Department of Chemistry

Development of Fungal Sterol Biosynthesis Inhibitors as New Drug Leads for the Treatment of Chagas Disease

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgement has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

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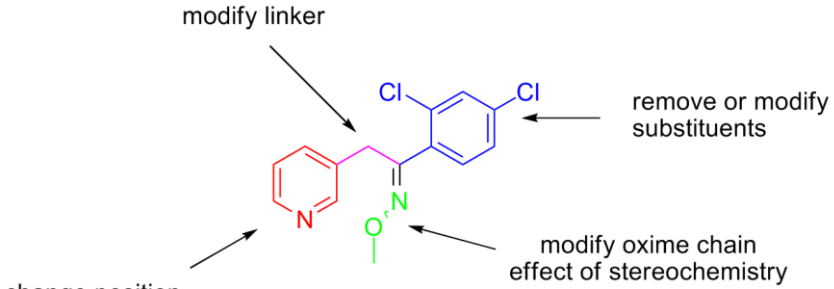
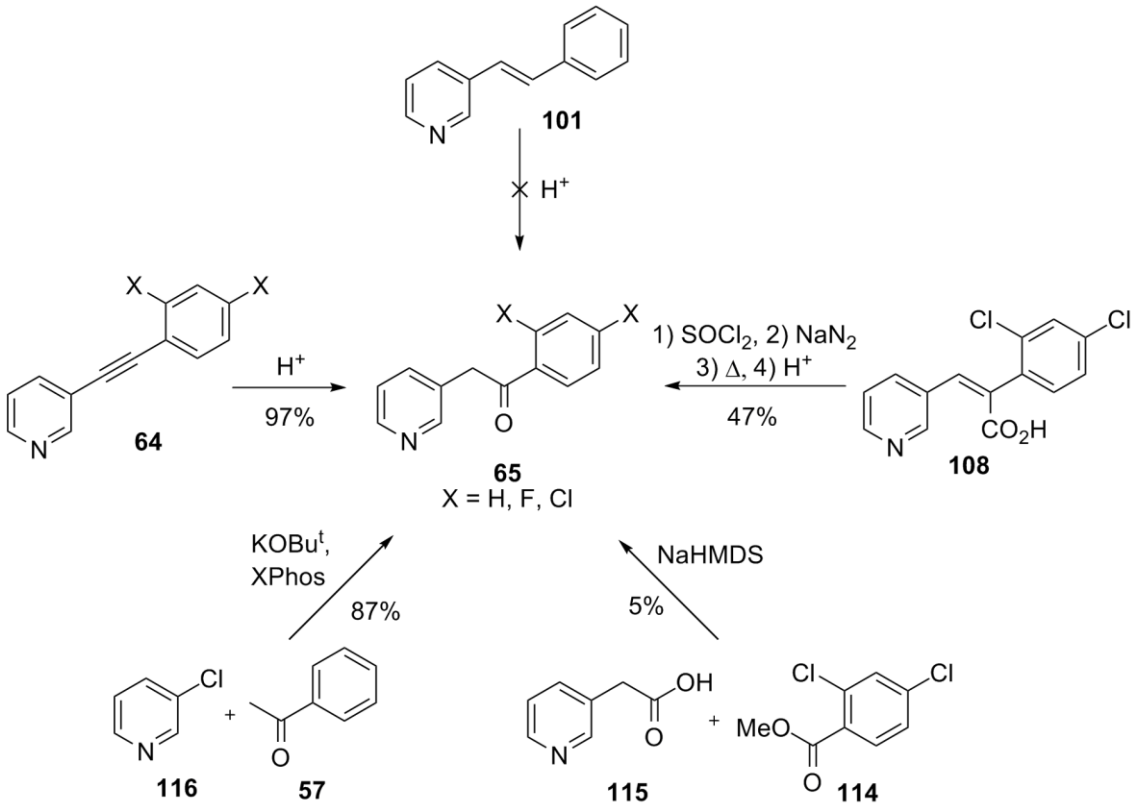
Abstract

Chagas disease is a neglected disease endemic in Central and South America but is becoming more widespread. The current treatments for Chagas disease, nifurtimox and benznidazole, were developed over 40 years ago and have detrimental side effects which inhibit the treatment process. In search for a new treatment for Chagas disease, an *in vitro* screening of commercially available fungicides and pesticides against *T. cruzi* was conducted and identified pyrifenox as having good inhibition against the parasites. This research focussed on the synthesis of pyrifenox analogues to improve the inhibition against the parasite.

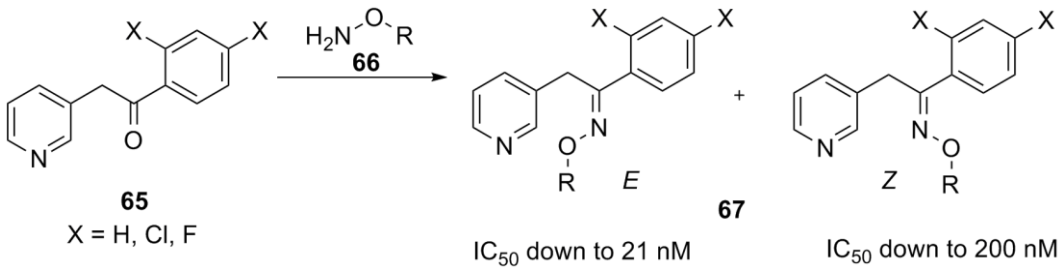


42
Pyrifenox
IC_{50} 290 nM

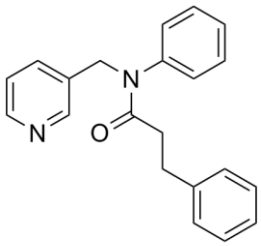
As there were limited routes to synthesise pyrifenox, a new modular synthetic method was developed. A variety of methods were used to obtain the ketone intermediate **65**. The hydration of an alkyne proved to be the most effective method using an acid and a gold catalyst. The alkene **101** was inactive towards hydration and alkylation reactions. The carboxylic acid **108** could be converted to the ketone **65** via a Curtius rearrangement in 47% yield but was rapidly oxidised to a diketone. A condensation reaction of a carboxylic acid **114** and methyl ester **115** produced the ketone **65** in low yield. The final method was a palladium catalysed cross-coupling reaction which produced the unsubstituted ketone in high yields but no reaction occurred when using halogenated acetophenones.



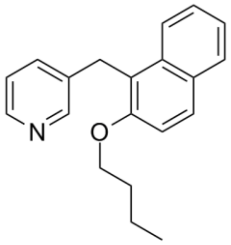
A variety of oximes were synthesised from the ketone **65** to produce 24 different analogues that were tested against *T. cruzi*. The substituents on the phenyl ring appeared to be important with the most active analogue containing chlorine substituents on the phenyl ring. The *E* oxime was also shown to be more potent than the *Z* oxime. Extension of the methyl oxime of pyrifenox to larger alkyl chains gave more potent analogues, with the benzyl oxime being the most potent.



The backbone of pyrifenox was modified to examine the contribution of structural rigidity to the biological activity of the compound. The stereochemistry of the oxime was removed by incorporating a nitrogen atom between the two rings creating a more flexible compound. The amide **168** showed decreased anti-trypanosomal activity. The structural rigidity of pyrifenox was increased. This compound was based on a naphthol system to produce a compound that mimicked the preferred *E* isomer. Unfortunately the increased rigidity in this compound decreased the anti-trypanosomal activity.

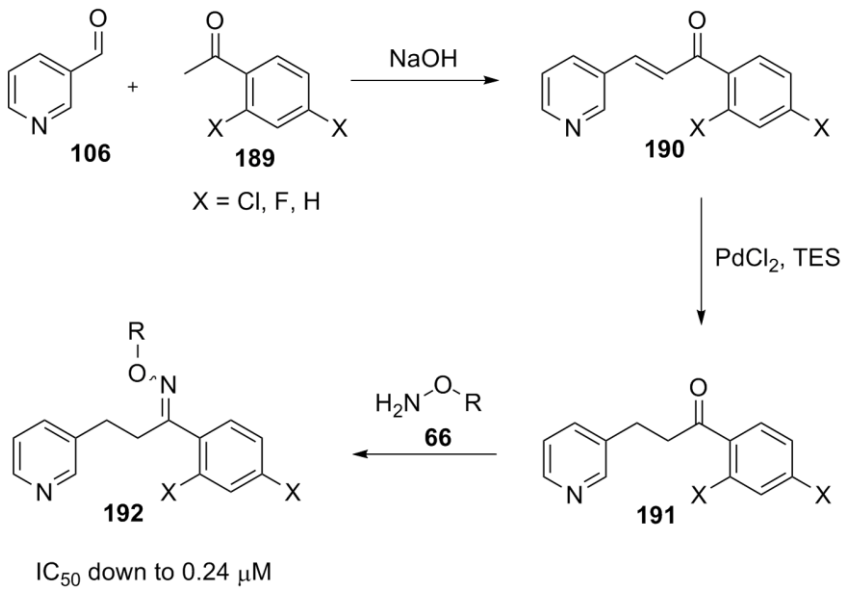


168
IC_{50} 5.75 μM

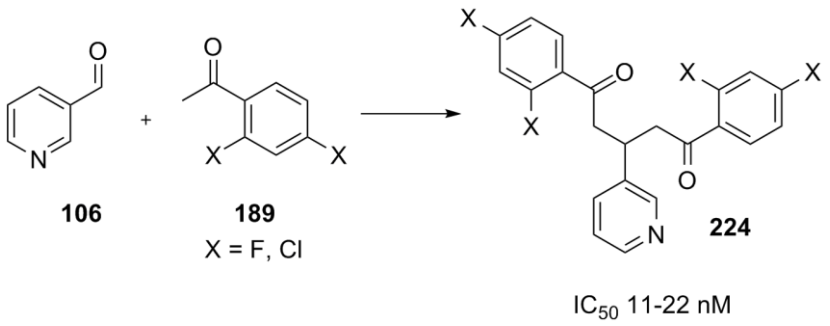


178
IC_{50} 6.98 μM

The linker between the pyridyl ring and the benzyl ring was also investigated. The longer linker was based on a hydrogenated chalcone. The longer linker series showed a diminished activity compared to the pyrifenox derivatives, however the substituents on the phenyl ring and the length of the oxime showed a similar trend to the first series of pyrifenox analogues. The chloro substituents on the phenyl ring appeared to be the most potent and a bulkier oxime was shown to improve the biological activity to 0.24 μM.



A side product of the chalcone synthesis was shown to have excellent inhibition against *T. cruzi*. The additional substituents on the phenyl ring were shown to be the most effective with the difluoro compound exhibiting the most promising results. This series of compounds provide a new lead as a drug target for the treatment of Chagas disease.



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Firstly, I would like to thank my Curtin supervisor, Dr Alan Payne. I will always be grateful to Alan for his constant advice, patience and believing in me before I had even finished honours. I would not have even thought of doing a PhD without his support and optimism.

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Finally, thanks to my family for their patience and support when I decided to go back to studying again. I couldn’t have done this without you.

Contents

Abbreviation

AUD	Australian dollars
Bn	benzyl
CPI	cysteine protease inhibitors
CSA	camphorsulfonic acid
CYP3A4	cytochrome P450 3A4
CYP51	sterol 14α-demethylase
DCE	1,2-dichloroethane
DCM	dichloromethane
DIPEA	diisopropylethylamine
DMAP	dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DND*i*	Drugs for Neglected Diseases *initiative*
e^-	electron
EC_{50}	half maximal effective concentration
ED_{50}	median effective dose
Et	ethyl
ether	diethyl ether
eq	equivalence
FDA	food and drug administration
h	hour(s)
Hz	hertz
IC_{50}	half maximal inhibitory concentration
IR	infra-red
J	coupling constant
*m*CPBA	*m*-chloroperbenzoic acid
Me	methyl
mp	melting point
NaHMDS	sodium bis(trimethylsilyl) amide
nM	nano molar
NOE	nuclear Overhauser effect
NTR	nitroreductases
o/n	overnight
PFT	protein farnesyltransferase
ppm	parts per million
PTC	phase transfer catalyst
qd	quaque die (one per day)
rt	room temperature
sec	second
SI	selectivity index
SM	starting material
T. cruzi	*Trypanosoma cruzi*
TES	triethylsilane
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography

1 Introduction

Diseases that affect poverty-stricken communities are often neglected by industrial and academic research as they are not considered of commercial importance.[1] Most of these neglected diseases are found in areas with unsafe drinking water, poor sanitation, substandard housing, and little or no access to healthcare. As these diseases mainly occur in poor or rural areas, there is a lack of government commitment for drug development even though there are high rates of mortality and morbidity caused by these diseases.[2–5] Recently, research has been sponsored by not-for-profit organisations (eg. World Health Organisation and Drugs for the Neglected Diseases *initiative*) to develop new drugs and vaccines, diagnostics, prevention and provide access to affordable medications. One major neglected disease is *American trypanosomiasis*, more commonly known as Chagas disease.[6,7]

1.1 Chagas Disease

Chagas disease is caused by a parasite, *Trypanosoma cruzi*, which affects approximately 16 to 18 million people[8] and kills greater than 15,000 people each year.[2,9] *T. cruzi* is endemic in Central and South America[10] affecting 21 countries[1]

but is becoming more widespread due to international travel.[11] Chagas disease is most commonly transmitted through the faeces of triatomine insects, also known as kissing bugs (Figure 1), which carry the parasite.[12] The parasite enters the body of the mammalian host through broken skin from the insect bite or other cuts and abrasions, and soft skin around the eyes and mouth from the faeces of infected insects.[8,9] Other modes of infection include blood transfusions, organ transplantation, perinatal infection and importation of infected livestock.[8] Accurate assays have been developed to help screen donated blood for the *T. cruzi* infection but organ transplantation still remains an issue.

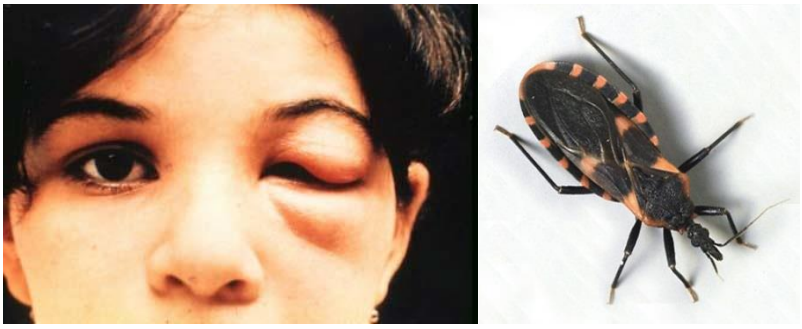


Figure 1. Swelling at the site of infection (left) and the triatomine insect that carries the parasite (right).[13]

The life cycle of *T. cruzi* is complex and involves two intermediate hosts and three morphological forms (Figure 2).[14] The triatomine bug is infected with the parasite when it takes a blood meal from mammals that are infected with the non-dividing bloodstream trypomastigotes. The insect appears to be unaffected by the parasite.[15] The trypomastigotes then transform into epimastigotes in the gut of the insect.[16] The epimastigotes form replicates in the midgut of the bug and develop into nonreplicative metacyclic trypomastigotes.[8,17] *T. cruzi* is then transmitted to mammals when the faeces of the infected insect come into contact with broken skin.[16] Once the trypomastigotes enter the host cells, they transform into amastigotes and multiply.[16] The cell then bursts, releasing the trypomastigotes to infect other cells and enter the blood stream.[8] The life cycle is complete when a triatomine insect takes another blood meal of the infected blood.[16]

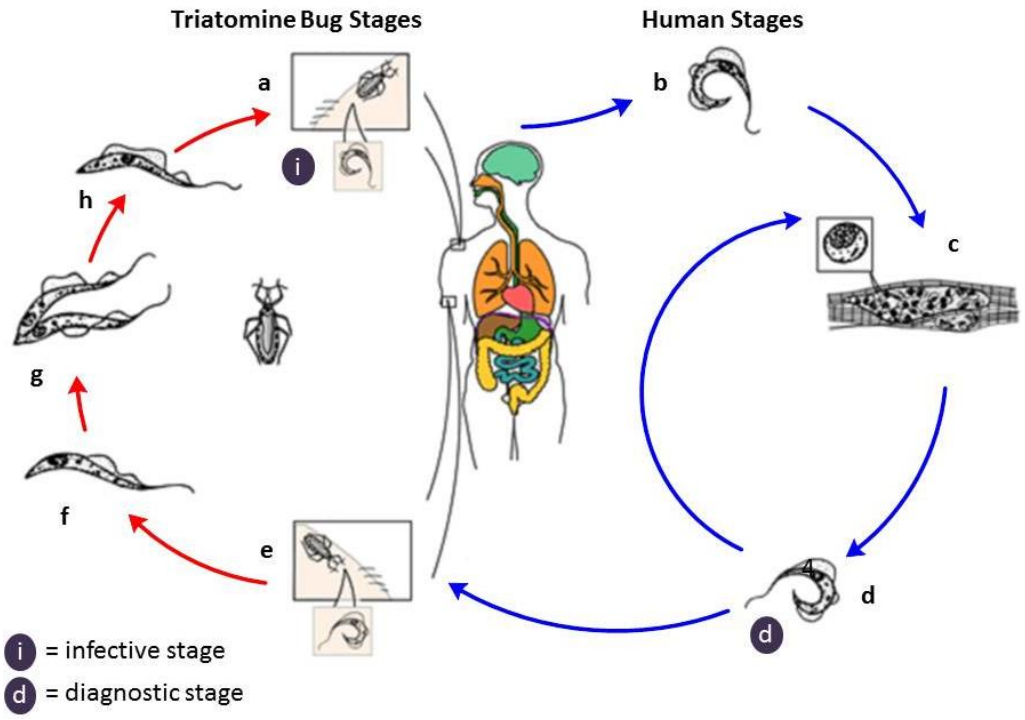


Figure 2. Representation of the lifecycle of *T. cruzi*. a) Triatomine bug takes a blood meal. b) Metacyclic trypomastigotes penetrate cells and transform into amastigotes. c) Amastigotes mutiply in cells. d) Intracellular amastigotes transform into trypomastigotes and enter bloodstream. e) Triatomine bug takes a blood meal. f) Epimastigotes in midgut. g) Multiply in midgut. h) Metacyclic trypomastigotes in hindgut. Adapted from Clayton.[9]

There are three phases of infection: acute, indeterminate and chronic phases.[2,10] The acute phase normally occurs in children with 75% of cases in patients less than 15 years old.[18] The acute phase begins one to two weeks after infection and usually lasts six to eight weeks.[19] Many people experience no symptoms in the acute phase or the symptoms are very mild and non-specific.[17,20] Therefore they may not realise they have been infected and they go untreated.[9] Acute symptoms include local swelling of the site of infection (Figure 1), fever, anorexia and myocarditis with diagnosis being made by detecting parasites in the blood by microscopy (Figure 3).[9] The mortality rate in the acute phase is approximately 10%, with the majority being children.[2,8] After the acute phase, if untreated, patients appear to be immune with no symptoms observed but they will still remain infected. This is known as the indeterminate stage and can last 10 to 30 years before chronic symptoms appear. Chronic symptoms include cardiac abnormalities, digestive tract problems and neurological problems which tend to be fatal.[9] Mortality in the chronic stage is mainly due to cardiomyopathy.[2] One in three carriers develop chronic symptoms[21] and it is often at

this stage that they are first diagnosed with the infection.[9] Although these are typical symptoms of Chagas disease, the pathology is still not fully understood and can vary between regions.[22]

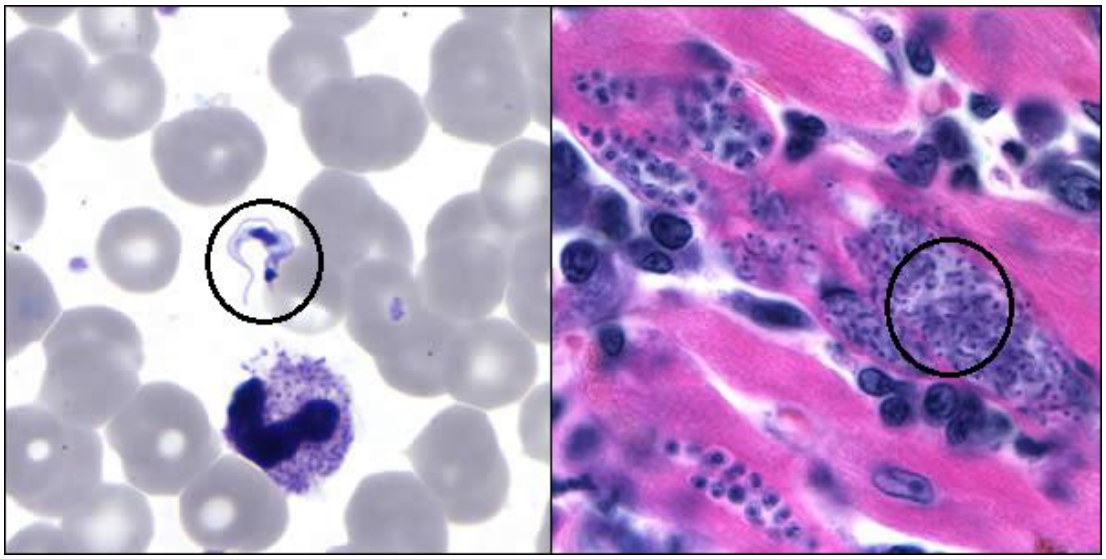


Figure 3. *T. cruzi* trypomastigote in a thin blood smear stained with Giemsa (left). *T. cruzi* amastigotes in heart tissue stained with hematoxylin and eosin (right).[23]

1.2 Current therapies

There are currently two drugs available to treat Chagas disease: nifurtimox **1** and benznidazole **2** (Figure 4).[20] In the acute phase both drugs reduce severity of the symptoms and are thought to cure 60 to 80% of patients treated.[19] Both compounds function as prodrugs and undergo enzyme-mediated activation within the cell.[1,2,24] These reactions are catalysed by nitroreductases (NTRs) and are divided into two groups based on oxygen sensitivity (Figure 5).[1,2,8] Type 1 NTRs are oxygen insensitive and function through a two electron reduction of the nitro group to generate the nitroso intermediate.[25] The nitroso intermediate rapidly undergoes reduction to a hydroxylamine derivative. This leads to compounds that promote DNA damage. Type 2 NTRs are oxygen-sensitive and mediate one electron reduction of the nitro group generating an unstable nitro radical. In the presence of oxygen, the nitro radical undergoes futile cycling to produce superoxides and regeneration of the parent nitro compound.

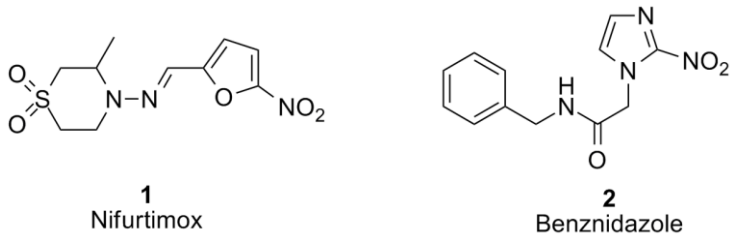


Figure 4. Chemical structures for Nifurtimox and Benznidazole.

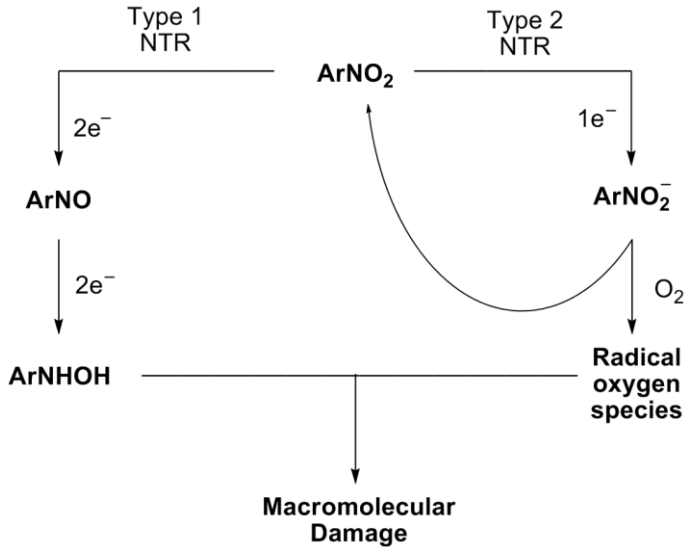


Figure 5. Activation of antichagasic nitroheterocyclic drugs. Adapted from Salas *et al*.[2]

Nifurtimox **1** was the first drug commercialised to treat Chagas disease and was released under the name Lampit® in 1972.[1] The production of nifurtimox was discontinued in 1997[1] and the treatment is no longer used due to serious gastrointestinal tract and central nervous system side effects, and its limited efficacy and genotoxicity.[24] Other side effects include seizures and other nervous system disorders, anorexia, weight loss, digestive problems and skin rashes.[2,3,8,25] The more serious effects are usually resolved when the treatment is stopped. Children appear to tolerate the treatment better than adults, however there is no known correct dosage for children.[19] Nifurtimox contains a nitrofuran ring that inhibits the pyruvic acid synthesis in *T. cruzi* during glycolysis. It appears to be catalysed by both types of the NTRs (Figure 6).[25] In type 1, the nitrofuran undergoes a two electron reduction to the nitroso **4** intermediate which readily undergoes another two electron reduction to a hydroxylamine derivative **5**. The hydroxylamine **5** can then be processed further to

generate the amine **7** which is inert or the nitronium ions **6** that promote DNA breakage.[25] Fragmentation of the furan ring may also occur giving open chain nitriles **9** that are toxic.[25] The nitrofuran can also react with oxygen via type 2 NTRs to form the superoxide anion and the nitro anion radical **10**.[8]

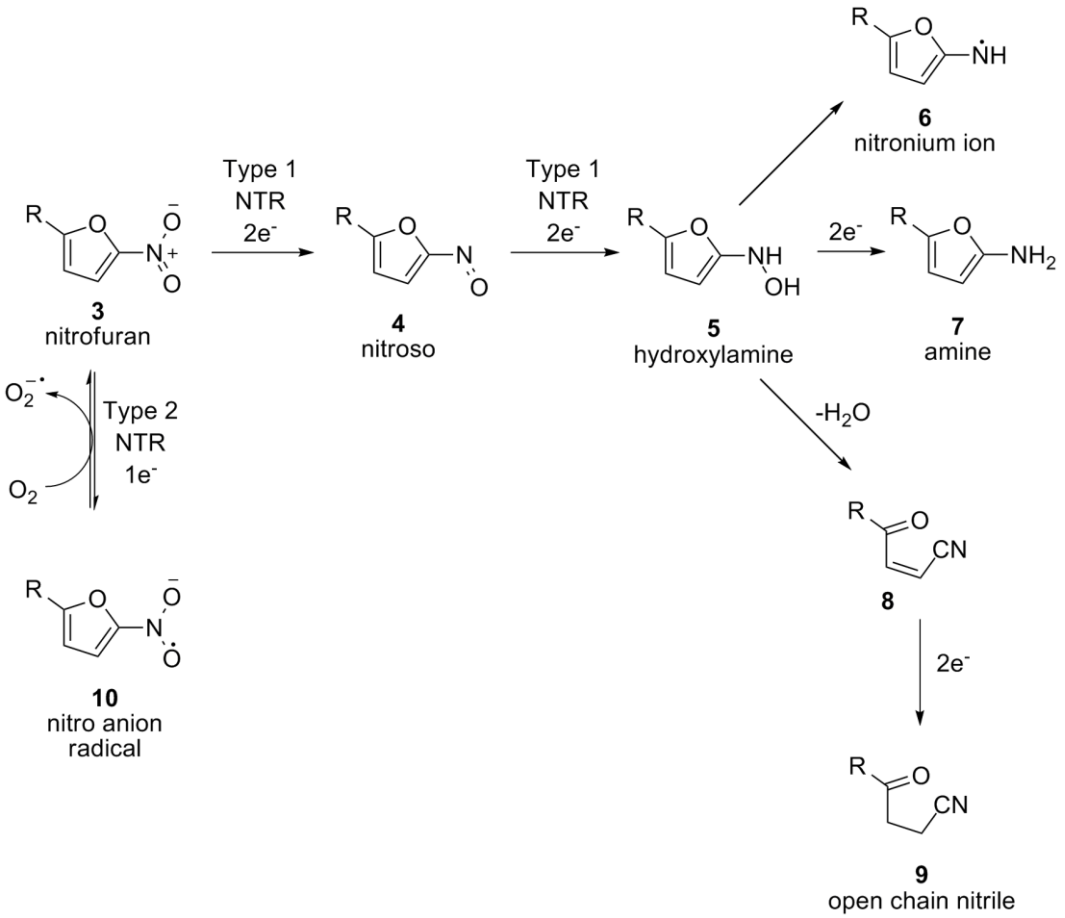


Figure 6. Reactions of the nifurtimox nitrofuran ring are catalysed by type 1 and type 2 NTRs.

Benznidazole **2**, also known commercially as Rochagan®, was discovered in 1974 but was not introduced for clinical use until 1978.[1] It is a nitroimidazole derivative considered more trypanocidal than nifurtimox.[19] Benznidazole inhibits RNA synthesis and generates the accumulation of superoxides.[26,27] The reduced metabolites of benznidazole interact with the DNA of the parasite and inhibit the respiratory chain.[8] It appears to be catalysed only by type 1 NTRs (Figure 7). The nitroimidazole **11** undergoes a two electron reduction to the nitroso intermediate **12** which readily undergoes another two electron reduction to a hydroxylamine derivative **13**. The hydroxylamine metabolite can undergo rearrangement and

hydration to produce a dihydro-dihydroxyimidazole **15** that can decompose to release glyoxal **17**. Both products can interact with the biomolecules forming adducts with DNA and thiols.[24]

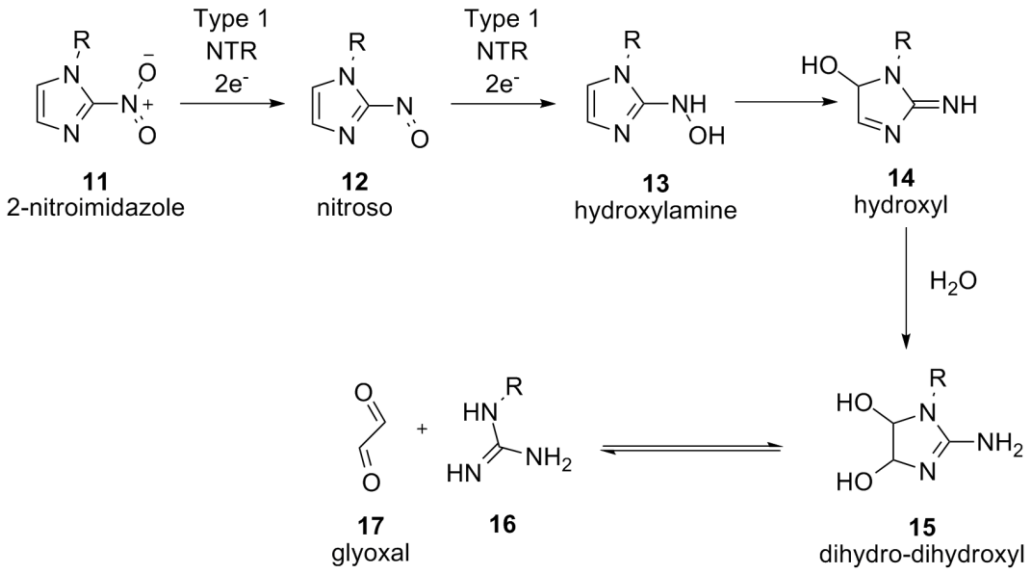


Figure 7. Reactions of the benznidazole nitrofuran ring are catalysed by type 1 NTRs.

Benznidazole is still used to treat the early acute stage, curing 16−76% of patients compared to only 8−9% of chronic patients.[28] It is thought the effectiveness of benznidazole occurs in the infected extracellular forms of *T. cruzi* in the acute phase rather than the intracellular forms in the chronic phase.[8] The side effects are severe and can lead to resistance.[19] Side effects include skin rashes, nausea and kidney and liver failure.[3,5,8] The effectiveness of the drug also varies according to geographical area.[29] Children are known to tolerate the treatment better than adults but there is no known correct dosages or formulation for children with benznidazole tablets being split by hand into half and quarters.[20] This is not ideal as there is a high risk of delivering incorrect dosage and raises concerns about safety, efficacy and decreased stability.[30] This is seen as a major issue as most patients in the acute stage are children.[20]

Both nifurtimox and benznidazole have shown positive results in treating the early acute stage but they have low effectiveness in the chronic phase of the disease[10] and resistance of different strains against the drugs has been documented.[29] They also required prolonged treatments (greater than 60 days) and the detrimental side effects often lead to abandonment of treatment. As there is limited efficacy of nifurtimox and benznidazole, undesirable side effects and drug resistances, *there is a need to develop new drugs for the treatment of Chagas disease*.

1.3 Advances in Chagas disease drug development

The current treatments for Chagas disease have problems with toxicity, efficacy, length of treatment and the administration of drugs to children. Therefore new drugs need to be developed that are less toxic but provide the same or better inhibition than benznidazole and nifurtimox. As the disease targets poorer communities, the new drug also needs to be relatively cheap to produce. The development of new drugs to treat Chagas disease has been a challenge because *T. cruzi* is an intracellular parasite. This means that the drugs are required to cross the cell membrane in order to kill the parasite.[9] Early stage research into other compound classes to treat Chagas disease might show promising activity but there is no standardised assays for the parasites used.[1] Therefore the results are not reproducible between the testing laboratories and few compounds have advanced to clinical trials. The most promising compounds have been shown to inhibit cysteine proteases and sterol biosynthesis.

1.3.1 Cysteine protease inhibitors (CPI)

The toxicity and lack of efficacy of nifurtimox and benznidazole has prompted interest into the development of a vaccine based on the cruzipain antigen (also known as cruzain). Cruzain is a cathespin of L-cysteine protease that is responsible for the proteolytic activity in all life stages of *T. cruzi*.[27,31,32] The genes that code for this protein have been cloned and expressed, and drugs have been studied to specifically target the inhibition of the CPI protease *in vitro* by blocking the growth of the cells.[27]

It has been demonstrated that drugs can block the development of cruzain and its transport of lysomes.[31,33] The lack of cruzain weakens the parasite and it is therefore unable to survive.[32] The cysteine protease is expressed on the surface of the parasite and has shown some promising results. One cruzain inhibitor that has shown the most promising results is K777 **18** (Figure 8).[33,34]

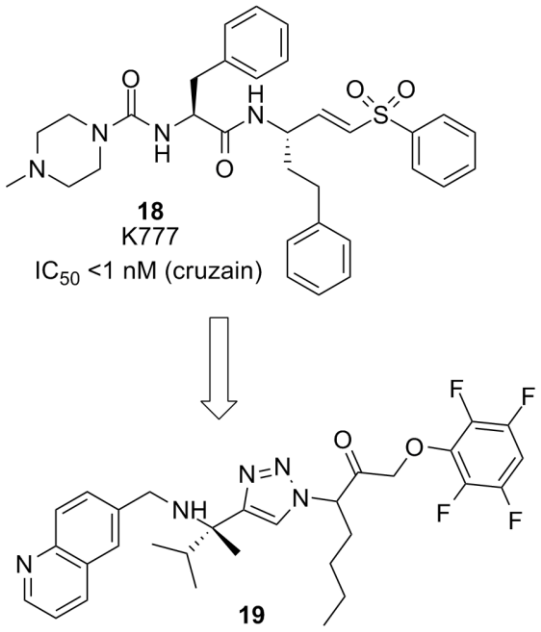


Figure 8. Structure of K777 and nonpeptidic cruzain inhibitor

K777 **18** is a peptidic vinyl sulfone protease inhibitor of cruzain that is irreversible (Figure 9).[34,35] The vinyl sulfone serves as a Michael acceptor for the nucleophilic cysteine residue within the active site of the protease. The peptidic backbone contains several hydrogen bond acceptors that interact with complimentary residues in the active site. Studies have shown that K777 **18** is effective in selectively targeting *T. cruzi* in acute and non-acute models of the infection in mice and treating cardiac damage in dogs.[31] The development of K777 **18** was halted in 2005 due to hepatotoxicity and manufacturing problems.[31] Further investigations into these problems are currently being assessed.[31]

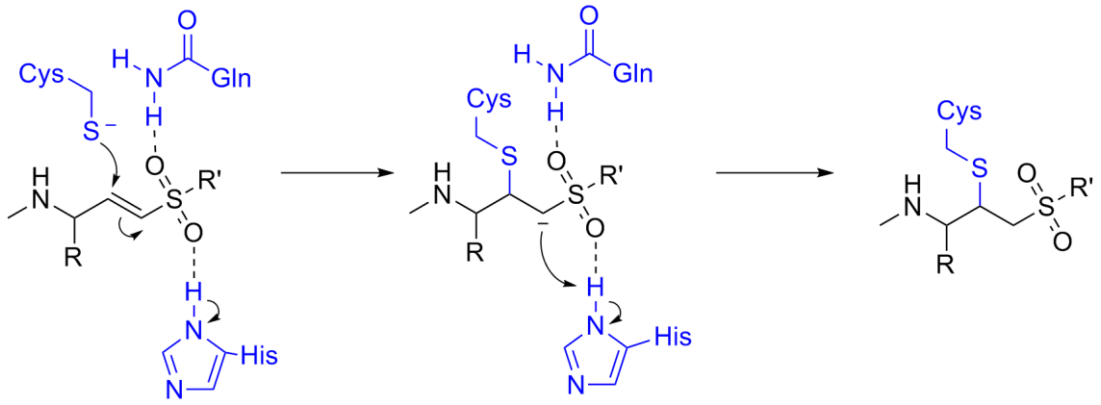


Figure 9. Mechanism of irreversible inhibition of cysteine peptidase by vinyl sulfones.[36]

Nonpeptidic analogues of K777 **18** have been investigated and a 1,2,3-triazole based tetrafluorophenoxymethyl ketone **19** (Figure 8) has shown suppressive activity in the mouse *T. cruzi* model and did not show any toxicity.[33] This analogue does not contain the vinyl sulfone and therefore the mechanism for inhibition differs from K777 **18**. The mode of action is still under investigation.

Although cruzain is an ideal target, the short half-life of the drug requires high and increasing doses which inhibits its use in clinical practice.[27] Nonpeptidic compounds may help improve pharmacological properties of cruzain inhibitors and are still under investigation.[33]

1.3.2 Sterol biosynthesis inhibitors

Sterol biosynthesis inhibitors have been shown to be some of the most promising anti-*T. cruzi* agents.[37,38] Sterols, such as cholesterol **24** in animals and ergosterol **26** in fungi, are essential structural components of cell membranes and serve as precursors for biologically active molecules.[39] In the *T. cruzi* biosynthetic pathway lanosterol **22** is converted to eburicol **25** which is the preferred substrate of sterol 14α-demethylase (CYP51) and eventually will give ergosterol **26** in fungi (Figure 10) and cholesterol **24** in humans.[39–41] The parasite has an essential requirement for ergosterol for survival and multiplying, and cannot survive solely on cholesterol from the host.[42] Ergosterol **26** differs from cholesterol **24** by the presence of a C-24

methyl group and a double bond at C-7 and C-22.[27] The enzymes in CYP51 which produce the demethylation and double bonds to form ergosterol **26** in fungi differ from those found in humans.[27] This makes the sterol biosynthesis pathway in the parasite an attractive target for drug development.[39] Several steps in the sterol biosynthesis pathway have been investigated as potential targets, with CYP51 being the most favourable.[39]

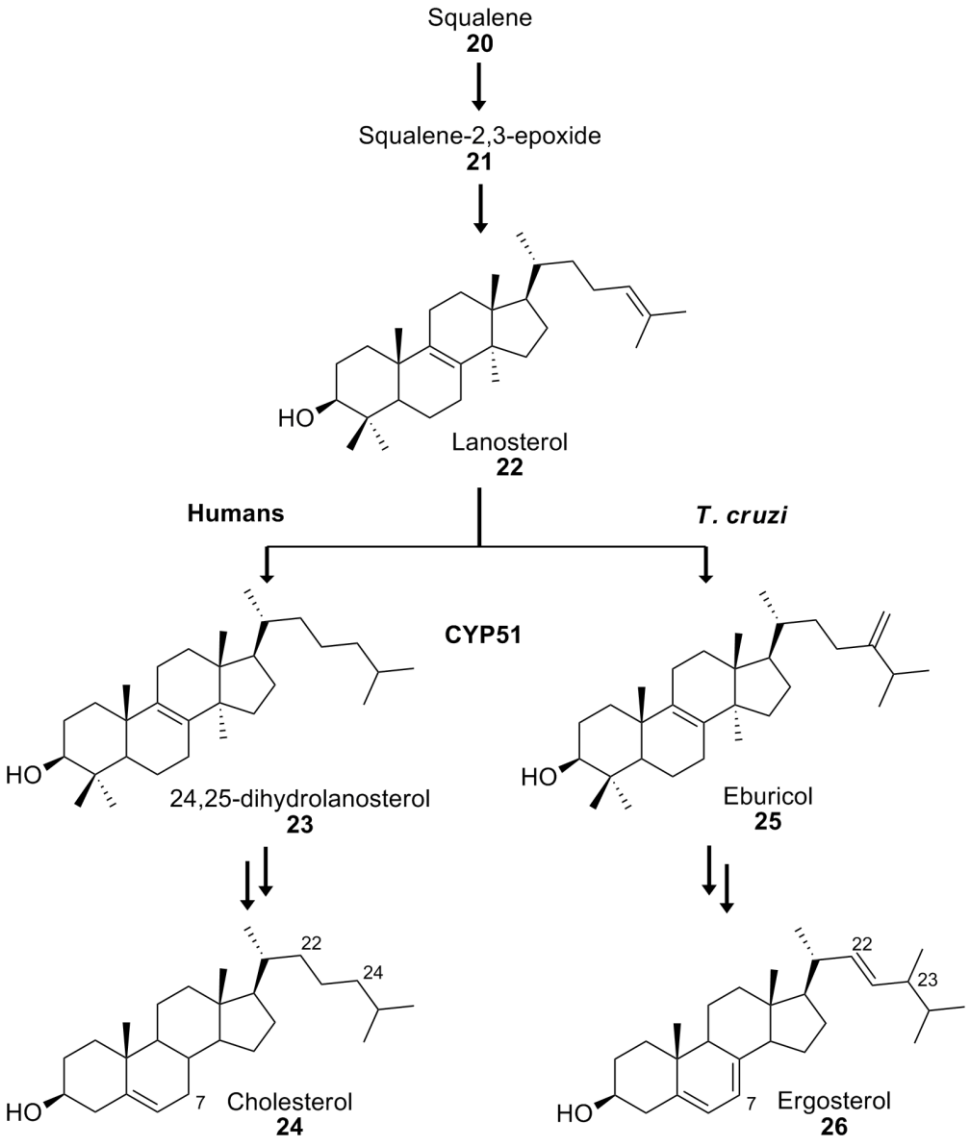


Figure 10. Biosynthetic pathway of cholesterol in humans and ergosterol in *T. cruzi*.

The CYP51 is the cytochrome P450 monooxygenase that catalyses the removal of the 14α-methyl group from eburicol.[39] When the CYP51 is disrupted there is an alteration in the ultrastructure of several organelles, decline of endogenous sterols in the parasites and accumulation of various 14α-methyl sterols with cytostatic and cytotoxic consequences. Although humans have CYP51, it is much less sensitive to the drugs than *T. cruzi*.[27] The azole compounds appear to be the most potent inhibitors in the *in vitro* studies against *T. cruzi* cultures.[37] It has been thought that the potency could be due to the CYP51 structure, which has been elucidated by x-ray crystallography (Figure 11). The substrate binding cavity is quite rigid creating an actual binding cavity rather than a flexible pocket. It also contains a heme where azole heterocycles can coordinate to whilst the rest of the molecule occupies the active site cavity.[21] The coordination to the iron in the heme alone is insufficient for a strong inhibitor as small molecules, like imidazole, have a low binding affinity. There is also a long hydrophobic tunnel connecting the chamber adjacent to the heme with the protein surface.[42] The hydrophobic part of the inhibitor may enhance the molecules interaction with the cavity and increase the strength of the binding.

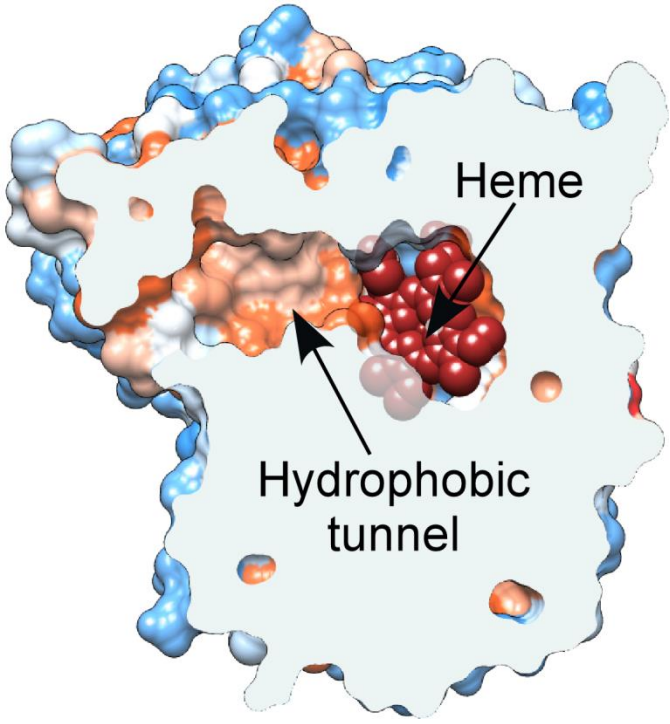


Figure 11. Representation of the binding cavity of CYP51.[42]

Commercially available ergosterol biosynthesis inhibitors such as ketoconazole **27**, fluconazole **29** and itraconazole are highly successful at treating fungal diseases but fail to inhibit *T. cruzi* in animal models and human patients.[6,31] They exhibit suppressive effects but are unable to stop the progression of the disease.[6] When the dosing of the drug stops, the parasite will start to replicate again. Other azole antifungal agents posaconazole **28**, ravuconazole **30** and TAK-187 **32** have demonstrated potent *in vivo* activity with IC_{50} values in the picomolar range.[31] These are thought to inhibit the CYP51 of lanosterol biosynthesis pathway.[37]

Posaconazole **28** is a structural analogue of itraconazole that was developed from ketoconazole **29**. It is a broad spectrum antifungal drug that has been FDA approved for systemic fungal infection and targets CYP51.[6,42] Posaconazole **28** contains a similar backbone to ketoconazole **29** (Figure 12). The triazole binds to the heme iron in the cavity (Figure 13) similar to fluconazole.[42] The binding is strengthened by van der Waals contacts with amino acid residues in the binding cavity and in the substrate access channel surrounding the long arm.[43] This is important in the potency of the drug inhibition. It has been reported to have 100% suppression of *T. cruzi* in mice treated with 20 mg/kg/day of posaconazole **28** at 54 days post infection, compared to only 50% suppression of mice treated with benznidazole.[44] Evidence also suggests that there is less cardiac damage obtained than when treated with benznidazole **2**.[6] Although posaconazole **28** is potent, it has a complex structure that makes it expensive and difficult to synthesise.[39,42] There is also limited selectivity over other CYP enzymes, rapid appearance of laboratory induced resistance to azoles and has significant toxicity.[45,46]

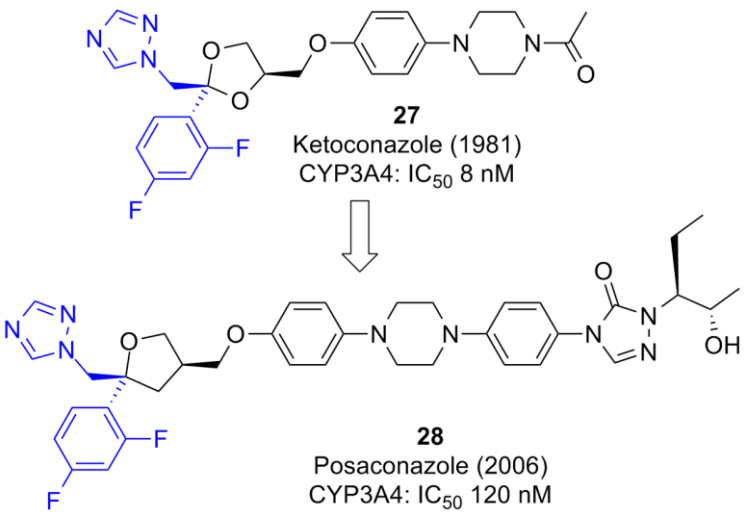


Figure 12. Structures of ketoconazole **27** and its analogue, posaconazole **28**.

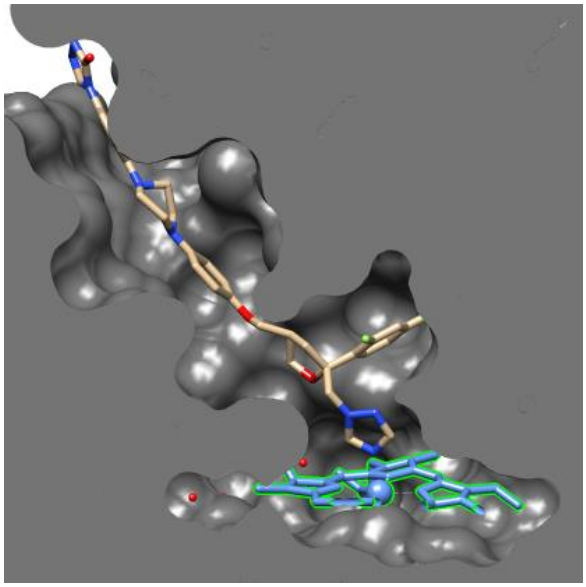


Figure 13. X-ray structure of posaconazole **28** binding in CYP51 active site.[42]

Another triazole that is in development is ravuconazole **30**. Ravuconazole **30** is an investigational triazole that is based on fluconazole **29** and has shown potent and broad spectrum antifungal activity (Figure 14).[38] It is currently in phase II trials as an anti-fungal agent and is an option for clinical development for Chagas disease. Ravuconazole **30** has been shown to be very active against *T. cruzi in vitro*, but only has limited activity *in vivo* in mice.[38] It has a short half-life in mice (4.5 hours) which limited its activity in the experimental model but appears to have a long terminal

half-life (120 hours) in humans.[37,38] The drug also appears more tolerable by humans than other drugs investigated.

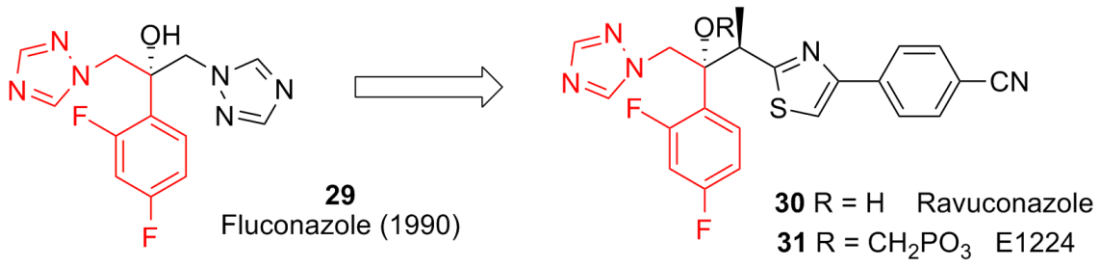


Figure 14. Strutures of fluconazole **29** and its derivatives ravuconazole **30** and E1224 **31**.

A Japanese company, Eisai, has developed a new antifungal prodrug E1224 **31** which is water soluble and converts to the active drug ravuconazole in the body.[33,47] This improved the absorption and bioavailability of the drug. In 2009, the phase II trial was started in conjunction with DND*i* to evaluate its potential as an oral, easy-to-use, safe and affordable treatment for chronic human Chagas disease.[37] Initial results showed good clearing of the parasite and a long terminal half-life but little sustained efficacy after one year.[22] Less than one third of the patients remained parasite free after one year compared with 80% of patients treated with benznidazole. The study showed promise for combined treatment using E1224 with benznidazole.

Another Japanese company, Takeda Chemical Company, has discovered a long lasting triazole derivative with a broad-spectrum anti-fungal activity known as TAK-187 **32** (Figure 15).[6] It has been shown to have *in vitro* and *in vivo* activity against *T. cruzi*, is capable of curing both acute and chronic infections in animals, and has no detectable toxicity.[6,38] TAK-187 **32** also has a long half-life (35.6 h in mice) leading to longer drug exposure time for the same number of doses.

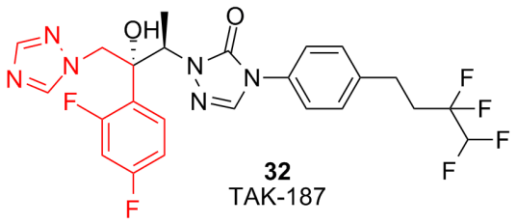


Figure 15. Structure of TAK-187 **32**.

An imidazole derivative, VNI **33**, was recently shown to inhibit CYP51 with high selectivity to fungal CYP51 and not the human CYP51 (Figure 16).[48,49] It was the first compound not to come from an antifungal agent but from studying the inhibition of *T. cruzi* CYP51 activity.[48] It appears to bind to CYP51 in a similar manner to posaconazole **28**.[21,49] Research has suggested that the potency for the inhibition of CYP51 by VNI **33** is enhanced by the hydrogen bond interactions found in the active site.[48] As well as showing good inhibition against *T. cruzi*, VNI **33** has a low toxicity and is cheap to synthesise (<USD $0.10/mg).[21,49] This makes it a good candidate for clinical trials and it is currently undergoing further investigation.[48–50]

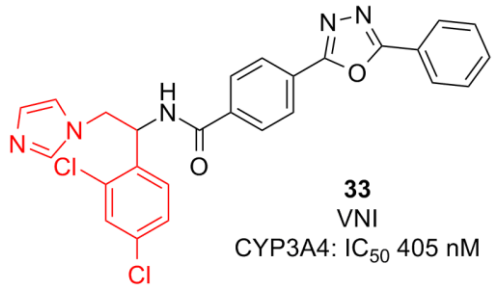
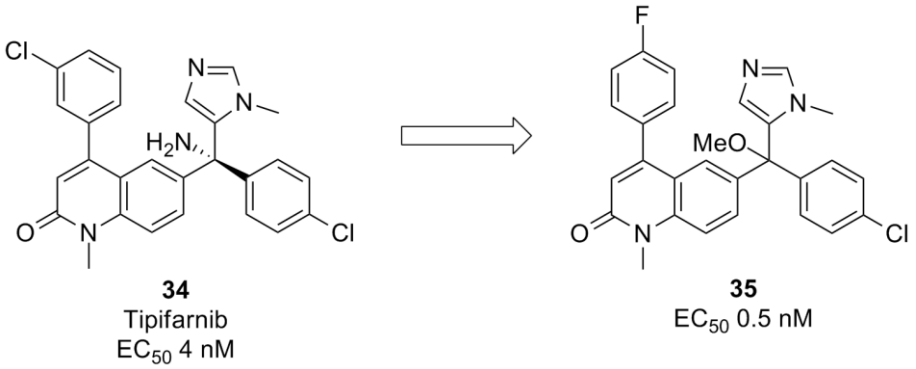


Figure 16. Structure of VNI **33**.

It has been reported that a preclinical anticancer drug, tipifarnib **34**, has shown potent activity against *T. cruzi* (EC_{50} 4 nM). Tipifarnib is an inhibitor of human protein farnesyltransferase (PFT) but has recently been shown to inhibit CYP51 in *T.cruzi*.[51] As tipifarnib would target human PFT and lead to undesirable side effects there have been investigations into analogues that avoid the inhibition of human PFT whilst retaining good inhibition of CYP51. Analogues have been designed to minimise the anti-PFT activity whilst improving CYP51 inhibitory activity (Figure 17).[6,7] The

racemic mixture of the tipifarnib analogue **35** has an EC_{50} 0.5 nM which is similar to tipifarnib (4 nM) but does not bind to the human CYP51.[51] It only has a short half-life in mice of 2 hours. Therefore there is no drug accumulation making it a good candidate for further investigation.[51]

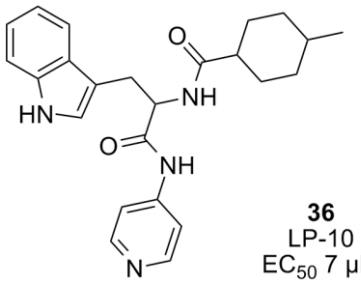


34
Tipifarnib
EC_{50} 4 nM

35
EC_{50} 0.5 nM

Figure 17. Structure of tipifarnib **34** and its analogue **35**.

Although azole derivatives remain the most potent inhibitors, other non-azole compounds have also been considered. A high-throughput screening identified a pyridine derivative LP-10 **36** as a potential lead for development with an EC_{50} 7 µM.[52,53] LP-10 inhibits the synthesis of endogenous sterol and is curative in 60% of cases of acute Chagas disease in murine models.[47] It has been found to selectively bind to the *T. cruzi* CYP51 and is not cytotoxic in the cell.[47]



36
LP-10
EC_{50} 7 µM

Figure 18. Structure of LP-10 **36**.

Amiodarone **37** is currently used for its anti-arrhythmic effect in cardiomyopathy for chronic Chagas disease patients.[3,6,21] Some observations have shown that it kills *T. cruzi* in cell cultures and animals cells. Reports suggest that it may reduce the levels of parasitemia in infected humans as well. It is active against *T. cruzi* by interfering with parasite calcium homeostasis by inducing the release of calcium from intracellular stores and by blocking ergosterol biosynthesis by inhibiting oxidosqualene cyclase.[3,6,21,47] A study on mice has shown that using amiodarone **37** alone resulted in a 0% cure rate, but when combined with posaconazole **28** it resulted in an 80% cure rate. Further clinical studies are being conducted on the anti-parasitic activity of this drug.

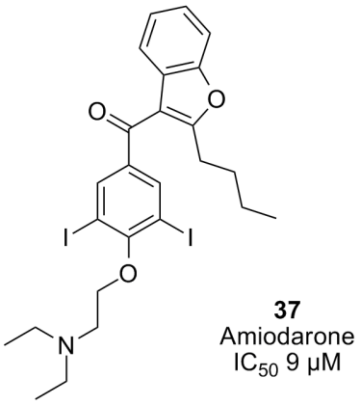


Figure 19. Structure of amiodarone **37**.

The high selectivity of azole inhibitors for the pathogenic sterol 14α-demethylase of *T. cruzi* are desirable to prevent harmful methylated sterols in the human body and avoid negative side effects.[39] This area is a prime target for further investigations.

1.4 Initial investigation of the lead target compound

DND*i* has a goal of developing a superior drug candidate and has provided funding to a team of experts from Epichem Pty Ltd (medicinal chemistry), Murdoch University (parasitology) and the Centre for Drug Candidate Optimisation at Monash University (DMPK evaluation) for this to occur. The Murdoch University Parasitology group conducted *in vitro* screening of various commercially available

pesticides and herbicides against the *T. cruzi* strain Tulahuèn.[54] The plant fungicides fenarimol **38** and pyrifenox **42** showed good inhibition against *T. cruzi* parasites with an IC_{50} of 350 nM and 290 nM respectively. These fungicides are used to treat powdery mildew and fungus on fruits and vegetables. Fenarimol has recently been reported to show inhibition against a related protozoan parasite *Leishmania donovani*[55] and has demonstrated activity against *T. cruzi in vitro* and orally in an acute mouse model.[54]

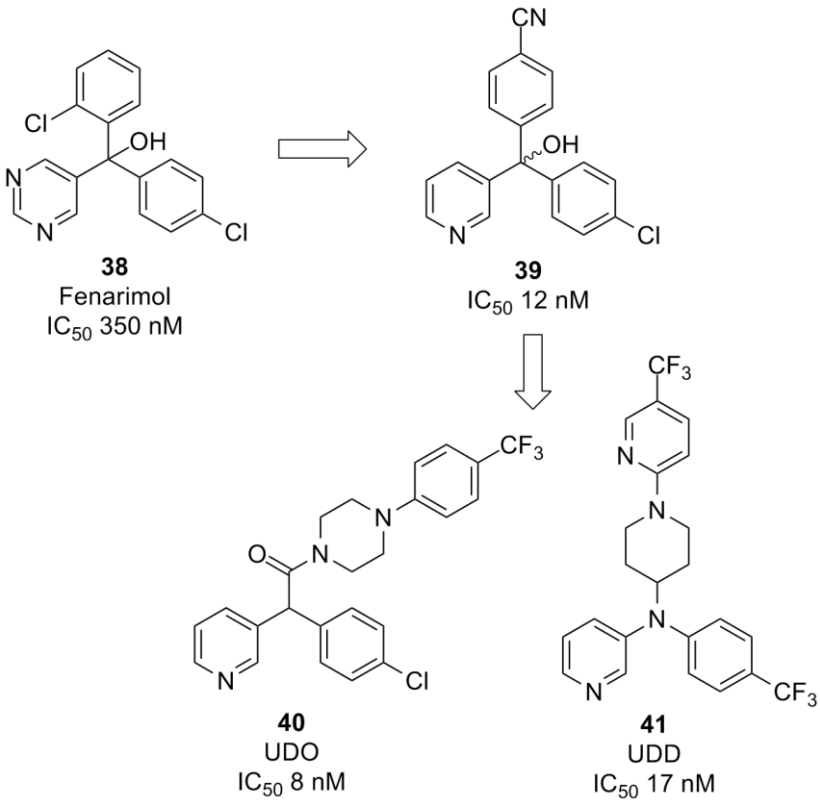


Figure 20. Fenarimol **38** and its analogues UDO **40** and UDD **41**.

Under the DND*i*, fenarimol **38** was used as a lead compound and developed further (Figure 20). Initial results showed the replacement of the 5-pyrimidine with a pyridine ring led to significant improvements in potency when the nitrogen is in the 3-position (compound **39**).[22,54] This compound suppressed the parasitemia to negligible levels in an *in vivo* mouse model of *T. cruzi* infection. However, the parasites re-emerged in the blood after three cycles of immunosuppression after dosing had ceased, which indicated that it was not curative.[54,56] Further

functionalization of the two aromatic rings produced two candidates UDO **40** and UDD **41**, which have been identified for possible preclinical development.[22,54,56] They can be prepared by a short synthetic route, have potent *in vitro* activity and are noncytotoxic. UDO **40** has an EC_{50} of 7.5 nM against *T. cruzi* and in the acute Chagas mouse model an ED_{50} of 20 mg/kg/qd for 20 days. UDD **41** has an EC_{50} of 15 nM and ED_{50} of 20 mg/kg/qd for 20 days. The effectiveness is comparable to posaconazole **28** and better than benznidazole **2**.[56] Both UDO **40** and UDD **41** have been shown to inhibit the CYP51 in *T. cruzi* by the nitrogen in the pyridine ring binding to the heme (Figure 21) but do not inhibit the CYP3A4 in microsomes.[41] This furthers the investigation of non-azole compounds to be highly potent CYP51 inhibitors of *T. cruzi*.

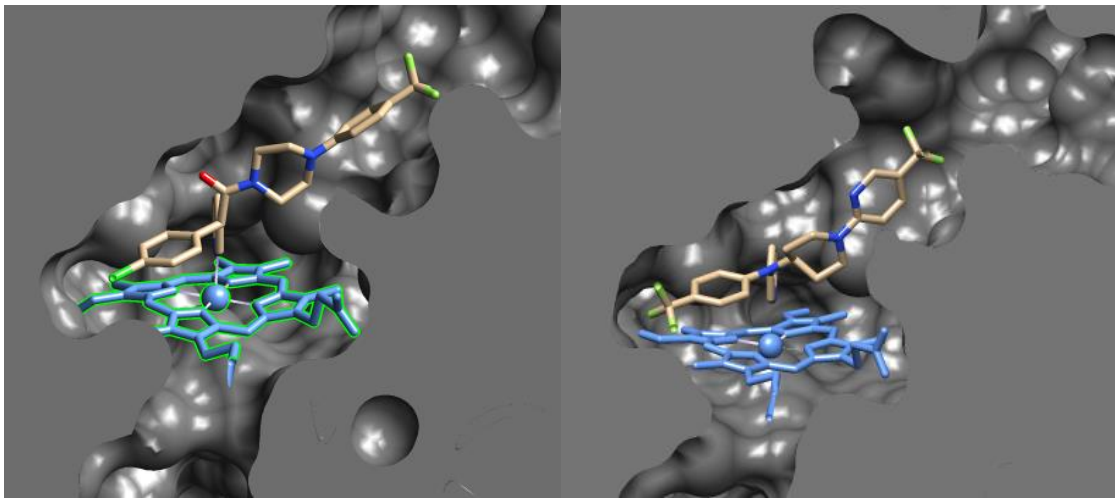
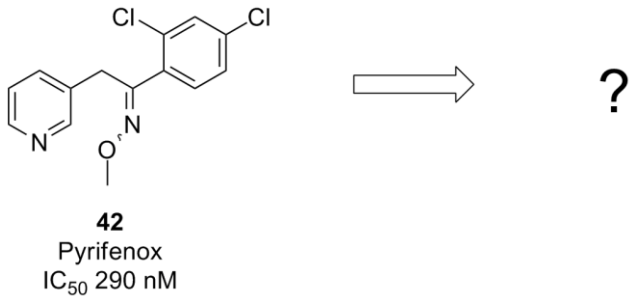


Figure 21. X-ray structure of UDO **40** and UDD **41** bound to the heme in the binding site of CYP51.

The other fungicide, pyrifenox **42** (Figure 22), was also identified as a good CYP51 inhibitor of *T. cruzi* by Witschel *et al*. with an IC_{50} of 491 nM.[57] Although pyrifenox **42** has been identified in two different high-throughput screenings of commercially available agrochemicals there has been *no further investigations published in the literature into analogues of this compound as a new drug candidate for Chagas disease*. Pyrifenox **42** has been reported to block the enzyme required for the biosynthesis of ergosterol and is thought to act on the enzyme CYP51.[42,58] It has low toxicity and has no allergenic or mutagenic effects as an antifungal making it a suitable target for further research.[59]



42
Pyrifenox
IC_{50} 290 nM

Figure 22. Analogues of pyrifenox have not been investigated.

The structure of pyrifenox **42** has three distinct components− the pyridine ring, the oxime and the substituted phenyl ring (Figure 23). Initial investigations conducted at Epichem Pty Ltd have shown the aromatic nitrogen in the pyridine ring is essential for activity.[*] The compounds synthesised without the pyridine ring (**43**) showed a decrease in biological activity with an IC_{50} greater than 10 µM. Therefore it appears the nitrogen in the pyridine ring is essential for binding to the heme in the active site. Having at least two carbons in the linker between the pyridyl ring and the phenyl ring also appears crucial. Where there is only one carbon between the two aromatic rings the biological activity is greater than 1 µM (**44**). The oxime group was also briefly investigated. The longer oxime **45** improved the activity slightly with one isomer showing an IC_{50} of 0.023 µM whilst the other isomer was only 0.26 µM. The stereochemistry of the two isomers was not identified. This indicates that the stereochemistry could also be important and requires investigation. Further research is still required into the structure activity relationship of pyrifenox to effectively inhibit CYP51 of *T. cruzi*.

[*] Alexander, P; Keenan, M and Best, W. Epichem Ltd Pty. Personal communication, 2011

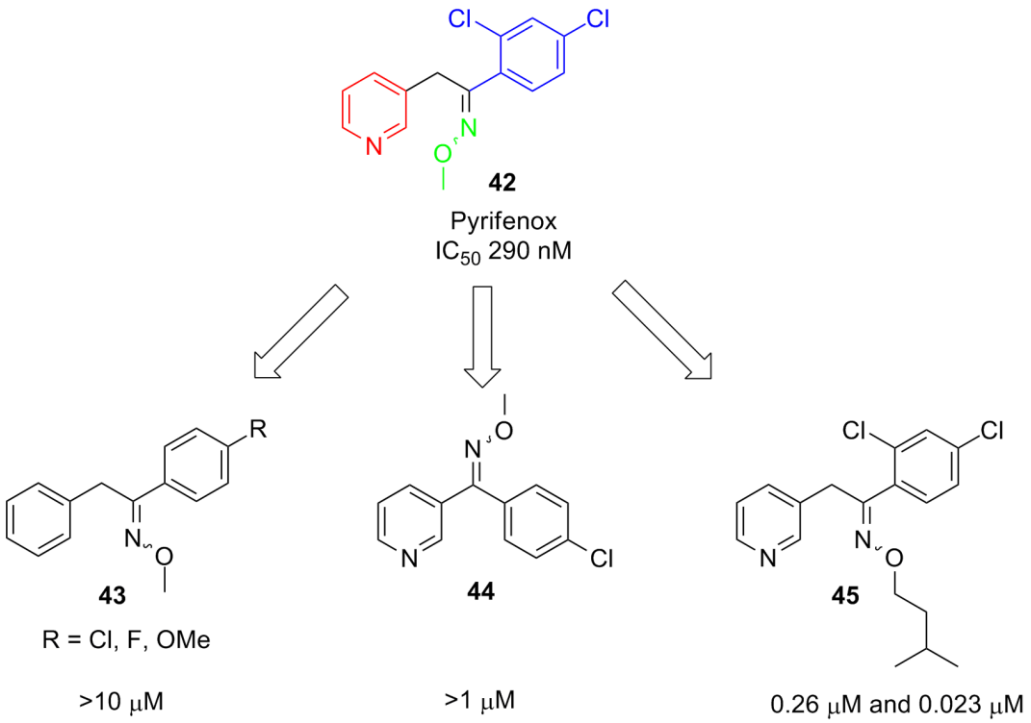
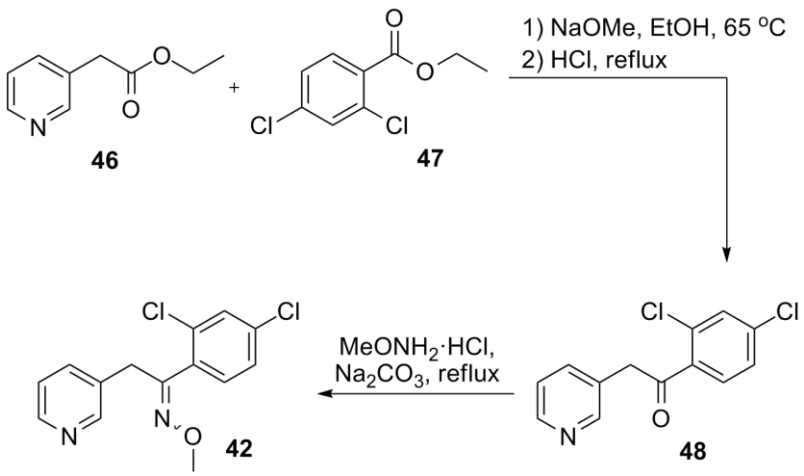


Figure 23. Pyrifenox made of 3 components- pyridine ring (red), oxime (green) and substituted phenyl ring (blue) and the initial analogues.

1.5 Synthesis of pyrifenox

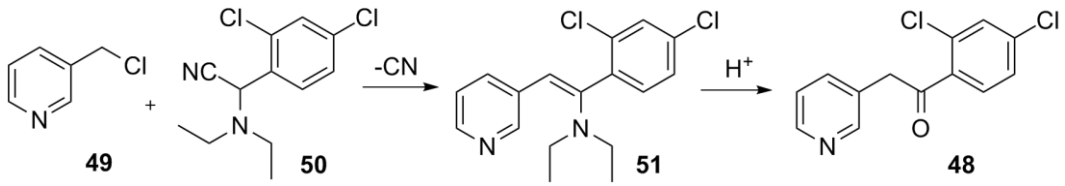
The synthesis of pyrifenox **42** and related fungicides have been reported in a patent.[60] Dorn identified ketone **48** as the key intermediate to synthesise pyrifenox (Scheme 1).[60,61] The ketone intermediate **48** was synthesised from the reaction between ethyl 3-pyridylacetate **46** and ethyl 2,4-dichlorobenzoate **47** with sodium methoxide. The ketone **48** was then reacted with methylhydroxylamine hydrochloride to give pyrifenox **42** as two isomers. The yield for the ketone formation was low (30%) and the starting materials are expensive. This procedure was also not repeatable in the initial investigations at Epichem Ltd Pty.*

* Alexander, P; Keenan, M and Best, W. Epichem Ltd Pty. Personal communication, 2011



Scheme 1. Reported patent for the synthesis of pyrifenox **42.**

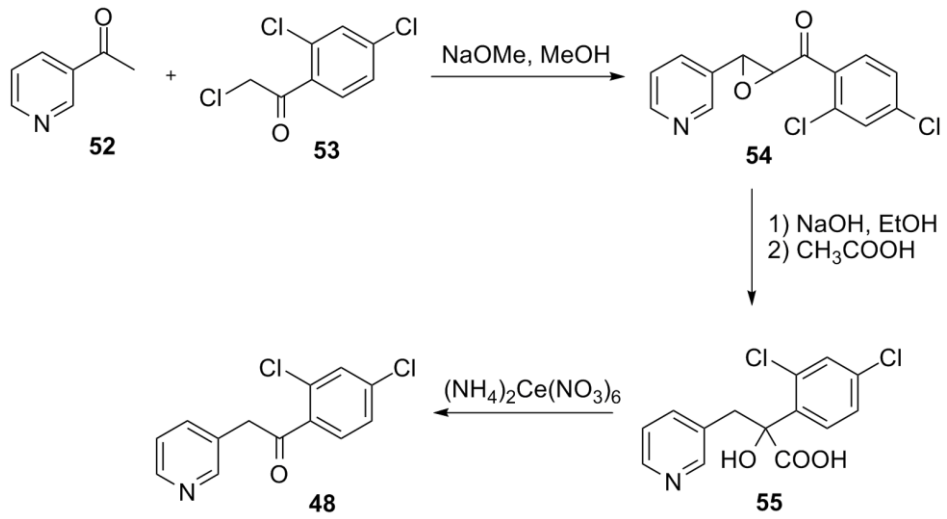
As the ketone **48** has been identified as the key intermediate this will be synthesised. An extensive literature search showed limited routes to the ketone **48**. The other methods to synthesise this compound were also developed by Dorn *et al*.[61] The first technique condensed 3-chloromethylpyridine **49** with **50** under phase transfer conditions to eliminate hydrogen cyanide to give compound **51** (Scheme 2). Acid hydrolysis of **51** gives the desired ketone **48**. This method was also low yielding (30−50%)[61] and therefore not economical on a large scale.



Scheme 2

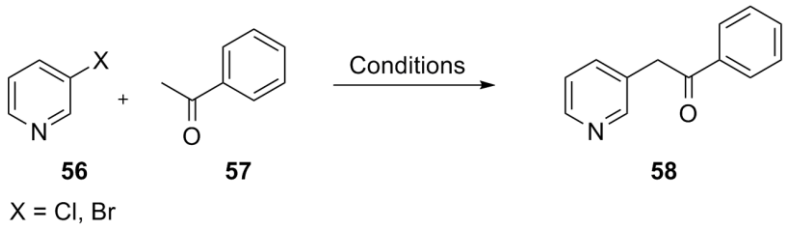
Another technique for the synthesis of the ketone intermediate **48** is shown in Scheme 3.[61] 3-Pyridineacetophenone **52** and 2-chloro-1-(2,4-dichlorophenyl)-ethanone **53** were reacted with sodium methoxide to give the epoxyketone **54**. The carbon chain was then shortened by a benzylic acid type rearrangement before an oxidative decarboxylation with ceric ammonium nitrate to give the desired ketone **48**. Although this method was higher yielding, the ceric ammonium nitrate is

expensive and the authors found that it could not be replaced with a cheaper reagent.[61]



Scheme 3

Due to the limited routes to synthesise the ketone intermediate **48**, the literature search was broadened to produce a ketone (Table 1). This search also produced limited results.[61–66] In recent publications, the use of palladium catalysts have be shown to be effective in cross coupling reactions. Biscoe and Buchwald developed a palladium precatalyst **59** that was used to synthesise similar ketones in one step from an aryl halide **56** and an acetophenone **57** (entry 1).[67] Cao *et al*. then developed another palladium catalyst, (SIPr)Pd(Py)Cl_2 **60** (entry 2) that could be used under similar conditions.[66] Both catalysts were reported to work in high yields (90% and 70% respectively) but the use of the catalysts appeared temperamental. Crawford *et al*. developed a method using a DalPhos-based (**61**) catalyst system with [Pd(cinnamyl)Cl]$_2$ in dioxane (entry 3).[62] Although this system showed high yields with some ketones, the use of a 3-pyridyl ring was low yielding (21%).

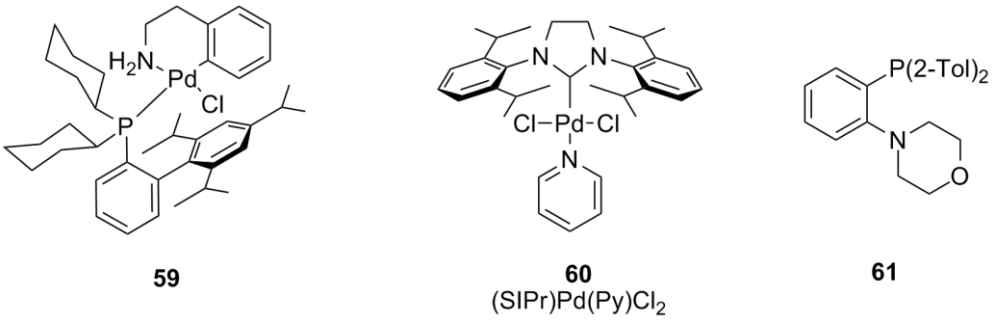


56 **57** **58**

X = Cl, Br

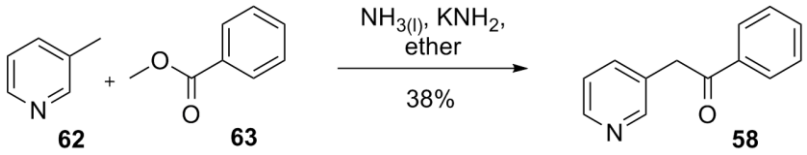
Table 1

Entry	Conditions	Yield
1	$KOBu^t$, toluene, **59**	90%
2	$KOBu^t$, toluene, $(SIPr)Pd(Py)Cl_2$ **60**	70%
3	$NaOBu^t$, 1,4-dioxane, $[Pd(cinnamyl)Cl]_2$, **61**	21%



59 **60** $(SIPr)Pd(Py)Cl_2$ **61**

Other methods published in the literature appear quite dated. Miller *et al*. reported a procedure in 1956 where the ketone was prepared from 3-picoline **62**, methyl benzoate **63** and potassium in liquid ammonia (Scheme 4).[68] The reaction was low yielding (38%) and the strong base used restricts a variety of functional groups.



62 **63** **58**

Scheme 4

Due to the published procedures for the synthesis of a ketone requiring expensive catalysts and low yielding reactions, a new synthetic route to synthesise pyrifenox is required.

1.6 Aim of project

The commercially available antifungal pyrifenox **42** has been reported to have good inhibition against *T. cruzi in vitro* but has not been investigated further.[54,57] This could be due to limited routes available to synthesise pyrifenox[60,61] or the simplified ketone intermediate.[62–66] This research will focus on a new and economical synthesis of the desired ketone intermediate and therefore a new synthesis for pyrifenox **42**.

Once an efficient method has been developed to synthesise pyrifenox **42**, other analogues will be synthesised. The possible areas for investigation to increase the trypanosomal activity are shown in Figure 24. The areas for investigation will follow on from the initial investigations conducted by Epichem Pty Ltd mentioned in Section 1.4. The oxime chain appears to be an ideal target for investigation. The length and bulk of the oxime chain will be explored to determine the steric bulk required to bind effectively in the enzyme. The substituents on the phenyl ring can also be modified to explore the steric bulk required to fit into the hydrophobic pocket of the enzyme. The linker between the phenyl ring and the pyridine ring can also be investigated. Initial results have shown that having only one carbon linker between the pyridyl ring and the phenyl ring decreases biological activity (>1 μM). Other modifications of the linker include increasing the length of the carbon chain between the pyridyl ring and the phenyl ring, changing the position of the oxime and alkylation of the CH_2 linker.

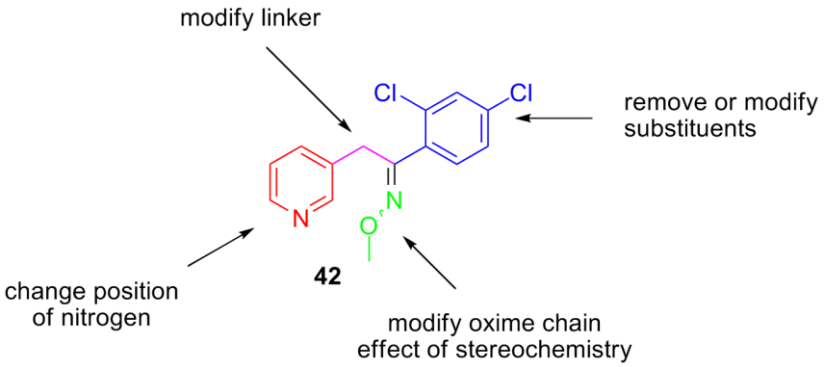
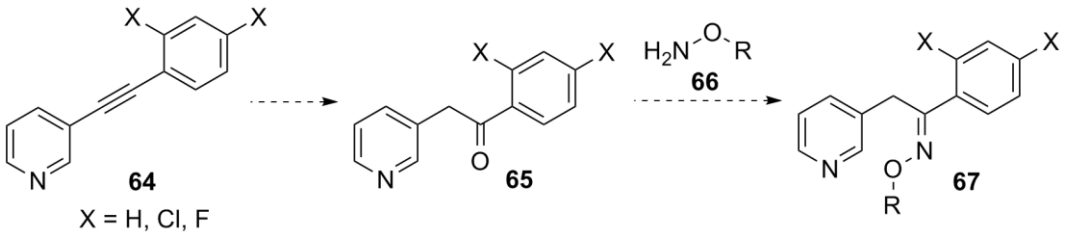


Figure 24. Proposed changes to pyrifenox.

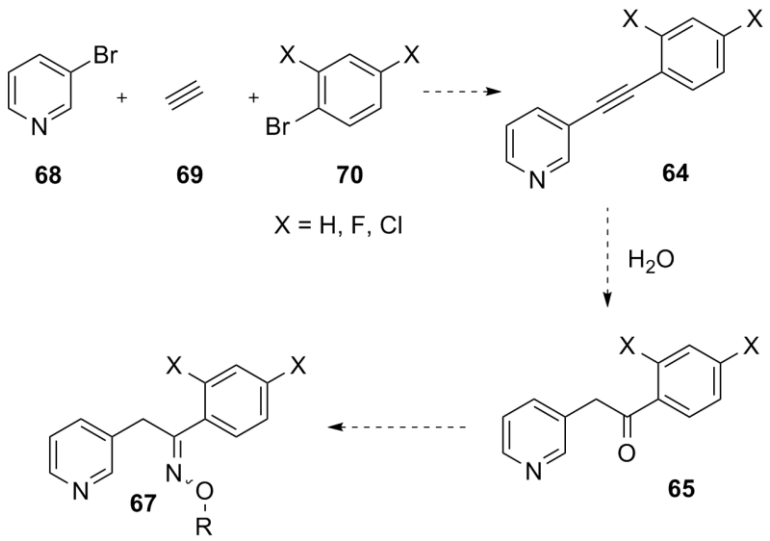
Initially the synthesis of pyrifenox will be examined. There have been reports in the literature that show the hydration of an alkyne can achieve the desired ketone **65** (Scheme 5).[69–72] Once the ketone has been synthesised a simple condensation of the ketone with a variety of alkoxyamine derivatives **66** will give a variety of pyrifenox analogues **67**. The substituents X on the phenyl ring will also be varied. The main substituents that will be focussed on are hydrogen, fluorine and chlorine.



Scheme 5. Proposed method to synthesise pyrifenox analogues from an alkyne.

2 Synthesis of 3-(pyridyl)acetophenones

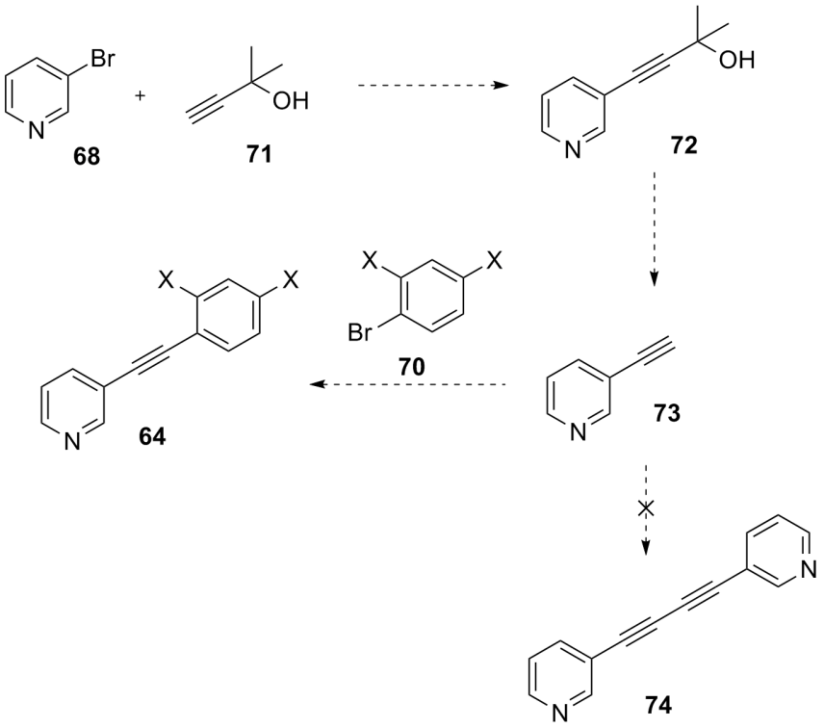
In order to access derivatives of pyrifenox, the synthesis of the ketone **65** was the initial focus of attention. Surprisingly, the published synthesis of the ketone **65** was sparse (Section 1.5) and a new more modular synthesis was mapped out. The plan was to make the key intermediate **65**, containing an internal ketone, as shown in Scheme 6. 3-Bromopyridine **68** and an aryl bromide **70** would be connected to an acetylene equivalent **69** using two sequential Sonogashira coupling reactions.[73,74] The alkyne **64** could then be hydrated to form the key ketone intermediate **65**. There were two key variations that were investigated: the substituents X and R on the pyrifenox analogue **67**. The substituents X that were focussed on were hydrogen, fluorine and chlorine. The variation of the substituents on the phenyl would explore the steric bulk to see how large the pocket is where the aryl group binds inside the enzyme.



Scheme 6. General synthetic plan for the synthesis of pyrifenox derivatives.

2.1 Synthesis of alkyne 64

The alkyne **64**, identified as the precursor to the ketone intermediate **65**, could be made by two Sonogashira coupling reactions (Scheme 7). One side of the alkyne would need to be protected for the first Sonogashira reaction to stop other reactions from occurring. There are two common compounds that could be used. The most common protected alkyne used in literature was trimethylsilylacetylene.[75–77] The trimethylsilyl protecting group could easily be removed under mild conditions in the presence of a base or fluoride ion.[75] The main drawback with using trimethylsilylacetylene was the cost. A cheaper alternative would be to use 2-methyl-3-butyn-2-ol **71**, as the protecting group can readily be removed with the addition of a strong base to give the unprotected alkyne **73**.[75,78] Reaction of a phenyl substituted ring **70** and an unprotected alkyne **73** would give the desired alkyne **65**. An unwanted by-product in Sonogashira reactions is the formation of a diyne **74** formed by a Glaser coupling between two terminal alkynes.[73,79]

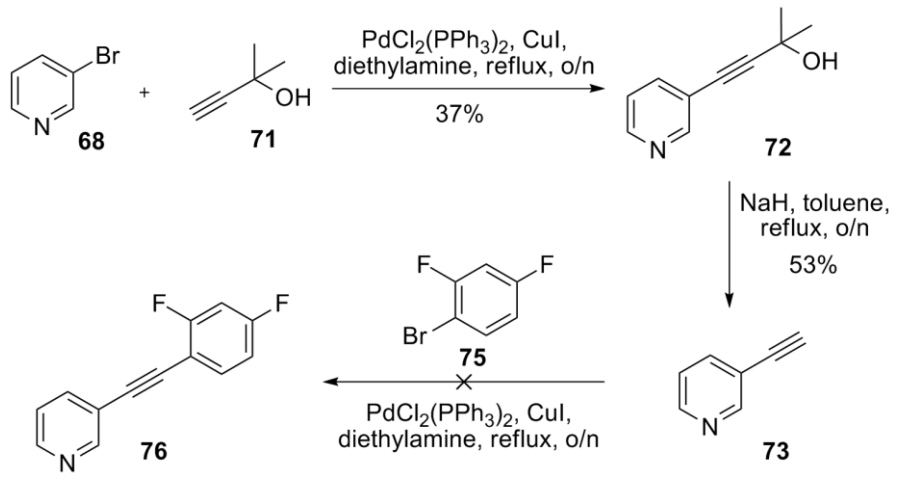


Scheme 7. Proposed synthesis of the alkyne **64**.

The first target that was investigated was the difluoronated alkyne **76**. The preparation of the alkyne **76** was more challenging than expected. Using the conditions outlined by Cosford *et al*,[74] 3-bromopyridine **68** and 2-methyl-3-butyne-2-ol **71** were heated with $PdCl_2(PPh_3)_2$ and copper(I) iodide in 1,2-dimethoxyethane under reflux (Scheme 8), no reaction was observed. On changing the solvents to neat diethylamine, the reaction gave the expected coupled product **72** in 37% yield.[80] The spectral data matched that in the literature.[81] The low reactivity of 3-bromopyridine could be attributed to the aromatic ring being electron-deficient. A study by Shoji *et al*. showed that in electron rich systems the Sonogashira reaction occurs in excellent yields but in electron-deficient systems the cross coupling product occurs in lower yields.[82]

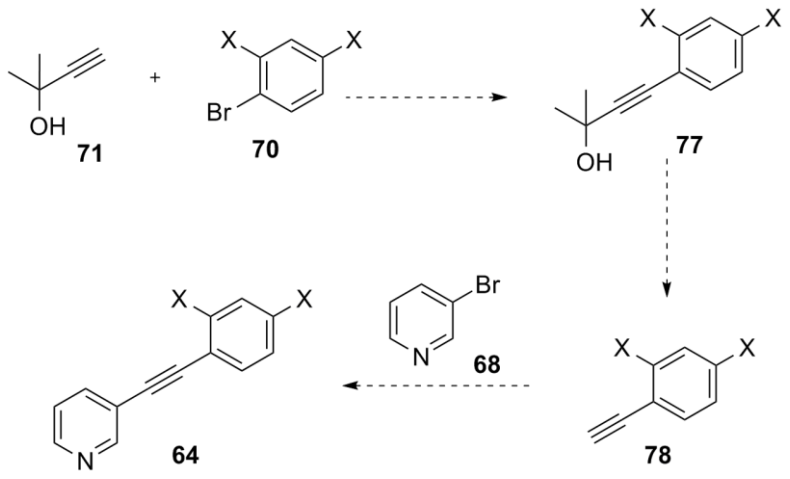
The alkyne **72** was then deprotected with toluene and sodium hydride to form the terminal alkyne **73**. The spectral data was consistent with that in the literature.[74] The ^{1}H NMR spectrum showed the terminal alkyne signal at 3.20 ppm which was similar to phenylacetylene (3.06 ppm).[83] The product did not require any further purification

and was used in the subsequent Sonogashira reaction. When the terminal alkyne **73** and 1-bromo-2,4-difluorobenzene **75** were heated under reflux in the presence of $PdCl_2(PPh_3)_2$ and copper(I) iodide in diethylamine, a complex mixture was produced.



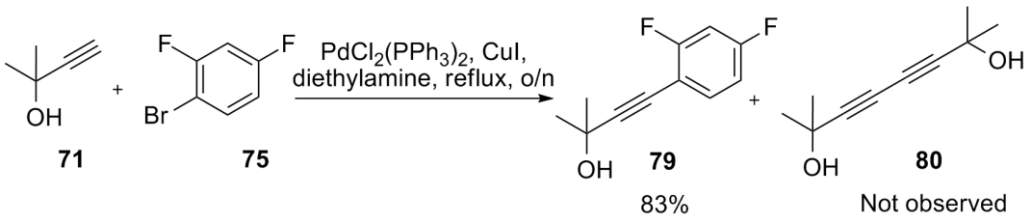
Scheme 8

The nitrogen on the pyridine ring was suspected to interfere with the reactions. A solution to this problem was to rearrange the order of the two Sonogashira reactions (Scheme 9). The aryl bromide **70** could be reacted with 2-methyl-3-butyne-2-ol **71** to give the protected alkyne **77**. The alkyne **77** could then be deprotected and reacted with 3-bromopyridine **68** to give the desired product **64**. This approach would introduce the problematic pyridyl group at a later stage in the synthesis.



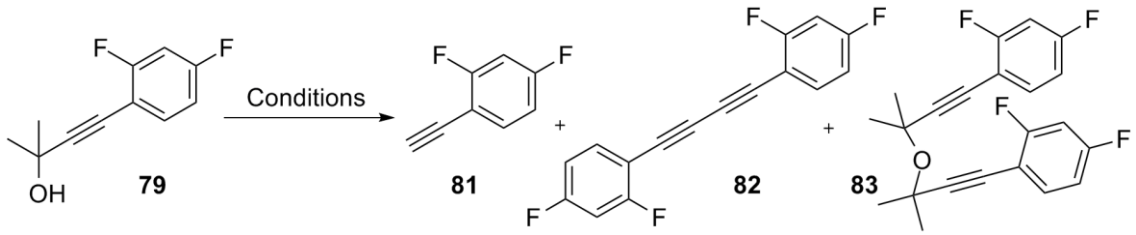
Scheme 9. Alternate proposed synthesis of the alkyne **64**.

1-Bromo-2,4-difluorobenzene **75** was heated with 2-methyl-3-butyne-2-ol **71**, $PdCl_2(PPh_3)_2$ and copper(I) iodide in diethylamine under reflux to give **79** (Scheme 10). The ^{1}H NMR spectrum showed two multiplets at 6.77−6.85 ppm and 7.33−7.41 ppm with the integration of three hydrogens which corresponded to the aromatic hydrogens. There was also a singlet at 1.62 ppm with the integration of six hydrogens which corresponded to the two methyl groups. The signal at 3371 cm^{-1} in the IR spectrum corresponds to an OH stretch. This reaction was high yielding (83%) and was performed on a large scale (17 g). Surprisingly the solvent did not need to be dried and only stirred under nitrogen for one hour without purging the system. The Glaser by-product **80** was not observed with this reaction.



Scheme 10

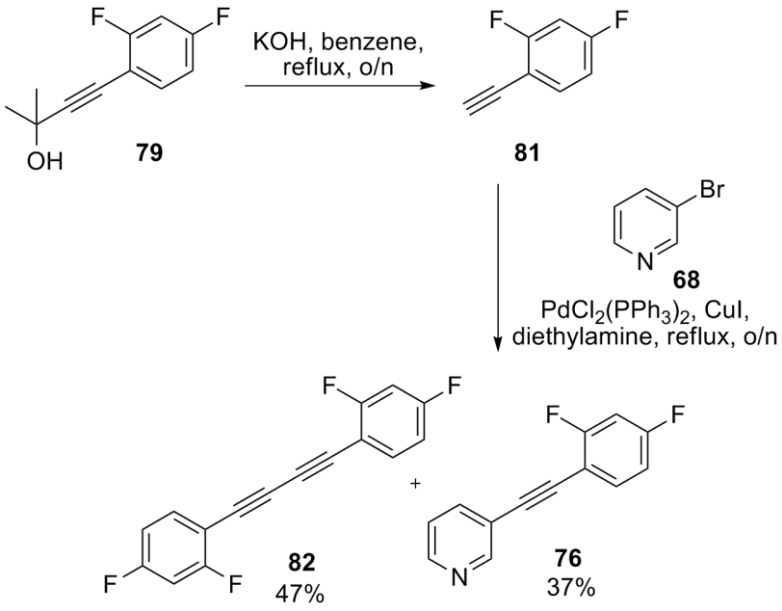
The removal of the alkyne protecting group proved to be difficult (Scheme 11). The use of sodium hydride and toluene produced a complex mixture. There was no signal at approximately 3 ppm in the ^{1}H NMR spectrum corresponding to the terminal alkyne. Isolation by flash chromatography separated starting material and a compound that was not observed in the ^{1}H NMR spectrum of the crude material. This suggested that the crude reaction mixture was decomposing during isolation and purification. The base was changed from sodium hydride to potassium hydroxide. The deprotection of the alkyne **79** with powdered potassium hydroxide in toluene[84] also did not produce the desired terminal alkyne **81**. The ^{1}H NMR spectrum of the crude material showed a complex mixture that was different from the previous attempt. Wang *et al*. reported that a mixture of terminal alkyne **81**, dimer **82** and arylethynyl isopropyl ether **83** were obtained upon the deprotection using sodium hydroxide or potassium hydroxide in toluene on a large scale.[80] Examination of the ^{1}H NMR spectrum of the complex mixture showed there was a small singlet at 3.28 ppm which was consistent with the terminal alkyne signal. This suggested that the terminal alkyne **81** was produced but was only a minor product. There were signals in the aromatic region which could be associated with the dimer **82** and two singlets between 1 and 2 ppm which could be compound **83** and starting material **79**. Wang *et al.* also reported that upon changing the solvent from toluene to benzene and having a dilute solution improved the yield without any of the by-products occurring.[80] The protected alkyne **79** was heated with potassium hydroxide in benzene under reflux. A Dean-Stark apparatus was used to remove the acetone generated. This gave the desired terminal alkyne **81** but the compound decomposed upon workup. The ^{1}H NMR spectrum of the reaction mixture in benzene suggested that the reaction did occur by the terminal alkyne signal at 2.40 ppm which was consistent with literature spectral data.[85] As the terminal alkyne **81** was decomposing upon workup it was not isolated and used directly in the next reaction.



Scheme 11

Entry	Conditions	Product
1	NaH, toluene, reflux, 3 h	Decomposition
2	KOH, toluene, 80 °C, 2 h	Complex mixture
3	KOH, benzene, reflux, o/n	Decomposed on workup

The crude reaction mixture in benzene was used in the second Sonogashira reaction with 3-bromopyridine **68**, $PdCl_2(PPh_3)_2$ and copper(I) iodide in diethylamine under reflux to give the alkyne **76** (Scheme 12) in moderate yield (37%). The ^{1}H NMR spectrum showed two broad singlets at 8.59 ppm and 8.79 ppm corresponding to the hydrogens next to the nitrogen in the pyridine ring. The ^{13}C NMR spectrum showed two signals at 85.0 ppm and 90.7 ppm corresponding to the alkyne carbons. The lower yield (37%) was due to the low reactivity of 3-bromopyridine as it is electron deficient. The slower reaction allows the Glaser coupling to occur forming **82**. Elangovan *et al*. found when oxygen is present in the reaction mixture the Pd(0) may reoxidise back to Pd(II) which is thought to catalyse the homocoupling of the terminal alkyne.[86] To prevent the formation of the Glaser by-product from occurring, the reaction mixture was completely degassed using the freeze-pump-thaw method to remove oxygen from the system.

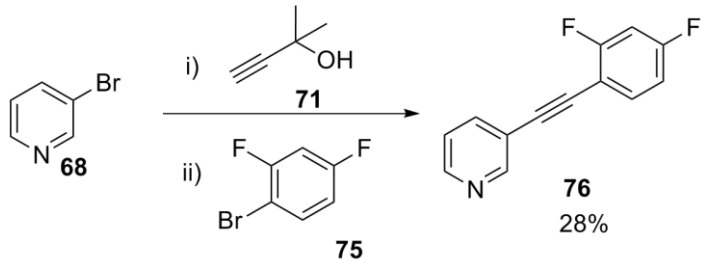


Scheme 12

The other factor that could be affecting the yield of the second Sonogashira reaction was the solvent.[80] The benzene was dried before use to see if that would affect the reaction and was found not to have any effect on the yield. If there was any water present in the solvent the Dean-Stark apparatus would help dry the reaction. It could be assumed that not removing the benzene from the deprotection step could be affecting the second Sonogashira reaction. By having a minimal amount of benzene in the reaction the yield improved slightly but there needed to be enough benzene for the deprotection to occur. Thus by diluting the reaction further with diethylamine, it was possible to obtain a maximum yield of 41%. This reaction was done on a 2 gram scale.

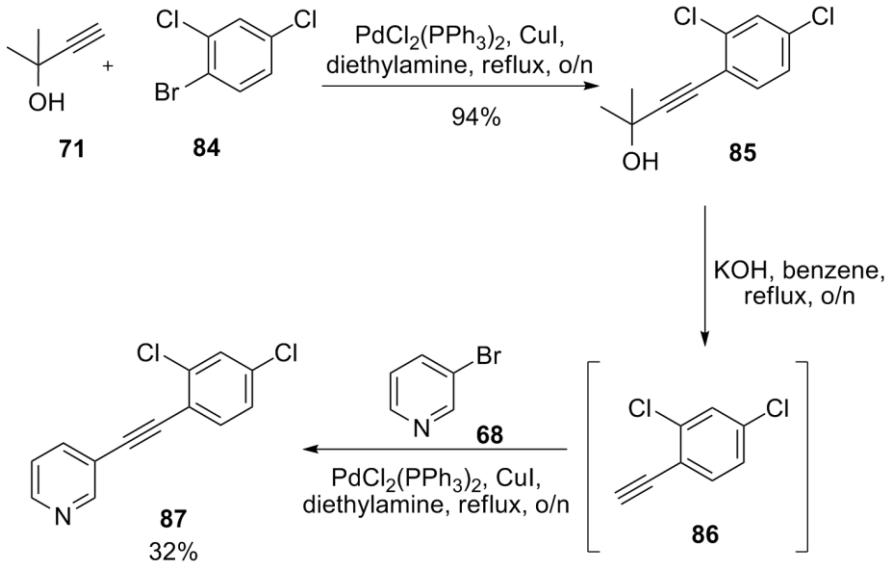
A sequential Sonogashira-deprotection-Sonogashira one-pot synthesis was also investigated (Scheme 13).[75] 3-Bromopyridine **68** and 2-methyl-3-butyne-2-ol **71** in neat diisopropylamine were heated in an 50 °C oil bath with $PdCl_2(PPh_3)_2$ and copper(I) iodide. Once TLC analysis showed no remaining starting material, 1-bromo-2,4-difluorobenzene **75** and potassium hydroxide were added into the reaction mixture with additional $PdCl_2(PPh_3)_2$ and copper(I) iodide and increased the temperature of the oil bath to 110 °C. This method produced the desired alkyne **76** in

low yield (28%) which could be due to more by-products being produced than in the previous two step sequence. Thus, the best procedure for preparing the alkyne **76** was by isolating the first Sonogashira product **79** (Scheme 10) before the deprotection and second Sonogashira reaction (Scheme 12). This method produced a compound that was easier to purify and gave a slightly better overall yield. The optimised conditions were used to synthesise a library of alkynes.



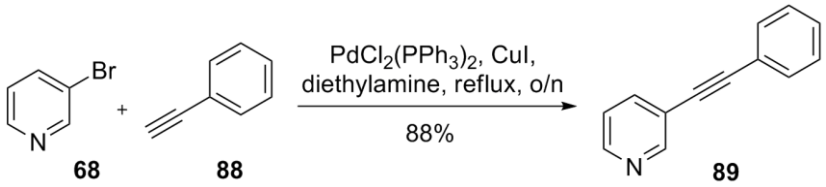
Scheme 13. Conditions: i) $PdCl_2(PPh_3)_2$, CuI, diisopropylamine, 50 °C, 2 days; ii) KOH, $PdCl_2(PPh_3)_2$, CuI, 110 °C, 3 days.

Using the optimised method developed for compound **76** the chloro-substituted alkyne **87** was synthesised (Scheme 14). 1-Bromo-2,4-dichlorobenzene **84** and the protected alkyne **71** were reacted with $PdCl_2(PPh_3)_2$ and copper(I) iodide in diethylamine to give **85**. The ^{1}H NMR spectrum showed a doublet of doublets at 7.16 ppm, a doublet at 7.34 ppm and a doublet at 7.38 ppm corresponding to the aromatic hydrogens and a singlet at 1.62 ppm with the integration of six hydrogens corresponding to the two methyl groups. The IR spectrum showed a signal at 3362 cm^{-1} corresponding to an OH stretch. This reaction was high yielding (94%) and was done on a large scale (15 g). The alkyne **85** was then sequentially deprotected with potassium hydroxide in benzene followed by a Sonogashira reaction with 3-bromopyridine **68**, $PdCl_2(PPh_3)_2$ and copper(I) iodide in diethylamine to give the desired alkyne **87**. The ^{1}H NMR spectrum showed a doublet of doublets at 8.50 ppm and a broad singlet at 8.71 ppm corresponding to the hydrogens next to the nitrogen on the pyridine ring. The ^{13}C NMR spectrum showed two signals at 88.6 ppm and 91.9 ppm that corresponded to the alkyne carbons. The reaction yield was modest (32 %), but acceptable.



Scheme 14

Since the halogenated alkynes produced low yields, a model unhalogenated system was synthesised using the optimised Sonogashira reaction conditions (Scheme 15). 3-Bromopyridine **68** was heated under reflux with phenylacetylene **88**, $PdCl_2(PPh_3)_2$ and copper(I) iodide in diethylamine to give the alkyne **89**. The spectral data matched that in the literature.[87] The ^{13}C NMR spectrum showed two peaks at 86.0 ppm and 92.7 ppm corresponding to the alkyne carbons. This reaction was done on a large scale (3.5 g) and in high yield (88%).

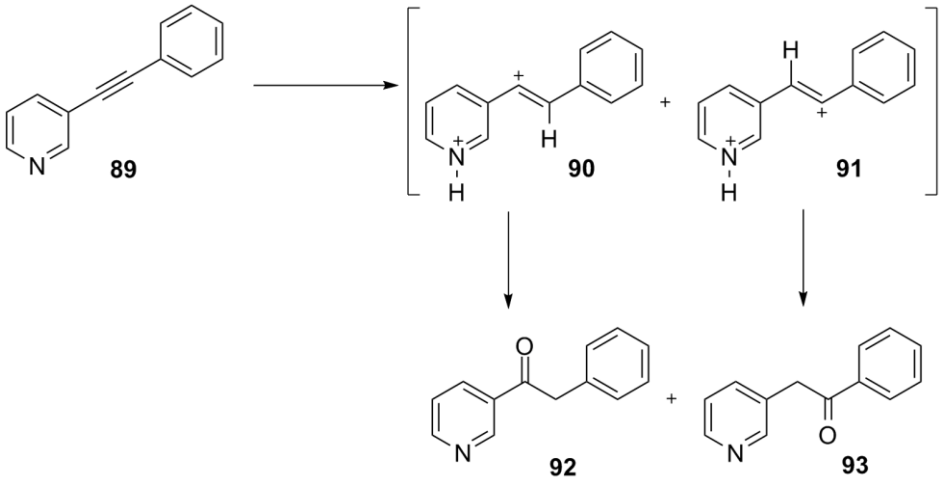


Scheme 15

Three different alkyne systems have been successfully synthesised from the optimised reaction conditions. Having effectively produced three different alkyne systems the next key step was to form the ketone.

2.2 Hydration of the alkyne

The next key reaction was to convert the alkyne **89** to the ketone **93**. As this system has an internal alkyne there are two possible positions where the ketone can be formed: **92** and **93** (Scheme 16). Under acidic conditions, the nitrogen in the pyridine ring would be protonated generating a positive charge. For the ketone to form next to the pyridyl ring, compound **92**, the carbocation would need to form on the carbon next to the pyridyl ring. This intermediate would be unstable due to the close proximity of two positive charges. The favoured position under acidic conditions would be compound **93**. Under specific conditions the reaction can be regiospecific to form only compound **93**.[71] There has been limited work done on the hydration of disubstituted alkynes, particularly in biaryl systems. This could be due to the biaryl systems being electron deficient making it less reactive.[71] Another drawback is the nitrogen in the pyridine ring can co-ordinate to metals involved in the hydrolysis reaction. Mercury(II) salts are effective for the hydration of alkynes but usually require strongly acidic conditions. Recent interest for the hydration of alkynes lies in using transition-metal-complex catalysts.[88] These include ruthenium, rhodium, platinum and gold. The most common of these methods uses gold as the catalyst.[71,89–91] Brønsted acids and microwave irradiation are also common methods for hydration.



Scheme 16

2.2.1 Brønsted acid promoted hydrations

A recent review by Hintermann and Labonne[71] discussed various methods where Brønsted acids are used to hydrate alkynes to form ketones. However, it was previously reported that the use of Brønsted acids required a large excess of the acidic reagent and would generally react in low yields with only electron-rich acetylene systems reacting in high yields.[92] The unsubstituted alkyne **89** was used as a model compound to test the most efficient hydration reactions (Table 2).

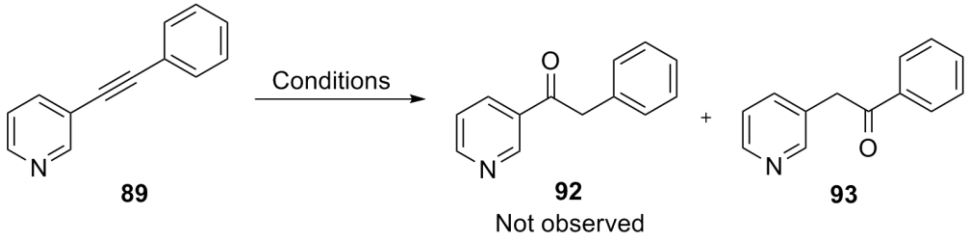


Table 2

Entry	Conditions	Product
1	Formic acid 98%, reflux, o/n	No reaction
2	CF_3COOH, H_2O, reflux, o/n	34%
3	0.5 M HCl, microwave, 180 °C, 60 min	20%

Formic acid has been shown to hydrate terminal and internal alkynes in moderate to high yields.[71,72,93] On heating the alkyne **89** in a large excess of formic acid under reflux[72] no reaction occurred. It has been noted in the literature that less activated alkynes require a ruthenium catalyst to facilitate the reaction in formic acid.[71,93] The formic acid might not be strong enough to hydrate this internal triple bond. Therefore the acid was changed from formic acid to trifluoroacetic acid.[94] Trifluoroacetic acid is a much stronger acid (pK_a −0.5) compared with formic acid (pK_a 3.75). When the alkyne **89** was stirred at room temperature in trifluoroacetic acid and water, no reaction occurred, but heating the reaction under reflux gratifyingly gave the ketone **93** in 34% yield. The spectral data matched that in the literature.[66] The ^{1}H NMR spectrum showed a singlet at 4.27 ppm integrating for two hydrogens. The key diagnostic signal at 4.27 ppm corresponded to the CH_2 between the pyridyl ring and the ketone. The ^{13}C NMR spectrum showed a peak at 42.3 ppm corresponding to the

CH_2 and a peak at 196.4 ppm corresponding to a carbonyl carbon. The IR spectrum showed a signal at 1679 cm^{-1} which suggested that a carbonyl group was present. Although the reaction with trifluoroacetic acid afforded the ketone **93**, the reaction did not go to completion and was therefore low yielding (34%). On a larger scale (4 mmol) the yield decreased further to only 4%. Due to the low yield it was not a reasonable method to use to hydrate the alkyne.

The use of microwave irradiation in water[69,95] or in conjunction with a Brønsted acid has shown good results in the literature for the hydration of alkynes.[69,71,95,96] It has been thought that in pure water the H_3O^+ catalyses the reaction and the addition of protic acids increases the reaction rate.[71] The alkyne **89** was reacted with a solution of hydrochloric acid (0.5 M) by microwave irradiation at 180 °C for 60 minutes. The ^{1}H NMR spectrum of the crude material showed the key diagnostic signal at 4.27 ppm. The desired ketone was isolated by column chromatography in 20% yield. Initial studies have shown the alkyne **89** was only slightly soluble under these conditions and the reaction does not go to completion. Therefore this reaction required further investigation.

2.2.2 *N*-Oxides

As the previous reactions were low yielding, the formation of the *N*-oxide was investigated to prevent the protonation of the nitrogen in the pyridine ring (Table 3). The use of hydrogen peroxide and acetic acid at 70 °C to oxidise the 3-bromopyridine **68** gave no reaction. Using *m*-chloroperbenzoic acid in dichloromethane at room temperature produced the *N*-oxide **94** in high yield (82%). The ^{1}H NMR spectrum showed a multiplet at 8.09−8.14 ppm and a triplet at 8.32 ppm corresponding to the hydrogens next to the nitrogen in the pyridine ring. There was also a doublet of doublets at 7.13 ppm and a multiplet at 7.35−7.39 ppm corresponding to the other two aromatic hydrogens. The reaction product was pure and did not require further purification.

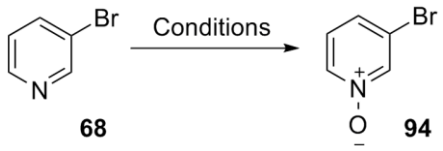
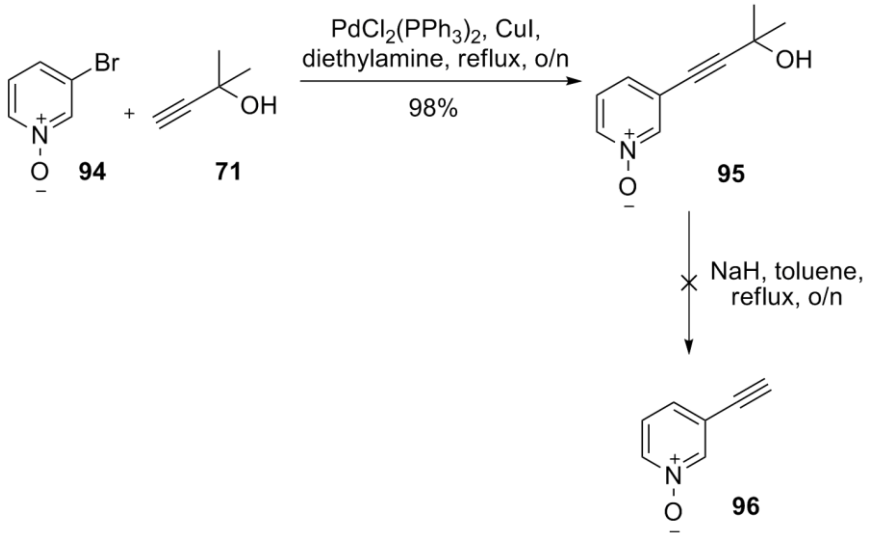


Table 3

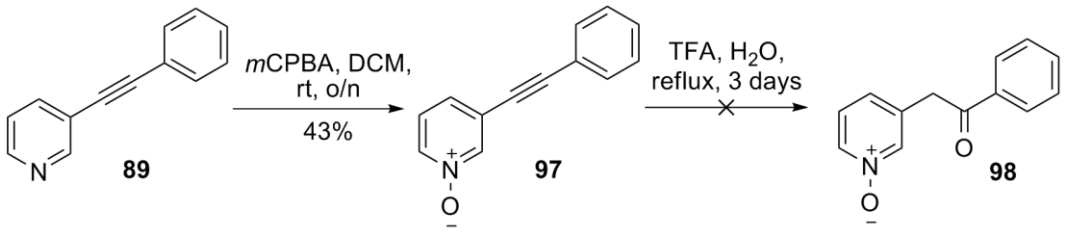
Entry	Conditions	Product
1	H_2O_2, CH_3COOH, 70 °C, 1 day	No reaction
2	*m*CPBA, DCM, rt, o/n	82%

The *N*-oxide **94** and 2-methyl-3-butyn-2-ol **71** were heated under reflux with $PdCl_2(PPh_3)_2$ and copper iodide in diethylamine to give the protected alkyne **95** in high yield (98%) (Scheme 17). The ^{1}H NMR spectrum showed broad peaks at 7.21, 8.12, and 8.22 ppm corresponding to the aromatic hydrogens. The singlet at 1.59 ppm with the integration of six hydrogens corresponded to the two methyl groups and the singlet at 3.47 ppm corresponded to the OH group. The protected alkyne **95** was difficult to purify and was used without further purification in the next step using the previously established deprotection conditions. The alkyne **95** was heated with sodium hydride in toluene under reflux, but under these conditions gave a complex mixture of products.



Scheme 17

An alternate approach was to form the *N*-oxide of the alkyne **89** in the step before the hydration reaction. The alkyne **89** was heated with *m*-chloroperbenzoic acid in dichloromethane under reflux to give the *N*-oxide **97** (Scheme 18). The ^{1}H NMR spectrum showed multiplets at 7.21−7.28 ppm, 7.33−7.41 ppm and 7.49−7.56 ppm corresponding to the aromatic hydrogens. The doublet at 8.18 ppm and singlet at 8.34 ppm corresponded to the hydrogens next to the nitrogen on the pyridine ring. The yield (43%) was much lower than with 3-bromopyridine (Table 3, 82%). This could be due to the formation of the *N*-oxide being a more complicated system and the alkyne being oxidised to form unwanted by-products. The next step was the formation of the ketone. Using the previously established hydration condition of trifluoroacetic acid in water gave no reaction. As the *N*-oxides **94**−**97** were difficult to purify, this route was abandoned.



Scheme 18

2.2.3 Optimisation study on the hydration of alkynes using microwave irradiation

The hydration of the alkyne **89** showed promising results by microwave irradiation (Section 2.2.1). The hydration reaction using microwave irradiation was investigated by heating 0.5 mmol of the alkyne and changing the solvent system (Table 4). The use of only water at the maximum temperature (195 °C) for 20 minutes produced a mixture of degradation products (entry 2). The addition of ethanol was used to increase the solubility. The alkyne **89** was reacted with a solution of hydrochloric acid (0.5 M) and ethanol by microwave irradiation at 180 °C for 90 minutes (entry 3), but only trace amounts of the desired ketone was observed in the ^{1}H NMR spectrum of the crude material. Another similar method used on internal aryl alkynes involved the use of camphorsulfonic acid and ethanol.[96] The reaction of the alkyne

89 with camphorsulfonic acid and ethanol in a microwave at 150 °C for 30 minutes was ineffective with no reaction occurring (entry 4). The use of 2 M hydrochloric acid was also ineffective with only starting material being recovered (entry 5). The concentration of the solution of hydrochloric acid was decreased from 2 M to 0.5 M and was heated at 170 °C for 20 minutes under microwave irradiation (entry 6). This produced trace amounts of the product as seen in the ^{1}H NMR spectrum of the crude material. This suggested that a dilute acid needs to be present for the reaction to occur. It also appears that the key temperature of 180 °C is required for the reaction to occur. The low yielding reaction could be due to the solubility of the alkyne in the solution of hydrochloric acid.

Table 4. Standard condition: 0.5 mmol alkyne

Entry	Conditions		Yield
1	0.5 M HCl	180 °C 60 min	20%
2	Water	195 °C 20 min	Degradation
3	0.5 M HCl, EtOH	180 °C 90 min	Trace
4	CSA, EtOH	150 °C 30 min	SM
5	2 M HCl	170 °C 20 min	SM
6	0.5M HCl	170 °C 20 min	Trace
7	TFA, H_2O, Sodium dodecyl sulfate	170 °C 20 min	Trace

In the previous study on Brønsted acids (Section 2.2.1), it was identified that trifluoroacetic acid hydrated the alkyne in low yields. Therefore trifluoroacetic acid was substituted for the 0.5 M hydrochloric acid to see if microwave irradiation could assist the reaction to go to completion (Table 4, entry 7). Unfortunately only trace amounts of the product was observed in the ^{1}H NMR spectrum of the crude material.

As the alkyne **89** is insoluble in water a few surfactants were explored (Table 5). The addition of benzyltriethylammonium bromide and tetrabutylammonium bromide to the reaction mixture before microwave irradiation produced trace amounts of product whilst the use of sodium dodecyl sulfate gave 14% yield. These reactions were done at a high temperature (190 °C) and were reacted for a longer period of time (60 min). The surfactant sodium dodecyl sulfate was used to investigate the reaction further.

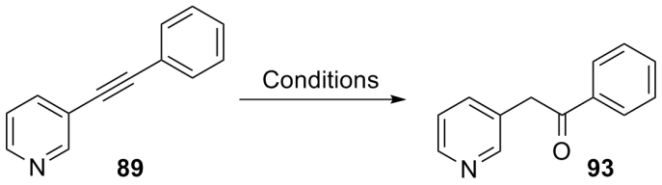


Table 5. Standard conditions: 0.5 mmol alkyne, 0.5 M HCl, PTC, microwave irradiation 190 °C, 60 min.

Entry	PTC	Yield
1	Benzyltriethylammonium bromide	Trace
2	Tetrabutylammonium bromide	Trace
3	Sodium dodecyl sulfate	14%

The temperature and time of the microwave reaction were investigated further using a solution of hydrochloric acid (0.5 M) and sodium dodecyl sulfate as a surfactant (Table 6). At 150 °C for 10 minutes only starting material was observed in the ^{1}H NMR spectrum (entry 2). By increasing the temperature to 170 °C for 10 minutes trace amounts of the ketone **93** was observed in the ^{1}H NMR spectrum of the crude material but the majority was starting material (entry 3). Upon increasing the time of the reaction to 20 minutes the yield increased to 27% (entry 4) but decreased to 3% when reacting for 60 minutes (entry 5). This was due to the increase of degradation by-products forming. The reaction was heated to the maximum temperature (195 °C) to get the reaction to go to completion (entry 6). Signals corresponding to the starting material were no longer present in the ^{1}H NMR spectrum and the ketone yield increased (20%) but there were more degradation and side reactions occurring. The best conditions observed were heating 0.5 M hydrochloric acid and sodium dodecyl sulfate to 170 °C for 20 minutes by microwave irradiation. The yield (27%) was still too low and therefore this method did not seem a viable way of synthesising the ketone.

Table 6. Standard conditions: 0.5 mmol alkyne, 0.5 M HCl, sodium dodecyl sulfate.

Entry	Temperature/Time	Yield
1	190 °C 60 min	14%
2	150 °C 10 min	SM
3	170 °C 10 min	Trace
4	170 °C 20 min	27%
5	170 °C 60 min	3%
6	195 °C 60 min	20%

2.2.4 Transition metals

The use of Brønsted acids and microwave irradiation were shown to be ineffective for the hydration of the alkyne **89** in high yields. Another method that has shown recent interest in the literature for the hydration of alkynes uses transition-metal-complexes as catalysts. The most common catalyst used in hydration reactions are mercury catalysts, especially mercury(II) sulfate under acidic conditions.[71] This can be obtained by using mercury(II) oxide in aqueous sulfuric acid and diluted in an organic solvent.[71] The alkyne **89** was heated under reflux with mercury(II) oxide in sulfuric acid, water and methanol (Table 7, entry 1). The ^{1}H NMR spectrum of the crude material showed a complex mixture. This suggested that under these reaction conditions the starting material could be decomposing. This could be due to the nitrogen in the pyridine ring affecting the reaction

Table 7

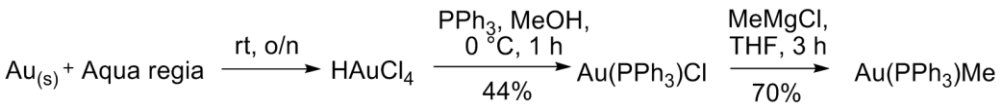
Entry	Conditions	Product
1	HgO 0.8 eq., MeOH, H_2O, H_2SO_4, reflux, o/n	Degradation
2	$RuCl_3 \cdot H_2O$ 0.6 eq., H_2O, HCl, reflux, o/n	Degradation
3	$K[AuBr_4]$ 0.2 eq., MeOH, H_2O, reflux, o/n	No reaction
4	$Au(PPh_3)Me$ 7.6 eq., MeOH, H_2O, H_2SO_4, reflux, o/n	28%

Ruthenium catalysts have also been investigated for the hydration of alkynes.[71,97] The alkyne **89** was heated with ruthenium(III) chloride in hydrochloric acid and water under reflux (Table 7, entry 2). The ^{1}H NMR spectrum of the crude material showed a complex mixture of products. The ^{1}H NMR spectrum of the crude reaction mixture clearly did not show the key signal for the CH_2 peak at 4.27 ppm, suggesting the reaction did not occur.

One of the more recent investigations in the literature was the use of a gold catalyst which has shown significant results in the hydration of internal alkynes.[71] Gold(III) catalysts have also been shown to be successful in unactivated alkynes.[89] The alkyne **89** was heated under reflux with $K[AuBr_4]$ in methanol and water,[89] however no reaction occurred. Using an alternate gold(I) catalyst, $Au(PPh_3)Me$,[70] the alkyne **89** dissolved in sulfuric acid, water and methanol under reflux produced the desired ketone **93**. The spectral data matched that in the literature.[66] The reaction was low yielding (28%) and was not reproducible. Nevertheless, this reaction was promising and was investigated further.

2.2.5 Synthesis of methyl(triphenylphosphine) gold

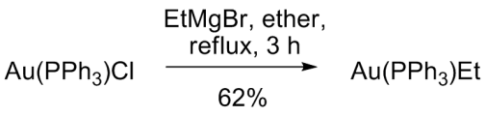
The hydration of the alkyne was investigated using $Au(PPh_3)Me$ as the catalyst. As the gold catalyst can be expensive to purchase (AU$233 for 500 mg),[98] the synthesis was investigated. Although $Au(PPh_3)Me$ has been used extensively in the literature,[70,90–92,99–102] a clear synthesis to prepare the catalyst has yet to be reported from gold. The preparation of the gold catalyst is reported here from a gold coin (Scheme 19). The gold coin (shown in Figure 25a) was dissolved in a mixture of nitric acid and hydrochloric acid (1:4) to give tetrachloroauric acid as an orange solution (Figure 25b).[100] The gold had nearly dissolved after two hours but was left overnight to ensure complete dissolution. The tetrachloroauric acid was dissolved in methanol and was added dropwise to a solution of triphenylphosphine in methanol.[101] A white precipitate formed immediately on the addition of the chloroauric acid and the solution slowly turned yellow as the mixture became more concentrated. The precipitate was filtered off to give $Au(PPh_3)Cl$ as a fluffy white solid (Figure 25c). The ^{1}H NMR spectrum showed a multiplet at 7.45−7.55 ppm corresponding to the triphenylphosphine hydrogens. The ^{13}C NMR spectrum showed six carbon signals between 129−134 ppm and were in the aromatic region. The ^{31}P NMR spectrum showed only one signal at 33.75 ppm that was different from the uncoordinated triphenylphosphine unit (−4.78 ppm). This compound was stable at room temperature under normal atmospheric conditions after two years. $Au(PPh_3)Cl$ was converted to $Au(PPh_3)Me$ by reacting with methylmagnesium chloride in anhydrous tetrahydrofuran.[100] The spectral data matched that in the literature.[90] The ^{31}P NMR spectrum showed a peak at 47.96 ppm which was different from the starting material (33.75 ppm) and the uncoordinated triphenylphosphine unit (−4.78 ppm). On a small scale fine white crystals were obtained (Figure 25d) but when done on a larger scale the crystals were slightly purple. This could be due to the colloidal gold not being fully removed in the first filtration step, however this did not appear to affect the catalyst.



Scheme 19

Figure 25. Gold coin (top left); $HAuCl_4$ (top right); $Au(PPh_3)Cl$ (bottom left); $Au(PPh_3)Me$ (bottom right).

The ethyl analogue was also synthesised by heating $Au(PPh_3)Cl$ with ethylmagnesium bromide in anhydrous ether to give $Au(PPh_3)Et$ as white crystals (Scheme 20). The yield (62%) was similar to the methyl analogue (59%).



Scheme 20

2.2.6 Gold catalysed hydration

As mentioned previously, the alkyne **89** was reacted with $Au(PPh_3)Me$ in a mixture of sulfuric acid, methanol and water to give the desired ketone **93** in 28% yield but the reaction was not reproducible. A variety of different conditions were investigated to optimise the reaction using the gold catalyst (Table 8). It has been reported that the addition of ethyl diphenylphosphinite or phosphite prevents catalyst deterioration.[70] In this case the addition of triethylphosphite did not improve the reaction as only trace amounts of the product were observed in the ^1H NMR spectrum of the crude

material (entry 2). The addition of unbound triphenylphosphine also gave no reaction (entry 3) which is consistent with that reported in the literature.[70] Replacing sulfuric acid with triflic acid[70] or hydrochloric acid also gave no reaction (entry 4 and 5). Doubling the volume of sulfuric acid led to degradation (entry 6). The system also appeared to require oxygen free conditions. When there was oxygen present in the reaction only trace amounts of the product was observed in the ^{1}H NMR spectrum of the crude material (entry 7) but on sparging the reaction mixture with nitrogen before the addition of the catalyst, the yield improved dramatically (entry 8, 90%). It has been reported in the literature that silver salts are useful in gold hydration reactions as an alternate method of forming the active catalyst.[103] The alkyne **89** was heated with $Au(PPh_3)Cl$ and silver triflate in dioxane and water under reflux, however these conditions returned unreacted starting material (entry 9).

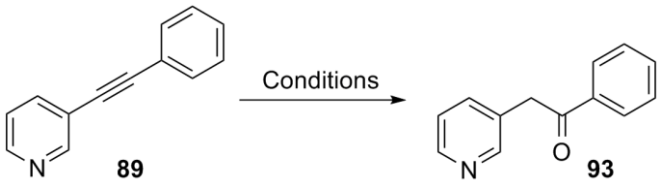
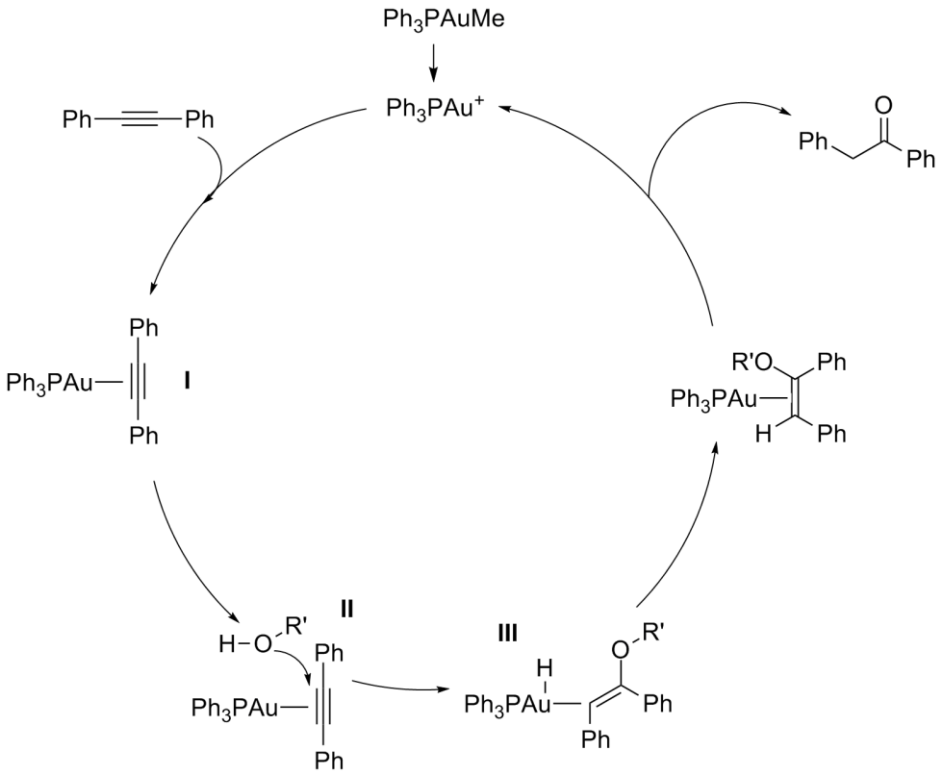


Table 8

Entry	Conditions	Product
1	MeOH, H_2O, H_2SO_4, $Au(PPh_3)Me$, reflux, o/n	28%
2	Addition of triethylphosphite	Trace
3	Addition of triphenylphosphine	No reaction
4	Change acid to triflic acid	No reaction
5	Change acid to hydrochloric acid	No reaction
6	Double volume of acid	Degradation
7	No degassing	Trace
8	Completely degassed system	90%
9	$Au(PPh_3)Cl$, AgOTf, dioxane, H_2O	No reaction
10	*N*,*N*,*N*-Trimethylethylenediamine polymer bound	97%

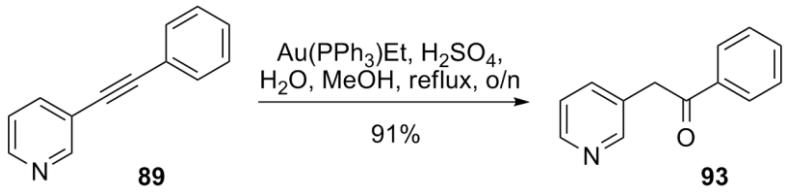
The ideal conditions for the hydration reaction with the gold catalyst $Au(PPh_3)Me$ appeared to be the alkyne dissolved in a mixture of methanol, sulfuric acid and water and an oxygen free system. However the reaction was still not reliable as it would only occasionally work. Sometimes the reaction would be finished in 30 minutes; in other cases it would take overnight for the reaction to go to completion. Therefore other impurities could be poisoning the catalyst. Kumar *et al.* have reported that trace amounts of halides and basic components present in the solvent or starting material could poison the gold catalyst.[104] Therefore HPLC grade methanol and MilliQ water were utilised to improve the purity of the solvent. The ^{31}P NMR spectrum of the alkyne **89** showed a signal at 29.09 ppm which was not $PdCl_2(PPh_3)_2$, triphenylphosphine or triphenylphosphine oxide. This could indicate that there was some of the catalyst by-products present in the starting material from the Sonogashira reaction which could be inhibiting the reactivity of the gold catalyst. This was consistent with literature which discussed the product from Sonogashira reactions might have a high palladium contamination.[81] A metal scavenger was investigated to clean the alkyne before the reaction. *N*,*N*,*N*-Trimethylethylenediamine polymer bound (Sigma-Aldrich, catalogue #656836) is a metal scavenger used to remove trace metals from solution, including Cu(I), Cu(II) and Pd(II). The alkyne was dissolved in dichloromethane and stirred overnight with the metal scavenger before being filtered off. The metal scavenger started off as a pale cream beads but after filtering off they had swollen slightly and were dark brown. This indicated that although the alkyne looked pure in the ^1H NMR spectrum, there were possible metal contaminants that could be interfering with the gold catalyst. Upon reacting the clean alkyne with $Au(PPh_3)Me$, HPLC grade methanol, sulfuric acid and MilliQ water the desired ketone was produced in high yields (97%).

The mechanism for hydration by gold catalysis has been proposed by Mazzone *et al*. (Scheme 21).[105] The Au(I) complex coordinates to the triple bond to give the Au-π-alkyne complex (I). The R'OH adds to the alkyne forming the intermediate (III). Protodeauration of the intermediate gives the *E* isomer of the enolic product and releases the catalyst. Hydrolysis of the enol ether under the reaction conditions leads to the formation of the ketone product.[105]



Scheme 21. Proposed mechanism for the Au(I)-catalysed hydration of an alkyne. Adapted from Mazzone *et al*.[105]

The ethyl gold catalyst was also trialled under the optimised conditions. The alkyne **89** was heated with Au(PPh_3)Et in HPLC grade methanol, sulfuric acid and MilliQ water under reflux in an oxygen free system (Scheme 22). This produced the desired ketone **93** in 91% yield which was similar to the methyl catalyst (Table 8, entry 10, 97%).



Scheme 22

2.2.7 Scope of gold catalysed hydration of internal alkynes

Using the optimised conditions for the hydration of the alkyne **89**, other internal alkynes were explored (Table 9). The alkynes were heated under reflux with $Au(PPh_3)Me$, HPLC grade methanol, sulfuric acid and MilliQ water to give the desired ketones. The fluoro ketone **99** was high yielding (96%) and there were no observable impurities in the ^1H NMR spectrum. The chloro ketone **48** had a slightly lower yield (88%). This was due to impurities present in the sample. Column chromatography was unable to isolate the impurities and the compound was used in the subsequent reaction.

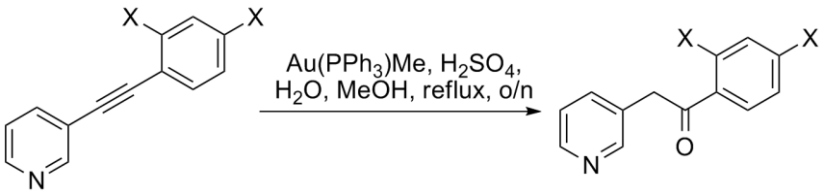


Table 9

Compound	X	Yield
93	H	97%
99	F	96%
48	Cl	88%

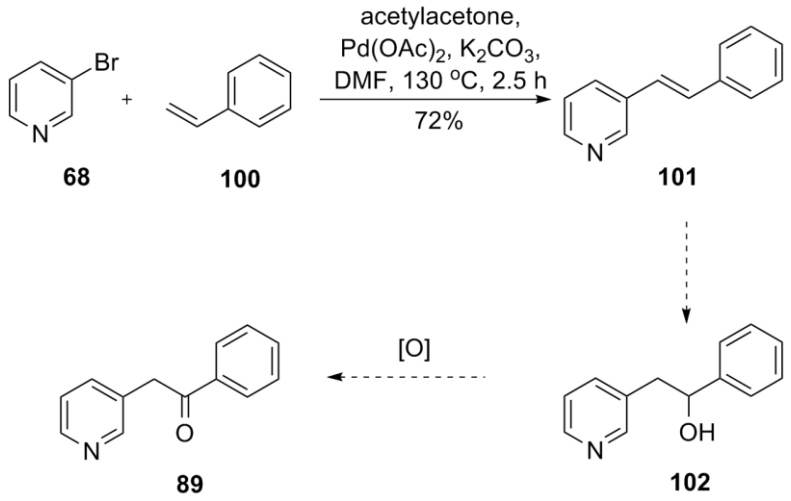
2.3 Alternative reactions

In view of the challenges in producing desired ketone intermediate, a series of alternative synthetic sequences were investigated to generate the key intermediate **65**. These methods include a Heck reaction, a Curtius rearrangement and cross-coupling reactions to form the ketone **65**.

2.3.1 Synthesis of an alkene

One potential route to form the desired ketone was to form the alkene instead of the alkyne. The alkene **101** could be formed via a Heck reaction. The alkene could then be hydrolysed to **102** and then oxidised to the ketone **89**. Following the procedure by Cui *et al*,[106] 3-bromopyridine **68** and styrene **100** were heated at 130 °C with

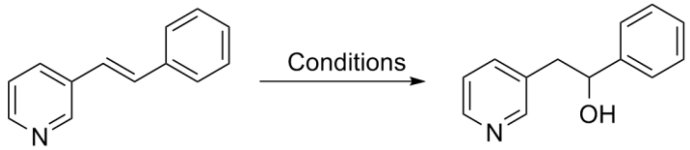
acetylacetone, palladium(II) acetate and potassium carbonate in dry *N*,*N*-dimethylformamide to give the alkene **101** (Scheme 23). The spectral data matched that in the literature.[107] The ^{1}H NMR spectrum showed two doublets at 7.06 ppm and 7.16 ppm corresponding to the alkene. The doublets had a *J* coupling of 16.4 Hz which suggested the double bond was in the *trans* configuration which was the expected product. This reaction worked well and in high yields (72%).



Scheme 23

2.3.2 Hydrolysis of the alkene

The next step was to hydrolyse the double bond. The alkene **101** was subjected to a variety of hydration conditions to form compound **102** (Table 10). Based on the previous result of the hydration of the alkyne, the alkene **101** was heated under reflux with trifluoroacetic acid and water. This returned unreacted starting material. The trifluoroacetic acid might not be a strong enough acid to hydrolyse the alkene. Therefore the acid was changed from trifluoroacetic acid to sulfuric acid. Sulfuric acid is a stronger acid (pK_a −3) compared to trifluoroacetic acid (pK_a −0.5). The alkene **101** was heated in sulfuric acid and water under reflux also produced no reaction. As there had been some results in Section 2.2.3 for the hydration of the alkyne with microwave irradiation this technique was also trialled. The alkene **101** was treated with 0.5 M hydrochloric acid by microwave irradiation at 180 °C for one hour. Unfortunately, only starting material was recovered.

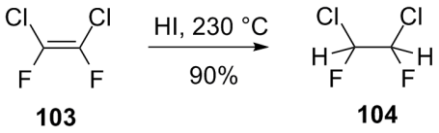


101 **102**

Table 10

Entry	Conditions	Product
1	CF_3COOH, H_2O, reflux, 3 days	No reaction
2	H_2SO_4, H_2O, reflux, 4 days	No reaction
3	HCl, H_2O, microwave 180 °C, 1 h	No reaction

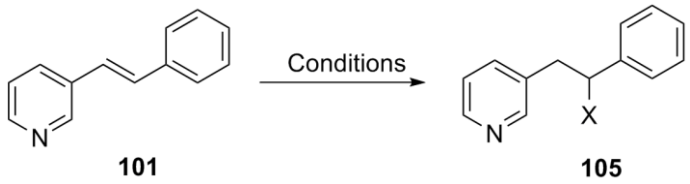
An alternative to hydrolysing the alkene was the addition of a halogen which could then be hydrolysed (Table 11). The alkene **101** was heated to 60 °C with hydrobromic acid (48%) (entry 1). This gave no reaction. Since the bromide gave no reaction, iodide was trialled. Iodides are better nucleophiles than bromides. I^- could be formed *in situ* from potassium iodide and phosphoric acid.[108] When the alkene **101** was heated with potassium iodide and phosphoric acid, a new compound was isolated in 72% yield (entry 2). The ^1H NMR spectrum showed a broad singlet at 8.47 ppm corresponding to the hydrogens next to the nitrogen in the pyridine ring and signals relating to the other seven aromatic hydrogens between 7.14 ppm and 7.47 ppm. There was a multiplet at 2.94−2.96 ppm with the integration of four hydrogens. The ^{13}C NMR spectrum showed seven signals in the aromatic region and two CH_2 signals at 34.9 ppm and 37.5 ppm. This suggested that the alkane was being formed. The spectral data matched that in the literature for the alkane.[109] This suggested the alkene is being hydrogenated under these conditions. This could be due to I^- being oxidised to I_2 thereby reducing the alkene to the alkane. There is a patent which describes a reaction of reducing an alkene **103** to an alkane **104** using anhydrous hydrogen iodide and heating to 230 °C in a sealed vessel for 15 hours (Scheme 24).[110] This procedure was high yielding but used harsh conditions of heating the reaction to high temperatures in a sealed vessel. In comparison, the conditions of potassium iodide and phosphoric acid are less harsh than those described in the patent.



103 **104**

Scheme 24

There have been reports that the surfaces of silica gel and alumina facilitates hydrohalogenation of alkenes by promoting ionic addition through hydrogen-bonding interactions.[111,112] The alkene **101** was treated with oxalyl chloride in the presence of silica gel (Table 11, entry 3). [112] This produced no reaction in this system. The reaction was also trialled with alumina which also gave no reaction.



101 **105**

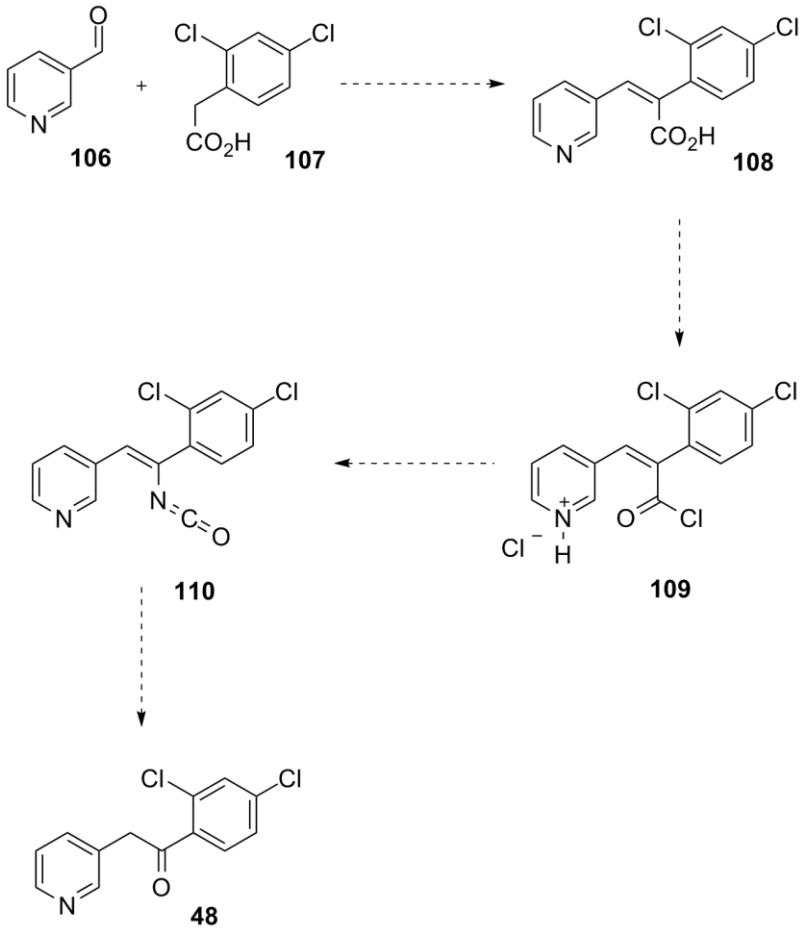
Table 11

Entry	Conditions	Product
1	HBr 48%, 60 °C, o/n	No reaction
2	KI, H_3PO_4, 150 °C, 2 days	X = H, 72%
3	$(COCl)_2$, SiO_2, DCM, rt, 4 h	No reaction

The alkene **101** appears to be fairly unreactive. Therefore this route was abandoned. Nevertheless, an interesting hydrogenation of an alkene was observed with potassium iodide and phosphoric acid reducing the alkene to an alkane.

2.3.3 Curtius rearrangement

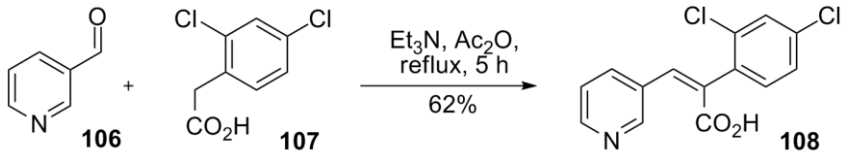
Another potential procedure to form the desired ketone **42** is shown in Scheme 25. An aldol condensation reaction between 3-pyridinecarboxaldehyde **106** and 2,4-dichlorophenylacetic acid **107** would form the reported the carboxylic acid intermediate **108**. Conversion of the carboxylic acid **108** to an acid chloride using thionyl chloride would form the salt **109**. This could be converted to the ketone **48** via a Curtius rearrangement.



Scheme 25. Proposed synthesis of the ketone **48**.

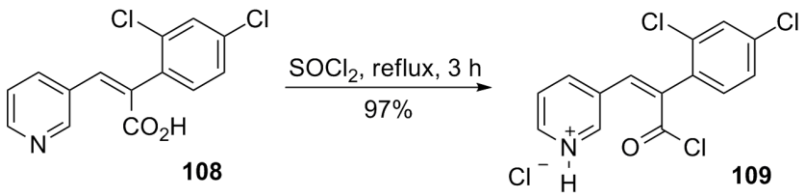
Following a patented procedure, 3-pyridinecarboxaldehyde **106** and 2,4-dichlorophenylacetic acid **107** were heated with triethylamine in acetic anhydride under reflux to give a solid product upon workup (Scheme 26).[113] Recrystallisation of the crude material produced the carboxylic acid **108** in 62% yield. The spectral

data was consistent with that in the literature.[113] A singlet in the ^{1}H NMR spectrum at 7.86 ppm corresponded to the vinylic proton. The ^{13}C NMR spectrum showed a peak at 166.9 ppm corresponding to the carbonyl carbon.



Scheme 26

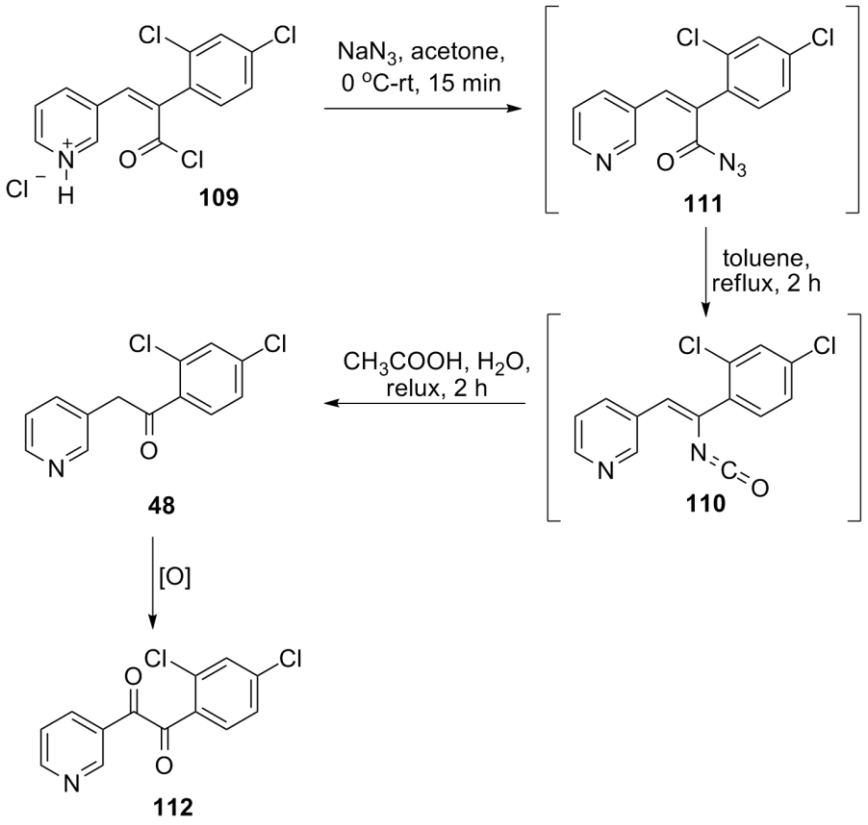
Treating the forgoing acid **108** with an excess of thionyl chloride at reflux produced the acid chloride as the hydrochloride salt (Scheme 27).[113] The excess thionyl chloride was removed via distillation. Trituration of the residue with diethyl ether removed the remaining traces of thionyl chloride. This reaction was high yielding (97%) and was used in the next step without further purification.



Scheme 27

To convert the acid chloride **109** to the ketone **48** required a 3-step synthesis (Scheme 28). The acid chloride **109** was dissolved in acetone and reacted with a solution of sodium azide and water via a substitution reaction to give the azide **111**. The reaction mixture was made basic with a solution of sodium carbonate and the neutral compounds were extracted with toluene. The toluene extract was heated under reflux to give compound **110**. The solvent was removed under reduced pressure and the residue was heated under reflux with acetic acid and water to give the ketone **48** in 44% yield. The spectral data matched that previously synthesised. The key peak of a singlet at 4.25 ppm in the ^{1}H NMR spectrum corresponded to the

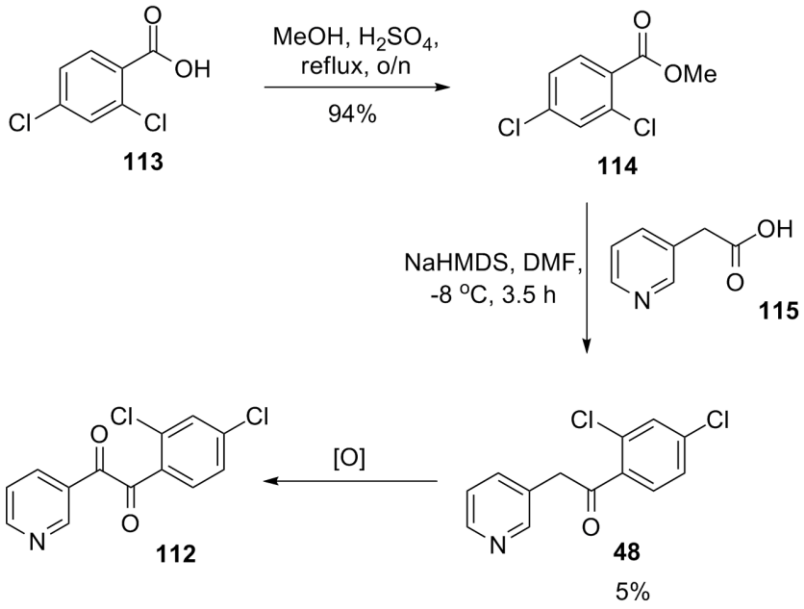
CH_2 between the pyridyl ring and the ketone. There was an impurity observed in the ^{1}H NMR spectrum even after purification which was similar to that observed in the gold method (Section 2.2.7). Over time this impurity increased until the ketone was fully converted to the impurity. Analysis of the spectral data of the isolated impurity suggested that the ketone **48** was converting to form the diketone **112**. The ^{1}H NMR spectrum showed a doublet at 9.24 ppm and a doublet of doublets at 8.90 ppm corresponding to the pyridyl ring and there was an absence of the key CH_2 signal at 4.25 ppm. In the ^{13}C spectrum there were two peaks in the carbonyl region at 191.8 ppm and 190.2 ppm. The absence of the CH_2 peak in the ^{1}H NMR spectrum and the two signals in the carbonyl region for the ^{13}C NMR spectrum suggested the diketone **112** was being formed. Therefore, ketone **48** could not be purified as it was continually being oxidised to the diketone. However, the oxidation was not as rapid in the gold method. The rapid oxidation in this system could be due to an acid or an oxidant present in the ketone **48** during isolation and purification. The best storage conditions to slow down the rapid oxidation were to store the ketone at −20 °C under nitrogen in the dark.



Scheme 28

2.3.4 Condensation reaction

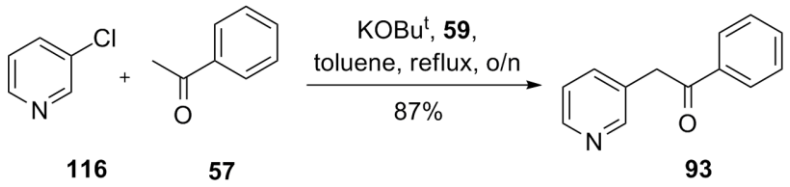
Recently, Wu *et al*. published a direct Claisen-decarboxylation cascade reaction between an α-carboxylic acid and an ester to produce an internal ketone similar to compound **48**.[114] This method was investigated to synthesise the ketone **48** (Scheme 29). The ester was made by according to Potter *et al*.[115] by heating 2,4-dichlorobenzoic acid **113**, methanol and sulfuric acid under reflux to give the desired methyl ester **114** in 94% yield. The spectral data matched that in the literature. The product obtained was pure without further purification. The methyl ester **114** and 2,4-dichlorophenylacetic acid **115** were reacted with sodium bis(trimethylsilyl)amide (1.0 M in THF) in *N*,*N*-dimethylformamide at −8 °C. The ^{1}H NMR spectrum of the crude material showed mainly the methyl ester **114** and trace amounts of the desired ketone **48**. The ^{1}H NMR spectrum of the ketone after column chromatography showed additional peaks not observed in the spectrum of the crude material. The impurity signals match that of the diketone **112** that was identified in Section 2.3.3. This suggested the ketone was being formed and then being oxidised to the diketone **112**. Due to the very low yield of the unstable ketone, this reaction was not investigated further.



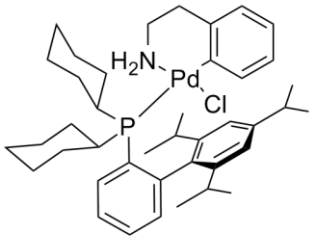
Scheme 29

2.3.5 Palladium catalysed cross-coupling

The final method examined has recently been reported in the literature and involved a cross coupling reaction between an aryl halide and an enolate using a precatalyst as a palladium source.[65] The use of the precatalyst, chloro(2-dicyclohexylphosphino-2′,4′,6′-tri-*iso*-propyl-1,1′-biphenyl)[2-(2-aminoethyl)phenyl]Pd(II) methyl-*tert*-butyl ether adduct **59** and potassium *tert*-butoxide in dry toluene would give the desired monoarylation.[65] Potassium *tert*-butoxide was used as a base to activate the catalyst.[67] 3-Chloropyridine **116** and acetophenone **57** were heated under reflux with **59** and potassium *tert*-butoxide in dry toluene to give the desired ketone **93**. The spectral data matched that previously synthesised. The reaction was high yielding (87%) on a small scale (1 mmol) but on increasing the scale the yield decreased to only 10%. A variety of other acetophenones were also investigated, however no reaction occurred. Due to the high cost of the catalyst and the inconsistent results this method was not investigated further.



116 **57** **93**



59

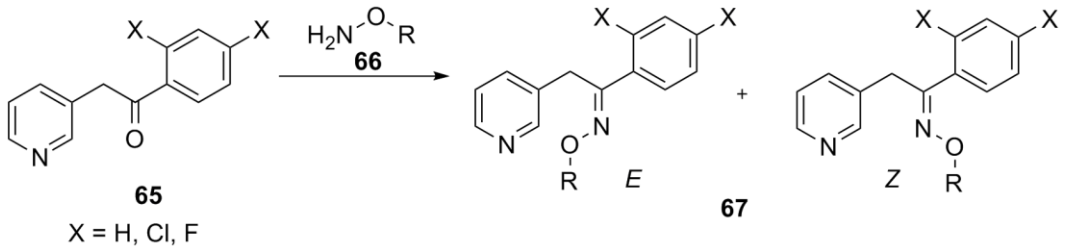
Scheme 30

2.4 Conclusions

A variety of different methods were investigated to synthesise the desired ketone intermediate. The use of the Sonogashira coupling reaction was optimised to synthesise an alkyne which was then hydrated to form the ketone. The alkyne appeared relatively unreactive to many hydration methods. The use of the gold catalyst, $Au(PPh_3)Me$, showed optimum results with the ketone being synthesised in high yields (99%). However, the dichloro ketone **48** could not be purified as it continued to be oxidised to the dione **112**. This could be why there are limited procedures in the literature to synthesise the ketone **48** and pyrifenox **42**.

3 Preparation and anti-trypanosomal activity of pyrifenox analogues

In the previous chapter, a simple synthetic route was established to synthesise the ketone intermediate **65**. The final step to form the pyrifenox analogues was the formation of the oxime **67** (Scheme 31). A condensation reaction of the ketone **65** with a range of alkoxyamines **66** would give the desired oxime **67** as two isomers.



Scheme 31

There are three features to the binding of the molecule. These include the binding of the molecule to the heme, the hydrophobic pocket and the access tunnel. Hargrove *et al*. obtained a crystal structure of UDO **40** bound to the active site CYP51 (Figure

26).[41] The nitrogen in the pyridine ring was observed binding to the iron in the heme and the phenyl ring twisting perpendicular to the pyridine ring in the hydrophobic pocket. The longer chain was orientated down the access channel. It was thought that pyrifenox and its analogues would bind to CYP51 in a similar way.

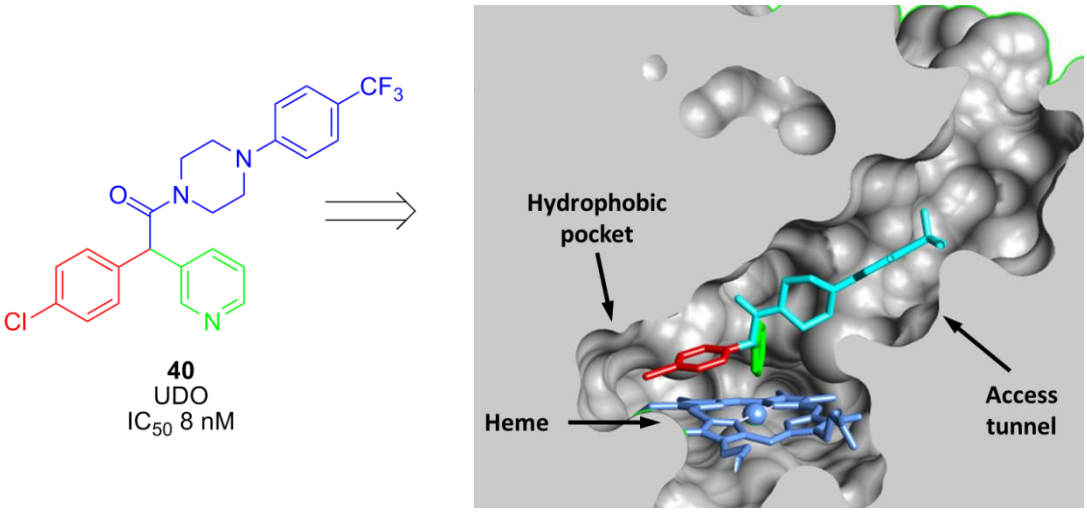


Figure 26

Initially this research focused on changing the substituents on the phenyl ring and having a methyl oxime. The substituents that were investigated were hydrogen, fluorine and chlorine to determine the steric bulk required to fit into the hydrophobic pocket. The steric bulk and length of the oxime chain were then investigated to determine the fit inside the access tunnel. As two isomers should be formed the stereochemistry of the oxime would also be considered.

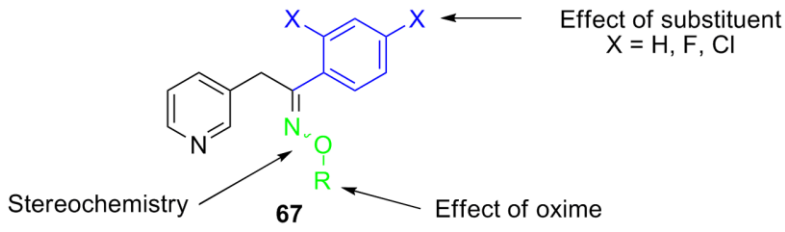
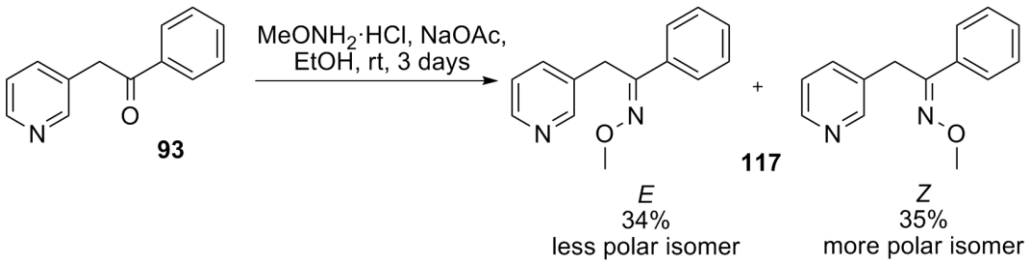


Figure 27. Modification of pyrifenox.

3.1 Methyloxime analogues

The final step in the synthesis was the formation of the oxime. To synthesise pyrifenox, Dorn[60] heated a mixture of the dichloro ketone **42**, *O*-methylhydroxylamine hydrochloride and sodium carbonate in ethanol under reflux to give the methyl oxime as two isomers. This appeared to be the most popular method in the literature for the formation of oximes.[60,116–118] The ketone **93** was stirred with *O*-methylhydroxylamine hydrochloride and sodium acetate in ethanol at room temperature (Scheme 32).[117] The ^{1}H NMR spectrum of the crude material showed four singlets between 3.8 ppm and 4.2 ppm. These peaks corresponded to the CH_2 and the methyl groups of two isomers. The two isomers were isolated by column chromatography due to slightly different polarities. The ^{1}H NMR spectrum of the less polar isomer showed a singlet at 4.02 ppm with the integration for three hydrogens and a singlet at 4.12 ppm with the integration of two hydrogens (Figure 29). These peaks corresponded to the methyl oxime and the CH_2. The signals in the ^{1}H NMR spectrum of the more polar isomer are more shielded. The methyl signal was seen at 3.88 ppm and the CH_2 signal at 3.86 ppm. The high resolution mass spectrometry showed that both compounds have the same accurate mass, thus indicating that both isomers were synthesised.



Scheme 32

The two isomers were successfully synthesised and isolated, and the identification of the stereochemistry was investigated. NOE experiments were conducted but it was not possible to determine the configuration of the two isomers unambiguously as there were no enhancements between the methyl group and any other hydrogen. The stereochemistry was determined based on the chemical shifts in a similar system in

the literature where a biphenyl system was used.[119] The ^{1}H NMR spectra of the methyl and CH_2 signals were compared (Figure 28). In compound **118** the less polar *E* isomer showed the CH_2 singlet at 4.05 ppm and the methyl singlet at 3.92 ppm. This was similar to the less polar isomer in the pyrifenox analogue **117**. The more polar *Z* isomer in the biphenyl system **118** showed the CH_2 singlet at 3.73 ppm and the methyl singlet at 3.80 ppm. This was similar to the *Z* isomer of the pyrifenox analogue **117**. Therefore it could be proposed that the less polar isomer was the *E* isomer and the more polar isomer was the *Z* isomer. In the biphenyl system **118** for the *E* isomer the CH_2 signal was more downfield than the methyl signal. For the *Z* isomer both signals were more shielded and the methyl signal was shifted slightly downfield to the CH_2. This was similar to what was seen in the *E* and *Z* isomers of compound **117** (Figure 29). The overall yield was 67% with a ratio of the isomers 3:1 as an *E*/*Z* mixture as observed in the ^{1}H NMR spectrum of the crude material. Although the *E* isomer was the favoured isomer only 34% was isolated compared to the *Z* isomer 35%. This suggested that some of the *E* isomer was lost during purification.

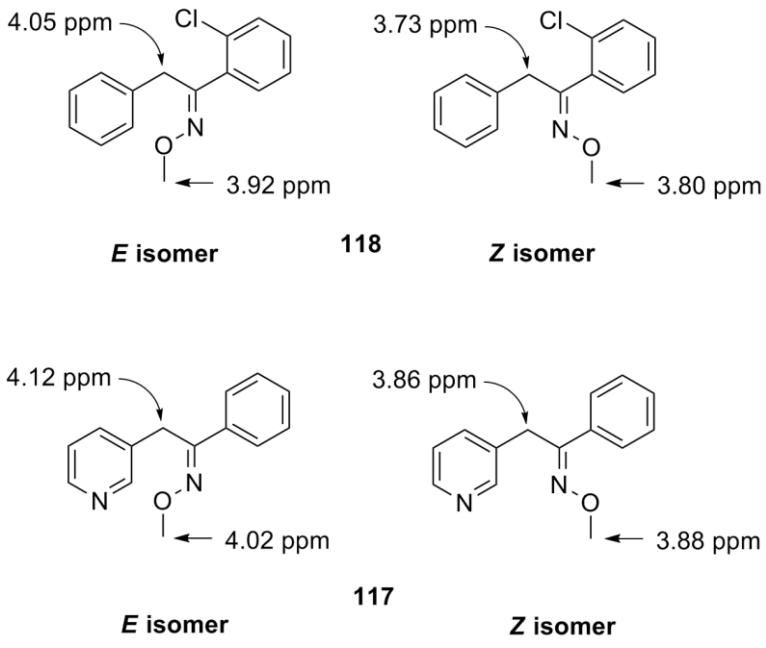


Figure 28. Comparison of a biphenyl system **118** ^{1}H NMR signals for the CH_2 and methyl to compound **117**.

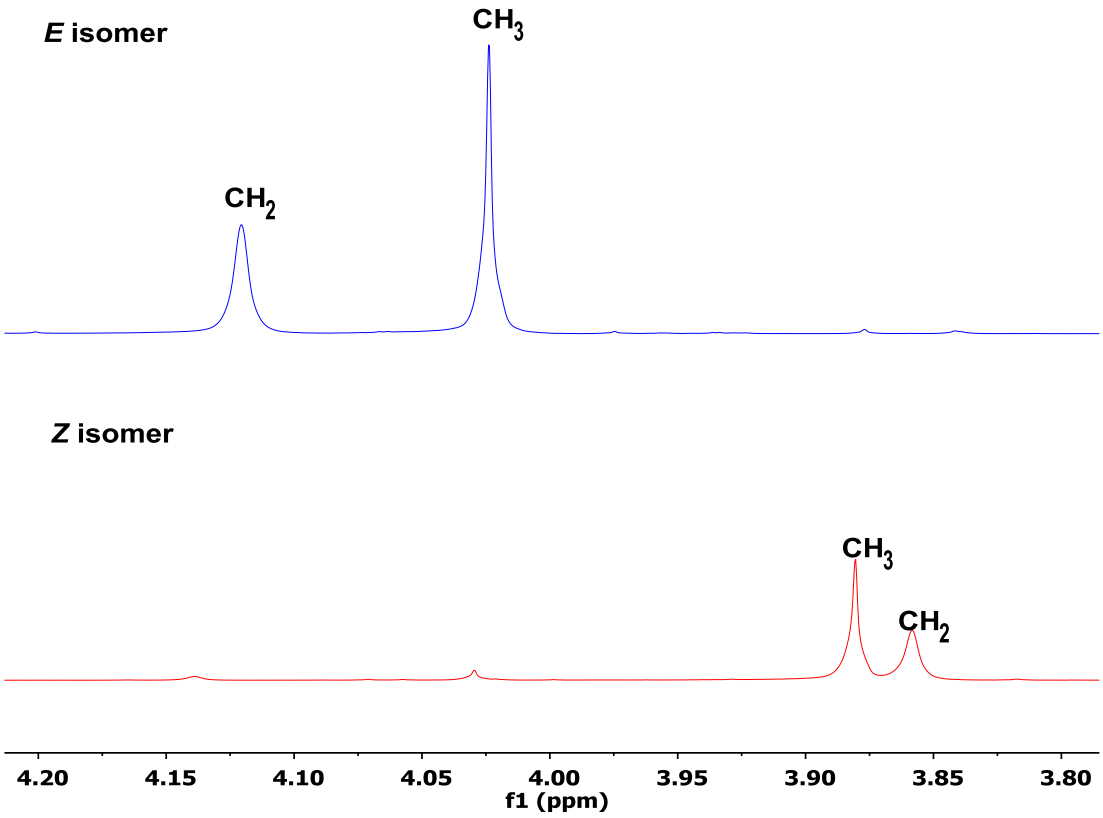
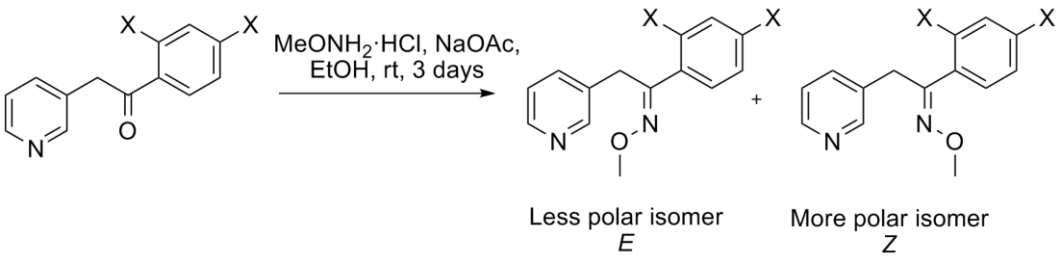


Figure 29. Chemical shifts of the *E* and *Z* isomers of compound **117** for the CH_2 and methyl signals.

The same conditions were used to synthesise the methyl oxime for the fluoro and chloro substituted compounds. The ketone was stirred with *O*-methylhydroxylamine hydrochloride and sodium acetate in ethanol at room temperature (Scheme 33). The fluoro methyl oxime **119** was successfully isolated with a ratio of isomers 2.4:1 with the less polar isomer being the major isomer. The ^{1}H NMR spectrum of the less polar isomer showed a singlet at 4.00 ppm with the integration of three hydrogens and a singlet at 4.08 ppm with the integration of two hydrogens, which corresponded to the methyl oxime and the CH_2 respectively. The ^{1}H NMR spectrum of the more polar isomer showed a singlet at 3.83 ppm with the integration of two hydrogens and a singlet at 3.87 ppm with the integration of three hydrogens, corresponding to the CH_2 and the methyl oxime respectively. Based on the proposed structure outlined for compound **117**, it can be proposed that the *E* isomer was the less polar isomer, and was favoured synthetically over the more polar *Z* isomer.



Scheme 33

Compound	X	Yield	
		E	***Z***
119	F	45%	19%
42	Cl	Complex mixture	

The synthesis of pyrifenox **42** was problematic. The *E* and *Z* isomers were the major products, however extra singlets were seen in the ^{1}H NMR spectrum associating to methyl groups. Spontaneous oxidation of the ketone **42** to the diketone **112** was observed in Chapter 2 and the diketone could then react with *O*-methylhydroxylamine forming the methyl oximes on both ketones as a mixture of isomers (Figure 30). Due to the very similar polarities of the mixture of compounds the *E* and *Z* isomers were unable to be purified.

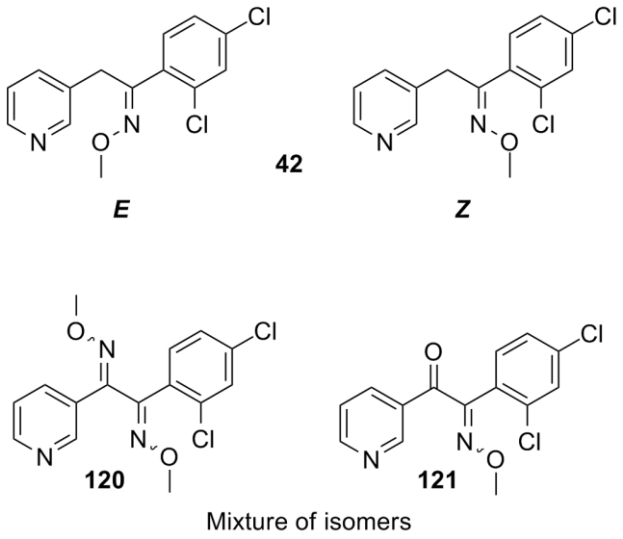


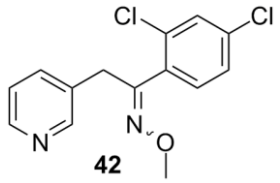
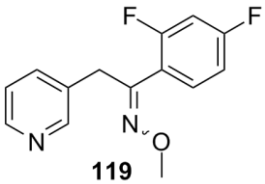
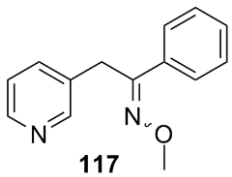
Figure 30

The methyl oximes **117** and **119** were successfully synthesised and were analysed *in vitro T. cruzi* assays. Unfortunately pyrifenox **42** was unable to be purified and was not investigated further.

3.1.1 Methyl oxime inhibition of *T. cruzi*

The pyrifenox analogues were assayed in an *in vitro* whole cell parasite assay by the School of Veterinary and Biomedical Science at Murdoch University (Table 12). The compounds were incubated in L6 cells against *T. cruzi* Tulahuèn strain that expresses the β-galactosidase gene. Benznidazole (Epichem Pty Ltd) was used as a control. The cytotoxicity was determined as a counter-screen in L6 cells. The same conditions were used in subsequent testing throughout this thesis.

Table 12. Inhibition and toxicity of the methyl oxime analogues against *T. cruzi*.

Structure	IC_{50} (toxicity)	
	E	***Z***
 **42**	0.29 µM	
 **119**	0.67 µM (>100 µM)	2.31 µM (>100 µM)
 **117**	>10 µM (>100 µM)	>10 µM (>100 µM)

The methyl oximes synthesised were tested for their ability to inhibit *T. cruzi* (Table 12). The pyrifenox that was initially analysed was a mixture of *E* and *Z* isomers with a ratio 2:1 and had an IC_{50} of 0.29 µM. The fluoro methyl oximes **119** were isolated and both *E* and *Z* isomers were tested. The *E* isomer had an IC_{50} of 0.67 µM and the

Z isomer had an IC_{50} of 2.31 µM. This showed that changing the chlorine substituents on the phenyl ring to fluorine substituents increased the IC_{50}, but not significantly. The fluoro compound was also non-cytotoxic (>100 µM). The stereochemistry also appeared to be important. The *E* isomer was three times more potent than the *Z* isomer. The unsubstituted phenyl ring methyl oxime **117** had an IC_{50} greater than 10 µM for both isomers. This suggested that the phenyl ring requires bulky substituents to bind better into the hydrophobic pocket of CYP51.

Visualisation of the compounds bound to CYP51 provided some insight to the above results. As can be seen in Figure 31, the nitrogen in the pyridine ring binds to the heme iron in the enzyme. The phenyl ring has twisted slightly to become perpendicular to the pyridyl ring. The substituted phenyl ring appears to orientate into the hydrophobic pocket in the enzyme. The chloro substituents are larger than fluoro and hydrogen atoms. As the pyrifenox showed a better biological activity than compounds **117** and **119**, the chlorines must be required to fill the hydrophobic pocket to interact better within the enzyme. The *Z* isomer also appears to not fit into the active site. The results obtained for the *Z* isomer could be due to the isomerisation of the *Z* isomer to the *E* isomer within the parasite by an acid catalysed conversion.

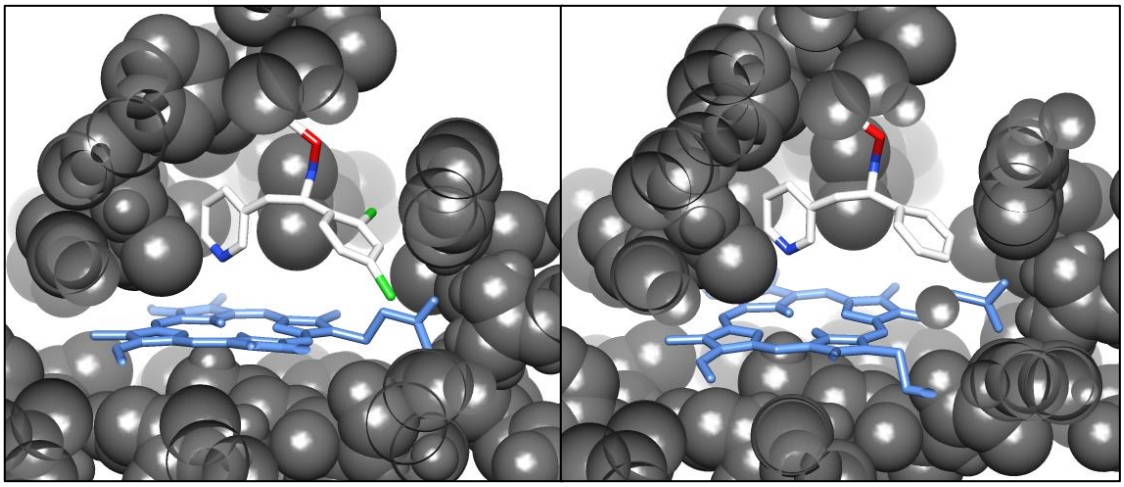
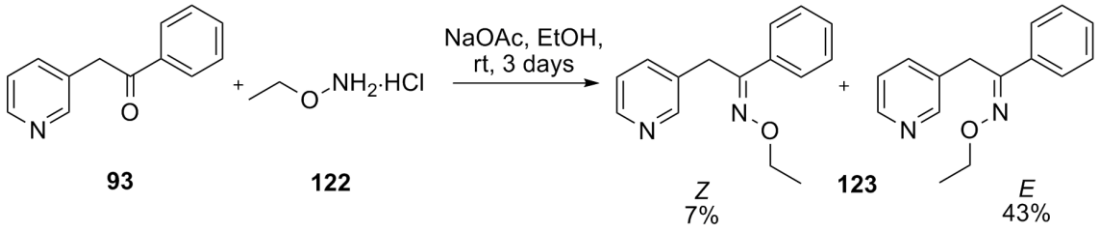


Figure 31. Visualisation of the methyl oxime analogues **70** and **69** binding in CYP51.

3.2 Longer chain oximes

To take advantage of extra hydrophobic interactions in the access tunnel, larger, bulkier oximes could be utilised. The longer oximes could potentially fit into the enzyme better than the methyl oxime. The increased interactions with the hydrophobic tunnel could be used to increase the selectivity and potency of the compound. Having successfully synthesised the methyl oximes **117** and **119** more complicated oximes were sought. In Section 3.1.1, it was shown that the chloro substituents on the phenyl ring showed the best activity but the ketone intermediate was difficult to synthesise due to it being oxidised to the diketone. Therefore the simpler unhalogenated ketone **93** was used to synthesise other oximes.

The first oxime that was investigated was increasing the chain from a methyl to an ethyl. The ketone **93** was stirred with *O*-ethoxylamine hydrochloride **122** and sodium acetate in ethanol (Scheme 34). The ethyl oxime **123** was seen as two isomers in the ^{1}H NMR spectrum of the crude material as a ratio of 2:1 (Figure 32). It was only possible to fully purify and characterise the less polar isomer. The ^{1}H NMR spectrum showed a singlet at 4.22 ppm corresponding to the CH_2, a quartet at 4.29 ppm and a triplet at 1.32 ppm corresponding to the ethyl oxime. Based on the proposed structure for the methyl oxime it can be suggested that the *E* isomer was isolated. The *E* isomer and a mixture of *E/Z* isomers were tested against *T. cruzi*.



Scheme 34

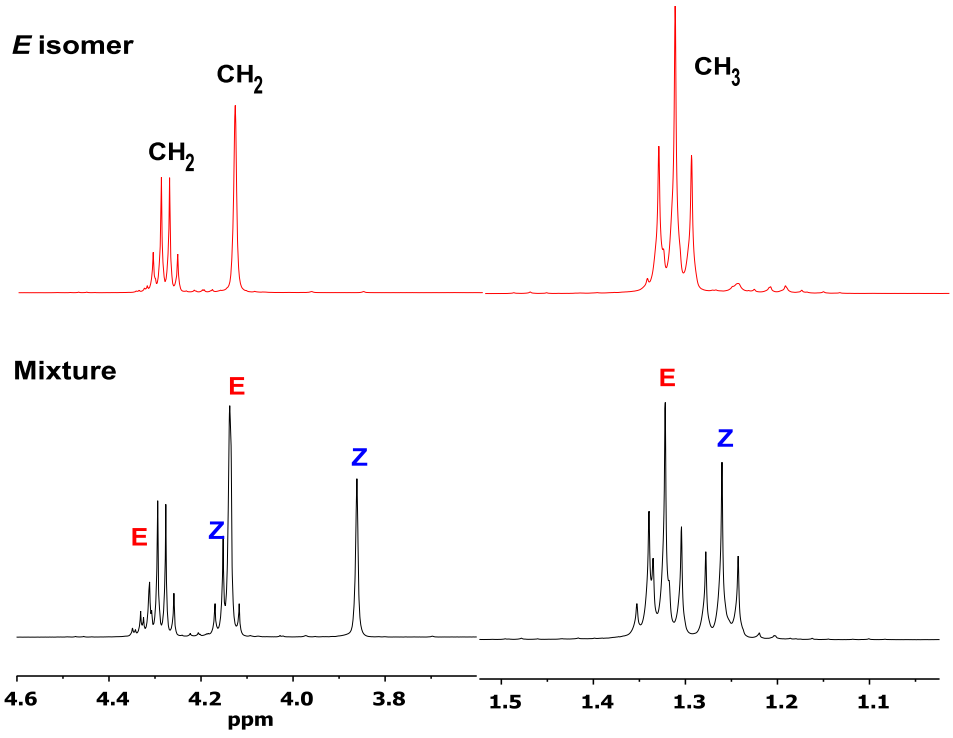
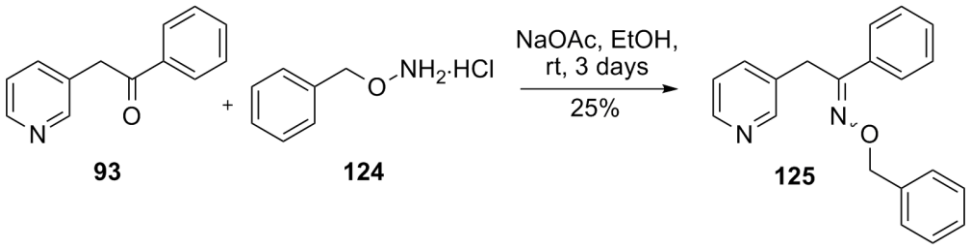


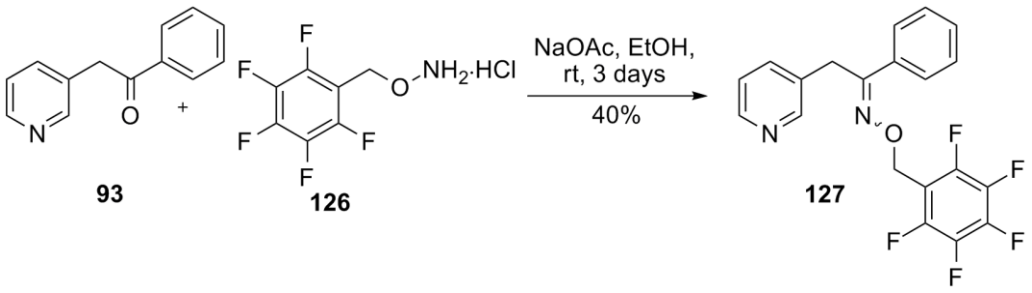
Figure 32. The ^{1}H NMR spectrum of the purified *E* isomer **123** (red) and a mixture of the two isomers.

The bulkiness of the oxime was also considered. The ketone **93** was stirred with *O*-benzylhydroxylamine hydrochloride **124** and sodium acetate in ethanol (Scheme 35). The ^{1}H NMR spectrum of the crude material suggested there were two isomers synthesised by the presence of two singlets at 5.18 ppm and 5.05 ppm corresponding to the CH_2 of the oxime and two singlets at 4.06 ppm and 3.74 ppm corresponding to the CH_2 between the oxime and pyridyl ring. It was only possible to purify one isomer by column chromatography, which was identified as the *E* isomer.



Scheme 35

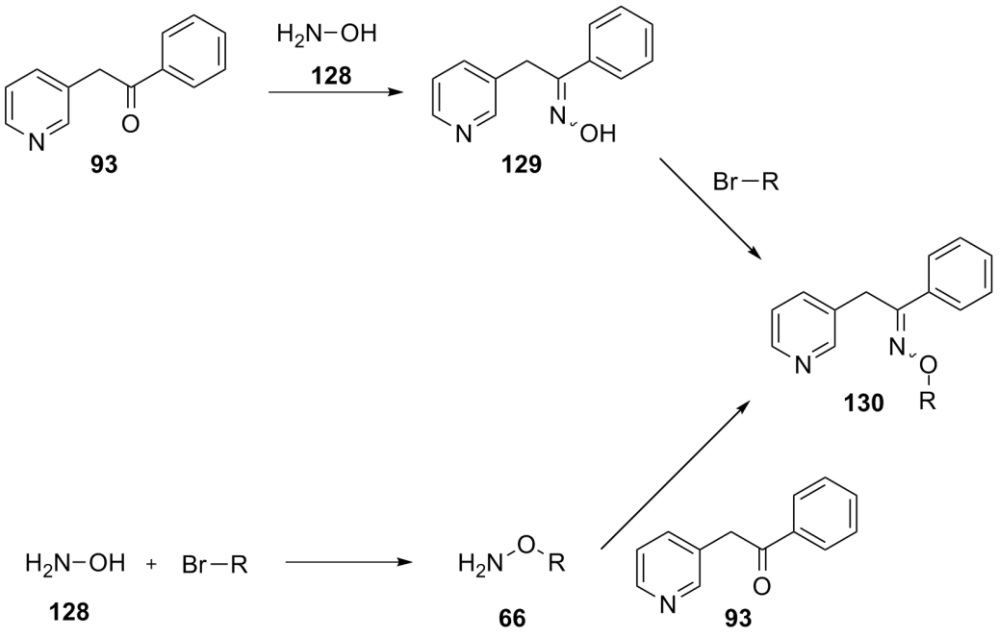
To increase the bulk of the oxime further the pentafluorobenzyl oxime was synthesised (Scheme 36). The ketone **93** was stirred with *O*-pentafluorobenzylhydroxylamine hydrochloride **126** and sodium acetate in ethanol. Column chromatography isolated the ketone starting material **93** (34%) and a mixture of the two isomers **127** as a 2.9:1 ratio (40%). The two isomers were seen in the ^{1}H NMR spectrum as two singlets at 3.81 ppm and 4.09 ppm corresponding to the CH_2 between the pyridyl ring and the oxime, and two singlets at 5.14 ppm and 5.32 ppm corresponding to the CH_2 of the oxime. The two isomers were unable to be separated and were tested as a mixture against *T. cruzi*.



Scheme 36

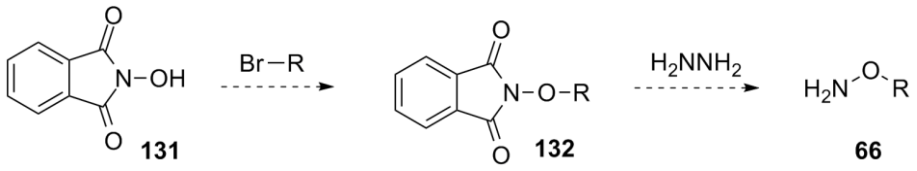
3.3 Synthesis of alkoxyamines

Only simple alkoxyamines are commercially available and since a variety of alkoxyamines were going to be investigated the precursors needed to be synthesised. There were two possible methods that could be used (Scheme 37). The ketone **93** could be reacted with the hydroxylamine hydrochloride **128** forming the hydroxyloxime **129**. This could then be alkylated with a variety of alkyl chains to give the more complicated oximes **130**. The downfall with this method was the potential for the pyridyl nitrogen to be alkylated. Another method that could be used was to first synthesise the alkoxyamines **66** from hydroxylamine **128**. This could then react with the ketone **93** to form the oxime **130**. As the ketone **93** was difficult to synthesise and wanting to limit potential alkylation of the pyridyl nitrogen, the latter method was used.



Scheme 37

Alkoxyamines can be made by alkylating the hydroxylamine. The reaction could occur on the nitrogen or the oxygen. The nitrogen would be the favoured position for the reaction to occur due to it being a better nucleophile than oxygen. Therefore the nitrogen needed to be protected for the reaction to occur on the oxygen. The nitrogen could be protected by using *N*-hydroxyphthalimide **131** as the starting material (Scheme 38). The carbonyl groups are electron withdrawing and protect the nitrogen so the alkylation could occur on the alcohol forming an ether type compound **132**. The protecting group could then be cleaved using hydrazine to form the alkoxyamine derivatives **66**.[120]



Scheme 38

Following a procedure by Dendane *et al*,[120] 1-bromobutane **133** was reacted with *N*-hydroxyphthalimide **131** and sodium carbonate in *N*,*N*-dimethylformamide at room temperature (Table 13, entry 1). The product **134** precipitated out of the reaction mixture after the addition of water and was dried under vacuum. The spectral data matched that in the literature.[121] The product obtained was pure and could be used without further purification but the yield was very low (2.4%). Upon heating to 45 °C the yield improved dramatically to 41%. Therefore heat must be required for the reaction to progress. The reaction was heated to 90 °C and the yield improved to 79% and could be done on a large scale (13 g).

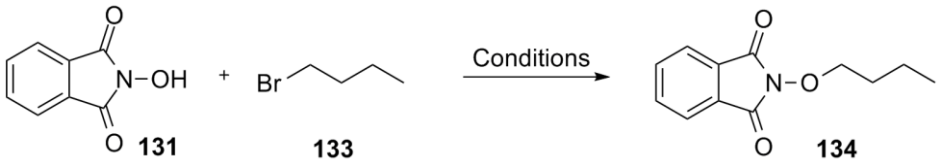


Table 13

Entry	Conditions	Product
1	Na_2CO_3, DMF, 1.5 days, r.t.	2.4%
2	Na_2CO_3, DMF, 2 days, 45 °C	41%
3	Na_2CO_3, DMF, 2 days, 90 °C	79%

The optimised conditions were used to synthesise other alkoxyamine precursors (Table 14). The alkyl halide was heated to 90 °C with *N*-hydroxyphthalimide **131** and sodium carbonate in *N*,*N*-dimethylformamide. The reactions occurred in moderate to high yield (34−85%). The majority of the products precipitated out of the reaction mixture upon the addition of water. The 1-bromo-3-methylbutane **137** and ethyl 4-bromobutyrate **141** derivatives were more soluble and required extraction from the reaction mixture with dichloromethane first before a solid was formed.

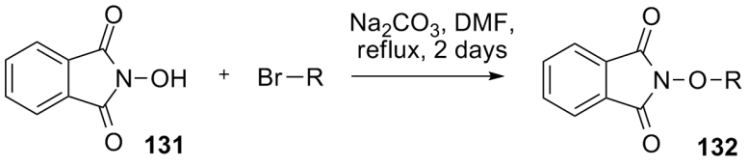
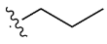
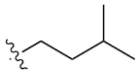
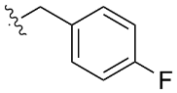
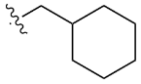
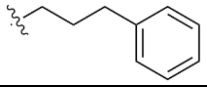
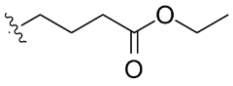
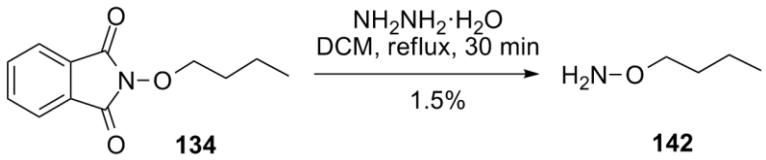


Table 14

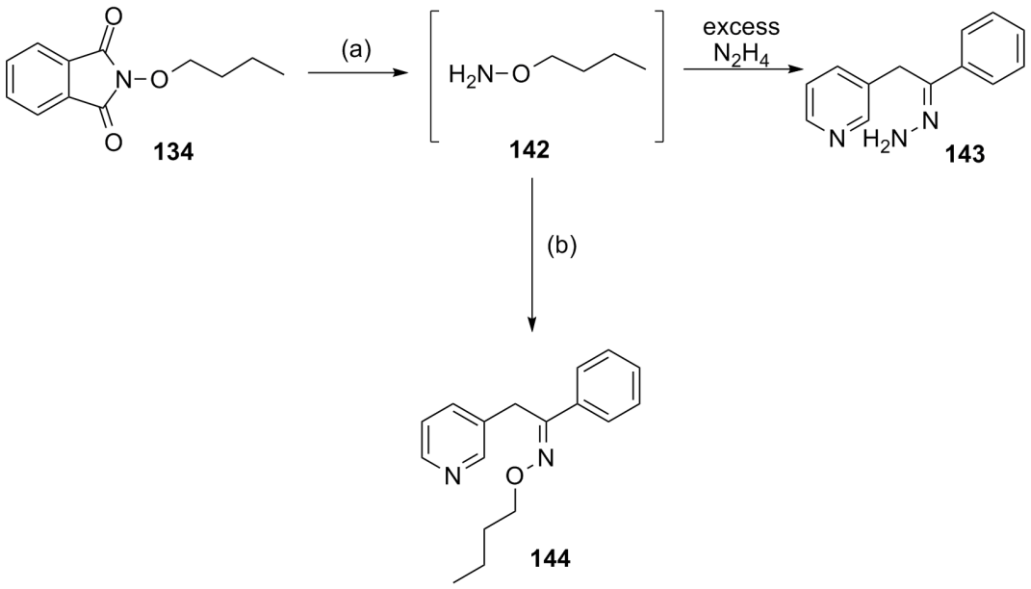
Compound	R	Yield
135		69%
136		68%
137		59%
138		51%
139		85%
140		48%
141		34%

The hydroxylamines were synthesised from the precursors by reacting with hydrazine hydrate. *N*-Butoxyphthalimide **134** was heated with hydrazine hydrate and dichloromethane under reflux (Scheme 39).[117,118] The ^{1}H NMR spectrum of the isolated compound showed a triplet at 0.87 ppm corresponding to the CH_3 and a triplet at 3.61 corresponding to the CH_2 next to the oxime. The yield obtained was low (1.5%) which could be due to the suspected volatility of butoxyamine **142**. Mesitylene was added to the reaction mixture as an internal standard to determine the reaction yield before isolation. The ^{1}H NMR spectrum of the crude material showed 90% yield of product and LC-MS of the reaction mixture showed full conversion after 30 minutes.



Scheme 39

As the hydroxylamine **142** synthesised was volatile, a one-pot synthesis was used to convert the protected alkoxyamine **134** to the alkoxyamine **142**, before the addition of the ketone **93**. As the unhalogenated ketone **93** was the simplest to make, this was used to synthesise the other oximes. *N*-Butoxyphthalimide **134** was heated under reflux with hydrazine hydrate in ethanol for 30 minutes. The ketone **93** and acetic acid in ethanol was then added to the reaction mixture (Scheme 40). The ^{1}H NMR spectrum of the crude material showed trace amounts of one isomer and an unknown compound. The two compounds were isolated by column chromatography. The desired isomer was observed in the ^{1}H NMR spectrum as a singlet at 4.13 ppm corresponding to the CH_2 between the oxime and the pyridyl ring, and a triplet at 4.23 ppm corresponding to the CH_2 next to the oxime. Although the yield was low (3.4%) the reaction was not optimised as there was enough material for biological testing. The other compound isolated had a similar ^{1}H NMR spectrum to the ketone **93**. The key signal at 4.21 ppm corresponded to the CH_2 and the signals at 8.45 ppm and 8.59 ppm corresponded to the hydrogens next to the nitrogen in the pyridine ring. There was also a broad singlet at 9.05 ppm with the integration of one hydrogen and a singlet at 2.02 ppm with the integration of two hydrogens. It has been observed in the literature that hydrazine could also add to the ketone. Therefore it can be suggested that compound **143** is forming. The GC-MS showed a molecular ion of *m/z* 212, which supported the theory that the hydrazine had reacted with the ketone to form compound **143**. To prevent this from occurring, the hydrazine hydrate was only used in a slight excess.



Scheme 40. Conditions: a) $NH_2NH_2 \cdot H_2O$, EtOH, reflux, 30 min; b) **93**, CH_3COOH, EtOH, reflux, 1 day.

These conditions were used to synthesise a variety of oximes (Table 15). The hydroxylamine precursors **132** were reacted with hydrazine hydrate in ethanol for 30 minutes before the ketone **93** in acetic acid and ethanol were added. This produced the desired oximes in a variety of yields. In all cases the *E* isomer was the synthetically favoured isomer.

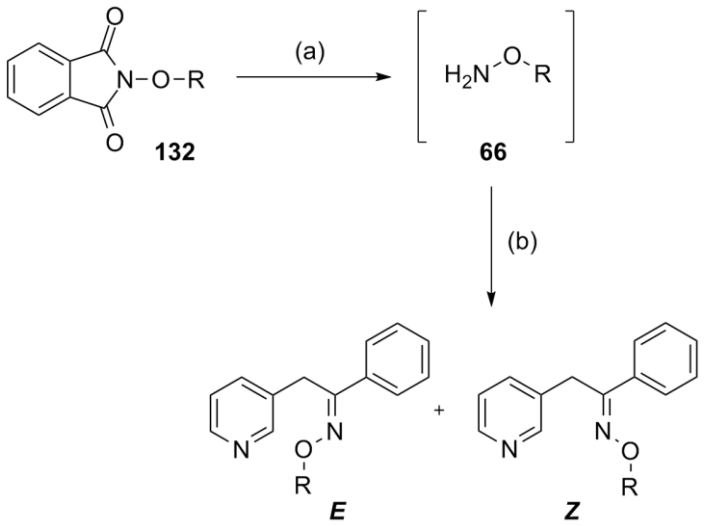
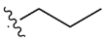
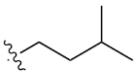
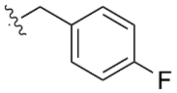
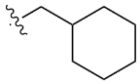
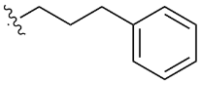
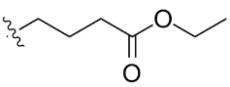


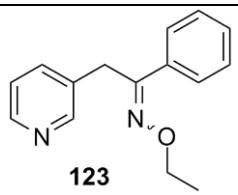
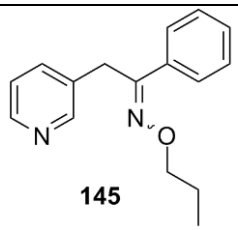
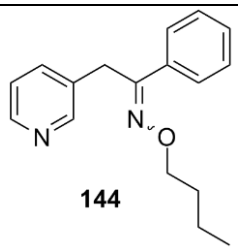
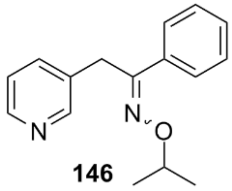
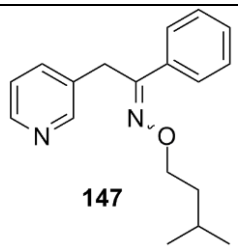
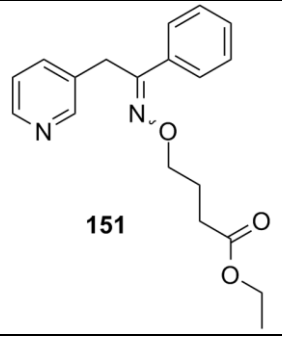
Table 15. Conditions: a) $NH_2NH_2 \cdot H_2O$, EtOH, reflux, 30 min; b) **93**, CH_3COOH, EtOH, reflux, 1 day.

Compound	R	Yield	
		E	***Z***
145		2%	0%
146		4%	0%
147		32%	0%
148		44%	3%
149		36%	9%
150		48%	9%
151		12%	1.1%

3.3.1 Anti-trypanosomal activity

All the oximes synthesised were tested for their ability to inhibit *T. cruzi* following the conditions outlined in Section 3.1.1. Initially the length of the alkyl chain was investigated (Table 16). As seen previously, the methyl oxime **117** had an IC_{50} greater than 10 µM for both the *E* and *Z* isomers. The ethyl oxime **123** was tested as a single *E* isomer and a mixture of *E*/*Z* as a ratio of 1.68:1. The *E* isomer had an IC_{50} of 4.91 µM whilst the mixture of isomers had an IC_{50} greater than 10 µM. This suggested the *E* isomer was the preferred orientation over the *Z* isomer. The length of the oxime also appeared to play a role in the inhibition of the site. The ethyl oxime **123** contained an extra carbon in its chain and the anti-trypanosomal activity has doubled. This continued to be the trend with the propyl and butyl oximes. The propyl oxime **145** had an IC_{50} of 2.00 µM whilst the butyl oxime **144** had an IC_{50} of 1.04 µM for the *E* isomers. This suggested that a longer chain was required to interact better in the access tunnel. Having a branched chain also appeared to improve the activity as well. Compound **146** had an IC_{50} of 1.55 µM compared to the ethyl oxime **123** which was only 4.91 µM. The longer branched chain oxime **147** was also synthesised and is currently awaiting results. A long chain ester group was also investigated. Compound **151** had an IC_{50} of 4.55 µM for the *E* isomer and greater than 10 µM for the *Z* isomer. This suggested that having the ester group decreases the biological activity. This could be due to the carbonyl group reacting unfavourably within the enzyme active site. All the compounds also appeared to be non-cytotoxic except for compound **151** which had a toxicity of 57.41 µM for the *E* isomer.

Table 16

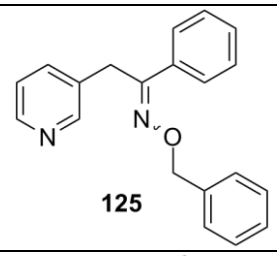
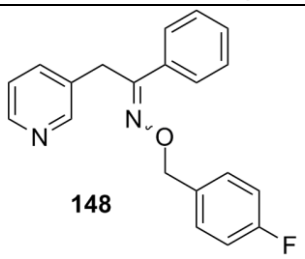
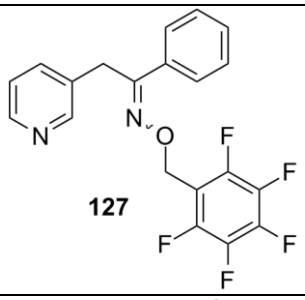
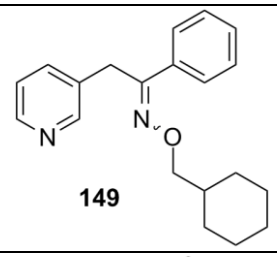
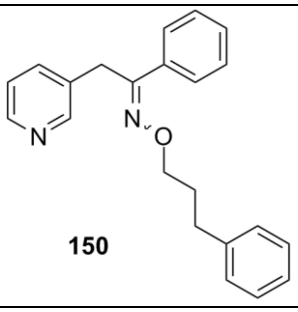
Structure	IC_{50} (toxicity)	
	E	***Z***
 **123**	4.91 µM (>100 µM)	>10 µM (>100 µM)*
 **145**	2.00 µM (>100 µM)	–
 **144**	1.04 µM (>100 µM)	–
 **146**	1.55 µM (>100 µM)	–
 **147**	In testing	–
 **151**	4.55 µM (57.41 µM) SI = 13	>10 µM (>100 µM)

* Mixture of isomers

As the biological activity improved with a longer alkyl chain, a bulkier oxime was considered. On changing the oxime to a bulkier group the biological activity drastically improved (Table 17). Compound **125** showed an IC_{50} of 0.57 μM for the *E* isomer which was ten times more potent than the ethyl oxime (*E* 4.91 μM). The increased anti-trypanosomal activity suggested the chain that orientates into the hydrophobic access tunnel could be bulky to bind more effectively. The addition of a fluorine substituent on the para position of the phenyl oxime **148** did not appear to improve the IC_{50} (0.66 μM) but increased the cytotoxicity (34.22 μM, SI = 173). The pentafluorobenzyl oxime **127** had an IC_{50} of 0.37 μM for a mixture of isomers and was also toxic (64.06 μM). The increased hydrophobicity tends to be more potent but also more toxic. Although the addition of the fluorine substituents did not improve the potency, they were significantly more toxic.

Another way to increase the bulk of the oxime was to have a cyclohexane ring instead of the phenyl ring on the oxime. The phenyl ring is planar whereas the cyclohexane would occur in the chair conformation adding to the bulk of the oxime. This improved the anti-trypanosomal activity slightly with an IC_{50} of 0.22 μM for the *E* isomer. Although it was showed some toxicity (61.10 μM), the SI value is very high (278) meaning a large dose would be required for the compound to have toxic effects. Having a longer alkyl chain before the bulky phenyl ring was also considered. Compound **150** had an IC_{50} of 0.48 μM for the *E* isomer and greater than 1 μM for the *Z* isomer. The longer alkyl chain between the phenyl ring and the oxime in compound **150** did not improve the activity compared to compound **125**. This could be due to the phenyl ring of the oxime fitting into the access tunnel best with compound **125**.

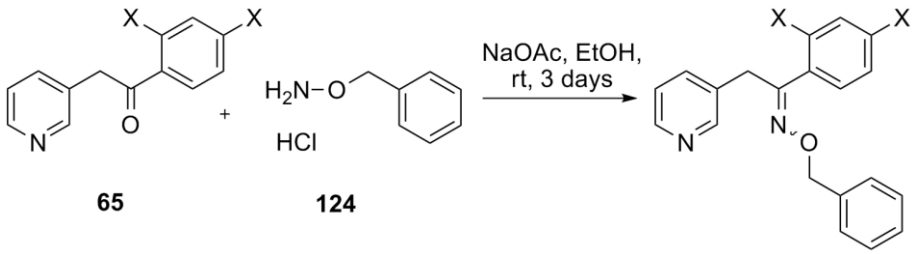
Table 17

Structure	IC_{50} (toxicity)	
	E	***Z***
 **125**	0.57 µM (>100 µM)	–
 **148**	0.66 µM (34.22 µM) SI = 52	0.95 µM (34.21 µM) SI = 36
 **127**	0.37 µM (64.06 µM)* SI= 173	
 **149**	0.22 µM (61.10 µM) SI = 278	In testing
 **150**	0.48 µM (35.08 µM) SI = 73	>1 µM (20.36 µM)

* Mixture of isomers

3.4 Substituted phenyl ring oximes

Substituents on the phenyl ring had a greater inhibition against *T. cruzi* than the unsubstituted phenyl ring and a bulky oxime had better anti-trypanosomal activity, therefore the benzyl oximes for the fluoro and chloro substituted phenyl ring were synthesised. The ketone **65** was stirred at room temperature with *O*-benzylhydroxylamine hydrochloride **124** and sodium acetate in ethanol (Table 18). The ^{1}H NMR spectrum of the crude material for the fluoro substituted compound **152** showed a mixture of the two isomers as 1.7:1 ratio. Unfortunately it was only possible to purify the *E* isomer in 21% yield. The chloro substituted compound **153** showed a mixture of the two isomers as 1:1 ratio in the ^{1}H NMR spectrum of the crude material and both isomers were separated.



65 **124**

Table 18

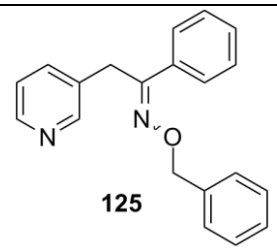
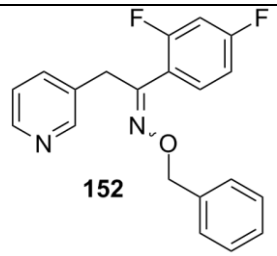
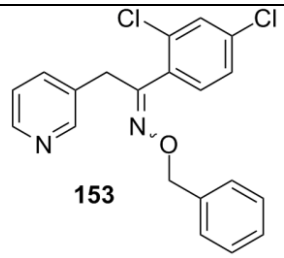
Compound	X	Overall Yield
152	F	*E* 21%
153	Cl	70%

3.4.1 Anti-trypanosomal activity

It has been shown that the substituents on the phenyl ring were important in improving the potency of the compound against *T. cruzi*. The fluoro and chloro substituted phenyl oximes were tested for their ability to inhibit *T. cruzi* and compared to compound **125** (Table 19). These compounds appear to follow the same trend as seen in Section 3.1.1. The fluoro substituted compound **152** showed an IC_{50} of 0.021 µM for the *E* isomer whilst the chloro substituted compound **153** showed an IC_{50} of 0.20 µM for the *Z* isomer. The fluoro substituted compound **152** was ten times more potent than compound **125** and the *Z* isomer of compound **153**. It was

shown in Section 3.1 that the chloro substituted compound had a better inhibition than the fluoro and the *E* isomer was the preferred isomer (Table 12). This would suggest the *E* isomer of compound **153** would show a similar or better activity than compound **152**. The *E* isomer of the dichloro compound **153** is currently in testing.

Table 19

Structure	**IC_{50} (toxicity)**	
	E	***Z***
 **125**	0.57 µM (>100 µM)	In testing
 **152**	0.021 µM (45.65 µM) SI = 2174	–
 **153**	In testing	0.20 µM (46.89 µM) SI = 234

3.5 Conclusions

A library of twenty four pyrifenox analogues were successfully synthesised and tested in whole cell assays for their ability to inhibit *T. cruzi*. The summary of key findings is shown in Figure 33. The substituents on the phenyl ring were shown to be important. The unsubstituted phenyl ring was easier to synthesise but was the least active. The chloro substituents were the most active which could be due to the bulky chlorine atoms filling out the hydrophobic pocket more effectively. The oxime was also shown to play a role in improving the activity. The *E* isomer was the preferred isomer as it was able to fit better into the access tunnel. A bulky oxime was shown to improve the biological activity over a straight alkyl chain. Compound **152** was shown to have the best inhibition with the *E* isomer being fourteen times more potent than pyrifenox. It would be expected that the *E* isomer of compound **153** would have similar or better activity. Further investigations into these two compounds are required.

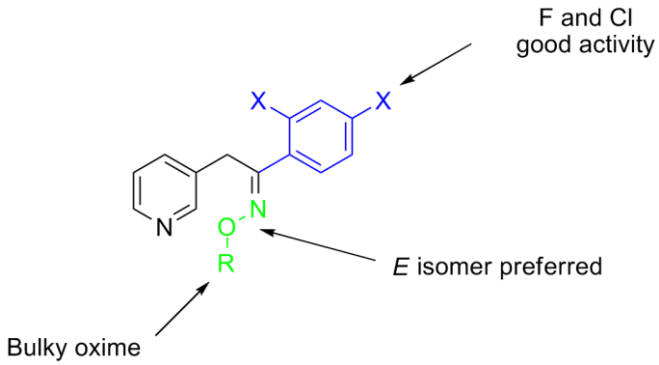


Figure 33. Diagram of key findings

4 3-Anilinemethylpyridine analogues

The main difficulty in the pyrifenox analogues was the separation of the two oxime isomers. The separation of the two isomers was necessary as the *E* isomer was shown to be more potent (Chapter 3). To overcome this, a nitrogen atom could be incorporated into the chain between the two rings to remove the stereochemistry (Figure 34). Having a nitrogen at this position is not ideal as the nitrogen is electron rich and can react within the enzyme. However it would provide information on how important it is to have a rigid system, like the *E* oxime in the pyrifenox analogues. The plan was to use an alkyl chain that is of a similar length to the substituted oxime in order to allow comparison with pyrifenox.

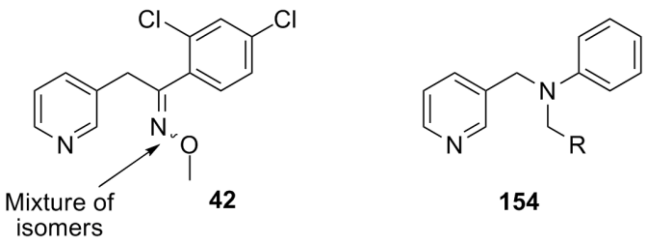
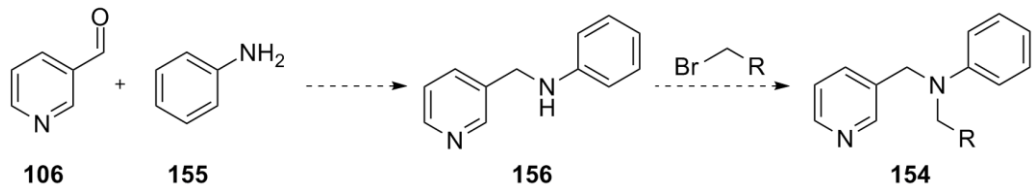


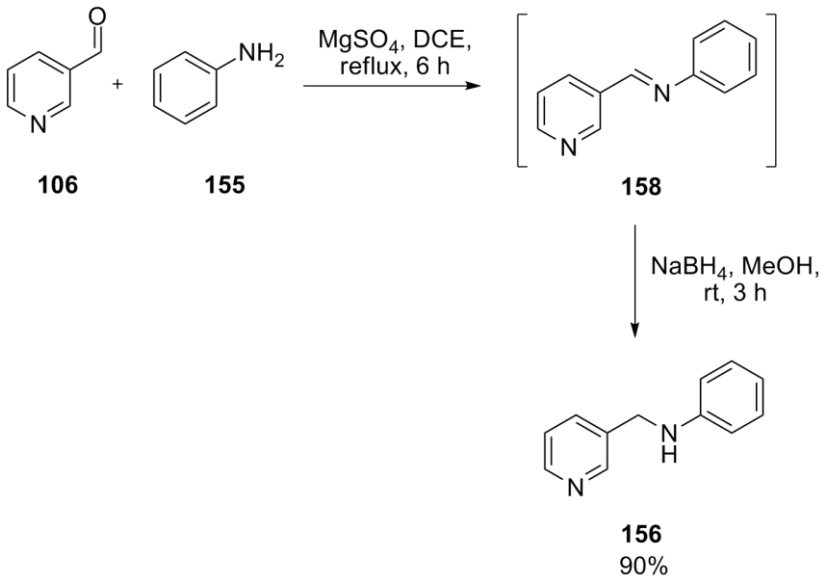
Figure 34

The proposed synthesis of these analogues is shown in Scheme 41. The nitrogen could be incorporated between the two rings by a reductive amination reaction to give **156**. This could then be reacted with an alkyl halide to give **154**. This has a similar backbone to pyrifenox without the stereochemistry of the oxime.



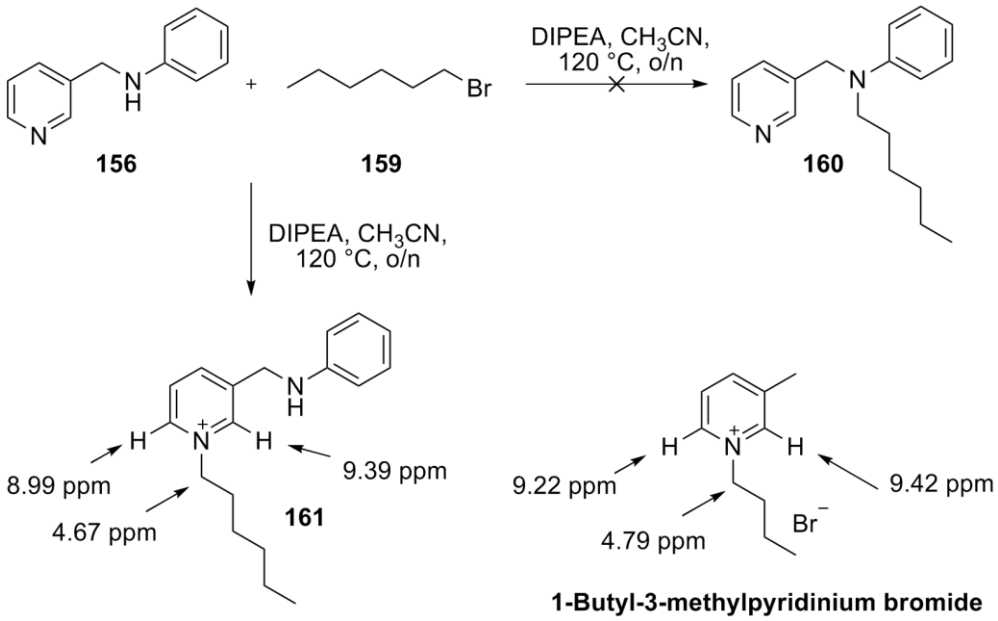
Scheme 41. Proposed synthesis of compound 106.

The intermediate **156** was prepared according to Dickson *et al*. (Scheme 42).[122] Aniline **155** was heated under reflux with 3-pyridinecarboxaldehyde **106** in the presence of anhydrous magnesium sulfate and dry 1,2-dichloroethane to give the imine **158**. The ^{1}H NMR spectrum of the crude residue matched that in the literature.[122] The spectrum showed the key imine singlet at 8.47 ppm. The crude residue was stirred at room temperature with sodium borohydride in methanol to give the secondary amine **156**. The spectral data matched that in the literature.[122] The ^{1}H NMR spectrum showed a singlet at 4.35 ppm corresponding to the CH_2 and a broad singlet at 4.09 ppm corresponding to the amine. The IR spectrum showed signals at 3248 cm^{-1} and 1600 cm^{-1} suggesting a secondary amine was present. This reaction was high yielding (90%) on a 2 mmol scale. On increasing the scale to 4 mmol the yield decreased dramatically (25%). Therefore the reaction was only done on a small scale.



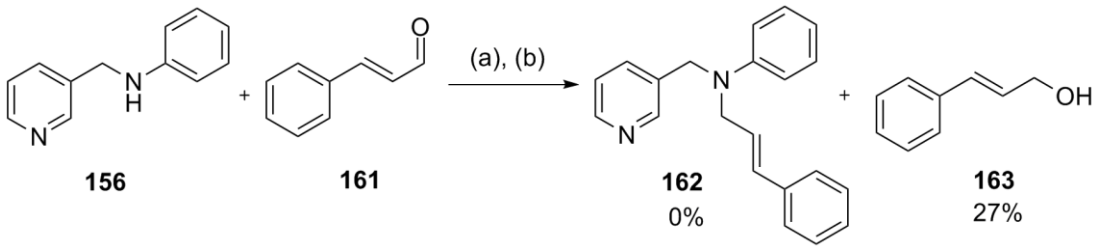
Scheme 42

The final step was an alkylation reaction. The alkylation could occur on the amine or on the nitrogen in the pyridine ring. The amine **156** was heated at 120 °C with 1-bromohexane **159** in *N*,*N*-diisopropylethylamine and acetonitrile (Scheme 43). The ^{1}H NMR spectrum showed the compound isolated was not the desired product **160**. The peaks corresponding to the pyridine were shifted downfield to 9.39, 8.99, 8.45 and 7.88 ppm. This suggested that the environment around the nitrogen in the pyridine ring had changed which is typical of an alkylated pyridine. A known compound, 1-butyl-3-methylpyridinium, showed the hydrogens next to the pyridine are shifted downfield with values similar to compound **161**. The ^{1}H NMR spectrum also showed signals relating to the hexane chain. This suggested the nitrogen in the pyridine ring was alkylated instead of the amine to give **161**. This implied that the nitrogen in the pyridine ring is more reactive towards alkylation than the amine.



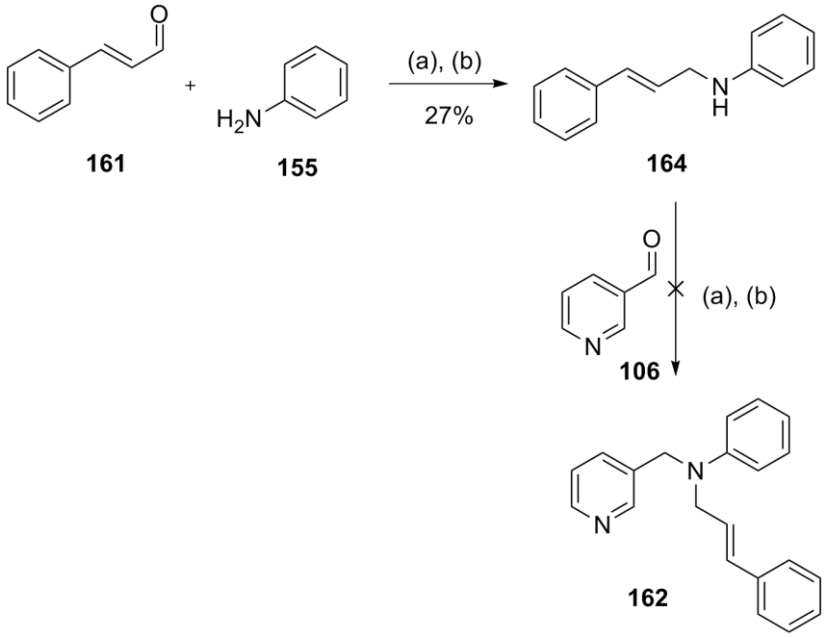
Scheme 43

As the nitrogen on the pyridine ring appeared to be the preferred alkylation position other methods were investigated to add a group onto the amine. A reductive amination on the amine was conducted under the same conditions used to synthesise **156** (Scheme 42). Compound **156** was heated under reflux with *trans*-cinnamaldehyde **161** and anhydrous magnesium sulfate in dry 1,2-dichloroethane (Scheme 44). Upon removal of the solvent, the crude residue was stirred at room temperature with sodium borohydride in methanol. This did not produce the desired compound with mainly starting material **156** (60%) and cinnamyl alcohol **163** being isolated. It appeared the aldehyde was not condensing with the amine in the first step and was being reduced to the alcohol with sodium borohydride in the second step.



Scheme 44. Conditions: a) $MgSO_4$, 1,2-dichloroethane, reflux, 6 h; b) $NaBH_4$, MeOH, rt, 3 h.

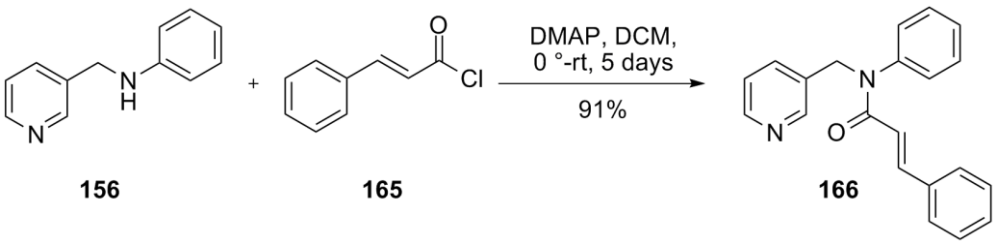
An alternate approach was to add the pyridine ring on last (Scheme 45). Aniline **155** and *trans*-cinnamaldehyde **161** were heated under reflux with anhydrous magnesium sulfate in dry 1,2-dichloroethane. The crude residue was stirred at room temperature with sodium borohydride in methanol. The ^{1}H NMR spectrum showed an AB pattern centred around 3.85 ppm corresponding to the CH_2 and a singlet at 4.24 ppm corresponding to the amine. This reaction was low yielding (27%) due to an incomplete reaction and degradation. Separation of the crude material by column chromatography resulted in the recovery of 48% of the aniline **155**. The cinnamyl alcohol **163** was not observed. The amine **164** was then heated under reflux with 3-pyridinecarboxaldehyde **106** and anhydrous magnesium sulfate in dry 1,2-dichloroethane. The crude residue was then stirred with sodium borohydride in methanol at room temperature. Flash chromatography separated starting material (16%) and degradation products. Due to the low yield of the amine **164** this route was abandoned.



Scheme 45. Conditions: a) $MgSO_4$, 1,2-dichloroethane, reflux, 6 h; b) $NaBH_4$, MeOH, rt, 3 h.

Another approach to add a group onto the amine was to form an amide from the amine and an acid chloride (Scheme 46). The amine **156** was stirred with cinnamoyl chloride **165** and 4-dimethylaminopyridine in dichloromethane at 0 °C before

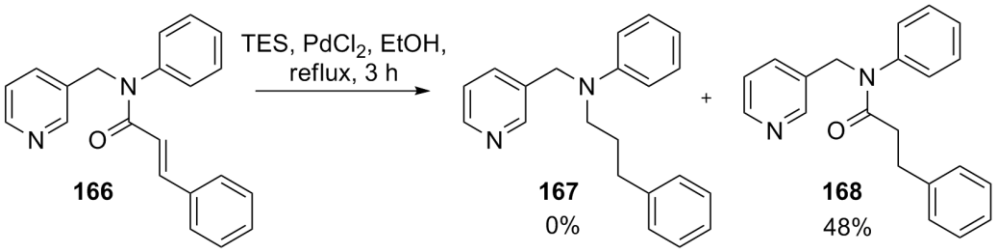
warming to room temperature[123] to produce the desired amide **166** in 91% yield. The ^{1}H NMR spectrum showed a singlet at 5.03 ppm corresponding to the CH_2 and a doublet at 6.30 ppm corresponding to the alkene. The doublet had a *J* coupling of 15.6 Hz corresponding to the expected *trans* alkene. The other doublet overlaps with some aromatic signals between 7.69−7.73 ppm.



156 **165** **166**

Scheme 46

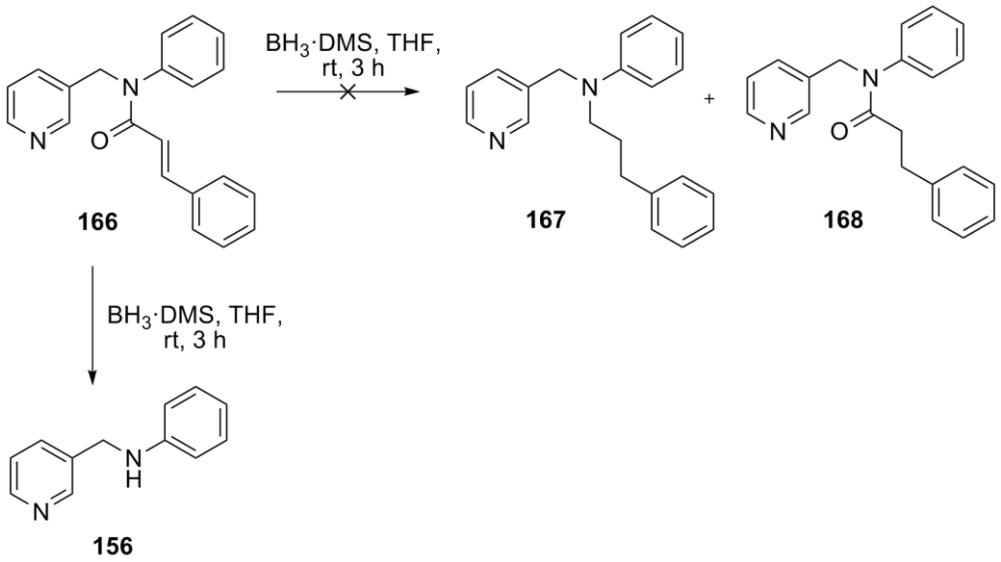
The next step was to reduce the amide to an amine. The amide **166** was heated under reflux with 2.65 equivalents of triethylsilane and palladium chloride in ethanol (Scheme 47). The ^{13}C NMR spectrum showed a signal at 172.3 ppm corresponding to a carbonyl carbon, showing the amide has not been reduced. The ^{1}H NMR spectrum showed two triplets at 2.29 ppm and 2.85 ppm each with the integration of two hydrogens. This suggested that compound **168** was produced as there was still an amide present and no alkene. The amount of triethylsilane used was increased to 12 equivalents to try and reduce the amide. The ^{1}H NMR spectrum showed only the alkene being hydrogenated.



166 **167** 0% **168** 48%

Scheme 47

As triethylsilane and palladium chloride were unable to reduce the amide other methods were explored. The amide **166** was treated with sodium borohydride and methanesulfonic acid in dimethylsulfoxide. The ^{1}H NMR spectrum of the crude material showed only the alkene being hydrogenated and degradation. Another method attempted was by stirring the amide **166** with borane dimethyl sulfide complex (2 M in THF) under nitrogen at room temperature (Scheme 48). This reduced the amide back to the amine **156** in 49% yield and the rest of the compound was degraded.



Scheme 48

Due to the difficulties in synthesising the tertiary amine analogues of pyrifenox, the reduction of the amide and the incorporation of the nitrogen between the two rings were abandoned. Although the target compound was not synthesised, compounds **166** and **168** still had a similar structure to the target compound and were tested for their ability to inhibit *T. cruzi*.

4.1 Anti-trypanosomal activity

Compounds **166** and **168** were assayed in an *in vitro* whole cell assay. Compound **166** showed an IC_{50} of 1.29 µM whilst compound **168** showed an IC_{50} of 5.75 µM against *T. cruzi*. In comparing the two structures the only difference is the conjugated double bond in the amide chain in compound **166**. This double bond could be increasing the rigidity making the structure fit better into the enzyme. The results of these two compounds were compared against compound **125** that was synthesised in Section 3.3. This compound has a similar structure to compounds **166** and **168**. The amide has a similar rigidity to the oxime, however the addition of the amide group decreases the anti-trypanosomal activity. This could be due to the oxygen present in the amide group not fitting into the binding cavity.

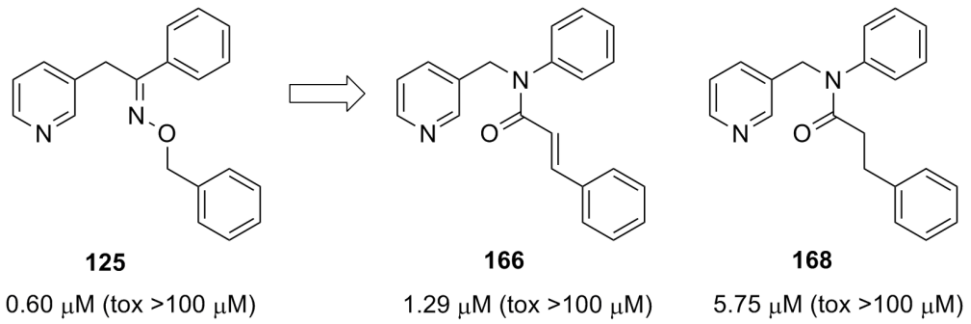


Figure 35. Comparison of the biological activity of compound **125** to **166** and **168.**

5 Investigation of a naphthol system

The structural rigidity of new biological compounds has been known to reduce side effects and to enhance the biological activity in medicinal chemistry.[124,125] This is due to the drug being designed to specifically target the desired active site. In the pyrifenox model, the nitrogen in the pyridyl ring binds to the heme iron in the active site and the substituted benzyl ring is free to rotate. In Chapter 3, the *E* isomer was the biologically preferred isomer. A more rigid system mimicking the *E* isomer could be created that prevents the phenyl ring from rotating. This could be achieved by enclosing the area between the nitrogen of the oxime and the chlorine substituent on the phenyl ring (Figure 36). This would mimic the preferred *E* isomer of the oxime and create a more rigid system.

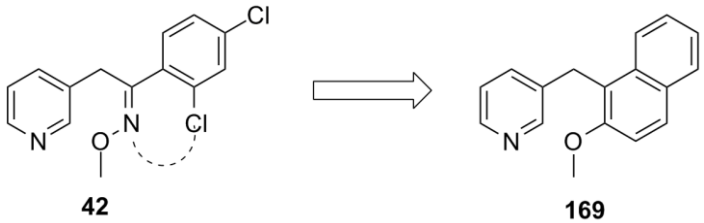
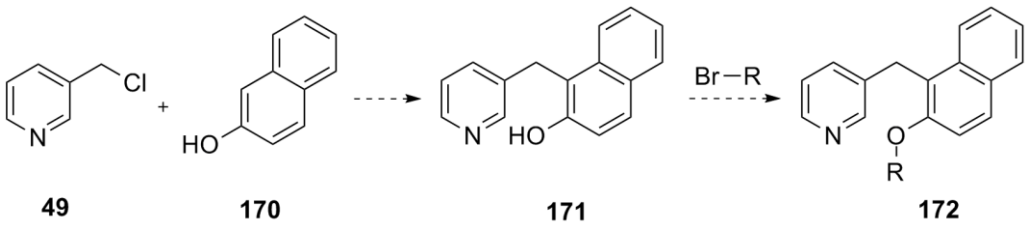


Figure 36

The proposed structure of the more rigid system is based around a naphthol motif. The planned synthesis is shown in Scheme 49. By reacting 3-chloromethylpyridine **49** with 2-napthol **170** a rigid backbone would be created. A simple alkylation of the hydroxyl group would then give compound **172**.



Scheme 49. Proposed synthesis of a more rigid analogue.

There are two possible alkylation sites in 2-naphthol, the C2 position or the hydroxyl group (Figure 37).[126,127] The position of the alkylation can be controlled by changing the solvent system of the reaction.[126,127] Solvents such as *N*,*N*-dimethylformamide and dimethyl sulfoxide give predominantly the ether product whilst strong hydrogen bonding solvents, such as water, give the carbon alkylated product.[127] It has been proposed that this observation could be due to the dielectric constant of the solvent.[127] Aprotic solvents such as *N*,*N*-dimethylformamide and dimethyl sulfoxide have relatively high dielectric constants and strongly favour oxygen alkylation.[127] Solvents with a low dielectric constant favour carbon alkylation by disfavouring the oxygen alkylation.[127]

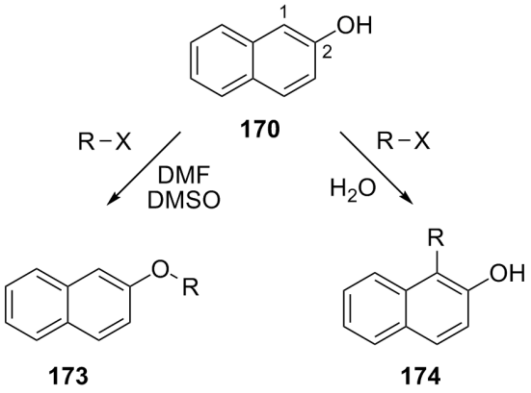


Figure 37. There are two possible alkylation sites on 2-naphthol.

Following the procedure from Kornblum *et al.*[127] 3-chloromethyl pyridine hydrochloride **49** was stirred with 2-naphthol **170** in the presence of sodium hydroxide and water at room temperature for three days (Table 20, entry 1). Separation of the crude material resulted in the recovery of the unreacted 2-naphthol **170** (50%) and a new compound. The ^{1}H NMR spectrum of the new compound suggested that it contained two pyridine rings. There were two signals at 4.56 ppm and 5.22 ppm integrating for two hydrogens each and there were extra signals in the aromatic region. This suggested that the dialkylated compound **176** had formed. Since the pyridine **49** had alkylated 2-naphthol at the C2 position and on the hydroxyl group, the reaction time was shortened and the 2-naphthol **170** was used in a slight excess (2.1 eq.). Under these reaction conditions, 2-naphthol **170** (30%) and another compound, that was not the desired product or compound **176**, were isolated. The ^{1}H NMR spectrum showed one singlet at 5.05 ppm, a doublet at 8.89 ppm and a singlet at 8.63 ppm indicating only one pyridine ring was present. The absence of an OH stretch in the IR spectrum at ~3200 cm^{-1} suggested that the ether compound **175** had formed. This indicated that under these conditions the hydroxyl group was more reactive than the C2 position. The solvent of the reaction was changed from water to 2,2,2-trifluoroethanol.[127] 2,2,2-Trifluoroethanol is a better hydrogen bond donor than water. The major product obtained from this reaction was 2-naphthol **170** (83%) and there were trace amounts of compound **176** (13%). This method was not a viable way to synthesise compound **171**.

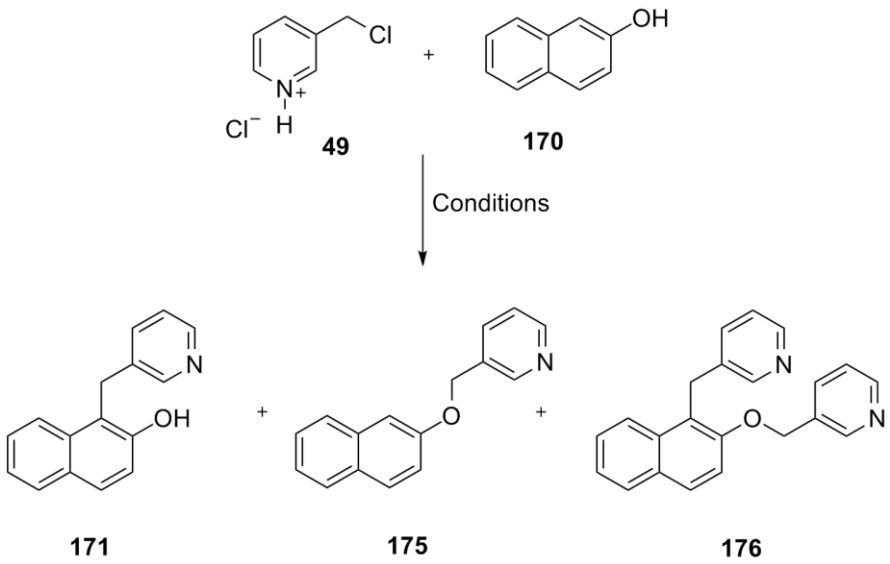
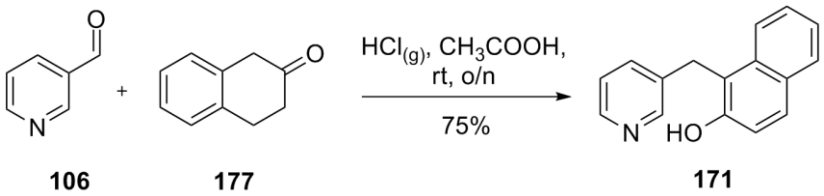


Table 20

Entry	Conditions	Product			
		170	**171**	**175**	**176**
1	NaOH 2 eq., H_2O, rt, 3 days	50%	–	–	19%
2	NaOH 2.7 eq., H_2O, rt, o/n	30%	–	16%	–
3	NaOH, 2,2,2-trifluoroethanol, rt, o/n	83%	–	–	13%

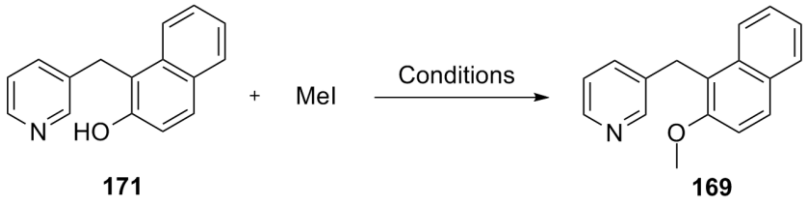
An alternate procedure developed by Huang *et al.* was investigated.[128] 3-Pyridine carboxaldehyde **106** and β-tetralone **177** were reacted in the presence of freshly prepared dry hydrogen chloride gas and acetic acid (Scheme 50). The hydrogen chloride gas was generated by adding sulfuric acid dropwise to ammonium chloride salt. The gas was bubbled directly into the reaction mixture. This reaction gave the desired compound **171** in 75% yield. The spectral data of compound **171** was similar to that in the literature.[128] The ^{1}H NMR spectrum showed a singlet at 4.47 ppm corresponding to the CH_2 between the pyridyl ring and the naphthol. The IR spectrum showed a broad signal at 2868 cm^{-1} indicating an OH group was present. The compound degraded upon purification by column chromatography. Therefore it was used without further purification.



106 **177** **171**

Scheme 50

The last step to form the desired compound was an alkylation reaction. The alkylation could occur on the nitrogen of the pyridine ring or the hydroxyl group to form an ether. Under basic conditions and using an aprotic solvent with a relatively high dielectric constant, the oxygen alkylation should be the favoured product. The naphthol **171** was stirred with 12 equivalents of methyl iodide and sodium carbonate in dry *N*,*N*-dimethylformamide at room temperature (Scheme 51). The recovery of starting material suggested no reaction had occurred. The base was changed from sodium carbonate to sodium hydride and methyl iodide was used in a large excess (29 eq.). The ^{1}H NMR of the crude material showed a singlet at 4.57 ppm with the integration of two hydrogens and a singlet at 4.52 ppm with the integration of three hydrogens. This suggested that a methyl group could have been present but due to the low yield it was difficult to determine whether the methylation had occurred on the oxygen or the nitrogen. As the peaks in the ^{1}H NMR spectrum corresponding to the hydrogens next to the nitrogen in the pyridine ring were not shifted downfield it could be thought that the oxygen was alkylated to form compound **169**.

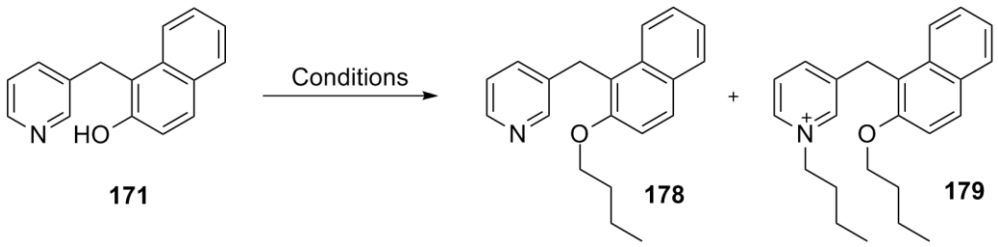


171 **169**

Scheme 51

Entry	Conditions	Product
1	Na_2CO_3, 2.9 eq., DMF, rt, 3 days	No reaction
2	NaH 2.2 eq., DMF, rt, o/n	Trace

A longer alkyl chain was considered instead. The naphthol **171** was stirred with 1-bromobutane and potassium carbonate in *N*,*N*-dimethylformamide (Scheme 52). On the first attempt a large excess of bromobutane was used (10 eq.). This gave the desired ether **178** in low yield (3%) and compound **179** as the major product. The ^{1}H NMR spectrum of **178** showed a singlet at 4.46 ppm with an integration of two hydrogens corresponding to the CH_2 between the pyridyl ring and the naphthol. The signals at 8.38 ppm and 8.60 ppm corresponded to the pyridyl ring. There was also a triplet at 4.12 ppm, multiplets at 1.73−184 ppm and 1.40−1.54 ppm and a triplet at 0.92 ppm corresponding to the butyl chain. The IR spectrum showed the lack of a signal at 2868 cm^{-1} suggesting that the ether had formed. The ^{1}H NMR spectrum of the major product **179** showed extra signals due to the butyl chain that were shifted slightly downfield. This suggested that although the desired ether is forming the large excess of 1-bromobutane was reacting with the nitrogen in the pyridine ring. Reducing the amount of 1-bromobutane to two equivalents gave the desired product **178** in 84% yield without the formation of compound **179**.



Scheme 52

Entry	Conditions	Product	
		178	179
1	1-Bromobutane 10 eq., K_2CO_3 3.7 eq., DMF, 80 °C, 3 days	3%	85%
2	1-Bromobutane 2 eq., K_2CO_3 2.9 eq., DMF, 80 °C, 3 days	84%	0%

The desired compound **178** was successfully synthesised and was tested for its ability to inhibit *T. cruzi*.

5.1 Anti-trypanosomal activity

Compound **178** was the target compound which was similar to the *E* isomer of compound **144**. It had an IC_{50} of 6.98 µM compared to **144** which had an IC_{50} of 1.04 µM. This suggested the more rigid system in this particular compound decreased the biological activity. The ether compound **175** was also assayed and showed an IC_{50} greater than 10 µM. As the results for these compounds were not biologically active they were not investigated further.

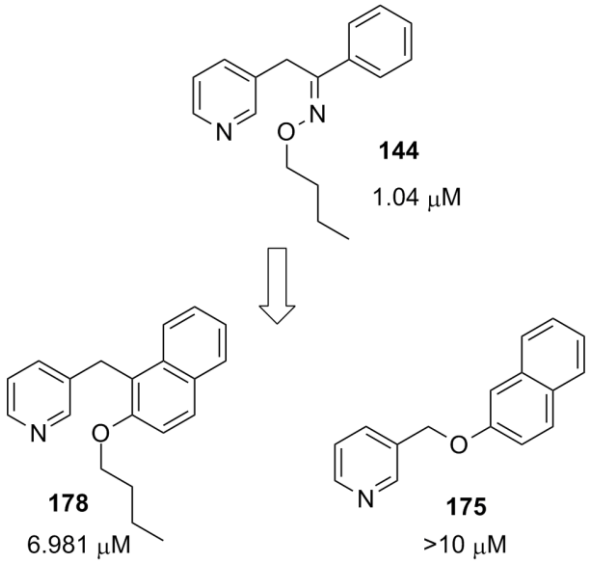


Figure 38

6 Further modification of pyrifenox

Although the majority of pyrifenox derivatives modified the oxime and the halogens on the substituted phenyl ring, further modifications were sought. Two potential analogues based on the modification of the oxime fragment were considered (Figure 39). The position of the oxime in the molecule could be modified such that the methyl oxime is next to the pyridyl ring (compound **180**). The effect of the oxime at this position could be further investigated by substituting it for a cyclopropyl group (compound **181**). Removal of the oxime from the molecule alleviates the problems associated with the separation of the oxime stereoisomers.

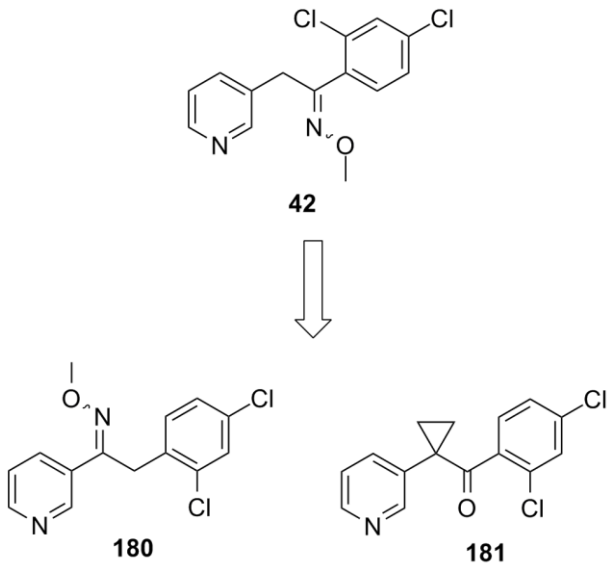
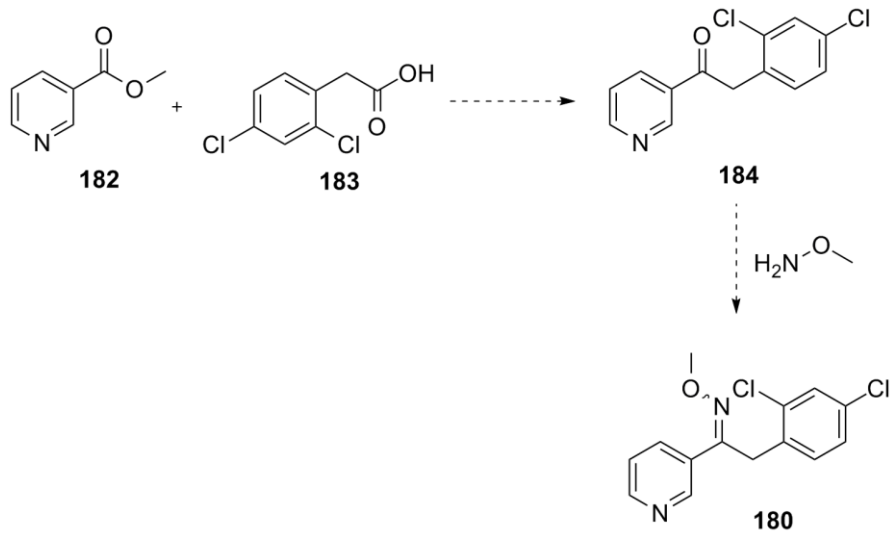


Figure 39. Modification of the oxime fragment.

6.1 Synthesis of the oxime next to the pyridyl ring

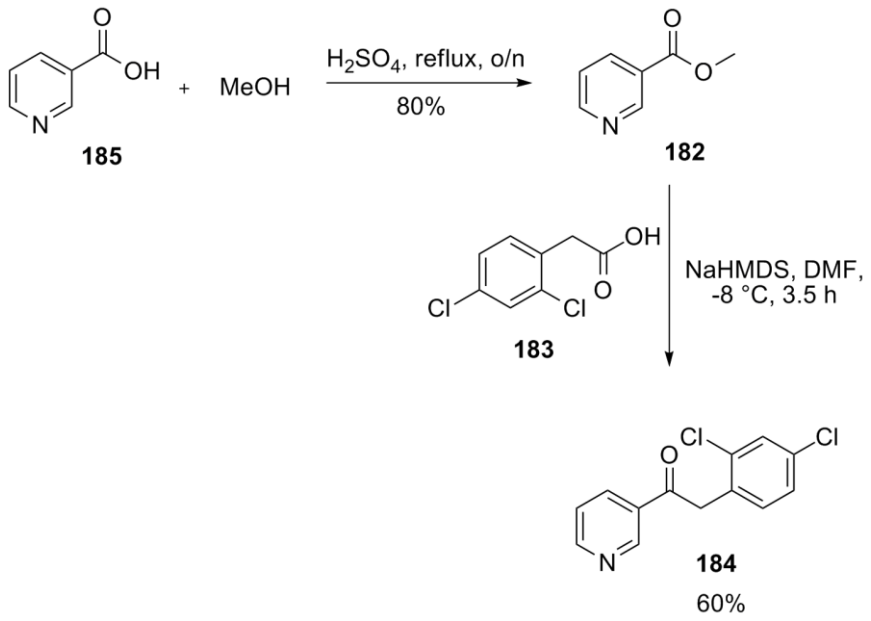
As in the pyrifenox series, ketone **184** was identified as the key intermediate to synthesise the oxime next to the pyridyl ring. Initial investigations conducted at Epichem Pty Ltd produced the desired ketone in a two-step synthesis from a Grignard reaction of benzyl chloride with 3-pyridyl Weinreb amide (*N*,methoxy-*N*-methyl-3-pyridinecarboxamide).[*] The synthesis of 3-pyridyl Weinreb amide was low yielding and expensive to make. An alternate method was explored (Scheme 53). The reaction between methyl nicotinoate **182** and 2,4-dichlorophenylacetic acid **183** would form the ketone in the position next to the pyridyl ring in one step. A simple oxime formation used previously (Section 3.1) would then form the desired product **180** as two isomers.

[*] Alexander, P; Keenan, M and Best, W. Epichem Ltd Pty. Personal communication, 2011



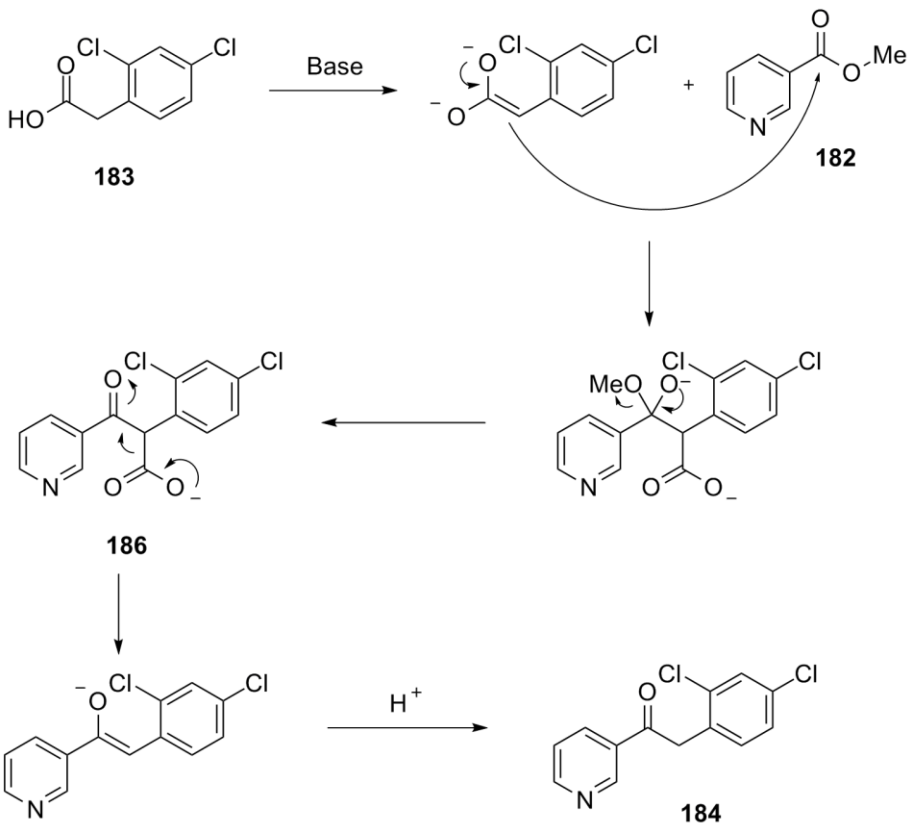
Scheme 53. Proposed synthesis of compound 129.

Wu *et al*. optimised a direct Claisen-decarboxylation cascade reaction between an α-carboxylic acid and an ester of a similar system.[114] This method was used to synthesise ketone **184** (Scheme 54). The ester **182** was made according to Potter *et al*.[115] by heating nicotinic acid **185**, methanol and sulfuric acid under reflux to give the desired nicotinic acid methyl ester **182** in 80% yield. The spectral data of compound **182** matched those in the literature.[115,129] The product obtained was pure and was used in the subsequent reaction without purification. Nicotinic acid methyl ester **182** was stirred with 2,4-dichlorophenylacetic acid **183** and sodium bis(trimethylsilyl)amide (1.0 M in THF) in *N*,*N*-dimethylformamide at −8 °C to give the ketone **184** in 60% yield. The spectral data of compound **184** matched that in the literature.[114] A singlet at 4.98 ppm in the ^{1}H NMR spectrum was assigned to the methylene protons and a signal at 194.7 ppm in the ^{13}C NMR spectrum was assigned to a ketone. This provided a simple method of synthesising the desired ketone **184** in moderate yield (60%) and the ketone did not oxidise in air to the diketone like the pyrifenox system.



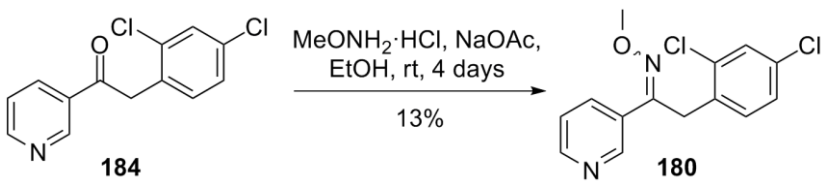
Scheme 54

The mechanism for this reaction was thought to proceed according to Scheme 55.[114] 2,4-Dichlorophenylacetic acid **183** undergoes a Claisen condensation with nicotinic acid methyl ester **182** to form the condensation intermediate **186**. The condensation intermediate **186** was not observed in the reaction as it spontaneously loses carbon dioxide to give the desired decarboxylation product **184**.



Scheme 55. Proposed mechansim for the direct Claisen-decarboxylation cascade reaction.[114]

The final step was the formation of the oxime **180**. The ketone **184** was stirred with *O*-methylhydroxylamine hydrochloride and sodium acetate in ethanol at room temperature (Scheme 56). The two isomers were observed in the ^{1}H NMR spectrum as two separate singlets at 3.82 ppm and 3.99 ppm for the methyl groups whilst the CH_2 signals appeared as two singlets at 3.92 ppm and 4.12 ppm. The two isomers were inseparable by flash chromatography, however the ratio of the *E*:*Z* isomers were estimated to be 1.5:1 from the ^{1}H NMR spectrum of the product.

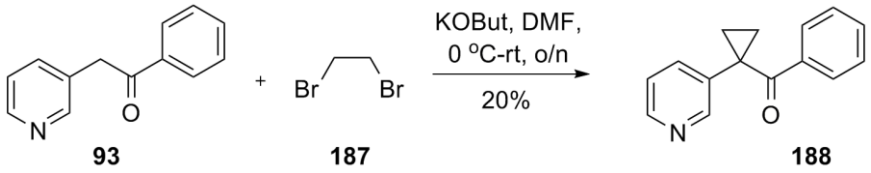


Scheme 56

The oxime **180** was successfully synthesised as 1.5:1 mixture of the two isomers and is currently being tested against *T. cruzi*. Initial investigations conducted by Epichem Ltd Pty* showed an IC_{50} greater than 1 µM.

6.2 Synthesis of an isopropyl group

The effect of the oxime position next to the pyridyl ring was further studied by substituting the oxime for a cyclopropyl group. Since the dichloro-substituted ketone was difficult to synthesise the unhalogenated ketone **93** was used. The ketone **93** was stirred at 0 °C with 1,2-dibromoethane **187** and potassium *tert*-butoxide in *N*,*N*-dimethylformamide for 2.5 hours and then at room temperature overnight.[130] The ^{1}H NMR spectrum showed two apparent doublets of doublets at 1.32 ppm and 1.68 ppm corresponding to the cyclopropyl ring. The lack of singlet at 4.27 ppm in the ^{1}H NMR spectrum suggested the cyclopropyl ring was on the carbon between the ketone and the pyridyl ring. The signal at 199.5 ppm in the ^{13}C NMR spectrum corresponded to the carbonyl carbon. The low yield (20%) was due to the reaction not going to completion and other unidentified by-products being formed. Although the yield was low, the reaction conditions were not optimised as there was enough material for biological testing.



93 **187** **188**

Scheme 57

Compound **188** was successfully synthesised and is currently being tested against *T. cruzi*.

* Alexander, P; Keenan, M and Best, W. Epichem Ltd Pty. Personal communication, 2011

7 Preparation and anti-trypanosomal activity of hydrogenated chalcone analogues

An aspect that was considered important in the structure activity relationship series was the linker between the pyridyl ring and the phenyl ring. Preliminary studies[*] showed the condensed analogue (B) was less active in the Chagas model, suggesting that there needed to be at least one CH_2 linkage between the oxime and the pyridyl ring to achieve good binding (Figure 40). An alternative was to look at the extended analogues (C) which have an extra CH_2 between the oxime functionality and the pyridyl ring compared to pyrifenox. The core of these extended analogues was based on a hydrogenated chalcone, making these compounds readily accessible. As well as extending the chain between the oxime and the pyridyl ring, the substituents X on the phenyl ring and the oxime R group were also varied. Changing the substituents on the phenyl ring from hydrogen to fluorine to chlorine would explore the steric bulk required to bind effectively inside the hydrophobic pocket of CYP51. The oxime R group was also varied to determine how well the oxime chain interacts with the access channel.

[*] Alexander, P; Keenan, M and Best, W. Epichem Ltd Pty. Personal communication, 2011

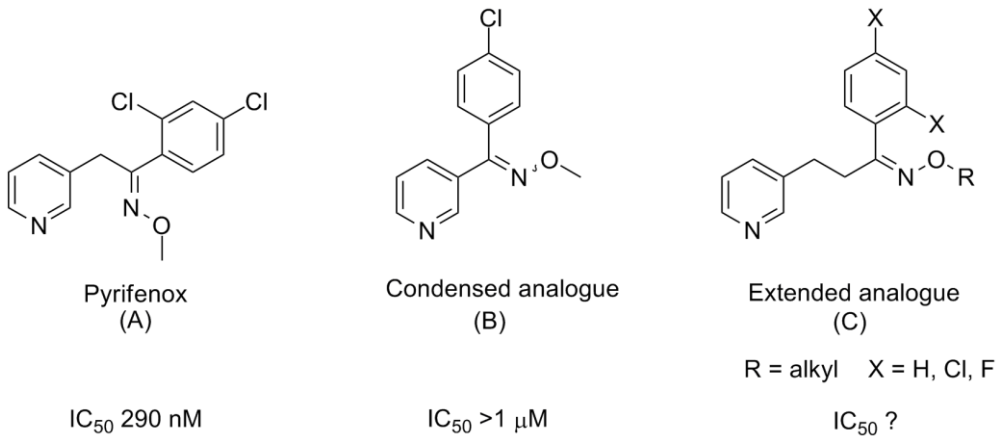
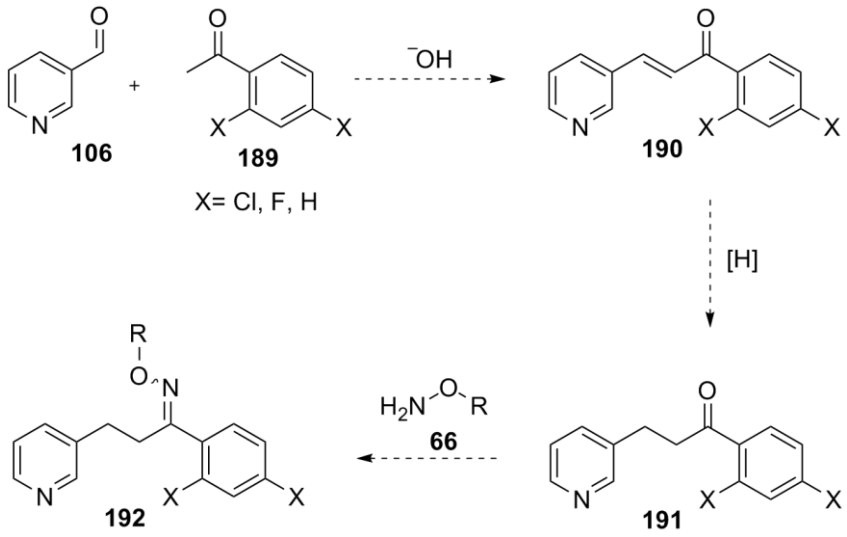


Figure 40

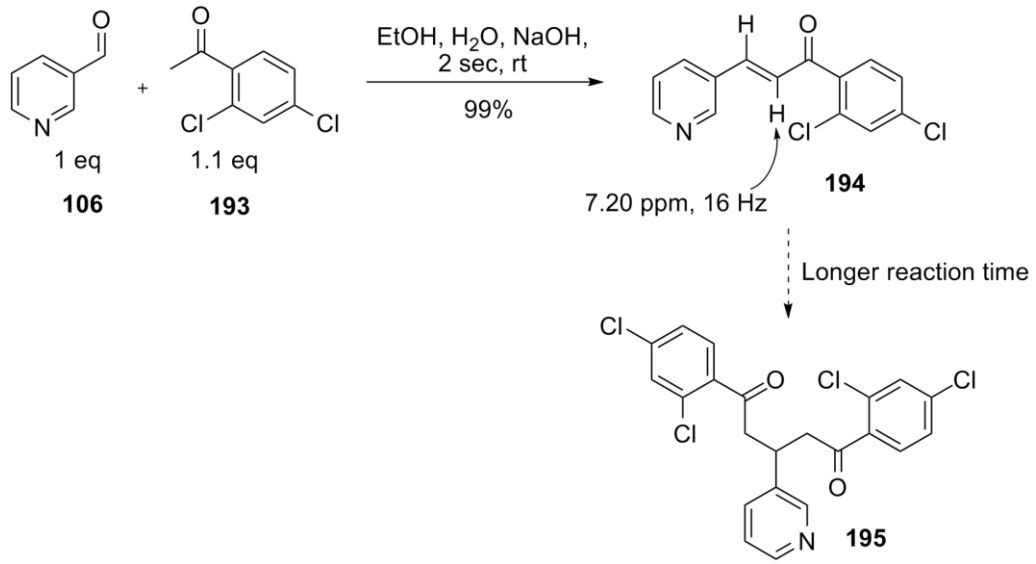
The extended analogues (C) could be made using a 3 step synthesis (Scheme 58). An aldol condensation between 3-pyridinecarboxaldehyde **106** and an appropriate acetophenone **189** would give the chalcone **190**. The alkene of the chalcone could then be hydrogenated to form the intermediate **191**. Finally, a condensation reaction between the ketone **191** and a variety of alkoxyamines **66** would give the desired oxime **192** as two isomers.



Scheme 58. Proposed scheme to synthesise the longer linker analogues.

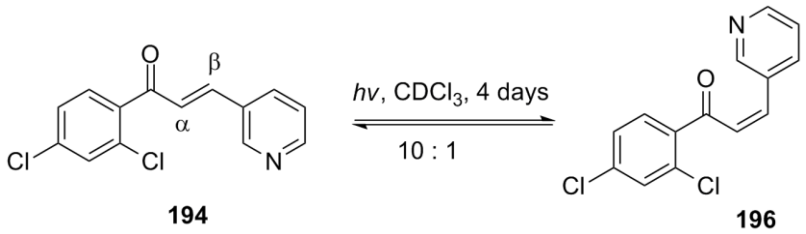
7.1 Synthesis of chalcones

The most closely related extended analogue to pyrifenox would require the 2,4-dichloro substituted chalcone **194**. This compound was previously prepared by Shekarchi *et al.*[131], however the procedure used in this work was based on that by Attar *et al.*[132] 3-Pyridinecarboxaldehyde **106** and 2′,4′-dichloroacetophenone **193** were stirred in a solution of sodium hydroxide in ethanol and water (Scheme 59). Deionised water was added immediately causing a solid to precipitate. The solid was collected to give the pure chalcone **194**. The spectral data of compound **194** match that in the literature.[131] The ^{1}H NMR spectrum showed a doublet at 7.20 ppm with a *J* coupling of 16.1 Hz which corresponded to a *trans* alkene. The corresponding doublet was in the middle of the aromatic signals between 7.45−7.53 ppm. The signal at 1678 cm^{-1} in the IR spectrum was assigned as a C=O stretch in conjugation with the phenyl ring. If the chalcone was left in solution for too long (5 min), 2′,4′ -dichloroacetophenone **193** reacted with the double bond of the chalcone **194** via a Michael addition to give the disubstituted product **195.** This phenomenon has been reported in the literature.[133] To prevent the Michael addition from occurring, 3-pyridinecarboxaldehyde **106** was used in a slight excess (1.1 eq.) and a large excess of water (400 mL) was added to precipitate out the product immediately after the addition of the base. This reaction could be performed on large scales (10 g) with high yields (99%) and did not require purification.



Scheme 59

The synthesis of the chalcone **194** produced exclusively the *trans* isomer. This was confirmed by ^{1}H NMR spectroscopy by the two vinylic protons coupling constants (16.1 Hz) observed in the spectrum. When the chalcone **194** was allowed to stand in solution ($CDCl_3$) for two days, the *cis* isomer **196** was observed in the ^{1}H NMR spectrum (Scheme 60). In Figure 42, the initial ^{1}H NMR spectrum showed the *trans* alkene at 7.20 ppm. After one week two doublets were observed in the ^{1}H NMR spectrum at 6.68 ppm and 7.00 ppm both with a *J* value of 12.5 Hz corresponding to a *cis* alkene as a 10:1 ratio. When the solution was left in the dark for the same amount of time the *cis* alkene was not observed. This was consistent with the literature which states that irradiation of chalcones in solution by sunlight converts the chalcone from the *trans* to the *cis* conformation.[134] The ratio of the two isomers was 10:1 after one week and did not change after a further two weeks. This suggested the isomerisation of the *trans* to *cis* alkene is in equilibrium. As the alkene is hydrogenated in the next step both isomers can be used.



Scheme 60

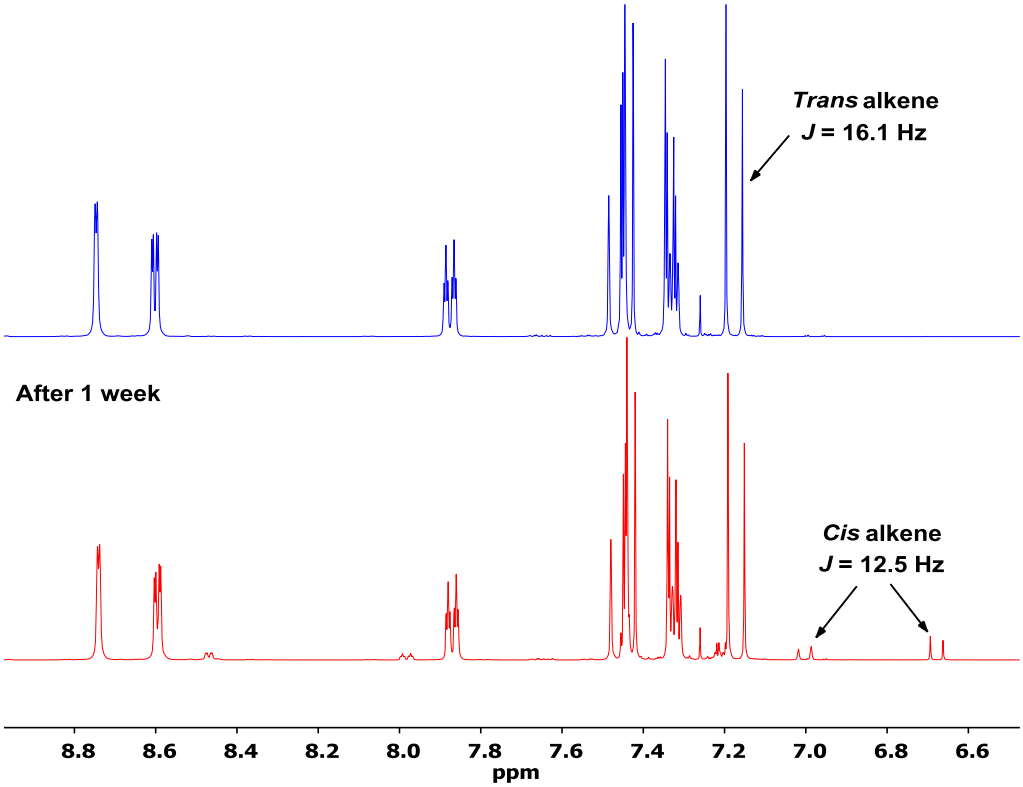
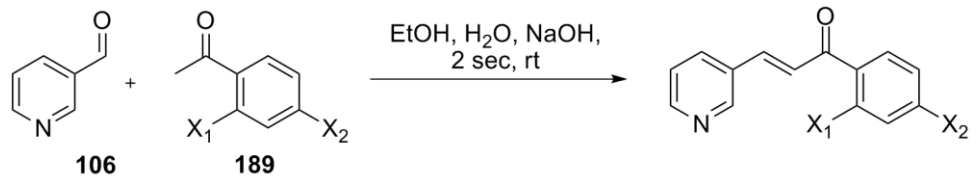


Figure 41. ^{1}H NMR spectrum of compound **194** (top) and after 1 week in $CDCl_3$ solution (bottom).

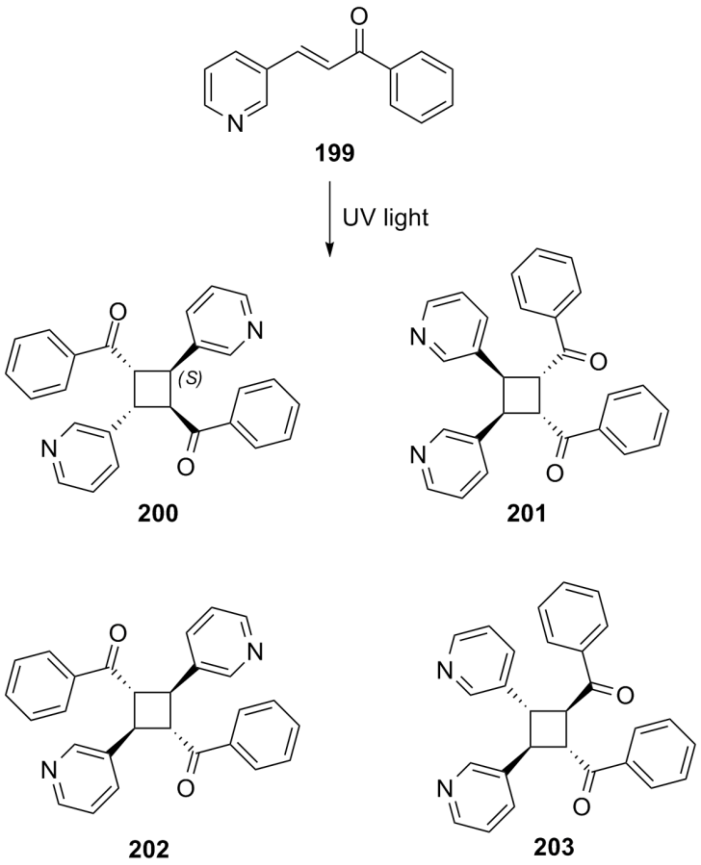
A variety of pyridyl chalcones were also synthesised under the optimised conditions (Scheme 61). The acetophenones **189** and 3-pyridinecarboxaldehyde **106** were reacted with a solution of sodium hydroxide in ethanol and water. The 4-fluoro and 4-chloro chalcones (**197** and **198**) were synthesised on a large scale (10 g) in high yields (90% and 92% respectively) and did not require any further purification. However, the reaction to form the unsubstituted chalcone was low yielding (26%) and the ^{1}H NMR spectrum of the crude material showed a complex mixture. The mixture was separated by column chromatography to give the unsubstituted chalcone and the Michael addition dimer.



Scheme 61

Compound	X_1	X_2	Yield
197	H	F	90%
198	H	Cl	92%
199	H	H	26%

Another side reaction that could occur is the cyclisation of the chalcone in the solid state, molten state and solution by UV irradiation (Scheme 62).[135,136] The chalcone can undergo intermolecular [2+2] photocycloaddition to give a cyclobutane.[135] Recrystallisation of the unsubstituted chalcone **199** from dichloromethane and petroleum spirits gave fine white crystals. The ^{1}H NMR spectrum of the crystals showed the absence of an alkene signal and therefore was not the desired compound **199** but could be the cyclised form. Chalcones similar to this compound have been known to dimerize by photochemical pathways.[134,136–138] There are four possible configurations that could form (Scheme 62) and the different configurations may be dependent on the physical state of the substrate and the conditions under which it absorbs light.[135] The configurations are *syn*-head-to-tail **200**, *syn*-head-to-head **201**, *anti*-head-to-tail **202**, and *anti*-head-to-head **203**.[135,136,138] The ^{1}H NMR spectrum showed only one compound was present. X-ray crystallography of the white crystals confirmed the configuration as compound **200** (Figure 42). The photodimerisation of the chalcone only gave the *syn*-head-to-tail dimer out of the four possible dimers. To prevent the cyclisation of the chalcone **199** from occurring it was stored in a brown glass vial.



Scheme 62

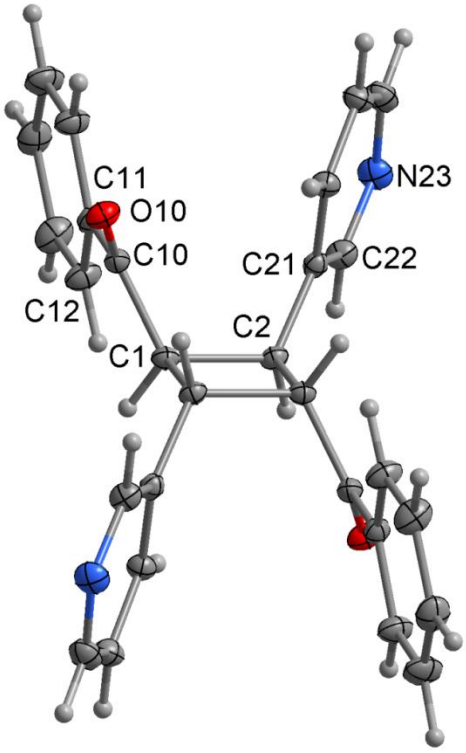


Figure 42. Crystal structure of compound **200**.

7.2 Hydrogenation of an alkene

The next reaction was a selective conjugated reduction of the α,β-unsaturated carbonyl of the chalcones. The methods investigated are outlined in Table 21. A common form of hydrogenation in the literature was using a highly reactive form of zinc.[139,140] Li *et al*. developed a method using zinc powder and ammonium chloride in water and ethanol.[140] The authors stated that the way the zinc was added to the reaction mixture was critical. It had to be added in three equal portions in order to obtain the best yield. The chalcone **197** was stirred with zinc powder and ammonium chloride in water and ethanol at room temperature (entry 1). This produced the desired compound **204** in 30% yield. The ^{1}H NMR spectrum showed two multiplets at 3.03−3.09 ppm and 3.24−3.31 ppm each with an integration of two hydrogens and were assigned to the methylenes. The ^{13}C NMR spectrum showed two CH_2 carbons at 27.2 ppm and 39.7 ppm corresponding to the reduced alkene. The ketone was still present due to the ^{13}C NMR spectrum showing a peak at 196.9 ppm and the C=O peak in the IR spectrum (1682 cm^{-1}). The low yield was due to the reaction not going to completion with 25% starting material being recovered. An alternate method to prepare the activated zinc was sought to improve the yield. Activated zinc can also be prepared *in situ* from magnesium and zinc chloride.[139] The chalcone **197** was reacted with magnesium turnings and zinc chloride in water at room temperature (entry 2). Unfortunately, only starting material was recovered.

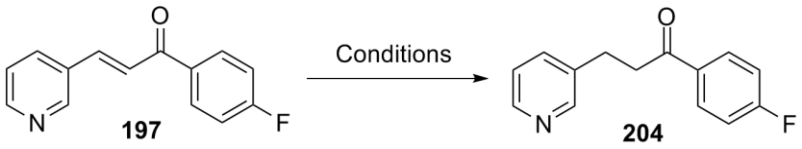


Table 21

Entry	Conditions	Product
1	Zn, NH_4Cl, H_2O, EtOH, 3 h, rt.	30%
2	Mg, $ZnCl_2$, H_2O, 3 days, rt.	No reaction
3	Pd/C, CH_3COOH, $NaBH_4$, toluene, o/n, rt.	Complex mixture

The other metal that is commonly used for the selective reduction of alkenes is palladium.[141] The chalcone **197** was stirred at room temperature with palladium on carbon, sodium borohydride and acetic acid in toluene (Table 21, entry 3). The ^{1}H NMR spectrum of the crude material showed the desired hydrogenated product and a complex mixture of other side products. These could be other common reductive products such as the ketone being reduced to the alcohol (Figure 43).[141] The ^{1}H NMR spectrum of the crude material showed two broad signals in the 4.5−5.5 ppm region. These could be due to the hydrogen on the carbon with the hydroxyl group (**205** or **206**). There were also mutliplets in the same region as the desired compound. This suggested that compound **204** was produced but as part of a complex mixture.

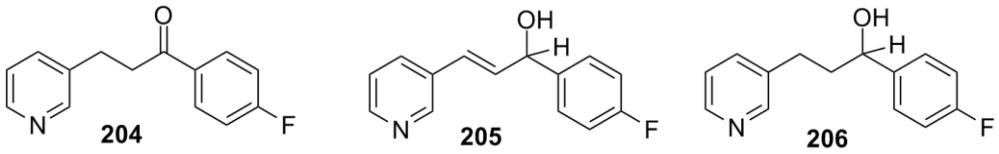
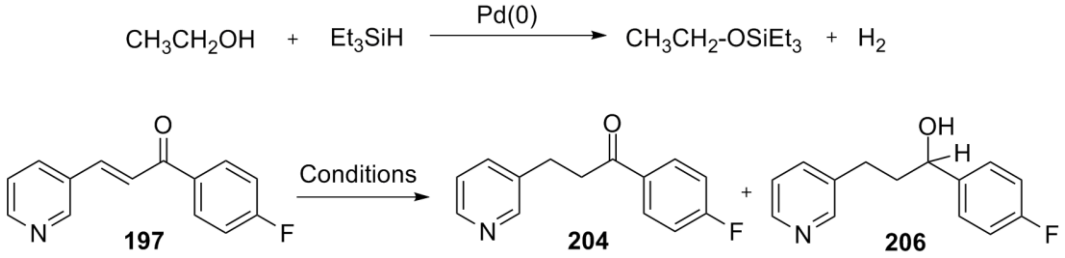


Figure 43

Hydrogenation of α,β-unsaturated carbonyl compounds has been shown to occur with triethylsilane and palladium chloride in ethanol. The palladium would catalyse the reaction between ethanol and triethylsilane to generate molecular hydrogen, which could then reduce the alkene (Scheme 63).[142] The chalcone **197** was heated with triethylsilane and palladium chloride in ethanol under reflux to give the desired hydrogenated product **204** and the ethoxysilane by-product. The by-product was seen in the ^{1}H NMR spectrum of the crude material as a quartet at 3.67 ppm corresponding to the CH_2 next to the oxygen, a triplet at 1.19 ppm corresponding to the CH_3 on the ether chain and multiplets at 0.93 ppm and 0.56 ppm corresponding to the CH_3 and CH_2 of the triethylsilane. On a small scale the product could be purified by the extraction of the hydrogenated chalcone **204** with a solution of hydrochloric acid (1 M), to separate it from the ethoxytriethylsilane by-product, and then neutralising the acidic layer with a solution of sodium bicarbonate. On a larger scale (2 g) column chromatography was required, as the impurities were not able to be removed with the acid/base washings. The use of column chromatography caused the yield to decrease.

If an excess of triethylsilane was used compound **197** was hydrogenated further to form the alcohol **206**. This was observed in the ^{1}H NMR spectrum as a doublet of doublets at 4.54 ppm corresponding to the hydrogen next to the hydroxyl group and two multiplets at 2.59 and 1.83 ppm corresponding to the CH_2 groups. This is consistent with that reported in the literature[143] where both alkene and carbonyl groups were indiscriminately reduced in the presence of excess triethylsilane due to the increased hydrogen content in solution. Therefore, only one mole equivalent of triethylsilane was required to obtain compound **204** in optimum yield (94%).



Scheme 63

Entry	Conditions	Product	
		204	**206**
1	$PdCl_2$, TES 2.8 eq., EtOH, reflux, o/n	31%	32%
2	$PdCl_2$, TES 1 eq., EtOH, reflux, o/n	94%	0%

The optimised conditions for the selective hydrogenation using palladium chloride and triethylsilane in ethanol were also used to hydrogenate the chloro- and dichloro-substituted compounds (Table 22). The chalcones **190** were heated under reflux with palladium chloride and one equivalent of triethylsilane in ethanol. The lower yields obtained for the chloro- and dichloro-substituted compounds (**208** 71% and **209** 78%) compared with the fluoro-substituted compound **204** (94%) were due to the reaction being done on a larger scale (1.6 g) and thus column chromatography was required to purify them. Both compounds showed the key alkane signals in the ^{1}H NMR (3.0−3.3 ppm) and the ^{13}C NMR (27−43 ppm) spectra. When an excess of triethylsilane (3 eq.) was used the chloro-substituted compound was hydrogenated further to remove the ketone giving compound **207** (22%) along with **191** (32%). This was seen in the ^{1}H NMR spectrum as multiplets integrating for four hydrogens

at 2.60−2.70 ppm and a multiplet at 1.90−2.05 ppm integrating for two hydrogens. The ^{13}C NMR spectrum showed three signals at 32.4, 32.6 and 35.2 ppm corresponding to the methylenes. This was different to what is seen in Scheme 63 where the carbonyl group is hydrogenated to the alcohol. The fluoro substituted compound is more electron withdrawing pulling electrons out of the system. Therefore it cannot support the carbocation and results only in the formation of the alcohol. With the chlorinated systems the ketone is completely hydrogenated.

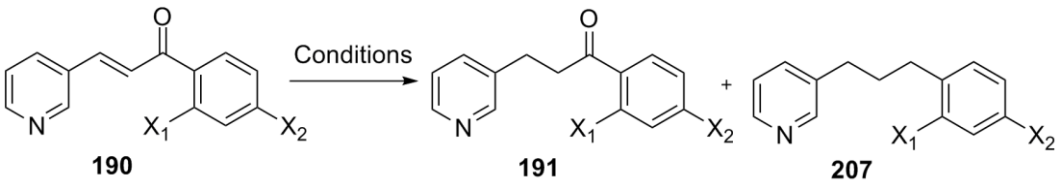


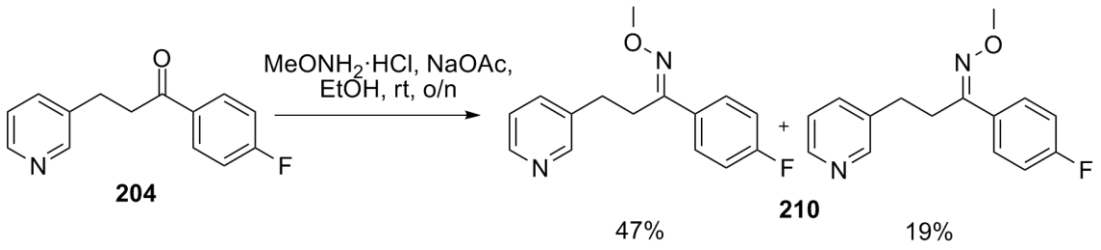
Table 22. Conditions- $PdCl_2$, TES, EtOH, reflux, 7 h.

Entry	Eq. TES	X_1	X_2	Yield	
				191	**207**
1	1	H	F	94%	0%
2	1	H	Cl	71%	0%
3	1	Cl	Cl	78%	0%
4	3	H	Cl	32%	22%

7.3 Methyloxime analogues

The final step in the synthesis was the formation of the oxime. The ketone **204** was stirred with *O*-methylhydroxylamine hydrochloride and sodium acetate in ethanol at room temperature (Scheme 64).[117] Two stereoisomers were seen in the ^1H NMR spectrum of the crude material as two singlets at 3.90 ppm and 3.74 ppm corresponding to the methyl groups. The two isomers were isolated by column chromatography due to the slightly different polarities of the two compounds. The major compound was the less polar isomer. The ^1H NMR spectrum of the less polar isomer showed the singlet at 3.90 ppm corresponding to the methyl group and two separate multiplets at 2.73−2.79 ppm and 3.00−3.06 ppm corresponding to the CH_2 groups. The signals in the ^1H NMR spectrum for the more polar isomer are more shielded. The methyl signal was seen at 3.74 ppm and the CH_2 signals were merged

into one large multiplet at 2.72−2.77 ppm. The high resolution mass spectrometry showed that both compounds have the same accurate mass, thus indicating both isomers were synthesised.



Scheme 64

The identification of the *E* and *Z* isomers proved to be difficult, however they were assigned based on evidence obtained from the polarity of previous compounds synthesised and NOE experiments. The pyrifenox series suggested the *E* isomer was the less polar isomer and the *Z* isomer was the more polar isomer. It could be thought the less polar isomer of compound **210** could be the *E* isomer and the more polar isomer could be the *Z* isomer. NOE experiments were conducted to investigate this further. All of the enhancements were very small, however they suggest a slight difference in the enhancements. The irradiation of H_A in the less polar isomer showed NOE effects with H_C (1.2%) and H_D (0.2%) whilst in the more polar isomer showed a NOE effect of 0.9% (Figure 44). This suggested that in the less polar isomer the phenyl ring was closer to the alkyl chain and the more polar isomer is perpendicular to the pyridine ring. Irradiation of H_A showed no enhancements to the methyl group in the less polar isomer but a slight enhancement (0.1%) in the more polar isomer. This suggested the more polar isomer could be the *Z* isomer. Irradiation of H_C showed a slight signal enhancement for the less polar isomer (0.2%) but there was no effect with the more polar isomer. This suggested that the less polar isomer could be the *E* isomer.

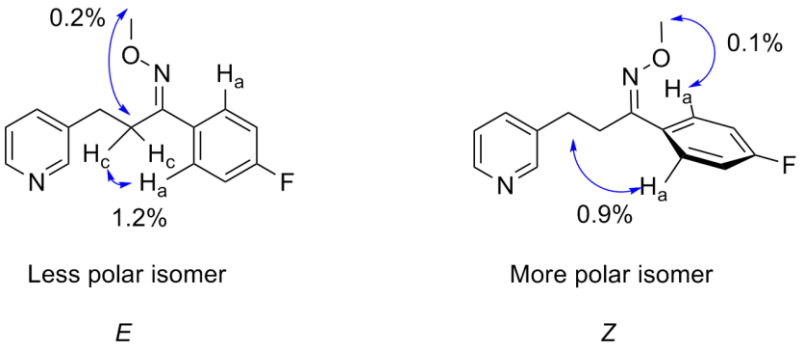


Figure 44. NOE effects of the two isomers used to determine the configuration.

The same conditions were used to synthesise the methyloximes for the chloro- and dichloro-substituted compounds. The ketone **191** was stirred with *O*-methylhydroxylamine hydrochloride and sodium acetate in ethanol (Table 23). The ^{1}H NMR spectra for the chloro- and dichloro-substituted methyl oximes showed similar peaks to the fluoro substituted methyl oxime. This was used to identify the *E* and *Z* isomers. In all three cases the *E* isomer was the major product.

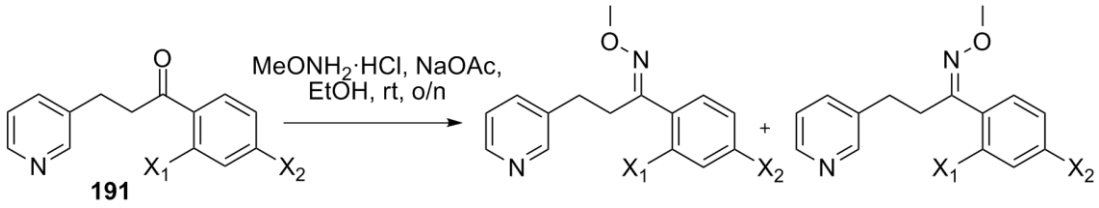


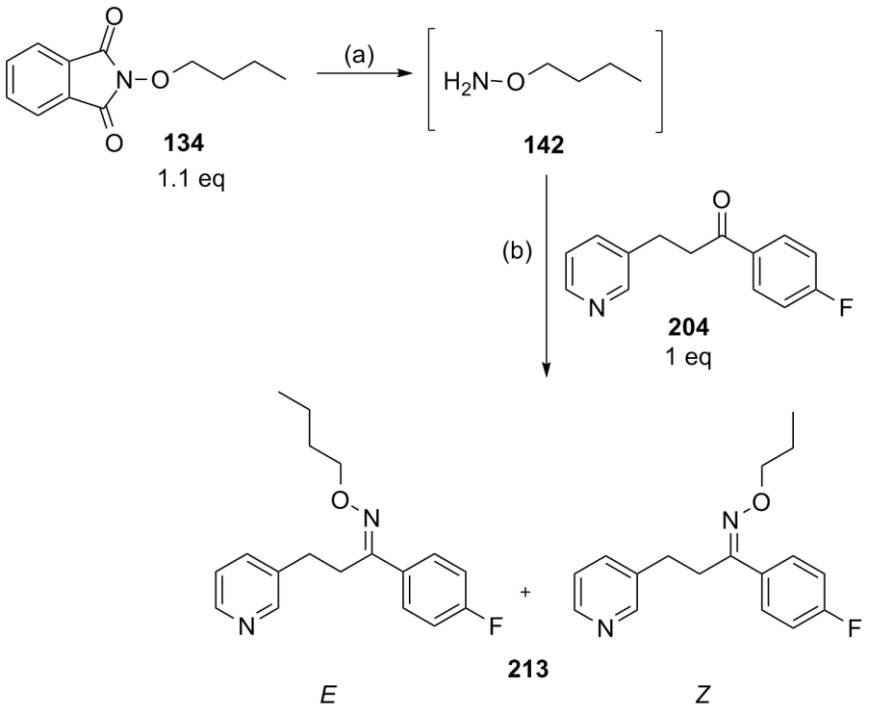
Table 23

Compound	X_1	X_2	*E*	*Z*
210	H	F	47%	19%
211	H	Cl	42%	11%
212	Cl	Cl	38%	13%

.

7.4 Longer chain oximes

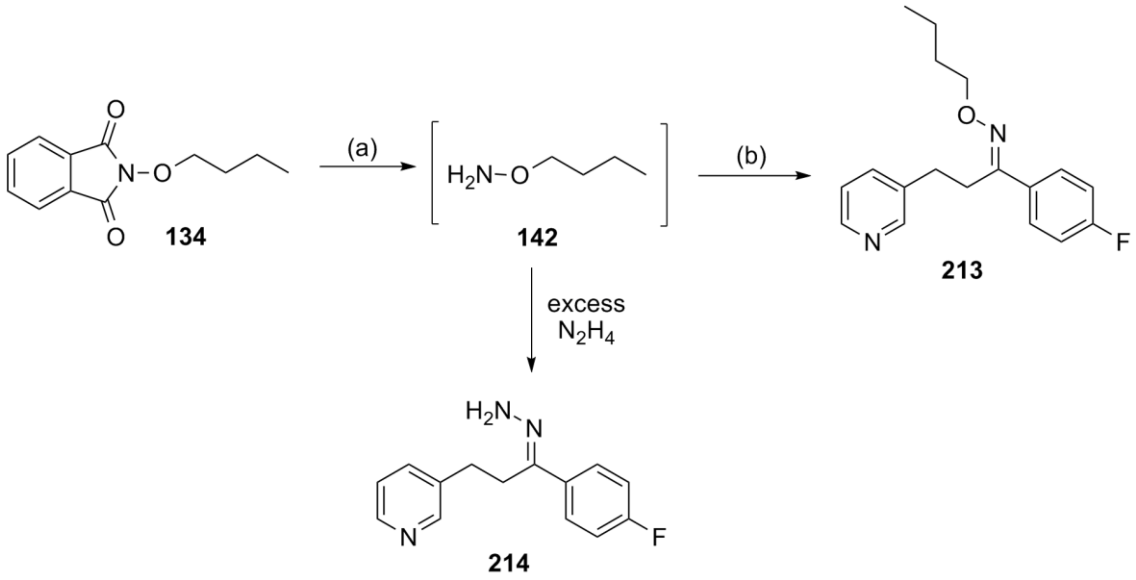
Having successfully synthesised the methyl oximes **210**, **211** and **212**, more complicated oximes were sought. The butyl and isopropyl alkoxyamine precursors **83** that were synthesised in Section 3.3 were used. As already mentioned in Section 3.3, the alkoxyamines were volatile and were difficult to isolate. A one-pot synthesis was used to convert the alkoxyamine precursors to the alkoxyamines before the addition of the ketone (Scheme 65). *N*-Butoxyphthalimide **134** and hydrazine hydrate in dichloromethane were heated under reflux until a white precipitate formed.[118] A solution of the fluoro ketone **204** in acetic acid and ethanol was added to the reaction mixture with continued heating under reflux. The ^{1}H NMR spectrum of the crude material showed mainly the ketone **204** and trace amounts of the oxime as two isomers. Separation of the crude material by column chromatography afforded the starting material **204** (67% yield) and one isomer (2% yield). The ^{1}H NMR spectrum of the isolated product **213** showed two multiplets at 2.80−2.87 ppm and 2.97−3.05 ppm corresponding to the linker. The CH_2 next to the oxime appeared as a triplet at 4.14 ppm. This was shifted downfield compared to the free hydroxyl amine and was in a similar region to the methyl group of the previous methyl oximes. Based on the CH_2 groups of the linker between the pyridyl ring and the oxime being seen as two multiplets, it was concluded that the *E* isomer was synthesised. As the yield was very low (2%), the initial solvent was changed from dichloromethane to ethanol.[117] Although the yield remained low (13%), the *Z* isomer was also observed in the ^{1}H NMR spectrum of the crude material (Table 24). Unfortunately the *Z* isomer could not be purified for analysis as it was not isolated from the column.



Scheme 65

Entry	Conditions	Yield		
		Starting Material	*E*	*Z*
1	a) $NH_2NH_2 \cdot H_2O$, DCM, reflux, o/n; b) CH_3COOH, EtOH, reflux, 1 day	67%	2%	0%
2	a) $NH_2NH_2 \cdot H_2O$, EtOH, reflux, 2.5 h; b) CH_3COOH, EtOH, reflux, 2 days	64%	9%	4%

If the hydrazine hydrate was used in a large excess it would react with the ketone **204** to form the hydrazone **214** (Scheme 66). The ^1H NMR spectrum showed a product similar to the ketone except for the shifting of the peaks associated with the CH_2 linker. The signals at 2.82 ppm and 3.17 ppm had moved upfield and there was a larger gap between the two signals (0.35 ppm compared to 0.18 ppm for compound **213**). The mass spectrum showed a mass similar to the molecular weight of the hydrazine adding to the ketone. To prevent this from occurring, the hydrazine hydrate was used only in a slight excess (1.1 eq.).



Scheme 66. Conditions- a) $NH_2NH_2 \cdot H_2O$, EtOH, reflux, 30 min; b) **204**, CH_3COOH, EtOH, reflux, 2 days.

The optimised conditions were used to synthesise other analogues (Table 24). The precursor **132** was heated with hydrazine hydrate in ethanol for 30 minutes under reflux before the ketone **191** in acetic acid and ethanol were added. The isomers were separated by flash chromatography except for compounds **215** and **216**, where the *Z* isomer was inseparable from the *E* isomer. Although the isolated yields for all the oximes were low (Table 24), sufficient product was obtained for biological testing, and the reaction was not optimised further.

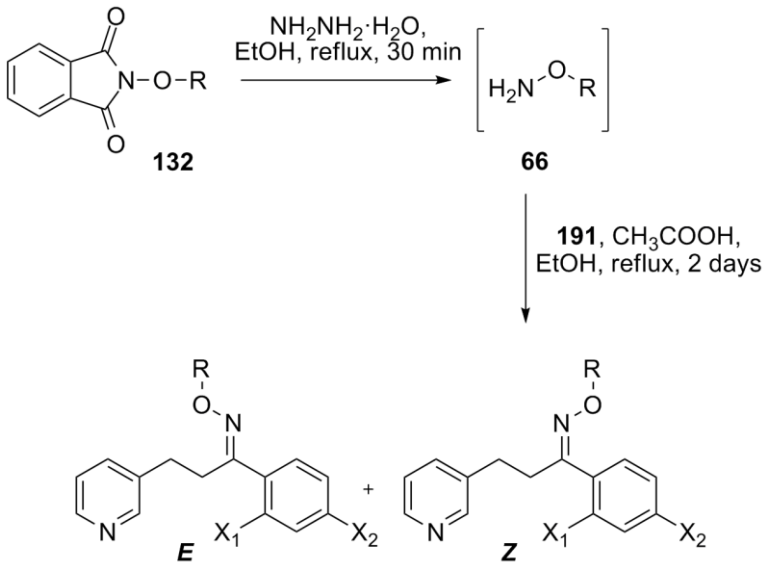
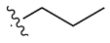
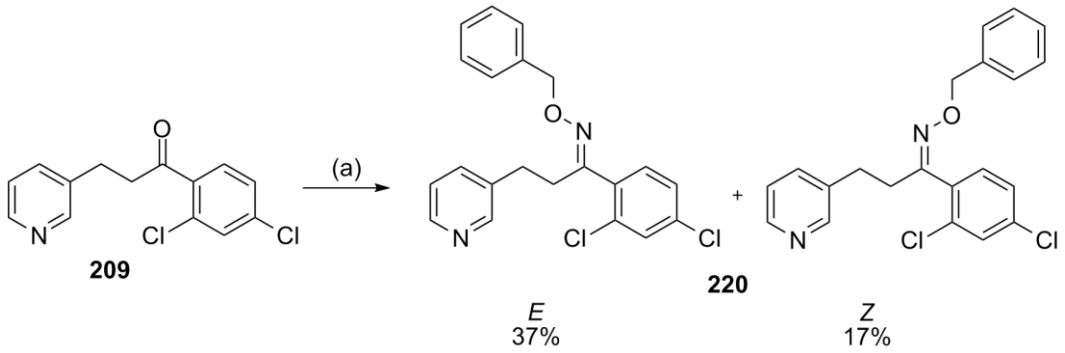


Table 24

Compound	X_1	X_2	R	Yield	
				E	***Z***
213	H	F		9%	2%
215	H	Cl		20%	7%*
216	Cl	Cl		1.7%	4%*
217	H	F		28%	4%
218	H	Cl		7%	3%
219	Cl	Cl		16%	5%

*Mixture of isomers

To investigate the impact of steric bulk on the oxime binding within the access tunnel, the benzyl analogue was also synthesised. The dichloro ketone **209** was stirred at room temperature with *O*-benzylhydroxylamine hydrochloride and sodium acetate in ethanol (Scheme 67). The ^{1}H NMR spectrum of the crude material showed two singlets at 4.98 ppm and 5.22 ppm indicating two isomers were formed. The *E* isomer was isolated but, unfortunately, the *Z* isomer could not be purified.

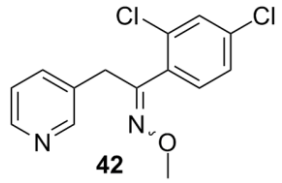
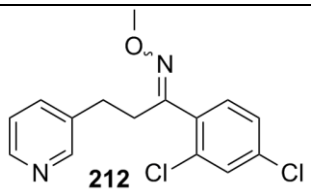
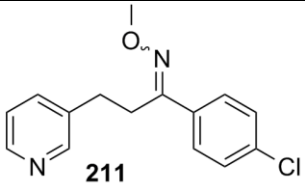
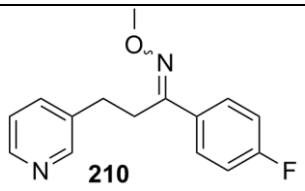


Scheme 67. Conditions- a) $BnONH_2 \cdot HCl$, NaOAc, EtOH, o/n, rt.

7.5 Anti-trypanosomal activity

All the oximes synthesised were tested for their ability to inhibit *T. cruzi*. The methyl oxime was the most structurally similar compound to pyrifenox. The *E* isomer of the dicholoro methyl oxime **212** had an IC_{50} of 1.87 µM and the *Z* isomer had an IC_{50} of 1.43 µM. In comparison, the IC_{50} value of pyrifenox **42** was 0.29 µM which suggested that increasing the carbon chain by one carbon decreases the anti-trypanosomal activity. Although the *Z* isomer had a slightly lower IC_{50} than the *E* isomer, they were very similar. This suggested one isomer might not be favoured over the other, although the *Z* isomer had a greater cytotoxicity (77.73 µM, SI = 54). On changing the phenyl ring to the 4-chloro-substituted ring (compound **211**), the inhibition also decreased. The *E* isomer had an inhibition of 3.18 µM whilst the *Z* isomer had an inhibition of 4.56 µM. The *E* isomer of the fluoro methyl oxime **210** also had a lower IC_{50} value (6.01 µM) as compared to the *Z* isomer which a slightly better inhibition of 3.62 µM.

Table 25

Structure	IC_{50} (toxicity)	
	E	***Z***
 **42**	0.29 µM	
 **212**	1.87 µM (>100 µM)	1.43 µM (77.43 µM) SI = 54
 **211**	3.18 µM (>100 µM)	4.57 µM (100 µM)
 **210**	6.01 µM (>100 µM)	3.62 µM (>100 µM)

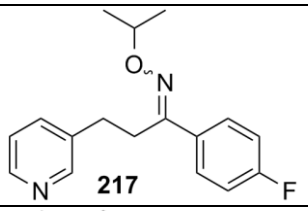
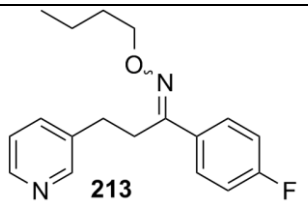
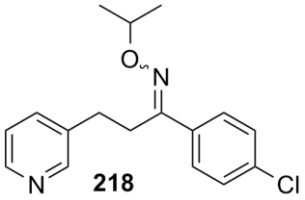
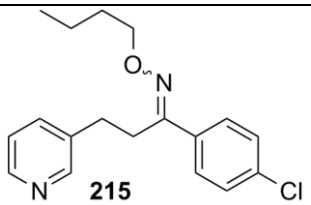
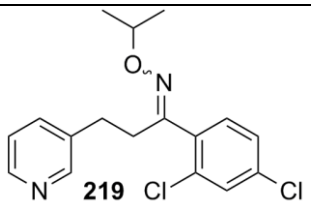
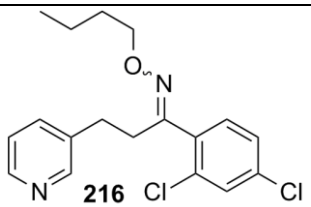
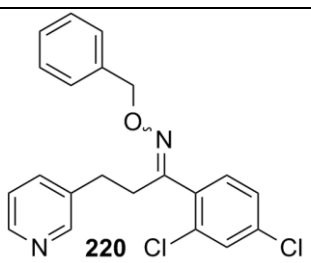
The length of the oxime was also investigated (Table 26). The fluoro isopropyl oxime **217** had an IC_{50} of 1.57 µM and 6.80 µM for the *E* and *Z* isomers respectively. This was an increase in activity for the *E* isomer but a decrease for the *Z* isomer. On changing the oxime to a butyl chain (compound **213**), only the *E* isomer was isolated. This showed an IC_{50} of 1.23 µM which was an improvement on the biological activity as compared to the methyl oxime. This suggested that the butyl chain interacts better into the CYP51 pocket. The chloro isopropyl oxime **218** IC_{50} for the *E* isomer was 1.45 µM and the Z isomer was 1.17 µM. Both the *E* and *Z* isomers showed very similar IC_{50} to the fluoro *E* isomer (**217** IC_{50} 1.57 µM). The chloro butyl oxime **215** showed a slightly better inhibition. The *E* isomer had an IC_{50} of 0.49 µM and the *Z* isomer an IC_{50} of 2.10 µM. In this case the *E* isomer had a better inhibition which was approaching the activity of pyrifenox **42** (0.29 µM). The dichloro isopropyl oxime **219** had a similar inhibition to the chloro compound. The *E* isomer had an IC_{50} of 1.82 µM and the *Z* isomer had an IC_{50} of 1.17 µM. On increasing the length of the oxime chain to a butyl chain the inhibition was also

similar to the chloro. The *E* isomer had an IC_{50} of 0.55 µM and the *Z* isomer had an IC_{50} of 0.90 µM. This suggested that compounds with chloro substituents have a slightly better activity than compounds with fluoro substituents; however, there was not much difference between the chloro and dichloro derivatives.

In comparing the toxicities, all the fluoro and chloro compounds had a toxicity greater than 100 µM except for the fluoro butyl oxime **213**. The dichloro compounds were starting to show some toxicity with the dichloro butyl oxime having a toxicity of 83.52 µM for both isomers. Although some compounds had a slight toxicity, the SI values were very large.

A benzyl oxime was also synthesised for the dichloro substituted compound to investigate the steric bulk required to fit into the access tunnel. The *E* isomer of compound **220** produced an IC_{50} of 0.24 µM which was similar to pyrifenox (0.29 µM). Although the longer chain between the phenyl and pyridyl rings decreased the biological activity, having a larger oxime gave a compound with similar inhibition. This suggested that the access tunnel requires a bulky group on the oxime to increase the binding affinity.

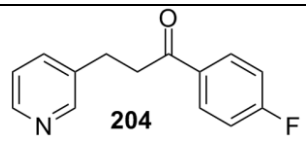
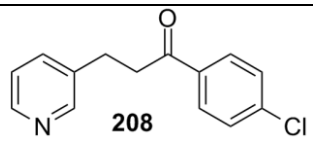
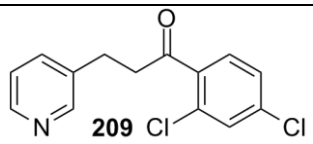
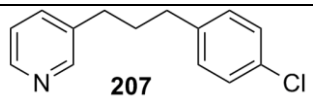
Table 26

Structure	IC_{50} (toxicity)	
	E	***Z***
 **217**	1.57 µM (>100 µM)	6.80 µM (>100 µM)
 **213**	1.23 µM (75.09 µM) SI = 61	–
 **218**	1.45 µM (>100 µM)	1.17 µM (>100 µM)
 **215**	0.49 µM (>100 µM)	2.10 µM (>100 µM)*
 **219**	1.82 µM (>100 µM)	1.17 µM (>100 µM)
 **216**	0.55 µM (83.52 µM) SI = 151	0.90 µM (83.52 µM)* SI = 93
 **220**	0.24 µM (>100 µM)	–

*Mixture of isomers

As a comparison the ketones were analysed against *T. cruzi* to see if the oxime was required. As can be seen in Table 27, the inhibitions of the ketones were similar to the methyl oximes. The fluoro ketone **204** had an IC_{50} of 6.20 µM compared with the *E* isomer which had an IC_{50} of 6.01 µM. The dichloro ketone **209** had an IC_{50} of 1.54 µM compared with the methyl oxime **212** which had an IC_{50} of 1.87 µM and 1.43 µM. The chloro ketone **208** is still awaiting results but from the other ketones analysed it can be predicted that it will be similar in activity to the methyl oxime **211**. The fully hydrogenated chloro compound **207** was also analysed. It had an IC_{50} of 2.77 µM which was slightly better than the chloro methyl oxime (3.18 µM and 4.57 µM). This could be due to the compound being more flexible than the methyl oxime or the compounds are more lipophilic.

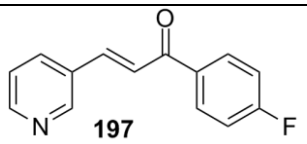
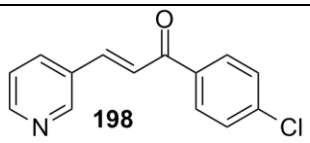
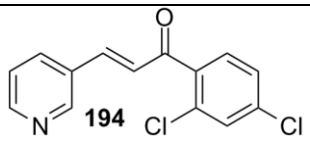
Table 27

Structure	IC_{50} (toxicity)
 **204**	6.20 µM (>100 µM)
 **208**	In testing
 **209**	1.54 µM (>100 µM)
 **207**	2.77 µM (>100 µM)

The chalcones inhibition of *T. cruzi* was also investigated (Table 28). In most cases the chalcone was more active than the ketone. This could be due to the chalcone being more rigid than the ketone and therefore binding differently. Although the chalcones are more active, they are also more toxic. The double bond makes the compound a good Michael acceptor and therefore it may be reacting indiscriminately with proteins within the cell causing a higher cytotoxicity. Previously the dichloro-

substituted compounds appeared to be the most toxic. With the chalcones, the fluoro chalcone **197** was shown to be the most toxic (16.33 µM, SI = 8). This was most likely due to the fluoro substituent being more electron withdrawing than the chloro substituents making it a better Michael acceptor. Therefore it can react more readily with the biomolecules in the parasite and in human cells.

Table 28

Structure	IC_{50} (toxicity)
 **197**	2.12 µM (16.33 µM) SI = 8
 **198**	0.69 µM (17.46 µM) SI = 25
 **194**	1.88 µM (19.14 µM) SI = 10

Chalcones have recently been reported to show good inhibition against *T. cruzi* (Figure 45).[144,145] Lunardi *et al*. reported the unhalogenated chalcone showed the best activity of their series with an IC_{50} of 24.8 µM.[144] The chalcones synthesised in this chapter showed a better inhibition than those reported with the IC_{50} ten times that in the literature. This could be due to the pyridine ring making the compound more water soluble. The nitrogen in the pyridine ring can interact with enzymes either through hydrogen bonding or through coordination with metal centres, such as iron in the heme.

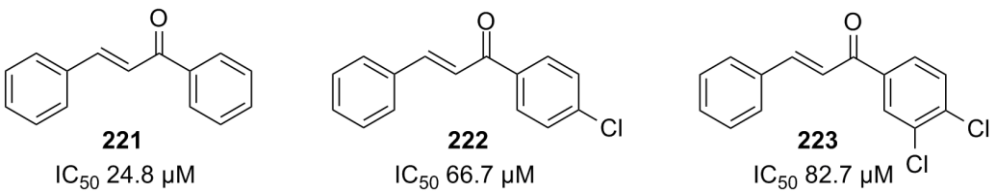


Figure 45. Chalcones that showed good inhibition against *T. cruzi*.[144]

7.6 Conclusions

The longer linker series was successfully synthesised via a condensation reaction, hydrogenation and finally the oxime formation. All compounds were tested *in vitro* against *T. cruzi* cell lines. Biological testing showed that overall one extra carbon in the linker between the phenyl and pyridyl rings retained but decreased the anti-trypanosomal activity. The substituent was also shown to be important. A chloro substituent was more potent than a fluoro substituent. The chalcone appeared more reactive than the ketone most likely due to the unsaturated alkene undergoing indiscriminate Michael addition in the cell. Finally the size of the oxime also appeared important. Having a longer, bulkier group improved the biological activity. The dichloro benzyl compound was synthesised and had a similar activity as pyrifenox.

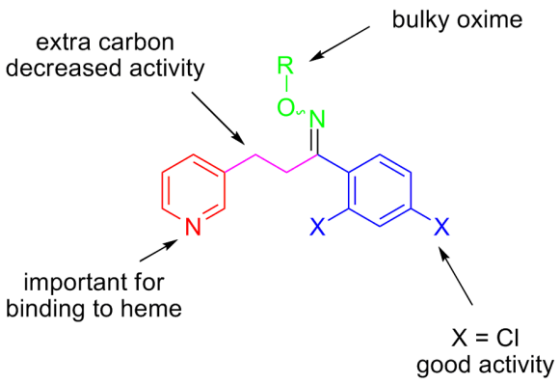


Figure 46. Diagram of key findings.

8 Development of a new trypanosomal lead

In the previous chapter, the initial lack of potency of the hydrogenated chalcone was overcome by using bulky oximes (e.g. compound **220**). One of the side products in the preparation of the chalcones was the Michael addition product **195**. Compounds such as **195** will be referred to as dimers as there are two symmetrical acetophenones attached to the pyridyl ring. This compound looked similar to the benzyl oxime **220** (Figure 47). They both have a similar backbone of the hydrogenated chalcone where the nitrogen on the pyridine ring could bind to the heme iron in CYP51 and the substituted phenyl ring would fit into the hydrophobic pocket. The main difference between the two compounds would be the oxime. Compound **220** has a benzyl oxime adjacent to the substituted phenyl ring whereas the dimer **195** lacks the oxime. Instead, compound **195** has a similar phenyl ring that can orientate towards the access tunnel on the carbon next to the pyridyl ring. This also removes the problematic stereochemistry that was in the oxime compounds.

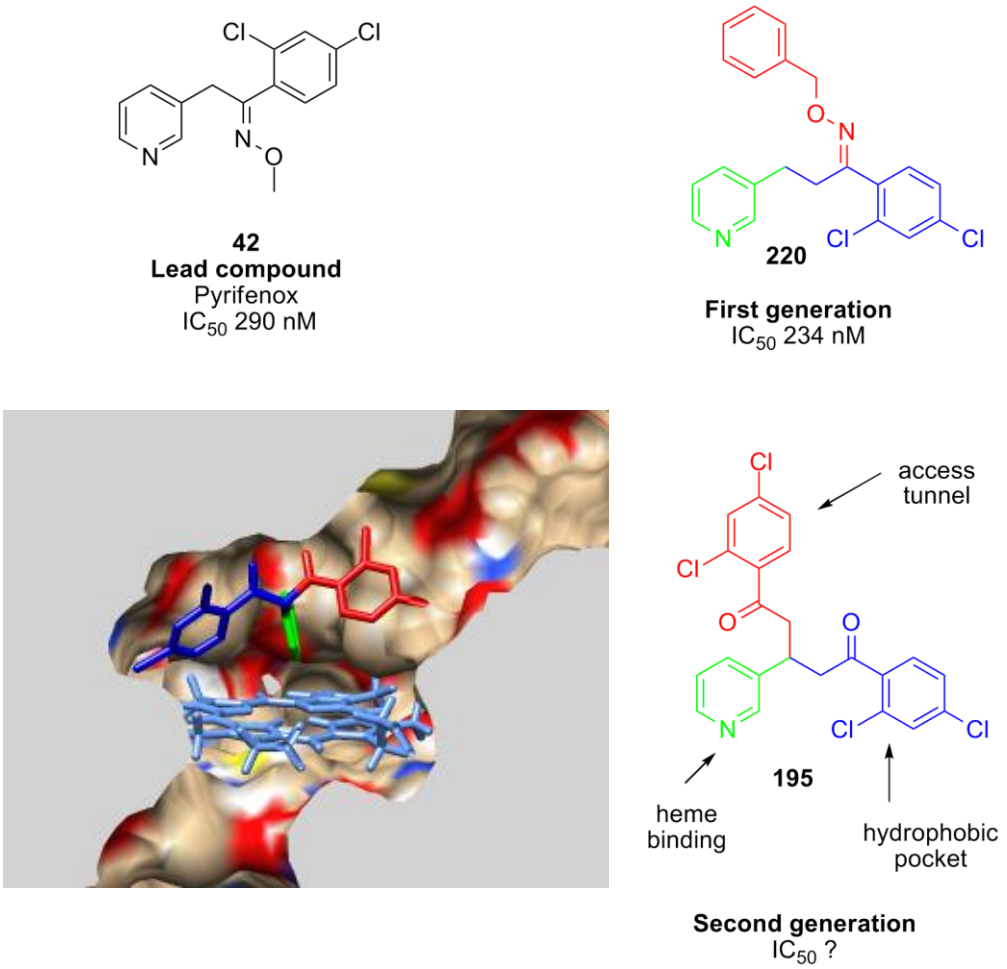
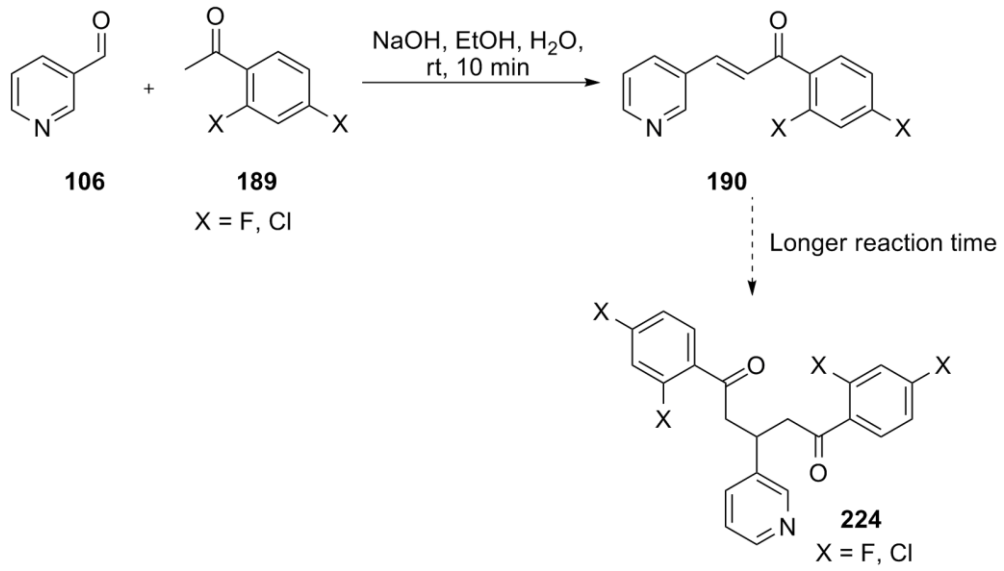


Figure 47

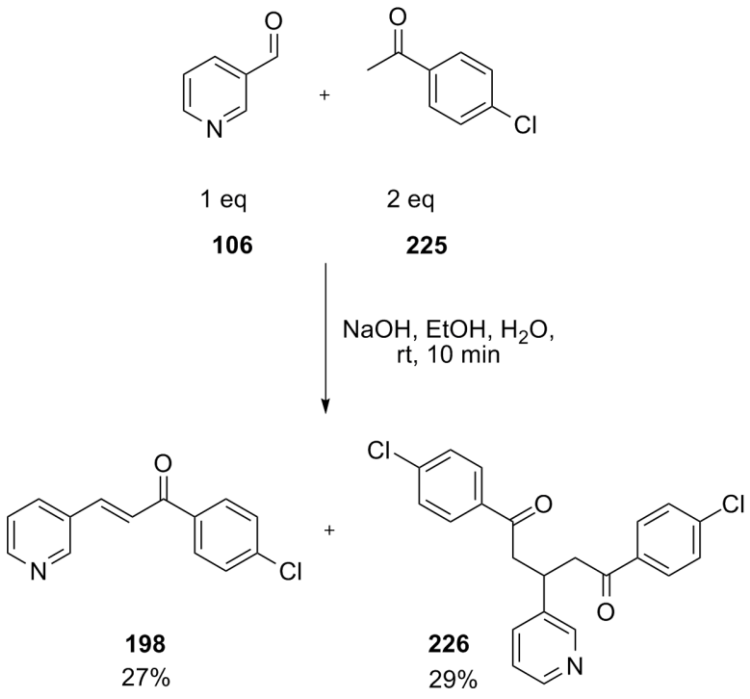
8.1 Proof of concept studies

The dimers could be readily synthesised in one step by stirring 3-pyridinecarboxaldehyde **106** and a substituted acetophenone **189** in the presence of a base with a longer reaction time than used to synthesise the chalcones in Section 7.1 (Scheme 68). The substituents X on the phenyl ring would be varied. The use of fluorine and chlorine substituents would help explore the steric bulk required to fit better into the access tunnel.



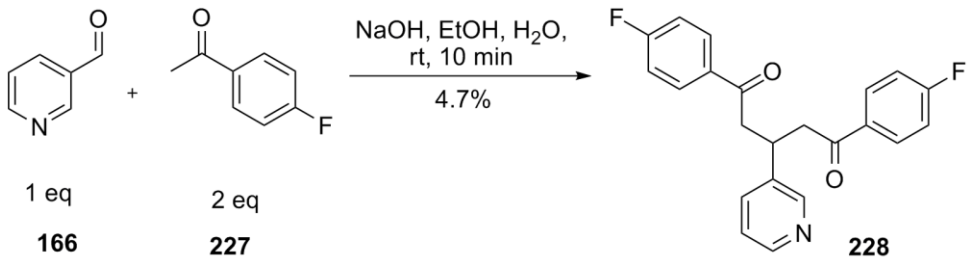
Scheme 68

3-Pyridinecarboxaldehyde **106** and 4′-chloroacetophenone **225** were stirred with a solution of sodium hydroxide in ethanol and minimal water and the reaction was cooled for 14 hours. When additional water was added a precipitate formed. The ^{1}H NMR spectrum showed a complex mixture of degradation by-products. The longer reaction time appeared to degrade the product. Therefore the reaction times were changed. 3-Pyridinecarboxaldehyde and 4′-chloroacetophenone were reacted with a solution of sodium hydroxide in ethanol and water at room temperature (Scheme 69). After 5 minutes a precipitate started to form in the solution. Excess water was added after 10 minutes to precipitate the rest of the product. Column chromatography separated minimal degradation by-products and a mixture of the chalcone **198** (27%), dimer **226** (29%) and starting material (13%). The ^{1}H NMR spectrum of the dimer showed two signals at 7.42 ppm and 7.87 ppm each with the integration of four hydrogens which corresponded to the chlorophenyl rings. There were two apparent doublet of doublets at 3.35 ppm and 3.51 ppm corresponding to two methylenes and a quintet at 4.06 ppm corresponding to the CH.



Scheme 69

Using the optimised conditions, the fluoro dimer was also synthesised (Scheme 70). 3-Pyridinecarboxaldehyde **166** and 4′-fluoroacetophenone **227** were stirred with a solution of sodium hydroxide in ethanol and minimal water. Additional water was added after 30 minutes until a precipitate had formed. The ^{1}H NMR spectrum of the product showed the key signals at 3.37, 3.54 and 4.10 ppm. Although the yield was low (4.7%) there was sufficient sample for biological testing.

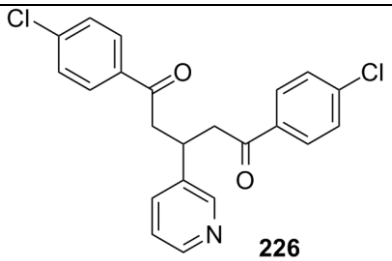
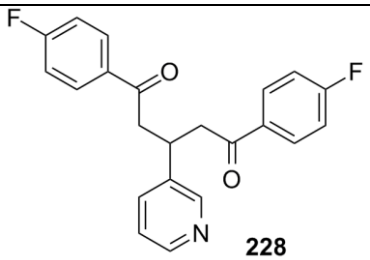


Scheme 70

Compounds **226** and **228** were tested for their ability to inhibit *T. cruzi* (Table 29). Surprisingly, these compounds showed excellent inhibition against *T. cruzi*. The

chloro dimer **226** had an IC_{50} of 0.022 µM and the fluoro dimer **228** had an IC_{50} of 0.029 µM. This was ten times more potent than pyrifenox (0.29 µM). Although they are slightly toxic (40.35 µM and 67.22 µM), the SI values are large. This means the drug dose can be low. These compounds establish a new class of compounds for further research. They can be synthesised in one step, using cheap starting materials and eliminated the problem of the separation of the stereoisomers in the oxime analogues.

Table 29

Structure	IC_{50} (toxicity)
 **226**	0.022 µM (40.35 µM) SI = 1834
 **228**	0.029 µM (67.22 µM) SI = 2317

As these compounds were derived from a known antifungal, they were also tested against *Candida albicans* by Eurofins Panlabs Inc. Taiwan.[*] *Candida albicans* (ATCC 10231) is a common yeast infection in humans. Both compounds were shown to be inactive at concentrations less than 128 µg/mL. This suggested that these compounds are selective for Chagas disease, unlike other azole antifungals that are active in yeast samples.

[*] Screening was done according to CLSI. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard -Second Edition. CLSI document M27-A2 (ISBN 1-56238-469-4). Vol. 22 No. 15. Clinical and Laboratory Standards Institute; Wayne, PA 19087 USA, 2002.

8.2 Further investigations into the 1,5-diaryl-3-pyridyl-pentane-1,5-dione system

Due to the surprising effectiveness of the fluoro and chloro substituted dimers at inhibiting *T. cruzi*, further investigations were conducted. Using the conditions outlined in Section 8.1, 3-pyridinecarboxaldehyde **106** and disubstituted acetophenones **229** were stirred with a solution of sodium hydroxide in ethanol and water (Table 30). The 3,4-difluoro dimer **230** produced a complex mixture. Column chromatography isolated the desired product **230** in 7.5% yield. As only a small amount of compound was required for biological testing the reaction was not optimised further. The 2,4-dichloro dimer **195** was observed in trace quantities in the ^{1}H NMR spectrum of the crude material, however it was not possible to isolate the desired product. As it has been shown the dichloro analogues were the most biologically active, the synthesis of the dichloro dimer was investigated further.

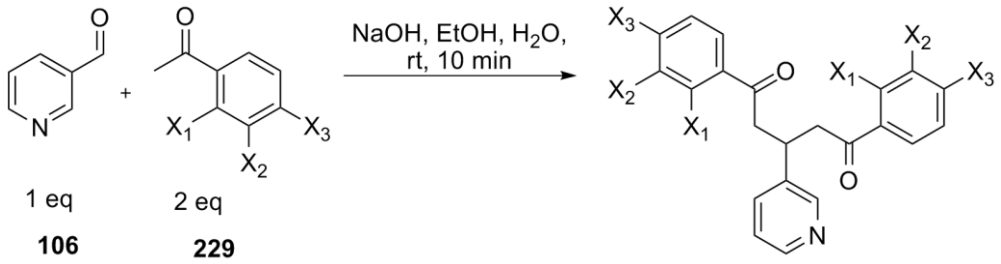
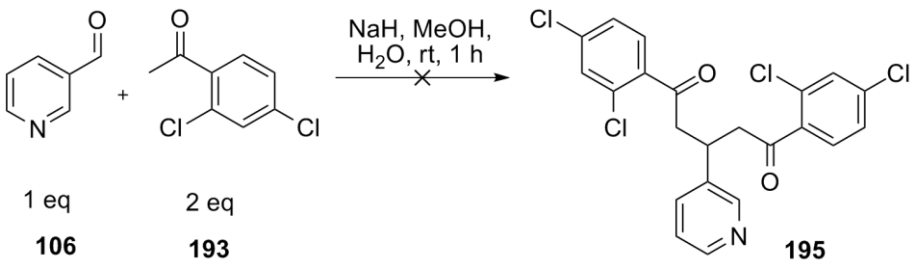


Table 30

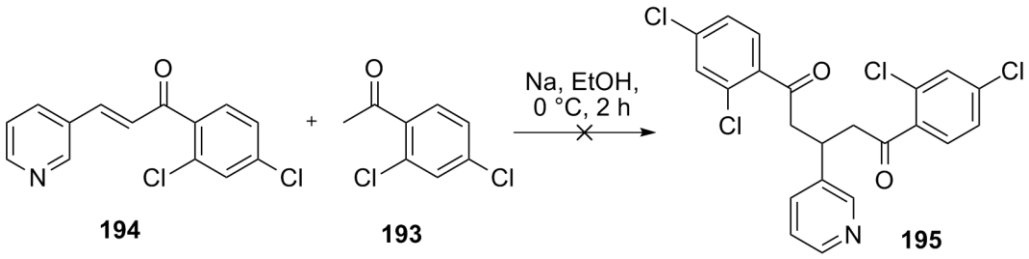
Compound	X_1	X_2	X_3	Yield
230	H	F	F	7.5%
195	Cl	H	Cl	Trace

As the previous synthesis produced the dichloro dimer **195** in trace amounts that could not be purified, alternative methods were sought. 2′,4′-Dichloroacetophenone **193** was dissolved in methanol and sodium hydride was added at 0 °C before 3-pyridinecarboxaldehyde **106** was added dropwise. Water was added until the solution became cloudy and stirred for 1 hour. The ^{1}H NMR spectrum of the crude material showed that only the chalcone had formed. The chalcone appeared to be precipitating out of solution before the Michael addition occurred.



Scheme 71

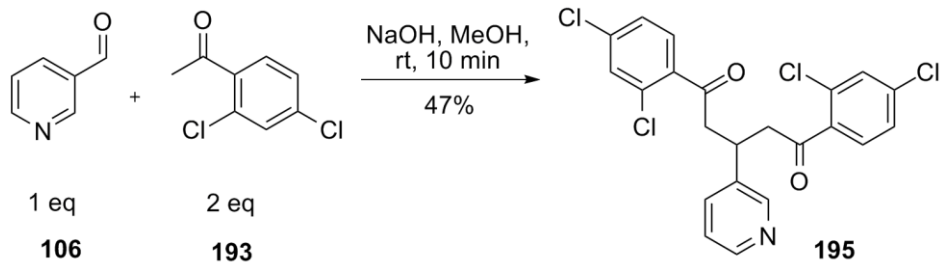
Another method to synthesise the dimer was to start with the chalcone. The dichloro chalcone **194** was stirred with a solution of 2′,4′-dichloroacetophenone **193** and sodium ethoxide in ethanol at 0 °C for 2 hours (Scheme 72). The ^{1}H NMR spectrum of the crude material showed a complex mixture and the key signals for the methylene and the CH were not observed. Sodium ethoxide was substituted for sodium hydride and methanol. The use of sodium ethoxide mainly returned unreacted chalcone but trace amounts of the dimer were visible in the ^{1}H NMR spectrum.



Scheme 72

The last method investigated was based on a procedure by Kudernac *et al.*[146] 3-Pyridinecarboxaldehyde **106**, 2′,4′-dichloroacetophenone **193** (2 eq.) and powdered sodium hydroxide were ground together in methanol for 10 minutes (Scheme 73). The colour changed overtime from a blood red to brown. The ^{1}H NMR spectrum of the crude material showed a complex mixture of compounds. Column chromatography isolated the desired dichloro dimer **195** in 42% yield and degradation by-products. The ^{1}H NMR spectrum showed the key signals at 3.34 ppm

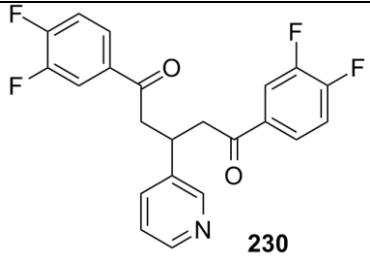
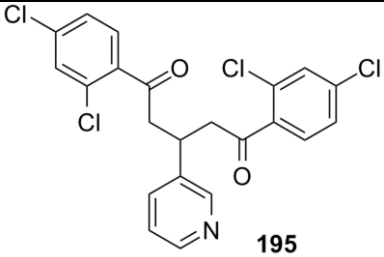
and 3.44 ppm corresponding to the methylenes and at 3.96 ppm corresponding to the CH.



Scheme 73

The dimers **195** and **230** were successfully synthesised and were tested for their ability to inhibit *T. cruzi* (Table 31). As already mentioned the chloro dimer **226** and the fluoro dimer **228** were ten times more potent than pyrifenox. The difluoro dimer **230** had an IC_{50} of 0.011 µM which was slightly better than compound **228**. Although the results for the dichloro dimer **195** have not been obtained, it would be expected, based on previous results, that it would have similar activity or better than the other dimers.

Table 31

Structure	IC_{50} (toxicity)
 **230**	0.011 µM (53.35 µM) SI = 4850
 **195**	In testing

Compounds **195** and **230** were also tested against the yeast *Candida albicans* (ATCC 10231).[*] As with the other dimers, these compounds were also shown to be inactive at concentrations less than 128 µg/mL.

The dichloro dimer **195** was also tested against another parasite, *Trypanosoma brucei*. *T. brucei* is another vector borne parasitic disease known as Human African trypanosomiasis, or sleeping sickness. The dichloro dimer **195** showed only less than 100% activity at the highest dose in the assay (69.8% activity at 83.33 µM)[†]. The inactivity of compound **195** against *Candida albicans* and *T. brucei* suggest that this series of compounds could be selective to CYP51 in *T. cruzi*.

8.3 Conclusions

A series of four dimers were successfully synthesised and tested in whole cell assays for their ability to inhibit *T. cruzi*. All four dimers showed surprising results at inhibiting *T. cruzi* with IC_{50} in the nano molar range and were inactive against *Candida albicans* and *T. brucei*. The substituents on the phenyl ring show slightly different inhibitions and the two substituents proved to be the most effective. The most biologically active molecule synthesised was **230** and it would be expected the dichloro dimer **195** would show equal or better activity. These compounds have provided a new lead compound for future investigations.

[*] Screening was done according to CLSI. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard -Second Edition. CLSI document M27-A2 (ISBN 1-56238-469-4). Vol. 22 No. 15. Clinical and Laboratory Standards Institute; Wayne, PA 19087 USA, 2002.

[†] The compound activity against T. brucei was assessed in an Alamar blue® viability assay as previously descrived by Sykes and Avery.[161]

9 Conclusions

A new synthetic method was designed to synthesise pyrifenox **42** and its analogues. The ketone intermediate was identified as the key compound to synthesising the analogues. Sequential Sonogashira reactions were utilised to obtain an alkyne which was hydrated to form the ketone. A variety of different conditions were investigated which involved the hydration of an alkyne and alkene, a Curtius rearrangement, a condensation reaction and a cross coupling reaction. The hydration of an alkyne using a gold catalyst, $Au(PPh_3)Me$, provided the optimum procedure in synthesising the key ketone intermediate. Analogues were then synthesised using a simple condensation reaction of a variety of alkoxyamines and were tested for their ability to inhibit *T. cruzi*. Out of 24 different analogues synthesised, it was shown the chloro substituents on the phenyl ring were the most active compared to hydrogen and fluorine. The stereochemistry of the oxime was also shown to be important. The *E* isomer is the synthetically and biologically favoured isomer. The analogues of pyrifenox were improved by adding an oxime with a phenyl ring to give compound **153** that was ten times more potent than pyrifenox **42** (Figure 48).

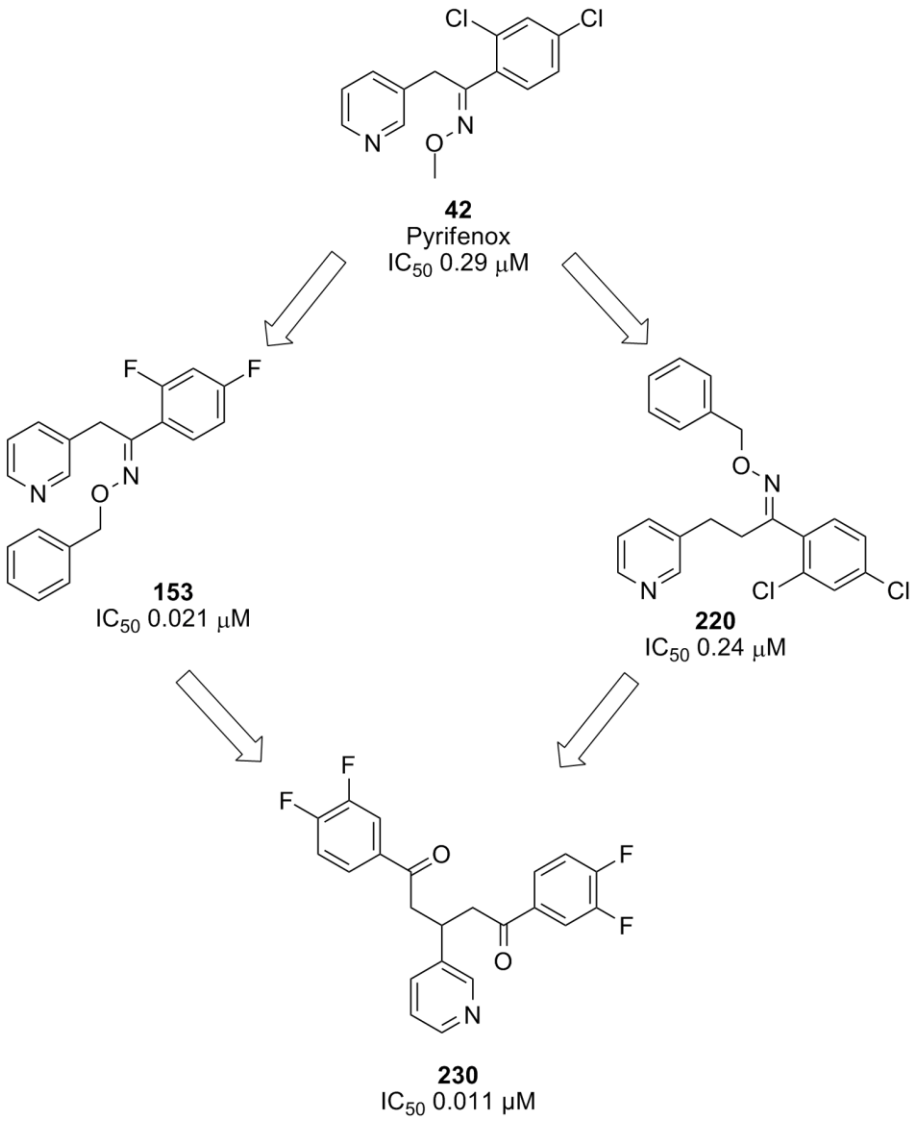


Figure 48

The rigidity of the backbone of pyrifenox was also explored. The attempt at trying to remove the stereochemistry to create a more flexible compound by incorporating a nitrogen between the two rings was unsuccessful. The anti-trypanosomal activity of the compounds tested showed a decrease in activity. The increased rigidity was also investigated and was shown to decrease the biological activity.

The linker between the substituted phenyl ring and the pyridyl ring was shown to be important. Increasing the carbon chain by an extra CH_2 decreased the biological activity in comparison to pyrifenox. The analogues synthesised showed a similar

trend to the initial pyrifenox analogues. The substituents on the phenyl ring showed the best activity with the bulkier chlorine groups and the bulkier oxime was also preferred. The preferred stereochemistry of these compounds was not fully identified and requires further investigations.

Finally, a new lead compound was identified from a by-product in the chalcone reaction. The dimers showed excellent activity against *T. cruzi* and were shown to be inactive against *Candida albicans* and *T. brucei*. This suggested that these compounds could be selective in their activity to *T. cruzi*. The dimers could be synthesised in one step from inexpensive starting materials. This class of compounds provide a new area of investigation to provide a new drug for the treatment of Chagas disease.

10 Experimental

10.1 General procedure

Reactions were conducted under a positive pressure of nitrogen at room temperature unless otherwise stated. Materials were obtained from commercial sources and used without further purification unless otherwise stated. Dry solvents were prepared according to Armarego and Chai.[147] Melting points were determined using Barnstead Electrothermal 9100 melting point apparatus. NMR spectra were recorded on a Bruker AVANCE III 400 MHz spectrometer. All ^{1}H NMR spectra were recorded at a frequency of 400.1 MHz, ^{13}C NMR spectra were recorded at a frequency of 100.6 MHz and ^{31}P NMR spectra were recorded at a frequency of 161.9 MHz. NMR spectra were referenced to their respective solvents: chloroform-*d* ($CDCl_3$, ^{1}H δ 7.26 ppm, ^{13}C δ 77.16 ppm); acetone d_6 (^{1}H δ 2.05 ppm, ^{13}C δ 29.84 ppm); dimethylsulfoxide-d_6 (DMSO-d_6, ^{13}C δ 39.5). Multiplicity was assigned as follows: s = singlet, d = doublet, t = triplet, q = quarter, m = multiplet and br = broad. Infra-red (IR) spectra were obtained on a Perkin Elmer Spectrometer 100 using an attenuated total reflectance attachment with a ZnSe/diamond composite crystal. High resolution ESI and ASAP ionisation (HRMS) mass measurements were carried were recorded in positive mode on a Walters Xovo Q-TOF instrument and were carried out at the

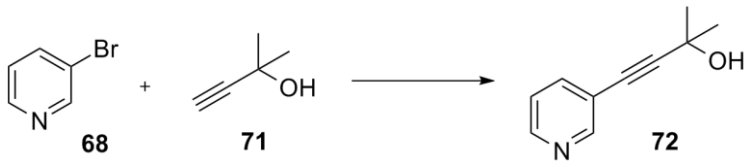
University of Wollongong or in positive and negative ionisation mode on a LTQ Orbitrap XL instrument carried out at Curtin University. Thin layer chromatography (TLC) was run on Merck aluminium backed silica gel 60 F_{254} sheets and visualised under ultraviolet light. Compounds were purified by flash chromatography on Davisil silica gel 40−63 µm or on a Flashmaster II system using cartridges prepared in house. Petroleum spirits refers to the fraction of boiling point 40−60 °C. All organic extracts were dried using anhydrous magnesium sulfate. Microwave reactions were carried out using a Biotage Initiator EXP machine.

10.2 Synthesis

10.2.1 Synthesis of dichlorobis(triphenylphosphine)palladium(II) complex

A solution of palladium chloride (2.003 g, 11.30 mmol) and triphenylphosphine (6.904 g, 26.32 mmol) in *N*,*N*-dimethylformamide (70 mL) was heated under reflux for 3 hours under nitrogen. Yellow crystals formed upon cooling and were collected by vacuum filtration, washed with ether and dried under vacuum (1 mmHg) to give dichlorobis(triphenylphosphine)palladium(II) as yellow crystals (7.795 g, 96%): m.p. 270 °C decomp. (lit.[148] m.p. 288−290 °C); ^{1}H NMR ($CDCl_3$) δ 7.34−7.47 (18H, m), 7.67−7.76 (12H, m); ^{31}P NMR ($CDCl_3$) δ 23.24 ppm; IR 3051, 1481, 1435, 1097, 744, 691 cm^{-1}.

10.2.2 Synthesis of 2-methyl-4-(3-pyridinyl)-3-butyn-2-ol (72)

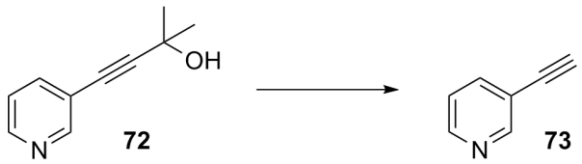


68 **71** **72**

3-Bromopyridine **68** (0.525 g, 3.3 mmol) and 2-methyl-3-butyn-2-ol **71** (0.279 g, 3.3 mmol) were dissolved in diethylamine (12 mL). The reaction mixture was stirred under nitrogen for 1 hour before $PdCl_2(PPh_3)_2$ (0.074 g, 0.11 mmol) and copper(I) iodide (0.010 g, 0.052 mmol) were added and the reaction mixture was heated under reflux overnight. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was dissolved in ethyl acetate (10

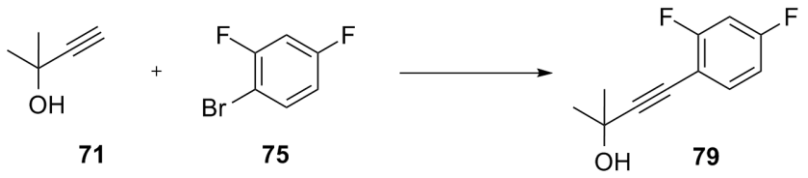
mL), washed with 10% citric acid solution (10 mL), brine (10 mL) and water (3 × 10 mL). The organic layer was dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The resulting oil was subjected to flash chromatography. Elution with 15% ethyl acetate in petroleum afforded 2-methyl-4-(3-pyridinyl)-3-butyn-2-ol **72** as a brown solid (0.195 g, 37%): m.p. 42−43 °C (lit.[149] m.p. 55−56 °C). The spectroscopic properties were similar to those reported.[149] ^{1}H NMR ($CDCl_3$) δ 1.58 (6H, s), 7.21 (1H, dd, *J* = 7.9, 5.0 Hz), 7.66 (1H, ddd, *J* = 7.9, 1.8, 1.8), 8.46 (1H, br. s), 8.70 (1H, br. s); ^{13}C NMR ($CDCl_3$) δ 31.4 (CH_3), 65.0 (C), 78.3 (C), 98.5 (C), 120.5 (CH), 123.3 (C), 139.0 (CH), 148.0 (CH), 151.9 (CH); IR 3258, 2981, 2933, 1587, 1568, 1477, 1165 cm^{-1}.

10.2.3 Synthesis of 3-ethynylpyridine (73)



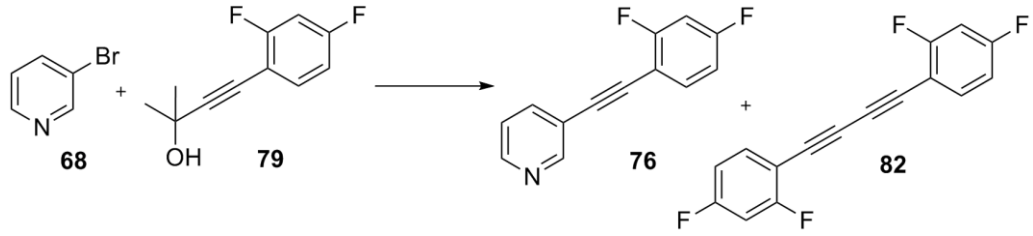
2-Methyl-4-(3-pyridinyl)-3-butyn-2-ol **72** (0.195 g, 1.21 mmol) was dissolved in dry toluene (4 mL) and stirred under nitrogen for 30 minutes. Sodium hydride (60% in mineral oil) (50 mg) was added and the reaction mixture was heated under reflux for 20 minutes. The reaction mixture was allowed to cool to room temperature before hydrochloric acid (1 M, 10 mL) was added. The acidic layer was extracted with ethyl acetate (2 × 10 mL), basified with a solution of sodium hydrogen carbonate (1 M, 10 mL) and extracted with dichloromethane (2 × 10 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure to give 3-ethynylpyridine **73** as a brown oil (0.075 g, 53%). The spectroscopic properties were similar to those reported.[85] ^{1}H NMR ($CDCl_3$) δ 3.20 (1H, s), 7.23 (1H, dd, *J* = 7.7, 4.6 Hz) 7.73 (1H, d, *J* = 7.8 Hz), 8.54 (1H, br. s), 8.70 (1H, br. s); ^{13}C NMR ($CDCl_3$) 80.1, 80.8, 119.0, 123.1, 139.1, 149.2, 152.8.

10.2.4 Synthesis of 4-(2,4-difluorophenyl)-2-methyl-3-butyn-2-ol (79)



1-Bromo-2,4-difluorobenzene **75** (16.762 g, 86.85 mmol) and 2-methyl-3-butyn-2-ol **71** (7.266 g, 86.77 mmol) were dissolved in diethylamine (120 mL) and stirred under nitrogen. After 1 hour, $PdCl_2(PPh_3)_2$ (0.801 g, 1.14 mmol) and copper(I) iodide (0.060 g, 0.32 mmol) were added and the reaction mixture was heated under reflux for 4 days. The reaction mixture was concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 20% ethyl acetate in petroleum spirits produced 4-(2,4-difluorophenyl)-2-methyl-3-butyn-2-ol **79** as a brown oil (14.043 g, 83%): ^{1}H NMR ($CDCl_3$) δ 1.62 (6H, s), 2.30 (1H, s), 6.77−6.85 (2H, m), 7.33−7.41 (1H, m); ^{13}C NMR ($CDCl_3$) δ 31.4 (CH_3), 65.7 (C), 74.6 (C), 99.0 (C, dd, *J* = 3.5, 1.9 Hz), 104.2 (CH, dd, *J* = 24.9, 0.7 Hz), 107.8 (C, dd, *J* = 15.8, 4.0 Hz), 111.5 (CH, dd, *J* = 18.2, 3.7 Hz), 134.4 (CH, dd, *J* = 9.6, 2.9 Hz), 162.7 (C, dd, *J* = 240.7, 11.1 Hz), 163.1 (C, dd, *J* = 241.7, 12.8 Hz); IR 3371, 2983, 1615, 1589, 1502, 1142 cm^{-1}.

10.2.5 Synthesis of 3-(2,4-difluorophenylethynyl)pyridine (76)



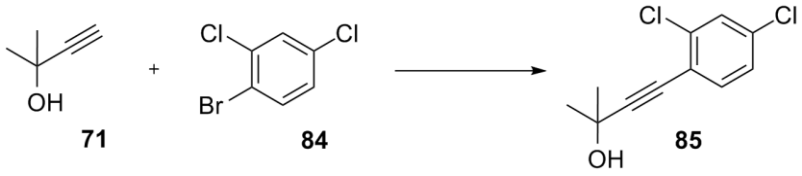
4-(2,4-Difluorophenyl)-2-methyl-3-butyn-2-ol **79** (2.107 g, 10.7 mmol) and potassium hydroxide (0.814 g, 14.5 mmol) were mixed with benzene (6 mL) and the reaction mixture was heated under reflux overnight. 3-Bromopyridine **68** (1.40 mL, 2.30 g, 14.53 mmol) in diethylamine (80 mL) was added to the reaction mixture. The resulting mixture was degassed with nitrogen by the freeze-pump-thaw method. $PdCl_2(PPh_3)_2$ (0.097 g) and copper(I) iodide (0.026 g) were added and the mixture was degassed with nitrogen again. The reaction mixture was heated under reflux for 2 days. After cooling to room temperature the reaction mixture was concentrated

under reduced pressure. The residue was subjected to flash chromatography. Elution with 25% ethyl acetate in petroleum spirits afforded 3-(2,4-difluorophenylethynyl)pyridine **76** as a brown oil (0.983 g, 41%) and 1,4-Bis(2,4-difluorophenyl)buta-1,3-diyne **82** as a brown oil (0.580 g, 19%).

3-(2,4-Difluorophenylethynyl)pyridine (**76**): HRMS *m/z* Calcd for $C_{13}H_8F_2N$ 216.0625, found 216.0617; ^{1}H NMR ($CDCl_3$) δ 6.84−6.95 (2H, m), 7.29 (1H, m), 7.46−7.54 (1H, m), 7.81 (1H, d, *J* = 7.8 Hz), 8.59 (1H, br. s), 8.79 (1H, br. s); ^{13}C NMR ($CDCl_3$) 85.0 (C), 90.7 (C), 104.4 (CH, dd, *J* = 24.6, 1.0 Hz), 107.5 (C, d, *J* = 4.0 Hz), 107.7 (C, d, *J* = 4.0 Hz), 111.6 (CH, d, *J* = 3.7), 111.8 (CH, d, *J* = 3.7 Hz), 134.3 (CH, dd, *J* = 9.8, 2.4 Hz), 138.4 (CH), 148.9 (CH), 152.2 (CH), 163.0 (C, dd, *J* = 255.1, 4.4 Hz), 163.12 (C, dd, *J* = 249.8, 5.7 Hz); IR 3064, 2228 1588, 1500, 1266, 806 cm^{-1}.

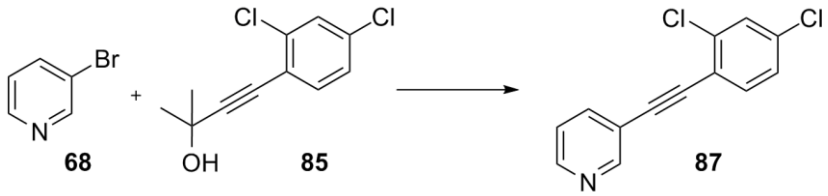
1,4-Bis(2,4-difluorophenyl)buta-1,3-diyne **82**: ^{1}H NMR ($CDCl_3$) δ 6.83−6.95 (4H, m), 7.46−7.56 (2H, m).

10.2.6 Synthesis of 4-(2,4-dichlorophenyl)-2-methyl-3-butyn-2-ol (85)



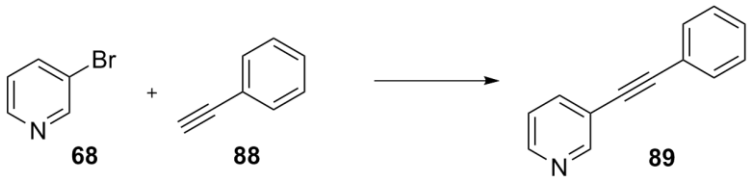
1-Bromo-2,4-dichlorobenzene **84** (15.260 g, 67.55 mmol) and 2-methyl-3-butyn-2-ol **71** (5.75 g, 68.35 mmol) were dissolved in diethylamine (110 mL) and stirred under nitrogen. After 1 hour, $PdCl_2(PPh_3)_2$ (0.760 g, 1.08 mmol) and copper(I) iodide (0.060 g) were added and the reaction mixture was heated under reflux for 3 days. The reaction mixture was concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 20% ethyl acetate in petroleum spirits produced 4-(2,4-dichlorophenyl)-2-methyl-3-butyn-2-ol **85** as a brown oil (14.48 g, 94%): ^{1}H NMR ($CDCl_3$) δ 1.62 (6H, s), 2.32 (1H, s), 7.16 (1H, dd, *J* = 8.3, 2.1 Hz), 7.34 (1H, d, *J* = 8.3 Hz) 7.38 (1H, d, *J* = 2.1 Hz); ^{13}C NMR ($CDCl_3$) δ 31.4 (CH_3), 65.7 (C), 78.1 (C), 100.2 (C), 121.4 (C), 127.0 (CH), 129.3 (CH), 133.9 (CH), 134.6 (C), 136.8 (C); IR 3362, 2982, 2933, 2233, 1583, 1545, 1474, 1100 cm^{-1}.

10.2.7 Synthesis of 3-(2,4-dichlorophenylethynyl)pyridine (87)



4-(2,4-Dichlorophenyl)-2-methyl-3-butyn-2-ol **85** (0.857 g, 3.74 mmol) and potassium hydroxide (0.385 g, 6.86 mmol) were dissolved in benzene (10 mL) and the reaction mixture was heated under reflux overnight. 3-Bromopyridine **68** (0.36 mL, 0.590 g, 3.74 mmol) in diethylamine (40 mL) was added to the reaction mixture and the reaction mixture was degassed with nitrogen. $PdCl_2(PPh_3)_2$ (0.047 g, 0.067 mmol) and copper(I) iodide (0.013 g, 0.068 mmol) were added and the reaction mixture was heated under reflux for 2 days. The reaction mixture was filtered through Celite and the filtrate was concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 25% ethyl acetate in petroleum spirits afforded 3-(2,4-dichlorophenylethynyl)pyridine **87** as a brown oil (0.297 g, 32%): HRMS *m/z* Calcd for $C_{13}H_8Cl_2N$ requires 248.0034, found 248.0027; ^{1}H NMR ($CDCl_3$) δ 7.14−7.24 (2H, m), 7.36−7.43 (2H, m), 7.71−7.78 (1H, m), 8.50 (1H, dd, *J* = 4.8, 1.5 Hz), 8.71 (1H, br. s); ^{13}C NMR ($CDCl_3$) δ 88.6 (C), 91.9 (C), 119.9 (C), 121.2 (C), 123.3 (CH), 127.2 (CH), 129.5 (CH), 133.9 (CH), 135.3 (C), 136.9 (C), 138.8 (CH), 149.0 (CH), 152.2 (CH); IR 3062, 3025, 1577, 1558, 1542, 1478, 1101, 803, 698 cm^{-1}.

10.2.8 Synthesis of 3-(2-phenylethynyl)-pyridine (89)



3-Bromopyridine **68** (1.057 g, 6.69 mmol) and phenyl acetylene **88** (0.661 g, 6.47 mmol) were dissolved in diethylamine (40 mL) and the solution was degassed with nitrogen. $PdCl_2(PPh_3)_2$ (0.127 g, 0.181 mmol) and copper(I) iodide (0.049 g, 0.252 mmol) were added and the mixture was heated under reflux for 2 days. The reaction mixture was filtered through Celite and concentrated under reduced pressure. The

residue was subjected to flash chromatography. Elution with 25% ethyl acetate in petroleum spirits produced 3-(2-phenylethynyl)pyridine **89** as brown solid (1.025g, 88%): m.p. 37 °C. The spectroscopic properties were similar to those reported.[87] ^{1}H NMR ($CDCl_3$) δ 7.24−7.31 (1H, m), 7.34−7.41 (3H, m), 7.53−7.60 (2H, m), 7.81 (1H, ddd, *J* = 7.9, 1.9, 1.9 Hz), 8.56 (1H, d, *J* = 3.8 Hz), 8.80 (1H, br. s); ^{13}C NMR ($CDCl_3$) δ 86.0 (C), 92.7 (C), 120.5 (C), 122.5 (C), 123.1 (CH), 128.4 (CH), 128.8 (CH), 131.6 (CH), 138.2 (CH), 148.3 (CH), 152.1 (CH); IR 3048, 3007, 2956, 2220, 1963, 1819, 1560, 1489, 1021, 756, 689 cm^{-1}.

10.2.9 Synthesis of methyltriphenylphosphine gold

A mixture of concentrated nitric acid (2 mL) and concentrated hydrochloric acid (8 mL) were added to a gold ingot (1.00 g, 5.08 mmol). The mixture was allowed to stand at room temperature overnight until all the gold had dissolved.

The acid solution was diluted with methanol (25 mL) and was added dropwise to a solution of triphenylphosphine (2.630 g, 10.03 mmol) in methanol (75 mL) with a white precipitate forming immediately. The reaction mixture was left to stand at 0 °C for 1 hour. The precipitate was collected by vacuum filtration to give triphenylphosphine gold(I) chloride as white crystals (2.222 g, 44%): m.p. 201 °C decomp; ^{1}H NMR ($CDCl_3$) δ 7.45−7.55 (m); ^{31}P NMR (161 MHz, $CDCl_3$) δ 33.75; IR 3058, 1479, 1433, 1101, 747, 690 cm^{-1}.

Triphenylphosphinegold(I) chloride (2.220 g, 4.5 mmol) was suspended in dry tetrahydrofuran (50 mL) and nitrogen was bubbled through the mixture. A solution of methylmagnesium iodide (3 M in THF) (10 mL, 30 mmol, 6.7 eq.) was added dropwise to the reaction mixture over 10 minutes. The resulting mixture was heated under reflux for 3 hours and then allowed to cool to room temperature before cooling to 0 °C. A degassed and cooled (0 °C) solution of sulfuric acid (0.5%, 100 mL) was added to the reaction mixture to form a purple precipitate. Tetrahydrofuran (30 mL) was added and the solid was removed by vacuum filtration and the residue was washed with toluene (10 mL). The filtrate was separated and the organic layer was washed with water, dried over anhydrous magnesium sulfate and concentrated under reduced pressure until about 10 mL of solvent remained. Hexanes (15 mL) was added slowly to the surface of the reaction mixture and the reaction mixture was

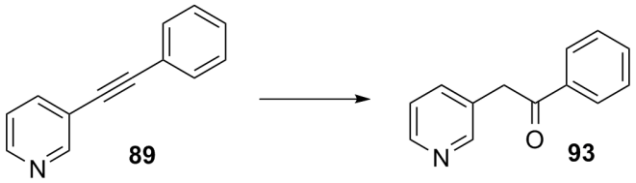
cooled (−20 °C) overnight. The resulting precipitate was collected to give white crystals (1.499 g, 70%): m.p. 259 °C decomp. (lit.[101] m.p. 173−175 °C); ^{1}H NMR ($CDCl_3$) δ 0.54 (3H, d, *J* = 7.9), 7.41−7.47 (9H, m), 7.51−7.56 (6H, m); ^{13}C NMR ($CDCl_3$)[*] δ 7.3 (CH_3, d, *J* = 95.3 Hz), 129.0 (CH), 129.1 (CH), 130.9 (CH), 131.0 (CH), 131.5 (C), 134.4 (CH), 134.5 (CH); ^{31}P NMR ($CDCl_3$) δ 47.96; IR 3053, 2928, 2869, 1477, 1432, 1098, 742, 690 cm^{-1}.

10.2.10 Synthesis of ethyltriphenylphosphine gold

Triphenylphosphinegold(I) chloride (0.499 g, 1.01 mmol) was suspended in anhydrous ether (12 mL). A solution of ethylmagnesium bromide (3 M in ether) (2 mL, 6 mmol, 6 eq.) was added dropwise and the resulting mixture was heated under reflux for 3 hours. The reaction was cooled to room temperature and a solution of sulfuric acid (0.5%, 12 mL) was added. The resulting precipitate was vacuum filtered and washed with toluene (3 mL). The filtrate was separated and the organic layer was washed with water (30 mL). The organics were dried over anhydrous magnesium sulfate and concentrated under reduced pressure until 5 mL remained. Hexanes (12 mL) were added to the surface of the reaction mixture and the mixture was cooled (−20 °C) for 7 days. The resulting precipitate was collected to give white crystals (0.310 g, 62%): m.p. 125.5 °C (lit.[100] m.p. 134.5−135.5 °C); ^{1}H NMR ($CDCl_3$) δ 1.34−1.48 (5H, m), 7.40−7.49 (9H, m), 7.49−7.57 (6H, m); ^{13}C NMR ($CDCl_3$)[†] δ 16.5 (CH_3), 22.1 (CH_2), 23.0 (CH_2), 129.0 (CH), 129.1 (CH), 130.9 (CH), 131.7 (C), 132.2 (C), 134.4 (CH), 134.5 (CH); ^{31}P NMR ($CDCl_3$) δ 45.61 ppm; IR 2872, 2853, 1477, 1433, 1099, 742, 691 cm^{-1}.

[*] This is a description of the spectra. The peaks were not assigned.
[†] This is a description of the spectra. The peaks were not assigned.

10.2.11 Synthesis of 1-phenyl-2(3-pyridinyl)ethanone (93)



Procedure 1

3-(2-Phenylethynyl)pyridine **89** (0.163 g, 0.91 mmol) was dissolved in methanol (3 mL), water (1 mL) and concentrated sulfuric acid (0.5 mL). $Au(PPh_3)Me$ (0.033 g, 0.069 mmol, 7.6 eq.) was added and nitrogen was bubbled through the solution. The reaction mixture was heated under reflux for 1 hour. The reaction mixture was cooled to room temperature and poured into a solution of sodium hydrogen carbonate (1 M, 25 mL) and extracted with dichloromethane (3 × 20 mL). The combined extracts were washed with brine (25 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits afforded 1-phenyl-2-(3-pyridinyl)ethanone **93** as a colourless oil (0.174 g, 97%). The spectroscopic properties were similar to those reported.[66] ^{1}H NMR ($CDCl_3$) δ 4.27 (2H, s), 7.23 (1H, ddd, *J* = 7.8, 4.8, 0.7 Hz), 7.42−7.48 (2H, m), 7.52−7.60 (2H, m), 7.96−8.01 (2H, m), 8.46−8.51 (2H, m); ^{13}C NMR ($CDCl_3$) δ 42.3 (CH_2), 123.5 (CH), 128.4 (CH), 128.8 (CH), 130.3 (C), 133.6 (CH), 136.2 (C), 137.4 (CH), 148.2 (CH), 150.6 (CH), 196.4 (C); IR 3058, 1679, 1579, 1209, 687 cm^{-1}.

Procedure 2

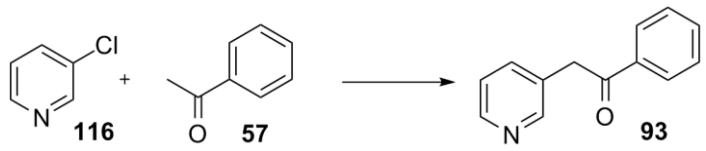
3-(2-Phenylethynyl)pyridine **89** (0.055 g, 0.329 mmol) was dissolved in trifluoroacetic acid (1 mL) and water (0.10 mL) and the reaction mixture was heated under reflux overnight. The reaction mixture was cooled to room temperature and poured into a solution of sodium hydrogen carbonate (1 M, 10 mL). The aqueous layer was extracted with dichloromethane (2 × 10 mL). The combined extracts were washed with brine (15 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash

chromatography. Elution with 25% ethyl acetate in petroleum spirits produced 1-phenyl-2(3-pyridinyl)ethanone **93** as a colourless oil (0.021 g, 34 %).

Procedure 3

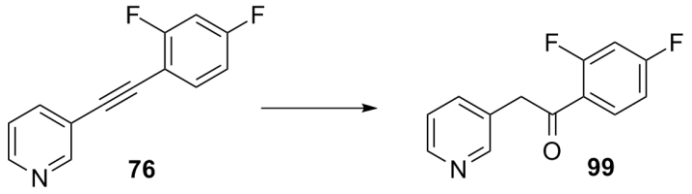
Sodium dodecyl sulfate (0.015 g, 0.05 mmol, 0.13 eq.) was added to a solution of 3-(2-phenylethynyl)pyridine **89** (0.072 g, 0.40 mmol) in a solution of hydrochloric acid (2 M, 0.25 mL) and water (1.75 mL). The reaction mixture was heated in a microwave oven at 170 °C for 20 minutes with 10 seconds prestirring. The reaction mixture was cooled to room temperature and poured into a solution of sodium carbonate (1 M, 10 mL). The aqueous layer was extracted with dichloromethane (5 × 10 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits afforded 1-phenyl-2(3-pyridinyl)ethanone **93** as a colourless oil (0.021 g, 27%).

10.2.12 Synthesis of 1-phenyl-2-(3-pyridinyl)ethanone (93)



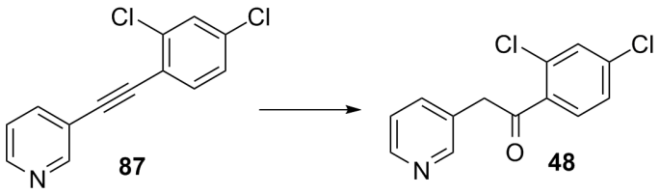
3-Chloropyridine **116** (0.1 mL, 0.121 g, 1.06 mmol), acetophenone **57** (0.15 mL, 0.15 g, 1.25 mmol) and dry toluene (4 mL) were added via syringe to chloro(2-dicyclohexylphosphino-2′,4′,6′-tri-*iso*-propyl-1,1′-biphenyl)[2-(2-aminoethyl)-phenyl]Pd(II) methyl-*tert*-butylether adduct **59** (0.008 g) and potassium *tert*-butoxide (0.262 g, 2.33 mmol) under nitrogen. The reaction mixture was heated under reflux overnight. Upon cooling, the reaction mixture was poured into a solution of ammonium chloride (1 M, 40 mL) and extracted with ethyl acetate (3 × 40 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 0−40% ethyl acetate in hexanes afforded 1-phenyl-2-(3-pyridinyl)ethanone **93** as a colourless oil (0.181 g, 87%).

10.2.13 Synthesis of 1-(2,4-difluorophenyl)-2-(3-pyridinyl)ethanone (99)



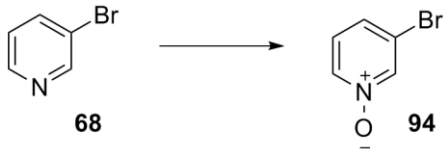
$Au(PPh_3)Me$ (0.082 g, 0.17 mmol) was added to a solution of 4-(2,4-Difluorophenyl)ethynylpyridine **76** (0.454 g, 2.11 mmol) in methanol (9 mL), water (3 mL) and concentrated sulfuric acid (1.5 mL) and nitrogen was bubbled through the solution. The reaction mixture was heated under reflux overnight. The reaction mixture was cooled to room temperature and poured into a solution of sodium hydrogen carbonate (1 M, 25 mL) and extracted with dichloromethane (3 × 20 mL). The combined extracts were washed with brine (25 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits afforded 1-(2,4-difluorophenyl)-2-(3-pyridinyl)ethanone **99** as a yellow oil (0.470 g, 96%): HRMS *m/z* Calcd for $C_{13}H_9F_2NO$ requires 234.0730, found 234.0732; ^{1}H NMR ($CDCl_3$) δ 4.19 (2H, s), 7.17−7.22 (2H, m), 7.26−7.41 (1H, m), 7.49−7.54 (1H, m), 7.8−7.88 (1H, m), 8.43−8.46 (2H, m); ^{13}C NMR ($CDCl_3$) δ 46.6 (CH_2, d, J = 8.4 Hz), 104.8 (CH, dd, J = 25.2, 2.4 Hz), 112.4 (CH, dd, J = 18.2, 3.4 Hz), 121.5 (C, dd, J = 13.1, 3.7), 123.3 (CH), 129.6 (C, d, J = 1.7 Hz), 133.0 (CH, J = 10.6, 4.2 Hz), 137.3 (CH), 148.3 (CH), 150.6 (CH), 162.6 (C, dd, J = 244.4, 12.8 Hz), 166.0 (C, dd, J = 245.4, 11.8 Hz), 193.2 (C, d, J = 5.0 Hz); IR 3100, 2476, 1664, 1608, 1589, 1428 cm^{-1}.

10.2.14 Synthesis of 1-(2,4-dichlorophenyl)-2-(3-pyridinyl)ethanone (48)



$Au(PPh_3)Me$ (0.136 g, 0.29 mmol) was added to a solution of 3-(2,4-dichlorophenylethynyl)pyridine **87** (0.700 g, 2.82 mmol) was dissolved in methanol (10 mL), water (3 mL) and concentrated sulfuric acid (1.5 mL). Nitrogen was bubbled through the solution and the reaction mixture was heated under reflux overnight. The reaction was cooled to room temperature and poured into a solution of sodium hydrogen carbonate (1 M, 25 mL) and extracted with dichloromethane (3 × 20 mL). The combined extracts were washed with brine (25 mL), dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits afforded 1-(2,4-dichlorophenyl)-2(3-pyridinyl)-ethanone **42** as a yellow oil (0.662 g, 88%): ^{1}H NMR ($CDCl_3$) δ 4.25 (2H, s), 7.24−7.29 (1H, m), 7.31 (1H, d, *J* = 1.9 Hz), 7.41 (1H, d, *J* = 8.2 Hz), 7.44 (1H, d, *J* = 1.9 Hz), 7.59 (1H, m), 8.49 (2H, br. s); ^{13}C NMR ($CDCl_3$) δ 46.5 (CH_2), 123.5 (CH), 127.5 (CH), 129.4 (C), 130.4 (CH), 130.5 (CH), 132.1 (C), 136.8 (C), 137.4 (CH), 137.8 (C), 148.4 (CH), 150.4 (CH), 198.3 (C); IR 3086, 2922, 1683, 1580, 1552, 1102 cm^{-1}.

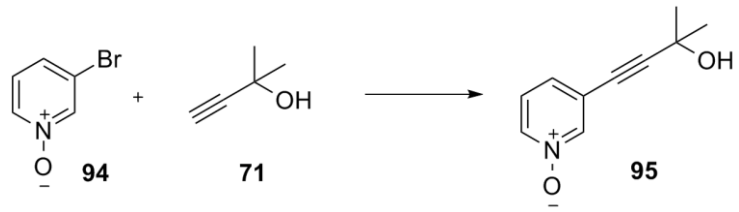
10.2.15 Synthesis of 3-bromopyridine *N*-oxide (94)



3-Chloroperoxybenzoic acid (0.292 g, 1.27 mmol) was added to a solution of 3-bromopyridine **68** (0.166 g, 1.05 mmol) in dichloromethane (1 mL) and the reaction mixture was stirred at room temperature overnight. The reaction mixture was poured into a solution of sodium hydrogen carbonate (1 M, 5 mL) and extracted with dichloromethane (3 × 5 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure to give 3-bromopyridine *N*-oxide **94** as a yellow oil (0.149 g, 82%): ^{1}H NMR ($CDCl_3$) δ 7.13 (1 H, dd, *J* = 8.2,

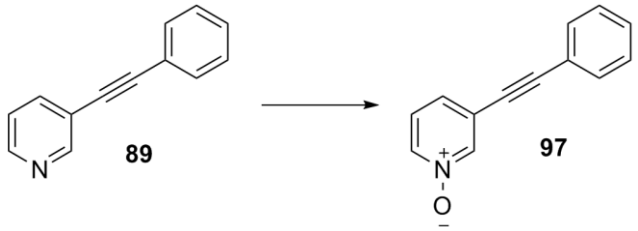
6.6 Hz), 7.35−7.39 (1 H, m), 8.09−8.14 (1 H, m), 8.31−8.33 (1 H, m); IR 3390, 3098, 1592, 1466, 1421 cm^{-1}.

10.2.16 Synthesis of 2-methyl-4-(3-pyridinyl)-3-butyn-2-ol *N*-oxide (95)



3-Bromopyridine *N*-oxide **94** (0.149 g, 0.86 mmol) and 2-methyl-3-butyn-2-ol **71** (0.081 g, 0.96 mmol) were dissolved in diethylamine (10 mL) and the solution was degassed with nitrogen. $PdCl_2(PPh_3)_2$ (0.021 g) and copper(I) iodide (0.010 g, 0.052 mmol) were added and the reaction mixture was heated under reflux for 1 day. The reaction mixture was filtered through Celite and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits before increasing to methanol afforded 2-methyl-4-(3-pyridinyl)-3-butyn-2-ol *N*-oxide **95** as a brown oil (0.150 g, 98%): HRMS *m/z* Calcd for $C_{10}H_{11}NO_2$ requires 178.0868, found 178.0869; ^{1}H NMR ($CDCl_3$) δ 1.59 (6H, s), 3.47 (1H, s), 7.21 (2H, br. s), 8.12 (1H, br. s), 8.22 (1H, br. s); IR 3398, 3129, 3073, 2979, 1627, 1594, 1477, 1150, 984, 880 cm^{-1}.

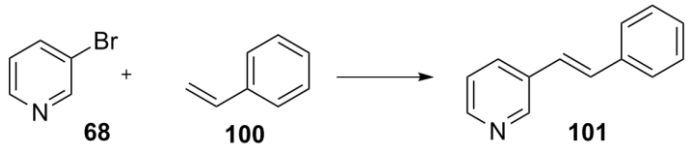
10.2.17 Synthesis of 1-phenyl-2-(3-pyridinyl)ethanone *N*-oxide (97)



3-Chloroperoxybenzoic acid (0.300 g, 1.74 mmol) was added to a solution of 3-(2-phenylethynyl)pyridine **89** (0.188 g, 1.049 mmol) in dichloromethane (1 mL) and the reaction mixture was stirred at room temperature for 1 day. The reaction mixture was poured into a solution of sodium hydrogen carbonate (1 M, 10 mL) and extracted with dichloromethane (3 × 10 mL). The combined extracts were dried over

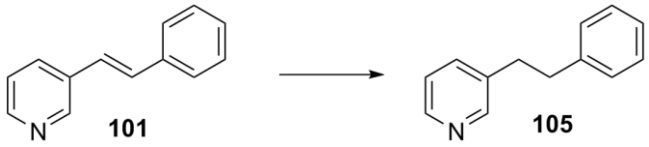
magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 25% ethyl acetate in petroleum spirits and increasing gave the product **97** as a brown oil (0.089 g, 43%): HRMS *m/z* Calcd for $C_{13}H_{10}NO$ requires 196.0762, found 196.0762; ^{1}H NMR ($CDCl_3$) δ 7.21−7.28 (1H, m), 7.33−7.41 (4H, m), 7.49−7.56 (2H, m), 8.18 (1H, d, *J* = 6.3 Hz), 8.34 (1H, s); ^{13}C NMR ($CDCl_3$) δ 83.2 (C), 94.9 (C), 121.6 (C), 128.5 (CH), 128.7 (CH), 129.1 (CH), 129.7 (CH), 132.0 (CH), 132.2 (CH); IR 3406, 2219, 1598, 1558, 1494, 1213 cm^{-1}.

10.2.18 Synthesis of 3-(2-phenylethynyl)pyridine (101)



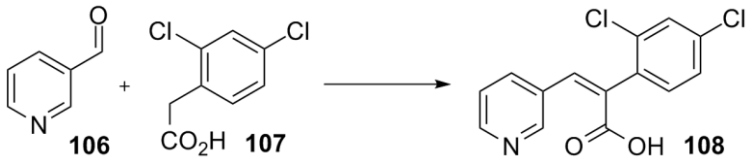
Acetylacetone **100** (0.10 mL, 0.97 mmol), $Pd(OAc)_2$ (0.004 g, 0.018 mmol) and potassium carbonate (5.102 g, 36.92 mmol) were added to a solution of 3-bromopyridine **68** (2.856 g, 18.08 mmol) and styrene (2.847 g, 27.34 mmol) in dry *N*,*N*-dimethylformamide (38 mL). The reaction mixture was heated to 130 °C for 2.5 hours. The reaction mixture was cooled to room temperature. A solution of sodium hydroxide (1 M, 10 mL) was added to the reaction mixture and extracted with ethyl acetate (3 × 10 mL). The combined extracts were washed with water (20 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits gave 3-(2-phenylethenyl)pyridine **101** as a pale brown solid (2.566 g, 78%): m.p. 72 °C (lit.[107] m.p. 81−83 °C). The spectroscopic properties were similar to those reported.[107] ^{1}H NMR ($CDCl_3$) δ 7.06 (1H, d, *J* = 16.4 Hz), 7.16 (1H, d, *J* = 16.4 Hz), 7.25−7.32 (2H, m), 7.35−7.41 (2H, m), 7.53 (2H, m), 7.82 (1H, m), 8.49 (1H, d, *J* = 3.7 Hz), 8.73 (1H, br. s); ^{13}C NMR ($CDCl_3$) δ 123.7 (CH), 125.0 (CH), 126.8 (CH), 128.3 (CH), 128.9 (CH), 131.0 (CH), 132.8 (CH), 133.1 (C), 136.8 (C), 148.7 (CH); IR 3054, 3025, 2999, 1636, 1565, 1492, 1479, 1448, 962 cm^{-1}.

10.2.19 Synthesis of 3-(2-phenylethyl)pyridine (105)



3-(2-Phenylethenyl)pyridine **101** (0.088 g, 0.486 mmol) and potassium iodide (1.388 g, 8.06 mmol) were mixed in phosphoric acid (2 mL) and the reaction mixture was heated to 150 °C for 48 hours. The reaction mixture was poured into a solution of sodium hydrogen carbonate (1 M, 10 mL) and extracted with dichloromethane (2 × 10 mL). The combined extracts were washed with brine (10 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits gave 3-(2-phenylethyl)pyridine **105** as a colourless oil (0.064 g, 72%). The spectroscopic properties were similar to those reported.[109] ^{1}H NMR ($CDCl_3$) δ 2.94−2.96 (4H, m), 7.14−7.18 (2H, m), 7.19−7.25 (2H, m), 7.27−7.33 (2H, m), 7.47 (1H, ddd, *J* = 7.8, 1.7, 1.7 Hz), 8.47 (2H, br. s); ^{13}C NMR ($CDCl_3$) δ 34.9 (CH_2), 37.5 (CH_2), 123.4 (CH), 126.2 (CH), 128.5 (CH), 136.4 (CH), 137.1 (C), 140.7 (C), 147.1 (CH), 149.6 (CH); IR 3027, 2924, 2858, 1602, 1575, 1494, 1478, 1453, 1422, 697 cm^{-1}.

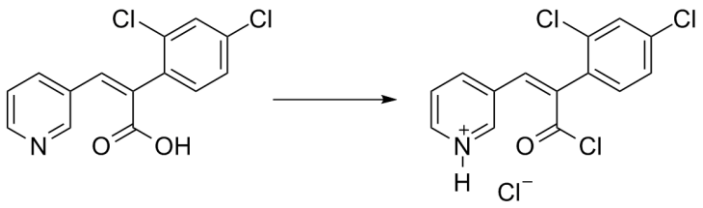
10.2.20 Synthesis of 2,4-dichloro-α-(3-pyridinylmethylene)phenylacetic acid (108)



3-Pyridinecarboxaldehyde **106** (5.6 mL, 6.39 g, 59.65 mmol), 2,4-dichlorophenylacetic acid **107** (12.102 g, 59.02 g) and triethylamine (6 mL) were suspended in acetic anhydride (48 mL) and heated under reflux for 5 hours. The reaction mixture was cooled to room temperature and water (200 mL) was added. The resulting mixture was left to crystallise for 3 days. The resulting precipitate was filtered. Recrystallisation with ethanol afforded the product **108** as a pale brown solid (10.704 g, 62%): m.p. 205−207°C (lit.[113] m.p. 208−211 °C); ^{1}H NMR (d_6-acetone) δ

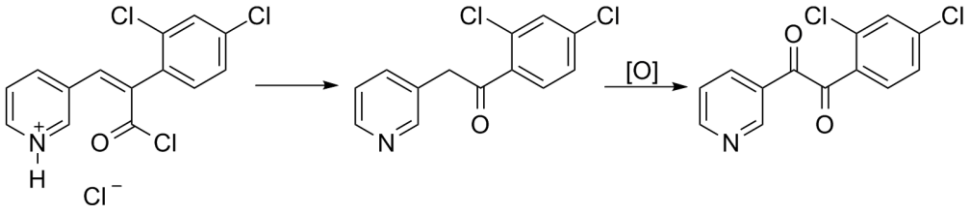
7.11−7.16 (1H, m), 7.19 (1H, d, *J* = 8.3 Hz), 7.27 (1H, m), 7.28−7.30 (1H, m), 7.31 (1H, d, *J* = 2.1 Hz), 7.50 (1H, d, *J* = 2.0 Hz), 7.86 (1H, s), 8.29 (1H, d, *J* = 2.1 Hz), 8.34 (1H, dd, *J* = 4.8, 1.7 Hz); ^{13}C NMR (d_6-acetone) δ 124.3 (CH), 128.7 (CH), 130.2 (CH), 131.2 (C), 132.8 (C), 133.5 (CH), 135.3 (C), 135.3 (C), 135.6 (C), 137.0 (CH), 139.2 (CH), 151.0 (CH), 151.8 (CH), 167.0 (C); IR 2446, 1694, 1610, 1244, 1192, 801, 695 cm^{-1}.

10.2.21 Synthesis of 2,4-dichloro-α-(3-pyridinylmethylene)benzeneacetyl chloride hydrochloride (109)



2,4-Dichloro-α-(3-pyridinylmethylene)phenylacetic acid **108** (4.40 g, 14.96 mmol) was suspended in thionyl chloride (26 mL) and heated under reflux for 3 hours. The solvent was distilled off leaving a brown solid. The residue was washed with ether and collected via vacuum filtration to give the product **109** as a pale brown solid (5.043 g, 97%).

10.2.22 Synthesis of 1-(2,4-dichlorophenyl)-2-(3-pyridinyl)-1,2-ethanedione (112)

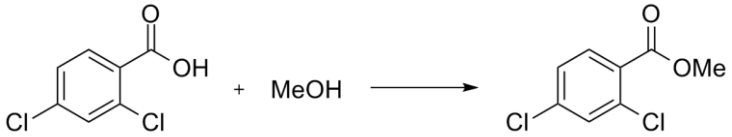


2,4-Dichloro-α-(3-pyridinylmethylene)phenylacetyl chloride hydrochloride **109** (2.05 g, 5.87 mmol) was dissolved in acetone (5 mL) and cooled to 0 °C. A solution of sodium azide (0.467 g, 7.18 mmol, 1.2 eq.) in water (5 mL) was added and the reaction mixture was stirred at 0 °C for 10 minutes and at room temperature for 5 minutes. A solution of sodium carbonate (0.85 g) in water (40 mL) was added until a

cloudy precipitate formed. The aqueous phase was extracted with toluene (3 × 30 mL). The combined extracts were dried over anhydrous magnesium sulfate and filtered. The filtrate was heated under reflux for 2 hours. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was dissolved in acetic acid (12 mL) and water (6 mL) and heated under reflux for 2 hours. The reaction was cooled to room temperature and concentrated under reduced pressure until half its volume remained. A solution of saturated sodium hydrogen carbonate (250 mL) was added until pH 8 and was extracted with dichloromethane (4 × 40 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits afforded 1-(2,4-dichlorophenyl)-2(3-pyridinyl)ethanone **48** as an oil (0.700 g).

After 1 week under normal atmospheric conditions was converted to 1-(2,4-dichlorophenyl)-2-(3-pyridinyl)-1,2-ethanedione **112** as a yellow oil (0.682 g, 44%): HRMS *m/z* Calc for $C_{13}H_7Cl_2NO_2$ requires 280.9824, found 279.9922; ^{1}H NMR ($CDCl_3$) δ 7.46 (1H, d, *J* = 1.8 Hz), 7.47−7.49 (1H, m), 7.52 (1H, ddd, *J* = 7.8, 4.9, 0.8 Hz), 7.86 (1H, dd, *J* = 7.8, 0.5 Hz), 8.35 (1H, ddd, *J* = 8.1, 2.0, 2.0 Hz), 8.90 (1H, dd, *J* = 4.9, 1.7 Hz), 9.24 (1H, d, *J* = 1.5 Hz); ^{13}C NMR ($CDCl_3$) δ 124.0 (CH), 128.1 (C), 128.3 (CH), 130.5 (CH), 132.2 (C), 133.1 (CH), 134.8 (C), 137.3 (CH), 141.0 (C), 151.7 (CH), 154.8 (CH), 190.2 (C), 191.8 (C); IR 3088, 2924, 1677, 1578, 1206, 871 cm^{-1}.

10.2.23 Synthesis of 2,4dichlorobenzoic acid methyl ester (114)

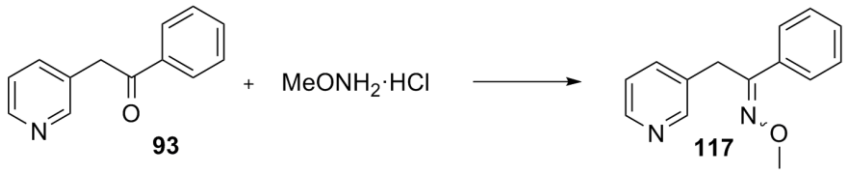


113 **114**

2,4-Dichlorobenzoic acid (1.524 g, 7.98 mmol) and concentrated sulfuric acid (3.6 mL) in methanol (20 mL) were heated under reflux overnight. The reaction mixture was cooled to room temperature, poured onto water (10 mL) and made basic with a solution of sodium carbonte (1 M, 130 mL). The aqueous layer was extracted with dichloromethane (5 × 25 mL). The combined extracts were dried over anhydrous

magnesium sulfate and concentrated under reduced pressure to give 2,4-dichlorobenzoic acid methyl ester **114** as a colourless oil (1.549 g, 94%): ^{1}H NMR ($CDCl_3$) δ 3.84 (3H, s), 7.21 (1H, dd, *J* = 8.4, 2.0 Hz), 7.38 (1H, d, *J* = 2.0 Hz), 7.71 (1H, d, *J* = 8.4 Hz); ^{13}C NMR ($CDCl_3$) δ 52.5 (CH_3), 127 (CH), 128.2 (C), 131.0 (CH), 132.0 (CH), 134.9 (C), 138.3 (C), 165.2 (C).

10.2.24 Synthesis of 1-phenyl-2-(3-pyridinyl)ethanone methyloxime (117)

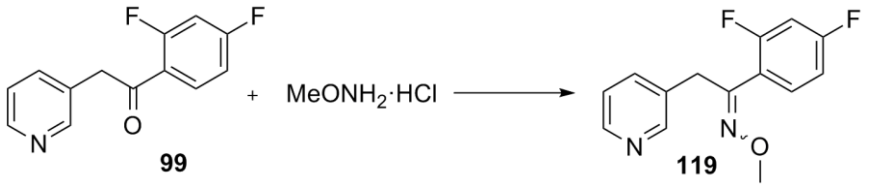


O-Methylhydroxylamine hydrochloride (0.084 g, 1.01 mmol) and sodium acetate (0.195 g, 2.38 mmol) were added to a solution of 1-phenyl-2-(3-pyridinyl)ethanone **93** (0.180 g, 0.91 mmol) in ethanol (4 mL). The reaction mixture was stirred at room temperature for 3 days. The reaction mixture was poured into a solution of sodium hydrogen carbonate (1 M, 20 mL). The aqueous layer was extracted with dichloromethane (4 × 20 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 0−25% ethyl acetate in hexanes afforded the two isomers as an oil (0.142 g, 69%, *E*:*Z* 3:1).

E isomer (colourless oil, 0.070 g, 34%) HRMS *m/z* Calcd for $C_{14}H_{15}N_2O$ requires 227.1184, found 227.1186; ^{1}H NMR ($CDCl_3$) δ 4.02 (3H, s), 4.12 (2H, s), 7.18 (1H, dd, *J* = 7.8, 4.8 Hz), 7.31−7.35 (3H, m), 7.51 (1H, d, *J* = 7.8 Hz), 7.59−7.65 (2H, m), 8.43 (1H, br. s), 8.52 (1H, br. s); ^{13}C NMR ($CDCl_3$) δ 30.1 (CH_2), 62.3 (CH_3), 123.7 (CH), 126.5 (CH), 128.7 (CH), 129.5 (CH), 132.8 (C), 135.2 (C), 136.3 (CH), 147.5 (CH), 149.8 (CH), 155.1 (C); IR 2936, 1574, 1423, 1043, 893, 691 cm^{-1}.

Z isomer (colourless oil, 0.072 g, 35%) HRMS *m/z* Calcd for $C_{14}H_{15}N_2O$ requires 227.1184, found 227.1183; ^{1}H NMR ($CDCl_3$ δ 3.86 (2H, s), 3.88 (3H, s), 7.19 (1H, dd, *J* = 7.8, 4.8 Hz), 7.27−7.37 (5H, m), 7.54 (1H, d, *J* = 7.8 Hz), 8.41−8.47 (2H, br. s); ^{13}C NMR ($CDCl_3$) δ 39.1 (CH_2), 62.1 (CH_3), 123.5 (CH), 128.1 (CH), 128.3 (CH), 129.1 (CH), 132.7 (C), 133.0 (C), 136.6 (CH), 148.0 (CH), 150.2 (CH), 155.3 (C); IR 3056, 2935, 2817, 1677, 1575, 1423, 1036, 1021, 696 cm^{-1}.

10.2.25 Synthesis of 1-(2,4-fluorophenyl)-2-(3-pyridinyl)ethanone methyloxime (119)

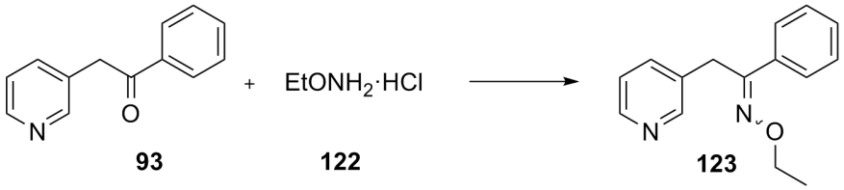


O-Methylhydroxylamine hydrochloride (0.094 g, 1.13 mmol) and sodium acetate (0.195 g, 2.38 mmol) were added to a solution of 1-(2,4-difluorophenyl)-2-(3-pyridinyl)-ethanone **99** (0.221 g, 0.95 mmol) in ethanol (4 mL). The reaction mixture was stirred at room temperature for 4 days. The reaction mixture was poured into a solution of sodium hydrogen carbonate (1 M, 20 mL) and extracted with dichloromethane (4 × 20 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 25% ethyl acetate in petroleum spirits afforded the two isomers as oils (0.153 g, 64%, *E*:*Z* 1.5:1).

E isomer (colourless oil, 0.103 g, 40%) HRMS *m/z* Calcd for $C_{14}H_{13}F_2N_2O$ requires 263.0996, found 263.0989; ^{1}H NMR ($CDCl_3$) δ 4.00 (3H, s), 4.08 (2H, s), 6.73−6.83 (2H, m), 7.14 (1H, dd, *J* = 7.8, 4.8 Hz), 7.35 (1H, m), 7.41−7.46 (1H, m), 8.39 (2H, br. s); ^{13}C NMR ($CDCl_3$) δ 32.1 (CH_2, d, *J* = 4.7 Hz), 62.4 (CH_3), 104.6 (CH, dd, *J* = 25.6, 25.6 Hz), 111.8 (CH, dd, *J* = 18.9, 4.0 Hz), 119.9 (C, dd, *J* = 9.1, 4.0 Hz) 123.4 (CH), 131.5 (CH, dd, *J* = 9.8, 5.0 Hz), 132.1 (C), 136.4 (CH), 147.8 (CH), 150.1 (CH), 153.0 (C, d, *J* = 2.0 Hz), 160.6 (C, dd, *J* = 240.7, 12.7 Hz), 163.6 (C, dd, *J* = 239.7, 12.5 Hz); IR 2940, 2821, 1673, 1609, 1503, 1479, 1421, 1042, 968 cm^{-1}.

Z isomer (colourless oil, 0.020 g, 8%) HRMS *m/z* Calcd for $C_{14}H_{13}F_2N_2O$ requires 263.0996, found 263.0993; ^{1}H NMR ($CDCl_3$) δ 3.83 (2H, s), 3.87 (3H, s), 6.74−6.83 (2H, m), 6.96−7.03 (1H, m), 7.21 (1H, dd, *J* = 7.5, 4.8 Hz), 7.53 (1H, d, *J* = 7.8 Hz), 8.43 (2H, br. s); ^{13}C NMR ($CDCl_3$) δ 38.7 (CH_2), 62.3 (CH_3), 104.5 (CH, dd, *J* = 25.0, 25.0 Hz), 107.9 (C), 111.6 (CH, dd, *J* = 17.3, 4.2 Hz), 117.3 (CH, dd, *J* = 13.5, 3.5 Hz), 130.4 (CH, dd, *J* = 9.8, 6.0 Hz), 137.8 (CH), 147.3 (CH), 149.2 (CH), 150.4 (C), 158.0 (C, dd, *J* = 239.3, 13.1 Hz), 163.3 (C, dd, *J* = 242.0, 13.9 Hz); IR 2939, 2820, 1611, 1592, 1503, 1478, 1422, 1026, 966 cm^{-1}.

10.2.26 Synthesis of 1-phenyl-2-(3-pyridinyl)ethanone ethyloxime (123)

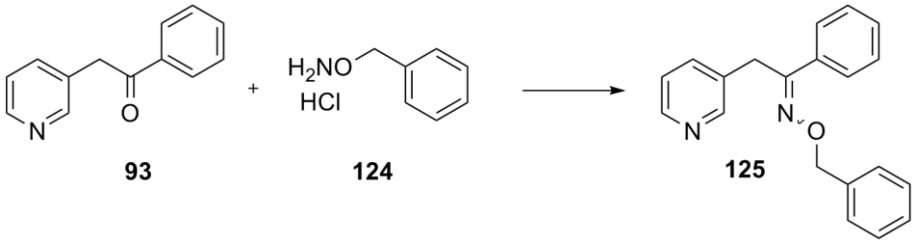


O-Ethylhydroxylamine hydrochloride **122** (0.078 g, 0.80 mmol) and sodium acetate (0.195 g, 2.38 mmol) were added to a solution of 1-phenyl-2-(3-pyridinyl)ethanone **93** (0.140 g, 0.71 mmol) in ethanol (4 mL). The reaction mixture was stirred at room temperature for 3 days. The reaction mixture was poured into a solution of sodium hydrogen carbonate (1 M, 20 mL) and was extracted with dichloromethane (4 × 20 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 0−25% ethyl acetate in hexanes afforded the two isomers as an oil (0.093 g, 55%, *E*:*Z* 2:1).

E isomer (colourless oil, 0.062 g, 43%) HRMS *m/z* Calcd for $C_{15}H_{17}N_2O$ requires 241.1341, found 241.1340; ^{1}H NMR ($CDCl_3$) δ 1.32 (3H, t, *J* = 7.0 Hz), 4.12 (2H, s), 4.29 (2H, q, *J* = 7.0 Hz), 7.16 (1H, dd, *J* = 7.7, 4.8 Hz), 7.29−7.35 (3H, m), 7.48−7.53 (1H, m), 7.60−7.65 (2H, m), 8.42 (1H, d, *J* = 4.1 Hz), 8.53 (1H, s); ^{13}C NMR ($CDCl_3$) δ 14.8 (CH_3), 30.1 (CH_2), 70.1 (CH_2), 123.5 (CH), 126.4 (CH), 123.6 (CH), 129.3 (CH), 132.8 (C), 135.4 (C), 136.1 (CH), 147.7 (CH), 150.1 (CH), 154.7 (C); IR 3030, 2977, 2931, 1669, 1574, 1042, 691 cm^{-1}.

Z isomer (colourless oil, 0.012 g, 7%) ^{1}H NMR ($CDCl_3$) δ 1.26 (3H, t, *J* = 7.0 Hz), 3.86 (2H, s), 4.14 (2H, q, *J* = 7.0 Hz), 7.20 (1H, dd, *J* = 7.6, 5.0 Hz), 7.27−7.34 (5H, m), 7.53−7.57 (1H, m), 8.41−8.47 (2H, m); ^{13}C NMR ($CDCl_3$) δ 14.8 (CH_3), 39.1 (CH_2), 69.9 (CH_2), 123.7 (CH), 128.3 (CH), 129.1 (CH), 133.2 (C), 133.4 (C), 137.2 (CH), 147.5 (CH), 149.7 (CH), 154.5 (C); IR 3031, 2978, 2932, 1666, 1575, 1041, 693 cm^{-1}.

10.2.27 Synthesis of 1-phenyl-2-(3-pyridinal)ethanone benzyloxime (125)



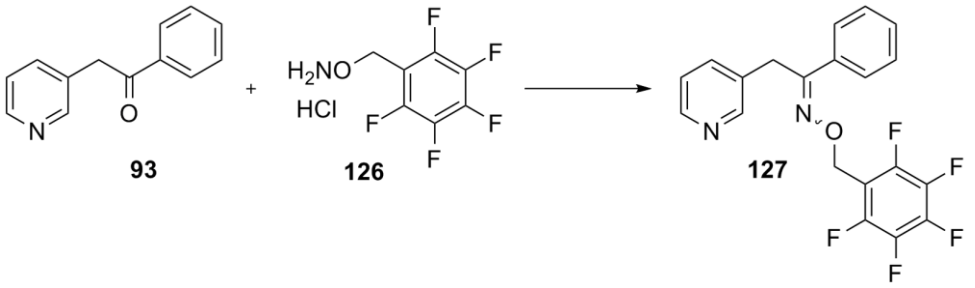
O-Benzylhydroxylamine hydrochloride **124** (0.142 g, 0.89 mmol) and sodium acetate (0.183 g, 2.23 mmol) were added to a solution of 1-phenyl-2-(3-pyridinyl)ethanone **93** (0.162 g, 0.82 mmol) in ethanol (4 mL). The reaction mixture was stirred at room temperature for 2 days. The reaction mixture poured into a solution of sodium hydrogen carbonate (1 M, 20 mL) and was extracted with dichloromethane (4 x 20 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits afforded the two isomers as an oil (0.083 g, 33%, *E*:*Z* 2.8:1).

E isomer (colourless oil, 0.063 g, 25%) HRMS *m/z* Calcd for $C_{20}H_{18}N_2O$ requires 303.1497, found 303.1498; ^{1}H NMR ($CDCl_3$) δ 4.16 (2H, s), 5.28 (2H, s), 7.12 (1H, dd, *J* = 7.8, 4.7 Hz), 7.29−7.38 (8H, m), 7.42−7.47 (1H, m), 7.60−7.66 (2H, m), 8.43 (1H, d, *J* = 3.7 Hz), 8.52 (1H, br. s); ^{13}C NMR ($CDCl_3$) δ 30.2 (CH_2), 76.7 (CH_2), 123.6 (CH), 126.5 (CH), 128.1 (CH), 128.4 (CH), 128.5 (CH), 128.7 (CH), 129.5 (CH), 132.7 (C), 135.2 (C), 136.1 (CH), 137.7 (C), 147.7 (CH), 150.1 (CH), 155.5 (C); IR 3030, 2917, 2849, 1701, 1574, 1014, 692 cm^{-1}.

Z isomer (colourless oil, selected peaks)[*] $C_{20}H_{18}N_2O$ requires 303.1497, found 303.1486; ^{1}H NMR ($CDCl_3$) δ 3.83 (2H, s), 5.14 (2H, s), 7.14 (1H, dd, *J* = 7.7, 4.6 Hz), 7.28−7.38 (8 H, m), 7.46 (1H, d, *J* = 7.7 Hz), 7.60−7.65 (2H, m), 8.43 (2H, br. s).

[*] The *Z* isomer was not isolated. Peaks were assigned based on the integration in the ^{1}H NMR spectrum.

10.2.28 Synthesis of 1-phenyl-2-(3-pyridinal)ethanone pentafluorobenzyloxime (127)

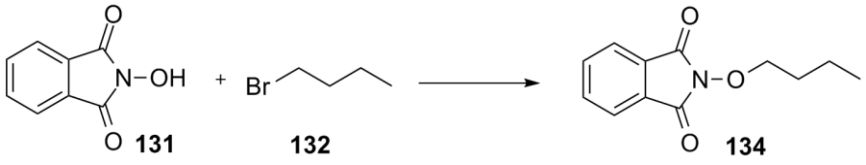


O-Pentafluorobenzylhydroxylamine hydrochloride **126** (0.074 g, 0.291 mmol) and sodium acetate (0.076 g, 0.93 mmol) were added to a solution of 1-phenyl-2-(3-pyridinyl)ethanone **93** (0.083 g, 0.42 mmol) in ethanol (3 mL). The mixture was stirred at room temperature for 3 days. The reaction mixture was poured into a solution of sodium hydrogen carbonate (1 M, 20 mL). The aqueous layer was extracted with dichloromethane (4 × 20 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 40% ethyl acetate in petroleum spirits afforded the product **127** as an oil (0.047 g, 40%, *E*:*Z* 2.9:1).

E isomer (colourless oil, selected peaks)[*] ^{1}H NMR ($CDCl_3$) δ 4.09 (2H, s), 5.32 (2H, t, *J* = 1.6 Hz), 8.47 (1H, br. s.), 8.50 (1H, br. s.).

Z isomer (colourless oil, selected peaks)[*] ^{1}H NMR ($CDCl_3$) δ 3.81 (2H, s), 5.14 (2H, t, *J* = 1.6 Hz), 8.37 (2H, br. s.).

10.2.29 Synthesis of *N*-butoxyphthalimide (134)

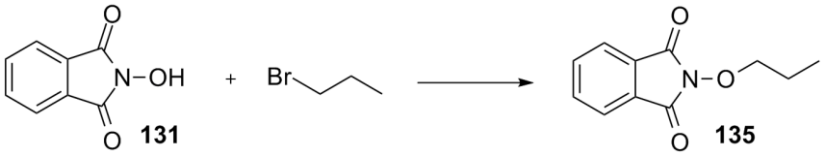


Sodium carbonate (0.952 g, 8.98 mmol) and 1-bromobutane **132** (15.39 g, 112 mmol) were added to a solution of *N*-hydroxyphthalimide **131** (18.01 g, 110 mmol) in *N*,*N*-dimethylformamide (25 mL) and the reaction mixture heated to 90 °C for 5 days. The reaction mixture was cooled to room temperature and water (50 mL) was

[*] Isomers were not isolated. Peaks were assigned based on the integration in the ^{1}H NMR spectrum

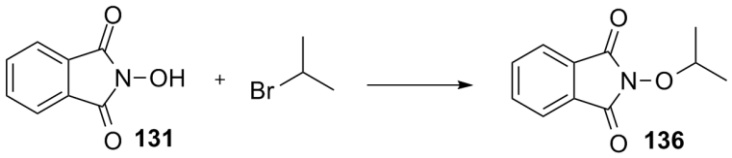
added. The resulting precipitate was collected by vacuum filtration and dried under vacuum to give *N*-butoxyphthalimide **134** as a cream solid (19.03 g, 79%): m.p. 193.2−194 °C (lit.[121] m.p. 29.2−30.0 °C). The spectroscopic properties were similar to those reported.[121] ^{1}H NMR ($CDCl_3$) δ 0.97 (3H, t, *J* = 7.4 Hz), 1.51 (2H, q, *J* = 7.4 Hz), 1.72−1.81 (2H, m), 4.20 (2H, t, *J* = 6.8 Hz), 7.71−7.76 (2H, m), 7.79−7.85 (2H, m); ^{13}C NMR (DMSO-d_6) δ 13.6 (CH_3), 18.4 (CH_2), 29.7 (CH_2), 77.4 (CH_2), 123.0 (CH), 128.8 (C), 134.6 (CH), 164.1 (C); IR 3128, 2959, 1787, 1705, 1607, 1463 cm^{-1}.

10.2.30 Synthesis of *N*-propoxyphthalimide (135)



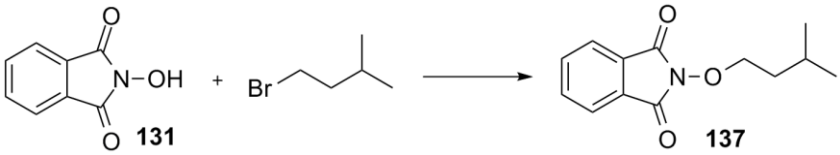
N-Hydroxyphthalimide **131** (8.919 g, 54.67 mmol) was dissolved in *N*,*N*-dimethylformamide (20 mL). Sodium carbonate (0.460 g, 4.34 mmol) and 1-bromopropane (7.581 g, 61.64 mmol) were added and the mixture was heated to 90 °C for 3 days. The reaction mixture was cooled to room temperature and water (50 mL) was added. The resulting precipitate was collected by vacuum filtration and dried under vacuum to give *N*-propoxyphthalimide **135** as an off-white powder (7.720 g, 69%): m.p. 210−216 °C (lit. m.p. 57.1−58.3 °C). The spectroscopic properties were similar to those reported.[121,150] ^{1}H NMR ($CDCl_3$) δ 1.06 (3H, t, *J* = 7.5 Hz), 1.78−1.87 1.82 (2H, m), 4.17 (2H, t, *J* = 6.5 Hz), 7.75 (2H, m), 7.84 (2H, m); ^{13}C NMR ($CDCl_3$) δ 10.0 (CH_3), 21.6 (CH_2), 80.1 (CH_2), 123.5 (CH), 129.0 (C), 134.4 (CH), 163.7 (C); IR 3128, 2971, 1786, 1706, 1607, 1463 cm^{-1}.

10.2.31 Synthesis of *N*-isopropoxyphthalimide (136)



Sodium carbonate (0.977 g, 9.22 mmol) and 2-bromopropane (13.184 g, 112 mmol) were added to a solution of *N*-hydroxyphthalimide **131** (18.042 g, 111 mmol) in *N*,*N*-dimethylformamide (25 mL) and the reaction mixture was heated to 90 °C for 5 days. The reaction mixture was cooled to room temperature and water (75 mL) was added. The resulting precipitate was collected by vacuum filtration and dried under vacuum to give *N*-isopropoxyphthalimide **136** as a white powder (15.37 g, 68%): m.p. 188.5−191 °C (lit.[150] m.p. 58−59 °C). The spectroscopic properties were similar to that reported.[150] ^{1}H NMR ($CDCl_3$) δ 1.38 (6H, d, *J* = 6.2 Hz), 4.55 (1H, hept, *J* = 6.2 Hz), 7.75 (2H, m), 7.84 (2H, m); ^{13}C NMR ($CDCl_3$) δ 21.0 (CH_3), 80.9 (CH), 123.6 (CH), 129.2 (C), 134.6 (CH), 164.5 (C); IR 3477, 3132, 2983, 1787, 1705, 1607, 1463 cm^{-1}.

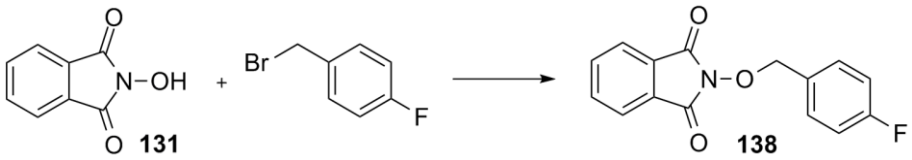
10.2.32 Synthesis of *N*-isopentoxyphthalimide (137)



Sodium carbonate (0.527 g, 4.97 mmol) and 1-bromo-3-methylbutane (1.00 g, 6.67 mmol) were added to a solution of *N*-hydroxyphthalimide **131** (1.12 g, 6.87 mmol) in *N*,*N*-dimethylformamide (10 mL) and the mixture was heated to 90 °C for 2 days. The reaction mixture was cooled to room temperature and water (50 mL) was added. The aqueous layer was extracted with dichloromethane (3 × 25 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. Water was added to the residue and cooled to −23 °C overnight. The resulting precipitate was collected by vacuum filtration and dried under vacuum to give *N*-isopentoxyphthalimide **137** as white powder (0.919 g, 59%): m.p. 48.1−48.5 °C (lit.[151] m.p. 42−43 °C); HRMS *m/z* Calcd for $C_{13}H_{16}NO_3$ requires 234.1130, found 234.1132; ^{1}H NMR ($CDCl_3$) δ 0.97 (6H, d, *J* = 6.6 Hz), 1.69 (2H, q,

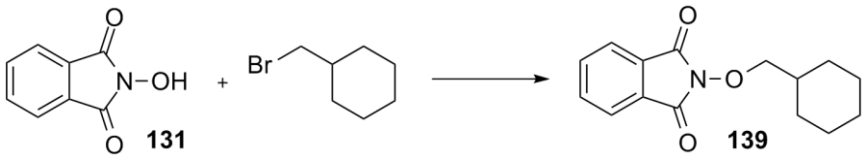
J = 6.9 Hz), 1.82−187 (1H, m), 4.23 (2H, t, *J* = 6.8 Hz), 7.73−7.76 (2H, m), 7.82−7.85 (2H, m); ^{13}C NMR ($CDCl_3$) δ 22.5 (CH_3), 24.9 (CH), 36.7 (CH_2), 76.7 (CH_2), 123.5 (CH), 129.0 (C), 134.4 (CH), 163.7 (C); IR 2958, 1786, 1726 cm^{-1}.

10.2.33 Synthesis of *N*-(4-fluorobenzyloxy)phthalimide (138)



Sodium carbonate (0.506 g, 4.77 mmol) and 4-fluorobenzyl bromide (1.000 g, 5.29 mmol) were added to a solution of *N*-hydroxyphthalimide **131** (0.909 g, 5.57 mmol) in *N*,*N*-dimethylformamide (10 mL) and the reaction mixture was heated to 90 °C for 2 days. The reaction mixture was cooled to room temperature and water (50 mL) was added. The resulting precipitate was collected by vacuum filtration and dried under vacuum to give *N*-(4-fluorobenzyloxy)phthalimide **138** as an off-white powder (0.733 g, 51%): m.p. 154−156 °C (lit.[152] m.p. 155−156 °C). The spectroscopic properties were similar to those reported.[152] ^{1}H NMR ($CDCl_3$) δ 5.18 (2H, s), 7.02−7.10 (2H, m), 7.50−7.55 (2H, m), 7.71−7.77 (2H, m), 7.78−7.85 (2H, m); ^{13}C NMR ($CDCl_3$) δ 79.2 (CH_2), 115.7 (CH, d, *J* = 21.2 Hz), 123.7 (CH), 129.0 (C), 129.8 (C, d, *J* = 3.0 Hz), 132.0 (CH, d, *J* = 8.0 Hz), 134.6 (CH), 163.5 (C, d, *J* = 248.1 Hz), 163.6 (C); IR 1776, 1722, 1604 cm^{-1}.

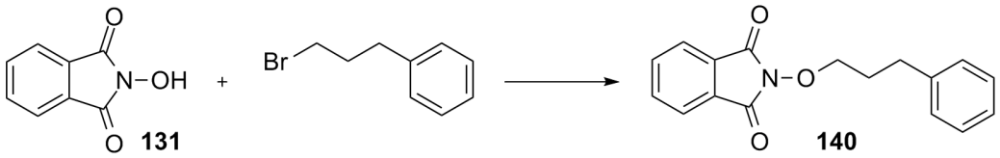
10.2.34 Synthesis of *N*-cyclohexylmethoxyphthalimide (139)



Sodium carbonate (0.247 g, 2.33 mmol) and bromomethylcyclohexane (0.500 g, 2.84 mmol) were added to a solution of *N*-hydroxyphthalimide **131** (0.525 g, 3.22 mmol) in *N*,*N*-dimethylformamide (10 mL) and the reaction mixture was heated to 90 °C for 2 days. The reaction mixture was cooled to room temperature and water (50 mL) was added. The resulting precipitate was collected by vacuum filtration and dried under

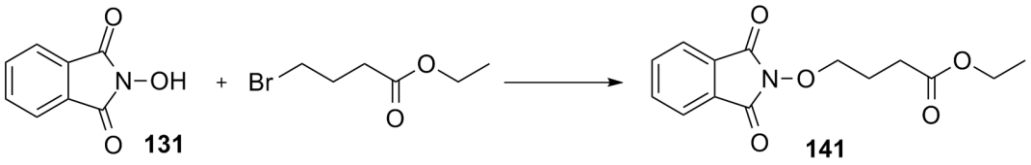
vacuum to give *N*-cyclohexylmethoxyphthalimide **139** as an off-white powder (0.548 g, 85%): m.p. 95.3−96.2 °C (lit.[153] m.p. 91−93 °C). The spectroscopic properties were similar to those reported.[154] ^{1}H NMR ($CDCl_3$) δ 1.00−1.36 (5H, m), 1.64−1.98 (6H, m), 4.00 (2H, d, *J* = 6.6 Hz), 7.70−7.76 (2H, m), 7.79−7.86 (2H, m); ^{13}C NMR ($CDCl_3$) δ 25.7 (CH_2), 26.5 (CH_2), 29.6 (CH_2), 37.0 (CH), 84.0 (C), 123.6 (CH), 129.2 (C), 134.5 (CH), 163.8 (C); IR 3388, 2925, 2854, 1781, 1720 cm^{-1}.

10.2.35 Synthesis of *N*-(3-phenylpropoxy)phthalimide (140)



Sodium carbonate (1.017 g, 9.60 mmol) and 3-bromopropylbenzene (1.8 mL, 2.358 g, 11.84 mmol) were added to a solution of *N*-hydroxyphthalimide **131** (1.962 g, 12.02 mmol) in *N*,*N*-dimethylformamide (20 mL) and the reaction mixture was heated to 90 °C for 4 days. The reaction mixture was cooled to room temperature and water (50 mL) was added. The resulting precipitate was collected by vacuum filtration and dried under vacuum to give *N*-(3-phenylpropoxy)phthalimide **140** as an off-white powder (1.590 g, 48%): m.p. 59.5−61 °C; HRMS *m/z* Calcd for $C_{17}H_{16}NO_3$ requires 282.1130, found 282.1133. The spectroscopic properties were similar to those reported.[155] ^{1}H NMR ($CDCl_3$) δ 2.06−2.15 (2H, m), 2.84−2.91 (2H, m), 4.23 (2H, t, *J* = 6.4 Hz), 7.17−7.22 (1H, m), 7.24−7.32 (4H, m), 7.72−7.77 (2H, m), 7.81−7.87 (2H, m); ^{13}C NMR ($CDCl_3$) δ 30.1 (CH_2), 31.9 (CH_2), 77.7 (CH_2), 123.6 (CH), 126.2 (CH), 128.6 (CH), 128.7 (CH), 129.1 (C), 134.6 (CH), 141.3 (C), 163.8 (C); IR 2951, 1790, 1717 cm^{-1}.

10.2.36 Synthesis of Ethyl 4-[(1,3-dihydro-1,3-dioxo-2-isoindol-2-yl)oxy]-butanoate (141)

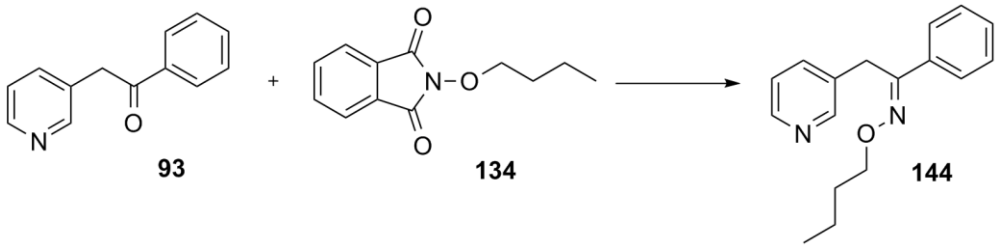


Sodium carbonate (1.043 g, 9.84 mmol) and ethyl-4-bromobutyrate (1.8 mL, 2.45 g, 12.58 mmol) were added to a solution of *N*-hydroxyphthalimide **131** (1.976 g, 12.11 mmol) in *N*,*N*-dimethylformamide (20 mL) and the reaction mixture was heated to 90 °C for 4 days. The reaction mixture was cooled to room temperature and water (50 mL) was added. The aqueous layer was extracted with dichloromethane (3 × 25 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. Water was added to the residue and cooled to −23 °C overnight. The resulting precipitate was collected by vacuum filtration and dried under vacuum to give the product **141** as white crystals (1.129 g, 34%): m.p. 44.8 °C; HRMS *m/z* Calcd for $C_{14}H_{15}NO_5$ requires 278.1028, found 278.1030; ^{1}H NMR ($CDCl_3$) δ 1.27 (3H, t, *J* = 7.2 Hz), 2.05−2.14 (2H, m), 2.64 (2H, t, *J* = 7.3 Hz), 4.16 (2H, q, *J* = 7.2 Hz), 4.27 (2H, t, *J* = 6.1 Hz), 7.72−7.77 (2H, m), 7.81−7.86 (2H, m); ^{13}C NMR ($CDCl_3$) δ 14.4 (CH_3), 23.7 (CH_2), 30.4 (CH_2), 60.7 (CH_2), 77.5 (CH_2), 123.7 (CH), 129.1 (C), 134.6 (CH), 163.7 (C); IR 1979, 1786, 1722 cm^{-1}.

10.2.37 Synthesis of *O*-butylhydroxylamine (142)

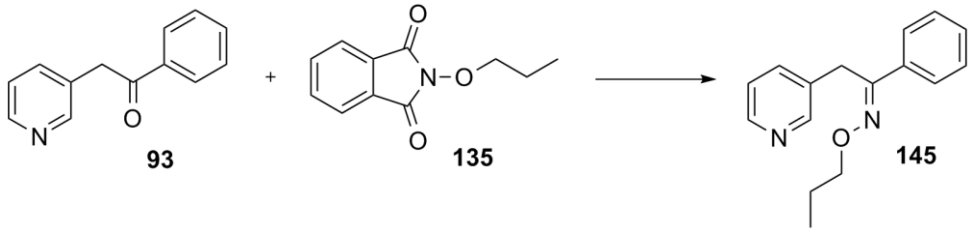
Hydrazine hydrate (600 μL, 0.613 g, 19.13 mmol) was added to a solution of *N*-butoxyoxyphthalimide **134** (0.982 g, 4.48 mmol) in dichloromethane (17 mL) and the mixture was heated under reflux for 2 days. The reaction mixture was cooled to room temperature and filtered. Quantitative ^{1}H NMR experiments of the reaction mixture showed 90% yield of product **142**. ^{1}H NMR ($CDCl_3$) δ 0.87 (3H, t, *J* = 7.6 Hz), 1.25−1.37 (2H, m), 1.46−1.55 (2H, m), 3.61 (2H, t, *J* = 6.6 Hz).

10.2.38 Synthesis of 1-phenyl-2-(3-pyridinyl)ethanone butyloxime (144)



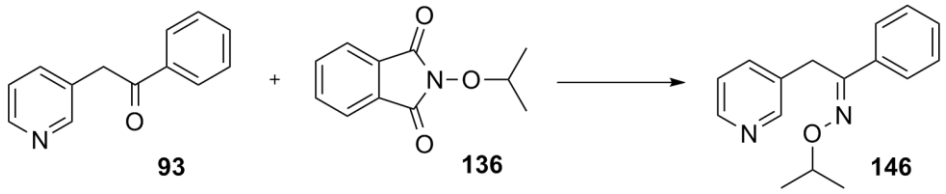
Hydrazine hydrate (0.15 mL, 0.015 g, 0.31 mmol) was added to a solution of *N*-butoxyphthalimide **134** (0.501 g, 2.28 mmol) in ethanol (6 mL) and the reaction mixture was heated under reflux for 30 minutes. A solution of 1-phenyl-2-(3-pyridinyl)-ethanone **93** (0.200 g, 1.01 mmol) in acetic acid (0.5 mL) and ethanol (1 mL) was added to the reaction mixture and the reaction mixture was heated under reflux for 1 day. The reaction mixture was cooled to room temperature and poured into a solution of sodium hydrogen carbonate (1 M, 20 mL). The aqueous layer was extracted with dichloromethane (3 × 20 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 0−25% ethyl acetate in hexanes afforded the *E* isomer as a colourless oil (0.009 g, 3.4%): ^{1}H NMR ($CDCl_3$) δ 0.92 (3H, t, *J* = 7.8 Hz), 1.32−1.43 (2H, m), 1.65−1.75 (2H, m), 4.13 (2H, s), 4.23 (2H, t, *J* = 6.7 Hz), 7.18 (1H, dd, *J* = 7.8, 4.8 Hz), 7.31−7.36 (3H, m), 7.49−7.53 (1H, m), 7.60−7.66 (2H, m), 8.43 (1H, d, *J* = 3.5 Hz), 8.53 (1H, br. s); ^{13}C NMR ($CDCl_3$) δ 14.1 (CH_3), 19.3 (CH_2), 30.2 (CH_2), 31.4 (CH_2), 74.6 (CH_2), 123.9 (CH), 126.4 (CH), 128.8 (CH), 129.5 (CH), 132.3 (C), 135.4 (C), 136.9 (CH), 146.9 (CH), 149.3 (CH), 154.5 (C).

10.2.39 Synthesis of 1-phenyl-2-(3-pyridinyl)ethanone propyloxime (145)



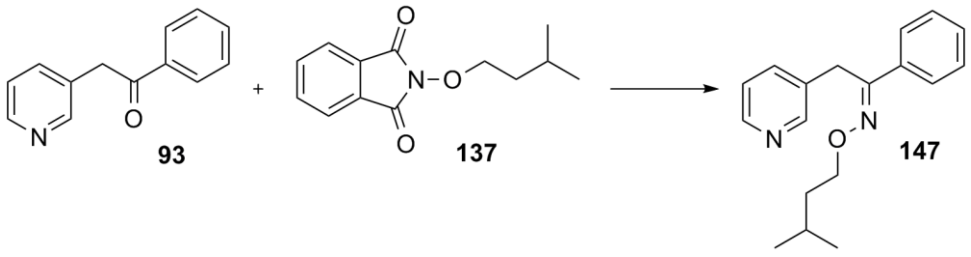
Hydrazine hydrate (0.15 mL, 0.015 g, 0.309 mmol) was added to a solution of *N*-propoxyphthalimide **135** (0.507 g, mmol) in ethanol (6 mL) and the reaction mixture was heated under reflux for 30 minutes. A solution of 1-phenyl-2-(3-pyridinyl)-ethanone **93** (0.200 g, 1.01 mmol) in acetic acid (0.5 mL) and ethanol (1 mL) was added to the reaction mixture and the reaction mixture was heated under reflux for 1 day. The reaction mixture was cooled to room temperature and poured into a solution of sodium hydrogen carbonate (1 M, 20 mL). The aqueous layer was extracted with dichloromethane (3 × 20 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 0−25% ethyl acetate in hexanes afforded the product as the *E* isomer as a colourless oil (0.005 g, 2%): ^{1}H NMR ($CDCl_3$) δ 0.97 (3H, t, *J* = 7.3 Hz), 1.69−1.78 (1H, m), 4.14 (2H, s), 4.19 (2H, t, *J* = 6.7 Hz), 7.19−7.22 (1H, m), 7.32−7.37 (3H, m), 7.55 (1H, d, *J* = 7.9 Hz), 7.61−7.65 (2H, m), 8.44 (1H, br. s), 8.54 (1H, br. s).

10.2.40 Synthesis of 1-phenyl-2-(3-pyridinyl)ethanone isopropyloxime (146)



Hydrazine hydrate (0.15 mL, 0.015 g, 0.31 mmol) was added to a solution of *N*-isopropoxyphthalimide **136** (0.496 g, 2.42 mmol) in ethanol (6 mL) and the reaction mixture was heated under reflux for 30 minutes. A solution of 1-phenyl-2-(3-pyridinyl)ethanone **93** (0.200 g, 1.01 mmol) in acetic acid (0.5 mL) and ethanol (1 mL) was added to the reaction mixture and the reaction mixture was heated under reflux for 1 day. The reaction mixture was cooled to room temperature and poured into a solution of sodium hydrogen carbonate (1 M, 20 mL). The aqueous layer was extracted with dichloromethane (3 × 20 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 0−25% ethyl acetate in hexanes afforded the product **146** as the *E* isomer as a colourless oil (0.009 g, 4%): ^{1}H NMR ($CDCl_3$) δ 1.30 (6H, d, *J* = 6.2 Hz), 4.12 (2H, s), 4.51 (1H, sept, *J* = 6.2 Hz), 7.19 (1H, dd, *J* = 7.7, 5.1 Hz), 7.32−7.35 (3H, m), 7.52−7.56 (1H, m), 7.62−7.66 (2H, m), 8.43 (1H, d, *J* = 4.6 Hz), 8.54 (1H, s).

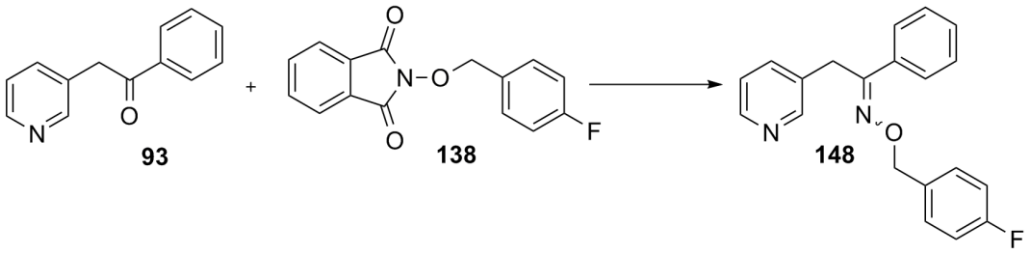
10.2.41 Synthesis of 1-phenyl-2-(3-pyridinyl)ethanone isopentyloxime (147)



Hydrazine hydrate (0.1 mL) was added to a solution of *N*-isopentoxyphthalimide **137** (0.247 g, 1.06 mmol) in ethanol (5 mL) and the reaction mixture was heated to 80 °C for 1 hour. The reaction mixture was cooled to room temperature. A solution of 1-phenyl-2-(3-pyridinyl)-ethanone **93** (0.169 g, 0.86 mmol) in acetic acid (0.5 mL) and ethanol (1 mL) was added to the reaction mixture and the reaction mixture was heated to 80 °C for 2 days. The reaction mixture was then cooled to room

temperature and poured into a solution of sodium hydrogen carbonate (1 M, 20 mL). The aqueous layer was extracted with dichloromethane (3 × 20 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits with 1% triethylamine afforded the product **147** as the *E* isomer as a colourless oil (0.077g, 32%): HRMS *m/z* Calcd for $C_{18}H_{23}N_2O$ requires 283.1810, found 283.1802; ^{1}H NMR ($CDCl_3$) δ 0.92 (6H, d, *J* = 6.7 Hz), 1.56−1.65 (2H, m), 4.11 (2H, s), 4.26 (2H, t, *J* = 6.7 Hz), 7.13−7.17 (1H, m), 7.30−7.34 (3H, m), 7.47−7.52 (1H, m), 7.60−7.65 (2H, m), 8.39−8.44 (1H, m), 8.52 (1H, d, *J* = 1.3 Hz); ^{13}C NMR ($CDCl_3$) δ 22.7 (CH_3), 25.2 (CH), 30.2 (CH_2), 38.1 (CH_2), 73.2 (CH_2), 123.5 (CH), 126.4 (CH), 128.7 (CH), 129.3 (CH), 132.9 (C), 135.5 (C), 136.0 (CH), 147.7 (CH), 150.1 (CH), 154.7 (C); IR 2955, 2932, 1574, 1052, 691 cm^{-1}.

10.2.42 Synthesis of 1-phenyl-2-(3-pyridinyl)ethanone 4-fluorobenzyloxime (148)



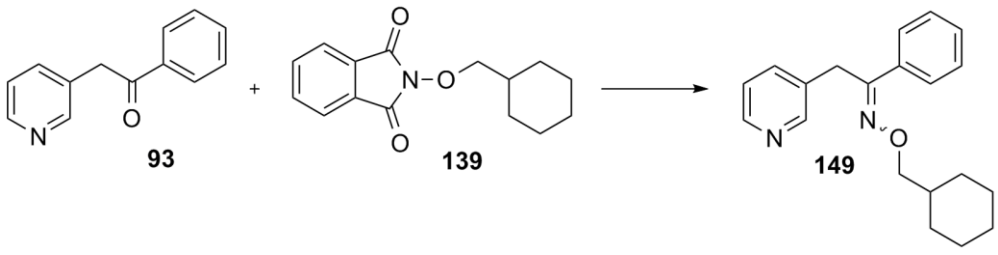
Hydrazine hydrate (0.07 mL) was added to a solution of *N*-(4-fluorobenzyloxy)phthalimide **138** (0.228 g, 0.84 mmol) in ethanol (4 mL) and the reaction mixture was heated to 80 °C for 30 minutes. The reaction mixture was cooled to room temperature. A solution of 1-phenyl-2-(3-pyridinyl)-ethanone **93** (0.150 g, 0.76 mmol) in acetic acid (0.5 mL) and ethanol (1 mL) was added to the reaction mixture and the reaction mixture was heated to 80 °C for 3 days. The reaction mixture was cooled to room temperature and poured into a solution of sodium hydrogen carbonate (1 M, 20 mL). The aqueous layer was extracted with dichloromethane (3 × 20 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum

spirits with 1% triethylamine afforded the product **148** as two isomers (0.131 g, 53%, *E*:*Z* 7.5:1)

E isomer (colourless oil, 0.108 g, 44%) HRMS *m/z* Calcd for $C_{20}H_{18}FN_2O$ requires 321.1403, found 321.1390; ^{1}H NMR ($CDCl_3$) δ 4.03 (2H, s), 5.12 (2H, s), 6.90−6.96 (2H, m), 7.00−7.05 (1H, m), 7.19−7.26 (5H, m), 7.31−7.34 (1H, m), 7.49−7.56 (2H, m), 8.31 (1H, d, *J* = 4.0 Hz), 8.40 (1H, s); ^{13}C NMR ($CDCl_3$) δ 30.1 (CH_2), 75.8 (CH_2), 115.3 (CH, d, *J* = 21.5 Hz), 123.5 (CH), 126.4 (CH), 128.6 (CH), 129.5 (CH), 130.2 (CH, d, *J* = 8.1 Hz), 132.5 (C), 133.4 (C, d, *J* = 3.4 Hz), 135.0 (C), 135.9 (CH), 147.6 (CH), 149.9 (CH), 155.6 (C), 162.6 (C, d, *J* = 246.0 Hz); IR 3055, 2927, 1603, 1509, 1220, 692 cm^{-1}.

Z isomer (colourless oil, 0.008 g, 3%) HRMS *m/z* Calcd for $C_{20}H_{18}FN_2O$ requires 321.1403, found 321.1389; ^{1}H NMR ($CDCl_3$) δ 3.77 (2H, s), 5.00 (2H, s), 6.91−6.98 (2H, m), 7.12 (1H, dd, *J* = 7.8, 5.0 Hz), 7.17−7.26 (7H, m), 7.39−7.42 (1H, m), 8.33 (1H, br. s), 8.37 (1H, d, *J* = 4.1 Hz); ^{13}C NMR ($CDCl_3$) δ 39.0 (CH_2), 75.4 (CH_2), 115.2 (CH, d, *J* = 21.2 Hz), 123.6 (CH), 127.9 (CH), 128.2 (CH), 129.1 (CH), 129.8 (CH, d, *J* = 8.1 Hz), 132.9 (C), 133.8 (C, d, *J* = 3.0 Hz), 134.9 (C), 137.2 (CH), 147.3 (CH), 149.4 (CH), 155.7 (C), 162.4 (C, d, *J* = 245.0 Hz); IR 3055, 2927, 2869, 1603, 1509, 1220, 695 cm^{-1}.

10.2.43 Synthesis of 1-phenyl-2-(3-pyridinyl)ethanone cyclohexylmethyloxime (149)



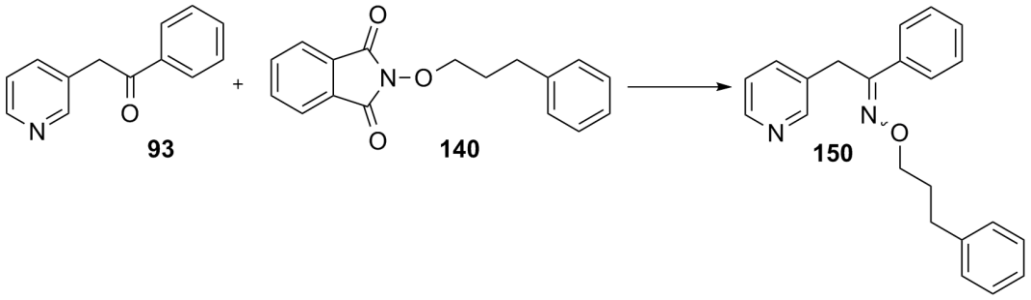
Hydrazine hydrate (0.07 mL) was added to a solution of *N*-cyclohexylmethoxyphthalimide **139** (0.253 g, 0.98 mmol) in ethanol (4 mL) and the reaction mixture was heated to 80 °C for 30 minutes. The reaction mixture was cooled to room temperature. A solution of 1-phenyl-2-(3-pyridinyl)-ethanone **93** (0.150 g, 0.76 mmol) in acetic acid (0.5 mL) and ethanol (1 mL) was added to the

reaction mixture and the reaction mixture was heated to 80 °C for 3 days. The reaction mixture was cooled to room temperature and poured into a solution of sodium hydrogen carbonate (1 M, 20 mL). The aqueous layer was extracted with dichloromethane (3 × 20 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits with 1% triethylamine afforded the product **149** as two isomers (0.103 g, 45%, *E*:*Z*, 4:1)

E isomer (colourless oil, 0.083 g, 36%) HRMS *m/z* Calcd for $C_{20}H_{25}N_2O$ requires 309.1967, found 309.1952; ^{1}H NMR ($CDCl_3$) δ 0.82−1.03 (2H, m), 1.05−1.32 (4H, m), 1.60−1.80 (5H, m), 4.04 (2H, d, *J* = 6.3 Hz), 4.11 (2H, s), 7.15 (1H, ddd, *J* = 7.9, 4.8, 0.7 Hz), 7.29−7.34 (3H, m), 7.46−7.51 (1H, m), 7.58−7.65 (2H, m), 8.40 (1H, dd, *J* = 4.8, 1.5 Hz), 8.52 (1H, d, *J* = 1.6 Hz); ^{13}C NMR ($CDCl_3$) δ 25.8 (CH_2), 26.6 (CH_2), 29.9 (CH_2), 30.1 (CH_2), 37.6 (CH), 80.2 (CH_2), 123.4 (CH), 126.3 (CH), 128.6 (CH), 129.2 (CH), 132.8 (C), 135.4 (C), 135.9 (CH), 147.6 (CH), 150.0 (CH), 154.5 (C); IR 2921, 2851, 1574, 1023, 691 cm^{-1}.

Z isomer (colourless oil, 0.020 g, 9%) HRMS *m/z* Calcd for $C_{20}H_{25}N_2O$ requires 309.1967, found 309.1953; ^{1}H NMR ($CDCl_3$) δ 0.81−1.00 (2H, m), 1.10−1.32 (4H, m), 1.62−1.76 (4H, m), 3.85 (2H, s), 3.90 (2H, d, *J* = 6.3 Hz), 7.16 (1H, ddd, *J* = 7.8, 4.8, 0.6 Hz), 7.20−7.35 (6H, m), 7.47−7.53 (1H, m), 8.40−8.46 (2H, m); ^{13}C NMR ($CDCl_3$) δ 25.8 (CH_2), 26.6 (CH_2), 29.8 (CH_2), 37.5 (CH), 38.9 (CH_2), 80.0 (CH_2), 123.3 (CH), 127.8 (CH), 128.1 (CH), 128.9 (CH), 132.9 (C), 133.1 (C), 136.3 (CH), 148.0 (CH), 150.2 (CH), 154.3 (C); IR 2921, 2851, 1574, 1023, 691 cm^{-1}.

10.2.44 Synthesis of 1-phenyl-2-(3-pyridinyl)ethanone phenylpropyloxime (150)



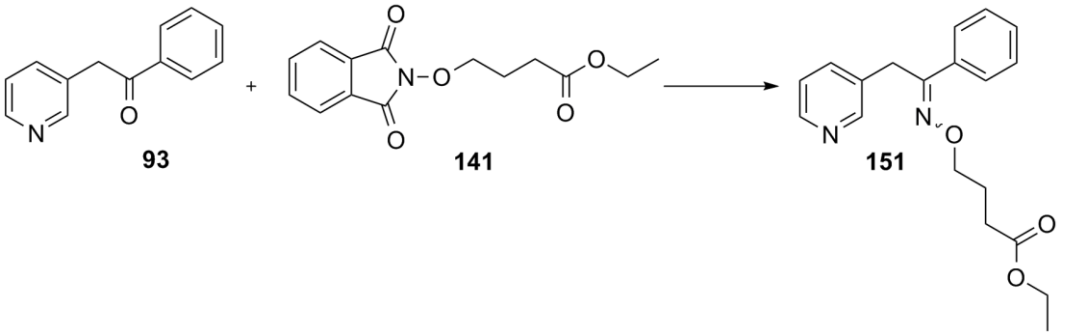
Hydrazine hydrate (0.15 mL, 0.30 g, 0.31 mmol) was added to a solution of *N*-(3-phenylpropoxy)phthalimide **140** (0.490 g, 1.74 mmol) in ethanol (5 mL) and the reaction mixture was heated to 80 °C in a sealed vessel for 1 hour. The reaction mixture was cooled to room temperature. A solution of 1-phenyl-2-(3-pyridinyl)-ethanone **93** (0.205 g, 1.04 mmol) in acetic acid (0.5 mL) and ethanol (1 mL) was added to the reaction mixture and the reaction mixture was heated to 80 °C for 5 days. The reaction mixture was cooled to room temperature and poured into a solution of sodium hydrogen carbonate (1 M, 20 mL). The aqueous layer was extracted with dichloromethane (3 × 20 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits with 1% triethylamine afforded the product **150** as two isomers (0.182 g, 57%, *E*:*Z*, 5.5:1).

E isomer (colourless oil, 0.154 g, 48%) HRMS *m/z* Calcd for $C_{22}H_{23}N_2O$ requires 331.1810, found 331.1793; ^{1}H NMR ($CDCl_3$) δ 1.91−2.00 (2H, m), 2.58 (2H, t, *J* = 7.5 Hz), 4.02 (2H, s), 4.17 (2H, t, *J* = 6.5 Hz), 7.04−7.12 (4H, m), 7.15−7.21 (3H, m), 7.22−7.27 (3H, m), 7.39−7.47 (1H, m), 7.52−7.58 (1H, m), 8.33 (1H, d, *J* = 3.8 Hz), 8.45 (1H, s); ^{13}C NMR ($CDCl_3$) δ 30.1 (CH_2), 30.9 (CH_2), 32.3 (CH_2), 73.8 (CH_2), 123.5 (CH), 125.9 (CH), 126.4 (CH), 128.4 (CH), 128.5 (CH), 128.6 (CH), 129.4 (CH), 132.8 (C), 135.3 (CH), 136.0 (C), 141.7 (C), 147.6 (CH), 150.0 (CH), 154.9 (C); IR 3026, 2931, 1602, 1574, 1025, 692 cm^{-1}.

Z isomer (colourless oil, 0.028 g, 9%) HRMS *m/z* Calcd for $C_{22}H_{23}N_2O$ requires 331.1810, found 331.1791; ^{1}H NMR ($CDCl_3$) δ 1.96−2.06 (2H, m), 2.63−2.70 (2H, m), 3.89 (2H, s), 4.14 (2H, t, *J* = 6.6 Hz), 7.14−7.23 (4H, m), 7.26−7.37 (7H, m), 7.52−7.57 (1H, m), 8.47 (2H, br. s); ^{13}C NMR ($CDCl_3$) δ 30.8 (CH_2), 32.2 (CH_2),

39.0 (CH_2), 73.4 (CH_2), 123.4 (CH), 125.8 (CH), 128.1 (CH), 128.2 (CH), 128.4 (CH), 128.5 (CH), 129.0 (CH), 132.9 (C), 133.1 (C), 136.6 (CH), 141.8 (C), 147.8 (CH), 150.1 (CH), 154.9 (C); IR 3026, 2927, 1601, 1575, 1024, 695 cm^{-1}.

10.2.45 Synthesis of ethyl-4-(((1-phenyl-2-(3-pyridinyl)ethylidene)amino)oxy)-butanoate (151)



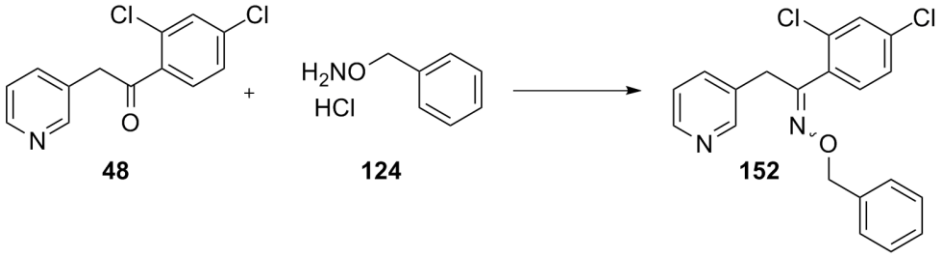
Hydrazine hydrate (0.14 mmol) was added to a solution of ethyl 4-[(1,3-dihydro-1,3-dioxo-2-isoindol-2-yl)oxy]-butanoate **141** (0.520 g, 1.88 mmol) in ethanol (5 mL) and the reaction mixture was heated to 80 °C for 1 hour. The reaction mixture was then cooled to room temperature. A solution of 1-phenyl-2-(3-pyridinyl)-ethanone **93** (0.224 g, 1.14 mmol) in acetic acid (0.5 mL) and ethanol (1 mL) was added to the reaction mixture and the reaction mixture was heated to 80 °C for 5 days. The reaction mixture was cooled to room temperature and poured into a solution of sodium hydrogen carbonate (1 M, 20 mL). The aqueous layer was extracted with dichloromethane (3 × 20 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits with 1% triethylamine afforded the product **151** as two isomers (0.085 g, 14%, *E*:*Z*, 10:1).

E isomer (colourless oil, 0.073 g, 12%) HRMS *m/z* Calcd for $C_{19}H_{23}N_2O_3$ requires 327.1709, found 327.1700; ^{1}H NMR ($CDCl_3$) δ 1.19−1.26 (3H, m), 1.98−2.08 (2H, m), 2.32−2.37 (2H, m), 4.05−4.13 (4H, m), 4.25 (2H, t, *J* = 6.2 Hz), 7.14−7.20 (1H, m), 7.29−7.33 (3H, m), 7.50 (1H, d, *J* = 7.8 Hz), 7.59−7.62 (2H, m), 8.42 (1H, br. s), 8.51 (1H, br. s); ^{13}C NMR ($CDCl_3$) δ 14.3 (CH_3), 24.7 (CH_2), 30.1 (CH_2), 30.9 (CH_2), 60.5 (CH_2), 73.4 (CH_2), 123.7 (CH), 126.4 (CH), 128.7 (CH), 129.5 (CH),

132.8 (C), 135.2 (C), 136.2 (CH), 147.5 (CH), 149.8 (CH), 155.1 (C), 173.3 (C); IR 2953, 1729, 1176, 1026, 693 cm^{-1}.

Z isomer (colourless oil, 0.007 g, 1.1%) ^{1}H NMR ($CDCl_3$) δ 1.20−1.27 (3H, m), 1.93−2.04 (2H, m), 2.30−2.36 (2H, m), 3.89 (2H, s), 4.08−4.14 (4H, m), 7.27−7.37 (6H, m), 7.68 (1H, d, *J* = 7.8 Hz), 8.48 (2H, br. s); ^{13}C NMR ($CDCl_3$) δ 14.2 (CH_3), 24.5 (CH_2), 30.9 (CH_2), 38.9 (CH_2), 60.4 (CH_2), 73.2 (CH_2), 126.4 (CH), 128.0 (CH), 128.3 (CH), 128.9 (CH), 129.2 (CH), 132.7 (C), 138.6 (CH), 143.5 (CH), 154.5 (C), 173.2 (C).

10.2.46 Synthesis of 1-(2,4-dichlorophenyl)-2-(3-pyridinyl)ethanone benzyloxime (152)



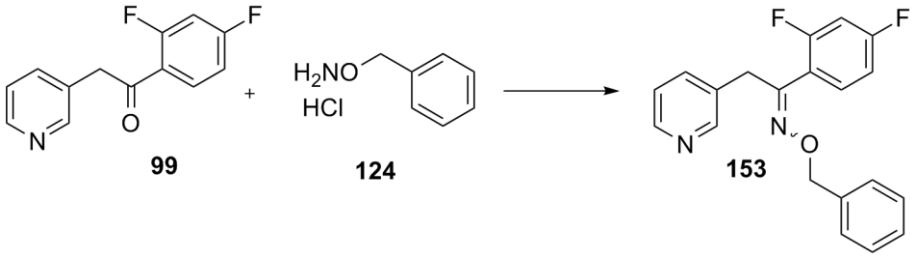
O-Benzylhydroxylamine hydrochloride **124** (0.137 g, 0.86 mmol) and sodium acetate (0.183 g, 2.23 mmol) were added to a solution of 1-(2,4-dichlorophenyl)-2-(3-pyridinyl)-ethanone **42** (0.217 g, 0.82 mmol) in ethanol (4 mL). The reaction mixture was stirred at room temperature for 2 days. The reaction mixture poured into a solution of sodium hydrogen carbonate (1 M, 20 mL). The aqueous layer was extracted with dichloromethane (4 × 20 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits afforded the two isomers as an oil (0.211 g, 70 %, *E*:*Z*, 1.5:1).

E isomer (colourless oil, 0.093 g, 31%) HRMS *m/z* Calcd for $C_{20}H_{17}Cl_2N_2O$ requires 372.0718, found 371.0708; ^{1}H NMR ($CDCl_3$) δ 3.79 (2H, s), 5.09 (2H, s), 6.66 (1H, d, *J* = 8.2 Hz), 7.13 (1H, dd, *J* = 8.2, 1.9 Hz), 7.18 (1H, dd, *J* = 7.3, 4.9 Hz), 7.23−7.46 (7H, m), 8.34 (1H, br. s), 8.47 (1H, br. s); ^{13}C NMR ($CDCl_3$) δ 38.6 (CH_2), 76.4 (CH_2), 123.5 (CH), 127.1 (CH), 127.9 (CH), 128.2 (CH), 128.4 (CH), 129.7 (CH), 129.9 (CH), 132.1 (C), 132.3 (C), 135.2 (C), 137.3 (CH), 137.8 (C),

148.2 (CH), 150.4 (CH), 153.9 (C) ; IR 3031, 2924, 1585, 1554, 1472, 1424, 997, 696 cm^{-1}.

Z isomer (colourless oil, 0.059 g, 18%) HRMS *m/z* Calcd for $C_{20}H_{17}Cl_2N_2O$ requires 372.0718, found 371.0710; ^1H NMR ($CDCl_3$) δ 3.80 (2H, s), 5.06 (2H, s), 6.67 (1H, d, *J* = 8.3 Hz), 7.12 (1H, dd, *J* = 8.3, 2.1 Hz), 7.20−7.38 (6H, m), 7.51 (2H, d, *J* = 7.6 Hz), 8.69 (2H, br. s); ^{13}C NMR ($CDCl_3$) δ 24.0 (CH_2), 76.4 (CH_2), 123.5 (CH), 127.2 (CH), 127.9 (CH), 128.2 (CH), 128.4 (CH), 129.7 (CH), 129.8 (CH), 131.6 (C), 132.2 (C), 132.3 (C), 135.3 (C), 137.3 (CH), 137.7 (C), 148.2(CH), 150.4 (CH), 153.7 (C).

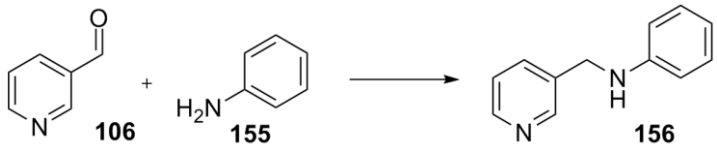
10.2.47 Synthesis of 1-(2,4-difluorophenyl)-2-(3-pyridinyl)ethanone benzyloxime (153)



O-Benzylhydroxylamine hydrochloride **124** (0.115 g, 0.72 mmol) and sodium acetate (0.189 g, 2.30 mmol) were added to a solution of 1-(2,4-difluorophenyl)-2-(3-pyridinyl)-ethanone **99** (0.170 g, 0.73 mmol) in ethanol (4 mL). The reaction mixture was stirred at room temperature for 4 days. The reaction mixture was poured into a solution of sodium hydrogen carbonate (1 M, 20 mL). The aqueous layer was extracted with dichloromethane (4 × 20 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 25% ethyl acetate in petroleum spirits afforded the *E* isomer as a colourless oil (0.053 g, 21%): HRMS *m/z* Calcd for $C_{20}H_{17}F_2N_2O$ requires 339.1309, found 339.1304; ^1H NMR ($CDCl_3$) δ 4.13 (2H, s), 5.25 (2H, s), 6.74−6.85 (2H, m), 7.13 (1H, dd, *J* = 7.8, 4.8 Hz), 7.30−7.44 (7H, m), 8.41 (2H, br. s); ^{13}C NMR ($CDCl_3$) δ 32.4 (CH_2, d, *J* = 5.0 Hz), 76.8 (CH_2), 104.6 (CH, t, *J* = 25.9), 111.9 (CH, dd, *J* = 17.8, 3.4 Hz), 119.9 (C, dd, *J* = 12.8, 3.7 Hz), 123.6 (CH), 128.2 (CH), 128.4 (CH), 128.6 (CH), 131.6 (CH, dd *J* = 9.6, 4.9 Hz), 132.5 (C), 136.7 (CH), 137.3 (C), 147.3 (CH), 149.6 (CH), 153.4 (C, d, *J* = 2.0 Hz),

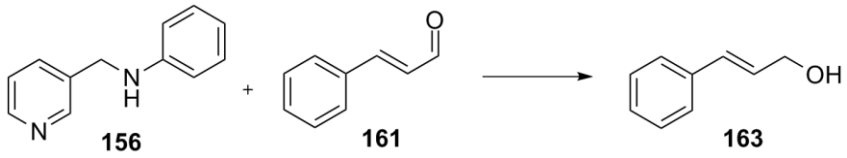
160.7 (C, dd, *J* = 240.7, 12.1 Hz), 163.7 (C, dd, *J* = 240.0, 12.5 Hz); IR 3032, 2930, 1610, 1575, 1502, 1478, 1421, 968, 696 cm^{-1}.

10.2.48 Synthesis of *N*-phenyl-*N*-(3-pyridinemethyl)amine (156)



Anhydrous magnesium sulfate (0.330 g, 2.74 mmol) was added to a solution of aniline **155** (0.195 mL, 0.199 g, 2.14 mmol) and 3-pyridinecarboxaldehyde **106** (0.175 mL, 0.20 g, 1.86 mmol) in dry 1,2-dichloroethane (25 mL) and the reaction mixture was heated under reflux for 6 hours. Upon cooling, the reaction mixture was filtered and the filtrate was concentrated under reduced pressure. ^{1}H NMR ($CDCl_3$) δ 7.17−7.30 (3H, m), 7.34−7.44 (3H, m), 8.27 (1H, m), 8.47 (1H, s), 8.69 (1H, d, *J* = 3.81 Hz), 9.01 (1H, br. s). The residue was dissolved in methanol (6 mL) and sodium borohydride (0.527 g, 13.93 mmol) was added in portions. The reaction mixture was stirred at room temperature for 3 hours. The reaction mixture was acidified with a solution of hydrochloric acid (5 M) and then adjusted to pH 9 with a solution of sodium hydrogen carbonate. The aqueous layer was extracted with dichloromethane (3 × 20 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 25% ethyl acetate in petroleum spirits with 1% triethylamine gave the product **156** as a colourless oil (0.308 g, 90%). The spectroscopic properties were similar to those reported.[156] ^{1}H NMR ($CDCl_3$) δ 4.09 (1H, br. s), 4.35 (2H, s), 6.60−6.65 (2H, m), 6.74 (1H, m), 7.15−7.21 (2H, m), 7.23−7.28 (1H, m), 7.68−7.71 (1H, m), 8.52 (1H, d, *J* = 4.1 Hz), 8.63 (1H, s); ^{13}C NMR ($CDCl_3$) δ 45.9 (CH_2), 113.1 (CH), 118.2 (CH), 123.7 (CH), 129.4 (CH), 135.1 (C), 135.3 (CH), 147.7 (C), 148.6 (CH), 149.1 (CH); IR 3248, 3110, 3030, 2921, 1917, 1600, 1578, 1497, 1423, 1311 cm^{-1}.

10.2.49 Synthesis of 3-phenyl-2-propenol 163



156 161 163

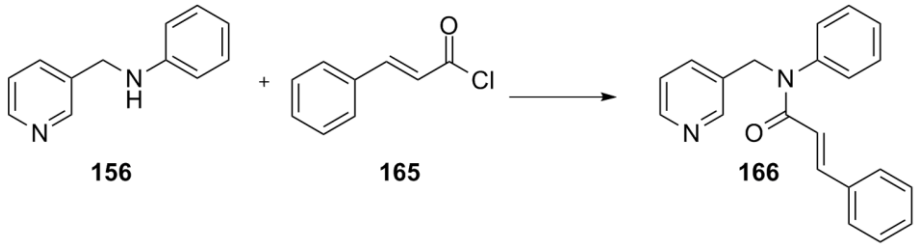
Anhydrous magnesium sulfate (0.309 g, 2.57 mmol) was added to a solution of *N*-phenyl-*N*-(3-pyridinemethyl)amine **156** (0.347 g, 1.88 mmol) and trans-cinnamaldehyde **161** (0.256 g, 1.94 mmol) in dry 1,2-dichloroethane (15 mL) and the reaction mixture was heated under reflux for 4 days. Upon cooling, the reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was dissolved in methanol (6 mL) and sodium borohydride (0.406 g, 10.73 mmol) was added in portions. The reaction mixture was stirred at room temperature for 4 days. The reaction mixture was acidified with a solution of hydrochloric acid (5 M) and then adjusted to pH 9 with a solution of sodium hydrogen carbonate. The aqueous layer was extracted with dichloromethane (3 × 20 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits with 1% triethylamine afforded the starting material **156** (0.209 g, 60%) and 3-phenyl-2-propenol **163** as a colourless oil (0.069 g, 27%).

^{1}H NMR ($CDCl_3$) δ 1.87 (1H, br. s.), 4.35 (2H, dd, *J* = 5.7, 1.3 Hz), 6.34−6.44 (1 H, m), 6.64 (1 H, d, *J* = 15.9 Hz), 7.23−7.45 (H, m); ^{13}C NMR ($CDCl_3$) δ 63.8 (CH_2), 112.8 (CH), 126.6 (CH), 127.8 (CH), 128.7 (CH), 131.2 (CH), 136.8 (C).

10.2.50 Synthesis of *N*-(3-phenyl-2-propen-1-yl)-benzenamine (164)

Anhydrous magnesium sulfate (0.330 g, 2.74 mmol) was added to a solution of aniline **155** (0.20 mL, 0.204 g, 2.19 mmol) and *trans*-cinnamaldehyde **161** (0.28 mL, 0.294 g, 2.22 mmol) in dry 1,2-dichloroethane (25 mL) and the reaction mixture was heated under reflux for 1 day. Upon cooling the reaction was filtered and the filtrate was concentrated under reduced pressure. The residue was dissolved in methanol (7 mL) and sodium borohydride (0.620 g, 16.39 mmol) was added in portions. The reaction mixture was stirred at room temperature for 4 hours. The reaction mixture was acidified with a solution of hydrochloric acid (5 M) and then adjusted to pH 9 with a solution of sodium hydrogen carbonate. The organics were extracted with dichloromethane (3 × 20 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 25% ethyl acetate in petroleum spirits with 1% triethylamine gave the product **164** as a colourless oil (0.122 g, 27%). The spectroscopic properties were similar to those reported.[157] ^{1}H NMR ($CDCl_3$) δ 3.85 (2H, dd, *J* = 5.7, 1.6 Hz), 4.24 (1H, s), 6.25 (1H, dt, *J* = 15.9, 5.7 Hz), 6.51−6.68 (5H, m), 7.06−7.31 (6H, m); ^{13}C NMR ($CDCl_3$) δ 46.4 (CH_2), 113.2 (CH), 117.8 (CH), 126.5 (CH), 127.1 (CH), 127.7 (CH), 128.7 (CH), 129.4 (CH), 131.7 (CH), 137.0 (C), 148.1 (C); IR 3404, 3054, 3025, 1683, 1599, 1501, 689 cm^{-1}.

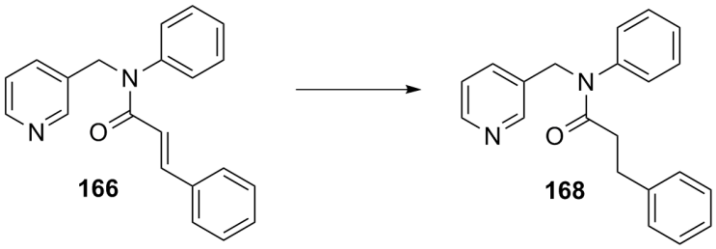
10.2.51 Synthesis of *N*-phenyl-*N*-(3-pyridylmethyl)cinnamamide (166)



156 165 166

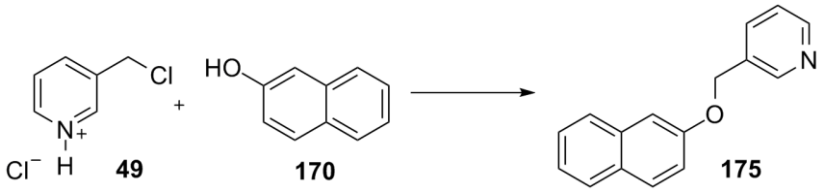
N-Phenyl-*N*-(3-pyridinemethyl)amine **156** (0.255 g, 1.38 mmol) and DMAP (0.003 g, 0.03 mmol) were dissolved in dry dichloromethane (3 mL) and the reaction mixture was cooled to 0 °C. Cinnamoyl chloride **165** (0.320 g, 1.92 mmol) was added and the reaction mixture was stirred at room temperature for 5 days. The reaction mixture was concentrated under reduced pressure and a solution of sodium hydrogen carbonate (1 M) was added. The aqueous layer was extracted with dichloromethane (2 × 10 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits with 1% triethylamine afforded the product **166** as a yellow oil (0.395 g, 91%): HRMS *m/z* Calcd for $C_{21}H_{19}N_2O$ requires 315.1497, found 315.1499; ^{1}H NMR ($CDCl_3$) δ 5.03 (2H, s), 6.30 (1H, d, *J* = 15.6 Hz), 7.02−7.08 (2H, m), 7.20−7.32 (6H, m), 7.33−7.41 (3H, m), 7.69−7.77 (2H, m), 8.41 (1H, br. s), 8.50 (1H, d, *J* = 3.1 Hz); ^{13}C NMR ($CDCl_3$) δ 50.9 (CH_2), 118.4 (CH), 123.7 (CH), 128.0 (CH), 128.3 (CH), 128.4 (CH), 128.8 (CH), 129.8 (CH), 197.9 (CH), 133.3 (C), 135.1 (C), 136.8 (CH), 141.7 (C), 142.9 (CH), 148.9 (CH), 149.9 (CH), 166.3 (C); IR 3057, 3028, 2927, 1651, 1492, 1380, 760, 698 cm^{-1}.

10.2.52 Synthesis of *N*-phenyl-*N*-(3-pyridinylmethyl)dihydrocinnamamide (168)



N-Phenyl-*N*-(3-pyridylmethyl)cinnamamide **166** (0.147 g, 0.47 mmol) was dissolved in dry ethanol (6 mL) under nitrogen. Triethylsilane (0.20 mL, 0.146 g, 1.25 mmol, 2.65 eq.) and palladium(II) chloride (0.020 g, 0.17 mmol) were added and the mixture was heated under reflux for 3 hours under nitrogen. The reaction mixture was poured into a solution of hydrochloric acid (3 M, 20 mL) and washed with ethyl acetate (2 × 20 mL). The aqueous layer was made basic with a solution of sodium hydroxide (3 M, 20 mL). The organics were extracted with dichloromethane (3 × 20 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits afforded the product **168** as a colourless oil (0.068 g, 48%): HRMS *m/z* Calcd for $C_{21}H_{21}N_2O$ requires 317.1654, found 317.1641; ^{1}H NMR ($CDCl_3$) δ 2.29 (2H, t, *J* = 7.6 Hz), 2.85 (2H, t, *J* = 7.6 Hz), 4.77 (2H, s), 6.64−6.71 (2H, m), 6.94−6.99 (2H, m), 7.04−7.16 (4H, m), 7.17−7.23 (3H, m), 7.48 (1H, d, *J* = 7.8 Hz), 8.25 (1H, br. s), 8.41 (1H, br. s); ^{13}C NMR ($CDCl_3$) δ 31.7 (CH_2), 36.0 (CH_2), 50.6 (CH_2), 123.9 (CH), 126.3 (CH), 128.4 (CH), 128.5 (CH), 128.6 (CH), 130.0 (CH), 133.7 (CH), 137.6 (CH), 141.0 (C), 141.9 (C), 148.2 (CH), 149.3 (C), 172.4 (C); IR 3028, 2927, 1651, 1593, 1494, 1264, 698 cm^{-1}.

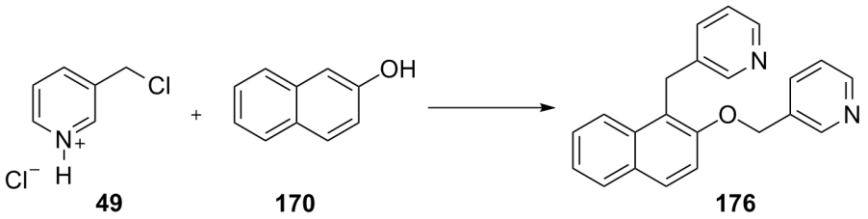
10.2.53 Synthesis of 3-(2-(naphthoxy)methyl)pyridine (175)



3-Chloromethylpyridine **49** (0.378 g, 2.30 mmol) and 2-naphthol **170** (0.334g, 2.32 mmol) were dissolved in a solution of sodium hydroxide (0.190 g, 4.75 mmol) and water (3 mL) and the mixture was stirred at room temperature overnight. The mixture was poured into a solution of hydrochloric acid (1 M, 10 mL) and made basic to pH 8 with a solution of sodium hydrogen carbonate (1 M). The aqueous layer was extracted with dichloromethane (5 × 20 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits with 1% triethylamine afforded starting material **170** as a white solid (0.098 g, 30%) and compound **175** as a colourless oil (0.087 g, 16%).

3-(2-(naphthoxy)methyl)pyridine (**175**): ^{1}H NMR ($CDCl_3$) δ 5.18 (2H, s), 7.19−7.25 (2H, m), 7.31−7.40 (2H, m), 7.46 (1H, ddd, *J* = 8.2, 6.9, 1.2 Hz), 7.71−7.80 (3H, m), 7.81−7.86 (1H, m), 8.62 (1H, dd, *J* = 4.8, 1.3 Hz), 8.76 (1H, d, *J* = 1.7 Hz); ^{13}C NMR ($CDCl_3$) δ 67.5 (CH_2), 107.3 (CH), 118.8 (CH), 123.6 (CH), 124.0 (CH), 126.6 (CH), 126.9 (CH), 127.7 (CH), 129.3 (C), 129.7 (CH), 132.6 (C), 134.5 (C), 135.5 (CH), 148.9 (CH), 149.4 (CH), 158.4 (C); IR 3027, 1625, 1597, 1255, 1212, 1177, 1021, 838 cm^{-1}.

10.2.54 Synthesis of 3-((2-(pyridin-3-ylmethoxy)naphthalen-1-yl)methyl)-pyridine (176)

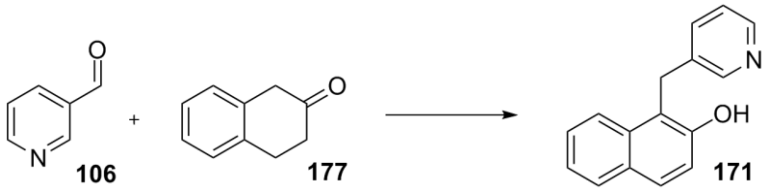


49 **170** **176**

2-Napthol **170** (0.694 g, 4.814 mmol) and 3-chloromethylpyridine hydrochloride (0.789 g, 4.81 mmol) were added to a solution of sodium hydroxide (0.393 g, 9.83 mmol) in water (5 mL). The reaction mixture was stirred at room temperature for 60 hours. The reaction mixture was poured onto a solution of hydrochloric acid (1 M, 10 mL) and made basic to pH 8 with a solution of sodium hydrogen carbonate (1 M). The aqueous layer was extracted with dichloromethane (2 × 15 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits afforded naphthol **170** (0.349 g, 50%) and 3-((2-(pyridin-3-ylmethoxy)naphthalen-1-yl)methyl)pyridine **176** as a brown oil (0.296 g, 19 %).

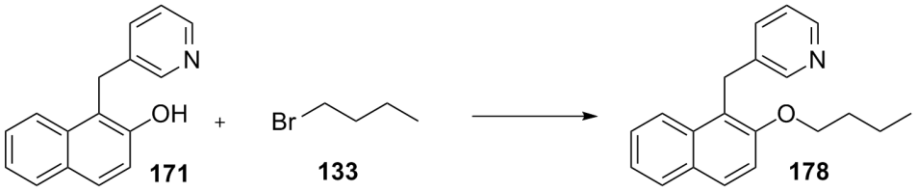
3-((2-(pyridin-3-ylmethoxy)naphthalen-1-yl)methyl)pyridine **176**: ^{1}H NMR ($CDCl_3$) δ 4.56 (2H, s), 5.22 (2H, s), 7.08−7.13 (3H, m), 7.15−7.25 (2H, m), 7.29−7.35 (2H, m), 7.45 (1H, d, *J* = 7.8 Hz), 7.55−7.61 (3H, m), 7.65 (2H, d, *J* = 9.1 Hz), 8.46 (1H, dq, *J* = 2.6, 0.7 Hz), 8.51 (1H, d, *J* 4.3 Hz); 13C NMR ($CDCl_3$) δ 46.7 (CH2), 70.6 (CH2), 118.8 (CH), 121.4 (CH), 122.7 (CH), 122.8 (CH), 123.1 (CH), 123.9 (CH), 126.4 (CH), 126.9 (CH), 127.6 (CH), 129.1 (C), 129.6 (CH), 134.5 (C), 136.9 (CH), 137.1 (CH), 149.2 (CH), 149.4 (CH), 156.3 (C), 156.5 (C), 157.1 (C).

10.2.55 Synthesis of 1-(3-pyridinylmethyl)-2-naphthol (171)



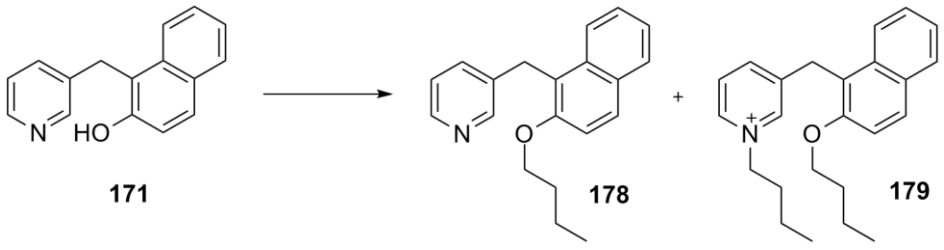
3-Pyridine carboxaldehyde **106** (0.55 mL, 0.628 g, 5.86 mmol) and β-tetralone **177** (0.66 mL, 0.73 g, 4.99 mmol) were dissolved in acetic acid (20 mL). Freshly prepared anhydrous hydrogen chloride gas was bubbled through the reaction mixture until saturated. The mixture was stirred at room temperature overnight and then concentrated under reduced pressure. The residue was dissolved in ethyl acetate (20 mL) and washed with a solution of sodium hydrogen carbonate (1 M, 20 mL). The aqueous layer was extracted with ethyl acetate (3 × 20 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. Diethyl ether was added to the residue and the resulting crystals were collected by vacuum filtration and dried under vacuum to give 1-(3-pyridinylmethyl)-2-naphthol **171** as yellow crystals (0.876 g, 75%): m.p. 202−203 °C (lit.[128] m.p. 197−200 °C). The spectroscopic properties were similar to those reported.[128] ^{1}H NMR ($CDCl_3$) δ 4.47 (2H, s), 7.20 (1H, d, *J* = 8.6 Hz), 7.27−7.33 (2H, m), 7.44 (1H, ddd, *J* = 8.6, 6.9, 1.3 Hz), 7.62 (1H, d, *J* = 8.6 Hz), 7.74−7.80 (2H, m), 7.87 (1H, d, *J* = 8.6 Hz), 8.42 (1H, d, *J* = 4.4 Hz), 8.65 (1H, s); ^{13}C NMR ($CDCl_3$) δ 28.3 (CH_2), 117.9 (C), 118.4 (CH), 122.8 (CH), 123.1 (CH), 123.7 (CH), 126.8 (CH), 128.5 (CH), 128.8 (CH), 129.3 (C), 133.5 (CH), 136.7 (CH), 137.5 (C), 146.3 (CH), 149.5 (CH), 150.8 (C); IR 3041, 2868, 2598, 1626, 1578, 1435, 1273, 997, 706 cm^{-1}.

10.2.56 Synthesis of 2-butoxy-1-(pyridylmethyl)naphthalene (178)



1-(3-Pyridinylmethyl)-2-naphthol **171** (0.146 g, 0.62 mmol) was dissolved in *N*,*N*-dimethylformamide (11 mL). Potassium carbonate (0.264 g, 1.91 mmol) was added to the solution followed by 1-bromobutane **133** (0.2 mL, 0.253 g, 1.85 mmol). The reaction mixture was heated to 80 °C for 4 days under nitrogen. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 5% methanol in dichloromethane afforded the product **178** as a pale brown oil (0.153 g, 84%): HRMS *m/z* Calcd for $C_{20}H_{22}NO$ requires 292.1701, found 292.1697; ^{1}H NMR ($CDCl_3$) δ 0.96 (3H, t, *J* = 7 Hz), 1.40−1.54 (2H, m), 1.73−1.84 (2H, m), 4.12 (2H, t, *J* = 6.5 Hz), 4.46 (2H, s), 7.09 (1H, dd, *J* = 7.6, 4.8 Hz), 7.28−7.36 (2H, m), 7.40−7.47 (2H, m), 7.76−7.82 (2H, m), 7.86−7.92 (1H, m), 8.38 (1H, br. s), 8.60 (1H, br. s); ^{13}C NMR ($CDCl_3$) δ 13.9 (CH_3), 19.4 (CH_2), 28.2 (CH_2), 31.7 (CH_2), 68.9 (CH_2), 114.3 (CH), 120.5 (C), 123.2 (CH), 123.4 (CH), 126.8 (CH), 128.7 (CH), 128.8 (CH), 129.3 (C), 133.2 (C), 136.0 (CH), 137.1 (C), 147.0 (CH), 150.0 (CH), 154.4 (C); IR 2951, 2935, 2869, 1623, 1594, 1262, 1250, 1079, 803, 750 cm^{-1}.

10.2.57 Synthesis of 3-((2-butoxynaphthalen-1-yl)methyl)-1-butylpyridin-1-ium (179)

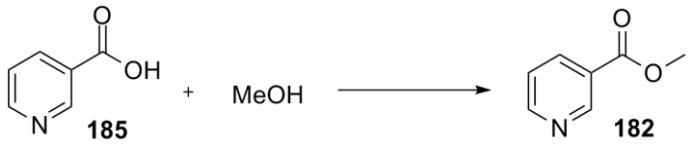


1-(3-Pyridinylmethyl)-2-naphthol **171** (0.148 g, 0.63 mmol) was dissolved in dimethylformamide (15 mL). Potassium carbonate (0.238 g, 1.70 mmol) was added to the solution followed by 1-bromobutane (1.5 mL, 1.905 g, 13.9 mmol). The reaction mixture was heated to 80 °C for 3 days under nitrogen. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The

residue was subjected to flash chromatography. Elution with 5% methanol in dichloromethane afforded 2-butoxy-1-(pyridylmethyl)naphthalene **178** as a pale brown oil (0.006 g, 3.3%) and 3-((2-butoxynaphthalen-1-yl)methyl)-1-butylpyridin-1-ium **179** as a pale brown oil (0.186 g, 85%).

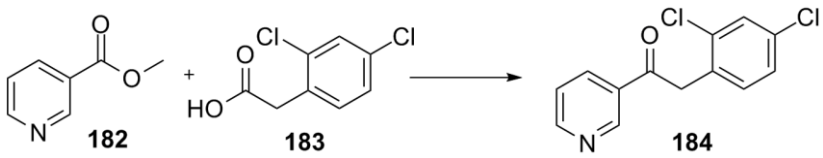
3-((2-butoxynaphthalen-1-yl)methyl)-1-butylpyridin-1-ium **179**: ^{1}H NMR ($CDCl_3$) δ 0.86 (3H, t, *J* = 7.3 Hz), 0.92 (3H, t, *J* = 7.3 Hz), 1.24−1.47 (4H, m), 1.68−1.78 (2H, m), 1.89−2.00 (2H, m), 4.10 (2H, t, *J* = 6.5 Hz), 4.68 (2H, s), 4.85 (2H, t, *J* = 7.3 Hz), 7.26−7.31 (2H, m), 7.45 (1H, ddd, *J* = 8.4, 7.0, 1.2 Hz), 7.76 (1H, d, *J* = 7.9 Hz), 7.80 (1H, d, *J* = 9.1 Hz), 7.83−7.92 (2H, m), 8.03 (1H, d, *J* = 8.1 Hz), 9.35 (1H, s), 9.41 (1H, d, *J* = 6.0 Hz); ^{13}C NMR ($CDCl_3$) δ 13.4 (CH3), 13.8 (CH3), 19.2 (CH2), 19.3 (CH2), 27.8 (CH2), 31.5 (CH2), 33.7 (CH2), 61.5 (CH2), 68.9 (CH2), 113.8 (CH), 117.5 (C), 122.6 (CH), 123.8 (CH), 127.6 (CH), 128.0 (CH), 128.8 (CH), 129.1 (C), 129.9 (CH), 132.5 (C), 142.8 (CH), 143.3 (C), 143.7 (CH), 144.5 (CH), 154.2 (C).

10.2.58 Synthesis of nicotinic acid methyl ester (182)



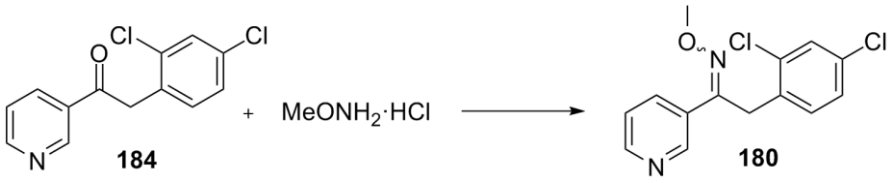
Nicotinic acid **185** (1.064 g, 7.87 mmol) and concentrated sulfuric acid (3.4 mL) in methanol (17 mL) were heated under reflux overnight. The reaction mixture was cooled to room temperature, poured into water (10 mL) and made basic with a solution of sodium carbonate (1 M). The aqueous layer was extracted with dichloromethane (5 × 25 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure to give nicotinic acid methyl ester **182** as a white solid (0.939 g, 80%): ^{1}H NMR ($CDCl_3$) δ 3.81 (3H, s), 7.26 (1H, ddd, *J* = 7.9, 4.8, 0.88 Hz), 8.14 (1H, m), 8.63 (1H, dd, *J* = 4.8, 1.8 Hz), 9.07 (1H, dd, *J* = 2.2, 0.9 Hz); ^{13}C NMR ($CDCl_3$) δ 51.9 (CH_3), 122.9 (CH), 125.6 (C), 136.5 (CH), 150.4 (CH), 153.0 (CH), 165.2 (C).

10.2.59 Synthesis of 2-(2,4-dichlorophenyl)-1-(3-pyridinyl)ethanone (184)



Nicotinic acid methyl ester **182** (0.147 g, 0.99 mmol) and 2,4-dichlorophenylacetic acid **183** (0.206 g, 1.00 mmol) were dissolved in anhydrous *N*,*N*-dimethylformamide (3 mL). The mixture was cooled to −8 °C and sodium bis(trimethylsilyl)amide (1 M in THF, 2 mL) was added dropwise. The resulting mixture was stirred for 3.5 hours. The reaction mixture was poured into a solution of ammonium chloride (1 M, 20 mL). The aqueous layer was extracted with dichloromethane (4 × 20 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 20% ethyl acetate in hexanes afforded the ketone **184** as a yellow oil (0.158 g, 60%): ^{1}H NMR ($CDCl_3$) δ 4.39 (2H, s), 7.10−7.20 (2H, m), 7.35 (1H, d, *J* = 2.0 Hz), 7.40−7.46 (1H, m), 8.20−8.24 (1H, m), 8.80 (1H, d, *J* = 3.5 Hz), 9.24 (1H, s); ^{13}C NMR ($CDCl_3$) δ 43.0 (CH_2), 124.0 (CH), 127.5 (CH), 129.5 (CH), 130.9 (C), 131.9 (C), 132.2 (CH), 134.1 (C), 135.2 (C), 135.9 (CH), 149.6 (CH), 153.7 (CH), 194.7 (C); IR 3071, 1692, 1548, 1473, 1228, 1100, 823, 699 cm^{-1}.

10.2.60 Synthesis of 2-(2,4-dichlorophenyl)-1-(3-pyridinyl)methyloxime ethanone (180)



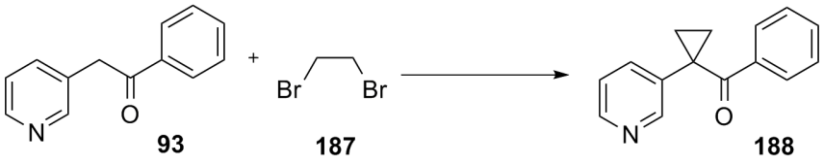
A mixture of 2-(2,4-dichlorophenyl)-1-(3-pyridinyl)ethanone **184** (0.150 g, 0.56 mmol), *O*-methylhydroxylamine hydrochloride (0.054 g, 0.65 mmol) and sodium acetate (0.186 g, 22.27 mmol) in ethanol (4 mL) was stirred at room temperature for 4 days. The reaction mixture was poured into a solution of sodium hydrogen carbonate (1 M, 20 mL) and extracted with dichloromethane (4 × 20 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution

with 25% ethyl acetate in petroleum spirits afforded the two isomers as a coloulress oil (0.021 g, 13%).

Data for major isomer (selected peaks)* ^{1}H NMR ($CDCl_3$) δ 3.99 (3H, s), 4.12 (2H, s), 6.92 (1H, d, *J* = 8.4 Hz), 7.88 (1H, m), 8.58 (1H, br. s.), 8.78 (1H, br. s.).

Data for minor isomer (selected peaks)* ^{1}H NMR ($CDCl_3$) δ 3.82 (3H, s), 3.92 (2H, s), 7.62−7.67 (1H, m), 8.50 (2H, br. s.).

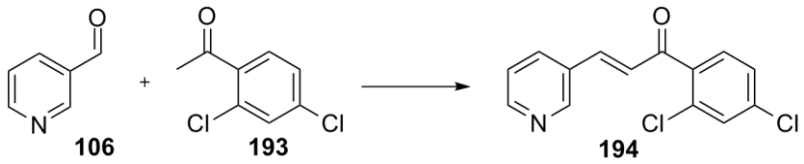
10.2.61 Synthesis of 1-benzoyl-1-(3-pyridyl)cyclopropane (181)



Potassium *t*-butoxide (0.106 g, 0.94 mmol) was added to a cooled (0 °C) solution of 1-phenyl-2-(3-pyridinyl)ethanone **93** (0.153 g, 0.78 mmol) in anhydrous *N*,*N*-dimethylformamide (4 mL). The reaction mixture was stirred at 0 °C under nitrogen for 30 minutes. 1,2-Dibromoethane **187** (0.1 mL, 0.22 g, 1.16 mmol) was added to the reaction mixture and the reaction mixture was stirred at 0 °C for 2.5 hours. Additional potassium *t*-butoxide (0.110 g, 0.98 mmol) was added and the reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated under reduced pressure to give an oil. The residue was dissolved in ethyl acetate (20 mL) and washed with water. The organic layer was dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 50% ethyl acetate in hexanes afforded the product **188** as a colourless oil (0.034 g, 20%): HRMS *m/z* Calcd for $C_{15}H_{14}NO$ requires 224.1075, found 224.1064; ^{1}H NMR ($CDCl_3$) δ 1.32 (2H, dd, *J* = 4.5, 2.6 Hz), 1.68 (2H, dd, *J* = 4.4, 2.6 Hz), 7.08 (1H, ddd, *J* = 7.9, 4.8, 0.8 Hz), 7.18−7.24 (2H, m), 7.31 (1H, m), 7.40−7.44 (1H, m), 7.60−7.65 (2H, m), 8.33 (1H, dd, *J* = 4.8, 1.7 Hz), 8.42 (1H, d, *J* = 1.8 Hz); ^{13}C NMR ($CDCl_3$) δ 16.2 (CH_2), 33.1 (C), 123.3 (CH), 128.2 (CH), 129.2 (CH), 132.2 (CH), 135.7 (CH), 136.7 (C), 136.8 (C), 147.9 (CH), 149.5 (CH), 199.5 (C); IR 3035, 1719, 1670, 1284, 988, 707 cm^{-1}.

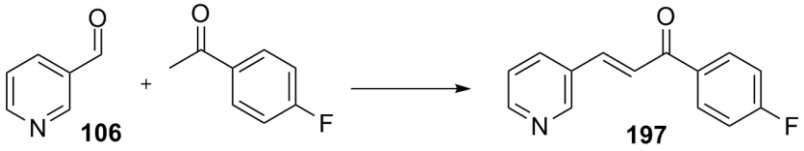
* Isomers were not to be isolated. Peaks were assigned based on the integration in the ^{1}H NMR spectrum

10.2.62 Synthesis of 1-(2,4-dichlorophenyl)-3-(3-pyridinyl)-2-propen-1-one (194)



A solution of sodium hydroxide (2.264 g, 56.6 mmol) in water (80 mL) and ethanol (80 mL) was added to a solution of 3-pyridinecarboxaldehyde **106** (4.38 mL, 4.9 g, 46.7 mmol) and 2′,4′-dichloroacetophenone **193** (6.69 mL, 8.82 g, 46.7 mmol) in ethanol (160 mL). Water (600 mL) was added immediately until a solid precipitated out. The solid was collected via vacuum filtration and dried under vacuum to give 1-(2,4-dichlorophenyl)-3-(3-pyridinyl)-2-propen-1-one **194** as a pale yellow solid (9.544 g, 99%): m.p. 115.5−116.5 °C (lit.[131] m.p. 120−122 °C). The spectroscopic properties were similar to those reported.[131] ^{1}H NMR ($CDCl_3$) δ 7.20 (1H, d, *J* = 16.1 Hz), 7.33−7.40 (2H, m), 7.45−7.53 (3H, m), 7.90 (1H, m), 8.64 (1H, dd, *J* = 4.8, 1.4 Hz), 8.78 (1H, d, *J* = 1.6 Hz); ^{13}C NMR ($CDCl_3$) δ 124.0 (CH), 127.6 (CH), 127.6 (CH), 130.3 (C), 130.5 (CH), 130.8 (CH), 132.6 (C), 134.7 (CH), 137.2 (C), 137.6 (C), 142.3 (CH), 150.4 (CH), 151.7 (CH), 191.9 (C); IR 3087, 3060, 3034, 1678, 1608, 1582, 1568, 1551, 1211 cm^{-1}.

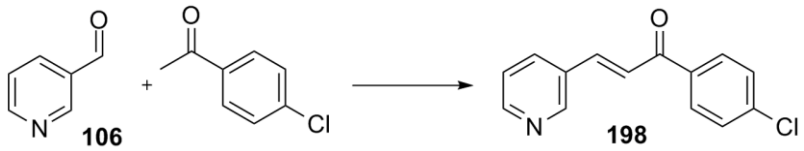
10.2.63 Synthesis of 1-(4-fluorophenyl)-3-(3-pyridinyl)-2-propen-1-one (197)



A solution of sodium hydroxide (2.27 g, 56.7 mmol) in water (80 mL) and ethanol (80 mL) was added to a solution of 3-pyridinecarboxaldehyde **106** (4.38 mL, 5 g, 46.7 mmol) and 4′-fluoroacetophenone (5.67 mL, 6.45 g, 46.7 mmol) in ethanol (160 mL). Water (600 mL) was added immediately until a solid precipitated out. The solid was collected via vacuum filtration and dried under vacuum to give 1-(4-fluorophenyl)-3-(3-pyridinyl)-2-propen-1-one **197** as a pale yellow solid (9.35 g, 90%): m.p. 125 °C (lit.[158] m.p. 126−127 °C). The spectroscopic properties were similar to those reported.[131,158] ^{1}H NMR ($CDCl_3$) δ 7.13−7.21 (2H, m), 7.35 (1H, dd,

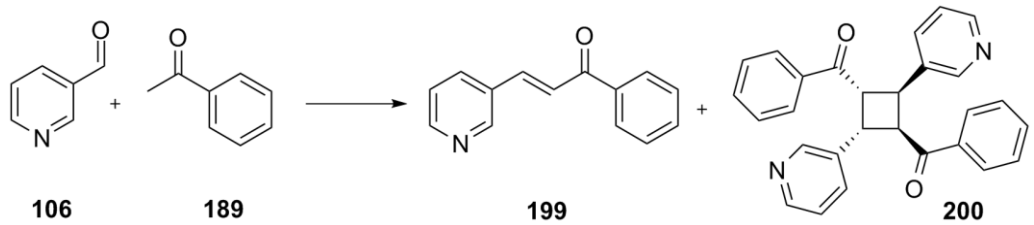
J = 7.9, 4.8 Hz), 7.56 (1H, d, *J* = 15.8 Hz), 7.78 (1H, d, *J* = 15.8 Hz), 7.93 (1H, m), 8.01−8.09 (2H, m), 8.62 (1H, br. s), 8.85 (1H, br. s); ^{13}C NMR ($CDCl_3$) δ 116.0 (CH, d, *J* = 21.9 Hz), 123.5 (CH), 123.9 (CH), 130.7 (C), 131.2 (CH, d, *J* = 9.1 Hz), 134.2 (C, d, *J* = 3.0 Hz), 134.7 (CH), 141.3 (CH), 150.1 (CH), 151.3 (CH), 165.9 (C, d, *J* = 255.1 Hz), 188.2 (C); IR 3069, 1686, 1664, 1603, 1587, 1567, 1217 cm^{-1}.

10.2.64 Synthesis of 1-(4-chlorophenyl)-3-(3-pyridinyl)-2-propen-1-one (198)



A solution of sodium hydroxide (2.27 g, 56.7 mmol) in water (80 mL) and ethanol (80 mL) was added to a solution of 3-pyridinecarboxaldehyde **106** (4.38 mL, 5 g, 46.7 mmol) and 4′-chloroacetophenone (6 mL, 7.152 g, 46.2 mmol) in ethanol (160 mL). Water (600 mL) was added immediately until a solid precipitated out. The solid was collected via vacuum filtration and dried under vacuum to give 1-(4-chlorophenyl)-3-(3-pyridinyl)-2-propen-1-one **198** as a pale yellow solid (10.47 g, 92%): m.p. 120 °C; HRMS Calcd for $C_{14}H_{11}ClNO$ requires 244.0529, found 244.0520; ^{1}H NMR ($CDCl_3$) δ 7.37 (1H, dd, *J* = 7.9, 4.8 Hz), 7.46−7.51 (2H, m), 7.55 (1H, d, *J* = 15.8 Hz), 7.79 (1H, d, *J* = 15.8 Hz), 7.93−7.99 (3H, m), 8.64 (1H, d, *J* = 3.8 Hz), 8.86 (1H, br. s); ^{13}C NMR ($CDCl_3$) δ 123.6 (CH), 124.0 (CH), 129.2 (CH), 130.1 (CH), 130.7 (C), 134.9 (C), 136.2 (C), 139.8 (C), 141.5 (CH), 150.0 (CH), 151.3 (CH), 188.6 (C); IR 3063, 3032, 1666, 1604, 1580, 1568, 796 cm^{-1}.

10.2.65 Synthesis of ((1*R*,2*R*,3*S*,4*S*)-2,4-di(pyridin-3-yl)cyclobutane-1,3-diyl)bis-(phenylmethanone) (200)

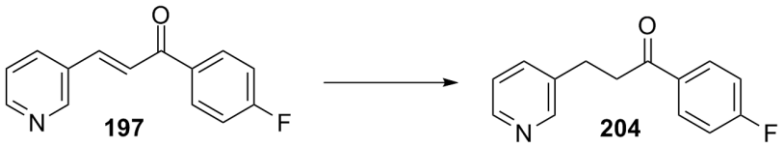


106 189 199 200

A solution of sodium hydroxide (0.113 g, 2.83 mmol) in water (1.5 mL) and ethanol (1.5 mL) was added to a solution of 3-pyridinecarboxaldehyde **106** (0.512 g, 4.78 mmol) and acetophenone (0.579 g, 4.82 mmol) in ethanol (1.5 mL). Water (10 mL) was added immediately. The aqueous layer was extracted with dichloromethane (5 × 10 mL). The combined organics were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits afforded 1-phenyl-3-(3-pyridinyl)-2-propen-1-one **199** as a white solid (0.259 g, 26 %): ^{1}H NMR ($CDCl_3$) δ 7.34−7.39 (1H, m), 7.49−7.56 (2H, m), 7.57−7.64 (2H, m), 7.79 (1H, d, *J* = 15.3 Hz), 7.92−7.98 (1H, m), 8.00−8.05 (2H, m), 8.64 (1H, dd, *J* = 4.7, 1.6 Hz), 8.86 (1H, d, *J* = 2.1 Hz); ^{13}C NMR ($CDCl_3$) δ 123.9 (CH), 124.0 (CH), 128.7 (CH), 128.9 (CH), 130.8 (C), 133.3 (CH), 134.7 (CH), 137.9 (C), 141.1 (CH), 150.1 (CH), 151.3 (CH), 190.0 (C); IR 3062, 3039, 1650, 1603, 1220, 679 cm^{-1}.

Recrystallisation of 1-phenyl-3-(3-pyridinyl)-2-propen-1-one **199** from dichloromethane and petroleum spirits afforded ((1R,2R,3S,4S)-2,4-di(pyridin-3-yl)cyclobutane-1,3-diyl)bis-(phenylmethanone) **200** as a white solid: ^{1}H NMR ($CDCl_3$) δ 4.89−4.95 (2H, m), 4.97−5.03 (2H, m), 7.21 (2H, dd, *J* = 7.7, 5.2 Hz), 7.34−7.41 (4H, m), 7.48−7.53 (2H, m), 7.69−7.77 (6H, m), 8.35−8.39 (2H, m), 8.52 (2H, d, *J* = 1.6 Hz); ^{13}C NMR ($CDCl_3$) δ 39.8 (CH), 49.7 (CH), 123.4 (CH), 128.1 (CH), 128.7 (CH), 133.7 (CH), 134.5 (C), 135.6 (CH), 135.9 (C), 147.9 (CH), 149.0 (CH), 197.6 (C); IR 3059, 3037, 1660, 1595, 1579, 1428, 1025 cm^{-1}.

10.2.66 Synthesis of 1-(4-fluorophenyl)-3-(3-pyridinyl)-1-propanone (204)



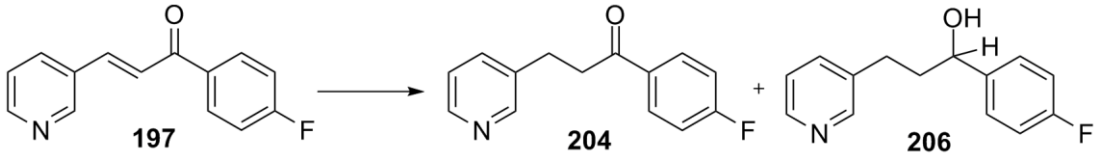
Procedure 1

Ammonium chloride (1.495 g, 27.9 mmol) in water (11 mL) was added to a solution of 1-(4-fluorophenyl)-3-(3-pyridinyl)-2-propen-1-one **197** (0.158 g, 0.7 mmol) in ethanol (70 mL). Zinc powder (0.277 g, 4.24 mmol) was added in portions over 15 minutes whilst stirring. After 2 hours the reaction mixture was gravity filtered and the filtrate was concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits afforded 1-(4-fluorophenyl)-3-(3-pyridinyl)-1-propanone **204** as a yellow oil (0.048 g, 30%): HRMS *m/z* Calcd for $C_{14}H_{13}FNO$ requires 230.0981, found 230.0971; ^{1}H NMR ($CDCl_3$) δ 2.90 (2H, t, *J* = 7.3 Hz), 3.13 (2H, t, *J* = 7.3 Hz), 7.07−7.15 (2H, m), 7.20 (1H, dd, *J* = 7.7, 4.8 Hz), 7.54−7.59 (1H, m), 7.93−7.99 (2H, m), 8.44 (1H, d, *J* = 3.7 Hz), 8.51 (1H, br. s); ^{13}C NMR ($CDCl_3$) δ 27.2 (CH_2), 39.7 (CH_2), 115.9 (CH, d, *J* = 22.6 Hz), 123.6 (CH), 130.8 (CH, d, *J* = 9.1 Hz), 133.2 (C, d, *J* = 3.4 Hz), 136.0 (CH), 136.4 (C), 147.6 (CH), 149.8 (CH), 165.8 (C, d, *J* = 254.8 Hz), 196.9 (C); IR 3057, 2919, 1682, 1597, 1506, 1225 cm^{-1}.

Procedure 2

Triethylsilane (1.11 mL, 0.808 g, 6.95 mmol) and palladium(II) chloride (0.072 g, 0.41 mmol) were added to a solution of 1-(4-fluorophenyl)-3-(3-pyridinyl)-2-propen-1-one **197** (0.699 g, 3.15 mmol) in dry ethanol (37 mL). The mixture was heated under reflux for 1 day. On cooling the mixture was filtered through Celite and concentrated under reduced pressure. The residue was acidified with a solution of hydrochloric acid (1 M, 20 mL) and washed with ethyl acetate (10 mL). The aqueous layer was made basic with a solution of sodium hydrogen carbonate (1 M, 30 mL) and extracted with dichloromethane (3 × 30 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure to give 1-(4-fluorophenyl)-3-(3-pyridinyl)-1-propanone **204** as a yellow oil (0.676 g, 94%).

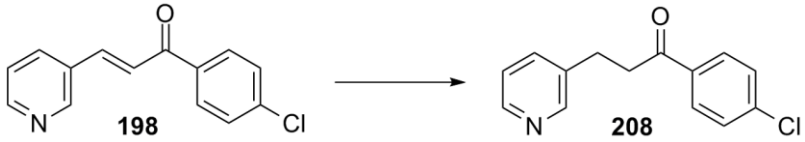
10.2.67 Synthesis of 1-(4-fluorophenyl)-3-(3-pyridinyl)-1-propanol (206)



Triethylsilane (4.00 mL, 2.912 g, 25.0 mmol) and palladium(II) chloride (0.238 g, 1.34 mmol) were added to a solution of 1-(4-fluorophenyl)-3-(3-pyridinyl)-2-propen-1-one **197** (1.998 g, 8.99 mmol) in dry ethanol (150 mL). The mixture was heated under reflux for 2 days. On cooling the mixture was filtered through Celite and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 50% ethylacetate in petroleum spirits afforded 1-(4-fluorophenyl)-3-(3-pyridinyl)-1-propanone **204** as a yellow oil (0.634 g, 31%) and 1-(4-fluorophenyl)-3-(3-pyridinyl)-1-propanol **206** as a yellow oil (0.612 g, 32%).

1-(4-fluorophenyl)-3-(3-pyridinyl)-1-propanol **206**: ^{1}H NMR ($CDCl_3$) δ 1.77−2.02 (2H, m), 2.49−2.68 (2H, m), 4.44 (1H, br. s.), 4.54 (1H, dd, *J* = 7.8, 5.1 Hz), 6.86−6.93 (2H, m), 7.07 (1H, dd, *J* = 7.8, 5.1 Hz), 7.17−7.23 (2H, m), 7.39 (1H, dt, *J* = 7.8, 1.9 Hz), 8.16 (1H, d, *J* = 3.8 Hz), 8.17−8.21 (1H, m).

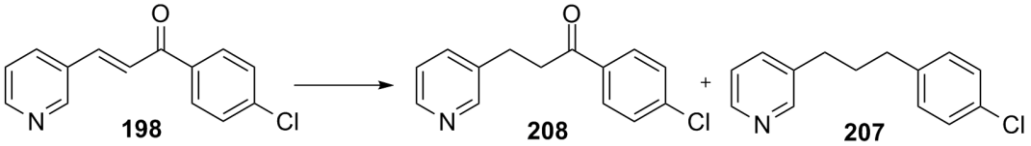
10.2.68 Synthesis of 1-(4-chlorophenyl)-3-(3-pyridinyl)-1-propanone (208)



Triethylsilane (1.3 mL, 0.946 g, 8.14 mmol) and palladium(II) chloride (0.132 g, 0.74 mmol) were added to a solution of 1-(4-chlorophenyl)-3-(3-pyridinyl)-2-propen-1-one **198** (1.603 g, 6.58 mmol) in dry ethanol (50 mL).. The mixture was heated under reflux for 2 days. The reaction mixture was filtered through Celite and concentrated under reduced pressure. The resulting oil was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits afforded 1-(4-chlorophenyl)-3-(3-pyridinyl)-1-propanone **208** as a yellow oil (1.153 g, 71%): HRMS *m/z* Calcd for $C_{14}H_{13}ClNO$ requires 246.0686, found 246.0680; ^{1}H NMR ($CDCl_3$) δ 2.98 (2H, t, *J* = 7.3 Hz), 3.20 (2H, t, *J* = 7.3 Hz), 7.18−7.24 (1H, m), 7.38−7.48 (2H, m), 7.52−7.61 (1H, m), 7.84−7.96 (2H, m), 8.38 (1H, d, *J* = 4.7

Hz), 8.45 (1H, br. s); ^{13}C NMR ($CDCl_3$) δ 27.1 (CH_2), 39.8 (CH_2), 127.9 (CH), 128.9 (CH), 129.3 (CH), 133.4 (CH), 135.0 (C), 136.3 (C), 139.8 (C), 147.7 (CH), 149.9 (CH), 197.3 (C); IR 3031, 2917, 2849, 1681, 1588, 1573, 1089 cm^{-1}.

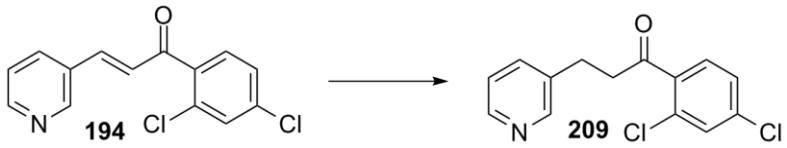
10.2.69 Synthesis of 3-[4-(4-chlorophenyl)propyl]pyridine (207)



Triethylsilane (4.0 mL, 2.912 g, 25.0 mmol) and palladium(II) chloride (0.238 g, 1.34 mmol) were added to a solution of 1-(4-chlorophenyl)-3-(3-pyridinyl)-2-propen-1-one **198** (2.001 g, 8.20 mmol) in dry ethanol (100 mL). The mixture was heated under reflux for 2 days. The reaction mixture was filtered through Celite and concentrated under reduced pressure. The resulting oil was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits afforded 1-(4-chlorophenyl)-3-(3-pyridinyl)-1-propanone **208** as a yellow oil (0.639 G, 32%) and 3-[4-(4-chlorophenyl)propyl]pyridine **207** as an oil (0.412 g, 22%)

3-[4-(4-chlorophenyl)propyl]pyridine **207**: ^{1}H NMR ($CDCl_3$) δ 1.91−2.01 (2H, m), 2.60−2.69 (4H, m), 7.15−7.22 (4H, m), 7.25−7.32 (2H, m), 7.44−7.50 (1H, m), 8.41−8.49 (2H, m); ^{13}C NMR ($CDCl_3$) δ 32.4 (CH_2), 32.6 (CH_2), 35.2 (CH_2), 123.3 (CH), 125.9 (CH), 128.4 (CH), 135.9 (CH), 1317.4 (C), 141.7 (C), 147.3 (CH), 149.9 (CH).

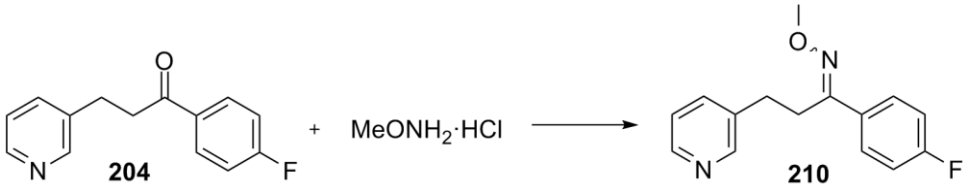
10.2.70 Synthesis of 1-(2,4-chlorophenyl)-3-(3-pyridinyl)-1-propanone (209)



Triethylsilane (1.3 mL, 0.946 g, 8.14 mmol) and palladium(II) chloride (0.136 g, 0.77 mmol) were added to a solution of 1-(2,4-dichlorophenyl)-3-(3-pyridinyl)-2-propen-1-one **194** (1.673 g, 6.02 mmol) in dry ethanol (50 mL).. The mixture was heated under reflux for 2 days. On cooling the mixture was filtered through Celite

and concentrated under reduced pressure. The resulting oil was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits afforded 1-(2,4-chlorophenyl)-3-(3-pyridinyl)-1-propanone **209** as a yellow oil (1.315 g, 78%): HRMS *m/z* Calcd for $C_{14}H_{12}Cl_2NO$ requires 280.0296, found 280.0291; ^{1}H NMR ($CDCl_3$) δ 3.07 (2H, t, *J* = 7.3 Hz), 3.29 (2H, t, *J* = 7.3 Hz), 7.26−7.31 (2H, m), 7.38−7.44 (2H, m), 7.63 (1H, d, *J* = 7.3 Hz), 8.46 (1H, d, *J* = 3.81 Hz), 8.52 (1H, br. s); ^{13}C NMR ($CDCl_3$) δ 27.2 (CH_2), 43.7 (CH_2), 123.7 (CH), 127.5 (CH), 130.3 (CH), 130.5 (CH), 132.1 (C), 136.5 (C), 136.9 (C), 137.0 (CH), 137.7 (C), 146.9 (CH), 149.0 (CH), 200.1 (C); IR 3029, 2931, 1694, 1580, 1552, 1423 cm^{-1}.

10.2.71 Synthesis of 1-(4-fluorophenyl)-3-(3-pyridinyl)-1-propanone methyloxime (210)



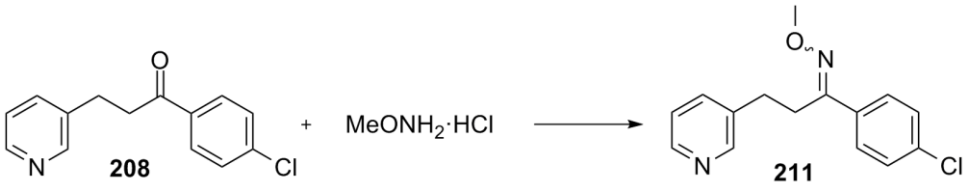
1-(4-Fluorophenyl)-3-(3-pyridinyl)-1-propanone **204** (0.661 g, 2.88 mmol), *O*-methylhydroxylamine hydrochloride (0.253 g, 3 mmol) and sodium acetate (0.557 g, 6.8 mmol) were stirred in ethanol (9 mL) at room temperature for 4 days. The reaction mixture was poured into a solution of sodium hydrogen carbonate (1 M, 10 mL) and the aqueous layer was extracted with ethyl acetate (3 × 10 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The resulting oil was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits with 1% triethylamine afforded the two isomers (0.492 g, 66%, *E*:*Z* 2.5:1).

E isomer (colourless oil, 0.353 g, 47%) HRMS *m/z* Calcd for $C_{15}H_{16}FN_2O$ requires 259.1247, found 259.1247; ^{1}H NMR ($CDCl_3$) δ 2.76−2.82 (2H, m), 2.95−3.01 (2H, m), 3.90 (3H, s), 6.97−7.04 (2H, m), 7.12−7.18 (1H, m), 7.44−7.48 (1H, m), 7.49−7.56 (2H, m), 8.38−8.44 (2H, m); ^{13}C NMR ($CDCl_3$) δ 28.1 (CH_2), 29.6 (CH_2), 62.0 (CH_3), 115.6 (CH, d, *J* = 21.2 Hz), 123.3 (CH), 128.6 (CH, d, *J* = 8.1 Hz), 131.6 (C, d, *J* = 3.4 Hz), 135.9 (CH), 147.7 (CH), 149.8 (CH), 156.1 (C), 163.4 (C, d, *J* =

249.1 Hz); IR 3393, 3058, 3042, 3021, 2938, 2823, 1630, 1608, 1510, 1457, 1222, 1049 cm^{-1}.

Z isomer (colourless oil, 0.139 g, 19%) HRMS *m/z* Calcd for $C_{15}H_{16}FN_2O$ requires 259.1247, found 259.1243; ^1H NMR ($CDCl_3$) δ 2.72−2.77 (4H, m), 3.74 (3H, s), 6.97−7.04 (2H, m), 7.13 (1H, dd, *J* = 7.7, 4.8 Hz), 7.28−7.34 (2H, m), 7.47−7.50 (1H, m), 8.41 (1H, br. s), 8.43 (1H, br. s); ^{13}C NMR ($CDCl_3$) δ 30.1 (CH_2), 36.6 (CH_2), 61.9 (CH_3), 115.5 (CH, d, *J* = 20.9 Hz), 123.5 (CH), 129.4 (C, d, *J* = 3.7 Hz), 130.1 (CH, d, *J* = 8.4 Hz), 136.1 (CH), 147.6 (CH), 150.0 (CH), 154.8 (C), 162.8 (C, d, *J* = 249.1 Hz); IR 3386, 2937, 2818, 1685, 1601, 1576, 1507, 1423, 1227, 1045 cm^{-1}.

10.2.72 Synthesis of 1-(4-chlorophenyl)-3-(3-pyridinyl)-1-propanone methyloxime (211)



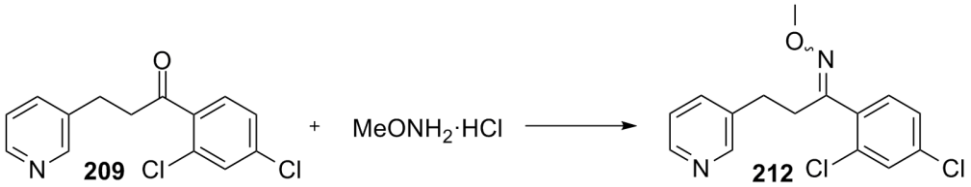
1-(4-Chlorophenyl)-3-(3-pyridinyl)-1-propanone **208** (0.134 g, 0.55 mmol), *O*-methylhydroxylamine hydrochloride (0.048 g, 0.57 mmol) and sodium acetate (0.140 g, 1.71 mmol) were stirred in ethanol (3 mL) at room temperature for 4 days. The reaction mixture was poured into a solution of sodium hydrogen carbonate (1 M, 10 mL) and the aqueous layer was extracted with ethyl acetate (3 × 10 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The resulting oil was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits with 1% triethylamine afforded the two isomers (0.081 g, 53%, *E*:*Z* 3.8 :1).

E isomer (colourless oil, 0.069 g, 42%) HRMS *m/z* Calcd for $C_{15}H_{16}ClN_2O$ requires 275.0951, found 275.0949; ^1H NMR ($CDCl_3$) δ 2.79−2.87 (2H, m), 2.97−3.06 (2H, m), 3.93 (3H, s), 7.14−7.21 (1H, m), 7.29−7.37 (2H, m), 7.45−7.53 (3H, m), 8.46 (2H, br. s); ^{13}C NMR ($CDCl_3$) δ 28.7 (CH_2), 30.5 (CH_2), 62.2 (CH_3), 123.6 (CH),

127.2 (CH), 129.8 (CH), 131.6 (CH), 133.6 (C), 134.0 (C), 135.5 (C), 147.1 (CH), 149.2 (CH), 157.2 (C); IR 3029, 2936, 2817, 1563, 1575, 1046 cm^{-1}.

Z isomer (colourless oil, 0.017 g, 11%) ^{1}H NMR ($CDCl_3$) δ 2.78−2.87 (4H, m), 3.82 (3H, s), 7.21 (1H, d, *J* = 1.61 Hz), 7.29−7.34 (2H, m), 7.35−7.40 (2H, m), 7.49 (1H, d, *J* = 7.6 Hz), 8.35−8.58 (2H, m); ^{13}C NMR ($CDCl_3$) δ 27.3 (CH_2), 29.7 (CH_2), 62.4 (CH_3), 126.2 (CH), 127.5 (CH), 129.0 (CH), 129.1 (CH), 129.3 (CH), 129.6 (CH), 133.0 (C), 135.8 (C), 142.6 (C), 155.1 (C); IR 2933, 1684, 1492, 1092, 1047 cm^{-1}.

10.2.73 Synthesis of 1-(2,4-dichlorophenyl)-3-(3-pyridinyl)-1-propanone methyloxime (212)

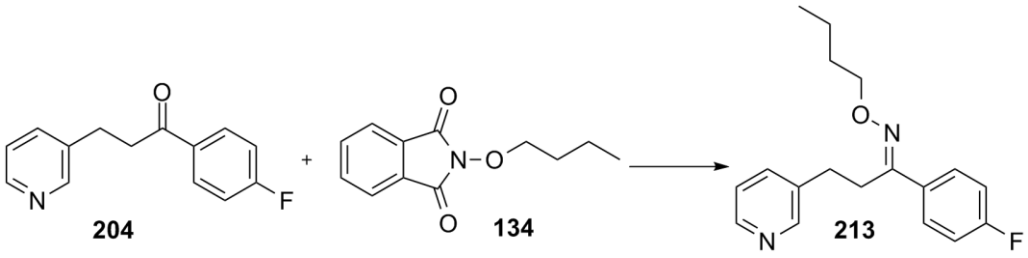


1-(2,4-Dichlorophenyl)-3-(3-pyridinyl)-1-propanone **209** (0.346 g, 1.24 mmol), *O*-methylhydroxylamine hydrochloride (0.114 g, 1.36 mmol) and sodium acetate (0.173 g, 2.11 mmol) were stirred in ethanol (4 mL) at room temperature for 3 days. The reaction mixture was poured into a solution of sodium hydrogen carbonate (1 M, 10 mL) and the aqueous layer was extracted with ethyl acetate (3 × 10 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The resulting oil was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits with 1% triethylamine afforded the two isomers (0.193 g, 51%, *E*:*Z* 2.9:1).

E isomer (colourless oil, 0.144 g, 38%) HRMS *m/z* Calcd for $C_{15}H_{15}Cl_2N_2O$ requires 309.0561, found 309.0559; ^{1}H NMR ($CDCl_3$) δ 2.73−2.79 (2H, m), 3.00−3.06 (2H, m), 3.96 (3H, s), 7.03 (1H, d, *J* = 8.2 Hz), 7.15−7.22 (2H, m), 7.40 (1H, d, *J* = 1.9 Hz), 7.43−7.48 (1H, m), 8.38 (1H, s), 8.43 (1H, d, *J* = 4.1 Hz); ^{13}C NMR ($CDCl_3$) δ 28.8 (CH_2), 30.5 (CH_2), 62.2 (CH_3), 123.5 (CH), 127.3 (CH), 129.8 (CH), 131.6 (CH), 133.6 (C), 134.0 (C), 135.5 (C), 136.2 (CH), 136.5 (C), 147.3 (CH), 149.3 (CH), 157.3 (C); IR 2936, 2818, 1587, 1552, 1473, 1043 cm^{-1}.

Z isomer (colourless oil, 0.049 g, 13%) HRMS *m/z* Calcd for $C_{15}H_{15}Cl_2N_2O$ requires 309.0561, found 309.0554; ^{1}H NMR ($CDCl_3$) δ 2.76−2.84 (2H, m), 2.86−2.94 (2H, m), 3.83 (3H, s), 7.01 (1H, d, *J* = 8.2 Hz), 7.20−7.25 (2H, m), 7.26−7.31 (1H, m), 7.55 (1H, d, *J* = 7.8 Hz), 8.47 (2H, br. s); ^{13}C NMR ($CDCl_3$) δ 29.4 (CH_2), 36.0 (CH_2), 62.2 (CH_3), 127.3 (CH), 128.3 (CH), 129.5 (CH), 129.8 (CH), 132.3 (C), 132.8 (C), 135.2 (C), 136.8 (CH), 137.2 (C), 146.9 (CH), 149.2 (CH), 153.7 (C); IR 2935, 1630, 1585, 1554, 1470, 1055, 814 cm^{-1}.

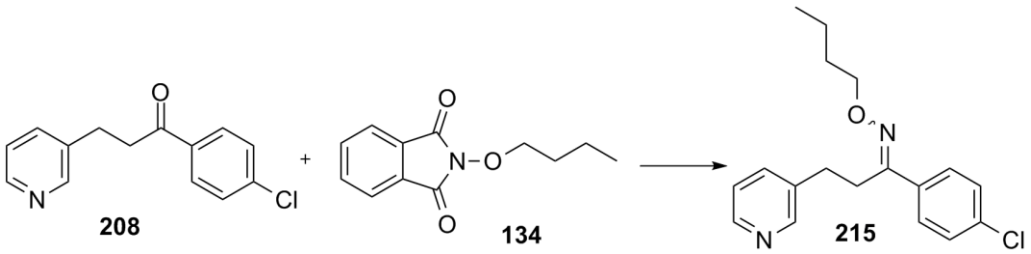
10.2.74 Synthesis of 1-(4-fluorophenyl)-3-(3-pyridinyl)-1-propanone butyloxime (213)



Hydrazine hydrate (117 μL, 0.120 g, 3.76 mmol) was added to a solution of *N*-butoxyphthalimide **134** (0.525 g, 2.39 mmol) in ethanol (7 mL) and the reaction mixture was heated under reflux for 2.5 hours. The reaction mixture was allowed to cool to room temperature before 1-(4-fluorophenyl)-3-(3-pyridinyl)-1-propanone **204** (0.492 g, 2.15 mmol) in ethanol (1 mL) and acetic acid (0.270 mL) were added. The mixture was then heated under reflux for 3 days. The reaction mixture was poured into a solution of sodium hydrogen carbonate (1 M, 10 mL) and extracted with dichloromethane (5 × 10 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The resulting residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits with 1% triethylamine afforded the *E* isomer as a colourless oil (0.061 g, 9%): HRMS *m/z* Calcd for $C_{18}H_{22}FN_2O$ requires 301.1716, found 301.1702; ^{1}H NMR ($CDCl_3$) δ 0.95 (3H, t, *J* = 7.4 Hz), 1.35−1.47 (2H, m), 1.62−1.71 (2H, m), 2.80−2.87 (2H, m), 2.97−3.05 (2H, m), 4.14 (2H, t, *J* = 6.7 Hz), 6.99−7.07 (2H, m), 7.18 (1H, dd, *J* = 7.6, 4.8 Hz), 7.48−7.50 (1H, m), 7.52−7.58 (2H, m), 8.45 (2H, br. s); ^{13}C NMR ($CDCl_3$) δ 14.0 (CH_3), 19.4 (CH_2), 28.3 (CH_2), 29.7 (CH_2), 31.4 (CH_2), 74.3 (CH_2), 115.6 (CH, d, *J* = 21.2 Hz), 123.6 (CH), 128.0 (CH, d, *J* = 8.1 Hz), 131.7

(C, d, J = 3.4 Hz), 136.8 (CH), 137.0 (C) 146.9 (CH), 149.1 (CH), 155.5 (C), 163.3 (C, d, J = 250.1 Hz); IR 2958, 2932, 2872, 1734, 1602, 1576, 1509, 1230 cm^{-1}.

10.2.75 Synthesis of 1-(4-chlorophenyl)-3-(3-pyridinyl)-1-propanone butyl oxime (215)

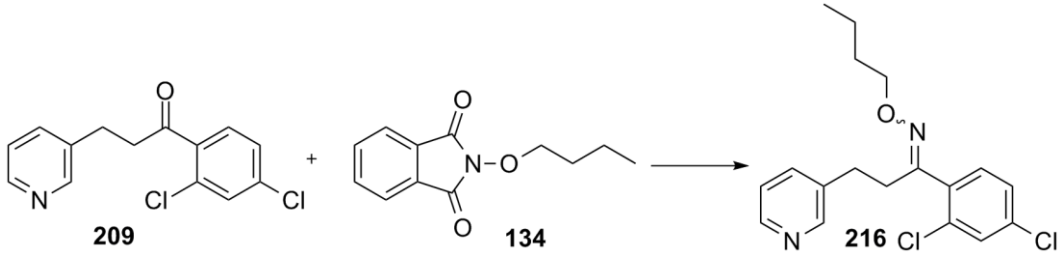


Hydrazine hydrate (117 µL, 0.120 g, 3.76 mmol) was added to a solution of *N*-butoxyphthalimide **134** (0.525 g, 2.39 mmol) in ethanol (5 mL) and the reaction mixture was heated under reflux for 4.5 hours. The reaction mixture was allowed to cool to room temperature before 1-(4-chlorophenyl)-3-(3-pyridinyl)-1-propanone **208** (0.492 g, 2.15 mmol) in ethanol (1 mL) and acetic acid (0.270 mL) were added. The mixture was then heated under reflux for 3 days. The reaction mixture was poured into a solution of sodium hydrogen carbonate (1 M, 10 mL) and extracted with dichloromethane (5 × 10 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The resulting residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits afforded two isomers (0.102 g, 39%, *E*:*Z* 6:1).

E isomer (colourless oil, 0.065 g, 20%) HRMS *m/z* Calcd for $C_{18}H_{21}ClN_2O$ requires 317.1421, found 317.1421; ^{1}H NMR ($CDCl_3$) δ 0.96 (3H, t, J = 7.4 Hz), 1.35−1.48 (2H, m), 1.62−1.72 (2H, m), 2.80−2.89 (2H, m), 2.98−3.08 (2H, m), 4.12−4.19 (2H, m), 7.19 (1H, dd, J = 7.6, 5.0 Hz), 7.29−7.38 (2H, m), 7.47−7.54 (2H, m), 7.56−7.61 (1H, m), 8.45 (2H, br.s.); ^{13}C NMR ($CDCl_3$) δ 14.1 (CH_3), 19.4 (CH_2), 28.2 (CH_2), 29.7 (CH_2), 31.4 (CH_2), 74.4 (CH_2), 123.7 (CH), 126.3 (CH), 127.5 (CH), 128.9 (CH), 134.4 (C), 135.2 (C), 136.4 (CH), 149.4 (CH), 155.5 (C), 156.6 (C); IR 3029, 2958, 2931, 2871, 1601, 1575, 1027, 712 cm^{-1}.

Z isomer (colourless oil, selected peaks)* ^{1}H NMR ($CDCl_3$) δ 0.86−0.92 (3 H, m), 1.27−1.35 (2 H, m), 1.51−1.62 (2 H, m), 4.01 (2 H, t, *J* = 6.7 Hz).

10.2.76 Synthesis of 1-(2,4-dichlorophenyl)-3-(3-pyridinyl)-1-propanone butyloxime (216)



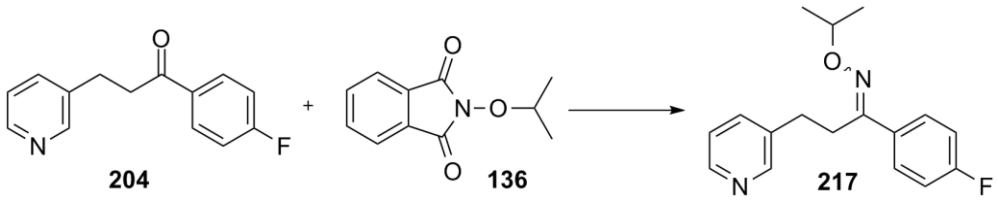
Hydrazine hydrate (117 µL, 0.120 g, 3.76 mmol) was added to a solution of *N*-butoxyphthalimide **134** (0.500 g, 2.28 mmol) in ethanol (5 mL) and the mixture was heated under reflux for 1 hour. The reaction mixture was allowed to cool to room temperature before 1-(2,4-dichlorophenyl)-3-(3-pyridinyl)-1-propanone **209** (0.250 g, 1.02 mmol) in ethanol (1 mL) and acetic acid (0.5 mL) were added. The reaction mixture was then heated under reflux for 3 days. The reaction mixture was poured into a solution of sodium hydrogen carbonate (1 M, 10 mL) and extracted with dichloromethane (5 × 10 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The resulting residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits afforded two isomers as an oil (0.028 g, 6%, *E*:*Z* 1.8:1).

E isomer (colourless oil, 0.008 g, 2%) HRMS *m/z* Calcd for $C_{18}H_{21}Cl_2N_2O$ requires 351.1031, found 351.1024; ^{1}H NMR ($CDCl_3$) δ 0.93−1.00 (3H, m), 1.38−1.48 (2H, m), 1.64−1.74 (2H, m), 2.77−2.83 (2H, m), 3.01−3.07 (2H, m), 4.17 (2H, t, *J* = 6.7 Hz), 7.04 (1H, d, *J* = 8.2 Hz), 7.21 (2H, dd, *J* = 8.2, 2.1 Hz), 7.41 (1H, d, *J* = 2.1 Hz), 7.48−7.53 (1H, m), 8.43 (2H, br. s); ^{13}C NMR ($CDCl_3$) δ 14.1 (CH_3), 19.4 (CH_2), 28.9 (CH_2), 30.8 (CH_2), 31.4 (CH_2), 74.5 (CH_2), 127.4 (CH), 128.9 (CH), 129.9 (CH), 131.7 (CH), 133.8 (C), 134.4 (C), 135.5 (C), 136.4 (CH), 136.7 (C), 147.3 (CH), 149.4 (CH), 156.9 (C); IR 2932, 2871, 1576, 1477, 1423, 1027, 908, 730 cm^{-1}.

* The *Z* isomer was not isolated. Peaks were assigned based on the integration in the ^{1}H NMR spectrum

Z isomer (colourless oil, selected peaks)* HRMS *m/z* Calcd for $C_{18}H_{21}Cl_2N_2O$ requires 351.1031, found 351.1025; ^{1}H NMR ($CDCl_3$) δ 0.89 (3H, t, *J* = 7.2 Hz), 1.26−1.36 (2H, m), 1.50−1.62 (2H, m), 4.01 (2H, t, *J* = 6.7 Hz).

10.2.77 Synthesis of 1-(4-fluorophenyl)-3-(3-pyridinyl)-1-propanone isopropyloxime (217)



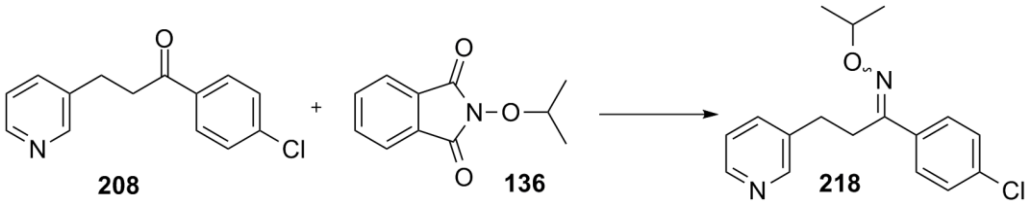
Hydrazine hydrate (135 µL, 0.159 g, 4.33 mmol) was added to a solution of *N*-isopropoxyphthalimide **136** (0.506 g, 2.47 mmol) in ethanol (5 mL) and the reaction mixture was heated under reflux for 5 hours. The reaction mixture was allowed to cool to room temperature before 1-(4-fluorophenyl)-3-(3-pyridinyl)-1-propanone **204** (0.410 g, 1.79 mmol) in ethanol (1 mL) and acetic acid (0.5 mL) were added. The mixture was then heated under reflux for 5 days. The reaction mixture was poured into a solution of sodium hydrogen carbonate (1 M, 10 mL) and extracted with dichloromethane (5 × 10 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The resulting residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits afforded two isomers (0.331 g, 32%, *E*:*Z* 7.4:1).

E isomer (yellow oil, 0.141 g, 28%) HRMS *m/z* Calcd for $C_{17}H_{20}FN_2O$ requires 287.1560, found 287.1554; ^{1}H NMR ($CDCl_3$) δ 1.24 (6H, d, *J* = 6.2 Hz), 2.78−2.86 (2H, m), 2.96−3.03 (2H, m), 4.38 (1H, sept, *J* = 6.2 Hz), 6.98−7.05 (2H, m), 7.16 (1H, dd, *J* = 7.8, 4.8 Hz), 7.47−7.49 (1H, m), 7.52−7.58 (2H, m), 8.43 (2H, br. s); ^{13}C NMR ($CDCl_3$) δ 21.8 (CH_3), 28.2 (CH_2), 29.7 (CH_2), 75.9 (CH), 115.6 (CH, d, *J* = 21.5 Hz), 123.5 (CH), 128.0 (CH, d, *J* = 8.5 Hz), 131.9 (C, d, *J* = 3.4 Hz), 136.3 (CH), 136.8 (C), 147.3 (CH), 149.6 (CH), 155.0 (C), 163.2 (C, d, *J* = 248.7 Hz); IR 2974, 2931, 1734, 1602, 1576, 1509, 1226, 960 cm^{-1}.

* The *Z* isomer was not isolated. Peaks were assigned based on the integration in the ^{1}H NMR spectrum

Z isomer (yellow oil, 0.019 g, 4%) ^{1}H NMR ($CDCl_3$) δ 1.18 (6H, d, *J* = 6.3 Hz), 2.73−2.82 (2H, m), 2.91−2.99 (2H, m), 4.32 (1H, sept, *J* = 6.2 Hz), 6.92−7.00 (2H, m), 7.13 (1H, dd, *J* = 7.8, 4.7 Hz), 7.44 (1H, d, *J* = 7.8 Hz), 7.46−7.52 (2H, m), 8.39 (2H, br. s); ^{13}C NMR ($CDCl_3$) δ 21.7 (CH_3), 28.1 (CH_2), 29.6 (CH_2), 75.8 (CH), 115.6 (CH, d, *J* = 21.5 Hz), 123.4 (CH), 128.0 (CH, d, *J* = 8.1 Hz), 131.9 (C, d, *J* = 8.1 Hz), 136.2 (CH), 136.8 (C), 147.2 (CH), 149.5 (CH), 154.9 (C), 163.5 (C, d, *J* = 248.7 Hz); IR 2974, 2931, 1734, 1602, 1576, 1509, 1227, 960 cm^{-1}.

10.2.78 Synthesis of 1-(4-chlorophenyl)-3-(3-pyridinyl)-1-propanone isopropyloxime (218)

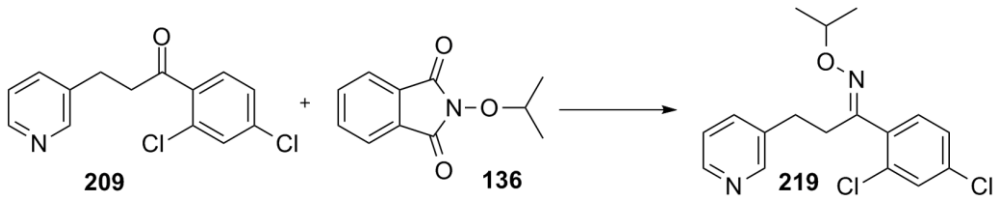


Hydrazine hydrate (117 µL, 0.120 g, 3.76 mmol) was added to a solution of *N*-isopropoxyphthalimide **136** (0.500 g, 2.28 mmol) in ethanol (5 mL) and the reaction mixture was heated under reflux for 1 hour. The reaction mixture was cooled to room temperature before 1-(4-chlorophenyl)-3-(3-pyridinyl)-1-propanone **208** (0.250 g, 1.02 mmol) in ethanol (1 mL) and acetic acid (0.5 mL) were added. The reaction mixture was then heated under reflux for 3 days. The reaction mixture was poured into a solution of sodium hydrogen carbonate (1 M, 10 mL) and extracted with dichloromethane (5 × 10 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The resulting residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits afforded the two isomers (0.033g, 10%, 3:1).

E isomer (colourless oil, 0.029 g, 7%) HRMS *m/z* Calcd for $C_{17}H_{20}ClN_2O$ requires 303.1264, found 303.1267; ^{1}H NMR ($CDCl_3$) δ 1.26 (6H, d, *J* = 6.3 Hz), 2.84−2.90 (2H, m), 2.97−3.08 (2H, m), 4.40 (1H, sept, *J* = 6.1 Hz), 7.16−7.23 (1H, m), 7.29−7.38 (2H, m), 7.47−7.55 (2H, m), 7.57−7.62 (1H, m), 8.45 (2H, br. s); ^{13}C NMR ($CDCl_3$) δ 21.8 (CH_3), 28.1 (CH_2), 29.7 (CH_2), 76.1 (CH), 126.3 (CH), 127.5 (CH), 128.8 (CH), 134.4 (C), 135.0 (C), 136.2 (CH), 147.6 (CH), 149.8 (CH), 154.9 (C), 156.0 (C); IR 3029, 2973, 2928, 1601, 1575, 960 cm^{-1}.

Z isomer (colourless oil, selected peaks)* ^{1}H NMR ($CDCl_3$) 1.13−1.18 (6H, m), 4.12 (1H, sept, *J* = 7.2 Hz).

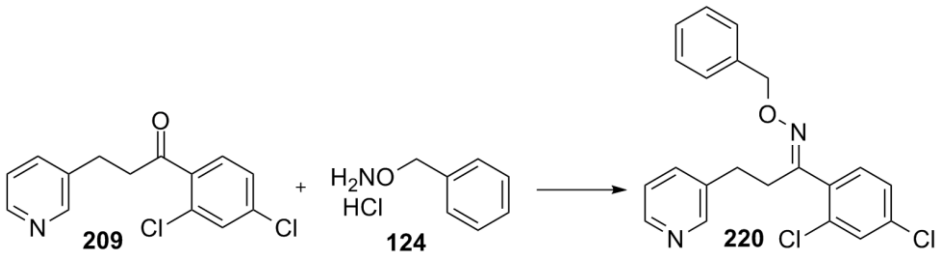
10.2.79 Synthesis of 1-(2,4-dichlorophenyl)-3-(3-pyridinyl)-1-propanone isopropyloxime (219)



Hydrazine hydrate (135 µL, 0.159 g, 4.33 mmol) was added to a solution of *N*-isopropoxyphthalimide **136** (0.103 g, 0.75 mmol) in ethanol (5 mL) and the reaction mixture was heated under reflux for 2 hours. The reaction mixture was cooled to room temperature before 1-(2,4-dichlorophenyl)-3-(3-pyridinyl)-1-propanone **209** (0.204 g, 0.73 mmol) in ethanol (1 mL) and acetic acid (0.5 mL) were added. The reaction mixture was then heated under reflux for 3 days. The reaction mixture was poured into a solution of sodium hydrogen carbonate (1 M, 10 mL) and extracted with dichloromethane (5 × 10 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The resulting residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits afforded the *E* isomer as a yellow oil (0.006 g, 3%): ^{1}H NMR ($CDCl_3$) δ 1.29 (6H, d, *J* = 6.2 Hz), 2.84−2.91 (2H, m), 3.02−3.10 (2H, m), 4.42 (1H, sept, *J* = 5.9 Hz), 7.07 (1H, d, *J* = 8.2 Hz), 7.23 (1H, dd, *J* = 8.2, 2.1 Hz), 7.42 (1H, d, *J* = 2.1 Hz), 7.70 (1H, d, *J* = 7.8 Hz), 8.32−8.68 (2H, m); ^{13}C NMR ($CDCl_3$) δ 21.9 (CH_3), 28.9 (CH_2), 29.9 (CH_2), 76.4 (CH), 127.4 (CH), 130.0 (CH), 130.0 (CH), 131.7 (CH), 134.4 (C), 135.5 (C), 138.8 (CH), 155.8 (C); IR 2926, 1719, 1588, 961, 906, 727 cm^{-1}.

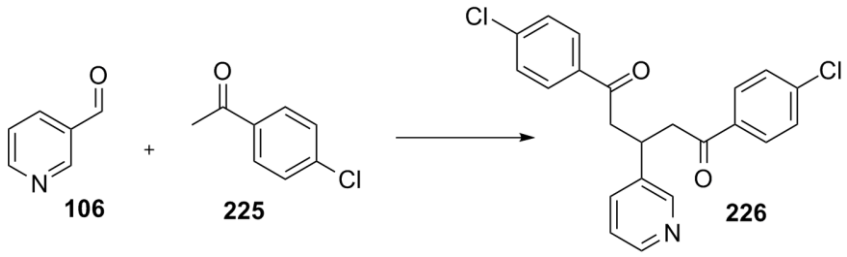
* The Z isomer was not isolated. Peaks were assigned based on the integration in the ^{1}H NMR spectrum

10.2.80 Synthesis of 1-(2,4-dichlorophenyl)-3-(3-pyridinyl)-1-propanone *O*-benzyloxime (220)



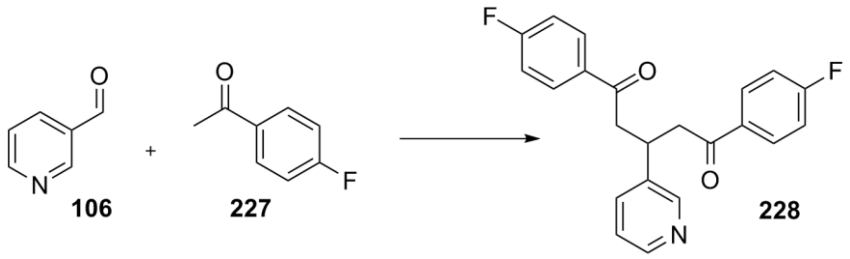
1-(2,4-Dichlorophenyl)-3-(3-pyridinyl)-1-propanone **209** (0.261 g, 0.932 mmol), *O*-benzylhydroxylamine hydrochloride **124** (0.150 g, 0.94 mmol) and sodium acetate (0.110 g, 1.34 mmol) were stirred in ethanol (5 mL) at room temperature for 4 days. The reaction mixture was concentrated under reduced pressure. The residue was washed with a solution of sodium hydrogen carbonate (1 M, 10 mL) and extracted with ethyl acetate (3 × 10 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The resulting oil was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits with 1% triethylamine afforded one isomer as a colourless oil (0.087 g, 31%). HRMS *m/z* Calcd for $C_{21}H_{19}Cl_2N_2O$ requires 385.0874, found 385.0874; ^{1}H NMR ($CDCl_3$) δ 2.74−2.81 (2H, m), 3.04−3.11 (2H, m), 5.22 (2H, s), 6.98 (1H, d, *J* = 8.2 Hz), 7.13 (1H, dd, *J* = 7.8, 4.84 Hz), 7.19 (1H, dd, *J* = 8.2, 2.1 Hz), 7.32−7.42 (7H, m), 8.33 (1H, br. s), 8.42 (1H, d, *J* = 3.7 Hz); ^{13}C NMR ($CDCl_3$) δ 28.7 (CH_2), 30.7 (CH_2), 76.6 (CH_2), 123.5 (CH), 127.2 (CH), 128.1 (CH), 128.2 (CH), 128.5 (CH), 129.8 (CH), 131.6 (CH), 133.6 (C), 134.0 (C), 135.5 (C), 136.5 (CH), 136.7(C), 137.4 (C), 147.0 (CH), 149.0 (CH), 157.7 (C); IR 3030, 2928, 2864, 1587, 1551, 1473, 1024, 696 cm^{-1}.

10.2.81 Synthesis of 1,5-bis(4-chlorophenyl)-3-(3-pyridinyl)-1,5-pentanedione (226)



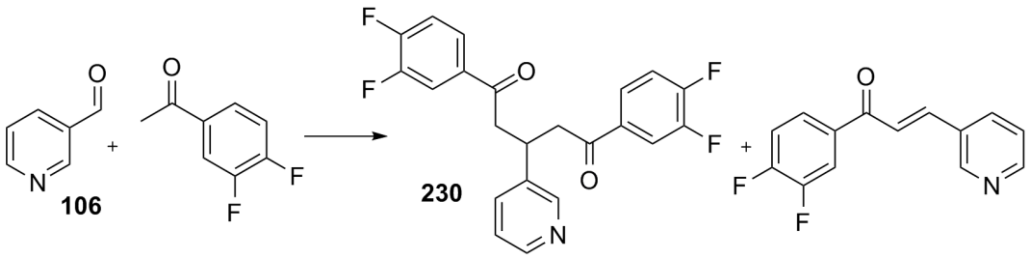
A solution of sodium hydroxide (0.198 g, 4.95 mmol) in water (7.5 mL) and ethanol (7.5 mL) was added to 3-pyridinecarboxaldehyde **106** (0.465 g, 4.34 mmol) and 4′-chloroacetophenone **225** in ethanol (15 mL). The reaction mixture was left to stand for 10 minutes before water (400 mL) was added until the precipitate had formed. The solid was collected via vacuum filtration. The solid was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits afforded the 1, 5-bis(4-chlorophenyl)-3-(3-pyridinyl)-1,5-pentanedione **226** as a pale yellow solid (0.712 g, 29%): HRMS *m/z* Calcd for $C_{22}H_{18}Cl_2NO_2$ requires 398.0715, found 398.0702; ^{1}H NMR ($CDCl_3$) δ 3.35 (2H, dd, *J* = 10.0, 6.9 Hz), 3.51 (2H, dd, *J* = 10.0, 6.9 Hz), 4.06 (1H, quin, *J* = 6.9 Hz), 7.22 (1H, dd, *J* = 7.1, 4.9 Hz), 7.42 (4H, d, *J* = 8.5 Hz), 7.65 (1H, d, *J* = 7.9 Hz), 7.87 (4H, d, *J* = 8.5 Hz), 8.45 (1H, br. s), 8.55 (1H, br. s); ^{13}C NMR ($CDCl_3$) 34.6 (CH), 44.3 (CH_2), 129.1 (CH), 129.2 (CH), 129.6 (CH), 135.0 (C), 136.1 (CH), 140.0 (C), 147.8 (CH), 148.7 (CH), 171.2 (C), 196.7 (C); IR 3035, 1879, 1679, 1588, 1266, 1090 cm^{-1}.

10.2.82 Synthesis of 1,5-bis(4-fluorophenyl)-3-(3-pyridinyl)-1,5-pentanedione (228)



A solution of sodium hydroxide (0.200 g, 5.00 mmol) in water (7.5 mL) and ethanol (7.5 mL) was added 3-pyridinecarboxaldehyde **106** (0.449 g, 4.19 mmol) and 4′-fluoroacetophenone **227** (1.158 g, 8.38 mmol) in ethanol (15 mL). The mixture was stirred for 30 minutes before water (200 mL) was added until the solution was opaque. The mixture was left to stand at room temperature overnight. The solid was collected via vacuum filtration. The solid was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits afforded 1,5-bis(4-fluorophenyl)-3-(3-pyridinyl)-1,5-pentanedione **228** as a pale yellow solid (0.072 g, 4.7%): HRMS *m/z* Calcd for $C_{22}H_{18}F_2NO_2$ requires 366.1306, found 366.1292; ^{1}H NMR ($CDCl_3$) δ 3.37 (2H, dd, *J* = 10.0, 6.9 Hz), 3.54 (2H, dd, *J* = 10.0, 6.9 Hz), 4.10 (1H, quin, *J* = 6.9 Hz), 7.09−7.17 (4H, m), 7.26 (1H, dd, *J* = 7.6, 4.8 Hz), 7.70−7.72 (1H, m), 7.93−8.01 (4H, m), 8.46 (1H, br. s), 8.60 (1H, br. s); ^{13}C NMR ($CDCl_3$) δ 34.5 (CH), 44.2 (CH_2), 115.8 (CH, d, *J* = 21.5 Hz), 123.6 (CH), 130.8 (CH, d, *J* = 9.4 Hz), 133.0 (C, d, *J* = 3.0 Hz), 135.8 (CH), 139.3 (C), 147.8 (CH), 148.8 (CH), 165.88 (C, d, *J* = 255.5 Hz), 196.2 (C); IR 2923, 1685, 1608, 1514, 1278, 1107, 766, 714 cm^{-1}.

10.2.83 Synthesis of 1,5-bis(3,4-difluorophenyl)-3-(3-pyridinyl)-1,5-pentanedione (230)

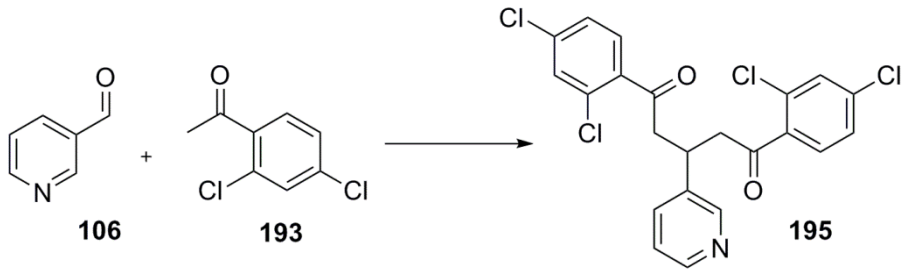


A solution of sodium hydroxide (0.200 g, 5.00 mmol) in water (3 mL) and ethanol (3 mL) was added to 3-pyridinecarboxaldeyde **106** (0.23 mL, 0.260 g, 2.43 mmol) and 3′,4′-difluoroacetophenone (0.772 g, 4.94 mmol) in ethanol (8 mL). The reaction mixture was stirred for 5 minutes before water (150 mL) was added until a solid precipitated out. The mixture was left to stand at room temperature for 30 minutes. The solid was collected via vacuum filtration. The solid was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits afforded 1,5-bis(3,4-difluoroophenyl)-3-(3-pyridinyl)- 1,5-pentanedione **230** (0.073 g 7.5%) and 1-(3,4-fluorophenyl)-3-(3-pyridinyl)-2-propen-1-one (0.061 g, 10%).

1-(3,4-fluorophenyl)-3-(3-pyridinyl)-2-propen-1-one (pale yellow solid) HRMS *m/z* Calcd for $C_{14}H_{10}F_2NO$ requires 246.0730, found 246.0721; ^{1}H NMR ($CDCl_3$) δ 7.28−7.31 (1H, m), 7.37 (1H, dd, *J* = 8.0, 4.7 Hz), 7.52 (1H, d, *J* = 15.7 Hz), 7.76−7.90 (3H, m), 7.93−7.96 (1H, m), 8.64 (1H, d, *J* = 4.1 Hz), 8.85 (1H, s); ^{13}C NMR ($CDCl_3$) δ 117.7 (CH, d, *J* = 17.8 Hz), 117.9 (CH, dd, *J* = 17.8, 1.7 Hz), 122.7 (CH), 123.8 (CH), 125.5 (CH, dd, *J* = 7.4, 3.7), 130.4 (C), 134.7 (CH), 134.8 (C), 141.9 (CH), 150.0 (CH), 150.5 (C, dd, *J* = 238.3, 13.1 Hz), 151.4 (CH), 153.7 (C, dd, *J* = 245.7, 11.8 Hz), 187.0 (C); IR 3047, 2924, 1661, 1539, 799 cm^{-1}.

1,5-bis(3,4-difluorophenyl)-3-(3-pyridinyl)-1,5-pentanedione **230** (pale yellow solid) ^{1}H NMR ($CDCl_3$) δ 3.26−3.37 (2H, m), 3.44−3.53 (2H, m), 4.04 (1H, quin, *J* = 6.9 Hz), 7.19−7.28 (3H, m), 7.62−7.65 (1H, m), 7.69−7.79 (4H, m), 8.44 (1H, dd, *J* = 4.8, 1.5 Hz), 8.54 (1H, d, *J* = 2.0 Hz); ^{13}C NMR ($CDCl_3$) δ 34.4 (CH), 44.0 (CH_2), 117.4 (CH, dd, *J* = 17.8, 1.7 Hz), 117.6 (CH, d, *J* = 17.8 Hz), 123.6 (CH), 125.1 (CH, dd, *J* = 7.6, 3.5 Hz), 133.6 (C, dd, *J* = 3.9, 3.9 Hz), 135.3 (CH), 138.7 (C), 148.4 (CH), 149.1 (CH), 150.5 (C, dd, *J* = 238.6, 12.8 Hz), 153.8 (C, dd, *J* = 245.0, 12.5 Hz), 195.2 (C); IR 2924, 1691, 1677, 1513, 1274, 1123 cm^{-1}.

10.2.84 Synthesis of 1,5-bis(2,4-dichlorophenyl)-3-(3-pyridinyl)-1,5-pentanedione (195)



3-Pyridinecarboxaldehyde **106** (0.18 mL, 0.205 g, 1.92 mmol), 2′,4′-dichloroacetophenone **193** (0.67 mL, 0.884 g, 4.68 mmol) and sodium hydroxide (0.233 g, 3.82 mmol) in methanol (1 mL) were ground together for 10 minutes at room temperature. The mixture was dissolved in dichloromethane (20 mL) and was washed with water (2 × 20 mL) and brine (20 mL). The organic layer was dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was subjected to flash chromatography. Elution with 50% ethyl acetate in petroleum spirits afforded the 1,5-bis(2,4-dichlorophenyl)-3-(3-pyridinyl)-1,5-pentanedione **195** as a brown oil (0.240 g, 27%): HRMS *m/z* Calcd for $C_{22}H_{16}Cl_4NO_2$ requires 465.9935, found 465.9915; ^{1}H NMR ($CDCl_3$) δ 3.34 (2H, dd, *J* = 9.0, 7.8 Hz), 3.44 (2H, dd, *J* = 9.0, 7.8 Hz), 3.96 (1H, quin, *J* = 7.8 Hz), 7.17 (1H, dd, *J* = 7.8, 4.7 Hz), 7.20−7.28 (4H, m), 7.37 (2H, d, *J* = 1.7 Hz), 7.55−7.58 (1H, m), 8.42 (1H, d, *J* = 3.8 Hz), 8.47 (1H, br. s); ^{13}C NMR ($CDCl_3$) δ 38.4 (CH), 48.3 (CH_2), 123.5 (CH), 127.5 (CH), 130.2 (CH), 130.6 (CH), 132.1 (C), 135.4 (CH), 137.1 (C), 137.8 (C), 138.2 (C), 148.5 (CH), 149.4 (CH), 199.53 (C); IR 3086, 1693, 1580, 1371, 817 cm^{-1}.

10.3 Biology

The inhibition of *T. cruzi* in an in vitro whole cell parasite assay was carried out by the School of Veterinary and Biomedical Science, Murdoch University in accordance to the procedure described by Buckner *et al*.[159] and in the literature.[54,56]

10.3.1 *In vitro T. cruzi assay for the determination of IC_{50}*

L6 cells were plated into 96-well, flat bottom tissue culture plates and incubated at 37 °C in 5% CO_2 for 24 hours to allow cells to adhere. *T. cruzi* trypomastigotes (Tulahuèn strain expressing the β-galactosidase gene) were then added at a multiplicity of infection of 3 and plates were incubated for a further 48 hours. Extracellular trypomastigotes were then removed and compounds were added in 7 point serial dilutions performed in triplicate. Benznidazole (Epichem Pty Ltd) was included as a control. After 96 hours of incubation with the compounds, the colorimetric agent CPRG was added. Absorbance was read on a Dynex microplate reader at 530 nm. The % inhibition was calculated by the following equation:

$$\%\text{Inhibition} = 100 - \frac{(T.cruzi \text{ with compound} - \text{compound only})}{(T.cruzi \text{ only} - \text{media only})} \times 100$$

For each compound, % inhibition values were used to generate a standard curve from which the IC_{50} was calculated. Each assay was performed twice and the average was used.

10.3.2 *In vitro* assay for determination of cytotoxicity

L6 cells were plated into 96-well flat bottom tissue culture plates and exposed to compounds for 72 hours in total. Compounds were initially screened at 100 μM, and those that showed toxicity were carried through to a dose response curve analysis to determine an IC_{50} value. Podophylotoxin was used as a control. Alamar Blue® (AbD Serotec) was added to the plates and absorbance was read on a Dynex microplate

reader at 570 nm and 630 nm. Each assay was performed twice and an average was used to determine the IC_{50}.

10.4 Crystallography

The crystal data for compound **149** are summarized in Appendix with the structure depicted in Figure 42 where ellipsoids have been drawn at the 50% probability level.

The crystallography was carried out by Professor Brian Skelton at the University of Western Australia. Crystallographic data for the structure were collected at 100(2) K on an Oxford Diffraction Xcalibur diffractometer fitted with Mo Kα radiation. Following multi-scan absorption corrections and solution by direct methods, the structure was refined against F^2 with full-matrix least-squares using the program SHELXL-97.[160] All hydrogen atoms were added at calculated positions and refined by use of a riding model with isotropic displacement parameters based on those of the parent atoms. Anisotropic displacement parameters were employed throughout for the non-hydrogen atoms.

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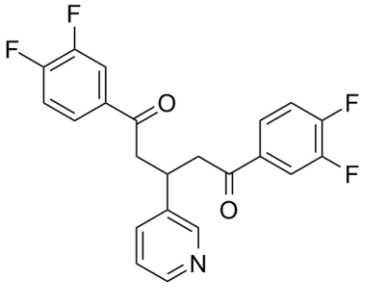
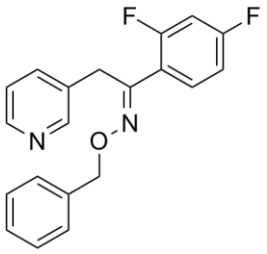
Appendix A – Biological Results

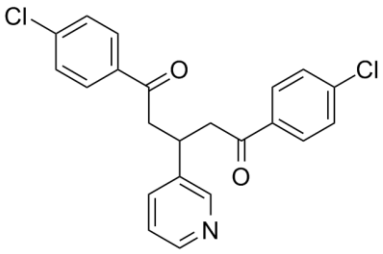
All compounds tested against *T. cruzi* in order of biological activity



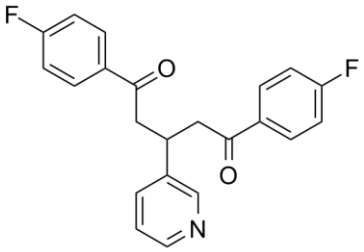
230
IC_{50} 0.011 μM
(toxicity 53.35 μM)



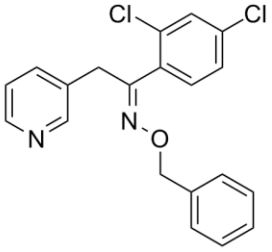
153
***E* isomer**
IC_{50} 0.021 μM
(toxicity 45.65 μM)



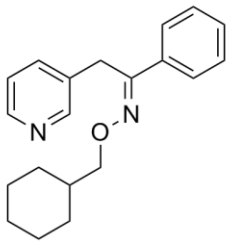
226
IC_{50} 0.022 μM
(toxicity 40.35 μM)



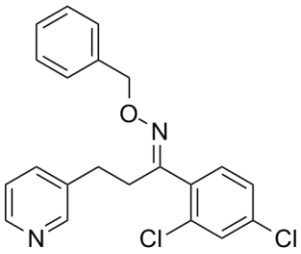
228
IC_{50} 0.029 μM
(toxicity 67.22 μM)



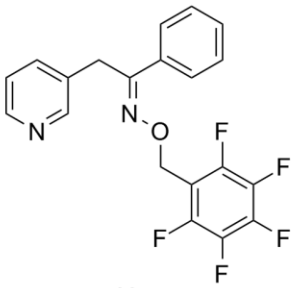
152
***Z* isomer**
IC_{50} 0.20 μM
(toxicity 46.89 μM)



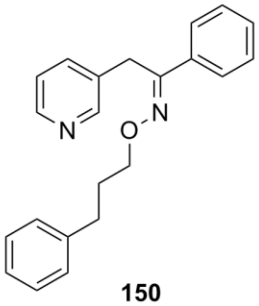
149
***E* isomer**
IC_{50} 0.22 μM
(toxicity 61.10 μM)



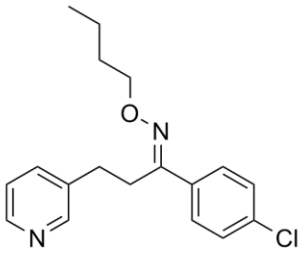
220
***E* isomer**
IC_{50} 0.24 μM
(toxicity >100 μM)



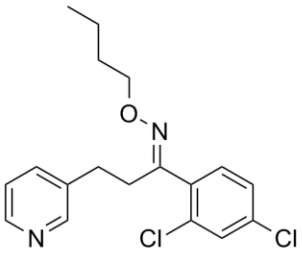
127
Mixture *E*/*Z* isomer
IC_{50} 0.37 μM
(toxicity 64.06 μM)



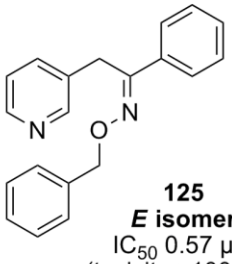
150
***E* isomer**
IC_{50} 0.48 μM
(toxicity 35.08 μM)



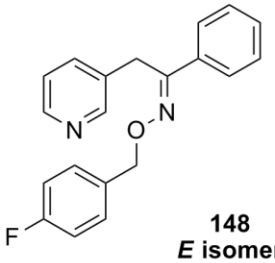
215
***E* isomer**
IC_{50} 0.49 μM
(toxicity >100 μM)



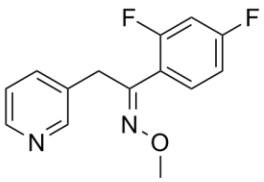
216
***E* isomer**
IC_{50} 0.55 μM
(toxicity 83.52 μM)



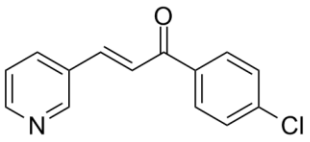
125
***E* isomer**
IC_{50} 0.57 μM
(toxicity >100 μM)



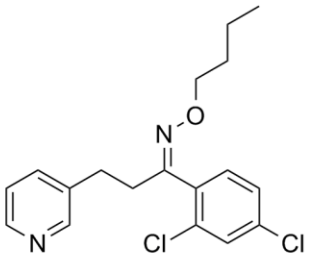
148
***E* isomer**
IC_{50} 0.66 μM
(toxicity 34.22 μM)



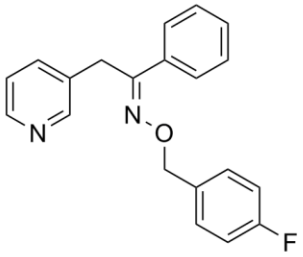
119
***E* isomer**
IC_{50} 0.67 μM
(toxicity >100 μM)



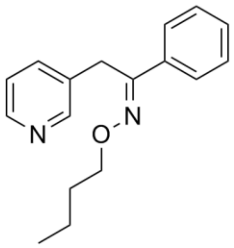
198
IC_{50} 0.69 μM
(toxicity 17.46 μM)



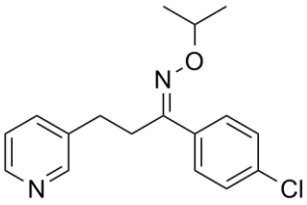
216
Mixture *E*/*Z* isomers
IC_{50} 0.90 μM
(toxicity 83.52 μM)



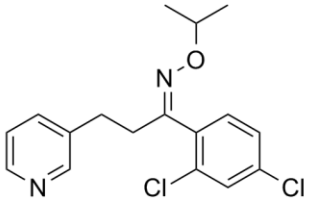
148
***Z* isomer**
IC_{50} 0.95 μM
(toxicity 34.21 μM)



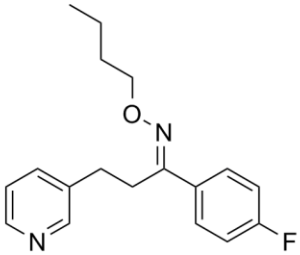
144
***E* isomer**
IC_{50} 1.04 μM
(toxicity >100 μM)



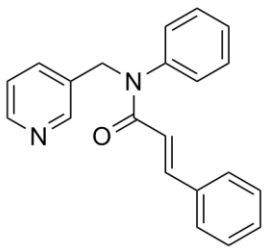
218
***Z* isomer**
IC_{50} 1.17 μM
(toxicity >100 μM)



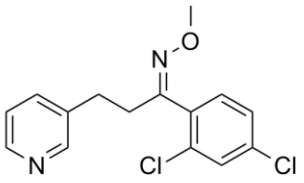
219
***Z* isomer**
IC_{50} 1.17 μM
(toxicity >100 μM)



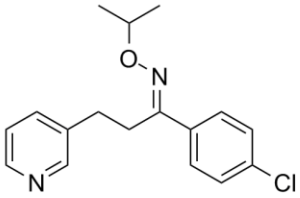
213
***E* isomer**
IC_{50} 1.23 μM
(toxicity 75.09 μM)



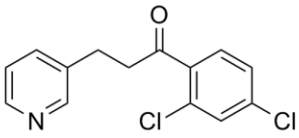
166
IC_{50} 1.29 μM
(toxicity >100 μM)



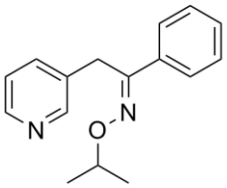
212
***Z* isomer**
IC_{50} 1.43 μM
(toxicity 77.43 μM)



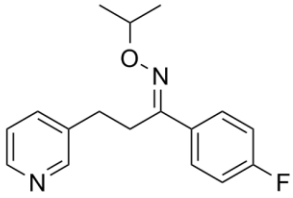
218
***E* isomer**
IC_{50} 1.45 μM
(toxicity >100 μM)



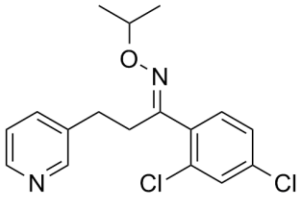
208
IC_{50} 1.54 μM
(toxicity >100 μM)



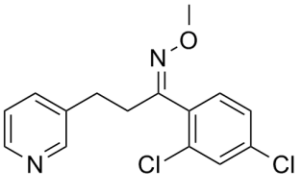
146
***E* isomer**
IC_{50} 1.55 μM
(toxicity >100 μM)



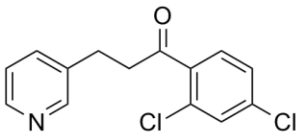
217
***E* isomer**
IC_{50} 1.57 μM
(toxicity >100 μM)



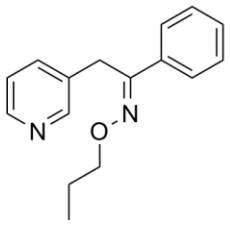
219
***E* isomer**
IC_{50} 1.82 μM
(toxicity >100 μM)



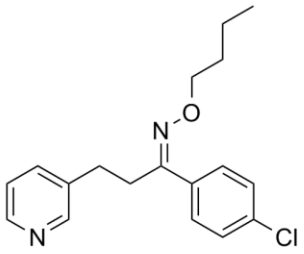
212
***E* isomer**
IC_{50} 1.87 μM
(toxicity >100 μM)



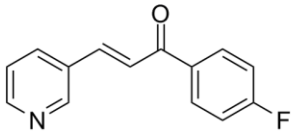
209
IC_{50} 1.88 μM
(toxicity 19.14 μM)



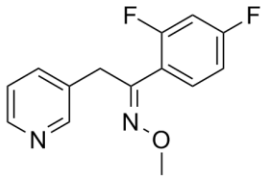
145
***E* isomer**
IC_{50} 2.00 μM
(toxicity >100 μM)



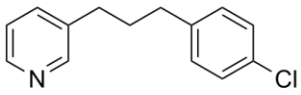
215
***Z* isomer**
IC_{50} 2.10 μM
(toxicity >100 μM)



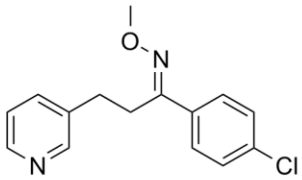
204
IC_{50} 2.12 μM
(toxicity 16.33 μM)



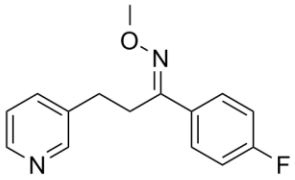
119
***Z* isomer**
IC_{50} 2.31 μM
(toxicity >100 μM)



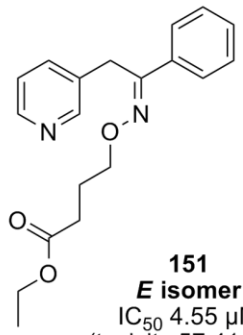
207
IC_{50} 2.77 μM
(toxicity >100 μM)



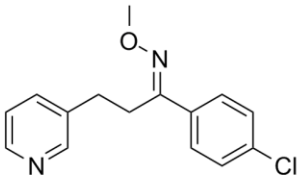
211
***E* isomer**
IC_{50} 3.18 μM
(toxicity >100 μM)



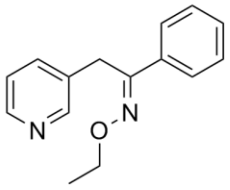
210
***Z* isomer**
IC_{50} 3.62 μM
(toxicity >100 μM)



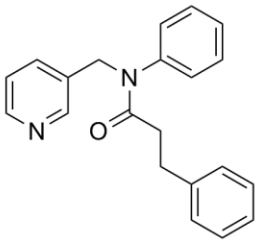
151
***E* isomer**
IC_{50} 4.55 μM
(toxicity 57.41 μM)



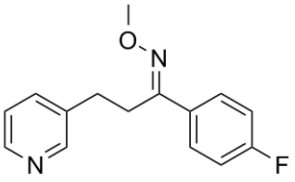
211
***Z* isomer**
IC_{50} 4.57 μM
(toxicity >100 μM)



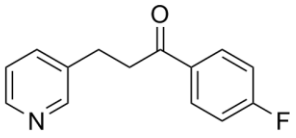
123
***E* isomer**
IC_{50} 4.91 μM
(toxicity >100 μM)



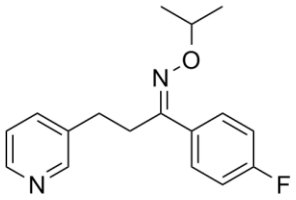
168
IC_{50} 1.29 μM
(toxicity >100 μM)



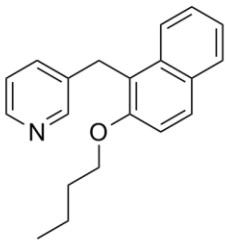
210
***E* isomer**
IC_{50} 6.01 μM
(toxicity >100 μM)



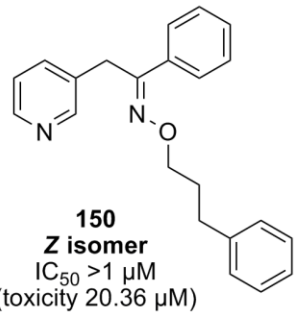
204
IC_{50} 6.20 μM
(toxicity >100 μM)



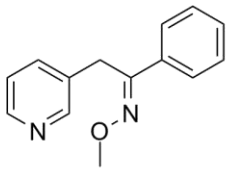
217
***Z* isomer**
IC_{50} 6.80 μM
(toxicity >100 μM)



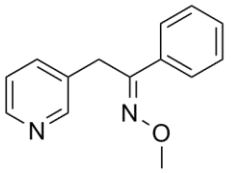
171
IC_{50} 6.981 μM
(toxicity >100 μM)



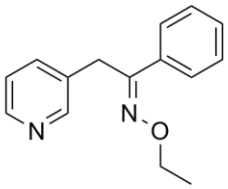
150
***Z* isomer**
IC_{50} >1 μM
(toxicity 20.36 μM)



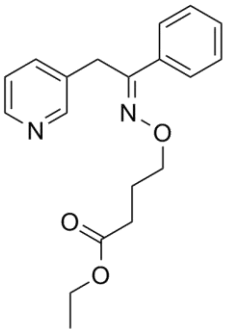
117
***E* isomer**
IC_{50} >10 µM
(toxicity >100 µM)



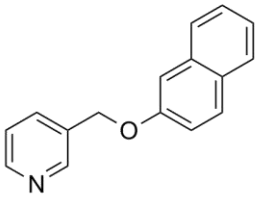
117
***Z* isomer**
IC_{50} >10 µM
(toxicity >100 µM)



123
Mixture *E*/*Z* isomers
IC_{50} >10 µM
(toxicity >100 µM)



100
***Z* isomer**
IC_{50} >10 µM
(toxicity >100 µM)



175
IC_{50} >10 µM
(toxicity >100 µM)

Appendix B – Crystal data and structure refinement information

Table 22. Crystal data and structure refinement for compound **149**

Empirical formula	$C_{28}H_{22}N_2O_2$
Formula weight	418.48
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1/c$
Unit cell dimensions	a = 11.6361(5) Å
	b = 5.9352(3) Å
	c = 15.2509(8) Å
	β = 102.444(5)°
Volume	1028.52(9) Å^3
Z	2
Density (calculated)	1.351 Mg/m^3
μ	0.086 mm^{-1}
Crystal size	0.50 × 0.19 × 0.16 mm^3
θ range for data collection	2.74 to 32.03°
Index ranges	−17<=h<=16, −8<=k<=8, −22<=l<=18
Reflections collected	6464
Independent reflections	3321 [R(int) = 0.0205]
Completeness to θ = 30.50°	99.9 %
Absorption correction	Semi-empirical from equivalents
Max./ min. transmission	0.9864/0.9585
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3321 / 0 / 145
Goodness-of-fit on F^2	1.029
Final R indices [I>2σ(I)]	R_1 = 0.0446, wR_2 = 0.1125
R indices (all data)	R_1 = 0.0526, wR_2 = 0.1186
Largest diff. peak and hole	0.416 and −0.215 e.Å^{-3}