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**THE SYNTHESIS OF POLYFUNCTIONAL
PYRROLES AND THE INVESTIGATION OF THE
CHEMOSELECTIVITY OF THEIR REACTIONS**

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A thesis submitted in partial fulfilment of the requirements of the
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Symbols and Abbreviations

a.q.	Aqueous
ADP	Adenosin 5'-diphosphate
AIBN	Azobisisobutyronitrile
ATP	Adenosine triphosphate
Boc	<i>t</i> -Butoxycarbonyl
bp	Boiling point
br	Broad
CDCl ₃	Chloroform
CNS	Central nervous system
COSY	Correlated spectroscopy
d	Doublet
DBU	Diaza(1,3)bicyclo[5.4.0]undecane
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
dd	Doublet of doublets
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DEPT	Distortionless Enhancement by Polarization Transfer
DIBAL-H	Diisobutylaluminium hydride
DMF	<i>N,N</i> -Dimethylformamide
DMSO	Dimethylsulphoxide
DNA	Deoxyribonucleic acid
dppp	1,3- <i>Bis</i> (diphenylphosphino)propane
EDG	Electron donating group

EGF	Epidermal growth factor
Et ₂ O	Diethyl ether
EWG	Electron withdrawing group
FBS	Fetal bovine serum
FDA	Food and drug administration
FGF	Fibroblast growth factor
GI ₅₀	50% Growth inhibition
h	Hours
HMBC	Heteronuclear multiple bond correlation
HMQC	Heteronuclear multiple quantum correlation
Hz	Hertz
IC ₅₀	50% Inhibition
IR	Infrared spectroscopy
J	Coupling constant
KSF	A type of montmorillonite clay
lit.	Literature
Log	Logarithm
m	Multiplet
<i>m/z</i>	Mass to charge ratio
M ⁺	Mass of molecular ion
MHz	Megahertz
min	Minutes
ml	Millilitres
mol	Moles
mp	Melting point

MTBE	Methyl <i>t</i> -butyl ether
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NBS	<i>N</i> -Bromosuccinimide
NMR	Nuclear magnetic resonance
NSAID	Non-steroidal anti-inflammatory drug
<i>p</i>	Para
PBS	Phosphate buffered saline
PDB	Protein Data Bank
PDGF	Platelet-derived growth factor
PDT	Photodynamic therapy
ppm	Parts per million
PPy	Polypyrrole
PTK	Protein tyrosine kinase
<i>p</i> -TsOH	<i>Para</i> -toluenesulphonic acid
q	Quartet
QSAR	Quantitative structure-activity relationship
r.t.	Room temperature
RTK	Receptor tyrosine kinase
s	Singlet
SAR	Structure-activity relationship
SBF	Structure based focusing
SET	Single electron transfer
t	Triplet
TBAF	Tetra- <i>n</i> -butylammonium fluoride
TEA	Triethylamine

TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TIPS	Tri <i>isopropyl</i> silyl
TLC	Thin layer chromatography
VEGF	Vascular endothelial growth factor
δ	Chemical shift

ABSTRACT

Polyfunctional pyrroles are interesting heterocyclic intermediates as they have a range of reactive centres and the chemoselectivity of their reactions under a range of conditions, is therefore, of much interest. Polyfunctionalised heterocycles are relatively difficult to prepare, but the reactions of these substituted pyrroles allow access to a wide variety of new substituted heterocyclic compounds *via* these intermediates.

The aim of this project was to synthesise polyfunctional pyrroles in order to investigate their use in the preparation of libraries and compounds with known biological activity. The synthesis and initial investigation of the regioselectivity of polyfunctional pyrroles, such as 3,5-dichloro-1*H*-pyrrole-2,4-dicarboxaldehyde, has previously been described; this work investigated only nucleophilic substitutions. We have investigated the chemoselectivity of the reaction of these pyrroles with a range of reagents and a number of pyrrole derivatives were synthesised *via* selective functional group transformations. All new compounds were fully characterised by spectroscopic and elemental analysis.

Another aim of this project was to discover novel agents that inhibit VEGF receptors using structure based drug design. We have identified hit compounds and synthesised them using regioselective reactions of functional groups present on the pyrrole ring. The compounds were tested for anti-proliferative activity against the HaCaT, human keratinocyte cell line, and also against HT29 and CaCo-2, human colon cell lines using the MTT assay.

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Chapter One

Introduction

1. INTRODUCTION

1.1 Background

About half of all known compounds contain a heterocyclic ring, and many of these contain an aromatic heterocyclic ring. Heterocyclic rings can be found in many of the products of both primary and secondary metabolism, as well as in many synthetic compounds of commercial interest, such as drugs, pest control agents, colouring agents and flavourings.¹

In the last few decades, the chemistry of pyrrole and its derivatives has received growing interest. Pyrroles are widely used intermediates in pharmaceuticals,² agrochemicals² and dyes² and are also highly flexible building blocks for a wide variety of other compounds, including natural products.³ For example, the pyrrole ring is the main component of naturally occurring tetrapyrroles such as haeme and chlorophyll, while the pentasubstituted pyrroles, Atorvastatin (Lipitor^R)⁴ **1** and Fluvastatin (Lescol^R)⁴ **2** are the most common prescription drugs for lowering cholesterol levels, Figure 1.

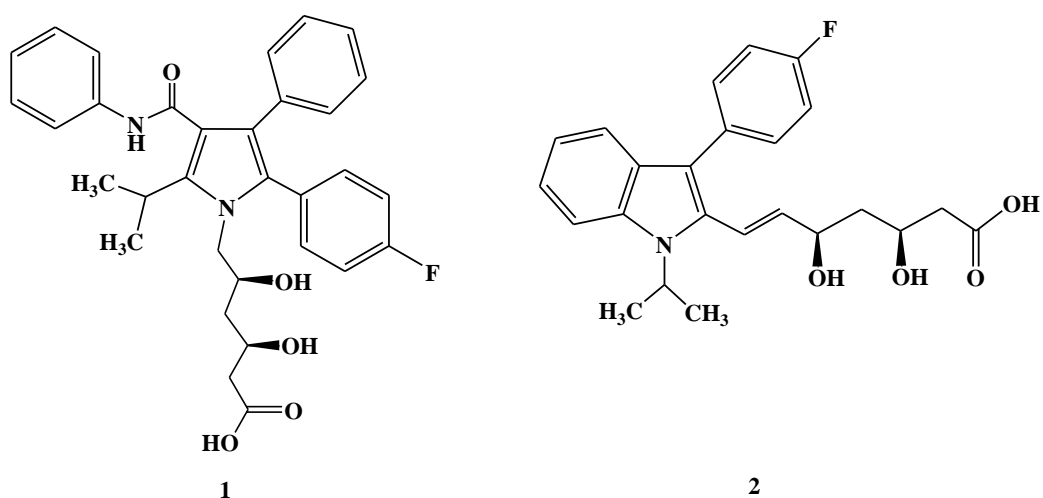
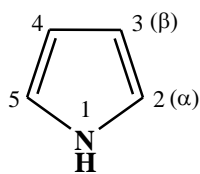


Figure 1. Structures of the clinically used pyrroles Atorvastatin **1** and Fluvastatin **2**

Pyrrole⁵ **3** is an electron rich, five-membered aromatic heterocycle which was discovered in 1834 by Runge,^{3b} who identified it in coal tar, and was first isolated in 1857 by Anderson through the dry distillation of bone material. In the 1870s, after the description of their structure, chemists became increasingly interested in pyrroles and their aromatic properties.⁶



In the 19th Century, Paal and Knorr published highly effective synthetic routes to pyrrole and its derivatives,^{7,8} using efficient cyclisation reactions for the direct synthesis of pyrroles from easily accessible starting materials such as acetoacetates, ketones and amines.

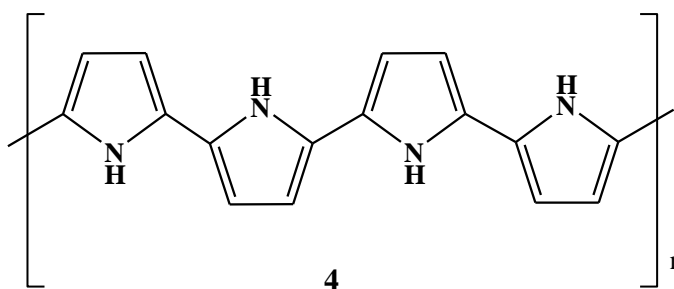
During the 20th Century extensive studies continued on the synthesis and chemical behaviour of pyrroles and particular effort was directed towards the study of the reactions of such systems with electrophiles.⁹

Today, although different synthetic approaches to substituted pyrroles exist, the synthesis of highly functionalised pyrroles and the study of modified pyrroles remains challenging.¹⁰

1.2 Importance of pyrroles

Pyrrole and its derivatives are important heterocyclic compounds, not only because of their interesting chemical reactions, but they are also essential building blocks for several natural products such as haemoglobin, chlorophyll, bile pigments or vitamin B₁₂.¹¹ Pyrroles are widely used as intermediates in the synthesis of pharmaceuticals, medicines, agrochemicals, perfumes, compounds in many foods and also exhibit a wide variety of optical and electronic properties.¹² Pyrroles are also used as catalysts for polymerisation processes and as corrosion inhibitors.¹³

Polypyrroles (PPy) **4** are among the most extensively studied conducting polymers, since monomeric pyrrole is easily oxidised, water-soluble and commercially available. They exhibit special interest because of their high conductivity and stability, easy preparation and good mechanical properties and they are suitable for use in batteries, electronic devices or sensors.¹⁴



Polypyrrole

One of the most studied applications of PPy is in the manufacture of capacitor devices. For example, a PPy-aluminum solid electrolytic capacitor shows good frequency and temperature features, as well as good thermal and moisture stabilities. This capacitor can function continuously for more than 3600 h at 150 °C without

deterioration.¹⁵ Porphyrins¹⁶ are an extremely important group of organic compounds and their basic structure contains four pyrrole molecules joined together by methene bridges, forming the tetrapyrrole structure. The parent compound of this class is porphine **5**, Figure 2. Each of the nitrogen atoms can form a bond with small metal cations such as Mg^{2+} , Fe^{2+} , Zn^{2+} , and Co^{2+} .

Haeme **6** is a porphyrin derivative in which the ferrous ion is held in the centre of the macrocycle, Figure 2. When haeme combines with the protein globin it forms haemoglobin (contained in red blood cells), which is responsible for oxygen transport from the lung to the tissues through the blood and also plays an important role in the transport of carbon dioxide from the tissues back to the lung.

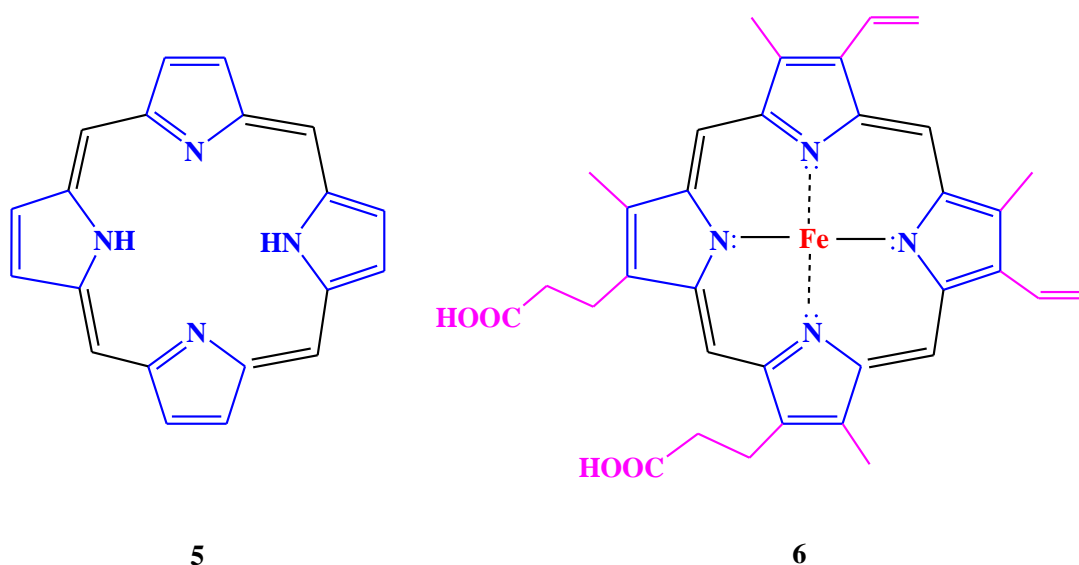


Figure 2. The structure of Porphin **5** and Haeme **6** where the four pyrrole rings are highlighted in blue, the side groups which were added to the porphine in purple and the central atom in red

Another important porphyrin, called chlorophyll, a green pigment, occurs in most plants and algae and is responsible for the absorption of energy from sunlight – it

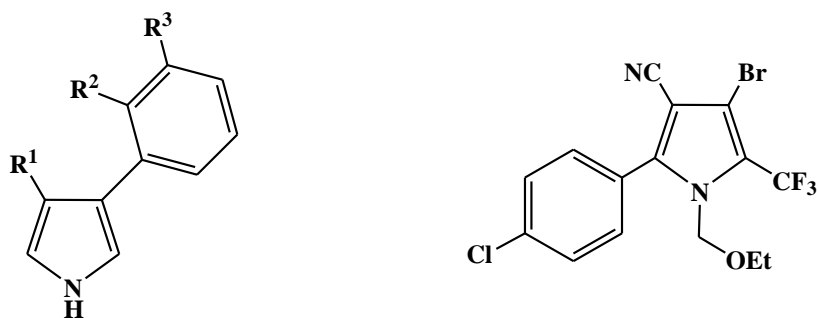
absorbs the red and blue / violet parts of the spectrum but reflects the green. Chlorophyll has a similar structure to haeme, with a magnesium ion at the centre of the complex. Vitamin B₁₂ or cobalamin is also a porphyrin derivative and plays an important role in the nervous system and in blood.¹⁷

1.2.1 Importance of halogenated pyrroles in agrochemistry and pharmaceuticals

It is well known that halogenated pyrroles, isolated from Nature, are lead compounds in agrochemistry and pharmaceuticals.

1.2.1.1 Agrochemistry

Fenpiclonil **7a** and Fludioxonil **7b** are phenylpyrrole fungicides derived from the natural antibiotic pyrrolnitrin **7c** which was isolated from the bacterium *Pseudomonas pyrociniae*.¹⁸ Phenylpyrroles are used to control a variety of important plant-pathogenic fungi¹⁹ and, together with anilinopyrimidines and dicarboximides, the phenylpyrroles belong to the most powerful botryticides. Chlorofenapyr **8** was the first commercialised pyrrole insecticide for the control of agricultural pests and termites. In addition, substituted analogues with cyano- or carboxylic acid moieties at the α -position are important intermediates in porphyrin syntheses,²⁰ Figure 3.



7a Fenpiclonil, Beret^R ($R^1 = \text{CN}$, $R^2, R^3 = \text{Cl}$)

7b Fludioxonil, Celeste^R ($R^1 = \text{CN}$, $R^2 = R^3 = \text{OCF}_2\text{O}$)

7c Pyrrolnitrin ($R^1 = R^3 = \text{Cl}$, $R^2 = \text{NO}_2$)

8 Chlorofenapyr

Figure 3. Pyrrolnitrin and its derivatives

Pyoluteorin²¹ **9** is an antibiotic substance, produced naturally by certain strains of *Pseudomonas* species, which led to the discovery of synthetic analogues **10** and **11** with herbicidal activity, Figure 4.

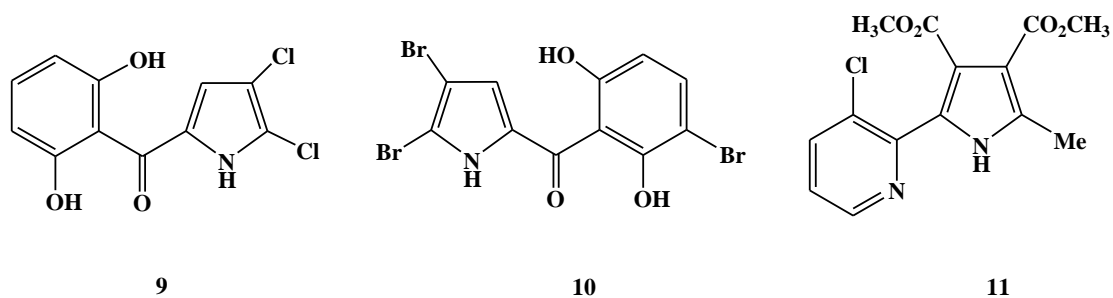


Figure 4. Pyoluteorin and its derivatives

The 2-aryl-3-cyanopyrrole derivatives **12**, **13** and **14** exhibit molluscicidal, insecticidal, fungicidal and herbicidal activity, Figure 5.²¹⁻²²

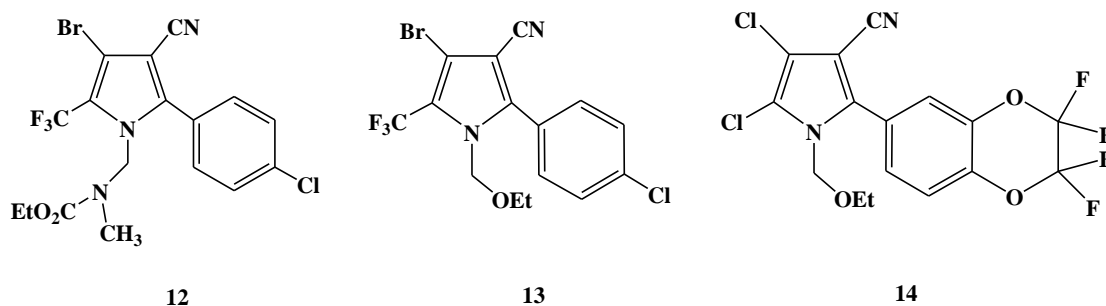


Figure 5. Halopyrroles with molluscidal, insecticidal, fungicidal and herbicidal activity

1.2.1.2 Pharmaceuticals

Pyrrole containing compounds are a promising starting point in drug research in view of their various pharmacological activities.

Zomepirac **15** and Tolmetin **16** are non-steroidal anti-inflammatory drugs (NSAID) and they exhibit anti-inflammatory, analgesic and antipyretic properties,²³ Figure 6.

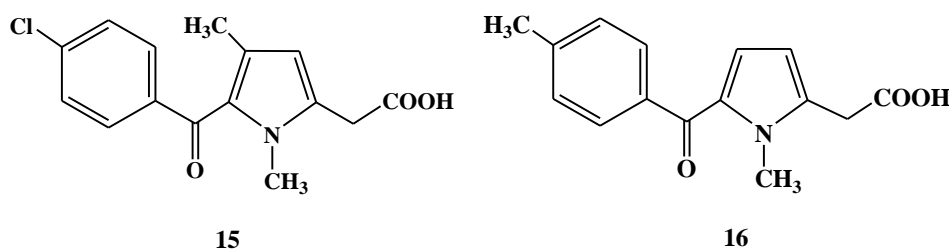


Figure 6. The anti-inflammatory agents, Zomepirac and Tolmetin

Atorvastatin (Lipitor^R) **1** is one of the most prescribed drugs in the US and Europe for the lowering of cholesterol levels and it has been shown that Atorvastatin, like other statin drugs, has potential in the treatment of Alzheimer's disease.²⁴

In the last few decades, fungal infections have increased significantly in number, mainly due to the growing number of immunocompromised individuals suffering

from cancer, AIDS or tuberculosis. A small number of agents are currently available to treat fungal infections. Antifungal azole agents such as fluconazole and voriconazole²⁵ **17** have some drawbacks, such as poor central nervous system (CNS) penetration or high cost. Onnis *et al.* reported on the synthesis and antifungal activity of new potential pyrrole derivatives **18** and **19** which also have a wide spectrum of activity against breast, lung and CNS cancer, Figure 7.²⁶

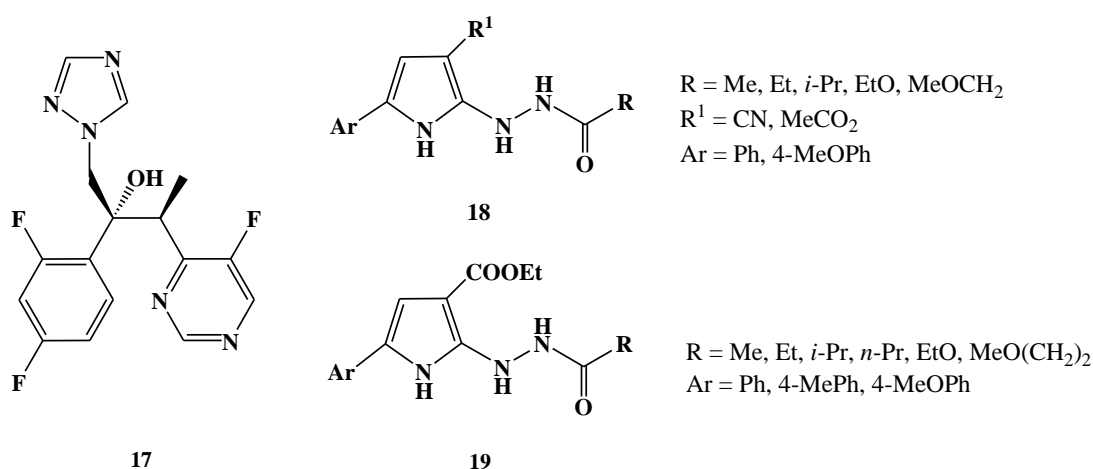


Figure 7. Voriconazole and new potential antifungal activity pyrrole derivatives

3-Halopyrroles, isolated from micro-organisms, have special importance in both pharmaceuticals and agrochemistry, although their synthesis is difficult because of problems with overhalogenation. 3-Chloropyrrole **20** is a fibrosis inhibitor, while roseophilin **21**, isolated from *Streptomyces griseoviridis* in 1992 by Seto *et al.*, has antileukemic and antibiotic properties, Figure 8.²⁷

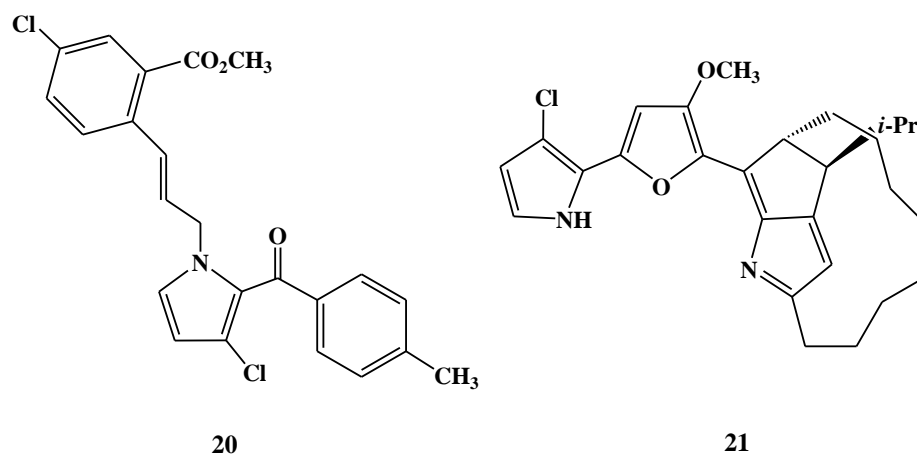


Figure 8. Halopyrroles

Pentabromopseudilin **22** was first isolated from the marine bacterium *Alteromonas luteoviolaceus* and exhibits antitumour and antibacterial activities, Figure 9. This polybrominated pyrrole also inhibits a number of different enzyme systems and cholesterol biosynthesis.²⁸ Tetrapyrrolic compounds are commonly used as therapeutic agents in photodynamic therapy (PDT) for the treatment of cancer.²⁹

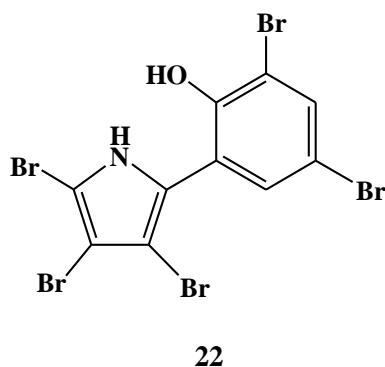


Figure 9. Pentabromopseudilin

1.2.2 Importance of pyrroles in dyes

Several pyrrole derivatives have been found to be useful as both laser and textile dyes. For example, the boron pyrromethene-BF₂ complex **23** are well-known as laser dyes (broadband laser activity in the region 530-580 nm under flash lamp excitation) and fluorescent labels in biology.³⁰ The alkaloid ageladine A **24** is a pyrrole-pyridoimidazole and shows fluorescence in the blue-green region during excitation with UV light at 370 nm, Figure 10.³¹

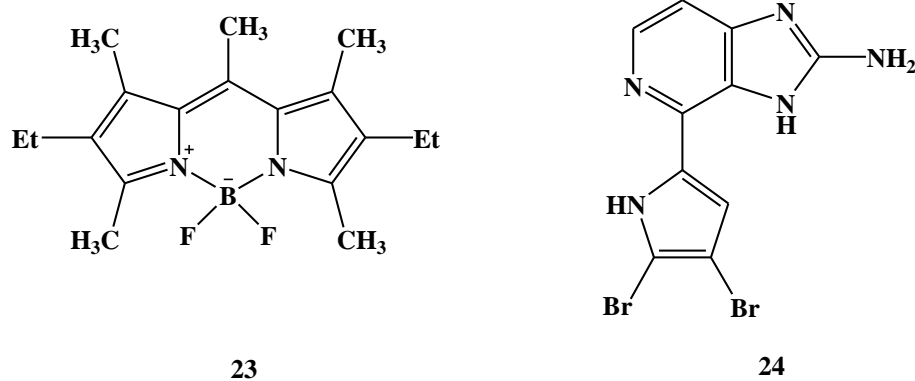
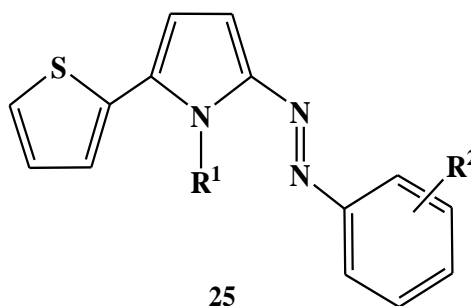


Figure 10. Laser dyes

Raposo *et al.* reported the first synthesis of a series of thienylpyrrole azo dyes **25**, Figure 11.³² Azo dyes with heterocyclic diazo components have been widely investigated for the creation of bright and strong colour shades, ranging from red to greenish blue, on synthetic fabrics.



R^1 = alkyl, aryl

R^2 = NO_2 , CN, CO_2Me

Figure 11. Thienylpyrrole azo dyes

1.2.3 Importance of pyrroles in food chemistry

Acetylpyrrole is found in many foods as a component of baked, fried and roasted flavourings.³³ Siegmund *et al.* reported on the importance of 2-acetylpyrrole **26**, which is responsible for the roasted flavour of the pumpkin oil, while *N*-methyl-2-acetylpyrrole **27** is responsible for some of the sweet aromas in coffee, Figure 12. In addition, pyrroles are important components of cosmetics and alcoholic perfumery.³⁴

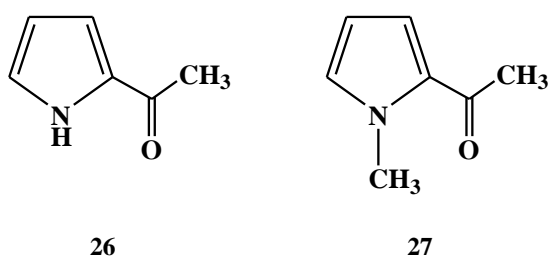
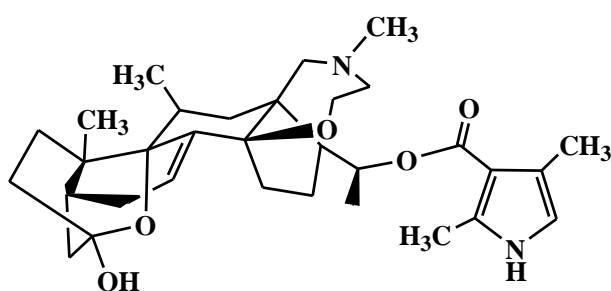


Figure 12. Acetylpyrroles in food chemistry

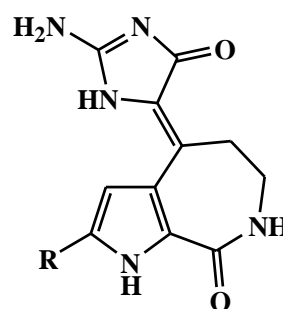
1.3 Pyrrole-containing natural products

Natural products are an important source of new therapeutic agents and an increasing number are being discovered from sources ranging from insects, sponges and plants to bacteria. Pyrrole alkaloids represent one of the most important groups of natural products – they exhibit various biological properties and are important as lead compounds for drug development.^{35,36} Several monopyrroles have been isolated from birds and frogs. An interesting example is batrachotoxin **28**, which was first isolated from the skin of poison arrow frogs from Columbian rainforests and is one of the most toxic substances known, Figure 13.³⁶

One of the growing classes of pyrrole alkaloids are the bromopyrroles, derived from marine sponges, and several members of this group have interesting biological properties.³⁷ For example, hymenialdisine **29** and its debrominated analogue **30**, collected from tropical regions, have anti-inflammatory properties and several other bromopyrroles show antibacterial properties, Figure 13.³⁷



28 Batrachotoxin



29 R = Br Hymenialdisine
30 R = H Debromohymenialdisine

Figure 13. Bromopyrrole alkaloids

A number of compounds containing the indole-2-one structure show important biological properties. For example, indole-2-one³⁸ **31** has antitumour activity while indole-2-ones **32** and **33** have phosphodiesterase³⁹ and tyrosine kinase inhibitory activity, Figure 14.⁴⁰

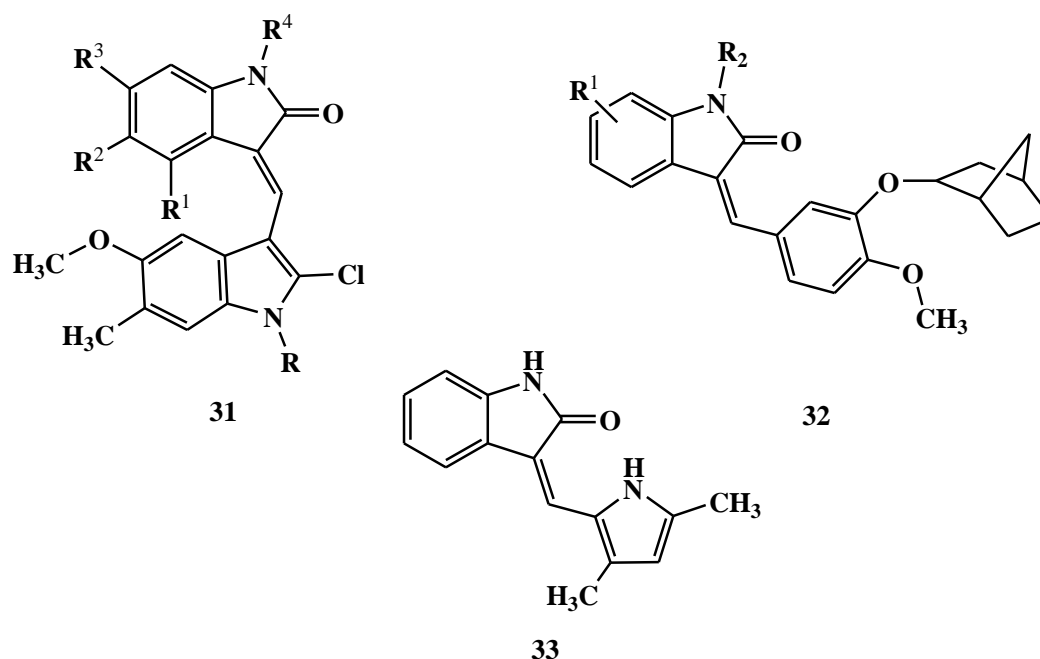


Figure 14. Indole-2-one containing natural products

Pyrrole derivatives with two aryl groups are especially important classes of natural products and some of them exhibit remarkable biological and pharmacological properties. For example, lamellarin natural products are specially interesting due to their high biological activities and a great deal of attention has thus been focused on the synthesis of lamellarins and related 3,4-diarylpyrrole derivatives. Lamellarins O **34**, P **35**, Q **36** and R **37** are 2-carboxylic acid esters and they belong to a large group of DOPA-derived pyrrole alkaloids.⁴¹ Most of the lamellarins show cytotoxic properties against a large range of cancer cell lines and the most effective of these compounds are lamellarins D **38**, M **39**, and K **40** (GI₅₀ 38-110 μM), Figure 15.⁴²

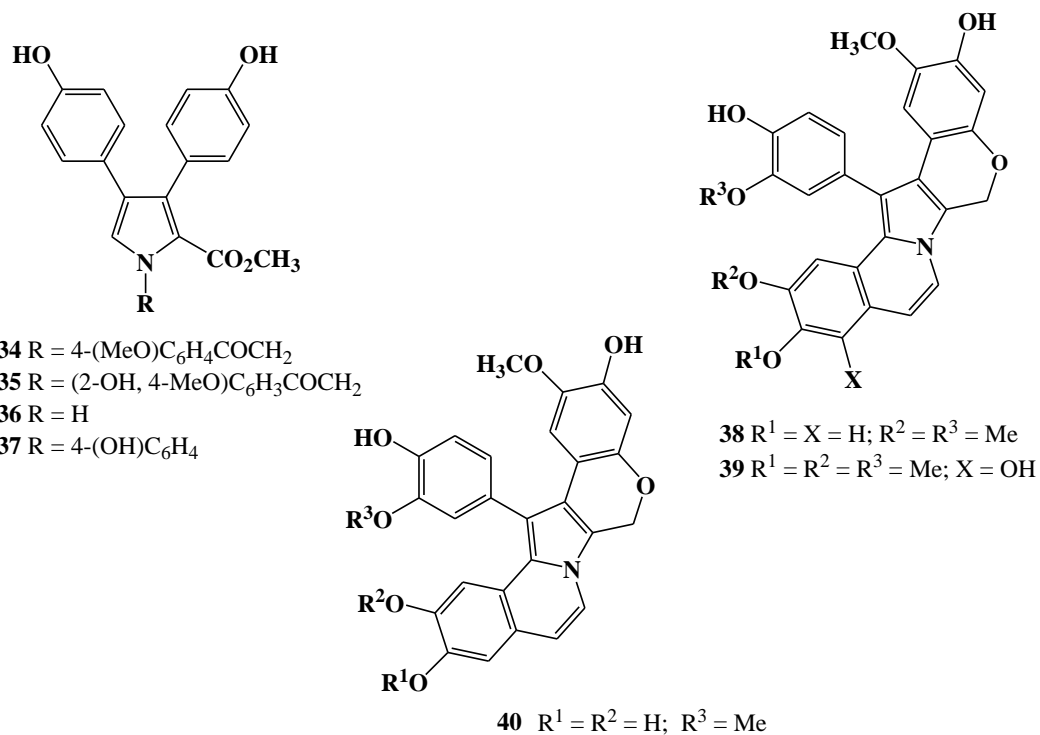
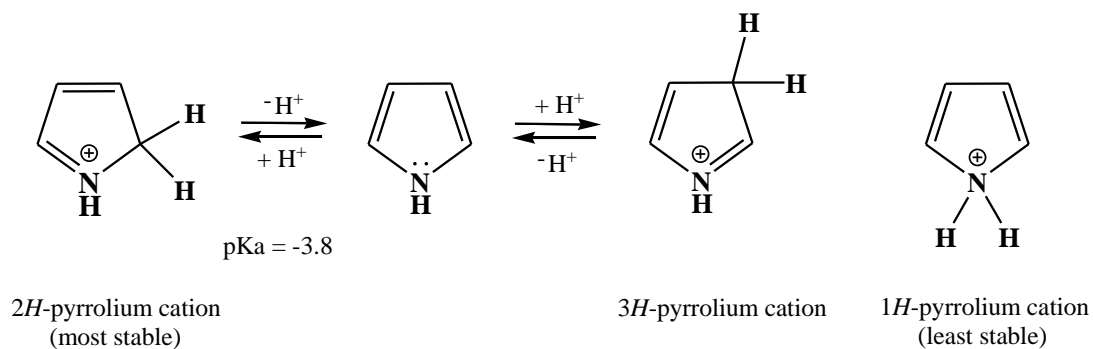


Figure 15. Pyrrole derivatives with aryl groups

1.4 Chemical reactions and synthesis of pyrroles

1.4.1 Protonation of pyrroles

Chiang *et al.* investigated the pK_a values of a huge range of pyrroles.⁴³ Pyrrole itself is a very weak base (pK_a -3.8) compared to amines or pyridine, in which the ring nitrogen is not bonded to a hydrogen atom. In pyrrole the lone pair of the nitrogen is part of the 6π aromatic ring and protonation destroys the aromaticity. In very acidic solutions, protonation takes place most readily on the carbon atoms of the ring and not on the nitrogen, Scheme 1.



Scheme 1. Protonation of the pyrrole ring

Basicity can be increased markedly with the introduction of alkyl substituents on the ring as these have a stabilising effect on cations; for example, 2,3,4,5-tetramethylpyrrole has a pK_a of +3.7.

1.4.2 Substitution of pyrroles

Pyrrole is an electron-rich heteroaromatic compound and so its major chemical reactivity is through attack by electrophiles and subsequent substitution reactions.^{11b} There are three possible positions for substitution in pyrrole **3**, Figure 16 to give the *N*-, α - and β -substituted products. The difference in these positions is their distance from the nitrogen heteroatom, which represents the polar centre of the ring, and the possibilities for resonance. All three of these products can be obtained, depending upon the reaction conditions used^{5a} and the regioselectivity can be controlled by varying the reaction conditions or the use of protecting groups.⁴⁴

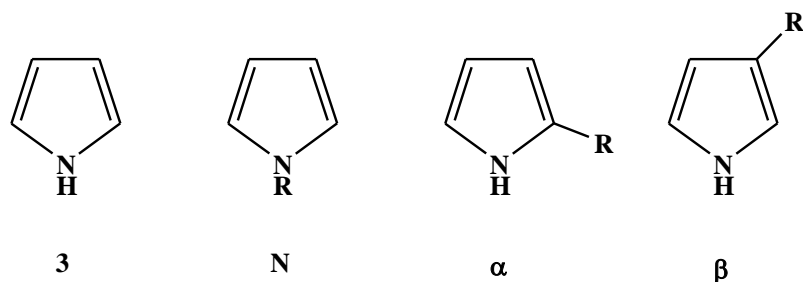
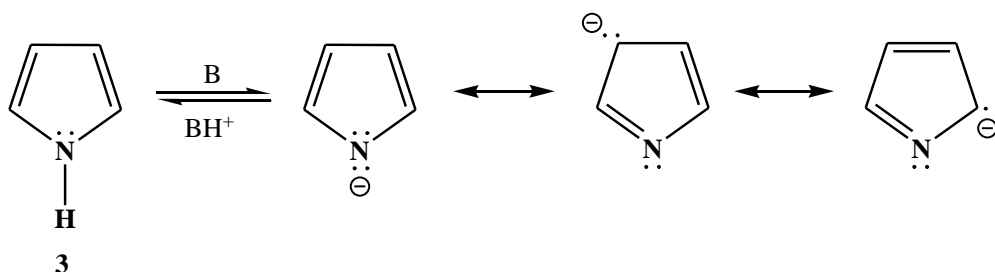


Figure 16. Different positions for substitution of pyrrole **3**

1.4.2.1 Substitution on nitrogen

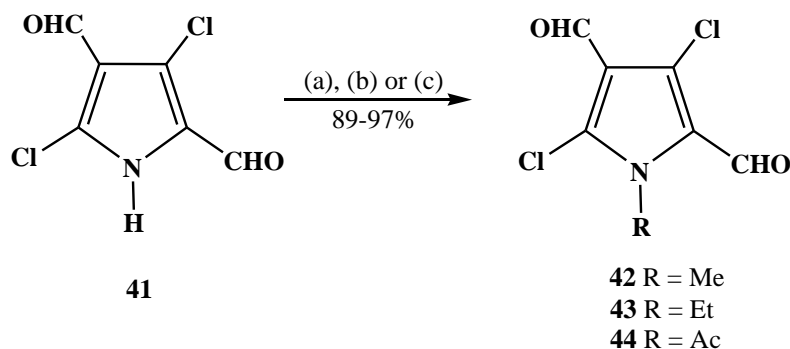
The lone pair on the nitrogen in pyrrole is involved in the aromatic π -system and is thus, not easily available for reaction with electrophiles. *N*-Substitution of pyrroles can, however, be readily achieved after deprotonation, to give the corresponding anion, followed by reaction with an electrophile.^{44,45}

Pyrrole is much more acidic (pK_a 17.7) than comparable saturated amines; for example, the pK_a of pyrrolidine is ~ 35 , while the pK_a of anilines is 30. The unsubstituted pyrrole **3** can be deprotonated easily with a strong base, such as NaH or butyllithium, to form the corresponding anion, Scheme 2.⁴⁵



Scheme 2. Pyrrole anion

For example, the pyrrole anion of **41** can be *N*-alkylated to give **42**, **43** or *N*-acetylated to give **44** in excellent yield; the *N*-alkyl analogues of pyrroles can be readily prepared using alkyl iodides as the electrophiles and sodium hydride in DMF as the base, Scheme 3.⁴⁶

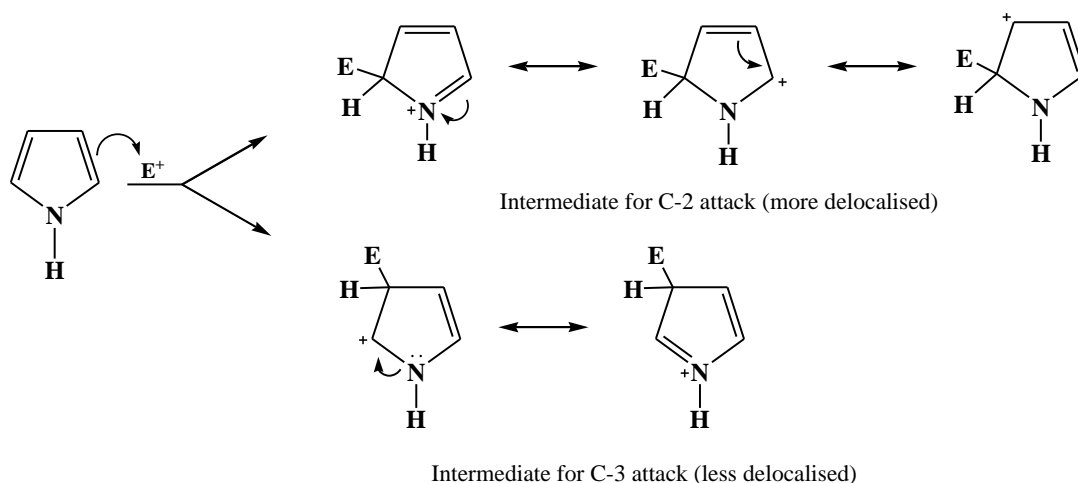


Scheme 3. Examples of the *N*-alkylation and acylation of pyrrole **41**. Reagents and conditions; a) NaH, DMF, MeI, r.t., 89%; b) NaH, DMF, EtBr, r.t., 97%; c) KH, THF, AcCl, r.t., 90%

1.4.2.2 Electrophilic substitution at the C-2 and C-3 positions

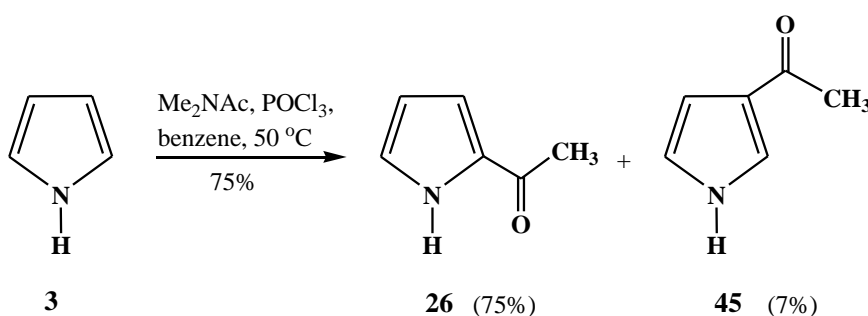
Good yields in the *N*-substitution of pyrroles depend upon the selective deprotonation of the nitrogen. Without previous deprotonation pyrrole normally reacts with electrophiles (E^+) at the kinetically preferred C-2 (α) position in preference to the thermodynamically more stable C-3 (β). The explanation of the α -selectivity of the substitution reactions is clear from the mechanism outlined in Scheme 4. The intermediate formed by electrophilic attack at C-2 is stabilised by charge delocalisation to a greater degree than the intermediate from C-3 attack. From the Hammond postulate, the activation energy for substitution at the former position is

less than the latter substitution. Attack at nitrogen is inhibited because no delocalisation of charge is possible in the resulting intermediate.⁴⁷



Scheme 4. Electrophilic substitution at C-2 and C-3 position of pyrroles

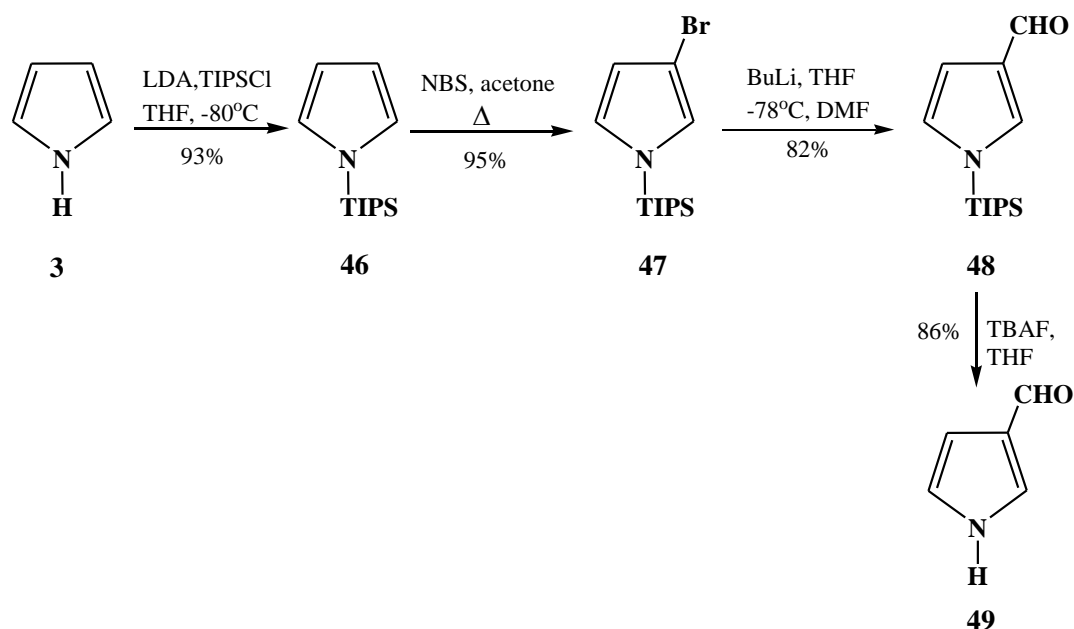
Electrophilic substitution at the C-3 position is possible but the β -isomer is usually formed only in minor quantities; for example, β -acetylpyrrole **45** is a by-product of the α -acetylation of pyrrole **26**, Scheme 5.⁴⁸



Scheme 5. Example of electrophilic substitution at the β -position

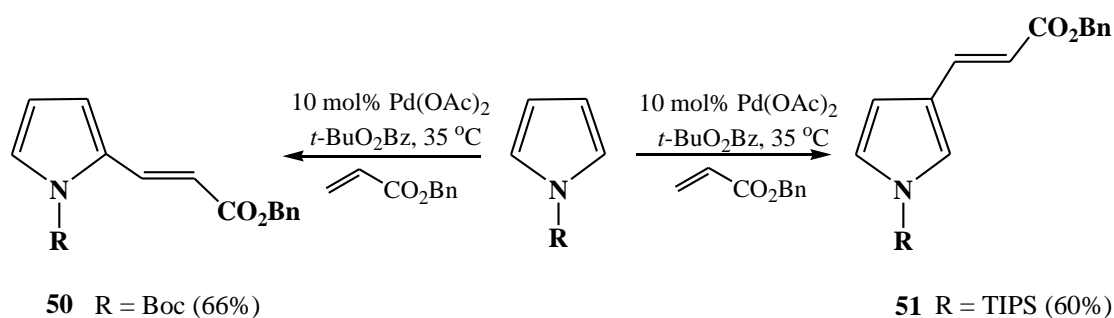
The position of substitution can be controlled by the protection of the pyrrole nitrogen with sterically bulky groups, such as the *tert*-butyl or triisopropylsilyl groups, which block substitution at the C-2 position.^{44a,45a} For example, the triisopropylsilyl (TIPS)

protecting group was found to be useful for the preparation of 3-formylpyrrole **49**. The first step in this synthesis is to protect the pyrrole **3** with TIPS, followed by the bromination at the C-3 position with *N*-bromosuccinimide (NBS) to result in the brominated product **47**. Halogen-metal exchange then allows selective functionalisation at the C-3 position. Reaction of the carbanion with *N,N*-dimethylformamide provided β -formyl derivative **48**, the desilylation of which was performed using tetrabutylammonium fluoride (TBAF) in THF to afford 3-formylpyrrole **49** in good overall yield from **3**, Scheme 6.⁴⁹



Scheme 6. Synthesis of β -formylpyrrole **49**

Gaunt *et al.* investigated the regioselective alkenylation of pyrroles under mild, aerobic, palladium catalysed conditions. Electron withdrawing *N*-protecting groups (*N*-Ac, *N*-Ts, *N*-Boc) decrease the reactivity of pyrroles and result only in C-2 substituted product e.g. **50**, in contrast with the reaction with *N*-TIPS pyrrole which afforded the C-3 product **51**, Scheme 7.⁵⁰



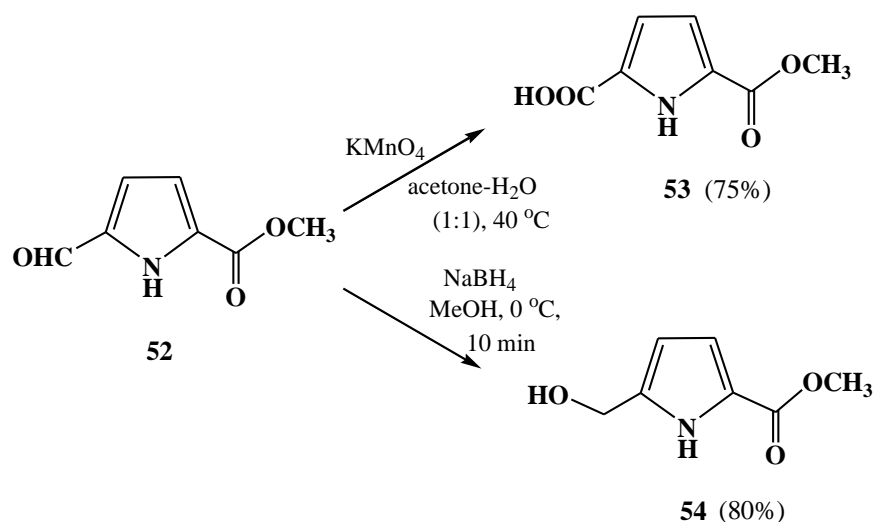
Scheme 7. Regioselective C-H alkenylation of pyrrole

1.4.3 Interconversion of substituents

Interconversion of functional groups is another feasible method if the direct introduction of the corresponding substituent is not possible because of instability under the reaction conditions or unfavourable regiochemistry.

1.4.3.1 Transformation of the formyl group

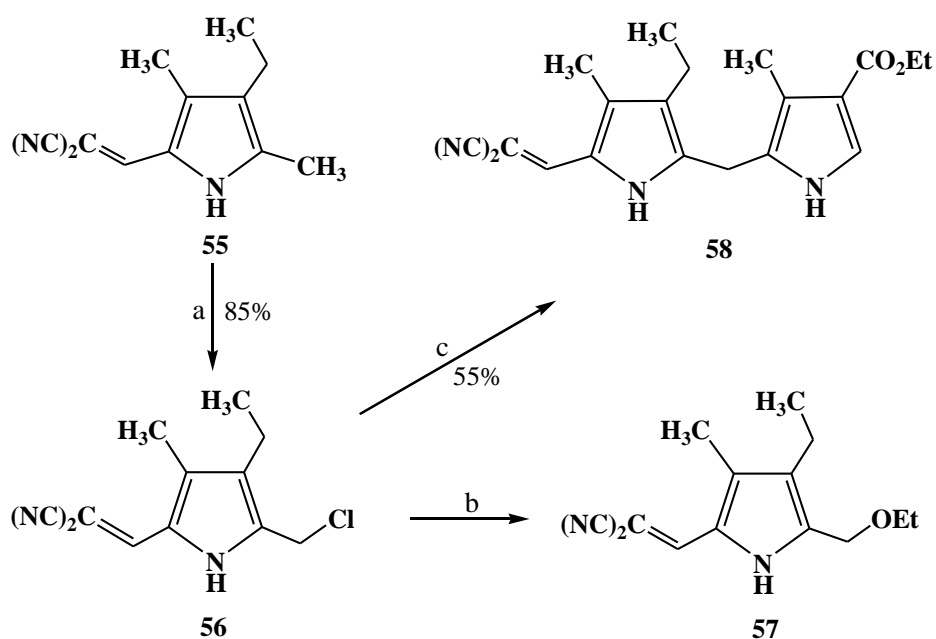
The formyl group is the most reactive centre in 5-formyl-1*H*-pyrrole-2-carboxylic acid methyl ester **52** and this can undergo functional group interconversion in a number of ways. Oxidation with potassium permanganate results in the corresponding carboxylic acid **53** in 75% yield,⁵¹ while selective reduction with sodium borohydride provides the alcohol **54** in good yield, without affecting the ester group, Scheme 8.^{52,53}



Scheme 8. Selective oxidation and reduction of aldehyde

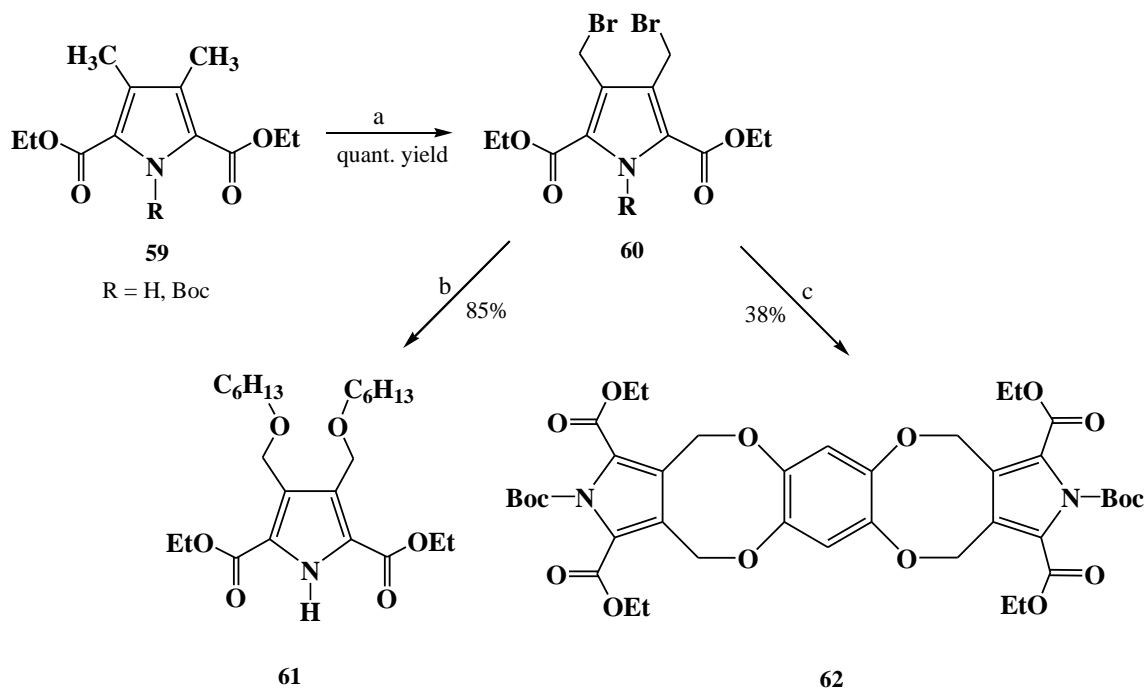
1.4.3.2 Halogenation *via* radical reactions

The α -methyl group of pyrrole **55** can be chlorinated selectively through a radical substitution reaction using sulphuryl chloride as the halogenating agent, to result in α -chloromethylpyrrole **56**, despite the other alkyl groups in the molecule. The alcoholysis of **56**, in ethanol, results **57**, while condensation in acidic ethanol with another pyrrole results in the formation of the dipyrrole **58**, which is a key intermediate in chlorophyll synthesis, Scheme 9.⁵⁴



Scheme 9. Chlorination of α -methylpyrrole **55**. Reagents and conditions; a) SO_2Cl_2 , AcOH, 55°C , 1h; b) EtOH, Δ ; c) 3-ethoxycarbonyl-4-methylpyrrole, HCl, EtOH, reflux, 1h

Radical β -halogenation can also be achieved if the substituent on the α -position is unreactive toward radical reactions and there is no free hydrogen on the ring. For example, both methyl groups of pyrrole **59** can be brominated selectively using NBS to afford the dibromide **60**, Scheme 10.⁵⁵ The dibromopyrrole **60** can then be used in further reactions as an electrophile. For example, the reaction with *n*-hexanol produces compound **61**, and cyclization of **60**, with 1,2,4,5-tetrahydroxybenzene as nucleophile, can be achieved to provide the pentacycle **62**.^{47,55}



Scheme 10. Halogenation of the β -alkyl group of pyrroles. Reagent and conditions; a) NBS, AIBN, CCl₄, reflux, 2.5 h; b) *n*-hexane-1-ol, toluene, Et₃N, reflux, 20 h; c) DMSO, Cs₂CO₃, r.t., 3 h

1.4.4 Synthesis of substituted pyrroles *via* cyclisation reactions

Considering the importance of this class of heterocycle, it is not a great surprise that a huge number of procedures have been developed for the synthesis of pyrroles.^{2c, 5a, 56} Most chemical research in this area involves substituted pyrroles rather than the parent compound itself. Different synthetic routes to these substituted derivatives exist and they can be readily obtained by substitution reactions of simple pyrroles, while alternative routes utilise suitable starting materials for direct cyclisation into substituted pyrroles, the functionalisation of already substituted pyrroles, or the interconversion of substituent groups.⁴⁷

Several different methods exist for the construction of a pyrrole ring using classical condensation reactions, the most common disconnections for which are shown in Figure 17.

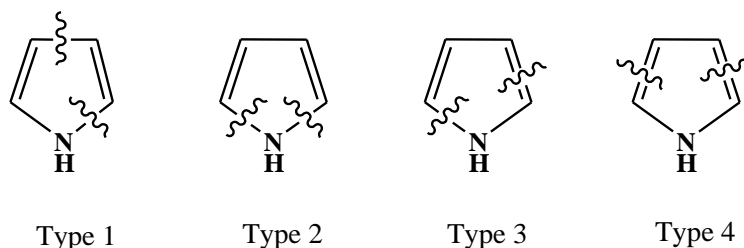
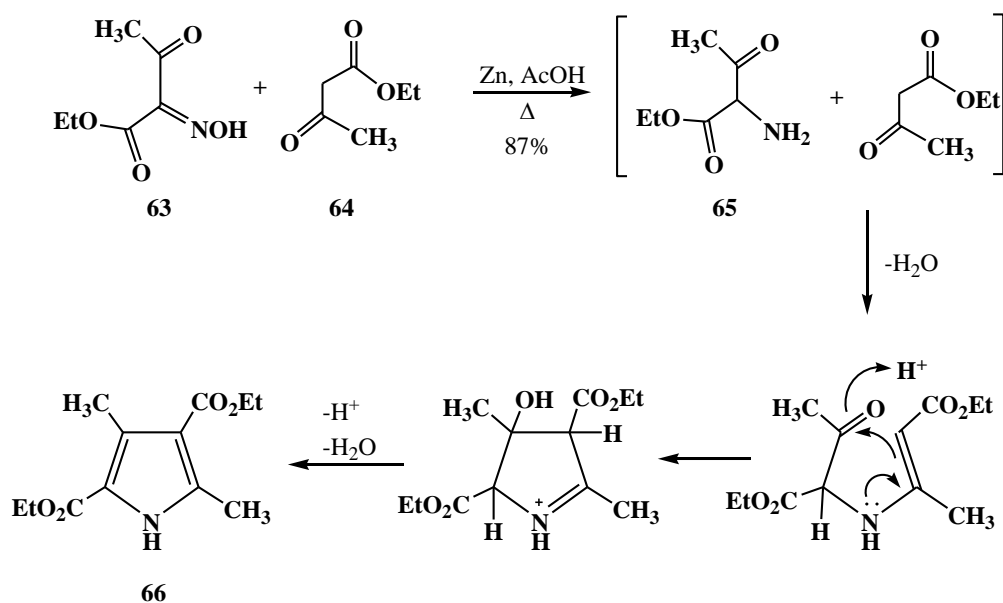


Figure 17. Different methods for the retrosynthetic cleavage of pyrroles

For each cyclisation method, a huge number of modifications have been developed in addition to the classical examples, so it is often difficult to predict which synthetic approach will be the most suited for the preparation of any specific pyrrole.

1.4.4.1 Knorr pyrrole synthesis

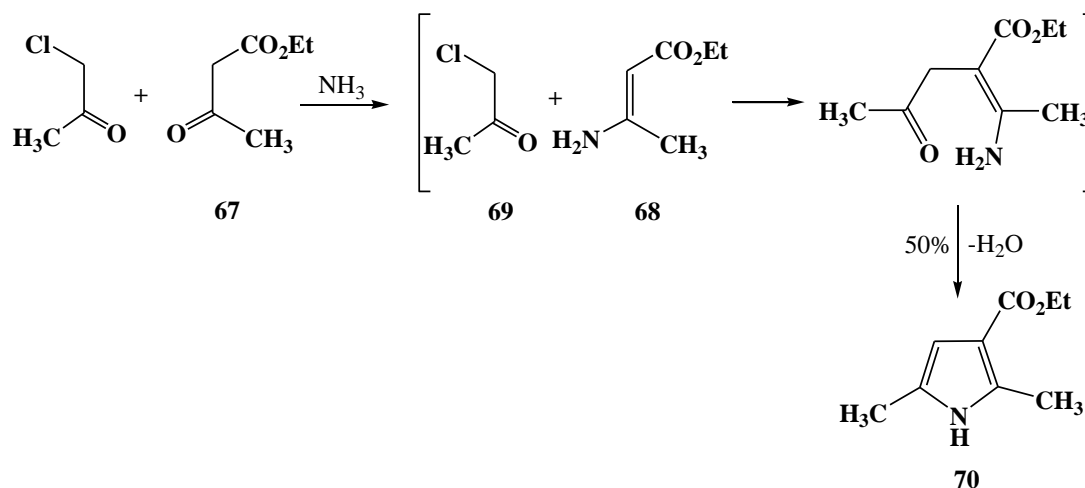
One of the most common syntheses of pyrroles is the classical Knorr reaction⁷ (retrosynthetic cleavage of type 1), which for e.g. results in the formation of pyrrole **66** after condensation of a ketone **64** with an α -aminoketone **65**, Scheme 11. The α -aminoketones must be prepared *in situ*, by the reduction of an oxime **63** (using zinc and acetic acid or sodium dithionite), because they self condense very readily (to form the corresponding pyrazines). The reaction proceeds rapidly at room temperature in ethanol, and provides the pyrrole **66** in high yield.⁵⁷ *N*-Substituted pyrroles can be prepared using secondary amines, which again have to be synthesised prior to the Knorr reaction.



Scheme 11. The Knorr pyrrole synthesis

1.4.4.2 Hantzsch pyrrole synthesis

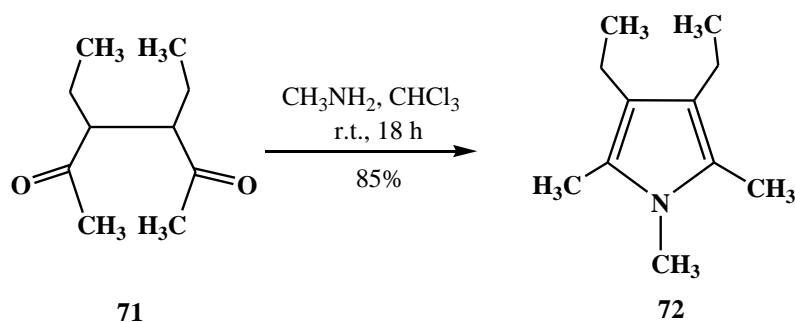
Another widely used reaction is the Hantzsch pyrrole synthesis (same retrosynthetic cleavage as the Knorr reaction, Type 1). Substituted 2-alkylpyrrole-3-carboxylic esters are easily prepared from the reaction of a dicarbonyl compound **67** with ammonia to give for e.g. the corresponding enamine **68**, followed by condensation with chloroacetone **69** to provide the pyrrole **70**, Scheme 12. This is an interesting alternative route to the Knorr reaction as the use of primary amines instead of ammonia gives *N*-substituted pyrroles.⁵⁸



Scheme 12. The Hantzsch pyrrole synthesis

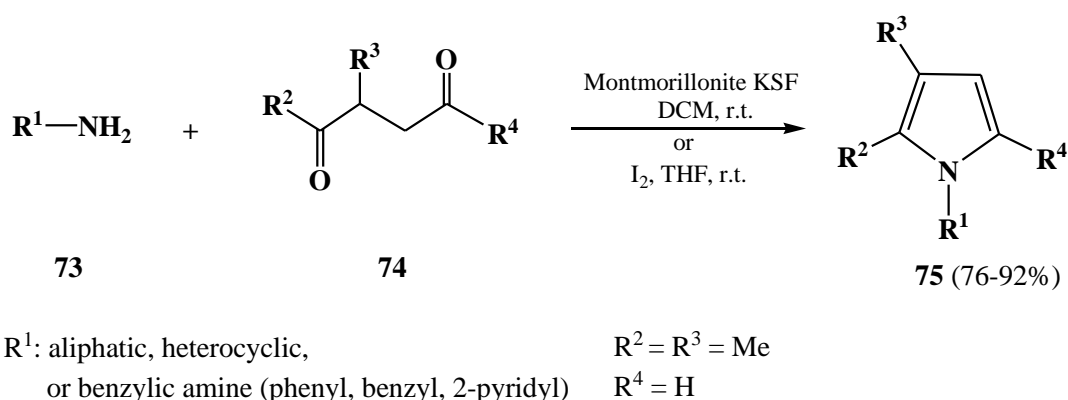
1.4.4.3 Paal-Knorr pyrrole synthesis

N-Substituted pyrroles can also be prepared by the Paal-Knorr synthesis, in which 1,4-dicarbonyl compounds react with ammonia or primary amines to give 3,4-disubstituted or 1,3,4-trisubstituted pyrroles. As an example of this method, 1,4-diketone **71** was reacted with methylamine at room temperature to provide pyrrole **72** in high yield, Scheme 13.⁵⁹



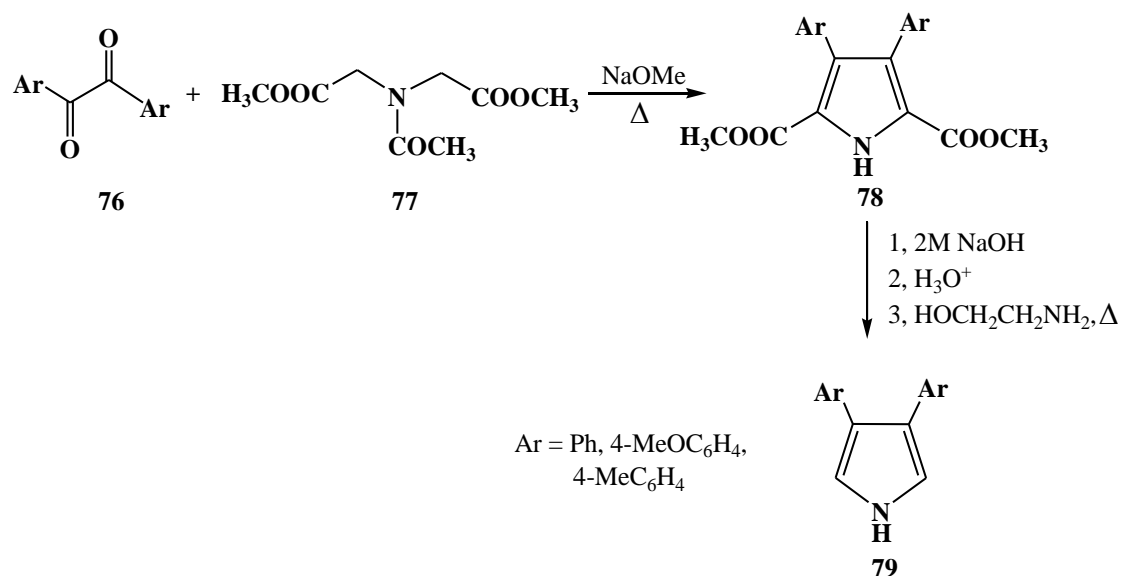
Scheme 13. Example of the Paal-Knorr synthesis of pyrroles

Banik and co-workers have reported a simple method for the synthesis of substituted pyrroles using iodine or montmorillonite KSF clay as the catalyst. The reaction was carried out at room temperature by mixing the catalyst with different amines **73** and substituted diketones **74** in the appropriate solvent, and then the solution was kept at room temperature for a specified time and resulted in pyrroles **75** in good yield, Scheme 14.⁶⁰



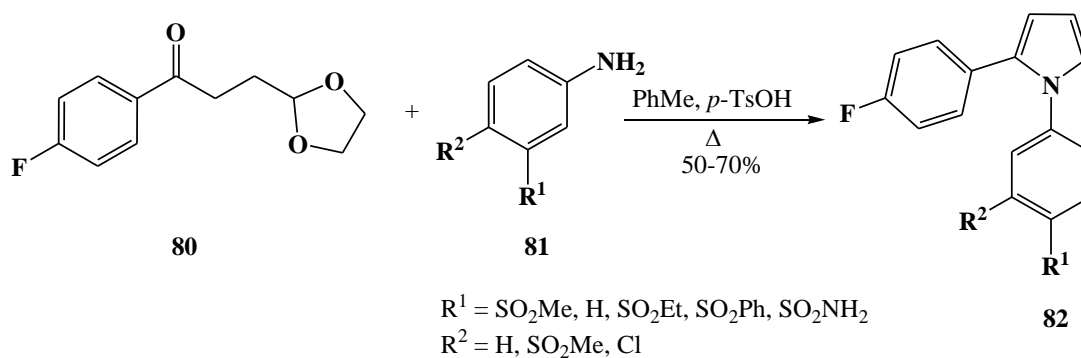
Scheme 14. Synthesis of substituted pyrroles

Several synthetic methods have been described for the synthesis of pyrrole derivatives with two aryl groups on adjacent positions.⁶¹ 3,4-Diarylpyrroles are the building blocks for the naturally occurring lamellarins^{61e} or ningalins.^{61f} The 3,4-diarylpyrroles **79** were prepared from dimethyl *N*-acetylaminodiacetate **77** and diketone **76** in the presence of sodium methoxide, followed by hydrolysis and decarboxylation, Scheme 15.^{61g}



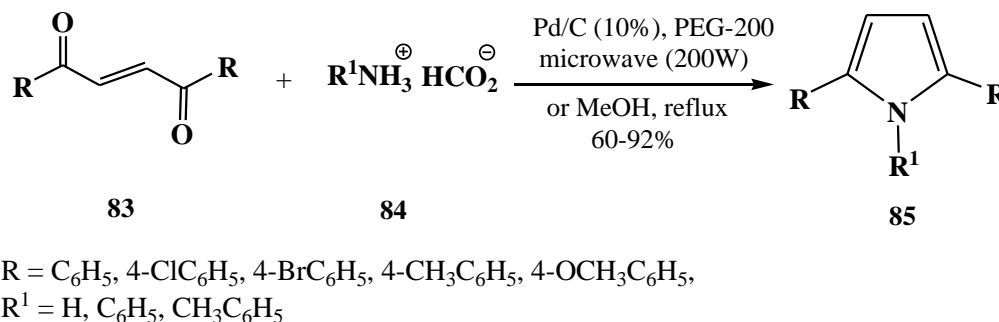
Scheme 15. Synthesis of 3,4-diarylpyrroles

1-(4-Fluorophenyl)-2-aryl-1*H*-pyrrole derivatives **82** were synthesized by Khanna and co-workers by the reaction of a 1,4-ketoacetal **80** and anilines **81**, in toluene in the presence of *p*-toluenesulfonic acid, Scheme 16.^{61c}

Scheme 16. Synthesis of 1,2-diaryl-1*H*-pyrroles

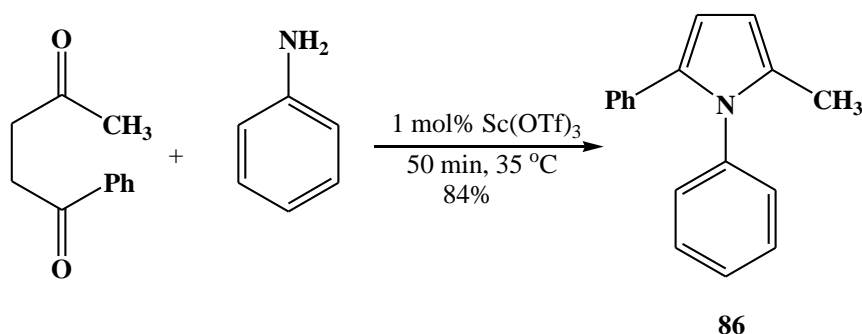
Rao *et al.* investigated the simple one-pot synthesis of 2,5-di- and 1,2,5-trisubstituted pyrrole derivatives **85** from (*E*)-1,4-diaryl-2-butene-1,4-diones **83** using ammonium

formates **84** in the presence of Pd/C in different solvents, under microwave irradiation, Scheme 17.⁶²



Scheme 17. Reaction of (*E*)-1,4-diaryl-2-butene-1,4-diones with ammonium formates

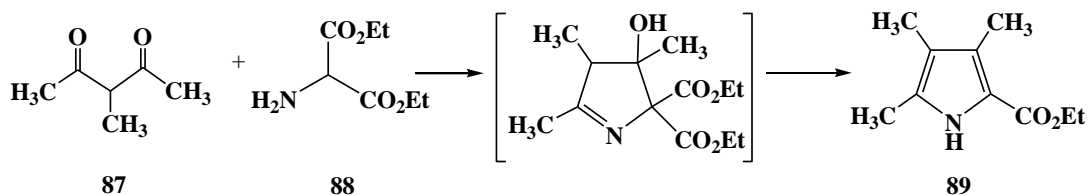
Su *et al.* have reported a new catalytic procedure for the synthesis of 1,2,5-trisubstituted pyrrole derivatives **86**. Most of the existing methods suffer from disadvantages such as long reaction times, harmful organic solvents or the use of an excess of acid. These workers reported an environmental friendly synthesis of pyrroles using metal triflates which are inexpensive, have low toxicity, high stability and can be easily recovered from water, Scheme 18.⁶³



Scheme 18. Sc(OTf)₃ catalysed synthesis of trisubstituted pyrroles under solvent-free conditions

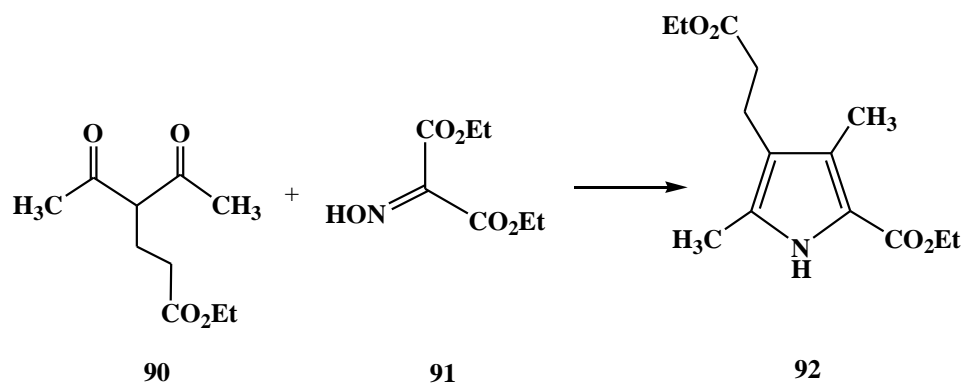
1.4.4.4 Modified Knorr syntheses

An interesting example of the Type 3 cyclisation method (Figure 17) is the modified Knorr synthesis, in which the β -diketone **87** reacts with an α -amino carbonyl compound **88**.⁶⁴ In the classical Knorr reaction, both reactants contribute two carbon atoms to the heterocyclic ring, while using the modified Knorr reaction three carbon atoms are derived from the 1,3-dicarbonyl compound and the amino compound donates one, in addition to the nitrogen, Scheme 19.⁶⁴



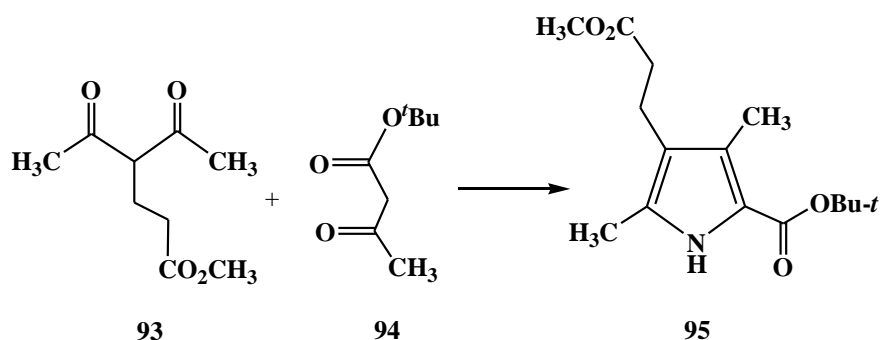
Scheme 19. Modified Knorr pyrrole synthesis of compound **89**. Reagents and conditions; AcOH, H₂O, reflux, 89%

Appropriately substituted 1,3-dicarbonyl compounds are required as the starting materials for the preparation of unsymmetrical β -substituted compounds because differentiation in the β -positions is not possible. The amino derivative was prepared *in situ* by the reduction of oxime **91** then reaction with one of the ketone functions of **90**, and a final cyclisation resulted in pyrrole **92** in good yield, Scheme 20.⁶⁵



Scheme 20. The modified Knorr reaction of the diester compound **92**. Reagents and conditions; Zn, AcOH, NaOAc, reflux, 2 h, 75%

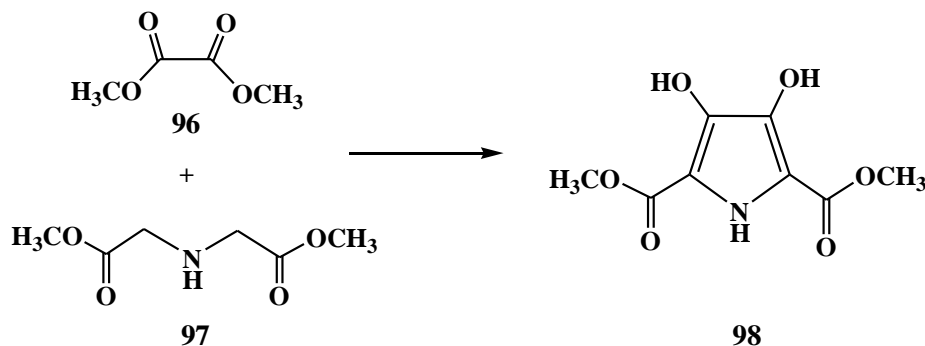
Using suitable starting materials, pyrroles can be prepared with two different ester groups. The reaction of acetoacetate **94** with sodium nitrite results in a β -diketo- α -oxime, then an *in situ* reduction with zinc results in an amine which undergoes the modified Knorr reaction with diketone **93** to result in pyrrole **95**, Scheme 21.⁶⁶



Scheme 21. Modified Knorr synthesis for the preparation of **95**. Reagents and conditions; a) NaNO_2 , AcOH, H_2O , 0°C , 12h, b) Zn, 65°C , 12 h

Another interesting process is the synthesis of different 3,4-disubstituted pyrroles (according to the type 4 retrosynthetic cleavage). The reaction of an α -dicarbonyl

compound **96** with a secondary amine **97** under basic conditions results in pyrrole **98** in good yield, Scheme 22.⁶⁷

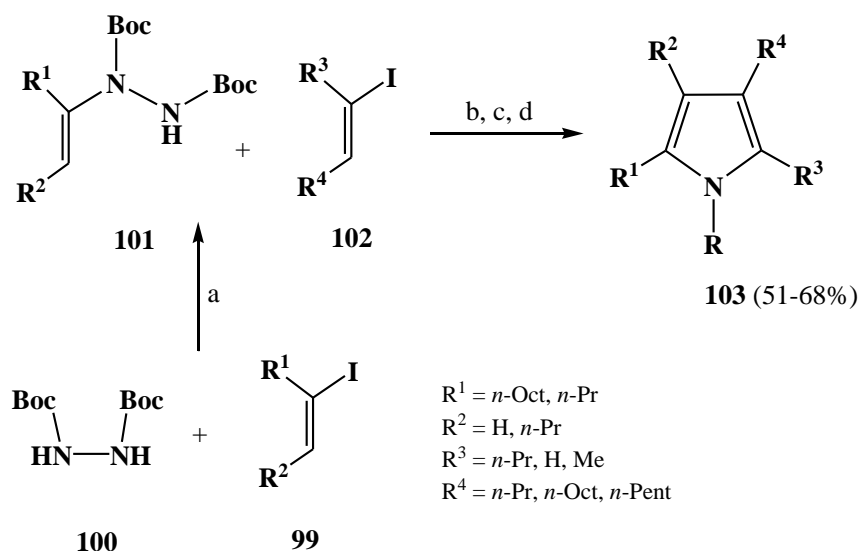


Scheme 22. Synthesis of 3,4-disubstituted pyrroles. Reagents and conditions;
NaOMe, MeOH, reflux, 5 h, 61%

1.4.4.5 Unconventional pyrrole syntheses

The synthesis of polysubstituted pyrrole rings is usually based on the classical condensation methods, as stated above, although these approaches suffer from a limitation in the substituents which can be introduced. Recently, several novel syntheses have been described;⁶¹ however, efficient multi-component coupling reactions, with methods involving fewer steps or regioselective approaches are still an extremely attractive area in the synthesis of multi-substituted pyrroles.

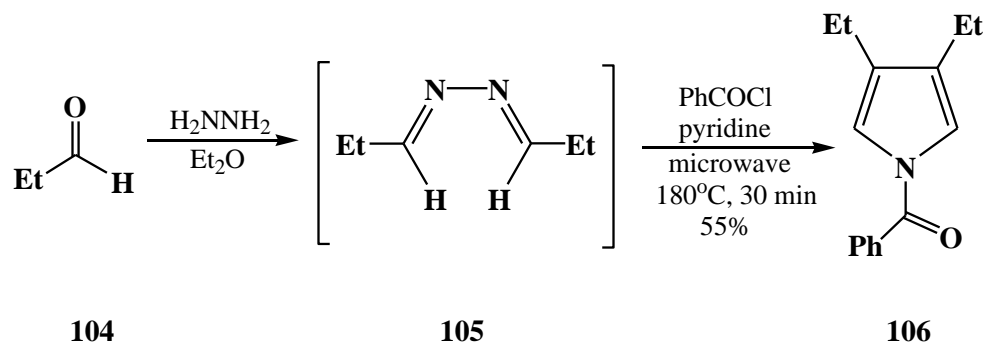
Buchwald and co-workers described a convenient and selective Piloty-Robinson synthesis of highly substituted pyrroles.⁶⁸ The reaction involves two sequential Cu-catalysed couplings of the corresponding vinyl iodides **99** and **102** with bis-Boc-hydrazine **100**, then a cyclisation to produce the substituted pyrrole **103**, Scheme 23.



Scheme 23. Synthesis of pyrroles through copper-catalysed vinylation of hydrazides.

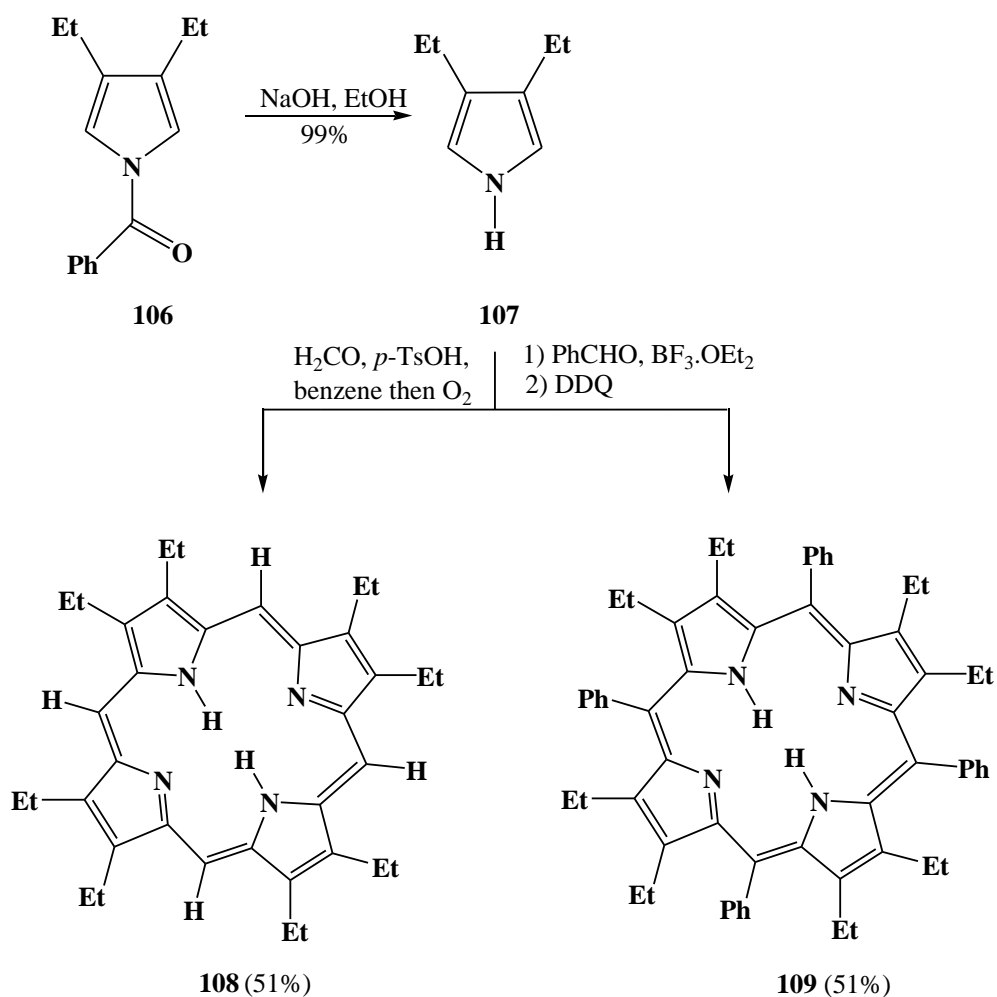
Reagents and conditions; a) CuI (5 mol%), 1,10-phenanthroline (10 mol%), Cs₂CO₃ (1.2 equiv), DMF, 80 °C, 12-13h; b) CuI (10 mol%), 1,10-phenanthroline (20 mol%), Cs₂CO₃ (1.2 equiv), DMF, 80 °C, 22-36h; c) xylene, 140 °C, 24-48 h; d) *p*-TsOH (2 equiv), r.t., 1-6 h

Scheidt *et al.* have recently devised a new and efficient method for the synthesis of *N*-acyl-3,4-disubstituted pyrroles which, compared to the previously reported method,⁶⁹ avoids high temperatures and long reaction times and produces high yields.⁷⁰ The process requires only two purification steps, uses inexpensive starting materials, and involves the reaction of a symmetric azine **105** from hydrazine and a saturated aldehyde **104**. Benzoyl chloride is then used, under microwave irradiation, for the cyclisation to afford the disubstituted *N*-acylpyrrole **106** in good yield, Scheme 24.⁷⁰



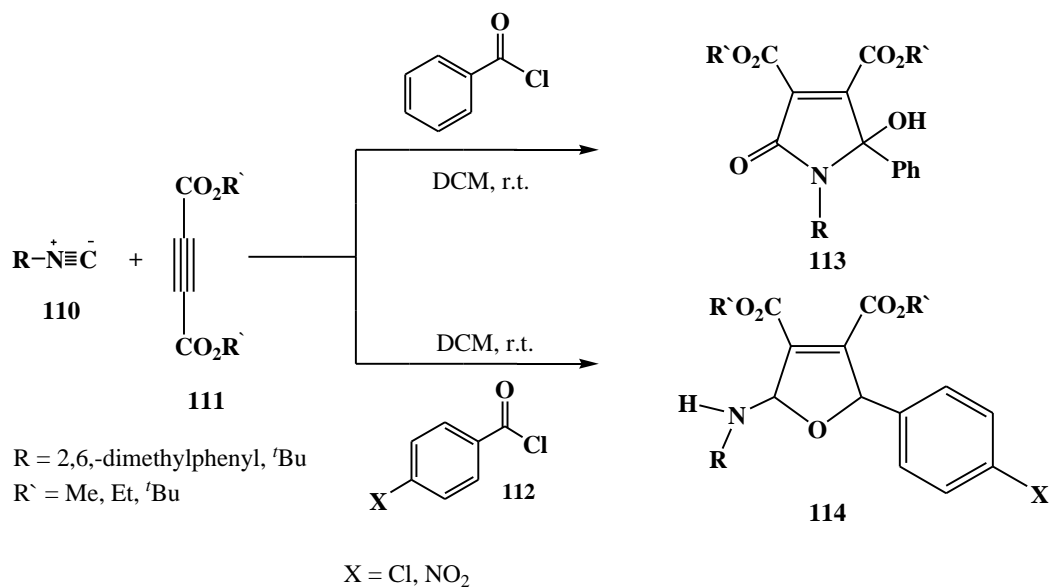
Scheme 24. Synthesis of 3,4-disubstituted pyrroles

In recent years symmetric 3,4-disubstituted pyrroles have received special interest since they are the basic building blocks for highly substituted porphyrins. For e.g. the product **106** could be converted to the free N-H pyrrole **107** by simple basic hydrolysis and then used directly in the synthesis of porphyrins **108** and **109**, Scheme 25.⁷¹



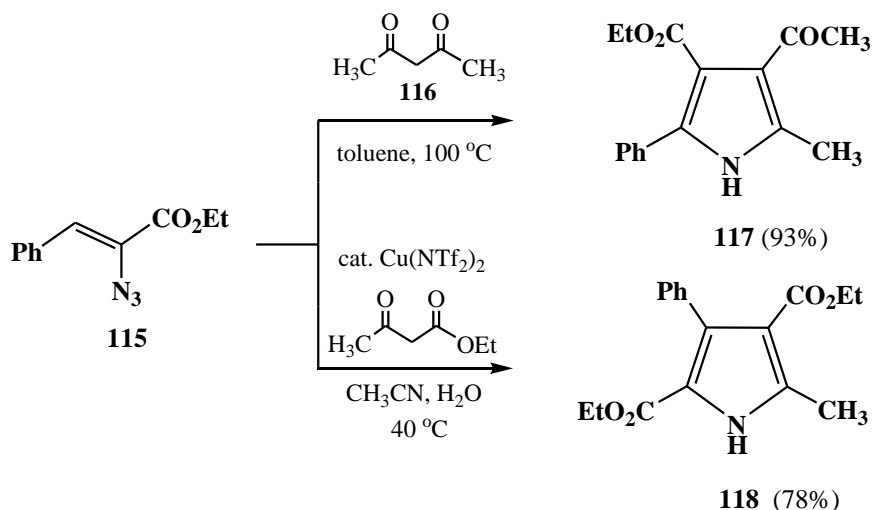
Scheme 25. Synthesis of porphyrin derivatives **108** and **109** from 3,4-disubstituted pyrrole **107**

Yavari and co-workers recently reported on a novel synthesis of functionalised 2,5-dihydro-1*H*-pyrroles **113**, based on the reaction of benzoyl chloride and dialkyl acetylenedicarboxylates **111** in the presence of isocyanides **110**. From the reaction with benzoyl chlorides **112**, which had electron-withdrawing groups at the *para* position, tetrasubstituted furans **114** were obtained, but the presence of electron donating Me or OMe groups afforded complex reaction mixtures, Scheme 26.⁷²



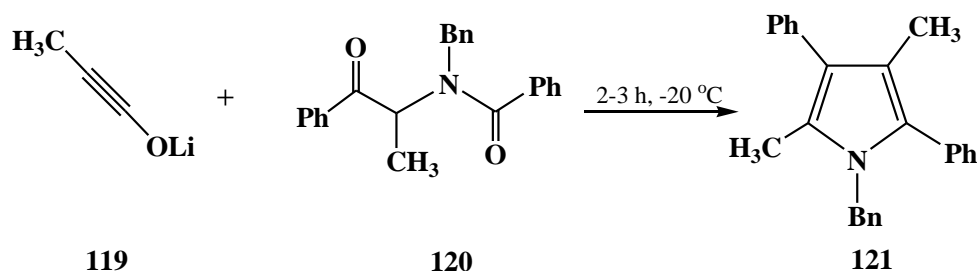
Scheme 26. Synthesis of functionalised pyrrole and furan derivatives

Narasaka *et al.* described new synthetic routes for the preparation of tetra- and trisubstituted pyrroles from vinyl azides and 1,3-dicarbonyl compounds. The reaction of the corresponding vinyl azide **115** with acetylacetone **116**, in toluene at 100°C, afforded several pyrrole derivatives **117**. To improve the yield these workers decided to use different additives, such as acids or bases, but the results did not show any significant improvement, although the reaction in the presence of a catalytic amount of Cu(OTf)₂, in CH₃CN gave the unexpected formation of pyrrole **118**, Scheme 27.⁷³



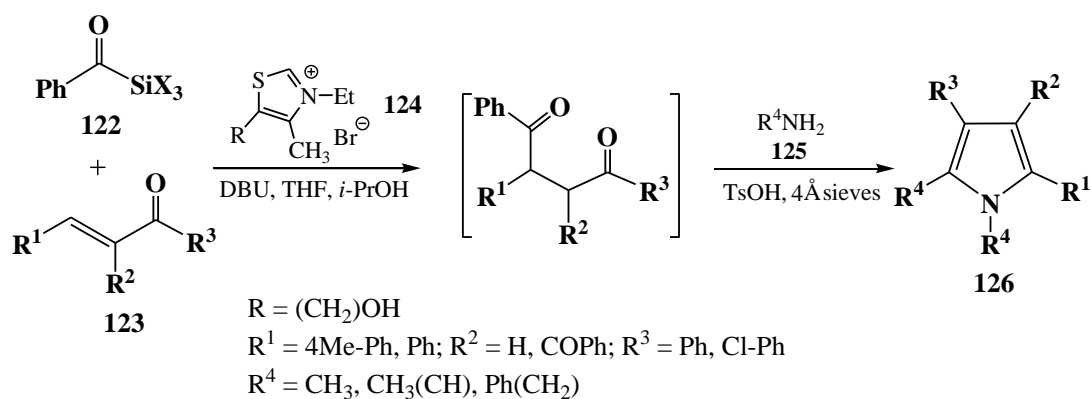
Scheme 27. Reaction of vinyl azides with acetylacetone

Shindo and co-workers reported an efficient one pot synthesis of pyrroles using ynolates **119** and α -acylaminoketones **120** which was carried out at -20°C over 2-3h. The reaction with aromatic, aliphatic and cyclic ketones resulted in penta- and tetrasubstituted pyrroles and sterically hindered, electron-withdrawing and functionalised acyl groups afforded the expected pyrroles **121**, Scheme 28.⁷⁴



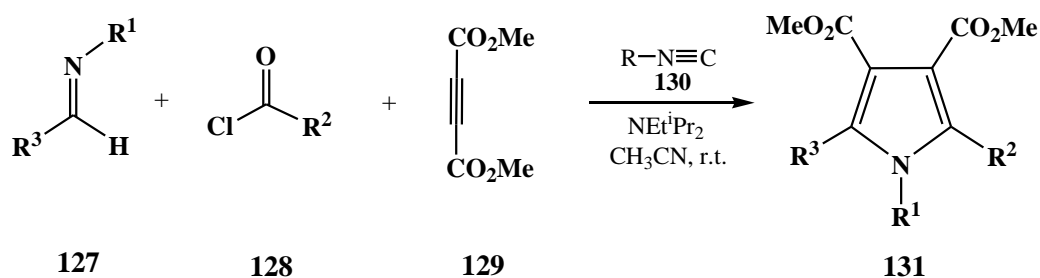
Scheme 28. One-pot synthesis of pyrroles

An interesting multi-component reaction has been reported by Scheidt *et al.* The one pot reaction of acylsilane **122**, as an acyl anion precursor, unsaturated carbonyl compound **123** and amine **125**, catalysed by a thiazolium salt **124**, gave highly substituted pyrroles **126** in over 80% yield, Scheme 29.⁷⁵



Scheme 29. Multi-component pyrrole synthesis

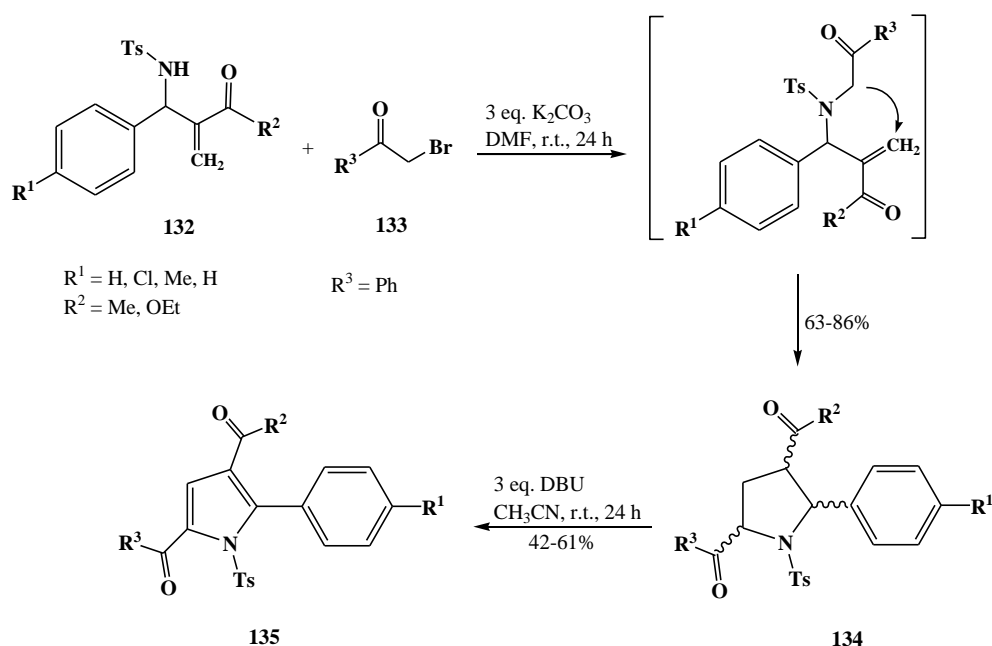
Arndsten and co-workers described a palladium-catalysed multi-component coupling of imines **127**, acid chlorides **128** and alkynes **129** to generate a number of substituted pyrroles **131**.⁷⁶ These workers found some limitations in using this approach, such as the slow rate of catalysis or using alkyl-substituted imines or acid chlorides which underwent rapid decomposition. Recently, these workers have developed an alternative route involving isocyanides **130** instead of the palladium-catalyst, and this allowed the use of a wide range of imines of aromatic and heteroaromatic aldehydes and a number of acid chlorides, Scheme 30.⁷⁷



$\text{R} = ^t\text{Bu, Cy, pentyl}$
 $\text{R}^1 = \text{Bn, Hex}$
 $\text{R}^2 = \text{Ph, Tol}$
 $\text{R}^3 = \text{Tol, MeO, Ph}$

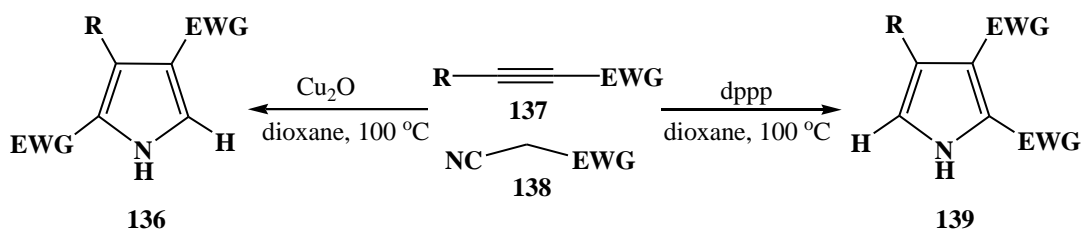
Scheme 30. Direct pyrrole synthesis

Kim *et al.* synthesised several polysubstituted pyrroles **135** from Baylis-Hillman adduct **132**, which was *N*-alkylated with phenacyl bromide **133**, in DMF and in the presence of K_2CO_3 , to result in a mixture of diastereoisomeric tetrahydropyrroles **134**. The elimination of *p*-toluenesulfonic acid was carried out with DBU in CH_3CN to give the pyrroles **135**, Scheme 31.⁷⁸



Scheme 31. Synthesis of polyfunctionalised pyrroles

Yamamoto *et al.* have developed a new regioselective synthesis of substituted pyrroles via [3+2] cycloadditions between isocyanides **138** and electron deficient alkynes **137**. The reaction in the presence of Cu_2O afforded 2,4-disubstituted pyrroles **136**, while the phosphine-catalysed reaction gave 2,3-disubstituted pyrroles **139** regioselectively, Scheme 32.⁷⁹

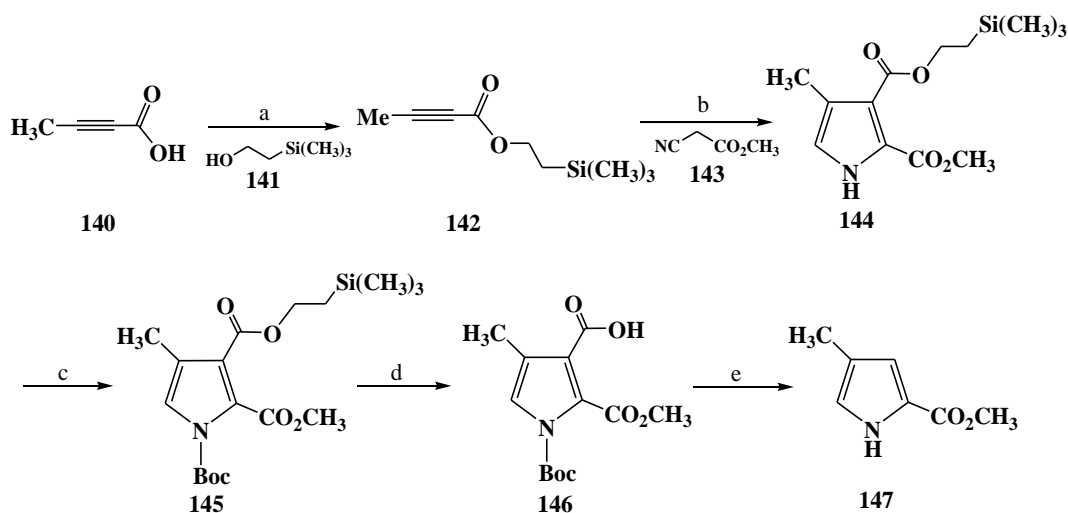


EWG = CO₂Et, COMe, CONEt₂, CN, SO₂Ph

R = Me, ^tBu, Ph, H

Scheme 32. Regioselective pyrrole synthesis

These workers applied the phosphine-catalysed condensation to the synthesis of the trail pheromone **147** of a leaf-cutting ant. This synthesis starts with the condensation of 2-butynoic acid **140** and 2-(trimethylsilyl)ethanol **141**, followed by the phosphine-catalysed reaction of ester **142** and methyl isocyanoacetate **143** to result in pyrrole **144**. The Boc-protected pyrrole **145** was then treated with TBAF to result in the carboxylic acid **146**, which was decarboxylated by Cu(OAc)₂ in ⁱPr₂NEt / anisole, Scheme 33.⁸⁰



Scheme 33. Ant trail pheromone synthesis. Reagents and conditions; a) DCC, pyridine, DCM, 0 °C, 1h; b) dppp, 1,4-dioxane, 100°C, 7 h; c) (Boc)₂O, 4-dimethylaminopyridine, CH₃CN, r.t., 13 h; d) TBAF, THF, 8 h; e) Cu(OAc)₂, anisole, ⁱPr₂NEt, 130 °C, 12 h

1.5 References

1. Yasuhiko, H.; Takayuki, S.; *Applied Catalysis A: General*, **2004**, 260, 251.
2. a) Baumann, K. L.; Butler, D. E.; Deering, C. F.; Mennen, K. E.; Millar, A.; Nanninga, T. N.; Palmer, C. W.; Roth, B. D.; *Tetrahedron Lett.*, **1992**, 33, 2283; b) Verniest, G.; Bombeke, F.; Claessens, S.; Van Thienen, T.; De Kimpe, N.; *Tetrahedron*, **2005**, 61, 2879; c) Katritzky, A.; Rees, C. W.; Scriven, E. F. V.; *Comprehensive Heterocyclic Chemistry*; Eds.; Pergamon: Oxford, **1996**; 2, 1.
3. a) John, T. G.; *Top Heterocycl Chem.*, **2006**, 2, 53; b) Banwell, M. G.; Goodwin, T. E.; Ng, S.; Smith, J. A.; Wong, D. J.; *Eur. J. Org. Chem*, **2006**, 3043; c) Hoffmann, H.; Lindel, T.; *Synthesis*, **2003**, 1753.
4. Holub, J. M.; Toole-Colin, K.; Getzel, A.; Argenti, A.; Evans, M. A.; Smith, D. C.; Dalglish, G. A.; Rifat, S.; Wilson, D. L.; Taylor, B. M.; Miott, U.; Glersaye, J.; Lam, K. S.; McCranor, B. J.; Berkowitz, J. D.; Miller, R. B.; Lukens, J. R.; Krumpe, K.; Gupton, J. T.; Burnham, B. S.; *Molecules*, **2004**, 9, 135.
5. a) Jones, R. A.; Bean, G. P.; *The Chemistry of Pyrroles*; Academic Press: London, **1977**, 209; b) Jones, R. A.; In *Pyrroles, Chemistry of Heterocyclic Compounds*; John Wiley & Sons: New York, **1990**; 48, 1, 499.
6. Pozharskii, A. F.; Soldatenkov, A. T.; Katritzky, A. R.; *Heterocycles in Life and Society*; **1997**.
7. a) Knorr, L.; *Ber. Dtsch. Chem. Ges.*, **1885**, 18, 299; b) Katritzky, A. R.; Ostercamp, D. L.; Yousaf, T. I.; *Tetrahedron*, **1987**, 43, 5171.
8. Paal, C.; *Ber. Dtsch. Chem. Ges.*, **1885**, 18, 367.
9. Black, D. St. C.; *Science of Synthesis*, **2000**, 9, 441.

10. Gilchrist, T. L.; *Heterocyclic Chemistry*, 3rd ed.; Longman Scientific & Technical; Essex, **1997**.
11. a) Woodward, R. B.; Ayer, W. A.; Beaton, J. M.; Bickelhaupt, F.; Bonnett, R.; Buchschacher, P.; Closs, G. L.; Dutler, H.; Hannah, J.; Hauck, F. P.; Ito, S.; Langemann, A.; Le Goff, E.; Leimgruber, W.; Lwowski, W.; Sauer, J.; Valenta, Z.; Volz, H.; *Tetrahedron*, **1990**, *46*, 7599; b) Joule, J. A.; Mills, K.; *Heterocyclic Chemistry*, 4th ed.; Blackwell Publishing, **2000**.
12. Sternberg, E. D.; Dolphin, D.; Bruckner, C.; *Tetrahedron*, **1998**, *54*, 4151.
13. Fenelon, A.; Breslin, C. B.; *Corrosion Science*, **2003**, *45*, 2837.
14. Reza, A.; *E-Journal of Chemistry*, **2006**, *3*, 186.
15. Hideo, Y.; Masashi, O.; Minoru, F.; Isao, I.; Katsumi, Y.; *Journal of Power Sources*, **1996**, *60*, 173.
16. Frydman, R. B.; Frydman, B.; *The Porphyrins*, vol. 4., Academic Press, New York, **1979**, 1.
17. Reynolds, E.; *The Lancet Neurology*, **2006**, *5*, 949.
18. a) Nakano, H.; Umio, S.; Kariyone, K.; Tanaka, K.; Kishimoto, T.; Noguchi, H.; Ueda, I.; Nakamura, H.; Morimoto, Y.; *Tetrahedron Lett.*, **1966**, 737.
19. Irmeler, S.; Rogniaux, H.; Hess, D.; Pillonel, C.; *Pesticide Biochemistry and Physiology*, **2006**, *84*, 25.
20. Guido, V.; Sven, C.; Filip, B.; Tinneke, V. T.; Norbert, De-K.; *Tetrahedron*, **2005**, *61*, 2879.
21. De Kimpe, N.; Tehrani, K. A.; Stevens, C.; De Cooman, P.; *Tetrahedron*, **1997**, *53*, 3693.

22. Nakano, H.; Umio, S.; Kariyone, K.; Tanaka, K.; Kishimoto, T.; Noguchi, H.; Ueda, I.; Nakamura, H.; Morimoto, Y.; *Tetrahedron Lett.*, **1966**, 737; b) Black, B. C.; Hollingworth, R. M.; Ahammadsahib, K. I.; Kukel, C. D.; Donovan, S.; *Chem. Abstr.*, **1995**, 122, 25831.
23. a) Ming-Thau, S.; Jender, W.; Chih-Jui, C.; Su-Hui, C.; An-Bang, W.; *Tetrahedron Lett.*, **2004**, 45, 8107; b) Cannell, G. R.; Vesey, D. A.; Dickinson, R. G.; *Life Science*, **2001**, 70, 37.
24. Flavio, C.; Marina, D-G.; Maria, R. I.; Sara, M.; Lucio, P.; Fabio, T.; *Tetrahedron*, **2006**, 62, 7390.
25. a) Yigal, K.; Christopher, A., H.; *Current Opinion in Chemical Biology*, **1997**, 1, 176; b) Qing-Y., S.; Jian-M. X.; Yong-B. C.; Wan-N. Z.; Qiu-Y., W.; Da-Z., Z.; Jun Z.; Hui-Q., Z.; Yuan-Y., J.; *Eur. J. Med. Chem.*, **2007**, 42, 1226.
26. Onnis, V.; Alessandro, De L.; Maria, T. C.; Roberta, F.; Rita, M.; Cenzo, C.; *Eur. J. Med. Chem.*, **2008**, Article in press.
27. Seto, H.; Hayakawa, Y.; Kawakami, K.; *Tetrahedron Lett.*, **1992**, 33, 2701.
28. a) Laatsch, H.; Pudleiner, H.; *Liebigs Ann. Chem.*, **1989**, 863; b) Ohri, R. V.; Radosevich, A. T.; Hrovat, K. J.; Musich, C.; Huang, D.; Holman, T. R.; Toste, F. T.; *Org. Lett.*, **2005**, 7, 2501; c) Raimondi, M. V.; Cascioferro, S.; Schillachi, D.; Petruso, S.; *Eur. J. Med. Chem.*, **2006**, 41, 1439.
29. Nonell, S.; Bou, N.; Borrell, J. I.; Teixido, J.; Villanueva, A.; Juarranz, A.; Canete, M.; *Tetrahedron Lett.*, **1995**, 36, 3405.
30. Brothers, P. J.; *Chem. Commun.*, **2008**, 2090.

31. Ulf, B.; Achim, G.; Karl-W., K.; Matthias, K.; *Biochemical and Biophysical Research Communications*, **2008**, 373, 419.
32. Raposo, M. M. M.; Sousa, A. M. C.; Fonseca, A. M. C.; Kirschb, G.; *Tetrahedron*, **2005**, 61, 8249.
33. Joseph, A. M.; *Journal of Agricultural and Food Chemistry*, **1981**, 29.
34. Barbara, S.; Michael, M.; *Food Chemistry*, **2004**, 84, 367.
35. Butler, M. S.; *J. Nat. Prod.*, **2004**, 67, 2141.
36. Gossauer, A.; *Prog. Chem. Org. Nat. Prod.*, **2003**, 86, 1.
37. a) Cafieri, F.; Fatturoso, E.; Tagliatela, S. O.; *J. Nat. Prod.*, **1998**, 61, 122; b) Sosa, A. C. B.; Yakushijin, K.; Horne, D. A.; *J. Org. Chem.*, **2000**, 65, 610.
38. a) Andreani, A.; Granaiolo, M.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M.; Geraline, V.; Farruggia, G.; Masotti, L.; *Bioorg. Med. Chem.*, **2004**, 12, 1121; b) Andreani, A.; Granaiolo, M.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M.; Geraline, V.; *J. Med. Chem.*, **2002**, 45, 2666.
39. Masamune, H.; Cheng, J. B.; Cooper, K.; Eggler, J. F.; Marfat, A.; Marshall, S. C.; Shirley, J. T.; Tickner, J. E.; Umland, J. P.; Vazquez, E. *Bioorg. Med. Chem. Lett.*, **1995**, 5, 1965.
40. Sun, L.; Tran, N.; Liang, C.; Hubbard, S.; Tang, F.; Lipson, K.; Schreck, R.; Zhou, Y.; McMahon, G.; Tang, C.; *J. Med. Chem.*, **2000**, 43, 2655; Sun, L.; Tran, N.; Liang, C.; Shirazian, S.; Zhou, Y.; Miller, T.; Cui, J.; Fukuda, J. Y.; Chu, J. Y.; Nematalla, A.; Wang, X.; Chen, H.; Sistla, A.; Luu, T. C.; Tang, F.; Wei, J.; Tang, C.; *J. Med. Chem.*, **2003**, 46, 1116.
41. Bailly, C.; *Curr. Med. Chem – AntiCancer Agents*, **2004**, 4, 364.

42. a) Quesada, A. R.; Gravalos, M. D. G.; Puentes, J. L. F. Br.; *J. Cancer*, **1996**, 74, 677; b) Iwao, M.; Takeuchi, T.; Fujikawa, N.; Fukuda, T.; Ishibashi, F.; *Tetrahedron Lett.*, **2003**, 44, 4443.
43. Chiang, Y.; Hinman, R. L.; Theodoropoulos, S.; Whipple, E. B.; *Tetrahedron*, **1967**, 23, 745.
44. a) Lubell, W. D.; Thompson, A.; Chapman, E. E.; Jolicueur, B.; *Tetrahedron*, **2006**, 62, 11531; b) Green, W. T.; Wuts, M. G. P.; *Protective Groups in Organic Synthesis*, 3rd ed., John Wiley & Sons, **1999**.
45. a) Muchowski, J. M.; Bray, B. L.; Mathies, P. H.; Naef, R.; Solas, D. R.; Tidwell, T. T.; Artis, D. R.; *J. Org. Chem.*, **1990**, 55, 6317; b) Wong, H. N. C.; Liu, J-H., Chan, H-W.; *J. Org. Chem.*, **2000**, 65, 3274.
46. a) Balasundaram, B.; Venugopal, M.; Perumal, P. T.; *Tetrahedron Lett.*, **1993**, 34, 4249; b) Hongbo, D.; Joseph, P. K.; *Organic Lett.*, **2001**, 3, 3001; c) Drew, M. G. B.; George, A. V.; Isaacs, N. S.; *J. Chem. Soc., Perkin Trans. 1*, **1985**, 1277.
47. Schmuck, C.; Rupprecht D.; *Synthesis*, **2007**, 20, 3095.
48. a) Yadav, J. S.; Reddy, B. V. S.; Kondaji, G.; Srinivasa, R. R.; Kumar, P. S.; *Tetrahedron Lett.*, **2002**, 43, 8133; b) Firouzabadi, H.; Iranpoor, N.; Nowrouzi, F.; *Tetrahedron*, **2004**, 60, 10843.
49. Purkarthofer, T.; Gruber, K.; Fechter, M. H.; Griengl, H.; *Tetrahedron*, **2005**, 61, 7661; b) Gilow, H. M.; Burton, D. E.; *J. Org. Chem.*, **1981**, 46, 2221.
50. Gaunt, J. M.; Beck, E. M.; Grimster, N. P.; Hatley, R.; *J. Am. Chem. Soc.*, **2006**, 128, 2528.
51. Schmuck, C.; *Eur. J. Org. Chem.*, **1999**, 2397.

52. Chakraborty, T. K.; Mohan, B. K.; Kumar, S. K.; Kunwar, A., C.; *Tetrahedron Lett.*, **2002**, *43*, 2589.
53. Schmuck, C.; Graupner, S.; *Tetrahedron, Lett.*, **2005**, *46*, 1295.
54. Woodward, R. B.; Ayer, W. A.; Beaton, J. M.; Bickelhaupt, F.; Bonnett, R.; Buchschacher, P.; Closs, G. L.; Dutler, H.; Hannah, J.; Hauck, F. P.; Ito, S.; Langemann, A.; Le Goff, E.; Leimgruber, W.; Lwowski, W.; Sauer, J.; Valenta, Z.; Volz, H.; *Tetrahedron*, **1990**, *46*, 7599.
55. Patterson, J., M.; *Synthesis*, **1976**, 281.
56. a) Schmuck, C.; Wienand, W.; *J. Am. Chem. Soc.*, **2003**, *125*, 452; b) Bachiocchi, E.; Muraglia, E.; Sleiter, G.; *J. Org. Chem.* **1992**, *57*, 2486.
57. Fabiano, E.; Golding, B., T.; *J. Chem. Soc. Perkin Trans. 1*, **1991**, 3371.
58. Agosta, W. C.; *J. Org. Chem.*, **1961**, *26*, 1724.
59. Amarnath, V.; Anthony, D. C.; Amarnath, K.; Valentine, W. M.; Wetterau, L. A.; Graham, D. C.; *J. Org. Chem.*, **1991**, *56*, 6924.
60. Banik, B. K.; Samajdar, S.; Banik, I.; *J. Org. Chem.*, **2004**, *69*, 213.
61. Biava, M.; Fioravanti, M.; Porretta, G. C.; Deidda, D.; Maullu, C.; Pompei, R.; *Bioorg. Med. Chem. Lett.*, **1999**, *9*, 2983; b) Biava, M.; Porretta, G. C.; Capelli, A.; Vomero, S.; Manetti, F.; Botta, M.; Sautebin, L.; Rossi, A.; Makovec, F.; Anzini, M.; *J. Med. Chem.*, **2005**, *48*, 3428; c) Khanna, I. K.; Weier, R. M.; Yu, Y.; Collins, P. W.; Miyashiro, J. M.; Koboldt, C. M.; Veenhuizen, A., W., Currie, J. L.; Seibert, K.; Isakson, P. C.; *J. Med. Chem.*, **1997**, *40*, 1619; d) Rossi, R.; Fabio, B.; *Tetrahedron*, **2006**, *62*, 7213; e) Gilchrist, T.; *J. Chem. Soc. Perkin Trans. 1*, **2001**, 2491; f) Kang, H.; Fenical, W.; *J. Org. Chem.*, **1997**, *62*, 3254; g) Light, M. E.; Coles, S. J.; Hursthouse, M., B. J.; *J. Org. Chem.*, **2001**, *66*, 7849.

62. Rao, H.; Surya, P.; Jothilingam, S.; *Tetrahedron Lett.*, **2001**, 42, 6595.
63. Su, W.; Chen, J.; Wu, H.; Zheng, Z.; Jin, C.; Zhang, X.; *Tetrahedron Lett.*, **2006**, 47, 5383.
64. Paine, J. B.; Dolphin, D.; *J. Org. Chem.*, **1985**, 50, 5598.
65. Xie, M.; Lightner, D.; *Tetrahedron*, **1993**, 49, 2185.
66. Harbuck, J. W.; Rapoport, H.; *J. Org. Chem.*, **1971**, 36, 853.
67. Merz, A.; Schropp, R.; Dotterl, E.; *Synthesis*, **1995**, 795.
68. Buchwald, S. L.; Rivero, M. R.; *Organic Lett.*, **2007**, 9, 973.
69. Baldwin, J. E.; Bottaro, J. C.; *J. Chem. Soc., Chem. Commun.*, **1982**, 624.
70. Scheidt, K. A.; Benjamin, C. M.; Katrine, E.; Steven, M. R.; Scheidt, W. R.; *J. Org. Chem.*, **2007**, 72, 3941.
71. a) Hinze, C.; Kreipl, A.; Terpin, A.; Steglich, W.; *Synthesis*, **2007**, 608; b) Nishizawa, T.; Gruschow, S.; Jayamaha, D. H. E.; Nishizawa-H., C.; Sherman, D. H.; *J. Am. Chem. Soc.*, **2006**, 128, 724.
72. Yavari, I.; Sanandaj-M, A.; Moradi, L.; Mirzaei, A.; *Tetrahedron*, **2008**, 64, 5221.
73. Narasaka, K.; Chiba, S.; Wang, Y-F.; Lapointe, G.; *Org. Lett.*, **2008**, 10, 313.
74. Shindo, M.; Yoshimura, Y.; Hayashi, M.; Soejima, H.; Yoshikawa, T.; Matsumoto, K.; Shishido, K.; *Org. Lett.*, **2007**, 9, 1963.
75. Scheidt, K. A.; Bharadwaj, A. R.; *Org. Lett.*, **2004**, 6, 2465.
76. Arndsten, B. A.; Dhawan R.; *J. Am. Chem. Soc.*, **2004**, 126, 468.
77. Arndsten, B. A.; Cyr, D.; J., St.; Martin, N.; *Org. Lett.*, **2007**, 9, 449.
78. Hyuan, S. L.; Jeong, M. K.; Jae, N. K.; *Tetrahedron Lett.*, **2007**, 48, 4119.

79. Yamamoto, Y.; Kamijo, S.; Kanazawa, C.; *J. Am. Chem. Soc.*, **2005**, *127*, 9260.
80. Yamamoto, Y.; Kamijo, S.; Kanazawa, C.; *Tetrahedron Lett.*, **2005**, *46*, 2563.

Chapter Two

Results and Discussion

2. Results and Discussion

2.1 Aims

The initial aims of this research project were the synthesis of polyfunctional pyrroles and the further investigation of the chemoselectivity of the reactions of these pyrroles with a range of reagents in order to determine the regioselectivity of the reactions of these heterocyclic building blocks and to investigate the use of these substituted pyrroles in libraries. The first investigations of the chemoselectivity of the reactions of multifunctional pyrroles with a range of nucleophiles were previously undertaken in this Department, and our aim was to continue this work. These pyrroles are interesting heterocyclic intermediates as they have a range of reactive centres, and the chemoselectivity of their reactions under a range of conditions is, therefore, of much interest. Polyfunctional pyrroles are relatively difficult to prepare, but the reactions of these substituted pyrroles allows the preparation of a wide variety of new substituted heterocyclic compounds *via* these intermediates.

2.2 Synthesis of 3,5-dichloro-1*H*-pyrrole-2,4-dicarboxaldehyde

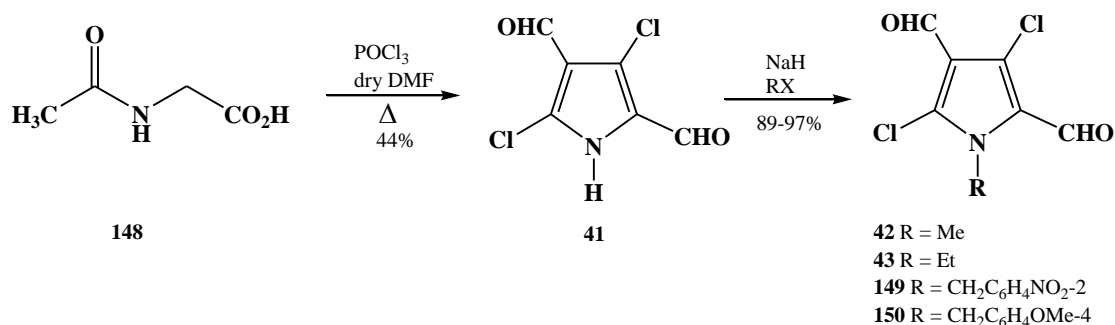
3,5-Dichloro-1*H*-pyrrole-2,4-dicarboxaldehyde **41** was first synthesised by Balasundaram and co-workers in 1993 using the Vilsmeier reaction of *N*-acetylglycine **148**, Scheme 34.¹ and it was also successfully synthesized in this Department, in 44% yield.² We initially attempted to improve the yield of the pyrrole from the Vilsmeier reaction of *N*-acetylglycine **148** and a chlorinating agent. In the original method, DMF, *N*-acetylglycine and POCl₃ were added together at 0 °C but

we prepared the Vilsmeier reagent first, from DMF and POCl₃ at 0 °C, and then stirred for half an hour at ambient temperature. *N*-Acetylglycine was added to the reaction at room temperature and, after stirring for 1 hour, the mixture was refluxed, Scheme 34. This small variation led to an increase in the yield (56% instead of 44%). Replacement of the chlorinating agent used initially, POCl₃, by oxalyl chloride (thus allowing isolation of a crystalline Vilsmeier reagent before the reaction with *N*-acetylglycine) did not lead to an improvement in the yield.

We then found another method to increase the yield, based on the continuous extraction of the product from the aqueous phase into the organic layer, and this variation gave the desired pyrrole in 70% yield.

3,5-Dichloro-1*H*-pyrrole-2,4-dicarboxaldehyde **41** is polyfunctional, with 5 reactive centres (2 aldehyde groups, 2 electrophilic carbons of the pyrrole ring bonded to chlorine atoms, and an NH) and there are, therefore, a range of potential reactions of this pyrrole with nucleophiles.

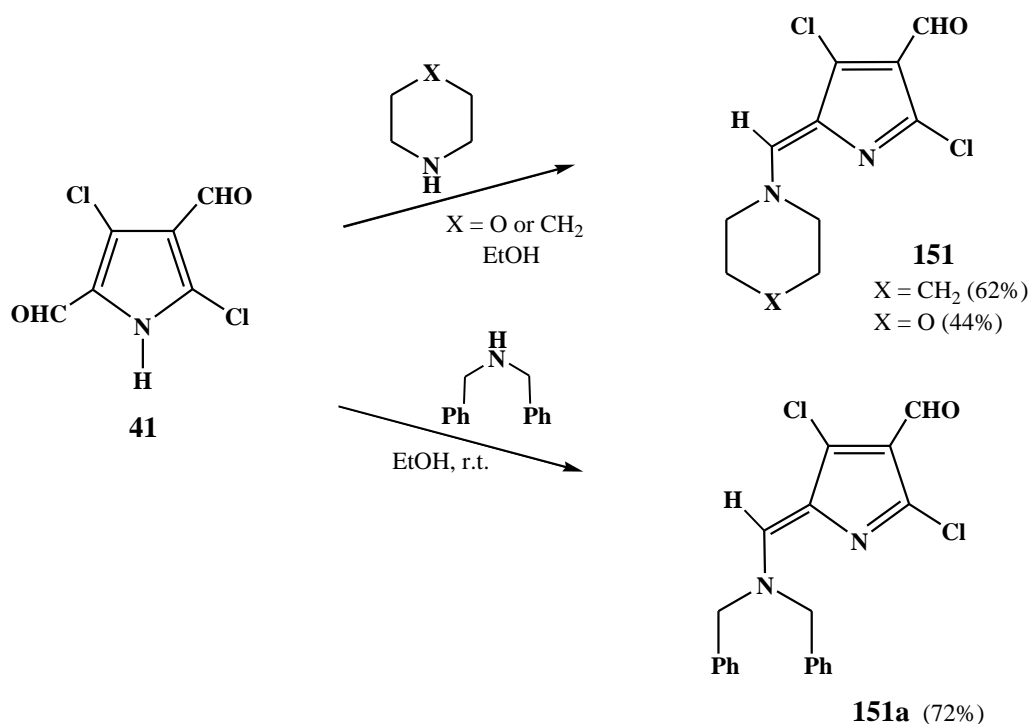
Substituted analogues (**42**, **43**, **149** and **150**) were readily prepared by alkylation of the pyrrole anion, Scheme 34.²



Scheme 34

2.2.1 Nucleophilic substitution of 3,5-dichloro-1*H*-pyrrole-2,4-dicarboxaldehyde

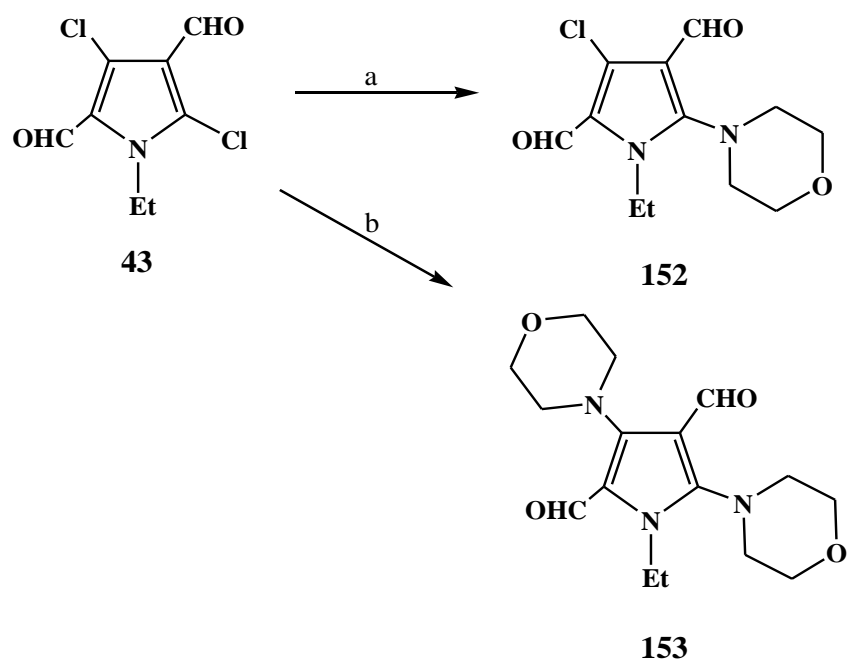
The reaction of polyfunctional pyrroles with nucleophiles has already been investigated² and the initial results indicate that these pyrroles undergo chemoselective reactions with nucleophiles. The reaction of the parent pyrrole **41** with morpholine or piperidine gives the 5-methylenepyrroles **151** *via* nucleophilic attack on the 2-formyl group, and the reaction with dibenzylamine resulted in the novel compound **151a** which is more stable than the piperidine and morpholine analogues, Scheme 35.



Scheme 35

In order to facilitate nucleophilic substitution of the chloro-substituents, the labile NH proton was replaced by an alkyl group. For the substituted pyrrole **43**, attack by sulphur or amino nucleophiles takes place at C-5, presumably due to the reduced

electrophilic nature of the C-3 'enamine-like' position, to give the pyrroles **152**. Substitution of both chloro groups, to give pyrrole **153**, requires more forcing conditions, Scheme 36.²



Scheme 36.² Reagents and conditions; (a) 2.5 equiv. morpholine, DMSO, r.t., 3 days, 45%; (b) 5 equiv. morpholine, EtOH, Δ , 70 h, 21%

2.3 Reaction of the aldehyde groups

Carbonyl groups in indoles maintain their characteristic properties due to the inductive effect of the nitrogen and the aldehyde group in the α -position increases the electrophilicity of the aldehyde carbon, Figure 18.

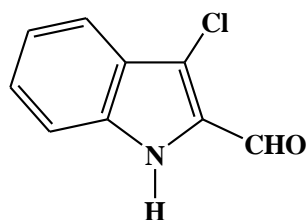
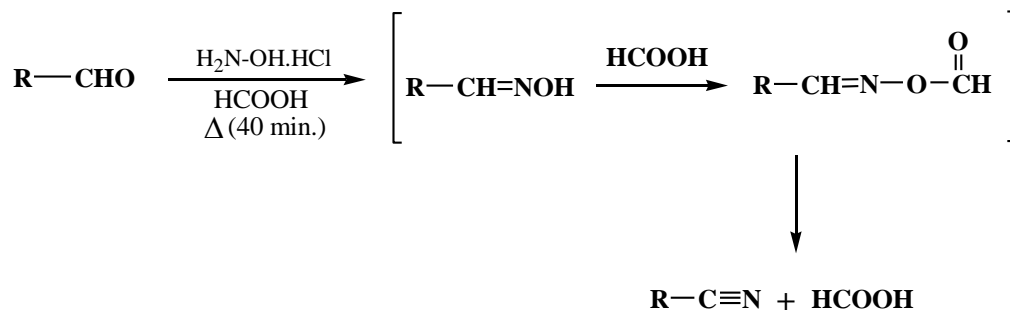


Figure 18

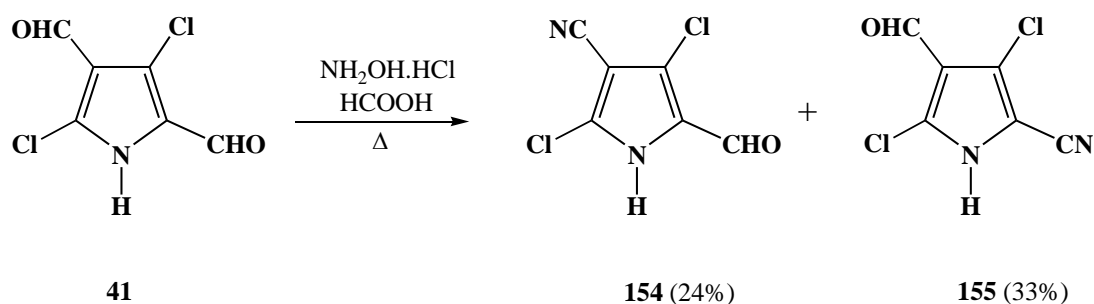
2.3.1 Conversion of aldehydes into nitriles

The transformation of an aldehyde into a nitrile is an important process in organic chemistry,³ and nitriles are especially useful starting materials for the synthesis of various bioactive molecules.⁴ Several procedures are available for the one-step conversion of aldehydes into nitriles using different chemical reagents,⁵ but most of these methods suffer from serious drawbacks which include the use of hazardous / expensive / commercially non-available reagents, long reaction times and low yields. A useful procedure for the direct conversion of aromatic aldehydes into the corresponding nitriles involves refluxing a solution of the aldehyde and hydroxylamine hydrochloride in 95-98% formic acid and this has been reported to result in the nitrile in 1 hour, in excellent yield, Scheme 37.⁶



Scheme 37

It was hoped that the reaction of 1 equivalent of the unsubstituted pyrrole **41** with 1.2 equivalents of hydroxylamine hydrochloride in 95-98% formic acid would give the 2-carbonitrile, however, the reaction afforded a mixture of the 2- **155** and 4-carbonitriles **154**, Scheme 38. The main product was the 2-carbonitrile **155**, presumably because the carbon of the aldehyde group in the 2-position of the pyrrole possesses a greater positive charge (is more electrophilic) than the carbon of the aldehyde group in 4-position.

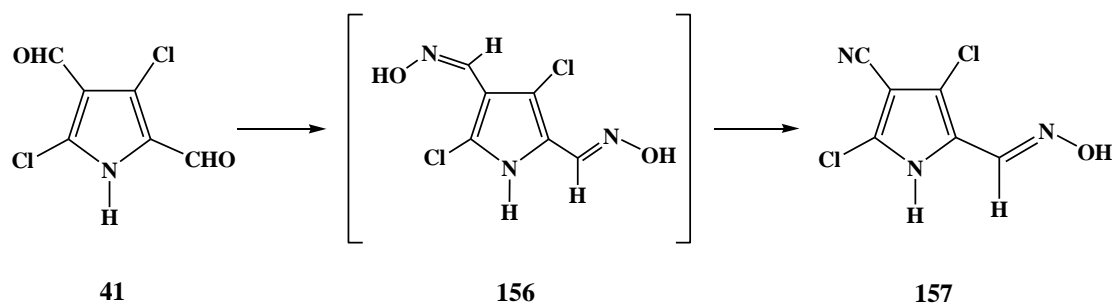


Scheme 38

The separation of these products proved to be difficult so we decided to use the hydroxylamine hydrochloride in excess (2.4 equivalents). The unsubstituted pyrrole was heated at 85°C in the presence of NH₂OH.HCl and formic acid for 1 hour. Work up gave the crude oxime **156** *via* reaction at both aldehyde groups. Further reaction with formic acid afforded the mononitrile compound **157** in 44% yield, Scheme 39, Method A.

An alternative method was proposed in order to improve the yield, using hydroxylamine hydrochloride and ethanol in the presence of pyridine, and after 2 hours at reflux the crude oxime **156** was obtained. This oxime was dehydrated in

refluxing acetic anhydride (Ac_2O) to give 3,5-dichloro-4-cyano-1*H*-pyrrole-2-carboxaldehyde oxime **157** in 72% yield, Scheme 39, Method B.



Scheme 39. Reagent and conditions; *Method A*: 1) $\text{NH}_2\text{OH}\cdot\text{HCl}$, HCOOH , 40 min, Δ ; 2) HCOOH , 1 h, Δ , 44%; *Method B*: 1) $\text{NH}_2\text{OH}\cdot\text{HCl}$, EtOH, pyridine, 2 h, Δ ; 2) Ac_2O , 1,5 h, Δ , 72%

The structure of the 3,5-dichloro-4-cyano-1*H*-pyrrole-2-carboxaldehyde oxime **157** was confirmed by its infra-red spectrum, with a broad NH and OH stretch at 3170 cm^{-1} and a CN stretch at 2234 cm^{-1} , whilst the ^1H NMR spectrum showed the disappearance of the protons of the aldehyde groups, and the appearance of a new CH proton at $\delta 7.89$ and a carbon signal at $\delta 139.2$. After the reaction with formic acid it is not obvious which oxime has been transformed to the nitrile group to give the mononitrile but this was determined using the HMBC spectrum, in which two carbon atoms (C-2 and C-3) and the NH gave cross peak signals to the hydrogen of the oxime in **157**, Figure 19.

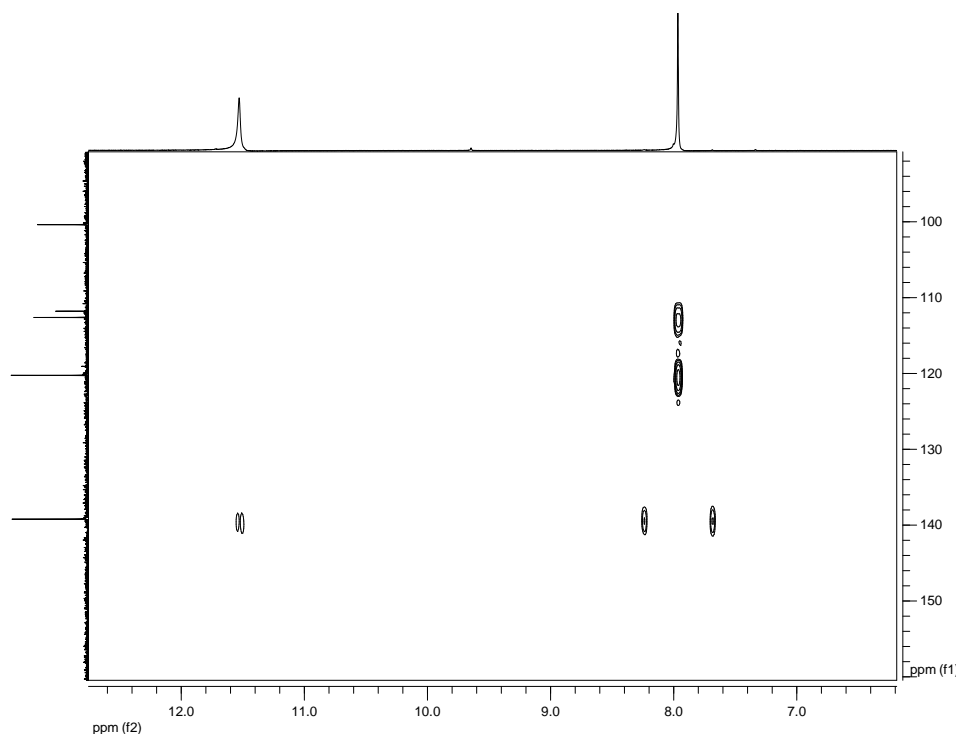
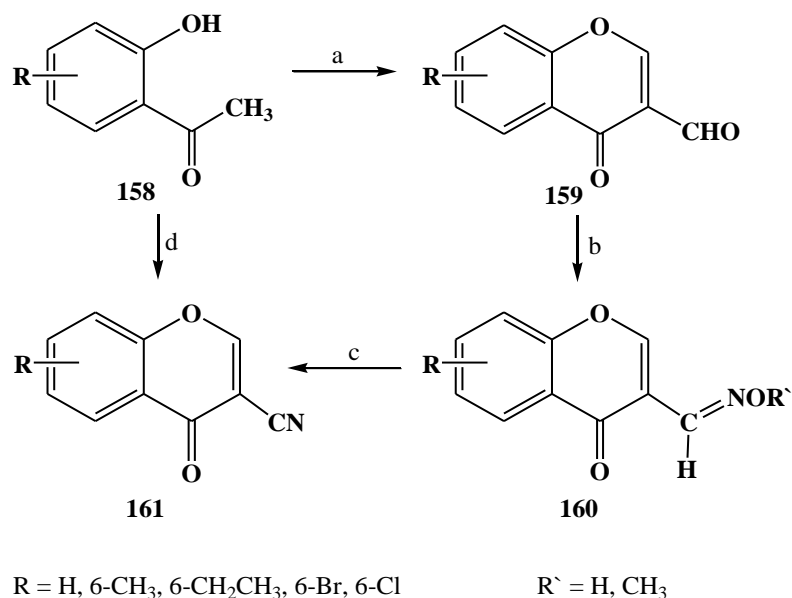


Figure 19. HMBC spectrum of 3,5-dichloro-4-cyano-1*H*-pyrrole-2-carboxaldehyde oxime **157** (300 Hz, *d*₆-DMSO)

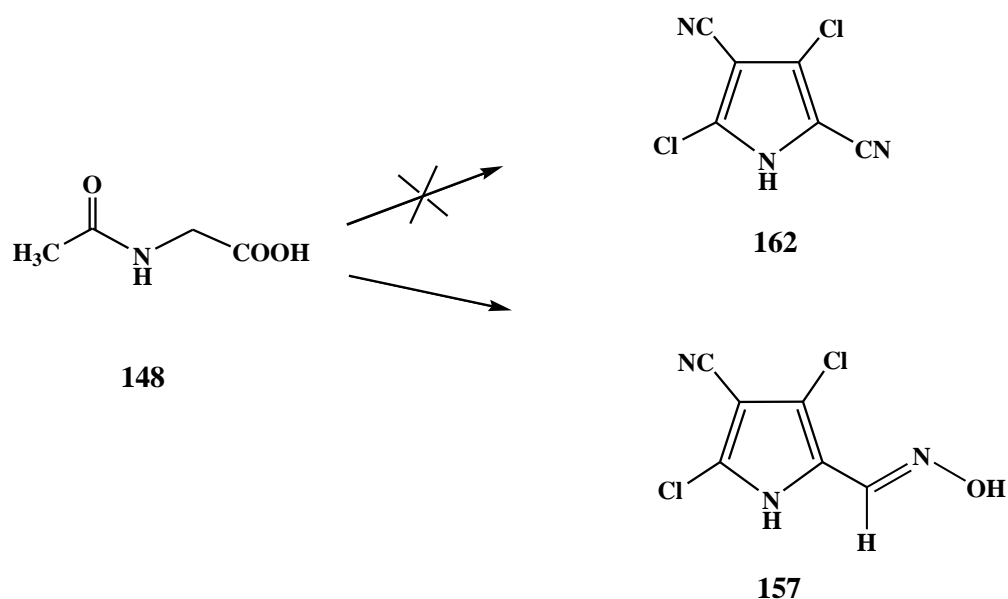
Reddy *et al.* reported a simple one-pot synthesis of benzopyrone derivatives from 2-hydroxyacetophenones under mild conditions, in which 3-cyano-4-benzopyrones **161** are generally prepared in 3 steps, starting from 2-hydroxyacetophenone **158**.⁷ The Vilsmeier-Haack reaction of the starting material results in 3-formylbenzopyrones **159**, which then react with hydroxylamine-hydrochloride in ethanol to give the corresponding oximes **160**. Finally, the dehydration of the oximes results in 3-cyano-4-benzopyrones **161**, using different dehydrating agents, such as hydrochloric acid, acetic anhydride or sodium formate in formic acid. These methods have some drawbacks, such as the isolation of the intermediate 3-formylbenzopyrones **159** and oximes **160**, the use of strongly acidic conditions, long reaction times, and in some cases, low yields in the last dehydration step. In view of these difficulties, Reddy and

co-workers developed an efficient procedure for the synthesis of cyanobenzopyrones and their method has great potential in the preparation of a number of cyano-derivatives under mild conditions, Scheme 40.⁸



Scheme 40.⁸ Reagents and conditions; (a) DMF/ POCl_3 , Δ , 4 h, 40-70%; (b) $\text{NH}_2\text{OR}'\cdot\text{HCl}$ / EtOH, Δ ; (c) EtOH/HCl or acetic anhydride, 50-70%; (d) DMF / POCl_3 / DCM / $\text{NH}_2\text{OH}\cdot\text{HCl}$, 51-72%

Following the method of Reddy, we attempted to synthesise a 3,5-dichloro-1*H*-pyrrole-2,4-dicarbonitrile **162**. *N*-Acetylglycine **148** was subjected to the Vilsmeier reaction, with DMF and POCl_3 , and the reaction mixture was subsequently treated *in situ* with hydroxylamine hydrochloride at room temperature. Analysis of the product indicated that instead of the expected dicarbonitrile **162**, only 3,5-dichloro-4-(hydroxyiminomethyl)-1*H*-pyrrole-2-carbonitrile **157** was obtained, in 45% yield, Scheme 41.



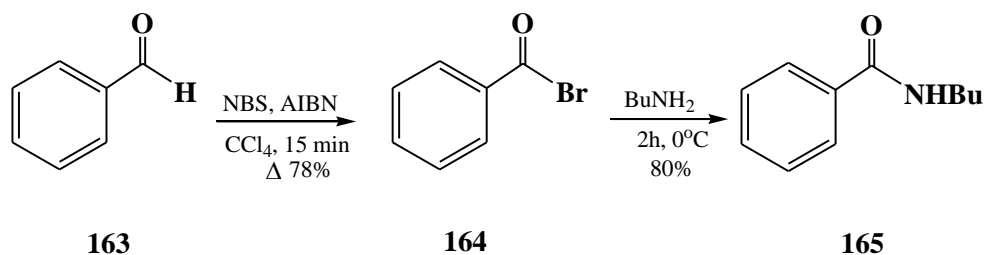
Scheme 41. Reagent and conditions; a) POCl_3 , dry DMF, Δ ; b) NH_2OH , HCl ,
DCM, $0\text{ }^\circ\text{C}$, 45%

All attempts at the preparation of dicyanitrile **162** failed, even with the alkyl substituted pyrroles (methyl, ethyl) and despite varying the reaction conditions. In addition, the direct transformation of aldehydes to nitriles with iodine in ammonia / water (Fang-method) did not result in any new compounds.

2.3.2 Transformation of aldehydes into amides

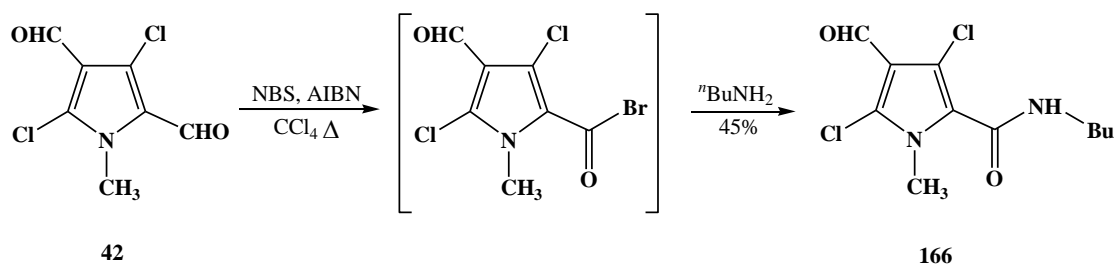
An extensive literature search has shown that there are only a few efficient methods for the transformation of aldehydes into amides. Aromatic aldehydes can be converted to the corresponding amides in a rapid reaction, in two steps, by reaction with a primary or secondary amine in the presence of an equimolar amount of *N*-bromosuccinimide (NBS) and AIBN, Scheme 42.⁹ The aldehyde **163** and NBS were dissolved in CCl_4 and heated in the presence of a catalytic amount of AIBN as a

radical initiator. A rapid reaction resulted in the formation of a precipitate of succinimide and the acid bromide **164**, which is thermally and moisture sensitive. It is normally easier, therefore, to use the acyl bromides **164** directly to prepare amides **165** without isolation, Scheme 42.



Scheme 42.⁹ Preparation of amide from aldehyde

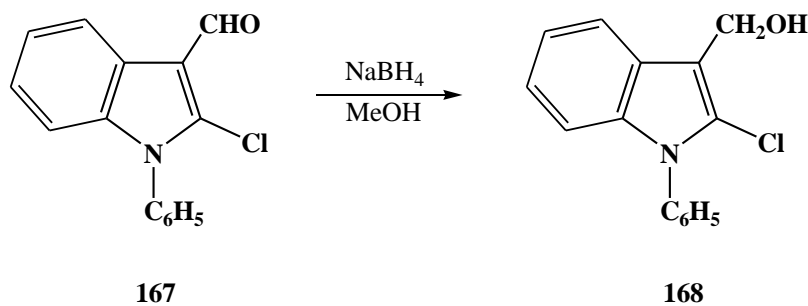
Following the previous method, methyl substituted pyrrole **42** was dissolved in CCl_4 in the presence of NBS and AIBN and refluxed for 15 minutes, then *n*-butylamine was added dropwise at 0°C . After stirring at room temperature for 20 minutes, *N*-butyl-3,5-dichloro-4-formyl-1-methyl-1*H*-pyrrole-2-carboxamide **166** was obtained, Scheme 43. Evidence for the formation of the amide was obtained from both the ^1H NMR and ^{13}C NMR spectra. The aldehyde signal was obvious at $\delta 9.66$, and a broad signal indicated the presence of an NH at $\delta 8.21$. New CH_2 signals appeared at $\delta 3.20$, $\delta 1.47$ and $\delta 1.34$, and a CH_3 peak at $\delta 0.89$. The ^{13}C NMR spectrum showed the new carbon signals for the butyl group at $\delta 14.1$, $\delta 19.9$, $\delta 31.5$, and $\delta 39.2$. We attempted to try different amines, including allylamine and *N*-methylallylamine but the highest yield was only 6%, making it clear that an alternative approach to other substituted amines was required.



Scheme 43

2.3.3 Reduction of the aldehyde groups

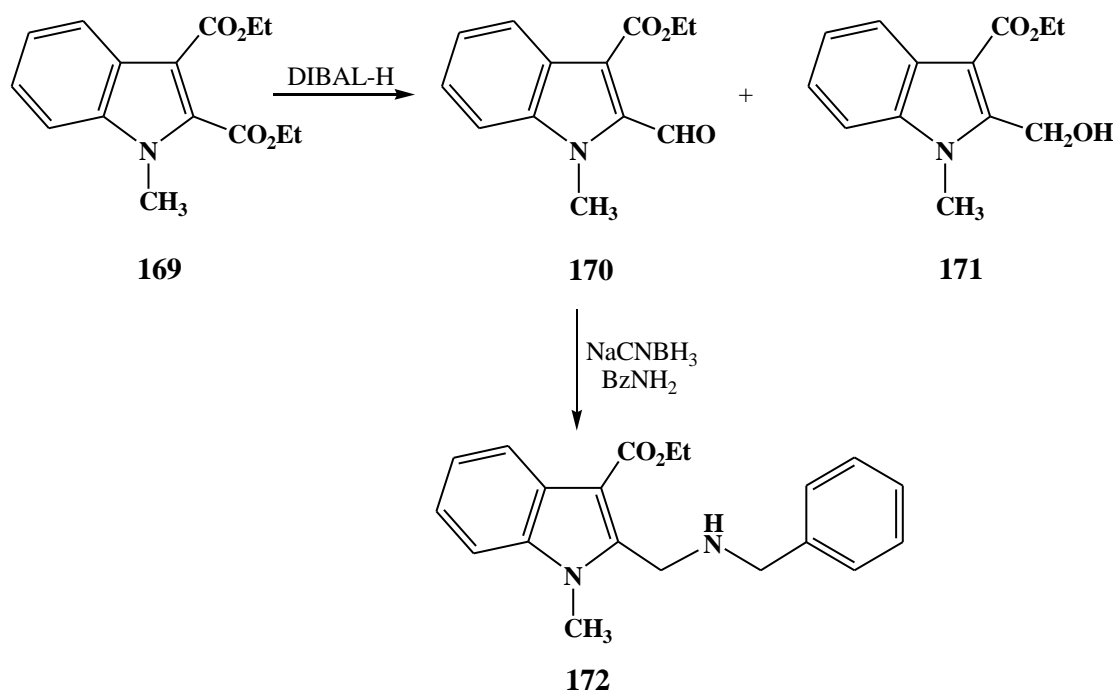
Aldehydes can be reduced to primary alcohols by a number of reducing agents¹⁰ and the utility of sodium cyanoborohydride as a selective reducing agent has been reviewed. The reduction under neutral conditions, in water or methanol, is negligible, however, at pH 3-4 the rate of reduction is sufficiently rapid.¹¹ The reduction of 1-phenyl-2-chloro-3-formylindole **167** with sodium borohydride yields the corresponding alcohol **168**, Scheme 44.¹²



Scheme 44.¹²

Beller *et al.* were interested in the synthesis and further functionalisation of indoles as they occur in numerous natural products and are important building blocks for several alkaloids.¹³ They reported a simple one-pot synthesis of indole-2,3-dicarboxylates from arylhydrazines and acetylene dicarboxylates and, in continuation of this work, they investigated the unreported chemoselective reduction of these indoles.¹⁴ The

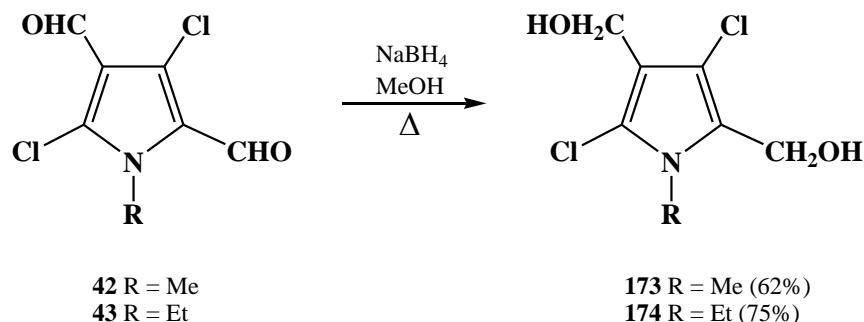
reduction of indole-2,3-diester **169** with either NaBH_4 or NaCNBH_3 did not result in the expected products, only starting material was recovered. The reaction with LiAlH_4 afforded a complex mixture of different products. Next, these workers decided to investigate this reduction in the presence of 2.0 equiv. of DIBAL-H, which resulted in the 2-formylindole-3-carboxylate **170** in 90% yield. Using 2.5 equiv. of DIBAL-H, however, led to the formation of a major product **171** in 60% yield and the aldehyde **170** was only obtained in minor quantities. Reductive amination of 2-formylindole **170** with benzylamine in the presence of NaBH_3CN gave the secondary amine **172** in 80% yield, Scheme 45.



Scheme 45.¹⁴ Reduction of indole derivatives

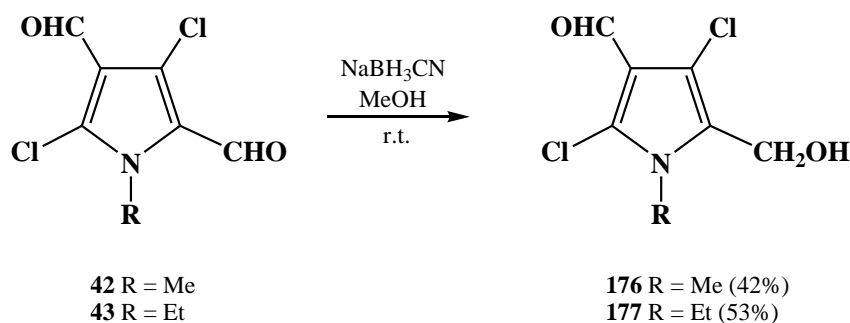
We attempted the reduction of the aldehyde groups in the parent pyrrole **41** with metal hydrides. Lithium aluminium hydride gave an uncharacterisable product upon reaction with either *N*-methylpyrrole **42** or *N*-ethylpyrrole **43**, while the reduction with sodium borohydride in methanol gave the products **173**, **174**, respectively, from

reduction of both aldehyde groups (even when using only 0.25 equivalents of NaBH_4), Scheme 46.



Scheme 46. Reduction of aldehyde groups using NaBH_4

The structure of diol **174** was confirmed by its infra-red spectrum, with a broad OH stretch at 3338 cm^{-1} , whilst the ^1H NMR spectrum showed a broad signal for the OH groups at $\delta 4.71$ and $\delta 5.13$, the expected triplet at $\delta 1.25$ and quartet at $\delta 4.01$ ($J = 7.2$ Hz) for the ethyl group and two singlets for the CH_2 protons at $\delta 4.24$ and $\delta 4.42$. The spectroscopic data for compound **173** showed similar results but, in this case, high resolution mass spectrometry did not confirm the formation of the expected product. Selective reduction of the 2-formyl group in the methyl- **42** and ethyl-substituted pyrroles **43** was, however, achieved, using sodium cyanoborohydride¹⁵ in methanol (pH 3-4), to give the mono-hydroxymethylpyrrolecarboxaldehydes **176** and **177**, respectively, Scheme 47.



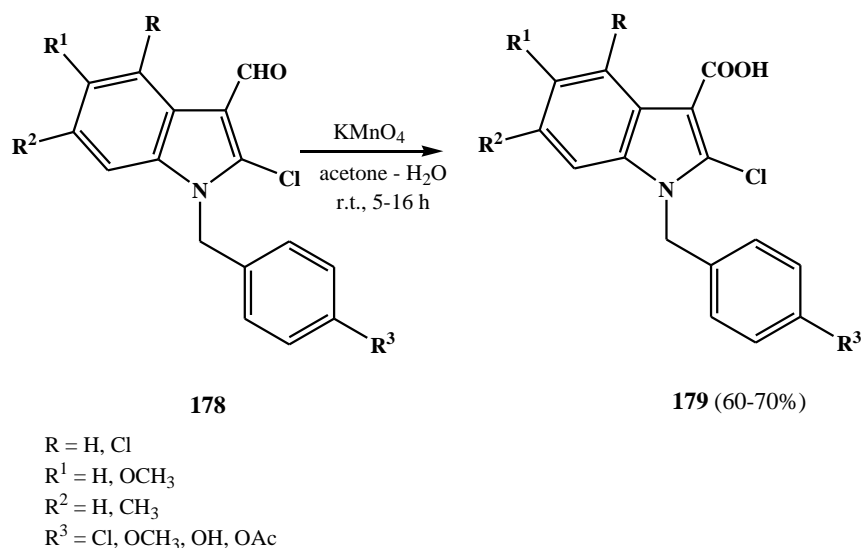
Scheme 47. Selective reduction of aldehyde using NaBH_3CN

The structure of aldehyde **176** was confirmed by high resolution mass spectrometry and ^1H NMR spectroscopy, with singlets at δ 3.72, δ 4.54 and δ 9.84 for the CH_3 , CH_2 and CHO protons, together with a broad signal at δ 5.38 for the OH . From the HMBC spectrum it was obvious that the 2-formyl group had been reduced to the alcohol, since the 2D spectrum showed that the CH_2 had connectivity to C-2 and C-3. Compound **177** was identified in a similar manner.

2.3.4 Oxidation of aldehyde groups

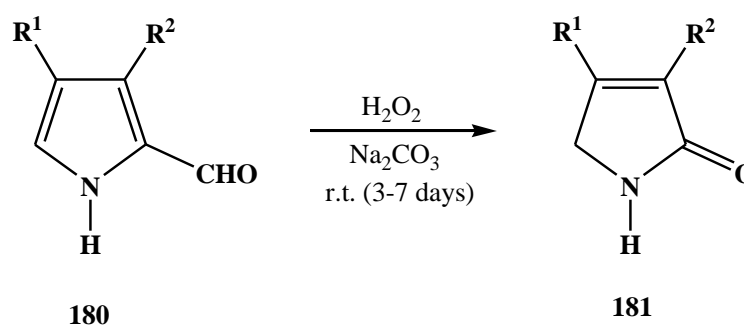
Oxidation of aldehydes into the corresponding carboxylic acids has been an extensively studied area, and a variety of methods have been reported using many different reagents.¹⁶

Andreani *et al.* reported the oxidation of *N*-benzyl-2-chloroindole-3-carboxaldehydes **178** to the corresponding carboxylic acids **179** with potassium permanganate in a mixture of acetone-water,¹⁷ Scheme 48, whilst Liebscher and Showalter *et al.* used sodium chlorite / H_2O_2 for the conversion to the 3-carboxylic acid.¹⁸



Scheme 48.¹⁷ Oxidation of compound **178** to the corresponding carboxylic acid

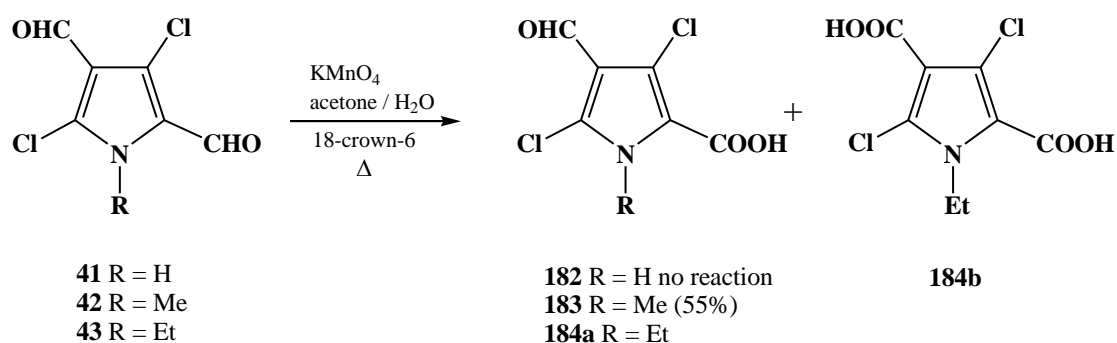
The oxidation of several α -formylpyrroles to pyrrolin-2-ones was carried out by the Scott research group using H_2O_2 under mild conditions.¹⁹ In 2007, Rhee *et al.* investigated the oxidation of various aldehydes under mild and facile conditions using a Pd/C catalyst, sodium borohydride and potassium hydroxide in aqueous methanol.²⁰ Regioselective oxidation of the pyrrole-2-carboxaldehydes **180** to the corresponding 3-pyrrolin-2-ones **181** was achieved by Elky *et al.*, utilising hydrogen peroxide and sodium bicarbonate at ambient temperature, Scheme 49.²¹ This reaction possibly proceeds *via* a Bayer-Villiger-type oxidation of the formyl group, followed by hydrolysis of the intermediate formate ester.



Scheme 49.²¹

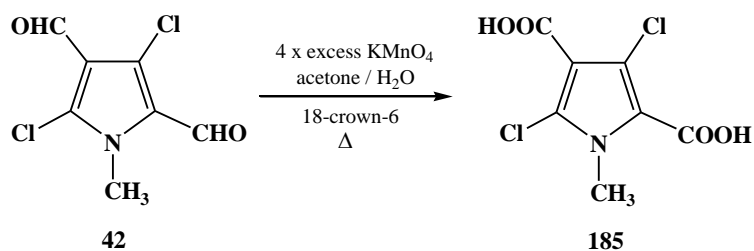
After the successful selective reduction of compound **174**, **176**, **177** we next turned our attention to the investigation of the selective oxidation of the 2-formyl group of the unsubstituted pyrrole **41**. The reaction with KMnO_4 in aqueous acetone did not result in the expected product **182**, and only starting material was obvious from the ¹H NMR spectrum. Assuming that the reason for this failure was the unprotected nitrogen, we decided to solve this problem by introduction of a protecting group. After an extensive literature search, the oxidation in the presence of an EWG acyl group afforded the expected acid in low yield (3%),¹⁷ so we decided to investigate the reaction with the alkyl substituted pyrroles. The initial attempt involved the reaction

of pyrrole **42** with KMnO_4 in aqueous acetone at room temperature, in the presence of crown ether but after acidic work up, no product was observed. Subsequent experiments at reflux temperature without using the crown ether resulted in the formation of the desired monocarboxyl-pyrrolicarboxaldehyde **183**. The reaction with ethyl substituted pyrrole **43** always gave a mixture of the mono- **184a** and dicarboxylic acids **184b**, Scheme 50.



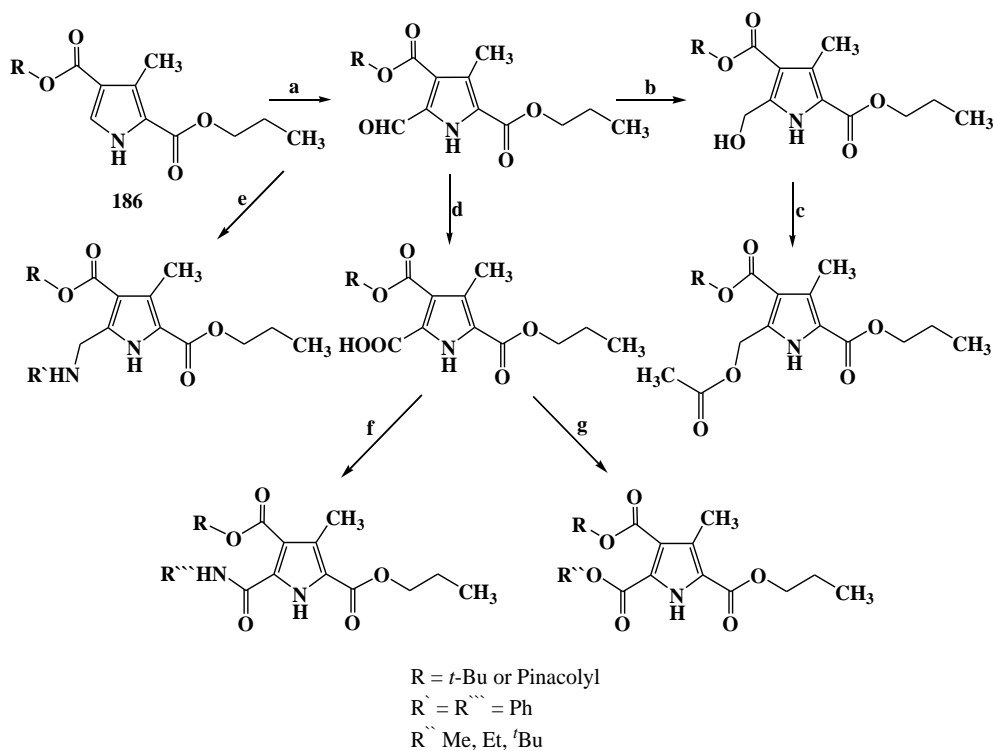
Scheme 50

Broad stretches in the IR spectrum at 2588 and 1662 cm^{-1} for the OH and C=O bonds respectively, together with a high resolution mass spectrum, confirmed the presence of the mono-carboxylic acid. The ^1H NMR spectrum also gave confirmation of the structure, with two singlets at $\delta 3.87$ and $\delta 9.72$, for the CH_3 and CHO, together with a broad signal at $\delta 13.15$ for the OH proton. When the reaction was carried out with four equivalents of KMnO_4 , oxidation of both aldehydes gave **185**, Scheme 51, with elemental analysis confirming the desired product and the ^1H NMR spectrum also showing that the signal from the protons of both aldehyde groups had disappeared.



Scheme 51

Micheli and co-workers have reported the synthesis and biological properties of 3,5-dimethylpyrrole-2,4-dicarboxylic acid-2-propyl ester²² and their excellent results inspired them to continue their study on this class of pyrroles. They then prepared several pyrrole derivatives starting from compound **186**, Scheme 52.²³

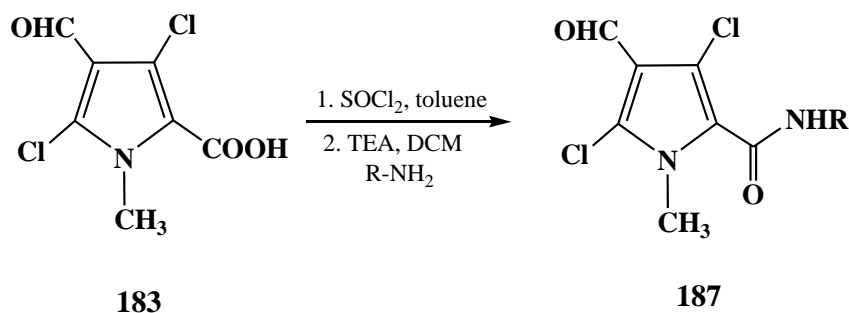


Scheme 52.²³ Reagent and conditions; a) POCl_3 , DMF, CH_2Cl_2 , from 0°C to r.t., 50%;
 b) NaBH_4 , MeOH, 0°C to r.t., 95%; c) CH_3COCl , Py, THF, r.t., 95%;
 d) NaOClO , CH_3CN , H_2O , r.t., 70%; e) (i) NaCNBH_3 , $\text{R}'\text{NH}_2$, THF, 0°C , 30%; (ii) H_2 , Pd/C, 95%; f) $\text{R}'''\text{NH}_2$, DCC, THF, r.t., 80%; g) $(\text{CF}_3\text{CO})_2\text{O}$, r.t., $\text{R}'''\text{OH}$, THF, 80%

2.3.4.1 Synthesis of amides from carboxylic acid

Having successfully devised a selective oxidation, we next turned our attention to the preparation of a number of pyrrole derivatives, starting from 3,5-dichloro-4-formyl-1-methyl-1*H*-pyrrole-2-carboxylic acid. We had already studied the transformation of aldehydes into amides in a rapid reaction in the presence of NBS and AIBN in 45% yield, so we now aimed to improve the yield of the amide through the conversion of the carboxylic acid into the corresponding amide since this is a well established functional group transformation in organic chemistry.²⁴

The monocarboxyl-pyrrolecarboxaldehyde **183** and SOCl₂ were refluxed in toluene for 4 hours and this reaction resulted in the formation of the acid chloride. Without isolation of the unstable intermediate, the crude mixture was dissolved in DCM then a solution of *n*-butylamine and TEA in DCM was added dropwise at 0 °C. After stirring at room temperature for 2 hours, *N*-butyl-3,5-dichloro-4-formyl-1-methyl-1*H*-pyrrole-2-carboxamide **187e** was obtained, but the overall yield was only 37%. We then investigated the reaction of the methyl substituted pyrrole in the presence of different amines, giving the corresponding derivatives **187a-d** in moderate yields. The results are summarized in Table 1. The reaction of methylallylamine did not give any characterisable product, Scheme 53.



Scheme 53

Characterisation of the compounds was achieved by elemental analysis or high resolution mass spectra, and ^1H NMR and ^{13}C NMR spectroscopy was also used to confirm the structures.

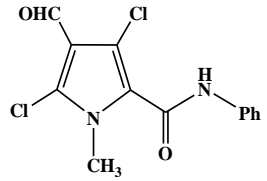
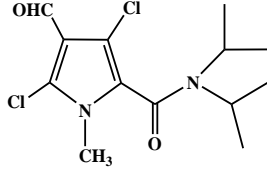
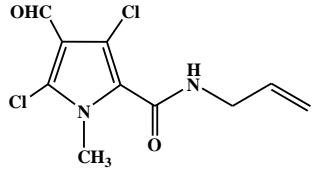
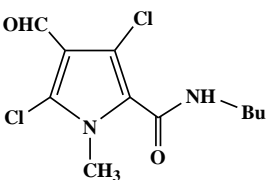
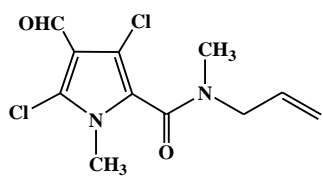
Entry	Amine	Product	Yield (%) ^{a,b}
a	PhNH ₂		80
b	NH[CH(CH ₃) ₂] ₂		68
c	NH ₂ CH ₂ CHCH ₂		54
d	NH ₂ Bu		68
e	CH ₃ NHCH ₂ CHCH ₂		0

Table 1. a) (i) Reaction conditions; pyrrole **183** (1.35 mmol), SOCl₂ (0.49 ml),

toluene, reflux, 4h; (ii) CH₂Cl₂, amine (2.01 mmol), TEA (0.19 ml), r.t., 0°C

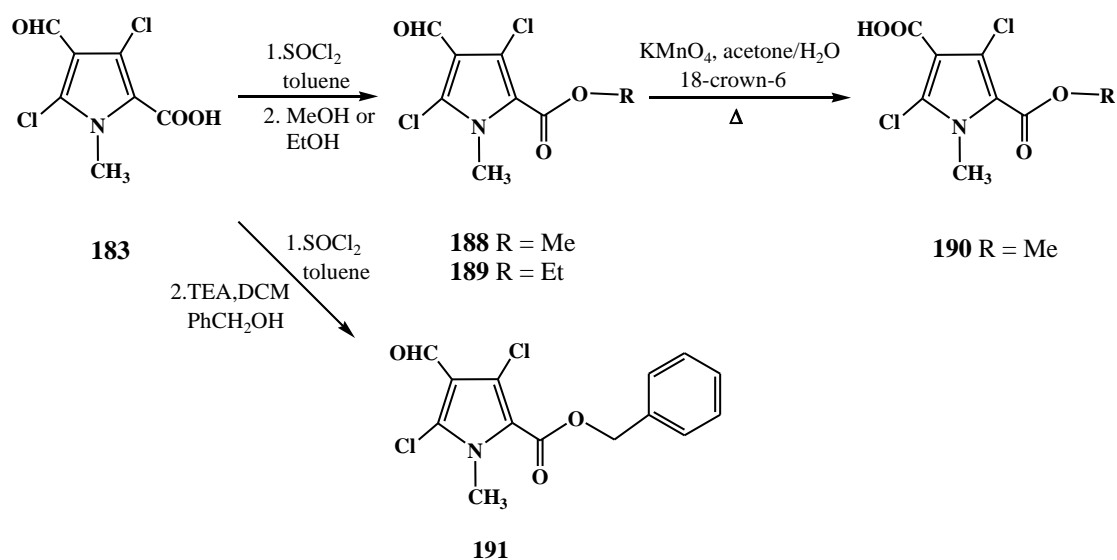
b) Isolated yield

2.3.4.2 Synthesis of esters from carboxylic acids

Effective esterification of carboxylic acids with alcohols is one of the most fundamental reactions in organic synthesis.²⁵

The preparation of the corresponding ester derivatives of acid **183** was achieved using SOCl₂, and dry MeOH or EtOH to give the expected compounds **188** and **189** respectively. Further oxidation of the 4-formyl-2-carboxylic acid methyl ester **188** resulted in the dicarboxylic acid monoester **190**, Scheme 54. The structure of these esters was confirmed by spectroscopic methods and elemental analysis. The ¹H NMR spectrum of compound **189** showed the presence of the ethyl group, with the expected triplet at δ1.35 and quartet at δ4.35. The signal for the *N*-CH₃ appeared at δ3.93 and the CHO proton at δ9.76.

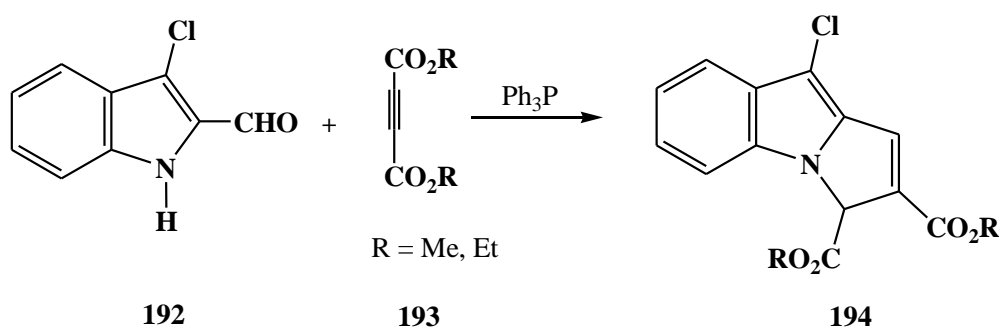
The reaction of the carboxylic acid with benzyl alcohol resulted in the benzyl 3,5-dichloro-4-formyl-1-methyl-1*H*-pyrrole-2-carboxylate **191** in 38%. Evidence for the formation of the product **191** was given by elemental analysis and spectroscopic data. The ¹H NMR spectrum gave the conformation of the structure, with the appearance of new aromatic protons at δ7.41 and a singlet at δ5.34 which can be attributed to the CH₂ protons. The ¹³C NMR also confirmed the structure of the product with the presence of aromatic carbons at δ128.3, δ128.5, δ128.9 and δ136.1 respectively.



Scheme 54

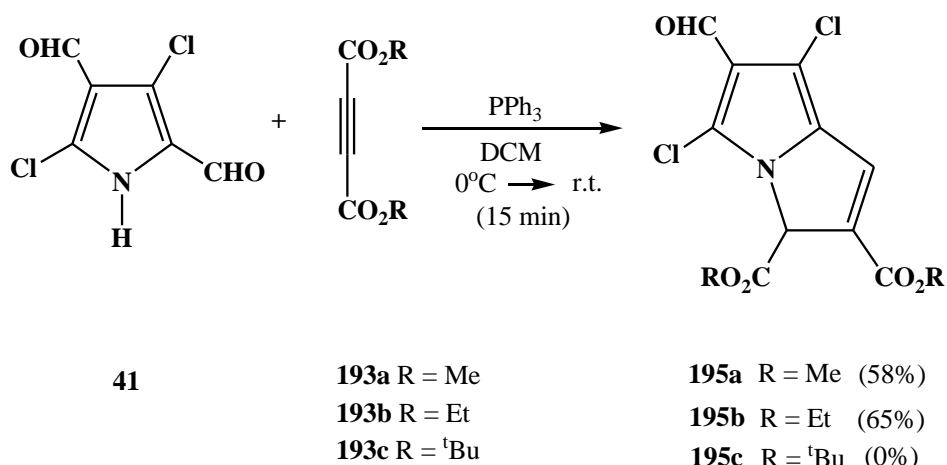
2.4 Reactions of two electrophilic centres

There are two functional groups in 3-chloroindole-2-carboxaldehyde **192** which are close enough to each other to allow the reaction of both groups simultaneously and Yavari and co-workers have observed an interesting reaction between 3-chloroindole-2-carboxaldehyde **192** and dialkyl acetylenedicarboxylates **193**, in the presence of triphenylphosphine, which proceeds smoothly, in DCM at ambient temperature, to give dialkyl 9-chloro-3*H*-pyrrolo[1,2-*a*]indole-2,3-dicarboxylates **194** in 96-98% yields, Scheme 55.²⁶



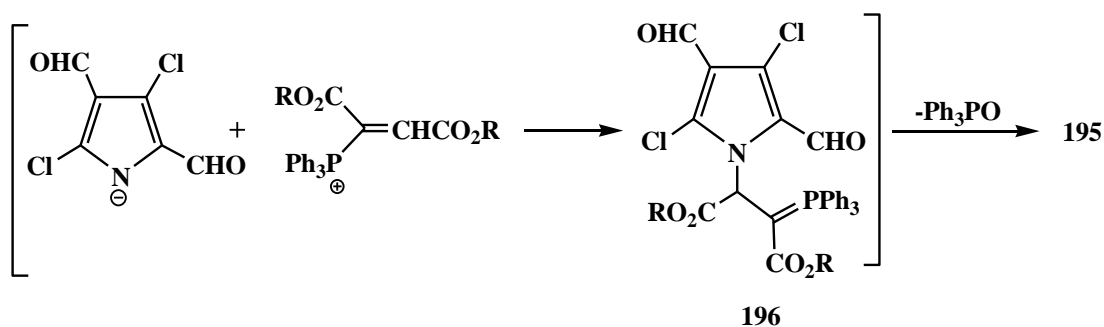
Scheme 55.²⁶

A one-pot synthesis was subsequently attempted on the parent pyrrole **41** following the Yavari method. The reaction of 3,5-dichloro-1*H*-pyrrole-2,4-dicarboxaldehyde with acetylenic esters **193 a-e**, in the presence of triphenylphosphine, proceeded smoothly in DCM at ambient temperature, to produce 5,7-dichloro-6-formyl-3*H*-pyrrolizine-2,3-dicarboxylic acid esters **195 a-e**, Scheme 56. The reaction with the methyl and ethyl esters results in the products **195a** and **195b** in moderate yield, while using the *t*-butyl ester gave no isolated product, only starting material was recovered.



Scheme 56

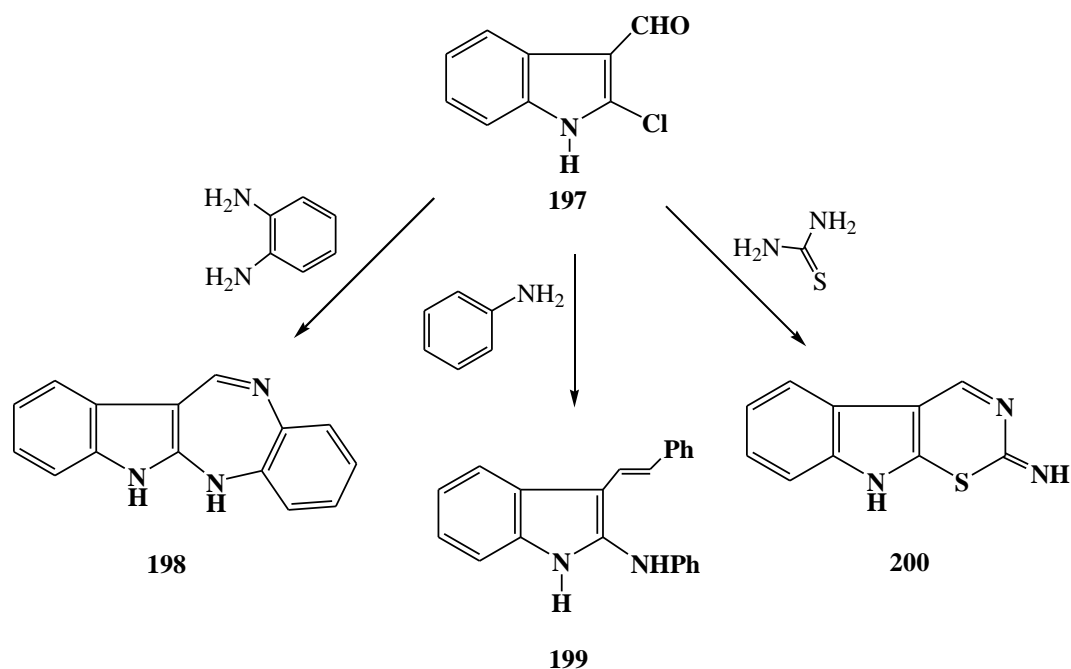
On the basis of the chemistry of trivalent phosphorus nucleophiles, it is reasonable to assume that the initial addition of triphenylphosphine to the acetylenic ester, followed by protonation of the 1:1 adduct by the NH of pyrrole **41** will result in compound **195**. The nitrogen atom of the conjugate base of the pyrrole will attack on the positively charged ion to form phosphorane **196**, which undergoes an intramolecular Wittig reaction to result in the bicyclic pyrrole derivative **195**, Scheme 57.



Scheme 57

The spectroscopic data confirmed the structure of compound **195a**, with the ^1H NMR spectrum exhibiting a single sharp singlet for the two methoxy group (at δ 3.78) protons. The two CH groups appear as two doublets, at δ 6.04 and δ 7.85, with allylic coupling of $J = 1.8$ Hz. The ^{13}C NMR spectrum of pyrrolizine **195a** includes a signal at δ 65.1 for the N-CH moiety. The ^1H and ^{13}C NMR spectra of **195b** are similar to those of the methyl derivative **195a**.

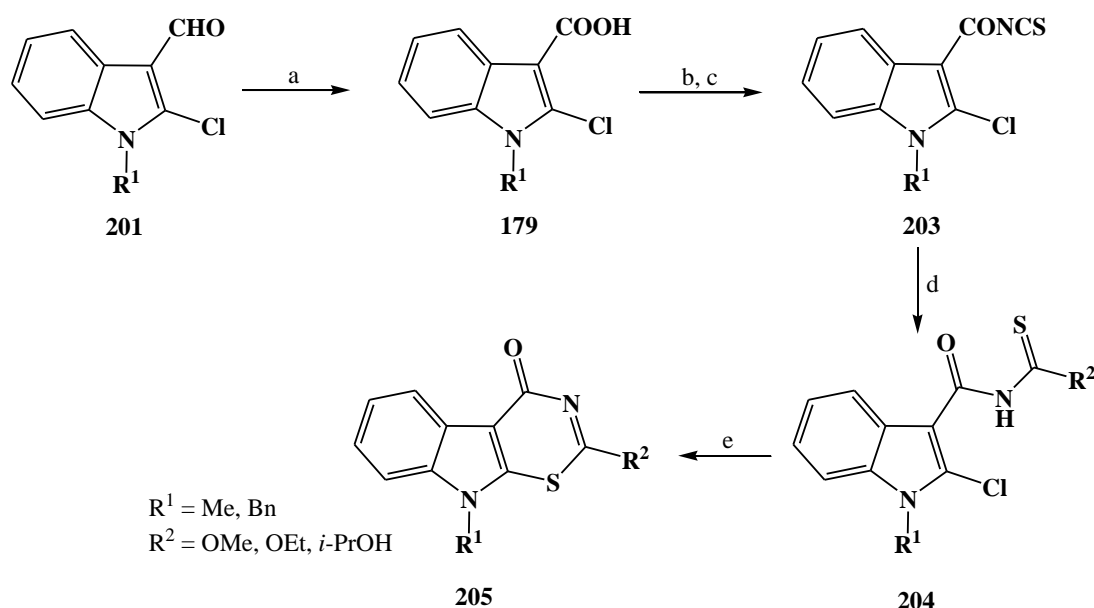
Schulte *et al.* reported the reaction of 2-chloroindole-3-carboxaldehyde **197** with *o*-phenylenediamine in MeOH to produce 5,6-dihydrobenzo[2,3][1,4]diazepino[5,6-*b*]indole **198**. Treatment of the same indole with an excess of aniline results in the corresponding 2-phenylaminoindole **199**, while the reaction with thiourea gives 2-imino-9*H*-1,3-thiazino[6,5-*b*]indole hydrochloride **200** in excellent yield (97%), Scheme 58.²⁷



Scheme 58.²⁷

Suchy and his research group decided to study indole phytoalexins since several isolated compounds of this family have been shown to have antifungal and antitumour activity.²⁸ These workers were interested in the synthesis of cyclobrassinon since its isolation from plants is relatively difficult and time consuming, and a synthetic route to cyclobrassinon had not previously been described.²⁹ Further work was focused on a synthesis of cyclobrassinon analogues as interesting synthetic targets and on the investigation of their biological properties.

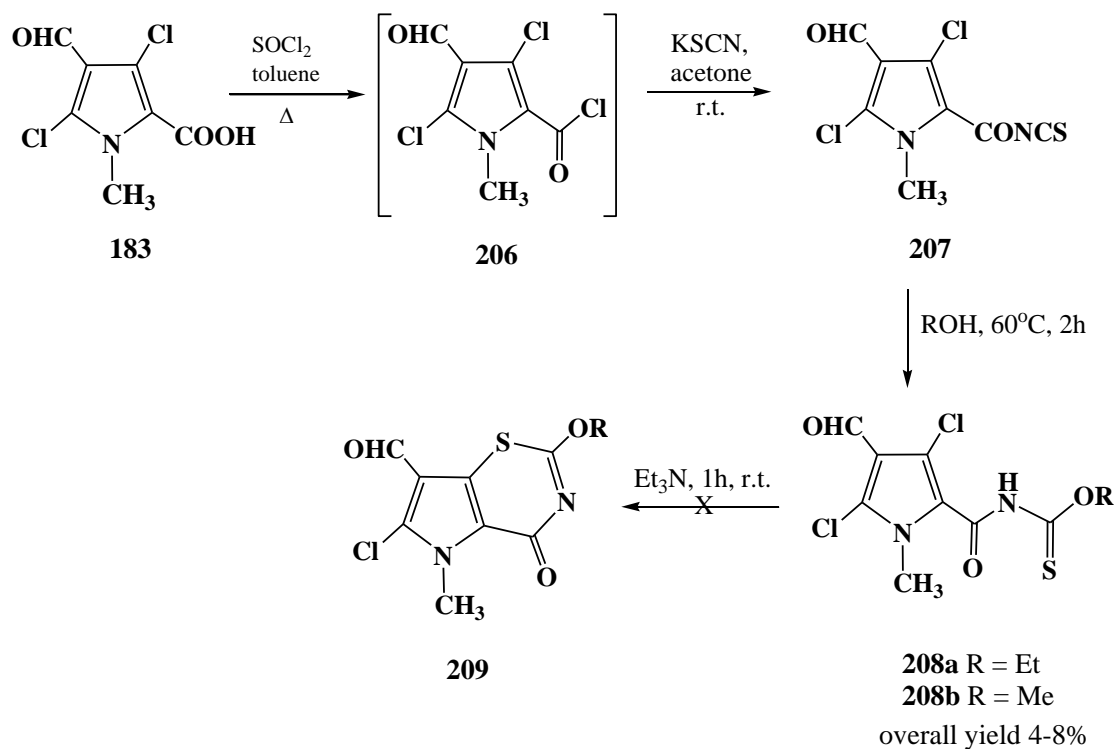
The acid **202** was prepared by oxidation of 2-chloroindole-3-carboxaldehyde **201** with KMnO₄ in aqueous acetone. Heating of this acid with PCl₃ in benzene resulted in an unstable acid chloride which, after immediate treatment with KSCN, gave the stable isothiocyanate **203**. The reaction of this isothiocyanate with methanol, ethanol or 2-propanol afforded thiocarbamoyl compounds **204**, which were cyclised upon treatment with Et₃N and afforded **205**, Scheme 59.²⁹



Scheme 59.²⁹ Total synthesis of phytoalexin cyclobrassinin. Reagent and conditions;

- a) KMnO_4 , acetone/water, r.t.; b) PCl_3 , benzene, 85-90°C; c) KSCN , acetone, r.t.; d) MeOH or EtOH or *i*-PrOH, 60°C; e) Et_3N , r.t., 1-2h

Following the Suchy method, we attempted to synthesise compound **209** since a literature search did not disclose any similar analogues. The synthesis of the expected compound **209** was achieved by starting from the monocarboxyl-pyrrolecarboxaldehyde **183**, which was prepared by the oxidation of the methyl substituted pyrrole **42** with KMnO_4 . The acid chloride **206** was then prepared by heating acid **183** with SOCl_2 in toluene, and treatment of the acid chloride **206** with KSCN in acetone afforded the surprisingly stable isothiocyanate **207**. The conformation of the identity of this product was obtained by the IR spectrum, which contained a peak for the $\text{N}=\text{C}=\text{S}$ group at 1954 cm^{-1} . The next step was the nucleophilic addition of the ethanol or methanol to the crude isothiocyanate to give the corresponding thiocarbamate **208**, Scheme 60.

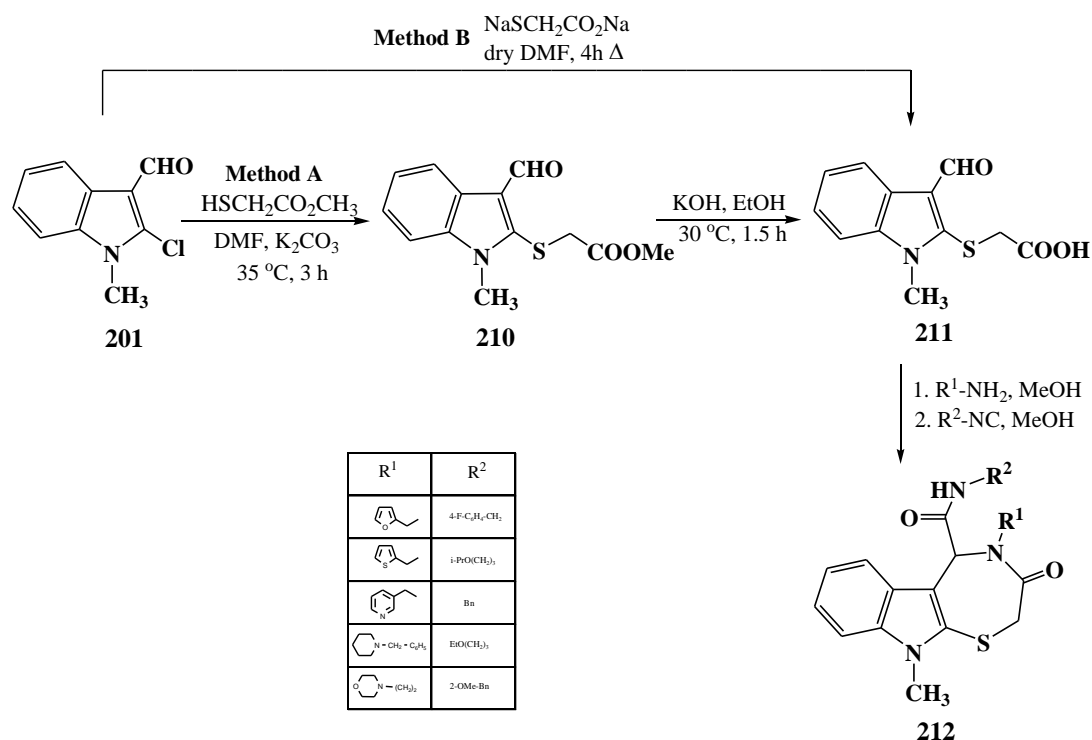


Scheme 60

Characterisation of the 3,5-dichloro-4-formyl-1-methyl-1H-pyrrole-2-carbonyl thiocarbamic acid *O*-ethyl ester **208** was achieved by high resolution mass spectrometry and spectroscopic data. The ^1H NMR spectrum showed a broad signal for the NH group at $\delta 9.22$, while the ^{13}C NMR spectrum showed the C=S and C=O groups at $\delta 155.3$ and $\delta 177.6$, respectively. We hoped that the reaction of the thiocarbamate **208** with triethylamine would result in the bicycle **209**, but the analysis of the reaction mixture gave no conclusive evidence for this structure, therefore the cyclisation to the bicycle requires further investigation.

Ivachtchenko *et al.* were interested in the synthesis of heteroaryl-fused carboxamide derivatives of 3(5)-oxo-1,4-thiazepine heterocycles³⁰ since the 1,4-thiazepine fragment is present several natural and synthetic biologically active compounds. They initially described two synthetic routes for the preparation of bifunctional reagent **211** and its use in the modified four component Ugi reaction. According to method A, the reaction of chloroindole **201** with methyl mercaptoacetate, in the presence of K_2CO_3

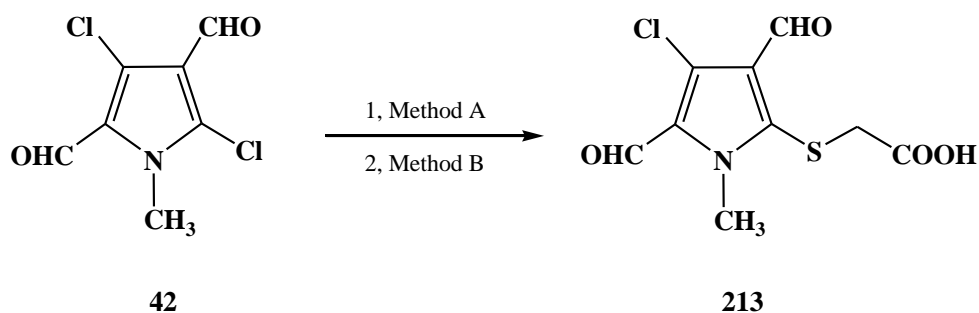
in DMF, gave the intermediate ester **210**, which was then hydrolysed to the expected aldehyde-substituted acid **211**. The relatively low overall yield of this reaction inspired them to try an alternative route for the preparation of acid **212** and they found that the reaction of chloroindole **201** with disodium mercaptoacetate in methanol afforded the desired product in better yield and in fewer steps, Scheme 61. In a continuation of this study, these workers investigated a synthetic approach to the 3(5)-oxo-1,4-thiazepine **212** derivatives. The reaction of acid **211** with different amines and isocyanides in methanol led to the desired product **212**.



Scheme 61.³⁰ Synthesis of heteroaryl fused 3(5)-oxo-1,4-thiazepine heterocycles

We next turned our attention to the investigation of the synthesis of pyrrole fused thiazepins using the modified Ugi reaction. Following the Ivachtchenko method, formation of the bifunctional reagents was initially attempted, using the methyl

substituted pyrrole **42** and methyl mercaptoacetate, in the presence of K_2CO_3 in DMF. The intermediate ester was then hydrolysed to the expected acid **213**, Scheme 62, Method A, but analysis of the sample did not show any evidence for the formation of the desired product.



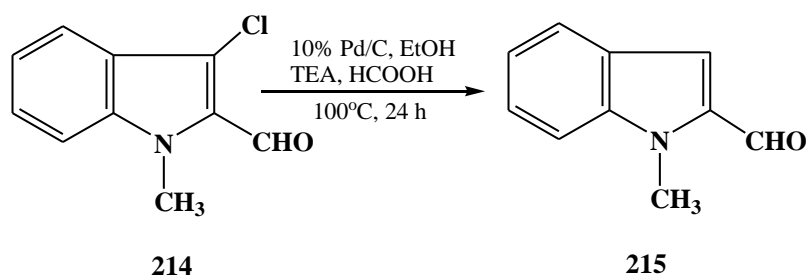
Scheme 62. Reagent and conditions; Method A: $HSCH_2CO_2CH_3$, DMF, K_2CO_3 , 35 °C, 3h then KOH, EtOH, 30 °C, 1.5h; Method B: $NaSCH_2CO_2Na$, dry DMF, 4 h, Δ

An alternative route involved the reaction of substituted pyrrole **42** with the previously prepared disodium mercaptoacetate in DMF, to afford the 4-chloro-3,5-diformyl-1-methyl-1*H*-pyrrole-2-ylsulfanylacetic acid **213**, Scheme 62, Method B, but despite the 1H NMR and ^{13}C NMR spectrum suggesting the presence of the expected compound, the high resolution mass spectrum did not confirm the structure and the subsequent reaction was abandoned.

2.5 Dehalogenation

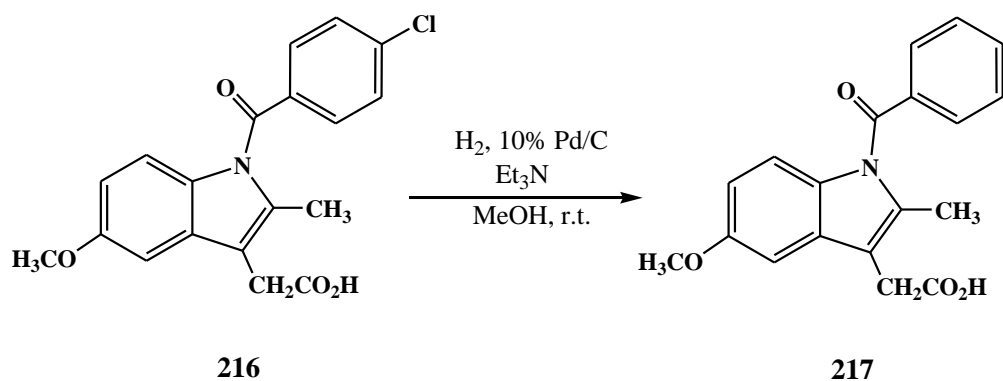
Dehalogenation of aromatic halides is an important chemical transformation in organic synthesis³² and a great number of methods have been developed over the years³³ but there are only a few efficient methods for the dechlorination of aromatic chlorides, as it is well known that they are much less reactive than aromatic bromides and iodides.

Heck *et al.* reported that aromatic halide groups can be removed at 50-100 °C by palladium-catalysed reduction with triethylammonium formate, Scheme 63.³⁴



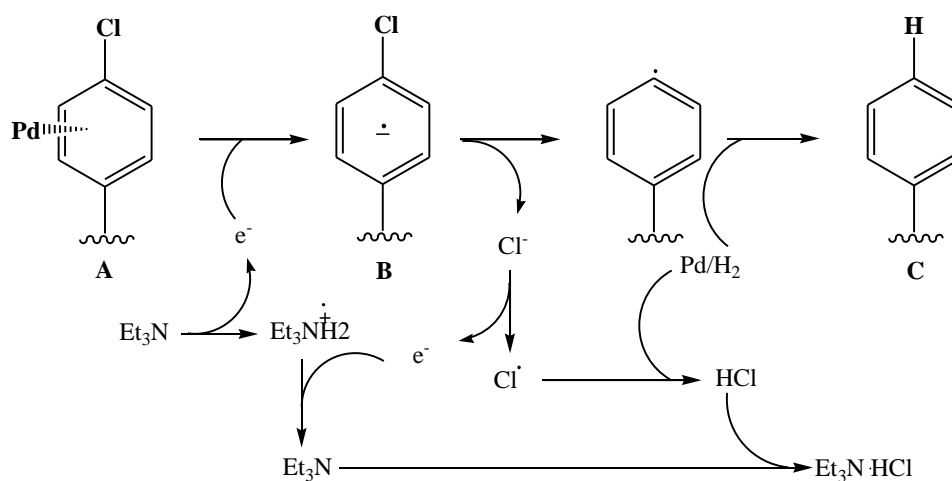
Scheme 63.³⁴

Sajiki and co-workers described a mild and efficient one-pot method for the Pd/C-catalysed hydrodechlorination of aromatic chlorides at room temperature under ambient hydrogen pressure and in the presence of Et₃N, which involves a single electron transfer (SET), Scheme 64.³⁵ A few years later these workers published an extensive study outlining the optimised reaction conditions, in which they investigated various nitrogen-containing bases, and also optimised the solvent and the reaction temperature.³⁶



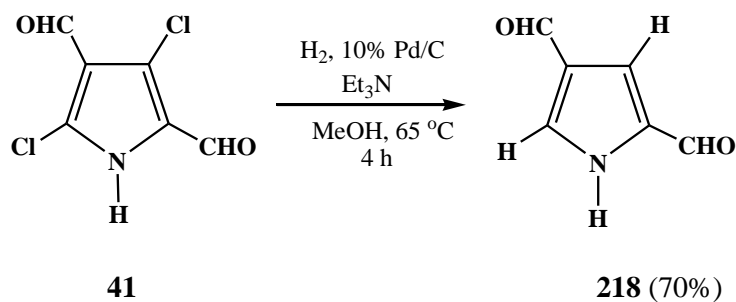
Scheme 64.³⁶ Hydrodechlorination of aromatic chloride

A possible mechanism for the dehalogenation of aromatic chlorides (Pd/C-Et₃N) involves the SET mechanism – the initial step is the single electron transfer from Et₃N to the palladium activated benzene ring of **A**, which results in the anion radical **B**. Elimination of the chloride anion and then hydrogenation of the benzene radical will result in the dehalogenated benzene ring **C**, Scheme 65.



Scheme 65. Possible mechanism of the hydrodechlorination

The complete dechlorination of 3,5-dichloro-1*H*-pyrrole-2,4-dicarboxaldehyde **41** was carried out with 10% Pd/C and Et₃N in MeOH and gave 1*H*-pyrrole-2,4-dicarbaldehyde **218** in 4 hours at 65 °C, in 70% yield, Scheme 66.



Scheme 66

Characterisation of the dialdehyde **218** was achieved from the spectroscopic data; the ¹H NMR spectrum of 1*H*-pyrrole-2,4-dicarboxaldehyde **218** showed the presence of two new CH signals, at δ7.42 and 7.97, with a coupling constant of $J = 2.1$ Hz, thus indicating the disappearance of the chloro substituents. The HH-COSY spectrum (Figure 20) showed that H-3 is coupled to H-5 and, in addition, 2 CH signals appeared in the DEPT 135 spectrum.

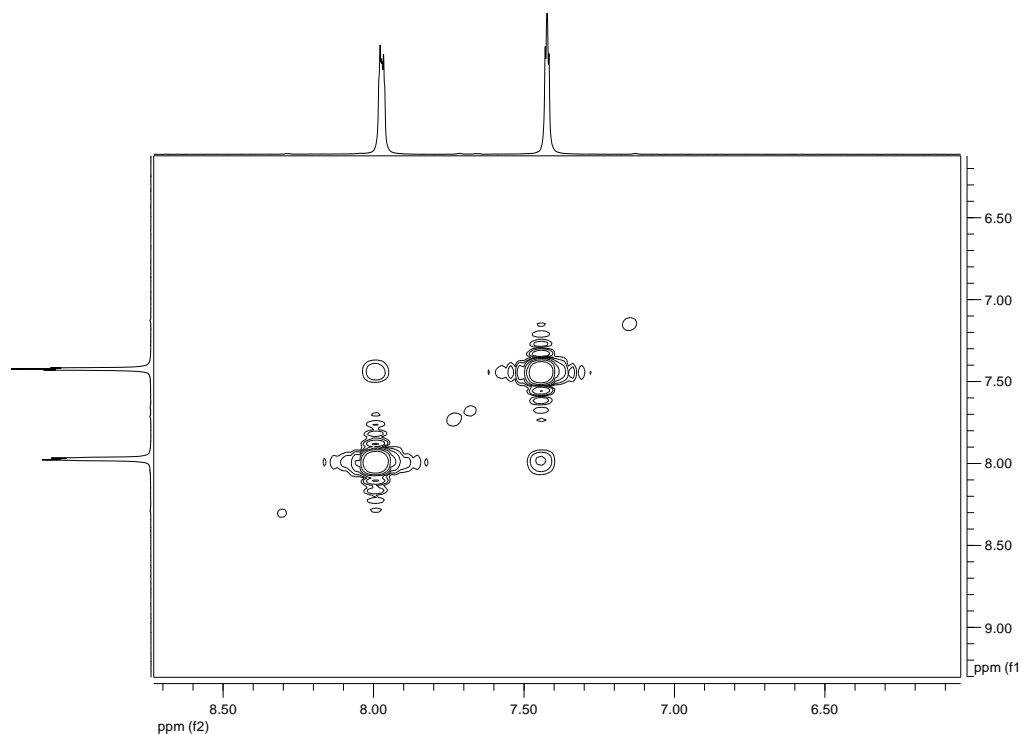
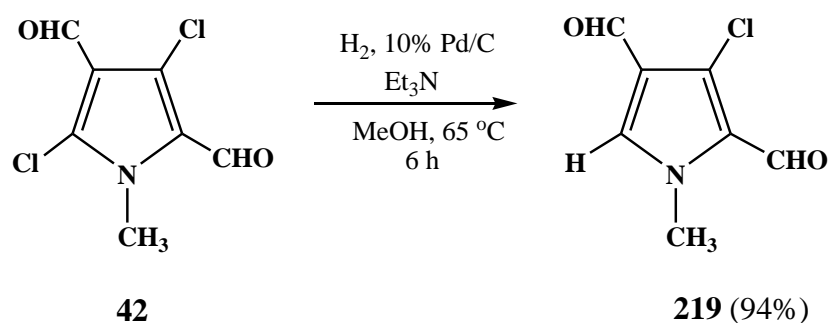


Figure 20. HH-COSY spectrum of 1*H*-pyrrole-2,4-dicarboxaldehyde **218** (300 MHz, DMSO-*d*₆)

The reaction of the methyl substituted pyrrole **42** under the same conditions resulted in the selective dehalogenation at C-5 in 6 hours, in high 94% yield, Scheme 67.



Scheme 67

The structure of the monochloropyrrole **219** was confirmed by elemental analysis and spectroscopic data. The ¹H NMR spectrum showed the appearance of H-5 at δ7.36

and the DEPT 135 spectrum showed a new CH signal at δ 132.7. It is obvious from the HMBC spectrum that the dehalogenation has occurred at the C-5 position since the H-5 proton shows connectivity to C-4 (at δ 127.0) and the CH₃ (at δ 38.4), Figure 21.

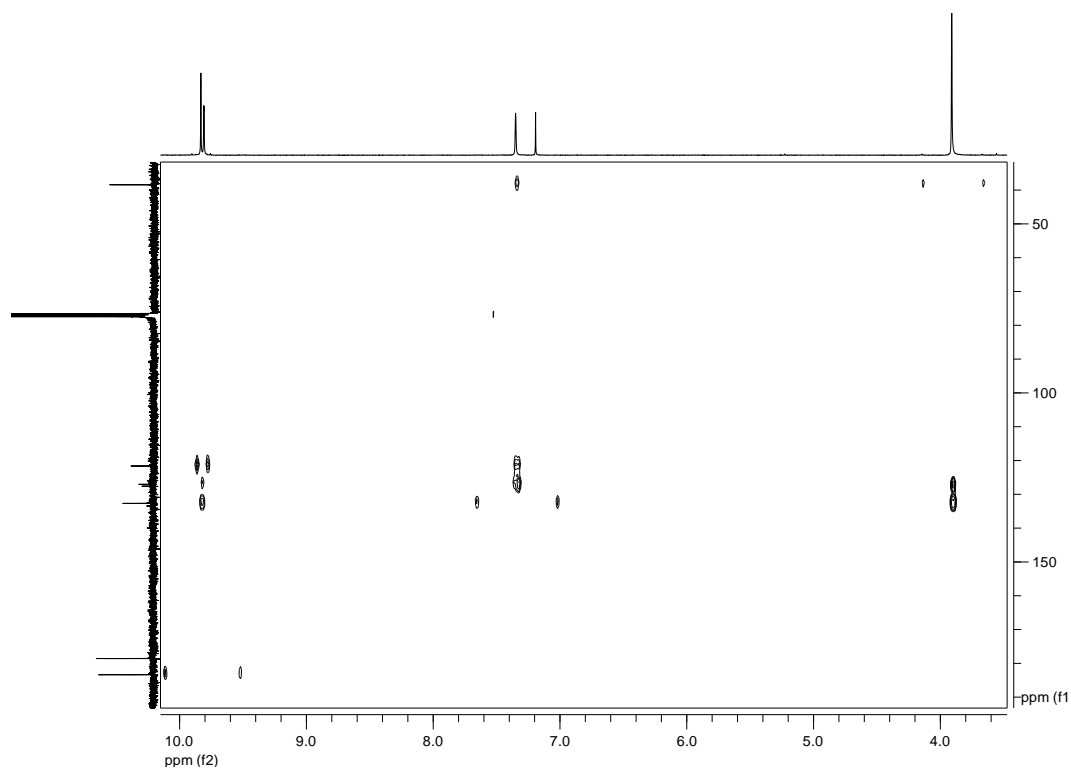
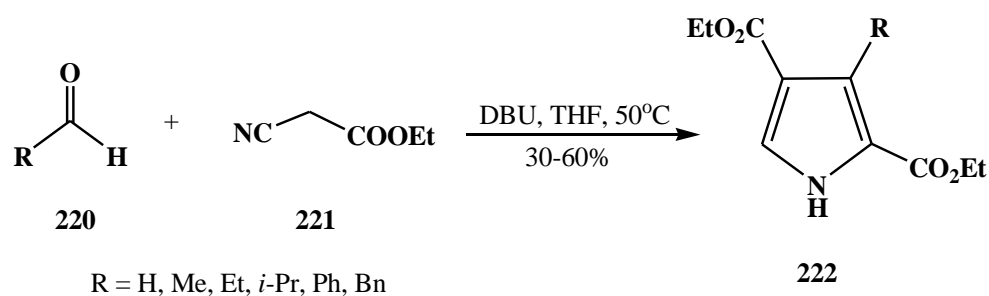


Figure 21. HMBC spectrum of compound **219** (300 MHz, CDCl₃)

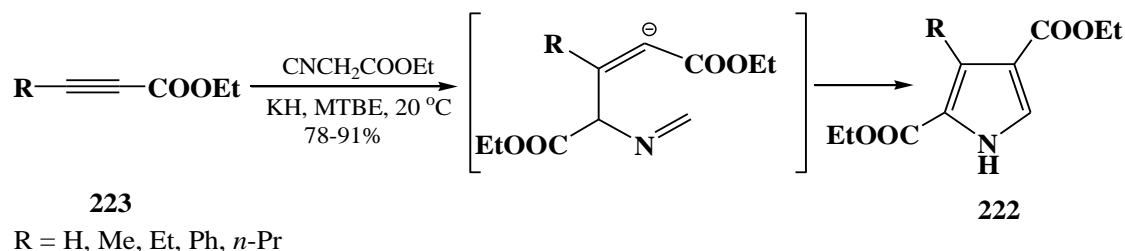
2.6 Synthesis of pyrrole-2,4-dicarboxylate derivatives

Matsumoto *et al.* investigated the reaction of alkyl isocyanoacetate **221** with a variety of aliphatic and aromatic aldehydes **220** in THF, using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as the base, Scheme 68.³⁷



Scheme 68.³⁷ Synthesis of pyrrole-2,4-dicarboxylate derivatives **222** by reaction of aldehydes and isocyanoacetates

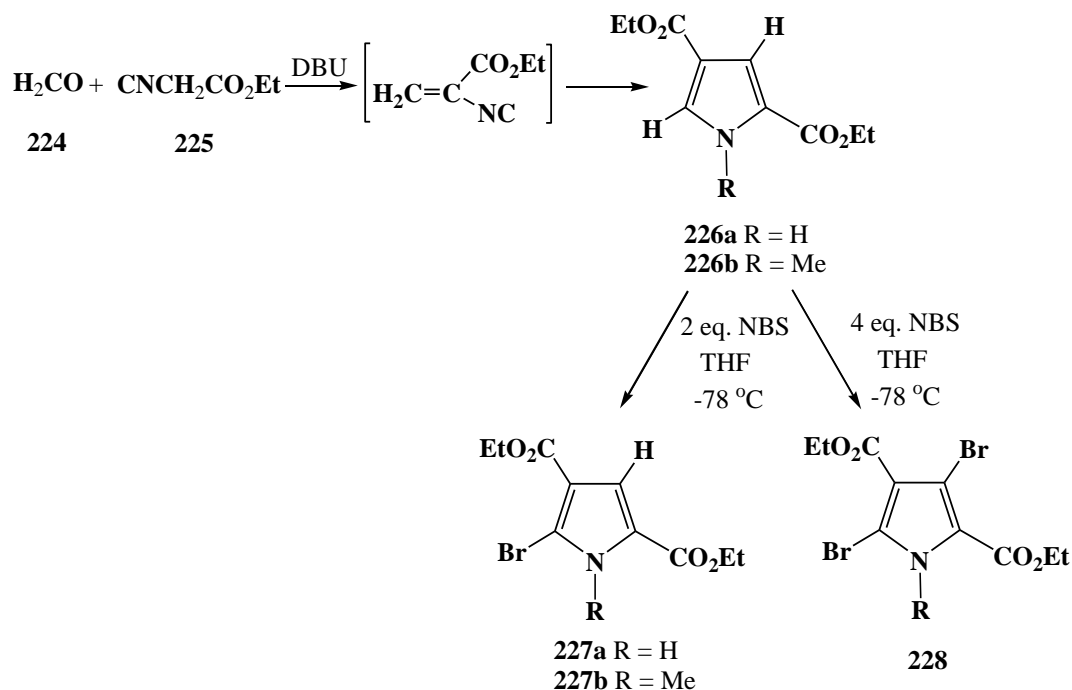
Bhattacharya and his group also described an efficient one-pot synthesis of pyrrole-2,4-dicarboxylate derivatives **222**³⁸ by treatment of a mixture of acetylenic esters **223** and ethyl isocyanoacetate **221** with KH in MTBE for 4-20 hours at room temperature. The pyrrole derivatives were obtained in good to excellent yields, Scheme 69.



Scheme 69.³⁸ Synthesis of pyrrole-2,4-dicarboxylate derivatives **222** from acetylenic esters

Our initial idea was to synthesise diethyl 1*H*-pyrrole-2,4-dicarboxylate **226**, following the method of Matsumoto. The condensation of two equivalents of ethyl isocyanoacetate **225** with formaldehyde **224**, in the presence of DBU in THF, afforded a known pyrrolodiester **226**, Scheme 70. Comparison of the analysis obtained with that of the analysis acquired from the original synthesis showed clearly

that the product was the desired pyrrolodiester. Next, we decided to investigate the selective halogenation of this pyrrole ester, since only a few examples exist in the literature.³⁹ The brominated pyrroles **227** and **228** were prepared from compound **226** using NBS in THF at -78 °C. On addition of 1 and 1.5 equivalents of NBS, it became evident that no reaction occurred. Using 2 equivalents of NBS we obtained the 5-bromo-1*H*-pyrrole-2,4-dicarboxylic acid diethyl ester **227** as the major product, together with the dibromo derivative **228**. In order to optimise the formation of the dibromo compound **228**, and especially to obtain only the 3,5-dibromo-1*H*-pyrrole-2,4-dicarboxylic acid diethyl ester **228**, the bromination was attempted with 4 equivalents of NBS, Scheme 70. Evidence for the formation of the expected product **228** was given by the ¹H NMR spectrum, with the disappearance of the 2 CH signals at δ7.23 and δ7.47 and also the ¹H NMR spectrum of the monobromo compound **227** showed a singlet of the H-3 at δ7.03.



Scheme 70. Selective halogenation of pyrrolodiester **226**

2.7 Palladium catalysed cross-coupling reactions

Heterocycles have been widely functionalised by using palladium(0)-catalysed cross-coupling reactions⁴⁰ and metal catalysed carbon-carbon bond cross-coupling reactions play an increasingly important role in the preparation of polyfunctionalised heterocycles, especially the Suzuki, Negishi, Stille and Sonogashira reactions.

A typical cross-coupling reaction includes four major steps, Figure 22. The first step in this cycle is the oxidative addition of the halide component with a palladium(0) complex to give a palladium-(II) species, followed by metathesis, and transmetallation to form an intermediate which must undergo isomerisation to the *cis* complex before reductive elimination can occur. The final step is the reductive elimination of the desired products and the regeneration of the Pd(0) complex.

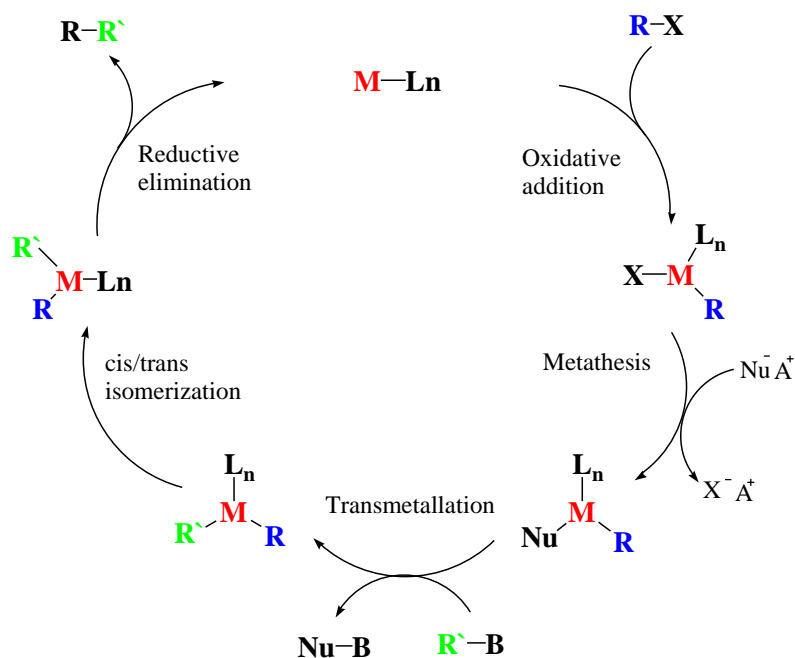
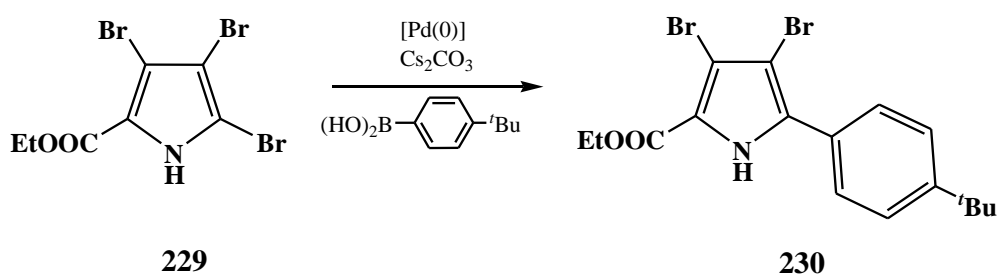


Figure 22. The general catalytic cycle of Pd(0)-catalysed cross-coupling reactions

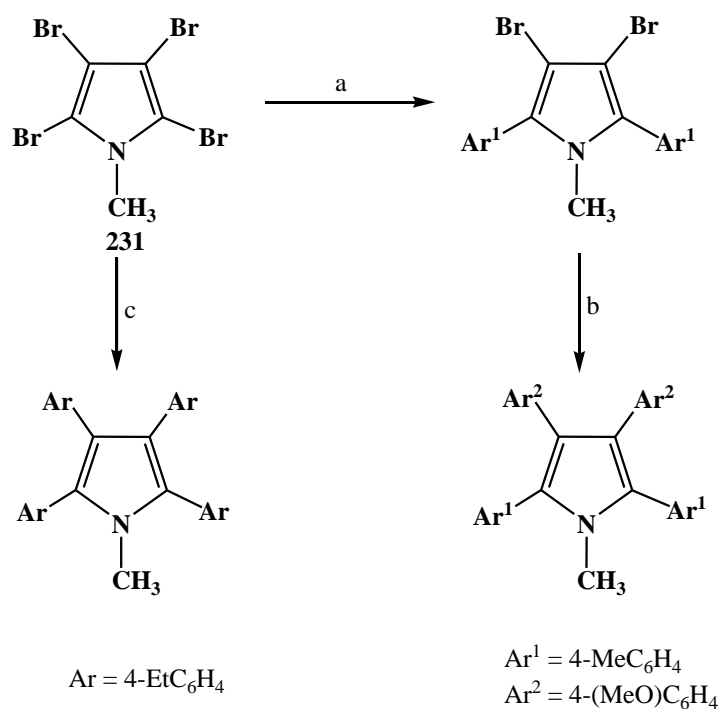
2.7.1 Suzuki reaction

Bach and Schroter investigated the regioselective Suzuki cross-coupling reactions of halogenated nitrogen-, oxygen-, and sulfur-containing heterocycles, Scheme 71.⁴¹ They focused on the optimization of the reaction conditions for ethyl 2,3,4-tribromopyrrole-5-carboxylate **229** and found Pd(PPh)₃, Pd₂(dba)₃/P(2-furyl)₃ to be the best catalysts for the cross-coupling reaction to give phenylpyrrole **230**. They also established the optimum reaction temperature to be between 130-150 °C, in the presence of Cs₂CO₃ as base and the best solvent system to consist of an aromatic hydrocarbon (xylene or mesitylene), ethanol and water in a ratio of 5:1:1.⁴²



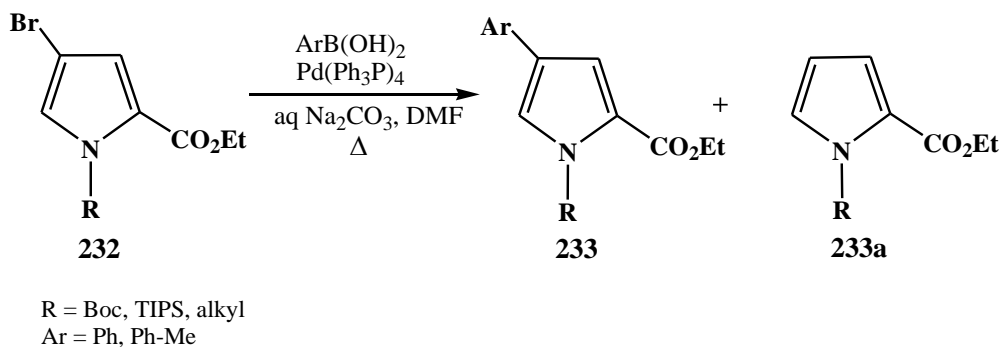
Scheme 71.⁴² Suzuki cross coupling reaction of polyhalogenated pyrrole

Langer and his research group were interested in the palladium(0)-catalyzed cross-coupling reactions of tetrahalopyrroles **231** as this had not previously been reported because of the unstable nature of these compounds.⁴³ These workers demonstrated that the stoichiometry, temperature, solvent and the presence of water play an important role in terms of yield, Scheme 72. They reported the best yields (57-78%) were obtained using a solvent mixture (DMF/toluene/EtOH/H₂O = 4:1:1:1) and an increased amount of catalyst (10-20 mol%).



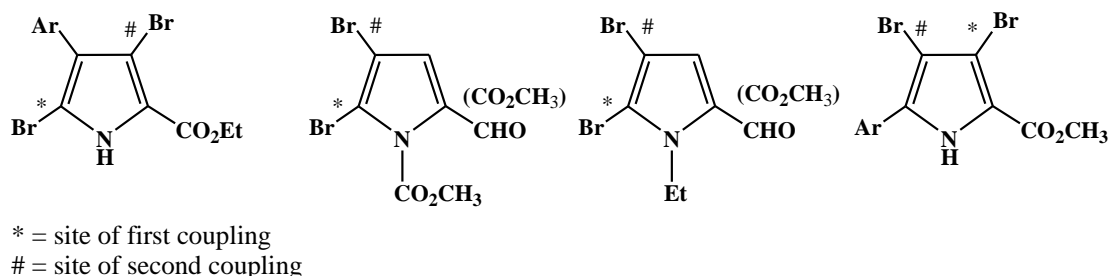
Scheme 72.⁴³ Synthesis of 2,5-diaryl-3,4-dibromo- and tetraarylpyrroles, Reagent and conditions; a) $\text{Ar}^1\text{-B(OH)}_2$, $\text{Pd(PPh}_3)_4$ (10 mol%), K_3PO_4 , Toluene- H_2O (5:1), 90 °C; b) $\text{Ar}^2\text{-B(OH)}_2$, $\text{Pd(PPh}_3)_4$ (20 mol%), K_3PO_4 , DMF, Toluene, EtOH, H_2O (4:1:1:1), 90 °C, 96 h; c) Ar-B(OH)_2 , $\text{Pd(PPh}_3)_4$ (20 mol%), K_3PO_4 , DMF, Toluene, EtOH, H_2O , (4:1:1:1), 90 °C, 96 h;

Handy and co-workers reported an unusual dehalogenation of 4-bromopyrrole-2-carboxylate.⁴⁴ The coupling reaction with phenylboronic acid resulted in a mixture of the desired coupling adduct (55%) and the debrominated compound (28%) but when the *N*-protected pyrrole **232** (BOC, TIPS, alkyl) was reacted as the starting material with 2-3 equivalents of boronic acid, the expected compound **233** was the main product and only a slight amount (<5%) of dehalogenated compound was obtained, Scheme 73.



Scheme 73.⁴⁴ Suzuki coupling reaction of *N*-protected pyrrole

Handy and Zhang later established a simple guide for predicting regioselectivity in the coupling of polyheteroaromatics using a ¹H NMR method in which they investigated a series of dibromo compounds under Suzuki coupling and found that the more electron deficient site undergoes coupling first, Scheme 74.⁴⁵

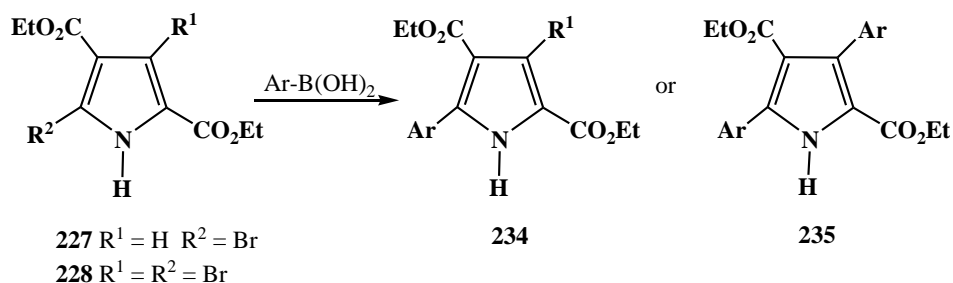


Scheme 74.⁴⁵

2.7.2 Preparation of biaryl compounds

The Suzuki coupling reaction is one of the most extensively studied methods for the preparation of biaryls and several applications have been described in pyrrole chemistry.⁴⁶ We were also interested in the study of the palladium-catalysed C-C bond forming reactions of 3,5-dihalogenated pyrroles. Our initial study involved the

coupling of mono-bromosubstituted pyrrole **227** with commercially available boronic acids, Scheme 75. Our initial attempt involved the conversion of bromopyrrole **227** to the corresponding diethyl-5-phenyl-1*H*-pyrrole-2,4-dicarboxylate with phenylboronic acid in the presence of Pd(OAc)₂, PPh₃ and K₂CO₃. Following the reaction by TLC did not show any expected product and only starting material was observed. A repeat of the reaction was attempted by changing the reaction solvent and base, but again, only starting material was recovered. We thus assumed that the problem was associated with the catalyst, therefore, the experiment was repeated in the presence of Pd(PPh₃)₄ and fortunately, TLC indicated the appearance of new compound. As a result of the optimisation of the reaction, we identified Pd(PPh₃)₄ as a good catalyst for the cross-coupling reactions and Na₂CO₃ was used as the base since other carbonates, such as K₂CO₃, Cs₂CO₃, did not promote the reaction. The coupling reaction of mono- and dibromopyrroles **227** and **228** was then carried out under the optimised Suzuki conditions with various boronic acids, Table 2. We hoped that by using carefully controlled conditions and equimolar quantities of phenylboronic acids, a regioselective Suzuki-Miyaura cross-coupling reaction with the dibromide might occur. Unfortunately, when equimolar quantities of the substrate were heated at 90 °C a complex mixture of mono- and dibromo compounds was produced.



Scheme 75. Suzuki reaction of bromo-substituted pyrrole

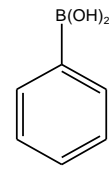
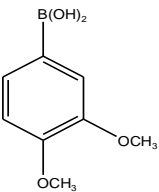
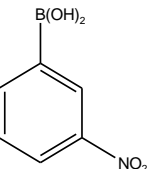
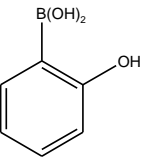
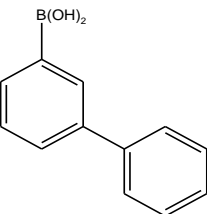
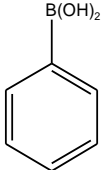
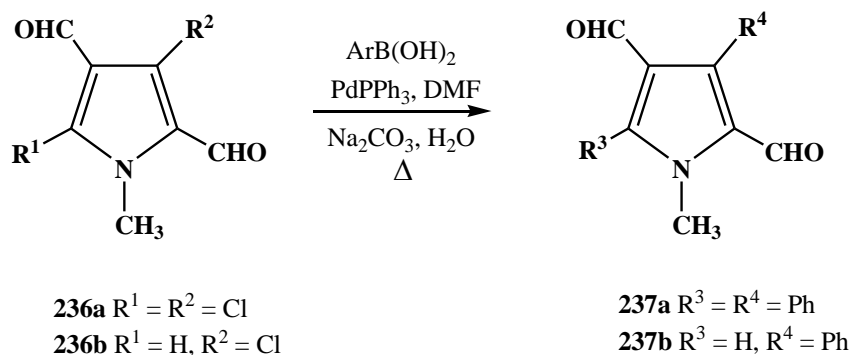
Starting Material	R ¹	R ²	Boronic acid	Time	Product	Yield (%)
227	H	Br		14h	234a	58% ^a
227	H	Br		6h	234b	88% ^a
227	H	Br		18h	234c	0% ^a
227	H	Br		18h	234d	0% ^a
228	Br	Br		9h	235a	68% ^b
228	Br	Br		14h	235b	71% ^b

Table 2. Suzuki products **234-235**. a) using 1.2 equivalents of boronic acid, b) using 3 equivalents of boronic acid

All the structures were confirmed by NMR and IR spectroscopy and high resolution mass spectrometry or elemental analysis.

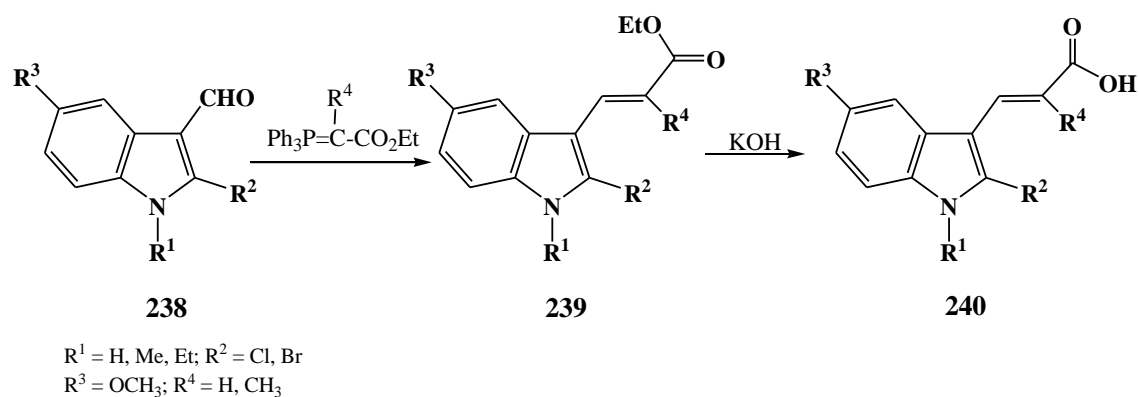
The Suzuki reaction was also successfully carried out on the chloro substituted pyrrole **236**, using the same conditions as above, Scheme 76, and the structure of the compounds was again confirmed by high resolution mass spectrometry and ^1H and ^{13}C NMR spectroscopy; new aromatic signals appeared on the ^1H spectra of compound **237a** at $\delta 7.38$ and $\delta 7.46$ as multiplets



Scheme 76

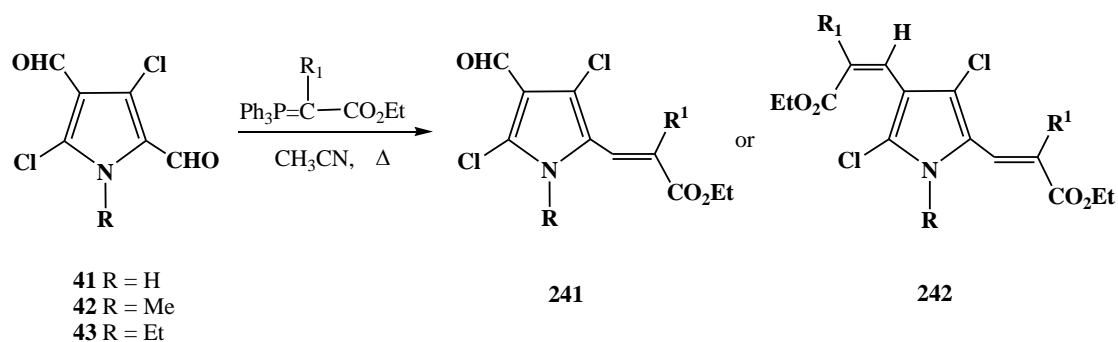
2.8 Wittig reaction

Rambaldi *et al.* investigated the synthesis of indolecarboxylic acids as potential anti-inflammatory agents and they reported a new series of indoleacrylic and methylacrylic acids.⁴⁷ The starting aldehyde **238** was reacted with (carboethoxyethylidene)- or (carboethoxymethylene)triphenylphosphorane in acetonitrile. Hydrolysis of the crude intermediate ester **239** resulted in the expected indolecarboxylic acid **240** in 70-80% yield, Scheme 77.



Scheme 77.⁴⁷ Synthesis of indolecarboxylic acids

In addition to the synthesis of indolecarboxylic acids **240**, we decided to attempt to prepare some pyrrole analogues. The appropriate aldehydes **41**, **42**, **43** were reacted under Wittig conditions for 9-12 h then, following the standard work up, the pyrrole acrylates **241**, **242** were obtained in 35-64% yield, Scheme 78.



Scheme 78. Preparation of pyrrole acrylates. For definition of R, R¹ see Table 3.

Entry	R	R ¹	Product ^{a,b}	Yield (%)
a	Me	H	241a ^a	35%
b	Me	H	242a ^b	45%
c	H	Me	241b ^a	35%
d	H	H	242b ^b	39%
e	Et	H	241c ^a	43%
f	Et	H	242c ^b	64%

Table 3. a) using 1.05 equivalent of Ph₃P=CR¹CO₂Et, b) using 1.75 equivalent of Ph₃P=CR¹CO₂Et

Identification of the products was achieved by spectroscopic data and high resolution mass spectrometry. In the ¹H NMR spectra, the coupling constant ($J = 16$ Hz) of the olefinic protons indicated the *trans* configuration.

2.9 Conclusion

The aim of this part of the work was to investigate the chemoselectivity of the reactions of polyfunctional pyrroles with a range of reagents in order to examine the use of these multi-substituted pyrroles as starting materials for a range of pyrrole libraries and in the generation of other heterocyclic libraries.

First we studied the synthesis of 3,5-dichloro-1*H*-pyrrole-2,4-dicarboxaldehyde and we successfully improved the yield from 44% to 70% using continuously extraction. Next, we turned our attention to investigate the selective oxidation and reduction of the aldehyde functions in the parent pyrrole, selective transformation into nitrile and amide and also selective dehalogenation. We explored interesting reactions of the two electrophilic centres in the parent pyrrole which are close enough to each other to allow the reaction of both groups simultaneously.

During this project we successfully synthesised pyrrole-2,4-dicarboxylate derivatives and we investigated the selective bromination of these pyrrole esters.

The structures of the synthesised novel compounds were fully characterised by ¹H NMR, ¹³C NMR and IR spectroscopy and high resolution mass spectroscopy or elemental analysis. We used 2D-NMR spectra to identify the regioisomers which formed in the oxidation and reduction of these polyfunctionalized pyrroles, as well as the products of other reactions, including the Suzuki, Wittig, and dehalogenation reactions.

2.10 References

1. Balasundaram, B.; Venugopal, M.; Perumal, P. T.; *Tetrahedron Lett.* **1993**, *34*, 4249.
2. Zaytsev, A. V.; Anderson, R. J.; Meth-Cohn, O.; Groundwater, P. W.; *Tetrahedron*, **2005**, *61*, 5831.
3. Smith, R. F.; Albright, J. A.; Waring, A. M.; *J. Org. Chem.*, **1966**, *31*, 4100.
4. Judkins, B. D.; Allen, D. G.; Cook, T. A.; Evans, B.; Shardharwala, T. E.; *Synth. Commun.*, **1996**, *26*, 4351.
5. a) Blatter, H. M.; Lukaszewski, H.; Stevens, G.; *J. Am. Chem. Soc.*, **1961**, *83*, 2203; b) Fizet, C.; Streith, J.; *Tetrahedron Lett.*, **1974**, 3187.
6. George, A.; Olah, T. K.; *Synthesis*, **1979**, 112.
7. Nohara, A.; Umetani, T.; Sanno, Y.; *Tetrahedron Lett.*, **1973**, *22*, 1995.
8. Reddy, G. J.; Latha, D.; Thirupathaiiah, C.; Rao, S. K.; *Tetrahedron Lett.*, **2004**, *45*, 847.
9. a) Cheung, Y.-F.; *Tetrahedron Lett.*, **1979**, 3809; b) Istvan, E. M.; Abdelaziz, Mekhalfia; *Tetrahedron Lett.*, **1990**, *31*, 7237.
10. Hudlicky, M.; *Reduction in Organic Chemistry*, **1984**.
11. Borch, R. F.; Bernstein, M. D.; Durst, H. D.; *J. Am. Chem. Soc.*, **1971**, *93*, 2897.
12. Andreani, D. B.; Rambaldi, M.; Guarnieri, A.; *J. Med. Chem.*, **1977**, *20*, 1344.
13. Beller, M.; Sayyed, I. A.; Alex, K.; Tillack, A.; Schwarz, N.; Maichalik, D.; *Eur. J. Org. Chem.*, **2007**, 4525.
14. Sayyed, I. A.; Alex, K.; Tillack, A.; Schwarz, N.; Spannenberg, A.; Maichalik, D.; Beller, M.; *Tetrahedron*, **2008**, *64*, 4590.

15. a) Clinton, F. L.; *Synthesis*, **1975**, 135; b) Shinya, S.; Takeshi, S.; Etsuko, M.; Yasuo, K.; *Tetrahedron*, **2004**, *60*, 7899.
16. a) Hollingworth, G. J.; *Comprehensive Organic Functional Group Transformations*; Katritzky, A. R.; Meth-Cohn, O.; Rees, C. W.; Pattenden, G.; Eds.; Elsevier Science: Oxford, **1995**; 5; b) Hudlicky, M.; *Oxidations in Organic Chemistry*; ACS Monograph Series 186; American Chemical Society: Washington, DC, **1990**.
17. Andreani, A.; Massimiliano, G.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M.; Roda, A.; Assimo, G.; Traniello, S.; Spisani, S.; *Eur. J. Med. Chem.*, **2004**, *39*, 785.
18. a) Radspieler, A.; Liebscher, J.; *Synthesis*, **2001**, *5*, 745; b) Showalter, H.; Sercel, A. D.; Leja, B. M.; Wolfangel, C. D.; Ambroso, L. A.; Elliott, W. L.; Fry, D. W.; Kraker, A. J.; Howard, C. T.; Lu, G. H.; Moore, C. W.; Nelson, J. M.; Roberts, B. J.; Vincent, P. W.; Denny, W. A.; Thompson, A. M.; *J. Med. Chem.*, **1997**, *40*, 413.
19. Scott, I.; Clotilde, P. S.; *Tetrahedron Lett.*, **2000**, *41*, 2825.
20. Minkyung, L.; Yoon, C. M.; Gwangil, A.; Hakjune, R.; *Tetrahedron Lett.*, **2007**, *48*, 3835.
21. Coffin, R. A.; Roussell, A. M.; Tserlin, E.; Pelkey, T. E.; *J. Org. Chem.*, **2006**, *71*, 6678.
22. Micheli, F.; Fabio, R.; Cavanni, P.; Rimland, J. M.; Capelli, A. M.; Chiamulera, C.; Corsi, M.; Corti, C.; Donati, D.; Feriani, A.; Ferraguti, F.; Maffeis, M.; Missio, A.; Ratti, E.; Paio, A.; Pachera, R.; Quartaroli, M.; Reggiani, A.; Sabatini, F. M.; Trist, D. G.; Ugolini, A.; Vitulli, G.; *Bioorg. Med. Chem.*, **2003**, *11*, 171.

23. Micheli, F.; Fabio, R.; Cavallini, P.; Cavanni, P.; Donati, D.; Faedo, S.; Maffei, M.; Sabbatini, F. M.; Tarzia, G.; Tranquillini, M. E.; *Bioorg. Med. Chem. Lett.*, **2003**, *13*, 2113.
24. a) March, J.; *Advanced Organic Chemistry*; John Wiley & Sons: New York, **1992**; b) *Comprehensive Organic Synthesis*; Trost, B. M., Ed.; Pergamon Press: Oxford, **1991**; Vol. 6.
25. a) Crawley, M. L.; Goljer, I.; Jenkins, D. J.; Mehlmann, J. F.; Nogle, L.; Dooley, R.; Mahaney, P. E.; *Org. Lett.*, **2006**, *8*, 5837; b) Minetto, G.; Raveglia, L. F., Sega, A.; Taddei, M.; *Eur. J. Org. Chem.*, **2005**, 5277; c) Huang, X.; Shen, R.; Zhang, T.; *J. Org. Chem.*, **2007**, *72*, 1534.
26. Yavari, M. A.; Sayahi, M. H.; *J. Chem. Soc., Perkin Trans 1*, **2002**, 1517.
27. Schulte, K. E.; Reisch, J.; Stoess, U.; *Arch. Pharmaz.*, **1971**, 523.
28. Suchy, M.; Peter, K.; Milan, D.; Vladimir, K.; Aldo, A.; Juraj, A.; *Tetrahedron Lett.*, **2001**, *42*, 6961.
29. Suchy, M.; Peter, K.; Milan, D.; Vladimir, K.; Aldo, A.; Juraj, A.; Maddalena, R.; Maria, G.; *Tetrahedron*, **2002**, *58*, 9029.
30. Ivachtchenko, A. V.; Ilyn, A. P.; Loseva, M. V.; Vvedensky, V. Y.; Putsykina, E., B.; Tkachenko, S. E.; Kravchenko, D. V.; Khvat, A. V.; Krasavin, M. Y.; *J. Org. Chem.*, **2006**, *71*, 2811.
31. Dehaen, W.; Bert, M.; Maarten, K.; Gitte, B.; Mario, S.; *Tetrahedron*, **2006**, *62*, 6018.
32. Hudlicky, M.; *Comprehensive Organic Synthesis*; Trost, B. M; Eds.; Pergamon: Oxford, **1991**; Vol. 8.
33. Alonso, F.; Beletskaya, I. P.; Yus, M.; *Chem. Rev.* **2002**, *102*, 4009.
34. Heck, F.; Cortese, N. A.; *J. Org. Chem.*, **1977**, *42*, 3491.

35. Sajiki, H.; Kume, A.; Hattori, K.; Hirota, K.; *Tetrahedron Lett.*, **2002**, 43, 7247.
36. Sajiki, H.; Monguchi, Y.; Kume, A.; Hattori, K.; Maegawa, T.; *Tetrahedron*, **2006**, 62, 7926.
37. a) Matsumoto, K.; Miyoshi, M.; Suzuki, M.; *J. Org. Chem.*, **1974**, 39, 1980; b) Fink, B. E.; Vite, G. D.; Mastalerz, H.; Kadow, J. F.; Kim, S-H.; Leavitt, K. J.; Du, K.; Crews, D.; Mitt, T.; Wong, T. W.; Hunt, J. T.; Vyas, D. M.; Tokarski, J. S.; *Bioorg. Med. Chem Lett.*, **2005**, 15, 4774.
38. Bhattacharya, A.; Cherukuri, S.; Plata, R. E.; Patel, N.; Tamez, V.; Grosso, J. A.; Peddicord, M.; Palaniswamy, V. A.; *Tetrahedron Lett.*, **2006**, 47, 5481.
39. a) Belanger, P.; *Tetrahedron, Lett.*, **1979**, 27, 2505; b) Balsamini, C.; Bedini, A.; Diamantini, G.; Spadoni, G.; Tontini, A.; Tarzia, G.; Di Fabio, R.; Feriani, R.; Reggiani, A.; Tedesco, G.; Valigi, R.; *J. Med. Chem.*, **1998**, 41, 808.
40. Li, J. J.; Gribble, G. W.; *Palladium in Heterocyclic Chemistry*; Pergamon Press: Oxford, **2000**.
41. Bach, T.; Schroter, S.; Stock, S.; *Tetrahedron*, **2005**, 61, 2245.
42. Bach, T.; Schroter, S.; *Synlett*, **2005**, 12, 1957.
43. Dang, T. T.; Ahmad, R.; Reinke, H.; Langer, P.; *Tetrahedron Lett.*, **2008**, 49, 1698.
44. Handy, T. S.; Howard, B.; Jennifer, L.; Xiaolei, Z.; Yanan, Z.; *Tetrahedron Lett.*, **2003**, 44, 427.
45. Handy, T. S.; Yanan, Z.; *Chem Commun.*, **2006**, 299.
46. a) Banwell, M. G.; Goodwin, T. E.; NG, S.; Smith, J. A.; Wong, D. J.; *Eur. J. Org. Chem.*, **2006**, 3043; b) Stanforth, S. P.; *Tetrahedron*, **1998**, 54, 263.
47. Rambaldi, M.; Andreani, A.; Locatelli, A.; Pifferi, G.; *Eur. J. Med. Chem.*, **1994**, 29, 903.

Chapter Three

Molecular Modelling

3. Molecular Modelling

3.1 Introduction

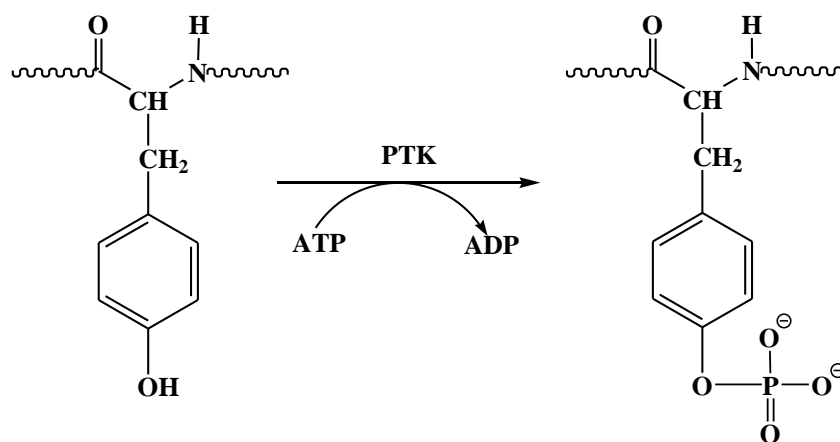
During the last decade molecular modelling has become an increasingly popular method in drug discovery, partly due to the increased speed of today's computers. This technique allows the computer aided generation of molecular structures, as well as the computation of molecular properties. As stated earlier, molecular modelling makes it possible to construct models of already known compounds, but molecules which have not yet been synthesised can also be investigated. The prediction of three-dimensional structures and molecular surface properties, and the optimisation of drug-receptor interactions by visual inspection can all be achieved through the use of molecular modelling.¹

A basic theory in pharmacology is that drugs should bind to a specific macromolecule, called a receptor (which is present on either on the surface of the cell membrane or in the cytoplasm) and thus prevent cellular biochemical processes, for example enzymatic activity, DNA transcription or protein phosphorylation.

Any potential molecules (*e.g.* drug, hormone, or neurotransmitter) which can bind to a receptor are called ligands. Receptors have an active site with a specific shape and accept only a specific type of ligand with the correct size, shape and charge into the binding site, which contains chemical groups that direct by participating in the binding of the ligand.² The ligand can activate (agonist, *e.g.* nicotine or morphine) or deactivate (antagonist, *e.g.* naloxone) the receptor, and the activation can therefore increase or decrease a particular function of the cell.

3.1.1 Protein tyrosine kinase (PTK)

In the past two decades a main topic of research in the area of cancer biochemistry has been the understanding of the role of PTKs in the uncontrolled proliferation of malignant cells and the development of inhibitors which are designed to block the activity of tyrosine kinases (unregulated activation of these enzymes can result in a number of different forms of cancer). Protein tyrosine kinases (PTK) are enzymes which catalyse the process of phosphate group transfer from a donor molecule, such as adenosine triphosphate (ATP), to an amino acid (tyrosine) residue of a protein, Scheme 79. The PTK family can be divided into two major groups, the transmembrane receptor PTKs and the non-receptor PTKs.³



Scheme 79. Phosphate group transfer

3.1.2 Receptor tyrosine kinase (RTK)

Receptor tyrosine kinases play an important role in different cellular functions including cell growth, cellular differentiation and angiogenesis.^{4a} All known RTKs contain a glycosylated extracellular ligand binding domain, which is connected to the

cytoplasmic domain by a transmembrane region.^{4b} The signalling pathway involving receptor tyrosine kinases in normal cells starts with the binding of the ligand, *e.g.* hormone or growth factor, to a specific site within the extracellular domain of the receptor and this initiates the binding of two receptor molecules to one another (dimerisation). The nascent signal crosses the membrane and activates the intracellular domain, which catalyses the phosphorylation of the tyrosine residue of a protein and modulates various cellular responses, *e.g.* angiogenesis.^{4c} Growth factors (*e.g.* vascular endothelial growth factors (VEGFs), platelet-derived growth factors (PDGFs) and fibroblast growth factors (FGFs)) are the main regulators of angiogenesis.^{4d}

3.1.3 The Vascular Endothelial Growth Factor (VEGF)

The VEGF is an endothelial, cell-specific, growth stimulator which acts by binding the VEGF receptor-1 (VEGFR-1 or Flt-1) and VEGF receptor-2 (VEGFR-2 or Flk-1)⁵ and also binds to the VEGFR receptor-3 (VEGFR-3, Flt-4). These receptors are mainly expressed on endothelial cells but have recently been found to be over-expressed on non-endothelial cells, such as malignant melanoma or ovarian carcinoma tumour cells.⁶

Each receptor has an extracellular part, with seven immunoglobulin-like domains, a single transmembrane region and an intracellular domain. The ligand binding site of these receptors is located in their second and third immunoglobulin-like loops, and VEGF binding leads to the dimerisation of the receptor in which the immunoglobulin-like domains are held close to each other, in order to help stabilise the receptor dimers. The receptor–ligand complexes instigate a signal being passed to

the intracellular tyrosine kinase domains and the activated tyrosine kinase initiates processes within the endothelial cells leading to cell migration, proliferation and survival, Figure 23.⁷

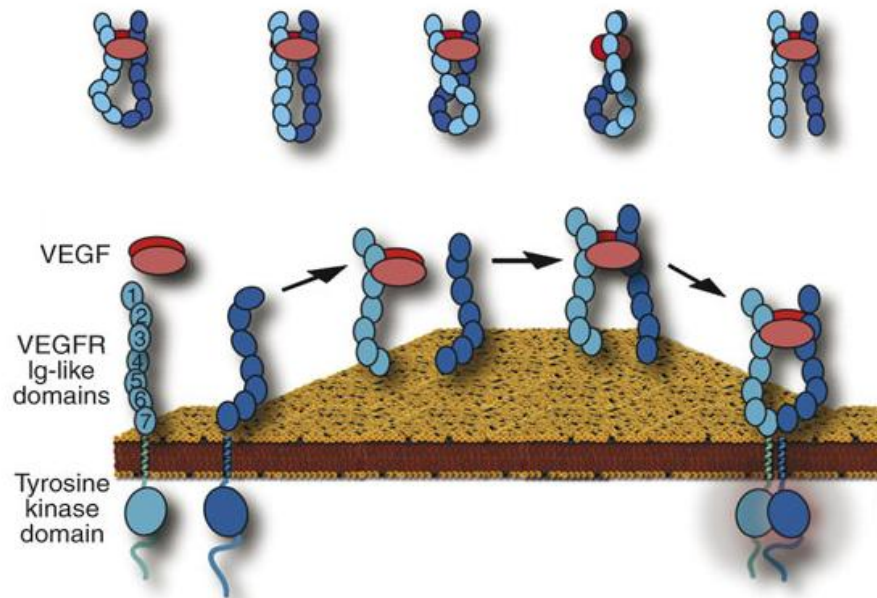


Figure 23. Suggested model for the activation of the RTKs of the VEGF receptor family⁷

The VEGFR-1 plays an important role in the development of angiogenesis while the VEGFR-2 is the major mediator of endothelial cell proliferation, microvascular permeability, migration and survival.⁸ The VEGFR-3 is located mainly on the surface of the lymphatic endothelial cells and this receptor is involved in tumour lymphangiogenesis. The importance of the VEGF in tumour angiogenesis and the pathogenesis of human cancers is well-established and so different strategies have been developed for the inhibition of VEGF-mediated tumour growth.⁸

Several agents target the VEGF, including soluble VEGFRs or VEGF antibodies. For example, VEGF-Trap is a high affinity soluble VEGF receptor, which can block the

biological activity of VEGF by preventing it from binding to its normal receptor, while Bevacizumab (Avastin) is a monoclonal antibody, which inhibits the interaction of the VEGF with the corresponding receptor.⁹

An alternative method of blocking VEGF-mediated processes uses small-molecule kinase inhibitors, with several VEGFR inhibitors in preclinical and clinical evaluation, for example the anilinoquinazoline derivatives ZD4190, ZD6474 and AZD2171.^{9,10} There are, however, only a few examples in the literature of pyrrole-containing inhibitors, such as Semaxanib (SU5416), SU6668, SU10944¹¹ (Figure 24) and Sunitinib (SU11248),¹² (Figure 25).

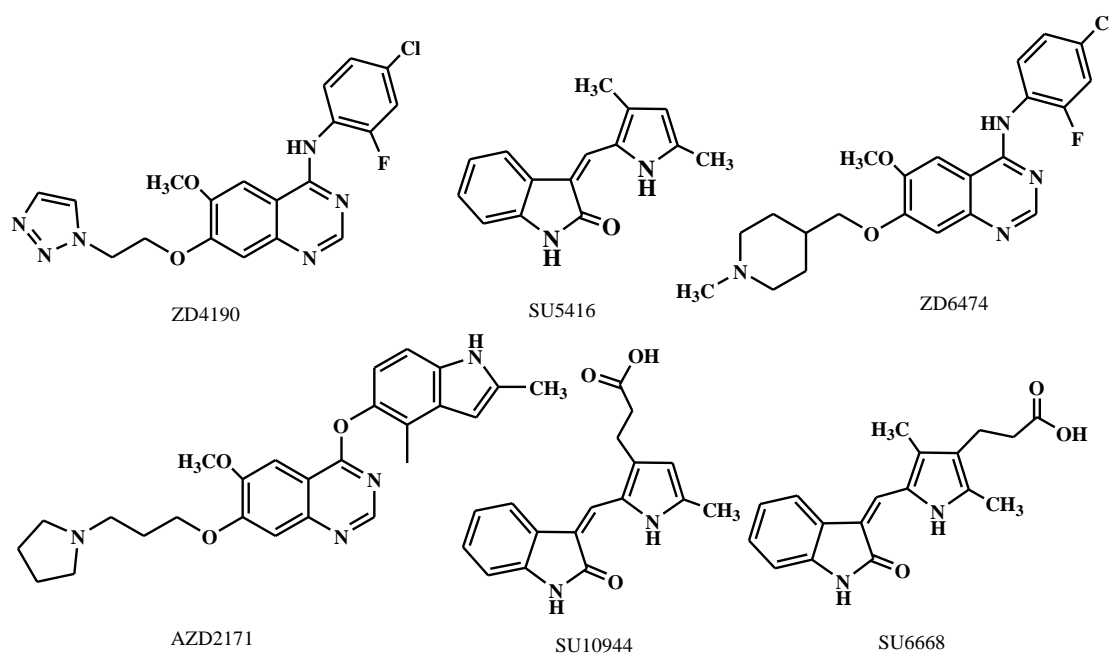


Figure 24. Examples of VEGFR inhibitors

An attractive starting point for the design of novel inhibitors of the VEGFR-2 kinase domain is the Sutent (SU11248) structure, Figure 25.

3.1.4 Sutent

Sutent (previously known as SU11248; chemical name sunitinib malate) **243** is a novel, oral, multi-targeted receptor tyrosine kinase (RTK) inhibitor that exhibits anti-cancer and anti-angiogenic effects, Figure 25.^{12a}

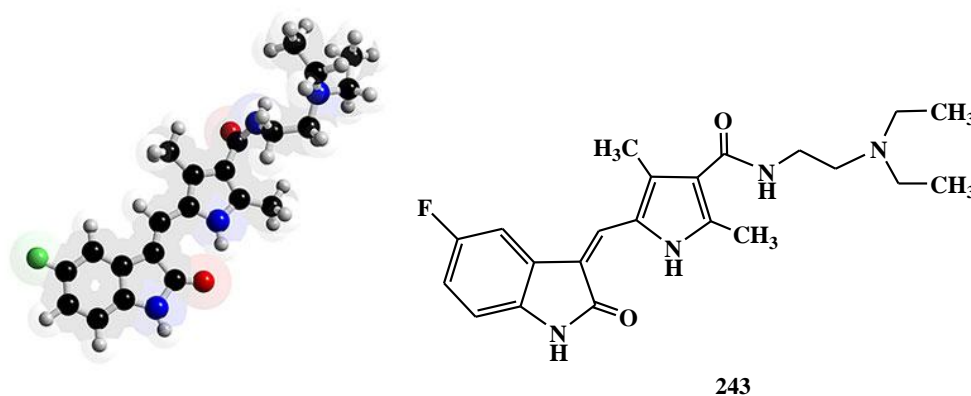


Figure 25. The 3D^{12b} - and 2D-chemical structures of Sutent

Sutent was approved by the FDA (Food and Drug Administration) for the treatment of renal cell carcinoma (RCC) and gastrointestinal stromal tumor (GIST) in 2006, becoming the first cancer drug simultaneously approved for two different indications. The cellular targets of this drug are the multiple RTKs, including the vascular endothelial growth factor receptor (VEGFR), and the platelet-derived growth factor receptor (PDGFR).¹³ Sutent exhibits competitive inhibition of VEGFR-2 and PDGF-dependent PDGFR- β phosphorylation, with an $IC_{50} = 10$ nM for both RTKs, and also inhibits the VEGF- induced proliferation of the HUVEC cell line, with an $IC_{50} = 40$ nM.¹⁴ The simultaneous inhibition of these targets leads to reduced tumour vascularisation, cancer cell death, and also tumour shrinkage.

3.2 Structure Based Drug Design (SBDD)

The process of designing a new drug and then bringing it to the market is very time consuming, and it takes around 10-15 years and \$1 billion for the average new drug to reach the clinic. Structure based drug design has been around since the early to mid – 1980s and success stories are only just starting to appear.^{2,15} There are numerous examples of current pharmaceuticals that were developed by structure based design, including for example Zanamivir (Relenza¹⁶, a neuraminidase inhibitor for the treatment of the influenza virus), Sildenafil (Viagra¹⁷, a phosphodiesterase-5 inhibitor) and Saquinavir (Fortovase¹⁸, an HIV protease inhibitor), Figure 26.

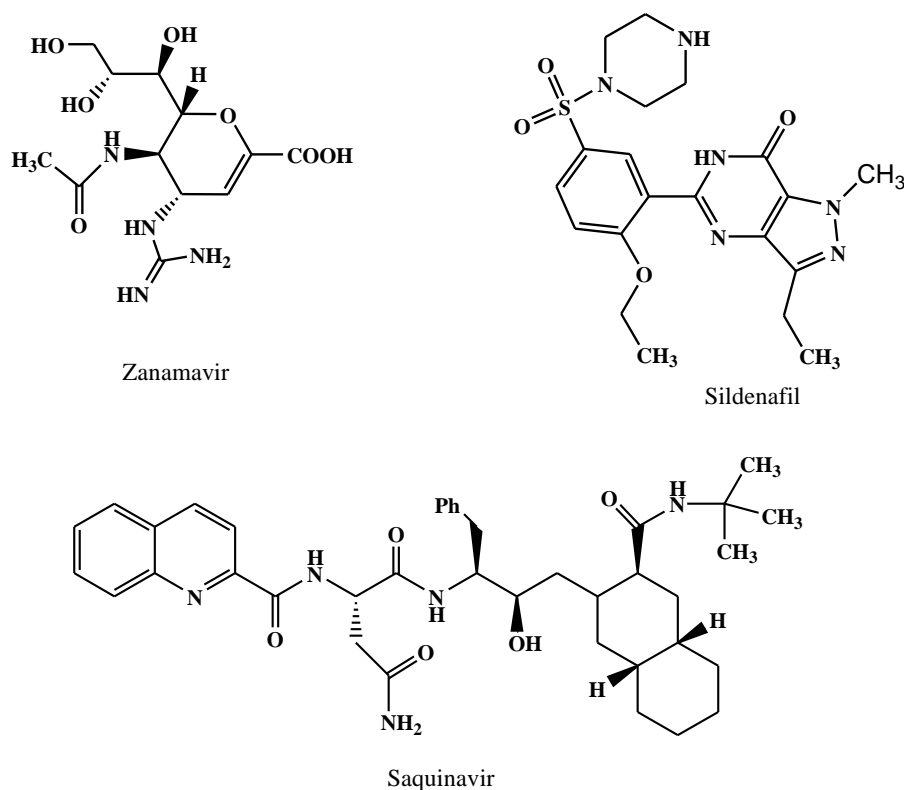


Figure 26. Structure of Zanamivir, Sildenafil and Saquinavir

Structure Based Drug Design is an iterative process, which starts with the identification of the potential target (receptor or an enzyme), followed by the characterisation of the target (X-ray crystallography, Nuclear Magnetic Resonance) and identification of a possible ligand binding site (ideally, the target site is a pocket with a variety of potential hydrogen bond donors and acceptors, hydrophobic characteristics, etc.). The next step is to design an inhibitor which will bind to the active site of the target and prevent the usual chemical reaction.

Once the inhibitor hit compound is identified, it can be synthesised and a small library can be prepared (five to ten compounds) around the proposed ligand in order to obtain structure-activity relationship (SAR) data. After they have been synthesised, the target compounds can be tested in a relevant biological assay in order to determine if the SBDD has been successful. The for possible scenarios for structure based drug design are shown in the figure below, Figure 27.¹⁹

The first approach involves the structure of both the ligand and the protein being known. Another method is combinatorial chemistry if the structures of the ligand and proteins are not known, while a third scenario is the *de novo* design technique, which is used if the protein structure is known and the ligand structure unknown. Finally, QSAR and pharmacophore generation can be used when the ligand structure is known and the protein structure is unknown.

		Protein structure	
		known	unknown
Ligand structure	known	Structure based design (SBD)	QSAR Pharmacophore generation
	unknown	de novo design	Library Design/Analysis

Figure 27. Scenarios for structure based drug design¹⁹

3.2.1 The process of structure based drug design

The crystal structure of the VEGFR-2 (1ywn)²⁰ and EGFR kinase domain (2ity)²¹ (Figure 28) were downloaded from the RSCB Protein Data Bank (PDB) and molecular modelling was carried out using InsightII, Cerius² and Catalyst (Accelrys, San Diego). InsightII was used to minimise the protein and correct the structure, structure based drug design was performed on Cerius², and Catalyst was used to view the pharmacophores.

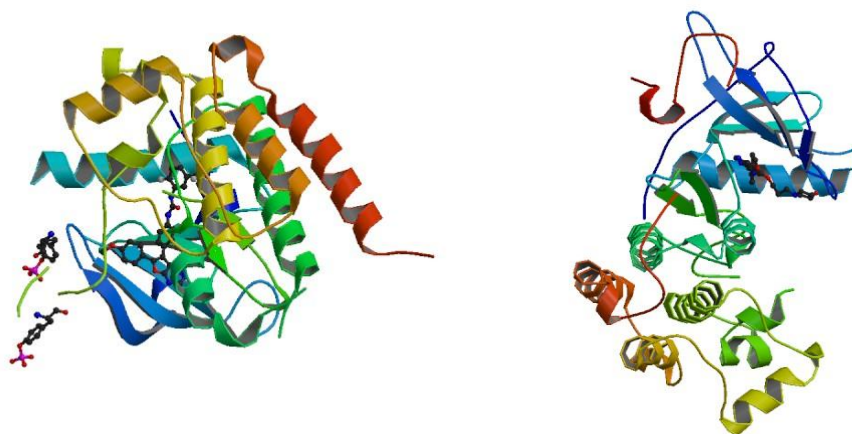


Figure 28. Crystal structure of VEGFR-2 (1ywn) and EGFR (2ity)^{20,21}

The process of structure based drug design is shown in the flow diagram in, Figure 29.

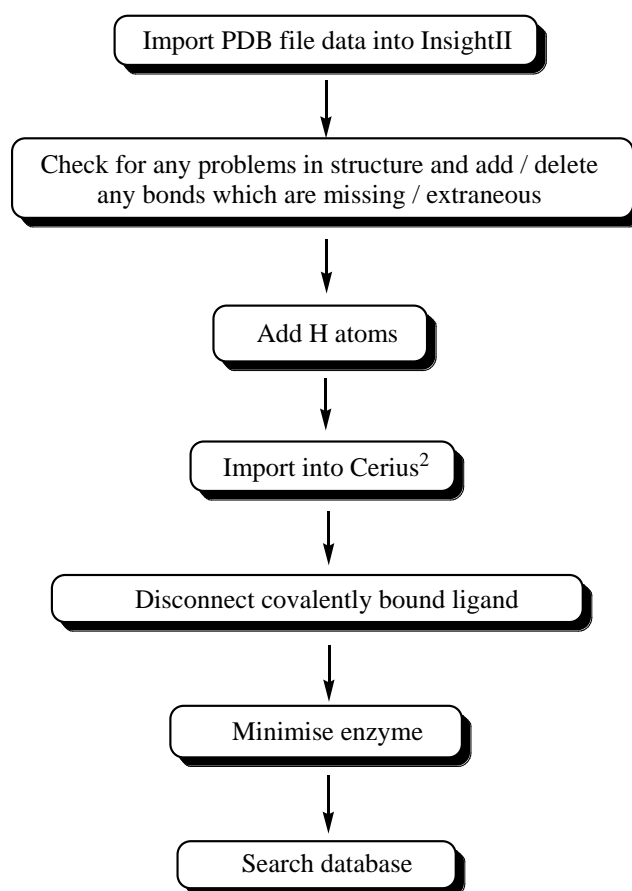


Figure 29. Structure based drug design

3.2.2 InsightII

The crystal structure of the VEGFR-2 (1ywn) in complex with a novel 4-aminofuro[2,3-*d*]pyrimidine was downloaded into InsightII, Figure 30. Hydrogens are not resolved in the PDB (Protein Data Bank) files obtained from X-ray crystal structures, as they are difficult to observe by X-ray crystallography, so they were first added to the crystal structure using the Builder section, then the pH was set to 7, the potentials were fixed (using the CFF91 forcefield) and all atoms and bonds of the

residue were corrected. Next, the crystal structure was transferred to Cerius², still in complex with the ligand, and used in the LigandFit and structure-based focusing (SBF) modules.

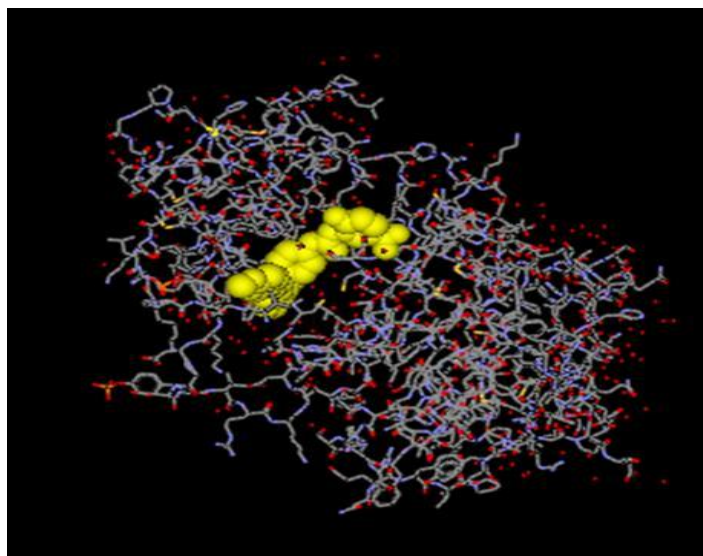


Figure 30. The VEGF receptor-2 complexed with the ligand (yellow) and solvent (red)

3.2.3 Cerius²

3.2.3.1 LigandFit

LigandFit²² is designed to investigate the docking of a ligand into a protein binding site based upon its shape. During this process, the protein is rigid while the ligand remains flexible, so allowing different ligand conformations to be searched and docked within the binding site.

There are three key steps in this process:

- the definition of the active site of the protein based upon the protein shape or a docked ligand,
- the generation of possible ligand conformations for docking, using a Monte Carlo algorithm, and
- the docking of the conformations into the active site and the computation of the docking scores.

The protein was imported into Cerius² as a PDB file, and the first step involved was to remove the solvent, then to separate the ligand atoms from the protein atoms before minimising both structures to find the local minimum of the system. Atomic motion in the protein was allowed only for the hydrogen atoms in order to maintain the shape of the active site. Next, the site model was generated in order to define the active site and then modified in order to remove parts of the active site that could not reasonably bind a ligand. PDB files containing a ligand docked into the active site allow for a more accurate search for a possible binding site than those without a ligand.

After a flexible docking process, a Monte Carlo algorithm was employed to generate ligand conformers. The shape matching method selected the conformers from the database (Maybridge 2005) which fitted into the binding site and, finally, their energies were optimised.

Once the docking was complete, from the hits obtained, pyrrole-containing molecules (and other 5-membered heterocycles) were chosen then clustered, and the top conformers were prioritised using the scoring functions.

Several scoring functions have been developed to rank hits relative to one another. For the best docked conformers we computed scores using the empirical based LigScore1, LigScore2,²³ Jain,²⁴ Dockscore, and Ludi1, Ludi2 scoring functions.²⁵

Scoring functions are used to describe various types of interactions between the two binding partners (*e.g.* ligand-receptor) such as hydrogen-bond or aliphatic- and aromatic-liphophilic interactions. The 12 top scoring 5-membered heterocycle compounds are shown in Figure 31.

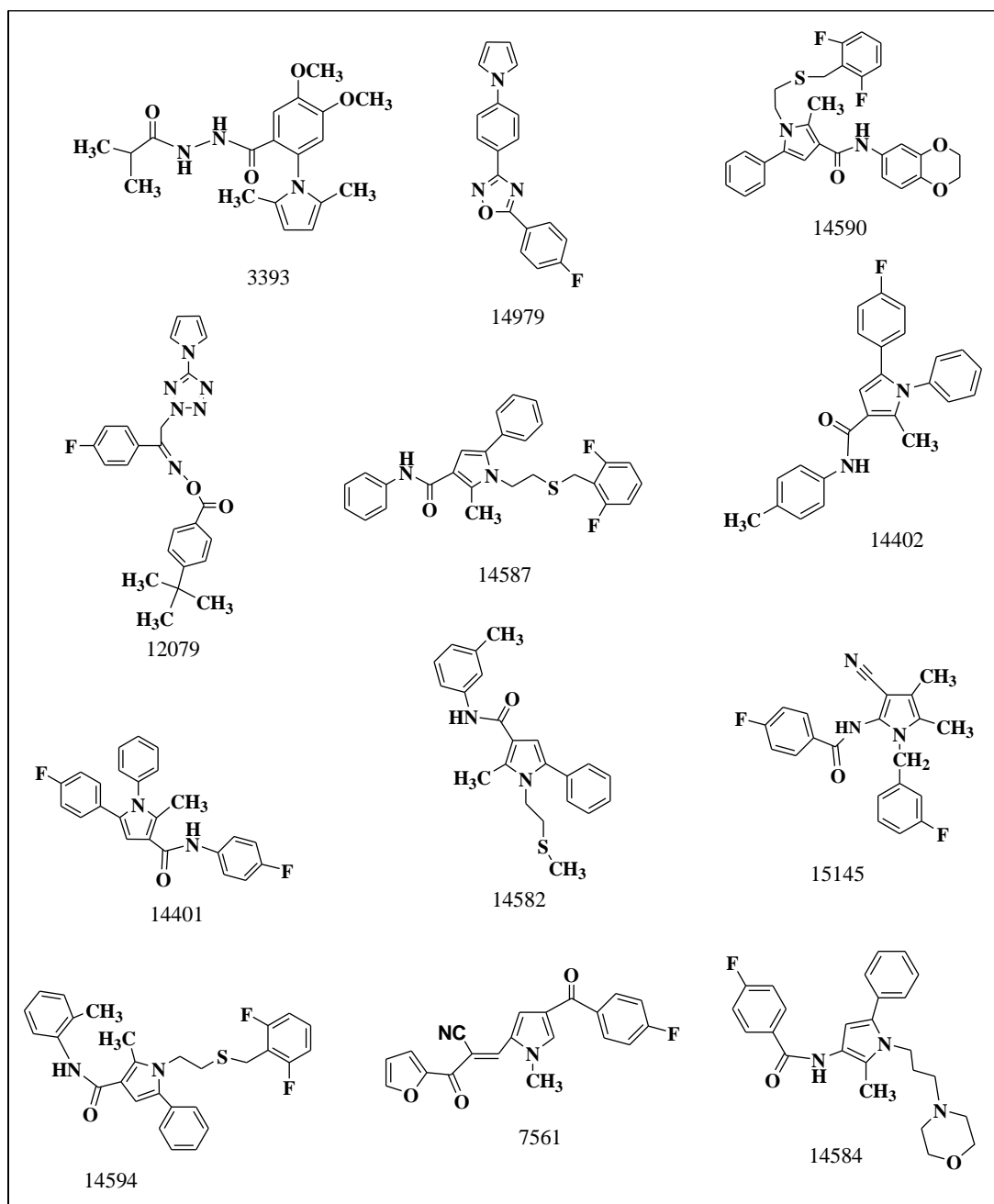


Figure 31. Highest scoring 5-membered heterocycle hits

3.2.3.2 Structure Based Focusing

Structure Based Focusing (SBF) is a method that uses the known active site of a protein to select compounds which are likely to bind within the defined active site. The first step was to define the active site of the protein. The centre of the bound ligand atoms was marked and, starting from this point, the radius (including Asp1044, Glu883, Glu915, and Cys917 residues) was defined, within 7.5 Å, to assign the active site, Figure 32.

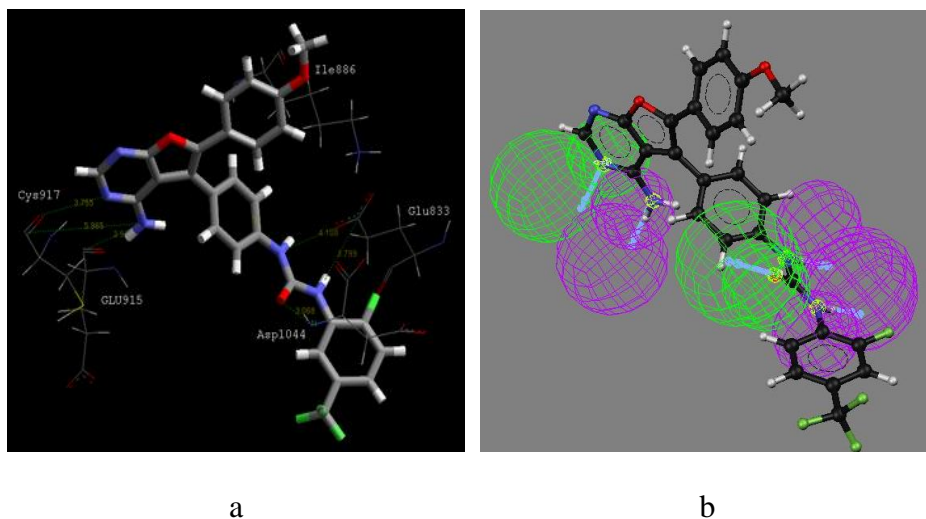


Figure 32. a) Structure of the ligand complexed with VEGFR-2; b) Five feature query in the active site of 1ywn. The green spheres show the hydrogen bond acceptor, and purple hydrogen bond donor interactions

From the defined active site, an interaction map was generated (Figure 33) using the Ludi program, which contains a list of features, such as lipophilic regions, hydrogen bond donors, and acceptors that a ligand is expected to satisfy in order to have a reasonable interaction with the protein.

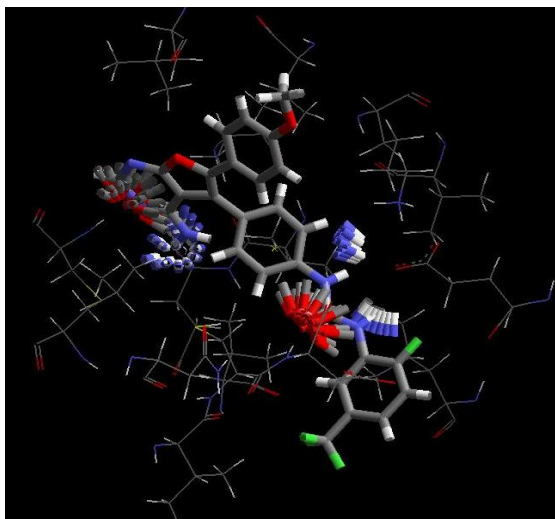


Figure 33. Interaction map of VEGFR complexed with the ligand. Hydrogen bond donors are shown in blue / white and hydrogen bond acceptors are shown in red / grey

Once the interaction model was complete, the next step was to generate the volume exclusion model, which defines regions within the active site that a ligand may not overlap. This process makes the search more specific and precludes ligands that would clash with protein atoms in the active site. The exclusion model does not include hydrogen atoms, which allows for some flexibility in the protein when fitting a ligand to the search query, Figure 34.

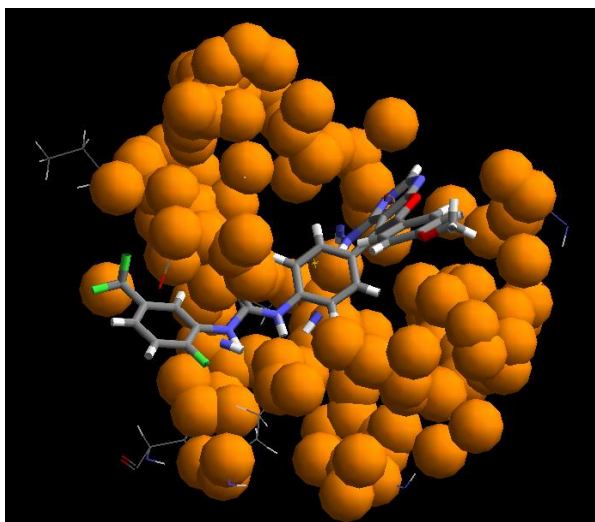


Figure 34. Volume exclusion model

The next step was to generate the 3D queries and then import them into Catalyst to check for any overlaying features and that the exclusion model did not interfere with any of the features. Ten of the top queries were then chosen to search in the previously downloaded Maybridge 2005 database, and the results are summarised in Figure 35.

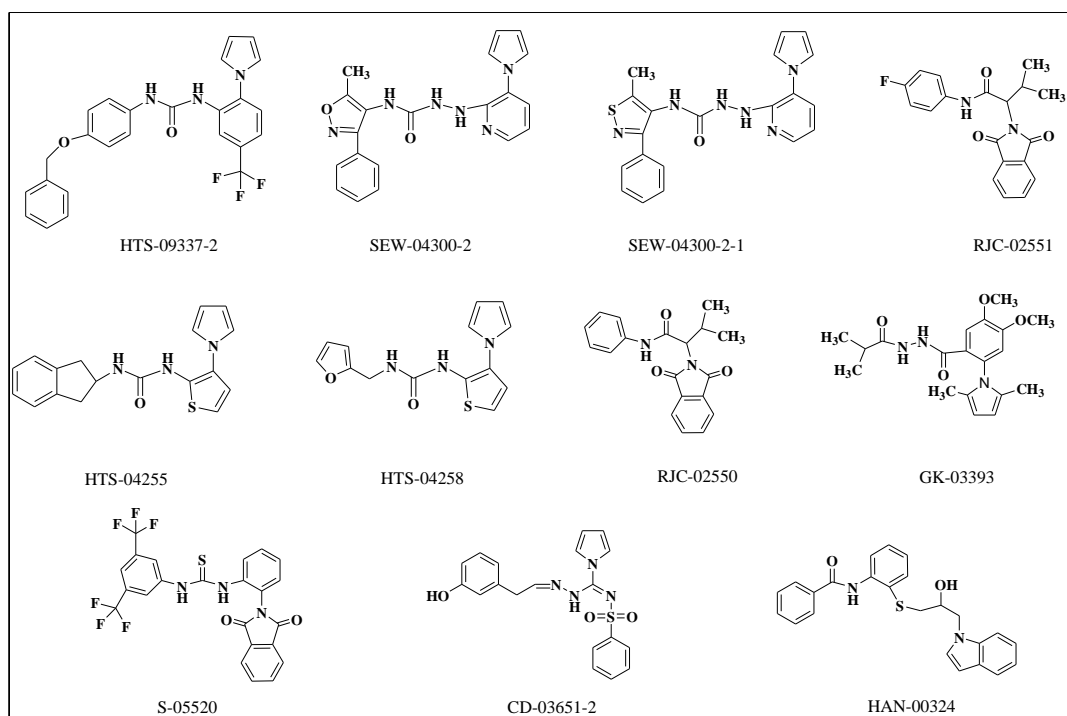


Figure 35. The structure of the 11 highest scoring heterocycles from SBF

In the Sunderland Pharmacy School, several current projects are aimed at the development of novel anti-cancer agents through the testing of inhibitors of the dimerisation of the epidermal growth factor receptor (EGFR), so we also chose to study this process.

The crystal structure of the EGFR kinase domain (2ity) was downloaded into InsightII and LigandFit was performed on the EGFR in the same manner as for the VEGFR-2. The difference between these two processes was only in the searching of the database – for the VEGFR, the Maybridge 2005 database (which contains around 60,000 molecules) was used, while in the second part (EGFR) a virtual library was created from previously found hit molecules. Once the active site of the protein was defined, a Monte Carlo search was used to generate different ligand conformers for docking and then shape matching was applied to select conformers which are similar to the shape of the active site. After fitting, the docked conformers were clustered and, according to the selected method and criteria, the redundant conformers were removed. The top conformers of each ligand were saved, then prioritised with Ligand Scoring and the results are summarised in Table 4.

	Ligscore1	Ligscore2	Jain	Dockscore	Ludi1	Ludi2	Conscore
3026	2.25	4.6	2.11	50.328	531	445	6
6074	2	5.48	1.6	53.477	493	399	6
SEW-04300-01	2.11	4.85	1.57	47.29	510	401	6
SEW-04300-03	2.02	4.92	1.01	47.241	552	435	6
SEW-04300-04	1.77	5.16	3.22	57.207	536	423	6
SEW-04300-02	1.76	5.28	0.89	55.991	546	434	6
13282	1.75	5.27	2.1	45.194	450	427	5
14401	1.63	4.63	1.76	43.437	466	422	5
14402	1.94	4.68	2.6	27.882	555	488	5
14590	2.19	5.59	1.53	-3.62	494	425	5
HTS-04255	1.49	4.57	2.31	45.411	500	462	5
HTS-04258-03	3.32	4.59	3.28	50.418	450	392	5
SEW-04300-08	2.3	4.46	1.23	42.137	554	455	5
SEW-04300-05	1.73	4.66	0.97	37.956	543	470	5
SEW-04300-06	2.31	5.05	0.72	55.574	549	439	5
SEW-04300-07	1.86	4.68	1.46	52.081	460	358	5
13282-01	1.67	4.99	0.99	46.182	405	389	4
14401-01	2.12	5.4	0.58	44.007	468	380	4
14402-01	2.06	5.49	-0.06	41.971	473	418	4
14584	3.19	5.03	2.37	45.753	401	356	4
15145	1.46	4.54	1.42	43.749	437	400	4
7561	1.47	5.1	0.97	42.124	454	383	4
9502	1.73	4.75	0.48	44.153	437	392	4

Table 4. Prioritisation of ligand hits for EGFR

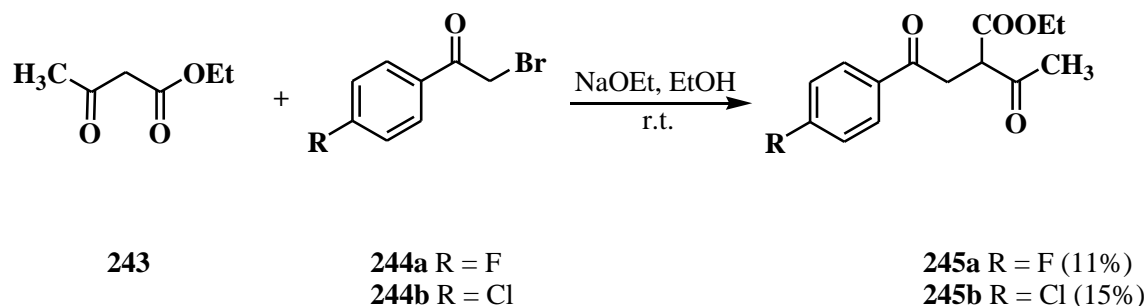
Consensus Scoring²⁵ is a fast means of identifying ligands that score very highly in more than one scoring function. For each scoring function the ligands were prioritised by the score (in descending order) then a value of 1 was assigned to ligands in the top 40%. The remaining ligands were given a value of 0 in the ranking list. For each ligand, the rank value (either 0 or 1) was added across the different scoring functions

to obtain the consensus score for the ligand, with the maximum consensus score being equal to the number of scoring functions used.

The evaluation of the results of the LigandFit and Structure Based Focusing methods suggested the synthesis of compound **249 (14402)** and its derivatives as they are potential inhibitors of both the VEGF and EGF receptors.

3.3 Synthesis of 5-(3'-fluoro-phenyl)-2-methyl-1-phenyl-1*H*-pyrrole-3-carboxylic acid *p*-tolylamide and its derivatives

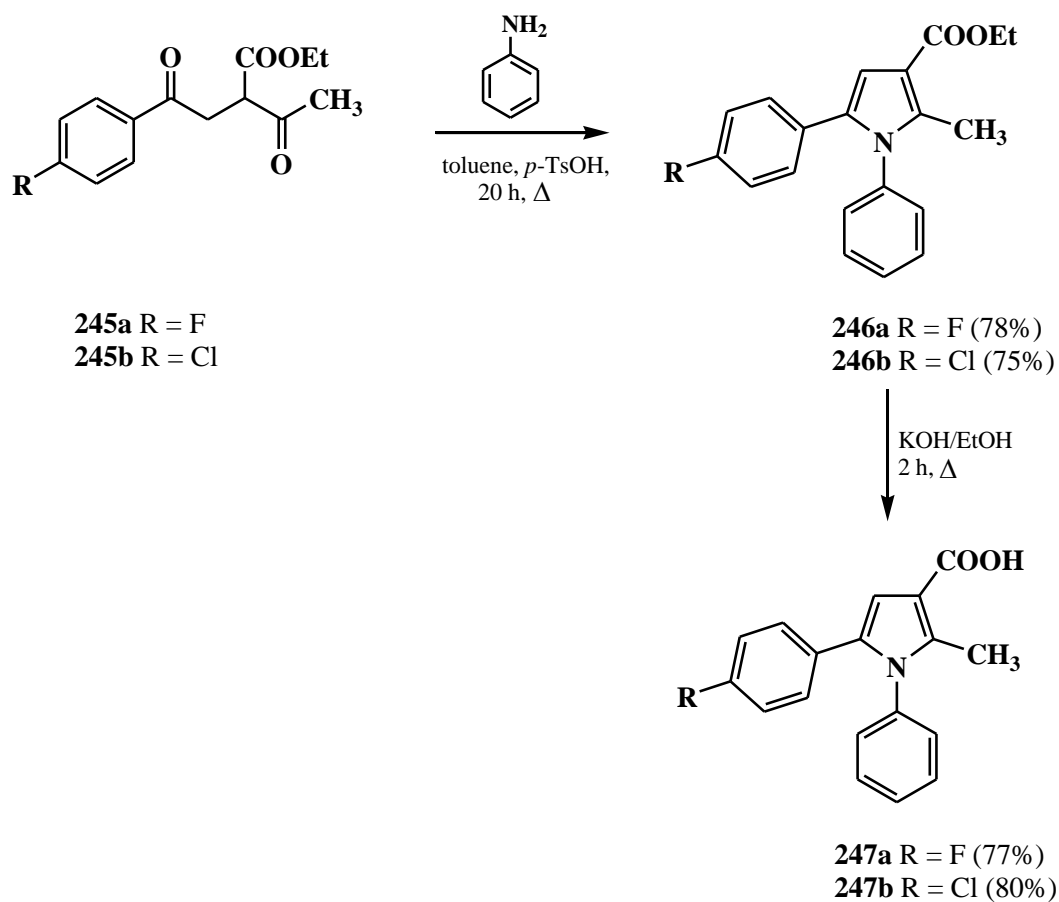
The synthetic route to the target compounds is outlined in Schemes 80-82. The first step in this synthesis is the preparation of 1,4-diketone **245** from the commercially available ketoester **243** and an appropriate halophenacyl bromide **244**, Scheme 80.²⁶ After stirring for 20 hours at room temperature, the TLC indicated the completion of the reaction and chromatographic purification gave the products **245** in low yield (11-15%). Increasing the temperature and the reaction time did not give a significant improvement in the yields. Characterisation of the compounds was achieved by spectroscopic data. In the ¹H NMR spectrum of ethyl 2-[2-(4-fluorophenyl)-2-oxoethyl]-3-oxobutanoate **245a**, the singlet of the methylene of the halophenacyl bromide had disappeared and the CH₂ protons were found to exhibit geminal coupling as part of an ABX spin system at δ3.39 ppm (*J* = 18 Hz, CH₂-a) and δ3.60 ppm (*J* = 18 Hz, CH₂-b). In the IR spectrum of this 1,4-dicarbonyl the three C=O peaks were seen at 1682, 1716, and 1733 cm⁻¹.



Scheme 80. Preparation of 1,4-diketones from ketoester **243** and halophenacyl bromide **244**

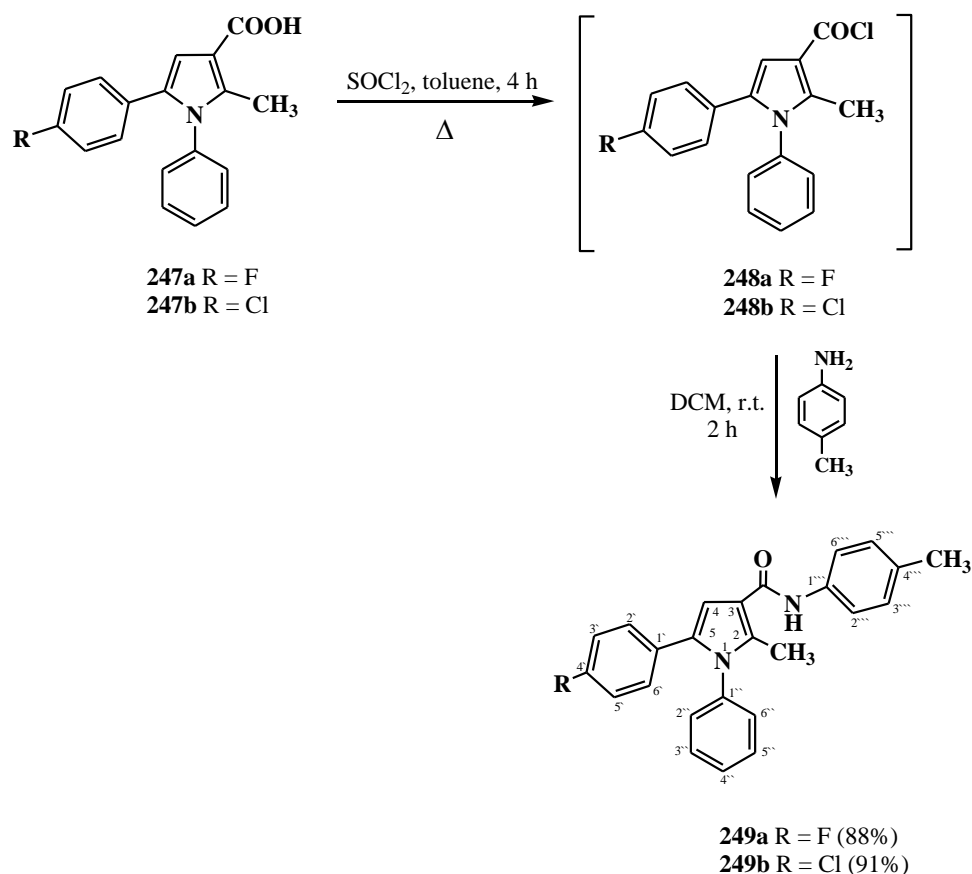
The condensation of the 1,4-diketones **245** with aniline in methanol²⁷ did not result in any products, only starting materials were obtained, but changing the solvent, to toluene, gave the expected pyrroles **246** in good 75-78% yield. Compounds **246** were then hydrolysed in a solution of potassium hydroxide in ethanol / water to afford the acids **247** in 77-80% yields, Scheme 81.

The structure of compound **247a** was confirmed by its infra-red spectrum, with a broad OH stretch at 2584 and carbonyl stretch at 1686 cm^{-1} and its ^1H NMR spectrum with the presence of a CH group at $\delta 6.71$ ppm (singlet) belonging to the pyrrole ring, and the disappearance of the ester group.



Scheme 81. Preparation of the pyrrole acids

The acid chlorides **248** were then prepared by heating compounds **247**, with SOCl_2 in toluene. Thus crude **248** were stirred in the presence of *p*-toluidine at room temperature, to obtain the desired pyrrole derivatives **249** in good yield (88-91%). As the acid chlorides **248** were unstable, they were used directly in the next step without further purification, Scheme 82.



Scheme 82. Synthesis of the potential inhibitors

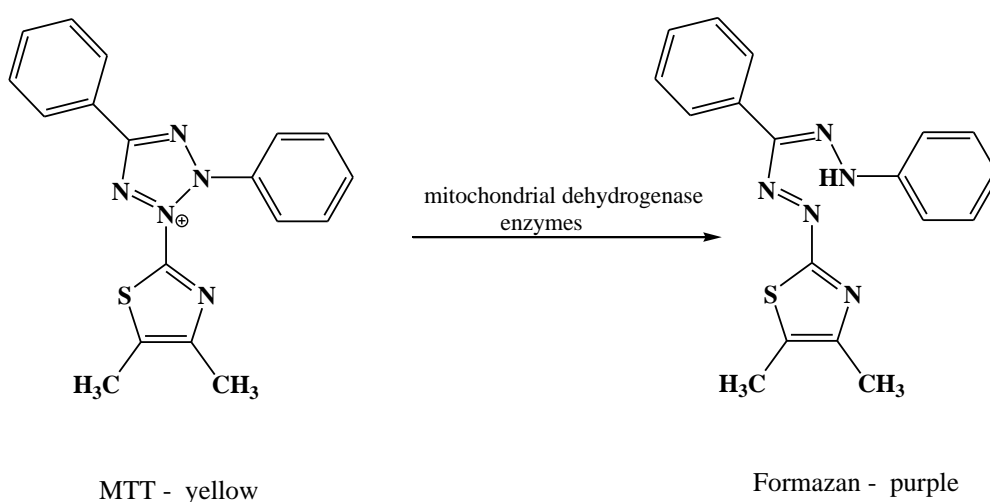
The identification of the final compounds was relatively straightforward, and involved the comparison of their spectra with those of the previous intermediates in the synthetic route. The ^1H NMR spectra of both the Cl and F substituted compounds were similar, but the ^{13}C NMR spectrum was different as the peaks for carbons close to the fluoro group are doublets. From the coupling constants for these peaks it was easy to define the position in the ring of the carbon atoms, because the carbon atom with the larger ^{13}C - ^{19}F coupling constant is located closer to the fluoro group. For example, the coupling constant for C-1' at $\delta 133.3$ is $J = 3.2$ Hz, while that for C-4' at $\delta 161.7$ is $J = 245.5$ Hz. A similar approach was used to determine C-2', C-6' at $\delta 129.9$ with $J = 7.9$ Hz and C-3', C-5' at $\delta 115.2$ with $J = 22.5$ Hz.

3.4 Biological activity assay

3.4.1 MTT assay

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell proliferation assay is a simple method for the determination of cell number using standard microplate absorbance readers.

The MTT assay was first described by Mosmann in 1983²⁸ and it is based on the cleavage of the yellow tetrazolium salt, MTT, to form dark purple formazan crystals by metabolically active cells, Scheme 83. This purple formazan product accumulates within the cell since it cannot pass through the cell membrane. Upon addition of DMSO, or another suitable solvent, the cell is solubilised and the dark purple formazan crystals are liberated. The resulting coloured solution can be quantified by measuring (usually between 500 and 600 nm) the absorbance with a UV spectrophotometer.



Scheme 83. Conversion of the tetrazolium salt MTT to an insoluble purple formazan

3.4.2 Materials and Methods

3.4.2.1 Cell cultures

Human colon cancer cell lines, HT29, CaCo-2, and HaCaT (keratinocytes) cell lines were obtained from Sunderland University. Fetal Bovine Serum (FBS), McCoy's 5a Medium Modified and RPMI 1640 Medium were obtained from Sigma-Aldrich Chemicals.

HT29 cells were cultured in McCoy's 5A medium, supplemented with 10% Fetal Bovine Serum (FBS), 100 µg/ml penicillin and 100 µg/ml streptomycin. The CaCo-2 cell line was maintained in RPMI 1640 medium supplemented with 5% Fetal Bovine Serum, 100 µg/ml penicillin and 100 µg/ml streptomycin.

HaCaT cell lines were cultured in Dulbecco's MEM medium supplemented with 10% Fetal Bovine Serum (FBS), 100 µg/ml penicillin and 100 µg/ml streptomycin.

3.4.2.2 Cell proliferation assay

The MTT proliferation assays were carried out in 96 well plates with 1×10^4 cells/well (see Appendix A). Cells were grown to 60-80% confluence in a serum-free medium then trypsinized (in which the cells are detached from the flask surface) and counted on a haemocytometer under a microscope. Cells were then re-suspended in 10 ml of medium, plated and initially incubated for 24 hours at 37 °C under 5% CO₂. The media was removed from each well before the assay was started. Drug samples (**249a**, **249b**) were dissolved in dimethylsulphoxide (DMSO) to give 10 mg/ml stock solution then a required amount of this stock was added to the media for preparation of the final 200 µg/ml concentration. A serial dilution was performed across the plate

and all samples were tested at concentration of 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563 $\mu\text{g/ml}$. Plates were incubated for 3 days at 37 °C with 5% CO_2 then the culture media was removed again and the cells were rinsed twice with PBS (phosphate buffered saline) buffer solution. MTT working solution (0.5 mg/ml in PBS buffer) was added to each well and the plates were incubated at 37 °C. After 2 hours, the MTT was removed then each well washed carefully with PBS solution.

Cells were dissolved in DMSO-isopropanol solution to solubilise the purple formazan crystals, the plates were incubated for 10 minutes in the dark and the absorption was read at 595 nm with a spectrophotometric micro-plate reader. The percentage of growth was calculated by the following formula:

$$\% \text{Growth} = \frac{\text{Compound} - \text{Blank}}{\text{Control} - \text{Blank}} \times 100$$

Where:

- Compound is the mean absorbance of the cells treated with compounds
- Blank is the mean absorbance of the wells which contain only media
- Control is the mean absorbance of the wells which contain only cells (no compound)

The calculation was carried out with Microsoft Excel then the results were transferred to the GraphPad Prism program to analyse graphically the percentage of growth vs the log of tested compound concentration.

3.5 Results

Two synthetic compounds, *N*-(4-methylphenyl)-5-(3'-fluorophenyl)-2-methyl-1-phenyl-1*H*-pyrrole-3-carboxamide (**249a**) and *N*-(4-methylphenyl)-5-(3'-chlorophenyl)-2-methyl-1-phenyl-1*H*-pyrrole-3-carboxamide (**249b**) were tested *in vitro* on HT29 and CaCo-2 cell lines and the results are summarised in Table 5 (Appendix B). The cells were treated with various concentrations of the compounds for 3 days and cell growth inhibition was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The GI₅₀ is the concentration of the drug that inhibits 50% of cell growth and the GI₅₀ values were obtained from curves which were generated by a nonlinear regression curve fitting, Figure 35. The results are expressed as the average of two experiments for each compound.

Cell line	Tumour type	GI ₅₀ (μM)		
		Sutent (lit.data)	249a	249b
HT29	colon	no effect	23	11
CaCo-2	colon	N/A	102	19

Table 5. GI₅₀ values of the tested compounds tested for antiproliferative activity

In order to establish the quality of the results, we have to draw a comparison with data taken from the literature. The initial plan was to test the synthesised compounds in the HUVEC cell line under MTT assay conditions, then compare the results with Sutent, which has a GI₅₀ = 40 nM in the HUVEC cell line.¹⁴ Due to unforeseen

problems with the growing of the HUVEC cells, we were not able to perform the planned tests; therefore, we decided to study the activity of **249a** and **249b** on two human colon cancer cell lines (HT29 and CaCo-2), which are also endothelial cells, and were readily available from Sunderland University. A literature search showed that Sutent did not have any activity in the HT29 cell line.²⁹ However, compounds **249a** and **249b** both showed activity, with GI₅₀ values of 23 μ M and 11 μ M in the HT29 cell line using the MTT assay. Both compounds also showed activity, with GI₅₀ values of 102 μ M and 19 μ M, in the CaCo-2 cell line, respectively, Figure 36. Unfortunately, no literature data is available on the effect of Sutent on the CaCo-2 cell line.

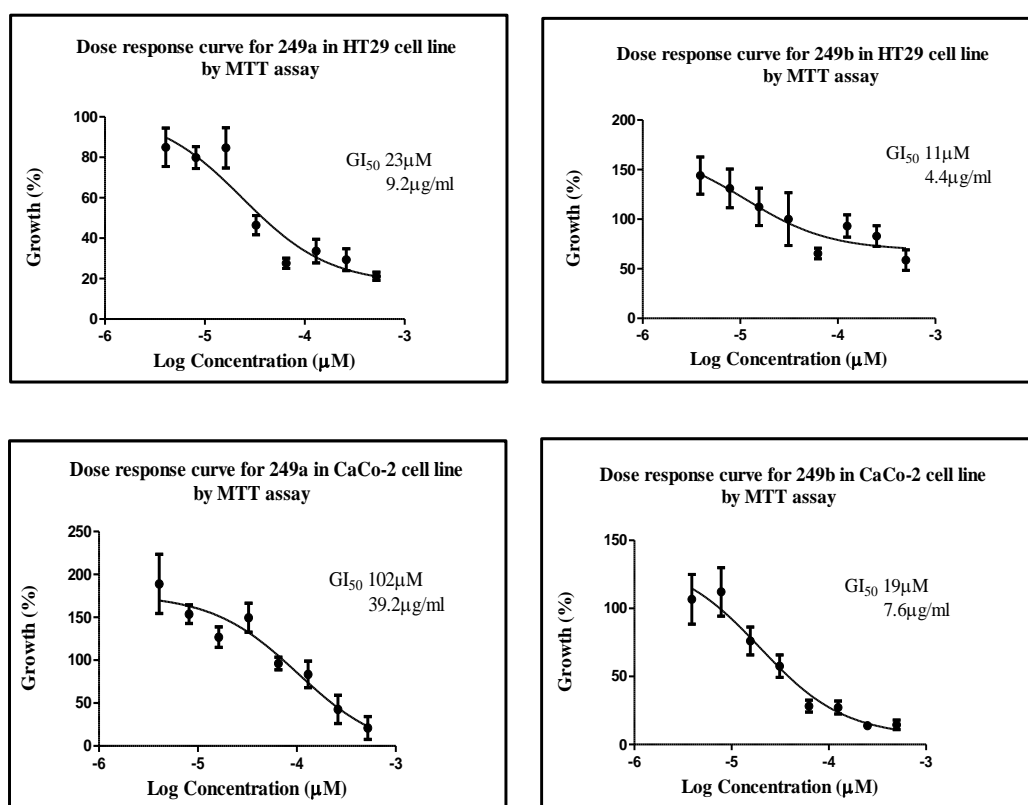


Figure 36. Dose response curves for **249a** and **249b**

We were also interested in the study of the activity of the designed compounds on the HaCaT cell line, which over-expresses the EGF receptor. The results of the cytotoxicity assay on this cell line are summarised in Table 6.

Compound	GI ₅₀	
	HaCaT	
	µg/ml	µM
249a	103	41
249b	63	24.2

Table 6. GI₅₀ values of the tested compounds on HaCaT cell line

Both compounds inhibited the proliferation of HaCaT cells at high concentration and both showed activity, with GI₅₀ values of 103 and 63 µM, Figure 37.

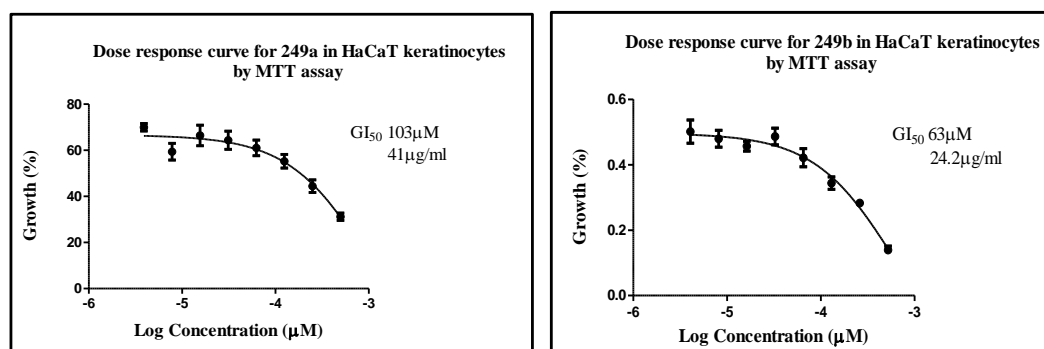
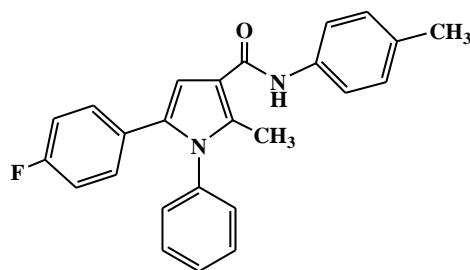
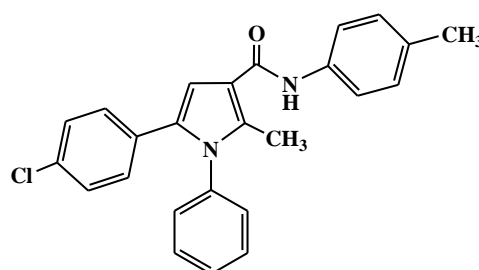


Figure 37. Dose response curve for **249** inhibition of the HaCaT cell line

3.6 Conclusion

In this part of the work we have identified small molecules **249a** and **249b** as a novel class of potential VEGFR-2 inhibitors using structure based drug design. We prepared these compounds in a 5 steps synthesis using regioselective reactions of functional groups present on the pyrrole ring. The synthesised compounds were fully characterized by spectroscopic data.

**249a****249b**

The antiproliferative activities of the synthesized compounds were tested in three different cell lines, HT29, CaCo-2 and HaCaT, using the MTT assay. The tested compounds showed antiproliferative activity, with GI_{50} values of 102 μ M and 19 μ M, in the CaCo-2 cell line and 23 μ M and 11 μ M in the HT29 cell line, Table 5. The lower growth rate indicated that compound **249b** has stronger activity in the colon cancer cell lines compare to compound **249a**.

We also investigated the activity of the designed compounds in the HaCaT cell line, and found that both inhibited proliferation, with GI_{50} values of 41 μ M and 24.2 μ M, Table 6. These studies could be used as an initial screen for identifying new VEGFR-2 antagonist molecules.

3.7 References

1. Andrew, R. L.; *Molecular Modelling: Principles and Applications*, **2001**.
2. Anderson, A. C.; *Chem. Biology*, **2003**, *10*, 787.
3. Levitzki, A.; Gazit, A.; *Science*, **1995**, *267*, 1782.
4. a) Schlessinger, J.; *Cell*, **2000**, *103*, 211; b) Geer, P. V.; Hunter, T.; Lindberg, R. A.; *Annual Review of Cell Biology*, **1994**, *10*, 251; c) Risau, W.; *Nature*, **1997**, *386*, 671; d) De Vries, C.; Escobedo, J. A.; Ueno, H.; Houck, K.; Ferrara, N.; Williams, L., T.; *Science*, **1992**, *255*, 989.
5. Napoleana F.; *Endocrine Reviews*, **2004**, *4*, 25, 581.
6. Harmey, J. H.; *VEGF and Cancer*, **2004**, Springer.
7. Rutch, C.; Skiniotis, G.; Steinmetz, M., O.; Walz, T.; Ballmer-Hofer, K.; *Nature Structural & Molecular Biology*, **2007**, *14*, 249.
8. Gerald, M.; *The Oncologist*, **2000**, *5*, 3.
9. Veeravagu, A.; Hsu, A. R.; Cai, W.; Hou, L. C.; Tse, V. C. K.; Chen, X.; *Recent Patents on Anti-Cancer Drug Discovery*, **2007**, *2*, 59.
10. a) Harris, P. A.; Cheung, M.; Hunter, R. M.; Brown, M. L.; Veal, J. M.; Nolte, R. T.; Wang, L.; Liu, W.; Crosby, R. M.; Johnson, J. H.; Epperly, A. H.; Kumar, R.; Luttrell, D. K.; Stafford, J. A.; *J. Med. Chem.*, **2005**, *48*, 1610; b) Troiani, T.; Lockerbie, O.; Morrow, M.; Ciardiello, F.; Eckhardt, S. G.; *J. Clinical Oncology*, **2006**, *24*, 13171.
11. Srikala, S. S.; Frances, A. S.; *Lung Cancer*, **2003**, *42*, 581.
12. a) Dirk, B. M.; Douglas, L. A.; Xiaohua, X.; *Clinical Cancer Research*, **2003**, *9*, 327; b) <http://en.wikipedia.org/wiki/Sunitinib>

13. Robert, J. M.; Bruce, G. R.; Gary, R. H.; George, W.; Robert, A. F.; Michelle, S. G.; Sindy, T. K.; Charles, M. B.; Samuel, E. D.; Jim, Z. L.; Carlo, L. B.; Charles, P. T.; Daniel, J. G.; Brian, I. R.; *J. Clinical Oncology*, **2006**, *24*, 16.
14. Morabito, A.; De Maio, E.; Di Maio, M., Normanno, N.; Perrone, F.; *The Oncologist*, **2006**, *11*, 753.
15. Paul, D. L.; *Drug Discov. Today*, **2002**, *7*, 1047.
16. John, C.; *Current Biology*, **1999**, *9*, 796.
17. Raymond, C. R.; John, B. K.; *The American. Journal of Cardiology*, **2003**, *92*, 9.
18. Dorsey, B. D.; Levin, R. B.; McDaniel, S. L.; Vacca, J. P.; Guare, J. P.; Darke, P. L.; Zugay, J. A.; Emini, E. A.; Schleif, W. A.; Quintero, J. C.; *J. Med. Chem*, **1994**, *37*, 3443.
19. Accelry Software Inc., San Diego; Cerius² Modeling Environment, **2003**.
20. Miyazaki, Y.; Matsunaga, S.; Tang, J.; Maeda, Y.; Nakano, M.; Philippe, R. J.; Shibahara, M.; Liu, W.; Sato, H.; Wang, L.; Nolte, R. T; *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2203.
21. Yun, C. H.; Boggon, T. J.; Li, Y.; Woo, S.; Greulich, H.; Meyerson, M.; Eck, M. J.; *Cancer Cell*, **2007**, *11*, 217.
22. Venkatachalam, C., M.; Jiang, X.; Oldfield, T.; Waldman, M.; *J. Mol. Graphics and Modelling*, **2003**, *21*, 289.
23. Krammer, A.; Kirchhoff, P. D.; Jiang, X.; Venkatachalam, C. M.; Marvin W.; *J. Mol. Graphics and Modelling*, **2005**, *23*, 395.
24. Oprea, T. I.; *Perspect. Drug Discovery Des.*, **1998**, 35.
25. Lyne, P. D.; *Drug Discov. Today*, **2002**, *7*, 1047.

26. Duus, F.; *Tetrahedron*, **1976**, 32, 2817; Biava, M.; Porretta, G. C.; Cappelli, A.; Vomero, S.; Manetti, F.; Botta, M.; Sautebin, L.; Rossi, A.; Makovec, F.; Anzini, M.; *J. Med. Chem.*, **2005**, 48, 3428.
27. Porretta, G. C.; Scalzo, M.; Chimenti, F.; Bolasco, A.; Biava, M.; *Il Farmaco*, **1987**, 629; Scalzo, M.; Porretta, G. C.; Chimenti, F.; Bolasco, A.; Casanova, M. C.; *Il Farmaco*, **1988**, 677; Cerreto, F.; Scalzo, M.; *Il Farmaco*, **1993**, 48, 1735.
28. Mosmann, T.; *J. Immunol. Meth.* **1983**, 65, 55.
29. Patel, N.; Sun, L.; Moshinsky, D.; Chen, H.; Leahy, K. M.; Le, P.; Moss, G. K.; Wang, X.; Rice, A.; Tam, D.; Laird, A. D.; Yu, X.; Zhang, Q.; Tang, C.; McMahon, G.; Howlett, A.; *Molecular Cancer Therapeutics*, **2006**, 5, 1280.

Chapter Four

Experimental Part

4. Experimental Part

Chemicals were purchased from Aldrich, Fluka, Lancaster and Johnson Matthey, and used without further purification. The structures of the synthesised compounds were characterised by ^1H NMR, ^{13}C NMR and IR spectroscopy, and melting point, mass spectrometry, high resolution mass spectrometry and elemental analysis.

4.1 Instruments and Techniques

4.1.1 Nuclear Magnetic Resonance Spectroscopy

NMR spectra were obtained using a Bruker 300 NMR spectrometer operating at 300 MHz for proton and 75 MHz for carbon, or using a Bruker 500 NMR spectrometer at 500 MHz for proton and 125 MHz for carbon.

Spectra were referenced to residual protonated solvent (δ_{H} CDCl_3 7.25, δ_{C} CDCl_3 77.16; δ_{H} DMSO 2.50, δ_{C} DMSO 39.52) and all chemical shifts are relative to an internal standard (tetramethylsilane, TMS). Coupling constants are reported in Hz.

4.1.2 Infra-red Spectroscopy

Infra-red spectra of liquid or solid samples were obtained on a SpectrumBX fitted with Pike Miracle.

4.1.3 Mass Spectrometry

Low-resolution electrospray mass spectra were obtained on an Esquire 3000+ ion trap mass spectrometer (ChemiSPEC, University of Sunderland) and high-resolution spectra were obtained by means of ESI-MS on a Synapt HDMS instrument

(University of Warwick). An internal standard of sodiated maltose in methanol was added at an appropriate level for mass correction using the ion at m/z 365.1060.

4.1.4 Elemental Analysis

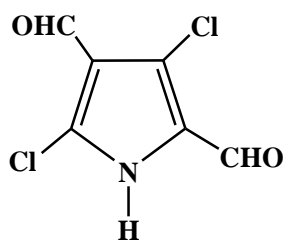
Elemental Analysis (C, H, N) for new compounds was performed on an Exeter Analytical CE 440 Elemental Analyzer instrument, and all elements for all analyses were within ± 0.4 % of the theoretical values.

4.1.5 Melting Points

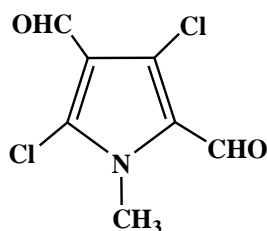
Melting points were determined on an Electrothermal 9100, a Gallenkamp melting point apparatus, or a Reichert hot stage microscope.

4.1.6 Thin Layer and Flash Column Chromatography

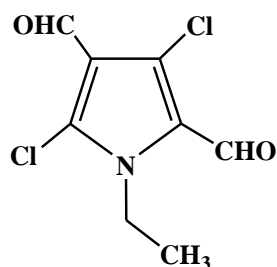
Thin layer chromatography (TLC) was performed on Merck silica gel 60F₂₅₄ plates and the components were detected under UV light (254 nm). Kieselgel 60 (Merck) was used for flash column chromatography

4.2 3,5-Dichloro-1*H*-pyrrole-2,4-dicarboxaldehyde **41**

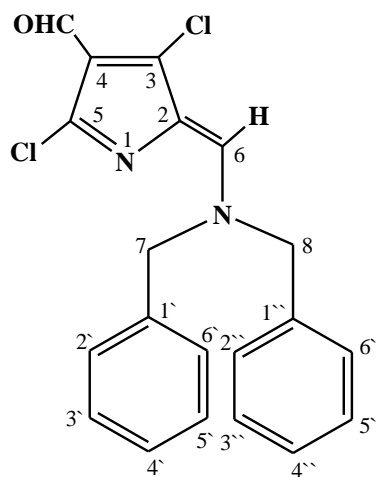
Dry DMF (100 ml) was cooled to 0 °C in an ice bath then POCl₃ (29.2 ml) was added dropwise over 1 h. *N*-Acetylglycine (10 g, 85.4 mmol) was added to the solution and the resulting mixture stirred for 1 h at room temperature then 6 h at 90 °C. After completion of the reaction, as indicated by TLC, the mixture was poured onto a mixture of crushed ice (1000 ml), sodium acetate (29 g) and water (100 ml). The product was continuously extracted overnight with diethyl ether (1000 ml), and dried over anhydrous MgSO₄. After filtration, the solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica, eluting with ethyl acetate: petroleum ether (60-80 °C) (50:50) to give pyrrole **41** as a yellow solid (11.5 g, 70 %); mp 166-168 °C (lit.¹ mp 170 °C); $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 1671 (C=O), 1635 (C=O), 1539 (C=C); ¹H NMR (300 MHz, CDCl₃) δ_{H} 9.63 (1H, s, CHO), 9.92 (1H, s, CHO), 11.12 (1H, br s, NH); ¹³C NMR (75 MHz, CDCl₃) δ_{C} 118.3 (quat.), 125.5 (quat.), 127.6 (quat.), 130.6 (quat.), 177.4 (CHO), 182.7 (CHO); *m/z* Found: [M-H⁺]⁻, 190. Calc. for C₆H₃³⁵Cl₂NO₂: (M-H⁺)⁻, 190.

4.3 3,5-Dichloro-1-methyl-1*H*-pyrrole-2,4-dicarboxaldehyde **42**

A solution of pyrrole **41** (5 g, 26 mmol) in dry DMF (55 ml) was added dropwise to a stirred solution of NaH (60 % in oil, 1.35 g, 56 mmol) in dry DMF (55 ml) and the mixture stirred for 30 min at room temperature. Methyl iodide (6.55 ml, 105 mmol) was then added to the resulting solution and stirring was continued for an additional 3 h at room temperature. The reaction mixture was quenched with water (100 ml), extracted with diethyl ether (3 × 100 ml) and dried over anhydrous MgSO₄. After filtration, the solvent was evaporated under reduced pressure and the crude product purified by column chromatography on silica, eluting with ethyl acetate: petroleum ether (60-80 °C) (5:5) to give pyrrole **42** as a yellow solid (4.98 g, 92 %); mp 109-111 °C (lit.² mp 108.5-109.5 °C); $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 1664 (C=O), 1510 (C=C); ¹H NMR (300 MHz, CDCl₃) δ_{H} 3.91 (3H, s, CH₃), 9.75 (1H, s, 2-CHO), 9.89 (1H, s, 4-CHO); ¹³C NMR (75MHz, CDCl₃) δ_{C} 33.2 (CH₃), 117.0 (quat., C-4), 126.7 (quat., C-3), 127.0 (quat., C-5), 132.0 (quat., C-2), 177.7 (2-CHO), 182.2 (4-CHO); *m/z* Found: MNa⁺, 229. Calc. for C₇H₅³⁵Cl₂NO₂Na: MNa⁺, 229.

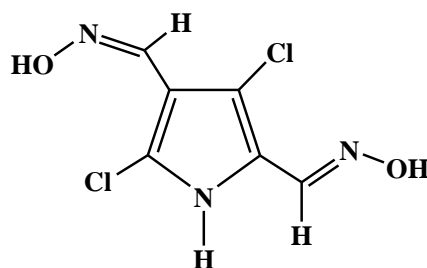
4.4 3,5-Dichloro-1-ethyl-1*H*-pyrrole-2,4-dicarboxaldehyde **43**

This pyrrole **43** was prepared as described above, and the crude product was purified by column chromatography on silica, eluting with ethyl acetate: petroleum ether (60-80 °C) (50:50) to give pyrrole **43** as a yellow solid (4.90 g, 86 %); mp 69-71 °C (lit.² mp 68-69 °C); $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 1657 (C=O), 1508 (C=C); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ_{H} 1.28 (3H, t, $J = 7.2$ Hz, CH_3), 4.45 (2H, q, $J = 7.2$ Hz, CH_2), 9.74 (1H, s, 2-CHO), 9.89 (1H, s, 4-CHO); $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ_{C} 15.1 (CH_3), 41.7 (CH_2), 117.0 (quat., C-4), 125.9 (quat., C-3), 127.4 (quat., C-5), 131.1 (quat., C-2), 177.3 (2-CHO), 182.2 (4-CHO).

4.5 3,5-Dichloro-2-[(dibenzylamino)methylene]-1*H*-pyrrole-4-carboxaldehyde**151a**

Pyrrole **41** (0.60 g, 3.13 mmol) was dissolved in ethanol (12 ml) and dibenzylamine (2.9 ml, 15.46 mmol) was added slowly to the solution. The reaction mixture was stirred at room temperature for 4 h then quenched with brine (30 ml), extracted with DCM (3 × 30 ml) and the combined organics dried over MgSO₄. After filtration, the solvent was evaporated under reduced pressure and the crude product purified by column chromatography on silica, eluting with CH₂Cl₂ / EtOAc (95:5) to give a yellow solid **151a** (0.84 g, 72 %); mp 149-150 °C; [Found: C, 64.6; H, 4.4; N 7.5. C₂₀H₁₆Cl₂N₂O requires C, 64.7; H, 4.3; N, 7.6 %]; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 1671 (C=O), 1606 (enamine C=C); ¹H NMR (300 MHz, CDCl₃) δ_{H} 4.51 (2H, s, CH₂), 5.54 (2H, s, CH₂), 7.13 (2H, m, ArH), 7.23 (2H, m, ArH), 7.29 (3H, m, ArH), 7.35 (3H, m, ArH), 7.67 (1H, s, CH), 9.85 (1H, s, CHO); ¹³C NMR (75 MHz, CDCl₃) δ_{C} 53.4 (CH₂), 60.7 (CH₂), 121.2 (quat., C-4), 126.9 (quat., C-2), 128.3 (CH), 128.8 (CH), 129.2 (CH), 129.5 (CH), 130.3 (CH), 132.7 (quat., C-1' or C-1''), 134.0 (quat., C-1' or C-1''), 137.1 (quat., C-3), 144.6 (quat., C-5), 147.6 (CH), 183.5 (CHO); *m/z* Found: MNa⁺, 393. Calc. for C₂₀H₁₆³⁵Cl₂N₂O: MNa⁺, 393.

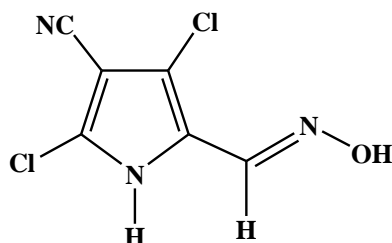
4.6 3,5-Dichloro-1*H*-pyrrole-2,4-dicarboxaldehyde bisoxime **156**



A solution of pyrrole **41** (1.00 g, 5.2 mmol) and hydroxylamine hydrochloride (1.10 g 15.86 mmol) in 95-98 % formic acid (10.2 ml) was refluxed for 40 min and then

allowed to cool. The mixture was diluted with ice-water (50 ml) then neutralised with 5% sodium hydroxide solution, and extracted with ethyl acetate (3 × 40 ml). The combined organic layer was dried over MgSO₄ and evaporated. After filtration, the residue was purified by column chromatography on silica, eluting with ethyl acetate: petroleum ether (60-80 °C) (4:6) to give pyrrole **156** as a pale yellow solid (0.91 g, 76 %); mp 147-148 °C; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3277 (broad NH and OH); ¹H NMR (300 MHz, DMSO-*d*₆) δ_{H} 7.21 (1H, s, CH), 7.83 (1H, s, CH), 11.19 (1H, s, OH), 11.75 (1H, s, OH), 12.22 (1H, s, NH); ¹³C NMR (75 MHz, DMSO-*d*₆) δ_{C} 111.1 (quat., C-4), 112.6 (quat., C-3), 117.8 (quat., C-5), 120.5 (quat., C-2), 133.0 (CH, 2-CH=N), 140.0 (CH, 4-CH=N); HRMS Found: MH⁺, 221.9836. Calc. for C₆H₆Cl₂N₃O₂: MH⁺, 221.9832.

4.7 3,5-Dichloro-4-cyano-1H-pyrrole-2-carboxaldehyde oxime **157**



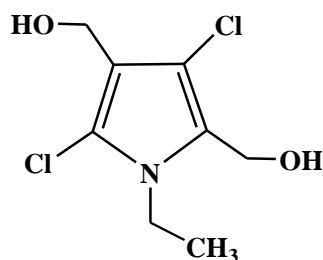
Method A

POCl₃ (2.92 ml) was added dropwise to dry DMF (10 ml) at 0 °C. To this solution, *N*-acetylglycine (1.00 g, 8.54 mmol) was added and the mixture stirred for 1 h at room temperature then 4 h at 90 °C. After completion of the reaction, as indicated by TLC, the reaction mixture was diluted with DCM (12 ml), cooled to 0 °C and hydroxylamine hydrochloride (1.77 g, 25.5 mmol) in DMF (5 ml) was added. The mixture was stirred for 4 h at room temperature. After the reaction was complete, it was diluted with water (8 ml) and extracted with DCM (2 × 15 ml). The combined

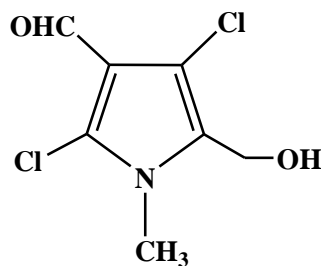
organic phases were washed with water (2×10 ml), saturated NaHCO_3 solution (8 ml), and water (15 ml) and dried over MgSO_4 . After filtration, the solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica, eluting with ethyl acetate: petroleum ether (60-80 °C) (3:7) to give a yellow solid **157** (0.72 g, 45 %), spectral data given below.

Method B

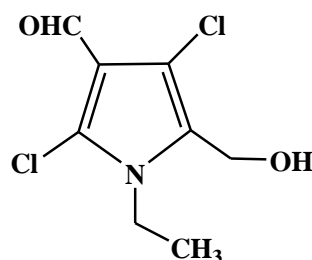
3,5-Dichloro-1*H*-pyrrole-2,4-dicarboxaldehyde **41** (1.00 g, 5.21 mmol), hydroxylamine hydrochloride (0.38 g, 5.47 mmol) and pyridine (0.43 g, 5.43 mmol) were refluxed in EtOH for 2 h, to give the crude oxime. To this solution was added Ac_2O (15 ml), then the mixture was heated under reflux for 1.5 h, cooled, stirred with water (100 ml), extracted with DCM (3×30 ml) and dried over MgSO_4 . After filtration, the solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica, eluting with ethyl acetate: petroleum ether (60-80 °C) (3:7) to give a yellow solid **157** (0.78 g, 72 %); mp 158-159 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3170 (broad NH and OH), 2234 ($\text{C}\equiv\text{N}$); ^1H NMR (300 MHz, $\text{DMSO-}d_6$) δ_{H} 4.98 (1H, s, NH), 7.89 (1H, s, CH), 11.53 (1H, s, OH); ^{13}C NMR (75 MHz, $\text{DMSO-}d_6$) δ_{C} 100.3 (quat., C-4 or C-5), 111.7 (quat., C-4 or C-5), 112.6 (quat., C-2 or C-3), 120.2 (quat., C-2 or C-3), 139.2 (CH); HRMS Found: MH^+ , 203.9732. Calc. for $\text{C}_6\text{H}_4\text{Cl}_2\text{N}_3\text{O}$: MH^+ , 203.9727.

4.8 3,5-Dichloro-1-ethyl-2,4-bis(hydroxymethyl)-1*H*-pyrrole 174

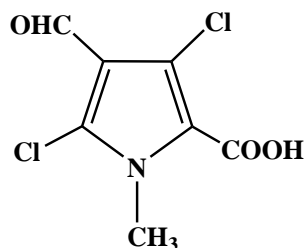
Methanol (15 ml) was added dropwise to sodium borohydride (37 mg, 0.97 mmol) then the reaction mixture was stirred for 5 min at room temperature. 3,5-Dichloro-1-ethyl-1*H*-pyrrole-2,4-dicarboxaldehyde **43** (0.40 g, 1.82 mmol) was added to the solution which was then refluxed for 4 h. After completion of the reaction, as indicated by TLC, the solvent was evaporated under reduced pressure, and the residue quenched with water (20 ml), extracted with ether (3 × 30 ml) and the combined organics dried over MgSO₄. After filtration, the solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica, eluting with ethyl acetate: petroleum ether (60-80 °C) (5:5) to give pyrrole **174** as a white solid (0.23 g, 75 %); mp 139-140 °C; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3338 (broad OH); ¹H NMR (300 MHz, DMSO-*d*₆) δ_{H} 1.25 (3H, t, *J* = 7.2 Hz, CH₃), 4.01 (2H, q, *J* = 7.2 Hz, CH₂), 4.24 (2H, s, CH₂-C4), 4.42 (2H, s, CH₂-C2), 4.71 (1H, br s, OH), 5.13 (1H, br s, OH); ¹³C NMR (75 MHz, DMSO-*d*₆) δ_{C} 16.3 (CH₃), 39.9 (CH₂), 52.3 (CH₂-C4), 53.1 (CH₂-C2), 109.8 (quat., C-3), 114.2 (quat., C-5), 116.8 (quat., C-4), 128.1 (quat., C-2); HRMS Found: MH⁺, 224.0244. Calc. for C₈H₁₂Cl₂NO₂: MH⁺, 224.0240.

4.9 3,5-Dichloro-1-methyl-2-hydroxymethyl-1H-pyrrole-4-carboxaldehyde **176**

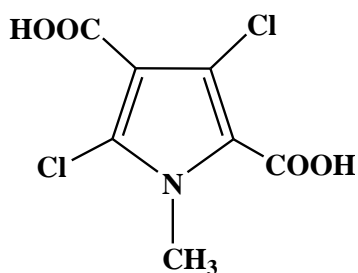
3,5-Dichloro-1-methyl-1H-pyrrole-2,4-dicarboxaldehyde **42** (0.50 g, 2.43 mmol) and sodium cyanoborohydride (0.15 g, 2.3 mmol) were dissolved in methanol (10 ml) and 2M HCl-methanol (3 ml, 20:80) added dropwise, with stirring, to the solution. Stirring was continued for an additional 1 h then the methanol was evaporated under reduced pressure, the residue was taken up in water (7 ml), saturated with sodium chloride, extracted with ether (3 × 20 ml) and the combined organics dried over MgSO₄. After filtration, the solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica, eluting with ethyl acetate: petroleum ether (60-80 °C) (3:7) to give pyrrole **176** as a yellow solid (0.21 g, 42 %); mp 129-130 °C; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3353 (broad OH), 1710 (C=O), 1511 (C=C); ¹H NMR (300 MHz, DMSO-*d*₆) δ_{H} 3.72 (3H, s, CH₃), 4.54 (2H, s, CH₂), 5.38 (1H, br s, OH), 9.84 (1H, s, CHO); ¹³C NMR (75 MHz, DMSO-*d*₆) δ_{C} 31.9 (CH₃), 51.8 (CH₂), 109.8 (quat., C-3), 115.3 (quat., C-4), 124.7 (quat., C-5), 131.8 (quat., C-2), 182.7 (CHO); HRMS Found: MH⁺, 207.9937. Calc. for C₇H₈Cl₂NO₂: MH⁺, 207.9927.

4.10 3,5-Dichloro-1-ethyl-2-hydroxymethyl-1*H*-pyrrole-4-carboxaldehyde **177**

3,5-Dichloro-1-ethyl-1*H*-pyrrole-2,4-dicarboxaldehyde **43** (0.40 g, 1.82 mmol) and sodium cyanoborohydride (0.08 g, 1.84 mmol) were dissolved in methanol (15 ml) and 2M HCl-methanol (3 ml, 2:8) was added dropwise, with stirring, to the solution. Stirring was continued for an additional 1 h then the methanol was evaporated under reduced pressure, the residue was taken up in water (10 ml), saturated with sodium chloride, extracted with ether (3 × 20 ml) and the combined organics dried over MgSO₄. After filtration, the solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica, eluting with ethyl acetate: petroleum ether (60-80 °C) (3:7) to give pyrrole **177** as a yellow solid (0.21 g, 53 %); mp 122-123 °C; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3350 (broad OH), 1662 (C=O); ¹H NMR (300 MHz, CDCl₃) δ_{H} 1.24 (3H, t, $J = 7.2$ Hz, CH₃), 4.38 (2H, q, $J = 7.2$ Hz, CH₂), 4.50 (2H, s, CH₂), 9.60 (1H, s, CHO); ¹³C NMR (75 MHz, CDCl₃) δ_{C} 15.8 (CH₃), 41.3 (CH₂CH₃), 52.5 (CH₂), 119.1 (C-4 or C-5), 120.3 (C-3), 124.7 (C-4 or C-5), 126.3 (C-2), 176.9 (CHO); HRMS Found: MH⁺, 222.0099. Calc. for C₈H₁₀Cl₂NO₂: MH⁺, 222.0084.

4.11 3,5-Dichloro-4-formyl-1-methyl-1*H*-pyrrole-2-carboxylic acid **183**

3,5-Dichloro-1-methyl-1*H*-pyrrole-2,4-dicarboxaldehyde **42** (0.50 g, 2.43 mmol) was dissolved in acetone (40 ml) and treated with a solution of KMnO_4 (0.78 g, 4.9 mmol) in H_2O (13 ml). The reaction mixture was refluxed for 12 h then decolourised with charcoal. After filtration, the solvent was evaporated under reduced pressure, acidified with 2M HCl and the crude product was recrystallised from methanol to give pyrrole **183** as a white solid (0.30 g, 55 %); mp 173-175 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 2588 (broad OH), 1662 (C=O); $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ_{H} 3.87 (3H, s, CH_3), 9.72 (1H, s, CHO), 13.15 (1H, br s, OH); $^{13}\text{C NMR}$ (75 MHz, $\text{DMSO-}d_6$) δ_{C} 33.6 (CH_3), 111.4 (C-4), 125.1 (C-3), 126.5 (C-5), 130.7 (C-2), 162.1 (C=O), 178.4 (CHO); HRMS Found: MH^+ , 259.9261. Calc. for $\text{C}_7\text{H}_6\text{NO}_3\text{Cl}$: MH^+ , 259.9278.

4.12 3,5-Dichloro-1-methyl-1*H*-pyrrole-2,4-dicarboxylic acid **185**

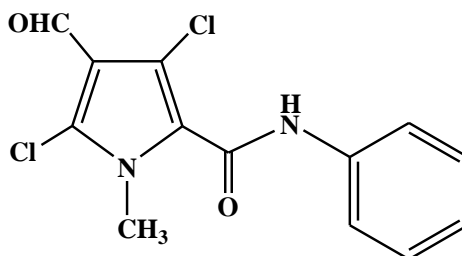
This pyrrole was prepared, as described above, to give a white solid **185** (0.23 g, 39 %); mp 179-180 °C; [Found: C, 35.9; H, 2.3; N, 5.5. C₇H₅Cl₂NO₄ requires C, 35.5; H, 2.1; N, 5.8 %]; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 2591 (broad OH), 1661 (C=O); ¹H NMR (300 MHz, DMSO-*d*₆) δ_{H} 3.84 (3H, s, CH₃); ¹³C NMR (75 MHz, DMSO-*d*₆) δ_{C} 34.6 (CH₃), 111.3 (C-4), 118.9 (C-3), 121.5 (C-5), 126.7 (C-2), 160.8 (COOH), 162.6 (COOH); *m/z* Found: MNa⁺ 260. Calc. for C₇H₅³⁵Cl₂NO₄Na: MNa⁺, 260.

4.13 General procedure for preparation of compounds **187a-e** and **191**

A solution of 3,5-dichloro-4-formyl-1-methyl-1*H*-pyrrole-2-carboxylic acid **183** (1.35 mmol) and SOCl₂ (0.49 ml) in toluene (5 ml) was refluxed for 4 h. After evaporation of the solvent, the crude mixture was dissolved in DCM (5 ml) and a solution of amine or benzyl alcohol (2.01 mmol) and TEA (0.19 ml) in DCM (1.6 ml) was added dropwise at 0 °C. The mixture was stirred for 2 h at room temperature then washed sequentially with 5 % aq. HCl (10 ml) and 5 % aq. NaOH (10 ml). The organic layer was dried over MgSO₄ and, after filtration, the solvent was evaporated under reduced pressure and purified by column chromatography or recrystallised.

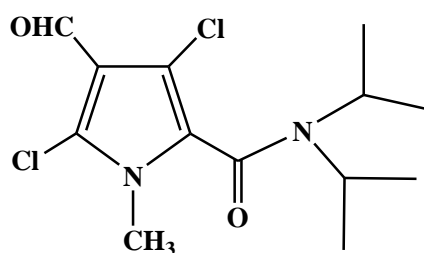
4.13.1 *N*-Phenyl-3,5-dichloro-4-formyl-1-methyl-1*H*-pyrrole-2-carboxamide

187a

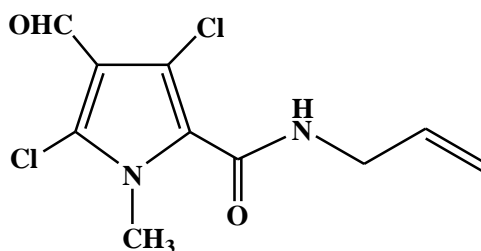


The crude product was recrystallised from methanol to give colourless needles **187a** (0.32 g, 80 %); mp 165-167 °C; [Found: C, 52.9; H, 3.8; N, 9.0. C₁₃H₁₀Cl₂N₂O₂ requires C, 52.6; H, 3.4; N, 9.4 %]; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3276 (NH), 1713 (C=O), 1660 (C=O); ¹H NMR (300 MHz, CDCl₃) δ_{H} 3.91 (3H, s, CH₃), 7.11 (1H, m, Ar-H), 7.31 (2H, m, Ar-H), 7.53 (2H, m, Ar-H), 7.91 (1H, br s, NH), 9.70 (1H, s, CHO); ¹³C NMR (75 MHz, CDCl₃) δ_{C} 33.3 (CH₃), 114.5 (quat.), 120.2 (CH, Ar), 120.4 (quat.), 120.5 (CH, Ar), 122.8 (quat.), 124.9 (CH, Ar), 125.2 (quat.), 125.9 (quat.), 129.1 (CH, Ar), 129.2 (CH, Ar), 137.4 (C=O), 177.6 (CHO).

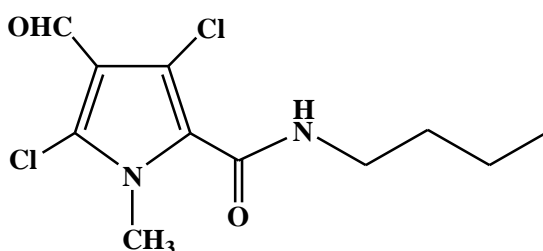
4.13.2 *N,N*-Diisopropyl-3,5-dichloro-4-formyl-1-methyl-1*H*-pyrrole-2-carboxamide **187b**



The crude product was recrystallised from methanol give white crystals **187b** (0.28 g, 68 %); mp 141-142 °C; [Found: C, 51.1; H, 5.9; N, 8.9. C₁₃H₁₈Cl₂N₂O₂ requires C, 51.1; H, 5.9; N, 9.1 %]; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 1675 (C=O), 1635 (C=O), 1536 (C=C); ¹H NMR (300 MHz, CDCl₃) δ_{H} 1.13 (6H, m, Hz, 2 × CH₃), 1.47 (6H, m, 2 × CH₃), 3.45 (1H, m, CH), 3.76 (1H, m, CH), 3.85 (3H, s, CH₃), 9.62 (1H, s, CHO); ¹³C NMR (75 MHz, CDCl₃) δ_{C} 20.4 (CH₃), 20.5 (CH₃), 21.2 (CH₃), 21.3 (CH₃), 33.2 (NCH₃), 46.3 (CH), 51.7 (CH), 119.5 (quat., C-3 or C-4), 122.4 (quat., C-3 or C-4), 124.5 (quat., C-5), 125.4 (quat., C-2), 160.9 (C=O), 177.3 (CHO); *m/z* Found: MNa⁺, 327. Calc. for C₁₃H₁₈³⁵Cl₂N₂O₂Na: MNa⁺, 327.

4.13.3 *N*-Allyl-3,5-dichloro-4-formyl-1-methyl-1*H*-pyrrole-2-carboxamide **187c**

This pyrrole was prepared, as described above and the crude product was purified by column chromatography on silica, ethyl acetate: petroleum ether (60-80 °C) (4:6) to give an orange solid **187c** (0.19 g, 54 %); mp 125-126 °C; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3262 (NH), 1668 (C=O), 1635 (C=O), 1535 (C=C); $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ_{H} 3.88 (3H, s, CH_3), 4.01 (2H, m, CH_2), 5.13 (1H, t, $J = 1.8$ Hz, = CH_2 -a), 5.19 (1H, t, $J = 1.8$ Hz, = CH_2 -b), 5.86 (1H, m, CH), 6.27 (1H, br s, NH), 9.69 (1H, s, CHO); $^{13}\text{C NMR}$ (75 MHz, $\text{DMSO-}d_6$) δ_{C} 33.2 (CH_3), 41.9 (CH_2), 114.3 (quat.), 116.7 (=CH₂), 123.0 (quat.), 125.7 (quat.), 129.4 (quat.), 133.8 (CH), 160.1 (C=O), 177.6 (CHO); m/z Found: MH^+ , 261. Calc. for $\text{C}_{10}\text{H}_{10}^{35}\text{Cl}_2\text{N}_2\text{O}_2$: MH^+ , 261. HRMS Found: MH^+ , 260.0203. Calc. for $\text{C}_{10}\text{H}_{11}\text{Cl}_2\text{N}_2\text{O}_2$: MH^+ , 261.0193.

4.13.4 *N*-Butyl-3,5-dichloro-4-formyl-1-methyl-1*H*-pyrrole-2-carboxamide **187d**

Method A

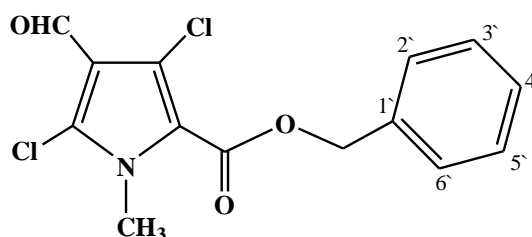
3,5-Dichloro-1-methyl-1*H*-pyrrole-2,4-dicarboxaldehyde **42** (0.40 g, 1.96 mmol) was dissolved in dry CCl₄ (10 ml). To this solution was added AIBN (0.005 g, 0.033 mmol) and NBS (0.45 g, 2.52 mmol). The reaction mixture was refluxed for 15 min then cooled to 0 °C (ice-water bath) and *n*-butylamine (0.33 g, 4.5 mmol) was added dropwise. The ice-bath was removed and the suspension was stirred at room temperature for 10 min. The solid material was removed by filtration and washed with CCl₄ (10 ml). The filtrate was extracted with water (2 × 10 ml) and the combined organics dried over MgSO₄. After filtration, the solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica, eluting with ethyl acetate: petroleum ether (60-80 °C) (2:8) to give a yellow solid **187d** (0.24 g, 45 %).

Method B

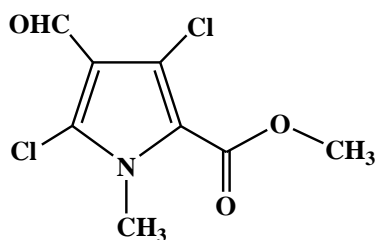
A solution of 3,5-dichloro-4-formyl-1-methyl-1*H*-pyrrole-2-carboxylic acid **183** (0.30 g, 1.35 mmol) and SOCl₂ (0.49 ml) in toluene (5 ml) was refluxed for 4 h. After evaporation of the solvent, the crude mixture was dissolved in DCM (5 ml) and a solution of *n*-butylamine (0.31 ml, 3.1 mmol) and TEA (0.19 ml) in DCM (2 ml) was added dropwise at 0 °C. The mixture was stirred for 2 h at room temperature then washed sequentially with 5 % aq. HCl (10 ml) and 5 % aq. NaOH (10 ml). The organic layer was dried over MgSO₄ and, after filtration, the solvent was evaporated under reduced pressure and the residue purified by column chromatography on silica, eluting with ethyl acetate: petroleum ether (60-80 °C) (3:7) and give a yellow solid **187d** (0.28 g, 68 %); mp 134-135 °C; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3273 (NH), 1671 (C=O), 1637 (C=O), 1554 (C=C); ¹H NMR (300 MHz, DMSO-*d*₆) δ_{H} 0.89 (3H, t, *J* = 7.2 Hz,

CH₃), 1.34 (2H, q, $J = 7.2$ Hz, CH₂), 1.47 (2H, q, $J = 6.9$ Hz, CH₂), 3.20 (2H, q, $J = 6.9$ Hz, CH₂), 3.86 (3H, s, CH₃), 8.21 (1H, br s, NH), 9.66 (1H, s, CHO); ¹³C NMR (75 MHz, DMSO-*d*₆) δ_C 14.1 (CH₃), 19.9 (CH₂), 31.5 (CH₂), 33.4 (NCH₃), 39.0 (CH₂), 118.1 (quat.), 122.1 (quat.), 125.5 (quat.), 126.2 (quat.), 160.0 (C=O), 177.9 (CHO); HRMS Found: MH⁺ 277.0515. Calc. for C₁₁H₁₅Cl₂N₂O₂: MH⁺, 277.0506.

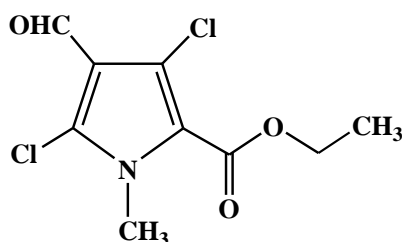
4.13.5 Benzyl 3,5-dichloro-4-formyl-1-methyl-1*H*-pyrrole-2-carboxylate **191**



This pyrrole was prepared, as described above, and the crude product was purified by column chromatography on silica, eluting with ethyl acetate: petroleum ether (60-80 °C) (5:5) to give pyrrole **191** as a white solid (0.15 g, 38 %); mp 124-125 °C; [Found: C, 53.5; H, 3.8; N, 4.2. C₁₄H₁₁NO₃Cl₂ requires C, 53.8; H, 3.6; N, 4.5 %]; ν_{max}(KBr)/cm⁻¹ 1707 (C=O), 1664 (C=O); ¹H NMR (300 MHz, DMSO-*d*₆) δ_H 3.89 (3H, s, CH₃), 5.34 (2H, s, CH₂), 7.39-7.45 (5H, m, Ar-H), 9.73 (1H, s, CHO); ¹³C NMR (75 MHz, DMSO-*d*₆) δ_C 33.7 (CH₃), 66.4 (CH₂), 110.2 (quat.), 124.9 (quat.), 126.7 (quat.), 128.3 (2 × CH), 128.5 (CH), 128.9 (2 × CH), 130.9 (quat.), 136.3 (quat., C-1'), 160.5 (C=O), 178.5 (CHO).

4.14 Methyl 3,5-dichloro-4-formyl-1-methyl-1*H*-pyrrole-2-carboxylate **188**

This pyrrole was prepared, as described above, and recrystallised from methanol to give a white solid **188** (0.22 g, 80 %); mp 108-110 °C; [Found: C, 40.5; H, 2.9; N, 5.7. C₈H₇Cl₂NO₃ requires C, 40.7 ; H, 2.9; N, 5.9 %]; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 1711 (C=O), 1654 (C=O), 1511 (C=C); ¹H NMR (300 MHz, DMSO-*d*₆) δ_{H} 3.87 (3H, s, *N*CH₃), 3.94 (3H, s, *O*CH₃), 9.79 (1H, s, CHO); ¹³C NMR (75 MHz, DMSO-*d*₆) δ_{C} 33.7 (*N*CH₃), 52.2 (*O*CH₃), 110.4 (quat.), 124.8 (quat.), 126.7 (quat.), 130.7 (quat.), 161.1 (C=O), 178.5 (CHO).

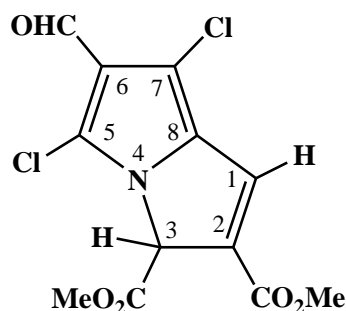
4.15 Ethyl 3,5-dichloro-4-formyl-1-methyl-1*H*-pyrrole-2-carboxylate **183**

A solution of 3,5-dichloro-4-formyl-1-methyl-1*H*-pyrrole-2-carboxylic acid **183** (0.30 g, 1.35 mmol) and SOCl₂ (0.49 ml) in toluene (5 ml) was refluxed for 4 h. After evaporation of the solvent, the crude mixture was cooled to 0 °C and treated with dry ethanol (10 ml) at 40 °C for 2 h. The solvent was evaporated under reduced pressure

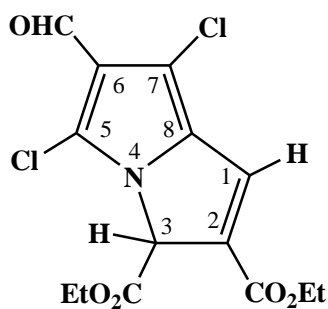
and the crude mixture was diluted with water (10 ml), extracted with EtOAc (3 × 20 ml) and the combined organics dried over MgSO₄. The product was purified by column chromatography on silica, eluting with petroleum ether (60-80 °C): diethyl ether (60:40) to give ester **189** (0.25 g, 86 %) as white solid; mp 78-80 °C; [Found: C, 43.3; H, 3.8; N, 5.4. C₉H₉Cl₂NO₃ requires C, 43.2; H, 3.6; N, 5.6 %]; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 1706 (C=O), 1662 (C=O), 1512 (C=C); ¹H NMR (300 MHz, DMSO-*d*₆) δ_{H} 1.35 (3H, t, *J* = 7.2 Hz, CH₂CH₃), 3.93 (3H, s, NCH₃), 4.35 (2H, q, *J* = 7.2 Hz, CH₂), 9.76 (1H, s, CHO); ¹³C NMR (75 MHz, DMSO-*d*₆) δ_{C} 14.5 (CH₂CH₃), 33.6 (NCH₃), 60.9 (CH₂), 110.5 (quat., C-3 or C-4), 124.8 (quat., C-3 or C-4), 126.6 (quat., C-5), 130.6 (quat., C-2), 160.6 (C=O), 178.4 (CHO); *m/z* Found: MNa⁺, 272. Calc. for C₉H₉³⁵Cl₂NO₃Na : MNa⁺, 272.

4.16 General procedure for the preparation of compounds 195a-b

Triphenylphosphine (0.41 g, 1.56 mmol) and pyrrole **41** (0.30 g, 1.56 mmol) were dissolved in DCM (16 ml). To this solution was added dropwise a mixture of dimethyl acetylenedicarboxylate (0.22 g, 1.56 mmol) in DCM (5.45 ml), at 0 °C over 10 min. The reaction mixture was then allowed to warm to room temperature and stirred for a further 20 min. The solvent was removed under reduced pressure and the residue was extracted with ether (4 × 30 ml). The combined ether layers were dried over anhydrous MgSO₄. After filtration, the solvent was removed under reduced pressure and the crude product was purified by column chromatography or by recrystallisation.

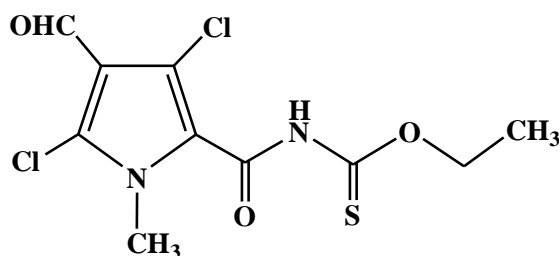
4.16.1 Dimethyl 5,7-dichloro-6-formyl-3*H*-pyrrolizine-2,3-dicarboxylate **195a**

The crude product was purified by column chromatography on silica, eluting with ethyl acetate: petroleum ether (60-80 °C) (3:7), to give a yellow solid **195a** (0.29 g, 58 %); mp 121-122 °C; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 1741 (C=O), 1714 (C=O), 1677 (C=O); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ_{H} 3.78 (6H, s, $2 \times \text{CH}_3$), 6.05 (1H, d, $J = 1.8$ Hz, CH-3), 7.85 (1H, d, $J = 1.8$ Hz, CH-1), 9.79 (1H, s, CHO); $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ_{C} 52.8 (OCH_3), 54.1 (OCH_3), 65.1 (CH, C-3), 106.8 (C-3), 120.2 (C-4), 123.7 (C-5), 130.3 (CH, C-1), 133.2 (C-2), 135.7 (C-7), 161.9 (C=O), 165.9 (C=O), 183.2 (CHO); HRMS Found: MH^+ , 339.9770. Calc. for $\text{C}_{12}\text{H}_{10}\text{Cl}_2\text{NO}_5$: MH^+ , 339.9749.

4.16.2 Diethyl 5,7-dichloro-6-formyl-3*H*-pyrrolizine-2,3-dicarboxylate **195b**

Purified by column chromatography on silica, eluting with ethyl acetate: petroleum ether (60-80 °C) (3:7) to give a yellow solid **195b** (1.17 g, 65 %); mp 129-131 °C; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 1725 (C=O), 1703 (C=O), 1663 (C=O); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ_{H} 1.29 (3H, t, $J = 6.9$ Hz, OCH_2CH_3), 1.36 (3H, t, $J = 7.2$ Hz, OCH_2CH_3), 4.27 (2H, q, $J = 6.9$ Hz, OCH_2CH_3), 4.38 (2H, q, $J = 7.2$ Hz, OCH_2CH_3), 6.79 (1H, s, H-3), 6.87 (1H, s, H-1), 9.84 (1H, s, CHO); $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ_{C} 13.9 (CH_3), 14.2 (CH_3), 61.3 (CH_2), 62.2 (CH_2), 67.8 (CH, C-3), 109.9 (CH, C-1), 125.6 (quat.), 126.6 (quat.), 136.1 (quat.), 136.8 (quat.), 139.5 (quat.), 158.3 (C=O), 162.4 (C=O), 181.4 (CHO); HRMS Found: MH^+ , 368.0073. Calc. for $\text{C}_{14}\text{H}_{14}\text{Cl}_2\text{NO}_5$: MH^+ , 368.0062.

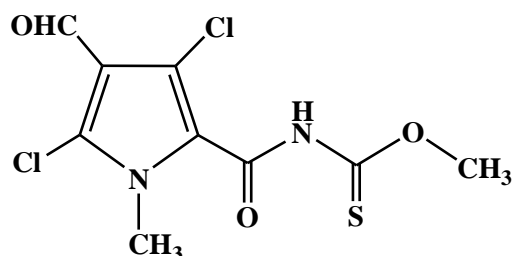
4.17 *N*-Ethoxythiocarbonyl 3,5-dichloro-4-formyl-1-methyl-1*H*-pyrrole-2-carboxamide **208a**



A solution of 3,5-dichloro-4-formyl-1-methyl-1*H*-pyrrole-2-carboxylic acid **183** (0.57 g, 2.57 mmol) and SOCl_2 (0.92 ml) in toluene (10 ml) was refluxed for 4 h. After evaporation of the solvent, the crude mixture was cooled to 0 °C and treated with a solution of KSCN (0.27 g, 2.70 mmol) in dry acetone (15 ml). After stirring for 2 h at room temperature, the mixture was filtered, then the solvent was evaporated under reduced pressure and the crude product was used in the next reaction without any

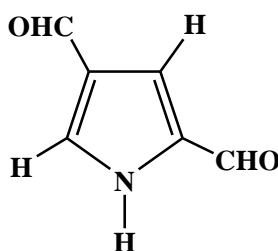
further purification **207** (0.21 g, 31 %). The isothiocyanate (0.21 g, 0.79 mmol) in dry ethanol (15 ml) was refluxed for 2 h at 60 °C. After completion of the reaction, as indicated by TLC, the solvent was evaporated under reduced pressure, the crude mixture was diluted with water (8 ml) and extracted with EtOAc (2 × 15 ml). The combined organic phases were washed with 4 % aqueous NaHCO₃ solution (8 ml), then with water (15 ml) and dried over MgSO₄. After filtration, the solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica, eluting with ethyl acetate: petroleum ether (60-80 °C) (3:7) to give the thiocarbamic acid ester **208a** (0.065 g, 26 %) as a white solid, mp 115-116 °C; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3377 (NH), 1703 (C=O), 1670 (C=O), 1170 (C=S); ¹H NMR (300 MHz, CDCl₃) δ_{H} 1.38 (3H, t, $J = 7.2$ Hz, OCH₂CH₃), 3.91 (3H, s, CH₃), 4.59 (2H, q, $J = 7.2$ Hz, OCH₂CH₃), 9.22 (1H, s, NH), 9.73 (1H, s, CHO); ¹³C NMR (75 MHz, CDCl₃) δ_{C} 13.7 (CH₃), 33.4 (NCH₃), 69.5 (OCH₂), 112.8 (quat.), 123.3 (quat.), 126.2 (quat.), 130.9 (quat., C-4), 155.3 (C=S), 177.6 (C=O), 188.6 (CHO); HRMS Found: MH⁺, 308.9866. Calc. for C₁₀H₁₁Cl₂N₂O₃S: MH⁺, 308.9862.

4.18 *N*-Methoxythiocarbonyl 3,5-dichloro-4-formyl-1-methyl-1*H*-pyrrole-2-carboxamide **208b**



This pyrrole was prepared in a similar way as described above, to give a yellow solid **208b** (0.89 g, 45 %); mp 120-121 °C; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 1719 (C=O), 1649 (C=O), 1163 (C=S); $^1\text{H NMR}$ (300 MHz, CDCl_3) δ_{H} 3.92 (3H, s, NCH_3), 4.12 (3H, s, OCH_3), 9.30 (1H, s, NH), 9.73 (1H, s, CHO); $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ_{C} 33.5 (NCH_3), 59.4 (OCH_3), 112.6 (quat.), 123.2 (quat.), 126.2 (quat.), 131.1 (quat., C-4), 155.3 (C=S), 177.6 (C=O), 189.4 (CHO); HRMS Found: MH^+ , 294.9716. Calc. for $\text{C}_9\text{H}_9\text{Cl}_2\text{N}_2\text{O}_3\text{S}$: MH^+ , 294.9706.

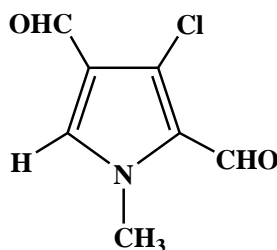
4.19 1H-Pyrrole-2,4-dicarboxaldehyde **218**



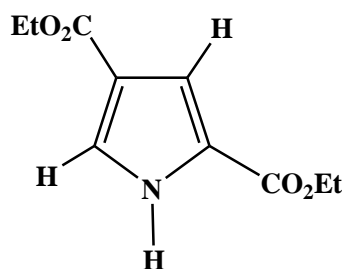
3,5-Dichloro-1H-pyrrole-2,4-dicarboxaldehyde **41** (0.80 g, 4.16 mmol), 10 % palladium on carbon (24 mg) and Et_3N (0.71 ml, 5.1 mmol) were dissolved in methanol (80 ml) then stirred under hydrogen (1 atmosphere) at ambient temperature (*ca.* 23 °C). After 4 h the reaction mixture was filtered through celite, and the methanol was concentrated *in vacuo*. The residue was extracted with ethyl acetate (3 × 40 ml), and the combined organic layer was washed with brine (70 ml) and dried over MgSO_4 . After filtration, the solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica, eluting with ethyl acetate: petroleum ether (60-80 °C) (3:7) to give a white solid **218** (0.36 g, 70 %); mp 103-104 °C; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3117 (NH), 1666 (C=O), 1637 (C=O), 1540 (C=C); $^1\text{H NMR}$ (300 MHz, $\text{DMSO}-d_6$) δ_{H} 7.42 (1H, s, H-3), 7.97 (1H, s, H-5), 9.62

(1H, s, CHO), 9.81 (1H, s, CHO), 12.85 (1H, br s, NH); ^{13}C NMR (75 MHz, DMSO- d_6) δ_{C} 118.9 (CH, C-3), 127.6 (quat., C-2), 133.5 (CH, C-5), 134.6 (quat., C-4), 181.5 (CHO), 186.4 (CHO); HRMS Found: MH^+ , 124.0395. Calc. for $\text{C}_6\text{H}_6\text{NO}_2$: MH^+ , 124.0394.

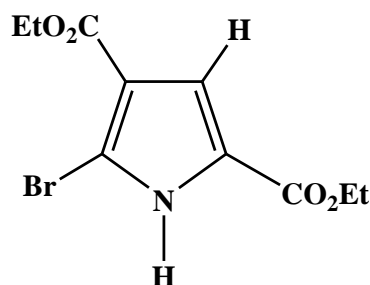
4.20 3-Chloro-1-methyl-1H-pyrrole-2,4-dicarboxaldehyde **219**



3,5-Dichloro-1-methyl-1H-pyrrole-2,4-dicarboxaldehyde **42** (0.8 g, 4.16 mmol), 5% Pd on charcoal (24 mg) and Et_3N (0.71 ml, 5.1 mmol) were dissolved in methanol (80 ml) then stirred under hydrogen (4 bar) at 60 °C. After 4 h the reaction mixture was filtered through celite, and the methanol was concentrated *in vacuo*. The residue was extracted with ethyl acetate (3 × 40 ml) and the combined organic layer was washed with brine (70 ml) and dried over MgSO_4 . After filtration, the solvent was evaporated under reduced pressure and recrystallised from petroleum ether (60-80 °C) to give a white powder **219** (0.79 g, 94 %); mp 100-101 °C; [Found: C, 48.8; H, 3.5; N, 8.0. $\text{C}_7\text{H}_6\text{NO}_2\text{Cl}$ requires C, 49.0; H, 3.5; N, 8.2 %]; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 1715 (C=O), 1653 (C=O), 1508 (C=C); ^1H NMR (300 MHz, CDCl_3) δ_{H} 3.91 (1H, s, CH_3), 7.36 (1H, s, H-5), 9.80 (1H, s, CHO), 9.83 (1H, s, CHO); ^{13}C NMR (75 MHz, CDCl_3) δ_{C} 38.4 (CH_3), 121.6 (quat., C-3), 127.0 (quat., C-4), 127.6 (quat., C-2), 132.7 (CH, C-5), 178.6 (4-CHO), 183.4 (2-CHO).

4.21 Diethyl 1*H*-pyrrole-2,4-dicarboxylate **226**

Diethyl 1*H*-pyrrole-2,4-dicarboxylate was prepared by the method of Kazuo.³ To a mixture of ethyl isocyanoacetate (2.19 ml, 0.02 mol) and DBU (2.99 ml, 0.02 mol) in THF (30 ml), formaldehyde (0.27 ml, 0.01 mol) was added dropwise at 45-50 °C. After stirring for an additional 4 h at the same temperature, the reaction mixture was neutralised with acetic acid then the solvent was removed under reduced pressure. The residue was diluted with water (8 ml) and extracted with ethyl acetate (2 × 15 ml). The combined organic phases were washed with 5 % aq. HCl (2 × 15 ml) and dried over MgSO₄. After filtration, the solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica, eluting with ethyl acetate: petroleum ether (60-80 °C) (50:50) to give diester **226** (0.39 g, 19 %) as a white solid; mp 186-188 °C (lit.⁵ mp 183-185 °C); $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3277 (NH), 1707 (C=O), 1673 (C=O), 1566 (C=C); ¹H NMR (300 MHz, CDCl₃) δ_{H} 6.14 (6H, t, $J = 7.2$ Hz, 2 × CH₃), 4.25 (4H, q, $J = 7.2$ Hz, 2 × CH₂), 7.23 (1H, dd, $J = 3.9$ and 1.5 Hz, CH), 7.47 (1H, dd, $J = 3.9$ and 1.5 Hz, CH), 9.81 (1H, br s, NH); ¹³C NMR (75 MHz, CDCl₃) δ_{C} 14.3 (CH₃), 14.4 (CH₃), 60.2 (CH₂), 60.9 (CH₂), 115.9 (CH, C-3), 118.4 (quat., C-4), 123.8 (quat., C-2), 127.0 (CH, C-5), 161.1 (C=O), 164.1 (C=O); m/z Found: MH⁺, 212. Calc. for C₁₀H₁₄NO₄: MH⁺, 212.

4.22 Diethyl 5-bromo-1*H*-pyrrole-2,4-dicarboxylate **227a****Method A**

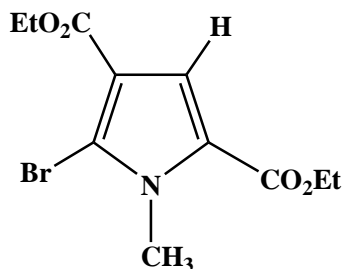
A solution of *N*-bromosuccinimide (0.60 g, 3.39 mmol) in THF (6 ml) was added dropwise to the 1*H*-pyrrole-2,4-dicarboxylic acid diethyl ester **226** (0.34 g, 1.61 mmol) in THF (5 ml) at -78 °C, under argon. The mixture was warmed to room temperature and stirred for a further 4 h. The solvent was evaporated under reduced pressure, the crude product diluted with CCl₄ (5 ml) and the precipitate formed was filtered off. The filtrate was evaporated under reduced pressure and the crude product was purified by column chromatography on silica, eluting with ethyl acetate: petroleum ether (60-80 °C) (4:6) to give diester **227** as a white solid (0.15 g, 32 %); spectral data given below.

Method B

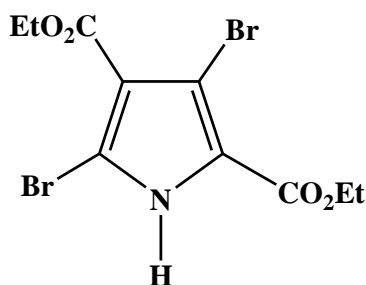
Bromine (0.72 ml, 14.05 mmol) in chloroform (5 ml) was added dropwise to a solution of pyrrole **226** (1.5 g, 7.11 mmol) in chloroform (40 ml) at room temperature and stirred for 4 h at the same temperature. The reaction mixture was then poured into 0.1 % w/v aqueous sodium metabisulphite, extracted with DCM (3 × 30 ml) and the combined organics dried over MgSO₄. After filtration, the solvent was evaporated under reduced pressure and the residue recrystallised from hexane to give the diester

227 as white solid (1.89 g, 91 %); mp 133-134 °C; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3213 (NH), 1709 (C=O), 1665 (C=O); ^1H NMR (300 MHz, DMSO- d_6) δ_{H} 1.22 (6H, t, $J = 7.2$ Hz, 2 \times CH₃), 4.17 (4H, q, $J = 7.2$ Hz, 2 \times CH₂), 7.03 (1H, s, CH), 13.27 (NH); ^{13}C NMR (75 MHz, DMSO- d_6) δ_{C} 14.7 (2 \times CH₃), 60.2 (CH₂), 60.8 (CH₂), 111.0 (quat.), 115.4 (quat.), 117.4 (CH, C-3), 124.5 (quat.), 159.6 (C=O), 162.4 (C=O); HRMS Found: MH^+ , 290.0033. Calc. for C₁₀H₁₃BrNO₄: MH^+ , 290.0023.

4.23 Diethyl 5-bromo-1-methyl-1H-pyrrole-2,4-dicarboxylate **227b**



This pyrrole was prepared in a similar way as described above, and recrystallised from hexane to give diester **227b** as a white solid (1.47 g, 93 %); mp 92-93 °C; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 1697 (C=O), 1540 (C=C); ^1H NMR (300 MHz, DMSO- d_6) δ_{H} 1.35 (6H, m, 2 \times CH₃), 3.97 (3H, s, NCH₃), 4.29 (4H, m, 2 \times CH₂), 7.31 (1H, s, CH); ^{13}C NMR (75 MHz, DMSO- d_6) δ_{C} 14.6 (CH₃), 14.7 (CH₃), 33.3 (NCH₃), 60.3 (CH₂), 60.9 (CH₂), 114.4 (quat., C-4), 116.9 (quat., C-5), 118.9 (CH, C-3), 124.4 (quat., C-2), 159.8 (C=O), 162.1 (C=O); HRMS Found: MH^+ , 304.0199. Calc. for C₁₁H₁₅BrNO₄: MH^+ , 304.0179.

4.24 Diethyl 3,5-dibromo-1H-pyrrole-2,4-dicarboxylate 228

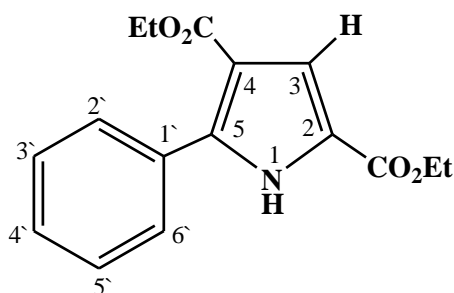
This pyrrole was prepared in a similar way as described above, and recrystallised from methanol to give the diester **228** as white crystals (1.49 g, 85 %); mp 159-160 °C; [Found: C, 32.4; H, 2.9 ; N, 3.6. C₁₀H₁₁Br₂NO₄ requires C, 32.5; H, 3.0; N, 3.8 %]; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3210 (NH), 1698 (C=O), 1661 (C=O), 1530 (C=C); ¹H NMR (300 MHz, DMSO-*d*₆) δ_{H} 1.31 (6H, t, *J* = 7.2 Hz, 2 × CH₃), 4.28 (4H, q, *J* = 7.2 Hz, 2 × CH₂), 13.61 (1H, br s, NH); ¹³C NMR (75 MHz, DMSO-*d*₆) δ_{C} 15.0 (CH₃), 15.1 (CH₃), 61.2 (CH₂), 61.5 (CH₂), 104.2 (quat.), 111.6 (quat.), 116.3 (quat.), 123.5 (quat.), 159.2 (C=O), 162.1 (C=O); *m/z* Found: (M-H)⁻, 368. Calc. for C₁₀H₁₀Br₂NO₄: (M-H)⁻, 368.

4.25 General procedure for the Suzuki reaction of bromo derivatives

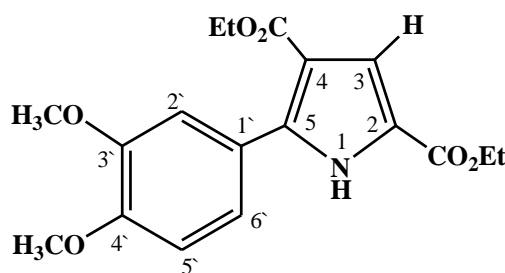
Pyrrole (1.04 mmol) was dissolved in DMF (10 ml) and the mixture was stirred under argon. Palladium tetrakis(triphenylphosphine) (0.053 mmol) and a boronic acid (1.25 mmol) were added to this solution, sequentially, at room temperature. The reaction mixture was heated to 70 °C and sodium carbonate (9.3 mmol) dissolved in the minimum of water was added to the solution. The mixture was refluxed at 110 °C then the reaction mixture was allowed to cool to room temperature, diluted with water

(48 ml), extracted with Et₂O (3 × 50 ml), and the combined organics dried over MgSO₄. After filtration, the solvent was evaporated under reduced pressure and the crude product was purified by column chromatography or recrystallised.

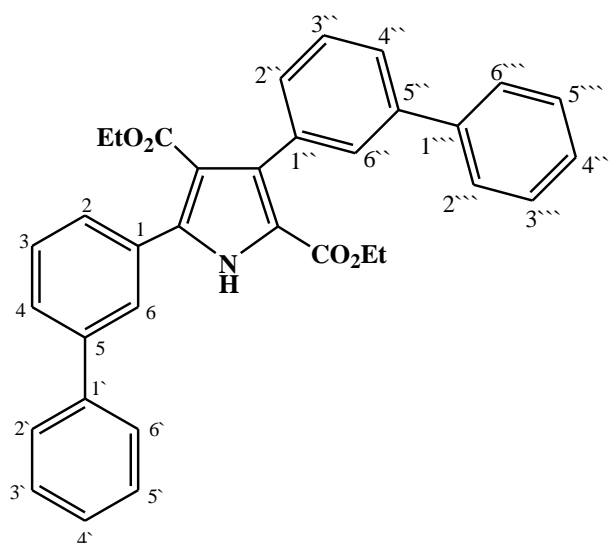
4.25.1 Diethyl 5-phenyl-1*H*-pyrrole-2,4-dicarboxylate **234a**



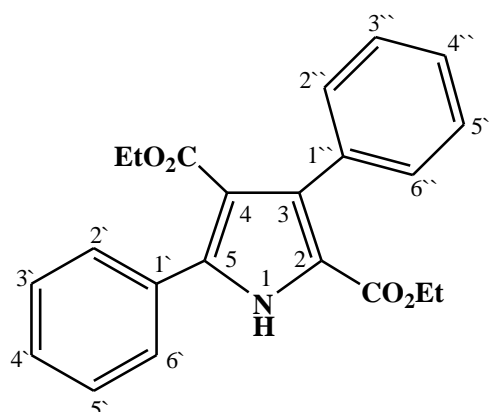
The crude product was purified by column chromatography on silica, eluting with ethyl acetate: petroleum ether (60-80 °C) (2:8) to give diester **234a** as a white solid (0.29 g, 58 %); mp 117-118 °C; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3275 (NH), 1714 (C=O), 1668 (C=O); ¹H NMR (300 MHz, DMSO-*d*₆) δ_{H} 1.22 (3H, t, $J = 6.9$ Hz, CH₃), 1.35 (3H, t, $J = 7.2$ Hz, CH₃), 4.16 (2H, q, $J = 6.9$ Hz, CH₂), 4.32 (2H, q, $J = 7.2$ Hz, CH₂), 7.25 (1H, s, CH), 7.35-7.47 (3H, m, ArH), 7.62 (2H, m, ArH), 12.60 (1H, br s, NH); ¹³C NMR (75 MHz, DMSO-*d*₆) δ_{C} 14.5 (CH₃), 14.6 (CH₃), 59.8 (CH₂), 60.5 (CH₂), 113.5 (quat.), 118.2 (CH, C-3), 122.9 (quat.), 127.9 (2 × CH), 129.5 (CH), 130.4 (2 × CH), 160.4 (C=O), 163.7 (C=O); HRMS Found: MH⁺, 288.1235, Calc. for C₁₆H₁₈NO₄: MH⁺, 288.1231.

4.25.2 Diethyl 5-(3,4-dimethoxyphenyl)-1*H*-pyrrole-2,4-dicarboxylate **234b**

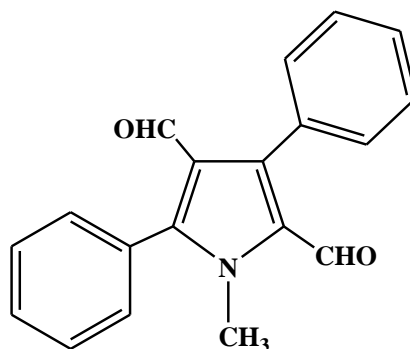
The crude product was recrystallised from petroleum ether (60-80 °C) to give diester **234b** as white crystals (0.42 g, 88 %); mp 157-158 °C; [Found: C, 62.2; H, 6.1; N, 4.0. C₁₈H₂₁NO₆ requires C, 62.2; H, 6.1; N, 4.0 %]; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3275 (NH), 1715 (C=O), 1679 (C=O); ¹H NMR (300 MHz, CDCl₃) δ_{H} 1.22 (3H, t, $J = 7.2$ Hz, CH₃), 1.28 (3H, t, $J = 6.9$ Hz, CH₃), 3.85 (6H, s, 2 × OCH₃), 4.17 (2H, q, $J = 7.2$ Hz, CH₂), 4.23 (2H, q, $J = 6.9$ Hz, CH₂), 6.85 (1H, d, $J = 8.4$ Hz, H-5'), 7.12 (1H, dd, $J = 8.4$ and 1.8 Hz, H-6'), 7.17 (1H, d, $J = 1.8$ Hz, H-2'), 7.31 (1H, s, H-3), 9.32 (1H, s, NH); ¹³C NMR (75 MHz, CDCl₃) δ_{C} 14.3 (CH₃), 14.4 (CH₃), 56.0 (OCH₃), 56.1 (OCH₃), 60.0 (CH₂), 60.9 (CH₂), 110.9 (CH), 112.8 (CH), 113.8 (quat.), 118.4 (CH, C-3), 121.8 (CH), 121.9 (quat.), 123.5 (quat.), 140.6 (quat.), 148.6 (quat., C-3' or C-4'), 149.9 (quat., C-3' or C-4'), 161.0 (C=O), 164.1 (C=O); m/z Found: MNa⁺, 370. Calc. for C₁₈H₂₁NO₆Na: MNa⁺, 370.

4.25.3 Diethyl 3,5-bis(biphenyl-3-yl)-1*H*-pyrrole-2,4-dicarboxylate **235a**

The crude product was recrystallised from methanol to give diester **235a** as white crystals (0.47 g, 68 %); mp 154-155 °C; [Found: C, 78.8; H, 5.7; N, 2.6. C₃₄H₂₉NO₄ requires C, 79.2; H, 5.7; N, 2.7 %]; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3258 (NH), 1708 (C=O), 1664 (C=O); ¹H NMR (300 MHz, CDCl₃) δ_{H} 0.73 (3H, t, $J = 7.2$ Hz, CH₃), 0.90 (3H, t, $J = 6.9$ Hz, CH₃), 3.89 (2H, q, $J = 6.9$ Hz, CH₂), 3.96 (2H, q, $J = 7.2$ Hz, CH₂), 7.25 (1H, m, ArH), 7.28 (1H, m, ArH), 7.33 (5H, m, ArH), 7.40 (1H, m, ArH), 7.41-7.45 (1H, m, ArH), 7.46-7.48 (1H, m, ArH), 7.49-7.51 (1H, m, ArH), 7.52-7.55 (6H, m, ArH), 7.78 (1H, s, ArH), 9.50 (1H, s, NH); ¹³C NMR (75 MHz, CDCl₃) δ_{C} 13.5 (CH₃), 13.9 (CH₃), 60.0 (CH₂), 60.6 (CH₂), 115.1 (quat.), 119.9 (quat.), 125.8 (CH), 127.11 (CH), 127.15 (2 × CH), 127.2 (2 × CH), 127.5 (CH), 127.6 (CH), 127.7 (CH), 127.8 (2 × CH), 127.9 (CH), 128.7 (2 × CH), 128.8 (CH), 128.9 (2 × CH), 128.9 (CH), 129.0 (CH), 131.7 (quat.), 133.3 (quat.), 135.1 (quat.), 138.9 (quat.), 139.9 (quat.), 140.5 (quat.), 141.4 (quat.), 161.1 (C=O), 164.5 (C=O); m/z Found: MNa⁺, 538. Calc. for C₃₄H₂₉NO₄Na: MNa⁺, 538.

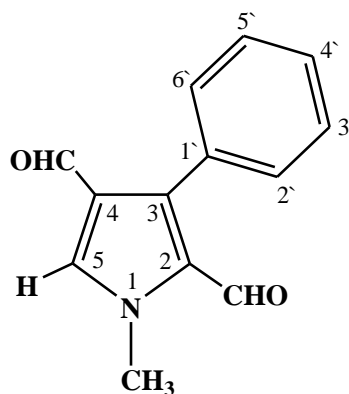
4.25.4 Diethyl 3,5-diphenyl-1*H*-pyrrole-2,4-dicarboxylate **235b**

The crude product was recrystallised from petroleum ether (60-80 °C) – ethyl acetate to give diester **235b** as white crystals (0.21 g, 71 %); mp 110-112 °C; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3288 (NH), 1712 (C=O), 1661 (C=O); $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ_{H} 0.83 (3H, t, $J = 7.2$ Hz, CH_3), 1.09 (3H, t, $J = 6.9$ Hz, CH_3), 3.90 (2H, q, $J = 7.2$ Hz, CH_2), 4.11 (2H, q, $J = 6.9$ Hz, CH_2), 7.29-7.34 (5H, m, ArH), 7.43-7.49 (3H, m, ArH), 7.59-7.61 (2H, m, ArH), 12.49 (1H, s, NH); $^{13}\text{C NMR}$ (75 MHz, $\text{DMSO-}d_6$) δ_{C} 13.8 (CH_3), 14.3 (CH_3), 59.8 (CH_2), 60.1 (CH_2), 114.8 (quat.), 119.9 (quat.), 127.0 (CH), 127.4 (CH), 128.0 (CH), 128.3 (CH), 128.9 (CH), 129.8 (CH), 130.5 (CH), 131.3 (quat.), 132.6 (quat.), 135.1 (quat.), 138.9 (quat.), 160.5 (C=O), 164.7 (C=O); HRMS Found: MH^+ , 364.1547. Calc. for $\text{C}_{22}\text{H}_{21}\text{NO}_4$: MH^+ , 364.1544.

4.25.5 1-Methyl-3,5-diphenyl-1*H*-pyrrole-2,4-dicarboxaldehyde **237a**

The crude product was recrystallised from petroleum ether (60-80 °C) – ethyl acetate to give pyrrole **237a** as a yellow solid (0.31 g, 74 %); mp 117-119 °C; [Found: C, 78.8; H, 5.3; N, 4.8. C₁₉H₁₅NO₂ requires C, 78.9; H, 5.2; N, 4.8 %]; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 1657 (C=O); ¹H NMR (300 MHz, CDCl₃) δ_{H} 3.78 (3H, s, CH₃), 7.29-7.38 (7H, m, ArH), 7.41-7.46 (3H, m, ArH), 9.46 (1H, s, 4-CHO), 9.56 (1H, s, 2-CHO); ¹³C NMR (75 MHz, CDCl₃) δ_{C} 34.5 (CH₃), 120.8 (C-3 or C-4), 128.1 (CH), 128.6 (CH), 128.8 (CH), 129.1 (C-3 or C-4), 130.1 (CH), 130.6 (CH), 131.1 (CH), 140.2 (C-5), 147.2 (C-2), 181.7 (CHO), 185.9 (CHO); *m/z* Found: MNa⁺, 312. Calc. for C₁₉H₁₅NO₂Na: MNa⁺, 312.

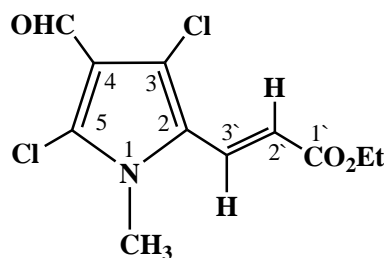
4.25.6 1-Methyl-3-phenyl-1*H*-pyrrole-2,4-dicarboxaldehyde **237b**



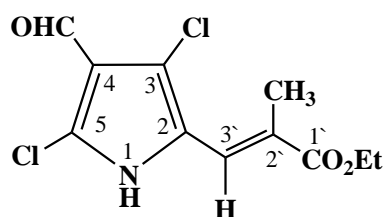
The crude product was purified by column chromatography on silica, eluting with ethyl acetate: petroleum ether (60-80 °C) (3:7) to give the dialdehyde **237b** as a yellow solid (0.082 g, 66 %); mp 120-122 °C; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 1654 (C=O); $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ_{H} 3.99 (3H, s, CH_3), 7.42-7.49 (5H, m, ArH), 8.03 (1H, s, CH), 9.44 (1H, s, CHO), 9.64 (1H, s, CHO); $^{13}\text{C NMR}$ (75 MHz, $\text{DMSO-}d_6$) δ_{C} 37.9 (CH_3), 122.6 (C-4), 128.7 (2 \times CH), 128.8 (CH), 129.1 (quat., C-2), 130.6 (quat., C-3), 131.4 (2 \times CH), 135.9 (CH, C-5), 139.1 (quat., C-1'), 180.9 (CHO), 185.6 (CHO). HRMS Found: MH^+ 214.0876, Calc. for $\text{C}_{13}\text{H}_{12}\text{NO}_2$: MH^+ , 214.0869.

4.26 General procedure for the Wittig reaction

The appropriate aldehyde (2.91 mmol) was dissolved in CH_3CN (30 ml) and treated with (carbethoxymethylene)triphenylphosphorane (3.06 mmol) or (carbethoxyethylidene)triphenylphosphorane (3.06 mmol). The reaction mixture was refluxed for 9-12 h then the solvent was removed under reduced pressure and the residue was treated with water (20 ml), extracted with EtOAc (3 \times 30 ml) and the combined organics dried over MgSO_4 . After filtration, the solvent was evaporated under reduced pressure and the crude product was purified by column chromatography or recrystallised.

4.26.1 Ethyl 3'-(3,5-dichloro-4-formyl-1-methyl-1*H*-pyrrole-2-yl)acrylate **241a**

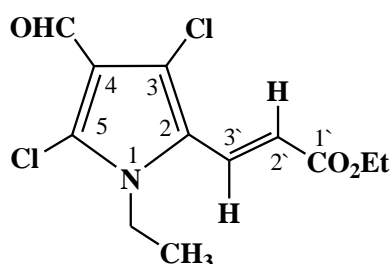
The crude product was purified by column chromatography on silica, eluting with ethyl acetate: petroleum ether (60-80 °C) (2:8), to give the acrylate **241a** as a pink solid (0.24 g, 35 %); mp 114-116 °C; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 1701 (C=O), 1660 (C=O), 1634 (C=O), 1511 (C=C); ^1H NMR (300 MHz, CDCl_3) δ_{H} 1.27 (3H, t, $J = 7.2$ Hz, CH_3), 3.89 (3H, s, NCH_3), 4.19 (2H, q, $J = 7.2$ Hz, CH_2), 6.61 (1H, d, $J = 16$ Hz, =CH-3'), 7.48 (1H, d, $J = 16$ Hz, =CH-2'), 9.69 (1H, s, CHO); ^{13}C NMR (75 MHz, CDCl_3) δ_{C} 14.4 (CH_3), 33.5 (CH_3), 60.6 (CH_2), 114.6 (quat., C-3), 119.1 (CH-3'), 125.3 (quat., C-5), 126.5 (quat., C-2), 128.8 (quat., C-4), 131.8 (CH-2'), 167.1 (C=O), 177.5 (CHO); HRMS Found: MH^+ , 276.0196. Calc. for $\text{C}_{11}\text{H}_{12}\text{Cl}_2\text{NO}_3$: MH^+ , 276.0190.

4.26.2 Ethyl-3'-(3,5-dichloro-4-formyl-1*H*-pyrrole-2-yl)-2'-methylacrylate **241b**

The crude product was purified by column chromatography on silica, eluting with ethyl acetate: petroleum ether (60-80 °C) (2:8), to give ester **241b** a yellow solid (0.2 g, 35 %); mp 149-150 °C; [Found: C, 47.8; H, 4.0; N, 5.0. $\text{C}_{11}\text{H}_{11}\text{Cl}_2\text{NO}_3$ requires C,

47.9; H, 4.0; N, 5.1 %]; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3180 (NH), 1717 (C=O), 1666 (C=O); ^1H NMR (300 MHz, DMSO- d_6) δ_{H} 1.35 (3H, t, $J = 7.2$ Hz, CH₃), 2.13 (3H, d, $J = 1.5$ Hz, CH₃), 4.28 (2H, q, $J = 7.2$ Hz, CH₂), 7.37 (1H, q, $J = 1.5$ Hz, =CH), 9.88 (1H, s, CHO), 12.96 (1H, br s, NH); ^{13}C NMR (75 MHz, DMSO- d_6) δ_{C} 14.6 (CH₃), 15.2 (CH₃), 61.3 (CH₂), 114.3 (quat., C-4), 116.7 (quat., C-3), 123.8 (CH), 125.5 (quat., C-5), 126.1 (quat., C-2), 128.5 (quat., =C), 167.5 (C=O), 182.9 (CHO); m/z Found: MH⁺, 276. Calc. for C₁₁H₁₁³⁵Cl₂NO₃: MH⁺, 276.

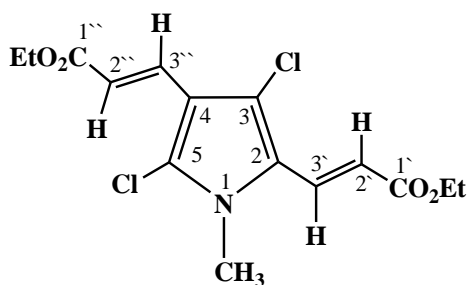
4.26.3 Ethyl 3-(3,5-dichloro-1-ethyl-4-formyl-1H-pyrrole-2-yl)acrylate **241c**



The crude product was purified by column chromatography on silica, eluting with ethyl acetate: petroleum ether (60-80 °C) (2:8), to give ester **241c** as a pale yellow solid (0.2 g, 43 %); mp 108-109 °C; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 1706 (C=O), 1665 (C=O), 1634 (C=O), 1525 (C=C); ^1H NMR (300 MHz, CDCl₃) δ_{H} 1.25 (3H, t, $J = 7.2$ Hz, OCH₂CH₃), 1.28 (3H, t, $J = 6.9$ Hz, NCH₂CH₃), 4.19 (2H, q, $J = 7.2$ Hz, OCH₂CH₃), 4.41 (2H, q, $J = 6.9$ Hz, NCH₂CH₃), 6.66 (1H, d, $J = 16.2$ Hz, H-2'), 7.49 (1H, d, $J = 16.2$ Hz, H-3'), 9.68 (1H, s, CHO); ^{13}C NMR (75 MHz, CDCl₃) δ_{C} 14.4 (CH₃), 15.3 (CH₃), 41.8 (CH₂), 60.6 (CH₂), 114.6 (quat., C-2), 119.1 (CH, C-2'), 125.6 (quat., C-3 or C-5), 125.8 (quat., C-3 or C-5), 127.9 (quat., C-4), 131.8 (CH, C-3'), 167.1

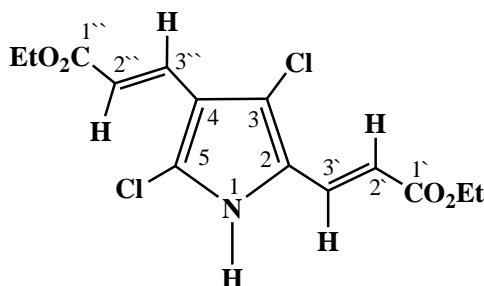
(C=O), 177.2 (CHO); HRMS Found: MH^+ , 290.0359. Calc. for $C_{12}H_{13}Cl_2NO_3$: MH^+ , 290.0346.

4.26.4 3,5-Dichloro-2,4-bis(2-ethoxycarbonyl-1H-pyrrole-2-yl)-1-methyl-1H-pyrrole 242a



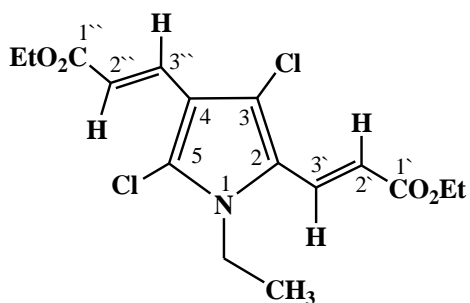
The crude product was purified by column chromatography on silica, eluting with ethyl acetate: petroleum ether (60-80 °C) (2:8), to give the diester **242a** as a yellow solid (0.38 g, 45 %); mp 113-114 °C; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 1698 (C=O), 1624 (C=O); ^1H NMR (300 MHz, $\text{DMSO-}d_6$) δ_{H} 1.30-1.36 (6H, m, 2 \times CH_3), 3.79 (3H, s, $N\text{CH}_3$), 4.24-4.28 (4H, m, 2 \times CH_2), 6.64 (1H, d, $J = 16.2$ Hz, =CH), 6.67 (1H, d, $J = 16.2$ Hz, =CH), 7.49 (1H, d, $J = 16.2$ Hz, =CH), 7.57 (1H, d, $J = 16.2$ Hz, =CH); ^{13}C NMR (75 MHz, $\text{DMSO-}d_6$) δ_{C} 14.7 (2 \times CH_3), 33.1 ($N\text{CH}_3$), 60.6 (CH_2), 60.7 (CH_2), 113.6 (quat.), 114.6 (quat.), 116.9 (CH), 117.4 (CH), 123.8 (quat.), 125.8 (quat.), 129.4 (CH), 132.6 (CH), 166.7 (2 \times C=O); HRMS Found: MH^+ , 346.0618. Calc. for $C_{15}H_{18}Cl_2NO_4$: MH^+ , 346.0608.

4.26.5 3,5-Dichloro-2,4-bis(2-ethoxycarbonyl-1H-pyrrole-2-yl)pyrrole 242b



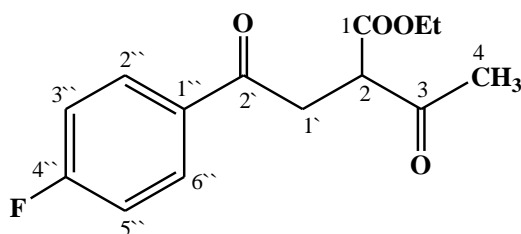
The crude product was purified by column chromatography on silica, eluting with ethyl acetate: petroleum ether (60-80 °C) (2:8), to give diester **242b** as a pink solid (0.42 g, 39 %); mp 168-169 °C; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3208 (NH), 1704 (C=O), 1667 (C=O), 1624 (C=O), 1542 (C=C); $^1\text{H NMR}$ (300 MHz, DMSO- d_6) δ_{H} 1.29 (6H, t, $J = 6.9$ Hz, $2 \times \text{CH}_3$), 4.22 (4H, q, $J = 6.9$ Hz, $2 \times \text{CH}_2$), 6.46 (1H, d, $J = 16.0$ Hz, =CH), 6.59 (1H, d, $J = 16.0$ Hz, =CH), 7.39 (2H, m, =CH), 13.18 (1H, s, NH); $^{13}\text{C NMR}$ (75 MHz, DMSO- d_6) δ_{C} 14.6 ($2 \times \text{CH}_3$), 60.5 ($2 \times \text{CH}_2$), 113.7 (quat.), 115.5 (CH), 115.6 (quat.), 116.7 (CH), 122.3 (quat.), 125.5 (quat.), 128.7 (CH), 132.6 (CH), 166.5 (C=O), 166.7 (C=O); HRMS Found: MH^+ , 332.0438. Calc. for $\text{C}_{14}\text{H}_{15}\text{Cl}_2\text{NO}_4$: MH^+ , 332.0452.

4.26.6 3,5-Dichloro-2,4-bis(2'-ethoxycarbonyl-1H-pyrrole-2-yl)-1-ethyl-1H-pyrrole 242c



The crude product was purified by column chromatography on silica, eluting with ethyl acetate: petroleum ether (60-80 °C) (2:8), to give diester **242c** as an orange solid (0.42 g, 64 %); mp 84-86 °C; [Found: C, 53.5; H, 5.4; N, 3.8. C₁₆H₁₉Cl₂NO₄ requires C, 53.4; H, 5.3; N, 3.9 %]; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 1704 (C=O), 1624 (C=O); ¹H NMR (300 MHz, DMSO-*d*₆) δ_{H} 1.30-1.36 (9H, m, 3 × CH₃), 4.24-4.28 (6H, m, 3 × CH₂), 6.65 (1H, d, *J* = 16.0 Hz, =CH), 6.68 (1H, d, *J* = 16.4 Hz, =CH), 7.49 (1H, d, *J* = 16.0 Hz, =CH), 7.57 (1H, d, *J* = 16.4 Hz, =CH); ¹³C NMR (75 MHz, DMSO-*d*₆) δ_{C} 14.6 (2 × CH₃), 15.6 (CH₃), 40.8 (CH₂), 60.6 (CH₂), 60.8 (CH₂), 113.8 (quat.), 114.8 (quat.), 117.2 (CH), 117.6 (CH), 122.8 (quat.), 124.6 (quat.), 129.1 (CH), 132.5 (CH), 166.7 (2 × C=O).

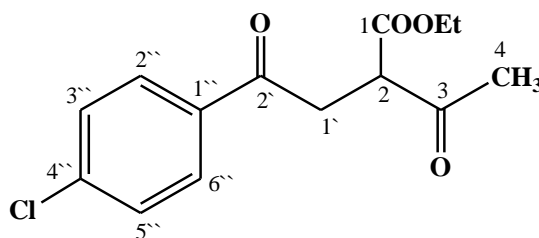
4.27 Ethyl 2-[2'-(4''-fluorophenyl)-2'-oxoethyl]-3-oxobutanoate **245a**⁴



To a refluxing solution of sodium ethoxide (1.72 g, 25.72 mmol) in EtOH (24 ml) was added, dropwise, ethyl acetoacetate **243** (3.26 ml, 25.72 mmol). Stirring was continued for an additional 1 h then the reaction was allowed to cool to room temperature. 4-Fluorophenacyl bromide **244a** (6 g, 25.72 mmol) was added to the solution in small portions and stirring was continued overnight at room temperature. After the reaction was complete, the solvent was removed under reduced pressure, the residue was diluted with water (16 ml), extracted with diethyl ether (2 × 18 ml) and the combined organics dried over MgSO₄. After filtration, the solvent was evaporated

under reduced pressure and the crude product was purified by column chromatography on silica, eluting with petroleum ether (60-80 °C): diethyl ether (80:20) to give ester **245a** (1.2 g, 11 %) as a white solid; mp 55-56 °C (lit.⁴ mp 52-53 °C); $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 1733 (C=O), 1715 (C=O), 1680 (C=O), 1592 (C=C); ¹H NMR (300 MHz, CDCl₃) δ_{H} 1.22 (3H, t, $J = 6.9$ Hz, OCH₂CH₃), 2.36 (3H, s, CH₃), 3.39 (1H, dd, $J = 18$ and 5.7 Hz, CH₂-a), 3.60 (1H, dd, $J = 18$ and 8.1 Hz, CH₂-b), 4.12-4.19 (3H, m, OCH₂CH₃, H-2), 7.06 (2H, t, $J = 8.7$ Hz, CH-3'',5''), 7.93 (2H, dd, $J = 8.7$ and 5.4 Hz, CH-2'',6''); ¹³C NMR (75 MHz, CDCl₃) δ_{C} 14.1 (CH₃), 30.2 (CH₃), 37.3 (CH₂), 53.9 (CH), 61.9 (OCH₂), 115.8 (2 × CH, C-3'',5''), d, $J = 21.9$ Hz), 130.8 (2 × CH, C-2'',6''), d, $J = 9.4$ Hz), 132.6 (quat., C-1''), d, $J = 2.9$ Hz), 165.9 (quat., C-4''), d, $J = 253.8$ Hz), 168.8 (C=O, C-2'), 195.6 (C=O, COOEt), 202.2 (C=O, C-3).

4.28 Ethyl 2-[2'-(4''-chlorophenyl)-2'-oxoethyl]-3-oxobutanoate **245b**⁴

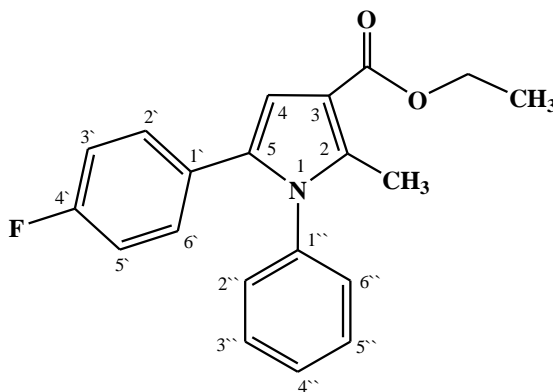


The diketone was prepared, as described above, from 4-chlorophenacyl bromide **244b**, to give a white solid **245b** (1.05 g, 15 %); mp 59-60 °C (lit.⁴ mp 58-59 °C); $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 1737 (C=O), 1714 (C=O), 1685 (C=O), 1589 (C=C); ¹H NMR (300 MHz, CDCl₃) δ_{H} 1.22 (3H, t, $J = 7.2$ Hz, OCH₂CH₃), 2.36 (3H, s, CH₃), 3.39 (1H, dd, $J = 18.3$ and 5.7 Hz, CH₂-a), 3.59 (1H, dd, $J = 18.3$ and 8.1 Hz, CH₂-b), 4.12-4.19 (3H, m, OCH₂CH₃, H-2), 7.37 (2H, d, $J = 8.7$ Hz), 7.85 (2H, d, $J = 8.7$ Hz); ¹³C NMR (75 MHz, CDCl₃) δ_{C} 14.1 (CH₃), 30.2 (CH₃), 37.3 (CH₂), 53.9 (CH), 61.9 (OCH₂),

129.0 (2 × CH, C-3'',5''), 129.6 (2 × CH, C-2'',6''), 134.5 (quat., C-4''), 140.0 (quat., C-1''), 168.7 (C=O, C-2''), 195.9 (C=O, COOEt), 202.1 (C=O, C-3).

4.29 Ethyl 5-(4'-fluorophenyl)-2-methyl-1-phenyl-1H-pyrrole-3-carboxylate

246a⁵

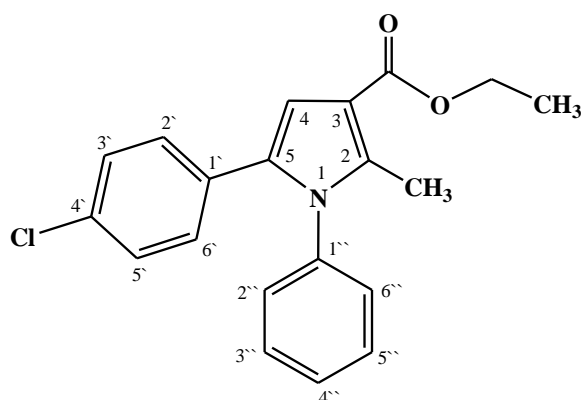


A solution of ethyl 2-[2'-(4'-fluorophenyl)-2'-oxoethyl]-3-oxobutanoate **245a** (1 g, 3.76 mmol), aniline (0.42 ml) and *p*-TsOH (0.1 g, 0.53 mmol) in toluene (53 ml) was refluxed for 20 h. After the reaction was complete, the solvent was evaporated under reduced pressure and the crude residue was diluted with water (20 ml) then extracted with diethyl ether (2 × 20 ml) and the combined organics dried over MgSO₄. After filtration, the solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica, eluting with petroleum ether (60-80 °C): ethyl acetate (70:30) to give ester **246a** (0.95 g, 78 %) as a white solid; mp 95-96 °C (lit.⁵ mp 99-100 °C); $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 1686 (C=O); ¹H NMR (300 MHz, CDCl₃) δ_{H} 1.29 (3H, t, *J* = 7.2 Hz, OCH₂CH₃), 2.32 (3H, s, CH₃), 4.25 (2H, q, *J* = 7.2 Hz, OCH₂CH₃), 6.68 (1H, s, H-4), 6.75 (2H, t, *J* = 8.7 Hz, ArH), 6.93 (2H, m, ArH), 7.04 (2H, m, ArH), 7.30 (3H, m, ArH); ¹³C NMR (75 MHz, CDCl₃) δ_{C} 12.5 (CH₃), 14.6 (OCH₂CH₃), 59.6 (CH₂), 109.9 (CH, C-4), 112.9 (quat., C-3), 115.0 (2 × CH, C-3',5'), d, *J* = 21.4 Hz), 128.4 (CH), 128.5 (CH), 128.6 (quat., C-1''), 129.2 (CH),

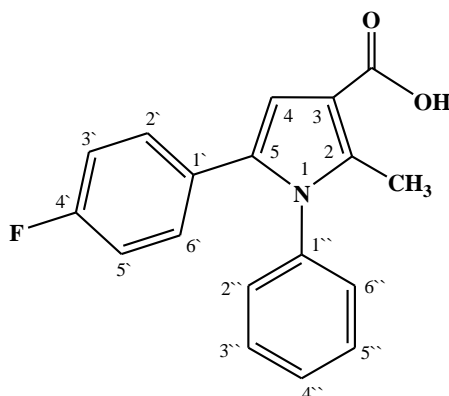
129.9 ($2 \times \text{CH}$, C-2'',6'', d, $J = 7.95$ Hz), 132.9 (quat., C-5), 137.9 (quat., C-1'), 161.6 (quat., C-4', d, $J = 245.0$ Hz), 165.5 (C=O); m/z Found: MH^+ , 324. Calc. for $\text{C}_{20}\text{H}_{19}\text{FNO}_2$: MH^+ , 324.

4.30 Ethyl 5-(4'-chlorophenyl)-2-methyl-1-phenyl-1H-pyrrole-3-carboxylate

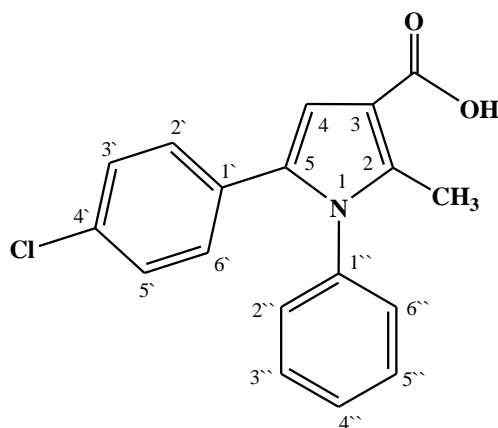
246b⁵



This pyrrole was prepared, as described above, from 1,4-diketone **245b** and aniline, to give a white solid **246b** (0.89 g, 75 %); mp 98-99 °C (lit.⁵ 103-105 °C); $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 1705 (C=O); ^1H NMR (300 MHz, CDCl_3) δ_{H} 1.30 (3H, t, $J = 7.2$ Hz, OCH_2CH_3), 2.32 (3H, s, CH_3), 4.26 (2H, q, $J = 7.2$ Hz, OCH_2CH_3), 6.72 (1H, s, H-4), 6.89 (2H, d, $J = 8.4$ Hz, ArH), 7.04 (4H, m, ArH), 7.32 (3H, m, ArH); ^{13}C NMR (75 MHz, CDCl_3) δ_{C} 12.5 (CH_3), 14.6 (CH_3), 59.6 (CH_2), 110.4 (CH, C-4), 113.1 (quat.), 128.2 (CH), 128.5 (CH), 129.2 (CH), 129.3 (CH), 130.9 (quat.), 132.5 (quat.), 132.7 (quat.), 137.9 (quat.), 138.4 (quat.), 165.4 (C=O); m/z Found: MH^+ , 340. Calc. for $\text{C}_{20}\text{H}_{20}^{35}\text{ClNO}_2$: MH^+ , 340.

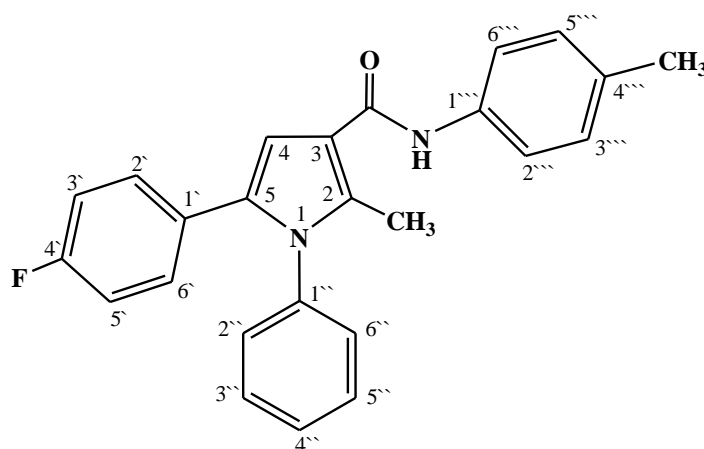
4.31 5-(4'-Fluorophenyl)-2-methyl-1-phenyl-1*H*-pyrrole-3-carboxylic acid **247a**⁵

To a solution of ethyl 5-(4'-fluorophenyl)-2-methyl-1-phenyl-1*H*-pyrrole-3-carboxylate **246a** (0.8 g, 2.48 mmol) in EtOH (19 ml) was added KOH (0.7 g) in water (4 ml) and the solution was refluxed for 2 h. After the reaction was complete, the solvent was evaporated under reduced pressure then the crude residue was diluted with 1M aq. HCl (15 ml), extracted with DCM (2 × 20 ml) and the combined organics dried over MgSO₄. After filtration, the solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica, eluting with petroleum ether (60-80 °C): ethyl acetate (70:30) to give acid **247a** (0.56 g, 77 %) as a white solid; mp 53-54 °C (lit.⁵ 57-59 °C); $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 1654 (C=O); ¹H NMR (300 MHz, DMSO-*d*₆) δ_{H} 2.34 (3H, s, CH₃), 6.70 (1H, s, H-4), 7.08 (4H, m, ArH), 7.29 (2H, d, *J* = 7.5 Hz, ArH), 7.51 (3H, m, ArH), 11.96 (1H, s, OH); ¹³C NMR (75 MHz, DMSO-*d*₆) δ_{C} 12.6 (CH₃), 110.4 (CH, C-4), 113.3 (quat., C-3), 115.6 (CH, C-3',5', d, *J* = 21.3 Hz), 128.9 (CH), 129.0 (CH), 129.1 (quat., C-1''), 129.9 (CH), 130.3 (CH, C-2',6', d, *J* = 8.1 Hz), 132.6 (quat., C-5), 137.8 (quat., C-1', d, *J* = 1.4 Hz), 161.3 (quat., C-4', d, *J* = 243 Hz), 166.5 (C=O); *m/z* Found: MH⁺, 296. Calc. for C₁₈H₁₅FNO₂: MH⁺, 296.

4.32 5-(4'-Chlorophenyl)-2-methyl-1-phenyl-1*H*-pyrrole-3-carboxylic acid **247b**⁵

This pyrrole was prepared, as described above, to give a white solid **247b** (0.22 g, 80 %); mp 249-250 °C (lit.⁵ mp 249-250 °C); $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 1657 (C=O); $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$) δ_{H} 2.34 (3H, s, CH_3), 6.76 (1H, s, H-4), 7.08 (2H, d, $J = 8.4$ Hz, ArH), 7.28 (4H, m, ArH), 7.51 (3H, m, ArH), 11.99 (1H, s, OH); $^{13}\text{C NMR}$ (75 MHz, $\text{DMSO-}d_6$) δ_{C} 12.6 (CH_3), 110.9 (CH), 113.5 (quat.), 128.6 (CH), 128.9 (CH), 129.1 (CH), 129.8 (CH), 129.9 (CH), 131.4 (quat.), 131.7 (quat.), 132.7 (quat.), 137.8 (quat.), 138.2 (quat.), 166.4 (C=O); m/z Found: $[\text{M-H}^+]$, 310. Calc. for $\text{C}_{18}\text{H}_{13}^{35}\text{ClNO}_2$: $[\text{M-H}^+]$, 310.

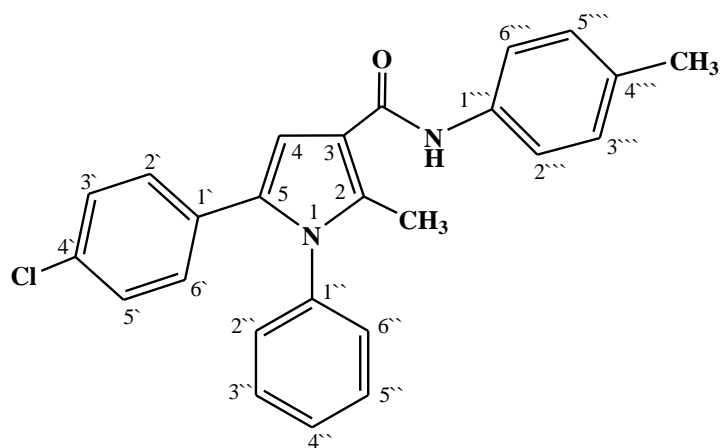
4.33 *N*-(4'''-Methylphenyl)-5-(4'-fluorophenyl)-2-methyl-1-phenyl-1*H*-pyrrole-3-carboxamide **249a**



A solution of 5-(4'-fluorophenyl)-2-methyl-1-phenyl-1*H*-pyrrole-3-carboxylic acid **247a** (0.4 g, 1.36 mmol) and SOCl_2 (0.47 ml) in toluene (7.5 ml) was refluxed for 4 h and, after evaporation of the solvent, the crude mixture was dissolved in DCM (8 ml) and a solution of *p*-toluidine (0.26 g, 2.43 mmol) and TEA (0.2 ml) in DCM (1.5 ml) was added dropwise at 0 °C. The mixture was stirred for 2 h at room temperature then washed with 5 % aq. HCl (12 ml) and 5 % aq. NaOH (12 ml). The organic layer was dried over MgSO_4 and, after filtration, the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica, eluting with petroleum ether (60-80°C): ethyl acetate (70:30) to give a yellow solid **249a** (0.46 g, 88 %); mp 205-206 °C; [Found: C, 76.8; H, 5.4; N, 7.0. $\text{C}_{25}\text{H}_{21}\text{ON}_2\text{F}$ requires C, 76.5; H, 5.7; N, 7.0 %]; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 3281 (NH), 1633 (C=O); ^1H NMR (300 MHz, CDCl_3) δ_{H} 2.26 (3H, s, C-2- CH_3), 2.37 (3H, s, CH_3), 6.45 (1H, s, H-4), 6.78 (2H, t, $J = 8.7$ Hz ArH), 6.94 (2H, m, ArH), 7.06 (4H, m, ArH), 7.32 (3H, m, ArH), 7.45 (2H, d, $J = 8.4$ Hz, ArH), 7.49 (1H, br s, NH); ^{13}C NMR (75 MHz, CDCl_3) δ_{C} 12.5 (CH_3), 20.9 (C-2- CH_3), 106.7 (CH, C-4), 115.2 (CH, C-3',5', d, $J = 22.5$ Hz), 115.8 (quat., C-3), 120.1 (CH), 128.4 (CH), 128.5 (CH), 129.3 (CH), 129.5

(CH), 129.9 (CH, C-2',6', d, $J = 7.9$ Hz), 133.3 (quat., C-1', d, $J = 3.1$ Hz), 135.9 (quat.), 136.7 (quat.), 137.8 (quat.), 161.7 (quat., C-4', d, $J = 245.5$ Hz), 163.9 (C=O).

4.34 *N*-(4'''-Methylphenyl)-5-(4'-chlorophenyl)-2-methyl-1-phenyl-1*H*-pyrrole-3-carboxamide **249b**



This pyrrole was prepared, as described above, from 5-(4'-chlorophenyl)-2-methyl-1-phenyl-1*H*-pyrrole-3-carboxylic acid, to give a white solid **249b** (0.35 g, 91 %); mp 214-215 °C; [Found: C, 74.4; H, 5.3; N, 6.9. C₂₅H₂₁ON₂Cl requires C, 74.9; H, 5.3; N, 6.9 %]; $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$ 3352 (NH), 1704 (C=O); ¹H NMR (300 MHz, CDCl₃) δ_{H} 2.25 (3H, s, C-2-CH₃), 2.37 (3H, s, CH₃), 6.49 (1H, s, H-4), 6.89 (2H, d, $J = 8.7$ Hz, ArH), 7.06 (6H, m, ArH), 7.34 (3H, m, ArH), 7.43 (2H, d, $J = 8.4$ Hz, ArH), 7.52 (1H, br s, NH); ¹³C NMR (75 MHz, CDCl₃) δ_{C} 12.5 (CH₃), 20.9 (C-2-CH₃), 107.1 (CH, C-4), 116.1 (quat.), 120.2 (CH), 128.4 (CH), 128.5 (CH), 129.3 (CH), 129.4 (CH), 129.5 (CH), 130.8 (quat.), 132.6 (quat.), 132.8 (quat.), 133.5 (quat.), 135.9 (quat.), 137.2 (quat.), 137.8 (quat.), 163.8 (C=O).

4.35 References

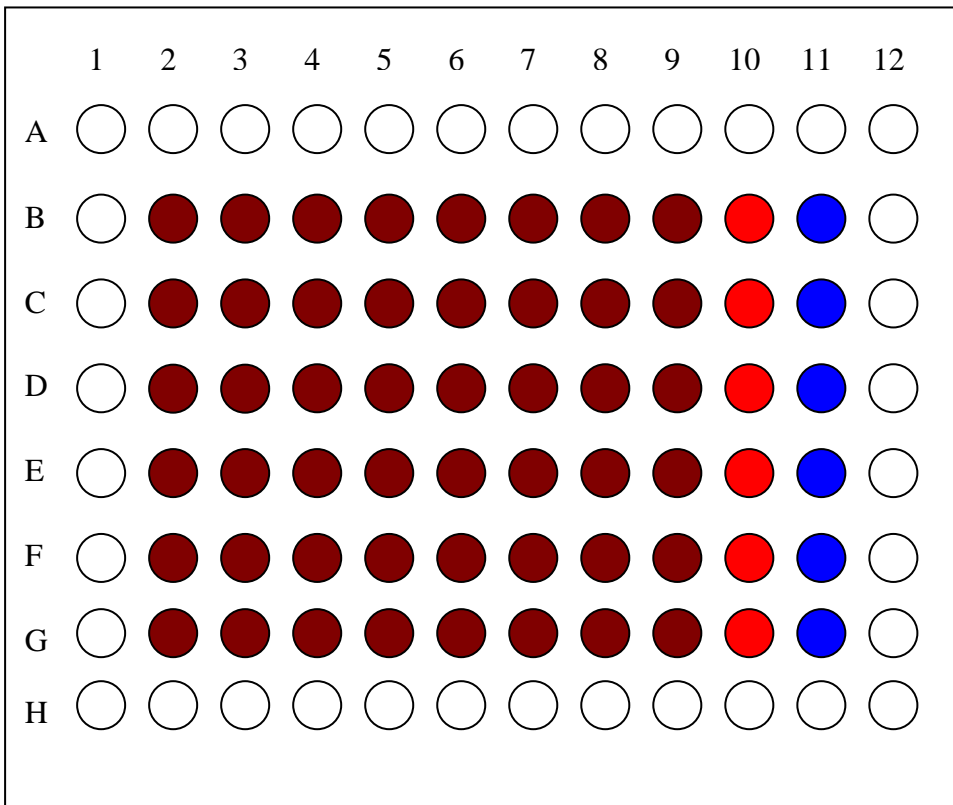
1. Balasundaram, B.; Venugopal, M.; Perumal, P. T.; *Tetrahedron Lett.*, **1993**, *34*, 4249.
2. Zaytsev, A. V.; Anderson, R. J.; Meth-Cohn, O.; Groundwater, P. W.; *Tetrahedron*, **2005**, *61*, 5831.
3. Mamoru, S.; Muneji, M.; Kazuo, M.; *J. Org. Chem.*, **1980**, *39*, 1974.
4. Poretta, G. C.; Scalzo, M.; Chimenti, F.; Bolasco, A.; Biava, M.; *Farmaco*, **1987**, *42*, 629.
5. Poretta, G. C.; Cerreto, F.; Fioravanti, R.; Biava, M.; Scalzo, M.; *Farmaco*, **1989**, *44*, 1.

Appendix

APPENDIX A

The layout of a 96-well plate, used for the antiproliferative assay.

- Unused wells
- Test wells with 1×10^4 cells
- Media with cells (Media Control)
- Media without cells (Blank Control)

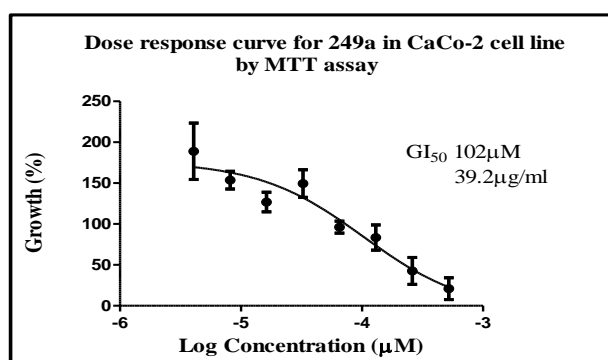


APPENDIX B

MTT 96-well Assay Calculation Forms

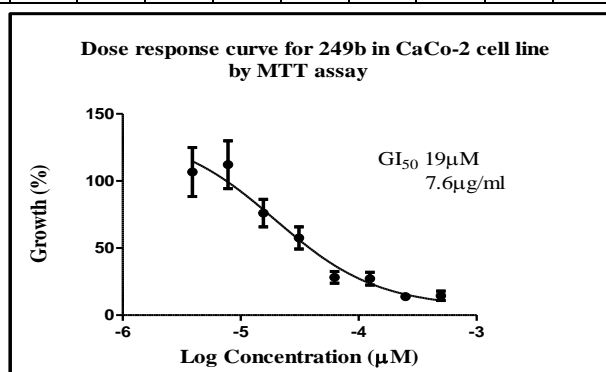
MTT 96-well Assay Calculation Form Compound 249a / CaCo-2

	Conc	Conc	Absorbances (at 595nm), n=6						Mean	SD	% SD	Corrected Ab	%Growth
Blank	µg/mL	µM	0,105	0,099	0,123	0,121	0,123	0,146	0,120	0,016	13,80	N/A	N/A
Control			0,237	0,204	0,158	0,170	0,145	0,209	0,187	0,035	18,76	0,068	100
1	200	521,00	0,136	0,128	0,156	0,160	0,102	0,120	0,134	0,022	16,46	0,014	20,94
2	100	260,50	0,200	0,151	0,133	0,141	0,122	0,143	0,148	0,027	18,31	0,029	42,61
3	50	130,25	0,195	0,161	0,146	0,210	0,155	0,189	0,176	0,012	6,88	0,057	83,50
4	25	65,13	0,169	0,189	0,172	0,198	0,183	0,196	0,185	0,012	6,56	0,065	96,06
5	12,5	32,56	0,226	0,254	0,183	0,226	0,192	0,243	0,221	0,028	12,67	0,101	149,51
6	6,25	16,28	0,222	0,187	0,208	0,193	0,187	0,235	0,205	0,020	9,71	0,086	126,85
7	3,125	8,14	0,227	0,200	0,213	0,213	0,245	0,243	0,224	0,018	8,07	0,104	153,69
8	1,5625	4,07	0,244	0,240	0,210	0,229	0,202	0,359	0,247	0,057	23,10	0,128	188,92



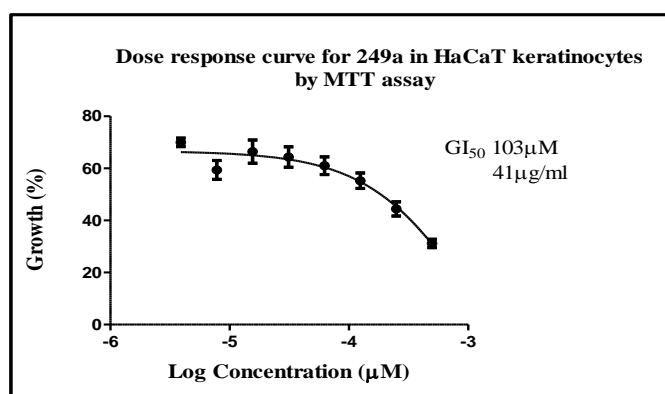
MTT 96-well Assay Calculation Form Compound 249b / CaCo-2

	Conc	Conc	Absorbances (at 595nm), n=6						Mean	SD	% SD	Corrected Ab	%Growth
Blank	µg/mL	µM	0,109	0,114	0,135	0,126	0,127	0,145	0,126	0,013	10,51	N/A	N/A
Control			0,543	0,355	0,259	0,426	0,58	0,636	0,467	0,145	31,00	0,341	100
1	200	499,00	0,217	0,147	0,186	0,195	0,161	0,144	0,175	0,029	16,61	0,049	14,39
2	100	249,50	0,155	0,189	0,164	0,191	0,162	0,176	0,173	0,015	8,64	0,047	13,75
3	50	124,75	0,180	0,194	0,190	0,213	0,278	0,256	0,219	0,036	16,68	0,093	27,17
4	25	62,38	0,216	0,209	0,201	0,246	0,178	0,281	0,222	0,036	16,43	0,096	28,14
5	12,5	31,19	0,263	0,253	0,364	0,377	0,267	0,408	0,322	0,068	21,27	0,196	57,56
6	6,25	15,59	0,262	0,365	0,422	0,502	0,433	0,325	0,385	0,085	22,20	0,259	76,02
7	3,125	7,80	0,306	0,600	0,416	0,708	0,430	0,587	0,508	0,148	29,21	0,382	112,14
8	1,5625	3,90	0,664	0,397	0,279	0,667	0,466	0,462	0,489	0,152	31,16	0,363	106,66



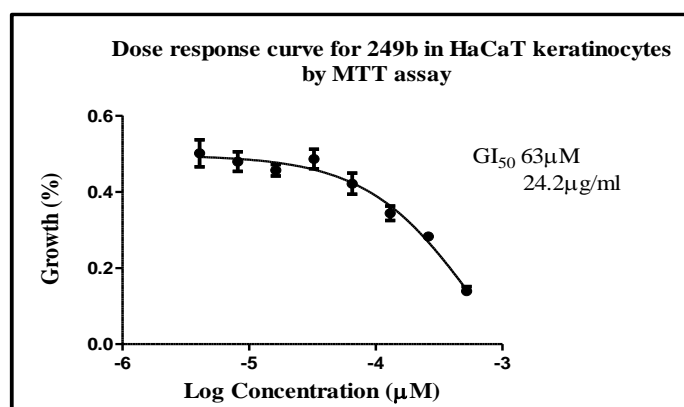
**MTT 96-well Assay Calculation Form
Compound 249a / HaCaT**

	Conc	Conc	Absorbances (at 595nm), n=6						Mean	SD	% SD	Corrected Ab	%Growth
Blank	µg/mL	µM	0,072	0,076	0,097	0,089	0,094	0,116	0,091	0,016	17,50	N/A	N/A
Control			0,655	0,5	0,579	0,533	0,561	0,539	0,561	0,053	9,48	0,471	100
1	200	499,00	0,263	0,229	0,209	0,237	0,249	0,237	0,237	0,018	7,69	0,147	31,17
2	100	249,50	0,237	0,309	0,315	0,311	0,325	0,301	0,300	0,032	10,58	0,209	44,42
3	50	124,75	0,286	0,346	0,376	0,378	0,363	0,355	0,351	0,040	11,27	0,260	55,26
4	25	62,38	0,356	0,366	0,416	0,395	0,418	0,316	0,378	0,040	10,46	0,287	61,03
5	12,5	31,19	0,349	0,393	0,400	0,466	0,412	0,342	0,394	0,045	11,51	0,303	64,40
6	6,25	15,59	0,341	0,426	0,444	0,383	0,470	0,355	0,403	0,051	12,77	0,313	66,42
7	3,125	7,80	0,314	0,382	0,425	0,398	0,372	0,329	0,370	0,042	11,32	0,279	59,37
8	1,5625	3,90	0,408	0,411	0,418	0,45	0,434	0,399	0,420	0,019	4,47	0,329	70,00



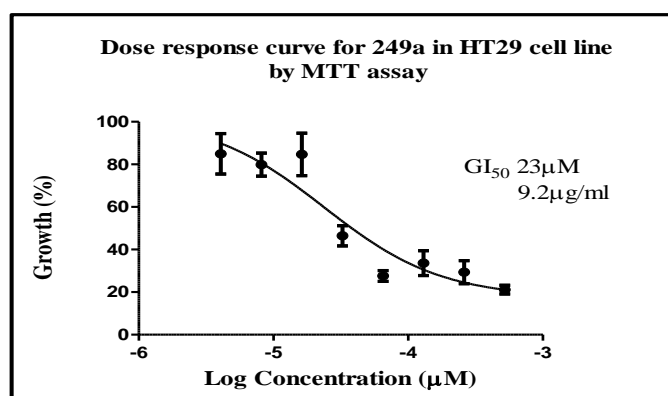
**MTT 96-well Assay Calculation Form
Compound 249b / HaCaT**

	Conc	Conc	Absorbances (at 595nm), n=6						Mean	SD	% SD	Corrected Ab	%Growth
Blank	µg/mL	µM	0,072	0,086	0,097	0,094	0,113	0,135	0,100	0,022	22,10	N/A	N/A
Control			0,461	0,507	0,532	0,515	0,665	0,508	0,531	0,070	13,10	0,432	100
1	200	521,00	0,104	0,125	0,135	0,150	0,133	0,189	0,139	0,029	20,54	0,040	9,22
2	100	260,50	0,294	0,320	0,275	0,263	0,278	0,266	0,283	0,021	7,53	0,183	42,42
3	50	130,25	0,272	0,359	0,330	0,349	0,415	0,337	0,344	0,068	19,79	0,244	56,54
4	25	65,13	0,376	0,399	0,409	0,511	0,495	0,338	0,421	0,068	16,14	0,322	74,53
5	12,5	32,56	0,576	0,417	0,475	0,532	0,496	0,422	0,486	0,062	12,77	0,387	89,58
6	6,25	16,28	0,409	0,453	0,482	0,471	0,504	0,421	0,457	0,036	7,98	0,357	82,71
7	3,125	8,14	0,397	0,584	0,488	0,502	0,457	0,449	0,480	0,063	13,10	0,380	88,00
8	1,5625	4,07	0,562	0,467	0,439	0,488	0,644	0,408	0,501	0,087	17,39	0,402	93,05



**MTT 96-well Assay Calculation Form
Compound 249a / HT29**

	Conc	Conc	Absorbances (at 595nm), n=6						Mean	SD	% SD	Corrected Ab	%Growth
Blank	µg/mL	µM	0,099	0,109	0,109	0,129	0,116	0,145	0,118	0,017	14,08	N/A	N/A
Control			0,357	0,374	0,416	0,559	0,595	0,58	0,480	0,109	22,80	0,362	100
1	200	521,00	0,172	0,202	0,183	0,181	0,215	0,214	0,195	0,018	9,42	0,077	21,16
2	100	260,50	0,189	0,170	0,203	0,218	0,281	0,285	0,224	0,048	21,46	0,107	29,39
3	50	130,25	0,203	0,185	0,204	0,264	0,260	0,322	0,240	0,022	9,38	0,122	33,62
4	25	65,13	0,210	0,212	0,237	0,183	0,247	0,218	0,218	0,022	10,32	0,100	27,60
5	12,5	32,56	0,271	0,214	0,301	0,305	0,340	0,286	0,286	0,042	14,75	0,168	46,46
6	6,25	16,28	0,329	0,342	0,574	0,448	0,425	0,43	0,425	0,088	20,76	0,307	84,68
7	3,125	8,14	0,372	0,447	0,379	0,357	0,481	0,407	0,407	0,048	11,82	0,289	79,85
8	1,5625	4,07	0,315	0,493	0,549	0,424	0,399	0,374	0,426	0,084	19,76	0,308	84,96



**MTT 96-well Assay Calculation Form
Compound 249b / HT29**

	Conc	Conc	Absorbances (at 595nm), n=6						Mean	SD	% SD	Corrected Ab	%Growth
Blank	µg/mL	µM	0,111	0,111	0,138	0,118	0,168	0,217	0,144	0,042	29,16	N/A	N/A
Control			0,222	0,209	0,271	0,246	0,327	0,305	0,263	0,046	17,66	0,120	100
1	200	499,00	0,178	0,246	0,221	0,183	0,249	0,207	0,214	0,030	14,18	0,070	58,72
2	100	249,50	0,224	0,219	0,213	0,250	0,293	0,259	0,243	0,030	12,54	0,099	82,98
3	50	124,75	0,225	0,214	0,241	0,271	0,293	0,287	0,255	0,016	6,18	0,111	93,17
4	25	62,38	0,201	0,209	0,224	0,232	0,245	0,221	0,222	0,016	7,11	0,078	65,41
5	12,5	31,19	0,230	0,187	0,211	0,403	0,253	0,297	0,264	0,078	29,60	0,120	100,14
6	6,25	15,59	0,200	0,235	0,288	0,357	0,281	0,308	0,278	0,055	19,81	0,134	112,41
7	3,125	7,80	0,233	0,285	0,252	0,392	0,322	0,319	0,301	0,057	19,02	0,157	131,10
8	1,5625	3,90	0,228	0,266	0,352	0,354	0,351	0,345	0,316	0,055	17,36	0,172	144,07

