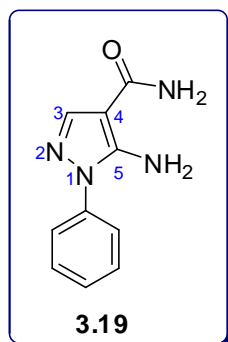
^{13}C NMR (100 MHz, CDCl_3): δ 141.9 (d, C-3), 135 (s, C-5), 129.3 (s, C-1'), 125.5 (d, C-3'/C-5'), 124.1 (d, C-4'), 121.3 (d, C-2'/C-6'), 115.6 (s, $\text{C}\equiv\text{N}$) and 86.2 (s, C-4).

APCI-MS: m/z (%) 226 ($[\text{M} + \text{MeCNH}]^+$, 100) and 185 ($[\text{MH}]^+$, 64).

6.15.2 Method B:

A solution of phenylhydrazine (**3.16a**; 0.45 mL, 4.2 mmol) and ethoxymethylenemalononitrile (**3.17**; 0.514 g, 4.2 mmol) in a toluene–acetic acid mixture (5:1 by volume; 4 mL) was irradiated at 120 °C for 60 min in a pressure-rated glass tube (10 mL) using a CEM Discover microwave synthesizer by moderating the initial power (100 W). After cooling in a flow of compressed air, the reaction mixture was neutralised with saturated aqueous NaHCO_3 solution and then extracted with ethyl acetate (3×15 mL). The organic layers were combined, washed successively with water and brine, dried (MgSO_4) and evaporated *in vacuo*. Purification of the crude product by crystallization from hexane–ethyl acetate (9:1 by volume) gave the *title compound* (0.42 g, 54%) as a light brown solid. The physical properties and the NMR spectral data of the product obtained was consistent in all respects with data produced from using **Method A** (Section 6.15.1).

6.16 Synthesis of 5-amino-4-aminocarbonyl-1-phenyl-1H-pyrazole (3.19)



A mixture of 5-amino-4-cyano-1-phenyl-1H-pyrazole (**3.18**; 0.47 g, 2.55 mmol) and H_2SO_4 (4 mL) was irradiated for 60 min at 80 °C in a sealed pressure-rated glass tube (10 mL) using a CEM Discover microwave synthesizer by moderating the initial power (150 W). After cooling in a flow of compressed air, the mixture was extracted with chloroform (2×20 mL), washed with brine, dried over MgSO_4 and evaporated in *vacuo* to give the *title compound* (**3.19**; 0.43 g, 85%) as a colourless solid

mp 170–172 °C (lit.⁷ 172–173 °C) (Found: 203.0931. $\text{C}_{10}\text{H}_{11}\text{N}_4\text{O}$ [MH] requires 203.0927).

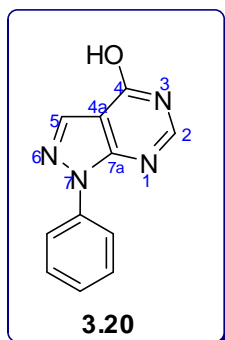
FT-IR (KBr)/ cm^{-1} ν_{max} : 3420 (NH), 3230 (NH), 1680 (C=O), 1611, 1505 and 1430.

^1H NMR (400 MHz, CDCl_3): δ 7.53 (s, 1 H, H-3), 7.49–7.37 (m, 5 H, Ph), 5.48 (br s, exch. D_2O , 2 H, NH_2) and 4.23 (br, s, exch. D_2O , 2 H, NH_2).

^{13}C NMR (100 MHz, CDCl_3): δ 168.0 (s, C=O), 141.9 (s, C-4), 135.5 (s, C-5), 129.3 (s, C-1'), 125.5 (d, C-3), 124.0 (d, C-3'/C-5'), 122.9 (d, C-4') and 121.3 (d, C-2'/C-6').

ES⁺-MS: m/z (%) 244 ($[\text{M} + \text{MeCNH}]^+$, 7), 203 ($[\text{MH}]^+$, 100) and 186 (21).

6.17 Synthesis of 4-hydroxy-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (3.20)



A mixture of 5-amino-4-aminocarbonyl-1-phenyl-1H-pyrazole (**3.19**; 0.15 g, 0.74 mmol) in formamide (0.50 mL, 5.55 mmol) was irradiated at 150 °C for 45 min in a pressure-rated glass tube (10 mL) using a CEM Discover microwave synthesizer by moderating the initial power (150 W). After cooling in a flow of compressed air the mixture was diluted with water and allowed to stand in a refrigerator overnight. The solid obtained was filtered, washed with water and dried to give the *title compound* (**3.20**; 0.152 g, 97%) as a colourless solid.

mp 296–298 °C (lit.⁷ 299 °C) (Found: MH^+ , 213.0678. $C_{11}H_9N_4O$ requires $[MH]$: 213.0771).

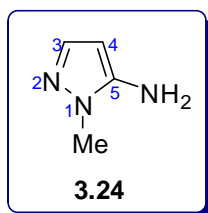
FT-IR (KBr)/ cm^{-1} ν_{max} : 3223 (OH), 2850 (CH), 1560, 1510 and 1170.

1H NMR (400 MHz, $CDCl_3$): δ 9.71 (s, exch. D_2O , 1 H, OH), 8.23 (s, 1 H, H-5), 7.96 (d, $J = 8.0$ Hz, 2 H, H-2'/H-6'), 7.94 (s, 1 H, H-2), 7.47 (t, $J = 8.0$ Hz, 2 H, H-3'/H-5') and 7.19 (t, $J = 8.0$ Hz, 1 H, H-4').

^{13}C NMR (100 MHz, $CDCl_3$): δ 158.0 (s, C-4), 150.1 (d, C-2), 148.3 (s, C-7a), 137.5 (s, C-1'), 130.9 (d, C-5), 127.6 (d, C-3'/C-5'), 124.5 (d, C-4'), 118.9 (d, C-2'/C-6') and 103.9 (s, C-4a).

ES⁺–MS: m/z (%) 212 ([M]⁺, 100), 184 ([M – N₂]⁺, 69), 157 (75), 142 (20) 130 (32), 103 (22), 91 (51) and 77 ([Ph]⁺, 83).

6.18 Synthesis of 5-amino-1-methyl-1H-pyrazole (3.24)



A solution of methylhydrazine (**3.26**; 0.25 mL, 2.3 mmol) and 3-methoxyacrylonitrile (**3.25**; 0.20 mL, 4.6 mmol) in a toluene–acetic acid mixture (4:1 by volume; 5 mL) was irradiated at 120 °C (initial power 100 W) for 60 min in a sealed pressure-rated reaction tube (10 mL), using a CEM Discover microwave synthesizer. The solution was neutralised with saturated aqueous NaHCO₃ solution and then extracted with ethyl acetate (3 × 20 mL). The organic layers were combined, washed successively with water and brine, dried (MgSO₄) and evaporated *in vacuo* to give the *title compound* (**3.24**; 0.22 g, 97%) as a colourless solid.

mp 71–73 °C (lit.⁸ 70 °C) (Found: M⁺, 97.0640. C₄H₇N₃ [M] requires 97.0611).

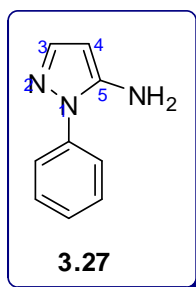
FT–IR (KBr)/cm^{–1} ν_{\max} : 3340 (NH), 3120 (NH), 2965 (CH), 1575, 1560, 1490, 1450, 1371, and 1099.

¹H NMR (400 MHz; DMSO–*d*₆): δ 7.60 (d, J = 2 Hz, 1 H, H-3), 5.15 (d, J = 2 Hz, 1 H, H-4) 3.85 (br s, exch. D₂O, 2 H, NH₂) and 3.50 (s, 3 H, CH₃).

¹³C NMR (125 MHz; DMSO–*d*₆): δ 145.6 (s, C-5), 138.5 (d, C-3), 90.8 (d, C-4) and 34.0 (q, CH₃).

APCI-MS: m/z (%) 97 (M^{++} , 100).

6.19 Synthesis of 5-amino-1-phenyl-1H-pyrazole (3.27)



A solution of phenylhydrazine (**3.16a**; 0.12 mL, 1.15 mmol) and 3-methoxyacrylonitrile (**3.26**; 0.10 mL, 1.15 mmol) in a toluene–acetic acid mixture (1:1 by volume; 5 mL) was irradiated at 120 °C (initial power 100 W) for 60 min in a sealed pressure-rated reaction tube (10 mL), using a CEM Discover microwave synthesizer. The solution was neutralised with saturated aqueous NaHCO_3 solution and then extracted with ethyl acetate (3×20 mL). The organic layers were combined, washed successively with water and brine, dried (MgSO_4) and evaporated *in vacuo*. Purification of the crude product by crystallization from a mixture of hexane–ethyl acetate (3:1 by volume) gave the *title compound* (**3.27**; 0.17 g, 92%) as pale yellow crystals.

mp 48–50 °C (lit.⁸ 50 °C) (Found: 159.0796. $\text{C}_9\text{H}_9\text{N}_3$ requires $[M]$ 159.0780).

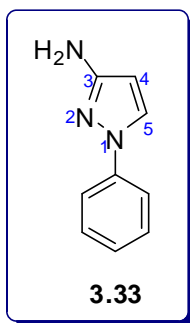
FT-IR (KBr)/ cm^{-1} ν_{max} : 3420 (NH), 3240 (NH), 1540 (C=N), 1510, 1415 and 1137.

^1H NMR (400 MHz; $\text{DMSO}-d_6$): δ 9.60 (br s, exch. D_2O , 2 H, NH_2), 7.30–6.95 (m, 6 H, H-3 and Ph) and 6.18 (d, $J = 1.8$ Hz, 1 H, H-4).

^{13}C NMR (125 MHz; $\text{DMSO}-d_6$): δ 148.0 (s, C-5), 140.9 (d, C-3), 139.9 (s, C-1'), 129.5 (d, C-3'/C-5'), 126.5 (d, C-4'), 120.4 (d, C-2'/C-6') and 94.1 (d, C-4).

APCI–MS: m/z (%) 159 (M^{++} , 100).

6.20 Synthesis of 3-amino-1-phenyl-1H-pyrazole (3.33)



A solution of phenylhydrazine hydrochloride (**3.16b**; 0.17 g, 1.20 mmol), 3-methoxyacrylonitrile (**3.26**; 0.20 mL, 2.40 mmol) and NaOEt (0.41 g, 4.8 mmol) in EtOH (4 mL) was irradiated at 150 °C for 2 h in a sealed, pressure-rated glass tube (10 mL) using a CEM Discover microwave synthesizer by moderating the initial power (150 W). The mixture was cooled by passing a stream of compressed air through the microwave cavity and water was added (10 mL). The aqueous layer was extracted with ethyl acetate (2 × 20 mL) and the organic extracts were combined, washed with brine, dried over MgSO₄ and evaporated *in vacuo*. Purification by column chromatography [silica gel; hexane–ethyl acetate (1:3 by volume)] gave the *title compound* (**3.33**; 0.19 g, 85%) as a yellow solid.

mp 90–92 °C (lit.⁹ 91 °C) (Found: 159.0796. C₉H₉N₃ [M] requires 159.0796).

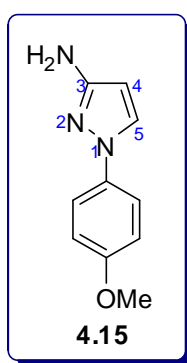
FT–IR (KBr)/cm^{–1} ν_{\max} : 3477 (NH), 3297 (NH), 1490, 1415, 1367 and 1172.

¹H NMR (400 MHz; DMSO–*d*₆): δ 8.13 (d, J = 2.4 Hz, 1 H, H-5), 7.64 (d, J = 7.7 Hz, 2 H, H-2'/H-6'), 7.39 (t, J = 7.7 Hz, 2 H, H-3'/H-5'), 7.11 (t, J = 7.7 Hz, 1 H, H-4'), 5.74 (d, J = 2.4 Hz, 1 H, H-4) and 5.09 (s, exch. D₂O, 2 H, NH₂).

^{13}C NMR (125 MHz; $\text{DMSO}-d_6$): δ 157.5 (s, C-3), 140.4 (s, C-1'), 129.7 (d, C-5), 128.2 (d, C-3'/C-5'), 124.3 (d, C-4'), 116.8 (d, C-2'/C-6') and 96.8 (d, C-4).

EI-MS: m/z (%) 160 ($[\text{MH}]^+$, 12), 159 (M^+ , 100), 158 ($[\text{M} - 1]^+$, 30), 131 ($[\text{M} - \text{N}_2]^+$, 13), 104 (16), 92 (8), 84 (27) and 77 ($[\text{Ph}]^+$, 29).

6.21 Synthesis of 3-amino-1-(4-methoxyphenyl)-1H-pyrazole (4.15)



A solution of 4-methoxyphenyl hydrazine hydrochloride (**4.6**; 0.210 g, 1.2 mmol), 3-methoxyacrylonitrile (0.20 mL, 2.43 mmol) and NaOEt (0.41 g, 4.8 mmol) in EtOH (3 mL) was irradiated at 150 °C for 2 h in a sealed pressure-rated glass tube (10 mL) using a CEM Discover microwave synthesizer by moderating the initial power (150 W). The mixture was cooled by passing a stream of compressed air through the microwave cavity and water was added (10 mL). The aqueous layer was extracted with EtOAc (2 × 20 mL) and the organic extracts were combined, washed with brine, dried over MgSO_4 and evaporated *in vacuo*. Purification by column chromatography (silica gel; hexane–EtOAc in 1:3 by volume) gave the *title compound* (**4.15**; 0.19 g, 85%) as a yellow solid. mp 146–149 °C (Found: M^+ , 189.0900. $\text{C}_{10}\text{H}_{11}\text{N}_3\text{O}$ [M] requires 189.0902).

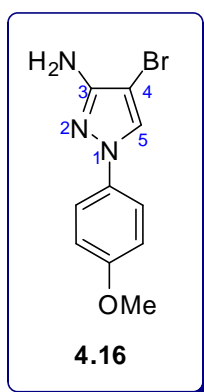
FT-IR (KBr)/ cm^{-1} : ν_{max} 3360 (NH), 1556, 1310, 1299 and 1069.

^1H NMR (400 MHz; $\text{DMSO}-d_6$): δ 8.00 (d, $J = 2.5$ Hz, 1 H, H-5), 7.55 (d, $J = 9.0$ Hz, 2 H, H-2'/H-6'), 6.95 (d, $J = 9.0$ Hz, 2 H, H-3'/H-5'), 5.67 (d, $J = 2.5$ Hz, 1 H, H-4), 4.97 (s, exch. D_2O , 2 H, NH_2) and 3.76 (s, 3 H, OCH_3).

^{13}C NMR (125 MHz; $\text{DMSO}-d_6$): δ 157.1 (s, C-4'), 156.5 (s, C-3), 134.5 (s, C-1'), 127.9 (d, C-5), 118.3 (d, C-3'/C-5'), 114.8 (d, C-2'/C-6'), 95.8 (d, C-4) and 55.8 (q, OCH_3).

EI-MS: m/z (%) 189 (M^+ , 100) and 174 (81).

6.22 Synthesis of 3-amino-4-bromo-1-(4-methoxyphenyl)-1H-pyrazole (4.16)



6.22.1 Method A:

A mixture of 3-amino-1-(4-methoxyphenyl)-1H-pyrazole (**3.15**; 0.13 g, 0.68 mmol) and NBS (0.12 g, 0.68 mmol) in THF (4 mL) was irradiated at 150 °C for 2 h in a sealed pressure-rated glass tube (10 mL) using a CEM Discover microwave synthesizer by moderating the initial power (150 W). The mixture was cooled by passing a stream of compressed air through the microwave cavity and evaporated *in vacuo*. The residue obtained was dissolved in EtOAc, filtered through Celite and the solvent was

evaporated *in vacuo*. Purification by column chromatography (silica gel; hexane–EtOAc in 3:1 by volume) gave the *title compound* (**3.16**; 0.14 g, 77%) as a brown solid.

mp 93–96 °C (Found: M^+ , 267.0015. $C_{10}H_{10}N_3O^{79}Br$ [M] requires 267.0007).

FT–IR (KBr)/ cm^{-1} ν_{max} 3353 (NH), 1548, 1516, 1403, 1245, 1084 and 1041.

1H NMR (400 MHz; DMSO– d_6): δ 8.34 (s, 1 H, H-5), 7.57 (d, $J = 9.0$ Hz, 2 H, H-2'/H-6'), 6.98 (d, $J = 9.0$ Hz, 2 H, H-3'/H-5'), 5.15 (s, exch D_2O , 2 H, NH_2) and 3.76 (s, 3 H, OCH_3).

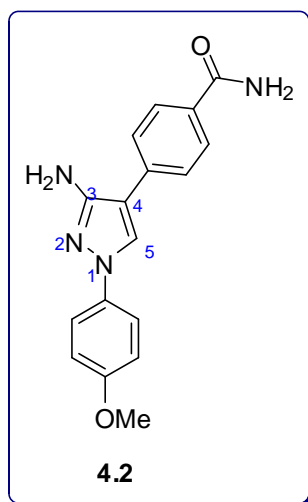
^{13}C NMR (125 MHz; DMSO– d_6): δ 157.0 (s, C-4'), 155.5 (s, C-3), 134.2 (s, C-1'), 127.8 (d, C-5), 118.5 (d, C-3'/C-5'), 114.9 (d, C-2'/C-6'), 95.3 (s, C-4) and 55.8 (q, OCH_3).

EI–MS: m/z (%) 269 ($^{81}BrM^{+}$, 97) and 267 ($^{79}BrM^{+}$, 100).

6.22.2 Method B:

A mixture of 3-amino-1-(4-methoxyphenyl)-1*H*-pyrazole (**3.15**; 0.07 g, 0.37 mmol) and NBS (0.06 g, 0.37 mmol) in THF (10 mL) was stirred at room temperature for 16 hours. The solvent was removed under reduced pressure and the residue obtained was dissolved in EtOAc, filtered through Celite and the solvent was evaporated *in vacuo*. Purification by column chromatography (silica gel; hexane–EtOAc in 3:1 by volume) gave the *title compound* (0.08 g, 82%) as a brown solid. The product was identical in all respects with data produced *via* **Method A** (Section 6.22.1).

6.23 Synthesis of 3-amino-4-(4-aminocarbonylphenyl)-1-(4-methoxyphenyl)-1H-pyrazole (4.2)



A mixture of 3-amino-4-bromo-1-(4-methoxyphenyl)-1H-pyrazole (**4.16**; 0.15 g, 0.55 mmol), 4-carbamoylphenylboronic acid (**4.26**; 0.090 g, 0.54 mmol), K₂CO₃ (0.20 g, 1.44 mmol) and PdCl₂(PPh₃)₂ (0.04 g, 0.05 mmol) in 2-PrOH–H₂O (5 mL; 1:1 by volume) was irradiated at 150 °C for 2 h in a sealed pressure-rated glass tube (10 mL) using a CEM Discover microwave synthesizer by moderating the initial power (150 W). The mixture was cooled by passing a stream of compressed air through the microwave cavity and water was added (10 mL). The aqueous layer was extracted with EtOAc (2 × 20 mL) and the organic extracts were combined, washed with brine, dried over MgSO₄ and evaporated *in vacuo*. Purification by column chromatography (silica gel; hexane–EtOAc in 1:3 by volume) gave the *title compound* (**4.2**; 0.09 g, 54%) as a cream solid.

mp 251–253 °C (Found: M⁺, 308.1281. C₁₇H₁₆N₄O₂ [*M*] requires 308.1273).

FT–IR (KBr)/cm^{–1} ν_{max} : 3389 (NH), 1653 (C=O), 1554, 1522, 1399 and 1106.

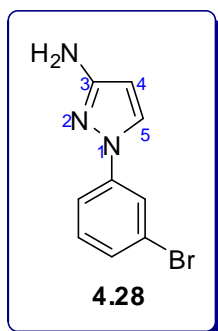
¹H NMR (400 MHz; DMSO–*d*₆): δ 8.59 (s, 1 H, H-5), 7.96 (br s, exch. D₂O, 1 H, *N*HH), 7.90 (d, *J* = 8.4 Hz, 2 H, H-2'/H-6'), 7.69 (d, *J* = 7.6 Hz, 2 H, H-3''/H-5''), 7.67

(d, $J = 7.6$ Hz, 2 H, H-2''/H-6''), 7.31 (br s, exch. D₂O, 1 H, NHH), 7.02 (d, $J = 8.4$ Hz, 2 H, H-3'/H-5'), 5.18 (s, exch. D₂O, 2 H, NH₂) and 3.51 (s, 3H, OCH₃).

¹³C NMR (125 MHz; DMSO-*d*₆): δ 168.2 (s, C=O), 158.3 (s, C-4'), 155.5 (s, C-3), 135.1 (s, C-1'), 133.6 (s, C-4''), 132.1 (s, C-1''), 128.9 (d, C-3''/C''-5), 126.5 (d, C-5), 125.5 (d, C-2''/C-6''), 119.5 (d, C-3'/C-5'), 116.7 (d, C-2'/C-6') and 56.0 (q, OCH₃).

EI-MS: m/z (%) 308 (M⁺, 100).

6.24 Synthesis of 3-amino-1-(3-bromophenyl)-1H-pyrazole (4.28)



6.24.1 Method A:

A solution of 3-bromophenyl hydrazine hydrochloride (**4.26**; 0.136 g, 0.60mmol), 3-methoxyacrylonitrile (0.10 mL, 1.20 mmol) and KO^tBu (0.30 g, 2.60 mmol) in *t*-BuOH (3 mL) was irradiated at 150 °C for 2 h in a sealed pressure-rated glass tube (10 mL) using a CEM Discover microwave synthesizer by moderating the initial power (150 W). The mixture was cooled by passing a stream of compressed air through the microwave cavity and water was added (10 mL). The aqueous layer was extracted with EtOAc (3 × 15 mL) and the organic extracts were combined, washed with brine, dried over MgSO₄ and evaporated *in vacuo*. Purification by column chromatography (silica gel; petroleum ether–EtOAc in 3:1 by volume) gave the *title compound* (**4.28**; 0.10 g, 68%) as a yellow solid.

mp 85–88 °C (Found: M^+ , 236.9902. $C_9H_8N_3^{79}Br$ [M] requires 236.9898).

FT-IR (KBr)/ cm^{-1} ν_{max} : 3351 (NH), 2921, 2348, 2205, 1627 and 1587.

1H NMR (400 MHz; DMSO- d_6): δ 8.21 (d, $J = 2.5$ Hz, 1 H, H-5), 7.86 (m, 1 H, H-2'), 7.65 (m, 1 H, H-4'), 7.33 (app t, $J = 8.0$ Hz, 1 H, H-5'), 7.28 (m, 1 H, H-6') 5.77 (d, $J = 2.5$ Hz, 1 H, H-4) and 5.17 (s, exch. D_2O , 2 H, NH_2).

^{13}C NMR (125 MHz; DMSO- d_6): δ 157.9 (s, C-3), 141.7 (s, C-1'), 131.7 (d, C-5'), 128.8 (d, C-4'), 126.6 (d, C-5), 122.7 (s, C-3'), 119.2 (d, C-6'), 115.4 (d, C-2') and 97.6 (d, C-4).

EI-MS: m/z (%) 239 ($[^{81}Br]M^+$, 96), 237 ($[^{79}Br]M^+$, 100), 211 ($[^{81}Br]M - N_2]^+$, 5), 209 ($[^{79}Br]M - N_2]^+$, 6), 170 (7), 155 (12), 131 (21), 109 (15), 93 (18), 84 (24) and 69 (45).

6.24.2 Method B:

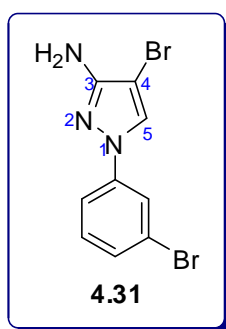
A solution of 3-bromophenylhydrazine hydrochloride (**4.26**; 0.136 g, 0.60 mmol), 3-methoxyacrylonitrile (0.10 mL, 1.20 mmol) and NaOEt (0.18 g, 2.60 mmol) in dry EtOH (3 mL) was irradiated at 150 °C for 1 hour in a sealed pressure-rated glass tube (10 mL) using a CEM Discover microwave synthesizer by moderating the initial power (150 W). The mixture was cooled by passing a stream of compressed air through the microwave cavity and water was added (3 mL). The pH of the mixture was adjusted to *ca.* 2–3 by the addition of HCl (6 M) and the mixture was stirred for 10 min at room temperature. The mixture was neutralised with saturated aqueous $NaHCO_3$ solution. The aqueous layer was extracted with EtOAc (3×15 mL) and the organic extracts were combined, washed successively with water and brine, dried ($MgSO_4$) and evaporated *in vacuo*. Purification by crystallization from a mixture of petroleum hexane–EtOAc (3:1 by volume) gave the *title compound* (**4.28**; 0.13 g, 89%) as a yellow solid. The spectral

data of the product were consistent in all respects with data obtained *via* **Method A** (Section 6.24.1).

6.19.3 Method C:

A solution of 3-bromophenyl hydrazine hydrochloride (**4.26**; 1.36 g, 6.00 mmol), 3-methoxyacrylonitrile (1.00 mL, 12.00 mmol) and NaOEt (1.77 g, 26.0 mmol) in dry EtOH (30 mL) was heated at reflux for 20 hours. After cooling the mixture to room temperature, water was added and the formed precipitate was filtered, washed with water and finally dissolved in CH₂Cl₂. The residual water was removed in a separating funnel and the organic extract was then washed successively with water and brine, dried (MgSO₄) and evaporated *in vacuo* to obtain the *title compound* (**4.28**; 1.29 g, 90%) as a yellow solid after drying under high vacuum. The spectral data of the product were consistent in all respects with data obtained *via* **Method A** (Section 6.24.1).

6.25 Synthesis of 3-amino-4-bromo-1-(3-bromophenyl)-1H-pyrazole (**4.31**)



A mixture of 3-amino-4-bromo-1-(3-bromophenyl)-1H-pyrazole (**4.28**; 0.16 g, 0.68 mmol) and NBS (0.120 g, 0.68 mmol) in THF (10 mL) was stirred at room temperature for 16 hours. The solvent was removed *in vacuo*. The crude residue was dissolved in EtOAc, filtered through Celite and evaporated *in vacuo*. Purification by column

chromatography on SiO₂, eluting with hexane–EtOAc (3:1 by volume), gave the *title compound* (**4.31**; 0.18 g, 83%) as a brown solid.

mp 93–95 °C (Found: [⁷⁹Br₂]M⁺, 314.9005. C₉H₇Br₂N₃ [{⁷⁹Br₂}M] requires 314.9007).

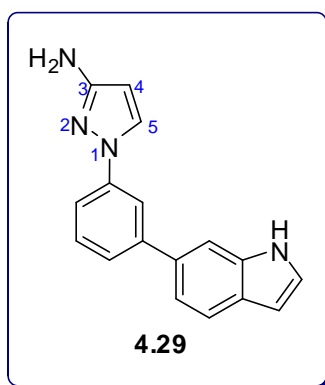
FT–IR (KBr)/cm^{–1} ν_{\max} : 3983 (NH), 3415 (C=N), 3312, 3213, 1629, 1594 and 1556.

¹H NMR (400 MHz; DMSO–*d*₆): δ 8.55 (s, 1 H, H-5), 7.88 (m, 1 H, H-2'), 7.67 (m, 1 H, H-5'), 7.37–7.35 (m, 2 H, H-4'/H-6') and 5.38 (s, exch. D₂O, 2 H, NH₂).

¹³C NMR (125 MHz; DMSO–*d*₆): δ 159.9 (s, C-3), 142.5 (s, C-1'), 131.8 (d, C-5'), 128.6 (d, C-4'), 127.5 (d, C-5), 122.7 (s, C-3'), 119.3 (d, C-6'), 115.1 (d, C-2') and 85.2 (s, C-4).

EI-MS: *m/z* (%) 319 ([⁸¹Br₂]M⁺, 61) 317 ([⁸¹Br⁷⁹Br]M⁺, 30), 315 ([⁷⁹Br₂]M⁺, 17, 220 (42), 205 (100), 189 (8) 177 (14), 145 (15) and 105 (12).

6.26 Synthesis of 3-amino-1-(3-(1*H*-indol-6-yl)phenyl)-1*H*-pyrazole (**4.29**)



A solution of 3-amino-1-(3-bromophenyl)-1*H*-pyrazole (**4.28**; 0.20 g, 0.84 mmol), 6-indolylboronic acid (**4.27**; 0.13 g, 0.25 mmol), caesium carbonate (0.55 g, 1.68 mmol) and Pd(PPh₃)₄ (0.10 g, 0.08 mmol) in DMF (4 mL) was irradiated at 150 °C for 2 hours in a sealed pressure-rated glass tube (10 mL) using a CEM Discover microwave

synthesizer by moderating the initial power (150 W). The mixture was cooled by passing a stream of compressed air through the microwave cavity and water was added (10 mL). The aqueous layer was extracted with EtOAc (3×20 mL) and the organic extracts were combined, washed with brine, dried over MgSO_4 and evaporated *in vacuo*. Purification of the crude product by column chromatography (silica gel; hexane–EtOAc in 1:1 by volume) gave the *title compound* (**4.29**; 0.19 g, 84%) as a colourless solid.

mp 97–99 °C (Found: M^+ , 274.1218. $\text{C}_{17}\text{H}_{14}\text{N}_2$ requires [M] 274.1222).

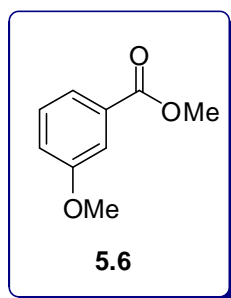
FT–IR (KBr)/ cm^{-1} ν_{max} : 3411 (NH), 2922 (CH), 1626, 1604, 1586 and 1479.

^1H NMR (400 MHz; $\text{DMSO}-d_6$) δ : 11.18 (s, exch., D_2O , 1 H, NH), 8.26 (d, $J = 2.6$ Hz, 1 H, H-5), 7.96 (m, 1 H, H-2'), 7.69 (m, 1 H, H-7'), 7.63 (d, $J = 8.3$ Hz, 1 H, H-6'), 7.58 (m, 1 H, H-5'), 7.46–7.41 (m, 3 H, H-4', H-2'' and H-4''), 7.35 (dd, $J = 8.3, 1.6$ Hz, 1 H, H-4'), 6.47 (m, 1 H, H-3''), 5.76 (d, $J = 2.6$ Hz, 1 H, H-4) and 5.17 (s, exch., D_2O , 2 H, NH_2).

^{13}C NMR (100 MHz; $\text{DMSO}-d_6$) δ : 157.5 (s, C-3), 134.3 (s, C-1'), 141.0 (s, C-6''), 137.0 (s, C-3') 133.5 (s, C-7''a), 130.2 (s, C-3''a), 128.5 (d, C-5'), 127.9 (d, C-5), 126.8 (d, C-4'), 122.7 (d, C-2''), 120.9 (d, C-4''), 118.8 (d, C-6'), 115.2 (d, C5''), 114.9 (d, C-2'), 110.0 (d, C7''), 101.5 (C-3'') and 96.7 (d, C-4).

EI–MS: m/z (%) 275 ($[\text{MH}]^+$, 17), 274 (M^+ , 100), 273 ($[\text{M} - 1]^+$, 19), 246 ($[\text{M} - \text{N}_2]^+$, 7), 205 (6), 191 (9), 133 (14) and 84 (21).

6.27 Synthesis of 3-methoxybenzoic acid methyl ester (5.6)



A solution of 3-methoxybenzoic acid (**5.5**) (1.00 g, 6.57 mmol) and concentrated sulfuric acid (0.075 mL, 0.165 mmol) in methanol (6 mL, 3.41 mmol) was stirred at reflux for 2.5 h. The residue was evaporated and then the mixture was extracted with ether. The ethereal solution was washed with saturated aqueous NaHCO_3 solution, dried (Na_2SO_4) and evaporated *in vacuo* to afford the *title compound* (**5.6**; 0.87 g, 80%)¹⁰ as a colourless oil.

[R_f = 0.64 (petroleum ether–EtOAc, 6:4)] (Found: MNa^+ , 189.0522. $\text{C}_9\text{H}_{10}\text{O}_3\text{Na}$ requires [MNa^+], 189.0522).

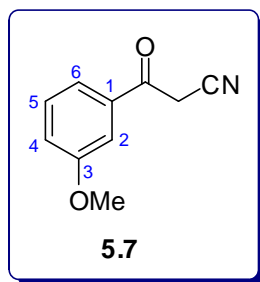
FT–IR (nujol)/ cm^{-1} ν_{max} 2939, 2902, 2886, 1732, 1725, 1695, 1653, 1453, 1429, 1326, 1284, 1103, 1048 and 758.

^1H NMR (400 MHz, CDCl_3): δ 7.63 (1H, d, J = 8.0 Hz, 6-ArH), 7.56 (1H, s, 2-ArH), 7.35 (1H, t, J = 8.0 Hz, 5-ArH), 7.10 (1H, d, J = 8.0 Hz, 4-ArH), 3.91 (3H, s, COOCH_3) and 3.85 (3H, s, ArOCH_3).

^{13}C NMR (100 MHz, CDCl_3): δ 167.0 (s, C), 159.6 (s, C), 131.4 (s, C), 129.4 (d, CH), 122.0 (d, CH), 119.5 (d, CH), 114.0 (d, CH), 55.4 (q, CH_3) and 52.2 (q, CH_3).

APCI–MS: m/z 167 (MH^+ , 100%).

6.28 Synthesis of 3-methoxybenzoylacetonitrile (**5.7**)



6.28.1 Method A:

A mixture of dry tetrahydrofuran (4 mL), sodium hydride (0.50 g, 60 % in mineral oil) and 3-methoxybenzoic acid methyl ester (**5.6**; 0.75 g, 3.93 mmol) was stirred at room temperature for 10 min. A dry acetonitrile (0.13 mL, 3.93 mmol) then was added dropwise to the mixture and then was irradiated at 150 °C for 1 hour in a pressure-rated glass tube (10 mL) using a CEM Discover microwave synthesizer by moderating the initial power (100 W). After cooling in a flow of compressed air, the product was diluted with ether (15 mL) and the resultant precipitate was washed with ether, dissolved in water (15 mL) and diluted with hydrochloric acid. The mixture was neutralised with saturated aqueous NaHCO₃ solution and the aqueous layer was extracted with EtOAc (3 × 20 mL). The organic extracts were combined, washed with brine, dried over MgSO₄ and evaporated *in vacuo* to afford the *title compound* as a colourless solid (**5.7**; 0.23 g, 35%).

mp 57–58 °C (lit.¹⁰ 55 °C) (Found: M⁺, 175.0635. C₁₀H₉NO₂ requires [M] 175.0633).

FT-IR (KBr)/cm⁻¹ ν_{\max} : 3103 (CH), 2343 (C≡N), 1945, 1750 (C=O), 1697, 1533, 1487 and 1435.

¹H NMR (400 MHz, CDCl₃): δ 7.43–7.35 (m, 2 H, H-4 and H-6), 7.13 (m, 1 H, H-4), 4.10 (s, 3H, OCH₃) and 3.89 (s, 2 H, CH₂).

^{13}C NMR (100 MHz, CDCl_3): δ 187.1 (s, C=O), 160.2 (s, C-3), 136.7 (s, C-1), 130.3 (d, C-2), 121.3 (d, C-5), 119.5 (d, C-6), 115.9 (s, $\text{C}\equiv\text{N}$), 113.6 (d, C-4), 55.7 (q, CH_3) and 29.5 (t, CH_2).

ES^+ -MS: m/z (%) 175 (M^+ , 37), 135 ($[\text{M} - \text{CH}_2\text{CN}]^+$, 87), 107 ($[\text{M} - \text{COCH}_2\text{CN}]^+$, 33), 92 ($[\text{M} - \text{COCH}_2\text{CN} - \text{OH}]^+$, 15), 85 (92), 84 (100) and 77 (23).

6.28.2 Method B:

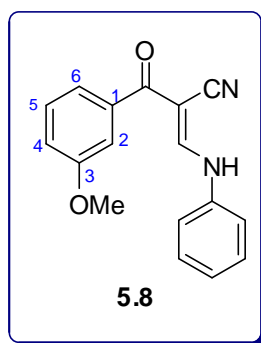
A mixture of dry tetrahydrofuran (50 mL), sodium hydride (2.00 g, 60% in mineral oil) and 3-methoxybenzoic acid methyl ester (**5.6**; 4.20 g, 29.06 mmol) was stirred at room temperature for 20 min. Dry acetonitrile (1.20 mL) was then added dropwise and the mixture was heated at reflux for 24 h. After cooling, the product was diluted with ether (50 mL) and the resultant precipitate was washed with ether, dissolved in water (35 mL) and diluted with hydrochloric acid. The solution was neutralised with saturated aqueous NaHCO_3 solution and the aqueous layer was extracted with EtOAc (3×60 mL). The organic extracts were combined, washed with brine, dried over MgSO_4 and evaporated *in vacuo* to afford the *title compound* as a colourless solid (**5.7**; 3.83 g, 75%). The spectral data of the product were consistent in all respects with the data obtained *via* **Method A** (Section 6.28.1).

6.28.3 Method C:

A solution of 3-iodoanisole (**5.12**; 0.25 mL, 1.90 mmol), 3-methoxyacrylonitrile (0.16 mL, 1.90 mmol), K_2CO_3 (0.35 g, 1.91 mmol), xantphos (0.11 g, 0.19 mmol) and palladium(II) acetate (0.05 g, 0.19 mmol) in a mixture of acetonitrile–water (2:1 by volume; 4 mL) was irradiated at 150 °C for 1.5 h in a pressure-rated glass tube (10 mL) using a CEM Discover microwave synthesizer by moderating the initial power (150 W). After cooling in a flow of compressed air, HCl (3M) was added to the mixture to adjust

the pH to *ca.* 2–3 and the mixture was stirred for 10 min at room temperature. The mixture was neutralised with saturated aqueous NaHCO₃ solution. The aqueous layer was extracted with EtOAc (3 × 25 mL) and the organic extracts were combined, washed successively with water and brine, dried (MgSO₄) and evaporated *in vacuo*. Purification by column chromatography (silica gel; petroleum ether–ethyl acetate in 3:1 by volume) gave the *title compound* (**5.7**; 0.25 g, 76%) as a colourless solid. The spectral data of the product were consistent in all respects with data obtained *via* **Method A** (Section 6.28.1).

6.29 Synthesis of 2-(3-methoxybenzoyl)-3-phenylaminoacrylonitrile (**5.8**)



A solution of 3-methoxybenzoylacetonitrile (**5.8**; 0.05 g, 0.29 mmol) and *N,N'*-diphenylformamidine (0.06 g, 0.29 mmol) in dry xylenes (2 mL) was irradiated at 180 °C for 20 min in a pressure-rated glass tube (10 mL) using a CEM Discover microwave synthesizer by moderating the initial power (200 W). After cooling in a stream of compressed air, the mixture was diluted with ether. The precipitate was filtered and washed with ether to afford the *title compound* (**5.8**; 0.07 g, 90%) as a colourless solid.

mp 102–104 °C (lit.¹⁰ 105 °C) (Found: M⁺, 278.1061. C₁₇H₁₄N₂O₂ requires [M] 278.1061).

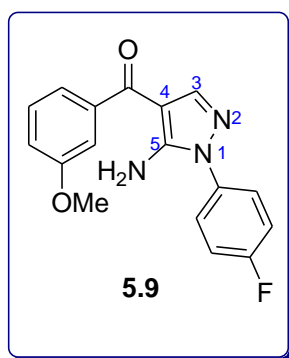
FT-IR (KBr)/ cm^{-1} : ν_{max} 3120 (NH), 3093 (CH), 2971, 2206 ($\text{C}\equiv\text{N}$), 1638 ($\text{C}=\text{O}$) and 1574.

^1H NMR (400 MHz, CDCl_3): δ 8.06 (d, $J = 13.0$ Hz, 1 H, $\text{C}=\text{CHNH}$), 7.57 (d, $J = 7.4$ Hz, 1 H, H-6), 7.36–7.47 (m, 4 H, H-2, H-5 and H-2'/H-6'), 7.28 (t, $J = 7.2$ Hz, 1 H, H-4), 7.13 (d, $J = 8.0$ Hz, 2 H, H-3'/H-5'), 7.10 (d, $J = 8.0$ Hz, 1 H, H-4), 4.10 (d, $J = 13.0$ Hz, exch. D_2O , 1 H, NH) and 3.89 (s, 3 H, OCH_3).

^{13}C NMR (100 MHz, CDCl_3): δ 192.3 (s, $\text{C}=\text{O}$), 159.5 (s, C-3), 154.1 (d, $\text{C}=\text{CHNH}$), 139.1 (s, C-1), 138.0 (s, C-1'), 130.2 (d, C-3'/C-5'), 129.5 (d, C-4'), 126.7 (d, C-6), 120.5 (d, C-4), 120.4 (s, $\text{C}\equiv\text{N}$), 118.9 (d, C-2'/C-6' of Ph), 117.9 (d, C-5), 112.6 (d, C-2), 55.5 (q, OCH_3) and 83.4 (s, $\text{C}=\text{CHNH}$).

EI-MS: m/z (%) 278 (M^+ , 78), 277 ($[\text{M} - 1]^+$, 100), 247 ($[\text{M} - \text{OMe}]^+$, 10), 209 (30), 175 (92), 135 (98), 107 (92), 77 ($[\text{Ph}]^+$, 95) and 64 (47).

6.30 Synthesis of [5-amino-1-(4-fluorophenyl)-1H-pyrazol-4-yl]-3-methoxyphenyl ketone (5.9)



A mixture of 2-(3-methoxybenzoyl)-3-phenylaminoacrylonitrile (**5.8**; 0.11 g, 0.40 mmol), 4-fluorophenylhydrazine hydrochloride (0.06 g, 0.40 mmol) and triethylamine (0.010 mL, 0.09 mmol) in ethanol (4 mL) was irradiated in a sealed tube at 140 °C for 1

h using a CEM Discover single-mode microwave synthesizer, by moderating the initial microwave power (150 W). After cooling in a stream of compressed air, the mixture was evaporated *in vacuo* and purified by column chromatography on silica, eluting with petroleum ether–EtOAc (3:2; by volume), to afford the *title compound* (**5.9**; 0.01 g, 87%) as a colourless solid.

mp 116–117 °C (Ref.¹⁰ 114 °C) (Found: MH^+ , 312.1143. $C_{17}H_{15}N_3O_2F$ requires [MH] 312.1143).

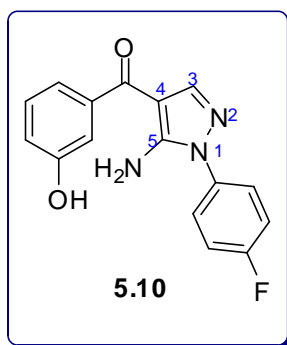
FT–IR (KBr)/ cm^{-1} ν_{max} : 3996 (NH), 3312 (NH), 2980 (CH), 1636, 1654 (C=O) and 1577.

1H NMR (400 MHz, $CDCl_3$): δ 7.83 (s, 1 H, H-3), 7.56–7.58 (m, 2 H, H-2'/H-6'), 7.44–7.40 (m, 2 H, H-5'' and H-6''), 7.28 (m, 1 H, H-2'), 7.24–7.29 (m, 2 H, H-3'/H-5'), 7.13 (m, 1 H, H-4'), 6.07 (br s, exch. D_2O , 2 H, NH_2) and 3.90 (s, 3 H, OCH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ 189.5 (s, C=O), 162.1 (s [d, J = 248.0 Hz], C-4'), 159.7 (s, C-4''), 150.5 (s, C-5), 142.0 (d, C-3), 140.9 (s, C-1''), 133.1 (s [d, J = 3.3 Hz, C-1'), 129.5 (d, C-5), 126.1 (d [d, J = 8.8 Hz], C-2'/C-6'), 117.8 (d, C-6''), 120.6 (d, C-4''), 116.9 (d [d, J = 23.0 Hz], C-3'/C-5'), 112.9 (d, C-2''), 104.8 (s, C-4) and 55.4 (q, OCH_3).

APcI–MS: m/z (%) 312 (MH^+ , 100).

6.31 Synthesis of [5-amino-1-(4-fluorophenyl)-1*H*-pyrazol-4-yl]-3-hydroxyphenyl ketone (**5.10**)



A solution of [5-amino-1-(4-fluorophenyl)-1*H*-pyrazol-4-yl]-3-methoxyphenyl ketone (**5.9**; 0.10 g, 0.32 mmol) in dry CH₂Cl₂ (15 mL) was added at 0 °C to a solution of boron tribromide (BBr₃; 0.45 mL, 1.22 mmol) in CH₂Cl₂ under nitrogen and the mixture was stirred overnight at room temperature. Water (10 mL) was added and the mixture was extracted with CH₂Cl₂ (2 x 15 mL). The organic extracts were combined, dried (MgSO₄) and evaporated *in vacuo* to afford the *title compound* (**5.10**; 0.073 g, 77%) as a colourless solid.

mp 153–154 °C (Ref.¹⁰ 153 °C) (Found: *M*⁺ 297.0914. C₁₆H₁₂N₃O₂F requires [*M*] 297.0909).

FT-IR (KBr)/cm⁻¹ *v*_{max}: 3520 (NH/OH), 3470 (NH/OH), 3337, 2980 (CH), 1625 (C=O), 1587, 1513 and 1487.

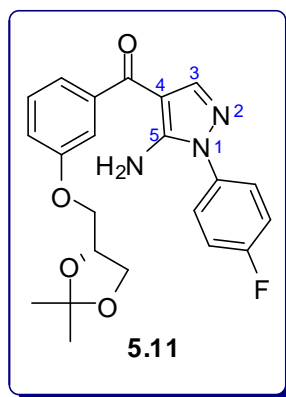
¹H NMR (400 MHz, CD₃OD): δ 7.72 (s, 1 H, H-3), 7.51-7.47 (m, 2 H, H-2'/H-6'), 7.22 (m, 3 H, H-5'', H-6'' and OH), 7.07 (m, 1 H, H-2''), 6.90 (m, 1 H, H-4'') and 7.02 (d, *J* = 8.1 Hz, 1 H, H-4'').

¹³C NMR (100 MHz, CD₃OD) δ: 189.9 (s, C=O), 162.3 (s [d, *J* = 246.6 Hz], C-4'), 157.5 (s, C-3''), 151.6 (s, C-5), 141.9 (d, C-3), 141.0 (s, C-1''), 133.4 (d [d, *J* = 3.3 Hz],

C-1'), 129.3 (d, C-5'), 126.7 (d [d, $J = 8.8$ Hz], C-2'/C-6'), 118.9 (d, C-6'), 118.3 (d, C-4'), 116.3 (d [d, $J = 23.4$ Hz], C-3'/C-5'), 114.3 (d, C-2') and 103.9 (s, C-4).

EI-MS: m/z (%) 298 ($[MH]^+$, 12), 297 (M^+ , 71), 296 ($[M - 1]^+$, 100), 280 ($[M - OH]^+$, 15), 204 ($[M - C_6H_4OH]^+$, 25), 149 (6), 121 (4) and 95 (12).

6.32 Synthesis of [5-amino-1-(4-fluorophenyl)-1*H*-pyrazol-4-yl]-3-[[*(R)*-2,2-dimethyl-1,3-dioxolan-4-yl]methoxy]phenyl ketone (**5.11**)



To a solution of [5-amino-1-(4-fluorophenyl)-1*H*-pyrazol-4-yl]-3-hydroxyphenyl ketone (**5.10**; 0.05 g, 0.16 mmol) in dry dimethylsulfoxide (DMSO; 5 mL) was added (*S*)-2,2-dimethyl-1,3-dioxolane-4-methanol *p*-toluenesulfonate (0.06 g, 0.21 mmol) followed by anhydrous potassium carbonate (0.07 g, 0.38 mmol). The reaction mixture was warmed to 100 °C under nitrogen. After 24 hours, the reaction was cooled to room temperature, diluted with water (20 mL) and the product was extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with brine, dried ($MgSO_4$) and then concentrated *in vacuo* to give the crude product as a brown oil. Purification by crystallization using a mixture of hexane–EtOAc (3:1 by volume)] gave the *title compound* (**5.11**; 0.062 g, 68%) as colourless crystals.¹¹

mp 58–61 °C (Found: MH^+ 412.1673. $C_{22}H_{23}N_3O_4F$ requires $[MH]$, 412.1669).

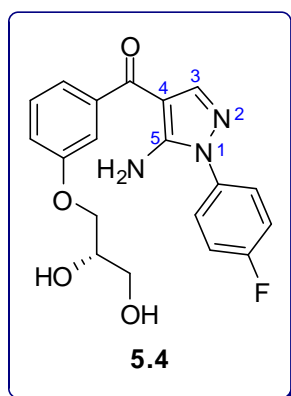
FT-IR (KBr)/cm⁻¹ ν_{\max} : 3412 (NH), 3309 (NH), 2986 (CH), 1621 (C=O), 1599, 1578, 1512 and 1310.

¹H NMR (400 MHz, CDCl₃): δ 7.66 (s, 1 H, H-3), 7.44–7.48 (m, 2 H, H-2'/H-6'), 7.32 (app. t, J = 8.0 Hz, 1 H, H-5'), 7.26–7.81 (m, 4 H, H-2, H-6 and H-3'/H-5'), 7.06 (m, 1 H, H-4'), 4.80 (br s, exch. D₂O, 2 H, NH₂), 4.38 (m, 1 H, CH), 4.14 (m, 1 H, 1 H of CH₂), 4.12 (m, 1 H, 1 H of CH₂), 4.02 (m, 1 H, 1 H of CH₂), 3.92 (m, 1 H, 1 H of CH₂), 1.31 (s, 3 H, CH₃) and 1.26 (s, 3 H, CH₃).

¹³C NMR (100 MHz, CDCl₃): δ 189.2 (s, C=O), 162.2 (s [d, J = 249.6 Hz], C-4'), 158.8 (s, C-3'), 150.6 (s, C-5), 142.0 (d, C-3), 141.1 (s, C-1'), 133.2 (s [d, J = 3.3 Hz], C-1'), 129.6 (d, C-5'), 126.2 (d [d, J = 8.8 Hz], C-2'/C-6'), 121.1 (d, C-6'), 118.4 (d, C-4'), 117.0 (d [d, J = 23.4 Hz], C-3'/C-5'), 113.6 (d, C-2'), 109.9 (s, C-4), 104.8 (s, C(CH₃)₂), 74.0 (d, CH), 70.0 (t, CH₂), 66.8 (t, CH₂), 26.8 (q, CH₃) and 25.4 (q, CH₃).

APCI-MS: m/z (%) 412 ([MH]⁺, 100), 372 (5), 354 (6) and 312 (4).

6.33 Synthesis of 5-amino-1-(4-fluorophenyl)-4-{3-[2(*S*)-3-dihydroxypropoxy]benzoyl}pyrazole (RO3201195) (5.4)



To a solution of [5-amino-1-(4-fluorophenyl)-1*H*-pyrazol-4-yl]-3-{[(*R*)-2,2-dimethyl-1,3-dioxolan-4-yl]methoxy}phenyl ketone (**5.11**; 0.02 g, 0.05 mmol) in dry methanol

Chapter Six: Experimental

(2 mL) was added distilled water (0.03 mL) and *p*-toluenesulfonic acid monohydrate (0.60 g, 3.15 mmol). The mixture was warmed to 50 °C under nitrogen and stirred overnight. The reaction mixture was cooled to room temperature and concentrated *in vacuo* to give a yellow oil. The oil was dissolved in ethyl acetate and washed with aqueous sodium bicarbonate solution (NaHCO₃). The organic layer was separated, dried (MgSO₄) and concentrated *in vacuo*. Purification by column chromatography (silica gel; petroleum ether–EtOAc in 3:2 by volume) afforded the *title compound* as a colourless solid¹¹ (**5.4**; 0.013 g, 56%).

mp 77–78 °C (Ref¹⁰ 75 °C) (Found: MH⁺, 372.1354. C₁₉H₁₉N₃O₄F requires [MH] 372.1352).

FT–IR (KBr)/cm^{–1} ν_{\max} : 3520 (OH), 3110 (NH), 2380 (CH), 1970, 1635 (C=O), 1567, 1515, 1470, 1324 and 1295.

¹H NMR (400 MHz, DMSO–*d*₆): δ 7.81 (s, 1 H, H-3), 7.60–7.63 (m, 2 H, H-2'/H-6'), 7.25–7.43 (m, 3 H, H-5'' and H-3'/H-5'), 7.26 (m, 1 H, H-6''), 7.24 (s, 1 H, H-2''), 7.13–7.20 (m, 3 H, NH₂ and H-4'), 5.02 (d, *J* = 4.8 Hz, exch. D₂O, 1 H, CHOH), 4.71 (t, *J* = 5.7 Hz, exch. D₂O, 1 H, CH₂OH), 4.09 (m, 1 H, 1 H of CH₂O), 3.94 (m, 1 H, 1 H of CH₂O), 3.83 (m, 1 H, CHOH) and 3.46–3.47 (m, 2 H, CH₂OH).

¹³C NMR (125 MHz, DMSO–*d*₆): δ 188.1 (s, C=O), 161.2 (s [d, *J* = 245.9 Hz], C-4'), 159.3 (s, C-3''), 151.7 (s, C-5), 141.9 (d, C-3), 141.4 (s, C-1'), 134.3 (s [d, *J* = 3.0 Hz], C-1'), 130.2 (d, C-5'), 126.9 (d [d, *J* = 9.1 Hz], C-2'/C-6'), 120.6 (d, C-6''), 118.4 (d, C-4'), 116.9 (d [d, *J* = 21.2 Hz], C-3'/C-5'), 113.8 (d, C-2'), 104.0 (s, C-4), 70.4 (d, CH), 70.3 (t, CH₂) and 63.1 (t, CH₂).

APCI–MS: *m/z* (%) 372 (MH⁺, 100).

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