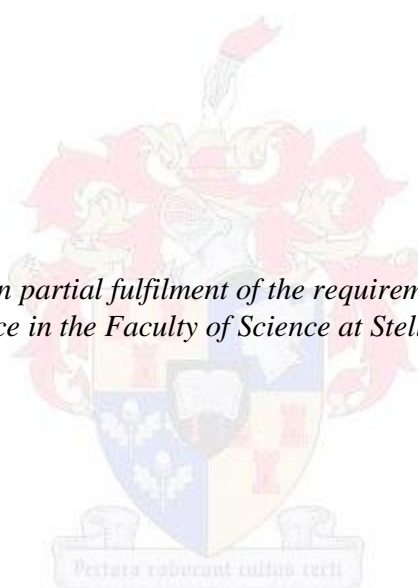


Synthesis of potentially irreversible Akt inhibitors

by
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of Master of Science in the Faculty of Science at Stellenbosch University*



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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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ABSTRACT

Cancer is a leading cause of disease worldwide, with an increasing number of cases reported annually. A member of the phosphatidylinositol 3-kinase-Akt (PI3K-Akt) pathway, Akt, has been found to be dysregulated in certain cancers, aiding in cancer development and progression. There are several known reversible inhibitors of this kinase, but there have been no irreversible inhibitors reported thus far. The aim of this project was therefore to design and synthesise irreversible inhibitors of Akt.

Two sets of potential inhibitors were designed, with the first based on the structure of the Type V reversible inhibitor, Akti-1/2. The structure of this inter-domain inhibitor was partly altered, keeping its piperidinyl benzimidazolone functionality intact and replacing the other half of the compound with reported heterocyclic inhibitors of Akt. The second set included the same heterocyclic moieties as the first set, with the piperidinyl benzimidazolone functionality replaced by a 1,2,3-triazole ring-containing structure.

Various synthetic routes were explored towards these two sets of compounds, with the key reaction towards the first set being the metal-catalysed synthesis of the required benzimidazolone functionality. Unfortunately, this reaction was found to be exceedingly difficult and no target compounds could be obtained.

The key reaction towards the second set of compounds was a click reaction, specifically a copper(I)-catalysed azide alkyne cycloaddition, enabling the convenient synthesis of the 1,4-disubstituted 1,2,3-triazole ring. This route proved more successful, with two target compounds successfully synthesised. These are *N*-[2-(1-{1-[4-(3-phenylimidazo[1,2-*a*]pyridin-2-yl)benzyl]piperidin-4-yl}-1*H*-1,2,3-triazol-4-yl)phenyl]acrylamide and *N*-[3-(1-{1-[4-(3-phenylimidazo[1,2-*a*]pyridin-2-yl)benzyl]piperidin-4-yl}-1*H*-1,2,3-triazol-4-yl)phenyl]acrylamide.

These target compounds were biochemically evaluated for activity against wild-type Akt using iFLiK and HTRF[®] KinEASE[™] assays. Encouragingly, both target compounds had activity against wild-type Akt1, with IC₅₀ values in the nanomolar range. Further evaluation, by LC-MS, revealed the latter compound to be an irreversible inhibitor of wild-type Akt1. This is the first reported irreversible inhibitor of Akt and is proof that Akt can be covalently modified by an appropriate inhibitor. This discovery has allowed for many possibilities for future research towards potent irreversible inhibitors of Akt.

UITTREKSEL

Kanker is een van die vernaamste oorsake van siekte ter wêreld, met 'n groeiende aantal gevalle wat jaarliks aangemeld word. Daar is bevind dat Akt, 'n lid van die fosfatidielinositol 3-kinase-Akt (PI3K-Akt) roete, gedisreguleer is in sekere vorme van kankers en daardeur kankerontwikkeling en – voortsetting bevorder. Daar is verskeie bekende omkeerbare onderdrukkers van hierdie kinase, maar daar is tot dusver nog geen onomkeerbare onderdrukkers gerapporteer nie. Die doel van hierdie projek was dus om onomkeerbare onderdrukkers van Akt te ontwerp en te sintetiseer.

Twee stelle potensiële onderdrukkers is ontwerp, waarvan die eerste stel gebaseer op die struktuur van die bekende Tipe V omkeerbare onderdrukker, Akti-1/2. Die struktuur van hierdie interdomein onderdrukker is deels verander, met die piperidinielbensimidasoloon funksionaliteit onaangeraak en die ander helfte daarvan vervang deur berigte heterosikliese onderdrukkers van Akt. Die tweede stel verbindings het dieselfde heterosikliese gedeeltes gehad, met die piperidinielbensimidasoloon funksionaliteit vervang deur 'n 1,2,3-triasoolring-bevattende raamwerk.

Verskeie sintetiese roetes is ondersoek ten einde hierdie twee stelle verbindings te sintetiseer, met die metaalgekataliseerde sintese van die vereiste bensimidasoloon funksionaliteit as die kernstap in die sintese van die eerste stel verbindings. Ongelukkig is hierdie reaksie as uiters moeilik bevind en geen teikenverbindings kon verkry word nie.

Die sleutelreaksie na die tweede stel verbindings was 'n kliekreaksie, spesifiek 'n koper(I)gekataliseerde asied-alkyn sikloaddisie, wat die gerieflike sintese van die 1,4-digesubstitueerde 1,2,3-triasool moontlik gemaak het. Hierdie roete was meer suksesvol as die eerste, met twee teikenverbindings suksesvol gesintetiseer. Hierdie verbindings is *N*-[2-(1-{1-[4-(3-fenielimidaso[1,2-*a*]piridin-2-iel)bensiel]piperidin-4-iel}-1*H*-1,2,3-triasol-4-iel)feniel]akriëlamied en *N*-[3-(1-{1-[4-(3-fenielimidaso[1,2-*a*]piridin-2-iel)bensiel]piperidin-4-iel}-1*H*-1,2,3-triasol-4-iel)feniel]akriëlamied.

Deur die gebruik van iFLiK en HTRF[®] KinEASE[™] assessering, is hierdie teikenverbindings biochemies geëvalueer vir aktiwiteit teenoor wilde-tipe Akt1. Beide teikenverbindings het aktiwiteit teenoor wilde-tipe Akt getoon, met IC₅₀ waardes in die nanomolaarruimte. Verdere evaluasie, met behulp van vloeistofchromatografie-massaspektrometrie, het daarop gewys dat die laasgenoemde verbinding 'n onomkeerbare onderdrukker van wilde-tipe Akt1 is. Dit is die eerste bekende onomkeerbare onderdrukker van Akt en bewys dat Akt wel kovalent gewysig kan word deur 'n geskikte onderdrukker. Hierdie resultaat maak die deur oop vir verskeie moontlikhede vir toekomstige navorsing met die oog op meer kragtige onderdrukkers van Akt.

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LIST OF COMMON ABBREVIATIONS

AcOH – Acetic acid

ADMET – Absorption into and Distribution in the body, Metabolism and Excretion from the body and its Toxicity to the body

ALL – Acute lymphoblastic leukaemia

ATP – Adenosine triphosphate

BCR-ABL – Breakpoint cluster region-Abelson protein kinase

Boc₂O – Di-*tert*-butyl dicarbonate

BTK – Bruton's tyrosine kinase

CC – Column chromatography

CCK4 – Colon carcinoma kinase 4

CDK – Cyclin dependent kinase

CML – Chronic myeloid leukaemia

CuAAC – Copper(I)-catalysed azide alkyne cycloaddition

Cu(acac)₂ – Copper(II) acetylacetonate

DBU – 1,8-Diazabicyclo[5.4.0]undec-7-ene

DCM – Dichloromethane

DEPT – Distortionless Enhancement by Polarization Transfer

DIPEA – *N,N*-Diisopropylethylamine (Hünig's base)

DMAP – 4-Dimethylaminopyridine

DMF – *N,N*-Dimethylformamide

DMSO – Dimethyl sulfoxide

DMSO-d₆ – Deuterated dimethyl sulfoxide (D₆)

DNA – Deoxyribonucleic acid

EDTA – Ethylenediaminetetraacetic acid

EGFR – Epidermal growth factor receptor

EtOAc – Ethyl acetate

FDA – Food and Drug Administration

FGFR – Fibroblast growth factor receptor

FGI – Functional group interconversion

FLT3 – Fms-like tyrosine kinase 3

FRET – Förster resonance energy transfer

gHMBC – gradient Heteronuclear Multiple Bond Coherence

gHSQC – gradient Heteronuclear Single Quantum Coherence

GIST – Gastrointestinal stromal tumour

GPCR – G protein-coupled receptor

HC – Hepatocellular carcinoma

HER2 – Human epidermal growth factor receptor 2

hERG – human Ether-a-go-go-Related Gene

HTRF – Homogeneous time-resolved fluorescence

IAA – Iodoacetamide

ILK – Integrin-linked kinase

IR – Infrared

MAOS – microwave-assisted organic synthesis

MAPK – Mitogen activated protein kinase

MCL – Mantle cell lymphoma

Mp – Melting point

MS – Mass spectrometry

MTC – Medullary thyroid cancer

mTOR – Mammalian target of rapamycin

NMR – Nuclear magnetic resonance

NOESY – Nuclear Overhauser effect spectroscopy

NSCLC – Non-small-cell lung carcinoma

Oop – Out of plane bending

PC – Pancreatic cancer

PDGFR – Platelet-derived growth factor receptor

PDK1 – Phosphoinositide dependent kinase 1

PH – Pleckstrin homology

PH domain leucine-rich repeat protein phosphatase (PHLPP α)

PI3K – Phosphatidylinositol 3-kinase

PKB – Protein kinase B

PP2A – Protein phosphatase 2A

PTEN – Phosphatase and tensin homolog

PyMPO – 4-[5-(4-Methoxyphenyl)oxazol-2-yl]pyridinium

RCC – Renal cell carcinoma

RNA – Ribonucleic acid

RTKs – Receptor tyrosine kinases

RuAAC – Ruthenium-catalysed azide alkyne cycloaddition

S473K – Ser473 kinase

SAR – Structure-activity relationship

SHIP – SH2-domain-containing inositol polyphosphate 5-phosphatase

S_N1 – Substitution nucleophilic (unimolecular)

S_N2 – Substitution nucleophilic (bi-molecular)

STS – Advanced soft tissue sarcoma

TCN – Tricyclic nucleoside

TEA – Triethylamine

THF – Tetrahydrofuran

TLC – Thin layer chromatography

VEGFR – Vascular endothelial growth factor receptor

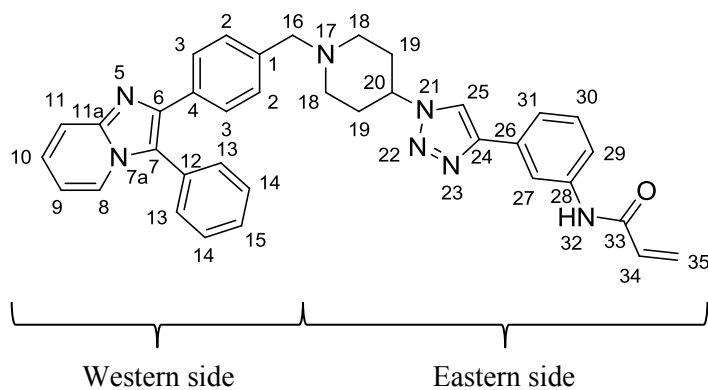
wtAkt1 – Wild-type Akt1

PREFACE

A note to the reader on the interpretation of NMR spectra

An unconventional labelling system was used for the interpretation of the ^1H and ^{13}C NMR spectra, with the final target compounds numbered first, and this numbering system followed to all the intermediates and compounds involved in its synthesis. To this end, the western side of the compounds were numbered from 1 to 16, with the eastern side numbered from 17 upwards to either 31 or 35, depending on the target compound. Any additional functional groups, used during the synthesis, were given numbers above that up to a maximum of 39. This was done in order to assist in the analysis of spectra and to increase the ease of reading when referring to the same atom in different substrates.

An example of the labelling system used:



CHAPTER 1 – CANCER

1.1 CANCER

1.1.1 Cancer statistics

Cancer is a leading cause of disease worldwide with an estimated 12.7 million new cancer cases occurring in 2008.¹ It is consequently also the universal leading cause of death and accounted for 7.6 million deaths in 2008 alone, which is approximately 13% of all deaths that year.^{1; 2} Of this, 56% of the new cancer cases and 63% of the cancer-related deaths occurred in less developed regions of the world. Lung cancer is the most common cancer in the world, accounting for 1.6 million cases and 1.4 million deaths in 2008. This is followed in terms of highest incidence by breast cancer, colorectal cancer, stomach cancer, prostate cancer and liver cancer and in terms of deaths, by stomach cancer, colorectal cancer, prostate cancer, and breast cancer.¹ If recent trends in major cancers are extrapolated to 2030, the burden of cancer is estimated to increase to a projected 20.3 million new cases and 13.2 million cancer-related deaths. This is a rise of 75% in number of people affected in 2030 when compared to new cases and cancer-related deaths in 2008.³

1.1.2 A brief introduction to cancer

Cancer is caused when cells develop defects in the regulatory systems that govern normal cell proliferation and homeostasis.⁴ Hanahan and Weinberg of the Whitehead Institute for Biomedical Research have suggested six hallmarks of cancer and proposed that these capabilities are shared by most, if not all, types of human tumours.⁴ These hallmarks are six essential alterations that cell physiology must undergo in order for them to form into malignant growths:⁴

1. Self-sufficiency in growth signals;
2. Insensitivity to growth-inhibitory (antigrowth) signals;
3. Evasion of programmed cell death (apoptosis);
4. Limitless replicative potential;
5. Sustained angiogenesis and;
6. Tissue invasion and metastasis.

Each of these changes is seen as a capability acquired during tumour development and represents the successful breaching of the anticancer defence mechanism hardwired into cells and tissues.⁴ Genome integrity is maintained by a complex array of DNA monitoring and repair enzymes, but when these malfunction, cells acquire increased mutability and the genomes of the cells are altered. With enough mutations, this can in turn enable the alterations needed for tumour development.^{4; 5} Research has

shown that the majority of cancer cases are caused by lifestyle factors and the environment, leading to genetic defects, and ultimately to cancer development. It is estimated that hereditary genetic defects only account for a maximum of 10% of human cancer cases.⁶

There are more than 100 distinct types of cancer and subtypes of tumours can also be found within specific organs. This makes it difficult to treat cancer as what is effective against one type of cancer, is not necessarily effective against other types of cancer.^{4; 7} There are many different forms of cancer treatment available in the armamentarium against cancer and they include surgery, radiation, chemotherapy, hormone therapy, gene therapy and immunotherapy.⁷⁻¹⁰ It has been found that a combination of therapies often improves survival and may even have a complementary or synergistic effect, leading to a greater antitumour effect than either treatment would have alone.^{8; 9} The standard treatment for many cancers is thus surgery, radiation and chemotherapy, whilst other treatment methods are chosen for their ability to support these three.^{7; 10}

1.2 CHEMOTHERAPY

1.2.1 A brief history of chemotherapy

The term chemotherapy has been used as early as the beginning of the twentieth century and was defined in 1910 by Schweitzer as “the science dealing with the treatment of internal parasitic diseases by means of preparations synthesised with the object of combining the maximum power of efficiency in the destruction of the greatest variety of protozoa with the minimum poisonous action upon the patient's tissues”.^{11; 12} Although outdated, this describes the key criteria of chemotherapeutic agents even today, namely obtaining maximum efficacy with minimum toxicity. Even with chemotherapy then still in its infancy, the hope was expressed that cancer may ultimately be found susceptible to chemotherapy agents. It is interesting to note that during that time, many researchers regarded the cause of cancer as organisms resembling the spirochete of syphilis.¹² Less than fifteen years later, a more concise description of chemotherapy was given by Dale in 1924, namely “the specific treatment of infections by artificial remedies”.¹³

One of the first instances where chemotherapy was found to have an effect on cancer was in 1938 when *The Lancet* reported Strong's work in the treatment of spontaneous mammary carcinoma in mice using oleum gaultheriae.^{14; 15} It was not until 1942 however, that lasting success was achieved with chemical substances used to influence the course of cancer progression.¹⁶ This came from a series of biochemical studies, followed by animal experiments and clinical trials, in 1942 and 1943. These tests afforded the first substance which gave promising results against cancer and thus initiated the development of cancer chemotherapy into what it has become today.¹⁶ This compound, called

nitrogen mustard, later became the first cytotoxic agent in routine clinical use and is now more commonly known as mechlorethamine.^{16; 17}

From these beginnings, chemotherapeutic agents, and their use, have grown considerably and are nowadays seen as chemical agents that can increase the length and the quality of the lives of patients with various cancers. They are thus one of the main tools currently used for the treatment of cancers.¹⁸ As mentioned previously, there should be a balance between the antitumour effect and toxicity of chemotherapeutic agents, and these drugs should therefore be administered in such a way as to achieve maximal efficacy with acceptable toxicity.¹⁸ To achieve optimal efficacy, drugs are often given at the maximally tolerated dose and the shortest possible dosing interval.¹⁸ The shortest possible dosing interval is in part determined by the toxicity that will arise from administering the drug and it may rise to unacceptable levels if the intervals are too short. Toxicity is usually dealt with by either treating it symptomatically or by acting to prevent it, should it be known before treatment.¹⁸ The potential for severe toxicity is still a very real obstacle and each patient must be matched with the appropriate drugs to ensure minimum side-effects.¹⁸

1.2.2 The different types of chemotherapeutic agents

There are many different types of chemotherapeutic agents currently in use and these are sorted into different groups and subgroups according to their molecular targets, as well as their origins.

Here follows a list of the most common chemotherapy agents:¹⁸⁻²²

- cytotoxic agents
 - alkylating agents
 - nitrogen mustard derivatives
 - ethylenimines
 - alkyl sulfonates
 - nitrosoureas
 - triazenes
 - antimetabolites
 - microtubule-targeted agents
 - vinca alkaloids
 - taxanes
 - topoisomerase inhibitors
 - topoisomerase I inhibitors
 - topoisomerase II inhibitors

- epipodophyllotoxins
 - anthracyclines
 - anthraquinones
 - acridines
-
- platinum complexes
 - antitumour antibiotics
 - biologically active agents

Alkylating agents target DNA, forming DNA-alkyl adducts which then leads to apoptosis.^{18; 20} These chemotherapeutic agents are divided into several classes according to their origin and structure and consist of nitrogen mustard derivatives (e.g. cyclophosphamide, ifosfamide and melphalan), ethylenimines (e.g. *N,N,N'*-triethylenethiophosphoramidate also known as thioTEPA), alkyl sulfonates (e.g. busulfan), nitrosoureas (e.g. carmustine and lomustine) and the triazenes (e.g. dacarbazine and temozolomide) as the main classes.²⁰

Antimetabolites resemble ordinary metabolites and many of these are structural analogues of folic acid, purines or pyrimidines.^{16; 18} Since these compounds resemble normal metabolites closely, they can substitute for normally occurring metabolites in intracellular biochemical reactions.¹⁶ They are however, adequately dissimilar that they distort these important reactions, thereby interfering with DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) synthesis.¹⁹ 5-Fluorouracil, fludarabine, gemcitabine and cytarabine are examples of antimetabolites approved for use.¹⁹

Microtubule-targeted agents include mainly two different groups of drugs, the vinca alkaloids and the taxanes.¹⁹ The vinca alkaloids were derived from the common periwinkle plant (e.g. vincristine, vinorelbine and vinblastine), whereas the original taxanes were isolated from yew trees such as the Pacific yew tree (paclitaxel) and the English yew tree (docetaxel).^{18; 19} Both these agents interfere with microtubule function, thereby causing cell cycle arrest. This ultimately leads to apoptosis, necrosis or post-mitotic cell death.^{18; 19}

Topoisomerase inhibitors consist of different chemotherapeutic agents either targeting topoisomerase I or topoisomerase II. Disrupting the normal action of either of these enzymes leads to an interference in DNA coiling and therefore indirectly leads to inhibition of transcription and replication, and eventual cell-death.¹⁹ Topoisomerase I inhibitors stabilise the enzyme-DNA complex, leading to inhibition of religation of DNA and thereby leading to single-strand breaks.²³ Topotecan and irinotecan are examples of topoisomerase I inhibitors.²⁴ The majority of topoisomerase II agents also stabilise the intermediate state of the enzyme-DNA complex, disrupting the normal catalytic cycle of the enzyme and eventually leading to drug-induced apoptosis and/or cell cycle arrest.²² Other compounds that target topoisomerase II are those that disrupt (inhibit) the activity of topoisomerase II

and this also leads to eventual apoptosis.²² Topoisomerase II agents can be divided into four major chemical families. These include epipodophyllotoxins (e.g. etoposide), anthracyclines (natural products which were isolated from microorganisms, e.g. doxorubicin, daunorubicin and idarubicin), anthraquinones (e.g. mitoxantrone) and acridines (e.g. amsacrine).²²

Platinum complexes work in a similar fashion to the cytotoxic alkylating agents, forming DNA adducts (both intrastrand and interstrand DNA cross-links), thereby inducing apoptosis.²⁰ Platinum complexes include cis-diammine dichloroplatinum II (cisplatin) and carboplatin.¹⁸

Antitumour antibiotics have multiple mechanisms of action and also include topoisomerase II inhibitors.¹⁸ Their mechanisms include intercalating into the DNA helix, the production of free radicals and also interference of the function of topoisomerase II.¹⁸ Examples include doxorubicin, mitoxantrone and mitomycin.^{18; 21}

Biologically active agents compass several approaches such as the inhibition of ornithine metabolism (e.g. mitoguanzone or diethyl norspermine), the inhibition of angiogenesis (e.g. pentosan and linomide), the induction of apoptosis and/or differentiation and also inhibition of cell signalling pathways.²¹ Two current ways to affect the cell signalling pathways is by inhibiting kinases (e.g. sunitinib) or autocrine growth factors (e.g. bevacizumab).^{19; 21} The focus of this project lies at these biologically active kinase inhibitors.

As can be noted, most of the traditional anticancer agents target the proliferative mechanism such as DNA replication or chromosome segregation. These agents can demonstrate only partial selectivity for tumour cells compared with normal proliferating tissues such as the gut and bone marrow, and therein lays the origin of toxicity mentioned above [see Section 1.2.1].²⁵

1.3 KINASES AS TARGETS

1.3.1 Kinases and their relation to cancer

The sequencing of the human genome has enabled the identification of 518 protein kinases.²⁶ Kinases are transferase enzymes that catalyse the transfer of phosphate groups from adenosine triphosphate (ATP) to serine, threonine and/or tyrosine residues of proteins.^{27; 28} By means of this, protein kinases are able to facilitate the majority of the signal transduction in eukaryotic cells and also to regulate most aspects of normal cellular function. These processes include metabolism, transcription, cell cycle progression, cytoskeletal rearrangement, cell movement, apoptosis and differentiation.²⁶ Protein phosphorylation, and by implication kinases, is also crucial to intercellular communication during development, in physiological responses and homeostasis, as well as the proper functioning of the nervous and immune systems.^{26; 29}

The first link between cancer and these enzymes was revealed in 1975 by Varmus and Bishop when they discovered the first cellular oncogene, *src*, which was later found to encode a tyrosine kinase.³⁰ Since then, it has been found that many cancers, and several other diseases, are caused by the dysfunction of protein kinase signalling pathways.²⁸

Abnormal signal transduction through activated growth factor receptors such as the ErbB family of receptor tyrosine kinases is a common feature of many solid tumours.³¹ One of these receptor tyrosine kinases is epidermal growth factor receptor (EGFR). This kinase is known to be overexpressed, mutated, or both, in various solid tumours and is thus implicated as an important factor in supporting the oncogenesis and progression of these cancers.^{32; 33}

Numerous other tyrosine kinases have also been found to be dysregulated in cancer cells in different ways, including loss of autoregulation, overexpression or being constitutively active.³² Some of the tyrosine kinases involved that have been identified include breakpoint cluster region-Abelson protein (BCR-ABL), which have been branded as the direct cause of chronic myeloid leukaemia, c-KIT, which is associated with most gastrointestinal stromal tumours, platelet-derived growth factor receptor (PDGFR), which has been associated in certain solid tumours and also others such as colon carcinoma kinase 4 (CCK4), Fms-like tyrosine kinase 3 (FLT3), fibroblast growth factor receptors 1 to 4 (FGFR1 to FGFR4) and human epidermal growth factor receptor 2 (HER2).^{29; 32; 34}

Serine/threonine kinases such as the cyclin dependent kinases (CDKs) have also been implicated in cancer.²⁵ Hyperactivity and dysregulation in the form of mutations, deletions and overexpression of this group of kinases and their corresponding genes have been identified as the primary causes for uncontrolled proliferation of cells in various human cancers.²⁵ Another serine/threonine kinase thus involved is the mitogen-activated protein kinase (MAP kinase) implicated in breast cancer.³⁵ It has been found that breast cancer tumours frequently contain an increased amount of cells with the activated form of this kinase.³⁵

As can be gathered from the aforementioned examples, there are a wide range of targetable protein kinases and this enables a broad therapeutic potential.

1.3.2 The targeting of kinases

Almost all eukaryotic protein phosphorylation is conducted by a single superfamily of eukaryotic protein kinases. The mechanism of protein phosphorylation is very similar across kinases and they have a high degree of conservation, not only in the catalytic domain, but also in other key domains of the kinases.^{25; 28; 29} This makes it complex to design an inhibitor which is not only potent, but also selective for the specific kinase over all of the other protein kinases.²⁵ Selectivity is especially

important as a lack thereof can have undesired and potentially toxic side effects by acting on other kinases. Another point to consider is that targeting a certain kinase may be beneficial in the system or organ where it is dysregulated, but harmful in other systems or organs where it is functioning as normal. An example is the inhibition of the overexpressed cell-surface HER2 tyrosine kinase receptor in patients with breast cancer. The inhibition leads to a beneficial outcome with regards to the breast cancer, but can also lead to severe cardiac dysfunction in selected patients.²⁸ The long term safety of kinase inhibitors is critical for its usefulness as most small-molecule kinase inhibitors do not entirely eradicate clonogenic cells and will therefore have to be taken for life.³⁰

Targeting protein kinases implicated in cancer is a relatively recent plan of action against cancer, but from this, novel therapeutic agents have been gleaned that are more specific and efficacious and less toxic than other, more traditional chemotherapeutic agents, regardless of the possible drawbacks mentioned here.²⁵

1.4 DIFFERENT TYPES OF KINASE INHIBITORS

1.4.1 Inhibitors and their mechanism of action

Kinase inhibitors can be categorised based on their mechanisms of action and are divided into five different groups, namely Type I, Type II, Type III, Type IV and Type V.³⁶

Type I inhibitors are ATP-competitive and interact directly with the ATP binding pocket and typically mimic the binding of ATP to the target, forming hydrogen bonds with the hinge region of the kinase domain.^{36; 37} These inhibitors bind to the active conformation of the kinase in which the activation loop is phosphorylated (Figure 1).³⁶ In addition to binding to the hinge region of the kinase domain, Type II kinase inhibitors also bind to an allosteric site adjacent to the hinge region. This type of inhibitor targets the inactive conformation of the kinase, as the allosteric site is not present when the kinase is in its active conformation.³⁷

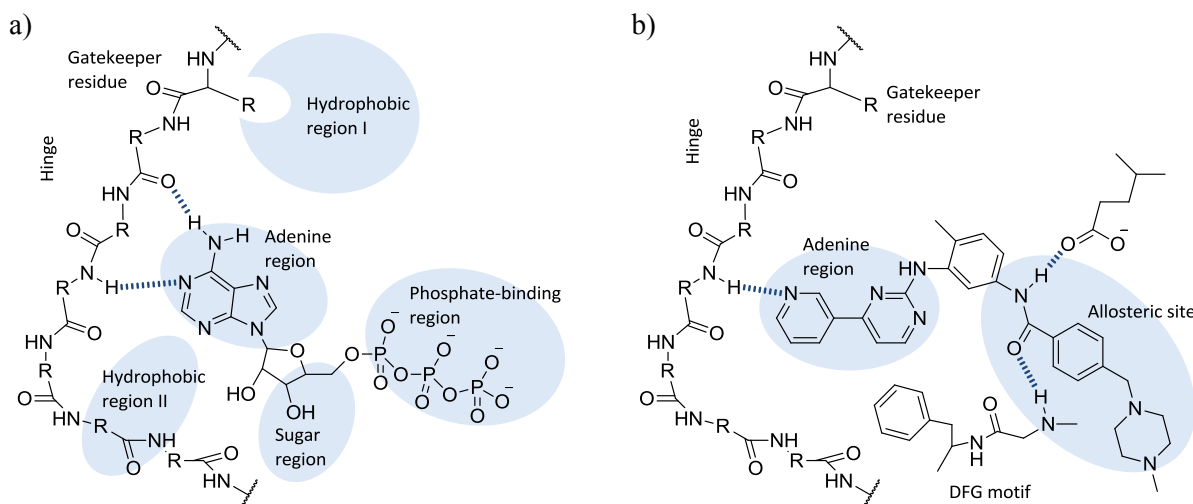


Figure 1

An adapted figure, from Liu and Gray, depicting the ATP (a) and allosteric (b) binding sites.³⁸ The ATP binding site (a) is occupied by ATP and the two hydrogen bonds are indicated by dark blue hashed bonds. Type I inhibitors would bind to this site in a similar manner. The allosteric site (b) is occupied by a Type II inhibitor, showing intermolecular interactions with both the ATP binding site and the allosteric site, also as blue hashed bonds. A Type III inhibitor will have similar interactions with the allosteric site, but none with the ATP binding site.

Type III kinase inhibitors only bind to the aforementioned allosteric site adjacent to the hinge region of the kinase domain.³⁷ This inhibitor therefore also targets the inactive conformation of the kinase. Inhibitors that bind to other available allosteric sites which are unrelated to the ATP binding pocket and the allosteric site involved in Type II and III inhibitors, are called Type IV inhibitors.³⁷ When bound, these inhibitors induce conformational changes in the kinase which makes it inactive.³⁶ Type V kinase inhibitors are bi-substrate inhibitors. They have two conjugated inhibitory fragments of which each is targeted to a different binding site of a bi-substrate kinase, thus binding twice to the kinase.^{36; 39} Type V inhibitors therefore have the potential to have increased potency and selectivity over that of the mono-substrate inhibitors.³⁶

1.4.2 Reversible and irreversible inhibitors

It has been proposed that one of the most crucial factors for sustained drug efficacy is not just the affinity of the drug for its target, but additionally the residence time of the drug molecule on its target.⁴⁰ This is because the drug action (e.g. inhibition) can only occur when the drug is bound to its target and therefore the longer there is such a drug-target complex, the longer the drug exerts its effect.⁴⁰ Kinase inhibitors can bind to the kinase in a reversible or irreversible manner and while reversible inhibitors only have transient inhibition due to temporary residence time, irreversible

inhibitors can potentially grant terminal inhibition of the target.^{34; 41} Irreversible inhibitors bind to the inhibitor in a covalent fashion and can thus have a so-called infinite residence time.⁴¹

Irreversible inhibitors contain a driving portion and a warhead. The driving portion is commonly a heterocyclic core structure, generally similar in structure to a known reversible inhibitor for the particular target, and the warhead is a moderately electrophilic functional group which is attached to the driving portion in an optimal position, so as to allow it to covalently interact with an available nucleophilic residue on the target (Figure 2).^{42; 43} Irreversible inhibition occurs in at least two steps, first the compound's driving portion must bind to the target in a non-covalent fashion, similar to that of a reversible inhibitor binding to its target.⁴³ This places the electrophilic warhead in close proximity to the targeted nucleophilic residue, which in turn facilitates the formation of the covalent bond between the drug and the target, yielding the irreversibly inhibited drug-target complex.⁴³

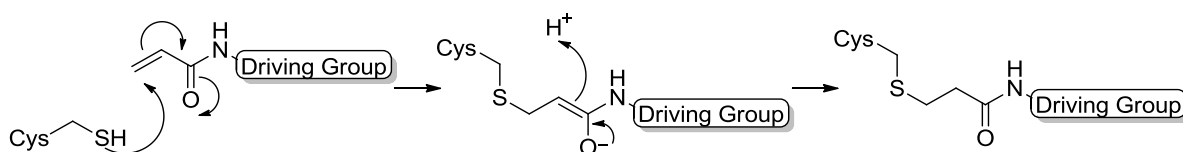


Figure 2

A schematic representation of how irreversible inhibition is achieved.⁴²

To date, the most robust approach has been to utilise a nucleophilic cysteine thiol group in the target kinase, and a study in 2000 indicated acrylamide and substituted acrylamides to be the electrophilic warheads of choice (Figure 2).^{41; 44} These electrophiles are highly suitable as they are weak enough to only show minimal reactivity towards other thiol functionalities, yet strong enough that they react efficiently with the target cysteine residue when brought in its proximity by the non-covalent interactions of the driving part of the inhibitor.⁴³ This, coupled with targeting nonconserved cysteine residues by preference, limits non-selective reactions with off-target proteins, thereby reducing the concern of increased toxicity when using irreversible inhibitors.^{42; 43} Lysine has also been used as the nucleophilic group, but it is not as attractive a target as cysteine due to its well-conserved nature and its common occurrence, residing in virtually every ATP-binding pocket of the human kinome.⁴⁵

Theoretically, covalent irreversible kinase inhibitors have several advantages over that of reversible inhibitors and one of these is a prolonged pharmacologic effect relative to its systemic exposure.⁴² Through terminal inhibition of the target, it is projected that the inhibition effect of this inhibitor should persist even after it has left the system.⁴² This means that no excessive circulating levels are required to maintain drug efficacy and that rapid clearance of the drug may even be preferred, thereby minimising the time the drug spends in the system and thus also minimising off-target toxicity.⁴⁵ Activity of the target will only be restored following re-synthesis of the protein target once the unbound drug has left the system and this may therefore make these inhibitors suitable for less

frequent dosing regimens.⁴⁵ These two factors will allow for a lower body burden to the patient and owing to their different inhibition methods, these inhibitors also have sustained potency in the face of resistance mutations, something which many ATP-competitive inhibitors lack.⁴⁴

A potential drawback of covalent irreversible inhibition is that the electrophilic warhead can have potential toxicity issues involving other reactive cysteine groups from off-target proteins.⁴² As previously mentioned, this effect is limited by the use of weak electrophilic warheads, which need to be properly positioned in close proximity to the nucleophile.⁴⁴ Only those kinases which have the ability to form the critical covalent bond to the drug should therefore be inhibited and this essentially leads to another advantage, notable selectivity gains over reversible inhibitors.^{42; 44} The therapeutic use of these inhibitors therefore relies on adequate selectivity for the protein kinase of interest.⁴⁵

1.4.3 A comparison between ATP-competitive and allosteric inhibitors

The ATP binding pocket is highly conserved throughout the kinome and these inhibitors are therefore less selective than those that have an allosteric site as target.⁴¹ They also have an increased the risk of toxicity brought about by the lower selectivity.^{29; 30} In addition, tumours invariably acquire mutations in the ATP-binding pocket that interfere with drug binding and thus cause drug resistance.³⁰

ATP-competitive inhibitors (Type I and II) inhibit their targets in a reversible manner by binding to the ATP binding pocket of the target kinase *via* weak interactions such as hydrogen bonds, van der Waals attractions and hydrophobic interactions.⁴² They also have to compete with high intracellular ATP concentrations under physiological conditions.⁴⁵ This allows non-ATP competitive inhibitors (Types III to V) to potentially offer higher selectivity and efficacy than their ATP-competitive counterparts.⁴⁵ These effects can theoretically be seen in both reversible and irreversible allosteric inhibitors.

From the inhibitor types discussed, based on binding area and type of inhibition, it can be reasoned that an irreversible inhibitor which binds to one or two allosteric sites unrelated to the ATP binding pocket (either Type IV or Type V) could potentially be a potent inhibitor with favourable properties, such as strong and selective inhibition.

1.5 KINASE INHIBITORS AS CHEMOTHERAPEUTIC AGENTS

1.5.1 The FDA approved kinase inhibitors

As of 13 Nov 2013, there are 24 FDA approved small molecule protein kinase inhibitors (Table 1 and Table 2). Of these, 11 drugs were approved from 2001 to 2010 and 13 drugs were approved from 2011 to 2013. This shows a promising trend for the future of kinase inhibitors as chemotherapeutic agents against cancer.

Kinases can also be inhibited by compounds other than small molecules, namely monoclonal antibodies and RNA aptamers.^{46; 47} There are FDA approved representatives of both of these compounds, with more in clinical trials, but these will not be discussed here as the focus of this project lies exclusively at small molecule inhibitors.^{46; 47}

Table 1

FDA approved small molecule protein kinase inhibitors (2001 to 2009)⁴⁵

Generic (brand) name	Year of approval	Company	Indication	Target kinase
imatinib (Gleevec [®])	2001	Novartis	CML	BCR-ABL, c-Kit, PDGFR α/β
gefitinib (Iressa [®])	2003	AstraZeneca	NSCLC	EGFR
erlotinib (Tarceva [®])	2005	Genetech, OSI	NSCLC, PC	EGFR
	2013	Genetech, OSI	NSCLC with EGFR (exon 19 deletion or L858R)	EGFR
sorafenib (Nexavar [®])	2005	Bayer, Onyx	HC, RCC	Raf, VEGFR2/3, c-Kit, PDGFR β
sunitinib (Sutent [®])	2006	Pfizer	GIST, RCC	c-Kit, VEGFR, PDGFR, FLT3
dasatinib (Sprycel [®])	2006	Bristol-Myers Squibb	CML, ALL	BCR-ABL, c-Kit, PDGFR, Src
nilotinib (Tasigna [®])	2007	Novartis	CML	BCR-ABL, c-Kit, PDGFR, Src, ephrin
lapatinib (Tykerb [®])	2007	GlaxoSmithKline	breast cancer	EGFR, ErbB2 (HER2)
temsirolimus (Torisel [®]) ⁴⁸	2007	Wyeth Pharmaceuticals	RCC	FKBP12/mTOR
everolimus (Afinitor [®]) ⁴⁹	2009	Novartis	RCC	FKBP12/mTOR
pazopanib (Votrient [®])	2009	GlaxoSmithKline	RCC, STS	VEGFR, PDGFR α/β , c-Kit

Table 2

FDA approved small molecule protein kinase inhibitors (2011 until mid-November 2013)⁴⁵

Generic (brand) name	Year of approval	Company	Indication	Target kinase
vandetanib (Caprelsa [®])	2011	AstraZeneca	MTC	VEGFR, EGFR, RET
vemurafenib (Zelboraf [®])	2011	Roche, Plexxicon	CML	BCR-ABL, c-Kit, PDGFR, Src, ephrin
crizotinib (Xalkori [®])	2011	Pfizer	NSCLC (ALK +ve)	ALK, MET
ruxolitinib (Jakafi [®])	2011	Incyte	Myelofibrosis	JAK1/2
axitinib (Inlyta [®]) ⁴⁹	2012	Pfizer	RCC	VEGFR
bosutinib (Bosulif [®]) ⁴⁹	2012	Pfizer	CML	BCR-ABL
cabozantinib (Cometriq [®]) ⁵⁰	2012	Exelixis	MTC	RET, MET, VEGFR, c-Kit, FLT3
ponatinib (Iclusig [®]) ⁵⁰	2012	Ariad	CML, ALL	BCR-ABL, c-Kit, RET, FLT3
regorafenib (Stivarga [®]) ⁵⁰	2012	Bayer	Colorectal cancer	RET, VEGFR, c-Kit
dabrafenib (Tafinlar [®]) ⁵¹	2013	GlaxoSmithKline	Melanoma with BRAF(V600E)	BRAF
trametinib (Mekinist [®]) ⁵²	2013	GlaxoSmithKline	Melanoma with BRAF(V600E/V600K)	BRAF, MEK
afatinib (Gilotrif [®]) ⁵³	2013	Boehringer Ingelheim	NSCLC with EGFR (exon 19 deletion or L858R)	EGFR
ibrutinib (Imbruvica [®]) ^{54; 55}	2013	Pharmacyclics and Janssen Biotech	MCL	BTK

Of the FDA approved small molecule kinase inhibitors, the two most recent approvals are irreversible inhibitors, afatinib (Gilotrif[®]) and ibrutinib (Imbruvica[®]) (Figure 3).^{56; 57} Each inhibitor consists of a driving portion selective for its target kinase, as well as the crucial warhead, either an acrylamide (ibrutinib) or a substituted acrylamide (afatinib). The approval of these two compounds is promising as it indicates that the disadvantages of irreversible inhibitors can be overcome, leading to potent inhibitors of their respective targets.

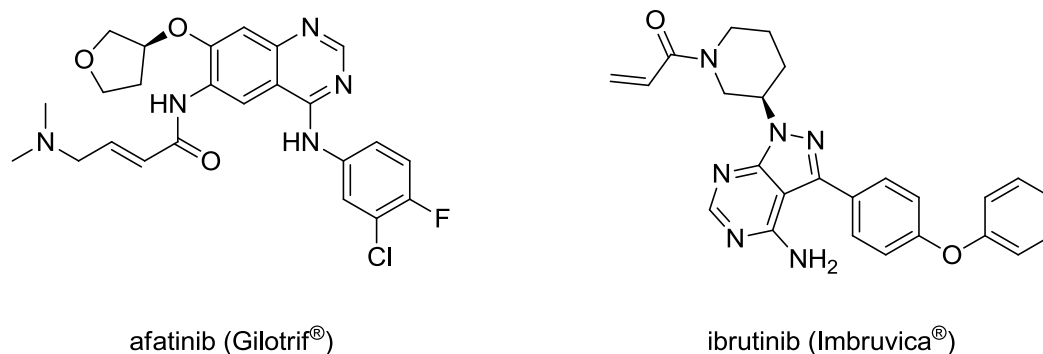


Figure 3

The two FDA approved covalent kinase inhibitors, afatinib and ibrutinib.

1.5.2 Potential obstacles

The 24 FDA approved kinase inhibitors listed above are only a small fraction of the multitude of known kinase inhibitors, with most inhibitors never reaching clinical trials and many failing during preclinical or clinical development.³⁰ Reasons for this can include a lack of potency or selectivity, but can also involve an inhibitor having potent and selective inhibition of its target when tested *in vitro*, but lacking cellular potency, possibly from poor physicochemical properties and pharmacokinetic behaviour [See 1.3.2].^{45; 58; 59} The physicochemical properties of a compound include its absorption into and distribution in the body, metabolism and excretion from the body and its toxicity to the body (ADMET).^{58; 59} These are the properties which influence the compound's pharmacokinetic behaviour and therefore play a large role in its success, or failure, as a drug.⁵⁹

Targeting kinases for chemotherapeutic use is difficult and it has been found that the FDA approved kinase inhibitors work well in a small percentage of patients in certain cancers, but that most patients benefit little and those that do respond, ultimately relapse.³⁰ One such an example is Astra-Zeneca's small-molecule EGFR inhibitor gefitinib (Iressa[®]) which was FDA approved in 2003, but showed no survival benefit to patients in phase 3 after being successful in phase 2 clinical trials.³⁰ There is therefore a large room for improvement, especially in tailor-made therapies for patients, based on the evaluation of biomarkers.^{60; 61} A different approach to combat this is by facilitating cancer treatment in a similar way as what HIV therapy is conducted, by simultaneously hitting multiple tumour-

promoting signalling pathways.³⁰ This can be done by either using a combination of drugs or with a single multi-targeted compound to effectively block several cell growth pathways and possibly overwhelm tumours before they can become resistant to treatment.³⁰ Garber stated in 2006 that kinase drug combinations were in early testing, by combining novel kinase inhibitors with either Herceptin or Erbitux.³⁰ Unfortunately this is a double-edged sword, potentially leading to increased side effects and therefore greater toxicity.³⁰

1.5.3 The road forward

Along with the aforementioned strategies, different kinases can also be targeted. One such a target is Akt, also known as protein kinase B (PKB). To date, there are no FDA approved Akt kinase inhibitors even though it is a validated target for cancer therapy.⁶² This is therefore a starting point as good as any other.

CHAPTER 2 – AKT KINASE

2.1 AKT KINASE

2.1.1 Akt and its function

The phosphatidylinositol 3-kinase-Akt (PI3K-Akt) pathway, downstream of receptor tyrosine kinases (RTKs) is an important regulator of mammalian cell proliferation and survival, responsible for the regulation of various cellular processes such as proliferation, growth, apoptosis and cytoskeletal rearrangement.^{63; 64} Research has shown that several components of this pathway are dysregulated in a wide range of human cancers which include glioblastoma, ovarian, breast, endometrial, hepatocellular carcinoma, melanoma, digestive tract, lung, renal-cell carcinoma, thyroid and lymphoid cancers. Its role in cancer is further confirmed by gain- or loss-of-function mutants of several components of this pathway leading to neoplastic transformations in model systems.⁶³

One of the key members of this pathway is Akt. This serine/threonine protein kinase belongs to the AGC subfamily of the protein kinase superfamily and is conserved from primitive metazoans to humans.^{63; 64} There are three mammalian isoforms of Akt, namely Akt1, Akt2 and Akt3 (also known as PKB α , PKB β and PKB γ , respectively) expressed by the *Akt1/PKB α* , *Akt2/PKB β* and *Akt3/PKB γ* genes, respectively.⁶⁴ Akt plays a crucial role in the PI3K-Akt pathway, helping to regulate biological processes such as metabolism, cell survival, motility, transcription and cell-cycle progression.⁶⁴ With mouse gene knockout studies it was found that knockout of alternate isoforms of Akt gave rise to distinct phenotypes, suggesting that each isoform may have a unique function.⁶⁵ The study revealed Akt1 to regulate placental development and maintenance, with its absence resulting in mice smaller than the wild-type mice and with a 40% increase in neonatal mortality. Akt2 was found to be primarily involved with glucose homeostasis, with *Akt2/PKB β* knockout leading to severely insulin resistant diabetic mice. Lastly, it was found that Akt3 deficient mice had a uniform reduction in brain size and weight, with all major brain regions being affected, indicating an involvement in postnatal brain growth.⁶⁵

The three Akt isoforms share up to 80% of amino acid homology, each consisting of an N-terminal pleckstrin homology (PH) domain, a central kinase domain and a C-terminal regulatory domain.⁶⁴ Akt is activated by a dual regulatory mechanism, requiring translocation to the plasma membrane as well as phosphorylation at its two regulatory phosphorylation sites, a threonine residue (T308 in Akt1) in the kinase domain and a serine residue (S473 in Akt1) in the C-terminal domain.^{63; 66} Akt membrane translocation is controlled by phosphoinositides *via* interactions with Akt's PH domain and whilst phosphorylation of the threonine residue is sufficient for Akt activation, additional phosphorylation at the serine residue enables maximal Akt activation.^{63; 66} The threonine residue is phosphorylated by

phosphoinositide-dependent kinase 1 (PDK1) and the serine residue is phosphorylated by the rictor-mTOR complex.^{64; 67}

Under basal conditions, Akt is maintained in its inactive conformation in the cytoplasm by intramolecular interactions between the PH and kinase domains (Figure 4).⁶⁸ This so-called “PH in” conformer forms a complex with PDK1 and the associated and dissociated forms are in dynamic equilibrium. With growth factor stimulation, the Akt-PDK1 complex is recruited to the plasma membrane where it associates and interacts with phosphoinositides. This induces a change to the “PH out” Akt conformer where the regulatory threonine residue is accessible to phosphorylation by PDK1.⁶⁸ After additional phosphorylation of the regulatory serine residue and loading of ATP, the complex can dissociate from the plasma membrane in its active conformation and return to the cytoplasm (and cell nucleus) to perform its function. Akt then readopts the inactive, “PH in” conformation upon dephosphorylation of the regulatory threonine and serine residues.⁶⁸

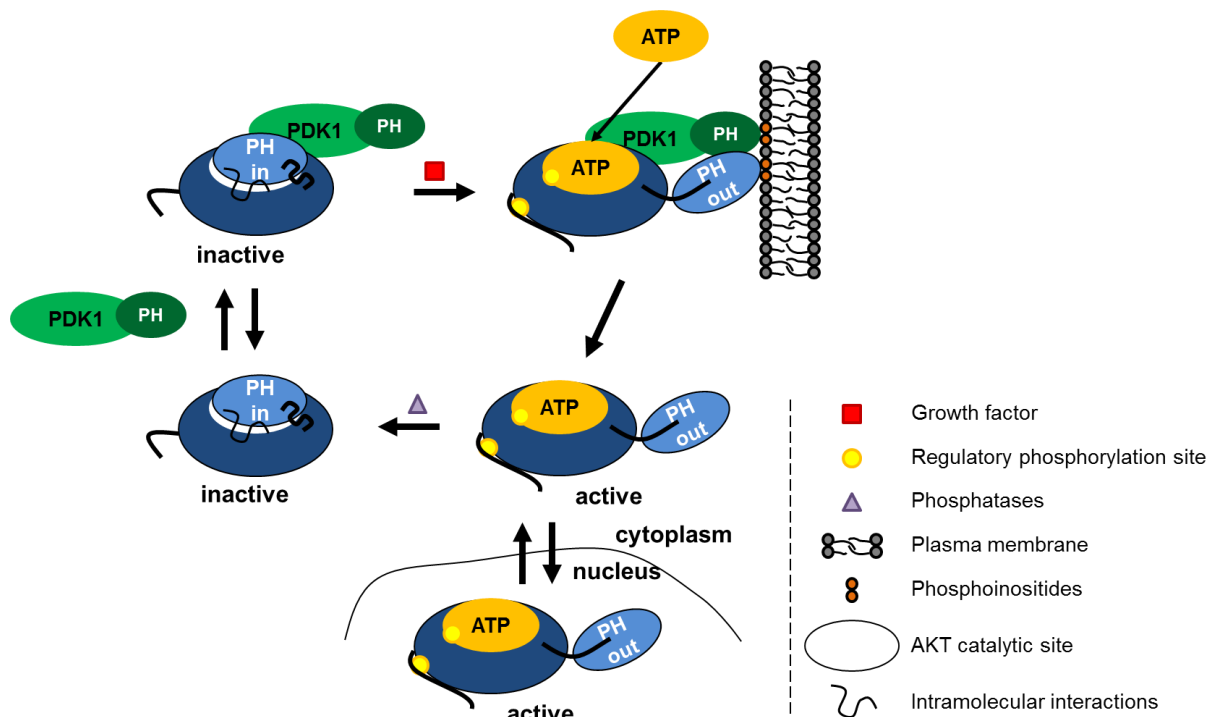


Figure 4

An adapted pictorial representation from Larijani and co-workers, illustrating the process of Akt activation and inactivation as discussed in the text.⁶⁸

Akt activity can be inhibited directly with protein phosphatase 2A (PP2A) and PH domain leucine-rich repeat protein phosphatase (PHLPP α), or indirectly by the tumour suppressor phosphatase and tensin homolog (PTEN) and the SH2-domain-containing inositol polyphosphate 5-phosphatase (SHIP) doing so in an indirect manner.^{62; 64}

2.1.2 Akt and its relation to cancer

It has been shown that the PI3K-Akt pathway is frequently hyperactivated in many human cancers and that its components are often altered, with irregularity not only playing a major role in tumour development, but also in the tumour's potential response to cancer treatment.^{66; 69} Virtually all normal tissues (with the exception of adult liver tissue) express Akt1, 2 and 3, as do the majority of human tumour cell lines, with dysregulation of all three isoforms of Akt having been implicated in the progression of human tumours.^{62; 70} Akt2 is the most frequently dysregulated Akt isoform in human cancer.⁶²

Tumour cells have been found to be more resistant to chemotherapeutic drugs if they have overexpressed or constitutively active Akt, and knockdown of Akt by antisense or siRNA reduces tumour growth and induces apoptosis and cell growth arrest in only tumour cells overexpressing Akt.⁶² Akt dysregulation has consequently been associated with poor clinical outcome in many cancer types such as melanoma, breast, prostate, endometrial, gastric, pancreatic and brain cancer.⁷¹

Dysregulation of Akt is seen as it either being overexpressed or constitutively active.^{70; 72} This can influence downstream effectors and mediate pathways that favour tumourigenesis by promoting cell survival, cell proliferation (increased cell number) and cell growth (increased cell size).⁶³ Various mechanisms can account for the dysregulation of Akt, including amplification of the *Pkb/Akt* genes, responsible for their expression, or mutations in components of the PI3K-Akt signalling pathway such as PTEN, one of the proteins responsible for its inhibition.⁶⁴ Clinical evidence of PI3K-Akt pathway dysregulation has provided potential targets for cancer therapy, including Akt and other pathway components such as mTOR (mammalian target of rapamycin), PDK1 and ILK (Integrin-linked kinase).⁶³

As the PI3K-Akt pathway components, including Akt, is present in most tissues and involved in so many different biological processes, Akt inhibition may affect normal cellular functions, which could limit its therapeutic use.^{71; 73} A therapeutic window would be necessary wherein tumours are more sensitive to the inhibitor than what normal tissues are.^{71; 73} Fortunately, a few studies have reported positive results pertaining to this potential problem, including an *in vivo* study using a tumour xenograft mouse model with an Akt inhibitor named “tricyclic nucleoside” (TCN).⁷⁴ This study showed that Akt inhibition had a selective antitumour effect for those tumours with elevated levels of Akt, leading to the induction of apoptosis and cell growth arrest, whilst having little effect against tumours with low levels of Akt.⁷⁴ It was further reported that the antitumour effect was observed with a high degree of efficacy at low doses of TCN, and no compound-related side effects were observed at these doses.⁷⁴ This, and many other studies not discussed here, provides validation for the development of Akt inhibitors, pointing to Akt inhibition being central in reducing the apoptotic

threshold of cancer cells and preferentially killing these cells, either as a single therapeutic agent or in combination with other chemotherapeutic agents.^{62; 74-79}

2.1.3 Akt and its target validation

Studies, such as the one mentioned above, which investigate Akt's potential as a therapeutic target for cancer chemotherapy, have been hampered by two key shortcomings with regards to the available Akt inhibitors.⁸⁰ The first is the lack of small molecule inhibitors with a specific preference for only Akt, whilst the other is the lack of isoform specificity which could help contribute in the elucidation of each isoform's role in tumour formation and maintenance.⁸⁰ The design of selective and isoform specific inhibitors is therefore a priority.

There are a number of different types of known Akt inhibitors and these include Akt antibodies, lipid-based inhibitors, peptide-based inhibitors and the focus of this project, small molecule inhibitors.^{62; 81} Several possible binding sites on Akt can be targeted by small molecule inhibitors, including the ATP binding pocket, the phosphoinositide binding pocket of the PH domain, an allosteric hinge region lying between the PH and protein kinase domains and the substrate binding groove adjacent to the ATP binding pocket.⁷¹ Due to the high degree of conservation between kinases (especially their catalytic domains), as well as between the three isoforms of Akt, lack of selectivity was generally anticipated from ATP-competitive inhibitors.^{25; 64; 80} This study therefore focuses on the allosteric hinge region and small molecule inhibitors of that particular site, in order to design and synthesise new potential allosteric Akt inhibitors which possess both kinase selectivity and isoform specificity.

2.2 AKT INHIBITION

2.2.1 The first allosteric Akt inhibitors

This section closely follows the information set out in two reviews, by Lindsley and co-workers, about small molecule inhibitors of Akt.^{80; 82} Allosteric inhibition of Akt is a relatively new concept, with the first small molecule allosteric inhibitor, NGD-28835 **1**, (Figure 5) reported by NeoGenesis Pharmaceuticals in 2004.⁸³ This was closely followed by Merck in 2005 when they published their research presenting their allosteric inhibitors, which not only show an increase in specificity for Akt, but also display the sought-after isoform selectivity.^{80; 84}

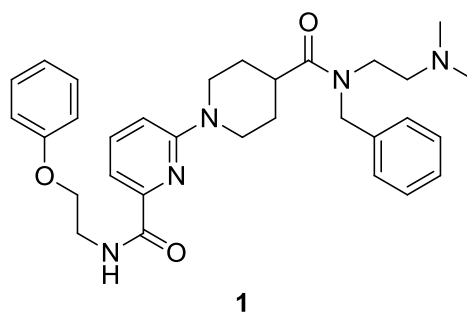


Figure 5

NGD-28835, the first reported allosteric inhibitor of Akt.⁸³

Barnett and colleagues from Merck originally screened approximately 270 000 compounds for their ability to inhibit Akt. Aside from compounds belonging to classes known for ATP-competitive inhibition, they also identified two isoform selective inhibitors of Akt, inhibitors **2** and **3**, which displayed unique and unpredicted inhibition properties (Figure 6).⁸⁴ These compounds were further studied and inhibitor **2** was found to be a selective inhibitor of only Akt1 while inhibitor **3** was selective for both Akt1 and Akt2, with neither displaying significant activity against Akt3. Both of these compounds were found to be inactive against mutants lacking the PH domain and it was thus postulated that these inhibitors bind *via* direct interaction with the PH domain, or to a site which is only formed in the presence of the PH domain.⁸⁴ They were also tested for activity against other closely related protein kinases such as PDK1 and protein kinase A and C, and were found to be inactive, possessing specificity towards Akt with respect to these closely related kinases.⁸⁴ It was determined that inhibitors **2** and **3** are non-competitive with respect to ATP and peptide substrate, rather binding to a novel allosteric site. From cell-based experiments, it was established that binding led to a decrease in phosphorylation of the regulatory phosphorylation sites, as well as to a drop in Akt kinase activity. Inhibitor binding was thus postulated to stabilise Akt in a formation that hindered its activation.⁸⁴ Downstream effects were only discerned with inhibitor **3** and not inhibitor **2**, indicating that Akt1 inhibition alone may not be sufficient to block downstream signalling in the cell types used, instead requiring inhibition of both Akt1 and Akt2.

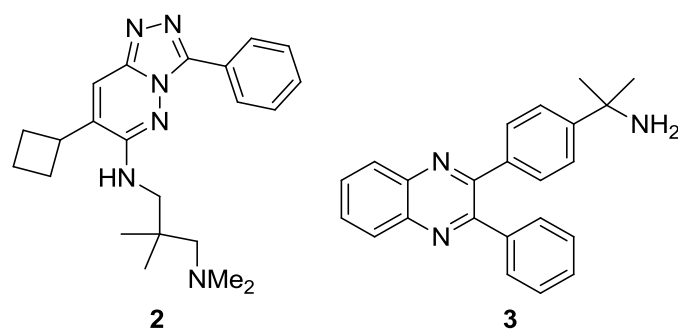


Figure 6

The two allosteric Akt inhibitors published by Merck.⁸⁴

Merck explored the structure-activity relationship of allosteric Akt inhibitors, using the quinoxaline-based scaffold of inhibitor **3** as a point of departure and designing and synthesising more potential isoform selective allosteric inhibitors of Akt (Figure 7 to Figure 9, discussed below).^{85; 86} The most promising compounds were more potent than inhibitors **2** and **3**, having varying specificity for the different Akt isoforms, with Akt1, Akt2 or both Akt1 and Akt2 being selectively inhibited.⁸⁵

The initial strategy was to modify the eastern side of inhibitor **3**, eliminating its *gem*-dimethyl functionality and replacing the primary amine with a number of functionalised amines.⁸⁶ This first round of compounds showed promise, with the best representative of the group being inhibitor **4** (Figure 7). Inhibitor **4** gained potency by over 10-fold relative to inhibitor **3** whilst retaining isoform selectivity.⁸⁶ Interestingly, the piperidiny benzimidazolone functionality introduced on the eastern side of inhibitor **4** is a G protein-coupled receptor (GPCR) privileged structure.^{86; 87} This promising result indicates that the *gem*-dimethyl functionality is not essential for either potency or isoform selectivity, and that the piperidiny benzimidazolone functionality is also amenable to interacting with Akt. Regrettably, inhibitor **4** displayed poor solubility and a lack of cell activity and thus required further optimisation.⁸⁶

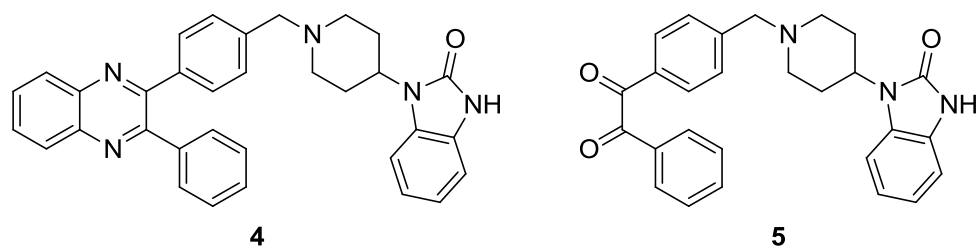


Figure 7

The allosteric Akt inhibitor **4**, with a piperidiny benzimidazolone functionality, and an intermediate in the process of synthesising additional library compounds, **5**.⁸⁶

To this end, intermediate **5**, containing the key piperidiny benzimidazolone privileged structure, was synthesised and used as the starting material for subsequent libraries in this study (Figure 7).⁸⁶ It was

converted into a reasonable number of library compounds, utilising microwave-assisted organic synthesis (MAOS) by reacting it with 24 commercially available α -aminocarboxamides to yield 48 regioisomeric 5,6-diphenylpyrazin-2(1*H*)-ones, represented by compounds **6** and **7**, as well as 50 differently functionalised aryl-1,2-diamines to deliver C6/C7-functionalised and tricyclic quinoxalines represented by compound **8** (Figure 8).^{86; 88}

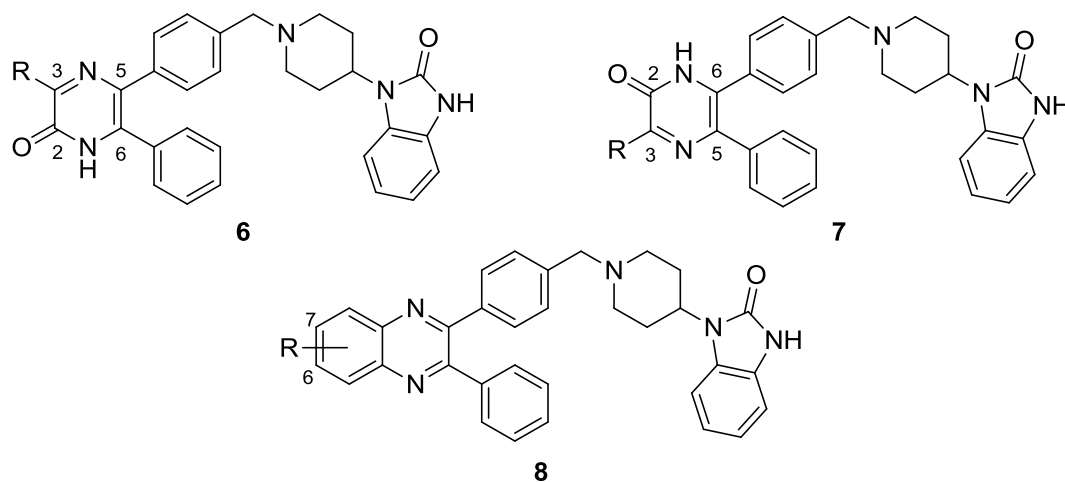


Figure 8

General structures of the regioisomeric 5,6-diphenylpyrazin-2(1*H*)-ones and C6/C7-functionalised and tricyclic quinoxalines, compounds **6,7** and **8**, where R represents different alkyl, aryl, carbonyl and heterocyclic functionalities.⁸⁶

From investigating the 98 library compounds obtained, they found that the degree of inhibition of the different isoforms was highly variable, comprising of a combination of Akt1 selective, Akt2 selective and dual Akt1/2 selective inhibitors (Figure 9). The regioisomeric 5,6-diphenylpyrazin-2(1*H*)-ones, **6** and **7**, each displayed a distinct inhibition profile with the nature of the substituents on the functionalised α -aminocarboxamides playing a role in isoform selectivity.⁸⁶ Interestingly the C6/C7 functionalised and tricyclic quinoxalines **10**, had a conserved degree of inhibition of the individual Akt isoforms, not excessively influenced by the different functionalised aryl-1,2-diamines.⁸⁶ Of these compounds, inhibitor **11** (known as Inhibitor VIII or Akti-1/2) was selected for further evaluation as it displayed improved potency, solubility and cell permeability compared to the other library compounds. The most favourable selective Akt1 inhibitor (inhibitor **9**) and selective Akt2 inhibitor (inhibitor **10**) was also earmarked for use in further studies into the role of Akt in cancer.^{84; 86} Unfortunately, no selective Akt3 inhibitors were identified to help elucidate its role in cancer development.

It was determined that with these three inhibitors (inhibitors **9**, **10** and **11**), the allosteric mode of inhibition, seen in inhibitors **2** and **3**, is conserved as they are similarly non-competitive with ATP and displayed the expected PH domain-dependent binding.⁸⁵ Binding was further investigated and it was

proposed that it does not occur directly to only the PH domain, but rather that the binding site is formed from an interaction between multiple domains within Akt.⁸⁵

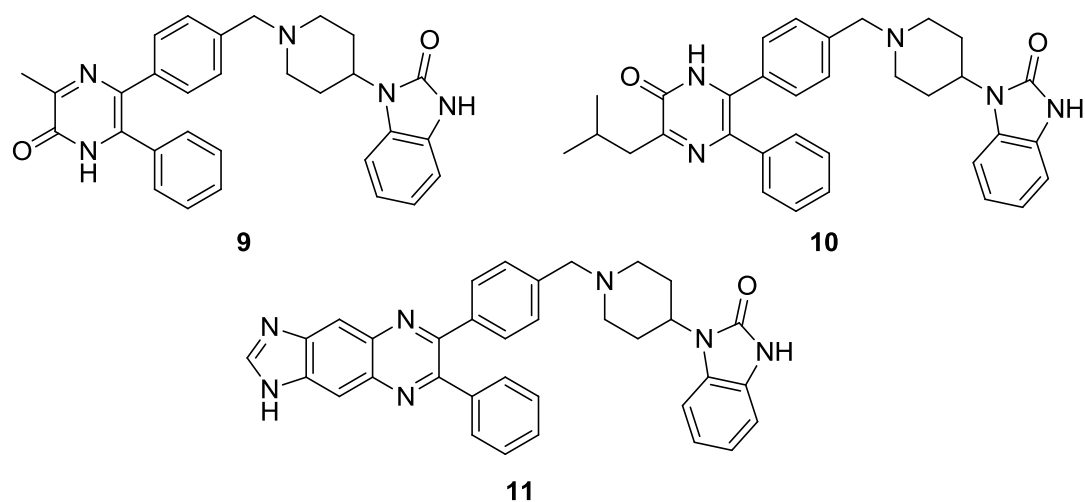


Figure 9

Allosteric Akt inhibitors of Akt1, Akt2 and a dual Akt1/2 inhibitor, inhibitors **9**, **10** and **11**.⁸⁶

These compounds were further studied using various tumour cell lines to test their ability to act as successful chemotherapeutic agents.⁸⁵ Once more, it was found that Akt1/2 dual inhibitors such as Akti-1/2 **11** act cooperatively with other chemotherapeutic agents and γ -radiation, whilst inhibitors of only Akt1 or Akt2 (isoform selective inhibitors **9** and **10**), show less cooperativity, suggestive of inhibition of both Akt1 and Akt2 being required for a maximal chemotherapeutic response.⁸⁵ Promisingly, these inhibitors could have a potentially large therapeutic window for cancer therapy as they only sensitised tumour cells, and not normal cells, to the apoptotic stimuli used in the tests.⁸⁵

Following these promising results, a mouse pharmacodynamic study was run to determine the ability of Akti-1/2 **11** to inhibit Akt phosphorylation *in vivo*. The results were encouraging, showing inhibition of Akt1 and Akt2 phosphorylation, whilst having no effect on Akt3 phosphorylation.^{85; 86} Unfortunately, further *in vivo* studies on this compound were ceased due to its poor physical properties, solubility and pharmacokinetics.⁸⁰

In general, incorporating the quinoxaline scaffold, as found in inhibitors **3**, **4** and **11**, results in poor physical properties and limited to no aqueous solubility and this translates to poor cellular potency.⁸⁹ As additional *in vivo* studies into allosteric Akt inhibition were desirable, some effort was put into modifying inhibitor **4** to obtain a dual Akt1/2 inhibitor with improved physical properties and cellular potency.⁸⁹ This was done by incorporating a more basic nitrogen into the core ring system, converting it from a quinoxaline core to a quinoline core (Figure 10).⁸⁹ Interestingly quinoline derivative **12** was

almost equal to inhibitor **4** with regards to *in vitro* inhibition of Akt1 and more potent with regards to inhibition of Akt2. On the contrary, quinoline derivative **13** possessed only a fraction of quinoline derivative **12**'s activity, suggesting an important role for the nitrogen in that location of the quinolone core.

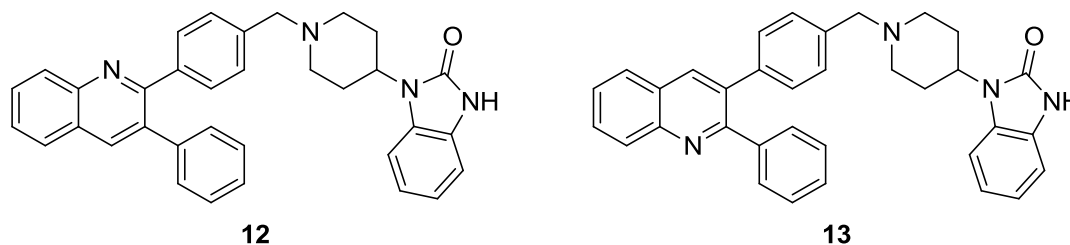


Figure 10

Quinoline derivatives **12** and **13** which displayed Akt inhibition.⁸⁹

The idea to increase basicity was taken even further with the quinoline core of quinoline derivatives **12** and **13** being replaced by a pyridine template. This also helped decrease the overall molecular weight of the compound, possibly further improving solubility.⁸⁹ This idea led to the eventual identification of tetrazolylpyridine derivative **14**, which is a potent dual Akt1/2 allosteric inhibitor with excellent aqueous solubility at low pH (Figure 11). It was, however, not cell permeable and afforded no inhibition of any of the Akt isoforms in a cell-based assay. This effect was ascribed to the zwitterionic character of the tetrazole and was verified by methylating the tetrazole to give compound **15**. The resulting compound was cell-permeable and active in the cell-based assay, but unfortunately displayed a drop in potency against both Akt1 and Akt2.⁸⁹ Optimisation of tetrazolylpyridine derivative **14** produced inhibitor **16**, lacking the piperidinyl benzimidazolone functionality and possessing an amine-substituted purine moiety in its place. This compound was slightly more potent than tetrazolylpyridine derivative **14**, but like inhibitor **14**, it also lacked cellular activity, further pointing to the tetrazole's involvement in this problem.⁹⁰

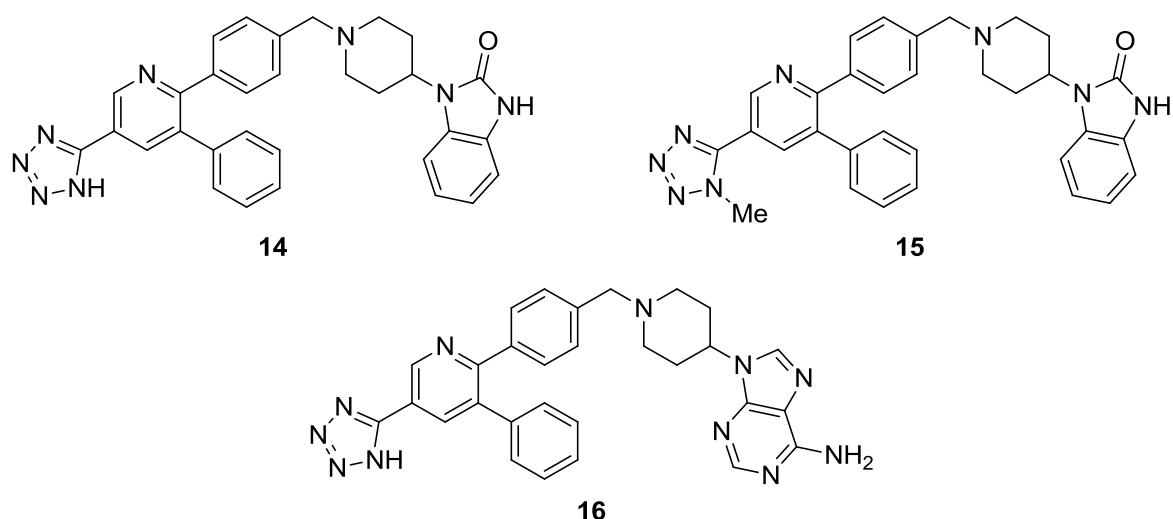


Figure 11

The tetrazolylpyridine derivative **14**, its methylated derivative **15** and a derivative lacking the piperidinyl benzimidazolone functionality seen in previous Akt inhibitors, **16**.^{89; 90}

Of note, most of the reported tetrazolylpyridine derivatives, such as inhibitor **14**, was more potent inhibitors of Akt2 than of Akt1, while the reverse was true of the inhibitors previously reported, with them inhibiting Akt1 more potently (Figure 6 to Figure 10). Although the compounds identified in this study possessed improved aqueous solubility and cell permeability and reduced molecular weight relative to the previous reported allosteric inhibitors, the nagging lack of cellular activity warranted further optimisation.^{89; 90}

The focus of the optimisation was to improve the overall physical properties of the compounds in order to gain cellular activity. The two strategies employed were to replace the acidic tetrazole moiety with non-acidic heterocycles and to modify the amine moiety on the eastern portion of the compound, replacing the piperidinyl benzimidazolone functionality with other promising heterocycles.⁹⁰ Firstly, the aminothiadiazole moiety was found to be a suitable replacement of the tetrazole moiety, facilitating the gain of cellular activity without compromising on potency. Further optimisation of the eastern amine moiety led to the discovery of inhibitor **17** (Figure 12), a compound having a similar eastern portion to inhibitor **16**, but with greater cellular activity.⁹⁰

This compound showed selectivity for Akt against an assortment of other kinases, including PKA and PKC and also displayed *in vitro* antitumour activity. In addition, it displayed a desired pharmacokinetic profile in dogs when administered intravenously.⁹⁰

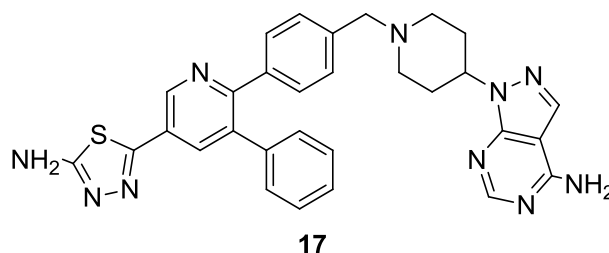


Figure 12

The result of optimisation of compound **16**, compound **17**.⁹⁰

2.2.2 More on Akti-1/2 **11**

Whilst the search for allosteric Akt inhibitors with improved physical properties, isoform selectivity and potency was continued, further studies were also conducted into how Akti-1/2 **11** binds to Akt. Calleja and co-workers established that Akti-1/2 **11** binds to Akt1 in a PH domain-induced cavity which is negligible in Akt3 (explaining its lack of efficacy against Akt3) and that a tryptophan amino acid residue (W80 in Akt1) located in the PH domain is an essential part of inhibitor binding.⁹¹ These findings are consistent with previous studies which showed the PH domain to be crucial for inhibitor binding.^{84; 85; 92} Additionally, they discovered that Akti-1/2 **11** works by locking Akt into its inactive “PH in” conformation, thus prohibiting the phosphorylation of its regulatory threonine and serine residues.⁹¹

In 2010, a co-crystal structure of Akt1 with Akti-1/2 **11** was reported by Brandhuber and colleagues.⁹³ This revealed Akt to be in an inactive state with the PH domain nestled between the N- and C-lobes of the kinase domain (“PH in”) and Akti-1/2 **11** binding to all three of these regions in a cavity as predicted earlier.^{91; 93} When comparing this Akti-1/2-bound crystal structure with a crystal structure of an ATP-competitive inhibitor bound to Akt, it can be seen that the PH domain in the inactive state fills part of the space occupied by the α C-helix and phosphorylated activation loop in the activated Akt kinase domain, thus sterically preventing the kinase domain from attaining an active conformation.⁹³ In addition, ATP binding site residues are seen to interact with PH domain residues and this further hinders activation of Akt by increasing the energy cost of changing conformation.⁹³

The aforementioned interaction between Akti-1/2 **11** and Trp80 is visible as an aromatic ring-stacking, with the imidazoquinoline’s aromatic components having π - π interactions with tryptophan’s aromatic ring.^{91; 93} There is also a hydrogen bond present between Akti-1/2 **11** and Ser205.⁹³ This interaction may be the reason why Akti-1/2 **11** is more active against Akt1 than Akt2, as Akt2 (and Akt3) has a threonine in that position, rendering it unable to hydrogen bond to Akti-1/2 **11** in a similar fashion.⁹³ There are several more hydrophobic contacts, as well as a number of polar

contacts, present between Akti-1/2 **11** and Akt1 and not all of these are necessarily present or as strong between Akti-1/2 **11** and Akt2.⁹³

From the crystal structure, the cysteine residue Cys296 can be seen positioned in close proximity to the aromatic ring of the benzimidazolone part of Akti-1/2 **11**. This makes Cys296 an attractive target for the design of a potential irreversible inhibitor which binds in the same site as Akti-1/2 **11**.⁹³

2.2.3 Further studies into allosteric Akt inhibitors

Another approach to drug discovery is called diversity-oriented synthesis and this was also utilised for the potential discovery of novel Akt inhibitors.^{92; 94} Diversity-oriented synthesis was applied to the canthine alkaloid template and two promising dual Akt1/2 allosteric inhibitors, inhibitors **18** and **19**, were identified from the library compounds synthesised using MAOS (Figure 13). These two compounds not only had good potency and induced apoptosis in tumour cells, but they also had the same inter-domain, PH domain-dependant and ATP non-competitive mode of inhibition as the compounds discussed previously in Sections 2.2.1 and 2.2.2.⁹²

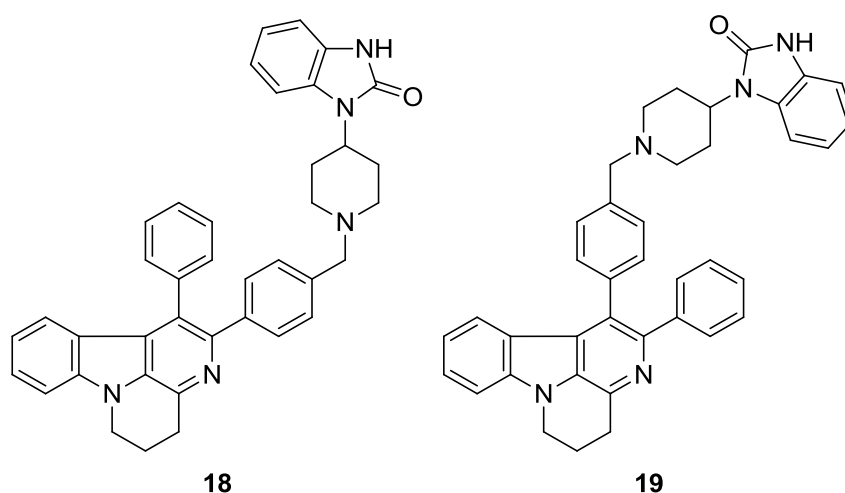


Figure 13

Unnatural canthine alkaloids **18** and **19**.⁹²

Following their successes discussed in Section 2.2.1, Merck continued their search for a promising allosteric Akt inhibitor, described in seven articles published during 2008 and 2009.⁹⁵⁻¹⁰¹ These will not be discussed in detail for the sake of brevity, but a similar system was followed as the one discussed before, with Akti-1/2 **11** being their starting point. During this study, some inhibitors displayed off-target activity, binding human Ether-a-go-go-Related Gene (hERG), a potassium channel.¹⁰⁰ This is extremely unfavourable as it can trigger the development of a fatal cardiac condition and some effort was made to modify their libraries so they did not display hERG activity.¹⁰⁰

These seven articles led to the eventual discovery of [1,2,4]triazolo[3,4-f][1,6]naphthyridine inhibitor **20**, which had improved cellular activity and thus improved inhibition of Akt1 and Akt2 in a mouse model, while having no discernable interaction with hERG (Figure 14).¹⁰¹

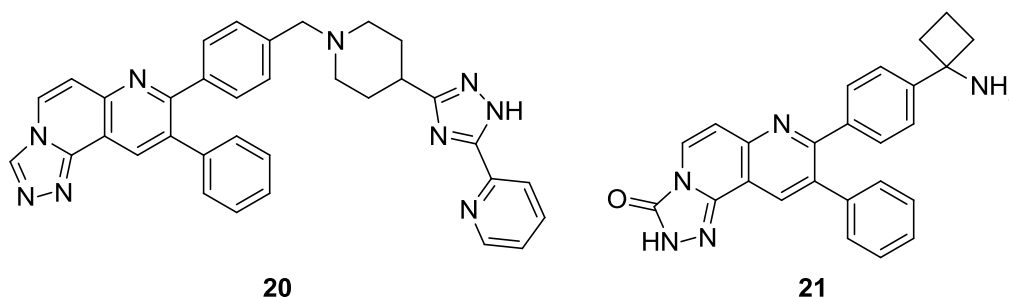


Figure 14

A [1,2,4]triazolo[3,4-f][1,6]naphthyridine inhibitor of Akt and MK-2206, **20** and **21**.¹⁰¹

From this study, MK-2206 **21** was designed and synthesised, a novel allosteric inhibitor of Akt (Figure 14).^{102; 103} MK-2206 **21** has a similar western side to inhibitor **20**, but lacks functionality on the eastern amine. Similar to the *gem*-dimethyl functionality of inhibitor **3**, it possesses a cyclobutyl functionality on the carbon *alpha* to the unsubstituted eastern amine. Inhibitor **20** passed its first-in-man phase I trial, showing efficacy against solid tumours in patients, whilst being well tolerated at the biologically active doses sufficient to inhibit Akt signalling.¹⁰⁴

MK-2206 **21** is equally potent towards Akt1 and Akt2, while being approximately 5-fold less potent against Akt3.¹⁰³ This compound was shown to be effective against tumour cell lines when used as a monotherapy as well as when used in combination with other chemotherapeutic agents (with targets other than Akt).¹⁰³ Preclinical trials found MK-2206 to work synergistically with the MEK inhibitor, AZD6244, the two used together having a greater antitumour effect than with each used individually.^{82; 105} It has passed phase I of other clinical trials, including as part of a combination therapy and more clinical trials are ongoing.^{104; 106; 107}

In another unrelated study, indole-3-carbinol **22** (Figure 15), was found to have anticancer properties against various cell lines. This compound is found in *Brassica* vegetables such as broccoli and cabbage and was identified as a novel allosteric Akt inhibitor.¹⁰⁸ Derivatives of indole-3-carbinol were synthesised and evaluated and (3-chloroacetyl)-indole **23**, was identified as a potent and specific inhibitor of Akt1 and Akt2, suppressing colon cancer growth both *in vitro* and *in vivo*.¹⁰⁸

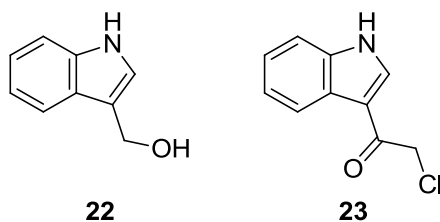


Figure 15

Allosteric inhibitors of Akt indole-3-carbinol and (3-chloroacetyl)-indole, **22** and **23**.¹⁰⁸

AstraZeneca investigated other allosteric Akt inhibitors similar to MK-2206 **21**, also lacking the key piperidinyl benzimidazolone structure found in potent inhibitors such as Akti-1/2 **11**.¹⁰⁹ Their findings were published in 2012 and included a range of diverse heterocyclic scaffolds designed to replace the quinoxaline based core of Akti-1/2 **11**. Their investigation of much of the literature reported above led to their design of a basic SAR for allosteric inhibition, **24**, and also a strategy, **25**, for the design of promising heterocyclic compounds, hoped to display Akt inhibition (Figure 16).¹⁰⁹

As discussed above in Section 2.2.1, the central scaffold often contains groups such as pyridines or quinolines with a sp^2 nitrogen atom being kept conserved throughout the more active inhibitors (although its function is still unknown).¹⁰⁹ The rest of the central scaffold can vary greatly, consisting of monocyclic, bicyclic or tricyclic ring systems. The lower pendant phenyl ring remains unsubstituted in all reported allosteric inhibitors of Akt, hinting at the small lipophilic pocket seen in the Akt1/**11** co-crystal structure discussed in Section 2.2.2. The upper phenyl ring frequently carries a basic benzylic amine functionality, but this can also be a cyclic amine, such as seen in Akti-1/2 **11**, indicating the tolerance of large substituents on the eastern side of the inhibitor.¹⁰⁹

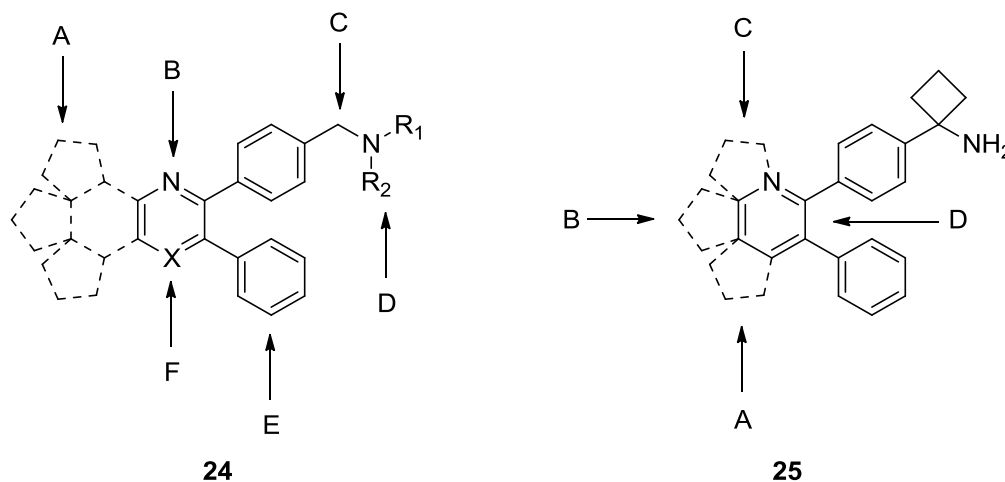


Figure 16

SAR of allosteric Akt inhibitors, **24** (A - Mono-, bi- or tricyclic linear fused rings, angular tricycles; B - N Critical; C - Substitution; D - Varied amines, cyclic, large size tolerated; E - Unsubstituted; F - N or C) and a representation of the potential inhibitors planned, **25** (A - Fusion “South”; B - Linear cycles with bridgehead N; C - Fusion “North”; D - Can also be a 5-membered ring).¹⁰⁹

For their heterocyclic scaffolds, they truncated Akti-1/2 **11** in a similar fashion as what was done with **21**, leaving the eastern amine unsubstituted, but with a cyclobutane functionality attached to the carbon *alpha* to it. They then designed a diverse array of heterocyclic cores, staying in line with what they perceived to be important as laid out in their SAR diagram, **24**.¹⁰⁹ Their designed heterocyclic compounds can be divided into three groups: scaffolds with a bridgehead nitrogen (scaffolds **26** and **27**), those with a fused “South” ring (scaffolds **28-31**) and the rest with a fused “North” ring (scaffolds **32** and **33**) (Figure 17). This strategy proved fruitful and they identified a number of scaffolds, not only with increased potency (the most potent being scaffold **29**), but also with improved physical properties when compared to many previous inhibitors.¹⁰⁹

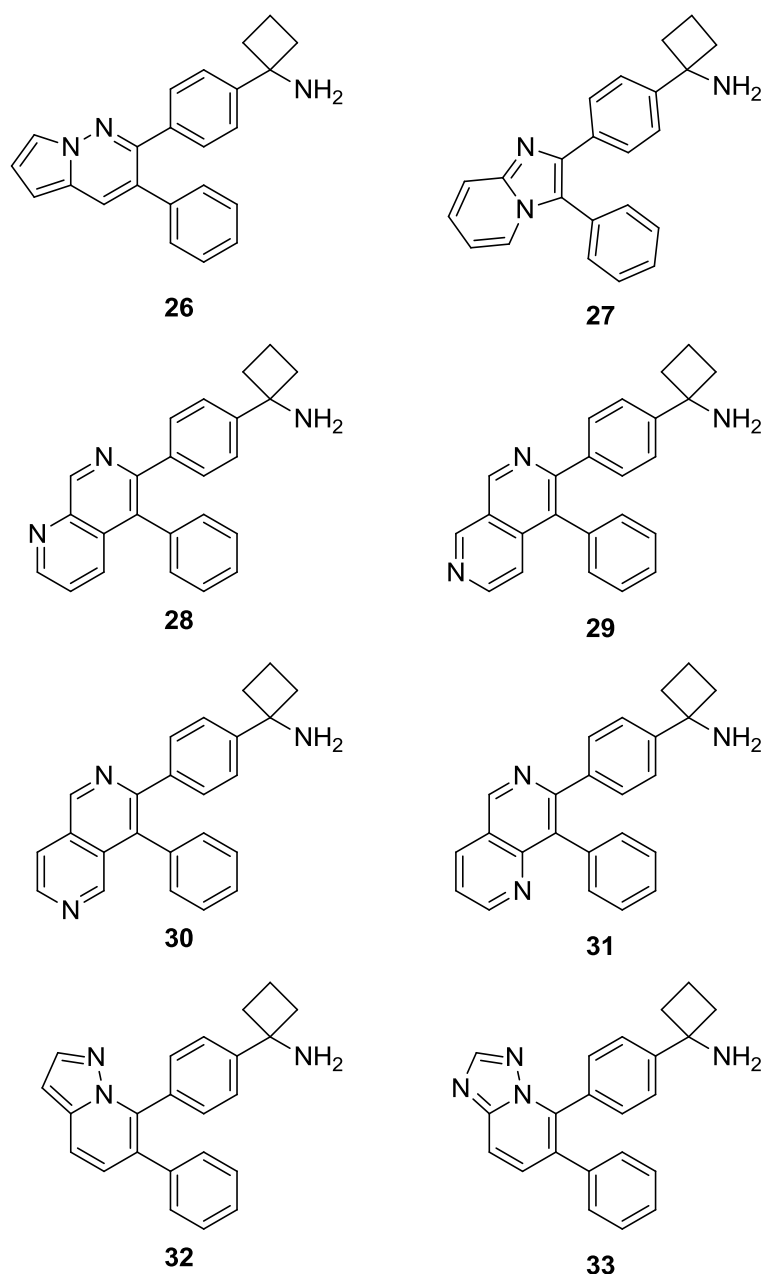


Figure 17

A number of the heterocyclic scaffolds reported by Kettle and co-workers, scaffolds **26-33**.¹⁰⁹

Of the data published, heterocyclic core structures **27-31** look most promising in terms of physical properties (mainly aqueous solubility), whilst still maintaining good potency.

Studies into finding more novel allosteric inhibitors continue, including kinase binding assays and computational studies, but this is outside the scope of this mini-review.^{110; 111}

2.3 THE TARGET COMPOUNDS

All of the data discussed in Sections 2.2.1 to 2.2.3 has led to the design of the first target compounds of this project. The aim was to combine the piperidinyl benzimidazolone functionality found in Akti-1/2 **11** with the most promising heterocyclic core structures reported by Kettle and co-workers, in order to have the prospect of synthesising a potent allosteric inhibitor of Akt with improved physical properties. In addition to this, an appropriate warhead such as acrylamide will be attached in different positions deemed most likely to interact with Cys296, thereby making a potential allosteric irreversible inhibitor of Akt. The original targets were represented by compounds **34** and **35** (and included compounds containing eastern portions like those of scaffolds **28**, **29**, **30** and **31**) (Figure 18).

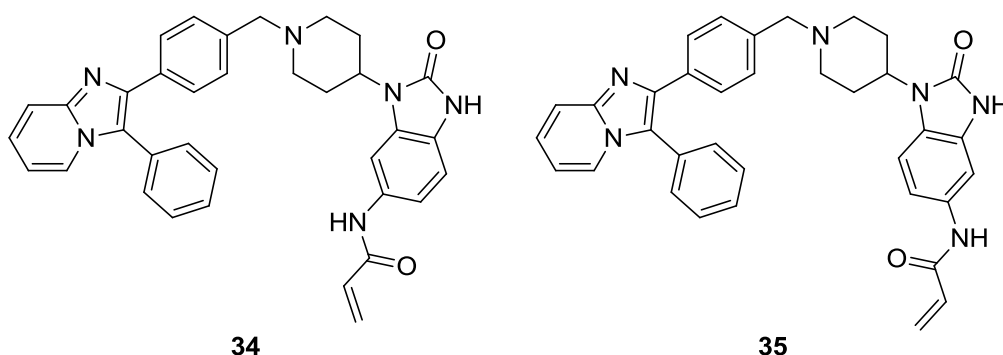


Figure 18

Representatives of the original target compounds, **34** and **35**.

Due to difficulties in synthesising the eastern side of the original target molecules, compounds **34** and **35**, (as discussed below in Chapter 3, the original idea was adapted with the new target compounds represented by compounds **36**, **37** and **38** (Figure 19). These deviate from the piperidinyl benzimidazolone functionality, instead having a 1,2,3-triazole connecting the piperidine to an aromatic ring bearing the acrylamide functionality, reminiscent of an eastern amine functionality reported by Merck Pharmaceuticals in compound **20** (Figure 14).⁹⁸

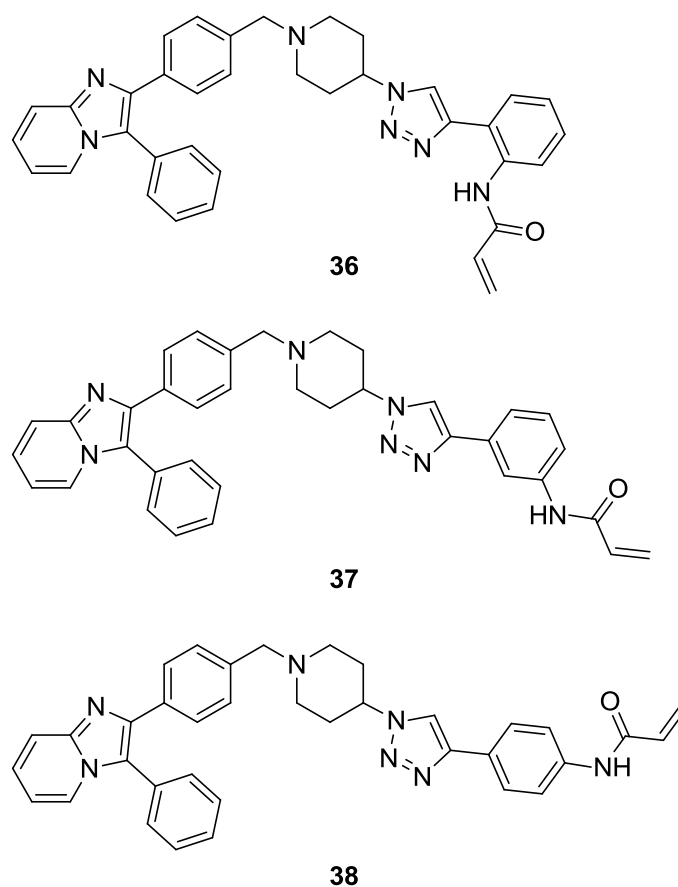


Figure 19

Representatives of the adapted target compounds, **36-38**.

2.3.1 The significance of the triazole and click chemistry

The triazole functional group has promising biological features and is an increasingly common structural unit of bioactive molecules, including antifungal, antibacterial, anti-allergic, anti-HIV, anti-tubercular, anti-inflammatory agents and anticancer agents.¹¹²⁻¹¹⁴ One such an example is carboxyamidotriazole **39**, a small molecule with anticancer properties, currently available on the market as a chemotherapeutic agent (Figure 20).^{112; 114-116}

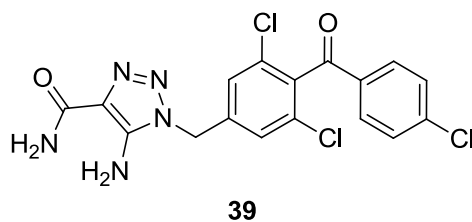
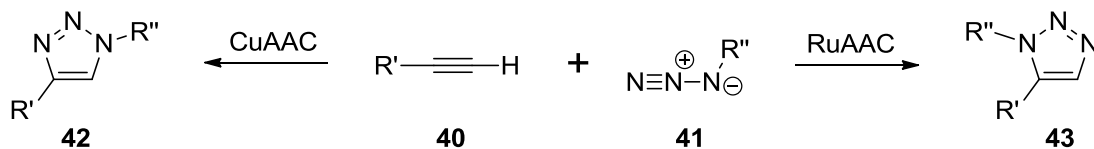


Figure 20

Carboxyamidotriazole, a calcium channel blocker with anti-tumour properties against certain cancers.¹¹⁴

1,2,3-Triazoles have a high dipole moment and are capable of hydrogen bonding as well as dipole–dipole and π -stacking interactions, potentially binding a biological target through either of these interactions.^{112; 117} To sweeten the deal, they are stable under various conditions including, acidic and basic hydrolysis and also severe reductive and oxidative conditions.¹¹⁷ A common click reaction, the copper(I)-catalysed azide alkyne cycloaddition (CuAAC), is employed to afford 1,4-disubstituted 1,2,3-triazoles (**42**) in very high yields from suitable azides (**41**) and terminal alkynes (**40**) (Scheme 1).¹¹⁴ In addition to the stability of the 1,2,3-triazole, the azides and alkynes used are also commonly stable in a variety of organic reaction conditions.¹¹³ While CuAAC is regioselective for the synthesis of 1,4-disubstituted 1,2,3-triazoles, a different click reaction, named ruthenium-catalysed azide alkyne cycloaddition (RuAAC), is regioselective for the synthesis of 1,5-disubstituted 1,2,3-triazoles (**43**).^{118;}

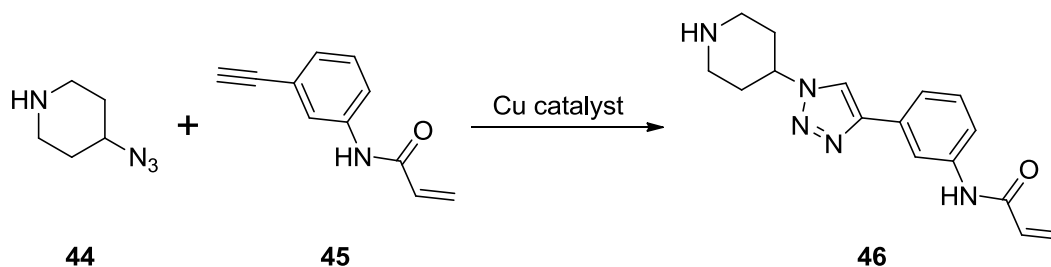
119



Scheme 1

Schematic representation of generic CuAAC and RuAAC, affording 1,4- and 1,5-disubstituted 1,2,3-triazoles respectively.^{119; 120}

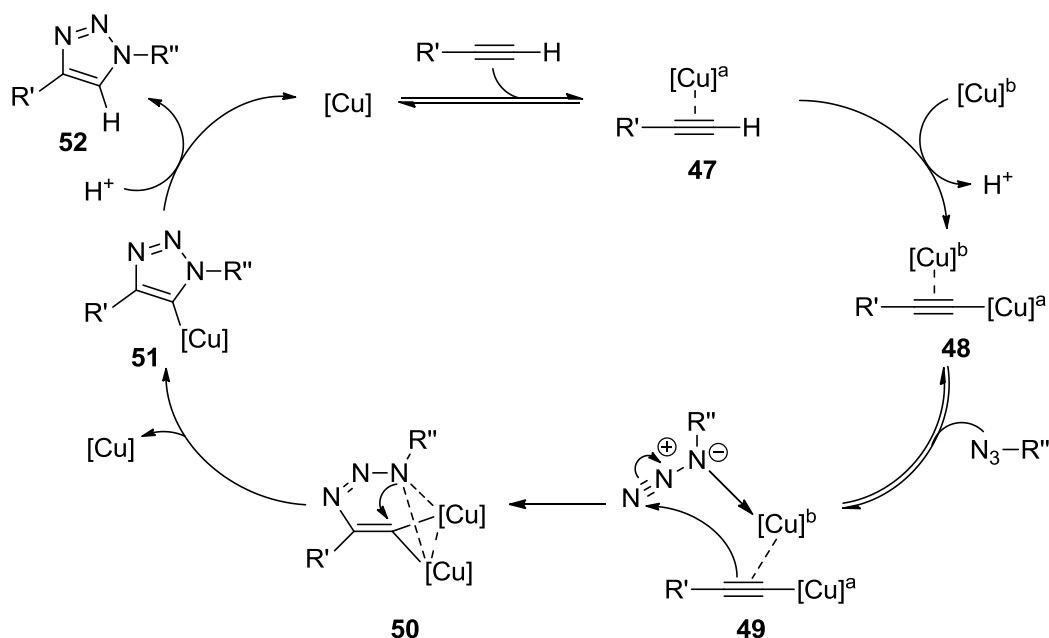
The synthesis of the eastern moieties, will each require the correct match of azide and alkyne, for example, 4-azidopiperidine **44** and *N*-(3-ethynylphenyl)acrylamide **45** will undergo CuAAC to afford *N*-{3-[1-(piperidin-4-yl)-1*H*-1,2,3-triazol-4-yl]phenyl}acrylamide **46** (Scheme 2).



Scheme 2

Copper(I)-catalysed azide alkyne cycloaddition towards an eastern moiety, compound **46**.

Using computational methods, Fokin and co-workers first suggested a possible mechanism for CuAAC in 2005.¹²⁰ This mechanism has since been updated, with Fokin and co-workers suggesting that the mechanism involves a dinuclear copper intermediate, previously not seen in the mechanism (Scheme 3).^{120; 121} The reaction sequence is initiated with the formation of a π -alkyne copper complex, intermediate **47**. This complex can then coordinate to a second copper atom, displacing one of its ligands, to form the crucial copper(I) acetylide **48**.¹²¹ Next, this copper(I) acetylide **48** reversibly coordinates an organic azide to form the complex **49**. The first C-N bond is then formed by nucleophilic attack at the terminal site of the azide from the β -carbon of the acetylide, producing the intermediate **50**. The second C-N bond is thus formed, resulting in ring-closure to give the triazolide **51**. Proteolysis of this triazolide **51** completes the cycle, affording the 1,4-disubstituted 1,2,3-triazole product **52** and the regenerated copper-catalyst.¹²¹



Scheme 3

The proposed reaction mechanism for CuAAC.¹²¹

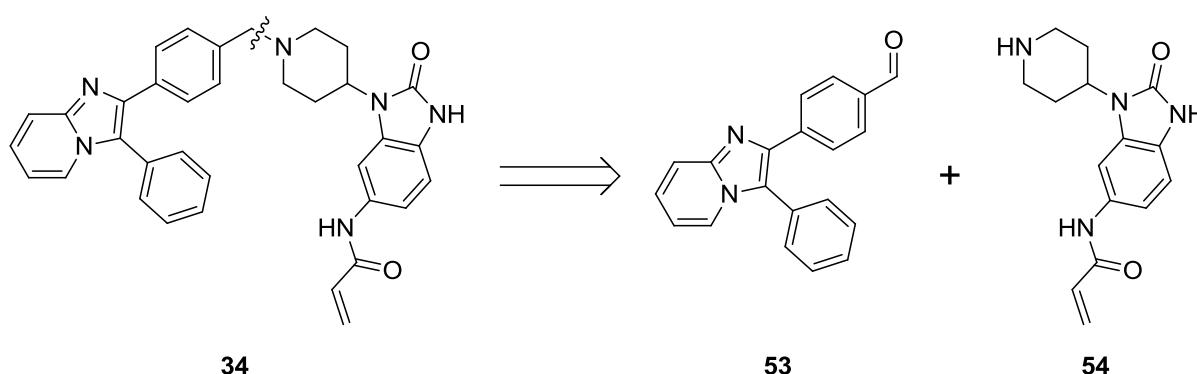
Herewith follows the synthetic endeavours which comprise this project.

CHAPTER 3 – TOWARDS THE PIPERIDINYL BENZIMIDAZOLONE

TARGET

3.1 RETROSYNTHETIC PLANNING – ORIGINAL ROUTE

With the target compounds **34** and **35** in mind, a possible synthetic route was laid out, which involved separate synthesis of the eastern and the western moieties, followed by linking them *via* reductive amination to afford the target compound (Figure 18 and Scheme 4). This would be achieved by incorporating an aldehyde on the western moiety, thereby allowing for the reductive amination with the secondary amine of the eastern moiety piperidine. This convergent approach would potentially save time as a number of the eastern and the western moieties could be synthesised which may then be connected in various combinations, enabling the synthesis of a maximum number of possible target compounds.

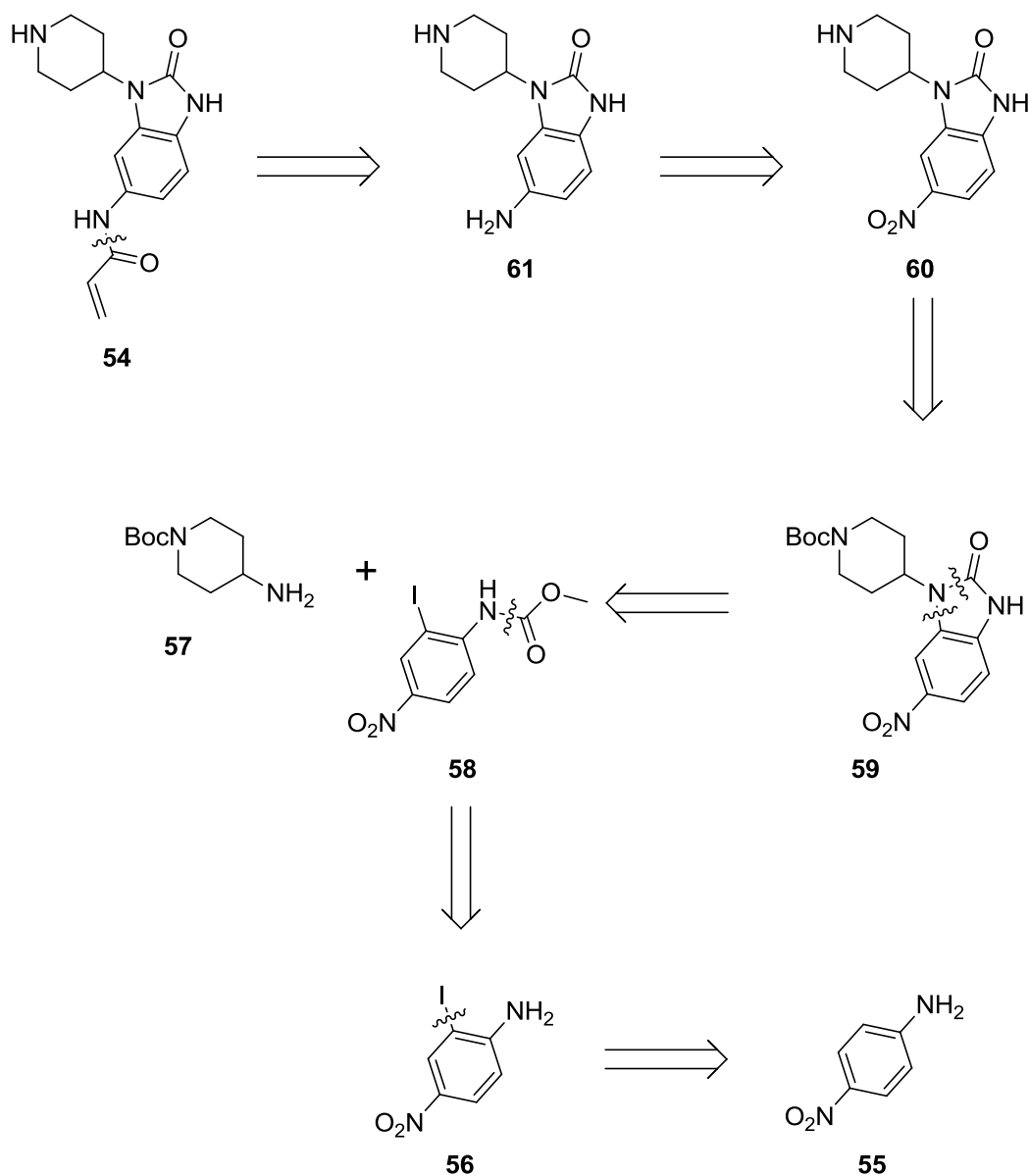


Scheme 4

Retrosynthetic step to achieve the target compounds.

The synthetic procedures towards the cyclobutylamine versions for the majority of the chosen western moieties were available in the literature and the original focus was in synthesising the eastern moieties.¹⁰⁹ Retrosynthetic analysis of the eastern moiety **54**, led to a fairly linear approach, beginning with commercially available 4-nitroaniline **55** (Scheme 5). Synthesis commenced by regioselective iodination of **55** to produce 2-iodo-4-nitroaniline **56**. Acylation of **56** would afford **58**, and this would be followed by a metal-catalysed coupling and cyclization reaction between **58** and commercially available *tert*-butyl 4-aminopiperidine-1-carboxylate **57** to afford the benzimidazolone derivative, *tert*-butyl 4-(6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)piperidine-1-carboxylate **59**. Subsequent Boc-deprotection of the protected amine and reduction of the nitro group could then afford 6-amino-1-(piperidin-4-yl)-1*H*-benzo[*d*]imidazol-2(3*H*)-one **61**. Finally, the intermediate could

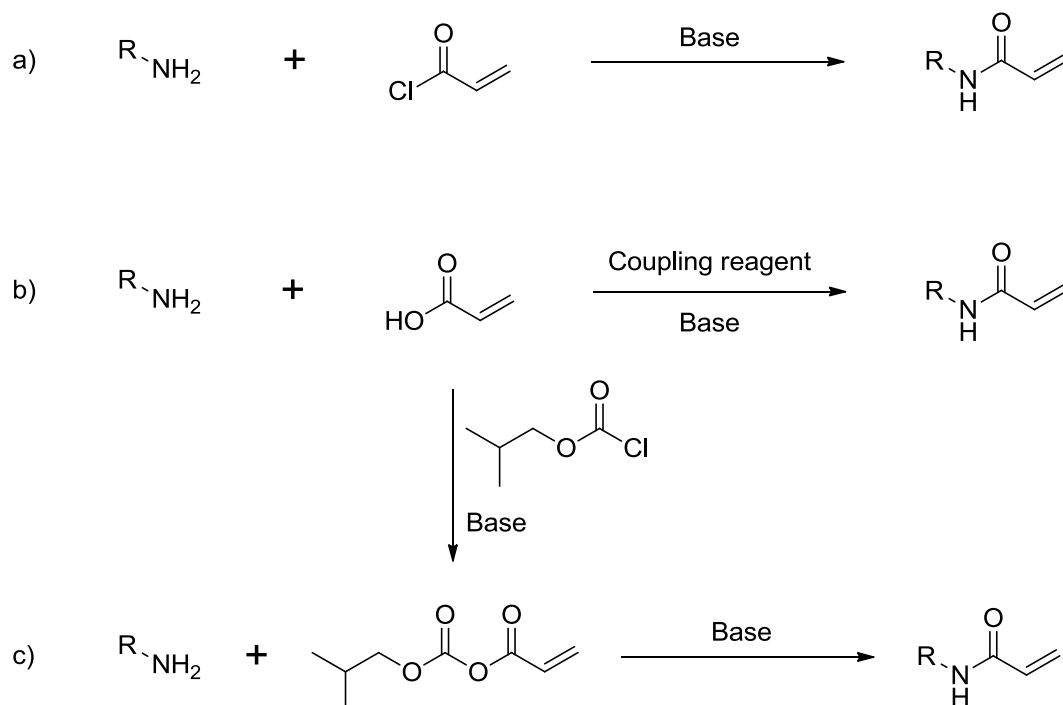
then be acylated to afford the completed eastern moiety, *N*-[2-oxo-3-(piperidin-4-yl)-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl]acrylamide **54**, ready for linking to the available western moieties.



Scheme 5

The retrosynthetic strategy to generate eastern moiety **54**.

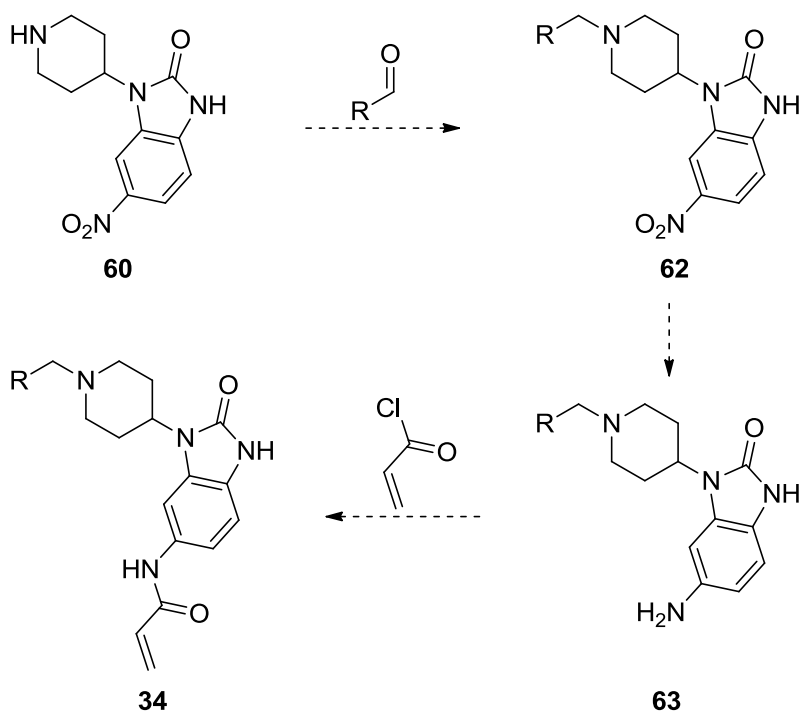
There are various methods to incorporate the acrylamide *via* an amine of which the most commonly used is by use of acryloyl chloride in the presence of base (Scheme 6, a).¹²²⁻¹²⁵ Other methods include coupling of an amine and acrylic acid in the presence of a coupling reagent such as *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride and a base (Scheme 6, b), or reacting an amine and reactive mixed anhydride, from acrylic acid and isobutyl chloroformate, in the presence of base (Scheme 6, c).¹²⁶ It was decided to employ the first method for acrylamide synthesis, using acryloyl chloride, as it was the more simple approach.



Scheme 6

Different possible techniques for incorporating an acrylamide into a molecule *via* an amine functional group.

There was a potential regioselectivity concern with this step as the acryloyl chloride could react with either the secondary amine (of the piperidine) or the aniline nitrogen of compound **61** (Scheme 5). The expectation was that it would favour the less hindered aniline nitrogen and only one molar equivalent of acryloyl chloride was to be used in this step, minimising unwanted side-products. If this reaction was not to proceed in the expected manner, the order of the reactions could have been exchanged, with the reductive amination to be done before introducing the acrylamide to eliminate the possibility of competing reactions between the two amines (Scheme 7). The reductive amination would be carried out between the western moiety and the uncompleted eastern moiety **60**. Following the reductive amination, the nitro functionality would then be reduced and the acrylamide incorporated, by use of acryloyl chloride in the same manner as planned, to afford target compound **34**.



Scheme 7

A proposal of how the acrylamide could be incorporated as the final synthetic step, where R represents a completed western moiety.

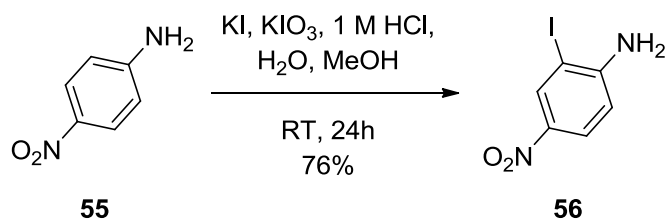
Another concern was potential reactivity problems with the acrylamide functionality and it was decided to follow common practice and incorporate it in the final step of the eastern moiety's synthesis (Scheme 5).¹²²⁻¹²⁶ This precautionary measure safeguards the acrylamide against unwelcome side-reactions, such as 1,4-addition (Michael addition) and possible reduction of the terminal carbon-carbon double bond. If the acrylamide was to be compromised during the final reductive amination step, the synthetic procedure could have been modified in the same manner as mentioned above, with acylation reserved for the last step of the synthesis (Scheme 7). This route was chosen as an alternative method, and not as the preferred route, due to its lack of efficiency (Scheme 6, Scheme 5 and Scheme 7).

The retrosynthetic schemes discussed here deal with the making the final compound, *N*-(2-oxo-3-{1-[4-(3-phenylimidazo[1,2-*a*]pyridin-2-yl)benzyl]piperidin-4-yl}-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)acrylamide **34** (Figure 18 and Scheme 5 and Scheme 7). For its regioisomer, *N*-(2-oxo-1-{1-[4-(3-phenylimidazo[1,2-*a*]pyridin-2-yl)benzyl]piperidin-4-yl}-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)acrylamide **35**, the planned synthetic route was similar as that of compound **34**, except that it started from 3-nitroaniline **70** and not 4-nitroaniline **55** (Scheme 5).

Herewith follows a discussion of the synthetic work towards **34** and **35**.

3.2 SYNTHETIC WORK – ORIGINAL ROUTE

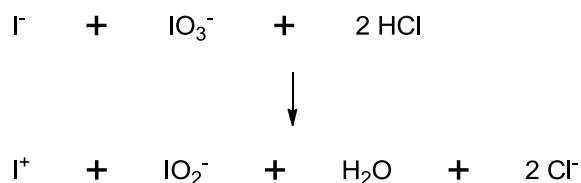
3.2.1 2-Iodo-4-nitroaniline



Scheme 8

Iodination of aryl **55** to afford aryl iodide **56**.

The first reaction was the regioselective iodination of 4-nitroaniline **55**, following a procedure by Adimurthy and co-workers.¹²⁷ This procedure involved the *in situ* formation of an electrophilic iodonium species through the oxidation of the iodide (Scheme 9). The iodonium could then iodinate the aromatic ring at the most appropriate position, or positions, by electrophilic aromatic substitution. As it is known for an aromatic system, the amine functionality is an *ortho*- and *para*-directing activator, whereas the nitro functionality is a *meta*-directing deactivator. In 4-nitroaniline **55**, the amine and nitro functionalities work together, both substituents promoting the iodination of the positions *ortho* to the amine. By generating only one equivalent of the electrophilic iodonium species, only one position would be iodinated, theoretically leading to 2-iodo-4-nitroaniline **56**.



Scheme 9

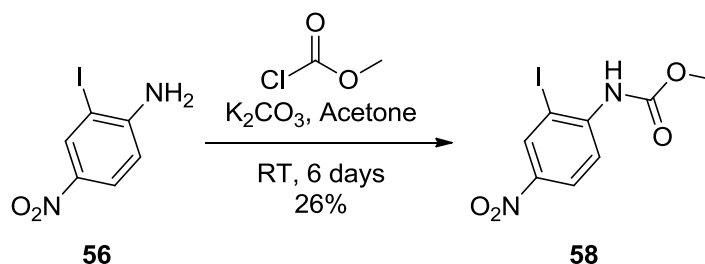
Formation of the iodonium species through oxidation of the iodide anion in acidic media.

Following the procedure by Adimurthy and co-workers, 4-nitroaniline **55**, potassium iodide and potassium iodate were added to a mixture of water and methanol (Scheme 8).¹²⁷ 1M hydrochloric acid was added and the reaction mixture was stirred at room temperature for three hours. The crude material was purified by recrystallisation from ethanol. Unfortunately, the reaction time was insufficient for a full conversion of starting material **55**, with only a 50% conversion noted. Increasing the reaction time from three to 24 hours allowed for a full conversion of starting material **55**, and product **56** was obtained in a reasonable yield of 76%. This did not compare well with the reported yield of 96%, but purification by crystallisation could have led to a significant loss of the uncrystallised compound in the mother liquor.¹²⁷ Purification by column chromatography, using

triethylamine as modifier, can therefore be considered as an alternative method to limit crystallisation losses.

The recorded ^1H and ^{13}C NMR spectra of 2-iodo-4-nitroaniline **56** correlated well to that of the literature, but there were slight deviations in chemical shifts as a result of using a different deuterated solvent.¹²⁸ In the ^1H NMR spectrum, the typical 1,2,4-substitution pattern was easily identified, confirming a single iodination in the required position.

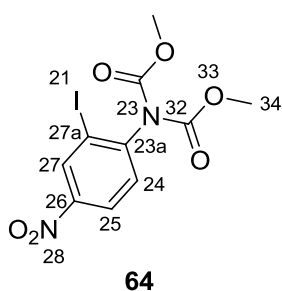
3.2.2 Methyl (2-iodo-4-nitrophenyl)carbamate



Scheme 10

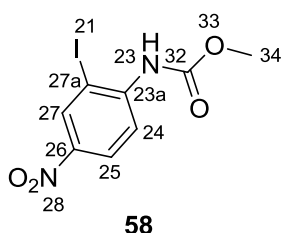
Acylation of aniline **56** to afford product **58**.

In the following step, the aniline of 2-iodo-4-nitroaniline **56** was *N*-acylated by using methyl chloroformate. No literature procedure was available for this reaction, but there was precedence for the acylation of starting material **56** using ethyl chloroformate.¹²⁹ From this, a reaction mixture of **56**, methyl chloroformate and potassium carbonate was stirred at room temperature in acetone.¹²⁹ Starting material **56** was found to be only partially soluble in acetone and the resultant turbid yellow reaction mixture was stirred at room temperature for 48 hours until complete consumption of the starting material, as monitored by TLC. This was followed by purification *via* column chromatography. NMR spectroscopic data of this compound did not match what was expected of methyl (2-iodo-4-nitrophenyl)carbamate **58** and analysis showed that the *N*-acylation had occurred twice, affording dimethyl 2-iodo-4-nitrophenylimidodicarbonate **64** in a yield of 92% (Scheme 12).



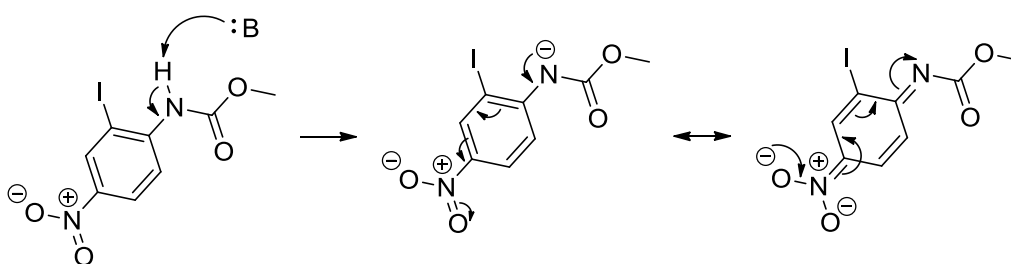
The ^1H NMR spectrum of this compound **64** had a similar aromatic region to that of starting material **56**, with the addition of the signal for the aliphatic methoxy protons, H_{34} . This singlet integrated for six protons, showing the symmetry of the identical acyl groups on the aniline nitrogen, N_{23} . The ^{13}C NMR spectrum had the trademark carbonyl signal for C_{32} at 151.2 ppm and a signal at 54.5 ppm, representing the two identical methoxy carbons, C_{34} . In addition, the mass spectroscopic analysis result of 402.9389 amu corresponded with the calculated value of 402.9403 amu for the sodium adduct.

Since the methyl chloroformate was initially used in excess, it was decided to attempt a single acylation using only 1.1 equivalents of methyl chloroformate. This reaction proceeded slowly and more methyl chloroformate was added to aid in starting material consumption. After a reaction time of six days, product **58** was obtained in a 26% yield (Scheme 10). In two additional runs, triethylamine was used as base with dichloromethane or tetrahydrofuran as solvent. The starting material readily dissolved in these two solvents, but neither of the reactions offered an improved yield. A final attempt was made using a procedure found for the methyl formylation of unsubstituted aniline, using pyridine as base and chloroform as solvent.¹³⁰ Unfortunately, this procedure did not work satisfactorily, affording a poor yield of less than 8%.



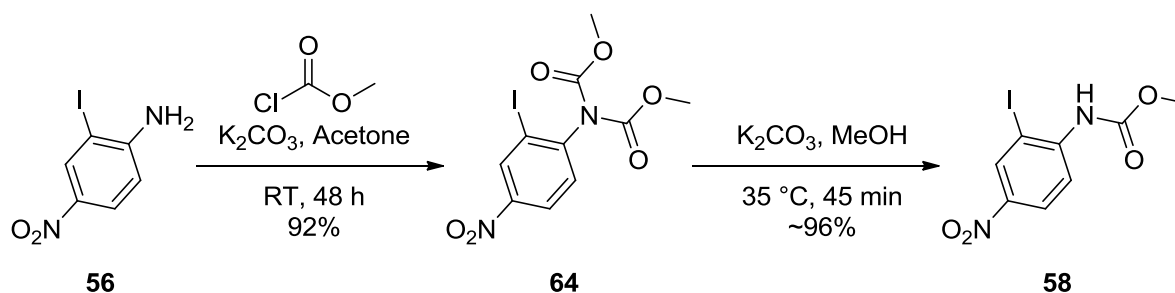
The ¹H and ¹³C NMR spectra of compound **58** correlated well with that of the literature and this time, the H₃₄ signal integrated for the correct three protons.¹³¹ In addition, the mass spectroscopic analysis result of 322.9542 amu corresponded with the calculated value of 322.9529 amu.

Obtaining compound **64** was a rather surprising result as one would not expect the carbamate nitrogen of **58** to be nucleophilic enough to perform a second acylation. However, in retrospect, it is not surprising that in the presence of the strong base, the now acidic carbamate proton could be abstracted, leading to a second nucleophilic attack of the carbamate nitrogen. Indeed, the anion formed in this manner could further be stabilised by resonance into the aromatic system (Scheme 11). This could also be seen with the methyl and ethyl formylation of substrates similar to compound **56** (but lacking the nitro functionality *para* to the amine) as these were reported to proceed more readily, affording the singly acylated products in yields ranging between 67 and 95%.^{129; 130}



Scheme 11

The stabilising effect of the electron-withdrawing nitro functionality on the deprotonated carbamate.



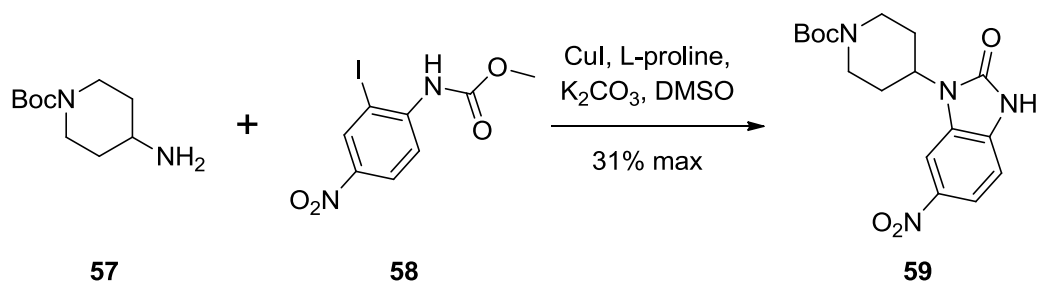
Alternate synthesis of product **58**.

This problem could not be solved satisfactorily in a reasonable timeframe and it was decided to work with the hurdle, using the dimethyl 2-iodo-4-nitrophenylimidodicarbonate **64** and selectively removing one of the acyl groups. This was carried out using a procedure for the removal of one Boc-protecting group from a double protected naphthylamine.¹³² Following this procedure, compound **64** was treated with three molar equivalents of potassium carbonate in methanol at 50 °C for 20 hours (Scheme 12).¹³² Unfortunately, the combined long reaction time and high temperature was too harsh for this system, resulting in both acyl groups being removed, affording 2-iodo-4-nitroaniline **56**. Different reaction conditions were thus tested in order to find the ideal combination for the selective removal of one acyl group (Table 3). It was determined that a four to one molar ratio of starting material **64** to potassium carbonate at 35 °C for 45 minutes, afforded the best results, with a yield of ca. 96%.

Table 3

Different reaction conditions tested for the removal of one acyl group from dimethyl 2-iodo-4-nitrophenylimidodicarbonate **64**.

Entry	Scale mg	Solvent mL	Compound 64 :Base	Temp °C	Time	Compound yields	
						58	56
1	430	20	1:3	50	20 h	-	quantitative
2	12	10	1:3	25	60 min	57%	35%
3	220	10	1:3	0	150 min	62%	38%
4	200	10	1:1	-14	170 min	8%	16%
5	56	10	4:1	50	19 h	54%	trace
6	100	10	4:1	50	30 min	72%	22%
7	100	10	4:1	35	45 min	~96%	trace (also trace 64)
8	340	30	5:1	35	3 h	50%	17%

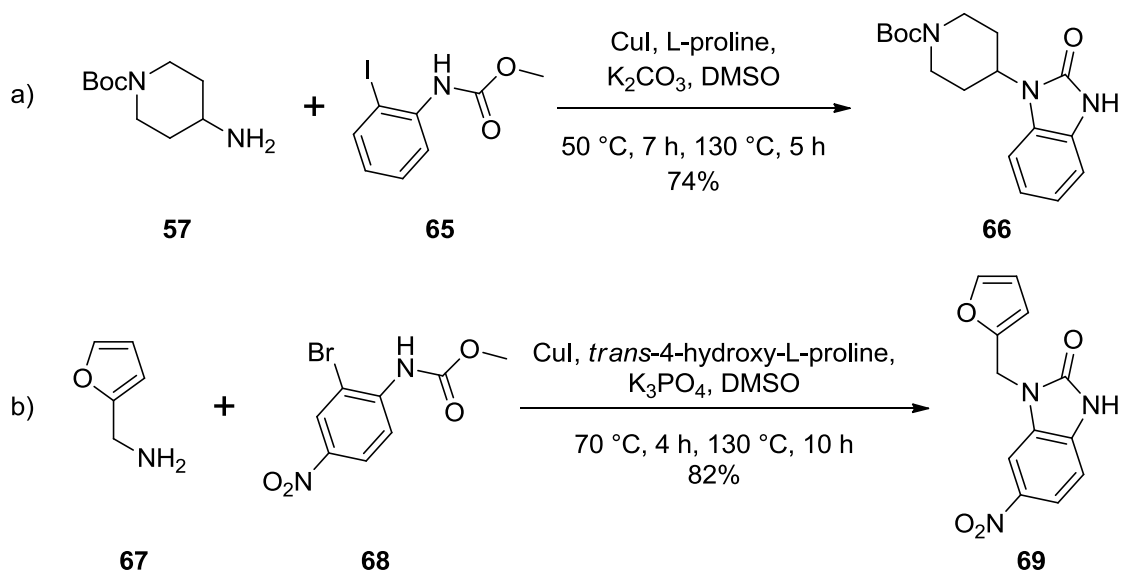
3.2.3 *tert*-Butyl 4-(6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)piperidine-1-carboxylate

Scheme 13

Cascade coupling/cyclisation using compounds **57** and **58** to afford benzimidazolone **59**.

The successful synthesis of methyl (2-iodo-4-nitrophenyl)carbamate **58** allowed for the attempted synthesis of the first compound containing the benzimidazolone functionality found in the eastern moiety **59**. The reaction used was from a procedure reported by Ma and co-workers.¹³³ These researchers reported a one-pot procedure for the synthesis of *N*-substituted 1,3-dihydrobenzimidazolones from different methyl *o*-haloarylcarbamates and amines. They reported that the electronic nature of the methyl *o*-haloarylcarbamate had little observable influence on the reaction, as it worked equally well with electron-rich and electron-deficient methyl *o*-haloarylcarbamates. A variety of functional group substituents were tolerated during the reactions, including ester, ketone, amide, nitro and silyl ether groups. Sterically hindered amines were noted to require longer reaction times.¹³³

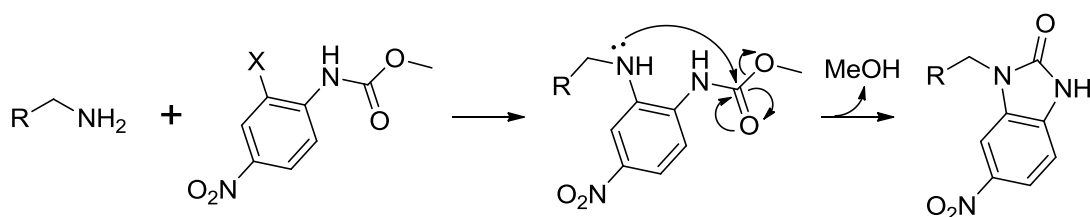
Ma and co-workers did not report doing the reaction between the substrates methyl (2-iodo-4-nitrophenyl)carbamate **58** and *tert*-butyl 4-aminopiperidine-1-carboxylate **57**, but they successfully used *tert*-butyl 4-aminopiperidine-1-carboxylate **57** with a different methyl *o*-haloarylcarbamate **65**, as well as the nitro-containing methyl *o*-haloarylcarbamate, methyl (2-bromo-4-nitrophenyl)carbamate **68** with a different amine **67** (Scheme 14, a and b respectively). Both of these reactions offered the products in good yields, similar to the other combinations reported, and this was considered promising for the combination employed to afford *tert*-butyl 4-(6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)piperidine-1-carboxylate **59**. As it can be noted with these two examples, they used a different set of reagents (ligand and base) and different conditions for the methyl *o*-iodoarylcarbamates and the methyl *o*-bromoarylcarbamates (Scheme 14).



Scheme 14

Examples of the synthesis of two different N-substituted 1,3-dihydrobenzimidazolones, following the procedure by Ma and co-workers.¹³³

To begin, the reaction mixture was heated to 50 °C, enabling the copper(I) iodide-ligand complex to catalyse cross-coupling with the amine and methyl *o*-haloarylcarbamate, forming an intermediate with the coupled amine in close proximity to the neighbouring carbamate (Scheme 15). The reaction mixture was then heated to 130 °C, and with the necessary elements in close proximity, cyclisation can occur, resulting in the release of a methoxy anion and formation of the desired N-substituted 1,3-dihydrobenzimidazol-one.¹³³ This reaction is therefore suitably called a cascade coupling/cyclisation reaction, and this term will be used to refer to it from here on.



Scheme 15

The sequence of events in the cascade coupling/cyclisation between a methyl *o*-haloarylcarbamate and an amine. The methoxy anion is protonated by a reaction intermediate.

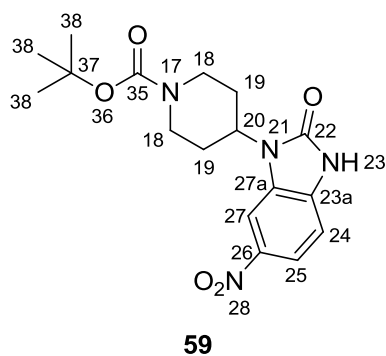
The initial attempt closely followed the procedure described by Ma and co-workers, with methyl (2-iodo-4-nitrophenyl)carbamate **58**, commercially available *tert*-butyl 4-aminopiperidine-1-carboxylate **57** and the other reagents added together in a Schlenk tube (Scheme 13).¹³³ The reaction was carried out at 50 °C for several hours, awaiting the complete consumption of starting material **58**, which did not occur even after 26 hours. Since none of the literature examples exceeded 22 hours at 50 °C for

this first part of the reaction procedure, we decided to similarly press forward and heat the mixture to 130 °C.¹³³ The reaction mixture was stirred at 130 °C for four and a half hours followed by heating at 80 °C for a further 16 hours with starting material **58** still visible by TLC. The crude material was subsequently purified by column chromatography to afford the product, *tert*-butyl 4-(6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)piperidine-1-carboxylate **59** in a poor yield of 6%.

The reaction yield needed to be improved significantly and different reaction conditions were tested (Table 4). It was found that heating the reaction at 50 °C for 10 minutes and then at 130 °C for 30 minutes (Table 4, entry 4) gave a similar yield to a higher catalyst loading at longer reaction times (Table 4, entry 3). The yield of the reaction was never significantly higher than the mole percentage of catalyst added and this suggested the possibility that the catalyst performed only one cycle before being deactivated. It was proposed that the reaction was possibly exposed to air, thereby compromising the catalyst cycle. This possibility was eliminated by using the freeze-pump-thaw technique to prepare the Schlenk tube with its reaction contents. This did not increase the reaction yield (Table 4, entry 6). The highest yield obtained was 31%, when the amine was added only after heating the reaction mixture for ca. five hours (Table 4, entry 5).

Table 4
 Different reaction conditions tested for the synthesis of **59**.

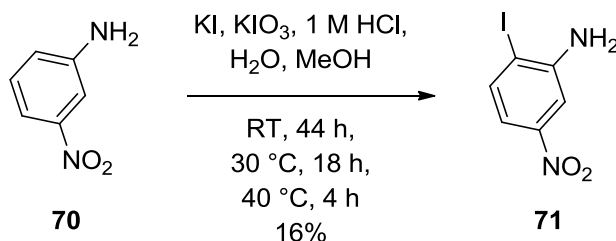
Entry	Compounds 57:58	Time at 50 °C	Time at 130 °C	% CuI	% L-proline	Yield
1	1:1	26 h	4.5 h (and 16 h @ 80 °C)	26	20	6%
2	1:1	47 h	4 h	30	60	12%
3	2:1	22 h	3.5 h	50	100	21%
4	1:1	10 min	30 min	20	40	20%
5	2:1	30 min @ 55 °C, 140 min @ 60 °C, 70 min @ 65 °C, 340 min @ 70 °C then 35 min @ 130 °C (add amine after first 55 min at 70°C)		50	100	31%
6	1:1	25 h	2 h	20	40	~23%



No literature data was found for compound **59**, but the ^1H and ^{13}C NMR spectra correlated well to what would be expected of this compound. In addition, the mass spectroscopic analysis result of 363.1666 amu corresponded with the calculated value of 363.1688 amu, confirming the isolation of product.

Unfortunately, conditions for affording a reasonable yield of compound **59** could not be determined and it was decided to abandon this route temporarily, instead focusing on obtaining compound **59**, by somewhat different routes. Before returning to that, the original synthetic work towards the regioisomer of target compound **34**, target **35**, is discussed (Figure 18).

3.2.4 2-Iodo-5-nitroaniline



Scheme 16

Iodination of aryl **70** to afford aryl iodide **71**.

With the iodination of 3-nitroaniline **70**, there was a slight challenge with clashing between the directing effects of the amine and nitro substituents (Figure 21, a). Due to steric considerations, one could safely assume that a single iodination would not occur at C_2 between the two substituents, but that still left three possible positions of iodination, C_4 , C_5 and C_6 . The question was thus: how would one distinguish between these three potential products?

If the C_5 position is iodinated, the product will be 1,3,5-substituted, affording a ^1H NMR spectrum with three doublet of doublets, each with long-range coupling to both neighbours on either side (Figure 21, b). Iodination of C_4 and C_6 , however, will give a 1,2,4-substituted aromatic ring, which will have the typical 1,2,4-substitution pattern, not unlike that of 2-iodo-4-nitroaniline **56** (Figure 21, c). A ^1H NMR spectrum would also be able to assist in the differentiation between iodination at C_4 and iodination at C_6 , with the doublet of doublets either more upfield (iodination at C_4) or more downfield (iodination at C_6), depending on its neighbouring functional group. This should help in determining which carbon was iodinated.

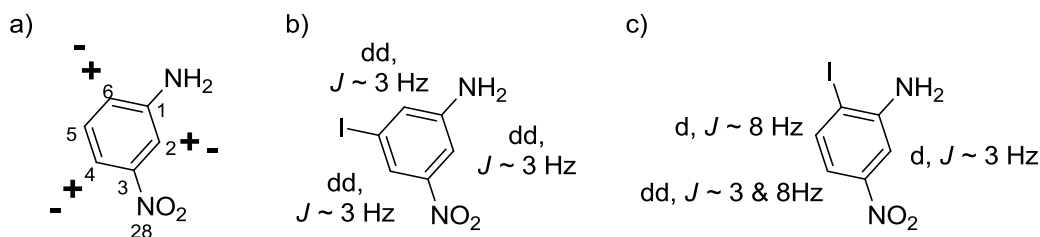
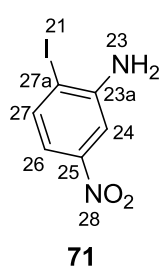


Figure 21

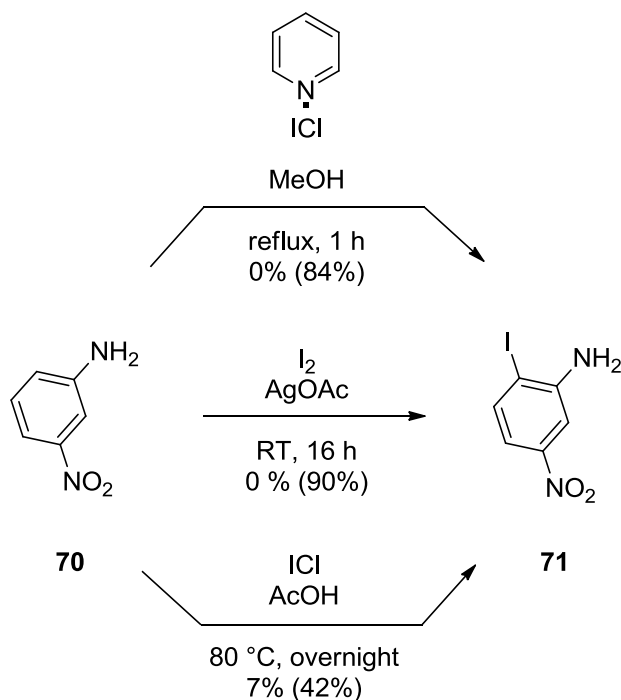
To consider with the iodination of 3-nitroaniline **70**: a) the directing effects of the nitro and amine substituents; b and c) the most likely iodination products and their predicted ¹H NMR spectra signals.

Originally, the same procedure used for iodinating 4-nitroaniline **55** was applied even though 3-nitroaniline **70** was not as electron-rich as aryl **55** and the other compounds iodinated by this procedure (Scheme 16).¹²⁷ It was expected that this could cause aniline **70** to be less readily iodinated than those reported by Adimurthy and co-workers and this was confirmed when the reaction mixture needed to be heated to 40 °C to encourage full consumption of the starting material **70**. A poor yield of 16% was obtained and two subsequent attempts to increase the yield, resulted in an equivalent yield of 17%.



The ¹H NMR spectrum of product **71** had a 1,2,4-substitution pattern, pointing to the correct carbon being iodinated. The ¹³C spectrum of this compound also supported iodination, with one of the carbon signals found in an upfield position at 91.0 ppm. These spectra correlated well to that of the literature, supporting iodination at the correct position.¹³⁴

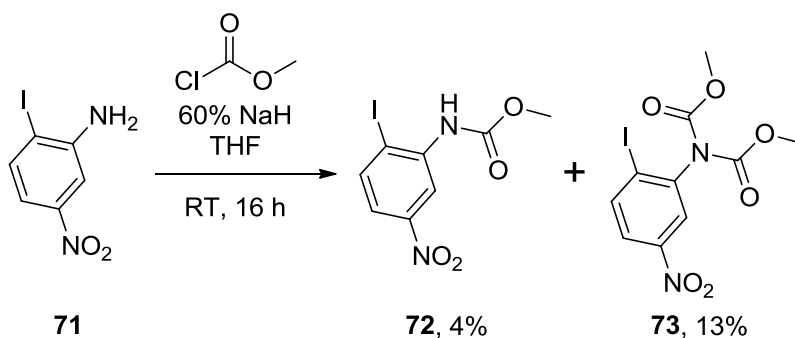
Due to the limited success with this procedure, it was decided to employ other procedures reported in the literature, specifically for the iodination of aniline **70** to afford 2-iodo-5-nitroaniline **71**. Three procedures were tried, and not one of them gave a satisfactory yield (Scheme 17).¹³⁴⁻¹³⁶ With all of these reactions, the complete consumption of starting material was never achieved, pointing to the expected problem of iodinating an aromatic position which is deactivated by a nitro functionality *para* to it.



Scheme 17

Different procedures attempted for the iodination 3-nitroaniline **70**. Reported literature yields are given in brackets.¹³⁴⁻¹³⁶

3.2.5 Methyl (2-iodo-5-nitrophenyl)carbamate

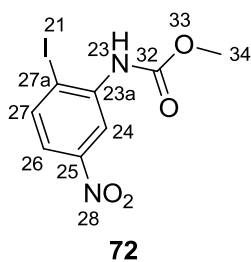


Scheme 18

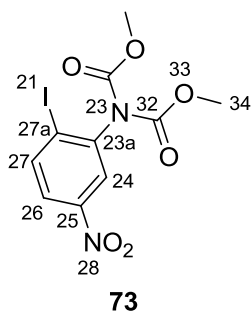
Acylation of aniline **71** to afford product **72** and side-product **73**.

As with 2-iodo-4-nitroaniline **56**, no literature precedent for the acylation of 2-iodo-5-nitroaniline **71** using methyl chloroformate was found. The search was therefore extended to similar compounds and a procedure was obtained from a patent in which 2-chloro-5-nitroaniline was acylated using methyl chloroformate and sodium hydride.¹³⁷ The product was isolated in a yield of 37%, again lower than what one would expect from a relatively simple procedure. Following this procedure, starting material **71**, methyl chloroformate and 60% sodium hydride in mineral oil was added to tetrahydrofuran

(Scheme 18).¹³⁷ The reaction mixture was stirred at room temperature for 16 hours and starting material was still visible on TLC, but the reaction was stopped for fear of possible double acylation, as in the previous acylation procedure [see Section 3.2.2]. The crude material was purified using column chromatography and the product, methyl (2-iodo-5-nitrophenyl)carbamate **72**, was isolated in a poor yield of 4%. As before, the double acylated compound, dimethyl 2-iodo-5-nitrophenylimidodicarbonate **73**, was also isolated, this time in a lower yield of 13%.



The ¹H NMR spectrum of compound **72** displayed the expected singlet at 3.71 ppm for the three methoxy protons, H₃₄. A ¹³C NMR spectrum could not be obtained as an insufficient amount of compound was isolated.



The ¹H and ¹³C NMR spectra of compound **73** were as was expected, including the important ¹H NMR singlet at 3.68 ppm, integrating for six protons.

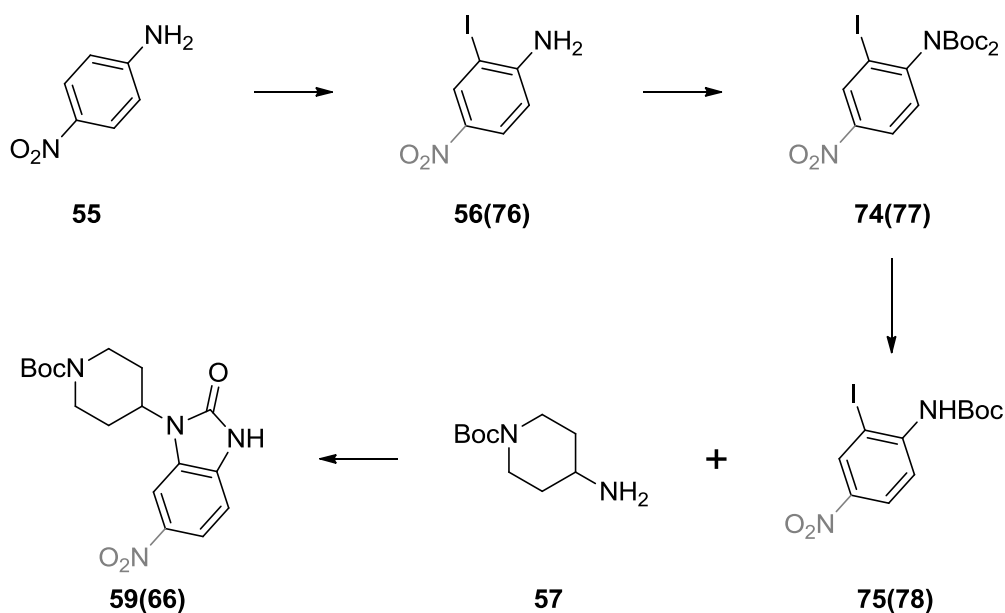
As with the synthesis of methyl (2-iodo-4-nitrophenyl)carbamate **58**, this reaction also requires optimisation, since both of these reactions have limited consumption of starting material, long reaction times and the requirement to add excess methyl chloroformate. The nitro functionality may also have played a role in this reaction, but it would not have had the same effect as before, as it is *meta* to the amine functionality and not *para*. Full characterisation of these compounds will be required if this route is to be undertaken again.

3.3 EFFORT TOWARDS THE CASCADE COUPLING/CYCLISATION PRODUCT **59**

The major hurdle in the original synthetic route (Scheme 5), was the cascade coupling/cyclisation towards *tert*-butyl 4-(6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)piperidine-1-carboxylate **59** [see Section 3.2.3]. Efforts were thus focussed on either modifying the reaction conditions in search of an increased yield or by modifying the original route in order to work around this problem.

Different routes were designed, with the first on the assumption that the coupling does occur in a reasonable amount and that the problem lies at the cyclisation reaction. Looking at the proposed mechanism, one can reason that the small methoxy anion could attack the carbonyl carbon of the product **59**, initiating the reverse reaction towards the non-cyclised intermediate (Scheme 15). This could be minimized, or even prevented, if the leaving group was a more bulky compound, such as *tert*-butoxy anion. The first idea, Plan A1, was therefore to synthesise *tert*-butyl (2-iodo-4-nitrophenyl)carbamate **75** and then attempt the cascade coupling/cyclisation procedure described by

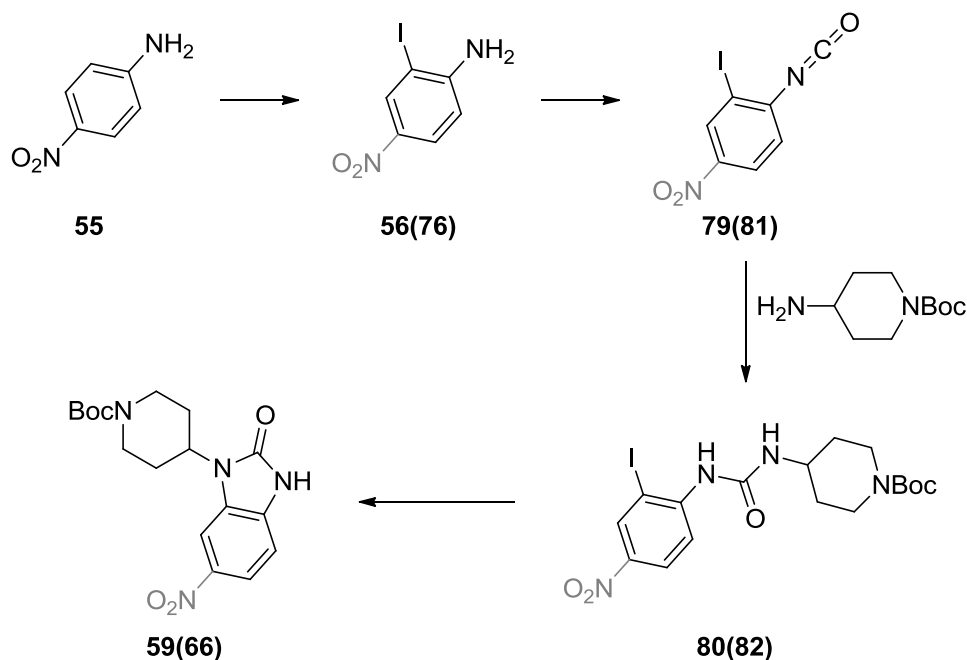
Ma and co-workers, using this substrate in the place of the original *o*-haloarylcarbamate **58** (Scheme 19).¹³³ The second idea, Plan A2 was formed on the assumption that the coupling reaction did not occur as readily as hoped, in part, due to the presence of the electron-withdrawing nitro functionality. The methyl *o*-haloarylcarbamate was thus also to be synthesised without the nitro functionality (compound **78**), in order to determine whether this change would improve the yield of the cascade coupling/cyclisation reaction. This adaption was called Plan A2.



Scheme 19

Plan A, the first modified plan for the synthesis of *tert*-butyl 4-(6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)piperidine-1-carboxylate **59**. In the absence of the grey nitro functionality, benzimidazolone **66** will be synthesised (these compound numbers in brackets). These are called Plan A1 and A2, respectively.

In addition to this, a different route, Plan B1, was also investigated, not going *via* the cascade coupling/cyclisation, but rather connecting compounds **58** and **57**, which then only need to undergo a coupling reaction, reported by Li and co-workers, to give the product **59** (Scheme 20).¹³⁸ This route was also followed using the nitro-free starting material, in order to determine its effect on the reaction, and was called Plan B2.

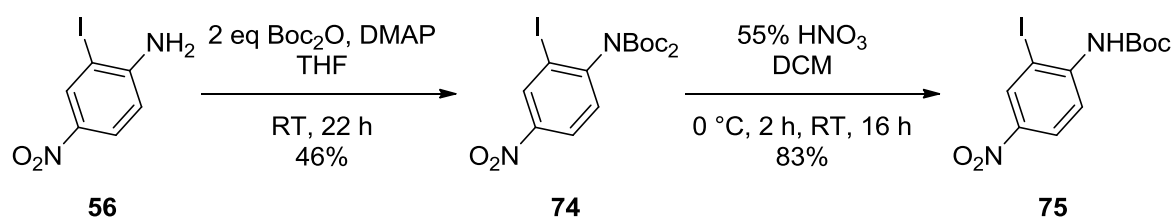


Scheme 20

Plan B, the second modified plan for the synthesis of **59**. In the absence of the grey nitro functionality, compound **66** will be synthesised (compound numbers in brackets). These are called Plan B1 and B2, respectively.

For all of the following reactions, the 2-iodo-4-nitroaniline **56** synthesised earlier was used [see Section 3.2.1].

3.3.1 Plan A1, synthesis of *tert*-butyl (2-iodo-4-nitrophenyl)carbamate

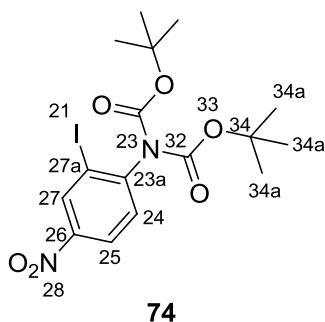


Scheme 21

Two-step synthesis of Boc-protected aniline **75** from aniline **56**.

Due to the unwanted double acylation experienced with aniline **56**, using methyl chloroformate, it was decided to start with a double Boc-protection of the aniline, following a procedure by Wensbo and co-workers [see Section 3.2.2].¹³⁹ This was to be subsequently followed by the selective removal of one *tert*-butyloxycarbonyl group (Boc-deprotection), in a procedure described by Strazzolini and co-workers, to afford the single Boc-protected product, *tert*-butyl (2-iodo-4-nitrophenyl)carbamate **75**.¹⁴⁰

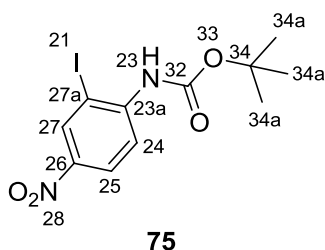
The procedure from Wensbo and co-workers was thus followed, stirring 2-iodo-4-nitroaniline **56** with di-*tert*-butyl dicarbonate and 4-dimethylaminopyridine in tetrahydrofuran (Scheme 21).¹³⁹ The reaction mixture was stirred at room temperature for 22 hours, until starting material was consumed according to TLC. The product was then purified by means of column chromatography to afford the target compound **74** in a yield of 46%. This does not compare well with their reported yield of 99%, but they did not purify their product, instead making use of the crude material for the following reaction.¹³⁹



The ¹H and ¹³C NMR spectra of compound **74** correlated well to that of the literature.¹⁴¹ In the ¹H NMR spectrum, the presence of the Boc-protecting groups was indicated, with the singlet, for H_{34a}, at 1.42 ppm. The ¹³C NMR spectrum of compound **74** further confirmed successful Boc-protection by the addition of the appropriate carbon signals.

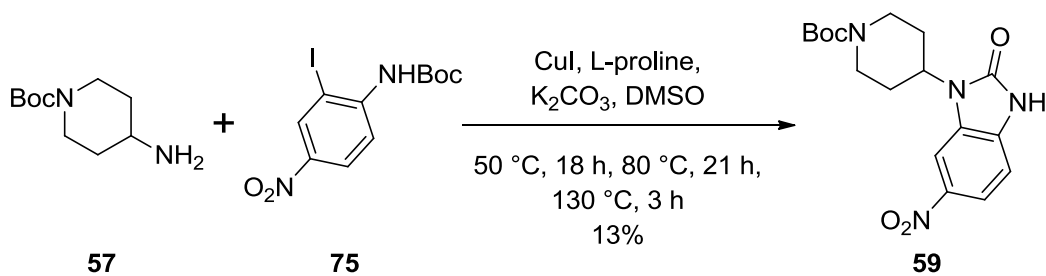
For the selective Boc-deprotection of compound **74**, the procedure from Strazzolini and co-workers was followed, treating compound **74** with nitric acid in dichloromethane (Scheme 21).¹⁴⁰ 55% Nitric acid was used in the place of the reported 100% nitric acid, lengthening the reaction time from two hours at 0 °C, to 16 hours at room temperature. The resulting crude material was purified and the title compound **75** was isolated in an 83% yield. This compares reasonably well to the reported yield of 94%.¹⁴⁰ The overall yield for these two reactions was 38%.

The reaction was repeated with the reaction time extended to three days for the first step. The prepared crude material **74** was then used for the second step, with the reaction mixture stirred at 40 °C for 75 min, followed by twelve hours at room temperature. For this sequence, the isolated yield over the two steps was 58%. This 20% increase could be attributed to the increased reaction times and temperature, and the elimination of the purification step.



The ¹H and ¹³C NMR spectra correlated well to that of the literature.¹³⁹ The successful removal of only one Boc-protecting group was indicated by integration of the H_{34a} signal at 1.56 ppm, now integrating for three protons and not six as before.

3.3.2 Plan A1, synthesis of *tert*-butyl 4-(6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)piperidine-1-carboxylate



Scheme 22

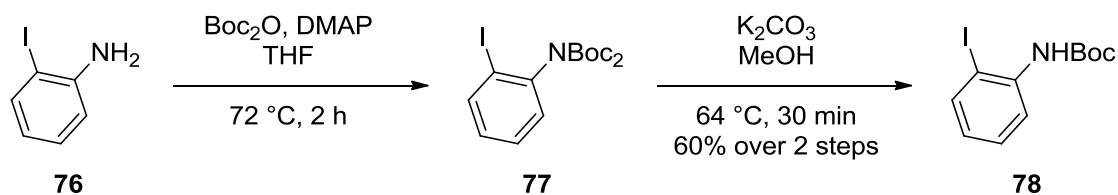
Cascade coupling/cyclisation of compounds **57** and **75** to afford benzimidazolone **59**.

Compound **75** was subsequently used in the cascade coupling/cyclisation procedure from Ma and co-workers to afford product **59**.¹³³ The reaction mixture was initially stirred at 50 °C for 18 hours, but the starting material **75** was not fully consumed during this time and the temperature was increased to 80 °C for a further 21 hours. The starting material was still not completely consumed after this time, but new spots were visible by TLC, indicating that some starting material may have been converted into the coupled intermediate. The temperature was then further increased to 130 °C for three hours, followed by purification of the crude mixture to offer the product **59** in a poor yield of 13%.

When this reaction was compared to that of the previous ones using the intended starting material **58**, it could be seen that it corresponded with entry two (of Table 4) with regards to yield [see Section 3.2.3]. This result was promising as this reaction used 11 mole percent (11 mol%) of copper(I) iodide, whereas entry two (of Table 4) used a threefold amount of the catalyst, with the reaction times and temperatures being reasonably similar. Following this promising result, the reaction was repeated using one equivalent of copper(I) iodide and two equivalents of L-proline, with the intent to substantially increase the yield. Unfortunately, this was not the case, resulting in a yield of only 12%. The increased amounts of the catalyst and ligand did not affect the yield, but seemed to have increased the rate of the reaction, since the reaction was complete in less than five hours. The iodine-free compound, *tert*-butyl (4-nitrophenyl)carbamate, was also isolated (39%), indicating that the coupling reaction was initiated with a good amount of starting material, but that something interfered with its completion, affording compound lacking the iodine of the starting material and the amine it was meant to be coupled to.

With these results in mind, the next step was to repeat the reaction with the compound lacking the nitro functionality, in order to compare the results.

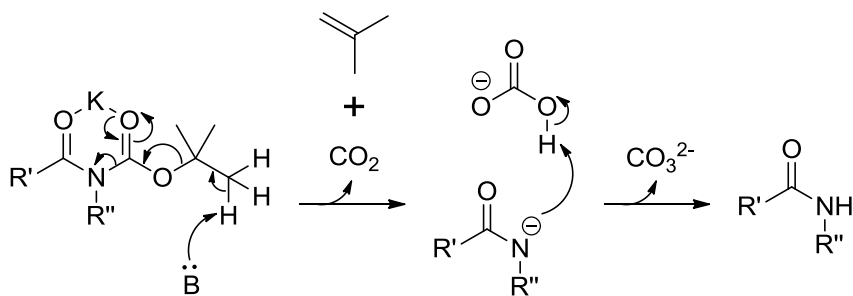
3.3.3 Plan A2, synthesis of *tert*-butyl (2-iodophenyl)carbamate



Scheme 23

Two-step synthesis of Boc-protected aniline **78** from aniline **76**.

A procedure by Darnbrough and co-workers was followed for the synthesis of compound **78**.¹⁴² The Boc-protection procedure was similar as before, but was done at a higher temperature, thereby enabling the reaction to proceed at a faster rate. The single Boc-deprotection was different from before, with Darnbrough and co-workers reportedly using potassium carbonate in methanol to remove the normally base-resistant protecting group. This procedure differs from the acidic conditions used previously to synthesise compound **75** [see Section 3.3.1]. A possible mechanism for this base-mediated Boc-deprotection was reported by Mohapatra and Durugkar (Scheme 24).¹⁴³ To start, the potassium cation and the two Boc-protecting groups form a six-membered chelate. The base (methoxy anion) can then deprotonate one of the methyl groups, leading to the loss of isobutene and carbon dioxide and having the mono-Boc-protected compound as product. The beauty of this method of deprotection, is that the second Boc-protecting group will not be removed, as the six-membered chelate is needed as starting point.

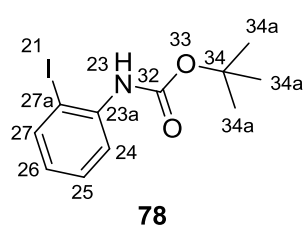


Scheme 24

Suggested mechanism for selective Boc-deprotection under basic conditions.¹⁴³

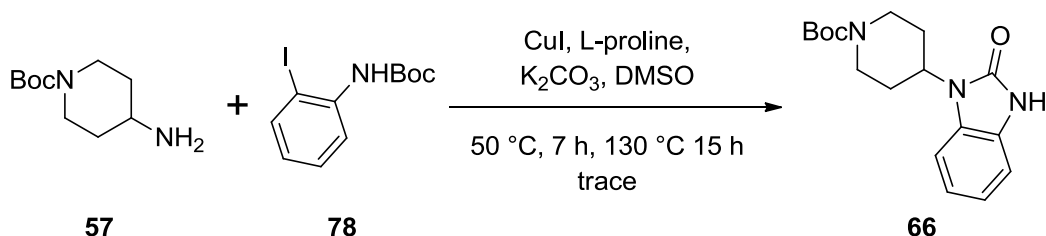
The literature procedure was followed with no deviations for the synthesis of *tert*-butyl (2-iodophenyl)carbamate **78**, stirring 2-iodoaniline **76**, di-*tert*-butyl dicarbonate and catalytic 4-dimethylaminopyridine together in tetrahydrofuran at $70\text{ }^\circ\text{C}$ (Scheme 23).¹⁴² According to TLC, the starting material was consumed within 105 minutes. Learning from previous results, the crude material was used for the following reaction, thereby minimising loss of product [see Section 3.3.1]. The deprotection step was also performed following the literature procedure, adding the crude material **77** to potassium carbonate and methanol.¹⁴² The reaction mixture was heated at $62\text{ }^\circ\text{C}$ for

four hours, stirred at room temperature for 14 hours, followed by heating at 62 °C for 23 hours. Once starting material was consumed according to TLC, the crude material was taken and purified *via* column chromatography. Product **78** was isolated in a yield of 45%, lower than the reported yield of 76%.¹⁴² The procedure was repeated, heating the first reaction mixture at 72 °C for 120 minutes and the second reaction mixture at 64 °C for 30 minutes. This time, the product **78** was isolated in a more acceptable yield of 60%, indicating that the first reaction should perhaps be left for longer, allowing for the complete consumption of starting material **77**, including compound below the sensitivity of TLC.



The ¹H and ¹³C NMR spectra of compound **78** correlated well to that found in the literature.¹⁴⁴ In the ¹H NMR spectrum, one could see the expected singlet for the Boc-protecting group protons, H_{34a}, at 1.55 ppm, integrating for nine protons. The ¹³C NMR spectrum had one absent aromatic signal (from approximately 148.5 ppm according to literature), suspected to be the quaternary C_{23a}.¹⁴⁴

3.3.4 Plan A2, synthesis of *tert*-butyl 4-(2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)piperidine-1-carboxylate



Scheme 25

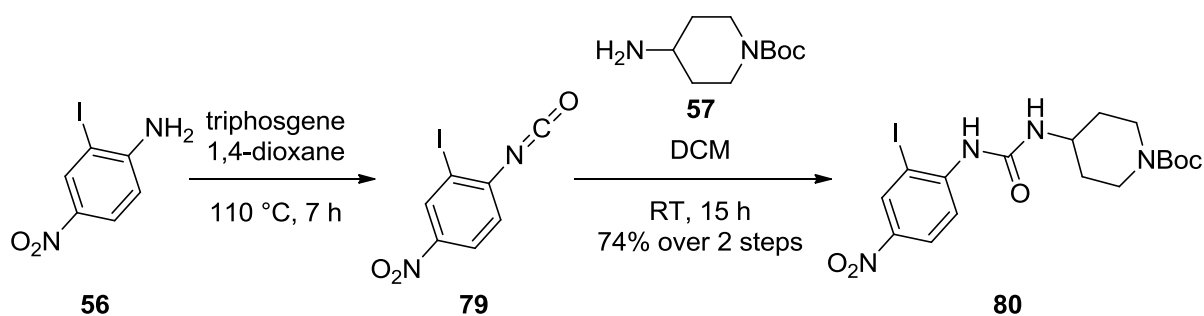
Cascade coupling/cyclisation of compounds **57** and **78** to afford benzimidazolone **66**.

Again, the procedure of Ma and co-workers was followed for the crucial cascade coupling/cyclisation reaction. They reported that this procedure afforded a yield of 74% when it was done with methyl (2-iodophenyl)carbamate (similar to *tert*-butyl (2-iodophenyl)carbamate **78**) and the amine **57**, heating the reaction mixture at 50 °C for seven hours and then at 130 °C for five hours.¹³³ The reaction conditions were slightly adapted, with the reaction components heated at 50 °C for seven hours, followed by 15 hours 130 °C. Starting material was not consumed after five hours at 130 °C and the reaction mixture was stirred at 130 °C for an additional ten hours. The crude material, containing various compounds according to TLC, was purified and only a trace amount of product was isolated. The mass spectroscopic result of 318.1814 amu corresponded with the calculated value of 318.1818 amu. No further characterisation was done as there was not sufficient amount of product **66**.

This result is confusing to some degree as the Boc-containing starting material reacted relatively successfully when using the nitro-containing compound **75** [see Section 3.3.2]. The reaction was not repeated, but it could be that for this particular reaction, it was exposed to air, interfering and leading to side-effects and almost no isolated product **66**. This is supported by the observation that the reaction mixture turned blue during the heating process, indicating possible oxidation of Cu(I) to Cu(II).

This concluded the implementation of Plan A1 and A2, with limited success.

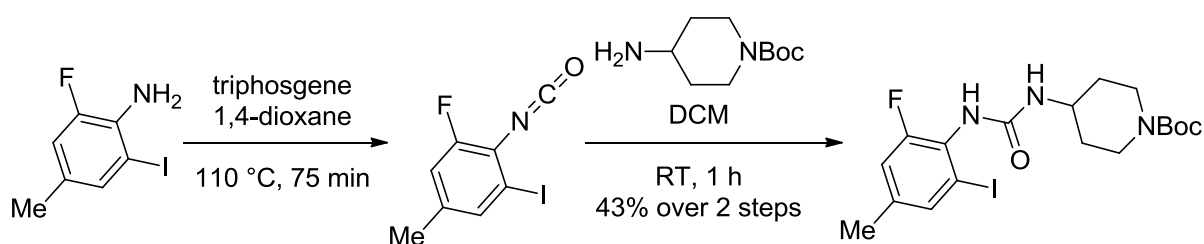
3.3.5 Plan B1, synthesis of *tert*-butyl 4-[3-(2-iodo-4-nitrophenyl)ureido]piperidine-1-carboxylate



Scheme 26

Synthesis of product **80** from aniline **56**.

For the start of Plan B, 2-iodo-4-nitroaniline **56** and *tert*-butyl 4-aminopiperidine-1-carboxylate **57** were connected *via* an isocyanate intermediate following a procedure from a patent by Budzik and co-workers (Scheme 26 and Scheme 27).¹⁴⁵



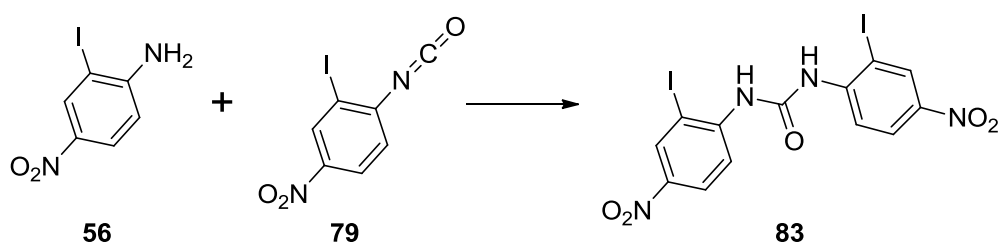
Scheme 27

The procedure reported by Budzik and co-workers.¹⁴⁵

Isocyanate compounds are generally synthesised and used for the next reaction step without purification, perhaps chiefly for the fact that an isocyanate is a good electrophile, prone to unwanted side reactions.^{145; 146} The isocyanate, 2-iodo-1-isocyanato-4-nitrobenzene **79** was synthesised using the literature procedure, stirring 2-iodo-4-nitroaniline **56** and triphosgene together in 1,4-dioxane at 110 °C (Scheme 26).¹⁴⁵ This reaction did not proceed as readily as the literature example and the reaction

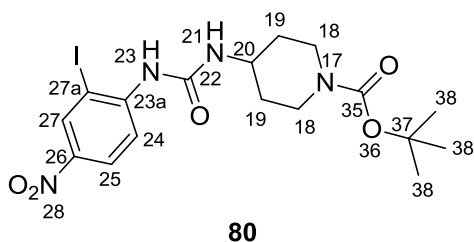
mixture was heated for a total 21 hours at 110 °C, with starting material still present according to TLC. The unpurified isocyanate **79** was used for the next reaction step and was stirred with the amine **57** in dichloromethane. This step was also monitored by TLC and was stirred at room temperature for 17 hours, which is also a longer reaction time compared to the one hour as mentioned in the literature. The crude material was purified by column chromatography and compound **80** was isolated in a 42% yield, comparable to that obtained in the reported procedure.¹⁴⁵

This procedure was repeated with the reaction mixture in the first step stirred at 110 °C for seven hours and in the second step at room temperature for 15 hours. This time, the product **80** was isolated in an increased yield of 74%. The main problem with this reaction was the incomplete consumption of starting material **56** for the first step, possibly due to the aniline being deactivated by the nitro functionality *para* to it. From these results it can, however, be seen that the reaction should not be left for too long either, with the shorter reaction time of seven hours affording more product compared to the longer reaction time of 21 hours. This phenomenon could be explained by the fact that the starting material **56** has the ability to react with the intermediate **79** to form the side-product, 1,3-bis(2-iodo-4-nitrophenyl)urea **83** (Scheme 28). This would be expected to account for more loss of compound **79** if the reaction was left for longer, thus explaining the increase in yield when left for a shorter amount of time.



Scheme 28

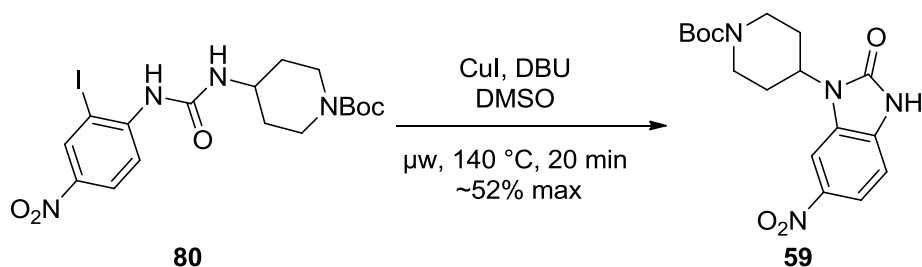
A possible explanation for the low yield of product **83** when the reaction is carried out for a longer time period.



The ¹H and ¹³C NMR spectra of compound **80** looked like expected, namely a combination between the spectra of the starting materials, compounds **56** and **57**. In the ¹³C NMR spectrum, the one piperidine signal, C₁₈, was absent, possibly obscured by the dimethyl sulfoxide peak, but the rest correlated well with the expected signals, including the

two downfield carbonyl carbon signals for C₂₂ and C₃₅. IR spectroscopy also revealed two carbonyl C=O stretches, at 1710 and 1649 cm⁻¹. Finally, the mass spectroscopic analysis result of 491.0778 amu corresponded with the calculated value of 491.0791 amu.

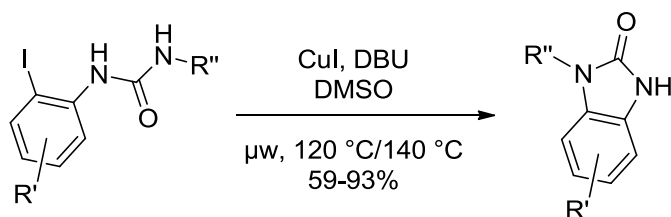
3.3.6 Plan B1, synthesis of *tert*-butyl 4-(6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)piperidine-1-carboxylate



Scheme 29

Reaction of compound **80** to afford benzimidazolone **59**.

For this reaction, a microwave coupling procedure from Li and co-workers was followed (Scheme 30).¹³⁸ Like Ma and co-workers with their reported cascade coupling/cyclisation reaction, Li and co-workers found that both electron-donating and electron-withdrawing functional groups were tolerated.^{133; 138} The researchers predominantly used aromatic amines, but found that aliphatic amines were also successfully coupled if the temperature was increased from 120 °C to 140 °C.¹³⁸



Scheme 30

General procedure for the intramolecular cyclisation, where R' can be an electron-donating or electron-withdrawing functional group and R'' can be an aromatic or aliphatic amine.¹³⁸

The literature procedure was followed, heating the reaction mixture at 120 °C for 20 minutes (Scheme 29).¹³⁸ The crude material was purified by column chromatography to afford the product **59** in a yield of 46%. ¹H NMR spectroscopy indicated the presence of a small amount of impurity. The compound **59** could not be further purified due to the polar nature of the product and the small amount of sample available. The reaction was repeated, this time with at 140 °C for twenty minutes, as recommended for aliphatic amines.¹³⁸ Purification of the crude material afforded the product in a maximum yield of 52%. This too had impurities as detected by ¹H NMR spectroscopy. For this second run, 45% 2-iodo-4-nitroaniline **56** was recovered, potentially indicating that the high temperature was too harsh for this reaction, leading to the decomposition of starting material **80**. Three more reaction conditions were thus tried, each having a lower temperature than 120 °C (Table 5). Due to the small scale of these reactions and the difficulty involved with isolating small amounts of polar compound, the crude material of these reactions were all combined with the isolated product of the first two reactions and

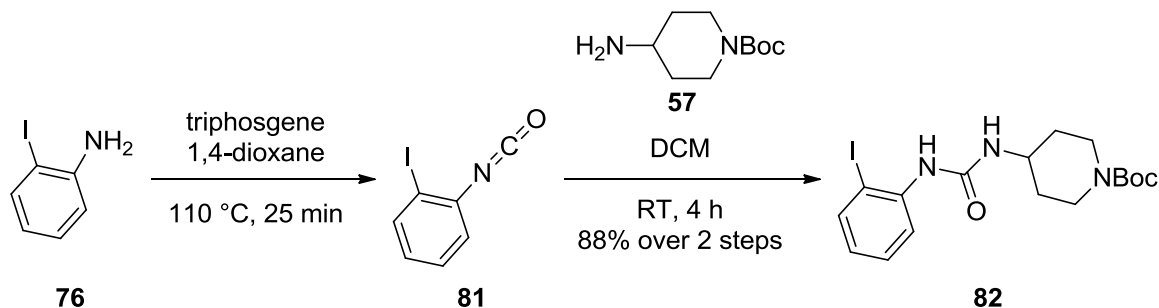
purified with column chromatography. An overall yield of 8% was obtained. For all five of these reactions, TLC analysis indicated the presence of possible decomposed starting material **80**, such as that identified by the second reaction. However, if this reaction was optimised by carrying the reactions out on a larger scale, it could be a viable method for the preparation of *tert*-butyl 4-(6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)piperidine-1-carboxylate **59**.

Table 5

Different reaction conditions tested for the synthesis of *tert*-butyl 4-(6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)piperidine-1-carboxylate **59**. The overall isolated yield was 8% and no individual yields were obtained for entries 3 to 5.

Entry	Scale (mg)	Heating	Conditions	% CuI	Yield
1	50	microwave	20 min @ 120 °C	23	<46%
2	20	microwave	20 min @ 140 °C	23	<52%
3	20	microwave	20 min @ 100 °C	23	n.d.
4	20	conventional	48 h @ 60 °C	100	n.d.
5	20	conventional	26 h @ 40 °C then 23 h @ 70 °C	23	n.d.

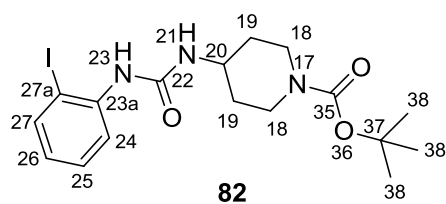
3.3.7 Plan B2, synthesis of *tert*-butyl 4-[3-(2-iodophenyl)ureido]piperidine-1-carboxylate



Scheme 31

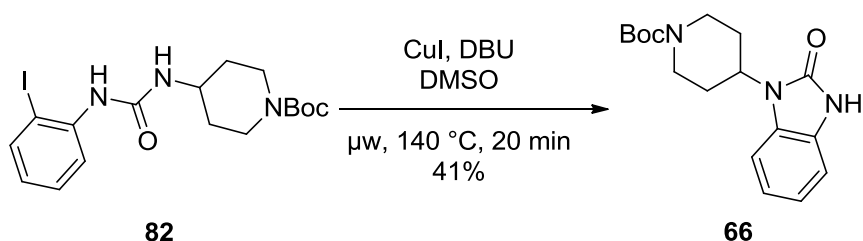
Synthesis of product **82** from aniline **76**.

To test the coupling reaction in the absence of the nitro functionality, compound **82** was prepared using the procedure from the patent by Budzik and co-workers.¹⁴⁵ It was noted that this substrate needs a much shorter reaction time for complete consumption than the starting material with the nitro functionality, with the first part being complete within 25 minutes and the second within four hours (Scheme 31) [see Section 3.3.5]. This observation supports the idea that the nitro functionality was reducing the aniline's nucleophilicity in the first step. The product **82** was isolated in an overall yield of 88%.



For compound **82**, the ^1H and ^{13}C NMR spectra looked as was expected. The two carbonyl carbons, C_{22} and C_{35} , were again visible in the downfield region of the ^{13}C NMR spectrum. IR spectroscopy revealed the two carbonyl $\text{C}=\text{O}$ stretches at 1683 and 1642 cm^{-1} . One of these had moved 27 cm^{-1} compared to the stretch in compound **80** [see Section 3.3.5]. This must be the signal for C_{22} , the carbonyl most influenced by the nitro functionality. Also, the mass spectroscopic analysis result of 446.0938 amu corresponded with the calculated value of 446.0941 amu .

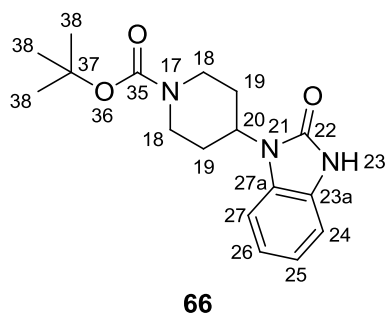
3.3.8 Plan B2, synthesis of *tert*-butyl 4-(2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)piperidine-1-carboxylate



Scheme 32

Reaction of compound **82** to afford benzimidazolone **66**.

Again, the coupling procedure by Li and co-workers was followed.¹³⁸ The reaction mixture was heated at $140\text{ }^\circ\text{C}$ for 20 minutes and then for another 20 minutes when starting material was not consumed during the first 20 minutes (Scheme 32). The crude material was then purified by column chromatography to afford the product in a yield of 39%. This reaction was also repeated, using 1.1 molar equivalents (110 mol%) of copper(I) iodide. This reaction mixture was heated at $140\text{ }^\circ\text{C}$ for 10 minutes and then heated for another 10 minutes to allow for complete consumption of the starting material. The crude material was purified to afford the product in a yield of 41%. Addition of more copper(I) iodide did not significantly increase the yield, but may have increased the rate of reaction, with starting material consumed in half the time.



The ^1H and ^{13}C NMR of compound **66** indicated a successful ring-closure with the signal for $\text{C}_{27\text{a}}$ shifted more downfield than before, as was expected with the substitution of the iodine. This alone was not conclusive, but mass spectroscopic analysis confirmed that this was product **66**, with the result of 340.1631 amu corresponding with the calculated value of 340.1637 amu for the sodium adduct.

The literature also reports the identification of the product as a sodium adduct when using mass spectroscopic analysis.¹⁴⁷

The reaction yields of this reaction are comparable to when the nitro-containing compound **80** is used, to afford benzimidazolone **59**, and indicates Plan B to be a more feasible route towards benzimidazolone **59** than what Plan A is [see Section 3.3.6]. These routes were however, put on hold while alternate routes were also attempted, as discussed in the following section.

3.4 FURTHER WORK TOWARDS THE PIPERIDINYL BENZIMIDAZOLONE TARGET

As mentioned, this section contains alternate routes attempted which did not involve a metal-catalysed coupling or cyclisation reaction. To do this, the benzimidazolone functionality was incorporated *via* other commercially available starting materials. Three different routes were undertaken, with each having the potential to give both regioisomers of the target (Figure 22). In order to keep it simple, schemes will include only one of the regioisomers and its building blocks, namely *N*-(2-oxo-3-{1-[4-(3-phenylimidazo[1,2-*a*]pyridin-2-yl)benzyl]piperidin-4-yl}-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)acrylamide **34** (Figure 22), unless it was known which regioisomer was present. Provided the two regioisomers were separable, it was in fact preferred that both were formed during these reactions, as this would have enabled the synthesis of both regioisomers with minimum additional effort, albeit with the additional challenge of separation.

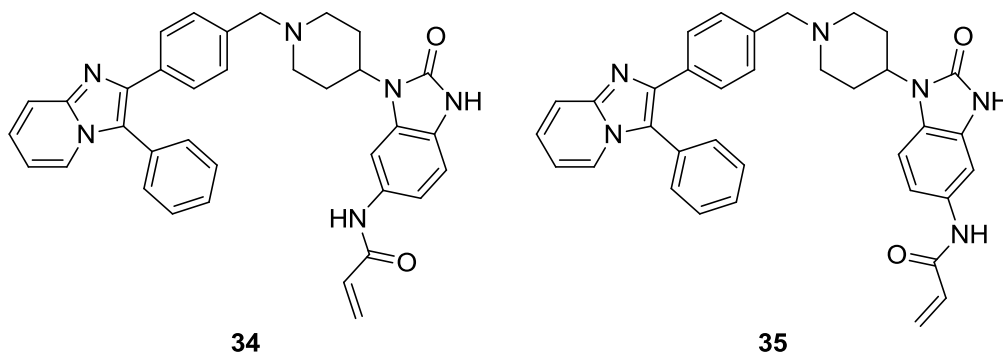
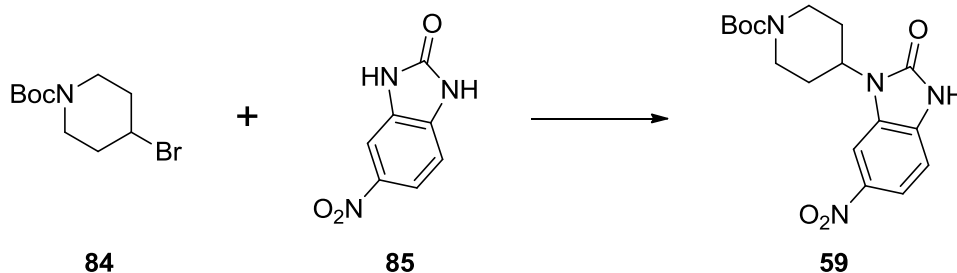


Figure 22

The two target compounds, **34** and **35**.

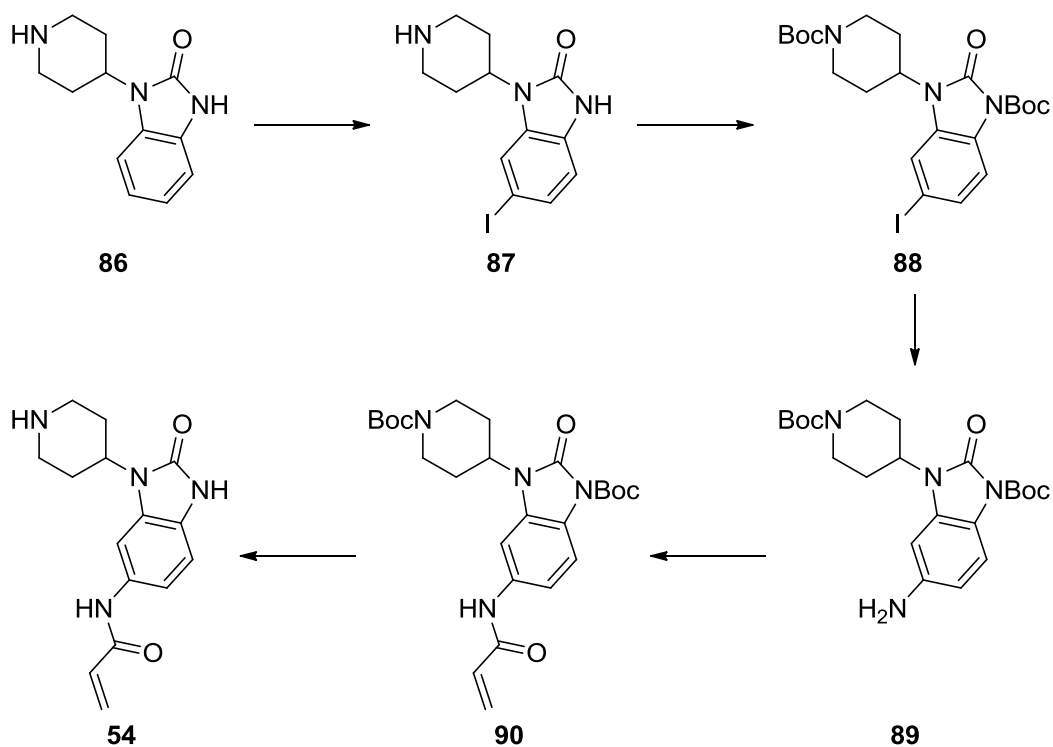
The first plan was to start with commercially available 5-nitro-1*H*-benzo[*d*]imidazol-2(3*H*)-one **85** and having it undergo nucleophilic substitution with the alkyl halide, *tert*-butyl 4-bromopiperidine-1-carboxylate **84** (Scheme 33). This would afford *tert*-butyl 4-(6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)piperidine-1-carboxylate **59**, which could be used to continue the original synthetic route as discussed previously (Scheme 5). This reaction was recognised as potentially problematic due to the position of the halide, on a piperidine ring, and the steric bulk of the nucleophile.



Scheme 33

Planning for the nucleophilic substitution route, both regioisomers may be synthesised.

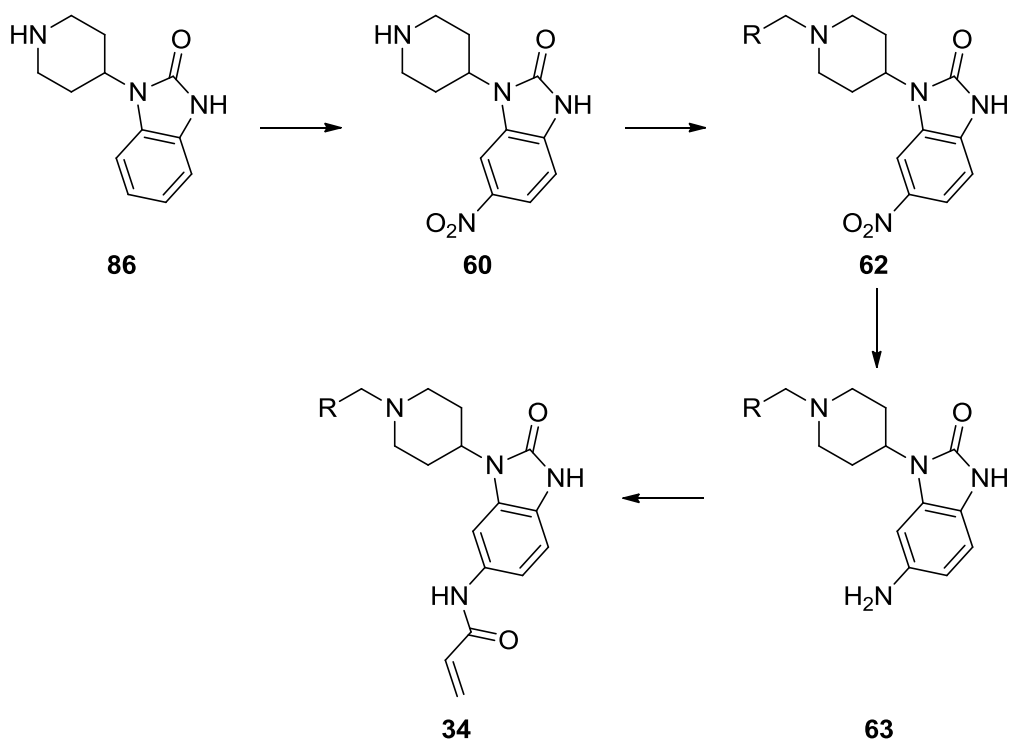
Another plan involved using commercially available 1-(piperidin-4-yl)-1*H*-benzo[*d*]imidazol-2(3*H*)-one **86** and to iodinate its aromatic ring at the appropriate position (Scheme 34). The assumption was that either the 5- or the 6-positions would be iodinated, and not 4- or 7-positions, due to steric reasons. For consistency by showing only one specific regioisomer throughout, we show 6-iodo-1-(piperidin-4-yl)-1*H*-benzo[*d*]imidazol-2(3*H*)-one **87** which could lead to the target compound **54**. After iodination, both nitrogen atoms (amine and urea) would be Boc-protected and the aryl iodide could then be converted to an aniline *via* a suitable metal-catalysed reaction. This route would then proceed in a similar fashion to the original plan, except with the Boc-protecting groups kept on further towards the target compound, only being removed after incorporation of the acrylamide functionality. If this route gave problems, either by the acrylamide being affected by Boc-deprotection or in a different manner, it could also be modified in such a way that Boc-deprotection occurs at a different, more suitable, time.



Scheme 34

Planning for the iodination route, both regioisomers may be synthesised.

The final idea was to nitrate the starting material also involved above, compound **86** (Scheme 35). This could be followed by a similar plan as originally discussed (Scheme 5), or it could follow a route somewhat different, first incorporating the western moiety *via* reductive amination and then reducing the nitro functionality in order to introduce the acrylamide *via* the amine, affording the target compound **54**.

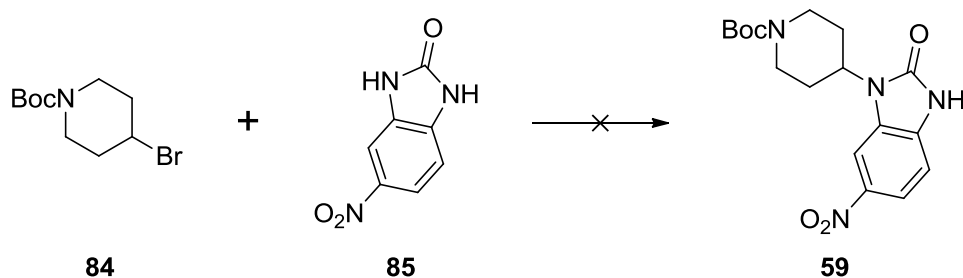


Scheme 35

Planning for the nitration route, both regioisomers may be synthesised.

These routes were thus investigated, with various levels of success, as described in the following sections.

3.4.1 Nucleophilic substitution route, synthesis of *tert*-butyl 4-(6-nitro-2-oxo-2,3-dihydro-1H-benzo[*d*]imidazol-1-yl)piperidine-1-carboxylate



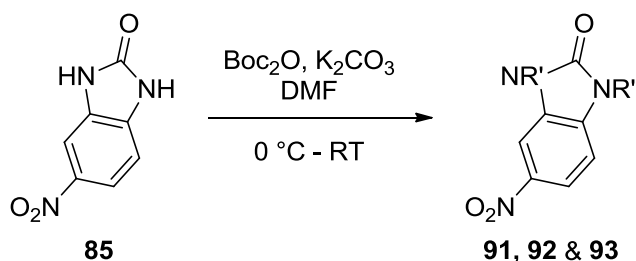
Scheme 36

Attempted synthesis of benzimidazolone 59.

For the nucleophilic substitution involving the starting material, 5-nitro-1H-benzo[*d*]imidazol-2(3H)-one 85, a procedure by Rawson and co-workers was used.¹⁴⁸ Their reaction involved a nucleophile similar to benzimidazolone 85, with 2-bromopropane as their much less hindered alkyl bromide, reacting readily to afford the product in a reported yield of 92%.¹⁴⁸

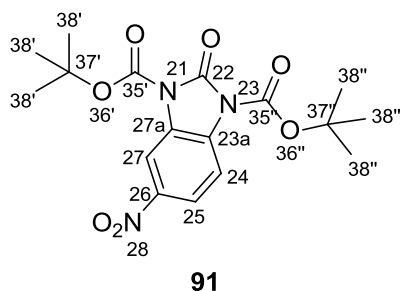
The reaction was initiated, closely following the literature procedure, by stirring equimolar amounts of the two starting materials and sodium hydride together in *N,N*-dimethylformamide at 60 °C (Scheme 36).¹⁴⁸ Starting material was not readily consumed at 60 °C and the reaction mixture was heated to 80 °C for three days. The starting materials did not react and no product was isolated. The reaction was repeated, using a different base and solvent, namely potassium carbonate and acetonitrile, but this was also unsuccessful, with no product isolated.

The lack of any product formed during these attempts posed the question of whether either of the nitrogen atoms was nucleophilic enough to react. It was decided to test this, by attempting a Boc-protection of both of these nitrogen atoms. A procedure described by Meanwell and co-workers, for the Boc-protecting of both nitrogen atoms of substituted benzimidazolones, was used.¹⁴⁹ Following this procedure, the product, di-*tert*-butyl 5-nitro-2-oxo-1*H*-benzo[*d*]imidazole-1,3(2*H*)-dicarboxylate **91**, was isolated in a yield of 60%. Two mono-protected side-products were also isolated, namely *tert*-butyl 5-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-1-carboxylate **92** and *tert*-butyl 6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-1-carboxylate **93**, in yields of 1% and 5% respectively. The identities of these two compounds were determined by obtaining ¹H and ¹³C NMR spectra of each, as well as a 2D Nuclear Overhauser effect spectroscopy (NOESY) spectrum of one of the two regioisomers, compound **93**, in order to determine which regioisomer was which. The reaction was repeated twice, once using *N,N*-dimethylformamide as solvent and the other time using dimethyl sulfoxide as solvent (Table 6).

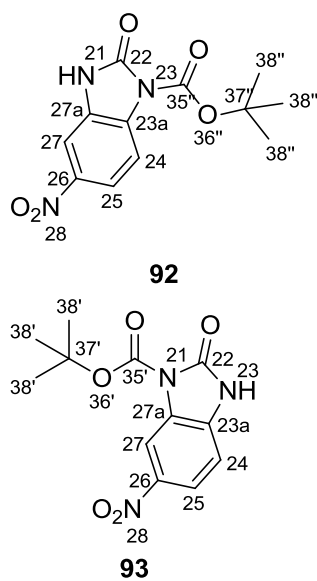


Scheme 37

The three possibilities, with R' and R'' being H or Boc-protecting group.



For compound **91**, the ¹H and ¹³C NMR spectra were as expected, with the addition of the Boc-protecting groups visible in both spectra. Furthermore, mass spectroscopic analysis confirmed successful synthesis of the product, with the result of 402.1280 amu corresponding with the calculated value of 402.1277 amu for the sodium adduct.



The ^1H spectra of compounds **92** and **93** correlated to that found in the literature.¹⁵⁰ In order to confirm which regioisomer was which, Nuclear Overhauser effect spectroscopy (NOESY) was utilised and two-dimensional and one-dimensional ^1H NMR spectra of compound **93** was obtained. In the 2D NOESY spectrum, a clear correlation between H_{27} and $\text{H}_{38'}$ was visible, indicating that the Boc-protecting group was on the same side of the aromatic ring as H_{27} (Figure 23). The two 1D NOESY spectra was obtained by irradiating the chemical shifts of H_{24} and H_{27} , respectively. From these two spectra, the correlation between H_{27} and $\text{H}_{38'}$ was again visible, with H_{24} and $\text{H}_{38'}$ having a far weaker correlation, indicating a longer distance between them. This is supported by the ^1H NMR spectra of each reported in the literature.¹⁵⁰

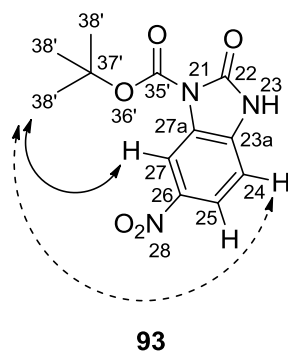


Figure 23

A schematic diagram illustrating the correlations to the Boc-protecting group protons of compound **93**, as identified using NOE. Note, the dashed correlation between H_{24} and $\text{H}_{38'}$ indicate a weak correlation compared to that of the correlation between H_{27} and $\text{H}_{38'}$.

This result could be rationalised by looking at the position of the nitro functionality and keeping in mind that it withdraws electron density from the substituents *ortho* and *para* to it. Compound **92** would thus be expected to be present in a lower yield than compound **93**, as in this compound, the deactivated nitrogen atom, N_{23} , which is *para* to the nitro functionality, acted as the nucleophile. This was indeed what was observed, with compound **93** present in larger quantities in all three runs.

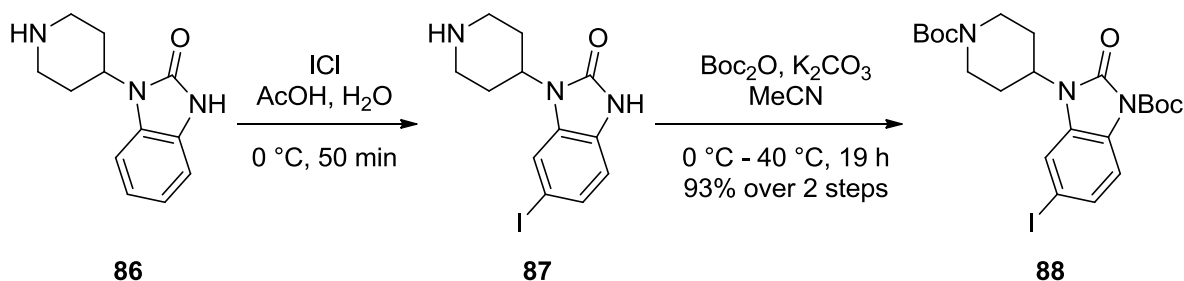
Table 6

Different reaction conditions tested for the Boc-protection of 5-nitro-1*H*-benzo[*d*]imidazol-2(3*H*)-one **85**.

Entry	Solvent	Time	Compound yields			Overall yield
			91	92	93	
1	Acetonitrile	48 h	60%	1%	5%	66%
2	DMF	18 h	42%	1%	31%	74%
3	DMSO	21 h	30%	14%	25%	69%

The question of the nucleophilic nature of compound **85** had thus been answered, with it proven that the benzimidazolone nitrogen atoms were indeed nucleophilic. They were, however, bulky and the steric nature of the electrophilic site possibly did not allow for nucleophilic substitution with a bulky nucleophile such as this. Nucleophilic substitution with smaller nucleophile would be more viable, such as the reaction between *N,N*-dimethylamine as nucleophile and a substituted bromocyclohexane as electrophile, reported by Tormena and co-workers.¹⁵¹ It was decided to abandon this idea and look to more promising avenues.

3.4.2 Iodination route, synthesis of *tert*-butyl 3-[1-(*tert*-butoxycarbonyl)piperidin-4-yl]-5-iodo-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-1-carboxylate



Scheme 38

Iodination of Boc-protection of compound **86** to afford product **88**.

For the iodination of the commercially available 1-(piperidin-4-yl)-1*H*-benzo[*d*]imidazol-2(3*H*)-one **86**, a procedure by Sudalai and co-workers was followed.¹⁵² This procedure described the iodination of a variety of activated aromatic systems, by the *in situ* generation of one molar equivalent of iodine monochloride using sodium periodate, potassium iodate and sodium chloride.¹⁵² The procedure was followed loosely, adding benzimidazolone **86** and iodine monochloride to a nine-to-one mixture of acetic acid to water at room temperature (Scheme 38).¹⁵² The starting material **86** was very insoluble in the reaction solvent mixture, but TLC indicated complete consumption of the starting material after

the addition of iodine monochloride over 50 minutes. The organic matter could not be extracted using ethyl acetate or dichloromethane, as the product was insoluble in these solvents. The resulting precipitate was therefore filtered and washed with a minimum amount of cold water. The insolubility of the starting material **86** and potential product could be attributed to it possibly being protonated by the acetic acid, thus affording highly polar and charged compounds and were also found to be insoluble in methanol. It was decided to assume that the iodination had worked and to use the material unpurified in the following reaction, using the results of that reaction to determine whether the reaction was successful or not.

This reaction involved the Boc-protection of both nitrogen atoms of the potentially iodinated product. This would not only allow for safe manipulation of the aryl iodide, but also increase compound solubility in organic solvents, thereby enabling purification by column chromatography and ultimately, characterisation. For this reaction, the procedure by Meanwell and co-workers was again utilised, with the crude material **86** being added to di-*tert*-butyl dicarbonate and potassium carbonate in acetonitrile [see Section 3.4.1].¹⁴⁹ Additional equivalents of both di-*tert*-butyl dicarbonate and potassium carbonate were required in order to ensure complete Boc-protection of both nitrogen atoms. The reaction mixture was heated at 40 °C for 19 hours, and the crude material was purified by column chromatography to afford the product in a good yield of 93% over two steps. NOESY was utilised in order to identify which of the two possible regioisomers was present, obtaining two one-dimensional ¹H NMR spectra of the product by irradiation of the chemical shift values of H₂₄ and H₂₇, respectively. The ¹H NMR spectra of previously synthesised **93** were used to identify which proton signals correlated to which Boc-protecting groups, with H_{38''} being the more downfield signal at 1.65 ppm [see Section 3.4.1]. The other Boc-protecting group, H_{38'}, was visible as a more upfield singlet, at 1.50 ppm. It was assumed that the H_{38'} protons would more readily correlate with both H₂₄ and H₂₇ as it was attached to the benzimidazolone core *via* piperidine, allowing for some flexibility and reach. The correlations of H_{38''} with the other protons was therefore to be used as guide. H₂₄ was seen to have correlations with H₂₅, H_{38'} and H_{38''}, whereas H₂₇ had correlations only with H₂₅ and H_{38'} (Figure 24). This indicated that H₂₇ was out of reach of the protons of the more constrained Boc-protecting with H_{38''} being closer to H₂₄. In this manner, the compound was identified as *tert*-butyl 3-[1-(*tert*-butoxycarbonyl)piperidin-4-yl]-5-iodo-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-1-carboxylate **88**.

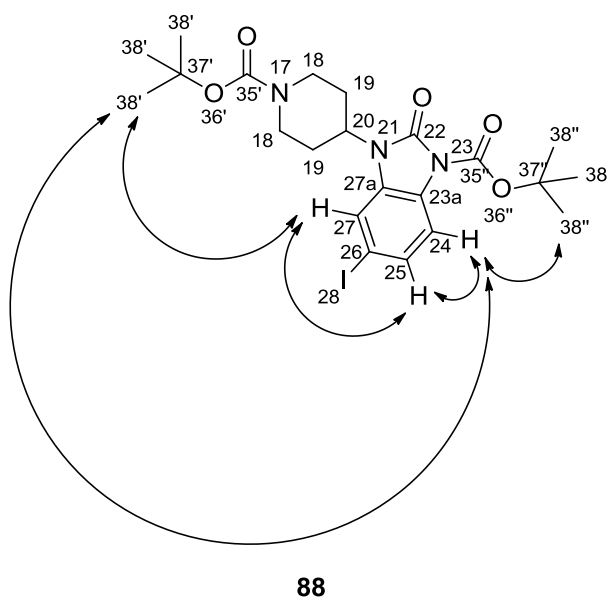
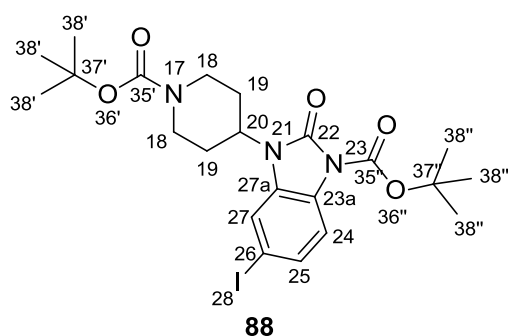
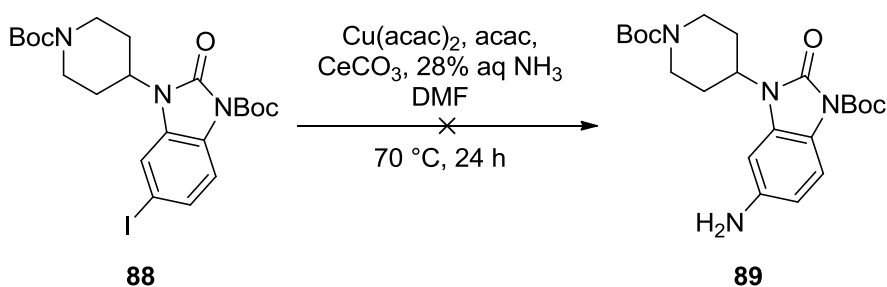


Figure 24

A schematic diagram illustrating the correlations between protons in compound **88** as identified using NOE.



The ^1H and ^{13}C NMR spectra of compound **88** indicated successful iodination and Boc-protection, with only three protons signals present in the aromatic region of the ^1H NMR spectrum. These were in the typical 1,2,4-substitution pattern and supported iodination at C_{26} as shown by NOE experiments. Further confirmation was the mass spectroscopic result of 566.1133 amu, which corresponded to the calculated value of 566.1128 amu for the sodium adduct.

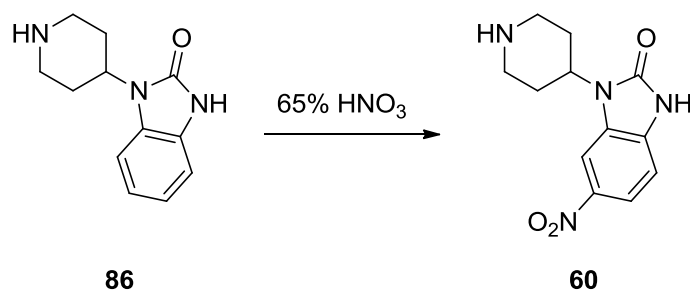


Scheme 39

Attempted amination of aryl iodide **88** to afford aniline **89**.

Following this success, aryl iodide **88** was to be converted into *tert*-butyl 5-amino-3-[1-(*tert*-butoxycarbonyl)piperidin-4-yl]-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-1-carboxylate **89** using a procedure by Xia and Taillefer for the copper-catalysed amination of aryl halides (Scheme 39).¹⁵³ This reaction was unsuccessful with no product isolated, but is something that could be pursued further.

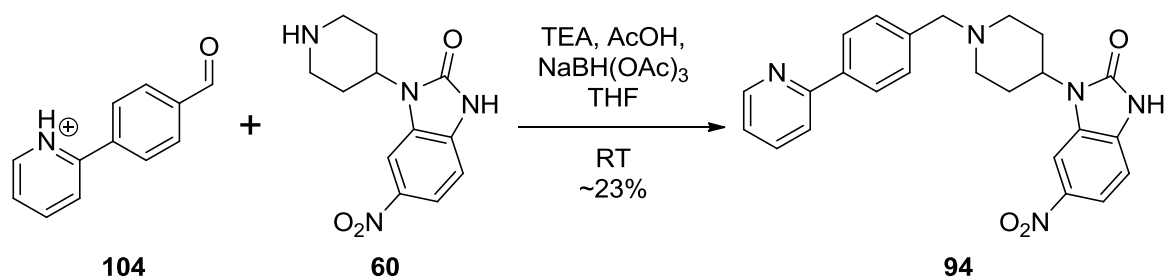
3.4.3 Nitration route, synthesis of 5-nitro-1-{1-[4-(pyridin-2-yl)benzyl]piperidin-4-yl}-1H-benzo[d]imidazol-2(3H)-one and 6-nitro-1-{1-[4-(pyridin-2-yl)benzyl]piperidin-4-yl}-1H-benzo[d]imidazol-2(3H)-one



Scheme 40

Nitration of benzimidazolone **86** to afford product **60**. Both regioisomers were present.

The final alternate route started with the nitration of benzimidazolone **86** to afford 6-nitro-1-(piperidin-4-yl)-1H-benzo[d]imidazol-2(3H)-one **60** and/or its regioisomer, 5-nitro-1-(piperidin-4-yl)-1H-benzo[d]imidazol-2(3H)-one. For this, a procedure from James and Turner was followed, wherein they reported the nitration of 1H-benzo[d]imidazol-2(3H)-one to afford 5-nitro-1H-benzo[d]imidazol-2(3H)-one **85**.¹⁵⁴ The starting material **86** was stirred with 65% aqueous nitric acid at 0 °C and the product was instantly formed as a precipitate (Scheme 40). NMR and IR spectroscopic analysis of the crude material **86** indicated successful nitration with the two regioisomers present in a ratio of three-to-four. The crude material was used unpurified for the following reaction as compound **60** was very polar and expected to be difficult to purify or separate using column chromatography.

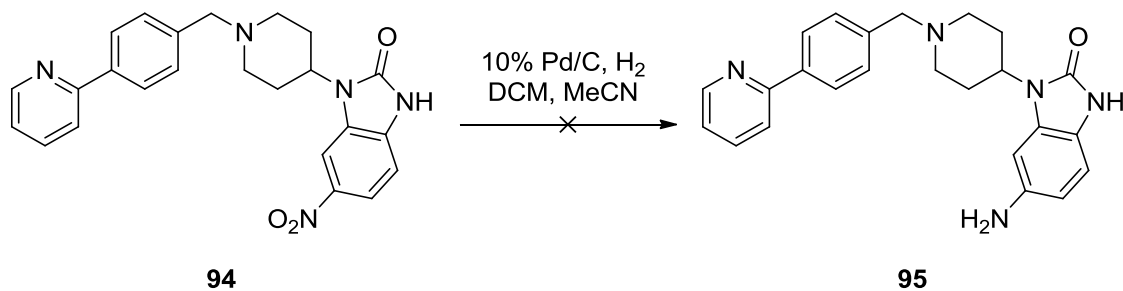


Scheme 41

Reductive amination between **104** and **60** to afford product **94**. Both regioisomers were present.

The next reaction was the reductive amination of 4-(pyridin-2-yl)benzaldehyde **104** and **60**, using the procedure reported by Hoelder and co-workers.¹⁵⁵ One equivalent of 4-(pyridin-2-yl)benzaldehyde **104** was used in the place of a complete western moiety in order to allow for optimisation of the reaction before the western moiety was used (**104** to be discussed later, see Section 4.1). Following the procedure by Hoelder and co-workers afforded the title compound **94** in a poor yield of 23% (Scheme 41). Starting material **60** and reduced aldehyde, [4-(pyridin-2-yl)phenyl]methanol, were also

isolated in yields of 41% and 23%, respectively. This procedure was repeated, but the title compound **94** was isolated in a reduced yield of 12%.



Scheme 42

Attempted reduction of compound **94** to afford product **95**. Both regioisomers were present.

Next, the nitro functionality was to be reduced into an amine to afford **95**. This reaction was performed using palladium on charcoal with the addition of hydrogen, following a procedure by Park and Standaert.¹⁵⁶ Unfortunately, this did not work and no product was obtained.

With no completed eastern moiety to show after a significant amount of effort, it was decided to shift the focus to the alternate, adapted target compounds (Figure 19). The discussion follows in the ensuing chapters.

CHAPTER 4 – TOWARDS THE 1,2,3-TRIAZOLE TARGET

THE MAKING OF THE ROLE-PLAYERS

4.1 RETROSYNTHETIC PLANNING – ADAPTED ROUTE

With the limited success in synthesising the original targets, such as **34** (Figure 25), the focus was shifted towards the adapted targets discussed in Chapter 2 [see Section 2.3]. Once again, the planning and initial synthetic work will focus on one of the regioisomers, namely *N*-[3-(1-{1-[4-(3-phenylimidazo[1,2-*a*]pyridin-2-yl)benzyl]piperidin-4-yl}-1*H*-1,2,3-triazol-4-yl)phenyl]acrylamide **37** (Figure 25Figure 19).

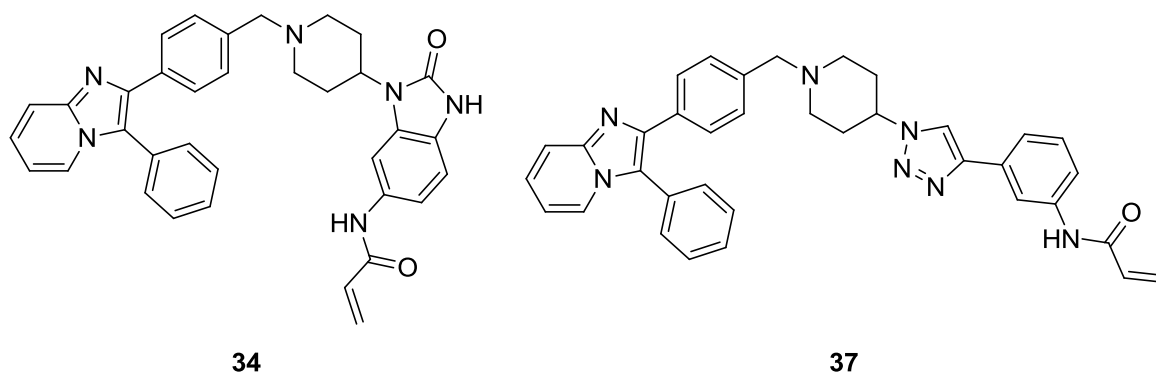
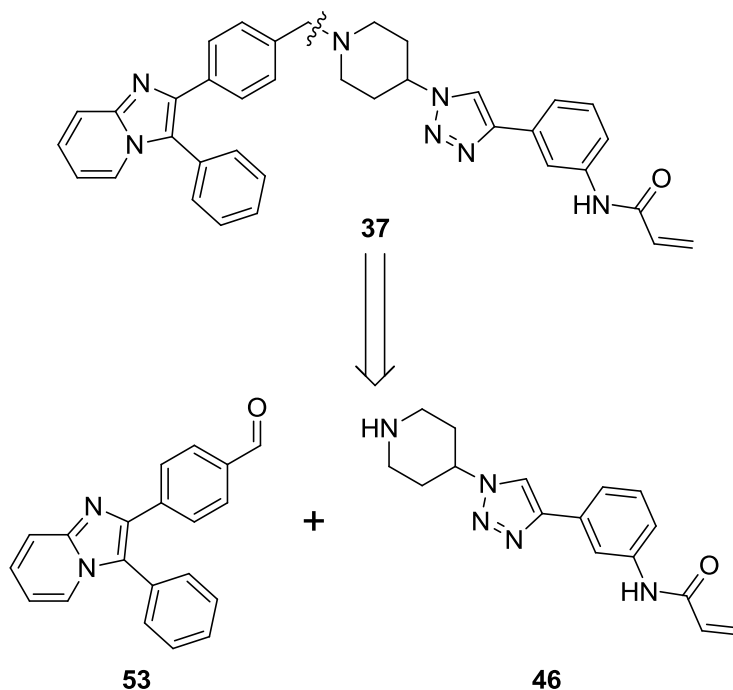


Figure 25

A representations of the original target compounds **34** and of the adapted target compounds **37**.

As described before, the final step was to be linking of the completed eastern and western moieties *via* reductive amination (Scheme 43) [see Section 3.1].

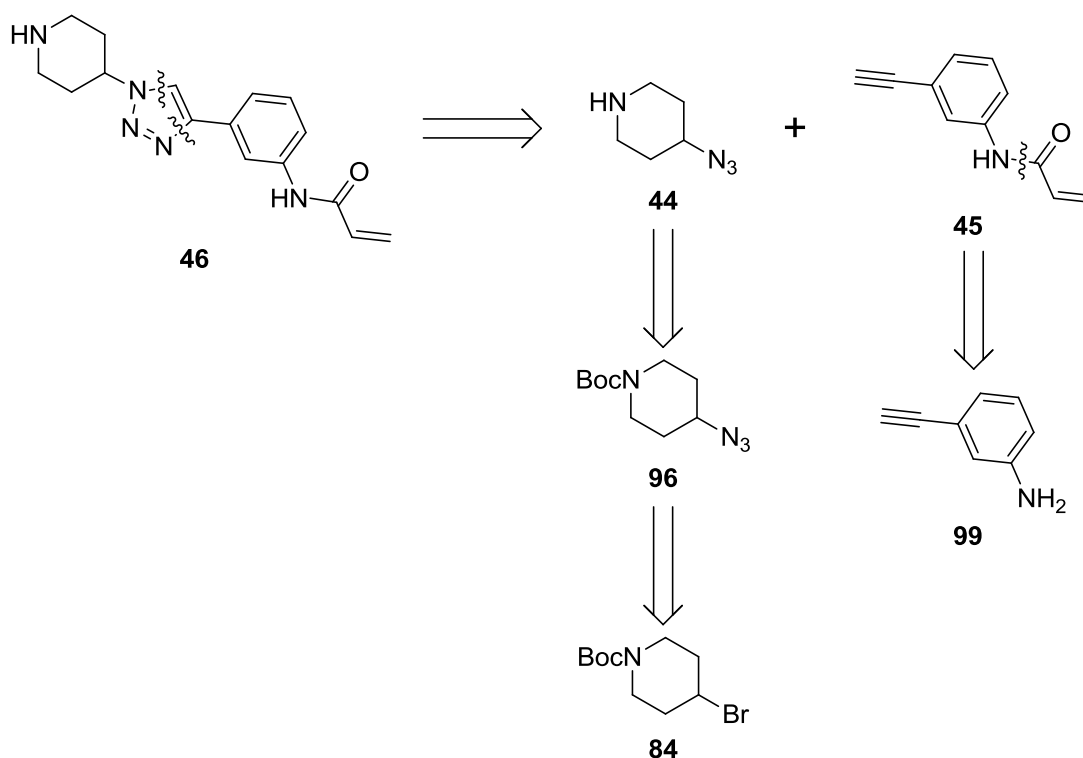


Scheme 43

The final step (reductive amination) towards the final compound(s).

Retrosynthetic analysis of this particular regioisomer of the eastern moiety, *N*-{3-[1-(piperidin-4-yl)-1*H*-1,2,3-triazol-4-yl]phenyl}acrylamide **46**, revealed a convergent approach, involving the synthesis of the two components for the click reaction, 4-azidopiperidine **44** and *N*-(3-ethynylphenyl)acrylamide **45**, followed by the click reaction itself to afford the completed eastern moiety, **46** (Scheme 44). For the two remaining eastern moieties, *N*-(2-(1-(piperidin-4-yl)-1*H*-1,2,3-triazol-4-yl)phenyl)acrylamide and *N*-(4-(1-(piperidin-4-yl)-1*H*-1,2,3-triazol-4-yl)phenyl)acrylamide, the synthesis of *N*-(2-ethynylphenyl)acrylamide **101** and *N*-(4-ethynylphenyl)acrylamide **134** will respectively be required. As will be seen later, the initial work was done with **45**, followed with **101** at a later stage.

For the synthesis of the first component, 4-azidopiperidine **44**, commercially available *tert*-butyl 4-bromopiperidine-1-carboxylate **84** could be converted into *tert*-butyl 4-azidopiperidine-1-carboxylate **96** using sodium azide in a straightforward nucleophilic substitution reaction. 4-Azidopiperidine **44** would then be obtained by Boc-deprotection of compound **97**. On the other side, *N*-(3-ethynylphenyl)acrylamide **45** could be synthesised by the acylation of 3-ethynylaniline **99** using acryloyl chloride, as discussed earlier with the planning towards the first target [see Section 3.1].



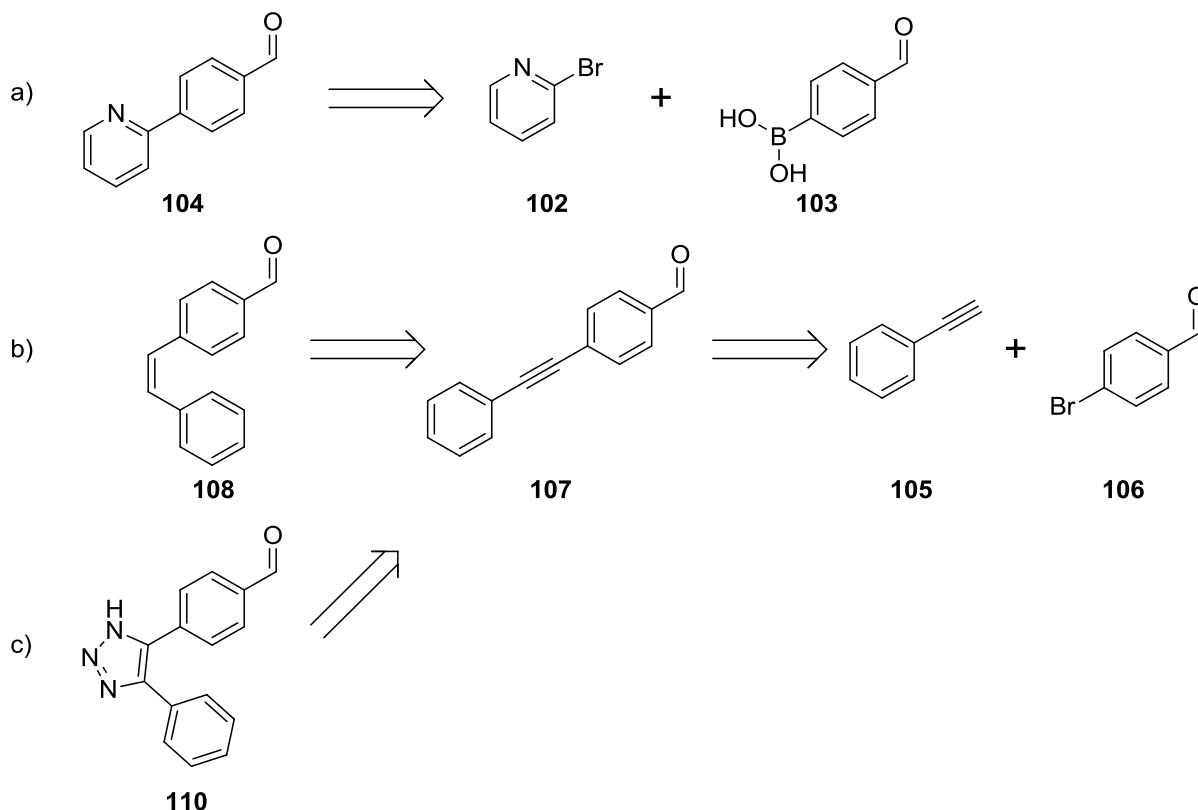
Scheme 44

The retrosynthetic strategy to generate the chosen representative eastern moiety.

If the acrylamide was to interfere with the final reductive amination step, it would be incorporated after reductive amination, circumventing any possible side-reactions or degradation of the acrylamide. This alternate route would have to include protection of the aniline before reductive amination and deprotection thereafter, lengthening the synthetic route by two additional steps, but thus preventing the very possible side-reaction where the aniline undergoes reductive amination with the western moiety.

As synthesis of the western moieties were expected to be complicated processes, involving a number of metal-catalysed coupling reactions, it was decided to first use smaller aldehydes as representatives of the western moieties before attempting the reductive amination with the more valuable western moieties themselves.¹⁰⁹ These compounds were 4-(pyridin-2-yl)benzaldehyde **104**, (*Z*)-4-styrylbenzaldehyde **108** and 4-(4-phenyl-1*H*-1,2,3-triazol-5-yl)benzaldehyde **110** (Scheme 45). They were chosen after investigating the SAR of allosteric Akt inhibitors, as discussed by Kettle and co-workers [see Section 2.2.3].¹⁰⁹ 4-(Pyridin-2-yl)benzaldehyde **104** had the pyridine nitrogen in the position deemed important for Akt inhibition, (*Z*)-4-styrylbenzaldehyde **108** had the two aryl components fixed relative to all the chosen western moieties, but lacked the heterocyclic rings attached to these. Lastly, 4-(4-phenyl-1*H*-1,2,3-triazol-5-yl)benzaldehyde **110** was similar to a number of the western moieties, but lacked the second heterocyclic ring. In addition, to be used as

frontiers in the synthesis towards target compounds, it would also be interesting to compare their potential activity against Akt to that of the target compounds.



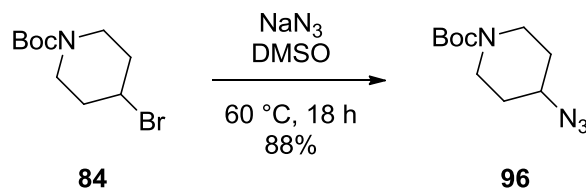
Scheme 45

Retrosynthetic strategies to generate the truncated western moieties.

All of the building blocks needed to be synthesised first before the main work towards the target **37** could begin. This chapter will therefore cover synthesis of all of these building-blocks (as shown in Scheme 45).

4.2 SYNTHESIS OF THE COMPONENTS FOR THE CLICK REACTION(S)

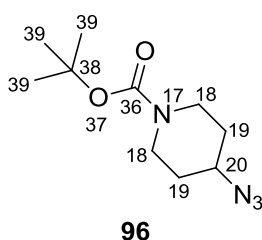
4.2.1 *tert*-Butyl 4-azidopiperidine-1-carboxylate



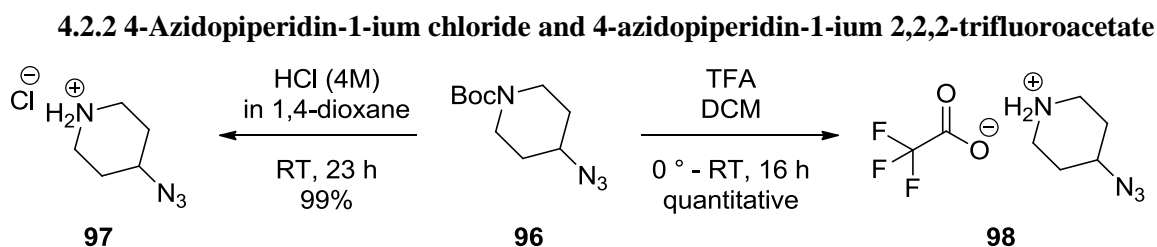
Scheme 46

Nucleophilic substitution between alkyl bromide **84** and sodium azide to afford alkyl azide **96**.

A procedure reported by Moore and co-workers was used for the synthesis of *tert*-butyl 4-azidopiperidine-1-carboxylate **96**, synthesising it from the nucleophilic substitution reaction between *tert*-butyl 4-bromopiperidine-1-carboxylate **84** and sodium azide in *N,N*-dimethylformamide.¹⁵⁷ Their procedure was followed, heating compound **84** and sodium azide in a different polar aprotic solvent, dimethyl sulfoxide, at 60 °C until complete consumption of the starting material, as detected by TLC (Scheme 46).¹⁵⁷ The reaction was complete after six hours and the crude material was purified by column chromatography to afford the title compound **96** in a yield of 79%. This compared reasonably well with the reported yield of 99%.¹⁵⁷ The reaction was repeated eight more times, with an average yield of 76% proving the reproducibility of the reaction.



The ¹H and ¹³C NMR spectra of alkyl azide **96** looked similar to that of the starting material alkyl bromide **84**, with the ¹H NMR singlet at 1.43 ppm, integrating for nine protons, indicating that the Boc-protecting group was still intact. The two signals at 154.5 and 28.3 ppm in the ¹³C NMR spectrum confirmed this. As the reaction involved a C-Br bond being exchanged for that of a C-N bond, NMR alone could not be used to determine whether the reaction was successful or not. For this, IR spectroscopy was utilised and the strong azide stretch was visible at 2090 cm⁻¹, indicating a successful nucleophilic substitution reaction. The mass spectroscopic result of 171.0872 amu was different from the calculated value of 227.1508 amu for the protonated molecular ion. This loss of 55 amu pointed to possible cleavage of the carbon-oxygen bond between the tertiary butyl group and the rest of the Boc-protecting group. This cleavage would result in a loss of 57 amu, but the compound may have also been protonated twice in the ionisation process, affording an eventual difference of 55 amu from the calculated value. The NMR spectra clearly indicated the presence of the Boc-protecting group and confirmed the possibility that the loss occurred during the mass spectroscopic analysis process, and not during the reaction.



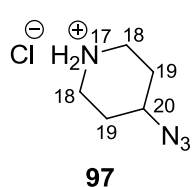
Scheme 47

Boc-deprotection procedures to afford ammonium salts **97** and **98** from compound **96**.

For the removal of the *tert*-butyloxycarbonyl group (Boc-deprotection), a procedure by Rauh and co-workers was followed.¹⁵⁸ They reported the quantitative Boc-deprotection of substituted

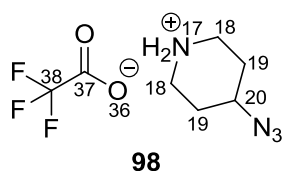
aminothiazoles using hydrochloric acid in 1,4-dioxane at room temperature. It was expected that the neutral product, 4-azidopiperidine **44**, would be highly polar and possibly soluble in water, as it is small and its ratio of nitrogen atoms to carbon atoms, is four to five. This procedure was thus chosen as no aqueous work-up was necessary, ending with the isolation of the ammonium salt instead.

Following the literature procedure, *tert*-butyl 4-azidopiperidine-1-carboxylate **96** was added to 4M hydrochloric acid in 1,4-dioxane (Scheme 47). The reaction mixture was stirred at room temperature for 18 hours until complete consumption of starting material according to TLC. The solvent was removed *in vacuo* and the crude 4-azidopiperidin-1-ium chloride **97** was obtained as a dark yellow oil. The oil weighed more than the product **97** should have weighed with a quantitative yield and increased in mass when left in open air, suggesting the material was hygroscopic. The same procedure was repeated, taking care not to unnecessarily expose the reaction contents to air, and the crude material was obtained as a yellow solid in a yield of 99%.



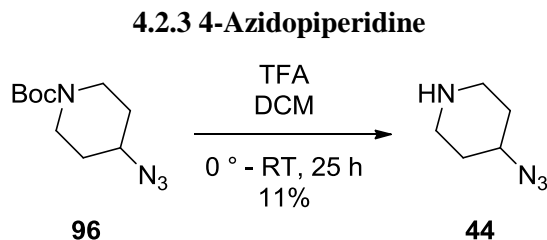
The ^1H and ^{13}C NMR spectra of the product **97** were as expected, with the Boc-protecting group signals absent. The IR spectrum supported this, with no carbonyl C=O stretch visible in the area of 1700 cm^{-1} and the azide stretch still visible at 2094 cm^{-1} . Lastly, the mass spectroscopic result of 127.0977 amu corresponded well with the calculated value for 4-azidopiperidin-1-ium, lacking the chloride ion, 127.1676 amu .

It was decided to also remove the *tert*-butyloxycarbonyl group using a different acid to determine if this made the product less hygroscopic and easier to handle in air. The reaction was performed in a similar manner as above, this time stirring the starting material **96** and trifluoroacetic acid in dichloromethane at room temperature, until complete consumption of starting material as detected by TLC (Scheme 47). This was again not neutralised and the solvent was removed *in vacuo* after addition of toluene. Toluene was added so it would azeotrope with the trifluoroacetic acid, thereby assisting in its complete removal.^{159; 160} The crude product, 4-azidopiperidin-1-ium 2,2,2-trifluoroacetate **98**, was isolated in a quantitative yield. This procedure was repeated three more times and care had to be taken with the removal of the solvent, ensuring its complete removal, to obtain the product **98** as a dry yellow solid, and not a thick yellow oil.



As with the hydrochloric acid salt, the Boc-protecting group signals were absent from the ^1H and ^{13}C NMR spectra of **98**, indicating a successful removal of the protecting group. IR spectroscopy could not be used to confirm the removal of the Boc-protecting group in the same manner as before, as the trifluoroacetate **98**'s carbonyl C=O stretch was visible at 1666 cm^{-1} , potentially obscuring the carbonyl C=O stretch of the Boc-protecting group, had it not been successfully removed. Thankfully, the mass spectroscopic result of 127.0989 amu corresponded with the

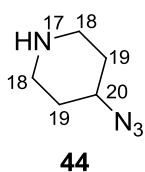
calculated value for 4-azidopiperidin-1-ium, 127.0984 amu, thus confirming the removal of the Boc-protecting group.



Scheme 48

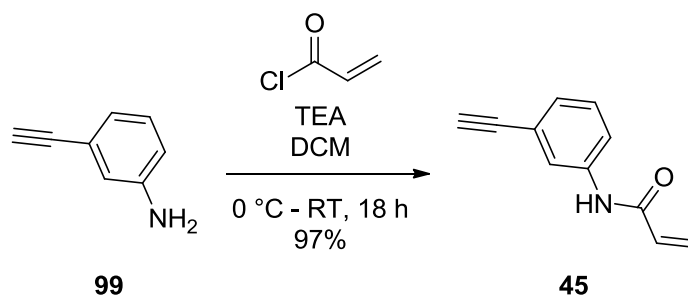
Boc-deprotection of compound **96** to afford amine **44**.

Due to the difficulty in handling the 4-azidopiperidin-1-ium salts, **97** and **98**, it was decided to attempt the neutralisation of this compound in order to obtain 4-azidopiperidine **44**. This would also be beneficial if this compound needed to be used in reactions where the addition of base to neutralise the acidic salt would interfere with the reaction. The Boc-protecting group was removed using trifluoroacetic acid as discussed above (Scheme 48) [see Section 4.2.2]. The acidic crude material was then neutralised with an aqueous solution of 2 M sodium hydroxide, using universal indicator paper to confirm a basic reaction mixture. This neutralised solution was extracted with both dichloromethane and ethyl acetate, but no product was isolated. The reaction was repeated, and this time round, neutralised using an aqueous solution of 0.1 M sodium hydroxide, care being taken to keep the volume of the aqueous layer to a minimum, using only enough sodium hydroxide until the mixture was slightly basic according to universal indicator paper. Extraction was done with ethyl acetate and the title compound was recovered in a low yield of 11%. It was decided that obtaining the neutral compound **44** was not worth the loss of so much compound and **97** and **98** were thus used as their respective salts.



The ^1H and ^{13}C NMR spectra for alkyl azide **44** looked much like that of its two salt counterparts, alkyl azides **97** and **98**, with the only significant difference being the upfield shift of the amine proton signal, H_{17} , now visible at 6.33 ppm in the ^1H NMR spectrum.

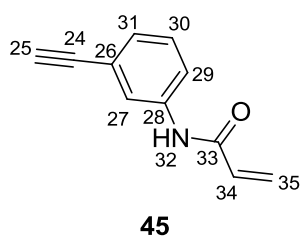
4.2.4 N-(3-Ethynylphenyl)acrylamide



Scheme 49

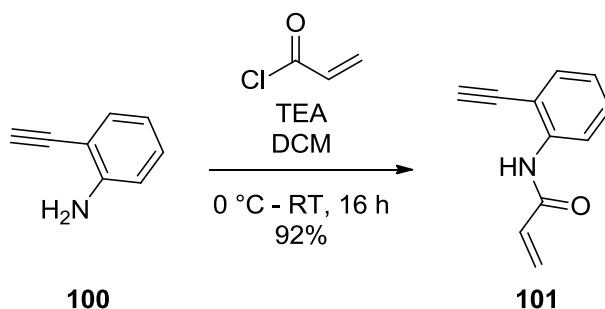
Acylation of aniline **99** to afford acrylamide **45**.

This particular reaction was carried out following a procedure reported by Li and co-workers for the acylation of numerous pteridin-7(8*H*)-one derivatives using acryloyl chloride.¹²² They reported yields between 30 and 87% and it was decided to use additional equivalents of both acryloyl chloride and triethylamine, in order to obtain the maximum yield possible.¹²² The starting material, 3-ethynylaniline **99**, was combined with five molar equivalents of both acryloyl chloride and triethylamine and stirred at room temperature in dichloromethane (Scheme 49). The starting material was consumed after 21 hours and the crude material was purified by column chromatography to afford the pure title compound **45** in a yield of 36%. This was lower than expected as the starting material was consumed and no by-products were visible by TLC. The reaction was thus repeated, taking care to extract the aqueous layer with more extractions of dichloromethane. The yield was increased when doing this, with an acceptable average yield of 74% from five runs.



The ¹H and ¹³C NMR spectra of alkyne **45** pointed to a successful reaction, with the ¹H NMR spectrum having the appropriate three acrylamide proton signals, at 6.42, 6.27 and 5.76 ppm, and the amine now integrating for one, and not two, protons. The ¹³C NMR spectrum also contained the relevant carbon signals for the acrylamide, including the carbonyl carbon at 163.8 ppm. Finally, the mass spectroscopic analysis result of 172.0766 amu corresponded with the calculated value of 172.0762 amu, further confirming a successful reaction.

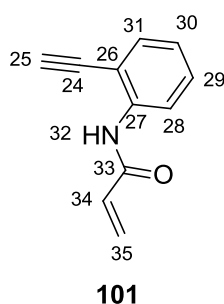
4.2.5 *N*-(2-Ethynylphenyl)acrylamide



Scheme 50

Acylation of aniline **100** to afford acrylamide **101**.

The same procedure by Li and co-workers was used for the acylation of 2-ethynylaniline **100**, this time with 4.9 molar equivalents of acryloyl chloride and triethylamine being added to the 2-ethynylaniline **100**, and stirring it together at room temperature for 24 hours (Scheme 50).¹²² The crude material was purified by column chromatography and the title compound **101** was isolated in a yield of 76%. The procedure was repeated once, this time using 2.8 molar equivalents of acryloyl chloride and triethylamine and stirring the reaction mixture for 16 hours. The yield of this run was 92%, indicating that the reaction does not need such a large excess of reagents as previously thought [see Section 4.2.4].

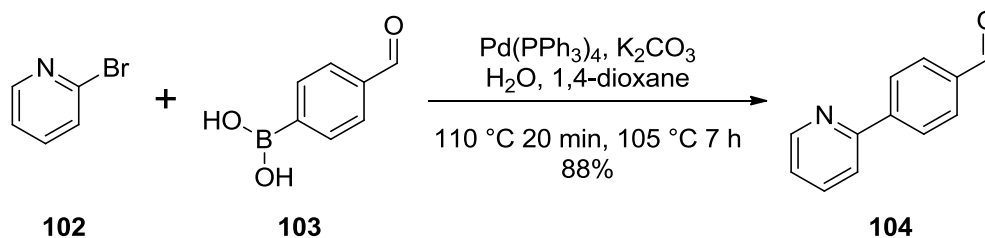


As with the previous regioisomer acrylamide **45**, the ¹H and ¹³C NMR spectra of acrylamide **101** in deuterated chloroform confirmed the presence of the newly-attached acrylamide. However, these ¹H and ¹³C NMR spectra were different from that of the previous regioisomer, having two sets of proton and carbon signals, respectively. This is caused by the two different possible rotamers of this compound. The acrylamide is able to twist around, either being close to or far from the neighbouring alkyne. This places the atoms in two slightly different

environments, causing each atom to have two chemical shift values. Integration of the proton signals indicated the rotamers to be present in a three-to-one ratio. This was not observed with *N*-(3-ethynylphenyl)acrylamide **45** as the two substituents are further from each other, effectively out of electronic reach. In addition, the mass spectroscopic analysis result of 172.0764 amu corresponded with the calculated value of 172.0762 amu, further confirming a successful reaction.

4.4 SYNTHESIS OF THE TRUNCATED WESTERN MOIETIES

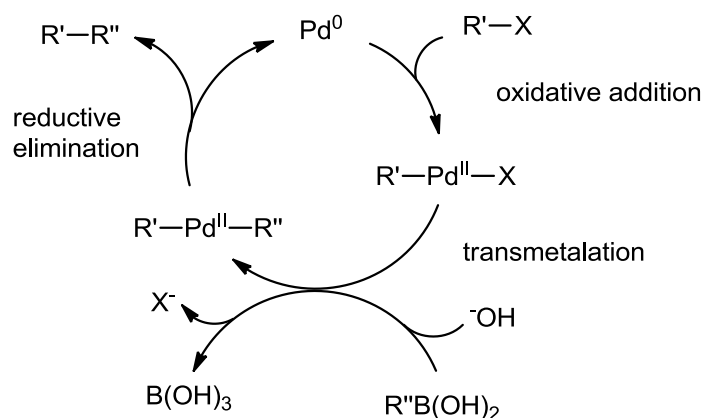
4.4.1 4-(Pyridin-2-yl)benzaldehyde



Scheme 51

Suzuki reaction between compound **102** and **103** to afford aldehyde **104**.

A literature procedure by Reißig and co-workers was followed for the synthesis of 4-(pyridin-2-yl)benzaldehyde **104** via the Suzuki reaction.¹⁶¹ The Suzuki reaction is a palladium-mediated cross-coupling reaction between an organo-boric acid or -borate ester and an aryl or vinyl organohalide. The mechanism is believed to involve oxidative addition, transmetalation, and reductive elimination in the catalytic cycle (Scheme 52).¹⁶²

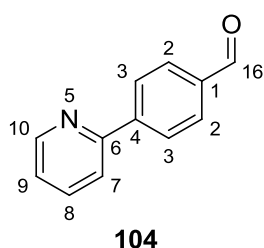


Scheme 52

The proposed catalytic cycle of the Suzuki reaction with an organo-boric acid.¹⁶²

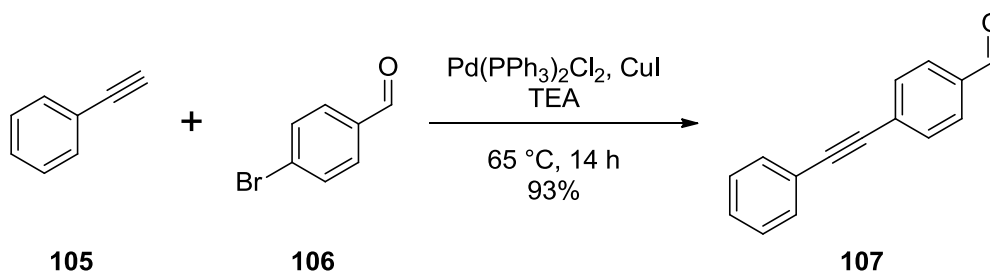
Reißig and co-workers reported on the synthesis of various 2,6-disubstituted pyridine derivatives, including the synthesis of aldehyde **104**, using (4-formylphenyl)boronic acid **103** and 2-bromopyridine **102** in a three-to-two ratio.¹⁶¹ Following their procedure, (4-formylphenyl)boronic acid **103**, 6.6 molar equivalents of 2-bromopyridine **102**, three mole percent (3 mol%) of palladium catalyst and two molar equivalents of potassium carbonate were added to a one-to-four mixture of water and 1,4-dioxane (Scheme 51). This reaction mixture was stirred at 110 °C for 20 minutes and then at 105 °C for a further seven hours. The crude material was purified by column chromatography and the title compound **104** was isolated in a fair yield of 88% compared to the reported quantitative yield.¹⁶¹ The 2-bromopyridine **102** was accidentally used in vast excess and the reaction was repeated

using the correct ratio of the two components. This time, the title compound **104** was understandably obtained in a reduced yield of 73%. The reaction was done a total of three times, with an average yield of 79%. This is not as high as was reported by Reißig and co-workers and could be improved on, possibly by preparing fresh palladium catalyst, using a slightly higher catalyst loading or by degassing the solvent for longer, thereby ensuring the exclusion of oxygen.



The ^1H and ^{13}C NMR spectra for compound **104** matched that of the spectra reported in the literature, with the aldehyde proton and carbon signals visible at 10.09 and 192.0 ppm, respectively.¹⁶¹

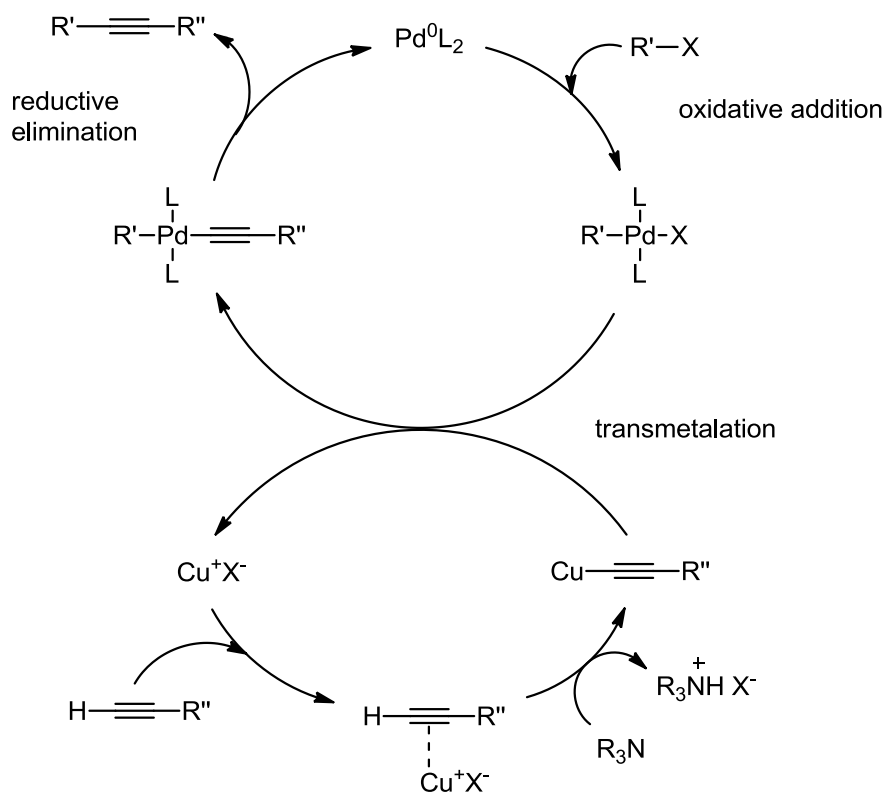
4.4.2 4-(Phenylethynyl)benzaldehyde



Scheme 53

Sonogashira between compounds **105** and **106** to afford internal alkyne **107**.

4-(Phenylethynyl)benzaldehyde **107** was also synthesised *via* a cross-coupling reaction, this time *via* the Sonogashira reaction, as reported by Ho and co-workers.¹⁶³ The Sonogashira reaction is also a palladium-mediated cross-coupling reaction, in this case occurring between aryl or vinyl halides or triflates, and terminal alkynes. This reaction takes place in two independent catalytic cycles when copper is added as co-catalyst, once again involving oxidative addition, transmetalation, and reductive elimination steps to afford the coupled product (Scheme 54).¹⁶⁴



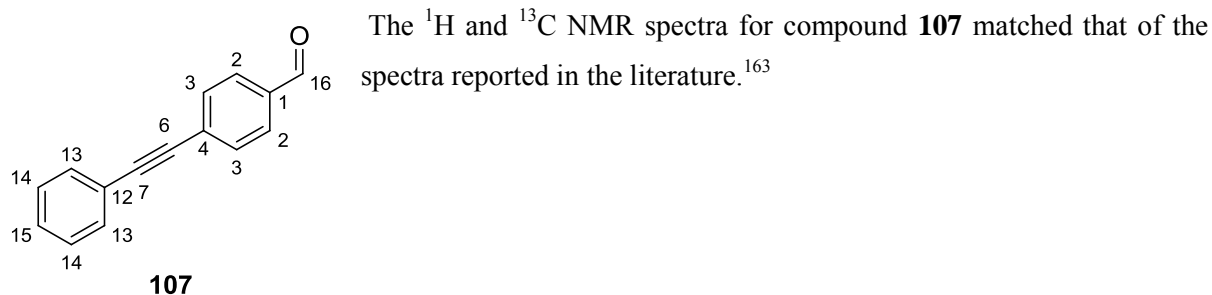
Scheme 54

The proposed catalytic cycle of the Sonogashira reaction.¹⁶⁴

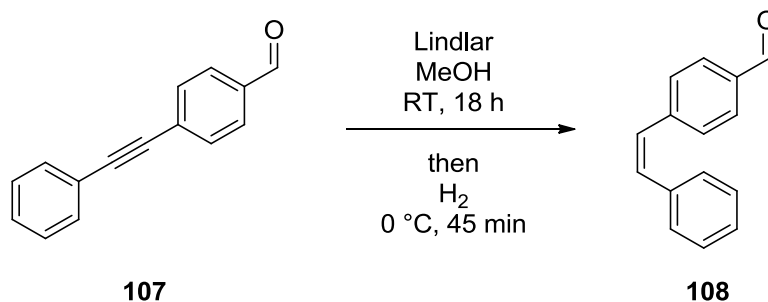
Ho and co-workers reported the synthesis of a number of terminal arylethyne, diarylethyne and arylpyridylethyne, including that of aldehyde **107**. To this end, they used equimolar amounts of phenylacetylene **105** and 4-bromobenzaldehyde **106**, catalytic amounts of the palladium and copper catalysts, an excess of triethylamine and *N,N*-dimethylformamide as solvent. The reaction was stirred for three hours at 100 °C to afford an impressive yield of 95%.¹⁶³

Their procedure was adapted and done in slightly a different manner (Scheme 53). Firstly, double the reported phenylacetylene **105** and metal-catalysts were added, relative to 4-bromobenzaldehyde **106**. In addition, triethylamine was used both as base and as solvent, with no *N,N*-dimethylformamide present. This modified reaction mixture was heated at 65 °C for 20 hours until complete consumption of the starting material according to TLC. The crude material was subsequently purified by column chromatography and pure title compound **107** was isolated in a yield of 72%. This yield was lower than expected, but this was in part due to the difficulty of purifying compound **107** by column chromatography, leading to the loss of product. The title compound **107** and 4-bromobenzaldehyde **106** have similar retention factors (0.44 vs 0.51 in 10% EtOAc/Hexane) and in order to separate the title compound **107** from the small amount of unreacted **106**, the column had to be run at a low polarity, allowing for the separation of the two compounds before eluting. This was not as simple in practice and resulted in tailing of 4-bromobenzaldehyde **106**, with both compounds eventually eluting

together. The slow elution of the compounds also allowed for other impurities to co-elute with the title compound **107**, making purification increasingly difficult. The reaction was carried out a total of five times, with an average yield of 82%. On average, the purification of the product involved two or three runs of column chromatography. To remedy this loss by column chromatography, purification by crystallisation from ethyl acetate was attempted. This was repeated three times, with an average yield of 57%. This evidently did not solve the problem, but was a more convenient procedure, with less time spent on purification and no wastage of column chromatography solvent and silica gel.



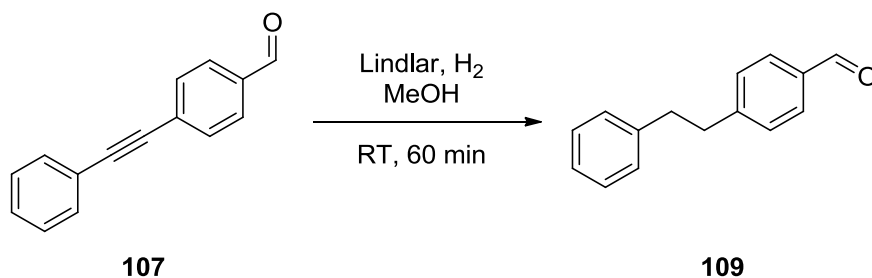
4.4.3 (Z)-4-Styrylbenzaldehyde



Scheme 55

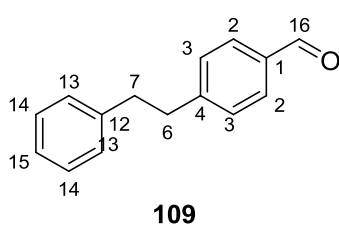
Lindlar reduction of alkyne **107** to afford alkene **108**.

For the partial reduction of the alkyne, it was important that the reaction afforded only the *Z*-isomer and no *E*-isomer. It was therefore decided to use the Lindlar catalyst, palladium on calcium carbonate, poisoned with lead.¹⁶⁵ This would allow for stereoselective reduction of the alkyne **107**, affording (*Z*)-4-styrylbenzaldehyde **108**, and not (*E*)-4-styrylbenzaldehyde. The procedure by Crombie and Harper was loosely followed, as they had a different substrate.¹⁶⁵ For the first run, 4-(phenylethynyl)benzaldehyde **107** was stirred with 0.090 g/mmol Lindlar catalyst in methanol (Scheme 55). The flask was fitted with a hydrogen-filled balloon and was stirred at room temperature for 60 minutes. After the reaction was deemed complete, the crude material was identified as the overreduced compound, 4-phenethylbenzaldehyde **109** (Scheme 56).



Scheme 56

Overreduction of alkyne **107** to afford alkane **109**.



The ^1H NMR spectrum of the material indicated overreduction of the alkyne by the presence of the two sets of aliphatic protons, H_6 and H_7 , each integrating for two protons. They were found as a multiplet between 3.04 and 2.90 ppm, rather than two more downfield doublets each integrating for one proton.

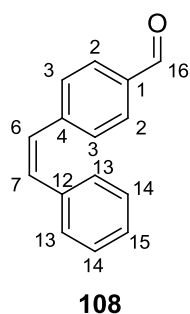
The reaction was repeated a few more times, with conditions varied, in order to find the optimum conditions where starting material was consumed, but no 4-phenethylbenzaldehyde **109** was present (Table 7). Unfortunately, the amount of hydrogen introduced could not be controlled as it was introduced *via* a filled balloon.

Table 7

Different reaction conditions tested for the Lindlar reduction of 4-(phenylethynyl)benzaldehyde **107** to afford **108**. Note: entry six was stirred at room temperature for 18 hours before cooling to 0 °C and attachment of the H_2 balloon.

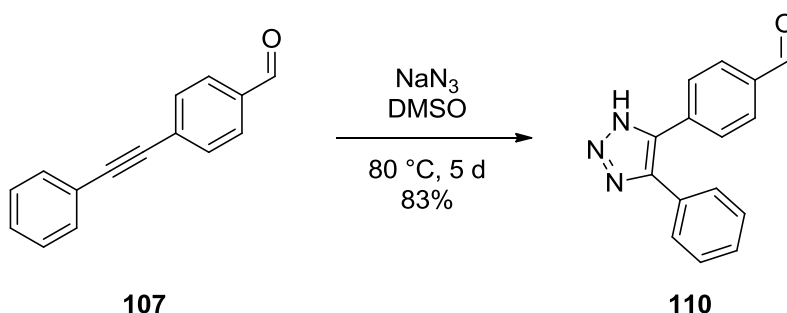
Entry	Lindlar g/mmol	Temperature	Time	Compounds afforded
1	0.090	RT	60 min	109
2	0.040	0 °C	30 min	5:6 of 108:107
3	0.046	0 °C	70 min	4:1 of 108:107
4	0.053	RT	35 min	4:3 of 108:107 (trace 109)
5	0.046	0 °C	60 min	15:2:5 of 108:107: 109
6	0.040	0 °C	45 min	108 (trace 107 & 109)

Eventually, the ideal conditions were found (Table 7, entry 6), and the title compound **108** was obtained with only trace amounts of unreacted **107** and overreduced compound **109**. Note, for this last reaction no purification was done and the slightly contaminated product was used as is for the following reaction.



The ^1H NMR spectrum of **108** showed trace amounts of unreacted starting material and overreduced alkane product, but of particular interest were the two doublets for H_6 and H_7 at 6.80 and 6.65 ppm, each integrating for one proton, indicating a successful reduction. The coupling constant, between these two protons, was 12.3 Hz. This was closer to the average J value for Z -isomers, 10 Hz, than for E -isomers, 16 Hz, suggesting the successful synthesis of the Z -isomer.¹⁶⁶ The ^{13}C NMR spectrum showed only one set of carbon signals, namely for the product. This supports the assumption that the other two compounds are present in trace amounts, only visible in the more sensitive ^1H NMR spectrum.

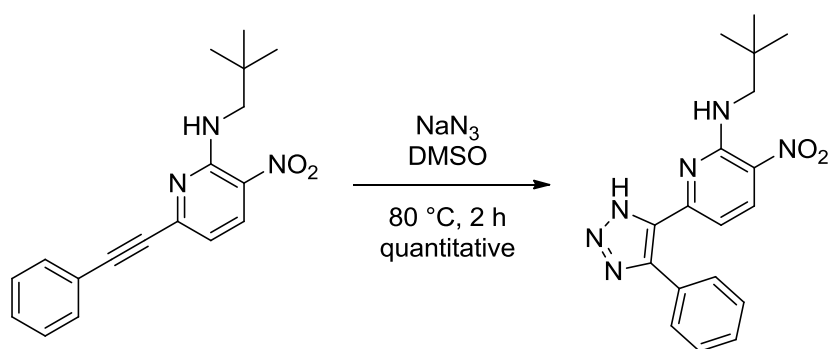
4.4.4 4-(4-Phenyl-1*H*-1,2,3-triazol-5-yl)benzaldehyde



Scheme 57

Thermal click reaction of alkyne **107** to afford triazole **110**.

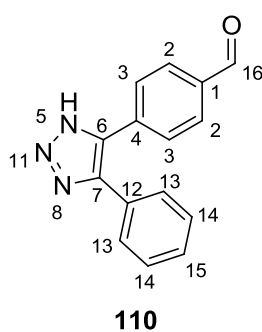
The synthesis of this compound, 4-(4-phenyl-1*H*-1,2,3-triazol-5-yl)benzaldehyde **110**, involved a metal-free thermal click reaction. Bonjouklian and co-workers reported such a procedure using a substrate similar to **110** (Scheme 58).¹⁶⁷



Scheme 58

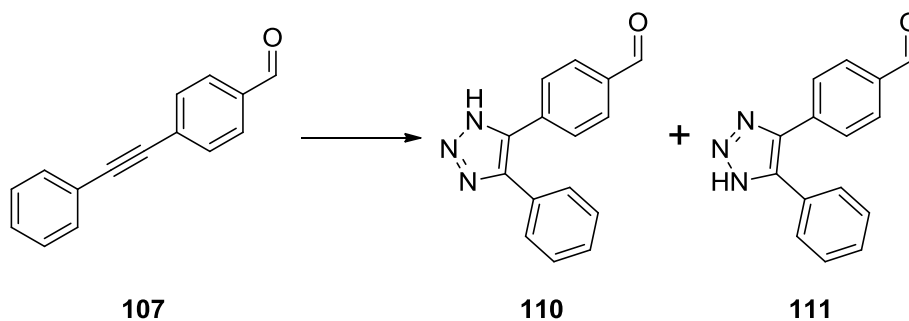
Thermal click reaction procedure reported by Bonjouklian and co-workers.¹⁶⁷

Following the procedure by Bonjouklian and co-workers, 4-(phenylethynyl)benzaldehyde **107** was stirred with sodium azide in dimethyl sulfoxide (Scheme 57). The reaction mixture was then heated to 80 °C for two hours. According to TLC, there was still starting material present after two hours and the reaction mixture was therefore left for longer. The reaction was stopped after three days, finally showing consumption of starting material. The crude material was purified using column chromatography and the title compound **110** was obtained in a yield of 42%. This was significantly less than the reported quantitative yield and it also proved difficult to remove dimethyl sulfoxide from the title compound. The solvent was therefore changed to *N,N*-dimethylformamide, in the hope that it would make the purification less problematic. This was not the case, but the title compound **110** was obtained in a better yield of 72%. Due to the same problems with purification, dimethyl sulfoxide was used again and the reaction was done a total of six times, using dimethyl sulfoxide, with an average yield of 69%, similar to the yield obtained when *N,N*-dimethylformamide was used.



The ^1H and ^{13}C NMR spectra of this compound **110** was not sufficient to indicate a successful reaction as there would only be minor changes expected in the ^1H NMR spectrum and in the ^{13}C NMR spectrum, the important carbon signals, C_6 and C_7 , were not visible above the signal to noise ratio. IR spectroscopy helped determine the success of the reaction by the presence of a N-H stretch band at 3178 cm^{-1} , a N-H bend band at 1605 and a C-N stretch band at 1214 . The mass spectroscopic result of 250.0982 amu corresponded very well with the calculated value of 250.0980 amu , further confirming that the reaction did occur as expected.

This reaction could, in reality, afford both 4-(4-(phenyl-1*H*-1,2,3-triazol-5-yl)benzaldehyde **110** and its tautomeric form 4-(5-(phenyl-1*H*-1,2,3-triazol-4-yl)benzaldehyde **111** (Scheme 59). It was not determined which tautomer was formed, and it was decided to determine the correct form if the eventual target compound showed activity against Akt. This could be done either by obtaining a crystal structure or by using long-range coupling NMR spectroscopy, determining whether the triazole proton interacts with H_3 or H_{13} . It is also entirely possible that these two forms readily interconvert in solution, affording a mixture of the two. To keep things simple, further reactions will be discussed using only tautomer **110**.



Scheme 59

A representation of the reaction occurring to afford both possible tautomers.

4.5 THE WESTERN MOIETY: 4-(3-PHENYLIMIDAZO[1,2-*A*]PYRIDIN-2- YL)BENZALDEHYDE

4-(3-Phenylimidazo[1,2-*a*]pyridin-2-yl)benzaldehyde **53** was the only full western moiety synthesised. The procedure reported by Kettle and co-workers was followed, but had to be adapted as they synthesised the cyclobutanamine version, namely 1-[4-(3-phenylimidazo[1,2-*a*]pyridin-2-yl)phenyl]cyclobutanamine **27** (Figure 26).¹⁰⁹ The synthesis of this compound, **53**, was kindly undertaken by a postdoctoral researcher in the Group of Medicinal and Organic Chemistry of Stellenbosch, Doctor Abu Taher. The synthesis of this and other western moieties will be included in future work [Chapter 8 – Future work].

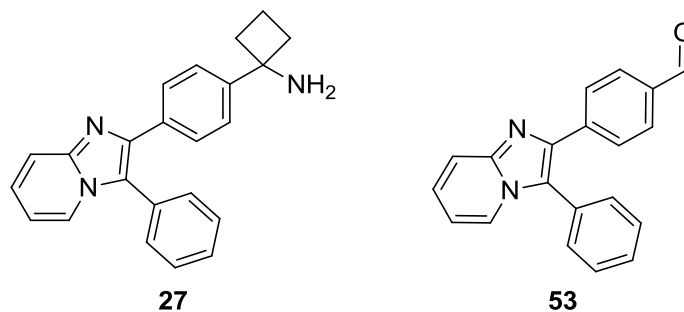


Figure 26

The inspiration for the first western moiety, **27**, and the western moiety itself, **53**.¹⁰⁹

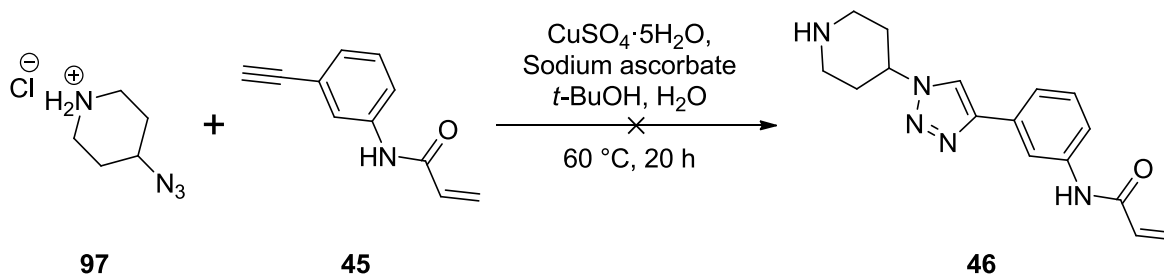
CHAPTER 5 – TOWARDS THE 1,2,3-TRIAZOLE TARGET

THE REAL WORK

5.1 ORIGINAL STRATEGY

As discussed above, a 1,2,3-triazole functionality was introduced with the modification of the original target compounds [see Sections 2.3 and 4.1]. With the synthesised components in hand, the click reaction for the formation of this 1,2,3-triazole functionality could be attempted next (Scheme 44). If successful, this was to be followed by the reductive amination between the product and any of the truncated western moieties and later, the so-called western moiety **53** [see Sections 4.4 and 4.5].

5.1.1 *N*-{3-[1-(Piperidin-4-yl)-1*H*-1,2,3-triazol-4-yl]phenyl}acrylamide



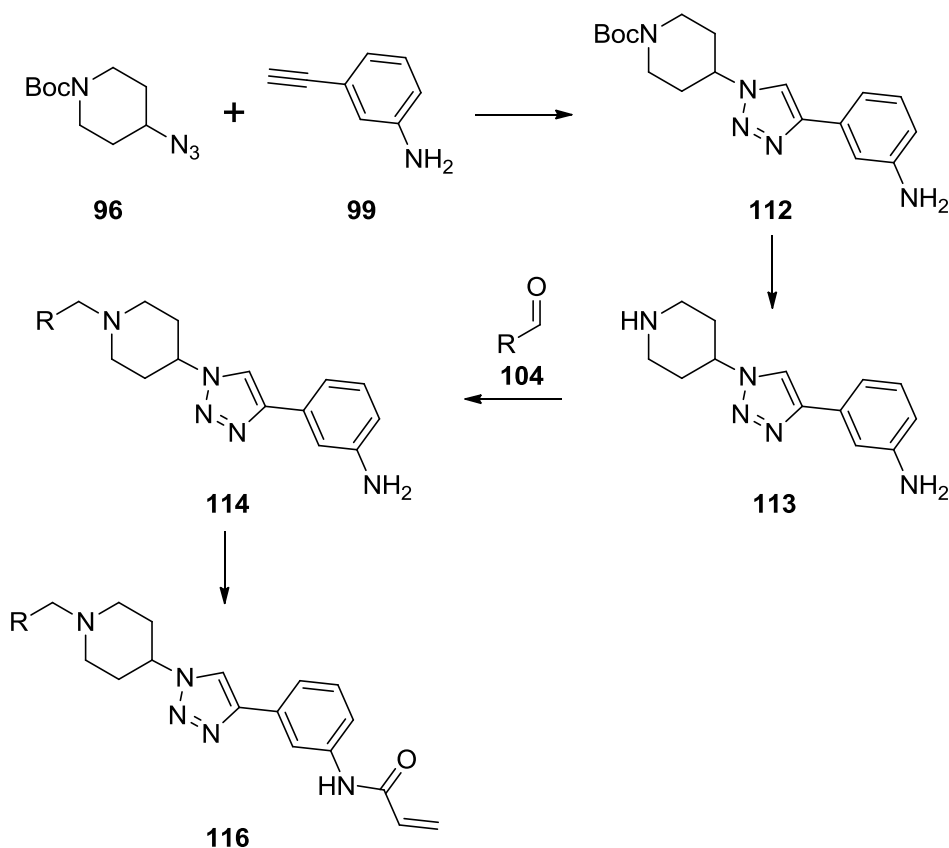
Scheme 60

Attempted click reaction between compounds **97** and **45** to afford 1,2,3-triazole **46**.

A procedure by Sharpless and co-workers was used for the copper(I)-catalysed azide alkyne cycloaddition (CuAAC) towards the eastern moiety, **46**.¹⁶⁸ These researchers reported synthesising a number of different 1,4-disubstituted 1,2,3-triazoles in yields of 92 - 100%, using copper(II) sulphate pentahydrate as copper source and sodium ascorbate as reductant in order to generate copper(I) *in situ*.¹⁶⁸ This literature procedure was followed, heating the azide **97** and alkyne **45** with catalytic copper(II) sulphate pentahydrate and sodium ascorbate in a mixture of water and *tert*-butanol (Scheme 60). The reaction was monitored by TLC, following alkyne **45**, as azide **97** was highly polar and difficult to follow by TLC. This would be the case for all further click reactions using similar substrates. Alkyne **45** was not consumed in three hours, when following the procedure by Sharpless and co-workers, and the reaction mixture was heated at 60 °C for a further 17 hours. Unfortunately, starting material **45** was still not consumed and no product was obtained, with only 69% of starting material **45** recovered.

5.2 FIRST MODIFIED ROUTE TOWARDS THE TARGET COMPOUNDS

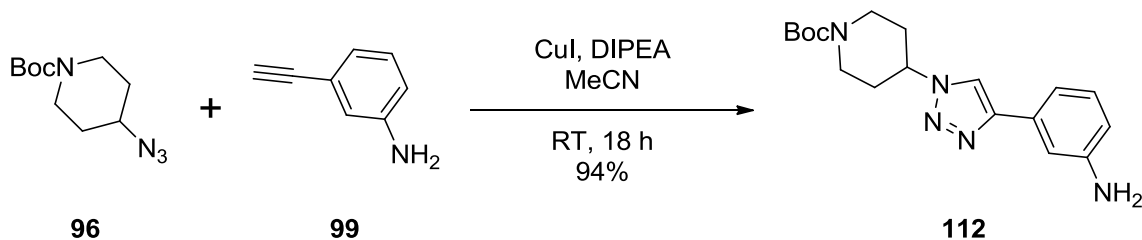
With the failure of the first click reaction, it was decided to tread more cautiously and modify the route in such a manner that the click reaction had an improved chance of succeeding. With so much of the *N*-(3-ethynylphenyl)acrylamide **45** recovered, it was believed that the 4-azidopiperidin-1-ium chloride **97** interfered with the reaction, possibly because it made the reaction mixture slightly acidic. To be safe however, it was decided to next do the click reaction with the Boc-protected amine, *tert*-butyl 4-azidopiperidine-1-carboxylate **96**, and the acrylamide-free alkyne, 3-ethynylaniline **99**, effectively cutting out both possible interferences. This would change the route towards the final compound, but with potentially fewer problems (Scheme 61). Following a successful click reaction, the Boc-protecting group would be removed from **112** and the deprotected compound **113** would subsequently undergo the reductive amination with the truncated western moiety, 4-(pyridin-2-yl)benzaldehyde **104**, followed by acylation with acryloyl chloride to afford the truncated target compound **116**. The reductive amination reaction could have the same selectivity issue as mentioned previously, potentially occurring at both the secondary alkylamine and the aniline amine [see Section 3.1]. Any product from this reaction would therefore have to be scrutinised carefully in order to determine whether it is the correct product or not.



Scheme 61

The modified route towards truncated target compound **116**.

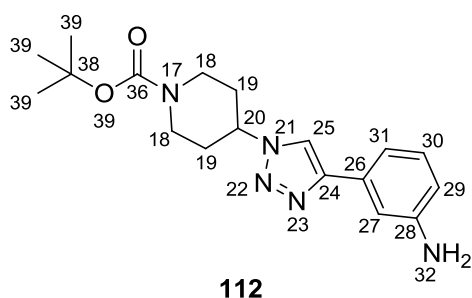
5.2.1 *tert*-Butyl 4-[4-(3-aminophenyl)-1*H*-1,2,3-triazol-1-yl]piperidine-1-carboxylate



Scheme 62

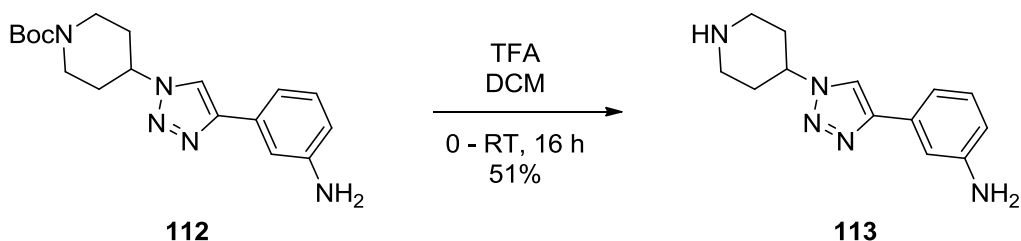
Click reaction between compounds **96** and **99** to afford 1,2,3-triazole **112**.

The click reaction was again performed following the procedure by Sharpless and co-workers, this time affording the purified product **112** in a yield of 58% after 20 hours at 60 °C (Scheme 62).¹⁶⁸ Another set of reagents was then tried, this time from a procedure by Dory and co-workers.¹⁶⁹ These researchers reported a successful click reaction on a nevirapine analogue with attached alkyne functionality, isolating the product in a yield of 97% after 18 hours at room temperature.¹⁶⁹ For this reaction, ten mole percent (10 mol%) copper(I) iodide was used, in conjunction with one molar equivalent of each of two different bases, *N,N*-diisopropylethylamine and 2,6-lutidine. Following this procedure, the azide **96** and alkyne **99** were stirred at room temperature with ten mole percent (10 mol%) copper(I) iodide and two molar equivalents of *N,N*-diisopropylethylamine (Hünig's base) in acetonitrile. Starting material was consumed within 18 hours and the crude material was purified by column chromatography to afford the product in an improved yield of 94%. As can be seen, an extra molar equivalent of *N,N*-diisopropylethylamine was used in the place of the one molar equivalent of 2,6-lutidine. This did not seem to affect the yield in a significant manner.



The ¹H and ¹³C NMR spectra of compound **112** both indicated a successful reaction, with the easily identified singlet from the triazole proton, H₂₅, visible at 7.72 ppm in the ¹H NMR spectrum. The absence of the azide stretch confirmed a successful reaction, as did the mass spectroscopic analysis result of 344.2095 amu, which corresponded with the calculated value of 344.2087 amu.

5.2.2 3-[1-(Piperidin-4-yl)-1*H*-1,2,3-triazol-4-yl]aniline

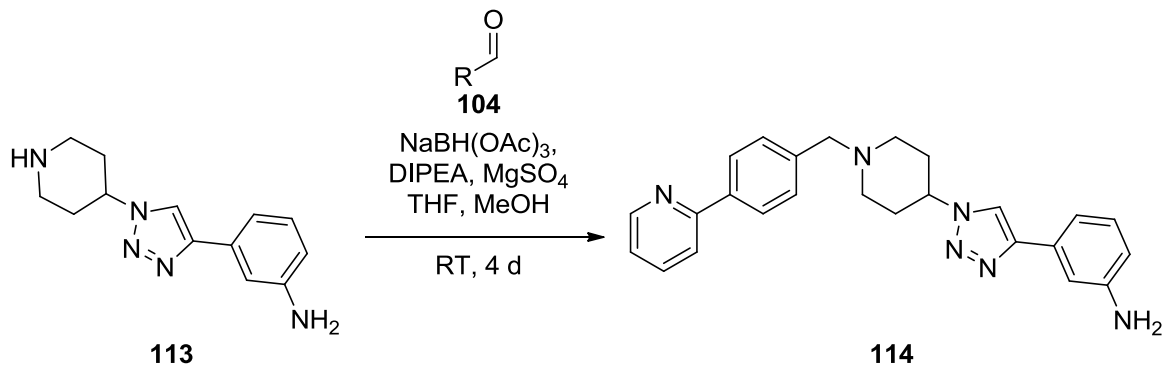


Scheme 63

Boc-deprotection of compound **112** to afford product **113**.

Removal of the Boc-protecting group from compound **112** took place using the same procedure as before, by stirring the Boc-protected starting material **112** with trifluoroacetic acid in dichloromethane [see Section 4.2.2]. The reaction mixture was neutralised with aqueous sodium bicarbonate, affording the neutral 3-[1-(piperidin-4-yl)-1*H*-1,2,3-triazol-4-yl]aniline **113** in a crude yield of 51% (Scheme 63). The reaction was also done without neutralising the reaction mixture, obtaining the trifluoroacetate salt instead. Neither of these compounds was purified and was used as is in the following reaction. The ¹H NMR spectra of the impure compounds were promising, indicating successful Boc-removal by the absence of proton peaks for the *tert*-butyloxycarbonyl group. IR spectroscopy also confirmed this, with no carbonyl C=O stretch found in the spectra.

5.2.3 3-(1-{1-[4-(Pyridin-2-yl)benzyl]piperidin-4-yl}-1*H*-1,2,3-triazol-4-yl)aniline



Scheme 64

Reductive amination between compounds **113** and **104** to afford product **114**.

The reductive amination was carried out using a procedure by Hudson and Sweeney.¹⁷⁰ These researchers reported an efficient reductive amination in basic medium that worked for various aldehydes and ketones with a variety of amines, affording yields between 65 - 97%.¹⁷⁰ The researchers used the bulky borohydride, sodium triacetoxyborohydride as reducing agent, *N,N*-diisopropylethylamine as base and tetrahydrofuran as solvent.¹⁷⁰ They also included anhydrous

magnesium sulphate to act as desiccant, absorbing water released during iminium formation, thereby helping drive the reaction forwards. This procedure was chosen specifically as it was done in basic medium, allowing for the *in situ* neutralisation of the trifluoroacetate salt.

4-(Pyridin-2-yl)benzaldehyde **104**, the first of the truncated western moieties, was used as aldehyde and the reaction was originally performed using the neutral amine compound **113**, adding that and magnesium sulphate and three molar equivalents of *N,N*-diisopropylethylamine to tetrahydrofuran and stirring the mixture at room temperature (Scheme 64). Neither of the starting materials was soluble in tetrahydrofuran so methanol was added to the reaction mixture, thus solubilising the starting materials. Two molar equivalents of sodium triacetoxyborohydride were added to the reaction mixture and it was stirred overnight, as described by Hudson and Sweeney.¹⁷⁰ However, the starting material was not consumed during this time and the reaction mixture was left for a further three days, after which the crude material was purified, affording the product **114** in a low yield of 13%. This reaction was repeated with the trifluoroacetate salt of compound **113**, adding enough *N,N*-diisopropylethylamine to neutralise the ammonium salt, and using four molar equivalents of triacetoxyborohydride with methanol as solvent. This reaction proceeded in a similar manner than the first, with starting material **104** still visible by TLC after four days. Ultimately, a mixture of product **114** and aldehyde **104** was isolated, accounting for less than 20% of **104** used. The product, **114**, was difficult to purify due to a high polarity and was reasonably insoluble in the common solvents used in column chromatography. ¹H NMR spectroscopy revealed that this could be the desired product **114**, or the expected side-product **115**, with the reductive amination taking place at the aniline (Figure 27). Owing to the low yields obtained in both of these reactions, the route was abandoned and further analysis was therefore not done to determine if either of these reactions gave the product **114**, or the side-product **115** (Figure 27).

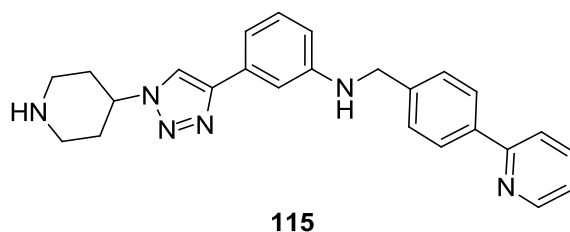


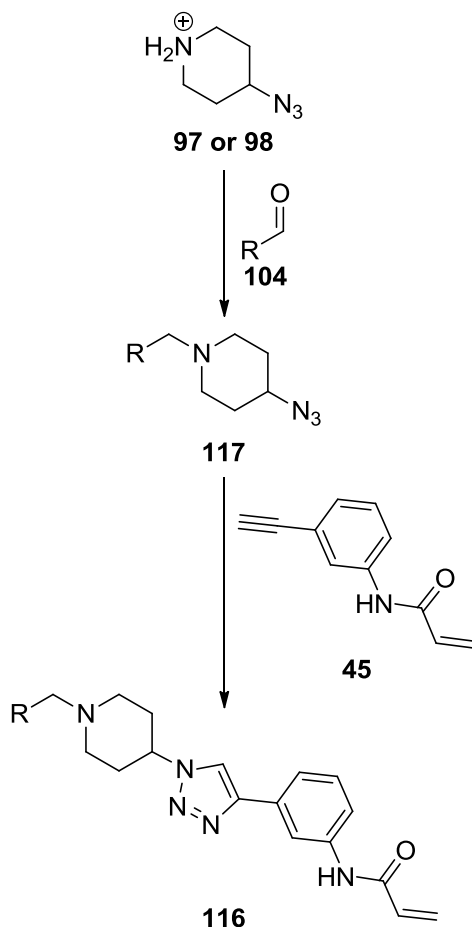
Figure 27

The potential side-product **115** for the reductive amination attempted in this section.

5.3 A BREAKTHROUGH

Due to the limited success with the above reductive amination procedure, it was decided to attempt switching the reductive amination and click reactions, thus first doing the reductive amination and

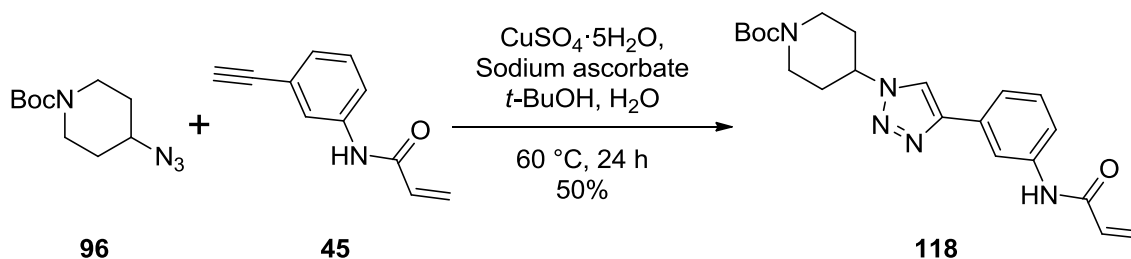
then following with the click reaction (Scheme 65). In order to keep the reaction steps to a minimum, it was decided to attempt the click reaction with the acrylamide already present. A test reaction was first necessary to determine whether this would be successful [see Section 5.3.1].



Scheme 65

A modified route towards **116**, now with the reductive amination prior to the click reaction.

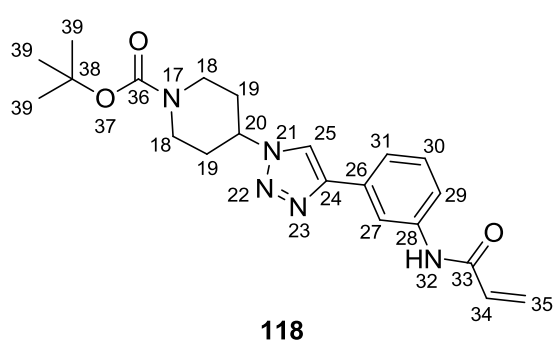
5.3.1 *tert*-Butyl 4-[4-(3-acrylamidophenyl)-1*H*-1,2,3-triazol-1-yl]piperidine-1-carboxylate



Scheme 66

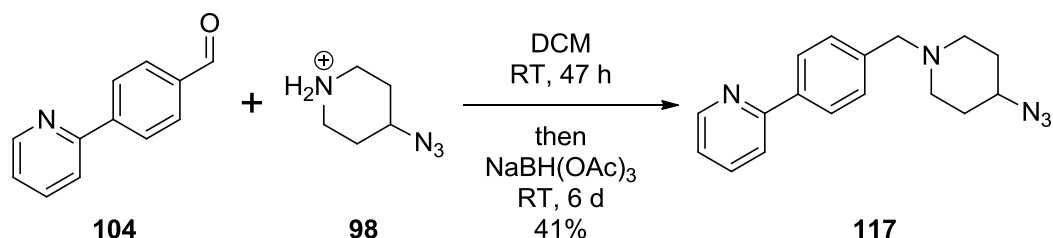
Click reaction between compounds **96** and **45** to afford 1,2,3-triazole **118**.

tert-Butyl 4-azidopiperidine-1-carboxylate **96** and *N*-(3-ethynylphenyl)acrylamide **45** were used in the test reaction in order to determine whether the click reaction would proceed satisfactorily in the presence of an acrylamide and that the acrylamide would stay intact during the reaction conditions. Following the procedure described by Sharpless and co-workers, the reaction mixture was stirred at 60 °C for 24 hours (Scheme 66).¹⁶⁸ The reaction was indeed successful, with the product **118** purified and isolated in a moderate yield of 50%.



The ¹H and ¹³C NMR spectra for this compound indicated a positive click reaction, with the triazole singlet, H₂₅, visible at 7.73 ppm in the ¹H NMR spectrum and the acrylamide signals visible in both of the NMR spectra. The IR spectrum also confirmed this, with the acrylamide's carbonyl C=O stretch at 1663 cm⁻¹, distinct from that of the Boc-protecting group at 1693 cm⁻¹. Furthermore, the mass spectroscopic analysis result of 398.2193 amu corresponded well with the calculated value of 398.2192 amu.

5.3.2 2-{4-[(4-Azidopiperidin-1-yl)methyl]phenyl}pyridine



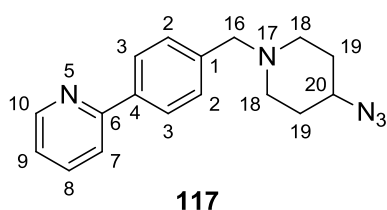
Scheme 67

Reductive amination between compounds **104** and **98** to afford product **117**.

Following the success of the test reaction, the first reductive amination of this route could be attempted, once again using the truncated western moiety, 4-(pyridin-2-yl)benzaldehyde **104**, thereby ensuring consistency. This time, a procedure from a patent by Benesh and Blanco-Pillado was used, wherein the researchers describe the reductive amination between a substituted piperidine and benzaldehyde.¹⁷¹ In this procedure, sodium triacetoxyborohydride was used as reducing agent and the reaction mixture was stirred overnight at room temperature in dichloromethane to afford the product in a yield of 95%.¹⁷¹ The aldehyde and amine, compounds **104** and **98** respectively, were thus stirred in dichloromethane for six days, with the first sodium triacetoxyborohydride added after 47 hours and another addition three days later. The delay in the addition of the reducing agent was to allow for the formation of the iminium intermediate, before adding the borohydride, possibly minimising reduction

of the aldehyde starting material to an alcohol as was seen previously [see Section 3.4.3]. The reaction mixture was left for such a long time period as complete consumption of starting material was once again a problem, with starting material still visible by TLC, even after eight days. The crude material was purified and the product, **117**, was isolated in a yield of 41%. Delaying the addition of the reducing agent did not help, with an indeterminate amount of reduced aldehyde also identified.

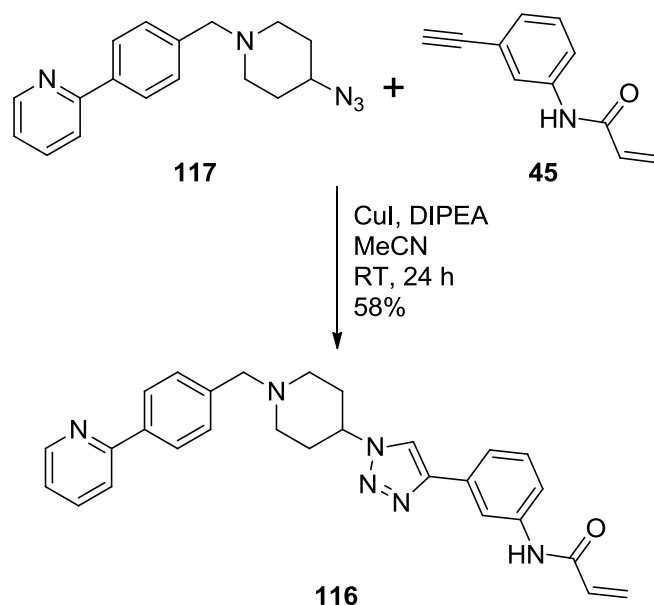
A second procedure was used in an attempt to improve the yield, this time using a procedure by Moreira and co-workers.¹⁷² These researchers reported a reductive amination protocol using similar substrates as Benesh and Blanco-Pillado, but with sodium cyanoborohydride as reducing agent and MeOH as solvent, stirring the reaction mixture at room temperature for 20 hours and isolating their product in a yield of 66%.^{171; 172} The substrates were thus dissolved in MeOH and stirred at 0 °C during the addition of the sodium cyanoborohydride. The reaction mixture was then allowed to warm up to room temperature and was stirred for three days, once again with sluggish starting material consumption. The crude material was purified and the product **117** was isolated in a yield of 36%. This was slightly less than was achieved with the previous reaction, but was obtained in a considerably shorter timeframe.



The ¹H and ¹³C NMR spectra for compound **117** were as expected, with the methylene signal visible at 3.56 and 62.6 ppm in the ¹H and ¹³C NMR spectra, respectively. IR spectroscopy showed that the azide was still intact, with the azide stretch visible at 2088 cm⁻¹. Furthermore, mass spectroscopic analysis confirmed a successful

reaction, with the result of 294.1714 amu corresponding to the calculated value of 294.1719 amu.

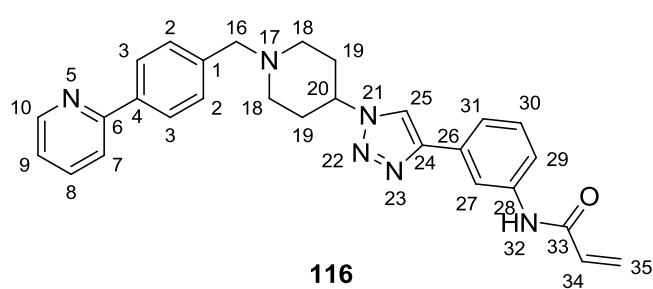
5.3.3 *N*-[3-(1-{1-[4-(Pyridin-2-yl)benzyl]piperidin-4-yl}-1*H*-1,2,3-triazol-4-yl)phenyl]acrylamide



Scheme 68

Click reaction between compounds **117** and **45** to afford 1,2,3-triazole **116**.

For the click reaction towards the first target compound, it was decided to use the procedure by Dory and co-workers as this gave a comparatively higher yield than that of Sharpless and co-workers when using the same substrates [see Section 5.2.1].¹⁶⁹ *N*-(3-Ethynylphenyl)acrylamide **45** was used as the alkyne and it was hoped that the acrylamide would survive these reaction conditions, as it did during the procedure by Sharpless and co-workers at the beginning of this section (Scheme 68). The reaction was successful, affording the first completed target compound, product **116**, in a yield of 58%.



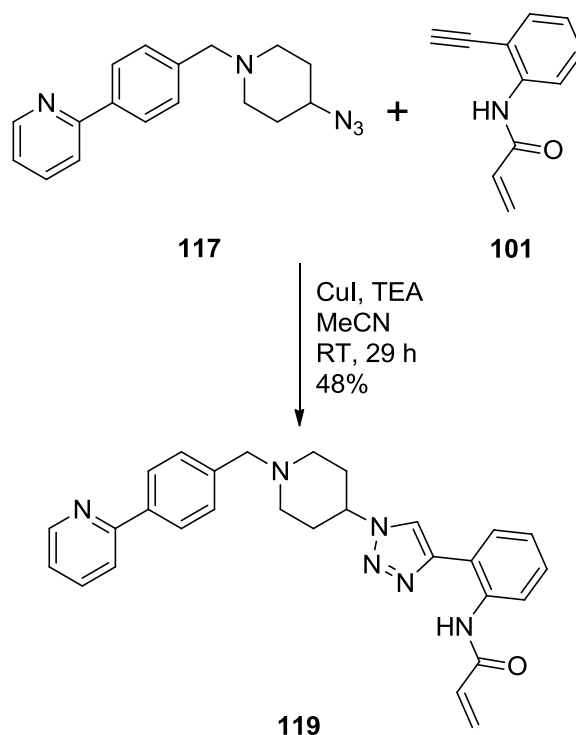
The ¹H and ¹³C NMR spectra of compound **116** correlated well with the spectra of the two building blocks, compounds **45** and **117**. The triazole proton, H₂₅, was visible as a characteristic singlet at 7.80 ppm and the presence of the acrylamide proton and carbon signals pointed to it surviving the reaction conditions intact. The amide proton, H₃₂, was not observed in the ¹H NMR spectrum. This was the case for a number of related compounds synthesised as discussed below. With the absence of the starting material azide stretch, IR spectroscopy also pointed to a successful reaction. This was confirmed when the mass spectroscopic analysis result of 465.2406 amu corresponded with the expected value of 465.2403 amu. A two-dimensional NMR spectrum was obtained using gradient Heteronuclear Single Quantum Coherence (gHSQC), allowing for the identification of which protons were bonded to which carbon atoms. Additionally, ¹³C NMR

For the click reaction towards the first target compound, it was decided to use the procedure by Dory and co-workers as this gave a comparatively higher yield than that of Sharpless and co-workers when using the same substrates [see Section 5.2.1].¹⁶⁹ *N*-(3-Ethynylphenyl)acrylamide **45** was used as the alkyne and it was hoped that the acrylamide would survive these reaction conditions, as it did during the procedure by Sharpless and co-workers at the beginning of this section (Scheme 68). The reaction was successful, affording the first completed target compound, product **116**, in a yield of 58%.

spectra were obtained using Distortionless Enhancement by Polarization Transfer (DEPT), thereby allowing for complete assignment of the ^1H and ^{13}C NMR spectra.

With the first completed target compound **116** in hand, it was decided to synthesise one of its regioisomers, namely compound **119**, by doing the click reaction with a different alkyne, namely *N*-(2-ethynylphenyl)acrylamide **101**.

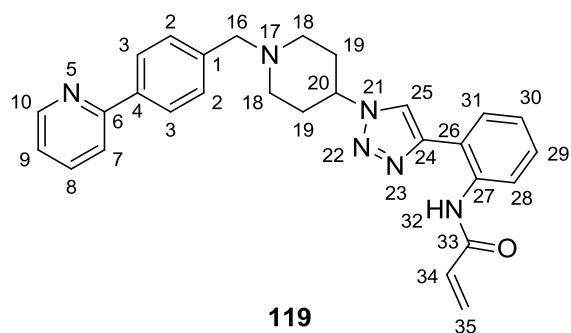
5.3.4 *N*-[2-(1-{1-[4-(Pyridin-2-yl)benzyl]piperidin-4-yl}-1*H*-1,2,3-triazol-4-yl)phenyl]acrylamide



Scheme 69

Click reaction between compounds **117** and **101** to afford 1,2,3-triazole **119**.

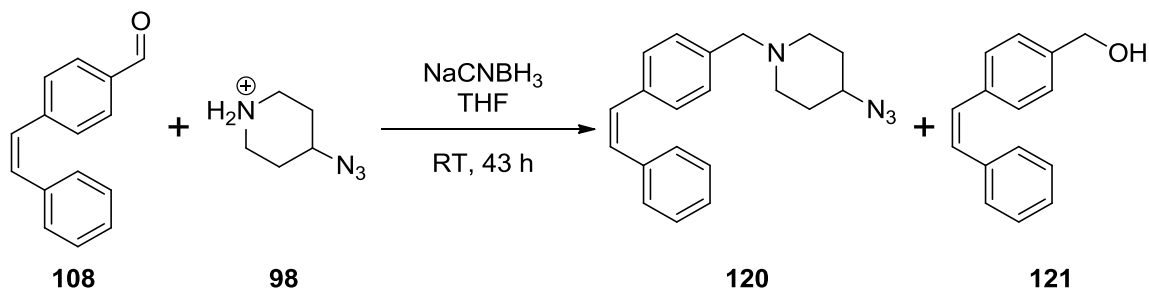
The alkyne *N*-(2-ethynylphenyl)acrylamide **101** was used for the synthesis of the regioisomer of **116**, with the acrylamide now in an *ortho* position to the triazole. The procedure by Dory and co-workers was again followed, but this time the less sterically hindered amine base, triethylamine, was used in the place of the previously used *N,N*-diisopropylethylamine (Scheme 69). This change did not appear to have a negative influence on the reaction, with product **119** isolated in a reasonable yield of 48%.¹⁶⁹



The ^1H and ^{13}C NMR spectra of **119** looked similar to that of its regioisomer, **116**, with the triazole's proton visible as a singlet at 7.90 ppm of the ^1H NMR spectrum. Once again, the starting material azide stretch was absent from the IR spectrum and the mass spectroscopic analysis result of 465.2408 amu corresponded favourably with the calculated value of 465.2403 amu.

Following the successful synthesis of these two isomers, it was time to move on to another of the truncated western moieties, namely (*Z*)-4-styrylbenzaldehyde **108**.

5.3.5 (*Z*)-4-Azido-1-(4-styrylbenzyl)piperidine



Scheme 70

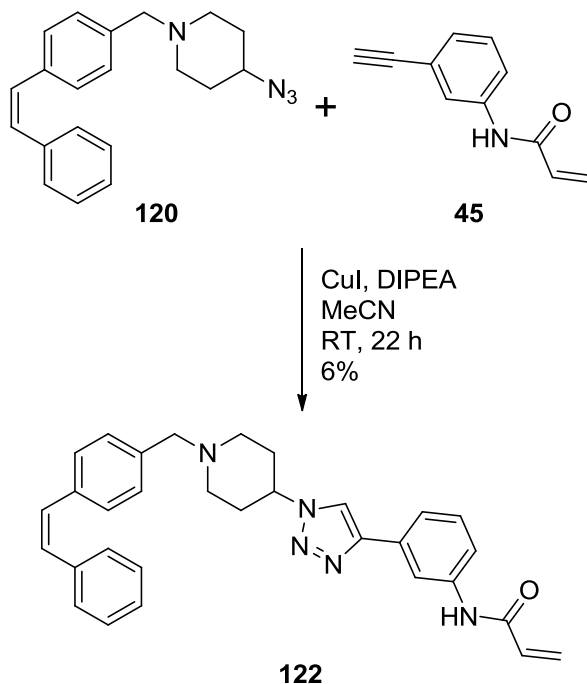
Reductive amination between compounds **108** and **98** to afford product **120** and side-product **121**.

As the procedure by Moreira and co-workers afforded a comparable yield in a much shorter time with the synthesis of **117**, it was decided to follow this procedure with the preparation of **120**, with (*Z*)-4-styrylbenzaldehyde **108** as aldehyde.¹⁷² The reaction mixture was stirred at room temperature for 43 hours, after which the starting material was consumed and the crude material was purified by column chromatography (Scheme 70). Unfortunately, the product **120** was not isolated in a good yield. In fact, it was isolated as a mixture with the reduced aldehyde, (*Z*)-(4-styrylphenyl)methanol **121**, whose retention factor overlapped with that of the product, **120**. ^1H NMR analysis of this mixture indicated the presence of both these compounds and IR spectroscopy confirmed this, with both the alcohol O-H stretch of side-product **121** and the azide stretch of product **120** visible at 3357 and 2091 cm^{-1} , respectively.

Once again, the starting material aldehyde was reduced to the alcohol, either pointing to the fact that these aldehydes are readily reduced or, more likely, that the ammonium salt **98** is not reacting as well as was hoped, thus allowing for the reduction of the aldehyde in the presence of the excess reducing

agent. Instead of attempting purification and potentially losing the small amount of product present, it was decided to continue with the mixture to the last reaction, the click reaction.

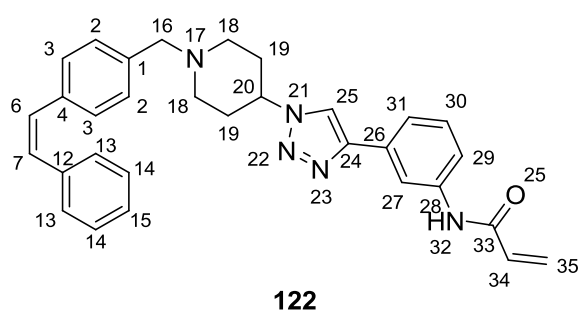
5.3.6 (Z)-N-(3-{1-[1-(4-Styrylbenzyl)piperidin-4-yl]-1H-1,2,3-triazol-4-yl}phenyl)acrylamide



Scheme 71

Click reaction between compounds **120** and **45** to afford 1,2,3-triazole **122**.

Using the mixture containing (Z)-4-azido-1-(4-styrylbenzyl)piperidine **120** and alcohol **121**, the procedure by Dory and co-workers was followed, affording the title compound **122** in a very poor yield of 6% over two steps (Scheme 71).¹⁶⁹

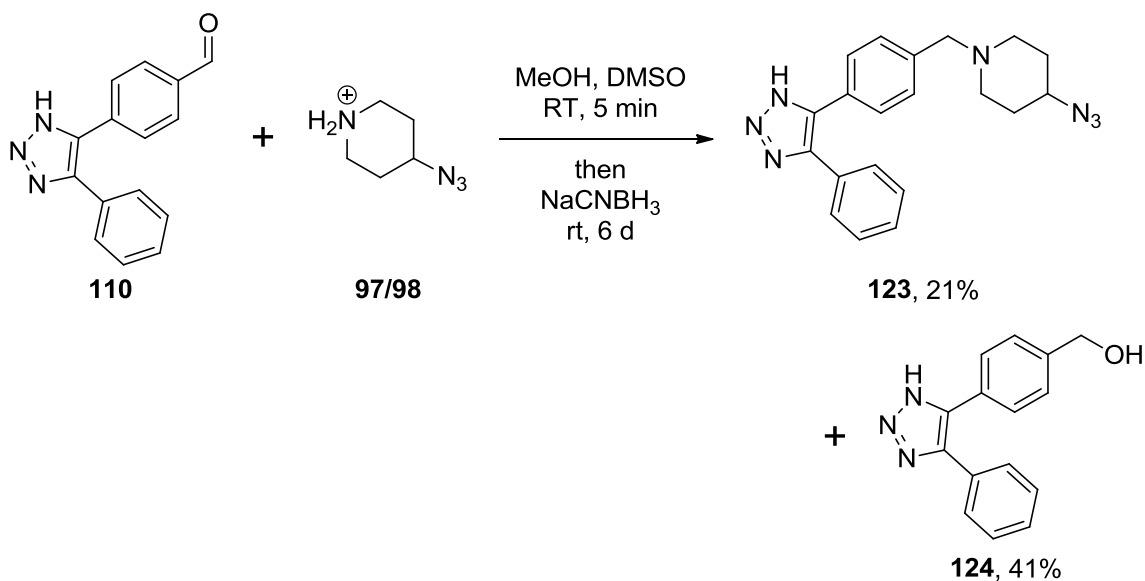


The ¹H and ¹³C NMR spectra of compound **122** looked as expected, and the triazole proton singlet was identified at 7.82 ppm. IR spectroscopy indicated the absence of the azide stretch and mass spectroscopic analysis confirmed a successful reaction, as the result of 490.2603 amu corresponded with the calculated value of

490.2607 amu.

With the successful synthesis of truncated target compound **122**, the next step was to utilise the third and final truncated western moiety, aldehyde **110**.

5.3.7 4-Azido-1-[4-(4-phenyl-1H-1,2,3-triazol-5-yl)benzyl]piperidine



Scheme 72

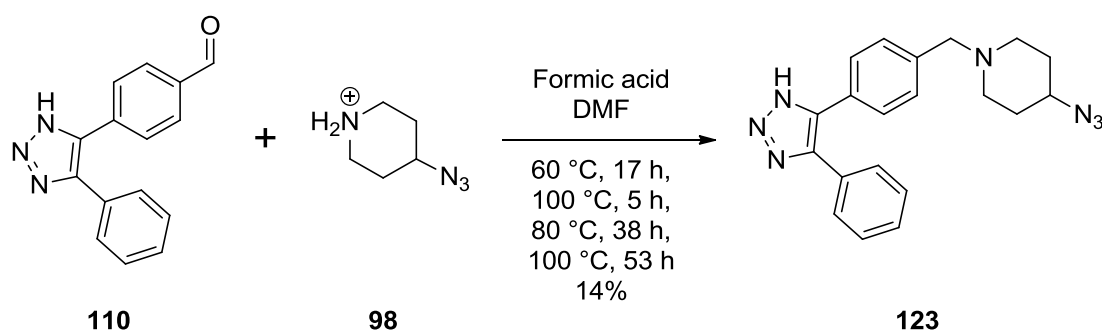
Reductive amination between compounds **110** and **97** (or **98**) to afford product **123** and side-product **124**.

Following the procedure by Moreira and co-workers, 4-azido-1-[4-(4-phenyl-1H-1,2,3-triazol-5-yl)benzyl]piperidine **123** was to be synthesised using 4-(4-phenyl-1H-1,2,3-triazol-5-yl)benzaldehyde **110** as aldehyde.¹⁷² This procedure was slightly modified from those used before, as the aldehyde **110** was not soluble in methanol so dimethyl sulfoxide was added to help solubilise it (Scheme 72). This time, the product **123** was isolated in a poor yield of 8% (as well as 18% reduced aldehyde, [4-(4-phenyl-1H-1,2,3-triazol-5-yl)phenyl]methanol **124**) and the reaction was repeated, using the previously discussed procedure by Benesh and Blanco-Pillado.¹⁷¹ This did not improve the yield, again affording a mixture of product **123** and alcohol **124**. Various combinations of reagents and reaction conditions were attempted and are summarised in Table 8. These included the procedure by Hudson and Sweeney (entry 3), the use of a Dean-Stark apparatus (entry 8) and also the addition of zinc chloride as Lewis acid, following a procedure by Galley and co-workers (entry 9).^{170; 173} The Dean-Stark apparatus was employed to remove water from the reaction mixture as it was formed as by-product in the formation of the iminium intermediate, thereby preventing the reverse reaction. The reaction was carried out in benzene at 100 °C for 31 hours, before the reducing agent, sodium cyanoborohydride was introduced. Unfortunately, this did not improve the yield or prevent aldehyde reduction, with the mixture of both obtained with a mass-recovery of 26%. The use of a Lewis acid had a similar function as the Dean-Stark apparatus. The zinc chloride had to act as Lewis acid, activating the aldehyde and this promoting attack by the amine. This did not go as planned, once again a mixture of product and alcohol was obtained, with a low mass-recovery of 26%.

Table 8

Different reaction conditions tested for the synthesis of 4-azido-1-[4-(4-phenyl-1*H*-1,2,3-triazol-5-yl)benzyl]piperidine **123**. Note: A number of reactions afforded a mixture of compounds **123** and **124** and the yields were not determined in these cases.

Entry	Compounds 110:97/98	Compound 97 or 98	Reductant and its equivalents		Additional and its equivalents		Solvent	Compound(s) isolated
1	1.5:1	98	NaCNBH ₃	3			MeOH	8% of 123
2	1:1.2	98	NaCNBH ₃	3			THF	123&124
3	1:1.2	98	NaBH(OAc) ₃	2	DIPEA & MgSO ₄	3	THF	110
4	1:1.2	98	NaCNBH ₃	3			THF	124
5	1:1.2	98	NaCNBH ₃	3			MeOH (+DMSO)	21% of 123
6	1:1.2	98	NaCNBH ₃	5	AcOH	<6	MeOH	110&124
7	1:1	98	NaBH(OAc) ₃	3.5+	TEA	2.2	NMP	-
8	1:1.1	98	NaCNBH ₃	3.6			Benzene then THF	123&124
9	1:1	97	NaCNBH ₃	2.9	ZnCl ₂	4.2	MeOH	123&124

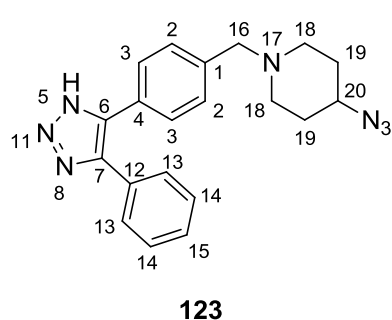


Scheme 73

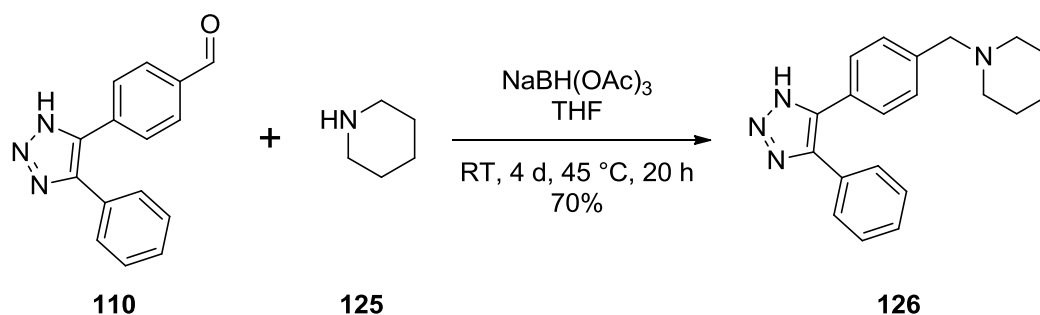
Reductive amination between compounds **110** and **98** to afford product **123**.

None of the reactions attempted had a reasonable yield, with the maximum being 21% (Table 8, entry 5) and it was thus decided to try entirely different reaction conditions, using the Leuckart-Wallach reaction as per a procedure reported by Yang and co-workers.¹⁷⁴ This reaction involves the reductive amination using formic acid as reducing agent for the reduction of the imine/iminium intermediate.¹⁷⁵ Yang and co-workers reported doing the Leuckart-Wallach reaction with a substituted piperidine as the hydrochloric acid salt and various aldehydes, by stirring the reagents together in *N,N*-dimethylformamide at 100 °C for 2 to 24 hours, and obtaining the products in yields from 23 to

81%.¹⁷⁴ This reaction was then attempted, heating the reaction mixture at elevated temperatures for several days in the attempt to have the majority of the aldehyde starting material **110** consumed (Scheme 73). After this, purification of the crude material by column chromatography, the elusive product **123**, was obtained still in a poor 14% yield. This reaction was repeated, but unfortunately did not result in an increase in yield.



The ¹H and ¹³C NMR spectra of **123** were as expected, with the signal for the methylene protons, H₁₆, visible at 3.56 ppm and the carbon signal, C₁₆, visible at 62.5 ppm. IR spectroscopy supported this, with the azide stretch visible at 2089 cm⁻¹. The mass spectroscopic results further supported a positive reaction with the result of 360.1940 amu corresponding well with the calculated value of 360.1937 amu.



Scheme 74

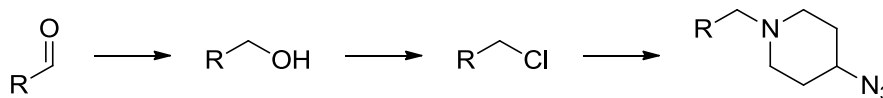
Reductive amination between compounds **110** and **125** to afford product **126**.

To help determine whether the aldehyde **110** or the ammonium salts **97** and **98** were the problem, a reductive amination reaction was carried out using the aldehyde **110** and piperidine **125** (Scheme 74). The product, 1-[4-(4-phenyl-1*H*-1,2,3-triazol-5-yl)benzyl]piperidine **126** was isolated in a good yield of 70%. This indicated that the use of the positively-charged ammonium salts, **97** and **98**, with this aldehyde **110**, was less than ideal with regards to their activity towards the aldehydes. Unfortunately, as discussed, 4-azidopiperidine **44** could not be reliably isolated and a different route was to be undertaken [see Section 4.2.3].

5.4 A FURTHER MODIFICATION

With the failure to obtain 4-azido-1-[4-(4-phenyl-1*H*-1,2,3-triazol-5-yl)benzyl]piperidine **123** in a yield adequate for the following click reaction, the route was modified, once more, in order to exclude the problematic reductive amination step (Scheme 75). To this end, the substituted benzaldehyde was to be reduced to a benzyl alcohol, and then converted into an benzyl chloride using the Appel

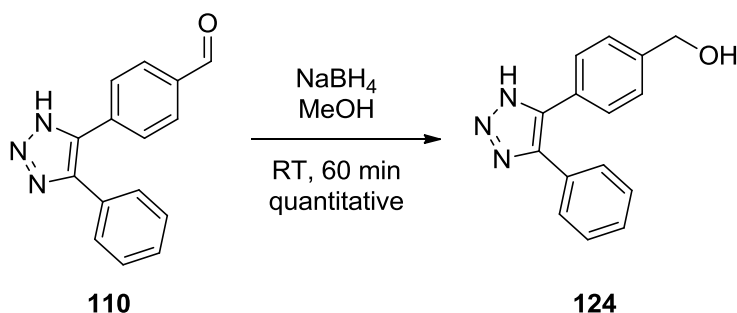
reaction.¹⁷⁶ This benzyl chloride could then undergo nucleophilic substitution with a 4-azidopiperidin-1-ium salt, affording the same product as the reductive amination would, ready for the final step, the click reaction.



Scheme 75

A schematic representation of the new planned route, where RCO can be any of the truncated or full western moieties.

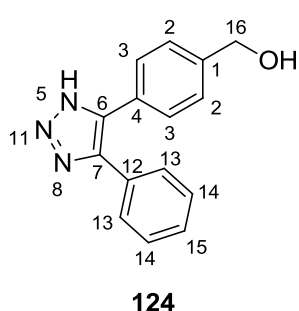
5.4.1 [4-(4-Phenyl-1H-1,2,3-triazol-5-yl)phenyl]methanol



Scheme 76

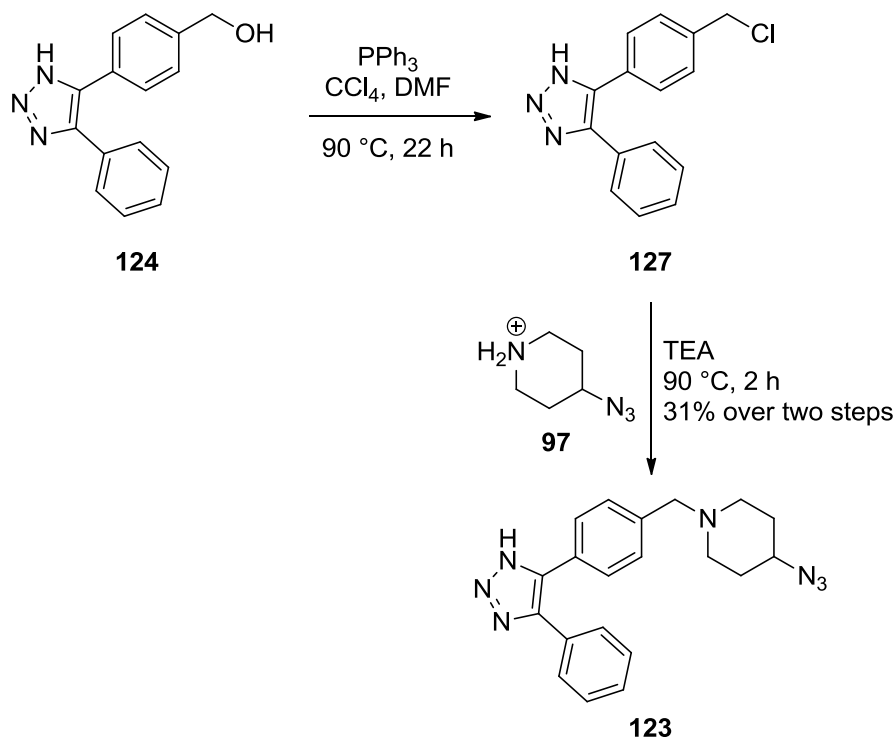
Reduction of aldehyde **110** to afford alcohol **124**.

For the synthesis of the alcohol **124**, 4-(4-phenyl-1H-1,2,3-triazol-5-yl)benzaldehyde **110** was reduced following a procedure by Zhao and co-workers.¹⁷⁷ These researchers reported the reduction of substituted benzaldehydes, using sodium borohydride as reductant, stirring the sodium borohydride with the aldehyde at room temperature for 30 minutes to afford the corresponding alcohols in an average yield of 80%.¹⁷⁷ Following this procedure, the reaction mixture was stirred at room temperature for 60 minutes (Scheme 76). The crude alcohol **124** was obtained in a quantitative yield and was used as is for the following reaction.



The ¹H and ¹³C NMR spectra for compound **124** indicated a positive result, with the methylene proton and carbon signals clearly visible in the spectra at 4.66 and 64.0 ppm respectively. IR spectroscopy confirmed this with the presence of the alcohol O-H stretch at 3124 cm⁻¹, as did the mass spectroscopic analysis result of 252.1138 amu, which corresponded with the calculated value of 252.1137 amu.

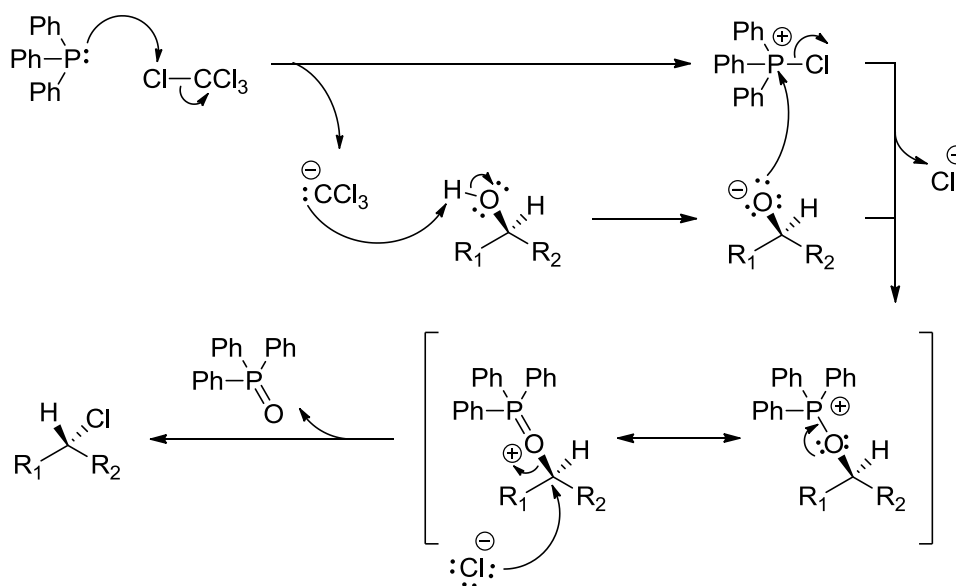
5.4.2 4-Azido-1-[4-(4-phenyl-1*H*-1,2,3-triazol-5-yl)benzyl]piperidine



Scheme 77

Appel reaction using alcohol **124** and amine **97** to afford compound **123**.

With the benzyl alcohol, [4-(4-phenyl-1*H*-1,2,3-triazol-5-yl)phenyl]methanol **124**, in hand, the next step was the Appel reaction to convert alcohol **124** into the benzyl chloride **127** and then to subsequently have the benzyl chloride **127** undergo nucleophilic substitution to afford 4-azido-1-[4-(4-phenyl-1*H*-1,2,3-triazol-5-yl)benzyl]piperidine **123**. The Appel reaction is initiated by the halogenation of triphenylphosphine and the subsequent formation of an alkoxide from the alcohol starting material (Scheme 78).¹⁷⁸ This alkoxide then attacks the positively charged phosphorous-species to afford the intermediate whose resonance form is attacked by a previously released chloride anion to result in the formation of the alkyl chloride product and triphenylphosphine oxide by-product. For secondary alcohols the alkyl halide will have inverted stereochemistry at the carbon atom of relevance.¹⁷⁸



Scheme 78

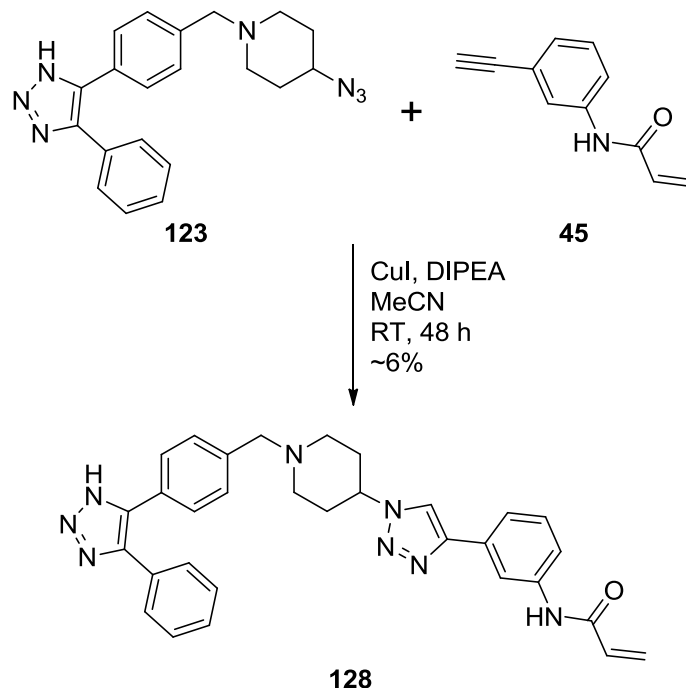
The proposed mechanism for the Appel reaction to afford an alkyl chloride from an alcohol.¹⁷⁸

A similar procedure to the one to be carried out was reported by Qing and Takacs, involving the synthesis of benzyl azides from benzyl alcohols.¹⁷⁶ During this reaction, the benzyl alcohol was stirred at 90 °C with triphenylphosphine and sodium azide in a mixture of carbon tetrachloride and *N,N*-dimethylformamide, first forming the benzyl chloride, which was then attacked by the sodium azide to form the product, a benzyl azide (Scheme 77). The researchers reported that the reaction was completed within five hours, affording the benzyl azides in yields from 75 - 88%.¹⁷⁶

This procedure was followed in a similar manner, but with using 4-azidopiperidin-1-ium chloride **97** as the nucleophile. Care had to be taken that the azide functionality of compound **97** was not reduced by the triphenylphosphine, as seen in the Staudinger reaction, and only one equivalent of this reagent was therefore used.¹⁷⁹ This was not an unnecessarily high risk, with sodium azide and the resulting product not being affected by the reaction conditions reported, but it was additionally decided to only add the amine **97** after the starting material had been consumed, indicating the conversion of the alcohol to the benzyl chloride **127** and therefore consumption of the triphenylphosphine. The starting material **124** was consumed within 40 minutes, indicating the successful formation of the benzyl halide. The amine **97** was then added and the reaction mixture was heated at 90 °C for a further 16 hours. TLC analysis indicated that the newly-formed benzyl chloride **127** had not been consumed during this time, and triethylamine was added, thereby ensuring that **97** was neutralised so the nitrogen lone pair was available for nucleophilic attack. After the addition of triethylamine, the reaction was complete within two hours and the product **123** was again isolated in a similar yield of 30%. IR spectroscopy of compound **123** revealed that the azide was still intact, with the azide stretch visible at 2089 cm^{-1} . This reaction was repeated using similar conditions, but with the addition of

triethylamine at the same time as addition of the amine, and the product was isolated in a yield of 31%. This was not a great yield, but with the quantitative reduction of the aldehyde **110**, this sequence allowed for a 10% increase in yield, compared to the most successful reductive amination from the aldehyde **100** towards the product **123** [see Section 5.3.7].

5.4.3 *N*-[3-(1-{1-[4-(4-Phenyl-1*H*-1,2,3-triazol-5-yl)benzyl]piperidin-4-yl}-1*H*-1,2,3-triazol-4-yl)phenyl]acrylamide



Scheme 79

Click reaction between compounds **123** and **45** to afford 1,2,3-triazole **128**.

With the reasonably successful synthesis of 4-azido-1-[4-(4-phenyl-1*H*-1,2,3-triazol-5-yl)benzyl]piperidine **123**, it was time to attempt the synthesis of the target compound, *N*-[3-(1-{1-[4-(4-phenyl-1*H*-1,2,3-triazol-5-yl)benzyl]piperidin-4-yl}-1*H*-1,2,3-triazol-4-yl)phenyl]acrylamide **128**, via a click reaction with alkyne **45**. This first procedure attempted was that of Dory and co-workers (Scheme 79).¹⁶⁹ Unfortunately, this proved to be a difficult reaction, with the target compound isolated in a poor yield of 6%, even after extending the reaction time to allow for starting material consumption. This attempt was followed by various repeats, using the procedure by Dory and co-workers, as well as that of Sharpless and co-workers (Table 9).^{168; 169} Unfortunately, none of the attempts afforded any improvement in yield, with most not even affording trace amount of product.

A third procedure was thus attempted, this one from a procedure by Lal and Díez-González.¹⁸⁰ These researchers reported a versatile click reaction using tris(triphenylphosphine)copper(I) bromide as

catalyst, either neat or in water, with yields greater than 95%.¹⁸⁰ This procedure was therefore performed using a small volume of acetonitrile as solvent, as neither starting material was liquid and nor were they soluble in water. Regrettably, none of the title compound **128** was isolated, with starting material still present even after 41 hours of reaction time, or after an additional 25 hours at 40 °C with the addition of triethylamine.

Table 9

Different reaction conditions tested for the synthesis of **128**. Note: “+” indicates additional reagent was added during the reaction.

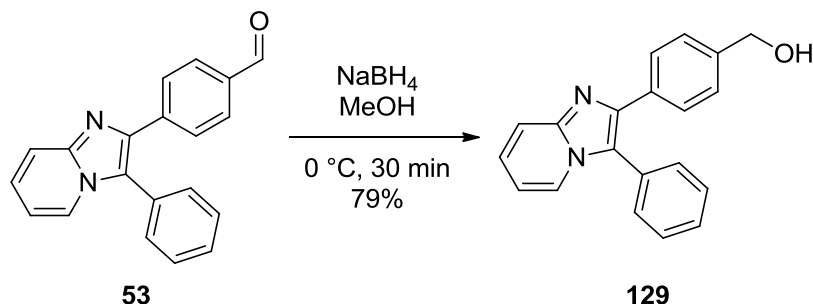
Entry	Compounds 123:45	Catalyst and mol%		Extra and molar equivalent added		Solvent	Yield
1	1:1.2	CuI	57+	DIPEA	2.6+	MeCN	6%
2	1:1.3	CuI	23+	TEA	2.0	MeCN	Trace
3	1:1.3	CuI	15+	DIPEA	3.3	MECN	-
4	1:1.2	CuSO ₄ ·5H ₂ O	15	Sodium ascorbate	19 mol%	<i>t</i> -BuOH & H ₂ O	Trace
5	1:1.1	CuSO ₄ ·5H ₂ O	24	Sodium ascorbate	42 mol%	THF & H ₂ O	-
6	1:1.4	CuSO ₄ ·5H ₂ O	10	Ascorbic acid	23 mol%	<i>t</i> -BuOH & H ₂ O	-
7	1:1.1	CuBr(PPh ₃) ₃	5+	TEA	1.1	MeCN	-

Synthesis of the title compound, **128**, was temporarily abandoned.

5.5 SUCCESSFUL SYNTHESIS OF THE TARGET COMPOUNDS

With the successful synthesis of **116**, **119** and **122**, it was decided to finally attempt the synthesis of one of the target compounds using one of the originally chosen western moieties, namely 4-(3-phenylimidazo[1,2-*a*]pyridin-2-yl)benzaldehyde **53** which was synthesised by Dr Abu Taher, as mentioned above [see Section 4.5].

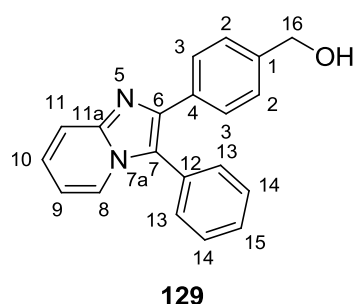
5.5.1 [4-(3-Phenylimidazo[1,2-*a*]pyridin-2-yl)phenyl]methanol



Scheme 80

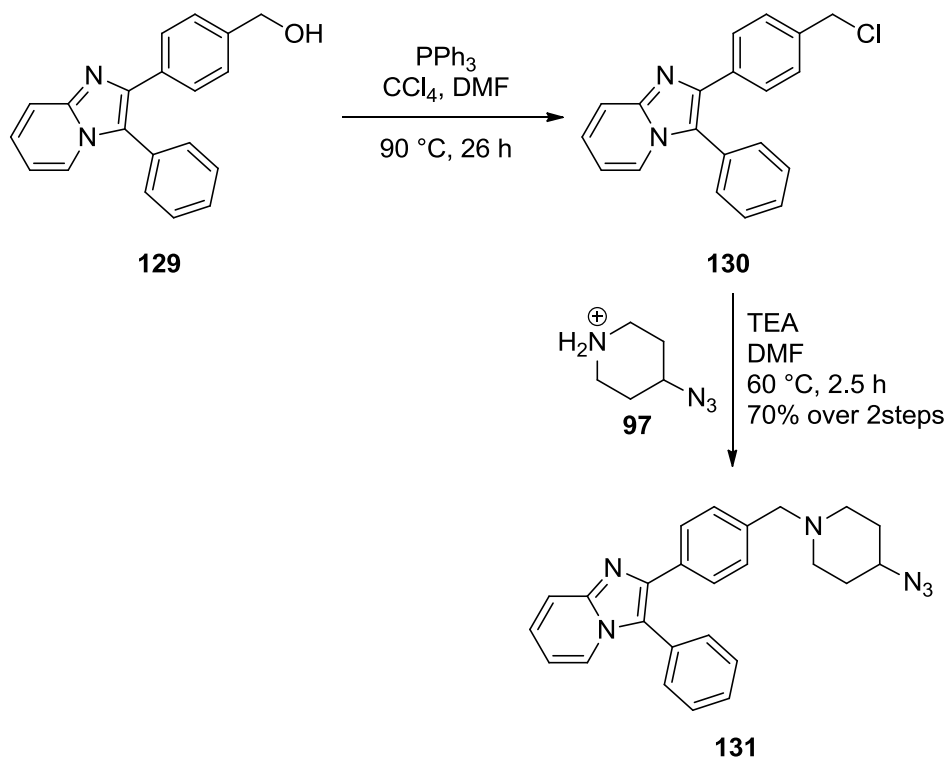
Reduction of aldehyde **53** to afford alcohol **129**.

4-(3-Phenylimidazo[1,2-*a*]pyridin-2-yl)benzaldehyde **53** was reduced using the same procedure as before, as carried out by Zhao and co-workers.¹⁷⁷ This reaction was performed in the same manner as before, but the reaction mixture was kept at 0 °C, instead of room temperature (Scheme 80) [see Section 5.4.1]. The reduction proceeded readily, with the starting material consumed within 30 minutes. The purified compound was isolated in a yield of 79%. This is lower than the quantitative yield of [4-(4-phenyl-1*H*-1,2,3-triazol-5-yl)phenyl]methanol **124**, but could be accounted for as a loss during purification by column chromatography.



The ¹H and ¹³C NMR spectra of compound **129** were as expected, with the methylene proton and carbon signals visible at 4.67 and 64.9 ppm, respectively. IR spectroscopy also confirmed a successful reaction with the alcohol O-H stretch visible at 3217 cm⁻¹. Finally, the mass spectroscopic analysis result of 301.1344 amu corresponded with the expected value of 301.1341 amu.

5.5.2 2-{4-[(4-Azidopiperidin-1-yl)methyl]phenyl}-3-phenylimidazo[1,2-*a*]pyridine



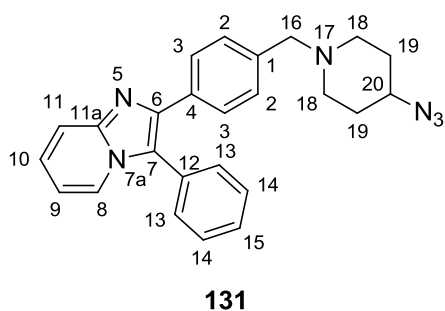
Scheme 81

Appel reaction using alcohol **129** and amine **97** to afford compound **131**.

Following the successful synthesis of [4-(3-phenylimidazo[1,2-*a*]pyridin-2-yl)phenyl]methanol **129**, the modified procedure by Qing and Takacs was used to obtain 2-{4-[(4-azidopiperidin-1-yl)methyl]phenyl}-3-phenylimidazo[1,2-*a*]pyridine **131**.¹⁷⁶ This reaction was carried out in two steps in order to purify benzyl chloride **130** before using it to synthesise title compound **131** (Scheme 81). This was to ensure that there was as little chance for the azide to be reduced during this penultimate reaction.

For the synthesis of title compound **131**, [4-(3-phenylimidazo[1,2-*a*]pyridin-2-yl)phenyl]methanol **129** was heated at 90 °C with triphenylphosphine in a mixture of carbon tetrachloride and *N,N*-dimethylformamide. Starting material was still visible after 21 hours so another two equivalents of triphenylphosphine was added. The reaction was then heated at 90 °C for an additional five hours until complete consumption of starting material **129**. The crude material was purified by column chromatography, but the ¹H NMR spectrum indicated that the material was contaminated by an impurity seen in the aliphatic region, so a percentage yield could not be calculated. We were happy that the reaction was successful as the methylene signals were recognisable at 4.58 and 46.2 ppm in the ¹H and ¹³C NMR spectra respectively, indicating the expected upfield shift from the exchange of the electron-withdrawing oxygen atom to the chloride atom, which will shield the protons more through its inductive effect.

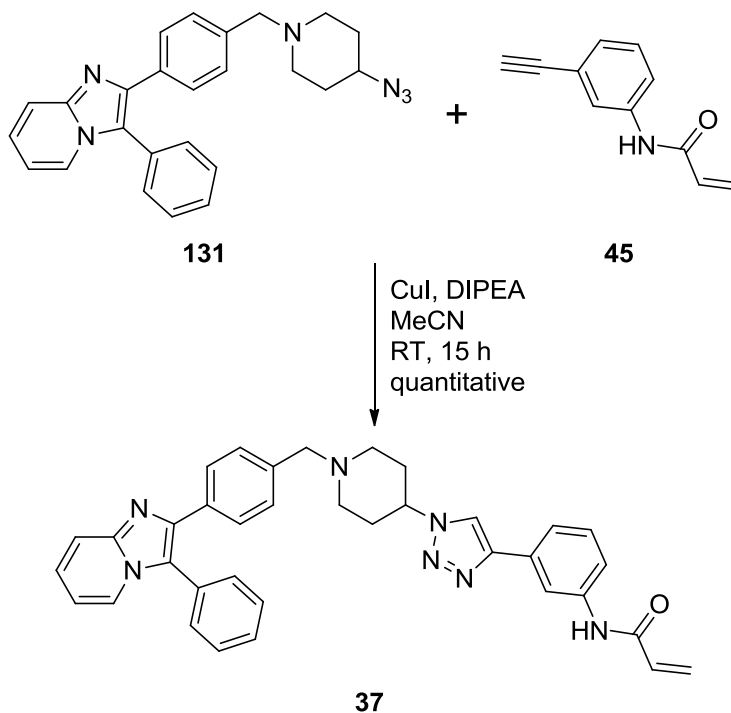
For the nucleophilic substitution, the 4-azidopiperidin-1-ium chloride **97** was first stirred with 1.8 equivalents of triethylamine in *N,N*-dimethylformamide. This was to ensure that the amine was neutral and not positively-charged. The reasonably pure benzyl chloride **130** was then added to the reaction mixture at room temperature, after which it was heated at 60 °C whilst being closely monitored by TLC. Starting material was consumed after two and a half hours and after workup, the crude material was purified to afford the title compound **131** in a good yield of 70% over the two steps.



The ^1H and ^{13}C NMR spectra of compound **131** looked as expected, with the attachment of the azidopiperidine confirmed by the addition of the aliphatic proton and carbon signals characteristic of this compound. IR spectroscopy conformed that the azide was still intact, with the azide stretch discernible at 2094 cm^{-1} . In addition, the mass spectroscopic analysis result of 409.2138 amu corresponded

with the calculated value of 409.2141 amu.

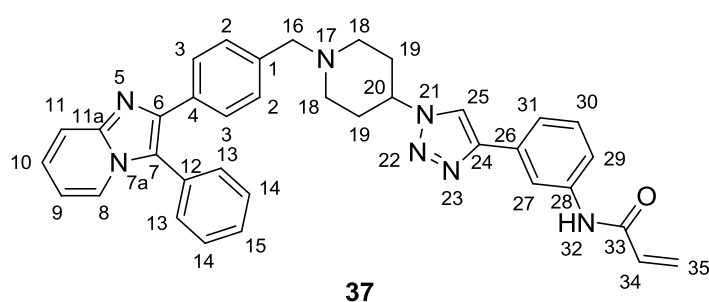
5.5.3 *N*-[3-(1-{1-[4-(3-Phenylimidazo[1,2-*a*]pyridin-2-yl)benzyl]piperidin-4-yl}-1*H*-1,2,3-triazol-4-yl)phenyl]acrylamide



Scheme 82

Click reaction between compounds **131** and **45** to afford 1,2,3-triazole **37**.

Finally the stage had arrived for the synthesis of the target compound, *N*-[3-{1-(1-[4-(3-phenylimidazo[1,2-*a*]pyridin-2-yl)benzyl]piperidin-4-yl)-1*H*-1,2,3-triazol-4-yl]phenyl]acrylamide **37**. Again, the procedure by Dory and co-workers was followed, as this had thus far been the most successful.¹⁶⁹ The newly-synthesised azide **131** was stirred at 25 °C with the alkyne **45** and the reagents, copper(I) iodide and triethylamine, in acetonitrile (Scheme 82). The reaction was complete after 15 hours and the crude material was purified by column chromatography, this time to afford the title compound **37** in a quantitative yield. This was the successful synthesis of the first of the real target compounds.



For this compound **37**, the ¹H and ¹³C NMR spectra looked as expected, with the triazole proton visible at 7.89 ppm, indicating the fusion of the two components, **45** and **131**. The IR spectrum confirmed this with the presence of the amide N-H stretch at

3278 cm⁻¹ and the carbonyl C=O stretch at 1615 cm⁻¹. Importantly, the mass spectroscopic analysis result of 580.2805 amu corresponded with the calculated value of 580.2825 amu. Two-dimensional NMR spectra was obtained using gradient Heteronuclear Single Quantum Coherence (gHSQC) and gradient Heteronuclear Multiple Bond Coherence (gHMBC) and supplementary ¹³C NMR spectra were obtained using Distortionless Enhancement by Polarization Transfer (DEPT), allowing for assignment of the ¹H and ¹³C NMR spectra. The gHMBC ¹³C NMR spectrum did not display multiple bond correlation between position 25 of the triazole and the piperidinyll functionality and could not confirm synthesis of the required 1,4-disubstituted 1,2,3-triazole and not the 1,5-disubstituted 1,2,3-triazole. A 2D NOESY spectrum was therefore obtained to confirm this and this showed correlation between the triazole proton, H₂₅, and the piperidinyll protons, H₁₉ and H₂₀, confirming successful synthesis of the 1,4-disubstituted 1,2,3-triazole (Figure 28). The triazole proton in the 1,5-disubstituted 1,2,3-triazole would be too far from the piperidinyll protons to correlate.

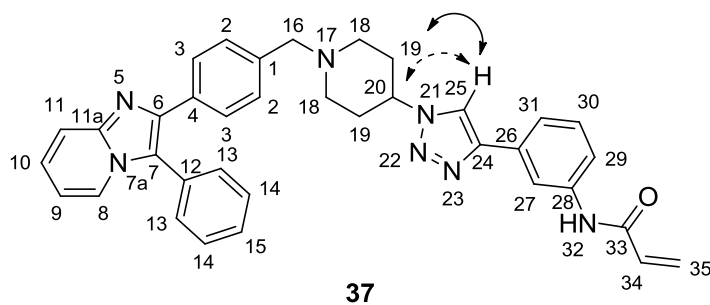
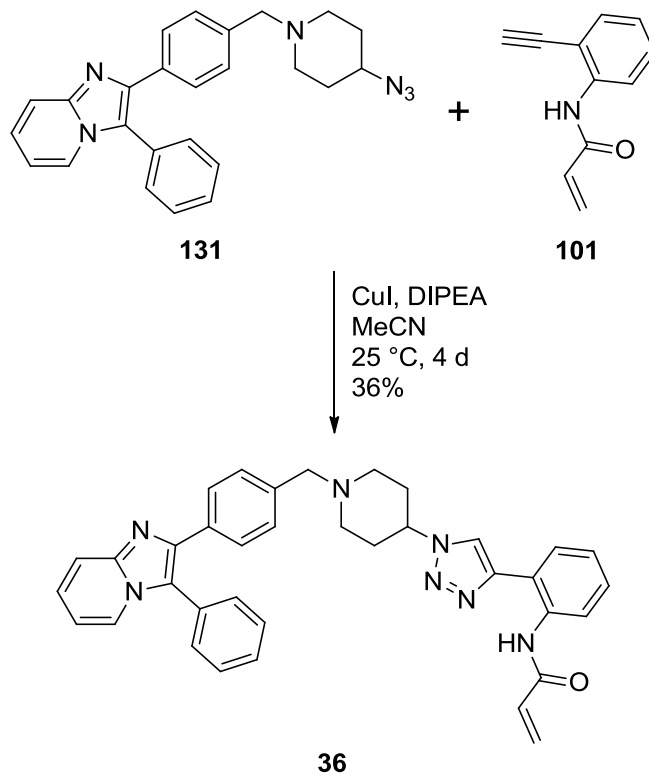


Figure 28

A schematic diagram illustrating the piperidinyll correlations to the triazole proton of target compound **37**.

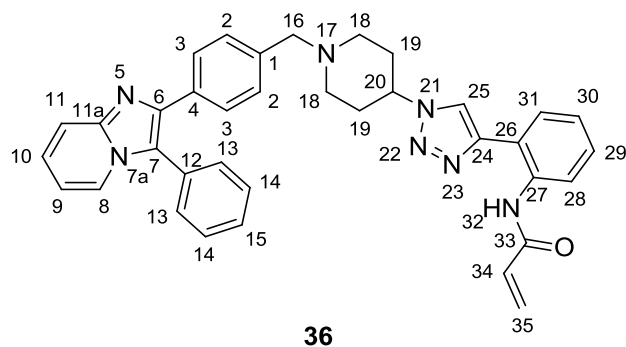
5.5.4 *N*-[2-(1-{1-[4-(3-Phenylimidazo[1,2-*a*]pyridin-2-yl)benzyl]piperidin-4-yl}-1*H*-1,2,3-triazol-4-yl)phenyl]acrylamide



Scheme 83

Click reaction between compounds **131** and **101** to afford 1,2,3-triazole **36**.

In order to obtain the regioisomer of **37**, *N*-[2-(1-{1-[4-(3-phenylimidazo[1,2-*a*]pyridin-2-yl)benzyl]piperidin-4-yl}-1*H*-1,2,3-triazol-4-yl)phenyl]acrylamide **36**, the procedure carried out by Dory and co-workers was, once again, employed.¹⁶⁹ The starting materials, compounds **131** and **101**, were stirred with the reagents in MeCN and, after 24 hours at room temperature, more copper(I) iodide was added (Scheme 83). Stirring was continued at 25 °C for a further three days. This increase in reaction time compared to the synthesis of **37** was due to the consumption of starting material not being noticed in due time. This is as both of these molecules are very polar and had the same retention factor in ethyl acetate ($R_f = 0.18$). It was only at a later stage that this was realised, when a better solvent system was used, namely a mixture of methanol and dichloromethane (**131** & **36** = 0.47 & 0.54 in 5% MeOH/DCM). The product **36** was eventually isolated in a reasonable yield of 36%. This may potentially have been higher had the reaction been stopped sooner, especially before additional copper(I) iodide was added to the reaction mixture. Nevertheless, this concluded the successful synthesis of the second of the real target compounds.



The ^1H and ^{13}C NMR of **36** looked as expected, with the triazole singlet visible at 7.89 ppm, the same as with **37**. IR spectroscopy further confirmed the success of the reaction, with the amide N-H stretch visible at 3074 cm^{-1} and the carbonyl C=O stretch at 1684 cm^{-1} . In addition, the mass spectroscopic result of 580.2831 amu

corresponded with the calculated value of 580.2825 amu.

This concluded the synthetic work of this project, with the successful synthesis of two target compounds, **36** and **37** as well as three truncated target compounds, **116**, **119** and **122**.

CHAPTER 6 – BIOCHEMICAL EVALUATION OF THE TARGET COMPOUNDS

6.1 COMPOUNDS EVALUATED

Six compounds were available for biochemical evaluation to test for inhibitory activity against Akt (Figure 29). These included the three so-called truncated target compounds, **116**, **119** and **122**, the two target compounds, **36** and **37**, as well as a fourth truncated target compound, **132**, made by an honours student at Stellenbosch University, Jaques Buys, as part of his practical work in the synthetic laboratory. This compound was made using benzaldehyde as the western moiety. The small amount of synthesised compound **128** was regrettably not sufficient to send for evaluation.

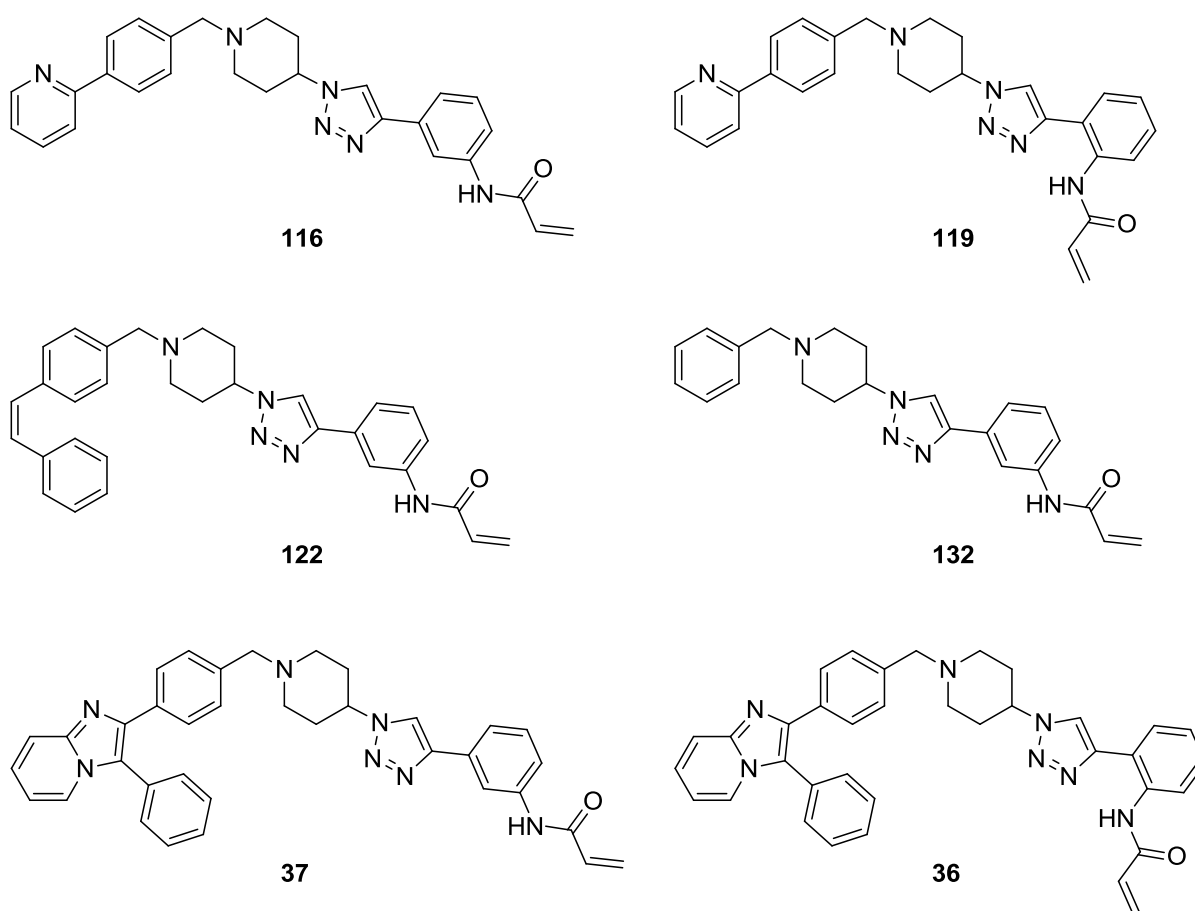


Figure 29

The six target compounds sent for biochemical evaluation for activity against Akt.

6.2 THE ASSAYS USED

Biochemical evaluation was undertaken by Jörn Weisner from the Technical University of Dortmund in Germany, under the supervision of Professor Daniel Rauh.

Two different assays were used, firstly, interface-Fluorescent Labels in Kinases (iFLiK) to determine whether the potential inhibitors bound to Akt in the inter-domain allosteric site discussed in Chapter 2, and secondly, homogeneous time-resolved fluorescence KinEASE™ (HTRF® KinEASE™), to determine whether binding of the potential inhibitors had an inhibitory effect on kinase activity, or not [see Section 2.2.2]. The equilibrium dissociation constant (K_d) and the half-maximal inhibitory concentration (IC_{50}) values were obtained from these assays, respectively. For both of these assays, wild-type Akt1 (wtAkt1) was used. The wtAkt1 needed for the iFLiK assays was acquired from insect cells and purified *via* immobilized metal affinity chromatography and size exclusion chromatography, whereas the wtAkt1 needed for the HTRF® KinEASE™ assays was acquired from Merck Millipore. Akti-1/2 **11** and GSK690693 **133** were used as control inhibitors. Akti-1/2 **11** is the previously discussed dual allosteric inhibitor of both Akt1 and Akt2, and GSK690693 **133** is a potent and selective, ATP-competitive inhibitor of all three isoforms of Akt (Figure 30) [see Section 2.2.2].^{181; 182}

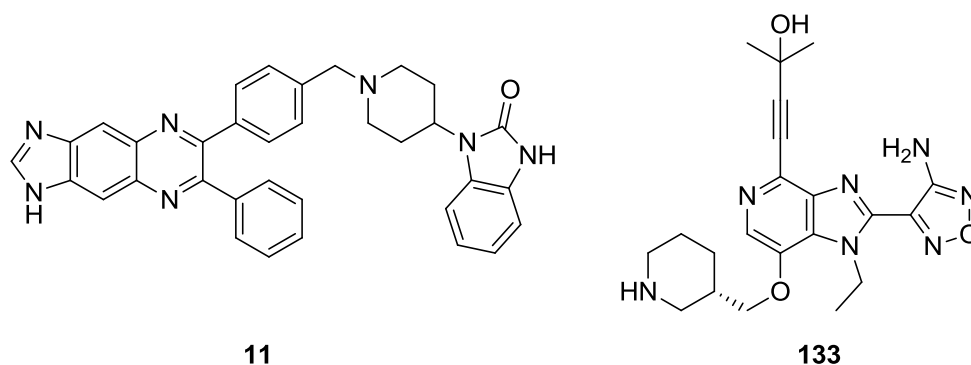


Figure 30

The control inhibitors used for the biochemical assays, allosteric inhibitor, Akti-1/2 **11**, and ATP-competitive inhibitor, GSK690693 **133**.

Herewith follows a short description of the two assays, iFLiK and HTRF® KinEASE™.

6.2.1 iFLiK

The first assay, iFLiK, is an adaption of the biochemical assay, FLiK developed by Professor Rauh of the Technical University of Dortmund (Figure 31).¹⁸³ FLiK is a fluorescence-based binding assay that can monitor conformational changes in kinases upon ligand binding, thereby allowing for the identification of potential inhibitors.^{184; 185} iFLiK was developed, from FLiK, to specifically monitor inter-domain interactions at the interface of the PH and kinase domains of Akt, thus allowing for the

identification of Type V inter-domain inhibitors of Akt through the resulting conformational changes in the kinase.¹⁸³ For iFLiK, these changes are followed by the environmentally sensitive solvatochromic fluorophore, 4-[5-(4-methoxyphenyl)oxazol-2-yl]pyridinium (PyMPO) which reacts to the binding of inter-domain inhibitors by a bathochromic shift of the emission spectrum.¹⁸³ Following fluorophore excitation, this shift is followed by measuring the emission at two different wavelengths, namely 638 and 508 nm, and using the ratio of emission at the wavelengths.¹⁸³ By incubating different concentrations of the potential inhibitor with Akt, a dose-response curve can be plotted, following the shift in emission, or lack thereof, with increasing concentration of potential inhibitor. This method allows for the direct measurement of the dissociation constant (K_d) between the potential inhibitor and Akt, which is an indication of the affinity of the potential inhibitor for Akt.¹⁸⁴

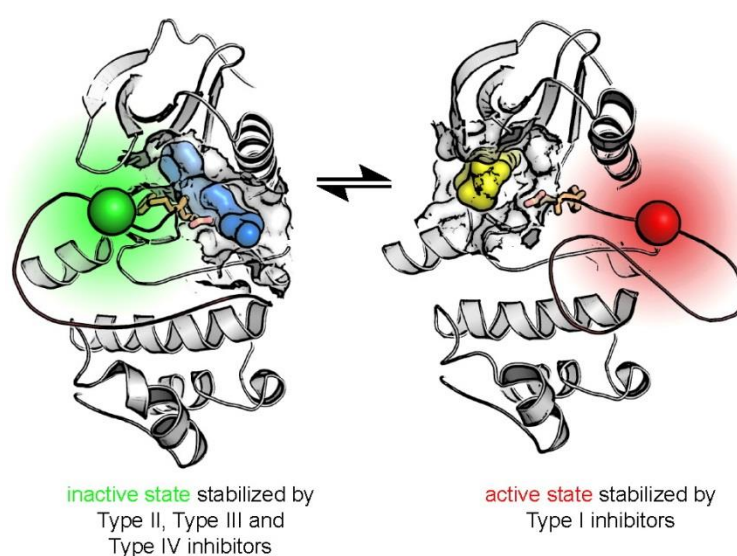


Figure 31

A pictorial representation from Rauh and co-workers, illustrating the principle of the FliK assay.¹⁸⁶ The introduced fluorophore (coloured sphere) has an altered emission spectrum depending on the conformation of the kinase. This allows for identification of the type of binding, whether ATP-competitive or non-ATP competitive.

6.2.2 HTRF[®] KinEASE[™]

Homogeneous time-resolved fluorescence (HTRF) is a generic assay technology frequently used in drug discovery and high-throughput screening, assisting in the *in vitro* detection of molecular interactions in GPCRs, kinases, new biomarkers, protein-protein interactions, and other targets of interest.¹⁸⁷ HTRF[®] KinEASE[™] is an extension of this technology, developed for the assessment of kinase activity using antibody-based detection (Figure 32).¹⁸⁸ To assess the inhibitory effect of a potential inhibitor on kinase activity, the kinase is first incubated for a set time with different concentrations of the potential inhibitor to allow for ligand binding. This is followed by the addition

of a biotinylated substrate and the other components essential for enzymatic reaction.¹⁸⁸ After a set time, the reaction is stopped and an antibody, specific for the phosphorylated site, and labelled with europium-cryptate (donor fluorophore) and an XL665-labeled streptavidin (acceptor fluorophore) is added. The XL665-labeled streptavidin binds to the biotinylated substrate and, if phosphorylation has occurred, the europium-cryptate antibody binds to the phosphorylated site of the substrate.¹⁸⁸ This brings these two compounds in close proximity to each other and, upon the external excitation of the donor, energy is transferred from donor to acceptor *via* Förster resonance energy transfer (FRET). This results in a decreased emission of the donor and an increased emission of the acceptor, measured at 620 and 665, respectively.¹⁸⁷ For low kinase activity, there would be a low degree of phosphorylation of the substrate and thus limited binding of the europium-cryptate-labelled donor fluorophore. This will lead to a low incidence of FRET and thus low emission of the acceptor fluorophore. Conversely, for high kinase activity, increased emission of the acceptor fluorophore will be measured at 665 nm. The ratio of emission at these two wavelengths is taken, allowing for the calculation of various enzyme kinetic parameters, including the half-maximal inhibitory concentration (IC₅₀), which is the inhibitor concentration where 50% inhibition of kinase is achieved.¹⁸⁹

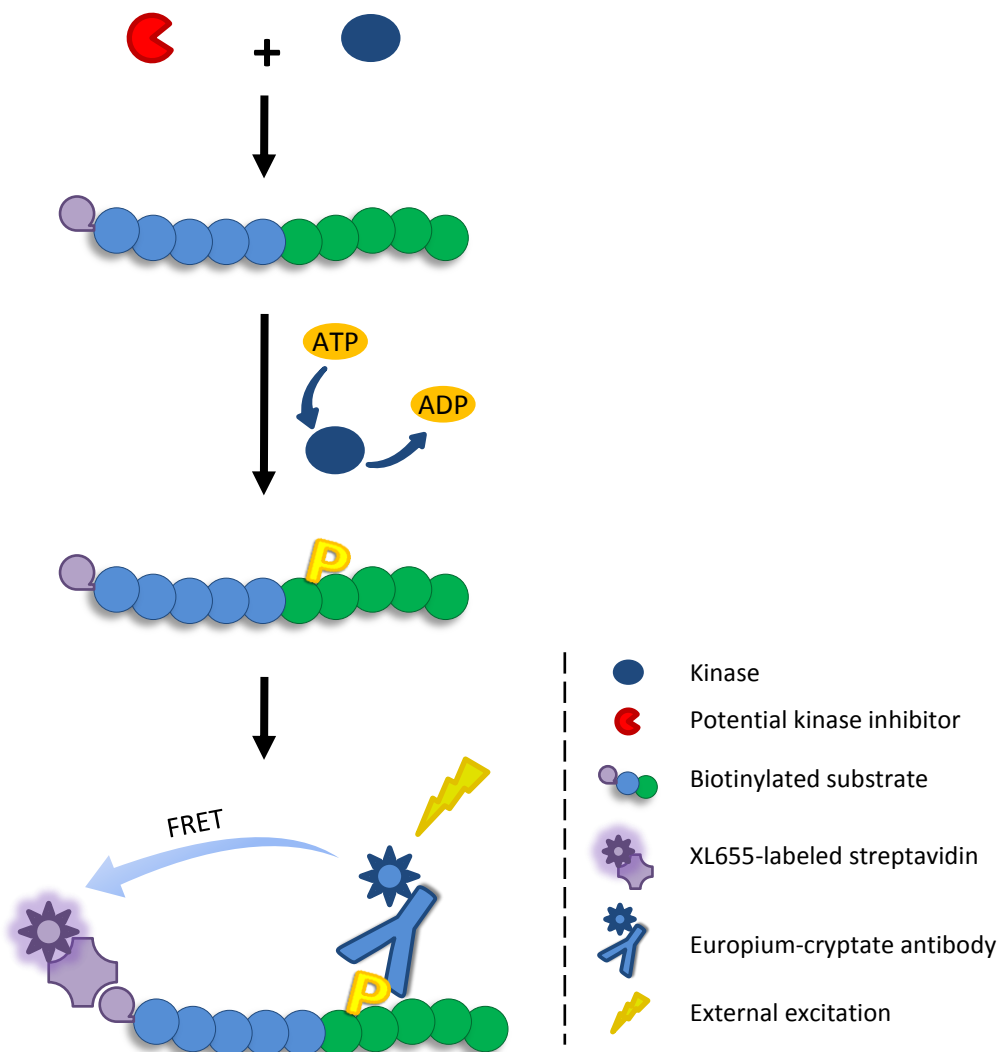


Figure 32

An adapted pictorial representation from Drexler, illustrating the HTRF[®] KinEASE[™] protocol utilised.¹⁸⁸

6.3 EVALUATION RESULTS AND DISCUSSION

Of the four truncated target compounds, not one showed binding to or inhibition of wtAkt1. Encouragingly, both target compounds **36** and **37**, showed not only direct binding to Akt1 in the targeted allosteric site (from the iFLiK assay), but also displayed an inhibitory effect on the kinase activity (from the HTRF[®] KinEASE[™] assay). The dissociation constants and IC₅₀ values for these and the control compounds are given in Table 10, with compounds **36** and **37** both having IC₅₀ values below 1 μM. Compound **37** was the most promising, with an IC₅₀ value of 438 nM, twice as potent as compound **36**. The *in vitro* equilibrium dissociation constant can sometimes be directly related to the *in vivo* efficacy of an inhibitor, provided the compound has favourable ADMET properties.⁴⁰ This is a further sign of the potential of compound **37** as Akt inhibitor, with a dissociation constant roughly

seven times that of Akti-1/2 **11**. Note that GSK690693 **133** has no dissociation constant from the iFLiK assay as the assay is specific for inter-domain Type V allosteric inhibitors.

Table 10

Results of the iFLiK and HTRF[®] KinEASE[™] assays, reporting the dissociation constants en IC₅₀ values of the active target compounds, **36** and **37**, and the two control compounds, **11** and **133**.

Inhibitor	K_d [nM]	IC₅₀ [nM]
36	1856 ± 240	931 ± 110
37	649 ± 58	438 ± 56
Akti-1/2 11	98 ± 5	4.8 ± 0.2
GSK690693 133	n.d.	<1

To determine whether the identified inhibitors bound to Akt1 in a covalent manner by targeting the previously discussed Cys296, liquid chromatography-mass spectrometry (LC-MS) experiments were conducted. WtAkt1 was incubated in dimethyl sulfoxide with two molar equivalents of either compound **36** or **37**, with Akt incubated in dimethyl sulfoxide as a negative control. These samples were subsequently serially diluted and examined by LC-MS (Figure 33). From the negative control, the mass for the free wtAkt1 was determined as ca. 59246 Da. With the addition of compound **36**, there was no increase in mass observed, signifying that compound **36** was a reversible inhibitor of Akt. For compound **37** however, an increase of 580 Da, corresponding to the mass of compound **37**, was observed. This was a splendid result, indicating that compound **37** was a covalent modifier of Akt1. Along with the other results, K_d and IC₅₀, this could be labelled as the first irreversible inhibitor of Akt1.

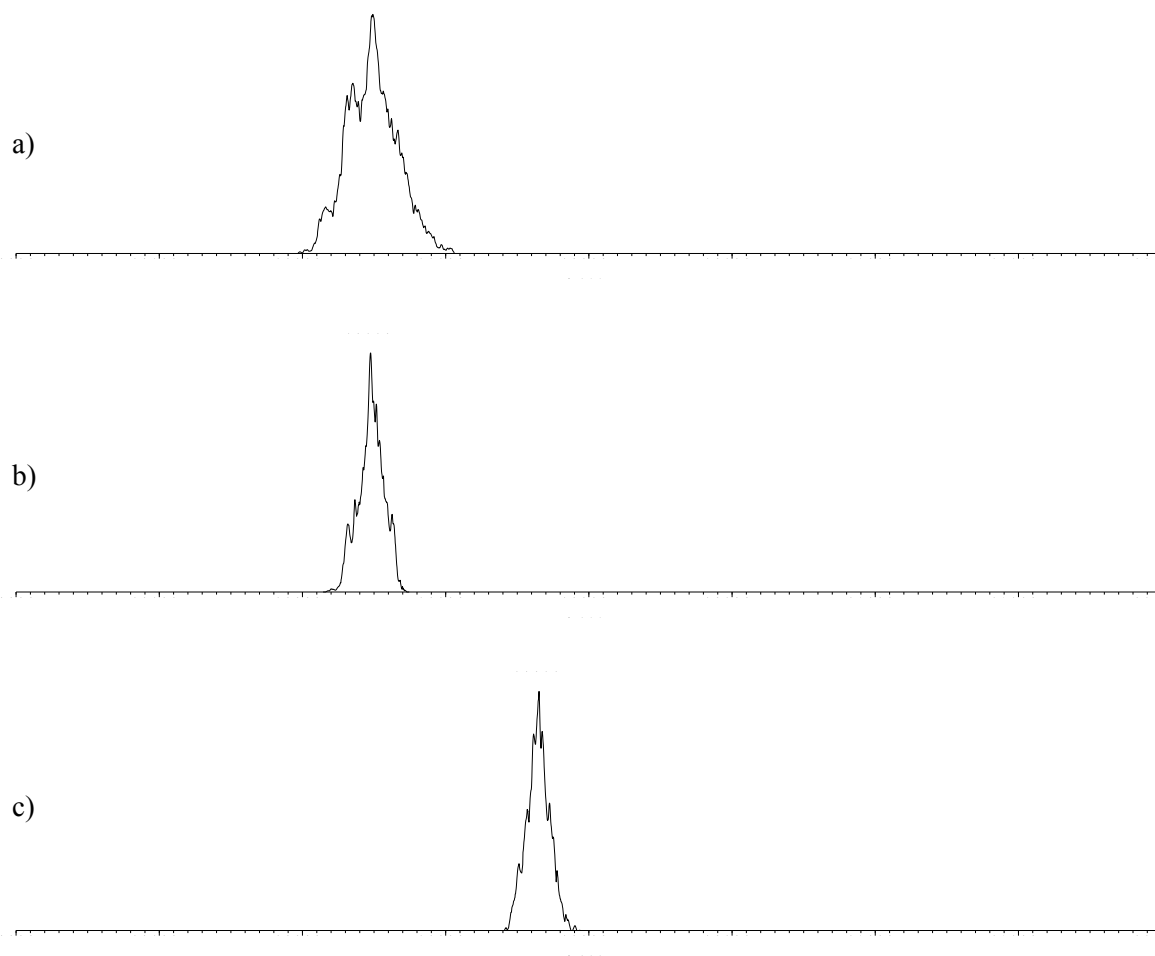


Figure 33

LC-MS results for the following mixtures incubated in dimethyl sulfoxide: a) wtAkt1, 59246 Da; b) wtAkt1 + 2 molar equivalents of compound **36**, 59238 Da; c) wtAkt1 + 2 molar equivalents of compound **37**, 59826 Da.

These results were very promising, allowing for the identification of the first allosteric irreversible inhibitor of Akt1. The results also showed that the choice of western moiety (or driving portion) is very important for successful activity against Akt1, with the four truncated compounds having the same eastern moiety as compound **37**, displaying no inter-domain binding to or inhibition of Akt1. Furthermore, the correct position of the acrylamide is essential, with compound **36** having an IC_{50} twice that of compound **37** and displaying no covalent binding to Akt1.

CHAPTER 7 – CONCLUSION

With the worldwide concern about the increase in cancer incidence, this project focussed on synthesising compounds which could potentially lead to the discovery of novel chemotherapeutic agents. These compounds were to target Akt, a kinase which has been found to be dysregulated in numerous cancers. No irreversible inhibitors of Akt have been reported thus far and the aim was therefore to design and synthesise potential irreversible inhibitors of Akt and to evaluate these compounds for activity against Akt.

The initial targets were based on the allosteric reversible inhibitor, Akti-1/2 **11**, and heterocyclic scaffolds **27** to **31**, as reported by Kettle and co-workers.^{93; 109} This led to the first target compounds, represented by compounds **34** and **35**. These compounds were hoped to not only be allosteric inhibitors of Akt, but also to react with the nearby Cys296 residue, thus potentially allowing for irreversible inhibition of Akt. Various synthetic routes were attempted towards the synthesis of these compounds, but none were successful in affording the target compounds.

The synthesis of the original target compounds was thus abandoned in favour of novel, adapted target compounds, represented by compounds **36**, **37** and **38**. These compounds differed from the original target compounds with respect to their eastern moiety, with the original piperidinyl benzimidazolone functionality replaced by a 1,2,3-triazole ring-containing structure, to be synthesised using click chemistry. After some trial and error, synthesis of these compounds proved much more fruitful than that of the original compounds, with two of the three target compounds (**36** and **37**) successfully synthesised, along with three truncated target compounds obtained (**116**, **119** and **122**).

These potential inhibitors were consequently sent for biochemical evaluation, with both compounds **36** and **37** displaying allosteric inhibition of wtAkt1, and with IC₅₀ values in the nanomolar range. In addition to this, further tests revealed compound **37** to be an irreversible inhibitor of wtAkt1. This is the first known irreversible inhibitor of Akt, supplying us with a good starting point for further research into more potent irreversible inhibitors of Akt.

CHAPTER 8 – FUTURE WORK

As much as this project has answered some questions, other questions remain and have also been supplemented with additional questions. The future work described here was chosen to specifically answer the most pertinent of these questions.

8.1 SYNTHETIC WORK TOWARDS THE ORIGINAL TARGET COMPOUNDS, **34** AND **35**

Despite the struggle to synthesise target compounds **34** and **35**, these are still sought-after for their potential as irreversible inhibitors of Akt (Figure 34). This is even more pertinent after the successful synthesis, evaluation and discovery of Akt irreversible inhibitor, compound **37** (Figure 35, see Section 8.2). A substantial amount of time and effort was put into synthesising the key piperidiny benzimidazolone functionality from scratch, but this was the least successful of the routes attempted [see Sections 3.2 and 3.3]. The focus for the synthesis of these compounds will therefore be on the last three routes attempted [see Section 3.4]. Different possibilities towards their synthesis are discussed below.

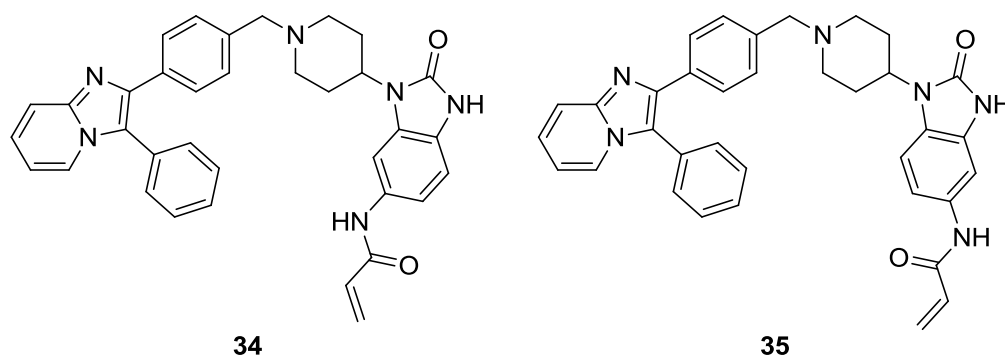


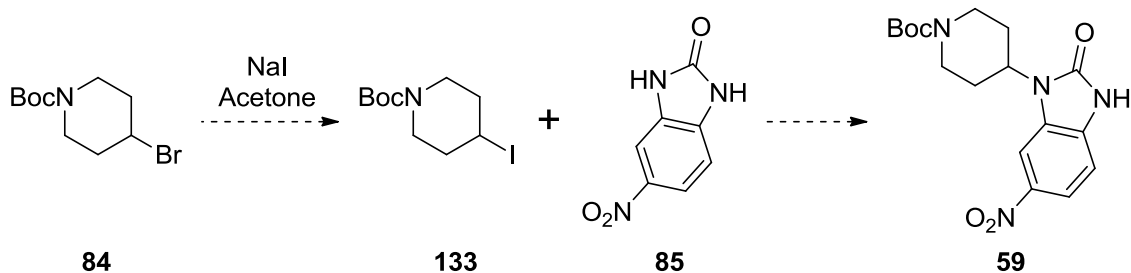
Figure 34

Representatives of the original target compounds, **34** and **35**.

8.1.1 Nucleophilic substitution route

The bulky nucleophile compound **85**, and sterically hindered electrophilic site of compound **84**, prevented bi-molecular nucleophilic substitution (S_N2) from taking place between these two substrates [see Section 3.4.1]. Unfortunately, the nucleophile's steric bulk cannot be addressed and it would therefore be required to enhance the electrophilicity of alkyl bromide **84**. One possible way of doing this, is by making unimolecular nucleophilic substitution (S_N1) more favourable by halogen exchange, turning alkyl bromide **84** into alkyl iodide **133** (Scheme 84). Alkyl iodides are more reactive than alkyl bromides, with the C-I bond being weaker than the C-Br bond and iodine anions being more stable than that of bromine.¹⁹⁰ This could encourage an S_N1 reaction, neatly side-stepping the issue of

the nucleophile's steric bulk. The halogen exchange to alkyl iodide **133** could thus be achieved using the Finkelstein reaction.¹⁹¹

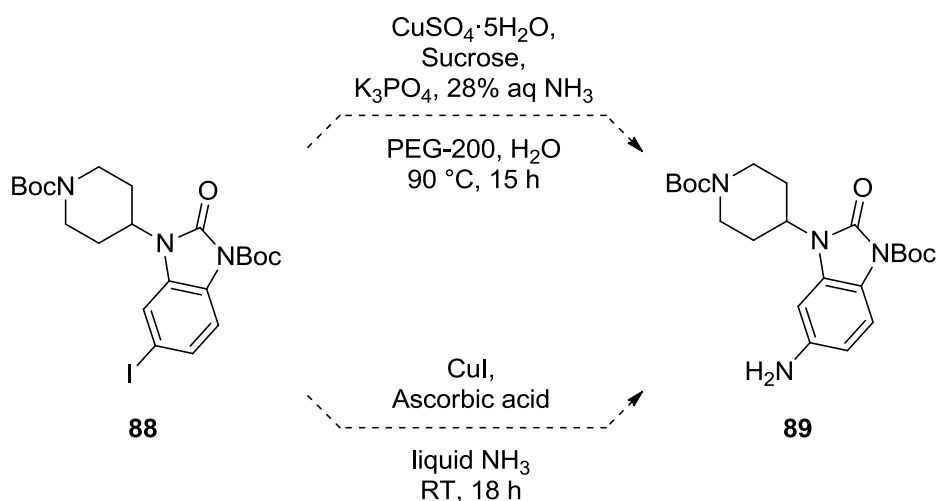


Scheme 84

A possible solution to the failure of the nucleophilic substitution reaction towards **59**.¹⁹¹

8.1.2 Iodination route

The iodination route was reasonably successful, only falling flat at the conversion of the aryl iodide **88** into the aryl amine **89** using 28% aqueous ammonia and copper(II) acetylacetonate. [see Section 3.4.2]. Other procedures could be followed for this problematic conversion, such as those described by Wan and co-workers, as well as Page and co-workers (Scheme 85).^{192; 193} Wan and co-workers reported the successful amination of aryl halides in high yields, using 28% aqueous ammonia as nitrogen source and copper(II) sulphate pentahydrate as catalyst.¹⁹² In contrast, Page and co-workers discussed the use of anhydrous liquid ammonia and copper(I) iodide, maintaining a water-free system for the amination of aryl halides.¹⁹³ It is hoped that either of these, or other, methods would allow for the successful synthesis of benzimidazolone **89**, enabling the continuation of this route.

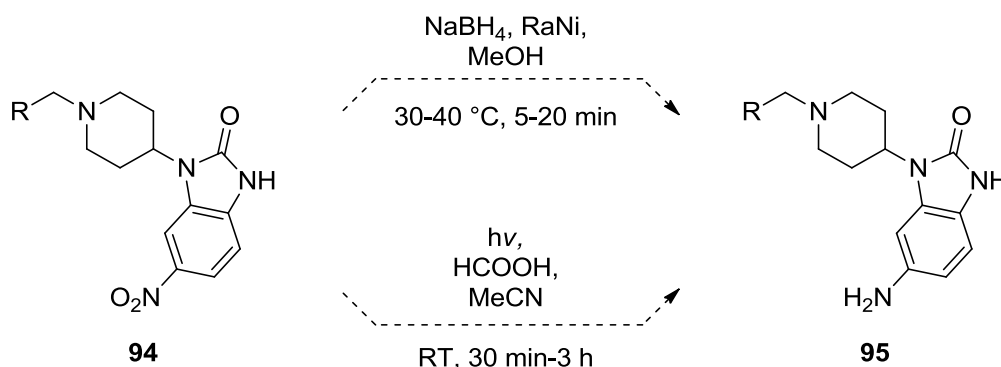


Scheme 85

Two possible methods for aminating aryl iodide **88**.^{192; 193}

8.1.3 Nitration route

This route was equally promising as the iodination route, with the problem lying at the reduction of the nitro functionality of regioisomers **94** to afford aniline **95** [see Section 3.4.3]. To this end, procedures such as those reported by Litvić and co-workers, or Bonesi and co-workers could be undertaken (Scheme 86).^{194; 195} Litvić and co-workers discussed the rapid reduction of aromatic nitro compounds, using a combination of sodium borohydride and Raney nickel, with yields in excess of 85%.¹⁹⁴ Bonesi and co-workers reported the formic acid-mediated photoreduction of these compounds, also with good to excellent reported yields.¹⁹⁵



Scheme 86

Two possible methods for the reduction of the nitro functionality of compound **94**.^{194; 195}

In addition to reducing the nitro functionality, the two regioisomers, obtained from nitration, will need to be separated at a convenient stage of this route. These compounds have similar retention factors, but it is hoped that they would be separable by column chromatography if they could temporarily be made soluble enough for column chromatography with as little as possible tailing. This could be done after successful reduction of compound **94**, attaching Boc-protecting groups to the available nitrogen atoms of aniline **95**, N₂₃ and N₂₈, thus potentially increasing its solubility in the common column chromatography solvents, ethyl acetate and hexane, and by implication, increasing the likelihood of successfully separating the regioisomers. Due to the potential difficulty in separating these regioisomers, this route would be the last of the three to attempt.

8.2 SYNTHETIC WORK EXPANDING THE ADAPTED TARGET COMPOUNDS

After the success of compound **37** as an irreversible inhibitor of Akt1, it is pertinent that this compound and additional derivatives are further explored (Figure 35). This includes smaller, less obvious modifications, as well as larger, more drastic changes. Both of these are described below.

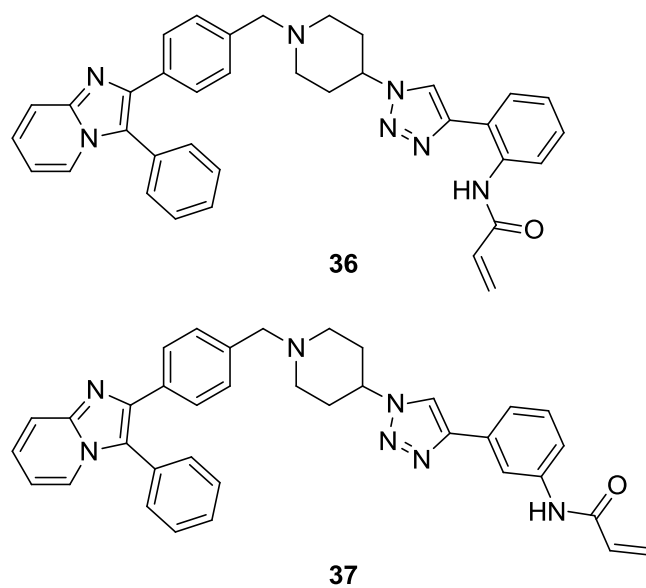
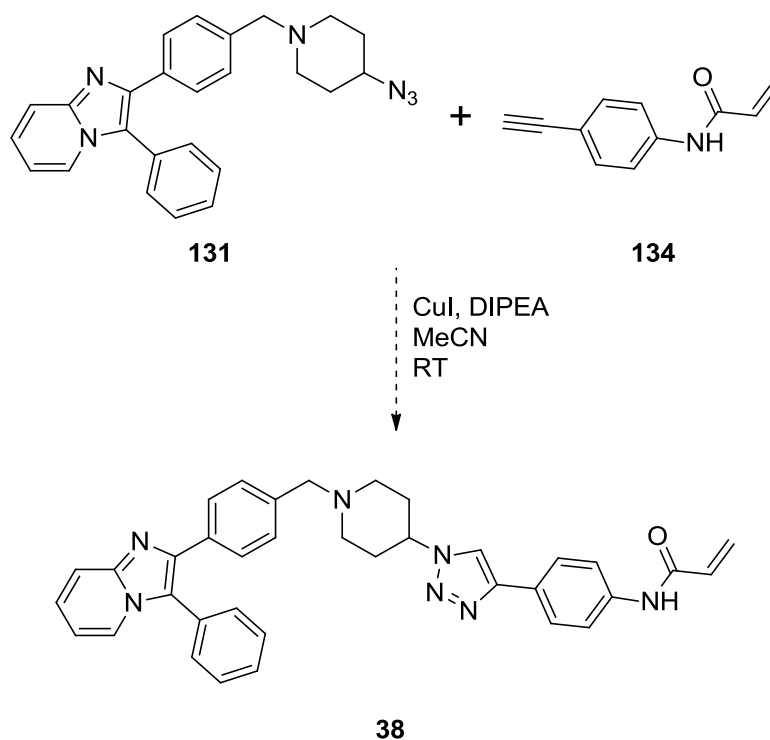


Figure 35

The two successfully synthesised target compounds **36** and **37**.

8.2.1 Modifications of compound 37

In order to complete the regioisomeric set of compounds **36** and **37**, synthesis of the *para* regioisomer **38** will be undertaken (Figure 35). This will be done by using *N*-(4-ethynylphenyl)acrylamide **134** as alkyne for the final click reaction (Scheme 87).



Scheme 87

Final planned step towards the synthesis of regioisomer **38**.

Additionally, **37** will be modified to contain a propionamide group in place of its current acrylamide functionality, giving **135** (Figure 36). Replacing the electrophilic acrylamide with the non-reactive propionamide would help determine whether the improved activity of compound **37**, over that of compound **36**, is due to the irreversible inhibition it imparts *via* the acrylamide or from the *meta* position of the acrylamide functionality, perhaps allowing for additional non-covalent interactions to Akt, not considered before and not possible with the *ortho* acrylamide of inhibitor **36**.

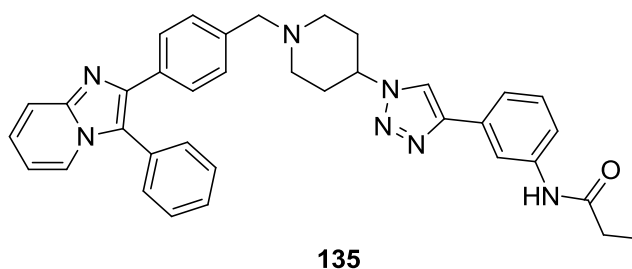


Figure 36

An adapted target of compound **37**, with the warhead rendered ineffective.

Another possible modification would be to attach different electrophilic warheads on compound **37**, such as the substituted acrylamide functionality which is present in a number of compounds in clinical development (Figure 37).⁴⁴ Acryloyl chloride will be replaced by (*E*)-4-(dimethylamino)but-2-enoyl chloride **137** in order to synthesise this compound. Using this functionality may completely alter its

activity against Akt1. This could be a favourable modification, allowing for additional non-covalent interactions with Akt1, but it could also be highly unfavourable if this functionality was too big for the space occupied by compound **37**'s smaller unsubstituted acrylamide.

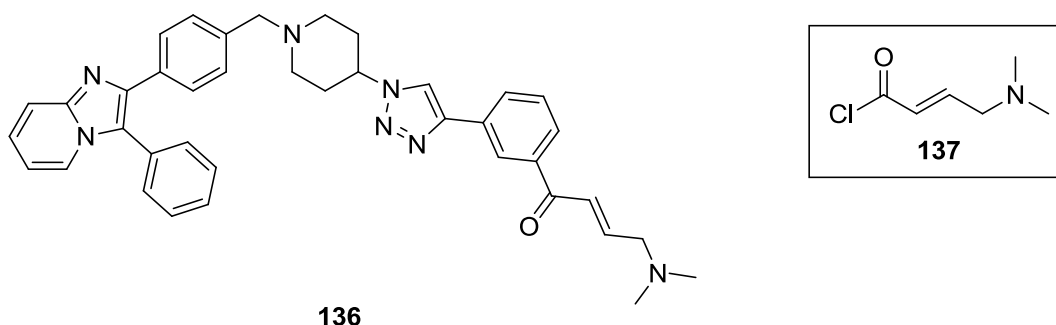


Figure 37

An adapted target of compound **37**, with a substituted acrylamide as warhead.

A smaller warhead could also be used, synthesising compound **138** in order to determine whether this allows for improved activity against Akt1, or not (Figure 38). This functionality would have to be introduced in a different manner than the others, by ways of Friedel-Crafts acylation, using acryloyl chloride and a suitable Lewis acid.¹⁹⁶ Optimum reaction conditions and reagents would need to be determined for this system, imparting control on where the functionality will be attached and without compromise of the C-C double-bond present. One idea would be to attach a small directing group on the aryl ring, assisting in directing acylation to the preferred acylation site. For example, an electron-donating group such as a methoxy functionality *ortho* or *para* to the preferred acylation site may improve the incidence of the desired regioisomer. For this functionality, the electrophilic carbon would be spatially related to Cyc296 in a different manner than that of compound **37**, as it is now closer to the aryl ring. Additionally, the electronic nature of this functionality could potentially be significantly different from the previous two, as it lacks the nitrogen atom, N₃₂, and thus the influence from its lone-pair electrons which donate electron density into the warhead carbonyl group of compound **37**. The functionality is now also closer to the aryl ring and may be more sensitive to electronic effects from it and its substituents.

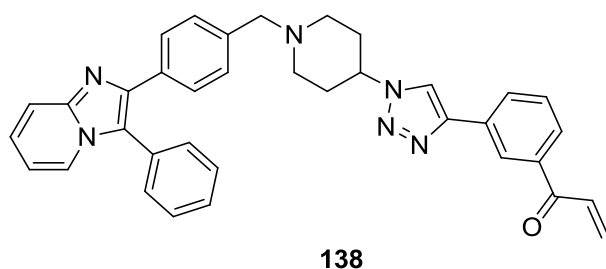


Figure 38

An adapted target of compound **37**, with a smaller warhead.

8.2.2 Further, more drastic modifications of compound **37**

Since the current target compounds, **36**, **37** and the modified ones to be synthesised, all have the same western moiety, effort will be put into synthesising deviations of these, containing other western moieties. The first western moieties will be adaptations from the heterocyclic scaffolds discussed earlier, and could include, but not be limited to aldehydes from scaffolds **28**, **29**, **30** and **31** (Figure 39).¹⁰⁹

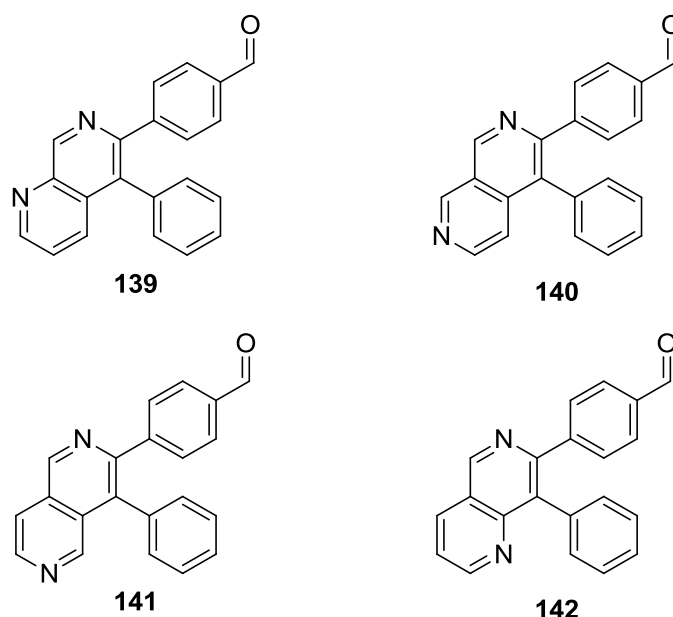


Figure 39

The western moieties to be synthesised next, aldehydes **139-142**.

8.3 BIOCHEMICAL EVALUATION

Irreversible inhibitor **37** will undergo further biochemical evaluation, firstly in order to attempt pinpointing which part of the kinase houses the covalent modification site. This could allow for the

identification of the specific cysteine residue being modified, thus determining whether it is Cys296 or a different reactive cysteine. Modification of another nucleophilic amino acid residue (tyrosine or serine), is possible, but not very likely. This test will be done by incubating compound **37** with wtAkt1 in dimethyl sulfoxide and then obtaining tryptic digests of this protein with the inhibitor. For the negative control, tryptic digests will be obtained from wtAkt1 incubated in dimethyl sulfoxide in the absence of inhibitor. LC-MS analysis of these digests will allow for the identification of which fraction of the kinase has the added mass of compound **37**. Furthermore, *in vivo* work can be done to determine its potency against Akt1 in cellular medium.

In addition to doing tests using wtAkt1, the other isoforms could also be used for evaluating inhibitor **37**. It would also be interesting to see whether this inhibitor, **37**, has activity against a mutated form of Akt1, E17K, which is found in a number of different cancers.^{197; 198}

Last, but not least, any new target compounds synthesised can also be evaluated using the techniques described previously, with the results compared to that of reversible inhibitor **36** and irreversible inhibitor **37**. Work towards a co-crystal structure between Akt and a bound inhibitor will be initiated if any of these compounds are irreversible inhibitors of Akt and display increased potency over that of compound **37**.

CHAPTER 9 – EXPERIMENTAL WORK

9.1 GENERAL INFORMATION

The chemicals used were purchased from either Sigma-Aldrich or Merck or obtained from the De Beers Building chemical stores. Tetrahydrofuran was distilled under nitrogen from sodium flakes with benzophenone as an indicator. Toluene was also distilled under nitrogen from sodium, but with no benzophenone as indicator. Dichloromethane and acetonitrile was distilled under nitrogen from calcium hydride. Triethylamine was distilled under potassium hydroxide. Other reaction solvents were dried over the appropriate size activated molecular sieves.¹⁹⁹ The ethyl acetate, hexane and dichloromethane, used for column chromatography, were distilled before use. If necessary, reagents were purified using literature procedures.¹⁹⁹

Reactions were performed in anhydrous conditions with a positive pressure of nitrogen, unless water was used as solvent or it was stated otherwise. Glassware was either dried by heating whilst under a positive nitrogen pressure or dried in an oven at 110 °C for a minimum of two hours. Heating of reaction mixtures was accomplished by placing the reaction vial in a paraffin oil bath, which was heated at the correct temperature. Solvents were removed *in vacuo*, using a rotary evaporator followed by removal of trace amounts of remaining solvent using a high vacuum pump at ca. 0.08 mm Hg.

Thin layer chromatography (TLC) was performed using Macherey-Nagel ALUGRAM® Xtra SIL G UV254 aluminium sheets. Visualisation of the plates was achieved using a 254 nm UV light or stains appropriate to the compounds, including *p*-anisaldehyde, iodine, ninhydrin and potassium permanganate. Column chromatography was performed under gravity using Merck silica gel 60 (particle size 0.063-0.200 mm, 60 Å). Solvents used included different combinations of ethyl acetate, hexane, dichloromethane, methanol and triethylamine.

NMR spectra (¹H, ¹³C, NOESY, gHSQC and gHMBC) were recorded on either one of three instruments; a 300 MHz Varian VNMRS (75 MHz for ¹³C), a 400 MHz Varian Unity Inova (100 MHz for ¹³C), or a 600 MHz Varian Unity Inova (150 MHz for ¹³C). All spectra were obtained at 25 °C. Chemical shifts (δ) are reported in ppm and J-values are given in Hz. Mass spectrometry was performed on a Waters SYNAPT G2. Infrared spectra were recorded on a Thermo Nicolet Nexus 470 FT-IR apparatus, using the Attenuated Total Reflectance (ATR) mode. Melting points were obtained using a Gallenkamp Melting Point Apparatus and are uncorrected.

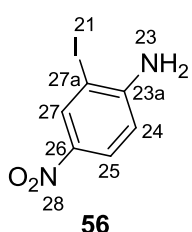
Biochemical evaluation of target compounds was carried out at the Technical University of Dortmund under supervision of Professor Daniel Rauh.

9.2 EXPERIMENTAL WORK PERTAINING TO CHAPTER 3

9.2.1 Original work towards regioisomer 34

9.2.1.1 2-Iodo-4-nitroaniline - 56

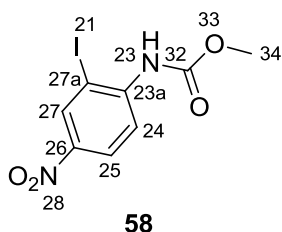
A 250 mL one-neck round-bottomed flask was charged with 4-nitroaniline **55** (4.00 g, 29.0 mmol), KI (0.67 equiv, 3.21 g, 19.3 mmol) and KIO₃ (0.34 equiv, 2.09 g, 9.78 mmol) in H₂O (90 mL) and MeOH (15 mL). The resulting milky yellow reaction mixture was stirred at RT whilst 1M HCl (1.0 equiv, 29 mL, 29 mmol) was added in a dropwise manner over 70 min. The reaction mixture turned first orange and later brown upon addition of the acid. The reaction mixture was stirred for 24 h during which it turned green. The reaction mixture was diluted with H₂O (200 ml) and extraction was done with EtOAc (3 × 50 mL). The combined organic layers were washed with 5% aqueous Na₂S₂O₃ (150 mL), H₂O (150 mL) and brine (150 mL) after which it was dried over anhydrous Na₂SO₄ and filtered. The solvent was removed in vacuo to yield the crude material as a dark orange solid. The crystals were washed with hexane and then purified by crystallisation from a minimum amount of hot EtOH. The resulting yellow crystals were filtered and redissolved in a minimum amount of hot EtOH and left to recrystallise to afford the title compound **56** (5.81 g, 22.0 mmol, 76%) (R_f = 0.38, 30% EtOAc/Hexane) as yellow plate-like crystals.



¹H NMR (300 MHz, DMSO-*d*₆) δ 8.39 (d, *J* = 2.6 Hz, 1H, H₂₇), 7.97 (dd, *J* = 9.2, 2.6 Hz, 1H, H₂₅), 6.76 (d, *J* = 9.2 Hz, 1H, H₂₄), 6.71 (br s, 1H, H₂₃). ¹³C NMR (75 MHz, DMSO-*d*₆) δ 155.1 (C_{23a}), 136.6, 135.1, 125.6, 112.1, 79.8 (C_{27a}).

9.2.1.2 Methyl (2-iodo-4-nitrophenyl)carbamate - 58

A 100 mL one-neck round-bottomed flask was charged with 2-iodo-4-nitroaniline **56** (0.32 g, 1.2 mmol), methyl chloroformate (1.1 equiv, 0.10 mL, 0.12 g, 1.3 mmol) and K₂CO₃ (6.6 equiv, 1.1 g, 8.0 mmol) in acetone (9 mL). The resulting yellow reaction mixture was stirred at RT for 6 days. Additional methyl chloroformate (4.2 equiv, 0.04 mL, 0.49 g, 5.2 mmol) and acetone (14 mL) added during this time and the reaction mixture changed to orange. The reaction was quenched with a saturated solution of aqueous NH₄Cl (100 mL) after which extraction was done with EtOAc (3 × 25 mL). The combined organic layers were washed with brine (100 mL), dried over anhydrous MgSO₄ and filtered. Solvent was removed *in vacuo* to afford the crude material as a pale yellow solid which was purified by CC (20 to 40% EtOAc/Hexane) to afford the title compound **58** (0.10 g, 0.31 mmol, 26%) (R_f = 0.54, 30% EtOAc/Hexane) as a yellow solid.

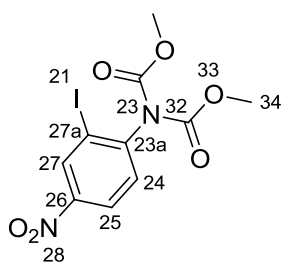


58

Mp 156-159 °C. **IR (ATR, cm⁻¹):** 3351 (N-H str), 1746 (C=O str), 1582 (Ar C=C str), 1501 (nitro N=O), 1455 (Ar C=C str), 1333 (nitro N=O), 1243 (C-O str), 1207 (C-O str), 1057, 740. **¹H NMR (300 MHz, DMSO-d₆)** δ 9.10 (br s, H₂₃), 8.60 (d, *J* = 2.6 Hz, 1H, H₂₇), 8.24 (dd, *J* = 9.0, 2.6 Hz, 1H, H₂₅), 7.79 (d, *J* = 9.0 Hz, 1H, H₂₄), 3.72 (s, 3H, H₃₄). **¹³C NMR (75 MHz, DMSO-d₆)** δ 154.0 (C₃₂), 145.7 (C_{23a/26}), 143.9 (C_{23a/26}), 134.0 (C_{24/25/27}), 124.1 (C_{24/25/27}), 123.9 (C_{24/25/27}), 93.1 (C_{27a}), 52.5 (C₃₄). **HRMS:** calcd for C₈H₈IN₂O₄⁺ [M+H]⁺, 322.9529, found 322.9542.

9.2.1.3 Two-step synthesis of methyl (2-iodo-4-nitrophenyl)carbamate - 58

This reaction was not performed under positive pressure of N₂, but was equipped with a CaCl₂ drying tube. A 100 mL one-neck round-bottomed flask was charged with 2-iodo-4-nitroaniline **56** (0.325 g, 1.23 mmol), methyl chloroformate (4.2 equiv, 0.40 mL, 0.49 g, 5.2 mmol) and K₂CO₃ (6.5 equiv, 1.1 g, 8.0 mmol) in acetone (9 mL). The resulting yellow reaction mixture was stirred at RT for 48 h. More methyl chloroformate (4.2 equiv, 0.40 mL, 0.49 g, 5.2 mmol) and acetone (6 mL) was added after 24 h and the reaction mixture turned light orange with time. The reaction was quenched with a saturated solution of aqueous NH₄Cl (100 mL) and stirred for 60 min after which extraction was done with EtOAc (4 × 25 mL). The combined organic layers were washed with brine (100 mL), dried over anhydrous MgSO₄ and filtered. Solvent was removed *in vacuo* to afford the crude product as a pale yellow solid. This was identified as dimethyl 2-iodo-4-nitrophenylimidodicarbonate **64** (0.430 g, 1.13 mmol, 92%) (R_f = 0.24, 30% EtOAc/Hexane).



64

Mp 214-219 °C. **IR (ATR, cm⁻¹):** 3086 (Ar C-H str), 3062 (Ar C-H str), 1787 (C=O str), 1531 (Ar C=C str), 1435 (Ar C=C str), 1345 (C-N str), 1267 (C-O str), 1193 (C-O str), 1102, 774. **¹H NMR (300 MHz, CDCl₃)** δ 8.73 (dd, *J* = 2.6, 0.6 Hz, 1H, H₂₇), 8.27 (dd, *J* = 8.7, 2.6 Hz, 1H, H₂₅), 7.43 (d, *J* = 8.7 Hz, 1H, H₂₄), 3.81 (s, 6H, H₃₄). **¹³C NMR (75 MHz, CDCl₃)** δ 151.2 (C₃₂), 147.3 (C_{23a/26}), 146.4 (C_{23a/26}), 134.5 (C_{24/25/27}), 129.6 (C_{24/25/27}), 124.3 (C_{24/25/27}), 99.5 (C_{27a}), 54.5 (C₃₄). **HRMS:** calcd for C₁₀H₉IN₂NaO₆⁺ [M+Na]⁺, 402.9403, found 402.9389.

Next, a 50 mL two-neck round-bottomed flask was charged with dimethyl 2-iodo-4-nitrophenylimidodicarbonate **64** (0.101 g, 0.265 mmol) and K₂CO₃ (0.25 equiv, 9 mg, 0.07 mmol) in MeOH (10 mL). The reaction mixture was heated to 35 °C and stirred for 45 min. The reaction was allowed to cool to RT and quenched with a saturated solution of aqueous NH₄Cl (50 mL). Extraction was done using DCM (3 × 25 mL). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄ and filtered. Solvent was removed *in vacuo* to yield the crude title compound **58**

(0.082 g, 0.254 mmol, 96%) ($R_f = 0.54$, 30% EtOAc/Hexane) as a pale yellow solid. ^1H NMR analysis indicated the presence of trace amounts of compounds **64** and **56**.

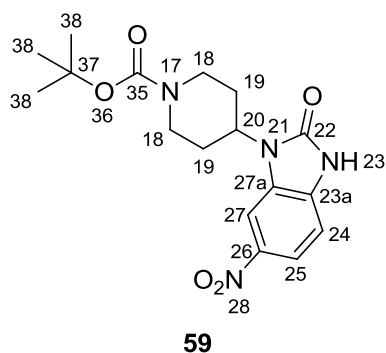
Note: Other conditions were also used, see Table 3.

9.2.1.4 Attempted synthesis of methyl (2-iodo-4-nitrophenyl)carbamate - **58**

A 100 mL one-neck round-bottomed flask was charged with 2-iodo-4-nitroaniline **56** (0.504 g, 1.91 mmol) and CHCl_3 (10 mL) to yield a yellow reaction mixture. Pyridine (1.3 equiv, 0.20 mL, 0.20 g, 2.5 mmol) was added to the reaction mixture and it was then cooled to 0 °C by means of an ice bath. Methyl chloroformate (1.2 equiv, 0.18 mL, 0.22 g, 2.3 mmol) was added to CHCl_3 (2 mL) and this was added to the milky yellow reaction mixture over 60 min. The reaction mixture went clear after the first few additions of methyl chloroformate and it was stirred at RT for 18 h. The reaction mixture was added to a mixture of crushed ice and H_2O and the organic layer was separated from the aqueous layer. The organic layer was then washed with H_2O and brine, dried over MgSO_4 and filtered. The solvent was removed *in vacuo* to yield the crude product which was purified by CC (20% EtOAc/Hexane) to afford the title compound **58** (0.047 g, 0.15 mmol, <8%) ($R_f = 0.54$, 30% EtOAc/Hexane) as a still impure yellow solid.

9.2.1.5 *tert*-Butyl 4-(6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)piperidine-1-carboxylate - **59**

A Schlenk tube was charged with methyl (2-iodo-4-nitrophenyl)carbamate **58** (0.101 g, 0.312 mmol), CuI (50 mol%, 0.030 g, 0.016 mmol), *L*-proline (1.0 equiv, 0.036 g, 0.31 mmol) and K_2CO_3 (2 equiv, 0.087 g, 0.63 mmol) and then evacuated and backfilled with N_2 . DMSO (0.60 mL) was subsequently added to yield a dark brown reaction mixture. The reaction mixture was heated to 55 °C and stirred for 30 min. It was then heated at 60 °C for 140 min, 65 °C for 70 min and 70 °C for 55 min. *tert*-Butyl 4-aminopiperidine-1-carboxylate **57** (2.0 equiv, 0.125 g, 0.622 mmol) was added to the reaction mixture and it was heated at 70 °C for a further 285 min, followed by 35 min at 130 °C. The reaction mixture was allowed to cool to RT and quenched with a saturated solution of aqueous NH_4Cl (150 mL). Extraction was done with EtOAc (40 & 2 × 25 mL) and the combined organic layers were washed with brine (200 mL), dried over anhydrous Na_2SO_4 and filtered. The solvent was removed *in vacuo* to yield the crude material as a dark brown oil. This was purified by CC (20 to 40% EtOAc/Hexane) to afford the title compound **59** (0.035 g, 0.097 mmol, 31%) ($R_f = 0.45$, 60% EtOAc/Hexane) as a yellow solid.



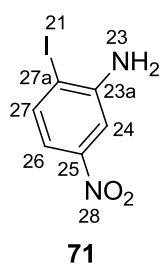
Mp 254-257 °C. **IR (ATR, cm⁻¹):** 2929 (alkane C-H str), 2324, 2164, 1696 (C=O str), 1426 (Ar C=C str), 1327 (C-N str), 1133 (C-O str). **¹H NMR (300 MHz, DMSO-d₆)** δ 11.65 (br s, 1H, H₂₃), 8.09 (d, J = 2.2 Hz, 1H, H₂₇), 7.98 (dd, J = 8.7, 2.2 Hz, 1H, H₂₅), 7.14 (d, J = 8.7 Hz, 1H, H₂₄), 4.56 – 4.41 (m, 1H, H₂₀), 4.08 (d, J = 12.6 Hz, 2H, H₁₈), 3.01 – 2.80 (m, 2H, H₁₈), 2.21 (m, J = 12.6, 4.3 Hz, 2H, H₁₉), 1.73 (dd, J = 12.1, 2.1 Hz, 2H, H₁₉), 1.44 (s, 10H, H₃₈ & impurities), 1 proton signal absent; H₂₃. **¹³C NMR (75 MHz, DMSO-d₆)** δ 154.0 (C_{22/35}), 153.9 (C_{22/35}), 141.4, 134.2, 129.6, 117.9, 108.4, 103.7, 78.9 (C₃₇), 50.5 (C₁₈), 28.3 (C₁₉), 28.1 (C₃₈), 1 carbon signal absent; C₂₀. **HRMS:** calcd for C₁₇H₂₃N₄O₅⁺ [M+H]⁺, 363.1668, found 363.1666.

Note: Other conditions were also used, see Table 4.

9.2.2 Original work towards regioisomer 35

9.2.2.1 2-Iodo-5-nitroaniline - 71

A 100 mL one-neck round-bottomed flask was charged with 3-nitroaniline **70** (0.622 g, 4.50 mmol), KI (0.67 equiv, 0.498 g, 3.00 mmol) and KIO₃ (0.33 equiv, 0.321 g, 1.50 mmol) in H₂O (15 mL) and MeOH (2.5 mL). The resulting milky yellow reaction mixture was stirred at RT whilst 1M HCl (1.1 equiv, 5 mL, 5 mmol) was added in a dropwise manner over 40 min. The reaction mixture turned dark green upon addition of the acid. It was stirred for at RT for 44 h, followed by 18 h at 30 °C and 4 h at 40 °C. The reaction mixture was allowed to cool to RT and diluted with H₂O (100 mL). Extraction was done with EtOAc (3 × 30 mL) and the combined organic layers were washed with 5% aqueous Na₂S₂O₃ (80mL), H₂O (80 mL) and brine (80 mL) after which it was dried over anhydrous Na₂SO₄ and filtered. The solvent was removed *in vacuo* to yield the crude product which was purified by CC (5 - 10% EtOAc/Hexane) to afford the title compound **71** (0.192 g, 0.728 mmol, 16%) (R_f = 0.38, 20% EtOAc/Hexane) as a yellow solid.



¹H NMR (300 MHz, CDCl₃) δ 7.80 (d, J = 8.6 Hz, 1H, H₂₇), 7.54 (d, J = 2.7 Hz, 1H, H₂₄), 7.30 (dd, J = 8.6, 2.7 Hz, 1H, H₂₆), 4.46 (br s, 1H, H₂₃). **¹³C NMR (75 MHz, CDCl₃)** δ 147.6, 139.7, 113.7, 108.0, 91.1 (C_{27a}), 1 carbon signal absent.

¹H NMR (300 MHz, DMSO-d₆) δ 7.82 (d, J = 8.6 Hz, 1H, H₂₇), 7.54 (d, J = 2.7 Hz, 1H, H₂₄), 7.10 (dd, J = 8.6, 2.7 Hz, 1H, H₂₆), 5.90 (br s, 2H, H₂₃). **¹³C NMR (75 MHz, DMSO-d₆)** δ 149.9 (C_{23a/28}), 148.5 (C_{23a/28}), 139.6, 111.2, 107.0, 91.0 (C_{27a}).

9.2.2.2 Attempted synthesis of 2-iodo-5-nitroaniline - 71

A 250 mL one-neck round-bottomed flask was charged with pyridine (4.0 mL, 3.9 g, 49 mmol) and AcOH (20 mL) and then cooled to 0 °C by means of an ice-bath. A solution of ICl (1.0 equiv, 2.5 mL, 8.1 g, 50 mmol) in AcOH (10 mL) was added to the reaction mixture in a dropwise manner. The reaction mixture changed from yellow to milky orange with the addition of ICl and more AcOH (100 mL) was added to the thickening mixture. The precipitate was filtered to yield a yellow solid which was purified by crystallisation from a minimum amount of hot EtOH. The resulting yellow crystals (4.0 g, 17 mmol, 34%) were dried and used as is for the iodination of 3-nitroaniline **70**. A 100 mL two-neck round-bottomed flask was charged with 3-nitroaniline **70** (0.14 g, 1.0 mmol) and crude synthesised pyridinium iodochloride (1.0 equiv, 0.24 g, 0.99 mmol) in MeOH (15 mL). The reaction flask was fitted with a reflux condenser and the yellow reaction mixture was heated at 70 °C for 3 h during which it turned a dark orange. The reaction was allowed to cool to RT and diluted with cold distilled H₂O. A precipitate was formed and filtered. No product was isolated.

9.2.2.3 Attempted synthesis of 2-iodo-5-nitroaniline - 71

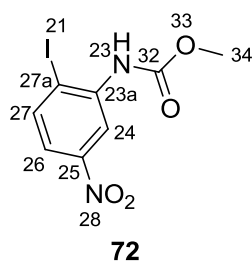
A 100 mL two-neck round-bottomed flask was charged with silver(I) acetate (2.0 equiv, 1.21 g, 7.26 mmol), iodine (1.0 equiv, 0.935 g, 3.68 mmol) and EtOH (35 mL). 3-Nitroaniline **70** (0.503 g, 3.64 mmol) was added to this dark orange reaction mixture and it was stirred at RT for 3 days. A precipitate formed during this time. Additional iodine (0.5 equiv, 0.463 g, 1.82 mmol) dissolved in EtOH (5 mL) was added after the first 44 h. The reaction mixture was filtered and the precipitate discarded. The solvent of the filtrate was removed *in vacuo* and the crude material was purified by CC (20 to 40% EtOAc/Hexane). No product was isolated.

9.2.2.4 Alternate synthesis of 2-iodo-5-nitroaniline - 71

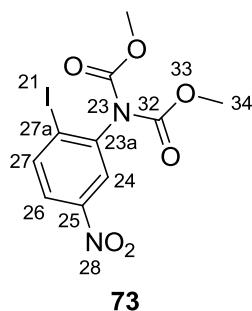
A 50 mL two-neck round-bottomed flask was charged with 3-nitroaniline **70** (0.500 g, 3.62 mmol) and AcOH (3 mL). A mixture of ICl (1.1 equiv, 0.20 mL, 0.65 g, 4.0 mmol) and AcOH (2 mL) was added to the reaction mixture in a dropwise manner. It was then heated at 80 °C for 16 h. The reaction mixture was allowed to cool to RT and 1M sodium hydroxide (250 mL) was added to it. Extraction was done with EtOAc (50 mL, 2 × 25 mL). The combined organic layers were washed with an aqueous solution of Na₂S₂O₃ (100 mL) and brine (100 mL), dried over anhydrous MgSO₄ and filtered. The solvent was removed *in vacuo* and the crude material was purified by CC (5% EtOAc/Hexane) to afford the title compound **71** (0.070 g, 0.27 mmol, 7%) (*R*_f = 0.38, 20% EtOAc/Hexane) as an orange solid.

9.2.2.5 Methyl (2-iodo-5-nitrophenyl)carbamate - 72

A Schlenk tube was charged with 2-iodo-5-nitroaniline **71** (0.105 g, 0.397 mmol), methyl chloroformate (1.3 equiv, 0.04 mL, 0.05 g, 0.5 mmol) and THF (1 mL). The reaction mixture was stirred at RT and 60% sodium hydride in mineral oil (1.2 equiv, 19 mg, 0.49 mmol) was added to the yellow reaction mixture. The reaction mixture was stirred at RT for 16 h. The reaction was quenched with H₂O (10 mL) and the organic solvent was removed *in vacuo*. Extraction was done with EtOAc (3 × 10 mL) and the combine organic layers were washed with H₂O and brine after which the solvent was removed *in vacuo*. The crude material was purified by CC (10% EtOAc/Hexane) to afford the pure title compound **72** (5 mg, 0.01 mmol, 4%) ($R_f = 0.50$, 40% EtOAc/Hexane) as a pale yellow solid. Dimethyl 2-iodo-5-nitrophenylimidodicarbonate **73** (20 mg, 0.052 mmol, 13%) ($R_f = 0.38$, 40% EtOAc/Hexane) was also isolated.



¹H NMR (300 MHz, DMSO-*d*₆) δ 9.22 (br s, 1H, H₂₃), 8.25 (d, $J = 2.7$ Hz, 1H, H₂₄), 8.17 (d, $J = 8.8$ Hz, 1H, H₂₇), 7.76 (dd, $J = 8.8, 2.7$ Hz, 1H, H₂₆), 3.71 (s, 3H, H₃₄). **¹³C NMR (75 MHz, DMSO-*d*₆)** δ No peaks visible, too little sample.

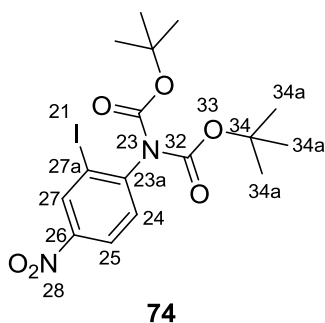


¹H NMR (300 MHz, DMSO-*d*₆) δ 8.47 (d, $J = 2.7$ Hz, 1H, H₂₄), 8.24 (d, $J = 8.8$ Hz, 1H, H₂₇), 7.96 (dd, $J = 8.8, 2.7$ Hz, 1H, H₂₆), 3.68 (s, 6H, H₃₄). **¹³C NMR (75 MHz, DMSO-*d*₆)** δ 151.0 (C₃₂), 148.2, 142.0, 140.2, 140.1, 124.2, 110.6, 54.17 (C₃₄).

9.2.3 Plan A1 towards compound 59

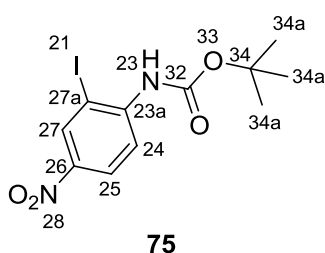
9.2.3.1 *tert*-Butyl (2-iodo-4-nitrophenyl)carbamate - 75

A Schlenk tube was charged with 2-iodo-4-nitroaniline **56** (0.206 g, 0.779 mmol), Boc₂O (2.2 equiv, 0.40 mL, 0.38 g, 1.74 mmol), DMAP (10 mol%, 10 mg, 0.079 mmol) and THF (5 mL). The orange reaction mixture was stirred at RT for 22 h. The solvent was removed *in vacuo* and the material was redissolved in DCM (10 mL). This was filtered through silica and the silica was washed with additional DCM (20 mL). The solvent was removed *in vacuo* to afford di(*tert*-butyl) 2-iodo-4-nitrophenylimidodicarbonate **75** (0.167 g, 0.360 mmol, 46%) ($R_f = 0.74$, 30% EtOAc/Hexane) as an off-white solid.



¹H NMR (300 MHz, CDCl₃) δ 8.71 (d, *J* = 2.6 Hz, 1H, H₂₇), 8.24 (dd, *J* = 8.7, 2.6 Hz, 1H, H₂₅), 7.38 (d, *J* = 8.7 Hz, 1H, H₂₄), 1.42 (s, 21H, H_{34a} and impurities). **¹³C NMR (75 MHz, CDCl₃)** δ 149.2 (C_{23a/32}), 148.0 (C_{23a/32}), 146.7 (C₂₆), 134.1 (C_{24/25/27}), 129.4 (C_{24/25/27}), 124.0 (C_{24/25/27}), 99.7 (C_{27a}), 84.0 (C₃₄), 27.8 (C_{34a}).

Next, a Schlenk tube was charged with di(*tert*-butyl) 2-iodo-4-nitrophenylimidodicarbonate **74** (0.102 g, 0.219 mmol) and DCM (2 mL) and was cooled to 0 °C by means of an ice-bath. 55% Nitric acid (2.0 equiv, 0.05 mL, 0.03 g, 0.4 mmol) was added to DCM (2 mL) and the reaction mixture too was cooled to 0 °C by means of an ice bath. This acidic solution was added to the Schlenk in a dropwise manner and the resulting reaction mixture was stirred at 0 °C for 2 h. There was still starting material visible by TLC and the reaction mixture was left to stir at RT for 16 h. The reaction mixture was diluted with DCM (10 mL) and washed with 0.1 M HCl (2 × 8 mL) and an aqueous solution of Na₂SO₄, followed by drying over anhydrous Na₂SO₄ and filtering. The solvent was removed *in vacuo* to afford the title compound **75** (0.067 g, 0.18 mmol, 83%) (*R_f* = 0.80, 30% EtOAc/Hexane) as a pale yellow solid.



IR (ATR, cm⁻¹): 3384 (N-H str), 3078 (Ar C-H str), 2986 (alkane C-H str), 1730 (C=O str), 1580 (Ar C=C str), 1497 (nitro N=O str), 1450, 1389, 1369, 1340 (nitro N=O str), 1244 (C-O str), 1218, 1140 (C-O str). **¹H NMR (300 MHz, CDCl₃)** δ 8.64 (d, *J* = 2.5 Hz, 1H, H₂₇), 8.34 (d, *J* = 9.2 Hz, 1H, H₂₄), 8.21 (dd, *J* = 9.2, 2.5 Hz, 1H, H₂₅), 7.20 (br s, 1H, H₂₃), 1.56 (s, 11H, H_{34a} & impurities). **¹³C NMR (75 MHz, CDCl₃)** δ

151.6 (C_{23a/32}), 144.6 (C_{23a/32}), 142.6, 134.4, 124.9, 117.7, 85.8 (C_{27a/34}), 82.6 (C_{27a/34}), 28.2 (C_{34a}).

Note: This reaction was repeated without purification after the first step and this afforded a slightly increased overall yield of 58% (*vs* 38%).

9.2.3.2 *tert*-Butyl 4-(6-nitro-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-1-yl)piperidine-1-carboxylate - **59**

A Schlenk tube was charged with *tert*-butyl (2-iodo-4-nitrophenyl)carbamate **75** (0.100 g, 0.276 mmol), CuI (11 mol%, 6 mg, 0.03 mmol), L-proline (20 mol%, 6 mg, 0.06 mmol) and K₂CO₃ (2.0 equiv, 0.077 g, 0.55 mmol) and then evacuated and backfilled with N₂. *tert*-Butyl 4-aminopiperidine-1-carboxylate **57** (1.0 equiv, 0.056 g, 0.28 mmol) and DMSO (0.60 mL) were subsequently added to yield a dark brown reaction mixture. The reaction mixture was heated at 50 °C for 18 h. More DMSO (0.80 mL) was subsequently added and it was heated at 80 °C for 21 h followed by 3 h at 130 °C. The reaction mixture was allowed to cool to RT and quenched with a saturated solution of aqueous NH₄Cl

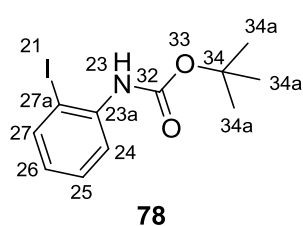
(100 mL). Extraction was done with EtOAc (40 and 2 × 20 mL) and the combined organic layers were washed with brine (100 mL), dried over anhydrous MgSO₄ and filtered. The solvent was removed *in vacuo* to yield the crude material as a dark brown oil. This was purified by CC (20 to 40% EtOAc/Hexane) to afford the title compound **59** (13 mg, 0.035 mmol, 13%) ($R_f = 0.45$, 60% EtOAc/Hexane) as a pale yellow solid.

Note: This reaction was repeated using 1 equiv of CuI and 2 equiv of L-proline. The isolated yield was 12%.

9.2.4 Plan A2 towards compound 66

9.2.4.1 *tert*-Butyl (2-iodophenyl)carbamate - 78

A 50 mL two-neck flask was charged with 2-iodoaniline **76** (0.505 g, 2.30 mmol) and THF (15 mL). Boc₂O (3.0 equiv, 1.60 mL, 1.52 g, 6.96 mmol) and DMAP (10 mol%, 0.029 g, 0.24 mmol) was added to the brown reaction mixture and it was heated at 72 °C for 2 h. The reaction mixture was allowed to cool to RT and it was diluted with H₂O (50 mL). Extraction was done with EtOAc (3 × 25 mL) and the combined organic layers were washed with brine (50 mL), dried over anhydrous MgSO₄ and filtered. Next, the solvent was removed *in vacuo* to afford the crude material. Potassium carbonate (3.0 equiv, 0.949 g, 6.87 mmol) and MeOH (10 mL) was added to the crude material and it was heated at 64 °C for 30 min. The reaction mixture was allowed to cool to RT and quenched with 0.1 M HCl (100 mL). Extraction was done with EtOAc (3 × 100 mL) and the combined organic layers were washed with brine (100 mL), dried over anhydrous MgSO₄ and filtered. The solvent was removed *in vacuo* and the crude material was purified by CC (2% EtOAc/Hexane) to afford the title compound **78** (0.445 g, 1.39 mmol, 60%) ($R_f = 0.37$, 10% EtOAc/Hexane) as a clear oil.

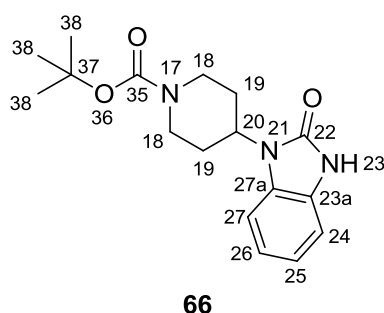


¹H NMR (300 MHz, CDCl₃) δ 8.06 (dd, $J = 8.6, 1.6$ Hz, 1H, H₂₄), 7.75 (dd, $J = 7.9, 1.5$ Hz, 1H, H₂₇), 7.32 (ddd, $J = 8.6, 7.2, 1.5$ Hz, 1H, H₂₅), 6.83 (br s, 1H, H₂₃), 6.77 (ddd, $J = 7.9, 7.2, 1.6$ Hz, 1H, H₂₆), 1.55 (s, 9H, H_{34a}). **¹³C NMR (75 MHz, CDCl₃)** δ 152.6 (C₃₂), 138.8, 129.2, 124.7, 120.2, 88.7 (C_{27a}), 81.1 (C₃₄), 28.3 (C_{34a}), 1 aromatic carbon absent.

9.2.4.2 *tert*-Butyl 4-(2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)piperidine-1-carboxylate - 66

A Schlenk tube was charged with *tert*-butyl (2-iodophenyl)carbamate **78** (0.083 g, 0.260 mmol), CuI (11 mol%, 5 mg, 0.03 mmol), L-proline (23 mol%, 7 mg, 0.06 mmol), K₂CO₃ (2.0 equiv, 0.073 g, 0.52 mmol) and DMSO (0.40 mL) and then evacuated and backfilled with N₂. *tert*-Butyl 4-aminopiperidine-1-carboxylate **57** (1.0 equiv, 0.051 g, 0.25 mmol) and DMSO (0.20 mL) were subsequently added to yield a grey reaction mixture. The reaction mixture was heated at 50 °C for 7 h

and the reaction mixture turned a blue colour. It was then heated at 130 °C for 15 h during which the mixture turned brown. The reaction mixture was allowed to cool to RT and quenched with a saturated solution of aqueous NH₄Cl (100 mL). Extraction was done with EtOAc (40 and 2 × 20 mL) and the combined organic layers were washed with brine (100 mL), dried over anhydrous MgSO₄ and filtered. The solvent was removed *in vacuo* to yield the crude material as a dark brown oil which was purified by CC (20 to 40% EtOAc/Hexane) to afford a trace amount of the title compound **66**.

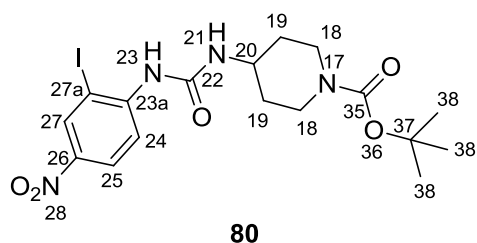


HRMS: calcd for C₁₇H₂₄N₃O₃⁺ [M+H]⁺, 318.1818 found 318.1814.

9.2.5 Plan B1 towards compound 59

9.2.5.1 *tert*-Butyl 4-[3-(2-iodo-4-nitrophenyl)ureido]piperidine-1-carboxylate - **80**

A 50 mL two-neck round-bottomed flask was charged with 2-iodo-4-nitroaniline **56** (0.201 g, 0.762 mmol) and 1,4-dioxane (1.5 mL) and stirred until dissolved. Triphosgene (0.53 equiv, 0.12 g, 0.40 mmol) was added to the yellow reaction mixture and the reaction flask was fitted with a reflux condenser. The reaction mixture was subsequently heated at 110 °C for 7 h. Next, solvent was removed *in vacuo* and *tert*-butyl 4-aminopiperidine-1-carboxylate **57** (1.0 equiv, 0.152 g, 0.759 mmol) and DCM (2 mL) were added to the yellow oil. The reaction mixture was stirred at RT for 15 h. Solvent was removed *in vacuo* and the crude yellow oil was purified by CC (30 to 40% EtOAc/Hexane) to afford the title compound **80** (0.275 g, 0.560 mmol, 74%) (R_f = 0.20, 30% EtOAc/Hexane) as a pale yellow solid.



Mp 208-210 °C. **IR (ATR, cm⁻¹):** 3339 (N-H str), 2952 (alkane C-H str), 1710 (C=O str), 1649 (C=O str), 1544 (Ar C=C str), 1503 (nitro N=O str) 1424, 1337 (nitro N=O str), 1243 (C-O str), 1212, 1154 (C-O str), 1016. **¹H NMR (300 MHz, DMSO-d₆)** δ 8.56 (d, *J* = 2.7 Hz, 1H, H₂₇), 8.27 (d, *J* = 9.4 Hz, 1H, H₂₄), 8.17 (dd, *J* = 9.4, 2.7 Hz, 1H, H₂₅),

7.94, (br s, 1H, H_{21/23}), 7.66 (d, 1H, H_{21/23}), 3.80 (d, *J* = 13.3 Hz, 2H, H₁₈), 3.72 – 3.62 (m, 1H, H₂₀), 3.03 – 2.85 (m, 2H, H₁₈), 1.88 – 1.79 (m, 2H, H₁₉), 1.40 (s, 10H, H₃₈ & impurities), 1.33 – 1.21 (m, 2H, H₁₉). **¹³C NMR (75 MHz, DMSO-d₆)** δ 153.9 (C_{22/35}), 153.2 (C_{22/35}), 146.9 (C_{23a/26}), 141.2

(C_{23a/26}), 134.3, 124.3, 118.5, 86.7 (C_{27a/37}), 78.7 (C_{27a/37}), 46.4 (C₂₀), 31.6 (C₁₉), 28.1 (C₃₈), C₁₈ absent.
HRMS: calcd for C₁₇H₂₄IN₄O₅⁺ [M+H]⁺, 491.0791, found 491.0778.

Note: This reaction was repeated, doing the first step at 110 °C for 21 h and the second step at RT for 17 h. The product was isolated in a decreased yield of 42%.

9.2.5.2 *tert*-Butyl 4-(6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)piperidine-1-carboxylate - 59

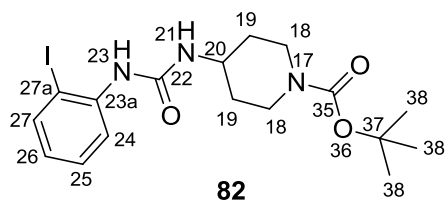
A 0.5 – 2.0 mL microwave vial was charged with *tert*-butyl 4-[3-(2-iodo-4-nitrophenyl)ureido]piperidine-1-carboxylate **80** (21 mg, 0.041 mmol), CuI (23 mol%, 2 mg, 0.009 mmol), 1,8-diazabicyclo[5.4.0]undec-7-ene (2.4 equiv, 0.015 mL, 0.015 g, 0.10 mmol) and DMSO (0.50 mL). The vial was placed in the microwave reactor and heated at 140 °C for 20 min. The reaction mixture was allowed to cool to RT and quenched with a saturated solution of aqueous NH₄Cl (25 mL). Extraction was done with EtOAc (3 × 25 mL) and the combined organic layers were washed with brine (50 mL), dried over anhydrous Na₂SO₄ and filtered. The solvent was removed *in vacuo* and the crude material was purified by CC (40 to 60% EtOAc/Hexane) to afford the still impure title compound **59** (8 mg, 0.022 mmol, <52%) (R_f = 0.45, 60% EtOAc/Hexane) as a pale yellow solid. Decomposed starting material (5 mg, 0.019 mmol, 45%) was also recovered and identified as 2-iodo-4-nitroaniline **56**.

Note: This reaction was done five times, with an overall yield of 8%, see Table 5.

9.2.6 Plan B2 towards compound 66

9.2.6.1 *tert*-Butyl 4-[3-(2-iodophenyl)ureido]piperidine-1-carboxylate - 82

A 50 mL two-neck round-bottomed flask was charged with 2-iodoaniline **76** (0.106 g, 0.482 mmol) and 1,4-dioxane (1.5 mL). Triphosgene (0.48 equiv, 0.069 g, 0.23 mmol) was added to the clear brown reaction mixture, turning it milky brown. It was heated at 110 °C for 25 min and turned clear during this time. Next, the reaction mixture was allowed to cool to RT and solvent was removed *in vacuo*. The flask was charged with *tert*-butyl 4-aminopiperidine-1-carboxylate **57** (0.96 equiv, 0.093 g, 0.46 mmol) and DCM (2 mL) and stirred at RT for 4 h. The solvent was removed *in vacuo* and the crude material was purified by CC (40% EtOAc/Hexane) to afford the title compound **82** (0.188 g, 0.423 mmol, 88%) (R_f = 0.32, 40% EtOAc/Hexane) as an off-white solid.



82

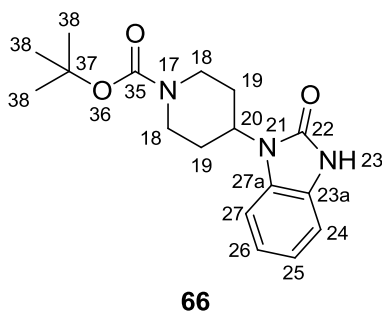
Mp 174-179 °C. **IR (ATR, cm⁻¹):** 3320 (N-H str), 3292, 2974-2928 (alkane C-H str), 1683 (C=O str), 1642 (C=O str), 1557 (Ar C=C str), 1522, 1430 (Ar C=C str), 1365, 1307, 1275, 1224 (C-O str), 1176, 1144 (C-O str), 741. **¹H NMR (300 MHz, CDCl₃)** δ 7.89 (dd, *J* = 8.3, 1.6 Hz, 1H, H₂₄), 7.76

(dd, *J* = 8.0, 1.5 Hz, 1H, H₂₇), 7.31 (ddd, *J* = 8.3, 7.4, 1.5 Hz, 1H, H₂₅), 6.79 (ddd, *J* = 8.0, 7.4, 1.6 Hz, 1H, H₂₆), 6.60 (br s, 1H, H_{21/23}), 5.08 (d, 1H, H_{21/23}), 4.02 (br d, *J* = 12.9 Hz, 2H, H₁₈), 3.89 – 3.75 (m, 1H, H₂₀), 2.86 (t, *J* = 12.6 Hz, 2H, H₁₈), 2.01 – 1.90 (m, 2H, H₁₉), 1.46 (s, H₉, H₃₈), 1.31 (m, *J* = 11.7, 4.2 Hz, 2H, H₁₉). **¹³C NMR (75 MHz, CDCl₃)** δ 154.7 (C_{22/35}), 154.3 (C_{22/35}), 139.3, 139.0, 129.2, 125.3, 122.5, 91.2 (C_{27a}), 79.7 (C₃₇), 47.7 (C₂₀), 42.7 (C₁₈), 31.5 (C₁₉), 28.5 (C₃₈). **HRMS:** calcd for C₁₇H₂₅IN₃O₃⁺ [M+H]⁺, 446.0941, found 446.0938.

Note: This reaction was also carried out with the first part at 110 °C for 15 min and the second part at RT for 18 h. No product was isolated.

9.2.6.2 *tert*-Butyl 4-(2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)piperidine-1-carboxylate - **66**

A 0.5 – 2.0 mL microwave vial was charged with *tert*-butyl 4-[3-(2-iodophenyl)ureido]piperidine-1-carboxylate **82** (0.050 g, 0.11 mmol), CuI (1.1 equiv, 23 mg, 0.12 mmol), 1,8-diazabicyclo[5.4.0]undec-7-ene (2.0 equiv, 0.034 mL, 0.035 g, 0.23 mmol) and DMSO (1 mL). The vial was placed in the microwave reactor and heated at 140 °C for 10 min. Starting material was still present as detected by TLC and the reaction mixture was heated at 140 °C for another 10 min. The reaction mixture was allowed to cool to RT and quenched with 0.1M HCl (15 mL). Extraction was done with EtOAc (3 × 25 mL) and the combined organic layers were washed with brine (50 mL), dried over anhydrous MgSO₄ and filtered. The solvent was removed *in vacuo* and the crude material was purified by CC (20 to 50% EtOAc/Hexane) to afford the pure title compound **66** (15 mg, 0.046 mmol, 41%) (*R_f* = 0.43, 50% EtOAc/Hexane) as a white solid.



66

¹H NMR (300 MHz, DMSO-*d*₆) δ 10.84 (br s, 1H, H₂₃), 7.21 – 7.14 (m, 1H), 7.02 – 6.93 (m, 3H), 4.40 – 4.26 (m, 1H, H₂₀), 4.09 (br d, *J* = 12.6 Hz, 2H, H₁₈), 2.99 – 2.75 (m, 2H, H₁₈), 2.19 (m, *J* = 12.6, 4.4 Hz, 2H, H₁₉), 1.67 (dd, *J* = 12.4, 2.6 Hz, 2H, H₁₉), 1.43 (s, 9H, H₃₈). **¹³C NMR (75 MHz, DMSO-*d*₆)** δ 153.9 (C_{22/35}), 153.7 (C_{22/35}), 129.2, 128.3, 120.6, 120.4, 108.8, 108.4, 78.8 (C₃₇), 49.9 (C₂₀), 43.1 (C₁₈), 28.5 (C₁₉), 28.1 (C₃₈). **HRMS:** calcd for C₁₇H₂₃N₃NaO₃⁺ [M+Na]⁺, 340.1637, found 340.1631.

Note: This reaction was also done using 22 mol% CuI and heating the reaction mixture at 140 °C in two 20 min stints. The yield of this run was 38%.

9.2.7 Nucleophilic substitution plan

9.2.7.1 Attempted synthesis of *tert*-butyl 4-(6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)piperidine-1-carboxylate - 59

A Schlenk tube was evacuated and backfilled with N₂ and subsequently charged with 5-nitro-1*H*-benzo[*d*]imidazol-2(3*H*)-one **85** (0.102 g, 0.567 mmol) and DMF (2 mL). The yellow reaction mixture was cooled to 0 °C by means of an ice-bath and 60% sodium hydride (1.5 equiv, 0.034 g, 0.84 mmol) was then added, turning the reaction mixture red. *tert*-Butyl 4-bromopiperidine-1-carboxylate **84** (3.0 equiv, 0.446 g, 1.69 mmol) was added after stirring for 20 min and the reaction mixture was then heated to 60 °C for 2.5 h after which it was heated to 80 °C for 3 days. Monitoring by TLC showed almost exclusively starting material so the reaction mixture was allowed to cool to RT and more 60% sodium hydride (1.5 equiv, 0.034 g, 0.84 mmol) was added. The reaction mixture was then heated again at 80 °C for 80 min. The starting material was still not consumed according to TLC. No product was isolated.

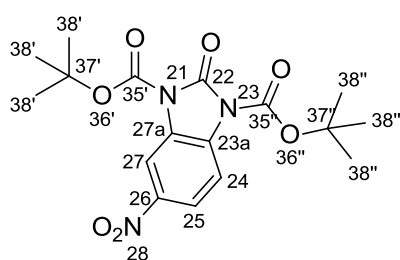
9.2.7.2 Attempted synthesis of *tert*-butyl 4-(6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)piperidine-1-carboxylate - 59

A Schlenk tube was charged with MeCN (1.5 mL) and subsequently evacuated and backfilled with N₂. 5-Nitro-1*H*-benzo[*d*]imidazol-2(3*H*)-one **85** (0.101 g, 0.565 mmol), *tert*-butyl 4-bromopiperidine-1-carboxylate **84** (1.1 equiv, 0.164 g, 0.620 mmol) and K₂CO₃ (5.0 equiv, 0.387 g, 2.80 mmol) was added and it was again evacuated and backfilled with N₂. The yellow reaction mixture was stirred at RT for 20 min and then heated at 50 °C for 4 h, followed by stirring at RT for 16 h. More MeCN (6 mL) was added during this time and the reaction mixture turned dark orange over time. The reaction mixture was filtered to remove undissolved K₂CO₃. The solvent was removed *in vacuo* and NMR analysis of the crude material showed unreacted starting materials. No product was isolated.

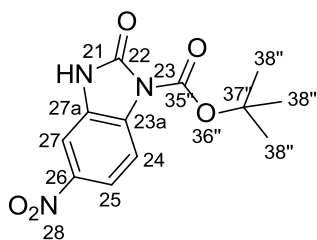
9.2.7.3 Di-*tert*-butyl 5-nitro-2-oxo-1*H*-benzo[*d*]imidazole-1,3(2*H*)-dicarboxylate, *tert*-butyl 5-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-1-carboxylate and *tert*-butyl 6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-1-carboxylate - 91, 92 & 93

A Schlenk tube was charged with 5-nitro-1*H*-benzo[*d*]imidazol-2(3*H*)-one **85** (0.101 g, 0.564 mmol), K₂CO₃ (2.0 equiv, 0.155 g, 1.12 mmol) and degassed DMF (1.5 mL). The reaction mixture was then

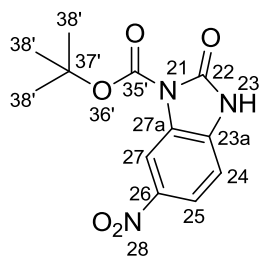
cooled to 0 °C by means of an ice-bath. Boc₂O (1.0 equiv, 0.13 mL, 0.12 g, 0.57 mmol) was added to degassed DMF (1.5 mL) and this was added to the cooled reaction mixture contents in a portionwise manner over 35 min. The reaction mixture was stirred at RT for 18 h. The reaction was quenched with H₂O (20 mL) and extracted with EtOAc (40, 20 and 10 mL). The combined organic fractions were washed with brine (100 mL), dried over anhydrous magnesium and filtered. The solvent was removed *in vacuo* and purification was done with CC (20 to 40% EtOAc/Hexane) to afford **91** (0.090 g, 0.24 mmol, 42%) (*R_f* = 0.85, 50% EtOAc/Hexane), **92** (2 mg, 0.008 mmol, 1%) (*R_f* = 0.63, 50% EtOAc/Hexane) and **93** (0.048 g, 0.17 mmol, 31%) (*R_f* = 0.45, 50% EtOAc/Hexane) as pale yellow solids.



91



92



93

91: Mp 129-131 °C. **IR (ATR, cm⁻¹):** 2981 (alkane C-H str), 1820 (C=O str), 1800 (C=O str), 1740, 1535 (nitro N=O), 1483 (Ar C=C str), 1372, 1339 (nitro N=O), 1312 (C-N str), 1251 (C-O str), 1212, 1106 (C-O str), 1071, 1000. **¹H NMR (300 MHz, DMSO-d₆)** δ 8.55 (d, *J* = 2.5 Hz, 1H, H₂₇), 8.19 (dd, *J* = 9.1, 2.5 Hz, 1H, H₂₅), 7.94 (d, *J* = 9.1 Hz, 1H, H₂₄), 1.62 & 1.61 (s, 20H, H_{38'} & H_{38''} & impurities). **¹³C NMR (75 MHz, DMSO-d₆)** δ 147.5 (C_{22/35'/35''}), 147.3 (C_{22/35'/35''}), 146.2 (C_{22/35'/35''}), 145.9, 143.4, 131.3, 126.4, 120.3, 113.8, 108.8, 85.6 (C_{37'/37''/impurity}), 85.6 (C_{37'/37''/impurity}), 85.5 (C_{37'/37''/impurity}), 66.9 (*tert*-Butyl alcohol), 31.3 (*tert*-Butyl alcohol), 27.5 (C_{38'/38''}), 26.8 (C_{38'/38''}). **HRMS:** calcd for C₁₇H₂₁N₃NaO₇⁺ [M+Na]⁺, 402.1277 found 402.1280.

92: ¹H NMR (300 MHz, DMSO-d₆) δ 11.73 (br s, H₂₁), 8.04 (dd, *J* = 9.0, 2.3 Hz, 1H, H₂₅), 7.83 (d, *J* = 9.0 Hz, 1H, H₂₄), 7.73 (d, *J* = 2.3 Hz, 1H, H₂₇), 1.60 (s, 13H, H_{38'} & impurities).

93: ¹H NMR (300 MHz, DMSO-d₆) δ 11.97 (s, H₂₃), 8.47 (d, *J* = 2.3 Hz, 1H, H₂₇), 8.12 (dd, *J* = 8.6, 2.3 Hz, 1H, H₂₅), 7.17 (d, *J* = 8.6 Hz, 1H, H₂₄), 1.61 (s, 12H, H_{38'} & impurities).

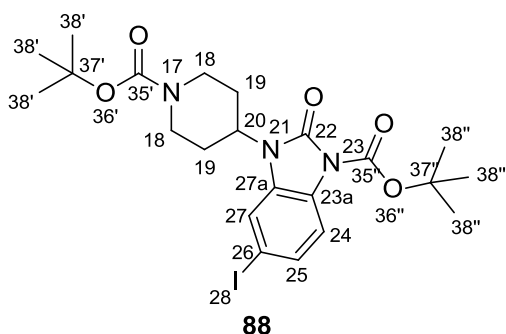
Note: This reaction was done three times in total, see Table 6.

Also note that the total mole of the Boc-protecting group in the three isolated products exceeded that of the Boc₂O used in the reaction, indicating contamination by an unidentified impurity.

9.2.8 Iodination plan

9.2.8.1 *tert*-Butyl 3-[1-(*tert*-butoxycarbonyl)piperidin-4-yl]-5-iodo-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-1-carboxylate - **88**

A 50 mL two-neck round-bottomed flask was charged with 1-(piperidin-4-yl)-1*H*-benzo[*d*]imidazol-2(3*H*)-one **86** (0.108 g, 0.499 mmol), AcOH (2.7 mL) and H₂O (0.3 mL) and cooled to 0 °C by means of an ice-bath. The starting material was insoluble in the solvent mixture. ICl (1.0 equiv, 0.025 mL, 0.081 g, 0.50 mmol) was added to AcOH (1.8 mL) and H₂O (0.2 mL) and this was added to the milky white reaction mixture in a dropwise manner over 50 min. Starting material was consumed after the addition of the iodinating agent. The reaction mixture was filtered and the yellow precipitate was washed with cold H₂O. Next, a 50 mL two-neck round-bottomed flask was charged with a portion of the crude material (0.119 g, ~0.346 mmol), K₂CO₃ (3.0 equiv, 0.144 g, 1.04 mmol) and MeCN (5 mL). Boc₂O (3.1 equiv, 0.25 mL, 0.24 g, 1.1 mmol) was added to the milky yellow solution while it was kept at 0 °C by means of an ice-bath. The reaction mixture was then heated at 40 °C for 19 h. The reaction mixture was allowed to cool to RT and diluted with H₂O (50 mL). Extraction was done with EtOAc (3 × 50 mL) and the combined organic layers were washed with brine (50 mL), dried over anhydrous MgSO₄ and filtered. Solvent was removed *in vacuo* and the crude material was purified by CC (40% EtOAc/Hexane) to afford the title compound **88** (0.175 g, 0.322, 93%) (*R*_f = 0.52, 20% EtOAc/Hexane) as an off-white oil.



IR (ATR, cm⁻¹): 2979-2932 (alkane C-H str), 1794, 1749 (C=O str), 1693 (C=O str), 1480 (Ar C=C str), 1367, 1310 (C-N str), 1242 (C-O str), 1211, 1154 (C-O str), 1111, 1062, 843 (1,2,4-substituted Ar C-H oop bend), 809 (1,2,4-substituted Ar C-H oop bend), 771. **¹H NMR (300 MHz, CDCl₃)** δ 7.62 (d, *J* = 8.5 Hz, 1H, H₂₄), 7.42 (dd, *J* = 8.5, 1.6 Hz, 1H, H₂₅), 7.36 (d, *J* = 1.6

Hz, 1H, H₂₇), 4.44 – 4.22 (m, 3H, H₁₈ & H₂₀), 2.72 (impurity), 2.82 (t, *J* = 12.8 Hz, 2H, H₁₈), 2.30 (m, *J* = 12.7, 4.6 Hz, 2H, H₁₉), 1.81 – 1.68 (m, 3H, H₁₉ & EtOAc), 1.65 (s, 10H, H_{38''} & impurity), 1.50 (s, 9H, H_{38'}). **¹³C NMR (75 MHz, CDCl₃)** δ 154.6, 149.8, 148.5, 146.7, 130.8, 129.7, 126.0, 117.2, 116.2, 86.6, 85.1, 80.0, 51.5 (C₂₀), 28.4, 28.0, 27.3. **HRMS:** calcd for C₂₂H₃₀IN₃NaO₅⁺ [M+Na]⁺, 566.1128, found 566.1133.

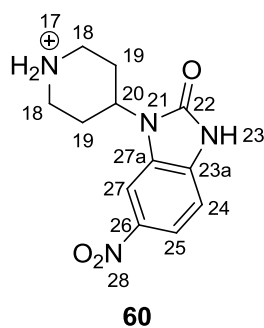
9.2.8.2 Attempted synthesis of *tert*-butyl 5-amino-3-[1-(*tert*-butoxycarbonyl)piperidin-4-yl]-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-1-carboxylate - 89

A Schlenk tube was evacuated and backfilled with N₂ and charged with Cu(acac)₂ (11 mol%, 3 mg, 0.01 mmol), caesium carbonate (2.1 equiv, 0.068 g, 0.21 mmol), *tert*-butyl 3-[1-(*tert*-butoxycarbonyl)piperidin-4-yl]-5-iodo-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazole-1-carboxylate **88** (0.056 g, 0.10 mmol) and DMF (0.4 mL). It was then evacuated and backfilled with N₂ for a second time and 2,4-pentadione (1 equiv, 0.01 mL, 10 mg, 0.1 mmol) and 28% aqueous ammonia (2.3 equiv, 0.03 mL, 8 mg, 0.2 mmol) was added to the reaction mixture, followed by a third evacuation and backfill. The pale green reaction mixture was heated at 70 °C for 24 h with additional DMF (0.4 mL) added to prevent it running dry. The colour of the reaction mixture changed to a dark orange with time. The reaction was allowed to cool to RT and diluted with DCM (50 mL). This was filtered through Celite and the Celite was washed with more DCM (100 mL). The filtered DCM was then washed with H₂O (50 mL), dried over anhydrous MgSO₄ and filtered. The solvent was removed *in vacuo* and the crude material was purified by CC to afford recovered starting material **88** (11 mg, 0.020 mmol, 19%) (R_f = 0.52, 20% EtOAc/Hexane) as a yellow oil. No product was isolated.

9.2.9 Nitration plan

9.2.9.1 4-(5-Nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)piperidin-1-ium nitrate and 4-(6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)piperidin-1-ium nitrate - 60

A 20 mL polytop was charged with 1-(piperidin-4-yl)-1*H*-benzo[*d*]imidazol-2(3*H*)-one **86** (0.501 g, 2.31 mmol) and 65% aqueous nitric acid (11 equiv, 2.50 mL, 1.63 g, 25.8 mmol). A thick dark orange mixture forms on addition of the acid. The reaction mixture is cooled to 0 °C by means of an ice-bath and the precipitate is filtered. The precipitate is washed with cold H₂O and then dried under vacuum. NMR analysis of the crude material (0.767 g) revealed a 4:3 mixture of regioisomers.



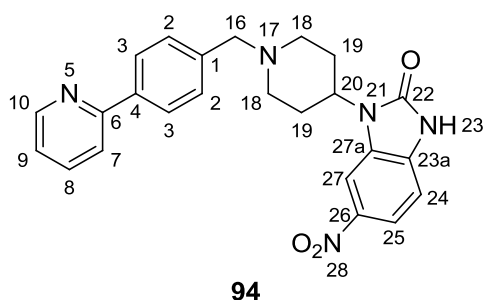
60

IR (ATR, cm⁻¹): 3017 (N-H str), 1694 (C=O str), 1615 (N-H bend), 1492 (N=O str), 1334 (N=O str). **¹H NMR (300 MHz, DMSO-*d*₆)** δ 11.73 (br s, 1H), 11.53 (br s, 1H), 8.21 (d, *J* = 2.1 Hz, 1H), 8.07 (dd, *J* = 8.8, 2.4 Hz, 1H), 8.01 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.78 (d, *J* = 2.4 Hz, 1H), 7.47 (d, *J* = 8.8 Hz, 1H), 7.18 (d, *J* = 8.7 Hz, 1H), 4.70 – 4.54 (m, 2H), 3.46 (d, *J* = 12.0 Hz, 4H), 3.20 – 3.01 (m, 4H), 2.64 – 2.45 (m), 1.92 (d, *J* = 12.8 Hz, 4H). **¹³C NMR (75 MHz, DMSO-*d*₆)** δ 153.9, 153.9, 141.5, 134.7, 134.4, 129.2, 128.5,

118.2, 108.6, 107.9, 104.1, 103.9, 47.7, 43.1, 25.3.

9.2.9.2 5-Nitro-1-{1-[4-(pyridin-2-yl)benzyl]piperidin-4-yl}-1H-benzo[d]imidazol-2(3H)-one and 6-nitro-1-{1-[4-(pyridin-2-yl)benzyl]piperidin-4-yl}-1H-benzo[d]imidazol-2(3H)-one - 94

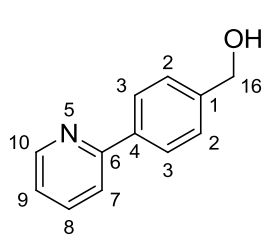
A 50 mL two-neck round-bottomed flask was charged with 4-(pyridin-2-yl)benzaldehyde **104** (1.0 equiv, 0.057 g, 0.31 mmol), TEA (2.3 equiv, 0.10 mL, 0.073 g, 0.72 mmol) and THF (5 mL). This was stirred for 5 min followed by addition of 4-(6-nitro-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-1-yl)piperidin-1-ium nitrate / 4-(5-nitro-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-1-yl)piperidin-1-ium nitrate **60** (0.100 g, 0.306 mmol), DMSO (0.5 mL) and AcOH (3 equiv, 0.05 mL, 0.05 g, 0.9 mmol). NaBH(OAc)₃ (6.1 equiv, 0.393 g, 1.86 mmol) was added portionwise over 35 min, followed by addition of more 4-(pyridin-2-yl)benzaldehyde **104** (0.47 equiv, 0.027 g, 0.14 mmol). The reaction mixture was stirred at RT for 4 days, with more THF (4 mL) added after the first 22 h. The reaction was quenched with MeOH (20 mL) and the solvent removed *in vacuo*. H₂O was added to the crude material and extraction was done with DCM (4 × 25 mL). The combined organic layers were dried over anhydrous MgSO₄ and filtered. The solvent was removed *in vacuo* and the crude material was purified by CC (20% MeOH/EtOAc with 1% TEA) to afford the title compound **94** (0.031 g, 0.072 mmol, 23%) as a yellow oil. Starting material **104** (0.034 g, 0.19 mmol, 41%) was recovered and reduced starting material, [4-(pyridin-2-yl)phenyl]methanol (20 mg, 0.10 mmol, 23%) (R_f = 0.70, 90% EtOAc/MeOH with 2.5% TEA) was isolated as a colourless oil.



94

¹H NMR (600 MHz, DMSO-d₆) δ 8.74 – 8.71 (m, 1H), 8.16 (d, *J* = 2.1 Hz, 0.2H), 8.09 – 8.05 (m, 0.2H), 8.04 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.97 (d, *J* = 8.4 Hz, 0.4H), 7.96 (d, *J* = 8.2 Hz, 2H, H₃), 7.91 (d, *J* = 2.3 Hz, 1H), 7.81 – 7.75 (m, 1H & 0.2H), 7.73 (dd, *J* = 8.1, 1.0 Hz, 1H, H₇), 7.62 (d, *J* = 8.4 Hz, 0.2H), 7.51 (br s, 0.2H), 7.49 (d, *J* = 8.1 Hz, 2H), 7.36 (d, *J* = 8.8 Hz, 1H), 7.27 – 7.23 (m, 1H),

7.10 (d, *J* = 8.6 Hz), 4.46 – 4.34 (m, 1H, H₂₀), 3.71 (s, H₁₆), 3.70 (s, H₁₆), 3.20 – 3.12 (m, 2H, H₁₈), 2.60 – 2.43 (m, 2H, H₁₈), 2.27 (t, *J* = 11.1 Hz, 2H, H₁₉), 1.85 (d, *J* = 12.3 Hz, 2H, H₁₉) (both regioisomers visible). **¹³C NMR (150 MHz, DMSO-d₆)** δ 175.5, 171.2, 157.2, 157.2, 155.6, 155.4, 149.5, 142.4, 142.3, 138.5, 138.2, 136.9, 136.9, 134.2, 133.5, 129.8, 129.7, 129.0, 128.1, 127.5, 127.2, 127.0, 122.2, 122.1, 120.9, 120.7, 118.3, 118.0, 108.9, 108.7, 105.4, 105.2, 62.2, 60.4, 52.6, 52.5, 51.5, 51.2, 28.9, 21.5, 21.0, 14.1 (both regioisomers visible).



$^1\text{H NMR}$ (600 MHz, CDCl_3) δ 8.66 (d, $J = 4.7$ Hz, 1H, H_{10}), 7.92 (d, $J = 8.2$ Hz, 2H, H_3), 7.74 (td, $J = 7.7, 1.8$ Hz, 1H, H_8), 7.70 (d, $J = 7.9$ Hz, 1H, H_7), 7.42 (d, $J = 8.1$ Hz, 2H, H_2), 7.24 – 7.21 (m, 1H, H_9), 4.71 (s, 2H, H_{16}).
 $^{13}\text{C NMR}$ (150 MHz, CDCl_3) δ 157.2, 149.5, 142.0, 138.4, 136.8, 127.2, 127.0, 122.1, 120.7, 64.7 (C_{16}).

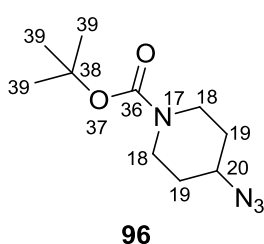
Note: This reaction was repeated, using similar conditions, and the product was isolated in a yield of 12%.

9.3 EXPERIMENTAL WORK PERTAINING TO CHAPTER 4

9.3.1 Synthesis of the components for the click reaction(s)

9.3.1.1 *tert*-Butyl 4-azidopiperidine-1-carboxylate - 96

A 50 mL two-neck round-bottomed flask was charged with DMSO (5 mL) and *tert*-butyl 4-bromopiperidine-1-carboxylate **84** (1.01 g, 3.82 mmol). This was followed by stirring at RT until the solution became clear and the starting material had dissolved. Sodium azide (1.2 equiv, 0.301 g, 4.64 mmol) was then added and the colourless reaction mixture was stirred at 60 °C for 18 h. The reaction mixture was diluted with distilled H_2O and the product was extracted with EtOAc (5×80 mL). The combined organic layers were then washed with brine, dried over MgSO_4 and filtered. The solvent was removed *in vacuo* to yield the crude product which was purified by CC (5% EtOAc/Hexane, 20% EtOAc/Hexane) to afford the title compound **96** (0.762 g, 3.37 mmol, 88%) ($R_f = 0.47$, 10% EtOAc/Hexane) as a colourless oil.

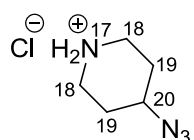


IR (ATR, cm^{-1}): 2976-2861 (C-H str), 2090 (N=N=N str), 1687 (C=O str), 1417, 1365, 1231, 1164 (C-O str), 1129, 1019. $^1\text{H NMR}$ (600 MHz, CDCl_3) δ 3.84 – 3.74 (m, 2H, H_{18}), 3.57 – 3.52 (m, 1H, H_{20}), 3.09 – 3.03 (m, 2H, H_{18}), 1.86 – 1.81 (m, 2H, H_{19}), 1.56 – 1.49 (m, 2H, H_{19}), 1.43 (s, 9H, H_{39}).
 $^{13}\text{C NMR}$ (150 MHz, CDCl_3) δ 154.5 (C_{36}), 79.7 (C_{38}), 57.48 (C_{20}), 41.4 (C_{18}), 30.5 (C_{19}), 28.3 (C_{39}). **HRMS**: calcd for $\text{C}_{10}\text{H}_{19}\text{N}_4\text{O}_2^+$ $[\text{M}+\text{H}]^+$, 227.1508, found 171.0872 $[\text{M}-55]^+$.

Note: This reaction was done a total of nine times, all in a similar fashion, and the average yield was 76%.

9.3.1.2 4-Azidopiperidin-1-ium chloride - 97

A one-neck round-bottomed flask was charged with *tert*-butyl 4-azidopiperidine-1-carboxylate **96** (0.714 g, 3.16 mmol) and 4 M HCl in 1,4-dioxane (6 mL). The reaction mixture was stirred at RT for 23 h after which solvent was removed *in vacuo* to afford the crude title compound **97** (0.508 g, 3.12 mmol, 99%) as a pale yellow solid.

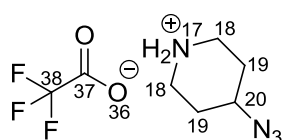


97

IR (ATR, cm⁻¹): 2935 (C-H str), 2793, 2718 (N-H str), 2633, 2499, 2094 (N=N=N str), 1591, 1445 (-CH₂- bend), 1373, 1254 (C-N str). **¹H NMR (300 MHz, CDCl₃)** δ 9.59 (br s, 1H, H₁₇), 3.93 – 3.81 (m, 1H, H₂₀), 3.41 – 3.10 (m, 4H, H₁₈), 2.33 – 2.15 (m, 2H, H₁₉), 2.04 – 1.89 (m, 2H, H₁₉). **¹³C NMR (75 MHz, CDCl₃)** δ 54.0 (C₂₀), 40.3 (C₁₈), 27.0 (C₁₉). **HRMS:** calcd for C₅H₁₁N₄⁺ [M+H]⁺, 127.1676, found 127.0977 (so without Cl).

9.3.1.3 4-Azidopiperidin-1-ium 2,2,2-trifluoroacetate - 98

A 50 mL one-neck round-bottomed flask was charged with *tert*-butyl 4-azidopiperidine-1-carboxylate **96** (0.708 g, 3.13 mmol) and DCM (6 mL). The colourless reaction mixture was cooled to 0 °C by means of an ice-bath and TFA (4.4 equiv, 1.05 mL, 1.56 g, 13.7 mmol) was added in a dropwise manner. The reaction was left to stir at RT for 16 h. Toluene (10 mL) was added to the reaction mixture and the solvent was removed *in vacuo*. Toluene was added twice more and each time as much as possible solvent was removed *in vacuo*. Diethyl ether (10 mL) was then added to the reaction mixture and also removed *in vacuo* to afford the title compound **98** (0.758 g, 3.16 mmol, quantitative) as a pale yellow solid.



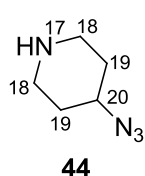
98

Mp 75-76 °C. **IR (ATR, cm⁻¹):** 2978, 2821, 2738, 2543, 2140, 2104 (N=N=N str), 1666 (C=O str), 1627, 1426 (-CH₂- bend), 1333, 1281, 1170 (C-F str), 1121 (C-F str), 1034, 832, 794, 720. **¹H NMR (300 MHz, CDCl₃)** δ 11.78 (s, 1H), 8.71 (s, 2H, H₁₇), 3.95 – 3.77 (m, 1H, H₂₀), 3.46 – 3.06 (m, 4H, H₁₈), 2.24 – 1.82 (m, 4H, H₁₉). **¹³C NMR (75 MHz, CDCl₃)** δ 161.6 (q, C₃₇), 115.7 (q, C₃₈), 53.8 (C₂₀), 40.8 (C₁₈), 27.1 (C₁₉). **HRMS:** calcd for C₇H₁₂O₂N₄F₃⁺ [M+H]⁺, 241.0912 and for C₅H₁₁N₄⁺, 127.0984, found 127.0989.

9.3.1.4 4-Azidopiperidine - 44

A 50 mL one-neck round-bottomed flask was charged with *tert*-butyl 4-azidopiperidine-1-carboxylate **96** (0.374 g, 1.64 mmol) and DCM (4 mL) and cooled to 0 °C by means of an ice-bath. TFA (6 equiv, 0.75 mL, 1.1 g, 9.8 mmol) was added dropwise to the cooled reaction mixture and then stirred at RT

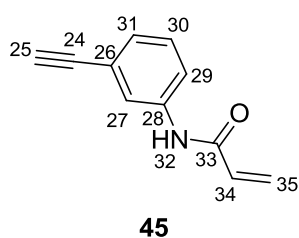
for 25 h. The reaction was quenched with a 0.1 M sodium hydroxide until the pH was basic according to universal indicator paper. It was then extracted with EtOAc (3 × 150 mL) and the combined organic layers were dried over MgSO₄ and filtered. The solvent was removed in vacuo to afford the crude title compound **44** (40 mg, 0.18 mmol, 11%) as a pale white solid which was not purified. Sodium chloride was added to the aqueous phase, but no more product was isolated.



¹H NMR (300 MHz, CDCl₃) δ 6.33 (br s, H₁₇), 3.89 – 3.79 (m, 1H, H₂₀), 3.34 – 3.22 (m, 2H, H₁₈), 3.15 – 3.03 (m, 2H, H₁₈), 2.21 – 2.08 (m, 2H, H₁₉), 1.96 – 1.83 (m, 2H, H₁₉). **¹³C NMR (75 MHz, CDCl₃)** δ 54.4 (C₂₀), 40.7 (C₁₈), 27.5 (C₁₉).

9.3.1.5 *N*-(3-ethynylphenyl)acrylamide - **45**

A Schlenk tube was charged with DCM (15 mL), 3-ethynylaniline **99** (0.20 mL, 0.22 g, 1.9 mmol) and TEA (4.5 equiv, 1.20 mL, 0.871 g, 8.61 mmol). The colourless reaction mixture was cooled to 0 °C by means of an ice-bath and acryloyl chloride (4.5 equiv, 0.70 mL, 0.78 g, 8.6 mmol) was added in a dropwise manner. The reaction mixture turned a milky yellow colour over time. After addition of the acryloyl chloride, the reaction mixture was left to stir at RT for 18 h. The reaction was quenched with a saturated solution of aqueous NaHCO₃ and the product was extracted with DCM (3 × 150 mL). The combined organic layers were dried over MgSO₄ and filtered. The solvent was removed *in vacuo* to yield the crude product which was purified by CC (20% EtOAc/Hexane) to afford the title compound **45** (0.317 g, 1.85 mmol, 97%) (R_f = 0.20, 20% EtOAc/Hexane) as a pale yellow solid.



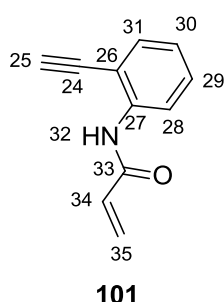
Mp 111 °C. **IR (ATR, cm⁻¹):** 3296 (alkyne C-H str), 3106 (Ar C-H str), 1667 (C=O), 1625 (alkene C=C str), 1607 (Ar C=C str), 1556, 1478 (Ar C=C str), 1409, 1332, 1289, 1215, 878 (Ar C-H out-of-plane bend), 734 (alkene C-H out-of-plane bend). **¹H NMR (600 MHz, CDCl₃)** δ 7.77 (s, 1H, H₃₂), 7.70 (s, 1H, H₂₇), 7.62 (d, *J* = 7.6 Hz, 1H, H₂₉), 7.27 (t, *J* = 7.6

Hz, 1H, H₃₀), 7.24 (d, *J* = 7.6 Hz, 1H, H₃₁), 6.42 (dd, *J* = 17.0, 1.2 Hz, 1H, H₃₅), 6.27 (dd, *J* = 17.0, 10.3 Hz, 1H, H₃₄), 5.76 (dd, *J* = 10.3, 1.2 Hz, 1H, H₃₅), 3.06 (s, 1H, H₂₅). **¹³C NMR (150 MHz, CDCl₃)** δ 163.8 (C₃₃), 137.8, 130.9, 129.0, 128.2, 128.1, 123.5, 122.8, 120.7, 83.06, 77.5. **HRMS:** calcd for C₁₁H₁₀NO⁺ [M+H]⁺, 172.0762, found 172.0766.

Note: This reaction was done a total of five times, all in a similar fashion, and the average yield was 74%.

9.3.1.6 *N*-(2-ethynylphenyl)acrylamide - 101

A Schlenk tube was charged with 2-ethynylaniline **100** (0.35 mL, 0.36 g, 3.1 mmol), DCM (20 mL), and TEA (2.8 equiv, 1.20 mL, 0.871 g, 8.61 mmol). The clear yellow reaction mixture was cooled to 0 °C by means of an ice-bath and the acryloyl chloride (2.8 equiv, 0.70 mL, 0.78 g, 8.6 mmol) was added in a dropwise manner. The reaction mixture turned a milky yellow colour over time. After addition of the acryloyl chloride, the reaction mixture was left to stir at RT for 16 h. The reaction was quenched with a saturated solution of aqueous NaHCO₃ and the product was extracted with DCM (3 × 250 mL). The combined organic layers were dried over MgSO₄ and filtered. The solvent was removed *in vacuo* to yield the crude product which was purified by CC (20% EtOAc/Hexane) to afford the title compound **101** (0.486 g, 2.84 mmol, 92%) (R_f = 0.35, 20% EtOAc/Hexane) as a pale yellow solid.



Mp 76-78 °C. **IR (ATR, cm⁻¹):** 3251 (alkyne C-H str), 3211, 1687 (alkene C=C str), 1665 (C=O str), 1613 (Ar C=C str), 1576, 1520, 1443 (Ar C=C str), 1401, 1313, 1287, 1217, 1193, 1145, 972 (alkene C-H oop bend), 761 (*ortho*-substituted Ar C-H oop bend). **¹H NMR (400 MHz, CDCl₃)** δ 8.52 (d, *J* = 8.3 Hz, 1H, H_{A28}), 8.07 (s, 1H, H_{A32}), 7.48 (dd, *J* = 7.7, 1.5 Hz, 1H, H_{A31}), 7.38 (dd, *J* = 8.3, 1.5 Hz, 1H, H_{A29}), 7.07 (td, *J* = 7.7, 0.9 Hz, 1H, H_{A30}), 6.44 (dd, *J* = 16.9, 1.1 Hz, 1H, H_{A35}), 6.30 (dd, *J* = 16.9, 10.2 Hz, 1H, H_{A34}), 5.81 (dd, *J* = 10.2, 1.1 Hz, 1H, H_{A35}), 3.54 (s, 1H, H_{A25}), for A the most abundant rotamer. Also, 7.63 (dd, *J* = 7.4, 2.0 Hz, 1H, H_{A'}), 7.47 (d, *J* = 7.8 Hz, H_{A'}), 7.44 (dd, *J* = 4.5, 1.8 Hz, 1H, H_{A'}), 7.42 (t, *J* = 1.6 Hz, 1H, H_{A'}), 7.21 (dd, *J* = 7.8, 1.6 Hz, 1H, H_{A'}), 6.51 (d, *J* = 6.9 Hz, 1H, H_{A35'}), 6.50 (d, *J* = 2.5 Hz, 1H, H_{A35'}), 5.76 (dd, *J* = 6.9, 2.5 Hz, 1H, H_{A34'}), 3.23 (s, 1H, H_{A25'}), for A' the least abundant rotamer. **¹³C NMR (100 MHz, CDCl₃)** δ 167.7 (C₃₃), 163.4 (C₃₃), 139.9, 139.4, 133.7, 132.2, 131.3, 130.3, 130.3, 130.1, 130.1, 129.7, 129.0, 127.9, 123.5, 122.4, 119.4, 110.8, 84.6 (C_{24/25}), 83.2 (C_{24/25}), 79.2 (C_{24/25}), 79.0 (C_{24/25}), for both rotamers. **HRMS:** calcd for C₁₁H₁₀NO⁺ [M+H]⁺, 172.0762, found 172.0764.

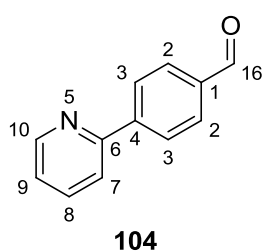
Note: This reaction was carried out one other time, using 4.9 equiv of acryloyl chloride and TEA. It was done in 24 h and a yield of 76% was obtained.

9.3.2 Synthesis of the truncated western moieties

9.3.2.1 4-(Pyridin-2-yl)benzaldehyde - 104

A two-neck round-bottomed flask was degassed and charged with degassed 1,4-dioxane (20 mL) and distilled H₂O (5 mL) followed by (4-formylphenyl)boronic acid **103** (0.359 g, 2.40 mmol), K₂CO₃ (2 equiv, 0.663 g, 4.80 mmol) and 2-bromopyridine **102** (6.6 equiv, 1.5 mL, 2.49 g, 15.7 mmol). The reaction mixture was degassed and Pd(PPh₃)₄ (3 mol%, 0.095 g, 0.082 mmol) was added to it. It was then heated at 110 °C for 20 min and then 105 °C for 7 h. During this time the reaction mixture

underwent colour changes from a milky pale yellow to a clear pale yellow to an intense yellow and finally to orange. The reaction was allowed to cool to RT and then diluted with distilled H₂O which turned the reaction mixture a bright red colour. The product was extracted with DCM (4 × 200 mL) and the combined organic layers were dried over MgSO₄ and filtered. The solvent was removed *in vacuo* to yield the crude product which was purified by CC (10% EtOAc/Hexane 20% EtOAc/Hexane) to afford the title compound **104** (0.384 g, 2.10 mmol, 88%) (R_f = 0.49, 40% EtOAc/Hexane) as an off-white solid.

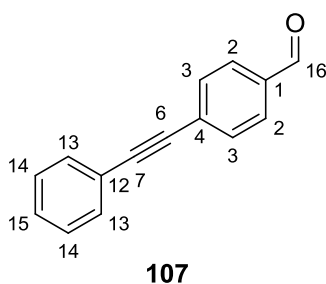


Mp 55-57 °C. **¹H NMR (300 MHz, CDCl₃)** δ 10.09 (s, 1H, H₁₆), 8.75 (dt, *J* = 4.8, 1.4 Hz, H₁₀), 8.18 (d, *J* = 8.4 Hz, 2H, H₃), 7.99 (d, *J* = 8.4 Hz, 2H, H₂), 7.83 – 7.79 (m, 2H, H₇ & H₈), 7.35 – 7.27 (m, 1H, H₉). **¹³C NMR (75 MHz, CDCl₃)** δ 192.0 (C₁₆), 155.9, 150.0, 144.9, 137.0, 136.4, 130.1, 127.4, 123.1, 121.2.

Note: This reaction was repeated twice, using the correct ratios of starting material as reported in the literature procedure.¹⁶¹ An average yield of 74% was obtained.

9.3.2.2 4-(Phenylethynyl)benzaldehyde - **107**

A Schlenk tube was charged with TEA (20 mL), PdCl₂(PPh₃)₂ (4 mol%, 0.071 g, 0.10 mmol) and CuI (2 mol%, 11 mg, 0.059 mmol). The reaction mixture was then stirred for 2 min, charged with 4-bromobenzaldehyde **106** (0.454 g, 2.51 mmol), stirred another 2 min and lastly charged with phenylacetylene **105** (2.0 equiv, 0.55 mL, 0.51 g, 5.0 mmol). The reaction went from yellow to dark brown moments after the addition of phenylacetylene. The reaction was stirred at 65 °C for 14 h. The reaction was allowed to cool to RT, quenched with saturated aqueous NH₄Cl and extracted with DCM (5 × 150 mL). The combined organic layers were washed with brine, dried over MgSO₄ and filtered. The solvent was removed *in vacuo* to yield the crude product which was purified by CC (2.5% then 5% EtOAc/Hexane) to afford still impure title compound **107** which was then further purified by a second round of CC (Hexane then 1.25% EtOAc/Hexane) to afford the title compound **107** (0.479 g, 2.32 mmol, 93%) (R_f = 0.44, 10% EtOAc/Hexane) as a yellow solid.



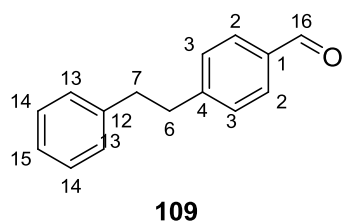
Mp 96-99 °C. **¹H NMR (300 MHz, CDCl₃)** δ 10.03 (s, 1H, H₁₆), 7.90 – 7.85 (m, 2H, H₂), 7.72 – 7.66 (m, 2H, H₃), 7.61 – 7.53 (m, 2H, H₁₃), 7.42 – 7.36 (m, 3H, H₁₄ & H₁₅). **¹³C NMR (75 MHz, CDCl₃)** δ 191.4 (C₁₆), 135.4, 132.1, 131.8, 129.6, 129.6, 129.0, 128.5, 122.5, 93.4, 88.5.

Note: This reaction was done in this manner a total of five times, with

an average yield of 82%. It was also repeated three times in a similar manner, but with the title compound purification by crystallisation. And average yield of 57% was obtained.

9.3.2.3 Attempted synthesis of (Z)-4-styrylbenzaldehyde - 108

A 50 mL two-neck round-bottomed flask was charged with 4-(phenylethynyl)benzaldehyde **107** (0.111 g, 0.538 mmol), Lindlar's catalyst (0.090 g/mmol, 0.049 g) and MeOH (5 mL). The flask was fitted with a hydrogen balloon and the reaction mixture was stirred at RT for 60 min. The reaction mixture was filtered through Celite and the solvent was removed *in vacuo* to yield a bright yellow oil. NMR analysis identified the crude material (0.117 g) as the overreduced compound, 4-phenethylbenzaldehyde **109** ($R_f = 0.41$, 10% EtOAc/Hexane).

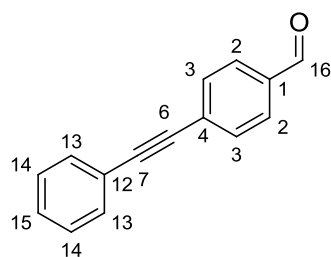


109

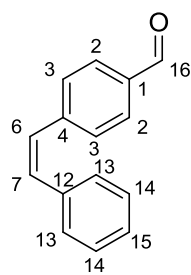
IR (ATR, cm^{-1}): 1696 (C=O str), 1604 (Ar C=C str), 1574, 1494, 1443 (Ar C=C str), 1305, 1212, 1167, 839, 813 (*para*-substituted Ar C-H oop bend), 742 (monosubstituted Ar C-H oop bend), 689 (monosubstituted Ar C-H oop bend). **$^1\text{H NMR}$ (300 MHz, CDCl_3) δ** 9.96 (s, 1H, H_{16}), 7.78 (d, $J = 8.2$ Hz, 2H, H_2), 7.33 – 7.11 (m, 7H, H_3 & H_{13} & H_{14} & H_{15}), 3.04 – 2.90 (m, 4H, H_6 & H_7).

9.3.2.4 Attempted synthesis of (Z)-4-styrylbenzaldehyde - 108

A 50 mL two-neck round-bottomed flask was charged with 4-(phenylethynyl)benzaldehyde **107** (0.049 g, 0.24 mmol), Lindlar's catalyst (0.040 g/mmol, 0.010 g) and MeOH (2.5 mL). The reaction mixture was cooled to 0 °C by means of an ice-bath and the flask was fitted with a hydrogen balloon. The reaction mixture was stirred at 0 °C for 30 min. The reaction mixture was filtered through Celite and the solvent was removed *in vacuo* to yield a bright yellow solid. NMR analysis identified the crude material (0.039 g) as a 5:6 mixture of the title compound **108** and 4-(phenylethynyl)benzaldehyde **107**.



107



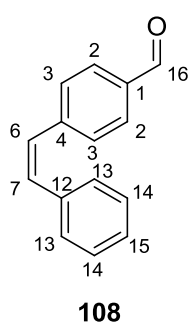
108

$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 10.03 (s, 1H, $\text{H}_{16(107)}$), 9.96 (s, 1H, $\text{H}_{16(108)}$), 7.88 (dt, $J = 8.1, 1.6$ Hz, 2H, $\text{H}_{2(107)}$), 7.74 (dt, $J = 8.1, 1.7$ Hz, 2H, $\text{H}_{2(108)}$), 7.69 (dt, $J = 8.1, 1.6$ Hz, 2H, $\text{H}_{3(107)}$), 7.60 – 7.54 (m, 2H, $\text{H}_{13(107)}$), 7.43 – 7.36 (m, 5H, $\text{H}_{3(108)}$ & $\text{H}_{14(107)}$ & $\text{H}_{15(107)}$), 7.26 – 7.22 (m, 5H, $\text{H}_{13(108)}$, $\text{H}_{14(108)}$, $\text{H}_{15(108)}$), 6.77 (d, $J = 12.3$ Hz, 1H, $\text{H}_{6/7(108)}$), 6.63 (d, $J = 12.3$ Hz, 1H, $\text{H}_{6/7(108)}$).

Note: This reaction was repeated testing several different reaction conditions, see Table 7.

9.3.2.5 (Z)-4-Styrylbenzaldehyde - 108

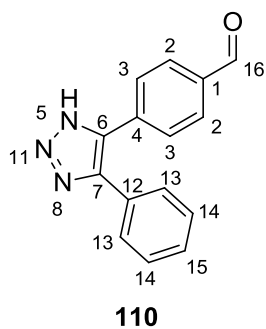
A 50 mL two-neck round-bottomed flask was charged with 4-(phenylethynyl)benzaldehyde **107** (0.190 g, 0.920 mmol) and MeOH (12 mL) and heated to dissolve the alkyne starting material. The reaction mixture was then cooled to 0 °C by means of an ice-bath and the starting material crashed out of solution. Lindlar's catalyst (0.040 g/mmol, 0.037 g) was added and the reaction was stirred at RT for 18 h. The reaction mixture was then cooled to 0 °C by means of an ice-bath and the flask was fitted with a hydrogen balloon. It was stirred at 0 °C for 45 min. The reaction mixture was filtered through Celite and the solvent was removed *in vacuo* to yield a yellow solid. NMR analysis identified the crude material (0.194 g) as predominantly the title compound **108** ($R_f = 0.47$, 10% EtOAc/Hexane) with trace amounts of starting material 4-(phenylethynyl)benzaldehyde **107** and 4-phenethylbenzaldehyde **109**.



¹H NMR (300 MHz, CDCl₃) δ 9.99 (s, 1H, H₁₆), 7.77 (dt, $J = 8.2, 1.7$ Hz, 2H, H₂), 7.43 (d, $J = 8.2$ Hz, 2H, H₃), 7.38 – 7.16 (m, 3H, H₁₃, H₁₄, H₁₅), 6.80 (d, $J = 12.3$ Hz, 1H, H_{6/7}), 6.65 (d, $J = 12.3$ Hz, 1H, H_{6/7}). **¹³C NMR (150 MHz, CDCl₃)** δ 191.7 (C₁₆), 143.7, 136.4, 132.9, 129.6, 129.4, 128.9, 128.8, 128.4, 127.6, 126.8.

9.3.2.6 4-(4-Phenyl-1H-1,2,3-triazol-5-yl)benzaldehyde - 110

A two-neck round-bottomed flask was charged with 4-(phenylethynyl)benzaldehyde **107** (0.840 g, 4.07 mmol), sodium azide (2.0 equiv, 0.532 g, 8.19) and DMSO (15 mL). The reaction was stirred at 80 °C for 5 days. The reaction mixture was diluted with H₂O and a few drops of 1 M HCl. The product was extracted with EtOAc (3 × 150 mL) and the combined organic layers were then dried over MgSO₄ and filtered. The solvent was removed *in vacuo* to yield the crude product which was purified by CC (30% EtOAc/Hexane) to afford the title compound **110** (0.84 g, 3.4 mmol, 83%) ($R_f = 0.50$, 50% EtOAc/Hexane) as a yellow solid.



Mp 172-174 °C. **IR (ATR, cm⁻¹):** 3178 (N-H str), 2988, 2165, 1665 (C=O str), 1605 (N-H bend), 1569, 1503, 1214 (C-N str), 1172, 981, 834 (*para*-substituted Ar C-H oop bend), 781 (monosubstituted Ar C-H oop bend), 703 (monosubstituted Ar C-H oop bend). **¹H NMR (600 MHz, CDCl₃)** δ 10.01 (s, 1H, H₁₆), 7.93 (d, *J* = 8.2 Hz, 2H, H₃), 7.73 (d, *J* = 8.2 Hz, 2H, H₂), 7.52 – 7.48 (m, 2H, H₁₃), 7.48 – 7.42 (m, 3H, H₁₄ & H₁₅). **¹³C NMR (125 MHz, CDCl₃)** δ 192.6 (C₁₆), 136.6, 135.5, 129.9, 128.8, 128.9, 128.2, 128.1, 1 aromatic carbon and C₆ & C₇ absent. **HRMS:** calcd for C₁₅H₁₂N₃O⁺ [M+H]⁺, 250.0980, found 250.0982.

Did reaction six times using DMSO as solvent, average yield of 69%. Once in DMF, similar conditions, yield of 72%.

9.4 EXPERIMENTAL WORK PERTAINING TO CHAPTER 5

9.4.1 Original strategy

9.4.1.1 *N*-{3-[1-(Piperidin-4-yl)-1*H*-1,2,3-triazol-4-yl]phenyl}acrylamide - 46

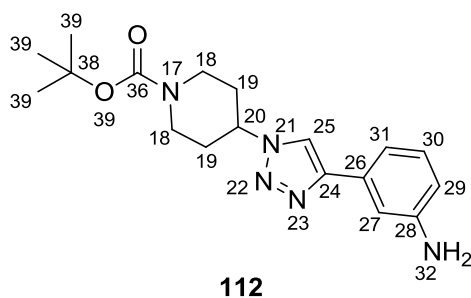
A 50 mL two-neck round-bottomed flask was charged with distilled H₂O (1 mL), *tert*-butanol (1 mL) and 4-azidopiperidine hydrochloride (0.074 g, 0.46 mmol). *N*-(3-ethynylphenyl)acrylamide **45** (1.2 equiv, 0.091 g, 0.53 mmol) was added to the yellow reaction mixture followed by CuSO₄·5H₂O (7 mol%, 8 mg, 0.030 mmol) and sodium ascorbate (11 mol%, 10 mg, 0.051 mmol). The reaction mixture was then stirred at 60 °C for 20 h. The reaction was allowed to cool to RT and quenched with a saturated solution of aqueous NH₄Cl. This was then neutralised with the addition of NaHCO₃. Extraction was done using DCM (3 × 100 mL) and the combined organic layers were washed with brine, dried over MgSO₄ and filtered. The solvent was removed *in vacuo* to yield the crude material which was identified as the starting material, *N*-(3-ethynylphenyl)acrylamide **45** (0.062 g, 69%).

9.4.2 First modified route towards the target compound(s)

9.4.2.1 *tert*-Butyl 4-[4-(3-aminophenyl)-1*H*-1,2,3-triazol-1-yl]piperidine-1-carboxylate - 112

A 50 mL two-neck round-bottomed flask was charged with distilled H₂O (2 mL), *tert*-butanol (2 mL) and *tert*-butyl 4-azidopiperidine-1-carboxylate **96** (0.279 g, 1.23 mmol). 3-Ethynylaniline **99** (1.2 equiv, 0.16 mL, 0.18 g, 1.5 mmol) was added to the colourless reaction mixture and it turned a milky white colour. CuSO₄·5H₂O (5 mol%, 16 mg, 0.064 mmol) and sodium ascorbate (11 mol%, 26 mg, 0.13 mmol) was added to the reaction mixture. The reaction mixture was then stirred at 60 °C for 20

h, turning progressively darker, first yellow, then orange and lastly dark brown. The reaction was allowed to cool to RT and quenched with a saturated solution of aqueous NH_4Cl . Extraction was done using DCM (3×100 mL) and the combined organic layers were dried over MgSO_4 and filtered. The solvent was removed *in vacuo* to yield the crude ‘triazole product’ which was purified by CC (20 to 50% EtOAc/Hexane followed by 1% MeOH/EtOAc) to afford still impure title compound **112** which was then further purified by a second round of CC (1% MeOH/EtOAc) to afford the title compound **112** (0.244 g, 0.711 mmol, 58%) ($R_f = 0.33$, 50% DCM/EtOAc) as a light orange solid.



Mp 171-172 °C. **IR (ATR, cm^{-1}):** 3423 (N-H str), 3353, 2928 (alkene C-H str), 2179, 1678 (C=O str), 1647, 1612 (Ar C=C str), 1433 (Ar C=C str), 1244 (C-N str), 1164 (C-O str), 1071, 1002, 782. **$^1\text{H NMR}$ (400 MHz, CDCl_3) δ** 7.72 (s, 1H, H_{25}), 7.28 (s, 1H, H_{27}), 7.20 (br t, $J = 7.5$ Hz, 1H, H_{30}), 7.14 (d, $J = 7.5$ Hz, 1H, H_{31}), 6.69 (d, $J = 7.5$ Hz, 1H, H_{29}), 4.69 – 4.58 (m, 1H, H_{20}), 4.28 (br s, 2 H, H_{18}),

3.53 (br s, 2 H, H_{32}), 2.95 (br t, $J = 11.1$ Hz, 2H, H_{18}), 2.22 (d, $J = 12.1$ Hz, 2H, H_{19}), 1.98 (m, $J = 12.1$, 3.8 Hz, 2H, H_{19}), 1.49 (s, 9H, H_{39}). **$^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ** 154.5 (C_{36}), 147.6, 146.4, 131.5, 129.8, 117.4, 116.2, 115.2, 112.4, 80.1 (C_{38}), 58.1 (C_{20}), 42.5 (C_{18}), 31.4 (C_{19}), 28.4 (C_{39}). **HRMS:** calcd for $\text{C}_{18}\text{H}_{26}\text{N}_5\text{O}_2^+$ [$\text{M}+\text{H}$] $^+$, 344.2087, found 344.2095.

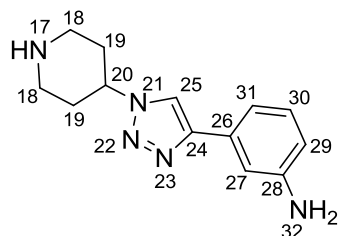
9.4.2.2 *tert*-Butyl 4-[4-(3-aminophenyl)-1*H*-1,2,3-triazol-1-yl]piperidine-1-carboxylate - **112**

A 50 mL one-neck round-bottomed flask was charged with *tert*-butyl 4-azidopiperidine-1-carboxylate **96** (0.366 g, 1.62 mmol) and MeCN (5 mL) and stirring at RT commenced. 3-Ethynylaniline **99** (1.1 equiv, 0.16 mL, 0.18 g, 1.5 mmol), *N,N*-diisopropylethylamine (Hünig's base) (2 equiv, 0.48 mL, 0.36 g, 2.8 mmol) and CuI (10 mol%, 26 mg, 0.14 mmol) was added in this order and the reaction mixture was stirred at RT for 18 h. Saturated aqueous NH_4Cl was added to the reaction mixture and the product was extracted with DCM (3×100 mL). The combined organic layers were dried over MgSO_4 and filtered. The solvent was removed *in vacuo* to yield the crude product which was purified by CC (50% EtOAc/DCM then 1% MeOH/EtOAc) to afford the title compound **112** (0.496 g, 1.44 mmol, 94%) ($R_f = 0.33$, 50% DCM/EtOAc) as a light orange solid.

9.4.2.3 3-[1-(Piperidin-4-yl)-1*H*-1,2,3-triazol-4-yl]aniline - **113**

A 50 mL two-neck round-bottomed flask was charged with *tert*-butyl 4-[4-(3-aminophenyl)-1*H*-1,2,3-triazol-1-yl]piperidine-1-carboxylate **112** (0.210 g, 0.612 mmol) and DCM (1.2 mL). The reaction mixture was cooled to 0 °C by means of an ice-bath and TFA (8 equiv, 0.38 mL, 0.56 g, 4.9 mmol)

was added dropwise to the cooled reaction mixture. After addition of the TFA, the reaction mixture was stirred at RT for 16 h. The solvent was removed *in vacuo* to yield the crude deprotected triazole salt which was then neutralised with saturated aqueous NaHCO₃ and extracted using CHCl₃. The CHCl₃ was removed *in vacuo* to afford the crude title compound **113** (0.076 g, 0.31 mmol, 51%) (R_f = 0.21, 10% MeOH/DCM with 2.5% TEA) as a thick white oil.



113

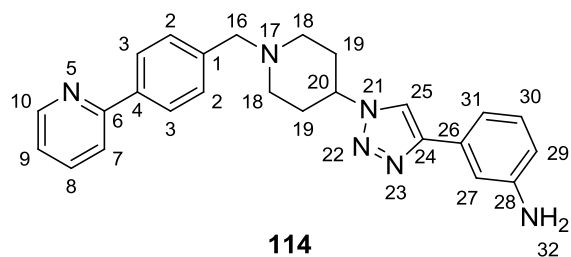
IR (ATR, cm⁻¹): 3353 (N-H str), 3146, 2942 (alkene C-H str), 2361, 2161, 1606 (Ar C=C str), 1586, 1488 (Ar C=C str), 1450, 776. **¹H NMR (300 MHz, MeOD-d₄)** δ 8.26 (s, 1H, H₂₅), 7.18 (br t, *J* = 1.6 Hz, 1H, H₂₇), 7.15 (dd, *J* = 7.6, 0.52 Hz, 1H, H₃₁), 7.11 (dt, *J* = 7.6, 1.5 Hz, 1H, H₃₀), 6.71 (ddd, *J* = 7.6, 2.3, 1.6 Hz, 1H, H₂₉), 4.71 – 4.59 (m, 1H, H₂₀), 3.21 (br td, *J* = 13.2, 3.0 Hz, 2H, H₁₈), 2.80 (dt, *J* = 12.5, 2.5 Hz,

2H, H₁₈), 2.20 (br d, *J* = 12.5 Hz, 2H, H₁₉), 2.00 (m, *J* = 12.5, 4.2 Hz, 2H, H₁₉).

Note: This reaction was also carried out without neutralising the reaction mixture, thus isolating the crude trifluoroacetate salt, 4-[4-(3-aminophenyl)-1H-1,2,3-triazol-1-yl]piperidin-1-ium 2,2,2-trifluoroacetate **113**.

9.4.2.4 3-(1-{1-[4-(Pyridin-2-yl)benzyl]piperidin-4-yl}-1H-1,2,3-triazol-4-yl)aniline - **114**

A 50 mL one-neck round-bottomed flask was charged with 3-[1-(piperidin-4-yl)-1H-1,2,3-triazol-4-yl]aniline **113** (0.066 g, 0.27 mmol), 4-(pyridin-2-yl)benzaldehyde **104** (1.1 equiv, 0.055 g, 0.30 mmol), MgSO₄ (0.6 equiv, 0.020 g, 0.17 mmol), *N,N*-diisopropylethylamine (3.2 equiv, 0.15 mL, 0.11 g, 0.86 mmol) and THF (6.5 mL). MeOH (2 mL) was added to the milky yellow reaction mixture. The reaction turned a clear yellow as its contents dissolved and NaBH(OAc)₃ (2.1 equiv, 0.118 g, 0.554 mmol) was subsequently added. The reaction mixture was stirred for 4 days at RT and a white precipitate became visible over time. The solvent was removed *in vacuo* and the resulting yellow precipitate was diluted with saturated solution of aqueous NaHCO₃. Extraction was done using DCM (3 × 100 mL) and the combined organic layers were dried over MgSO₄ and filtered after which the solvent was removed *in vacuo* to afford the crude product which was purified by CC (5% MeOH/DCM with 1% TEA) to afford still impure title compound **114** which was then further purified by a second round of CC (40% EtOAc/Hexane then 60% EtOAc/Hexane with 6% TEA) to afford what was perceived as the title compound **114** (14 mg, 0.34 mmol, ~13%) (R_f = 0.22, 60% EtOAc/Hexane) as a pale yellow solid, with the presence of a contaminant, according to ¹H NMR spectroscopy.



114

If Product **114**: $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 8.72 (ddt, $J = 13.2, 4.9, 1.4$ Hz, 2H, H_{10}), 8.61 (s, 1H, H_{25}), 8.14 (d, $J = 8.5$ Hz, 2H, H_3), 8.03 (d, $J = 8.5$ Hz, 2H, H_2), 7.97 (d, $J = 8.2$ Hz, 2H, H_7 & H_8), 7.85 (s, 1H, H_{27}), 7.82 – 7.70 (m, 7H), 7.50 – 7.43 (m, 3H), 7.30 – 7.20 (m, 4H), 4.63 – 4.50 (m, 1H,

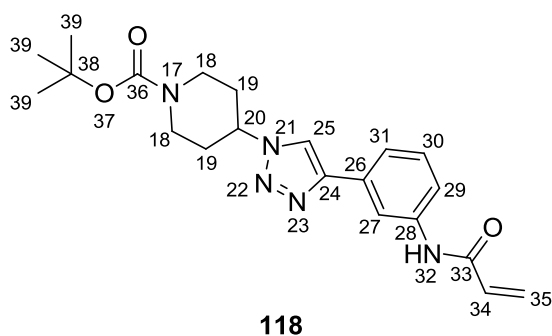
H_{20}), 3.64 (s, 2H, H_{16}), 3.08 (d, $J = 10.7$ Hz, 2H, H_{18}), 2.64 – 2.46 (m, 2H, H_{18}), 2.42 – 2.04 (m, 8H, H_{19} & impurity).

Note: This reaction was repeated using the trifluoroacetate salt, 4-[4-(3-aminophenyl)-1*H*-1,2,3-triazol-1-yl]piperidin-1-ium 2,2,2-trifluoroacetate, with 4 molar equiv of triacetoxyborohydride and MeOH as solvent. A mixture of product **114** and aldehyde **104** was isolated, accounting for less than 20% of **104** used. This route was abandoned and further analysis was not done to ascertain if either of these reactions gave the product **114** or a side-product, with the reductive amination taking place at the aniline amine.

9.4.3 A breakthrough

9.4.3.1 *tert*-Butyl 4-[4-(3-acrylamidophenyl)-1*H*-1,2,3-triazol-1-yl]piperidine-1-carboxylate - **118**

A 50 mL two-neck round-bottomed flask was charged with distilled H_2O (1 mL), *tert*-butanol (1 mL) and *tert*-butyl 4-azidopiperidine-1-carboxylate **96** (0.101 g, 0.446 mmol). *N*-(3-ethynylphenyl)acrylamide **45** (0.8 equiv, 0.060 g, 0.35 mmol), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (6 mol%, 6 mg, 0.026 mmol) and sodium ascorbate (11 mol%, 10 mg, 0.051 mmol) was added to the colourless reaction mixture which then turned a bright yellow colour. The reaction mixture was stirred at 60 °C for 24 h, turning a pale yellow over time. More distilled H_2O (1 mL), *tert*-butanol (1 mL) was added once as the reaction progressed. The reaction was allowed to cool to RT and quenched with ice H_2O and 25% aqueous ammonia. The resulting yellow precipitate was diluted with H_2O and extraction was done using DCM (3 × 100 mL). The combined organic layers were dried over MgSO_4 and filtered after which the solvent was removed *in vacuo* to yield the crude ‘triazole product’ which was purified by CC (60 - 100% EtOAc/Hexane) to afford the title compound **118** (0.070 g, 0.18 mmol, 50%) ($R_f = 0.38$, 60% EtOAc/Hex) as a thick colourless oil.

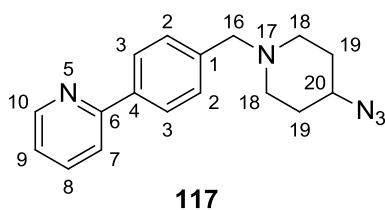


IR (ATR, cm⁻¹): 3065, 2929 (alkene C-H str), 2160, 1693 (ester C=O str), 1663 (amide C=O str), 1589 (alkene C=C str), 1537 (Ar C=C str), 1442 (Ar C=C str), 1423, 1246 (C-N str), 1156 (C-O str), 1004, 938, 791. **¹H NMR (300 MHz, CDCl₃)** δ 8.84 (s, 1H, H₃₂), 8.10 (s, 1H, H₂₇), 7.73 (s, 1H, H₂₅), 7.57 (t, *J* = 8.7 Hz, 2H, H₂₉ & H₃₁), 7.29 (t, *J* = 7.9 Hz, 1H, H₃₀), 6.40 (br s, 1H, H₃₅), 6.38 (d, *J* = 4.2 Hz, 1H,

H₃₅), 5.68 (dd, *J* = 7.8, 4.2 Hz, 1H, H₃₄), 4.55 (tt, *J* = 11.7, 4.0 Hz, 1H, H₂₀), 4.22 (br d, *J* = 11.8 Hz, 2H, H₁₈), 2.89 (br t, *J* = 12.4 Hz, 2H, H₁₈), 2.13 (br dd, *J* = 12.4, 2.3 Hz, 2H, H₁₉), 1.89 (m, *J* = 11.8, 3.9 Hz, 2H, H₁₉), 1.47 (s, 9H, H₃₉). Acrylamide protons more clear in **¹H NMR (300 MHz, DMSO)**: 6.46 (dd, *J* = 16.9, 10.0 Hz, 1H, H₃₄), 6.27 (dd, *J* = 16.9, 2.1 Hz, 1H, H₃₅), 5.77 (dd, *J* = 10.0, 2.1 Hz, 1H, H₃₅). **¹³C NMR (75 MHz, CDCl₃)** δ 164.1 (C₃₃), 154.4 (C₃₆), 147.1, 138.7, 131.2, 131.1, 129.4, 127.6, 121.4, 119.8, 118.0, 117.1, 80.2 (C₃₈), 58.1 (C₂₀), 42.5 (C₁₈), 32.2 (C₁₉), 28.3 (C₃₉). **HRMS:** calcd for C₂₁H₂₈N₅O₃⁺ [M+H]⁺, 398.2192, found 398.2193.

9.4.3.2 2-{4-[(4-Azidopiperidin-1-yl)methyl]phenyl}pyridine - 117

A Schlenk tube was charged with 4-(pyridin-2-yl)benzaldehyde **104** (0.5 equiv, 0.096 g, 0.52 mmol) as well as 4-azidopiperidin-1-ium 2,2,2-trifluoroacetate **98** (0.237 g, 0.987 mmol) which was dissolved in DCM (1 mL). More DCM (7 mL) was added to the pale yellow reaction mixture and it was stirred at RT for 47 h after which NaBH(OAc)₃ (1.1 equiv, 0.224 g, 1.06 mmol) was added in small portions over 1 h. The reaction mixture was stirred for another 6 days and more 4-(pyridin-2-yl)benzaldehyde **104** (0.4 equiv, 0.079 g, 0.43 mmol) and NaBH(OAc)₃ (1.1 equiv, 0.222g, 1.05 mmol) was added after the third day and the reaction mixture turned a brown colour over time. The reaction was quenched with a saturated solution of aqueous NaHCO₃ and the product was extracted with DCM (3 × 150 mL). The combined organic layers were dried over MgSO₄ and filtered. The solvent was removed *in vacuo* to yield the crude product which was purified by CC (20% EtOAc/Hexane with 2% TEA then 60% EtOAc/Hexane with 2% TEA) to afford the title compound **117** (0.114 g, 0.389 mmol, 41%) (R_f = 0.28, 20% EtOAc/Hexane with 1% TEA) as a yellow solid.



Mp 58-59 °C. **IR (ATR, cm⁻¹):** 2950, 2928, 2801, 2761, 2088 (N=N=N str), 1585 (Ar C=C str), 1463 (-CH₂- bend), 1434 (Ar C=C str), 1254 (C-N str), 769. **¹H NMR (300 MHz, CDCl₃)** δ 8.69 (dt, *J* = 4.6, 1.4 Hz, 1H, H₁₀), 7.95 (d, *J* = 8.5 Hz, 2H, H₃), 7.76 – 7.72 (m, 2H, H₇ & H₈), 7.42 (d, *J* = 8.5 Hz, H₂), 7.22 (ddd, *J* = 6.8, 4.6, 1.9 Hz, 1H, H₉), 3.56 (s, 2H, H₁₆), 3.47 – 3.33 (m, 1H, H₂₀), 2.85 – 2.74 (m, 2H, H₁₈),

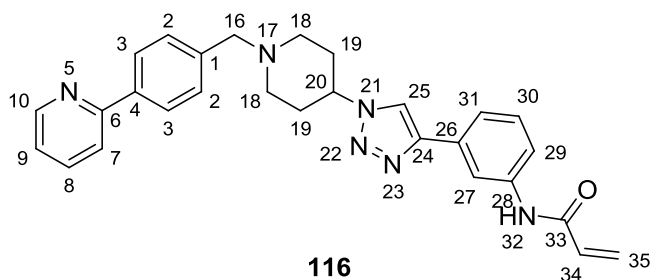
2.25 – 2.14 (m, 2H, H₁₈), 1.96 – 1.85 (m, 2H, H₁₉), 1.76 – 1.62 (m, 2H, H₁₉). ¹³C NMR (75 MHz, CDCl₃) δ 157.3, 149.7, 139.2, 138.3, 136.7, 130.2, 129.4, 127, 5, 126.8, 122.0, 120.4, 62.6 (C₁₆), 57.8 (C₂₀), 51.2 (C₁₈), 30.9 (C₁₉). HRMS: calcd for C₁₇H₂₀N₅⁺ [M+H]⁺, 294.1719, found 294.1714.

9.4.3.3 2-{4-[(4-Azidopiperidin-1-yl)methyl]phenyl}pyridine - 117

A Schlenk tube was charged with 4-azidopiperidin-1-ium 2,2,2-trifluoroacetate **98** (0.084 g, 0.348 mmol) and 4-(pyridin-2-yl)benzaldehyde **104** (2.1 equiv, 0.133 g, 0.727 mmol) in MeOH (1.5 mL). The pale yellow reaction mixture was cooled to 0 °C by means of an ice-bath and NaCNBH₃ (2 equiv, 0.045 g, 0.723 mmol) was added to the cooled reaction mixture which was then stirred at RT for 3 days. The reaction was diluted with H₂O and the product was extracted with DCM (3 × 150 mL). The combined organic layers were dried over MgSO₄ and filtered. The solvent was removed *in vacuo* to yield the crude product which was purified by CC (20% EtOAc/Hexane with 2% TEA then 60% EtOAc/Hexane with 2% TEA) to afford the title compound **117** (37 mg, 0.124 mmol, 36%) (R_f = 0.28, 20% EtOAc/Hexane with 1% TEA) as a yellow solid.

9.4.3.4 N-[3-(1-{1-[4-(Pyridin-2-yl)benzyl]piperidin-4-yl}-1H-1,2,3-triazol-4-yl)phenyl]acrylamide - 116

A 50 mL one-neck round-bottomed flask was charged with 2-{4-[(4-azidopiperidin-1-yl)methyl]phenyl}pyridine **117** (0.088 g, 0.30 mmol), N-(3-ethynylphenyl)acrylamide **45** (1.1 equiv, 0.057 g, 0.33 mmol) and MeCN (2 mL) and stirring at RT commenced. N,N-Diisopropylethylamine (1.9 equiv, 0.10 mL, 0.074 g, 0.58 mmol) and CuI (15 mol%, 9 mg, 0.05 mmol) was then added and the reaction was stirred at RT for 24 h. The reaction mixture was diluted with DCM and washed with saturated aqueous NH₄Cl (3 × 50 mL). The organic layer was then dried over MgSO₄ and filtered. The solvent was removed *in vacuo* to yield the crude product which was purified by CC (60% EtOAc/Hexane then 5% MeOH/EtOAc) to afford still impure title compound **116** which was then further purified by a second round of CC (2% MeOH/EtOAc) to afford the title compound **116** (0.080 g, 0.17 mmol, 58%) (R_f = 0.23, 60% EtOAc/Hexane) as a white solid.



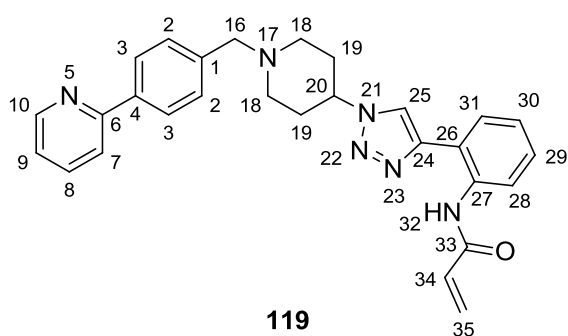
Mp 161-163 °C. **IR** (ATR, cm⁻¹): 3275, 3120 (Ar C-H str), 3080 (alkene C-H str) 2946, 2794, 2360, 1663 (C=O str), 1614 (Ar C=C str), 1587, 1535, 1467 (Ar C=C str), 1435 (-CH₂- bend), 1403, 791, 769. **¹H NMR** (300 MHz, CDCl₃) δ 8.69 (dt, *J* =

5.0, 1.3 Hz, 1H, H₁₀) 8.09 – 8.02 (m, 1H, H₂₇), 7.96 (dt, *J* = 8.5, 1.8 Hz, 2H, H₃), 7.80 (s, 1H, H₂₅),

7.77 – 7.73 (m, 2H, H₇ & H₈), 7.65 – 7.57 (m, 2H, H₂₉ & H₃₁), 7.45 (d, *J* = 8.5 Hz, 2H, H₂), 7.35 (t, *J* = 7.9 Hz, 1H, H₃₀), 7.23 (ddd, *J* = 6.8, 4.3, 1.9 Hz, 1H, H₉), 6.44 (dd, *J* = 16.9, 1.7 Hz, 1H, H₃₅), 6.30 (dd, *J* = 16.9, 9.9 Hz, 1H, H₃₄), 5.74 (dd, *J* = 9.9, 1.7 Hz, 1H, H₃₅), 4.56 – 4.44 (m, 1H, H₂₀), 3.62 (s, 2H, H₁₆), 3.05 (d, *J* = 11.6 Hz, 2H, H₁₈), 2.29 – 2.06 (m, 6H, H₁₈ & H₁₉). ¹³C NMR (75 MHz, CDCl₃) δ 163.8 (C₃₃), 157.2 (C₆), 149.6 (C₁₀), 147.1 (C₂₄), 138.9 (C₂₈), 138.4 (C₄), 138.4 (C₁), 136.8 (C₈), 131.4 (C₂₆), 131.1 (C₃₄), 129.5 (C₃₀), 129.4 (C₂), 127.8 (C₃₅), 126.9 (C₃), 122.1 (C₉), 121.6 (C₂₉), 120.5 (C₇), 119.5 (C₃₁), 117.7 (C₂₅), 117.0 (C₂₇), 62.3 (C₁₆), 58.4 (C₂₀), 52.1 (C₁₈), 32.6 (C₁₉). HRMS: calcd for C₂₈H₂₉N₆O⁺ [M+H]⁺, 465.2403, found 465.2406.

9.4.3.5 *N*-[2-(1-{1-[4-(Pyridin-2-yl)benzyl]piperidin-4-yl}-1*H*-1,2,3-triazol-4-yl)phenyl]acrylamide - **119**

A 5 mL one-neck round-bottomed flask was charged with 2-{4-[(4-azidopiperidin-1-yl)methyl]phenyl}pyridine **117** (0.037 g, 0.12 mmol), *N*-(2-ethynylphenyl)acrylamide **101** (1.2 equiv, 0.025 g, 0.14 mmol) and MeCN (1 mL) and stirring at RT commenced. TEA (2.9 equiv, 0.05 mL, 0.04 g, 0.4 mmol) and CuI (20 mol%, 4 mg, 0.03 mmol) was then added and the reaction was stirred at RT for 29 h. The reaction mixture was diluted with DCM and washed with saturated aqueous NH₄Cl (3 × 50 mL). The organic layer was then dried over MgSO₄ and filtered. The solvent was removed *in vacuo* to yield the crude product which was purified by CC (60% EtOAc/Hexane then 5% MeOH/EtOAc) to afford the title compound **119** (0.028 g, 0.060 mmol, 48%) (R_f = 0.10, 80% EtOAc/Hexane) as a white solid. The ¹H NMR spectrum indicated the presence of a small amount of contamination.



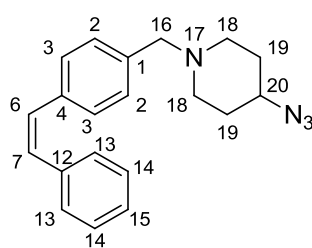
Mp 156 °C. **IR** (ATR, cm⁻¹): 3127 (Ar C-H str), 2958, 2820, 2361, 1681 (C=O str), 1617 (Ar C=C str), 1586, 1555, 1453 (Ar C=C str), 1400, 1374, 1327, 1257, 1079, 772, 752. ¹H NMR (600 MHz, CDCl₃) δ 8.77 (d, *J* = 7.9 Hz, 1H, H₃₁), 8.71 (d, *J* = 4.1 Hz, 1H, H₁₀), 7.98 (d, *J* = 8.2 Hz, 2H, H₃), 7.90 (s, 1H, H₂₅), 7.79 – 7.73 (m, 2H, H₇ & H₈), 7.49

(dd, *J* = 7.8, 1.2 Hz, 1H, H₂₈), 7.47 (d, *J* = 8.2 Hz, 2H, H₂), 7.36 (t, *J* = 7.8 Hz, 1H, H₂₉), 7.25 (ddd, *J* = 6.7, 5.0, 1.8 Hz, 1H, H₉), 7.12 (td, *J* = 7.9, 1.2 Hz, 1H, H₃₀), 6.47 (dd, *J* = 17.0, 1.8 Hz, 1H, H₃₅), 6.42 (dd, *J* = 17.0, 9.4 Hz, 1H, H₃₄), 5.77 (dd, *J* = 9.4, 1.8 Hz, 1H, H₃₅), 4.60 – 4.53 (m, 1H, H₂₀), 3.66 (s, 2H, H₁₆) 3.11 (br d, *J* = 11.7 Hz, 2H, H₁₈), 2.33 – 2.24 (m, 5H, H₁₈ & H₁₉ & impurity), 2.24 – 2.16 (m, 2H, H₁₉), H₃₂ absent. ¹³C NMR (125 MHz, CDCl₃) δ 164.3 (C₃₃), 157.2 (C₆), 149.7 (C₁₀), 147.5 (C₂₄), 138.9 (C₁), 138.6, 136.8 (C₄), 136.6 (C₈), 132.8, 129.4 (C₂), 129.1 (C₃₄), 127.0 (C₃), 126.8 (C₃₅),

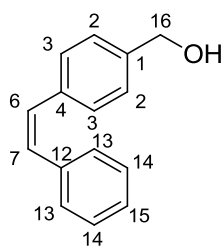
126.6, 123.5, 122.1 (C₉), 121.5 (C₂₆), 120.5 (C₇), 118.6, 117.5 (C₂₅), 62.4 (C₁₆), 58.9 (C₂₀), 52.1 (C₁₈), 32.7 (C₁₉). **HRMS:** calcd for C₂₈H₂₉N₆O⁺ [M+H]⁺, 465.2403, found 465.2408.

9.4.3.6 (Z)-4-Azido-1-(4-styrylbenzyl)piperidine - 120

A 50 mL two-neck round-bottomed flask was charged with (Z)-4-styrylbenzaldehyde **108** (0.094 g, 0.45 mmol) dissolved in THF (1 mL) and 4-azidopiperidin-1-ium 2,2,2-trifluoroacetate **98** (1.3 equiv, 0.136 g, 0.568 mmol) also dissolved in THF (0.95 mL). More THF (7 mL) was added and the reaction mixture was stirred for a few min followed by portionwise addition of NaCNBH₃ (3.3 equiv, 0.095 g, 1.5 mmol). It was stirred at RT for 43 h. The reaction mixture was diluted with H₂O and the pH was increased to approximately 7 using a saturated solution of aqueous NaHCO₃ and monitoring it with universal indicator paper. The product was extracted with DCM (3 × 150 mL). The combined organic layers were dried over MgSO₄ and filtered. The solvent was removed *in vacuo* to yield the crude product which was purified by CC (20% EtOAc/Hexane) to afford a mixture of the title compound **120** and side-product **121** (0.071 g) (For mixture, R_f ~ 0.28, 20% EtOAc/Hexane). ¹H NMR spectroscopy confirmed the presence of both title compound **120** and the reduced aldehyde, (Z)-(4-styrylphenyl)methanol **121**.



120



121

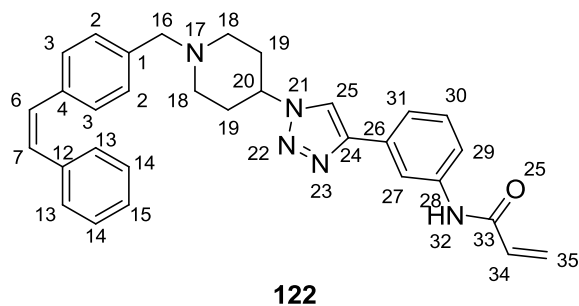
IR (ATR, cm⁻¹): 3357, 3019, 2938, 2091 (N=N=N str), 1770, 1510, 1492, 1446 (-CH₂-bend), 1418, 1366, 1256 (C-N str), 1032, 920, 874, 820 (*para*-substituted Ar C-H oop bend), 773 (monosubstituted Ar C-H oop bend), 695 (monosubstituted Ar C-H oop bend). **¹H NMR**

(300 MHz, CDCl₃) δ 7.30 – 7.12 (m, H_{Ar(120)} & H_{Ar(121)}), 6.59 (d, *J* = 1.5 Hz, 2H, H₆₍₁₂₁₎ & H₇₍₁₂₁₎), 6.57 (d, *J* = 0.9 Hz, 2H, H₆₍₁₂₀₎ & H₇₍₁₂₀₎), 4.63 (s, 2H, H₁₆₍₁₂₁₎), 3.45(s, 2H, H₁₆₍₁₂₀₎), 3.43 – 3.32 (m, 1H, H₂₀₍₁₂₀₎), 2.79 – 2.67 (m, 2H, H₁₈₍₁₂₀₎), remaining H₁₈₍₁₂₀₎ and H₁₉₍₁₂₀₎ obscured by aliphatic impurities. **HRMS:** calcd for C₂₀H₂₃N₄⁺ [M+H]⁺, 319.1923, found 319.1930.

9.4.3.7 (Z)-N-(3-{1-[1-(4-Styrylbenzyl)piperidin-4-yl]-1H-1,2,3-triazol-4-yl}phenyl)acrylamide - 122

A 50 mL one-neck round-bottomed flask was charged with a mixture of (Z)-4-azido-1-(4-styrylbenzyl)piperidine **120** and (Z)-(4-styrylphenyl)methanol **121** (0.071 g), MeCN (1.5 mL) and *N*-(3-ethynylphenyl)acrylamide **45** (1.1 equiv, 0.042 g, 0.25 mmol) and stirring at RT commenced. *N,N*-Diisopropylethylamine (2.1 equiv, 0.080 mL, 0.060 g, 0.46 mmol) and CuI (21 mol%, 9 mg, 0.05 mmol) was then added and the reaction was stirred at RT for 22 h. The reaction mixture was diluted

with DCM and washed with saturated aqueous NH_4Cl (3×50 mL) which was then extracted with one portion of EtOAc. The combined organic layers were then dried over MgSO_4 and filtered. The solvent was removed *in vacuo* to yield the crude product which was purified by CC (40% then 60% EtOAc/Hexane) to afford still impure title compound **122** which was then further purified by a second round of CC (60% EtOAc/Hexane) to afford the title compound **122** (0.014 g, 0.028 mmol, 6% over two steps) ($R_f = 0.32$, 60% EtOAc/Hexane) as a pale yellow solid.



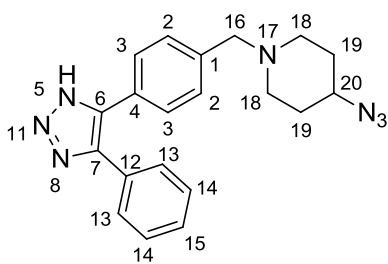
Mp <140 °C. **IR (ATR, cm^{-1}):** 3272, 3065 (Ar C-H str), 3025 (alkene C-H str), 2949, 2767, 2356, 1663 (C=O str), 1590, 1537, 1482 (Ar C=C str), 1443 (-CH₂- bend), 1421, 1253 (C-N str), 784, 691. **¹H NMR (300 MHz, CDCl_3)** δ 8.07 (br s, 1H, H₂₇), 7.82 (s, 1H, H₂₅), 7.62 (dd, $J = 7.6, 1.2$ Hz, 2H, H₃), 7.57 (br s, 1H, H₃₂), 7.39 (t, $J = 7.9$

Hz, 1H, H₃₀), 7.31 – 7.16 (m, 9H, H₂ & H₁₃ & H₁₄ & H₁₅ & H₂₉ & H₃₁), 6.60 (s, 2H, H₆ & H₇), 6.46 (dd, $J = 16.9, 1.5$ Hz, 1H, H₃₅), 6.28 (dd, $J = 16.9, 10.1$ Hz, 1H, H₃₄), 5.79 (dd, $J = 10.1, 1.5$ Hz, 1H, H₃₅), 4.59 – 4.46 (m, 1H, H₂₀), 3.54 (s, 2H, H₁₆), 3.04 (d, $J = 10.7$ Hz, 2H, H₁₈), 2.28 – 2.03 (m, 6H, H₁₈ & H₁₉). **¹³C NMR (100 MHz, CDCl_3)** δ 163.6 (C₃₃), 147.1 (C₂₄), 138.3 (C₂₈), 137.3, 136.9, 136.2, 131.5 (C₂₆), 131.1 (C₃₄), 130.2, 129.9, 129.6 (C₃₀), 128.9, 128.8, 128.8, 128.2, 127.9 (C₃₅), 127.1, 121.7 (C₂₉), 119.4 (C₃₁), 117.6 (C₂₅), 116.9 (C₂₇), 62.4 (C₁₆), 58.4 (C₂₀), 52.1 (C₁₈), 32.7 (C₁₉). **HRMS:** calcd for $\text{C}_{31}\text{H}_{32}\text{N}_5\text{O}^+$ $[\text{M}+\text{H}]^+$, 490.2607, found 490.2603.

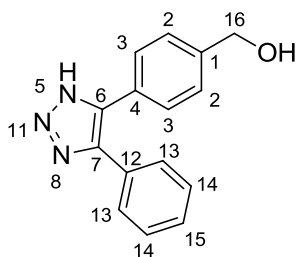
9.4.3.8 4-Azido-1-[4-(4-phenyl-1H-1,2,3-triazol-5-yl)benzyl]piperidine - 123

A 50 mL two-neck round-bottomed flask was charged with 4-azidopiperidin-1-ium 2,2,2-trifluoroacetate **98** (1.2 equiv, 0.148 g, 0.617 mmol) dissolved in MeOH (2.2 mL) as well as 4-(4-Phenyl-1H-1,2,3-triazol-5-yl)benzaldehyde **110** (0.124 g, 0.499 mmol) dissolved in MeOH (2 mL) and DMSO (0.5 mL). More MeOH (2 mL) was added and the reaction mixture was stirred for a few min followed by addition of NaCNBH_3 (1.9 equiv, 0.060 g, 0.95 mmol). It was stirred at RT for 44 h, followed by addition of more NaCNBH_3 (1.3 equiv, 0.041 g, 0.65 mmol). The reaction mixture was then stirred at RT for 4 days. This was diluted with distilled H_2O and the pH was increased to approximately 7 using a saturated solution of aqueous NaHCO_3 and monitoring it with universal indicator paper. The product was extracted with EtOAc (3×150 mL) after adding brine to increase separation of the aqueous and organic layers. The combined organic layers were dried over MgSO_4 and filtered. The solvent was removed *in vacuo* to yield the crude product which was purified by CC (50% EtOAc/Hexane) to afford the title compound **123** (0.038 g, 0.11 mmol, 21%) ($R_f = 0.42$, 60% EtOAc/Hexane) as a pale yellow oil. Additionally, [4-(4-phenyl-1H-1,2,3-triazol-5-

yl)phenyl]methanol **124** (0.051 g, 0.20 mmol, 41%) ($R_f = 0.42$, 60% EtOAc/Hexane) was isolated as a yellow oil.



123



124

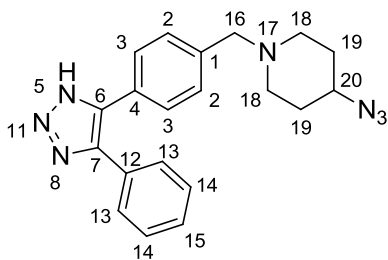
$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.60 – 7.56 (m, 2H, H_{13}), 7.53 (dt, $J = 8.2, 1.8$ Hz, 2H, H_3), 7.42 – 7.36 (m, 3H, H_{14} & H_{15}), 7.33 (d, $J = 8.2$ Hz, 2H, H_2), 3.56 (s, 2H, H_{16}), 3.50 – 3.37 (m, 1H, H_{20}), 2.86 – 2.74 (m, 2H, H_{18}), 2.33 – 2.17 (m, 2H, H_{18}), 1.99 – 1.88 (m, 2H, H_{19}), 1.79 – 1.63 (m, 2H, H_{19}).

$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.60 – 7.52 (m, 4H, H_2 & H_3), 7.42 – 7.35 (m, 5H, H_{13} & H_{14} & H_{15}), 4.75 (s, 2H, H_{16}).

Note: The reaction was repeated several times and these are summarised in Table 8.

9.4.3.9 4-Azido-1-[4-(4-phenyl-1H-1,2,3-triazol-5-yl)benzyl]piperidine - **123**

A 50 mL two-neck round-bottomed flask was charged with 4-(4-Phenyl-1H-1,2,3-triazol-5-yl)benzaldehyde **110** (0.081 g, 0.32 mmol), 4-azidopiperidin-1-ium 2,2,2-trifluoroacetate **98** (2 equiv, 0.155 g, 0.645 mmol) dissolved in DMF (1.5 mL) and formic acid of unknown purity (0.040 mL). The reaction was heated at 60 °C for 17 h and then allowed to cool to RT. More formic acid (0.10 mL) and DMF (3.5 mL) was added to the reaction mixture at RT after which it was heated at 100 °C for 5 h, 80 °C for 38 h and 100 °C for 53 h. The reaction mixture was allowed to cool to RT and quenched with a saturated solution of aqueous NaHCO_3 . Extraction was done with DCM (3 \times 150 mL) and EtOAc (100 mL) and the combined organic layers were dried over MgSO_4 and filtered. The solvent was removed *in vacuo* to yield the crude product which was purified by CC (50% EtOAc/Hexane) to afford the title compound **123** (0.016 g, 0.044 mmol, 14%) ($R_f = 0.42$, 60% EtOAc/Hexane) as a pale yellow oil. There was contamination visible in both the ^1H and the ^{13}C NMR spectra.



123

IR (ATR, cm^{-1}): 2925, 2812, 2089 (N=N=N str), 1448 (-CH₂- C-H bend), 1365, 1253 (C-N str), 985, 854, 775, 697. $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.61 – 7.55 (m, 2H, H_{13}), 7.53 (dt, $J = 8.2, 1.8$ Hz, 2H, H_3), 7.41 – 7.35 (m, 3H, H_{14} & H_{15}), 7.32 (d, $J = 8.4$ Hz, 2H, H_2), 3.56 (s, 2H, H_{16}), 3.50 – 3.38 (m, 1H, H_{20}), 2.87 – 2.74 (m, 2H, H_{18}), 2.28 (t, $J = 10.1$ Hz, 2H, H_{18}), 1.99 – 1.87 (m, 2H,

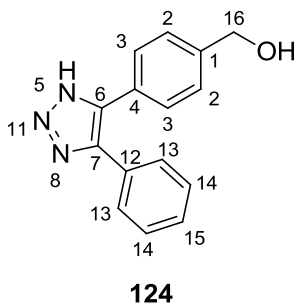
H₁₉), 1.78 – 1.64 (m, 2H, H₁₉). ¹³C NMR (75 MHz, CDCl₃) δ 142.8 (C_{6/7}), 142.7 (C_{6/7}), 138.2 (C₁), 130.4, 129.5, 129.3, 128.7, 128.5, 128.3, 128.1, 62.5 (C₁₆), 57.5 (C₂₀), 51.0 (C₁₈), 30.6 (C₁₉). HRMS: calcd for C₂₀H₂₂N₇⁺ [M+H]⁺, 360.1937, found 360.1940.

Note: This reaction was repeated, using similar conditions, and the title compound **123**, was isolated in a yield of 11%.

9.4.4 A further modification

Z.4.4.1 [4-(4-Phenyl-1H-1,2,3-triazol-5-yl)phenyl]methanol - **124**

A 50 mL one-neck round-bottomed flask was charged with 4-(4-phenyl-1H-1,2,3-triazol-5-yl)benzaldehyde **110** (0.278 g, 1.12 mmol) and MeOH (5 mL). Sodium borohydride (2.1 equiv, 0.087 g, 2.30 mmol) was added to this milky yellow reaction mixture and it increased in temperature with the addition. The resulting clear yellow reaction mixture was left to stir at RT for 60 min. The reaction was allowed to cool to RT and the solvent was removed *in vacuo* to yield a yellow oil to which distilled H₂O was added. Extraction was done using EtOAc (4 × 150 mL) and the combined organic layers were dried over MgSO₄ and filtered. The solvent was removed *in vacuo* to yield the crude title compound **124** (0.292 g, quantitative) (R_f = 0.39, 60% EtOAc/Hexane) as a pale yellow solid. The title compound weighted slightly too much for a quantitative yield, an unidentified contaminant was suspected.



124

Mp 130-131 °C. **IR** (ATR, cm⁻¹): 3124 (O-H str), 2920, 2867, 1446, 1412, 1205, 984, 823 (*para*-substituted Ar C-H oop bend), 773 (monosubstituted Ar C-H oop bend), 696 (monosubstituted Ar C-H oop bend). **¹H NMR** (300 MHz, CDCl₃ & MeOD-d₄) δ 7.54 – 7.46 (m, 4H, H₃ & H₁₃), 7.37 – 7.30 (m, 5H, H₂ & H₁₄ & H₁₅), 4.66 (s, 2H, H₁₆). **¹³C NMR** (75 MHz, CDCl₃ & MeOD-d₄) δ 141.3, 129.8, 128.8, 128.5, 128.4, 128.0, 128.0, 127.0, 64.0, 48.9 (MeOH). **HRMS**: calcd for C₁₅H₁₄N₃O⁺ [M+H]⁺, 252.1137 found 252.1138.

Z.4.4.2 4-Azido-1-[4-(4-phenyl-1H-1,2,3-triazol-5-yl)benzyl]piperidine - **123**

A 50 mL two-neck round-bottomed flask was charged with [4-(4-phenyl-1H-1,2,3-triazol-5-yl)phenyl]methanol **124** (0.160 g, 0.637 mmol), triphenylphosphine (1.0 equiv, 0.166 g, 0.634 mmol), carbon tetrachloride (1.5 mL) and DMF (2.5 mL). The flask was fitted with a reflux condenser and the reaction mixture was heated at 90 °C for 22 h, with more triphenylphosphine (0.19 equiv, 0.032 g, 0.121 mmol) added after the first 3 h. 4-Azidopiperidin-1-ium chloride **97** (1.3 equiv, 0.130 g, 0.799

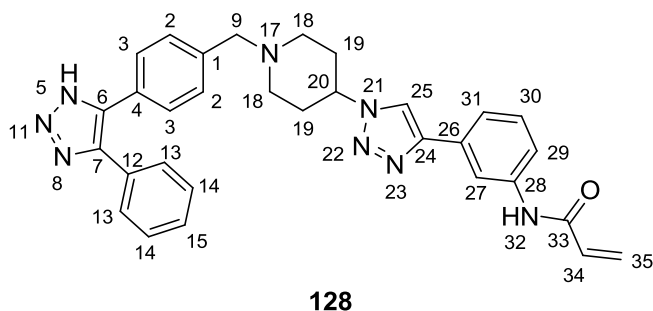
mmol), TEA (1.3 equiv, 0.11 mL, 0.080 g, 0.79 mmol) and more DMF (3 mL) was subsequently added and the reaction mixture was stirred at 90 °C for a further 2 h. The reaction mixture was diluted with distilled H₂O and the product was extracted with EtOAc (3 × 150 mL). The combined organic layers were then dried over MgSO₄ and filtered and the solvent was removed *in vacuo* to yield the crude product which was purified by CC (25 to 40% EtOAc/Hexane) to afford the title compound **123** (0.069 g, 0.19 mmol, 30%) ($R_f = 0.42$, 60% EtOAc/Hexane) as a yellow oil.

IR (ATR, cm⁻¹): 2928, 2810, 2089 (N=N=N str), 1736, 1448 (-CH₂- C-H bend), 1370, 1243 (C-N str), 1120, 1037, 985, 776. 725, 697.

Note: This reaction was also done with the TEA added 16 h later than azidopiperidin-1-ium chloride **97** addition. Starting material was only consumed after the addition of the TEA and the reaction was complete in 2 more h, affording the title compound **123** in a yield of 30%.

9.4.4.3 *N*-[3-(1-[4-(4-Phenyl-1*H*-1,2,3-triazol-5-yl)benzyl]piperidin-4-yl)-1*H*-1,2,3-triazol-4-yl)phenyl]acrylamide - **128**

A 50 mL one-neck round-bottomed flask was charged with 4-azido-1-[4-(4-phenyl-1*H*-1,2,3-triazol-5-yl)benzyl]piperidine **123** (0.038 g, 0.11 mmol), MeCN (1 mL) and *N,N*-Diisopropylethylamine **45** (1.2 equiv, 0.022 g, 0.13), and stirring at RT commenced. *N,N*-Diisopropylethylamine (2.6 equiv, 0.050 mL, 0.037 g, 0.29 mmol) and CuI (57 mol%, 12 mg, 0.060 mmol) was then added and the reaction mixture changed colour from pale yellow to bright yellow. It was stirred at RT for 17 h, followed by addition of more *N,N*-diisopropylethylamine (2.6 equiv, 0.050 mL, 0.037 g, 0.29 mmol) and CuI (20 mol%, 4 mg, 0.02 mmol) and MeCN (1 mL). The reaction mixture was then stirred at RT for another 31 h. The reaction mixture was diluted with DCM and washed with saturated aqueous NH₄Cl (3 × 50 mL) which was then extracted with one portion of EtOAc. The combined organic layers were then dried over MgSO₄ and filtered. The solvent was removed *in vacuo* to yield the crude product which was purified by CC (85% EtOAc/Hexane) to afford still impure title compound **128** (3 mg, 0.006 mmol, 6%) ($R_f = 0.21$, 80% EtOAc/Hexane) as a pale yellow solid. There was contamination visible in the ¹H NMR spectrum.



128

¹H NMR (300 MHz, CDCl₃ & MeOD-d₄)
 δ 7.93 (d, $J = 7.6$ Hz, 1H), 7.89 (s, 1H, H₂₅),
7.84 (t, $J = 1.9$ Hz, 1H, H₂₇), 7.58 – 7.51 (m,
4H), 7.46 – 7.32 (m, 7H), 6.45 (dd, $J = 16.9$,
1.8 Hz, 1H, H₃₅), 6.32 (dd, $J = 16.9$, 9.8 Hz,
1H, H₃₄), 5.75 (dd, $J = 9.8$, 1.8 Hz, H₃₅),
3.61 (s, 2H, H₁₆), 3.43 – 3.39 (m, 1H, H₂₀),

3.11 – 3.03 (m, 1H, H₁₈), 2.31 – 2.14 (m, 5H, H₁₈ & H₁₉).

The title compound was not characterised further as too little compound was available.

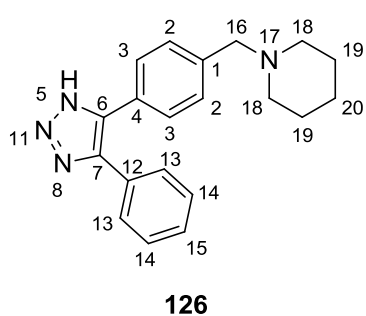
Note: This reaction was carried out several times, testing different reaction conditions. See Table 9.

9.4.4.4 *N*-[3-(1-[4-(4-Phenyl-1*H*-1,2,3-triazol-5-yl)benzyl]piperidin-4-yl)-1*H*-1,2,3-triazol-4-yl)phenyl]acrylamide - **128**

A Schlenk tube was charged with 4-azido-1-[4-(4-phenyl-1*H*-1,2,3-triazol-5-yl)benzyl]piperidine **123** (0.116 g, 0.322 mmol), *N*-(3-ethynylphenyl)acrylamide **45** (1.1 equiv, 0.061 g, 0.36 mmol) and MeCN (0.5 mL). Stirring commenced at RT and tris(triphenylphosphine)copper(I) bromide (5 mol%, 0.016 g, 0.018 mmol) was added, changing the colour of the reaction mixture from pale yellow to dark yellow. It was stirred at RT for 41 h. Tris(triphenylphosphine)copper(I) bromide (10 mol%, 0.030 g, 0.032 mmol), TEA (1.1 equiv, 0.050 mL, 0.036 g, 0.36 mmol) and MeCN (1.5 mL) was then added to the reaction mixture and it was heated at 40 °C for 25 h. The reaction mixture was allowed to cool to RT and quenched with an aqueous solution of ethylenediaminetetraacetic acid. This was extracted with diethyl ether, EtOAc and CHCl₃ and the combined organic layers were then dried over MgSO₄ and filtered. The solvent was removed *in vacuo* to yield the crude material which was purified by CC (60% EtOAc/Hexane to 80% EtOAc/MeOH). No product was isolated.

9.4.4.5 1-[4-(4-Phenyl-1*H*-1,2,3-triazol-5-yl)benzyl]piperidine - **126**

A 50 mL two-neck round-bottomed flask was charged with 4-(4-phenyl-1*H*-1,2,3-triazol-5-yl)benzaldehyde **110** (0.053 g, 0.21 mmol), piperidine **124** (1.1 equiv, 0.022 mL, 0.019 g, 0.22 mmol) and THF (2.5 mL). The reaction was stirred at RT after which NaBH(OAc)₃ (1.9 equiv, 0.085 g, 0.40 mmol) was added. The reaction was then stirred at RT for 4 days and at 45 °C for an additional 20 h. The reaction was allowed to cool to RT and then quenched with a saturated solution of aqueous NaHCO₃. The product was extracted with EtOAc (3 × 150 mL) and the combined organic layers were dried over MgSO₄ and filtered. The solvent was removed *in vacuo* to yield the crude product which was purified by CC (60% EtOAc/Hexane) to afford the title compound **126** (0.047 g, 0.149 mmol, 70%) (R_f = 0.08, 60% EtOAc/Hexane) as a pale yellow solid.



126

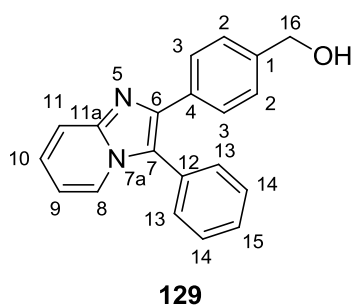
IR (ATR, cm^{-1}): 2933, 2856, 1736, 1651, 1605 (Ar C=C str), 1445 (Ar C=C str), 1370, 1243, 1113, 1039, 985, 850 (*para*-substituted Ar C-H oop bend), 775 (monosubstituted Ar C-H oop bend), 730, 697 (monosubstituted Ar C-H oop bend). **$^1\text{H NMR}$ (300 MHz, CDCl_3) δ** 7.62 – 7.40 (m, 4H, H_3 & H_{13}), 7.40 – 7.22 (m, 5H, H_2 & H_{14} & H_{15}), 3.61 (s, 2H, H_{16}), 2.52 (br s, 3H, H_{18}), 1.74 – 1.32 (m, 7H, H_{18} & H_{19} & H_{20}). **HRMS:** calcd for $\text{C}_{20}\text{H}_{23}\text{N}_4^+$ $[\text{M}+\text{H}]^+$, 319.1923 found

319.1931.

9.4.5 Successful synthesis of the target compounds

9.4.5.1 [4-(3-Phenylimidazo[1,2-*a*]pyridin-2-yl)phenyl]methanol - 129

A 50 mL one-neck round-bottomed flask was charged with 4-(3-phenylimidazo[1,2-*a*]pyridin-2-yl)benzaldehyde **53** (0.170 g, 0.569 mmol) and MeOH (3.5 mL). The milky yellow reaction mixture was cooled to 0 °C by means of an ice-bath and the sodium borohydride (2.1 equiv, 0.045 g, 2.30 mmol) was added. The resulting clear yellow reaction mixture was left to stir at 0 °C temperature for 30 min. The reaction was allowed to cool to RT and the solvent was removed *in vacuo* to yield a pale yellow oil to which distilled H_2O was added. Extraction was done using EtOAc (4 × 150 mL) and the combined organic layers were dried over MgSO_4 and filtered. The solvent was removed *in vacuo* to yield the crude product which was purified by CC (40% to 50% to 80% EtOAc/Hexane) to afford the title compound **129** (0.135 g, 0.449 mmol, 79%) (R_f = 0.11, 50% EtOAc/Hexane) as a white solid.



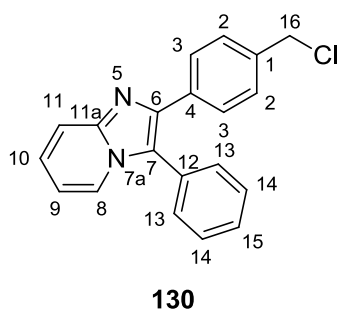
129

Mp 150-151 **IR (ATR, cm^{-1}):** 3217 (O-H str), 2917, 2209, 2048, 1962, 1353, 1038, 752, 704. **$^1\text{H NMR}$ (300 MHz, CDCl_3) δ** 7.96 (dt, J = 6.9, 1.2 Hz, 1H, H_8), 7.68 (dt, J = 9.1, 1.1 Hz, 1H, H_{11}), 7.60 (dt, J = 8.3, 1.7 Hz, 2H, H_3), 7.56 – 7.42 (m, 5H, H_{13} & H_{14} & H_{15}), 7.25 (dt, J = 8.3, 1.7 Hz, 2H, H_2), 7.21 (ddd, J = 9.1, 6.9, 1.2 Hz, 1H, H_{10}), 6.74 (td, J = 6.9, 1.2 Hz, 1H, H_9), 4.67 (s, 2H, H_{16}). **$^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ** 144.8, 142.1, 140.4, 133.2, 130.7, 129.7, 129.5, 128.9, 128.2, 126.9, 124.8, 123.2, 121.1, 117.4, 112.3, 64.9 (C_{16}). **HRMS:** calcd for $\text{C}_{20}\text{H}_{17}\text{N}_2\text{O}^+$ $[\text{M}+\text{H}]^+$, 301.1341 found 301.1344.

9.4.5.2 2-[4-(Chloromethyl)phenyl]-3-phenylimidazo[1,2-*a*]pyridine - 130

A 50 mL two-neck round-bottomed flask was charged with [4-(3-phenylimidazo[1,2-*a*]pyridin-2-yl)phenyl]methanol **129** (0.121 g, 0.403 mmol) and DMF (5 mL). Carbon tetrachloride (2.5 mL) and triphenylphosphine (2.1 equiv, 0.223 g, 0.850 mmol) was added to the reaction mixture and the reaction flask was fitted with a reflux condenser. It was subsequently heated at 90 °C for 21 h.

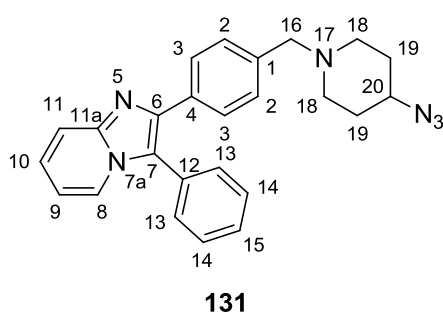
Additional triphenylphosphine (2.0 equiv, 0.216 g, 0.823 mmol) was then added to the reaction mixture and it was heated at 90 °C for an additional 5 h. The reaction mixture was diluted with distilled H₂O and the product was extracted with EtOAc (3 × 150 mL). The combined organic layers were then dried over MgSO₄ and filtered and the solvent was removed *in vacuo* to yield the crude product which was purified by CC (30% EtOAc/Hexane) to afford the title compound **130** (0.149 g, still impure) (R_f = 0.40, 40% EtOAc/Hexane) as a yellow oil.



¹H NMR (300 MHz, CDCl₃) δ 7.95 (dt, *J* = 6.9, 1.3, 1H, H₈), 7.72 – 7.65 (m, 3H, H₃ & H₁₁), 7.59 – 7.43 (m, 5H, H₁₃ & H₁₄ & H₁₅), 7.31 (dt, *J* = 8.4, 1.7 Hz, 2H, H₂), 7.22 (ddd, *J* = 9.1, 6.6, 1.3 Hz, 1H, H₁₀), 6.75 (td, *J* = 6.9, 1.3 Hz, 1H, H₉), 4.58 (s, 2H), aliphatic impurities present. **¹³C NMR (75 MHz, CDCl₃)** δ 144.8, 141.6, 136.4, 134.3, 130.7, 129.7, 129.6, 129.0, 128.5, 128.2, 124.8, 123.8, 121.3, 117.5, 112.3, 46.2 (C₁₆), aliphatic impurities present.

9.4.5.3 2-[4-[(4-Azidopiperidin-1-yl)methyl]phenyl]-3-phenylimidazo[1,2-a]pyridine - **131**

A 50 mL two-neck round-bottomed flask was charged with 4-azidopiperidin-1-ium chloride **97** (1.7 equiv, 0.114 g, 0.702 mmol), DMF (1 mL) and TEA (1.8 equiv, 0.10 mL, 0.073 g, 0.717 mmol). These were stirred together at RT and then 2-[4-(chloromethyl)phenyl]-3-phenylimidazo[1,2-a]pyridine **130** (0.128 g, 0.403 mmol) dissolved in DMF (2 mL) was added. The reaction mixture was heated at 60 °C for 2.5 h. The reaction mixture was allowed to cool to RT and diluted with distilled H₂O. The product was extracted with EtOAc (3 × 150 mL) and the combined organic layers were then dried over MgSO₄ and filtered. The solvent was removed *in vacuo* to afford the crude product which was diluted with brine and extracted with diethyl ether and then purified by CC (80% EtOAc/Hexane) to afford the title compound **131** (0.115 g, 0.282 mmol, 70%) (R_f = 0.12, 80% EtOAc/Hexane) as a thick yellow oil.

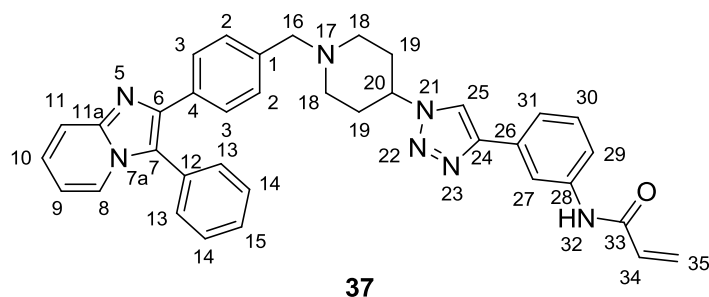


IR (ATR, cm⁻¹): 3069, 2928, 2826, 2094 (N=N=N str), 1542, 1448, 1385, 1365, 1341, 1317, 1256, 1237, 753, 743, 696. **¹H NMR (300 MHz, CDCl₃)** δ 7.94 (dd, *J* = 6.9, 0.4 Hz, 1H, H₈), 7.67 (dd, *J* = 9.1, 0.6 Hz, 1H, H₁₁), 7.63 (d, *J* = 8.1 Hz, 2H, H₃), 7.58 – 7.42 (m, 5H, H₁₃ & H₁₄ & H₁₅), 7.21 (d, *H* = 8.1 Hz, 2H, H₂), 7.20 – 7.16 (m, 1H, H₁₀), 6.72 (t, *J* = 6.9 Hz, 1H, H₉), 3.47 (s, 2H, H₁₆), 3.43 – 3.30 (m, 1H, H₂₀), 2.81 – 2.68 (m, 2H, H₁₈), 2.22 – 2.07 (m, 2H, H₁₈), 1.94 – 1.81 (m, 2H, H₁₉), 1.73 – 1.57 (m, 2H, H₁₉). **¹³C NMR (75 MHz, CDCl₃)** δ 144.72, 142.2, 137.3, 133.0, 130.7, 129.9, 129.5, 128.9, 128.8, 127.8,

124.6, 123.2, 120.9, 117.4, 112.2, 62.6 (C₁₆), 57.7 (C₂₀), 51.1 (C₁₈), 30.8 (C₁₉). **HRMS:** calcd for C₂₅H₂₅N₆⁺ [M+H]⁺, 409.2141, found 409.2138.

9.4.5.4 *N*-[3-(1-[1-[4-(3-Phenylimidazo[1,2-*a*]pyridin-2-yl)benzyl]piperidin-4-yl]-1*H*-1,2,3-triazol-4-yl)phenyl]acrylamide - **37**

A Schlenk tube was charged with 2-{4-[(4-azidopiperidin-1-yl)methyl]phenyl}-3-phenylimidazo[1,2-*a*]pyridine **131** (0.038 g, 0.094 mmol), *N*-(3-ethynylphenyl)acrylamide **45** (2.2 equiv, 0.035 g, 0.20 mmol), *N,N*-diisopropylethylamine (3.1 equiv, 0.050 mL, 0.037 g, 0.29 mmol) and MeCN (1.5 mL). The milky yellow reaction mixture was heated to 25 °C to dissolve the contents and the reaction mixture turned an almost clear yellow. CuI (25 mol%, 5 mg, 0.02 mmol) was added and the reaction was stirred at 25 °C for 15 h. The reaction mixture was diluted with DCM, filtered through a cotton wool plug and concentrated *in vacuo*. The resulting orange crude material was purified by CC (1% then 2.5% MeOH/DCM) to afford the title compound **37** (0.068 g, quantitative) (R_f = 0.19, EtOAc) as a pale yellow solid. For characterisation, EtOAc and diethyl ether was visible in the ¹H NMR spectrum, causing the excess mass of the product. It was trapped by the compound which was originally an oil before further drying under vacuum. The sample sent for biochemical evaluation was spread open for a high surface area to volume ratio and further dried under vacuum, ensuring a completely dry sample.

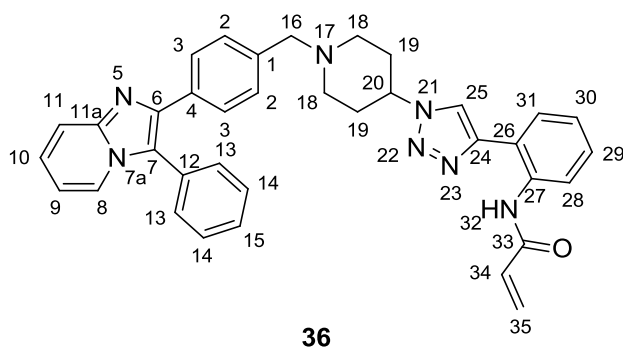


Mp ~180 °C. **IR (ATR, cm⁻¹):** 3278 (N-H str), 2946, 2364, 2165, 1685 (C=O str), 1615 (Ar C=C str), 1567, 1533, 1480 (Ar C=C str), 1441 (-CH₂-bend), 1343, 1236 (C-N str), 753, 732, 699. **¹H NMR (600 MHz, CDCl₃) δ** 7.92 (d, *J* = 6.6 Hz, 1H, H₈), 7.90 (d, *J*

= 8.1 Hz, 1H, H₃₁), 7.87 (s, 1H, H₂₅), 7.80 (s, 1H, H₂₇), 7.63 (d, *J* = 9.4 Hz, 1H, H₁₁), 7.59 (d, *J* = 8.2 Hz, 2H, H₃), 7.55 – 7.45 (m, 3H, H₁₃ & H₂₉), 7.44 – 7.39 (m, 3H, H₁₄ & H₁₅), 7.32 (t, *J* = 8.1 Hz, 1H, H₃₀), 7.23 (d, *J* = 8.2 Hz, 2H, H₂), 7.20 (t, *J* = 7.9 Hz, 1H, H₁₀), 6.74 (t, *J* = 6.6 Hz, 1H, H₉), 6.40 (dd, *J* = 17.0, 1.4 Hz, 1H, H₃₅), 6.33 (dd, *J* = 17.0, 10.1 Hz, 1H, H₃₄), 5.70 (dd, *J* = 10.1, 1.4 Hz, 1H, H₃₅), 4.51 – 4.44 (m, 1H, H₂₀), 3.55 (s, 2H, H₁₆), 3.03 (d, *J* = 11.7 Hz, 2H, H₁₈), 2.27 – 2.05 (m, 6H, H₁₈ & H₁₉). **¹³C NMR (150 MHz, CDCl₃) δ** 164.2 (C₃₃), 147.2 (C₂₄), 144.8 (C_{11a}), 142.0 (C₆), 139.0 (C₂₈), 138.9 (C₇), 136.8 (C₁), 133.3 (C₄), 131.4 (C₃₄), 131.2 (C₂₆), 130.8 (C₁₃), 129.8 (C₁₂), 129.7 (C₁₄), 129.7 (C₃₀), 129.2 (C₂), 129.1 (C₁₅), 128.1 (C₃), 127.6 (C₃₅), 125.1 (C₁₀), 123.5 (C₃₁), 121.4 (C₂₉), 119.8 (C₂₇), 118.0 (C₂₅), 117.4 (C₁₁), 116.9 (C₈), 112.5 (C₉), 62.4 (C₁₆), 58.3 (C₂₀), 52.1 (C₁₈), 32.5 (C₁₉). **HRMS:** calcd for C₃₆H₃₄N₇O⁺ [M+H]⁺, 580.2825, found 580.2805.

9.4.5.5 *N*-[2-(1-[1-[4-(3-Phenylimidazo[1,2-*a*]pyridin-2-yl)benzyl]piperidin-4-yl]-1*H*-1,2,3-triazol-4-yl)phenyl]acrylamide - **36**

A Schlenk tube was charged with 2-{4-[(4-azidopiperidin-1-yl)methyl]phenyl}-3-phenylimidazo[1,2-*a*]pyridine **131** (0.043 g, 0.11 mmol), *N*-(2-ethynylphenyl)acrylamide **101** (1.7 equiv, 0.031 g, 0.18 mmol), *N,N*-diisopropylethylamine (2.7 equiv, 0.050 mL, 0.037 g, 0.29 mmol) and MeCN (1 mL). The milky yellow reaction mixture was heated to 45 °C to dissolve the contents and the reaction mixture turned a clear yellow. The reaction mixture was then allowed to cool to RT before CuI (38 mol%, 8 mg, 0.04 mmol) was added. The reaction was stirred at RT for 24 h and more MeCN (1 mL) was added after the first 4.5 h. The reaction mixture was then heated to 25 °C and more CuI (20 mol%, 4 mg, 0.02 mmol) was added. It was stirred at 25 °C for 3 days and turned a light orange colour with time. The reaction mixture was diluted with DCM, filtered through a cotton wool plug and concentrated *in vacuo*. The resulting orange crude material was purified by CC (2.5% MeOH/DCM) to afford the still impure title compound **36** which was then further purified by a second round of CC (2.5% MeOH/DCM) to afford the title compound **36** (0.022 g, 0.038 mmol, 36%) ($R_f = 0.54$, 5% MeOH/DCM) as a pale yellow solid.



36

Mp 114-116 °C. **IR (ATR, cm^{-1}):** 3074, 2943, 1684 (C=O str), 1615 (Ar C=C str), 1589, 1540, 1476 (Ar C=C str), 1448 (-CH₂- bend), 1399, 1344, 1320, 1270 (C-N str), 1192, 978, 753, 700. **¹H NMR (400 MHz, CDCl₃) δ** 11.68 (s, 1H, H₃₂), 8.76 (d, $J = 7.7$ Hz, 1H, H₃₁), 7.95 (dt, $J = 6.9, 1.2$ Hz, 1H, H₈), 7.89 (s, 1H, H₂₅), 7.73 (dt, $J = 9.1, 1.2$ Hz, 1H, H₁₁),

7.65 (d, 8.6 Hz, 2H, H₃), 7.57 – 7.44 (m, 6H, H₁₃ & H₁₄ & H₁₅ & H₂₈), 7.35 (t, $J = 8.0$ Hz, 1H, H₂₉), 7.25 (d, $J = 8.6$ Hz, 2H, H₂), 7.21 (ddd, $J = 9.1, 6.8, 1.3$ Hz, 1H, H₁₀), 7.10 (td, $J = 7.7, 1.2$ Hz, 1H, H₃₀), 6.76 (td, $J = 6.9, 1.3$ Hz, 1H, H₉), 6.44 (d, $J = 2.5$ Hz, 1H, H₃₅), 6.43 (d, $J = 9.1$ Hz, 1H, H₃₄), 5.76 (dd, $J = 9.1, 2.5$ Hz, 1H, H₃₅), 4.57 – 4.48 (m, 1H, H₂₀), 3.55 (s, 2H, H₁₆), 3.06 (d, $J = 9.8$ Hz, 2H, H₁₈), 2.27 – 2.12 (m, 7H, H₁₈ & H₁₉ & Acetone). **¹³C NMR (100 MHz, CDCl₃) δ** 164.2 (C₃₃), 147.4, 144.7, 141.9, 137.1, 136.5, 133.0, 132.7, 130.7, 130.4, 129.6, 129.5, 129.0, 128.9, 128.9, 128.0, 126.8, 126.5, 124.9, 123.4, 123.2, 121.4, 121.0, 118.5, 117.4, 112.4, 62.3 (C₁₆), 58.7 (C₂₀), 52.0 (C₁₈), 32.6 (C₁₉). **HRMS:** calcd for C₃₆H₃₄N₇O⁺ [M+H]⁺, 580.2825, found 580.2831.

CHAPTER 10 – ADDENDUM A

The NMR spectra of final compounds **36** and **37**, as well as truncated compounds **116**, **119** and **122**, can be found in the attached compact disk.

CHAPTER 11 – REFERENCES

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